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### CELL-SPECIFIC TRANSCRIPTIONAL REGULATORY SEQUENCES AND USES THEREOF

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#### Abstract

New synthetic expression cassettes comprising a minimal promoter and a cell-specific enhancer for expression of a nucleic acid of interest in one or more specific cell subtypes are disclosed. Vectors and host cells comprising such synthetic expression cassettes are also disclosed. The application also discloses methods for expressing a nucleic acid of interest, such as a nucleic acid encoding a chimeric antigen receptor (CAR), in a cell and for treating diseases or conditions such as cancers and genetic diseases using the synthetic expression cassettes, vectors and cells.

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## Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS [0001] The present application is divisional application of U.S. application Ser. No. 17/310,107, filed Jul. 16, 2021, which is a National Entry Application of PCT application no. PCT/CA2020/050084 filed on Jan. 24, 2020, which claims the benefit of U.S. provisional application Ser. No. 62/796,254 filed on Jan. 24, 2019. All documents above are incorporated herein in their entirety by reference.

## SEQUENCE LISTING

[0002] Pursuant to 37 C.F.R. 1.831-1.834, a sequence listing is submitted herewith as an XML file named G17685\_00022-Seq listing\_ST26.xml, created on May 1, 2025, and having a size of ~ 94,353 bytes. The content of the aforementioned file is hereby incorporated by reference in its entirety.

## TECHNICAL FIELD

[0003] The present invention generally relates to the targeted expression of genes in specific cell subtypes, for example immune cells such as T cells, B cells and natural killer (NK) cells, which may be used in hematopoietic stem cell (HSC) engineering and cell-based therapy.

## BACKGROUND ART

[0004] The targeted expression of a transduced gene in a given cell subtype or tissue is challenging. With the growing fields of stem cell engineering and inducible pluripotent stem cells (iPS) research, there is a need for the ability to express a given protein only in targeted populations stemming from the parent cell. However, the use of traditional/natural promoter is faced with the technical issue of size and sometimes specificity. For example, currently, gene therapy for hematopoietic-related disorders relies on the transduction of HSC with a transgene under the control of a strong promoter.<sup>sup.1,2</sup> With this type of construct, the cells that originate from the modified stem cells will express the new gene, irrespectively of the cell subtype, which could potentially lead to hazardous consequences.

[0005] Chimeric Antigen Receptor (CAR) immune cell therapy has emerged as a promising new therapeutic tool against various cancer. In CAR immune cell therapy, patient's immune cells (e.g., T cells, NK cells) are engineered to express CARs that binds to tumor antigens, which permits the specific killing of tumor cells expressing the antigen. Currently, this strategy, although potent, typically does not last because of T-cell exhaustion and loss of engineered T cells in vivo. Moreover, the infusion of a large number of CAR-T cells may lead to high toxicity due to a massive release of cytokines (cytokine release syndrome). There is thus a need for an approach that permits the continuous and progressive replenishment of CAR-modified cells in the circulation, and which limits the expression of the CAR only to specific cells (e.g., T cells, NK cells).

[0006] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

## SUMMARY OF THE INVENTION

[0007] The present disclosure provides the following items:

[0008] 1. A synthetic expression cassette for expressing a nucleic acid of interest in a cell

comprising: [0009] (i) a minimal promoter; and [0010] (ii) a transcriptional enhancer operatively coupled to the minimal promoter for expression of the nucleic acid of interest in the cell, wherein the transcriptional enhancer comprises a sequence having at least 70% sequence identity with at least 50 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0011] 2. The synthetic expression cassette of item 1, wherein the transcriptional enhancer comprises a sequence having at least 70% sequence identity with at least 100 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0012] 3. The synthetic expression cassette of item 2, wherein the transcriptional enhancer comprises a sequence having at least 70% sequence identity with any one of the sequences set forth in SEQ ID NOs: 7-47.

[0013] 4. The synthetic expression cassette of item 1, wherein the transcriptional enhancer comprises a sequence having at least 80% sequence identity with at least 50 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0014] 5. The synthetic expression cassette of item 4, wherein the transcriptional enhancer comprises a sequence having at least 80% sequence identity with at least 100 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0015] 6. The synthetic expression cassette of item 5, wherein the transcriptional enhancer comprises a sequence having at least 80% sequence identity with any one of the sequences set forth in SEQ ID NOs: 7-47.

[0016] 7. The synthetic expression cassette of item 1, wherein the transcriptional enhancer comprises a sequence having at least 90% sequence identity with at least 50 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0017] 8. The synthetic expression cassette of item 7, wherein the transcriptional enhancer comprises a sequence having at least 90% sequence identity with at least 100 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0018] 9. The synthetic expression cassette of item 8, wherein the transcriptional enhancer comprises a sequence having at least 90% sequence identity with any one of the sequences set forth in SEQ ID NOs: 7-47.

[0019] 10. The synthetic expression cassette of item 1, wherein the transcriptional enhancer comprises a sequence having at least 95% sequence identity with at least 50 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0020] 11. The synthetic expression cassette of item 10, wherein the transcriptional enhancer comprises a sequence having at least 95% sequence identity with at least 100 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0021] 12. The synthetic expression cassette of item 11, wherein the transcriptional enhancer comprises a sequence having at least 95% sequence identity with any one of the sequences set forth in SEQ ID NOs: 7-47.

[0022] 13. The synthetic expression cassette of item 1, wherein the transcriptional enhancer comprises or consists of at least 50 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0023] 14. The synthetic expression cassette of item 13, wherein the transcriptional enhancer comprises or consists of at least 100 consecutive nucleotides from any one of the sequences set forth in SEQ ID NOs: 7-47.

[0024] 15. The synthetic expression cassette of item 13, wherein the transcriptional enhancer comprises or consists of any one of the sequences set forth in SEQ ID NOs: 7-47.

[0025] 16. The synthetic expression cassette of any one of items 1 to 15, wherein the minimal promoter is a human cytomegalovirus CMV minimal promoter (miniCMV).

[0026] 17. The synthetic expression cassette of item 16, wherein the minimal promoter comprises or consists of the sequence of SEQ ID NO: 6.

[0027] 18. The synthetic expression cassette of any one of items 1 to 17, wherein the transcriptional

enhancer is upstream of the minimal promoter in the synthetic expression cassette.

[0028] 19. The synthetic expression cassette of any one of items 1 to 18, further comprising a polyadenylation (poly(A)) signal.

[0029] 20. The synthetic expression cassette of any one of items 1 to 19, further comprising a transcriptional termination signal.

[0030] 21. The synthetic expression cassette of any one of items 1 to 20, further comprising the nucleic acid of interest operatively coupled to the minimal promoter and transcriptional enhancer.

[0031] 22. The synthetic expression cassette of any one of items 1 to 21, further comprising a selectable marker.

[0032] 23. The synthetic expression cassette of any one of items 1 to 22, wherein the cell is a stem cell.

[0033] 24. The synthetic expression cassette of item 23, wherein the stem cell is a hematopoietic stem cell (HSC), an embryonic stem cell, a totipotent stem cell, a pluripotent stem cell, a multipotent stem cell or an induced pluripotent stem cell (iPSC).

[0034] 25. The synthetic expression cassette of any one of items 1 to 22, wherein the cell is an immune cell.

[0035] 26. The synthetic expression cassette of item 25, wherein the immune cell is a T cell, a natural killer (NK) cell, or a B cell.

[0036] 27. The synthetic expression cassette of any one of items 1 to 26, wherein the nucleic acid of interest encoded a chimeric antigen receptor (CAR).

[0037] 28. A vector comprising the synthetic expression cassette of any one of items 1 to 27.

[0038] 29. The vector of item 28, wherein the vector is a viral vector.

[0039] 30. A host cell comprising the synthetic expression cassette of any one of items 1 to 27 or the vector of item 28 or 29.

[0040] 31. The host cell of item 30, wherein said cell is a hematopoietic stem cell, a T cell, a natural killer (NK) cell, or a B cell.

[0041] 32. A composition comprising the host cell of item 30 or 31.

[0042] 33. A method for inducing the expression of a nucleic acid of interest by a cell, the method comprising introducing the synthetic expression cassette of any one of items 1 to 27 or the vector of item 28 or 29 in the cell.

[0043] 34. The method of item 33, wherein the nucleic acid of interest encodes a protein that is absent or defective in said cell.

[0044] 35. The method of item 33 or 34, wherein the nucleic acid of interest encodes a chimeric antigen receptor (CAR).

[0045] 36. The method of any one of items 33 to 35, wherein said cell is a hematopoietic stem cell, a T cell, a natural killer (NK) cell, or a B cell.

[0046] 37. A method for treating a disease, condition or disorder in a subject, the method comprising administering an effective amount of the cell of item 30 or 31, or the composition of item 32, to said subject.

[0047] 38. The method of item 37, wherein the disease, condition or disorder is associated with the absence of expression of a protein or the expression of a defective protein, and wherein the nucleic acid of interest encodes a functional form of the protein.

[0048] 39. The method of item 37, wherein the disease, condition or disorder is associated with expression of an antigen, and wherein the nucleic acid of interest encodes a recombinant receptor that specifically binds to the antigen.

[0049] 40. The method of item 39, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

[0050] 41. The method of item 39 or 40, wherein the disease, condition or disorder is a cancer, an autoimmune or inflammatory disease, or an infectious disease.

[0051] 42. The method of item 41, wherein the disease, condition or disorder is a cancer.

[0052] 43. The method of item 42, wherein the cancer is a hematological cancer.

[0053] 44. The method of any one of items 37 to 43, wherein said cell is a hematopoietic stem cell, a T cell, a natural killer (NK) cell, or a B cell.

[0054] 45. The method of any one of items 37 to 44, wherein said method comprises administering at least  $1 \times 10^2$ ,  $1 \times 10^3$  or  $1 \times 10^4$  cells to said subject.

[0055] 46. The method of item 45, wherein said method comprises administering  $1 \times 10^6$  to  $1 \times 10^8$  cells to said subject.

[0056] 47. The method of any one of items 37 to 46, wherein said cells are autologous cells.

[0057] 48. The method of any one of items 37 to 46, wherein said cells are allogeneic cells.

[0058] 49. The cell of item 30 or 31, or the composition of item 32, for use in treating a disease, condition or disorder in a subject.

[0059] 50. The cell or composition for use according to item 50, wherein the disease, condition or disorder is associated with the absence of expression of a protein or the expression of a defective protein, and wherein the nucleic acid of interest encodes a functional form of the protein.

[0060] 51. The cell or composition for use according to item 50, wherein the disease, condition or disorder is associated with expression of an antigen, and wherein the nucleic acid of interest encodes a recombinant receptor that specifically binds to the antigen.

[0061] 52. The cell or composition for use according to item 51, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

[0062] 53. The cell or composition for use according to item 51 or 52, wherein the disease, condition or disorder is a cancer, an autoimmune or inflammatory disease, or an infectious disease.

[0063] 54. The cell or composition for use according to item 53, wherein the disease, condition or disorder is a cancer.

[0064] 55. The cell or composition for use according to item 54, wherein the cancer is a hematological cancer.

[0065] 56. The cell or composition for use according to any one of items 49 to 55, wherein said cell is a hematopoietic stem cell, a T cell, a natural killer (NK) cell, or a B cell.

[0066] 57. The cell or composition for use according to any one of items 49 to 56, wherein said method comprises administering at least  $1 \times 10^2$ ,  $1 \times 10^3$  or  $1 \times 10^4$  cells to said subject.

[0067] 58. The cell or composition for use according to item 57, wherein said method comprises administering  $1 \times 10^6$  to  $1 \times 10^8$  cells to said subject.

[0068] 59. The cell or composition for use according to any one of items 49 to 58, wherein said cells are autologous cells.

[0069] 60. The cell or composition for use according to any one of items 49 to 58, wherein said cells are allogeneic cells.

[0070] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

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## Description

### BRIEF DESCRIPTION OF DRAWINGS

[0071] In the appended drawings:

[0072] FIGS. 1A and 1B depict the cloning strategy used to create the T-cell specific- (Tspe)-promoter from Chr16-445. History is depicted from the bottom-up.

[0073] FIG. 2 depicts the cloning strategy used to create a plasmid to produce lentiviral particles for Tenh (Chr16-445). History is depicted from the bottom-up.

[0074] FIG. 3 shows the results of in vitro experiments for assessing the expression pattern of GFP under the control of the T cell-specific synthetic promoter Chr16-445-minCMV. FIG. 3A: Jurkat

(T-cell) and K562 (myeloid) cell lines were transfected with a vector coding for the GFP under a control promoter versus the synthetic T-cell specific promoter. FIG. 3B: PBMC transfected with a vector coding for the GFP under a control spleen focus forming virus (SFFV) promoter versus the synthetic T-cell specific promoter.

[0075] FIG. 4 shows the results of in vitro experiments for assessing the expression pattern of GFP under the control of the NK cell-specific synthetic promoter (NK6, SEQ ID NO:11). Various cell lines were transfected with a vector coding for the GFP under a control promoter versus the synthetic NK-cell specific promoter.

[0076] FIG. 5 shows the results of in vitro experiments aiming at determining the timing and functional expression of GFP under a synthetic NK cell-specific (NKspe-NK8, SEQ ID NO:14) promoter. In vitro differentiation system allowing for maturation of CD34.sup.+ cells into NK cells (OP9-DL4 with NK-specific media, box on the left) or B cells (OP9, box on the right) shows that the GFP is expressed early in NK cells progeny but not in B cells, showing its NK cell specificity.

[0077] FIG. 6 shows the expression of GFP in Nalm6 cell line (B cell line) when transduced with a lentivirus coding for the GFP under the control of a B-cell specific promoter (B-enh-1, SEQ ID NO: 23, left panel), or the minimal CMV promoter sequence as a negative control (SEQ ID NO: 6, right panel).

[0078] FIG. 7 shows the results of in vivo experiments for assessing the expression pattern of GFP under the control of the Chr16-445 T cell-specific synthetic promoter. In vivo validation of the synthetic promoter was assessed by injecting human CD34.sup.+ cells transduced with GFP under the control of the synthetic promoter in a sublethally irradiated NSG mouse without (FIG. 7A) or with the engraftment of a human thymus (BLT model) (FIG. 7B). Blood analysis show that engineered-HSC can give rise to different immune populations and that only T cells express the GFP protein, validating the specificity of our promoter. FIG. 7C: GFP expression in various cell types in BLT mice engrafted with CD34.sup.+ cells transduced with GFP under the control of a strong non-specific promoter.

[0079] FIGS. 8A-B show the results of in vivo experiments aiming at testing the differentiation of CD34.sup.+ cells modified with a GFP under the control of a NK-cell specific promoter (NK8, SEQ ID NO: 14). FIG. 8A shows the percentage of human CD45.sup.+ cells that express GFP in the blood (left bars), spleen (middle bars) and bone marrow (right bars) (results from 2 humanized mice). FIG. 8B shows the expression of GFP in human CD45.sup.+ cells isolated from the bone marrow of 2 humanized mice. Dot plots show that NK cells (CD56.sup.+), but not B cells (CD19.sup.+CD3.sup.-) or T cells (CD3.sup.+CD19.sup.-), express the GFP when the mice were humanized with CD34.sup.+ transduced with the GFP under the control of the NK8-cell specific promoter (SEQ ID NO:14).

[0080] FIG. 9 shows the results of in vivo experiments aiming at testing the differentiation of CD34.sup.+ cells modified with a GFP under the control of a B-cell specific promoter (B-enh-1, SEQ ID NO: 23). GFP expression was monitored in the blood of a humanized mice 4 weeks post-humanization. Cells were gated based on the expression of hCD45.sup.+. Dot plots show that B cells (CD19.sup.+), but not monocytes (CD14.sup.+), express the GFP when the mice were humanized with CD34.sup.+ transduced with the GFP under the control of the B-cell specific promoter (SEQ ID NO: 23, middle line). Mice humanized with CD34.sup.+ expressing the GFP under the control of a strong and unspecific promoter (SFFV, bottom panels) show expression of GFP in all human subpopulations (hCD45.sup.+), while no GFP expression is observed in untransduced CD34.sup.+ (negative control). At this time post-humanization (week 4), no T-cell are found in the blood of mice.

[0081] FIGS. 10A-D show the results of experiments for assessing the ability of the T-cell specific promoter for expressing a functional CAR. FIG. 10A: CAR-CD33 expression measured by flow cytometry following transduction of primary T-cells with the CAR-CD33 construct under the control of the non-specific SFFV promoter or the T-cell specific promoter. FIG. 10B: Cytotoxicity

against CD33.sup.+ or CD33 AML cells of T-cells expressing CAR-CD33 under the control of the SFFV (strong) promoter or the T-cell specific promoter (Tspe) at a 2:1 ratio (no significant difference). \*\*\*  $p < 0.001$ . FIG. 10C: CAR-CD22 expressed under the Tenh Chr16-445 (Tspe) promoter induced a CAR expression strong enough to result in a similar CAR-specific cytotoxicity against RS4;11 ALL-cell line to that of SFFV (strong) promoter (ratios of 0.5:1/1:1/2:1/4:1). FIG. 10D: Primary T cells transduced with a CAR-GD2 under the control of the T-cell specific promoter (Chr16-445) (squares) induced a cytotoxicity against a GD2.sup.+ NB-cell line (SK-N-DZ), at levels similar to those of primary T cell transduced with a non-specific strong promoter (SFFV, circles). In contrast, unmodified primary T-cells (diamonds) did not kill the target cell line. \*\*\*\*  $p < 0.0001$

[0082] FIGS. 11A-C show the results of in vitro experiments aiming at determining the T cell differentiation stage at which the T-cell specific promoter is expressed. In vitro differentiation system allowing for maturation of CD34.sup.+ cell into T cells (OP9-DL4-, FIG. 11A) or B cells (OP9, FIG. 11B) shows that the CAR is expressed early in T cells differentiation process (CD1aCD7.sup.+ stage) but is not expressed in B cells, confirming its T cell specificity. FIG. 11C shows the percentage of expression of the CAR-CD22 in the different sub-populations obtained in the OP9-DL4 and OP9 systems when the CAR-CD22 was expressed under the control of the T-cell specific promoter (Chr16-445, middle bars), or of a strong non-specific promoter (UCOE-SFFV, left bars). Black bars (left) depict the results obtained with untransduced cells as a negative control. DP: Double-positive.

[0083] FIG. 12 shows the results of in vivo experiments aiming at testing the differentiation of CD34.sup.+ cells transduced with a CAR-CD22 under the control of the T-cell specific promoter (Chr16-445). CAR-CD22 expression was monitored in hCD45.sup.+ cells of the blood of BLT mice 30 weeks post-humanization. Histogram plots shows that T cells (CD3.sup.+), but not B cells (CD19.sup.+) or monocytes (CD14.sup.+), express the CAR-CD22 when the mice were humanized with CD34.sup.+ modified with the CAR-CD22 under the control of the T-cell specific promoter (Chr16-445) (left column). This expression was not observed when the CD34.sup.+ were not transduced (negative control, right column).

#### DISCLOSURE OF INVENTION

[0084] Unless otherwise defined herein, scientific and technical terms used in connection with this disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the specification unless otherwise indicated. See, e.g.: Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3.sup.rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

[0085] The use of the terms "a" and "an" and "the" and similar referents in the context of

describing the technology (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

[0086] The terms “comprising”, “having”, “including”, and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”) unless otherwise noted.

[0087] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All subsets of values within the ranges are also incorporated into the specification as if they were individually recited herein.

[0088] The use of any and all examples, or exemplary language (“e.g.”, “such as”) provided herein, is intended merely to better illustrate the technology and does not pose a limitation on the scope of the claimed invention unless otherwise claimed.

[0089] No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the claimed invention.

[0090] Herein, the term “about” has its ordinary meaning. The term “about” is used to indicate that a value includes an inherent variation of error for the device or the method being employed to determine the value, or encompass values close to the recited values, for example within 10% of the recited values (or range of values).

[0091] The present inventors have developed an alternative strategy to the use of traditional promoters by designing specific synthetic regulatory elements to target the expression of a gene of interest in a cell subtype-specific manner. By engineering the transduced gene to be under the control of this synthetic regulatory element, and by transducing this construct into stem cells, it has been possible to direct gene expression exclusively in a specific and targeted cell subtype (or subtypes) that derive(s) from these genetically-modified stem cells, which is a significant refinement of the actual methods. Several cell-specific transcriptional enhancer candidates have been identified. As a proof-of concept, a first “synthetic regulatory element” comprising a T cell-specific transcriptional enhancer candidate that induced the expression of the transgene specifically in the human T cell population was designed, and the same methodology was successfully applied to design other cell-specific promoters, notably two human NK cell-specific promoters and one B cell-specific promoter. The designed human T/NK/B-cell specific promoters have a reduced size and showed good specificity, and are thus amenable for use in human gene therapy and HSC engineering.

