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Office de la Propriété Intellectuelle du Canada

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Canadian Intellectual Property Office

CA 3207166 A1 2022/08/04

(21) **3 207 166**

(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2022/02/01
(87) **Date publication PCT/PCT Publication Date:** 2022/08/04
(85) **Entrée phase nationale/National Entry:** 2023/08/01
(86) **N° demande PCT/PCT Application No.:** EP 2022/052372
(87) **N° publication PCT/PCT Publication No.:** 2022/162247
(30) **Priorité/Priority:** 2021/02/01 (EP21154639.5)

(51) **Cl.Int./Int.Cl.** ***C07K 14/47*** (2006.01), ***C12N 15/113*** (2010.01), ***C12N 9/10*** (2006.01), ***C12N 9/22*** (2006.01)

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(54) **Titre : SILENCAGE GENIQUE**
(54) **Title: GENE SILENCING**

(57) **Abrégé/Abstract:**
An engineered transcriptional modulator (ETM) comprising: (a) at least one epigenetic effector domain; operably linked to (b) an endonuclease.

Date Submitted: 2023/08/01

CA App. No.: 3207166

Abstract:

An engineered transcriptional modulator (ETM) comprising: (a) at least one epigenetic effector domain; operably linked to (b) an endonuclease.

GENE SILENCING

FIELD OF THE INVENTION

[0001] The present invention relates to engineered transcriptional modulators (ETM), for example engineered transcriptional repressors (ETRs), for gene editing and epigenetic modification. More specifically, the present invention relates to ETMs (e.g., ETRs) for use in multiplexing methods for modifying the expression of at least two target genes, wherein the expression of a first target gene is modified by gene editing and the expression of second target gene is modified by epigenetic modification, including during gene therapy applications.

BACKGROUND TO THE INVENTION

[0002] Adoptive immunotherapy using engineered T cells has emerged as a powerful approach to treat cancer. These cells can be prepared from the patient's own blood (autologous) or derived from a different donor (allogeneic) and are redirected against cancer cells by ectopic expression of a transgenic T Cell Receptor (TCR) or a Chimeric Antigen Receptor (CAR) recognizing tumour-related antigens. TCRs and CARs may be introduced into *ex vivo* expanded T cells by different means, including lentiviral and retroviral vectors. These vectors, however, tend to integrate semi-randomly in the genome of T cells, posing safety concerns related to transcriptional deregulation of tumour-promoting genes. To avoid this risk, genome editing with artificial nucleases, such as CRISPR/Cas9, has been used to drive insertion of the CAR sequence into the endogenous *TCR* locus (J. Eyquem *et al.,* Nature 2017 Mar 2;543(7643):113-117), an approach that also enhances T-cell potency.

[0003] Genome editing has been further used to improve efficiency and reduce toxicity of T cell therapy *via* the knockout of additional key genes. In this regard, the most common targets are the TCR genes (encoded by *TRAC* and *TRBC*, with the latter present in two copies *in cis* on the same chromosome), the β-2 microglobulin (*B2M*) gene, and the programmed cell death 1 (*PDCD1*, also referred to as *PD1*) gene. Inactivation of *TRAC* and *B2M* is believed to reduce graft-versus-host reactions, whereas inactivation of *PDCD1* is used to desensitize transplanted T cells to immune dampening signals originating from the cancer cells/microenvironment.

[0004] While promising, these multiplexing gene editing approaches (i.e., disruption of multiple genes *per* cell) come with two related issues:

(i) Induction of multiple DNA breaks *per* cell may over-activate cellular DNA damage responses, ultimately leading to apoptosis or poor performance/fitness of the transplanted cells. In this regard, triple editing has been posed as the upper limit for multiplexing, above which significant cell toxicity can be observed.

(ii) Chromosomal translocations may occur between or among multiple DNA breaks (including on- and off-target sites of the nucleases and spontaneous breaks, the latter occurring at a relatively high rate in cultured T cells), further jeopardizing safety of the approach. Clinical and preclinical studies of multiplexing in CAR-T cell products have reported alarming levels of genomic translocations (up to 5%), even when dual-gene editing approaches were used (L. Poirot *et al.,* Cancer Res. 2015 Sep 15;75(18):3853-64; W. Qasim *et al.,* Sci Transl Med; 2017 Jan 25;9(374); E. Stadtaumer *et al.,* Science 2020 Feb 28;367(6481)).

[0005] Targeted epigenetic modification (such as epi-silencing) may represent a safer alternative to gene editing approaches for multiplexing in T cells. Epi-silencing exploits epigenetics, rather than DNA breaks, to inactivate its intended target gene, for example through DNA methylation at CpG sites (A. Amabile *et al.,* Cell. 2016 Sep 22;167(1):219-232).

[0006] Epi-silencing may be achieved by the transient delivery of Engineered Transcriptional Repressors (ETRs), proteins comprising, for example, a catalytically disabled Cas9 (dCas9) or a transcription activator-like effector (TALE) or a Zinc-finger protein (ZFP) fused to epigenetic domains from naturally occurring epigenetic effector proteins (such as KRAB, DNMT3L and DNMT3A). The application of ETRs in silencing individual as well as multi-copy genes in cell lines and in primary T lymphocytes was reported by A. Amabile *supra* and T. Mlambo *et al.,* Nucleic Acids Res. 2018 May 18;46(9):4456-4468. However, the activity of ETRs appears to preferably occur at genes that possess a CpG island (CGI), thus excluding several potentially relevant targets (e.g., *TCR* genes and *PD1* amongst others).

[0007] Accordingly, there remains a need for the development of technologies capable of modifying multiple genes within the same cell. Technologies which reduce the number of multiple DNA breaks per cell, compared to multiplexing gene editing strategies, may be a safer approach and may avoid cellular DNA damage responses and undesired chromosomal translocations.

SUMMARY OF THE INVENTION

[0008] The present invention relates to the development of a combined gene and epigenetic editing strategy to modify multiple genes within the same cell. In particular, it exploits an engineered transcriptional modulator (ETM), for example an engineered transcriptional repressor (ETR), which comprises an epigenetic effector domain operably linked to an endonuclease (such as a catalytically active Cas9) and guide ribonucleic acids (gRNAs) of different lengths to promote permanent epigenetic editing (e.g., silencing) of one or more genes and genetic editing (e.g., inactivation) of another gene.

[0009] This orthogonal approach overcomes the genotoxic risks associated with the use of nuclease-mediated genome editing technologies to inactivate multiple genes per cell. Advantageously, the present invention enables targeting of genes that may be more challenging to achieve with targeted epigenetic modification, enabling targeting of both genes having a CpG island (CGI) and genes which do not have a CGI in one multiplexing strategy.

[0010] Thus, the present invention provides a combined strategy of gene editing coupled to epigenetic modification, such as epigenetic silencing. This combination will:

(i) reduce the burden of genomic translocations compared to multiplexing gene editing methods. The target selected for gene editing will typically lack a CGI. This gene may be also used as a target site for insertion of exogenous expression cassettes encoding, for example, tumour restricted TCRs or CARs introduced with homologous recombination; and

(ii) utilise epigenetics to modify, e.g., silence, one or more CGI-containing genes.

(iii) allow the use of the same construct (an ETM) to achieve silencing in two different modalities, thus reducing the amount of gene editor-encoding RNA that needs to be added to the cell for correct silencing. An advantage of the present invention is to reduce the number of constructs required for multiplex modification, thus improving efficiency and decreasing manufacturing costs.

[0011] Suitably, gene editing may be limited to one gene (which lacks CGI) and at least one gene (such as at least two, or at least three or more genes) comprising a CGI may be modified epigenetically.

[0012] Overall, development of such a combined strategy will result in safer and more efficient T cell products for adoptive immunotherapy of cancer.

[0013] In one aspect, the present invention provides an engineered transcriptional modulator (ETM) comprising: a) at least one epigenetic effector domain; operably linked to b) an endonuclease.

[0014] In certain embodiments, the ETM is an engineered transcriptional repressor (ETR). In some embodiments, the ETM is an engineered transcriptional activator (ETA).

[0015] In some embodiments, the ETM (e.g., ETR) comprises one, two or three epigenetic effector domains. In some embodiments, the ETM (e.g., ETR) comprises one epigenetic effector domain. In some embodiments, the ETM (e.g., ETR) comprises two epigenetic effector domains. In some embodiments, the ETM (e.g., ETR) comprises three epigenetic effector domains.

[0016] In some embodiments, the at least one epigenetic effector domain comprises a Krüppel-associated box (KRAB) domain, a DNA methyltransferase (DNMT) domain, a DNMT-like domain, and/or a histone methyltransferase (HMT) domain. In some embodiments, the epigenetic effector domain is a transcriptional repressor domain (e.g., a Krüppel-associated box (KRAB) domain).

[0017] In some embodiments, the at least one epigenetic effector domain is selected from the group consisting of: DNMT1, DNMT3A, DNMT3B, DNMT3L and SETDB1.

[0018] In some embodiments, the ETM (e.g., ETR) comprises a first epigenetic effector domain comprising a KRAB domain and a second epigenetic effector domain comprising a DNMT domain. In some embodiments, the ETM (e.g., ETR) comprises a first epigenetic effector domain comprising a KRAB domain and a second epigenetic effector domain comprising a DNMT-like domain. In some embodiments, the ETM (e.g., ETR) comprises a first epigenetic effector domain comprising a KRAB domain, a second epigenetic effector domain comprising a DNMT domain, and a third epigenetic effector domain comprising a DNMT-like domain. In certain embodiments, the ETM may comprise as epigenetic effector domains KRAB and DNMT3A; KRAB and DNMT3L; or KRAB, DNMT3A, and

DNMT3L. In some embodiments, the ETM (e.g., ETR) comprises a transcriptional repressor domain (e.g., a Krüppel-associated box (KRAB) domain) and a DNMT3L domain. In some embodiments, the ETM (e.g., ETR) comprises a transcriptional repressor domain (e.g., a Krüppel-associated box (KRAB) domain), a DNMT3A domain and a DNMT3L domain.

[0019] In some embodiments, the endonuclease comprises an RNA binding domain.

[0020] In some embodiments, the endonuclease is a *Clustered Regularly Interspaced Short Palindromic Repeats* (CRISPR)/Cas system.

[0021] In some embodiments, the endonuclease is a Cas endonuclease.

[0022] In certain embodiments, the endonuclease is a Cas9 endonuclease. In certain embodiments, the endonuclease is a SpCas9 endonuclease

[0023] In some embodiments, the ETM (e.g., ETR) comprises or consists of a Cas9-KRAB, Cas9-DNMT3A or Cas9-DNMT3L fusion protein, which can be used together.

[0024] In some embodiments, the ETM (e.g., ETR) is a bi- or tri-partite fusion protein.

[0025] In another aspect, the present invention provides a gRNA which comprises a spacer sequence which comprises or consists of the sequence of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, or 4553-4565 or a homologue or fragment thereof. In another aspect, the present invention provides a gRNA which comprises a spacer sequence which comprises or consists of the sequence of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565 or a homologue or fragment thereof.

[0026] In another aspect, the spacer sequence consists of a fragment of any one of SEQ ID NOs: 23-46, 562-1076 or 2778-4478, such as a 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide fragment of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478 or 4553-4565. In another aspect, the spacer sequence consists of a fragment of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, such as a 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide fragment of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565. The fragment may be a truncation of the sequence from the 5' end.

[0027] In another aspect, the spacer sequence consists of a fragment of any one of SEQ ID NOs: 23-46, 562-1076 or 2778-4478, such as at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 continuous nucleotides of any one of SEQ ID NOs: 23-46, 562-1076 or 2778-4478. In another aspect, the spacer sequence consists of a fragment of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, such as at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 continuous nucleotides of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565.

[0028] In another aspect, the present invention provides a combination (e.g., a system) comprising an ETM (e.g., ETR) according to the present invention, and at least one gRNA. The gRNA(s) may target the ETM (e.g., ETR) to one or more target gene(s). In another aspect, the present invention provides a combination (e.g., a system) comprising an ETM (e.g., ETR) according to the present invention, or polynucleotide(s) encoding therefor, and at least one gRNA, or polynucleotides coding therefor. The combination may comprise one or more ETMs (e.g., ETRs) according to the present invention, such as one, two or three ETMs (e.g., ETRs), or polynucleotides encoding therefor.

[0029] In some embodiments, each ETM is a fusion protein comprising a catalytically active CRISPR/Cas endonuclease domain.

[0030] In another aspect, the present invention provides a combination for modifying transcription, expression and/or activity of one or more (e.g. two or more) gene in a cell, the combination comprising: (A) one or more fusion proteins each comprising a catalytically active CRISPR/Cas endonuclease domain, wherein the one or more fusion proteins collectively comprise a transcriptional repressor domain and a DNMT3L domain, or polynucleotide(s) encoding the one or more fusion proteins; (B) one or more guide RNAs (gRNAs) having a spacer sequence with a length that allows epigenetic editing and not gene editing of a first gene in the cell, wherein the first gene comprises a CpG island (CGI), or polynucleotide(s) coding for the one or more gRNAs; and (C) one or more gRNAs having a spacer sequence with a length that allows gene editing of a second gene in the cell, or polynucleotide(s) coding for the one or more gRNAs.

[0031] In some embodiments, at least one epigenetic effector domain is a transcriptional repressor domain (e.g. a Krüppel-associated box (KRAB) domain), and/or at least one epigenetic effector domain is a DNMT3L domain. In some embodiments, at least one epigenetic effector domain is a transcriptional repressor domain (e.g. a Krüppel-associated box (KRAB) domain), at least one epigenetic effector domain is a DNMT3A domain, and/or at least one epigenetic effector domain is a DNMT3L domain.

[0032] In some embodiments, the one or more ETMs collectively comprise a transcriptional repressor domain (e.g. a Krüppel-associated box (KRAB) domain) and a DNMT3L domain. In some embodiments, the one or more ETMs collectively comprise a transcriptional repressor domain (e.g. a Krüppel-associated box (KRAB) domain), a DNMT3A domain and a DNMT3L domain.

[0033] In some embodiments, the spacer sequence is less than or equal to 16 nucleotides in length. In some embodiments, the spacer sequence is 11 to 16 nucleotides in length, such as 12 to 16, 13 to 16, 14 to 16 or 15 to 16 nucleotides in length.

[0034] In some embodiments, the spacer sequence is 17 or more nucleotides in length, such as 18 or more, 19 or more, or 20 or more nucleotides in length. In some embodiments, the spacer sequence is 17 to 30 nucleotides in length, such as 18 to 30, 19 to 30 or 20 to 30 nucleotides in length. In some embodiments, the spacer sequence is 17 to 25 nucleotides in length, such as 18 to 25, 19 to 25 or 20 to 25 nucleotides in length. In some embodiments, the spacer sequence is 17 to 20 nucleotides in length, such as 18 to 20 or 19 to 20 nucleotides in length.

[0035] In some embodiments, the spacer sequence is less than or equal to 17 nucleotides in length. In some embodiments, the spacer sequence is 11 to 17 nucleotides in length, such as 12 to 17, 13 to 17, 14 to 17, 15 to 17, 16 to 17, 12 to 16, 13 to 16, 14 to 16, or 15 nucleotides in length. In some embodiments, the one or more gRNAs in (B) has a spacer sequence of less than or equal to 17 nucleotides. In some embodiments, the one or more gRNAs in (B) has a spacer sequence of 11 to 17 nucleotides, such as 12 to 17, 13 to 17, 14 to 17, 15 to 17, 16 to 17, 12 to 16, 13 to 16, 14 to 16, or 15 nucleotides.

[0036] In some embodiments, the spacer sequence is 18 or more nucleotides in length, such as 19 or more, or 20 or more nucleotides in length. In some embodiments, the spacer sequence is 18 to 30 nucleotides in length, such as 19 to 30 or 20 to 30 nucleotides in length. In some embodiments, the spacer sequence is 18 to 25 nucleotides in length, such as 19 to 25 or 20 to 25 nucleotides in length. In some embodiments, the spacer sequence is 18 to 21 nucleotides in length, such as 19 to 21 or 20 to 21 nucleotides in length. In some embodiments, the spacer sequence is 18 to 20 nucleotides in length, such as 19 to 20 nucleotides in length. In some embodiments, the one or more gRNAs in (C) has a spacer sequence of 18 or more nucleotides, such as 19 or more, or 20 or more nucleotides. In some embodiments, the one or more gRNAs in (C) has a spacer sequence of 18 to 30 nucleotides, such as 19 to 30 or 20 to 30 nucleotides. In some embodiments, the one or more gRNAs in (C) has a spacer sequence of 18 to 25 nucleotides, such as 19 to 25 or 20 to 25 nucleotides. In some embodiments, the one or more gRNAs in (C) has a spacer sequence of 18 to 21 nucleotides, such as 19 to 21 or 20 to 21 nucleotides. In some embodiments, the one or more gRNAs in (C) has a spacer sequence of 18 to 20 nucleotides, such as 19 to 20 nucleotides.

[0037] In certain embodiments, the combination comprises at least two gRNAs. Suitably, the combination may comprise two gRNAs. Suitably, the combination may comprise three, four, five, six, seven or eight gRNAs.

[0038] The at least two gRNAs may target the ETM (e.g., ETR) to different target genes. For example, a first gRNA may target the ETM (e.g., ETR) to a first target gene and a second gRNA may target the ETM (e.g., ETR) to a second target gene. A third gRNA may, for example, target the ETM (e.g., ETR) to a third target gene. Additional gRNAs may target the ETM (e.g., ETR) to additional target genes.

[0039] In some embodiments, one target gene may be targeted with two or more gRNAs. For example, it may be beneficial to target the same gene with several gRNAs for optimal epigenetic modification e.g., epigenetic silencing. A second target gene may be targeted with another gRNA.

[0040] In particular embodiments, the at least two gRNAs comprise spacer sequences of different lengths.

[0041] In some embodiments, at least one gRNA (e.g., one, two, three or more gRNAs) may have a spacer sequence with a length that allows epigenetic editing of a target gene by the ETM and/or at least one gRNA may have a spacer sequence with a length that allows gene editing of a target gene by the ETM.

[0042] In some embodiments, a first gRNA may have a spacer sequence with a length that allows epigenetic editing of a first target gene by the ETM and a second gRNA may have a spacer sequence with a length that allows gene editing of a second target gene by the ETM.

[0043] In some embodiments, at least one gRNA (e.g., one, two, three or more gRNAs) may have a spacer sequence with a length that allows epigenetic editing and not gene editing of a target gene by the ETM and/or at least one gRNA may have a spacer sequence with a length that allows gene editing of another target gene by the ETM.

[0044] In some embodiments, a first gRNA may have a spacer sequence with a length that allows epigenetic editing and not gene editing of a first target gene by the ETM and a second gRNA may have a spacer sequence with a length that allows gene editing of a second target gene by the ETM.

[0045] Suitably, at least one gRNA(s) may comprise a spacer sequence which is 15, 16, 17, 18, 19 or 20 nucleotides in length.

[0046] Suitably, one of the at least two gRNAs may comprise a spacer sequence which is less than or equal to 17 (e.g., less than or equal to 16) nucleotides in length.

[0047] In some embodiments, the combination comprises:

(a) a first gRNA comprises a spacer sequence which is less than or equal to 16 nucleotides in length, such as less than or equal to 15, less than or equal to 14, less than or equal to 13 or less than or equal to 12 nucleotides in length; and/or

(b) a second gRNA comprises a spacer sequence which is 17 or more nucleotides in length, such as 18 or more, 19 or more, or 20 or more nucleotides in length.

[0048] In some embodiments, the combination comprises:

(a) a first gRNA comprises a spacer sequence which is 11 to 16 nucleotides in length, such as 12 to 16, 13 to 16, 14 to 16 or 15 to 16 nucleotides in length; and/or

(b) a second gRNA comprises a spacer sequence which is 17 to 30 nucleotides in length, such as 18 to 30, 19 to 30, 20 to 30, 17 to 25, 18 to 25, 19 to 25, 20 to 25, 17 to 20, 18 to 20 or 19 to 20 nucleotides in length.

[0049] In some embodiments, the combination comprises:

(a) a first gRNA comprises a spacer sequence which is less than or equal to 17 nucleotides in length, such as less than or equal to 16, less than or equal to 15, less than or equal to 14, less than or equal to 13, less than or equal to 12 nucleotides, or equal to 11 nucleotides in length; and/or

(b) a second gRNA comprises a spacer sequence which is 18 or more nucleotides in length, such as 19 or more, or 20 or more nucleotides in length.

[0050] In some embodiments, the combination comprises:

(a) a first gRNA comprises a spacer sequence which is 11 to 17 nucleotides in length, such as 12 to 17 (e.g., 12 or 16), 13 to 17 (e.g., 13 to 16), 14 to 17 (e.g., 14 to 16), 15 to 17 (e.g., 16), or 17 nucleotides in length; and/or
(b) a second gRNA comprises a spacer sequence which is 18 to 30 nucleotides in length, such as 19 to 30, 20 to 30, 18 to 25, 19 to 25, 20 to 25, 18 to 20, or 19 to 20 nucleotides in length.

[0051] In some embodiments, the one or more guide RNAs (gRNAs) having a spacer sequence with a length that allows epigenetic editing and not gene editing of a first gene in the cell has a spacer sequence of:

(a) less than or equal to 17 nucleotides (e.g., less than or equal to 16 nucleotides), such as less than or equal to 15, less than or equal to 14, less than or equal to 13, less than or equal to 12 nucleotides, or equal to 11 nucleotides; or
(b) 11 to 17 nucleotides (e.g., 11 to 16 nucleotides), such as 12 to 17 (e.g., 12 or 16), 13 to 17 (e.g., 13 to 16), 14 to 17 (e.g., 14 to 16), 15 to 17 (e.g., 16), or 17 nucleotides.

[0052] In some embodiments, the one or more gRNAs having a spacer sequence with a length that allows gene editing of a second gene in the cell has a spacer sequence of:

(a) 17 or more nucleotides (e.g., 18 or more nucleotides), such as 19 or more, or 20 or more nucleotides; or
(b) 17 to 30 nucleotides, such as 18 to 30, 19 to 30, 20 to 30, 18 to 25, 19 to 25, 20 to 25, 18 to 20, or 19 to 20 nucleotides, optionally 18 to 25 nucleotides (e.g., 18 to 21 nucleotides).

[0053] In some embodiments, the at least one target gene is selected from: genes without CpG Islands (CGI), such as: *TRAC*; *TRBC*; *PDCD1*; *TIM-3*; *TIGIT*; *LAG3*; *CTLA4*; *AAVS1* and *CCR5*; and/or genes having CGI, such as: *B2M*; *TET2*; *TGFBR2*; *A2AR*; *CISH*; *PTPN11*; *PTPN6*; *PTPA*; *PTPN2*; *JUNB*; *TOX*; *TOX2*; *NR4A1*; *NR4A2*; *NR4A3*; *MAP4K1*; *REL*; *IRF4*; *DGKA*; *PIK3CD*; *HLA-A*; *USP16*; *DCK*; and *FAS*. For example, the target genes may comprise one or more of *B2M*, *TRAC*, *TET2*, and *TGFBR2*. In some embodiments, the target genes may comprise, e.g., *B2M* and *TRAC*. In some embodiments, the target genes may comprise, e.g., *B2M*, *TRAC*, *TET2*, and *TGFBR2*. In some embodiments, the target genes may comprise a combination of *B2M*, *TET2*, and *TRAC*; a combination of *B2M*, *TET2*, and *TGFBR2*; a combination of *B2M*, *TGFBR2* and *TRAC*; or a combination of *TET2*, *TGFBR2*, and *TRAC*.

[0054] In some embodiments, the first gene is selected from *B2M, TET2, TGFBR2, A2AR, CISH, PTPN11, PTPN6, PTPA, PTPN2, JUNB, TOX, TOX2, NR4A1, NR4A2, NR4A3, MAP4K1, REL, IRF4, DGKA, PIK3CD, HLA-A, USP16, DCK*, and *FAS*; and/or the second gene is selected from *TRAC, TRBC, PDCD1, TIM-3, TIGIT, LAG3, CTLA4, AAVS1*, and *CCR5*.

[0055] In some embodiments, the second gene is a *TRAC* gene, optionally wherein the one or more gRNAs targeting the *TRAC* gene comprise a spacer having the sequence of one of SEQ ID NOs: 562-611, optionally SEQ ID NO: 604.

[0056] In some embodiments, the first gene is a *B2M* gene, optionally wherein the one or more gRNAs targeting the *B2M* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 28-33 and 39-44; or the sequence of one of SEQ ID NOs: 2778-2878 with a 3 to 9 nucleotide truncation at the 5' end, optionally one of SEQ ID NOs: 2778, 2780, 2801, and 2863 with a 3 to 9 nucleotide truncation at the 5' end, selected from SEQ ID NOs: 4486-4492, 4497-4503, 4508-4514, and 4519-4525.

[0057] In some embodiments, the first gene is a *TGFBR2* gene, optionally wherein the one or more gRNAs targeting the *TGFBR2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 2929-2978 and 4553-4559 with a 3 to 9 nucleotide truncation at the 5' end.

[0058] In some embodiments, the first gene is a *TET2* gene, optionally wherein the one or more gRNAs targeting the *TET2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 4429-4478 and 4560-4565 with a 3 to 9 nucleotide truncation at the 5' end.

[0059] In some embodiments, the combination is for modifying transcription, expression and/or activity of one or more (e.g. two or more) gene in a cell, wherein the cell is a mammalian cell, optionally a human cell, optionally wherein the cell is a human immune cell or human T cell.

[0060] In some embodiments, the combination, further comprises a donor DNA comprising 5' and 3' arms that are homologous to sequences in the second gene.

[0061] In some embodiments, the combination further comprises an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination; and/or

iii) which enables selection of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination. In some embodiments, the agent is a CAR or transgenic TCR. In some embodiments, the agent is FIX.

[0062] In another aspect the invention provides a combination for regulating one or more gene in a human cell, optionally an immune cell or a T cell, the combination comprising:

one or more (e.g. one to three) fusion proteins each comprising a catalytically inactive Cas9, optionally SpCas9, endonuclease domain, wherein the one or more (e.g. one to three) fusion proteins collectively comprise a transcriptional repressor and a DNMT3L domain, or polynucleotide(s) encoding the one ore more (e.g. one to three) fusion proteins, wherein the gene comprises a CpG island (CGI) and is

(i) a *B2M* gene and the combination further comprises two or more gRNAs each comprising a spacer having the sequence of one of SEQ ID NOs: 2778-2878

CA 03207166 2023-8-1

optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNAs;

(ii) a *TGFBR2* gene and the combination further comprises a gRNA that comprises a spacer having the sequence of any one of SEQ ID NOs: 2929-2978 and 4553-4559 optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNA; or

(iii) a *TET2* gene and the combination further comprises a gRNA that comprises a spacer having the sequence of any one of SEQ ID NOs: 4429-4478 and 4560-4565 optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNA.

[0063] In some embodiments, the combination comprises at least one gRNA according to the present invention. In some embodiments, the combination comprises one or more gRNAs comprising one or more gRNA sequences shown in Table 8. In some embodiments, the present disclosure provides a combination for regulating a gene comprising one or more gRNAs comprising one or more gRNA sequences shown in Table 8.

[0064] In some embodiments, the gene comprising a CGI is a *B2M* gene and the gRNAs targeting it are two or three gRNAs each independently comprising a spacer having the sequence of: C8 (SEQ ID NO: 35), F4 (SEQ ID NO: 24), H8 (SEQ ID NO: 2780), H10 (SEQ ID NO: 2863), H11 (SEQ ID NO: 2778), or H12 (SEQ ID NO: 2801), optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0065] In some embodiments, the *B2M*-targeting gRNAs comprise a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0066] In some embodiments, the *B2M*-targeting gRNAs comprise a gRNA comprising a spacer having the sequence of C8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the

5' end, and a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0067] In some embodiments, the *B2M*-targeting gRNAs comprise a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0068] In some embodiments, the *B2M*-targeting gRNAs comprise a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0069] In some embodiments, the *B2M*-targeting gRNAs comprise a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0070] In some embodiments, the gene comprising a CGI is a *TGFBR2* gene and the combination comprises one or more gRNAs targeting it, or coding sequences of the one or more gRNAs, the one or more gRNAs each independently comprising a spacer having the sequence of

TG1 (SEQ ID NO: 4553),
TG2 (SEQ ID NO: 4554),
TG3 (SEQ ID NO: 4555),
TG4 (SEQ ID NO: 4556),
TG5 (SEQ ID NO: 4557),
TG6 (SEQ ID NO: 2940),
TG7 (SEQ ID NO: 2937),
TG8 (SEQ ID NO: 2930),
TG9 (SEQ ID NO: 2955),
TG10 (SEQ ID NO: 4558),
TG11 (SEQ ID NO: 2957),
TG12 (SEQ ID NO: 2929),
TG13 (SEQ ID NO: 4559),

TG14 (SEQ ID NO: 2945),

TG15 (SEQ ID NO: 2931),

TG16 (SEQ ID NO: 2942),

TG17 (SEQ ID NO: 2939),

TG18 (SEQ ID NO: 2935),

TG19 (SEQ ID NO: 2938), or

TG20 (SEQ ID NO: 2932),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0071] In some embodiments, the *TGFBR2*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of TG7 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TG8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(ii) a gRNA comprising a spacer having the sequence of TG19 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TG20 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0072] In some embodiments, the gene comprising a CGI is a *TET2* gene and the combination comprises one or more gRNAs targeting it, or coding sequences of the one or more gRNAs, the one or more gRNAs each independently comprising a spacer having the sequence of

TE1 (SEQ ID NO: 4560),

TE2 (SEQ ID NO: 4561),

TE3 (SEQ ID NO: 4562),

TE4 (SEQ ID NO: 4563),

TE5 (SEQ ID NO: 4443),

TE6 (SEQ ID NO: 4434),

TE7 (SEQ ID NO: 4466),

TE8 (SEQ ID NO: 4438),

TE9 (SEQ ID NO: 4429),

TE10 (SEQ ID NO: 4469),

TE11 (SEQ ID NO: 4564),

TE12 (SEQ ID NO: 4449),

TE13 (SEQ ID NO: 4433),

TE14 (SEQ ID NO: 4442),
TE15 (SEQ ID NO: 4430),
TE16 (SEQ ID NO: 4431),
TE17 (SEQ ID NO: 4474),
TE18 (SEQ ID NO: 4432),
TE19 (SEQ ID NO: 4565), or
TE20 (SEQ ID NO: 4478),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0073] In some embodiments, the *TET2*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of TE13 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TE14 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(ii) a gRNA comprising a spacer having the sequence of TE19 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TE20 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

[0074] In some embodiments, the ETM(s) (e.g., one or more fusion proteins) collectively further comprise a DNMT1, DNMT3A, DNMT3B, or SETDB1 domain, optionally DNMT3A.

[0075] In some embodiments, the combination comprises: (i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, and a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, or (ii) a fusion protein comprising, optionally from N-terminus to C-terminus, a transcriptional repressor domain, a Cas endonuclease domain, and a DNMT3L domain.

[0076] In some embodiments, the combination comprises (i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, and a third fusion protein comprising a DNMT3A domain and a Cas endonuclease domain, or (ii) a fusion protein comprising a transcriptional repressor domain, a Cas endonuclease domain, a DNMT3L domain, and a DNMT3A domain.

[0077] In some embodiments, the epigenetic effector domain (e.g. transcriptional repressor domain) is a Krüppel-associated box (KRAB) domain, optionally derived from human Kox1 or ZIM3.

[0078] In some embodiments, the combination comprises a fusion protein comprising, optionally from N terminus to C terminus, a KRAB domain derived from ZIM3, a catalytically active Cas9 domain, and a DNMT3L domain, optionally comprising an amino acid sequence of SEQ ID NO: 4482.

[0079] In some embodiments, the combination further comprises gRNAs for targeting one or more additional genes in the cell, optionally wherein the combination comprises gRNAs targeting the following genes, or comprises polynucleotides coding for the gRNAs: (i) *B2M* and *TRAC*, (ii) *B2M*, *TRAC*, and *TGFBR2*, (iii) *B2M*, *TRAC*, and *TET2*, (iv) *B2M*, *TGFBR2*, and *TET2*, or (v) *B2M*, *TGFBR2*, *TET2*, and *TRAC*.

[0080] In some embodiments, the gRNA(s) are chemically modified, optionally wherein the chemically modified gRNA(s) comprise phosphorothioate internucleoside linkages at the 5' and/or 3' ends, and/or 2'-O-methyl nucleotides.

[0081] In a further aspect, the present invention provides a polynucleotide encoding at least one ETM (e.g., ETR) according to the present invention.

[0082] In another aspect, the present invention provides a nucleic acid construct comprising a nucleic acid sequence encoding at least one ETM (e.g., ETR) according to the present invention.

[0083] In some embodiments, the nucleic acid construct further comprises a nucleic acid sequence:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

iii) which enables selection of a cell, such as a cell which comprises the nucleic acid construct or a cell which does not comprise the construct.

[0084] In one aspect, the present invention provides a vector comprising a polynucleotide according to the present invention or a nucleic acid construct according to the present invention.

[0085] In another aspect, the present invention provides a kit of polynucleotides comprising:

a) at least one polynucleotide encoding at least one ETM (e.g., ETR) according to the present invention; and

b) a polynucleotide providing at least one gRNA disclosed herein; and optionally,

c) a further polynucleotide comprising a nucleic acid sequence which encodes an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises said polynucleotides or a cell which does not comprise said polynucleotides; and/or

iii) which enables selection of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides.

[0086] In another aspect, the present invention provides a cell (such as an engineered cell) comprising an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention. In another aspect, the invention provides a progeny of the cell.

[0087] In another aspect, the invention provides a cell obtained by the use or method of the invention, or a progeny thereof.

[0088] In some embodiments, the cell is a human T cell, optionally engineered to express a recombinant antigen receptor, optionally selected from a recombinant T cell receptor (TCR) or a chimeric antigen receptor (CAR).

[0089] In a further aspect, the present invention provides a composition comprising an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention or a cell according to the present invention.

[0090] In another aspect, the present invention provides a pharmaceutical composition comprising an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention or a cell according to the present invention.

[0091] In a further aspect, the present invention provides the use of an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention or a cell according to the present invention for modifying the transcription, expression and/or activity at least one target gene. The use may, for example, be *in vitro* or *ex vivo* use.

[0092] In another aspect, the present invention provides a method of modifying the transcription, expression and/or activity of at least one target gene in a cell comprising the step of administering an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention to a cell. The cell may be, for example, a T cell.

[0093] In some embodiments, the modifying the transcription, expression and/or activity is repressing transcription, expression and/or activity, e.g., silencing.

[0094] In some embodiments, the method comprises repressing the transcription and/or expression of at least two different target genes in a cell.

[0095] In some embodiments, the method comprises silencing at least two different target genes in a cell.

[0096] Suitably, transcription and/or expression of at least one of the at least two target genes may be epigenetically repressed (e.g., silenced) and at least one of the at least two target genes may be repressed (e.g., silenced) by gene editing, wherein at least one ETM (e.g., ETR) and at least two gRNAs are administered to said cell simultaneously, sequentially, or separately.

[0097] In one aspect, an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention, a cell according to the present invention or a pharmaceutical composition according to the present invention may be for use in therapy.

[0098] In another aspect the invention provides use of an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention, a cell according to the present invention or a pharmaceutical composition according to the present invention in the manufacture of medicament for treating a human in need thereof.

[0099] Suitably, at least one ETM (e.g., ETR) and at least two gRNAs may be administered to a subject simultaneously, sequentially, or separately.

[0100] In another aspect, the present invention provides a method for treating and/or preventing a disease, which comprises the step of administering an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention, a cell according to the present invention or a pharmaceutical composition according to the present invention to a subject in need thereof.

[0101] Suitably, at least one ETM (e.g., ETR) and at least two gRNAs may be administered to a subject simultaneously, sequentially, or separately.

[0102] In one aspect, the present invention provides a method of gene therapy which comprises the steps:

(i) isolation of a cell containing sample;

(ii) introduction of an ETM (e.g. ETR) according to the present invention, at least one gRNA according to the present invention, a polynucleotide according the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention to the cell(s); and

(iii) administering the cell(s) from step (ii) to a subject.

[0103] The polynucleotide, nucleic acid construct or vector may, for example, be introduced by transduction or transfection.

[0104] In some embodiments, the cell is autologous. In some embodiments, the cell is allogeneic.

[0105] It is understood that an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention, a kit of polynucleotides according to the present invention, a cell according to the present invention or a pharmaceutical composition according to the present invention may be used in a method of treatment described herein, may be for use in a treatment described herein, or may be used in the manufacture of a medicament for a treatment described herein.

[0106] Other features, objects, and advantages of the invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments and aspects of the invention, is given by way of illustration only, not limitation. Various changes and modification within the scope of the invention will become apparent to those skilled in the art from the detailed description.

DESCRIPTION OF THE DRAWINGS

[0107] **Figure 1** shows (A) the sequence (SEQ ID NOs: 21 and 22 for the sense and antisense strands, respectively) within the *B2M* gene which may be targeted by Cas9 or dCas9-ETRs and which is targeted in the Examples herein (the protospacer adjacent motif (PAM) sequence is underlined), and (B) the sequences of spacers which may be used in gRNAs to target *B2M* and which are used in the Examples herein (SED ID NOs: 23-34, in order of appearance).

[0108] **Figure 2** shows a histogram illustrating the percentage of mutated *B2M* alleles in cells transfected with Cas9 and the indicated gRNAs. Data are represented as % of non-homologous end-joining (NHEJ) at B2M (n=3; mean±s.d.). UT: untreated.

[0109] **Figure 3** shows a histogram illustrating the percentage of tdTomato-negative cells 44 days upon transfection with the triple combination of dCas9-based ETRs and the indicated gRNAs (n=3; mean±s.d.).

[0110] **Figure 4** shows histograms illustrating the percentage of tdTomato-negative cells 20 days upon transfection with the triple combination of dCas9-based ETRs (left panel) or Cas9 (right panel) and the indicated gRNAs H8, C8, and H10, which were either full length or truncated as indicated. The full-length sequences of H8, C8, and H10 are SEQ ID NOs: 2780, 35, and 2863, respectively. The truncated versions (19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleotide versions) are truncated at the 5' end of the full-length sequence by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides, respectively.

[0111] **Figure 5** shows a histogram illustrating the percentage of mutated *TRAC* alleles in cells transfected with Cas9 and the *TRAC* gRNA. Data are represented as % of NHEJ at *TRAC* (n=3; mean±s.d.).

[0112] **Figure 6** shows a histogram illustrating the percentage of B2M-negative cells ($B2M^{-}$ cells) 25 days after transfection with the indicated Cas9 constructs (i.e., Cas9, dCas9-ETRs, Cas9-ETRs (namely ETM)) and gRNA combinations (n=3; mean±s.d.).

[0113] **Figure 7** shows representative flow cytometry dot plots analyses of the cells treated with the 16 nt *B2M* gRNA and either Cas9-ETRs (namely ETM) or dCas9-ETRs. Analysis was performed at day 25 post-treatment.

[0114] **Figure 8** shows time-course flow cytometric analysis of cells treated as indicated. Data are shown as % of B2M-negative cells normalized to Untreated (UT) cells. Analysis was performed at day 25 post-treatment (n=3; mean±s.d.).

[0115] **Figure 9** shows a histogram illustrating the percentage of gene editing at the *B2M* or *TRAC* gene for the indicated treatment conditions (n=3; mean±s.d.).

[0116] **Figure 10** shows polymerase chain reaction (PCR) analysis of the indicated treatment conditions for reciprocal chromosomal translocations between the *B2M* and the *TRAC* locus. Top: it shows a schematic diagram of the PCR strategy indicating the primers used (arrows) for the analysis. Bottom: it shows a picture of the agarose-stained gel loaded with the PCR products from the indicated treatment conditions (each in triplicate). Translocations were detected only in samples treated with Cas9 or Cas9-ETR (namely ETM) in combination with the 20nt *B2M* gRNA. MW: molecular weight.

[0117] **Figure 11** is a diagram of the *B2M* gene showing the CpG island (CGI) and the distribution of gRNAs H8 (SEQ ID NO: 2780), C8 (SEQ ID NO: 35), F4 (SEQ ID NO: 2878), H10 (SEQ ID NO: 2863), H11 (SEQ ID NO: 2778), and H12 (SEQ ID NO: 2801).

[0118] **Figure 12** shows the percentage of *B2M* silencing by the triple combination of dCas9-based ETRs at days 12 and 25 post-treatment with the indicated gRNAs, either alone (first row of each table) or in combinations (second and third row of each table). Data are shown as heatmap.

[0119] **Figure 13** shows representative flow cytometry analyses of T cells treated with the indicated gRNA combinations (namely C8+F4, C8+H8 or H8+F4) and the triple combination of dCas9-based ETRs at days 12 and 25 post-treatment. The fold increase in terms of efficiency of *B2M* epi-silencing between the C8+F4 and H8+F4 conditions is indicated.

[0120] **Figure 14** shows a time-course flow cytometry analysis of T cells treated with the triple combination of dCas9-based ETRs and the indicated gRNAs combinations. Data are shown as % of B2M-negative cells. UT: untreated T cells. Vertical dashed red lines indicate the days at which T cells were restimulated.

[0121] **Figure 15** shows a histogram illustrating the fold change in the percentage of B2M negative T cells between day 25 and 12 post-treatment, calculated based on the data shown in Figure 14. Data are represented as fold decrease in B2M negative cells.

[0122] **Figure 16** shows a time-course flow cytometry analysis of T cells treated with the triple combination of dCas9-based ETRs and the indicated gRNAs combinations. Data are shown as % of B2M-negative cells. UT: untreated T cells. Vertical dashed red lines indicate the days at which T cells were restimulated.

[0123] **Figure 17** shows a histogram illustrating the fold change in the percentage of B2M negative T cells between day 25 and 12 post-treatment, calculated based on the data shown in Figure 16. Data are represented as fold decrease in B2M negative cells.

[0124] **Figure 18A** shows a time-course flow cytometry analysis of T cells treated with the indicated ETR combinations and the gRNA combination C8+F4. Data are shown as % of B2M-negative cells. UT: untreated T cells. K+3A+3L: standard triple ETR combination; K: KRAB-based ETR alone: 3A+3L: double ETR combination containing DNMT3A and DNMT3L; K+3A: double ETR combination containing KRAB and DNMT3A; K+3L: double ETR combination containing KRAB and DNMT3L; triple Vertical dashed red lines indicate the days at which T cells were restimulated.

[0125] **Figure 18B** shows representative flow cytometry analyses of T cells from **Figure 18A** and treated with the indicated ETR combinations and the gRNA combination C8+F4. K+3A+3L: standard triple ETR combination; K: KRAB-based ETR alone: 3A+3L: double ETR combination containing DNMT3A and DNMT3L; K+3A: double ETR combination containing KRAB and DNMT3A; K+3L: double ETR combination containing KRAB and DNMT3L.

[0126] **Figure 19** shows a time-course flow cytometry analysis of T cells treated with the double ETR combination containing KRAB and DNMT3L, plus the indicated gRNAs combinations. Data are shown as % of B2M-negative cells. UT: untreated T cells. Vertical dashed red lines indicate the days at which T cells were restimulated.

[0127] **Figure 20A** shows on the left a schematic of the ZIM3:dCas9:3L fusion ETR and on the right a time-course flow cytometry analysis of T cells co-treated with ether the double ETR combination containing DNMT3A and DNMT3L or ZIM3:dCas9:3L, plus the indicated gRNAs combinations. Data are shown as % of

B2M-negative cells. UT: untreated T cells. Vertical dashed red lines indicate the days at which T cells were restimulated.

[0128] **Figure 20B** shows representative flow cytometry analyses of T cells from Figure 20A and treated with the indicated ETRs and gRNA combinations. Indicated is also the fold change increase in the efficiency of epi-silencing between sample treated with the double ETR combination and the ETR fusion.

[0129] **Figure 21** shows representative flow cytometry analyses of T cells treated with decreasing doses (in micrograms) of the mRNA encoding for ZIM3:dCas9:3L fusion ETR and the indicated gRNA combination.

[0130] **Figure 22** shows representative flow cytometry analyses of T cells treated or not with Cas9, a gRNA against *TRAC* (see Figure 5) and transduced with an AAV6 for targeted integration into *TRAC* of the NY-ESO engineered TCR. Upper left quadrant shows wild-type, un-edited cells. Bottom left quadrant shows cells with genetically disrupted TCR. Upper right quadrant shows T cells with targeted integration of the NY-ESO TCR.

[0131] **Figure 23** shows on the left a schematic representation of the double ETM combination containing the catalytically active Cas9 and the KRAB and DNMT3L effectors, while, on the right, it shows representative flow cytometry analyses of T cells treated with these ETMs and the indicated truncated gRNA against *B2M*, plus the full-length gRNA against *TRAC* and the AAV6 for targeted integration of the NY-ESO TCR into *TRAC*. The flow cytometry dot plot on the left reports the expression levels of B2M. The flow cytometry dot plot on the middle reports the expression levels of the endogenous TCR and the targeted NY-ESO. The flow cytometry dot plot on the right reports the expression level of NY-ESO and B2M. SSCH: side scatter height.

[0132] **Figure 24** shows on the left a schematic representation of the ETM containing the catalytically active Cas9 and the ZIM3 and DNMT3L effectors (namely ZIM3:Cas9:3L), while, on the right, it shows representative flow cytometry analyses of T cells treated with this ETM and the indicated truncated gRNA against *B2M*, plus the full-length gRNA against *TRAC* and the AAV6 for targeted integration of the NY-ESO TCR into *TRAC*. The flow cytometry dot plot on the top left reports the expression levels of B2M. The flow cytometry dot plot on the top middle reports the expression levels of the endogenous TCR and the targeted NY-ESO. The flow cytometry dot plot on the bottom reports the expression level of NY-ESO and B2M.

The flow cytometry dot plot on the top right shows, within the NY-ESO positive cells, the expression levels of B2M. The flow cytometry dot plot on the bottom right shows, within the endogenous TCR negative cells, the expression levels of B2M.

[0133] **Figure 25** shows a polymerase chain reaction (PCR) analysis of the indicated treatment conditions for reciprocal chromosomal translocations between the *B2M* and *TRAC*. Top: it shows a schematic diagram of the PCR strategy indicating the primers used (arrows) for the analysis. Bottom: it shows a picture of the agarose-stained gel loaded with the PCR products from the indicated treatment conditions. Expected position of the *B2M-TRAC* translocation band is shown by the asterisks. MW: molecular weight. Translocations were detected only in samples treated with the ETM in combination with the 20nt gRNAs for *B2M* and *TRAC*.

[0134] **Figure 26** shows schematics of the *TGFBR2* (top) and *TET2* (bottom) genes, in which are indicated the relative positions of each gRNA and their pairing (P). The CpG Island (CGI) of each gene are also indicated.

[0135] **Figure 27** shows the percentages of *TGFBR2* epi-silencing for the indicated combinations of gRNA pairs. Percentages are reported in the boxes. Unlabeled boxes indicate combinations that were already present in the matrix. np: not performed.

[0136] **Figure 28** shows the percentages of *TET* epi-silencing for the indicated combinations of gRNA pairs. Percentages are reported in the boxes. Unlabeled boxes indicate combinations that were already present in the matrix. Negative data indicate upregulation of TET2. np: not performed.

[0137] **Figure 29** shows histograms illustrating the percentages of epi-silencing of *TGFBR2* (left) and *TET2* (right) in T cells treated with the triple ETR combination and the indicated pairs (P) of gRNAs, either alone or in combination. The pairs used in these studies correspond to those described in **Figures 27** and **28**.

[0138] **Figure 30** shows a histogram illustrating the percentage of epigenetic silencing of the indicated genes as measured by ddPCR.

[0139] **Figure 31** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and on the right a histogram illustrating the percentage of epigenetic silencing of *TGFBR2*.

[0140] **Figure 32** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and

CA 03207166 2023-8-1

on the right a histogram illustrating the percentage of epigenetic silencing of *TGFBR2*.

[0141] **Figure 33** shows polymerase chain reaction (PCR) analyses of the indicated treatment conditions for reciprocal chromosomal translocations among *B2M*, *TGFBR2* and *TRAC*. Top: a schematic diagram of the PCR strategy for two hypothetical genes (X and Y), where arrows indicate the primers used for analysis. Bottom: pictures of the agarose-stained gels loaded with the PCR products from the indicated treatment conditions. Expected positions of translocations bands are indicated by the asterisks. MW: molecular weight. Translocations were detected only in samples treated with the ETM in combination with the 20nt gRNAs for *B2M*, *TGFBR2* and *TRAC*.

[0142] **Figure 34** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and on the right a histogram illustrating the percentage of epigenetic silencing of *TET2*.

[0143] **Figure 35** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and on the right a histogram illustrating the percentage of epigenetic silencing of *TET2*.

[0144] **Figure 36** shows polymerase chain reaction (PCR) analyses of the indicated treatment conditions for reciprocal chromosomal translocations among *B2M*, *TET2* and *TRAC*. Top: a schematic diagram of the PCR strategy for two hypothetical genes (X and Y), where arrows indicate the primers used for analysis. Bottom: pictures of the agarose-stained gels loaded with the PCR products from the indicated treatment conditions. Expected positions of translocations bands are indicated by the asterisks. MW: molecular weight. Translocations were detected only in samples treated with the ETM in combination with the 20nt gRNAs for *B2M*, *TET2* and *TRAC*.

[0145] **Figure 37** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and on the right a histogram illustrating the percentage of epigenetic silencing of *TGFBR2* and *TET2*.

[0146] **Figure 38** shows on the left representative flow cytometry analyses for B2M (left plot) and TRAC (right plot) expression by T cells treated as indicated and on the right a histogram illustrating the percentage of epigenetic silencing of *TGFBR2* and *TET2*.

[0147] **Figure 39** shows polymerase chain reaction (PCR) analyses of the indicated treatment conditions for reciprocal chromosomal translocations among *B2M*, *TGFBR2*, *TET2* and *TRAC*. Top: a schematic diagram of the PCR strategy for two hypothetical genes (X and Y), where arrows indicate the primers used for analysis. Bottom: pictures of the agarose-stained gels loaded with the PCR products from the indicated treatment conditions. Expected positions of translocations bands are indicated by the asterisks. MW: molecular weight. Translocations were detected only in samples treated with the ETM in combination with the 20nt gRNAs for *B2M*, *TGFBR2*, *TET2* and *TRAC*.

DETAILED DESCRIPTION OF THE INVENTION

Engineered transcriptional modulator (ETM)

[0148] In one aspect, the present invention provides an engineered transcriptional modulator (ETM), for example an engineered transcriptional repressor (ETR), comprising: a) at least one epigenetic effector domain; operably linked to b) an endonuclease.

[0149] The ETMs of the invention may be ETRs. ETRs may repress transcription and/or expression of target gene(s).

[0150] The ETMs (e.g., ETRs) of the invention are agents that may enable multiplexing of gene editing and epigenetic editing of different target genes. For example, the ETMs (e.g., ETRs) according to the present invention may enable repression of transcription and/or expression (e.g., silencing) of multiple different target genes, wherein one gene is repressed (e.g., silenced) by genetic editing and at least one gene is repressed (e.g., silenced) by epigenetic repression (e.g., silencing). An advantage of this poly-functional editing system is that there is no reciprocal translocation between the simultaneously edited genes, thus greatly improving the safety of multiplex gene editing. Furthermore, application of such a poly-functional editing approach allows performance of orthogonal edits in one step, without the need for sequential engineering procedures, thus greatly facilitating product manufacturing and reducing associated costs and cell toxicity. The target gene selected for gene editing also may be used as a target site for insertion of exogenous expression cassettes.

[0151] The ETMs may be referred to as programmable multi-editors (ProMEs). For example, the design of gRNAs may allow an ETM to be programmed to modify transcription, expression and/or activity of multiple targets in the same cell. The ETMs (e.g., ETRs) may be chimeric or fusion proteins that are comprised of at least one (such as one) endonuclease operably linked to at least one effector domain (e.g., a KRAB domain, a SETDB1 domain, a DNMT3A, DNMT3B or DNMT1 domain or a DNMT3L domain, or homologues thereof; wherein the domains may be full-length proteins or functional fragments thereof and may be referred to herein as "KRAB," "SETDB1," "DNMT3A," "DNMT3B," "DNMT1," or "DNMT3L," respectively). The endonuclease may enable cleavage of specific DNA sequence(s), and may be chosen or engineered to bind to nucleic acid sequence(s) of choice. The epigenetic effector domain may harbour a catalytic activity which enables modification (such as repression) of transcription of a target gene. Alternatively, or additionally, the effector domain may recruit additional agents within a cell to a target gene, which results in the modification (such as repression) of transcription of the target gene. The present invention also envisages ETMs that are engineered transcription activators (ETAs). ETAs may increase transcription and/or expression of target gene(s).

[0152] By "operably linked", it is to be understood that the individual components are linked together in a manner which enables them to carry out their function (e.g., cleavage of DNA, binding to DNA, catalysing a reaction or recruiting additional agents from within a cell) substantially unhindered. For example, an endonuclease may be conjugated to an epigenetic effector domain, for example to form a fusion protein. Methods for conjugating polypeptides are known in the art, for example through the provision of a linker amino acid sequence connecting the polypeptides (e.g., a linker comprising glycine and/or serine residues). Alternative methods of conjugating polypeptides known in the art include chemical and light-induced conjugation methods (e.g., using chemical cross-linking agents). In an example, the endonuclease and epigenetic effector domain (e.g., KRAB domain, DNMT3A, DNMT3B or DNMT1 domain or DNMT3L domain, or homologue thereof) of the ETM form a fusion protein.

[0153] In one aspect, the ETM (e.g., ETR) comprises an RNA binding domain. The RNA binding domain may bind to a gRNA which is complementary to a genomic target site. Thus, the RNA binding domain may direct the ETM (e.g., ETR) to a target gene.

[0154] In one aspect, the ETM (e.g., ETR) is a fusion protein comprising a) at least one epigenetic effector domain; and b) an endonuclease.

[0155] In some aspects, the ETM (e.g., ETR) is a bi-partite fusion protein. For example, the ETM (e.g., ETR) may comprise two effector domains fused to the same endonuclease.

[0156] In some aspects, the ETM (e.g., ETR) is a tri-partite fusion. For example, the ETM (e.g., ETR) may comprise three effector domains fused to the same endonuclease.

[0157] In some aspects, the ETM (e.g., ETR) may comprise four or five or six or more effector domains fused to the same endonuclease.

[0158] Suitably, where the ETM (e.g., ETR) comprises multiple effector domains, the effector domains may be different. Suitably, where the ETM (e.g., ETR) comprises multiple effector domains, the effector domains may be the same.

[0159] In one aspect, an ETM (e.g., ETR) according to the present invention comprises or consists of a Cas9-KRAB, Cas9-DNMT3A or Cas9-DNMT3L fusion protein.

[0160] Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of endonuclease, KRAB and DNMT3A domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of endonuclease, DNMT3L and DNMT3A domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of endonuclease, DNMT3L and KRAB domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of endonuclease, DNMT3L, KRAB and DNMT3A domains.

[0161] Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of Cas (e.g., Cas9), KRAB, and DNMT3A domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of Cas (e.g., Cas9), DNMT3L and DNMT3A domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of Cas (e.g., Cas9), DNMT3L and KRAB domains. Suitably, an ETM (e.g., ETR) according to the present invention may be a fusion protein comprising or consisting of Cas (e.g., Cas9), DNMT3L, KRAB and DNMT3A domains.

CA 03207166 2023-8-1

[0162] In one aspect, the ETM (e.g., ETR) comprises or consists of an endonuclease-KRAB fusion protein such as a Cas-KRAB, e.g., Cas9-KRAB domain fusion protein.

[0163] An exemplary sequence of an ETM according to the present invention comprising a KRAB domain (**ETM-KRAB**) is set forth below in SEQ ID NO: 18:

M**YPYDVPDYA**SPKKKRKVEAS*DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHS*
IKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEES
FLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRG
HFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQ
LPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLF
LAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQI
HLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRL
SRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI
ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEE
GIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK
DDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVK
KTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK
SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL
QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVIL
ADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
*ATLIHQSITGLYETRIDLSQLGGDS*RADPKKKRKV**GSGGG***ALSPQHSAVTQGSIIKNKEGMD*
AKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVIL
RLEKGEEPWLVEREIHQETHPDSETAFEIKSSV (SEQ ID NO: 18)

[0164] In the above sequence, the Cas9 domain is shown in italics, a haemagglutinin (HA) tag is shown in bold, a linker domain is shown in bold and double-underlined, and the KRAB domain is in italics and underlined. Nuclear localization signal (NLS) sequences are boxed.

[0165] It will be appreciated that alternatives to the HA tag and glycine-serine linker shown in these exemplary ETMs may be used in ETMs according to the present invention, or they may be absent.

[0166] In one aspect, the ETM (e.g., ETR) comprises or consists of an endonuclease-DNMT3A fusion protein such as a Cas-DNMT3A, e.g., a Cas9-DNMT3A domain fusion protein.

[0167] An exemplary sequence of an ETM according to the present invention comprising a DNMT3A domain (**ETM-D3A**) is set forth below in SEQ ID NO: 19:

M**YPYDVPDYA**SPKKKRKVEAS*DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHS*
IKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEES
FLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRG
HFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQ
LPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLF
LAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQI
HLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRL
SRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI
ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEE
GIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK
DDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVK
KTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK
SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL
QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVIL
ADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
*ATLIHQSITGLYETRIDLSQLGGDS*RADPKKKRKV**GSGGG***TYGLLRRREDWPSRLQMFFANN*

HDQEFDPPKVYPPVPAEKRKPIRVLSLFDGIATGLLVLKDLGIQVDRYIASEVCEDSITVGMVRHQGKIMYVGDVRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFENVVAMGVSDKRDISRFLESNPVMIDAKEVSAAHRARYFWGNLPGMNRPLASTVNDKLELQECLEHGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGRSWSVPVIRHLFAPLKEYFACV (SEQ ID NO: 19)

[0168] In the above sequence, the Cas9 domain is shown in italics, an HA tag is shown in bold, a linker domain is shown in bold and double-underlined, and the DNMT3A domain is in italics and underlined. NLS sequences are boxed.

[0169] In one aspect, the ETM (e.g., ETR) comprises or consists of an endonuclease-DNMT3L fusion protein such as a Cas-DNMT3L, e.g., a Cas9-DNMT3L domain fusion protein.

[0170] An exemplary sequence of an ETM according to the present invention comprising a DNMT3L domain (**ETM-D3L**) is set forth below in SEQ ID NO: 20:

M**YPYDVPDYA**SPKKKRKVEAS*DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK*

*SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL
QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVIL
ADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
ATLIHQSITGLYETRIDLSQLGGDS*RADPKKKRKV**GSGGG***MAAIPALDPEAEPSMDVILVGS
SELSSSVSPGTGRDLIAYEVKANQRNIEDICICCGSLQVHTQHPLFEGGICAPCKDKFLDAL
FLYDDDGYQSYCSICCSGETLLICGNPDCTRCYCFECVDSLVGPGTSGKVHAMSNWVCYLCL
PSSRSGLLQRRRKWRSQLKAFYDRESENPLEMFETVPVWRRQPVRVLSLFEDIKKELTSLGF
LESGSDPGQLKHVVDVTDTVRKDVEEWGPFDLVYGATPPLGHTCDRPPSWYLFQFHRLLQYA
RPKPGSPRPFFWMFVDNLVLNKEDLDVASRFLEMEPVTIPDVHGGSLQNAVRVWSNIPAIRS
RHWALVSEEELSLLAQNKQSSKLAAKWPTKLVKNCFLPLREYFKYFSTELTSSL* (SEQ ID NO: 20)

[0171] The Cas9 domain is shown in italics, an HA tag is shown in bold, a linker domain is shown in bold and double-underlined, and the DNMT3L domain is in italics and underlined. NLS sequences are boxed.

[0172] A fusion protein may, for example, comprise an amino acid sequence that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 18, 19, 20, 4481 or 4482, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 18, 19, 20, 4481 or 4482.

[0173] A fusion protein may, for example, be encoded by a polynucleotide comprising a nucleic acid sequence which encodes the protein of SEQ ID NO: 18, 19, 20, 4481 or 4482, or a protein that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid identity to SEQ ID NO: 18, 19, 20, 4481 or 4482, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 18, 19, 20, 4481 or 4482, respectively. The coding sequence may be codon-optimized for optimal expression in human cells.

Epigenetic effector domains

[0174] The term "epigenetic effector domain", is to be understood as referring to the part of the ETM which provides for the epigenetic effect on a target gene, for example by catalysing a reaction on the DNA or chromatin (e.g., methylation of DNA), or by recruiting an additional agent from within a cell, e.g., resulting in the repression of the transcription of a gene.

[0175] "Domain" is to be understood in this context as referring to a part of the ETM that harbours a certain function. The domain may be an individual domain (e.g., a catalytic domain) isolated from a natural protein or it may be an entire, full-length natural protein. Put another way, either the full-length protein or a functional fragment thereof can be used as an effector domain. Therefore, for example, "Krüppel-associated box (KRAB) domain" or "KRAB domain" refers to the part of the ETM that comprises an amino acid sequence with the function of a KRAB domain.

[0176] Chromatin remodeling enzymes that are known to be involved in the permanent epigenetic silencing of endogenous retroviruses (ERVs; Feschotte, C. *et al.* (2012) *Nat. Rev. Genet.* 13: 283-96; Leung, D.C. *et al.* (2012) *Trends Biochem. Sci.* 37: 127-33) may provide suitable effector domains for exploitation in the present invention.

[0177] In one aspect, the epigenetic effector domain is capable of repressing transcription and/or expression of at least one target gene. A factor capable of repressing transcription of a gene is also called a transcriptional repressor. In one aspect, the epigenetic effector domain is a repressor domain, e.g., a transcriptional repressor domain.

[0178] In one aspect, the epigenetic effector domain initiates chemical modification of chromatin and/or chromatin remodeling.

[0179] In one aspect, the epigenetic effector domain initiates DNA modification, such as DNA methylation. In one aspect, the epigenetic effector domain is a DNA methyltransferase and/or is capable of recruiting a DNA methyltransferase.

[0180] In one aspect, the epigenetic effector domain initiates histone modification, such as histone methylation or histone acetylation. In one aspect, the epigenetic effector domain is a histone methyltransferase or histone acetyltransferase.

[0181] In one aspect, the at least one epigenetic effector domain comprises a Krüppel-associated box (KRAB) domain, a DNA methyltransferase (DNMT) domain, a DNMT-like domain, or a histone methyltransferase (HMT) domain.

[0182] In one aspect, the at least one epigenetic effector domain is an antibody or derivative thereof, such as a nanobody, which binds an epigenetic regulator, such as a chromatin regulator which may chemically modify chromatin and/or remodel chromatin.

[0183] See, for example, Van *et al.,* Nat Commun. 2021 Jan 22;12(1)537, which describes nanobody-mediated control of gene expression and epigenetic memory.

KRAB

[0184] In some aspects, the at least one epigenetic effector domain comprises a KRAB domain. The family of the Krüppel-associated box containing zinc finger proteins (KRAB-ZFP; Huntley, S. *et al.* (2006) *Genome Res.* 16: 669-77) plays an important role in the silencing of endogenous retroviruses. These transcription factors bind to specific ERV sequences through their ZFP DNA binding domain, while they recruit the KRAB Associated Protein 1 (KAP1) with their conserved KRAB domain. KAP1 in turn binds a large number of effectors that promote the local formation of repressive chromatin (Iyengar, S. *et al.* (2011) *J. Biol. Chem.* 286: 26267-76).

[0185] An ETM of the present invention may, for example, comprise a KRAB domain. Various KRAB domains are known in the family of KRAB-ZFP proteins. For example, an ETM of the present invention may comprise the KRAB domain of human zinc finger protein 10 (ZNF10; Szulc, J. *et al.* (2006) *Nat. Methods* 3: 109-16):

ALSPQHSAVTQGSIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQI
VYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIK
SSV (SEQ ID NO: 1)

[0186] Further examples of suitable KRAB domains for use in the present invention include:

MNNSQGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQGETTKPDV
ILRLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKESL

(the KRAB domain of the human ZIM3 protein; SEQ ID NO: 2)

ITLEDVAVDFTWEEWQLLGAAQKDLYRDVMLENYSNLVAVGYQASKPDALFKLEQGE
QLWTIEDGIHSGACS

(the KRAB domain of the ZNF350 protein; SEQ ID NO: 3)

VMFEEVSVCFTSEEWACLGPIQRALYWDVMLENYGNVTSLEWETMTENEEVTSKPSS
SQRADSHKGTSKRLQG

(the KRAB domain of the ZNF197 protein; SEQ ID NO: 4)

VSFKDVAVDFTQEEWQQLDPDEKITYRDVMLENYSHLVSVGYDTTKPNVIIKLEQGE
EPWIMGGEFPCQHSP

(the KRAB domain of the RBAK protein; SEQ ID NO: 5)

VKIEDMAVSLILEEWGCQNLARRNLSRDNRQENYGSAFPQGGENRNENEESTSKAET
SEDSASRGETTGRSQKE

(the KRAB domain of the ZKSCAN1 protein; SEQ ID NO: 6)

LTFKDVFVDFTLEEWQQLDSAQKNLYRDVMLENYSHLVSVGYLVAKPDVIFRLGPGE
ESWMADGGTPVRTCA

(the KRAB domain of the KRBOX4 protein; SEQ ID NO: 7)

VTFEDVTLGFTPEEWGLLDLKQKSLYREVMLENYRNLVSVEHQLSKPDVVSQLEEAE
DFWPVERGIPQDTIP

(the KRAB domain of the ZNF274 protein; SEQ ID NO: 8)

[0187] The above KRAB domains are illustrative only. Functional variants thereof are also contemplated herein. For example, the ZIM3 KRAB domain shown in SEQ ID NO: 4481 and 4482 (see Examples 3 and 4 below) may also be used. That ZIM3 KRAB domain has the following sequence:

MGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQGETTKPDVILRL
EQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKESL (SEQ ID NO: 4637)

DNMT

[0188] In some aspects, the epigenetic effector domain comprises a DNA methyltransferase (DNMT) domain. DNMTs catalyse the transfer of a methyl group to DNA. Examples of DNMTs are DNMT1, DNMT3A and DNMT3B.

