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(54) **COMPOSITIONS AND METHODS FOR REDUCING MHC CLASS I IN A CELL**

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(73) Assignee: **Intellia Therapeutics, Inc.**, Cambridge, MA (US)

(21) Appl. No.: **18/980,462**

(22) Filed: **Dec. 13, 2024**

Related U.S. Application Data

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C12N 15/90	(2006.01)

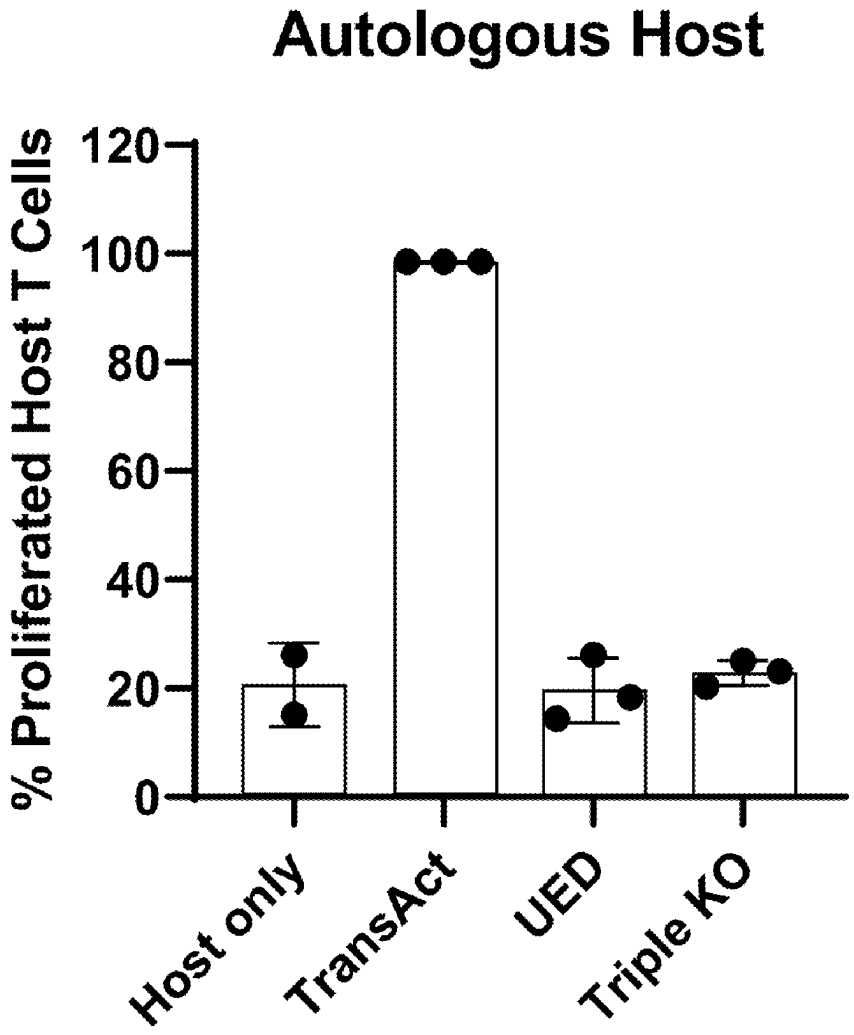
(52) **U.S. Cl.**

CPC ***A61K 40/22*** (2025.01); ***A61K 40/31*** (2025.01); ***C12N 9/226*** (2025.05); ***C12N 15/11*** (2013.01); ***C12N 15/907*** (2013.01); *C12N 2310/20* (2017.05)

(57) **ABSTRACT**

Compositions and methods for reducing MHC class I protein expression in a cell comprising genetically modifying MHC class I for use e.g., in adoptive cell transfer therapies.

Specification includes a Sequence Listing.



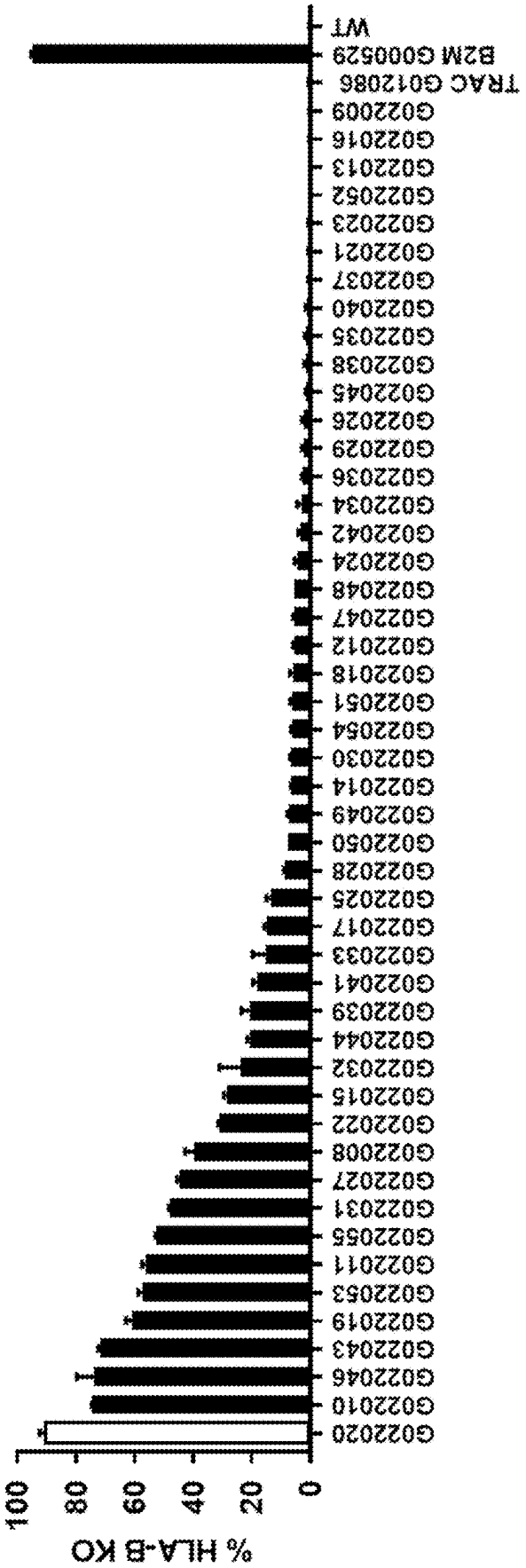

% HLA-B KO
100
80
60
40
20
0
G022020
G022010
G022046
G022043
G022019
G022053
G022011
G022055
G022031
G022027
G022008
G022022
G022015
G022032
G022044
G022039
G022041
G022033
G022017
G022025
G022028
G022050
G022049
G022014
G022030
G022054
G022051
G022018
G022012
G022047
G022048
G022024
G022042
G022034
G022036
G022029
G022026
G022045
G022038
G022035
G022040
G022037
G022021
G022023
G022052
G022013
G022016
G022009
TRAC G012086
B2M G000529
WT

Fig. 1

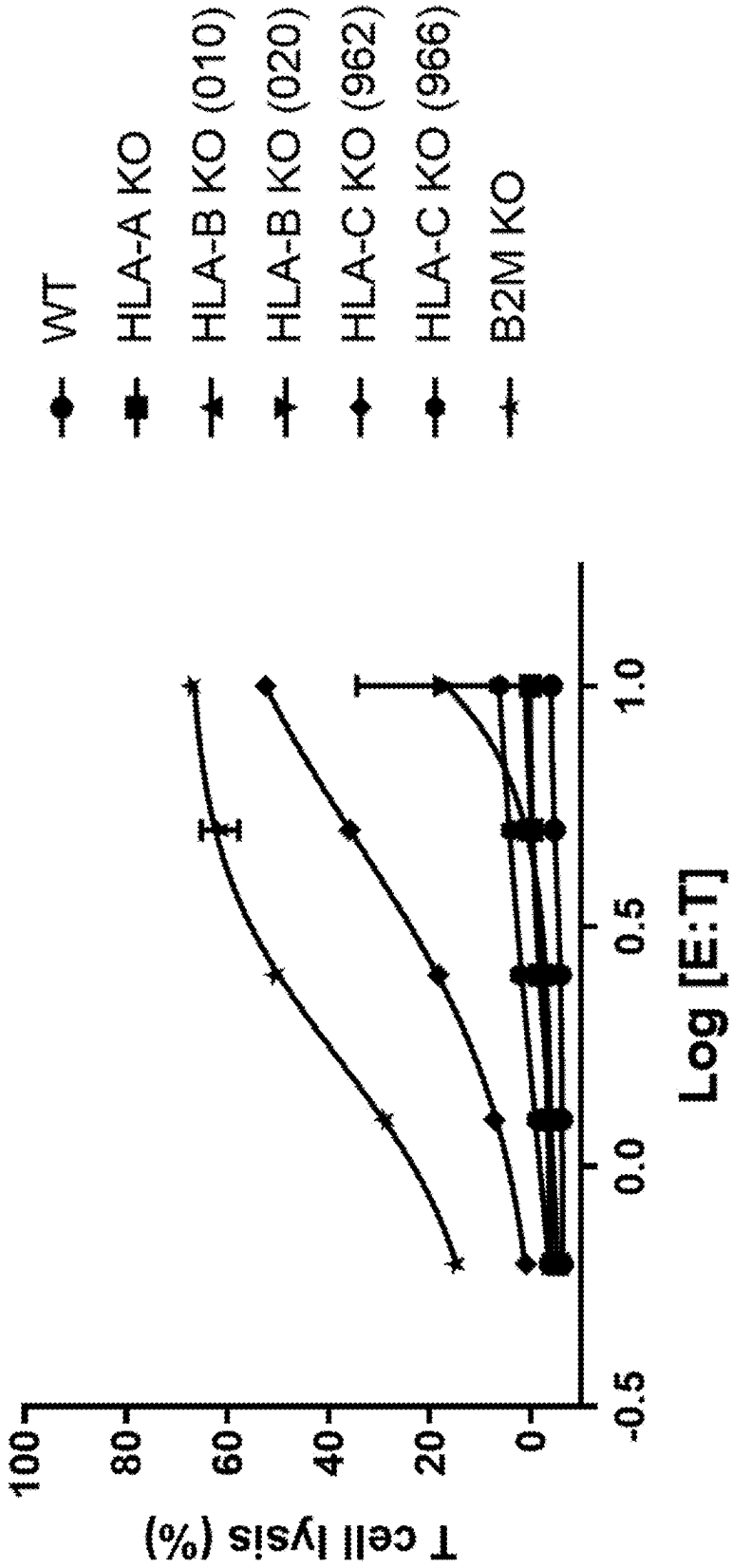

WT
HLA-A KO
HLA-B KO (010)
HLA-B KO (020)
HLA-C KO (962)
HLA-C KO (966)
B2M KO
T cell lysis (%)
100
80
60
40
20
0
-0.5
0.0
0.5
1.0
Log [E:T]

Fig. 2

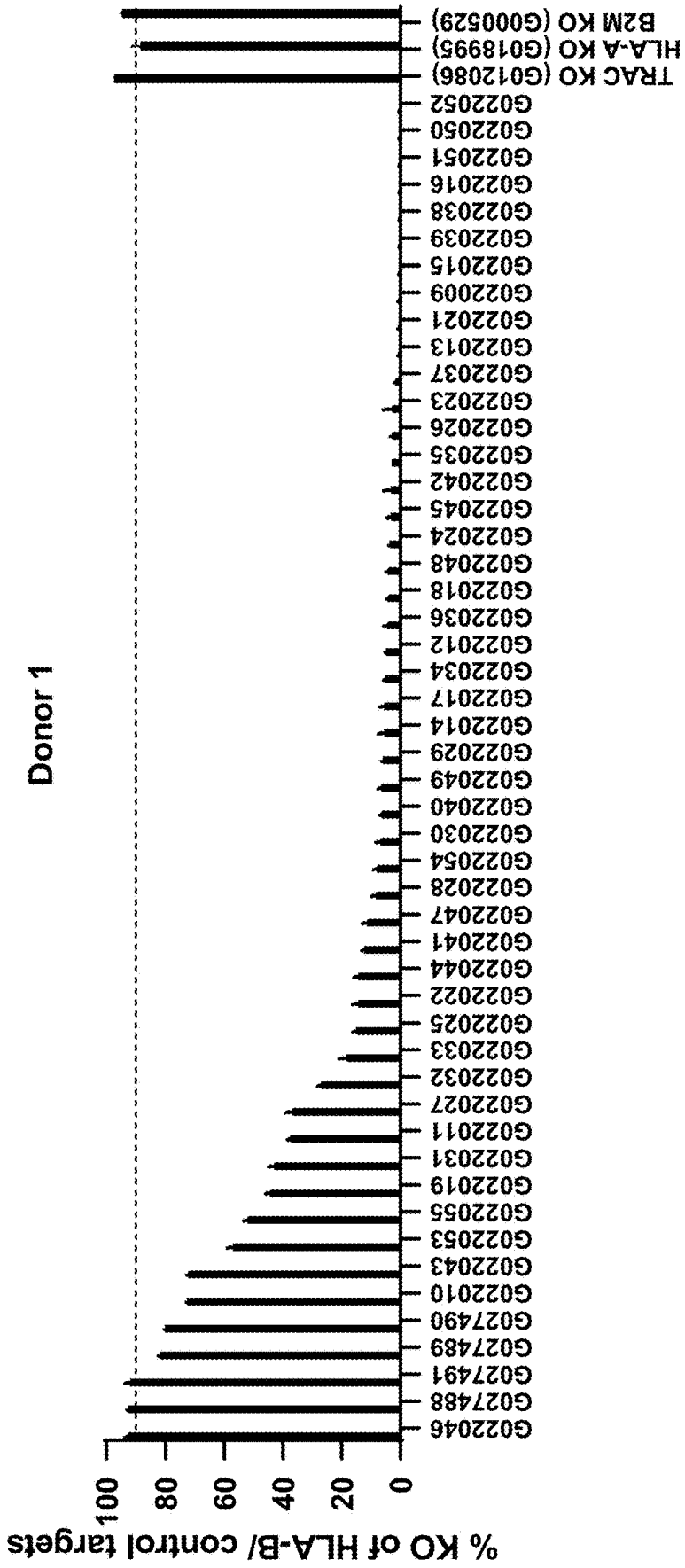

Donor 1
% KO of HLA-B/ control targets
100
80
60
40
20
0
G022046
G027488
G027491
G027489
G027490
G022010
G022043
G022053
G022055
G022019
G022031
G022011
G022027
G022032
G022033
G022025
G022022
G022044
G022041
G022047
G022028
G022054
G022030
G022040
G022049
G022029
G022014
G022017
G022034
G022012
G022036
G022018
G022048
G022024
G022045
G022042
G022035
G022026
G022023
G022037
G022013
G022021
G022009
G022015
G022039
G022038
G022016
G022051
G022050
G022052
TRAC KO (G012086)
HLA-A KO (G018995)
B2M KO (G000529)

Fig. 3A

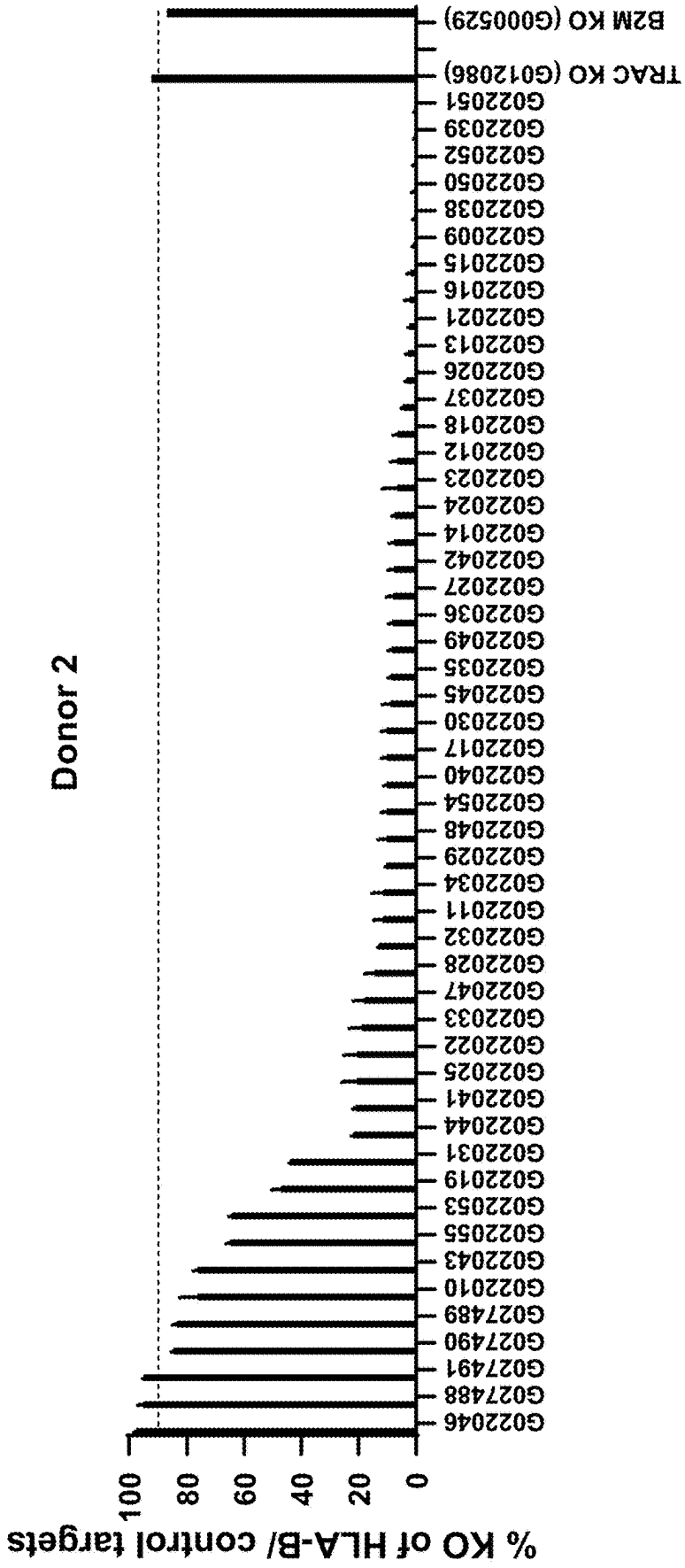

Donor 2
% KO of HLA-B/ control targets
100
80
60
40
20
0
G022046
G027488
G027491
G027490
G027489
G022010
G022043
G022055
G022053
G022019
G022031
G022044
G022041
G022025
G022022
G022033
G022047
G022028
G022032
G022011
G022034
G022029
G022048
G022054
G022040
G022017
G022030
G022045
G022035
G022049
G022036
G022027
G022042
G022014
G022024
G022023
G022012
G022018
G022037
G022026
G022013
G022021
G022016
G022015
G022009
G022038
G022050
G022052
G022039
G022051
TRAC KO (G012086)
B2M KO (G000529)

Fig. 3B

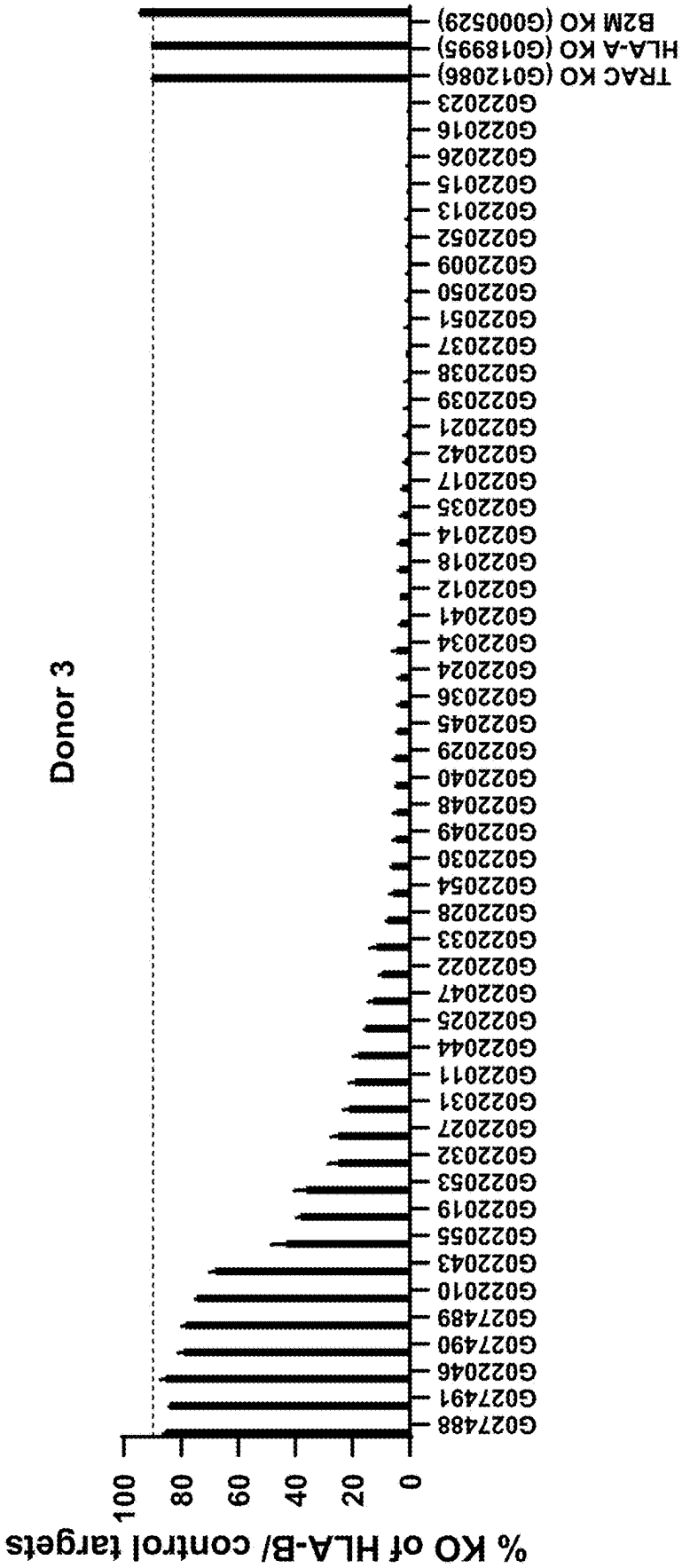

Donor 3
% KO of HLA-B/ control targets
100
80
60
40
20
0
G027488
G027491
G022046
G027490
G027489
G022010
G022043
G022055
G022019
G022053
G022032
G022027
G022031
G022011
G022044
G022025
G022047
G022022
G022033
G022028
G022054
G022030
G022049
G022048
G022040
G022029
G022045
G022036
G022024
G022034
G022041
G022012
G022018
G022014
G022035
G022017
G022042
G022021
G022039
G022038
G022037
G022051
G022050
G022009
G022052
G022013
G022015
G022026
G022016
G022023
TRAC KO (G012086)
HLA-A KO (G018995)
B2M KO (G000529)

Fig. 3C

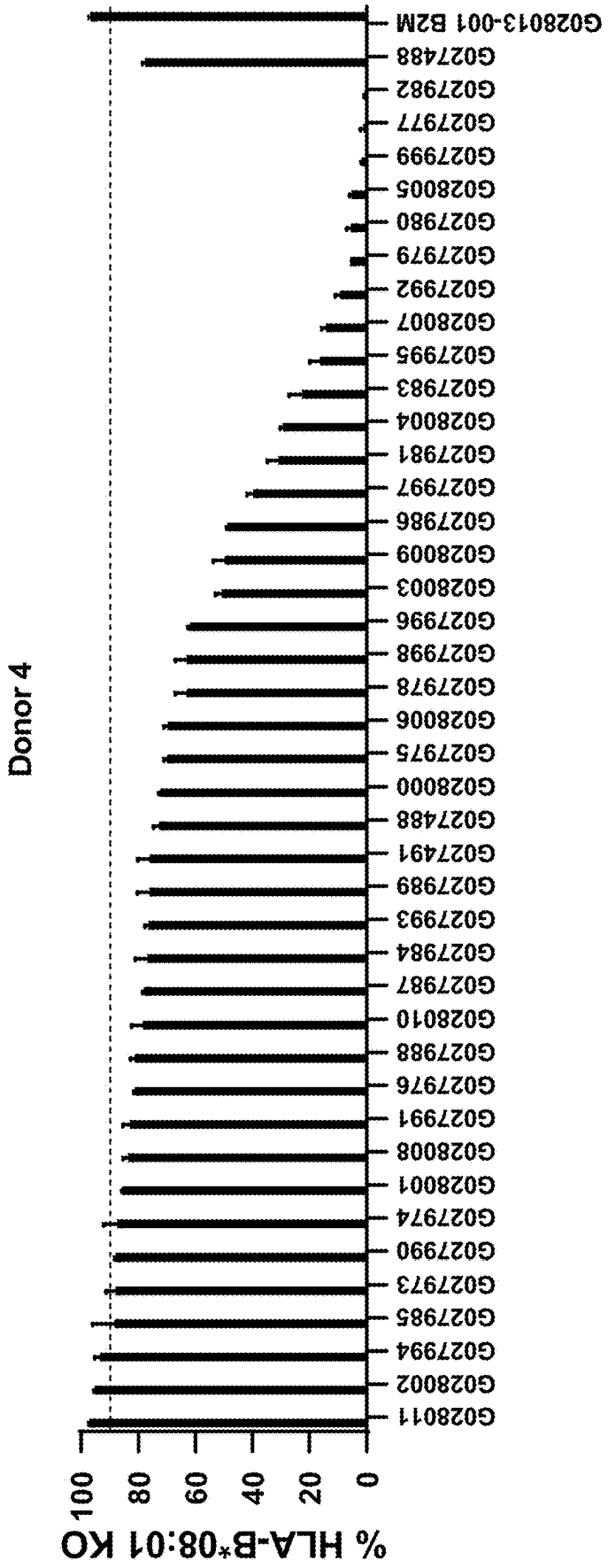

Donor 4
% HLA-B*08:01 KO
100
80
60
40
20
0
G028011
G028002
G027994
G027985
G027973
G027990
G027974
G028001
G028008
G027991
G027976
G027988
G028010
G027987
G027984
G027993
G027989
G027491
G027488
G028000
G027975
G028006
G027978
G027998
G027996
G028003
G028009
G027986
G027997
G027981
G028004
G027983
G027995
G028007
G027992
G027979
G027980
G028005
G027999
G027977
G027982
G027488
G028013-001 B2M

Fig. 3D

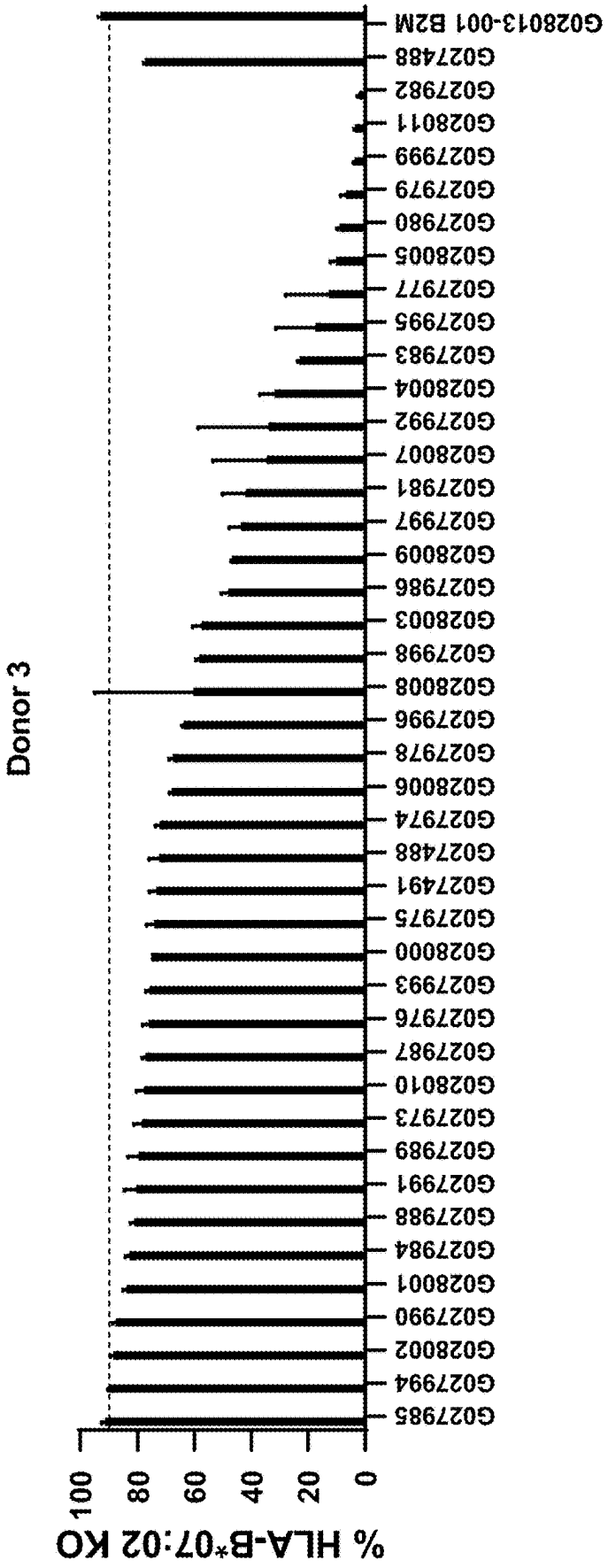

Donor 3
% HLA-B*07:02 KO
100
80
60
40
20
0
G027985
G027994
G028002
G027990
G028001
G027984
G027988
G027991
G027989
G027973
G028010
G027987
G027976
G027993
G028000
G027975
G027491
G027488
G027974
G028006
G027978
G027996
G028008
G027998
G028003
G027986
G028009
G027997
G027981
G028007
G027992
G028004
G027983
G027995
G027977
G028005
G027980
G027979
G027999
G028011
G027982
G027488
G028013-001 B2M

Fig. 3E

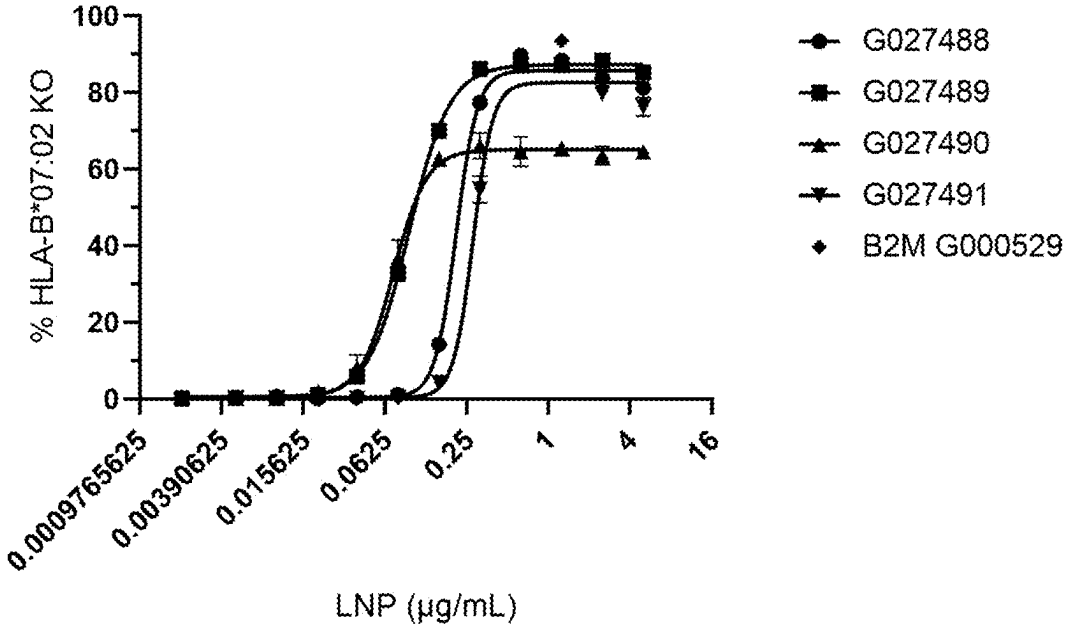

100
80
60
40
20
0
% HLA-B*07:02 KO
0.0009765625
0.00390625
0.015625
0.0625
0.25
1
4
16
LNP (µg/mL)
G027488
G027489
G027490
G027491
B2M G000529

Fig. 4A

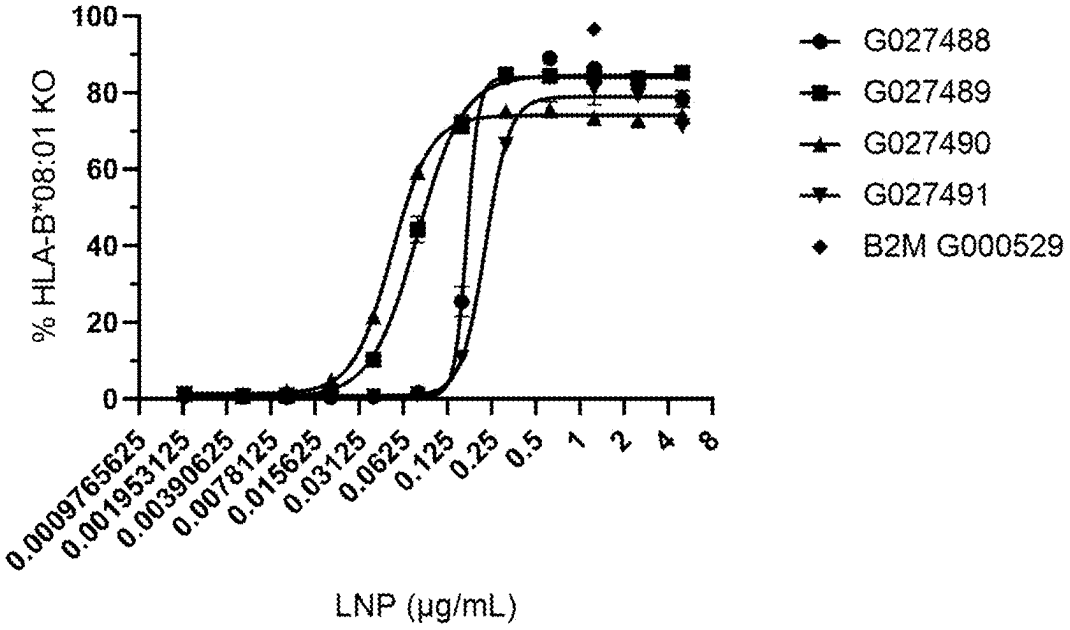

100
80
60
40
20
0
% HLA-B*08:01 KO
0.0009765625
0.001953125
0.00390625
0.0078125
0.015625
0.03125
0.0625
0.125
0.25
0.5
1
2
4
8
LNP (µg/mL)
G027488
G027489
G027490
G027491
B2M G000529

Fig. 4B

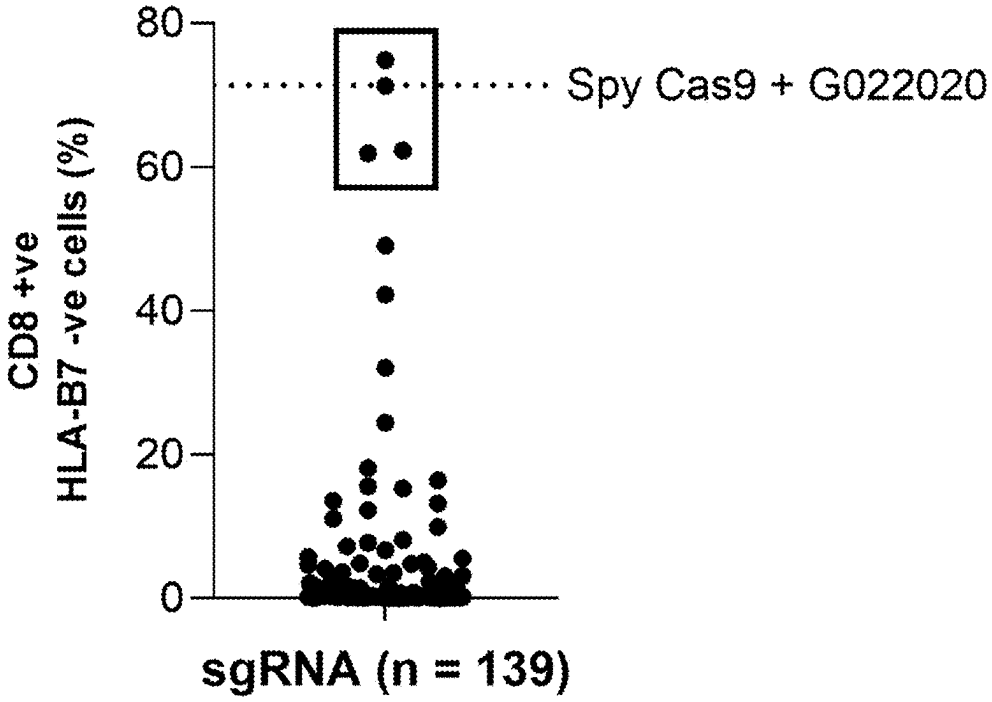


Fig. 5A

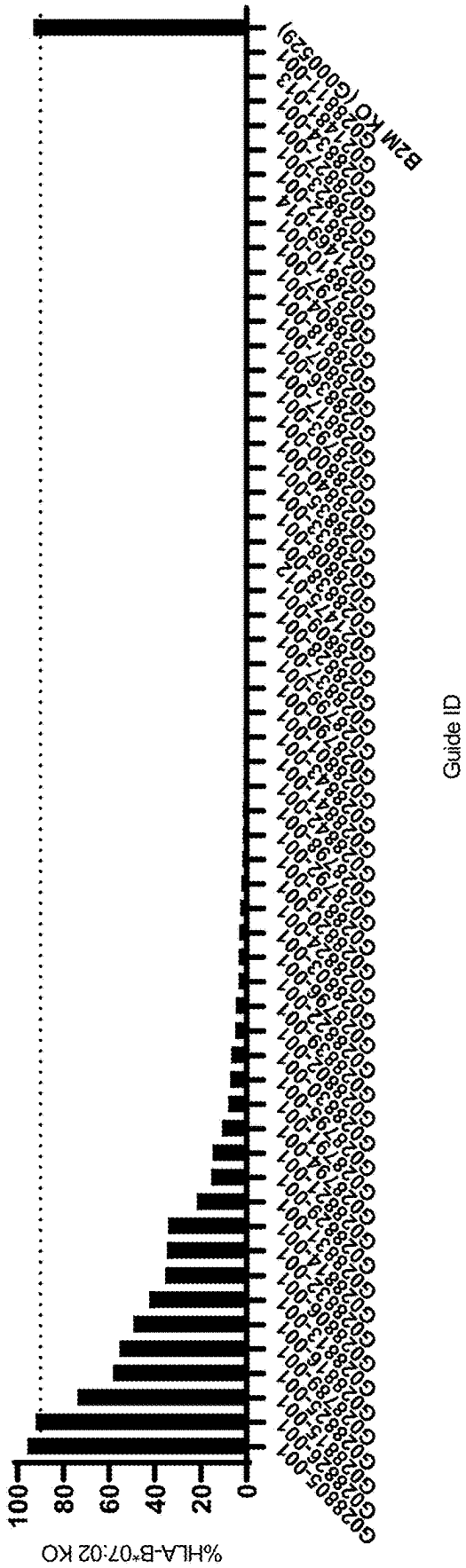


Fig. 5B

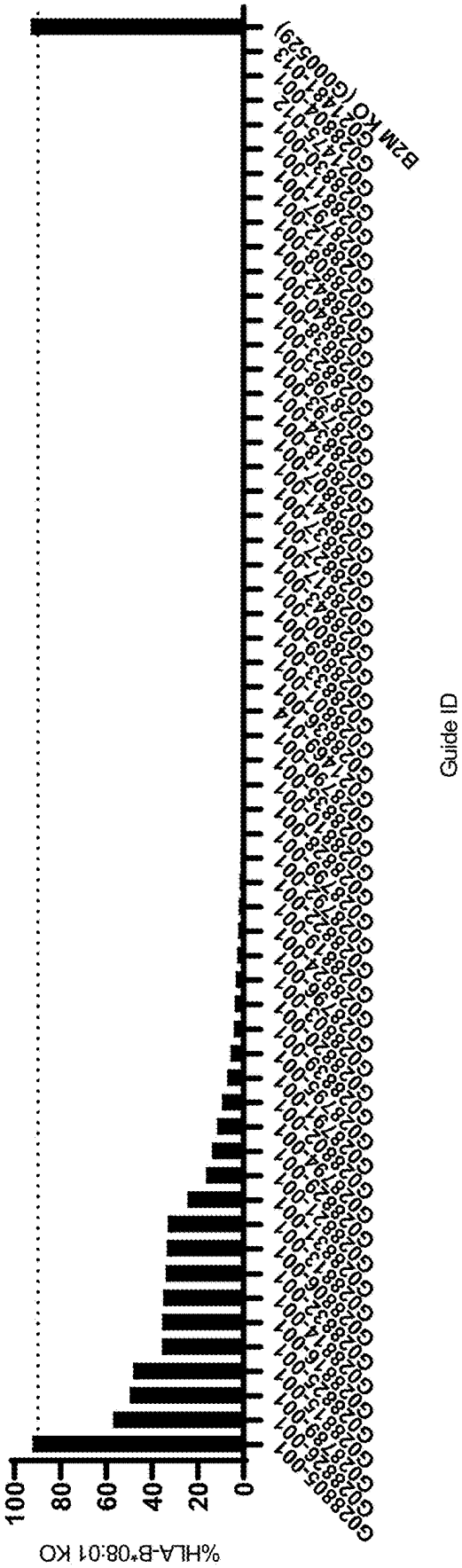


Fig. 5C

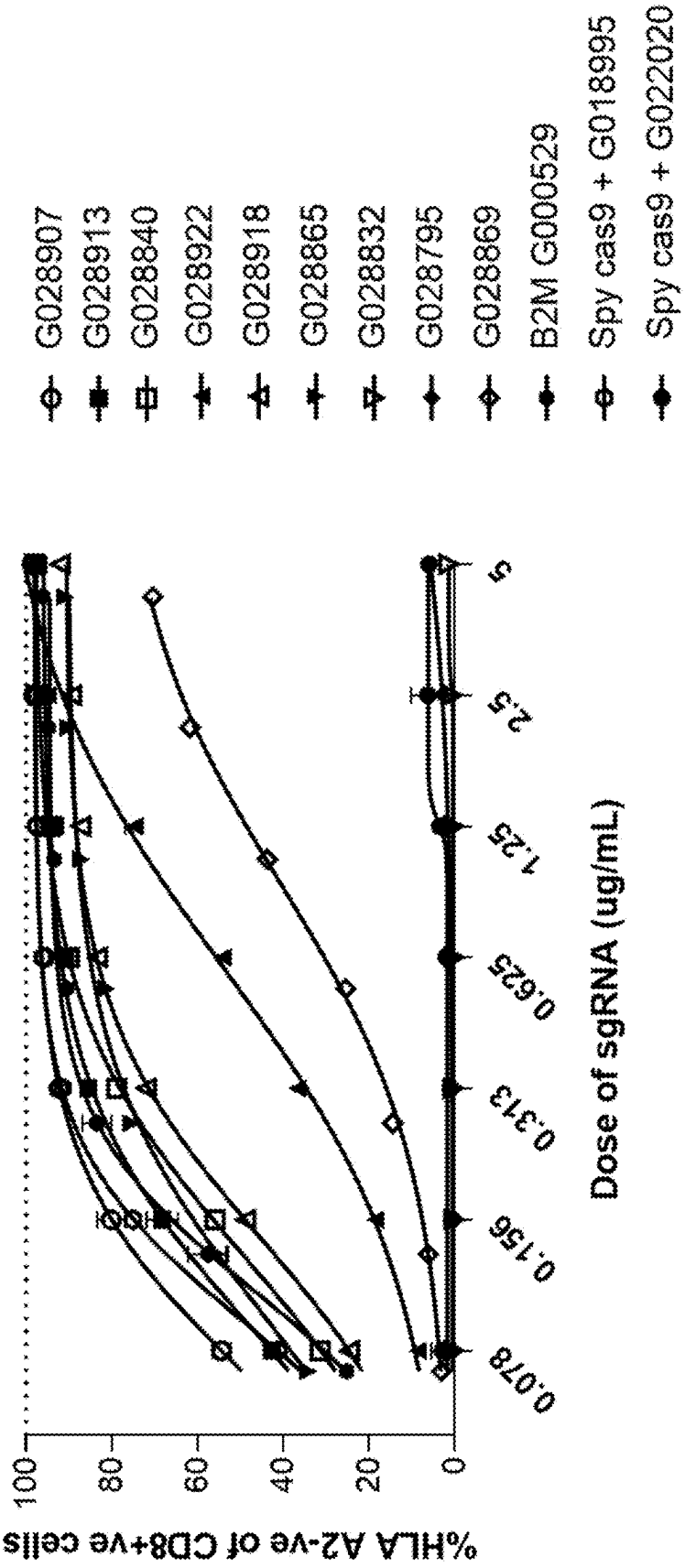

%HLA A2-ve of CD8+ve cells
100
80
60
40
20
0
0.078
0.156
0.313
0.625
1.25
2.5
5
Dose of sgRNA (ug/mL)
G028907
G028913
G028840
G028922
G028918
G028865
G028832
G028795
G028869
B2M G000529
Spy cas9 + G018995
Spy cas9 + G022020

Fig. 6

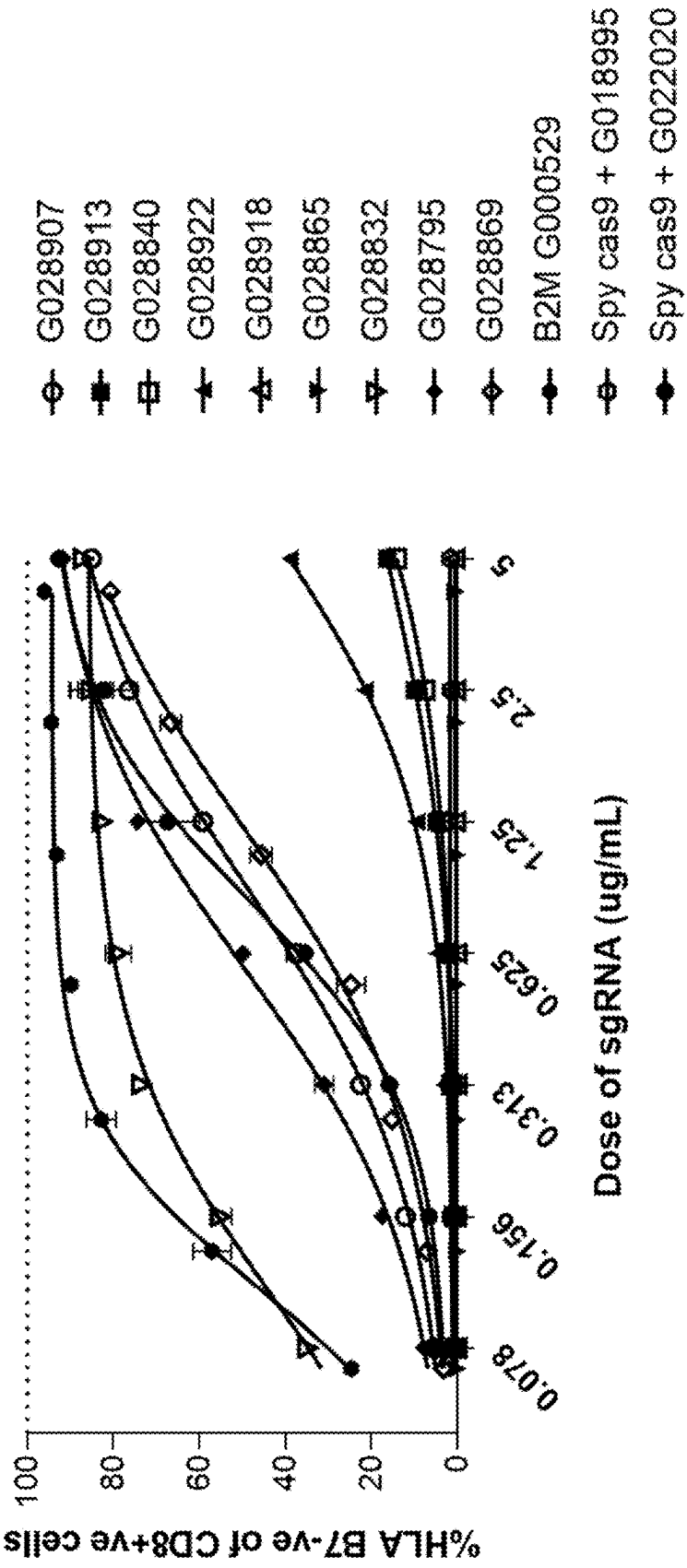

%HLA B7-ve of CD8+ve cells
100
80
60
40
20
0
0.078
0.156
0.313
0.625
1.25
2.5
5
Dose of sgRNA (ug/mL)
G028907
G028913
G028840
G028922
G028918
G028865
G028832
G028795
G028869
B2M G000529
Spy cas9 + G018995
Spy cas9 + G022020

Fig. 7

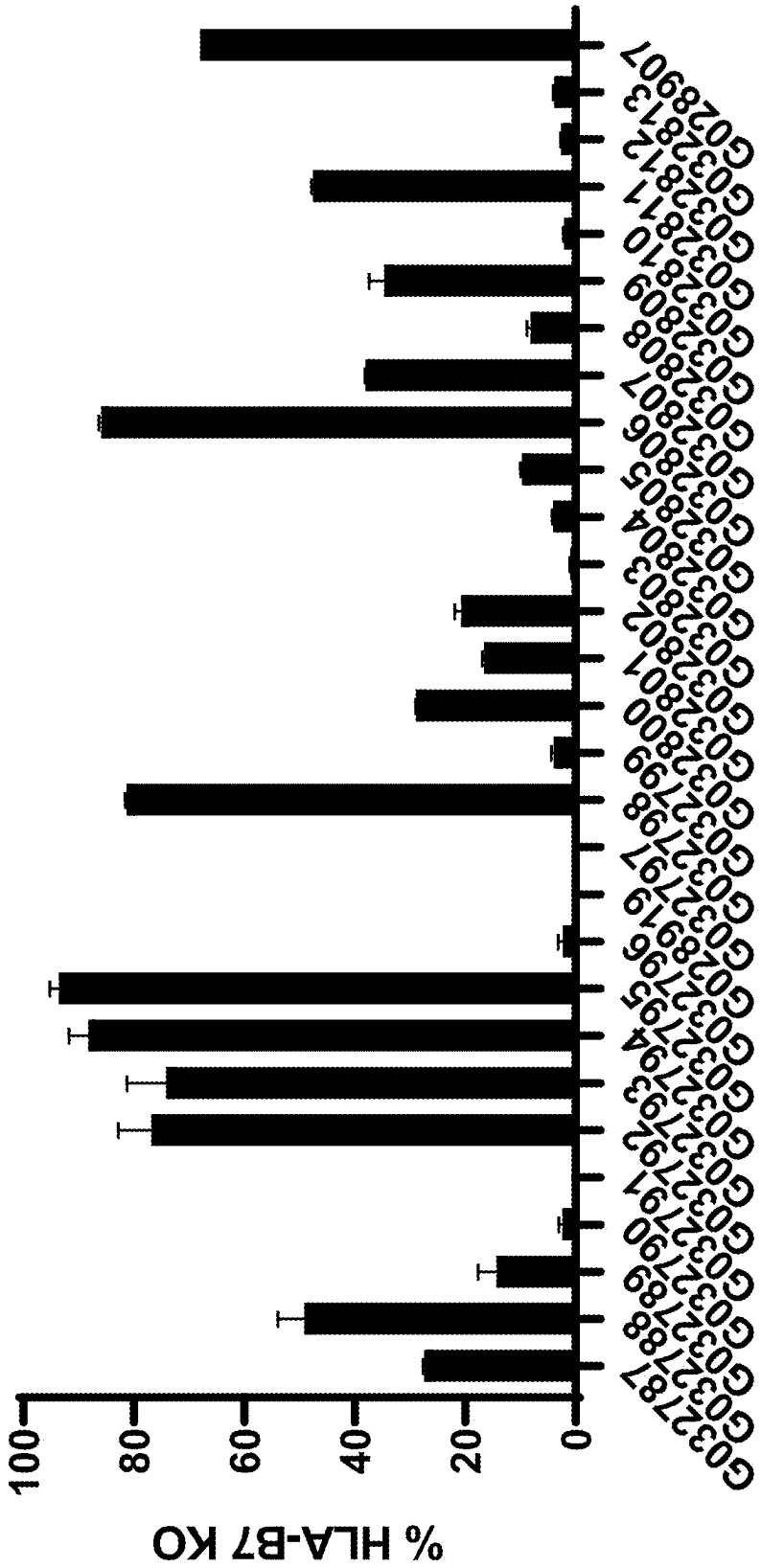

% HLA-B7 KO
100
80
60
40
20
0
G032781
G032788
G032789
G032790
G032791
G032792
G032793
G032794
G032795
G032796
G032797
G032798
G032799
G032800
G032801
G032802
G032803
G032804
G032805
G032806
G032807
G032808
G032809
G032810
G032811
G032812
G032813
G032890
G032891

Fig. 8A

% HLA-B8 KO
100
80
60
40
20
0
G032187
G032188
G032189
G032190
G032191
G032192
G032193
G032194
G032195
G032196
G032197
G032198
G032199
G032800
G032801
G032802
G032803
G032804
G032805
G032806
G032807
G032808
G032809
G032810
G032811
G032812
G032813
G028907

Fig. 8B

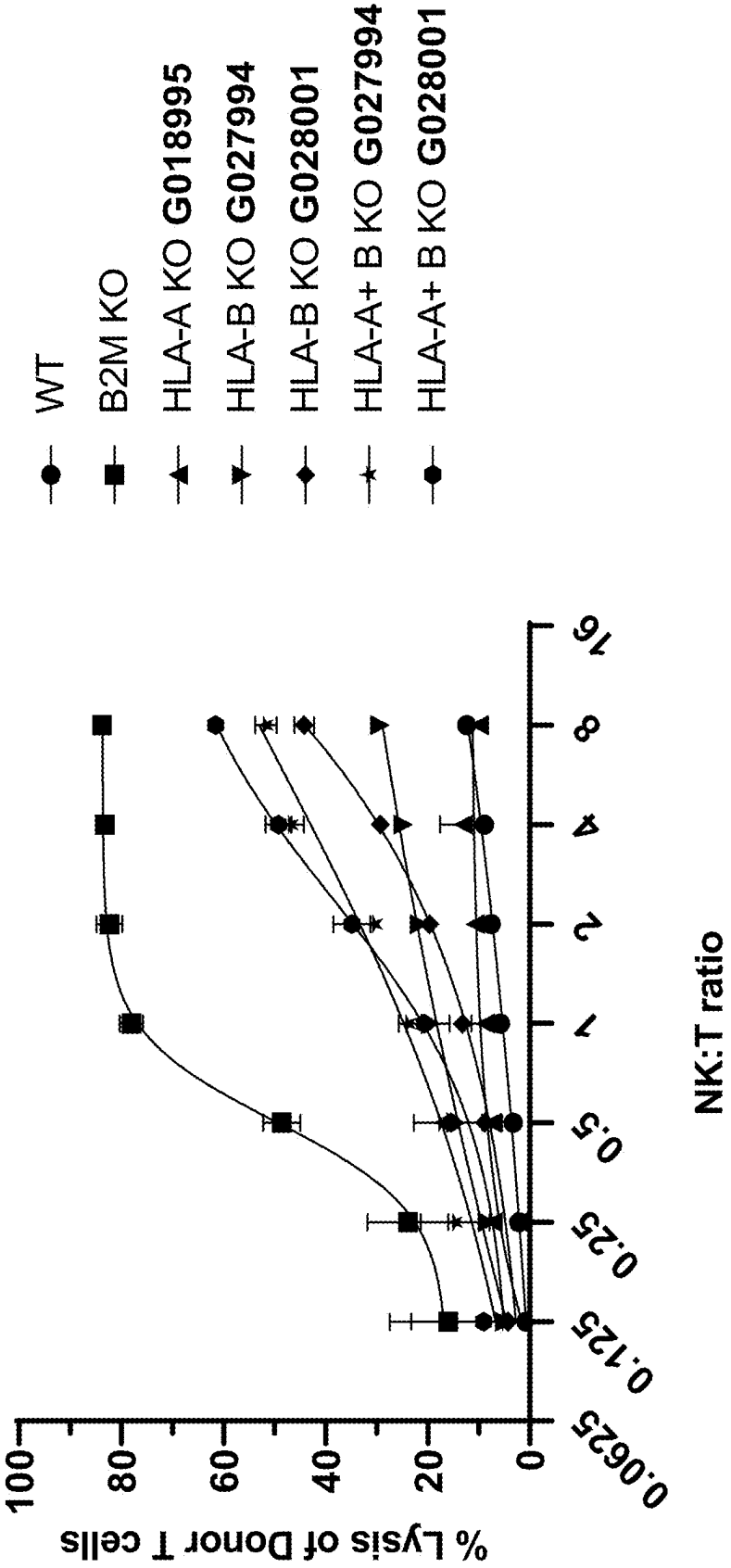

WT
B2M KO
HLA-A KO G018995
HLA-B KO G027994
HLA-B KO G028001
HLA-A+ B KO G027994
HLA-A+ B KO G028001
% Lysis of Donor T cells
100
80
60
40
20
0
0.0625
0.125
0.25
0.5
1
2
4
8
16
NK:T ratio

Fig. 9

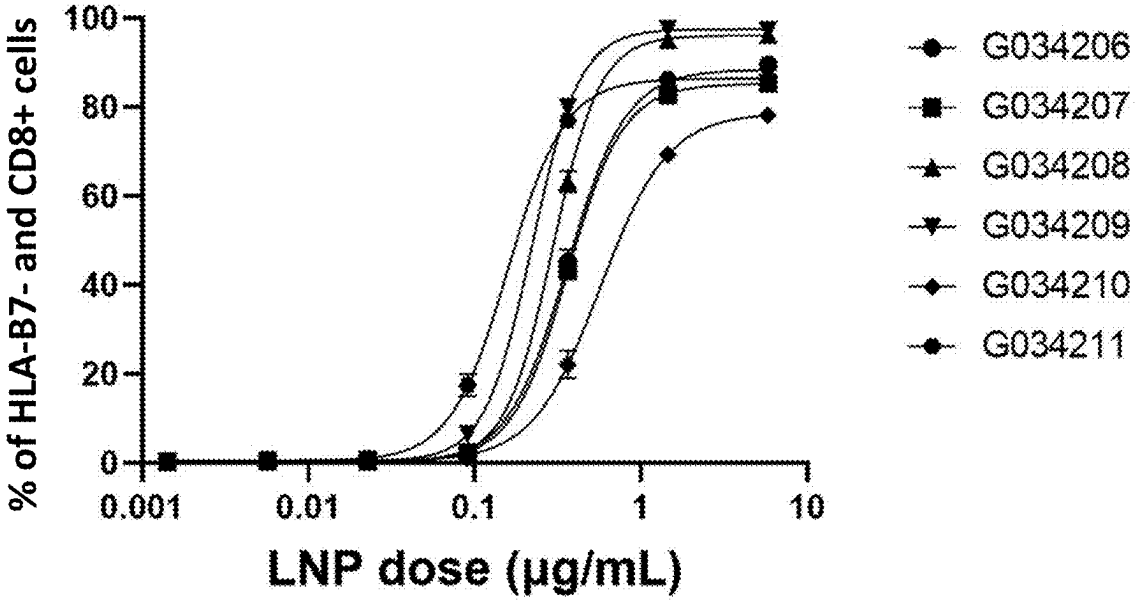


Fig. 10A

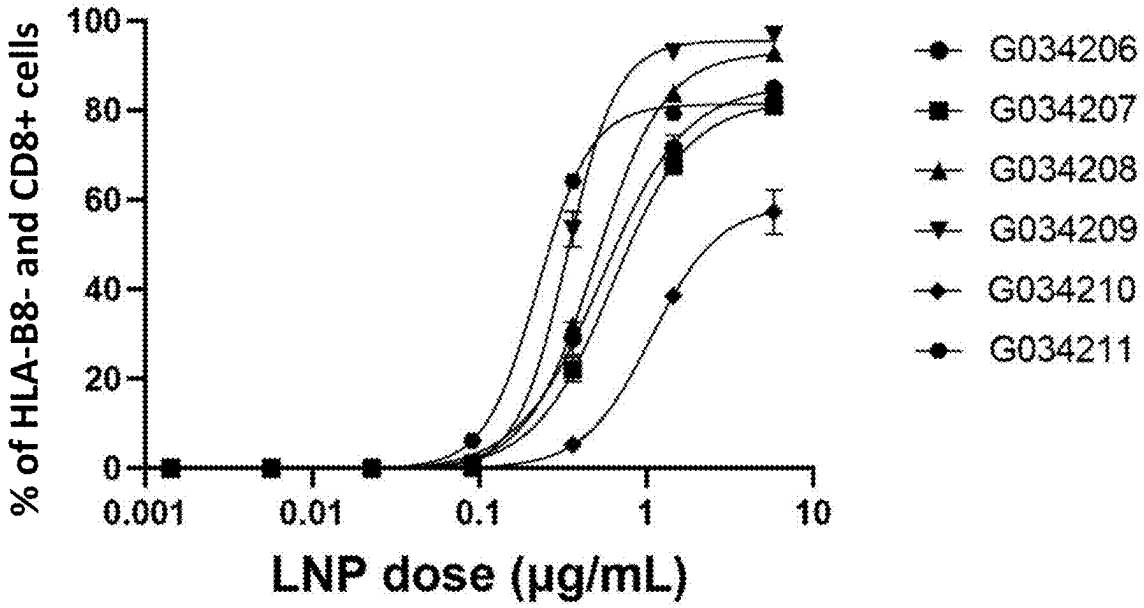


Fig. 10B

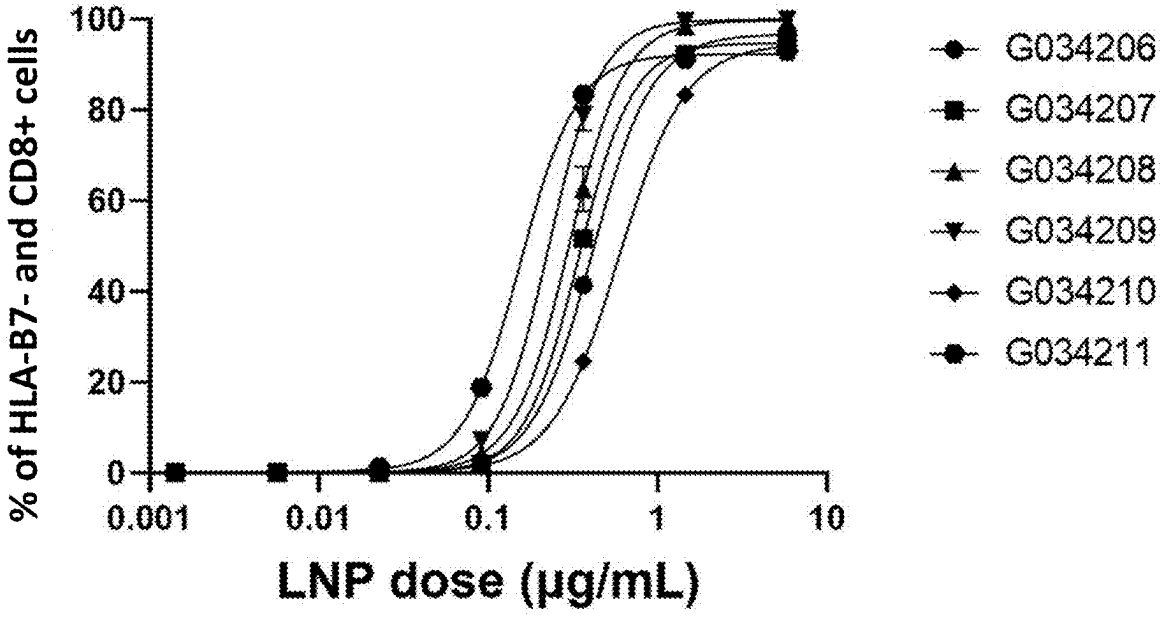


Fig. 10C

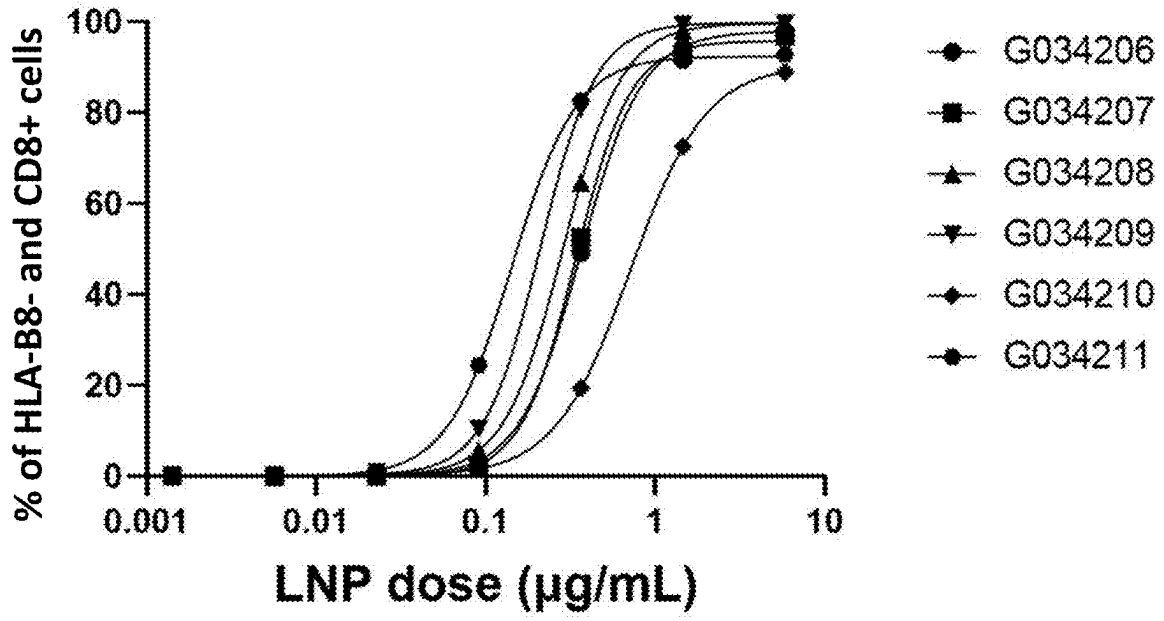


Fig. 10D

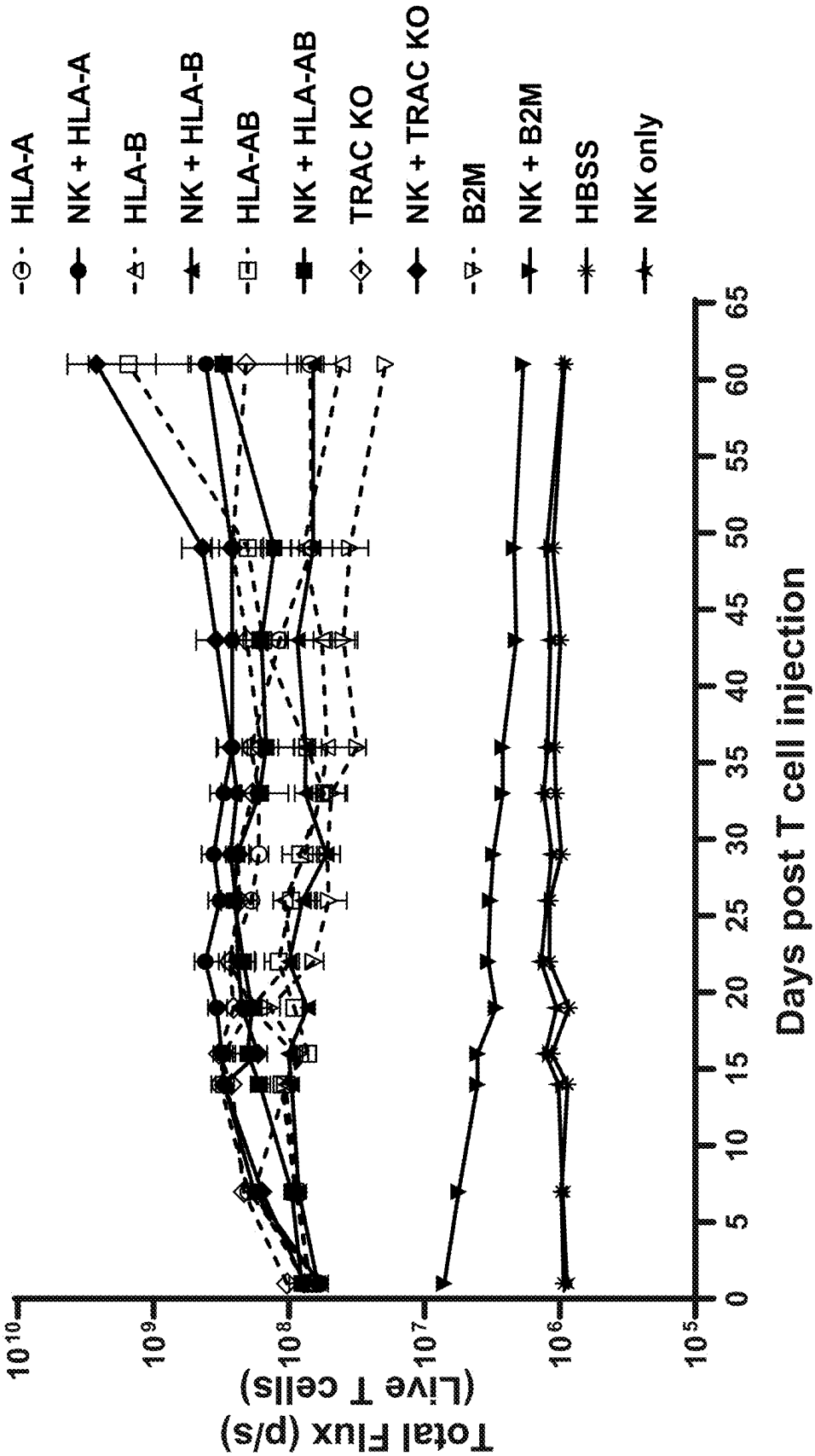

HLA-A
NK + HLA-A
HLA-B
NK + HLA-B
HLA-AB
NK + HLA-AB
TRAC KO
NK + TRAC KO
B2M
NK + B2M
HBSS
NK only
Total Flux (p/s)
(Live T cells)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Days post T cell injection

Fig. 11

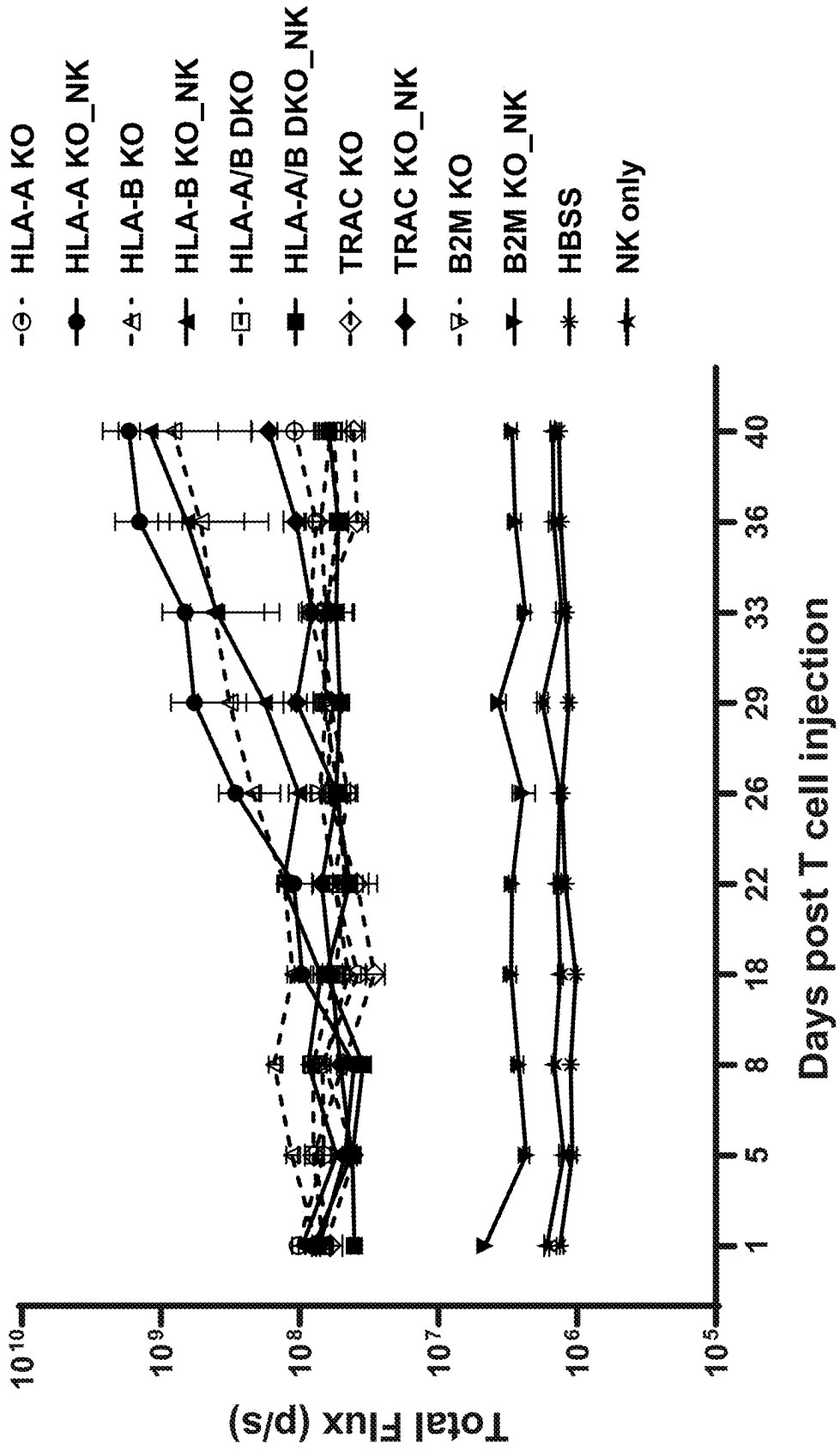

HLA-A KO
HLA-A KO_NK
HLA-B KO
HLA-B KO_NK
HLA-A/B DKO
HLA-A/B DKO_NK
TRAC KO
TRAC KO_NK
B2M KO
B2M KO_NK
HBSS
NK only
Total Flux (p/s)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
1
5
8
18
22
26
29
33
36
40
Days post T cell injection

Fig. 12

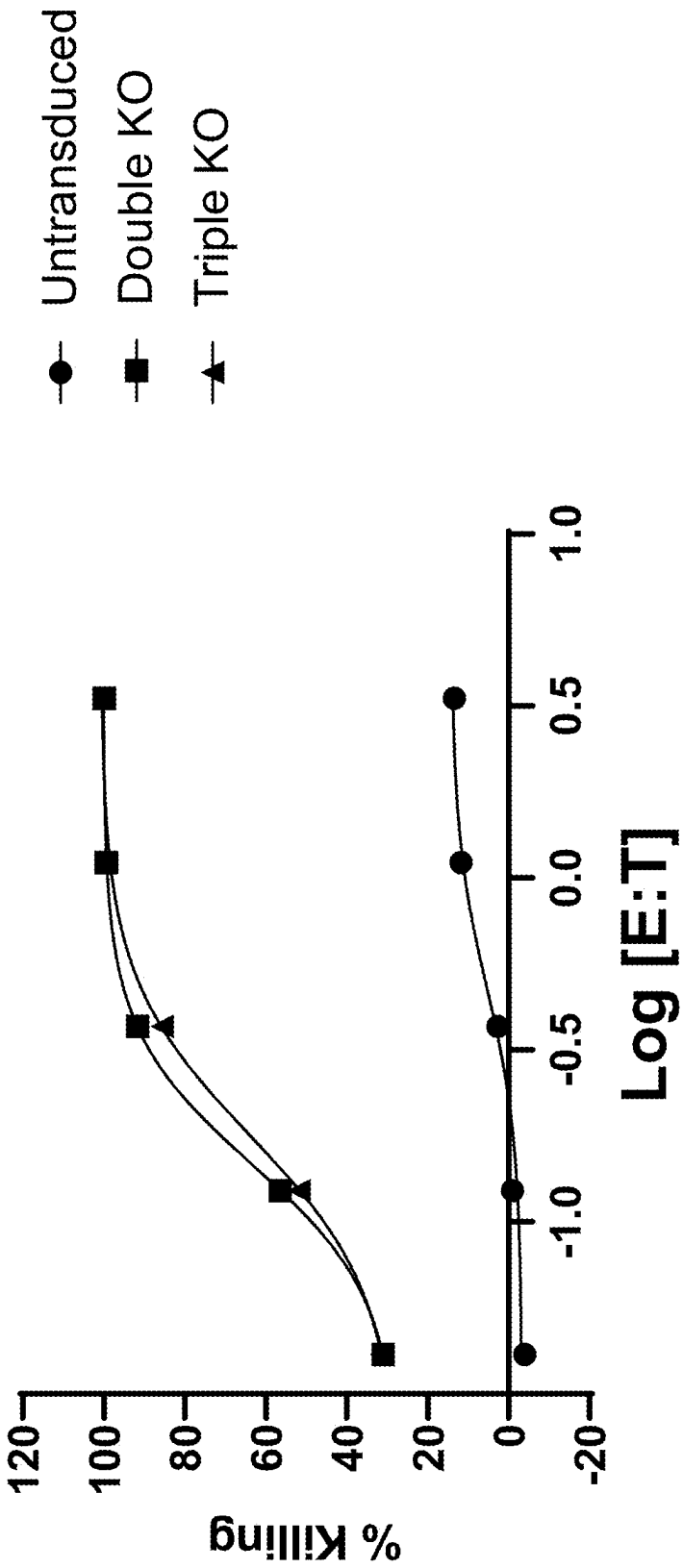

Untransduced
Double KO
Triple KO
% Killing
120
100
80
60
40
20
0
-20
-1.0
-0.5
0.0
0.5
1.0
Log [E:T]

Fig. 13A

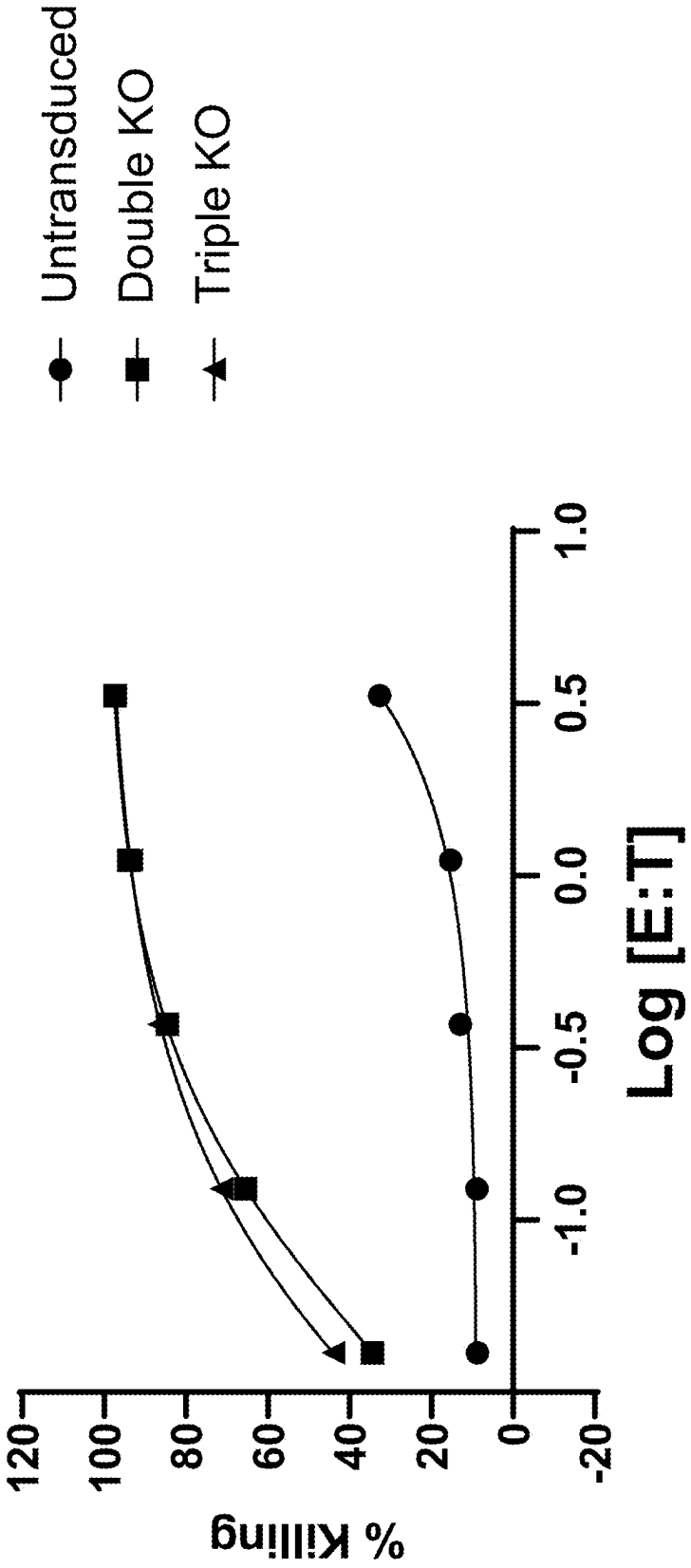

Untransduced
Double KO
Triple KO
% Killing
120
100
80
60
40
20
0
-20
-1.0
-0.5
0.0
0.5
1.0
Log [E:T]

Fig. 13B

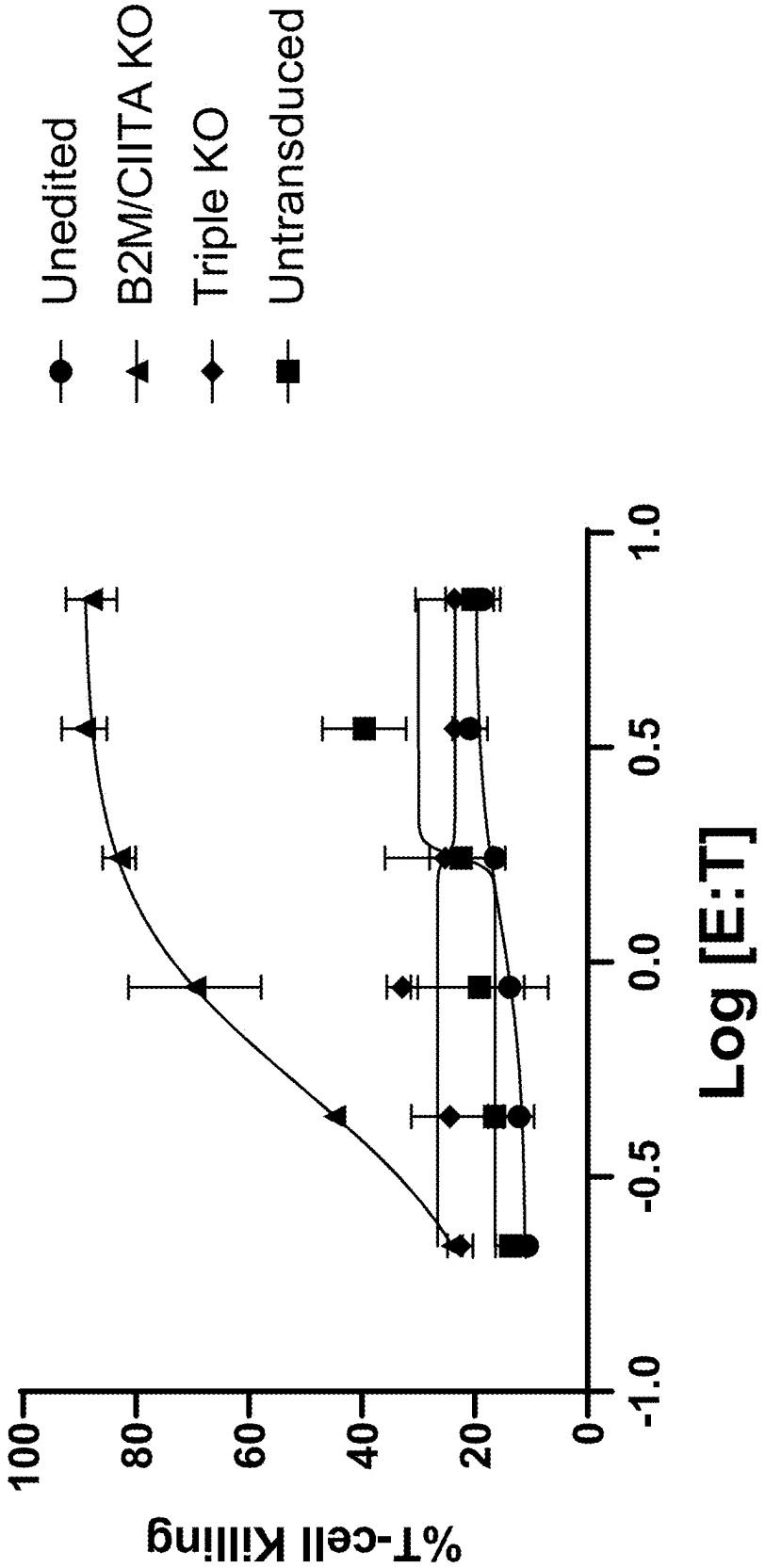

Unedited
B2M/CIITA KO
Triple KO
Untransduced
%T-cell Killing
100
80
60
40
20
0
-1.0
-0.5
0.0
0.5
1.0
Log [E:T]

Fig. 14

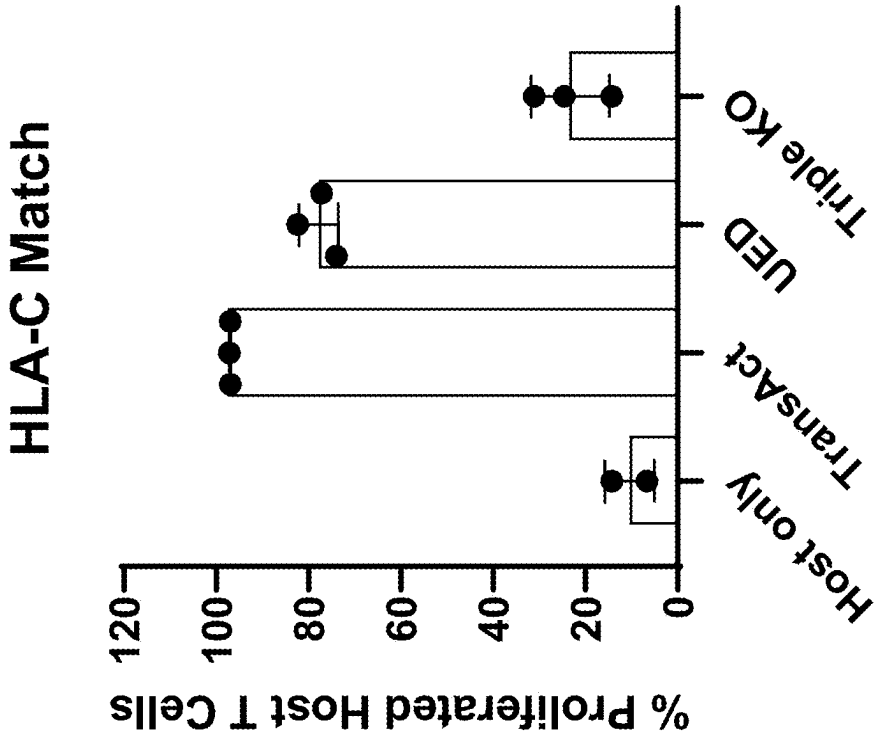


Fig. 15B

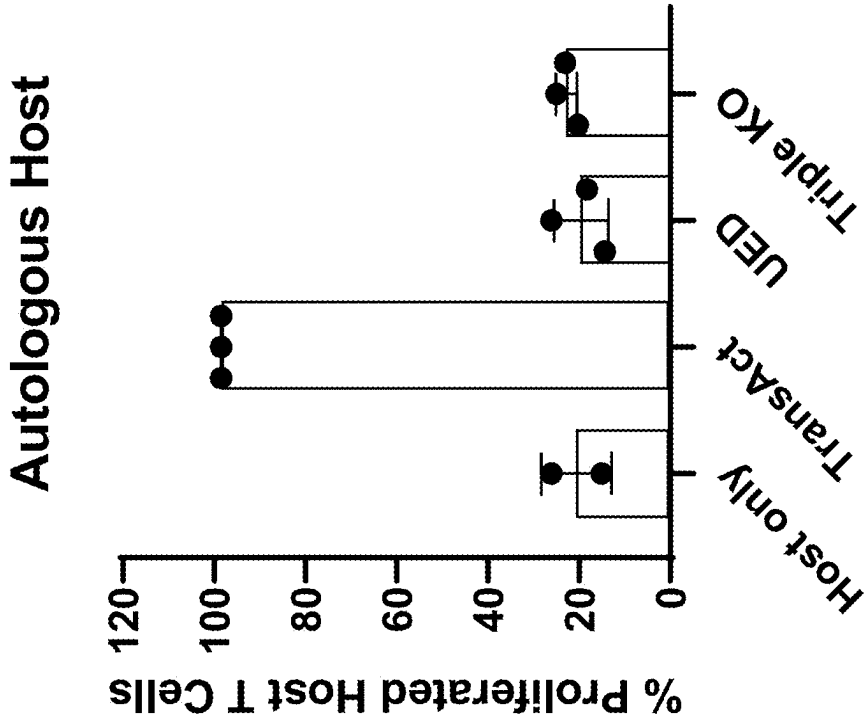


Fig. 15A

COMPOSITIONS AND METHODS FOR REDUCING MHC CLASS I IN A CELL

I. CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of International Application No. PCT/US2023/068498, filed on Jun. 15, 2023, which claims the benefit under 35 USC 119(e) of U.S. Provisional Application No. 63/352,991, filed Jun. 16, 2022, and U.S. Provisional Application No. 63/494,208, filed Apr. 4, 2023, the content of each of which is herein incorporated by reference in its entirety.

II. REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] This application contains a sequence listing, which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML file, created on Jun. 13, 2023, is named "01155-0054-OOPCT_SL.xml" and is 14,122,813 bytes in size.

III. INTRODUCTION AND SUMMARY

[0003] The ability to downregulate MHC class I is critical for many in vivo and ex vivo utilities, e.g., when using allogeneic cells (originating from a donor) for transplantation or e.g., for creating a cell population in vitro that does not activate T cells. In particular, the transfer of allogeneic cells into a subject is of great interest to the field of cell therapy. The use of allogeneic cells has been limited due to the problem of rejection by the recipient subject's immune cells, which recognize the transplanted cells as foreign and mount an attack. To avoid the problem of immune rejection, cell-based therapies have focused on autologous approaches that use a subject's own cells as the cell source for therapy, an approach that is time-consuming and costly.

[0004] Typically, immune rejection of allogeneic cells results from a mismatching of major histocompatibility complex (MHC) molecules between the donor and recipient. Within the human population, MHC molecules exist in various forms, including e.g., numerous genetic variants of any given MHC gene, i.e., alleles, encoding different forms of MHC protein. The primary classes of MHC molecules are referred to as MHC class I and MHC class II. MHC class I molecules (e.g., HLA-A, HLA-B, and HLA-C in humans) are expressed on all nucleated cells and present antigens to activate cytotoxic T cells (CD8+ T cells or CTLs). MHC class II molecules (e.g., HLA-DP, HLA-DQ, and HLA-DR in humans) are expressed on only certain cell types (e.g., B cells, dendritic cells, and macrophages) and present antigens to activate helper T cells (CD4+ T cells or Th cells), which in turn provide signals to B cells to produce antibodies.

[0005] Slight differences, e.g., mismatches in MHC alleles between individuals can cause the T cells in a recipient to become activated. During T cell development, an individual's T cell repertoire is tolerized to one's own MHC molecules, but T cells that recognize another individual's MHC molecules may persist in circulation and are referred to as alloreactive T cells. Alloreactive T cells can become activated e.g., by the presence of another individual's cells expressing MHC molecules in the body, causing e.g., graft versus host disease and transplant rejection.

[0006] While fully matching HLA types between donor and recipient is theoretically possible as a means of reducing transplant rejection, such an approach is logistically and practically challenging given the diversity of HLA alleles across the population to fully match e.g., 10 out 10 alleles (i.e., 2 alleles for each of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1).

[0007] Methods and compositions for reducing the susceptibility of an allogeneic cell to rejection are of interest, including e.g., reducing the cell's expression of MHC protein to avoid recipient T cell responses. In practice, the ability to genetically modify an allogeneic cell for transplantation into a subject has been hampered by the requirement for multiple gene edits to reduce all MHC protein expression, while at the same time, avoiding other harmful recipient immune responses. For example, while strategies to deplete MHC class I protein may reduce activation of CTLs, cells that lack MHC class I on their surface are susceptible to lysis by natural killer (NK) cells of the immune system because NK cell activation is regulated by MHC class I-specific inhibitory receptors. Moreover, while several groups have studied the NK protective effects of the different MHC class I components, some inconsistencies remain in the field. See, e.g., Keystone 2022 presentation "Multiplex Base Editing Mitigates CAR-T Cell Allorejection" by Beam Therapeutics—Workshop: Therapeutic Applications session, Apr. 29, 2022 ("Keystone Presentation"). For example, the Keystone Presentation concluded that retention of HLA-A, not HLA-B or —C, afforded protection against NK killing in vitro, while other groups have shown that HLA-C is important in inhibiting NK activity (Xu et al. 2019, Cell Stem Cell 24, 566-578). Further still, earlier studies suggest HLA-E and HLA-G provide some but not complete protection. Therefore, safely reducing or eliminating expression of MHC class I has proven challenging.

[0008] Gene editing strategies to deplete MHC class II molecules have also proven difficult particularly in certain cell types for reasons including low editing efficiencies and low cell survival rates, preventing practical application as a cell therapy.

[0009] Thus, there exists a need for improved methods and compositions for modifying allogeneic cells to overcome the problem of recipient immune rejection and the technical difficulties associated with the multiple genetic modifications required to produce a safer cell for transplant.

[0010] The present disclosure provides engineered human cells with reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and HLA-C, or wherein the cell has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell and is homozygous for HLA-C. The engineered human cells disclosed herein therefore provide a "partial matching" approach to the problem of allogeneic cell transfer and MHC class I compatibility. The use of cells that have reduced or eliminated expression of HLA-B and are homozygous for HLA-A and HLA-C, or that have reduced or eliminated expression of HLA-A and HLA-B and are homozygous for HLA-C, limits the number of donors that are necessary to provide a therapy that covers a majority of recipients in population because the disclosed partial matching approach requires only one matching HLA-A allele (as opposed to two) and only one HLA-C allele (as opposed to two), or requires only one matching HLA-C allele (as opposed to two). Surprisingly, the engineered human cells that have

reduced or eliminated surface expression of HLA-B protein only or both HLA-A and HLA-B protein relative to an unmodified cell, disclosed herein, demonstrate persistence and are protective against NK-mediated rejection, especially as compared to engineered cells with reduced or eliminated B2M expression. The disclosure provides methods and compositions for generating such engineered human cells with reduced or eliminated surface expression of HLA-B protein only or both HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for both HLA-A and HLA-C or HLA-C only. In some embodiments, the disclosure provides engineered human cells, and methods and compositions for generating engineered human cells, wherein the cell further has reduced or eliminated expression of MHC class II protein on the surface of the cell, e.g., wherein the cell has a genetic modification in the CIITA gene. In some embodiments, the disclosure provides for further engineering of the cell, including to reduce or eliminate the expression of endogenous T cell receptor proteins (e.g., TRAC, TRBC), and to introduce an exogenous nucleic acid, e.g., encoding a polypeptide expressed on the cell surface or a polypeptide that is secreted by the cell. Thus, the disclosure thus provides a flexible platform for genetically engineering human cells for a variety of desired adoptive cell therapy purposes.

[0011] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and homozygous for HLA-C. Also provided is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6:31354623-31357108 or 31354497-31357157, wherein the cell is homozygous for HLA-A and homozygous for HLA-C.

[0012] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising (i) a genetic modification in the HLA-A gene, wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from chr6: 29942854-chr6:29942913 and chr6: 29943518-chr6:29943619, and (ii) a genetic modification in the HLA-B gene, wherein the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6: 31354623-31357108 or 31354497-31357157, wherein the cell is homozygous for HLA-C.

[0013] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355182-31355202; chr6:31355348-31355368; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355410-31355430; chr6: 31355414-31355434; or chr6:31355409-31355429.

[0014] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355182-31355202; chr6:31355349-31355369; chr6: 31355348-31355368; or chr6:31355145-31355165.

[0015] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355348-31355368; chr6:31355349-31355369; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355182-31355202; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355145-31355165; chr6: 31355432-31355452; chr6:31355340-31355360; or chr6: 31355414-31355434.

[0016] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355348-31355368; chr6:31355349-31355369; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355205-31355225; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355182-31355202; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355145-31355165; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355410-31355430; chr6: 31355414-31355434; or chr6:31355409-31355429.

[0017] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355348-31355368, chr6:31355349-31355369, chr6: 31355192-31355212, chr6:31355347-31355367, chr6: 31355340-31355360, chr6:31355409-31355429.

[0018] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355349-31355369 or chr6:31355348-31355368.

[0019] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide

within the genomic coordinates chosen from: chr6: 31355192-31355212 or chr6:31355347-31355367.

[0020] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355347-31355367; chr6:31355340-31355360; or chr6: 31355409-31355429.

[0021] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355348-31355368; chr6:31355145-31355165; chr6: 31355347-31355367; chr6:31355432-31355452; or chr6: 31355340-31355360.

[0022] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; chr6: 31355469-31355493; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; or chr6:31356764-31356788.

[0023] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31356777-31356801; chr6:31355492-31355516; chr6: 31355361-31355385; chr6:31355379-31355403; chr6: 31355491-31355515; chr6:31355356-31355380; chr6: 31355366-31355390; chr6:31355417-31355441; chr6: 31357078-31357102; chr6:31355460-31355484; chr6: 31355415-31355439; chr6:31355166-31355190; chr6: 31355378-31355402; chr6:31355401-31355425; chr6: 31356262-31356286; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; or chr6:31356764-31356788.

[0024] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; or chr6: 31356426-31356450.

[0025] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; or chr6: 31355441-31355465.

[0026] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; or chr6:31355441-31355465.

[0027] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6: 31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; chr6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791.

[0028] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6: 31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410.

[0029] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a

genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0030] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355349-31355369; chr6:31355348-31355368; or chr6:31355145-31355165.

[0031] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; or chr6:31355414-31355434.

[0032] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0033] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355347-31355367, chr6:31355340-31355360, chr6:31355409-31355429.

[0034] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355349-31355369 or chr6:31355348-31355368.

[0035] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355192-31355212 or chr6:31355347-31355367.

[0036] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355347-31355367; chr6:31355340-31355360; or chr6:31355409-31355429.

[0037] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355347-31355367; chr6:31355432-31355452; or chr6:31355340-31355360.

[0038] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; chr6:31355409-31355429.

[0039] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; chr6:

31355469-31355493; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; or chr6:31356764-31356788.

[0040] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355361-31355385; chr6:31355379-31355403; chr6: 31355491-31355515; chr6:31355356-31355380; chr6: 31355366-31355390; chr6:31355417-31355441; chr6: 31357078-31357102; chr6:31355460-31355484; chr6: 31355415-31355439; chr6:31355166-31355190; chr6: 31355378-31355402; chr6:31355401-31355425; chr6: 31356262-31356286; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; or chr6:31356764-31356788.

[0041] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; or chr6: 31356426-31356450.

[0042] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385.

[0043] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; chr6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791.

[0044] Provided herein is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410.

[0045] Provided herein is a method of making an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C, comprising contacting a cell with a composition comprising: (a) an HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, or 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0046] Provided herein is a method of making an engineered human cell, which as reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-C, comprising contacting a cell with a first composition comprising (a) an HLA-A guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 301-590; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs 429-462 and 512-590; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 429-462 and 512-590; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 4, Table 5B or Table 6; or a guide sequence that is

complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 5A or Table 7; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and contacting a cell with a second composition comprising (a) an HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0047] Provided herein is a method of reducing surface expression of HLA-B protein in a human cell relative to an unmodified cell, comprising contacting a cell with composition comprising: (a) a HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0048] Provided herein is a method of reducing surface expression of HLA-A and HLA-B protein in a human cell relative to an unmodified cell, comprising contacting a cell with composition comprising: contacting a cell with a first composition comprising (a) an HLA-A guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 301-590; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 429-462 and 512-590; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 429-462 and 512-590; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 4, 5B or 6; or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 5A or Table 7; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and contacting a cell with a second composition comprising (a) an HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0049] Provided herein is a method of administering an engineered cell to a recipient subject in need thereof, the method comprising: (a) determining the HLA-A and HLA-C alleles of the recipient subject; (b) selecting an engineered cell or cell population of any one of the preceding embodiments, or engineered cell or cell population produced by the method of any one of the preceding embodiments, wherein the engineered cell comprises at least one of the same HLA-A or HLA-C alleles as the recipient subject; (c) administering the selected engineered cell to the recipient subject.

[0050] Provided herein is a method of administering an engineered cell to a recipient subject in need thereof, the method comprising (a) determining the HLA-C alleles of the recipient subject; (b) selecting an engineered cell or cell population of any one of the preceding embodiments, or engineered cell or cell population produced by the method of any one of the preceding embodiments, wherein the engineered cell comprises at least one of the same HLA-C alleles as the recipient subject; (c) administering the selected engineered cell to the recipient subject.

[0051] Further embodiments are provided throughout and described in the claims and Figures.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

[0052] FIG. **1** shows the mean percentage of cells negative for HLA-B7 following editing at the HLA-B locus using 100-mer Spy guides.

[0053] FIG. **2** shows the percentage of T cell lysis following NK cell challenge after editing with various Spy sgRNAs.

[0054] FIGS. **3**A-**3**E show the mean percentage of cells negative for HLA-B following editing at the HLA-B locus. FIG. **3**A-**3**C show the mean percentage of HLA-B-cells across three donors in 100-mer Spy guides and four 91-mer Spy guides. FIG. **3**D-**3**E show the mean percentage of HLA-B-cells across two donors in 91-mer Spy guides following editing at the HLA-B locus.

[0055] FIGS. **4**A-B show the mean percentage of HLA-B knockout. FIG. **4**A shows the mean percentage of HLA-B*07:02 knockout and FIG. **4**B shows the mean percentage of HLA*B08:01 knockout.

[0056] FIGS. **5**A-C shows the mean percentage of cells negative for HLA-B7 following editing at the HLA-B locus with various Nine sgRNAs. FIG. **5**A shows HLA-B7 negative cells in cells with Nme2 BC22n guides. FIGS. **5**B-C show HLA-B7 negative cells in cells treated with Nme2 Cleavase guides. FIG. **5**B shows the mean percentage of HLA-B*07:02 knockout and FIG. **5**C shows the mean percentage of HLA-B*08:01 knockout.

[0057] FIG. **6** shows the dose response curve for the percent of HLA-A2− of CD8+ cells with various doses of Nine sgRNA following editing at the HLA-B locus.

[0058] FIG. **7** shows the dose response curve for the percent of HLA-B7− of CD8+ cells with various doses of Nine sgRNA following editing at the HLA-B locus.

[0059] FIG. **8**A shows the mean percentage of cells negative for HLA-B7 following editing using candidate guides at the HLA-B locus with an Nme2 base editor (deaminase, also referred to as BC22n). G028907 was used as a control.

[0060] FIG. **8**B shows the mean percentage of cells negative for and HLA-B8 following editing using candidate guides at the HLA-B locus with an Nme2 base editor (deaminase, also referred to as BC22n). G028907 was used as a control.

[0061] FIG. **9** shows the percentage of T cell lysis following NK cell challenge to engineered T cells with HLA-A, HLA-B, or HLA-A/B knockout.

[0062] FIGS. **10**A-**10**D show the percent editing at each sgRNA dose in either HLA-B homozygous or heterozygous donors. FIG. **10**A shows percent of HLA-B7− and CD8+ cells in an HLA-B7 homozygous donor. FIG. **10**B shows percent of HLA-B8− and CD8+ cells in an HLA-B7 homozygous donor. FIG. **10**C shows percent of HLA-B7− and CD8+ cells in an HLA-B7 heterozygous donor. FIG. **10**D shows percent of HLA-B8− and CD8+ cells in an HLA-B7 heterozygous donor.

[0063] FIG. **11** shows the total flux (photons/s) from luciferase expressing T cells present at the various time points after injection for cells edited with HLA-A, HLA-B, CIITA, TRAC, and/or B2M.

[0064] FIG. **12** shows the total flux (photons/s) from luciferase expressing T cells present at the various time points after injection for cells edited with HLA-A, HLA-B, CIITA, TRAC, and/or B2M.

[0065] FIGS. **13**A and **13**B show the percentage killing results in tumor cells. FIG. **13**A shows the percentage killing results in HH cells for double and triple KO edits. FIG. **13**B shows the percentage killing results in MOLT-4 cells for double and triple KO edits.

[0066] FIG. **14** shows the % T cell killing results with NK cells for T cells with different edits or controls of B2M/CIITA KO, unedited, or untransduced T cells.

[0067] FIGS. **15**A and **15**B show the percentage of host T cell proliferation when co-cultured with engineered donor T cells.

V. DETAILED DESCRIPTION

[0068] The present disclosure provides engineered human cells, as well as methods and compositions for genetically modifying a human cell to make engineered human cells that are useful, for example, for adoptive cell transfer (ACT) therapies. The disclosure provides engineered human cells with reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C. Additionally, the disclosure provides engineered human cells with reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-C. Thus, the engineered human cells disclosed herein provide a "partial matching" solution to hurdles associated with allogeneic cell transfer.

[0069] In some embodiments, the disclosure provides engineered human cells with reduced or eliminated surface expression of HLA-B protein as a result of a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and HLA-C. In some embodiments, the disclosure provides compositions and methods for reducing or eliminating expression of HLA-B protein relative to an unmodified cell and compositions and methods to reduce the cell's susceptibility to immune rejection. In some embodiments, the engineered human cells with reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell are not susceptible to lysis by NK cells, a problem observed with other approaches that reduce or eliminate MHC class I protein expression. In some embodiments, the methods and compositions comprise reducing or eliminating surface expression of HLA-B protein by genetically modifying HLA-B with a gene editing system, and inserting an exogenous nucleic acid encoding a targeting receptor, or other polypeptide (expressed on the cell surface or secreted) into the cell by genetic modification. The engineered cell compositions produced by the methods disclosed herein have desirable properties, including e.g., reduced or eliminated expression of HLA-B, reduced immunogenicity in vitro and in vivo, increased survival, and increased genetic compatibility with greater subjects for transplant.

[0070] In some embodiments, the disclosure provides engineered human cells with reduced or eliminated surface expression of HLA-A and HLA-B protein as a result of a genetic modification in the HLA-A and HLA-B genes, wherein the cell is homozygous for HLA-C. In some embodiments, the disclosure provides compositions and methods for reducing or eliminating expression of HLA-A and HLA-B protein relative to an unmodified cell and compositions and methods to reduce the cell's susceptibility to immune rejection. In some embodiments, the engineered human cells with reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell are not susceptible to lysis by NK cells, a problem observed with other approaches that reduce or eliminate MHC class I protein expression. In some embodiments, the methods and

compositions comprise reducing or eliminating surface expression of HLA-A and HLA-B protein by genetically modifying HLA-A and HLA-B with a gene editing system, and inserting an exogenous nucleic acid encoding a targeting receptor, or other polypeptide (expressed on the cell surface or secreted) into the cell by genetic modification. The engineered cell compositions produced by the methods disclosed herein have desirable properties, including e.g., reduced or eliminated surface expression of HLA-A and HLA-B protein, reduced immunogenicity in vitro and in vivo, increased survival, and increased genetic compatibility with greater subjects for transplant.

[0071] The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined, or a degree of variation that does not substantially affect the properties of the described subject matter, or within the tolerances accepted in the art, e.g., within 10%, 5%, 2%, or 1%. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0072] Provided herein are the following numbered embodiments:

[0073] Embodiment 1 is an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-A gene and a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-C.

[0074] Embodiment 2 is an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-A gene and a genetic modification in the HLA-B gene, wherein (i) the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:29942854-chr6:29942913 and chr6:29943518-chr6: 29943619; and (b) chr6:29942540-29945459; (ii) the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6: 31354497-31357157; wherein the cell is homozygous for HLA-C.

[0075] Embodiment 3 is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and homozygous for HLA-C.

[0076] Embodiment 4 is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6: 31354497-31357157; wherein the cell is homozygous for HLA-A and homozygous for HLA-C.

[0077] Embodiment 5 is the engineered human cell of any one of embodiments 1-4, wherein the cell has reduced or eliminated expression of at least one HLA-B allele selected from HLA-B7, HLA-B8, HLA-B35, HLA-B40, HLA-B44, HLA-B15, HLA-B14, HLA-B18 and HLA-B51.

[0078] Embodiment 6 is the engineered human cell of any one of embodiments 1, 2, or 5, wherein the cell has reduced or eliminated expression of at least one HLA-A allele selected from: HLA-A1, HLA-A2, HLA-A3, HLA-AT1, HLA-A29, HLA-A26, HLA-A33, and HLA-A24.

[0079] Embodiment 7 is the engineered cell of any one of embodiments 1-6, wherein the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355182-31355596 or (b) chr6: 31355203-31356461.

[0080] Embodiment 8 is the engineered cell of any one of embodiments 1-7, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; or chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6: 31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791.

[0081] Embodiment 9 is the engineered cell of any of embodiments 1-8, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0082] Embodiment 10 is the engineered cell of any of embodiments 1-9, wherein the genetic modification in the

HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355349-31355369; chr6:31355348-31355368; or chr6:31355145-31355165.

[0083] Embodiment 11 is the engineered cell of any of embodiments 1-10, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; or chr6:31355414-31355434.

[0084] Embodiment 12 is the engineered cell of any of embodiments 1-11, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0085] Embodiment 13 is the engineered cell of any of embodiments 1-12, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368, chr6:31355347-31355367, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355340-31355360, chr6:31355409-31355429.

[0086] Embodiment 14 is the engineered cell of any of embodiments 1-13, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: (i) chr6:31355349-31355369 or chr6:31355348-31355368; (ii) chr6:31355192-31355212 or chr6:31355347-31355367; (iii) chr6: 31355347-31355367; chr6:31355340-31355360; or chr6: 31355409-31355429; or (iv) chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355347-31355367; chr6: 31355432-31355452; or chr6:31355340-31355360.

[0087] Embodiment 15 is the engineered cell of any of embodiments 1-14, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0088] Embodiment 16 is the engineered cell of any of embodiments 1-15, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355361-31355385; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355356-31355380; chr6:31355366-31355390; chr6:31355417-31355441; chr6:31357078-31357102; chr6:31355460-31355484; chr6:31355415-31355439; chr6:31355166-31355190; chr6:31355378-31355402; chr6:31355401-31355425; chr6:31356262-31356286; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0089] Embodiment 17 is the engineered cell of any of embodiments 1-16, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; ch6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; or chr6:31356426-31356450.

[0090] Embodiment 18 is the engineered cell of any of embodiments 1-17, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; ch6:31355491-31355515; chr6:31355361-31355385; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0091] Embodiment 19 is the engineered cell of any of embodiments 1-18, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0092] Embodiment 20 is the engineered cell of any one of embodiments 1-19, wherein the genetic modification in the HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6: 31355417-31355441; or chr6: 31356386-31356410.

[0093] Embodiment 21 is the engineered cell of any one of embodiments 1-2 and 5-20, wherein the genetic modification in HLA-A comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; and chr6:29942883-29942903.

[0094] Embodiment 22 is the engineered cell of any one of embodiments 1-2 and 5-21, wherein the genetic modification in HLA-A comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942891-29942915; chr6:29942609-29942633; chr6:29942864-29942884; chr6:29944266-29944290; chr6:29942889-29942913; chr6:29942891-29942915chr6:29944471-29944495; chr6:29944470-29944494.

[0095] Embodiment 23 is the engineered cell of any one of embodiments 1-2 and 5-22, wherein the genetic modification in HLA-A comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942891-29942915; chr6:29942609-29942633.

[0096] Embodiment 24 is an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or chr6:31355347-31355367; chr6:31355182-31355202; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; or chr6:31355409-31355429; or (b) chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; or chr6:31356767-31356791.

[0097] Embodiment 25 is an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising (i) a genetic modification in the HLA-A gene comprising an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; chr6:29944026-29944046; chr6:29934330-29934350, chr6:29943115-29943135, chr6:29943135-29943155, chr6:29943140-29943160, chr6:29943590-29943610, chr6:29943824-29943844, chr6:29943858-29943878, chr6:29944478-29944498, and chr6:29944850-29944870; or (b) chr6:29942891-29942915; chr6:29942609-29942633; chr6:29944266-29944290; chr6:29942889-29942913; chr6:29944471-29944495; and chr6:29944470-29944494; and (ii) a genetic modification in the HLA-B gene comprising an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or chr6:31355347-31355367; chr6:31355182-31355202; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; and chr6:31355409-31355429; or (b) chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; or chr6:31356767-31356791.

[0098] Embodiment 26 is the engineered cell of any one of embodiment 24 or 25, wherein the genetic modification in the HLA-B comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31355390-31355414.

[0099] Embodiment 27 is the engineered cell of any one of embodiments 24-26, wherein the genetic modification in the HLA-A or the genetic modification in the HLA-B comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates, or wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates.

[0100] Embodiment 28 is the engineered cell of any one of embodiments 24-27, wherein the genetic modification in the HLA-A or the genetic modification in the HLA-B comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates.

[0101] Embodiment 29 is the engineered cell of any one of embodiments 24-28, wherein the genetic modification in the HLA-A or the genetic modification in the HLA-B comprises

at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[0102] Embodiment 30 is the engineered cell of any one of embodiments 1-29, wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355348-31355368; or chr6:31355347-31355367; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; chr6:31355409-31355429.

[0103] Embodiment 31 is the engineered cell of any one of embodiments 1-30, wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; or chr6:31356767-31356791.

[0104] Embodiment 32 is the engineered cell of any one of embodiments 1-2, 5-23, and 25-31, wherein HLA-A expression is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942891-29942915; chr6:29942609-29942633; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; chr6:29944026-29944046; chr6:29934330-29934350, chr6:29943115-29943135, chr6:29943135-29943155, chr6:29943140-29943160, chr6:29943590-29943610, chr6:29943824-29943844, chr6:29943858-29943878, and chr6:29944478-29944498, chr6:29944850-29944870.

[0105] Embodiment 33 is the engineered cell of any one of embodiments 1, 2, 5-23, and 25-32, wherein HLA-A expression is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942891-29942915; chr6:29942609-29942633; chr6:29944266-29944290; chr6:29942889-29942913; chr6:29944471-29944495; and chr6:29944470-29944494.

[0106] Embodiment 34 is the engineered cell of any one of embodiments 1, 2, 5-23, and 25-33, wherein HLA-A expression is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942891-29942915 or chr6:29942609-29942633.

[0107] Embodiment 35 is the engineered cell of any one of embodiments 30-34, wherein the HLA-A genomic target sequence or the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates.

[0108] Embodiment 36 is the engineered cell of any one of embodiments 30-35, wherein the HLA-A genomic target sequence or the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0109] Embodiment 37 is the engineered cell of any one of embodiments 30-36, wherein the HLA-A genomic target sequence or the HLA-B genomic target sequence comprises at least 17, 18, 19, or 20 contiguous nucleotides within the genomic coordinates.

[0110] Embodiment 38 is the engineered cell of any one of embodiments 30-36, wherein the HLA-A genomic target sequence or the HLA-B genomic target sequence comprises at least 17, 18, 19, 20, 21, 22, 23, 24 or 25 contiguous nucleotides within the genomic coordinates.

[0111] Embodiment 39 is the engineered cell of any one of embodiments 1-38, wherein the cell is homozygous for HLA-C.

[0112] Embodiment 40 is the engineered cell of any one of embodiments 1-39, wherein the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:04; HLA-C*04:01; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

[0113] Embodiment 41 is the engineered cell of any one of embodiments 1-40, wherein the HLA-C allele is HLA-C*07:02.

[0114] Embodiment 42 is the engineered cell of any one of embodiments 1-40, wherein the HLA-C allele is HLA-C*07:01.

[0115] Embodiment 43 is the engineered cell of any one of embodiments 1-40, wherein the HLA-C allele is HLA-C*05:01.

[0116] Embodiment 44 is the engineered cell of any one of embodiments 1-40, wherein the HLA-C allele is HLA-C*04:01.

[0117] Embodiment 45 is the engineered cell of any one of embodiments 1-40, wherein the HLA-C allele is HLA-C*06:02.

[0118] Embodiment 46 is the engineered cell of any one of embodiments 3-24 and 26-45, wherein the engineered cell is homozygous for HLA-A, the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02.

[0119] Embodiment 47 is the engineered cell of any one of embodiments 3-24 and 26-45, wherein the engineered cell is homozygous for HLA-A and wherein the engineered cell is homozygous for HLA-C wherein the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*16:01; HLA-C*15:02; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

[0120] Embodiment 48 is the engineered cell of any one of embodiments 1-47, wherein the cell has reduced or eliminated surface expression of MHC class II protein.

[0121] Embodiment 49 is the engineered cell of any one of embodiments 1-48, wherein the cell has a genetic modification of a gene selected from CIITA, HLA-DR, HLA-DQ, HLA-DP, RFX5, RFXB/ANK, RFXAP, CREB, NF-YA, NF-YB, and NF-YC.

[0122] Embodiment 50 is the engineered cell of any one of embodiments 1-49, wherein the cell has a genetic modification in the CIITA gene.

[0123] Embodiment 51 is the engineered cell of any one of embodiments 1-50, wherein the cell has reduced or eliminated surface expression of TRAC protein.

[0124] Embodiment 52 is the engineered cell of any one of embodiments 1-51, wherein the cell has reduced or eliminated surface expression of TRBC protein.

[0125] Embodiment 53 is the engineered cell of any one of embodiments 1-52, wherein the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates, or wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates.

[0126] Embodiment 54 is the engineered cell of any one of embodiments 1-53, wherein the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates.

[0127] Embodiment 55 is the engineered cell of any one of embodiments 1-54, wherein the genetic modification comprises an indel.

[0128] Embodiment 56 is the engineered cell of any one of embodiments 1-55, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[0129] Embodiment 57 is a pharmaceutical composition comprising the engineered cell of any one of embodiments 1-56.

[0130] Embodiment 58 is a population of cells comprising the engineered cell of any one of embodiments 1-57.

[0131] Embodiment 59 is a pharmaceutical composition comprising the population of cells of embodiment 58.

[0132] Embodiment 60 is the population of embodiment 58 or the pharmaceutical composition of embodiment 59, wherein the population of cells is at least 65%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% HLA-A negative or HLA-B negative as measured by flow cytometry.

[0133] Embodiment 61 is the population or pharmaceutical composition of any one of embodiments 58-60, wherein at least 65%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the population of cells comprises the genetic modification in the HLA-A gene or the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS).

[0134] Embodiment 62 is the population or pharmaceutical composition of any one of embodiments 58-61, wherein the population of cells is at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% CIITA negative as measured by flow cytometry.

[0135] Embodiment 63 is the population or pharmaceutical composition of any one of embodiments 58-62, wherein the population of cells is at least 95%, at least 97%, at least 98%, at least 99%, or at least 99.5% endogenous TCR protein negative as measured by flow cytometry.

[0136] Embodiment 64 is a method of administering the engineered cell, population of cells, pharmaceutical composition of any one of embodiments 1-63 to a subject in need thereof.

[0137] Embodiment 65 is a method of administering the engineered cell, population of cells, or pharmaceutical composition of any one of embodiments 1-63 to a subject as an adoptive cell transfer (ACT) therapy.

[0138] Embodiment 66 is a method of treating a disease or disorder comprising administering the engineered cell, population of cells, or pharmaceutical composition of any one of embodiments 1-63 to a subject in need thereof.

[0139] Embodiment 67 is a composition, comprising an HLA-B guide RNA, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91, 101-164, 167-176, 178-185; ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 101-185; iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence

selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3.

[0140] Embodiment 68 is a composition, comprising an HLA-B guide RNA and an HLA-A guide RNA, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91 and 101-164, 167-176, 178-185; ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 101-185; iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3, and wherein the HLA-A guide RNA comprises: i. a guide sequence selected from SEQ ID Nos: 576, 571, 301-570, 572-575, 577-590; or ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 429-462 and 512-590; or iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 512-590; or iv. a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or v. a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Tables 4-7.

[0141] Embodiment 69 is a method of making an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C, comprising: contacting a cell with a composition comprising (i) an HLA-B guide RNA and (ii) optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91, 101-164, 167-176, 178-185; ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 101-185; iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3.

[0142] Embodiment 70 is a method of reducing surface expression of HLA-B protein in a human cell relative to an unmodified cell, comprising contacting a cell with a composition comprising (i) an HLA-B guide RNA and (ii) optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91 and 101-164, 167-176, 178-185; ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs; and 101-185; iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3.

[0143] Embodiment 71 is a method of making an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-C, comprising: (a) contacting a cell with a first composition comprising an HLA-B guide RNA and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91 and 101-164, 167-176, 178-185; or ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 101-185; or iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; and (b) contacting a cell with a second composition comprising an HLA-A guide RNA and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-A guide RNA comprises: i. a guide sequence selected from SEQ ID Nos: 576, 571, 301-570, 572-575, 577-590; or ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 429-462 and 512-590; or iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected

from SEQ ID NOs: 301-428 and 463-511; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 512-590; or iv. a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Tables 4-7.

[0144] Embodiment 72 is a method of reducing surface expression of HLA-A protein and HLA-B protein in a human cell relative to an unmodified cell, comprising (a) contacting a cell with a first composition comprising an HLA-B guide RNA and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-B guide RNA comprises: i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91 and 101-164, 167-176, 178-185; ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs; and 101-185; iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; and (b) contacting a cell with a second composition comprising an HLA-A guide RNA and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, wherein the HLA-A guide RNA comprises: i. a guide sequence selected from SEQ ID Nos: 576, 571, 301-570, 572-575, 577-590; or ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected form SEQ ID NOs: 429-462 and 512-590; or iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 429-462 and 512-590; or iv. a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or v. a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Tables 4-7.

[0145] Embodiment 73 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-72, wherein the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is SpyCas9, and the HLA-B guide RNA comprises: (i) a guide sequence selected from SEQ ID NOs: 13, 74, 1-12, 14-73, 75-91; or (ii) a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or (iii) a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2; or (vi) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91.

[0146] Embodiment 74 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-72, wherein the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is NmeCas9, and the HLA-B guide RNA comprises: (i) a guide sequence selected from SEQ ID NOs: 165, 166, 177, 101-164, 167-176, and 178-185; or (ii) a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185.

[0147] Embodiment 75 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-72, wherein the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is NmeCas9, and the HLA-B guide RNA comprises: (i) a guide sequence selected from SEQ ID NOs: 165, 166, 163, 164, 169, and 177; or (ii) a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 165, 166, 163, 164, and 177; or (iii) a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 165, 166, 163, 164, and 177.

[0148] Embodiment 76 is the composition or method of any one of embodiments 67-75, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification.

[0149] Embodiment 77 is the composition or method of any one of embodiments 67-76, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[0150] Embodiment 78 is the composition or method of any one of embodiments 67-77, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a phosphorothioate (PS) bond between nucleotides.

[0151] Embodiment 79 is the composition or method of any one of embodiments 67-78, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a 2'-fluoro (2'-F) modified nucleotide.

[0152] Embodiment 80 is the composition or method of any one of embodiments 67-79, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a modification at one or more of the first five nucleotides at the 5' end of the guide RNA.

[0153] Embodiment 81 is the composition or method of any one of embodiments 67-80, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one

modification, comprising a modification at one or more of the last five nucleotides at the 3' end of the guide RNA.

[0154] Embodiment 82 is the composition or method of any one of embodiments 67-81, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a PS bond between the first four nucleotides of the guide RNA.

[0155] Embodiment 83 is the composition or method of any one of embodiments 67-82, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a PS bond between the last four nucleotides of the guide RNA.

[0156] Embodiment 84 is the composition or method of any one of embodiments 67-83, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a 2'-O-Me modified nucleotide at the first three nucleotides at the 5' end of the guide RNA.

[0157] Embodiment 85 is the composition or method of any one of embodiments 67-84, wherein the HLA-B guide RNA or the HLA-A guide RNA comprises at least one modification, comprising a 2'-O-Me modified nucleotide at the last three nucleotides at the 3' end of the guide RNA.

[0158] Embodiment 86 is the method of any one of embodiments 67-85, further comprising reducing or eliminating the surface expression of MHC class II protein in the cell relative to an unmodified cell, for example by contacting the cell with a gene editing system targeting a gene selected from CIITA, HLA-DR, HLA-DQ, HLA-DP, RFX5, RFXB/ANK, RFXAP, CREB, NF-YA, NF-YB, and NF-YC.

[0159] Embodiment 87 is the method of any one of embodiments 67-86, further comprising contacting the cell with a CIITA guide RNA.

[0160] Embodiment 88 is the method of any one of embodiments 67-87, further comprising reducing or eliminating the surface expression of a TCR protein in the cell relative to an unmodified cell.

[0161] Embodiment 89 is the method of any one of embodiments 67-88, further comprising contacting the cell with an exogenous nucleic acid.

[0162] Embodiment 90 is the method of embodiment 89, further comprising contacting the cell with an exogenous nucleic acid encoding a targeting receptor.

[0163] Embodiment 91 is the method of embodiment 89, further comprising contacting the cell with an exogenous nucleic acid encoding a polypeptide that is secreted by the cell.

[0164] Embodiment 92 is the method of embodiment 89, further comprising contacting the cell with a DNA-dependent protein kinase inhibitor (DNAPKi).

[0165] Embodiment 93 is the method of embodiment 92, wherein the DNAPKi is Compound 1.

[0166] Embodiment 94 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-93, wherein the cell is an allogeneic cell.

[0167] Embodiment 95 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a primary cell.

[0168] Embodiment 96 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a CD4+ T cell.

[0169] Embodiment 97 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a CD8+ T cell.

[0170] Embodiment 98 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a memory T cell.

[0171] Embodiment 99 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a B cell.

[0172] Embodiment 100 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a plasma B cell.

[0173] Embodiment 101 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a memory B cell.

[0174] Embodiment 102 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a natural killer (NK) cell.

[0175] Embodiment 103 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a macrophage.

[0176] Embodiment 104 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a stem cell.

[0177] Embodiment 105 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a pluripotent stem cell (PSC).

[0178] Embodiment 106 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a hematopoietic stem cell (HSC).

[0179] Embodiment 107 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is an induced pluripotent stem cell (iPSC).

[0180] Embodiment 108 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a mesenchymal stem cell (MSC).

[0181] Embodiment 109 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a neural stem cell (NSC).

[0182] Embodiment 110 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a limbal stem cell (LSC).

[0183] Embodiment 111 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a progenitor cell, e.g. an endothelial progenitor cell or a neural progenitor cell.

[0184] Embodiment 112 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a tissue-specific primary cell.

[0185] Embodiment 113 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a chosen from: chondrocyte, myocyte, and keratinocyte.

[0186] Embodiment 114 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is an activated cell.

[0187] Embodiment 115 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-94, wherein the cell is a non-activated cell.

[0188] Embodiment 116 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an antibody or antibody fragment.

[0189] Embodiment 117 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-116, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a full-length IgG antibody.

[0190] Embodiment 118 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-116, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a single chain antibody.

[0191] Embodiment 119 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-118, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a neutralizing antibody.

[0192] Embodiment 120 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an enzyme.

[0193] Embodiment 121 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a cytokine.

[0194] Embodiment 122 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-121, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a fusion protein.

[0195] Embodiment 123 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-122, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide comprises a soluble receptor.

[0196] Embodiment 124 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a T cell receptor (TCR).

[0197] Embodiment 125 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a genetically modified TCR.

[0198] Embodiment 126 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a WT1 TCR.

[0199] Embodiment 127 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a CAR.

[0200] Embodiment 128 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-115, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a universal CAR.

[0201] Embodiment 129 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-127, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is an anti-CD30 CAR.

[0202] Embodiment 130 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-129, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a proliferation-inducing ligand (APRIL).

[0203] Embodiment 131 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-129, wherein the cells are engineered with a gene editing system.

[0204] Embodiment 132 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 131, wherein the gene editing system comprises a transcription activator-like effector nuclease (TALEN).

[0205] Embodiment 133 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 131, wherein the gene editing system comprises a zinc finger nuclease.

[0206] Embodiment 134 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 131, wherein the gene editing system comprises an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0207] Embodiment 135 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-134, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid comprises a Cas9 protein.

[0208] Embodiment 136 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is *S. pyogenes* Cas9.

[0209] Embodiment 137 is the engineered cell, population of cells, pharmaceutical composition, or method of any one

of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is *N. meningitidis* Cas9, optionally Nme2Cas9.

[0210] Embodiment 138 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is *S. thermophilus* Cas9.

[0211] Embodiment 139 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is *S. aureus* Cas9.

[0212] Embodiment 140 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is Cpf1 from *F. novicida.*

[0213] Embodiment 141 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is Cpf1 from *Acidaminococcus* sp.

[0214] Embodiment 142 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is Cpf1 from *Lachnospiraceae bacterium* ND2006.

[0215] Embodiment 143 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is Cas12a.

[0216] Embodiment 144 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is CasX.

[0217] Embodiment 145 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is Mad7 nuclease.

[0218] Embodiment 146 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is an ARCUS nucleases.

[0219] Embodiment 147 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is an A to G base editor.

[0220] Embodiment 148 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid is a C to T base editor.

[0221] Embodiment 149 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-135, wherein the RNA-guided DNA-binding agent or the RNA-guided DNA-binding agent encoded by the nucleic acid comprises a cytidine deaminase and an RNA-guided nickase.

[0222] Embodiment 150 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 1-149, wherein the cell is engineered by a base editing system comprising a C to T base editor or an A to G base editor.

[0223] Embodiment 151 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the immediately preceding embodiment, wherein the base editing system comprises a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, or a nucleic acid encoding the polypeptide.

[0224] Embodiment 152 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 149 or 151 wherein the cytidine deaminase comprises APOBEC3A deaminase (A3A).

[0225] Embodiment 153 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 151, wherein the polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 811 or 976.

[0226] Embodiment 154 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 151, wherein the nucleic acid encoding the polypeptide comprises a sequence that is at least 80%, 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 804 or SEQ ID NO: 822.

[0227] Embodiment 155 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiment 148-154, wherein the base editing system further comprises a uracil glycosylase inhibitor (UGI) in a polypeptide different from the polypeptide comprising a cytidine deaminase and an RNA-guided nickase.

[0228] Embodiment 156 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 148-152, wherein the polypeptide comprising the cytidine deaminase and the RNA-guided nickase further comprises a uracil glycosylase inhibitor (UGI).

[0229] Embodiment 157 is the engineered cell, population of cells, pharmaceutical composition, or method of embodiment 156, wherein the polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 100% identical to any one of SEQ ID NO: 977, 978, 979, and 980.

[0230] Embodiment 158 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-157, wherein the HLA-A guide RNA or the HLA-B guide RNA is provided to the cell in a vector.

[0231] Embodiment 159 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-158, wherein the RNA-guided DNA binding agent is provided to the cell in a vector, optionally in the same vector as the HLA-A guide RNA or the HLA-B guide RNA.

[0232] Embodiment 160 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-159, wherein the exogenous nucleic acid is provided to the cell in a vector.

[0233] Embodiment 161 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 158-160, wherein the vector is a viral vector.

[0234] Embodiment 162 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 158-160, wherein the vector is a non-viral vector.

[0235] Embodiment 163 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 158-160, wherein the vector is a lentiviral vector.

[0236] Embodiment 164 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 158-160, wherein the vector is a retroviral vector.

[0237] Embodiment 165 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 158-160, wherein the vector is an AAV.

[0238] Embodiment 166 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-165, wherein the guide RNA is provided to the cell in a lipid nanoparticle (LNP).

[0239] Embodiment 167 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-166, wherein the guide RNA is provided to the cell in a same lipid nanoparticle (LNP) as an RNA-guided DNA binding agent.

[0240] Embodiment 168 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-167, wherein the exogenous nucleic acid is provided to the cell in a lipid nanoparticle (LNP).

[0241] Embodiment 169 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-168, wherein the exogenous nucleic acid is integrated into the genome of the cell.

[0242] Embodiment 170 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-169, wherein the exogenous nucleic acid is integrated into the genome of the cell by homologous recombination (HR).

[0243] Embodiment 171 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-170, wherein the exogenous nucleic acid is integrated into a safe harbor locus in the genome of the cell.

[0244] Embodiment 172 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 87-171, wherein the exogenous nucleic acid is integrated into the gene of the cell by nonhomologous end joining (NHEJ).

[0245] Embodiment 173 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 3, 13, 18, 32, 36, 39, 48-56, 58, 64-71, 73-74, 80-82, 86, and 88-91.

[0246] Embodiment 174 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 3, 13, 36, 39, 49-56, 64-71, 74, 80-82, 88, and 90-91.

[0247] Embodiment 175 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 13, 39, 49, 52, 65, 74, 82, and 91.

[0248] Embodiment 176 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 3, 39, and 49-52.

[0249] Embodiment 177 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 3, 36, 39, 49, 50, 51, and 52.

[0250] Embodiment 178 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 39, 49, and 52.

[0251] Embodiment 179 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 49, 52-54, 55, 56, 64, 65, 67-71, 73-74, 80-82, and 90.

[0252] Embodiment 180 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 49, 51, 74, 81, and 82.

[0253] Embodiment 181 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 103, 106, 107, 114, 117, 118, 125-129, 137, 138, 141, 143, 144, 145, 159, 160, 163, 164, 165, 166, 169, 171, 172, 173, 176, 177, 178, 179, and 180.

[0254] Embodiment 182 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 65 and 74.

[0255] Embodiment 183 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 49, 52-54, 56, 64-65, 67-71, 73-74, 80-82, 88, and 90-91.

[0256] Embodiment 184 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 74, 82, and 91.

[0257] Embodiment 185 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 3, 13, 18, 32, 36, 39, 48-56, 58, 64-71, 73-74, 80-82, 86, and 88-90.

[0258] Embodiment 186 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 3.

[0259] Embodiment 187 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 13.

[0260] Embodiment 188 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 18.

[0261] Embodiment 189 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 32.

[0262] Embodiment 190 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 36.

[0263] Embodiment 191 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 39.

[0264] Embodiment 192 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 48.

[0265] Embodiment 193 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 49.

[0266] Embodiment 194 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 50.

[0267] Embodiment 195 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 51.

[0268] Embodiment 196 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 52.

[0269] Embodiment 197 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 53.

[0270] Embodiment 198 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 54.

[0271] Embodiment 199 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 55.

[0272] Embodiment 200 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 56.

[0273] Embodiment 201 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 58.

[0274] Embodiment 202 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 64.

[0275] Embodiment 203 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 65.

[0276] Embodiment 204 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 66.

[0277] Embodiment 205 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 67.

[0278] Embodiment 206 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 68.

[0279] Embodiment 207 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 69.

[0280] Embodiment 208 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 70.

[0281] Embodiment 209 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 71.

[0282] Embodiment 210 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 73.

[0283] Embodiment 211 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 74.

[0284] Embodiment 212 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 80.

[0285] Embodiment 213 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 81.

[0286] Embodiment 214 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 82.

[0287] Embodiment 215 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 86.

[0288] Embodiment 216 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 88.

[0289] Embodiment 217 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 89.

[0290] Embodiment 218 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 90.

[0291] Embodiment 219 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 91.

[0292] Embodiment 220 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 103, 106, 107, 114, 117, 118, 125-129, 133, 137, 138, 141, 143, 144, 145, 159, 160, 163, 164, 165, 166, 169, 171, 172, 173, 176, 177, 178, 179, and 180.

[0293] Embodiment 221 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 103, 106, 117, 118, 125-128, 133, 137-138, 141, 143-144, 159, 163, 164, 165, 166, 169, 171, 173, 177, 178, and 180.

[0294] Embodiment 222 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 106, 114, 117-118, 125-128, 133, 137-138, 141, 143-144, 159, 163, 164, 165, 166, 169, 171, 173, 177, 178, and 180.

[0295] Embodiment 223 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-181172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 117-118, 125-128, 137-138, 144, 159, 163, 164, 165, 166, 169, 177, 178, and 180.

[0296] Embodiment 224 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 117, 127, 137-138, 163, 164, 165, 166, 169, and 177.

[0297] Embodiment 225 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101, 103, 106, 107, 117, 125-129, 137, 138, 141, 143, 144, 145, 159, 160, 163, 164, 165, 166, 169, 171, 172, 173, 176, 177, 178, 179, and 180.

[0298] Embodiment 226 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises a guide sequence comprising a sequence of any one of SEQ ID NOs: 163-166, 169, and 177.

[0299] Embodiment 227 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 101.

[0300] Embodiment 228 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 103.

[0301] Embodiment 229 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 106.

[0302] Embodiment 230 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 107.

[0303] Embodiment 231 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 117.

[0304] Embodiment 232 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 125.

[0305] Embodiment 233 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 126.

[0306] Embodiment 234 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 127.

[0307] Embodiment 235 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 128.

[0308] Embodiment 236 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 129.

[0309] Embodiment 237 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 137.

[0310] Embodiment 238 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 138.

[0311] Embodiment 239 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 141.

[0312] Embodiment 240 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 143.

[0313] Embodiment 241 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 144.

[0314] Embodiment 242 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 145.

[0315] Embodiment 243 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 159.

[0316] Embodiment 244 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 160.

[0317] Embodiment 245 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 163.

[0318] Embodiment 246 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 164.

[0319] Embodiment 247 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 165.

[0320] Embodiment 248 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 166.

[0321] Embodiment 249 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 169.

[0322] Embodiment 250 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 171.

[0323] Embodiment 251 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 172.

[0324] Embodiment 252 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 173.

[0325] Embodiment 253 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 176.

[0326] Embodiment 254 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 177.

[0327] Embodiment 255 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 178.

[0328] Embodiment 256 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 179.

[0329] Embodiment 257 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 180.

[0330] Embodiment 258 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises the sequence of any one of SEQ ID NOs: 2186-2191.

[0331] Embodiment 259 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2186.

[0332] Embodiment 260 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2187.

[0333] Embodiment 261 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2188.

[0334] Embodiment 262 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2189.

[0335] Embodiment 263 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2190.

[0336] Embodiment 264 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 67-172, wherein the HLA-B guide RNA comprises SEQ ID NO: 2191.

[0337] Embodiment 265 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 313 or 314.

[0338] Embodiment 266 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 314.

[0339] Embodiment 267 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 315.

[0340] Embodiment 268 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 316.

[0341] Embodiment 269 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 317.

[0342] Embodiment 270 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 318.

[0343] Embodiment 271 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 326.

[0344] Embodiment 272 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 337.

[0345] Embodiment 273 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 338.

[0346] Embodiment 274 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 339.

[0347] Embodiment 275 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 341.

[0348] Embodiment 276 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 343.

[0349] Embodiment 277 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 345.

[0350] Embodiment 278 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-264, wherein the HLA-A guide RNA comprises SEQ ID NO: 362.

[0351] Embodiment 279 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-257 wherein the HLA-A guide RNA comprises SEQ ID NO: 576.

[0352] Embodiment 280 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of embodiments 67-257 wherein the HLA-A guide RNA comprises SEQ ID NO: 571.

[0353] Embodiment 281 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 1-280, for use to express a TCR with specificity for a polypeptide expressed by cancer cells.

[0354] Embodiment 282 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 1-281, for use in administering to a subject as an adoptive cell transfer (ACT) therapy.

[0355] Embodiment 283 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 1-282, for use in treating a subject with cancer.

[0356] Embodiment 284 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 1-283, for use in treating a subject with an infectious disease.

[0357] Embodiment 285 is the engineered cell, population of cells, pharmaceutical composition, composition, or method of any one of embodiments 1-284, for use in treating a subject with an autoimmune disease.

[0358] Embodiment 286 is a cell bank comprising: (a) the engineered cells of any one of embodiments 1-56, 73-75, 94-285, or the engineered cells produced by the method of any one of embodiments 69-285; and (b) a catalogue comprising information documenting the HLA-A and HLA-C alleles of the donor cells in the cell bank.

[0359] Embodiment 287 is the cell bank of embodiment 286, wherein the cell bank comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40 donor cells that have a unique combination of HLA-A and HLA-C alleles as compared to other donor cells in the cell bank.

[0360] Embodiment 288 is a method of administering an engineered cell to a recipient subject in need thereof, the method comprising: (a) determining the HLA-A and HLA-C alleles of the recipient subject; (b) selecting an engineered cell or cell population of embodiments 1-56, 58, 60-63, 73-75, 94-285, or an engineered cell or cell population produced by the method of any one of embodiments 69-285, wherein the engineered cell comprises at least one of the same HLA-A or HLA-C alleles as the recipient subject; (c) administering the selected engineered cell to the recipient subject.

[0361] Embodiment 289 is the method of embodiment 288, wherein the subject has the HLA-A and HLA-C alleles of the engineered cell.

[0362] Embodiment 290 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 1-285, for use in administering to a partially matched subject for an adoptive cell transfer (ACT) therapy, wherein the partially matched subject has the HLA-A and HLA-C alleles of the engineered cell or cell population.

[0363] Embodiment 291 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 64-290, wherein the engineered cell or cell population comprises HLA-A and HLA-C alleles shared with the subject.

[0364] Embodiment 292 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 64-290, wherein the HLA-A and HLA-C alleles of the engineered cell or cell population consist of alleles that match one or more HLA-A and HLA-C alleles of the subject.

[0365] Embodiment 293 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of the preceding embodiments 64-290, wherein the HLA-C alleles of the engineered cell or cell population consist of alleles that match one or both HLA-C alleles of the subject.

[0366] Embodiment 294 is a cell bank comprising: (a) the engineered cells of any one of embodiments 1-56, 73-75, 94-285, or the engineered cells produced by the method of any one of any one of embodiments 69-285; and (b) a catalogue comprising information documenting the HLA-C alleles of the donor cells in the cell bank.

[0367] Embodiment 295 is a method of administering an engineered cell to a recipient subject in need thereof, the method comprising: (a) determining the HLA-C alleles of the recipient subject; (b) selecting an engineered cell or cell population of any one of embodiments 1-56, 58, 60-63, 73-75, 94-285, or engineered cell or cell population produced by the method of any one of embodiments 69-285, wherein the engineered cell is homozygous for one of the HLA-C alleles of the recipient subject; (c) administering the selected engineered cell to the recipient subject.

[0368] Embodiment 296 is the method of embodiment 295, wherein the subject is homozygous or heterozygous for the HLA-C allele of the engineered cell.

[0369] Embodiment 297 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 1-285, for use in administering to a partially matched subject for an adoptive cell transfer (ACT) therapy, wherein the partially matched subject is homozygous or heterozygous for the HLA-C allele of the engineered cell or cell population.

[0370] Embodiment 298 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 64-297, wherein the engineered cell or cell population comprises HLA-C alleles shared with the subject.

[0371] Embodiment 299 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 64-298, wherein the HLA-C alleles of the engineered cell or cell population consist of alleles that match one or more HLA-C alleles of the subject.

[0372] Embodiment 300 is the engineered cell, population, composition, pharmaceutical composition, or method of any one of embodiments 64-299, wherein the HLA-C alleles of the engineered cell or cell population consist of alleles that match one or both HLA-C alleles of the subject.

A. Definitions

[0373] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0374] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed terms preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, ACB, CBA, BCA, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AAB, BBC, CBBA, CABA, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0375] As used herein, the term "kit" refers to a packaged set of related components, such as one or more polynucleotides or compositions and one or more related materials such as delivery devices (e.g., syringes), solvents, solutions, buffers, instructions, or desiccants.

[0376] An "allogeneic" cell, as used herein, refers to a cell originating from a donor subject of the same species as a recipient subject, wherein the donor subject and recipient subject have genetic dissimilarity, e.g., genes at one or more loci that are not identical. Thus, e.g., a cell is allogeneic with respect to the subject to be administered the cell. As used herein, a cell that is removed or isolated from a donor, that will not be re-introduced into the original donor, is considered an allogeneic cell.

[0377] An "autologous" cell, as used herein, refers to a cell derived from the same subject to whom the material will later be re-introduced. Thus, e.g., a cell is considered autologous if it is removed from a subject and it will then be re-introduced into the same subject.

[0378] "β2M" or "B2M," as used herein, refers to nucleic acid sequence or protein sequence of "3-2 microglobulin"; the human gene has accession number NC_000015 (range 44711492..44718877), reference GRCh38.p13. The B2M protein is associated with MHC class I molecules as a heterodimer on the surface of nucleated cells and is required for MHC class I protein expression.

[0379] "CIITA" or "CIITA" or "C2TA," as used herein, refers to the nucleic acid sequence or protein sequence of "class II major histocompatibility complex transactivator;" the human gene has accession number NC_000016.10 (range 10866208..10941562), reference GRCh38.p13. The CIITA protein in the nucleus acts as a positive regulator of MHC class II gene transcription and is required for MHC class II protein expression.

[0380] As used herein, "MHC" or "MHC molecule(s)" or "MHC protein" or "MHC complex(es)," refers to a major histocompatibility complex molecule (or plural), and includes e.g., MHC class I and MHC class II molecules. In humans, MHC molecules are referred to as "human leukocyte antigen" complexes or "HLA molecules" or "HLA protein." The use of terms "MHC" and "HLA" are not meant to be limiting; as used herein, the term "MHC" may be used to refer to human MHC molecules, i.e., HLA molecules. Therefore, the terms "MHC" and "HLA" are used interchangeably herein.

[0381] The term "HLA-A," as used herein in the context of HLA-A protein, refers to the MHC class I protein molecule, which is a heterodimer consisting of a heavy chain (encoded by the HLA-A gene) and a light chain (i.e., beta-2 microglobulin). The term "HLA-A" or "HLA-A gene," as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-A protein molecule. The HLA-A gene is also referred to as "HLA class I histocompatibility, A alpha chain;" the human gene has accession number NC_000006.12 (29942532..29945870). The HLA-A gene is known to have thousands of different genotypic versions of the HLA-A gene across the population (and an individual may receive two different alleles of the HLA-A gene). A public database for HLA-A alleles, including sequence information, may be accessed at IPD-IMGT/HLA: www.ebi.ac.uk/ipd/imgt/hla/. All alleles of HLA-A are encompassed by the terms "HLA-A" and "HLA-A gene."

[0382] The term "HLA-B," as used herein in the context of HLA-B protein, refers to the MHC class I protein molecule, which is a heterodimer consisting of a heavy chain (encoded by the HLA-B gene) and a light chain (i.e., beta-2 microglobulin). "HLA-B" as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-B protein molecule. The HLA-B is also referred to as "HLA class I histocompatibility, B alpha chain;" the human gene has accession number NC_000006.12 (31353875..31357179). The HLA-B gene is known to have thousands of different genotypic versions of the HLA-B gene across the population (and an individual may receive two different alleles of the HLA-A gene). A public database for HLA-B alleles, including sequence information, may be accessed at IPD-IMGT/HLA: www.ebi.ac.uk/ipd/imgt/hla/. All alleles of HLA-B are encompassed by the terms "HLA-B" and "HLA-B gene."

[0383] "HLA-C" as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-C protein molecule. The HLA-C is also referred to as "HLA class I histocompatibility, C alpha chain;" the human gene has accession number NC_000006.12 (31268749..31272092).

[0384] As used herein, the term "within the genomic coordinates" includes the boundaries of the genomic coordinate range given. For example, if chr6:29942854-chr6:29942913 is given, the coordinates chr6:29942854-chr6:29942913 are encompassed. Throughout this application, the referenced genomic coordinates are based on genomic annotations in the GRCh38 (also referred to as hg38) assembly of the human genome from the Genome Reference Consortium, available at the National Center for Biotechnology Information website. Tools and methods for converting genomic coordinates between one assembly and another are known in the art and can be used to convert the genomic coordinates provided herein to the corresponding coordinates in another assembly of the human genome, including conversion to an earlier assembly generated by the same institution or using the same algorithm (e.g., from GRCh38 to GRCh37), and conversion of an assembly generated by a different institution or algorithm (e.g., from GRCh38 to NCBI33, generated by the International Human Genome Sequencing Consortium). Available methods and tools known in the art include, but are not limited to, NCBI Genome Remapping Service, available at the National Center for Biotechnology Information website, UCSC LiftOver, available at the UCSC Genome Brower website, and Assembly Converter, available at the Ensembl.org website.

[0385] As used herein, the term "homozygous" refers to having two identical alleles of a particular gene.

[0386] As used herein, an HLA "allele" can refer to a named HLA-A, HLA-B, or HLA-C gene wherein the first four digits (or the first two sets of digits separated by a colon, e.g., HLA-A*02:101:01:02N where the first two sets of digits are bolded and in italics) of the name following "HLA-A", HLA-B", or "HLA-C" are specified. As known in the art, the first four digits (or first two sets of digits separated by a colon) specify the protein of the allele. For example, HLA-A*02:01 and HLA-A*01:02 are distinct HLA-A alleles. Further genotypes of each allele exist, such as, e.g., HLA-A*02:01:02:01. Further genotypes of a given allele are considered to be identical alleles, e.g., HLA-A*02:01:02:01 and HLA-A*02:01 are identical alleles. Thus, HLA alleles are homozygous when the alleles are identical (i.e., when the alleles have the same first four digits or same first two sets of digits separated by a colon).

[0387] "Matching" or "matched" refers to shared alleles between the donor and the recipient, e.g., identical alleles.

[0388] "Polynucleotide" and "nucleic acid" are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid "backbone" can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds ("peptide nucleic acids" or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or NT-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., N^4-methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O^6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O^4-alkyl-pyrimidines; U.S. Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more "abasic" residues where the backbone includes no nitrogenous base for position(s) of the polymer (U.S. Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes "locked nucleic acid" (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42): 13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[0389] "Guide RNA", "gRNA", and simply "guide" are used herein interchangeably to refer to, for example, the guide that directs an RNA-guided DNA binding agent to a target DNA and can be a single guide RNA, or the combination of a crRNA and a trRNA (also known as tracrRNA). Exemplary gRNAs include Class II Cas nuclease guide RNAs, in modified or unmodified forms. The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA strands (dual guide RNA, dgRNA). "Guide RNA" or "gRNA" refers to each type. The trRNA may be a naturally occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences.

[0390] As used herein, a "guide sequence" refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A "guide sequence" may also be referred to as a "targeting sequence," or a "spacer sequence." A guide sequence can be 20 nucleotides in length, e.g., in the case of *Streptococcus pyogenes* (i.e., Spy Cas9 (SpCas9)) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[0391] Accordingly, in the case of *Neisseria meningitides* (i.e., Nine Cas9 (NmeCas9)) and related Cas9 homologs/orthologs, a guide sequence may be 19, 20, 21, preferably 22, 23, or 24 nucleotides in length, or may be 20-25 nucleotides in length. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence is at least 80%, 85%, preferably 90%, or 95%. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch, i.e., one nucleotide that is not identical or not complementary, depending on the reference sequence. For example, the guide sequence and the target sequence may contain 1-2, preferably no more than 1 mismatch, where the total length of the target sequence is 19, 20, 21, 22, preferably 23, or 24, nucleotides, or more. In some embodiments, the guide sequence and the target region may contain 1-2 mismatches where the guide sequence comprises at least 24

nucleotides, or more. In some embodiments, the guide sequence and the target region may contain 1-2 mismatches where the guide sequence comprises 24 nucleotides. That is, the guide sequence and the target region may form a duplex region having at least 2× base pairs, or more. In certain embodiments, the duplex region may include 1-2 mismatches such that guide strand and target sequence are not fully complementary. Mismatch positions are known in the art as provided in, for example, PAM distal mismatches tend to be better tolerated than PAM proximal matches. Mismatch tolerances at other positions are known in the art (see, e.g., Edraki et al., 2019. Mol. Cell, 73:1-13).

[0392] Target sequences for RNA-guided DNA binding agents include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse compliment), as a nucleic acid substrate for an RNA-guided DNA binding agent is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[0393] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the presence of a PAM and the sequence of the guide RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. The dCas DNA binding agent may be a dead nuclease comprising non-functional nuclease domains (RuvC or HNH domain). In some embodiments the Cas cleavase or Cas nickase encompasses a dCas DNA binding agent modified to permit DNA cleavage, e.g. via fusion with a FokI domain. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases.

[0394] As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity. Class 2 Cas nucleases include Class 2 Cas cleavases/nickases (e.g., H840A or D10A variants of Spy Cas9 and D16A and H588A of Nine Cas9, e.g., Nme2 Cas9), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., Cell, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. See, e.g., Makarova et al., Nat Rev Microbiol, 13(11): 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015).

[0395] Several Cas9 orthologs have been obtained from *N. meningitidis* (Esvelt et al., NAT. METHODS, vol. 10, 2013, 1116-1121; Hou et al., PNAS, vol. 110, 2013, pages 15644-15649) (Nme1Cas9, Nme2Cas9, and Nme3Cas9). The Nme2Cas9 ortholog functions efficiently in mammalian cells, recognizes an N4CC PAM, and can be used for in vivo editing with cognate gRNAs (Ran et al., NATURE, vol. 520, 2015, pages 186-191; Kim et al., NAT. COMMUN., vol. 8, 2017, pages 14500). Nme2Cas9 can be specific and selective, e.g. capable of low off-target editing (Lee et al., MOL. THER., vol. 24, 2016, pages 645-654; Kim et al., 2017). See also e.g., WO/2020081568 (e.g., pages 28 and 42), describing an Nme2Cas9 D16A nickase, the contents of which are hereby incorporated by reference in its entirety. Throughout, "NmeCas9" or "Nine Cas9" is generic and encompasses any type of NmeCas9, including, Nme1Cas9, Nme2Cas9, and Nme3Cas9.

[0396] As used herein, the term "editor" refers to an agent comprising a polypeptide that is capable of making a modification within a DNA sequence. In some embodiments, the editor is a cleavase, such as a Cas9 cleavase. In some embodiments, the editor is capable of deaminating a base within a DNA molecule, and it may be called a base editor. In some embodiments, the editor is capable of deaminating a cytosine (C) in DNA. In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to a cytidine deaminase. In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to an APOBEC3A deaminase (A3A). In some embodiments, the editor comprises a Cas9 nickase fused to an APOBEC3A deaminase (A3A). In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to a cytidine deaminase and a UGI. In some embodiments, the editor lacks a UGI.

[0397] As used herein, a "cytidine deaminase" means a polypeptide or complex of polypeptides that is capable of cytidine deaminase activity, that is catalyzing the hydrolytic deamination of cytidine or deoxycytidine, typically resulting in uridine or deoxyuridine. Cytidine deaminases encompass enzymes in the cytidine deaminase superfamily, and in particular, enzymes of the APOBEC family (APOBEC1, APOBEC2, APOBEC4, and APOBEC3 subgroups of enzymes), activation-induced cytidine deaminase (AID or AICDA) and CMP deaminases (see, e.g., Conticello et al., Mol. Biol. Evol. 22:367-77, 2005; Conticello, Genome Biol. 9:229, 2008; Muramatsu et al., J. Biol. Chem. 274: 18470-6, 1999); Carrington et al., Cells 9:1690 (2020)).

[0398] As used herein, the term "APOBEC3" refers to a APOBEC3 protein, such as an APOBEC3 protein expressed by any of the seven genes (A3A-A3H) of the human APOBEC3 locus. The APOBEC3 may have catalytic DNA or RNA editing activity. An amino acid sequence of APOBEC3A has been described (UniPROT accession ID: p31941) and is included herein as SEQ ID NO: 799. In some embodiments, the APOBEC3 protein is a human APOBEC3 protein or a wild-type protein. Variants include proteins having a sequence that differs from wild-type APOBEC3 protein by one or several mutations (i.e. substitutions, deletions, insertions), such as one or several single point substitutions. For instance, a shortened APOBEC3 sequence could be used, e.g. by deleting several N-term or C-term amino acids, preferably one to four amino acids at the

C-terminus of the sequence. As used herein, the term "variant" refers to allelic variants, splicing variants, and natural or artificial mutants, which are homologous to a APOBEC3 reference sequence. The variant is "functional" in that it shows a catalytic activity of DNA or RNA editing. In some embodiments, an APOBEC3 (such as a human APOBEC3A) has a wild-type amino acid position 57 (as numbered in the wild-type sequence). In some embodiments, an APOBEC3 (such as a human APOBEC3A) has an asparagine at amino acid position 57 (as numbered in the wild-type sequence).

[0399] As used herein, a "nickase" is an enzyme that creates a single-strand break (also known as a "nick") in double strand DNA, i.e., cuts one strand but not the other of the DNA double helix. As used herein, an "RNA-guided DNA nickase" means a polypeptide or complex of polypeptides having DNA nickase activity, wherein the DNA nickase activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA nickases include Cas nickases. Cas nickases include nickase forms of a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. Class 2 Cas nickases include variants in which only one of the two catalytic domains is inactivated, which have RNA-guided DNA nickase activity. Class 2 Cas nickases include polypeptides in which either the HNH or RuvC catalytic domain is inactivated, for example, Cas9 for example, Cas9 (e.g., H840A, D10A, or N863A variants of SpyCas9 or D16A variant of NmeCas9). Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain or RuvC or RuvC-like domains for *N. meningitidis* include Nme2Cas9 D16A (HNH nickase) and Nme2Cas9 H588A (RuvC nickase), Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g, K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., Cell, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like protein domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. "Cas9" encompasses *S. pyogenes* (Spy) Cas9, the variants of Cas9 listed herein, and equivalents thereof. See, e.g., Makarova et al., Nat Rev Microbiol, 13(11): 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015).

[0400] As used herein, the term "fusion protein" refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein may be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an "amino-terminal fusion protein" or a "carboxy-terminal fusion protein," respectively. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

[0401] The term "linker," as used herein, refers to a chemical group or a molecule linking two adjacent molecules or moieties. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein) such as a 16-amino acid residue "XTEN" linker, or a variant thereof (See, e.g., the Examples; and Schellenberger et al. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. Nat. Biotechnol. 27, 1186-1190 (2009)). In some embodiments, the XTEN linker comprises the sequence

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                                    (SEQ ID NO: 900)
SGSETPGTSESATPES,

                                    (SEQ ID NO: 901)
SGSETPGTSESA,
or

                                    (SEQ ID NO: 902)
SGSETPGTSESATPEGGSGGS.
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[0402] As used herein, the term "uracil glycosylase inhibitor" or "UGI" refers to a protein that is capable of inhibiting a uracil-DNA glycosylase (UDG) base-excision repair enzyme.

[0403] As used herein, "open reading frame" or "ORF" of a gene refers to a sequence consisting of a series of codons that specify the amino acid sequence of the protein that the gene codes for. The ORF begins with a start codon (e.g., ATG in DNA or AUG in RNA) and ends with a stop codon, e.g., TAA, TAG or TGA in DNA or UAA, UAG, or UGA in RNA.

[0404] As used herein, "ribonucleoprotein" (RNP) or "RNP complex" refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[0405] As used herein, a first sequence is considered to "comprise a sequence with at least X % identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X % or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as

pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[0406] "mRNA" is used herein to refer to a polynucleotide and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof.

[0407] As used herein, "indel" refers to an insertion or deletion mutation consisting of a number of nucleotides that are either inserted, deleted, or inserted and deleted, e.g. at the site of double-stranded breaks (DSBs), in a target nucleic acid. As used herein, when indel formation results in an insertion, the insertion is a random insertion at the site of a DSB and is not generally directed by or based on a template sequence.

[0408] As used herein, "reduced or eliminated" expression of a protein on a cell refers to a partial or complete loss of expression of the protein relative to an unmodified cell. In some embodiments, the surface expression of a protein on a cell is measured by flow cytometry and has "reduced" or "eliminated" surface expression relative to an unmodified cell as evidenced by a reduction in fluorescence signal upon staining with the same antibody against the protein. A cell that has "reduced" or "eliminated" surface expression of a protein by flow cytometry relative to an unmodified cell may be referred to as "negative" for expression of that protein as evidenced by a fluorescence signal similar to a cell stained with an isotype control antibody. The "reduction" or "elimination" of protein expression can be measured by other known techniques in the field with appropriate controls known to those skilled in the art.

[0409] As used herein, "knockdown" refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both), e.g., as compared to expression of an unedited target sequence. Knockdown of a protein can be measured by detecting total cellular amount of the protein from a sample, such as a tissue, fluid, or cell population of interest. It can also be measured by measuring a surrogate, marker, or activity for the protein. Methods for measuring knockdown of mRNA are known and include analyzing mRNA isolated from a sample of interest. In some embodiments, "knockdown" may refer to some loss of expression of a particular gene product, for example a decrease in the amount of mRNA transcribed or a decrease in the amount of protein expressed by a cell or population of cells (including in vivo populations such as those found in tissues).

[0410] As used herein, "knockout" (or "KO") refers to a loss of expression from a particular gene or of a particular protein in a cell. Knockout can result in a decrease in expression below the level of detection of the assay. Knockout can be measured either by detecting total cellular amount of a protein in a cell, a tissue or a population of cells.

[0411] As used herein, a "target sequence" or "genomic target sequence" refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[0412] As used herein, "treatment" refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing one or more symptoms of the disease, including recurrence of the symptom.

[0413] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention is described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims and included embodiments.

[0414] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality of cells and the like.

[0415] Numeric ranges are inclusive of the numbers defining the range. Measured and measurable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[0416] Unless specifically noted in the specification, embodiments in the specification that recite "comprising" various components are also contemplated as "consisting of" or "consisting essentially of" the recited components; embodiments in the specification that recite "consisting of" various components are also contemplated as "comprising" or "consisting essentially of" the recited components; and embodiments in the specification that recite "consisting essentially of" various components are also contemplated as "consisting of" or "comprising" the recited components (this interchangeability does not apply to the use of these terms in the claims). The term "or" is used in an inclusive sense, i.e., equivalent to "and/or," unless the context clearly indicates otherwise.

[0417] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of

this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

B. Genetically Modified Cells

1. Engineered Human Cell Compositions

[0418] The present disclosure provides engineered human cell compositions which have reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and homozygous for HLA-C. Additionally, the disclosure provides engineered human cell compositions which have reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising (i) a genetic modification in the HLA-A gene and (ii) a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-C. In some embodiments, the engineered human cell is an allogeneic cell. In some embodiments, the engineered human cell with reduced or eliminated HLA-B expression or HLA-A and HLA-B expression is useful for adoptive cell transfer therapies. In some embodiments, the engineered human cell comprises additional genetic modifications in the genome of the cell (e.g., reducing or elimination of MHC class II proteins, or reducing or eliminating endogenous T cell receptor (TCR) proteins, or introduction of an exogenous nucleic acid for expression) to yield a cell that is desirable for allogeneic transplant purposes.

[0419] In some embodiments, the engineered human cell is an allogeneic cell therapy. In some embodiments, the engineered human cell is transferred to a recipient that has the same HLA-A allele as the engineered human cell. In some embodiments, the engineered human cell is transferred to a recipient that has the same HLA-C allele as the engineered human cell. In some embodiments, the engineered human cell is transferred to a recipient that has the same HLA-A and HLA-C alleles as the engineered human cell. Thus, the engineered human cells disclosed herein provide a partial HLA match to a recipient, thereby reducing the risk of an adverse immune response.

[0420] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and HLA-C.

[0421] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-C.

[0422] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6:31354480-31357174 or (b) chr6:31357084-31354647; wherein the cell is homozygous for HLA-A and HLA-C.

[0423] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-A and HLA-B genes, (i) wherein the genetic modification in HLA-A comprises at least one nucleotide within the genomic coordinates chosen from chr6:29942854-chr6:29942913 and chr6:29943518-chr6:29943619; and (ii) wherein the genetic modification in HLA-B comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6: 31354623-31357108 or 31354497-31357157; and wherein the cell is homozygous for HLA-C.

[0424] In some embodiments, for each given range of genomic coordinates, a range may encompass +/−10 nucleotides on either end of the specified coordinates. For example, if chr6:29942854-chr6:29942913 is given, in some embodiments the genomic target sequence or genetic modification may fall within chr6:29942844-chr6:29942923. In some embodiments, for each given range of genomic coordinates, the range may encompass +/−5 nucleotides on either end of the range.

[0425] In some embodiments, a given range of genomic coordinates may comprise a target sequence on both strands of the DNA (i.e., the plus (+) strand and the minus (−) strand).

[0426] Genetic modifications in the HLA-A or HLA-B gene are described further herein. In some embodiments, a genetic modification in the HLA-A or HLA-B genes comprises any one or more of an insertion, deletion, substitution, or deamination of at least one nucleotide in a target sequence.

[0427] The engineered human cells described herein may comprise a genetic modification in any HLA-B allele of the HLA-B gene or a genetic modification in any HLA-A allele of the HLA-A gene. The HLA gene is located in chromosome 6 in a genomic region referred to as the HLA superlocus; hundreds of HLA-A and HLA-B alleles have been reported in the art (see e.g., Shiina et al., Journal of Human Genetics 54:15-39 (2009). Sequences for HLA-A and HLA-B alleles are available in the art (see e.g., IPD-IMGT/HLA database for retrieving sequences of specific HLA-A and HLA-B alleles https://www.ebi.ac.uk/ipd/imgt/hla/allele.html).

[0428] In some embodiments, the cell has reduced or eliminated expression of at least one HLA-A allele selected from: HLA-A1, HLA-A2, HLA-A3, HLA-AT1, and HLA-A24. In some embodiments, the cell has reduced or eliminated expression of HLA-A1. In some embodiments, the cell has reduced or eliminated expression of HLA-A2. In some embodiments, the cell has reduced or eliminated expression of HLA-A3. In some embodiments, the cell has reduced or eliminated expression of HLA-A11. In some embodiments, the cell has reduced or eliminated expression of HLA-A24.

[0429] In some embodiments, the cell has reduced or eliminated expression of at least one HLA-B allele selected from: HLA-B7, HLA-B8, HLA-B13, HLA-B21, HLA-B27, HLA-B35, HLA-B37, HLA-B38, HLA-B39, HLA-B40, HLA-B41, HLA-B42, HLA-B44, HLA-B45, HLA-B46, HLA-B47, HLA-B48, HLA-B49, HLA-B50, HLA-B51, HLA-B52, HLA-B56, HLA-B67, HLA-B73, HLA-B81, and HLA-B83. In some embodiments, the cell has reduced or eliminated expression of HLA-B7. In some embodiments, the cell has reduced or eliminated expression of HLA-B8. In

some embodiments, the cell has reduced or eliminated expression of HLA-B13. In some embodiments, the cell has reduced or eliminated expression of HLA-B21. In some embodiments, the cell has reduced or eliminated expression of HLA-B27. In some embodiments, the cell has reduced or eliminated expression of HLA-B35. In some embodiments, the cell has reduced or eliminated expression of HLA-B37. In some embodiments, the cell has reduced or eliminated expression of HLA-B38. In some embodiments, the cell has reduced or eliminated expression of HLA-B39. In some embodiments, the cell has reduced or eliminated expression of HLA-B40. In some embodiments, the cell has reduced or eliminated expression of HLA-B41. In some embodiments, the cell has reduced or eliminated expression of HLA-B42. In some embodiments, the cell has reduced or eliminated expression of HLA-B44. In some embodiments, the cell has reduced or eliminated expression of HLA-B45. In some embodiments, the cell has reduced or eliminated expression of HLA-B46. In some embodiments, the cell has reduced or eliminated expression of HLA-B47. In some embodiments, the cell has reduced or eliminated expression of HLA-B48. In some embodiments, the cell has reduced or eliminated expression of HLA-B49. In some embodiments, the cell has reduced or eliminated expression of HLA-B50. In some embodiments, the cell has reduced or eliminated expression of HLA-B51. In some embodiments, the cell has reduced or eliminated expression of HLA-B52. In some embodiments, the cell has reduced or eliminated expression of HLA-B56. In some embodiments, the cell has reduced or eliminated expression of HLA-B57. In some embodiments, the cell has reduced or eliminated expression of HLA-B67. In some embodiments, the cell has reduced or eliminated expression of HLA-B73. In some embodiments, the cell has reduced or eliminated expression of HLA-B81. In some embodiments, the cell has reduced or eliminated expression of HLA-B83.

[0430] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0431] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355349-31355369; chr6:31355348-31355368; or chr6:31355145-31355165.

[0432] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; or chr6:31355414-31355434.

[0433] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0434] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355347-31355367, chr6:31355340-31355360, chr6:31355409-31355429.

[0435] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355349-31355369 or chr6:31355348-31355368.

[0436] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355192-31355212 or chr6:31355347-31355367.

[0437] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355347-31355367; chr6:31355340-31355360; or chr6:31355409-31355429.

[0438] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from:

chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355347-31355367; chr6:31355432-31355452; or chr6:31355340-31355360.

[0439] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; chr6:31355409-31355429.

[0440] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; and chr6:31355469-31355493.

[0441] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355361-31355385; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355356-31355380; chr6:31355366-31355390; chr6:31355417-31355441; chr6:31357078-31357102; chr6:31355460-31355484; chr6:31355415-31355439; chr6:31355166-31355190; chr6:31355378-31355402; chr6:31355401-31355425; chr6:31356262-31356286; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0442] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; or chr6:31356426-31356450.

[0443] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0444] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0445] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; chr6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; or chr6:31356767-31356791.

[0446] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410.

[0447] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene,

wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355145-31356401 or (b) chr6:31357084-31354647. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0448] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-A and HLA-B gene, wherein the genetic modification in HLA-A comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from chr6:29942854-chr6:29942913 and chr6:29943518-chr6:29943619; and wherein the genetic modification in HLA-B comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355145-31356401 or (b) chr6:31357084-31354647. In some embodiments, the cell is homozygous for HLA-C.

[0449] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0450] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355349-31355369; chr6:31355348-31355368; or chr6:31355145-31355165. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0451] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; or chr6:31355414-31355434. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0452] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0453] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355347-31355367, chr6:31355340-31355360, chr6:31355409-31355429. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0454] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355349-31355369 or chr6:31355348-31355368. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0455] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31355192-31355212 or chr6:31355347-31355367. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0456] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355347-31355367; chr6:31355340-31355360; or chr6:31355409-31355429. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0457] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene,

wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355347-31355367; chr6:31355432-31355452; or chr6:31355340-31355360. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0458] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; chr6:31355469-31355493; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0459] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355361-31355385; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355356-31355380; chr6:31355366-31355390; chr6:31355417-31355441; chr6:31357078-31357102; chr6:31355460-31355484; chr6:31355415-31355439; chr6:31355166-31355190; chr6:31355378-31355402; chr6:31355401-31355425; chr6:31356262-31356286; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0460] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; or chr6:31356426-31356450. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0461] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0462] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0463] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410. In some embodiments, the cell is homozygous for HLA-A and HLA-C.