#### Synthetic Expression Cassette

[0092] Accordingly, in a first aspect, the present disclosure provides synthetic expression cassette for expressing a nucleic acid (e.g., gene, gRNA, miRNA, shRNA) of interest in a cell comprising: a minimal promoter; and a transcriptional enhancer operatively coupled to the minimal promoter for expression of the nucleic acid of interest in the cell, wherein the transcriptional enhancer comprises a sequence having at least 70% sequence identity with at least 50 consecutive/contiguous nucleotides, preferably at least 100, 150, 200 or 250 consecutive/contiguous nucleotides, from one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23.

[0093] The term “enhancer” or “transcriptional enhancer” or “transcriptional regulatory element” refers to a cis-acting sequence that comprises one or more binding sites for transcription factors or transcriptional activators and that increases the activity of a promoter (e.g. a minimal promoter) in an orientation- and position-independent manner. The transcriptional enhancer may be located upstream or downstream of the minimal promoter. In an embodiment, the transcriptional enhancer is located upstream of the promoter.

[0094] In an embodiment, the transcriptional enhancer is a cell type- or subtype-specific transcriptional enhancer, i.e. the transcriptional enhancer specifically increases the activity of the promoter (and in turn the expression of the peptide/protein, or nucleic acid (e.g., miRNA, shRNA, gRNA), of interest) in a particular cell type or subtype. The term “specifically increases” as used



herein means that the increase in activity of the minimal promoter in the target cell type or subtype is higher than that in the other cell types or subtypes. In embodiment, the transcriptional enhancer is an immune cell-specific transcriptional enhancer, i.e. it specifically increases the activity of the promoter in one or more immune cell type(s), such as T cells, NK cells, B cells, macrophages, dendritic cells, basophils, neutrophils, etc. In an embodiment, the immune cell-specific transcriptional enhancer comprises a sequence having at least 70% sequence identity with at least 50 consecutive/contiguous nucleotides, preferably at least 100, 150, 200 or 250 consecutive/contiguous nucleotides, from one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23, and maintains transcriptional enhancing activity (i.e. exhibits transcriptional enhancing activity that is similar or better than the native sequence). In an embodiment, the immune cell-specific transcriptional enhancer comprises a sequence having at least 70% sequence identity with one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23, and maintains transcriptional enhancing activity (i.e. exhibits transcriptional enhancing activity that is similar or better than the native sequence).

[0095] In further embodiments, the immune cell-specific transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with at least 50 consecutive/contiguous nucleotides, preferably at least 100, 150, 200 or 250 consecutive/contiguous nucleotides, from one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23. In further embodiments, the immune cell-specific transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23.

[0096] In further embodiments, the immune cell-specific transcriptional enhancer comprises or consists of at least 50 consecutive/contiguous nucleotides, preferably at least 100, 150, 200 or 250 consecutive/contiguous residues, from one of the sequences set forth in SEQ ID NOs: 7-47, preferably SEQ ID NOs: 7-17 and 23.

[0097] Some of the sequences set forth in SEQ ID NOs: 7-47 comprise repetitive domains/motifs. For example, the sequence set forth in SEQ ID NO: 7 comprises a repetitive domain/motif of about 50 nucleotides (sequence:

GGTGTGGAGGGCCGGGTGGTGACX.sup.1CTX.sup.2AGTGACAGGTGAGGATGTGGCAX.sup.3 (SEQ ID NO: 63), wherein X.sup.1 is G or A, preferably G; X.sup.2 is G or C, preferably G, and X.sup.3 is C or T, preferably C. In an embodiment, the cell-specific transcriptional enhancer comprises at least one, preferably at least 2, 3, 4, 5, 6, 7, or 8 repetitive domains/motifs. The list of putative repetitive motifs that are present in each of the sequences set forth in SEQ ID NOs: 7-47 are depicted in Table IV (SEQ ID Nos: 64-82 and AAACCACA). Thus, in an embodiment, the transcriptional enhancer sequence comprises one or more of the motif(s) depicted in Table IV (SEQ ID Nos: 64-82 and AAACCACA). For example, SEQ ID NO:8 includes one or more of motifs #3 (SEQ ID NO:66), #4 (SEQ ID NO:67), #17 (SEQ ID NO: 80), and 20 (AAACCACA) depicted in Table IV. In a further embodiment, the transcriptional enhancer sequence comprises the motif(s) and repeats depicted in Table IV for each of SEQ ID NOS: 7-47.

[0098] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in a T cell, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 7-10, 13, 14, 18-22, 24-31, 33-43, 45 and 47, preferably SEQ ID NOs: 7-10, 13, 14. In a further embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in a T cell, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 7, 9, 27, 33, 34, 36, 37, 42, 43 and 45, preferably SEQ ID NOs: 7 and 9. In an embodiment, the cell is a CD4<sup>+</sup> cells and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%,

95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 22, 34, 37, 38, 43, 45 and 47.

[0099] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in an NK cell, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 8, 10-14, 18-22, 24-26, 28-31, 35, 38-41, 44 and 47, preferably SEQ ID NOs: 11, 12 and 14.

[0100] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in NK and T cells, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with the sequence set forth in SEQ ID NOs: 8, 10, 13, 14, 18-21, 22, 24-26, 28-31, 35, 38, 39-41 and 47, preferably SEQ ID NOs: 8, 10, 13 and 14.

[0101] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in B cells, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 15-17, 23, 32, 44 and 46, preferably SEQ ID NOs: 15-17 and 23, and more preferably SEQ ID NO: 23.

[0102] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in B and NK cells, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NO: 44.

[0103] In an embodiment, the synthetic expression cassette is for expressing the nucleic acid of interest in immune cells, such as NK cells, T cells, basophils and monocytes/macrophages, and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with the sequence set forth in SEQ ID NO: 14.

[0104] In an embodiment, the cell is a CD4.sup.+ cells (e.g., a CD4.sup.+ T cell) and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 22, 34, 37, 38, 43, 45 and 47.

[0105] In another embodiment, the cell is a CD8.sup.+ cells (e.g., a CD8.sup.+ T cell) and the transcriptional enhancer comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with one of the sequences set forth in SEQ ID NOs: 33, 35 and 39-41.

[0106] In an embodiment, the transcriptional enhancer sequence comprises one or more binding sites for transcription factor(s). In an embodiment, the transcriptional enhancer sequence comprises binding sites for at least two transcription factor(s). In an embodiment, the transcriptional enhancer sequence comprises binding sites for at least three transcription factor(s). In an embodiment, the transcriptional enhancer sequence comprises binding sites for at least four transcription factor(s). For example, the sequence set forth in SEQ ID NO:13 comprises binding sites for the transcription factors RUNX3, GATA2, FOS and JUN, and thus in an embodiment the sequence of the cell-specific transcriptional enhancer comprises 1, 2, 3 or all of these binding sites. Putative binding sites for transcription factors in each of the sequences set forth in SEQ ID NOs: 7-47 are depicted in Table V. Thus, in an embodiment, the transcriptional enhancer sequence comprises one or more of the binding sites for transcription factor(s) depicted in Table V.

[0107] The term “minimal promoter” refers to a promoter that only comprises the minimal elements of a promoter, namely the TATA box (also called the Goldberg-Hogness box) and a transcription initiation site, and which is inactive (or poorly active) at inducing/driving gene expression in the absence of properly located (usually upstream) one or more regulatory elements that enhance promoter activity (transcriptional enhancers). Any minimal promoter sequence known

to those of ordinary skill in the art is contemplated for inclusion in the minimal promoter sequences of the present disclosure. Minimal promoter sequences are often derived from viruses or are truncated eukaryotic promoters, and thus the minimal promoter may be a proopiomelanocortin minimal promoter (POMC), an adenoviral minimal promoter, a baculoviral minimal promoter, a CMV minimal promoter, a parvovirus minimal promoter, a herpesvirus minimal promoter, a poxvirus minimal promoter, an adeno-associated virus minimal promoter, a semiliki forest virus minimal promoter, an SV40 minimal promoter, a vaccinia virus minimal promoter, or a retrovirus minimal promoter. Examples of minimal promoters include the human simplex virus thymidine kinase (HSV TK or miniTK) minimal promoter, the cauliflower mosaic virus (CaMV) 35S minimal promoter, the human cytomegalovirus CMV minimal promoter (miniCMV), CMV53 (minCMV with the addition of an upstream GC box), the minimal simian virus 40 promoter (minSV40), MLP (the -38 to +6 region of the adenovirus major late promoter), the minP (synthetic minimal promoter composed of TATA box and transcription start site—from Promega), pJB42CAT5 (a minimal promoter derived from the human junB gene), YB\_TATA, and the super core promoter 1 (SCP1) minimal promoter (see Table I below). Several minimal promoters (also sometimes referred to as “core promoters”) are described in Ede et al., *ACS Synth Biol.* 2016 May 20; 5 (5): 395-404.

TABLE-US-00001 TABLE I Sequences of representative minimal promoters

| Name      | Sequence  |
|-----------|---|
| SCP1      | GTACT <b>TATATA</b> AAGGGGGTGGGGGCGCGTTCGTCCTCAGTCGCGATCGAACA         |
| miniCMV   | CTCGAGCCGAGCAGACGTGCCTACGGACCG (SEQ ID NO: 48)                        |
| CMV53     | GTAGGCGTGTACGGTGGGAGGTCT <b>TATATA</b> AAGCAGAGCTCGTTT                |
| minP      | AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 50)                       |
| HSV TK    | TTCGCAT <b>ATTA</b> AGGTGACGCGTGTGGCCTCGAACACCGAGCGACCTGCAG (miniTK)  |
| minSV40   | TGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCCC                |
| MLP       | GGGGGGGCT <b>TATAAA</b> AAGGGGGTGGGGGCGTTCGTCCTCACTCT (SEQ ID NO: 53) |
| pJB42CAT5 | CTGACAAATTCAGTATAAAAGCTTGGGGCTGGGGCCGAGCACTGGGGACTTT                  |
| YB_TATA   | TCTAGAGGG <b>TATATA</b> ATGGGGG CCA (SEQ ID NO: 55)                   |

GTACT**TATATA**AAGGGGGTGGGGGCGCGTTCGTCCTCAGTCGCGATCGAACA  
 CTCGAGCCGAGCAGACGTGCCTACGGACCG (SEQ ID NO: 48) miniCMV  
 GTAGGCGTGTACGGTGGGAGGTCT**TATATA**AAGCAGAGCTCGTTT  
 GTCAGATC (SEQ ID NO: 6) CMV53  
 CAACAAAATGTCGTAACAAGGGCGGTAGGCGTGTACGGTGGGAGGTCT**TATA**  
**TAAGCAGAGCTCGTTT**AGTGAACCG (SEQ ID NO: 49) minP  
 AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 50) HSV TK  
 TTCGCAT**ATTA**AGGTGACGCGTGTGGCCTCGAACACCGAGCGACCTGCAG (miniTK)  
 C GACCCGCTTAA (SEQ ID NO: 51) minSV40  
 TGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCCC  
 GCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATCGCTGACTAATT  
 TTTTT**TATTTAT**GCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGA  
 AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT (SEQ ID  
 NO: 52) MLP GGGGGGCT**TATAAA**AAGGGGGTGGGGGCGTTCGTCCTCACTCT (SEQ ID  
 NO: 53) pJB42CAT5  
 CTGACAAATTCAGTATAAAAGCTTGGGGCTGGGGCCGAGCACTGGGGACTTT  
 GAGGGTGGCCAGGCCAGCGTAGGAGGCCAGCGTAGGATCCTGCTGGGAGC  
 GGGGAAGCTCCGGAAGCCCAGCAGCG (SEQ ID NO: 54) YB\_TATA  
 TCTAGAGGG**TATATA**ATGGGGG CCA (SEQ ID NO: 55) Bold = TATA box  
 consensus sequence Underlined = consensus GC box sequence Italics = additional B-  
 recognition element found in pJB42CAT5

[0108] Sequence identity between two nucleotide sequences may be determined by comparing each position in the aligned sequences. A degree of identity between nucleotide sequences is a function of the number of identical nucleotides at positions shared by the sequences. As used herein, a given percentage of identity between sequences denotes the degree of sequence identity in optimally aligned sequences. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms and sequence alignment tools, such as the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math* 2:482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85:2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics

Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence identity may also be determined using the BLAST algorithm, described in Altschul et al., 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software/tools for performing BLAST analysis may be available through the National Center for Biotechnology Information. Other sequence alignment tools such as Needle, Stretcher, Clustal Omega and Kalign are available through the European Bioinformatics Institute (EMBL-EBI).

[0109] The terms “operatively positioned”, “operatively linked” and “operatively coupled” mean that a promoter (and/or enhancer) is in a correct functional location and orientation in relation to a nucleic acid sequence to control transcriptional initiation and expression of that nucleic acid. An enhancer is “operatively coupled” to a promoter (e.g. a minimal promoter) when it is in a correct functional location and orientation for increasing the transcriptional activity of the promoter.

[0110] The terms “synthetic” mean that the expression cassette is an artificial or recombinant construct that is not found in nature, i.e. that the combination of the minimal promoter and the transcriptional enhancer is not naturally found in the native genome of a cell. In an embodiment, the minimal promoter is heterologous with the transcriptional enhancer, i.e. it is not normally associated with the transcriptional enhancer in its natural environment, e.g., they do not control the expression of the same genes in the native genome of a cell. In an embodiment, the minimal promoter and the transcriptional enhancer are from different cell types or from different organisms (e.g., virus vs. eukaryotic cell). In an embodiment, the minimal promoter and/or the transcriptional enhancer is/are heterologous with the nucleic acid of interest, i.e. they are not normally associated with the nucleic acid of interest in its natural environment. In an embodiment, the transcriptional enhancer is of human origin. In an embodiment, the minimal promoter is of viral origin.

[0111] In an embodiment, the synthetic expression cassette further comprises a polyadenylation (poly(A)) signal. The poly(A) signal effects proper polyadenylation of the nucleic acid of interest (transcript). The nature of the poly(A) signal not believed to be crucial to the successful practice of the invention, and thus any such sequence may be employed. Examples of representative poly(A) signals include the SV40 poly(A) signal and/or the bovine growth hormone poly(A) signal, convenient and/or known to function well in various target cells. In an embodiment, the synthetic expression cassette further comprises a Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE). Such element is commonly used to increase expression of genes delivered by viral vectors, has been shown to increase mRNA stability and protein yield (see, e.g., Lee, Y B, et al. 2005. *Exp Physiol.* 90(1): 33-7). In an embodiment, the WPRE is used in combination with the poly(A) signal. In another embodiment, the WPRE replaces the poly(A) signal.

[0112] In an embodiment, the synthetic expression cassette further comprises a transcriptional termination signal. A “termination signal” or “terminator” is comprised of the DNA sequences involved in specific termination of an RNA transcript by an RNA polymerase. Thus, in certain embodiments, a termination signal that ends the production of an RNA transcript is contemplated.

[0113] In an embodiment, the synthetic expression cassette further comprises a nucleic acid of interest. The term “nucleic acid of interest” or “gene of interest” is used to refer to a nucleic acid that encodes a functional peptide or polypeptide (protein) of interest (native or modified peptides/proteins). In an embodiment, the functional peptide or polypeptide is a therapeutic peptide or polypeptide, i.e. a peptide or polypeptide that can be administered to a subject for the purpose of treating or preventing a disease. Any nucleic acid encoding a peptide or polypeptide of interest known to those of ordinary skill in the art is contemplated for inclusion in the synthetic expression cassette. The peptide or polypeptide of interest may be an enzyme, a signaling molecule (e.g., kinase, phosphatase), a receptor, a growth factor (e.g., cytokines), a chemotactic protein (e.g., chemokines), a structural protein (cytoskeletal proteins), a transcription factor, a cell adhesion protein, an antibody or antigen-binding fragment thereof, etc. The peptide or polypeptide may be a naturally-occurring peptide or polypeptide, a fragment or variant thereof, chimeric versions thereof,

etc.

[0114] In an embodiment, the nucleic acid of interest encodes a recombinant receptor, such as a chimeric antigen receptor (CAR). Such CAR typically comprises a ligand-binding domain (e.g. antibody or antibody fragment such as a single-chain variable fragment (scFv)) that provides specificity for a desired antigen (e.g., tumor antigen) linked to an activating intracellular domain portion, such as a T cell or NK cell activating domain, providing a primary activation signal, in some aspects via linkers and/or transmembrane domain(s).

[0115] In particular embodiments, the recombinant receptor (e.g., CAR) comprises an intracellular signaling domain, which includes an activating cytoplasmic signaling domain (also interchangeably called an intracellular signaling region), such as an activating cytoplasmic (intracellular) domain capable of inducing a primary activation signal in an immune cell (T cell, NK cell, for example), a cytoplasmic signaling domain of a T cell receptor (TCR) component (e.g. a cytoplasmic signaling domain of a CD3-zeta (CD3  $\zeta$ ) chain or a functional variant or signaling portion thereof) and/or that comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[0116] In some embodiments, the recombinant receptor (e.g., CAR) further comprises an extracellular ligand-binding domain that specifically binds to a ligand (e.g., antigen) antigen. In some embodiments, the ligand, such as an antigen, is a protein expressed on the surface of cells. In some embodiments, the CAR is a TCR-like CAR and the antigen is a processed peptide antigen, such as a peptide antigen of an intracellular protein, which is recognized on the cell surface in the context of a major histocompatibility complex (MHC) molecule.

[0117] Exemplary recombinant receptors, including CARs and recombinant TCRs, as well as methods for engineering and introducing the receptors into cells, include those described, for example, in international patent application publication numbers WO 2000/14257, WO 2013/126726, WO 2012/129514, WO 2014/031687, WO 2013/166321, WO 2013/071154, WO 2013/123061, US patent application publication numbers US 2002/131960, US 2013/287748, US 2013/0149337, U.S. Pat. Nos. 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application number EP2537416, and/or those described by Sadelain et al., *Cancer Discov.* 2013 April; 3 (4): 388-398; Davila et al. (2013) *PLoS ONE* 8 (4): e61338; Turtle et al., *Curr. Opin. Immunol.* 2012 October; 24 (5): 633-39; Wu et al., *Cancer*, 2012 Mar. 18 (2): 160-75. In some embodiments, the genetically engineered antigen receptors include a CAR as described in U.S. Pat. No. 7,446,190, and those described in International Patent Application Publication No.: WO 2014/055668.

[0118] In some embodiments, the recombinant receptor (e.g. CAR) includes in its extracellular portion an antigen- or ligand-binding domain that binds (specifically binds) to an antigen (or a ligand), such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable domains, and/or antibody molecules. In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (V.sub.H) and variable light (V.sub.L) chains of a monoclonal antibody (mAb). The term “antibody” herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab').sub.2 fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy chain (VH) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The

term also encompasses intact or full-length antibodies, including antibodies of any class or subclass, including IgG and subclasses thereof, IgM, IgE, IgA, and IgD.

[0119] In some embodiments, the antigen-binding proteins, antibodies and antigen binding fragments thereof specifically recognize an antigen of a full-length antibody. In some embodiments, the heavy and light chains of an antibody can be full-length or can be an antigen-binding portion (a Fab, F(ab').sub.2, Fv or a single chain Fv fragment (scFv)). In other embodiments, the antibody heavy chain constant region is chosen from, e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE, particularly chosen from, e.g., IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (e.g., human IgG1). In another embodiment, the antibody light chain constant region is chosen from, e.g., kappa or lambda, particularly kappa.

[0120] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V.sub.H and V.sub.L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, e.g., Kindt et al. *Kuby Immunology*, 6.sup.th ed., W.H. Freeman and Co., page 91 (2007). A single V.sub.H or V.sub.L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V.sub.H or V.sub.L domain from an antibody that binds the antigen to screen a library of complementary V.sub.L or V.sub.H domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0121] Single-domain antibodies (sdAbs) are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, the single-domain antibody is a human single-domain antibody.