[0189] An ETM of the present invention may, for example, comprise a domain of human DNA methyltransferase 3A (DNMT3A; Law, J.A. *et al.* (2010) *Nat. Rev. Genet.* 11: 204-20), e.g., the catalytic domain. For example, an ETM of the present invention may comprise the sequence:

TYGLLRRREDWPSRLQMFFANNHDQEFDPPKVYPPVPAEKRKPIRVLSLFDGIATGL
LVLKDLGIQVDRYIASEVCEDSITVGMVRHQGKIMYVGDVRSVTQKHIQEWGPFDLV
IGGSPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFENVVAM
GVSDKRDISRFLESNPVMIDAKEVSAAHRARYFWGNLPGMNRPLASTVNDKLELQEC
LEHGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYT
DVSNMSRLARQRLLGRSWSVPVIRHLFAPLKEYFACV

(the catalytic domain of human DNMT3A; SEQ ID NO: 9)

[0190] DNA methyltransferases 3B and 1 (DNMT3B and DNMT1), similarly to DNMT3A, are also responsible for the deposition and maintenance of DNA methylation, and may also be used in an ETM of the present invention. For example, an ETM of the present invention may comprise any of the sequences:

CHGVLRRRKDWNVRLQAFFTSDTGLEYEAPKLYPAIPAARRRPIRVLSLFDGIATGY
LVLKELGIKVGKYVASEVCEESIAVGTVKHEGNIKYVNDVRNITKKNIEEWGPFDLV
IGGSPCNDLSNVNPARKGLYEGTGRLFFEFYHLLNYSRPKEGDDRPFFWMFENVVAM
KVGDKRDISRFLECNPVMIDAIKVSAAHRARYFWGNLPGMNRPVIASKNDKLELQDC
LEYNRIAKLKKVQTITTKSNSIKQGKNQLFPVVMNGKEDVLWCTELERIFGFPVHYT
DVSNMGRGARQKLLGRSWSVPVIRHLFAPLKDYFACE

(the catalytic domain of human DNMT3B; SEQ ID NO: 10)

MVAELISEEDLEFMKGDTRHLNGEEDAGGREDSILVNGACSDQSSDSPPILEAIRTP
EIRGRRSSSRLSKREVSSLLSYTQDLTGDGDGEDGDGSDTPVMPKLFRETRTRSESP
AVRTRNNNSVSSRERHRPSPRSTRGRQGRNHVDESPVEFPATRSLRRRATASAGTPW
PSPPSSYLTIDLTDDTEDTHGTPQSSSTPYARLAQDSQQGGMESPQVEADSGDGDSS
EYQDGKEFGIGDLVWGKIKGFSWWPAMVVSWKATSKRQAMSGMRWVQWFGDGKFSEV
SADKLVALGLFSQHFNLATFNKLVSYRKAMYHALEKARVRAGKTFPSSPGDSLEDQL
KPMLEWAHGGFKPTGIEGLKPNNTQPENKTRRRTADDSATSDYCPAPKRLKTNCYNN
GKDRGDEDQSREQMASDVANNKSSLEDGCLSCGRKNPVSFHPLFEGGLCQTCRDRFL
ELFYMYDDDGYQSYCTVCCEGRELLLCSNTSCCRCFCVECLEVLVGTGTAAEAKLQE
PWSCYMCLPQRCHGVLRRRKDWNVRLQAFFTSDTGLEYEAPKLYPAIPAARRRPIRV
LSLFDGIATGYLVLKELGIKVGKYVASEVCEESIAVGTVKHEGNIKYVNDVRNITKK
NIEEWGPFDLVIGGSPCNDLSNVNPARKGLYEGTGRLFFEFYHLLNYSRPKEGDDRP
FFWMFENVVAMKVGDKRDISRFLECNPVMIDAIKVSAAHRARYFWGNLPGMNRPVIA
SKNDKLELQDCLEYNRIAKLKKVQTITTKSNSIKQGKNQLFPVVMNGKEDVLWCTEL
ERIFGFPVHYTDVSNMGRGARQKLLGRSWSVPVIRHLFAPLKDYFACE

(human DNMT3B: SEQ ID NO: 11)

LRTLDVFSGCGGLSEGFHQAGISDTLWAIEMWDPAAQAFRLNNPGSTVFTEDCNILL
KLVMAGETTNSRGQRLPQKGDVEMLCGGPPCQGFSGMNRFNSRTYSKFKNSLVVSFL
SYCDYYRPRFFLLENVRNFVSFKRSMVLKLTLRCLVRMGYQCTFGVLQAGQYGVAQT
RRRAIILAAAPGEKLPLFPEPLHVFAPRACQLSVVVDDKKFVSNITRLSSGPFRTIT
VRDTMSDLPEVRNGASALEISYNGEPQSWFQRQLRGAQYQPILRDHICKDMSALVAA
RMRHIPLAPGSDWRDLPNIEVRLSDGTMARKLRYTHHDRKNGRSSSGALRGVCSCVE
AGKACDPAARQFNTLIPWCLPHTGNRHNHWAGLYGRLEWDGFFSTTVTNPEPMGKQG
RVLHPEQHRVVSVRECARSQGFPDTYRLFGNILDKHRQVGNAVPPPLAKAIGLEIKL
CMLAKARESASAKIKEEEAAKD

(the catalytic domain of human DNMT1; SEQ ID NO: 12)

DNMT-like

[0191] In some aspects, the epigenetic effector domain may be a DNMT-like domain. A “DNMT-like” domain refers to a protein, or a functional fragment thereof, wherein the protein is a member of a DNMT family but does not possess DNA methylation activity. The DNMT-like protein typically activates or recruits other epigenetic effector domains.

[0192] An ETM of the present invention may, for example, comprise DNA (cytosine-5)-methyltransferase 3-like (DNMT3L), a catalytically inactive DNA methyltransferase that activates DNMT3A by binding to its catalytic domain. For example, an ETM of the present invention may comprise the sequence:

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MAAIPALDPEAEPSMDVILVGSSELSSSVSPGTGRDLIAYEVKANQRNIEDICICCG
SLQVHTQHPLFEGGICAPCKDKFLDALFLYDDDGYQSYCSICCSGETLLICGNPDCT
RCYCFECVDSLVGPGTSGKVHAMSNWVCYLCLPSSRSGLLQRRRKWRSQLKAFYDRE
SENPLEMFETVPVWRRQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVVDVTDTV
RKDVEEWGPFDLVYGATPPLGHTCDRPPSWYLFQFHRLLQYARPKPGSPRPFFWMFV
DNLVLNKEDLDVASRFLEMEPVTIPDVHGGSLQNAVRVWSNIPAIRSRHWALVSEEE
LSLLAQNKQSSKLAAKWPTKLVKNCFLPLREYFKYFSTELTSSL
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(human DNMT3L; SEQ ID NO: 13)

HMT

[0193] In some aspects, the epigenetic effector domain may be a histone methyltransferase (HMT) domain, e.g., the catalytic domain. HMTs are histone modifying enzymes which catalyse the transfer of methyl groups to lysine and arginine residues of histone proteins.

[0194] Lysine-specific HMTs may contain a SET (Su(var)3-9, Enhancer of Zeste, Trithorax) domain or may be non-SET domain containing.

[0195] An example of an HMT is SET domain bifurcated 1 (SETDB1).

[0196] In early embryonic development, KAP1 is known to recruit SETDB1, a histone methyltransferase that deposits histone H3 lysine-9 di- and tri-methylation (H3K9me2 and H3K9me3, respectively), two histone marks associated with transcriptional repression. Concurrently, KAP1 binds to Heterochromatin Protein 1 alpha (HP1α), which reads H3K9me2 and H3K9me3 and stabilises the KAP1-containing complex. KAP1 can also interact with other well-known epigenetic silencers, such as lysine-specific histone demethylase 1 (LSD1) that inhibits transcription by removing histone H3 lysine-4 methylation, and the nucleosome remodeling and deacetylase complex (NURD), which removes acetyl groups from histones. Finally, the KAP1-containing complex contributes to the recruitment of the de novo DNA methyltransferase 3A (DNMT3A), which methylates cytosines at CpG sites (Jones, P.A. (2012) *Nat. Rev. Genet.* 13: 484-92). Together, these data suggest a model in which, in the pre-implantation embryo, the KAP1 complex ensures ERV silencing through the concerted action of histone modifying enzymes and DNA methylation. Then, after implantation, the DNA methylation previously targeted by KRAB-ZFPs to the ERVs becomes stable (Reik, W. (2007) *Nature* 447: 425-32), being inherited throughout mitosis and somatic cell differentiation without the need for continuous expression of ERVs-specific KRAB-ZFPs. Unlike in embryonic stem cells, the KAP1 complex is not able to efficiently induce DNA methylation in somatic cells, being only able to deposit H3K9 methylation. However, this histone mark is not maintained without continuous deposition at the targeted site by the KRAB-ZFPs (Hathaway, N.A. *et al.* (2012) *Cell* 149: 1447-60).

[0197] In some aspects, at least two epigenetic effector domains may be utilised, one based on, for example, the KRAB domain (e.g., the initiator of the epigenetic cascade occurring at ERVs in embryonic stem cells), and the other based on, for example, DNMT3A (e.g., the final lock of this process). This approach may allow recapitulating on a pre-selected target gene those repressive chromatin states established at ERVs in the pre-implantation embryo and then permanently inherited throughout mammalian development and adult life.

[0198] An ETM of the present invention may, for example, comprise a SETDB1 domain. For example, an ETM of the present invention may comprise any of the sequences:

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MSSLPGCIGLDAATATVESEEIAELQQAVVEELGISMEELRHFIDEELEKMDCVQQR
KKQLAELETWVIQKESEVAHVDQLFDDASRAVTNCESLVKDFYSKLGLQYRDSSSED
ESSRPTEIIEIPDEDDDVLSIDSGDAGSRTPKDQKLREAMAALRKSAQDVQKFMDAV
NKKSSSQDLHKGTLSQMSGELSKDGDLIVSMRILGKKRTKTWHKGTLIAIQTVGPGK
KYKVKFDNKGKSLLSGNHIAYDYHPPADKLYVGSRVVAKYKDGNQVWLYAGIVAETP
NVKNKLRFLIFFDDGYASYVTQSELYPICRPLKKTWEDIEDISCRDFIEEYVTAYPN
RPMVLLKSGQLIKTEWEGTWWKSRVEEVDGSLVRILFLDDKRCEWIYRGSTRLEPMF
SMKTSSASALEKKQGQLRTRPNMGAVRSKGPVVQYTQDLTGTGTQFKPVEPPQPTAP
PAPPFPPAPPLSPQAGDSDLESQLAQSRKQVAKKSTSFRPGSVGSGHSSPTSPALSE
NVSGGKPGINQTYRSPLGSTASAPAPSALPAPPAPPVFHGMLERAPAEPSYRAPMEK
LFYLPHVCSYTCLSRVRPMRNEQYRGKNPLLVPLLYDFRRMTARRRVNRKMGFHVIY
KTPCGLCLRTMQEIERYLFETGCDFLFLEMFCLDPYVLVDRKFQPYKPFYYILDITY
GKEDVPLSCVNEIDTTPPPQVAYSKERIPGKGVFINTGPEFLVGCDCKDGCRDKSKC
ACHQLTIQATACTPGGQINPNSGYQYKRLEECLPTGVYECNKRCKCDPNMCTNRLVQ
HGLQVRLQLFKTQNKGWGIRCLDDIAKGSFVCIYAGKILTDDFADKEGLEMGDEYFA
NLDHIESVENFKEGYESDAPCSSDSSGVDLKDQEDGNSGTEDPEESNDDSSDDNFCK
DEDFSTSSVWRSYATRRQTRGQKENGLSETTSKDSHPPDLGPPHIPVPPSIPVGGCN
PPSSEETPKNKVASWLSCNSVSEGGFADSDSHSSFKTNEGGEGRAGGSRMEAEKAST
SGLGIKDEGDIKQAKKEDTDDRNKMSVVTESSRNYGYNPSPVKPEGLRRPPSKTSMH
QSRRLMASAQSNPDDVLTLSSSTESEGESGTSRKPTAGQTSATAVDSDDIQTISSGS
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EGDDFEDKKNMTGPMKRQVAVKSTRGFALKSTHGIAIKSTNMASVDKGESAPVRKNT
RQFYDGEESCYIIDAKLEGNLGRYLNHSCSPNLFVQNVFVDTHDLRFPWVAFFASKR
IRAGTELTWDYNYEVGSVEGKELLCCCGAIECRGRLL

(human SETDB1; SEQ ID NO: 14)

VGCDCKDGCRDKSKCACHQLTIQATACTPGGQINPNSGYQYKRLEECLPTGVYECNK
RCKCDPNMCTNRLVQHGLQVRLQLFKTQNKGWGIRCLDDIAKGSFVCIYAGKILTDD
FADKEGLEMGDEYFANLDHIESVENFKEGYESDAPCSSDSSGVDLKDQEDGNSGTED
PEESNDDSSDDNFCKDEDFSTSSVWRSYATRRQTRGQKENGLSETTSKDSHPPDLGP
PHIPVPPSIPVGGCNPPSSEETPKNKVASWLSCNSVSEGGFADSDSHSSFKTNEGGE
GRAGGSRMEAEKASTSGLGIKDEGDIKQAKKEDTDDRNKMSVVTESSRNYGYNPSPV
KPEGLRRPPSKTSMHQSRRLMASAQSNPDDVLTLSSSTESEGESGTSRKPTAGQTSA
TAVDSDDIQTISSGSEGDDFEDKKNMTGPMKRQVAVKSTRGFALKSTHGIAIKSTNM
ASVDKGESAPVRKNTRQFYDGEESCYIIDAKLEGNLGRYLNHSCSPNLFVQNVFVDT
HDLRFPWVAFFASKRIRAGTELTWDYNYEVGSVEGKELLCCCGAIECRGRLL

(the catalytic domain of human SETDB1; SEQ ID NO: 15)

[0199] The ETM of the present invention may, for example, comprise an amino acid sequence that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, respectively.

[0200] The ETM of the present invention may, for example, be encoded by a polynucleotide comprising a nucleic acid sequence which encodes the protein of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, or a protein that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, respectively. The coding sequence may be codon-optimized for optimal expression in human cells.

[0201] The ETM of the present invention may, for example, comprise an amino acid sequence that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 4637, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 4637.

[0202] The ETM of the present invention may, for example, be encoded by a polynucleotide comprising a nucleic acid sequence which encodes the protein of

SEQ ID NO: 4637, or a protein that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid identity to SEQ ID NO: 4637, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 4637. The coding sequence may be codon-optimized for optimal expression in human cells.

Endonuclease

[0203] The ETM (e.g., ETR) of the invention may comprise an endonuclease.

[0204] The endonuclease may be, for example, site-specific. As used herein, "site-specific endonuclease" may refer to an enzyme which induces site-directed double-strand breaks in DNA. The site-specific endonuclease enables the activity of the ETM (e.g., ETR) to be targeted to specific sites in a polynucleotide, for example the genome of a cell. For example, the endonuclease may be site-specific when used in combination with gRNAs, in other words, the endonuclease is capable of inducing site-directed DNA breaks when used in combination with gRNAs.

[0205] In one aspect, the endonuclease has exonuclease activity in addition to endonuclease activity.

[0206] The endonuclease may, e.g., bind to binding sites within a target gene or within regulatory sequences for the target gene, for example promoter or enhancer sequences.

[0207] The endonuclease may, e.g., bind to binding sites within splicing sites. Splicing variants of a given gene may be regulated by DNA methylation/ demethylation at splicing sites. In turn, these modifications may cause exon exclusion/inclusion in the mature transcript. This exclusion/inclusion may have therapeutic relevance, such as in the case of Duchenne Muscular Dystrophy, in which exclusion (by genetic ablation or exon skipping) from the mature mRNA of an exon bearing the most frequent disease-causing mutation has been proposed for therapy (Ousterout, D.G. et al. (2015) Mol. Ther. 23: 523-32; Ousterout, D.G. et al. (2015) Nat. Commun. 6: 6244; Kole, R. et al. (2015) Adv. Drug Deliv. Rev. 87: 104-7; Touznik, A. et al. (2014) Expert Opin. Biol. Ther. 14: 809-19).

[0208] A number of suitable endonucleases are known in the art. For example, CRISPR/Cas systems (Sander, J.D. *et al.* (2014) *Nat. Biotechnol.* 32: 347-55) may be employed as suitable endonucleases in the ETMs (e.g., ETRs) of the present invention.

[0209] "CRISPR/Cas system" refers to a clustered regularly interspaced short palindromic repeats/CRISPR associated nuclease system.

[0210] Clustered Regularly Interspaced Short Palindromic Repeats consist of short sequences that originate from viral genomes and have been incorporated into the bacterial genome. CRISPR associated proteins (Cas) process these sequences and cut matching viral DNA sequences. By introducing Cas and specifically constructed CRISPRs into eukaryotic cells, the eukaryotic genome can be cut at any desired position.

[0211] The CRISPR/Cas system is an RNA-guided DNA binding system (van der Oost *et al.* (2014) *Nat. Rev. Microbiol.* 12: 479-92), wherein the guide RNA (gRNA) may be selected to enable an ETM (e.g., ETR) comprising a Cas domain to be targeted to a specific sequence. Thus, to employ the CRISPR/Cas system as an endonuclease in the present invention, it is to be understood that an epigenetic effector domain may be operably linked to a Cas endonuclease such as a Cas9 endonuclease. The ETM (e.g., ETR) comprising the Cas endonuclease may be delivered to a target cell in combination with one or more gRNAs. The gRNAs are designed to target the ETM (e.g., ETR) to a target gene of interest or a regulatory element (e.g., a promoter, enhancer, or splicing site) of the target gene. Methods for the design of gRNAs are known in the art. Furthermore, fully orthogonal Cas9 proteins, as well as Cas9/gRNA ribonucleoprotein complexes and modifications of the gRNA structure/composition to bind different proteins, have been developed to simultaneously and directionally target different effector domains to desired genomic sites of cells (Esvelt *et al.* (2013) *Nat. Methods* 10: 1116-21; Zetsche, B. *et al.* (2015) Cell pii: S0092-8674(15)01200-3; Zalatan, J.G. *et al.* (2015) Cell 160: 339-50; Paix, A. et al. (2015) Genetics 201: 47-54), and are suitable for use in the present invention.

[0212] In one aspect, the ETM (e.g., ETR) comprises at least one endonuclease derived from type II CRISPR bacterial immune systems. In other words, the ETM (e.g., ETR) may comprise a Type II Cas.

[0213] Examples of Cas Type II enzymes include Cas9, Csn2 and Cas4.

[0214] Cas9 endonucleases typically comprise RecI, RecII, bridge helix, RuvC, HNH and PAM interacting domains.

[0215] The HNH and RuvC domains are nuclease domains. The RecI domain binds gRNA. The bridge helix initiates cleavage upon binding of target DNA. The PAM–interacting domain confers PAM specificity and is responsible for initiating binding to target DNA.

[0216] The endonuclease may comprise or consist of a Cas endonuclease. Thus, the endonuclease may have nuclease activity. For example, the endonuclease may be a catalytically active nuclease, bind gRNA, and bind to target DNA.

[0217] The endonuclease comprised in an ETM (e.g., ETR) according to the invention is a catalytically active endonuclease. In other words, the ETM (e.g., ETR) is capable of cleaving a target sequence, such as target DNA.

[0218] In one aspect, the endonuclease is catalytically active Cas nuclease.

[0219] In one aspect, the endonuclease is a modified or a variant endonuclease, such as a modified Cas or modified Cas9 enzyme. For example, it will be appreciated that the enzyme may be modified to recognise a specific PAM site suitable for a target gene. The modified PAM may be different to the PAM naturally recognised by the enzyme.

[0220] In one aspect, the ETM (e.g., ETR) according to the present invention does not comprise only catalytically inactive, or catalytically dead (dCas) nuclease. In one aspect, the ETM (e.g., ETR) according to the present invention does not comprise a catalytically inactive, or catalytically dead (dCas) nuclease, such as dCas9.

[0221] In one aspect, the endonuclease is a catalytically active Cas9 nuclease.

[0222] In one aspect, the endonuclease is a catalytically active Cas9 nuclease from *Streptococcus pyogenes* (SpCas9).

[0223] Methods for determining whether a protein is a catalytically active nuclease are known in the art, for example using gel assays, Kunitz assays, radiolabel assays and fluorescence-based methods. Gel assays may be performed using purified recombinant target DNA as a substrate in an assay buffer. The protein to be tested may be incubated with the substrate, for example incubated at 37°C for 1 hour. The reaction products can be separated by electrophoresis, for example, on an agarose gel with ethidium bromide to visualize the products of the nuclease reaction. Other methods include, for example, fluorescence real-time quantification of DNA and RNA nuclease activity as reported in Sheppard, E.C., *et al. Sci Rep* **9,** 8853 (2019) and cell free detection of Cas nucleases as reported in J. Cox *et al.,* Chem Sci. 2019 Mar 7; 10(9): 2653–2662.

[0224] For example, an ETM (e.g., ETR) of the present invention may comprise the following catalytically active Cas9 sequence:

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET
AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER
HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL
PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQY
ADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL
PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLAR
GNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKK
IECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS
DGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVK
VVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEH
PVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDK
AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDF
QFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVR
KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAY
SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKL
PKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK
QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS

(SEQ ID NO: 16)

[0225] The ETM (e.g., ETR) of the present invention may, for example, comprise an amino acid sequence that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 16, e.g., wherein the amino acid sequence substantially retains the natural function (e.g., endonuclease function) of the protein represented by SEQ ID NO: 16.

[0226] The ETM (e.g., ETR) of the present invention may, for example, be encoded by a polynucleotide comprising a nucleic acid sequence which encodes the protein of SEQ ID NO: 16, or a protein that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid identity to SEQ ID NO: 16, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 16. The coding sequence may be codon-optimized for optimal expression in human cells.

[0227] For comparison, the sequence of a catalytically dead Cas9 (dCas9) is:

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET
AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER
HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL
PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQY
ADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL
PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLAR
GNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKK
IECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS
DGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVK
VVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEH
PVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDK
AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDF
QFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVR
KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAY
SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKL
PKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK
QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS

(catalytically dead Cas9; dCas9; SEQ ID NO: 17)

[0228] The above sequence contains D9A and H839A substitutions relative to its catalytically active (i.e., live) counterpart (SEQ ID NO: 16). A catalytically dead Cas9 (e.g., the above dCas9) may be used in the ETM for epi-editing of one or more target genes, without simultaneous genetic editing of another gene in a cell. For this use, the ETM (e.g., ETR) may, for example, comprise an amino acid sequence that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 16, except for the endonuclease function. The ETM (e.g., ETR) may, for example, be encoded by a polynucleotide comprising a nucleic acid sequence which encodes the protein of SEQ ID NO: 17, or a protein that has at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid identity to SEQ ID NO: 17, e.g., wherein the amino acid sequence substantially retains the natural function of the protein represented by SEQ ID NO: 16 but for the endonuclease function. The coding sequence may be codon-optimized for optimal expression in human cells.

gRNA

[0229] In one aspect, the present invention provides guide RNAs (gRNAs).

[0230] The gRNA targets the ETM (e.g., ETR) to a target gene. The gRNA may, for example, be an RNA sequence which recognises the target DNA region of interest and directs the endonuclease within the ETM (e.g., ETR) to that region.

[0231] A gRNA is typically made up of two parts:

a) a spacer sequence (which may also be referred to as a targeting domain, guide sequence, or complementarity region, and which may constitute a CRISPR RNA (crRNA)); and

b) a scaffold sequence (which may also be referred to as a tracrRNA in a CRISPR/Cas system).

[0232] The spacer and the scaffold sequences may, for example, be provided as separate molecules, or they may be linked, such as via a linker loop or other sequence or may be fused together.

[0233] For example, the gRNA may be constituted by two separate molecules, e.g., the spacer (crRNA) and the scaffold (tracrRNA). The 3' end of the spacer (crRNA) may be complementary to the 5' end of the scaffold (tracrRNA), which complementarity may lead to dimerization of the two molecules.

[0234] In another example, the spacer (crRNA) and the scaffold (tracrRNA) may be fused, for example via a linker loop. This artificial configuration may also be known as a single guide RNA (sgRNA).

[0235] In some aspects, variants of the scaffold (tracrRNA) may be used. For example, the tetraloop and stem loop of the scaffold (tracrRNA) sequence may be modified to include RNA aptamers, which can be bound by specific protein domains. In some aspects, such modified gRNAs can be used to facilitate the recruitment of repressive or activating domains fused to the protein-interacting RNA aptamers.

[0236] Exemplary tracrRNA sequences include, without limitation:

5'-GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUA
GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU-3'
(SEQ ID NO: 4566), and

5′-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU-3′ (SEQ ID NO: 4567)

[0237] A “spacer” or “spacer sequence” refers to a sequence that may be fully complementary to a target domain (i.e., region) within a target sequence.

[0238] The 3’ end of the genomic target sequence generally comprises a proto-spacer adjacent motif (PAM) sequence. A “PAM” sequence is typically a 2 to 6 base pair DNA sequence immediately following the DNA sequence targeted by the nuclease. The PAM sequence is required for cleavage but is not part of the target of the gRNA sequence. The PAM sequence varies depending on the species of the nuclease. For example, the canonical PAM associated with the Cas9 nuclease of *Streptococcus pyogenes* is the sequence 5’-NGG-3’ where “N” is any nucleobase. Nuclease enzymes derived from different organisms or which have been engineered may recognise different PAM sequences.

[0239] For example, the Cas9 of *Francisella novicida* recognizes the canonical PAM sequence 5'-NGG-3', but has been engineered to recognize 5'-YG-3' (where "Y" is a pyrimidine), thus adding to the range of possible Cas9 targets. The Cas12a (or Cpf1) nuclease of *Francisella novicida* recognizes the PAM 5'-TTTN-3' or 5'-YTN-3'.

[0240] The nucleotides upstream (towards the 5’ end of the target sequence) of the PAM sequence is the protospacer sequence.

[0241] A Cas9 nuclease will typically cleave approximately three bases upstream of the PAM.

[0242] It will be appreciated that one may choose a suitable nuclease of a particular context based on PAM specificity and the genomic target.

[0243] A “scaffold” or “scaffold sequence” is a sequence necessary for endonuclease binding e.g., Cas binding.

[0244] In one aspect, the present invention provides single guide RNAs (sgRNAs). In one aspect, the gRNA according to the present invention is a sgRNA. sgRNAs are single RNA molecules which contain a crRNA sequence fused to the scaffold tracrRNA sequence. In nature, crRNAs and tracrRNAs exist as two separate RNA molecules, but sgRNAs have become a common format for CRISPR gRNAs in research.

[0245] In one aspect the gRNA comprises a spacer sequence which is 10 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 11 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 12 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 13 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 14 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 15 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 16 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 17 nucleotides in length. In one aspect the gRNA/ comprises a spacer sequence which is 18 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 19 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 20 nucleotides in length. In one aspect the gRNA comprises a spacer sequence which is 21 nucleotides in length.

[0246] Without wishing to be bound by theory, certain gRNAs (e.g., gRNAs comprising a spacer sequence of around 20 nucleotides in length) may be used to induce gene editing by an ETM (e.g., ETR) whilst gRNAs comprising shorter spacer sequences (e.g., gRNAs comprising spacer sequences of around 16 nucleotides in length) may favour epigenetic editing such as epi-silencing by an ETM (e.g., ETR). See, for example, **Figure 2**, which shows that gRNAs comprising spacer sequences of about 18 to 20 nucleotides in length induce NHEJ whilst gRNAs comprising spacer sequences of about 16 nucleotides in length or less do not induce NHEJ. **Figure 3** shows that gRNAs comprising spacer sequences of about 11 to 16 nucleotides in length are capable of inducing epigenetic modification, e.g., epi-silencing of *B2M*.

[0247] In some embodiments, the gRNA comprises a spacer sequence which is less than or equal to 15, 16, or 17 (e.g., less than or equal to 17 or 16) nucleotides in length. In some embodiments, the gRNA comprises a spacer sequence which is 11 to 16 nucleotides in length, such as 12 to 16, 13 to 16, 14 to 16, 15 to 16, 12 to 17, 13 to 17, 14 to 17, 15 to 17, 16, or 17 nucleotides in length.

[0248] In some embodiments, the gRNA comprises a spacer sequence which is greater than or equal to 16, 17, or 18 (e.g., greater than or equal to 17 or 18) nucleotides in length, such as 18 or more, 19 or more, or 20 or more nucleotides in length. In some embodiments, the gRNA comprises a spacer sequence which is 17 to 30 nucleotides in length, such as 18 to 30, 19 to 30 or 20 to 30 nucleotides in length. In some embodiments, the gRNA comprises a spacer sequence which is 17 to 25 nucleotides in length, such as 18 to 25, 19 to 25 or 20 to 25 nucleotides in length. In some embodiments, the gRNA comprises a spacer sequence which is 17 to 20 nucleotides in length, such as 18 to 20 or 19 to 20 nucleotides in length.

[0249] The ETM according to the present invention may be capable of modifying the transcription, expression and/or activity (e.g., repressing transcription and/or expression) of multiple target genes within the same cell by epigenetic editing and by gene editing.

[0250] The present invention enables the selection of gRNAs which promote either gene editing or epigenetic editing of a target. In this manner, it is possible to choose to perform gene editing on gene targets which are not susceptible to epigenetic editing whilst simultaneously epigenetically targeting genes which are susceptible to epigenetic editing in a multiplexing approach.

[0251] In one aspect, a gRNA is capable of promoting epigenetic editing of a target. Epigenetic editing may be measured using methods known in the art. For example, as described in Example 2, the level of expression of a reporter gene may be measured as a model of epigenetic editing.

[0252] In one aspect, a gRNA is capable of promoting gene editing of a target. Gene editing may be measured using methods known in the art. For example, as described in Example 1, the level of non-homologous end joining may be measured as a model of gene editing.