[0464] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:

31355166-31355190; chr6:31355401-31355425; ch6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791, wherein the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the cell is homozygous for HLA-A. In some embodiments, the cell is homozygous for HLA-C. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0465] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31355157-31355177; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355185-31355205; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355190-31355210; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355576-31355596; chr6: 31355410-31355430; chr6:31355419-31355439; chr6: 31355414-31355434; chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; ch6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791; wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the cell is homozygous for HLA-A. In some embodiments, the cell is homozygous for HLA-C. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0466] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31355157-31355177; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355185-31355205; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355190-31355210; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355576-31355596; chr6: 31355410-31355430; chr6:31355419-31355439; chr6: 31355414-31355434; chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; ch6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791, wherein the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 7 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 8 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 9 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the cell is homozygous for HLA-A. In some embodiments, the cell is homozygous for HLA-C. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0467] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in an HLA-B gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31355157-31355177; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355185-31355205; chr6: 31355191-31355211; chr6:31355192-31355212; chr6:

31355190-31355210; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355576-31355596; chr6: 31355410-31355430; chr6:31355419-31355439; chr6: 31355414-31355434; chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; ch6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791; wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates. In some embodiments, the cell is homozygous for HLA-A. In some embodiments, the cell is homozygous for HLA-C. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0468] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; and chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0469] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0470] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6:

31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0471] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791.

[0472] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791. Due to allelic polymorphism, in some embodiments, the target sequences may comprise 1, 2, or 3 mismatches from the genomic sequence of hg38. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0473] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6:

31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA binding agent, such as an *S. pyogenes* Cas9, an *N. meningitidis* Cas9, or a base editor that comprises an *S. pyogenes* or *N. meningitidis* Cas9 nickase.

[0474] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA binding agent, such as an *S. pyogenes* Cas9.

[0475] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; chr6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA binding agent, such as an *N. meningitidis* Cas9 or Nme2Cas9.

[0476] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA binding agent, such as a base editor comprising a deaminase and an *S. pyogenes* Cas9 nickase.

[0477] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355180-31355200; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31355157-31355177; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355185-31355205; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355190-31355210; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355576-31355596; chr6:31355410-31355430; chr6: 31355419-31355439; chr6:31355414-31355434; chr6: 31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6:

31355401-31355425; ch6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; or chr6:31356767-31356791. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0478] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: (a) chr6:31355145-31356401 or (b) chr6:31357084-31354647. In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0479] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; and chr6:31355409-31355429.

[0480] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355182-31355202.

[0481] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355348-31355368.

[0482] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355180-31355200.

[0483] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355145-31355165.

[0484] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355349-31355369.

[0485] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355157-31355177.

[0486] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356381-31356401.

[0487] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356380-31356400.

[0488] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355204-31355224.

[0489] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355205-31355225.

[0490] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355185-31355205.

[0491] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355191-31355211.

[0492] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355192-31355212.

[0493] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B

genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355190-31355210.

[0494] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355193-31355213.

[0495] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355198-31355218.

[0496] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355320-31355340.

[0497] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355319-31355339.

[0498] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355178-31355198.

[0499] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355347-31355367.

[0500] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355432-31355452.

[0501] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355340-31355360.

[0502] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355576-31355596.

[0503] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355410-31355430.

[0504] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355419-31355439.

[0505] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355414-31355434.

[0506] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355409-31355429.

[0507] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356777-31356801.

[0508] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355492-31355516.

[0509] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355491-31355515.

[0510] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355469-31355493.

[0511] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355460-31355484.

[0512] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355419-31355443.

[0513] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355415-31355439.

[0514] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B

genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355417-31355441.

[0515] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355401-31355425.

[0516] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355390-31355414.

[0517] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355379-31355403.

[0518] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates 31355378-31355402.

[0519] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355369-31355393.

[0520] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr 6:31355361-31355385.

[0521] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr 6:31355366-31355390.

[0522] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr 6:31355356-31355380.

[0523] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355221-31355245.

[0524] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355222-31355246.

[0525] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355205-31355229.

[0526] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355446-31355470.

[0527] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356425-31356449.

[0528] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355441-31355465.

[0529] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355203-31355227.

[0530] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356437-31356461.

[0531] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356426-31356450.

[0532] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356763-31356787.

[0533] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356764-31356788.

[0534] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356762-31356786.

[0535] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31355204-31355228.

[0536] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356436-31356460.

[0537] In some embodiments, an engineered human cell is provided wherein the HLA-B expression is reduced or eliminated by a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr6:31356767-31356791.

[0538] In some embodiments, the HLA-B genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the HLA-B genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[0539] In some embodiments, the HLA-B genomic target sequence comprises at least 17, 18, 19 20, 21, 22, 23, or 24 contiguous nucleotides within the genomic coordinates.

[0540] In some embodiments, the gene editing system comprises a transcription activator-like effector nuclease (TALEN). In some embodiments, the gene editing system comprises a zinc finger nuclease. In some embodiments, the gene editing system comprises a CRISPR/Cas system, such as a class 2 system. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0541] Exemplary RNA-guided DNA binding agents are shown in Table 1A below.

TABLE 1A

Exemplary RNA-guided DNA binding agents.

RNA-guided DNA binding agent	PAM	Guide Length
Cas9 nuclease from *S. pyogenes*	NGG	20 bp
Cas9 nuclease from *Neisseria meningitidis*	NNNNG[A/C]TT	20 bp
Cas9 nuclease from *Streptococcus thermophilus*	NNAGAAW	20 bp
Cas9 nuclease is from *Staphylococcus aureus*	NNG(A/G)(A/G)T	20 bp
Cpf1 nuclease from *Francisella novicida*	TTTN	23 bp
Cpf1 nuclease from *Acidaminococcus* sp.	TTTV	23 bp
Cpf1 nuclease from *Lachnospiraceae bacterium*	TTTV	23 bp
C-to-T base editor*	NGG	20 bp
A-to-G base editor*	NGG	20 bp
Cas12a	same as Cpf1	
CasX	TTCN	20 bp
NME2	NNNNCC	24 bp

*Exemplary base editor based on deaminase-SpyCas9 nickase. As is apparent, the base editor specificity, including PAM, will vary with its nickase.

[0542] In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent comprises a Cas9 protein. In some embodiments, the RNA-guided DNA binding agent is selected from one of: *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, Mad7 nuclease, ARCUS nucleases, and CasX. In some embodiments, the RNA-guided DNA binding agent comprises a polypeptide selected from one of: *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, and CasX.

[0543] In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid

encoding the RNA-guided DNA binding agent is *N. meningitidis* Cas9, e.g. Nme2Cas9. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is *S. thermophilus* Cas9. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is *S. aureus* Cas9. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is Cpf1 from *F. novicida*. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is Cpf1 from *Acidaminococcus* sp. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is Cpf1 from *Lachnospiraceae bacterium* ND2006. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is a C to T base editor. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is a A to G base editor. In some embodiments, the base editor comprises a deaminase and an RNA-guided nickase. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent comprises a APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the RNA-guided nickase is a Spy-Cas9 nickase. In some embodiments, the RNA-guided nickase comprises an NmeCas9 nickase. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is Cas12a. In some embodiments, the RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent is CasX.

[0544] In any of the above embodiments, the C comprises an RNA-guided DNA binding agent, or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent comprises a Cas9. In some embodiments, the RNA-guided DNA binding agent is an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent is a base editor. In some embodiments the base editor comprises a C to T deaminase and an RNA-guided nickase such as an *S. pyogenes* Cas9 nickase. In some embodiments the base editor comprises a A to G deaminase and an RNA-guided nickase such as an *S. pyogenes* Cas9 nickase.

[0545] In any of the above embodiments, the gene editing system comprises an RNA-guided DNA binding agent, or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent comprises a Cas9. In some embodiments, the RNA-guided DNA binding agent is an *N. meningitidis* or Nme2 Cas9. In some embodiments, the RNA-guided DNA binding agent is a base editor. In some embodiments the base editor comprises a C to T deaminase and an RNA-guided nickase such as an *N. meningitidis* or Nme2 Cas9 nickase. In some embodiments the base editor comprises a A to G deaminase and an RNA-guided nickase such as an *N. meningitidis* or Nme2 Cas9 nickase.

[0546] In some embodiments, the gene editing system further comprises a uracil glycosylase inhibitor (UGI), and the UGI and the base editor are comprised in a single polypeptide. In some embodiments, the gene editing system comprises a UGI, and the UGI and the base editor are comprised in different polypeptides. In some embodiments, the base editor comprises a cytidine deaminase and an RNA-guided nickase. In some embodiments, the cytidine deaminase, the RNA-guided nickase, and the UGI are comprised in a single polypeptide. In some embodiments, the cytidine deaminase, the RNA-guided nickase, and the UGI are comprised in different polypeptides. In some embodiments, the cytidine deaminase and the RNA-guided nickase are comprised in a single polypeptide, and wherein the UGI is comprised in a different polypeptide.

[0547] In some embodiments, when the engineered cell is homozygous for HLA-A, the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02.

[0548] In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*16:01; HLA-C*15:02; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

[0549] In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is HLA-C*03:04. In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is HLA-C*06:02. In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is HLA-C*01:02. In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is HLA-C*08:01. In some embodiments, when the engineered cell is homozygous for HLA-C, the HLA-C allele is HLA-C*03:02.

[0550] In some embodiments, the engineered cell is homozygous for HLA-A and homozygous for HLA-C, the HLA-A and HLA-C allele pair is selected from the following: HLA-A*01:01 and HLA-C*07:01; HLA-A*02:01 and HLA-C*07:02; HLA-A*02:01 and HLA-C*05:01; HLA-A*03:01 and HLA-C*07:02; HLA-A*02:01 and HLA-C*04:01; HLA-A*02:01 and HLA-C*03:04; HLA-A*01:01 and HLA-C*06:02; HLA-A*03:01 and HLA-C*04:01; HLA-A*02:01 and HLA-C*07:01; HLA-A*24:02 and HLA-C*04:01; HLA-A*29:02 and HLA-C*16:01; HLA-A*02:01 and HLA-C*06:02; HLA-A*24:02 and HLA-C*07:02; HLA-A*26:01 and HLA-C*12:03; HLA-A*11:01 and HLA-C*04:01; HLA-A*25:01 and HLA-C*12:03; HLA-A*02:01 and HLA-C*02:02; HLA-A*24:02 and HLA-C*03:03; HLA-A*30:01 and HLA-C*06:02; HLA-A*02:01 and HLA-C*01:02; HLA-A*11:01 and HLA-C*07:02; HLA-A*03:01 and HLA-C*07:01; HLA-A*23:01 and HLA-C*04:01; HLA-A*24:02 and HLA-C*07:01; HLA-A*31:01 and HLA-C*03:04; HLA-A*33:01 and HLA-C*08:02; HLA-A*02:01 and HLA-C*03:03; HLA-A*11:01 and HLA-C*01:02; HLA-A*01:01 and HLA-C*04:01; HLA-A*03:01 and HLA-C*06:02.

[0551] The HLA-A and HLA-C allele pairs disclosed herein cumulatively cover about 810% of the population. The cumulative frequency of HLA-A and HLA-C allele pairs is shown in Table 1B below.

TABLE 1B

Cumulative Frequency of HLA-A and HLA-C Alleles in the Population.

Cumulative Frequency	Alleles
0.136	HLA-A*01:01 and HLA- C*07:01
0.231	HLA-A*02:01 and HLA-C*07:02
0.299	HLA-A*02:01 and HLA-C*05:01
0.361	HLA-A*03:01 and HLA-C*07:02
0.417	HLA-A*02:01 and HLA-C*04:01
0.460	HLA-A*02:01 and HLA-C*03:04
0.493	HLA-A*01:01 and HLA-C*06:02
0.524	HLA-A*03:01 and HLA-C*04:01
0.554	HLA-A*02:01 and HLA-C*07:01
0.579	HLA-A*24:02 and HLA-C*04:01
0.600	HLA-A*29:02 and HLA-C*16:01
0.621	HLA-A*02:01 and HLA-C*06:02
0.640	HLA-A*24:02 and HLA-C*07:02
0.657	HLA-A*26:01 and HLA-C*12:03
0.673	HLA-A*11:01 and HLA-C*04:01
0.686	HLA-A*25:01 and HLA-C*12:03
0.698	HLA-A*02:01 and HLA-C*02:02
0.710	HLA-A*24:02 and HLA-C*03:03
0.720	HLA-A*30:01 and HLA-C*06:02
0.730	HLA-A*02:01 and HLA-C*01:02
0.740	HLA-A*11:01 and HLA-C*07:02
0.749	HLA-A*03:01 and HLA-C*07:01
0.758	HLA-A*23:01 and HLA-C*04:01
0.766	HLA-A*24:02 and HLA-C*07:01
0.773	HLA-A*31:01 and HLA-C*03:04
0.780	HLA-A*33:01 and HLA-C*08:02
0.787	HLA-A*02:01 and HLA-C*03:03
0.794	HLA-A*11:01 and HLA-C*01:02
0.800	HLA-A*01:01 and HLA-C*04:01
0.806	HLA-A*03:01 and HLA-C*06:02

[0552] In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-A and homozygous for HLA-C, further has reduced or eliminated surface expression of MHC class II protein. In some embodiments, the engineered human cell has a genetic modification in a gene that reduces or eliminates surface expression of MHC class II protein. In some embodiments, the engineered human cell has a genetic modification in the CIITA gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DR gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DQ gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DP gene. In some embodiments, the engineered human cell has a genetic modification in the RFX gene. In some embodiments, the engineered human cell has a genetic modification in the CREB gene. In some embodiments, the engineered human cell has a genetic modification in the Nuclear Factor (NF)-gamma gene.

[0553] In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-C, further has reduced or eliminated surface expression of MHC class II protein. In some embodiments, the engineered human cell has a genetic modification in a gene that reduces or eliminates surface expression of MHC class II protein. In some embodiments, the engineered human cell has a genetic modification in the CIITA gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DR gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DQ gene. In some embodiments, the engineered human cell has a genetic modification in the HLA-DP gene. In some embodiments, the engineered human cell has a genetic modification in the RFX gene. In some embodiments, the engineered human cell has a genetic modification in the CREB gene. In some embodiments, the engineered human cell has a genetic modification in the Nuclear Factor (NF)-gamma gene.

[0554] In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-A and homozygous for HLA-C, further has reduced or eliminated surface expression of TRAC protein. In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-A and HLA-C, further has reduced or eliminated surface expression of TRBC protein.

[0555] In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-A and homozygous for HLA-C, further has reduced or eliminated surface expression of TRAC protein. In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-C, further has reduced or eliminated surface expression of TRBC protein.

[0556] In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-C, further has reduced or eliminated surface expression of TRAC protein. In some embodiments, an engineered human cell which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell is provided, that is homozygous for HLA-C, further has reduced or eliminated surface expression of TRBC protein.

[0557] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6:31355145-31356401 or (b) chr6: 31357084-31354647, and wherein the engineered cell further comprises a genetic modification in a gene that reduces or eliminates the surface expression of MHC class II protein. In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6:31355182-31355596 or (b) chr6: 31355203-31356461, and wherein the engineered cell further comprises a genetic modification in the CIITA gene.

[0558] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene,

wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6:31355182-31355596 or (b) chr6: 31355203-31356461, and wherein the engineered cell further comprises a genetic modification in the TRAC gene. In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6: 31355182-31355596 or (b) chr6: chr6: 31355203-31356461, and wherein the engineered cell further comprises a genetic modification in the TRBC gene.

[0559] In some embodiments, an engineered human cell is provided which has reduced or eliminated surface expression of HLA-A B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from (a) chr6:31354480-31357174; chr631355145-31356401 or (b) chr6: chr6:31355203-31356461, and wherein the engineered cell further comprises an exogenous nucleic acid. In some embodiments, the engineered cell comprises an exogenous nucleic acid encoding a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a CAR or a universal CAR. In some embodiments, the targeting receptor is a TCR. In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a ligand for the receptor. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain) and a subunit of a TCR. In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the engineered cell further comprises an exogenous nucleic acid encoding a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some embodiments, the secreted polypeptide is an antibody. In some embodiments, the secreted polypeptide is an enzyme. In some embodiments, the exogenous nucleic acid encodes an antibody encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein.

[0560] The engineered human cell may be any of the exemplary cell types disclosed herein. Further, because MHC class I molecules are expressed on all nucleated cells, the engineered human cell may be any nucleated cell. In some embodiments, the engineered cell is an immune cell. In some embodiments, the engineered cell is a stem cell such as a hematopoietic stem cell (HSC). In some embodiments, the engineered cell is an induced pluripotent stem cell (iPSC). In some embodiments, the engineered cell is a mesenchymal stem cell (MSC). In some embodiments, the engineered cell is a neural stem cell (NSC). In some embodiments, the engineered cell is a limbal stem cell (LSC). In some embodiments, the engineered cell is a progenitor cell, e.g. an endothelial progenitor cell or a neural progenitor cell. In some embodiments, the engineered cell is a tissue-specific primary cell. In some embodiments, the engineered cell is chosen from: chondrocyte, myocyte, and keratinocyte. In some embodiments, the engineered cell is a monocyte, macrophage, mast cell, dendritic cell, or granulocyte. In some embodiments, the engineered cell is monocyte. In some embodiments, the engineered cell is a macrophage. In some embodiments, the engineered cell is a mast cell. In some embodiments, the engineered cell is a dendritic cell. In some embodiments, the engineered cell is a granulocyte. In some embodiments, the engineered cell is a lymphocyte. In some embodiments, the engineered cell is a T cell. In some embodiments, the engineered cell is a CD4+ T cell. In some embodiments, the engineered cell is a CD8+ T cell. In some embodiments, the engineered cell is a memory T cell. In some embodiments, the engineered cell is a B cell. In some embodiments, the engineered cell is a plasma B cell. In some embodiments, the engineered cell is a memory B cell. In some embodiments, the engineered cell is a macrophage.

[0561] In some embodiments, the disclosure provides a pharmaceutical composition comprising any one of the engineered human cells disclosed herein. In some embodiments, the pharmaceutical composition comprises a population of any one of the engineered cells disclosed herein. In some embodiments, the population of engineered cells is at least 65% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 70% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 80% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 90% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 91% negative as measured by flow cytometry. In some embodiments, the population of engineered cells that is at least 92% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 93% HLA-B negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 94% HLA-B negative as measured by flow cytometry.

[0562] In some embodiments, the population of cells is at least 94% HLA-A negative or at least 94% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 95% HLA-A negative or at least 95% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 96% HLA-A negative or at least 96% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 97% HLA-A negative or at least 97% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 98% HLA-A negative or at least 98% HLA-B negative as measured by flow cytometry. In some embodiments, the population of cells is at least 99% HLA-A negative or at least 98% HLA-B negative as measured by flow cytometry.

[0563] In some embodiments, the population of cells is at least 94% HLA-A negative and at least 94% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 95% HLA-A negative and at least 95% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 96% HLA-A negative and at least 96% HLA-B negative, as measured by flow cytometry. In some embodiments, the population of cells is at least 97% HLA-A negative and at least 97% HLA-B negative, as measured by flow cytometry.

In some embodiments, the population of cells is at least 98% HLA-A negative and at least 98% HLA-B negative as measured by flow cytometry. In some embodiments, the population of cells is at least 99% HLA-A negative and at least 98% HLA-B negative as measured by flow cytometry.

[0564] In some embodiments, at least 92% of the population of cells comprises the genetic modification in the HLA-A gene or 92% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, the population of cells is at least 93% HLA-A negative or at least 93% HLA-B negative, as measured by flow cytometry. In some embodiments, at least 93% of the population of cells comprises the genetic modification in the HLA-A gene or at least 93% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 94% of the population of cells comprises the genetic modification in the HLA-A gene or at least 94% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 95% of the population of cells comprises the genetic modification in the HLA-A gene or at least 95% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 96% of the population of cells comprises the genetic modification in the HLA-A gene or at least 96% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 96% of the population of cells comprises the genetic modification in the HLA-A gene or at least 97% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 96% of the population of cells comprises the genetic modification in the HLA-A gene or at least 98% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS). In some embodiments, at least 96% of the population of cells comprises the genetic modification in the HLA-A gene or at least 99% of the population of cells comprises the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS).

[0565] In some embodiments, the population of cells is at least 95% CIITA negative as measured by flow cytometry. In some embodiments, the population of cells is at least 96% CIITA negative as measured by flow cytometry. In some embodiments, the population of cells is at least 97% CIITA negative as measured by flow cytometry. In some embodiments, the population of cells is at least 98% CIITA negative as measured by flow cytometry. In some embodiments, the population of cells is at least 99% CIITA negative as measured by flow cytometry.

[0566] In some embodiments, the population of engineered cells is at least 95% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 97% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 98% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 99% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the population of engineered cells is at least 99.5% endogenous TCR protein negative as measured by flow cytometry.

[0567] In some embodiments, methods are provided for administering the engineered human cells or pharmaceutical compositions disclosed herein to a subject in need thereof. In some embodiments, methods are provided for administering the engineered human cells or pharmaceutical compositions disclosed herein to a subject as an ACT therapy. In some embodiments, methods are provided for administering the engineered human cells or pharmaceutical compositions disclosed herein to a subject as a treatment for cancer. In some embodiments, methods are provided for administering the engineered human cells or pharmaceutical compositions disclosed herein to a subject as a treatment for an autoimmune disease. In some embodiments, methods are provided for administering the engineered human cells or pharmaceutical compositions disclosed herein to a subject as a treatment for an infectious disease.

C. Methods and Compositions for Reducing or Eliminating Surface Expression of HLA-B

[0568] The present disclosure provides methods and compositions for reducing or eliminating surface expression of HLA-B protein relative to an unmodified cell by genetically modifying the HLA-B gene. The disclosure also provides methods and compositions for reducing or eliminating surface expression of both HLA-A and HLA-B protein relative to an unmodified cell by genetically modifying the HLA-A and HLA-B genes. The resultant genetically modified cell may also be referred to herein as an engineered cell. In some embodiments, an already-genetically modified (or engineered) cell may be the starting cell for further genetic modification using the methods or compositions provided herein. In some embodiments, the cell is an allogeneic cell. In some embodiments, a cell with reduced or eliminated surface expression of HLA-B protein only or HLA-A and HLA-B protein is useful for adoptive cell transfer therapies. In some embodiments, editing of the HLA-A or HLA-B gene is combined with additional genetic modifications to yield a cell that is desirable for allogeneic transplant purposes.

[0569] In some embodiments, the methods comprise reducing surface expression of HLA-B protein in a human cell relative to an unmodified cell, comprising contacting a cell with composition comprising (a) a guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence

selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0570] In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent comprises a Cas9 protein. In some embodiments, the RNA-guided DNA binding agent is selected from one of *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, and CasX. In some embodiments, the RNA-guided DNA binding agent comprises a polypeptide selected from one of: *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, and CasX. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments the RNA-guided DNA binding agent is *N. meningitidis* Cas9, e.g., Nme2Cas9. In some embodiments the RNA-guided DNA binding agent is *S. thermophilus* Cas9. In some embodiments the RNA-guided DNA binding agent is *S. aureus* Cas9. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *F. novicida*. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *Acidaminococcus* sp. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *Lachnospiraceae bacterium* ND2006. In some embodiments the RNA-guided DNA binding agent is a C to T base editor. In some embodiments the RNA-guided DNA binding agent is a A to G base editor. In some embodiments, the base editor comprises a deaminase and an RNA-guided nickase. In some embodiments the RNA-guided DNA binding agent comprises a APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the RNA-guided nickase is a SpyCas9 nickase. In some embodiments, the RNA-guided nickase comprises an NmeCas9 nickase. In some embodiments the RNA-guided DNA binding agent is Cas12a. In some embodiments the RNA-guided DNA binding agent is CasX. In some embodiments, the surface expression of HLA-A protein (i.e., engineered cell) is thereby reduced or eliminated.

[0571] In some embodiments, the methods comprise reducing surface expression of HLA-A and HLA-B protein in a human cell relative to an unmodified cell, comprising contacting a cell with composition comprising (a) an HLA-A guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 301-590; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs 429-462 and 512-590; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 429-462 and 512-590; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 4, Table 5B, or Table 6, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 5A or Table 7; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and contacting a cell with a second composition comprising (a) an HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent comprises a Cas9 protein. In some embodiments, the RNA-guided DNA binding agent is selected from one of: *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, and CasX. In some embodiments, the RNA-guided DNA binding agent comprises a polypeptide selected from one of: *S. pyogenes* Cas9, *Neisseria meningitidis* Cas9, e.g. an Nme2Cas9, *S. thermophilus* Cas9, *S. aureus* Cas9, *Francisella novicida* Cpf1, *Acidaminococcus* sp. Cpf1, *Lachnospiraceae bacterium* Cpf1, C-to-T base editor, A-to-G base editor, Cas12a, and CasX. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments the RNA-guided DNA binding agent is *N. meningitidis* Cas9, e.g., Nme2Cas9. In some embodiments the RNA-guided DNA binding agent is *S. thermophilus* Cas9. In some embodiments the RNA-guided DNA binding agent is *S. aureus* Cas9. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *F. novicida*. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *Acidamino-*

coccus sp. In some embodiments the RNA-guided DNA binding agent is Cpf1 from *Lachnospiraceae bacterium* ND2006. In some embodiments the RNA-guided DNA binding agent is a C to T base editor. In some embodiments the RNA-guided DNA binding agent is a A to G base editor. In some embodiments, the base editor comprises a deaminase and an RNA-guided nickase. In some embodiments the RNA-guided DNA binding agent comprises a APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the RNA-guided nickase is a SpyCas9 nickase. In some embodiments, the RNA-guided nickase comprises an NmeCas9 nickase. In some embodiments the RNA-guided DNA binding agent is Cas12a. In some embodiments the RNA-guided DNA binding agent is CasX. In some embodiments, the surface expression of HLA-A protein (i.e., engineered cell) is thereby reduced or eliminated.

[0572] In some embodiments, the methods comprise making an engineered human cell, which has reduced or eliminated surface expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C, comprising contacting a cell with composition comprising (a) a guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* or *N. meningitidis* (e.g., Nme2) Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the surface expression of HLA-A B protein (i.e., engineered cell) is thereby reduced or eliminated.

[0573] In some embodiments, the methods comprise making an engineered human cell, which has reduced or eliminated surface expression of HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C, comprising contacting a cell with composition comprising (a) an HLA-A guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 301-590; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs 429-462 and 512-590; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 429-462 and 512-590; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Tables 4, 5B and 6, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 5A or Table 7; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and contacting a cell with a second composition comprising (a) an HLA-B guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-91 and 101-185; or (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally (b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* or *N. meningitidis* (e.g., Nme2) Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the surface expression of HLA-B protein (i.e., engineered cell) is thereby reduced or eliminated.

[0574] In some embodiments, the methods of reducing or eliminating surface expression of HLA-A or HLA-B protein comprise contacting a cell with any one or more of the HLA-A or HLA-B guide RNAs disclosed herein.

[0575] In some embodiments, compositions are provided comprising a) an HLA-B guide RNA comprising: (i) a guide sequence selected from SEQ ID NOs: 1-91 or 101-185; or (ii). at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a

sequence selected from SEQ ID NOs: 101-185; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185; or (iv) a guide sequence that binds a target site comprising a genomic region listed in Tables 2-3; or (v) a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; or (vi) a guide sequence that is at least 95%, 90%, or 85%, 80%, 75%, or 70% identical to a sequence selected from (v); and optionally b) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the composition further comprises an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the composition comprises an RNA-guided DNA binding agent that is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[0576] In some embodiments, the composition further comprises a uracil glycosylase inhibitor (UGI). In some embodiments, the composition comprises an RNA-guided DNA binding agent that the RNA-guided DNA binding agent generates a cytosine (C) to thymine (T) conversion with the HLA-A or HLA-B genomic target sequence. In some embodiments, the composition comprises an RNA-guided DNA binding agent that generates an adenosine (A) to guanine (G) conversion with the HLA-A or HLA-B genomic target sequence.

[0577] In some embodiments, an engineered human cell produced by the methods described herein is provided. In some embodiments, the engineered human cell produced by the methods and compositions described herein is an allogeneic cell. In some embodiments, the methods produce a composition comprising an engineered human cell having reduced or eliminated surface expression of HLA-A or HLA-B protein. In some embodiments, the engineered human cell produced by the methods disclosed herein elicits a reduced response from CD8+ T cells as compared to an unmodified cell as measured in an in vitro cell culture assay containing CD8+ T cells.

[0578] In some embodiments, the compositions disclosed herein further comprise a pharmaceutically acceptable carrier. In some embodiments, a cell produced by the compositions disclosed herein comprising a pharmaceutically acceptable carrier is provided. In some embodiments, compositions comprising the cells disclosed herein are provided.

1. HLA-B Guide RNAs

[0579] The methods and compositions provided herein disclose guide RNAs useful for reducing or eliminating the surface expression of HLA-B protein. In some embodiments, such guide RNAs direct an RNA-guided DNA binding agent to an HLA-A genomic target sequence and may be referred to herein as "HLA-B guide RNAs." In some embodiments, the HLA-B guide RNA directs an RNA-guided DNA binding agent to a human HLA-B genomic target sequence. In some embodiments, the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 1-91. In some embodiments, the HLA-B guide RNA comprises a guide sequence selected from SEQ ID NOs: 101-185.

[0580] In some embodiments, a composition is provided comprising an -B guide RNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0581] In some embodiments, a composition is provided comprising an HLA-B single-guide RNA (sgRNA) comprising a guide sequence selected from SEQ ID NOs: 1-91 or 101-185. In some embodiments, a composition is provided comprising HLA-B sgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0582] In some embodiments, a composition is provided comprising an HLA-B dual-guide RNA (dgRNA) comprising a guide sequence selected from SEQ ID NOs: 1-91 or 101-185. In some embodiments, a composition is provided comprising an HLA-B dgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0583] In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 1-91 or 101-185. Exemplary HLA-B guide sequences are shown below in Table 2 (SEQ ID NOs: 1-91), and Table 3 (SEQ ID NOs: 101-185).

[0584] In some embodiments, the HLA-A gRNA is a sgRNA comprising a sequence as shown below in Table 2 (SEQ ID NOs: 1001-1091 and 2001-2091), Table 3 (SEQ ID NOs: 1101-1185, and 2101-2185), and Table 3A (SEQ ID NOs: 2186-2191).

TABLE 2

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
G022008	1	CCAGCCUGGACG CAGGCACC	CCAGCCUGG ACGCAGGCA CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU	mC*mC*mA*GCCUG GACGCAGGCACCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm	chr6:31355718-31355738

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022009	2	AGGCGCUUUGCA UCUCUCAU	AGGCGCUUU GCAUCUCUC AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mG*mG*CGCUU UGCAUCUCUCAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355616-31355636
G022010	3	AACAAUGCCCAC GAUGGGGA	AACAAUGCC CACGAUGGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mA*mC*AAUGC CCACGAUGGGGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355182-31355202
G022011	4	CCAGACACCAGC CUGGACGC	CCAGACACC AGCCUGGAC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mA*GACAC CAGCCUGGACGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355711-31355731
G022012	5	CCAUCUCCUAUA GGUCGCCG	CCAUCUCCU AUAGGUCGC CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mA*UCUCC UAUAGGUCGCCGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356120-31356140
G022013	6	CUUCUCUCUAGG ACAAUUAA	CUUCUCUCU AGGACAAUU AAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mC*mU*mU*CUCUC UAGGACAAUUAAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC	chr6:31270893-31270913

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022014	7	CCCCAUCUCCUA UAGGUCGC	CCCCAUCUC CUAUAGGUC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mC*CAUCU CCUAUAGGUCGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356122-31356142
G022015	8	GGAUCUCGGACC CGGAGACU	GGAUCUCGG ACCCGGAGA CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mG*mA*UCUCG GACCCGGAGACUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354563-31354583
G022016	9	CUUCCUCCCAGU CCCCUCAC	CUUCCUCCC AGUCCCCUC ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mU*CCUCC CAGUCCCCUCACG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354682-31354702
G022017	10	CUCCUGUCCCAC GUCUCCUG	CUCCUGUCC CACGUCUCC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mC*CUGUC CCACGUCUCCUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355900-31355920
G022018	11	CCAUUUUCCUCC UCUUCUCG	CCAUUUUCC UCCUCUUCU CGGUUUUAG AGCUAGAAA UAGCAAGUU	mC*mC*mA*UUUUC CUCCUCUUCUCGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA	chr6:31356086-31356106

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022019	12	GGUUCCCAAGGC UGCUGCAG	GGUUCCCAA GGCUGCUGC AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mG*mU*UCCCA AGGCUGCUGCAGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356623-31356643
G022020	13	ACAUGCCAUGUA CAGCAUGA	ACAUGCCAU GUACAGCAU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mC*mA*UGCCA UGUACAGCAUGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355348-31355368
G022021	14	CGGUUCCCAAGG CUGCUGCA	CGGUUCCCA AGGCUGCUG CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mG*mG*UUCCC AAGGCUGCUGCAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355899-31355919
G022022	15	AACGCGCCUGGG GCUCUCGC	AACGCGCCU GGGGCUCUC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mA*mC*GCGCC UGGGGCUCUCGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356539-31356559
G022023	16	UGGUGGGGGUGG GAGUGUGG	UGGUGGGGG UGGGAGUGU GGGUUUUAG AGCUAGAAA	mU*mG*mG*UGGG GGUGGGAGUGUGG GUUUUAGAmGmCm UmAmGmAmAmAm	chr6:31357031-31357051

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	
G022024	17	GAGUCUCUGAGC GGGGAACA	GAGUCUCUG AGCGGGGAA CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mA*mG*UCUCU GAGCGGGGAACAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355713-31355733
G022025	18	GGGAGGAGCGAG GGGACCGC	GGGAGGAGC GAGGGGACC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mG*mG*AGGA GCGAGGGGACCGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31355180-31355200
G022026	19	UGAGGGGACUGG GAGGAAGC	UGAGGGGAC UGGGAGGAA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mU*mG*mA*GGGG ACUGGGAGGAAGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31356601-31356621
G022027	20	CUGCGUCCAGGC UGGUGUCU	CUGCGUCCA GGCUGGUGU CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mG*CGUCC AGGCUGGUGUCUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31270992-31271012
G022028	21	CCUCGACCGGCG AGAGCCCC	CCUCGACCG GCGAGAGCC CCGUUUUAG	mC*mC*mU*CGACC GGCGAGAGCCCCG UUUUAGAmGmCmU	chr6:31356548-31356568

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022029	22	AACACGAGGAAA GCAAGUGU	AACACGAGG AAAGCAAGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mA*mC*ACGAG GAAAGCAAGUGUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354795-31354815
G022030	23	ACUUUACCUCCA CUCAGAUC	ACUUUACCU CCACUCAGA UCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mC*mU*UUACC UCCACUCAGAUCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355803-31355823
G022031	24	UCUGCUCCUGAU CUGAGUGG	UCUGCUCCU GAUCUGAGU GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mU*mC*mU*GCUCC UGAUCUGAGUGGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356486-31356506
G022032	25	AUCUCGGACCCG GAGACUCG	AUCUCGGAC CCGGAGACU CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU	mA*mU*mC*UCGGA CCCGGAGACUCGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354820-31354840
G022033	26	CCUGGGGCUCUC GCCGGUCG	CCUGGGGCU CUCGCCGGU	mC*mC*mU*GGGGC UCUCGCCGGUCGG	chr6:31356545-31356565

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022034	27	CUGGUCACAUGG GUGGUCCU	CUGGUCACA UGGGUGGUC CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mG*GUCAC AUGGGUGGUCCUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356501-31356521
G022035	28	CUCCUAUAGGUC GCCGGGGA	CUCCUAUAG GUCGCCGGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mC*CUAUA GGUCGCCGGGGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356116-31356136
G022036	29	GUAAAGUGACUC AGAAGUGC	GUAAAGUGA CUCAGAAGU GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mU*mA*AAGU GACUCAGAAGUGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31355816-31355836
G022037	30	ACACAGGGGCUA ACGCAGCC	ACACAGGGG CUAACGCAG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mC*mA*CAGGG GCUAACGCAGCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354983-31355003

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
G022038	31	AUACUUCUGGAA AUUCCUUU	AUACUUCUG GAAAUUCCU UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mU*mA*CUUCU GGAAAUUCCUUUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354748-31354768
G022039	32	GUCCUAGCAGUU GUGGUCAU	GUCCUAGCA GUUGUGGUC AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mU*mC*CUAGC AGUUGUGGUCAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355150-31355170
G022040	33	CCCAUCUCCUAU AGGUCGCC	CCCAUCUCC UAUAGGUCG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mC*AUCUC CUAUAGGUCGCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356121-31356141
G022041	34	CGACCUAUAGGA GAUGGGGA	CGACCUAUA GGAGAUGGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mG*mA*CCUAU AGGAGAUGGGGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356123-31356143
G022042	35	GAAGUAUGACUA CAGACCCA	GAAGUAUGA CUACAGACC CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG	mG*mA*mA*GUAU GACUACAGACCCA GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG	chr6:31354761-31354781

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			GCACCGAGU CGGUGCUUU U	mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	
G022043	36	CUCCGAUGACCA CAACUGCU	CUCCGAUGA CCACAACUG CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mU*mC*CGAUG ACCACAACUGCUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355145-31355165
G022044	37	AGUUGUGGUCAU CGGAGCUG	AGUUGUGGU CAUCGGAGC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mG*mU*UGUG GUCAUCGGAGCUG GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31355142-31355162
G022045	38	UACUUCUGGAAA UUCCUUUU	UACUUCUGG AAAUUCCUU UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mU*mA*mC*UUCUG GAAAUUCCUUUUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31354747-31354767
G022046	39	CACAUGCCAUGU ACAGCAUG	CACAUGCCA UGUACAGCA UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mA*mC*AUGCC AUGUACAGCAUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355349-31355369
G022047	40	GGAGCGAGGGGA CCGCAGGC	GGAGCGAGG GGACCGCAG GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU	mG*mG*mA*GCGA GGGGACCGCAGGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA	chr6:31357027-31357047

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			GAAAAAGUG GCACCGAGU CGGUGCUUU U	mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	
G022048	41	AGGAAAAGUCAC GGUUCCCA	AGGAAAAGU CACGGUUCC CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mA*mG*mG*AAAA GUCACGGUUCCCA GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31355888-31355908
G022049	42	GAGCCUUCCCCA UCUCCUAU	GAGCCUUCC CCAUCUCCU AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mA*mG*CCUUC CCCAUCUCCUAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356129-31356149
G022050	43	GACUCCCCAUCC CCCACGUA	GACUCCCCA UCCCCCACG UAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mA*mC*UCCCC AUCCCCCACGUAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356636-31356656
G022051	44	CCCGGCGACCUA UAGGAGAU	CCCGGCGAC CUAUAGGAG AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mC*GGCGA CCUAUAGGAGAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31356118-31356138
G022052	45	ACGGUUCCCAAG GCUGCUGC	ACGGUUCCC AAGGCUGCU GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU	mA*mC*mG*GUUCC CAAGGCUGCUGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm	chr6:31355898-31355918

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	
G022053	46	GGGAGUCGUGAC CUGCGCCC	GGGAGUCGU GACCUGCGC CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mG*mG*AGUC GUGACCUGCGCCC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31356649-31356669
G022054	47	GAGCGAGGGGAC CGCAGGCG	GAGCGAGGG GACCGCAGG CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mG*mA*mG*CGAG GGGACCGCAGGCG GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCAmAmC mUmUmGmAmAmA mAmAmGmUmGmG mCmAmCmCmGmA mGmUmCmGmGmU mGmCmU*mU*mU* mU	chr6:31357026-31357046
G022055	48	CCUGGCUGUCCU AGCAGUUG	CCUGGCUGU CCUAGCAGU UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGU CGGUGCUUU U	mC*mC*mU*GGCUG UCCUAGCAGUUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCAmAmCm UmUmGmAmAmAm AmAmGmUmGmGm CmAmCmCmGmAm GmUmCmGmGmUm GmCmU*mU*mU*m U	chr6:31355157-31355177
G027488	49	ACAUGCCAUGUA CAGCAUGA	ACAUGCCAU GUACAGCAU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mA*mC*mA*UGCCA UGUACAGCAUGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355348-31355368
G027489	50	AACAAUGCCCAC GAUGGGGA	AACAAUGCC CACGAUGGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mA*mA*mC*AAUGC CCACGAUGGGGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC	chr6:31355182-31355202

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	
G027490	51	CUCCGAUGACCA CAACUGCU	CUCCGAUGA CCACAACUG CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mU*mC*CGAUG ACCACAACUGCUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355145-31355165
G027491	52	CACAUGCCAUGU ACAGCAUG	CACAUGCCA UGUACAGCA UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mA*mC*AUGCC AUGUACAGCAUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355349-31355369
G027973	53	CCGGACGGGCGC CUCCUCCG	CCGGACGGG CGCCUCCUC CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mG*GACGG GCGCCUCCUCCGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31356381-31356401
G027974	54	CGGACGGGCGCC UCCUCCGC	CGGACGGGC GCCUCCUCC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mG*mG*ACGGG CGCCUCCUCCGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31356380-31356400
G027975	55	GAGCCGUCUUCC CAGUCCAC	GUGGACUGG GAAGACGGC UCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mU*mG*GACU GGGAAGACGGCUC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355204-31355224
G027976	56	AGAGCCGUCUUC CCAGUCCA	UGGACUGGG AAGACGGCU CUGUUUUAG	mU*mG*mG*ACUG GGAAGACGGCUCU GUUUUAGAmGmCm	chr6:31355205-31355225

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	
G027977	57	GUACGGCUGCGA CGUGGGGC	GUACGGCUG CGACGUGGG GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mU*mA*CGGCU GCGACGUGGGGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31356400-31356420
G027978	58	CCGUCCCCAUCG UGGGCAUU	AAUGCCCAC GAUGGGGAC GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mA*mA*mU*GCCCA CGAUGGGGACGGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355185-31355205
G027979	59	UCGCUGCUGUGA UGUGUAGG	UCGCUGCUG UGAUGUGUA GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mU*mC*mG*CUGCU GUGAUGUGUAGGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355119-31355139
G027980	60	UGGUCGCUGCUG UGAUGUGU	UGGUCGCUG CUGUGAUGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mU*mG*mG*UCGCU GCUGUGAUGUGUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355122-31355142
G027981	61	CCAGGCAGCGAC AGUGCCCA	CCAGGCAGC GACAGUGCC CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mA*GGCAG CGACAGUGCCCAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31354510-31354530

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
G027982	62	GGCAGCGACAGU GCCCAGGG	CCCUGGGCA CUGUCGCUG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mC*UGGGC ACUGUCGCUGCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31354507-31354527
G027983	63	UCCAGGCAGCGA CAGUGCCC	UCCAGGCAG CGACAGUGC CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mU*mC*mC*AGGCA GCGACAGUGCCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31354511-31354531
G027984	64	AGUCCACCGUCC CCAUCGUG	CACGAUGGG GACGGUGGA CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mA*mC*GAUGG GGACGGUGGACUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355191-31355211
G027985	65	CAGUCCACCGUC CCCAUCGU	CAGUCCACC GUCCCCAUC GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mA*mG*UCCAC CGUCCCCAUCGUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355192-31355212
G027986	66	GUCCACCGUCCC CAUCGUGG	CCACGAUGG GGACGGUGG ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mA*CGAUG GGGACGGUGGACG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355190-31355210
G027987	67	CCAGUCCACCGU CCCCAUCG	CCAGUCCAC CGUCCCCAU CGGUUUUAG AGCUAGAAA	mC*mC*mA*GUCCA CCGUCCCCAUCGG UUUUAGAmGmCmU mAmGmAmAmAmU	chr6:31355193-31355213

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	
G027988	68	UCUUCCCAGUCC ACCGUCCC	GGGACGGUG GACUGGGAA GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mG*mG*ACGG UGGACUGGGAAGA GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355198-31355218
G027989	69	CGAAGCCCCUCA CCCUGAGA	CGAAGCCCC UCACCCUGA GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mG*mA*AGCCC CUCACCCUGAGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355320-31355340
G027990	70	GAAGCCCCUCAC CCUGAGAU	GAAGCCCCU CACCCUGAG AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mA*mA*GCCCC UCACCCUGAGAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355319-31355339
G027991	71	UCCCCAUCGUGG GCAUUGUU	AACAAUGCC CACGAUGGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mA*mA*mC*AAUGC CCACGAUGGGGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355182-31355202
G027992	72	AGUUGUGGUCAU CGGAGCUG	AGUUGUGGU CAUCGGAGC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mA*mG*mU*UGUG GUCAUCGGAGCUG GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355142-31355162

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
G027993	73	CAUCGUGGGCAU UGUUGCUG	CAGCAACAA UGCCCACGA UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mA*mG*CAACA AUGCCCACGAUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355178-31355198
G027994	74	CAUGCCAUGUAC AGCAUGAG	CAUGCCAUG UACAGCAUG AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mA*mU*GCCAU GUACAGCAUGAGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355347-31355367
G027995	75	GGCUGUCCUAGC AGUUGUGG	CCACAACUG CUAGGACAG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mA*CAACU GCUAGGACAGCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355154-31355174
G027996	76	AUCGUGGGCAUU GUUGCUGG	CCAGCAACA AUGCCCACG AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mA*GCAAC AAUGCCCACGAUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355177-31355197
G027997	77	CCCAUCGUGGGC AUUGUUGC	CCCAUCGUG GGCAUUGUU GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mC*mC*AUCGU GGGCAUUGUUGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355180-31355200
G027998	78	CCUGGCUGUCCU AGCAGUUG	CCUGGCUGU CCUAGCAGU UGGUUUUAG AGCUAGAAA UAGCAAGUU	mC*mC*mU*GGCUG UCCUAGCAGUUGG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA	chr6:31355157-31355177

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	
G027999	79	CGUGGGCAUUGU UGCUGGCC	CGUGGGCAU UGUUGCUGG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mG*mU*GGGCA UUGUUGCUGGCCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355175-31355195
G028000	80	AGCAGUUGUGGU CAUCGGAG	CUCCGAUGA CCACAACUG CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mU*mC*CGAUG ACCACAACUGCUG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355145-31355165
G028001	81	CUUGUGGAGACC AGACCAGC	CUUGUGGAG ACCAGACCA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mU*mU*GUGG AGACCAGACCAGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355432-31355452
G028002	82	UGUACAGCAUGA GGGGCUGC	GCAGCCCCU CAUGCUGUA CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mC*mA*GCCCC UCAUGCUGUACAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355340-31355360
G028003	83	UCGUGGGCAUUG UUGCUGGC	GCCAGCAAC AAUGCCCAC GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mC*mC*AGCAA CAAUGCCCACGAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31355176-31355196
G028004	84	GUCCUAGCAGUU GUGGUCAU	GUCCUAGCA GUUGUGGUC	mG*mU*mC*CUAGC AGUUGUGGUCAUG	chr6:31355150-31355170

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
			AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	
G028005	85	GUGUCUCUCACA GCUUGAAA	GUGUCUCUC ACAGCUUGA AAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mU*mG*UCUCU CACAGCUUGAAAG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31354480-31354500
G028006	86	UCAGACCCCCCA AAGACACA	UGUGUCUUU GGGGGGUCU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mU*mG*mU*GUCU UUGGGGGGUCUGA GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355576-31355596
G028007	87	CUCCUCAGACGC CGAGAUGC	CUCCUCAGA CGCCGAGAU GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mC*mU*mC*CUCAG ACGCCGAGAUGCG UUUUAGAmGmCmU mAmGmAmAmAmU mAmGmCAAGUUAA AAUAAGGCUAGUC CGUUAUCACGAAA GGGCACCGAGUCG GmU*mG*mC*mU	chr6:31357154-31357174
G028008	88	GAGAUAGAACCU UCCAGAAG	GAGAUAGAA CCUUCCAGA AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mA*mG*AUAG AACCUUCCAGAAG GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355410-31355430
G028009	89	GACCAGCAGGAG AUAGAACC	GGUUCUAUC UCCUGCUGG UCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mG*mG*mU*UCUA UCUCCUGCUGGUC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355419-31355439

TABLE 2-continued

Exemplary HLA-B Spy guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOS: 1001-1091)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2001-2091)	Genomic Coordinates (hg38)
G028010	90	GCAGGAGAUAGA ACCUUCCA	UGGAAGGUU CUAUCUCCU GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mU*mG*mG*AAGG UUCUAUCUCCUGC GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355414-31355434
G028011	91	AGAUAGAACCUU CCAGAAGU	AGAUAGAAC CUUCCAGAA GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCACGAA AGGGCACCG AGUCGGUGC U	mA*mG*mA*UAGA ACCUUCCAGAAGU GUUUUAGAmGmCm UmAmGmAmAmAm UmAmGmCAAGUUA AAAUAAGGCUAGU CCGUUAUCACGAA AGGGCACCGAGUC GGmU*mG*mC*mU	chr6:31355409-31355429

TABLE 3

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
G028789	101	CUUCUGGAG AAGAGCAGA GAUACA	CUUCUGGAG AAGAGCAGA GAUACAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mU*mU*mCmUG GmAmGAmAGmAGC AGmAGAUmACAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355361-31355385
G028790	102	CACAUGCCAU GUACAGCAU GAGGG	CACAUGCCAU GUACAGCAU GAGGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mC*mAmUG CmCmAUmGUmACA GCmAUGAmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355339-31355363
G028791	103	CACUGACCUG GCAGCGGGA UGGCG	CACUGACCUG GCAGCGGGA UGGCGGUUG UAGCUCCCUG AAACCGUUG	mC*mA*mC*mUmGA CmCmUGmGCmAGC GGmGAUGmGCGmG UUGmUmAmGmCUC CCmUmGmAmAmAm	chr6: 31355469-31355493

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028792	104	CCAUGUACA GCAUGAGGG GCUGCC	CCAUGUACA GCAUGAGGG GCUGCCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mC*mA*mUmGU AmCmAGmCAmUGA GGmGGCUmGCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355333- 31355357
G028793	105	CUUACACGCA GCCUGAGAG UAGCU	CUUACACGCA GCCUGAGAG UAGCUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mU*mU*mAmCA CmGmCAmGCmCUG AGmAGUAmGCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31354627- 31354651
G028794	106	AGCUGCCCAC UUCUGGAAG GUUCU	AGCUGCCCAC UUCUGGAAG GUUCUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mC*mUmGC CmCmACmUUmCUG GAmAGGUmUCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355401- 31355425
G028795	107	CUUCCAGAA GUGGGCAGC UGUGGU	CUUCCAGAA GUGGGCAGC UGUGGUGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mU*mU*mCmCA GmAmAGmUGmGGC AGmCUGUmGGUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355390- 31355414
G028796	108	CCAUCUCAGG GUGAGGGGC UUCGG	CCAUCUCAGG GUGAGGGGC UUCGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC	mC*mC*mA*mUmCU CmAmGGmGUmGAG GGmGCUUmCGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm	chr6: 31355317- 31355341

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			UGCCUUCUG GCAUCGUU	CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028797	109	CAUCUCAGG GUGAGGGGC UUCGGC	CAUCUCAGG GUGAGGGGC UUCGGCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mU*mCmUC AmGmGGmUGmAGG GGmCUUCmGGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355318- 31355342
G028798	110	CAGCAUGAG GGGCUGCCG AAGCCC	CAGCAUGAG GGGCUGCCG AAGCCCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mG*mCmAU GmAmGGmGGmCUG CCmGAAGmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355326- 31355350
G028799	111	CCCAUCUCAG GGUGAGGGG CUUCG	CCCAUCUCAG GGUGAGGGG CUUCGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mC*mAmUC UmCmAGmGGmUGA GGmGGCUmUCGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355316- 31355340
G028800	112	AUGUACAGC AUGAGGGGC UGCCGA	AUGUACAGC AUGAGGGGC UGCCGAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mA*mU*mG*mUmAC AmGmCAmUGmAGG GGmCUGCmCGAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355331- 31355355
G028801	113	CAUGUACAG CAUGAGGGG CUGCCG	CAUGUACAG CAUGAGGGG CUGCCGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mU*mGmUA CmAmGCmAUmGAG GGmGCUGmCCGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355332- 31355356

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
G028802	114	AGAUCACCCA GCGCAAGUG GGAGG	AGAUCACCCA GCGCAAGUG GGAGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mA*mUmCA CmCmCAmGCmGCA AGmUGGGmAGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356262-31356286
G028803	115	CAGAUCACCC AGCGCAAGU GGGAG	CAGAUCACCC AGCGCAAGU GGGAGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mG*mAmUC AmCmCCmAGmCGC AAmGUGGmGAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356263-31356287
G028804	116	GCCUCCCACU UGCGCUGGG UGAUC	GCCUCCCACU UGCGCUGGG UGAUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mC*mC*mUmCC CmAmCUmUGmCGC UGmGGUGmAUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356267-31356291
G028805	117	CCCAAUACUC CGGCCCCUCC UGCU	CCCAAUACUC CGGCCCCUCC UGCUGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mC*mAmAU AmCmUCmCGmGCC CCmUCCUmGCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356777-31356801
G028806	118	CACUCACCGG CCCAGGUCUC GGUC	CACUCACCGG CCCAGGUCUC GGUCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mA*mC*mUmCA CmCmGGmCCmCAG GUmCUCGmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31357078-31357102
G028807	119	UAGAGCAGG AGGGGCCGG AGUAUU	UAGAGCAGG AGGGGCCGG AGUAUUGUU	mU*mA*mG*mAmGC AmGmGAmGGmGGC CGmGAGUmAUUmG	chr6: 31356774-31356798

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028808	120	CCGGCCCAGG UCUCGGUCA GGGCC	CCGGCCCAGG UCUCGGUCA GGGCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mG*mGmCC CmAmGGmUCmUCG GUmCAGGmGCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31357084-31357108
G028809	121	ACCACUUACA CGCAGCCUGA GAGU	ACCACUUACA CGCAGCCUGA GAGUGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mA*mC*mC*mAmCU UmAmCAmCGmCAG CCmUGAGmAGUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31354623-31354647
G028810	122	CCACUUACAC GCAGCCUGA GAGUA	CCACUUACAC GCAGCCUGA GAGUAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mA*mCmUU AmCmACmGCmAGC CUmGAGAmGUAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31354624-31354648
G028811	123	UAUCUGCGG AGCCACUCCA CGCAC	UAUCUGCGG AGCCACUCCA CGCACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mA*mU*mCmUG CmGmGAmGCmCAC UCmCACGmCACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356201-31356225
G028812	124	AGCCUGAGA GUAGCUCCCU CCUUU	AGCCUGAGA GUAGCUCCCU CCUUUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA	mA*mG*mC*mCmUG AmGmAGmUAmGCU CCmCUCCmUUUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC	chr6: 31354636-31354660

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028813	125	CCUGAGUUU	CCUGAGUUU	mC*mC*mU*mGmAG	chr6: 31355460-
		GGUCCUCGCC	GGUCCUCGCC	UmUmUGmGUmCCU	31355484
		AUCCC	AUCCCGUUG	CGmCCAUmCCCmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028814	126	GUGUAUCUC	GUGUAUCUC	mG*mU*mG*mUmA	chr6: 31355366-
		UGCUCUUCUC	UGCUCUUCUC	UCmUmCUmGCmUC	31355390
		CAGAA	CAGAAGUUG	UUCmUCCAmGAAm	
			UAGCUCCCUG	GUUGmUmAmGmCU	
			AAACCGUUG	CCCmUmGmAmAmA	
			CUACAAUAA	mCmCGUUmGmCUA	
			GGCCGUCGA	mCAAU*AAGmGmC	
			AAGAUGUGC	CmGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028815	127	CUUCUCCAGA	CUUCUCCAGA	mC*mU*mU*mCmUC	chr6: 31355379-
		AGGCACCACC	AGGCACCACC	CmAmGAmAGmGCA	31355403
		ACAG	ACAGGUUGU	CCmACCAmCAGmG	
			AGCUCCCUGA	UUGmUmAmGmCUC	
			AACCGUUGC	CCmUmGmAmAmAm	
			UACAAUAAG	CmCGUUmGmCUAm	
			GCCGUCGAA	CAAU*AAGmGmCC	
			AGAUGUGCC	mGmUmCmGmAmA	
			GCAACGCUCU	mAmGmAmUGUGCm	
			GCCUUCUGGC	CGmCAAmCGCUCU	
			AUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028816	128	UACAUGGCA	UACAUGGCA	mU*mA*mC*mAmUG	chr6: 31355356-
		UGUGUAUCU	UGUGUAUCU	GmCmAUmGUmGUA	31355380
		CUGCUC	CUGCUCGUU	UCmUCUGmCUCmG	
			GUAGCUCCCU	UUGmUmAmGmCUC	
			GAAACCGUU	CCmUmGmAmAmAm	
			GCUACAAUA	CmCGUUmGmCUAm	
			AGGCCGUCG	CAAU*AAGmGmCC	
			AAAGAUGUG	mGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028817	129	UAUCUCUGC	UAUCUCUGC	mU*mA*mU*mCmUC	chr6: 31355369-
		UCUUCUCCAG	UCUUCUCCAG	UmGmCUmCUmUCU	31355393
		AAGGC	AAGGCGUUG	CCmAGAAmGGCmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028818	130	UGCUAGGAC	UGCUAGGAC	mU*mG*mC*mUmAG	chr6: 31355161-
		AGCCAGGCCA	AGCCAGGCCA	GmAmCAmGCmCAG	31355185
		GCAAC	GCAACGUUG	GCmCAGCmAACmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
				CGmCAAmCGCUCU	
			UGCCUUCUG	mGmCCmUmUmCmU	
			GCAUCGUU	GGCAUCG*mU*mU	
G028819	131	GCUAGGACA	GCUAGGACA	mG*mC*mU*mAmGG	chr6: 31355162-
		GCCAGGCCAG	GCCAGGCCAG	AmCmAGmCCmAGG	31355186
		CAACA	CAACAGUUG	CCmAGCAmACAmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028820	132	GCUCCGAUG	GCUCCGAUG	mG*mC*mU*mCmCG	chr6: 31355144-
		ACCACAACUG	ACCACAACUG	AmUmGAmCCmACA	31355168
		CUAGG	CUAGGGUUG	ACmUGCUmAGGmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028821	133	UCGUGGGCA	UCGUGGGCA	mU*mC*mG*mUmGG	chr6: 31355166-
		UUGUUGCUG	UUGUUGCUG	GmCmAUmUGmUUG	31355190
		GCCUGG	GCCUGGGUU	CUmGGCCmUGGmG	
			GUAGCUCCCU	UUGmUmAmGmCUC	
			GAAACCGUU	CCmUmGmAmAmAm	
			GCUACAAUA	CmCGUUmGmCUAm	
			AGGCCGUCG	CAAU*AAGmGmCC	
			AAAGAUGUG	mGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028822	134	GAUGACCAC	GAUGACCAC	mG*mA*mU*mGmAC	chr6: 31355149-
		AACUGCUAG	AACUGCUAG	CmAmCAmACmUGC	31355173
		GACAGC	GACAGCGUU	UAmGGACmAGCmG	
			GUAGCUCCCU	UUGmUmAmGmCUC	
			GAAACCGUU	CCmUmGmAmAmAm	
			GCUACAAUA	CmCGUUmGmCUAm	
			AGGCCGUCG	CAAU*AAGmGmCC	

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AAAGAUGUG	mGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028823	135	GUCCUCGUUC	GUCCUCGUUC	mG*mU*mC*mCmUC	chr6: 31356326-
		AGGGCGAUG	AGGGCGAUG	GmUmUCmAGmGGC	31356350
		UAAUC	UAAUCGUUG	GAmUGUAmAUCmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028824	136	CGCAGCCUGA	CGCAGCCUGA	mC*mG*mC*mAmGC	chr6: 31354633-
		GAGUAGCUC	GAGUAGCUC	CmUmGAmGAmGUA	31354657
		CCUCC	CCUCCGUUGU	GCmUCCCmUCCmG	
			AGCUCCCUGA	UUGmUmAmGmCUC	
			AACCGUUGC	CCmUmGmAmAmAm	
			UACAAUAAG	CmCGUUmGmCUAm	
			GCCGUCGAA	CAAU*AAGmGmCC	
			AGAUGUGCC	mGmUmCmGmAmA	
			GCAACGCUCU	mAmGmAmUGUGCm	
			GCCUUCUGGC	CGmCAAmCGCUCU	
			AUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028825	137	GGUCAGUGU	GGUCAGUGU	mG*mG*mU*mCmAG	chr6: 31355491-
		GAUCUCCGCA	GAUCUCCGCA	UmGmUGmAUmCUC	31355515
		GGGUA	GGGUAGUUG	CGmCAGGmGUAmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028826	138	GUCAGUGUG	GUCAGUGUG	mG*mU*mC*mAmGU	chr6: 31355492-
		AUCUCCGCAG	AUCUCCGCAG	GmUmGAmUCmUCC	31355516
		GGUAG	GGUAGGUUG	GCmAGGGmUAGmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028827	139	UACAUCGCCC	UACAUCGCCC	mU*mA*mC*mAmUC	chr6: 31356317-
		UGAACGAGG	UGAACGAGG	GmCmCCmUGmAAC	31356341
		ACCUG	ACCUGGUUG	GAmGGACmCUGmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028828	140	GGCUCCCACU CCAUGAGGU AUUUC	GGCUCCCACU CCAUGAGGU AUUUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mG*mC*mUmCC CmAmCUmCCmAUG AGmGUAUmUUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356928- 31356952
G028829	141	UCUUCUCCAG AAGGCACCAC CACA	UCUUCUCCAG AAGGCACCAC CACAGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mU*mUmCU CmCmAGmAAmGGC ACmCACCmACAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355378- 31355402
G028830	142	AGCAUGAGG GGCUGCCGA AGCCCC	AGCAUGAGG GGCUGCCGA AGCCCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mC*mAmUG AmGmGGmGCmUGC CGmAAGCmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355325- 31355349
G028831	143	AGACCAGACC AGCAGGAGA UAGAA	AGACCAGACC AGCAGGAGA UAGAAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mA*mCmCA GmAmCCmAGmCAG GAmGAUAmGAAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355415- 31355439
G028832	144	AAGGUUCUA UCUCCUGCUG GUCUG	AAGGUUCUA UCUCCUGCUG GUCUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mA*mG*mGmU UCmUmAUmCUmCC UGCmUGGUmCUGm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355417- 31355441

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
G028833	145	GUGGAGACC AGACCAGCA GGAGAU	GUGGAGACC AGACCAGCA GGAGAUGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mU*mG*mGmA GAmCmCAmGAmCC AGCmAGGAmGAUm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355419-31355443
G028834	146	GCAAGGAUU ACAUCGCCCU GAACG	GCAAGGAUU ACAUCGCCCU GAACGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mC*mA*mAmGG AmUmUAmCAmUCG CCmCUGAmACGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356325-31356349
G028835	147	ACCAUGAGG CCACCCUGAG GUGCU	ACCAUGAGG CCACCCUGAG GUGCUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mC*mAmUG AmGmGCmCAmCCC UGmAGGUmGCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355523-31355547
G028836	148	GACCAUGAG GCCACCCUGA GGUGC	GACCAUGAG GCCACCCUGA GGUGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mA*mC*mCmAU GmAmGGmCCmACC CUmGAGGmUGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355524-31355548
G028837	149	ACCACCCCAU CUCUGACCAU GAGG	ACCACCCCAU CUCUGACCAU GAGGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mA*mC*mC*mAmCC CmCmAUmCUmCUG ACmCAUGmAGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355538-31355562
G028838	150	CACCACCCCA UCUCUGACCA UGAG	CACCACCCCA UCUCUGACCA UGAGGUUGU	mC*mA*mC*mCmAC CmCmCAmUCmUCU GAmCCAUmGAGmG	chr6: 31355539-31355563

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028839	151	AUGCCCGCGG AGGAGGCGC CCGUC	AUGCCCGCGG AGGAGGCGC CCGUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mU*mG*mCmCC GmCmGGmAGmGAG GCmGCCCmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356374-31356398
G028840	152	GAGGCGCCCG UCCGGCCCCA CGUC	GAGGCGCCCG UCCGGCCCCA CGUCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mG*mA*mG*mGmCG CmCmCGmUCmCGG CCmCCACmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356386-31356410
G028841	153	GCUGCGACG UGGGGCCGG ACGGGC	GCUGCGACG UGGGGCCGG ACGGGCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mC*mU*mGmCG AmCmGUmGGmGGC CGmGACGmGGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356385-31356409
G028842	154	GCGACGUGG GGCCGGACG GGCGCC	GCGACGUGG GGCCGGACG GGCGCCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mC*mG*mAmCG UmGmGGmGCmCGG ACmGGGCmGCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356382-31356406
G028843	155	CGUGAGGUU CGACAGCGAC GCCGC	CGUGAGGUU CGACAGCGAC GCCGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA	mC*mG*mU*mGmAG GmUmUCmGAmCAG CGmACGCmCGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC	chr6: 31356830-31356854

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028931	156	UCCUUUUCCA	UCCUUUUCCA	mU*mC*mC*mUmUU	chr6: 31354654-
		CCUGUGGGA	CCUGUGGGA	UmCmCAmCCmUGU	31354678
		AGAAA	AGAAAGUUG	GGmGAAGmAAAmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028932	157	CAGAGCGAG	CAGAGCGAG	mC*mA*mG*mAmGC	chr6: 31356670-
		GCCGGUGAG	GCCGGUGAG	GmAmGGmCCmGGU	31356694
			UGACCCGUU	GAmGUGAmCCCmG	
			GUAGCUCCCU	UUGmUmAmGmCUC	
			GAAACCGUU	CCmUmGmAmAmAm	
		UGACCC	GCUACAAUA	CmCGUUmGmCUAm	
			AGGCCGUCG	CAAU*AAGmGmCC	
			AAAGAUGUG	mGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032787	158	GGUGUAGAA	GGUGUAGAA	mG*mG*mU*mGmU	chr6: 31356928-
		AUACCUCAU	AUACCUCAU	AGmAmAAmUAmCC	31356952
		GGAGUG	GGAGUGGUU	UCAmUGGAmGUGm	
			GUAGCUCCCU	GUUGmUmAmGmCU	
			GAAACCGUU	CCCmUmGmAmAmA	
			GCUACAAUA	mCmCGUUmGmCUA	
			AGGCCGUCG	mCAAU*AAGmGmC	
			AAAGAUGUG	CmGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032788	159	AGACCCUGGC	AGACCCUGGC	mA*mG*mA*mCmCC	chr6: 31356437-
		CCCGGCCCCG	CCCGGCCCCG	UmGmGCmCCmCGG	31356461
		CGGU	CGGUGUUGU	CCmCCGCmGGUmG	
			AGCUCCCUGA	UUGmUmAmGmCUC	
			AACCGUUGC	CCmUmGmAmAmAm	
			UACAAUAAG	CmCGUUmGmCUAm	
			GCCGUCGAA	CAAU*AAGmGmCC	
			AGAUGUGCC	mGmUmCmGmAmA	
			GCAACGCUCU	mAmGmAmUGUGCm	
			GCCUUCUGGC	CGmCAAmCGCUCU	
			AUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032789	160	GAGACCCUG	GAGACCCUG	mG*mA*mG*mAmCC	chr6: 31356436-
		GCCCCGGCCC	GCCCCGGCCC	CmUmGGmCCmCCG	31356460
		CGCGG	CGCGGGUUG	GCmCCCGmCGGmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G032790	161	CUCACCGGCC UCGCUCUGG UUGUA	CUCACCGGCC UCGCUCUGG UUGUAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mU*mC*mAmCC GmGmCCmUCmGCU CUmGGUUmGUAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356682- 31356706
G032791	162	CUGCGCGGCU ACUACAACCA GAGC	CUGCGCGGCU ACUACAACCA GAGCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mU*mG*mCmGC GmGmCUmACmUAC AAmCCAGmAGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356694- 31356718
G032792	163	CAAACUCAG GACACUGAG CUUGUG	CAAACUCAG GACACUGAG CUUGUGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mA*mAmCU CmAmGGmACmACU GAmGCUUmGUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355446- 31355470
G032793	164	UCAGGACAC UGAGCUUGU GGAGAC	UCAGGACAC UGAGCUUGU GGAGACGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mC*mA*mGmGA CmAmCUmGAmGCU UGmUGGAmGACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355441- 31355465
G032794	165	UCUGGGAAA GGAGGGGAA GAUGAG	UCUGGGAAA GGAGGGGAA GAUGAGGUU GUAGCUCCCU GAAACCGUU	mU*mC*mU*mGmGG AmAmAGmGAmGGG GAmAGAUmGAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm	chr6: 31355222- 31355246

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G032795	166	CUCUGGGAA AGGAGGGGA AGAUGA	CUCUGGGAA AGGAGGGGA AGAUGAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mU*mC*mUmGG GmAmAAmGGmAGG GGmAAGAmUGAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355221-31355245
G032796	167	ACCCAGUUCG UGAGGUUCG ACAGC	ACCCAGUUCG UGAGGUUCG ACAGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mC*mCmAG UmUmCGmUGmAGG UUmCGACmAGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356844-31356868
G032797	168	CGCUGCCUGG AGUAGAACA AAAAC	CGCUGCCUGG AGUAGAACA AAAACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mG*mC*mUmGC CmUmGGmAGmUAG AAmCAAAmAACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31354520-31354544
G032798	169	CUGGAGGGU GUGAGACCC UGGCCC	CUGGAGGGU GUGAGACCC UGGCCCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mU*mG*mGmAG GmGmUGmUGmAGA CCmCUGGmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356425-31356449
G032799	170	GCCCAGGUCU CGGUCAGGG CCAGG	GCCCAGGUCU CGGUCAGGG CCAGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC	mG*mC*mC*mCmAG GmUmCUmCGmGUC AGmGGCCmAGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm	chr6: 31357087-31357111

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			UGCCUUCUG GCAUCGUU	CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G032800	171	CUGUGUGUU CCGGUCCCAA UACUC	CUGUGUGUU CCGGUCCCAA UACUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mU*mG*mUmGU GmUmUCmCGmGUC CCmAAUAmCUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356763-31356787
G032801	172	UCUGUGUGU UCCGGUCCCA AUACU	UCUGUGUGU UCCGGUCCCA AUACUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mC*mU*mGmUG UmGmUUmCCmGGU CCmCAAUmACUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356762-31356786
G032802	173	UGUGUGUUC CGGUCCCAAU ACUCC	UGUGUGUUC CGGUCCCAAU ACUCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mG*mU*mGmU GUmUmCCmGGmUC CCAmAUACmUCCm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356764-31356788
G032803	174	CGAACCGUCC UCCUGCUGCU CUCG	CGAACCGUCC UCCUGCUGCU CUCGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mG*mA*mAmCC GmUmCCmUCmCUG CUmGCUCmUCGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31357116-31357140
G032804	175	UCUGGAGGG UGUGAGACC CUGGCC	UCUGGAGGG UGUGAGACC CUGGCCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mC*mU*mGmGA GmGmGUmGUmGAG ACmCCUGmGCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356424-31356448

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
G032805	176	CCCAGAGCCG UCUUCCCAGU CCAC	CCCAGAGCCG UCUUCCCAGU CCACGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mC*mAmGA GmCmCGmUCmUUC CCmAGUCmCACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355204-31355228
G032806	177	UCCCAGAGCC GUCUUCCCAG UCCA	UCCCAGAGCC GUCUUCCCAG UCCAGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mCmAG AmGmCCmGUmCUU CCmCAGUmCCAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355205-31355229
G032807	178	CCAGAGCCGU CUUCCCAGUC CACC	CCAGAGCCGU CUUCCCAGUC CACCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mA*mGmAG CmCmGUmCUmUCC CAmGUCCmACCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31355203-31355227
G032808	179	GUGUUCCGG UCCCAAUACU CCGGC	GUGUUCCGG UCCCAAUACU CCGGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mU*mG*mUmUC CmGmGUmCCmCAA UAmCUCCmGGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356767-31356791
G032809	180	UGGAGGGUG UGAGACCCU GGCCCC	UGGAGGGUG UGAGACCCU GGCCCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mG*mG*mAmG GGmUmGUmGAmGA CCCmUGGCmCCCm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6: 31356426-31356450
G032810	181	CCGGCCCCUC CUGCUCUAUC CACG	CCGGCCCCUC CUGCUCUAUC CACGGUUGU AGCUCCCUGA	mC*mC*mG*mGmCC CmCmUCmCUmGCU CUmAUCCmACGmG UUGmUmAmGmCUC	chr6: 31356786-31356810

TABLE 3-continued

Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1101-1185)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2101-2185)	Genomic Coordinates (hg38)
			AACCGUUGC	CCmUmGmAmAmAm	
			UACAAUAAG	CmCGUUmGmCUAm	
			GCCGUCGAA	CAAU*AAGmGmCC	
			AGAUGUGCC	mGmUmCmGmAmA	
			GCAACGCUCU	mAmGmAmUGUGCm	
			GCCUUCUGGC	CGmCAAmCGCUCU	
			AUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032811	182	UCCCACUUGC	UCCCACUUGC	mU*mC*mC*mCmAC	chr6: 31356270-
		GCUGGGUGA	GCUGGGUGA	UmUmGCmGCmUGG	31356294
		UCUGA	UCUGAGUUG	GUmGAUCmUGAmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032812	183	CACAGACUG	CACAGACUG	mC*mA*mC*mAmGA	chr6: 31356723-
		ACCGAGAGA	ACCGAGAGA	CmUmGAmCCmGAG	31356747
		GCCUGC	GCCUGCGUU	AGmAGCCmUGCmG	
			GUAGCUCCCU	UUGmUmAmGmCUC	
			GAAACCGUU	CCmUmGmAmAmAm	
			GCUACAAUA	CmCGUUmGmCUAm	
			AGGCCGUCG	CAAU*AAGmGmCC	
			AAAGAUGUG	mGmUmCmGmAmA	
			CCGCAACGCU	mAmGmAmUGUGCm	
			CUGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G032813	184	UGCUGGUCA	UGCUGGUCA	mU*mG*mC*mUmGG	chr6: 31357133-
		UGGCGCCCCG	UGGCGCCCCG	UmCmAUmGGmCGC	31357157
		AACCG	AACCGGUUG	CCmCGAAmCCGmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	
G028919	185	CACAUCAGA	CACAUCAGA	mC*mA*mC*mAmUC	chr6: 31354497-
		GCCCUGGGCA	GCCCUGGGCA	AmGmAGmCCmCUG	31354521
		CUGUC	CUGUCGUUG	GGmCACUmGUCmG	
			UAGCUCCCUG	UUGmUmAmGmCUC	
			AAACCGUUG	CCmUmGmAmAmAm	
			CUACAAUAA	CmCGUUmGmCUAm	
			GGCCGUCGA	CAAU*AAGmGmCC	
			AAGAUGUGC	mGmUmCmGmAmA	
			CGCAACGCUC	mAmGmAmUGUGCm	
			UGCCUUCUG	CGmCAAmCGCUCU	
			GCAUCGUU	mGmCCmUmUmCmU	
				GGCAUCG*mU*mU	

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

TABLE 3A

Additional Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs

Guide ID	Guide Sequence	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2186-2191)
G034206	CAAACUCAG GACACUGAG CUUGUG (SEQ ID NO: 163)	mC*mA*mA*mAmCUCmAmGGmACmACUGAmGCUUmGUGmGUUGm UmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2186)
G034207	UCAGGACAC UGAGCUUGU GGAGAC (SEQ ID NO: 164)	mU*mC*mA*mGmGACmAmCUmGAmGCUUGmUGGAmGACmGUUGm UmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2187)
G034208	UCUGGGAAA GGAGGGGAA GAUGAG (SEQ ID NO: 165)	mU*mC*mU*mGmGGAmAmAGmGAmGGGGAmAGAUmGAGmGUUG mUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAG mGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCU mGmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2188)
G034209	CUCUGGGAA AGGAGGGGA AGAUGA (SEQ ID NO: 166)	mC*mU*mC*mUmGGGmAmAAmGGmAGGGGmAAGAmUGAmGUUGm UmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2189)
G034210	CUGGAGGGU GUGAGACCC UGGCCC (SEQ ID NO: 169)	mC*mU*mG*mGmAGGmGmUGmUGmAGACCmCUGGmCCCmGUUGm UmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2190)
G034211	UCCCAGAGCC GUCUUCCCAG UCCA (SEQ ID NO: 177)	mU*mC*mC*mCmAGAmGmCCmGUmCUUCCmCAGUmCCAmGUUGm UmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2191)

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

[0585] In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 1-91. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 3, 13, 18, 32, 36, 39, 48-56, 58, 64-71, 73-73, 80-82, 86, and 88-91. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 3, 13, 36, 39, 49-56, 64-71, 74, 80-82, 88, and 90-91. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 13, 39, 49, 52, 65, 74, 82, and 91. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 3, 39, and 49-52. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 3, 36, 39, 49, 50, 51, and 52. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 39, 49, and 52. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 49, 52-54, 55, 56, 64, 65, 67-71, 73-74, 80-82, and 90. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 49, 51, 74, 81, and 82. In some embodiments, the HLA-B gRNA comprises a guide sequence of SEQ ID NO: 13 or 74. In some embodiments, the HLA-B gRNA comprises a guide sequence of SEQ ID NO: 13. In some embodiments, the HLA-B gRNA comprises a guide sequence of SEQ ID NO: 74.

[0586] In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101-185. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 103, 106, 107, 114, 117, 118, 125-129,137, 138, 141, 143, 144, 145, 159, 160, 163, 164, 165, 166, 169, 171, 172, 173, 176, 177, 178, 179, and 180. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 65 and 74. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 49, 52-54, 56, 64-65, 67-71, 73-74, 80-82, 88, and 90-91. In some embodiments, the HLA-B gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 74, 82, and 91.

[0587] In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 1-91 or 101-185. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 103, 106, 107, 117, 125-129, 137, 138, 141, 143, 144, 145, 159, 160, 163, 164, 165, 166, 169, 171, 172, 173, 176, 177, 178, 179, and 180. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 103, 106, 117, 118, 125-128, 133, 137-138, 141, 143-144, 159, 163, 164, 165, 166, 169, 171, 173, 177, 178, and 180. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 106, 114, 117-118, 125-128, 133, 137-138, 141, 143-144, 159, 163, 164, 165, 166, 169, 171, 173, 177, 178, and 180. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 117-118, 125-128, 137-138, 144, 159, 163, 164, 165, 166,

169, 177, 178, and 180. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 101, 117, 127, 137-138, 163, 164, 165, 166, 169, and 177. In some embodiments, the gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 163-166, 169, and 177. In some embodiments, the gRNA comprises a sequence selected from any one of SEQ ID NOs: 2186-2191. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 163. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 164. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 165. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 166. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 169. In some embodiments, the gRNA comprises a guide sequence comprising a sequence of SEQ ID NO: 177.

[0588] In some embodiments, the HLA-B guide RNA comprises a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91 or 101-185. In some embodiments, the HLA-B guide RNA comprises a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 1-91 or 101-185. In some embodiments, the HLA-B guide RNA comprises a guide sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 1-91 or 101-185.

[0589] In some embodiments, the HLA-B guide RNA comprises a guide sequence that comprises at least 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 2-3. As used herein, at least 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate means, for example, at least 10 contiguous nucleotides within the genomic coordinates wherein the genomic coordinates include 10 nucleotides in the 5' direction and 10 nucleotides in the 3' direction from the ranges listed in Tables 2-3. For example, an HLA-B guide RNA may comprise 10 contiguous nucleotides within the genomic coordinates (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410, including the boundary nucleotides of these ranges. In some embodiments, the HLA-B guide RNA comprises a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 3. In some embodiments, the HLA-B guide RNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from a sequence that is 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 2, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 3.

[0590] In some embodiments, the Tables 2-3 guide RNA comprises a guide sequence that comprises at least 15 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 2-3. In some embodiments, the HLA-B guide RNA comprises a guide sequence that comprises at least 20 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 2-3. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 1. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 3. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 4. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 5. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 6. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 7. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 8. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 9. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 10. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 11. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 12. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 13. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 14. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 15. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 16. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 17. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 18. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 19. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 20. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 21. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 22. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 23. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 24. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 25. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 26. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 27. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 28. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 29. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 30. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 31. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 32. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 33. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 34. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 35. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 36. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 37. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 38. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 39. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 40. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 41. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 42. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 43. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 44. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 45. In some embodiments, the

HLA-B guide RNA comprises SEQ ID NO: 46. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 47. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 48. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 49. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 50. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 51. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 52. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 53. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 54. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 55. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 56. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 57. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 58. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 59. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 60. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 61. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 62. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 63. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 64. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 65. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 66. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 67. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 68. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 69. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 70. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 71. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 72. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 73. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 74. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 75. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 76. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 77. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 78. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 79. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 80. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 81. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 82. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 83. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 84. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 85. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 86. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 87. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 88. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 89. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 90. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 91.

[0591] In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 101. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 102. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 103. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 104. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 105. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 106. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 107. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 108. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 109. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 110. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 111. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 112. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 113. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 114. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 115. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 116. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 117. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 118. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 119. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 120. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 121. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 122. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 123. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 124. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 125. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 126. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 127. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 128. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 129. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 130. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 131. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 132. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 133. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 134. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 135. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 136. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 137. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 138. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 139. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 140. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 141. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 142. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 143. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 144. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 145. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 146. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 147. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 148. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 149. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 150. In some

embodiments, the HLA-B guide RNA comprises SEQ ID NO: 151. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 152. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 153. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 154. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 155. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 156. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 157. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 158. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 159. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 160. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 161. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 162. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 163. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 164. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 165. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 166. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 167. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 168. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 169. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 170. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 171. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 172. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 173. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 174. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 175. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 176. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 177. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 178. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 179. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 180. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 181. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 182. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 183. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 184. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 185. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2186. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2187. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2188. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2189. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2190. In some embodiments, the HLA-B guide RNA comprises SEQ ID NO: 2191.

[0592] Additional embodiments of HLA-B guide RNAs are provided herein, including e.g., exemplary modifications to the guide RNA. 2. Genetic modifications to HLA-B

[0593] In some embodiments, the methods and compositions disclosed herein genetically modify at least one nucleotide in the HLA-B gene in a cell. Genetic modifications encompass the population of modifications that results from contact with a gene editing system (e.g., the population of edits that result from Cas9 and an HLA-B guide RNA, or the population of edits that result from BC22 and an HLA-B guide RNA).

[0594] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6: 31355182-31355596 or (b) chr6:31355203-31356461.

[0595] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6: 31355348-31355368; chr6:31355145-31355165; chr6: 31355349-31355369; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355347-31355367; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355410-31355430; chr6:31355414-31355434; or chr6: 31355409-31355429.

[0596] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355182-31355202; chr6: 31355349-31355369; chr6:31355348-31355368; or chr6: 31355145-31355165.

[0597] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6: 31355349-31355369; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355191-31355211; chr6: 31355192-31355212; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355182-31355202; chr6: 31355178-31355198; chr6:31355347-31355367; chr6: 31355145-31355165; chr6:31355432-31355452; chr6: 31355340-31355360; or chr6:31355414-31355434.

[0598] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6: 31355349-31355369; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355205-31355225; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355182-31355202; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355145-31355165; chr6: 31355432-31355452; chr6:31355340-31355360; chr6: 31355410-31355430; chr6:31355414-31355434; or chr6: 31355409-31355429.

[0599] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355348-31355368; chr6: 31355349-31355369; chr6:31355192-31355212; chr6: 31355347-31355367; chr6:31355340-31355360; and chr6: 31355409-31355429. In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355349-31355369 or chr6:31355348-31355368. In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355192-31355212 or chr6:31355347-31355367. In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen

from: chr6:31355347-31355367; chr6:31355340-31355360; or chr6:31355409-31355429. In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from; chr6: 31355348-31355368; chr6:31355145-31355165; chr6: 31355347-31355367; chr6:31355432-31355452; or chr6: 31355340-31355360.

[0600] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6: 31355492-31355516; chr6: 31355379-31355403; chr6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355415-31355439; chr6: 31355378-31355402; chr6:31355166-31355190; chr6: 31355401-31355425; ch6:31355469-31355493; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; or chr6: 31356764-31356788.

[0601] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6: 31355492-31355516; chr6:31355361-31355385; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355356-31355380; chr6:31355366-31355390; chr6: 31355417-31355441; chr6:31357078-31357102; chr6: 31355460-31355484; chr6:31355415-31355439; chr6: 31355166-31355190; chr6:31355378-31355402; chr6: 31355401-31355425; chr6:31356262-31356286; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; or chr6: 31356764-31356788.

[0602] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6: 31355492-31355516; chr6:31355379-31355403; ch6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355356-31355380; chr6:31355460-31355484; chr6: 31357078-31357102; chr6:31355417-31355441; chr6: 31355366-31355390; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; or chr6:31356764-31356788.

[0603] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31356777-31356801; chr6: 31355492-31355516; chr6:31355379-31355403; ch6: 31355491-31355515; chr6:31355361-31355385; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; or chr6:31355441-31355465.

[0604] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; or chr6: 31355441-31355465.

