United States Patent Application Publication 20250262302
Kind Code A1
Publication Date August 21, 2025
Inventor(s) Schultes; Birgit et al.

Compositions and Methods for Reducing MHC Class I in a Cell

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.

Inventors: **Schultes; Birgit (Arlington, MA), Zhang; Yong (Bedford, MA), Zhang; Boning (Cambridge, MA), Kiliç; Özgün (Cambridge, MA), Balwani; Ishina (Cambridge, MA), Liu; Biao (Cambridge, MA)**

Applicant: **Intellia Therapeutics, Inc.** (Cambridge, MA)

Family ID: **1000008613191**

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

Appl. No.: **18/980462**

Filed: **December 13, 2024**

Related U.S. Application Data

parent US continuation PCT/US23/68498 20230615 PENDING child US 18980462
us-provisional-application US 63494208 20230404
us-provisional-application US 63352991 20220616

Publication Classification

Int. Cl.: **A61K40/22** (20250101); **A61K40/31** (20250101); **C12N9/22** (20060101); **C12N15/11** (20060101); **C12N15/90** (20060101)

U.S. Cl.:

CPC **A61K40/22** (20250101); **A61K40/31** (20250101); **C12N9/226** (20250501); **C12N15/11** (20130101); **C12N15/907** (20130101); C12N2310/20 (20170501)

Background/Summary

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.

Description

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.sup.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.sup.6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O.sup.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., 11.sup.th 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 TABLE-US-00001 (SEQ ID NO: 900) SGSETPGTSESATPES, (SEQ ID NO: 901) SGSETPGTSESA, or (SEQ ID NO: 902) SGSETPGTSESATPEGGSGGS.

[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-US-00002 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* NNNNG[A/C]TT 20 bp *meningitidis* Cas9 nuclease from *Streptococcus* NNAGAAW 20 bp *thermophilus* Cas9 NNG(A/G)(A/G)T 20 bp nuclease is from *Staphylococcus aureus* Cpf1 nuclease TTTN 23 bp from *Francisella novicida* Cpf1 nuclease TTTV 23 bp from *Acidaminococcus* sp. Cpf1 nuclease TTTV 23 bp from *Lachnospiraceae bacterium* 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 SpyCas9 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-US-00003 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 *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.

[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-US-00004 TABLE 2 Exemplary HLA-B Spy guide RNAs SEQ ID Exemplary Exemplary Guide NO to Guide RNA RNA Modified the Full Sequence Sequence Genomic Guide Guide Guide (SEQ ID NOS: (SEQ ID NOs: Coordinates ID Sequence Sequence 1001-1091) 2001-2091) (hg38) G022008 1 CCAGCCUGGACG CCAGCCUGG mC*mC*mA*GCCUG chr6:31355718- CAGGCACC ACGCAGGCA GACGCAGGCACCG 31355738 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022009 2 AGGCGCUUUGCA AGGCGCUUU mA*mG*mG*CGCUU chr6:31355616- UCUCUCAU GCAUCUCUC UGCAUCUCUCAUG 31355636 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022010 3 AACAAUGCCCAC AACAAUGCC mA*mA*mC*AAUGC chr6:31355182- GAUGGGGA CACGAUGGG CCACGAUGGGGAG 31355202 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022011 4 CCAGACACCAGC CCAGACACC mC*mC*mA*GACAC chr6:31355711- CUGGACGC AGCCUGGAC CAGCCUGGACGCG 31355731 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022012 5 CCAUCUCCUAUA CCAUCUCCU mC*mC*mA*UCUCC chr6:31356120- GGUCGCCG AUAGGUCGC UAUAGGUCGCCGG 31356140 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022013 6 CUUCUCUCUAGG CUUCUCUCU mC*mU*mU*CUCUC chr6:31270893- ACAAUUAA AGGACAAUU UAGGACAAUUAAG 31270913 AAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022014 7 CCCCAUCUCCUA CCCCAUCUC mC*mC*mC*CAUCU chr6:31356122- UAGGUCGC CUAUAGGUC CCUAUAGGUCGCG 31356142 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022015 8 GGAUCUCGGACC GGAUCUCGG mG*mG*mA*UCUCG chr6:31354563- CGGAGACU ACCCGGAGA GACCCGGAGACUG 31354583 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022016 9 CUUCCUCCCAGU CUUCCUCCC mC*mU*mU*CCUCC chr6:31354682- CCCCUCAC AGUCCCCUC CAGUCCCCUCACG 31354702 ACGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022017 10 CUCCUGUCCCAC CUCCUGUCC mC*mU*mC*CUGUC chr6:31355900- GUCUCCUG CACGUCUCC CCACGUCUCCUGG 31355920 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022018 11 CCAUUUUCCUCC CCAUUUUCC mC*mC*mA*UUUUC chr6:31356086- UCUUCUCG UCCUCUUCU CUCCUCUUCUCGG 31356106 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022019 12 GGUUCCCAAGGC GGUUCCCAA mG*mG*mU*UCCCA chr6:31356623- UGCUGCAG GGCUGCUGC AGGCUGCUGCAGG 31356643 AGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022020 13 ACAUGCCAUGUA ACAUGCCAU mA*mC*mA*UGCCA chr6:31355348- CAGCAUGA GUACAGCAU UGUACAGCAUGAG 31355368 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022021 14 CGGUUCCCAAGG CGGUUCCCA mC*mG*mG*UUCCC chr6:31355899- CUGCUGCA AGGCUGCUG AAGGCUGCUGCAG 31355919 CAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU

UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022022 15 AACGCGCCUGGG AACGCGCCU mA*mA*mC*GCGCC chr6:31356539- GCUCUCGC
GGGGCUCUC UGGGGCUCUCGCG 31356559 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022023 16 UGGUGGGGGUGG UGGUGGGGG mU*mG*mG*UGGG chr6:31357031- GAGUGUGG
UGGGAGUGU GGUGGGAGUGUGG 31357051 GGGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm
UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU
mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U
mGmCmU*mU*mU* mU G022024 17 GAGUCUCUGAGC GAGUCUCUG mG*mA*mG*UCUCU chr6:31355713- GGGGAACA
AGCGGGGAA GAGCGGGGAACAG 31355733 CAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022025 18 GGGAGGAGCGAG GGGAGGAGC mG*mG*mG*AGGA chr6:31355180- GGGACCGC
GAGGGGACC GCGAGGGGACCGC 31355200 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm
UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU
mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U
mGmCmU*mU*mU* mU G022026 19 UGAGGGGACUGG UGAGGGGAC mU*mG*mA*GGGG chr6:31356601- GAGGAAGC
UGGGAGGAA ACUGGGAGGAAGC 31356621 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm
UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU
mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U
mGmCmU*mU*mU* mU G022027 20 CUGCGUCCAGGC CUGCGUCCA mC*mU*mG*CGUCC chr6:31270992- UGGUGUCU
GGCUGGUGU AGGCUGGUGUCUG 31271012 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022028 21 CCUCGACCGGCG CCUCGACCG mC*mC*mU*CGACC chr6:31356548- AGAGCCCC
GCGAGAGCC GGCGAGAGCCCCG 31356568 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022029 22 AACACGAGGAAA AACACGAGG mA*mA*mC*ACGAG chr6:31354795- GCAAGUGU
AAAGCAAGU GAAAGCAAGUGUG 31354815 GUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022030 23 ACUUUACCUCCA ACUUUACCU mA*mC*mU*UUACC chr6:31355803- CUCAGAUC
CCACUCAGA UCCACUCAGAUCG 31355823 UCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022031 24 UCUGCUCCUGAU UCUGCUCCU mU*mC*mU*GCUCC chr6:31356486- CUGAGUGG
GAUCUGAGU UGAUCUGAGUGGG 31356506 GGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022032 25 AUCUCGGACCCG AUCUCGGAC mA*mU*mC*UCGGA chr6:31354820- GAGACUCG
CCGGAGACU CCCGGAGACUCGG 31354840 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm
GmCmU*mU*mU*m U G022033 26 CCUGGGGCUCUC CCUGGGGCU mC*mC*mU*GGGGC chr6:31356545- GCCGGUCG
CUCGCCGGU UCUCGCCGGUCGG 31356565 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022034 27 CUGGUCACAUGG CUGGUCACA mC*mU*mG*GUCAC chr6:31356501- GUGGUCCU
UGGGUGGUC AUGGGUGGUCCUG 31356521 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022035 28 CUCCUAUAGGUC CUCCUAUAG mC*mU*mC*CUAUA chr6:31356116- GCCGGGGA
GUCGCCGGG GGUCGCCGGGGAG 31356136 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022036 29 GUAAAGUGACUC GUAAAGUGA mG*mU*mA*AAGU chr6:31355816- AGAAGUGC
CUCAGAAGU GACUCAGAAGUGC 31355836 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm
UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU
mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U
mGmCmU*mU*mU* mU G022037 30 ACACAGGGGCUA ACACAGGGG mA*mC*mA*CAGGG chr6:31354983- ACGCAGCC
CUAACGCAG GCUAACGCAGCCG 31355003 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU
UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022038 31 AUACUUCUGGAA AUACUUCUG mA*mU*mA*CUUCU chr6:31354748- AUUCCUUU
GAAAUUCCU GGAAAUUCCUUUG UUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU
mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm
31354768 GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U
GmCmU*mU*mU*m U G022039 32 GUCCUAGCAGUU GUCCUAGCA mG*mU*mC*CUAGC chr6:31355150- GUGGUCAU
GUUGUGGUC AGUUGUGGUCAUG 31355170 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU
UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU

UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022040 33 CCCAUCUCCUAU CCCAUCUCC mC*mC*mC*AUCUC chr6:31356121- AGGUCGCC UAUAGGUCG CUAUAGGUCGCCG 31356141 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022041 34 CGACCUAUAGGA CGACCUAUA mC*mG*mA*CCUAU chr6:31356123- GAUGGGGA GGAGAUGGG AGGAGAUGGGGAG 31356143 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022042 35 GAAGUAUGACUA GAAGUAUGA mG*mA*mA*GUAU chr6:31354761- CAGACCCA CUACAGACC GACUACAGACCCA 31354781 CAGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022043 36 CUCCGAUGACCA CUCCGAUGA mC*mU*mC*CGAUG chr6:31355145- CAACUGCU CCACAACUG ACCACAACUGCUG 31355165 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022044 37 AGUUGUGGUCAU AGUUGUGGU mA*mG*mU*UGUG chr6:31355142- CGGAGCUG CAUCGGAGC GUCAUCGGAGCUG 31355162 UGGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022045 38 UACUUCUGGAAA UACUUCUGG mU*mA*mC*UUCUG chr6:31354747- UUCCUUUU AAAUUCCUU GAAAUUCCUUUUG 31354767 UUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022046 39 CACAUGCCAUGU CACAUGCCA mC*mA*mC*AUGCC chr6:31355349- ACAGCAUG UGUACAGCA AUGUACAGCAUGG 31355369 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022047 40 GGAGCGAGGGGA GGAGCGAGG mG*mG*mA*GCGA chr6:31357027- CCGCAGGC GGACCGCAG GGGGACCGCAGGC 31357047 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022048 41 AGGAAAAGUCAC AGGAAAAGU mA*mG*mG*AAAA chr6:31355888- GGUUCCCA CACGGUUCC GUCACGGUUCCCA 31355908 CAGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022049 42 GAGCCUUCCCCA GAGCCUUCC mG*mA*mG*CCUUC chr6:31356129- UCUCCUAU CCAUCUCCU CCCAUCUCCUAUG 31356149 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022050 43 GACUCCCCAUCC GACUCCCCA mG*mA*mC*UCCCC chr6:31356636- CCCACGUA UCCCCCACG AUCCCCCACGUAG 31356656 UAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022051 44 CCCGGCGACCUA CCCGGCGAC mC*mC*mC*GGCGA chr6:31356118- UAGGAGAU CUAUAGGAG CCUAUAGGAGAUG 31356138 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022052 45 ACGGUUCCCAAG ACGGUUCCC mA*mC*mG*GUUCC chr6:31355898- GCUGCUGC AAGGCUGCU CAAGGCUGCUGCG 31355918 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G022053 46 GGGAGUCGUGAC GGGAGUCGU mG*mG*mG*AGUC chr6:31356649- CUGCGCCC GACCUGCGC GUGACCUGCGCCC 31356669 CCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022054 47 GAGCGAGGGGAC GAGCGAGGG mG*mA*mG*CGAG chr6:31357026- CGCAGGCG GACCGCAGG GGGACCGCAGGCG 31357046 CGGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCAmAmC UAUCAACUU mUmUmGmAmAmA GAAAAAGUG mAmAmGmUmGmG GCACCGAGU mCmAmCmCmGmA CGGUGCUUU mGmUmCmGmGmU U mGmCmU*mU*mU* mU G022055 48 CCUGGCUGUCCU CCUGGCUGU mC*mC*mU*GGCUG chr6:31355157- AGCAGUUG CCUAGCAGU UCCUAGCAGUUGG 31355177 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCAmAmCm UAUCAACUU UmUmGmAmAmAm GAAAAAGUG AmAmGmUmGmGm GCACCGAGU CmAmCmCmGmAm CGGUGCUUU GmUmCmGmGmUm U GmCmU*mU*mU*m U G027488 49 ACAUGCCAUGUA ACAUGCCAU mA*mC*mA*UGCCA chr6:31355348- CAGCAUGA GUACAGCAU UGUACAGCAUGAG 31355368 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027489 50 AACAAUGCCCAC AACAAUGCC mA*mA*mC*AAUGC chr6:31355182- GAUGGGGA CACGAUGGG CCACGAUGGGGAG 31355202 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027490 51 CUCCGAUGACCA CUCCGAUGA mC*mU*mC*CGAUG chr6:31355145- CAACUGCU CCACAACUG ACCACAACUGCUG

31355165 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027491 52 CACAUGCCAUGU CACAUGCCA mC*mA*mC*AUGCC chr6:31355349-ACAGCAUG UGUACAGCA AUGUACAGCAUGG 31355369 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027973 53 CCGGACGGGCGC CCGGACGGG mC*mC*mG*GACGG chr6:31356381- CUCCUCCG CGCCUCCUC GCGCCUCCUCCGG 31356401 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027974 54 CGGACGGGCGCC CGGACGGGC mC*mG*mG*ACGGG chr6:31356380- UCCUCCGC GCCUCCUCC CGCCUCCUCCGCG 31356400 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027975 55 GAGCCGUCUUCC GUGGACUGG mG*mU*mG*GACU chr6:31355204- CAGUCCAC GAAGACGGC GGGAAGACGGCUC 31355224 UCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G027976 56 AGAGCCGUCUUC UGGACUGGG mU*mG*mG*ACUG chr6:31355205- CCAGUCCA AAGACGGCU GGAAGACGGCUCU 31355225 CUGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G027977 57 GUACGGCUGCGA GUACGGCUG mG*mU*mA*CGGCU chr6:31356400- CGUGGGGC CGACGUGGG GCGACGUGGGGCG 31356420 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027978 58 CCGUCCCCAUCG AAUGCCCAC mA*mA*mU*GCCCA chr6:31355185- UGGGCAUU GAUGGGGAC CGAUGGGGACGGG 31355205 GGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027979 59 UCGCUGCUGUGA UCGCUGCUG mU*mC*mG*CUGCU chr6:31355119- UGUGUAGG UGAUGUGUA GUGAUGUGUAGGG 31355139 GGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027980 60 UGGUCGCUGCUG UGGUCGCUG mU*mG*mG*UCGCU chr6:31355122- UGAUGUGU CUGUGAUGU GCUGUGAUGUGUG 31355142 GUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027981 61 CCAGGCAGCGAC CCAGGCAGC mC*mC*mA*GGCAG chr6:31354510- AGUGCCCA GACAGUGCC CGACAGUGCCCAG 31354530 CAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027982 62 GGCAGCGACAGU CCCUGGGCA mC*mC*mC*UGGGC chr6:31354507-GCCCAGGG CUGUCGCUG ACUGUCGCUGCCG 31354527 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027983 63 UCCAGGCAGCGA UCCAGGCAG mU*mC*mC*AGGCA chr6:31354511- CAGUGCCC CGACAGUGC GCGACAGUGCCCG 31354531 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027984 64 AGUCCACCGUCC CACGAUGGG mC*mA*mC*GAUGG chr6:31355191- CCAUCGUG GACGGUGGA GGACGGUGGACUG 31355211 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027985 65 CAGUCCACCGUC CAGUCCACC mC*mA*mG*UCCAC chr6:31355192- CCCAUCGU GUCCCCAUC CGUCCCCAUCGUG 31355212 GUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027986 66 GUCCACCGUCCC CCACGAUGG mC*mC*mA*CGAUG chr6:31355190- CAUCGUGG GGACGGUGG GGGACGGUGGACG 31355210 ACGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027987 67 CCAGUCCACCGU CCAGUCCAC mC*mC*mA*GUCCA chr6:31355193- CCCCAUCG CGUCCCCAU CCGUCCCCAUCGG 31355213 CGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027988 68 UCUUCCCAGUCC GGGACGGUG mG*mG*mG*ACGG chr6:31355198- ACCGUCCC GACUGGGAA UGGACUGGGAAGA 31355218 GAGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G027989 69 CGAAGCCCCUCA CGAAGCCCC mC*mG*mA*AGCCC chr6:31355320- CCCUGAGA UCACCCUGA CUCACCCUGAGAG 31355340 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027990 70 GAAGCCCCUCAC GAAGCCCCU mG*mA*mA*GCCCC chr6:31355319- CCUGAGAU CACCCUGAG UCACCCUGAGAUG 31355339 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027991 71 UCCCCAUCGUGG AACAAUGCC mA*mA*mC*AAUGC chr6:31355182- GCAUUGUU CACGAUGGG CCACGAUGGGGAG 31355202 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027992 72 AGUUGUGGUCAU AGUUGUGGU mA*mG*mU*UGUG chr6:31355142- CGGAGCUG CAUCGGAGC GUCAUCGGAGCUG 31355162 UGGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G027993 73 CAUCGUGGGCAU CAGCAACAA mC*mA*mG*CAACA chr6:31355178- UGUUGCUG UGCCCACGA

AUGCCCACGAUGG 31355198 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027994 74 CAUGCCAUGUAC CAUGCCAUG mC*mA*mU*GCCAU chr6:31355347- AGCAUGAG UACAGCAUG GUACAGCAUGAGG 31355367 AGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027995 75 GGCUGUCCUAGC CCACAACUG mC*mC*mA*CAACU chr6:31355154- AGUUGUGG CUAGGACAG GCUAGGACAGCCG 31355174 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027996 76 AUCGUGGGCAUU CCAGCAACA mC*mC*mA*GCAAC chr6:31355177- GUUGCUGG AUGCCCACG AAUGCCCACGAUG 31355197 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027997 77 CCCAUCGUGGGC CCCAUCGUG mC*mC*mC*AUCGU chr6:31355180- AUUGUUGC GGCAUUGUU GGGCAUUGUUGCG 31355200 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027998 78 CCUGGCUGUCCU CCUGGCUGU mC*mC*mU*GGCUG chr6:31355157- AGCAGUUG CCUAGCAGU UCCUAGCAGUUGG 31355177 UGGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G027999 79 CGUGGGCAUUGU CGUGGGCAU mC*mG*mU*GGGCA chr6:31355175- UGCUGGCC UGUUGCUGG UUGUUGCUGGCCG 31355195 CCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028000 80 AGCAGUUGUGGU CUCCGAUGA mC*mU*mC*CGAUG chr6:31355145- CAUCGGAG CCACAACUG ACCACAACUGCUG 31355165 CUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028001 81 CUUGUGGAGACC CUUGUGGAG mC*mU*mU*GUGG chr6:31355432- AGACCAGC ACCAGACCA AGACCAGACCAGC 31355452 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G028002 82 UGUACAGCAUGA GCAGCCCCU mG*mC*mA*GCCCC chr6:31355340- GGGGCUGC CAUGCUGUA UCAUGCUGUACAG 31355360 CAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028003 83 UCGUGGGCAUUG GCCAGCAAC mG*mC*mC*AGCAA chr6:31355176- UUGCUGGC AAUGCCCAC CAAUGCCCACGAG 31355196 GAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028004 84 GUCCUAGCAGUU GUCCUAGCA mG*mU*mC*CUAGC chr6:31355150- GUGGUCAU GUUGUGGUC AGUUGUGGUCAUG 31355170 AUGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028005 85 GUGUCUCUCACA GUGUCUCUC mG*mU*mG*UCUCU chr6:31354480- GCUUGAAA ACAGCUUGA CACAGCUUGAAAG 31354500 AAGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028006 86 UCAGACCCCCCA UGUGUCUUU mU*mG*mU*GUCU chr6:31355576- AAGACACA GGGGGGUCU UUGGGGGGUCUGA 31355596 GAGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G028007 87 CUCCUCAGACGC CUCCUCAGA mC*mU*mC*CUCAG chr6:31357154- CGAGAUGC CGCCGAGAU ACGCCGAGAUGCG 31357174 GCGUUUUAG UUUUAGAmGmCmU AGCUAGAAA mAmGmAmAmAmU UAGCAAGUU mAmGmCAAGUUAA AAAAUAAGG AAUAAGGCUAGUC CUAGUCCGU CGUUAUCACGAAA UAUCACGAA GGGCACCGAGUCG AGGGCACCG GmU*mG*mC*mU AGUCGGUGC U G028008 88 GAGAUAGAACCU GAGAUAGAA mG*mA*mG*AUAG chr6:31355410- UCCAGAAG CCUUCCAGA AACCUUCCAGAAG 31355430 AGGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G028009 89 GACCAGCAGGAG GGUUCUAUC mG*mG*mU*UCUA chr6:31355419- AUAGAACC UCCUGCUGG UCUCCUGCUGGUC 31355439 UCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G028010 90 GCAGGAGAUAGA UGGAAGGUU mU*mG*mG*AAGG chr6:31355414- ACCUUCCA CUAUCUCCU UUCUAUCUCCUGC 31355434 GCGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U G028011 91 AGAUAGAACCUU AGAUAGAAC mA*mG*mA*UAGA chr6:31355409- CCAGAAGU CUUCCAGAA ACCUUCCAGAAGU 31355429 GUGUUUUAG GUUUUAGAmGmCm AGCUAGAAA UmAmGmAmAmAm UAGCAAGUU UmAmGmCAAGUUA AAAAUAAGG AAAUAAGGCUAGU CUAGUCCGU CCGUUAUCACGAA UAUCACGAA AGGGCACCGAGUC AGGGCACCG GGmU*mG*mC*mU AGUCGGUGC U

TABLE-US-00005 TABLE 3 Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs SEQ ID Exemplary Exemplary Guide NO to Guide RNA Full RNA Modified the Sequence Sequence Genomic Guide (SEQ ID NOs: (SEQ ID NOs: Coordinates Guide ID Sequence Guide Sequence 1101-1185) 2101-2185) (hg38) G028789 101 CUUCUGGAG CUUCUGGAG mC*mU*mU*mCmUG chr6: 31355361- AAGAGCAGA AAGAGCAGA GmAmGAmAGmAGC 31355385 GAUACA GAUACAGUU AGmAGAUmACAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028790 102 CACAUGCCAU CACAUGCCAU mC*mA*mC*mAmUG chr6: 31355339- GUACAGCAU GUACAGCAU CmCmAUmGUmACA 31355363 GAGGG GAGGGGUUG GCmAUGAmGGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm

UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028791 103 CACUGACCUG
CACUGACCUG mC*mA*mC*mUmGA chr6: 31355469- GCAGCGGGA GCAGCGGGA CmCmUGmGCmAGC 31355493 UGGCG
UGGCGGUUG GGmGAUGmGCGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA
CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm
UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028792 104 CCAUGUACA
CCAUGUACA mC*mC*mA*mUmGU chr6: 31355333- GCAUGAGGG GCAUGAGGG AmCmAGmCAmUGA 31355357 GCUGCC
GCUGCCGUU GGmGGCUmGCCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA
CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028793 105
CUUACACGCA CUUACACGCA mC*mU*mU*mAmCA chr6: 31354627- GCCUGAGAG GCCUGAGAG CmGmCAmGCmCUG
31354651 UAGCU UAGCUGUUG AGmAGUAmGCUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028794 106
AGCUGCCCAC AGCUGCCCAC mA*mG*mC*mUmGC chr6: 31355401- UUCUGGAAG UUCUGGAAG CmCmACmUUmCUG
31355425 GUUCU GUUCUGUUG GAmAGGUmUCUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028795 107
CUUCCAGAA CUUCCAGAA mC*mU*mU*mCmCA chr6: 31355390- GUGGGCAGC GUGGGCAGC GmAmAGmUGmGGC 31355414
UGUGGU UGUGGUGUU AGmCUGUmGGUmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028796 108
CCAUCUCAGG CCAUCUCAGG mC*mC*mA*mUmCU chr6: 31355317- GUGAGGGGC GUGAGGGGC CmAmGGmGUmGAG
31355341 UUCGG UUCGGGUUG GGmGCUUmCGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028797 109
CAUCUCAGG CAUCUCAGG mC*mA*mU*mCmUC chr6: 31355318- GUGAGGGGC GUGAGGGGC AmGmGGmUGmAGG 31355342
UUCGGC UUCGGCGUU GGmCUUCmGGCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028798 110
CAGCAUGAG CAGCAUGAG mC*mA*mG*mCmAU chr6: 31355326- GGGCUGCCG GGGCUGCCG GmAmGGmGGmCUG 31355350
AAGCCC AAGCCCGUU CCmGAAGmCCCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
CmCGUUmGmCUAm GCUACAAUA CAAU*AAGmGmCC AGGCCGUCG mGmUmCmGmAmA AAAGAUGUG mAmGmAmUGUGCm
CCGCAACGCU CGmCAAmCGCUCU CUGCCUUCUG mGmCCmUmUmCmU GCAUCGUU GGCAUCG*mU*mU G028799 111
CCCAUCUCAG CCCAUCUCAG mC*mC*mC*mAmUC chr6: 31355316- GGUGAGGGG GGUGAGGGG UmCmAGmGGmUGA
31355340 CUUCG CUUCGGUUG GGmGGCUmUCGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028800 112
AUGUACAGC AUGUACAGC mA*mU*mG*mUmAC chr6: 31355331- AUGAGGGGC AUGAGGGGC AmGmCAmUGmAGG 31355355
UGCCGA UGCCGAGUU GGmCUGCmCGAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028801 113
CAUGUACAG CAUGUACAG mC*mA*mU*mGmUA chr6: 31355332- CAUGAGGGG CAUGAGGGG CmAmGCmAUmGAG 31355356
CUGCCG CUGCCGGUU GGmGCUGmCCGmG GUAGCUCCCU GAAACCGUU UUGmUmAmGmCUC GCUACAAUA
CCmUmGmAmAmAm AGGCCGUCG CmCGUUmGmCUAm AAAGAUGUG CAAU*AAGmGmCC CCGCAACGCU
mGmUmCmGmAmA CUGCCUUCUG mAmGmAmUGUGCm GCAUCGUU CGmCAAmCGCUCU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028802 114 AGAUCACCCA AGAUCACCCA mA*mG*mA*mUmCA chr6: 31356262- GCGCAAGUG
GCGCAAGUG CmCmCAmGCmGCA 31356286 GGAGG GGAGGGUUG AGmUGGGmAGGmG UAGCUCCCUG UUGmUmAmGmCUC
AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC
mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028803 115 CAGAUCACCC CAGAUCACCC mC*mA*mG*mAmUC chr6: 31356263- AGCGCAAGU
AGCGCAAGU AmCmCCmAGmCGC 31356287 GGGAG GGGAGGUUG AAmGUGGmGAGmG UAGCUCCCUG UUGmUmAmGmCUC
AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC
mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028804 116 GCCUCCCACU GCCUCCCACU mG*mC*mC*mUmCC chr6: 31356267- UGCGCUGGG
UGCGCUGGG CmAmCUmUGmCGC 31356291 UGAUC UGAUCGUUG UGmGGUGmAUCmG UAGCUCCCUG UUGmUmAmGmCUC
AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC
mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028805 117 CCCAAUACUC CCCAAUACUC mC*mC*mC*mAmAU chr6: 31356777- CGGCCCCUCC
CGGCCCCUCC AmCmUCmCGmGCC 31356801 UGCU UGCUGUUGU CCmUCCUmGCUmG AGCUCCCUGA UUGmUmAmGmCUC
AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC
mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028806 118 CACUCACCGG CACUCACCGG mC*mA*mC*mUmCA chr6: 31357078- CCCAGGUCUC
CCCAGGUCUC CmCmGGmCCmCAG 31357102 GGUC GGUCGUUGU GUmCUCGmGUCmG AGCUCCCUGA UUGmUmAmGmCUC
AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC
mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028807 119 UAGAGCAGG UAGAGCAGG mU*mA*mG*mAmGC chr6: 31356774- AGGGGCCGG
AGGGGCCGG AmGmGAmGGmGGC 31356798 AGUAUU AGUAUUGUU CGmGAGUmAUUmG GUAGCUCCCU
UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC
AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028808 120 CCGGCCCAGG CCGGCCCAGG mC*mC*mG*mGmCC chr6: 31357084-
UCUCGGUCA UCUCGGUCA CmAmGGmUCmUCG 31357108 GGGCC GGGCCGUUG GUmCAGGmGCCmG UAGCUCCCUG
UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC
AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU

mGmCCmUmUmCmU GGCAUCG*mU*mU G028809 121 ACCACUUACA ACCACUUACA mA*mC*mC*mAmCU chr6: 31354623-
CGCAGCCUGA CGCAGCCUGA UmAmCAmCGmCAG 31354647 GAGU GAGUGUUGU CCmUGAGmAGUmG AGCUCCCUGA
UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC
AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028810 122 CCACUUACAC CCACUUACAC mC*mC*mA*mCmUU chr6: 31354624-
GCAGCCUGA GCAGCCUGA AmCmACmGCmAGC 31354648 GAGUA GAGUAGUUG CUmGAGAmGUAmG UAGCUCCCUG
UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC
AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028811 123 UAUCUGCGG UAUCUGCGG mU*mA*mU*mCmUG chr6: 31356201-
AGCCACUCCA AGCCACUCCA CmGmGAmGCmCAC 31356225 CGCAC CGCACGUUG UCmCACGmCACmG UAGCUCCCUG
UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC
AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028812 124 AGCCUGAGA AGCCUGAGA mA*mG*mC*mCmUG chr6: 31354636-
GUAGCUCCCU GUAGCUCCCU AmGmAGmUAmGCU 31354660 CCUUU CCUUUGUUG CCmCUCCmUUUmG UAGCUCCCUG
UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC
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
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 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 AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028828 140
GGCUCCCACU GGCUCCCACU mG*mG*mC*mUmCC chr6: 31356928- CCAUGAGGU CCAUGAGGU CmAmCUmCCmAUG
31356952 AUUUC AUUUCGUUG AGmGUAUmUUCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028829 141
UCUUCUCCAG UCUUCUCCAG mU*mC*mU*mUmCU chr6: 31355378- AAGGCACCAC AAGGCACCAC CmCmAGmAAmGGC
31355402 CACA CACAGUUGU ACmCACCmACAmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028830 142
AGCAUGAGG AGCAUGAGG mA*mG*mC*mAmUG chr6: 31355325- GGCUGCCGA GGCUGCCGA AmGmGGmGCmUGC 31355349
AGCCCC AGCCCCGUUG CGmAAGCmCCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028831 143
AGACCAGACC AGACCAGACC mA*mG*mA*mCmCA chr6: 31355415- AGCAGGAGA AGCAGGAGA GmAmCCmAGmCAG
31355439 UAGAA UAGAAGUUG GAmGAUAmGAAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028832 144
AAGGUUCUA AAGGUUCUA mA*mA*mG*mGmU chr6: 31355417- UCUCCUGCUG UCUCCUGCUG UCmUmAUmCUmCC
31355441 GUCUG GUCUGGUUG UGCmUGGUmCUGm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028833 145
GUGGAGACC GUGGAGACC mG*mU*mG*mGmA chr6: 31355419- AGACCAGCA AGACCAGCA GAmCmCAmGAmCC 31355443
GGAGAU GGAGAUGUU AGCmAGGAmGAUm GUAGCUCCCU GUUGmUmAmGmCU GAAACCGUU CCCmUmGmAmAmA
GCUACAAUA mCmCGUUmGmCUA AGGCCGUCG mCAAU*AAGmGmC AAAGAUGUG CmGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CGmCAAmCGCUCU CUGCCUUCUG mGmCCmUmUmCmU GCAUCGUU GGCAUCG*mU*mU G028834 146
GCAAGGAUU GCAAGGAUU mG*mC*mA*mAmGG chr6: 31356325- ACAUCGCCCU ACAUCGCCCU AmUmUAmCAmUCG
31356349 GAACG GAACGGUUG CCmCUGAmACGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028835 147
ACCAUGAGG ACCAUGAGG mA*mC*mC*mAmUG chr6: 31355523- CCACCCUGAG CCACCCUGAG AmGmGCmCAmCCC
31355547 GUGCU GUGCUGUUG UGmAGGUmGCUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028836 148
GACCAUGAG GACCAUGAG mG*mA*mC*mCmAU chr6: 31355524- GCCACCCUGA GCCACCCUGA GmAmGGmCCmACC
31355548 GGUGC GGUGCGUUG CUmGAGGmUGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028837 149
ACCACCCCAU ACCACCCCAU mA*mC*mC*mAmCC chr6: 31355538- CUCUGACCAU CUCUGACCAU CmCmAUmCUmCUG
31355562 GAGG GAGGGUUGU ACmCAUGmAGGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028838 150
CACCACCCCA CACCACCCCA mC*mA*mC*mCmAC chr6: 31355539- UCUCUGACCA UCUCUGACCA CmCmCAmUCmUCU
31355563 UGAG UGAGGUUGU GAmCCAUmGAGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028839 151
AUGCCCGCGG AUGCCCGCGG mA*mU*mG*mCmCC chr6: 31356374- AGGAGGCGC AGGAGGCGC GmCmGGmAGmGAG
31356398 CCGUC CCGUCGUUG GCmGCCCmGUCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028840 152
GAGGCGCCCG GAGGCGCCCG mG*mA*mG*mGmCG chr6: 31356386- UCCGGCCCCA UCCGGCCCCA CmCmCGmUCmCGG
31356410 CGUC CGUCGUUGU CCmCCACmGUCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028841 153
GCUGCGACG GCUGCGACG mG*mC*mU*mGmCG chr6: 31356385- UGGGGCCGG UGGGGCCGG AmCmGUmGGmGGC 31356409
ACGGGC ACGGGCGUU CGmGACGmGGCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028842 154
GCGACGUGG GCGACGUGG mG*mC*mG*mAmCG chr6: 31356382- GGCCGGACG GGCCGGACG UmGmGGmGCmCGG 31356406
GGCGCC GGCGCCGUU ACmGGGCmGCCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028843 155
CGUGAGGUU CGUGAGGUU mC*mG*mU*mGmAG chr6: 31356830- CGACAGCGAC CGACAGCGAC GmUmUCmGAmCAG
31356854 GCCGC GCCGCGUUG CGmACGCmCGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC 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 GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm CGmCAAmCGCUCU UGCCUUCUG mGmCCmUmUmCmU GCAUCGUU GGCAUCG*mU*mU G032790 161
CUCACCGGCC CUCACCGGCC mC*mU*mC*mAmCC chr6: 31356682- UCGCUCUGG UCGCUCUGG GmGmCCmUCmGCU
31356706 UUGUA UUGUAGUUG CUmGGUUmGUAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032791 162
CUGCGCGGCU CUGCGCGGCU mC*mU*mG*mCmGC chr6: 31356694- ACUACAACCA ACUACAACCA GmGmCUmACmUAC
31356718 GAGC GAGCGUUGU AAmCCAGmAGCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032792 163
CAAACUCAG CAAACUCAG mC*mA*mA*mAmCU chr6: 31355446- GACACUGAG GACACUGAG CmAmGGmACmACU 31355470
CUUGUG CUUGUGGUU GAmGCUUmGUGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032793 164
UCAGGACAC UCAGGACAC mU*mC*mA*mGmGA chr6: 31355441- UGAGCUUGU UGAGCUUGU CmAmCUmGAmGCU 31355465
GGAGAC GGAGACGUU UGmUGGAmGACmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032794 165
UCUGGGAAA UCUGGGAAA mU*mC*mU*mGmGG chr6: 31355222- GGAGGGGAA GGAGGGGAA AmAmAGmGAmGGG 31355246
GAUGAG GAUGAGGUU GAmAGAUmGAGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032795 166
CUCUGGGAA CUCUGGGAA mC*mU*mC*mUmGG chr6: 31355221- AGGAGGGGA AGGAGGGGA GmAmAAmGGmAGG 31355245
AGAUGA AGAUGAGUU GGmAAGAmUGAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032796 167
ACCCAGUUCG ACCCAGUUCG mA*mC*mC*mCmAG chr6: 31356844- UGAGGUUCG UGAGGUUCG UmUmCGmUGmAGG
31356868 ACAGC ACAGCGUUG UUmCGACmAGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032797 168
CGCUGCCUGG CGCUGCCUGG mC*mG*mC*mUmGC chr6: 31354520- AGUAGAACA AGUAGAACA CmUmGGmAGmUAG
31354544 AAAAC AAAACGUUG AAmCAAAmAACmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032798 169
CUGGAGGGU CUGGAGGGU mC*mU*mG*mGmAG chr6: 31356425- GUGAGACCC GUGAGACCC GmGmUGmUGmAGA 31356449
UGGCCC UGGCCCGUU CCmCUGGmCCCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032799 170
GCCCAGGUCU GCCCAGGUCU mG*mC*mC*mCmAG chr6: 31357087- CGGUCAGGG CGGUCAGGG GmUmCUmCGmGUC
31357111 CCAGG CCAGGGUUG AGmGGCCmAGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032800 171
CUGUGUGUU CUGUGUGUU mC*mU*mG*mUmGU chr6: 31356763- CCGGUCCCAA CCGGUCCCAA GmUmUCmCGmGUC
31356787 UACUC UACUCGUUG CCmAAUAmCUCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032801 172
UCUGUGUGU UCUGUGUGU mU*mC*mU*mGmUG chr6: 31356762- UCCGGUCCCA UCCGGUCCCA UmGmUUmCCmGGU
31356786 AUACU AUACUGUUG CCmCAAUmACUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032802 173
UGUGUGUUC UGUGUGUUC mU*mG*mU*mGmU chr6: 31356764- CGGUCCCAAU CGGUCCCAAU GUmUmCCmGGmUC
31356788 ACUCC ACUCCGUUG CCAmAUACmUCCm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032803 174
CGAACCGUCC CGAACCGUCC mC*mG*mA*mAmCC chr6: 31357116- UCCUGCUGCU UCCUGCUGCU GmUmCCmUCmCUG
31357140 CUCG CUCGGUUGU CUmGCUCmUCGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032804 175
UCUGGAGGG UCUGGAGGG mU*mC*mU*mGmGA chr6: 31356424- UGUGAGACC UGUGAGACC GmGmGUmGUmGAG 31356448
CUGGCC CUGGCCGUU ACmCCUGmGCCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm

GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CGmCAAmCGCUCU CUGCCUUCUG mGmCCmUmUmCmU GCAUCGUU GGCAUCG*mU*mU G032805 176
CCCAGAGCCG CCCAGAGCCG mC*mC*mC*mAmGA chr6: 31355204- UCUUCCCAGU UCUUCCCAGU GmCmCGmUCmUUC
31355228 CCAC CCACGUUGU CCmAGUCmCACmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032806 177
UCCCAGAGCC UCCCAGAGCC mU*mC*mC*mCmAG chr6: 31355205- GUCUUCCCAG GUCUUCCCAG AmGmCCmGUmCUU
31355229 UCCA UCCAGUUGU CCmCAGUmCCAmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032807 178
CCAGAGCCGU CCAGAGCCGU mC*mC*mA*mGmAG chr6: 31355203- CUUCCCAGUC CUUCCCAGUC CmCmGUmCUmUCC
31355227 CACC CACCGUUGU CAmGUCCmACCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032808 179
GUGUUCCGG GUGUUCCGG mG*mU*mG*mUmUC chr6: 31356767- UCCCAAUACU UCCCAAUACU CmGmGUmCCmCAA
31356791 CCGGC CCGGCGUUG UAmCUCCmGGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032809 180
UGGAGGGUG UGGAGGGUG mU*mG*mG*mAmG chr6: 31356426- UGAGACCCU UGAGACCCU GGmUmGUmGAmGA 31356450
GGCCCC GGCCCCGUUG CCCmUGGCmCCCm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G032810 181
CCGGCCCCUC CCGGCCCCUC mC*mC*mG*mGmCC chr6: 31356786- CUGCUCUAUC CUGCUCUAUC CmCmUCmCUmGCU
31356810 CACG CACGGUUGU CUmAUCCmACGmG AGCUCCCUGA UUGmUmAmGmCUC 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-US-00006 TABLE 3A Additional Exemplary *N. meningitidis* (Nme) HLA-B guide RNAs Exemplary Guide RNA Modified Sequence Guide ID Guide Sequence (SEQ ID NOs: 2186-2191) G034206 CAAACUCAG mC*mA*mA*mAmCUCmAmGGmACmACUGAmGCUUmGUGmGUUGm GACACUGAG UmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAGm CUUGUG (SEQ GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm ID NO: 163) GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2186) G034207 UCAGGACAC mU*mC*mA*mGmGACmAmCUmGAmGCUUGmUGGAmGACmGUUGm UGAGCUUGU UmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAGm GGAGAC (SEQ GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm ID NO: 164) GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2187) G034208 UCUGGGAAA mU*mC*mU*mGmGGAmAmAGmGAmGGGGAmAGAUmGAGmGUUG GGAGGGGAA mUmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAG GAUGAG (SEQ mGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCU ID NO: 165) mGmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2188) G034209 CUCUGGGAA mC*mU*mC*mUmGGGmAmAAmGGmAGGGGmAAGAmUGAmGUUGm AGGAGGGGA UmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAGm AGAUGA (SEQ GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm ID NO: 166) GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2189) G034210 CUGGAGGGU mC*mU*mG*mGmAGGmGmUGmUGmAGACCmCUGGmCCCmGUUGm GUGAGACCC UmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAGm UGGCCC (SEQ GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm ID NO: 169) GmCCmUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2190) G034211 UCCCAGAGCC mU*mC*mC*mCmAGAmGmCCmGUmCUUCCmCAGUmCCAmGUUGm GUCUUCCCAG UmAmGmCUCCCmUmGmAmAmAmAmCmCGUUmGmCUAmCAAUAAGm UCCA (SEQ ID GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUm NO: 177) 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-US-00007 TABLE 4 Exemplary Spy HLA-A guide RNAs Exemplary Mod Sequence (four terminal U SEQ ID Exemplary residues are optional NO to the Full Sequence and may include 0, 1, Genomic Guide (SEQ ID NOS: 2, 3, 4, or more Us) Coordinates Guide ID Sequence Guide Sequence 1301-1428) (SEQ ID NOs: 2301-2428) (hg38) G018983 301 UGGAGGGCC UGGAGGGCC mU*mG*mG*AGG chr6: 29945290- UGAUGUGUG UGAUGUGUG GCCUGAUGUGUG 29945310 UU UUGUUUUAG UUGUUUUAGAmG (mismatch to AGCUAGAAA mCmUmAmGmAm hg38 = 2) UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018984 302 GCCUGAUGU GCCUGAUGU mG*mC*mC*UGAU chr6: 29945296- GUGUUGGGU GUGUUGGGU GUGUGUUGGGUG 29945316 GU GUGUUUUAG UGUUUUAGAmGm (mismatch to AGCUAGAAA CmUmAmGmAmA hg38 = 2) UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018985 303 CCUGAUGUG CCUGAUGUG mC*mC*mU*GAUG chr6: 29945297- UGUUGGGUG UGUUGGGUG UGUGUUGGGUGU 29945317 UU UUGUUUUAG UGUUUUAGAmGm (mismatch to AGCUAGAAA CmUmAmGmAmA hg38 = 1) UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018986 304 CCCAACACCC CCCAACACCC mC*mC*mC*AACA chr6: 29945300- AACACACAUC AACACACAUC CCCAACACACAU 29945320 GUUUUAGAG CGUUUUAGAmGm (mismatch to CUAGAAAUA CmUmAmGmAmA hg38 = 1) GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018965 305 UCAGGAAAC UCAGGAAAC mU*mC*mA*GGA chr6: 29890117- AUGAAGAAA AUGAAGAAA AACAUGAAGAAA GC GCGUUUUAG GCGUUUUAGAmG AGCUAGAAA mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU UAUCAACUU AUCAmAmCmUmU 29890137 GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019018 306 AGGCGCCUG AGGCGCCUG mA*mG*mG*CGCC chr6: 29927058- GGCCUCUCCC GGCCUCUCCC UGGGCCUCUCCC 29927078 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018937 307 CGGGCUGGCC CGGGCUGGCC mC*mG*mG*GCUG chr6: 29934330- UCCCACAAGG UCCCACAAGG GCCUCCCACAAG 29934350 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA UCAACUUGA UCAmAmCmUmU AAAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018990 308 ACGGCCAUCC ACGGCCAUCC mA*mC*mG*GCCA chr6: 29942541- UCGGCGUCU UCGGCGUCU UCCUCGGCGUCU 29942561 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018991 309 GACGGCCAUC GACGGCCAUC mG*mA*mC*GGCC chr6: 29942542- CUCGGCGUCU CUCGGCGUCU AUCCUCGGCGUC 29942562 GUUUUAGAG UGUUUUAGAmGm CUAGAAAUA CmUmAmGmAmA GCAAGUUAA

mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA UCAACUUGA UCAmAmCmUmU
AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU
mGmCmU*mU*mU *mU G018992 310 GACGCCGAG GACGCCGAG mG*mA*mC*GCCG chr6: 29942543- GAUGGCCGU
GAUGGCCGU AGGAUGGCCGUC 29942563 CA CAGUUUUAG AGUUUUAGAmGm AGCUAGAAA CmUmAmGmAmA
UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA UAUCAACUU
UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm
GmUmCmGmGmU mGmCmU*mU*mU *mU G018993 311 UGACGGCCA UGACGGCCA mU*mG*mA*CGGC chr6: 29942543-
UCCUCGGCGU UCCUCGGCGU CAUCCUCGGCGU 29942563 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAm UmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018994 312 GGCGCCAUG GGCGCCAUG mG*mG*mC*GCCA
chr6: 29942550- ACGGCCAUCC ACGGCCAUCC UGACGGCCAUCC 29942570 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018995 313 ACAGCGACGC ACAGCGACGC mA*mC*mA*GCGA
chr6: 29942864- CGCGAGCCAG CGCGAGCCAG CGCCGCGAGCCA 29942884 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018996 314 CGACGCCGCG CGACGCCGCG mC*mG*mA*CGCC
chr6: 29942868- AGCCAGAGG AGCCAGAGG GCGAGCCAGAGG 29942888 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018997 315 CGAGCCAGA CGAGCCAGA mC*mG*mA*GCCA
chr6: 29942876- GGAUGGAGC GGAUGGAGC GAGGAUGGAGCC 29942896 CG CGGUUUUAG GGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018998 316 CGGCUCCAUC CGGCUCCAUC mC*mG*mG*CUCC
chr6: 29942876- CUCUGGCUCG CUCUGGCUCG AUCCUCUGGCUC 29942896 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018999 317 GAGCCAGAG GAGCCAGAG mG*mA*mG*CCAG
chr6: 29942877- GAUGGAGCC GAUGGAGCC AGGAUGGAGCCG 29942897 GC GCGUUUUAG CGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019000 318 GCGCCCGCGG GCGCCCGCGG mG*mC*mG*CCCG
chr6: 29942883- CUCCAUCCUC CUCCAUCCUC CGGCUCCAUCCU 29942903 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019001 319 GCCCGUCCGU GCCCGUCCGU mG*mC*mC*CGUC
chr6: 29943062- GGGGGAUGA GGGGGAUGA CGUGGGGGAUGA 29943082 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019002 320 UCAUCCCCCA UCAUCCCCCA mU*mC*mA*UCCC
chr6: 29943063- CGGACGGGCC CGGACGGGCC CCACGGACGGGC 29943083 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019003 321 AUCUCGGACC AUCUCGGACC mA*mU*mC*UCGG
chr6: 29943092- CGGAGACUG CGGAGACUG ACCCGGAGACUG 29943112 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019004 322 GGGGUCCCGC GGGGUCCCGC mG*mG*mG*GUCC
chr6: 29943115- GGCUUCGGG GGCUUCGGG CGCGGCUUCGGG 29943135 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019005 323 CUCGGGGUCC CUCGGGGUCC mC*mU*mC*GGGG
chr6: 29943118- CGCGGCUUCG CGCGGCUUCG UCCCGCGGCUUC 29943138 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019006 324 UCUCGGGGU UCUCGGGGU mU*mC*mU*CGGG
chr6: 29943119- CCCGCGGCUU CCCGCGGCUU GUCCCGCGGCUU 29943139 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019007 325 GUCUCGGGG GUCUCGGGG mG*mU*mC*UCGG
chr6: 29943120- UCCCGCGGCU UCCCGCGGCU GGUCCCGCGGCU 29943140 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019008 326 GCAAGGGUC GCAAGGGUC mG*mC*mA*AGG
chr6: 29943126- UCGGGGUCCC UCGGGGUCCC GUCUCGGGGUCC 29943146 G GGUUUUAGA CGGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019009 327 GGACCCCGAG GGACCCCGAG mG*mG*mA*CCCC
chr6: 29943128- ACCCUUGCCC ACCCUUGCCC GAGACCCUUGCC 29943148 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA

UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019010 328 GACCCCGAGA GACCCCGAGA mG*mA*mC*CCCG
chr6: 29943129- CCCUUGCCCC CCCUUGCCCC AGACCCUUGCCC 29943149 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019011 329 CGAGACCCUU CGAGACCCUU mC*mG*mA*GACC
chr6: 29943134- GCCCCGGGAG GCCCCGGGAG CUUGCCCCGGGA 29943154 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019012 330 CUCCCGGGGC CUCCCGGGGC mC*mU*mC*CCGG
chr6: 29943134- AAGGGUCUC AAGGGUCUC GGCAAGGGUCUC 29943154 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019013 331 UCUCCCGGGG UCUCCCGGGG mU*mC*mU*CCCG
chr6: 29943135- CAAGGGUCU CAAGGGUCU GGGCAAGGGUCU 29943155 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019014 332 CUCUCCCGGG CUCUCCCGGG mC*mU*mC*UCCC
chr6: 29943136- GCAAGGGUC GCAAGGGUC GGGGCAAGGGUC 29943156 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019015 333 CCUUGCCCCG CCUUGCCCCG mC*mC*mU*UGCC
chr6: 29943140- GGAGAGGCC GGAGAGGCC CCGGGAGAGGCC 29943160 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019016 334 CUGGGCCUCU CUGGGCCUCU mC*mU*mG*GGCC
chr6: 29943142- CCCGGGGCAA CCCGGGGCAA UCUCCCGGGGCA 29943162 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019017 335 CCUGGGCCUC CCUGGGCCUC mC*mC*mU*GGGC
chr6: 29943143- UCCCGGGGCA UCCCGGGGCA CUCUCCCGGGGC 29943163 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G019019 336 UUUAGGCCA UUUAGGCCA mU*mU*mU*AGG chr6:
29943188- AAAAUCCCCC AAAAUCCCCC CCAAAAAUCCCC 29943208 C CGUUUUAGA CCGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021208 337 CGCUGCAGCG CGCUGCAGCG mC*mG*mC*UGCA
chr6: 29943528- CACGGGUACC CACGGGUACC GCGCACGGGUAC 29943548 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021209 338 GCUGCAGCGC GCUGCAGCGC mG*mC*mU*GCAG
chr6: 29943529- ACGGGUACC ACGGGUACC CGCACGGGUACC 29943549 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021210 339 CUGCAGCGCA CUGCAGCGCA mC*mU*mG*CAGC
chr6: 29943530- CGGGUACCA CGGGUACCA GCACGGGUACCA 29943550 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018932 340 CGCACGGGU CGCACGGGU mC*mG*mC*ACGG
chr6: 29943536- ACCAGGGGCC ACCAGGGGCC GUACCAGGGGCC 29943556 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018933 341 GCACGGGUA GCACGGGUA mG*mC*mA*CGGG
chr6: 29943537- CCAGGGGCCA CCAGGGGCCA UACCAGGGGCCA 29943557 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG AAAAAGUGG UCAmAmCmUmU CACCGAGUCG mGmAmAmAmAm GUGCUUUU AmGmUmGmGmC
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018934 342 CACGGGUACC CACGGGUACC mC*mA*mC*GGGU
chr6: 29943538- AGGGGCCAC AGGGGCCAC ACCAGGGGCCAC 29943558 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018935 343 GGGAGGCGC GGGAGGCGC mG*mG*mG*AGG
chr6: 29943549- CCCGUGGCCC CCCGUGGCCC CGCCCCGUGGCC 29943569 C CGUUUUAGA CCGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018936 344 GCGAUCAGG GCGAUCAGG mG*mC*mG*AUCA
chr6: 29943556- GAGGCGCCCC GAGGCGCCCC GGGAGGCGCCCC 29943576 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021211 345 UCCUUGUGG UCCUUGUGG mU*mC*mC*UUGU
chr6: 29943589- GAGGCCAGCC GAGGCCAGCC GGGAGGCCAGCC 29943609 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU

mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018938 346 CUCCUUGUG CUCCUUGUG mC*mU*mC*CUUG
chr6: 29943590- GGAGGCCAG GGAGGCCAG UGGGAGGCCAGC 29943610 CC CCGUUUUAG CGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018939 347 GGCUGGCCUC GGCUGGCCUC mG*mG*mC*UGGC
chr6: 29943590- CCACAAGGA CCACAAGGA CUCCCACAAGGA 29943610 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018940 348 UUGUCUCCCC UUGUCUCCCC mU*mU*mG*UCUC
chr6: 29943599- UCCUUGUGG UCCUUGUGG CCCUCCUUGUGG 29943619 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018941 349 CCACAAGGA CCACAAGGA mC*mC*mA*CAAG
chr6: 29943600- GGGGAGACA GGGGAGACA GAGGGGAGACAA 29943620 AU AUGUUUUAG UGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018942 350 CACAAGGAG CACAAGGAG mC*mA*mC*AAGG
chr6: 29943601- GGGAGACAA GGGAGACAA AGGGGAGACAAU 29943621 UU UUGUUUUAG UGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018943 351 CAAUUGUCU CAAUUGUCU mC*mA*mA*UUG
chr6: 29943602- CCCCUCCUUG CCCCUCCUUG UCUCCCCUCCUU 29943622 U UGUUUUAGA GUGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018944 352 CCAAUUGUC CCAAUUGUC mC*mC*mA*AUUG
chr6: 29943603- UCCCCUCCUU UCCCCUCCUU UCUCCCCUCCUU 29943623 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018945 353 AUCCCUCGAA AUCCCUCGAA mA*mU*mC*CCUC
chr6: 29943774- UACUGAUGA UACUGAUGA GAAUACUGAUGA 29943794 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018946 354 AACCACUCAU AACCACUCAU mA*mA*mC*CACU
chr6: 29943779- CAGUAUUCG CAGUAUUCG CAUCAGUAUUCG 29943799 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018947 355 GAACCACUCA GAACCACUCA mG*mA*mA*CCAC
chr6: 29943780- UCAGUAUUC UCAGUAUUC UCAUCAGUAUUC 29943800 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018948 356 GAGGAAAAG GAGGAAAAG mG*mA*mG*GAA
chr6: 29943822- UCACGGGCCC UCACGGGCCC AAGUCACGGGCC 29943842 A AGUUUUAGA CAGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018949 357 GGCCCGUGAC GGCCCGUGAC mG*mG*mC*CCGU
chr6: 29943824- UUUUCCUCUC UUUUCCUCUC GACUUUUCCUCU 29943844 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018950 358 UGCUUCACAC UGCUUCACAC mU*mG*mC*UUCA
chr6: 29943857- UCAAUGUGU UCAAUGUGU CACUCAAUGUGU 29943877 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018951 359 GCUUCACACU GCUUCACACU mG*mC*mU*UCAC
chr6: 29943858- CAAUGUGUG CAAUGUGUG ACUCAAUGUGUG 29943878 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018952 360 CUUCACACUC CUUCACACUC mC*mU*mU*CACA
chr6: 29943859- AAUGUGUGU AAUGUGUGU CUCAAUGUGUGU 29943879 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018953 361 UUCACACUCA UUCACACUCA mU*mU*mC*ACAC
chr6: 29943860- AUGUGUGUG AUGUGUGUG UCAAUGUGUGUG 29943880 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018954 362 UUGAGAAUG UUGAGAAUG mU*mU*mG*AGA
chr6: 29944026- GACAGGACA GACAGGACA AUGGACAGGACA 29944046 CC CCGUUUUAG CCGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021205 363 AGGCAUUUU AGGCAUUUU mA*mG*mG*CAU
chr6: 29944077- GCAUCUGUC GCAUCUGUC UUUGCAUCUGUC 29944097 AU AUGUUUUAG AUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021206 364 CAGGCAUUU CAGGCAUUU mC*mA*mG*GCAU

chr6: 29944078- UGCAUCUGU UGCAUCUGU UUUGCAUCUGUC 29944098 CA CAGUUUUAG AGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018955 365 AGGGGCCCU AGGGGCCCU mA*mG*mG*GGCC
chr6: 29944458- GACCCUGCUA GACCCUGCUA CUGACCCUGCUA 29944478 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018956 366 UGGGAAAAG UGGGAAAAG mU*mG*mG*GAA
chr6: 29944478- AGGGGAAGG AGGGGAAGG AAGAGGGGAAGG 29944498 UG UGGUUUUAG UGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018957 367 UGGAGGAGG UGGAGGAGG mU*mG*mG*AGG
chr6: 29944597- AAGAGCUCA AAGAGCUCA AGGAAGAGCUCA 29944617 GG GGGUUUUAG GGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018958 368 UGAGAUUUC UGAGAUUUC mU*mG*mA*GAU
chr6: 29944642- UUGUCUCAC UUGUCUCAC UUCUUGUCUCAC 29944662 UG UGGUUUUAG UGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018959 369 GAGAUUUCU GAGAUUUCU mG*mA*mG*AUU
chr6: 29944643- UGUCUCACU UGUCUCACU UCUUGUCUCACU 29944663 GA GAGUUUUAG GAGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018960 370 UAAAGCACC UAAAGCACC mU*mA*mA*AGC chr6:
29944772- UGUUAAAAU UGUUAAAAU ACCUGUUAAAAU 29944792 GA GAGUUUUAG GAGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018961 371 AAUCUGUCC AAUCUGUCC mA*mA*mU*CUG chr6:
29944782- UUCAUUUUA UUCAUUUUA UCCUUCAUUUUA 29944802 AC ACGUUUUAG ACGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAm UmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018962 372 GUCACAGGG GUCACAGGG mG*mU*mC*ACAG
chr6: 29944850- GAAGGUCCC GAAGGUCCC GGGAAGGUCCCU 29944870 UG UGGUUUUAG GGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018964 373 AAACAUGAA AAACAUGAA mA*mA*mA*CAU chr6:
29944907- GAAAGCAGG GAAAGCAGG GAAGAAAGCAGG 29944927 UG UGGUUUUAG UGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018966 374 UGUCCUGUG UGUCCUGUG mU*mG*mU*CCUG
chr6: 29945024- AGAUACCAG AGAUACCAG UGAGAUACCAGA 29945044 AA AAGUUUUAG AGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018967 375 AUGAAGGAG AUGAAGGAG mA*mU*mG*AAG
chr6: 29945097- GCUGAUGCC GCUGAUGCC GAGGCUGAUGCC 29945117 UG UGGUUUUAG UGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018968 376 AGGCUGAUG AGGCUGAUG mA*mG*mG*CUG
chr6: 29945104- CCUGAGGUCC CCUGAGGUCC AUGCCUGAGGUC 29945124 U UGUUUUAGA CUGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAm UmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018969 377 GGCUGAUGC GGCUGAUGC mG*mG*mC*UGA chr6:
29945105- CUGAGGUCC CUGAGGUCC UGCCUGAGGUCC 29945125 UU UUGUUUUAG UUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018970 378 CACAAUAUCC CACAAUAUCC mC*mA*mC*AAUA
chr6: 29945116- CAAGGACCUC CAAGGACCUC UCCCAAGGACCU 29945136 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018971 379 GGUCCUUGG GGUCCUUGG mG*mG*mU*CCUU
chr6: 29945118- GAUAUUGUG GAUAUUGUG GGGAUAUUGUGU 29945138 UU UUGUUUUAG UGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018972 380 GUCCUUGGG GUCCUUGGG mG*mU*mC*CUUG
chr6: 29945119- AUAUUGUGU AUAUUGUGU GGAUAUUGUGUU 29945139 UU UUGUUUUAG UGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018973 381 CUCCCAAACA CUCCCAAACA mC*mU*mC*CCAA
chr6: 29945124- CAAUAUCCCA CAAUAUCCCA ACACAAUAUCCC 29945144 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018974 382 UCCUCUAGCC UCCUCUAGCC mU*mC*mC*UCUA
chr6: 29945176- ACAUCUUCU ACAUCUUCU GCCACAUCUUCU 29945196 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU

CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018975 383 ACAGAAGAU ACAGAAGAU mA*mC*mA*GAA chr6:
29945177- GUGGCUAGA GUGGCUAGA GAUGUGGCUAGA 29945197 GG GGGUUUUAG GGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018976 384 CCUCUAGCCA CCUCUAGCCA mC*mC*mU*CUAG
chr6: 29945177- CAUCUUCUG CAUCUUCUG CCACAUCUUCUG 29945197 U UGUUUUAGA UGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018977 385 CCCACAGAAG CCCACAGAAG mC*mC*mC*ACAG
chr6: 29945180- AUGUGGCUA AUGUGGCUA AAGAUGUGGCUA 29945200 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018978 386 GUCAGAUCCC GUCAGAUCCC mG*mU*mC*AGA
chr6: 29945187- ACAGAAGAU ACAGAAGAU UCCCACAGAAGA 29945207 G GGUUUUAGA UGGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018979 387 AUCUUCUGU AUCUUCUGU mA*mU*mC*UUCU
chr6: 29945188- GGGAUCUGA GGGAUCUGA GUGGGAUCUGAC 29945208 CC CCGUUUUAG CGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018980 388 CCCAGGCAGU CCCAGGCAGU mC*mC*mC*AGGC
chr6: 29945228- GACAGUGCCC GACAGUGCCC AGUGACAGUGCC 29945248 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018981 389 CUGGGCACU CUGGGCACU mC*mU*mG*GGCA
chr6: 29945230- GUCACUGCCU GUCACUGCCU CUGUCACUGCCU 29945250 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018982 390 CCUGGGCACU CCUGGGCACU mC*mC*mU*GGGC
chr6: 29945231- GUCACUGCCU GUCACUGCCU ACUGUCACUGCC 29945251 GUUUUAGAG UGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021207 391 CCCUGGGCAC CCCUGGGCAC mC*mC*mC*UGGG
chr6: 29945232- UGUCACUGCC UGUCACUGCC CACUGUCACUGC 29945252 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018987 392 UUGGGUGUU UUGGGUGUU mU*mU*mG*GGU
chr6: 29945308- GGGCGGAAC GGGCGGAAC GUUGGGCGGAAC 29945328 AG AGGUUUUAG AGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018988 393 UGGAUGUAU UGGAUGUAU mU*mG*mG*AUG
chr6: 29945361- UGAGCAUGC UGAGCAUGC UAUUGAGCAUGC 29945381 GA GAGUUUUAG GAGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018989 394 GGAUGUAUU GGAUGUAUU mG*mG*mA*UGU
chr6: 29945362- GAGCAUGCG GAGCAUGCG AUUGAGCAUGCG 29945382 AU AUGUUUUAG AUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G018963 395 AACAUGAAG AACAUGAAG mA*mA*mC*AUG
chr6: 31382543- AAAGCAGGU AAAGCAGGU AAGAAAGCAGGU 31382563 GU GUGUUUUAG GUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021885 396 UAGCCCACGG UAGCCCACGG mU*mA*mG*CCCA
chr6: 29942815- CGAUGAAGC CGAUGAAGC CGGCGAUGAAGC 29942835 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021886 397 GUAGCCCACG GUAGCCCACG mG*mU*mA*GCCC
chr6: 29942816- GCGAUGAAG GCGAUGAAG ACGGCGAUGAAG 29942836 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021887 398 CGUAGCCCAC CGUAGCCCAC mC*mG*mU*AGCC
chr6: 29942817- GGCGAUGAA GGCGAUGAA CACGGCGAUGAA 29942837 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021888 399 CUUCAUCGCC CUUCAUCGCC mC*mU*mU*CAUC
chr6: 29942817- GUGGGCUAC GUGGGCUAC GCCGUGGGCUAC 29942837 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021889 400 CGUGUCGUCC CGUGUCGUCC mC*mG*mU*GUCG
chr6: 29942828- ACGUAGCCCA ACGUAGCCCA UCCACGUAGCCC 29942848 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA

UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021890 401 UGGACGACA UGGACGACA mU*mG*mG*ACG chr6:
29942837- CGCAGUUCG CGCAGUUCG ACACGCAGUUCG 29942857 UG UGGUUUUAG UGGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021891 402 GGAUGGAGC GGAUGGAGC mG*mG*mA*UGG
chr6: 29942885- CGCGGGCGCC CGCGGGCGCC AGCCGCGGGCGC 29942905 G GGUUUUAGA CGGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021892 403 GCGGGCGCCG GCGGGCGCCG mG*mC*mG*GGCG
chr6: 29942895- UGGAUAGAG UGGAUAGAG CCGUGGAUAGAG 29942915 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021893 404 UGCUCUAUCC UGCUCUAUCC mU*mG*mC*UCUA
chr6: 29942896- ACGGCGCCCG ACGGCGCCCG UCCACGGCGCCC 29942916 GUUUUAGAG GGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021894 405 GGCGCCGUG GGCGCCGUG mG*mG*mC*GCCG
chr6: 29942898- GAUAGAGCA GAUAGAGCA UGGAUAGAGCAG 29942918 GG GGGUUUUAG GGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021895 406 GCGCCGUGG GCGCCGUGG mG*mC*mG*CCGU
chr6: 29942899- AUAGAGCAG AUAGAGCAG GGAUAGAGCAGG 29942919 GA GAGUUUUAG AGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021896 407 CGCCGUGGA CGCCGUGGA mC*mG*mC*CGUG
chr6: 29942900- UAGAGCAGG UAGAGCAGG GAUAGAGCAGGA 29942920 AG AGGUUUUAG GGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021897 408 GUGGAUAGA GUGGAUAGA mG*mU*mG*GAU
chr6: 29942904- GCAGGAGGG GCAGGAGGG AGAGCAGGAGGG 29942924 GC GCGUUUUAG GCGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021898 409 GGCCCCUCCU GGCCCCUCCU mG*mG*mC*CCCU
chr6: 29942905- GCUCUAUCCA GCUCUAUCCA CCUGCUCUAUCC 29942925 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021899 410 AGCAGGAGG AGCAGGAGG mA*mG*mC*AGG
chr6: 29942912- GGCCGGAGU GGCCGGAGU AGGGGCCGGAGU 29942932 AU AUGUUUUAG AUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021900 411 GCAGGAGGG GCAGGAGGG mG*mC*mA*GGA
chr6: 29942913- GCCGGAGUA GCCGGAGUA GGGGCCGGAGUA 29942933 UU UUGUUUUAG UUGUUUUAGAmG AGCUAGAAA
mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU
UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021901 412 GGAGUGGCU GGAGUGGCU mG*mG*mA*GUG
chr6: 29943490- CCGCAGAUAC CCGCAGAUAC GCUCCGCAGAUA 29943510 C CGUUUUAGA CCGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021902 413 CUCCGCAGAU CUCCGCAGAU mC*mU*mC*CGCA
chr6: 29943497- ACCUGGAGA ACCUGGAGA GAUACCUGGAGA 29943517 A AGUUUUAGA AGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021903 414 UCCGCAGAU UCCGCAGAU mU*mC*mC*GCAG
chr6: 29943498- ACCUGGAGA ACCUGGAGA AUACCUGGAGAA 29943518 AC ACGUUUUAG CGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021904 415 CAGAUACCU CAGAUACCU mC*mA*mG*AUAC
chr6: 29943502- GGAGAACGG GGAGAACGG CUGGAGAACGGG 29943522 GA GAGUUUUAG AGUUUUAGAmGm AGCUAGAAA
CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA
UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021905 416 UCCCGUUCUC UCCCGUUCUC mU*mC*mC*CGUU
chr6: 29943502- CAGGUAUCU CAGGUAUCU CUCCAGGUAUCU 29943522 G GGUUUUAGA GGUUUUAGAmGm GCUAGAAAU
CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA
AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021906 417 GCGUCUCCUU GCGUCUCCUU mG*mC*mG*UCUC
chr6: 29943511- CCCGUUCUCC CCCGUUCUCC CUUCCCGUUCUC 29943531 GUUUUAGAG CGUUUUAGAmGm CUAGAAAUA
CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA
UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU
mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021907 418 GAAGGAGAC GAAGGAGAC mG*mA*mA*GGA
chr6: 29943520- GCUGCAGCGC GCUGCAGCGC GACGCUGCAGCG 29943540 A AGUUUUAGA CAGUUUUAGAmG GCUAGAAAU
mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU
AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU

mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021908 419 AAGGAGACG AAGGAGACG mA*mA*mG*GAG chr6: 29943521- CUGCAGCGCA CUGCAGCGCA ACGCUGCAGCGC 29943541 C CGUUUUAGA ACGUUUUAGAmG GCUAGAAAU mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021909 420 AGAUCUACA AGAUCUACA mA*mG*mA*UCU chr6: 29943566- GGCGAUCAG GGCGAUCAG ACAGGCGAUCAG 29943586 GG GGGUUUUAG GGGUUUUAGAmG AGCUAGAAA mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021910 421 UGAUCGCCU UGAUCGCCU mU*mG*mA*UCGC chr6: 29943569- GUAGAUCUC GUAGAUCUC CUGUAGAUCUCC 29943589 CC CCGUUUUAG CGUUUUAGAmGm AGCUAGAAA CmUmAmGmAmA UAGCAAGUU mAmUmAmGmCA AAAAUAAGG AGUUAAAAUAAG CUAGUCCGU GCUAGUCCGUUA UAUCAACUU UCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021911 422 GGGAGAUCU GGGAGAUCU mG*mG*mG*AGA chr6: 29943569- ACAGGCGAU ACAGGCGAU UCUACAGGCGAU 29943589 CA CAGUUUUAG CAGUUUUAGAmG AGCUAGAAA mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021912 423 CGGGAGAUC CGGGAGAUC mC*mG*mG*GAG chr6: 29943570- UACAGGCGA UACAGGCGA AUCUACAGGCGA 29943590 UC UCGUUUUAG UCGUUUUAGAmG AGCUAGAAA mCmUmAmGmAm UAGCAAGUU AmAmUmAmGmC AAAAUAAGG AAGUUAAAAUAA CUAGUCCGU GGCUAGUCCGUU UAUCAACUU AUCAmAmCmUmU GAAAAAGUG mGmAmAmAmAm GCACCGAGUC AmGmUmGmGmC GGUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021913 424 CGCCUGUAG CGCCUGUAG mC*mG*mC*CUGU chr6: 29943573- AUCUCCCGGG AUCUCCCGGG AGAUCUCCCGGG 29943593 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021914 425 GGCCAGCCCG GGCCAGCCCG mG*mG*mC*CAGC chr6: 29943578- GGAGAUCUA GGAGAUCUA CCGGGAGAUCUA 29943598 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021915 426 UCCCGGGCUG UCCCGGGCUG mU*mC*mC*CGGG chr6: 29943585- GCCUCCCACA GCCUCCCACA CUGGCCUCCCAC 29943605 GUUUUAGAG AGUUUUAGAmGm CUAGAAAUA CmUmAmGmAmA GCAAGUUAA mAmUmAmGmCA AAUAAGGCU AGUUAAAAUAAG AGUCCGUUA GCUAGUCCGUUA UCAACUUGA UCAmAmCmUmU AAAAGUGGC mGmAmAmAmAm ACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021916 427 GGGCUGGCC GGGCUGGCC mG*mG*mG*CUG chr6: 29943589- UCCCACAAGG UCCCACAAGG GCCUCCCACAAG 29943609 A AGUUUUAGA GAGUUUUAGAmG GCUAGAAAU mCmUmAmGmAm AGCAAGUUA AmAmUmAmGmC AAAUAAGGC AAGUUAAAAUAA UAGUCCGUU GGCUAGUCCGUU AUCAACUUG AUCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU G021917 428 CUGAUCGCCU CUGAUCGCCU mC*mU*mG*AUCG chr6: 29943568- GUAGAUCUC GUAGAUCUC CCUGUAGAUCUC 29943588 C CGUUUUAGA CGUUUUAGAmGm GCUAGAAAU CmUmAmGmAmA AGCAAGUUA mAmUmAmGmCA AAAUAAGGC AGUUAAAAUAAG UAGUCCGUU GCUAGUCCGUUA AUCAACUUG UCAmAmCmUmU AAAAAGUGG mGmAmAmAmAm CACCGAGUCG AmGmUmGmGmC GUGCUUUU mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*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-US-00008 TABLE 5A Additional exemplary HLA-A guide sequences SEQ ID RNA-guided DNA Genomic NO Guide Sequence PAM binding agent Coordinates (hg38) 429 AGGAUGGAGCCGCGGGCGCC GTGGA *S. aureus* Cas9 chr6: 29942884- T 29942904 430 GGAAGGAGACGCUGCAGCGC ACGGG *S. aureus* Cas9 chr6: 29943519- T 29943539 431 GACAGCGACGCCGCGAGCCA GAGGA *S. aureus* Cas9 chr6: 29942863- T 29942883 432 CGGGAAGGAGACGCUGCAGC TTCT CasX chr6: 29943517- 29943537 433 CCGUGCGCUGCAGCGUCUCC TTCC CasX chr6: 29943523- 29943543 434 ACGCAGUUCGUGCGGUUCGA NNNNC NME2 chr6: 29942845- CAGC C 29942869 435 UCGUGCGGUUCGACAGCGAC NNNNC NME2 chr6: 29942852- GCCG C 29942876 436 CAGCGACGCCGCGAGCCAGA NNNNC NME2 chr6: 29942865- GGAU C 29942889 437 GCUCUAUCCACGGCGCCCGC NNNNC NME2 chr6: 29942891- GGCU C 29942915 438 UCCUGCUCUAUCCACGGCGC NNNNC NME2 chr6: 29942895- CCGC C 29942919 439 CCGGCCCCUCCUGCUCUAUC NNNNC NME2 chr6: 29942903- CACG C 29942927 440 UCCGGCCCCUCCUGCUCUAU NNNNC NME2 chr6: 29942904- CCAC C 29942928 441 GGGAAGGAGACGCUGCAGCG NNNNC NME2 chr6: 29943518- CACG C 29943542 442 AGACGCUGCAGCGCACGGGU NNNNC NME2 chr6: 29943525- ACCA C 29943549 443 GCGCACGGGUACCAGGGGCC NNNNC NME2 chr6: 29943535- ACGG C 29943559 444 CACGGGUACCAGGGGCCACG NNNNC NME2 chr6: 29943538- GGGC C 29943562 445 ACGGGUACCAGGGGCCACGG NNNNC NME2 chr6: 29943539- GGCG C 29943563 446 CAGGGGCCACGGGGCGCCUC NNNNC NME2 chr6: 29943547- CCUG C 29943571 447 CAGGGAGGCGCCCCGUGGCC NNNNC NME2 chr6: 29943547- CCUG C 29943571 448 UCAGGGAGGCGCCCCGUGGC NNNNC NME2 chr6: 29943548- CCCU C 29943572 449 CAGGCGAUCAGGGAGGCGCC NNNNC NME2 chr6: 29943555- CCGU C 29943579 450 ACAGGCGAUCAGGGAGGCGC NNNNC NME2 chr6: 29943556- CCCG C 29943580 451 UACAGGCGAUCAGGGAGGCG NNNNC NME2 chr6: 29943557- CCCC C 29943581 452 GGGCGCCUCCCUGAUCGCCU NNNNC NME2 chr6: 29943558- GUAG C 29943582 453 GGCGCCUCCCUGAUCGCCUG NNNNC NME2 chr6: 29943559- UAGA C 29943583 454 GAGAUCUACAGGCGAUCAGG NNNNC NME2 chr6: 29943563- GAGG C 29943587 455 GGAGAUCUACAGGCGAUCAG NNNNC NME2 chr6: 29943564- GGAG C 29943588 456 GGGAGAUCUACAGGCGAUCA NNNNC NME2 chr6: 29943565- GGGA C 29943589 457 CUGAUCGCCUGUAGAUCUCC NNNNC NME2 chr6: 29943568- CGGG C 29943592 458 AUCGCCUGUAGAUCUCCCGG NNNNC NME2 chr6: 29943571- GCUG C 29943595 459 UCGCCUGUAGAUCUCCCGGG NNNNC NME2 chr6: 29943572- CUGG C 29943596 460 UUGUCUCCCCUCCUUGUGGG NNNNC NME2 chr6: 29943595- AGGC C 29943619 461 AUUGUCUCCCCUCCUUGUGG NNNNC NME2 chr6: 29943596- GAGG C 29943620 462 CCCAAUUGUCUCCCCUCCUU NNNNC NME2 chr6: 29943600- GUGG C 29943624

TABLE-US-00009 TABLE 5B Exemplary Spy HLA-A guide sequences 463 GGAUGGAGCCGCGGGCGCCG NGG Spy + chr6: Base_ 29942885- Editor 29942905 464 GCGGGCGCCGUGGAUAGAGC NGG Spy + chr6: Base_ 29942895- Editor 29942915 465 UGCUCUAUCCACGGCGCCCG NGG Spy + chr6: Base_ 29942896- Editor 29942916 466 GGCGCCGUGGAUAGAGCAGG NGG Spy + chr6: Base_ 29942898- Editor 29942918 467 GCGCCGUGGAUAGAGCAGGA NGG Spy + chr6: Base_ 29942899- Editor 29942919 468

CGCCGUGGAUAGAGCAGGAG NGG Spy + chr6: Base_ 29942900- Editor 29942920 469 GUGGAUAGAGCAGGAGGGGC NGG Spy + chr6: Base_ 29942904- Editor 29942924 470 GCGUCUCCUUCCCGUUCUCC NGG Spy + chr6: Base_ 29943511- Editor 29943531 471 GAAGGAGACGCUGCAGCGCA NGG Spy + chr6: Base_ 29943520- Editor 29943540 472 AAGGAGACGCUGCAGCGCAC NGG Spy + chr6: Base_ 29943521- Editor 29943541 473 GCUGCAGCGCACGGGUACCA NGG Spy + chr6: Base_ 29943529- Editor 29943549 474 AGAUCUACAGGCGAUCAGGG NGG Spy + chr6: Base_ 29943566- Editor 29943586 475 CUGAUCGCCUGUAGAUCUCC NGG Spy + chr6: Base_ 29943568- Editor 29943588 476 UGAUCGCCUGUAGAUCUCCC NGG Spy + chr6: Base_ 29943569- Editor 29943589 477 GGGAGAUCUACAGGCGAUCA NGG Spy + chr6: Base_ 29943569- Editor 29943589 478 CGGGAGAUCUACAGGCGAUC NGG Spy + chr6: Base_ 29943570- Editor 29943590 479 CGCCUGUAGAUCUCCCGGGC NGG Spy + chr6: Base_ 29943573- Editor 29943593 480 GGCCAGCCCGGGAGAUCUAC NGG Spy + chr6: Base_ 29943578- Editor 29943598 481 UCCCGGGCUGGCCUCCCACA NGG Spy + chr6: Base_ 29943585- Editor 29943605 482 GGGCUGGCCUCCCACAAGGA NGG Spy + chr6: Base_ 29943589- Editor 29943609

TABLE-US-00010 [000632] Table 6. Additional Exemplary HLA-A guide sequences. Exemplary Guide RNA Exemplary Modified Sequence Guide RNA Full (four terminal U Sequence residues are optional SEQ ID NO with PAM and may include 0, to the (SEQ ID NOs: 1, 2, 3, 4, or more Us) Genomic Guide Guide Guide 1483-1498 and (SEQ ID NOs: 2483-2498 Coordinates ID Sequence Sequence 1500-1511) and 2500-2511) (hg38) G021857 483 ACGACAC ACGACACUGAUU mA*mC*mG*ACACUGAU chr6: 29942469- UGAUUGG GGCUUCUCGUUU UGGCUUCUCGUUUUAG 29942489 CUUCUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021858 484 ACCCCUC ACCCCUCAUCCC mA*mC*mC*CCUCAUCC chr6: 29943058- AUCCCCC CCACGGACGUUU CCCACGGACGUUUUAG 29943078 ACGGAC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021859 485 GGCCCGU GGCCCGUCCGUG mG*mG*mC*CCGUCCGU chr6: 29943063- CCGUGGG GGGGAUGAGUUU GGGGGAUGAGUUUUAG 29943083 GGAUGA UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021860 486 GCCAGGU GCCAGGUCGCCC mG*mC*mC*AGGUCGCC chr6: 29943080- CGCCCAC ACAGUCUCGUUU CACAGUCUCGUUUUAG 29943100 AGUCUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021861 487 GUUUAGG GUUUAGGCCAAA mG*mU*mU*UAGGCCAA chr6: 29943187- CCAAAAA AAUCCCCCGUUU AAAUCCCCCGUUUUAG 29943207 UCCCCC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021862 488 GGCCAAA GGCCAAAAAUCC mG*mG*mC*CAAAAAUC chr6: 29943192- AAUCCCC CCCCGGGUGUUU CCCCCGGGUGUUUUAG 29943212 CCGGGU UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021863 489 GACCAAC GACCAACCCGGG mG*mA*mC*CAACCCGG chr6: 29943197- CCGGGGG GGGAUUUUGUUU GGGGAUUUUGUUUUAG 29943217 GAUUUU UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021864 490 CACGGGC CACGGGCCCAAG mC*mA*mC*GGGCCCAA chr6: 29943812- CCAAGGC GCUGCUGCGUUU GGCUGCUGCGUUUUAG 29943832 UGCUGC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021865 491 ACCCUCA ACCCUCAUGCUG mA*mC*mC*CUCAUGCU chr6: 29944349- UGCUGCA CACAUGGCGUUU GCACAUGGCGUUUUAG 29944369 CAUGGC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021866 492 CCUCUAG CCUCUAGGACCU mC*mC*mU*CUAGGACC chr6: 29944996- GACCUUA UAAGGCCCGUUU UUAAGGCCCGUUUUAG 29945016 AGGCCC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021867 493 GCUCCUU GCUCCUUUCUGG mG*mC*mU*CCUUUCUG chr6: 29945018- UCUGGUA UAUCUCACGUUU GUAUCUCACGUUUUAG 29945038 UCUCAC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021868 494 GCUAUGG GCUAUGGGGUUU mG*mC*mU*AUGGGGUU chr6: 29945341- GGUUUCU CUUUGCAUGUUU UCUUUGCAUGUUUUAG 29945361 UUGCAU UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021869 495 GCCUUUG GCCUUUGCAGAA mG*mC*mC*UUUGCAGA chr6: 29945526- CAGAAAC ACAAAGUCGUUU AACAAAGUCGUUUUAG 29945546 AAAGUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021870 496 UGGACCA UGGACCAACCGC mU*mG*mG*ACCAACCG chr6: 29944880- ACCGCCC CCUCCUGAGUUU CCCUCCUGAGUUUUAG 29944900 UCCUGA UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 2) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021871 497 AGCCUCU AGCCUCUCUGAC mA*mG*mC*CUCUCUGA Na CUGACCU CUUUAGCAGUUU CCUUUAGCAGUUUUAG UUAGCA UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm

GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021872 498 CGCCCUC CGCCCUCCUGAA mC*mG*mC*CCUCCUGA Na CUGAAGG GGUCCUCAGUUU AGGUCCUCAGUUUUAG UCCUCA UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021873 500 CCGCCCU CCGCCCUCCUGA mC*mC*mG*CCCUCCUG Na CCUGAAG AGGUCCUCGUUU AAGGUCCUCGUUUUAG GUCCUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021874 501 UGGUUCC UGGUUCCCUUUG mU*mG*mG*UUCCCUUU chr6: 29943794- CUUUGAC ACACACACGUUU GACACACACGUUUUAG 29943814 ACACAC UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 3) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021875 502 GACCCUG GACCCUGCUAAA mG*mA*mC*CCUGCUAA na CUAAAGG GGUCAGAGGUUU AGGUCAGAGGUUUUAG UCAGAG UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021876 503 AGGACCU AGGACCUUCAGG mA*mG*mG*ACCUUCAG na UCAGGAG AGGGCGGUGUUU GAGGGCGGUGUUUUAG GGCGGU UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021877 504 GCACACU GCACACUUCUAC mG*mC*mA*CACUUCUA chr6: 29944671- UCUACCU CUGGGUCUGUUU CCUGGGUCUGUUUUAG 29944691 GGGUCU UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 3) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021878 505 GAGCCUC GAGCCUCUCUGA mG*mA*mG*CCUCUCUG na UCUGACC CCUUUAGCGUUU ACCUUUAGCGUUUUAG UUUAGC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021879 506 ACACUCC ACACUCCUCCAG mA*mC*mA*CUCCUCCA chr6: 29944054- UCCAGCA CACACAUGGUUU GCACACAUGGUUUUAG 29944074 CACAUG UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 2) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAm AmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021880 507 CUCUGAC CUCUGACCUUUA mC*mU*mC*UGACCUUU na CUUUAGC GCAGGGUCGUUU AGCAGGGUCGUUUUAG AGGGUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm UAGCAAGUUAAA AmUmAmGmCAAGUUAA AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021881 508 CAAGAUA CAAGAUAGCCAC mC*mA*mA*GAUAGCCA chr6: 29944043- GCCACAU AUGUGUGCGUUU CAUGUGUGCGUUUUAG 29944063 GUGUGC UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 2) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021882 509 UCUGACC UCUGACCUUUAG mU*mC*mU*GACCUUUA chr6: 29944450- UUUAGCA CAGGGUCAGUUU GCAGGGUCAGUUUUAG 29944470 GGGUCA UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 3) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021883 510 UGUAAAG UGUAAAGGUGAG mU*mG*mU*AAAGGUGA chr6: 29945274- GUGAGAG AGCCUGGAGUUU GAGCCUGGAGUUUUAG 29945294 CCUGGA UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 1) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*mU*mU G021884 511 GAAGGUC GAAGGUCCCUGA mG*mA*mA*GGUCCCUG chr6: 29944859- CCUGAGG GGACCUUCGUUU AGGACCUUCGUUUUAG 29944879 ACCUUC UAGAGCUAGAAA AmGmCmUmAmGmAmAm (mismatch to UAGCAAGUUAAA AmUmAmGmCAAGUUAA hg38 = 3) AUAAGGCUAGUC AAUAAGGCUAGUCCGU CGUUAUCAACUU UAUCAmAmCmUmUmGm GAAAAAGUGGCA AmAmAmAmAmGmUmG CCGAGUCGGUGC mGmCmAmCmCmGmAmG UUUU mUmCmGmGmUmGmCmU *mU*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-US-00011 TABLE 7 Additional Exemplary HLA-A Nme guide sequences SEQ ID Exemplary Exemplary Guide NO to Guide RNA Full RNA Modified the Sequence Sequence Genomic Guide Guide Guide (SEQ ID NOs: (SEQ ID NOs: Coordinates ID Sequence Sequence 1512-1590) 2512-2590) (hg38) G028854 512 CCUGGGUCU CCUGGGUCU mC*mC*mU*mGmGG chr6:29944223- GGUCCUCCCC GGUCCUCCCC UmCmUGmGUmCCU 29944247 AUCCC AUCCCGUUG CCmCCAUmCCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028855 513 GUGGAGACC GUGGAGACC mG*mU*mG*mGmA chr6:29944264- AGGCCUGCA AGGCCUGCA GAmCmCAmGGmCC 29944288 GGGGAU GGGGAUGUU UGCmAGGGmGAUm GUAGCUCCCU GUUGmUmAmGmCU GAAACCGUU CCCmUmGmAmAmA GCUACAAUA mCmCGUUmGmCUA AGGCCGUCG mCAAU*AAGmGmC AAAGAUGUG CmGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028856 514 CCGUGUCCUG CCGUGUCCUG mC*mC*mG*mUmGU chr6:29944229- GGUCUGGUC GGUCUGGUC CmCmUGmGGmUCU 29944253 CUCCC CUCCCGUUGU GGmUCCUmCCCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028857 515 UCCGUGUCCU UCCGUGUCCU mU*mC*mC*mGmUG chr6:29944230- GGGUCUGGU GGGUCUGGU UmCmCUmGGmGUC 29944254 CCUCC CCUCCGUUGU UGmGUCCmUCCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG

CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028858 516 CACAUGGCA CACAUGGCA mC*mA*mC*mAmUG chr6:29944327- GGUGUAUCU GGUGUAUCU GmCmAGmGUmGUA 29944351 CUGCUC CUGCUCGUU UCmUCUGmCUCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028859 517 UGCUGCACA UGCUGCACA mU*mG*mC*mUmGC chr6:29944332- UGGCAGGUG UGGCAGGUG AmCmAUmGGmCAG 29944356 UAUCUC UAUCUCGUU GUmGUAUmCUCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028860 518 CCGCACGAAC CCGCACGAAC mC*mC*mG*mCmAC chr6:29942830- UGCGUGUCG UGCGUGUCG GmAmACmUGmCGU 29942854 UCCAC UCCACGUUG GUmCGUCmCACmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028861 519 CGCACGAACU CGCACGAACU mC*mG*mC*mAmCG chr6:29942829- GCGUGUCGU GCGUGUCGU AmAmCUmGCmGUG 29942853 CCACG CCACGGUUG UCmGUCCmACGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028862 520 GUGCGCUGC GUGCGCUGC mG*mU*mG*mCmGC chr6:29943511- AGCGUCUCCU AGCGUCUCCU UmGmCAmGCmGUC 29943535 UCCCG UCCCGGUUG UCmCUUCmCCGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028863 521 CCGAGGAUG CCGAGGAUG mC*mC*mG*mAmGG chr6:29942547- GCCGUCAUG GCCGUCAUG AmUmGGmCCmGUC 29942571 GCGCCC GCGCCCGUUG AUmGGCGmCCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028864 522 CGAGGAUGG CGAGGAUGG mC*mG*mA*mGmGA chr6:29942548- CCGUCAUGGC CCGUCAUGGC UmGmGCmCGmUCA 29942572 GCCCC GCCCCGUUGU UGmGCGCmCCCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028865 523 AAGGUUCCA AAGGUUCCA mA*mA*mG*mGmU chr6:29944266- UCCCCUGCAG UCCCCUGCAG UCmCmAUmCCmCC 29944290 GCCUG GCCUGGUUG UGCmAGGCmCUGm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028866 524 AGACCAGGCC AGACCAGGCC mA*mG*mA*mCmCA chr6:29944268- UGCAGGGGA UGCAGGGGA GmGmCCmUGmCAG 29944292 UGGAA UGGAAGUUG GGmGAUGmGAAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028867 525 ACUUCUGGA ACUUCUGGA mA*mC*mU*mUmCU chr6:29944274- AGGUUCCAU AGGUUCCAU GmGmAAmGGmUUC 29944298 CCCCUG CCCCUGGUUG CAmUCCCmCUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028868 526 CGAUGAAGC CGAUGAAGC mC*mG*mA*mUmGA chr6:29942795- GGGGCUCCCC GGGGCUCCCC AmGmCGmGGmGCU 29942819 GCGGC GCGGCGUUG CCmCCGCmGGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028869 527 UCCGUGUCCC UCCGUGUCCC mU*mC*mC*mGmUG chr6:29942785- GGCCCGGCCG GGCCCGGCCG UmCmCCmGGmCCC 29942809 CGGG CGGGGUUGU GGmCCGCmGGGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028870 528 CCGUGUCCCG CCGUGUCCCG mC*mC*mG*mUmGU chr6:29942786- GCCCGGCCGC GCCCGGCCGC CmCmCGmGCmCCG 29942810 GGGG GGGGGUUGU GCmCGCGmGGGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028871 529 CAGACGCCGA CAGACGCCGA mC*mA*mG*mAmCG chr6:29942541- GGAUGGCCG GGAUGGCCG CmCmGAmGGmAUG 29942565 UCAUG UCAUGGUUG GCmCGUCmAUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028872 530 CCAGACGCCG CCAGACGCCG mC*mC*mA*mGmAC chr6:29942540- AGGAUGGCC AGGAUGGCC GmCmCGmAGmGAU 29942564 GUCAU GUCAUGUUG GGmCCGUmCAUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028873 531 AGACGCCGA AGACGCCGA mA*mG*mA*mCmGC chr6:29942542- GGAUGGCCG GGAUGGCCG CmGmAGmGAmUGG 29942566 UCAUGG UCAUGGGUU CCmGUCAmUGGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028874 532 CUGUCGAACC CUGUCGAACC mC*mU*mG*mUmCG chr6:29942838- GCACGAACU GCACGAACU AmAmCCmGCmACG 29942862 GCGUG GCGUGGUUG AAmCUGCmGUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028875 533 UCGUGCGGU UCGUGCGGU mU*mC*mG*mUmGC chr6:29942852- UCGACAGCG UCGACAGCG GmGmUUmCGmACA 29942876 ACGCCG ACGCCGGUU GCmGACGmCCGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU

mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028876 534
CCAUCCCCAU CCAUCCCCAU mC*mC*mA*mUmCC chr6:29944517- CGUGGGCAU CGUGGGCAU CmCmAUmCGmUGG 29944541
CAUUG CAUUGGUUG GCmAUCAmUUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028877 535
UCCUCUGGCU UCCUCUGGCU mU*mC*mC*mUmCU chr6:29942858- CGCGGCGUCG CGCGGCGUCG GmGmCUmCGmCGG
29942882 CUGU CUGUGUUGU CGmUCGCmUGUmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028878 536
CUCUGGUUG CUCUGGUUG mC*mU*mC*mUmGG chr6:29942988- UAGUAGCCG UAGUAGCCG UmUmGUmAGmUAG 29943012
CGCAGG CGCAGGGUU CCmGCGCmAGGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028879 537
ACCUGGGGA ACCUGGGGA mA*mC*mC*mUmGG chr6:29942984- CCCUGCGCGG CCCUGCGCGG GmGmACmCCmUGC 29943008
CUACU CUACUGUUG GCmGGCUmACUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028880 538
CGCUCUGGU CGCUCUGGU mC*mG*mC*mUmCU chr6:29942990- UGUAGUAGC UGUAGUAGC GmGmUUmGUmAGU 29943014
CGCGCA CGCGCAGUU AGmCCGCmGCAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028881 539
GCUCUGGUU GCUCUGGUU mG*mC*mU*mCmUG chr6:29942989- GUAGUAGCC GUAGUAGCC GmUmUGmUAmGUA 29943013
GCGCAG GCGCAGGUU GCmCGCGmCAGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028882 540
UAUUUUUUU UAUUUUUUU mU*mA*mU*mUmU chr6:29945435- CUAUAGUGU CUAUAGUGU UUmUmUCmUAmUA 29945459
GAGACA GAGACAGUU GUGmUGAGmACAm GUAGCUCCCU GUUGmUmAmGmCU GAAACCGUU CCCmUmGmAmAmA
GCUACAAUA mCmCGUUmGmCUA AGGCCGUCG mCAAU*AAGmGmC AAAGAUGUG CmGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028883 541
ACGCCUACGA ACGCCUACGA mA*mC*mG*mCmCU chr6:29943342- CGGCAAGGA CGGCAAGGA AmCmGAmCGmGCA 29943366
UUACA UUACAGUUG AGmGAUUmACAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028884 542
UUUGUUCUA UUUGUUCUA mU*mU*mU*mGmU chr6:29945218- CCCCAGGCAG CCCCAGGCAG UCmUmACmCCmCA 29945242
UGACA UGACAGUUG GGCmAGUGmACAm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028885 543
UUUUGUUCU UUUUGUUCU mU*mU*mU*mUmG chr6:29945217- ACCCCAGGCA ACCCCAGGCA UUmCmUAmCCmCC 29945241
GUGAC GUGACGUUG AGGmCAGUmGACm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028886 544
AGCGACCACA AGCGACCACA mA*mG*mC*mGmAC chr6:29944558- GCUCCAGUG GCUCCAGUG CmAmCAmGCmUCC 29944582
AUCAC AUCACGUUG AGmUGAUmCACmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028887 545
GAGCUCCGU GAGCUCCGU mG*mA*mG*mCmUC chr6:29944234- GUCCUGGGU GUCCUGGGU CmGmUGmUCmCUG 29944258
CUGGUC CUGGUCGUU GGmUCUGmGUCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028888 546
UCCACGAGCU UCCACGAGCU mUmC*mC*mAmCG chr6:29944239- CCGUGUCCUG CCGUGUCCUG AmGmCUmCCmGUG
29944263 GGUC GGUCGUUGU UCmCUGGmGUCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028889 547
ACGAGCUCCG ACGAGCUCCG mA*mC*mG*mAmGC chr6:29944236- UGUCCUGGG UGUCCUGGG UmCmCGmUGmUCC 29944260
UCUGG UCUGGGUUG UGmGGUCmUGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028890 548
CGAGCUCCGU CGAGCUCCGU mC*mG*mA*mGmCU chr6:29944235- GUCCUGGGU GUCCUGGGU CmCmGUmGUmCCU 29944259
CUGGU CUGGUGUUG GGmGUCUmGGUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028891 549
GCAGGCCUG GCAGGCCUG mG*mC*mA*mGmGC chr6:29944251- GUCUCCACGA GUCUCCACGA CmUmGGmUCmUCC 29944275
GCUCC GCUCCGUUG ACmGAGCmUCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028892 550
UCCCCUGCAG UCCCCUGCAG mU*mC*mC*mCmCU chr6:29944257- GCCUGGUCUC GCCUGGUCUC GmCmAGmGCmCUG
29944281 CACG CACGGUUGU GUmCUCCmACGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028893 551
AUCCGUGUCC AUCCGUGUCC mA*mU*mC*mCmGU chr6:29942784- CGGCCCGGCC CGGCCCGGCC GmUmCCmCGmGCC
29942808 GCGG GCGGGUUGU CGmGCCGmCGGmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028894 552