[0253] An exemplary sequence of a genomic target site (i.e., protospacer and PAM) recognised by gRNAs for use in targeting the β2-microglobulin (B2M) gene includes:

```
5′-AGGGTAGGAGAGACTCACGCTGG-3′   (SEQ ID NO: 21)
   |||||||||||||||||||||||
3′-TCCCATCCTCTCTGAGTGCGACC-5′  (SEQ ID NO: 22)
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The underlined nucleotides are the PAM.

[0254] In one aspect, the present invention provides gRNAs which target the β2-microglobulin gene region set forth in SEQ ID NO: 21 or SEQ ID NO: 22 above.

[0255] Examples of spacer sequences which may be used in gRNAs targeting the β2-microglobulin gene, and in particular the target site above, include:

GAGGGUAGGAGAGACUCACGC -21-nt (SEQ ID NO: 23)

AGGGUAGGAGAGACUCACGC -20-nt (SEQ ID NO: 24) - This spacer sequence may be incorporated in a gRNA and may be used for gene editing of B2M when used in combination with an ETM as shown in Example 2.

GGGUAGGAGAGACUCACGC -19-nt (SEQ ID NO: 25)

GGUAGGAGAGACUCACGC -18-nt (SEQ ID NO: 26)

GUAGGAGAGACUCACGC -17-nt (SEQ ID NO: 27)

UAGGAGAGACUCACGC -16-nt (SEQ ID NO: 28)

AGGAGAGACUCACGC -15-nt (SEQ ID NO: 29)

GGAGAGACUCACGC -14-nt (SEQ ID NO: 30)

GAGAGACUCACGC -13-nt (SEQ ID NO: 31)

AGAGACUCACGC -12-nt (SEQ ID NO: 32)

GAGACUCACGC -11-nt (SEQ ID NO: 33)

AGACUCACGC -10-nt (SEQ ID NO: 34)

[0256] In some aspects, the spacer sequence comprises a “G” nucleotide at the 5’ end. This “G” may, for example, not be part of the targeting sequence and may be necessary when the promoter that drives its expression is a U6 promoter.

[0257] For example, the “G” at the 5’ end of SEQ ID NO: 23 is used herein to drive expression from a U6 promoter. Thus, it will be understood that if the spacer sequence in SEQ ID NO: 23 is not driven by a U6 promoter, the “G” at the 5’ end may not be necessary.

[0258] In some aspects the spacer sequences according to the present invention comprise a “G” nucleotide at the 5’ end.

[0259] Examples of a gRNA according to the present invention are:

AGGGUAGGAGAGACUCACGCGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4479), and

AGGGUAGGAGAGACUCACGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4568), which comprise the spacer sequence SEQ ID NO: 24 (underlined above).

[0260] Alternative gRNAs for epi-silencing of *B2M* may be found, e.g., in Amabile *et al.*, *supra*.

[0261] For example, an alternative spacer sequence which may be used in a gRNA according to the present invention is:

GAGUAGCGCGAGCACAGCUA -20-nt (SEQ ID NO: 35)

[0262] Examples of gRNA according to the present invention is:

GAGUAGCGCGAGCACAGCUAGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4480), and

GAGUAGCGCGAGCACAGCUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4569).

which comprise the spacer sequence SEQ ID NO: 35 (underlined above).

[0263] Truncated spacer sequences based on SEQ ID NO: 35 suitable for use in gRNAs according to the present invention include:

AGUAGCGCGAGCACAGCUA -19-nt (SEQ ID NO: 36)

GUAGCGCGAGCACAGCUA -18-nt (SEQ ID NO: 37)

UAGCGCGAGCACAGCUA -17-nt (SEQ ID NO: 38)

AGCGCGAGCACAGCUA -16-nt (SEQ ID NO: 39)

GCGCGAGCACAGCUA -15-nt (SEQ ID NO: 40)

CGCGAGCACAGCUA -14-nt (SEQ ID NO: 41)

GCGAGCACAGCUA -13-nt (SEQ ID NO: 42)

CGAGCACAGCUA -12-nt (SEQ ID NO: 43)

GAGCACAGCUA -11-nt (SEQ ID NO: 44)

AGCACAGCUA -10-nt (SEQ ID NO: 45)

[0264] Another spacer sequence (H8) which may be used in a gRNA according to the present invention is:

CAUCGGCGCCCUCCGAUCUG -20-nt (SEQ ID NO: 2780)

Examples of gRNAs having this spacer (underlined) are:

CAUCGGCGCCCUCCGAUCUGGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4483), and

CAUCGGCGCCCUCCGAUCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4570).

Truncated spacer sequences based on SEQ ID NO: 2780 suitable for use in gRNAs according to the present invention include:

AUCGGCGCCCUCCGAUCUG -19-nt (SEQ ID NO: 4484)

UCGGCGCCCUCCGAUCUG -18-nt (SEQ ID NO: 4485)

CGGCGCCCUCCGAUCUG -17-nt (SEQ ID NO: 4486)

GGCGCCCUCCGAUCUG -16-nt (SEQ ID NO: 4487)

GCGCCCUCCGAUCUG -15-nt (SEQ ID NO: 4488)

CGCCCUCCGAUCUG -14-nt (SEQ ID NO: 4489)

GCCCUCCGAUCUG -13-nt (SEQ ID NO: 4490)

CCCUCCGAUCUG -12-nt (SEQ ID NO: 4491)

CCUCCGAUCUG -11-nt (SEQ ID NO: 4492)

CUCCGAUCUG -10-nt (SEQ ID NO: 4493)

[0265] Another spacer sequence (H10) which may be used in a gRNA according to the present invention is:

GCGGGCCACCAAGGAGAACU -20-nt (SEQ ID NO: 2863)

Examples of gRNAs having this spacer (underlined) are:

GCGGGCCACCAAGGAGAACUGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4494), and

CA 03207166 2023-8-1

GCGGGCCACCAAGGAGAACUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4571).

Truncated spacer sequences based on SEQ ID NO: 2863 suitable for use in gRNAs according to the present invention include:

CGGGCCACCAAGGAGAACU -19-nt (SEQ ID NO: 4495)

GGGCCACCAAGGAGAACU -18-nt (SEQ ID NO: 4496)

GGCCACCAAGGAGAACU -17-nt (SEQ ID NO: 4497)

GCCACCAAGGAGAACU -16-nt (SEQ ID NO: 4498)

CCACCAAGGAGAACU -15-nt (SEQ ID NO: 4499)

CACCAAGGAGAACU -14-nt (SEQ ID NO: 4500)

ACCAAGGAGAACU -13-nt (SEQ ID NO: 4501)

CCAAGGAGAACU -12-nt (SEQ ID NO: 4502)

CAAGGAGAACU -11-nt (SEQ ID NO: 4503)

AAGGAGAACU -10-nt (SEQ ID NO: 4504)

[0266] Another spacer sequence (H11) which may be used in a gRNA according to the present invention is:

CGAUAAGCGUCAGAGCGCCG -20-nt (SEQ ID NO: 2778)

Examples of gRNAs having this spacer (underlined) are:

CGAUAAGCGUCAGAGCGCCGGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4505), and

CGAUAAGCGUCAGAGCGCCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4572).

Truncated spacer sequences based on SEQ ID NO: 2778 suitable for use in gRNAs according to the present invention include:

GAUAAGCGUCAGAGCGCCG -19-nt (SEQ ID NO: 4506)

AUAAGCGUCAGAGCGCCG -18-nt (SEQ ID NO: 4507)

UAAGCGUCAGAGCGCCG -17-nt (SEQ ID NO: 4508)

AAGCGUCAGAGCGCCG -16-nt (SEQ ID NO: 4509)

AGCGUCAGAGCGCCG -15-nt (SEQ ID NO: 4510)

GCGUCAGAGCGCCG -14-nt (SEQ ID NO: 4511)

CGUCAGAGCGCCG -13-nt (SEQ ID NO: 4512)

GUCAGAGCGCCG -12-nt (SEQ ID NO: 4513)

UCAGAGCGCCG -11-nt (SEQ ID NO: 4514)

CAGAGCGCCG -10-nt (SEQ ID NO: 4515)

[0267] Another spacer sequence (H12) which may be used in a gRNA according to the present invention is:

GAACGCGUGGAGGGGCGCUU -20-nt (SEQ ID NO: 2801)

Examples of gRNAs having this spacer (underlined) are:

GAACGCGUGGAGGGGCGCUUGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4516), and

GAACGCGUGGAGGGGCGCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4573).

Truncated spacer sequences based on SEQ ID NO: 2801 suitable for use in gRNAs according to the present invention include:

AACGCGUGGAGGGGCGCUU -19-nt (SEQ ID NO: 4517)

ACGCGUGGAGGGGCGCUU -18-nt (SEQ ID NO: 4518)

CGCGUGGAGGGGCGCUU -17-nt (SEQ ID NO: 4519)

GCGUGGAGGGGCGCUU -16-nt (SEQ ID NO: 4520)

CGUGGAGGGGCGCUU -15-nt (SEQ ID NO: 4521)

CA 03207166 2023-8-1

GUGGAGGGGCGCUU -14-nt (SEQ ID NO: 4522)

UGGAGGGGCGCUU -13-nt (SEQ ID NO: 4523)

GGAGGGGCGCUU -12-nt (SEQ ID NO: 4524)

GAGGGGCGCUU -11-nt (SEQ ID NO: 4525)

AGGGGCGCUU -10-nt (SEQ ID NO: 4526)

[0268] An example of a spacer sequence for use in a gRNA targeting the *TRAC* gene, includes:

AGAGUCUCUCAGCUGGUACA (SEQ ID NO: 46).

Examples of gRNAs having this spacer (underlined) are:

AGAGUCUCUCAGCUGGUACAGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 4574), and

AGAGUCUCUCAGCUGGUACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 4575).

[0269] The present disclosure also provides variations of the above exemplified gRNAs in which the spacer sequences (those underlined) are truncated by, e.g., 1 to 9 (e.g., 3 to 9) nucleotides at the 5' end. The present disclosure also provides gRNAs in which the spacers (full-length or truncated versions) described herein are linked to the above-exemplified tracr RNA (the portions of the above gRNAs, e.g., SEQ ID NOs: 4574 and 4575, that are not underlined).

[0270] In one aspect, the present invention provides a gRNA which comprises a spacer sequence which comprises or consists of a sequence set forth in any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, or a homologue thereof.

[0271] In one aspect, the present invention provides a gRNA which comprises a spacer sequence wherein the spacer sequence comprises or consists of a sequence set forth in any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565 having one or more (such as two, or three, or four, or five) conservative substitutions. The spacer sequence comprising one or more conservative substitution(s) retains substantially the same activity as the spacer sequence having a sequence set forth in any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565.

[0272] In one aspect, the present invention provides a gRNA which comprises a spacer sequence which comprises or consists of a sequence set forth in any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, or a fragment thereof.

[0273] Suitably, the spacer sequence may comprise or consist of a sequence set forth in any one of SEQ ID NO: 23-46, 562-1076, 2778-4478, and 4553-4565, and is 21 nucleotides in length or less (such as 20 nucleotides, such as 19 nucleotides, such as 18 nucleotides, such as 17 nucleotides, such as 16 nucleotides, such as 15 nucleotides, such as 14 nucleotides, such as 13 nucleotides, such as 12 nucleotides, such as 11 nucleotides, or such as 10 nucleotides).

[0274] In one aspect, the spacer sequence may comprise a sequence set forth in any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, or a fragment thereof that comprises or consists of 21 continuous nucleotides in length or less (such as 20 continuous nucleotides, such as 19 continuous nucleotides, such as 18 continuous nucleotides, such as 17 continuous nucleotides, such as 16 continuous nucleotides, such as 15 continuous nucleotides, such as 14 continuous nucleotides, such as continuous 13 nucleotides, such as 12 continuous nucleotides, such as 11 continuous nucleotides, or such as 10 continuous nucleotides) of SEQ ID NO: 23-46, 562-1076, 2778-4478, and 4553-4565. The fragment may be, e.g., a truncation of SEQ ID NO: 23-46, 562-1076, 2778-4478, and 4553-4565 from the 5' end (i.e., nucleotides at the 5' end are removed).

[0275] In some aspects, gRNA can be chemically modified. For example, chemical modification may increase the stability of the gRNA once administrated in a target cell as described for example in (Yin *et al.,* Nat Biotechnol. 2017 Dec;35(12):1179-1187). Such chemical modifications are known in the literature and can comprise but are not limited to locked nucleic acids (LNA), phosphorothioate modified oligonucleotides, 2'-O-methoxyethyl modified oligonucleotides, and 2' O-methyl modified oligonucleotides.

[0276] In some aspects, the first three nucleosides and the last three nucleosides of a gRNA, regardless of the gRNA's length, are 2'-O-methyl modified nucleosides. In some aspects, the first three internucleoside linkages and the last three internucleoside linkages of a gRNA, regardless of the gRNA's length, are phosphorothioate linkages.

[0277] For gRNA sequences having the tracr RNA of Seq ID No: 4567 (which is 80 nucleotides in length), the tracr sequence portion of the full-length gRNA may be modified as follows (with nucleoside 1 being at the 5' end of the tracr RNA sequence, and nucleoside 80 being at the 3' end of the tracr RNA sequence):

nucleosides 1-8: unmodified RNA nucleosides,

nucleosides 9-20: 2'-O-Me modified nucleosides,

nucleosides 21-48: unmodified RNA nucleosides, and

nucleosides 49-80: 2'-O-Me modified nucleosides.

In such a modified tracr sequence, the internucleoside linkages between nucleosides 77 and 78, 78 and 79, and 79 and 80 (i.e., the last three internucleoside linkages) may be phosphorothioate linkages. A spacer RNA may be attached at the 5' end of this modified tracr sequence to form a full-length gRNA. In this full-length gRNA, the tracr portion of the gRNA sequence is modified as described above, and the spacer portion of the gRNA sequence is modified as follows:

the first three nucleosides of the spacer sequence are 2'-O-Me nucleosides, and

the first three internucleoside linkages are phosphorothioate linkages.

The general schematic for this full-length gRNA is shown below, wherein lowercase letters represent 2'-O-Me nucleosides, capital letters represent unmodified RNA nucleosides, s represents a phosphorothioate linkage, each X independently represents an A, C, G, or U nucleoside, and each x represents a 2'-O-Me A, C, G, or U nucleoside:

5'-xsxsxs[X_7-X_{17}]GUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGU CCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4638)

[0278] More specifically, for gRNA sequences having full-length spacer RNAs (i.e., 20 nucleotides) and the tracr RNA of Seq ID No: 4567 (which is 80 nucleotides in length, for a gRNA of 100 nucleotides in length), the gRNA may be modified as follows (with nucleoside 1 being at the 5' end of the oligonucleotide, and nucleotide 100 being at the 3' end of the oligonucleotide):

nucleosides 1-3: 2'-O-Me modified nucleosides,

nucleosides 4-28: Unmodified RNA nucleosides,

nucleosides 29-40: 2'-O-Me modified nucleosides,

nucleosides 41-68: Unmodified RNA nucleosides, and nucleosides 79-100: 2'-O-Me modified nucleosides.

In such a modified gRNA, the internucleoside linkages between nucleosides 1 and 2, 2 and 3, 3 and 4, 97 and 98, 98 and 99, and 99 and 100 (i.e., the first three internucleoside linkages and the last three internucleoside linkages) may be phosphorothioate linkages. The remainder of the internucleoside linkages are phosphate linkages.

[0279] Similar modifications may be made to truncated gRNAs (e.g., a gRNA with a spacer that is 11 to 19 nucleotides). For example, the first three and the last three internucleoside linkages of the gRNA may be phosphorothioate linkages, and/or some or all of the nucleotides may be chemically modified, e.g., 2'-O-methyl nucleotides.

[0280] For example, the sequence of SEQ ID NO: 4568 can be modified as follows:

5'-asgsgsGUAGGAGAGACUCACGCGUUUUAGAgcuagaaauagcAAGUUAAAAU AAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'

(gRNA ID F4_20)

where:

N: RNA nucleosides; n: 2'-O-methyl nucleosides; s: phosphorothioate backbone modification between two nucleosides.

[0281] Another example is the modification of the sequence of SEQ ID NO: 4569

5'-gsasgsUAGCGCGAGCACAGCUAGUUUUAGAgcuagaaauagcAAGUUAAAAU AAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'

(gRNA ID C8_20)

[0282] Exemplary full-length modified gRNAs targeting *B2M* are shown below:

5'-csasusCGGCGCCCUCCGAUCUGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (modified version of SEQ ID NO: 4570; gRNA ID H8_20)

5'-gscsgsGGCCACCAAGGAGAACUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (modified version of SEQ ID NO: 4571; gRNA ID H10_20)

5'-csgsasUAAGCGUCAGAGCGCCGGUUUUAGAgcuagaaauagcAAGUUAAAA

UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (modified version of SEQ ID NO: 4572; gRNA ID H11_20)

5'-gsasasCGCGUGGAGGGGCGCUUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (modified version of SEQ ID NO: 4573; gRNA ID H12_20)

[0283] Exemplary truncated modified gRNAs targeting *B2M* are shown below:

5'-csascsCAAGGAGAACUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4576; gRNA ID H10_14)

5'-gscsgsCCCUCCGAUCUGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4577; gRNA ID H8_15)

5'-asgscsGCGAGCACAGCUAGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4578; gRNA ID C8_16)

5'-usasgsGAGAGACUCACGCGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4579; gRNA ID F4_16)

[0284] An exemplary full-length modified gRNA targeting *TRAC* is shown below:

5'-asgsasGUCUCUCAGCUGGUACAGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (modified version of SEQ ID NO: 4575)

[0285] Exemplary truncated modified gRNAs targeting *TET2* are shown below:

5'-cscsgsUGCAGUGGCGCGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4580; gRNA ID sgRNA TE13_15)

5'-csgscsCGGCCUUUGUGCGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4581; gRNA ID sgRNA TE14_15)

5'-gscsgsGGGCCGGCGUCUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4582; gRNA ID sgRNA TE19_15)

5'-usgsasAUAUUGAUGCGGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' SEQ ID NO: 4583; gRNA ID sgRNA TE20_15)

[0286] Exemplary truncated modified gRNAs targeting *TGFBR2* are shown below:

5'-uscscsUCGCCAACAGCUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4584; gRNA ID sgRNA TG7_15)

5'-asgsusCACUCGCGCAGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4585; gRNA ID sgRNA TG8_15)

5'-ascsusCCCGUAGCUGCAGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4586; gRNA ID sgRNA TG19_15)

5'-usgsusUGGCCGCGUUCGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4587; gRNA ID sgRNA TG20_15)

[0287] Exemplary full-length modified gRNAs targeting *TET2* are shown below:

5'-gsgsasAUUAGCUCUGUAUCGGUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4588; gRNA ID TE1_20)

5'-asasasGUAAGGGCUCUUACGAGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4589; gRNA ID TE2_20)

5'-gsgscsGUCUCACAGAUUGAAAUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4590; gRNA ID TE3_20)

5'-csgsgsUCAAUUUCCCAGUUUGUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4591; gRNA ID TE4_20)

5'-asgscsGCUCCCCUGUUUCACCGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4592; gRNA ID TE5_20)

5'-csgscsGGGCAACGGGAUCUAAAGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4593; gRNA ID TE6_20)

5'-csgscsAAGCGGAGGUGUGGUGCGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4594; gRNA ID TE7_20)

5'-gsusgsCGGGUACACUCCGGAGGGUUUUAGAgcuagaaauagcAAGUUAAAA

UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4595; gRNA ID TE8_20)

5'-usgscsGCGGGACCUCGAAGUGGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4596; gRNA ID TE9_20)

5'-asgscsAGAGCAAGCGCGAAGGUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4597; gRNA ID TE10_20)

5'-usgscsAGCCCUCGGGAACCCCGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4598; gRNA ID TE11_20)

5'-gsusgsGUGCGCCCGGACCAGCGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4599; gRNA ID TE12_20)

5'-uscsasCGCCGUGCAGUGGCGCGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4600; gRNA ID TE13_20)

5'-gsgsusGCCGCCGGCCUUUGUGCGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4601; gRNA ID TE14_20)

5'-gscsasCCGGGCGUCCAGCACAAGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4602; gRNA ID TE15_20)

5'-asgsgsGAAUUAGCCCCCCGCACGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4603; gRNA ID TE16_20)

5'-asgsusGGCAGCGGCGAGAGCUUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4604; gRNA ID TE17_20)

5'-ascsusUGCAUGCGAGCGGGACCGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4605; gRNA ID TE18_20)

5'-ascsusCAGCGGGGCCGGCGUCUGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4606; gRNA ID TE19_20)

5'-cscsusUAUGAAUAUUGAUGCGGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4607; gRNA ID TE20_20)

[0288] Exemplary full-length modified gRNAs targeting *TGFBR2* are shown below:

5'-ususcsUUUAGGUCGAAGUCUAGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4608; gRNA ID TG1_20)

5'-gsusgsCUCGCGACUCAAUAGAUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4609; gRNA ID TG2_20)

5'-asascsGCAUCUCUAAAGCACCUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4610; gRNA ID TG3_20)

5'-csusgsAUCUACUAGGGAAAACGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4611; gRNA ID TG4_20)

5'-ususgsAGUAAAUACUUGGAGCGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4612; gRNA ID TG5_20)

5'-asgsusCGGCCAAAGCUCUCGGAGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4613; gRNA ID TG6_20)

5'-gsasasACUCCUCGCCAACAGCUGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4614; gRNA ID TG7_20)

5'-gsasgsUGAGUCACUCGCGCGCAGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4615; gRNA ID TG8_20)

5'-csgscsGUGCACCCGCUCGGGACGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4616; gRNA ID TG9_20)

5'-gsgsgsGCCUCCCCGCGCCUCGCGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4617; gRNA ID TG10_20)

5'-usgsgsCGAGCGGGCGCCACAUCGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4618; gRNA ID TG11_20)

5'-uscsgsGUCUAUGACGAGCAGCGGUUUUAGAgcuagaaauagcAAGUUAAAA
UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'
(SEQ ID NO: 4619; gRNA ID TG12_20)

5'-cscsusGAGCAGCCCCCGACCCAGUUUUAGAgcuagaaauagcAAGUUAAAA

UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4620; gRNA ID TG13_20)

5'-gsgsasCGAUGUGCAGCGGCCACGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4621; gRNA ID TG14_20)

5'-usgscsUGGCGAUACGCGUCCACGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4622; gRNA ID TG15_20)

5'-asascsGUGCGGUGGGAUCGUGCGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4623; gRNA ID TG16_20)

5'-gsascsUGUCAAGCGCAGCGGAGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4624; gRNA ID TG17_20)

5'-csususUCCUCGUUUCCGCCCGGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4625; gRNA ID TG18_20)

5'-gscscsCGACUCCCGUAGCUGCAGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4626; gRNA ID TG19_20)

5'-csgsusUGUGUUGGCCGCGUUCGGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4627; gRNA ID TG20_20)

[0289] An exemplary full-length modified gRNA targeting *GFP* is shown below:

5'-csuscsCUCGCCCUUGCUCACCAGUUUUAGAgcuagaaauagcAAGUUAAAA UAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3' (SEQ ID NO: 4628; gRNA ID GFP1)

[0290] In some aspects, the present invention utilizes two or more gRNAs.

[0291] Suitably, the two or more gRNAs may target the ETM (e.g., ETR) to different target genes. Suitably, the two or more gRNAs may comprise spacer sequences of different lengths. For example, the spacer sequences of different lengths may target the endonuclease of the ETM (e.g., ETR) to different target genes.

[0292] In some aspects, a two or more gRNAs may target the same target gene. For example, it may be beneficial to target the same gene with two gRNAs for optimal epigenetic modification e.g., epigenetic silencing.

[0293] In one aspect, at least one of the at least two gRNAs comprises a spacer sequence which is 18, 19 or 20 nucleotides in length.

[0294] In one aspect, at least one of the at least two gRNAs comprises a spacer sequence which is less than or equal to 17 nucleotides in length, such as 16 nucleotides in length, 15 nucleotides in length, such as 14 nucleotides in length, such as less than 13 nucleotides in length, such as 12 nucleotides in length, such as 11 nucleotides in length, or such as 10 nucleotides in length.

Multiplexing – modifying multiple genes in the same cell

[0295] The present invention relates to the development of a combined gene editing and epigenetic editing strategy to modify the expression and/or activity of multiple target genes within the same cell. In particular, it may exploit an ETM (e.g., ETR) which comprises an epigenetic effector domain and an endonuclease and gRNAs comprising spacer sequences of different lengths to promote epigenetic editing of one or more genes and genetic editing of another gene.

[0296] As used herein "modify the expression and/or activity" refers to increasing or decreasing (e.g., decreasing) the expression and/or activity of a target gene.

[0297] In one aspect, transcription and/or expression of a target gene may be repressed.

[0298] In one aspect, a target gene may be silenced.

[0299] In one aspect, a target gene may be enhanced. In other words, the expression of the target gene may be increased. For example, the expression of an endogenous target gene may be increased.

[0300] In another example, an endogenous target (e.g., gene) may be modified (e.g., mutated) by gene editing and the expression of the modified target (e.g., gene) may be increased.

[0301] The effect of an ETM or combination of ETMs may be studied by comparing the transcription or expression of the target gene, for example a gene endogenous to a cell, in the presence and absence of the ETM or combination of ETMs. Methods of analysing transcription or expression of a gene are well known in the art.

[0302] The effect of an ETM or a combination of ETM and gRNAs may also be studied using a model system wherein the expression of a reporter gene, for example a gene encoding a fluorescent protein, is monitored. Suitable methods for monitoring expression of such reporter genes include flow cytometry, fluorescence-activated cell sorting (FACS) and fluorescence microscopy.

[0303] For example, a population of cells may be transfected with a vector which harbours a reporter gene. The vector may be constructed such that the reporter gene is expressed when the vector transfects a cell. Suitable reporter genes include genes encoding fluorescent proteins, for example green, yellow, cherry, cyan or orange fluorescent proteins. In addition, the population of cells may be transfected with vectors encoding the ETMs of interest and/or gRNAs. Subsequently, the number of cells expressing and not-expressing the reporter gene, as well as the level of expression of the reporter gene may be quantified using a suitable technique, such as FACS. The level of reporter gene expression may then be compared in the presence and absence of the ETM and/or gRNAs.

[0304] Methods for determining the transcription of a gene, for example the target of an ETM, are known in the art. Suitable methods include reverse transcription PCR and Northern blot-based approaches. In addition to the methods for determining the transcription of a gene, methods for determining the expression of a gene are known in the art. Suitable additional methods include Western blot-based or flow cytometry approaches.

Target gene transcription and expression

[0305] In some aspects, the product (e.g., ETM and/or gRNA) according to the present invention is used in a method which represses transcription and/or expression of at least one target gene. Suitably, the target gene may be an endogenous gene.

[0306] In one aspect, the target gene transcription and/or expression is repressed by epigenetic editing. In one aspect, the target gene transcription and/or expression is repressed by gene editing.

[0307] In some aspects, the product (e.g., ETM and/or gRNA) according to the present invention is used in a method which represses transcription and/or expression of at least two target genes. Suitably, at least one or both of the target genes may be an endogenous gene.

CA 03207166 2023-8-1

[0308] In one aspect, transcription and/or expression of only one gene is repressed by gene editing.

[0309] Following administration of an ETM (e.g., ETR) of the invention (e.g., with suitable gRNA(s)), the level of transcription or expression of the target gene may be reduced by, for example, at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100% compared to the level of transcription or expression in the absence of the ETM (e.g., ETR).

[0310] In some aspects, the product (e.g., ETM and/or gRNA) according to the present invention is used in a method which silences at least one target gene. Suitably, the target gene may be an endogenous gene. Suitably, the target gene may be an exogenous gene, such as a viral gene.

[0311] In one aspect, the target gene is silenced by epigenetic editing. In one aspect, the target gene is silenced by gene editing.

[0312] In some aspects, the product (e.g., ETM and/or gRNA) according to the present invention is used in a method which silences at least two target genes. Suitably, at least one or both of the target genes may be an endogenous gene.

[0313] In one aspect, only one gene is silenced by gene editing.

[0314] Without wishing to be bound by theory, restricting gene editing activity to one gene may reduce the potential for undesirable genomic translocations.

[0315] By “silencing a target gene”, it is to be understood that the expression of the target gene is reduced to an extent sufficient to achieve a desired effect. The reduced expression may be sufficient to achieve a therapeutically relevant effect, such as the prevention or treatment of a disease. For example, a dysfunctional target gene which gives rise to a disease may be repressed to an extent that there is either no expression of the target gene, or the residual level of expression of the target gene is sufficiently low to ameliorate or prevent the disease state. Furthermore, the reduced expression may allow for purification of the cells harbouring gene silencing.

[0316] The reduced expression may be sufficient to enable investigations to be performed into the gene’s function by studying cells reduced in or lacking that function.

[0317] The repression of the target gene may occur, e.g., following transient delivery or expression of the ETMs (e.g., ETRs) of the present invention to or in a cell (e.g., along with suitable gRNAs).

Enhancing a target gene

[0318] By "enhancing a target gene", it is to be understood that the expression of the target gene is increased to an extent sufficient to achieve a desired effect. The increased expression may be sufficient to achieve a therapeutically relevant effect, such as the prevention or treatment of a disease. For example, a dysfunctional target gene which gives rise to a disease may be enhanced to an extent that there is sufficient expression of the target gene to ameliorate or prevent the disease state. Alternatively, increased expression of the target gene may compensate for the dysfunctional activity of a disease-related gene. Furthermore, increased expression of the target gene may allow for selection of the cells expressing *de novo* that specific target gene.

[0319] Following administration of an ETM of the invention (e.g., with suitable gRNA(s)), the level of transcription or expression of the target gene may be increased by, for example, at least 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100%, 200%, 300%, 400% or 500% compared to the level of transcription or expression in the absence of the ETM.

[0320] The enhancement of the target gene may occur, e.g., following transient delivery or expression of the ETMs of the present invention to or in a cell (along with suitable gRNAs).

Transient expression

[0321] By "transient expression", it is to be understood that the expression of the ETM (e.g., ETR) is not stable over a prolonged period of time. For example, the polynucleotide encoding the ETM (e.g., ETR) may not integrate into the host genome. More specifically, transient expression may be expression which is substantially lost within 20 weeks following introduction of the polynucleotide encoding the ETM (e.g., ETR) into the cell. For example, expression may be substantially lost within 12, 6, 4, or 2 weeks following introduction of the polynucleotide encoding the ETM (e.g., ETR) into the cell.

[0322] Similarly, by "transient delivery", it is to be understood that the ETM (e.g., ETR) substantially does not remain in the cell (i.e., is substantially lost by the cell) over a prolonged period of time. More specifically, transient delivery may result in the ETM (e.g., ETR) being substantially lost by the cell within 20 weeks following introduction of the ETM (e.g., ETR) into the cell. For example, the ETM (e.g., ETR) may be substantially lost within 12, 6, 4, or 2 weeks following introduction of the ETM (e.g., ETR) into the cell.

[0323] In one aspect, the ETM and/or gRNA may be delivered transiently. Transient delivery may result in permanent changes for example; transient delivery of the ETM and/or gRNA may lead to DNA methylation of a repressive regulatory element which in turn may lead to gene activation (e.g., given the stability of this epigenetic modification, permanent gene activation).

[0324] The target gene may, for example, be repressed, silenced, or enhanced permanently. By “permanent repression”, “permanent silencing” or “permanent enhancement” of a target gene, it is to be understood that transcription or expression of the target gene is reduced or increased (e.g., reduced or increased by at least 60%, at least 70%, at least 80%, at least 90% or 100%) compared to the level of transcription or expression in the absence of the ETM (e.g., ETR) for at least 2 months, 6 months, 1 year, 2 year or the entire lifetime of the cell/organism. For example, a permanently repressed, silenced, or enhanced target gene may remain repressed, silenced, or enhanced for the remainder of the cell’s life.

[0325] In one aspect, the ETM and/or gRNA is stably expressed. For example, stable expression may be required to achieve permanent gene activation of some targets. The target gene may, for example, remain repressed, silenced, or enhanced in the progeny of the cell to which the product of the invention has been administered (i.e., the repression, silencing or enhancement of the target gene is inherited by the cell’s progeny). For example, the ETM (e.g., ETR) and gRNAs of the invention may be administered to a stem cell (e.g., a haematopoietic stem cell) to repress or silence a target gene in a stem cell and also in the stem cell’s progeny, which may include cells that have differentiated from the stem cell.

Target gene

[0326] The target gene may, for example, give rise to a therapeutic effect when modified, e.g., repressed or silenced.

[0327] The products, of the present invention may be used to modify, e.g., repress or silence, genes without CpG islands (CGI). Genes without CGI include: *TRAC*; *TRBC*; *PDCD1*; *TIM-3*; *TIGIT*; *LAG3*; *CTLA4*; *AAVS1* and *CCR5*.

[0328] For example, targeting genes, such as genes without a CGI, may:

produce allogenic products (e.g., by targeting *TRAC* and/or *TRABC*);
alter resistance to an immunosuppressive tumour microenvironment (e.g., by targeting of *PDCD1*, *TIM-3*, *TIGIT*, *LAG3* and/or *CTLA4*); and/or
allow CAR/transgenic TCR integration in a safe site (e.g., by targeting of *AAVS1* and/or *CCR5*).

[0329] In one aspect, the present invention provides gRNAs which target a sequence set forth in any one of SEQ ID NOs: 47 to 561.

[0330] By way of example, target genes without CGI islands and exemplary gRNAs suitable for targeting said genes are presented in Table 1 below (SEQ: SEQ ID NO).

Table 1. Target genes without CGI islands and exemplary gRNAs

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
TRAC	GATTAAACCCGGCCACTTTCAGG	47	GAUUAAACCCGGCCACUUUC	562
	CGTCATGAGCAGATTAAACCCGG	48	CGUCAUGAGCAGAUUAAACC	563
	CTCGACCAGCTTGACATCACAGG	49	CUCGACCAGCUUGACAUCAC	564
	AAGTTCCTGTGATGTCAAGCTGG	50	AAGUUCCUGUGAUGUCAAGC	565
	TTCGGAACCCAATCACTGACAGG	51	UUCGGAACCCAAUCACUGAC	566
	TCAGGGTTCTGGATATCTGTGGG	52	UCAGGGUUCUGGAUAUCUGU	567
	GAGAATCAAAATCGGTGAATAGG	53	GAGAAUCAAAAUCGGUGAAU	568
	CTCTCAGCTGGTACACGGCAGGG	54	CUCUCAGCUGGUACACGGCA	569
	TAAACCCGGCCACTTTCAGGAGG	55	UAAACCCGGCCACUUUCAGG	570
	GGTAAGACAGGGGTCTAGCCTGG	56	GGUAAGACAGGGGUCUAGCC	571
	TGGATTTAGAGTCTCTCAGCTGG	57	UGGAUUUAGAGUCUCUCAGC	572
	GCACCAAAGCTGCCCTTACCTGG	58	GCACCAAAGCUGCCCUUACC	573
	GTAAGACAGGGGTCTAGCCTGGG	59	GUAAGACAGGGGUCUAGCCU	574
	ACCCGGCCACTTTCAGGAGGAGG	60	ACCCGGCCACUUUCAGGAGG	575
	TCTCTCAGCTGGTACACGGCAGG	61	UCUCUCAGCUGGUACACGGC	576
	GTCGAGAAAAGCTTTGAAACAGG	62	GUCGAGAAAAGCUUUGAAAC	577
	ACACGGCAGGGTCAGGGTTCTGG	63	ACACGGCAGGGUCAGGGUUC	578
	CTGGATATCTGTGGGACAAGAGG	64	CUGGAUAUCUGUGGGACAAG	579
	CCGAATCCTCCTCCTGAAAGTGG	65	CCGAAUCCUCCUCCUGAAAG	580
	AAAGTCAGATTTGTTGCTCCAGG	66	AAAGUCAGAUUUGUUGCUCC	581
	GCTGGTACACGGCAGGGTCAGGG	67	GCUGGUACACGGCAGGGUCA	582
	TGTGCTAGACATGAGGTCTATGG	68	UGUGCUAGACAUGAGGUCUA	583
	ACAAAACTGTGCTAGACATGAGG	69	ACAAAACUGUGCUAGACAUG	584
	CTGACAGGTTTTGAAAGTTTAGG	70	CUGACAGGUUUUGAAAGUUU	585
	ATCCTCCTCCTGAAAGTGGCCGG	71	AUCCUCCUCCUGAAAGUGGC	586

CA 03207166 2023-8-1

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TAGGCAGACAGACTTGTCACTGG	72	UAGGCAGACAGACUUGUCAC	587
	AGCTTTGAAACAGGTAAGACAGG	73	AGCUUUGAAACAGGUAAGAC	588
	AAGCTGCCCTTACCTGGGCTGGG	74	AAGCUGCCCUUACCUGGGCU	589
	TTCAAAACCTGTCAGTGATTGGG	75	UUCAAAACCUGUCAGUGAUU	590
	TCAAGGCCCCTCACCTCAGCTGG	76	UCAAGGCCCCUCACCUCAGC	591
	GCTTTGAAACAGGTAAGACAGGG	77	GCUUUGAAACAGGUAAGACA	592
	GTCAGGGTTCTGGATATCTGTGG	78	GUCAGGGUUCUGGAUAUCUG	593
	CTTCAAGAGCAACAGTGCTGTGG	79	CUUCAAGAGCAACAGUGCUG	594
	AAAGCTGCCCTTACCTGGGCTGG	80	AAAGCUGCCCUUACCUGGGC	595
	ATCTGTGGGACAAGAGGATCAGG	81	AUCUGUGGGACAAGAGGAUC	596
	TTAATCTGCTCATGACGCTGCGG	82	UUAAUCUGCUCAUGACGCUG	597
	AGCCCAGGTAAGGGCAGCTTTGG	83	AGCCCAGGUAAGGGCAGCUU	598
	CTGCGGCTGTGGTCCAGCTGAGG	84	CUGCGGCUGUGGUCCAGCUG	599
	TGCTCATGACGCTGCGGCTGTGG	85	UGCUCAUGACGCUGCGGCUG	600
	CATCACAGGAACTTTCTAAAAGG	86	CAUCACAGGAACUUUCUAAA	601
	TCTGTGGGACAAGAGGATCAGGG	87	UCUGUGGGACAAGAGGAUCA	602
	TTCGTATCTGTAAAACCAAGAGG	88	UUCGUAUCUGUAAAACCAAG	603
	AGAGTCTCTCAGCTGGTACACGG	89	AGAGUCUCUCAGCUGGUACA	604
	AGGTGAGGGGCCTTGAAGCTGGG	90	AGGUGAGGGGCCUUGAAGCU	605
	TTCTTCCCCAGCCCAGGTAAGGG	91	UUCUUCCCCAGCCCAGGUAA	606
	CACCAAAGCTGCCCTTACCTGGG	92	CACCAAAGCUGCCCUUACCU	607
	GAGGTGAGGGGCCTTGAAGCTGG	93	GAGGUGAGGGGCCUUGAAGC	608
	TCCTCCTCCTGAAAGTGGCCGGG	94	UCCUCCUCCUGAAAGUGGCC	609
	AGCTGGTACACGGCAGGGTCAGG	95	AGCUGGUACACGGCAGGGUC	610
	AACAAATGTGTCACAAAGTAAGG	96	AACAAAUGUGUCACAAAGUA	611
TRBC1 (ENST00000 633705)	AGGAAGGGCTTACTTACCCGAGG	97	AGGAAGGGCUUACUUACCCG	612
	TCAAACACAGCGACCTCGGGTGG	98	UCAAACACAGCGACCUCGGG	613
	CGGGTGGGAACACCTTGTTCAGG	99	CGGGUGGGAACACCUUGUUC	614
	GTAGGACACTGTTGGCACGGAGG	100	GUAGGACACUGUUGGCACGG	615
	CACCCAGATCGTCAGCGCCGAGG	101	CACCCAGAUCGUCAGCGCCG	616
	ATCGTCAGCGCCGAGGCCTGGGG	102	AUCGUCAGCGCCGAGGCCUG	617
	AGTCCAGTTCTACGGGCTCTCGG	103	AGUCCAGUUCUACGGGCUCU	618
	GACGGGTTTGGCCCTATCCTGGG	104	GACGGGUUUGGCCCUAUCCU	619
	TGACGGGTTTGGCCCTATCCTGG	105	UGACGGGUUUGGCCCUAUCC	620
	GAACAAGGTGTTCCCACCCGAGG	106	GAACAAGGUGUUCCCACCCG	621
	TCTCCGAGAGCCCGTAGAACTGG	107	UCUCCGAGAGCCCGUAGAAC	622
	GGCTCTCGGAGAATGACGAGTGG	108	GGCUCUCGGAGAAUGACGAG	623
	AGACAGGACCCCTTGCTGGTAGG	109	AGACAGGACCCCUUGCUGGU	624
	GGCGCTGACGATCTGGGTGACGG	110	GGCGCUGACGAUCUGGGUGA	625
	CAAACACAGCGACCTCGGGTGGG	111	CAAACACAGCGACCUCGGGU	626
	TGACAGCGGAAGTGGTTGCGGGG	112	UGACAGCGGAAGUGGUUGCG	627
	TGACGAGTGGACCCAGGATAGGG	113	UGACGAGUGGACCCAGGAUA	628
	CGCCCTTGTGTTGATGGCCATGG	114	CGCCCUUGUGUUGAUGGCCA	629
	ATGACGAGTGGACCCAGGATAGG	115	AUGACGAGUGGACCCAGGAU	630
	CTTTCCAGAGGACCTGAACAAGG	116	CUUUCCAGAGGACCUGAACA	631
	GGCCTCGGCGCTGACGATCTGGG	117	GGCCUCGGCGCUGACGAUCU	632
	CGCTGTCAAGTCCAGTTCTACGG	118	CGCUGUCAAGUCCAGUUCUA	633
	GGTCAGCGCCCTTGTGTTGATGG	119	GGUCAGCGCCCUUGUGUUGA	634

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AACACCTTGTTCAGGTCCTCTGG	120	AACACCUUGUUCAGGUCCUC	635
	TTGACAGCGGAAGTGGTTGCGGG	121	UUGACAGCGGAAGUGGUUGC	636
	GACGATCTGGGTGACGGGTTTGG	122	GACGAUCUGGGUGACGGGUU	637
	GACCAGCACAGCATACAGGGTGG	123	GACCAGCACAGCAUACAGGG	638
	TCCCTAGCAGGATCTCATAGAGG	124	UCCCUAGCAGGAUCUCAUAG	639
	TGTTGATGGCCATGGTAAGCAGG	125	UGUUGAUGGCCAUGGUAAGC	640
	CTGGTAGGACACTGTTGGCACGG	126	CUGGUAGGACACUGUUGGCA	641
	AGGCCTCGGCGCTGACGATCTGG	127	AGGCCUCGGCGCUGACGAUC	642
	CGTAGAACTGGACTTGACAGCGG	128	CGUAGAACUGGACUUGACAG	643
	CCAACAGTGTCCTACCAGCAAGG	129	CCAACAGUGUCCUACCAGCA	644
	TGAGGGTCTCGGCCACCTTCTGG	130	UGAGGGUCUCGGCCACCUUC	645
	GTATCTGGAGTCATTGAGGGCGG	131	GUAUCUGGAGUCAUUGAGGG	646
	TATCTGGAGTCATTGAGGGCGGG	132	UAUCUGGAGUCAUUGAGGGC	647
	GGCTCAAACACAGCGACCTCGGG	133	GGCUCAAACACAGCGACCUC	648
	GGCCACCCTGTATGCTGTGCTGG	134	GGCCACCCUGUAUGCUGUGC	649
	GCGGCTGCTCAGGCAGTATCTGG	135	GCGGCUGCUCAGGCAGUAUC	650
	TAGCAGGATCTCATAGAGGATGG	136	UAGCAGGAUCUCAUAGAGGA	651
	CTTGTTCAGGTCCTCTGGAAAGG	137	CUUGUUCAGGUCCUCUGGAA	652
	GTTGCGGGGGTTCTGCCAGAAGG	138	GUUGCGGGGGUUCUGCCAGA	653
	TCAGACTGTGGCTTTACCTCGGG	139	UCAGACUGUGGCUUUACCUC	654
	CTTGACAGCGGAAGTGGTTGCGG	140	CUUGACAGCGGAAGUGGUUG	655
	GCTGTCAAGTCCAGTTCTACGGG	141	GCUGUCAAGUCCAGUUCUAC	656
	CAGCTCAGCTCCACGTGGTCAGG	142	CAGCUCAGCUCCACGUGGUC	657
	CAACAGTGTCCTACCAGCAAGGG	143	CAACAGUGUCCUACCAGCAA	658
	AGATCGTCAGCGCCGAGGCCTGG	144	AGAUCGUCAGCGCCGAGGCC	659
	GATCGTCAGCGCCGAGGCCTGGG	145	GAUCGUCAGCGCCGAGGCCU	660
	AACAGTGTCCTACCAGCAAGGGG	146	AACAGUGUCCUACCAGCAAG	661
TRBC2 (ENST00000 466254)	GACCAGCACGGCATACAAGGTGG	147	GACCAGCACGGCAUACAAGG	662
	CACCCAGATCGTCAGCGCCGAGG	148	CACCCAGAUCGUCAGCGCCG	663
	ATCGTCAGCGCCGAGGCCTGGGG	149	AUCGUCAGCGCCGAGGCCUG	664
	AGTCCAGTTCTACGGGCTCTCGG	150	AGUCCAGUUCUACGGGCUCU	665
	ACTGACCAGCACGGCATACAAGG	151	ACUGACCAGCACGGCAUACA	666
	AGGAGAGACTCACTTACCGGAGG	152	AGGAGAGACUCACUUACCGG	667
	TCTCCGAGAGCCCGTAGAACTGG	153	UCUCCGAGAGCCCGUAGAAC	668
	GGCTCTCGGAGAATGACGAGTGG	154	GGCUCUCGGAGAAUGACGAG	669
	GGCCACCTTGTATGCCGTGCTGG	155	GGCCACCUUGUAUGCCGUGC	670
	TACCATGGCCATCAGCACGAGGG	156	UACCAUGGCCAUCAGCACGA	671
	TCAACAGAGTCTTACCAGCAAGG	157	UCAACAGAGUCUUACCAGCA	672
	TGACAGCGGAAGTGGTTGCGGGG	158	UGACAGCGGAAGUGGUUGCG	673
	CTATGAGATCTTGCTAGGGAAGG	159	CUAUGAGAUCUUGCUAGGGA	674
	TGACGAGTGGACCCAGGATAGGG	160	UGACGAGUGGACCCAGGAUA	675
	ATGACGAGTGGACCCAGGATAGG	161	AUGACGAGUGGACCCAGGAU	676
	GACAGGTTTGGCCCTATCCTGGG	162	GACAGGUUUGGCCCUAUCCU	677
	TGACAGGTTTGGCCCTATCCTGG	163	UGACAGGUUUGGCCCUAUCC	678
	GGCCTCGGCGCTGACGATCTGGG	164	GGCCUCGGCGCUGACGAUCU	679
	CGCTGTCAAGTCCAGTTCTACGG	165	CGCUGUCAAGUCCAGUUCUA	680
	GGCTCAAACACAGCGACCTTGGG	166	GGCUCAAACACAGCGACCUU	681
	TTGACAGCGGAAGTGGTTGCGGG	167	UUGACAGCGGAAGUGGUUGC	682

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TGGGTGGGAACACGTTTTTCAGG	168	UGGGUGGGAACACGUUUUUC	683
	TTACCATGGCCATCAGCACGAGG	169	UUACCAUGGCCAUCAGCACG	684
	CAAACACAGCGACCTTGGGTGGG	170	CAAACACAGCGACCUUGGGU	685
	TCAAACACAGCGACCTTGGGTGG	171	UCAAACACAGCGACCUUGGG	686
	ATGGTTTTGGAGCTAGCCTCTGG	172	AUGGUUUUGGAGCUAGCCUC	687
	CAACAGAGTCTTACCAGCAAGGG	173	CAACAGAGUCUUACCAGCAA	688
	AGGCCTCGGCGCTGACGATCTGG	174	AGGCCUCGGCGCUGACGAUC	689
	CGTAGAACTGGACTTGACAGCGG	175	CGUAGAACUGGACUUGACAG	690
	CACGAGGGCACTGACCAGCACGG	176	CACGAGGGCACUGACCAGCA	691
	TCGTGCTGATGGCCATGGTAAGG	177	UCGUGCUGAUGGCCAUGGUA	692
	AACAGAGTCTTACCAGCAAGGGG	178	AACAGAGUCUUACCAGCAAG	693
	TGAGGGTCTCGGCCACCTTCTGG	179	UGAGGGUCUCGGCCACCUUC	694
	TCCCTAGCAAGATCTCATAGAGG	180	UCCCUAGCAAGAUCUCAUAG	695
	GTATCTGGAGTCATTGAGGGCGG	181	GUAUCUGGAGUCAUUGAGGG	696
	TATCTGGAGTCATTGAGGGCGGG	182	UAUCUGGAGUCAUUGAGGGC	697
	CCGACCACGTGGAGCTGAGCTGG	183	CCGACCACGUGGAGCUGAGC	698
	AGGCTTCTACCCCGACCACGTGG	184	AGGCUUCUACCCCGACCACG	699
	GCGGCTGCTCAGGCAGTATCTGG	185	GCGGCUGCUCAGGCAGUAUC	700
	GAAAAACGTGTTCCCACCCAAGG	186	GAAAAACGUGUUCCCACCCA	701
	CAAGATCTCATAGAGGATGGTGG	187	CAAGAUCUCAUAGAGGAUGG	702
	TCCTCTATGAGATCTTGCTAGGG	188	UCCUCUAUGAGAUCUUGCUA	703
	GTTGCGGGGGTTCTGCCAGAAGG	189	GUUGCGGGGGUUCUGCCAGA	704
	AACACGTTTTTCAGGTCCTCTGG	190	AACACGUUUUUCAGGUCCUC	705
	CTTGACAGCGGAAGTGGTTGCGG	191	CUUGACAGCGGAAGUGGUUG	706
	ATCCTCTATGAGATCTTGCTAGG	192	AUCCUCUAUGAGAUCUUGCU	707
	GCTGTCAAGTCCAGTTCTACGGG	193	GCUGUCAAGUCCAGUUCUAC	708
	CAGCTCAGCTCCACGTGGTCGGG	194	CAGCUCAGCUCCACGUGGUC	709
	AGATCGTCAGCGCCGAGGCCTGG	195	AGAUCGUCAGCGCCGAGGCC	710
	GATCGTCAGCGCCGAGGCCTGGG	196	GAUCGUCAGCGCCGAGGCCU	711
PDCD1	ACCGCCCAGACGACTGGCCAGGG	197	ACCGCCCAGACGACUGGCCA	712
	TGACGTTACCTCGTGCGGCCCGG	198	UGACGUUACCUCGUGCGGCC	713
	ATGTGGAAGTCACGCCCGTTGGG	199	AUGUGGAAGUCACGCCCGUU	714
	TGGGATGACGTTACCTCGTGCGG	200	UGGGAUGACGUUACCUCGUG	715
	GTCTGGGCGGTGCTACAACTGGG	201	GUCUGGGCGGUGCUACAACU	716
	GACGTTACCTCGTGCGGCCCGGG	202	GACGUUACCUCGUGCGGCCC	717
	CGTCTGGGCGGTGCTACAACTGG	203	CGUCUGGGCGGUGCUACAAC	718
	GCGTGACTTCCACATGAGCGTGG	204	GCGUGACUUCCACAUGAGCG	719
	CGACTGGCCAGGGCGCCTGTGGG	205	CGACUGGCCAGGGCGCCUGU	720
	TGTAGCACCGCCCAGACGACTGG	206	UGUAGCACCGCCCAGACGAC	721
	CACGAAGCTCTCCGATGTGTTGG	207	CACGAAGCUCUCCGAUGUGU	722
	TGACACGGAAGCGGCAGTCCTGG	208	UGACACGGAAGCGGCAGUCC	723
	TCAGTGGCTGGGCACTCCGAGGG	209	UCAGUGGCUGGGCACUCCGA	724
	CGGAGAGCTTCGTGCTAAACTGG	210	CGGAGAGCUUCGUGCUAAAC	725
	AGGTGCCGCTGTCATTGCGCCGG	211	AGGUGCCGCUGUCAUUGCGC	726
	AGCTTGTCCGTCTGGTTGCTGGG	212	AGCUUGUCCGUCUGGUUGCU	727
	CACCTACCTAAGAACCATCCTGG	213	CACCUACCUAAGAACCAUCC	728
	CGCCCACGACACCAACCACCAGG	214	CGCCCACGACACCAACCACC	729
	ATTGTCTTTCCTAGCGGAATGGG	215	AUUGUCUUUCCUAGCGGAAU	730

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GTGGCATACTCCGTCTGCTCAGG	216	GUGGCAUACUCCGUCUGCUC	731
	CCCCTTCGGTCACCACGAGCAGG	217	CCCCUUCGGUCACCACGAGC	732
	AGGCGCCCTGGCCAGTCGTCTGG	218	AGGCGCCCUGGCCAGUCGUC	733
	AGCCGGCCAGTTCCAAACCCTGG	219	AGCCGGCCAGUUCCAAACCC	734
	ACTTCCACATGAGCGTGGTCAGG	220	ACUUCCACAUGAGCGUGGUC	735
	CGTTGGGCAGTTGTGTGACACGG	221	CGUUGGGCAGUUGUGUGACA	736
	CCCTTCGGTCACCACGAGCAGGG	222	CCCUUCGGUCACCACGAGCA	737
	ATCTGCTCCCGGGCCGCACGAGG	223	AUCUGCUCCCGGGCCGCACG	738
	ACCCTGGTGGTTGGTGTCGTGGG	224	ACCCUGGUGGUUGGUGUCGU	739
	CACCGCCCAGACGACTGGCCAGG	225	CACCGCCCAGACGACUGGCC	740
	GGGCGGTGCTACAACTGGGCTGG	226	GGGCGGUGCUACAACUGGGC	741
	CAGCTTGTCCGTCTGGTTGCTGG	227	CAGCUUGUCCGUCUGGUUGC	742
	CATGTGGAAGTCACGCCCGTTGG	228	CAUGUGGAAGUCACGCCCGU	743
	CGTGTCACACAACTGCCCAACGG	229	CGUGUCACACAACUGCCCAA	744
	AGGGCCCGGCGCAATGACAGCGG	230	AGGGCCCGGCGCAAUGACAG	745
	GGTGACAGGTGCGGCCTCGGAGG	231	GGUGACAGGUGCGGCCUCGG	746
	GTGTCACACAACTGCCCAACGGG	232	GUGUCACACAACUGCCCAAC	747
	AGGGTTTGGAACTGGCCGGCTGG	233	AGGGUUUGGAACUGGCCGGC	748
	TGGCGGCCAGGATGGTTCTTAGG	234	UGGCGGCCAGGAUGGUUCUU	749
	CGACACCAACCACCAGGGTTTGG	235	CGACACCAACCACCAGGGUU	750
	AGGCGGCCAGCTTGTCCGTCTGG	236	AGGCGGCCAGCUUGUCCGUC	751
	CTACAACTGGGCTGGCGGCCAGG	237	CUACAACUGGGCUGGCGGCC	752
	GCTCTCTTTGATCTGCGCCTTGG	238	GCUCUCUUUGAUCUGCGCCU	753
	CTCTCTTTGATCTGCGCCTTGGG	239	CUCUCUUUGAUCUGCGCCUU	754
	TCGGTCACCACGAGCAGGGCTGG	240	UCGGUCACCACGAGCAGGGC	755
	TCCGCTAGGAAAGACAATGGTGG	241	UCCGCUAGGAAAGACAAUGG	756
	GATGAGGTGCCCATTCCGCTAGG	242	GAUGAGGUGCCCAUUCCGCU	757
	ACCTCATCCCCCGCCCGCAGGGG	243	ACCUCAUCCCCCGCCCGCAG	758
	GATCTGCGCCTTGGGGGCCAGGG	244	GAUCUGCGCCUUGGGGGCCA	759
	GGTGCCGCTGTCATTGCGCCGGG	245	GGUGCCGCUGUCAUUGCGCC	760
	AGGATGGTTCTTAGGTAGGTGGG	246	AGGAUGGUUCUUAGGUAGGU	761
TIM-3/HAVCR2	ATAGGCATCTACATCGGAGCAGG	247	AUAGGCAUCUACAUCGGAGC	762
	TCTCTCTGCCGAGTCGGTGCAGG	248	UCUCUCUGCCGAGUCGGUGC	763
	ATGAGAATACCCTAGTAAGGGGG	249	AUGAGAAUACCCUAGUAAGG	764
	CGACAACCCAAAGGTTGTGAGGG	250	CGACAACCCAAAGGUUGUGA	765
	CCGTAACTCATTGGCCAATGTGG	251	CCGUAACUCAUUGGCCAAUG	766
	TATGAGAATACCCTAGTAAGGGG	252	UAUGAGAAUACCCUAGUAAG	767
	TGAGTTACGGGACTCTAGATTGG	253	UGAGUUACGGGACUCUAGAU	768
	TCTAGAGTCCCGTAACTCATTGG	254	UCUAGAGUCCCGUAACUCAU	769
	GCCAATGACTTACGGGACTCTGG	255	GCCAAUGACUUACGGGACUC	770
	GACGGGCACGAGGTTCCCTGGGG	256	GACGGGCACGAGGUUCCCUG	771
	AGACGGGCACGAGGTTCCCTGGG	257	AGACGGGCACGAGGUUCCCU	772
	TCTGGAGCAACCATCAGAATAGG	258	UCUGGAGCAACCAUCAGAAU	773
	CAGACGGGCACGAGGTTCCCTGG	259	CAGACGGGCACGAGGUUCCC	774
	CTGGTTTGATGACCAACTTCAGG	260	CUGGUUUGAUGACCAACUUC	775
	GGCCCAGGTAACTATGCATGGGG	261	GGCCCAGGUAACUAUGCAUG	776
	ATTGCAAAGCGACAACCCAAAGG	262	AUUGCAAAGCGACAACCCAA	777
	TGGTCATCAAACCAGGTGAGTGG	263	UGGUCAUCAAACCAGGUGAG	778

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTTACAAGTAAGTCTCGGCATGG	264	CUUACAAGUAAGUCUCGGCA	779
	CTAAATGGGGATTTCCGCAAAGG	265	CUAAAUGGGGAUUUCCGCAA	780
	CATGCAAATGTCCACTCACCTGG	266	CAUGCAAAUGUCCACUCACC	781
	GCTATGAGAATACCCTAGTAAGG	267	GCUAUGAGAAUACCCUAGUA	782
	CTCTCTGCCGAGTCGGTGCAGGG	268	CUCUCUGCCGAGUCGGUGCA	783
	GAACCTCGTGCCCGTCTGCTGGG	269	GAACCUCGUGCCCGUCUGCU	784
	GTGAAGTCTCTCTGCCGAGTCGG	270	GUGAAGUCUCUCUGCCGAGU	785
	TCCAGAGTCCCGTAAGTCATTGG	271	UCCAGAGUCCCGUAAGUCAU	786
	AATGTGACTCTAGCAGACAGTGG	272	AAUGUGACUCUAGCAGACAG	787
	TAGGCATCTACATCGGAGCAGGG	273	UAGGCAUCUACAUCGGAGCA	788
	GTTGTTTCTGACATTAGCCAAGG	274	GUUGUUUCUGACAUUAGCCA	789
	TGCTGCCGGATCCAAATCCCAGG	275	UGCUGCCGGAUCCAAAUCCC	790
	GCCAATGTGGATATTTGCTATGG	276	GCCAAUGUGGAUAUUUGCUA	791
	CTAGATTGGCCAATGACTTACGG	277	CUAGAUUGGCCAAUGACUUA	792
	CTGCCCCATGCATAGTTACCTGG	278	CUGCCCCAUGCAUAGUUACC	793
	TGTGTTTGAATGTGGCAACGTGG	279	UGUGUUUGAAUGUGGCAACG	794
	AGAAGTGGAATACAGAGCGGAGG	280	AGAAGUGGAAUACAGAGCGG	795
	TGGCCCAGGTAACTATGCATGGG	281	UGGCCCAGGUAACUAUGCAU	796
	TAGATTGGCCAATGACTTACGGG	282	UAGAUUGGCCAAUGACUUAC	797
	GGAACCTCGTGCCCGTCTGCTGG	283	GGAACCUCGUGCCCGUCUGC	798
	ACGTTGCCACATTCAAACACAGG	284	ACGUUGCCACAUUCAAACAC	799
	TGCCCCAGCAGACGGGCACGAGG	285	UGCCCCAGCAGACGGGCACG	800
	ATGGCCCAGGTAACTATGCATGG	286	AUGGCCCAGGUAACUAUGCA	801
	AGGTCACCCCTGCACCGACTCGG	287	AGGUCACCCCUGCACCGACU	802
	AATGTGGCAACGTGGTGCTCAGG	288	AAUGUGGCAACGUGGUGCUC	803
	GAGTCACATTCTCTATGGTCAGG	289	GAGUCACAUUCUCUAUGGUC	804
	ATCCCCATTTAGCCAGTATCTGG	290	AUCCCCAUUUAGCCAGUAUC	805
	CATCCAGATACTGGCTAAATGGG	291	CAUCCAGAUACUGGCUAAAU	806
	ATGTGACTCTAGCAGACAGTGGG	292	AUGUGACUCUAGCAGACAGU	807
	GATGTAGATGCCTATTCTGATGG	293	GAUGUAGAUGCCUAUUCUGA	808
	CTTACTGTTAGATTTATATCAGG	294	CUUACUGUUAGAUUUAUAUC	809
	ATCAGAATAGGCATCTACATCGG	295	AUCAGAAUAGGCAUCUACAU	810
	ATTATTGCTATGTCAGCAGCAGG	296	AUUAUUGCUAUGUCAGCAGC	811
TIGIT	GTACTCCCCTGTATCGTTCACGG	297	GUACUCCCCUGUAUCGUUCA	812
	TATCGTTCACGGTCAGCGACTGG	298	UAUCGUUCACGGUCAGCGAC	813
	TCGCTGACCGTGAACGATACAGG	299	UCGCUGACCGUGAACGAUAC	814
	TGGGGCCACTCGATCCTTGAAGG	300	UGGGGCCACUCGAUCCUUGA	815
	CGTTCACGGTCAGCGACTGGAGG	301	CGUUCACGGUCAGCGACUGG	816
	ACCCTGATGGGACGTACACTGGG	302	ACCCUGAUGGGACGUACACU	817
	GCGGCCATGGCTCCAAGCAATGG	303	GCGGCCAUGGCUCCAAGCAA	818
	CGCTGACCGTGAACGATACAGGG	304	CGCUGACCGUGAACGAUACA	819
	TCCCAGTGTACGTCCCATCAGGG	305	UCCCAGUGUACGUCCCAUCA	820
	CCCATCCTTCAAGGATCGAGTGG	306	CCCAUCCUUCAAGGAUCGAG	821
	CGCGTTGACTAGAAAGGTAATGG	307	CGCGUUGACUAGAAAGGUAA	822
	CTCCCAGTGTACGTCCCATCAGG	308	CUCCCAGUGUACGUCCCAUC	823
	AGTGTACGTCCCATCAGGGTAGG	309	AGUGUACGUCCCAUCAGGGU	824
	TTCAAGGATCGAGTGGCCCCAGG	310	UUCAAGGAUCGAGUGGCCCC	825
	GAAAGCTCAGGTATTCCTGCTGG	311	GAAAGCUCAGGUAUUCCUGC	826

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GGTGGTCGCGTTGACTAGAAAGG	312	GGUGGUCGCGUUGACUAGAA	827
	GACCACCAGCGTCGCGGCCATGG	313	GACCACCAGCGUCGCGGCCA	828
	GCCACTCGATCCTTGAAGGATGG	314	GCCACUCGAUCCUUGAAGGA	829
	GCAGATGACCACCAGCGTCGCGG	315	GCAGAUGACCACCAGCGUCG	830
	GTTCACGGTCAGCGACTGGAGGG	316	GUUCACGGUCAGCGACUGGA	831
	CAGGCACAATAGAAACAACGGGG	317	CAGGCACAAUAGAAACAACG	832
	TGGAGCCATGGCCGCGACGCTGG	318	UGGAGCCAUGGCCGCGACGC	833
	CACAAGTGACCCAGGTCAACTGG	319	CACAAGUGACCCAGGUCAAC	834
	TAGCAACCAGAGGCATCTTCTGG	320	UAGCAACCAGAGGCAUCUUC	835
	GCTGACCGTGAACGATACAGGGG	321	GCUGACCGUGAACGAUACAG	836
	GACCTGGGTCACTTGTGCCGTGG	322	GACCUGGGUCACUUGUGCCG	837
	TACCCTGATGGGACGTACACTGG	323	UACCCUGAUGGGACGUACAC	838
	GACTAGAAAGGTAATGGCTCCGG	324	GACUAGAAAGGUAAUGGCUC	839
	TCTATCACACCTACCCTGATGGG	325	UCUAUCACACCUACCCUGAU	840
	AGGTTCCAGATTCCATTGCTTGG	326	AGGUUCCAGAUUCCAUUGCU	841
	ATTGAAGTAGTCATGCAGCTCGG	327	AUUGAAGUAGUCAUGCAGCU	842
	CACCACGGCACAAGTGACCCAGG	328	CACCACGGCACAAGUGACCC	843
	TTTGTAATGCTGACTTGGGGTGG	329	UUUGUAAUGCUGACUUGGGG	844
	TCAGGCCTTACCTGAGGCGAGGG	330	UCAGGCCUUACCUGAGGCGA	845
	GATTCCATTGCTTGGAGCCATGG	331	GAUUCCAUUGCUUGGAGCCA	846
	CTGCACAGCAGTCATCGTGGTGG	332	CUGCACAGCAGUCAUCGUGG	847
	GATCGAGTGGCCCCAGGTCCCGG	333	GAUCGAGUGGCCCCAGGUCC	848
	AGCCATGGCCGCGACGCTGGTGG	334	AGCCAUGGCCGCGACGCUGG	849
	ATCTATCACACCTACCCTGATGG	335	AUCUAUCACACCUACCCUGA	850
	AGAGACTGGTTAGCAACCAGAGG	336	AGAGACUGGUUAGCAACCAG	851
	ACAAGTGACCCAGGTCAACTGGG	337	ACAAGUGACCCAGGUCAACU	852
	CGGTCAGCGACTGGAGGGTGAGG	338	CGGUCAGCGACUGGAGGGUG	853
	GTACACTGGGAGAATCTTCCTGG	339	GUACACUGGGAGAAUCUUCC	854
	ATTCTGTGGAAGGTGACCTCAGG	340	AUUCUGUGGAAGGUGACCUC	855
	TACCCAGGCTTCTGTAACTCAGG	341	UACCCAGGCUUCUGUAACUC	856
	CCATTTGTAATGCTGACTTGGGG	342	CCAUUUGUAAUGCUGACUUG	857
	CAGGCCTTACCTGAGGCGAGGGG	343	CAGGCCUUACCUGAGGCGAG	858
	GTCCAGCTGATTTTCTCCTGAGG	344	GUCCAGCUGAUUUUCUCCUG	859
	CACTCGATCCTTGAAGGATGGGG	345	CACUCGAUCCUUGAAGGAUG	860
	GCCATTTGTAATGCTGACTTGGG	346	GCCAUUUGUAAUGCUGACUU	861
LAG3	GTGCATTGGTTCCGGAACCGGGG	347	GUGCAUUGGUUCCGGAACCG	862
	CGACTTTACCCTTCGACTAGAGG	348	CGACUUUACCCUUCGACUAG	863
	TCGACTAGAGGATGTGAGCCAGG	349	UCGACUAGAGGAUGUGAGCC	864
	GCTTTCCGCTAAGTGGTGATGGG	350	GCUUUCCGCUAAGUGGUGAU	865
	CGCTACACGGTGCTGAGCGTGGG	351	CGCUACACGGUGCUGAGCGU	866
	GCGTACACTGTCAAGGGAGTTGG	352	GCGUACACUGUCAAGGGAGU	867
	AGCGCGGGGACTTCTCGCTATGG	353	AGCGCGGGGACUUCUCGCUA	868
	GCTCCAGCGTACACTGTCAAGGG	354	GCUCCAGCGUACACUGUCAA	869
	GCTCACATCCTCTAGTCGAAGGG	355	GCUCACAUCCUCUAGUCGAA	870
	GGCTCACATCCTCTAGTCGAAGG	356	GGCUCACAUCCUCUAGUCGA	871
	CGCCCCACATACTCGAGGCCTGG	357	CGCCCCACAUACUCGAGGCC	872
	CTGTGCATTGGTTCCGGAACCGG	358	CUGUGCAUUGGUUCCGGAAC	873
	TTGGTTCCGGAACCGGGGCCAGG	359	UUGGUUCCGGAACCGGGGCC	874

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CGCTCATCCAGCTGGACGCGGGG	360	CGCUCAUCCAGCUGGACGCG	875
	TTCCGCTAAGTGGTGATGGGGGG	361	UUCCGCUAAGUGGUGAUGGG	876
	CAGGCCTCGAGTATGTGGGGCGG	362	CAGGCCUCGAGUAUGUGGGG	877
	GCAAGGGATTCACCCTCCGCAGG	363	GCAAGGGAUUCACCCUCCGC	878
	AGCTTTCCGCTAAGTGGTGATGG	364	AGCUUUCCGCUAAGUGGUGA	879
	CGCTCAGCACCGTGTAGCGGCGG	365	CGCUCAGCACCGUGUAGCGG	880
	CGTACACTGTCAAGGGAGTTGGG	366	CGUACACUGUCAAGGGAGUU	881
	ACCGTGTAGCGGCGGGGCCTGGG	367	ACCGUGUAGCGGCGGGGCCU	882
	GCCGGCCGCGCTCATCCAGCTGG	368	GCCGGCCGCGCUCAUCCAGC	883
	CGTCCCGCCCCACATACTCGAGG	369	CGUCCCGCCCCACAUACUCG	884
	ACTCCCTTGACAGTGTACGCTGG	370	ACUCCCUUGACAGUGUACGC	885
	CGCCGGCGAGTACCGCGCCGCGG	371	CGCCGGCGAGUACCGCGCCG	886
	GTTCCGGAACCAATGCACAGAGG	372	GUUCCGGAACCAAUGCACAG	887
	CGCGTCCAGCTGGATGAGCGCGG	373	CGCGUCCAGCUGGAUGAGCG	888
	GTACGCTGGAGCAGGTTCCAGGG	374	GUACGCUGGAGCAGGUUCCA	889
	TCTAAGGCAGAAAATCGTCTTGG	375	UCUAAGGCAGAAAAUCGUCU	890
	AAGCGTTCTTGTCCAGATACTGG	376	AAGCGUUCUUGUCCAGAUAC	891
	GCGAGAAGTCCCCGCGCTGCCGG	377	GCGAGAAGUCCCCGCGCUGC	892
	CACCGCGGCGCGGTACTCGCCGG	378	CACCGCGGCGCGGUACUCGC	893
	TCCATAGGTGCCCAACGCTCTGG	379	UCCAUAGGUGCCCAACGCUC	894
	CACCGTGTAGCGGCGGGGCCTGG	380	CACCGUGUAGCGGCGGGGCC	895
	GGGTGGCTCCAGGTAAAACGGGG	381	GGGUGGCUCCAGGUAAAACG	896
	GACGTTGAAGCCATCTCTGTAGG	382	GACGUUGAAGCCAUCUCUGU	897
	GATGGGGGGACTCCCGGACAGGG	383	GAUGGGGGGACUCCCGGACA	898
	AGTATGTGGGGCGGGACGATGGG	384	AGUAUGUGGGGCGGGACGAU	899
	CCAGGTAAAACGGGGATGGCGGG	385	CCAGGUAAAACGGGGAUGGC	900
	GGGCCAGGCCTCGAGTATGTGGG	386	GGGCCAGGCCUCGAGUAUGU	901
	GGTAAAACGGGGATGGCGGGAGG	387	GGUAAAACGGGGAUGGCGGG	902
	ACCGCGCCGCGGTGCACCTCAGG	388	ACCGCGCCGCGGUGCACCUC	903
	ACTCGCCGGCGTCCGCGCGCCGG	389	ACUCGCCGGCGUCCGCGCGC	904
	GATCTCTCAGAGCCTCCGACTGG	390	GAUCUCUCAGAGCCUCCGAC	905
	GCGGTCCCTGAGGTGCACCGCGG	391	GCGGUCCCUGAGGUGCACCG	906
	GTCCCCCCATCACCACTTAGCGG	392	GUCCCCCCAUCACCACUUAG	907
	AGAGGAAGCTTTCCGCTAAGTGG	393	AGAGGAAGCUUUCCGCUAAG	908
	TGCTCCAGCGTACACTGTCAAGG	394	UGCUCCAGCGUACACUGUCA	909
	TTGACAGTGTACGCTGGAGCAGG	395	UUGACAGUGUACGCUGGAGC	910
	AGGCCTCGAGTATGTGGGGCGGG	396	AGGCCUCGAGUAUGUGGGGC	911
CTLA4	ACACCGCTCCCATAAAGCCATGG	397	ACACCGCUCCCAUAAAGCCA	912
	GTGCGGCAACCTACATGATGGGG	398	GUGCGGCAACCUACAUGAUG	913
	TACCCACCGCCATACTACCTGGG	399	UACCCACCGCCAUACUACCU	914
	CCGCCATACTACCTGGGCATAGG	400	CCGCCAUACUACCUGGGCAU	915
	GTACCCACCGCCATACTACCTGG	401	GUACCCACCGCCAUACUACC	916
	GGGTTCCGTTGCCTATGCCCAGG	402	GGGUUCCGUUGCCUAUGCCC	917
	CATAGACCCCTGTTGTAAGAGGG	403	CAUAGACCCCUGUUGUAAGA	918
	TGCCCAGGTAGTATGGCGGTGGG	404	UGCCCAGGUAGUAUGGCGGU	919
	AGGTCCGGGTGACAGTGCTTCGG	405	AGGUCCGGGUGACAGUGCUU	920
	TGAACCTGGCTACCAGGACCTGG	406	UGAACCUGGCUACCAGGACC	921
	TTGCCTATGCCCAGGTAGTATGG	407	UUGCCUAUGCCCAGGUAGUA	922

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTGTGCGGCAACCTACATGATGG	408	CUGUGCGGCAACCUACAUGA	923
	TGTGCGGCAACCTACATGATGGG	409	UGUGCGGCAACCUACAUGAU	924
	CCGGGTGACAGTGCTTCGGCAGG	410	CCGGGUGACAGUGCUUCGGC	925
	ACATAGACCCCTGTTGTAAGAGG	411	ACAUAGACCCCUGUUGUAAG	926
	CCTTGGATTTCAGCGGCACAAGG	412	CCUUGGAUUUCAGCGGCACA	927
	GTTCACTTGATTTCCACTGGAGG	413	GUUCACUUGAUUUCCACUGG	928
	GGCCACGTGCATTGCTAGCATGG	414	GGCCACGUGCAUUGCUAGCA	929
	TACTACCTGGGCATAGGCAACGG	415	UACUACCUGGGCAUAGGCAA	930
	GCTCACCAATTACATAAATCTGG	416	GCUCACCAAUUACAUAAAUC	931
	ACTGGAGGTGCCCGTGCAGATGG	417	ACUGGAGGUGCCCGUGCAGA	932
	TTCCATGCTAGCAATGCACGTGG	418	UUCCAUGCUAGCAAUGCACG	933
	AAGGCAAGCCATGGCTTTATGGG	419	AAGGCAAGCCAUGGCUUUAU	934
	CAAGGCAAGCCATGGCTTTATGG	420	CAAGGCAAGCCAUGGCUUUA	935
	ATCTGCACGGGCACCTCCAGTGG	421	AUCUGCACGGGCACCUCCAG	936
	CACTGTCACCCGGACCTCAGTGG	422	CACUGUCACCCGGACCUCAG	937
	CCTCACTATCCAAGGACTGAGGG	423	CCUCACUAUCCAAGGACUGA	938
	CTAGATGATTCCATCTGCACGGG	424	CUAGAUGAUUCCAUCUGCAC	939
	GCTTCGGCAGGCTGACAGCCAGG	425	GCUUCGGCAGGCUGACAGCC	940
	CACGGGACTCTACATCTGCAAGG	426	CACGGGACUCUACAUCUGCA	941
	ATGCCCAGGTAGTATGGCGGTGG	427	AUGCCCAGGUAGUAUGGCGG	942
	AAGAAGCCCTCTTACAACAGGGG	428	AAGAAGCCCUCUUACAACAG	943
	GCAAAGGTGAGTGAGACTTTTGG	429	GCAAAGGUGAGUGAGACUUU	944