[0605] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355182-31355202; chr6: 31355348-31355368; chr6:31355180-31355200; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31355157-31355177; chr6:31356381-31356401; chr6: 31356380-31356400; chr6:31355204-31355224; chr6: 31355205-31355225; chr6:31355185-31355205; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355190-31355210; chr6:31355193-31355213; chr6: 31355198-31355218; chr6:31355320-31355340; chr6: 31355319-31355339; chr6:31355178-31355198; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355576-31355596; chr6: 31355410-31355430; chr6:31355419-31355439; chr6: 31355414-31355434; and chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6: 31355361-31355385; chr6:31355356-31355380; chr6: 31355460-31355484; chr6:31357078-31357102; chr6: 31355417-31355441; chr6:31355366-31355390; chr6: 31355415-31355439; chr6:31355378-31355402; chr6: 31355166-31355190; chr6:31355401-31355425; ch6: 31355469-31355493; chr6:31356262-31356286; chr6: 31355419-31355443; chr6:31355390-31355414; chr6: 31355369-31355393; chr6:31355221-31355245; chr6: 31355222-31355246; chr6:31355205-31355229; chr6: 31355446-31355470; chr6:31356425-31356449; chr6: 31355441-31355465; chr6:31355203-31355227; chr6: 31356437-31356461; chr6:31356426-31356450; chr6: 31356763-31356787; chr6:31356764-31356788; chr6: 31356762-31356786; chr6:31355204-31355228; chr6: 31356436-31356460; or chr6:31356767-31356791.

[0606] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410.

[0607] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355348-31355368; chr6: 31355145-31355165; chr6:31355349-31355369; chr6: 31356381-31356401; chr6:31356380-31356400; chr6: 31355204-31355224; chr6:31355205-31355225; chr6: 31355191-31355211; chr6:31355192-31355212; chr6: 31355193-31355213; chr6:31355198-31355218; chr6: 31355320-31355340; chr6:31355319-31355339; chr6: 31355347-31355367; chr6:31355432-31355452; chr6: 31355340-31355360; chr6:31355410-31355430; chr6: 31355414-31355434; or chr6:31355409-31355429.

[0608] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355349-31355369; chr6: 31355348-31355368; or chr6:31355145-31355165.

[0609] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6: 31356381-31356401; chr6:31356380-31356400; chr6:

31355204-31355224; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; or chr6:31355414-31355434.

[0610] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368; chr6:31355349-31355369; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355205-31355225; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355182-31355202; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355145-31355165; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355410-31355430; chr6:31355414-31355434; or chr6:31355409-31355429.

[0611] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355348-31355368, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355347-31355367, chr6:31355340-31355360, chr6:31355409-31355429. In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31355349-31355369 or chr6:31355348-31355368. In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31355192-31355212 or chr6:31355347-31355367. In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31355347-31355367; chr6:31355340-31355360; or chr6:31355409-31355429. In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:31355348-31355368; chr6:31355145-31355165; chr6:31355347-31355367; chr6:31355432-31355452; or chr6:31355340-31355360.

[0612] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0613] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355361-31355385; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355356-31355380; chr6:31355366-31355390; chr6:31355417-31355441; chr6:31357078-31357102; chr6:31355460-31355484; chr6:31355415-31355439; chr6:31355166-31355190; chr6:31355378-31355402; chr6:31355401-31355425; and chr6:31356262-31356286; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0614] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; ch6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; or chr6:31356764-31356788.

[0615] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; ch6:31355491-31355515; chr6:31355361-31355385; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0616] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465.

[0617] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; and chr6:31355409-31355429; or (b) chr6:31356777-31356801; chr6:31355492-31355516; chr6:31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:

31355401-31355425; ch6:31355469-31355493; chr6: 31356262-31356286; chr6:31355419-31355443; chr6: 31355390-31355414; chr6:31355369-31355393; chr6: 31355221-31355245; chr6:31355222-31355246; chr6: 31355205-31355229; chr6:31355446-31355470; chr6: 31356425-31356449; chr6:31355441-31355465; chr6: 31355203-31355227; chr6:31356437-31356461; chr6: 31356426-31356450; chr6:31356763-31356787; chr6: 31356764-31356788; chr6:31356762-31356786; chr6: 31355204-31355228; chr6:31356436-31356460; or chr6: 31356767-31356791.

[0618] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or (b) chr6:31355390-31355414; chr6:31355417-31355441; or chr6: 31356386-31356410.

[0619] In some embodiments, the modification to HLA-B comprises any one or more of an insertion, deletion, substitution, or deamination of at least one nucleotide in a target sequence. In some embodiments, the modification to HLA-B comprises an insertion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-B comprises a deletion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In other embodiments, the modification to HLA-B comprises an insertion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In other embodiments, the modification to HLA-B comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-B comprises an indel, which is generally defined in the art as an insertion or deletion of less than 1000 base pairs (bp). In some embodiments, the modification to HLA-B comprises an indel which results in a frameshift mutation in a target sequence. In some embodiments, the modification to HLA-B comprises a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-B comprises one or more of an insertion, deletion, or substitution of nucleotides resulting from the incorporation of a template nucleic acid. In some embodiments, the modification to HLA-B comprises an insertion of a donor nucleic acid in a target sequence. In some embodiments, the modification to HLA-B is not transient.

3. HLA-A Guide RNAs

[0620] The methods and compositions provided herein disclose guide RNAs useful for reducing or eliminating the surface expression of HLA-A protein. In some embodiments, such guide RNAs direct an RNA-guided DNA binding agent to an HLA-A genomic target sequence and may be referred to herein as "HLA-A guide RNAs." In some embodiments, the HLA-A guide RNA directs an RNA-guided DNA binding agent to a human HLA-A genomic target sequence. In some embodiments, the HLA-A guide RNA comprises a guide sequence selected from SEQ ID NO: 301-428, 429-462, 463-511 and 512-590. Further detailed description of the guide RNAs for reducing or eliminating the surface expression of HLA-A protein and for genetic modifications of HLA-A are provided in PCT/US2021/064930, the entire contents of which is incorporated herein by reference.

[0621] In some embodiments, a composition is provided comprising an HLA-A guide RNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0622] In some embodiments, a composition is provided comprising an HLA-A single-guide RNA (sgRNA) comprising a guide sequence selected from SEQ ID NO: 301-590. In some embodiments, a composition is provided comprising HLA-A sgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0623] In some embodiments, a composition is provided comprising an HLA-A dual-guide RNA (dgRNA) comprising a guide sequence selected from SEQ ID NO: 301-590. In some embodiments, a composition is provided comprising an HLA-A dgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0624] In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 301-590. Exemplary HLA-A guide sequences are shown below in Table 4 (SEQ ID NOs: 301-428), Table 5A and Table 5B (SEQ ID NOs: 429-482), and Table 6 (SEQ ID NOs:483-498, 500-511), and Table 7 (SEQ ID NOs: 512-590). In some embodiments, the HLA-A gRNA is a sgRNA comprising a sequence as shown below in Table 4 (SEQ ID NOs: 1301-1428 and 2301-2428), Table 6 (SEQ ID NOs: 1483-1498, 1500-1511, 2483-2498, 2500-2511), Table 7 (SEQ ID NOs: 1512-1590 and 2512-2590), and Table 9A (SEQ ID NOs: 3111 and 3112).

TABLE 4

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G018983	301	UGGAGGGCC UGAUGUGUG UU	UGGAGGGCC UGAUGUGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU	mU*mG*mG*AGG GCCUGAUGUGUG UUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU	chr6: 29945290-29945310 (mismatch to hg38 = 2)

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			GAAAAAGUG GCACCGAGUC GGUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018984	302	GCCUGAUGU GUGUUGGGU GU	GCCUGAUGU GUGUUGGGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mC*UGAU GUGUGUUGGGUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945296- 29945316 (mismatch to hg38 = 2)
G018985	303	CCUGAUGUG UGUUGGGUG UU	CCUGAUGUG UGUUGGGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mC*mU*GAUG UGUGUUGGGUGU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945297- 29945317 (mismatch to hg38 = 1)
G018986	304	CCCAACACCC AACACACAUC	CCCAACACCC AACACACAUC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*AACA CCCAACACACAU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945300- 29945320 (mismatch to hg38 = 1)
G018965	305	UCAGGAAAC AUGAAGAAA GC	UCAGGAAAC AUGAAGAAA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mC*mA*GGA AACAUGAAGAAA GCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29890117- 29890137

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G019018	306	AGGCGCCUG GGCCUCUCCC G	AGGCGCCUG GGCCUCUCCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*CGCC UGGGCCUCUCCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29927058-29927078
G018937	307	CGGGCUGGCC UCCCACAAGG	CGGGCUGGCC UCCCACAAGG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mG*GCUG GCCUCCCACAAG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29934330-29934350
G018990	308	ACGGCCAUCC UCGGCGUCU G	ACGGCCAUCC UCGGCGUCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mC*mG*GCCA UCCUCGGCGUCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942541-29942561
G018991	309	GACGGCCAUC CUCGGCGUCU	GACGGCCAUC CUCGGCGUCU GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mA*mC*GGCC AUCCUCGGCGUC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942542-29942562
G018992	310	GACGCCGAG GAUGGCCGU CA	GACGCCGAG GAUGGCCGU CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU	mG*mA*mC*GCCG AGGAUGGCCGUC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29942543-29942563

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018993	311	UGACGGCCA UCCUCGGCGU C	UGACGGCCA UCCUCGGCGU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mG*mA*CGGC CAUCCUCGGCGU CGUUUUAGAmGm CmUmAmGmAmA mAm UmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942543-29942563
G018994	312	GGCGCCAUG ACGGCCAUCC U	GGCGCCAUG ACGGCCAUCC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mC*GCCA UGACGGCCAUCC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942550-29942570
G018995	313	ACAGCGACGC CGCGAGCCAG	ACAGCGACGC CGCGAGCCAG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mC*mA*GCGA CGCCGCGAGCCA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942864-29942884
G018996	314	CGACGCCGCG AGCCAGAGG A	CGACGCCGCG AGCCAGAGG AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mA*CGCC GCGAGCCAGAGG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942868-29942888

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G018997	315	CGAGCCAGA GGAUGGAGC CG	CGAGCCAGA GGAUGGAGC CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mG*mA*GCCA GAGGAUGGAGCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942876-29942896
G018998	316	CGGCUCCAUC CUCUGGCUCG	CGGCUCCAUC CUCUGGCUCG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mG*CUCC AUCCUCUGGCUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942876-29942896
G018999	317	GAGCCAGAG GAUGGAGCC GC	GAGCCAGAG GAUGGAGCC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mA*mG*CCAG AGGAUGGAGCCG CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942877-29942897
G019000	318	GCGCCCGCGG CUCCAUCCUC	GCGCCCGCGG CUCCAUCCUC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mC*mG*CCCG CGGCUCCAUCCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942883-29942903
G019001	319	GCCCGUCCGU GGGGGAUGA G	GCCCGUCCGU GGGGGAUGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mG*mC*mC*CGUC CGUGGGGGAUGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943062-29943082

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019002	320	UCAUCCCCCA CGGACGGGCC	UCAUCCCCCA CGGACGGGCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mA*UCCC CCACGGACGGGC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943063- 29943083
G019003	321	AUCUCGGACC CGGAGACUG U	AUCUCGGACC CGGAGACUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mU*mC*UCGG ACCCGGAGACUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943092- 29943112
G019004	322	GGGGUCCCGC GGCUUCGGG G	GGGGUCCCGC GGCUUCGGG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mG*GUCC CGCGGCUUCGGG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943115- 29943135
G019005	323	CUCGGGGUCC CGCGGCUUCG	CUCGGGGUCC CGCGGCUUCG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA	mC*mU*mC*GGGG UCCCGCGGCUUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29943118- 29943138

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019006	324	UCUCGGGGU CCCGCGGCUU C	UCUCGGGGU CCCGCGGCUU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mU*CGGG GUCCCGCGGCUU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943119-29943139
G019007	325	GUCUCGGGG UCCCGCGGCU U	GUCUCGGGG UCCCGCGGCU UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mU*mC*UCGG GGUCCCGCGGCU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943120-29943140
G019008	326	GCAAGGGUC UCGGGGUCCC G	GCAAGGGUC UCGGGGUCCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mA*AGG GUCUCGGGGUCC CGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943126-29943146
G019009	327	GGACCCCGAG ACCCUUGCCC	GGACCCCGAG ACCCUUGCCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mG*mA*CCCC GAGACCCUUGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943128-29943148

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G019010	328	GACCCCGAGA CCCUUGCCCC	GACCCCGAGA CCCUUGCCCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mA*mC*CCCG AGACCCUUGCCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943129-29943149
G019011	329	CGAGACCCUU GCCCCGGGAG	CGAGACCCUU GCCCCGGGAG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mA*GACC CUUGCCCCGGGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943134-29943154
G019012	330	CUCCCGGGGC AAGGGUCUC G	CUCCCGGGGC AAGGGUCUC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mC*CCGG GGCAAGGGUCUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943134-29943154
G019013	331	UCUCCCGGGG CAAGGGUCU C	UCUCCCGGGG CAAGGGUCU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mU*CCCG GGGCAAGGGUCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943135-29943155
G019014	332	CUCUCCCGGG GCAAGGGUC U	CUCUCCCGGG GCAAGGGUC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mC*mU*mC*UCCC GGGGCAAGGGUC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943136-29943156

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019015	333	CCUUGCCCCG GGAGAGGCC C	CCUUGCCCCG GGAGAGGCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mU*UGCC CCGGGAGAGGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943140- 29943160
G019016	334	CUGGGCCUCU CCCGGGGCAA	CUGGGCCUCU CCCGGGGCAA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mG*GGCC UCUCCCGGGGCA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943142- 29943162
G019017	335	CCUGGGCCUC UCCCGGGGCA	CCUGGGCCUC UCCCGGGGCA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mU*GGGC CUCUCCCGGGGC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943143- 29943163
G019019	336	UUUAGGCCA AAAAUCCCCC C	UUUAGGCCA AAAAUCCCCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mU*mU*mU*AGG CCAAAAAUCCCC CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA	chr6: 29943188- 29943208

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021208	337	CGCUGCAGCG CACGGGUACC	CGCUGCAGCG CACGGGUACC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mC*UGCA GCGCACGGGUAC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943528-29943548
G021209	338	GCUGCAGCGC ACGGGUACC A	GCUGCAGCGC ACGGGUACC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mU*GCAG CGCACGGGUACC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943529-29943549
G021210	339	CUGCAGCGCA CGGGUACCA G	CUGCAGCGCA CGGGUACCA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mG*CAGC GCACGGGUACCA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943530-29943550
G018932	340	CGCACGGGU ACCAGGGGCC A	CGCACGGGU ACCAGGGGCC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mC*mG*mC*ACGG GUACCAGGGGCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943536-29943556

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018933	341	GCACGGGUA CCAGGGGCCA C	GCACGGGUA CCAGGGGCCA CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mA*CGGG UACCAGGGGCCA CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943537-29943557
G018934	342	CACGGGUACC AGGGGCCAC G	CACGGGUACC AGGGGCCAC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mC*GGGU ACCAGGGGCCAC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943538-29943558
G018935	343	GGGAGGCGC CCCGUGGCCC C	GGGAGGCGC CCCGUGGCCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mG*AGG CGCCCCGUGGCC CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943549-29943569
G018936	344	GCGAUCAGG GAGGCGCCCC G	GCGAUCAGG GAGGCGCCCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mG*mC*mG*AUCA GGGAGGCGCCCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943556-29943576

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021211	345	UCCUUGUGG GAGGCCAGCC C	UCCUUGUGG GAGGCCAGCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*UUGU GGGAGGCCAGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943589-29943609
G018938	346	CUCCUUGUG GGAGGCCAG CC	CUCCUUGUG GGAGGCCAG CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mU*mC*CUUG UGGGAGGCCAGC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943590-29943610
G018939	347	GGCUGGCCUC CCACAAGGA G	GGCUGGCCUC CCACAAGGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mC*UGGC CUCCCACAAGGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943590-29943610
G018940	348	UUGUCUCCCC UCCUUGUGG G	UUGUCUCCCC UCCUUGUGG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU	mU*mU*mG*UCUC CCCUCCUUGUGG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29943599-29943619

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018941	349	CCACAAGGA GGGGAGACA AU	CCACAAGGA GGGGAGACA AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mC*mA*CAAG GAGGGGAGACAA UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943600-29943620
G018942	350	CACAAGGAG GGGAGACAA UU	CACAAGGAG GGGAGACAA UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mC*AAGG AGGGGAGACAAU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943601-29943621
G018943	351	CAAUUGUCU CCCCUCCUUG U	CAAUUGUCU CCCCUCCUUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mA*UUG UCUCCCCUCCUU GUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943602-29943622
G018944	352	CCAAUUGUC UCCCCUCCUU G	CCAAUUGUC UCCCCUCCUU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mA*AUUG UCUCCCCUCCUU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943603-29943623

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G018945	353	AUCCCUCGAA UACUGAUGA G	AUCCCUCGAA UACUGAUGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mU*mC*CCUC GAAUACUGAUGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943774-29943794
G018946	354	AACCACUCAU CAGUAUUCG A	AACCACUCAU CAGUAUUCG AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mA*mC*CACU CAUCAGUAUUCG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943779-29943799
G018947	355	GAACCACUCA UCAGUAUUC G	GAACCACUCA UCAGUAUUC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mA*CCAC UCAUCAGUAUUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943780-29943800
G018948	356	GAGGAAAAG UCACGGGCCC A	GAGGAAAAG UCACGGGCCC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mG*GAA AAGUCACGGGCC CAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943822-29943842
G018949	357	GGCCCGUGAC UUUUCCUCUC	GGCCCGUGAC UUUUCCUCUC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU	mG*mG*mC*CCGU GACUUUUCCUCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943824-29943844

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018950	358	UGCUUCACAC UCAAUGUGU G	UGCUUCACAC UCAAUGUGU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mG*mC*UUCA CACUCAAUGUGU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943857-29943877
G018951	359	GCUUCACACU CAAUGUGUG U	GCUUCACACU CAAUGUGUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mU*UCAC ACUCAAUGUGUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943858-29943878
G018952	360	CUUCACACUC AAUGUGUGU G	CUUCACACUC AAUGUGUGU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mU*CACA CUCAAUGUGUGU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943859-29943879
G018953	361	UUCACACUCA AUGUGUGUG G	UUCACACUCA AUGUGUGUG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU	mU*mU*mC*ACAC UCAAUGUGUGUG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29943860-29943880

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018954	362	UUGAGAAUG GACAGGACA CC	UUGAGAAUG GACAGGACA CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mU*mG*AGA AUGGACAGGACA CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944026-29944046
G021205	363	AGGCAUUUU GCAUCUGUC AU	AGGCAUUUU GCAUCUGUC AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mG*CAU UUUGCAUCUGUC AUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944077-29944097
G021206	364	CAGGCAUUU UGCAUCUGU CA	CAGGCAUUU UGCAUCUGU CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*GCAU UUUGCAUCUGUC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944078-29944098
G018955	365	AGGGGCCCU GACCCUGCUA A	AGGGGCCCU GACCCUGCUA AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*GGCC CUGACCCUGCUA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944458-29944478

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G018956	366	UGGGAAAAG AGGGGAAGG UG	UGGGAAAAG AGGGGAAGG UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*GAA AAGAGGGGAAGG UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944478-29944498
G018957	367	UGGAGGAGG AAGAGCUCA GG	UGGAGGAGG AAGAGCUCA GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*AGG AGGAAGAGCUCA GGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944597-29944617
G018958	368	UGAGAUUUC UUGUCUCAC UG	UGAGAUUUC UUGUCUCAC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mA*GAU UUCUUGUCUCAC UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944642-29944662
G018959	369	GAGAUUUCU UGUCUCACU GA	GAGAUUUCU UGUCUCACU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mA*mG*AUU UCUUGUCUCACU GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944643-29944663
G018960	370	UAAAGCACC UGUUAAAAU GA	UAAAGCACC UGUUAAAAU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mU*mA*mA*AGC ACCUGUUAAAAU GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA	chr6: 29944772-29944792

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018961	371	AAUCUGUCC UUCAUUUUA AC	AAUCUGUCC UUCAUUUUA ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mU*CUG UCCUUCAUUUUA ACGUUUUAGAmG mCmUmAmGmAm AmAm UmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944782-29944802
G018962	372	GUCACAGGG GAAGGUCCC UG	GUCACAGGG GAAGGUCCC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mU*mC*ACAG GGGAAGGUCCCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944850-29944870
G018964	373	AAACAUGAA GAAAGCAGG UG	AAACAUGAA GAAAGCAGG UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mA*CAU GAAGAAAGCAGG UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29944907-29944927
G018966	374	UGUCCUGUG AGAUACCAG AA	UGUCCUGUG AGAUACCAG AAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU	mU*mG*mU*CCUG UGAGAUACCAGA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29945024-29945044

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018967	375	AUGAAGGAG GCUGAUGCC UG	AUGAAGGAG GCUGAUGCC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mU*mG*AAG GAGGCUGAUGCC UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945097- 29945117
G018968	376	AGGCUGAUG CCUGAGGUCC U	AGGCUGAUG CCUGAGGUCC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*CUG AUGCCUGAGGUC CUGUUUUAGAmG mCmUmAmGmAm AmAm UmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945104- 29945124
G018969	377	GGCUGAUGC CUGAGGUCC UU	GGCUGAUGC CUGAGGUCC UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mC*UGA UGCCUGAGGUCC UUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945105- 29945125
G018970	378	CACAAUAUCC CAAGGACCUC	CACAAUAUCC CAAGGACCUC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mC*AAUA UCCCAAGGACCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945116- 29945136

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G018971	379	GGUCCUUGG GAUAUUGUG UU	GGUCCUUGG GAUAUUGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mU*CCUU GGGAUAUUGUGU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945118-29945138
G018972	380	GUCCUUGGG AUAUUGUGU UU	GUCCUUGGG AUAUUGUGU UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mU*mC*CUUG GGAUAUUGUGUU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945119-29945139
G018973	381	CUCCCAAACA CAAUAUCCCA	CUCCCAAACA CAAUAUCCCA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mC*CCAA ACACAAUAUCCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945124-29945144
G018974	382	UCCUCUAGCC ACAUCUUCU G	UCCUCUAGCC ACAUCUUCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*UCUA GCCACAUCUUCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945176-29945196
G018975	383	ACAGAAGAU GUGGCUAGA GG	ACAGAAGAU GUGGCUAGA GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mA*mC*mA*GAA GAUGUGGCUAGA GGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA	chr6: 29945177-29945197

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018976	384	CCUCUAGCCA CAUCUUCUG U	CCUCUAGCCA CAUCUUCUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mU*CUAG CCACAUCUUCUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945177-29945197
G018977	385	CCCACAGAAG AUGUGGCUA G	CCCACAGAAG AUGUGGCUA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mC*ACAG AAGAUGUGGCUA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945180-29945200
G018978	386	GUCAGAUCCC ACAGAAGAU G	GUCAGAUCCC ACAGAAGAU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mU*mC*AGA UCCCACAGAAGA UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945187-29945207
G018979	387	AUCUUCUGU GGGAUCUGA CC	AUCUUCUGU GGGAUCUGA CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mA*mU*mC*UUCU GUGGGAUCUGAC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29945188-29945208

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018980	388	CCCAGGCAGU GACAGUGCCC	CCCAGGCAGU GACAGUGCCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*AGGC AGUGACAGUGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945228- 29945248
G018981	389	CUGGGCACU GUCACUGCCU G	CUGGGCACU GUCACUGCCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mG*GGCA CUGUCACUGCCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945230- 29945250
G018982	390	CCUGGGCACU GUCACUGCCU	CCUGGGCACU GUCACUGCCU GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mU*GGGC ACUGUCACUGCC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945231- 29945251
G021207	391	CCCUGGGCAC UGUCACUGCC	CCCUGGGCAC UGUCACUGCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU	mC*mC*mC*UGGG CACUGUCACUGC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29945232- 29945252

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018987	392	UUGGGUGUU GGGCGGAAC AG	UUGGGUGUU GGGCGGAAC AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mU*mG*GGU GUUGGGCGGAAC AGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945308-29945328
G018988	393	UGGAUGUAU UGAGCAUGC GA	UGGAUGUAU UGAGCAUGC GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*AUG UAUUGAGCAUGC GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945361-29945381
G018989	394	GGAUGUAUU GAGCAUGCG AU	GGAUGUAUU GAGCAUGCG AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mA*UGU AUUGAGCAUGCG AUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29945362-29945382
G018963	395	AACAUGAAG AAAGCAGGU GU	AACAUGAAG AAAGCAGGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mA*mA*mC*AUG AAGAAAGCAGGU GUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA	chr6: 31382543-31382563

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021885	396	UAGCCCACGG CGAUGAAGC G	UAGCCCACGG CGAUGAAGC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mA*mG*CCCA CGGCGAUGAAGC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942815-29942835
G021886	397	GUAGCCCACG GCGAUGAAG C	GUAGCCCACG GCGAUGAAG CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mU*mA*GCCC ACGGCGAUGAAG CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942816-29942836
G021887	398	CGUAGCCCAC GGCGAUGAA G	CGUAGCCCAC GGCGAUGAA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mU*AGCC CACGGCGAUGAA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942817-29942837
G021888	399	CUUCAUCGCC GUGGGCUAC G	CUUCAUCGCC GUGGGCUAC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mU*CAUC GCCGUGGGCUAC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942817-29942837

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G021889	400	CGUGUCGUCC ACGUAGCCCA	CGUGUCGUCC ACGUAGCCCA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mU*GUCG UCCACGUAGCCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942828-29942848
G021890	401	UGGACGACA CGCAGUUCG UG	UGGACGACA CGCAGUUCG UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*ACG ACACGCAGUUCG UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942837-29942857
G021891	402	GGAUGGAGC CGCGGGCGCC G	GGAUGGAGC CGCGGGCGCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mA*UGG AGCCGCGGGCGC CGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942885-29942905
G021892	403	GCGGGCGCCG UGGAUAGAG C	GCGGGCGCCG UGGAUAGAG CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mG*GGCG CCGUGGAUAGAG CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942895-29942915
G021893	404	UGCUCUAUCC ACGGCGCCCG	UGCUCUAUCC ACGGCGCCCG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU	mU*mG*mC*UCUA UCCACGGCGCCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29942896-29942916

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021894	405	GGCGCCGUG GAUAGAGCA GG	GGCGCCGUG GAUAGAGCA GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mC*GCCG UGGAUAGAGCAG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942898-29942918
G021895	406	GCGCCGUGG AUAGAGCAG GA	GCGCCGUGG AUAGAGCAG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mG*CCGU GGAUAGAGCAGG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942899-29942919
G021896	407	CGCCGUGGA UAGAGCAGG AG	CGCCGUGGA UAGAGCAGG AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mG*mC*CGUG GAUAGAGCAGGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942900-29942920
G021897	408	GUGGAUAGA GCAGGAGGG GC	GUGGAUAGA GCAGGAGGG GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mG*mU*mG*GAU AGAGCAGGAGGG GCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA	chr6: 29942904-29942924

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021898	409	GGCCCCUCCU GCUCUAUCCA	GGCCCCUCCU GCUCUAUCCA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mG*mC*CCCU CCUGCUCUAUCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942905-29942925
G021899	410	AGCAGGAGG GGCCGGAGU AU	AGCAGGAGG GGCCGGAGU AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mC*AGG AGGGGCCGGAGU AUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942912-29942932
G021900	411	GCAGGAGGG GCCGGAGUA UU	GCAGGAGGG GCCGGAGUA UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mA*GGA GGGGCCGGAGUA UUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29942913-29942933
G021901	412	GGAGUGGCU CCGCAGAUAC C	GGAGUGGCU CCGCAGAUAC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU	mG*mG*mA*GUG GCUCCGCAGAUA CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU	chr6: 29943490-29943510

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021902	413	CUCCGCAGAU ACCUGGAGA A	CUCCGCAGAU ACCUGGAGA AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mC*CGCA GAUACCUGGAGA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943497-29943517
G021903	414	UCCGCAGAU ACCUGGAGA AC	UCCGCAGAU ACCUGGAGA ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mC*mC*GCAG AUACCUGGAGAA CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943498-29943518
G021904	415	CAGAUACCU GGAGAACGG GA	CAGAUACCU GGAGAACGG GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*AUAC CUGGAGAACGGG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943502-29943522
G021905	416	UCCCGUUCUC CAGGUAUCU G	UCCCGUUCUC CAGGUAUCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*CGUU CUCCAGGUAUCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943502-29943522

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
G021906	417	GCGUCUCCUU CCCGUUCUCC	GCGUCUCCUU CCCGUUCUCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mC*mG*UCUC CUUCCCGUUCUC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943511-29943531
G021907	418	GAAGGAGAC GCUGCAGCGC A	GAAGGAGAC GCUGCAGCGC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mA*GGA GACGCUGCAGCG CAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943520-29943540
G021908	419	AAGGAGACG CUGCAGCGCA C	AAGGAGACG CUGCAGCGCA CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mA*mG*GAG ACGCUGCAGCGC ACGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943521-29943541
G021909	420	AGAUCUACA GGCGAUCAG GG	AGAUCUACA GGCGAUCAG GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mA*UCU ACAGGCGAUCAG GGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943566-29943586
G021910	421	UGAUCGCCU GUAGAUCUC CC	UGAUCGCCU GUAGAUCUC CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG	mU*mG*mA*UCGC CUGUAGAUCUCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG	chr6: 29943569-29943589

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021911	422	GGGAGAUCU ACAGGCGAU CA	GGGAGAUCU ACAGGCGAU CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mG*AGA UCUACAGGCGAU CAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943569-29943589
G021912	423	CGGGAGAUC UACAGGCGA UC	CGGGAGAUC UACAGGCGA UCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mG*mG*GAG AUCUACAGGCGA UCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943570-29943590
G021913	424	CGCCUGUAG AUCUCCCGGG C	CGCCUGUAG AUCUCCCGGG CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mC*CUGU AGAUCUCCCGGG CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943573-29943593
G021914	425	GGCCAGCCCG GGAGAUCUA C	GGCCAGCCCG GGAGAUCUA CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU	mG*mG*mC*CAGC CCGGGAGAUCUA CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA	chr6: 29943578-29943598

TABLE 4-continued

Exemplary Spy HLA-A guide RNAs

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOS: 1301-1428)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2301-2428)	Genomic Coordinates (hg38)
			AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021915	426	UCCCGGGCUG GCCUCCCACA	UCCCGGGCUG GCCUCCCACA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mC*CGGG CUGGCCUCCCAC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943585-29943605
G021916	427	GGGCUGGCC UCCCACAAGG A	GGGCUGGCC UCCCACAAGG AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mG*CUG GCCUCCCACAAG GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943589-29943609
G021917	428	CUGAUCGCCU GUAGAUCUC C	CUGAUCGCCU GUAGAUCUC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mG*AUCG CCUGUAGAUCUC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6: 29943568-29943588

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

TABLE 5A

Additional exemplary HLA-A guide sequences

SEQ ID NO	Guide Sequence	PAM	RNA-guided DNA binding agent	Genomic Coordinates (hg38)
429	AGGAUGGAGCCGCGGGCGCC	GTGGAT	*S. aureus* Cas9	chr6: 29942884-29942904

TABLE 5A-continued

Additional exemplary HLA-A guide sequences

SEQ ID NO	Guide Sequence	PAM	RNA-guided DNA binding agent	Genomic Coordinates (hg38)
430	GGAAGGAGACGCUGCAGCGC	ACGGGT	*S. aureus* Cas9	chr6: 29943519-29943539
431	GACAGCGACGCCGCGAGCCA	GAGGAT	*S. aureus* Cas9	chr6: 29942863-29942883
432	CGGGAAGGAGACGCUGCAGC	TTCT	CasX	chr6: 29943517-29943537
433	CCGUGCGCUGCAGCGUCUCC	TTCC	CasX	chr6: 29943523-29943543
434	ACGCAGUUCGUGCGGUUCGACAGC	NNNNCC	NME2	chr6: 29942845-29942869
435	UCGUGCGGUUCGACAGCGACGCCG	NNNNCC	NME2	chr6: 29942852-29942876
436	CAGCGACGCCGCGAGCCAGAGGAU	NNNNCC	NME2	chr6: 29942865-29942889
437	GCUCUAUCCACGGCGCCCGCGGCU	NNNNCC	NME2	chr6: 29942891-29942915
438	UCCUGCUCUAUCCACGGCGCCCGC	NNNNCC	NME2	chr6: 29942895-29942919
439	CCGGCCCCUCCUGCUCUAUCCACG	NNNNCC	NME2	chr6: 29942903-29942927
440	UCCGGCCCCUCCUGCUCUAUCCAC	NNNNCC	NME2	chr6: 29942904-29942928
441	GGGAAGGAGACGCUGCAGCGCACG	NNNNCC	NME2	chr6: 29943518-29943542
442	AGACGCUGCAGCGCACGGGUACCA	NNNNCC	NME2	chr6: 29943525-29943549
443	GCGCACGGGUACCAGGGGCCACGG	NNNNCC	NME2	chr6: 29943535-29943559
444	CACGGGUACCAGGGGCCACGGGGC	NNNNCC	NME2	chr6: 29943538-29943562
445	ACGGGUACCAGGGGCCACGGGGCG	NNNNCC	NME2	chr6: 29943539-29943563
446	CAGGGGCCACGGGGCGCCUCCCUG	NNNNCC	NME2	chr6: 29943547-29943571
447	CAGGGAGGCGCCCCGUGGCCCCUG	NNNNCC	NME2	chr6: 29943547-29943571
448	UCAGGGAGGCGCCCCGUGGCCCCU	NNNNCC	NME2	chr6: 29943548-29943572
449	CAGGCGAUCAGGGAGGCGCCCCGU	NNNNCC	NME2	chr6: 29943555-29943579
450	ACAGGCGAUCAGGGAGGCGCCCCG	NNNNCC	NME2	chr6: 29943556-29943580
451	UACAGGCGAUCAGGGAGGCGCCCC	NNNNCC	NME2	chr6: 29943557-29943581
452	GGGCGCCUCCCUGAUCGCCUGUAG	NNNNCC	NME2	chr6: 29943558-29943582
453	GGCGCCUCCCUGAUCGCCUGUAGA	NNNNCC	NME2	chr6: 29943559-29943583

TABLE 5A-continued

Additional exemplary HLA-A guide sequences

SEQ ID NO	Guide Sequence	PAM	RNA-guided DNA binding agent	Genomic Coordinates (hg38)
454	GAGAUCUACAGGCGAUCAGG GAGG	NNNNNC C	NME2	chr6: 29943563-29943587
455	GGAGAUCUACAGGCGAUCAG GGAG	NNNNNC C	NME2	chr6: 29943564-29943588
456	GGGAGAUCUACAGGCGAUCA GGGA	NNNNNC C	NME2	chr6: 29943565-29943589
457	CUGAUCGCCUGUAGAUCUCC CGGG	NNNNNC C	NME2	chr6: 29943568-29943592
458	AUCGCCUGUAGAUCUCCCGG GCUG	NNNNNC C	NME2	chr6: 29943571-29943595
459	UCGCCUGUAGAUCUCCCGGG CUGG	NNNNNC C	NME2	chr6: 29943572-29943596
460	UUGUCUCCCCUCCUUGUGGG AGGC	NNNNNC C	NME2	chr6: 29943595-29943619
461	AUUGUCUCCCCUCCUUGUGG GAGG	NNNNNC C	NME2	chr6: 29943596-29943620
462	CCCAAUUGUCUCCCCUCCUU GUGG	NNNNNC C	NME2	chr6: 29943600-29943624

TABLE 5B

Exemplary Spy HLA-A guide sequences

463	GGAUGGAGCCGCGGGCGCCG	NGG	Spy + Base_ Editor	chr6: 29942885-29942905
464	GCGGGCGCCGUGGAUAGAGC	NGG	Spy + Base_ Editor	chr6: 29942895-29942915
465	UGCUCUAUCCACGGCGCCCG	NGG	Spy + Base_ Editor	chr6: 29942896-29942916
466	GGCGCCGUGGAUAGAGCAGG	NGG	Spy + Base_ Editor	chr6: 29942898-29942918
467	GCGCCGUGGAUAGAGCAGGA	NGG	Spy + Base_ Editor	chr6: 29942899-29942919
468	CGCCGUGGAUAGAGCAGGAG	NGG	Spy + Base_ Editor	chr6: 29942900-29942920
469	GUGGAUAGAGCAGGAGGGGC	NGG	Spy + Base_ Editor	chr6: 29942904-29942924
470	GCGUCUCCUUCCCGUUCUCC	NGG	Spy + Base_ Editor	chr6: 29943511-29943531
471	GAAGGAGACGCUGCAGCGCA	NGG	Spy + Base_ Editor	chr6: 29943520-29943540

TABLE 5B-continued

Exemplary Spy HLA-A guide sequences

472	AAGGAGACGCUGCAGCGCAC	NGG	Spy + Base_ Editor	chr6: 29943521-29943541
473	GCUGCAGCGCACGGGUACCA	NGG	Spy + Base_ Editor	chr6: 29943529-29943549
474	AGAUCUACAGGCGAUCAGGG	NGG	Spy + Base_ Editor	chr6: 29943566-29943586
475	CUGAUCGCCUGUAGAUCUCC	NGG	Spy + Base_ Editor	chr6: 29943568-29943588
476	UGAUCGCCUGUAGAUCUCCC	NGG	Spy + Base_ Editor	chr6: 29943569-29943589
477	GGGAGAUCUACAGGCGAUCA	NGG	Spy + Base_ Editor	chr6: 29943569-29943589
478	CGGGAGAUCUACAGGCGAUC	NGG	Spy + Base_ Editor	chr6: 29943570-29943590
479	CGCCUGUAGAUCUCCCGGGC	NGG	Spy + Base_ Editor	chr6: 29943573-29943593
480	GGCCAGCCCGGGAGAUCUAC	NGG	Spy + Base_ Editor	chr6: 29943578-29943598

TABLE 5B-continued

Exemplary Spy HLA-A guide sequences				
481	UCCCGGGCUGGCCUCCCACA	NGG	Spy + Base_ Editor	chr6: 29943585-29943605

TABLE 5B-continued

Exemplary Spy HLA-A guide sequences				
482	GGGCUGGCCUCCCACAAGGA	NGG	Spy + Base_ Editor	chr6: 29943589-29943609

[000632] Table 6. Additional Exemplary HLA-A guide sequences.

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence with PAM (SEQ ID NOs: 1483-1498 and 1500-1511)	Exemplary Guide RNA Modified Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2483-2498 and 2500-2511)	Genomic Coordinates (hg38)
G021857	483	ACGACACUGAUUGGCUUC	ACGACACUGAUUGGCUUCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	mA*mC*mG*ACACUGAUUGGCUUCUCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	chr6: 29942469-29942489
G021858	484	ACCCCUCAUCCCCACGGAC	ACCCCUCAUCCCCCACGGACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	mA*mC*mC*CCUCAUCCCCCACGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	chr6: 29943058-29943078
G021859	485	GGCCCGUCCGUGGGGGA	GGCCCGUCCGUGGGGGAUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	mG*mG*mC*CCGUCCGUGGGGGAUGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	chr6: 29943063-29943083
G021860	486	GCCAGGUCGCCCACAGUC	GCCAGGUCGCCCACAGUCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	mG*mC*mC*AGGUCGCCCACAGUCUCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	chr6: 29943080-29943100
G021861	487	GUUUAGGCCAAAAUCCCC	GUUUAGGCCAAAAUCCCCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	mG*mU*mU*UAGGCCAAAAUCCCCCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	chr6: 29943187-29943207

-continued

[000632] Table 6. Additional Exemplary HLA-A guide sequences.

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence with PAM (SEQ ID NOs: 1483-1498 and 1500-1511)	Exemplary Guide RNA Modified Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2483-2498 and 2500-2511)	Genomic Coordinates (hg38)
G021862	488	GGCCAAA AAUCCCC CCGGGU	GGCCAAAAAUCC CCCCGGGUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mG*mC*CAAAAAUC CCCCCGGGUGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29943192-29943212
G021863	489	GACCAAC CCGGGGG GAUUUU	GACCAACCCGGG GGGAUUUUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mA*mC*CAACCCGG GGGGAUUUUGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29943197-29943217
G021864	490	CACGGGC CCAAGGC UGCUGC	CACGGGCCCAAG GCUGCUGCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mA*mC*GGGCCCAA GGCUGCUGCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29943812-29943832
G021865	491	ACCCUCA UGCUGCA CAUGGC	ACCCUCAUGCUG CACAUGGCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mA*mC*mC*CUCAUGCU GCACAUGGCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944349-29944369
G021866	492	CCUCUAG GACCUUA AGGCCC	CCUCUAGGACCU UAAGGCCCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mC*mU*CUAGGACC UUAAGGCCCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944996-29945016
G021867	493	GCUCCUU UCUGGUA UCUCAC	GCUCCUUUCUGG UAUCUCACGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mC*mU*CCUUUCUG GUAUCUCACGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29945018-29945038

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[000632] Table 6. Additional Exemplary HLA-A guide sequences.

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence with PAM (SEQ ID NOs: 1483-1498 and 1500-1511)	Exemplary Guide RNA Modified Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2483-2498 and 2500-2511)	Genomic Coordinates (hg38)
G021868	494	GCUAUGG GGUUUCU UUGCAU	GCUAUGGGGUUU CUUUGCAUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mC*mU*AUGGGGUU UCUUUGCAUGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29945341-29945361
G021869	495	GCCUUUG CAGAAAC AAAGUC	GCCUUUGCAGAA ACAAAGUCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mC*mC*UUUGCAGA AACAAAGUCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29945526-29945546
G021870	496	UGGACCA ACCGCCC UCCUGA	UGGACCAACCGC CCUCCUGAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mU*mG*mG*ACCAACCG CCCUCCUGAGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944880-29944900 (mismatch to hg38 = 2)
G021871	497	AGCCUCU CUGACCU UUAGCA	AGCCUCUCUGAC CUUUAGCAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mA*mG*mC*CUCUCUGA CCUUUAGCAGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	Na
G021872	498	CGCCCUC CUGAAGG UCCUCA	CGCCCUCCUGAA GGUCCUCAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mG*mC*CCUCCUGA AGGUCCUCAGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	Na
G021873	500	CCGCCCU CCUGAAG GUCCUC	CCGCCCUCCUGA AGGUCCUCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mC*mG*CCCUCCUG AAGGUCCUCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	Na

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[000632] Table 6. Additional Exemplary HLA-A guide sequences.

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence with PAM (SEQ ID NOs: 1483-1498 and 1500-1511)	Exemplary Guide RNA Modified Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2483-2498 and 2500-2511)	Genomic Coordinates (hg38)
G021874	501	UGGUUCC CUUUGAC ACACAC	UGGUUCCCUUUG ACACACACGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mU*mG*mG*UUCCCUUU GACACACACGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29943794-29943814 (mismatch to hg38 = 3)
G021875	502	GACCCUG CUAAAGG UCAGAG	GACCCUGCUAAA GGUCAGAGGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mA*mC*CCUGCUAA AGGUCAGAGGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	na
G021876	503	AGGACCU UCAGGAG GGCGGU	AGGACCUUCAGG AGGGCGGUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mA*mG*mG*ACCUUCAG GAGGGCGGUGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	na
G021877	504	GCACACU UCUACCU GGGUCU	GCACACUUCUAC CUGGGUCUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mC*mA*CACUUCUA CCUGGGUCUGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944671-29944691 (mismatch to hg38 = 3)
G021878	505	GAGCCUC UCUGACC UUUAGC	GAGCCUCUCUGA CCUUUAGCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mA*mG*CCUCUCUG ACCUUUAGCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	na
G021879	506	ACACUCC UCCAGCA CACAUG	ACACUCCUCCAG CACACAUGGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA	mA*mC*mA*CUCCUCCA GCACACAUGGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAm AmAmGmUmG	chr6: 29944054-29944074 (mismatch to hg38 = 2)

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[000632] Table 6. Additional Exemplary HLA-A guide sequences.

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence with PAM (SEQ ID NOs: 1483-1498 and 1500-1511)	Exemplary Guide RNA Modified Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 2483-2498 and 2500-2511)	Genomic Coordinates (hg38)
			CCGAGUCGGUGC UUUU	mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	
G021880	507	CUCUGAC CUUUAGC AGGGUC	CUCUGACCUUUA GCAGGGUCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mU*mC*UGACCUUU AGCAGGGUCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	na
G021881	508	CAAGAUA GCCACAU GUGUGC	CAAGAUAGCCAC AUGUGUGCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mC*mA*mA*GAUAGCCA CAUGUGUGCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944043-29944063 (mismatch to hg38 = 2)
G021882	509	UCUGACC UUUAGCA GGGUCA	UCUGACCUUUAG CAGGGUCAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mU*mC*mU*GACCUUUA GCAGGGUCAGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944450-29944470 (mismatch to hg38 = 3)
G021883	510	UGUAAAG GUGAGAG CCUGGA	UGUAAAGGUGAG AGCCUGGAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mU*mG*mU*AAAGGUGA GAGCCUGGAGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29945274-29945294 (mismatch to hg38 = 1)
G021884	511	GAAGGUC CCUGAGG ACCUUC	GAAGGUCCCUGA GGACCUUCGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	mG*mA*mA*GGUCCCUG AGGACCUUCGUUUUAG AmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAA AAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGm AmAmAmAmAmGmUmG mGmCmAmCmCmGmAmG mUmCmGmGmUmGmCmU *mU*mU*mU	chr6: 29944859-29944879 (mismatch to hg38 = 3)

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

TABLE 7

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
G028854	512	CCUGGGUCU GGUCCUCCCC AUCCC	CCUGGGUCU GGUCCUCCCC AUCCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mU*mGmGG UmCmUGmGUmCCU CCmCCAUmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944223-29944247
G028855	513	GUGGAGACC AGGCCUGCA GGGGAU	GUGGAGACC AGGCCUGCA GGGGAUGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mU*mG*mGmA GAmCmCAmGGmCC UGCmAGGGmGAUm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944264-29944288
G028856	514	CCGUGUCCUG GGUCUGGUC CUCCC	CCGUGUCCUG GGUCUGGUC CUCCCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mG*mUmGU CmCmUGmGGmUCU GGmUCCUmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944229-29944253
G028857	515	UCCGUGUCCU GGGUCUGGU CCUCC	UCCGUGUCCU GGGUCUGGU CCUCCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mGmUG UmCmCUmGGmGUC UGmGUCCmUCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944230-29944254
G028858	516	CACAUGGCA GGUGUAUCU CUGCUC	CACAUGGCA GGUGUAUCU CUGCUCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mC*mAmUG GmCmAGmGUmGUA UCmUCUGmCUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944327-29944351
G028859	517	UGCUGCACA UGGCAGGUG UAUCUC	UGCUGCACA UGGCAGGUG UAUCUCGUU GUAGCUCCCU	mU*mG*mC*mUmGC AmCmAUmGGmCAG GUmGUAUmCUCmG UUGmUmAmGmCUC	chr6:29944332-29944356

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028860	518	CCGCACGAAC UGCGUGUCG UCCAC	CCGCACGAAC UGCGUGUCG UCCACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mG*mCmAC GmAmACmUGmCGU GUmCGUCmCACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942830- 29942854
G028861	519	CGCACGAACU GCGUGUCGU CCACG	CGCACGAACU GCGUGUCGU CCACGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mG*mC*mAmCG AmAmCUmGCmGUG UCmGUCCmACGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942829- 29942853
G028862	520	GUGCGCUGC AGCGUCUCCU UCCCG	GUGCGCUGC AGCGUCUCCU UCCCGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mU*mG*mCmGC UmGmCAmGCmGUC UCmCUUCmCCGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943511- 29943535
G028863	521	CCGAGGAUG GCCGUCAUG GCGCCC	CCGAGGAUG GCCGUCAUG GCGCCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mG*mAmGG AmUmGGmCCmGUC AUmGGCGmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942547- 29942571
G028864	522	CGAGGAUGG CCGUCAUGGC GCCCC	CGAGGAUGG CCGUCAUGGC GCCCCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC	mC*mG*mA*mGmGA UmGmGCmCGmUCA UGmGCGCmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA	chr6:29942548- 29942572

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			GCAACGCUCU GCCUUCUGGC AUCGUU	mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028865	523	AAGGUUCCA UCCCCUGCAG GCCUG	AAGGUUCCA UCCCCUGCAG GCCUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mA*mG*mGmU UCmCmAUmCCmCC UGCmAGGCmCUGm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944266- 29944290
G028866	524	AGACCAGGCC UGCAGGGGA UGGAA	AGACCAGGCC UGCAGGGGA UGGAAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mA*mCmCA GmGmCCmUGmCAG GGmGAUGmGAAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944268- 29944292
G028867	525	ACUUCUGGA AGGUUCCAU CCCCUG	ACUUCUGGA AGGUUCCAU CCCCUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mU*mUmCU GmGmAAmGGmUUC CAmUCCCmCUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944274- 29944298
G028868	526	CGAUGAAGC GGGGCUCCCC GCGGC	CGAUGAAGC GGGGCUCCCC GCGGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mG*mA*mUmGA AmGmCGmGGmGCU CCmCCGCmGGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942795- 29942819
G028869	527	UCCGUGUCCC GGCCCGGCCG CGGG	UCCGUGUCCC GGCCCGGCCG CGGGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mGmUG UmCmCCmGGmCCC GGmCCGCmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942785- 29942809

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
G028870	528	CCGUGUCCCG GCCCGGCCGC GGGG	CCGUGUCCCG GCCCGGCCGC GGGGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mG*mUmGU CmCmCGmGCmCCG GCmCGCGmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942786-29942810
G028871	529	CAGACGCCGA GGAUGGCCG UCAUG	CAGACGCCGA GGAUGGCCG UCAUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mG*mAmCG CmCmGAmGGmAUG GCmCGUCmAUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942541-29942565
G028872	530	CCAGACGCCG AGGAUGGCC GUCAU	CCAGACGCCG AGGAUGGCC GUCAUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mA*mGmAC GmCmCGmAGmGAU GGmCCGUmCAUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942540-29942564
G028873	531	AGACGCCGA GGAUGGCCG UCAUGG	AGACGCCGA GGAUGGCCG UCAUGGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mA*mG*mA*mCmGC CmGmAGmGAmUGG CCmGUCAmUGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942542-29942566
G028874	532	CUGUCGAACC GCACGAACU GCGUG	CUGUCGAACC GCACGAACU GCGUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mU*mG*mUmCG AmAmCCmGCmACG AAmCUGCmGUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942838-29942862
G028875	533	UCGUGCGGU UCGACAGCG ACGCCG	UCGUGCGGU UCGACAGCG ACGCCGGUU	mU*mC*mG*mUmGC GmGmUUmCGmACA GCmGACGmCCGmG	chr6:29942852-29942876

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028876	534	CCAUCCCCAU CGUGGGCAU CAUUG	CCAUCCCCAU CGUGGGCAU CAUUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mA*mUmCC CmCmAUmCGmUGG GCmAUCAmUUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944517- 29944541
G028877	535	UCCUCUGGCU CGCGGCGUCG CUGU	UCCUCUGGCU CGCGGCGUCG CUGUGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mUmCU GmGmCUmCGmCGG CGmUCGCmUGUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942858- 29942882
G028878	536	CUCUGGUUG UAGUAGCCG CGCAGG	CUCUGGUUG UAGUAGCCG CGCAGGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mU*mC*mUmGG UmUmGUmAGmUAG CCmGCGCmAGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942988- 29943012
G028879	537	ACCUGGGGA CCCUGCGCGG CUACU	ACCUGGGGA CCCUGCGCGG CUACUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mC*mUmGG GmGmACmCCmUGC GCmGGCUmACUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942984- 29943008
G028880	538	CGCUCUGGU UGUAGUAGC CGCGCA	CGCUCUGGU UGUAGUAGC CGCGCAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG	mC*mG*mC*mUmCU GmGmUUmGUmAGU AGmCCGCmGCAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC	chr6:29942990- 29943014

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028881	539	GCUCUGGUU GUAGUAGCC GCGCAG	GCUCUGGUU GUAGUAGCC GCGCAGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mC*mU*mCmUG GmUmUGmUAmGUA GCmCGCGmCAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942989- 29943013
G028882	540	UAUUUUUUU CUAUAGUGU GAGACA	UAUUUUUUU CUAUAGUGU GAGACAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mA*mU*mUmU UUmUmUCmUAmUA GUGmUGAGmACAm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29945435- 29945459
G028883	541	ACGCCUACGA CGGCAAGGA UUACA	ACGCCUACGA CGGCAAGGA UUACAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mG*mCmCU AmCmGAmCGmGCA AGmGAUUmACAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943342- 29943366
G028884	542	UUUGUUCUA CCCCAGGCAG UGACA	UUUGUUCUA CCCCAGGCAG UGACAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mU*mU*mGmU UCmUmACmCCmCA GGCmAGUGmACAm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29945218- 29945242
G028885	543	UUUUGUUCU ACCCCAGGCA GUGAC	UUUUGUUCU ACCCCAGGCA GUGACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC	mU*mU*mU*mUmG UUmCmUAmCCmCC AGGmCAGUmGACm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA	chr6:29945217- 29945241

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			CGCAACGCUC UGCCUUCUG GCAUCGUU	mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028886	544	AGCGACCACA GCUCCAGUG AUCAC	AGCGACCACA GCUCCAGUG AUCACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mG*mC*mGmAC CmAmCAmGCmUCC AGmUGAUmCACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944558- 29944582
G028887	545	GAGCUCCGU GUCCUGGGU CUGGUC	GAGCUCCGU GUCCUGGGU CUGGUCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mG*mA*mG*mCmUC CmGmUGmUCmCUG GGmUCUGmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944234- 29944258
G028888	546	UCCACGAGCU CCGUGUCCUG GGUC	UCCACGAGCU CCGUGUCCUG GGUCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mUmC*mC*mAmCG AmGmCUmCCmGUG UCmCUGGmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944239- 29944263
G028889	547	ACGAGCUCCG UGUCCUGGG UCUGG	ACGAGCUCCG UGUCCUGGG UCUGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mG*mAmGC UmCmCGmUGmUCC UGmGGUCmUGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944236- 29944260
G028890	548	CGAGCUCCGU GUCCUGGGU CUGGU	CGAGCUCCGU GUCCUGGGU CUGGUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mG*mA*mGmCU CmCmGUmGUmCCU GGmGUCUmGGUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944235- 29944259

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
G028891	549	GCAGGCCUG GUCUCCACGA GCUCC	GCAGGCCUG GUCUCCACGA GCUCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mC*mA*mGmGC CmUmGGmUCmUCC ACmGAGCmUCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944251-29944275
G028892	550	UCCCCUGCAG GCCUGGUCUC CACG	UCCCCUGCAG GCCUGGUCUC CACGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mCmCU GmCmAGmGCmCUG GUmCUCCmACGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944257-29944281
G028893	551	AUCCGUGUCC CGGCCCGGCC GCGG	AUCCGUGUCC CGGCCCGGCC GCGGGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mA*mU*mC*mCmGU GmUmCCmCGmGCC CGmGCCGmCGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942784-29942808
G028894	552	CCUUCCCGUU CUCCAGGUA UCUGC	CCUUCCCGUU CUCCAGGUA UCUGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mU*mUmCC CmGmUUmCUmCCA GGmUAUCmUGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943495-29943519
G028895	553	CCGUUCUCCA GGUAUCUGC GGAGC	CCGUUCUCCA GGUAUCUGC GGAGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mG*mUmUC UmCmCAmGGmUAU CUmGCGGmAGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943490-29943514
G028896	554	ACCUGCCAUG UGCAGCAUG AGGGU	ACCUGCCAUG UGCAGCAUG AGGGUGUUG	mA*mC*mC*mUmGC CmAmUGmUGmCAG CAmUGAGmGGUmG	chr6:29944345-29944369

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028897	555	CACCUGCCAU GUGCAGCAU GAGGG	CACCUGCCAU GUGCAGCAU GAGGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mC*mCmUG CmCmAUmGUmGCA GCmAUGAmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944344- 29944368
G028898	556	ACCUGGCAGC GGGAUGGGG AGGAC	ACCUGGCAGC GGGAUGGGG AGGACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mC*mC*mUmGG CmAmGCmGGmGAU GGmGGAGmGACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944219- 29944243
G028899	557	CACUGACCUG GCAGCGGGA UGGGG	CACUGACCUG GCAGCGGGA UGGGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mC*mUmGA CmCmUGmGCmAGC GGmGAUGmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944214- 29944238
G028900	558	CCUGGCAGCG GGAUGGGGA GGACC	CCUGGCAGCG GGAUGGGGA GGACCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mU*mGmGC AmGmCGmGGmAUG GGmGAGGmACCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944220- 29944244
G028901	559	CCAUCUCAGG GUGAGGGGC UUGGG	CCAUCUCAGG GUGAGGGGC UUGGGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA	mC*mC*mA*mUmCU CmAmGGmGUmGAG GGmGCUUmGGGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC	chr6:29944366- 29944390

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028902	560	UGGCCGUCA UGGCGCCCCG AACCC	UGGCCGUCA UGGCGCCCCG AACCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mU*mG*mG*mCmCG UmCmAUmGGmCGC CCmCGAAmCCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942554- 29942578
G028903	561	AUGUCCGCCG CGGUCCAAG AGCGC	AUGUCCGCCG CGGUCCAAG AGCGCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mA*mU*mG*mUmCC GmCmCGmCGmGUC CAmAGAGmCGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943379- 29943403
G028904	562	GGUACCCGU GCGCUGCAGC GUCUC	GGUACCCGU GCGCUGCAGC GUCUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mG*mU*mAmCC CmGmUGmCGmCUG CAmGCGUmCUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943518- 29943542
G028905	563	GGGAAGGAG ACGCUGCAGC GCACG	GGGAAGGAG ACGCUGCAGC GCACGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mG*mG*mAmA GGmAmGAmCGmCU GCAmGCGCmACGm GUUGmUmAmGmCU CCCmUmGmAmAmA mCmCGUUmGmCUA mCAAU*AAGmGmC CmGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943518- 29943542
G028906	564	GCGGGCGCCG UGGAUAGAG CAGGA	GCGGGCGCCG UGGAUAGAG CAGGAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC	mG*mC*mG*mGmGC GmCmCGmUGmGAU AGmAGCAmGGAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA	chr6:29942895- 29942919

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			CGCAACGCUC UGCCUUCUG GCAUCGUU	mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028907	565	UCCUGCUCUA UCCACGGCGC CCGC	UCCUGCUCUA UCCACGGCGC CCGCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mU*mC*mC*mUmGC UmCmUAmUCmCAC GGmCGCCmCGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942889- 29942913
G028908	566	CCCCUGGUAC CCGUGCGCUG CAGC	CCCCUGGUAC CCGUGCGCUG CAGCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mC*mC*mCmUG GmUmACmCCmGUG CGmCUGCmAGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943523- 29943547
G028909	567	UGGUACCCG UGCGCUGCA GCGUCU	UGGUACCCG UGCGCUGCA GCGUCUGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mG*mG*mUmAC CmCmGUmGCmGCU GCmAGCGmUCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943519- 29943543
G028910	568	CAUCUCAGG GUGAGGGGC UUGGGC	CAUCUCAGG GUGAGGGGC UUGGGCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mA*mU*mCmUC AmGmGGmUGmAGG GGmCUUGmGGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944365- 29944389
G028911	569	ACGCAGUUC GUGCGGUUC GACAGC	ACGCAGUUC GUGCGGUUC GACAGCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mA*mC*mG*mCmAG UmUmCGmUGmCGG UUmCGACmAGCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942845- 29942869

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
G028912	570	CGCCCAGGUC UGGGUCAGG GCCAG	CGCCCAGGUC UGGGUCAGG GCCAGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mG*mC*mCmCA GmGmUCmUGmGGU CAmGGGCmCAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942595-29942619
G028913	571	CACUCACCCG CCCAGGUCUG GGUC	CACUCACCCG CCCAGGUCUG GGUCGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mC*mA*mC*mUmCA CmCmCGmCCmCAG GUmCUGGmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942609-29942633
G028914	572	CCGCCCAGGU CUGGGUCAG GGCCA	CCGCCCAGGU CUGGGUCAG GGCCAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mG*mCmCC AmGmGUmCUmGGG UCmAGGGmCCAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942596-29942620
G028915	573	CCCGCCCAGG UCUGGGUCA GGGCC	CCCGCCCAGG UCUGGGUCA GGGCCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mC*mGmCC CmAmGGmUCmUGG GUmCAGGmGCCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942597-29942621
G028916	574	CAGCGACGCC GCGAGCCAG AGGAU	CAGCGACGCC GCGAGCCAG AGGAUGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mG*mCmGA CmGmCCmGCmGAG CCmAGAGmGAUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942865-29942889
G028917	575	CGCGAGCCAG AGGAUGGAG CCGCG	CGCGAGCCAG AGGAUGGAG CCGCGGUUG	mC*mG*mC*mGmAG CmCmAGmAGmGAU GGmAGCCmGCGmG	chr6:29942874-29942898

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028918	576	GCUCUAUCCA CGGCGCCCGC GGCU	GCUCUAUCCA CGGCGCCCGC GGCUGUUGU AGCUCCCUGA AACCGUUGC UACAAUAAG GCCGUCGAA AGAUGUGCC GCAACGCUCU GCCUUCUGGC AUCGUU	mG*mC*mU*mCmUA UmCmCAmCGmGCG CCmCGCGmGCUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29942891- 29942915
G028919	577	CACAUCAGA GCCCUGGGCA CUGUC	CACAUCAGA GCCCUGGGCA CUGUCGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mC*mAmUC AmGmAGmCCmCUG GGmCACUmGUCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29945232- 29945256
G028920	578	CAGACCCAGG ACACGGAGC UCGUG	CAGACCCAGG ACACGGAGC UCGUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mG*mAmCC CmAmGGmACmACG GAmGCUCmGUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944243- 29944267
G028921	579	CCAGGACACG GAGCUCGUG GAGAC	CCAGGACACG GAGCUCGUG GAGACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mA*mGmGA CmAmCGmGAmGCU CGmUGGAmGACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944248- 29944272
G028922	580	CUCUGGGAA AAGAGGGGA AGGUGA	CUCUGGGAA AAGAGGGGA AGGUGAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG	mC*mU*mC*mUmGG GmAmAAmAGmAGG GGmAAGGmUGAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC	chr6:29944471- 29944495

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028923	581	UCUGGGAAA AGAGGGGAA GGUGAG	UCUGGGAAA AGAGGGGAA GGUGAGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mC*mU*mGmGG AmAmAAmGAmGGG GAmAGGUmGAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944470- 29944494
G028924	582	AGACGCUGC AGCGCACGG GUACCA	AGACGCUGC AGCGCACGG GUACCAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mA*mG*mA*mCmGC UmGmCAmGCmGCA CGmGGUAmCCAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943525- 29943549
G028925	583	UCCUUUUCU AUCUGUGGG AAGAAA	UCCUUUUCU AUCUGUGGG AAGAAAGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mC*mC*mUmUU UmCmUAmUCmUGU GGmGAAGmAAAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	Unavailable. Imperfect alignment to human genome.
G028926	584	CACUGCCUGG GGUAGAACA AAAAC	CACUGCCUGG GGUAGAACA AAAACGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mA*mC*mUmGC CmUmGGmGGmUAG AAmCAAAmAACmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29945209- 29945233
G028927	585	ACAACCAGA GCGAGGCCG GUGAGU	ACAACCAGA GCGAGGCCG GUGAGUGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU	mA*mC*mA*mAmCC AmGmAGmCGmAGG CCmGGUGmAGUmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm	chr6:29943008- 29943032

TABLE 7-continued

Additional Exemplary HLA-A Nme guide sequences

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 1512-1590)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 2512-2590)	Genomic Coordinates (hg38)
			CUGCCUUCUG GCAUCGUU	CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	
G028928	586	CUACAACCAG AGCGAGGCC GGUGA	CUACAACCAG AGCGAGGCC GGUGAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mU*mA*mCmAA CmCmAGmAGmCGA GGmCCGGmUGAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943006- 29943030
G028929	587	UACAACCAG AGCGAGGCC GGUGAG	UACAACCAG AGCGAGGCC GGUGAGGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mU*mA*mC*mAmAC CmAmGAmGCmGAG GCmCGGUmGAGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943007- 29943031
G028930	588	CCAGAGCGA GGCCGGUGA GUGACC	CCAGAGCGA GGCCGGUGA GUGACCGUU GUAGCUCCCU GAAACCGUU GCUACAAUA AGGCCGUCG AAAGAUGUG CCGCAACGCU CUGCCUUCUG GCAUCGUU	mC*mC*mA*mGmAG CmGmAGmGCmCGG UGmAGUGmACCmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943012- 29943036
G028933	589	CCAUCCCGCU GCCAGGUCA GUGUG	CCAUCCCGCU GCCAGGUCA GUGUGGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mC*mC*mA*mUmCC CmGmCUmGCmCAG GUmCAGUmGUGmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29944206- 29944230
G028934	590	GUAUCUGCG GAGCCACUCC ACGCA	GUAUCUGCG GAGCCACUCC ACGCAGUUG UAGCUCCCUG AAACCGUUG CUACAAUAA GGCCGUCGA AAGAUGUGC CGCAACGCUC UGCCUUCUG GCAUCGUU	mG*mU*mA*mUmCU GmCmGGmAGmCCA CUmCCACmGCAmG UUGmUmAmGmCUC CCmUmGmAmAmAm CmCGUUmGmCUAm CAAU*AAGmGmCC mGmUmCmGmAmA mAmGmAmUGUGCm CGmCAAmCGCUCU mGmCCmUmUmCmU GGCAUCG*mU*mU	chr6:29943479- 29943503

[0625] In some embodiments, the HLA-A guide RNA comprises a guide sequence selected from any one of SEQ ID NOs: 301-590. In some embodiments, the HLA-A guide RNA comprises a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-590. In some embodiments, the HLA-A guide RNA comprises a guide sequence that is at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 301-590. In some embodiments, the HLA-A guide RNA comprises a guide sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 301-590.

[0626] In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 301-395. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 307, 313-318, 322, 326, 331, 333, 337-341, 343, 345, 347, 357, 359, 362, 366, 387. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 313-318, 326, 337-339, 341, 343, 345, 362. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 313-318. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 313-317. n some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 337-339, 341, 343, and 345. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 337-339. In some embodiments, the HLA-A gRNA comprises a guide sequence selected from any one of SEQ ID NOs: 523, 565, 571, 576, 580, 581.

[0627] In some embodiments, the HLA-A guide RNA comprises a guide sequence that comprises at least 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4-7. As used herein, at least 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate means, for example, at least 10 contiguous nucleotides within the genomic coordinates wherein the genomic coordinates include 10 nucleotides in the 5' direction and 10 nucleotides in the 3' direction from the ranges listed in Tables 4-7. For example, an HLA-A guide RNA may comprise 10 contiguous nucleotides within the genomic coordinates chr6:29942864 to chr6: 29942903 or chr6:29943528 to chr6:29943609, including the boundary nucleotides of these ranges. In some embodiments, the HLA-A guide RNA comprises a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4, 5B and 6, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 5A and 7. In some embodiments, the HLA-A guide RNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from a sequence that is 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4, 5B and 6, or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 5A and 7.

[0628] In some embodiments, the guide RNA comprises a guide sequence that comprises at least 15 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4-7. In some embodiments, the HLA-A guide RNA comprises a guide sequence that comprises at least 20 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4-7.

[0629] In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 301. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 302. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 303. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 304. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 305. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 306. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 307. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 308. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 309. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 310. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 311. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 312. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 313. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 314. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 315. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 316. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 317. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 318. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 319. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 320. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 321. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 322. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 323. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 324. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 325. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 326. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 327. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 328. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 329. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 330. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 331. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 332. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 333. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 334. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 335. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 336. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 337. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 338. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 339. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 340. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 341. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 342. In some embodiments, the HLA-A guide RNA

comprises SEQ ID NO: 343. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 344. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 345. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 346. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 347. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 348. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 349. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 350. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 351. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 352. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 353. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 354. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 355. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 356. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 357. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 358. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 359. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 360. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 361. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 362. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 363. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 364. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 365. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 366. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 367. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 368. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 369. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 370. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 371. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 372. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 373. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 374. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 375. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 376. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 377. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 378. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 379. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 380. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 381. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 382. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 383. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 384. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 385. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 386. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 387. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 388. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 389. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 390. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 391. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 392. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 393. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 394. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 395. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 396. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 397. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 398. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 399. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 400. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 401. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 402. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 403. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 404. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 405. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 406. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 407. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 408. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 409. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 410. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 411. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 412. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 413. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 414. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 415. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 416. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 417. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 418. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 419. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 420. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 421. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 422. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 423. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 424. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 425. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 426. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 427. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 428. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 429. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 430. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 431. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 432. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 433. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 434. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 435. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 436. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 437. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 438. In some embodiments, the HLA-A guide RNA

comprises SEQ ID NO: 439. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 440. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 441. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 442. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 443. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 444. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 445. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 446. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 447. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 448. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 449. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 450. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 451. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 452. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 453. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 454. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 455. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 456. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 457. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 458. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 459. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 460. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 461. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 462. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 463. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 464. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 465. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 466. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 467. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 468. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 469. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 470. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 471. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 472. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 473. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 474. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 475. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 476. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 477. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 478. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 479. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 480. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 481. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 482. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 483. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 484. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 485. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 486. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 487. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 488. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 489. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 490. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 491. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 492. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 493. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 494. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 495. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 496. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 497. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 498. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 499. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 500. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 501. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 502. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 503. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 504. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 505. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 506. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 507. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 508. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 509. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 510. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 511. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 512. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 513. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 514. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 515. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 516. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 517. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 518. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 519. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 520. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 521. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 522. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 523. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 524. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 525. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 526. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 527. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 528. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 529. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 530. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 531. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 532. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 533. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 534. In some embodiments, the HLA-A guide RNA

comprises SEQ ID NO: 535. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 536. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 537. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 538. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 540. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 541. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 542. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 543. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 544. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 545. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 546. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 547. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 548. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 549. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 550. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 551. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 552. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 553. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 554. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 555. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 556. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 557. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 558. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 559. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 560. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 561. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 562. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 563. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 564. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 565. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 566. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 567. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 568. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 569. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 570. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 571. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 572. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 573. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 574. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 575. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 576. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 577. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 580. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 581. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 582. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 583. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 584. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 585. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 586. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 587. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 588. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 589. In some embodiments, the HLA-A guide RNA comprises SEQ ID NO: 590.

[0630] Additional embodiments of HLA-A guide RNAs are provided herein, including e.g., exemplary modifications to the guide RNA.

4. Genetic Modifications to HLA-A

[0631] In some embodiments, the methods and compositions disclosed herein genetically modify at least one nucleotide in the HLA-A gene in a cell. Genetic modifications encompass the population of modifications that results from contact with a gene editing system (e.g., the population of edits that result from Cas9 and an HLA-A guide RNA, or the population of edits that result from BC22 and an HLA-A guide RNA). Methods and compositions for genetic modification of the HLA-A gene are provided in PCT/US2021/064930, the entire contents of which is incorporated herein by reference.

[0632] The following embodiments are directed to the genetic modification in the HLA-A gene:

[0633] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854-chr6:29942913 and chr6:29943518-chr6: 29943619.

[0634] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942864-chr6: 29942903.

[0635] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29943528-chr6:29943609.

[0636] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; and chr6:29942883-29942903.

[0637] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942864-29942884; chr6:29942864-29942884; chr6:29944266-29944290; chr6:29942889-29942913; chr6:29942609-29942633; chr6:29942891-29942915; chr6:29944471-29944495; and chr6:29944470-29944494.

[0638] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942609-29942633; and chr6:29942891-29942915.

[0639] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; and chr6:29943589-29943609.

[0640] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942876-29942897.

[0641] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29943528-chr629943550.

[0642] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coor-

dinates chosen from: chr6:29942864-29942884, chr6:29942868-29942888, chr6:29942876-29942896, and chr6:29942877-29942897.

[0643] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528-29943548, chr6:29943529-29943549, and chr6:29943530-29943550.

[0644] In some embodiments, the genetic modification comprises at least one nucleotide within the genomic coordinates chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046.

[0645] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046.

[0646] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046, chr6:29934330-29934350, chr6:29943115-29943135, chr6:29943135-29943155, chr6:29943140-29943160, chr6:29943590-29943610, chr6:29943824-29943844, chr6:29943858-29943878, chr6:29944478-29944498, and chr6:29944850-29944870.

[0647] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046.

[0648] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; and chr6:29943589-29943609.

[0649] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; and chr6:29942883-29942903.

[0650] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; and chr6:29943589-29943609.

[0651] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29890117-29890137, chr6:29927058-29927078, chr6:29934330-29934350, chr6:29942541-29942561, chr6:29942542-29942562, chr6:29942543-29942563, chr6:29942543-29942563, chr6:29942550-29942570, chr6:29942864-29942884, chr6:29942868-29942888, chr6:29942876-29942896, chr6:29942876-29942896, chr6:29942877-29942897, chr6:29942883-29942903, chr6:29943062-29943082, chr6:29943063-29943083, chr6:29943092-29943112, chr6:29943115-29943135, chr6:29943118-29943138, chr6:29943119-29943139, chr6:29943120-29943140, chr6:29943126-29943146, chr6:29943128-29943148, chr6:29943129-29943149, chr6:29943134-29943154, chr6:29943134-29943154, chr6:29943135-29943155, chr6:29943136-29943156, chr6:29943140-29943160, chr6:29943142-29943162, chr6:29943143-29943163, chr6:29943188-29943208, chr6:29943528-29943548, chr6:29943529-29943549, chr6:29943530-29943550, chr6:29943536-29943556, chr6:29943537-29943557, chr6:29943538-29943558, chr6:29943549-29943569, chr6:29943556-29943576, chr6:29943589-29943609, chr6:29943590-29943610, chr6:29943590-29943610, chr6:29943599-29943619, chr6:29943600-29943620, chr6:29943601-29943621, chr6:29943602-29943622, chr6:29943603-29943623, chr6:29943774-29943794, chr6:29943779-29943799, chr6:29943780-29943800, chr6:29943822-29943842, chr6:29943824-29943844, chr6:29943857-29943877, chr6:29943858-29943878, chr6:29943859-29943879, chr6:29943860-29943880, chr6:29944026-29944046, chr6:29944077-29944097, chr6:29944078-29944098, chr6:29944458-29944478, chr6:29944478-29944498, chr6:29944597-29944617, chr6:29944642-29944662, chr6:29944643-29944663, chr6:29944772-29944792, chr6:29944782-29944802, chr6:29944850-29944870, chr6:29944907-29944927, chr6:29945024-29945044, chr6:29945097-29945117, chr6:29945104-29945124, chr6:29945105-29945125, chr6:29945116-29945136, chr6:29945118-29945138, chr6:29945119-29945139, chr6:29945124-29945144, chr6:29945176-29945196, chr6:29945177-29945197, chr6:29945177-29945197, chr6:29945180-29945200, chr6:29945187-29945207, chr6:29945188-29945208, chr6:29945228-29945248, chr6:29945230-29945250, chr6:29945231-29945251, chr6:29945232-29945252, chr6:29945308-29945328, chr6:29945361-29945381, chr6:29945362-29945382, and chr6:31382543-31382563.

[0652] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942815-29942835, chr6:29942816-29942836, chr6:29942817-29942837, chr6:29942817-29942837, chr6:29942828-29942848, chr6:29942837-29942857, chr6:

29942885-29942905, chr6:29942895-29942915, chr6:29942896-29942916, chr6:29942898-29942918, chr6:29942899-29942919, chr6:29942900-29942920, chr6:29942904-29942924, chr6:29942905-29942925, chr6:29942912-29942932, chr6:29942913-29942933, chr6:29943490-29943510, chr6:29943497-29943517, chr6:29943498-29943518, chr6:29943502-29943522, chr6:29943502-29943522, chr6:29943511-29943531, chr6:29943520-29943540, chr6:29943521-29943541, chr6:29943566-29943586, chr6:29943569-29943589, chr6:29943569-29943589, chr6:29943570-29943590, chr6:29943573-29943593, chr6:29943578-29943598, chr6:29943585-29943605, chr6:29943589-29943609, chr6:29943568-29943588, and chr6:29942815-29942835.

[0653] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942884-29942904, chr6:29943519-29943539, chr6:29942863-29942883.

[0654] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29943517-29943537, and chr6:29943523-29943543.

[0655] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942845-29942869, chr6:29942852-29942876, chr6:29942865-29942889, chr6:29942891-29942915, chr6:29942895-29942919, chr6:29942903-29942927, chr6:29942904-29942928, chr6:29943518-29943542, chr6:29943525-29943549, chr6:29943535-29943559, chr6:29943538-29943562, chr6:29943539-29943563, chr6:29943547-29943571, chr6:29943547-29943571, chr6:29943548-29943572, chr6:29943555-29943579, chr6:29943556-29943580, chr6:29943557-29943581, chr6:29943558-29943582, chr6:29943559-29943583, chr6:29943563-29943587, chr6:29943564-29943588, chr6:29943565-29943589, chr6:29943568-29943592, chr6:29943571-29943595, chr6:29943572-29943596, chr6:29943595-29943619, chr6:29943596-29943620, chr6:29943600-29943624.

[0656] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942885-29942905, chr6:29942895-29942915, chr6:29942896-29942916, chr6:29942898-29942918, chr6:29942899-29942919, chr6:29942900-29942920, chr6:29942904-29942924, chr6:29943511-29943531, chr6:29943520-29943540, chr6:29943521-29943541, chr6:29943529-29943549, chr6:29943566-29943586, chr6:29943568-29943588, chr6:29943569-29943589, chr6:29943569-29943589, chr6:29943570-29943590, chr6:29943573-29943593, chr6:29943578-29943598, chr6:29943585-29943605, and chr6:29943589-29943609.

[0657] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942469-29942489, chr6:29943058-29943078, chr6:29943063-29943083, chr6:29943080-29943100, chr6:29943187-29943207, chr6:29943192-29943212, chr6:29943197-29943217, chr6:29943812-29943832, chr6:29944349-29944369, chr6:29944996-29945016, chr6:29945018-29945038, chr6:29945341-29945361, chr6:29945526-29945546.

[0658] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates: chr6:29942876-29942897.

[0659] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29942864-29942884, chr6:29942868-29942888, chr6:29942876-29942896, and chr6:29942877-29942897.

[0660] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates: chr6:29943528-chr629943550.

[0661] In some embodiments, the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from; chr6:29943528-29943548, chr6:29943529-29943549, and chr6:29943530-29943550.

[0662] In some embodiments, the modification to HLA-A comprises any one or more of an insertion, deletion, substitution, or deamination of at least one nucleotide in a target sequence. In some embodiments, the modification to HLA-A comprises an insertion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-A comprises a deletion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In other embodiments, the modification to HLA-A comprises an insertion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In other embodiments, the modification to HLA-A comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-A comprises an indel, which is generally defined in the art as an insertion or deletion of less than 1000 base pairs (bp). In some embodiments, the modification to HLA-A comprises an indel which results in a frameshift mutation in a target sequence. In some embodiments, the modification to HLA-A comprises a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to HLA-A comprises one or more of an insertion, deletion, or substitution of nucleotides resulting from the incorporation of a template nucleic acid. In some embodiments, the modification to HLA-A comprises an insertion of a donor nucleic acid in a target sequence. In some embodiments, the modification to HLA-A is not transient.

5. Efficacy of HLA-A and HLA-B Guide RNAs

[0663] The efficacy of an HLA-B guide RNA may be determined by techniques available in the art that assess the editing efficiency of a guide RNA, and the surface expression of HLA-A or HLA-B protein. In some embodiments, the reduction or elimination of surface expression of HLA-A or HLA-B protein may be determined by comparison to an unmodified cell (or "relative to an unmodified cell"). An engineered cell or cell population may also be compared to a population of unmodified cells.

[0664] An "unmodified cell" (or "unmodified cells") refers to a control cell (or cells) of the same type of cell in an experiment or test, wherein the "unmodified" control cell has not been contacted with an HLA-A or HLA-B guide. Therefore, an unmodified cell (or cells) may be a cell that

has not been contacted with a guide RNA, or a cell that has been contacted with a guide RNA that does not target HLA-A or HLA-B.

[0665] In some embodiments, the efficacy of an HLA-A or HLA-B guide RNA is determined by measuring levels of surface expression of HLA-A or HLA-B protein. In some embodiments, HLA-A or HLA-B protein levels are measured by flow cytometry (e.g., with an antibody against HLA-B7/HLA-B8). Surface expression of HLA-A or HLA-B protein may be measured by flow cytometry as commonly known in the art. One skilled in the art will be familiar with techniques for measuring surface expression of protein such as HLA-A or HLA-B protein, by flow cytometry. An exemplary measurement of levels of surface expression of HLA-A or HLA-B protein by flow cytometry is discussed in Examples 2-3 and 5-8. In some embodiments, the population of cells is enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is not enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 65% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 70% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 80% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 90% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 95% MHC I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 100% HLA-A or HLA-B negative as measured by flow cytometry relative to a population of unmodified cells.

[0666] In some embodiments, an effective HLA-A or HLA-B guide RNA may be determined by measuring the response of immune cells in vitro or in vivo (e.g., CD8+ T cells) to the genetically modified target cell. For example, a reduced response from CD8+ T cells is indicative of an effective HLA-A or HLA-B guide RNA. A CD8+ T cell response may be evaluated by an assay that measures CD8+ T cell activation responses, e.g., CD8+ T cell proliferation, expression of activation markers, or cytokine production (IL-2, IFN-γ, TNF-α) (e.g., flow cytometry, ELISA). The CD8+ T cell response may be assessed in vitro or in vivo. In some embodiments, the CD8+ T cell response may be evaluated by co-culturing the genetically modified cell with CD8+ T cells in vitro. In some embodiments, CD8+ T cell activity may be evaluated in an in vivo model, e.g., a rodent model. In an in vivo model, e.g., genetically modified cells may be administered with CD8+ T cell; survival of the genetically modified cells is indicative of the ability to avoid CD8+ T cell lysis. In some embodiments, the methods produce a composition comprising a cell that survives in vivo in the presence of CD8+ T cells for greater than 1, 2, 3, 4, 5, or 6 weeks or more. In some embodiments, the methods produce a composition comprising a cell that survives in vivo in the presence of CD8+ T cells for at least one week to six weeks. In some embodiments, the methods produce a composition comprising a cell that survives in vivo in the presence of CD8+ T cells for at least two to four weeks. In some embodiments, the methods produce a composition comprising a cell that survives in vivo in the presence of CD8+ T cells for at least four to six weeks. In some embodiments, the methods produce a composition comprising a cell that survives in vivo in the presence of CD8+ T cells for more than six weeks.

[0667] The efficacy of an HLA-A or HLA-B guide RNA may also be assessed by the survival of the cell post-editing. In some embodiments, the cell survives post editing for at least one week to six weeks. In some embodiments, the cell survives post editing for at least two weeks. In some embodiments, the cell survives post editing for at least three weeks. In some embodiments, the cell survives post editing for at least four weeks. In some embodiments, the cell survives post editing for at least five weeks. In some embodiments, the cell survives post editing for at least six weeks. In some embodiments, the cell survives post editing for at least one week to twelve weeks. The viability of a genetically modified cell may be measured using standard techniques, including e.g., by measures of cell death, by flow cytometry live/dead staining, or cell proliferation.

[0668] In some embodiments, the engineered cell is assessed by the persistence of the engineered human cell which has reduced or eliminated surface expression of HLA-B protein and is homozygous for HLA-A and homozygous for HLA-C. In some embodiments, the engineered cell is assessed by the persistence of the engineered human cell which has reduced or eliminated HLA-A and HLA-B expression and is homozygous for HLA-C. As used herein, "persistence" refers to the ability of the engineered cell to exist in an in vitro or in vivo environment with reactive or responding T cells or NK cells present, e.g., the ability to exist in vivo after transfer into a recipient. In some embodiments, the engineered human T cells are protective against NK-mediated rejection. In some embodiments, the ratio of viable engineered cells in vivo in the presence of NK cells relative to viable engineered cells in vivo in the absence of NK cells is at least 0.3:1 or greater, at least 20 days, at least 30 days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, or at least 90 days after transfer into a recipient, as demonstrated herein. In some embodiments, at least 90 days after transfer into a recipient, the ratio of viable engineered cells in vivo in the presence of NK cells relative to viable engineered cells in vivo in the absence of NK cells is at least 0.4:1 or greater, 0.5:1 or greater, 0.6:1 or greater, 0.7:1 or greater, 0.8:1 or greater, or 0.9:1 or greater, as demonstrated herein. In some embodiments, the engineered human T cells are protective against CD8+ T cell-mediated rejection.

[0669] In some embodiments, the engineered cells may be assessed using a mixed lymphocyte reaction (MLR). (See e.g., DeWolf et al., Transplantation 100:1639-1649 (2017). In some embodiments, engineered human cells are mixed with labeled unedited (non-engineered) responding T cells, and the MLR assay measures proliferation of responding T cells activated by allorecognition (i.e., through mismatched HLA molecules on the surface of the engineered human cell).

D. Methods and Compositions for Reducing or Eliminating MHC Class II, Additional Modifications, and Edited Cells

[0670] In some embodiments, multiplex gene editing may be performed in a cell. In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-B protein comprising genetically modifying the HLA-B gene comprising contacting the cell with a composition comprising a HLA-B guide RNA disclosed herein; and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent, the method further comprising contacting with one or more compositions selected from: (a) a guide RNA that directs an RNA-guided DNA binding agent to the CIITA gene; (b) a guide RNA that directs an RNA-guided DNA binding agent to a locus in the genome of the cell other than HLA-B or CIITA; and (c) a donor nucleic acid for insertion in the genome of the cell.