[0122] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, e.g., peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some embodiments, the antibody fragment is a scFv.

[0123] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0124] In some embodiments, the CAR comprises an antibody or an antigen-binding fragment (e.g., scFv) that specifically recognizes an antigen, such as an intact antigen, expressed on the surface of a cell.

[0125] In some embodiments, the CAR comprises a TCR-like antibody, such as an antibody or an antigen-binding fragment (e.g., scFv) that specifically recognizes an intracellular antigen, such as a tumor-associated antigen, presented on the cell surface as a MHC-peptide complex. In some embodiments, an antibody or antigen-binding portion thereof that recognizes an MHC-peptide complex can be expressed on cells as part of a recombinant receptor, such as an antigen receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR comprising an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a

TCR-like CAR.

[0126] In some embodiments, the recombinant receptors include recombinant T cell receptors (TCRs) and/or TCRs cloned from naturally occurring T cells. In some embodiments, a T cell receptor (TCR) comprises a variable  $\alpha$  and  $\beta$  chains (also known as TCR $\alpha$  and TCR $\beta$ , respectively) or a variable  $\gamma$  and  $\delta$  chains (also known as TCR $\gamma$  and TCR $\delta$ , respectively), or a functional fragment thereof such that the molecule is capable of specifically binding to an antigen peptide bound to a MHC receptor. In some embodiments, the TCR is in the  $\alpha\beta$  form. Typically, TCRs that exist in  $\alpha\beta$  and  $\gamma\delta$  forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found on the surface of a cell or in soluble form. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens bound to MHC molecules. In some embodiments, a TCR also can comprise a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, e.g., Janeway et al., *Immunobiology: The Immune System in Health and Disease*, 3<sup>rd</sup> ed., Current Biology Publications, p. 4:33, 1997). For example, in some embodiments, each chain of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR is associated with invariant proteins of the CD3 complex involved in mediating signal transduction.

[0127] In some embodiments, a TCR for a target antigen (e.g., a cancer/tumor antigen) is identified and introduced into the cells. In some embodiments, nucleic acid encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of publicly available TCR DNA sequences. In some embodiments, the TCR is obtained from a biological source, such as from cells, such as from a T cell (e.g., cytotoxic T cell), T cell hybridomas or other publicly available source. In some embodiments, the T-cells can be obtained from in vivo isolated cells. In some embodiments, a high-affinity T cell clone can be isolated from a patient and the TCR isolated. In some embodiments, the T cells can be a cultured T cell hybridoma or clone. In some embodiments, the TCR clone for a target antigen has been generated in transgenic mice engineered with human immune system genes (e.g., the human leukocyte antigen system, or HLA). See, e.g., tumor antigens (see, e.g., Parkhurst et al. (2009) *Clin Cancer Res.* 15:169-180 and Cohen et al. (2005) *J Immunol.* 175:5799-5808. In some embodiments, phage display is used to isolate TCRs against a target antigen (see, e.g., Varela-Rohena et al. (2008) *Nat Med.* 14:1390-1395 and Li (2005) *Nat Biotechnol.* 23:349-354. In some embodiments, the TCR or antigen-binding portion thereof can be synthetically generated from knowledge of the sequence of the TCR of interest.

[0128] In some embodiments, the recombinant receptor (e.g., a CAR such as an antibody or antigen-binding fragment thereof), further includes a spacer, which may be or include at least a portion of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region, e.g., an IgG4 hinge region, and/or a CH1/CL and/or Fc region. In some embodiments, the constant region or portion is of a human IgG, such as IgG4 or IgG1. In some aspects, the portion of the constant region serves as a spacer region between the antigen-recognition component, e.g., scFv, and transmembrane domain. The spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer. Exemplary spacers include those having at least about 10 to 220 amino acids, about 10 to 200 amino acids, about 10 to 175 amino acids, about 10 to 150 amino acids, about 10 to 125 amino acids, about 10 to 100 amino acids, about 10 to 75 amino acids, about 10 to 50 amino acids, about 10 to 40 amino acids, about 10 to 30 amino acids, about 10 to 20 amino acids, or about 10 to 15 amino acids, and including any integer between the endpoints of any of the listed ranges. Exemplary spacers include IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. Exemplary spacers include, but are not limited to, those described in Hudecek et al. (2013) *Clin. Cancer Res.*, 19:3153 or PCT patent publication number

[0129] The antigen/ligand recognition domain generally is linked to one or more intracellular signaling components, such as signaling components that mimic activation through an antigen receptor complex, such as a TCR or NK receptor complex, in the case of a CAR, and/or signal via another cell surface receptor. Thus, in some embodiments, the antigen-binding component (e.g., antibody) is linked to one or more transmembrane and intracellular signaling domains. In some embodiments, the transmembrane domain is fused to the extracellular domain. In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, e.g., CAR, is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0130] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the TCR, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively, the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

[0131] Among the intracellular signaling domains are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one comprising glycines and serines, e.g., glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR.

[0132] The receptor, e.g., the CAR, generally includes at least one intracellular signaling component or components. In some embodiments, the receptor includes an intracellular component of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, e.g., CD3 Z chain. Thus, in some aspects, the CAR is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, e.g., CAR, further includes a portion of one or more additional molecules such as Fc receptor  $\gamma$ , CD8, CD4, CD25, or CD16. In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary activation of the TCR complex. Primary cytoplasmic signaling sequences that act in a stimulatory manner may comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM comprising primary cytoplasmic signaling sequences include those derived from TCR or CD3  $\zeta$ , FcR gamma or FcR beta. In some embodiments, cytoplasmic signaling molecule(s) in the CAR comprise(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3  $\zeta$ . In some embodiments, to promote full activation, a component for generating a secondary or co-stimulatory signal is also included in the CAR, such as the signaling domain of a costimulatory receptor such as CD28, 4-1BB, OX40, DAP10, and ICOS. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal. In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first-generation CAR is one that solely provides an antigen-receptor (e.g., CD3-chain) induced signal upon antigen binding; in some aspects, a second-generation CARs is one that provides such a signal and costimulatory signal, such as one including an intracellular signaling domain from a



costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR in some aspects is one that includes multiple costimulatory domains of different costimulatory receptors. [0133] In some embodiments, the CAR or other antigen receptor may further include a marker or the cell may further express a marker, such as a surrogate marker, which may be used to confirm transduction or engineering of the cell to express the receptor, such as a truncated version of a cell surface receptor, such as truncated EGFR (tEGFR). In some aspects, the marker includes all or part (e.g., truncated form) of CD34, a NGFR, or epidermal growth factor receptor (e.g., tEGFR). In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, e.g., T2A. See WO 2014/031687. In some embodiments, introduction of a construct encoding the CAR and EGFRt separated by a T2A ribosome switch can express two proteins from the same construct, such that the EGFRt can be used as a marker to detect cells expressing such construct. In some embodiments, a marker, and optionally a linker sequence, can be any as disclosed in published patent application No. WO 2014/031687. For example, the marker can be a truncated EGFR (tEGFR) that is, optionally, linked to a linker sequence, such as a T2A cleavable linker sequence.

[0134] Among the antigens that may be targeted by the chimeric receptors are those expressed in the context of a disease, condition, or cell type to be targeted via the adoptive cell therapy. Among the diseases and conditions are proliferative, neoplastic, and malignant diseases and disorders, including cancers and tumors, including hematologic cancers, cancers of the immune system, such as lymphomas, leukemias, and/or myelomas (e.g., B cell, T cell, and myeloid leukemias, lymphomas, and multiple myelomas).

[0135] In some embodiments, the antigen (or a ligand) is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen (or a ligand) is selectively expressed or overexpressed on cells of the disease or condition, e.g., the tumor/cancer or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0136] In some embodiments, the antigen (or a ligand) is a tumor antigen or cancer marker. In certain embodiments, the antigen is an integrin (e.g.,  $\alpha$ .sub.v $\beta$ .sub.6 integrin,  $\alpha$ .sub.v $\beta$ .sub.3 integrin, integrin  $\beta$ .sub.7), B cell maturation antigen (BCMA), B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen IB (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, fetal acetylcholine receptor, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), Her2/neu (receptor tyrosine kinase erbB2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13R $\alpha$ 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC 16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast

glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), galectins (galectin-1, galectin-7) a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens such as bacteria and parasites. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen targeted by the receptor is CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In an embodiment, a plurality of recombinant receptors targeting a plurality of antigens are used. In a further embodiment, two recombinant receptors targeting two antigens are used.

#### Vector/Plasmids

[0137] In an embodiment, the synthetic expression cassette is comprised in a plasmid or a vector. Thus, the present disclosure also relates to a vector or plasmid comprising the synthetic expression cassette described herein. The term “vector” is used to refer to a carrier into which a nucleic acid (e.g., the synthetic expression cassette defined herein) can be inserted for introduction into a cell where it can be replicated. The term “expression vector” or “nucleic acid vector” refers to a vector containing a nucleic acid or “expression cassette” coding for at least part of a gene product capable of being transcribed and “regulatory” or “control” sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In addition to control sequences that govern transcription and translation, expression vectors may contain nucleic acid sequences that serve other functions as well.

[0138] In an embodiment, the vector further comprises a nucleic acid encoding a selectable marker or reporter protein. A selectable marker or reporter is defined herein to refer to a nucleic acid encoding a polypeptide that, when expressed, confers an identifiable characteristic (e.g., a detectable signal, resistance to a selective agent) to the cell permitting easy identification, isolation and/or selection of cells containing the selectable marker from cells without the selectable marker or reporter. Any selectable marker or reporter known to those of ordinary skill in the art is contemplated for inclusion as a selectable marker in the vector of the present disclosure. For example, the selectable marker may be a drug selection marker, an enzyme, or an immunologic marker. Examples of selectable markers or reporters include, but are not limited to, polypeptides conferring drug resistance (e.g., kanamycin/geneticin resistance), enzymes such as alkaline phosphatase and thymidine kinase, bioluminescent and fluorescent proteins such as luciferase, green fluorescent protein (GFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), blue fluorescent protein (BFP), citrine and red fluorescent protein from discosoma (dsRED), membrane bound proteins to which high affinity antibodies or ligands directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane-bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin (HA) or Myc. The nucleic acid encoding the selectable marker or reporter protein may be under the control of the same promoter/enhancer as the nucleic acid of interest, or may be under the control of a distinct promoter/enhancer.

[0139] In embodiments, the vector may comprise additional elements, such as one or more origins of replication sites (often termed “ori”), restriction endonuclease recognition sites (multiple cloning sites, MCS) and/or internal ribosome entry site (IRES) elements.

[0140] In an embodiment, the vector is a viral vector. The term “viral vector” as used herein refers to a recombinant virus capable of transducing cells and introducing their genetic material into the cells. In an embodiment, the viral vector is suitable for use in gene therapy applications. Examples of viral vectors that may be used in gene therapy include retroviruses (lentiviruses), adenoviruses, adeno-associated viruses (AAV), herpesviruses (herpes simplex viruses), alphaviruses, and vaccinia viruses (Poxviruses). In an embodiment, the viral vector is a lentiviral vector. As will be evident to

one of skill in the art, the term “lentiviral vector” is used to refer to a lentiviral particle that mediates nucleic acid transfer. Lentiviral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). In particular aspects, the terms “lentiviral vector,” “lentiviral expression vector” are used to refer to lentiviral transfer plasmids and/or infectious lentiviral particles.

[0141] In an embodiment, the lentiviral vector is a pseudotyped lentiviral vector. Pseudotyped lentiviral vectors consist of vector particles bearing enveloped proteins (glycoproteins, GP) derived from other enveloped viruses. Such particles possess the tropism of the virus from which the enveloped proteins are derived. One of the widely used glycoproteins for pseudotyping lentiviral vectors is the vesicular stomatitis virus GP (VSV-G), due to the very broad tropism and stability of the resulting pseudotypes. Pseudotyped lentiviral vectors are well known in the art, and several examples are described, for example, in Cronin et al., *Curr. Gene Ther.* 5 (4): 387-398. It includes lentiviral vectors pseudotyped with lyssavirus GPs, lymphocytic choriomeningitis virus (LCMV) GPs, alphavirus GPs (e.g., Ross River virus (RRV), Semliki Forest virus (SFV) and Sindbis virus GPs), Filovirus GPs (e.g., Marburg virus and Ebola Zaire virus GPs), gammaretrovirus GPs (e.g., ecotropic MLV, amphotropic 4070A MLV, 10A1 MLV, xenotropic NZB MLV, mink cell focus-forming virus, gibbon ape leukemia (GALV) virus, RD1 14 GPs), Vesicular Stomatitis Virus type-G (VSV-G), Measles-Virus Lentiviral vector (MV-LV), Baboon envelop (BaEV)-LVs and baculovirus GPs (GP64).

[0142] In an embodiment, the vector is an episomally-maintained viral vector or non-integrating vector, such as a Sendai virus or vector. Such vectors are not integrated into the genome, but are maintained episomally with cell division due to scaffold/matrix attachment region presence inside vector (see, e.g., Giannakopoulos A et al., *J Mol Biol.* 2009 Apr. 17; 387 (5): 1239-49; and Haase et al., *BMC Biotechnol.* 2010; 10:20).

[0143] In another embodiment, the vector is a non-viral vector, for example nude DNA, a liposome, a polymerizer or a molecular conjugate.

#### Cells

[0144] In another aspect, the present disclosure provides a cell (host cell, engineered cell) comprising the synthetic expression cassette or vector/plasmid described herein. In an embodiment, the cell is a primary cell, for example a brain/neuronal cell, a peripheral blood cell (e.g., a B or T lymphocyte, a monocyte, a NK cell), a cord blood cell, a bone marrow cell, a cardiac cell, an endothelial cell, an epidermal cell, an epithelial cell, a fibroblast, hepatic cell or a lung/pulmonary cell. In an embodiment, the cell is a bone marrow cell, peripheral blood cell or cord blood cell. In a further embodiment, the cell is an immune cell, such as a T cell (e.g., a CD8.sup.+ T cell), a B cell or a NK cell.

[0145] In an embodiment, the cell is a stem cell. The term “stem cell” as used herein refers to a cell that has pluripotency which allows it to differentiate into a functional mature cell. It includes primitive hematopoietic cells, progenitor cells, as well as adult stem cells that are undifferentiated cells found in various tissue within the human body, which can renew themselves and give rise to specialized cell types and tissue from which the cells came (e.g., muscle stem cells, skin stem cells, brain or neural stem cells, mesenchymal stem cell, lung stem cells, liver stem cells).

[0146] In an embodiment, the cell is a primitive hematopoietic cell. As used herein, the term “primitive hematopoietic cell” is used to refers to cells having pluripotency which allows them to differentiate into functional mature blood cells of the myeloid and lymphoid lineages such as T cells, B cells, NK cells, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), and monocytes (e.g., monocytes, macrophages), and that may or may not the ability to regenerate while maintaining their pluripotency (self-renewal). It encompasses “hematopoietic stem cells” or “HSCs”, which are cells having both pluripotency which allows them to differentiate into functional mature cells such as granulocytes, erythrocytes,

thrombocytes, and monocytes, and the ability to regenerate while maintaining their pluripotency (self-renewal), as well as pluripotent hematopoietic cells that do not have self-renewal capacity. It also encompasses embryonic stem cells (ESCs), which are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. In an embodiment, the population of cells comprises ESCs. In another embodiment, the population of cells comprises HSCs. HSCs may be obtained from the body or an organ of the body containing cells of hematopoietic origin. Such sources include un-fractionated bone marrow (from femurs, hip, ribs, sternum, and other bones), umbilical cord blood, peripheral blood, liver, thymus, lymph and spleen. All of the aforementioned crude or un-fractionated blood products can be enriched for cells having HSC characteristics in ways known to those of skill in the art. HSCs are phenotypically identified by their small size, lack of lineage (lin) markers, low staining (side population) with vital dyes such as rhodamine 123 (rhodamine.sup.DULL, also called rho.sup.0) or Hoechst 33342, and presence/absence of various antigenic markers on their surface many of which belongs to the cluster of differentiation series, such as: CD34, CD38, CD90, CD133, CD105, CD45 and c-kit.

[0147] In an embodiment, the stem cell is an induced pluripotent stem cell (iPSC). The term iPSC refers to a pluripotent stem cell that can be generated directly from adult cells using appropriate factors to “reprogram” the cells.

[0148] In an embodiment, the cell is a mammalian cell, for example a human cell.

[0149] The synthetic expression cassette or vector/plasmid described herein may be introduced into the cell using standard techniques for introducing nucleic acids into a cell, e.g., transfection, transduction or transformation. In an embodiment, the vector is a viral vector, and the cell is transduced with the vector. As used herein, the term “transduction” refers to the stable transfer of genetic material from a viral particle (e.g., lentiviral) to a cell genome (e.g., hematopoietic cell genome). It also encompasses the introduction of non-integrating viral vectors into cells, which leads to the transient or episomal expression of the gene of interest present in the viral vector.

[0150] Viruses may be used to infect cells in vivo, ex vivo, or in vitro using techniques well known in the art. For example, when cells, for instance CD34.sup.+ cells or stem cells are transduced ex vivo, the vector particles may be incubated with the cells using a dose generally in the order of between 1 to 100 or 1 to 50 multiplicities of infection (MOI) which also corresponds to  $1 \times 10^{5.5}$  to 100 or  $50 \times 10^{5.5}$  transducing units of the viral vector per  $10^{5.5}$  cells. This, of course, includes amount of vector corresponding to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, and 50 MOI.

[0151] Prior to, during, and/or following transduction, the cells may be cultured in media suitable for the maintenance, growth, or proliferation of the cells. The culture conditions of the population of cells will vary depending on different factors, notably, the starting cell population. Suitable culture media and conditions are well known in the art. The culture may be carried out in natural medium, a semi-synthetic medium or a synthetic medium in terms of composition, and may be a solid medium, a semisolid medium or a liquid medium in terms of shape, and any nutrient medium used for cell culture, such as stem cell culture, which may be supplemented with one or more of growth factors. Such medium typically comprises sodium, potassium, calcium, magnesium, phosphorus, chlorine, amino acids, vitamins, cytokines, hormones, antibiotics, serum, fatty acids, saccharides or the like. In the culture, other chemical components or biological components may be incorporated singly or in combination, as the case requires. Such components to be incorporated in the medium may be fetal calf serum, human serum, horse serum, insulin, transferrin, lactoferrin, cholesterol, ethanolamine, sodium selenite, monothioglycerol, 2-mercaptoethanol, bovine serum albumin, sodium pyruvate, polyethylene glycol, various vitamins, various amino acids, agar, agarose, collagen, methylcellulose, various cytokines, various growth factors or the like. Examples of such basal medium appropriate for a method of expanding stem cells include, without limitation, StemSpan™ Serum-Free Expansion Medium (SFEM) (StemCell Technologies®, Vancouver, Canada), StemSpan™ H3000-Defined Medium (StemCell Technologies®, Vancouver, Canada),

CellGro™, SCGM (CellGenix™, Freiburg Germany), StemPro™-34 SFM (Invitrogen®), Dulbecco's Modified Eagle's Medium (DMEM), Ham's Nutrient Mixture H12 Mixture F12, McCoy's 5A medium, Eagle's Minimum Essential Medium (EMEM), MEM medium (alpha Modified Eagle's Minimum Essential Medium), RPMI 1640 medium, Isocove's Modified Dulbecco's Medium (IMDM), StemPro34™ (Invitrogen®), X-VIVO™ 10 (Cambrex®), X-VIVO™ 15 (Cambrex®) and Stemline™ II (Sigma-Aldrich®).

[0152] Following transduction, the transduced cells may be cultured under conditions suitable for their maintenance, growth and/or proliferation. In particular aspects, the transduced cells are cultured for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days before transplantation.

[0153] Culture conditions for maintaining and/or expanding stem cells are well known in the art. Typically, the culturing conditions comprise the use of factors like cytokines and growth factors, generally known in the art for stem cell expansion. Such cytokines and growth factors can be biologics or small molecules and they include without limitation IL-1, IL-3, IL-6, IL-11, G-CSF, GM-CSF, SCF, FLT3-L, thrombopoietin (TPO), erythropoietin, and analogs thereof. As used herein, “analogs” include any structural variants of the cytokines and growth factors having the biological activity as the naturally occurring forms, including without limitation, variants with enhanced or decreased biological activity when compared to the naturally occurring forms or cytokine receptor agonists such as an agonist antibody against the TPO receptor (for example, VB22B sc (Fv) 2 as detailed in patent publication WO 2007/145227, and the like). Cytokine and growth factor combinations are chosen to maintain/expand stem cells while limiting the production of terminally differentiated cells. In one specific embodiment, one or more cytokines and growth factors are selected from the group consisting of SCF, FLT3-L and TPO.