	GGGACTCTACATCTGCAAGGTGG	430	GGGACUCUACAUCUGCAAGG	945
	ACCTCACTATCCAAGGACTGAGG	431	ACCUCACUAUCCAAGGACUG	946
	CAAGTGAACCTCACTATCCAAGG	432	CAAGUGAACCUCACUAUCCA	947
	GCTGGCGATGCCTCGGCTGCTGG	433	GCUGGCGAUGCCUCGGCUGC	948
	CTCACCAATTACATAAATCTGGG	434	CUCACCAAUUACAUAAAUCU	949
	GGAACCCAGATTTATGTAATTGG	435	GGAACCCAGAUUUAUGUAAU	950
	CCTAGATGATTCCATCTGCACGG	436	CCUAGAUGAUUCCAUCUGCA	951
	AAAGAAGCCCTCTTACAACAGGG	437	AAAGAAGCCCUCUUACAACA	952
	GAGGTTCACTTGATTTCCACTGG	438	GAGGUUCACUUGAUUUCCAC	953
	CGGACCTCAGTGGCTTTGCCTGG	439	CGGACCUCAGUGGCUUUGCC	954
	TGTCCATGGCCCTCAGTCCTTGG	440	UGUCCAUGGCCCUCAGUCCU	955
	ACACAAAGCTGGCGATGCCTCGG	441	ACACAAAGCUGGCGAUGCCU	956
	AAGCCATGGCTTTATGGGAGCGG	442	AAGCCAUGGCUUUAUGGGAG	957
	CTCAGCTGAACCTGGCTACCAGG	443	CUCAGCUGAACCUGGCUACC	958
	GATGTAGAGTCCCGTGTCCATGG	444	GAUGUAGAGUCCCGUGUCCA	959
	AAAAGAAGCCCTCTTACAACAGG	445	AAAAGAAGCCCUCUUACAAC	960
	GCACGTGGCCCAGCCTGCTGTGG	446	GCACGUGGCCCAGCCUGCUG	961
AAVS1	GCTGCTCTGACGCGGCCGTCTGG	447	GCUGCUCUGACGCGGCCGUC	962
	TATAAGGTGGTCCCAGCTCGGGG	448	UAUAAGGUGGUCCCAGCUCG	963
	GACGCAAGGGAGACATCCGTCGG	449	GACGCAAGGGAGACAUCCGU	964
	AGGGAGACATCCGTCGGAGAAGG	450	AGGGAGACAUCCGUCGGAGA	965
	CTTAGGATGGCCTTCTCCGACGG	451	CUUAGGAUGGCCUUCUCCGA	966
	CTGGTGCGTTTCACTGATCCTGG	452	CUGGUGCGUUUCACUGAUCC	967
	CAGGTAAAACTGACGCACGGAGG	453	CAGGUAAAACUGACGCACGG	968
	GATCAGTGAAACGCACCAGACGG	454	GAUCAGUGAAACGCACCAGA	969
	GTCACCAATCCTGTCCCTAGTGG	455	GUCACCAAUCCUGUCCCUAG	970

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GAGAGGTGACCCGAATCCACAGG	456	GAGAGGUGACCCGAAUCCAC	971
	CCTCTAAGGTTTGCTTACGATGG	457	CCUCUAAGGUUUGCUUACGA	972
	TAAGGAATCTGCCTAACAGGAGG	458	UAAGGAAUCUGCCUAACAGG	973
	ATTCCCAGGGCCGGTTAATGTGG	459	AUUCCCAGGGCCGGUUAAUG	974
	CCCAAAGTACCCCGTCTCCCTGG	460	CCCAAAGUACCCCGUCUCCC	975
	ATATAAGGTGGTCCCAGCTCGGG	461	AUAUAAGGUGGUCCCAGCUC	976
	TAACCGGCCCTGGGAATATAAGG	462	UAACCGGCCCUGGGAAUAUA	977
	CTGCATCATCACCGTTTTTCTGG	463	CUGCAUCAUCACCGUUUUUC	978
	TAAGAAACGAGAGATGGCACAGG	464	UAAGAAACGAGAGAUGGCAC	979
	AGAGCTAGCACAGACTAGAGAGG	465	AGAGCUAGCACAGACUAGAG	980
	GGCTACTGGCCTTATCTCACAGG	466	GGCUACUGGCCUUAUCUCAC	981
	ACCCCGTTCTCCTGTGGATTCGG	467	ACCCCGUUCUCCUGUGGAUU	982
	CGGAGGAACAATATAAATTGGGG	468	CGGAGGAACAAUAUAAAUUG	983
	ACAGTGGGGCCACTAGGGACAGG	469	ACAGUGGGGCCACUAGGGAC	984
	CGGCCGCGTCAGAGCAGCTCAGG	470	CGGCCGCGUCAGAGCAGCUC	985
	ACGGAGGAACAATATAAATTGGG	471	ACGGAGGAACAAUAUAAAUU	986
	GGGACCACCTTATATTCCCAGGG	472	GGGACCACCUUAUAUUCCCA	987
	TGGGACCACCTTATATTCCCAGG	473	UGGGACCACCUUAUAUUCCC	988
	CCATCTCTCGTTTCTTAGGATGG	474	CCAUCUCUCGUUUCUUAGGA	989
	TAAGCAAACCTTAGAGGTTCTGG	475	UAAGCAAACCUUAGAGGUUC	990
	CGTCAGAGCAGCTCAGGTTCTGG	476	CGUCAGAGCAGCUCAGGUUC	991
	GACCCGAATCCACAGGAGAACGG	477	GACCCGAAUCCACAGGAGAA	992
	AGAGCCACATTAACCGGCCCTGG	478	AGAGCCACAUUAACCGGCCC	993
	TCACAGGTAAAACTGACGCACGG	479	UCACAGGUAAAACUGACGCA	994
	TTCTGGGAGAGGGTAGCGCAGGG	480	UUCUGGGAGAGGGUAGCGCA	995
	GGATCCTGTGTCCCCGAGCTGGG	481	GGAUCCUGUGUCCCCGAGCU	996
	TGGGGGTTAGACCCAATATCAGG	482	UGGGGGUUAGACCCAAUAUC	997
	GTCCCTAGTGGCCCCACTGTGGG	483	GUCCCUAGUGGCCCCACUGU	998
	TGTTAGGCAGATTCCTTATCTGG	484	UGUUAGGCAGAUUCCUUAUC	999
	AAACCTTAGAGGTTCTGGCAAGG	485	AAACCUUAGAGGUUCUGGCA	1000
	CTGGACACCCCGTTCTCCTGTGG	486	CUGGACACCCCGUUCUCCUG	1001
	GGGGGGATGCGTGACCTGCCCGG	487	GGGGGGAUGCGUGACCUGCC	1002
	GGTTAATGTGGCTCTGGTTCTGG	488	GGUUAAUGUGGCUCUGGUUC	1003
	TGATGCAGGCCTACAAGAAGGGG	489	UGAUGCAGGCCUACAAGAAG	1004
	TAGCTGAGCTCTCGGACCCCTGG	490	UAGCUGAGCUCUCGGACCCC	1005
	TGCTTACGATGGAGCCAGAGAGG	491	UGCUUACGAUGGAGCCAGAG	1006
	TGCTGTCCTGAAGTGGACATAGG	492	UGCUGUCCUGAAGUGGACAU	1007
	CTGTCCTGAAGTGGACATAGGGG	493	CUGUCCUGAAGUGGACAUAG	1008
	CAGGGAGACGGGGTACTTTGGGG	494	CAGGGAGACGGGGUACUUUG	1009
	ATGATGCAGGCCTACAAGAAGGG	495	AUGAUGCAGGCCUACAAGAA	1010
	ACCCGAATCCACAGGAGAACGGG	496	ACCCGAAUCCACAGGAGAAC	1011
	GCAAACATGCTGTCCTGAAGTGG	497	GCAAACAUGCUGUCCUGAAG	1012
	GACATAGGGGCCCGGGTTGGAGG	498	GACAUAGGGGCCCGGGUUGG	1013
	TGGGGGTGTGTCACCAGATAAGG	499	UGGGGGUGUGUCACCAGAUA	1014
	TGGCTAAAGCCAGGGAGACGGGG	500	UGGCUAAAGCCAGGGAGACG	1015
	TTGGTCCTGAGTTCTAACTTTGG	501	UUGGUCCUGAGUUCUAACUU	1016
	TCCCTAGTGGCCCCACTGTGGGG	502	UCCCUAGUGGCCCCACUGUG	1017
	CAGAAAAACGGTGATGATGCAGG	503	CAGAAAAACGGUGAUGAUGC	1018

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTTCCTAGTCTCCTGATATTGGG	504	CUUCCUAGUCUCCUGAUAUU	1019
	CACGGAGGAACAATATAAATTGG	505	CACGGAGGAACAAUAUAAAU	1020
	GAACCTGAGCTGCTCTGACGCGG	506	GAACCUGAGCUGCUCUGACG	1021
	GAGCCACATTAACCGGCCCTGGG	507	GAGCCACAUUAACCGGCCCU	1022
	ACCCCACAGTGGGGCCACTAGGG	508	ACCCCACAGUGGGGCCACUA	1023
	GTCCCGCCTCCCCTTCTTGTAGG	509	GUCCCGCCUCCCCUUCUUGU	1024
	CCCCGTTCTCCTGTGGATTCGGG	510	CCCCGUUCUCCUGUGGAUUC	1025
	CCACCTTATATTCCCAGGGCCGG	511	CCACCUUAUAUUCCCAGGGC	1026
CCR5	TCAGTTTACACCCGATCCACTGG	512	UCAGUUUACACCCGAUCCAC	1027
	AGTTTACACCCGATCCACTGGGG	513	AGUUUACACCCGAUCCACUG	1028
	TCATCCTCCTGACAATCGATAGG	514	UCAUCCUCCUGACAAUCGAU	1029
	CTTGTGACACGGACTCAAGTGGG	515	CUUGUGACACGGACUCAAGU	1030
	CAGTTTACACCCGATCCACTGGG	516	CAGUUUACACCCGAUCCACU	1031
	ACAATGTGTCAACTCTTGACAGG	517	ACAAUGUGUCAACUCUUGAC	1032
	GGTACCTATCGATTGTCAGGAGG	518	GGUACCUAUCGAUUGUCAGG	1033
	GTAAACTGAGCTTGCTCGCTCGG	519	GUAAACUGAGCUUGCUCGCU	1034
	GACAAGTGTGATCACTTGGGTGG	520	GACAAGUGUGAUCACUUGGG	1035
	TCTGAACTTCTCCCCGACAAAGG	521	UCUGAACUUCUCCCCGACAA	1036
	CCTGACAATCGATAGGTACCTGG	522	CCUGACAAUCGAUAGGUACC	1037
	CTCGCTCGGGAGCCTCTTGCTGG	523	CUCGCUCGGGAGCCUCUUGC	1038
	CAGGTTGGACCAAGCTATGCAGG	524	CAGGUUGGACCAAGCUAUGC	1039
	TGACCATGACAAGCAGCGGCAGG	525	UGACCAUGACAAGCAGCGGC	1040
	CACCCCAAAGGTGACCGTCCTGG	526	CACCCCAAAGGUGACCGUCC	1041
	TAAACTGAGCTTGCTCGCTCGGG	527	UAAACUGAGCUUGCUCGCUC	1042
	TCACTATGCTGCCGCCCAGTGGG	528	UCACUAUGCUGCCGCCCAGU	1043
	AGCGTTTGGCAATGTGCTTTTGG	529	AGCGUUUGGCAAUGUGCUUU	1044
	TTGACAGGGCTCTATTTTATAGG	530	UUGACAGGGCUCUAUUUUAU	1045
	CATCATCTATGCCTTTGTCGGGG	531	CAUCAUCUAUGCCUUUGUCG	1046
	CAATGTGTCAACTCTTGACAGGG	532	CAAUGUGUCAACUCUUGACA	1047
	TTGCAGTAGCTCTAACAGGTTGG	533	UUGCAGUAGCUCUAACAGGU	1048
	GCTGCCGCCCAGTGGGACTTTGG	534	GCUGCCGCCCAGUGGGACUU	1049
	AAGCCAGGACGGTCACCTTTGGG	535	AAGCCAGGACGGUCACCUUU	1050
	TGACACGGACTCAAGTGGGCTGG	536	UGACACGGACUCAAGUGGGC	1051
	CGACAAAGGCATAGATGATGGGG	537	CGACAAAGGCAUAGAUGAUG	1052
	ATAATTGCAGTAGCTCTAACAGG	538	AUAAUUGCAGUAGCUCUAAC	1053
	CAGGACGGTCACCTTTGGGGTGG	539	CAGGACGGUCACCUUUGGGG	1054
	AGCCAGGACGGTCACCTTTGGGG	540	AGCCAGGACGGUCACCUUUG	1055
	CAGAATTGATACTGACTGTATGG	541	CAGAAUUGAUACUGACUGUA	1056
	GACACCGAAGCAGAGTTTTTAGG	542	GACACCGAAGCAGAGUUUUU	1057
	GGTGACAAGTGTGATCACTTGGG	543	GGUGACAAGUGUGAUCACUU	1058
	AACACCAGTGAGTAGAGCGGAGG	544	AACACCAGUGAGUAGAGCGG	1059
	CTCACTATGCTGCCGCCCAGTGG	545	CUCACUAUGCUGCCGCCCAG	1060
	CTGTTCTATTTTCCAGCAAGAGG	546	CUGUUCUAUUUUCCAGCAAG	1061
	TGTCATGGTCATCTGCTACTCGG	547	UGUCAUGGUCAUCUGCUACU	1062
	CCATCATCTATGCCTTTGTCGGG	548	CCAUCAUCUAUGCCUUUGUC	1063
	GTCATGGTCATCTGCTACTCGGG	549	GUCAUGGUCAUCUGCUACUC	1064
	CATACAGTCAGTATCAATTCTGG	550	CAUACAGUCAGUAUCAAUUC	1065
	TTTACCAGATCTCAAAAAGAAGG	551	UUUACCAGAUCUCAAAAAGA	1066

Target gene (no CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	ACAGCATTTGCAGAAGCGTTTGG	552	ACAGCAUUUGCAGAAGCGUU	1067
	ATATCTGTGGGCTTGTGACACGG	553	AUAUCUGUGGGCUUGUGACA	1068
	AAGTGTGATCACTTGGGTGGTGG	554	AAGUGUGAUCACUUGGGUGG	1069
	TTGTATTTCCAAAGTCCCACTGG	555	UUGUAUUUCCAAAGUCCCAC	1070
	CCCATCATCTATGCCTTTGTCGG	556	CCCAUCAUCUAUGCCUUUGU	1071
	TGTATTTCCAAAGTCCCACTGGG	557	UGUAUUUCCAAAGUCCCACU	1072
	ATGCAGGTGACAGAGACTCTTGG	558	AUGCAGGUGACAGAGACUCU	1073
	TCAGCCTTTTGCAGTTTATCAGG	559	UCAGCCUUUUGCAGUUUAUC	1074
	AAAGATAGTCATCTTGGGGCTGG	560	AAAGAUAGUCAUCUUGGGGC	1075
	AAAGCCAGGACGGTCACCTTTGG	561	AAAGCCAGGACGGUCACCUU	1076

[0331] The products of the present invention may be used to modify, e.g., repress or silence, genes having CpG islands (CGI). Genes having CGI include: *B2M*; *TET2*; *TGFBR2*; *A2AR*; *CISH*; *PTPN11*; *PTPN6*; *PTPA*; *PTPN2*; *JUNB*; *TOX*; *TOX2*; *NR4A1*; *NR4A2*; *NR4A3*; *MAP4K1*; *REL*; *IRF4*; *DGKA*; *PIK3CD*; *HLA-A*; *USP16*; *DCK* and *FAS*.

[0332] For example, targeting genes, such as genes with a CGI, may:

produce allogenic products (e.g., by targeting *B2M* and/or *HLA-A*);

alter resistance to an immunosuppressive tumour microenvironment (e.g., by targeting of *TGFBR2*, *A2AR*, *PTPN11*, *PTPN6*, *PTPN2*, and/or *DGKA*);

allow CAR/transgenic TCR integration in a safe site (e.g., by targeting of *AAVS1* and/or *CCR5*);

provide resistance to exhaustion (e.g., by targeting of *FAS*, *CISH*, *PTPA*, *PIK3CD*, *MAP4K1*, *NR4A1*, *NR4A2*, *NR4A3*, *JUNB*, *REL*, *TOX*, *TOX2*, *IRF4* and/or *TET2*); and/or

delay T cell senescence (e.g., by targeting *USP16*).

[0333] Silencing of the TCR genes, *PDCD1* and *CTLA4* may be used to improve efficacy of cancer immunotherapy approaches.

[0334] Silencing of *B2M* may be used to generate allogeneic HSPCs, T cells or mesenchymal cells to be used for transplantation.

[0335] In one aspect, the present invention provides gRNAs which target a sequence set forth in any one of SEQ ID NOs: 1077 to 2777.

[0336] By way of example, target genes having CGI islands and exemplary gRNAs suitable for targeting said genes are presented in Table 2 below (SEQ: SEQ ID NO).

Table 2. Target genes having CGI islands and exemplary gRNAs

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
B2M	CGATAAGCGTCAGAGCGCCGAGG	1077	CGAUAAGCGUCAGAGCGCCG	2778
	TTTGGCCTACGGCGACGGGAGGG	1078	UUUGGCCUACGGCGACGGGA	2779
	CATCGGCGCCCTCCGATCTGGGG	1079	CAUCGGCGCCCUCCGAUCUG	2780
	CTTTGGCCTACGGCGACGGGAGG	1080	CUUUGGCCUACGGCGACGGG	2781
	TATAAGTGGAGGCGTCGCGCTGG	1081	UAUAAGUGGAGGCGUCGCGC	2782
	CTCCCGTCGCCGTAGGCCAAAGG	1082	CUCCCGUCGCCGUAGGCCAA	2783
	GACCTTTGGCCTACGGCGACGGG	1083	GACCUUUGGCCUACGGCGAC	2784
	AGACCTTTGGCCTACGGCGACGG	1084	AGACCUUUGGCCUACGGCGA	2785
	CGCTACTTGCCCCTTTCGGCGGG	1085	CGCUACUUGCCCCUUUCGGC	2786
	ACATCGGCGCCCTCCGATCTGGG	1086	ACAUCGGCGCCCUCCGAUCU	2787
	CGCGCGCTACTTGCCCCTTTCGG	1087	CGCGCGCUACUUGCCCCUUU	2788
	TACATCGGCGCCCTCCGATCTGG	1088	UACAUCGGCGCCCUCCGAUC	2789
	GTCCGAGCAGTTAACTGGCTGGG	1089	GUCCGAGCAGUUAACUGGCU	2790
	CACGCGTTTAATATAAGTGGAGG	1090	CACGCGUUUAAUAUAAGUGG	2791
	CGCGACGTTTGTAGAATGCTTGG	1091	CGCGACGUUUGUAGAAUGCU	2792
	GGGCACGCGTTTAATATAAGTGG	1092	GGGCACGCGUUUAAUAUAAG	2793
	GCTACTTGCCCCTTTCGGCGGGG	1093	GCUACUUGCCCCUUUCGGCG	2794
	AAGCGTCAGAGCGCCGAGGTTGG	1094	AAGCGUCAGAGCGCCGAGGU	2795
	GCGCTACTTGCCCCTTTCGGCGG	1095	GCGCUACUUGCCCCUUUCGG	2796
	AAGTGGAGGCGTCGCGCTGGCGG	1096	AAGUGGAGGCGUCGCGCUGG	2797
	GCCTACGGCGACGGGAGGGTCGG	1097	GCCUACGGCGACGGGAGGGU	2798
	GGTCCGAGCAGTTAACTGGCTGG	1098	GGUCCGAGCAGUUAACUGGC	2799
	AGCGTCAGAGCGCCGAGGTTGGG	1099	AGCGUCAGAGCGCCGAGGUU	2800
	GAACGCGTGGAGGGGCGCTTGGG	1100	GAACGCGUGGAGGGGCGCUU	2801
	GGCGCTCATTCTAGGACTTCAGG	1101	GGCGCUCAUUCUAGGACUUC	2802
	TTCGCATGTCCTAGCACCTCTGG	1102	UUCGCAUGUCCUAGCACCUC	2803
	AACCTCAGCGCCGCGCCTTTGGG	1103	AACCUCAGCGCCGCGCCUUU	2804
	CTCCTTGGTGGCCCGCCGTGGGG	1104	CUCCUUGGUGGCCCGCCGUG	2805
	ACTCACGCTGGATAGCCTCCAGG	1105	ACUCACGCUGGAUAGCCUCC	2806
	GGCGCGCACCCCAGATCGGAGGG	1106	GGCGCGCACCCCAGAUCGGA	2807
	TCGCATGTCCTAGCACCTCTGGG	1107	UCGCAUGUCCUAGCACCUCU	2808
	AACGCGTGGAGGGGCGCTTGGGG	1108	AACGCGUGGAGGGGCGCUUG	2809
	TTCTCTTCCGCTCTTTCGCGGGG	1109	UUCUCUUCCGCUCUUUCGCG	2810

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GACGGGTAGGCTCGTCCCAAAGG	1110	GACGGGUAGGCUCGUCCCAA	2811
	CCCGCCGTGGGGCTAGTCCAGGG	1111	CCCGCCGUGGGGCUAGUCCA	2812
	GAGTAGCGCGAGCACAGCTAAGG	1112	GAGUAGCGCGAGCACAGCUA	2813
	GGGGCAAGTAGCGCGCGTCCCGG	1113	GGGGCAAGUAGCGCGCGUCC	2814
	AGCGCCCGGTGTCCCAAGCTGGG	1114	AGCGCCCGGUGUCCCAAGCU	2815
	CCTACGGCGACGGGAGGGTCGGG	1115	CCUACGGCGACGGGAGGGUC	2816
	CAAGCCAGCGACGCAGTGCCAGG	1116	CAAGCCAGCGACGCAGUGCC	2817
	AAACCTCAGCGCCGCGCCTTTGG	1117	AAACCUCAGCGCCGCGCCUU	2818
	TGAACGCGTGGAGGGGCGCTTGG	1118	UGAACGCGUGGAGGGGCGCU	2819
	CTAACCTGGCACTGCGTCGCTGG	1119	CUAACCUGGCACUGCGUCGC	2820
	CGTCAGAGCGCCGAGGTTGGGGG	1120	CGUCAGAGCGCCGAGGUUGG	2821
	GGCCGAGATGTCTCGCTCCGTGG	1121	GGCCGAGAUGUCUCGCUCCG	2822
	GCTAGGACATGCGAACTTAGCGG	1122	GCUAGGACAUGCGAACUUAG	2823
	CGCTGAGGTTTGTGAACGCGTGG	1123	CGCUGAGGUUUGUGAACGCG	2824
	GGGCGCGCACCCCAGATCGGAGG	1124	GGGCGCGCACCCCAGAUCGG	2825
	AAACGCGTGCCCAGCCAATCAGG	1125	AAACGCGUGCCCAGCCAAUC	2826
	TGCAGGTCCGAGCAGTTAACTGG	1126	UGCAGGUCCGAGCAGUUAAC	2827
	GGACACCGGGCGCTCATTCTAGG	1127	GGACACCGGGCGCUCAUUCU	2828
	CCGCTCTTTCGCGGGGCCTCTGG	1128	CCGCUCUUUCGCGGGGCCUC	2829
	GTAGGCTCGTCCCAAAGGCGCGG	1129	GUAGGCUCGUCCCAAAGGCG	2830
	TCCGAGCAGTTAACTGGCTGGGG	1130	UCCGAGCAGUUAACUGGCUG	2831
	TAGTCCAGGGCTGGATCTCGGGG	1131	UAGUCCAGGGCUGGAUCUCG	2832
	CTAGGACATGCGAACTTAGCGGG	1132	CUAGGACAUGCGAACUUAGC	2833
	AGTGGAGGCGTCGCGCTGGCGGG	1133	AGUGGAGGCGUCGCGCUGGC	2834
	TCTATGTGGGGCCACACCGTGGG	1134	UCUAUGUGGGGCCACACCGU	2835
	CTATGTGGGGCCACACCGTGGGG	1135	CUAUGUGGGGCCACACCGUG	2836
	GCGTCAGAGCGCCGAGGTTGGGG	1136	GCGUCAGAGCGCCGAGGUUG	2837
	GCGCCCGGTGTCCCAAGCTGGGG	1137	GCGCCCGGUGUCCCAAGCUG	2838
	CGCAGCAGACAGGCTTACCCGGG	1138	CGCAGCAGACAGGCUUACCC	2839
	CAATCAGGACAAGGCCCGCAGGG	1139	CAAUCAGGACAAGGCCCGCA	2840
	GAGTCTCGTGATGTTTAAGAAGG	1140	GAGUCUCGUGAUGUUUAAGA	2841
	TGGATCTCGGGGAAGCGGCGGGG	1141	UGGAUCUCGGGGAAGCGGCG	2842
	CATCACGAGACTCTAAGAAAAGG	1142	CAUCACGAGACUCUAAGAAA	2843
	GGGCAAGTAGCGCGCGTCCCGGG	1143	GGGCAAGUAGCGCGCGUCCC	2844
	GAGGGTCGGGACAAAGTTTAGGG	1144	GAGGGUCGGGACAAAGUUUA	2845
	GCCCCTTTCGGCGGGGAGCAGGG	1145	GCCCCUUUCGGCGGGGAGCA	2846
	TCTCCTTGGTGGCCCGCCGTGGG	1146	UCUCCUUGGUGGCCCGCCGU	2847
	TCCCCTGCTCCCCGCCGAAAGGG	1147	UCCCCUGCUCCCCGCCGAAA	2848
	GCCCGCCGTGGGGCTAGTCCAGG	1148	GCCCGCCGUGGGGCUAGUCC	2849
	TGCCCCTTTCGGCGGGGAGCAGG	1149	UGCCCCUUUCGGCGGGGAGC	2850
	GAGGTTTGTGAACGCGTGGAGGG	1150	GAGGUUUGUGAACGCGUGGA	2851
	TGGGGTGCGCGCCCCAGCTTGGG	1151	UGGGGUGCGCGCCCCAGCUU	2852

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AGGTTTGTGAACGCGTGGAGGGG	1152	AGGUUUGUGAACGCGUGGAG	2853
	GCCCGAATGCTGTCAGCTTCAGG	1153	GCCCGAAUGCUGUCAGCUUC	2854
	GAGAGCTGTGGACTTCGTCTAGG	1154	GAGAGCUGUGGACUUCGUCU	2855
	CTAGCACCTCTGGGTCTATGTGG	1155	CUAGCACCUCUGGGUCUAUG	2856
	CCGGGTAAGCCTGTCTGCTGCGG	1156	CCGGGUAAGCCUGUCUGCUG	2857
	CGCAGTGCCAGGTTAGAGAGAGG	1157	CGCAGUGCCAGGUUAGAGAG	2858
	TGAGGTTTGTGAACGCGTGGAGG	1158	UGAGGUUUGUGAACGCGUGG	2859
	AGCCCCACGGCGGGCCACCAAGG	1159	AGCCCCACGGCGGGCCACCA	2860
	TGCGTCGCTGGCTTGGAGACAGG	1160	UGCGUCGCUGGCUUGGAGAC	2861
	GGCCACGGAGCGAGACATCTCGG	1161	GGCCACGGAGCGAGACAUCU	2862
	GCGGGCCACCAAGGAGAACTTGG	1162	GCGGGCCACCAAGGAGAACU	2863
	TTCTCCTTGGTGGCCCGCCGTGG	1163	UUCUCCUUGGUGGCCCGCCG	2864
	CCTGCGGGCCTTGTCCTGATTGG	1164	CCUGCGGGCCUUGUCCUGAU	2865
	GCCCCAGCCAGTTAACTGCTCGG	1165	GCCCCAGCCAGUUAACUGCU	2866
	CTGGATCTCGGGGAAGCGGCGGG	1166	CUGGAUCUCGGGGAAGCGGC	2867
	CTCGCGCTACTCTCTCTTTCTGG	1167	CUCGCGCUACUCUCUCUUUC	2868
	CGCGAGCACAGCTAAGGCCACGG	1168	CGCGAGCACAGCUAAGGCCA	2869
	GAAAGTCCCTCTCTCTAACCTGG	1169	GAAAGUCCCUCUCUCUAACC	2870
	GTCCCAAAGGCGCGGCGCTGAGG	1170	GUCCCAAAGGCGCGGCGCUG	2871
	CGGGCCTTGTCCTGATTGGCTGG	1171	CGGGCCUUGUCCUGAUUGGC	2872
	CGGAGCGAGAGAGCACAGCGAGG	1172	CGGAGCGAGAGAGCACAGCG	2873
	GTCTATGTGGGGCCACACCGTGG	1173	GUCUAUGUGGGGCCACACCG	2874
	CGGCTCTGCTTCCCTTAGACTGG	1174	CGGCUCUGCUUCCCUUAGAC	2875
	CTCATTCTAGGACTTCAGGCTGG	1175	CUCAUUCUAGGACUUCAGGC	2876
	CGCGCCCCAGCTTGGGACACCGG	1176	CGCGCCCCAGCUUGGGACAC	2877
	AGGGTAGGAGAGACTCACGCTGG	1177	AGGGUAGGAGAGACUCACGC	2878
HLA-A	GGCGCTTCCTCCGCGGGTACCGG	1178	GGCGCUUCCUCCGCGGGUAC	2879
	GAGTCCCGGTGGGTGCGTGCGGG	1179	GAGUCCCGGUGGGUGCGUGC	2880
	CAGACTGACCGAGTGGACCTGGG	1180	CAGACUGACCGAGUGGACCU	2881
	TACCGGCAGGACGCCTACGACGG	1181	UACCGGCAGGACGCCUACGA	2882
	TGGTACAGGATCTGGAACCCAGG	1182	UGGUACAGGAUCUGGAACCC	2883
	CGTCCTGCCGGTACCCGCGGAGG	1183	CGUCCUGCCGGUACCCGCGG	2884
	AGGCGTCCTGCCGGTACCCGCGG	1184	AGGCGUCCUGCCGGUACCCG	2885
	CTTCCTCCGCGGGTACCGGCAGG	1185	CUUCCUCCGCGGGUACCGGC	2886
	ACAGACTGACCGAGTGGACCTGG	1186	ACAGACUGACCGAGUGGACC	2887
	CCAGTCACAGACTGACCGAGTGG	1187	CCAGUCACAGACUGACCGAG	2888
	TGCCGTCGTAGGCGTCCTGCCGG	1188	UGCCGUCGUAGGCGUCCUGC	2889
	CAGGATGAAGGACCCTACGTAGG	1189	CAGGAUGAAGGACCCUACGU	2890
	GACCAACCCGGGGGGATTTTTGG	1190	GACCAACCCGGGGGGAUUUU	2891
	TACAGGATCTGGAACCCAGGAGG	1191	UACAGGAUCUGGAACCCAGG	2892
	ACGACACTGATTGGCTTCTCTGG	1192	ACGACACUGAUUGGCUUCUC	2893
	GGCCAAAAATCCCCCCGGGTTGG	1193	GGCCAAAAAUCCCCCCGGGU	2894

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GGCCCGTCCGTGGGGGATGAGGG	1194	GGCCCGUCCGUGGGGGAUGA	2895
	TCCTGGCGGGGGCGCAGGACCGG	1195	UCCUGGCGGGGGCGCAGGAC	2896
	GCGACCGCGACGACACTGATTGG	1196	GCGACCGCGACGACACUGAU	2897
	CTACGTAGGGTCCTTCATCCTGG	1197	CUACGUAGGGUCCUUCAUCC	2898
	TTTAGGCCAAAAATCCCCCCGGG	1198	UUUAGGCCAAAAAUCCCCCC	2899
	GAGGGTTCGGGGCGCCATGACGG	1199	GAGGGUUCGGGGCGCCAUGA	2900
	TGAAGGACCCTACGTAGGTTGGG	1200	UGAAGGACCCUACGUAGGUU	2901
	CGCCTCTGCGGGGAGAAGCAAGG	1201	CGCCUCUGCGGGGAGAAGCA	2902
	GCCCGTCCGTGGGGGATGAGGGG	1202	GCCCGUCCGUGGGGGAUGAG	2903
	ACCCCTCATCCCCCACGGACGGG	1203	ACCCCUCAUCCCCCACGGAC	2904
	TCAGGACCCCTCATCCCCCACGG	1204	UCAGGACCCCUCAUCCCCCA	2905
	TGGGCGACCTGGCCCGTCCGTGG	1205	UGGGCGACCUGGCCCGUCCG	2906
	GACCCTACGTAGGTTGGGAGAGG	1206	GACCCUACGUAGGUUGGGAG	2907
	GACGCCGAGGATGGCCGTCATGG	1207	GACGCCGAGGAUGGCCGUCA	2908
	TTCACATCCGTGTCCCGGCCCGG	1208	UUCACAUCCGUGUCCCGGCC	2909
	GACGGCCATCCTCGGCGTCTGGG	1209	GACGGCCAUCCUCGGCGUCU	2910
	ACCCTACGTAGGTTGGGAGAGGG	1210	ACCCUACGUAGGUUGGGAGA	2911
	ACGGCCATCCTCGGCGTCTGGGG	1211	ACGGCCAUCCUCGGCGUCUG	2912
	ATGAAGGACCCTACGTAGGTTGG	1212	AUGAAGGACCCUACGUAGGU	2913
	AAGCAAGGGGCCCTCCTGGCGGG	1213	AAGCAAGGGGCCCUCCUGGC	2914
	TGACGGCCATCCTCGGCGTCTGG	1214	UGACGGCCAUCCUCGGCGUC	2915
	AGGCGCCTGGGCCTCTCCCGGGG	1215	AGGCGCCUGGGCCUCUCCCG	2916
	ATTTCTTCACATCCGTGTCCCGG	1216	AUUUCUUCACAUCCGUGUCC	2917
	TCTCCCGGGGCAAGGGTCTCGGG	1217	UCUCCCGGGGCAAGGGUCUC	2918
	TCCCTCTCCCAACCTACGTAGGG	1218	UCCCUCUCCCAACCUACGUA	2919
	GGGCGACCTGGCCCGTCCGTGGG	1219	GGGCGACCUGGCCCGUCCGU	2920
	GAAGCAAGGGGCCCTCCTGGCGG	1220	GAAGCAAGGGGCCCUCCUGG	2921
	GTCTCGGGGTCCCGCGGCTTCGG	1221	GUCUCGGGGUCCCGCGGCUU	2922
	GCGGAGTTGGGGAATCCCCAAGG	1222	GCGGAGUUGGGGAAUCCCCA	2923
	GCGCCCGCGGCTCCATCCTCTGG	1223	GCGCCCGCGGCUCCAUCCUC	2924
	TCTCGGGGTCCCGCGGCTTCGGG	1224	UCUCGGGGUCCCGCGGCUUC	2925
	GACCCCTCATCCCCCACGGACGG	1225	GACCCCUCAUCCCCCACGGA	2926
	AGCAAGGGGCCCTCCTGGCGGGG	1226	AGCAAGGGGCCCUCCUGGCG	2927
	CATCCTGGATACTCACGACGCGG	1227	CAUCCUGGAUACUCACGACG	2928
TGFBR2	TCGGTCTATGACGAGCAGCGGGG	1228	UCGGUCUAUGACGAGCAGCG	2929
	GAGTGAGTCACTCGCGCGCACGG	1229	GAGUGAGUCACUCGCGCGCA	2930
	TGCTGGCGATACGCGTCCACAGG	1230	UGCUGGCGAUACGCGUCCAC	2931
	CGTTGTGTTGGCCGCGTTCGAGG	1231	CGUUGUGUUGGCCGCGUUCG	2932
	GTGGGGGCTCGCCTCGAACGCGG	1232	GUGGGGGCUCGCCUCGAACG	2933
	TGGGCACGCGGCATCGCCATGGG	1233	UGGGCACGCGGCAUCGCCAU	2934
	CTTTCCTCGTTTCCGCCCGGGGG	1234	CUUUCCUCGUUUCCGCCCGG	2935
	GCACGCGGCATCGCCATGGGCGG	1235	GCACGCGGCAUCGCCAUGGG	2936

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GAAACTCCTCGCCAACAGCTGGG	1236	GAAACUCCUCGCCAACAGCU	2937
	GCCCGACTCCCGTAGCTGCAGGG	1237	GCCCGACUCCCGUAGCUGCA	2938
	GACTGTCAAGCGCAGCGGAGAGG	1238	GACUGUCAAGCGCAGCGGAG	2939
	AGTCGGCCAAAGCTCTCGGAGGG	1239	AGUCGGCCAAAGCUCUCGGA	2940
	TGGTTATCTGAAGGCGGCCGGGG	1240	UGGUUAUCUGAAGGCGGCCG	2941
	AACGTGCGGTGGGATCGTGCTGG	1241	AACGUGCGGUGGGAUCGUGC	2942
	ACTTTCCTCGTTTCCGCCCGGGG	1242	ACUUUCCUCGUUUCCGCCCG	2943
	TCTCCGCTGCGCTTGACAGTCGG	1243	UCUCCGCUGCGCUUGACAGU	2944
	GGACGATGTGCAGCGGCCACAGG	1244	GGACGAUGUGCAGCGGCCAC	2945
	CTCGGTCTATGACGAGCAGCGGG	1245	CUCGGUCUAUGACGAGCAGC	2946
	GTGGGCACGCGGCATCGCCATGG	1246	GUGGGCACGCGGCAUCGCCA	2947
	GTCGGCCAAAGCTCTCGGAGGGG	1247	GUCGGCCAAAGCUCUCGGAG	2948
	TCACCCGACTTCTGAACGTGCGG	1248	UCACCCGACUUCUGAACGUG	2949
	ACGTTCAGAAGTCGGGTGAGTGG	1249	ACGUUCAGAAGUCGGGUGAG	2950
	GTTCAGTTGCAAGGGGCGCGGGG	1250	GUUCAGUUGCAAGGGGCGCG	2951
	CGGCATCGCCATGGGCGGAGTGG	1251	CGGCAUCGCCAUGGGCGGAG	2952
	GCTCGGTCTATGACGAGCAGCGG	1252	GCUCGGUCUAUGACGAGCAG	2953
	GACAGTCGGGCCCGGCAACCCGG	1253	GACAGUCGGGCCCGGCAACC	2954
	CGCGTGCACCCGCTCGGGACAGG	1254	CGCGUGCACCCGCUCGGGAC	2955
	CTCCGCTGCGCTTGACAGTCGGG	1255	CUCCGCUGCGCUUGACAGUC	2956
	TGGCGAGCGGGCGCCACATCTGG	1256	UGGCGAGCGGGCGCCACAUC	2957
	AACTTCAACTCAGCGCTGCGGGG	1257	AACUUCAACUCAGCGCUGCG	2958
	ACTTCAACTCAGCGCTGCGGGGG	1258	ACUUCAACUCAGCGCUGCGG	2959
	GTCCCGAGCGGGTGCACGCGCGG	1259	GUCCCGAGCGGGUGCACGCG	2960
	AGCCCGACTCCCGTAGCTGCAGG	1260	AGCCCGACUCCCGUAGCUGC	2961
	AACTTTCCTCGTTTCCGCCCGGG	1261	AACUUUCCUCGUUUCCGCCC	2962
	GCCTTTCCTGCTCGCACAAAGGG	1262	GCCUUUCCUGCUCGCACAAA	2963
	GGCCCGACTGTCAAGCGCAGCGG	1263	GGCCCGACUGUCAAGCGCAG	2964
	GTCGGGCTGCGTGAGTGTCGCGG	1264	GUCGGGCUGCGUGAGUGUCG	2965
	TTGGTCCCCTTTGTGCGAGCAGG	1265	UUGGUCCCCUUUGUGCGAGC	2966
	CGCAGCGGACGGCGCCTTCCCGG	1266	CGCAGCGGACGGCGCCUUCC	2967
	CTCGTTTCCGCCCGGGGGCCGGG	1267	CUCGUUUCCGCCCGGGGGCC	2968
	CCTCGTTTCCGCCCGGGGGCCGG	1268	CCUCGUUUCCGCCCGGGGGC	2969
	AGTCCGGCTCCTGTCCCGAGCGG	1269	AGUCCGGCUCCUGUCCCGAG	2970
	GTGGCCGTCTCCAGGAGCTAAGG	1270	GUGGCCGUCUCCAGGAGCUA	2971
	GGCAGCTACGAGAGAGCTAGGGG	1271	GGCAGCUACGAGAGAGCUAG	2972
	TCAAGCGCAGCGGAGAGGCGGGG	1272	UCAAGCGCAGCGGAGAGGCG	2973
	CCCACCGCACGTTCAGAAGTCGG	1273	CCCACCGCACGUUCAGAAGU	2974
	TGGCAGCTACGAGAGAGCTAGGG	1274	UGGCAGCUACGAGAGAGCUA	2975
	GAGCTGGCCTTTTGAACGGGTGG	1275	GAGCUGGCCUUUUGAACGGG	2976
	TGTCAAGCGCAGCGGAGAGGCGG	1276	UGUCAAGCGCAGCGGAGAGG	2977
	CGAGCAGCGGGGTCTGCCATGGG	1277	CGAGCAGCGGGGUCUGCCAU	2978

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TTCTTTAGGTCGAAGTCTAGAGG	4539	UUCUUUAGGUCGAAGUCUAG	4553
	GTGCTCGCGACTCAATAGATTGG	4540	GUGCUCGCGACUCAAUAGAU	4554
	AACGCATCTCTAAAGCACCTAGG	4541	AACGCAUCUCUAAAGCACCU	4555
	CTGATCTACTAGGGAAAACGTGG	4542	CUGAUCUACUAGGGAAAACG	4556
	TTGAGTAAATACTTGGAGCGAGG	4543	UUGAGUAAAUACUUGGAGCG	4557
	GGGGCCTCCCCGCGCCTCGCCGG	4544	GGGGCCUCCCCGCGCCUCGC	4558
	CCTGAGCAGCCCCCGACCCATGG	4545	CCUGAGCAGCCCCCGACCCA	4559
A2AR	AAGGTTCATGCGAGCGCGCGGGG	1278	AAGGUUCAUGCGAGCGCGCG	2979
	GAAGGTTCATGCGAGCGCGCGGG	1279	GAAGGUUCAUGCGAGCGCGC	2980
	ATTTGGCGCAAGGCGGCCCAAGG	1280	AUUUGGCGCAAGGCGGCCCA	2981
	TCCTGGAAGGACGATCCCGCAGG	1281	UCCUGGAAGGACGAUCCCGC	2982
	CGAAGGTTCATGCGAGCGCGCGG	1282	CGAAGGUUCAUGCGAGCGCG	2983
	GTCTGCGGCGCATGGACGGACGG	1283	GUCUGCGGCGCAUGGACGGA	2984
	TCCGTCCCCCGTCGTCTCCTGGG	1284	UCCGUCCCCCGUCGUCUCCU	2985
	TCCGTCCATGCGCCGCAGACCGG	1285	UCCGUCCAUGCGCCGCAGAC	2986
	AACTGCACCGGAAGGCGCGCAGG	1286	AACUGCACCGGAAGGCGCGC	2987
	GTGGCGGCTCTCGAGGGATTTGG	1287	GUGGCGGCUCUCGAGGGAUU	2988
	ACCTGCGGGATCGTCCTTCCAGG	1288	ACCUGCGGGAUCGUCCUUCC	2989
	ATCCGTCCCCCGTCGTCTCCTGG	1289	AUCCGUCCCCCGUCGUCUCC	2990
	CGCGCCTTCCGGTGCAGTTTGGG	1290	CGCGCCUUCCGGUGCAGUUU	2991
	CTCGGTTTCTCCGCGCAGCGGGG	1291	CUCGGUUUCUCCGCGCAGCG	2992
	CTGTCCCAAACTGCACCGGAAGG	1292	CUGUCCCAAACUGCACCGGA	2993
	CGAGCTGTCCCAAACTGCACCGG	1293	CGAGCUGUCCCAAACUGCAC	2994
	GTCGCGGCCTCGTCCTGACAGGG	1294	GUCGCGGCCUCGUCCUGACA	2995
	AGGACTCGGACCCCGCGCCGGGG	1295	AGGACUCGGACCCCGCGCCG	2996
	TCACGTCCCAGGCGCAGTTGCGG	1296	UCACGUCCCAGGCGCAGUUG	2997
	TGTCAGGACGAGGCCGCGACGGG	1297	UGUCAGGACGAGGCCGCGAC	2998
	ACCGGAAGGCGCGCAGGGGTAGG	1298	ACCGGAAGGCGCGCAGGGGU	2999
	CTCTCGAGGGATTTGGCGCAAGG	1299	CUCUCGAGGGAUUUGGCGCA	3000
	TTCATGCGAGCGCGCGGGGCCGG	1300	UUCAUGCGAGCGCGCGGGGC	3001
	TTTCTCCGCGCAGCGGGGCGGGG	1301	UUUCUCCGCGCAGCGGGGCG	3002
	GCCCGGGACGCGCCGAGAAAGGG	1302	GCCCGGGACGCGCCGAGAAA	3003
	TCGAGGGATTTGGCGCAAGGCGG	1303	UCGAGGGAUUUGGCGCAAGG	3004
	CGGCGGGAAAGGAACCCTGAGGG	1304	CGGCGGGAAAGGAACCCUGA	3005
	GCGCCGGGGAACTGGTCTCGGGG	1305	GCGCCGGGGAACUGGUCUCG	3006
	GAGACCAGTTCCCCGGCGCGGGG	1306	GAGACCAGUUCCCCGGCGCG	3007
	AGGATCGCCTGCGGGCCTCGCGG	1307	AGGAUCGCCUGCGGGCCUCG	3008
	CCGAGCGCTGGCGTCTTCCGTGG	1308	CCGAGCGCUGGCGUCUUCCG	3009
	CCGGGACGCGCCGAGAAAGGGGG	1309	CCGGGACGCGCCGAGAAAGG	3010
	AAAGTCTGAGTGCGGGACACAGG	1310	AAAGUCUGAGUGCGGGACAC	3011
	TTTGGCGCAAGGCGGCCCAAGGG	1311	UUUGGCGCAAGGCGGCCCAA	3012
	GGGAATGGTGGCGGCTCTCGAGG	1312	GGGAAUGGUGGCGGCUCUCG	3013

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CACGCCGGCTCCCGCTGTCTCGG	1313	CACGCCGGCUCCCGCUGUCU	3014
	CGTCGCGGCCTCGTCCTGACAGG	1314	CGUCGCGGCCUCGUCCUGAC	3015
	CGCCCGGGACGCGCCGAGAAAGG	1315	CGCCCGGGACGCGCCGAGAA	3016
	GGCCTCGCGGGCCGATGCCTCGG	1316	GGCCUCGCGGGCCGAUGCCU	3017
	TGCAGTTTGGGACAGCTCGGAGG	1317	UGCAGUUUGGGACAGCUCGG	3018
	CTGACCTGCCGCTCGCACGCCGG	1318	CUGACCUGCCGCUCGCACGC	3019
	GCCGGTCTGCGGCGCATGGACGG	1319	GCCGGUCUGCGGCGCAUGGA	3020
	GCGCGCCTTCCGGTGCAGTTTGG	1320	GCGCGCCUUCCGGUGCAGUU	3021
	GCCTCGGTTTCTCCGCGCAGCGG	1321	GCCUCGGUUUCUCCGCGCAG	3022
	GCCGGCTCCCGCTGTCTCGGCGG	1322	GCCGGCUCCCGCUGUCUCGG	3023
	ACCCGAGGCATCGGCCCGCGAGG	1323	ACCCGAGGCAUCGGCCCGCG	3024
	GGGACGGATGCGAGCCCGGGAGG	1324	GGGACGGAUGCGAGCCCGGG	3025
	GGCCCGCAGGCGATCCTGGAAGG	1325	GGCCCGCAGGCGAUCCUGGA	3026
	TAGTTGCCCCGACTGTACCATGG	1326	UAGUUGCCCCGACUGUACCA	3027
	AAGGCGCGCAGGGGTAGGCGGGG	1327	AAGGCGCGCAGGGGUAGGCG	3028
	GAGACGACGGGGGACGGATGGGG	1328	GAGACGACGGGGGACGGAUG	3029
	GCGGCGCCGCAACTGCGCCTGGG	1329	GCGGCGCCGCAACUGCGCCU	3030
	CCTCGGTTTCTCCGCGCAGCGGG	1330	CCUCGGUUUCUCCGCGCAGC	3031
	CGTGCGAGCGGCAGGTCAGCCGG	1331	CGUGCGAGCGGCAGGUCAGC	3032
	CGGGGGATGTGGCGCGGTCCAGG	1332	CGGGGGAUGUGGCGCGGUCC	3033
	CGGCGGGACGGATGCGAGCCCGG	1333	CGGCGGGACGGAUGCGAGCC	3034
	CGATGCCTCGGGTCCCCCTCCGG	1334	CGAUGCCUCGGGUCCCCCUC	3035
	ACTGCACCGGAAGGCGCGCAGGG	1335	ACUGCACCGGAAGGCGCGCA	3036
	GGAGACGACGGGGGACGGATGGG	1336	GGAGACGACGGGGGACGGAU	3037
	GTTTCTCCGCGCAGCGGGGCGGG	1337	GUUUCUCCGCGCAGCGGGGC	3038
	CTTCGCGAGCTCCTCCAGCAGGG	1338	CUUCGCGAGCUCCUCCAGCA	3039
	CTGGCGTCTTCCGTGGACAGTGG	1339	CUGGCGUCUUCCGUGGACAG	3040
	GCGGCGCATGGACGGACGGACGG	1340	GCGGCGCAUGGACGGACGGA	3041
	GCAGAGATACCCGAGCGCCCGGG	1341	GCAGAGAUACCCGAGCGCCC	3042
	CGGGCGGAGACCGGTTCCCCGGG	1342	CGGGCGGAGACCGGUUCCCC	3043
	GGTTTCTCCGCGCAGCGGGGCGG	1343	GGUUUCUCCGCGCAGCGGGG	3044
	TGCGAGCGGCAGGTCAGCCGGGG	1344	UGCGAGCGGCAGGUCAGCCG	3045
	ACCGGTTCCCCGGGAAGGTGAGG	1345	ACCGGUUCCCCGGGAAGGUG	3046
	GGAACTGGTCTCGGGGCGGCGGG	1346	GGAACUGGUCUCGGGGCGGC	3047
	AGACGACGGGGGACGGATGGGGG	1347	AGACGACGGGGGACGGAUGG	3048
	CGAGACCAGTTCCCCGGCGCGGG	1348	CGAGACCAGUUCCCCGGCGC	3049
	TGGGGCCCGGAGCGCTCCAAGGG	1349	UGGGGCCCGGAGCGCUCCAA	3050
	GTCAGCCGGGGTGCTAGGTCTGG	1350	GUCAGCCGGGGUGCUAGGUC	3051
	CCGGGCGGAGACCGGTTCCCCGG	1351	CCGGGCGGAGACCGGUUCCC	3052
	CTGCACCGGAAGGCGCGCAGGGG	1352	CUGCACCGGAAGGCGCGCAG	3053
	GCGTCTTCCGTGGACAGTGGTGG	1353	GCGUCUUCCGUGGACAGUGG	3054
	GGCAGAGATACCCGAGCGCCCGG	1354	GGCAGAGAUACCCGAGCGCC	3055

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CGCCATTCCTACCTCCGCTCCGG	1355	CGCCAUUCCUACCUCCGCUC	3056
	TTCCTCCCATGGTACAGTCGGGG	1356	UUCCUCCCAUGGUACAGUCG	3057
	GGTGCTAGGTCTGGCGTGCGGGG	1357	GGUGCUAGGUCUGGCGUGCG	3058
	GGAATGGTGGCGGCTCTCGAGGG	1358	GGAAUGGUGGCGGCUCUCGA	3059
	CTGTCAGGACGAGGCCGCGACGG	1359	CUGUCAGGACGAGGCCGCGA	3060
	CGGAGCCGCAGGTAGCGGGCGGG	1360	CGGAGCCGCAGGUAGCGGGC	3061
	GCCTACCCCTGCGCGCCTTCCGG	1361	GCCUACCCCUGCGCGCCUUC	3062
	GGCGCAGAGGCGCTTCCTGAGGG	1362	GGCGCAGAGGCGCUUCCUGA	3063
	TGCGCGCCTCGGACTGGCCCCGG	1363	UGCGCGCCUCGGACUGGCCC	3064
	TGCCCCGACTGTACCATGGGAGG	1364	UGCCCCGACUGUACCAUGGG	3065
	GCGCAGAGGCGCTTCCTGAGGGG	1365	GCGCAGAGGCGCUUCCUGAG	3066
	GTCCTTCCAGGATCGCCTGCGGG	1366	GUCCUUCCAGGAUCGCCUGC	3067
	CGCCCCCGGTCCATCCCTGCTGG	1367	CGCCCCCGGUCCAUCCCUGC	3068
	GGGTGCTAGGTCTGGCGTGCGGG	1368	GGGUGCUAGGUCUGGCGUGC	3069
	GGTGGGTGCGCGCCTCGGACTGG	1369	GGUGGGUGCGCGCCUCGGAC	3070
	CGCGGAGAAACCGAGGCCGGAGG	1370	CGCGGAGAAACCGAGGCCGG	3071
	CTGCAGGGGGCGCCCGTGAGCGG	1371	CUGCAGGGGGCGCCCGUGAG	3072
	GCCCGGAGCGCTCCAAGGGGCGG	1372	GCCCGGAGCGCUCCAAGGGG	3073
	AAGTCTGAGTGCGGGACACAGGG	1373	AAGUCUGAGUGCGGGACACA	3074
	CGTCCTTCCAGGATCGCCTGCGG	1374	CGUCCUUCCAGGAUCGCCUG	3075
	GCGGCGGGAAAGGAACCCTGAGG	1375	GCGGCGGGAAAGGAACCCUG	3076
	CGGTGCAGTTTGGGACAGCTCGG	1376	CGGUGCAGUUUGGGACAGCU	3077
	CCAGCGGCCGCCGAGACAGCGGG	1377	CCAGCGGCCGCCGAGACAGC	3078
FAS	CGGTTTACGAGTGACTTGGCTGG	1378	CGGUUUACGAGUGACUUGGC	3079
	AACTTGGCCTGCGCGCGGGTAGG	1379	AACUUGGCCUGCGCGCGGGU	3080
	GACCCGCTCAGTACGGAGTTGGG	1380	GACCCGCUCAGUACGGAGUU	3081
	CTATCCCCGGGACTAAGACGGGG	1381	CUAUCCCCGGGACUAAGACG	3082
	CGAAGCAGTGGTTAAGCCGGAGG	1382	CGAAGCAGUGGUUAAGCCGG	3083
	CCCGTCTTAGTCCCGGGGATAGG	1383	CCCGUCUUAGUCCCGGGGAU	3084
	GGACGCGTGCGGGATTGCGGCGG	1384	GGACGCGUGCGGGAUUGCGG	3085
	TGCCGTTCTTCCGAGCCCTCCGG	1385	UGCCGUUCUUCCGAGCCCUC	3086
	GTTGGTGGACCCGCTCAGTACGG	1386	GUUGGUGGACCCGCUCAGUA	3087
	CCAAAGGTCCGCTCCGGCGCGGG	1387	CCAAAGGUCCGCUCCGGCGC	3088
	ATGCGAAGTGCTGACCCCGCTGG	1388	AUGCGAAGUGCUGACCCCGC	3089
	GCCGGAGCGGACCTTTGGCTTGG	1389	GCCGGAGCGGACCUUUGGCU	3090
	CTCGCGCAAGAGTGACACACAGG	1390	CUCGCGCAAGAGUGACACAC	3091
	CTTACCCCGTCTTAGTCCCGGGG	1391	CUUACCCCGUCUUAGUCCCG	3092
	GAAGCGGTTTACGAGTGACTTGG	1392	GAAGCGGUUUACGAGUGACU	3093
	GGGGTCAGCACTTCGCATCAAGG	1393	GGGGUCAGCACUUCGCAUCA	3094
	ACTGCGCTCCACGTTGAGGTGGG	1394	ACUGCGCUCCACGUUGAGGU	3095
	TGCGAAGTGCTGACCCCGCTGGG	1395	UGCGAAGUGCUGACCCCGCU	3096
	GAGCGGGTCCACCAACCCGCGGG	1396	GAGCGGGUCCACCAACCCGC	3097

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AGACGGGGTAAGCCTCCACCCGG	1397	AGACGGGGUAAGCCUCCACC	3098
	ACCCGCTCAGTACGGAGTTGGGG	1398	ACCCGCUCAGUACGGAGUUG	3099
	GCCAAAGGTCCGCTCCGGCGCGG	1399	GCCAAAGGUCCGCUCCGGCG	3100
	GGACCCGCTCAGTACGGAGTTGG	1400	GGACCCGCUCAGUACGGAGU	3101
	GCCTATCCCCGGGACTAAGACGG	1401	GCCUAUCCCCGGGACUAAGA	3102
	GAGCTCACGAAAAGCCCCGGTGG	1402	GAGCUCACGAAAAGCCCCGG	3103
	TGAGCGGGTCCACCAACCCGCGG	1403	UGAGCGGGUCCACCAACCCG	3104
	GAAAAGCCCCGGTGGTCAGGAGG	1404	GAAAAGCCCCGGUGGUCAGG	3105
	GACGAGCTCACGAAAAGCCCCGG	1405	GACGAGCUCACGAAAAGCCC	3106
	GCGTTGGAGACTGGCTCCCGGGG	1406	GCGUUGGAGACUGGCUCCCG	3107
	AGGTCCGCTCCGGCGCGGGTGGG	1407	AGGUCCGCUCCGGCGCGGGU	3108
	GCTTACCCCGTCTTAGTCCCGGG	1408	GCUUACCCCGUCUUAGUCCC	3109
	CTCCACGTTGAGGTGGGCGTGGG	1409	CUCCACGUUGAGGUGGGCGU	3110
	GGCTTACCCCGTCTTAGTCCCGG	1410	GGCUUACCCCGUCUUAGUCC	3111
	GCGGGACGCGTGCGGGATTGCGG	1411	GCGGGACGCGUGCGGGAUUG	3112
	CACGAAAAGCCCCGGTGGTCAGG	1412	CACGAAAAGCCCCGGUGGUC	3113
	GTCAGGGTTCGTTGCACAAATGG	1413	GUCAGGGUUCGUUGCACAAA	3114
	GACGGGGTAAGCCTCCACCCGGG	1414	GACGGGGUAAGCCUCCACCC	3115
	CGTTGGAGACTGGCTCCCGGGGG	1415	CGUUGGAGACUGGCUCCCGG	3116
	TTCTGGCAGTTCTCAGACGTAGG	1416	UUCUGGCAGUUCUCAGACGU	3117
	GTCCCGGGGCGTTCCTGCAGTGG	1417	GUCCCGGGGCGUUCCUGCAG	3118
	GCCAAGCCAAAGGTCCGCTCCGG	1418	GCCAAGCCAAAGGUCCGCUC	3119
	AAGGTCCGCTCCGGCGCGGGTGG	1419	AAGGUCCGCUCCGGCGCGGG	3120
	GCGCACTCACCCACCCGCGCCGG	1420	GCGCACUCACCCACCCGCGC	3121
	CGGAAGTCTGGGAAGCTTTAGGG	1421	CGGAAGUCUGGGAAGCUUUA	3122
	CACCTCAACGTGGAGCGCAGTGG	1422	CACCUCAACGUGGAGCGCAG	3123
	AACCCGGGCGTTCCCCAGCGAGG	1423	AACCCGGGCGUUCCCCAGCG	3124
	TCAGCAACTTGGCCTGCGCGCGG	1424	UCAGCAACUUGGCCUGCGCG	3125
	GGGAAGCTCTTTCACTTCGGAGG	1425	GGGAAGCUCUUUCACUUCGG	3126
	CAGTGGTCTCCGAGGAGCGCCGG	1426	CAGUGGUCUCCGAGGAGCGC	3127
	GTTCCGCTCCTCTCTCCAACCGG	1427	GUUCCGCUCCUCUCUCCAAC	3128
DCK	CGGGGACCGCAGTCACCCCGTGG	1428	CGGGGACCGCAGUCACCCCG	3129
	GGTTTGACTTTGGCGCGCGGAGG	1429	GGUUUGACUUUGGCGCGCGG	3130
	CTTGCGTCCCACATTTCCGGAGG	1430	CUUGCGUCCCACAUUUCCGG	3131
	GCGCGCCTCACAGAGACCGCAGG	1431	GCGCGCCUCACAGAGACCGC	3132
	CTCCGGAAATGTGGGACGCAAGG	1432	CUCCGGAAAUGUGGGACGCA	3133
	CGGGGTTTGACTTTGGCGCGCGG	1433	CGGGGUUUGACUUUGGCGCG	3134
	GAACATCGGTAAGGAGCCTCCGG	1434	GAACAUCGGUAAGGAGCCUC	3135
	AGCTCACTAGCTGACCCGGCAGG	1435	AGCUCACUAGCUGACCCGGC	3136
	GGAAAACCCGCCTCTCTAGTGGG	1436	GGAAAACCCGCCUCUCUAGU	3137
	GTGGCGGCCCAGAGCTCGTCCGG	1437	GUGGCGGCCCAGAGCUCGUC	3138
	CATCGAAGGGAACATCGGTAAGG	1438	CAUCGAAGGGAACAUCGGUA	3139

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TTTGCGAGTTCCCAACAAAGAGG	1439	UUUGCGAGUUCCCAACAAAG	3140
	TGAGCTCACCGGCCCGCCGGCGG	1440	UGAGCUCACCGGCCCGCCGG	3141
	CAGAGCTCGTCCGGCAAAGAGGG	1441	CAGAGCUCGUCCGGCAAAGA	3142
	TAGTGAGCTCACCGGCCCGCCGG	1442	UAGUGAGCUCACCGGCCCGC	3143
	CTCGTCCGGCAAAGAGGGCTGGG	1443	CUCGUCCGGCAAAGAGGGCU	3144
	ACTAGCTGACCCGGCAGGTCAGG	1444	ACUAGCUGACCCGGCAGGUC	3145
	ATCTCCATCGAAGGGAACATCGG	1445	AUCUCCAUCGAAGGGAACAU	3146
	CACGGGGTGACTGCGGTCCCCGG	1446	CACGGGGUGACUGCGGUCCC	3147
	GAGGCCTTCCGCCACACGCGCGG	1447	GAGGCCUUCCGCCACACGCG	3148
	CTTTGTTGGGAACTCGCAAAGGG	1448	CUUUGUUGGGAACUCGCAAA	3149
	CGGGAAGAGGTTCCGGAGTCGGG	1449	CGGGAAGAGGUUCCGGAGUC	3150
	CTTACCGATGTTCCCTTCGATGG	1450	CUUACCGAUGUUCCCUUCGA	3151
	ACGGGGTGACTGCGGTCCCCGGG	1451	ACGGGGUGACUGCGGUCCCC	3152
	GAACTCGCAAAGGGAAGCGGGGG	1452	GAACUCGCAAAGGGAAGCGG	3153
	GAAGGGTATTAGATTTCTTGAGG	1453	GAAGGGUAUUAGAUUUCUUG	3154
	AGTCAAACCCCGACACCCGCCGG	1454	AGUCAAACCCCGACACCCGC	3155
	AGCTCCAGTGCGCGCACCCGTGG	1455	AGCUCCAGUGCGCGCACCCG	3156
	TCTGGGCCGCCACAAGACTAAGG	1456	UCUGGGCCGCCACAAGACUA	3157
	GCGGGAAGAGGTTCCGGAGTCGG	1457	GCGGGAAGAGGUUCCGGAGU	3158
	TGGAAAACCCGCCTCTCTAGTGG	1458	UGGAAAACCCGCCUCUCUAG	3159
	CGGGTGTCGGGGTTTGACTTTGG	1459	CGGGUGUCGGGGUUUGACUU	3160
	CTCTTTGCCGGACGAGCTCTGGG	1460	CUCUUUGCCGGACGAGCUCU	3161
	CCTCTTTGCCGGACGAGCTCTGG	1461	CCUCUUUGCCGGACGAGCUC	3162
	AAACCCCGACACCCGCCGGCGGG	1462	AAACCCCGACACCCGCCGGC	3163
	TGGCGCCCAGTCTGACCCCGGGG	1463	UGGCGCCCAGUCUGACCCCG	3164
	TTGCGAGTTCCCAACAAAGAGGG	1464	UUGCGAGUUCCCAACAAAGA	3165
	CAAACCCCGACACCCGCCGGCGG	1465	CAAACCCCGACACCCGCCGG	3166
	TCTTTGTTGGGAACTCGCAAAGG	1466	UCUUUGUUGGGAACUCGCAA	3167
	CGATGGAGATTTTCTTGATGCGG	1467	CGAUGGAGAUUUUCUUGAUG	3168
	CGCCTCTCTAGTGGGCCTGTTGG	1468	CGCCUCUCUAGUGGGCCUGU	3169
	GCCCCGGCCTTCACGTGACCTGG	1469	GCCCCGGCCUUCACGUGACC	3170
	GCTCGTCCGGCAAAGAGGGCTGG	1470	GCUCGUCCGGCAAAGAGGGC	3171
	TGCGGTCCCCGGGGTCAGACTGG	1471	UGCGGUCCCCGGGGUCAGAC	3172
	AGCCTTGCGTCCCACATTTCCGG	1472	AGCCUUGCGUCCCACAUUUC	3173
	CTGCAGAGAGATGCGGCGAAGGG	1473	CUGCAGAGAGAUGCGGCGAA	3174
	GCGGTCCCCGGGGTCAGACTGGG	1474	GCGGUCCCCGGGGUCAGACU	3175
	CAGCTAGGGAGCGCGGCTTGAGG	1475	CAGCUAGGGAGCGCGGCUUG	3176
	ACGCCAGGTCACGTGAAGGCCGG	1476	ACGCCAGGUCACGUGAAGGC	3177
	CGCCAGGTCACGTGAAGGCCGGG	1477	CGCCAGGUCACGUGAAGGCC	3178
DGKA	ATCCCTCCGAATGAGCGGGAGGG	1478	AUCCCUCCGAAUGAGCGGGA	3179
	ATCCCTCCCGCTCATTCGGAGGG	1479	AUCCCUCCCGCUCAUUCGGA	3180
	GTATCGAGAAGGGTCTGCGCTGG	1480	GUAUCGAGAAGGGUCUGCGC	3181

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTATGTCGTCAGGAACGGGGCGG	1481	CUAUGUCGUCAGGAACGGGG	3182
	GCAGCACGAACGCAGCCCGTGGG	1482	GCAGCACGAACGCAGCCCGU	3183
	CTTCGAAGTTCCCAGAGTCGGGG	1483	CUUCGAAGUUCCCAGAGUCG	3184
	CATCCCTCCGAATGAGCGGGAGG	1484	CAUCCCUCCGAAUGAGCGGG	3185
	GAAACGTTACCCACCGGGTTCGG	1485	GAAACGUUACCCACCGGGUU	3186
	ACGGGCTGCGTTCGTGCTGCTGG	1486	ACGGGCUGCGUUCGUGCUGC	3187
	GAACCTCCACAGTGCCGCACGGG	1487	GAACCUCCACAGUGCCGCAC	3188
	GTTCCTGGGACCCGACTCGGAGG	1488	GUUCCUGGGACCCGACUCGG	3189
	CATCCCTCCCGCTCATTCGGAGG	1489	CAUCCCUCCCGCUCAUUCGG	3190
	AACTTCGAAGTTCCCAGAGTCGG	1490	AACUUCGAAGUUCCCAGAGU	3191
	CTCCGAATGAGCGGGAGGGATGG	1491	CUCCGAAUGAGCGGGAGGGA	3192
	ACTTCGAAGTTCCCAGAGTCGGG	1492	ACUUCGAAGUUCCCAGAGUC	3193
	AAACGTTACCCACCGGGTTCGGG	1493	AAACGUUACCCACCGGGUUC	3194
	CTCCCGCTCATTCGGAGGGATGG	1494	CUCCCGCUCAUUCGGAGGGA	3195
	CCCCCATCGGAAAAGGACAGGGG	1495	CCCCCAUCGGAAAAGGACAG	3196
	CAGCTATGTCGTCAGGAACGGGG	1496	CAGCUAUGUCGUCAGGAACG	3197
	GGAACCTCCACAGTGCCGCACGG	1497	GGAACCUCCACAGUGCCGCA	3198
	GTCTGGGAAACGTTACCCACCGG	1498	GUCUGGGAAACGUUACCCAC	3199
	GGGCGGGCAGCTATGTCGTCAGG	1499	GGGCGGGCAGCUAUGUCGUC	3200
	CGCGGTCGCAGCTGAAGCGCCGG	1500	CGCGGUCGCAGCUGAAGCGC	3201
	TTCGAAGTTCCCAGAGTCGGGGG	1501	UUCGAAGUUCCCAGAGUCGG	3202
	TCTGGGAAACGTTACCCACCGGG	1502	UCUGGGAAACGUUACCCACC	3203
	TATCGAGAAGGGTCTGCGCTGGG	1503	UAUCGAGAAGGGUCUGCGCU	3204
	AGCTGGAGCGGGTATCGAGAAGG	1504	AGCUGGAGCGGGUAUCGAGA	3205
	CCTCCACAGTGCCGCACGGGTGG	1505	CCUCCACAGUGCCGCACGGG	3206
	CCGGTTCCTGGGACCCGACTCGG	1506	CCGGUUCCUGGGACCCGACU	3207
	GAACGCAGCCCGTGGGTCCTCGG	1507	GAACGCAGCCCGUGGGUCCU	3208
	TACCCCTGTCCTTTTCCGATGGG	1508	UACCCCUGUCCUUUUCCGAU	3209
	AAGAGGACTTCCCTCCGAGTCGG	1509	AAGAGGACUUCCCUCCGAGU	3210
	TCACCATCCCTCCGAATGAGCGG	1510	UCACCAUCCCUCCGAAUGAG	3211
	TTCCTGGGACCCGACTCGGAGGG	1511	UUCCUGGGACCCGACUCGGA	3212
	ACCGCGAGCCCTCTCAAGCAAGG	1512	ACCGCGAGCCCUCUCAAGCA	3213
	ACCCCTGTCCTTTTCCGATGGGG	1513	ACCCCUGUCCUUUUCCGAUG	3214
	AGGGTCTGCGCTGGGACGCGGGG	1514	AGGGUCUGCGCUGGGACGCG	3215
	ACTCGGAGGGAAGTCCTCTTCGG	1515	ACUCGGAGGGAAGUCCUCUU	3216
	CTCCCCCATCGGAAAAGGACAGG	1516	CUCCCCCAUCGGAAAAGGAC	3217
	AGAACCCTTCTCCACCCGTGCGG	1517	AGAACCCUUCUCCACCCGUG	3218
	GGTCTCTACCTTGCTTGAGAGGG	1518	GGUCUCUACCUUGCUUGAGA	3219
	CAGTGCCGCACGGGTGGAGAAGG	1519	CAGUGCCGCACGGGUGGAGA	3220
	GCAGCTATGTCGTCAGGAACGGG	1520	GCAGCUAUGUCGUCAGGAAC	3221
	CTGTCCTTTTCCGATGGGGGAGG	1521	CUGUCCUUUUCCGAUGGGGG	3222
	TTCCCTCCGAGTCGGGTCCCAGG	1522	UUCCCUCCGAGUCGGGUCCC	3223

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AGTGCCGCACGGGTGGAGAAGGG	1523	AGUGCCGCACGGGUGGAGAA	3224
	CACCATCCCTCCGAATGAGCGGG	1524	CACCAUCCCUCCGAAUGAGC	3225
	CGAGTCGGGTCCCAGGAACCGGG	1525	CGAGUCGGGUCCCAGGAACC	3226
	AGAGGACTTCCCTCCGAGTCGGG	1526	AGAGGACUUCCCUCCGAGUC	3227
	GGGTCTCTACCTTGCTTGAGAGG	1527	GGGUCUCUACCUUGCUUGAG	3228
USP16	TAACTGCTCCGATCCCACGGGGG	1528	UAACUGCUCCGAUCCCACGG	3229
	CTAACTGCTCCGATCCCACGGGG	1529	CUAACUGCUCCGAUCCCACG	3230
	TCTCGACCCCGTGGACCCAGAGG	1530	UCUCGACCCCGUGGACCCAG	3231
	GTCGCTCTCAATTCGTCACCAGG	1531	GUCGCUCUCAAUUCGUCACC	3232
	CGCAGTACCGGAAAGTAGCCGGG	1532	CGCAGUACCGGAAAGUAGCC	3233
	CTTCCCATAATGCCGCGTTCCGG	1533	CUUCCCAUAAUGCCGCGUUC	3234
	TTCCGGTACTGCGATCTCATTGG	1534	UUCCGGUACUGCGAUCUCAU	3235
	CGCCGGATGTTCGGGTTTAGGGG	1535	CGCCGGAUGUUCGGGUUUAG	3236
	ACTTCCGGAACGCGGCATTATGG	1536	ACUUCCGGAACGCGGCAUUA	3237
	GCGCCGGATGTTCGGGTTTAGGG	1537	GCGCCGGAUGUUCGGGUUUA	3238
	TGGCGGCCTTCTCGACCCCGTGG	1538	UGGCGGCCUUCUCGACCCCG	3239
	CGGAAGTTATTGCTTTCCAGGGG	1539	CGGAAGUUAUUGCUUUCCAG	3240
	GTAGCCGGGTTACGTGCTTAAGG	1540	GUAGCCGGGUUACGUGCUUA	3241
	TCGCAGTACCGGAAAGTAGCCGG	1541	UCGCAGUACCGGAAAGUAGC	3242
	AGCCAATGAGATCGCAGTACCGG	1542	AGCCAAUGAGAUCGCAGUAC	3243
	GCGGCTTGCGCCGGATGTTCGGG	1543	GCGGCUUGCGCCGGAUGUUC	3244
	TGCGCCGGATGTTCGGGTTTAGG	1544	UGCGCCGGAUGUUCGGGUUU	3245
	TGCGGCTTGCGCCGGATGTTCGG	1545	UGCGGCUUGCGCCGGAUGUU	3246
	CACGTAACCCGGCTACTTTCCGG	1546	CACGUAACCCGGCUACUUUC	3247
	GTTATGGGCTCTGTCGCCGTGGG	1547	GUUAUGGGCUCUGUCGCCGU	3248
	GGAGCATTTATATAACTTCGTGG	1548	GGAGCAUUUAUAUAACUUCG	3249
	TTTACTAGCGTCAGAGCCGATGG	1549	UUUACUAGCGUCAGAGCCGA	3250
	ATCCGGCGCAAGCCGCACGCAGG	1550	AUCCGGCGCAAGCCGCACGC	3251
	CTTCCGGAACGCGGCATTATGGG	1551	CUUCCGGAACGCGGCAUUAU	3252
	GGTTACGTGCTTAAGGAGAGCGG	1552	GGUUACGUGCUUAAGGAGAG	3253
	GCTCTCAATTCGTCACCAGGAGG	1553	GCUCUCAAUUCGUCACCAGG	3254
	AAGCAATAACTTCCGGAACGCGG	1554	AAGCAAUAACUUCCGGAACG	3255
	TCAGAGCCGATGGTCCCGGGAGG	1555	UCAGAGCCGAUGGUCCCGGG	3256
	GCCGTGTTCATACGGCTGGTAGG	1556	GCCGUGUUCAUACGGCUGGU	3257
	TCTAACTGCTCCGATCCCACGGG	1557	UCUAACUGCUCCGAUCCCAC	3258
	AGTAAATCTGCGCCTGCGTGCGG	1558	AGUAAAUCUGCGCCUGCGUG	3259
	CCGGAAGTTATTGCTTTCCAGGG	1559	CCGGAAGUUAUUGCUUUCCA	3260
	AACCCCTAAACCCGAACATCCGG	1560	AACCCCUAAACCCGAACAUC	3261
	GTGGCAGGCGCCGAGCAAATGGG	1561	GUGGCAGGCGCCGAGCAAAU	3262
	CGTGGCAGGGACTTCCCTATGGG	1562	CGUGGCAGGGACUUCCCUAU	3263
	TGCAGCCGTGTTCATACGGCTGG	1563	UGCAGCCGUGUUCAUACGGC	3264
	CCTGGCAGTCGTCGCTCGCCTGG	1564	CCUGGCAGUCGUCGCUCGCC	3265

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTGTCGCCGTGGGTGAGTTCTGG	1565	CUGUCGCCGUGGGUGAGUUC	3266
	AGCGACGACTGCCAGGCAGTGGG	1566	AGCGACGACUGCCAGGCAGU	3267
	TGGTGACGAATTGAGAGCGACGG	1567	UGGUGACGAAUUGAGAGCGA	3268
	GAACGCGGCATTATGGGAAGTGG	1568	GAACGCGGCAUUAUGGGAAG	3269
	TCCGGAAGTTATTGCTTTCCAGG	1569	UCCGGAAGUUAUUGCUUUCC	3270
	ATCGGAGCAGTTAGAAGGGGAGG	1570	AUCGGAGCAGUUAGAAGGGG	3271
	CTGGGTCCACGGGGTCGAGAAGG	1571	CUGGGUCCACGGGGUCGAGA	3272
	ATACGGCTGGTAGGAAAAGCAGG	1572	AUACGGCUGGUAGGAAAAGC	3273
	TTCTAACTGCTCCGATCCCACGG	1573	UUCUAACUGCUCCGAUCCCA	3274
	GCGCCGAGCAAATGGGTGGGTGG	1574	GCGCCGAGCAAAUGGGUGGG	3275
	GCGTCAGAGCCGATGGTCCCGGG	1575	GCGUCAGAGCCGAUGGUCCC	3276
	GTGGGATCGGAGCAGTTAGAAGG	1576	GUGGGAUCGGAGCAGUUAGA	3277
	CGCCTGCGTGCGGCTTGCGCCGG	1577	CGCCUGCGUGCGGCUUGCGC	3278
PTPN11	ACGGGTCGGTGGCGTAGACGCGG	1578	ACGGGUCGGUGGCGUAGACG	3279
	ACGGGGCTAACCGAACGCGGCGG	1579	ACGGGGCUAACCGAACGCGG	3280
	CGTCGCGAGCGGTGACATCACGG	1580	CGUCGCGAGCGGUGACAUCA	3281
	TCGGTTAGCCCCGTCCGGAAGGG	1581	UCGGUUAGCCCCGUCCGGAA	3282
	CGGTTAGCCCCGTCCGGAAGGGG	1582	CGGUUAGCCCCGUCCGGAAG	3283
	AACCGAACGCGGCGGTGGCCGGG	1583	AACCGAACGCGGCGGUGGCC	3284
	TAACCGAACGCGGCGGTGGCCGG	1584	UAACCGAACGCGGCGGUGGC	3285
	TCGCGAGCGGTGACATCACGGGG	1585	UCGCGAGCGGUGACAUCACG	3286
	TAGAGCCGCCGAGGGAACCACGG	1586	UAGAGCCGCCGAGGGAACCA	3287
	AACATGACATCGCGGAGGTGAGG	1587	AACAUGACAUCGCGGAGGUG	3288
	GGTTAGCCCCGTCCGGAAGGGGG	1588	GGUUAGCCCCGUCCGGAAGG	3289
	TCGCTCGGTCCTCCGCTGACGGG	1589	UCGCUCGGUCCUCCGCUGAC	3290
	GGGCTAACCGAACGCGGCGGTGG	1590	GGGCUAACCGAACGCGGCGG	3291
	CGCGTTCGGTTAGCCCCGTCCGG	1591	CGCGUUCGGUUAGCCCCGUC	3292
	CGAAATAACCCTGCTCACTTGGG	1592	CGAAAUAACCCUGCUCACUU	3293
	ACACGAGAGGGGAGTTGCGCGGG	1593	ACACGAGAGGGGAGUUGCGC	3294
	TTCGGTTAGCCCCGTCCGGAAGG	1594	UUCGGUUAGCCCCGUCCGGA	3295
	CGCGAGCGGTGACATCACGGGGG	1595	CGCGAGCGGUGACAUCACGG	3296
	GACACGAGAGGGGAGTTGCGCGG	1596	GACACGAGAGGGGAGUUGCG	3297
	TCTACGCCACCGACCCGTCCGGG	1597	UCUACGCCACCGACCCGUCC	3298
	CGGTGACATCACGGGGGCGACGG	1598	CGGUGACAUCACGGGGGCGA	3299
	GTCTACGCCACCGACCCGTCCGG	1599	GUCUACGCCACCGACCCGUC	3300
	GGAGGAACATGACATCGCGGAGG	1600	GGAGGAACAUGACAUCGCGG	3301
	GGCACCCGTGGTTCCCTCGGCGG	1601	GGCACCCGUGGUUCCCUCGG	3302
	AGCAAGGAGCGGGTCCGTCGCGG	1602	AGCAAGGAGCGGGUCCGUCG	3303
	CGGACGGGGCTAACCGAACGCGG	1603	CGGACGGGGCUAACCGAACG	3304
	CACGAGAGGGGAGTTGCGCGGGG	1604	CACGAGAGGGGAGUUGCGCG	3305
	ATGAGTGGAGCGGCGATTTGTGG	1605	AUGAGUGGAGCGGCGAUUUG	3306
	GACCGAGCGACGGCCGGGAATGG	1606	GACCGAGCGACGGCCGGGAA	3307

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GTCGCTCGGTCCTCCGCTGACGG	1607	GUCGCUCGGUCCUCCGCUGA	3308
	GTCCTCCGCTGACGGGAAGCAGG	1608	GUCCUCCGCUGACGGGAAGC	3309
	GTTATTTCGGAATCACCATGAGG	1609	GUUAUUUCGGAAUCACCAUG	3310
	TTCCTGCTTCCCGTCAGCGGAGG	1610	UUCCUGCUUCCCGUCAGCGG	3311
	AGAGCCGCCGAGGGAACCACGGG	1611	AGAGCCGCCGAGGGAACCAC	3312
	GGGTCCGTCGCGGAGCCGGAGGG	1612	GGGUCCGUCGCGGAGCCGGA	3313
	ATGTGGCAGCGGGCCCGGACGGG	1613	AUGUGGCAGCGGGCCCGGAC	3314
	AATCGATGTGGCAGCGGGCCCGG	1614	AAUCGAUGUGGCAGCGGGCC	3315
	TGCCATTCCCGGCCGTCGCTCGG	1615	UGCCAUUCCCGGCCGUCGCU	3316
	CCGAAATAACCCTGCTCACTTGG	1616	CCGAAAUAACCCUGCUCACU	3317
	TCCTGGAAACCGCGGCCGCCAGG	1617	UCCUGGAAACCGCGGCCGCC	3318
	TTCGGGCTCCCGCCCCGGGTCGG	1618	UUCGGGCUCCCGCCCCGGGU	3319
	TTCTCATGAGGCAATGGGTCAGG	1619	UUCUCAUGAGGCAAUGGGUC	3320
	GAGCGGGTCCGTCGCGGAGCCGG	1620	GAGCGGGUCCGUCGCGGAGC	3321
	GCGGGAGGAACATGACATCGCGG	1621	GCGGGAGGAACAUGACAUCG	3322
	CCCGATGTGACCGAGCCCAGCGG	1622	CCCGAUGUGACCGAGCCCAG	3323
	TCCGCTGGGCTCGGTCACATCGG	1623	UCCGCUGGGCUCGGUCACAU	3324
	CGGAGGACCGAGCGACGGCCGGG	1624	CGGAGGACCGAGCGACGGCC	3325
	GAAATGAATGGGGACCCGAGGGG	1625	GAAAUGAAUGGGGACCCGAG	3326
	GCTGCCGCAGCCGGAACTCGGGG	1626	GCUGCCGCAGCCGGAACUCG	3327
	TGACATCACGGGGGCGACGGCGG	1627	UGACAUCACGGGGGCGACGG	3328
PTPN6	GGGTACCGTCCTTCTAAGTGGGG	1628	GGGUACCGUCCUUCUAAGUG	3329
	CTACTGTACAAAACGCAACTCGG	1629	CUACUGUACAAAACGCAACU	3330
	GTCCGCCTCGACCCAACCGGCGG	1630	GUCCGCCUCGACCCAACCGG	3331
	AATCGTCCTAGTCAAGGCATAGG	1631	AAUCGUCCUAGUCAAGGCAU	3332
	CTACGTCCGCCGGGAAAATGGGG	1632	CUACGUCCGCCGGGAAAAUG	3333
	CCGGGTACCGTCCTTCTAAGTGG	1633	CCGGGUACCGUCCUUCUAAG	3334
	CTGTTCTCCGACGCCTACCCGGG	1634	CUGUUCUCCGACGCCUACCC	3335
	CAGCGCTCAAGGCCGCCGGTTGG	1635	CAGCGCUCAAGGCCGCCGGU	3336
	TGGGTCGAGGCGGACGCCATAGG	1636	UGGGUCGAGGCGGACGCCAU	3337
	AGCGCTCAAGGCCGCCGGTTGGG	1637	AGCGCUCAAGGCCGCCGGUU	3338
	GAAACAGCATCCGGCGCAGCCGG	1638	GAAACAGCAUCCGGCGCAGC	3339
	TAATTCCTTGCGCTCTCCGCTGG	1639	UAAUUCCUUGCGCUCUCCGC	3340
	ACGCCTGGATCACCTCCGCGAGG	1640	ACGCCUGGAUCACCUCCGCG	3341
	GATAACGCCTGCAACGACATGGG	1641	GAUAACGCCUGCAACGACAU	3342
	GGATAACGCCTGCAACGACATGG	1642	GGAUAACGCCUGCAACGACA	3343
	CAAGGCCGCCGGTTGGGTCGAGG	1643	CAAGGCCGCCGGUUGGGUCG	3344
	GGGTCGAGGCGGACGCCATAGGG	1644	GGGUCGAGGCGGACGCCAUA	3345
	CGGGTACCGTCCTTCTAAGTGGG	1645	CGGGUACCGUCCUUCUAAGU	3346
	GCTACGTCCGCCGGGAAAATGGG	1646	GCUACGUCCGCCGGGAAAAU	3347
	GCGCGCAGTGGTCCTCGCGGAGG	1647	GCGCGCAGUGGUCCUCGCGG	3348
	GGTGAGAATCGTCCTAGTCAAGG	1648	GGUGAGAAUCGUCCUAGUCA	3349

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GCCGCCGTGTGGCGAGAAAGGGG	1649	GCCGCCGUGUGGCGAGAAAG	3350
	AATTCCTTGCGCTCTCCGCTGGG	1650	AAUUCCUUGCGCUCUCCGCU	3351
	TTTTCCCGGCGGACGTAGCCAGG	1651	UUUUCCCGGCGGACGUAGCC	3352
	CGGAGAGCGCAAGGAATTAGTGG	1652	CGGAGAGCGCAAGGAAUUAG	3353
	CATCTTACCCATGTCGTTGCAGG	1653	CAUCUUACCCAUGUCGUUGC	3354
	TCTCCGGGGCGGAGAACGCCTGG	1654	UCUCCGGGGCGGAGAACGCC	3355
	TTTCCGCTCCCAGGGGCGTTGGG	1655	UUUCCGCUCCCAGGGGCGUU	3356
	CATGCGCACTGCATTCTCCGGGG	1656	CAUGCGCACUGCAUUCUCCG	3357
	AACTCGGACGCACAAGCTCAGGG	1657	AACUCGGACGCACAAGCUCA	3358
	TGAAGCTCTAGGTTCAGCGGAGG	1658	UGAAGCUCUAGGUUCAGCGG	3359
	GGCGTCGGAGAACAGACAGCGGG	1659	GGCGUCGGAGAACAGACAGC	3360
	AGTTGCGTTTTGTACAGTAGAGG	1660	AGUUGCGUUUUGUACAGUAG	3361
	GTCCGCCGGGAAAATGGGGTAGG	1661	GUCCGCCGGGAAAAUGGGGU	3362
	CTAGAGCTTCAGACGCCCTATGG	1662	CUAGAGCUUCAGACGCCCUA	3363
	ACAAACCTGGCTACGTCCGCCGG	1663	ACAAACCUGGCUACGUCCGC	3364
	TCCGCCGGGAAAATGGGGTAGGG	1664	UCCGCCGGGAAAAUGGGGUA	3365
	TTGGGAACGGTTGTAGGACGTGG	1665	UUGGGAACGGUUGUAGGACG	3366
	GAATGCAGTGCGCATGGACGAGG	1666	GAAUGCAGUGCGCAUGGACG	3367
	GGCGCTTGCCCCCAAGACTTGGG	1667	GGCGCUUGCCCCCAAGACUU	3368
	TCTGTTCTCCGACGCCTACCCGG	1668	UCUGUUCUCCGACGCCUACC	3369
	CAACTCGGACGCACAAGCTCAGG	1669	CAACUCGGACGCACAAGCUC	3370
	GGCTACGTCCGCCGGGAAAATGG	1670	GGCUACGUCCGCCGGGAAAA	3371
	TGCCGCCGTGTGGCGAGAAAGGG	1671	UGCCGCCGUGUGGCGAGAAA	3372
	AGATTGTGGCTGCCGCCGTGTGG	1672	AGAUUGUGGCUGCCGCCGUG	3373
	TCCCCTTTCTCGCCACACGGCGG	1673	UCCCCUUUCUCGCCACACGG	3374
	AAGCGCCCCACTTAGAAGGACGG	1674	AAGCGCCCCACUUAGAAGGA	3375
	TTGAGCGCTGAGCAAGCAAAGGG	1675	UUGAGCGCUGAGCAAGCAAA	3376
	GGCCGCCGGTTGGGTCGAGGCGG	1676	GGCCGCCGGUUGGGUCGAGG	3377
	AATTAGTGGATTGAGGCTGTAGG	1677	AAUUAGUGGAUUGAGGCUGU	3378
PTPA	GCGACTGCCACGATTGTGCGGGG	1678	GCGACUGCCACGAUUGUGCG	3379
	CGTTCCCGGACGCAACCGCACGG	1679	CGUUCCCGGACGCAACCGCA	3380
	TCTCGGTTTTCGGTTATAGCCGG	1680	UCUCGGUUUUCGGUUAUAGC	3381
	CGGACTGCGTGTCCGCGGACGGG	1681	CGGACUGCGUGUCCGCGGAC	3382
	CGTGCGGTTGCGTCCGGGAACGG	1682	CGUGCGGUUGCGUCCGGGAA	3383
	GCGGACTGCGTGTCCGCGGACGG	1683	GCGGACUGCGUGUCCGCGGA	3384
	GCCAACGGCCGCCAAGCGCTAGG	1684	GCCAACGGCCGCCAAGCGCU	3385
	GCCTATTAACGGCCGGCGCGCGG	1685	GCCUAUUAACGGCCGGCGCG	3386
	CGCGACTGCCACGATTGTGCGGG	1686	CGCGACUGCCACGAUUGUGC	3387
	CAGTCGCGGCGCCCGACGTTCGG	1687	CAGUCGCGGCGCCCGACGUU	3388
	CCGTGAGCGGTCCTAGCGCTTGG	1688	CCGUGAGCGGUCCUAGCGCU	3389
	AGTCGCGGCGCCCGACGTTCGGG	1689	AGUCGCGGCGCCCGACGUUC	3390
	CATGGCGGCCGTCTTCGCTGTGG	1690	CAUGGCGGCCGUCUUCGCUG	3391

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CTTGACGCCCCGCACAATCGTGG	1691	CUUGACGCCCCGCACAAUCG	3392
	GGGATCCGCCGCACTCACCACGG	1692	GGGAUCCGCCGCACUCACCA	3393
	ACATCTTCGCTGCCCGTCCGCGG	1693	ACAUCUUCGCUGCCCGUCCG	3394
	CGGAGCGGACTGCGTGTCCGCGG	1694	CGGAGCGGACUGCGUGUCCG	3395
	ACGGCCGCCATGTCGGTGCGGGG	1695	ACGGCCGCCAUGUCGGUGCG	3396
	GCTCACGGCCGCCCGAACGTCGG	1696	GCUCACGGCCGCCCGAACGU	3397
	TATTAACGGCCGGCGCGCGGCGG	1697	UAUUAACGGCCGGCGCGCGG	3398
	AGCCTACTGCGACCCGCTACCGG	1698	AGCCUACUGCGACCCGCUAC	3399
	GCCTACTGCGACCCGCTACCGGG	1699	GCCUACUGCGACCCGCUACC	3400
	GCGGGCCGTGCGGTTGCGTCCGG	1700	GCGGGCCGUGCGGUUGCGUC	3401
	ATTGTGCGGGGCGTCAAGTTTGG	1701	AUUGUGCGGGGCGUCAAGUU	3402
	GCCGCGCGCCGGCCGTTAATAGG	1702	GCCGCGCGCCGGCCGUUAAU	3403
	GCGACGGCCATGTCAGTGCGGGG	1703	GCGACGGCCAUGUCAGUGCG	3404
	AAACACAACCATGTTGACCGGGG	1704	AAACACAACCAUGUUGACCG	3405
	GGGACTGCAAGCATCCGGGTCGG	1705	GGGACUGCAAGCAUCCGGGU	3406
	CTGGAGGCCGGGTCGAACAGCGG	1706	CUGGAGGCCGGGUCGAACAG	3407
	GGAGCAAGCCTATTAACGGCCGG	1707	GGAGCAAGCCUAUUAACGGC	3408
	CCGCACAATCGTGGCAGTCGCGG	1708	CCGCACAAUCGUGGCAGUCG	3409
	TGAGCGGTCCTAGCGCTTGGCGG	1709	UGAGCGGUCCUAGCGCUUGG	3410
	ATGTCGGTGCGGGGCGCTCAGGG	1710	AUGUCGGUGCGGGGCGCUCA	3411
	GCCCGGTAGCGGGTCGCAGTAGG	1711	GCCCGGUAGCGGGUCGCAGU	3412
	TGCACCTTTCCAACTCCGTCTGG	1712	UGCACCUUUCCAACUCCGUC	3413
	TTGTGCGGGGCGTCAAGTTTGGG	1713	UUGUGCGGGGCGUCAAGUUU	3414
	TCCTAGCGCTTGGCGGCCGTTGG	1714	UCCUAGCGCUUGGCGGCCGU	3415
	CGGACTTTGCCCGGTGTGTGGGG	1715	CGGACUUUGCCCGGUGUGUG	3416
	AGACGGCCGCCATGTCGGTGCGG	1716	AGACGGCCGCCAUGUCGGUG	3417
	GGTTTTCGGTTATAGCCGGCCGG	1717	GGUUUUCGGUUAUAGCCGGC	3418
	GGGCGAGAGTCATGACACGGAGG	1718	GGGCGAGAGUCAUGACACGG	3419
	GCATTCAGTTCCAACGACCCAGG	1719	GCAUUCAGUUCCAACGACCC	3420
	GGAACGGAGACCGCGTCCTGCGG	1720	GGAACGGAGACCGCGUCCUG	3421
	ACTCAGCGACCTTGGCCCGAAGG	1721	ACUCAGCGACCUUGGCCCGA	3422
	GCGGGATTAAACCCACGTCCTGG	1722	GCGGGAUUAAACCCACGUCC	3423
	GGTAGCGGGTCGCAGTAGGCTGG	1723	GGUAGCGGGUCGCAGUAGGC	3424
	AGATGTTAGCCTTCGCTGCCAGG	1724	AGAUGUUAGCCUUCGCUGCC	3425
	CGGGCCGTGCGGTTGCGTCCGGG	1725	CGGGCCGUGCGGUUGCGUCC	3426
	CGCGGACTTTGCCCGGTGTGTGG	1726	CGCGGACUUUGCCCGGUGUG	3427
	GAGTCATGACACGGAGGAACTGG	1727	GAGUCAUGACACGGAGGAAC	3428
PTPN2	CTCTTCGAACTCCCGCTCGATGG	1728	CUCUUCGAACUCCCGCUCGA	3429
	ACGGCCGACAGGGCTTGGCGTGG	1729	ACGGCCGACAGGGCUUGGCG	3430
	TTCGAACTCCCGCTCGATGGTGG	1730	UUCGAACUCCCGCUCGAUGG	3431
	CGTGCCGCGCGCAGGGACCACGG	1731	CGUGCCGCGCGCAGGGACCA	3432
	GCGTGCCGGCGACTTCTCAGGGG	1732	GCGUGCCGGCGACUUCUCAG	3433

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GATGCGCCACCAGCGTTGCGCGG	1733	GAUGCGCCACCAGCGUUGCG	3434
	AGCGAGCTTCGCCTCGCAGAGGG	1734	AGCGAGCUUCGCCUCGCAGA	3435
	GAGGTCGGCGACTGCCGCGTGGG	1735	GAGGUCGGCGACUGCCGCGU	3436
	GCACGATCCGGGGAGAGCGCTGG	1736	GCACGAUCCGGGGAGAGCGC	3437
	AGCGTGCCGGCGACTTCTCAGGG	1737	AGCGUGCCGGCGACUUCUCA	3438
	GTACTTTCCCCACGGCCGACAGG	1738	GUACUUUCCCCACGGCCGAC	3439
	ACGAGTCCGGGTCTCGGAGGAGG	1739	ACGAGUCCGGGUCUCGGAGG	3440
	TAGCGCGGGGTTACTGGAATGGG	1740	UAGCGCGGGGUUACUGGAAU	3441
	CGGACGTCAGCGCGCAGACTCGG	1741	CGGACGUCAGCGCGCAGACU	3442
	AGCGTTGCGCGGCCCGGGTCTGG	1742	AGCGUUGCGCGGCCCGGGUC	3443
	GCGTTGCGCGGCCCGGGTCTGGG	1743	GCGUUGCGCGGCCCGGGUCU	3444
	CGTGGGTAGCGCGGGGTTACTGG	1744	CGUGGGUAGCGCGGGGUUAC	3445
	TGGTGAGTCGCGGACCCACGCGG	1745	UGGUGAGUCGCGGACCCACG	3446
	CCGCGACTCACCAAGTACAGCGG	1746	CCGCGACUCACCAAGUACAG	3447
	TCGGAAGACGCAAGCCCAAGGGG	1747	UCGGAAGACGCAAGCCCAAG	3448
	CGCCCCGAGCGAGAGGCTAGAGG	1748	CGCCCCGAGCGAGAGGCUAG	3449
	GTCGGAAGACGCAAGCCCAAGGG	1749	GUCGGAAGACGCAAGCCCAA	3450
	TTCATTTGTGACACCCGTCTGGG	1750	UUCAUUUGUGACACCCGUCU	3451
	CTCAGGCCCCGCACGATCCGGGG	1751	CUCAGGCCCCGCACGAUCCG	3452
	AGCGCTCTCCCCGGATCGTGCGG	1752	AGCGCUCUCCCCGGAUCGUG	3453
	ACGGCGAAGCTGCGGCCCGGGGG	1753	ACGGCGAAGCUGCGGCCCGG	3454
	AGACCCGGGCCGCGCAACGCTGG	1754	AGACCCGGGCCGCGCAACGC	3455
	TCGGCCGTGGGGAAAGTACCTGG	1755	UCGGCCGUGGGGAAAGUACC	3456
	CGAGCGGGAGTTCGAAGAGTTGG	1756	CGAGCGGGAGUUCGAAGAGU	3457
	CGCCAAGCCCTGTCGGCCGTGGG	1757	CGCCAAGCCCUGUCGGCCGU	3458
	TCGCCTCTAGCCTCTCGCTCGGG	1758	UCGCCUCUAGCCUCUCGCUC	3459
	AGCAAGAGAGCGGTCAGCGCAGG	1759	AGCAAGAGAGCGGUCAGCGC	3460
	ACGCCAAGCCCTGTCGGCCGTGG	1760	ACGCCAAGCCCUGUCGGCCG	3461
	TCGTTCCGGGAAGGTTCTATGGG	1761	UCGUUCCGGGAAGGUUCUAU	3462
	AGCGCAAGCGCAGTTAGTTCTGG	1762	AGCGCAAGCGCAGUUAGUUC	3463
	CACGAGGTGAGCCGCCCCTTGGG	1763	CACGAGGUGAGCCGCCCCUU	3464
	ATTCATTTGTGACACCCGTCTGG	1764	AUUCAUUUGUGACACCCGUC	3465
	GCCGACTTCGCGCCGCGCTCGGG	1765	GCCGACUUCGCGCCGCGCUC	3466
	CTGGCGGAGCCGCGGTGGTTGGG	1766	CUGGCGGAGCCGCGGUGGUU	3467
	CGGTGGTCCGTGGGTAGCGCGGG	1767	CGGUGGUCCGUGGGUAGCGC	3468
	GTCTTCCGACAAGAGAGAGGCGG	1768	GUCUUCCGACAAGAGAGAGG	3469
	GCGCAGTTAGTTCTGGAGGGCGG	1769	GCGCAGUUAGUUCUGGAGGG	3470
	CAGTAACCCCGCGCTACCCACGG	1770	CAGUAACCCCGCGCUACCCA	3471
	GCAAGCGCAGTTAGTTCTGGAGG	1771	GCAAGCGCAGUUAGUUCUGG	3472
	CGCCTCTAGCCTCTCGCTCGGGG	1772	CGCCUCUAGCCUCUCGCUCG	3473
	TGGTCCCTGCGCGCGGCACGAGG	1773	UGGUCCCUGCGCGCGGCACG	3474
	TACTTTCCCCACGGCCGACAGGG	1774	UACUUUCCCCACGGCCGACA	3475

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CGTTCCGGGAAGGTTCTATGGGG	1775	CGUUCCGGGAAGGUUCUAUG	3476
	TGTCGGAAGACGCAAGCCCAAGG	1776	UGUCGGAAGACGCAAGCCCA	3477
	GAGCGAGCTTCGCCTCGCAGAGG	1777	GAGCGAGCUUCGCCUCGCAG	3478
CISH	TCGCGATTGGTCAGCTCGCGGGG	1778	UCGCGAUUGGUCAGCUCGCG	3479
	ACCAATCGCGACGCTGAAGGTGG	1779	ACCAAUCGCGACGCUGAAGG	3480
	CGTCGCGATTGGTCAGCTCGCGG	1780	CGUCGCGAUUGGUCAGCUCG	3481
	CTGACCAATCGCGACGCTGAAGG	1781	CUGACCAAUCGCGACGCUGA	3482
	GTCGCGATTGGTCAGCTCGCGGG	1782	GUCGCGAUUGGUCAGCUCGC	3483
	CAACGACGCAGAATGCCAGAAGG	1783	CAACGACGCAGAAUGCCAGA	3484
	AGGGCCCTCTTATCTCGCGGTGG	1784	AGGGCCCUCUUAUCUCGCGG	3485
	CGGCTAAAGGAGGAACTCACAGG	1785	CGGCUAAAGGAGGAACUCAC	3486
	AATAGCAGCGCGTGGACCCGGGG	1786	AAUAGCAGCGCGUGGACCCG	3487
	TTATCTCGCGGTGGAACTCGTGG	1787	UUAUCUCGCGGUGGAACUCG	3488
	TCCACCTTCAGCGTCGCGATTGG	1788	UCCACCUUCAGCGUCGCGAU	3489
	GTGGCGCGGACCGCCTGCGAGGG	1789	GUGGCGCGGACCGCCUGCGA	3490
	TCGCCGCTGCCGCGGGGACATGG	1790	UCGCCGCUGCCGCGGGGACA	3491
	CCAATAGCAGCGCGTGGACCCGG	1791	CCAAUAGCAGCGCGUGGACC	3492
	AGTTCCACCGCGAGATAAGAGGG	1792	AGUUCCACCGCGAGAUAAGA	3493
	TCTGCGTTCAGGGGTAAGCGCGG	1793	UCUGCGUUCAGGGGUAAGCG	3494
	CCGGTTTCCCAATCCACAGTGGG	1794	CCGGUUUCCCAAUCCACAGU	3495
	GTTCTCCCGTGCGCCCCTCGTGG	1795	GUUCUCCCGUGCGCCCCUCG	3496
	TCGCGGTGGAACTCGTGGCAGGG	1796	UCGCGGUGGAACUCGUGGCA	3497
	GAGTTCCACCGCGAGATAAGAGG	1797	GAGUUCCACCGCGAGAUAAG	3498
	TAGAACCGCGGGCTGAGCGGTGG	1798	UAGAACCGCGGGCUGAGCGG	3499
	GGACCATGTCCCCGCGGCAGCGG	1799	GGACCAUGUCCCCGCGGCAG	3500
	AGTGGCGCGGACCGCCTGCGAGG	1800	AGUGGCGCGGACCGCCUGCG	3501
	GCGCGGAGCGCGTGCTGGGTAGG	1801	GCGCGGAGCGCGUGCUGGGU	3502
	CGTGTTGGGACGGCCGCTCCTGG	1802	CGUGUUGGGACGGCCGCUCC	3503
	CTTCTGGCATTCTGCGTCGTTGG	1803	CUUCUGGCAUUCUGCGUCGU	3504
	ACCGGGGGCTGGCCGGCTAAAGG	1804	ACCGGGGGCUGGCCGGCUAA	3505
	AGAACCGCGGGCTGAGCGGTGGG	1805	AGAACCGCGGGCUGAGCGGU	3506
	CGCAGAGGACCATGTCCCCGCGG	1806	CGCAGAGGACCAUGUCCCCG	3507
	GCAGCGTCTTCCTAGAACCGCGG	1807	GCAGCGUCUUCCUAGAACCG	3508
	GGAGCGGCCGTCCCAACACGGGG	1808	GGAGCGGCCGUCCCAACACG	3509
	GACCGCCGGCTTGACCTCAGTGG	1809	GACCGCCGGCUUGACCUCAG	3510
	GGAGGGCCAATAGCAGCGCGTGG	1810	GGAGGGCCAAUAGCAGCGCG	3511
	TCTGGCATTCTGCGTCGTTGGGG	1811	UCUGGCAUUCUGCGUCGUUG	3512
	ACGCCGACAGACCTCCTTGGAGG	1812	ACGCCGACAGACCUCCUUGG	3513
	AGGAGCGGCCGTCCCAACACGGG	1813	AGGAGCGGCCGUCCCAACAC	3514
	ACTGAGCGCAGACGGACCTCAGG	1814	ACUGAGCGCAGACGGACCUC	3515
	GCGACTCCGGAGTGGGGACTCGG	1815	GCGACUCCGGAGUGGGGACU	3516
	CTTTCCAGGAAAACGGGGCGGGG	1816	CUUUCCAGGAAAACGGGGCG	3517

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GTGTGCAAGCGCCCCGTGTTGGG	1817	GUGUGCAAGCGCCCCGUGUU	3518
	CTGTGTGTCGGGTGTCGGATTGG	1818	CUGUGUGUCGGGUGUCGGAU	3519
	CCCGCGCCCAGATTGCCTTCTGG	1819	CCCGCGCCCAGAUUGCCUUC	3520
	TTCTGGCATTCTGCGTCGTTGGG	1820	UUCUGGCAUUCUGCGUCGUU	3521
	TCGTGCTAGCTGCCGGGCATTGG	1821	UCGUGCUAGCUGCCGGGCAU	3522
	CGTCTGCGCTCAGTCACCTCTGG	1822	CGUCUGCGCUCAGUCACCUC	3523
	CACACGCCGACAGACCTCCTTGG	1823	CACACGCCGACAGACCUCCU	3524
	AGACCGGGTCGGGGAAGTTAAGG	1824	AGACCGGGUCGGGGAAGUUA	3525
	ATTGGCCCTCCCCGACCGCTCGG	1825	AUUGGCCCUCCCCGACCGCU	3526
	CAGGAGCGGCCGTCCCAACACGG	1826	CAGGAGCGGCCGUCCCAACA	3527
	CCCTCGTGGTGGCCGGGAAGGGG	1827	CCCUCGUGGUGGCCGGGAAG	3528
PI3KCD.1	GGTCCCGAAAAGTGCGCTGTGGG	1828	GGUCCCGAAAAGUGCGCUGU	3529
	AGGTCCCGAAAAGTGCGCTGTGG	1829	AGGUCCCGAAAAGUGCGCUG	3530
	GATCGCCGCTGGCTGCGTCAGGG	1830	GAUCGCCGCUGGCUGCGUCA	3531
	CAGTTCGCCTACCGCTAGAGGGG	1831	CAGUUCGCCUACCGCUAGAG	3532
	AGCAAACGCGGCGAGCAACGCGG	1832	AGCAAACGCGGCGAGCAACG	3533
	CGGTTTTGCCGGCGTAACCCCGG	1833	CGGUUUUGCCGGCGUAACCC	3534
	TTGCCGGCGTAACCCCGGCTCGG	1834	UUGCCGGCGUAACCCCGGCU	3535
	GGACGGTAAGCGATCGCCGCTGG	1835	GGACGGUAAGCGAUCGCCGC	3536
	GCGGCGATCGCTTACCGTCCCGG	1836	GCGGCGAUCGCUUACCGUCC	3537
	CCTCTAGCGGTAGGCGAACTGGG	1837	CCUCUAGCGGUAGGCGAACU	3538
	CGATCGCCGCTGGCTGCGTCAGG	1838	CGAUCGCCGCUGGCUGCGUC	3539
	CCCTCTAGCGGTAGGCGAACTGG	1839	CCCUCUAGCGGUAGGCGAAC	3540
	AGGTAGGGGCGAGATTTCCGGGG	1840	AGGUAGGGGCGAGAUUUCCG	3541
	GATGATGCCCCTCTAGCGGTAGG	1841	GAUGAUGCCCCUCUAGCGGU	3542
	CCGGATCTGCGGCCGAGCCGGGG	1842	CCGGAUCUGCGGCCGAGCCG	3543
	CCGCTCCGAGCGCTGACTAGAGG	1843	CCGCUCCGAGCGCUGACUAG	3544
	CCGAAAAGTGCGCTGTGGGTGGG	1844	CCGAAAAGUGCGCUGUGGGU	3545
	GGCGAGATTTCCGGGGTCGCGGG	1845	GGCGAGAUUUCCGGGGUCGC	3546
	CCCGAAAAGTGCGCTGTGGGTGG	1846	CCCGAAAAGUGCGCUGUGGG	3547
	AGGACCCGGCTCGCTAGACTCGG	1847	AGGACCCGGCUCGCUAGACU	3548
	GGAACTGGGACGACCTTTCGTGG	1848	GGAACUGGGACGACCUUUCG	3549
	GCAAACGCGGCGAGCAACGCGGG	1849	GCAAACGCGGCGAGCAACGC	3550
	GGTCCTCGCGTGGCACCCTTGGG	1850	GGUCCUCGCGUGGCACCCUU	3551
	CCCCCGTGGGCCCGCCGAGAGGG	1851	CCCCCGUGGGCCCGCCGAGA	3552
	CTCTAGTCAGCGCTCGGAGCGGG	1852	CUCUAGUCAGCGCUCGGAGC	3553
	CAGTTTGCGGATGGAGCGCGGGG	1853	CAGUUUGCGGAUGGAGCGCG	3554
	GAACTGGGACGACCTTTCGTGGG	1854	GAACUGGGACGACCUUUCGU	3555
	TGGGCGCGAGTGAGCCTCGAGGG	1855	UGGGCGCGAGUGAGCCUCGA	3556
	AACGCGGCGAGCAACGCGGGAGG	1856	AACGCGGCGAGCAACGCGGG	3557
	TAGACTCGGGGAGGCGCCCAGGG	1857	UAGACUCGGGGAGGCGCCCA	3558
	TCGCGCCTCAGCCGGCGCACCGG	1858	UCGCGCCUCAGCCGGCGCAC	3559

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CGCCTCAGCCGGCGCACCGGAGG	1859	CGCCUCAGCCGGCGCACCGG	3560
	GGACCCGGCTCGCTAGACTCGGG	1860	GGACCCGGCUCGCUAGACUC	3561
	CCAGTTTGCGGATGGAGCGCGGG	1861	CCAGUUUGCGGAUGGAGCGC	3562
	CTCTAGCGGTAGGCGAACTGGGG	1862	CUCUAGCGGUAGGCGAACUG	3563
	TAGGACTTCTCAGGAATCGGCGG	1863	UAGGACUUCUCAGGAAUCGG	3564
	CGAGATCAGCTCCGGATCTGCGG	1864	CGAGAUCAGCUCCGGAUCUG	3565
	GACCCGGCTCGCTAGACTCGGGG	1865	GACCCGGCUCGCUAGACUCG	3566
	TGGACCCCGCTGCCGTACAGAGG	1866	UGGACCCCGCUGCCGUACAG	3567
	CGCGAGTGAGCCTCGAGGGAGGG	1867	CGCGAGUGAGCCUCGAGGGA	3568
	GACGACCTTTCGTGGGCACCAGG	1868	GACGACCUUUCGUGGGCACC	3569
	GAGGGCTGCGCACAGTTCGCCGG	1869	GAGGGCUGCGCACAGUUCGC	3570
	AGTCTAGCGAGCCGGGTCCTGGG	1870	AGUCUAGCGAGCCGGGUCCU	3571
	CGAGCGCTGACTAGAGGACCAGG	1871	CGAGCGCUGACUAGAGGACC	3572
	TCCGGATCTGCGGCCGAGCCGGG	1872	UCCGGAUCUGCGGCCGAGCC	3573
	CGCGTTTGCTGCAGCGGCGCAGG	1873	CGCGUUUGCUGCAGCGGCGC	3574
	ACCGTCCCGGCGCAGCTGGCAGG	1874	ACCGUCCCGGCGCAGCUGGC	3575
	GCTCGCCGCGTTTGCTGCAGCGG	1875	GCUCGCCGCGUUUGCUGCAG	3576
	GTCCGGAAATGCAAAGCTGGGGG	1876	GUCCGGAAAUGCAAAGCUGG	3577
	GATCCCAAGGGTGCCACGCGAGG	1877	GAUCCCAAGGGUGCCACGCG	3578
	GTACCGGGTGTCGCTGCCGGGGG	1878	GUACCGGGUGUCGCUGCCGG	3579
	CTTGCCTGCACCTCGCGCGGCGG	1879	CUUGCCUGCACCUCGCGCGG	3580