[0671] In some embodiments, multiplex gene editing may be performed in a cell. In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-A and HLA-B protein comprising genetically modifying the HLA-A and HLA-B genes, comprising contacting the cell with a first composition comprising a HLA-A guide RNA disclosed herein; and optionally a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and a second composition comprising a HLA-B guide RNA disclosed herein; and optionally a second RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; the method further comprising contacting with one or more compositions selected from: (a) a guide RNA that directs an RNA-guided DNA binding agent to the CIITA gene; (b) a guide RNA that directs an RNA-guided DNA binding agent to a locus in the genome of the cell other than HLA-A and HLA-B or CIITA; and (c) a donor nucleic acid for insertion in the genome of the cell.

[0672] In some embodiments, multiplex gene editing may be performed in a cell. In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-A and HLA-B protein and reducing or eliminating expression of CIITA protein, comprising genetically modifying the HLA-A, HLA-B, and CIITA genes, comprising contacting the cell with a first composition comprising a HLA-A guide RNA disclosed herein; and optionally a first RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and a second composition comprising a HLA-B guide RNA disclosed herein; and optionally a second RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; and a third composition comprising a CIITA guide RNA disclosed herein; and optionally a third RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent; the method further optionally comprising contacting with one or more compositions selected from: (a) a guide RNA that directs an RNA-guided DNA binding agent to a locus in the genome of the cell other than HLA-A, HLA-B, and CIITA, such as TRAC, TRBC1, and/or TRBC2; and (b) a donor nucleic acid for insertion in the genome of the cell.

[0673] In some embodiments, one or more compositions for multiplex gene editing in a cell are provided. In some embodiments, the one or more compositions comprise a HLA-A guide RNA disclosed herein, a HLA-B guide RNA disclosed herein, and a CIITA guide RNA disclosed herein; and optionally (a) a guide RNA that directs an RNA-guided DNA binding agent to a locus in the genome of the cell other than HLA-A, HLA-B, and CIITA, such as TRAC, TRBC1, and/or TRBC2; and (b) a donor nucleic acid for insertion in the genome of the cell.

[0674] In some embodiments, in any of the methods and compositions disclosed herein, the HLA-A guide RNA is an HLA-A guide RNA that comprises a guide sequence disclosed herein, such as a guide sequence selected from SEQ ID NOs: 301-590. In some embodiments, the HLA-A guide RNA comprises a sequence selected from SEQ ID NOs: 571, 576, 1571, 1576, 2571, 2576, 3111, and 3112. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 571. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 576. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 1571. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 1576. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 2571. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 2576. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 3111. In some embodiments, the HLA-A guide RNA comprises the sequence of SEQ ID NO: 3112. In some embodiments, in any of the methods and compositions disclosed herein, the HLA-B guide RNA is an HLA-B guide RNA that comprises a guide sequence disclosed herein, such as a guide sequence selected from SEQ ID NOs: 1-91 and 101-185. In some embodiments, the HLA-B guide RNA comprises a sequence selected from SEQ ID NOs: 13, 74, 163-166, 169, 177, 1013, 1074, 1163-1166, 1169, 1177, 2013, 2074, 2163-2166, 2169, 2177, and 2186-2191. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 13. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 74. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 163. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 164. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 165. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 166. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 169. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 177. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1013. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1074. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1163. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1164. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1165. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1166. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1169. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 1177. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2013. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2074. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO:

2163. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2164. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2165. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2166. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2169. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2177. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2186. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2187. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2188. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2189. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2190. In some embodiments, the HLA-B guide RNA comprises the sequence of SEQ ID NO: 2191. In some embodiments, in any of the methods and compositions disclosed herein, the CIITA guide RNA is a CIITA guide RNA that comprises a guide sequence disclosed herein, such as SEQ ID NO: 608 or 609. In some embodiments, the CIITA guide RNA comprises a sequence selected from SEQ ID NOs: 608, 609, 1608, 1609, 2608, 2609, 3116, and 3117. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 608. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 609. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 1608. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 1609. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 2608. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 2609. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 3116. In some embodiments, the CIITA guide RNA comprises the sequence of SEQ ID NO: 3117. In some embodiments, in any of the methods and compositions disclosed herein, the guide RNA that directs an RNA-guided DNA binding agent to a locus in the genome of the cell other than HLA-A, HLA-B, and CIITA comprises a TRAC guide RNA and/or a TRBC guide RNA. In some embodiments, the TRAC guide RNA comprises a sequence selected from SEQ ID NOs: 605, 606, 613, 1605, 1606, 1613, 2605, 2606, 2613, 3113, and 3114. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 605. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 606. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 613. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 1605. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 1606. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 1613. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 2605. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 2606. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 2613. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 3113. In some embodiments, the TRAC guide RNA comprises the sequence of SEQ ID NO: 3114. In some embodiments, the TRBC guide RNA comprises a sequence selected from SEQ ID NOs: 607, 1607, 2607, and 3115. In some embodiments, the TRBC guide RNA comprises the sequence of SEQ ID NO: 607. In some embodiments, the TRBC guide RNA comprises the sequence of SEQ ID NO: 1607. In some embodiments, the TRBC guide RNA comprises the sequence of SEQ ID NO: 2607. In some embodiments, the TRBC guide RNA comprises the sequence of SEQ ID NO: 3115.

[0675] In some embodiments, edited cells obtained by the multiplex gene editing methods or compositions are provided. In some embodiments, the edited cells comprise a genetic modification in the HLA-A gene, a genetic modification in the HLA-B gene, and a genetic modification in the CIITA gene; and optionally a genetic modification in a gene other than HLA-A, HLA-B, and CIITA, such as TRAC, TRBC1, and/or TRBC2. In some embodiments, the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942891-29942915; or chr6:29942609-29942633. In some embodiments, the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chr6:29942891-29942915. In some embodiments, the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chr6:29942609-29942633. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355348-31355368; or chr6:31355347-31355367; or (b) chr6:31355221-31355245; chr6:31355222-31355246; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; or chr6:31355441-31355465. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355348-31355368. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355347-31355367. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355221-31355245. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355222-31355246. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355205-31355229. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355446-31355470. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31356425-31356449. In some embodiments, the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chr6:31355441-31355465. In some embodiments, the genetic modification in the CIITA gene comprises at least one nucleotide within the genomic coordinates chosen from: chr16:10907504-10907528 or chr16:10906643-10906667. In some embodiments, the genetic modification in the CIITA gene comprises at least one nucleotide within the genomic coordinates chr16:10907504-10907528. In some embodiments, the genetic modification in the CIITA gene comprises at least one nucleotide within the genomic coordinates chr16:10906643-10906667. In some embodiments, the genetic modification in the TRAC gene comprises at least one nucleotide within the genomic coordinates chosen from: chr14:22550574-22550598 or

chr14:22550544-22550568. In some embodiments, the genetic modification in the TRAC gene comprises at least one nucleotide within the genomic coordinates chr14:22550574-22550598. In some embodiments, the genetic modification in the TRAC gene comprises at least one nucleotide within the genomic coordinates chr14:22550544-22550568. In some embodiments, the genetic modification in the TRBC gene comprises at least one nucleotide within the genomic coordinates chr7:142792690-142792714.

1. MHC Class H Knock Out

[0676] In some embodiments, methods for reducing or eliminating surface expression of HLA-B by genetically modifying HLA-B as disclosed herein are provided, wherein the methods and compositions further provide for reducing or eliminating surface expression of MHC class II protein relative to an unmodified cell. In some embodiments, MHC class II protein expression is reduced or eliminated by contacting the cell with a CIITA guide RNA. In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0677] In some embodiments, methods for reducing or eliminating surface expression of HLA-A and HLA-B protein by genetically modifying HLA-A and HLA-B genes as disclosed herein are provided, wherein the methods and compositions further provide for reducing or eliminating surface expression of MHC class II protein relative to an unmodified cell. In some embodiments, MHC class II protein expression is reduced or eliminated by contacting the cell with a CIITA guide RNA. In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-C.

[0678] In some embodiments, methods are provided for reducing surface expression of MHC class II protein on the engineered human cell. MHC class II expression is impacted by a variety of proteins. (See e.g., Crivello et al., Journal Immunology 202:1895-1903 (2019).) For example, the CIITA protein functions as a transcriptional activator (activating the MHC class II promoter) and is essential for MHC class II protein expression. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying a gene selected from: CIITA, HLA-DR, HLA-DQ, HLA-DP, RFX5, RFXB/ANK, RFXAP, CREB, NF-YA, NF-YB, and NF-YC. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the CIITA gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the HLA-DR gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the HLA-DQ gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the HLA-DP gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the RFX5 gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the RFXB/ANK gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the RFXAP gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the CREB gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the NK-YA gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the NK-YB gene. In some embodiments, MHC class II protein expression is reduced or eliminated by genetically modifying the NK-YC gene.

[0679] In some embodiments, methods are provided for making an engineered human cell which has reduced or eliminated expression of HLA-B protein relative to an unmodified cell, wherein the cell is homozygous for HLA-A and homozygous for HLA-C, further comprising reducing or eliminating the surface expression of MHC class II protein in the cell relative to an unmodified cell. In some embodiments, the methods comprise contacting the cell with a CIITA guide RNA.

[0680] In some embodiments, methods are provided for making an engineered human cell which has reduced or eliminated expression of HLA-A and HLA-B protein relative to an unmodified cell, wherein the cell homozygous for HLA-C, further comprising reducing or eliminating the surface expression of MHC class II protein in the cell relative to an unmodified cell. In some embodiments, the methods comprise contacting the cell with a CIITA guide RNA.

[0681] In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA protein in a cell. The levels of CIITA protein may be detected by, e.g., cell lysate and western blot with an anti-CIITA antibody. In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA protein in the cell nucleus. In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA mRNA in a cell. The levels of CIITA mRNA may be detected by e.g., RT-PCR. In some embodiments, a decrease in the levels CIITA protein or CIITA mRNA in the target cell as compared to an unmodified cell is indicative of an effective CIITA guide RNA.

[0682] In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring the reduction or elimination of MHC class II protein expression by the target cells. The CIITA protein functions as a transactivator, activating the MHC class II promoter, and is essential for the expression of MHC class II protein. In some embodiments, MHC class II protein expression may be detected on the surface of the target cells. In some embodiments, MHC class II protein expression is measured by flow cytometry. In some embodiments, an antibody against MHC class II protein (e.g., anti-HLA-DR, -DQ, -DP) may be used to detect MHC class II protein expression e.g., by flow cytometry. In some embodiments, a reduction or elimination in MHC class II protein on the surface of a cell (or population of cells) as compared to an unmodified cell (or population of unmodified cells) is indicative of an effective CIITA guide RNA. In some embodiments, a cell (or population of cells) that has been contacted with a particular CIITA guide RNA and RNA-guided DNA binding agent that is negative for MHC class II protein by flow cytometry is indicative of an effective CIITA guide RNA.

[0683] In some embodiments, the MHC class II protein expression is reduced or eliminated in a population of cells using the methods and compositions disclosed herein. In some embodiments, the population of cells is enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified

cells. In some embodiments, the population of cells is not enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells.

[0684] In some embodiments, the population of cells is at least 65% MHC II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 70% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 80% MHC II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 90% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 91% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 92% MHC II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 93% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells.

[0685] In some embodiments, the population of cells elicits a reduced response from immune cells in vitro or in vivo (e.g., CD4+ T cells). A CD4+ T cell response may be evaluated by an assay that measures the activation response of CD4+ T cells e.g., CD4+ T cell proliferation, expression of activation markers, or cytokine production (IL-2, IL-12, IFN-γ) (e.g., flow cytometry, ELISA). The response of CD4+ T cells may be evaluated in in vitro cell culture assays in which the genetically modified cell is co-cultured with cells comprising CD4+ T cells. For example, the engineered cell may be co-cultured e.g., with PBMCs, purified CD3+ T cells comprising CD4+ T cells, purified CD4+ T cells, or a CD4+ T cell line. The CD4+ T cell response elicited from the engineered cell may be compared to the response elicited from an unmodified cell.

[0686] In some embodiments, an engineered human cell is provided wherein the cell has reduced or eliminated surface expression of HLA-B and MHC class II protein wherein the cell comprises a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and HLA-C, and wherein the cell comprises a modification in the CIITA gene. In some embodiments, the engineered cell elicits a reduced response from CD4+ T cells and elicits a reduced response from CD8+ T cells.

[0687] In some embodiments, an engineered human cell is provided wherein the cell has reduced or eliminated surface expression of HLA-A, HLA-B, and MHC class II protein, wherein the cell comprises a genetic modification in the HLA-A and HLA-B genes, wherein the cell is homozygous for HLA-C, and wherein the cell comprises a modification in the CIITA gene. In some embodiments, the engineered cell elicits a reduced response from CD4+ T cells and elicits a reduced response from CD8+ T cells.

2. Exogenous Nucleic Acids Knock in

[0688] In some embodiments, the present disclosure provides methods and compositions for reducing or eliminating surface expression of HLA-B protein by genetically modifying HLA-B as disclosed herein, wherein the methods and compositions further provide for expression of a protein encoded by an exogenous nucleic acid (e.g., an antibody, chimeric antigen receptor (CAR), T cell receptor (TCR), cytokine or cytokine receptor, chemokine or chemokine receptor, enzyme, fusion protein, or other type of cell-surface bound or soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a protein that is expressed on the cell surface. For example, in some embodiments, the exogenous nucleic acid encodes a targeting receptor expressed on the cell surface (described further herein). In some embodiments, the genetically modified cell may function as a "cell factory" for the expression of a secreted polypeptide encoded by an exogenous nucleic acid, including e.g., as a source for continuous production of a polypeptide in vivo (as described further herein). In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0689] In some embodiments, the present disclosure provides methods and compositions for reducing or eliminating surface expression of HLA-A and HLA-B protein by genetically modifying HLA-A and HLA-B as disclosed herein, wherein the methods and compositions further provide for expression of a protein encoded by an exogenous nucleic acid (e.g., an antibody, chimeric antigen receptor (CAR), T cell receptor (TCR), cytokine or cytokine receptor, chemokine or chemokine receptor, enzyme, fusion protein, or other type of cell-surface bound or soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a protein that is expressed on the cell surface. For example, in some embodiments, the exogenous nucleic acid encodes a targeting receptor expressed on the cell surface (described further herein). In some embodiments, the targeting receptor is a CAR. In some embodiments, the targeting receptor is a universal CAR. In some embodiments, the targeting receptor is an anti-CD30 CAR. In some embodiments, the anti-CD30 CAR is any one of the anti-CD30 CARs disclosed in International Application No. PCT/US2023/018946, the content of which is incorporated herein by reference. In some embodiments, the genetically modified cell may function as a "cell factory" for the expression of a secreted polypeptide encoded by an exogenous nucleic acid, including e.g., as a source for continuous production of a polypeptide in vivo (as described further herein). In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-C.

[0690] In some embodiments, the methods comprise reducing surface expression of HLA-B protein comprising genetically modifying the HLA-B gene comprising contacting the cell with a composition comprising an HLA-B guide RNA disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid.

[0691] In some embodiments, the methods comprise reducing surface expression of HLA-A and HLA-B protein comprising genetically modifying the HLA-A and HLA-B genes comprising contacting the cell with a first composition comprising an HLA-A guide RNA disclosed herein and a second composition comprising an HLA-B guide RNA disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid.

[0692] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-B pro-

tein, comprising genetically modifying the cell with one or more compositions comprising an HLA-B guide RNA as disclosed herein, an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor), and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0693] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-A and HLA-B protein, comprising genetically modifying the cell with one or more compositions comprising a first composition comprising an HLA-A guide RNA as disclosed herein, a second composition comprising an HLA-B guide RNA as disclosed herein, an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor), and one or more RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0694] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-B protein and MHC class II protein, comprising genetically modifying the cell with one or more compositions comprising a HLA-B guide RNA as disclosed herein, a CIITA guide RNA, an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor), and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0695] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-A and HLA-B protein and MHC class II protein, comprising genetically modifying the cell with one or more compositions comprising a first composition comprising an HLA-A guide RNA as disclosed herein, a second composition comprising an HLA-B guide RNA as disclosed herein, a CIITA guide RNA, an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor), and one or more RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0696] In some embodiments, the exogenous nucleic acid encodes a polypeptide that is expressed on the surface of the cell. In some embodiments, the exogenous nucleic acid encodes a soluble polypeptide. As used herein, "soluble" polypeptide refers to a polypeptide that is secreted by the cell. In some embodiments, the soluble polypeptide is a therapeutic polypeptide. In some embodiments, the soluble polypeptide is an antibody. In some embodiments, the soluble polypeptide is an enzyme. In some embodiments, the soluble polypeptide is a cytokine. In some embodiments, the soluble polypeptide is a chemokine. In some embodiments, the soluble polypeptide is a fusion protein.

[0697] In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an antibody fragment (e.g., Fab, Fab2). In some embodiments, the exogenous nucleic acid encodes is a full-length antibody. In some embodiments, the exogenous nucleic acid encodes is a single-chain antibody (e.g., scFv). In some embodiments, the antibody is an IgG, IgM, IgD, IgA, or IgE. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG4 antibody. In some embodiments, the heavy chain constant region contains mutations known to reduce effector functions. In some embodiments, the heavy chain constant region contains mutations known to enhance effector functions. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the antibody is a single-domain antibody (e.g., VH domain-only antibody).

[0698] In some embodiments, the exogenous nucleic acid encodes a neutralizing antibody. A neutralizing antibody neutralizes the activity of its target antigen. In some embodiments, the antibody is a neutralizing antibody against a virus antigen. In some embodiments, the antibody neutralizes a target viral antigen, blocking the ability of the virus to infect a cell. In some embodiments, a cell-based neutralization assay may be used to measure the neutralizing activity of an antibody. The particular cells and readout will depend on the target antigen of the neutralizing antibody. The half maximal effective concentration (EC_{50}) of the antibody can be measured in a cell-based neutralization assay, wherein a lower EC_{50} is indicative of more potent neutralizing antibody.

[0699] In some embodiments, the exogenous nucleic acid encodes an antibody that binds to an antigen associated with a disease or disorder (see e.g., diseases and disorders described in Section IV).

[0700] In some embodiments, the exogenous nucleic acid encodes a polypeptide that is expressed on the surface of the cell (i.e., a cell-surface bound protein). In some embodiments, the exogenous nucleic acid encodes a targeting receptor. A "targeting receptor" is a receptor present on the surface of a cell, e.g., a T cell, to permit binding of the cell to a target site, e.g., a specific cell or tissue in an organism. In some embodiments, the targeting receptor is a CAR. In some embodiments, the targeting receptor is a universal CAR (UniCAR). In some embodiments, the targeting receptor is a proliferation-inducing ligand (APRIL). In some embodiments, the targeting receptor is a TCR. In some embodiments, the targeting receptor is a TRuC. In some embodiments, the targeting receptor is a B cell receptor (BCR) (e.g., expressed on a B cell). In some embodiments, the targeting receptor is chemokine receptor. In some embodiments, the targeting receptor is a cytokine receptor.

[0701] In some embodiments, targeting receptors include a chimeric antigen receptor (CAR), a T-cell receptor (TCR), and a receptor for a cell surface molecule operably linked through at least a transmembrane domain in an internal signaling domain capable of activating a T cell upon binding of the extracellular receptor portion. In some embodiments, a CAR refers to an extracellular antigen recognition domain, e.g., an scFv, VHH, nanobody; operably linked to an intracellular signaling domain, which activates the T cell when an antigen is bound. CARs are composed of four regions: an antigen recognition domain, an extracellular hinge region, a transmembrane domain, and an intracellular T-cell signaling domain. Such receptors are well known in the art (see, e.g., WO2020092057, WO2019191114, WO2019147805, WO2018208837). A universal CAR (UniCAR) for recognizing various antigens (see, e.g., EP 2 990 416 A1) and a reversed universal CAR (RevCAR) that promotes binding of an immune cell to a target cell through an adaptor molecule (see, e.g., WO2019238722) are also contemplated. CARs can be targeted to any antigen to which an antibody can be developed and are typically directed to molecules displayed on the surface of a cell or tissue to be targeted. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR (e.g., a TRuC). (See Baeuerle et al. Nature Communications 2087 (2019).)

[0702] In some embodiments, the exogenous nucleic acid encodes a TCR. In some embodiments, the exogenous

nucleic acid encodes a genetically modified TCR. In some embodiments, the exogenous nucleic acid encodes is a genetically modified TCR with specificity for a polypeptide expressed by cancer cells. In some embodiments, the exogenous nucleic acid encodes a targeting receptor specific for Wilms' tumor gene (WT1) antigen. In some embodiments, the exogenous nucleic acid encodes the WT1-specific TCR (see e.g., WO2020/081613A1).

[0703] In some embodiments, an exogenous nucleic acid is inserted into the genome of the target cell. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by homologous recombination (HR). In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by blunt end insertion. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by non-homologous end joining. In some embodiments, the exogenous nucleic acid is integrated into a safe harbor locus in the genome of the cell. In some embodiments, the exogenous nucleic acid is integrated into one of the TRAC locus, B2M locus, AAVS1 locus, or CIITA locus. In some embodiments, the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

[0704] In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-B protein and comprising an exogenous nucleic acid. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-B protein and that secretes or expresses a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-B protein, or reduced or eliminated HLA-B levels in the cell nucleus, and having reduced or eliminated surface expression of MHC class II protein expression, and secreting or expressing a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the engineered cell elicits a reduced response from CD4+ T cells, or CD8+ T cells.

[0705] In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-A and HLA-B protein and comprising an exogenous nucleic acid. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-A and HLA-B protein and that secretes or expresses a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated surface expression of HLA-A and HLA-B protein, or reduced or eliminated HLA-A and HLA-B levels in the cell nucleus, and having reduced surface expression of MHC class II protein, and secreting or expressing a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the engineered cell elicits a reduced response from CD4+ T cells, or CD8+ T cells.

[0706] In some embodiments, an allogeneic cell is provided wherein the cell has reduced or eliminated surface expression of MHC class II and HLA-B protein, wherein the cell comprises a modification in the HLA-B gene as disclosed herein, wherein the cell comprises a modification in the CIITA gene, and wherein the cell further comprises an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor).

[0707] In some embodiments, an allogeneic cell is provided wherein the cell has reduced or eliminated surface expression of MHC class II, HLA-A, and HLA-B protein, wherein the cell comprises a modification in the HLA-A and HLA-B gene as disclosed herein, wherein the cell comprises a modification in the CIITA gene, and wherein the cell further comprises an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor).

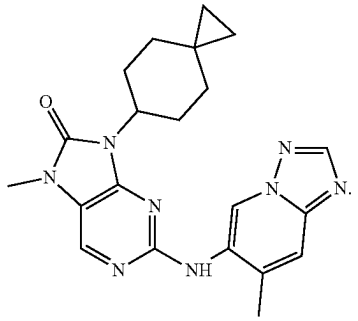
[0708] In some embodiments, the present disclosure provides methods for reducing or eliminating surface expression of HLA-B protein by genetically modifying HLA-B as disclosed herein, wherein the methods further provide for reducing expression of one or more additional target genes (e.g., TRAC, TRBC). In some embodiments, the additional genetic modifications provide further advantages for use of the genetically modified cells for adoptive cell transfer applications. In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C.

[0709] In some embodiments, the present disclosure provides methods for reducing or eliminating surface expression of HLA-A and HLA-B protein by genetically modifying HLA-A and HLA-B as disclosed herein, wherein the methods further provide for reducing expression of one or more additional target genes (e.g., TRAC, TRBC). In some embodiments, the additional genetic modifications provide further advantages for use of the genetically modified cells for adoptive cell transfer applications. In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is homozygous for HLA-C.

[0710] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-B protein, comprising genetically modifying the cell with one or more compositions comprising a HLA-B guide RNA as disclosed herein, a CIITA guide RNA, an exogenous nucleic acid encoding polypeptide (e.g., a targeting receptor), a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the additional target gene is TRAC. In some embodiments, the additional target gene is TRBC.

[0711] In some embodiments, the methods comprise reducing or eliminating surface expression of HLA-A and HLA-B protein, comprising genetically modifying the cell with one or more compositions comprising a HLA-B guide RNA as disclosed herein, a CIITA guide RNA, an exogenous nucleic acid encoding polypeptide (e.g., a targeting receptor), a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the additional target gene is TRAC. In some embodiments, the additional target gene is TRBC.

[0712] In some embodiments, the method disclosed herein further comprises contacting the cell with a DNA-dependent protein kinase inhibitor (DNAPK), optionally wherein the DNAPKi is Compound 1 or "DNAPKI Compound 1": 9-(4,4-difluorocyclohexyl)-7-methyl-2-((7-methyl-[1,2,4]triazolo[1,5-a]pyridin-6-yl)amino)-7,9-dihydro-8H-purin-8-one, also depicted as:



E. Exemplary Cell Types

[0713] In some embodiments, methods and compositions disclosed herein genetically modify a human cell. In some embodiments, the cell is an allogeneic cell. In some embodiments the genetically modified cell is referred to as an engineered cell. An engineered cell refers to a cell (or progeny of a cell) comprising an engineered genetic modification, e.g. that has been contacted with a gene editing system and genetically modified by the gene editing system. The terms "engineered cell" and "genetically modified cell" are used interchangeably throughout. The engineered human cell may be any of the exemplary cell types disclosed herein. Further, because MHC class I molecules are expressed on all nucleated cells, the engineered human cell may be any nucleated cell.

[0714] In some embodiments, when the cell is homozygous for HLA-A, the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02.

[0715] In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*16:01; HLA-C*15:02; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

[0716] In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is HLA-C*03:04. In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is HLA-C*06:02. In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is HLA-C*01:02. In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is HLA-C*08:01. In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is HLA-C*03:02.

[0717] In some embodiments, when the cell is homozygous for HLA-A and homozygous for HLA-C, and the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*16:01; HLA-C*15:02; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

[0718] In some embodiments, when the cell is homozygous for HLA-A and homozygous for HLA-C, and the HLA-A and HLA-C alleles are selected from any one of the following HLA-A and HLA-C alleles: HLA-A*01:01 and HLA-C*07:01; HLA-A*02:01 and HLA-C*07:02; HLA-A*02:01 and HLA-C*05:01; HLA-A*03:01 and HLA-C*07:02; HLA-A*02:01 and HLA-C*04:01; HLA-A*02:01 and HLA-C*03:04; HLA-A*01:01 and HLA-C*06:02; HLA-A*03:01 and HLA-C*04:01; HLA-A*02:01 and HLA-C*07:01; HLA-A*24:02 and HLA-C*04:01; HLA-A*29:02 and HLA-C*16:01; HLA-A*02:01 and HLA-C*06:02; HLA-A*24:02 and HLA-C*07:02; HLA-A*26:01 and HLA-C*12:03; HLA-A*11:01 and HLA-C*04:01; HLA-A*25:01 and HLA-C*12:03; HLA-A*02:01 and HLA-C*02:02; HLA-A*24:02 and HLA-C*03:03; HLA-A*30:01 and HLA-C*06:02; HLA-A*02:01 and HLA-C*01:02; HLA-A*11:01 and HLA-C*07:02; HLA-A*03:01 and HLA-C*07:01; HLA-A*23:01 and HLA-C*04:01; HLA-A*24:02 and HLA-C*07:01; HLA-A*31:01 and HLA-C*03:04; HLA-A*33:01 and HLA-C*08:02; HLA-A*02:01 and HLA-C*03:03; HLA-A*11:01 and HLA-C*01:02; HLA-A*01:01 and HLA-C*04:01; HLA-A*03:01 and HLA-C*06:02.

[0719] In some embodiments, the cell is homozygous for HLA-A and homozygous for HLA-C. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*01:01 and HLA-C*07:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*07:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*05:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*03:01 and HLA-C*07:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*04:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*03:04. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*01:01 and HLA-C*06:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*03:01 and HLA-C*04:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*07:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*24:02 and HLA-C*04:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*29:02 and HLA-C*16:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*06:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*24:02 and HLA-C*07:02. In some embodiments, the HLA-A and

HLA-C alleles are HLA-A*26:01 and HLA-C*12:03. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*11:01 and HLA-C*04:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*25:01 and HLA-C*12:03. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*02:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*24:02 and HLA-C*03:03. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*30:01 and HLA-C*06:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*01:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*11:01 and HLA-C*07:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*03:01 and HLA-C*07:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*23:01 and HLA-C*04:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*24:02 and HLA-C*07:01. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*31:01 and HLA-C*03:04. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*33:01 and HLA-C*08:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*02:01 and HLA-C*03:03. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*11:01 and HLA-C*01:02. In some embodiments, the HLA-A and HLA-C alleles are HLA-A*01:01 and HLA-C*04:01; HLA-A*03:01 and HLA-C*06:02.

[0720] In some embodiments, the cell is an immune cell. As used herein, "immune cell" refers to a cell of the immune system, including e.g., a lymphocyte (e.g., T cell, B cell, natural killer cell ("NK cell", and NKT cell, or iNKT cell)), monocyte, macrophage, mast cell, dendritic cell, or granulocyte (e.g., neutrophil, eosinophil, and basophil). In some embodiments, the cell is a primary immune cell. In some embodiments, the immune system cell may be selected from CD3+, CD4+ and CD8+ T cells, regulatory T cells (Tregs), B cells, NK cells, and dendritic cells (DC). In some embodiments, the immune cell is allogeneic.

[0721] In some embodiments, the cell is a lymphocyte. In some embodiments, the cell is an adaptive immune cell. In some embodiments, the cell is a T cell. In some embodiments, the cell is a B cell. In some embodiments, the cell is a NK cell. In some embodiments, the cell is a macrophage. In some embodiments, the lymphocyte is allogeneic.

[0722] As used herein, a T cell can be defined as a cell that expresses a T cell receptor ("TCR" or "αβ TCR" or "γδ TCR"), however in some embodiments, the TCR of a T cell may be genetically modified to reduce its expression (e.g., by genetic modification to the TRAC or TRBC genes), therefore expression of the protein CD3 may be used as a marker to identify a T cell by standard flow cytometry methods. CD3 is a multi-subunit signaling complex that associates with the TCR. Thus, a T cell may be referred to as CD3+. In some embodiments, a T cell is a cell that expresses a CD3+ marker and either a CD4+ or CD8+ marker. In some embodiments, the T cell is allogeneic.

[0723] In some embodiments, the T cell expresses the glycoprotein CD8 and therefore is CD8+ by standard flow cytometry methods and may be referred to as a "cytotoxic" T cell. In some embodiments, the T cell expresses the glycoprotein CD4 and therefore is CD4+ by standard flow cytometry methods and may be referred to as a "helper" T cell. CD4+ T cells can differentiate into subsets and may be referred to as a Th1 cell, Th2 cell, Th9 cell, Th17 cell, Th22 cell, T regulatory ("Treg") cell, or T follicular helper cells ("Tfh"). Each CD4+ subset releases specific cytokines that can have either proinflammatory or anti-inflammatory functions, survival or protective functions. A T cell may be isolated from a subject by CD4+ or CD8+ selection methods.

[0724] In some embodiments, the T cell is a memory T cell. In the body, a memory T cell has encountered antigen. A memory T cell can be located in the secondary lymphoid organs (central memory T cells) or in recently infected tissue (effector memory T cells). A memory T cell may be a CD8+ T cell. A memory T cell may be a CD4+ T cell.

[0725] As used herein, a "central memory T cell" can be defined as an antigen-experienced T cell, and for example, may expresses CD62L and CD45RO. A central memory T cell may be detected as CD62L+ and CD45RO+ by Central memory T cells also express CCR7, therefore may be detected as CCR7+ by standard flow cytometry methods.

[0726] As used herein, an "early stem-cell memory T cell" (or "Tscm") can be defined as a T cell that expresses CD27 and CD45RA, and therefore is CD27+ and CD45RA+ by standard flow cytometry methods. A Tscm does not express the CD45 isoform CD45RO, therefore a Tscm will further be CD45RO− if stained for this isoform by standard flow cytometry methods. A CD45RO− CD27+ cell is therefore also an early stem-cell memory T cell. Tscm cells further express CD62L and CCR7, therefore may be detected as CD62L+ and CCR7+ by standard flow cytometry methods. Early stem-cell memory T cells have been shown to correlate with increased persistence and therapeutic efficacy of cell therapy products.

[0727] In some embodiments, the cell is a B cell. As used herein, a "B cell" can be defined as a cell that expresses CD19 or CD20, or B cell mature antigen ("BCMA"), and therefore a B cell is CD19+, or CD20+, or BCMA+ by standard flow cytometry methods. A B cell is further negative for CD3 and CD56 by standard flow cytometry methods. The B cell may be a plasma cell. The B cell may be a memory B cell. The B cell may be a naïve B cell. The B cell may be IgM+ or may have a class-switched B cell receptor (e.g., IgG+, or IgA+). In some embodiments, the B cell is allogeneic.

[0728] In some embodiments, the cell is a mononuclear cell, such as from bone marrow or peripheral blood. In some embodiments, the cell is a peripheral blood mononuclear cell ("PBMC"). In some embodiments, the cell is a PBMC, e.g. a lymphocyte or monocyte. In some embodiments, the cell is a peripheral blood lymphocyte ("PBL"). In some embodiments, the mononuclear cell is allogeneic.

[0729] Cells used in ACT or tissue regenerative therapy are included, such as stem cells, progenitor cells, and primary cells. Stem cells, for example, include pluripotent stem cells (PSCs); induced pluripotent stem cells (iPSCs); embryonic stem cells (ESCs); mesenchymal stem cells (MSCs, e.g., isolated from bone marrow (BM), peripheral blood (PB), placenta, umbilical cord (UC) or adipose); hematopoietic stem cells (HSCs; e.g. isolated from BM or UC); neural stem cells (NSCs); tissue specific progenitor stem cells (TSPSCs); and limbal stem cells (LSCs). Progenitor and primary cells include mononuclear cells (MNCs, e.g., isolated from BM or PB); endothelial progenitor cells (EPCs, e.g. isolated from BM, PB, and UC); neural progenitor cells (NPCs); and tissue-specific primary cells or cells derived therefrom (TSCs) including chondrocytes,

myocytes, and keratinocytes. Cells for organ or tissue transplantations such as islet cells, cardiomyocytes, thyroid cells, thymocytes, neuronal cells, skin cells, and retinal cells are also included.

[0730] In some embodiments, the human cell is isolated from a human subject. In some embodiments, the cell is isolated from human donor PBMCs or leukopaks. In some embodiments, the cell is from a subject with a condition, disorder, or disease. In some embodiments, the cell is from a human donor with Epstein Barr Virus ("EBV").

[0731] In some embodiments, the methods are carried out ex vivo. As used herein, "ex vivo" refers to an in vitro method wherein the cell is capable of being transferred into a subject, e.g. as an ACT therapy. In some embodiments, an ex vivo method is an in vitro method involving an ACT therapy cell or cell population.

[0732] In some embodiments, the cell is from a cell line. In some embodiments, the cell line is derived from a human subject. In some embodiments, the cell line is a lymphoblastoid cell line ("LCL"). The cell may be cryopreserved and thawed. The cell may not have been previously cryopreserved.

[0733] In some embodiments, the cell is from a cell bank. In some embodiments, the cell is genetically modified and then transferred into a cell bank. In some embodiments the cell is removed from a subject, genetically modified ex vivo, and transferred into a cell bank. In some embodiments, a genetically modified population of cells is transferred into a cell bank. In some embodiments, a genetically modified population of immune cells is transferred into a cell bank. In some embodiments, a genetically modified population of immune cells comprising a first and second subpopulations, wherein the first and second sub-populations have at least one common genetic modification and at least one different genetic modification are transferred into a cell bank.

F. Exemplary Gene Editing Systems

[0734] Various suitable gene editing systems may be used to make the engineered cells disclosed herein, including but not limited to the CRISPR/Cas system; zinc finger nuclease (ZFN) system; and the transcription activator-like effector nuclease (TALEN) system. Generally, the gene editing systems involve the use of engineered cleavage systems to induce a double strand break (DSB) or a nick (e.g., a single strand break, or SSB) in a target DNA sequence. Cleavage or nicking can occur through the use of specific nucleases such as engineered ZFN, TALENs, or using the CRISPR/Cas system with an engineered guide RNA to guide specific cleavage or nicking of a target DNA sequence. Further, targeted nucleases are being developed based on the Argonaute system (e.g., from *T. thermophilus*, known as 'TtAgo', see Swarts et al (2014) Nature 507(7491): 258-261), which also may have the potential for uses in gene editing and gene therapy.

[0735] In some embodiments, the gene editing system is a TALEN system. Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). Transcription activator-like effectors (TALEs) can be engineered to bind to a desired DNA sequence, to promote DNA cleavage at specific locations (see, e.g., Boch, 2011, Nature Biotech). The restriction enzymes can be introduced into cells, for use in gene editing or for gene editing in situ, a technique known as gene editing with engineered nucleases. Such methods and compositions for use therein are known in the art. See, e.g., WO2019147805, WO2014040370, WO2018073393, the contents of which are hereby incorporated in their entireties.

[0736] In some embodiments, the gene editing system is a zinc-finger system. Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences to enables zinc-finger nucleases to target unique sequences within complex genomes. The non-specific cleavage domain from the type IIs restriction endonuclease FokI is typically used as the cleavage domain in ZFNs. Cleavage is repaired by endogenous DNA repair machinery, allowing ZFN to precisely alter the genomes of higher organisms. Such methods and compositions for use therein are known in the art. See, e.g., WO2011091324, the contents of which are hereby incorporated in their entireties.

[0737] In some embodiments, the gene editing system is a CRISPR/Cas system, including e.g., a CRISPR guide RNA comprising a guide sequence and RNA-guided DNA binding agent, and described further herein.

[0738] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the presence of a PAM and the sequence of the guide RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. The dCas DNA binding agent may be a dead nuclease comprising non-functional nuclease domains (RuvC or HNH domain). In some embodiments the Cas cleavase or Cas nickase encompasses a dCas DNA binding agent modified to permit DNA cleavage, e.g. via fusion with a FokI domain. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases.

[0739] As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity. Class 2 Cas nucleases include Class 2 Cas cleavases/nickases (e.g., H840A or D10A variants of Spy Cas9 and D16A and H588A of Nine Cas9, e.g., Nme2 Cas9), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., Cell, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. See, e.g., Makarova et al., Nat Rev Microbiol, 13(11): 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015).

[0740] In some embodiments the gene editing system comprises a base editor comprising a deaminase and an RNA-guided nickase. In some embodiments the gene editing system comprises a base editor comprising a cytidine deaminase and an RNA-guided nickase. In some embodiments, the gene editing system comprises a DNA polymerase. Further description of the gene editing system methods and compositions for use therein are known in the art. See e.g., WO2019/067910, WO2021/188840A1, WO2019/051097, and PCT/US2021/062922 filed Dec. 10, 2021, and U.S. Provisional Application No. 63/275,425 filed Nov. 3, 2021, the contents of each of which are hereby incorporated in their entireties.

[0741] Exemplary nucleotide and polypeptide sequences for the gene editing system disclosed herein are provided below in Table 9. Methods for identifying alternate nucleotide sequences encoding polypeptide sequences provided herein, including alternate naturally occurring variants, are known in the art. Sequences with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to any of the nucleic acid sequences, or nucleic acid sequences encoding the amino acid sequences provided herein are also contemplated.

G. CRISPR Guide RNA

[0742] Provided herein are guide sequences useful for modifying a target sequence, e.g., using a guide RNA comprising a disclosed guide sequence with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). Guide sequences are shown in Tables 2, 3, 3A, 4, 5A, 5B, 6, 7, and 9A (e.g., SEQ ID NOs: 1-91, 101-185, 301-498, and 500-590), as are the genomic coordinates that these guide RNAs target.

[0743] In some embodiments, a gRNA provided herein comprises a guide region (guide sequence) and a conserved region comprising a repeat/anti-repeat region, a hairpin 1 region, and a hairpin 2 region, wherein one or more of the repeat/anti-repeat region, the hairpin 1 region, and the hairpin 2 region are shortened. In some embodiments, the gRNA is from *S. pyogenes* Cas9 (SpyCas9). In some embodiments, the gRNA is from *N. meningitidis* Cas9 (NmeCas9).

[0744] An exemplary conserved region of an SpyCas9 guide RNA is shown in Table 8A (SEQ ID NO: 600). An exemplary conserved region of an NmeCas9 guide RNA is shown in Table 8B (SEQ ID NO: 3126). The first row shows the numbering of the nucleotides; the second row shows an exemplary sequence; and the third (and fourth) rows show the regions. "Shortened" with respect to an sgRNA means that its conserved region lacks at least one nucleotide shown in Table 8A-8B, as discussed in detail below.

[0745] Each of SpyCas9 guide RNAs disclosed herein may further comprise additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the guide sequence at its 3' end: GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 601) in 5' to 3' orientation. In the case of a sgRNA, the above guide sequences may further comprise additional nucleotides (scaffold sequence) to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence: GUUUUAGAGCUA-GAAAUAGCAAGUUAAAAUAAGGCUAGU-CCGUUAUCAACUU GAAAAAGUGGCACCGAGUCG-GUGCUUUU (SEQ ID NO: 602) or GUUUUAGAGCUA-GAAAUAGCAAGUUAAAAUAAGGCUAGU-CCGUUAUCAACUU GAAAAAGUGGCACCGAGUCG-GUGC (SEQ ID NO: 603, which is SEQ ID NO: 602 without the four terminal U's) in 5' to 3' orientation. In some embodiments, the four terminal U's of SEQ ID NO: 602 are not present. In some embodiments, only 1, 2, or 3 of the four terminal U's of SEQ ID NO: 602 are present.

[0746] In some embodiments, the SpyCas9 sgRNA comprises any one of the SpyCas9 guide sequences (e.g., HLA-B guide sequences of SEQ ID NOs: 1-91 or any one of the HLA-A guide sequences of SEQ ID NOs: 301-428 and 463-511) and additional nucleotides to form a crRNA, e.g., with the following exemplary scaffold nucleotide sequence following the guide sequence at its 3' end: GUUUUA-GAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGU-CCGUUAUCAACUU GGCACCGAGUCGGUGC (SEQ ID NO: 604) in 5' to 3' orientation. SEQ ID NO: 604 lacks 8 nucleotides with reference to a wild-type guide RNA conserved sequence: GUUUUAGAGCUA-GAAAUAGCAAGUUAAAAUAAGGCUAGU-CCGUUAUCAACUU GAAAAAGUGGCACCGAGUCG-GUGC (SEQ ID NO: 600). Other exemplary scaffold nucleotide sequences are provided in Table 9. In some embodiments, the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 1-91, 301-428, or 463-511 and additional guide scaffold sequences, in 5' to 3' orientation, in Table 9, including modified versions of the scaffold sequences, as shown.

[0747] In some embodiments, a gRNA provided herein comprises a guide region and a conserved region comprising a repeat/anti-repeat region, a hairpin 1 region, and a hairpin 2 region, wherein one or more of the repeat/anti-repeat region, the hairpin 1 region, and the hairpin 2 region are shortened. In some embodiments, the gRNA is from *N. meningitidis* Cas9 (NmeCas9).

[0748] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises any one of the modification patterns of the modified sgRNA sequences provided in Tables 2, 3, 3A, 4, 5A, 5B, 6, 7, 8A, 8B, 9, and 9A. In some embodiments, the conserved region comprises any one of modified conserved region Nine guide RNA motifs in Tables 8B, 9, and 9A, and wherein the conserved region is 3' of the guide region (guide sequence). In some embodiments, the conserved region comprises a modified sequence comprising any one of SEQ ID NOs: 715-723, and wherein the conserved region is 3' of the guide region (guide sequence). In some embodiments, the guide RNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 708 and 712-714, where the N's represent collectively any guide sequence disclosed herein, including the guide sequences provided in Tables 3, 3A, 5A, 7, and 9A. In certain embodiments, the N's represent collectively a guide sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to or complementary to any one of the guide sequences provided in Tables 3, 3A, 5A, 7, and 9A. In certain embodiments, the N's represent collectively any one of the guide sequences provided in Tables 3, 3A, 5A, 7, and 9A. In certain embodiments, when the N's represent collectively a guide sequence, within $(N)_{20-25}$, each N of the $(N)_{20-25}$ may be independently modified, e.g., modified with a 2'-OMe modification, optionally further with a PS modification, particularly at 1, 2, or 3 terminal

nucleotides. In certain embodiments, the $(N)_{20-25}$ has the following sequence and modification pattern:

mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNN.

[0749] An exemplary conserved region of an NmeCas9 single guide RNA (Nine sgRNA) is shown in Table 8B (SEQ ID NO: 3126). The first row shows the numbering of the nucleotides; the second row shows an exemplary sequence; and the third (and fourth) rows show the regions. "Shortened" with respect to an sgRNA means that its conserved region lacks at least one nucleotide shown in Table 8B, as discussed in detail below.

[0750] In some embodiments, the NmeCas9 sgRNA comprises any one of the Nine Cas9 guide sequences disclosed herein (e.g., SEQ ID NOs: 101-185) and additional nucleotides to form a crRNA, e.g., with the following exemplary scaffold nucleotide sequence following the guide sequence at its 3' end:

(SEQ ID NO: 699)
GUUGUAGCUCCCUUUCUCAUUUCGGAAACGAAAUGAGAACCGUUGCUAC
AAUAAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCUGCCCCUUAAAGCU
UCUGCUUUAAGGGGCAUCGUUUA.

[0751] In some embodiments, the NmeCas9sgRNA comprises any one of the guide sequences of SEQ ID NOs: 101-185 and additional nucleotides to form a crRNA with the following nucleotide sequence following the guide sequence at its 3' end:

(SEQ ID NO: 701)
GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGU
GCCGCAACGCUCUGCCUUCUGGCAUCGUU;

(SEQ ID NO: 702)
GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGU
GCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU;

(SEQ ID NO: 703)
GUUGUAGCUCCCUGGAAACCCGUUGCUACAAUAAGGCCGUCGAAAGA
UGUGCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU.

[0752] In some embodiments, the guide RNA is a chemically modified guide RNA. In some embodiments, the guide RNA is a chemically modified single guide RNA. The chemically modified guide RNAs may comprise one or more of the modifications as shown in Tables 2-7. The chemically modified guide RNAs may comprise one or more of modified nucleotides of any one of SEQ ID NOs: 705-714.

[0753] In some embodiments, the guide RNA is a sgRNA comprising the modification pattern shown in SEQ ID NO: 705-714.

[0754] In some embodiments, the guide RNA comprises a sgRNA comprising the modification pattern shown in SEQ ID NO: 705, 708, 711, 712, 713, or 714. In some embodiments, the guide RNA comprises a sgRNA comprising the modified nucleotides of SEQ ID NO: 705, 708, 711, 712, 713, or 714, including a guide sequence disclosed herein (e.g., SEQ ID NOs: 1-91). In some embodiments, the guide RNA is a sgRNA comprising a sequence of SEQ ID NO: 705, 708, 711, 712, 713, or 714 or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to SEQ ID NO: 705, 708, 711, 712, 713, or 714.

[0755] The guide RNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA) or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond. In some embodiments, a crRNA or trRNA sequence may be referred to as a "scaffold" or "conserved portion" of a guide RNA.

[0756] In each of the compositions, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a "dual guide RNA" or "dgRNA." The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Tables 2-3, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form an RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[0757] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a "single guide RNA" or "sgRNA". The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Tables 2-3, covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Tables 2-3. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[0758] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[0759] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in Tables 2-3 (for HLA-B SpyCas9 and NmeCas9 guides) and Tables 4, 5B and 6 (for HLA-A SpyCas9 guides) and Table 5A and 7 (for HLA-A NmeCas9 guides) is provided. In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in Tables 2-3 is provided, wherein the nucleotides of SEQ ID NO: 601-604 follow the guide sequence at its 3' end. In some embodiments, the one or more guide RNAs comprising a guide sequence of any one in Tables 2-3 (for HLA-B SpyCas9 and NmeCas9 guides) and Tables 4, 5B and 6 (for HLA-A SpyCas9 guides), wherein the nucleotides of SEQ ID NO: 601-604 follow the guide sequence at its 3' end, is modified according to the modification pattern of any one of SEQ ID NOs: 3003, 3007-3009, and 3011-3014.

[0760] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in Tables 2-3 (for HLA-B SpyCas9 and NmeCas9 guides) and Tables 4, 5B and 6 (for HLA-A SpyCas9 guides) is provided. In one aspect, a composition comprising one or more gRNAs is provided, comprising a guide sequence that

is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 1-91, 301-428, and 463-511.

[0761] In other embodiments, a composition is provided that comprises at least one, e.g., at least two gRNA's comprising guide sequences selected from any two or more of the guide sequences shown in Tables 2-3 (for HLA-B SpyCas9 and NmeCas9 guides) and Tables 4, 5B and 6 (for HLA-A SpyCas9 guides). In some embodiments, the composition comprises at least two gRNA's that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the guide sequences shown in Tables 2-3 (for HLA-B SpyCas9 and NmeCas9 guides) and Tables 4, 5B, and 6 (for HLA-A SpyCas9 and NmeCas9 guides).

[0762] In some embodiments, the guide RNA compositions of the present invention are designed to recognize (e.g., hybridize to) a target sequence in HLA-B. For example, the HLA-B target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence in HLA-B, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[0763] In some embodiments, the guide RNA compositions of the present invention are designed to recognize (or hybridize to) a target sequence in HLA-A and HLA-B. For example, the HLA-A and HLA-B target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence in HLA-A and HLA-B, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[0764] In some embodiments, the selection of the one or more HLA-B guide RNAs is determined based on target sequences within HLA-B. In some embodiments, the compositions comprising one or more guide sequences comprise a guide sequence that is complementary to the corresponding genomic region shown in Tables 2-3, according to coordinates from human reference genome hg38. Guide sequences of further embodiments may be complementary to sequences in the close vicinity of the genomic coordinate listed in any of the Tables 2-3 within HLA-B. For example, guide sequences of further embodiments may be complementary to sequences that comprise 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 2-3.

[0765] In some embodiments, the selection of the one or more HLA-A guide RNAs is determined based on target sequences within HLA-A. In some embodiments, the compositions comprising one or more guide sequences comprise a guide sequence that is complementary to the corresponding genomic region shown in Tables 4-7, according to coordinates from human reference genome hg38. Guide sequences of further embodiments may be complementary to sequences in the close vicinity of the genomic coordinate listed in any of the Tables 4-7 within HLA-A. For example, guide sequences of further embodiments may be complementary to sequences that comprise 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Tables 4-7. Without being bound by any particular theory, modifications (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the target gene may be less tolerable than mutations in other regions, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within the target gene used to direct an RNA-guided DNA binding agent to a particular location in the target gene.

[0766] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 85%, or 80% identical to a target sequence present in the target gene. In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 85%, or 80% identical to a target sequence present in the human HLA-A or HLA-B gene.

[0767] In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

[0768] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

H. Modified gRNAs and mRNAs

[0769] In some embodiments, the gRNA (e.g., sgRNA, short-sgRNA, dgRNA, or crRNA) is modified. The term "modified" or "modification" in the context of a gRNA described herein includes, the modifications described above, including, for example, (a) end modifications, e.g., 5' end modifications or 3' end modifications, including 5' or 3' protective end modifications, (b) nucleobase (or "base") modifications, including replacement or removal of bases, (c) sugar modifications, including modifications at the 2', 3', or 4' positions, (d) internucleoside linkage modifications, and (e) backbone modifications, which can include modification or replacement of the phosphodiester linkages or the ribose sugar. A modification of a nucleotide at a given position includes a modification or replacement of the phosphodiester linkage immediately 3' of the sugar of the nucleotide. Thus, for example, a nucleic acid comprising a phosphorothioate between the first and second sugars from the 5' end is considered to comprise a modification at position 1. The term "modified gRNA" generally refers to a gRNA having a modification to the chemical structure of one or more of the base, the sugar, and the phosphodiester

linkage or backbone portions, including nucleotide phosphates, all as detailed and exemplified herein.

[0770] Further description and exemplary patterns of modifications are provided in Table 1 of WO2019/237069 published Dec. 12, 2019, the entire contents of which are incorporated herein by reference.

[0771] In some embodiments, a gRNA comprises modifications at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more YA sites. In some embodiments, the pyrimidine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine). In some embodiments, the adenine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the adenine). In some embodiments, the pyrimidine and the adenine of the YA site comprise modifications, such as sugar, base, or internucleoside linkage modifications. The YA modifications can be any of the types of modifications set forth herein. In some embodiments, the YA modifications comprise one or more of phosphorothioate, 2'-OMe, or 2'-fluoro. In some embodiments, the YA modifications comprise pyrimidine modifications comprising one or more of phosphorothioate, 2'-OMe, 2'-H, inosine, or 2'-fluoro. In some embodiments, the YA modification comprises a bicyclic ribose analog (e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains one or more YA sites. In some embodiments, the YA modification comprises a bicyclic ribose analog (e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains a YA site, wherein the YA modification is distal to the YA site.

[0772] In some embodiments, the guide sequence (or guide region) of a gRNA comprises 1, 2, 3, 4, 5, or more YA sites ("guide region YA sites") that may comprise YA modifications. In some embodiments, one or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus (where "5-end", etc., refers to position 5 to the 3' end of the guide region, i.e., the most 3' nucleotide in the guide region) comprise YA modifications. A modified guide region YA site comprises a YA modification.

[0773] In some embodiments, a modified guide region YA site is within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region. For example, if a modified guide region YA site is within 10 nucleotides of the 3' terminal nucleotide of the guide region and the guide region is 20 nucleotides long, then the modified nucleotide of the modified guide region YA site is located at any of positions 11-20. In some embodiments, a modified guide region YA site is at or after nucleotide 4, 5, 6, 7, 8, 9, 10, or 11 from the 5' end of the 5' terminus.

[0774] In some embodiments, a modified guide region YA site is other than a 5' end modification. For example, a sgRNA can comprise a 5' end modification as described herein and further comprise a modified guide region YA site. Alternatively, a sgRNA can comprise an unmodified 5' end and a modified guide region YA site. Alternatively, a short-sgRNA can comprise a modified 5' end and an unmodified guide region YA site.

[0775] In some embodiments, a modified guide region YA site comprises a modification that at least one nucleotide located 5' of the guide region YA site does not comprise. For example, if nucleotides 1-3 comprise phosphorothioates, nucleotide 4 comprises only a 2'-OMe modification, and nucleotide 5 is the pyrimidine of a YA site and comprises a phosphorothioate, then the modified guide region YA site comprises a modification (phosphorothioate) that at least one nucleotide located 5' of the guide region YA site (nucleotide 4) does not comprise. In another example, if nucleotides 1-3 comprise phosphorothioates, and nucleotide 4 is the pyrimidine of a YA site and comprises a 2'-OMe, then the modified guide region YA site comprises a modification (2'-OMe) that at least one nucleotide located 5' of the guide region YA site (any of nucleotides 1-3) does not comprise. This condition is also always satisfied if an unmodified nucleotide is located 5' of the modified guide region YA site.

[0776] In some embodiments, the modified guide region YA sites comprise modifications as described for YA sites above. The guide region of a gRNA may be modified according to any embodiment comprising a modified guide region set forth herein. Any embodiments set forth elsewhere in this disclosure may be combined to the extent feasible with any of the foregoing embodiments.

[0777] In some embodiments, the 5' or 3' terminus regions of a gRNA are modified.

[0778] In some embodiments, the terminal (i.e., last) 1, 2, 3, 4, 5, 6, or 7 nucleotides in the 3' terminus region are modified. Throughout, this modification may be referred to as a "3' end modification". In some embodiments, the terminal (i.e., last) 1, 2, 3, 4, 5, 6, or 7 nucleotides in the 3' terminus region comprise more than one modification. In some embodiments, the 3' end modification comprises or further comprises any one or more of the following: a modified nucleotide selected from 2'-O-methyl (2'-O-Me) modified nucleotide, 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or combinations thereof. In some embodiments, the 3' end modification comprises or further comprises modifications of 1, 2, 3, 4, 5, 6, or 7 nucleotides at the 3' end of the gRNA. In some embodiments, the 3' end modification comprises or further comprises one PS linkage, wherein the linkage is between the last and second to last nucleotide. In some embodiments, the 3' end modification comprises or further comprises two PS linkages between the last three nucleotides. In some embodiments, the 3' end modification comprises or further comprises four PS linkages between the last four nucleotides. In some embodiments, the 3' end modification comprises or further comprises PS linkages between any one or more of the last 2, 3, 4, 5, 6, or 7 nucleotides. In some embodiments, the gRNA comprising a 3' end modification comprises or further comprises a 3' tail, wherein the 3' tail comprises a modification of any one or more of the nucleotides present in the 3' tail. In some embodiments, the 3' tail is fully modified. In some embodiments, the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, or 1-10 nucleotides, optionally where any one or more of these nucleotides are modified. In some embodiments, a gRNA is provided comprising a 3' protective end modification. In some embodiments, the 3' tail comprises between 1 and about 20 nucleotides, between 1 and about 15 nucleotides, between 1 and about 10 nucleotides, between 1 and about 5 nucleotides, between 1 and about 4 nucleotides, between 1 and about 3 nucleotides, and between 1 and about 2 nucleotides. In some embodiments, the gRNA does not comprise a 3' tail.

[0779] In some embodiments, the 5' terminus region is modified, for example, the first 1, 2, 3, 4, 5, 6, or 7 nucleotides of the gRNA are modified. Throughout, this modification may be referred to as a "5' end modification". In some embodiments, the first 1, 2, 3, 4, 5, 6, or 7 nucleotides of the 5' terminus region comprise more than one modification. In some embodiments, at least one of the terminal (i.e., first) 1, 2, 3, 4, 5, 6, or 7 nucleotides at the 5' end are modified. In some embodiments, both the 5' and 3' terminus regions (e.g., ends) of the gRNA are modified. In some embodiments, only the 5' terminus region of the gRNA is modified. In some embodiments, only the 3' terminus region (plus or minus a 3' tail) of the conserved portion of a gRNA is modified. In some embodiments, the gRNA comprises modifications at 1, 2, 3, 4, 5, 6, or 7 of the first 7 nucleotides at a 5' terminus region of the gRNA. In some embodiments, the gRNA comprises modifications at 1, 2, 3, 4, 5, 6, or 7 of the 7 terminal nucleotides at a 3' terminus region. In some embodiments, 2, 3, or 4 of the first 4 nucleotides at the 5' terminus region, or 2, 3, or 4 of the terminal 4 nucleotides at the 3' terminus region are modified. In some embodiments, 2, 3, or 4 of the first 4 nucleotides at the 5' terminus region are linked with phosphorothioate (PS) bonds. In some embodiments, the modification to the 5' terminus or 3' terminus comprises a 2'-O-methyl (2'-O-Me) or 2'-O-(2-methoxyethyl) (2'-O-moe) modification. In some embodiments, the modification comprises a 2'-fluoro (2'-F) modification to a nucleotide. In some embodiments, the modification comprises a phosphorothioate (PS) linkage between nucleotides. In some embodiments, the modification comprises an inverted abasic nucleotide. In some embodiments, the modification comprises a protective end modification. In some embodiments, the modification comprises a more than one modification selected from protective end modification, 2'-O-Me, 2'-O-moe, 2'-fluoro (2'-F), a phosphorothioate (PS) linkage between nucleotides, and an inverted abasic nucleotide. In some embodiments, an equivalent modification is encompassed.

[0780] In some embodiments, a gRNA is provided comprising a 5' end modification and a 3' end modification. In some embodiments, the gRNA comprises modified nucleotides that are not at the 5' or 3' ends.

[0781] In some embodiments, a sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region. In some embodiments, a sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises a modification of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 nucleotides in the upper stem region. In some embodiments, an sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises 1, 2, 3, 4, or 5 YA modifications in a YA site. In some embodiments, the upper stem modification comprises a 2'-OMe modified nucleotide, a 2'-O-moe modified nucleotide, a 2'-F modified nucleotide, or combinations thereof. Other modifications described herein, such as a 5' end modification or a 3' end modification may be combined with an upper stem modification.

[0782] In some embodiments, the sgRNA comprises a modification in the hairpin region. In some embodiments, the hairpin region modification comprises at least one modified nucleotide selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, or combinations thereof. In some embodiments, the hairpin region modification is in the hairpin 1 region. In some embodiments, the hairpin region modification is in the hairpin 2 region. In some embodiments, the hairpin modification comprises 1, 2, or 3 YA modifications in a YA site. In some embodiments, the hairpin modification comprises at least 1, 2, 3, 4, 5, or 6 YA modifications. Other modifications described herein, such as an upper stem modification, a 5' end modification, or a 3' end modification may be combined with a modification in the hairpin region.

[0783] In some embodiments, a gRNA comprises a substituted and optionally shortened hairpin 1 region, wherein at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9. "Watson-Crick pairing nucleotides" include any pair capable of forming a Watson-Crick base pair, including A-T, A-U, T-A, U-A, C-G, and G-C pairs, and pairs including modified versions of any of the foregoing nucleotides that have the same base pairing preference. In some embodiments, the hairpin 1 region lacks any one or two of H1-5 through H1-8. In some embodiments, the hairpin 1 region lacks one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10 or H1-4 and H1-9. In some embodiments, the hairpin 1 region lacks 1-8 nucleotides of the hairpin 1 region. In any of the foregoing embodiments, the lacking nucleotides may be such that the one or more nucleotide pairs substituted with Watson-Crick pairing nucleotides (H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9) form a base pair in the gRNA.

[0784] In some embodiments, the gRNA further comprises an upper stem region lacking at least 1 nucleotide, e.g., any of the shortened upper stem regions indicated in Table 7 of WO/2021/119275, the contents of which are hereby incorporated by reference in its entirety, or described elsewhere herein, which may be combined with any of the shortened or substituted hairpin 1 regions described herein.

[0785] In some embodiments, an sgRNA provided herein is a short-single guide RNAs (short-sgRNAs), e.g., comprising a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides or 6-10 nucleotides. In some embodiments, the 5-10 nucleotides or 6-10 nucleotides are consecutive.

[0786] In some embodiments, a short-sgRNA lacks at least nucleotides 54-58 (AAAAA) of the conserved portion of a spyCas9 sgRNA. In some embodiments, a short-sgRNA is a non-spyCas9 sgRNA that lacks nucleotides corresponding to nucleotides 54-58 (AAAAA) of the conserved portion of a spyCas9 as determined, for example, by pairwise or structural alignment.

[0787] In some embodiments, the short-sgRNA described herein comprises a conserved portion comprising a hairpin region, wherein the hairpin region lacks 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides. In some embodiments, the lacking nucleotides are 5-10 lacking nucleotides or 6-10 lacking nucleotides. In some embodiments, the lacking nucleotides are consecutive. In some embodiments, the lacking nucleotides span at least a portion of hairpin 1 and a portion of hairpin 2. In some embodiments, the 5-10 lacking nucleotides comprise or consist of nucleotides 54-58, 54-61, or 53-60 of SEQ ID NO: 600.

[0788] In some embodiments, the short-sgRNA described herein further comprises a nexus region, wherein the nexus

region lacks at least one nucleotide (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in the nexus region). In some embodiments, the short-sgRNA lacks each nucleotide in the nexus region.

[0789] In some embodiments, the SpyCas9 short-sgRNA described herein comprises a sequence of

(SEQ ID NO: 3002)
NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAA
UAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGCU.

[0790] In some embodiments, the short-sgRNA described herein comprises a modification pattern as shown in SEQ ID NO: 3003: mN*mN*mN*NNNNNNNNNNNN NNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAU-CACGAAAGGGCACCGAGUCG GmUmGmC*mU (SEQ ID NO: 3003), where A, C, G, U, and N are adenine, cytosine, guanine, uracil, and any ribonucleotide, respectively, unless otherwise indicated. An m is indicative of a 2'O-methyl modification, and an * is indicative of a phosphorothioate linkage between the nucleotides.

[0791] In certain embodiments, using SEQ ID NO: 600 ("Exemplary SpyCas9 sgRNA-1") as an example, the Exemplary SpyCas9 sgRNA-1 further includes one or more of:

[0792] A. a shortened hairpin 1 region, or a substituted and optionally shortened hairpin 1 region, wherein

[0793] 1. at least one of the following pairs of nucleotides are substituted in hairpin 1 with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9, and the hairpin 1 region optionally lacks

[0794] a. any one or two of H1-5 through H1-8,

[0795] b. one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and H1-4 and H1-9, or

[0796] c. 1-8 nucleotides of hairpin 1 region; or

[0797] 2. the shortened hairpin 1 region lacks 6-8 nucleotides, preferably 6 nucleotides; and

[0798] a. one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600) or

[0799] b. one or more of positions H1-6 through H1-10 is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600); or

[0800] 3. the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or n is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600); or

[0801] B. a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600); or

[0802] C. a substitution relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600) at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; or

[0803] D. Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 600) with an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region, wherein

[0804] 1. the modified nucleotide is optionally selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof; or

[0805] 2. the modified nucleotide optionally includes a 2'-OMe modified nucleotide.

[0806] In certain embodiments, Exemplary SpyCas9 sgRNA-1, or an sgRNA, such as an sgRNA comprising Exemplary SpyCas9 sgRNA-1, further includes a 3' tail, e.g., a 3' tail of 1, 2, 3, 4, or more nucleotides. In certain embodiments, the tail includes one or more modified nucleotides. In certain embodiments, the modified nucleotide is selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, and an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide. In certain embodiments, the modified nucleotide includes a PS linkage between nucleotides. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide and a PS linkage between nucleotides.

[0807] In some embodiments, the NmeCas9 gRNA described herein further comprises a nexus region, wherein the nexus region lacks at least one nucleotide.

[0808] In some embodiments, the HLA-B NmeCas9 sgRNA chosen from SEQ ID NOs 101-185 comprises a conserved portion comprising a repeat/anti-repeat region, a hairpin 1 region, and a hairpin 2 region, wherein one or more of the repeat/anti-repeat region, the hairpin 1 region, and the hairpin 2 region are shortened.

[0809] In some embodiments, the guide RNA is a Nine sgRNA comprising a conserved portion comprising a repeat/anti-repeat region, a hairpin 1 region, and a hairpin 2 region, wherein one or more of the repeat/anti-repeat region, the hairpin 1 region, and the hairpin 2 region are shortened. In some embodiments, the sgRNA described herein further comprises a guide region and a conserved region, wherein the conserved region comprises one or more of: (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 2-24 nucleotides, wherein (i) one or more of nucleotides 37-48 and 53-64 is deleted and optionally one or more of nucleotides 37-64 is substituted relative to SEQ ID NO: 3126; and (ii) nucleotide 36 is linked to nucleotide 65 by at least 2 nucleotides; or (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2-10, optionally 2-8 nucleotides, wherein one or more of nucleotides 82-86 and 91-95 is deleted and optionally one or more of positions 82-96 is substituted relative to SEQ ID NO: 3126; and nucleotide 81 is linked to nucleotide 96 by at least 4 nucleotides; or (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 2-18, optionally 2-16 nucleotides, wherein (i) one or more of nucleotides 113-121 and 126-134 is deleted and optionally one or more of nucleotides 113-134 is substituted relative to SEQ ID NO: 3126; and (ii) nucleotide 112 is linked to nucleotide 135 by at least 4 nucleotides; wherein one or both nucleotides 144-145 are

optionally deleted relative to SEQ ID NO: 3126; and wherein at least 10 nucleotides are modified nucleotides.

[0810] In some embodiment, the gRNA disclosed herein is a sgRNA.

[0811] In some embodiments, in the guide sequence, nucleotides 1-4 are modified nucleotides. In some embodiments, in the guide sequence, nucleotides 5, 8, 9, 11, 13, 18, and 22 are modified nucleotides. In some embodiments, in the guide sequence, nucleotides 1-5, 8, 9, 11, 13, 18, and 22 are modified nucleotides. In some embodiments, the modified nucleotides are 2'-O-methyl (2'-O-Me) modified nucleotides. In some embodiments, in the guide sequence, nucleotide 1 is linked to nucleotide 2 by a phosphorothioate (PS) linkage, nucleotide 2 is linked to nucleotide 3 by a PS linkage, and/or nucleotide 3 is linked to nucleotide 4 by a PS linkage.

[0812] In some embodiments, one or both nucleotides 144-145 are deleted relative to SEQ ID NO: 3126.

[0813] In some embodiments, at least 10 nucleotides of the conserved region are modified nucleotides.

[0814] In some embodiments, a repeat/anti-repeat region of a gRNA is a shortened repeat/anti-repeat region lacking 2-24 nucleotides, e.g., any of the repeat/anti-repeat regions indicated in the numbered embodiments above or Tables 3, 3A, 5A, 7, and 9A or described elsewhere herein, which may be combined with any of the shortened hairpin 1 region or hairpin 2 region described herein, including but not limited to combinations indicated in the numbered embodiments above and represented in the sequences of Tables 3, 3A, 5A, 7, and 9A or described elsewhere herein. In some embodiments, one or more of positions 49-52, 87-90, or 122-125 is substituted relative to SEQ ID NO: 3126. In some embodiments, all of positions 49-52, 87-90, or 122-125 are substituted relative to SEQ ID NO: 3126. In some embodiments, the 3' tail provided in Tables 3, 3A, 5A, 7, and 9A or described herein is deleted.

[0815] In some embodiments, the shortened repeat/anti-repeat region of the gRNA lacks 18 nucleotides. In some embodiments, the shortened repeat/anti-repeat region of the gRNA lacks 22 nucleotides.

[0816] In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 6 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 7 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 8 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 9 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 10 nucleotides.

[0817] In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotides 38, 41-48, 53-60, and 63 are deleted relative to SEQ ID NO: 3126.

[0818] In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 6 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126, and nucleotide 36 is linked to nucleotide 65 by nucleotides 37, 49-52, and 64.

[0819] In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotide 36 is linked to nucleotide 65 by 10 nucleotides. In some embodiments, in the shortened repeat/anti-repeat region of the gRNA, nucleotides 38, 41-48, 53-60, and 63 are deleted relative to SEQ ID NO: 3126, and nucleotide 36 is linked to nucleotide 65 by nucleotides 37, 39, 40, 49-52, 61, 62, and 64.

[0820] In some embodiments, all of nucleotides 38-48 and nucleotides 53-63 of the upper stem of the shortened repeat/anti-repeat region are deleted relative to SEQ ID NO: 3126.

[0821] In some embodiments, all of nucleotides 39-48 and nucleotides 53-62 of the upper stem of the shortened repeat/anti-repeat region are deleted relative to SEQ ID NO: 3126, and nucleotides 38 and 63 is substituted.

[0822] In some embodiments, the shortened repeat/anti-repeat region has 14 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 15 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 16 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 17 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 18 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 19 modified nucleotides. In some embodiments, the shortened repeat/anti-repeat region has 20 modified nucleotides. In some embodiments, in the shortened repeat/anti-repeat region, nucleotides 25, 29, 30, 31, 32, 37, 49-52, 64, 65, 69, 70, and 73 are modified nucleotides. In some embodiments, the modified nucleotides are 2'-O-Me modified nucleotides.

[0823] In some embodiments, between the shortened repeat/anti-repeat region and the shortened hairpin 1 region, nucleotide 76 is linked to nucleotide 77 by a PS linkage.

[0824] In some embodiments, the shortened hairpin 1 region lacks 2 nucleotides. In some embodiments, the shortened hairpin 1 region lacks 21 nucleotides. In some embodiments, the shortened hairpin 1 region lacks 2 nucleotides, and nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126. In some embodiments, the shortened hairpin 1 region lacks 2 nucleotides, and nucleotides 85 and 92 are deleted relative to SEQ ID NO: 3126. In some embodiments, in the shortened hairpin 1 region, nucleotide 81 is linked to nucleotide 96 by 12 nucleotides. In some embodiments, in the shortened hairpin 1 region, nucleotide 81 is linked to nucleotide 96 by 12 nucleotides. In some embodiments, in the shortened hairpin 1 region, nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126, and nucleotide 81 is linked to nucleotide 96 by nucleotides 82-85, 87-90, and 92-95. In some embodiments, in the shortened hairpin 1 region, nucleotides 85 and 92 are deleted relative to SEQ ID NO: 3126, and nucleotide 81 is linked to nucleotide 96 by nucleotides 82-84, 86-91, and 93-95.

[0825] In some embodiments, the shortened hairpin 1 region has a duplex portion of 7 base paired nucleotides in length. In some embodiments, the shortened hairpin 1 region has a duplex portion of 8 base paired nucleotides in length.

[0826] In the stem of the shortened hairpin 1 region is seven base paired nucleotides in length. In some embodiments, nucleotides 85-86 and nucleotides 91-92 of the shortened hairpin 1 region are deleted.

[0827] In some embodiments, the shortened hairpin 1 region has 13 modified nucleotides. In some embodiments,

in the shortened hairpin 1 region, nucleotides 80, 81, 83, 84, 85, 87-90, 92-94, and 99 are modified nucleotides. In some embodiments, the modified nucleotides are 2'-O-Me modified nucleotides.

[0828] In some embodiments, between the shortened hairpin 1 region and the shortened hairpin 2 region, nucleotide 101 is a modified nucleotide. In some embodiments, the modified nucleotide is a 2'-O-Me modified nucleotide.

[0829] In some embodiments, the shortened hairpin 2 lacks 18 nucleotides. In some embodiments, the shortened hairpin 2 has 24 nucleotides. In some embodiments, in the shortened hairpin 2 nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126. In some embodiments, the shortened hairpin 2 lacks 18 nucleotides, and nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126. In some embodiments, in the shortened hairpin 2 region, nucleotide 112 is linked to nucleotide 135 by 4 nucleotides. In some embodiments, in the shortened hairpin 2 region, nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126 and nucleotide 112 is linked to nucleotide 135 by nucleotides 122-125. In some embodiments, in the shortened hairpin 2 region, nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126. In some embodiments, the shortened hairpin 2 region lacks 18 nucleotides, and nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126.

[0830] In some embodiments, the shortened repeat/anti-repeat region has a length of 28 nucleotides. In some embodiments, the shortened repeat/anti-repeat region has a length of 32 nucleotides.

[0831] In some embodiments, the upper stem of the shortened repeat/anti-repeat region comprises no more than one base pair. In some embodiments, the upper stem of the shortened repeat/anti-repeat region comprises no more than three base pairs.

[0832] In some embodiments, the shortened hairpin 2 region has 8 modified nucleotides. In some embodiments, the shortened hairpin 2 region has 9 modified nucleotides. In some embodiments, the shortened hairpin 2 region has 13 modified nucleotides. In some embodiments, in the shortened hairpin 2 region, nucleotides 104, 110, 111, 122-125, 142, and 143 are modified nucleotides. In some embodiments, in the shortened hairpin 2 region, nucleotides 104, 106-111, 122-125, 142, and 143 are modified nucleotides. In some embodiments, the modified nucleotides are 2'-O-Me modified nucleotides.

[0833] In some embodiments, in the shortened hairpin 2 region, nucleotide 141 is linked to nucleotide 142 by a PS linkage, and/or nucleotide 142 is linked to nucleotide 143 by a PS linkage.

[0834] In some embodiments, a guide RNA (gRNA) comprises a guide region and a conserved region, the conserved region comprising:

- **[0835]** (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 18-22 nucleotides relative to SEQ ID NO: 3126, wherein
  - **[0836]** (i) nucleotides 38-48 and 53-63 are deleted; and
  - **[0837]** (ii) nucleotide 36 is linked to nucleotide 65 by 6-10 nucleotides;
- **[0838]** (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2 nucleotides, wherein nucleotides 86 and 91 are deleted or nucleotides 85 and 92 are deleted relative to SEQ ID NO: 3126; and
- **[0839]** (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 18 nucleotides, wherein nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126; and wherein nucleotides 144-145 are deleted relative to SEQ ID NO: 3126; wherein at least 10 nucleotides are modified nucleotides.

[0840] In some embodiments, a guide RNA (gRNA) comprises a guide region and a conserved region, the conserved region comprising:

- **[0841]** (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 18-22 nucleotides relative to SEQ ID NO: 3126, wherein
  - **[0842]** (i) nucleotides 38, 41-48, 53-60, and 63 are deleted; and
  - **[0843]** (ii) nucleotide 36 is linked to nucleotide 65 by 6-10 nucleotides;
- **[0844]** (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2 nucleotides, wherein nucleotides 86 and 91 are deleted or nucleotides 85 and 92 are deleted relative to SEQ ID NO: 3126;
- **[0845]** (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 18 nucleotides, wherein nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126; and
- **[0846]** wherein nucleotides 144-145 are deleted relative to SEQ ID NO: 3126;
- **[0847]** wherein at least 10 nucleotides are modified nucleotides.

[0848] In some embodiments, a guide RNA (gRNA) is provided, the gRNA comprising a guide region and a conserved region, the conserved region comprising one or more of:

- **[0849]** (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 18-22 nucleotides relative to SEQ ID NO: 3126, wherein
  - **[0850]** (i) nucleotides 37-48 and 53-64 are deleted; and
  - **[0851]** (ii) nucleotide 36 is linked to nucleotide 65 by 6-10 nucleotides; or
- **[0852]** (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2 nucleotides, wherein nucleotides 86 and 91 are deleted or nucleotides 85 and 92 are deleted relative to SEQ ID NO: 3126; or
- **[0853]** (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 18 nucleotides, wherein nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126; and
- **[0854]** wherein nucleotides 144-145 are deleted relative to SEQ ID NO: 3126;
- **[0855]** wherein at least 10 nucleotides are modified nucleotides.

[0856] In further embodiments, the shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 22 nucleotides relative to SEQ ID NO: 3126. In further embodiments, nucleotide 36 is linked to nucleotide 65 by a sequence comprising the nucleotide sequence UGAAAC. In further embodiments, the nucleotide 36 is linked to nucleotide 65 by 10 nucleotides. In further embodi-

ments, the nucleotide 36 is linked to nucleotide 65 by a sequence comprising the nucleotide sequence UUCGAAAGAC (SEQ ID NO: 3122).

[0857] In some embodiments, a guide RNA (gRNA) is provided, the gRNA comprising: a guide sequence comprising:

[0858] 2'-O-Me modified nucleotides at the first four nucleotides 1-4;

[0859] PS linkages between nucleotides 1-2, 2-3, and 3-4; and

[0860] 2'-O-Me modified nucleotides at nucleotides 5, 8, 9, 11, 13, 18, and 22 of the guide sequence;

a shortened repeat/anti-repeat region, wherein nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126, comprising:

[0861] 2'-O-Me modified nucleotides at nucleotides 25, 29, 30, 31, 32, 37, 49-52, 64, 65, 69, 70, and 73;

a PS linkage between nucleotides 76-77 between the shortened repeat/anti-repeat region and the shortened hairpin 1 region;

a shortened hairpin 1 region, wherein nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126, comprising:

[0862] 2'-O-Me modified nucleotides at nucleotides 80, 81, 83, 84, 85, 87-90, 92-94, and 99; 2'-O-Me modified nucleotide at nucleotide 101 between the shortened hairpin 1 region and the shortened hairpin 2 region;

a shortened hairpin 2 region, wherein nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126, comprising:

[0863] 2'-O-Me modified nucleotides at nucleotides 104, 110, 111, 122-125, 142, and 143,

[0864] PS linkages between nucleotides 141-142 and 142-143,

wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 3126.

[0865] In some embodiments, a guide RNA (gRNA) is provided, the gRNA comprising: a guide sequence comprising:

[0866] 2'-O-Me modified nucleotides at the first four nucleotides 1-4;

[0867] PS linkages between nucleotides 1-2, 2-3, and 3-4; and

[0868] 2'-O-Me modified nucleotides at nucleotides 5, 8, 9, 11, 13, 18, and 22 of the guide sequence;

a shortened repeat/anti-repeat region, wherein nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126, comprising:

[0869] 2'-O-Me modified nucleotides at nucleotides 25, 29, 30, 31, 32, 37, 49-52, 64, 65, 69, 70, and 73;

a shortened hairpin 1 region, wherein nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126, comprising:

[0870] 2'-O-Me modified nucleotides at nucleotides 80, 81, 83, 84, 85, 87-90, 92-94, and 99; 2'-O-Me modified nucleotide at nucleotide 101 between the shortened hairpin 1 region and the shortened hairpin 2 region;

a shortened hairpin 2 region, wherein nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126, comprising:

[0871] 2'-O-Me modified nucleotides at nucleotides 104, 110, 111, 122-125, 142, and 143, PS linkages between nucleotides 141-142 and 142-143, wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 3126.

[0872] In some embodiments, a guide RNA (gRNA) is provided, the gRNA comprising: a guide sequence comprising:

[0873] 2'-O-Me modified nucleotides at the first four nucleotides 1-4;

[0874] PS linkages between nucleotides 1-2, 2-3, and 3-4; and

[0875] 2'-O-Me modified nucleotides at nucleotides 5, 8, 9, 11, 13, 18, and 22 of the guide sequence;

a shortened repeat/anti-repeat region, wherein nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126, comprising:

[0876] 2'-O-Me modified nucleotides at nucleotides 25, 29, 30, 31, 32, 37, 49-52, 64, 65, 69, 70, and 73;

a PS linkage between nucleotides 76-77 between the shortened repeat/anti-repeat region and the shortened hairpin 1 region;

a shortened hairpin 1 region, wherein nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126, comprising:

[0877] 2'-O-Me modified nucleotides at nucleotides 80, 81, 83, 84, 85, 87-90, 92-94, and 99; 2'-O-Me modified nucleotide at nucleotide 101 between the shortened hairpin 1 region and the shortened hairpin 2 region;

a shortened hairpin 2 region, wherein nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126, comprising:

[0878] 2'-O-Me modified nucleotides at nucleotides 104, 106-111, 122-125, 142, and 143, PS linkages between nucleotides 141-142 and 142-143, wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 3126.

[0879] In some embodiments, a guide RNA (gRNA) is provided, the gRNA comprising: a guide sequence comprising:

[0880] 2'-O-Me modified nucleotides at the first four nucleotides 1-4;

[0881] PS linkages between nucleotides 1-2, 2-3, and 3-4; and

[0882] 2'-O-Me modified nucleotides at nucleotides 5, 8, 9, 11, 13, 18, and 22 of the guide sequence;

a shortened repeat/anti-repeat region, wherein nucleotides 38-48 and 53-63 are deleted relative to SEQ ID NO: 3126, comprising:

[0883] 2'-O-Me modified nucleotides at nucleotides 25, 29, 30, 31, 32, 37, 49-52, 64, 65, 69, 70, and 73;

a shortened hairpin 1 region, wherein nucleotides 86 and 91 are deleted relative to SEQ ID NO: 3126, comprising:

[0884] 2'-O-Me modified nucleotides at nucleotides 80, 81, 83, 84, 85, 87-90, 92-94, and 99; 2'-O-Me modified nucleotide at nucleotide 101 between the shortened hairpin 1 region and the shortened hairpin 2 region;

a shortened hairpin 2 region, wherein nucleotides 112-120 and 127-135 are deleted relative to SEQ ID NO: 3126, comprising:

[0885] 2'-O-Me modified nucleotides at nucleotides 104, 106-111, 122-125, 142, and 143,

[0886] PS linkages between nucleotides 141-142 and 142-143,

wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 3126.

[0887] In some embodiments, the guide RNA (gRNA) of the previous embodiment comprising a guide region and a conserved region, the conserved region comprising:

[0888] (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 18-22 nucleotides, wherein

[0889] (i) nucleotides 37-48 and 53-64 are deleted relative to SEQ ID NO: 3126; and

[0890] (ii) nucleotide 36 is linked to nucleotide 65 by 6-10 nucleotides;

[0891] (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2 nucleotides relative to SEQ ID NO: 3126, wherein nucleotides 86 and 91 are deleted or nucleotides 85 and 92 are deleted;

[0892] (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 18 nucleotides, wherein nucleotides 113-121 and 126-134 are deleted relative to SEQ ID NO: 3126; and

[0893] (d) wherein nucleotides 144-145 are deleted relative to SEQ ID NO: 3126;

[0894] wherein at least 10 nucleotides are modified nucleotides.

[0895] In further embodiments, the shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 22 nucleotides relative to SEQ ID NO: 3126. In further embodiments, nucleotide 36 is linked to nucleotide 65 by a sequence comprising the nucleotide sequence UGAAAC. In further embodiments, the nucleotide 36 is linked to nucleotide 65 by 10 nucleotides. In further embodiments, the nucleotide 36 is linked to nucleotide 65 by a sequence comprising the nucleotide sequence UUCGAAAGAC (SEQ ID NO: 3122).

[0896] In some embodiments, the sgRNA comprises one of the following sequences in 5' to 3' orientation: $(N)_{20-25}$ GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGC AACGCUCUGCCUUCUGGCAUCGUU (SEQ ID NO: 3119); $(N)_{20-25}$ GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGC AACGCUCUGCCUUCUGGCAUCGUUUAUU (SEQ ID NO: 3120); $(N)_{20-25}$ GUUGUAGCUCCCUGGAAACCCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCC GCAACGCUCUGCCUUCUGGCAUCGUUUAUU (SEQ ID NO: 3121), where A, C, G, U, and N are adenine, cytosine, guanine, uracil, and any ribonucleotide, respectively, unless otherwise indicated. In some embodiments, N equals 24. In some embodiments, N equals 25.

[0897] In some embodiments, at least 10 nucleotides of the conserved portion of the sgRNA are modified nucleotides.