[0154] Human IL-6 or interleukin-6, also known as B-cell stimulatory factor 2 has been described by (Kishimoto, *Ann. review of Immunol.* 23:1, 2005) and is commercially available. Human SCF or stem cell factor, also known as c-kit ligand, mast cell growth factor or Steel factor has been described (Smith, M A et al., *ACTA Haematologica*, 105(3): 143, 2001) and is commercially available. FLT3-L or FLT-3 Ligand, also referred as FL is a factor that binds to flt3-receptor. It has been described (Hannum C, *Nature* 368(6472): 643-8) and is commercially available. TPO or thrombopoietin, also known as megakaryocyte growth factor (MGDF) or c-Mpl ligand has been described (Kaushansky K (2006). *N. Engl. J. Med.* 354 (19): 2034-45) and is commercially available.

[0155] The chemical components and biological components mentioned above may be used not only by adding them to the medium but also by immobilizing them onto the surface of the substrate or support used for the culture, specifically speaking, by dissolving a component to be used in an appropriate solvent, coating the substrate or support with the resulting solution and then washing away an excess of the component. Such a component to be used may be added to the substrate or support preliminarily coated with a substance which binds to the component.

[0156] Stem cells may be cultured in a culture vessel generally used for animal cell culture such as a Petri dish, a flask, a plastic bag, a Teflon™ bag, optionally after preliminary coating with an extracellular matrix or a cell adhesion molecule. The material for such a coating may be collagens I to XIX, fibronectin, vitronectin, laminins 1 to 12, nitrogen, tenascin, thrombospondin, von Willebrand factor, osteopontin, fibrinogen, various elastins, various proteoglycans, various cadherins, desmocollin, desmoglein, various integrins, E-selectin, P-selectin, L-selectin, immunoglobulin superfamily, Matrigel®, poly-D-lysine, poly-L-lysine, chitin, chitosan, Sepharose®, alginate acid gel, hydrogel or a fragment thereof. Such a coating material may be a recombinant material having an artificially modified amino acid sequence. The stem cells may be cultured by using a bioreactor which can mechanically control the medium composition, pH and the like and obtain high density culture (Schwartz R M, *Proc. Natl. Acad. Sci. U.S.A.*, 88:6760, 1991; Koller M R, *Bone Marrow Transplant*, 21:653, 1998; Koller, M R, *Blood*, 82:378, 1993; Astori G, *Bone Marrow Transplant*, 35 (1) 101, 2005).

[0157] The cell population may then be washed to remove the compound or composition of invention and/or any other component of the cell culture and resuspended in an appropriate cell suspension medium for short term use or in a long-term storage medium, for example a medium suitable for cryopreservation, for example DMEM with 40% FCS and 10% DMSO. Other methods for preparing frozen stocks for cultured cells also are available to those skilled in the art.

#### Compositions

[0158] In another aspect, the present disclosure provides a composition comprising the synthetic expression cassette, vector or cell described herein. The composition may comprise one or more carrier or excipient, e.g. a buffer, a saline solution, a preservative, etc. In an embodiment, the composition is a pharmaceutical composition comprising at least one pharmaceutically acceptable carrier or excipient. An “excipient,” as used herein, has its normal meaning in the art and is any ingredient that is not an active ingredient (drug) itself. Excipients include for example binders, lubricants, diluents, fillers, thickening agents, disintegrants, plasticizers, coatings, barrier layer formulations, lubricants, stabilizing agent, release-delaying agents and other components.

“Pharmaceutically acceptable excipient” as used herein refers to any excipient that does not interfere with effectiveness of the biological activity of the active ingredients and that is not toxic to the subject, i.e., is a type of excipient and/or is for use in an amount which is not toxic to the subject. Excipients are well known in the art (see, e.g., *Remington: The Science and Practice of Pharmacy*, by Loyd V Allen, Jr, 2012, 22<sup>nd</sup> edition, *Pharmaceutical Press*; *Handbook of Pharmaceutical Excipients*, by Rowe et al., 2012, 7<sup>th</sup> edition, *Pharmaceutical Press*).

Pharmaceutical compositions may be prepared using standard methods known in the art by mixing the active ingredient having the desired degree of purity with one or more optional pharmaceutically acceptable carriers, excipients and/or stabilizers. The excipient may be selected for administration of the composition by any routes, for example, for intravenous, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, epidural, intracisternal, intraperitoneal, intranasal or pulmonary (e.g., aerosol) administration. In an embodiment, the pharmaceutical composition is formulated for injection, e.g. as a solution, suspension, or emulsion, including localized injection, catheter administration, systemic injection, intravenous injection, intraperitoneal injection, subcutaneous injection or parenteral administration.

[0159] Pharmaceutical compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium comprising, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0160] Sterile injectable solutions can be prepared by incorporating the cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can comprise auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0161] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of

the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0162] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers comprising the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0163] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

#### Methods/Uses

[0164] The present disclosure also relates to a method for inducing the expression of a gene of interest by a cell, the method comprising introducing the synthetic expression cassette or vector described herein in the cell. The present disclosure also relates to a use of the synthetic expression cassette or vector described herein for inducing the expression of a gene of interest by a cell. In an embodiment, the cell is a primary cell, for example a brain/neuronal cell, a peripheral blood cell (e.g., a B or T lymphocyte, a monocyte, a NK cell), a cord blood cell, a bone marrow cell, a cardiac cell, an endothelial cell, an epidermal cell, an epithelial cell, a fibroblast, hepatic cell or a lung/pulmonary cell. In an embodiment, the cell is a bone marrow cell, peripheral blood cell or cord blood cell. In a further embodiment, the cell is an immune cell, such as a T cell (e.g., a CD8.sup.+ T cell), a B cell or a NK cell.

[0165] In an embodiment, the gene of interest encodes a protein that is defective or absent in the cell. In an embodiment, the gene of interest encodes a recombinant receptor, such as a chimeric antigen receptor (CAR). In an embodiment, the gene of interest encodes a differentiation factor (for cell reprogramming).

[0166] The present disclosure also relates to a method for treating a disease, condition or disorder in a subject, the method comprising administering a cell comprising the synthetic expression cassette or vector described herein. The present disclosure also relates to the use of a cell comprising the synthetic expression cassette or vector described herein method for treating a disease, condition or disorder in a subject. The present disclosure also relates to the use of a cell comprising the synthetic expression cassette or vector described herein method for the manufacture of a medicament for treating a disease, condition or disorder in a subject. In an embodiment, the disease, condition or disorder is associated with the absence of expression of a protein or the expression of a defective (e.g., mutated) protein, and the synthetic expression cassette or vector comprises a nucleic acid encoding a functional (e.g., native) protein (e.g., gene therapy).

[0167] Examples of diseases/disorders associated with the absence of expression of a protein, or the expression of a defective (e.g., mutated) protein (e.g., genetic diseases/disorders), include certain hematologic and lysosomal storage diseases such as Wiskott-Aldrich syndrome (WAS) (Aiuti et al., *Science* 341 (6148)), metachromatic leukodystrophy (MLD) (Biffi et al., *Science* 341 (6148)), Leukocyte adherence deficiency, X-linked CGD, Fanconi anemia, adrenoleukodystrophy, Mucopolysaccharidosis IIIA, as well as immunodeficiencies such as severe combined immunodeficiency (SCID) and adenosine deaminase (ADA) deficiency.

[0168] The disease or condition that is treated can be any in which expression of an antigen is associated with and/or involved in the etiology of a disease condition or disorder, e.g. causes, exacerbates or otherwise is involved in such disease, condition, or disorder. Exemplary diseases and conditions can include diseases or conditions associated with malignancy or transformation of cells (e.g., cancer), autoimmune or inflammatory disease (e.g., arthritis, rheumatoid arthritis (RA), Type I diabetes, systemic lupus erythematosus (SLE), inflammatory bowel disease, psoriasis, scleroderma, autoimmune thyroid disease, Grave's disease, Crohn's disease, multiple sclerosis, asthma, and/or a disease or condition associated with transplant), or an infectious disease, e.g. caused by a bacterial, viral or other pathogen. In particular embodiments, the recombinant receptor,

e.g., the CAR, specifically binds to the antigen associated with the disease or condition. In an embodiment, the disease, condition or disorder is cancer or an infectious disease, and the nucleic acid of interest present in the synthetic expression cassette or vector encodes a recombinant receptor, such as a chimeric antigen receptor (CAR), that recognizes an antigen expressed by the tumor cell or infected cell. The tumor may be a solid tumor or a hematologic (blood) tumor. In an embodiment, the cancer is a hematologic cancer, such as a lymphoma, a leukemias, and/or a myeloma (e.g., B-cell, T-cell, and myeloid leukemias, lymphomas, and multiple myelomas). The infectious disease may be a disease caused by any pathogenic infection, such as a viral, bacterial, parasitic (e.g., protozoal) or fungal infection, for example human immunodeficiency virus (HIV) or cytomegalovirus (CMV) infection.

[0169] The cells (engineered cells comprising the synthetic expression cassette or vector described herein) or compositions comprising same may administered to a subject or patient having the particular disease or condition to be treated, e.g., via adoptive cell therapy such as adoptive T cell therapy, or stem cell therapy. Methods for administration of engineered cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in U.S. Patent Application Publication No. 2003/0170238 to Gruenberg et al; U.S. Pat. No. 4,690,915 to Rosenberg; Rosenberg (2011) *Nat Rev Clin Oncol*. 8 (10): 577-85). See, e.g., Themeli et al. (2013) *Nat Biotechnol*. 31 (10): 928-933; Tsukahara et al. (2013) *Biochem Biophys Res Commun* 438 (1): 84-9; Davila et al. (2013) *PLoS ONE* 8 (4): e61338.

[0170] As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0171] In some embodiments, the cell therapy, e.g., adoptive T cell therapy or stem cell therapy, is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, e.g., patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

[0172] In some embodiments, the cell therapy, e.g., adoptive T cell therapy or stem cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, e.g., a first subject. In such embodiments, the cells then are administered to a different subject, e.g., a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or super type as the first subject. The cells can be administered by any suitable means. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion.

[0173] In certain embodiments, the cells, or individual populations of sub-types of cells, are administered to the subject at a range of about one million to about 100 billion cells and/or that amount of cells per kilogram of body weight, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about



20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges and/or per kilogram of body weight. Dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

[0174] In some embodiments, for example, where the subject is a human, the dose of recombinant receptor (e.g., CAR)-expressing cells, stem cells, T cells, or peripheral blood mononuclear cells (PBMCs), is at least  $1 \times 10^2$ ,  $1 \times 10^3$ ,  $1 \times 10^4$  or  $1 \times 10^5$  cells, for example in the range of about  $1 \times 10^6$  to  $1 \times 10^8$  such cells, such as  $2 \times 10^6$ ,  $5 \times 10^6$ ,  $1 \times 10^7$ ,  $5 \times 10^7$ , or  $1 \times 10^8$  or total such cells, or the range between any two of the foregoing values.

[0175] In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents include a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

[0176] The cells may be used in combination with other therapy such as other chemotherapy, immunotherapy, radiotherapy, or surgery, according to the disease to be treated.

[0177] In some embodiments, the synthetic expression cassette is used as a research tool, for example as reporter tool or in a commercial detection method (assay development). For example, the synthetic expression cassette may be operably linked to a nucleic acid encoding a reporter protein, which may be used for the detection of the expression of a gene of interest in a specific cell type, e.g., to confirm that the gene of interest has been taken up by and is expressed by the cell. The term “reporter protein” refers to a protein that may be easily identified and measured such as fluorescent and luminescent proteins (e.g., GFP, YFP), as well as enzymes that are able to generate a detectable product from a substrate (e.g., luciferase). The synthetic expression cassette may also be used for the cell-specific expression of a gene of interest in vitro, e.g., to assess the effect of the expression of the gene of interest in the targeted cells.

#### MODE(S) FOR CARRYING OUT THE INVENTION

[0178] The present invention is illustrated in further details by the following non-limiting examples.

##### Example 1: Material and Methods

[0179] Experimental design. The experimental strategy to generate synthetic specific promoter (enhancer+minimal CMV promoter) was to first select specific enhancer sequences in silico, amplify the sequence(s) by PCR and then clone the endogenous enhancer sequences upstream of a minimal promoter, namely the CMV minimal promoter (minCMV) 5. To validate the specificity of the synthetic promoter (enhancer+minCMV) through expression pattern assays, it was cloned upstream of a GFP reporter gene. As proof-of-concept of functional uses, it was cloned upstream of a chimeric antigen receptor (CAR).

[0180] Design of specific promoter sequences. Enhancer sequences that are specific for a particular

cell type, and that are located upstream of a transcription start site, were selected in silico. To do so, the in silico method of selection was based on the “Functional ANnotation Of The Mammalian genome” FANTOM5 database (generated by RIKEN).<sup>sup.3,4</sup> The FANTOM5 database has systematically investigated exactly what are the sets of genes that are active in virtually all cell types across the human body, and the genomic regions, which determine where the genes are read from. Therefore, this database includes a large number of active enhancer sequences of different subtypes. In order to browse this data and to select enhancers, the PrESSTo (Promoter Enhancer Slider Selector Tool) for human enhancer was used (<http://enhancer.binf.ku.dk/enhancers.php>). PrESSTo allows for the selection of enhancers expressed in one of many cells or tissues based on sliders.

[0181] The first step to select specific enhancer sequences was based on the Cap Analysis of Gene Expression (CAGE) score reported in FANTOM5 database. A percentage for the cell type of interest (T or NK cell) was chosen, namely 60% for T cells 30% for NK cells. The percentage number for each cell type refers to the proportion of CAGE tag in the given cell population relatively to CAGE tag counts from all cells. The percentage number is a “lowest bound” value: only the enhancers that have higher or equal percentage of expression than the set value for the cell type were returned. The percentage was selected according to the number of hits in the results box. In average, the percentage was set to have less than 20 hits.

[0182] The enhancer sequence candidates identified were then selected based on the following criteria: [0183] a high score (over 0.15 tags per million) in samples representing the cellular population of interest (e.g., T cells or NK cells); [0184] a low score for all other populations (less than 0.15 tags per million); [0185] significantly overrepresented only in the given population(s), as defined by PreSSTo.

[0186] The selected enhancer sequence candidates were then validated based on ChIP-Seq data, available through the UCSC genome browser tool (Kent W J, et al. The human genome browser at UCSC. *Genome Res.* 2002 June; 12 (6): 996-1006-link available specifically for the selected sequence via the tab “View in UCSC” in the PrESSTo tool. The presence of transcription factor fixation sites that are related to the lineage of interest (e.g., POU2F2 in hematopoietic lineage, GATA3 for T cells) or that are indicative of active regions (ex.: POLR2A) were analyzed. The presence of such sites in the vicinity (within 2000-3000 bp) of the selected enhancer was considered to be indicative of a transcriptionally active region.<sup>sup.7</sup>

[0187] Additional bioinformatic analysis was used to impose upon the remaining candidate regulatory regions more stringent selection criteria using cell-specific epigenetic characteristics (ENCODE database): (1) chromatin accessibility (i.e DNase-seq, FAIRE-Seq) and (2) histone modifications differentiating active from inactive enhancer regions (ChIP-Seq for H3K27ac). For purposes of illustration, the following strategy was used for selecting the B cell-specific enhancer candidates. After using the PrESSTo tool to select B-cell-specific regulatory regions (i.e specific CAGE signal in genomic regions of B cells and much less in others), the genomic coordinates of those candidate regulatory regions were submitted to the Galaxy bioinformatic web portal (Enis Afgan et al., *Nucleic Acids Research*, Volume 46, Issue W1, 2 Jul. 2018, Pages W537-W544). The same procedure was followed in order to upload into the Galaxy portal the epigenomic data obtained from ENCODE for chromatin accessibility (FAIRE-Seq or/and DNase-Seq) and H3K27ac modifications (ChIP-Seq) for the human cell types of interest, that is B cells, T cells, CD4 cells, CD8 cells, NK cells, monocytes and/or neutrophils and/or CD34.<sup>sup.+</sup> cells. The next step was to intersect the ensemble of B cell regulatory regions discovered by PrESSTo for presence of B cell-specific H3K27Ac enrichment peaks, which restricts the list to CAGE regions that show an active H3K27Ac signature. The latter regions were then queried for presence of an open chromatin (occurrence of DNA-seq peaks) in B cells, which restrict the CAGE B cell enhancers to those that show evidence of an open chromatin state in B cells along with accompanying H3K27Ac modification (epigenetic signature of transcriptional activity; strongly associated with active

enhancers). Further purging of the latter list was performed to successively eliminate the regions that show evidence of chromatin opening and H3K27 acetylation in non-desired cell types, such as T cells, NK cells, monocytes and CD34<sup>sup.</sup> cells. The resulting final list was manually curated in the CAGE database for tag signal intensity and cell specificity, including further analysis of transcription factor binding, occurrence of over-represented DNA motifs (MEME tool) and proximity of known cell-specific genes.

[0188] Enhancer amplification for cloning. Once selected using the methodology described above, the enhancer sequence candidates were amplified by PCR from genomic DNA of cell lines (Jurkat T cell line or NK92 cells) and were then inserted in a cloning plasmid. To design PCR primers, a pair of primers of 18 to 22 nucleotides that have similar melting temperatures and that are spaced from the enhancer sequence of at least 10 nucleotides, in an effort to minimize the PCR amplicon size. PCR primers specificity was verified using UCSC genome browser tool. The primers were designed to add a restriction enzyme site and 6 bp randomly selected to allow an efficient cleavage (lowercase in Table II).

[0189] Table II lists all PCR primers (capital letters) designed to amplify the enhancer sequences from Jurkat T cell genomic DNA extracts, their apposed restriction site (in lowercase), and their PCR amplification conditions. The PCR was performed using the Q5 polymerase, according to the manufacturer's instructions (New England Biolabs, MA). The amplification conditions were the following: [0190] denaturation: 98° C. for 30 sec; [0191] amplification (35 cycles): 98° C. for 10 sec/Tm° C.\* for 30 sec/72° C. for 30 sec; and [0192] extension: 2 min at 72° C.

\*appropriate Tm for each reaction are indicated in Table I).

TABLE-US-00002 TABLE II PCR amplification of enhancers sequences selected in silico

| PCR Enhancer  | Restriction     | conditions | name              | PCR Primer | sequences | sites (Tm) | Tenh |
|---|-----------------|------------|-------------------|------------|-----------|------------|------|
| atcatcgcgccgcGGGTCTGACGTGCTCTGT                         | (SEQ NotI       | 72° C.     | (Chr16-445)       | ID NO: 1)  |           |            |      |
| NotI tattcagcgccgcCCTGGGTCAGTGCGTCA                     | (SEQ ID NO: 56) |            | NKspe-            |            |           |            |      |
| attcgggtaccGTGGGACACCAGTCATCTTA                         | (SEQ ID KpnI    | 63° C.     | promoter          | NO: 2)     |           |            |      |
| (NK6) gtacgaattcGAGAGCACACACAGTCA                       | (SEQ ID NO: 57) |            | NKspe-            |            |           |            |      |
| atatcgcgccgcGACCAGGTTTGGCCAATAGA                        | (SEQ NotI       | 68° C.     | candidate         | ID NO: 3)  |           |            |      |
| (NK20) gcgactagtTGCCAGCACCTGATTAAA                      | (SEQ ID NO: 58) |            | T-NK spe          |            |           |            |      |
| atcaggatccGAACTTGTCTGGGACCTA                            | (SEQ ID BamHI   | 63° C.     | candidate         | NO: 4)     |           |            |      |
| EcoRI (Chr14-591) cgcgcgaattcACCATACTGGTATATTCTCTCTC    | (SEQ ID NO: 59) |            |                   |            |           |            |      |
| NKspe- cagggaattcGCTTACTTGTTAGCATCCCTCTCatagc           | EcoRI 66° C.    | candidate  | (SEQ ID NO: 5)    |            |           |            |      |
| NotI (NK8) ggccgcTGACTTAGTCCTAAAGCATATCTTGG             | (SEQ ID NO: 60) |            | B-cell            |            |           |            |      |
| acggcaatcttggtaccCTCCAGTGCCAGATTTTTCAGG                 | KpnI 60° C.     | specific   | G (SEQ ID NO: 61) |            |           |            |      |
| SpeI candidate catcgcggtaccagtacgactagtGAACTGGCTGGGCTAT |                 |            |                   |            |           |            |      |
| TTTGTGC   | (SEQ ID NO: 62) |            |                   |            |           |            |      |

[0193] The PCR product length was verified on agarose gel and purified using the QIAquick™ PCR Purification Kit (Qiagen, Germany). The PCR product was then digested with the corresponding restriction enzymes (see Table I) for cloning.