CCUUCCCGUU CCUUCCCGUU mC*mC*mU*mUmCC chr6:29943495- CUCCAGGUA CUCCAGGUA CmGmUUmCUmCCA 29943519
UCUGC UCUGCGUUG GGmUAUCmUGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028895 553
CCGUUCUCCA CCGUUCUCCA mC*mC*mG*mUmUC chr6:29943490- GGUAUCUGC GGUAUCUGC UmCmCAmGGmUAU 29943514
GGAGC GGAGCGUUG CUmGCGGmAGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028896 554
ACCUGCCAUG ACCUGCCAUG mA*mC*mC*mUmGC chr6:29944345- UGCAGCAUG UGCAGCAUG CmAmUGmUGmCAG 29944369
AGGGU AGGGUGUUG CAmUGAGmGGUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028897 555
CACCUGCCAU CACCUGCCAU mC*mA*mC*mCmUG chr6:29944344- GUGCAGCAU GUGCAGCAU CmCmAUmGUmGCA 29944368
GAGGG GAGGGGUUG GCmAUGAmGGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028898 556
ACCUGGCAGC ACCUGGCAGC mA*mC*mC*mUmGG chr6:29944219- GGGAUGGGG GGGAUGGGG CmAmGCmGGmGAU
29944243 AGGAC AGGACGUUG GGmGGAGmGACmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028899 557
CACUGACCUG CACUGACCUG mC*mA*mC*mUmGA chr6:29944214- GCAGCGGGA GCAGCGGGA CmCmUGmGCmAGC 29944238
UGGGG UGGGGGUUG GGmGAUGmGGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028900 558
CCUGGCAGCG CCUGGCAGCG mC*mC*mU*mGmGC chr6:29944220- GGAUGGGGA GGAUGGGGA AmGmCGmGGmAUG 29944244
GGACC GGACCGUUG GGmGAGGmACCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028901 559
CCAUCUCAGG CCAUCUCAGG mC*mC*mA*mUmCU chr6:29944366- GUGAGGGGC GUGAGGGGC CmAmGGmGUmGAG 29944390
UUGGG UUGGGGUUG GGmGCUUmGGGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028902 560
UGGCCGUCA UGGCCGUCA mU*mG*mG*mCmCG chr6:29942554- UGGCGCCCCG UGGCGCCCCG UmCmAUmGGmCGC 29942578
AACCC AACCCGUUG CCmCGAAmCCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028903 561
AUGUCCGCCG AUGUCCGCCG mA*mU*mG*mUmCC chr6:29943379- CGGUCCAAG CGGUCCAAG GmCmCGmCGmGUC 29943403
AGCGC AGCGCGUUG CAmAGAGmCGCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028904 562
GGUACCCGU GGUACCCGU mG*mG*mU*mAmCC chr6:29943518- GCGCUGCAGC GCGCUGCAGC CmGmUGmCGmCUG 29943542
GUCUC GUCUCGUUG CAmGCGUmCUCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028905 563
GGGAAGGAG GGGAAGGAG mG*mG*mG*mAmA chr6:29943518- ACGCUGCAGC ACGCUGCAGC GGmAmGAmCGmCU 29943542
GCACG GCACGGUUG GCAmGCGCmACGm UAGCUCCCUG GUUGmUmAmGmCU AAACCGUUG CCCmUmGmAmAmA
CUACAAUAA mCmCGUUmGmCUA GGCCGUCGA mCAAU*AAGmGmC AAGAUGUGC CmGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028906 564
GCGGGCGCCG GCGGGCGCCG mG*mC*mG*mGmGC chr6:29942895- UGGAUAGAG UGGAUAGAG GmCmCGmUGmGAU
29942919 CAGGA CAGGAGUUG AGmAGCAmGGAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028907 565
UCCUGCUCUA UCCUGCUCUA mU*mC*mC*mUmGC chr6:29942889- UCCACGGCGC UCCACGGCGC UmCmUAmUCmCAC
29942913 CCGC CCGCGUUGU GGmCGCCmCGCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028908 566
CCCCUGGUAC CCCCUGGUAC mC*mC*mC*mCmUG chr6:29943523- CCGUGCGCUG CCGUGCGCUG GmUmACmCCmGUG
29943547 CAGC CAGCGUUGU CGmCUGCmAGCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028909 567
UGGUACCCG UGGUACCCG mU*mG*mG*mUmAC chr6:29943519- UGCGCUGCA UGCGCUGCA CmCmGUmGCmGCU GCGUCU
GCGUCUGUU GCmAGCGmUCUmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA
CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC 29943543 AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028910 568
CAUCUCAGG CAUCUCAGG mC*mA*mU*mCmUC chr6:29944365- GUGAGGGGC GUGAGGGGC AmGmGGmUGmAGG 29944389
UUGGGC UUGGGCGUU GGmCUUGmGGCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028911 569
ACGCAGUUC ACGCAGUUC mA*mC*mG*mCmAG chr6:29942845- GUGCGGUUC GUGCGGUUC UmUmCGmUGmCGG 29942869
GACAGC GACAGCGUU UUmCGACmAGCmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028912 570
CGCCCAGGUC CGCCCAGGUC mC*mG*mC*mCmCA chr6:29942595- UGGGUCAGG UGGGUCAGG GmGmUCmUGmGGU 29942619

GCCAG GCCAGGUUG CAmGGGCmCAGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028913 571
CACUCACCCG CACUCACCCG mC*mA*mC*mUmCA chr6:29942609- CCCAGGUCUG CCCAGGUCUG CmCmCGmCCmCAG
29942633 GGUC GGUCGUUGU GUmCUGGmGUCmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028914 572
CCGCCCAGGU CCGCCCAGGU mC*mC*mG*mCmCC chr6:29942596- CUGGGUCAG CUGGGUCAG AmGmGUmCUmGGG 29942620
GGCCA GGCCAGUUG UCmAGGGmCCAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028915 573
CCCGCCCAGG CCCGCCCAGG mC*mC*mC*mGmCC chr6:29942597- UCUGGGUCA UCUGGGUCA CmAmGGmUCmUGG 29942621
GGGCC GGGCCGUUG GUmCAGGmGCCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028916 574
CAGCGACGCC CAGCGACGCC mC*mA*mG*mCmGA chr6:29942865- GCGAGCCAG GCGAGCCAG CmGmCCmGCmGAG 29942889
AGGAU AGGAUGUUG CCmAGAGmGAUmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028917 575
CGCGAGCCAG CGCGAGCCAG mC*mG*mC*mGmAG chr6:29942874- AGGAUGGAG AGGAUGGAG CmCmAGmAGmGAU 29942898
CCGCG CCGCGGUUG GGmAGCCmGCGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028918 576
GCUCUAUCCA GCUCUAUCCA mG*mC*mU*mCmUA chr6:29942891- CGGCGCCCGC CGGCGCCCGC UmCmCAmCGmGCG
29942915 GGCU GGCUGUUGU CCmCGCGmGCUmG AGCUCCCUGA UUGmUmAmGmCUC AACCGUUGC CCmUmGmAmAmAm
UACAAUAAG CmCGUUmGmCUAm GCCGUCGAA CAAU*AAGmGmCC AGAUGUGCC mGmUmCmGmAmA GCAACGCUCU
mAmGmAmUGUGCm GCCUUCUGGC CGmCAAmCGCUCU AUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028919 577
CACAUCAGA CACAUCAGA mC*mA*mC*mAmUC chr6:29945232- GCCCUGGGCA GCCCUGGGCA AmGmAGmCCmCUG 29945256
CUGUC CUGUCGUUG GGmCACUmGUCmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028920 578
CAGACCCAGG CAGACCCAGG mC*mA*mG*mAmCC chr6:29944243- ACACGGAGC ACACGGAGC CmAmGGmACmACG 29944267
UCGUG UCGUGGUUG GAmGCUCmGUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028921 579
CCAGGACACG CCAGGACACG mC*mC*mA*mGmGA chr6:29944248- GAGCUCGUG GAGCUCGUG CmAmCGmGAmGCU 29944272
GAGAC GAGACGUUG CGmUGGAmGACmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm
CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC
mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028922 580
CUCUGGGAA CUCUGGGAA mC*mU*mC*mUmGG chr6:29944471- AAGAGGGGA AAGAGGGGA GmAmAAmAGmAGG 29944495
AGGUGA AGGUGAGUU GGmAAGGmUGAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028923 581
UCUGGGAAA UCUGGGAAA mU*mC*mU*mGmGG chr6:29944470- AGAGGGGAA AGAGGGGAA AmAmAAmGAmGGG 29944494
GGUGAG GGUGAGGUU GAmAGGUmGAGmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028924 582
AGACGCUGC AGACGCUGC mA*mG*mA*mCmGC chr6:29943525- AGCGCACGG AGCGCACGG UmGmCAmGCmGCA 29943549
GUACCA GUACCAGUU CGmGGUAmCCAmG GUAGCUCCCU UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm
GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU
mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028925 583
UCCUUUUCU UCCUUUUCU mU*mC*mC*mUmUU Unavailable. AUCUGUGGG AUCUGUGGG UmCmUAmUCmUGU Imperfect
AAGAAA AAGAAAGUU GGmGAAGmAAAmG alignment to GUAGCUCCCU UUGmUmAmGmCUC human genome. GAAACCGUU
CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA
CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028926 584 CACUGCCUGG CACUGCCUGG mC*mA*mC*mUmGC chr6:29945209- GGUAGAACA
GGUAGAACA CmUmGGmGGmUAG 29945233 AAAAC AAAACGUUG AAmCAAAmAACmG UAGCUCCCUG UUGmUmAmGmCUC
AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC
mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU
GGCAUCG*mU*mU G028927 585 ACAACCAGA ACAACCAGA mA*mC*mA*mAmCC chr6:29943008- GCGAGGCCG GCGAGGCCG
AmGmAGmCGmAGG 29943032 GUGAGU GUGAGUGUU CCmGGUGmAGUmG GUAGCUCCCU UUGmUmAmGmCUC
GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG
mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028928 586 CUACAACCAG CUACAACCAG mC*mU*mA*mCmAA chr6:29943006-
AGCGAGGCC AGCGAGGCC CmCmAGmAGmCGA 29943030 GGUGA GGUGAGUUG GGmCCGGmUGAmG UAGCUCCCUG
UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC
AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028929 587 UACAACCAG UACAACCAG mU*mA*mC*mAmAC chr6:29943007-
AGCGAGGCC AGCGAGGCC CmAmGAmGCmGAG 29943031 GGUGAG GGUGAGGUU GCmCGGUmGAGmG GUAGCUCCCU
UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC
AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU
mGmCCmUmUmCmU GGCAUCG*mU*mU G028930 588 CCAGAGCGA CCAGAGCGA mC*mC*mA*mGmAG chr6:29943012-
GGCCGGUGA GGCCGGUGA CmGmAGmGCmCGG 29943036 GUGACC GUGACCGUU UGmAGUGmACCmG GUAGCUCCCU

UUGmUmAmGmCUC GAAACCGUU CCmUmGmAmAmAm GCUACAAUA CmCGUUmGmCUAm AGGCCGUCG CAAU*AAGmGmCC AAAGAUGUG mGmUmCmGmAmA CCGCAACGCU mAmGmAmUGUGCm CUGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028933 589 CCAUCCCGCU CCAUCCCGCU mC*mC*mA*mUmCC chr6:29944206-GCCAGGUCA GCCAGGUCA CmGmCUmGCmCAG 29944230 GUGUG GUGUGGUUG GUmCAGUmGUGmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU G028934 590 GUAUCUGCG GUAUCUGCG mG*mU*mA*mUmCU chr6:29943479-GAGCCACUCC GAGCCACUCC GmCmGGmAGmCCA 29943503 ACGCA ACGCAGUUG CUmCCACmGCAmG UAGCUCCCUG UUGmUmAmGmCUC AAACCGUUG CCmUmGmAmAmAm CUACAAUAA CmCGUUmGmCUAm GGCCGUCGA CAAU*AAGmGmCC AAGAUGUGC mGmUmCmGmAmA CGCAACGCUC mAmGmAmUGUGCm UGCCUUCUG CGmCAAmCGCUCU GCAUCGUU mGmCCmUmUmCmU GGCAUCG*mU*mU

[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 coordinates 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 protein, 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.sub.50) of the antibody can be measured in a cell-based neutralization assay, wherein a lower EC.sub.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:
##STR00001##
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: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 602) or GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGC (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: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU 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: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGC (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).sub.20-25, each N of the (N).sub.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).sub.20-25 has the following sequence and modification pattern:

TABLE-US-00012 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:

TABLE-US-00013 (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:

TABLE-US-00014 (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 TABLE-US-00015 (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*NNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCG 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 embodiments, 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).sub.20-25 GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGC AACGCUCUGCCUUCUGGCAUCGUU (SEQ ID NO: 3119); (N).sub.20-25 GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGC AACGCUCUGCCUUCUGGCAUCGUUUAUU (SEQ ID NO: 3120); (N).sub.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: TABLE-US-00016 (SEQ ID NO: 707) GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCC mUmUmCmUGmGCmAmUC*mG*mU*mU; or (SEQ ID NO: 710) GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGm GmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU mUmCmUGGCAUCG*mU*mU;
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).sub.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.sub.2CH.sub.2O).sub.nCH.sub.2CH.sub.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.sub.2CH.sub.2OCH.sub.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.sub.2CH.sub.2NH).sub.nCH2CH.sub.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-US-00017 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-

TABLE-US-00018 TABLE 8B exemplary NmeCas9 sgRNA (SEQ ID NO: 3126) 1-24 25 26 27 28 29 30 31 32 33 34 NNNNNNNNNNNNNNN G U U G U A G C U C NNNNNNNNN Lower stem Guide region 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 Loop Hairpin 1 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) Lower stem Hairpin 1 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 Upper Stem Hairpin 2 119 120 121 122 123 124 125 126 127 128 129 130 131 132 A G C U U C U G C U U U A A Loop Upper Stem Hairpin 2 133 134 135 136 137 138 139 140 141 142 143 144 145 G G G G C A U C G U U U A Upper Stem Bulge Lower Stem Hairpin 2

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).sub.n (SEQ ID NO: 910), a (G).sub.n, an (EAAAK).sub.n (SEQ ID NO: 911), a (GGS).sub.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).sub.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.sup.2, 10.sup.3, 104, 105, 10.sup.6, 107, or 10.sup.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.sup.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-US-00019 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. SEQ ID Description NO Sequence Exemplary SpyCas9 600 GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC sgRNA scaffold Exemplary SpyCas 9 601 GUUUUAGAGCUAUGCUGUUUUG crRNA 3′ Sequence Exemplary SpyCas 9 602 GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU sgRNA Scaffold Exemplary SpyCas9 603 GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC sgRNA Scaffold Exemplary SpyCas9 604 GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGGUGC crRNA Scaffold 605-612 See Table 9A 613-698 Not used Exemplary NmeCas9 699 GUUGUAGCUCCCUUUCUCAUUUCGGAAACGAAAUGAGAACCGUUGCUACAAUAAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCU guide RNA scaffold GCCCCUUAAAGCUUCUGCUUUAAGGGGCAUCGUUUA Exemplary NmeCas 9 700 (N).sub.20-25 guide RNA GUUGUAGCUCCCUUUCUCAUUUCGGAAACGAAAUGAGAACCGUUGCUACAAUAAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCU GCCCCUUAAAGCUUCUGCUUUAAGGGGCAUCGUUUA Shortened/unmodified 3119 (N).sub.20-

25GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
NmeCas9 guide RNA motif Shortened/unmodified 3120 (N).sub.20-25 NmeCas9 guide RNA
GUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU
motif Shortened/unmodified 3121 (N).sub.20-25 NmeCas9 guide RNA
GUUGUAGCUCCCUGGAAACCCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUUUAUU
motif Shortened/unmodified 704 (N).sub.20-25 NmeCas9 guide RNA
GUUGUAGCUCCCUUCGAAAGACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCUGCCUUCUGGCAUCGUU
motif Shortened/modified 705
mN*mNNNNNNNmNNNmNNNNNNNNNNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmG
NmeCas9 guide RNA
mUmCmGmAmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU motif (101-mer)
Shortened/modified 706 (N).sub.20-
25GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGm NmeCas9 guide RNA
AmAmAmGmAmUGUGCmCGCmAmAmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU motif Shortened/modified 707
GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmAm
NmeCas9 guide RNA mCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU motif Shortened/modified 708
mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A
NmeCas9 guide RNA
AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU motif (101-
mer) Shortened/modified 709 (N).sub.20-
25GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGm NmeCas9 guide RNA
AmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU motif Shortened/modified 710
GUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAA
NmeCas9 guide RNA CGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU motif Shortened/modified 711
mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCU
NmeCas9 guide RNA
AmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU
motif (105-mer) Shortened/modified 712
mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAA
NmeCas9 guide RNA
GmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU motif (101-mer)
Shortened/modified 713
mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*A
NmeCas9 guide RNA
AGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU motif
(101-mer) Shortened/modified 714
mN*mN*mN*mNmNNNmNmNNmNNmNNNNNmNNNNmNNNmGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAA
NmeCas9 guide RNA
GmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU motif
(101-mer) Guide scaffold 715
mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGCmA
AmCmGCUCUmGmCCmUmUmCmUGmGCmAmUC*mG*mU*mU Guide scaffold 716
mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCA
mCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 717
mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAA
CGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 718
mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCA
mCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 719
mGUUGmUmAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAA
CGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 720
mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGU
CmCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 721
mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUC
mCGmCAAmCGCUCUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 722
mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAU*AAGmGmCCmGmUmCmGmAmAmAmGmAmUGU
CmCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU Guide scaffold 723
mGUUGmUmAmGmCUCCCmUmUmCmGmAmAmAmGmAmCmCGUUmGmCUAmCAAUAAGmGmCCmGmUmCmGmAmAmAmGmAmUGUC
mCGmCAAmCGmCmUmCmUmGmCCmUmUmCmUGGCAUCG*mU*mU 724-799 Not used Recombinant Cas9-NLS 800
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI
amino acid sequence
FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI
EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR
QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR
QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR
ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSELKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK
MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV

KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI
DLSQLGGDGGSPKKKRKV ORF encoding Spy Cas9 801
ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGA
GCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGA
AACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC
TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACG
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
AAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAG
AACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGG
AAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA
TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAG
AACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGA
CAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATC
GACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAG 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
AGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGA
CCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATC
GACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA Open reading frame 803
AUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCU for Spy Cas9 with
CCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUCCGGCGA Hibit tag
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
AAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCGCCGGCG

AGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCUGAAGGG
CUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUCUCCGAG
UUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGG
AGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCACCAUCGA
CCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACCCGGAUC
GACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCCGAGUCCGUGU
CCGGCUGGCGGCUGUUCAAGAAGAUCUCCUGA Open Reading frame 804
AUGgaggccucccccgccuccggcccccggcaccugauggaccccacaucuucaccuccAACUUCAACAACggcAUCggccggC for BC22n
ACAAGaccUACCUGUGCUACgagguggagcggCUGGACAACggcaccuccgugAAGAUGGACCAGCACcggggcUUCCUGCACAA
CCAGgccAAGAACCUGCUGUGCggcUUCUACggccggCACgccgagCUGcggUUCCUGGACCUGgugcccuccCUGCAGCUGGAC
cccgccCAGAUCUACcgggugaccUGGUUCAUCuccUGGucccccUGCUUCuccUGGggcUGCgccggcgaggugcgggccUUCC
UGCAGgagAACaccCACgugcggCUGcggAUCUUCgccgcccggAUCUACGACUACGACcccCUGUACAAGgaggccCUGCAGAU
GCUGcggGACgccggcgccCAGguguccAUCAUGaccUACGACgagUUCAAGCACUGCUGGGACaccUUCgugGACCACCAGggc
UGCcccUUCCAGcccUGGGACggcCUGGACgagCACuccCAGgccCUGuccggccggCUGcgggccAUCCUGCAGAACCAGggcA
ACuccggcuccgagacccccggcaccuccgaguccgccacccccgaguccgacaagaaguacuccaucggccuggCcaucggcac
caacuccguggggcugggccgugaucaccgacgaguacaaggugcccuccaagaaguucaaggugcugggcaacaccgaccggcac
uccaucaagaagaaccugaucggcgcccugcuguucgacuccggcgagaccgccgaggccacccggcugaagcggaccgcccggc
ggcgguacacccggcggaagaaccggaucugcuaccugcaggagaucuucuccaacgagauggccaagguggacgacuccuucuu
ccaccggcuggaggaguccuuccugguggaggaggacaagaagcacgagcggcacccccaucuucggcaacaucguggacgaggug
gccuaccacgagaaguaccccaccaucuaccaccugcggaagaagcuggugGacuccaccgacaaggccgaccugcggcugaucu
accuggcccuggcccacaugaucaaguuccggggccacuuccugaucgagggcgaccugaaccccgacaacuccgacguggacaa
gcuguucauccagcuggugcagaccuacaaccagcuguucgaggagaaccccaucaacgccuccggcguggacgccaaggccauc
cuguccgcccggcuguccaaguccggcggcuggagaaccugaucgcccagcugcccggcgagaagaagaacggccuguucggca
accugaucgcccuguccuggggccugaccccaacuucaaguccaacuucgaccuggccgaggacgccaagcugcagcuguccaa
ggacaccuacgacgacgaccuggacaaccugcuggcccagaucggcgaccaguacgccgaccuguuccuggccgccaagaaccug
uccgacgccauccugcuguccgacauccugcgggugaacaccgagaucaccaaggccccccuguccgccuccaugaucaagcggu
acgacgagcaccaccaggaccugacccugcugaaggcccuggugcggcagcagcugcccgagaaguacaaggagaucuucuucga
ccaguccaagaacggcuacgccggcuacaucgacggcggcgccucccaggaggaguucuacaaguucaucaagcccauccuggag
aagauggacggcaccgaggagcugcuggugaagcugaaccgggaggaccugcugcggaagcagcggaccuucgacaacggcucca
ucccccaccagauccaccugggcgagcugcacgccauccugcggcggcaggaggacuucuaccccuuccugaaggacaaccgggа
gaagaucgagaagauccugaccuuccggauccccuacuacgugggcccccuggcccggggcaacucccgguucgccuggaugacc
cggaaguccgaggagaccaucacccccuggaacuucgaggaggugguggacaagggcgccuccgcccaguccuucaucgagcgga
ugaccaacuucgacaagaaccugcccaacgagaaggugcugcccaagcacucccugcuguacgaguacuucaccguguacaacga
gcugaccaaggugaaguacgugaccgagggcaugcggaagcccgccuuccuguccggcgagcagaagaaggccaucguggaccug
cuguucaagaccaaccggaaggugaccgugaagcagcugaaggaggacuacuucaagaagaucgagugcuucgacuccguggaga
ucuccggcguggaggaccgguucaacgccucccugggcaccuaccacgaccugcugaagaucaucaaggacaaggacuuccugga
caacgaggagaacgaggacauccuggaggacaucgugcugacccugacccuguucgaggaccgggagaugaucgaggagcggcug
aagaccuacgcccaccuguucgacgacaaggugaugaagcagcugaagcggcggcgguacaccggcuggggccggcugucccgga
agcugaucaacggcauccgggacaagcaguccggcaagaccauccuggacuuccugaaguccgacggcuucgccaaccggaacuu
caugcagcugauccacgacgacucccugaccuucaaggaggacauccagaaggcccaggugucCggccagggcgacucccugcac
gagcacaucgccaaccuggccggcucccccgccaucaagaagggcauccugcagaccgugaaggugguggacgagcuggugaagg
ugaugggccggcacaagcccgagaacaucgugaucgagauggcccgggagaaccagaccacccagaagggccagaagaacucccg
ggagcggaugaagcggaucgaggagggcaucaaggagcugggcucccagauccugaaggagcaccccguggagaacacccagcug
cagaacgagaagcuguaccuguacuaccugcagaacggccgggacauguacguggaccaggagcuggacaucaaccggcuguccg
acuacgacguggaccacaucgugccccaguccuuccugaaggacgacuccaucgacaacaaggugcugacccgguccgacaagaa
ccggggcaaguccgacaacgugcccuccgaggaggugguguaagaagaugaagaacuacuggcggcagcugcugaacgccaagcug
aucacccagcggaaguucgacaaccugaccaaggccgagcggggCggccuguccgagcuggacaaggccggcuucaucaagcggc
agcuggugGagacccggcagaucaccaagcacguggcccagauccuggacucccggaugaacaccaaguacgacgagaacgacaa
gcugauccgggaggugaaggugaucacccugaaguccaagcugguguccgacuuccggaaggacuuccaguucuacaaggugcgg
gagaucaacaacuaccaccacgcccacgacgccuaccugaacgccgugguggggcaccgcccugaucaagaaguaccccaagcugg
aguccgaguucguguacggcgacuacaagguguacgacgugcggaagaugaucgccaaguccgagcaggagaucggcaaggccac
cgccaaguacuucuucuacuccaacaucaugaacuucuucaagaccgagaucacccuggccaacggcgagauccggaagcggccc
cugaucgagaccaacggcgagaccggcgagaucguguggGacaagggccgggacuucgccaccgugcggaaggugcuguccaugc
cccaggugaacaucgugaagaagaccgaggugcagaccggcggcuucuccaaggaguccaucCugcccaagcggaacuccgacaa
gcugaucgcccggaagaaggacugggacccaagaaguacggcggcuucgacucccccaccguggccuacuccgugcuggUggug
gccaagguggagaagggcaaguccaagaagcugaaguccgugaaggagcugcugggcaucaccaucauggagcggucccuccuucg
agaagaaccccaucgacuuccuggaggccaagggcuacaaggaggugaagaaggaccugaucaucaagcugcccaaguacucccu
guucgagcuggagaacggccggaagcggaugcuggccuccgccggcgagcugcagaagggcaacgagcuggcccugcccuccaag
uacgugaacuuccuguaccuggccucccacuacgagaagcugaagggcuccccccgaggacaacgagcagaagcagcuguucgugg
agcagcacaagcacuaccuggacgagaucaucgagcagaucuccgaguucuccaagcgggugauccuggccgacgccaaccugga
caaggugcuguccgccuacaacaagcaccgggacaagcccaucCgggagcaggccgagaacaucauccaccuguucacccugacc
aaccugggcgccccgccgccuucaaguacuucgacaccaccaucgaccggaagcgguacaccuccaccaaggaggugcuggacg
ccacccugauccaccaguccaucaccggccuguacgagacccggaucgaccugucccagcugggcggcgacggcggcggcucccc caagaagaagcggaaggugUgA Open reading
frame 805 AUGgaggccucccccgccuccggcccccggcaccugauggaccccacaucuucaccuccAACUUCAACAACggcAUCggccggC for BC22n with
Hibit ACAAGaccUACCUGUGCUACgagguggagcggCUGGACAACggcaccuccgugAAGAUGGACCAGCACcggggcUUCCUGCACAA tag
CCAGgccAAGAACCUGCUGUGCggcUUCUACggccggCACgccgagCUGcggUUCCUGGACCUGgugcccuccCUGCAGCUGGAC
cccgccCAGAUCUACcgggugaccUGGUUCAUCuccUGGucccccUGCUUCuccUGGggcUGCgccggcgaggugcgggccUUCC
UGCAGgagAACaccCACgugcggCUGcggAUCUUCgccgcccggAUCUACGACUACGACcccCUGUACAAGgaggccCUGCAGAU
GCUGcggGACgccggcgccCAGguguccAUCAUGaccUACGACgagUUCAAGCACUGCUGGGACaccUUCgugGACCACCAGggc
UGCcccUUCCAGcccUGGGACggcCUGGACgagCACuccCAGgccCUGuccggccggCUGcgggccAUCCUGCAGAACCAGggcA
ACuccggcuccgagacccccggcaccuccgaguccgccacccccgaguccgacaagaaguacuccaucggccuggCcaucggcac
caacuccguggggcugggccgugaucaccgacgaguacaaggugcccuccaagaaguucaaggugcugggcaacaccgaccggcac
uccaucaagaagaaccugaucggcgcccugcuguucgacuccggcgagaccgccgaggccacccggcugaagcggaccgcccggc

ggcgguacacccggcggaagaaccggaucugcuaccugcaggagaucuucuccaacgagauggccaagguggacgacuccuucuu
ccaccggcuggaggaguccuuccugguggaggaggacaagaagcacgagcggcacccccaucuucggcaacaucguggacgaggug
gccuaccacgagaaguaccccaccaucuaccaccugcggaagaagcugguggacuccaccgacaaggccgaccugcggcugaucu
accuggcccuggcccacaugaucaaguuccggggccacuuccugaucgagggcgaccugaaccccgacaacuccgacguggacaa
gcuguucauccagcuggugcagaccuacaaccagcuguucgaggagaaccccaucaacgccuccggcguggacgccaaggccauc
cuguccgcccggcuguccaagucccggcggcuggagaaccugaucgcccagcugcccggcgagaagaagaacggccuguucggca
accugaucgcccuguccccugggccugacccccaacuucaaguccaacuucgaccuggccgaggacgccaagcugcagcuguccaa
ggacaccuacgacgacgaccuggacaaccugcuggcccagaucggcgaccaguacgccgaccuguuccuggccgccaagaaccug
uccgacgccauccugcuguccgacauccugcgggugaacaccgagaucaccaaggccccccuguccgccuccaugaucaagcggu
acgacgagcaccaccaggaccugacccugcugaaggcccuggugcggcagcagcugcccgagaaguacaaggagaucuucuucga
ccaguccaagaacggcuacgccggcuacaucgacggcggcgccucccaggaggaguucuacaaguucaucaagcccauccuggag
aagauggacggcaccgaggagcugcuggugaagcugaaccgggaggaccugcugcggaagcagcggaccuucgacaacggcucca
ucccccaccagauccaccugggcgagcugcacgccauccugcggcggcaggaggacuucuaccccuuccugaaggacaaccgggа
gaagaucgagaagauccugaccuuccggauccccuacuacgugggcccccuggcccggggcaacucccgguucgccuggaugacc
cggaaguccgaggagaccaucacccccuggaacuucgaggaggugguggacaagggcgccuccgcccagcuccuucaucgagcgga
ugaccaacuucgacaagaaccugcccaacgagaaggugcugcccaagcacucccugcuguacgaguacuucaccguguacaacga
gcugaccaaggugaaguacgugaccgagggcaugcggaagcccgccuuccuguccggcgagcagaagaaggccaucguggaccug
cuguucaagaccaaccggaaggugaccgugaagcagcugaaggaggacuacuucaagaagaucgagugcuucgacuccguggaga
ucuccggcguggaggaccgguucaacgccuccccugggcaccuaccacgaccugcugaagaucaucaaggacaaggacuuccugga
caacgaggagaacgaggacauccuggaggacaucgugcugacccugacccuguucgaggaccgggagaugaucgaggagcggcug
aagaccuacgcccaccuguucgacgacaaggugaugaagcagcugaagcggcggcgguacaccggcuggggccggcugucccgga
agcugaucaacggcaucCgggacaagcaguccggcaagaccaucuggacuuccugaaguccgacggcuucgccaaccggaacuu
caugcagcugauccacgacgacucccugaccuucaaggaggacauccagaaggcccaggugucCggccagggcgacucccugcac
gagcacaucgccaaccuggccggcucccccgccaucaagaagggcauccugcagaccgugaaggugguggacgagcuggugaagg
ugaugggccggcacaagcccgagaacaucgugaucgagauggcccgggagaaccagaccacccagaagggccagaagaacucccg
ggagcggaugaagcggaucgaggagggcaucaaggagcugggcucccagauccugaaggagcaccccguggagaacacccagcug
cagaacgagaagcuguaccuguacuaccugcagaacggccgggacauguacguggaccaggagcuggacaucaaccggcuguccg
acuacgacguggaccacaucgugccccaguccuuccugaaggacgacuccaucgacaacaaggugcugacccgguccgacaagaa
ccggggcaaguccgacaacgugcccuccgaggaggugguguaagaagaugaagaacuacuggcggcagcugcugaacgccaagcug
aucacccagcggaaguucgacaaccugaccaaggccgagcggggcggccuguccgagcuggacaaggccggcuucaucaagcggc
agcugguggagacccggcagaucaccaagcacguggcccagauccuggacucccggaugaacaccaaguacgacgagaacgacaa
gcugauccgggaggugaaggugaucacccugaaguccaagcugguguccgacuuccggaaggacuuccaguucuacaaggugcgg
gagaucaacaacuaccaccacgcccacgacgccuaccugaacgccgugguggggcaccgcccugaucaagaaguaccccaagcugg
aguccgaguucguguacggcgacuacaagguguacgacgugcggaagaugaucgccaaguccgagcaggagaucggcaaggccac
cgccaaguacuucuucuacuccaacaucaugaacuucuucaagaccgagaucacccuggccaacggcgagauccggaagcggccc
cugaucgagaccaacggcgagaccggcgagaucguguggggacaagggccgggacuucgccaccgugcggaaggugcuguccaugc
cccaggugaacaucgugaagaagaccgaggugcagaccggcggcuucuccaaggaguccaucCugcccaagcggaacuccgacaa
gcugaucgcccggaagaaggacugggaccccaagaaguacggcggcuucgacucccccaccguggccuacuccgugcugguggug
gccaagguggagaagggcaaguccaagaagcugaaguccgugaaggagcugcugggcaucaccaucauggagcgguccuccuucg
agaagaaccccaucgacuuccuggaggccaagggcuacaaggaggugaagaaggaccugaucaucaagcugcccaaguacucccu
guucgagcuggagaacggccggaagcggaugcuggccuccgccggcgagcugcagaagggcaacgagcuggcccugcccuccaag
uacgugaacuuccuguaccuggccucccacuacgagaagcugaagggcuccccccgaggacaacgagcagaagcagcuguucgugg
agcagcacaagcacuaccuggacgagaucaucgagcagaucuccgaguucuccaagcgggugauccuggccgacgccaaccugga
caaggugcuguccgccuacaacaagcaccgggacaagcccaucCgggagcaggccgagaacaucauccaccuguucacccugacc
aaccugggcgcccccgccgccuucaaguacuucgacaccaccaucgaccggaagcgguacaccuccaccaaggaggugcuggacg
ccacccugauccaccaguccaucaccggccuguacgagacccggaucgaccugucccagcugggcggcgacggcggcggcucccc
caagaagaagcggaagguguccgaguccgccacccccgaguccguguccggcuggcggcuguucaagaagaucuccUgA ORF encoding Spy Cas9 806
ATGgaggccTcccccgccTccggcccccggcaccTgaTggacccccacaTcTTcaccTccAACTTCAACAACggcATCggccggC
ACAAGaccTACCTGTGCTACgaggTggagcggCTGGACAACggcaccTccgTgAAGATGGACCAGCACcggggcTTCCTGCACAA
CCAGgccAAGAACCTGCTGTGCggcTTCTACggccggCACgccgagCTGcggTTCCTGGACCTGgTgcccTccCTGCAGCTGGAC
cccgccCAGATCTACcgggTgaccTGGTTCATCTccTGGTCCCCCTGCTTCTccTGGggcTGCgccggcgaggTgcgggccTTCC
TGCAGgagAACaccCACgTgcggCTGcggATCTTCgccgcccggATCTACGACTACGACcccCTGTACAAGgaggccCTGCAGAT
GCTGcggGACgccggcgccCAGgTgTccATCATGaccTACGACgagTTCAAGCACTGCTGGGACaccTTCgTgGACCACCAGggc
TGCcccTTCCAGcccTGGGACggcCTGGACgagCACTccCAGgccCTGTccggccggCTGcgggccATCCTGCAGAACCAGggcA
ACTccggcTccgagacccccggcaccTccgagTccgccacccccgagTccgacaagaagTacTccaTcggccTggCcaTcggcac
caacTccgTgggcTgggccgTgaTcaccgacgagTacaaggTgcccTccaagaagTTcaaggTgcTgggcaacaccgaccggcac
TccaTcaagaagaaccTgaTcggcgcccTgcTgTTcgacTccggcgagaccgccgaggccacccggcTgaagcggaccgcccggc
ggcggTacacccggcggaagaaccggaTcTgcTaccTgcaggagaTcTTcTccaacgagaTggccaaggTggacgacTccTTcTT
ccaccggcTggaggagTccTTccTggTggaggaggacaagaagcacgagcggcaccccaTcTTcggcaacaTcgTggacgaggTg
gccTaccacgagaagTaccccaccaTcTaccaccTgcggaagaagcTggTggacTccaccgacaaggccgaccTgcggcTgaTcT
accTggcccTggcccacaTgaTcaagTTccggggccacTTccTgaTcgagggcgaccTgaaccccgacaacTccgacgTggacaa
gcTgTTcaTccagcTggTgcagaccTacaaccagcTgTTcgaggagaaccccaTcaacgccTccggcgTggacgccaaggccaTc
cTgTccgcccggcTgTccaagTcccggcggcTggagaaccTgaTcgcccagcTgcccggcgagaagaagaacggccTgTTcggca
accTgaTcgcccTgTcccTgggccTgacccccaacTTcaagTccaacTTcgaccTggccgaggacgccaagcTgcagcTgTccaa
ggacaccTacgacgacgaccTggacaaccTgcTggcccagaTcggcgaccagTacgccgaccTgTTccTggccgccaagaaccTg
TccgacgccaTccTgcTgTccgacaTccTgcgggTgaacaccgagaTcaccaaggccccccTgTccgccTccaTgaTcaagcggT
acgacgagcaccaccaggaccTgacccTgcTgaaggcccTggTgcggcagcagcTgcccgagaagTacaaggagaTcTTcTTcga
ccagTccaagaacggcTacgccggcTacaTcgacggcggcgccTcccaggaggagTTcTacaagTTcaTcaagcccaTccTggag
aagaTggacggcaccgaggagcTgcTggTgaagcTgaaccgggaggaccTgcTgcggaagcagcggaccTTcgacaacggcTcca
TcccccaccagaTccaccTgggcgagcTgcacgccaTccTgcggcggcaggaggacTTcTaccccTTccTgaaggacaaccgggа
gaagaTcgagaagaTccTgaccTTccggaTccccTacTacgTgggcccccTggcccggggcaacTcccggITcgccTggaTgacc
cggaagTccgaggagaccaTcacccccTggaacTTcgaggaggTggTggacaagggcgccTccgcccagTccTTcaTcgagcgga
TgaccaacTTcgacaagaaccTgcccaacgagaaggTgcTgcccaagcacTcccTgcTgTacgagTacTTcaccgTgTacaacga

gcTgaccaaggTgaagTacgTgaccgagggcaTgcggaagcccgccTTccTgTccggcgagcagaagaaggccaTcgTggaccTg
cTgTTcaagaccaaccggaaggTgaccgTgaagcagcTgaaggaggacTacTTcaagaagaTcgagTgcTTcgacTccgTggaga
TcTccggcgTggaggaccggTTcaacgccTcccTgggcaccTaccacgaccTgcTgaagaTcaTcaaggacaaggacTTccTgga
caacgaggagaacgaggacaTccTggaggacaTcgTgcTgacccTgacccTgTTcgaggaccgggagaTgaTcgaggagcggcTg
aagaccTacgcccaccTgTTcgacgacaaggTgaTgaagcagcTgaagcggcggcggTacaccggcTggggccggcTgTcccgga
agcTgaTcaacggcaTccgggacaagcagTccggcaagaccaTccTggacTTccTgaagTccgacggcTTcgccaaccggaacTT
caTgcagcTgaTccacgacgacTcccTgaccTTcaaggaggacaTccagaaggcccaggTgTccggccagggcgacTcccTgcac
gagcacaTcgccaaccTggccggcTccccccgccaTcaagaagggcaTccTgcagaccgTgaaggTggTggacgagcTggTgaagg
TgaTgggccggcacaagcccgagaacaTcgTgaTcgagaTggcccgggagaaccagaccacccagaagggccagaagaacTcccg
ggagcggaTgaagcggaTcgaggagggcaTcaaggagcTgggcTcccagaTccTgaaggagcaccccgTggagaacacccagcTg
cagaacgagaagcTgTaccTgTacTaccTgcagaacggccgggacaTgTacgTggaccaggagcTggacaTcaaccggcTgTccg
acTacgacgTggaccacaTcgTgccccagTccTTccTgaaggacgacTccaTcgacaacaaggTgcTgacccggTccgacaagaa
ccggggcaagTccgacaacgTgcccTccgaggaggTggTgaagaagaTgaagaacTacTggcggcagcTgcTgaacgccaagcTg
aTcacccagcggaagTTcgacaaccTgaccaaggccgagcggggcggccTgTccgagcTggacaaggccggcTTcaTcaagcggc
agcTggTggagacccggcagaTcaccaagcacgTggcccagaTccTggacTcccggaTgaacaccaagTacgacgagaacgacaa
gcTgaTccgggaggTgaaggTgaTcacccTgaagTccaagcTggTgTccgacTTccggaaggacTTccagTTcTacaaggTgcgg
gagaTcaacaacTaccaccacgcccacgacgccTaccTgaacgccgTggTgggcaccgcccTgaTcaagaagTaccccaagcTgg
agTccgagTTcgTgTacggcgacTacaaggTgTacgacgTgcggaagaTgaTcgccaagTccgagcaggagaTcggcaaggccac
cgccaagTacTTcTTcTacTccaacaTcaTgaacTTcTTcaagaccgagaTcacccTggccaacggcgagaTccggaagcggccc
cTgaTcgagaccaacggcgagaccggcgagaTcgTgTgggacaagggccgggacTTcgccaccgTgcggaaggTgcTgTccaTgc
cccaggTgaacaTcgTgaagaagaccgaggTgcagaccggcggcTTcTccaaggagTccaTccTgcccaagcggaacTccgacaa
gcTgaTcgcccggaagaaggacTgggaccccaagaagTacggcggcTTcgacTccccaccgTggccTacTccgTgcTggTggTg
gccaaggTggagaagggcaagTccaagaagcTgaagTccgTgaaggagcTgcTgggcaTcaccaTcaTggagcggTccTccTTcg
agaagaaccccaTcgacTTccTggaggccaaggcTacaaggaggTgaagaaggaccTgaTcaTcaagcTgcccaagTacTcccT
gTTcgagcTggagaacggccggaagcggaTgcTggccTccgccggcgagcTgcagaagggcaacgagcTggcccTgcccTccaag
TacgTgaacTTccTgTaccTggccTcccacTacgagaagcTgaaggcTcccccgaggacaacgagcagaagcagcTgTTcgTgg
agcagcacaagcacTaccTggacgagaTcaTcgagcagaTcTccgagTTcTccaagcgggTgaTccTggccgacgccaaccTgga
caaggTgcTgTccgccTacaacaagcaccgggacaagcccaTccgggagcaggccgagaacaTcaTccaccTgTTcacccTgacc
aaccTgggcgccccgccgccTTcaagTacTTcgacaccaccaTcgaccggaagcggTacaccTccaccaaggaggTgcTggacg
ccacccTgaTccaccagTccaTcaccggccTgTacgagacccggaTcgaccTgTcccagcTgggcggcgacggcggcggcTcccc caagaagaagcggaaggTgTgA Open
reading frame 807
AUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAAAGCAGCUGG
for UGI
UCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCUGGUCCACAC
AGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUGGUCAUCCAG
GACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGA Open reading frame 808
AUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAAGCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGG
for UGI
UGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUGGUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCU
GCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGGUGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCC
GGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUCCCCCAAGAAGAAGCGGAAGGUGGAGUGA Amino acid
sequence 809
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI for
Cas9 encoded by
FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI SEQ
ID Nos. 801-802
EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR
QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR
QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR
ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK
MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDELEAKGYKEV
KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI
DLSQLGGDGGGSPKKKRKV Amino acid sequence 810
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI for
Cas9 with Hibit
FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI tag
EGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVR
QQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR
QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR
ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL

DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK
MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV
KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI
DLSQLGGDGGGSPKKKRKVSESATPESVSGWRLFKKIS Amino acid sequence 811
MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGELHNQAKNLLCGFYGRHAELRFLDLVPSLQLD
for BC22n
PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG
CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRH
SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEV
AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKERGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI
LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT
RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL
LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNEMQLIHDDSLTFKEDIQKAQVSGQGDSLH
EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP
LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK
YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT
NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKV* Amino acid sequence 812
MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLD
for BC22n with Hibit
PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG tag
CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKEKVLGNTDRH
SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESELVEEDKKHERHPIFGNIVDEV
AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI
LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTEDNGSIPHQIHLGELHAILRRQEDFYPELKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT
RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL
LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH
EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP
LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK
YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLETLT
NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKVSESATPESVSGWRLFKKIS 813 Not
used Amino acid sequence 814
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS for UGI
GGSKRTADGSEFESPKKKRKVE 815-816 Not used Open reading frame 817
AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGA
for Cas9
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
GGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCA
AGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGA
AGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCA
GGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGA
AGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAG
CGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUC
AGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACAACAA
UCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAG
AAUCGACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG Open reading frame 818
AUGGAAGCAAGCCCGGCAAGCGGACCGAGACACCUGAUGGACCCGCACAUCUUCACAAGCAACUUCAACAACGGAAUCGGAAGAC
for BC22
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
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 819
AUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAAAGCAGCUGG
for UGI
UCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCUGGUCCACAC
AGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUGGUCAUCCAG
GACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGA mRNA encoding UGI 821
GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAA
GCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGGUGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUG
GUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCUGCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGG
UGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCCGGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUC
CCCCAAGAAGAAGCGGAAGGUGGAGUGAUAGCUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAA
CUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCG
AGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAA
AAAACGAAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUAAAAAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAA
AAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAUC
UAAAAAAAAAAAAACGAAAAAAAAAAAAACCCAAAAAAAAAAAAAGACAAAAAAAAAAAAAUAGAAAAAAAAAAAAAGUUAAAAAAAAAA
AACUGAAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG mRNA encoding 822
GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA
Nme2Cas9 base editor
GGUGGAGGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCUCCGGCGGCGGCGAGGCCUCC
CCCGCCUCCGGCCCCCGGCACCUGAUGGACCCCCACAUCUUCACCUCCAACUUCAACAACGGCAUCGGCCGGCACAAGACCUACC
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
CUGGGCAAGGAGAUCCGGCCCUGCCGGCUGAAGAAGCGGCCCCCCGUGCGGUAGUGACUAGCACCAGCCUCAAGAACACCCGAAU
GGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAA
AAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAUGGAAAAAAAAAAAACGGAAAAAAAAAAAGGUAAAAAAAAAAAAU
AUAAAAAAAAAAACAUAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAACUCAAAAAAAAAAAGAUAAAAAAAAAA
ACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAGGGAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAAAUGCAAAAAAA
AAAAAUCGAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAA
AAAAAAAAGUUAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG Open reading frame 823
ATGGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAATCGCATCGGTCGGATGGGCAATGGTCGAAA
for NmelCas9
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
Open reading frame 824
ATGACCAACCTGTCCGACATCATCGAGAAGGAGACCGGCAAGCAGCTGGTGATCCAGGAGTCCATCCTGATGCTGCCCGAGGAGG
for UGI encoded by
TGGAGGAGGTGATCGGCAACAAGCCCGAGTCCGACATCCTGGTGCACACCGCCTACGACGAGTCCACCGACGAGAACGTGATGCT
SEQ NO: 821
GCTGACCTCCGACGCCCCCGAGTACAAGCCCTGGGCCCTGGTGATCCAGGACTCCAACGGCGAGAACAAGATCAAGATGCTGTCC
GGCGGCTCCAAGCGGACCGCCGACGGCTCCGAGTTCGAGTCCCCCAAGAAGAAGCGGAAGGTGGAGTGATAG mRNA
encoding Nme2 825
GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA
Cas9
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
CCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAA
AAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAA
AAAAAAAAACUCAAAAAAAAAAAAGAUAAAAAAAAAAAAAACCUAAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACG
CAAAAAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAA
AACCCAAAAAAAAAAAAAGACAAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAA
AAAAAAUCUAG mRNA encoding Nme2 826
GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACGGCUCCGGCGGCGGCUCCCCCAAGAAGAAGCGGAA
Cas9 with Hibit tag
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
GCGGUCCGAGUCCGCCACCCCCGAGUCCGUGUCCGGCUGGCGGCUGUUCAAGAAGAUCUCCUAGCUAGCACCAGCCUCAAGAACA
CCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUC
CUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAUGGAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAA
AAAAAAAUAUAAAAAAAAAAAACAUAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAGAUA
AAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAA
AUGCAAAAAAAAAAAAAUCGAAAAAAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAA
AAAAAUAGAAAAAAAAAAAAAGUUAAAAAAAAAAAAACUGAAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG 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
AGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG
CCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTC
TCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCT
TCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGA
GCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG
AAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCG
AGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGG
CTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAG
TTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGG
AGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGA
CCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATC
GACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA ORF encoding Spy Cas9 828
aagctcagaataaacgctcaactttggccggatctgccaccATGGACGGCTCCGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGT
GGAGGACAAGCGGCCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCGGCTCCGGCGGCGGCGAGGCCTCCCCC
GCCTCCGGCCCCCGGCACCTGATGGACCCCCACATCTTCACCTCCAACTTCAACAACGGCATCGGCCGGCACAAGACCTACCTGT
GCTACGAGGTGGAGCGGCTGGACAACGGCACCTCCGTGAAGATGGACCAGCACCGGGGCTTCCTGCACAACCAGGCCAAGAACCT
GCTGTGCGGCTTCTACGGCCGGCACGCCGAGCTGCGGTTCCTGGACCTGGTGCCCTCCCTGCAGCTGGACCCCGCCCAGATCTAC
CGGGTGACCTGGTTCATCTCCTGGTCCCCCTGCTTCTCCTGGGGCTGCGCCGGCGAGGTGCGGGCCTTCCTGCAGGAGAACACCC
ACGTGCGGCTGCGGATCTTCGCCGCCCGGATCTACGACTACGACCCCCTGTACAAGGAGGCCCTGCAGATGCTGCGGGACGCCGG
CGCCCAGGTGTCCATCATGACCTACGACGAGTTCAAGCACTGCTGGGACACCTTCGTGGACCACCAGGGCTGCCCCTTCCAGCCC
TGGGACGGCCTGGACGAGCACTCCCAGGCCCTGTCCGGCCGGCTGCGGGCCATCCTGCAGAACCAGGGCAACTCCGGCTCCGAGA
CCCCCGGCACCTCCGAGTCCGCCACCCCCGAGTCCGCAGCGTTCAAACCAAATcccatcaactacatcctgggcctggccatcgg
catcgcctccgtgggctgggccatggtggagatcgacgaggaggagaaccccatccggctgatcgacctgggcgtgcgggtgttc
gagcgggccgaggtgcccaagaccggcgactccctggccatggcccggcggctggcccggtccgtgcggcggctgacccggcggc

gggcccaccggctgctgcgggcccggcggctgctgaagcgggagggcgtgctgcaggccgccgacttcgacgagaacggcctgat
caagtccctgcccaacacccccctggcagctgcgggccgccgccctggaccggaagctgacccccctggagtggtccgccgtgctg
ctgcacctgatcaagcaccggggctacctgtcccagcggaagaacgagggcgagaccgccgacaaggagctgggcgccctgctga
agggcgtggccaacaacgcccacgccctgcagaccggcgacttccggacccccgccgagctggccctgaacaagttcgagaagga
gtccggccacatccggaaccagcggggcgactactcccacaccttctcccggaaggacctgcaggccgagctgatcctgctgttc
gagaagcagaaggagttcggcaacccccacgtgtccggcggcctgaaggagggcatcgagaccctgctgatgacccagcggcccg
ccctgtccggcgacgccgtgcagaagatgctgggccactgcaccttcgagcccgccgagcccaaggccgccaagaacacctacac
cgccgagcggttcatctggctgaccaagctgaacaacctgcggatcctggagcagggctccgagcggccccgaccgacaccgag
cgggccaccctgatggacgagccctaccggaagtccaagctgacctacgcccaggcccggaagctgctgggcctggaggacaccg
ccttcttcaagggcctgcggtacggcaaggacaacgccgaggcctccaccctgatggagatgaaggcctaccacgccatctcccg
ggccctggagaaggagggcctgaaggacaagaagtccccccctgaacctgtcctccgagctgcaggacgagatcggcaccgccttc
tccctgttcaagaccgacgaggacatcaccggccggctgaaggaccgggtgcagcccgagatcctggaggccctgctgaagcaca
tctccttcgacaagttcgtgcagatctccctgaaggccctgcggcggatcgtgcccctgatggagcagggcaagcggtacgacga
ggcctgcgccgagatctacggcgaccactacggcaagaagaacaccgaggagaagatctacctgccccccatccccgccgacgag
atccggaaccccgtggtgctgcgggccctgtcccaggcccggaaggtgatcaacggcgtggtgcggcggtacggctcccccgccc
ggatccacatcgagaccgcccgggaggtgggcaagtccttcaaggaccggaaggagatcgagaagcggcaggaggagaaccggaa
ggaccgggagaaggccgccgccaagttccgggagtacttccccaacttcgtgggcgagcccaagtccaaggacatcctgaagctg
cggctgtacgagcagcagcacggcaagtgcctgtactccggcaaggagatcaacctggtgcggctgaacgagaagggctacgtgg
agatcgaccacgccctgcccttctcccggacctgggacgactccttcaacaacaaggtgctggtgctgggctccgagaaccagaa
caagggcaaccagacccccctacgagtacttcaacggcaaggacaactcccgggagtggcaggagttcaaggcccgggtggagacc
tcccggttcccccggtccaagaagcagcggatcctgctgcagaagttcgacgaggacggcttcaaggagtgcaacctgaacgaca
cccggtacgtgaaccgcttcctgtgccagttcgtggccgaccacatcctgctgaccggcaagggcaagcggcgggtgttcgcctc
caacggccagatcaccaacctgctgcggggcttctggggcctgcggaaggtgcgggccgagaacgaccggcaccacgccctggac
gccgtggtggtggcctgctccaccgtggccatgcagcagaagatcacccggttcgtgcggtacaaggagatgaacgccttcgacg
gcaagaccatcgacaaggagaccggcaaggtgctgcaccagaagacccacttcccccagccctgggagttcttcgcccaggaggt
gatgatccgggtgttcggcaagcccgacggcaagcccgagttcgaggaggccgacaccccgagaagctgcggaccctgctggcc
gagaagctgtcctcccggcccgaggccgtgcacgagtacgtgaccccctgttcgtgtcccgggcccccaaccggaagatgtccg
gcgcccacaaggacaccctgcggtccgccaagcggttcgtgaagcacaacgagaagatctccgtgaagcgggtgtggctgaccga
gatcaagctggccgacctggagaacatggtgaactacaagaacggccgggagatcgagctgtacgaggccctgaaggcccggctg
gaggcctacggcggcaacgccaagcaggccttcgaccccaaggacaaccccttctacaagaagggcggccagctggtgaaggccg
tgcgggtggagaagacccaggagtccggcgtgctgctgaacaagaagaacgcctacaccatcgccgacaacggcgacatggtgcg
ggtggacgtgttctgcaaggtggacaagaagggcaagaaccagtacttcatcgtgcccatctacgcctggcaggtggccgagaac
atcctgcccgacatcgactgcaagggctaccggatcgacgactcctacaccttctgcttctccctgcacaagtacgacctgatcg
ccttccagaaggacgagaagtccaaggtggagttcgcctactacatcaactgcgactcctccaacggccggttctacctggcctg
gcacgacaagggctccaaggagcagcagttccggatctccacccagaacctggtgctgatccagaagtaccaggtgaacgagctg
ggcaaggagatccggccctgccggctgaagaagcggccccccgtgcggtagTGActagcaccagcctcaagaacacccgaatgga
gtctctaagctacataataccaacttacactttacaaaatgttgtcccccaaaatgtagccattcgtatctgctcctaataaaaa gaaagtttcttcacattct 829-899 Not used Exemplary XTEN linker 900 SGSETPGTSESATPES sequence Exemplary XTEN linker 901 SGSETPGTSESA sequence Exemplary XTEN linker 902 SGSETPGTSESATPEGGSGGS sequence Exemplary NLS 903 PKKKRKV sequence Exemplary NLS 904 PKKKRRV sequence Exemplary bipartite 905 KRPAATKKAGQAKKKK NLS sequence exemplary XTEN 906 SGSETPGTSESATPES exemplary XTEN 907 SGSETPGTSESA exemplary XTEN 908 SGSETPGTSESATPEGGSGGS amino acid sequence 909 GGS for exemplary linker amino acid sequence 910 GGGGS for exemplary linker amino acid sequence 911 EAAAK for exemplary linker amino acid sequence 912 SEGSA for exemplary linker amino acid sequence 913 SEGSAGTST for exemplary linker amino acid sequence 914 GGGGSGGGGS for exemplary linker amino acid sequence 915 GGGGSEAAAK for exemplary linker amino acid sequence 916 EAAAKGGGGS for exemplary linker amino acid sequence 917 EAAAKEAAAK for exemplary linker amino acid sequence 918 SEGSAGTSTESEGSA for exemplary linker amino acid sequence 919 GGGGSGGGGSGGGGS for exemplary linker amino acid sequence 920 GGGGSGGGGSEAAAK for exemplary linker amino acid sequence 921 GGGGSEAAAKGGGGS for exemplary linker amino acid sequence 922 EAAAKGGGGSEAAAK for exemplary linker amino acid sequence 923 EAAAKEAAAKGGGGS for exemplary linker amino acid sequence 924 SEGSAGTSTESEGSAGTSTE for exemplary linker amino acid sequence 925 GGGGSGGGGSGGGGSEAAAK for exemplary linker amino acid sequence 926 GGGGSGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 927 GGGGSEAAAKGGGGSEAAAK for exemplary linker amino acid sequence 928 GGGGSEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 929 GGGGSEAAAKEAAAKEAAAK for exemplary linker amino acid sequence 930 EAAAKGGGGSGGGGSGGGGS for exemplary linker amino acid sequence 931 EAAAKGGGGSGGGGSEAAAK for exemplary linker amino acid sequence 932 EAAAKGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 933 EAAAKGGGGSEAAAKEAAAK for exemplary linker amino acid sequence 934 EAAAKEAAAKGGGGSGGGGS for exemplary linker amino acid sequence 935 EAAAKEAAAKGGGGSEAAAK for exemplary linker amino acid sequence 936 EAAAKEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 937 SEGSAGTSTESEGSAGTSTESEGSA for exemplary linker amino acid sequence 938 GGGGSGGGGSGGGGSGGGGSGGGGS for exemplary linker amino acid sequence 939 GGGGSGGGGSGGGGSGGGGSEAAAK for exemplary linker amino acid sequence 940 GGGGGGGGSGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 941 GGGGSGGGGSGGGGSEAAAKEAAAK for exemplary linker amino acid sequence 942 GGGGSGGGGSEAAAKGGGGSGGGGS for exemplary linker amino acid sequence 943 GGGGSGGGGSEAAAKGGGGSEAAAK for exemplary linker amino acid sequence 944 GGGGSGGGGSEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 945 GGGGSGGGGSEAAAKEAAAKEAAAK for exemplary linker amino acid sequence 946 GGGGSEAAAKGGGGSGGGGSGGGGS for exemplary linker amino acid sequence 947 GGGGSEAAAKGGGGSGGGGSEAAAK for exemplary linker amino acid sequence 948 GGGGSEAAAKGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 949 GGGGSEAAAKGGGGSEAAAKEAAAK for exemplary linker amino acid sequence 950 GGGGSEAAAKEAAAKGGGGSGGGGS for exemplary linker amino acid sequence 951 GGGGSEAAAKEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 952 GGGGSEAAAKEAAAKEAAAKEAAAK for exemplary linker amino acid sequence 953 EAAAKGGGGSGGGGSGGGGSGGGGS for exemplary linker amino acid sequence 954 EAAAKGGGGSGGGGGGGGSEAAAK for exemplary linker amino acid sequence 955 EAAAKGGGGSGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 956 EAAAKGGGGSGGGGSEAAAKEAAAK for exemplary linker amino acid sequence 957 EAAAKGGGGSEAAAKGGGGSGGGGS for exemplary linker amino acid sequence 958

EAAAKGGGGSEAAAKGGGGSEAAAK for exemplary linker amino acid sequence 959 EAAAKGGGGSEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 960 EAAAKGGGGSEAAAKEAAAKEAAAK for exemplary linker amino acid sequence 961 EAAAKEAAAKGGGGSEAAAKGGGGS for exemplary linker amino acid sequence 962 EAAAKEAAAKGGGGSEAAAKEAAAK for exemplary linker amino acid sequence 963 EAAAKEAAAKEAAAKGGGGSEAAAK for exemplary linker amino acid sequence 964 EAAAKEAAAKEAAAKEAAAKGGGGS for exemplary linker amino acid sequence 965 EAAAKEAAAKEAAAKEAAAKEAAAK for exemplary linker amino acid sequence 966 GTKDSTKDIPETPSKD for exemplary linker amino acid sequence 967 GRDVRQPEVKEEKPES for exemplary linker amino acid sequence 968 EGKSSGSGSESKSTAG for exemplary linker amino acid sequence 969 TPGS PAGS PTSTEEGT for exemplary linker amino acid sequence 970 GSEPATSGSETPGTST for exemplary linker 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
UGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAA
CGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUG
CGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCA
AGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGA
CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGC
GGCUUCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACG
GCGGCUUCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU
GAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAG
GAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCG
CCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCU
GAAGGGCUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC
UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCA
UCCGGGAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCAC
CAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACC
CGGAUCGACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUGACUAGCACCAGCCUCAAGAA
CACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGC
UCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAAAAAAACGGAAAAAAAAAAAAGGUAAA
AAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAGA
UAAAAAAAAAAAACCUAAAAAAAAAAAAAUGUAAAAAAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAA
AAAUGCAAAAAAAAAAAAUCGAAAAAAAAAAAAUCUAAAAAAAAAAAACGAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAA
AAAAAAAUAGAAAAAAAAAAAGUUAAAAAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG mRNA encoding BC22n 973

GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGAGGCCUCCCCCGCCUCCGGCCCCCGGCACCUGAUGGA
with HiBit tag
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
UGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAA
CGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUG
CGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCA
AGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGA
CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGC
GGCUUCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACG
GCGGCUUCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU
GAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAG
GAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCG
CCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCU
GAAGGGCUCCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC
UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCA
UCCGGGAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCAC
CAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACC
CGGAUCGACCUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCCGAGU
CCGUGUCCGGCUGGCGGCUGUUCAAGAAGAUCUCCUGACUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUA
AUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAU
UCUCUCGAGAAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUA
AAAAAAAAAAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUAAAAAAAAAAAAACCUAAAAAAAAAAAA
UGUAAAAAAAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAAAUGCAAAAAAAAAAAAUCGAAAAAAA
AAAAAUCUAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAA
AAAAAAAAACUGAAAAAAAAAAAAUUUAAAAAAAAAAAUCUAG 974 Not used mRNA encoding UGI 975
GGGAGACCCAAGCUGGCUAGCUCCCGCAGUCGGCGUCCAGCGGCUCUGCUUGUUCGUGUGUGUGUCGUUGCAGGCCUUAUUCGGA
UCCGCCACCAUGGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCACAAACCUGUCGGACAUCAUCGAAAAGGAAACAGGAA
AGCAGCUGGUCAUCCAGGAAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAACAAGCCGGAAUCGGACAUCCU
GGUCCACACAGCAUACGACGAAUCGACAGACGAAAACGUCAUGCUGCUGACAUCGGACGCACCGGAAUACAAGCCGUGGGCACUG
GUCAUCCAGGACUCGAACGGAGAAAACAAGAUCAAGAUGCUGUGAUAGUCUAGACAUCACAUUUAAAAGCAUCUCAGCCUACCAU
GAGAAUAAGAGAAAGAAAAUGAAGAUCAAUAGCUUAUUCAUCUCUUUUUCUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAAA
AAACAUAAAUUUCUUUAAUCAUUUUGCCUCUUUUCUCUGUGCUUCAAUUAAUAAAAAAUGGAAAGAACCUCGAGUCUAG
Exemplary amino acid 976

MDGSGGGSPKKKRKVEDKRPAATKKAGQAKKKKGGSGGGEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVK
sequence for NLS-NLS-
MDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDY
APOBEC3A-L070-
DPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGNGTKDSTKDIPETPSKDAA
Nme2D16A
FKPNPINYILGLAIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRLLRARRLLKR
EGVLQAADFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVANNAHALQTGD
FRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHC
TFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAE
ASTLMEMKAYHAISRALEKEGLKDKKSPLNLSSELQDEIGTAFSLEKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKAL
RRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSF
KDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLVRLNEKGYVEIDHALPFSRTWDD
SFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKECNLNDTRYVNRFLCQFVAD
HILLTGKGKRRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKVLHQ
KTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGAHKDTLRSAKRFV
KHNEKISVKRVWLTEIKLADLENMVNYKNGREIELYEALKARLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQESGVLLN
KKNAYTIADNGDMVRVDVFCKVDKKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSYTFCFSLHKYDLIAFQKDEKSKVEFAY
YINCDSSNGRFYLAWHDKGSKEQQFRISTQNLVLIQKYQVNELGKEIRPCRLKKRPPVR Amino acid sequence 977
MSSETGPVAVDPTLRRRIEPHEFEVFEDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSIT for BE3
WFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRY
PHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGTSESATPESDKKYSIGLAI
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDS
FFHRLEESELVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV
DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNFDLAEDAKLQL
SKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIF
FDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN
REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDF
LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR
NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD
KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN
DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADAN
LDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGG
STNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS
GGSPKKKRKV Amino acid sequence 978
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSIT for BE3
WFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRY
PHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGTSESATPESDKKYSIGLAI
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDS
FFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKERGHFLIEGDLNPDNSDV
DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSNEDLAEDAKLQL
SKDTYDDDLDNLLAQIGDQYADLELAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIF
FDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN
REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYETVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRENASLGTYHDLLKIIKDKDE
LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLEDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR
NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD
KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN
DKLIREVKVITLKSKLVSDERKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADAN
LDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYEDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGG
STNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLS
GGSPKKKRKV Amino acid sequence 979
MEASPASGPRHLMDPHIFTSNENNGIGRHKTYLCYEVERLDNGTSVKMDQHRGELHNQAKNLLCGFYGRHAELRELDLVPSLQLD
for BC22 with 2x UGI
PAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQG
CPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRH
SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESELVEEDKKHERHPIFGNIVDEV
AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI
LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNEDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT
RKSEETITPWNFEEVVDKGASAQSFIERMTNEDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDL
LFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRENASLGTYHDLLKIIKDKDELDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDELKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH

EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDERKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP
LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGEDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK
YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLETLT
NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKETGKQLVIQESILM
LPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSGGSGGSTNLSDIIEKETGKQL
VIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSKRTADGSEFEPKK KRKV
Amino acid sequence 980
MKRTADGSEFESPKKKRKVSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFI for
BE4MAX protein
EKFTTERYFCPNTRCSITWELSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYC
WRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGS
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
CCGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCCCCCCCGGGACTTCGCCGCCTACCGGAGCCTGCGGGTGAAGTTCAGCCG
GAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCCGGCGGGAGGAGTACGACGTG
CTGGACAAGCGGCGGGGCCGGGACCCCGAGATGGGCGGCAAGCCCCGGCGGAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGC
AGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGCGGCGGGGCAAGGGCCACGACGGCCTGTACCA
GGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCCGGTAA CD30 Construct (Amino
982 MDFQVQIFSFLLISASVIMSRMAQVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQ
Acid)
NFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPK
FMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTY
PLTFGGGTKLEIKRSDPATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDKDPKFWVLVVVGGVLACYSLLV
TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR* CD30
Construct Signal 983 ATGGACTTCCAGGTGCAGATCTTCAGCTTCCTGCTGATCAGCGCCAGCGTGATCATGAGCCGGATGGCC
Peptide (DNA) CD30 Construct Signal 984 MDFQVQIFSELLISASVIMSRMA Peptide (Amino Acid) CD30 Construct HRS3 985
CAGGTGCAGCTGCAGCAGAGCGGCGCCGAGCTGGCCCGGCCCGGCGCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCT
Binder (DNA)
TCACCACCTACACCATCCACTGGGTGCGGCGGCGGCCCGGCCACGACCTGGAGTGGATCGGCTACATCAACCCCAGCAGCGGCTG
CAGCGACTACAACCAGAACTTCAAGGGCAAGACCACCCTGACCGCCGACAAGAGCAGCAACACCGCCTACATGCAGCTGAACAGC
CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCCGGCGGGCCGACTACGGCAACTACGAGTACACCTGGTTCGCCTACTGGG
GCCAGGGCACCACCGTGACCGTGAGCAGCAGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGTGATCGAGCT
GACCCAGAGCCCCAAGTTCATGAGCACCAGCGTGGGCGACCGGGTGAACGTGACCTACAAGGCCAGCCAGAACGTGGGCACCAAC
GTGGCCTGGTTCCAGCAGAAGCCCGGCCAGAGCCCCAAGGTGCTGATCTACAGCGCCAGCTACCGGTACAGCGGCGTGCCCGACC
GGTTCACCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAACGTGCAGAGCGAGGACCTGGCCGAGTACTTCTGCCA
GCAGTACCACACCTACCCCCTGACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGCGG CD30 Construct HRS3 986
QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNEKGKTTLTADKSSNTAYMQLNS