[0898] In some embodiments, the sgRNA comprises a conserved region comprising one of the following sequences in 5' to 3' orientation:

(SEQ ID NO: 707)

```
GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGm
GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCC
mUmUmCmUGmGCmAmUC*mG*mU*mU;
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or

(SEQ ID NO: 710)

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GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGm
GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU
mUmCmUGGCAUCG*mU*mU;
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or any other modified conserved region motifs disclosed in Table 9, including any one of SEQ ID NOs: 715-723, where A, C, G, U, and N are adenine, cytosine, guanine, uracil, and any ribonucleotide, respectively, unless otherwise indicated. An m is indicative of a 2'O-methyl modification, and an * is indicative of a phosphorothioate linkage between the nucleotides.

[0899] In certain embodiments, the HLA-B guide sequence is 20-25 nucleotides in length ($(N)_{20-25}$), wherein each nucleotide may be independently modified. In certain embodiments, each of nucleotides 1-3 of the 5' end of the guide is independently modified. In certain embodiments, each of nucleotides 1-3 of the 5' end of the guide is independently modified with a 2'-OMe modification. In certain embodiments, each of nucleotides 1-3 of the 5' end of the guide is independently modified with a phosphorothioate linkage to the adjacent nucleotide residue. In certain embodiments, each of nucleotides 1-3 of the 5' end of the guide is independently modified with a 2'-OMe modification and a phosphorothioate linkage to the adjacent nucleotide residue.

[0900] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a "modified" gRNA or "chemically modified" gRNA, to describe the presence of one or more non-naturally or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. Modified nucleosides and nucleotides can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[0901] Chemical modifications such as those listed above can be combined to provide modified gRNAs comprising nucleosides and nucleotides (collectively "residues") that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, e.g., all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[0902] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%,

at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[0903] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, e.g., modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[0904] Examples of modified phosphate groups include phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters.

[0905] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[0906] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, i.e. at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, e.g. replaced with a number of different "oxy" or "deoxy" substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion. Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein "R" can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, e.g., H or optionally substituted alkyl, and n can be an integer from 0 to 20. In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include "locked" nucleic acids (LNA) in which the 2' hydroxyl can be connected, e.g., by a C1-6 alkylene or C1-6 heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges. In some embodiments, the 2' hydroxyl group modification can included "unlocked" nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), ($OCH_2CH_2OCH_3$, e.g., a PEG derivative).

[0907] "Deoxy" 2' modifications can include hydrogen (i.e. deoxyribose sugars, e.g., at the overhang portions of partially dsRNA); halo (e.g., bromo, chloro, fluoro, or iodo); amino (wherein amino can be, e.g., NH2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH2CH_2$— amino (wherein amino can be, e.g., as described herein), —NHC(O)R (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino as described herein.

[0908] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing e.g., arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form, e.g. L-nucleosides.

[0909] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[0910] In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifications. Such modifications may be at one or both ends of the crRNA or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification. In certain embodiments, one or more or all of the nucleotides in single stranded overhang of a gRNA molecule are deoxynucleotides.

[0911] In some embodiments, the gRNAs disclosed herein comprise one of the modification patterns disclosed in WO2018/107028 A1, published Jun. 14, 2018 the contents of which are hereby incorporated by reference in their entirety.

[0912] The terms "mA," "mC," "mU," or "mG" may be used to denote a nucleotide that has been modified with 2'-O-Me. The terms "TA," "fC," "fU," or "fG" may be used to denote a nucleotide that has been substituted with 2'-F. A "*" may be used to depict a PS modification. The terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond. The terms "mA*," "mC*," "mU*," or "mG*" may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

TABLE 8A

Exemplary spyCas9 sgRNA-1 (SEQ ID NO: 600)																													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
G	U	U	U	U	A	G	A	G	C	U	A	G	A	A	A	U	A	G	C	A	A	G	U	U	A	A	A	A	U
		LS1-LS6					B1-B2						US1-US12									B3-B6				LS7-LS12			
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
A	A	G	G	C	U	A	G	U	CC		G	U	U	A	U	C	A	A	C	U	U	G	A	A	A	A	A	G	U
								Nexus														H1-1 through H1-12							
61		62		63		64		65		66	67		68		69		70		71		72		73		74	75		76	
G		G		C		A		C		C	G		A		G		U		C		G		G		U	G		C	
N													H2-1 through H2-15																

TABLE 8B

exemplary NmeCas9 sgRNA (SEQ ID NO: 3126)													
1-24				25	26	27	28	29	30	31	32	33	34
NNNNNNNNNNNNNNNNNNNNNNNNN				G	U	U	G	U	A	G	C	U	C
Guide region								Lower stem Repeat/Anti-Repeat region					
35	36	37	38	39	40	41	42	43	44	45	46	47	48
C	C	U	U	U	C	U	C	A	U	U	U	C	G
							Upper stem Repeat/Anti-Repeat region						
49	50	51	52	53	54	55	56	57	58	59	60	61	62
G	A	A	A	C	G	A	A	A	U	G	A	G	A
		Loop						Upper stem Repeat/Anti-Repeat region					
63	64	65	66	67	68	69	70	71	72	73	74	75	76
A	C	C	G	U	U	G	C	U	A	C	A	A	U
							Lower Stem Repeat/Anti-Repeat region						
77	78	79	80	81	82	83	84	85	86	87	88	89	90
A	A	G	G	C	C	G	U	C	U	G	A	A	A
					Stem			Hairpin 1				Loop	
91	92	93	94	95	96	97	98	99	100	101	102	103	104
A	G	A	U	G	U	G	C	C	G	C	A	A	C
			Stem (96: unpaired) Hairpin 1									Lower stem Hairpin 2	
105	106	107	108	109	110	111	112	113	114	115	116	117	118
G	C	U	C	U	G	C	C	C	C	U	U	A	A
		Bulge				Hairpin 2		Upper Stem					
119	120	121	122	123	124	125	126	127	128	129	130	131	132
A	G	C	U	U	C	U	G	C	U	U	U	A	A
					Loop	Hairpin 2				Upper Stem			
133	134	135	136	137	138	139	140	141	142	143	144	145	
G	G	G	G	C	A	U	C	G	U	U	U	A	
		Upper Stem				Bulge Hairpin 2		Lower Stem					

I. Ribonucleoprotein Complex

[0913] In some embodiments, the disclosure provides compositions comprising one or more gRNAs comprising one or more guide sequences from Tables 2-7 and an RNA-guided DNA binding agent, e.g., a nuclease, such as a Cas nuclease, such as Cas9. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes, N. meningitidis, S. aureus*, and other prokaryotes (see e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., NAT. REV. MICROBIOL. 9:467-477 (2011); Makarova et al., NAT. REV. MICROBIOL, 13: 722-36 (2015); Shmakov et al., MOLECULAR CELL, 60:385-397 (2015). In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. In some embodiments, the RNA-guided nickase is modified or derived from a Cas protein, such as a Class 2 Cas nuclease (which may be, e.g., a Cas nuclease of Type II, V, or VI). Class 2 Cas nuclease include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins and modifications thereof.

[0914] Non-limiting exemplary species that the Cas nuclease or Cas nickase can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus* sp., *Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gamma proteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas* sp., *Crocosphaera watsonii, Cyanothece* sp., *Microcystis aeruginosa, Synechococcus* sp., *Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter* sp., *Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc* sp., *Arthrospira maxima, Arthrospira platensis, Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes, Oscillatoria* sp., *Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus* sp., *Lachnospiraceae bacterium* ND2006, and *Acaryochloris marina.*

[0915] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae bacterium* ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or Lachnospiraceae.

[0916] In some embodiments, the Cas nickase is derived from the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nickase is derived from the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nickase is a nickase form of the Cas9 nuclease from *Neisseria meningitidis*. See e.g., WO/2020081568, describing an Nme2Cas9 D16A nickase fusion protein. In some embodiments, the Cas nickase is derived from the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Lachnospiraceae bacterium* ND2006. In further embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nickase is derived from a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*. As discussed elsewhere, a nickase may be derived from a nuclease by inactivating one of the two catalytic domains, e.g., by mutating an active site residue essential for nucleolysis, such as D10, H840, of N863 in Spy Cas9. One skilled in the art will be familiar with techniques for easily identifying corresponding residues in other Cas proteins, such as sequence alignment and structural alignment, which is discussed in detail below.

[0917] In some embodiments, the gRNA together with an RNA-guided DNA binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II

CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[0918] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[0919] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as Fok1. In some embodiments, a Cas nuclease may be a modified nuclease.

[0920] In other embodiments, the Cas nuclease or Cas nickase may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[0921] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See e.g., U.S. Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain.

[0922] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[0923] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015) Cell October 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein. See, e.g., Zetsche et al. (2015). Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain or RuvC or RuvC-like domains for *N. meningitidis* include Nme2Cas9D16A (HNH nickase) and Nme2Cas9H588A (RuvC nickase). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB—A0Q7Q2 (CPF1_FRATN)).

[0924] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[0925] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1.

[0926] In some embodiments, the RNA-guided DNA binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[0927] In some embodiments, the RNA-guided DNA binding agent comprises a APOBEC3 deaminase. In some embodiments, a APOBEC3 deaminase is a APOBEC3A (A3A). In some embodiments, the A3A is a human A3A. In some embodiments, the A3A is a wild-type A3A.

[0928] In some embodiments, the RNA-guided DNA binding agent comprises a deaminase and an RNA-guided nickase. In some embodiments, the mRNA further comprises a linker to link the sequencing encoding A3A to the sequence sequencing encoding RNA-guided nickase. In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is a peptide linker. In some embodiments, the peptide linker is any stretch of amino acids having at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, or more amino acids. In some embodiments, the peptide linker is the 16 residue "XTEN" linker, or a variant thereof (See, e.g., the Examples; and Schellenberger et al. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. Nat. Biotechnol. 27, 1186-1190 (2009)). In some embodiments, the XTEN linker comprises the sequence

SGSETPGTSESATPES (SEQ ID NO: 900), SGSETPGTSESA (SEQ ID NO: 901), or SGSETPGTSESATPEGGSGGS (SEQ ID NO: 902).

[0929] In some embodiments, the peptide linker comprises a $(GGGGS)_n$ (SEQ ID NO: 910), a $(G)_n$, an $(EAAAK)_n$ (SEQ ID NO: 911), a $(GGS)_n$, an SGSETPGTSESATPES (SEQ ID NO: 906) motif (see, e.g., Guilinger J P, Thompson D B, Liu D R. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32(6): 577-82; the entire contents are incorporated herein by reference), or an $(XP)_n$ motif, or a combination of any of these, wherein n is independently an integer between 1 and 30 (SEQ DI NO: 3123). See, WO2015089406, e.g., paragraph [0012], the entire content of which is incorporated herein by reference.

[0930] In some embodiments, the peptide linker comprises one or more sequences selected from SEQ ID NOs: 906-970.

[0931] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be fused at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. In some embodiments, the NLS is not fused to the C-terminus. It may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two NLS sequences (e.g., SV40) fused at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, e.g., the SV40 NLS, PKKKRKV (SEQ ID NO: 903) or PKKKRRV (SEQ ID NO: 904). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 905). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 903) NLS may be fused at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

[0932] In some embodiments, the RNA-guided DNA binding agent comprises an editor. An exemplary editor is BC22n which includes a *H. sapiens* APOBEC3A fused to *S. pyogenes*-DIOA Cas9 nickase by an XTEN linker, and mRNA encoding BC22n. An mRNA encoding BC22n is provided (SEQ ID NO: 804).

[0933] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUBI), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFMI), and ubiquitin-like protein-5 (UBL5).

[0934] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (e.g., YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (e.g., EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6×His (SEQ ID NO: 3124), 8×His (SEQ ID NO: 3125), biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[0935] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[0936] In further embodiments, the heterologous functional domain may be an effector domain such as an editor domain. When the RNA-guided DNA-binding agent is directed to its target sequence, e.g., when a Cas nuclease is directed to a target sequence by a gRNA, the effector such as an editor domain may modify or affect the target sequence. In some embodiments, the effector such as an editor domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., U.S. Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell* 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Methods* 10:973-6 (2013); Mali et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *Cell* 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

J. Determination of Efficacy of Guide RNAs

[0937] In some embodiments, the efficacy of a guide RNA is determined when delivered or expressed together with other components (e.g., an RNA-guided DNA binding agent) forming an RNP. In some embodiments, the guide RNA is expressed together with an RNA-guided DNA binding agent, such as a Cas protein, e.g., Cas9. In some embodiments, the guide RNA is delivered to or expressed in a cell line that already stably expresses an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, e.g., Cas9 nuclease or nickase. In some embodiments the guide RNA is delivered to a cell as part of a RNP. In some embodiments, the guide RNA is delivered to a cell along with a mRNA encoding an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, e.g., Cas9 nuclease or nickase.

[0938] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to DSBs, SSBs, or site-specific binding that results in nucleic acid modification in the DNA or pre-mRNA which can produce errors in the form of insertion/deletion (indel) mutations upon repair by cellular machinery. Many mutations due to indels alter the reading frame, introduce premature stop codons, or induce exon skipping and, therefore, produce a non-functional protein.

[0939] In some embodiments, the efficacy of particular guide RNAs is determined based on in vitro models. In some embodiments, the in vitro model is T cell line. In some embodiments, the in vitro model is HEK293 T cells. In some embodiments, the in vitro model is HEK293 cells stably expressing Cas9 (HEK293_Cas9). In some embodiments, the in vitro model is a lymphoblastoid cell line. In some embodiments, the in vitro model is primary human T cells. In some embodiments, the in vitro model is primary human B cells. In some embodiments, the in vitro model is primary human peripheral blood lymphocytes. In some embodiments, the in vitro model is primary human peripheral blood mononuclear cells.

[0940] In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an in vitro model is determined, e.g., by analyzing genomic DNA from the cells transfected in vitro with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from cells transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[0941] In some embodiments, the efficacy of particular gRNAs is determined across multiple in vitro cell models for a guide RNA selection process. In some embodiments, a cell line comparison of data with selected guide RNAs is performed. In some embodiments, cross screening in multiple cell models is performed.

[0942] In some embodiments, the efficacy of a guide RNA is evaluated by on target cleavage efficiency. In some embodiments, the efficacy of a guide RNA is measured by percent editing at the target location, e.g., HLA-A, HLA-B, or CIITA. In some embodiments, deep sequencing may be utilized to identify the presence of modifications (e.g., insertions, deletions) introduced by gene editing. Indel percentage can be calculated from next generation sequencing "NGS."

[0943] In some embodiments, the efficacy of a guide RNA is measured by the number or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs are provided which produce indels at off target sites at very low frequencies (e.g., <5%) in a cell population or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (e.g., T cells or B cells), or which produce a frequency of off-target indel formation of <5% in a cell population or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs which do not exhibit any off target indel formation in the target cell type (e.g., T cells or B cells). In some embodiments, guide RNAs are provided which produce indels at less than 5 off-target sites, e.g., as evaluated by one or more methods described herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) e.g., as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (e.g., T cells or B cells) genome.

[0944] In some embodiments, linear amplification is used to detect gene editing events, such as the formation of insertion/deletion ("indel") mutations, translocations, and homology directed repair (HDR) events in target DNA. For example, linear amplification with a unique sequence-tagged primer and isolating the tagged amplification products (herein after referred to as "UnIT," or "Unique Identifier Tagmentation" method) may be used.

[0945] In some embodiments, the efficacy of a guide RNA is measured by the number of chromosomal rearrangements within the target cell type. Kromatid dGH assay may used to detect chromosomal rearrangements, including e.g., translocations, reciprocal translocations, translocations to off-target chromosomes, deletions (i.e., chromosomal rearrangements where fragments were lost during the cell replication cycle due to the editing event). In some embodiments, the target cell type has less than 10, less than 8, less than 5, less than 4, less than 3, less than 2, or less than 1 chromosomal rearrangement. In some embodiments, the target cell type has no chromosomal rearrangements.

K. Delivery of gRNA Compositions

[0946] Lipid nanoparticles (LNP compositions) are a well-known means for delivery of nucleotide and protein cargo and may be used for delivery of the guide RNAs, compositions, or pharmaceutical formulations disclosed herein. In some embodiments, the LNP compositions deliver nucleic acid, protein, or nucleic acid together with protein.

[0947] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to a subject, wherein the gRNA is formulated as an LNP. In some embodiments, the LNP comprises the gRNA and a Cas9 or an mRNA encoding Cas9.

[0948] In some embodiments, the invention comprises a composition comprising any one of the gRNAs disclosed and an LNP. In some embodiments, the composition further comprises a Cas9 or an mRNA encoding Cas9.

[0949] In some embodiments, the LNP compositions comprise cationic lipids. In some embodiments, the LNP compositions comprise (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate) (Lipid A) or another ionizable lipid. See, e.g., lipids of WO/2017/173054 and references described therein. In some embodiments, the LNP compositions comprise molar ratios of a cationic lipid amine to RNA phosphate (N:P) of about 4.5, 5.0, 5.5, 6.0, or 6.5. In some embodiments, the term cationic and ionizable in the context of LNP lipids is interchangeable, e.g., wherein ionizable lipids are cationic depending on the pH.

[0950] In some embodiments, the LNP comprises a lipid component, and the lipid component comprises: about 35 mol % Lipid A; about 15 mol % neutral lipid (e.g., distearoylphosphatidylcholine (DSPC)); about 47.5 mol % helper lipid (e.g., cholesterol); and about 2.5 mol % stealth lipid (e.g., 1,2-dimyristoyl-rac-gly cero-3-methylpolyoxyethylene glycol 2000 (PEG2k-DMG)), and wherein the N/P ratio of the LNP composition is about 3-7.

[0951] In some embodiments, the gRNAs disclosed herein are formulated as LNP compositions for use in preparing a medicament for treating a disease or disorder.

[0952] Electroporation is a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and Cas9 or an mRNA encoding Cas9.

[0953] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an ex vivo cell, wherein the gRNA is formulated as an LNP or not formulated as an LNP. In some embodiments, the LNP comprises the gRNA and a Cas9 or an mRNA encoding Cas9.

[0954] In some embodiments, the guide RNA compositions described herein, alone or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see e.g., WO/2017/173054 and WO 2019/067992, the contents of which are hereby incorporated by reference in their entirety.

[0955] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *S. pyogenes* (i.e., Spy Cas9). In one embodiment, the Cas9 nuclease is from *N. meningitidis* (i.e., Nine Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

L. Therapeutic Methods and Uses

[0956] Any of the engineered human cells and compositions described herein can be used in a method of treating a variety of diseases and disorders, as described herein. In some embodiments, the genetically modified cell (engineered cell) or population of genetically modified cells (engineered cells) and compositions may be used in methods of treating a variety of diseases and disorders. In some embodiments, a method of treating any one of the diseases or disorders described herein is encompassed, comprising administering any one or more composition described herein.

[0957] In some embodiments, the methods and compositions described herein may be used to treat diseases or disorders in need of delivery of a therapeutic agent. In some embodiments, the invention provides a method of providing an immunotherapy in a subject, the method including administering to the subject an effective amount of an engineered cell (or population of engineered cells) as described herein, for example, a cell of any of the aforementioned cell aspects and embodiments.

[0958] In some embodiments, the methods comprise administering to a subject a composition comprising an

engineered cell described herein as an adoptive cell transfer therapy. In some embodiments, the engineered cell is an allogeneic cell.

[0959] In some embodiments, the methods comprise administering to a subject a composition comprising an engineered cell described herein, wherein the cell produces, secretes, or expresses a polypeptide (e.g., a targeting receptor) useful for treatment of a disease or disorder in a subject. In some embodiments, the cell acts as a cell factory to produce a soluble polypeptide. In some embodiments, the cell acts as a cell factory to produce an antibody. In some embodiments, the cell continuously secretes the polypeptide in vivo. In some embodiments, the cell continuously secretes the polypeptide following transplantation in vivo for at least 1, 2, 3, 4, 5, or 6 weeks. In some embodiments, the cell continuously secretes the polypeptide following transplantation in vivo for more than 6 weeks. In some embodiments, the soluble polypeptide (e.g., an antibody) is produced by the cell at a concentration of at least 10^2, 10^3, 104, 105, 10^6, 107, or 10^8 copies per day. In some embodiments, the polypeptide is an antibody and is produced by the cell at a concentration of at least 10^8 copies per day.

[0960] In some embodiments of the methods, the method includes administering a lymphodepleting agent or immunosuppressant prior to administering to the subject an effective amount of the engineered cell (or engineered cells) as described herein, for example, a cell of any of the aforementioned cell aspects and embodiments. In another aspect, the invention provides a method of preparing engineered cells (e.g., a population of engineered cells).

[0961] Immunotherapy is the treatment of disease by activating or suppressing the immune system. Immunotherapies designed to elicit or amplify an immune response are classified as activation immunotherapies. Cell-based immunotherapies have been demonstrated to be effective in the treatment of some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer (NK) cells, cytotoxic T lymphocytes (CTLs), T helper cells, B cells, or their progenitors such as hematopoietic stem cells (HSC) or induced pluripotent stem cells (iPSC) can be programmed to act in response to abnormal antigens expressed on the surface of tumor cells. Thus, cancer immunotherapy allows components of the immune system to destroy tumors or other cancerous cells. Cell-based immunotherapies have also been demonstrated to be effective in the treatment of autoimmune diseases or transplant rejection. Immune effector cells such as regulatory T cells (Tregs) or mesenchymal stem cells can be programmed to act in response to autoantigens or transplant antigens expressed on the surface of normal tissues.

[0962] In some embodiments, the invention provides a method of preparing engineered cells (e.g., a population of engineered cells). The population of engineered cells may be used for immunotherapy.

[0963] In some embodiments, the invention provides a method of treating a subject in need thereof that includes administering engineered cells prepared by a method of preparing cells described herein, for example, a method of any of the aforementioned aspects and embodiments of methods of preparing cells.

[0964] In some embodiments, the engineered cells can be used to treat cancer, infectious diseases, inflammatory diseases, autoimmune diseases, cardiovascular diseases, neurological diseases, ophthalmologic diseases, renal diseases, liver diseases, musculoskeletal diseases, red blood cell diseases, or transplant rejections. In some embodiments, the engineered cells can be used in cell transplant, e.g., to the heart, liver, lung, kidney, pancreas, skin, or brain. (See e.g., Deuse et al., Nature Biotechnology 37:252-258 (2019).)

[0965] In some embodiments, the engineered cells can be used as a cell therapy comprising an allogeneic stem cell therapy. In some embodiments, the cell therapy comprises induced pluripotent stem cells (iPSCs). iPSCs may be induced to differentiate into other cell types including e.g., cardiomyocytes, beta islet cells, neurons, and blood cells. In some embodiments, the cell therapy comprises hematopoietic stem cells. In some embodiments, the stem cells comprise mesenchymal stem cells that can develop into bone, cartilage, muscle, and fat cells. In some embodiments, the stem cells comprise ocular stem cells. In some embodiments, the allogeneic stem cell transplant comprises allogeneic bone marrow transplant. In some embodiments, the stem cells comprise pluripotent stem cells (PSCs). In some embodiments, the stem cells comprise induced embryonic stem cells (ESCs).

[0966] The engineered human cells disclosed herein are suitable for further engineering, e.g., by introduction of further edited, or modified genes or alleles. Cells of the invention may also be suitable for further engineering by introduction of an exogenous nucleic acid encoding e.g., a targeting receptor, e.g., a TCR, CAR, UniCAR. CARs are also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors. In some embodiments, the TCR is a wild-type or variant TCR.

[0967] In some embodiments, the cell therapy is a transgenic T cell therapy. In some embodiments, the cell therapy comprises a Wilms' Tumor 1 (WT1) targeting transgenic T cell. In some embodiments, the cell therapy comprises a targeting receptor or a donor nucleic acid encoding a targeting receptor of a commercially available T cell therapy, such as a CAR T cell therapy. There are number of targeting receptors currently approved for cell therapy. The cells and methods provided herein can be used with these known constructs. Commercially approved cell products that include targeting receptor constructs for use as cell therapies include e.g., Kymriah® (tisagenlecleucel); Yescarta® (axicabtagene ciloleucel); Tecartus™ (brexucabtagene autoleucel); Tabelecleucel (Tab-cel®); Viralym-M (ALVR105); and Viralym-C.

[0968] In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is an injection. In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is an intravascular injection or infusion. In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is a single dose.

[0969] In some embodiments, the methods provide for reducing a sign or symptom associated of a subject's disease treated with a composition disclosed herein. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than one week. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than two weeks. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than three weeks. In some embodiments, the

subject has a response to treatment with a composition disclosed herein that lasts more than one month.

[0970] In some embodiments, the methods provide for administering the engineered cells to a subject, and wherein the subject has a response to the administered cell that comprises a reduction in a sign or symptom associated with the disease treated by the cell therapy. In some embodiments, the subject has a response that lasts more than one week. In some embodiments, the subject has a response that lasts more than one month. In some embodiments, the subject has a response that lasts for at least 1-6 weeks.

TABLE 9

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Exemplary SpyCas9 sgRNA scaffold	600	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC
Exemplary SpyCas 9 crRNA 3' Sequence	601	GUUUUAGAGCUAUGCUGUUUUG
Exemplary SpyCas 9 sgRNA Scaffold	602	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
Exemplary SpyCas9 sgRNA Scaffold	603	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC
Exemplary SpyCas9 crRNA Scaffold	604	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGGUGC
	605-612	See Table 9A
	613-698	Not used
Exemplary NmeCas9 guide RNA scaffold	699	GUUGUAGCUCCCUUUCUCAUUUCGGAAACGAAAUGAGAACCGUUGCUACAAUAAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCU GCCCCUUAAAGCUUCUGCUUUAAGGGGCAUCGUUUA
Exemplary NmeCas 9 guide RNA	700	$(N)_{20-25}$ GUUGUAGCUCCCUUUCUCAUUUCGGAAACGAAAUGAGAACCGUUGCUACAAUAAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCU GCCCCUUAAAGCUUCUGCUUUAAGGGGCAUCGUUUA
Shortened/unmodified NmeCas9 guide RNA motif	3119	$(N)_{20-25}$GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
Shortened/unmodified NmeCas9 guide RNA motif	3120	$(N)_{20-25}$ GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU
Shortened/unmodified NmeCas9 guide RNA motif	3121	$(N)_{20-25}$ GUUGUAGCUCCCUGGAAACCCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU
Shortened/unmodified NmeCas9 guide RNA motif	704	$(N)_{20-25}$ GUUGUAGCUCCCUUCGAAAGACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Shortened/modified NmeCas9 guide RNA motif (101-mer)	705	mN*mNNNNNNNNmNNNmNNNNNNNNNNNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmG mUmCmGmAmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU
Shortened/modified NmeCas9 guide RNA motif	706	$(N)_{20-25}$GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGm AmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU
Shortened/modified NmeCas9 guide RNA motif	707	GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmAmA mCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU
Shortened/modified NmeCas9 guide RNA motif (101-mer)	708	mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif	709	$(N)_{20-25}$GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGm AmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif	710	GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAm CGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif (105-mer)	711	mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCU AmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif (101-mer)	712	mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAA GmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif (101-mer)	713	mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
Shortened/modified NmeCas9 guide RNA motif (101-mer)	714	mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAA GmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	715	mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmAm AmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU
Guide scaffold	716	mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAA mCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Guide scaffold	717	mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAm CGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	718	mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAA mCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	719	mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAm CGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	720	mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUG CmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	721	mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGC mCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	722	mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUG CmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
Guide scaffold	723	mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGC mCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU
	724-799	Not used
Recombinant Cas9-NLS amino acid sequence	800	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSELKDD SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI DLSQLGGDGGGSPKKKRKV
ORF encoding Spy Cas9	801	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGA GCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGA AACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCT
		GGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC
		GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAA
		ACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGC
		ACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAAC
		TTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAG
		ACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAAT
		CACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGA
		CAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCC
		AGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGA
		CCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA
		CAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGAC
		CGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGT
		CGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAG
		CACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCAT
		TCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGA
		CTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCAC
		GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGA
		CACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAA
		GAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG
		GACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCC
		AGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAAT
		CCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGA
		GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCC
		AGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACAT
		GTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGAC
		AGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGA
		TGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGG
		ACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTG
		GACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCA
		GCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGT
		CGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAG
		ATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAG
		AAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGG
		AAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTC
		AGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGAT
		TCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGA
		ACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTC
		AAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAAGAGAATGCTGGCAAGCGCAGGAG
		AACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGG
		AAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA
		TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAG
		AACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGA
		CAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATC
		GACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
ORF encoding Spy Cas9	802	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCT CCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGA GACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATC TTCTCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCT GGTGGACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGA ACCCCATCAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGC CCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAAC TTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCG ACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGAT CACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGG CAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCC AGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGA CCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGG CAGGAGGACTTCTACCCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCC CCCTGGCCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGT GGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAG CACTCCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCT TCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGA CTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCAC GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGA CCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAA GCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTG GACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCC AGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCAT CCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGG GAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCC AGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACAT GTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGA TGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGG CCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTG GACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGT CCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGT GGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAG ATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCG AGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG CCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTC TCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCT TCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGA GCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG AAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCG AGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGG CTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAG TTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGA CCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATC GACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA
Open reading frame for Spy Cas9 with Hibit tag	803	AUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCU CCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUCCGGCGA GACCGCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUC UUCUCCAACGAGAUGGCCAAGGUGGACGACUCCUUCUUCCACCGGCUGGAGGAGUCCUUCCUGGUGGAGGAGGACAAGAAGCACG AGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUACCCCACCAUCUACCACCUGCGGAAGAAGCU GGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCCGGGGCCACUUCCUGAUC GAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUCCAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGA ACCCCAUCAACGCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCUGUCCAAGUCCCGGCGGCUGGAGAACCUGAUCGC CCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCCUGUCCCUGGGCCUGACCCCCAACUUCAAGUCCAAC UUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGCG ACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGUCCGACGCCAUCCUGCUGUCCGACAUCCUGCGGGUGAACACCGAGAU CACCAAGGCCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUGGUGCGG CAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCUCCC AGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCGGGAGGA CCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGG CAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUGACCUUCCGGAUCCCCUACUACGUGGGCC CCCUGGCCCGGGGCAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCACCCCCUGGAACUUCGAGGAGGUGGU GGACAAGGGCGCCUCCGCCCAGUCCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCUGCCCAAG CACUCCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCU UCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGA CUACUUCAAGAAGAUCGAGUGCUUCGACUCCGUGGAGAUCUCCGGCGUGGAGGACCGGUUCAACGCCUCCCUGGGCACCUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGACCCUGA CCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGACAAGGUGAUGAAGCAGCUGAA GCGGCGGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUG GACUUCCUGAAGUCCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACUCCCUGACCUUCAAGGAGGACAUCC AGAAGGCCCAGGUGUCCGGCCAGGGCGACUCCCUGCACGAGCACAUCGCCAACCUGGCCGGCUCCCCCGCCAUCAAGAAGGGCAU CCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCGG GAGAACCAGACCACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCUCCC AGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAU GUACGUGGACCAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCUUCCUGAAGGACGAC UCCAUCGACAACAAGGUGCUGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAGGUGGUGAAGAAGA UGAAGAACUACUGGCGGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGGCGG CCUGUCCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGGCCCAGAUCCUG GACUCCCGGAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAGUCCAAGCUGGUGU CCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAACGCCGU GGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAG AUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCAAGACCG AGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGACAAGGG CCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGCGGCUUC UCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACGGCGGCU UCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGUGAAGGA GCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAGGAGGUG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCGCCGGCG AGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCUGAAGGG CUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUCUCCGAG UUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGG AGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCACCAUCGA CCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACCCGGAUC GACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCCGAGUCCGUGU CCGGCUGGCGGCUGUUCAAGAAGAUCUCCUGA
Open Reading frame for BC22n	804	AUGgaggccucccccgccuccggcccccggcaccugauggacccccacaucuucaccuccAACUUCAACAACggcAUCggccggC ACAAGaccUACCUGUGCUACgaggguggagcggCUGGACAACggcaccuccgugAAGAUGGACCAGCACcggggcUUCCUGCACAA CCAGgccAAGAACCUGCUGUGCggcUUCUACggccggCACgccgagCUGcggUUCCUGGACCUGgugcccuccCUGCAGCUGGAC cccgccCAGAUCUACcgggugaccUGGUUCAUCuccUGGucccccUGCUUCuccUGGggcUGCgccggcgaggugcgggccUUCC UGCAGgagAACaccCACgugcggCUGcggAUCUUCgccgcccggAUCUACGACUACGACcccCUGUACAAGgaggccCUGCAGAU GCUGcggGACgccggcgccCAGguguccAUCAUGaccUACGACgagUUCAAGCACUGCUGGGACaccUUCgugGACCACCAGggc UGCcccUUCCAGcccUGGGACggcCUGGACgagCACuccCAGgccCUGuccggccggCUGcgggccAUCCUGCAGAACCAGggcA ACuccggcuccgagacccccggcaccuccgaguccgccacccccgaguccgacaagaaguacuccaucggccuggCcaucggcac caacuccgugggcugggccgugaucaccgacgaguacaaggugcccuccaagaaguucaaggugcugggcaacaccgaccggcac uccaucaagaagaaccugaucggcgcccugcuguucgacuccggcgagaccgccgaggccacccggcugaagcggaccgcccggc ggcgguacacccggcggaagaaccggaucugcuaccugcaggagaucuucuccaacgagauggccaagguggacgacuccuucuu ccaccggcuggaggaguccuuccuggugggaggaggacaagaagcacgagcggcaccccaucuucggcaacaucguggacgaggug gccuaccacgagaaguaccccaccaucuaccaccugcggaagaagcugguggacuccaccgacaaggccgaccugcggcugaucu accuggcccuggcccacaugaucaaguuccggggccacuuccugaucgagggcgaccugaaccccgacaacuccgacguggacaa gcuguucauccagcuggugcagaccuacaaccagcuguucgaggagaaccccaucaacgccuccggcguggacgccaaggccauc cuguccgcccggcuguccaaguccccggcggcuggagaaccugaucgcccagcugcccggcgagaagaagaacggccuguucggca accugaucgcccuguccccuggccugaccccccaacuucaaguccaacuucgaccuggccgaggacgccaagcugcagcuguccaa ggacaccuacgacgacgaccuggacaaccugcuggcccagaucggcgaccaguacgccgaccuguuccuggccgccaagaaccug uccgacgccauccugcuguccgacauccugcgggugaacaccgagaucaccaaggcccccccuguccgccuccaugaucaagcggu acgacgagcaccaccaggaccugacccugcugaaggcccuggugcggcagcagcugcccgagaaguacaaggagaucuucuucga ccaguccaagaacggcuacgccggcuacaucgacggcggcgcccuccaggaggaguucuacaaguucaucaagcccauccuggag aagauggacggcaccgaggagcugcuggugaagcugaaccgggaggaccugcugcggaagcagcggaccuucgacaacggcucca ucccccaccagauccaccuggcgagcugcacgccauccugcggcggcaggaggacuucuaccccuuccugaaggacaaccggga gaagaucgagaagauccugaccuuccggauccccuacuacguggggccccuggcccggggcaacucccgguucgccuggaugacc cggaaguccgaggagaccaucacccccuggaacuucgaggaggugguggacaagggcgccuccgcccaguccuucaucgagcgga ugaccaacuucgacaagaaccugcccaacgagaaggugcugcccaagcacucccugcuguacgaguacuucaccgguuacaacga gcugaccaaggugaaguacgugaccgagggcaugcggaagcccgccuuccuguccggcgagcagaagaaggccaucguggaccug cuguucaagaccaaccggaaggugaccgugaagcagcugaaggaggacuacuucaagaagaucgagugcuucgacuccguggaga ucuccggcguggaggaccgguucaacgccucccugggcaccuaccacgaccugcugaagaucaucaaggacaaggacuuccugga caacgaggagaacgaggacauccuggaggacaucgugcugacccugacccuguucgaggaccgggagaugaucgaggagcggcug aagaccuacgcccaccuguucgacgacaaggugaugaagcagcugaagcggcggcggcuacaccggcugggggccggcuguucccgga agcugaucaacggcauccgggacaagcaguccggcaagaccauccuggacuuccugaaguccgacggcuucgccaaccggaacuu caugcagcugauccacgacgacucccugaccuucaaggaggacauccagaaggcccaggugucccggccagggcgacucccugcac gagcacaucgccaaccuggccggcucccccgccaucaagaagggcauccugcagaccgugaaggugguggacgagcuggugaagg ugauggccggcacaagcccgagaacaucgugaucgagauggcccgggagaaccagaccacccagaagggccagaagaacucccg ggagcggaugaagcggaucgaggagggcaucaaggagcugggcucccagauccugaaggagcaccccguggagaacacccagcug cagaacgagaagcuguaccuguacuaccugcagaacggccgggacauguacguggaccaggagcuggacaucaaccggcuguccg

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		acuacgacguggaccacaucgugccccaguccuuccugaaggacgacuccaucgacaacaaggugcugacccgguccgacaagaa ccggggcaaguccgacaacgugcccuccgaggagguggugaagaagaugaagaacuacuggcggcagcugcugaacgccaagcug aucacccagcggaaguucgacaaccugaccaaggccgagcggggCggccuguccgagcuggacaaggccggcuucaucaagcggc agcugguggagacccggcagaucaccaagcacguggcccagauccuggacucccggaugaacaccaaguacgacgagaacgacaa gcugauccgggaggugaaggugaucacccugaaguccaagcuggugucccgacuuccggaaggacuuccaguucuacaaggugcgg gagaucaacaacuaccaccacgcccacgacgccuaccugaacgccgugguggggcaccgcccugaucaagaaguaccccaagcugg aguccgaguucguguacggcgacuacaagguguacgacgugcggaagaugaucgccaaguccgagcaggagaucggcaaggccac cgccaaguacuucuucuacuccaacaucaugaacuucuucaagaccgagaucacccuggccaacggcgagauccggaagcggccc cugaucgagaccaacggcgagaccggcgagaucguguggggacaagggccgggacuucgccaccgugcggaaggugcuguccaugc cccaggugaacaucgugaagaagaccgaggugcagaccggcggcuucuccaaggaguccauccugcccaagcggaacuccgacaa gcugaucgcccggaagaaggacugggaccccaagaaguacggcggcuucgacucccccaccguggccuacuccgugcugguggug gccaagguggagaagggcaaguccaagaagcugaaguccgugaaggagcugcugggcaucaccaucauggagcggucccuccuucg agaagaaccccaucgacuuccuggaggccaagggcuacaaggaggugaagaaggaccugaucaucaagcugcccaaguacucccu guucgagcuggagaacggccggaagcggaugcuggccuccgccggcgagcugcagaagggcaacgagcuggcccugcccuccaag uacgugaacuuccuguaccuggccucccacuacgagaagcugaagggcucccccgaggacaacgagcagaagcagcuguucgugg agcagcacaagcacuaccuggacgagaucaucgagcagaucuccgaguucuccaagcgggugauccuggccgacgccaaccugga caaggugcuguccgccuacaacaagcaccgggacaagcccauccgggagcaggccgagaacaucauccaccuguucacccugacc aaccugggcgccccccgccgccuucaaguacuucgacaccaccaucgaccggaagcgguacaccuccaccaaggaggugcuggacg ccacccugauccaccaguccaucaccggccuguacgagacccggaucgaccugucccagcugggcggcgacggcggcggcucccc caagaagaagcggaaggugUgA
Open reading frame for BC22n with Hibit tag	805	AUGgaggccucccccgccuccggcccccggcaccugauggacccccacaucuucaccuccAACUUCAACAACggcAUCggccggC ACAAGaccUACCUGUGCUACgaggugagcggCUGGACAACggcaccuccgugAAGAUGGACCAGCACcggggcUUCCUGCACAA CCAGgccAAGAACCUGCUGUGCggcUUCUACggccggCACgccgagCUGcggUUCCUGGACCUGgugcccuccCUGCAGCUGGAC cccgccCAGAUCUACcgggugaccUGGUUCAUCuccUGGucccccUGCUUCuccUGGggcUGCgccggcgaggugcgggccUUCC UGCAGgagAACaccCACgugcggCUGcggAUCUUCgccgcccggAUCUACGACUACGACcccCUGUACAAGgaggccCUGCAGAU GCUGcggGACgccggcgccCAGguguccAUCAUGaccUACGACgagUUCAAGCACUGCUGGGACaccUUCgugGACCACCAGggc UGCcccUUCCAGcccUGGGACggcCUGGACgagCACuccCAGgccCUGuccggccggCUGcgggccAUCCUGCAGAACCAGggcA ACuccggcuccgagacccccggcaccuccgaguccgccacccccgaguccgacaagaaguacuccaucggccuggCcaucggcac caacuccgugggcugggccgugaucaccgacgaguacaaggugcccuccaagaaguucaaggugcugggcaacaccgaccggcac uccaucaagaagaaccugaucggcgcccugcuguucgacuccggcgagaccgccgaggccacccggcugaagcggaccgcccggc ggcgguacacccggcggaagaaccggaucugcuaccugcaggagaucuucuccaacgagauggccaagguggacgacuccuucuu ccaccggcuggaggaguccuuccugguggaggaggacaagaagcacgagcggcaccccaucuucggcaacaucguggacgaggug gccuaccacgagaaguaccccaccaucuaccaccugcggaagaagcugguggacuccaccgacaaggccgaccugcggcugaucu accuggcccuggcccacaugaucaaguuccggggccacuuccugaucgagggcgaccugaaccccgacaacuccgacguggacaa gcuguucauccagcuggugcagaccuacaaccagcuguucgaggagaaccccaucaacgccuccggcguggacgccaaggccauc cuguccgcccggcuguccaagucccggcggcuggagaaccugaucgcccagcugcccggcgagaagaagaacggccuguucggca accugaucgcccuguccccuggccugacccccaacuucaaguccaacuucgaccuggccgaggacgccaagcugcagcuguccaa ggacaccuacgacgacgaccuggacaaccugcuggcccagaucggcgaccaguacgccgaccuguuccuggccgccaagaaccug uccgacgccauccugcuguccgacauccugcgggugaacaccgagaucaccaaggcccccuguccgccuccaugaucaagcggu acgacgagcaccaccaggaccugacccugcugaaggcccuggugcggcagcagcugcccgagaaguacaaggagaucuucuucga ccaguccaagaacggcuacgccggcuacaucgacggcggcgccucccaggaggaguucuacaaguucaucaagcccauccuggag aagauggacggcaccgaggagcugcuggugaagcugaaccgggaggaccugcugcggaagcagcggaccuucgacaacggcucca uccccaccagauccaccugggcgagcugcacgccauccugcggcggcaggaggacuucuaccccuuccugaaggacaaccggga gaagaucgagaagauccugaccuuccggauccccuacuacguggggccccuggcccggggcaacucccgguucgccuggaugacc cggaaguccgaggagaccaucacccccuggaacuucgaggaggugguggacaagggcgcccuccgcccaguccuucaucgagcgga

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		ugaccaacuucgacaagaaccugcccaacgagaaggugcugcccaagcacucccugcuguacgaguacuucaccguguacaacga gcugaccaaggugaaguacgugaccgagggcaugcggaagcccgccuuccuguccggcgagcagaagaaggccaucguggaccug cuguucaagaccaaccggaaggugaccgugaagcagcugaaggaggacuacuucaagaagaucgagugcuucgacuccguggaga ucuccggcguggaggaccgguucaacgccucccugggcaccuaccacgaccugcugaagaucaucaaggacaaggacuuccugga caacgaggagaacgaggacauccuggaggacaucgugcugacccugacccuguucgaggaccgggagaugaucgaggagcggcug aagaccuacgcccaccuguucgacgacaaggugaugaagcagcugaagcggcggcggcuacaccggcugggccggcuguucccgga agcugaucaacggcauccgggacaagcaguccggcaagaccauccuggacuuccugaaguccgacggcuucgccaaccggaacuu caugcagcugauccacgacgacucccugaccuucaaggaggacauccagaaggcccaggugucccggccagggcgacucccugcac gagcacaucgccaaccuggccggcucccccgccaucaagaagggcauccugcagaccgugaaggugguggacgagcuggugaagg ugaugggccggcacaagcccgagaacaucgugaucgagauggcccgggagaaccagaccacccagaagggccagaagaacucccg ggagcggaugaagcggaucgaggagggcaucaaggagcugggcucccagauccugaaggagcaccccguggagaacacccagcug cagaacgagaagcuguaccuguacuaccugcagaacggccgggacauguacguggaccaggagcuggacaucaaccggcuguccg acuacgacguggaccacaucgugccccaguccuuccugaaggacgacuccaucgacaacaaggugcugacccggucccgacaagaa ccggggcaaguccgacaacgugcccuccgaggaggugguguaagaagaugaagaacuacuggcggcagcugcugaacgccaagcug aucacccagcggaaguucgacaaccugaccaaggccgagcggggcggccuguccgagcuggacaaggccggcuucaucaagcggc agcugguggagacccggcagaucaccaagcacguggcccagauccuggacucccggaugaacaccaaguacgacgagaacgacaa gcugauccgggaggugaaggugaucacccugaagucccaagcuggugucccgacuucccggaaggacuucccaguucuacaaggugcgg gagaucaacaacuaccaccacgcccacgacgccuaccugaacgccguggugggcaccgcccugaucaagaaguaccccaagcugg aguccgaguucguguacggcgacuacaaggugguacgacgugcggaagaugaucgccaaguccgagcaggagaucggcaaggccac cgccaaguacuucuucuacuccaacaucaugaacuucuucaagaccgagaucacccuggccaacggcgagaucccggaagcggccc cugaucgagaccaacggcgagaccggcgagaucgugugggacaagggccgggacuucgccaccgugcggaaggugcuguccaugc cccaggugaacaucgugaagaagaccgaggugcagaccggcggcuucuccaaggaguccauccugcccaagcggaacuccgacaa gcugaucgcccggaagaaggacugggaccccaagaaguacggcggcuucgacucccccaccguggccuacuccgugcugguggug gccaagguggagaagggcaaguccaagaagcugaaguccgugaaggagcugcugggcaucaccaucauggagcgguccuccuucg agaagaaccccaucgacuuccuggaggccaagggcuacaaggaggugaagaaggaccugaucaucaagcugcccaaguacucccu guucgagcuggagaacggccggaagcggaugcuggccuccgccggcgagcugcagaagggcaacgagcuggcccugcccuccaag uacgugaacuuccuguaccuggccucccacuacgagaagcugaagggcucccccgaggacaacgagcagaagcaguguucgugg agcagcacaagcacuaccuggacgagaucaucgagcagaucuccgaguucuccaagcgggugauccuggccgacgccaaccugga caaggugcuguccgccuacaacaagcaccgggacaagcccauccgggagcaggccgagaacaucauccaccuguucacccugacc aaccugggcgcccccgccgccuucaaguacuucgacaccaccaucgaccggaagcgguacaccuccaccaaggaggugcuggacg ccacccugauccaccaguccaucaccggccuguacgagacccggaucgaccugucccagcugggcggcgacggcggcggcucccc caagaagaagcggaaggugucccgaguccgccacccccgaguccguguccggcuggcggcuguucaagaagaucuccUgA
ORF encoding Spy Cas9	806	ATGgaggccTccccgccTccggcccccggcaccTgaTggacccccacaTcTTcaccTccAACTTCAACAACggcATCggccggC ACAAGaccTACCTGTGCTACgaggTggagcggCTGGACAACggcaccTccgTgAAGATGGACCAGCACcggggcTTCCTGCACAA CCAGgccAAGAACCTGCTGTGCggcTTCTACggccggCACgccgagCTGcggTTCCTGGACCTGgTgcccTccCTGCAGCTGGAC cccgccCAGATCTACcgggTgaccTGGTTCATCTccTGGTCCCCCTGCTTCTccTGGggcTGCgccggcgaggTgcgggccTTCC TGCAGgagAACacccACgTgcggCTGcggATCTTCgccgcccggATCTACGACTACGACcccCTGTACAAGgaggccCTGCAGAT GCTGcggGACgccggcgccCAGgTgTccATCATGaccTACGACgagTTCAAGCACTGCTGGGACaccTTCgTgGACCACCAGggc TGCcccTTCCAGcccTGGGACggcCTGGACgagCACTcccAGgccCTGTccggccggCTGcgggccATCCTGCAGAACCAGggcA ACTccggcTccgagacccccggcaccTccgagTccgccaccccccgagTccgacaagaagTacTccaTcggccTggCcaTcggcac caacTccgTgggcTgggccgTgaTcaccgacgagTacaaggTgcccTccaagaagTTcaaggTgcTgggcaacaccgaccggcac TccaTcaagaagaaccTgaTcggcgcccTgcTgTTcgacTccggcgagaccgccgaggccacccggcTgaagcggaccgcccggc ggcggTacacccggcggaagaaccggaTcTgcTaccTgcaggagaTcTTcTccaacgagaTggccaaggTggacgacTccTTcTT ccaccggcTggaggagTccTTccTggTggaggaggacaagaagcacgagcggcaccccaTcTTcggcaacaTcgTggacgaggTg gccTaccacgagaagTaccccaccaTcTaccaccTgcggaagaagcTggTggacTccaccgacaaggccgaccTgcggcTgaTcT

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		accTggcccTggcccacaTgaTcaagTTccggggccacTTccTgaTcgagggcgaccTgaaccccgacaacTccgacgTggacaa gcTgTTcaTccagcTggTgcagaccTacaaccagcTgTTcgaggagaaccccaTcaacgccTccggcgTggacgccaaggccaTc cTgTccgcccggcTgTccaagTcccggcggcTggagaaccTgaTcgcccagcTgcccggcgagaagaagaacggccTgTTcggca accTgaTcgcccTgTcccTgggccTgacccccaacTTcaagTccaacTTcgaccTggccgaggacgccaagcTgcagcTgTccaa ggacaccTacgacgacgaccTggacaaccTgcTggcccagaTcggcgaccagTacgccgaccTgTTccTggccgccaagaaccTg TccgacgccaTccTgcTgTccgacaTccTgcgggTgaacaccgagaTcaccaaggcccccccTgTccgccTccaTgaTcaagcggT acgacgagcaccaccaggaccTgacccTgcTgaaggcccTggTgcggcagcagcTgcccgagaagTacaaggagaTcTTcTTcga ccagTccaagaacggcTacgccggcTacaTcgacggcggcgccTcccaggaggagTTcTacaagTTcaTcaagcccaTccTggag aagaTggacggcaccgaggagcTgcTggTgaagcTgaaccgggaggaccTgcTgcggaagcagcggaccTTcgacaacggcTcca TcccccaccagaTccaccTgggcgagcTgcacgccaTccTgcggcggcaggaggacTTcTaccccTTccTgaaggacaaccggga gaagaTcgagaagaTccTgaccTTccggaTccccTacTacgTgggcccccTggcccggggcaacTcccggITcgccTggaTgacc cggaagTccgaggagaccaTcacccccTggaacTTcgaggaggTggTggacaagggcgccTccgcccagTccTTcaTcgagcgga TgaccaacTTcgacaagaaccTgcccaacgagaaggTgcTgcccaagcacTcccTgcTgTacgagTacTTcaccgTgTacaacga gcTgaccaaggTgaagTacgTgaccgagggcaTgcggaagcccgccTTccTgTccggcgagcagaagaaggccaTcgTggaccTg cTgTTcaagaccaaccggaaggTgaccgTgaagcagcTgaaggaggacTacTTcaagaagaTcgagTgcTTcgacTccgTggaga TcTccggcgTggaggaccggTTcaacgccTcccTgggcaccTaccacgaccTgcTgaagaTcaTcaaggacaaggacTTccTgga caacgaggagaacgaggacaTccTggaggacaTcgTgcTgacccTgacccTgTTcgaggaccgggagaTgaTcgaggagcggcTg aagaccTacgcccaccTgTTcgacgacaaggTgaTgaagcagcTgaagcggcggcggTacaccggcTggggccggcTgTcccgga agcTgaTcaacggcaTccgggacaagcagTccggcaagaccaTccTggacTTccTgaagTccgacggcTTcgccaaccggaacTT caTgcagcTgaTccacgacgacTcccTgaccTTcaaggaggacaTccagaaggcccaggTgTccggccagggcgacTcccTgcac gagcacaTcgccaaccTggccggcTcccccgccaTcaagaagggcaTccTgcagaccgTgaaggTggTggacgagcTggTgaagg TgaTgggccggcacaagcccgagaacaTcgTgaTcgagaTggcccgggagaaccagaccacccagaagggccagaagaacTcccg ggagcggaTgaagcggaTcgaggagggcaTcaaggagcTgggcTcccagaTccTgaaggagcaccccgTggagaacacccagcTg cagaacgagaagcTgTaccTgTacTaccTgcagaacggccgggacaTgTacgTggaccaggagcTggacaTcaaccggcTgTccg acTacgacgTggaccacaTcgTgccccagTccTTccTgaaggacgacTccaTcgacaacaaggTgcTgacccggTccgacaagaa ccggggcaagTccgacaacgTgcccTccgaggaggTggTgaagaagaTgaagaacTacTggcggcagcTgcTgaacgccaagcTg aTcacccagcggaagTTcgacaaccTgaccaaggccgagcggggcggccTgTccgagcTggacaaggccggcTTcaTcaagcggc agcTggTggagacccggcagaTcaccaagcacgTggcccagaTccTggacTcccggaTgaacaccaagTacgacgagaacgacaa gcTgaTccgggaggTgaaggTgaTcacccTgaagTccaagcTggTgTccgacTTccggaaggacTTccagTTcTacaaggTgcgg gagaTcaacaacTaccaccacgcccacgacgccTaccTgaacgccgTggTgggcaccgcccTgaTcaagaagTaccccaagcTgg agTccgagTTcgTgTacggcgacTacaaggTgTacgacgTgcggaagaTgaTcgccaagTccgagcaggagaTcggcaaggccac cgccaagTacTTcTTcTacTccaacaTcaTgaacTTcTTcaagaccgagaTcacccTggccaacggcgagaTccggaagcggccc cTgaTcgagaccaacggcgagaccggcgagaTcgTgTgggacaagggccgggacTTcgccaccgTgcggaaggTgcTgTccaTgc cccaggTgaacaTcgTgaagaagaccgaggTgcagaccggcggcTTcTccaaggagTccaTccTgcccaagcggaacTccgacaa gcTgaTcgcccggaagaaggacTgggaccccaagaagTacggcggcTTcgacTcccccaccgTggccTacTccgTgcTggTggTg gccaaggTggagaagggcaagTccaagaagcTgaagTccgTgaaggagcTgcTgggcaTcaccaTcaTggagcggTccTccTTcg agaagaaccccaTcgacTTccTggaggccaagggcTacaaggaggTgaagaaggaccTgaTcaTcaagcTgcccaagTacTcccT gTTcgagcTggagaacggccggaagcggaTgcTggccTccgccggcgagcTgcagaagggcaacgagcTggcccTgcccTccaag TacgTgaacTTccTgTaccTggccTcccacTacgagaagcTgaagggcTcccccgaggacaacgagcagagaagcagcTgTTcgTgg agcagcacaagcacTacctggacgagaTcaTcgagcagaTcTccgagTTcTccaagcgggTgaTccTggccgacgccaaccTgga caaggTgcTgTccgccTacaacaagcaccgggacaagcccaTccgggagcaggccgagaacaTcaTccaccTgTTcacccTgacc aaccTgggcgcccccgccgccTTcaagTacTTcgacaccaccaTcgaccggaagcggTacaccTccaccaaggaggTgcTggacg ccacccTgaTccaccagTccaTcaccggccTgTacgagacccggaTcgaccTgTcccagcTgggcggcgacggcggcggcTcccc caagaagaagcggaaggTgTgA

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Open reading frame for UGI	807	AUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAAAGCAGCUGG UCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCUGGUCCACAC AGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUGGUCAUCCAG GACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGA
Open reading frame for UGI	808	AUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAAGCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGG UGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUGGUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCU GCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGGUGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCC GGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUCCCCCAAGAAGAAGCGGAAGGUGGAGUGA
Amino acid sequence for Cas9 encoded by SEQ ID Nos. 801-802	809	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLELAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDELEAKGYKEV KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI DLSQLGGDGGGSPKKKRKV
Amino acid sequence for Cas9 with Hibit tag	810	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI DLSQLGGDGGGSPKKKRKVSESATPESVSGWRLFKKIS

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Amino acid sequence for BC22n	811	MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGELHNQAKNLLCGFYGRHAELRFLDLVPSLQLD PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEV AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKERGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNEMQLIHDDSLTFKEDIQKAQVSGQGDSLH EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKV*
Amino acid sequence for BC22n with Hibit tag	812	MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLD PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKEKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESELVEEDKKHERHPIFGNIVDEV AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE KMDGTEELLVKLNREDLLRKQRTEDNGSIPHQIHLGELHAILRRQEDFYPELKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLETLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKVSESATPESVSGWRLFKKIS
	813	Not used

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Amino acid sequence for UGI	814	MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS GGSKRTADGSEFESPKKKRKVE
	815-816	Not used
Open reading frame for Cas9	817	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGA GCAAGAAGUUCAAGGUCCUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGA AACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACG AAAGACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCU GGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAA ACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGC ACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAG ACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAU CACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCC AGGAAGAAUUCUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGA CCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGAC CGCUGGCAAGAGGAAACAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGU CGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAU UCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGA CACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAA GAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCC AGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAU CCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAU GUACGUCGACCAGGAACUGGACAUCAACAGACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGG ACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACACUGAAGAGCAAGCUG GUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAG AAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAG ACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACA AGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGG AUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGA

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		GGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCA AGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGA AGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCA GGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGA AGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAG CGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUC AGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACAACAA UCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAG AAUCGACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG
Open reading frame for BC22	818	AUGGAAGCAAGCCCGGCAAGCGGACCGAGACACCUGAUGGACCCGCACAUCUUCACAAGCAACUUCAACAACGGAAUCGGAAGAC ACAAGACAUACCUGUGCUACGAAGUCGAAAGACUGGACAACGGAACAAGCGUCAAGAUGGACCAGCACAGAGGAUUCCUGCACAA CCAGGCAAAGAACCUGCUGUGCGGAUUCUACGGAAGACACGCAGAACUGAGAUUCCUGGACCUGGUCCCGAGCCUGCAGCUGGAC CCGGCACAGAUCUACAGAGUCACAUGGUUCAUCAGCUGGAGCCCGUGCUUCAGCUGGGGAUGCGCAGGAGAAGUCAGAGCAUUUC UGCAGGAAAACACACACGUCAGACUGAGAAUCUUCGCAGCAAGAAUCUAC GACUACGACCCGCUGUACAAGGAAGCACUGCAGAUGCUGAGAGACGCAGGAGCACAGGUCAGCAUCAUGACAUACGACGAAUUCA AGCACUGCUGGGACACAUUCGUCGACCACCAGGGAUGCCCGUUCCAGCCGUGGGACGGACUGGACGAACACAGCCAGGCACUGAG CGGAAGACUGAGAGCAAUCCUGCAGAACCAGGGAAACAGCGGAAGCGAAACACCGGGAACAAGCGAAAGCGCAACACCGGAAAGC GACAAGAAGUACAGCAUCGGACUGGCCAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCA AGAAGUUCAAGGUCCUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAAC AGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAA GACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGU CGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACC CGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACA GCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACC AGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCAC AAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGG AAGAAUUCUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCU GCUGAGAAAGCAGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGC UGGCAAGAGGAAACAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGA CAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCC UGAGCGGAGAACAGAAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUA CUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACAC UGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAG AAGAAGAUACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCU GCAGACAGUCAAGGUCGUCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGA

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		UCCUGAAGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUA CGUCGACCAGGAACUGGACAUCAACAGACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUC GACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGA ACUACUGGAGACAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGGACUGAG CGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGC AGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACU UCAGAAAGGACUUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGG AACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUC GCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCA CACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGA CUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAG GAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACA GCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCU GGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAG GACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGC AGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCC GGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGC AAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGG CAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAA GAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAUCUG AGCCAGCUGGGAGGAGACAGCGGAGGAAGCACAAACCUGAGCGACAUCAUCGAAAAGGAAACAGGAAAGCAGCUGGUCAUCCAGG AAAGCAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAAGCGACAUCCUGGUCCACACAGCAUACGA CGAAAGCACAGACGAAAACGUCAUGCUGCUGACAAGCGACGCACCGGAAUACAAGCCGUGGGCACUGGUCAUCCAGGACAGCAAC GGAGAAAACAAGAUCAAGAUGCUGAGCGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAA
Open reading frame for UGI	819	AUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAAAGCAGCUGG UCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCUGGUCCACAC AGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUGGUCAUCCAG GACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGA
mRNA encoding UGI	821	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAA GCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGGUGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUG GUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCUGCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGG UGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCCGGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUC CCCCAAGAAGAAGCGGAAGGUGGAGUGAUAGCUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAA CUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCG AGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAA AAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUAAAAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAA AAAAAAAAGGGAAAAAAAAAAAAACGCAAAAAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAUCGAAAAAAAAAAAAUC UAAAAAAAAAAAAACGAAAAAAAAAAAAACCCAAAAAAAAAAAAAGACAAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAA AACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG
mRNA encoding Nme2Cas9 base editor	822	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA GGUGGAGGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCUCCGGCGGCGGCGAGGCCUCC CCCGCCUCCGGCCCCCGGCACCUGAUGGACCCCCACAUCUUCACCUCCAACUUCAACAACGGCAUCGGCCGGCACAAGACCUACC

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		UGUGCUACGAGGUGGAGCGGCUGGACAACGGCACCUCCGUGAAGAUGGACCAGCACCGGGGCUUCCUGCACAACCAGGCCAAGAA
		CCUGCUGUGCGGCUUCUACGGCCGGCACGCCGAGCUGCGGUUCCUGGACCUGGUGCCCUCCCUGCAGCUGGACCCCGCCCAGAUC
		UACCGGGUGACCUGGUUCAUCUCCUGGUCCCCCUGCUUCUCCUGGGGCUGCGCCGGCGAGGUGCGGGCCUUCCUGCAGGAGAACA
		CCCACGUGCGGCUGCGGAUCUUCGCCGCCCGGAUCUACGACUACGACCCCCUGUACAAGGAGGCCCUGCAGAUGCUGCGGGACGC
		CGGCGCCCAGGUGUCCAUCAUGACCUACGACGAGUUCAAGCACUGCUGGGACACCUUCGUGGACCACCAGGGCUGCCCCUUCCAG
		CCCUGGGACGGCCUGGACGAGCACUCCCAGGCCCUGUCCGGCCGGCUGCGGGCCAUCCUGCAGAACCAGGGCAACUCCGGCUCCG
		AGACCCCCGGCACCUCCGAGUCCGCCACCCCCGAGUCCGCAGCGUUCAAACCAAAUCCCAUCAACUACAUCCUGGGCCUGGCCAU
		CGGCAUCGCCUCCGUGGGCUGGGCCAUGGUGGAGAUCGACGAGGAGGAGAACCCCAUCCGGCUGAUCGACCUGGGCGUGCGGGUG
		UUCGAGCGGGCCGAGGUGCCCAAGACCGGCGACUCCCUGGCCAUGGCCCGGCGGCUGGCCCGGUCCGUGCGGCGGCUGACCCGGC
		GGCGGGCCCACCGGCUGCUGCGGGCCCGGCGGCUGCUGAAGCGGGAGGGCGUGCUGCAGGCCGCCGACUUCGACGAGAACGGCCU
		GAUCAAGUCCCUGCCCAACACCCCCUGGCAGCUGCGGGCCGCCGCCCUGGACCGGAAGCUGACCCCCCUGGAGUGGUCCGCCGUG
		CUGCUGCACCUGAUCAAGCACCGGGGCUACCUGUCCCAGCGGAAGAACGAGGGCGAGACCGCCGACAAGGAGCUGGGCGCCCUGC
		UGAAGGGCGUGGCCAACAACGCCCACGCCCUGCAGACCGGCGACUUCCGGACCCCCGCCGAGCUGGCCCUGAACAAGUUCGAGAA
		GGAGUCCGGCCACAUCCGGAACCAGCGGGGCGACUACUCCCACACCUUCUCCCGGAAGGACCUGCAGGCCGAGCUGAUCCUGCUG
		UUCGAGAAGCAGAAGGAGUUCGGCAACCCCCACGUGUCCGGCGGCCUGAAGGAGGGCAUCGAGACCCUGCUGAUGACCCAGCGGC
		CCGCCCUGUCCGGCGACGCCGUGCAGAAGAUGCUGGGCCACUGCACCUUCGAGCCCGCCGAGCCCAAGGCCGCCAAGAACACCUA
		CACCGCCGAGCGGUUCAUCUGGCUGACCAAGCUGAACAACCUGCGGAUCCUGGAGCAGGGCUCCGAGCGGCCCCUGACCGACACC
		GAGCGGGCCACCCUGAUGGACGAGCCCUACCGGAAGUCCAAGCUGACCUACGCCCAGGCCCGGAAGCUGCUGGGCCUGGAGGACA
		CCGCCUUCUUCAAGGGCCUGCGGUACGGCAAGGACAACGCCGAGGCCUCCACCCUGAUGGAGAUGAAGGCCUACCACGCCAUCUC
		CCGGGCCCUGGAGAAGGAGGGCCUGAAGGACAAGAAGUCCCCCCUGAACCUGUCCUCCGAGCUGCAGGACGAGAUCGGCACCGCC
		UUCUCCCUGUUCAAGACCGACGAGGACAUCACCGGCCGGCUGAAGGACCGGGUGCAGCCCGAGAUCCUGGAGGCCCUGCUGAAGC
		ACAUCUCCUUCGACAAGUUCGUGCAGAUCUCCCUGAAGGCCCUGCGGCGGAUCGUGCCCCUGAUGGAGCAGGGCAAGCGGUACGA
		CGAGGCCUGCGCCGAGAUCUACGGCGACCACUACGGCAAGAAGAACACCGAGGAGAAGAUCUACCUGCCCCCCAUCCCCGCCGAC
		GAGAUCCGGAACCCCGUGGUGCUGCGGGCCCUGUCCCAGGCCCGGAAGGUGAUCAACGGCGUGGUGCGGCGGUACGGCUCCCCCG
		CCCGGAUCCACAUCGAGACCGCCCGGGAGGUGGGCAAGUCCUUCAAGGACCGGAAGGAGAUCGAGAAGCGGCAGGAGGAGAACCG
		GAAGGACCGGGAGAAGGCCGCCGCCAAGUUCCGGGAGUACUUCCCCAACUUCGUGGGCGAGCCCAAGUCCAAGGACAUCCUGAAG
		CUGCGGCUGUACGAGCAGCAGCACGGCAAGUGCCUGUACUCCGGCAAGGAGAUCAACCUGGUGCGGCUGAACGAGAAGGGCUACG
		UGGAGAUCGACCACGCCCUGCCCUUCUCCCGGACCUGGGACGACUCCUUCAACAACAAGGUGCUGGUGCUGGGCUCCGAGAACCA
		GAACAAGGGCAACCAGACCCCCUACGAGUACUUCAACGGCAAGGACAACUCCCGGGAGUGGCAGGAGUUCAAGGCCCGGGUGGAG
		ACCUCCCGGUUCCCCCGGUCCAAGAAGCAGCGGAUCCUGCUGCAGAAGUUCGACGAGGACGGCUUCAAGGAGUGCAACCUGAACG
		ACACCCGGUACGUGAACCGCUUCCUGUGCCAGUUCGUGGCCGACCACAUCCUGCUGACCGGCAAGGGCAAGCGGCGGGUGUUCGC
		CUCCAACGGCCAGAUCACCAACCUGCUGCGGGGCUUCUGGGGCCUGCGGAAGGUGCGGGCCGAGAACGACCGGCACCACGCCCUG
		GACGCCGUGGUGGUGGCCUGCUCCACCGUGGCCAUGCAGCAGAAGAUCACCCGGUUCGUGCGGUACAAGGAGAUGAACGCCUUCG
		ACGGCAAGACCAUCGACAAGGAGACCGGCAAGGUGCUGCACCAGAAGACCCACUUCCCCCAGCCCUGGGAGUUCUUCGCCCAGGA
		GGUGAUGAUCCGGGUGUUCGGCAAGCCCGACGGCAAGCCCGAGUUCGAGGAGGCCGACACCCCCGAGAAGCUGCGGACCCUGCUG
		GCCGAGAAGCUGUCCUCCCGGCCCGAGGCCGUGCACGAGUACGUGACCCCCCUGUUCGUGUCCCGGGCCCCCAACCGGAAGAUGU
		CCGGCGCCCACAAGGACACCCUGCGGUCCGCCAAGCGGUUCGUGAAGCACAACGAGAAGAUCUCCGUGAAGCGGGUGUGGCUGAC
		CGAGAUCAAGCUGGCCGACCUGGAGAACAUGGUGAACUACAAGAACGGCCGGGAGAUCGAGCUGUACGAGGCCCUGAAGGCCCGG
		CUGGAGGCCUACGGCGGCAACGCCAAGCAGGCCUUCGACCCCAAGGACAACCCCUUCUACAAGAAGGGCGGCCAGCUGGUGAAGG
		CCGUGCGGGUGGAGAAGACCCAGGAGUCCGGCGUGCUGCUGAACAAGAAGAACGCCUACACCAUCGCCGACAACGGCGACAUGGU
		GCGGGUGGACGUGUUCUGCAAGGUGGACAAGAAGGGCAAGAACCAGUACUUCAUCGUGCCCAUCUACGCCUGGCAGGUGGCCGAG
		AACAUCCUGCCCGACAUCGACUGCAAGGGCUACCGGAUCGACGACUCCUACACCUUCUGCUUCUCCCUGCACAAGUACGACCUGA
		UCGCCUUCCAGAAGGACGAGAAGUCCAAGGUGGAGUUCGCCUACUACAUCAACUGCGACUCCUCCAACGGCCGGUUCUACCUGGC
		CUGGCACGACAAGGGCUCCAAGGAGCAGCAGUUCCGGAUCUCCACCCAGAACCUGGUGCUGAUCCAGAAGUACCAGGUGAACGAG
		CUGGGCAAGGAGAUCCGGCCCUGCCGGCUGAAGAAGCGGCCCCCGUGCGGUAGUGACUAGCACCAGCCUCAAGAACACCCGAAU
		GGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAA

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAGGUAAAAAAAAAAAAAU AUAAAAAAAAAAACAUAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAGAUAAAAAAAAAAAA ACCUAAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAAAUGCAAAAAAA AAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAA AAAAAAAAGUUAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG
Open reading frame for NmelCas9	823	ATGGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAATCGCATCGGTCGGATGGGCAATGGTCGAAA TCGACGAAGAAGAAAACCCGATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCGAAGACAGGAGACTC GCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGAAGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTG CTGAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTGATCAAGTCGCTGCCGAACACACCGTGGCAGCTGA GAGCAGCAGCACTGGACAGAAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCACAGAGGATACCTGTC GCAGAGAAAGAACGAAGGAGAAACAGCAGACAAGGAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAG ACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACT ACTCGCACACATTCTCGAGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAATTCGGAAACCCGCACGT CTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTG GGACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACACAGCAGAAAGATTCATCTGGCTGACAAAGCTGA ACAACCTGAGAATCCTGGAACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGACGAACCGTACAGAAA GTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTGGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGAC AACGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCGAGAGCACTGGAAAAGGAAGGACTGAAGGACAAGA AGTCGCCGCTGAACCTGTCGCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGACGAAGACATCACAGG AAGACTGAAGGACAGAATCCAGCCGGAAATCCTGGAAGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTG AAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACGAAGCATGCGCAGAAATCTACGGAGACCACTACG GAAAGAAGAACACAGAAGAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGTCCTGAGAGCACTGTC GCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAGATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGA AAGTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAGGACAGAGAAAAGGCAGCAGCAAAGTTCAGAG AATACTTCCCGAACTTCGTCGGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCAGCACGGAAAGTGCCT GTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAACGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACA TGGGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAACAAGGGAAACCAGACACCGTACGAATACTTCA ACGGAAAGGACAACTCGAGAGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGATCGAAGAAGCAGAGAAT CCTGCTGCAGAAGTTCGACGAAGACGGATTCAAGGAAAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTC GTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCATCGAACGGACAGATCACAAACCTGCTGAGAGGAT TCTGGGGACTGAGAAAGGTCAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCATGCTCGACAGTCGCAAT GCAGCAGAAGATCACAAGATTCGTCAGATACAAGGAAATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTC CTGCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTCATGATCAGAGTCTTCGGAAAGCCGGACGGAA AGCCGGAATTCGAAGAAGCAGACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAGACCGGAAGCAGTCCA CGAATACGTCACACCGCTGTTCGTCTCGAGAGCACCGAACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCA AAGAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTGAAGCTGAAGGACCTGGAAAAGATGGTCAACAGAG AAAGAGAACCGAAGCTGTACGAAGCACTGAAGGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAGGCATTCGCAGAACCGTT CTACAAGTACGACAAGGCAGGAAACAGAACACAGCAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTC AGAAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTCTTCGAAAAGGGAGACAAGTACTACCTGGTCCCGA TCTACTCGTGGCAGGTCGCAAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGACTGGCAGCTGATCGA CGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAACGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTC GCATCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGACCACAAGATCGGAAAGAACGGAATCCTGGAAG GAATCGGAGTCAAGACAGCACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACCGTGCAGACTGAAGAA GAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGACGGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAATGA

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Open reading frame for UGI encoded by SEQ NO: 821	824	ATGACCAACCTGTCCGACATCATCGAGAAGGAGACCGGCAAGCAGCTGGTGATCCAGGAGTCCATCCTGATGCTGCCCGAGGAGG TGGAGGAGGTGATCGGCAACAAGCCCGAGTCCGACATCCTGGTGCACACCGCCTACGACGAGTCCACCGACGAGAACGTGATGCT GCTGACCTCCGACGCCCCCGAGTACAAGCCCTGGGCCCTGGTGATCCAGGACTCCAACGGCGAGAACAAGATCAAGATGCTGTCC GGCGGCTCCAAGCGGACCGCCGACGGCTCCGAGTTCGAGTCCCCCAAGAAGAAGCGGAAGGTGGAGTGATAG