[0194] Cloning strategy to create a synthetic promoter from the in silico selected enhancer. To generate a specific promoter from the selected and PCR-amplified enhancer, the endogenous enhancer sequences were cloned upstream of a minimal promoter, namely the CMV minimal promoter (minCMV: GTAGGCGTGTACGGTGGGAGG TCTATATAAGCAGAGC TCGTTTAGTGAACCGTCAGATC, SEQ ID NO:6).sup.5. To validate the specificity of the synthetic promoter (enhancer+minCMV) through expression pattern assays, it was cloned upstream of a GFP reporter gene. To perform these tests, the backbone of the pENTR1a vector (Addgene, #11813-011) digested with the appropriate restriction enzymes was used and treated with recombinant shrimp alkaline phosphatase (rSAP, New England Biolabs, MA) to prevent self-ligation of the pENTR1a vector. A ligation reaction using T3 Ligase enzyme (New England Biolabs, MA) was performed to create the final plasmids.

[0195] The detailed cloning strategy for the Tenh (Chr16-445) is illustrated in FIGS. 1A-B (history is depicted from the bottom-up). Briefly, pENTR1a-IRES-GFP was obtained by inserting the IRES-GFP sequence from pIRES2-AcGFP (ClonTech/Takara) in the pENTR1a gateway plasmid (Addgene, #11813-011). The minCMV was also excised from (ClonTech/Takara) and inserted in using NotI and XhoI enzyme to obtain the pENTR1a-minCMV-IRES-GFP. The IRES sequence was then removed by a digestion XhoI-NcoI, creating the pENTR1a-minCMV-GFP, in order to prevent this sequence to interfere with the synthetic promoter function and specificity. The SV40 poly(A) signal was inserted downstream of the GFP to stabilize the mRNA to create the pENTR1a-minCMV-GFP-SV40polyA. SV40 poly(A) sequence was amplified by PCR using the plasmid (Addgene #45461) as template. This pENTR1a-minCMV-GFP-SV40polyA plasmid was the plasmid used to clone all the enhancer sequences to perform the in vitro expression pattern analysis experiments. As described above, the PCR amplicon of the selected enhancer was digested using the appropriate restriction enzymes (listed in Table II) and inserted upstream of the minCMV sequence in pENTR1a-minCMV-GFP-SV40polyA. Plasmids were sequenced to ensure that the constructs correspond to the designed sequences.

[0196] Lentiviral particles were then produced in order to perform expression pattern tests in vivo and/or in NK cells. To produce the particles, a pHR-SIN vector backbone was first used. The cloning strategy is depicted in FIG. 2. Briefly, a pHR-SIN-Dest vector, that is devoid of the spleen focus-forming virus (SFFV)-GFP sequence originally comprised within the pHR-SIN-SFFV-GFP vector (kindly given by Els Veroheyn, France), was generated by introducing a LR cloning sites (attB1/attB2) amplified from the pLenti CMV/TO Puro DEST (Addgene, #17293) to replace the SFFV-GFP fragment. This plasmid was used to insert the “ENH-minCMV-GFP-SV40polyA” sequence from the pENTR1a (described above) using the Gateway LR clonase kit (Invitrogen, CA), using manufacturer's instructions. To produce viral particles, this vector was co-transfected in HEK293T cells (ATCC, CRL-3216) along with 3 others, the p8.91 that provides packaging enzymes and 2 vectors, pH830 and pF824, coding for measles virus proteins that optimize CD34.sup.+ transduction.sup.8,9. For NK cells transfection, lentiviral particles with a baboon retroviral envelope glycoprotein (pBaEV vector, kindly given by Els Veroheyn) were produced.sup.10.

[0197] In vitro Validation of Tenh (Chr16-445), NKspe (NK6) and Benh (B1, SEQ ID NO:23). To assess the specificity of the constructs, the vectors were transfected into human cell lines of various hematopoietic origin. First, for Tenh (Chr16-445), Jurkat T cells (T cell line, ATCC IB-152) and K-562 cells (erythroid myeloid cell line, CCL-243) were transfected. pENTR1a-Tenh-minCMV-GFP-SV40polyA was transfected using Lipofectamine™ 3000 (Invitrogen, CA) according to manufacturer's instructions. GFP signal was analyzed by flow cytometry (BD LSRII-Fortessa). It was observed that GFP was expressed only in Jurkat cells. These observations were then validated in primary human cells. Peripheral blood mononuclear cells (PBMC) (obtained from healthy controls with written consent, (REB #3527)) were nucleofected by electroporation using human monocyte nucleofector kit from Lonza, and the subpopulation expressing GFP was identified by flow cytometry. An anti-CD19 antibody was used to identify B cells (anti-CD19 PE clone HIB19 Biolegend), an anti-CD3 antibody was used to identify T cells (anti-CD3 PE clone HIT3a BD Pharmingen) and an anti-CD14 antibody was used to identify monocytes (anti-CD14 APC-Cy7, clone HCD14 Biolegend).

[0198] To test the specificity of the NKspe enhancer (NK6), a similar strategy was used. The vectors were transfected into cell lines of different hematopoietic origin with the pENTR1a-NK6-minCMV-GFP-SV40polyA. For this experiment, NK-92 (NK cell line, ATCC, CRL-2407), Jurkat (T cell line, ATCC IB-152), 697 (B-cell line, DSMZ ACC42) and K-562 (myeloid cell line, CCL-243) cells were transfected.

[0199] The B-cell specific enhancer (Benh, SEQ ID NO:23) capacity to induce the expression of a protein in a B cell line was tested by transducing Nalm6 cell line (B cell line, ATCC, CRL-3273)

with BaEV-lentiviral particles coding for Benh-minCMV-GFP-SV40polyA. The GFP expression pattern was analyzed by flow cytometry, using an anti-CD19 antibody co-staining B cells (anti-CD19 PE clone HIB19 Biolegend).

[0200] In vivo validation of the T cell-specific (T-specific/Chr16-445), NK cell-specific (NK8) and B cell-specific promoter constructs. To assess if the GFP expression pattern was similar in vivo, human HSC isolated from cord blood (CD34 MicroBead Kit UltraPure, Miltenyl Biotec, Germany) were transduced with measles or BaEV lentiviral particles coding either for the Tenh-minCMV-GFP-SV40polyA, Tenh-minCMV-CAR-CD22-SV40polyA, NK8-minCMV-GFP-SV40polyA or Benh-minCMV-GFP-SV40polyA. Cord bloods were obtained at the CHU Sainte-Justine Biobank of Cord Blood for research with the written consent of the mothers. Briefly, 200  $\mu$ L of concentrated measles lentiviral particles were coated in 12-well plates containing RetroNectin (Takara Bio USA) for 4 hours at 37° C. 250,000 purified CD34.sup.+ HSC in 150  $\mu$ L of StemSpan (StemCell, Canada) containing 5 nM Rapamycin and 3  $\mu$ M CIHR99021 were added to maintain stemness.sup.12. The plate were then centrifuged for 1 hour at 1,000 g; after which 700  $\mu$ L of StemSpan/Rapamycin/CIHR99021 media was added. Cells were cultured for 3 days and then injected in mice. NOD-scid IL2Ry.sup.null (NSG mice) were acquired from Jackson laboratory (#005557), bred and maintained under specific pathogen-free conditions. Mice were preconditioned using gamma radiation with 2 Gy. 10.sup.5 of CD34.sup.+ cells that were in contact with the lentiviral particles were injected intravenously (IV) into 7-11 weeks old NSG mice.

[0201] To study the activity of the T-cell specific promoter in a more physiological context where the maturation of engineered T-progeny is taking place in a human thymus, a group of mice was also engrafted with 3 pieces of pre-cultured human thymus in the quadricep muscle.sup.13. Thymus pieces were harvested from cardiac surgery procedures (where the thymus is removed for the sake of the surgery) following approval of a research protocol by Sainte-Justine Hospital institutional review board (and written informed consent from donors). Pieces of thymus (2-5 mm.sup.3) were cultured for 10 days on a GelFoam Sponge and an isopore membrane of 0.8- $\mu$ m.sup.13,14 in Ham's F-12 nutrient mix 1 $\times$  medium (F12) supplemented with 0.025 M HEPES pH 7.5 and 10% fetal bovine serum (FBS) (Life Technologies).

[0202] Mice were bled regularly to monitor human cell reconstitution. Reconstitution and GFP expression were analyzed by flow cytometry using anti-mouse CD45-PerCP-Cy5.5 (clone 30F11), anti-human CD45-PE-Cy7 (clone HI31), anti-human CD19-PE (clone HIB19), anti-human CD14-APC-Cy7 (clone HCD14), and anti-human CD3-APC (clone HIT3a) (all from Biolegend). Mice were maintained either in the animal facility of CHU Sainte-Justine Research Center.

[0203] Functional Validation of the T-specific promoter construct (T-specific/Chr16-445). To assess the potential therapeutic use of the Tenh-promoter, the eGFP sequence was replaced by a sequence coding for a CAR-CD33 or a CAR-CD22. CAR-CD33 was generated by synthesizing the ScFv sequence of gemtuzumab ozogamicin monoclonal antibody.sup.15 (IDT Technologies) and cloning this sequence in a second-generation CAR construct (CD28-CD3 $\zeta$ ). The CAR-CD22 construct was based on the m971 ScFv sequence fused to 28z and BBz (Haso W et al. *Blood*. 2013; 121 (7): 1165-1174). The CAR-GD2 construct was based on the 14g2a ScFv sequence (Louis C U et al., *Blood*. 2011; 118:6050-6) cloned in a second-generation CAR construct (CD28-CD34). To produce VSVg lentiviral particles, the construct was cloned in a pHR SIN vector and particles were produced in HEK293 as described above.

[0204] Primary T cells were isolated from a 10-ml blood sample of a healthy donor. PBMCs were isolated by Ficoll™ and T cells were purified using the T cell enrichment kit (#19051, StemCell Technologies, Canada). Five hundred thousand (500,000) T cells were then put in culture in 900  $\mu$ L of RPMI/10% FBS supplemented with 30 U/mL of human recombinant IL-2 and with Dynabeads in a 1:1 ratio (12.5 L/well; Life Technologies). On the second day, 100  $\mu$ L of concentrated lentiviral particles were added along with 8  $\mu$ g/mL of protamin sulfate and culture for the next 6 following days. Recombinant human IL-2 (30 U/mL) was added every odder day. Expression of the CAR-

CD33 on the surface of T cells was validated using a soluble CD33-Fc chimera protein (Siglec3/CD33 Fc R&D Systems, MI) and detected by a secondary staining using polyclonal anti-IgG PE (Jackson ImmunoResearch) by flow cytometry. Similarly, the detection of CAR-CD22 expression was performed by incubating cells with 2  $\mu$ l Siglec2 (CD22)-Fc chimera (50 mg/ml, R&D) for 30 minutes at 4° C., washed and stained with anti-Fc-PE (Jackson Immune), anti-CD56-APC and anti-CD3-FITC (Biolegend). The detection of the CAR-GD2 was performed using anti-mouse Fab (Jackson Immune 115-065-006) for 30 minutes at 4° C., washed and then stained with a streptavidin-PE (Biolegend).

[0205] Functionality of CAR-CD33-transduced T cells was then evaluated in a cytotoxic assay against native CD33.sup.+ (ATCC #CCL-240) or CD33 HL-60 cell line (generated using CRISP technology). Similarly, functionality of CAR-CD22-transduced T cells was tested in cytotoxic assay against RS4;11 (B-ALL cell line expressing CD22, ATCC #CRL-1873), and functionality of CAR-GD2-transduced T cells was assessed against the GD2-expressing SK-N-DZ neuroblastoma cell line (ATCC, CRL-2149). Briefly, HL-60 (for CAR-CD33), RS4;11 (for CAR-CD22) or SK-N-DZ (for CAR-GD2) target cells were stained with PKH26, a membrane labeling dye with long aliphatic tails which stably stains cell membrane. Following coincubation with effector T cells, the absolute count of living targets was calculated using CountBright™ Absolute Counting Beads (ThermoFisher) as well as the use of a viability dye (7-AAD). Briefly, 2×10<sup>6</sup> target cells were washed twice in RPMI 1640 or D-PBS and resuspended in 100  $\mu$ L of diluent C, then 100  $\mu$ L of PKH26 (8  $\mu$ M in diluent C) was added and cells were incubated 5 min at room temperature. The staining was stopped by adding FBS. Cells were then plated in different effector:target ratios (1:8, 1:4, 1:2, 1:1, 2:1, 4:1) and incubated for 24 hrs. After 24 hours, cells were harvested, stained with 7-AAD (BD Biosciences) and analyzed by flow cytometry. Cytotoxicity was calculated as follow: % Specific lysis=100-[absolute count of PKH26.sup.+ 7-AAD.sup.- targets after incubation with effector cells]/(absolute count of PKH26.sup.+ 7-AAD targets after incubation alone)×100].

[0206] Kinetic of expression during in vitro T-cell differentiation of a CAR under the T-specific promoter construct (T-specific/Chr16-445). First, human HSC isolated from cord blood (CD34 MicroBead Kit UltraPure™, Miltenyl Biotec, Germany) were transduced with BaEV lentiviral particles coding for the Tenh-minCMV-CAR-CD22-SV40polyA as described above. To test the expression of CAR by CD34.sup.+ cells progeny, engineered-CD34.sup.+ cells have been co-cultured with OP9-DL4 cells or OP9 (without DL4) to induce the differentiation of CD34.sup.+ into T and B cells, respectively (La Motte-Mohs R N et al., *Blood*. 2005; 105(4):1431-9. Epub 2004 Oct. 19). Cells were co-cultured in a medium containing: alpha MEM (Gibco), 20% HyClone™ Characterized FBS GE Healthcare), GlutaMAX-I™, PenStrep, 5 ng/ml IL-7 (Peprotech), 5 ng/ml FLT-3L (Peprotech) and 800  $\mu$ M L-Ascorbic acid 2-phosphate (Sigma). Cells were co-cultured for 2 weeks and medium was changed twice a week, and feeder cells (OP9 or OP9-DL4) were change each week. CAR expression on different sub-population was evaluated by flow cytometry using two antibody panels: 1) anti-CD1a-BV421 (clone HI49, Biolegend), anti-CD7-FITC (clone M-T701, BD Biosciences), anti-CD45-PeCy7 (clone HI30, Biolegend), anti-CD34-APC (clone 581, Biolegend), anti-CD19-APC-Cy7 (clone HIB19, Biolegend); and 2) anti-CD4-APC-Cy7 (clone RPA-T4, Biolegend), anti-CD8-APC (RPA-T8, BD Biosciences), anti-CD3-FITC (clone UCHT1, Biolegend), anti-CD45-PeCy7 (clone HI30, Biolegend). In both panels, the detection of CAR-CD22 expression was performed by incubating cells with 2  $\mu$ l Siglec2 (CD22)-Fc chimera (50 mg/ml, R&D) for 30 minutes at 4° C., washed and stained with anti-Fc-PE (Jackson Immune), and DAPI was used as a viability staining.

[0207] Assessment of the specificity of the NK8 promoter. The specificity of the NK8 promoter was also assessed in the OP9 co-culture system, in conditions favoring NK cell differentiation (Beck R C et al., *Biol Blood Marrow Transplant*. 2009, 15 (9): 1026-37). Human HSC isolated from cord blood (CD34 MicroBead Kit UltraPure™, Miltenyl Biotec, Germany) were transduced with BaEV lentiviral particles coding for the NK8-minCMV-GFP-SV40polyA as described above.

Transduced cells were co-cultured with OP9 cells was performed as described above with the addition of IL-15 (10 ng/ml) for the differentiation of NK cells. GFP expression was evaluated by flow cytometry. Co-staining of anti-CD56, anti-CD45, anti-CD4 and anti-CD8 (Biolegend) was performed to identify cell subpopulations.

#### Example 2: Promoter Sequences Tested and Validated

[0208] The first step was to identify a specific enhancer to create a synthetic promoter for T cells. 5 enhancers sequence candidates that fulfilled the above-described criteria were identified (Chr16-445 (SEQ ID NO:7); Chr 14-591 (SEQ ID NO:13); Chr 8-438 (SEQ ID NO:8); Chr 8-230 (SEQ ID NO: 9); Chr 12-199 (SEQ ID NO:10). Of those, the Chr16-445 sequence was thoroughly studied as each validation step was conclusive. This sequence, detailed in Table III, contains highly-repeated motifs, and is only significantly overexpressed in T cells (with a tags/million score of 0.511 in T cells). It is located in Chromosome 16 (position 88536883-88537327) and is 445-nucleotide long. Within 2.5 kb up-stream of the enhancer sequence, 23 putative binding sites for transcription factors such as Gata1, POLR2A, POU2F2 and MYC were identified, providing compelling evidence that it is located in a transcriptionally active region.

[0209] Similarly, 2 potentially NK-specific enhancer sequences were selected (NK6 and NK20—see Table IIIa), of which one (NK6) has resulted in a coherent pattern of expression in preliminary data. This sequence is located in Chromosome 6, is 379-nucleotide long and also contains highly repeated motifs. It was only significantly over-expressed in NK cells (tag score of 0.719 in NK cells). This sequence has 19 transcription factor binding site within less than 2 kb of the sequence. A second NK-specific candidate (NK20) located on Chromosome 20 has a tag score of 1.823 in NK cells.

[0210] An enhancer sequence that would induce the expression of a transgene in both T and NK cells could be interesting in the context of gene therapy of cytotoxic cells. A putative T and NK cell-specific promoter sequence was identified on Chromosome 14, position 61804524 to 61/805,115 (591 nucleotides). Its tags/million score is high in T cells (6.629) and in NK cells (3.327), with a significant expression only in these 2 cell subtypes. Four transcription binding sites—RUNX3, GATA2, FOS and JUN—are located within the enhancer sequence itself.

[0211] Also, 4 putative specific enhancers for B cells located on chromosomes 1, 3, 10 and 13 that matched the selection criteria were identified (B-spe candidates #1, 2, 3 and 4—see Table IIIa).

[0212] Finally, several other candidate enhancers for different cell type(s) were also identified (Table IIIb).