Binder (Amino Acid)
LTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTN
VAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKR CD30 Construct
CD8 987
ACCACCACCCCCGCCCCCCGGCCCCCCACCCCCGCCCCCACCATCGCCAGCCAGCCCCTGAGCCTGCGGCCCGAGGCCTGCCGGC
Hinge (DNA) CCGCCGCCGGCGGCGCCGTGCACACCCGGGGCCTGGACTTCGCCTGCGAC CD30 Construct CD8 988
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD Hinge (Amino Acid) CD30 Construct CD28 989
TTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGTGGCCTTCATCATCTTCTGGGTGCGGA
TM (DNA)
GCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCCCGGCGGCCCGGCCCCACCCGGAAGCACTACCAGCCCTA
CGCCCCCCCCCCGGGACTTCGCCGCCTACCGGAGC CD30 Construct CD28 990
FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS TM (Amino Acid) CD30
Construct 991
CTGCGGGTGAAGTTCAGCCGGAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCC
CD3zeta (DNA)
GGCGGGAGGAGTACGACGTGCTGGACAAGCGGCGGGGCCGGGACCCCGAGATGGGCGGCAAGCCCCGGCGGAAGAACCCCCAGGA
GGGCCTGTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGGGGGGGCAAG
GGCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCGG
CD30 Construct 992
LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
CD3zeta (Amino Acid) GHDGLYQGLSTATKDTYDALHMQALPPR B2M (G000529) 993
mG*mG*mC*CACGGAGCGAGACAUCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC
AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU 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
NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUC
GGUGCU Guide scaffold 3003
mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC
ACGAAAGGGCACCGAGUCGGmUmGmC*mU 3004 Not Used mRNA sequence 3005
GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGACCAACCUGUCCGACAUCAUCGAGAAGGAGACCGGCAA
encoding UGI
GCAGCUGGUGAUCCAGGAGUCCAUCCUGAUGCUGCCCGAGGAGGUGGAGGAGGUGAUCGGCAACAAGCCCGAGUCCGACAUCCUG
GUGCACACCGCCUACGACGAGUCCACCGACGAGAACGUGAUGCUGCUGACCUCCGACGCCCCCGAGUACAAGCCCUGGGCCCUGG
UGAUCCAGGACUCCAACGGCGAGAACAAGAUCAAGAUGCUGUCCGGCGGCUCCAAGCGGACCGCCGACGGCUCCGAGUUCGAGUC
CCCCAAGAAGAAGCGGAAGGUGGAGUGAUAGCUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAA
CUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCG
AGAAAAAAAAAAAAUGGAAAAAAAAAAAAACGGAAAAAAAAAAAAAGGUAAAAAAAAAAAAAUAUAAAAAAAAAAAAACAUAAAAAAAA
AAAACGAAAAAAAAAAAAACGUAAAAAAAAAAAAACUCAAAAAAAAAAAAAGAUAAAAAAAAAAAAACCUAAAAAAAAAAAAUGUAAAA
AAAAAAAAGGGAAAAAAAAAAAACGCAAAAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAUCGAAAAAAAAAAAAUC
UAAAAAAAAAAAACGAAAAAAAAAAAACCCAAAAAAAAAAAAGACAAAAAAAAAAAAUAGAAAAAAAAAAAAGUUAAAAAAAAAA
AACUGAAAAAAAAAAAAUUUAAAAAAAAAAAAUCUAG Guide scaffold 90-mer 3006
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC Guide scaffold
90-mer 3007
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG
with modification *mC Guide scaffold 90-mer 3008
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGmGmCmAmCmC
with modification GmAmGmUmCmGmG*mU*mG*mC Guide scaffold 88-mer 3009
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*m
with modification C Guide scaffold 88-mer 3010
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGGUGC Guide scaffold 88-
mer 3011
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*m
with modification C Guide scaffold 88-mer 3012
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmAmAmUmGmGmCmAmCmCmGmA
with modification GmUmCmGmG*mU*mG*mC Guide scaffold 3013
GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmU

GmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU Guide scaffold 3014
mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC
AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU 3015-3018 Not
Used G025420 TRAC 3019
mC*mU*mC*UCAGCUGGUACACGGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC
ACGAAAGGGCACCGAGUCGGmU*mG*mC*mU B2M (G000529) (full, 3100
GGCCACGGAGCGAGACAUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC
unmodified) CGAGUCGGUGCUUUU G021469 TRAC (full, 3101
AUAUCCAGAACCCUGACCCUGCCGGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCU
unmodified) GCCUUCUGGCAUCGUU G021475 TRAC (full, 3102
AACCCUGAUCCUCUUGUCCCACAGGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCU
unmodified) GCCUUCUGGCAUCGUU G021481 TRAC (full, 3103
GCCGUGUACCAGCUGAGAGACUCUGUUGUAGCUCCCUGAAACCGUUGCUACAAUAAGGCCGUCGAAAGAUGUGCCGCAACGCUCU
unmodified) GCCUUCUGGCAUCGUU G028013 B2M (full, 3104
GGCCACGGAGCGAGACAUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC
unmodified) CGAGUCGGUGCUUUU G018995 HLA-A (full, 3105
ACAGCGACGCCGCGAGCCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC
unmodified) CGAGUCGGUGCUUUU G012086 TRAC (full, 3106
AGAGUCUCUCAGCUGGUACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC
unmodified) CGAGUCGGUGCUUUU G000644 EMX1 target 3107 GAGUCCGAGCAGAAGAAGAA sequence G000645 VEGFA
target 3108 GACCCCCUCCACCCCGCCUC sequence G000646 RAG1B target 3109 GACUUGUUUUCAUUGUUCUC sequence
G013675 CIITA target 3110 CCCCCGGACGGUUCAAGCAA sequence G013675 CIITA sgRNA 3118
mC*mC*mC*CCGGACGGUUCAAGCAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUC
AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
TABLE-US-00020 TABLE 9A VII. Additional Exemplary Nme Guide RNAs Genomic Guide Guide Coordinates ID Target Sequence
Exemplary Guide RNA Full Sequence Exemplary Guide RNA Modified Sequence (hg38) G034202 HLA-A GCUCUAU
GCUCUAUCCACGGCGCCCGCGGCUGUUGU mG*mC*mU*mCmUAUmCmCAmCGmGCGCCmCGCGmGCUmGUUGmUm chr6:
CCACGGC AGCUCCCUGAAACCGUUGCUACAAUAAGG
AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC 29942891- GCCCGCG
CCGUCGAAAGAUGUGCCGCAACGCUCUGC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU 29942915
GCU (SEQ CUUCUGGCAUCGUU (SEQ ID NO: 1576) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3111) ID NO: 576)
G034617 HLA-A CACUCAC CACUCACCCGCCCAGGUCUGGGUCGUUGU
mC*mA*mC*mUmCACmCmCGmCCmCAGGUmCUGGmGUCmGUUGmUm chr6: CCGCCCA
AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC 29942609-
GGUCUGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC
mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU 29942633 GUC (SEQ CUUCUGGCAUCGUU (SEQ
ID NO: 1571) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3112) ID NO: 571) G028943 TRAC AAAACCU
AAAACCUGUCAGUGAUUGGGUUCCGUUGU mA*mA*mA*mAmCCUmGmUCmAGmUGAUUmGGGUmUCCmGUUGmUm chr14:
GUCAGUG AGCUCCCUGAAACCGUUGCUACAAUAAGG
AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 22550574- AUUGGG
CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm 22550598
UUCC CUUCUGGCAUCGUU (SEQ ID NO: 1605) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2605) (SEQ ID NO:
605) G034982 TRAC AAAACCU AAAACCUGUCAGUGAUUGGGUUCCGUUGU
mA*mA*mA*mAmCCUmGmUCmAGmUGAUUmGGGUmUCCmGUUGmUm chr14: GUCAGUG
AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC 22550574-
AUUGGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU
22550598 UUCC CUUCUGGCAUCGUU (SEQ ID NO: 1605) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3113) (SEQ ID
NO: 605) G028939 TRAC UUAGGU UUAGGUUCGUAUCUGUAAAACCAAGUUGU
mU*mU*mA*mGmGUUmCmGUmAUmCUGUAmAAACmCAAmGUUGmU chr14: UCGUAUC
AGCUCCCUGAAACCGUUGCUACAAUAAGG mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGm 22550544-
UGUAAA CCGUCGAAAGAUGUGCCGCAACGCUCUGC CCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCC
22550568 ACCAA CUUCUGGCAUCGUU (SEQ ID NO: 1606) mUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2606)
(SEQ ID NO: 606) G034981 TRAC UUAGGU UUAGGUUCGUAUCUGUAAAACCAAGUUGU
mU*mU*mA*mGmGUUmCmGUmAUmCUGUAmAAACmCAAmGUUGmU chr14: UCGUAUC
AGCUCCCUGAAACCGUUGCUACAAUAAGG mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmC 22550544-
UGUAAA CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm
22550568 ACCAA CUUCUGGCAUCGUU (SEQ ID NO: 1606) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3114)
(SEQ ID NO: 606) G013006 TRAC CUCUCAG CUCUCAGCUGGUACACGGCAGUUUUAGAG
mC*mU*mC*UCAGCUGGUACACGGCAGUUUUAGAmGmCmUmAmGmA chr14: CUGGUAC
CUAGAAAUAGCAAGUUAAAAUAAGGCUAG mAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCm
22547524- ACGGCA UCCGUUAUCAACUUGAAAAAGUGGCACCG
UmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGm 22547544 (SEQ ID AGUCGGUGCUUUU (SEQ ID
NO: 1613) GmUmGmCmU*mU*mU*mU (SEQ ID NO: 2613) NO: 613) G028986 TRBC1 GUGUCCU
GUGUCCUACCAGCAAGGGGUCCUGGUUGU mG*mU*mG*mUmCCUmAmCCmAGmCAAGGmGGUCmCUGmGUUGmUm chr7:
ACCAGCA AGCUCCCUGAAACCGUUGCUACAAUAAGG
AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 142792690- AGGGGUC
CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm
142792714 CUG (SEQ CUUCUGGCAUCGUU (SEQ ID NO: 1607) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2607)
ID NO: 607) G034618 TRBC1 GUGUCCU GUGUCCUACCAGCAAGGGGUCCUGGUUGU
mG*mU*mG*mUmCCUmAmCCmAGmCAAGGmGGUCmCUGmGUUGmUm chr7: ACCAGCA
AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC
142792690- AGGGGUC CCGUCGAAAGAUGUGCCGCAACGCUCUGC
mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU 142792714 CUG (SEQ CUUCUGGCAUCGUU (SEQ

ID NO: 1607) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3115) ID NO: 607) G026584 CIITA UCAAAGU UCAAAGUACCCUACAGGAGGACCAGUUGU mU*mC*mA*mAmAGUmAmCCmCUmACAGGmAGGAmCCAmGUUGmUm chr16: ACCCUAC AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 10907504- AGGAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm 10907528 ACCA CUUCUGGCAUCGUU (SEQ ID NO: 1608) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2608) (SEQ ID NO: 608) G034201 CIITA UCAAAGU UCAAAGUACCCUACAGGAGGACCAGUUGU mU*mC*mA*mAmAGUmAmCCmCUmACAGGmAGGAmCCAmGUUGmUm chr16: ACCCUAC AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC 10907504- AGGAGG CCGUCGAAAGAUGUGCCGCAACGCUCUGC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU 10907528 ACCA CUUCUGGCAUCGUU (SEQ ID NO: 1608) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3116) (SEQ ID NO: 608) G029131 CIITA AGCUGCC AGCUGCCGUUCUGCCCAGUCCGGGGUUGU mA*mG*mC*mUmGCCmGmUUmCUmGCCCAmGUCCmGGGmGUUGmUm chr16: GUUCUGC AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 10906643- CCAGUCC CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm 10906667 GGG (SEQ CUUCUGGCAUCGUU (SEQ ID NO: 1609) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2609) ID NO: 609) G034619 CIITA AGCUGCC AGCUGCCGUUCUGCCCAGUCCGGGGUUGU mA*mG*mC*mUmGCCmGmUUmCUmGCCCAmGUCCmGGGmGUUGmUm chr16: GUUCUGC AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAUAAGmGmCC 10906643- CCAGUCC CCGUCGAAAGAUGUGCCGCAACGCUCUGC mGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCmU 10906667 GGG (SEQ CUUCUGGCAUCGUU (SEQ ID NO: 1609) mUmCmUGGCAUCG*mU*mU (SEQ ID NO: 3117) ID NO: 609) G021557 VEGFA GCAUGGG GCAUGGGCAGGGGCUGGGGUGCACGUUGU mG*mC*mA*mUmGGGmCmAGmGGmGCUGGmGGUGmCACmGUUGmUm chr6: CAGGGGC AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 43774288- UGGGGU CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm 43774312 GCAC CUUCUGGCAUCGUU (SEQ ID NO: 1610) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2610) (SEQ ID NO: 610) G021558 VEGFA GAAUGGC GAAUGGCAGGCGGAGGUUGUACUGGUUGU mG*mA*mA*mUmGGCmAmGGmCGmGAGGUmUGUAmCUGmGUUGmU chr6: AGGCGGA AGCUCCCUGAAACCGUUGCUACAAUAAGG mAmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGm 43780852- GGUUGU CCGUCGAAAGAUGUGCCGCAACGCUCUGC CCmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCC 43780876 ACUG CUUCUGGCAUCGUU (SEQ ID NO: 1611) mUmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2611) (SEQ ID NO: 611) G021567 VEGFA GUGAGCA GUGAGCAGGCACCUGUGCCAACAUGUUGU mG*mU*mG*mAmGCAmGmGCmACmCUGUGmCCAAmCAUmGUUGmUm chr6: GGCACCU AGCUCCCUGAAACCGUUGCUACAAUAAGG AmGmCUCCCmUmGmAmAmAmCmCGUUmGmCUAmCAAU*AAGmGmC 43781113- GUGCCAA CCGUCGAAAGAUGUGCCGCAACGCUCUGC CmGmUmCmGmAmAmAmGmAmUGUGCmCGmCAAmCGCUCUmGmCCm 43781137 CAU (SEQ CUUCUGGCAUCGUU (SEQ ID NO: 1612) UmUmCmUGGCAUCG*mU*mU (SEQ ID NO: 2612) ID NO: 612)

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{circumflex over ( )}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-US-00021 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 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-US-00022 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 20.70 0.00 22.30 0.71 31.50 0.42 25.50 0.14 42.60 0.71 to 1 1.25 to 20.80 0.42 23.85 0.64 32.00 1.70 25.65 0.21 56.60 1.13 1 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-US-00023 TABLE 12 Off-Target Analysis Off- Target Guide Sequence Site gRNA ID Target (SEQ ID NO:) Count G022010 HLA-B AACAAUGCCCACGAUGGGGA 37 (SEQ ID NO: 3) G022020 HLA-B ACAUGCCAUGUACAGCAUGA 24 (SEQ ID NO: 13) G000644 EMX1 GAGUCCGAGCAGAAGAAGAA 58 (SEQ ID NO: 3107) G000645 VEGFA GACCCCCUCCACCCCGCCUC 793 (SEQ ID NO: 3108) G000646 RAG1B GACUUGUUUUCAUUGUUCUC 47 (SEQ ID NO: 3109)

[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{circumflex over ( )}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-US-00024 TABLE 13 Mean percentage of HLA-B knockout across 3 donors in 100-mers plus four 91-mers Donor 2 Donor 3 Donor 1 Mean % SD % Mean % SD % Mean % SD % Guide ID HLA-B KO HLA-B KO HLA-B KO HLA-B KO HLA-B KO 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-US-00025 TABLE 14 Mean percentage of HLA-B knockout across 2 donors in 91-mer format Donor 3 Donor 4 Mean % SD % Mean % SD % HLA-B HLA-B HLA-B HLA-B Guide ID KO KO KO 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{circumflex over ( )}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-US-00026 TABLE 15 G027488 G027489 G027490 Mean % SD % Mean % SD % Mean % SD % Guide HLA- HLA- HLA- HLA- HLA- HLA- sgRNA B*07:02 B*07:02 B*07:02 B*07:02 B*07:02 B*07:02 (μg/mL) KO KO N KO KO N KO KO 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 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 Mean % SD % Mean % SD % Guide HLA- HLA- HLA- HLA- sgRNA B*07:02 B*07:02 B*07:02 B*07:02 (μg/mL) KO KO N KO 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-US-00027 TABLE 16 G027488 G027489 G027490 Mean % SD % Mean % SD % Mean % SD % Guide HLA- HLA- HLA- HLA- HLA- HLA- sgRNA B*08:01 B*08:01 B*08:01 B*08:01 B*08:01 B*08:01 (μg/mL) KO KO N KO KO N KO 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 Mean % SD % Mean % SD % Guide HLA- HLA- HLA- HLA- sgRNA B*08:01 B*08:01 B*08:01 B*08:01 (μg/mL) KO KO N KO 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.sup.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.sup.6 T cells/mL in P3 electroporation buffer (Lonza Catalog #V4SP-3960). For each well to be electroporated, 1×10.sup.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-US-00028 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 0.27 0.170 2 G028828 2.13 0.834 2 HLA-A G021469 0.08 0.000 2 G028829 3.375 0.940 2 TRAC G021481 1.38 1.796 2 G028830 0.32 0.297 2 TRAC 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 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-US-00029 TABLE 18 Mean percentage HLA-B*07:02 or HLA-B*08:01 knockout following editing at the HLA-B locus % HLA-B*07:02 KO % HLA-B*08:01 KO Guide ID Mean 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 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{circumflex over ( )}.sub.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{circumflex over ( )}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-US-00030 TABLE 19 Dose response curve for the percent of HLA-A2.sup.− of CD8.sup.+ cells with various doses of sgRNA G028907 G028913 G028840 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells 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 Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells 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 Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells 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 Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells 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-US-00031 TABLE 20 Dose response curve for the percent of HLA-B7.sup.− of CD8+ cells with various doses of sgRNA G028907 G028913 G028840 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G028922 G028918 G028865 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G028832 G028795 G028869 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G000529 B2M G018995 Spy Cas G022020 Spy Cas Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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-US-00032 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.sup.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 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.sup.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.sup.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-US-00033 TABLE 22 Mean % HLA-B7.sup.− 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-US-00034 TABLE 23 Mean % HLA-B8.sup.− 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 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.sup.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-US-00035 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 HLA-B LNP (G027488) HLA-B KO HLA-B LNP (G027490) HLA-B KO HLA-B LNP (G027994) HLA-B KO HLA-B LNP (G028001) HLA-B KO HLA-B LNP (G028002) HLA-A + HLA-B HLA-A LNP HLA-B LNP KO (G027488) HLA-A + HLA-B HLA-A LNP HLA-B LNP KO (G027490) HLA-A + HLA-B HLA-A LNP HLA-B LNP KO (G027994) HLA-A + HLA-B HLA-A LNP HLA-B LNP KO (G028001) HLA-A + HLA-B HLA-A LNP HLA-B LNP KO (G028002)

[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.sup.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-US-00036 TABLE 25 Percentage T cell lysis following NK cell challenge to engineered T cells HLA-A KO HLA-B KO WT B2M KO G018995 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 0.070711 −5.225 7.389266 0 1.697056 0 0 2 0 HLA-B KO HLA-A+ B KO HLA-A+ B KO G028001 G027994 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 2.474874 0 0.353553 0 8.980256 2 0

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.sup.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.sup.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-US-00037 TABLE 26A Dose response curve for the percent of HLA-B7.sup.− and CD8.sup.+ cells in an HLA-B7 homozygous donor G034206 G034207 G034208 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G034209 G034210 G034211 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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-US-00038 TABLE 26B Dose response curve for the percent of HLA-B7.sup.− and CD8.sup.+ cells in an HLA-B7 heterozygous donor G034206 G034207 G034208 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+

% CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G034209 G034210 G034211 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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-US-00039 TABLE 27A Dose response curve for the percent of HLA-B8.sup.− and CD8.sup.+ cells in an HLA-B8 homozygous donor G034206 G034207 G034208 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G034209 G034210 G034211 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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-US-00040 TABLE 27B Dose response curve for the percent of HLA-B8.sup.− and CD8.sup.+ cells in an HLA-B8 heterozygous donor G034206 G034207 G034208 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 G034209 G034210 G034211 Guide Mean SD Mean SD Mean SD sgRNA % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ % CD8.sup.+ (μg/mL) Cells Cells N Cells Cells N Cells Cells 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 number 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-US-00041 TABLE 28 Off-Target Analysis Off- Target gRNA Guide Sequence Site ID Target (SEQ ID NO:) Count G034206 HLA-B CAAACUCAGGACACUGAGCUUGUG 1 (SEQ ID NO: 163) G034207 HLA-B UCAGGACACUGAGCUUGUGGAGAC 1 (SEQ ID NO: 164) G034208 HLA-B UCUGGGAAAGGAGGGGAAGAUGAG 1 (SEQ ID NO: 165) G034209 HLA-B CUCUGGGAAAGGAGGGGAAGAUGA 1 (SEQ ID NO: 166) G034210 HLA-B CUGGAGGGUGUGAGACCCUGGCCC 3 (SEQ ID NO: 169) G034211 HLA-B UCCCAGAGCCGUCUUCCCAGUCCA 2 (SEQ ID NO: 177) G021557 VEGFA GCAUGGGCAGGGGCUGGGGUGCAC 2 (SEQ ID NO: 610) G021558 VEGFA GAAUGGCAGGCGGAGGUUGUACUG 1 (SEQ ID NO: 611) G021567 VEGFA GUGAGCAGGCACCUGUGCCAACAU 1 (SEQ ID NO: 612)

[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.sup.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 1-1.5×10.sup.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-US-00042 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, TRAC LNP CIITA LNP HLA-B + CIITA CIITA LNP HLA-B LNP TRAC LNP HLA-A + HLA- HLA-A LNP, TRAC LNP B + CIITA CIITA LNP

[1031] On day 4, the T cells from each group were counted, re-plated at 2×10.sup.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.sup.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.sup.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.sup.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-US-00043 TABLE 30 Total Flux (photons/s) from luciferase expressing T cells in treated mice at intervals after T cell injection. Days post T cell injec- No NK injection NK injection Group tion Mean SD N Mean SD N TRAC 1 1.03E+08 1.70E+07 4 7.70E+07 1.04E+07 5 KO 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 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- 1 6.89E+07 5.43E+06 4 8.32E+07 6.69E+06 5 AB 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 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.sup.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-US-00044 TABLE 31 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 TRAC 1 60237500 19287091 4 78610000 5481726 5 KO 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 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.sup.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.sup.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-US-00045 TABLE 32 Editing scheme and viral transduction Condition Day 0 Day 1 Day 3 Day 5 CD30 CAR Thaw TransAct Nme2 BC22 HLA-A + GREX 6 Double KO CIITA + Spy Cas9 transfer TRAC + AAV CD30 CAR Thaw TransAct Nme2 BC22 HLA-A + GREX 6 Triple KO CIITA + HLA-B + transfer Spy Cas9 TRAC + AAV Untransduced/ Thaw TransAct Spy Cas9 TRAC GREX 6 TCR KO only 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.sup.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.sup.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.sup.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.sup.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-US-00046 TABLE 33 Order of editing and Harvest Condition Day 0 Day 1 Day 2 Day 3 Day 8 B2M + Thaw TransAct + B2M 24-well Grex Harvest CIITA CIITA transfer Unedited Thaw TransAct 24-well Grex Harvest transfer

[1054] 24 hrs post thaw (day 1), cells were counted and resuspended in TCAM with cytokines as described above at 2×10.sup.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.sup.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.sup.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.sup.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-US-00047 TABLE 34 percentage killing in HH cells for double and triple KO edits Untransduced Double KO Triple KO Mean % SD % Mean % SD % Mean % SD % E:T Killing Killing Killing Killing Killing 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-US-00048 TABLE 35 percentage killing in MOLT-4 cells for double and triple KO edits Untransduced Double KO Triple KO Mean % SD % Mean % SD % Mean % SD % E:T Killing Killing Killing Killing Killing 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.sup.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-US-00049 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 WT (unedited) Donor 1 A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 cells Triple KO Donor 1 A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 (HLA-A/HLA- B/CIITA) Host HLA-C Match Host 1 A*03:02 A*03:02 B*49:01 B*49:01 C*07:01 C*07:01 PBMCs Host Autologous Auto Host A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 (Donor 1)

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.sup.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.sup.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.sup.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.sup.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-US-00050 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 WT Donor 1 A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 Donor T B2M + CIITA Donor 1 A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 cells Triple KO Donor 1 A*02:01 A*02:01 B*08:01 B*08:01 C*07:01 C*07:01 (HLA-A/HLA- B/CIITA)
TABLE-US-00051 TABLE 38 T cell lysis following NK cell challenge B2M/ Unedited Untransduced CIITA KO Triple KO Mean Mean Mean Mean % SD % % SD % % SD % % SD % E:T Killing Killing Killing Killing Killing Killing Killing 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-US-00052 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.

Claims

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.