	GGCAGGCTGTTTACTTGTCGGGG	1880	GGCAGGCUGUUUACUUGUCG	3581
	ACTTGTCGGGGACCCAGCAGTGG	1881	ACUUGUCGGGGACCCAGCAG	3582
	GAGGCTCCGTCCCGAATAGGGGG	1882	GAGGCUCCGUCCCGAAUAGG	3583
	CGTACCGGGTGTCGCTGCCGGGG	1883	CGUACCGGGUGUCGCUGCCG	3584
	TCCGTCCCGAATAGGGGGCAGGG	1884	UCCGUCCCGAAUAGGGGGCA	3585
	CGTCCCGAATAGGGGGCAGGGGG	1885	CGUCCCGAAUAGGGGGCAGG	3586
	CAGGGGGTTGCGTTCGCGGTGGG	1886	CAGGGGGUUGCGUUCGCGGU	3587
	AGGAGGCTCCGTCCCGAATAGGG	1887	AGGAGGCUCCGUCCCGAAUA	3588
	TTCTCCGCTGCCGCCCTTGATGG	1888	UUCUCCGCUGCCGCCCUUGA	3589
	CGCGGTGGGATTCTCAGCTATGG	1889	CGCGGUGGGAUUCUCAGCUA	3590
	TCCGTACCGGGTGTCGCTGCCGG	1890	UCCGUACCGGGUGUCGCUGC	3591
	CGCACGAGGACGCGCCTGTTCGG	1891	CGCACGAGGACGCGCCUGUU	3592
	AGCGCCCGAGCTCACACGGGCGG	1892	AGCGCCCGAGCUCACACGGG	3593
	CCGCTTGCCTGCACCTCGCGCGG	1893	CCGCUUGCCUGCACCUCGCG	3594
	GGAGGCTCCGTCCCGAATAGGGG	1894	GGAGGCUCCGUCCCGAAUAG	3595
	CGACCCCCGCTGTTCTCGCCCGG	1895	CGACCCCCGCUGUUCUCGCC	3596
	GGGCCTCCGGGCGAGAACAGCGG	1896	GGGCCUCCGGGCGAGAACAG	3597
	AGGACGCGCCTGTTCGGGGCAGG	1897	AGGACGCGCCUGUUCGGGGC	3598
	CGTCCTCGTGCGAAGCCCGCTGG	1898	CGUCCUCGUGCGAAGCCCGC	3599
	TGACCCCCGGGGGGCACAAAAGG	1899	UGACCCCCGGGGGGCACAAA	3600
	TTGCCTGCACCTCGCGCGGCGGG	1900	UUGCCUGCACCUCGCGCGGC	3601

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GCACGAGGACGCGCCTGTTCGGG	1901	GCACGAGGACGCGCCUGUUC	3602
	CCAGCGTGCGCGCGCCGTCGGGG	1902	CCAGCGUGCGCGCGCCGUCG	3603
	GTAAACAGCCTGCCCCGAACAGG	1903	GUAAACAGCCUGCCCCGAAC	3604
	CGGTACGGAGCCCACCTGTGCGG	1904	CGGUACGGAGCCCACCUGUG	3605
	CCCGGCAGCGACACCCGGTACGG	1905	CCCGGCAGCGACACCCGGUA	3606
	GCGGTGGGATTCTCAGCTATGGG	1906	GCGGUGGGAUUCUCAGCUAU	3607
	CCTCCAGCGGGCTTCGCACGAGG	1907	CCUCCAGCGGGCUUCGCACG	3608
	GATCCCATCAAGGGCGGCAGCGG	1908	GAUCCCAUCAAGGGCGGCAG	3609
	TTTCCGCTCCCCGCTTTGCAAGG	1909	UUUCCGCUCCCCGCUUUGCA	3610
	CTCCGTCCCGAATAGGGGGCAGG	1910	CUCCGUCCCGAAUAGGGGGC	3611
	TCTCCGCTGCCGCCCTTGATGGG	1911	UCUCCGCUGCCGCCCUUGAU	3612
	AGCCAGCGTGCGCGCGCCGTCGG	1912	AGCCAGCGUGCGCGCGCCGU	3613
	GCTCAGGGTGCGAACCCCAAGGG	1913	GCUCAGGGUGCGAACCCCAA	3614
	TAGGAGAGGAGAGCGTCGCGCGG	1914	UAGGAGAGGAGAGCGUCGCG	3615
	CGCCCTTGATGGGATCCGTGAGG	1915	CGCCCUUGAUGGGAUCCGUG	3616
	CACCTGTGCGGGCGTCTGCGGGG	1916	CACCUGUGCGGGCGUCUGCG	3617
	TCTGCGGATGCCTTGCAAAGCGG	1917	UCUGCGGAUGCCUUGCAAAG	3618
	GCGCGAGGTGCAGGCAAGCGGGG	1918	GCGCGAGGUGCAGGCAAGCG	3619
	AGGCATCCGCAGAAAGGGCGGGG	1919	AGGCAUCCGCAGAAAGGGCG	3620
	GCCAGCGTGCGCGCGCCGTCGGG	1920	GCCAGCGUGCGCGCGCCGUC	3621
	CTCACGGATCCCATCAAGGGCGG	1921	CUCACGGAUCCCAUCAAGGG	3622
	CCTCCGGGCGAGAACAGCGGGGG	1922	CCUCCGGGCGAGAACAGCGG	3623
	AAGCGCCACCTGCAAAGCAAGGG	1923	AAGCGCCACCUGCAAAGCAA	3624
	GCCTCCGGGCGAGAACAGCGGGG	1924	GCCUCCGGGCGAGAACAGCG	3625
	AGAAGCGCCCGAGCTCACACGGG	1925	AGAAGCGCCCGAGCUCACAC	3626
	GCTTCCTTTTGTGCCCCCCGGGG	1926	GCUUCCUUUUGUGCCCCCCG	3627
	GCGAACGCAACCCCCTGCCTCGG	1927	GCGAACGCAACCCCCUGCCU	3628
MAP4K1	CACGACCCCCGTTCCCGCGGAGG	1928	CACGACCCCCGUUCCCGCGG	3629
	CGAGATGAGCACCGGTGAGTGGG	1929	CGAGAUGAGCACCGGUGAGU	3630
	ACGGCATCCCCCAAGACTTAGGG	1930	ACGGCAUCCCCCAAGACUUA	3631
	CGCTTAGCCTGAGGCACTACGGG	1931	CGCUUAGCCUGAGGCACUAC	3632
	GGGGGTCGTGACCTCCGAGTGGG	1932	GGGGGUCGUGACCUCCGAGU	3633
	ATGCCACCTTGGCGGCAGACGGG	1933	AUGCCACCUUGGCGGCAGAC	3634
	CGGACAGAGGCGTCGGCAGTGGG	1934	CGGACAGAGGCGUCGGCAGU	3635
	CCCGGTCAGCAGCGCGAACACGG	1935	CCCGGUCAGCAGCGCGAACA	3636
	AGGGCGGGGCTTATCAGATCCGG	1936	AGGGCGGGGCUUAUCAGAUC	3637
	GCGCGCCAAAGCGCACCGTGTGG	1937	GCGCGCCAAAGCGCACCGUG	3638
	AGCAGCGCGAACACGGACAGAGG	1938	AGCAGCGCGAACACGGACAG	3639
	GGTCACGACCCCCGTTCCCGCGG	1939	GGUCACGACCCCCGUUCCCG	3640
	CGGGGCTTATCAGATCCGGAGGG	1940	CGGGGCUUAUCAGAUCCGGA	3641
	CGGGGGTCGTGACCTCCGAGTGG	1941	CGGGGGUCGUGACCUCCGAG	3642
	CTGGTGCCTCCGCGGGAACGGGG	1942	CUGGUGCCUCCGCGGGAACG	3643

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GCGGGGCTTATCAGATCCGGAGG	1943	GCGGGGCUUAUCAGAUCCGG	3644
	TCCGTGTTCGCGCTGCTGACCGG	1944	UCCGUGUUCGCGCUGCUGAC	3645
	GCTTTGGCGCGCTCTCTTGCTGG	1945	GCUUUGGCGCGCUCUCUUGC	3646
	GGCCTGGGACTTCCGAACCAGGG	1946	GGCCUGGGACUUCCGAACCA	3647
	TGGTGCCTCCGCGGGAACGGGGG	1947	UGGUGCCUCCGCGGGAACGG	3648
	GACGGCATCCCCCAAGACTTAGG	1948	GACGGCAUCCCCCAAGACUU	3649
	CTCACGCCGATGCACACAGCGGG	1949	CUCACGCCGAUGCACACAGC	3650
	GGATGCCGTCTAGAAATGTCAGG	1950	GGAUGCCGUCUAGAAAUGUC	3651
	TCGCTTAGCCTGAGGCACTACGG	1951	UCGCUUAGCCUGAGGCACUA	3652
	CGAACCAGGGCCCTAAGTCTTGG	1952	CGAACCAGGGCCCUAAGUCU	3653
	ATGCCGTCTAGAAATGTCAGGGG	1953	AUGCCGUCUAGAAAUGUCAG	3654
	GCATCGGCGTGAGCCCCGGGCGG	1954	GCAUCGGCGUGAGCCCCGGG	3655
	GCGAACACGGACAGAGGCGTCGG	1955	GCGAACACGGACAGAGGCGU	3656
	GGGGCTTATCAGATCCGGAGGGG	1956	GGGGCUUAUCAGAUCCGGAG	3657
	TGGCCTGGGACTTCCGAACCAGG	1957	UGGCCUGGGACUUCCGAACC	3658
	AATGGCAGGTTTTAGTTAACTGG	1958	AAUGGCAGGUUUUAGUUAAC	3659
	TGGAAGCCACACCCACTCGGAGG	1959	UGGAAGCCACACCCACUCGG	3660
	GCTACAAGCCACGCCCCCTGAGG	1960	GCUACAAGCCACGCCCCCUG	3661
	TGGAAGAGCACCGACTTCCCCGG	1961	UGGAAGAGCACCGACUUCCC	3662
	TCGTGACCTCCGAGTGGGTGTGG	1962	UCGUGACCUCCGAGUGGGUG	3663
	CTCTTCCACACGGTGCGCTTTGG	1963	CUCUUCCACACGGUGCGCUU	3664
	AGGCACTACGGGACTGAGAAAGG	1964	AGGCACUACGGGACUGAGAA	3665
	GGCCCTGGTTCGGAAGTCCCAGG	1965	GGCCCUGGUUCGGAAGUCCC	3666
	CCTGGTGCCTCCGCGGGAACGGG	1966	CCUGGUGCCUCCGCGGGAAC	3667
	GCGAGATGAGCACCGGTGAGTGG	1967	GCGAGAUGAGCACCGGUGAG	3668
	GTCTCTTTGAGTGTCTAAGCAGG	1968	GUCUCUUUGAGUGUCUAAGC	3669
	CTAGGGGGTGGTTCAGGACGGGG	1969	CUAGGGGGUGGUUCAGGACG	3670
	GCCTGGTGCCTCCGCGGGAACGG	1970	GCCUGGUGCCUCCGCGGGAA	3671
	GCTTAGACACTCAAAGAGACAGG	1971	GCUUAGACACUCAAAGAGAC	3672
	GATAAGGCCTGGTGCCTCCGCGG	1972	GAUAAGGCCUGGUGCCUCCG	3673
	ACCTTGGCGGCAGACGGGCAGGG	1973	ACCUUGGCGGCAGACGGGCA	3674
	CAAGGTGGCATGCCCCCACATGG	1974	CAAGGUGGCAUGCCCCCACA	3675
	CATGCCACCTTGGCGGCAGACGG	1975	CAUGCCACCUUGGCGGCAGA	3676
	GGCGCGCTCTCTTGCTGGCTGGG	1976	GGCGCGCUCUCUUGCUGGCU	3677
	GATGCCGTCTAGAAATGTCAGGG	1977	GAUGCCGUCUAGAAAUGUCA	3678
NR4A1	CGCGGGGTTCCATTGACGCAGGG	1978	CGCGGGGUUCCAUUGACGCA	3679
	GGCGGAGGCTACGAAACTTGGGG	1979	GGCGGAGGCUACGAAACUUG	3680
	TAAGCGCTCCGTGACGCACGGGG	1980	UAAGCGCUCCGUGACGCACG	3681
	ACGCGGGGTTCCATTGACGCAGG	1981	ACGCGGGGUUCCAUUGACGC	3682
	AAGAACTTCGGGAGCGCACGCGG	1982	AAGAACUUCGGGAGCGCACG	3683
	TTTGGCCATACAAGGGCGCGGGG	1983	UUUGGCCAUACAAGGGCGCG	3684
	GTTTCGTAGCCTCCGCCACTGGG	1984	GUUUCGUAGCCUCCGCCACU	3685

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	ATCCGCGCTCCCTGCGTCAATGG	1985	AUCCGCGCUCCCUGCGUCAA	3686
	TTAAGCGCTCCGTGACGCACGGG	1986	UUAAGCGCUCCGUGACGCAC	3687
	GTGGCGGAGGCTACGAAACTTGG	1987	GUGGCGGAGGCUACGAAACU	3688
	GCGGAGGCTACGAAACTTGGGGG	1988	GCGGAGGCUACGAAACUUGG	3689
	ACAGATGCACGTTCCCCGAAGGG	1989	ACAGAUGCACGUUCCCCGAA	3690
	AACAGATGCACGTTCCCCGAAGG	1990	AACAGAUGCACGUUCCCCGA	3691
	TTGTATGGCCAAAGCTCGACGGG	1991	UUGUAUGGCCAAAGCUCGAC	3692
	CTGCGCGCGTGACGCACGCGGGG	1992	CUGCGCGCGUGACGCACGCG	3693
	CTTAAGCGCTCCGTGACGCACGG	1993	CUUAAGCGCUCCGUGACGCA	3694
	GTCACGCGCGCAGACATTCCAGG	1994	GUCACGCGCGCAGACAUUCC	3695
	TGCGTCACGGAGCGCTTAAGAGG	1995	UGCGUCACGGAGCGCUUAAG	3696
	CGCTCCGTGACGCACGGGGAGGG	1996	CGCUCCGUGACGCACGGGGA	3697
	TGAGACTCGGGGCGCCAGTCCGG	1997	UGAGACUCGGGGCGCCAGUC	3698
	GCGCTGTAGAGACGCGGCCGCGG	1998	GCGCUGUAGAGACGCGGCCG	3699
	TATGGCCAAAGCTCGACGGGCGG	1999	UAUGGCCAAAGCUCGACGGG	3700
	GTCGAGCTTTGGCCATACAAGGG	2000	GUCGAGCUUUGGCCAUACAA	3701
	TCACGGAGCGCTTAAGAGGAGGG	2001	UCACGGAGCGCUUAAGAGGA	3702
	GTCCAGAATAACCAGCGGGAGGG	2002	GUCCAGAAUAACCAGCGGGA	3703
	TGGGACCCGAGTCCGGTGCGGGG	2003	UGGGACCCGAGUCCGGUGCG	3704
	TTGGCCATACAAGGGCGCGGGGG	2004	UUGGCCAUACAAGGGCGCGG	3705
	AAGGAGATGGGTGTACGCGCGGG	2005	AAGGAGAUGGGUGUACGCGC	3706
	GCGCTCCGTGACGCACGGGGAGG	2006	GCGCUCCGUGACGCACGGGG	3707
	CGGGCAATTCGGACACACCCTGG	2007	CGGGCAAUUCGGACACACCC	3708
	CGTCGAGCTTTGGCCATACAAGG	2008	CGUCGAGCUUUGGCCAUACA	3709
	TGGCGGAGGCTACGAAACTTGGG	2009	UGGCGGAGGCUACGAAACUU	3710
	AGTTTCGTAGCCTCCGCCACTGG	2010	AGUUUCGUAGCCUCCGCCAC	3711
	AGGGCTCTAACTGACGTCTCAGG	2011	AGGGCUCUAACUGACGUCUC	3712
	GCAGGCCGCCCGTCGAGCTTTGG	2012	GCAGGCCGCCCGUCGAGCUU	3713
	GCGGGCTGAGGCGGGCAATTCGG	2013	GCGGGCUGAGGCGGGCAAUU	3714
	TCTGCGCGCGTGACGCACGCGGG	2014	UCUGCGCGCGUGACGCACGC	3715
	GTCTGCGCGCGTGACGCACGCGG	2015	GUCUGCGCGCGUGACGCACG	3716
	TAGGCTCCCCGCACCGGACTCGG	2016	UAGGCUCCCCGCACCGGACU	3717
	TTGTAGGGCCGGCATGCAAGAGG	2017	UUGUAGGGCCGGCAUGCAAG	3718
	GCTTTGGCCATACAAGGGCGCGG	2018	GCUUUGGCCAUACAAGGGCG	3719
	GGGCTCTAACTGACGTCTCAGGG	2019	GGGCUCUAACUGACGUCUCA	3720
	CTGTGCACTAGCTGCGCCTAGGG	2020	CUGUGCACUAGCUGCGCCUA	3721
	AGAGTGAGGAGATCCTCATCCGG	2021	AGAGUGAGGAGAUCCUCAUC	3722
	GCTCCGTGACGCACGGGGAGGGG	2022	GCUCCGUGACGCACGGGGAG	3723
	TCGGGGCGCCAGTCCGGGCAGGG	2023	UCGGGGCGCCAGUCCGGGCA	3724
	CGCAGCTAGTGCACAGGACGCGG	2024	CGCAGCUAGUGCACAGGACG	3725
	CGGCCGGGTAGGTTCCCTTCGGG	2025	CGGCCGGGUAGGUUCCCUUC	3726
	CTATTTTTAGCGGGCGCGGCGGG	2026	CUAUUUUUAGCGGGCGCGGC	3727

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CCCGCTGGTTATTCTGGACCTGG	2027	CCCGCUGGUUAUUCUGGACC	3728
NR4A2	CTCGAAACCGAAGAGCCCACAGG	2028	CUCGAAACCGAAGAGCCCAC	3729
	TCGAGGGCAAACGACCTCTCCGG	2029	UCGAGGGCAAACGACCUCUC	3730
	TAACTATACGACCCATTTGGAGG	2030	UAACUAUACGACCCAUUUGG	3731
	TCGGAAAAGCGGCGCTAACAGGG	2031	UCGGAAAAGCGGCGCUAACA	3732
	CTCGGAAAAGCGGCGCTAACAGG	2032	CUCGGAAAAGCGGCGCUAAC	3733
	AGCCGGGTTGGAGTCGACATGGG	2033	AGCCGGGUUGGAGUCGACAU	3734
	AGTCGACATGGGCCCTGACGAGG	2034	AGUCGACAUGGGCCCUGACG	3735
	AGACTCACCGGGGGCGAAGGGGG	2035	AGACUCACCGGGGGCGAAGG	3736
	CTTTAACTATACGACCCATTTGG	2036	CUUUAACUAUACGACCCAUU	3737
	GTCGACATGGGCCCTGACGAGGG	2037	GUCGACAUGGGCCCUGACGA	3738
	AGCGCCGCTTTTCCGAGCCCAGG	2038	AGCGCCGCUUUUCCGAGCCC	3739
	GGCCCATGTCGACTCCAACCCGG	2039	GGCCCAUGUCGACUCCAACC	3740
	ATGTGGACAAACCGACAGATGGG	2040	AUGUGGACAAACCGACAGAU	3741
	TGTGGGCTCTTCGGTTTCGAGGG	2041	UGUGGGCUCUUCGGUUUCGA	3742
	TCAGACTCACCGGGGGCGAAGGG	2042	UCAGACUCACCGGGGGCGAA	3743
	GTCTGATCAGTGCCCTCGTCAGG	2043	GUCUGAUCAGUGCCCUCGUC	3744
	GCACTGATCAGACTCACCGGGGG	2044	GCACUGAUCAGACUCACCGG	3745
	GACAGTTTAAAAGGCCGGAGAGG	2045	GACAGUUUAAAAGGCCGGAG	3746
	ATCAGACTCACCGGGGGCGAAGG	2046	AUCAGACUCACCGGGGGCGA	3747
	CAACCCGGCTATGACCAGCCTGG	2047	CAACCCGGCUAUGACCAGCC	3748
	GGCACTGATCAGACTCACCGGGG	2048	GGCACUGAUCAGACUCACCG	3749
	CTGAGAGTTAATGACGGATGTGG	2049	CUGAGAGUUAAUGACGGAUG	3750
	TCCAGGGTAAGAAGCTGGCGGGG	2050	UCCAGGGUAAGAAGCUGGCG	3751
	GTTCGCACAGACAGTTTAAAAGG	2051	GUUCGCACAGACAGUUUAAA	3752
	TACCCTGGAATAGTCCAGGCTGG	2052	UACCCUGGAAUAGUCCAGGC	3753
	TGACCAGCCTGGACTATTCCAGG	2053	UGACCAGCCUGGACUAUUCC	3754
	TTAACTCTCAGATTCAACGGGGG	2054	UUAACUCUCAGAUUCAACGG	3755
	ATTAACTCTCAGATTCAACGGGG	2055	AUUAACUCUCAGAUUCAACG	3756
	GTCTGTGCGAACCACTGCAAAGG	2056	GUCUGUGCGAACCACUGCAA	3757
	TGAGAGTTAATGACGGATGTGGG	2057	UGAGAGUUAAUGACGGAUGU	3758
	CAAATGGGTCGTATAGTTAAAGG	2058	CAAAUGGGUCGUAUAGUUAA	3759
	TAGCCGGGTTGGAGTCGACATGG	2059	UAGCCGGGUUGGAGUCGACA	3760
	TCTGATCAGTGCCCTCGTCAGGG	2060	UCUGAUCAGUGCCCUCGUCA	3761
	TTCTTACCCTGGAATAGTCCAGG	2061	UUCUUACCCUGGAAUAGUCC	3762
	CCCCCGCCAGCTTCTTACCCTGG	2062	CCCCCGCCAGCUUCUUACCC	3763
	CAGACTCACCGGGGGCGAAGGGG	2063	CAGACUCACCGGGGGCGAAG	3764
	ACTATTCCAGGGTAAGAAGCTGG	2064	ACUAUUCCAGGGUAAGAAGC	3765
	GACCAGCCTGGACTATTCCAGGG	2065	GACCAGCCUGGACUAUUCCA	3766
	CTGGCGGGGGGATATCATGTGG	2066	CUGGCGGGGGGAUAUCAUG	3767
	GAGAGTTAATGACGGATGTGGGG	2067	GAGAGUUAAUGACGGAUGUG	3768
	CAGGCTGGTCATAGCCGGGTTGG	2068	CAGGCUGGUCAUAGCCGGGU	3769

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TTAATGACGGATGTGGGGAGGGG	2069	UUAAUGACGGAUGUGGGGAG	3770
	CGTATAGTTAAAGGAGAGAAGGG	2070	CGUAUAGUUAAAGGAGAGAA	3771
	AGTTAATGACGGATGTGGGGAGG	2071	AGUUAAUGACGGAUGUGGGG	3772
	TTAATGCTTCTAGTCAGTGAAGG	2072	UUAAUGCUUCUAGUCAGUGA	3773
	GAGGGGTCCTGCCCATCTGTCGG	2073	GAGGGGUCCUGCCCAUCUGU	3774
	TCGTATAGTTAAAGGAGAGAAGG	2074	UCGUAUAGUUAAAGGAGAGA	3775
	CTGTGGGCTCTTCGGTTTCGAGG	2075	CUGUGGGCUCUUCGGUUUCG	3776
	TAGTCCAGGCTGGTCATAGCCGG	2076	UAGUCCAGGCUGGUCAUAGC	3777
	CATTAACTCTCAGATTCAACGGG	2077	CAUUAACUCUCAGAUUCAAC	3778
	CTACGCACATGATCGAGCAGAGG	2078	CUACGCACAUGAUCGAGCAG	3779
	GATCCCGGGTCGTCCCACATGGG	2079	GAUCCCGGGUCGUCCCACAU	3780
	CCGGGTCGGCTGAATGCGAGGGG	2080	CCGGGUCGGCUGAAUGCGAG	3781
	TGGACGCGGGCTTGCGAATGGGG	2081	UGGACGCGGGCUUGCGAAUG	3782
	AGTTGCCAGATGCGCTTCGACGG	2082	AGUUGCCAGAUGCGCUUCGA	3783
	GTTGCCAGATGCGCTTCGACGGG	2083	GUUGCCAGAUGCGCUUCGAC	3784
	GGGGCCCGTCGAAGCGCATCTGG	2084	GGGGCCCGUCGAAGCGCAUC	3785
	ATTCGCAAGCCCGCGTCCATGGG	2085	AUUCGCAAGCCCGCGUCCAU	3786
	CATGGACGCGGGCTTGCGAATGG	2086	CAUGGACGCGGGCUUGCGAA	3787
	CATTCGCAAGCCCGCGTCCATGG	2087	CAUUCGCAAGCCCGCGUCCA	3788
	CGGGTCGGCTGAATGCGAGGGGG	2088	CGGGUCGGCUGAAUGCGAGG	3789
	GGGCTTGTAGTAAACCGACCCGG	2089	GGGCUUGUAGUAAACCGACC	3790
	AGATCCCGGGTCGTCCCACATGG	2090	AGAUCCCGGGUCGUCCCACA	3791
	AGCCGGGTCGGCTGAATGCGAGG	2091	AGCCGGGUCGGCUGAAUGCG	3792
	GCCGGGTCGGCTGAATGCGAGGG	2092	GCCGGGUCGGCUGAAUGCGA	3793
	GAGACGCGTGGCCGATCTGCAGG	2093	GAGACGCGUGGCCGAUCUGC	3794
	ATGGACGCGGGCTTGCGAATGGG	2094	AUGGACGCGGGCUUGCGAAU	3795
	GCGTAGTGGCCACGTAGTTCTGG	2095	GCGUAGUGGCCACGUAGUUC	3796
	TTCGGCGGACCCCGGAGAGCTGG	2096	UUCGGCGGACCCCGGAGAGC	3797
	TACGGCGTGCGCACCTGTGAGGG	2097	UACGGCGUGCGCACCUGUGA	3798
	GCGCACGCCGTAGTGTTGGCAGG	2098	GCGCACGCCGUAGUGUUGGC	3799
	AGGTCTGCCCGTCCACCACGTGG	2099	AGGUCUGCCCGUCCACCACG	3800
	CGCATCTGGCAACTAGACACCGG	2100	CGCAUCUGGCAACUAGACAC	3801
	ATCCCGGGTCGTCCCACATGGGG	2101	AUCCCGGGUCGUCCCACAUG	3802
	ACTAGACACCGGGGTGCCAGGGG	2102	ACUAGACACCGGGGUGCCAG	3803
	GTGCCCTCACCGCCGTCGCGGGG	2103	GUGCCCUCACCGCCGUCGCG	3804
	TCGGCGGACCCCGGAGAGCTGGG	2104	UCGGCGGACCCCGGAGAGCU	3805
	CGGACAGCAGTCCTCCATTAAGG	2105	CGGACAGCAGUCCUCCAUUA	3806
	TGTCGAGCAGCTGAGACGCGTGG	2106	UGUCGAGCAGCUGAGACGCG	3807
	TAGTAAACCGACCCGGAGTGCGG	2107	UAGUAAACCGACCCGGAGUG	3808
	CTACGGCGTGCGCACCTGTGAGG	2108	CUACGGCGUGCGCACCUGUG	3809
	TCCGAGGTCCCGGGCACTAGGGG	2109	UCCGAGGUCCCGGGCACUAG	3810
	CCGGCTCCAGCAACTTCGGGCGG	2110	CCGGCUCCAGCAACUUCGGG	3811

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TCGCATTCAGCCGACCCGGCTGG	2111	UCGCAUUCAGCCGACCCGGC	3812
	GGCTCCAGCAACTTCGGGCGGGG	2112	GGCUCCAGCAACUUCGGGCG	3813
	AACTTCGGGCGGGGGCCAGCCGG	2113	AACUUCGGGCGGGGGCCAGC	3814
	AGGTGCGCACGCCGTAGTGTTGG	2114	AGGUGCGCACGCCGUAGUGU	3815
	CTAGACACCGGGGTGCCAGGGGG	2115	CUAGACACCGGGGUGCCAGG	3816
	TAGTGGCCACGTAGTTCTGGTGG	2116	UAGUGGCCACGUAGUUCUGG	3817
	GATGATGCCGCACTCCGGGTCGG	2117	GAUGAUGCCGCACUCCGGGU	3818
	GCGCACAGCCCCTCGTTGGAGGG	2118	GCGCACAGCCCCUCGUUGGA	3819
	GCGTGGCCGATCTGCAGGCCCGG	2119	GCGUGGCCGAUCUGCAGGCC	3820
	ATGCGAGGGGGATGCGACCCTGG	2120	AUGCGAGGGGGAUGCGACCC	3821
	GCTGTCCGGACAGGGGCATTTGG	2121	GCUGUCCGGACAGGGGCAUU	3822
	GCCAGGGGGCGATTGCTTAAAGG	2122	GCCAGGGGGCGAUUGCUUAA	3823
	CGGCATCATCTCCTCAGACTGGG	2123	CGGCAUCAUCUCCUCAGACU	3824
	CCGGGTTCATGGGGACGTGCAGG	2124	CCGGGUUCAUGGGGACGUGC	3825
	CGCGGGCTTGCGAATGGGGTTGG	2125	CGCGGGCUUGCGAAUGGGGU	3826
	AACGCGGCCTGCCAACACTACGG	2126	AACGCGGCCUGCCAACACUA	3827
	CTGCTCGATCATGTGCGTAGTGG	2127	CUGCUCGAUCAUGUGCGUAG	3828
NR4A3	GTACGGGTGGCTCTCAAGCGCGG	2128	GUACGGGUGGCUCUCAAGCG	3829
	CCACCTCGGCTACGACCCGACGG	2129	CCACCUCGGCUACGACCCGA	3830
	CATAACGCCCCCGCCTGCGGGGG	2130	CAUAACGCCCCCGCCUGCGG	3831
	CGCTTGAGAGCCACCCGTACGGG	2131	CGCUUGAGAGCCACCCGUAC	3832
	CGGCCGTCGGGTCGTAGCCGAGG	2132	CGGCCGUCGGGUCGUAGCCG	3833
	ACCGTGGGGACCGCCTTCATCGG	2133	ACCGUGGGGACCGCCUUCAU	3834
	GACGACGAGCTCCTGCTGGGCGG	2134	GACGACGAGCUCCUGCUGGG	3835
	GTGGGGACCGCCTTCATCGGCGG	2135	GUGGGGACCGCCUUCAUCGG	3836
	TCGGGTCGTAGCCGAGGTGGTGG	2136	UCGGGUCGUAGCCGAGGUGG	3837
	ATAACGCCCCCGCCTGCGGGGGG	2137	AUAACGCCCCCGCCUGCGGG	3838
	GCGCTTGAGAGCCACCCGTACGG	2138	GCGCUUGAGAGCCACCCGUA	3839
	CCCGCAGGCGGGGGCGTTATGGG	2139	CCCGCAGGCGGGGGCGUUAU	3840
	TACGGCGTGCGAACCTGCGAGGG	2140	UACGGCGUGCGAACCUGCGA	3841
	AGGTTCGCACGCCGTAGTGCTGG	2141	AGGUUCGCACGCCGUAGUGC	3842
	CAGGAGCTCGTCGTCTGGCGAGG	2142	CAGGAGCUCGUCGUCUGGCG	3843
	TGGGGACCGCCTTCATCGGCGGG	2143	UGGGGACCGCCUUCAUCGGC	3844
	CCCGGTTTGAGAGCTGTAATCGG	2144	CCCGGUUUGAGAGCUGUAAU	3845
	TCATCGGCGGGTCCAGCAGCGGG	2145	UCAUCGGCGGGUCCAGCAGC	3846
	CTACGGCGTGCGAACCTGCGAGG	2146	CUACGGCGUGCGAACCUGCG	3847
	TCGCACGCCGTAGTGCTGGCAGG	2147	UCGCACGCCGUAGUGCUGGC	3848
	CGGGTGGCTCTCAAGCGCGGCGG	2148	CGGGUGGCUCUCAAGCGCGG	3849
	TTCATCGGCGGGTCCAGCAGCGG	2149	UUCAUCGGCGGGUCCAGCAG	3850
	GCGCCCGGCTGCATCGCACCCGG	2150	GCGCCCGGCUGCAUCGCACC	3851
	TGAGCGCGGCAGCGGCCGTCGGG	2151	UGAGCGCGGCAGCGGCCGUC	3852
	AACGCCGCCTGCCAGCACTACGG	2152	AACGCCGCCUGCCAGCACUA	3853

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GATGAAGGCGGTCCCCACGGTGG	2153	GAUGAAGGCGGUCCCCACGG	3854
	AGGAGCTCGTCGTCTGGCGAGGG	2154	AGGAGCUCGUCGUCUGGCGA	3855
	GCCGATGAAGGCGGTCCCCACGG	2155	GCCGAUGAAGGCGGUCCCCA	3856
	GCGATGCAGCCGGGCGCCGAGGG	2156	GCGAUGCAGCCGGGCGCCGA	3857
	GCTGCTGGACCCGCCGATGAAGG	2157	GCUGCUGGACCCGCCGAUGA	3858
	GCCGATTACAGCTCTCAAACCGG	2158	GCCGAUUACAGCUCUCAAAC	3859
	GGGCACGTGTGCCGTGTGCGGGG	2159	GGGCACGUGUGCCGUGUGCG	3860
	CCACCCGTACGGGCTGCCGCTGG	2160	CCACCCGUACGGGCUGCCGC	3861
	CCCAGCAGGAGCTCGTCGTCTGG	2161	CCCAGCAGGAGCUCGUCGUC	3862
	CAGCAGGCTGGACGCGGTAGGGG	2162	CAGCAGGCUGGACGCGGUAG	3863
	CCCCGCAGGCGGGGGCGTTATGG	2163	CCCCGCAGGCGGGGGCGUUA	3864
	GGCGGCGTTGTCCCCGCACACGG	2164	GGCGGCGUUGUCCCCGCACA	3865
	CACGCCGTAGTGCTGGCAGGCGG	2165	CACGCCGUAGUGCUGGCAGG	3866
	TCCCATAACGCCCCCGCCTGCGG	2166	UCCCAUAACGCCCCCGCCUG	3867
	CCTACCGCGTCCAGCCTGCTGGG	2167	CCUACCGCGUCCAGCCUGCU	3868
	AGGGCACGTGTGCCGTGTGCGGG	2168	AGGGCACGUGUGCCGUGUGC	3869
	TTAGAAGCTCCCTTCAGTGAGGG	2169	UUAGAAGCUCCCUUCAGUGA	3870
	CTCGCCCAGCAGGCTGGACGCGG	2170	CUCGCCCAGCAGGCUGGACG	3871
	GGACTGCTTGAAGTACATGGAGG	2171	GGACUGCUUGAAGUACAUGG	3872
	TGGCCAGCGGCAGCCCGTACGGG	2172	UGGCCAGCGGCAGCCCGUAC	3873
	GGCTGGGACTCTCGCCCAGCAGG	2173	GGCUGGGACUCUCGCCCAGC	3874
	GGCTCTCAAGCGCGGCGGCCTGG	2174	GGCUCUCAAGCGCGGCGGCC	3875
	GGCGGGGGCGTTATGGGACGAGG	2175	GGCGGGGGCGUUAUGGGACG	3876
	TCCTGCTGGGCGGCGACGGCAGG	2176	UCCUGCUGGGCGGCGACGGC	3877
	CTTAGAAGCTCCCTTCAGTGAGG	2177	CUUAGAAGCUCCCUUCAGUG	3878
	CGCACCCAGTAAATGATGCGGGG	2178	CGCACCCAGUAAAUGAUGCG	3879
	CGAGGGGAACTCCTTCGTTGGGG	2179	CGAGGGGAACUCCUUCGUUG	3880
	TCTCCATTCAACGCCGCGCGGGG	2180	UCUCCAUUCAACGCCGCGCG	3881
	AAAAACCTCCGAGGTGCGCGGGG	2181	AAAAACCUCCGAGGUGCGCG	3882
	AGACGTCAATGTGACGCCATGGG	2182	AGACGUCAAUGUGACGCCAU	3883
	GTGATTCAAGCGGACCACATGGG	2183	GUGAUUCAAGCGGACCACAU	3884
	ACGAGCTCCGCCCGAATACGGGG	2184	ACGAGCUCCGCCCGAAUACG	3885
	ATTTCTTTACACGTACGGCGTGG	2185	AUUUCUUUACACGUACGGCG	3886
	GGTCATGCGAGCGCAGCCTGCGG	2186	GGUCAUGCGAGCGCAGCCUG	3887
	CGAGCTCCGCCCGAATACGGGGG	2187	CGAGCUCCGCCCGAAUACGG	3888
	ATCCGGCACTGGACTCGCGATGG	2188	AUCCGGCACUGGACUCGCGA	3889
	TAGGTAACCGGCCGCTTGTGGGG	2189	UAGGUAACCGGCCGCUUGUG	3890
	GCGAACGCTGGGCGCTCGAGGGG	2190	GCGAACGCUGGGCGCUCGAG	3891
	CTGGACTCGCGATGGAATGACGG	2191	CUGGACUCGCGAUGGAAUGA	3892
	AGCTTGCGCTCGATGTAGCGCGG	2192	AGCUUGCGCUCGAUGUAGCG	3893
	GGACGGCGTTAGCGGCTGATGGG	2193	GGACGGCGUUAGCGGCUGAU	3894
	CTATTAGCCGCGAGTTTCGAGGG	2194	CUAUUAGCCGCGAGUUUCGA	3895

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GACGAAGCGGACGGCGTTAGCGG	2195	GACGAAGCGGACGGCGUUAG	3896
	ACCGGCATGTCAGCGACGACAGG	2196	ACCGGCAUGUCAGCGACGAC	3897
	TTTCTTTACACGTACGGCGTGGG	2197	UUUCUUUACACGUACGGCGU	3898
	TTCCATCGCGAGTCCAGTGCCGG	2198	UUCCAUCGCGAGUCCAGUGC	3899
	TGCAGCGGAACCGCTCGCCAGGG	2199	UGCAGCGGAACCGCUCGCCA	3900
	ATGTTACTAAATTCGGCGGTTGG	2200	AUGUUACUAAAUUCGGCGGU	3901
	GCTTTTCGCCTCTTCGAGTGGGG	2201	GCUUUUCGCCUCUUCGAGUG	3902
	ATCAGAACCTACGGGCCGCTGGG	2202	AUCAGAACCUACGGGCCGCU	3903
	CCGACTATATTTGGTTCGGCCGG	2203	CCGACUAUAUUUGGUUCGGC	3904
	TCTATTAGCCGCGAGTTTCGAGG	2204	UCUAUUAGCCGCGAGUUUCG	3905
	GGCCCGGCGGTTCTACCACCCGG	2205	GGCCCGGCGGUUCUACCACC	3906
	AGCTGTCCCGAAATCTGCACTGG	2206	AGCUGUCCCGAAAUCUGCAC	3907
	CTCGAGCGCCCAGCGTTCGCGGG	2207	CUCGAGCGCCCAGCGUUCGC	3908
	GGACTCGGTTCGACCAGGTCTGG	2208	GGACUCGGUUCGACCAGGUC	3909
	CGGACGGCGTTAGCGGCTGATGG	2209	CGGACGGCGUUAGCGGCUGA	3910
	GATTCCGAGCTTACGAAGTCAGG	2210	GAUUCCGAGCUUACGAAGUC	3911
	GCGTTCCTCGGCCAGTCGCACGG	2211	GCGUUCCUCGGCCAGUCGCA	3912
	CGGCCGAACCAAATATAGTCGGG	2212	CGGCCGAACCAAAUAUAGUC	3913
	TTCTCCATTCAACGCCGCGCGGG	2213	UUCUCCAUUCAACGCCGCGC	3914
	TGCGGCCGTGCGACTGGCCGAGG	2214	UGCGGCCGUGCGACUGGCCG	3915
	CTGCCGGGTGGTAGAACCGCCGG	2215	CUGCCGGGUGGUAGAACCGC	3916
	ACTCCGCTTGAAAGGCCCTCAGG	2216	ACUCCGCUUGAAAGGCCCUC	3917
	CGGCTCTCTTCGTCCGGCGCGGG	2217	CGGCUCUCUUCGUCCGGCGC	3918
	AGAGAACGACTCCGCTTGAAAGG	2218	AGAGAACGACUCCGCUUGAA	3919
	TCGTCGCCGGTCACCAGACCTGG	2219	UCGUCGCCGGUCACCAGACC	3920
	AACGAGCTCCGCCCGAATACGGG	2220	AACGAGCUCCGCCCGAAUAC	3921
	TACTAAATTCGGCGGTTGGCCGG	2221	UACUAAAUUCGGCGGUUGGC	3922
	CTAGGTAACCGGCCGCTTGTGGG	2222	CUAGGUAACCGGCCGCUUGU	3923
	GGGCGCTATAGGCCGGAGTTTGG	2223	GGGCGCUAUAGGCCGGAGUU	3924
	TCGAGGGGAACTCCTTCGTTGGG	2224	UCGAGGGGAACUCCUUCGUU	3925
	AGCCGCGAGTTTCGAGGGCCAGG	2225	AGCCGCGAGUUUCGAGGGCC	3926
	CCCTCGAAGACACCGCCCTCTGG	2226	CCCUCGAAGACACCGCCCUC	3927
	GAACGAGCTCCGCCCGAATACGG	2227	GAACGAGCUCCGCCCGAAUA	3928
	ACCCTCGACGACCAGGAAATGGG	2228	ACCCUCGACGACCAGGAAAU	3929
	GGCGCTATAGGCCGGAGTTTGGG	2229	GGCGCUAUAGGCCGGAGUUU	3930
	CCGCACCACCGTGTCTGAATTGG	2230	CCGCACCACCGUGUCUGAAU	3931
	CTCGCACACGCGGAACCGGCTGG	2231	CUCGCACACGCGGAACCGGC	3932
	ATGTGCCCCCGCTAGGCCGCTGG	2232	AUGUGCCCCCGCUAGGCCGC	3933
	TAGCCAGGCCCGACTATATTTGG	2233	UAGCCAGGCCCGACUAUAUU	3934
	ACTGACCCCCCGTATTCGGGCGG	2234	ACUGACCCCCCGUAUUCGGG	3935
	ATAGCGGAGTAGGTTCCCCTCGG	2235	AUAGCGGAGUAGGUUCCCCU	3936
	TGGTCGAACCGAGTCCAAGATGG	2236	UGGUCGAACCGAGUCCAAGA	3937

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GATACCCTTCCCGGACGTCACGG	2237	GAUACCCUUCCCGGACGUCA	3938
	GAGCTCCGCCCGAATACGGGGGG	2238	GAGCUCCGCCCGAAUACGGG	3939
	CGTTGCAAAGTGAGCCCGGGAGG	2239	CGUUGCAAAGUGAGCCCGGG	3940
	ACAAGCCCAGCGGCTCCCGGAGG	2240	ACAAGCCCAGCGGCUCCCGG	3941
	GCGGTTTGTCTAGTCTCCCTCGG	2241	GCGGUUUGUCUAGUCUCCCU	3942
	TCCCGCACACTGACACGTGTGGG	2242	UCCCGCACACUGACACGUGU	3943
	CTCGGCTGGGTCAACTTTCGGGG	2243	CUCGGCUGGGUCAACUUUCG	3944
	TGCACCCGTGATGCAAGTGCAGG	2244	UGCACCCGUGAUGCAAGUGC	3945
	CGGCACGTCATTTATGCCACAGG	2245	CGGCACGUCAUUUAUGCCAC	3946
	TTTTCGCCTCTTCGAGTGGGGGG	2246	UUUUCGCCUCUUCGAGUGGG	3947
	ATCTTGGACTCGGTTCGACCAGG	2247	AUCUUGGACUCGGUUCGACC	3948
	GAGACGTCAATGTGACGCCATGG	2248	GAGACGUCAAUGUGACGCCA	3949
	GACCGGGATTTGTGCTATAGCGG	2249	GACCGGGAUUUGUGCUAUAG	3950
	TGCCGGGTGGTAGAACCGCCGGG	2250	UGCCGGGUGGUAGAACCGCC	3951
	CGATGTAGCGCGGGTAGAAGCGG	2251	CGAUGUAGCGCGGGUAGAAG	3952
	GAACCGCCGGGCCTTCCGCAGGG	2252	GAACCGCCGGGCCUUCCGCA	3953
	CGCGCCGCAGATAGCGGAGTAGG	2253	CGCGCCGCAGAUAGCGGAGU	3954
	AGCCGGTTCCGCGTGTGCGAGGG	2254	AGCCGGUUCCGCGUGUGCGA	3955
	GAACCAAATATAGTCGGGCCTGG	2255	GAACCAAAUAUAGUCGGGCC	3956
	CTTTCTCGTGGGCAGACGAAAGG	2256	CUUUCUCGUGGGCAGACGAA	3957
	GGAACTCCTTCGTTGGGGAGAGG	2257	GGAACUCCUUCGUUGGGGAG	3958
	CGTAAGCTCGGAATCAATTGTGG	2258	CGUAAGCUCGGAAUCAAUUG	3959
	GCAAGAGGGGTGTGAGCGCGCGG	2259	GCAAGAGGGGUGUGAGCGCG	3960
	TATTTCCCGGTCGTGGGAAAAGG	2260	UAUUUCCCGGUCGUGGGAAA	3961
	AGTGCCGGGGATACCCTTCCCGG	2261	AGUGCCGGGGAUACCCUUCC	3962
	TACACCCGATTTACCTCCTAAGG	2262	UACACCCGAUUUACCUCCUA	3963
	CCGCGCACCTCGGAGGTTTTTGG	2263	CCGCGCACCUCGGAGGUUUU	3964
	TCTCGGCTGGGTCAACTTTCGGG	2264	UCUCGGCUGGGUCAACUUUC	3965
	GGTTCCGCGTGTGCGAGGGAGGG	2265	GGUUCCGCGUGUGCGAGGGA	3966
	CGCGCGGCGTTGAATGGAGAAGG	2266	CGCGCGGCGUUGAAUGGAGA	3967
	CCTAGGTAACCGGCCGCTTGTGG	2267	CCUAGGUAACCGGCCGCUUG	3968
	TGCAGATGGTTCCGGGGATAAGG	2268	UGCAGAUGGUUCCGGGGAUA	3969
	GCAACCGAGTCTCTGCACTGCGG	2269	GCAACCGAGUCUCUGCACUG	3970
	CGACAGGCAGACGTGCCTAGTGG	2270	CGACAGGCAGACGUGCCUAG	3971
	GAGTTTGCAGACGCACTCGGAGG	2271	GAGUUUGCAGACGCACUCGG	3972
	TTAGCGGCTGATGGGACGAGCGG	2272	UUAGCGGCUGAUGGGACGAG	3973
	GAAACGCACCATTGTGACCGGGG	2273	GAAACGCACCAUUGUGACCG	3974
	TACGTACTTGAGTGCTGTGGCGG	2274	UACGUACUUGAGUGCUGUGG	3975
	GAAGAATTTCTGTGAGCGCACGG	2275	GAAGAAUUUCUGUGAGCGCA	3976
	GGCGATGTTACTAAATTCGGCGG	2276	GGCGAUGUUACUAAAUUCGG	3977
	ACCCTTCCCGGACGTCACGGAGG	2277	ACCCUUCCCGGACGUCACGG	3978
JUNB	GGGTAAAAGTACTGTCCCGGGGG	2278	GGGUAAAAGUACUGUCCCGG	3979

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TCTGCCCAGTGACGCGACCGCGG	2279	UCUGCCCAGUGACGCGACCG	3980
	GGACAGTACTTTTACCCCCGCGG	2280	GGACAGUACUUUUACCCCCG	3981
	ACTTCCGTGGCTGACTAGCGCGG	2281	ACUUCCGUGGCUGACUAGCG	3982
	GTCCCGTAGGATCCGAGTGACGG	2282	GUCCCGUAGGAUCCGAGUGA	3983
	TAGCGCGGTATAAAGGCGTGTGG	2283	UAGCGCGGUAUAAAGGCGUG	3984
	CTGACAGCCGTTGCTGACGTGGG	2284	CUGACAGCCGUUGCUGACGU	3985
	GACTAAGAGGTTACCATCGAGGG	2285	GACUAAGAGGUUACCAUCGA	3986
	GTACGAGCTCCCGGTCCCGACGG	2286	GUACGAGCUCCCGGUCCCGA	3987
	GCGCTTTGAGACTCCGGTAGGGG	2287	GCGCUUUGAGACUCCGGUAG	3988
	TCGCGCCAGAGAGGGCGACGGGG	2288	UCGCGCCAGAGAGGGCGACG	3989
	ATGCCTGCGCCGAACCGACGAGG	2289	AUGCCUGCGCCGAACCGACG	3990
	TGCGCACTCCAAGTCTCGGCCGG	2290	UGCGCACUCCAAGUCUCGGC	3991
	ATGTGTCCCCCTCGTCGGTTCGG	2291	AUGUGUCCCCCUCGUCGGUU	3992
	CGCCGCCCATATTAGGGCACAGG	2292	CGCCGCCCAUAUUAGGGCAC	3993
	ACTCAAGCCCGCGGGGACATTGG	2293	ACUCAAGCCCGCGGGGACAU	3994
	GTCGCGTCACTGGGCAGAATCGG	2294	GUCGCGUCACUGGGCAGAAU	3995
	TCCCCGCGGGCTTGAGTACCAGG	2295	UCCCCGCGGGCUUGAGUACC	3996
	TGTTCCATTGGCCCGACGGCGGG	2296	UGUUCCAUUGGCCCGACGGC	3997
	ATAGTCGGGTTCCCCGCTTCTGG	2297	AUAGUCGGGUUCCCCGCUUC	3998
	GGTCGCGCGTTCTCGGGGGCTGG	2298	GGUCGCGCGUUCUCGGGGGC	3999
	AACGTGTCCCTGGGCGCTACCGG	2299	AACGUGUCCCUGGGCGCUAC	4000
	CTCCGCTGCGGTGACCGGACTGG	2300	CUCCGCUGCGGUGACCGGAC	4001
	GGCTGACTAGCGCGGTATAAAGG	2301	GGCUGACUAGCGCGGUAUAA	4002
	AGTGACGCGACCGCGGTCTCTGG	2302	AGUGACGCGACCGCGGUCUC	4003
	CGCCGGGTGGCCACCGGCGAAGG	2303	CGCCGGGUGGCCACCGGCGA	4004
	TGACTAAGAGGTTACCATCGAGG	2304	UGACUAAGAGGUUACCAUCG	4005
	ACAGTACTTTTACCCCCGCGGGG	2305	ACAGUACUUUUACCCCCGCG	4006
	GTGCCCTAATATGGGCGGCGGGG	2306	GUGCCCUAAUAUGGGCGGCG	4007
	CCAATCGGAGCGCACTTCCGTGG	2307	CCAAUCGGAGCGCACUUCCG	4008
	GGGGCTTGTAAACGTCGAGGTGG	2308	GGGGCUUGUAAACGUCGAGG	4009
	TCAAGCAATGGTTCCGCCCGCGG	2309	UCAAGCAAUGGUUCCGCCCG	4010
	GTGTTCCATTGGCCCGACGGCGG	2310	GUGUUCCAUUGGCCCGACGG	4011
	TATCGCGCCAGAGAGGGCGACGG	2311	UAUCGCGCCAGAGAGGGCGA	4012
	TGCCTGCGCCGAACCGACGAGGG	2312	UGCCUGCGCCGAACCGACGA	4013
	ACACAGCTACGGGATACGGCCGG	2313	ACACAGCUACGGGAUACGGC	4014
	TATGAGTCGTCGTGGTAGAAGGG	2314	UAUGAGUCGUCGUGGUAGAA	4015
	CGACGACTCATACACAGCTACGG	2315	CGACGACUCAUACACAGCUA	4016
	AAAGGACCTCGGGGTACGCATGG	2316	AAAGGACCUCGGGGUACGCA	4017
	ACGCTCAAGGCCGAGAACGCGGG	2317	ACGCUCAAGGCCGAGAACGC	4018
	GCGGGGCTGTCGAGTACCGCCGG	2318	GCGGGGCUGUCGAGUACCGC	4019
	GACGCTCAAGGCCGAGAACGCGG	2319	GACGCUCAAGGCCGAGAACG	4020
	GAGACCGCGGTCGCGTCACTGGG	2320	GAGACCGCGGUCGCGUCACU	4021

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GGCGCTTTGAGACTCCGGTAGGG	2321	GGCGCUUUGAGACUCCGGUA	4022
	CCACACGCGCCGCCCTTCGCCGG	2322	CCACACGCGCCGCCCUUCGC	4023
	GTGCCCAGCCGTCCAAGCGAGGG	2323	GUGCCCAGCCGUCCAAGCGA	4024
	GTTCCATTGGCCCGACGGCGGGG	2324	GUUCCAUUGGCCCGACGGCG	4025
	GGGGTTTCTTCGCACATACTGGG	2325	GGGGUUUCUUCGCACAUACU	4026
	TGCCTGGTCGCGCGTTCTCGGGG	2326	UGCCUGGUCGCGCGUUCUCG	4027
	CACGACGACGCCTACACCCCCGG	2327	CACGACGACGCCUACACCCC	4028
	CGTTGCTGTTGGGGACAATCAGG	2328	CGUUGCUGUUGGGGACAAUC	4029
	CGTGTCCCTGGGCGCTACCGGGG	2329	CGUGUCCCUGGGCGCUACCG	4030
	TTCCATTGGCCCGACGGCGGGGG	2330	UUCCAUUGGCCCGACGGCGG	4031
	TACTTTTACCCCCGCGGGGGTGG	2331	UACUUUUACCCCCGCGGGGG	4032
	ACTCGACAGCCCCGCGTTCTCGG	2332	ACUCGACAGCCCCGCGUUCU	4033
	TGGCCCGCCTAGAGGGAGTCTGG	2333	UGGCCCGCCUAGAGGGAGUC	4034
	GTCCCCCGCCGTCGGGCCAATGG	2334	GUCCCCCGCCGUCGGGCCAA	4035
	TGTGCCCTAATATGGGCGGCGGG	2335	UGUGCCCUAAUAUGGGCGGC	4036
	TTTACGGACACCCCCTCGCTTGG	2336	UUUACGGACACCCCCUCGCU	4037
	TCTCGTATTCTGGGTACCTCAGG	2337	UCUCGUAUUCUGGGUACCUC	4038
	TGGTCGGGACTAGCAGTCTGGGG	2338	UGGUCGGGACUAGCAGUCUG	4039
	CGTGATCACGCCGTTGCTGTTGG	2339	CGUGAUCACGCCGUUGCUGU	4040
	TCTCCCCGCCGCCCATATTAGGG	2340	UCUCCCCGCCGCCCAUAUUA	4041
	AGAGACCGCGGTCGCGTCACTGG	2341	AGAGACCGCGGUCGCGUCAC	4042
	ACCCCCTCGCTTGGACGGCTGGG	2342	ACCCCCUCGCUUGGACGGCU	4043
	ACACCGGCGGCGTGGCGTCCCGG	2343	ACACCGGCGGCGUGGCGUCC	4044
	AGCGAGGGGGTGTCCGTAAAGGG	2344	AGCGAGGGGGUGUCCGUAAA	4045
	GGCAGAATCGGTCCTTGTATGGG	2345	GGCAGAAUCGGUCCUUGUAU	4046
	CTAAGAGGTTACCATCGAGGGGG	2346	CUAAGAGGUUACCAUCGAGG	4047
	TATACCGCGCTAGTCAGCCACGG	2347	UAUACCGCGCUAGUCAGCCA	4048
	GAAAGCTAGTAAGCGGCCTGGGG	2348	GAAAGCUAGUAAGCGGCCUG	4049
	TCCGTCTGACCTGACCGGGGCGG	2349	UCCGUCUGACCUGACCGGGG	4050
	ACGCCCAGGTTCCTCTTCCGAGG	2350	ACGCCCAGGUUCCUCUUCCG	4051
	GCCTGGTACTCAAGCCCGCGGGG	2351	GCCUGGUACUCAAGCCCGCG	4052
	GCCTGCGCCGAACCGACGAGGGG	2352	GCCUGCGCCGAACCGACGAG	4053
	CAGTACTTTTACCCCCGCGGGGG	2353	CAGUACUUUUACCCCCGCGG	4054
	GCCCCGGTCAGGTCAGACGGAGG	2354	GCCCCGGUCAGGUCAGACGG	4055
	ACTAAGAGGTTACCATCGAGGGG	2355	ACUAAGAGGUUACCAUCGAG	4056
	ACCCAGTCCGGTCACCGCAGCGG	2356	ACCCAGUCCGGUCACCGCAG	4057
	GACGACTCATACACAGCTACGGG	2357	GACGACUCAUACACAGCUAC	4058
	AGGCTCTCCCGTAAGCGGGAAGG	2358	AGGCUCUCCCGUAAGCGGGA	4059
	TCCTTGTAAACAGCGGCCACGGG	2359	UCCUUGUAAACAGCGGCCAC	4060
	GGTTCGGCGCAGGCATCTTGTGG	2360	GGUUCGGCGCAGGCAUCUUG	4061
	GGAAAGCTATCGCGCCAGAGAGG	2361	GGAAAGCUAUCGCGCCAGAG	4062
	CGCTCAAGGCCGAGAACGCGGGG	2362	CGCUCAAGGCCGAGAACGCG	4063

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CACAGCTACGGGATACGGCCGGG	2363	CACAGCUACGGGAUACGGCC	4064
	TGCCCAGCCGTCCAAGCGAGGGG	2364	UGCCCAGCCGUCCAAGCGAG	4065
	TGTGCCCAGCCGTCCAAGCGAGG	2365	UGUGCCCAGCCGUCCAAGCG	4066
	CGGCCAGACTCCCTCTAGGCGGG	2366	CGGCCAGACUCCCUCUAGGC	4067
	CGCCTGGTACTCAAGCCCGCGGG	2367	CGCCUGGUACUCAAGCCCGC	4068
	GGGGAACCCGACTATCTGCCAGG	2368	GGGGAACCCGACUAUCUGCC	4069
	CTCGTATTCTGGGTACCTCAGGG	2369	CUCGUAUUCUGGGUACCUCA	4070
	GACAGTACTTTTACCCCCGCGGG	2370	GACAGUACUUUUACCCCCGC	4071
	CCGGGGGCGAAGTCCGACCCAGG	2371	CCGGGGGCGAAGUCCGACCC	4072
	GGACTTCGCCCCCGGCCCGACGG	2372	GGACUUCGCCCCCGGCCCGA	4073
	ACTGTAAATCGGGAGGGTTAAGG	2373	ACUGUAAAUCGGGAGGGUUA	4074
	TAGCGCCCAGGGACACGTTGGGG	2374	UAGCGCCCAGGGACACGUUG	4075
	AGGGTGCTCCGGCCGAGACTTGG	2375	AGGGUGCUCCGGCCGAGACU	4076
	TGATCACGCCGTTGCTGTTGGGG	2376	UGAUCACGCCGUUGCUGUUG	4077
	GCCCGTGGCCGCTGTTTACAAGG	2377	GCCCGUGGCCGCUGUUUACA	4078
REL	GTGAGCCGCAAACCCAGCGGAGG	2378	GUGAGCCGCAAACCCAGCGG	4079
	CGACGGCCGGGGTTTTCGAGAGG	2379	CGACGGCCGGGGUUUUCGAG	4080
	CGTCGGGCCTACGTCAGCCGCGG	2380	CGUCGGGCCUACGUCAGCCG	4081
	CGGCCGGGGTTTTCGAGAGGTGG	2381	CGGCCGGGGUUUUCGAGAGG	4082
	CAGCGTCGCCGTCCACCGTACGG	2382	CAGCGUCGCCGUCCACCGUA	4083
	GGCGGGACGTTGCGCCCTGTAGG	2383	GGCGGGACGUUGCGCCCUGU	4084
	CGGGACGTTGCGCCCTGTAGGGG	2384	CGGGACGUUGCGCCCUGUAG	4085
	GCGGGACGTTGCGCCCTGTAGGG	2385	GCGGGACGUUGCGCCCUGUA	4086
	CGTACGGTGGACGGCGACGCTGG	2386	CGUACGGUGGACGGCGACGC	4087
	CGTCCACCGTACGGGAGCCAGGG	2387	CGUCCACCGUACGGGAGCCA	4088
	AGGGCGCAACGTCCCGCCGCTGG	2388	AGGGCGCAACGUCCCGCCGC	4089
	AGCGTCGCCGTCCACCGTACGGG	2389	AGCGUCGCCGUCCACCGUAC	4090
	CGCCGGGGGCGTATGCGTGGGGG	2390	CGCCGGGGGCGUAUGCGUGG	4091
	GGGTCCCCGTATGCAAATACAGG	2391	GGGUCCCCGUAUGCAAAUAC	4092
	GCGGCCGCAGTCAGTCAGTCAGG	2392	GCGGCCGCAGUCAGUCAGUC	4093
	GCAACGTCCCGCCGCTGGCGCGG	2393	GCAACGUCCCGCCGCUGGCG	4094
	ACGCAGCAACCCTCACCCGGAGG	2394	ACGCAGCAACCCUCACCCGG	4095
	CGCGCCCCATGAACACTCACCGG	2395	CGCGCCCCAUGAACACUCAC	4096
	GGGACGTTGCGCCCTGTAGGGGG	2396	GGGACGUUGCGCCCUGUAGG	4097
	GAATTTCCCGCGGCTGACGTAGG	2397	GAAUUUCCCGCGGCUGACGU	4098
	GTACGGTGGACGGCGACGCTGGG	2398	GUACGGUGGACGGCGACGCU	4099
	GCCGCAAACCCAGCGGAGGGCGG	2399	GCCGCAAACCCAGCGGAGGG	4100
	TGACTGACTGCGGCCGCCTCCGG	2400	UGACUGACUGCGGCCGCCUC	4101
	CAGTACCCTCGCAATTTAGATGG	2401	CAGUACCCUCGCAAUUUAGA	4102
	CGTATGCGTGGGGGCCGGCGGGG	2402	CGUAUGCGUGGGGGCCGGCG	4103
	AACGTCCCGCCGCTGGCGCGGGG	2403	AACGUCCCGCCGCUGGCGCG	4104
	AGCGCCGGGGGCGTATGCGTGGG	2404	AGCGCCGGGGGCGUAUGCGU	4105

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GCGTATGCGTGGGGGCCGGCGGG	2405	GCGUAUGCGUGGGGGCCGGC	4106
	CGGCGACGCTGGGTGACCCGGGG	2406	CGGCGACGCUGGGUGACCCG	4107
	TGACGGCTAGCAGCGTGAGAAGG	2407	UGACGGCUAGCAGCGUGAGA	4108
	GTCGGGCCTACGTCAGCCGCGGG	2408	GUCGGGCCUACGUCAGCCGC	4109
	GGCCCCCACGCATACGCCCCCGG	2409	GGCCCCCACGCAUACGCCCC	4110
	CCCCGCCGGCAGAGGTCCCTCGG	2410	CCCCGCCGGCAGAGGUCCCU	4111
	GAACCACCTCTCGAAAACCCCGG	2411	GAACCACCUCUCGAAAACCC	4112
	CCACACTCGGAAGAACAACCTGG	2412	CCACACUCGGAAGAACAACC	4113
	TTGCGCCCTGTAGGGGGAAGTGG	2413	UUGCGCCCUGUAGGGGGAAG	4114
	TACCCTCGCAATTTAGATGGAGG	2414	UACCCUCGCAAUUUAGAUGG	4115
	GTCCACCGTACGGGAGCCAGGGG	2415	GUCCACCGUACGGGAGCCAG	4116
	CCTGGCTCCCGTACGGTGGACGG	2416	CCUGGCUCCCGUACGGUGGA	4117
	CAACCCTCACCCGGAGGCGTGGG	2417	CAACCCUCACCCGGAGGCGU	4118
	CGCTGCTAGCCGTCACCTCCCGG	2418	CGCUGCUAGCCGUCACCUCC	4119
	GCGCCGGGGGCGTATGCGTGGGG	2419	GCGCCGGGGGCGUAUGCGUG	4120
	CAACGTCCCGCCGCTGGCGCGGG	2420	CAACGUCCCGCCGCUGGCGC	4121
	CGTAGAGAGGGCCGGCCGCTGGG	2421	CGUAGAGAGGGCCGGCCGCU	4122
	CCCGGGGTGCAAGAATTCAGGGG	2422	CCCGGGGUGCAAGAAUUCAG	4123
	TGAGCCGCAAACCCAGCGGAGGG	2423	UGAGCCGCAAACCCAGCGGA	4124
	GTTTAAAGTTCAGGAGCGGGCGG	2424	GUUUAAAGUUCAGGAGCGGG	4125
	GGCGGGAGGGGAATTTCCCGCGG	2425	GGCGGGAGGGGAAUUUCCCG	4126
	AGGGTGCGGATGACGTAGAGAGG	2426	AGGGUGCGGAUGACGUAGAG	4127
	CCGCCCACGCCTCCGGGTGAGGG	2427	CCGCCCACGCCUCCGGGUGA	4128
TOX	TTTCCCGTGGAATGCACCGAGGG	2428	UUUCCCGUGGAAUGCACCGA	4129
	GGTGGCGAGTCATCACCAAACGG	2429	GGUGGCGAGUCAUCACCAAA	4130
	AGGGACTCGAGCCGATCGAAGGG	2430	AGGGACUCGAGCCGAUCGAA	4131
	ACGTGTCTAAGCAGTCCCGTTGG	2431	ACGUGUCUAAGCAGUCCCGU	4132
	AAACGCCCCCCGGCAAACCTAGG	2432	AAACGCCCCCCGGCAAACCU	4133
	TTGAACGCGACGTGCTCGCCCGG	2433	UUGAACGCGACGUGCUCGCC	4134
	GTCGCGTGGTGCGGAGTCCAGGG	2434	GUCGCGUGGUGCGGAGUCCA	4135
	CGCGGTGTTTGGCAAGCCCCCGG	2435	CGCGGUGUUUGGCAAGCCCC	4136
	GATCCTTAGCCGCGAACAGCAGG	2436	GAUCCUUAGCCGCGAACAGC	4137
	TTCCGCACAATCGCGGTGTTTGG	2437	UUCCGCACAAUCGCGGUGUU	4138
	TCCGCGCGCACCCCTTAAACAGG	2438	UCCGCGCGCACCCCUUAAAC	4139
	GCGAGAGTTGGGCGTCTAAAAGG	2439	GCGAGAGUUGGGCGUCUAAA	4140
	AAGCCGCGGCGCGCACCCGTCGG	2440	AAGCCGCGGCGCGCACCCGU	4141
	ATATTGTGGAGTAGCTCCGGGGG	2441	AUAUUGUGGAGUAGCUCCGG	4142
	TACACTTCGAATCACCCCTGTGG	2442	UACACUUCGAAUCACCCCUG	4143
	AGTCCCAACGATTTTTCCCGTGG	2443	AGUCCCAACGAUUUUUCCCG	4144
	TTTACTACCCAAGCGCACGCAGG	2444	UUUACUACCCAAGCGCACGC	4145
	CGTCCAACTAGCCCTAGGCGTGG	2445	CGUCCAACUAGCCCUAGGCG	4146
	GTCCAACTAGCCCTAGGCGTGGG	2446	GUCCAACUAGCCCUAGGCGU	4147

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AGTGGGGCACGAATCTCGGAGGG	2447	AGUGGGGCACGAAUCUCGGA	4148
	GCCTTCGCAAACCGTCCAGTGGG	2448	GCCUUCGCAAACCGUCCAGU	4149
	TCAGCACACAATCCGGCTAAAGG	2449	UCAGCACACAAUCCGGCUAA	4150
	TGAAGTTACCTGCCCGGCGGCGG	2450	UGAAGUUACCUGCCCGGCGG	4151
	TGCGACTCGGTCGCGTGGTGCGG	2451	UGCGACUCGGUCGCGUGGUG	4152
	TTAGACGCCCAACTCTCGCTTGG	2452	UUAGACGCCCAACUCUCGCU	4153
	TAGAGCCCGAGCGCGTGTGCCGG	2453	UAGAGCCCGAGCGCGUGUGC	4154
	TTCGATCGGCTCGAGTCCCTCGG	2454	UUCGAUCGGCUCGAGUCCCU	4155
	GCGCTCGGGCTCTAGGTACTGGG	2455	GCGCUCGGGCUCUAGGUACU	4156
	TCCACTACGGGCCGGGAGTAGGG	2456	UCCACUACGGGCCGGGAGUA	4157
	CGAGTCGCAGCTCCGAGTCTTGG	2457	CGAGUCGCAGCUCCGAGUCU	4158
	GGCCGGACGCGGGCTCGTCAAGG	2458	GGCCGGACGCGGGCUCGUCA	4159
	CGCGGAAATTGCAAGTTTGTTGG	2459	CGCGGAAAUUGCAAGUUUGU	4160
	GCATGAAGTTACCTGCCCGGCGG	2460	GCAUGAAGUUACCUGCCCGG	4161
	TGTCACTTTCCGCACAATCGCGG	2461	UGUCACUUUCCGCACAAUCG	4162
	CAGTCCCGTTGGATGAACGTTGG	2462	CAGUCCCGUUGGAUGAACGU	4163
	CCAGCGCGTCGCACACAAAGGGG	2463	CCAGCGCGUCGCACACAAAG	4164
	GGAGCTGCGACTCGGTCGCGTGG	2464	GGAGCUGCGACUCGGUCGCG	4165
	GACCAGCGCGTCGCACACAAAGG	2465	GACCAGCGCGUCGCACACAA	4166
	CAAGACTCGGAGCTGCGACTCGG	2466	CAAGACUCGGAGCUGCGACU	4167
	CTAACTTGCCTAAACACCATCGG	2467	CUAACUUGCCUAAACACCAU	4168
	CCTTTGAGTGGGTCTCACACTGG	2468	CCUUUGAGUGGGUCUCACAC	4169
	ATTCCACGGGAAAAATCGTTGGG	2469	AUUCCACGGGAAAAAUCGUU	4170
	GAATGCACCGAGGGTCGCCATGG	2470	GAAUGCACCGAGGGUCGCCA	4171
	GGGTTCGGACACAGGTCCGCGGG	2471	GGGUUCGGACACAGGUCCGC	4172
	GAGGGACTCGAGCCGATCGAAGG	2472	GAGGGACUCGAGCCGAUCGA	4173
	TGGACGGTTTGCGAAGGCTGAGG	2473	UGGACGGUUUGCGAAGGCUG	4174
	CTCGAGCCGATCGAAGGGTGAGG	2474	CUCGAGCCGAUCGAAGGGUG	4175
	ATTTATCACCAAGCGAGAGTTGG	2475	AUUUAUCACCAAGCGAGAGU	4176
	GTTCGGCTGGGTCCACCTATAGG	2476	GUUCGGCUGGGUCCACCUAU	4177
	CAGCACACAATCCGGCTAAAGGG	2477	CAGCACACAAUCCGGCUAAA	4178
	GGCACGACTGCCCACGCCTAGGG	2478	GGCACGACUGCCCACGCCUA	4179
	AGAGCCCGAGCGCGTGTGCCGGG	2479	AGAGCCCGAGCGCGUGUGCC	4180
	CACGAATCTCGGAGGGGTGCGGG	2480	CACGAAUCUCGGAGGGGUGC	4181
	GCCCCCCGGCAAACCTAGGCAGG	2481	GCCCCCCGGCAAACCUAGGC	4182
	GAGAAAACCGTGGAATACTTTGG	2482	GAGAAAACCGUGGAAUACUU	4183
	CTCCGCGAGTGCGGGAGCTTTGG	2483	CUCCGCGAGUGCGGGAGCUU	4184
	CGCGGACCTGTGTCCGAACCCGG	2484	CGCGGACCUGUGUCCGAACC	4185
	AGCTCCCGCACTCGCGGAGCAGG	2485	AGCUCCCGCACUCGCGGAGC	4186
	CGCGCTCGGGCTCTAGGTACTGG	2486	CGCGCUCGGGCUCUAGGUAC	4187
	ACGGGTGCGCGCCGCGGCTTGGG	2487	ACGGGUGCGCGCCGCGGCUU	4188
	AATATTGTGGAGTAGCTCCGGGG	2488	AAUAUUGUGGAGUAGCUCCG	4189

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	TTCTCCGGATTAGTTGCCAGGGG	2489	UUCUCCGGAUUAGUUGCCAG	4190
	AGACACGTCCAACTAGCCCTAGG	2490	AGACACGUCCAACUAGCCCU	4191
	TCCCTGCCTAGGTTTGCCGGGGG	2491	UCCCUGCCUAGGUUUGCCGG	4192
	TGTGCGACGCGCTGGTCCCGAGG	2492	UGUGCGACGCGCUGGUCCCG	4193
	AAAGGGGTCGCACTCCCTGTGGG	2493	AAAGGGGUCGCACUCCCUGU	4194
	GCGACCCTCGGTGCATTCCACGG	2494	GCGACCCUCGGUGCAUUCCA	4195
	CGCTTGGGTAGTAAATATTGTGG	2495	CGCUUGGGUAGUAAAUAUUG	4196
	CGGGTTCGGACACAGGTCCGCGG	2496	CGGGUUCGGACACAGGUCCG	4197
	CATTCCACGGGAAAAATCGTTGG	2497	CAUUCCACGGGAAAAAUCGU	4198
	GTCCAAAGCTCCCGCACTCGCGG	2498	GUCCAAAGCUCCCGCACUCG	4199
	AGCGCATGAAGTTACCTGCCCGG	2499	AGCGCAUGAAGUUACCUGCC	4200
	CGCGCTGGTCCCGAGGAGCGCGG	2500	CGCGCUGGUCCCGAGGAGCG	4201
	ACAGTCAGGGGGTACGAGGGAGG	2501	ACAGUCAGGGGGUACGAGGG	4202
	CCCCACTGGACGGTTTGCGAAGG	2502	CCCCACUGGACGGUUUGCGA	4203
	CCTGTGTCCGAACCCGGGCTCGG	2503	CCUGUGUCCGAACCCGGGCU	4204
	GTACCCCCTGACTGTCCTATAGG	2504	GUACCCCCUGACUGUCCUAU	4205
	TCTGCCAACGTTCATCCAACGGG	2505	UCUGCCAACGUUCAUCCAAC	4206
	ACCCGAGGTCAGCGGGCCGTGGG	2506	ACCCGAGGUCAGCGGGCCGU	4207
	ACTACGGGCCGGGAGTAGGGAGG	2507	ACUACGGGCCGGGAGUAGGG	4208
	GTCGCCATGGATGTGCCTGCAGG	2508	GUCGCCAUGGAUGUGCCUGC	4209
	GGCCTTGACGAGCCCGCGTCCGG	2509	GGCCUUGACGAGCCCGCGUC	4210
	TGCCCACGCCTAGGGCTAGTTGG	2510	UGCCCACGCCUAGGGCUAGU	4211
	ACCAGCGCGTCGCACACAAAGGG	2511	ACCAGCGCGUCGCACACAAA	4212
	TGGTCCCGAGGAGCGCGGCACGG	2512	UGGUCCCGAGGAGCGCGGCA	4213
	TGTGCCCGTGCCGCGCTCCTCGG	2513	UGUGCCCGUGCCGCGCUCCU	4214
	CCGGGCTCGGCTGCCGGAACCGG	2514	CCGGGCUCGGCUGCCGGAAC	4215
	CGCAGCCCGGCACACGCGCTCGG	2515	CGCAGCCCGGCACACGCGCU	4216
	TTTTCCCGTGGAATGCACCGAGG	2516	UUUUCCCGUGGAAUGCACCG	4217
	CGACCCTCGGTGCATTCCACGGG	2517	CGACCCUCGGUGCAUUCCAC	4218
	CTCCACTACGGGCCGGGAGTAGG	2518	CUCCACUACGGGCCGGGAGU	4219
	GGTGATTCGAAGTGTAAATAGGG	2519	GGUGAUUCGAAGUGUAAAUA	4220
	TGCGCCCGACGCTCCCTGTCTGG	2520	UGCGCCCGACGCUCCCUGUC	4221
	CTTAGCCGCGAACAGCAGGAAGG	2521	CUUAGCCGCGAACAGCAGGA	4222
	TCCTGTTTAAGGGGTGCGCGCGG	2522	UCCUGUUUAAGGGGUGCGCG	4223
	CCCCCCGGCAAACCTAGGCAGGG	2523	CCCCCCGGCAAACCUAGGCA	4224
	TAGGACAGTCAGGGGGTACGAGG	2524	UAGGACAGUCAGGGGGUACG	4225
	AAGTCACCTCCACTACGGGCCGG	2525	AAGUCACCUCCACUACGGGC	4226
	CCCCCTGACTGTCCTATAGGTGG	2526	CCCCCUGACUGUCCUAUAGG	4227
	GCGGGCTCGTCAAGGCCCAATGG	2527	GCGGGCUCGUCAAGGCCCAA	4228
TOX2	CCACCCGTGCGACGACACAGTGG	2528	CCACCCGUGCGACGACACAG	4229
	TCATCCACACTCGCGCGTCGAGG	2529	UCAUCCACACUCGCGCGUCG	4230
	GTGACTCGTCTGTGGCGGTGAGG	2530	GUGACUCGUCUGUGGCGGUG	4231

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CGCAGCCTACTCGGAATCCGAGG	2531	CGCAGCCUACUCGGAAUCCG	4232
	TAAACCTCGACGCGCGAGTGTGG	2532	UAAACCUCGACGCGCGAGUG	4233
	AAGCGCGGGTTTTCGTCACTCGG	2533	AAGCGCGGGUUUUCGUCACU	4234
	CTGTCCGCGCGTCCGCCAGTCGG	2534	CUGUCCGCGCGUCCGCCAGU	4235
	GGTCTCGCGAAGAGTGGCGGTGG	2535	GGUCUCGCGAAGAGUGGCGG	4236
	TGTGAGCCGCCCGTGCCCGTCGG	2536	UGUGAGCCGCCCGUGCCCGU	4237
	TGCCGCCGTGGTAATAGTCCAGG	2537	UGCCGCCGUGGUAAUAGUCC	4238
	AGGGGACGCGGACTGCTTAGAGG	2538	AGGGGACGCGGACUGCUUAG	4239
	TACAGGCGGACGTCCATGGCGGG	2539	UACAGGCGGACGUCCAUGGC	4240
	TCGTCGCACGGGTGGCTGTCGGG	2540	UCGUCGCACGGGUGGCUGUC	4241
	TCGCAGTCCCGCTCGCACACTGG	2541	UCGCAGUCCCGCUCGCACAC	4242
	TAAGCAGTCCGCGTCCCCTTCGG	2542	UAAGCAGUCCGCGUCCCCUU	4243
	GGCCGGAACAATAGCGCGCGCGG	2543	GGCCGGAACAAUAGCGCGCG	4244
	GCCGGAACAATAGCGCGCGCGGG	2544	GCCGGAACAAUAGCGCGCGC	4245
	CTGTCAGGGGGACGCGAGTGAGG	2545	CUGUCAGGGGGACGCGAGUG	4246
	TCGATTGGCCGCAGCCTACTCGG	2546	UCGAUUGGCCGCAGCCUACU	4247
	GTCGTCGCACGGGTGGCTGTCGG	2547	GUCGUCGCACGGGUGGCUGU	4248
	CGGGACGGAAAAGCGCCGTCTGG	2548	CGGGACGGAAAAGCGCCGUC	4249
	AGCGCCGCAGCACACTAATTGGG	2549	AGCGCCGCAGCACACUAAUU	4250
	GGAACAATAGCGCGCGCGGGCGG	2550	GGAACAAUAGCGCGCGCGGG	4251
	CGCGCGAGTGTGGATGACCGAGG	2551	CGCGCGAGUGUGGAUGACCG	4252
	GTACAGGCGGACGTCCATGGCGG	2552	GUACAGGCGGACGUCCAUGG	4253
	GGACTATTACCACGGCGGCAAGG	2553	GGACUAUUACCACGGCGGCA	4254
	TCCCTCTCCGGCGACCGAAGGGG	2554	UCCCUCUCCGGCGACCGAAG	4255
	GCGTCCGCCAGTCGGTGCGTCGG	2555	GCGUCCGCCAGUCGGUGCGU	4256
	AACAACTCAGGGCGTGAGCGTGG	2556	AACAACUCAGGGCGUGAGCG	4257
	GCCTTTCGCCACCCACGGTGAGG	2557	GCCUUUCGCCACCCACGGUG	4258
	CTCGGATTCCGAGTAGGCTGCGG	2558	CUCGGAUUCCGAGUAGGCUG	4259
	GGACGTCCGCCTGTACCCCTCGG	2559	GGACGUCCGCCUGUACCCCU	4260
	TCCGGCGACCGAAGGGGACGCGG	2560	UCCGGCGACCGAAGGGGACG	4261
	TTCCCTCTCCGGCGACCGAAGGG	2561	UUCCCUCUCCGGCGACCGAA	4262
	GGTTTTCGTCACTCGGAGCCCGG	2562	GGUUUUCGUCACUCGGAGCC	4263
	AACCTCGCAGGCTTTTCGTCAGG	2563	AACCUCGCAGGCUUUUCGUC	4264
	CCCGGATTGAACAGCGCGCGTGG	2564	CCCGGAUUGAACAGCGCGCG	4265
	ACGCTCACGCCCTGAGTTGTTGG	2565	ACGCUCACGCCCUGAGUUGU	4266
	TTCGTCAGGCCCCTGGTAGTGGG	2566	UUCGUCAGGCCCCUGGUAGU	4267
	GCGCGCTATTGTTCCGGCCTCGG	2567	GCGCGCUAUUGUUCCGGCCU	4268
	GCCCGCGCGCGCTATTGTTCCGG	2568	GCCCGCGCGCGCUAUUGUUC	4269
	AGCGCCGCCTCAAATATTTAGGG	2569	AGCGCCGCCUCAAAUAUUUA	4270
	AACAATAGCGCGCGCGGGCGGGG	2570	AACAAUAGCGCGCGCGGGCG	4271
	CAGCGCCGCAGCACACTAATTGG	2571	CAGCGCCGCAGCACACUAAU	4272
	TAGCGCCGCCTCAAATATTTAGG	2572	UAGCGCCGCCUCAAAUAUUU	4273

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GTGGGCTCCGTGGCGATGCGGGG	2573	GUGGGCUCCGUGGCGAUGCG	4274
	CCGGATTGAACAGCGCGCGTGGG	2574	CCGGAUUGAACAGCGCGCGU	4275
	GAGTAGGCTGCGGCCAATCGAGG	2575	GAGUAGGCUGCGGCCAAUCG	4276
	GCGTGGGCTCCGTGGCGATGCGG	2576	GCGUGGGCUCCGUGGCGAUG	4277
	TATTACCACGGCGGCAAGGTAGG	2577	UAUUACCACGGCGGCAAGGU	4278
	GACCTGACGCCTCGGGTTCGGGG	2578	GACCUGACGCCUCGGGUUCG	4279
	ACCACCGCCCCACATCGCGCAGG	2579	ACCACCGCCCCACAUCGCGC	4280
	GAGTGACGAAAACCCGCGCTTGG	2580	GAGUGACGAAAACCCGCGCU	4281
	CGGGCGCCGAGGGGTACAGGCGG	2581	CGGGCGCCGAGGGGUACAGG	4282
	GTTCCCTCTCCGGCGACCGAAGG	2582	GUUCCCUCUCCGGCGACCGA	4283
	CGGAAAAGCGCCGTCTGGACAGG	2583	CGGAAAAGCGCCGUCUGGAC	4284
	TGACGAAAAGCCTGCGAGGTTGG	2584	UGACGAAAAGCCUGCGAGGU	4285
	GGGGTACAGGCGGACGTCCATGG	2585	GGGGUACAGGCGGACGUCCA	4286
	TCCCCTTCGGTCGCCGGAGAGGG	2586	UCCCCUUCGGUCGCCGGAGA	4287
	CGCACATCAGCCCCGCCGACGGG	2587	CGCACAUCAGCCCCGCCGAC	4288
	GTGAGTGCGCGTCCAGTGGCTGG	2588	GUGAGUGCGCGUCCAGUGGC	4289
	TTTCGTCAGGCCCCTGGTAGTGG	2589	UUUCGUCAGGCCCCUGGUAG	4290
	AACCCCGAACCCGAGGCGTCAGG	2590	AACCCCGAACCCGAGGCGUC	4291
	CACCTGGACTATTACCACGGCGG	2591	CACCUGGACUAUUACCACGG	4292
	ACGGCTTGTGAATGACTGCGAGG	2592	ACGGCUUGUGAAUGACUGCG	4293
	TGAGGTCTCGCGAAGAGTGGCGG	2593	UGAGGUCUCGCGAAGAGUGG	4294
	AAGTGAGGTCTCGCGAAGAGTGG	2594	AAGUGAGGUCUCGCGAAGAG	4295
	TGCCTGGAACCCCGAACCCGAGG	2595	UGCCUGGAACCCCGAACCCG	4296
	GCACTCACCCCGCATCGCCACGG	2596	GCACUCACCCCGCAUCGCCA	4297
	GCGCACCTGGACTATTACCACGG	2597	GCGCACCUGGACUAUUACCA	4298
	GCTGCGAGAGTGTGACTGTCGGG	2598	GCUGCGAGAGUGUGACUGUC	4299
	GTCCCCTTCGGTCGCCGGAGAGG	2599	GUCCCCUUCGGUCGCCGGAG	4300
	CGGGGCCTCGGATTCCGAGTAGG	2600	CGGGGCCUCGGAUUCCGAGU	4301
	TACCACGGCGGCAAGGTAGGCGG	2601	UACCACGGCGGCAAGGUAGG	4302
	TCGGTGCGTCGGTCCCGGGCCGG	2602	UCGGUGCGUCGGUCCCGGGC	4303
	GGTAATAGTCCAGGTGCGCCAGG	2603	GGUAAUAGUCCAGGUGCGCC	4304
	GGGTGACTTGCGTGGGACGGCGG	2604	GGGUGACUUGCGUGGGACGG	4305
	CAGCCCTGGCGCAGACGCGTGGG	2605	CAGCCCUGGCGCAGACGCGU	4306
	TGGCGGACGCGCGGACAGTCTGG	2606	UGGCGGACGCGCGGACAGUC	4307
	GCGGACGTCCATGGCGGGCGCGG	2607	GCGGACGUCCAUGGCGGGCG	4308
	GCGCACATCAGCCCCGCCGACGG	2608	GCGCACAUCAGCCCCGCCGA	4309
	TGGACAGGCGCGCCCCCCTCAGG	2609	UGGACAGGCGCGCCCCCCUC	4310
	GCCAGTCGGTGCGTCGGTCCCGG	2610	GCCAGUCGGUGCGUCGGUCC	4311
	GACGAAAAGCCTGCGAGGTTGGG	2611	GACGAAAAGCCUGCGAGGUU	4312
	CGCTCACGCCCTGAGTTGTTGGG	2612	CGCUCACGCCCUGAGUUGUU	4313
	TCCTCACCGTGGGTGGCGAAAGG	2613	UCCUCACCGUGGGUGGCGAA	4314
	TCAGACACAGATCGTCCTGACGG	2614	UCAGACACAGAUCGUCCUGA	4315

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	CAAAGTTAGAAGCCGATGAGGGG	2615	CAAAGUUAGAAGCCGAUGAG	4316
	CCACGGCGGCAAGGTAGGCGGGG	2616	CCACGGCGGCAAGGUAGGCG	4317
	TGTCAGGGGGACGCGAGTGAGGG	2617	UGUCAGGGGGACGCGAGUGA	4318
	TCGTCAGGCCCCTGGTAGTGGGG	2618	UCGUCAGGCCCCUGGUAGUG	4319
	AGGCTACCATCCCCCCTCATCGG	2619	AGGCUACCAUCCCCCCUCAU	4320
	GGGCCACTGTGTCGTCGCACGGG	2620	GGGCCACUGUGUCGUCGCAC	4321
	ACGAAAAGCCTGCGAGGTTGGGG	2621	ACGAAAAGCCUGCGAGGUUG	4322
	GGTGACTTGCGTGGGACGGCGGG	2622	GGUGACUUGCGUGGGACGGC	4323
	CCAGTCGGTGCGTCGGTCCCGGG	2623	CCAGUCGGUGCGUCGGUCCC	4324
	TCCGCGTCCCCTTCGGTCGCCGG	2624	UCCGCGUCCCCUUCGGUCGC	4325
	TGCGCGGCCCCGTGTGACCCCGG	2625	UGCGCGGCCCCGUGUGACCC	4326
	CACTAGGGCCTGCGCGATGTGGG	2626	CACUAGGGCCUGCGCGAUGU	4327
	TACCCCTCGGCGCCCGCGGTGGG	2627	UACCCCUCGGCGCCCGCGGU	4328
IRF4	ATGAGCTAACCGGACTGTCGGGG	2628	AUGAGCUAACCGGACUGUCG	4329
	ACGCGGGGCATGAACCTGGAGGG	2629	ACGCGGGGCAUGAACCUGGA	4330
	GCCGGAGACCTTGAAGAGCGCGG	2630	GCCGGAGACCUUGAAGAGCG	4331
	GGGGTCCTATTCGGGGCGAAGGG	2631	GGGGUCCUAUUCGGGGCGAA	4332
	GCGCGGAATCCCCCGTACTGGGG	2632	GCGCGGAAUCCCCCGUACUG	4333
	AACGACAAGTGGCGCAGACGCGG	2633	AACGACAAGUGGCGCAGACG	4334
	GTCGCTCCGAGCCTTGCGTGCGG	2634	GUCGCUCCGAGCCUUGCGUG	4335
	ACAGGCGCGGACGCACGGAGAGG	2635	ACAGGCGCGGACGCACGGAG	4336
	GCAGAGCGTGTAACGGAAGACGG	2636	GCAGAGCGUGUAACGGAAGA	4337
	TGCGGTGCCTCGTGGCTGAAGGG	2637	UGCGGUGCCUCGUGGCUGAA	4338
	CGTCTGCCGCCTCCGTCCGTGGG	2638	CGUCUGCCGCCUCCGUCCGU	4339
	GCGAATCTCGCCTTTGCGCCAGG	2639	GCGAAUCUCGCCUUUGCGCC	4340
	CCCGGTGATGGCCTTGCCGAGGG	2640	CCCGGUGAUGGCCUUGCCGA	4341
	CAAGACGAGCGGCGCGTGTCGGG	2641	CAAGACGAGCGGCGCGUGUC	4342
	GCGCGCGGAATCCCCCGTACTGG	2642	GCGCGCGGAAUCCCCCGUAC	4343
	CGCGCGGAATCCCCCGTACTGGG	2643	CGCGCGGAAUCCCCCGUACU	4344
	TTAGTGCGCGCTAGCTGGGCAGG	2644	UUAGUGCGCGCUAGCUGGGC	4345
	CCCACTTAGTGCGCGCTAGCTGG	2645	CCCACUUAGUGCGCGCUAGC	4346
	CCACTTAGTGCGCGCTAGCTGGG	2646	CCACUUAGUGCGCGCUAGCU	4347
	GCGATGTTCTCTAAACACCGCGG	2647	GCGAUGUUCUCUAAACACCG	4348
	GAGCGTGTAACGGAAGACGGAGG	2648	GAGCGUGUAACGGAAGACGG	4349
	CGGTGGGTCCCAAGATCGAGCGG	2649	CGGUGGGUCCCAAGAUCGAG	4350
	AGAGCGCGGCGTCCTCCTCGCGG	2650	AGAGCGCGGCGUCCUCCUCG	4351
	GCGAAGGTGCCTTCTTCCGGGGG	2651	GCGAAGGUGCCUUCUUCCGG	4352
	CTAAACACCGCGGAGAGGCAGGG	2652	CUAAACACCGCGGAGAGGCA	4353
	GCGAGGTCCTCCGCGCGTGGAGG	2653	GCGAGGUCCUCCGCGCGUGG	4354
	ATCGACAGCGGCAAGTACCCCGG	2654	AUCGACAGCGGCAAGUACCC	4355
	CGACAAGTGGCGCAGACGCGGGG	2655	CGACAAGUGGCGCAGACGCG	4356
	AGTACCCGCAGAGAGCTAGCAGG	2656	AGUACCCGCAGAGAGCUAGC	4357

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AATGGGGGGCGTGTAGTAGCGGG	2657	AAUGGGGGGCGUGUAGUAGC	4358
	ACGACAAGTGGCGCAGACGCGGG	2658	ACGACAAGUGGCGCAGACGC	4359
	GATGAGCTAACCGGACTGTCGGG	2659	GAUGAGCUAACCGGACUGUC	4360
	AGGGGTCCTATTCGGGGCGAAGG	2660	AGGGGUCCUAUUCGGGGCGA	4361
	CGAACCTCTGGTTCGCGCTCCGG	2661	CGAACCUCUGGUUCGCGCUC	4362
	CAGTTTCACCGCTCGATCTTGGG	2662	CAGUUUCACCGCUCGAUCUU	4363
	ACCTCGCCCTTCGCGGGAAACGG	2663	ACCUCGCCCUUCGCGGGAAA	4364
	AAGCGCGCGCGTGCCGTGTCAGG	2664	AAGCGCGCGCGUGCCGUGUC	4365
	TTGGGCTGCGGGTGCGTTACAGG	2665	UUGGGCUGCGGGUGCGUUAC	4366
	GCGACCCCGTCGCAGGAGCGCGG	2666	GCGACCCCGUCGCAGGAGCG	4367
	TAAGGGGCCCAAGCTCACGGCGG	2667	UAAGGGGCCCAAGCUCACGG	4368
	CTGATCGACCAGATCGACAGCGG	2668	CUGAUCGACCAGAUCGACAG	4369
	CAAGCAGGACTACAACCGCGAGG	2669	CAAGCAGGACUACAACCGCG	4370
	GTTCTCTAAACACCGCGGAGAGG	2670	GUUCUCUAAACACCGCGGAG	4371
	CGGAGAGTTCGGCATGAGCGCGG	2671	CGGAGAGUUCGGCAUGAGCG	4372
	TGCGTGGAAACGAGAACGCACGG	2672	UGCGUGGAAACGAGAACGCA	4373
	GTAACGCACCCGCAGCCCAAAGG	2673	GUAACGCACCCGCAGCCCAA	4374
	GGACCCGGAGCGCGAACCAGAGG	2674	GGACCCGGAGCGCGAACCAG	4375
	TAGCGGGAATCTGGTGCGAAGGG	2675	UAGCGGGAAUCUGGUGCGAA	4376
	GGGGTCGCCACAAGCTGGACGGG	2676	GGGGUCGCCACAAGCUGGAC	4377
	CTGGGGCCGTTTCCCGCGAAGGG	2677	CUGGGGCCGUUUCCCGCGAA	4378
	TCCGCGCGCAGAGCGTCCGCCGG	2678	UCCGCGCGCAGAGCGUCCGC	4379
	AGCTCATCCCGTCCAGCTTGTGG	2679	AGCUCAUCCCGUCCAGCUUG	4380
	TCCCGGTGATGGCCTTGCCGAGG	2680	UCCCGGUGAUGGCCUUGCCG	4381
	GCCGTTTCCCGCGAAGGGCGAGG	2681	GCCGUUUCCCGCGAAGGGCG	4382
	GCGACGGGGTCGCCACAAGCTGG	2682	GCGACGGGGUCGCCACAAGC	4383
	TTTCGCACCTCGCCCTTCGCGGG	2683	UUUCGCACCUCGCCCUUCGC	4384
	ACCCTCGGCAAGGCCATCACCGG	2684	ACCCUCGGCAAGGCCAUCAC	4385
	GGTACTTGCCGCTGTCGATCTGG	2685	GGUACUUGCCGCUGUCGAUC	4386
	TGCGTCCGCGCCTGTGCCGGCGG	2686	UGCGUCCGCGCCUGUGCCGG	4387
	CACGGACGGAGGCGGCAGACGGG	2687	CACGGACGGAGGCGGCAGAC	4388
	GGCGCGTGTCGGGAGCCTTTGGG	2688	GGCGCGUGUCGGGAGCCUUU	4389
	GCTTGTGGCGACCCCGTCGCAGG	2689	GCUUGUGGCGACCCCGUCGC	4390
	GCCTGCGGCCGGGCGTTCCAGGG	2690	GCCUGCGGCCGGGCGUUCCA	4391
	CGTGCCGTGTCAGGGTCGTCCGG	2691	CGUGCCGUGUCAGGGUCGUC	4392
	ACGAAAACAGCCGCCGGCACAGG	2692	ACGAAAACAGCCGCCGGCAC	4393
	TTCGCGCTCCGGGTCCTCTCTGG	2693	UUCGCGCUCCGGGUCCUCUC	4394
	AGCCGTCCGCCTTCCGAGCTCGG	2694	AGCCGUCCGCCUUCCGAGCU	4395
	CGGCGCGTGTCGGGAGCCTTTGG	2695	CGGCGCGUGUCGGGAGCCUU	4396
	AGGCACCTTCGCGGCCGGCCCGG	2696	AGGCACCUUCGCGGCCGGCC	4397
	GCGGTGAAACTGAGAGTGCGAGG	2697	GCGGUGAAACUGAGAGUGCG	4398
	CCTCGTGGTCACTGGCGCAGGGG	2698	CCUCGUGGUCACUGGCGCAG	4399

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	AGGGTACCCCGGCTTCGGAGCGG	2699	AGGGUACCCCGGCUUCGGAG	4400
	TCGACAGCGGCAAGTACCCCGGG	2700	UCGACAGCGGCAAGUACCCC	4401
	CGCGAAGGTGCCTTCTTCCGGGG	2701	CGCGAAGGUGCCUUCUUCCG	4402
	GCTCCGAGCCTTGCGTGCGGTGG	2702	GCUCCGAGCCUUGCGUGCGG	4403
	GCCGCGCTCTTCAAGGTCTCCGG	2703	GCCGCGCUCUUCAAGGUCUC	4404
	TCGCTTTGCAGAGCGTGTAACGG	2704	UCGCUUUGCAGAGCGUGUAA	4405
	CTCGGCTCTCAGCGGGACCGCGG	2705	CUCGGCUCUCAGCGGGACCG	4406
	GTGCCGTGTCAGGGTCGTCCGGG	2706	GUGCCGUGUCAGGGUCGUCC	4407
	GCAAGACGAGCGGCGCGTGTCGG	2707	GCAAGACGAGCGGCGCGUGU	4408
	AACTGACAGAGTCGCGGGGAAGG	2708	AACUGACAGAGUCGCGGGGA	4409
	CAGGCGGGTAGGAGCCTTCGCGG	2709	CAGGCGGGUAGGAGCCUUCG	4410
	GGGTACCCCGGCTTCGGAGCGGG	2710	GGGUACCCCGGCUUCGGAGC	4411
	GAGGCATCAGGTGGCGTCGCCGG	2711	GAGGCAUCAGGUGGCGUCGC	4412
	CCGTCTGCCGCCTCCGTCCGTGG	2712	CCGUCUGCCGCCUCCGUCCG	4413
	GCCGTCTTGTGTGGGTGCCTTGG	2713	GCCGUCUUGUGUGGGUGCCU	4414
	GAACCTCTGGTTCGCGCTCCGGG	2714	GAACCUCUGGUUCGCGCUCC	4415
	GCGCGGTGAGCTGCGGCAACGGG	2715	GCGCGGUGAGCUGCGGCAAC	4416
	GCCTCCGGCTCAGCGCAGATGGG	2716	GCCUCCGGCUCAGCGCAGAU	4417
	GGCGTGTAGTAGCGGGAATCTGG	2717	GGCGUGUAGUAGCGGGAAUC	4418
	GGAGGACGCCGCGCTCTTCAAGG	2718	GGAGGACGCCGCGCUCUUCA	4419
	CGGCACGCGGGGCATGAACCTGG	2719	CGGCACGCGGGGCAUGAACC	4420
	CCGCGAAGGTGCCTTCTTCCGGG	2720	CCGCGAAGGUGCCUUCUUCC	4421
	CGGGGTCGCCACAAGCTGGACGG	2721	CGGGGUCGCCACAAGCUGGA	4422
	TCCGGCGGACGCTCTGCGCGCGG	2722	UCCGGCGGACGCUCUGCGCG	4423
	AGCGCAGGGTACCCCGGCTTCGG	2723	AGCGCAGGGUACCCCGGCUU	4424
	CCTATTCGGGGCGAAGGGTCTGG	2724	CCUAUUCGGGGCGAAGGGUC	4425
	CTCTTCAAGGTCTCCGGCCTCGG	2725	CUCUUCAAGGUCUCCGGCCU	4426
	TCGGCTCTCAGCGGGACCGCGGG	2726	UCGGCUCUCAGCGGGACCGC	4427
	TGGAAACTGACAGAGTCGCGGGG	2727	UGGAAACUGACAGAGUCGCG	4428
TET2	TGCGCGGGACCTCGAAGTGGTGG	2728	UGCGCGGGACCUCGAAGUGG	4429
	GCACCGGGCGTCCAGCACAAAGG	2729	GCACCGGGCGUCCAGCACAA	4430
	AGGGAATTAGCCCCCCGCACCGG	2730	AGGGAAUUAGCCCCCCGCAC	4431
	ACTTGCATGCGAGCGGGACCCGG	2731	ACUUGCAUGCGAGCGGGACC	4432
	TCACGCCGTGCAGTGGCGCGGGG	2732	UCACGCCGUGCAGUGGCGCG	4433
	CGCGGGCAACGGGATCTAAAGGG	2733	CGCGGGCAACGGGAUCUAAA	4434
	GCGCGGGCAACGGGATCTAAAGG	2734	GCGCGGGCAACGGGAUCUAA	4435
	GACGTGACTTGCATGCGAGCGGG	2735	GACGUGACUUGCAUGCGAGC	4436
	ATAGAGACGCGGGCCTCTGAGGG	2736	AUAGAGACGCGGGCCUCUGA	4437
	GTGCGGGTACACTCCGGAGGAGG	2737	GUGCGGGUACACUCCGGAGG	4438
	CACGCCGTGCAGTGGCGCGGGGG	2738	CACGCCGUGCAGUGGCGCGG	4439
	GGCATGCCCTCGGTGAAACAGGG	2739	GGCAUGCCCUCGGUGAAACA	4440
	GGGAATTAGCCCCCCGCACCGGG	2740	GGGAAUUAGCCCCCCGCACC	4441

CA 03207166 2023-8-1

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	GGTGCCGCCGGCCTTTGTGCTGG	2741	GGUGCCGCCGGCCUUUGUGC	4442
	AGCGCTCCCCTGTTTCACCGAGG	2742	AGCGCUCCCCUGUUUCACCG	4443
	GCGCTCCCCTGTTTCACCGAGGG	2743	GCGCUCCCCUGUUUCACCGA	4444
	GTGTGCGCGGGACCTCGAAGTGG	2744	GUGUGCGCGGGACCUCGAAG	4445
	GTGCGGGGGGCTAATTCCCTGGG	2745	GUGCGGGGGGCUAAUUCCCU	4446
	ACCCGCACGTGCCCTCGCTCTGG	2746	ACCCGCACGUGCCCUCGCUC	4447
	CTCACGCCGTGCAGTGGCGCGGG	2747	CUCACGCCGUGCAGUGGCGC	4448
	GTGGTGCGCCCGGACCAGCGCGG	2748	GUGGUGCGCCCGGACCAGCG	4449
	CACGTGCGGGTACACTCCGGAGG	2749	CACGUGCGGGUACACUCCGG	4450
	GCGTCCAGCACAAAGGCCGGCGG	2750	GCGUCCAGCACAAAGGCCGG	4451
	TTTGTGCTGGACGCCCGGTGCGG	2751	UUUGUGCUGGACGCCCGGUG	4452
	TGTACGGCCCCAGGTGCCGCCGG	2752	UGUACGGCCCCAGGUGCCGC	4453
	CCGCGCCACTGCACGGCGTGAGG	2753	CCGCGCCACUGCACGGCGUG	4454
	GGGCATGCCCTCGGTGAAACAGG	2754	GGGCAUGCCCUCGGUGAAAC	4455
	TTGTGCTGGACGCCCGGTGCGGG	2755	UUGUGCUGGACGCCCGGUGC	4456
	GGGCACGTGCGGGTACACTCCGG	2756	GGGCACGUGCGGGUACACUC	4457
	GGACGTGACTTGCATGCGAGCGG	2757	GGACGUGACUUGCAUGCGAG	4458
	TCACGTCCGCCCCCTCGGCGCGG	2758	UCACGUCCGCCCCCUCGGCG	4459
	ACGCCGTGCAGTGGCGCGGGGGG	2759	ACGCCGUGCAGUGGCGCGGG	4460
	GCACCTGGGGCCGTACAGCGGGG	2760	GCACCUGGGGCCGUACAGCG	4461
	CGCGCCACTGCACGGCGTGAGGG	2761	CGCGCCACUGCACGGCGUGA	4462
	GGTAAGGTGGGCGCAAGCGGAGG	2762	GGUAAGGUGGGCGCAAGCGG	4463
	CTTGCATGCGAGCGGGACCCGGG	2763	CUUGCAUGCGAGCGGGACCC	4464
	GGAGACCCGCCGAGGTCCCCGGG	2764	GGAGACCCGCCGAGGUCCCC	4465
	CGCAAGCGGAGGTGTGGTGCGGG	2765	CGCAAGCGGAGGUGUGGUGC	4466
	GGTGCGGGGGGCTAATTCCCTGG	2766	GGUGCGGGGGGCUAAUUCCC	4467
	TAGATGTCACGTCTTTGTCCAGG	2767	UAGAUGUCACGUCUUUGUCC	4468
	AGCAGAGCAAGCGCGAAGGTTGG	2768	AGCAGAGCAAGCGCGAAGGU	4469
	GCATGCCCTCGGTGAAACAGGGG	2769	GCAUGCCCUCGGUGAAACAG	4470
	CTAAAGGGAGATAGAGACGCGGG	2770	CUAAAGGGAGAUAGAGACGC	4471
	CCACTGCGCGCCCCGCTGTACGG	2771	CCACUGCGCGCCCCGCUGUA	4472
	GACGCGGGCCTCTGAGGGTAAGG	2772	GACGCGGGCCUCUGAGGGUA	4473
	AGTGGCAGCGGCGAGAGCTTGGG	2773	AGUGGCAGCGGCGAGAGCUU	4474
	GCAGAGCAAGCGCGAAGGTTGGG	2774	GCAGAGCAAGCGCGAAGGUU	4475
	AAGCACTAAGGGCATGCCCTCGG	2775	AAGCACUAAGGGCAUGCCCU	4476
	TACAGGCCCCTAAAGCACTAAGG	2776	UACAGGCCCCUAAAGCACUA	4477
	CCTTATGAATATTGATGCGGAGG	2777	CCUUAUGAAUAUUGAUGCGG	4478
	GGAATTAGCTCTGTATCGGTCGG	4547	GGAAUUAGCUCUGUAUCGGU	4560
	AAAGTAAGGGCTCTTACGAGAGG	4548	AAAGUAAGGGCUCUUACGAG	4561
	GGCGTCTCACAGATTGAAATAGG	4549	GGCGUCUCACAGAUUGAAAU	4562
	CGGTCAATTTCCCAGTTTGTCGG	4550	CGGUCAAUUUCCCAGUUUGU	4563
	TGCAGCCCTCGGGAACCCCGGGG	4551	UGCAGCCCUCGGGAACCCCG	4564

Target gene (with CGI)	Exemplary target regions (including PAM)	SEQ	Exemplary gRNA spacer sequence	SEQ
	ACTCAGCGGGGCCGGCGTCTCGG	4552	ACUCAGCGGGGCCGGCGUCU	4565

[0337] It will be appreciated that it may be beneficial to increase the expression of certain targets. For example, *c-jun* is a gene that when activated, may be beneficial; for example, increased expression in T cells may increase cell viability.

Cell

[0338] In one aspect, the present invention provides a cell comprising an ETM (e.g., ETR) according to the present invention, at least one gRNA according the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention.

[0339] The cell may be any cell which can be used to express the product of the invention.

[0340] The cell may be an immune effector cell. An "immune effector cell" is a cell which has differentiated into a form capable of modulating or effecting a specific immune response. Immune effector cells may include alpha/beta T cells, gamma/delta T cells, B cells, natural killer (NK) cells, neutrophils, basophils, eosinophils, and macrophages. Suitably, the cell may be an alpha/beta T cell. Suitably, the cell may be a B cell. Suitably, the cell may be a gamma/delta T cell. Suitably, the cell may be a T cell, such as a cytolytic T cell, e.g., a CD8+ T cell. Suitably, the cell may be an NK cell, such as a cytolytic NK cell. Suitably, the cell may be a macrophage.

[0341] In one aspect, the cell may be a stem cell. A "stem cell" refers to an undifferentiated cell which is capable of indefinitely giving rise to more stem cells of the same type, and from which other, specialised cells may arise by differentiation. Adult stem cells are usually multipotent, while induced or embryonic-derived stem cells are pluripotent.

[0342] In another aspect, the cell may be a progenitor cell. A "progenitor cell" refers to a cell which is able to differentiate to form one or more types of cells but has limited self-renewal *in vitro and in vivo*.

[0343] Suitably, the cell may be capable of being differentiated into a T cell. Suitably, the cell may be capable of being differentiated into an NK cell. Suitably, the cell may be capable of being differentiated into a macrophage. Suitably, the cell may be an embryonic stem cell (ESC). Suitably, the cell may be a haematopoietic stem cell or haematopoietic progenitor cell. Suitably, the cell may be an induced pluripotent stem cell (iPSC). Suitably, the cell may be obtained from umbilical cord blood. Suitably, the cell may be obtained from adult peripheral blood or mobilized form the bone marrow.

[0344] A “hematopoietic stem and progenitor cell” or “HSPC” refers to a cell which expresses the antigenic marker CD34 (CD34+) and populations of such cells. In particular embodiments, the term “HSPC” refers to a cell identified by the presence of the antigenic marker CD34 (CD34+) and the absence of lineage (lin) markers. The population of cells comprising CD34+ and/or Lin(−) cells includes haematopoietic stem cells and hematopoietic progenitor cells.

[0345] HSPCs can be obtained or isolated from bone marrow of adults, which includes femurs, hip, ribs, sternum, and other bones. Bone marrow aspirates containing HSPCs can be obtained or isolated directly from the hip using a needle and syringe. Other sources of HSPCs include umbilical cord blood, placental blood, mobilized peripheral blood, Wharton's jelly, placenta, fetal blood, fetal liver, or fetal spleen. In particular embodiments, harvesting a sufficient quantity of HSPCs for use in therapeutic applications may require mobilizing the stem and progenitor cells in the subject.