TABLE-US-00003 TABLE IIIa Characteristics of candidate enhancer sequences for T cells, NK cells, T/NK cells and B cells identified herein

Position Validation/ Name (hg38) Sequence Characteristics T enh chr16: 88536883-CTGGTGGTGTGGAGGGCCGGGTGGTGA T-specificity (T-spe promoter/ 88537327

CACTCAGTGACAGGTGAGGATGTGGCAC confirmed in vitro Chr16-445)

GGTGTGGAGGGCCGGGTGGTGACGCTG and in vivo

AGTGACAGGTGAGGATGTGGCACGGTGT Highly repeated

GGAGGGCCGGGTGGTGACGCTGAGTGA motifs

CAGGTGAGGATGTGGCACGGTGTGGAG Tags/million = 0.511

GGCCGGGTGGTGACGCTGAGTGACAGG in T cells

TGAGGATGTGGCACGGTGTGGAGGGCC Only significantly

GGGTGGTGACGCTGAGTGACAGGTGAG overexpressed in T

GATGTGGCACGGTGTGGAGGGCCGGGT cells

GGTGACGCTGAGTGACACGTGAGGATGT GGCACGGTGTGGAGGGCCGGGTGGTGA

CGCTGAGTGACAGGTGAGGATGTGGCA CGGTGTGGAGGGCCGGGTGGTGACGCT

GAGTGACAGGTGAGGATGTGGCATAGG GAAACACATCCTCGCCGAGCGCACAGTG

GGAGCTCCG (SEQ ID NO: 7) T-NK spe chr8: 101819182-

CTGAGGACTTCTAGCTCTTCCTGGATCCT Significantly over- candidate 101819619

TATATGCCCTATTGTTAATAGCT expressed only in T (Chr8-438)  
ATATGACGTTGTGATACTATTCCAAACCC and NK cells  
TAGTCATAGCAACCACATACCTAATAGCC Tags/million = 3.082  
AAGGGATAACCATACTATCTTCCCTTTCT in T cells  
GAAGAACCTTTCAGCAAAAGATTTTCAGG Tags/million = 2.195  
GAATTTTACCAAGAAAACCATCCCATCCC in NK cells  
CTCCTCCTTCCATTTGACAGGTGGAGAA GTGAGGCACAGTGAAGCCAGAGGAGCC  
TGGTCAGACGGTGAGTCAGAAGTAGAGC AGGGCTGCACCTGGTGACACCTATTTCC  
TCCCTTGTGGTTTGGCCCCTGCCTCATA GGCTTCCTGGAAAGGTGTAGCTTCTTCA  
TGGCTTACTTGTGAGTAAACACTGCTAT GAGCTTTCAAATATTTCCCTAGGTTGCAG  
GAGGTTGTGTC (SEQ ID NO: 8) T-spe candidate chr8: 2153112-  
CTAAAAACAAACAAAGCCAAAAAACCATA Significantly over- (Chr8-230) 2153543  
GCACTTTATTTTAAGGATATTTCTACTTTA expressed only in T  
ATCCATAAATGGTTTCTAGTTCTTGTATTA cells  
ATGGCGACATCATGTTCCATGTTTTTCATG Tags/million = 1.348  
AACTGTGATTTATCAGAGAAGCCCTTGCA in T cells  
ACGGTGATCTAGGTTGTGTCATCGCTTCT GCGTAACAGCAAGGCCAAAGCTGCGGATT  
TATCGCCGTTCTACCCAGGTTTCCTGTTG ATTACGGCAGGGTTTGAGGTGCGGTCCC  
TGGTGTTTCATCATGACAAGTGGAGGTTT GCAGGAGAACTTCATAACCATCTGCAGA  
AAAGGTGAAGTCACATCTTGAGACCAGC TCTAGATATGTTACCGATGGGCTAATGGT  
TTTGATGTAAAATAAGTAAAAACATTAAA GGGCTAGAAGAAGCCAGAGGAAAAGTCA  
(SEQ ID NO: 9) T-NK spe chr12: 9106762-  
GTGAAATAAGACACACACAGAAAGAAAA Significantly over- candidate 9106960  
ATATGATATGATATCGGTTGTATGTGGAG expressed only in T (Chr12-199)  
TCTTGAAAAAATCACCATAGAAACAGTG and NK cells  
TAGGAAGATGGTTACCAGGGGGCGGGGT Tags/million = 1.294  
AGGGGAAATGGGAAGATGTAGGTCAAAA in T cells  
GGTACGAAGTTGCGGTTAGATTCTAATGT Tags/million = 1.551  
TCTAGTGTGTAACATAAGGACTATAGTTA in NK cells (SEQ ID NO: 10) NKspe-  
promoter chr6: GCACATTCATCTCTCTGAGAAACACCTCC Evidences of NK (NK6)  
168682215- CTATGCTGGGAAATGTGAAAGCAGGTGG specificity in vitro 168682594  
GACACCAGTCATCTTAGTACATCACATTG Highly repeated  
TCACTGCCGCGAATGTGTGGGACACCCA motifs  
TCATCTTACCACATCACATCGTCACTGCC Tags/million: 0.719 in  
GCGAACGTGTGGGACACCCATCATCTTA NK cells  
CCACATCACATCGTCACTGCCACGAACG Significantly over-  
TGTGGGACACCCATCATCTTACCACATCA expressed only in NK  
CATCGTCACTGCCGCGAACGTGTGGGAC cells  
ACCCATCATCTTACCACATCACATCGTCA CTGCCGCGAACGTGTGGGACACCCATCA  
TCTTACCACATCACATCGTCACTGCCGC GAACGTGTGGGACACCCATCATCTTACC  
ACATCACATCGTCACTGCGTGAATGTTTT TCTGAATACATCACATTTGGCTCCATA  
AAATCTGTTTTCTAATCTACTTTTTTAAGTT AGTATGTTGGCCTCAACTTTGCACTTTAT  
TTTTCTTCGTGACTGTGTGGTGCTCTCCT CTCTGGAGGTGCCTTCACTGAGGCTGCA  
TTGAAGGGCTGTGTGGGCCAGCGAGTG CTGTGGTCCG (SEQ ID NO: 11) NKspe-  
chr20: 24013863- CTCTTCATTGACCCTGAGTTTGACCAGGT Tags/million: 1.823  
candidate 24014201 TTGGCCAATAGAAGTCAGAGGAAGTGAC in NK cells (NK20)  
AGGTGCCAGTTACAAGAAGTTCCACAAC Significantly over-  
CCCTTTTAGAAATCAACTAAACCACTGTG expressed only in  
TGAATAAAGCCAGGTTAGCCTGCTGGAG NK cells  
AATGAGGCCCAATTGCTCCCATCATCTCA CATGACAGCCAACCAACCACAGTCATG



TGACTGTGCATCTTTGGACCACTTTTCCACCACTTTTCAGTAGACCTCAG  
ACACATGAACAAACCCCACTAAGATCAAC CAACCCTAGACAGTTCTGCAGAGTTGCC  
CAGTAACACACAGAATCATGACCAATAAT AAATGTGTACTGTTTGAAGTTGCCACATT  
GTTAAGTGGTTTGTATGCCACAAAACT AACTGACACATCAACTCTGAAACCTCCTT  
GGTTTAATCAGGGTGCTGGCAGGTACGG AGCAACTGTGCCCAGGGTAACATTACTT  
AGTCTCAGAATCATAATTAATTAAATTGC AAGTGACAGAAATATAATAAAATCT (SEQ  
ID NO: 12) T-NK spe chr14: 61804524- TGATTTTAAGTGAAAGGTTTCTGAACTGT  
Tags/millions: 6.629 candidate 61805115 ATATATACAATATTCAGAACAGTAATTC in  
Tcells; 3.327 in (Chr14-591) CTTTGCTTCATAGTACGGCACAACAGTGT NK cells  
TTGCACTTGATGTTTAGCGTAGCCTTGGT Significantly over-  
TTTGGTTGTTCAGAAGCCTTTGTAGCTC expressed only in T  
TTTGGTTGTGCCAAGAATATATTGTTTTT and NK cells  
GAGTCTCCTTGCCGGGCCTCCTCGCCTT CTTGTCATGCACATCCTGTACCTGGGT  
GTGGTTGTATTGATTTGCTGGGGCGCTG CTGTTGCAAATGTCTGGAGTGAATGAGA  
ATGTGTTTGTGGGTGTCTGCCCTCACCTT GGCCACTGATCTGCTTTCAGCCCTGAAG  
TACTGCCATCTGCATGAACATTAGGGAC CCCCAGCCTCCCCCACCCTTAGTAAAG  
TACCCTGCACATGTTTGGGATTCAGGAA ATGTTTGTAGAAAGGAAAAATTCCTTATC  
ATTCCTTACCATTCTCTGTCTTGAGTCTG AGTCATGATTCAAACACTTTTGCTAAAAG  
GTTTTCGCTTGAAAAATTCATATTCAGGT ACAGATTTACTTTTTTTTGGTGTGTGTGA  
GTTTAAGAGCTGCCTCAG (SEQ ID NO: 13) NKspe- chr8: 103109879-  
ATCAGAAGTTGCGTGGGTCATGAAGCC Tags/million: 31.336 candidate (NK8)  
103110286 CAAGGCCAGCAGTTCTGGATGCCTCCA in NK cells; 2.013 in  
TGAAGCTGGATATTGCTTACTTGTTAGC basophil; 1.503 in T  
ATCCCTCTCTGGCAATCATCAATAACCT cells; 0.835 in  
CTTTTCAAAAGTACCTCCTTATACAAGA monocytes  
CTCTTATCAACTGATTTTCATCATTTTTTAG Significantly over-  
CACTGAAACCTTGGTCTTCCTGCTCTCT expressed in NK,  
GCTCTGACAGCATCTCTCTGATAACCTG basophils, T cells  
AGAAATCAGAGTTTCACCCCTGAAACTA and monocytes  
AACAGGCCATTATTTCCCTAATTTTAAATG ATGACATGACAAGCCTTCACCCCTATCTG  
TTTCTTATTTCTTGAACCTCTCCCACCCT CACCAGCAGCCAACACAAATCACAACG  
CAATGCAAAGGCCAGGCCACAGAACAC GCTGTGAATCGACAGTTTCAGAAGACGT  
CATTACACAATGTGCAAGGCACTTCCT GCACAGCCATCTCTGTGCCCCTGCAAA  
GGGCATGTGGCATGAGGCAGTAAAATA AGTATAGTCTGTGTTTGGGTATGAAAGG  
TGGTGGGTGGGGCGTGAATACATCCAA GATATGCTTTAGGACTAAGTCAAAAGAG  
AACTGAGAGTGAGAAAGAAGATTG (SEQ ID NO: 14) B-spe candidate chr1:  
220219915- CTGACAAGATGCAAGCTCATCATTTTGT RAB3GAP2 #1 (B1) 220220218  
TTTGTGTCATAAGGGCATTTCCTACTA Tags/million: 5.001 in  
ATTAGCGTTTTGGAAACTTCCCCAATA B cells  
ATGGGGCCCACAGTCTCTAACACTGCC Negligible tags in  
CCTTTGAAGCTAGTGATCCAAAAATAAT other primary blood  
ATCATTTGACAAGGTAATTTCCCAAGCC cell types  
ACGCCTGAAATGTAAAACAAAAAAGCA CCTCACTGCTTAAAGGTGCTCCACCAG  
GGACCTTGGGTTTTCCACCAAACTTGC CTCCCCCACCCTGTTACTAAAGTTGACA  
CTGAATTTGCCTGCAGTCTCCCCCA (SEQ ID NO: 23) B-spe candidate chr3:  
112216852- TGGGCATGGTGGCTCATACCTGTAATC Tags/million: 2.541 in #2 (B3)  
112217043 CTAGCACTTTGGGAGGCTGAGGCAGGT B cells  
GGATCACTTGAGGTCAGGAGCTCCAGA Significantly  
CCAGCCTGCCCAACATGCATTGCATCC overrepresented in B  
ATAGCTTGGCTGACTTTCTTAAAAAACA cells GGTTGATGGCAAGAAAAGAGAACTGAG

TAGATTGATTAAGTTAAGTAACTTCTTCCGAAAATGCTCAG  
GCTGCCACACTGATGATGAATAGATAGC TCGTTCTTCCCAAAGTCTGAGTGCAGTAGA  
GTGTTGCAGTGCTCAGTTGGGTAGGAC AGATGTTGGATAATTGGGGTTATCCAAC  
ATCTCTACTAAAAATACAAAAATTAGCTG TCGGTGGTGGAAAGGTGCCTGTAATCCC  
AGCTA (SEQ ID NO: 15) B-spe candidate chr10: 49879351-  
AAGGAGGAATTGCTGTCTGAGAGGGTA Tags/million: 1.993 in #3 (B10) 49879455  
TTTTGTTCCCCAGTGACTGAGGGCAGG B cells  
GCAGGGGCAGGGAAGGCTTCCCCTCTC Significantly  
TTCTGGCCCTAGAGGCCCTGTAAATGC overrepresented in B  
ACTGTGCAGTCACTGACGTGCCCTCAG cells GCAGGGCCCTGGCGGGAAGGGGGCTG  
TTCCAGGTCTACCAGCTTCACACCCTTA TTCTATTGAATTCTCATGAAAACAAAATC  
TGTGAAACAGCTGTGATCTTCATTTTCT GATGAGGGAACAGAGCCTCCGTGCCCC  
CGAGATCACTGGTATGACTCCAAAGATT TTCC (SEQ ID NO: 16) B-spe candidate  
chr14: 74868763- GACTCACACCTTTGGCCACTGACCCCT Tags/million: 2.534 in #4  
(B13) 74868996 GCCCCACCTTCTGAGTGGGGTTCAGGG B cells  
ACTGTGCTGAGTCTGTCTCTGGAAGC Significantly  
AGCAGGGTGCAGGGGCACACTGATGAG overrepresented in B  
TGGTGCATGTGCCCAGGGGCAACATCA cells GAGCCGTTTAGCCACCAGGGCAGTCAG  
GCATGGACAGACGCATTTGGGAGGGGG CGGGGCCCTGTGTCAGCTGTAAACT  
TCAGTTCCTGAAGCAGAGAAGTCTGGA GTTCTGGGGAGGGGCCAGGGAGGCAG  
GAGAGGGAAACACTGGGAGGGTTTAGG GCTTGGCCTGTTTATCAACTACTGTGGA  
AGTATTTTCACTGTTCTGACAATCCCTG CAACTATGTCCATGGACCTGCTGTCTAT  
CCGCCCTGTTTCATCGGGAGGAAGAGAT GGAGAAGGCTGCCGGGGTTCTGGGAG T  
(SEQ ID NO: 17)

TABLE-US-00004 TABLE IIIb Characteristics of candidate enhancer sequences for  
various cell types identified herein Cell Coordinates Genome Specificity (hg38) Sequence  
Location T/NK chr1: 167489187- CCAACCCCCCTTCCCCACCCCCAAACTCTGCTTATA  
CD247 167489883 GGTTCTTATATGATAATTTAAACTATATATGATATTA  
AATTAGCACTCCAATAGCAAAAATAAAGTGTTCCC  
ACATCACCTTCTTTGAAAATATTTATTTAAATAGGA  
TGAAATGTTATTGAGGGCTTTTGTGTCTAGAAACA  
GGGTTAATTAAAGATTTCTGCCTGCTTTGTGGGT  
AGGTGACACTCTCATTGAGATAGCAGCAATGCCCT  
ATTTAGTGGTCTCTCAGCTTTCTTTCTATTCATCTG  
GTTTGGTAGTGGAATTCATGGGAAGTTTGACCCT  
ATTCAGTGATGAGAGAAGCAAAATACTGTTGTTCA  
CCTAGAGGCTGTTTGCTCAGAAAAACAGGGTGTCA  
GAGACATGGGACGCAAACCCAGAGTGACATAGCG  
CAGGGACTTGGCTCACTTTTCTGAGATCTGTAAAA  
TGTGATGTGACTGGCTCTCAGAAAGGAGGCTGAA  
GGGTTCTGCCCCGATTGGTTTTCAAAGGGCAAGA  
GCCCCGCTGCTGAGGAGCTGCGGCTTTGTTAGAC  
AAAAGCCCAGGGCAGCCCCCTCCTGGGCTCAGCG  
TTTTCCCCACCTCCCCCTTTCTCACAGGCGGTTTC  
CCTGAAATGAAAGAACTGTGATGCATTTGCGCCTT  
CAAAAGCAGTTTTTAAAATGCATTAAACCCA (SEQ ID NO: 18) T/NK chr1:  
167492555- TAGCAGGGAGGGGAATTTTCCTTTCTGGTCACGTA CD247 167492938  
GGCTGCTGTCTTCGGCTTCGATTCGTAAGCCACCT  
CCCAAGCCCCCATAGCCCCAGGTAGGGGACATC  
CCATCCCCCTAAACCTCCTTCTTGAAGTACAGTT

CACCCCTAGAAAGGGTGGGAAACCACTCAGATC  
CATCTCAAAGGGATGCCTACCAGGGTAGGTGTGT  
GGGAGGAAGGCTGAGCCCATGCTGTGTTTCAGGG  
GCCCCCCCAGAAGGTGTCAGCCTGGAGGAACATG  
CCCCACAGTTATAAAAGGCATCATTCCAGGAGCTA  
TCATGGCGTCTGCTAGGGGGCAGAGAGGAAGGGG  
AGGCAGGAAAGGGGCTGAGTATTTTGGGGCTGTA TGCTTAT(SEQ ID NO: 19) T/NK  
chr1: 167516527- GGCCTTTCCTCCGGGGAGACTGTGGAGGTGTCCC CD247 167516891  
CTCAGGCAGCAAAAGCACCATCGCAGCCCTCGGT  
GTCAAGGCCTCTCTGAGTCGCGCTTTGCCTTACC  
GTACCTCGTTTCTGGGCTAGGATATTTTGAAACAC  
TTTTGTCTATTAGCTTTATCTAAAATAGCTAAAATAT  
CAGTCTCCATAGAGGCCAAAGTAAGTGGCACTGAG  
AAGCAAAAAATCCAGCCAATGTGCAGTTTTCTCCT  
CCTGCCCCCTATTTGTGGAAATGAGGCACGGCCC  
CCATCTTGTCTGCTGGGTGGGGTTCTGTTTACCAC  
GCTTGGCAGCCACCGCCACCCAACAATCTTTCATT TTTCTTTCTAACTTCAC (SEQ  
ID NO: 20) T/NK chr1: 167516973-  
GAGCTCCCTGGTGTGGTGTCTCAAGCCTGAAGCT CD247 167517468  
CAGAGGCTGGAGCTATTGCCCCTGGCTGTCTCCC  
CTGAAGTCACTAAATTCCAGCGTTCTGGGTCCCCT  
TGTCCCCAGCACACAAGGATGGGCTGCTCTGAGC  
AAGCTCAGGCACCCTCACTCTGCCACACTGTGTGT  
GTGACTTAGACCCCACAAGGCTTTTCTGAGAAAAC  
AGCTGTGACCTGACAGAAACCCCTGCAGCTGCAC  
GGGCCTCAGAATCCCCCGAGGCGCTGGCCCGGG  
CTGTGCCTGGCTCCCTGGTGGGAGCAGAAGTGCC  
CATGGCCTCTCCTGCCCTCCCAACAGGGGCCCTA  
AACAAATTCCCACACCCAGGTGAGTTACCAGGAG  
CGATCAGGTGGGCCGAGGACATTTGCTTGCTTCA  
CCTTCCCTGTTTCTTTAGATTGAAATTCAGCCTGCC  
CCACTTCTCAGGAAGATGCCACGAGGCTGATCCC CCTGAGCAGTTTGTC (SEQ ID  
NO: 21) CD4/NK chr1: 193458645- TGGCTGGCAATCCTTTTTAGCCATCATATATAATAG  
intergenic 193459063 CTATCTAACTCTTGATTTTTGTGTAACATAAAGCGG  
TGTTTCATAATGAATCATTTTGTCTGTTCGTGTTCA  
AGTGCCCTATCTATTCTATCATTTGAAAAGCTAGTT  
ATGATAGTATGGTTTCAATTGTCAGACATTCTCTTT  
ATTCTAAAAAATAGGTTGACCCACATTTAGATGTT  
TTCCTACTCAAAGGACTTTGAAAGAACTTTTTATGT  
AACACTTAGTCATTGCCCTTTTCACAGTCCCTCTTC  
CGCTGCTTCTAATTAGGCTCTTTGGTAGCTGGAAT  
CGTCATTTCCCTGGCATGAAGGCACATGGTACCAA  
CTTGTTGGTTGCTTGATTTGGAGGGTCAAATATGG  
ACTTGCTTCTTTTGGTCTACCTTGC (SEQ ID NO: 22) B-cell chr1: 220219915-  
CTGACAAGATGCAAGCTCATCATTTTGTGTTTGTGTC RAB3GAP2 220220218  
ATAAGGGCATTTGCCTACTAATTAGCGTTTTGGAA  
AACTTCCCCAATAATGGGGCCCACAGTCTCTAACA  
CTGCCCCCTTGAAGCTAGTGATCCAAAAATAATAT  
CATTTGACAAGGTAATTTCCCAAGCCACGCCTGAA  
ATGTAAACAAAAAAAGCACCTCACTGCTTAAAGG