mRNA encoding Nme2 Cas9	825	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA GGUGGAGGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCUCCGGCGGCGGCGCCGCCUUC AAGCCCAACCCCAUCAACUACAUCCUGGGCCUGGACAUCGGCAUCGCCUCCGUGGGCUGGGCCAUGGUGGAGAUCGACGAGGAGG AGAACCCCAUCCGGCUGAUCGACCUGGGCGUGCGGGUGUUCGAGCGGGCCGAGGUGCCCAAGACCGGCGACUCCCUGGCCAUGGC CCGGCGGCUGGCCCGGUCCGUGCGGCGGCUGACCCGGCGGCGGGCCCACCGGCUGCUGCGGGCCCGGCGGCUGCUGAAGCGGGAG GGCGUGCUGCAGGCCGCCGACUUCGACGAGAACGGCCUGAUCAAGUCCCUGCCCAACACCCCCUGGCAGCUGCGGGCCGCCGCCC UGGACCGGAAGCUGACCCCCCUGGAGUGGUCCGCCGUGCUGCUGCACCUGAUCAAGCACCGGGGCUACCUGUCCCAGCGGAAGAA CGAGGGCGAGACCGCCGACAAGGAGCUGGGCGCCCUGCUGAAGGGCGUGGCCAACAACGCCCACGCCCUGCAGACCGGCGACUUC CGGACCCCCGCCGAGCUGGCCCUGAACAAGUUCGAGAAGGAGUCCGGCCACAUCCGGAACCAGCGGGGCGACUACUCCCACACCU UCUCCCGGAAGGACCUGCAGGCCGAGCUGAUCCUGCUGUUCGAGAAGCAGAAGGAGUUCGGCAACCCCCACGUGUCCGGCGGCCU GAAGGAGGGCAUCGAGACCCUGCUGAUGACCCAGCGGCCCGCCCUGUCCGGCGACGCCGUGCAGAAGAUGCUGGGCCACUGCACC UUCGAGCCCGCCGAGCCCAAGGCCGCCAAGAACACCUACACCGCCGAGCGGUUCAUCUGGCUGACCAAGCUGAACAACCUGCGGA UCCUGGAGCAGGGCUCCGAGCGGCCCCUGACCGACACCGAGCGGGCCACCCUGAUGGACGAGCCCUACCGGAAGUCCAAGCUGAC CUACGCCCAGGCCCGGAAGCUGCUGGGCCUGGAGGACACCGCCUUCUUCAAGGGCCUGCGGUACGGCAAGGACAACGCCGAGGCC UCCACCCUGAUGGAGAUGAAGGCCUACCACGCCAUCUCCCGGGCCCUGGAGAAGGAGGGCCUGAAGGACAAGAAGUCCCCCCUGA ACCUGUCCUCCGAGCUGCAGGACGAGAUCGGCACCGCCUUCUCCCUGUUCAAGACCGACGAGGACAUCACCGGCCGGCUGAAGGA CCGGGUGCAGCCCGAGAUCCUGGAGGCCCUGCUGAAGCACAUCUCCUUCGACAAGUUCGUGCAGAUCUCCCUGAAGGCCCUGCGG CGGAUCGUGCCCCUGAUGGAGCAGGGCAAGCGGUACGACGAGGCCUGCGCCGAGAUCUACGGCGACCACUACGGCAAGAAGAACA CCGAGGAGAAGAUCUACCUGCCCCCCAUCCCCGCCGACGAGAUCCGGAACCCCGUGGUGCUGCGGGCCCUGUCCCAGGCCCGGAA GGUGAUCAACGGCGUGGUGCGGCGGUACGGCUCCCCCGCCCGGAUCCACAUCGAGACCGCCCGGGAGGUGGGCAAGUCCUUCAAG GACCGGAAGGAGAUCGAGAAGCGGCAGGAGGAGAACCGGAAGGACCGGGAGAAGGCCGCCGCCAAGUUCCGGGAGUACUUCCCCA ACUUCGUGGGCGAGCCCAAGUCCAAGGACAUCCUGAAGCUGCGGCUGUACGAGCAGCAGCACGGCAAGUGCCUGUACUCCGGCAA GGAGAUCAACCUGGUGCGGCUGAACGAGAAGGGCUACGUGGAGAUCGACCACGCCCUGCCCUUCUCCCGGACCUGGGACGACUCC UUCAACAACAAGGUGCUGGUGCUGGGCUCCGAGAACCAGAACAAGGGCAACCAGACCCCCUACGAGUACUUCAACGGCAAGGACA ACUCCCGGGAGUGGCAGGAGUUCAAGGCCCGGGUGGAGACCUCCCGGUUCCCCCGGUCCAAGAAGCAGCGGAUCCUGCUGCAGAA GUUCGACGAGGACGGCUUCAAGGAGUGCAACCUGAACGACACCCGGUACGUGAACCGGUUCCUGUGCCAGUUCGUGGCCGACCAC AUCCUGCUGACCGGCAAGGGCAAGCGGCGGGUGUUCGCCUCCAACGGCCAGAUCACCAACCUGCUGCGGGGCUUCUGGGGCCUGC GGAAGGUGCGGGCCGAGAACGACCGGCACCACGCCCUGGACGCCGUGGUGGUGGCCUGCUCCACCGUGGCCAUGCAGCAGAAGAU CACCCGGUUCGUGCGGUACAAGGAGAUGAACGCCUUCGACGGCAAGACCAUCGACAAGGAGACCGGCAAGGUGCUGCACCAGAAG ACCCACUUCCCCCAGCCCUGGGAGUUCUUCGCCCAGGAGGUGAUGAUCCGGGUGUUCGGCAAGCCCGACGGCAAGCCCGAGUUCG AGGAGGCCGACACCCCCGAGAAGCUGCGGACCCUGCUGGCCGAGAAGCUGUCCUCCCGGCCCGAGGCCGUGCACGAGUACGUGAC CCCCCUGUUCGUGUCCCGGGCCCCCAACCGGAAGAUGUCCGGCGCCCACAAGGACACCCUGCGGUCCGCCAAGCGGUUCGUGAAG CACAACGAGAAGAUCUCCGUGAAGCGGGUGUGGCUGACCGAGAUCAAGCUGGCCGACCUGGAGAACAUGGUGAACUACAAGAACG GCCGGGAGAUCGAGCUGUACGAGGCCCUGAAGGCCCGGCUGGAGGCCUACGGCGGCAACGCCAAGCAGGCCUUCGACCCCAAGGA CAACCCCUUCUACAAGAAGGGCGGCCAGCUGGUGAAGGCCGUGCGGGUGGAGAAGACCCAGGAGUCCGGCGUGCUGCUGAACAAG AAGAACGCCUACACCAUCGCCGACAACGGCGACAUGGUGCGGGUGGACGUGUUCUGCAAGGUGGACAAGAAGGGCAAGAACCAGU ACUUCAUCGUGCCCAUCUACGCCUGGCAGGUGGCCGAGAACAUCCUGCCCGACAUCGACUGCAAGGGCUACCGGAUCGACGACUC CUACACCUUCUGCUUCUCCCUGCACAAGUACGACCUGAUCGCCUUCCAGAAGGACGAGAAGUCCAAGGUGGAGUUCGCCUACUAC AUCAACUGCGACUCCUCCAACGGCCGGUUCUACCUGGCCUGGCACGACAAGGGCUCCAAGGAGCAGCAGUUCCGGAUCUCCACCC AGAACCUGGUGCUGAUCCAGAAGUACCAGGUGAACGAGCUGGGCAAGGAGAUCCGGCCCUGCCGGCUGAAGAAGCGGCCCCCCGU GCGGUAGCUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCC

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		CCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAA AAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAA AAAAAAAAACUCAAAAAAAAAAAAGAUAAAAAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACG CAAAAAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAA AACCCAAAAAAAAAAAAAGACAAAAAAAAAAAAAUAGAAAAAAAAAAAAAGUUAAAAAAAAAAAAACUGAAAAAAAAAAAAAUUUAAAAAA AAAAAAUCUAG
mRNA encoding Nme2 Cas9 with Hibit tag	826	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA GGUGGAGGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCUCCGGCGGCGGCGCCGCCUUC AAGCCCAACCCCAUCAACUACAUCCUGGGCCUGGACAUCGGCAUCGCCUCCGUGGGCUGGGCCAUGGUGGAGAUCGACGAGGAGG AGAACCCCAUCCGGCUGAUCGACCUGGGCGUGCGGGUGUUCGAGCGGGCCGAGGUGCCCAAGACCGGCGACUCCCUGGCCAUGGC CCGGCGGCUGGCCCGGUCCGUGCGGCGGCUGACCCGGCGGCGGGCCCACCGGCUGCUGCGGGCCCGGCGGCUGCUGAAGCGGGAG GGCGUGCUGCAGGCCGCCGACUUCGACGAGAACGGCCUGAUCAAGUCCCUGCCCAACACCCCCUGGCAGCUGCGGGCCGCCGCCC UGGACCGGAAGCUGACCCCCCUGGAGUGGUCCGCCGUGCUGCUGCACCUGAUCAAGCACCGGGGCUACCUGUCCCAGCGGAAGAA CGAGGGCGAGACCGCCGACAAGGAGCUGGGCGCCCUGCUGAAGGGCGUGGCCAACAACGCCCACGCCCUGCAGACCGGCGACUUC CGGACCCCCGCCGAGCUGGCCCUGAACAAGUUCGAGAAGGAGUCCGGCCACAUCCGGAACCAGCGGGGCGACUACUCCCACACCU UCUCCCGGAAGGACCUGCAGGCCGAGCUGAUCCUGCUGUUCGAGAAGCAGAAGGAGUUCGGCAACCCCCACGUGUCCGGCGGCCU GAAGGAGGGCAUCGAGACCCUGCUGAUGACCCAGCGGCCCGCCCUGUCCGGCGACGCCGUGCAGAAGAUGCUGGGCCACUGCACC UUCGAGCCCGCCGAGCCCAAGGCCGCCAAGAACACCUACACCGCCGAGCGGUUCAUCUGGCUGACCAAGCUGAACAACCUGCGGA UCCUGGAGCAGGGCUCCGAGCGGCCCCUGACCGACACCGAGCGGGCCACCCUGAUGGACGAGCCCUACCGGAAGUCCAAGCUGAC CUACGCCCAGGCCCGGAAGCUGCUGGGCCUGGAGGACACCGCCUUCUUCAAGGGCCUGCGGUACGGCAAGGACAACGCCGAGGCC UCCACCCUGAUGGAGAUGAAGGCCUACCACGCCAUCUCCCGGGCCCUGGAGAAGGAGGGCCUGAAGGACAAGAAGUCCCCCCUGA ACCUGUCCUCCGAGCUGCAGGACGAGAUCGGCACCGCCUUCUCCCUGUUCAAGACCGACGAGGACAUCACCGGCCGGCUGAAGGA CCGGGUGCAGCCCGAGAUCCUGGAGGCCCUGCUGAAGCACAUCUCCUUCGACAAGUUCGUGCAGAUCUCCCUGAAGGCCCUGCGG CGGAUCGUGCCCCUGAUGGAGCAGGGCAAGCGGUACGACGAGGCCUGCGCCGAGAUCUACGGCGACCACUACGGCAAGAAGAACA CCGAGGAGAAGAUCUACCUGCCCCCCAUCCCCGCCGACGAGAUCCGGAACCCCGUGGUGCUGCGGGCCCUGUCCCAGGCCCGGAA GGUGAUCAACGGCGUGGUGCGGCGGUACGGCUCCCCCGCCCGGAUCCACAUCGAGACCGCCCGGGAGGUGGGCAAGUCCUUCAAG GACCGGAAGGAGAUCGAGAAGCGGCAGGAGGAGAACCGGAAGGACCGGGAGAAGGCCGCCGCCAAGUUCCGGGAGUACUUCCCCA ACUUCGUGGGCGAGCCCAAGUCCAAGGACAUCCUGAAGCUGCGGCUGUACGAGCAGCAGCACGGCAAGUGCCUGUACUCCGGCAA GGAGAUCAACCUGGUGCGGCUGAACGAGAAGGGCUACGUGGAGAUCGACCACGCCCUGCCCUUCUCCCGGACCUGGGACGACUCC UUCAACAACAAGGUGCUGGUGCUGGGCUCCGAGAACCAGAACAAGGGCAACCAGACCCCCUACGAGUACUUCAACGGCAAGGACA ACUCCCGGGAGUGGCAGGAGUUCAAGGCCCGGGUGGAGACCUCCCGGUUCCCCCGGUCCAAGAAGCAGCGGAUCCUGCUGCAGAA GUUCGACGAGGACGGCUUCAAGGAGUGCAACCUGAACGACACCCGGUACGUGAACCGGUUCCUGUGCCAGUUCGUGGCCGACCAC AUCCUGCUGACCGGCAAGGGCAAGCGGCGGGUGUUCGCCUCCAACGGCCAGAUCACCAACCUGCUGCGGGGCUUCUGGGGCCUGC GGAAGGUGCGGGCCGAGAACGACCGGCACCACGCCCUGGACGCCGUGGUGGUGGCCUGCUCCACCGUGGCCAUGCAGCAGAAGAU CACCCGGUUCGUGCGGUACAAGGAGAUGAACGCCUUCGACGGCAAGACCAUCGACAAGGAGACCGGCAAGGUGCUGCACCAGAAG ACCCACUUCCCCCAGCCCUGGGAGUUCUUCGCCCAGGAGGUGAUGAUCCGGGUGUUCGGCAAGCCCGACGGCAAGCCCGAGUUCG AGGAGGCCGACACCCCCGAGAAGCUGCGGACCCUGCUGGCCGAGAAGCUGUCCUCCCGGCCCGAGGCCGUGCACGAGUACGUGAC CCCCCUGUUCGUGUCCCGGGCCCCCAACCGGAAGAUGUCCGGCGCCCACAAGGACACCCUGCGGUCCGCCAAGCGGUUCGUGAAG CACAACGAGAAGAUCUCCGUGAAGCGGGUGUGGCUGACCGAGAUCAAGCUGGCCGACCUGGAGAACAUGGUGAACUACAAGAACG GCCGGGAGAUCGAGCUGUACGAGGCCCUGAAGGCCCGGCUGGAGGCCUACGGCGGCAACGCCAAGCAGGCCUUCGACCCCAAGGA CAACCCCUUCUACAAGAAGGGCGGCCAGCUGGUGAAGGCCGUGCGGGUGGAGAAGACCCAGGAGUCCGGCGUGCUGCUGAACAAG AAGAACGCCUACACCAUCGCCGACAACGGCGACAUGGUGCGGGUGGACGUGUUCUGCAAGGUGGACAAGAAGGGCAAGAACCAGU ACUUCAUCGUGCCCAUCUACGCCUGGCAGGUGGCCGAGAACAUCCUGCCCGACAUCGACUGCAAGGGCUACCGGAUCGACGACUC CUACACCUUCUGCUUCUCCCUGCACAAGUACGACCUGAUCGCCUUCCAGAAGGACGAGAAGUCCAAGGUGGAGUUCGCCUACUAC AUCAACUGCGACUCCUCCAACGGCCGGUUCUACCUGGCCUGGCACGACAAGGGCUCCAAGGAGCAGCAGUUCCGGAUCUCCACCC

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AGAACCUGGUGCUGAUCCAGAAGUACCAGGUGAACGAGCUGGGCAAGGAGAUCCGGCCCUGCCGGCUGAAGAAGCGGCCCCCCGU GCGGUCCGAGUCCGCCACCCCCGAGUCCGUGUCCGGCUGGCGGCUGUUCAAGAAGAUCUCCUAGCUAGCACCAGCCUCAAGAACA CCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUC CUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAA AAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUA AAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAA AUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAA AAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAUCUAG
ORF encoding Spy Cas9	827	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCT CCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGA GACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATC TTCTCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCT GGTGGACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGA ACCCCATCAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGC CCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAAC TTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCG ACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGAT CACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGG CAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCC AGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGA CCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGG CAGGAGGACTTCTACCCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCC CCCTGGCCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGT GGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAG CACTCCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCT TCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGA CTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCAC GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGA CCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAA GCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTG GACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCC AGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCAT CCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGG GAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCC AGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACAT GTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGA TGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGGG CCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTG GACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGT CCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGT GGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAG ATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		AGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG CCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTC TCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCT TCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGA GCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG AAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCG AGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGG CTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAG TTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGG AGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGA CCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATC GACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA
ORF encoding Spy Cas9	828	aagctcagaataaacgctcaactttggccggatctgccaccATGGACGGCTCCGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGT GGAGGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCTCCGGCGGCGGCGAGGCCTCCCCC GCCTCCGGCCCCCGGCACCTGATGGACCCCCACATCTTCACCTCCAACTTCAACAACGGCATCGGCCGGCACAAGACCTACCTGT GCTACGAGGTGGAGCGGCTGGACAACGGCACCTCCGTGAAGATGGACCAGCACCGGGGCTTCCTGCACAACCAGGCCAAGAACCT GCTGTGCGGCTTCTACGGCCGGCACGCCGAGCTGCGGTTCCTGGACCTGGTGCCCTCCCTGCAGCTGGACCCCGCCCAGATCTAC CGGGTGACCTGGTTCATCTCCTGGTCCCCCTGCTTCTCCTGGGGCTGCGCCGGCGAGGTGCGGGCCTTCCTGCAGGAGAACACCC ACGTGCGGCTGCGGATCTTCGCCGCCCGGATCTACGACTACGACCCCCTGTACAAGGAGGCCCTGCAGATGCTGCGGGACGCCGG CGCCCAGGTGTCCATCATGACCTACGACGAGTTCAAGCACTGCTGGGACACCTTCGTGGACCACCAGGGCTGCCCCTTCCAGCCC TGGGACGGCCTGGACGAGCACTCCCAGGCCCTGTCCGGCCGGCTGCGGGCCATCCTGCAGAACCAGGGCAACTCCGGCTCCGAGA CCCCCGGCACCTCCGAGTCCGCCACCCCCGAGTCCGCAGCGTTCAAACCAAATcccatcaactacatcctgggcctggccatcgg catcgcctccgtgggctgggccatggtggagatcgacgaggaggagaaccccatccggctgatcgacctgggcgtgcgggtgttc gagcgggccgaggtgcccaagaccggcgactccctggccatggcccggcggctggcccggtccgtgcggcggctgacccggcggc gggcccaccggctgctgcgggcccggcggctgctgaagcgggagggcgtgctgcaggccgccgacttcgacgagaacggcctgat caagtccctgcccaacacccccctggcagctgcgggccgccgccctggaccggaagctgaccccccctggagtggtccgccgtgctg ctgcacctgatcaagcaccggggctacctgtcccagcggaagaacgagggcgagaccgccgacaaggagctgggcgccctgctga agggcgtggccaacaacgcccacgccctgcagaccggcgacttccggacccccgccgagctggccctgaacaagttcgagaagga gtccggccacatccggaaccagcggggcgactactcccacaccttctcccggaaggacctgcaggccgagctgatcctgctgttc gagaagcagaaggagttcggcaacccccacgtgtccggcggcctgaaggagggcatcgagaccctgctgatgacccagcggcccg ccctgtccggcgacgccgtgcagaagatgctgggccactgcaccttcgagcccgccgagcccaaggccgccaagaacacctacac cgccgagcggttcatctggctgaccaagctgaacaacctgcggatcctggagcagggctccgagcggcccctgaccgacaccgag cgggccaccctgatggacgagccctaccggaagtccaagctgacctacgcccaggcccggaagctgctgggcctggaggacaccg ccttcttcaagggcctgcggtacggcaaggacaacgccgaggcctccaccctgatggagatgaaggcctaccacgccatctcccg ggccctggagaaggagggcctgaaggacaagaagtcccccctgaacctgtcctccgagctgcaggacgagatcggcaccgccttc tccctgttcaagaccgacgaggacatcaccggccggctgaaggaccgggtgcagcccgagatcctggaggccctgctgaagcaca tctccttcgacaagttcgtgcagatctccctgaaggccctgcggcggatcgtgcccctgatggagcagggcaagcggtacgacga ggcctgcgccgagatctacggcgaccactacggcaagaagaacaccgaggagaagatctacctgcccccatcccccgccgacgag atccggaaccccgtggtgctgcgggccctgtcccaggcccggaaggtgatcaacggcgtggtgcggcggtacggctcccccgccc ggatccacatcgagaccgcccgggaggtgggcaagtccttcaaggaccggaaggagatcgagaagcggcaggaggagaaccggaa ggaccgggagaaggccgccgccaagttccgggagtacttccccaacttcgtgggcgagcccaagtccaaggacatcctgaagctg cggctgtacgagcagcagcacggcaagtgcctgtactccggcaaggagatcaacctggtgcggctgaacgagaagggctacgtgg agatcgaccacgccctgcccttctcccggacctgggacgactccttcaacaacaaggtgctggtgctgggctccgagaaccagaa caagggcaaccagacccccctacgagtacttcaacggcaaggacaactcccgggagtggcaggagttcaaggcccgggtggagacc tcccggttcccccggtccaagaagcagcggatcctgctgcagaagttcgacgaggacggcttcaaggagtgcaacctgaacgaca

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		cccggtacgtgaaccgcttcctgtgccagttcgtggccgaccacatcctgctgaccggcaagggcaagcggcgggtgttcgcctc caacggccagatcaccaacctgctgcggggcttctggggcctgcggaaggtgcgggccgagaacgaccggcaccacgccctggac gccgtggtggtggcctgctccaccgtggccatgcagcagaagatcacccggttcgtgcggtacaaggagatgaacgccttcgacg gcaagaccatcgacaaggagaccggcaaggtgctgcaccagaagacccacttcccccagccctgggagttcttcgcccaggaggt gatgatccgggtgttcggcaagcccgacggcaagcccgagttcgaggaggccgacacccccgagaagctgcggaccctgctggcc gagaagctgtcctcccggcccgaggccgtgcacgagtacgtgaccccccctgttcgtgtcccgggcccccaaccggaagatgtccg gcgcccacaaggacaccctgcggtccgccaagcggttcgtgaagcacaacgagaagatctccgtgaagcgggtgtggctgaccga gatcaagctggccgacctggagaacatggtgaactacaagaacggccgggagatcgagctgtacgaggccctgaaggcccggctg gaggcctacggcggcaacgccaagcaggccttcgaccccaaggacaacccctctacaagaagggcggccagctggtgaaggccg tgcgggtggagaagacccaggagtccggcgtgctgctgaacaagaagaacgcctacaccatcgccgacaacggcgacatggtgcg ggtggacgtgttctgcaaggtggacaagaagggcaagaaccagtacttcatcgtgcccatctacgcctggcaggtggccgagaac atcctgcccgacatcgactgcaagggctaccggatcgacgactcctacaccttctgcttctccctgcacaagtacgacctgatcg ccttccagaaggacgagaagtccaaggtggagttcgcctactacatcaactgcgactcctccaacggccggttctacctggcctg gcacgacaagggctccaaggagcagcagttccggatctccacccagaacctggtgctgatccagaagtaccaggtgaacgagctg ggcaaggagatccggccctgccggctgaagaagcggccccccgtgcggtagTGActagcaccagcctcaagaacacccgaatgga gtctctaagctacataataccaacttacactttacaaaatgttgtcccccaaaatgtagccattcgtatctgctcctaataaaaa gaaagtttcttcacattct
	829-899	Not used
Exemplary XTEN linker sequence	900	SGSETPGTSESATPES
Exemplary XTEN linker sequence	901	SGSETPGTSESA
Exemplary XTEN linker sequence	902	SGSETPGTSESATPEGGSGGS
Exemplary NLS sequence	903	PKKKRKV
Exemplary NLS sequence	904	PKKKRRV
Exemplary bipartite NLS sequence	905	KRPAATKKAGQAKKKK
exemplary XTEN	906	SGSETPGTSESATPES
exemplary XTEN	907	SGSETPGTSESA
exemplary XTEN	908	SGSETPGTSESATPEGGSGGS
amino acid sequence for exemplary linker	909	GGS

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
amino acid sequence for exemplary linker	910	GGGGS
amino acid sequence for exemplary linker	911	EAAAK
amino acid sequence for exemplary linker	912	SEGSA
amino acid sequence for exemplary linker	913	SEGSAGTST
amino acid sequence for exemplary linker	914	GGGGSGGGGS
amino acid sequence for exemplary linker	915	GGGGSEAAAK
amino acid sequence for exemplary linker	916	EAAAKGGGGS
amino acid sequence for exemplary linker	917	EAAAKEAAAK
amino acid sequence for exemplary linker	918	SEGSAGTSTESEGSA
amino acid sequence for exemplary linker	919	GGGGSGGGGSGGGGS
amino acid sequence for exemplary linker	920	GGGGSGGGGSEAAAK
amino acid sequence for exemplary linker	921	GGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	922	EAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	923	EAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	924	SEGSAGTSTESEGSAGTSTE

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
amino acid sequence for exemplary linker	925	GGGGSGGGGSGGGGSEAAAK
amino acid sequence for exemplary linker	926	GGGGSGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	927	GGGGSEAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	928	GGGGSEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	929	GGGGSEAAAKEAAAKEAAAK
amino acid sequence for exemplary linker	930	EAAAKGGGGSGGGGSGGGGS
amino acid sequence for exemplary linker	931	EAAAKGGGGSGGGGSEAAAK
amino acid sequence for exemplary linker	932	EAAAKGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	933	EAAAKGGGGSEAAAKEAAAK
amino acid sequence for exemplary linker	934	EAAAKEAAAKGGGGSGGGGS
amino acid sequence for exemplary linker	935	EAAAKEAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	936	EAAAKEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	937	SEGSAGTSTESEGSAGTSTESEGSA
amino acid sequence for exemplary linker	938	GGGGSGGGGSGGGGSGGGGSGGGGS
amino acid sequence for exemplary linker	939	GGGGSGGGGSGGGGSGGGGSEAAAK

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
amino acid sequence for exemplary linker	940	GGGGGGGGSGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	941	GGGGSGGGGSGGGGSEAAAKEAAAK
amino acid sequence for exemplary linker	942	GGGGSGGGGSEAAAKGGGGSGGGGS
amino acid sequence for exemplary linker	943	GGGGSGGGGSEAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	944	GGGGSGGGGSEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	945	GGGGSGGGGSEAAAKEAAAKEAAAK
amino acid sequence for exemplary linker	946	GGGGSEAAAKGGGGSGGGGSGGGGS
amino acid sequence for exemplary linker	947	GGGGSEAAAKGGGGSGGGGSEAAAK
amino acid sequence for exemplary linker	948	GGGGSEAAAKGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	949	GGGGSEAAAKGGGGSEAAAKEAAAK
amino acid sequence for exemplary linker	950	GGGGSEAAAKEAAAKGGGGSGGGGS
amino acid sequence for exemplary linker	951	GGGGSEAAAKEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	952	GGGGSEAAAKEAAAKEAAAKEAAAK
amino acid sequence for exemplary linker	953	EAAAKGGGGSGGGGSGGGGSGGGGS
amino acid sequence for exemplary linker	954	EAAAKGGGGSGGGGGGGSEAAAK

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
amino acid sequence for exemplary linker	955	EAAAKGGGGSGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	956	EAAAKGGGGSGGGGSEAAAKEAAAK
amino acid sequence for exemplary linker	957	EAAAKGGGGSEAAAKGGGGSGGGGS
amino acid sequence for exemplary linker	958	EAAAKGGGGSEAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	959	EAAAKGGGGSEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	960	EAAAKGGGGSEAAAKEAAAKEAAAK
amino acid sequence for exemplary linker	961	EAAAKEAAAKGGGGSEAAAKGGGGS
amino acid sequence for exemplary linker	962	EAAAKEAAAKGGGGSEAAAKEAAAK
amino acid sequence for exemplary linker	963	EAAAKEAAAKEAAAKGGGGSEAAAK
amino acid sequence for exemplary linker	964	EAAAKEAAAKEAAAKEAAAKGGGGS
amino acid sequence for exemplary linker	965	EAAAKEAAAKEAAAKEAAAKEAAAK
amino acid sequence for exemplary linker	966	GTKDSTKDIPETPSKD
amino acid sequence for exemplary linker	967	GRDVRQPEVKEEKPES
amino acid sequence for exemplary linker	968	EGKSSGSGSESKSTAG
amino acid sequence for exemplary linker	969	TPGS PAGS PTSTEEGT

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
amino acid sequence for exemplary linker	970	GSEPATSGSETPGTST
mRNA encoding BC22n	972	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGAGGCCUCCCCCGCCUCCGGCCCCCGGCACCUGAUGGA CCCCCACAUCUUCACCUCCAACUUCAACAACGGCAUCGGCCGGCACAAGACCUACCUGUGCUACGAGGUGGAGCGGCUGGACAAC GGCACCUCCGUGAAGAUGGACCAGCACCGGGGCUUCCUGCACAACCAGGCCAAGAACCUGCUGUGCGGCUUCUACGGCCGGCACG CCGAGCUGCGGUUCCUGGACCUGGUGCCCUCCCUGCAGCUGGACCCCGCCCAGAUCUACCGGGUGACCUGGUUCAUCUCCUGGUC CCCCUGCUUCUCCUGGGGCUGCGCCGGCGAGGUGCGGGCCUUCCUGCAGGAGAACACCCACGUGCGGCUGCGGAUCUUCGCCGCC CGGAUCUACGACUACGACCCCCUGUACAAGGAGGCCCUGCAGAUGCUGCGGGACGCCGGCGCCCAGGUGUCCAUCAUGACCUACG ACGAGUUCAAGCACUGCUGGGACACCUUCGUGGACCACCAGGGCUGCCCCUUCCAGCCCUGGGACGGCCUGGACGAGCACUCCCA GGCCCUGUCCGGCCGGCUGCGGGCCAUCCUGCAGAACCAGGGCAACUCCGGCUCCGAGACCCCCGGCACCUCCGAGUCCGCCACC CCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCCAUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGG UGCCCUCCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUC CGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAG GAGAUCUUCUCCAACGAGAUGGCCAAGGUGGACGACUCCUUCUUCCACCGGCUGGAGGAGUCCUUCCUGGUGGAGGAGGACAAGA AGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUACCCCACCAUCUACCACCUGCGGAA GAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCCGGGGCCACUUC CUGAUCGAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUCCAGCUGGUGCAGACCUACAACCAGCUGUUCG AGGAGAACCCCAUCAACGCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCUGUCCAAGUCCCGGCGGCUGGAGAACCU GAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCCUGUCCCUGGGCCUGACCCCCAACUUCAAG UCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGA UCGGCGACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGUCCGACGCCAUCCUGCUGUCCGACAUCCUGCGGGUGAACAC CGAGAUCACCAAGGCCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUG GUGCGGCAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCG CCUCCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCG GGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUG CGGCGGCAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUGACCUUCCGGAUCCCCUACUACG UGGGCCCCCUGGCCCGGGGCAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCACCCCCUGGAACUUCGAGGA GGUGGUGGACAAGGGCGCCUCCGCCCAGUCCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCUG CCCAAGCACUCCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGC CCGCCUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAA GGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACUCCGUGGAGAUCUCCGGCGUGGAGGACCGGUUCAACGCCUCCCUGGGCACC UACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGA CCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGACAAGGUGAUGAAGCA GCUGAAGCGGCGGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACC AUCCUGGACUUCCUGAAGUCCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACUCCCUGACCUUCAAGGAGG ACAUCCAGAAGGCCCAGGUGUCCGGCCAGGGCGACUCCCUGCACGAGCACAUCGCCAACCUGGCCGGCUCCCCCGCCAUCAAGAA GGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUG GCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGG GCUCCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCG GGACAUGUACGUGGACCAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCUUCCUGAAG GACGACUCCAUCGACAACAAGGUGCUGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAGGUGGUGA AGAAGAUGAAGAACUACUGGCGGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCG GGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGGCCCAG AUCCUGGACUCCCGGAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAGUCCAAGC

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		UGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAA CGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUG CGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCA AGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGA CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGC GGCUUCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACG GCGGCUUCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU GAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAG GAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCG CCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCU GAAGGGCUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCA UCCGGGAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCAC CAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACC CGGAUCGACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUGACUAGCACCAGCCUCAAGAA CACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGC UCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAA AAAAAAAAAUAUAAAAAAAAAAAAAACAUAAAAAAAAAAAAAACGAAAAAAAAAAAAAACGUAAAAAAAAAAAAAACUCAAAAAAAAAAAAAGA UAAAAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAAACGCAAAAAAAAAAAAACACAAAAAAAAA AAAUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAA AAAAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG
mRNA encoding BC22n with HiBit tag	973	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGAGGCCUCCCCCGCCUCCGGCCCCCGGCACCUGAUGGA CCCCCACAUCUUCACCUCCAACUUCAACAACGGCAUCGGCCGGCACAAGACCUACCUGUGCUACGAGGUGGAGCGGCUGGACAAC GGCACCUCCGUGAAGAUGGACCAGCACCGGGGCUUCCUGCACAACCAGGCCAAGAACCUGCUGUGCGGCUUCUACGGCCGGCACG CCGAGCUGCGGUUCCUGGACCUGGUGCCCUCCCUGCAGCUGGACCCCGCCCAGAUCUACCGGGUGACCUGGUUCAUCUCCUGGUC CCCCUGCUUCUCCUGGGGCUGCGCCGGCGAGGUGCGGGCCUUCCUGCAGGAGAACACCCACGUGCGGCUGCGGAUCUUCGCCGCC CGGAUCUACGACUACGACCCCCUGUACAAGGAGGCCCUGCAGAUGCUGCGGGACGCCGGCGCCCAGGUGUCCAUCAUGACCUACG ACGAGUUCAAGCACUGCUGGGACACCUUCGUGGACCACCAGGGCUGCCCCUUCCAGCCCUGGGACGGCCUGGACGAGCACUCCCA GGCCCUGUCCGGCCGGCUGCGGGCCAUCCUGCAGAACCAGGGCAACUCCGGCUCCGAGACCCCCGGCACCUCCGAGUCCGCCACC CCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCCAUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGG UGCCCUCCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUC CGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAG GAGAUCUUCUCCAACGAGAUGGCCAAGGUGGACGACUCCUUCUUCCACCGGCUGGAGGAGUCCUUCCUGGUGGAGGAGGACAAGA AGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUACCCCACCAUCUACCACCUGCGGAA GAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCCGGGGCCACUUC CUGAUCGAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUCCAGCUGGUGCAGACCUACAACCAGCUGUUCG AGGAGAACCCCAUCAACGCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCUGUCCAAGUCCCGGCGGCUGGAGAACCU GAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCCUGUCCCUGGGCCUGACCCCCAACUUCAAG UCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGA UCGGCGACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGUCCGACGCCAUCCUGCUGUCCGACAUCCUGCGGGUGAACAC CGAGAUCACCAAGGCCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUG GUGCGGCAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCG CCUCCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCG GGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUG CGGCGGCAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUGACCUUCCGGAUCCCCUACUACG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		UGGGCCCCCUGGCCCGGGGCAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCACCCCCUGGAACUUCGAGGA GGUGGUGGACAAGGGCGCCUCCGCCCAGUCCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCUG CCCAAGCACUCCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGC CCGCCUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAA GGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACUCCGUGGAGAUCUCCGGCGUGGAGGACCGGUUCAACGCCUCCCUGGGCACC UACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGA CCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGACAAGGUGAUGAAGCA GCUGAAGCGGCGGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACC AUCCUGGACUUCCUGAAGUCCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACUCCCUGACCUUCAAGGAGG ACAUCCAGAAGGCCCAGGUGUCCGGCCAGGGCGACUCCCUGCACGAGCACAUCGCCAACCUGGCCGGCUCCCCCGCCAUCAAGAA GGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUG GCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGG GCUCCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCG GGACAUGUACGUGGACCAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCUUCCUGAAG GACGACUCCAUCGACAACAAGGUGCUGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAGGUGGUGA AGAAGAUGAAGAACUACUGGCGGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCG GGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGGCCCAG AUCCUGGACUCCCGGAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAGUCCAAGC UGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAA CGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUG CGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCA AGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGA CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGC GGCUUCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACG GCGGCUUCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU GAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAG GAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCG CCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCU GAAGGGCUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCA UCCGGGAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCAC CAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACC CGGAUCGACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCCGAGU CCGUGUCCGGCUGGCGGCUGUUCAAGAAGAUCUCCUGACUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUA AUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAU UCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUA AAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUAAAAAAAAAAAAACCUAAAAAAAAAAAA UGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAAAUGCAAAAAAAAAAAAUCGAAAAAAA AAAAAUCUAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAA AAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG
	974	Not used
mRNA encoding UGI	975	GGGAGACCCAAGCUGGCUAGCUCCCGCAGUCGGCGUCCAGCGGCUCUGCUUGUUCGUGUGUGUGUCGUUGCAGGCCUUAUUCGGA UCCGCCACCAUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAA AGCAGCUGGUCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCU

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		GGUCCACACAGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUG GUCAUCCAGGACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGAUAGUCUAGACAUCACAUUUAAAAGCAUCUCAGCCUACCAU GAGAAUAAGAGAAAGAAAAUGAAGAUCAAUAGCUUAUUCAUCUCUUUUUCUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAAA AAACAUAAAUUUCUUUAAUCAUUUUGCCUCUUUUCUCUGUGCUUCAAUUAAUAAAAAAUGGAAAGAACCUCGAGUCUAG
Exemplary amino acid sequence for NLS-NLS-APOBEC3A-L070-Nme2D16A	976	MDGSGGGSPKKKRKVEDKRPAATKKAGQAKKKKGGSGGGEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVK MDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDY DPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGNGTKDSTKDIPETPSKDAA FKPNPINYILGLAIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRLLRARRLLKR EGVLQAADFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVANNAHALQTGD FRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHC TFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAE ASTLMEMKAYHAISRALEKEGLKDKKSPLNLSSELQDEIGTAFSLEKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKAL RRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSF KDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLVRLNEKGYVEIDHALPFSRTWDD SFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKECNLNDTRYVNRFLCQFVAD HILLTGKGKRRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKVLHQ KTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGAHKDTLRSAKRFV KHNEKISVKRVWLTEIKLADLENMVNYKNGREIELYEALKARLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQESGVLLN KKNAYTIADNGDMVRVDVFCKVDKKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSYTFCFSLHKYDLIAFQKDEKSKVEFAY YINCDSSNGRFYLAWHDKGSKEQQFRISTQNLVLIQKYQVNELGKEIRPCRLKKRPPVR
Amino acid sequence for BE3	977	MSSETGPVAVDPTLRRRIEPHEFEVFEDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSIT WFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRY PHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGTSESATPESDKKYSIGLAI GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDS FFHRLEESELVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNFDLAEDAKLQL SKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIF FDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDF LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADAN LDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGG STNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS GGSPKKKRKV
Amino acid sequence for BE3	978	MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSIT WFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRY

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		PHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGTSESATPESDKKYSIGLAI GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDS FFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKERGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNEDLAEDAKLQL SKDTYDDDLDNLLAQIGDQYADLELAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIF FDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYETVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRENASLGTYHDLLKIIKDKDE LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDERKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNS DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADAN LDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGG STNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS GGSPKKKRKV
Amino acid sequence for BC22 with 2x UGI	979	MEASPASGPRHLMDPHIFTSNENNGIGRHKTYLCYEVERLDNGTSVKMDQHRGELHNQAKNLLCGFYGRHAELRELDLVPSLQLD PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESELVEEDKKHERHPIFGNIVDEV AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRENASLGTYHDLLKIIKDKDELDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDELKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDERKDFQFYKVR EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLETLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKETGKQLVIQESILM LPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSGGSGGSTNLSDIIEKETGKQL VIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSKRTADGSEFEPKK KRKV
Amino acid sequence for BE4MAX protein	980	MKRTADGSEFESPKKKRKVSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFI EKFTTERYFCPNTRCSITWELSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYC WRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGS

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
		SGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLEDSGETAEAT RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTD KADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE KKNGLFGNLIALSLGLTPNEKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPL SASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ RTFDNGSIPHQIHLGELHAILRRQEDFYPELKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGAS AQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI ECFDSVEISGVEDRENASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLEDDKVMKQLKRRRYT GWGRLSRKLINGIRDKQSGKTILDELKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVK VVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLTKAERGGLSELD KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDERKDFQFYKVREINNYHHAHDAYLNAVVGTAL IKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFAT VRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDN EQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYT STKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHT AYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKP ESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSKRTADGSEFEPKKKRKV**
CD30 Construct (DNA)	981	ATGGACTTCCAGGTGCAGATCTTCAGCTTCCTGCTGATCAGCGCCAGCGTGATCATGAGCCGGATGGCCCAGGTGCAGCTGCAGC AGAGCGGCGCCGAGCTGGCCCGGCCCGGCGCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACCACCTACACCAT CCACTGGGTGCGGCGGCGGCCCGGCCACGACCTGGAGTGGATCGGCTACATCAACCCCAGCAGCGGCTGCAGCGACTACAACCAG AACTTCAAGGGCAAGACCACCCTGACCGCCGACAAGAGCAGCAACACCGCCTACATGCAGCTGAACAGCCTGACCAGCGAGGACA GCGCCGTGTACTACTGCGCCCGGCGGGCCGACTACGGCAACTACGAGTACACCTGGTTCGCCTACTGGGGCCAGGGCACCACCGT GACCGTGAGCAGCAGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGTGATCGAGCTGACCCAGAGCCCCAAG TTCATGAGCACCAGCGTGGGCGACCGGGTGAACGTGACCTACAAGGCCAGCCAGAACGTGGGCACCAACGTGGCCTGGTTCCAGC AGAAGCCCGGCCAGAGCCCCAAGGTGCTGATCTACAGCGCCAGCTACCGGTACAGCGGCGTGCCCGACCGGTTCACCGGCAGCGG CAGCGGCACCGACTTCACCCTGACCATCAGCAACGTGCAGAGCGAGGACCTGGCCGAGTACTTCTGCCAGCAGTACCACACCTAC CCCCTGACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGCGGAGCGACCCCGCCACCACCACCCCCGCCCCCCGGCCCCCCACCC CCGCCCCCACCATCGCCAGCCAGCCCCTGAGCCTGCGGCCCGAGGCCTGCCGGCCCGCCGCCGGCGGCGCCGTGCACACCCGGGG CCTGGACTTCGCCTGCGACAAGGACCCCAAGTTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTG ACCGTGGCCTTCATCATCTTCTGGGTGCGGAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCCCGGCGGC CCGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCCCCCCGGGACTTCGCCGCCTACCGGAGCCTGCGGGTGAAGTTCAGCCG GAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCCGGCGGGAGGAGTACGACGTG CTGGACAAGCGGCGGGGCCGGGACCCCGAGATGGGCGGCAAGCCCCGGCGGAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGC AGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGCGGCGGGGCAAGGGCCACGACGGCCTGTACCA GGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCGGTAA
CD30 Construct (Amino Acid)	982	MDFQVQIFSFLLISASVIMSRMAQVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQ NFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPK FMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTY PLTFGGGTKLEIKRSDPATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDKDPKFWVLVVVGGVLACYSLLV TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
CD30 Construct Signal Peptide (DNA)	983	ATGGACTTCCAGGTGCAGATCTTCAGCTTCCTGCTGATCAGCGCCAGCGTGATCATGAGCCGGATGGCC
CD30 Construct Signal Peptide (Amino Acid)	984	MDFQVQIFSELLISASVIMSRMA
CD30 Construct HRS3 Binder (DNA)	985	CAGGTGCAGCTGCAGCAGAGCGGCGCCGAGCTGGCCCGGCCCGGCGCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCT TCACCACCTACACCATCCACTGGGTGCGGCGGCGGCCCGGCCACGACCTGGAGTGGATCGGCTACATCAACCCCAGCAGCGGCTG CAGCGACTACAACCAGAACTTCAAGGGCAAGACCACCCTGACCGCCGACAAGAGCAGCAACACCGCCTACATGCAGCTGAACAGC CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCCGGCGGGCCGACTACGGCAACTACGAGTACACCTGGTTCGCCTACTGGG GCCAGGGCACCACCGTGACCGTGAGCAGCAGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGTGATCGAGCT GACCCAGAGCCCCAAGTTCATGAGCACCAGCGTGGGCGACCGGGTGAACGTGACCTACAAGGCCAGCCAGAACGTGGGCACCAAC GTGGCCTGGTTCCAGCAGAAGCCCGGCCAGAGCCCCAAGGTGCTGATCTACAGCGCCAGCTACCGGTACAGCGGCGTGCCCGACC GGTTCACCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAACGTGCAGAGCGAGGACCTGGCCGAGTACTTCTGCCA GCAGTACCACACCTACCCCCTGACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGCGG
CD30 Construct HRS3 Binder (Amino Acid)	986	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNEKGKTTLTADKSSNTAYMQLNS LTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTN VAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKR
CD30 Construct CD8 Hinge (DNA)	987	ACCACCACCCCCGCCCCCCGGCCCCCCACCCCCGCCCCCACCATCGCCAGCCAGCCCCTGAGCCTGCGGCCCGAGGCCTGCCGGC CCGCCGCCGGCGGCGCCGTGCACACCCGGGGCCTGGACTTCGCCTGCGAC
CD30 Construct CD8 Hinge (Amino Acid)	988	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
CD30 Construct CD28 TM (DNA)	989	TTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGTGGCCTTCATCATCTTCTGGGTGCGGA GCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCCCGGCGGCCCGGCCCCACCCGGAAGCACTACCAGCCCTA CGCCCCCCCCCGGGACTTCGCCGCCTACCGGAGC
CD30 Construct CD28 TM (Amino Acid)	990	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
CD30 Construct CD3zeta (DNA)	991	CTGCGGGTGAAGTTCAGCCGGAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCC GGCGGGAGGAGTACGACGTGCTGGACAAGCGGCGGGGCCGGGACCCCGAGATGGGCGGCAAGCCCCGGCGGAAGAACCCCCAGGA GGGCCTGTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGGGGGGGCAAG GGCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCGG
CD30 Construct CD3zeta (Amino Acid)	992	LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
B2M (G000529)	993	mG*mG*mC*CACGGAGCGAGACAUCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
G021469 TRAC	994	mA*mU*mA*mUmCCAmGmAAmCCmCUGACmCCUGmCCGmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
G021475 TRAC	995	mA*mA*mC*mCmCUGmAmUCmCUmCUUGUmCCCAmCAGmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
G021481 TRAC	996	mG*mC*mC*mGmUGUmAmCCmAGmCUGAGmAGACmUCUmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
G028013 B2M	997	mG*mG*mC*CACGGAGCGAGACAUCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G018995 HLA-A	998	mA*mC*mA*GCGACGCCGCGAGCCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G012086 TRAC	999	mA*mG*mA*GUCUCUCAGCUGGUACAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
Guide Scaffold	3002	NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUC GGUGCU
Guide scaffold	3003	mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC ACGAAAGGGCACCGAGUCGGmUmGmC*mU
	3004	Not Used
mRNA sequence encoding UGI	3005	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAA GCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGGUGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUG GUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCUGCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGG UGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCCGGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUC CCCCAAGAAGAAGCGGAAGGUGGAGUGAUAGCUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAA CUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCG AGAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAA AAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAGAUAAAAAAAAAAAAACCUAAAAAAAAAAAAUGUAAAA AAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAAACACAAAAAAAAAAAAUGCAAAAAAAAAAAAUCGAAAAAAAAAAAUC UAAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAA AACUGAAAAAAAAAAAUUUAAAAAAAAAAAUCUAG

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
Guide scaffold 90-mer	3006	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC
Guide scaffold 90-mer with modification	3007	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 90-mer with modification	3008	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC
Guide scaffold 88-mer with modification	3009	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 88-mer	3010	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGGUGC
Guide scaffold 88-mer with modification	3011	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 88-mer with modification	3012	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmAmAmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC
Guide scaffold	3013	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
Guide scaffold	3014	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
	3015-3018	Not Used
G025420 TRAC	3019	mC*mU*mC*UCAGCUGGUACACGGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGmU*mG*mC*mU
B2M (G000529) (full, unmodified)	3100	GGCCACGGAGCGAGACAUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
G021469 TRAC (full, unmodified)	3101	AUAUCCAGAACCCUGACCCUGCCGGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
G021475 TRAC (full, unmodified)	3102	AACCCUGAUCCUCUUGUCCCACAGGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
G021481 TRAC (full, unmodified)	3103	GCCGUGUACCAGCUGAGAGACUCUGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
G028013 B2M (full, unmodified)	3104	GGCCACGGAGCGAGACAUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU

TABLE 9-continued

VI. ADDITIONAL SEQUENCES

*The guide sequence disclosed in this Table may be unmodified, modified with the exemplary modification pattern shown in the Table, or modified with a different modification pattern disclosed herein or available in the art.

Description	SEQ ID NO	Sequence
G018995 HLA-A (full, unmodified)	3105	ACAGCGACGCCGCGAGCCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC CGAGUCGGUGCUUUU
G012086 TRAC (full, unmodified)	3106	AGAGUCUCUCAGCUGGUACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC CGAGUCGGUGCUUUU
G000644 EMX1 target sequence	3107	GAGUCCGAGCAGAAGAAGAA
G000645 VEGFA target sequence	3108	GACCCCCUCCACCCCGCCUC
G000646 RAG1B target sequence	3109	GACUUGUUUUCAUUGUUCUC
G013675 CIITA target sequence	3110	CCCCCGGACGGUUCAAGCAA
G013675 CIITA sgRNA	3118	mC*mC*mC*CCGGACGGUUCAAGCAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

TABLE 9A

VII. Additional Exemplary Nme Guide RNAs

Guide ID	Target	Guide Sequence	Exemplary Guide RNA Full Sequence	Exemplary Guide RNA Modified Sequence	Genomic Coordinates (hg38)
G034202	HLA-A	GCUCUAU CCACGGC GCCCGCG GCU (SEQ ID NO: 576)	GCUCUAUCCACGGCGCCCGCGGCUGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1576)	mG*mC*mU*mCmUAUmCmCAmCGmGCGCCmCGCGmGCUmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3111)	chr6: 29942891-29942915
G034617	HLA-A	CACUCAC CCGCCCA GGUCUGG GUC (SEQ ID NO: 571)	CACUCACCCGCCCAGGUCUGGGUCGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1571)	mC*mA*mC*mUmCACmCmCGmCCmCAGGUmCUGGmGUCmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3112)	chr6: 29942609-29942633
G028943	TRAC	AAAACCU GUCAGUG AUUGGG UUCC (SEQ ID NO: 605)	AAAACCUGUCAGUGAUUGGGUUCCGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1605)	mA*mA*mA*mAmCCUmGmUCmAGmUGAUUmGGGUmUCCmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2605)	chr14: 22550574-22550598
G034982	TRAC	AAAACCU GUCAGUG AUUGGG UUCC (SEQ ID NO: 605)	AAAACCUGUCAGUGAUUGGGUUCCGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1605)	mA*mA*mA*mAmCCUmGmUCmAGmUGAUUmGGGUmUCCmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3113)	chr14: 22550574-22550598
G028939	TRAC	UUAGGU UCGUAUC UGUAAA ACCAA (SEQ ID NO: 606)	UUAGGUUCGUAUCUGUAAAACCAAGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1606)	mU*mU*mA*mGmGUUmCmGUmAUmCUGUAmAAACmCAAmGUUGmU mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGm CCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCC mUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2606)	chr14: 22550544-22550568
G034981	TRAC	UUAGGU UCGUAUC UGUAAA ACCAA (SEQ ID NO: 606)	UUAGGUUCGUAUCUGUAAAACCAAGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1606)	mU*mU*mA*mGmGUUmCmGUmAUmCUGUAmAAACmCAAmGUUGmU mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3114)	chr14: 22550544-22550568

TABLE 9A-continued

VII. Additional Exemplary Nme Guide RNAs

Guide ID	Target	Guide Sequence	Exemplary Guide RNA Full Sequence	Exemplary Guide RNA Modified Sequence	Genomic Coordinates (hg38)
G013006	TRAC	CUCUCAG CUGGUAC ACGGCA (SEQ ID NO: 613)	CUCUCAGCUGGUACACGGCAGUUUUAGAG CUAGAAAUAGCAAGUUAAAAUAAGGCUAG UCCGUUAUCAACUUGAAAAAGUGGCACCG AGUCGGUGCUUUU (SEQ ID NO: 1613)	mC*mU*mC*UCAGCUGGUACACGGCAGUUUUAGAmGmCmUmAmGmA mAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCm UmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGm GmUmGmCmU*mU*mU*mU (SEQ ID NO: 2613)	chr14: 22547524-22547544
G028986	TRBC1	GUGUCCU ACCAGCA AGGGGUC CUG (SEQ ID NO: 607)	GUGUCCUACCAGCAAGGGGUCCUGGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1607)	mG*mU*mG*mUmCCUmAmCCmAGmCAAGGmGGUCmCUGmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2607)	chr7: 142792690-142792714
G034618	TRBC1	GUGUCCU ACCAGCA AGGGGUC CUG (SEQ ID NO: 607)	GUGUCCUACCAGCAAGGGGUCCUGGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1607)	mG*mU*mG*mUmCCUmAmCCmAGmCAAGGmGGUCmCUGmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3115)	chr7: 142792690-142792714
G026584	CIITA	UCAAAGU ACCCUAC AGGAGG ACCA (SEQ ID NO: 608)	UCAAAGUACCCUACAGGAGGACCAGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1608)	mU*mC*mA*mAmAGUmAmCCmCUmACAGGmAGGAmCCAmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2608)	chr16: 10907504-10907528
G034201	CIITA	UCAAAGU ACCCUAC AGGAGG ACCA (SEQ ID NO: 608)	UCAAAGUACCCUACAGGAGGACCAGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1608)	mU*mC*mA*mAmAGUmAmCCmCUmACAGGmAGGAmCCAmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3116)	chr16: 10907504-10907528
G029131	CIITA	AGCUGCC GUUCUGC CCAGUCC GGG (SEQ ID NO: 609)	AGCUGCCGUUCUGCCCAGUCCGGGGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1609)	mA*mG*mC*mUmGCCmGmUUmCUmGCCCAmGUCCmGGGmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2609)	chr16: 10906643-10906667

TABLE 9A-continued

VII. Additional Exemplary Nme Guide RNAs

Guide ID	Target	Guide Sequence	Exemplary Guide RNA Full Sequence	Exemplary Guide RNA Modified Sequence	Genomic Coordinates (hg38)
G034619	CIITA	AGCUGCC GUUCUGC CCAGUCC GGG (SEQ ID NO: 609)	AGCUGCCGUUCUGCCCAGUCCGGGGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1609)	mA*mG*mC*mUmGCCmGmUUmCUmGCCCAmGUCCmGGGmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3117)	chr16: 10906643-10906667
G021557	VEGFA	GCAUGGG CAGGGGC UGGGGU GCAC (SEQ ID NO: 610)	GCAUGGGCAGGGGCUGGGGUGCACGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1610)	mG*mC*mA*mUmGGGmCmAGmGGmGCUGGmGGUGmCACmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2610)	chr6: 43774288-43774312
G021558	VEGFA	GAAUGGC AGGCGGA GGUUGU ACUG (SEQ ID NO: 611)	GAAUGGCAGGCGGAGGUUGUACUGGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1611)	mG*mA*mA*mUmGGCmAmGGmCGmGAGGUmUGUAmCUGmGUUGmU mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGm CCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCC mUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2611)	chr6: 43780852-43780876
G021567	VEGFA	GUGAGCA GGCACCU GUGCCAA CAU (SEQ ID NO: 612)	GUGAGCAGGCACCUGUGCCAACAUGUUGU AGCUCCCUGAAACCGUUGCUACAAUAAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CUUCUGGCAUCGUU (SEQ ID NO: 1612)	mG*mU*mG*mAmGCAmGmGCmACmCUGUGmCCAAmCAUmGUUGmUm AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2612)	chr6: 43781113-43781137

VII. EXAMPLES

[0971] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1. General Methods

1.1. T Cell Culture Media Preparation

[0972] T cell culture media compositions used below are described here. "TCAM Media" comprises of CTS OpTimizer T Cell Expansion SFM containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax, 1% (v/v) 10 1M HEPES buffer, and 1% of Penicillin-Streptomycin. In addition to the above mentioned components, media was supplemented with 100 U/mL of recombinant human interleukin-2, 5 ng/mL of human interleukin-7, and 5 ng/mL of human interleukin-15.

1.2. Preparation of Lipid Nanoparticles

[0973] The lipid components were dissolved in 100% ethanol at various molar ratios. The RNA cargos (e.g., Cas9 mRNA and sgRNA) were dissolved in 25 mM citrate buffer, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL.

[0974] The lipid nucleic acid assemblies contained ionizable Lipid A ((9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy) methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate), Lipid nanoparticles used 35% Lipid A, 47.5% cholesterol, 15% DSPC, and 2.5% PEG2k-DMG by molarity. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight.

[0975] Lipid nanoparticles (LNP compositions) were prepared using a cross-flow technique utilizing impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipids in ethanol were mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water was mixed with the outlet stream of the cross through an inline tee (See WO2016010840 FIG. **2**). The LNP compositions were held for 1 hour at room temperature (RT), and further diluted with water (approximately 1:1 v/v). LNP compositions were concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100 kD MWCO) and buffer exchanged using PD-10 desalting columns (GE) into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). Alternatively, the LNP's were optionally concentrated using 100 kDa Amicon spin filter and buffer exchanged using PD-10 desalting columns (GE) into TSS. The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at 4° C. or −80° C. until further use.

1.3. In Vitro Transcription ("IVT") of mRNA

[0976] Capped and polyadenylated mRNA containing N1-methyl pseudo-U was generated by in vitro transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter, a sequence for transcription, and a polyadenylation sequence was linearized by incubating at 37° C. for 2 hours with XbaI with the following conditions: 200 ng/μL plasmid, 2 U/μL XbaI (NEB), and 1× reaction buffer. The XbaI was inactivated by heating the reaction at 65° C. for 20 min. The linearized plasmid was purified from enzyme and buffer salts. The IVT reaction to generate modified mRNA was performed by incubating 50 ng/L linearized plasmid; 2-5 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10-25 mM ARCA (Trilink); 5 U/μL T7 RNA polymerase (NEB); 1 U/μL Murine RNase inhibitor (NEB); 0.004 U/μL Inorganic *E. coli* pyrophosphatase (NEB); and 1× reaction buffer at 37° C. for 1.5-4 hours. TURBO DNase (ThermoFisher) was added to a final concentration of 0.01 U/μL, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The mRNA was purified using a MegaClear Transcription Clean-up kit (ThermoFisher) or a RNeasy Maxi kit (Qiagen) per the manufacturers' protocols. Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by HPLC-based purification. Briefly, after the DNase digestion, mRNA is purified using LiCl precipitation, ammonium acetate precipitation and sodium acetate precipitation. For HPLC purified mRNA, after the LiCl precipitation and reconstitution, the mRNA was purified by RP-IP HPLC (see, e.g., Kariko, et al. Nucleic Acids Research, 2011, Vol. 39, No. 21 e142). The fractions chosen for pooling were combined and desalted by sodium acetate/ethanol precipitation as described above. In an alternative method, mRNA was purified with a LiCl precipitation method followed by further purification by tangential flow filtration. RNA concentrations were determined by measuring the light absorbance at 260 nm (Nanodrop), and transcripts were analyzed by capillary electrophoresis by Bioanlayzer (Agilent).

[0977] *S. pyogenes* ("Spy") Cas9 mRNA were generated from plasmid DNA encoding an open reading frame having a nucleic acid sequence of one of SEQ ID NOs: 801-803 and 806 (see sequences in Table 9). When SEQ ID NOs: 801-803 and 806 are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which were N1-methyl pseudouridines as described above). Messenger RNAs used in the Examples include a 5' cap and a 3' polyadenylation region, e.g., up to 100 nucleotides, and have a nucleic acid sequence of one of SEQ ID NOs: 801-803 and 806 in Table 9.

Example 2: Screening of HLA-B Guide RNAs with Spy Cas9

[0978] 48 sgRNAs in the 100-nt modified sgRNA format designed for the disruption of the HLA-B gene were screened for efficacy in T cells by assessing loss of HLA-B surface protein. The donor had an HLA-B phenotype of B*07:02 and B*07:02. The percentage of T cells negative for HLA-B7 was determined by flow cytometry following editing at the HLA-B locus by electroporation with Cas9 ribonucleoprotein (RNP) and each test guide.

2.1. RNP Electroporation of T Cells

[0979] Cas9 editing activity was assessed using electroporation of Cas9 ribonucleoprotein (RNP). Upon thaw, Pan CD3+ T cells (StemCell, HLA-B*07:02/B*07:02) were plated at a density of 0.5×10^6 cells/mL in T cell RPMI media composed of RPMI 1640 (Invitrogen, Cat. 22400-089) containing 5% (v/v) of fetal bovine serum, 1× Glutamax (Gibco, Cat. 35050-061), 50 μM of 2-Mercaptoethanol, 100 μM non-essential amino acids (Invitrogen, Cat. 11140-

050), 1 mM sodium pyruvate, 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02). T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). Cells were expanded in T cell RPMI media for 72 hours prior to RNP transfection.

[0980] HLA-B targeting sgRNAs and control B2M and HLA-A targeting sgRNAs were removed from their storage plates and denatured for 2 minutes at 95° C. before cooling at room temperature for 10 minutes. RNP mixture of 20 μM sgRNA and 10 μM Cas9-NLS protein (SEQ ID NO: 800) was prepared and incubated at 25° C. for 10 minutes. Five μL of RNP mixture was combined with 100,000 cells in 20 μL P3 electroporation Buffer (Lonza). 22 μL of RNP/cell mix was transferred to the corresponding wells of a Lonza shuttle 96-well electroporation plate. Cells were electroporated in duplicate with the manufacturer's pulse code. T cell RPMI media was added to the cells immediately post electroporation.

2.2. Flow Cytometry

[0981] On day 7 post-edit, T cells were phenotyped by flow cytometry to determine HLA-B protein expression following editing at the HLA-B locus. Briefly, T cells were incubated in antibody targeting HLA-B7, B27 (Miltenyi, Clone REA176, 130-120-234) surface protein corresponding the cells donor's genotype (HLA-B*07:02/B*07:02). Cells were subsequently washed, processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, and HLA-B7 expression. Table 10 and FIG. **1** shows the mean percentage of cells negative for HLA-B7 following editing at the HLA-B locus.

TABLE 10

Mean percentage of T cells HLA-B7 negative following editing at the HLA-B locus

Guide ID	Mean % B7−	SD % B7−
G022020	90.55	1.767767
G022010	74.55	0.212132
G022046	73.95	5.868986
G022043	71.65	0.777817
G022019	60.8	2.262742
G022053	57.25	1.626346
G022011	56.4	1.131371
G022055	52.8	0.141421
G022031	47.85	0.494975
G022027	44.55	1.06066
G022008	39.55	3.323402
G022022	31.1	0.424264
G022015	28.65	0.919239
G022032	23.8	7.353911

TABLE 10-continued

Mean percentage of T cells HLA-B7 negative following editing at the HLA-B locus

Guide ID	Mean % B7−	SD % B7−
G022044	20.7	0.848528
G022039	20.65	2.899138
G022041	17.9	1.697056
G022033	15.05	4.596194
G022017	14.8	0.848528
G022025	13.45	1.484924
G022028	8.575	0.360624
G022050	7.73	0.028284
G022049	7.01	0.876812
G022014	6.5	0.042426
G022030	6.37	0.579828
G022054	6.25	0.183848
G022051	6.225	0.586899
G022018	5.83	1.06066
G022012	5.495	0.26163
G022047	5.465	0.318198
G022048	5.44	0.028284
G022024	4.35	1.046518
G022042	3.255	0.855599
G022034	3.07	1.499066
G022036	2.595	0.205061
G022029	2.4	0.452548
G022026	2.08	0.692965
G022045	1.525	0.148492
G022038	1.47	0.523259
G022035	1.34	0.39598
G022040	1.165	0.487904
G022037	0.74	0.056569
G022021	0.64	0.155563
G022023	0.57	0.212132
G022052	0.43	0.028284
G022013	0.41	0.028284
G022016	0.195	0.06364
G022009	0.1845	0.120915

Example 3: NK Cell Functional Killing Assays

[0982] T cells edited to disrupt HLA-B (G022010 and G022020), HLA-A, or B2M were tested for their ability to resist natural killer (NK) cell mediated killing.

3.1 Flow Cytometry

[0983] NK cell mediated cytotoxicity towards engineered T cells was assayed. For this the T cells were co-cultured with the HLA-B/C matched CTV labelled NK cells at effector to target ratios (E:T) of 10:1, 5:1, 2.5:1, 1.25:1 and 0.625:1 for 21 hours. The cells were stained with 7AAD (BD Pharmingen, Cat. 559925), processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on CTV negativity, size, and shape and viability. Table 11 and FIG. **2** shows the percentage of T cell lysis following NK cell challenge.

TABLE 11

Percentage T cell lysis following NK cell challenge to engineered T cells

	WT		HLA-A KO		HLA-B KO (010)		HLA-B KO (020)		B2M KO	
E:T	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Basal	26.90	1.13	27.55	0.21	35.20	0.14	29.95	0.49	27.75	1.06
0.625 to 1	20.70	0.00	22.30	0.71	31.50	0.42	25.50	0.14	42.60	0.71

TABLE 11-continued

Percentage T cell lysis following NK cell challenge to engineered T cells										
	WT		HLA-A KO		HLA-B KO (010)		HLA-B KO (020)		B2M KO	
E:T	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.25 to 1	20.80	0.42	23.85	0.64	32.00	1.70	25.65	0.21	56.60	1.13
2.5 to 1	21.10	0.14	25.75	0.35	33.00	1.27	28.30	0.28	78.25	1.34
5 to 1	22.25	0.49	27.40	0.28	35.05	0.92	30.40	0.14	89.19	3.66
10 to 1	22.90	0.14	27.65	1.20	36.35	1.63	47.00	17.39	94.79	0.38

Example 4: Off-Target Analysis of HLA-B Human Guides

[0984] Screening for potential off-target genomic sites cleaved by Cas9 targeting HLA-B was performed. (See, e.g., Cameron et al., *Nature Methods*. 6, 600-606; 2017). In this experiment, 2 sgRNAs targeting human HLA-B and three control guides targeting EMX1, VEGFA, and RAG1B with known off-target profiles were screened using purified genomic DNA from lymphoblast cell line NA24385 (Coriell Institute). Genomic DNA was treated with Quick CIP (NEB M0525) prior to running SITE-Seq. The number of potential off-target sites were detected using a sgRNA as shown in Table 12 at a concentration of 48 nM sgRNA and 16 nM RNP in the biochemical assay. The assay identified potential off-target sites for the sgRNAs tested.

TABLE 12

Off-Target Analysis			
gRNA ID	Target	Guide Sequence (SEQ ID NO:)	Off-Target Site Count
G022010	HLA-B	AACAAUGCCCACGAUGGGGA (SEQ ID NO: 3)	37
G022020	HLA-B	ACAUGCCAUGUACAGCAUGA (SEQ ID NO: 13)	24
G000644	EMX1	GAGUCCGAGCAGAAGAAGAA (SEQ ID NO: 3107)	58
G000645	VEGFA	GACCCCCUCCACCCCGCCUC (SEQ ID NO: 3108)	793
G000646	RAG1B	GACUUGUUUUCAUUGUUCUC (SEQ ID NO: 3109)	47

[0985] In known off-target detection assays such as the biochemical method used above, a large number of potential off-target sites are typically recovered, by design, so as to "cast a wide net" for potential sites that can be validated in other contexts, e.g., in a primary cell of interest. For example, the biochemical method typically overrepresents the number of potential off-target sites as the assay utilizes purified high molecular weight genomic DNA free of the cell environment and is dependent on the dose of Cas9 RNP used. Accordingly, potential off-target sites identified by these methods may be validated using targeted sequencing of the identified potential off-target sites.

Example 5: Re-Screening of HLA-B Guide RNAs with Cas9

[0986] 91 sgRNAs in either 100-mer or 91-mer format designed for the disruption of the HLA-B gene were screened for efficacy in T cells by assessing loss of HLA-B surface protein. The donors had an HLA-B phenotype of B*07:02/B*08:01. The percentage of T cells negative for HLA-B7 or HLA-B8 was determined by flow cytometry following editing at the HLA-B locus by HTP-LNP delivery and each test guide.

5.1. Cell Activation and Transfection with HTP-LNP

[0987] One day post thaw, T cells (StemCell, HLA-B*07:02/B*08:01) were plated at a density of 0.5×10^6 cells/mL in TCAM media supplemented with cytokines: 100 U/mL of IL-2 (Peprotech, Cat. 200-02), 5 ng/mL of IL-7, and 5 ng/mL of IL-15. T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). HTP-LNPs of each guide or control guide were added to the respective wells in the 96 well-plate at a concentration of 2.5 ug/mL on the cells. A solution of ApoE3 in TCAM was added to all wells to make the final concentration of ApoE3 2.5 μg/mL, and plates were incubated at 37° C. Cell plates were split every 2-3 days and each replicate plate (as well as the original plate) were supplemented with TCAM supplemented with cytokines.

[0988] 8-11 days post transfection with LNPs, plates were spun down for 5 minutes at 500×g, media was aspirated, and cells were stained for flow cytometry readout.

5.2. Flow Cytometry

[0989] T cells were resuspended in a master mix of 100 μL of FACS buffer containing a 1:100 v/v solution of antibodies targeting HLA-A2 (Invitrogen, Cat. 17-9876-43), HLA-A3 (Invitrogen, Cat. 12-5754-42), HLA-B7 (Miltenyi, Cat. 130-120-234), or HLA-B8 (Miltenyi, Cat. 130-118-366) corresponding to the cells donor's HLA-A or HLA-B (HLA-B*07:02/B*08:01) genotype. Cells were subsequently washed to remove any excess unbound antibodies and run on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, and HLA-B7, HLA-B8 expression. Tables 13 and 14 and FIGS. **3**A-C and **3**D-E show the mean percentage of cells negative for HLA-B following editing at the HLA-B locus.

TABLE 13

Mean percentage of HLA-B knockout across 3 donors in 100-mers plus four 91-mers

	Donor 2		Donor 3		Donor 1	
Guide ID	Mean % HLA-B KO	SD % HLA-B KO	Mean % HLA-B KO	SD % HLA-B KO	Mean % HLA-B KO	SD % HLA-B KO
G022008 (100mer)	N/A	N/A	N/A	N/A	N/A	N/A
G022009 (100mer)	0.605	0.262	1.165	0.177	1.155	0.078
G022010 (100mer)	72.8	0.141	76.15	6.293	74.7	0.283
G022011 (100mer)	37.5	0.849	11.44	3.055	19.1	2.121
G022012 (100mer)	4.725	0.262	6.62	2.249	3.57	0.014
G022013 (100mer)	0.73	0.156	2.905	0.629	0.99	0.382
G022014 (100mer)	5.565	1.761	7.605	1.747	3.71	0.438
G022015 (100mer)	0.345	0.29	1.84	1.344	0.66	0.085
G022016 (100mer)	0.265	0.035	2.225	1.945	0.54	0.042
G022017 (100mer)	5.535	1.379	10.115	1.817	2.82	0.099
G022018 (100mer)	4.145	0.375	6.465	1.563	3.81	0.396
G022019 (100mer)	44.4	1.273	47.1	3.111	38.2	1.414
G022020 (100mer)	N/A	N/A	N/A	N/A	N/A	N/A
G022021 (100mer)	0.71	0.127	2.375	0.488	1.31	0.834
G022022 (100mer)	14.35	1.768	20.55	4.596	9.715	0.969
G022023 (100mer)	2.96	2.729	6.675	4.985	0.465	0.078
G022024 (100mer)	3.615	0.163	7.515	0.955	3.425	0.742
G022025 (100mer)	14.95	1.061	20.65	5.162	15.45	0.212
G022026 (100mer)	2.965	0.29	3.31	0.509	0.775	0.262
G022027 (100mer)	36.75	2.333	8.2	2.008	25.05	2.333
G022028 (100mer)	8.4	1.344	14.4	3.394	7.72	0.396
G022029 (100mer)	5.92	0.212	10.35	0.354	5.27	0.467
G022030 (100mer)	6.795	1.365	10.095	1.846	6.33	0.311
G022031 (100mer)	43.1	1.697	43.9	0.283	21.1	2.121
G022032 (100mer)	27	0.99	12.85	0.212	25.1	3.394
G022033 (100mer)	18.35	2.333	18.85	4.313	11.7	2.121
G022034 (100mer)	5.22	0.354	11.195	4.108	4.785	1.28
G022035 (100mer)	3.165	0.021	8.795	0.983	2.585	0.785
G022036 (100mer)	4.37	0.99	8.395	1.110	3.5	0.792
G022037 (100mer)	1.905	0.177	4.58	0.580	1.53	0.014
G022038 (100mer)	0.305	0.035	1.01	0.042	1.05	0.82
G022039 (100mer)	0.315	0.035	0.76	0.226	1.115	0.926
G022040 (100mer)	6.35	0.552	10.12	1.103	4.965	0.177
G022041 (100mer)	12.25	0.636	21.05	0.919	3.36	0.368
G022042 (100mer)	3.34	2.164	7.72	2.051	1.875	0.29
G022043 (100mer)	72.4	0.424	76	1.697	68.25	1.909
G022044 (100mer)	14.3	1.273	21.65	0.919	17.95	1.768
G022045 (100mer)	3.355	0.969	8.945	2.765	4.575	0.163
G022046 (100mer)	92.75	1.061	97.4	0.849	85.5	1.838
G022047 (100mer)	11.25	1.485	17.95	3.748	12.85	1.485
G022048 (100mer)	4.13	0.622	10.31	2.814	4.765	1.025
G022049 (100mer)	6.26	1.188	8.495	1.280	5.01	1.004
G022050 (100mer)	0.195	0.092	0.985	0.643	0.995	0.417
G022051 (100mer)	0.255	0.092	0.67	0.141	1.03	0.721
G022052 (100mer)	0.155	0.049	0.78	0.382	0.845	0.276
G022053 (100mer)	57.05	1.909	64.4	0.990	36.3	3.96
G022054 (100mer)	7.855	1.096	10.18	1.867	6.04	0.849
G022055 (100mer)	52.05	1.202	64.75	1.485	43.25	5.02
G027488 (91mer)	92.55	0.354	95.3	1.697	85.65	0.778
G027489 (91mer)	81.9	0.566	83.45	1.626	78.65	1.202
G027490 (91mer)	80.35	0.071	84.75	0.636	79.2	1.838
G027491 (91mer)	92	1.556	95.05	0.212	84.25	0.071
G000529 B2M	94.7	0.283	86.9	0	94.4	0.424
G028013 B2M	N/A	N/A	N/A	N/A	92.95	0.919
G018995 HLA-A	88.4	2.828	N/A	N/A	90.7	0
G012086 TRAC	97.5	0	92.4	0	90.4	0.283

TABLE 14

Mean percentage of HLA-B knockout across 2 donors in 91-mer format

Guide ID	Donor 3 Mean % HLA-B KO	Donor 3 SD % HLA-B KO	Donor 4 Mean % HLA-B KO	Donor 4 SD % HLA-B KO
G027488 (91mer)	72.4	3.536	72.85	1.909
G027488 (91mer)	77.35	0.495	77.85	0.778
G027491 (91mer)	73.35	2.475	76.15	4.172
G027973 (91mer)	78.6	2.687	88.1	3.111
G027974 (91mer)	72.2	1.414	87.55	4.596
G027975 (91mer)	74.2	2.687	69.95	1.202
G027976 (91mer)	76.05	2.192	81.4	0.424
G027977 (91mer)	12.65	15.203	1.415	0.898
G027978 (91mer)	67.65	1.344	63.2	3.96
G027979 (91mer)	6.46	2.093	5.725	0.007
G027980 (91mer)	8.945	0.856	5.51	1.471
G027981 (91mer)	41.9	8.202	30.95	3.889
G027982 (91mer)	2.28	0.566	0.875	0.134
G027983 (91mer)	23.05	0.495	22.6	4.525
G027984 (91mer)	82.85	1.485	76.95	4.313
G027985 (91mer)	91.5	0.99	88.45	7.566
G027986 (91mer)	48.2	2.404	48.9	0.283
G027987 (91mer)	77.3	1.131	78.05	0.636
G027988 (91mer)	81.2	1.414	81.3	1.556
G027989 (91mer)	79.65	3.748	76.3	4.243
G027990 (91mer)	87.75	1.061	88.1	0.283
G027991 (91mer)	80.45	4.313	83	2.546
G027992 (91mer)	33.75	24.961	9.25	1.768
G027993 (91mer)	75.85	1.202	76.5	1.273
G027994 (91mer)	90.05	0.354	93.45	1.768
G027995 (91mer)	17.4	14.001	16.3	3.677
G027996 (91mer)	63.75	0.636	62.05	0.778
G027997 (91mer)	43.7	4.101	39.8	2.121
G027998 (91mer)	58.35	1.061	63.2	3.96
G027999 (91mer)	3.78	0.424	1.77	0.24
G028000 (91mer)	75.3	0	72.2	0.849
G028001 (91mer)	83.85	1.344	85.8	0.141
G028002 (91mer)	88.6	0.99	95.3	0.283
G028003 (91mer)	57.45	3.182	50.9	2.121
G028004 (91mer)	31.75	5.162	29.25	1.061
G028005 (91mer)	10.13	1.937	5.19	0.75
G028006 (91mer)	67.95	0.778	69.8	1.273
G028007 (91mer)	34.5	18.95	14.1	1.556
G028008 (91mer)	60.35	34.578	83.75	1.768
G028009 (91mer)	46.9	0.283	49.9	3.96
G028010 (91mer)	77.7	2.687	78.5	3.96
G028011 (91mer)	3.675	0.247	97.15	0.212
G000529 B2M	94.4	0.424	N/A	N/A
G028013 B2M	92.95	0.919	96.85	0.636
G018995 HLA-A	90.7	0	N/A	N/A
G012086 TRAC	90.4	0.283	N/A	N/A

Example 6: LNP Dose Response Curves for Top HLA-B Spy Cleavase Guides

6.1 T Cell Preparation

[0990] One day post thaw, T cells (Cellex, HLA-B*07:02/B*08:01) were plated at a density of 0.5×10^6 cells/mL in TCAM media supplemented with 2× cytokines: 200 U/mL of IL-2 (Peprotech, Cat. 200-02), 10 ng/mL of IL-7, and 10 ng/mL of IL-15. T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). TCAM Media containing 4× ApoE3 (10 ug/mL) was prepared and filled in a reservoir for the Hamilton. To "Column 1" of a Hamilton compatible deep well dilution block, a 1 mL 4× stock solution (20 μg/mL) of each HLA-B LNP in TCAM (for guides G027488, G027489, G027490, G027491) was added in duplicates, according to the plate layout. Transfection was performed on Hamilton by diluting the LNP stock and ApoE3 media 4-fold to generate the highest point in the standard curve (5 ug/mL LNP), followed by a 2-fold serial dilution to obtain a 12-point DRC. The ApoE3 concentration was kept constant at 2.5 μg/mL in each well. The total volume of LNP+ ApoE3 media added to each well by the Hamilton to get the desired concentrations was 100 μL. Transfection of the B2M LNP was performed manually without the Hamilton, by adding B2M LNP to the cells at a concentration of 2.5 μg/mL and ApoE3 at a concentration of 2.5 ug/mL in TCAM. It is to be noted that since cells were plated with 2× cytokines, LNP and ApoE3 containing media was cytokine free to result in a 1× final concentration of cytokines on the cells (100 U/mL of IL-2 (Peprotech, Cat. 200-02), 5 ng/mL of IL-7, and 5 ng/mL of IL-15). Plates were transferred to at 37° C. incubator. Cell plates were split every 2-3 days and each replicate plate (as well as the original plate) were supplemented with TCAM supplemented with cytokines.

[0991] 9 days post transfection with LNPs, plates were spun down for 5 minutes at 500×g, media was aspirated, and cells were stained for flow cytometry readout.

6.2 Flow Cytometry

[0992] Flow cytometry was performed as in Example 5.2. Tables 15 and 16 and FIGS. **4**A and **4**B show the percent knockout at each LNP dose.

TABLE 15

Guide sgRNA (μg/mL)	G027488 Mean % HLA-B*07:02 KO	G027488 SD % HLA-B*07:02 KO	G027488 N	G027489 Mean % HLA-B*07:02 KO	G027489 SD % HLA-B*07:02 KO	G027489 N	G027490 Mean % HLA-B*07:02 KO	G027490 SD % HLA-B*07:02 KO	G027490 N
5	81.20	1.27	2	85.25	1.63	2	64.65	1.06	2
2.5	83.80	2.12	2	88.30	1.41	2	63.50	2.40	2
1.25	88.25	0.21	2	87.20	1.70	2	65.35	1.48	2
0.625	89.65	0.92	2	87.05	0.49	2	64.65	3.89	2
0.313	77.35	0.64	2	86.15	0.35	2	66.15	3.32	2
0.156	14.25	0.78	2	70.05	1.91	2	62.70	1.41	2
0.078	1.13	0.37	2	32.60	0.42	2	36.60	4.95	2
0.039	0.53	0.44	2	5.92	0.22	2	8.01	3.52	2
0.02	0.22	0.21	2	1.02	0.01	2	1.90	0.07	2

TABLE 15-continued

0.01	0.62	0.16	2	0.33	0.04	2	0.40	0.11	2
0.005	0.38	0.06	2	0.31	0.00	2	0.29	0.18	2
0.002	0.28	0.10	2	0.12	0.00	2	0.13	0.01	2

	G027491			G000529 B2M		
Guide sgRNA (μg/mL)	Mean % HLA-B*07:02 KO	SD % HLA-B*07:02 KO	N	Mean % HLA-B*07:02 KO	SD % HLA-B*07:02 KO	N
5	76.25	2.47	2			
2.5	79.80	0.14	2			
1.25	87.00	0.71	2	93.5		1
0.625	86.95	0.92	2			
0.313	54.70	3.54	2			
0.156	4.28	0.53	2			
0.078	0.33	0.01	2			
0.039	0.14	0.01	2			
0.02	0.13	0.00	2			
0.01	0.13	0.03	2			
0.005	0.10	0.00	2			
0.002	0.22	0.11	2			

TABLE 16

	G027488			G027489			G027490		
Guide sgRNA (μg/mL)	Mean % HLA-B*08:01 KO	SD % HLA-B*08:01 KO	N	Mean % HLA-B*08:01 KO	SD % HLA-B*08:01 KO	N	Mean % HLA-B*08:01 KO	SD % HLA-B*08:01 KO	N
5	78.45	2.05	2	85.20	0.14	2	74.05	2.19	2
2.5	82.20	1.27	2	83.80	0.14	2	72.75	0.49	2
1.25	86.25	0.07	2	84.10	0.28	2	73.35	1.91	2
0.625	89.00	0.71	2	84.45	0.78	2	75.60	2.12	2
0.313	84.05	1.63	2	84.75	0.92	2	75.20	1.27	2
0.156	25.45	3.89	2	71.80	2.40	2	71.60	1.41	2
0.078	1.64	0.35	2	44.30	3.39	2	59.25	1.91	2
0.039	0.61	0.22	2	10.30	1.00	2	21.55	0.21	2
0.02	0.47	0.18	2	1.88	0.23	2	4.84	0.59	2
0.01	0.48	0.20	2	0.90	0.02	2	1.98	0.35	2
0.005	0.61	0.11	2	0.81	0.04	2	1.02	0.22	2
0.002	0.76	0.11	2	1.37	0.65	2	1.36	0.76	2

	G027491			G000529 B2M		
Guide sgRNA (μg/mL)	Mean % HLA-B*08:01 KO	SD % HLA-B*08:01 KO	N	Mean % HLA-B*08:01 KO	SD % HLA-B*08:01 KO	N
5	71.25	0.49	2			
2.5	79.45	0.21	2			
1.25	81.10	4.24	2	96.6		1
0.625	84.00	1.84	2			
0.313	66.45	1.06	2			
0.156	10.75	0.64	2			
0.078	1.24	0.27	2			
0.039	0.73	0.08	2			
0.02	1.09	0.52	2			
0.01	0.95	0.23	2			
0.005	0.78	0.25	2			
0.002	0.72	0.13	2			

Example 7: Screening of HLA-B Guides with Nme2 BC22n and Nme2 Cleavase

[0993] 57 sgRNAs targeting HLA-B were screened at a fixed concentration of 100 μM. sgRNAs targeting TRAC and B2M were used as controls. Guides were either electroporated with mRNA encoding UGI (3490 ng/mL) (SEQ ID NO: 821) as well as either mRNA encoding Nme2 BC22n (1709 ng/mL) (SEQ ID NO: 822), an mRNA encoding Nme2-cleavase (1660 ng/mL) (SEQ ID NO: 825), or an mRNA encoding Spy-cleavase (2230 ng/mL) (SEQ ID NO: 827).

7.1 T cell Preparation

[0994] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and resuspended in in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. 130-070-525) and processed in a MultiMACS™ Cell 24 Separator Plus device (Miltenyi Biotec). T cells were isolated via positive selection using a Straight from Leukopak® CD4/CD8 MicroBead kit, human (Miltenyi Biotec Cat. 130-122-352). T cells were aliquoted and cryopreserved for future use in Cryostor® CS10 (StemCell Technologies Cat. 07930). Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell growth media (TCGM) composed of CTS OpTmizer T Cell Expansion SFM and T Cell Expansion Supplement (ThermoFisher Cat. A1048501) containing 5% human AB serum (GeminiBio, Cat. 100-512), 1× Penicillin-Streptomycin, 1× Glutamax, 10 mM HEPES, 1× cytokines (200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin 15 (Peprotech, Cat. 200-15)). T cells were rested in the T cell growth media for 24 hours at which time they were activated with TransAct™ (1:100 dilution, Miltenyi Biotec, Cat. 130-111-160). T cells were activated 48 hours prior to electroporation.

7.2 T Cell Editing with RNA Electroporation

[0995] T cells were edited at the HLA-B locus with Cas9 (SEQ ID NO: 827), mRNA encoding Nine BC22n (SEQ ID NO: 822) and UGI (SEQ ID NO: 821) to assess sgRNA editing efficacy and the corresponding loss of HLA-B7 expression.

[0996] A solution containing mRNA encoding BC22n (SEQ ID: 822) and UGI (SEQ ID NO: 821) was prepared in P3 electroporation buffer (Lonza Catalog #V4SP-3960). 100 μM HLA-B targeting sgRNAs included in Table 17 were removed from their storage plates and denatured for 2 minutes at 95° C. and incubated at room temperature for 5 minutes. Forty-eight hours post activation, T cells were harvested, centrifuged at 500 g for 5 minutes, and resuspended at a concentration of 12.5×10^6 T cells/mL in P3 electroporation buffer (Lonza Catalog #V4SP-3960). For each well to be electroporated, 1×10^5 T cells were mixed with 20 ng/μL of BC22n mRNAs, 20 ng/μL of UGI mRNA and 20 pmols of sgRNA in a final volume of 20 μL of P3 electroporation buffer. This mix was transferred in duplicate to a 96-well Nucleofector™ plate (manufacturer, catalog #) and electroporated using manufacturer's pulse code. Electroporated T cells were immediately rested in 80 μL of CTS Optimizer T cell growth media (manufacturer, catalog #) without cytokines for 15 minutes. After resting, T cells were transferred to flat-bottom 96-well plates (manufacturer, catalog #) containing 80 μL of CTS Optimizer T cell growth media (manufacturer, catalog #) supplemented with 2× cytokines (200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin-15 (Peprotech, Cat. 200-15) per well. The plates were incubated at 37° C. for 10 days.

[0997] On day 7 post-electroporation, cells were collected for flow cytometry analysis. NGS analysis of cells was run by a third-party facility using standard methods.

7.3 Flow Cytometry

[0998] T cells were phenotyped by flow cytometry to determine HLA-B7 protein expression. Briefly, T cells were incubated for 30 min at 4° C. with a mixture of antibodies against CD3 (BioLegend, Cat. No. 316314), CD4 (BioLegend, Cat. No. 317434), CD8 (BioLegend, Cat. No. 301046), Viakrome (Immunotech, Cat. No. C36628), HLA B7 (Milteny Biotech, Cat. No. 130-120-234) diluted at 1:200 in cell staining buffer. Cells were subsequently washed and resuspended in 100 μL of cell staining buffer. Cells were then processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, CD8, CD3, and HLA-B7 expression. Table 17 and FIG. **5**A shows the mean percentage of cells negative for HLA-B7 following editing at the HLA-B locus.

TABLE 17

Mean % HLA-B7 T cells following editing at the HLA-B locus.

	% HLA-B7-ve			Guide	% HLA-B7-ve		
Guide ID	Mean	SD	N	ID	Mean	SD	N
G018995 HLA-A	0.27	0.170	2	G028828	2.13	0.834	2
G021469 TRAC	0.08	0.000	2	G028829	3.375	0.940	2
G021481 TRAC	1.38	1.796	2	G028830	0.32	0.297	2
G028789	49.05	1.202	2	G028831	32.05	0.636	2
G028790	1.295	0.672	2	G028832	74.9	9.334	2
G028791	11.075	1.591	2	G028833	7.215	5.353	2
G028792	0.36	0.085	2	G028834	0.27	0.198	2
G028793	0.31	0.297	2	G028835	0.185	0.078	2
G028794	4.295	0.530	2	G028836	0.415	0.120	2
G028795	71.3	6.505	2	G028837	0.29	0.099	2
G028796	16.425	10.713	2	G028838	0.45	0.042	2
G028797	0.51	0.113	2	G028839	6.69	0.990	2
G028798	0.08	0.057	2	G028840	2.1	0.594	2
G028799	1.795	0.035	2	G028841	0.135	0.106	2
G028800	0.12	0.071	2	G028842	0.06	0.042	2
G028801	0.335	0.148	2	G028843	0.185	0.035	2
G028802	24.45	2.899	2	G028931	0.065	0.035	2
G028803	42.25	3.465	2	G028932	0.12	0.028	2
G028804	7.695	2.397	2				
G028805	9.915	0.686	2				
G028806	18.15	0.495	2				
G028807	4.615	0.742	2				
G028808	0.37	0.240	2				
G028809	0.03	0.000	2				
G028810	0.055	0.035	2				
G028811	0.24	0.156	2				
G028812	0.035	0.035	2				
G028813	1.625	0.134	2				
G028814	3.495	0.672	2				
G028815	12.2	0.849	2				
G028816	13.5	0.424	2				
G028817	1.52	0.778	2				
G028818	0.92	1.273	2				
G028819	0.1	0.014	2				
G028820	0.365	0.346	2				
G028821	0.085	0.007	2				

TABLE 17-continued

Mean % HLA-B7 T cells following editing at the HLA-B locus.							
	% HLA-B7-ve			Guide	% HLA-B7-ve		
Guide ID	Mean	SD	N	ID	Mean	SD	N
G028822	0.185	0.035	2				
G028823	1.49	0.071	2				
G028824	0.215	0.247	2				
G028825	1.585	0.205	2				
G028826	0.825	0.035	2				
G028827	0.605	0.375	2				

7.4 Nme2 Cleavase Screen

[0999] 55 sgRNAs targeting HLA-B were screened at a fixed concentration of 100 μM. Three sgRNAs targeting TRAC and a sgRNA targeting B2M were used as controls. The donors had an HLA-B phenotype of B*07:02/B*08:01. The percentage of T cells negative for HLA-B7 or HLA-B8 was determined by flow cytometry following editing at the HLA-B locus by HTP-LNP delivery and each test guide.