[0346] As used herein, the term “induced pluripotent stem cell” or “iPSC” refers to a non-pluripotent cell that has been reprogrammed to a pluripotent state. Once the cells of a subject have been reprogrammed to a pluripotent state, the cells can then be programmed to a desired cell type, such as a hematopoietic stem or progenitor cell (HSC and HPC respectively).

[0347] As used herein, the term “reprogramming” refers to a method of increasing the potency of a cell to a less differentiated state and “programming” refers to a method of decreasing the potency of a cell or differentiating the cell to a more differentiated state.

[0348] Suitably, the cell may be matched or is autologous to the subject. The cell may be generated *ex vivo* either from a patient’s own peripheral blood, or from donor peripheral blood.

[0349] Suitably, the cell may be autologous to the subject. In some aspects, the cell may be derived from *ex vivo* differentiation of inducible progenitor cells or embryonic progenitor cells to the immune cell.

[0350] In these instances, cells are generated by introducing DNA or RNA coding for the ETM (e.g., ETR) of the present invention by one of any means including transduction with a viral vector or transfection with DNA or RNA.

[0351] In some aspects, the cell further comprises a polynucleotide, such as an integrating vector, which encodes an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the polynucleotide or a cell which does not comprise the polynucleotide; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises the polynucleotide or a cell which does not comprise the polynucleotide and/or

iii) which enables selection of a cell, such as a cell which comprises the polynucleotide or a cell which does not comprise the polynucleotide.

Combinations

[0352] In one aspect, the present invention provides a combination (e.g., a system) comprising an ETM (e.g., ETR) according to the present invention, and at least one gRNA which targets the endonuclease of the ETM (e.g., ETR) to a target gene.

[0353] The combination may comprise at least two gRNAs (such as at least three, at least four, at least five, at least six, at least seven. or at least eight gRNAs).

[0354] The combination may comprise gRNAs which target the endonuclease to at least two different target genes.

[0355] In some embodiments, one target gene may be targeted with two or more gRNAs. For example, it may be beneficial to target the same gene with several gRNAs for optimal epigenetic modification, e.g., epigenetic silencing.

[0356] The combination may comprise at least two gRNAs which comprise spacer sequences of different lengths. Suitably, at least one gRNA comprises a spacer sequence which is 15, 16, 17, 18, 19 or 20 nucleotides in length. Suitably, at least one of the at least two gRNAs comprises a spacer sequence which is less than or equal to 17 (e.g., less than or equal to 16) nucleotides in length. Suitably, at least one of the at least two gRNAs comprises a spacer sequence which is less than or equal to 17 (e.g., less than or equal to 16) nucleotides in length and at least one of the at least two gRNAs comprises a spacer sequence which is more than 17 nucleotides in length.

[0357] Without wishing to be bound by theory, the gRNAs comprising spacer sequences of different lengths may target the ETM (e.g., ETR) to different target genes, wherein a first target gene is modified by gene editing and at least a second target gene is modified by epigenetic editing.

[0358] In one aspect, the combination comprises at least one gRNA according to the present invention. Suitably, the combination may comprise at least two gRNAs according to the present invention.

[0359] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of C8 and F4, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of H8, H10, H11, or H12.

[0360] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of C8 and H8, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of F4, H10, H11, or H12.

[0361] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of C8 and H10, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of F4, H8, H11, or H12.

[0362] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of C8 and H11, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of F4, H8, H10, or H12.

[0363] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of C8 and H12, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of F4, H8, H10, or H11.

CA 03207166 2023-8-1

[0364] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of F4 and H8, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, H10, H11, or H12.

[0365] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of F4 and H10, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, H8, H11, or H12.

[0366] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of F4 and H11, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, H8, H10, or H12.

[0367] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of F4 and H12, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, H8, H10, or H11.

[0368] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of H8 and H10, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, F4, H11, or H12.

[0369] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of H10 and H11, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, F4, H8, or H12.

[0370] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of H10 and H12, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, F4, H8, or H11.

[0371] Suitably, the combination may comprise a first gRNA and a second gRNA having the sequences of H11 and H12, respectively, optionally wherein the combination further comprises a third gRNA having the sequence of C8, F4, H8, or H10.

[0372] The combination may, for example, have gRNAs comprising or consisting of H8+F4, H8+H10, C8+H10, F4+H10, F4+H8+H10, or C8+F4+H10. In a particular case, the gRNAs may comprise or consist of F4+H8+H10.

[0373] In one aspect, the combination further comprises an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination; and/or

iii) which enables selection of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination.

[0374] The combination may further comprise an agent which modifies the tissue microenvironment.

[0375] The agent may be a protein, such as a cytokine or chemokine, which promotes the survival, proliferation and/or activity of a cell according to the present invention.

[0376] As used herein, "agent which promotes the survival, proliferation and/or activity of a cell" means that in the presence of the agent, the survival, proliferation, or activity of a cell which comprises a product according to the present invention is increased.

[0377] The agent may be, for example, beneficial for certain cells and detrimental to other cells.

[0378] The agent may play a role in homeostasis, for example, blood coagulation; an example of a suitable agent may be coagulation factor IX or FVIII.

[0379] The agent may, for example, allow selection of cells. An example of a suitable agent is Delta low-affinity nerve growth factor (LNGFR).

[0380] The agent may, for example, be detrimental for the cell. The agent may be a thymidine kinase (TK) or a caspase, such as CASP9. Activation of these agents can be used for *in vivo* removal of cells which comprise the agent, e.g., if it is desirable to remove engineered T cells from a subject.

[0381] Suitably, in the presence of the agent, the survival, proliferation and/or activity of the cell which comprises a product according to the present invention (e.g., a cell according to the present invention) may be increased by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99%.

[0382] The combination may comprise an agent which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell such as a tumour cell.

[0383] As used herein “agent which is detrimental to” means that in the presence of the agent, the survival, proliferation, or activity of a cell which does not comprise a product according to the present invention (e.g., a tumour cell) is compromised, reduced, or completely abolished.

[0384] Suitably, in the presence of the detrimental agent, the survival, proliferation and/or activity of the cell which does not comprise a product according to the present invention (e.g., a tumour cell) may be reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99%.

[0385] Cell survival and proliferation may be measured by methods known in the art. Suitable methods include measuring the size of the cell population (e.g., by counting cells using a marker specific for the cell population, i.e., a tumour specific marker or an engineered cell specific marker, such as a CAR or transgenic TCR); by performing cell cycle analysis using 5-bromo-2'-deoxyuridine (BrdU) which becomes incorporated into newly made DNA and/or propidium iodide (PI) and analysing by flow cytometry in combination with a cell population specific marker; and/or by measuring the number of viable cells, e.g., by measuring apoptosis by 7AAD and/or Annexin V staining using flow cytometry.

[0386] In one aspect, the combination further comprises a CAR. In one aspect, the combination further comprises a transgenic TCR.

[0387] The agent, e.g., which promotes the survival, proliferation and/or activity of a cell (or population of cells) or allows selection of the cell, such as the cell (or population of cells) which expresses an ETM (e.g., ETR); and/or which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell which does not express an ETM (e.g., ETR), may be introduced into the genome of the cell by any method. The method may include, for example, using an integrating vector (a procedure independent from the multiplexing strategy performed by the ETM (e.g., ETR) according to the invention); or by targeting the agent (e.g., CAR or transgenic TCR) within the site recognized by the nuclease (a procedure depending on the nuclease activity of the ETM (e.g., ETR) according to the present invention).

[0388] Thus in some aspects, the combination further comprises a polynucleotide, such as an integrating vector which encodes an agent which allows selection or promotes the survival, proliferation and/or activity of a cell (or population of cells), such as the cell (or population of cells) which comprises the polynucleotide; and/or which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell which does not comprise the polynucleotide; and/or which is beneficial for the survival, proliferation and/or activity of a cell, tissue or organ, such as a cell, tissue or organ which does not comprise the combination.

Polynucleotides

[0389] In one aspect, the present invention provides a polynucleotide encoding at least one ETM (e.g., ETR) according to the present invention.

[0390] Polynucleotides of the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that the skilled person may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

[0391] The polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or lifespan of the polynucleotides of the invention.

[0392] Polynucleotides such as DNA polynucleotides may be produced recombinantly, synthetically or by any means available to those of skill in the art. They may also be cloned by standard techniques.

[0393] Longer polynucleotides will generally be produced using recombinant means, for example using PCR cloning techniques. This will involve making a pair of primers (e.g., of about 15 to 30 nucleotides) flanking the target sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g., by purifying the reaction mixture with an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable vector.

Constructs

[0394] In one aspect, the present invention provides a nucleic acid construct comprising a nucleic acid sequence encoding at least one ETM (e.g., ETR) according to the present invention.

[0395] The nucleic acid construct may further comprise a nucleic acid sequence which encodes an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

iii) which enables selection of a cell, such as a cell which comprises the nucleic acid construct or a cell which does not comprise the construct.

Proteins

[0396] As used herein, the term “protein” includes single-chain polypeptide molecules as well as multiple-polypeptide complexes where individual constituent polypeptides are linked by covalent or non-covalent means. As used herein, the terms “polypeptide” and “peptide” refer to a polymer in which the monomers are amino acids and are joined together through peptide or disulfide bonds.

Variants, derivatives, analogues, homologues and fragments

[0397] In addition to the specific proteins and nucleotides mentioned herein, the present invention also encompasses the use of variants, derivatives, analogues, homologues, and fragments thereof.

[0398] In the context of the present invention, a variant of any given sequence is a sequence in which the specific sequence of residues (whether amino acid or nucleic acid residues) has been modified in such a manner that the polypeptide or polynucleotide in question substantially retains at least one of its endogenous functions. A variant sequence can be obtained by addition, deletion, substitution, modification, replacement and/or variation of at least one residue present in the naturally-occurring protein.

[0399] The term “derivative” as used herein, in relation to proteins or polypeptides of the present invention, includes any substitution of, variation of, modification of, replacement of, deletion of and/or addition of one (or more) amino acid residues from or to the sequence providing that the resultant protein or polypeptide substantially retains at least one of its endogenous functions.

[0400] The term “analogue” as used herein, in relation to polypeptides or polynucleotides, includes any mimetic, that is, a chemical compound that possesses at least one of the endogenous functions of the polypeptides or polynucleotides which it mimics.

[0401] Typically, amino acid substitutions may be made, for example from 1, 2 or 3 to 10 or 20 substitutions provided that the modified sequence substantially retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

[0402] Proteins used in the present invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine, and tyrosine.

[0403] Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and, in particular examples, in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R H
AROMATIC		F W Y

[0404] The term “homologue” as used herein means an entity having a certain homology with the wild type amino acid sequence or the wild type nucleotide sequence. The term “homology” can be equated with “identity”.

[0405] A homologous sequence may include an amino acid sequence which may be at least 50%, 55%, 65%, 75%, 85% or 90% identical, for example at least 95% or 97% or 99% identical, to the subject sequence. Typically, the homologues will comprise the same active sites, etc., as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e., amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

[0406] A homologous sequence may include a nucleotide sequence which may be at least 50%, 55%, 65%, 75%, 85% or 90% identical, for example at least 95% or 97% or 99% identical, to the subject sequence. Although homology can also be considered in terms of similarity, in the context of the present invention it is preferred to express homology in terms of sequence identity.

[0407] Reference to a sequence which has a percent identity to any one of the SEQ ID NOs detailed herein may refer, for example to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

[0408] Homology comparisons can be conducted by eye or, more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percentage homology or identity between two or more sequences.

[0409] Percentage homology may be calculated over contiguous sequences, i.e., one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

[0410] Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the nucleotide sequence may cause the following codons to be put out of alignment, thus potentially resulting in a large reduction in percent homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

[0411] However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example, when using the GCG Wisconsin Bestfit package, the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

[0412] Calculation of maximum percentage homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.* (1984) *Nucleic Acids Res.* 12: 387). Examples of other software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.* (1999) *ibid* – Ch. 18), FASTA (Atschul *et al.* (1990) *J. Mol. Biol.* 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.* (1999) *ibid*, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. Another tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequences (see *FEMS Microbiol. Lett.* (1999) 174: 247-50; *FEMS Microbiol. Lett.* (1999) 177: 187-8).

[0413] Although the final percentage homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix – the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see the user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

[0414] Once the software has produced an optimal alignment, it is possible to calculate percentage homology, e.g., percentage sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

[0415] "Fragments" are also variants and the term typically refers to a selected region of the polypeptide or polynucleotide that is of interest either functionally or, for example, in an assay. "Fragment" thus refers to an amino acid or nucleic acid sequence that is a portion of a full-length polypeptide or polynucleotide.

[0416] Such variants may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site may be made. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded protein. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

Codon optimisation

[0417] The polynucleotides used in the present invention may be codon-optimised. Codon optimisation has previously been described in WO 1999/41397 and WO 2001/79518. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available.

Vectors

[0418] In one aspect, the present invention provides a vector comprising a polynucleotide according the present invention, or a nucleic acid construct according to the present invention.

[0419] A vector is a tool that allows or facilitates the transfer of an entity from one environment to another. In accordance with the present invention, and by way of example, some vectors used in recombinant nucleic acid techniques allow entities, such as a segment of nucleic acid (e.g., a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell. The vector may serve the purpose of maintaining the heterologous nucleic acid (DNA or RNA) within the cell, facilitating the replication of the vector comprising a segment of nucleic acid, or facilitating the expression of the protein encoded by a segment of nucleic acid. Vectors may be non-viral or viral. Examples of vectors used in recombinant nucleic acid techniques include, but are not limited to, plasmids, mRNA molecules (e.g., *in vitro* transcribed mRNAs), chromosomes, artificial chromosomes, and viruses. The vector may also be, for example, a naked nucleic acid (e.g., DNA). In its simplest form, the vector may itself be a nucleotide of interest.

[0420] The vectors used in the invention may be, for example, plasmid, mRNA, or virus vectors and may include a promoter for the expression of a polynucleotide and optionally a regulator of the promoter.

[0421] Vectors comprising polynucleotides used in the invention may be introduced into cells using a variety of techniques known in the art, such as transfection, transformation, and transduction. Several such techniques are known in the art, for example infection with recombinant viral vectors, such as retroviral, lentiviral (e.g., integration-defective lentiviral), adenoviral, adeno-associated viral,

CA 03207166 2023-8-1

baculoviral and herpes simplex viral vectors; direct injection of nucleic acids and biolistic transformation.

[0422] Non-viral delivery systems include but are not limited to DNA or RNA transfection methods. Here, transfection includes a process using a non-viral vector to deliver a gene to a target cell. Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated transfection, cationic facial amphiphiles (CFAs) (*Nat. Biotechnol.* (1996) 14: 556) and combinations thereof.

[0423] The term "transfection" is to be understood as encompassing the delivery of polynucleotides to cells by both viral and non-viral delivery.

Protein transduction

[0424] As an alternative to the delivery of polynucleotides to cells, the products and ETMs (e.g., ETRs) of the present invention may be delivered to cells by protein transduction.

[0425] Protein transduction may be via vector delivery (Cai, Y. *et al.* (2014) *Elife* 3: e01911; Maetzig, T. *et al.* (2012) *Curr. Gene Ther.* 12: 389-409). Vector delivery involves the engineering of viral particles (e.g., lentiviral particles) to comprise the proteins to be delivered to a cell. Accordingly, when the engineered viral particles enter a cell as part of their natural life cycle, the proteins comprised in the particles are carried into the cell.

[0426] Protein transduction may be via protein delivery (Gaj, T. *et al.* (2012) *Nat. Methods* 9: 805-7). Protein delivery may be achieved, for example, by utilising a vehicle (e.g., liposomes) or even by administering the protein itself directly to a cell.

Composition

[0427] The products of the invention such as ETMs (e.g., ETRs), gRNAs, combinations, polynucleotides, nucleic acid constructs, vectors, cells, and kits of polynucleotides of the present invention may be provided in a composition.

[0428] The products of the invention such as combinations, ETMs (e.g., ETRs), gRNAs, polynucleotides, nucleic acid constructs, vectors, compositions, and cells of the present invention may be formulated for administration to subjects with a

CA 03207166 2023-8-1

pharmaceutically acceptable carrier, diluent, or excipient. Suitable carriers and diluents include isotonic saline solutions, for example, phosphate-buffered saline, and potentially contain human serum albumin.

[0429] Handling of the cell therapy products may be performed in compliance with the Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee – International Society Cell & Gene Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT) (FACT-JACIE) International Standards for cellular therapy.

[0430] In one aspect, there is provided a combination of chemically modified mRNA encoding for an ETM or ETR plus a chemically modified gRNA.

[0431] In another aspect, there is provided a ribonucleic complex of protein-RNA that includes the ETR protein attached to a chemically modified gRNA.

Kit

[0432] In one aspect, the present invention provides a kit of polynucleotides comprising:

a) at least one polynucleotide encoding at least one ETM (e.g., ETR) according to the present invention; and

b) a polynucleotide providing at least one gRNA as described herein; and optionally,

c) further comprising a nucleic acid sequence which encodes an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises said polynucleotides or a cell which does not comprise said polynucleotides; and/or

iii) which enables selection of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides.

[0433] The kit may also include instructions for use, for example instructions for the simultaneous, sequential, or separate administration of at least one ETM (e.g., ETR) and at least two gRNAs, to a subject in need thereof.

Use

[0434] In one aspect, the present invention provides the use of an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention for modifying the activity and/or expression of at least one target gene, e.g., wherein the use is *in vitro* or *ex vivo* use.

[0435] Suitably, the use may repress transcription and/or expression of (e.g., silence) at least one target gene. Suitably, the use may repress transcription and/or expression of (e.g., silence) at least two target genes. For example, transcription and/or expression of a first gene may be repressed (e.g., silenced) by gene editing and transcription and/or expression of a second target gene may be repressed (e.g., silenced) by epigenetic editing.

[0436] Suitably, the use may enhance at least one target gene.

[0437] In another aspect, the present invention provides a method of repressing transcription and/or expression of (e.g., silencing) at least one target gene in a cell comprising the step of administering an ETM (e.g., ETR) according to the present invention, at least one gRNA according to the present invention, a combination according to the present invention, a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention to a cell.

[0438] Suitably, transcription and/or expression of at least two target genes may be repressed (e.g., silenced), wherein at least one of the at least two target genes is epigenetically repressed (e.g., silenced) and at least one of the at least two target genes is repressed (e.g., silenced) by gene editing, wherein at least one ETM (e.g., ETR) and at least two gRNAs are administered to said cell simultaneously, sequentially, or separately.

[0439] In another aspect, the present invention provides the products, ETMs (e.g., ETRs), gRNAs, combinations, polynucleotides, nucleic acid constructs, vectors, kits of polynucleotides, cells, and pharmaceutical compositions of the present invention for use in therapy.

[0440] The use in therapy may, for example, be a use for the preparation of "universally" allogeneic transplantable cells (e.g., by the silencing of β2-microglobulin, B2M). This use may, for example, be applied to the preparation of haematopoietic stem and/or progenitor cells (HSPCs), whole organ transplantation and cancer immunotherapy.

[0441] The ETM (e.g., ETR) (or polynucleotide, nucleic acid construct, or vector encoding therefor) and gRNAs may be administered simultaneously, in combination, sequentially or separately (as part of a dosing regimen).

[0442] By "simultaneously", it is to be understood that the two or more agents are administered concurrently, whereas the term "in combination" is used to mean they are administered, if not simultaneously, then "sequentially" within a time frame that they both are available to act therapeutically within the same time frame. Thus, administration "sequentially" may permit one agent to be administered within 5 minutes, 10 minutes, or a matter of hours after the other provided the circulatory half-life of the first administered agent is such that they are both concurrently present in therapeutically effective amounts. The time delay between administration of the components will vary depending on the exact nature of the components, the interaction there-between, and their respective half-lives.

[0443] In contrast to "in combination" or "sequentially", "separately" is to be understood as meaning that the gap between administering one agent and the other agent is significant, i.e., the first administered agent may no longer be present in the bloodstream in a therapeutically effective amount when the second agent is administered.

[0444] In another aspect, the present invention provides a method for treating and/or preventing a disease or condition, which comprises the step of administering any of the products of the invention (e.g., ETMs (e.g., ETRs), gRNAs, combinations, polynucleotides, nucleic acid constructs, vectors, kits of polynucleotides, cells, or pharmaceutical compositions according to the present invention) to a subject in need thereof.

[0445] Suitably, the ETM (e.g., ETR) and gRNAs may be administered to a subject simultaneously, sequentially, or separately.

[0446] In one aspect, the present invention provides a method of gene therapy which comprises the steps of:

(i) isolation of a cell containing sample;

(ii) introduction of a polynucleotide according to the present invention, a nucleic acid construct according to the present invention, at least one gRNA according to the present invention, an ETM (e.g., ETR) according to the present invention, a vector according to the present invention or a kit of polynucleotides according to the present invention to the cell(s); and

(iii) administering the cell(s) from step (ii) to a subject.

[0447] The nucleic acid construct or vector may be introduced by transduction or transfection.

[0448] The cell may, for example, be autologous. The cell may, for example, be allogeneic.

[0449] It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment; although in the context of the present invention references to preventing are more commonly associated with prophylactic treatment. The treatment of mammals, particularly humans, is preferred. Both human and veterinary treatments are within the scope of the present invention.

Diseases and conditions

[0450] By way of example, the products, ETMs (e.g., ETRs), polynucleotides and cells of the present invention may be used in the treatment of, for example, Huntington's disease, spinocerebellar ataxias, collagenopathies, haemaglobinopathies, and diseases caused by trinucleotide expansions. Furthermore, the product of the present invention may be used in the treatment or prevention of certain infectious diseases (e.g., CCR5-tropic HIV infections) by inactivating either pathogen-associated gene products or host genes that are necessary for the pathogen life cycle.

[0451] In addition, or in the alternative, the products, ETMs (e.g., ETRs), polynucleotides and cells of the present invention may be useful in the treatment of the disorders listed in WO 1998/005635. For ease of reference, part of that list is now provided: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

[0452] In addition, or in the alternative, the products, ETMs (e.g., ETRs), polynucleotides and cells of the present invention may be useful in the treatment of the disorders listed in WO 1998/007859. For ease of reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g., for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g., treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g., for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration; inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g., for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g., for treating haemophilia and stroke); anti-inflammatory activity (for treating e.g., septic shock or Crohn's disease); as antimicrobials; modulators of e.g., metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g., psoriasis, in human or veterinary medicine.

CA 03207166 2023-8-1

[0453] In addition, or in the alternative, the products, ETMs (e.g., ETRs), polynucleotides and cells of the present invention may be useful in the treatment of the disorders listed in WO 1998/009985. For ease of reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e., inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g., retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g., following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g., due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g., leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

[0454] For example, the present invention may be used to treat inherited disease such as β-haemoglobinopathies by targeting hemoglobin F (HBF) or haemoglobin subunit beta (HBB); or to treat severe combined immunodeficiency disease (SCID), Wiskott-Aldrich syndrome protein (WASP), sickle cell disease (SCD) or adenosine deaminase deficiency (ADA).

[0455] The skilled person will understand that they can combine any or all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

FURTHER ASPECTS

[0456] The present invention also provides further aspects as defined in the following numbered paragraphs.

1. An engineered transcriptional modulator (ETM) comprising: (a) at least one epigenetic effector domain; operably linked to (b) an endonuclease.
2. An ETM according to paragraph 1, wherein the at least one epigenetic effector domain comprises a Krüppel-associated box (KRAB) domain, a DNA methyltransferase (DNMT) domain, a DNMT-like domain, and/or a histone methyltransferase (HMT) domain.
3. An ETM according to paragraph 1 or paragraph 2, wherein the at least one epigenetic effector domain is selected from the group consisting of: DNMT1, DNMT3A, DNMT3B, DNMT3L and SETDB1.
4. An ETM according to any preceding paragraph, wherein the endonuclease comprises an RNA binding domain.
5. An ETM according to any preceding paragraph, wherein the endonuclease is a *Clustered Regularly Interspaced Short Palindromic Repeats* (CRISPR)/Cas system.
6. An ETM according to any preceding paragraph, wherein the endonuclease is a Cas9 endonuclease.
7. An ETM according to any preceding paragraph, wherein the ETM comprises or consists of: a Cas9-KRAB, Cas9-DNMT3A or Cas9-DNMT3L fusion protein.
8. An ETM according to any preceding paragraph, wherein the ETM is bi- or tri-partite fusion protein.
9. A gRNA comprising a spacer sequence which comprises or consists of the sequence of any one of SEQ ID NOs: 23-46, 562-1076, 2778-4478, and 4553-4565, or a fragment thereof.
10. A combination comprising an ETM according to any one of paragraphs 1-8, and at least one guide RNA (gRNA).
11. A combination according to paragraph 10, which comprises one or more ETMs, wherein each ETM is a fusion protein comprising a catalytically active CRISPR/Cas endonuclease domain.
12. A combination according to paragraph 10 or paragraph 11, which comprises one to three ETMs.
13. A combination according to any one of paragraphs 10-12, wherein at least one epigenetic effector domain is a transcriptional repressor domain, and/or wherein at least one epigenetic effector domain is a DNMT3L domain.

14. A combination according to any one of paragraphs 10-13, wherein the one or more ETMs collectively comprise a transcriptional repressor domain and a DNMT3L domain.
15. A combination according to any one of paragraphs 10-14, which comprises at least two gRNAs.
16. A combination according to paragraph 15, wherein the gRNAs target the ETM to at least two different target genes.
17. A combination according to paragraph 15 or paragraph 16, wherein the at least two gRNAs comprise spacer sequences which are of different lengths.
18. A combination according to any one of paragraphs 10-13, wherein at least one gRNA comprises a spacer sequence which is 15, 16, 17, 18, 19 or 20 nucleotides in length.
19. A combination according to any one of paragraphs 15-18, wherein one of the at least two gRNAs comprises a spacer sequence which is less than or equal to 17 (e.g., less than or equal to 16) nucleotides in length.
20. A combination according to any one of paragraphs 10-19, wherein the at least one target gene is selected from: genes without CpG Islands (CGI), such as: *TRAC*; *TRBC*; *PDCD1*; *TIM-3*; *TIGIT*; *LAG3*; *CTLA4*; *AAVS1* and *CCR5*; and/or genes having CGI, such as: *B2M*; *TET2*; *TGFBR2*; *A2AR*; *CISH*; *PTPN11*; *PTPN6*; *PTPA*; *PTPN2*; *JUNB*; *TOX*; *TOX2*; *NR4A1*; *NR4A2*; *NR4A3*; *MAP4K1*; *REL*; *IRF4*; *DGKA*; *PIK3CD*; *HLA-A*; *USP16*; *DCK* and *FAS*.
21. A combination according to any one of paragraphs 10-20, which comprises: one or more guide RNAs (gRNAs) having a spacer sequence with a length that allows epigenetic editing and not gene editing of a first gene in the cell, optionally wherein the first gene comprises a CpG island (CGI); and one or more gRNAs having a spacer sequence with a length that allows gene editing of a second gene in the cell.
22. A combination according to paragraph 21, wherein the one or more guide RNAs (gRNAs) having a spacer sequence with a length that allows epigenetic editing and not gene editing of a first gene in the cell has a spacer sequence of:

(a) less than or equal to 17 nucleotides (e.g., less than or equal to 16 nucleotides); or

(b) 11 to 17 nucleotides (e.g., 11 to 16 nucleotides).

23. A combination according to paragraph 21 or paragraph 22, wherein the one or more gRNAs having a spacer sequence with a length that allows gene editing of a second gene in the cell has a spacer sequence of:

(a) 17 or more nucleotides (e.g., 18 or more nucleotides); or

(b) 17 to 30 nucleotides, optionally 18 to 25 nucleotides (e.g., 18 to 21 nucleotides).

24. A combination comprising one or more polynucleotides coding for the ETM(s) (e.g., fusion proteins) and/or gRNAs as defined in any one of paragraphs 10-23.

25. A combination according to any one of paragraphs 21-24, further comprising a donor DNA comprising 5' and 3' arms that are homologous to sequences in the second gene.

26. A combination according to any one of paragraphs 10-25, wherein the endonuclease domain is derived from a Cas9 protein, optionally SpCas9.

27. A combination according to any one of paragraphs 21-26, wherein

the first gene is selected from *B2M*, *TET2*, *TGFBR2*, *A2AR*, *CISH*, *PTPN11*, *PTPN6*, *PTPA*, *PTPN2*, *JUNB*, *TOX*, *TOX2*, *NR4A1*, *NR4A2*, *NR4A3*, *MAP4K1*, *REL*, *IRF4*, *DGKA*, *PIK3CD*, *HLA-A*, *USP16*, *DCK*, and *FAS*; and/or

the second gene is selected from *TRAC*, *TRBC*, *PDCD1*, *TIM-3*, *TIGIT*, *LAG3*, *CTLA4*, *AAVS1*, and *CCR5*.

28. A combination according to any one of paragraphs 21-27, wherein the second gene is a *TRAC* gene, optionally wherein the one or more gRNAs targeting the *TRAC* gene comprise a spacer having the sequence of one of SEQ ID NOs: 562-611, optionally SEQ ID NO: 604.

29. A combination according to any one of paragraphs 21-28, wherein the first gene is a *B2M* gene, optionally wherein the one or more gRNAs targeting the *B2M* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 28-33 and 39-44; or the sequence of one of SEQ ID NOs: 2778-2878 with a 3 to 9 nucleotide truncation at the 5' end, optionally one of SEQ ID NOs: 2778, 2780, 2801, and 2863 with a 3 to 9 nucleotide truncation at the 5' end, selected from SEQ ID NOs: 4486-4492, 4497-4503, 4508-4514, and 4519-4525.

30. A combination according to any one of paragraphs 21-28, wherein the first gene is a *TGFBR2* gene, optionally wherein the one or more gRNAs targeting the *TGFBR2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 2929-2978 and 4553-4559 with a 3 to 9 nucleotide truncation at the 5' end.

31. A combination according to any one of paragraphs 21-28, wherein the first gene is a *TET2* gene, optionally wherein the one or more gRNAs targeting the *TET2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 4429-4478 and 4560-4565 with a 3 to 9 nucleotide truncation at the 5' end.

32. A combination according to any one of paragraphs 10-31 for modifying transcription, expression and/or activity of one or more (e.g. two or more) gene in a cell, wherein the cell is a mammalian cell, optionally a human cell, optionally wherein the cell is a human immune cell, or a human T cell.

33. A combination according to any one of paragraphs 10 to 32, further comprising an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination and/or

iii) which enables selection of a cell, such as a cell which comprises the combination or a cell which does not comprise the combination.

34. A combination according to any one of paragraphs 10 to 33, comprising at least one gRNA according to paragraph 9.

35. The combination of any one of paragraphs 20-34, wherein the gene comprising a CGI is a *B2M* gene and the gRNAs targeting it are two or three gRNAs each independently comprising a spacer having the sequence of

C8 (SEQ ID NO: 35),

F4 (SEQ ID NO: 24),

H8 (SEQ ID NO: 2780),

H10 (SEQ ID NO: 2863),

H11 (SEQ ID NO: 2778), or

H12 (SEQ ID NO: 2801),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

36. The combination of paragraph 35, wherein the *B2M*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end,

CA 03207166 2023-8-1

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(ii) a gRNA comprising a spacer having the sequence of C8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end,

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(iii) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(iv) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(v) a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

37. The combination of any one of paragraphs 21-36, wherein the ETM(s) (e.g., one or more fusion proteins) collectively further comprise a DNMT1, DNMT3A, DNMT3B, or SETDB1 domain, optionally DNMT3A.

38. The combination of any one of paragraphs 10-37, wherein the combination comprises

(i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, and a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, or

(ii) a fusion protein comprising, optionally from N-terminus to C-terminus, a transcriptional repressor domain, a Cas endonuclease domain, and a DNMT3L domain.

39. The combination of any one of paragraphs 10-37, wherein the combination comprises

(i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, and a third fusion protein comprising a DNMT3A domain and a Cas endonuclease domain, or

(ii) a fusion protein comprising a transcriptional repressor domain, a Cas endonuclease domain, a DNMT3L domain, and a DNMT3A domain.

40. The combination of any one of paragraphs 10-39, wherein the epigenetic effector domain (e.g. transcriptional repressor domain) is a Krüppel-associated box (KRAB) domain, optionally derived from human Kox1 or ZIM3.

41. The combination of any one of paragraphs 10-40, wherein the combination comprises a fusion protein comprising, optionally from N terminus to C terminus, a KRAB domain derived from ZIM3, a catalytically active Cas9 domain, and a DNMT3L domain, optionally comprising an amino acid sequence of SEQ ID NO: 4482.

42. The combination of any one of paragraphs 10-41, further comprising gRNAs for targeting one or more additional genes in the cell.

43. The combination of any one of paragraphs 10-42, wherein the gRNA(s) are chemically modified, optionally wherein the chemically modified gRNA(s) comprise phosphorothioate internucleoside linkages at the 5' and/or 3' ends, and/or 2'-O-methyl nucleotides.

44. A polynucleotide encoding at least one ETM according to any one of paragraphs 1 to 8 or as defined in any one of paragraphs 10-43.

45. A nucleic acid construct comprising a nucleic acid sequence encoding at least one ETM according to any one of paragraphs 1 to 8 or as defined in any one of paragraphs 10-43.

46. A nucleic acid construct according to paragraph 45, further comprising a nucleic acid sequence:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which expresses said nucleic acid construct or a cell which does not express said nucleic acid construct; and/or

CA 03207166 2023-8-1

iii) which enables selection of a cell, such as a cell which comprises the nucleic acid construct or a cell which does not comprise the construct.

47. A vector comprising a polynucleotide according to paragraph 44 or a nucleic acid construct according to paragraph 45 or 46.

48. A kit of polynucleotides comprising:

a) at least one polynucleotide encoding at least one ETM according to any one of paragraphs 1 to 8 or as defined in any one of paragraphs 10-43; and

b) a polynucleotide providing at least one gRNA as described in any one of paragraphs 9 or 10 to 32 or 35 to 43; and optionally,

c) a further polynucleotide comprising a nucleic acid sequence which encodes an agent:

i) which promotes the survival, proliferation and/or activity of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides; and/or

ii) which is detrimental to the survival, proliferation, activity, chemoresistance and/or chemotaxis of a cell, such as a cell which comprises said polynucleotides or a cell which does not comprise said polynucleotides; and/or

iii) which enables selection of a cell, such as a cell which comprises the polynucleotides or a cell which does not comprise the polynucleotides.

49. A cell comprising an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47 or a kit of polynucleotides according to paragraph 48.

50. A cell wherein the cell is a progeny of the cell of paragraph 49.

51. A composition comprising an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48 or a cell according to paragraph 49 or paragraph 50.

52. A pharmaceutical composition comprising an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination

according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48 or a cell according to paragraph 49 or paragraph 50.

53. Use of an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48 or a cell according to paragraph 49 or paragraph 50 for modifying the transcription, expression and/or activity of (e.g. repressing or silencing) at least one target gene in a cell.

54. A method of modifying the transcription, expression and/or activity of (e.g. repressing or silencing) at least one target gene in a cell comprising the step of administering an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47 or a kit of polynucleotides according to paragraph 48 to a cell.

55. The use or method of paragraph 53 or 54, wherein the cell is a T cell.

56. The use or method of any one or paragraphs 53-55, wherein the ETM, at least one gRNA, combination, polynucleotide, nucleic acid construct, vector or a kit of polynucleotides is introduced into the cell *in vitro* or *ex vivo*.

57. A method according to any one of paragraphs 54-56, wherein at least two target genes are silenced, wherein at least one of the at least two target genes is epigenetically silenced and at least one of the at least two target genes is silenced by gene editing, wherein at least one ETM and at least two gRNAs are administered to said cell simultaneously, sequentially or separately.

58. A cell obtained by the use or method of any one of paragraphs 53-57, or a progeny of the cell.

59. The cell of any one of paragraphs 49, 50 or 58, wherein the cell is a human T cell, optionally engineered to express a recombinant antigen receptor, optionally selected from a recombinant T cell receptor (TCR) or a chimeric antigen receptor (CAR).

60. An ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48, a cell according to paragraph 49, 50, 58 or 59 or a pharmaceutical composition according to paragraph 52 for use in therapy (e.g. for use in treating a human in need thereof).

61. Use of an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48, a cell according to paragraph 49, 50, 58 or 59 or a pharmaceutical composition according to paragraph 52 in the manufacture of medicament for treating a human in need thereof.

62. An ETM, combination, polynucleotide, nucleic acid construct, vector, kit of polynucleotides, cell or pharmaceutical composition for use according to paragraph 60, or the use of paragraph 61, wherein at least one ETM (e.g. fusion protein) and at least two gRNAs are administered to a cell or subject simultaneously, sequentially or separately.

63. A method for treating and/or preventing a disease (e.g. in a human in need thereof), which comprises the step of administering an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, a combination according to any one of paragraphs 10 to 43, a polynucleotide according to paragraph 44, a nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47, a kit of polynucleotides according to paragraph 48, a cell according to paragraph 49, 50, 58 or 59 or a pharmaceutical composition according to paragraph 52 to a subject in need thereof.

64. A method for treating and/or preventing a disease according to paragraph 63, wherein at least one ETM (e.g. fusion protein) and at least two gRNAs are administered to a cell or subject simultaneously, sequentially or separately.

65. A method of gene therapy which comprises the steps:

(i) isolation of a cell containing sample;

(ii) introduction of an ETM according to any one of paragraphs 1 to 8, at least one gRNA according to paragraph 9, the combination according to any one of paragraphs 10 to 43, the polynucleotide as defined in paragraph 44, the nucleic acid construct according to paragraph 45 or paragraph 46, a vector according to paragraph 47 and/or a kit of polynucleotides according to paragraph 48 to the cell(s); and

(iii) administering the cell(s) from step (ii) to a subject.

66. The method according to paragraph 65, wherein the polynucleotide, nucleic acid construct and/or vector is introduced by transduction or transfection.

67. An ETM, combination, polynucleotide, nucleic acid construct, vector, kit of polynucleotides, cell or pharmaceutical composition for use according to paragraph 60 or 62, the use of paragraph 61 or 62, or the method according to any one of paragraphs 63-66, wherein the cell is autologous.

68. An ETM, combination, polynucleotide, nucleic acid construct, vector, kit of polynucleotides, cell or pharmaceutical composition for use according to paragraph 60 or 62, the use of paragraph 61 or 62, or the method according to any one of paragraphs 63-66, wherein the cell is allogeneic.

[0457] Unless otherwise defined herein, scientific, and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure. In case of conflict, the present specification, including definitions, will control. Generally, nomenclature used in connection with, and techniques of, medicine, medicinal and pharmaceutical chemistry, and cell biology described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words “have” and “comprise,” or variations such as “has,” “having,” “comprises,” or “comprising,” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. As used herein the term “about” refers to a numerical range that is 10%, 5%, or 1% plus or minus from a stated numerical value within the context of the particular usage. Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed embodiments.

[0458] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology, and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press; Ausubel, F.M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, Ch. 9, 13 and 16, John Wiley & Sons; Roe, B., Crabtree, J. and Kahn, A. (1996) DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; Polak, J.M. and McGee, J.O'D. (1990) In Situ Hybridization: Principles and Practice, Oxford University Press; Gait, M.J. (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; and Lilley, D.M. and Dahlberg, J.E. (1992) Methods in Enzymology: DNA Structures Part A: Synthesis and Physical Analysis of DNA, Academic Press.

[0459] All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0460] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

EXAMPLE 1 – gRNAs comprising truncated spacer sequences promote epigenetic silencing without causing mutagenesis

[0461] To assess the feasibility of using gRNAs comprising truncated spacer sequences to promote ETR-mediated epi-silencing of *B2M* while sparing the gene from mutagenesis, we first designed a 20nt-long gRNA against *B2M* (named F4; SEQ ID NO: 24) and a corresponding panel of 5'-truncated *B2M* gRNAs with spacer sequences of different lengths, and then we tested them in the *B2M*^{tdTomato} K-562 cell line (Amabile et al., *supra*).

[0462] In particular, the gRNAs comprising spacer sequences spanning from 21 to 10 nt in length and comprising the same seed and PAM sequence (**Figure 1**) were individually delivered into the cells together with either Cas9 or the dCas9-based ETR combination, the latter containing KRAB, DNMT3A or DNMT3L. The cells were then analysed for genetic traces of Cas9 activity at the *B2M* gene or expression of tdTomato, the latter used as a proxy for *B2M* epigenetic silencing.

[0463] gRNAs comprising the standard 20 nt-long *B2M* spacer sequence *plus* Cas9 or dCas9-ETRs were included as positive controls for gene disruption or epigenetic silencing, respectively. Molecular analyses of the *B2M* target site in Cas9-treated cells showed a threshold effect: gRNAs comprising a spacer sequence of ≥17nt in length mediated high and comparable levels of *B2M* editing (~30%) while gRNAs comprising a spacer sequence ≤16nt resulted in undetectable gene editing (**Figure 2**). Flow cytometry analyses of ETR-treated cells showed a different trend: all gRNAs except the gRNA comprising the 10nt-long spacer sequence were able to induce efficient epigenetic silencing of *B2M*, although at different levels (from 30 to 48% of tdTomato-negative cells; **Figure 3**). Importantly, the gRNAs comprising truncated spacer sequences that were ineffective in promoting gene editing with Cas9 (i.e., ≤16nt) were highly effective in mediating epigenetic silencing with the dCas9-ETRs. To assess if these findings were portable to other gRNAs, we performed a similar truncation experiment using three other gRNAs (named H8_20 (spacer SEQ ID NO: 2780; gRNA SEQ ID NO: 4570), C8_20 (spacer SEQ ID NO: 2813; gRNA SEQ ID NO: 4569) and H10_20 (spacer SEQ ID NO: 2863; gRNA SEQ ID NO: 4571) and found that the spacer length at which Cas9 lost its activity depended on the specific gRNA used, ranging between ≤15 and ≤17nt in length (**Figure 4**; left panel). In accordance to what we showed above, ETRs were able to induce epi-silencing of *B2M* even with truncated gRNAs (**Figure 4**; right panel).

[0464] Overall, these data indicate that gRNAs comprising a truncated spacer sequence of ≤17nt promote epigenetic silencing of *B2M* while sparing this gene from mutagenesis induced by Cas9-based ETRs. Furthermore, they provide the first demonstration that epi-silencing can be imposed also when using gRNAs comprising truncated spacer sequences. In parallel to these experiments, we also produced a gRNA comprising a 20nt spacer sequence capable of inducing gene editing at the *TRAC* locus (**Figure 5**).

EXAMPLE 2 – A combination of ETR and gRNAs enables simultaneous inactivation of two genes without inducing chromosomal translocations

[0465] Based on these data, we then constructed ETRs equipped with a catalytically active Cas9 (hereafter referred as to Cas9-ETRs, containing KRAB, DNMT3A or DNMT3L) and assessed their multiplexing efficiency with gRNAs comprising truncated or full-length spacer sequences in the $B2M^{tdTomato}$ K-562 cells. In particular, we co-transfected the cells with the triple Cas9-ETR combination plus the F4-derived *B2M* gRNA comprising 16nt-long spacer sequences (see **Figures 2** and **3**) and the *TRAC* gRNA with the 20nt-long spacer. The following controls were also included in the experiment: (i) cells co-transfected with the just mentioned gRNA combination plus either Cas9 or the standard triple dCas9-ETRs, used as positive control for either genetic disruption of *TRAC* or epi-silencing of *B2M* and disruption of *TRAC*, respectively; (ii) cells co-transfected with two gRNAs comprising 20nt-long spacer sequences, one against *B2M* and the other against *TRAC,* plus either Cas9 or Cas9-ETRs, were used here as positive controls for co-disruption of *B2M* and *TRAC.* The latter conditions were also included to assess if simultaneous gene editing of the two loci may lead to reciprocal chromosomal translocations. Upon transfection, the cells were longitudinally monitored by flow cytometry for tdTomato expression for up to 25 days.