TGCTCCACCGACCTTGGGTTTTCCACCAAAC  
TTGCCTCCCCACCCTGTTACTAAAGTTGACACTG AATTTGCCTGCAGTCTCCCCCA  
(SEQ ID NO: 23) T/NK chr10: 6530291-  
TGAGCTGTCGCCCTTGGGTACACCAGTAGCAAAA PRKCQ 6530474  
ACACTCCTGTCCTCCTATGCTGCTGTGACACCACA  
CCCCACTTCCTCCCGCGGGCGTGTGACACTTTTC  
AAAGAAAATACAGTATTTGGTAGTATCAATACAGCA  
AGCGGAAGCAGCAGTGCTCAGTCCGCAGTGAGCT AACAGTTTTA (SEQ ID NO:  
24) T/NK chr10: 6558358- CAGACATACAGTACTATTTTGTTTTTTTTAAAAAAG PRKCQ  
6558616 GCTTAGTAAACACAAAGAGGAGTTACATACTGAAA  
CCCACAGCTGATTTAATTTGCAAAACCACAGCGTT  
AGCTTGACTAAAGTAAAGATGACACAGATAAAATG  
CAACCAGAAAACCTGAGATAAAAGATACAGGATAAA  
TAACTTAAGCTGATGGTTTAGCAAGCAAACATCAT  
GGGTGTAACATGAAGATCTGAGAAGTGACTTCGGT CCAGGAAACTCA (SEQ ID  
NO: 25) T/NK chr10: 6592128- CTAAAAATTACACCTTCTGCTCACTCTAATTTATTG  
PRKCQ 6592406 CTGTAGAAGAAAGAATAAGTGAGATATTTCCATTTC  
TAGTGACAGGTGGCTGGCACTTTGAAACCTTCCAT  
TTCATTACTCACCTTAATGTTTTTCACTAAAACACAT  
GTGGTTTTTCAAACACAGGAAGGAAAACATGGTAAC  
CTGTGGTTACAATTTCCACTAGAAAATAGAAAAGG  
TGTAGGTCAGAAAGAATGTTTGTGGGGTGTTCCTT  
TTTTACTTTAAACATGAAAACCCATGTCAG (SEQ ID NO: 26) T chr14: 99250860-  
TTAAGAGAAAAAAAACACCCATTTATGTGACCA BCL11B 99251000  
GTATTGTCTGTCTTTTTTTTAAATCCAATAAACTTTC  
AACTACAATCGGTGCTGATGTCACCATGTTAGCGG  
CACACACTCTTGACTCTGGTTTGCACAGTTCAC (SEQ ID NO: 27) T/NK chr14:  
99253442- CGGCGGAGGAGGGAAAGCCGAGCGCCAGGAAAA BCL11B 99253634  
GCTCCTATTTGCAGCACACCTACCCCCACCGTGTG  
CCAGGCACTCATCATAAATGTCACAATGACAGATG  
AGGAAACCGCGGCTCAGAAGGTCAAGTGGCTGCC  
GGAGGCTGGCAGGGAGCAGGGTGGGGCTCTGAC TCGGGCTGTGTTCTTTCCCACA  
(SEQ ID NO: 28) T/NK chr14: 99259222-  
TGAAAACACAGAGGAACGGCGAGAAGGAATGGG BCL11B 99259750  
AGGGAGGAAAATACATTCCGTGGCAGTGAAGTTAT  
TGAAGTGCCAGAGCCAAAGAACAGATAATTTAAGG  
AAAAAATTCTGTGGCATCTCCCATCCTACACATAAA  
TCACTGTTCTCTATTTTCTGAACACGAGCCTATGCA  
GGGCCTAGGAGAGTCCATGTGTGAAACTGAATAC  
AGAATATACGACGATGTAAGACGTACAACGCGCAC  
GTATGAACTATGTGTGAATAGGTAGCGACGTAGGT  
ATCGCTGAGAAGAGAAACTACAATTTGAGATCCCA  
CCTGTGGTTACAGAAAAGCAGACAGAGCCCTCGA  
TGAATTAAATGCAGAATGCATCAGAAATGTGGCAG  
TACAGAAACGCCCCGCAGACGAGGAAATCCTAAA  
TCTGTTGTCTGCATCTCTCTAAGAAAAAGAACCTA  
CAAGGTAAAGAAACAGTCCTCACAAAGCCGGTCC  
CAGAAACCATTAATTACACTTTAGAAAGAAATAGGA GTTTA (SEQ ID NO: 29)  
T/NK chr14: 99260500- AATTTCAAACCTATTTTCTAATTCAAAAGAAACACA BCL11B  
99261397 GATGAATTTAAAAATTAGCAGAGGTCACACGTGCC

TGTTTCGCAAGCTCCTCCTCCTCAGCTTCGTCTT  
TTGCCAAGACTATGTACCAGCACTTTTTTCCTTTCTC  
TCCTCTTTTTTTTATTTTTTTTATTTTTTGGTAACATCT  
GGTATTCTTTTCCTTTTTTTTGGCAGTCTCTGCCTTTT  
CACTTTCCTCATGCAGCAGCCCTGCGAGCCCCCG  
TAATTGAGTTTGAAGGTGTCCAAAGCCGTTTGCTG  
TGCTGCAGCTCTGATTTCTGCGTCAAGTCCTAACA  
GCCAACCAACCTGGGAGCCAGGCCCTGTTGGCGT  
CCCCGAGACAGCCCCGGGATTATCCAGGCCTCCA  
GGGCTCAGTTCTGAGCTGGGATCTCCACGTCCCA  
GACCAGAGATCCAACCTCACTGCCTCTAAGGGAGT  
CTGGGAGGAAAAGAAACAAACAAAAAATATCTCCT  
TCCCCTCCCCTTTTTCAGCGTTGAAAGTTAAACCCC  
TGAGATGACAGGGTGTTCACCCGATTCCAAAGAAC  
GGGGTTTCCTCTCCCCACCACCCAGGCTGTGGCG  
CTGTGCCTTGGCCTGCTGTGTGCGACACTTCCCA  
GGAAGGTAGCAAGGTCACCCTCAACATCAGGCAG  
CAGAGTCACCCCCAGCCCCAAATTTCTGGGTCAG  
AGTAAGTGGACCAAGTGGGCCTGAAAGCTCACGT  
TCAAGGCCTTTGTAGACACCCCCAAAGACTGTGGT  
TTCACTTCCGCTGGCAGGCAGATACCAAGCTAGG  
CGTGTAGGGTGCCTGCATATGTGTGCATGTGTCC  
CTCCTTAGAACTGTATCGATAATGGAAGAAATAAA  
AGGAGAAAGCCAAGCCCTGGGAAGGGCAA (SEQ ID NO: 30) T/NK chr14:  
99269613- TGGCTCCTATGCTGGGTTTTTCAGGGGAGGGGAGA BCL11B 99270139  
ACCACTTTATATATTTATTTATTTATTTAAATTTTTTA  
AAATATAACATAAATATTCGGCTCTCGGCCGCCCCG  
GCAGCCAGTCCTCTGCGGTGACTGGGCGCGCAG  
CCCTCTCGAGCTCCGCGCGGGCAGCCCGGCCCC  
AGCCCGGCGAGGTGCGCGGGCGGATTGCAAGCAT  
ATAACCTGCCCCGCGGTCTCGATGGCACCCAGAGG  
ATGTTTTATTTCTATTGCAGTTAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAGGAACGGCGACCCAG  
GCACCGCGAGAGAAAGAACGGCGGGGAAATGTTC  
GCGCGCAGCGAAGAAGCCGCCCGCGGGCTGCG  
GCGGGCGGGGAGCGCCGCAAAGCCACCTTCCCG  
GTGCAAGTGTGCGGGGACTCGGGGCGGGTTCCC  
CTGCAAACACCGTACCTGGCCCCGCTCGCGCTCGC  
TTTTCCCCTCTGCTAAATAAACCCAACAGGGACGG TGGAAGCTGCTG (SEQ ID  
NO: 31) B-cell chr15: 70319616- CAGCTAGTGGGTGGTGGATCCGTGCTCTTGACCA  
intergenic 70319805 CCCCACTCTGTTGCCTTTCTGTTAGACAACACCTT  
GTCTACTTTCTCTTCCCCATTTACAGATGAGGAG  
ACAGGACCGGAACTGTGAGGACAATCTGTCTATA  
GTTAGGCAGTGAGCTGACTGCAGACTCAGGCTCT CCTGGGACCCTCTTCAG (SEQ  
ID NO: 32) CD8 chr16: 56708539-  
GCAAAAAGGCAGGGGCTGCAGGGGACTTTATAAA intergenic 56708977  
GTTGTGCTGTCTGGGCTGAAGGCTTGACAGACAGG  
AAGCTTGGGTGCAGGTGGGCTGTGAGCTGAATGC  
TTGCAACAGGATGTTTGGGTGCTAGTGAGCTGTTT  
GCTGTTGACCCTATTTCTCAGAACATTCACCTCCCC

TCTACCCTGTGTCTTCTTGCCAGCTAAGCTCA  
TTTCCAATTTTCTTTTAGCTCCTTAGGGCTCCACAT  
GCGTGACTTATTAGAGGAGCAAAAGAAGCCGAATA  
TGGAAGGGGAAAGCCTTGGCCTGATCCAGGGTGC  
TCTAGAACAAATTCCTCCCACTGAACTGTCCTGCT  
GTGGGGCAAGCAGGTGAGCACTTGTTCTGTTGTC  
AGTCACCATTTGCCCCCGGGGTGGGGTGTAACCT GCCAGGCATCCTCAAGGGATGCA  
(SEQ ID NO: 33) CD4 chr16: 84766016-  
CCCCCAGGCTGGTATTTAGTGACACTCTTAGTCT USP10 84766317  
GTTGTTGTCCCAAATGCTGCGTACCTGAAAGAAAG  
AGCACCCCTTTTGAGGCATCAAGACTTGATTGAGT  
CTCAGTTCTGAGACTGAATCAACAACCTTTCAACAG  
GTGAATAATAAGAACCTCAGAAACCTGCGCTGACG  
CCCTCAGAAGCTGGTTTCCCGTCCTCTGTGGAAGT  
GGATTTAGAAGCCAGTTGAGCAGCCATGTGACCTT  
GAACAAGTCACTTCTGTGTTCTGCACTGTGAGCTC TATTCCACTGGCCCCCTTTCTA  
(SEQ ID NO: 34) CD8/NK chr17: 35890611-  
TGAACCCAAGACTCTACAACATCCTGGCCTCGATT intergenic 35890953  
TTTGGTCCAGCTCTGGTTGGTTCTTTCTGTGTTATT  
CTGTTCTGCTCCTCAATTCCTCTCCCCTCCCCTCC  
CCCTCCTCTCCCCACCCCTTCCCCCACCCCTCC  
CCCTCCCCTCTTCTGTACCTTGCACCTGTGCACTT  
TAATGCACATTGCACTATGTCAAGGTACTAACTTTG  
ACAAGTCTCTCATAATTCCAACCACATTCAGGCT  
GAGGGTTGCAGCTTGGCTCCCCCTCACTCTTCTCA  
CCTTCACCACACAGGACATTGGCTGTGCACAGCC  
ACTTGTTTATTGCAGCTGGAGACCACA (SEQ ID NO: 35) T chr18: 13274085-  
CTTGCTGGCTCTCCTCGGTGGCTTCCTCTTGAATG LDLRAD4 13274184  
AACCTTTCTTCTGAAGGCTTGATTTCCCTTCCGGGA  
GGCTTAGTGTTTCTGCTCAGCCTTCTTTC (SEQ ID NO: 36) CD4 chr18: 13276705-  
TCTGTCACTCCCTCGTTGCGGACTCCTATGTAGGT LDLRAD4 13277363  
CGCCTGGTGTGGGGAGAAGCATTCTTTGTGGTATT  
CGGTTGGGTAGAAGCCAATCACAGCTTCCACCTA  
CACTCAAGGGGAGGGAGTTGTACAGGGTGTAAGC  
AATTTGGGGAGTCACCTTAGAATTCTGCCCACCAC  
AGTACCTTTCTGGTTATTTTCATGGACTTCCGTTCCC  
AAGAAATCTGAGCGTTTTCGTCCTAGGAAGACTG  
GTGAGAGCAGCCCCAGTGGAGGAATAAAACTAA  
GATCTGGAAAATCAGCAGTGGGTTGTTTTTCATCTC  
TCAGCAGGCAGGAAACAGGAGGAGAAAGGAATGT  
CTGTAGGCTCCCAACACTGATGGGAAGGAGGTGT  
TTGGCTGGGGTAGAGCTCCGCAGGAATCTCCCAG  
GCTCCTCTAGCTAGGTGCAGCCGTGTTTTATCCGG  
CTGTCTTTACATGGGCTGATCACTCAGGAGTGGCA  
TATGGCAGGAGCACGCTTTGGGGTACGAGCCTCC  
TGGAGAATGAACTGCAGCACACAAGCCAGACAGA  
TGGTGGCTTAGTCCCCCTTGGACAAGAGTGTGTC  
CTGCACACTGGAGGGCGGGGTGCTGAGAGGCGC  
TTGTGGTGTCTGAGGCCGAGCTTTGCTGAGTTCAC CTGA (SEQ ID NO: 37)  
CD4/NK chr2: 147449478- TGAAAGAAAGAGAATAAAGCACTGGGCTTTGCAAA

intergenic 147449829 CTAGGCTTAAGGCCCCCAAAACCAAGATTATCTTGA  
TGCATGTGATTGGGAAGGAGGACTAATTAGAAGA  
GAGGATGTCATTTTATTTTCTAACCAGCCCTCTCTG  
ATCTGAAGCCATGACACCCCCTCACTTGTTACCTA  
GCAACCCTTCTTTGTATATGTAAGGTTACTTCATTC  
CTGCATTGTTATTCAATTTATTTGCCATTTTAAGTG  
TCTTGATCCTCTCTAGGATCACCTGGTGTGATTG  
GCTGGCATTAGTCTCTAAAATACCGGAGGGCAGT  
CTATTAAGTGGATCCAAATGTCATTGTACAGCTCA (SEQ ID NO: 38) CD8/NK chr2:  
181142700- CTGAGATGTTCTGTAGTATTGTCATCTACATTTTCATA intergenic 181142853  
GAAGAAGAAACTGAAACAGGTTAGGTAACCTTACTC  
GTGGTCACAGAGGTAATTGGTGGATTTGAGGATTC  
ACAGGATTCTAAAATCGAGTTGCTGCACTGCCTTC TTAAAAAATTA (SEQ ID  
NO: 39) CD8/NK chr2: 86793857- TGTCAGCCAGTGGGGTGGCAGCCCCTTTGTACAG  
CD8a 86794067 AGCACCATGGGGGTGGGGTGGGGAGGATAAGG  
CAACATGTCAAACCCATCAAGGAGGCTTTGTGACC  
CCAGTGATATTTTTTGCAGAACGTGGTGGATTTTC  
GATGTGACCACAATGACATCCGCCTTGCAGTGGC  
AGAACAGATGCAATTGCACAAGTTCTGGAGAACT TTCT (SEQ ID NO: 40)  
CD8/NK chr2: 86794937- TGCTCTCTGCTTACCAAACCTCCTCTCCCTTAAGAC CD8a  
86795091 CCAGCTCAAGCATCTCCTTCCTGTGGGGTTTAGCT  
CCTTCCCCCTCCCCTCCAGACAGTACAGACCA  
CATCCTTCTCTTCTCTGTGTACCCGGACCTTGGG TATCTGCAGACTGGA (SEQ ID  
NO: 41) T chr20: 21548472- TAACTTAAGCGTGTTGGTATCTCTGTTAGTGGTGA  
intergenic 21548637 AGTCGGCTGCAAACCACAAAACACCTGACTGCTAC  
GGAAGATATTAACAGGGACTTTTTTCTTCTGCATA  
ACAGGAAGTCTTGAGTAGGTAGTCGAGGTTGGGT  
AGAGGACACTTCTGTTTCAATTCTA (SEQ ID NO: 42) CD4 chr20: 52975358-  
GCATTGCTCAGAATTGCTCGACTTTGATTATAATTA intergenic 52975749  
TCCTACGATCGATAAGGATCCAGGTGTACGTAACA  
TAACACTGTATCACATTATTTAAATCAGGTCCTTTT  
CATTAAAGCTGTGTCTGTTGGTGTGCTGGCTAGTTTAT  
TGGTGTGTGTGGGTGTGTGGGGGTGTGTGTGTGT  
GTGTGTTAGAACTTGTAAGTATAGTTTCAGTTTTT  
TGGCCTATTATATTCCTACTGTCTTTGTATGTTTGT  
TTTGTATGATTCTTATTATTTTCACCAGAAGCGGAA  
ACCCTTTTTAAGCTGAAAAAGGATGATTCATTTTCGT  
ACACAGTGAGGGCCCTCTTACCTTATTIATTTGCT  
CACATATTAATAGAGGAGACAGTTTTTTCATGCAGT G (SEQ ID NO: 43) B-cell/NK  
chr20: 57682799- TTCTGCATGAGCAGGCCCCGCTGAAAAGGAAGGC PMEPA1 57683080  
GGCTCGCCAGATTTGTTTCGAATTATGAAAATAGA  
TGTTGTCTCCCCACCACATCTGTTTTGCCTGACAA  
ATGAGCAGCAGCTCGCCTCCTAAATAAGGCAGCA  
CACCAAGACGGTCTTGAAACTCCGGCTTCTCCAAC  
TCTTCAGAAAAGGAGAAGAAGAAGAAAAAAGAGTC  
CAAGCCTCCCAGGTTTGAGCTCTAAAAGCCAGAC  
CTTTTTCAATGTCATCTCTCACGCCGAGTCTCCG GGGG (SEQ ID NO: 44) CD4  
chr22: 42183675- TATTAACAAGTTTGTGGTAAGGTGTTATGACAATGA TCF20 42183793  
TAGAAAATAATACACGGGGATTTCCCAAGACTCT  
CAAGTGATTGTTTCATTTTCCTTTTAGGTTCTTTTTTT TTTTTTTTGA (SEQ ID NO:

45) B-chr7: 1022952-1022952-GCCGGCTCTGTCGTCGCTCACAGGCAGG C7ORF50  
1023453 CACACGTGAGCTCCTGGAGGACAGGGAGAGCGG  
CCGCCCCGCCCCCTGCGGAGCACAGGACGCTTCCT  
GCCACCCCTGCAGAGCATGGGACGCTTCCTGCCA  
TGGTGCCGTGGCAATGGGTGGCACCTGCCTGTGG  
CCCCTTCTCAGAAGGACGTTTTAAACGCGTGAGGT  
CTGATGCACAGCCACAGGGAGACACAGACGAGCA  
GATGTGGGCATCCGAGTATTTACAAGGTCTGGTG  
GCTCCTGCAGCCGCGACACGGGCTGAGCGCAAG  
TGATGTGTGAGGTGTCCCCAACAGATGGCACGGG  
GAGCGCCACACCCGCCACCGCGGGGTCTGCGG  
AAGCTCGTGTGAGCTGGAGGTTAGGGAAGACGCA  
CAGGGGCTCATTCTCCACCCAGGTTCAAACCTCCCT  
GAACTCACGAGACCCAGGCTGAAGACATGGACAG  
ACTCCCAGCCCCAGCGCCCCTCTGAC (SEQ ID NO: 46) CD4/NK chr7: 70788396-  
CTGACTGATAAAAGAAAAACAAATTTTCCTTCAAGG AUTS2 70788551  
CAGTATGATAACAATCTCTTAACCACTGGGAGGAA  
TGTTTATGACATTCATCTCTGAGCTGTGAACTGCC  
AAAATGAGGCACGCCTAGCAACATAACCTGGCCG ACCCTAGGCCGCGAG (SEQ

ID NO: 47) Repeated motifs/domains in the sequences are underlined

[0213] Bioinformatics analyses were performed to further characterize the enhancer candidates listed in Tables IIIa and IIIB. First, the sequences were analyzed using the Multiple Em for Motif Elicitation (MEME) tool (Timothy L. Bailey and Charles Elkan, Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology, pp. 28-36, AAAI Press, Menlo Park, California, 1994) to identify repeated motifs in the sequences of the enhancer candidates. The results are depicted in Table IV.