[1000] T cells were transfected with HTP-LNP and flow cytometry was performed as in Example 5. Table 18 and FIGS. **5**B and **5**C show the mean percentage of knockout for HLA*B07:02 or HLAB*08:01.

TABLE 18

Mean percentage HLA-B*07:02 or HLA-B*08:01 knockout following editing at the HLA-B locus		
Guide ID	% HLA-B*07:02 KO Mean	% HLA-B*08:01 KO Mean
G000529 B2M	93.25	92.95
G028789	55.5	49.8
G028790	1.08	0.68
G028791	8.05	7.22
G028792	1.73	1.39
G028793	0.65	0.26
G028794	10.8	11.6
G028795	7.24	5.67
G028796	3.45	2.88
G028797	0.43	0.16
G028798	1.69	0.22
G028799	1.05	1.05
G028800	0.67	0.44
G028801	1.21	0.48
G028802	5.09	9.4
G028803	3.36	3.46
G028804	0.44	0.13
G028805	95.6	92.2
G028806	35.7	33.6
G028807	0.49	0.34
G028808	0.79	0.17
G028809	0.88	0.47
G028810	0.42	0.83
G028811	0.14	0.15
G028812	0.38	0.17
G028813	42.4	33.2
G028814	34.3	35.3
G028815	73.7	48.4
G028816	49.4	35.7
G028817	0.64	0.39
G028818	0.46	0.32
G028819	1.81	2.14
G028820	2.45	3.88
G028821	14.9	16.5
G028822	3.47	1.71
G028823	0.31	0.22
G028824	2.77	2.47

TABLE 18-continued

Mean percentage HLA-B*07:02 or HLA-B*08:01 knockout following editing at the HLA-B locus		
Guide ID	% HLA-B*07:02 KO Mean	% HLA-B*08:01 KO Mean
G028825	58.3	35.8
G028826	92.1	57.2
G028827	0.28	0.37
G028828	0.89	1.01
G028829	15.5	13.9
G028830	6.77	0.15
G028831	21.9	24.6
G028832	34.9	34.1
G028833	0.76	0.48
G028834	0.21	0.3
G028835	0.75	0.72
G028836	0.5	0.49
G028837	1.03	0.36
G028838	0.82	0.21
G028839	4.75	4.34
G028840	0.68	0.21
G028841	1.28	0.36
G028842	1.31	0.19
G028843	1.25	0.43
G021469 TRAC	0.42	0.63
G021475 TRAC	0.83	0.14
G021481 TRAC	0.19	0.12

Example 8: LNP Dose Response Curves (DRC) for Top HLA-A and HLA-B Nme2 Guides

[1001] A DRC was run for lead HLA-A and HLA-B sgRNAs along with Nme2 BC22 to determine the best guide for knocking out HLA genes. sgRNAs were titrated in 8-point DRC along with fixed concentration of an mRNA encoding UGI (SEQ ID NO: 821) (3490 ng/L) and an mRNA encoding Nme2 BC22n base editor (SEQ ID NO: 822) (1709 ng/L) or an mRNA encoding a Spy-cleavase (SEQ ID NO: 827) (2230 ng/L) in T cells using electroporation. T cells were then analyzed by flow cytometry to determine editing efficiencies. T cells were prepared as described in Example 1.

8.1 mRNA Electroporation

[1002] Solutions containing mRNA encoding BC22n (SEQ ID NO: 822) and UGI (SEQ ID NO: 821) were prepared in P3 buffer. 100 μM HLA-B targeting sgRNAs were removed from their storage plates and denatured for 2 minutes at 95° C. and incubated at room temperature for 5 minutes. Forty-eight hours post activation, T cells were harvested, centrifuged, and resuspended at a concentration of 12.5×10^{6} T cells/mL in P3 electroporation buffer (Lonza Catalog #V4SP-3960). Each sgRNA was serially diluted in ratio of 1:2 in P3 electroporation buffer starting from 5 μM in a 96 well PCR plate in duplicate as described in Table 19. Following dilution, 1×10^{5} T cells, 20 ng/μL of BC22n mRNAs and 20 ng/μL of UGI mRNA were mixed with sgRNA plate to make the final volume of 20 μL of P3 electroporation buffer. The mix was transferred to two 96-well Nucleofector™ plates. Cells were electroporated in duplicate using Lonza shuttle 96w using manufacturer's pulse code. Immediately post electroporation, cells were recovered in 80 μL of TCGM without cytokines at 37° C. for 15 minutes. Electroporated T cells were subsequently cultured in TCGM further supplemented with 2× cytokines (200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin-15 (Peprotech, Cat. 200-15) per well. The plates

were incubated at 37° C. for 10 days. On day 7 post-edit, edited T cells were collected for flow cytometry analysis.

8.2 Flow Cytometry

[1003] On day 10, cells were transferred to U bottom plates, spun and resuspended in master mix containing antibodies for PerCP/Cy5.5 CD3 (BioLegend, Cat. 317434), BV421 CD4 (BioLegend, Cat. 317434), BV785 CD8 (BioLegend, Cat. 301046), HLA A2 (BioLegend Inc., Cat. 343306), HLA B7 (Miltenyi Biotec Inc., Cat. 130-120-234) at a 1:200 dilution and Viakrome (Immunotech, Cat. C36628) at 1:100 final concentration in FACs buffer and then incubated at 4° C. for 30 minutes. After the incubation, the cells were washed and resuspended in 100 μL FACs buffer (PBS+2% FBS+2 mM EDTA) and processed by flow cytometry using a Beckman Coulter CytoflexS, and analyzed using the FlowJo software package. Tables 19 and 20 and FIGS. **6** and **7** show the percent editing at each sgRNA dose.

TABLE 19

Dose response curve for the percent of HLA-A2^{-} of CD8^{+} cells with various doses of sgRNA

	G028907			G028913			G028840		
Guide sgRNA (μg/mL)	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N
5	98.65	0.07	2	97.20	0.42	2	98.00	0.14	2
2.5	98.25	0.21	2	96.15	0.07	2	97.30	0.28	2
1.25	97.40	0.14	2	93.30	0.00	2	94.35	0.92	2
0.625	95.70	0.42	2	90.85	0.92	2	90.00	0.99	2
0.313	92.40	0.00	2	85.55	1.77	2	78.85	0.92	2
0.078	80.20	3.25	2	68.25	3.75	2	55.95	0.35	2
0	54.45	2.05	2	42.70	1.13	2	31.45	0.64	2
EC50		0.07038			0.08998			0.1289	

	G028922			G028918			G028865		
Guide sgRNA (μg/mL)	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N
5	98.65	0.21	2	92.15	0.07	2	90.95	1.06	2
2.5	95.00	0.99	2	89.45	1.20	2	90.20	1.27	2
1.25	74.90	2.12	2	87.25	1.06	2	87.20	0.42	2
0.625	54.20	0.42	2	83.40	0.71	2	81.35	1.34	2
0.313	36.80	0.85	2	72.05	0.64	2	75.45	1.06	2
0.078	18.60	0.42	2	48.80	0.85	2	55.60	2.26	2
0	8.73	0.02	2	24.65	1.20	2	34.30	1.56	2
EC50		0.6028			0.1429			0.09631	

	G028832			G028795			G028869		
Guide sgRNA (μg/mL)	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N
5	1.45	0.13	2	5.74	0.32	2	70.55	0.49	2
2.5	0.71	0.03	2	2.99	0.76	2	61.75	0.92	2
1.25	0.37	0.04	2	1.65	0.10	2	43.75	1.91	2
0.625	0.33	0.04	2	0.96	0.03	2	25.45	0.92	2
0.313	0.49	0.15	2	0.82	0.08	2	14.55	0.92	2
0.078	0.29	0.08	2	0.39	0.01	2	6.20	0.33	2
0	0.40	0.13	2	0.35	0.10	2	3.19	0.40	2
EC50		2.654			15.82			0.9213	

	G000529 B2M			G018995 Spy Cas			G022020 Spy Cas		
Guide sgRNA (μg/mL)	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N	Mean % CD8^{+} Cells	SD % CD8^{+} Cells	N
5	96.15	0.07	2	98.20	0.42	2	6.11	1.70	2
2.5	94.85	0.49	2	97.80	0.00	2	6.39	3.83	2
1.25	93.45	1.20	2	97.60	1.13	2	3.52	0.04	2
0.625	90.55	0.07	2	96.10	0.42	2	2.04	0.66	2
0.313	83.50	3.39	2	91.50	1.84	2	1.12	0.07	2
0.078	57.60	4.67	2	75.10	0.00	2	0.69	0.16	2
0	25.25	1.48	2	41.20	0.85	2	3.00	2.61	2
EC50		0.1087			0.09102			1.286	

TABLE 20

Dose response curve for the percent of HLA-B7⁻ of CD8+ cells with various doses of sgRNA

Guide sgRNA (μg/mL)	G028907 Mean % CD8⁺ Cells	G028907 SD % CD8⁺ Cells	G028907 N	G028913 Mean % CD8⁺ Cells	G028913 SD % CD8⁺ Cells	G028913 N	G028840 Mean % CD8⁺ Cells	G028840 SD % CD8⁺ Cells	G028840 N
5	85.15	0.35	2	16.35	0.21	2	14.20	1.84	2
2.5	76.45	0.49	2	10.00	1.41	2	7.65	0.90	2
1.25	59.25	0.64	2	4.87	0.23	2	3.69	1.02	2
0.625	37.50	2.26	2	2.62	0.04	2	2.11	0.56	2
0.313	22.65	0.92	2	1.27	0.25	2	1.34	0.11	2
0.078	12.15	1.63	2	0.79	0.12	2	0.82	0.06	2
0	5.44	0.15	2	0.43	0.08	2	0.64	0.08	2
EC50		0.8643			4.531			24.96	

Guide sgRNA (μg/mL)	G028922 Mean % CD8⁺ Cells	G028922 SD % CD8⁺ Cells	G028922 N	G028918 Mean % CD8⁺ Cells	G028918 SD % CD8⁺ Cells	G028918 N	G028865 Mean % CD8⁺ Cells	G028865 SD % CD8⁺ Cells	G028865 N
5	39.00	0.28	2	0.63	0.01	2	0.52	0.06	2
2.5	21.65	1.20	2	0.49	0.33	2	0.41	0.12	2
1.25	9.60	0.13	2	0.47	0.03	2	0.20	0.14	2
0.625	4.81	0.13	2	0.19	0.00	2	0.20	0.01	2
0.313	3.08	0.62	2	0.21	0.16	2	0.20	0.09	2
0.078	1.42	0.00	2	0.31	0.23	2	0.13	0.03	2
0	1.42	1.19	2	0.21	0.13	2	0.09	0.08	2
EC50		5.494			1.217			0.03500	

Guide sgRNA (μg/mL)	G028832 Mean % CD8⁺ Cells	G028832 SD % CD8⁺ Cells	G028832 N	G028795 Mean % CD8⁺ Cells	G028795 SD % CD8⁺ Cells	G028795 N	G028869 Mean % CD8⁺ Cells	G028869 SD % CD8⁺ Cells	G028869 N
5	87.05	1.77	2	91.75	1.34	2	80.80	1.70	2
2.5	85.20	5.09	2	84.50	3.82	2	66.70	2.40	2
1.25	82.60	1.84	2	74.40	1.27	2	45.70	2.55	2
0.625	78.85	3.04	2	49.90	0.85	2	24.75	3.32	2
0.313	73.50	0.42	2	31.05	2.19	2	15.30	0.85	2
0.078	55.05	2.47	2	17.60	1.56	2	7.17	0.26	2
0	34.85	1.63	2	7.86	0.65	2	3.25	0.13	2
EC50		0.1029			0.5729			1.205	

Guide sgRNA (μg/mL)	G000529 B2M Mean % CD8⁺ Cells	G000529 B2M SD % CD8⁺ Cells	G000529 B2M N	G018995 Spy Cas Mean % CD8⁺ Cells	G018995 Spy Cas SD % CD8⁺ Cells	G018995 Spy Cas N	G022020 Spy Cas Mean % CD8⁺ Cells	G022020 Spy Cas SD % CD8⁺ Cells	G022020 Spy Cas N
5	96.15	0.07	2	1.74	0.25	2	92.70	1.70	2
2.5	94.50	0.57	2	1.55	0.08	2	82.25	7.71	2
1.25	93.20	0.99	2	1.94	0.28	2	67.45	7.42	2
0.625	89.95	0.07	2	2.06	0.73	2	35.55	0.49	2
0.313	82.85	3.46	2	1.82	0.36	2	15.90	1.41	2
0.078	57.15	4.45	2	1.52	0.35	2	6.70	0.18	2
0	24.80	1.41	2	1.12	0.01	2	4.84	1.07	2
EC50		0.1091			0.1053			0.8285	

Example 9. HLA-B KO in Induced Pluripotent Stem Cells (iPSC)

[1004] Induced pluripotent stem cells were edited using HLA-B guide G022020 in a single KO experiment. Additionally, iPSCs were edited using HLA-B guide G022020, HLA-A guide 018995, and CIITA guide G013675 (CCCCCGGACGGUUCAAGCAA targeting sequence; SEQ ID NO: 3110) in a triple KO experiment. The results are shown below in Table 21.

TABLE 21

Editing efficiency in iPSC using HLA-B targeting guide RNA

ddPCR		Flow Cytometry	
Efficiency	HLA-B	Efficiency	HLA-B
Single KO	77%	Single KO	83%
Triple KO	79%	Triple KO	71%

Example 10: Screening of HLA-B Guides with Nme2 BC22n

[1005] 28 sgRNAs targeting HLA-B were screened at a fixed concentration of 3 μg/mL. Previously tested sgRNA (G028907) targeting HLA-B was used as a control. Guides, mRNA encoding UGI (0.5 μg/mL) (SEQ ID NO: 821), and mRNA encoding Nme2 BC22n (1 μg/mL) (SEQ ID NO: 828) were individually delivered using LNPs in parallel.

10.1 T cell Preparation

[1006] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and resuspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. 130-070-525) and processed in a MultiMACS™ Cell 24 Separator Plus device (Miltenyi Biotec).

[1007] T cells were isolated via positive selection

[1008] using a Straight from Leukopak® CD4/CD8 MicroBead kit,

[1009] human (Miltenyi Biotec Cat. 130-122-352). T cells were aliquoted and cryopreserved for future use in Cryostor® CS10 (StemCell Technologies Cat. 07930). Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell growth media (TCGM) composed of CTS OpTmizer T Cell Expansion SFM and T Cell Expansion Supplement (ThermoFisher, Cat. A1048501) containing 5% human AB serum (Gemini-Bio, Cat. 100-512), 1× Penicillin-Streptomycin, 1× Glutamax, 10 mM HEPES, 1× cytokines (200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin 15 (Peprotech, Cat. 200-15)). T cells were rested in the T cell growth media for 24 hours at which time they were activated with TransAct™ (1:100 dilution, Miltenyi Biotec, Cat. 130-111-160). T cells were activated 48 hours prior to LNP treatment.

10.2 T Cell Editing with HTP LNPs

[1010] T cells were edited at the HLA-B locus with mRNA encoding Nme2 BC22n (SEQ ID NO: 828) and UGI (SEQ ID NO: 821) to assess sgRNA editing efficacy and the corresponding loss of HLA-B7 and HLA-B8 expression.

[1011] Forty-eight hours post activation, T cells were harvested, centrifuged at 500 g for 5 minutes, and resuspended at a concentration of 1×10^6 T cells/mL in T cell growth media and plated in 96 well plates accordingly. For each well to be treated with LNPs, 0.5×10^5 T cells were mixed 2:1 ratio with LNP containing 4 μg/mL of Nme2 BC22n mRNA and LNP containing 2 μg/mL of UGI mRNA and 2:1 with LNP containing 12 μg/mL HLA-B sgRNA in a final volume of 100 μL of T cell growth media. The plates were incubated at 37° C. for 10 days. On day 10 post-thaw, T cells were collected for flow cytometry analysis.

10.3 Flow Cytometry

[1012] T cells were phenotyped by flow cytometry to determine HLA-B7 and HLA-B8 protein expression. Briefly, T cells were incubated for 30 minutes at 4° C. with a mixture of antibodies against CD3 (BioLegend, Cat. No. 317334), CD4 (BioLegend, Cat. No. 300536), CD8 (BioLegend, Cat. No. 344740), Viakrome (Immunotech, Cat. No. C36628), HLA-B7 (Miltenyi Biotec, Cat. No. 130-120-234), HLA-B8 (Miltenyi Biotec, Cat. No. 130-118-366), HLA-A2 (eBioscience, Cat. No. 17-9876-42), HLA-A3 (eBioscience, Cat. No. 12-5754-42). HLA-E (BioLegend, Cat. No. 342612), and HLA-C (BD Pharmingen Cat. No. 566372), diluted at 1:100 in cell staining buffer. Cells were subsequently washed and resuspended in 100 μL of cell staining buffer. Cells were then processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, CD8, CD3, HLA-E retention, HLA-C retention, and HLA-B7 and HLA-B8 expression. Table 22 and FIG. **8**A show the mean percentage of cells negative for HLA-B7 following editing at the HLA-B locus. Table 23 and FIG. **8**B show the mean percentage of cells negative for HLA-B8 following editing at the HLA-B locus.

TABLE 22

Mean % HLA-B7⁻ T cells following editing at the HLA-B locus

	% HLA-B7 −ve		
Guide ID	Mean	SD	N
G032787	27.46	0.226274	2
G032788	49.24	4.723473	2
G032789	14.4	3.323402	2
G032790	2.48	0.650538	2
G032791	0.035	0.007071	2
G032792	76.87	5.996266	2
G032793	74.19	7.113494	2
G032794	88.33	3.422397	2
G032795	93.61	1.668772	2
G032796	2.365	0.869741	2
G028919	0.165	0.176777	2
G032797	0.125	0.021213	2
G032798	81.355	0.304056	2
G032799	4.075	0.374767	2
G032800	28.985	0.049497	2
G032801	16.63	0.296985	2
G032802	20.805	1.152584	2
G032803	1.035	0.049497	2
G032804	4.1	0.19799	2
G032805	9.765	0.304056	2
G032806	85.975	0.374767	2
G032807	38.13	0.042426	2
G032808	8.245	0.657609	2
G032809	34.675	2.750645	2
G032810	2.105	0.162635	2
G032811	47.61	0.268701	2
G032812	2.72	0.113137	2
G032813	3.965	0.120208	2

TABLE 23

Mean % HLA-B8⁻ T cells following editing at the HLA-B locus

	% HLA-B8 −ve		
Guide ID	Mean	SD	N
G032787	4.215	0.799031	2
G032788	56.06	2.91328	2
G032789	31.46	3.563818	2
G032790	21.9	3.436539	2
G032791	0.7	0.056569	2
G032792	94.43	1.810193	2
G032793	92.05	3.719382	2
G032794	97.135	1.265721	2
G032795	98.2	1.117229	2
G032796	18.15	2.672864	2
G028919	1.64	0.141421	2
G032797	0.28	0.014142	2
G032798	88.635	0.431335	2
G032799	25.84	0.650538	2
G032800	51.57	0.070711	2
G032801	38.985	1.491995	2

TABLE 23-continued

Mean % HLA-B8⁻ T cells following editing at the HLA-B locus

Guide ID	% HLA-B8 −ve Mean	SD	N
G032802	44.305	0.544472	2
G032803	9.38	0.353553	2
G032804	19.48	0.551543	2
G032805	41.23	0.296985	2
G032806	91.28	0.46669	2
G032807	69.295	0.021213	2
G032808	37.38	0.876812	2
G032809	67.05	1.484924	2
G032810	46.075	0.275772	2
G032811	1.9	0.084853	2
G032812	16.26	0.509117	2
G032813	22.035	0.049497	2

Example 11: NK Cell Functional Killing Assays

[1013] T cells edited in various combinations to disrupt CIITA, HLA-A, HLA-B, or B2M were tested for their ability to resist natural killer (NK) cell mediated killing.

11.1. Engineering T Cells and Purification

[1014] Upon thaw, Pan CD3+ T cells (StemCell, HLA-A*02:01:01; B*08:01:01; C*07:01:01) were plated at a density of 0.5×10^6 cells/mL in T cell TCAM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and 1% (v/v) 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15).

[1015] On Day 1 (one day post-thaw), T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). As described in Table 24, T cells were edited to disrupt the HLA-A, HLA-B, or B2M gene. LNP compositions containing Spy Cas9 mRNA and one sgRNA G000529 targeting B2M, sgRNA G018995 targeting HLA-A, or sgRNAs G027488, G027490, G027994, G028001, and G028002 targeting HLA-B were formulated as described in Example 1. LNP compositions were incubated in TCAM with cytokines as described above supplemented with 5 μg/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37° C. An equal volume of LNP mix was added to one million activated T cells to yield a final concentration of 2.5 μg total LNP/mL for the B2M LNP; 1.25 μg total LNP/mL for the HLA-B LNPs, and 0.625 μg total LNP/mL for the HLA-A LNP.

TABLE 24

Order of sequential editing and viral transduction

Condition	Day 1	Day 2
Unedited		
B2M KO	B2M LNP	
HLA-A KO	HLA-A LNP	
HLA-B KO (G027488)	HLA-B LNP	

TABLE 24-continued

Order of sequential editing and viral transduction

Condition	Day 1	Day 2
HLA-B KO (G027490)	HLA-B LNP	
HLA-B KO (G027994)	HLA-B LNP	
HLA-B KO (G028001)	HLA-B LNP	
HLA-B KO (G028002)	HLA-B LNP	
HLA-A + HLA-B KO (G027488)	HLA-A LNP	HLA-B LNP
HLA-A + HLA-B KO (G027490)	HLA-A LNP	HLA-B LNP
HLA-A + HLA-B KO (G027994)	HLA-A LNP	HLA-B LNP
HLA-A + HLA-B KO (G028001)	HLA-A LNP	HLA-B LNP
HLA-A + HLA-B KO (G028002)	HLA-A LNP	HLA-B LNP

[1016] One day post activation (i.e., Day 2), additional T cells were edited with LNP compositions to disrupt the HLA-B gene (refer to Table 24, Day 2 column). This was performed for HLA-B editing using LNP compositions containing Spy Cas9 mRNA and sgRNAs G027488, G027490, G027994, G028001 and G028002 targeting HLA-B. LNP compositions were incubated in TCAM with cytokines as described above supplemented with 25 μg/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37° C. A volume equal to $1/10^{th}$ the volume of the cells was added to each well containing approximately 1 million cells to yield a final concentration of 1.25 μg total LNP/mL for each of the HLA-B LNPs.

[1017] Two days post activation (i.e, Day 3), all cells were transferred to GREX plate (Wilson Wolf, Cat. 80240M) for expansion with TCEM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 5% (v/v) of CTS™ Immune Cell SR (Gibco, Cat. A2596101), 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and 1% (v/v) 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15). Half of the media from the GREX wells was replaced with fresh TCEM supplemented with cytokines on Days 5, 7 and 9.

[1018] Five days post activation (i.e., Day 6) cells were stained by flow cytometry antibodies to determine HLA-A2 expression (HLA-A+), HLA-B8 expression (HLA-B8+), and HLA-Class-I expression (MHC I+) following knockout of B2M. T cells were incubated with an antibody cocktail targeting the following molecules: HLA-A2 (Biolegend, Cat. 343326), HLA-B8 (Miltenyi Biotec, Cat. 130-118-366), and B2M (Biolegend, Cat. 316304). Cells were subsequently washed, and analyzed on a Cytoflex LX instrument (Beckman Coulter) using the FlowJo software package.

11.2 Flow Cytometry

[1019] NK cell mediated cytotoxicity towards engineered T cells was assayed. T cells were co-cultured with the HLA-B/C matched CTV labelled NK cells at effector to target ratios (E:T) of 8:1, 4:1, 2:1, 1:1, 0.5:1, 0.25:1, and 0.125:1 for 21 hours. The cells were stained with 7AAD (BD

Pharmingen, Cat. 559925), processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on CTV negativity, size, and shape and viability. Table 25 and FIG. **9** show the mean percentage of T cell lysis following NK cell challenge.

TABLE 25

Percentage T cell lysis following NK cell challenge to engineered T cells

	WT		B2M KO		HLA-A KO G018995		HLA-B KO G027994		
NK:T	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
8	12.45	1.272792	83.78	0.19799	10	0.565685	29.4	0.424264	2
4	8.95	0.141421	83.29	0.226274	13.1	4.525483	24.85	0.353553	2
2	7.65	0.282843	82.34	2.545584	10.7	1.131371	21.65	1.06066	2
1	5.85	0.707107	78.07	2.291026	8.8	2.687006	19.45	0.494975	2
0.5	3.3	0.919239	48.6	3.606245	7.45	1.909188	14.5	1.131371	2
0.25	2.25	0.282843	23.95	7.919596	7.25	0.919239	8.4	0.848528	2
0.125	0.8	0.212132	16.05	11.45513	1.25	0.212132	5.05	0.919239	2
(Basal) 0	0	0.070711	−5.225	7.389266	0	1.697056	0	0	2

	HLA-B KO G028001		HLA-A+ B KO G027994		HLA-A+ B KO G028001		
NK:T	Mean	SD	Mean	SD	Mean	SD	n
8	44.25	1.979899	51.75	2.12132	61.6	0.070711	2
4	29.3	0.212132	46.85	2.545584	49.25	2.545584	2
2	19.7	1.484924	30.35	1.131371	34.85	3.676955	2
1	13.25	1.414214	23.85	1.555635	20.75	4.949747	2
0.5	8.9	1.626346	17.4	0.919239	15.5	7.2832	2
0.25	2	0.070711	14.65	6.788225	0.45	0.707107	2
0.125	4.35	5.23259	4.9	1.626346	9.1	14.21285	2
(Basal) 0	0	2.474874	0	0.353553	0	8.980256	2

Example 12: LNP Dose Response Curves (DRC) for Select HLA-B Nme2 BC22n Guides

[1020] A DRC was run for select HLA-B sgRNAs along with Nme2 BC22 to determine suitable guides for knocking out the HLA-B gene. sgRNAs were titrated in 8-point DRC along with fixed concentration of an mRNA encoding UGI (SEQ ID NO: 824) (3490 ng/μL) and an mRNA encoding Nme2 BC22n base editor (SEQ ID NO: 828) (1709 ng/μL) in T cells using LNPs. T cells were then analysed by flow cytometry to determine editing efficiencies. T cells were prepared as described in Example 1.

12.1 T Cell Editing with HPLC-Purified Guide LNPs

[1021] T cells were edited at the HLA-B locus with mRNA encoding Nme2 BC22n (SEQ ID NO: 828) and UGI (SEQ ID NO: 824) to assess sgRNA editing efficacy and the corresponding loss of HLA-B7 and HLA-B8 expression.

[1022] Forty-eight hours post activation, T cells were adjusted to a concentration of 1×10^6 T cells/mL in T cell growth media and plated in 96 well plates accordingly. For each well to be treated with LNPs, 0.5×10^5 T cells were mixed 2:1 ratio with LNP containing 10 μg/mL of Nme2 BC22n mRNA and HLA-B sgRNA; and LNP containing 0.4 μg/mL of UGI mRNA in a final volume of 100 μL of T cell growth media. The plates were incubated at 37° C. for 10 days with every other day media refreshing. On day 10 post-thaw, T cells were collected for flow cytometry analysis.

12.2 Flow Cytometry

[1023] On day 10, cells were transferred to U bottom plates, spun and resuspended in master mix containing antibodies for PerCP/Cy5.5 CD3 (BioLegend, Cat. 317336), BV605 CD4 (BioLegend, Cat. 300536), BV785 CD8 (BioLegend, Cat. 344740), BV510 HLA A2 (BioLegend Inc., Cat. 343320), APC HLA A3 (eBioscience, Cat. 12-5754-42), FITC HLA B7 (Miltenyi Biotec Inc., Cat. 130-120-234), FITC HLA B8 (Miltenyi Biotec Inc., Cat. 130-118-366), and BV421 HLA-E (Biolegend, Cat. 342612), PE HLA-C (BD Pharmigen, Cat. 566372) and Viakrome (Immunotech, Cat. C36628) at 1:100 final concentration in FACs buffer and then incubated at 4° C. for 30 minutes. After the incubation, the cells were washed and resuspended in 100 μL FACs buffer (PBS+2% FBS+2 mM EDTA) and processed by flow cytometry using a Beckman Coulter CytoflexS, and analyzed using the FlowJo software package. Tables 26A and 26B and FIGS. **10**A and **10**C show the percent editing at each sgRNA dose in an HLA-B7 homozygous or heterozygous donor. Tables 27A and 27B and FIGS. **10**B and **10**D show the percent editing at each sgRNA dose in an HLA-B8 homozygous or heterozygous donor.

TABLE 26A

Dose response curve for the percent of HLA-B7⁻ and CD8⁺ cells in an HLA-B7 homozygous donor

Guide sgRNA (μg/mL)	G034206 Mean % CD8⁺ Cells	G034206 SD % CD8⁺ Cells	G034206 N	G034207 Mean % CD8⁺ Cells	G034207 SD % CD8⁺ Cells	G034207 N	G034208 Mean % CD8⁺ Cells	G034208 SD % CD8⁺ Cells	G034208 N
5.83	89.65	0.13	2	85.47	1.44	2	96.39	0.17	2
1.46	84.24	1.87	2	82.77	0.01	2	95.17	0.13	2
0.36	45.40	2.73	2	43.17	0.74	2	63.27	2.40	2
0.09	1.83	0.42	2	2.40	0.08	2	2.95	0.49	2
0.02	0.43	0.10	2	0.46	0.06	2	0.53	0.19	2
0.005	0.52	0.18	2	0.52	0.32	2	0.50	0.43	2
0.001	0.48	0.15	2	0.39	0.11	2	0.37	0.23	2
0	0.43	0.02	2	0.30	0.06	2	0.49	0.23	2

Guide sgRNA (μg/mL)	G034209 Mean % CD8⁺ Cells	G034209 SD % CD8⁺ Cells	G034209 N	G034210 Mean % CD8⁺ Cells	G034210 SD % CD8⁺ Cells	G034210 N	G034211 Mean % CD8⁺ Cells	G034211 SD % CD8⁺ Cells	G034211 N
5.83	97.30	0.42	2	78.29	1.00	2	86.19	2.25	2
1.46	97.60	0.01	2	69.27	0.65	2	86.42	1.47	2
0.36	79.96	0.37	2	22.22	3.06	2	77.08	1.28	2
0.09	6.58	0.50	2	1.10	0.13	2	17.57	2.56	2
0.02	0.68	0.08	2	0.92	0.13	2	1.21	0.06	2
0.005	0.64	0.01	2	0.70	0.06	2	0.63	0.29	2
0.001	0.29	0.02	2	0.31	0.20	2	0.24	0.15	2
0	0.46	0.06	2	0.45	0.18	2	0.70	0.04	2

TABLE 26B

Dose response curve for the percent of HLA-B7⁻ and CD8⁺ cells in an HLA-B7 heterozygous donor

Guide sgRNA (μg/mL)	G034206 Mean % CD8⁺ Cells	G034206 SD % CD8⁺ Cells	G034206 N	G034207 Mean % CD8⁺ Cells	G034207 SD % CD8⁺ Cells	G034207 N	G034208 Mean % CD8⁺ Cells	G034208 SD % CD8⁺ Cells	G034208 N
5.83	96.82	0.08	2	95.45	0.01	2	99.55	0.07	2
1.46	92.36	1.27	2	92.25	0.88	2	98.53	0.32	2
0.36	41.46	0.18	2	51.70	1.21	2	62.67	4.94	2
0.09	2.02	0.45	2	2.06	0.37	2	4.28	0.81	2
0.02	0.20	0.06	2	0.15	0.02	2	0.35	0.04	2
0.005	0.13	0.08	2	0.11	0.01	2	0.14	0.02	2
0.001	0.18	0.01	2	0.18	0.03	2	0.15	0.08	2
0	0.11	0.01	2	0.21	0.08	2	0.17	0.01	2

Guide sgRNA (μg/mL)	G034209 Mean % CD8⁺ Cells	G034209 SD % CD8⁺ Cells	G034209 N	G034210 Mean % CD8⁺ Cells	G034210 SD % CD8⁺ Cells	G034210 N	G034211 Mean % CD8⁺ Cells	G034211 SD % CD8⁺ Cells	G034211 N
5.83	99.84	0.01	2	94.04	0.43	2	93.08	0.25	2
1.46	99.52	0.04	2	83.31	0.78	2	91.28	0.13	2
0.36	78.88	3.32	2	24.51	0.30	2	83.39	1.46	2
0.09	7.19	1.50	2	1.46	0.05	2	18.89	1.68	2
0.02	0.28	0.11	2	0.30	0.14	2	1.32	0.86	2
0.005	0.23	0.03	2	0.27	0.01	2	0.21	0.00	2
0.001	0.14	0.07	2	0.24	0.02	2	0.18	0.01	2
0	0.17	0.05	2	0.22	0.06	2	0.18	0.06	2

TABLE 27A

Dose response curve for the percent of HLA-B8⁻ and CD8⁺ cells in an HLA-B8 homozygous donor

Guide sgRNA (μg/mL)	G034206 Mean % CD8⁺ Cells	G034206 SD % CD8⁺ Cells	G034206 N	G034207 Mean % CD8⁺ Cells	G034207 SD % CD8⁺ Cells	G034207 N	G034208 Mean % CD8⁺ Cells	G034208 SD % CD8⁺ Cells	G034208 N
5.83	84.88	1.32	2	80.95	0.42	2	92.87	0.18	2
1.46	71.55	2.94	2	67.64	1.03	2	83.77	1.66	2
0.36	29.04	3.68	2	22.14	2.69	2	31.88	0.41	2
0.09	0.29	0.10	2	0.21	0.06	2	0.40	0.04	2
0.02	0.01	0.00	2	0.01	0.01	2	0.00	0.00	2
0.005	0.01	0.01	2	0.02	0.03	2	0.02	0.02	2
0.001	0.01	0.01	2	0.00	0.00	2	0.00	0.00	2
0	0.01	0.00	2	0.00	0.00	2	0.05	0.01	2

Guide sgRNA (μg/mL)	G034209 Mean % CD8⁺ Cells	G034209 SD % CD8⁺ Cells	G034209 N	G034210 Mean % CD8⁺ Cells	G034210 SD % CD8⁺ Cells	G034210 N	G034211 Mean % CD8⁺ Cells	G034211 SD % CD8⁺ Cells	G034211 N
5.83	96.75	0.34	2	57.29	4.96	2	82.85	0.35	2
1.46	93.01	0.52	2	38.65	0.18	2	79.35	0.54	2
0.36	53.42	3.96	2	5.28	0.22	2	64.19	0.30	2
0.09	1.17	0.16	2	0.04	0.01	2	6.25	0.87	2
0.02	0.03	0.01	2	0.02	0.01	2	0.09	0.07	2
0.005	0.01	0.01	2	0.02	0.03	2	0.02	0.01	2
0.001	0.00	0.00	2	0.01	0.00	2	0.02	0.01	2
0	0.00	0.00	2	0.00	0.00	2	0.01	0.01	2

TABLE 27B

Dose response curve for the percent of HLA-B8⁻ and CD8⁺ cells in an HLA-B8 heterozygous donor

Guide sgRNA (μg/mL)	G034206 Mean % CD8⁺ Cells	G034206 SD % CD8⁺ Cells	G034206 N	G034207 Mean % CD8⁺ Cells	G034207 SD % CD8⁺ Cells	G034207 N	G034208 Mean % CD8⁺ Cells	G034208 SD % CD8⁺ Cells	G034208 N
5.83	97.98	0.23	2	96.75	0.55	2	99.44	0.18	2
1.46	94.16	0.66	2	92.78	0.26	2	98.16	0.11	2
0.36	49.19	1.39	2	52.56	0.23	2	64.63	0.85	2
0.09	3.25	0.48	2	1.89	0.11	2	5.70	0.64	2
0.02	0.19	0.04	2	0.02	0.00	2	0.24	0.14	2
0.005	0.11	0.06	2	0.04	0.01	2	0.07	0.07	2
0.001	0.07	0.04	2	0.10	0.02	2	0.12	0.00	2
0	0.14	0.01	2	0.17	0.11	2	0.16	0.05	2

Guide sgRNA (μg/mL)	G034209 Mean % CD8⁺ Cells	G034209 SD % CD8⁺ Cells	G034209 N	G034210 Mean % CD8⁺ Cells	G034210 SD % CD8⁺ Cells	G034210 N	G034211 Mean % CD8⁺ Cells	G034211 SD % CD8⁺ Cells	G034211 N
5.83	99.66	0.11	2	88.96	0.16	2	92.88	0.25	2
1.46	99.31	0.04	2	72.50	0.57	2	91.49	0.72	2
0.36	80.98	0.74	2	19.39	1.07	2	82.62	0.29	2
0.09	10.34	0.35	2	1.59	0.06	2	24.49	0.51	2
0.02	0.79	0.52	2	0.14	0.03	2	0.74	0.07	2
0.005	0.13	0.13	2	0.14	0.01	2	0.13	0.05	2
0.001	0.11	0.06	2	0.11	0.03	2	0.10	0.06	2
0	0.14	0.01	2	0.17	0.11	2	0.16	0.05	2

Example 13: Off-Target Analysis of HLA-B Human Guides

[1024] Screening for potential off-target genomic sites cleaved by Cas9 targeting HLA-B was performed. (See, e.g., Cameron et al., *Nature Methods*. 6, 600-606; 2017). In this experiment, 6 sgRNA targeting human HLA-B and three control guides targeting VEGFA with known off-target profiles were screened using purified genomic DNA from lymphoblast cell line NA24385 (Coriell Institute). The num-

ber of potential off-target sites were detected using a sgRNA as shown in Table 28 at a concentration of 192 nM sgRNA and 64 nM RNP in the biochemical assay. The assay identified potential off-target sites for the sgRNAs tested.

TABLE 28

Off-Target Analysis

gRNA ID	Target	Guide Sequence (SEQ ID NO:)	Off-Target Site Count
G034206	HLA-B	CAAACUCAGGACACUGAGCUUGUG (SEQ ID NO: 163)	1
G034207	HLA-B	UCAGGACACUGAGCUUGUGGAGAC (SEQ ID NO: 164)	1
G034208	HLA-B	UCUGGGAAAGGAGGGGAAGAUGAG (SEQ ID NO: 165)	1
G034209	HLA-B	CUCUGGGAAAGGAGGGGAAGAUGA (SEQ ID NO: 166)	1
G034210	HLA-B	CUGGAGGGUGUGAGACCCUGGCCC (SEQ ID NO: 169)	3
G034211	HLA-B	UCCCAGAGCCGUCUUCCCAGUCCA (SEQ ID NO: 177)	2
G021557	VEGFA	GCAUGGGCAGGGGCUGGGGUGCAC (SEQ ID NO: 610)	2
G021558	VEGFA	GAAUGGCAGGCGGAGGUUGUACUG (SEQ ID NO: 611)	1
G021567	VEGFA	GUGAGCAGGCACCUGUGCCAACAU (SEQ ID NO: 612)	1

[1025] In known off-target detection assays such as the biochemical method used above, a large number of potential off-target sites are typically recovered, by design, so as to "cast a wide net" for potential sites that can be validated in other contexts, e.g., in a primary cell of interest. For example, the biochemical method typically overrepresents the number of potential off-target sites as the assay utilizes purified high molecular weight genomic DNA free of the cell environment and is dependent on the dose of Cas9 RNP used. Accordingly, potential off-target sites identified by these methods may be validated using targeted sequencing of the identified potential off-target sites.

Example 14: In Vivo NK Cell Killing of Engineered T Cells in a Mouse Model

[1026] Female NOG-hIL-15 mice were engrafted with 1.5×10^6 primary NK cells. Engineered T cells containing luciferase and edited according to Table 29 (various combinations of B2M/HLA-A/HLA-B/TRAC/CIITA KO) were injected 4 weeks later in order to assess protection of the engineered T cells from NK cell killing. 14.1. Preparation of T cells containing luciferase +/−B2M/HLA-A/HLA-B/TRAC/CIITA KO and HD1 TCR

[1027] On day 0, upon thaw, Pan CD3+ T cells (StemCell, HLA-A*02:01:01 A*11:01:01; B*08:01:01; C*07:01:01) were plated at a density of $1\text{-}1.5\times10^6$ cells/mL in T cell TCAM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and 1% (v/v) 10 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 200 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15).

[1028] On day 1, T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec).

[1029] On day 2, cells were transduced with a Lentivirus—LV-SFFV-Luc2-P2A-EmGFP to express GFP/Firefly luciferase (Imanis, Cat. LV050L) at a MOI of 0.5. This was performed by centrifuging the T cells at 500×g for 5 minutes, resuspending them at 2E6 cells/mL and transferring them to sterile 1.5 mL Eppendorf tubes such that each tube received 1E6 cells in 0.5 mL. 100 μL of LV-SFFV-Luc2-P2A-EmGFP (Imanis, Cat. LV050L) was added to each tube and the transduction was performed by centrifuging the tubes at 1000×g for 60 minutes at 37° C. Post centrifugation, cells were combined and transferred back to a cell culture flask and rested overnight in a 37° C. incubator.

[1030] On day 3, as described in Table 29, T cells were edited with LNPs to disrupt the HLA-A or CIITA genes. Briefly, LNPs for each of BC22 mRNA, UGI mRNA and sgRNA G028918 targeting HLA-A or sgRNA G026584 targeting CIITA were formulated as described in Example 1. LNP compositions were incubated in TCAM with cytokines as described above supplemented with 20 μg/mL recombinant human ApoE3 (Peprotech, Cat. 350-02). An equal volume of LNP mix was added to one million activated T cells to yield a final concentration of 0.266 μg total LNP/mL for the HLA-A LNP and 0.34 μg total LNP/mL for the CIITA LNP.

TABLE 29

Order of sequential editing and viral transduction

Condition	Day 3	Day 4	Day 8
Unedited			
B2M + CIITA KO	CIITA LNP	B2M LNP	TRAC LNP
HLA-A + CIITA	HLA-A LNP, CIITA LNP		TRAC LNP
HLA-B + CIITA	CIITA LNP	HLA-B LNP	TRAC LNP
HLA-A + HLA-B + CIITA	HLA-A LNP, CIITA LNP		TRAC LNP

[1031] On day 4, the T cells from each group were counted, re-plated at 2×10^6 cells/mL and edited with LNP compositions to disrupt the HLA-B or B2M genes (where noted in Table 29, Day 4 column). This was performed with LNPs co-formulated with either Nme2 BC22n mRNA and sgRNA G032795 targeting the HLA-B gene or Spy Cas9 mRNA and sgRNA G000529 targeting the B2M gene. LNP compositions were incubated in TCAM with cytokines as described above supplemented with 5 μg/mL recombinant human ApoE3 (Peprotech, Cat. 350-02). A volume equal to the volume of the cells was added to each well containing ~2 million cells to yield a final concentration of 1.25 μg total LNP/mL for the HLA-B LNP and 2.5 μg total LNP/mL for the B2M LNP.

[1032] On day 5, all cells were transferred to GREX plate (Wilson Wolf, Cat. 80240M) for expansion with TCAM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and

1% (v/v) 10 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15).

[1033] On day 7, each of the T cell groups was sorted on the GFP+ population, and cells were put back in culture in TCAM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and 1% (v/v) 10 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15).

[1034] On day 8, sorted cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec), and edited with LNP formulations to disrupt the TRAC gene. Briefly, LNPs for each Nme2 BC22 mRNA (mRNA100418), UGI mRNA (mRNA100032) and sgRNA G028939 targeting TRAC were formulated as described in Example 1. LNP compositions were incubated in TCAM with cytokines as described above supplemented with 20 μg/mL recombinant human ApoE3 (Peprotech, Cat. 350-02). An equal volume of LNP mix was added to one million activated T cells to yield a final concentration of 0.209 μg total LNP/mL for the TRAC LNP.

[1035] On day 10, all cells were transferred to GREX plate (Wilson Wolf, Cat. 80240M) for expansion with TCAM media composed of CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001) containing 2.5% (v/v) of Human AB Serum, 1% (v/v) Glutamax (Gibco, Cat. 35050-061) and 1% (v/v) 10 1M HEPES buffer (Gibco, Cat. 15630080), 1% of Penicillin-Streptomycin, and supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of human interleukin-7 (Peprotech, Cat. 200-07) and 5 ng/mL of human interleukin-15 (Peprotech, Cat. 200-15).

[1036] Half of the media from the GREX wells was replaced with fresh TCEM supplemented with cytokines on Days 12 and 14.

[1037] On day 17, an aliquot of cells was stained by flow cytometry antibodies to determine HLA-A2 expression (HLA-A+), HLA-B8 expression (HLA-B8+), HLA-Class-I expression (MHC I+) following knockout of B2M, HLA-Class-II expression (MHC II+) following knockout of CIITA, and CD3 expression following knockout of TRAC. T cells were incubated with an antibody cocktail targeting the following molecules: HLA-A2 (B eBioscience, Cat. 17-9876-42), HLA-B8 (Miltenyi Biotec, Cat. 130-118-960), CD3 (Biolegend, Cat. 317334), CD4 (Biolegend, Cat. 300536), CD8 (Biolegend, Cat. 344740), HLA-DR, DP, DQ (HLA Class-II) (Biolegend, Cat. 361712), HLA-E (Biolegend, Cat. 342612), and ViaKrome 808 Fixable Viability Dye (Beckman Coulter, Cat. C36628). Cells were subsequently washed, analyzed on a Cytoflex LX instrument (Beckman Coulter) using the FlowJo software package. The rest of the cells were frozen down for subsequent T cell injections in mice using a 1:1 dilution for CSB (Stemcells, 100-0237) and CS10 (Stemcells, 7930).

14.2. HLA-A and/or HLA-B with CIITA Knockout T Cells Show Greater Protection from NK Killing than B2M/CIITA KO T Cells

[1038] NK cells were isolated from a leukopak by methods known in the art, washed with HBSS (Gibco, Cat. No. 14025-092) and resuspended at 10×10^6 cells/mL for injection in 150 μL HBSS. Thirty female NOG-hIL-15 mice (Taconic) were dosed by tail vein injection with 1.5×10^6 isolated NK cells. An additional twenty-five female NOG-hIL-15 served NK-non-injected controls.

[1039] Twenty-seven days after NK cell injection, mice were injected with unedited or engineered T cells as described in Table 29. Briefly, engineered T cells were injected 16 days post first activation after washing in PBS and resuspending in HBSS solution at a concentration of 6.0×10^6 cells/150 μL.

[1040] IVIS imaging of live mice was performed to identify luciferase-positive T cells by IVIS spectrum. IVIS imaging was done at 24 hours, 7 days, 14 days, 16 days, 19 days, 22 days, 26 days, 29 days, 33 days, 36 days, 43 days, 49 days, and 61 days after T cell injection. Mice were prepared for imaging with an injection of D-luciferin i.p. at 10 μL/g body weight per the manufacturer's recommendation, about 150 μL per animal. Animals were anesthetized and then placed in the IVIS imaging unit. The visualization was performed with the exposure time set to auto, field of view D, medium binning, and F/stop set to 1. Table 30 and FIG. **11** shows total flux (photons/s) from luciferase expressing T cells present at the various time points after injection. In vivo, B32M edited cells showed sensitivity to NK killing, while HLA-A, HLA-B, TRAC and CIITA edited cells (as shown in Table 29) showed protection from NK mediated lysis.

TABLE 30

Total Flux (photons/s) from luciferase expressing T cells in treated mice at intervals after T cell injection.

Group	Days post T cell injection	No NK injection Mean	No NK injection SD	No NK injection N	NK injection Mean	NK injection SD	NK injection N
TRAC KO	1	1.03E+08	1.70E+07	4	7.70E+07	1.04E+07	5
	7	2.15E+08	1.36E+07	4	1.57E+08	2.85E+07	5
	14	2.66E+08	4.04E+07	4	3.01E+08	5.64E+07	5
	16	3.27E+08	4.84E+07	4	1.69E+08	4.40E+07	5
	19	2.57E+08	5.99E+07	4	2.22E+08	7.06E+07	5
	22	2.74E+08	3.40E+07	4	2.38E+08	1.19E+08	5
	26	2.33E+08	3.74E+07	4	2.45E+08	1.45E+08	5
	29	2.39E+08	5.41E+07	4	2.70E+08	1.46E+08	5
	33	1.85E+08	4.72E+07	4	2.42E+08	1.47E+08	5
	36	1.62E+08	1.71E+07	4	2.67E+08	1.51E+08	5
	43	2.05E+08	6.97E+07	4	3.45E+08	2.75E+08	5
	49	2.62E+08	1.85E+08	4	4.34E+08	3.66E+08	5
	61	2.07E+08	1.80E+08	4	2.63E+09	3.35E+09	5
B2M	1	7.23E+07	1.05E+07	4	7.14E+06	1.44E+06	5
	7	8.40E+07	1.00E+07	4	5.57E+06	7.80E+05	5
	14	1.07E+08	1.65E+07	4	4.06E+06	6.93E+05	5
	16	8.41E+07	2.04E+07	4	4.05E+06	5.02E+05	5
	19	1.38E+08	3.78E+07	4	2.96E+06	2.46E+05	5
	22	6.59E+07	1.77E+07	4	3.38E+06	4.43E+05	5
	26	5.09E+07	2.33E+07	4	3.26E+06	4.57E+05	5
	29	5.20E+07	1.76E+07	4	3.11E+06	5.11E+05	5

TABLE 30-continued

Total Flux (photons/s) from luciferase expressing T cells in treated mice at intervals after T cell injection.

Group	Days post T cell injection	No NK injection Mean	No NK injection SD	No NK injection N	NK injection Mean	NK injection SD	NK injection N
	33	4.86E+07	1.75E+07	4	2.65E+06	2.23E+05	5
	36	3.13E+07	7.28E+06	4	2.64E+06	2.87E+05	5
	43	3.96E+07	1.47E+07	4	2.10E+06	2.80E+05	5
	49	3.56E+07	3.23E+06	4	2.18E+06	2.29E+05	5
	61	1.94E+07	3.89E+06	4	1.86E+06	2.74E+05	5
HLA-A	1	5.93E+07	5.19E+06	4	5.92E+07	1.01E+07	5
	7	1.99E+08	2.97E+07	4	1.80E+08	4.31E+07	5
	14	3.23E+08	8.66E+07	4	3.01E+08	9.32E+07	5
	16	3.05E+08	9.72E+07	4	3.13E+08	8.01E+07	5
	19	1.88E+08	3.55E+07	4	3.41E+08	1.10E+08	5
	22	2.75E+08	2.41E+07	4	4.14E+08	1.68E+08	5
	26	1.89E+08	3.99E+07	4	3.24E+08	1.40E+08	5
	29	1.67E+08	4.29E+07	4	3.60E+08	1.65E+08	5
	33	1.67E+08	4.25E+07	4	3.03E+08	1.59E+08	5
	36	1.91E+08	5.23E+07	4	2.63E+08	1.43E+08	5
	43	1.17E+08	2.24E+07	4	2.62E+08	1.50E+08	5
	49	6.79E+07	1.14E+07	4	2.64E+08	1.27E+08	5
	61	6.99E+07	2.02E+07	4	4.09E+08	2.92E+08	5
HLA-B	1	7.56E+07	4.17E+07	4	6.13E+07	8.43E+06	5
	7	1.81E+08	1.85E+07	4	8.47E+07	1.53E+07	5
	14	1.04E+08	9.88E+06	4	9.54E+07	8.16E+06	5
	16	9.11E+07	5.68E+06	4	1.01E+08	2.80E+07	5
	19	1.90E+08	2.08E+07	4	7.30E+07	1.48E+07	5
	22	9.67E+07	3.17E+06	4	1.00E+08	2.37E+07	5
	26	1.08E+08	2.21E+07	4	7.96E+07	2.17E+07	5
	29	7.63E+07	1.59E+07	4	5.30E+07	8.34E+06	5
	33	5.77E+07	1.32E+07	4	7.60E+07	2.91E+07	5
	36	5.23E+07	9.16E+06	4	7.50E+07	3.48E+07	5
	43	5.72E+07	8.07E+06	4	8.64E+07	4.06E+07	5
	49	7.81E+07	3.27E+07	4	6.60E+07	3.65E+07	5
	61	4.10E+07	7.65E+06	4	6.60E+07	4.21E+07	5
HLA-AB	1	6.89E+07	5.43E+06	4	8.32E+07	6.69E+06	5
	7	8.96E+07	8.11E+06	4	1.53E+08	1.17E+08	5
	14	1.13E+08	1.06E+07	4	4.65E+08	6.03E+08	5
	16	7.28E+07	1.70E+07	4	6.94E+08	9.88E+08	5
	19	9.11E+07	2.35E+07	4	1.27E+09	2.15E+09	5
	22	1.20E+08	5.52E+07	4	2.58E+09	4.72E+09	5
	26	9.65E+07	5.94E+07	4	2.95E+09	5.40E+09	5
	29	8.27E+07	5.16E+07	4	3.10E+09	5.72E+09	5
	33	5.52E+07	3.10E+07	4	1.66E+08	1.11E+08	5
	36	7.34E+07	8.04E+07	4	1.47E+08	9.66E+07	5
	43	1.55E+08	2.13E+08	4	1.60E+08	9.99E+07	5
	49	2.02E+08	3.05E+08	4	1.29E+08	5.71E+07	5
	61	1.53E+09	2.56E+09	4	3.08E+08	3.14E+08	5
NK only	1				8.88E+05	8.15E+04	5
	7				9.56E+05	4.69E+04	5
	14				1.03E+06	9.95E+04	5
	16				1.28E+06	1.22E+05	5
	19				1.06E+06	5.24E+04	5
	22				1.38E+06	1.30E+05	5
	26				1.24E+06	1.03E+05	5
	29				1.14E+06	1.48E+05	5
	33				1.32E+06	1.81E+05	5
	36				1.24E+06	8.79E+04	5
	43				1.17E+06	9.95E+04	5
	49				1.25E+06	1.65E+05	5
	61				9.40E+05	3.32E+04	5
HBSS	1	9.41E+05	7.08E+04	5			
	7	9.77E+05	9.09E+04	5			
	14	8.80E+05	4.23E+04	5			
	16	1.15E+06	7.44E+04	5			
	19	8.56E+05	1.00E+05	5			
	22	1.19E+06	2.02E+05	5			
	26	1.19E+06	9.09E+04	5			
	29	9.74E+05	8.72E+04	5			
	33	1.07E+06	6.09E+04	5			
	36	1.10E+06	8.36E+04	5			

TABLE 30-continued

Total Flux (photons/s) from luciferase expressing T cells in treated mice at intervals after T cell injection.

Group	Days post T cell injection	No NK injection Mean	No NK injection SD	No NK injection N	NK injection Mean	NK injection SD	NK injection N
	43	9.89E+05	5.35E+04	5			
	49	1.12E+06	1.46E+05	5			
	61	9.24E+05	7.21E+04	5			

Example 15: In Vivo NK Cell Killing of Engineered T Cells in a Mouse Model

[1041] Female NOG-hIL-15 mice were engrafted with 1.5×10^6 primary NKI cells. Engineered T cells containing luciferase and edited according to Table 29 (various combinations of HLA-A/HLA-B/TRAC/CIITA KO) were injected 4 weeks later in order to assess protection of the engineered T cells from NKI cell killing.

15.1. Preparation of T Cells Containing Luciferase +/−B2M/HLA-A/HLA-B/TRAC/CIITA KO and HD1 TCR

[1042] T cells were prepared as in Example 14.1 except on day 4, the edit was performed with LNPs co-formulated with Spy Cas9 mRNA gRNA G027994 targeting the HLA-B gene.

15.2. HLA-A and/or HLA-B with CIITA Knockout T Cells Show Greater Protection from NK Killing than B2M/CIITA KO T Cells

[1043] The NK cells and mice were prepared as in Example 14.2.

[1044] IVIS imaging of live mice was performed to identify luciferase-positive T cells by IVIS spectrum. IVIS imaging was done at 24 hours, 7 days, 14 days, 16 days, 19 days, 22 days, 26 days, 29 days, 33 days, 36 days, and 40 days after T cell injection. Mice were prepared for imaging as in Example 14.2. Table 31 and FIG. **12** shows total flux (photons/s) from luciferase expressing T cells present at the various time points after injection. In vivo, B32M edited cells showed sensitivity to NK killing, while HLA-A, HLA-B, TRAC and CIITA edited cells (as shown in Table 29) showed protection from NK mediated lysis.

TABLE 31

Total Flux (photons/s) from luciferase expressing T cells in treated mice at indicated days after T cell injection.

Group	Days	No NK injection Mean	No NK injection SD	No NK injection N	NK injection Mean	NK injection SD	NK injection N
TRAC KO	1	60237500	19287091	4	78610000	5481726	5
	5	79142500	21375333	4	42522000	7716803	5
	8	48687500	4504023	4	50922000	5762317	5
	18	28987500	7896542	4	59024000	19425221	5
	22	38695000	11931246	4	69482000	23737977	5
	26	62917500	12062851	4	54070000	21862370	5
	29	59235000	17354556	4	1.05E+08	51444384	5
	33	70220000	18308445	4	81094000	30428047	5
	36	38415000	10687349	4	1.06E+08	50005246	5
	40	40777500	8983302	4	1.66E+08	1.14E+08	5

TABLE 31-continued

Total Flux (photons/s) from luciferase expressing T cells in treated mice at indicated days after T cell injection.

		No NK injection			NK injection		
Group	Days	Mean	SD	N	Mean	SD	N
B2M	1	61697500	3710178	4	4625200	1017123	5
	5	40126000	7705511	4	2337400	244229.9	5
	8	66758000	14162942	4	2650600	417803.6	5
	18	44676000	16960004	4	2997800	477095.1	5
	22	56008000	25277849	4	2953600	344565	5
	26	71536000	36732385	4	2469720	916718.8	5
	29	64236000	29804451	4	3688200	846342.1	5
	33	64786000	33498350	4	2378200	408716.7	5
	36	70582000	38523286	4	2803200	512631	5
	40	59696000	33547829	4	2952000	459698.6	5
HLA-A	1	1.03E+08	18126125	4	72340000	10886537	5
	5	67105000	14635427	4	47038000	4848218	5
	8	68767500	7969433	4	40606000	10750222	5
	18	38107500	9255540	4	96062000	25313313	5
	22	56917500	7445550	4	1.1E+08	60299107	5
	26	44572500	8581807	4	2.89E+08	1.86E+08	5
	29	60602500	18917399	4	5.74E+08	5.45E+08	5
	33	83882500	30944594	4	6.68E+08	6.13E+08	5
	36	75797500	29148427	4	1.42E+09	1.44E+09	5
	40	1.09E+08	61468496	4	1.7E+09	1.83E+09	5
HLA-B	1	75120000	13263039	4	95964000	11361716	5
	5	1.14E+08	2656478	4	54178000	11174948	5
	8	1.53E+08	30114147	4	86706000	15060945	5
	18	1.12E+08	19480373	4	70712000	17193704	5
	22	1.29E+08	23319638	4	1.31E+08	28386687	5
	26	2.18E+08	1.76E+08	4	1.02E+08	36123989	5
	29	3.19E+08	4.79E+08	4	1.79E+08	1.28E+08	5
	33	4.15E+08	6.04E+08	4	3.96E+08	4.33E+08	5
	36	5.16E+08	7.64E+08	4	6.5E+08	7.98E+08	5
	40	8.16E+08	1.3E+09	4	1.2E+09	1.63E+09	5
HLA-AB	1	54503800	31113691	4	40374000	5322889	5
	5	79315000	10423954	4	41694000	7467100	5
	8	79832500	12777999	4	34938000	8491715	5
	18	55077500	14565978	4	64404000	21336274	5
	22	46923250	33468493	4	43546000	16951128	5
	26	52755000	25167748	4	54498000	15582203	5
	29	66772500	37915812	4	51224000	14570855	5
	33	64415000	41401597	4	54594000	25814216	5
	36	52932500	30505738	4	52580000	16331823	5
	40	56737500	39401755	4	61682000	21887079	5
NK only	1				1659800	144107.5	5
	5				1252000	143766	5
	8				1468667	21746.01	5
	18				1321667	85253.87	5
	22				1393333	114106.2	5
	26				1329000	97857.04	5
	29				1786000	227694.2	5
	33				1290667	190931.6	5
	36				1475000	197293.4	5
	40				1505333	81667.35	5
HBSS	1	1335000	150939.7	5			
	5	1086560	154003	5			
	8	1116000	43446.52	5			
	18	1023540	60430.84	5			
	22	1214800	96373.03	5			
	26	1295000	109750.6	5			
	29	1145520	120455	5			
	33	1211400	60546.18	5			
	36	1319200	85889.23	5			
	40	1357000	112189.1	5			

Example 16: Functional Analysis of T Cells with Double/Triple Knockout (KO) Edits and Expressing Anti-CD30 CAR-T

16.1. Engineering T Cells

[1045] Upon thaw (day 0), CD4+CD8+ T cells (Cellex) at a ratio of 1:1 were plated at a density of ~1.2×10^6 cells/mL in T cell activation media (TCAM) composed of CTS OpTmizer T cell Expansion SFM (Gibco, Cat. A3705001) containing CTS Supplement, 2.5% (v/v) of Human AB Serum (Valley Biomedical, HP1022HI), 1× Glutamax (Gibco, Cat. 35050-061), 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 200 IU/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL of recombinant human interleukin-15 (Peprotech, Cat. 200-15). Cells were incubated in a 37° C. incubator overnight.

[1046] 24 hours post thaw (day 1), cells were counted and resuspended at 1×10^6 cells/mL in TCAM. Cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec).

[1047] Different T cell groups were edited as described in Table 32. Briefly, LNP compositions containing 1) Nme2 BC22 mRNA and 2) sgRNA G034202 targeting HLA-A; or sgRNA G034201 targeting CIITA; or sgRNA G025420 targeting TRAC; or sgRNA G034209 targeting HLA-B. UGI mRNA LNP was separately formulated. See Example 1 describing LNP formulation.

TABLE 32

Editing scheme and viral transduction

Condition	Day 0	Day 1	Day 3	Day 5
CD30 CAR Double KO	Thaw	TransAct	Nme2 BC22 HLA-A + CIITA + Spy Cas9 TRAC + AAV	GREX 6 transfer
CD30 CAR Triple KO	Thaw	TransAct	Nme2 BC22 HLA-A + CIITA + HLA-B + Spy Cas9 TRAC + AAV	GREX 6 transfer
Untransduced/ TCR KO only	Thaw	TransAct	Spy Cas9 TRAC	GREX 6 transfer
Unedited	Thaw	TransAct		GREX 6 transfer

[1048] On day 3, cells were counted and resuspended in TCAM with cytokines as described above at 1×10^6 cells/mL. A mixture of LNP, ApoE, AAV in TCAM was prepared at 2× concentration such that when equal volume of LNP mix was added to the T cells the concentration would be 1.5 μg/mL for each HLA-A, CIITA, or HLA-B sgRNA, with BC22 mRNA co-formulation LNPs, 0.2 μg/mL for UGI mRNA LNP, 1 μg/mL for TRAC sgRNA LNP, 10 μg/mL for ApoE, 1E5 GC/cell for AAV and the cells would be at final density 0.5×10^6 cells/mL. Cells were incubated in a 37° C. incubator overnight.

[1049] 24 hours post transfection (day 4), cells were counted and again brought to 0.5×10^6 cells/mL density.

[1050] 48 hours post transfection, (day 5) cells were cultured in 6-well GREX (Wilson Wolf, Cat. 80240M) in T cell expansion media (TCEM) composed of CTS OpTmizer T cell Expansion SFM (Gibco, Cat. A3705001) containing CTS Supplement, 5% (v/v) of Human AB Serum (Valley Biomedical, HP1022HI), 1× Glutamax (Gibco, Cat. 35050-061), 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 200 IU/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL of recombinant human interleukin-15 (Peprotech, Cat. 200-15). Cells were cultured till day 9 with regular media changes.

[1051] Additionally, T cells were also engineered to disrupt B2M and CIITA, to generate a double KO of these two genes.

[1052] Upon thaw (day 0), Donor DR26 T cells (Cellex) were plated at a density of ~1.5×10^6 cells/mL in T cell activation media (TCAM) composed of CTS OpTmizer T cell Expansion SFM (Gibco, Cat. A3705001) containing CTS Supplement, 2.5% (v/v) of Human AB Serum (Valley Biomedical, HP1022HI), 1× Glutamax (Gibco, Cat. 35050-061), 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 200 IU/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL of recombinant human interleukin-15 (Peprotech, Cat. 200-15). Cells were rested in a 37° C. incubator overnight.

[1053] Different T cell groups were edited as described in Table 33. Briefly, LNP compositions containing sgRNA G000529 (SEQ ID NO: 993) targeting B2M and sgRNA G013675 (SEQ ID NO: 3118) targeting CIITA.

TABLE 33

Order of editing and Harvest					
Condition	Day 0	Day 1	Day 2	Day 3	Day 8
B2M + CIITA	Thaw	TransAct + CIITA	B2M	24-well Grex transfer	Harvest
Unedited	Thaw	TransAct		24-well Grex transfer	Harvest

[1054] 24 hrs post thaw (day 1), cells were counted and resuspended in TCAM with cytokines as described above at 2×10^6 cells/mL. Cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). A mixture of LNP and ApoE3 in TCAM was prepared at 2× concentration such that when equal volume of LNP mix was added to T cells, the concentration would be 1.25 μg/mL for CIITA sgRNA formulation LNPs, and 2.5 μg/mL for ApoE and cell density would be 1×10^6 cells/mL. Cells were incubated in a 37° C. incubator overnight.

[1055] On day 2, cells were counted and again brought to 1×10^6 cells/mL density. A mixture of LNP and ApoE3 in TCAM was prepared at 10× concentration such that when equal volume of LNP mix was added to T cells resuspended at 1×10^6 cells/mL, the concentration would be 2.5 μg/mL for B2M and 2.5 μg/mL for ApoE3. Cells were incubated in a 37° C. incubator overnight

[1056] 24 hours later (on day 3) cells were cultured in 24-well GREX (Wilson Wolf, Cat. 80192M) in T cell expansion media (TCEM) composed of CTS OpTmizer T cell Expansion SFM (Gibco, Cat. A3705001) containing CTS Supplement, 5% (v/v) of Human AB Serum (Valley Biomedical, HP1022HI), 1× Glutamax (Gibco, Cat. 35050-061), 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 200 IU/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL of recombinant human interleukin-7 (Peprotech, Cat. 200-07), and 5 ng/mL of recombinant human interleukin-15 (Peprotech, Cat. 200-15). Cells were cultured until day 8 with regular media changes and frozen down on day 8.

Example 16.2. Tumor Cell Killing Assay Using Engineered T Cells Expressing Anti-CD30 CAR-T Cells and with Double and Triple Knockout Edits

[1057] T cells were engineered with anti-CD30 CAR constructs and different edits as described in Example 16.1 and were tested for their cytotoxicity against CD30 expressing HH and MOLT-4 tumor cell lines. HH cells and MOLT-4 cells were thawed and maintained in culture for at least 7 days before setting up the killing assay. CD30 CAR-T cells and control T-cells were removed from liquid nitrogen, thawed, and rested overnight in pre-warmed T cell media. The killing assay was setup the following day by first seeding 20,000 cells/well of HH or MOLT-4 cells in 100 μL in a 96 well plate. CD30-CAR T-cells and control T-cells were counted, centrifuged, and resuspended in T-cell media without any cytokines. They were then serially diluted 3-fold starting with E:T ratio of 3.3 and then diluted up to 5 points. They were then added to target cells to their respective wells and kept in incubator at 37° C.

[1058] Bright-Glo™ Luciferase Assay System (Promega, Cat. E2620) was pre-thawed in dark at room temperature. The killing assay plate was taken out from the incubator. 50 μL of Bright-Glo™ Luciferase Assay System was added to each well and the plate was shaken briefly on a shaker and then incubated in dark at room temperature for 5 minutes. The plate was then read for luminescence with a CLARIOstar plate reader. The percentage killing was calculated from the luminescence with the average of T cell to tumor cell ratio 0 as 0% killing. The percent killing results are shown in for HH cells in Table 34 and FIG. **13**A and the percent killing results for MOLT-4 cells are shown in Table 35 and FIG. **13**B.

TABLE 34

percentage killing in HH cells for double and triple KO edits						
	Untransduced		Double KO		Triple KO	
E:T	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing
3.33	13.48	2.23	99.94	0.02	99.92	0.04
1.11	11.84	0.42	99.53	0.12	99.26	0.13
0.37	2.71	1.91	91.74	0.65	85.62	0.81
0.12	−0.93	0.57	56.45	0.02	51.78	2.09
0.04	−3.92	1.43	31.10	2.18	31.44	2.59

TABLE 35

percentage killing in MOLT-4 cells for double and triple KO edits						
	Untransduced		Double KO		Triple KO	
E:T	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing
3.33	32.73	0.48	97.37	0.17	97.29	0.64
1.11	15.28	1.08	94.08	0.01	93.36	0.99
0.37	12.97	0.28	84.65	1.60	86.61	0.90
0.12	8.90	0.81	65.51	2.59	71.31	1.11
0.04	8.79	0.72	34.37	0.95	44.03	2.53

16.3. MLR Assay

Thawing & Resting Host PBMCs and Engineered Donor T Cells

[1059] Cryopreserved host PBMCs and engineered donor T cells as described in Table 36 were thawed at a cell concentration of 1.5×10^6 cells/mL into T cell growth media (TCGM) composed of OpTmizer TCGM (Gibco,

A1048501), Human Serum AB (GeminiBio, 100-512), HEPES 1M (Gibco, 15630-080), GlutaMAX Supplement (Gibco, 35050-061), and Penicillin-Streptomycin (Gibco, 15070-063) and further supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL IL-7 (Peprotech, Cat. 200-07), 5 ng/mL IL-15 (Peprotech, Cat. 200-15). Cells were rested in 37° C. incubator overnight.

TABLE 36

Donor T cells Editing Schematic and Host PBMCs

Cell Type		Donor	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
Donor T cells	WT (unedited)	Donor 1	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01
	Triple KO (HLA-A/HLA-B/CIITA)	Donor 1	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01
Host PBMCs	HLA-C Match Host	Host 1	A*03:02	A*03:02	B*49:01	B*49:01	C*07:01	C*07:01
	Autologous	Auto Host (Donor 1)	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01

Assay Setup with Engineered Donor T Cells and Host PBMCs

[1060] The following day, engineered donor T cells as described in Table 36 were irradiated at 5000 rad (Program C) and spun down, and each group was resuspended at 1×10^6/mL in TCGM without cytokines. Host PBMCs will undergo CD56 depletion using CD56 microbeads from miltenyi as described in manufacturer's protocol. CD56 depleted were then counted using Nexcelom Celleca cell counter and desired number of cells were collected in a 15 mL conical tube and then spun down in a centrifuge at 500×G for 5 minutes followed by resuspending at 1×10^6 cells/mL in phosphate buffered saline (Corning, Cat. No. 21-040-CV). A vial of Cell Trace Violet (Thermo Fisher, Cat. No. C34571) per donor was brought to room temperature and reconstituted using 20 μL DMSO to generate a stock of 5 mM CTV. A total of 10 million host PBMCs were transferred to a 50 mL conical tube and stained with 10 μL CTV at 5 μM concentration and incubated for 15 minutes in a 37° C. incubator. The labelled host cells were then spun down at 500×g for 5 minutes. CTV labelled host PBMCs were then resuspended at 1×10^6 cells/mL in pre-warmed TCGM media composed of OpTimizer TCGM as described in Example 16.1. For the Donor+Host co-culture at 3:1 donor: host ratio, 50 μL of host PBMCs and 150 μL of donor T cells were added per each well in a sterile 96 well round bottom plate. The plate was transferred to 37° C. incubator and incubated for 6 days. On day 6 post co-culture, half the media (~100 L) from each well was replaced with fresh media (TCGM without cytokines).

Alloreactivity Readout by Flow Cytometry

[1061] On day 8 post co-culture, the assay plate was stained and analyzed by flow cytometry. For the staining, the plate was spun at 600×g for 3 minutes, flicked to remove media, and 100 μL of a 1:100 v/v solution of Fc blocker (Biolegend, Cat #422302) in FACS buffer (Phosphate-buffered saline (Corning, Cat. 21-040-CV), Fetal Bovine Serum (Gibco, Cat. A3840201), UltraPure 0.5M EDTA (Invitrogen, Cat. 15575-020)) was added to each well. Cells were resuspended in the Fc blocker, and the plate was incubated at room temperature for 5 minutes. T cells were incubated with an antibody cocktail targeting the following molecules: CD3 (Biolegend, Cat. 317336), CD56 (Biolegend, Cat. 362546), CD4 (Biolegend, Cat. 300518), CD8 (Biolegend, Cat. 344742) and ViaKrome 808 Fixable Viability Dye (Beckman Coulter, Cat. C36628). Each antibody was prepared at a 1:100 v/v dilution, and 100 μL of this antibody mixture was added to each sample well. The plate was protected from light by covering with an aluminum foil and incubated at 2-8° C. for 20-30 minutes. After staining, the plate was spun at 600×g for 3 minutes, flicked to remove media and washed with 200 μL of FACS buffer. The plate was washed again, and the cell pellets were resuspended in 60 μL of FACS buffer. To each well, 10 μL of Count Bright Absolute Counting Beads was added and mixed well. The plate was run and recorded on fast mode with 70 μL total volume as a stopping rule on Cytoflex flow cytometer. FIGS. **15**A and **15**B show the percentage of host T cell proliferation as a result of donor T cell treatment. FIG. **15**A shows that, in an autologous context, host T cells co-cultured with unedited T cells (UED) or edited T cells (Triple KO) show similar proliferation as host T cells alone. FIG. **15**B shows host T cells co-cultured with unedited T cells (UED) show higher proliferation due to mismatch of HLA-A and B alleles (while C is matched) as compared to host only control. Additionally, host T cells co-cultured with unedited T cells (UED) show higher proliferation due to mismatch of HLA-A and B alleles (while C is matched), as compared to triple KO engineered cells, where HLA-A and HLA-B are disrupted, and HLA-C is matched.

In Vitro NK Cell Killing Assays

[1062] CD30-CAR T cells edited in various combinations to disrupt CIITA, HLA-A, HLA-B, and/or B2M as described in Example 16.1, were tested for their ability to resist natural killer (NK) cell mediated killing.

Thawing & Resting Host PBMCs and Engineered Donor T Cells

[1063] Cryopreserved NK cells and donor T cells as described in Table 36 were thawed at a cell concentration of 1.5×10^6 cells/ml into T cell growth media (TCGM) composed of OpTmizer TCGM as described in Example 16.3 and further supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL IL-7 (Peprotech, Cat. 200-07), 5 ng/mL IL-15 (Peprotech, Cat. 200-15) for T-cells and 500 U/mL of IL-2 only for NK cells. Cells were rested in 37° C. incubator overnight.

[1064] Flow cytometry was performed as in Example 11.2. Table 37 shows the genotypes of the donor T cells. Table 38 and FIG. **14** show the mean percentage of T cell lysis following NK cell challenge.

TABLE 37

Donor T cells and Host NK cells								
Cell Type		Donor ID	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
Donor T cells	WT	Donor 1	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01
	B2M + CIITA	Donor 1	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01
	Triple KO (HLA-A/HLA-B/CIITA)	Donor 1	A*02:01	A*02:01	B*08:01	B*08:01	C*07:01	C*07:01

TABLE 38

T cell lysis following NK cell challenge								
	Unedited		Untransduced		B2M/ CIITA KO		Triple KO	
E:T	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing	Mean % Killing	SD % Killing
7.00	18.65	1.91	20.35	4.88	87.98	4.50	23.60	6.93
3.50	20.90	3.11	39.65	7.42	89.18	4.07	23.55	1.48
1.75	16.45	0.21	22.45	5.59	83.00	2.97	25.25	10.68
0.88	13.85	2.62	19.20	12.16	69.65	11.81	32.85	2.76
0.44	12.20	2.69	16.50	1.70	44.90	0.42	24.45	6.86
0.22	10.70	0.28	13.70	2.69	24.00	0.00	22.60	2.26

Example 17: Double and Triple Knockout Edits in Differentiated iPSC Cells

[1065] iPSCs were reprogrammed from human PBMCs at CORM (Centre for Commercialization of Regenerative Medicine). After multiple clone characterization assays, including pluripotency marker expression and karyotyping, a single iPSC clone was selected for CRISPR/Cas9 mediated gene-editing. The selected iPSC cells were edited using HLA-A guide G018995, HLA-B guide G022020, and CIITA guide G013675, to generate double knockout (HLA-A and CIITA DKO) or triple knockout (HLA-A, HLA-B, and CIITA TKO) samples.

[1066] As shown in Table 39 below, ddPCR data showed high editing efficiency in all three targets in bulk iPSC cells.

TABLE 39

Editing Efficiencies for HLA-A, CIITA, and HLA-B			
	HLA-A Editing (%)	CIITA Editing (%)	HLA-B Editing (%)
WT unedited	−5	0	−8
DKO	87	84	N/A
TKO	87	84	79

[1067] After single cell plating, clonal expansion, and clone characterization, a DKO or TKO iPSC clone was further selected to differentiate into cardiomyocytes or pancreatic progenitors. Differentiation was also carried out at CCRM.

[1068] Differentiated cardiomyocytes and pancreatic progenitors were verified at CCRM. Briefly, the differentiated cardiomyocytes were assayed for a cardiac marker. More than 80% of the differentiated iPSCs from different groups (wild type, DKO, and TKO) were positive for cardiac Troponin (cTNT), a cardiac marker. Similarly, after differentiation, more than 80% wild type or TKO cells were observed to express pancreatic and duodenal homeobox 1 (PDX1) and NK6 homeobox 1 (NKX6.1), two key transcription factors that drive the development of beta cells. These data suggest DKO or TKO iPSCs have comparable differentiation capability as wild type iPSCs.

SEQUENCE LISTING

The patent application contains a lengthy sequence listing. A copy of the sequence listing is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20250262302A1). An electronic copy of the sequence listing will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. An engineered human cell, which has reduced or eliminated surface expression of:

I) HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-A gene and a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-C;

II) HLA-A and HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-A gene and a genetic modification in the HLA-B gene, wherein

(i) the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:29942854-chr6:29942913 and chr6:29943518-chr6:29943619, and (b) chr6:29942540-29945459, and

(ii) the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 or (b) chr6:31354497-31357157;

III) HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the cell is homozygous for HLA-A and homozygous for HLA-C: or

IV) HLA-B protein relative to an unmodified cell, comprising a genetic modification in the HLA-B gene, wherein the genetic modification comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31354480-31357174 and (b) chr6:31354497-31357157.

2. (canceled)

3. (canceled)

4. (canceled)

5. The engineered human cell of claim **1**, wherein the cell has reduced or eliminated expression of:

a) at least one HLA-B allele selected from HLA-B7, HLA-B8, HLA-B35, HLA-B40, HLA-B44, HLA-B15, HLA-B14, HLA-B18 and HLA-B51; and/or

b) at least one HLA-A allele selected from: HLA-A1, HLA-A2, HLA-A3, HLA-A11, HLA-A29, HLA-A26, HLA-A33, and HLA-A24.

6. (canceled)

7. The engineered human cell of claim **1**, wherein the genetic modification in the HLA-B gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:31355182-31355596; (b) chr6:31355203-31356461;

(c) chr6:31355182-31355202 chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; chr6:31355414-31355434; and chr6:31355409-31355429;

(d) chr6:31355222-31355246; chr6:31356777-31356801: chr6:31355492-31355516; chr6: 31355379-31355403; chr6:31355491-31355515; chr6:31355361-31355385; chr6:31355356-31355380; chr6:31355460-31355484; chr6:31357078-31357102; chr6:31355417-31355441; chr6:31355366-31355390; chr6:31355415-31355439; chr6:31355378-31355402; chr6:31355166-31355190; chr6:31355401-31355425; ch6:31355469-31355493; chr6:31356262-31356286: chr6:31355419-31355443: chr6:31355390-31355414 chr6:31355369-31355393; chr6:31355221-31355245; chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; chr6:31355441-31355465; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; and chr6:31356767-31356791;

(e) chr6:31355348-31355368, chr6:31355347-31355367, chr6:31355349-31355369, chr6:31355192-31355212, chr6:31355340-31355360, and chr6:31355409-31355429; and

(f) chr6:31355222-31355246; chr6:31355221-31355245: chr6:31355205-31355229; chr6:31355446-31355470; chr6:31356425-31356449; and chr6:31355441-31355465.

8. (canceled)

9. (canceled)

10. (canceled)

11. The engineered human cell of claim **1**, wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from:

a) chr6:29942891-29942915: chr6:29942609-29942633: chr6:29942864-29942884;

chr6:29944266-29944290; chr6:29942889-29942913; chr6:29944471-29944495; and

chr6:29944470-29944494;

b) chr6:29942891-29942915;

c) chr6:29942864-29942884; chr6:29942868-29942888 chr6:29942876-29942896 chr6:29942877-29942897; and chr6:29942883-29942903; and

d) chr6:29942609-29942633.

12. (canceled)

13. (canceled)

14. (canceled)

15. (canceled)

16. (canceled)

17. (canceled)

18. (canceled)

19. The engineered human cell of claim **1**, wherein the HLA-B expression is reduced or eliminated by:

a) a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 or at least 10 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355348-31355368; or chr6:31355347-31355367; chr6:31355182-31355202; chr6:31355348-31355368; chr6:31355180-31355200; chr6:31355145-31355165; chr6:31355349-31355369; chr6:31355157-31355177; chr6:31356381-31356401; chr6:31356380-31356400; chr6:31355204-31355224; chr6:31355205-31355225; chr6:31355185-31355205; chr6:31355191-31355211; chr6:31355192-31355212; chr6:31355190-31355210; chr6:31355193-31355213; chr6:31355198-31355218; chr6:31355320-31355340; chr6:31355319-31355339; chr6:31355178-31355198; chr6:31355347-31355367; chr6:31355432-31355452; chr6:31355340-31355360; chr6:31355576-31355596; chr6:31355410-31355430; chr6:31355419-31355439; and chr6:31355414-31355434; chr6:31355409-31355429; or

b) a gene editing system that binds to an HLA-B genomic target sequence comprising at least 5 or at least 10 contiguous nucleotides within the genomic coordinates chosen from: chr6:31355222-31355246: chr6:31355221-31355245: chr6:31355205-31355229; chr6:31355446-31355470: chr6:31356425-31356449: chr6:31355441-31355465; chr6:31356777-31356801: chr6:31355492-31355516: chr6: 31355379-31355403; chr6:31355491-31355515: chr6:31355361-31355385: chr6:31355356-31355380; chr6:31355460-31355484: chr6:31357078-31357102: chr6:31355417-31355441; chr6:31355366-31355390: chr6:31355415-31355439: chr6:31355378-31355402; chr6:31355166-31355190: chr6:31355401-31355425: ch6:31355469-31355493; chr6:31356262-31356286; chr6:31355419-31355443; chr6:31355390-31355414; chr6:31355369-31355393; chr6:31355203-31355227; chr6:31356437-31356461; chr6:31356426-31356450; chr6:31356763-31356787; chr6:31356764-31356788; chr6:31356762-31356786; chr6:31355204-31355228; chr6:31356436-31356460; and chr6:31356767-31356791.

20. (canceled)

21. The engineered human cell of claim **1**, wherein HLA-A expression is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 or at least 10 contiguous nucleotides within the genomic coordinates chosen from: (a) chr6:29942891-29942915; chr6:29942609-29942633; chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; chr6:29944026-29944046; chr6:29934330-29934350, chr6:29943115-29943135, chr6:29943135-29943155, chr6:29943140-29943160, chr6:29943590-29943610, chr6:29943824-29943844, chr6:29943858-29943878, chr6:29944478-29944498, and chr6:29944850-29944870; and (b) chr6:29942891-29942915 and chr6:29942609-29942633.

22. (canceled)

23. (canceled)

24. The engineered human cell of claim **1**, wherein the cell is homozygous for HLA-C.

25. The engineered human cell of claim **1**, wherein the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:04; HLA-C*04:01; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

26. The engineered human cell of claim **1**, wherein the engineered cell is homozygous for HLA-A, the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02.

27. The engineered human cell of claim **1**, wherein the engineered cell is homozygous for HLA-A and wherein the engineered cell is homozygous for HLA-C, wherein the HLA-A allele is selected from any one of the following HLA-A alleles: HLA-A*02:01; HLA-A*01:01; HLA-A*03:01; HLA-A*11:01; HLA-A*26:01; HLA-A*68:01; HLA-A*29:02; HLA-A*31:01; HLA-A*32:01; HLA-A*30:02; HLA-A*25:01; HLA-A*33:01; HLA-A*02:02; HLA-A*74:01; HLA-A*02:02; HLA-A*29:01; HLA-A*02:03; HLA-A*02:05; HLA-A*24:07; HLA-A*11:02; HLA-A*36:01; HLA-A*02:22; HLA-A*34:02; HLA-A*01:03; HLA-A*24:02; HLA-A*02:07; HLA-A*23:01; HLA-A*30:01; HLA-A*33:03; HLA-A*02:06; HLA-A*34:02; and HLA-A*68:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*08:01; HLA-C*03:02; HLA-C*16:01; HLA-C*15:02; HLA-C*03:04; HLA-C*12:03; HLA-C*02:10; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*17:01; HLA-C*01:02; and HLA-C*02:02.

28. The engineered human cell of claim **1**, wherein the cell has:

a) reduced or eliminated surface expression of MHC class II protein,

b) a genetic modification of a gene selected from CIITA, HLA-DR, HLA-DQ, HLA-DP, RFX5, RFXB/ANK, RFXAP, CREB, NF-YA, NF-YB, and NF-YC;

c) a genetic modification in the CIITA gene;

d) reduced or eliminated surface expression of TRAC protein; and/or

e) reduced or eliminated surface expression of TRBC protein.

29. (canceled)

30. (canceled)

31. (canceled)

32. (canceled)

33. The engineered human cell of claim **1**, wherein the genetic modification comprises:

a) at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates;

b) an indel; and/or

c) at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

34. (canceled)

35. (canceled)

36. A pharmaceutical composition comprising the engineered human cell of claim **1**.

37. A population of cells comprising the engineered human cell of claim **1**.

38. A pharmaceutical composition comprising the population of cells of claim **37**.

39. The population of claim **37**, wherein

a) at least 65%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the population of cells is HLA-A negative or HLA-B negative as measured by flow cytometry;

b) at least 65%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the population of cells comprises the genetic modification in the HLA-A gene or the genetic modification in the HLA-B gene, as measured by next-generation sequencing (NGS);

c) at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the population of cells is CIITA negative as measured by flow cytometry; and/or

d) at least 95%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the population of cells is endogenous TCR protein negative as measured by flow cytometry.

40. (canceled)

41. (canceled)

42. (canceled)

43. (canceled)

44. (canceled)

45. A method of treating a disease or disorder comprising administering the engineered human cell of claim **1** to a subject in need thereof, optionally wherein the disease or disorder is a cancer, an infectious disease, or an autoimmune disease.

46. A composition, comprising:

a) an HLA-B guide RNA; or

b) an HLA-B guide RNA and an HLA-A guide RNA,

wherein the HLA-B guide RNA comprises:

i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91, 101-164, 167-176, and 178-185;

ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185;

iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185;

iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or

v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3; and

wherein the HLA-A guide RNA, if present, comprises:

i. a guide sequence selected from SEQ ID NOs: 576, 571, 301-570, 572-575, and 577-590; or

ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511: or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 429-462 and 512-590; or

iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511: or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 512-590; or

iv. a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or

v. a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Tables 4-7.

47. (canceled)

48. A method of making an engineered human cell, wherein the engineered human cell has reduced or eliminated surface expression of:

I) HLA-B protein relative to an unmodified cell, the method comprising: contacting a cell with a composition comprising an HLA-B guide RNA; or

II) HLA-A and HLA-B protein relative to an unmodified cell, the method comprising: (a) contacting a cell with a first composition comprising an HLA-B guide RNA; and (b) contacting the cell with a second composition comprising an HLA-A guide RNA,

wherein the HLA-B guide RNA comprises:

i. a guide sequence selected from SEQ ID NOs: 165, 166, 177, 13, 74, 1-12, 14-73, 75-91, 101-164, 167-176, and 178-185;

ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-91; or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 101-185;

iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-91; or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 101-185;

iv. a guide sequence that binds a target site comprising a genomic region listed in Table 2 or 3; or

v. a guide sequence that is complementary to at least 17, 18, 19, or 20 contiguous nucleotides of a genomic

region listed in Table 2 or a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Table 3;

wherein the HLA-A guide RNA, if present, comprises:

i. a guide sequence selected from SEQ ID NOs: 576, 571, 301-570, 572-575, and 577-590; or

ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 301-428 and 463-511: or at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 429-462 and 512-590; or

iii. a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 301-428 and 463-511: or a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 512-590; or

iv. a guide sequence that binds a target site comprising a genomic region listed in Tables 4-7; or

v. a guide sequence that is complementary to at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a genomic region listed in Tables 4-7.

49. (canceled)

50. (canceled)

51. (canceled)

52. (canceled)

53. (canceled)

54. The engineered human cell of claim **1**, further comprising an RNA-guided DNA-binding agent or nucleic acid encoding the RNA-guided DNA binding agent, wherein the RNA-guided DNA-binding agent is NmeCas9, and the HLA-B guide RNA comprises: (i) a guide sequence selected from SEQ ID NOs: 165, 166, 163, 164, 169, and 177; or (ii) a guide sequence that is at least 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleotides of a sequence selected from SEQ ID NOs: 165, 166, 163, 164, and 177; or (iii) a guide sequence at least 95%, 90%, 85%, 80%, 75%, or 70% identical to a sequence selected from SEQ ID NOs: 165, 166, 163, 164, and 177.

55. (canceled)

56. (canceled)

57. (canceled)

58. (canceled)

59. (canceled)

60. (canceled)

61. (canceled)

62. (canceled)

63. (canceled)

64. (canceled)

65. (canceled)

66. The engineered human cell of claim **1**, wherein the cell is an allogeneic cell and/or a stem cell.

67. (canceled)

68. The engineered human cell of claim **1**, further comprising:

a) an exogenous nucleic acid encoding a polypeptide, wherein the polypeptide is an antibody or antibody fragment;

b) an exogenous nucleic acid encoding a polypeptide that is secreted by the cell, wherein the secreted polypeptide is an enzyme;

c) an exogenous nucleic acid encoding a polypeptide that is secreted by the cell, wherein the secreted polypeptide is a cytokine;

d) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a T cell receptor (TCR);

e) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a genetically modified TCR;

f) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a WT1 TCR;

g) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a CAR;

h) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a universal CAR; or

i) an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is an anti-CD30 CAR.

69. (canceled)

70. (canceled)

71. (canceled)

72. (canceled)

73. (canceled)

74. (canceled)

75. (canceled)

76. (canceled)

77. (canceled)

78. (canceled)

79. (canceled)

80. (canceled)

81. (canceled)

82. (canceled)

83. (canceled)

84. The engineered human cell of claim **68**, wherein the exogenous nucleic acid is provided to the cell in a vector, optionally wherein the vector is a viral vector.

85. (canceled)

86. (canceled)

87. (canceled)

88. (canceled)

89. (canceled)

90. (canceled)

91. (canceled)

92. (canceled)

93. (canceled)

94. (canceled)

95. (canceled)

96. (canceled)

97. (canceled)

98. (canceled)

99. (canceled)

100. (canceled)

101. (canceled)

102. (canceled)

103. (canceled)

104. (canceled)

105. (canceled)

106. (canceled)

107. (canceled)

108. (canceled)

109. (canceled)

110. (canceled)

111. A cell bank comprising: (a) the engineered human cell of claim **1**; and (b) a catalogue comprising information documenting the HLA-C alleles of the cell in the cell bank.

112. A method of administering an engineered human cell to a recipient subject in need thereof, the method comprising: (a) determining the HLA-C alleles of the recipient subject; (b) selecting the engineered human cell of claim **1**, wherein the engineered human cell is homozygous for one of the HLA-C alleles of the recipient subject; and (c) administering the selected engineered human cell to the recipient subject.

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