[0466] As shown in **Figure 6** and **Figure 7**, when delivered with the gRNA comprising a 16nt spacer sequence, Cas9- and dCas9-based ETRs performed equally in terms of *B2M* epi-silencing (14 vs. 21%, respectively). Cas9 promoted *B2M* inactivation only when coupled with the gRNA comprising a 20nt-long spacer sequence and not with its truncated counterpart (24% vs. 1.7% of tdTomato negative cells), further confirming the results of Figure 2 and Figure 3. The use of the gRNA comprising a 20nt-long *B2M* spacer sequence with Cas9-ETRs resulted in a percentage of tdTomato negative cells that was higher than that found in all other conditions (up to 44%), a finding expected considering the additive effect of gene and epigenetic editing on this locus. Of note, silencing was stable long-term in all analyzed conditions (**Figure 8**), indicating that also the Cas9-ETRs with the gRNA comprising a 16nt spacer sequence are able to instruct mitotically inherited epigenetic modifications.

[0467] We then analyzed the cells for gene editing (**Figure 9**) and found that both Cas9 and Cas9-ETRs induced efficient editing of TRAC (up to 37%). On the other hand, gene editing of *B2M* was limited to the conditions in which Cas9 or Cas9-ETRs were co-delivered with the gRNA comprising the 20nt-long *B2M* spacer sequence. Finally, we performed a PCR analysis with primers specific for reciprocal chromosomal translocations between *B2M* and *TRAC* and found occurrence of these events exclusively in the conditions co-treated with the two gRNAs comprising 20nt-long spacer sequences, but not when the gRNA comprising the 16nt spacer sequence was used (**Figure 10**).

[0468] Overall, these data show that Cas9-ETRs perform as their dCas9-based counterparts in terms of silencing efficiency and stability. Yet, adoption of Cas9-ETRs in combination with gRNAs comprising a truncated and a full-length spacer sequence can be safely used to inactivate simultaneously two genes without inducing chromosomal translocations.

EXAMPLE 3 – Optimization of the *B2M* epi-silencing procedure in human primary T lymphocytes

[0469] Inactivation of *B2M* is emerging as a promising approach to generate allogenic T cell products. To assess feasibility of *B2M* epi-silencing in human primary T cells, we first expanded our repertoire of gRNAs against this gene to include 2 other guides: H11_20 (spacer SEQ ID NO: 2778; gRNA SEQ ID NO: 4572) and H12_20 (spacer SEQ ID NO: 2801; gRNA SEQ ID NO: 4573) (**Figure 11**). We then delivered each of these 6 gRNAs with mRNAs encoding for the triple ETR combination in T cells. Time course flow cytometry analyses of treated cells were then used to assess efficiency and stability of *B2M* epi-silencing. Unexpectedly, at day 12 post-treatment, all but one of the tested gRNAs failed to induce epi-silencing of *B2M* (**Figure 12**). The only working gRNAs (namely gRNA C8) resulted in up to 2% of B2M-negative cells, which, however, were lost upon T cell restimulation (analysis at day 25).

[0470] We then tested whether combined delivery of gRNAs would improve epi-silencing efficiency. To this end, we combined either gRNA C8 or H8 with all other gRNAs and delivered these dual gRNA combinations together with the triple ETR combination in T cells. Flow cytometry analyses at day 12 post-treatment revealed that all gRNA combinations were able to induce epi-silencing of *B2M*, although at different levels (**Figure 12**). For instance, gRNA combination H12+H8 induced limited silencing, while the F4+H8 combination resulted in up to 28% of B2M-negative cells. Importantly, for some of these gRNA combinations, epi-silencing resisted the T cell restimulation process, ranging from 11 to 20% of long-term stable B2M-negative cells (**Figures 12** and **13**). Extended time course flow cytometry analyses over a timeframe of 37 days and spanning two rounds of T cell restimulations showed that most of the tested gRNA combinations induced an initial wave of B2M epi-silencing, which then declined after the first T cell restimulation (day 12) to reach near stability until the second round of T cell restimulation (day 25) (**Figure 14**). Then, the percentage of B2M increased until termination of the experiment (day 37). Of note, the efficiency of epi-silencing was dependent on the combination of gRNAs used, with H8+F4 being the most effective at long-term (up to 30% of B2M-negative cells) while H8+H11 and H8+H12 resulting in barely detectable, if any, epi-silencing.

[0471] Epi-silencing stability was also dependent on gRNA combination (**Figure 15**). Indeed, by comparing the percentage of B2M-negative cells between day 25 (just before to the second round of T cell restimulation) and day 12 (just before the first round of T cell restimulation), we found that some gRNA combinations were poorly resistant (fold reduction in B2M-negative cells <0.5) while others were more resistant (fold reduction in B2M-negative cells ≥ 0.5), although none of them were able to result in fully stable gene silencing. Among the most stable, combinations H8+F4 and H8+H10 were the best performing ones.

[0472] We then performed a similar experiment, in which we excluded the ineffective gRNA combinations H8+H11 and H8+H12 and included the new dual-gRNA combination F4+H10. Furthermore, we also included triple gRNA combinations (namely, C8+F4+H8, C8+F4+H10, C8+H8+H10 and F4+H8+H10) to assess if these were able to further improve epi-silencing efficiency and stability. Among the dual-gRNA combinations tested, the most effective at long-term (day 32) was F4+H10, reaching up to 36.5% of B2M-negative cells. Among the triple gRNA combinations tested, the F4+H8+H10 outperformed the others by 1.6-fold, reaching up to 66% of B2M-negative cells at termination of the experiment (**Figure 16**). As observed in the previous experiment, the first round of T cell restimulation caused a marked reduction in the percentage of B2M negative cells for most of the gRNA combinations (**Figure 16**). Noticeable exceptions to this were the gRNA combinations containing F4+H10 (including the triple C8+F4+H10 and F4+H8+H10), for which the percentage of B2M-negative cells at day 28 and 14 were nearly superimposable (fold reduction in B2M-negative cells ~1; **Figure 17**). Similar findings were obtained for the dual C8+H10 gRNA combination (**Figure 17**). Overall, these data show that epi-silencing efficiency and durability of *B2M* depends on which gRNA combination is used, with the triple based on the F4+H8+H10 being the best-preforming one.

[0473] With the aim of reducing the molecular complexity of the technology, we then asked whether all the components of the triple ETR combination were required for epi-silencing of *B2M*. To this end, we transiently delivered to T cells the dual-gRNA combination containing C8 and F4 together with mRNAs encoding either: (i) the triple ETR combination, taken here as reference for epi-silencing efficiency of *B2M*; (ii) the double ETR combination containing the KRAB and DNMT3L effector domains; (iii) the double ETR combination containing the DNMT3A and DNMT3L effector domains; or (iv) the double ETR combination containing the KRAB and DNMT3L effector domains. The T cells were then analysed for B2M expression by flow cytometry until day 37 post-treatment (**Figures 18A** and **18B**). This experiment showed that, among all the double ETR combinations tested, only the one based on the KRAB and DNMT3L effector domains induced long-term silencing, at efficiencies superimposable to those observed with the triple ETR combination (up to 14% of B2M-negative cells). The double ETR combination based on KRAB and DNMT3A induced only transient *B2M* repression, which, after the first round of T cell restimulation, returned to the levels observed in untreated T cells. Unexpectedly, the double ETR combination based on DNMT3A and DNMT3L failed to induce any *B2M* silencing, even at early time points post-treatment.

[0474] Based on these results, we then performed a similar experiment to that shown in **Figure 16** but using the double ETR combination containing KRAB and DNMT3L, confirming that this combination performed as efficiently as the triple one for all gRNA combinations tested (**Figure 19**). As for the triple ETR combination, the conditions in which the gRNAs F4 and H10 were co-present were the most resistant to T cell restimulation (**Figure 19**). Overall, these data show that the double ETR

CA 03207166 2023-8-1

combination containing KRAB and DNMT3L performs as efficiently as the canonical triple ETR combination in silencing *B2M* in T cells.

[0475] Based on these results, we then compared the efficiencies of *B2M* epi-silencing between the double ETR combination containing KRAB and DNMT3L and an all-in-one bi-partite ETR equipped with the KRAB domain homolog of the Zinc finger imprinted 3 (ZIM3) protein (Alerasool et al., Nat Methods (2020) 17(11):1093-6) and DNMT3L (**Figure 20A**, left schematic), hereafter referred as to the ZIM:dCas9:DNMT3L fusion. The amino acid sequence of this fusion protein is shown below, wherein the SV40 nuclear localization signals (NLSs) are in box, the ZIM3 KRAB repressor domain is in boldface, the flexible linkers are in underlined boldface, dCas9 is underlined and the DNMT3L domain is in italics (only):

MPKKKRKVGGGAS**MGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQGETTKPDVIL**
RLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKESLGGGGSGGGGSGGGGSGGGGSLEDKKYS
IGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTR
RKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKL
VDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKA
ILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL
LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEK
YKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQI
HLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVV
DKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILED
IVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSD
GFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRH
KPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDM
YVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK
LITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITL
KSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSE
QEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNI
VKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKE
LLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS
KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GDSPKKKRKVGVD**GSGGG***MAAIPALDPEAEPSMDVILVGSSELSSSVSPGTGRDLIAYEVKANQRNIE*
DICICCGSLQVHTQHPLFEGGICAPCKDKFLDALFLYDDDGYQSYCSICCSGETLLICGNPDCTRCYC
FECVDSLVGPGTSGKVHAMSNWVCYLCLPSSRSGLLQRRRKWRSQLKAFYDRESENPLEMFETVPVWR
RQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVVDVTDTVRKDVEEWGPFDLVYGATPPLGHTCDR
PPSWYLFQFHRLLQYARPKPGSPRPFFWMFVDNLVLNKEDLDVASRFLEMEPVTIPDVHGGSLQNAVR
VWSNIPAIRSRHWALVSEEELSLLAQNKQSSKLAAKWPTKLVKNCFLPLREYFKYFSTELTSSL
(SEQ ID NO: 4481)

[0476] In these experiments, the T cells were co-transfected with the mRNAs encoding the ETRs and (i) the gRNAs F4 or C8, to assess if the bi-partite ETR was able to rescue epi-silencing efficiency of individual gRNAs; (ii) the dual-gRNA combination C8+F4; or (iii) the best-performing triple gRNA combination F4+H8+H10. Cells were then analysed by flow cytometry until day 55. To avoid any confounding effects due to the delivery of different amounts of mRNAs encoding the ETRs, these experiments were performed by using 1.5 μg of each ETR for the double combination and 1.5 μg of the ZIM:dCas9:DNMT3L fusion. As such, matched amounts of epigenetic effectors were used. In accordance with our previous data, individual gRNAs were ineffective with the double ETR combination, and adoption of the ZIM:dCas9:DNMT3L fusion only slightly increased *B2M* epi-silencing efficiency and exclusively for gRNA C8 (**Figure 20A**, right graph). On the other hand, a marked increase in *B2M* epi-silencing was found when comparing the double ETR combination and the ZIM:dCas9:DNMT3L fusion with the dual-gRNA combination C8+F4 (from 11% to 70%, respectively; **Figures 20A-B**). Similar results were obtained for the triple gRNA combination F4+H8+H10, although the differences between the double ETR combination and the ZIM:dCas9:DNMT3L fusion were less pronounced (86 and 95% of B2M-negative cells for the double ETR combination and the ZIM:dCas9:DNMT3L fusion, respectively; **Figures 20A-B**). This effect was likely due to the already high epi-silencing efficiency of the double ETR combination. For all conditions with the triple gRNA combination, *B2M* epi-silencing proved to be durable, resisting 2 rounds of T cell restimulations. Notable was the stability observed with the ZIM:dCas9:DNMT3L fusion and the triple gRNA combination, which reached 95% of B2M-negative cells at day 8 post-treatment to then remain stable until day 55. Finally, to assess if the mRNA dose of the ZIM:dCas9:DNMT3L fusion was at saturation, we performed a dose titration experiment in T cells and found that one third of the standard doses (1 vs. 1.5 μg) was already sufficient to obtain efficient epi-silencing of *B2M* (**Figure 21**).

[0477] Overall, these data show that adoption of the fusion protein ZIM:dCas9:DNMT3L improves epi-silencing in T cells, achieving up to 95% of B2M-negative cells. Interesting features of this fusion protein include the reduced costs of production as compared to the triple or double ETR combinations and the fact that it can depose efficient silencing at one third of the dose of the double ETR combination.

EXAMPLE 4 – Orthogonal editing of *B2M* and *TRAC* in human primary T cells without inducing reciprocal chromosomal translocations

[0478] Based on the above data, we then tested if co-delivery of Cas9-based ETRs together with truncated gRNAs against *B2M* and the full-length gRNA against *TRAC* (SEQ ID NO: 4575) can induce orthogonal edits (namely epi-silencing of *B2M* and targeted integration into the *TRAC* gene) in human primary T cells without causing reciprocal chromosomal translocations. To mediate epi-silencing of *B2M*, we used some of the truncated gRNAs described above (see **Figures 2-4**), namely truncated C8 (C8_16; 16nt-long spacer; gRNA SEQ ID NO: 4578), truncated F4 (F4_16; 16nt-long spacer; gRNA SEQ ID NO: 4579) and truncated H8 (H8_15; 15nt-long spacer; gRNA SEQ ID NO: 4577), which we co-delivered in T cells as a triple combination. All truncations herein start from the 5' end of the full-length sequence. In these experiments, we also used the reduced ETR combination/architecture identified above, namely the double ETRs containing KRAB and DNMT3L or the cognate all-in-one fusion protein with a ZIM3 KRAB domain and DNMT3L, both of which were modified to contain the catalytically active Cas9. The all-in-one fusion with ZIM3 KRAB, active Cas9 and DNMT3L domains has the following amino acid sequence, wherein the SV40 NLSs are in box, the ZIM3 KRAB repressor domain is in boldface, the flexible linkers are in underlined boldface, Cas9 is underlined and the DNMT3L domain is in italics (only):

MPKKKRKVGGGASMGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQGETTKPDVILRLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKESLGGGGSGGGGSGGGGSGGGGSLEDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRH

KPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDM
YVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK
LITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITL
KSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSE
QEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNI
VKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKE
LLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS
KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GDSPKKKRKVGVD**GSGGG***MAAIPALDPEAEPSMDVILVGSSELSSSVSPGTGRDLIAYEVKANQRNIE*
DICICCGSLQVHTQHPLFEGGICAPCKDKFLDALFLYDDDGYQSYCSICCSGETLLICGNPDCTRCYC
FECVDSLVGPGTSGKVHAMSNWVCYLCLPSSRSGLLQRRRKWRSQLKAFYDRESENPLEMFETVPVWR
RQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVVDVTDTVRKDVEEWGPFDLVYGATPPLGHTCDR
PPSWYLFQFHRLLQYARPKPGSPRPFFWMFVDNLVLNKEDLDVASRFLEMEPVTIPDVHGGSLQNAVR
VWSNIPAIRSRHWALVSEEELSLLAQNKQSSKLAAKWPTKLVKNCFLPLREYFKYFSTELTSSL
(SEQ ID NO: 4482)

[0479] For Cas9-mediated targeted integration into the *TRAC* locus, we exploited a previously developed AAV6-based donor template, which contains the sequences encoding for a transgenic TCR against the tumour antigen NY-ESO embedded within TRAC homology arms (Roth et al., Nature (2018) 559(7714):405-9). Upon targeted integration, the transgenic TCR was expressed from the endogenous *TRAC* locus, and it can be measured by flow cytometry using a specific pentamer (**Figure 22**). Concerning T cells transfected with the double Cas9-based ETR combination, this treatment unexpectedly resulted in little, if any, epi-silencing of *B2M*, while editing of *TRAC* was highly efficient, resulting in up to 70% of NY-ESO-positive cells and up to 6% of endogenous TCR disrupted cells (**Figure 23**). Remarkably, the use of the ZIM3:Cas9:DNMT3L fusion protein rescued *B2M* epi-silencing efficiency, resulting in up to 70% of B2M-negative T cells (**Figure 24**). Also in these conditions, high levels of editing of the *TRAC* locus were measured. Further analyses of the NY-ESO-positive T cells showed that 65% of them were also B2M-negative. A similar analysis focused on the TCR disrupted cells showed comparable efficiencies of co-editing. Analyses at day 34 post-treatment showed that orthogonal edits were resistant to T cell restimulation.

[0480] We then evaluated by PCR analyses the presence of reciprocal chromosomal translocations between *B2M* and *TRAC*. Of note, no signs of reciprocal translocations were found (**Figure 25)**, indicating that (i) the *B2M* gene was silenced through epigenetic mechanisms rather than by genetic inactivation and

(ii) truncated gRNAs abolished Cas9 cleavage, confirming our previous findings in the *B2M*^{tdTomato} K-562 cell line (see Example 2). At variance with these data, T cells co-transfected with Cas9-based ETRs and full-length gRNAs against both *B2M* and *TRAC* displayed high levels of co-editing together with clear signs of reciprocal chromosomal translocations (**Figure 25**).

[0481] Overall, these data show that the co-adoption of gRNAs of different lengths and Cas9-based ETRs can promote orthogonal edits (i.e., epi-silencing and targeted integration or epi-silencing and gene disruption) at high efficiency in human primary T cells without inducing reciprocal chromosomal translocations.

EXAMPLE 5 – Identification of gRNAs to mediate high levels of epi-silencing of *TET2* and *TGFBR2* in human primary T lymphocytes

[0482] To expand the orthogonal editing approach to more than two genes, we designed a panel of gRNAs targeting *TET2* and *TGFBR2*. Inactivation of these genes represents a potential therapeutic approach to either increasing persistency or protecting T cell products from immune-dampening signals originating from the tumour microenvironment (see, e.g., Fraietta et al., Nature (2018) 558(7709):307-12; Nobles et al., J Clin Invest (2020) 130(2):673-85; Li et al., Nature (2020) 587(7832):121-5; Alishah et al., J Transl Med (2021) 19(1):482). For each of these genes, we designed 20 gRNAs in a genomic window of 1Kb around their transcription start site (**Figure 26**). We then set out to test epi-silencing efficacy of these gRNAs directly in T cells, using the standard triple ETR combination containing KRAB, DNMT3A and DNMT3L effector domains. We pool contiguous gRNAs, and then coupled each of these pairs with the others to obtain any possible pair combinations. The tested pairs are shown in Table 3 below (SEQ: SEQ ID NO). The gRNAs used in this experiment contained 20-nucleotide (full-length) spacer sequences.

Table 3. *TGFBR2* and *TET2* gRNA Pairs

gRNA Spacer ID	Pair No.	gRNA Target Sequence	Target Sequence SEQ	Spacer Sequence SEQ	gRNA SEQ
TGFBR2 gRNAs					
TG1	P1	TTCTTTAGGTCG AAGTCTAGAGG	4539	4553	4608

gRNA Spacer ID	Pair No.	gRNA Target Sequence	Target Sequence SEQ	Spacer Sequence SEQ	gRNA SEQ
TG2		GTGCTCGCGACTCAATAGATTGG	4540	4554	4609
TG3	P2	AACGCATCTCTAAAGCACCTAGG	4541	4555	4610
TG4		CTGATCTACTAGGGAAAACGTGG	4542	4556	4611
TG5	P3	TTGAGTAAATACTTGGAGCGAGG	4543	4557	4612
TG6		AGTCGCCCAAACCTCTCGGAGGG	1239	2940	4613
TG7	P4	GAAACTCCTCGCCAACAGCTGGG	1236	2937	4614
TG8		GAGTGAGTCACTCGCGCGCACGG	1229	2930	4615
TG9	P5	CGCGTGCACCCGCTCGGGACAGG	1254	2955	4616
TG10		GGGGCCTCCCCGCGCCTCGCCGG	4544	4558	4617
TG11	P6	TGGCGAGCGGGCGCCACATCTGG	1256	2957	4618
TG12		TCGGTCTATGACGAGCAGCGGGG	1228	2929	4619
TG13	P7	CCTGAGCAGCCCCCGACCCATGG	4545	4559	4620
TG14		GGACGATGTGCAGCGGCCACAGG	1244	2945	4621
TG15	P8	TGCTGGCGATACGCGTCCACAGG	1230	2931	4622
TG16		AACGTGCGGTGGGATCGTGCTGG	1241	2942	4623
TG17	P9	GACTGTCAAGCGCAGCGGAGAGG	1238	2939	4624
TG18		CTTTCCTCGTTTCCGCCCGGGGG	1234	2935	4625
TG19	P10	GCCCGACTCCCGTAGCTGCAGGG	1237	2938	4626
TG20		CGTTGTGTTGGCCGCGTTCGAGG	1231	2932	4627
TET2 gRNAs					
TE1	P1	GGAATTAGCTCTGTATCGGTCGG	4547	4560	4588
TE2		AAAGTAAGGGCTCTTACGAGAGG	4548	4561	4589
TE3	P2	GGCGTCTCACAGATTGAAATAGG	4549	4562	4590
TE4		CGGTCAATTTCCCAGTTTGTCGG	4550	4563	4591

gRNA Spacer ID	Pair No.	gRNA Target Sequence	Target Sequence SEQ	Spacer Sequence SEQ	gRNA SEQ
TE5	P3	AGCGCTCCCCTG TTTCACCGAGG	2742	4443	4592
TE6		CGCGGGCAACGG GATCTAAAGGG	2733	4434	4593
TE7	P4	CGCAAGCGGAGG TGTGGTGCGGG	2765	4466	4594
TE8		GTGCGGGTACAC TCCGGAGGAGG	2737	4438	4595
TE9	P5	TGCCGCCGCACCT CGAAGTGGTGG	2728	4429	4596
TE10		AGCAGAGCAAGC GCGAAGGTTGG	2768	4469	4597
TE11	P6	TGCAGCCCTCGG GAACCCCGGGG	4551	4564	4598
TE12		GTGGTGCGCCCG GACCAGCGCGG	2748	4449	4599
TE13	P7	TCACGCCGTGCA GTGGCGCGGGG	2732	4433	4600
TE14		GGTGCCGCCGGC CTTTGTGCTGG	2741	4442	4601
TE15	P8	GCACCGGGCGTC CAGCACAAAGG	2729	4430	4602
TE16		AGGGAATTAGCC CCCCGCACCGG	2730	4431	4603
TE17	P9	AGTGGCAGCGGC GAGAGCTTGGG	2773	4474	4604
TE18		ACTTGCATGCGA GCGGGACCCGG	2731	4432	4605
TE19	P10	ACTCAGCGGGGC CGGCGTCTCGG	4552	4565	4606
TE20		CCTTATGAATAT TGATGCGGAGG	2777	4478	4607

[0483] We then delivered these new pools individually to T cells together with the mRNAs encoding the triple ETR combination. The two genes were expressed at low levels and the detection of their protein products by flow cytometry was complicated by the nuclear localization of TET2 and the inducibility of TGFBR2. Thus, to quantify epi-silencing efficiencies, we used digital droplet PCR (ddPCR), a technique for measuring the expression profile of selected genes at high sensitivity. Finally, we analysed the cells at day 28 post-treatment. **Figure 27** shows the percentage of *TGFBR2* epi-silencing for each pair combination upon normalization to the levels of *TGFBR2* expression in mock-treated cells. This analysis shows that nearly all pair combinations were able to induce epi-silencing of *TGFBR2* at high efficiencies (≥60%). On the other hand, a similar analysis performed for *TET2* showed that epi-silencing of this gene was more variable, with some pair combinations displaying no activity while others being highly effective (**Figure 28**). In this regard, combinations containing either pair number 7 (P7) or number 10 (P10) were the most effective ones, leading to up to 92% of *TET2* reduction when coupled together.

[0484] With the aim of reducing to 2 the number of gRNAs required to silence each of these genes, we evaluated epi-silencing efficiency of selected gRNA pairs. In this regard, we chose pairs number 4 (gRNA IDs TG7_20 and TG8_20) and 10 (gRNA IDs TG19_20 and TG20_20) for *TGFBR2* and pairs number 7 (gRNAs TE13_20 and TE14_20) and 10 (gRNAs TE19_20 and TE20_20) for *TET2*. Co-delivery of these pairs individually together with the triple ETR combination followed by ddPCR analysis at day 22 post-treatment showed that pairs number 4 and number 10 were the most effective in promoting epi-silencing of *TGFBR2* and *TET2*, respectively, leading to up to 35% and 90% of reduction of the two transcripts (**Figure 29**). Of note, at variance with *TGFBR2* for which the pairs combination led to 89% reduction, for *TET2*, the epi-silencing efficiency of pair number 10 was comparable to those observed when delivering the parental pairs combination. Overall, these data show that *TET2* and *TGFBR2* can be efficiently silenced by the ETRs technology.

[0485] We then tested multiplexed epigenetic silencing of *B2M*, *TET2* and *TGFBR2*. To this end, we co-treated human primary T cells with: (i) the mRNA encoding for the ETR ZIM3:dCas9:DNMT3L fusion; (ii) the F4+H8+H10 combination of full-length gRNAs against *B2M* (i.e., gRNA IDs F4_20, H8_20, and H10_20); (iii) pair number 10 of full-length gRNAs against *TET2*; (iv) combination of pairs number 4 and 10 of full-length gRNAs against *TGFBR2*. We then measured the expression levels of these genes by ddPCR and found that they were all markedly downregulated, resulting in up to 47%, 92% and 67% of epi-silencing of *B2M*, *TET2* and *TGFBR2*, respectively (**Figure 30**). Overall, these data show that *B2M*, *TET2* and *TGFBR2* can be co-silenced by the ETRs technology.

EXAMPLE 6 – Poly-functional orthogonal editing of multiple genes with ETM without causing reciprocal chromosomal translocations in human primary T lymphocytes.

[0486] We decided to combine orthogonal editing of *B2M* and *TRAC* with epi-silencing of either *TGFBR2* or *TET2*. To this end, we first truncated the gRNAs against *TET2* and *TGFBR2* from **Figure 30** to 15nt in length. gRNAs with the truncated spacer are shown in the table below. We then co-transfected human primary T cells with the mRNA encoding for the ETM ZIM3:Cas9:DNMT3L fusion together with: (i) the truncated gRNAs against *B2M,* namely F4 (gRNA ID F4_16; 16nt-long spacer; gRNA SEQ ID NO: 4579), H8 (H8_15; 15nt-long spacer; gRNA SEQ ID NO: 4577) and H10 (H10_14; 14nt-long spacer; gRNA: SEQ ID NO: 4576); (ii) the full-length gRNA against *TRAC* (SEQ ID NO: 4575); (iii) the truncated gRNAs corresponding either to pair number 10 (TE19_15 and TE 20_15) for *TET2* or to pairs number 4 (TG7_15 and TG8_15) and 10 (TG19_15 and TG20_15) for *TGFBR2* (see **Table 4**; SEQ: SEQ ID NO). Cells were either transduced or not with the AAV6 donor template for targeted integration of the NY-ESO TCR into the *TRAC* locus. Treated T cells were then analysed by (i) flow cytometry to measure epigenetic silencing of *B2M* and genetic editing of *TRAC* (i.e., disruption or targeted integration of the NY-ESO TCR, according to the absence or not of the AAV6 donor) and (ii) ddPCR to quantify the expression levels of *TET2* and *TGFBR2*.

Table 4. Truncated *TGFBR2* and *TET2* gRNA Pairs

gRNA Spacer ID	Pair No.	gRNA ID	gRNA SEQ
TGFBR2 gRNAs			
TG7	P4	TG7_15	4584
TG8		TG8_15	4585
TG19	P10	TG19_15	4586
TG20		TG20_15	4587
TET2 gRNAs			
TE19	P10	TE19_15	4582
TE20		TE20_15	4583

[0487] Concerning the experimental conditions of poly-functional editing of *B2M*, *TRAC* and *TGFBR2* without the AAV6 donor, the analyses showed that ZIM3:Cas9:DNMT3L was able to induce up to 11% and 95% of cells negative for B2M and endogenous TCR, respectively (**Figure 31**). ddPCR analyses of bulk-treated cells showed that the expression levels of *TGFBR2* were markedly reduced in these samples, resulting up to 50% of epi-silencing (**Figure 31**). Concerning the samples treated with the AAV6 donor, we found that up to 16% of treated T cells were negative for B2M, while 59% and 26.7% turned negative for the endogenous TCR and positive for NY-ESO, respectively (**Figure 32**). In these cells, the epi-silencing efficiency of *TGFBR2* was 54% (**Figure 32**). Importantly, molecular analyses of treated T cells, either transduced or not with the AAV6 donor, did not show any sign of reciprocal chromosomal translocations among the three targeted genes (**Figure 33**). At variance with this latter data, experiments performed with ZIM3:Cas9:DNMT3L and full-length gRNAs against the investigated genes showed clear evidence of reciprocal chromosomal translocations among *B2M*, *TRAC* and *TGFBR2* (**Figure 33**).

[0488] Concerning the experimental conditions of poly-functional editing of *B2M*, *TRAC* and *TET2* without the AAV6 donor, the analyses showed that ZIM3:Cas9:DNMT3L was able to induce up to 46% and 99% of cells negative for B2M and endogenous TCR, respectively (**Figure 34**). ddPCR analyses of bulk-treated cells showed that the expression levels of *TET2* were markedly reduced in these samples, resulting up to 63% of epi-silencing (**Figure 34**). Concerning the samples treated with the AAV6 donor, we found that up to 40% of treated T cells were negative for B2M, while 53% and 29% turned negative for the endogenous TCR and positive for NY-ESO, respectively (**Figure 35**). In these cells, epi-silencing efficiency of *TET2* was 60% (**Figure 35**). Importantly, molecular analyses of treated T cells, either transduced or not with the AAV6 donor, did not show any sign of reciprocal chromosomal translocations among the three targeted genes (**Figure 36**). At variance with this latter data, experiments performed with ZIM3:Cas9:DNMT3L and full-length gRNAs against the investigated genes showed clear evidence of numerous reciprocal chromosomal translocations among *B2M*, *TRAC* and *TET2* (**Figure 36**).

[0489] Finally, we tested quadruple poly-functional editing of *B2M*, *TRAC*, *TGFBR2* and *TET2* using ZIM3:Cas9:DNMT3L, with or without the AAV6 donor. In this experiment we used truncated gRNAs for *B2M*, *TGFBR2* and *TET2* and the full-length gRNA for *TRAC*. In the conditions without the AAV6 donor, up to 5.7% and 93% of treated cells proved negative for B2M and the endogenous TCR, respectively (**Figure 37**). ddPCR analysis of these cells showed that the transcripts of *TGFBR2* and *TET2* were markedly reduced as compared to mock-treated samples, resulting in up to 70% and 71% of epi-silencing, respectively (**Figure 37**). Concerning the samples treated with the AAV6 donor, we found that up to 7% of treated T cells were negative for B2M, while 54% and 26% turned negative for the endogenous TCR and positive for NY-ESO, respectively (**Figure 38**). In these cells, the epi-silencing efficiencies of *TGFBR2* and *TET2* were 50% and 51%, respectively (**Figure 38**). Importantly, molecular analyses of treated T cells, either transduced or not with the AAV6 donor, did not show any sign of reciprocal chromosomal translocations among the three targeted genes (**Figure 39**). At variance with this latter data, experiments performed with ZIM3:Cas9:DNMT3L and full-length gRNAs against the investigated genes showed clear evidence of numerous reciprocal chromosomal translocations among *B2M*, *TRAC* and *TET2* (**Figure 39**).

[0490] Overall, these data show that Cas9-based ETRs (EMT) with truncated and full-length gRNAs can impose multiple orthogonal edits in T cells without inducing reciprocal chromosomal translocations.

[0491] Additional targets that may be silenced with epigenetic silencing include, for example: *A2AR*; *CISH*; *PTPN11*; *PTPN6*; *PTPA*; *PTPN2*; *JUNB*; *TOX*; *TOX2*; *NR4A1*; *NR4A2*; *NR4A3*; *MAP4K1*; *REL*; *IRF4*; *DGKA*; *PIK3CD*; *HLA-A*; *USP16*; *DCK* and *FAS*.

[0492] Epigenetic silencing of these targets may be coupled to gene editing of *TRAC*, *PD-1* and *CTLA4* genes that do not have CpG islands (CGIs).

CELL CULTURE CONDITIONS

[0493] Peripheral blood mononuclear cells (PBMCs) were freshly isolated from healthy donors using centrifugation on a Ficoll gradient (Lymphoprep™). CD3-positive lymphocytes were then purified by magnetic separation using Pan T cells isolation kit (Miltenyi Biotech), according to the manufacturer instructions. The purity of T lymphocytes was assessed by flow cytometry (FACSCanto™ II – BD Bioscience, Cytoflex – Beckman Coulter) using anti-CD3 (BD, 349201), CD4

(Biolegend, 317429) and -CD8 (Biolegend, 344708) antibodies. T lymphocytes were stimulated using anti-CD3/CD28 magnetic beads (Dynabeads human T-activator CD3/CD28, Thermo Fisher) in a 1:1 ratio and maintained in culture in RPMI (Corning) supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml), 2% glutamine, 10% FBS (Euroclone) and 5 ng/ml of each IL-7 and IL-15 (PeproTech). The K-562^{dTomato} reporter cell line was previously described (Amabile et al., *supra*) and maintained in culture in RPMI supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml), 2% glutamine and 10% FBS. All cells were cultured in a 5% CO_2 humidified atmosphere at 37°C.

mRNAs, gRNAs AND DONOR TEMPLATES

[0494] The gRNAs used in these studies were designed using CHOPCHOP (Labun et al., Nucleic Acids Res. (2019) 47(W1):W171-4). For T cell experiments, gRNAs were purchased highly chemically modified from IDT, including 2'-O-methyl residues and phosphorothioate modifications as previously described (Finn et al., Cell Rep (2018) 22(9):2227-35). mRNAs encoding for the ETRs, the Cas9-based ETRs and Cas9 were purchased from TriLink or produced in house using the MEGAscript™ T7 Transcription Kit (Invitrogen), according to the manufacturer instructions. In both cases, mRNAs were 5' capped using CleanCap® Reagent (TriLink) and UTP was completely substituted by N1-Methylpseudouridine-5'-Triphosphate (TriLink). In house produced mRNAs were also concentrated using Amicon® Ultra-15 Centrifugal Filter Unit (Sigma-Aldrich). The construct IG4 NY-ESO TCR alpha/beta with homology arms for the *TRAC* locus was obtained by Addgene (plasmid #112021) and cloned inside an AAV transfer construct containing AAV2 inverted terminal repeats. AAV6 was produced by TIGEM Vector Core by triple-transfection method and purified by ultracentrifugation. For the K-562^{dTomato} experiments, full-length or truncated gRNAs were cloned downstream the human U6 promoter as fusion transcripts with the tracrRNA (Amabile et al., *supra*). ETRs, Cas9-based ETRs and Cas9 sequences were cloned inside expression plasmids under the control of CMV promoter (Amabile et al., *supra*).

GENE EDITING PRODECURES

[0495] T cells were edited two days after purification. Dynabeads were removed prior to electroporation. 5 x 10^5 cells were electroporated with 1.5 µg (unless otherwise specified) of stabilized mRNA for each ETRs/Cas9-ETRs/Cas9 and 3 µg for each highly modified gRNA using the Lonza 4D-Nucleofector™ (P3 Primary Cell solution, EO-115 program). Immediately after nucleofection, 80 µl of RMPI were added directly to the cuvette and cells were incubated 15 minutes at 37°C. Cells were then moved in a 96-U bottom wells and 100 µl of complete 2X medium (RPMI with 20% FBS, 4 mM L-Glutamine, 2% P/S and 10 ng/ml of each IL-7 and IL-15) were added. In gene targeting experiments, AAV6 NY-ESO TCR was also added to the 2X medium at a dose of 10^5 vg/cell. Percentage of *B2M* negative cells was assessed by flow cytometry using an anti-B2M antibody (Biolegend, 316312) while NY-ESO/TCR positive events were assessed by using an anti-Vβ13.1 antibody (Beckman Coulter) or an anti-human TCR alpha/beta antibody (Biolegend). Complete fresh medium was added to the culture every third day. For the K-562^{dTomato} experiments, 5 x 10^5 cells were electroporated with 600 ng of each ETRs/Cas9-ETRs/Cas9 plasmid and 200 ng of the gRNA plasmid using the using the Lonza 4D-Nucleofector™ (SF Cell Line solution, FF-120 program). Immediately after nucleofection, cells were plated in 96-U bottom wells in complete RPMI. dTomato negative cells were analysed by flow cytometry. Cytofluorimetric analyses were performed using Flow Jo Software (FLOWJO, LLC).

MOLECULAR ANALYSIS

[0496] Genomic DNA from the cell line was extracted using Maxwell 16 LEV Blood DNA kit (Promega) for samples consisting of less than 2 x 10^6 cells. DNA from less than 5 x 10^5 cells was extracted using the QuickExtract™ DNA Extraction Solution (Epicentre). Genetic indels were detected by using Surveyor nuclease assay (Surveyor Mutation Kit, IDT), according to the manufacturer instructions. The following primers were used to measure mutations at the *B2M* locus:

Table 5. *B2M* Primers for Measurement of Mutations

Description	Sequence	SEQ ID NO
B2M (F4, C8) Forward	TACAGACAGCAAACTCACCCAGTC	4527

B2M (F4, C8) Reverse	AGAACTTGGAGAAGGGAAGTCACG	4528
B2M (H8) Forward	ATCTTCTGGGTTTCCGTTTTCT	4529
B2M (H8) Reverse	TCTCGTGATGTTTAAGAAGGCA	4530
B2M (H10) Forward	CGTGAGTCTCTCCTACCCTCC	4531
B2M (H10) Reverse	TTATCGACGCCCTAAACTTTGT	4532

The following primers were used to measure mutations on other loci of interest:

Table 6. Primers for Measurement of Mutations in Other Loci

Description	Sequence	SEQ ID NO
TRAC Forward	CCGTATAAAGCATGAGACCGTG	4533
TRAC Reverse	ATTCCTGAAGCAAGGAAACAGC	4534
TGFBR2 Forward	TCGGTCTATGACGAGCAGC	4535
TGFBR2 Reverse	GAAACTTTCCTCGTTTCCGC	4536
TET2 Forward	AACAAGGCAGTGCTAATGCCT	4537
TET2 Reverse	GCTTTGGAGGCAGCTCAGAG	4538

[0497] Translocation analyses were performed using GoTaq® DNA Polymerase (Promega) combining the forward and reverse primers listed above according to the gRNA employed in the experiment. Amplicons were run on a 1% agarose gel.
The following primers were used to detect genomic translocations of interest:

Table 7. Primers for Detection of Genomic Translocations

Description	Sequence	SEQ ID NO
B2M Forward	TACAGACAGCAAACTCACCCAGTC	4629
B2M Reverse	ACAAAGTTTAGGGCGTCGATAA	4630
TRAC Forward	CCGTATAAAGCATGAGACCGTG	4631
TRAC Reverse	ATTCCTGAAGCAAGGAAACAGC	4632
TGFBR2 Forward	CACGTTCAGAAGTCGGGTGAGT	4633
TGFBR2 Reverse	TCCAGGAGCTAAGGACTGAGGA	4634
TET2 Forward	TAATTCCCTGGGAGCCGGGG	4635
TET2 Reverse	TTGCTCCCCAGTCCCTGGAA	4636

[0498] For gene expression analysis, total RNA was extracted from 10^6 cells using the RNeasy Mini kit (QIAGEN) and reverse-transcribed using random hexamers according to the SuperScript III First-Strand Synthesis System (Invitrogen) manufacturer's instructions. Transcripts levels were determined by digital droplet PCR using from 0.2-10ng of template cDNA. The PCR reaction was carried out by adding 1X of TaqMan Gene Expression assays (Applied Biosystems) following manufacturer's instructions (Biorad), read with QX200 reader and analysed with

CA 03207166 2023-8-1

QuantaSoft software (Biorad). Data were normalized over HPRT and mock-treated samples. The reagents used are listed below:

B2M	Hs00187842_m1
TGFBR2	Hs00234253_m1
TET2	Hs00325999_m1
HPRT	Hs02800695_m1

CA 03207166 2023-8-1

LIST OF SEQUENCES

[0499] Sequences disclosed in the present disclosure are listed below.

Table 8. Sequence Description

SEQ ID NO	Description
1	ZNF10 KRAB domain
2	ZIM3 KRAB domain
3	ZNF350 KRAB domain
4	ZNF197 KRAB domain
5	RBAK KRAB domain
6	ZKSCAN1 KRAB domain
7	KRBOX4 KRAB domain
8	ZNF274 KRAB domain
9	DNMT3A catalytic domain
10	DNMT3B catalytic domain
11	DNMT3B
12	DNMT1 catalytic domain
13	DNMT3L
14	SETDB1
15	SETDB1 catalytic domain
16	Cas9 (catalytically active)
17	dCas9
18	exemplary ETM-KRAB
19	exemplary ETM-DNMT3A
20	exemplary ETM-ENMT3L
21	exemplary B2M target sequence, sense strand
22	exemplary B2M target sequence, antisense strand
23-45	B2M gRNA spacers 24: F4 25-34: F4 truncated from 5' end (19-10 nt sequences, respectively) 35: C8 36-45: C8 truncated from 5' end (19-10 nt sequences, respectively)
46	*TRAC* gRNA spacer
47-96	*TRAC* target sequences
97-146	*TRBC1* target sequences
147-196	*TRBC2* target sequences
197-246	*PDCD1* target sequences
247-296	*TIM-3/HAVCR2* target sequences
297-346	*TIGIT* target sequences
347-396	*LAG3* target sequences
397-446	*CTLA4* target sequences
447-511	*AAVS1* target sequences
512-561	*CCR5* target sequences
562-611	*TRAC* gRNA spacers

CA 03207166 2023-8-1

612-661	*TRBC1* gRNA spacers
662-711	*TRBC2* gRNA spacers
712-761	*PDCD1* gRNA spacers
762-811	*TIM-3/HAVCR2* gRNA spacers
812-861	*TIGIT* gRNA spacers
862-911	*LAG3* gRNA spacers
912-961	*CTLA4* gRNA spacers
962-1026	*AAVS*1 gRNA spacers
1027-1076	*CCR5* gRNA spacers
1077-1177	*B2M* target sequences
1178-1227	*HLA-A* target sequences
1228-1277	*TGFBR2* target sequences
1278-1377	*A2AR* target sequences
1378-1427	*FAS* target sequences
1428-1477	*DCK* target sequences
1478-1527	*DGKA* target sequences
1528-1577	*USP16* target sequences
1578-1627	*PTPN11* target sequences
1628-1677	*PTPN6* target sequences
1678-1727	*PTPA* target sequences
1728-1777	*PTPN2* target sequences
1778-1827	*CISH* target sequences
1828-1927	*PI3KCD.1* target sequences
1928-1977	*MAP4K1* target sequences
1978-2027	*NR4A1* target sequences
2028-2127	*NR4A2* target sequences
2128-2277	*NR4A3* target sequences
2278-2377	*JUNB* target sequences
2378-2427	*REL* target sequences
2428-2527	*TOX* target sequences
2528-2627	*TOX2* target sequences
2628-2727	*IRF4* target sequences
2728-2777	*TET2* target sequences
2778-2878	*B2M* gRNA spacers 2778: H11 2780: H8 2801: H12 2813: C8 2863: H10 2878: F4
2879-2928	*HLA-A* gRNA spacers
2929-2978	*TGFBR2* gRNA spacers
2979-3078	*A2AR* gRNA spacers
3079-3128	*FAS* gRNA spacers
3129-3178	*DCK* gRNA spacers
3179-3228	*DGKA* gRNA spacers
3229-3278	*USP16* gRNA spacers
3279-3328	*PTPN11* gRNA spacers

3329-3378	*PTPN6* gRNA spacers
3379-3428	*PTPA* gRNA spacers
3429-3478	*PTPN2* gRNA spacers
3479-3528	*CISH* gRNA spacers
3529-3628	*PI3KCD.1* gRNA spacers
3629-3678	*MAP4K1* gRNA spacers
3679-3728	*NR4A1* gRNA spacers
3729-3828	*NR4A2* gRNA spacers
3829-3978	*NR4A3* gRNA spacers
3979-4078	*JUNB* gRNA spacers
4079-4128	*REL* gRNA spacers
4129-4228	*TOX* gRNA spacers
4229-4328	*TOX2* gRNA spacers
4329-4428	*IRF4* gRNA spacers
4429-4478	*TET2* gRNA spacers
4479	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 24)
4480	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 35)
4481	ZIM:dCas9:DNMT3L
4482	ZIM:Cas9:DNMT3L
4483	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 2780)
4484-4493	H8 truncated from 5' end (19-10 nt sequences, respectively)
4494	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 2863)
4495-4504	H10 truncated from 5' end (19-10 nt sequences, respectively)
4505	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 2778)
4506-4515	H11 truncated from 5' end (19-10 nt sequences, respectively)
4516	Example of *B2M* gRNA designed for epigenetic editing (comprises spacer of SEQ ID NO: 2801)
4517-4526	H12 truncated from 5' end (19-10 nt sequences, respectively)
4527	*B2M* (F4, C8) forward primer
4528	*B2M* (F4, C8) reverse primer
4529	*B2M* (H8) forward primer
4530	*B2M* (H8) reverse primer
4531	*B2M* (H10) forward primer
4532	*B2M* (H10) reverse primer
4533	*TRAC* forward primer
4534	*TRAC* reverse primer
4535	*TGFBR2* forward primer
4536	*TGFBR2* reverse primer
4537	*TET2* forward primer
4538	*TET2* reverse primer
4539-4545	*TGFBR2* target sequences
4546-4552	*TET2* target sequences
4553-4559	*TGFBR2* gRNA spacers

4560-4565	*TET2* gRNA spacers
4566-4567	Examples of tracr sequences
4568-4573	Examples of *B2M* gRNAs (comprise spacers of SEQ ID NOs: 24, 35, 2780, 2863, 2778, and 2801, respectively)
4574-4575	Exemplary gRNAs targeting *TRAC*
4576-4579	Exemplary full-length modified gRNAs targeting *B2M*
4580-4583	Exemplary truncated modified gRNAs targeting *TET2*
4584-4587	Exemplary truncated modified gRNAs targeting *TGFBR2*
4588-4607	Exemplary full-length modified gRNAs targeting *TET2*
4608-4627	Exemplary full-length modified gRNAs targeting *TGFBR2*
4628	Exemplary full-length modified gRNA targeting GFP
4629	*B2M* forward primer for translocation assessment
4630	B2M reverse primer for translocation assessment
4631	*TRAC* forward primer for translocation assessment
4632	*TRAC* reverse primer for translocation assessment
4633	*TGFBR2* forward primer for translocation assessment
4634	*TGFBR2* reverse primer for translocation assessment
4635	*TET2* forward primer for translocation assessment
4636	*TET2* reverse primer for translocation assessment
4637	Alternative ZIM3 KRAB domain
4638	Exemplary chemically modified gRNA

CLAIMS

1. A combination for modifying transcription, expression and/or activity of one or more gene in a cell, the combination comprising:

(A) one or more fusion proteins each comprising a catalytically active CRISPR/Cas endonuclease domain, wherein the one or more fusion proteins collectively comprise a transcriptional repressor domain and a DNMT3L domain, or polynucleotide(s) encoding the one or more fusion proteins;

(B) one or more guide RNAs (gRNAs) having a spacer sequence with a length that allows epigenetic editing and not gene editing of a first gene in the cell, wherein the first gene comprises a CpG island (CGI), or polynucleotide(s) coding for the one or more gRNAs; and

(C) one or more gRNAs having a spacer sequence with a length that allows gene editing of a second gene in the cell, or polynucleotide(s) coding for the one or more gRNAs.

2. The combination of claim 1, wherein:

(a) the one or more gRNAs in (B) has a spacer sequence of 11 to 17 nucleotides; and/or

(b) the one or more gRNAs in (C) has a spacer sequence of 18 to 21 nucleotides.

3. The combination of claim 1 or 2, wherein the combination comprises one to three fusion proteins.

4. The combination of any one of claims 1-3, further comprising a donor DNA comprising 5' and 3' arms that are homologous to sequences in the second gene.

5. The combination of any one of claims 1-4, wherein the endonuclease domain is derived from a Cas9 protein, optionally SpCas9.

6. The combination of any one of claims 1-5, wherein

the first gene is selected from *B2M*, *TET2*, *TGFBR2*, *A2AR*, *CISH*, *PTPN11*, *PTPN6*, *PTPA*, *PTPN2*, *JUNB*, *TOX*, *TOX2*, *NR4A1*, *NR4A2*, *NR4A3*, *MAP4K1*, *REL*, *IRF4*, *DGKA*, *PIK3CD*, *HLA-A*, *USP16*, *DCK*, and *FAS*; and/or

the second gene is selected from *TRAC*, *TRBC*, *PDCD1*, *TIM-3*, *TIGIT*, *LAG3*, *CTLA4*, *AAVS1*, and *CCR5*.

7. The combination of claim 6, wherein the second gene is a *TRAC* gene, optionally wherein the one or more gRNAs targeting the *TRAC* gene comprise a spacer having the sequence of one of SEQ ID NOs: 562-611.

8. The combination of any one of claims 1-7, wherein the first gene is a *B2M* gene, optionally wherein the one or more gRNAs targeting the *B2M* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 28-33 and 39-44, or the sequence of one of SEQ ID NOs: 2778-2878 with a 3 to 9 nucleotide truncation at the 5' end.

9. The combination of any one of claims 1-7, wherein the first gene is a *TGFBR2* gene, optionally wherein the one or more gRNAs targeting the *TGFBR2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 2929-2978 and 4553-4559 with a 3 to 9 nucleotide truncation at the 5' end.

10. The combination of any one of claims 1-7, wherein the first gene is a *TET2* gene, optionally wherein the one or more gRNAs targeting the *TET2* gene each comprise a spacer having the sequence of one of SEQ ID NOs: 4429-4478 and 4560-4565 with a 3 to 9 nucleotide truncation at the 5' end.

11. The combination of any one of claims 1-10, wherein the cell is a mammalian cell, optionally a human cell, further optionally wherein the cell is a human immune cell, or a human T cell.

12. A combination for regulating one or more gene in a human cell, optionally an immune cell or a T cell, the combination comprising:

one to three fusion proteins each comprising a catalytically inactive Cas9, optionally SpCas9, endonuclease domain, wherein the one to three fusion proteins collectively comprise a transcriptional repressor and a DNMT3L domain, or polynucleotide(s) encoding the one to three fusion proteins, wherein the gene comprises a CpG island (CGI) and is

(i) a *B2M* gene and the combination further comprises two or more gRNAs each comprising a spacer having the sequence of one of SEQ ID NOs: 2778-2878 optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNAs;

(ii) a *TGFBR2* gene and the combination further comprises a gRNA that comprises a spacer having the sequence of any one of SEQ ID NOs: 2929-2978 and 4553-4559 optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNA; or

(iii) a *TET2* gene and the combination further comprises a gRNA that comprises a spacer having the sequence of any one of SEQ ID NOs: 4429-4478 and 4560-4565 optionally with a 1 to 9 nucleotide truncation at the 5' end, or comprises polynucleotide(s) coding for the gRNA.

13. The combination of any one of claims 1-12, wherein the gene comprising a CGI is a *B2M* gene and the gRNAs targeting it are two or three gRNAs each independently comprising a spacer having the sequence of

C8 (SEQ ID NO: 35),
F4 (SEQ ID NO: 24),
H8 (SEQ ID NO: 2780),
H10 (SEQ ID NO: 2863),
H11 (SEQ ID NO: 2778), or
H12 (SEQ ID NO: 2801),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

14. The combination of claim 13, wherein the *B2M*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end,

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(ii) a gRNA comprising a spacer having the sequence of C8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end,

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(iii) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end;

(iv) a gRNA comprising a spacer having the sequence of F4 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(v) a gRNA comprising a spacer having the sequence of H8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of H10 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

15. The combination on of any one of claims 1-12, wherein the gene comprising a CGI is a *TGFBR2* gene and the combination comprises one or more gRNAs targeting it, or coding sequences of the one or more gRNAs, the one or more gRNAs each independently comprising a spacer having the sequence of

TG1 (SEQ ID NO: 4553),

TG2 (SEQ ID NO: 4554),

TG3 (SEQ ID NO: 4555),

CA 03207166 2023-8-1

TG4 (SEQ ID NO: 4556),
TG5 (SEQ ID NO: 4557),
TG6 (SEQ ID NO: 2940),
TG7 (SEQ ID NO: 2937),
TG8 (SEQ ID NO: 2930),
TG9 (SEQ ID NO: 2955),
TG10 (SEQ ID NO: 4558),
TG11 (SEQ ID NO: 2957),
TG12 (SEQ ID NO: 2929),
TG13 (SEQ ID NO: 4559),
TG14 (SEQ ID NO: 2945),
TG15 (SEQ ID NO: 2931),
TG16 (SEQ ID NO: 2942),
TG17 (SEQ ID NO: 2939),
TG18 (SEQ ID NO: 2935),
TG19 (SEQ ID NO: 2938), or
TG20 (SEQ ID NO: 2932),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

16. The combination of claim 15, wherein the *TGFBR2*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of TG7 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TG8 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(ii) a gRNA comprising a spacer having the sequence of TG19 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TG20 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

17. The combination of any one of claims 1-12, wherein the gene comprising a CGI is a *TET2* gene and the combination comprises one or more gRNAs targeting it, or coding sequences of the one or more gRNAs, the one or more gRNAs each independently comprising a spacer having the sequence of

TE1 (SEQ ID NO: 4560),

TE2 (SEQ ID NO: 4561),
TE3 (SEQ ID NO: 4562),
TE4 (SEQ ID NO: 4563),
TE5 (SEQ ID NO: 4443),
TE6 (SEQ ID NO: 4434),
TE7 (SEQ ID NO: 4466),
TE8 (SEQ ID NO: 4438),
TE9 (SEQ ID NO: 4429),
TE10 (SEQ ID NO: 4469),
TE11 (SEQ ID NO: 4564),
TE12 (SEQ ID NO: 4449),
TE13 (SEQ ID NO: 4433),
TE14 (SEQ ID NO: 4442),
TE15 (SEQ ID NO: 4430),
TE16 (SEQ ID NO: 4431),
TE17 (SEQ ID NO: 4474),
TE18 (SEQ ID NO: 4432),
TE19 (SEQ ID NO: 4565), or
TE20 (SEQ ID NO: 4478),

optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

18. The combination of claim 17, wherein the *TET2*-targeting gRNAs comprise

(i) a gRNA comprising a spacer having the sequence of TE13 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TE14 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end; or

(ii) a gRNA comprising a spacer having the sequence of TE19 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end, and

a gRNA comprising a spacer having the sequence of TE20 optionally with a 1 to 9, optionally 3 to 9, nucleotide truncation at the 5' end.

19. The combination of any one of claims 1-18, further comprising gRNAs for targeting one or more additional genes in the cell, optionally wherein the combination

comprises gRNAs targeting the following genes, or comprises polynucleotides coding for the gRNAs:

(i) *B2M* and *TRAC*,

(ii) *B2M*, *TRAC*, and *TGFBR2*,

(iii) *B2M*, *TRAC*, and *TET2*,

(iv) *B2M*, *TGFBR2*, and *TET2*, or

(v) *B2M*, *TGFBR2*, *TET2*, and *TRAC*

20. The combination of any one of claims 1-19, wherein the one or more fusion proteins collectively further comprise a DNMT1, DNMT3A, DNMT3B, or SETDB1 domain, optionally DNMT3A.

21. The combination of any one of claims 1-20, wherein the combination comprises

(i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, and a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, or

(ii) a fusion protein comprising, optionally from N-terminus to C-terminus, a transcriptional repressor domain, a Cas endonuclease domain, and a DNMT3L domain.

22. The combination of any one of claims 1-20, wherein the combination comprises

(i) a first fusion protein comprising a transcriptional repressor domain and a Cas endonuclease domain, a second fusion protein comprising a DNMT3L domain and a Cas endonuclease domain, and a third fusion protein comprising a DNMT3A domain and a Cas endonuclease domain, or

(ii) a fusion protein comprising a transcriptional repressor domain, a Cas endonuclease domain, a DNMT3L domain, and a DNMT3A domain.

23. The combination of any one of claims 1-22, wherein the transcriptional repressor domain is a Krüppel-associated box (KRAB) domain, optionally derived from human Kox1 or ZIM3.

24. The combination of any one of claims 12-23, wherein the combination comprises a fusion protein comprising, optionally from N terminus to C terminus, a KRAB domain derived from ZIM3, a dCas9 domain, and a DNMT3L domain, optionally comprising an amino acid sequence of SEQ ID NO: 4481.

25. The combination of any one of claims 1-11 and 13-23, wherein the combination comprises a fusion protein comprising, optionally from N terminus to C terminus, a KRAB domain derived from ZIM3, a catalytically active Cas9 domain, and a DNMT3L domain, optionally comprising an amino acid sequence of SEQ ID NO: 4482.

26. The combination of any one of the claims 1-25, wherein the gRNA(s) are chemically modified, optionally wherein the chemically modified gRNA(s) comprise phosphorothioate internucleoside linkages at the 5' and/or 3' ends, and/or 2'-O-methyl nucleotides.

27. A pharmaceutical composition comprising the combination of any one of claims 1-26.

28. A cell comprising the combination of any one of claims 1-26, or a progeny of the cell.

29. A method of modifying transcription, expression and/or activity of one or more gene in a human T cell, comprising introducing the combination of any one of claim 1-26 into the cell *in vitro* or *ex vivo*.

30. A cell obtained by the method of claim 29, or a progeny of the cell.

31. The cell of claim 28 or 30, wherein the cell is a human T cell, optionally engineered to express a recombinant antigen receptor, optionally selected from a recombinant T cell receptor (TCR) or a chimeric antigen receptor (CAR).

32. The cell of claim 28, 30, or 31 for use in treating a human in need thereof.

33. Use of the cell of claim 28, 30, or 31 in the manufacture of medicament for treating a human in need thereof.

34. A method of treating a human in need thereof, comprising administering to the human the cell of claim 28, 30, or 31.

35. The cell for use according to claim 32, the use of claim 33, or the method of claim 34, wherein the cell is allogeneic or autologous to the human.

CA 03207166 2023-8-1

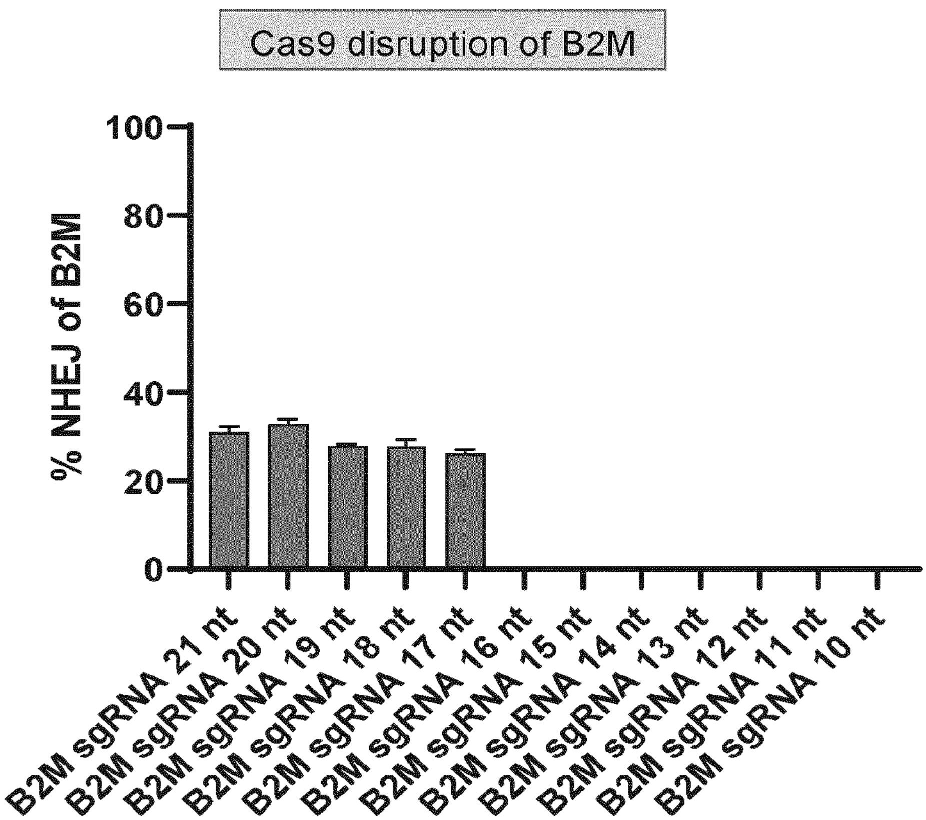
A. B2M target site