TABLE-US-00005 TABLE IV Motif ID SEQ. ID NO: (see sequences below) # of repeats  
SEQ ID NO: 7 1 8 SEQ ID NO: 8 3 2 4 1 17 1 20 2 SEQ ID NO: 9 4 2 12  
1 SEQ ID NO: 10 3 1 4 1 12 1 SEQ ID NO: 11 2 1 4 7 5 1 7 2 SEQ ID NO: 12 8 1  
18 1 20 2 SEQ ID NO: 13 3 3 4 1 7 2 12 1 13 1 20 1 SEQ ID NO: 14 3 1 4 2 7 3 14 1  
SEQ ID NO: 15 4 1 5 1 7 1 8 1 SEQ ID NO: 16 5 2 11 1 14 1 SEQ ID NO: 17 3 3  
5 2 7 1 11 1 18 1 SEQ ID NO: 18 3 2 4 1 7 3 9 1 11 1 12 2 13 1 SEQ ID NO: 19 3 3 13  
1 14 1 20 1 SEQ ID NO: 20 3 1 4 1 5 2 16 1 19 1 SEQ ID NO: 21 3 1 5 1 6 1 7 2 11 1  
13 1 16 1 SEQ ID NO: 22 4 1 12 2 SEQ ID NO: 23 4 1 11 1 12 1 SEQ ID NO: 24  
4 1 5 1 SEQ ID NO: 25 4 2 7 2 12 1 20 1 SEQ ID NO: 26 3 1 4 2 8 1 14 1 20 1 SEQ  
ID NO: 27 4 2 SEQ ID NO: 28 9 1 11 1 13 1 SEQ ID NO: 29 3 1 7 4 11 1 12 1 20 1  
SEQ ID NO: 30 3 4 4 4 6 1 7 3 8 2 9 1 16 2 20 1 SEQ ID NO: 31 3 1 4 4 5 1 6 2 9 1 15  
1 SEQ ID NO: 32 3 1 9 1 12 1 SEQ ID NO: 33 3 1 4 1 5 4 8 1 12 1 SEQ ID NO:  
34 7 2 SEQ ID NO: 35 3 3 20 1 SEQ ID NO: 36 14 1 SEQ ID NO: 37 3 1 5 1 6 2  
7 1 17 1 SEQ ID NO: 38 4 1 7 1 20 1 SEQ ID NO: 39 14 1 SEQ ID NO: 40 3 1 9  
1 SEQ ID NO: 41 3 1 7 1 SEQ ID NO: 42 3 1 12 1 20 1 SEQ ID NO: 43 4 2 5 1 7  
1 SEQ ID NO: 44 3 1 7 2 11 1 12 1 17 1 SEQ ID NO: 45 4 2 SEQ ID NO: 46 3 1  
8 1 9 2 10 2 13 2 15 1 19 1 SEQ ID NO: 47 4 1 7 1 Sequences of motif ID 1.

CGGTGTGGAGGGCCGGGTGGTGACGCTGAGTGACAGGTGAGGATGTGGCA (SEQ ID NO: 64) 2. GTGGGACACCCATCATCTTACCACATCACATCGTCACTGCC (SEQ ID NO: 65)  
3. YSCCTYCCCCWCCYCYTYCCH (SEQ ID NO: 66) 4. AAAADAAANAAARWA (SEQ ID NO: 67) 5. YTGGKGGSHRGGSGKSTGTG (SEQ ID NO: 68) 6.  
CTCVGVSCDGGNDGCCHGGCHMANVCCGGGCCWGBBBCGCGGVSG (SEQ ID NO: 69)  
7. TCWSTKTTCTG (SEQ ID NO: 70) 8. GTGDMASTGCCTG (SEQ ID NO: 71) 9.  
GCAGCCRCCYCRCKGKCTGAG (SEQ ID NO: 72) 10.



CCCTGCRGAGCAYRGGACGCTTCCTGCC (SEQ ID NO: 73) 11. TGKCCTCTMCCCACM (SEQ ID NO: 74) 12. GCCYTBHTGTYASRCAMAASM (SEQ ID NO: 75) 13. SWMTGACACMCTGTGKGTGTGMSYYWGMMSYASYWG (SEQ ID NO: 76) 14. ACYTKCTGCWCWGCCTTMTT (SEQ ID NO: 77) 15. CGGGGAGCGCC (SEQ ID NO: 78) 16. AGGHAGCAVAGKCAACCCTC (SEQ ID NO: 79) 17. TBTGGCGAGBCDCCTTNGNHTTCWGYGBGCCHCACT (SEQ ID NO: 80) 18. TGTGCCCAGGG (SEQ ID NO: 81) 19. GAGGTGTCCCC (SEQ ID NO: 82) 20. AAACCACA \* motifs 1 and 2 are highly repeated in SEQ. ID No. 7 and SEQ ID No 11, respectively (in bold in the table)

[0214] Second, the sequences were analyzed using the oPOSSUM tool (Kwon A T, Arenillas D J, Worsley Hunt R, Wasserman W W. G3. 2012 September; 2 (9): 987-1002. Epub 2012 Sep. 1) that permits the detection of over-represented conserved transcription factor binding sites and binding site combinations in sets of sequences. The results are depicted in Tables V and FIG. 13.

TABLE-US-00006 TABLE V Predicted conserved transcription factor binding site (bs) SEQ ID NO Cell Specificity Conserved binding site 7 Tspecific none (Chr16-445) 8 T/NK Zipper-type-Leucine Zipper-C9 binding site (bs): 2 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Beta-Hairpin-Ribbon-E2F T-C13 bs: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 9 T Zipper-type-Leucine Zipper-C9 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Other Alpha-Helix-Sand-C158: 1 time Helix-Turn-Helix-Homeo-C113 bs: 7 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 10 T/NK Zipper-type-Leucine Zipper-C9 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 1 time 11 NK (NK6) Winged Helix-Turn-Helix-Forkhead-C12 bs: 6 times Beta-Hairpin-Ribbon-E2F T-C13 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 12 NK (NK20) Beta-sheet-TATA-binding-C1 bs: 2 times Zipper-type-Leucine Zipper-C9 bs: 4 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Helix-Turn-Helix-Homeo-C167: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 8 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 13 T/NK Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 2 times Winged Helix-Turn-Helix-Forkhead-b: 3 times Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 7 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 5 times 14 NK (NK8) Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Helix-Turn-Helix-Homeo-C113 bs: 6 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 15 B Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 3 times Helix-Turn-Helix-Homeo-C113 bs: 8 times 16 B Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Other Alpha-Helix-Sand-C158: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 17 B Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 18 T/NK Beta-sheet-TATA-binding-C1 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Helix-Turn-Helix-Homeo-C167: 1 time Helix-Turn-Helix-Homeo-C113 bs: 8 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 19 T/NK Winged Helix-Turn-Helix-IRF-C134 bs: 2 times Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 20 T/NK Zipper-type-Leucine Zipper-C9 bs: 1 time Beta-Hairpin-Ribbon-E2F T-C13 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 4 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 21 T/NK Helix-Turn-Helix-Homeo-C113 bs: 4 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 22 CD4/NK Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 3 times Helix-Turn-Helix-Homeo-C167: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 6 times Winged Helix-Turn-Helix-

Forkhead-C57 bs: 2 times 23 B (b-enh-1) Zipper-type-Leucine Zipper-C9 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C167: 1 time Helix-Turn-Helix-Homeo-C113 bs: 5 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 1 time 24 T/NK Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 25 T/NK Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 6 times 26 T/NK Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 6 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 5 times 27 T Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 28 T/NK Zipper-type-Leucine Zipper-C9 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times 29 T/NK Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Other Alpha-Helix-Sand-C158: 1 time Winged Helix-Turn-Helix-IRE-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 7 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 30 T/NK Beta-sheet-TATA-binding-C1 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Winged Helix-Turn-Helix-IRF-C134 bs: 3 times Helix-Turn-Helix-Homeo-C113 bs: 11 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 31 T/NK Beta-sheet-TATA-binding-C1 bs: 2 times Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 6 times 32 B Zipper-type-Leucine Zipper-C9 bs: 1 time Beta-Hairpin-Ribbon-E2F T-C13 bs: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 2 times Helix-Turn-Helix-Homeo-C113 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 1 time 33 CD8 Zipper-type-Leucine Zipper-C9 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 34 CD4 none 35 CC8/NK Zipper-type-Leucine Zipper-C9 bs: 2 times Helix-Turn-Helix-Homeo-C113 bs: 3 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 1 time 36 T Helix-Turn-Helix-Homeo-C113 bs: 1 time 37 CD4 Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Other Alpha-Helix-Sand-C158: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 38 CD4-NK Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Helix-Turn-Helix-Homeo-C167: 1 time Helix-Turn-Helix-REX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 8 times 39 CD8/NK Beta-sheet-TATA-binding-C1 bs: 1 time Zipper-type-Leucine Zipper-C9 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times 40 CC8/NK Zipper-type-Leucine Zipper-C9 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 1 time 41 CD8/NK Winged Helix-Turn-Helix-Forkhead-C57 bs: 1 time 42 T Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 4 times 43 CD4 Beta-sheet-TATA-binding-C1 bs: 2 times Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C12 bs: 2 times Other Alpha-Helix-Sand-C158: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 6 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 4 times 44 BINK Zipper-type-Leucine Zipper-C9 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 45 CD4 Zipper-type-Leucine Zipper-C9 bs: 1 time Winged Helix-Turn-Helix-IRF-C134 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times Winged Helix-Turn-Helix-Forkhead-C57 bs: 3 times 46 B Beta-Hairpin-Ribbon-E2F T-C13 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-Homeo-C113 bs: 1 time Winged Helix-Turn-Helix-Forkhead-C57 bs: 2 times 47 CC4/NK Winged Helix-Turn-Helix-Forkhead-C12 bs: 1 time Zinc-coordinating-BetaBetaAlpha-zinc finger-C29: 1 time Helix-Turn-Helix-RFX-C154 bs: 1 time Helix-Turn-Helix-Homeo-C113 bs: 2 times

Example 3: In Vitro Validation of a T Cell-Specific Promoter Construct (Chr16-445, SEQ ID NO: 7)

[0215] A T cell-specific synthetic promoter was created by the juxtaposition of a selected enhancer sequence identified above and a minimal promoter such as CMV. The pENTR1a-Chr16-445-minCMV-GFP-SV40polyA vector was transfected in a T cell line (Jurkat) and myeloid cell line (K562), and GFP expression was only detected in the Jurkat T cell line by flow cytometry. In opposition, when transfected with a vector coding for a non-specific strong promoter (spleen focus-forming virus (SFFV) promoter) instead of the Chr16-445-minCMV promoter, both cell lines expressed GFP (FIG. 3A). Then, human PBMCs were transfected with the T cell-specific synthetic promoter and it was observed that GFP protein was only expressed in T cells, but not in monocytes or B cells (FIG. 3B), whereas all cell types expressed GFP when the PBMCs were transfected with a non-specific promoter.

Example 4: In Vitro Validation of a NK-Specific Promoter Construct (NK6, SEQ ID NO:11)

[0216] A NK cell line (NK92), myeloid cell line (K562), B cell line (697) and T cell line (Jurkat) were transfected with the pENTR1a-NK6-minCMV-GFP-SV40polyA vector. The NK6 synthetic promoter induced the expression of the GFP only in NK92 cells, whereas the SFFV strong promoter resulted in GFP expression in all cell lines (FIG. 4), confirming the specificity of the NK6 promoter.

Example 5: In Vitro Validation of Another NK-Specific Promoter Construct (NK8, SEQ ID NO:14)

[0217] The NK8 sequence specificity was tested in an OP9 co-culture system with CD34.sup.+ cells transduced or not with the NK8-minCMV-GFP-SV40polyA or the SFFV-GFP-SV40polyA. GFP expression was observed in the NK cells, but not in the B cells, harvested after co-culture in the well containing the NK8-transduced cells (FIG. 5). Cells transduced with the strong unspecific promoter (SFFV) led to both NK and B cells expressing GFP, while untransduced cells were not GFP positive. These results provide compelling evidence that the NK8-based synthetic promoter is specific to NK cells.

Example 6: In Vitro Validation of a B Cell-Specific Promoter Construct (SEQ ID NO: 23)

[0218] A B cell line (Nalm6) was transduced with a BaEV-LV particles coding for the Benh-minCMV-GFP-SV40polyA. The B cell-specific synthetic promoter was shown to induce the expression of the GFP in the B cells (FIG. 6) suggesting that this promoter can induce the expression of a protein of interest in a B cell-specific manner.

Example 7: In Vivo Validation of the T-Specific Promoter Construct (Chr16-445)

[0219] Human CD34.sup.+ cells were transduced with the Chr16-445-minCMV T cell specific promoter or the non-specific SFFV promoter and engrafted in NSG mice with or without the co-engraftment of human thymus (huNSG and BLT models, see methods). In both models, the development of GFP.sup.+ T cells was observed, while no other human cells expressed GFP (FIGS. 7A, B) when CD34.sup.+ cells were transduced with our Chr16-445-minCMV T cell specific promoter. In contrast, all lineages expressed GFP when CD34.sup.+ were transduced with the non-specific strong promoter SFFV (FIG. 7C). These results also show that engineered HSC are able to differentiate into various lineages both myeloid (monocytes) and lymphoid (T and B cells).

Example 8: In Vivo Validation of a NK-Specific Promoter Construct (NK8)

[0220] Human CD34.sup.+ cells were transduced with the NK8-minCMV driving the GFP expression to test the specificity of NK cell specific promoter in vivo. Human NK cells expressing the GFP were found in blood, spleen and bone marrow of mice (FIGS. 8A, B). In contrast, very few human B and T cells harvested from the same mice expressed the GFP, witnessing the specificity of the NK8 synthetic specific promoter.

Example 9: In Vivo Validation of a B Cell Specific Promoter Construct (SEQ ID NO:23)

[0221] Human CD34.sup.+ cells were transduced with the Benh-minCMV driving the GFP expression to test the specificity of B cell specific promoter in vivo. Four weeks after humanization, human cells circulating in the blood were analyzed by flow cytometry. At this

timepoint, only B cells (CD19.sup.+) and monocytes (CD14.sup.+) are developed in the humanized mice. While the strong unspecific promoter (SFFV) induced the GFP expression in all cell subtypes, GFP positive cells were only found in the B cell population (FIG. 9). These results suggest that the B cell synthetic specific promoter is induced specifically in the B cell population.

#### Example 10: Functional Validation of the T-Specific Promoter Construct (Chr16-445)

[0222] The therapeutic potential of the construct was explored through cytotoxic assays using CAR-CD33, CAR-CD22 or CAR-GD2. A sequence encoding a CAR-CD33, CAR-CD22 or CAR-GD2 was put under the control of a strong non-specific promoter (SFFV) or under the control of the synthetic T-cell specific promoter (Tenh Chr16-445). Its ability to drive the expression of a functional transgene was assessed by transducing primary T-cells with the CAR and performing cytotoxic assays against human AML cell lines expressing or not CD33, against human ALL cell line expressing CD22 (RS4;11), or against a GD2.sup.+ neuroblastoma (NB)-cell line (SK-N-DZ). Primary T-cells transduced with the CAR-CD33 construct under the control of the non-specific SFFV promoter or the T-cell specific promoter showed CAR-CD33 expression, as confirmed by flow cytometry (FIG. 10A). The results of cytotoxicity experiments show that the CAR-CD33 construct efficiently triggered lysis of CD33.sup.+ AML cells, even when its expression was driven by the specific T cell promoter (FIG. 10B). Unspecific lysis measured in CD33<sup>-</sup> cells (right bars) was minimal and significantly lower than CAR-mediated lysis (\*\*\* p<0.001). Similarly, CAR-CD22 expressed under the Tenh Chr16-445 promoter induced a CAR expression strong enough to induce a similar CAR-specific cytotoxicity against RS4;11 ALL-cell line to that of SFFV (strong) promoter (FIG. 10C). Similarly, the results depicted in FIG. 10D show that in a context of solid tumor, a CAR-GD2 expressed under the Tenh Chr16-445 promoter construct also induced target lysis at levels significantly higher than untransduced primary T cells (\*\*\*\* p<0.0001), and comparable to the levels of a CAR-GD2 driven by the strong unspecific promoter SFFV.

#### Example 11: Kinetic of Expression During T-Cell Differentiation of a CAR Under the T-Specific Promoter Construct (Chr16-445)

[0223] Using OP9-DL4 cells to mimic in vitro thymic differentiation and using a CAR-CD22 construct, it was observed that the specific promoter induced the expression of CAR-CD22 early in the T-cell differentiation process (as soon as CD7.sup.+CD1.sup.+ stage) (FIG. 11A). As a control, HSC engineered cells were co-cultured with OP9 cells, which results in B cell differentiation, and no CAR expression was detected (FIG. 11B). This data further evidences the specificity of the T-specific/Chr16-445 synthetic promoter. The results depicted in FIG. 11C shows that the Tenh Chr16-445 promoter is active in all stages of T cell differentiation: in pro-T, CD7.sup.+, CD7.sup.+CD1a.sup.+, CD4.sup.+CD8.sup.+ double-positive, as well as CD4.sup.+ and CD8.sup.+ single-positive T cells. Similar results were obtained when Chr16-445 was inserted in the reverse orientation, confirming that this sequence shares this feature of transcription enhancers (i.e. orientation-independent).

#### Example 12: In Vivo T-Cell Differentiation of CD34.SUP.+ Cells Transduced with a CAR Under the T-Specific Promoter Construct

[0224] The in vivo T-cell differentiation of CD34.sup.+ cells transduced with a CAR was assessed in a mouse in which transduced CD34.sup.+ cells were engrafted along with a fetal human thymus (BLT model). Blood sampling of the mice 30 weeks after humanization shows that T cells expressed the CAR-CD22 at their surface (FIG. 12). Consistent with the previous findings, B cells and monocytes did not express the GFP. Again, similar results were obtained when Chr16-445 was inserted in the reverse orientation. These results confirm the specificity of the T-specific/Chr16-445 promoter, but also suggest that thymic selection does not interfere with CAR-positive T cell development.

[0225] Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims. In the claims, the word “comprising” is used as an

open-ended term, substantially equivalent to the phrase “including, but not limited to”. The singular forms “a”, “an” and “the” include corresponding plural references unless the context clearly dictates otherwise.

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## Claims

1. A synthetic expression cassette for expressing a nucleic acid of interest in a T cell and natural killer (NK) cell comprising: (i) a minimal promoter; and (ii) a transcriptional enhancer operatively coupled to the minimal promoter for expression of the nucleic acid of interest in the cell, wherein the transcriptional enhancer comprises a sequence having at least 95% sequence identity with the nucleotide sequence set forth in SEQ ID NO: 26.
2. The synthetic expression cassette of claim 1, wherein the transcriptional enhancer comprises a sequence having at least 98% sequence identity with the nucleotide sequence set forth in SEQ ID NO:26.
3. The synthetic expression cassette of claim 1, wherein the transcriptional enhancer comprises a sequence having at least 99% sequence identity with the nucleotide sequence set forth in SEQ ID NO:26.
4. The synthetic expression cassette of claim 1, wherein the transcriptional enhancer comprises the nucleotide sequence set forth in SEQ ID NO:26.
5. The synthetic expression cassette of claim 1, wherein the minimal promoter is a human cytomegalovirus CMV minimal promoter (miniCMV), CMV53, MinP, MLP, pJB42CAT5, or MiniTK.
6. The synthetic expression cassette of claim 5, wherein the minimal promoter is a human cytomegalovirus CMV minimal promoter (miniCMV).
7. The synthetic expression cassette of claim 6, wherein the minimal promoter comprises or consists of the sequence of SEQ ID NO: 6.
8. The synthetic expression cassette of claim 1, wherein the transcriptional enhancer is upstream of the minimal promoter in the synthetic expression cassette.
9. The synthetic expression cassette of claim 1, further comprising a polyadenylation (poly(A)) signal and/or a transcriptional termination signal.
10. The synthetic expression cassette of claim 1, further comprising the nucleic acid of interest operatively coupled to the minimal promoter and transcriptional enhancer.
11. The synthetic expression cassette of claim 1, wherein the cell is a stem cell.
12. The synthetic expression cassette of claim 1, wherein the nucleic acid of interest encoded a chimeric antigen receptor (CAR).

- 13.** A vector comprising the synthetic expression cassette of claim 1.
  - 14.** An isolated host cell comprising the synthetic expression cassette of claim 1.
  - 15.** The isolated host cell of claim 14, wherein said cell is a hematopoietic stem cell, a T cell, or a natural killer (NK) cell.
  - 16.** A method for inducing the expression of a nucleic acid of interest by a cell, the method comprising introducing the synthetic expression cassette of claim 10 into the cell.
  - 17.** The method of claim 16, wherein the nucleic acid of interest encodes a protein that is absent or defective in said cell.
  - 18.** The method of claim 16, wherein the nucleic acid of interest encodes a chimeric antigen receptor (CAR).
  - 19.** The method of claim 16, wherein said cell is a hematopoietic stem cell, a T cell, or a natural killer (NK) cell.
  - 20.** The method of claim 16, wherein said introducing is performed in vivo.
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