```
AGGGTAGGAGAGACTCACGCTGG
|||||||||||||||||||||||
TCCCATCCTCTCTGAGTGCGACC
```

B. B2M sgRNA

```
TAGGGTAGGAGAGACTCACGC -21-nt
 AGGGTAGGAGAGACTCACGC -20-nt
  GGGTAGGAGAGACTCACGC -19-nt
   GGTAGGAGAGACTCACGC -18-nt
    GTAGGAGAGACTCACGC -17-nt
     TAGGAGAGACTCACGC -16-nt
      AGGAGAGACTCACGC -15-nt
       GGAGAGACTCACGC -14-nt
        GAGAGACTCACGC -13-nt
         AGAGACTCACGC -12-nt
          GAGACTCACGC -11-nt
           AGACTCACGC -10-nt
```

FIGURE 1



Cas9 disruption of B2M
% NHEJ of B2M
100
80
60
40
20
0
B2M sgRNA 21 nt
B2M sgRNA 20 nt
B2M sgRNA 19 nt
B2M sgRNA 18 nt
B2M sgRNA 17 nt
B2M sgRNA 16 nt
B2M sgRNA 15 nt
B2M sgRNA 14 nt
B2M sgRNA 13 nt
B2M sgRNA 12 nt
B2M sgRNA 11 nt
B2M sgRNA 10 nt

FIGURE 2

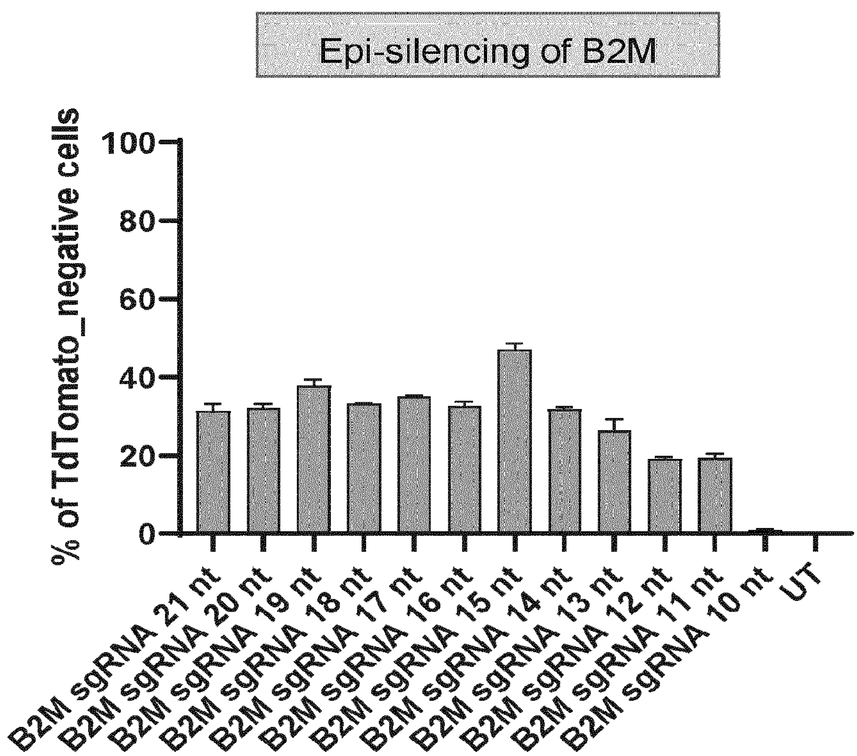

Epi-silencing of B2M
% of TdTomato_negative cells
100
80
60
40
20
0
B2M sgRNA 21 nt
B2M sgRNA 20 nt
B2M sgRNA 19 nt
B2M sgRNA 18 nt
B2M sgRNA 17 nt
B2M sgRNA 16 nt
B2M sgRNA 15 nt
B2M sgRNA 14 nt
B2M sgRNA 13 nt
B2M sgRNA 12 nt
B2M sgRNA 11 nt
B2M sgRNA 10 nt
UT

FIGURE 3

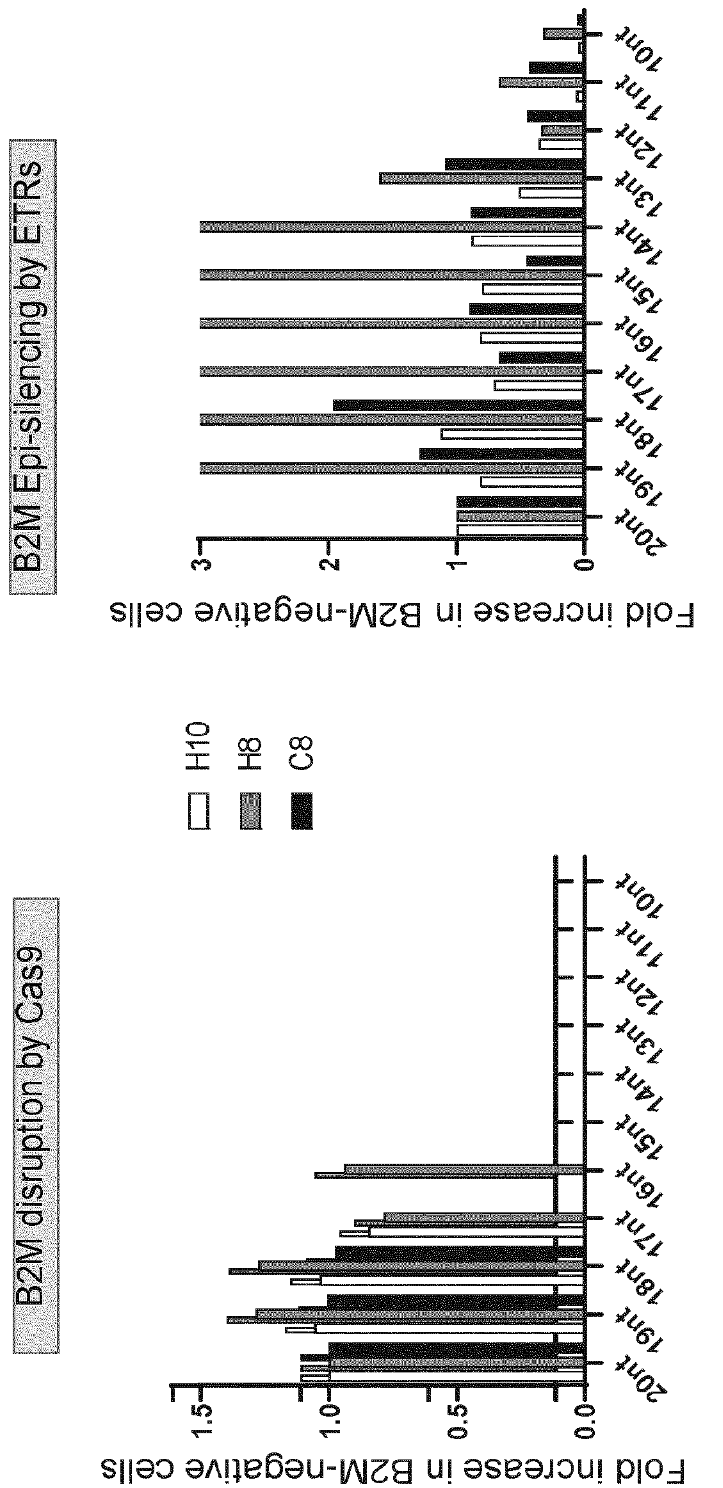

B2M Epi-silencing by ETRs
Fold increase in B2M-negative cells
3
2
1
0
20nt
19nt
18nt
17nt
16nt
15nt
14nt
13nt
12nt
11nt
10nt
H10
H8
C8
B2M disruption by Cas9
Fold increase in B2M-negative cells
1.5
1.0
0.5
0.0

FIGURE 4

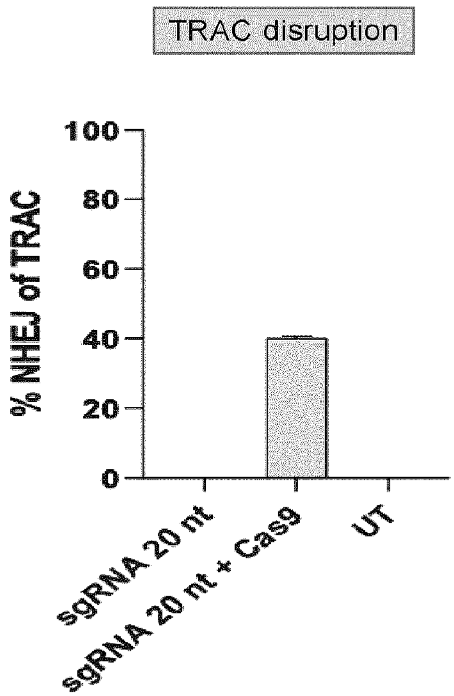


FIGURE 5

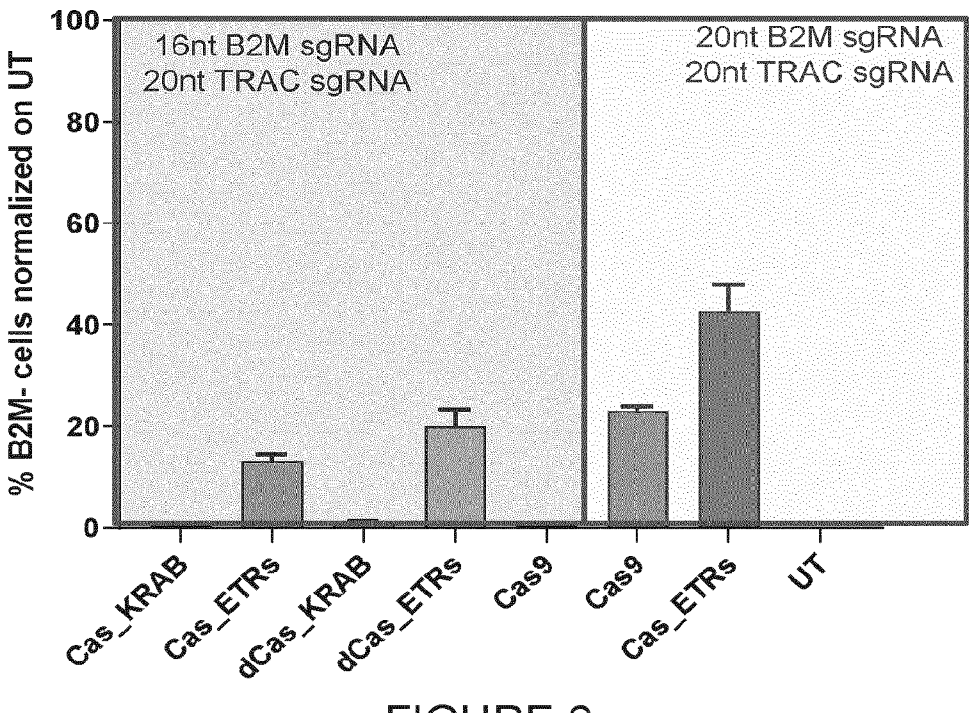


FIGURE 6

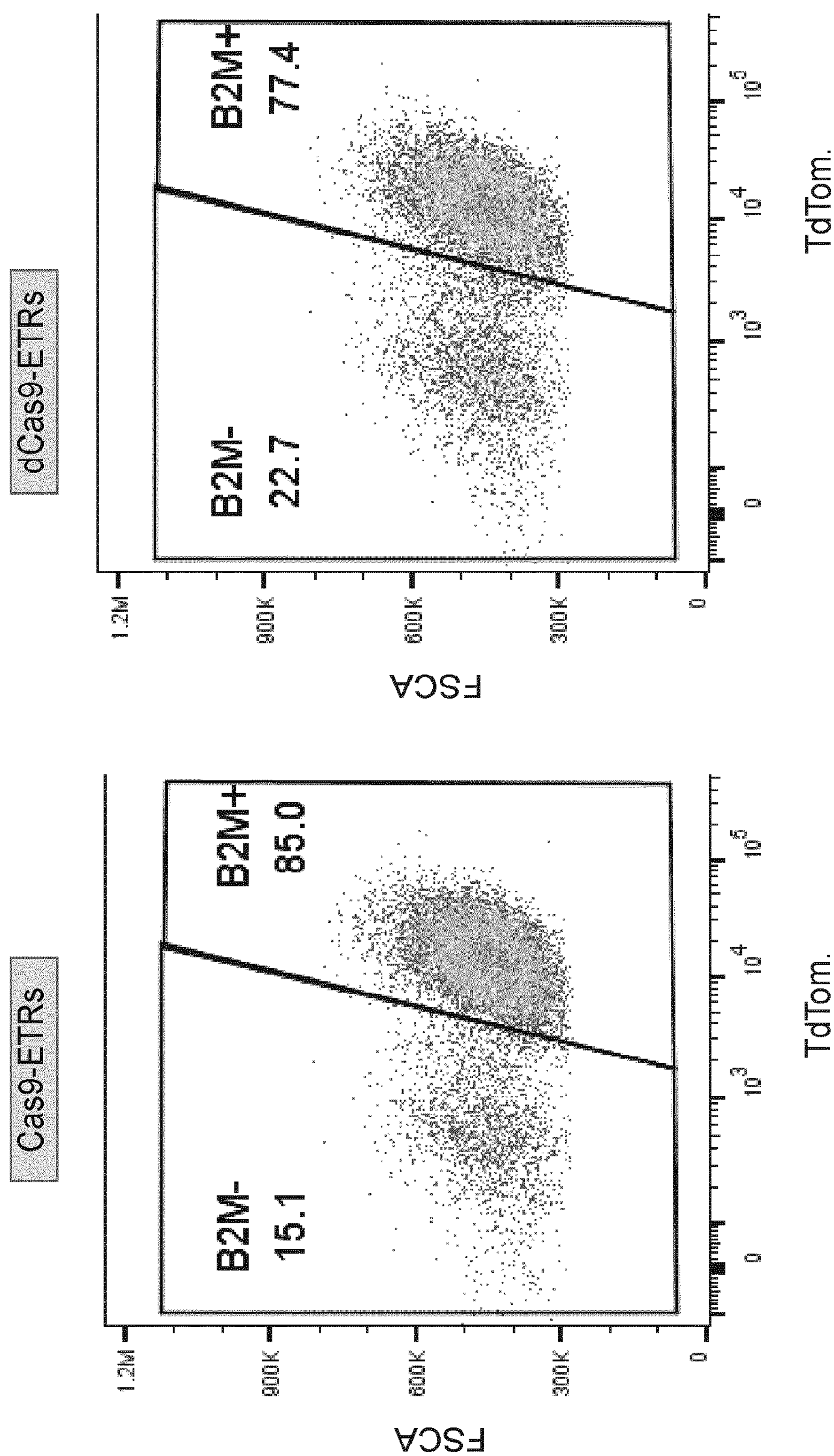

Cas9-ETRs
B2M-
15.1
B2M+
85.0
FSCA
TdTom.
dCas9-ETRs
B2M-
22.7
B2M+
77.4
FSCA
TdTom.
1.2M
900K
600K
300K
0

FIGURE 7

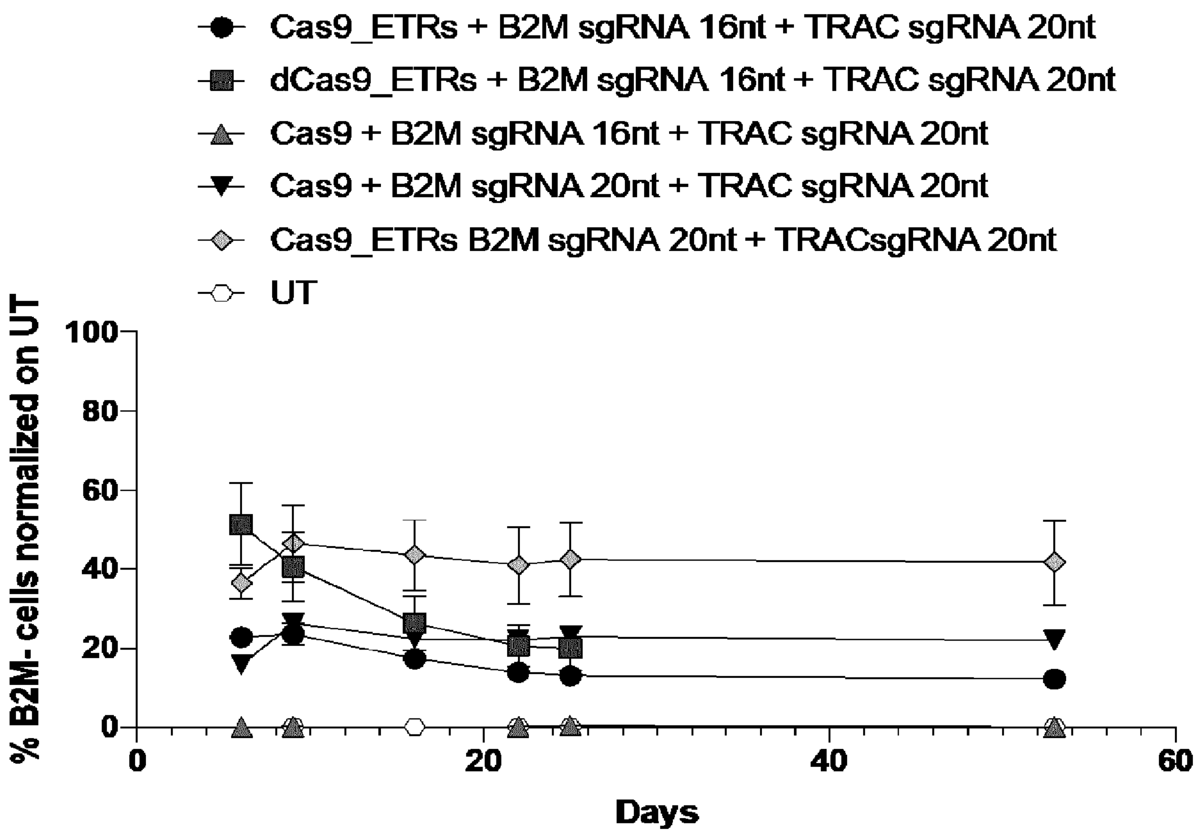
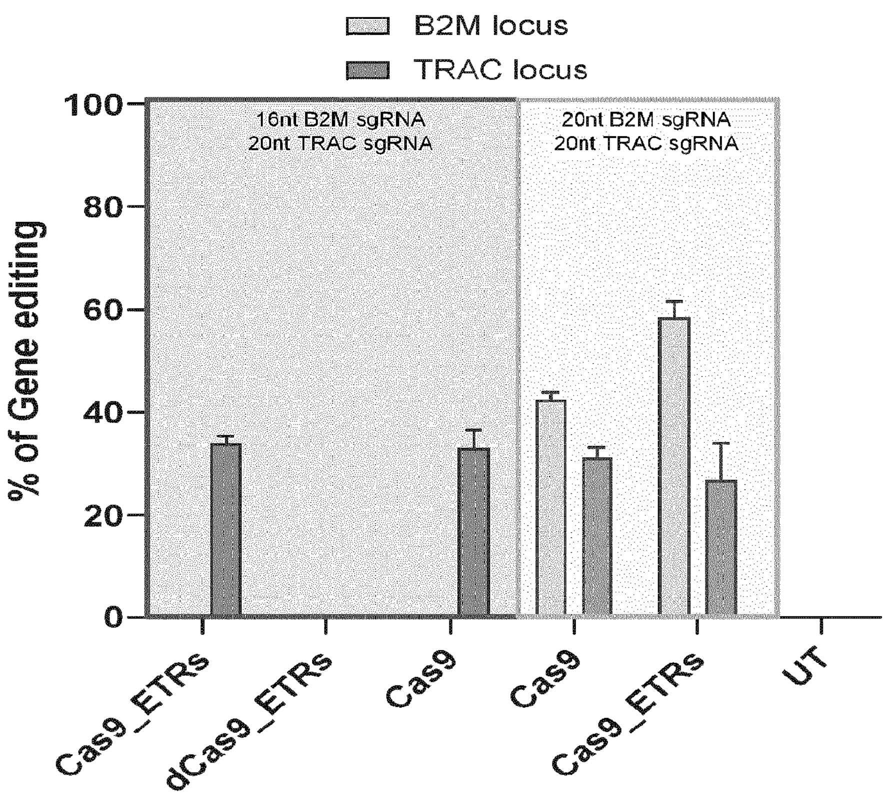
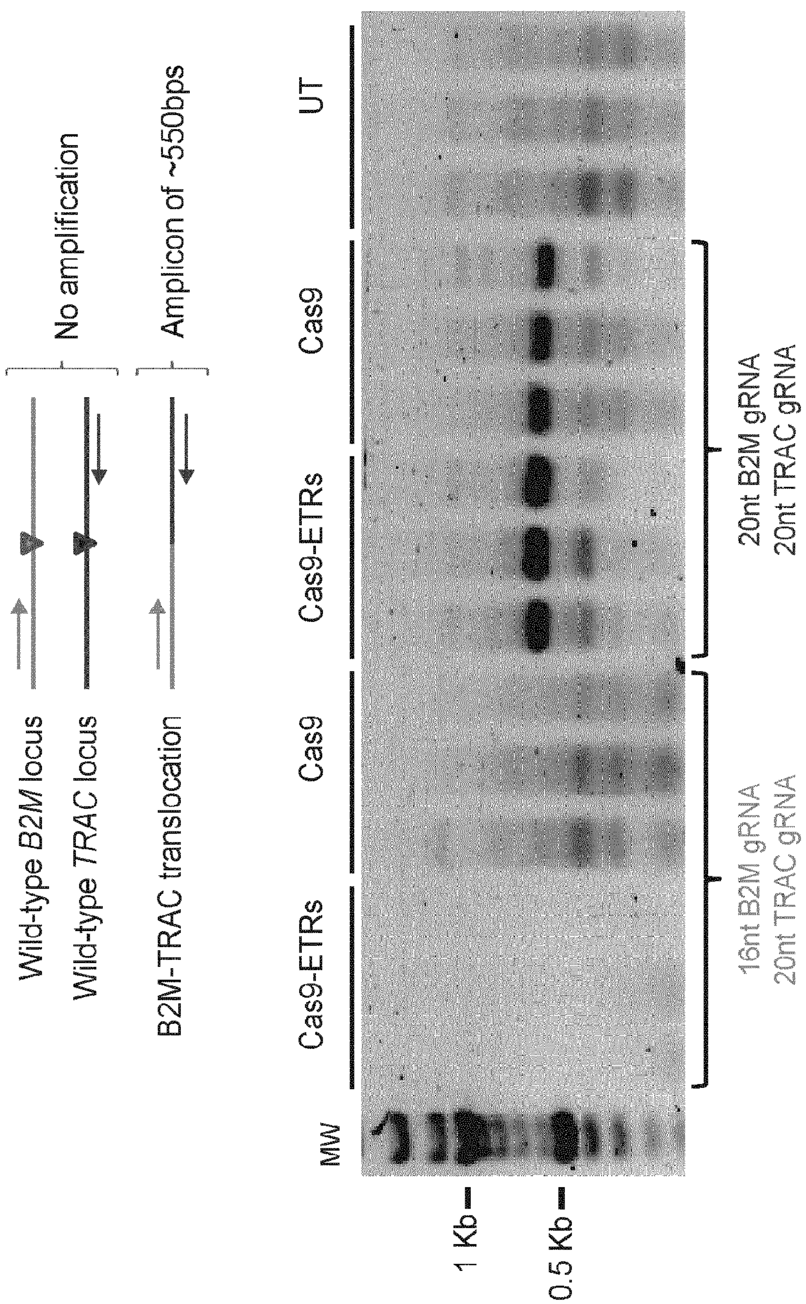

Cas9_ETRs + B2M sgRNA 16nt + TRAC sgRNA 20nt
dCas9_ETRs + B2M sgRNA 16nt + TRAC sgRNA 20nt
Cas9 + B2M sgRNA 16nt + TRAC sgRNA 20nt
Cas9 + B2M sgRNA 20nt + TRAC sgRNA 20nt
Cas9_ETRs B2M sgRNA 20nt + TRACsgRNA 20nt
UT
% B2M- cells normalized on UT
100
80
60
40
20
0
0
20
40
60
Days

FIGURE 8



B2M locus
TRAC locus
% of Gene editing
100
80
60
40
20
0
16nt B2M sgRNA
20nt TRAC sgRNA
20nt B2M sgRNA
20nt TRAC sgRNA
Cas9_ETRs
dCas9_ETRs
Cas9
Cas9
Cas9_ETRs
UT

FIGURE 9



Wild-type B2M locus
Wild-type TRAC locus
B2M-TRAC translocation
No amplification
Amplicon of ~550bps
MW
Cas9-ETRs
Cas9
Cas9-ETRs
Cas9
UT
1 Kb
0.5 Kb
16nt B2M gRNA
20nt TRAC gRNA
20nt B2M gRNA
20nt TRAC gRNA

FIGURE 10

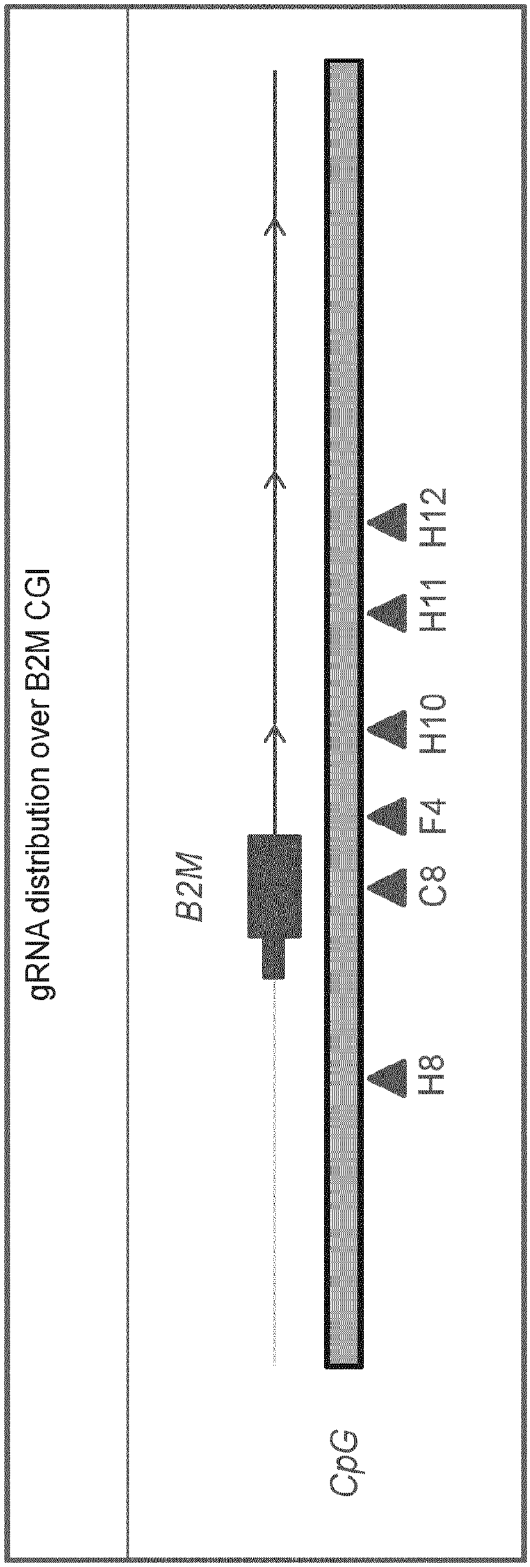
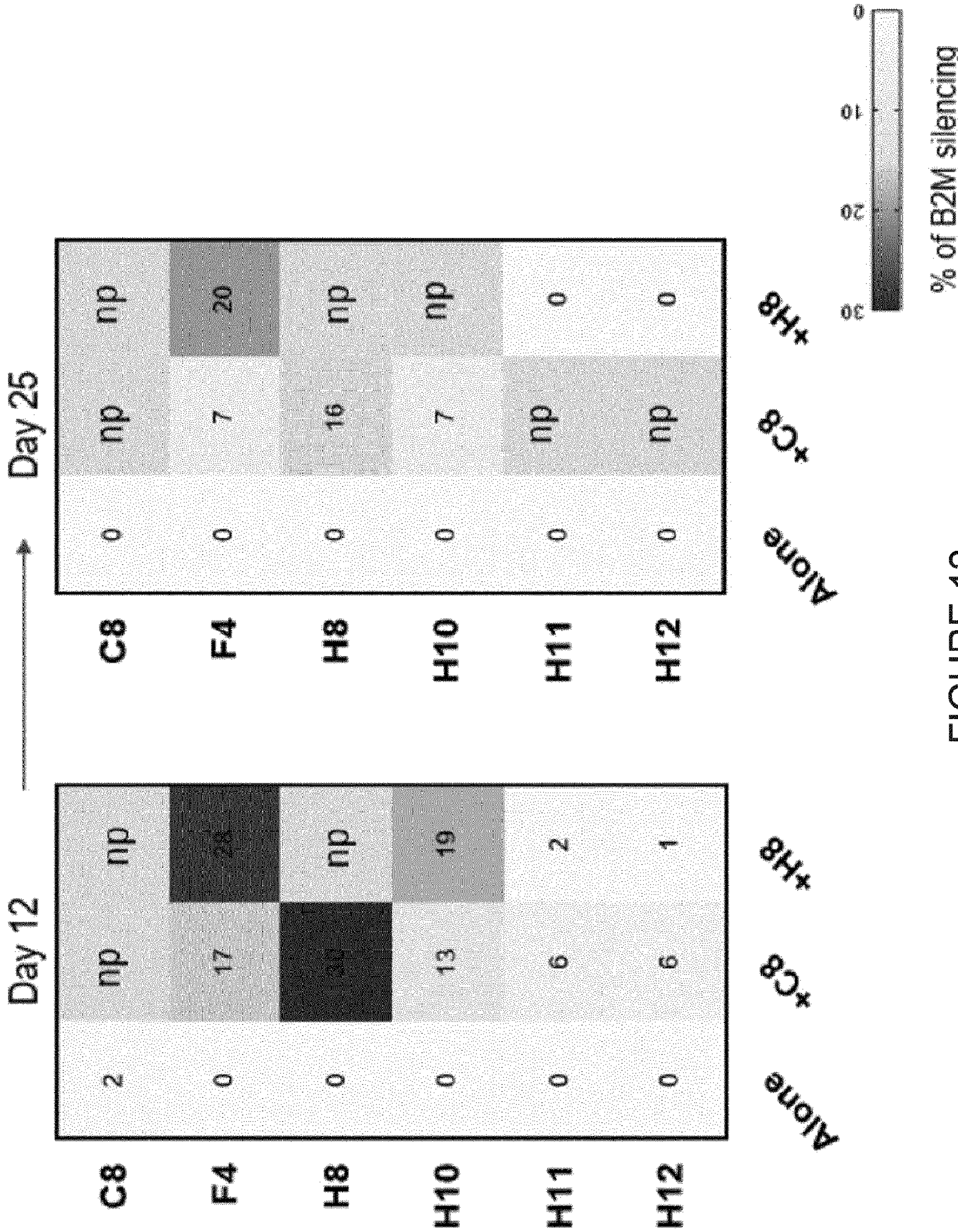


FIGURE 11



Day 12
Day 25
C8
F4
H8
H10
H11
H12
Alone
+C8
+H8
2 np np
0 17 28
0 30 np
0 13 19
0 6 2
0 6 1
0 np np
0 7 20
0 16 np
0 7 np
0 np 0
0 np 0
% of B2M silencing
0
10
20
30

FIGURE 12

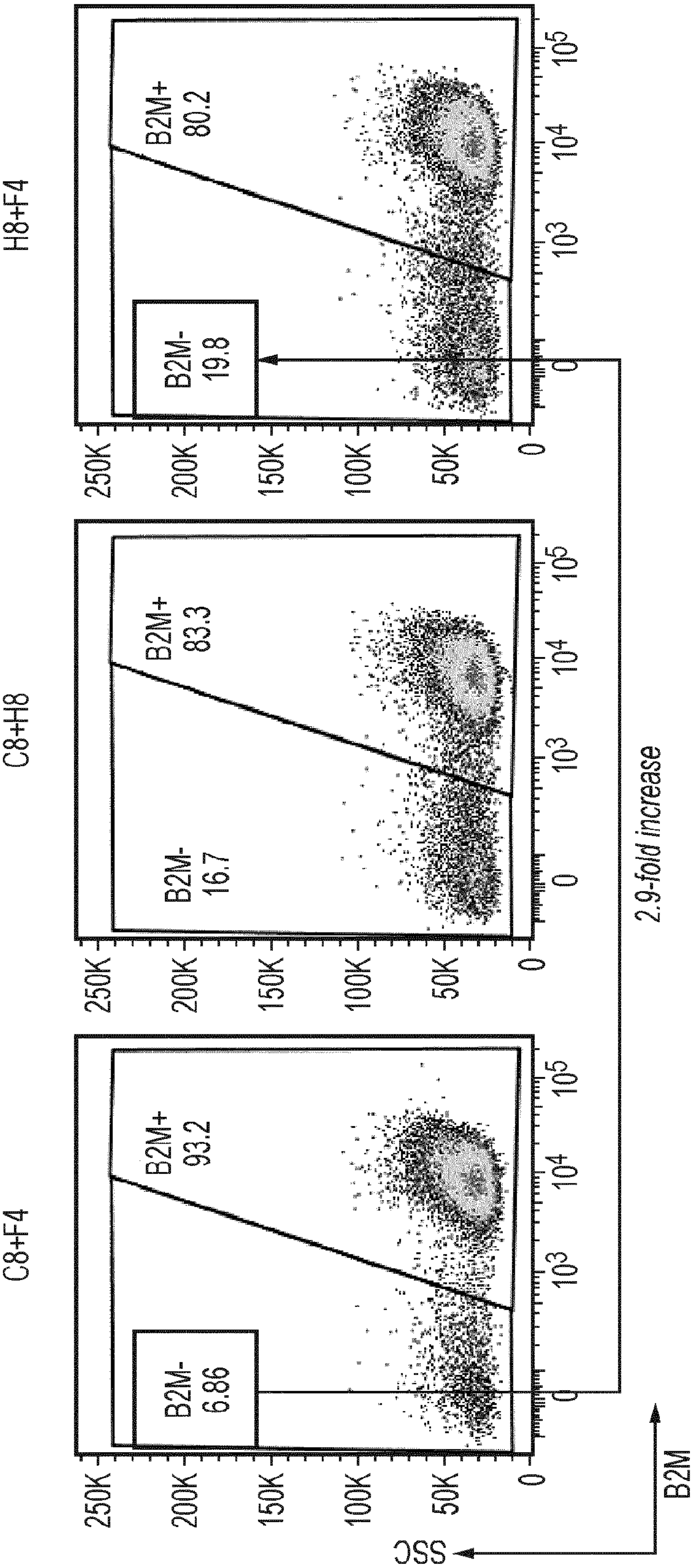

C8+F4
C8+H8
H8+F4
B2M+
93.2
B2M-
6.86
B2M+
83.3
B2M-
16.7
B2M+
80.2
B2M-
19.8
2.9-fold increase
SSC
B2M

FIGURE 13

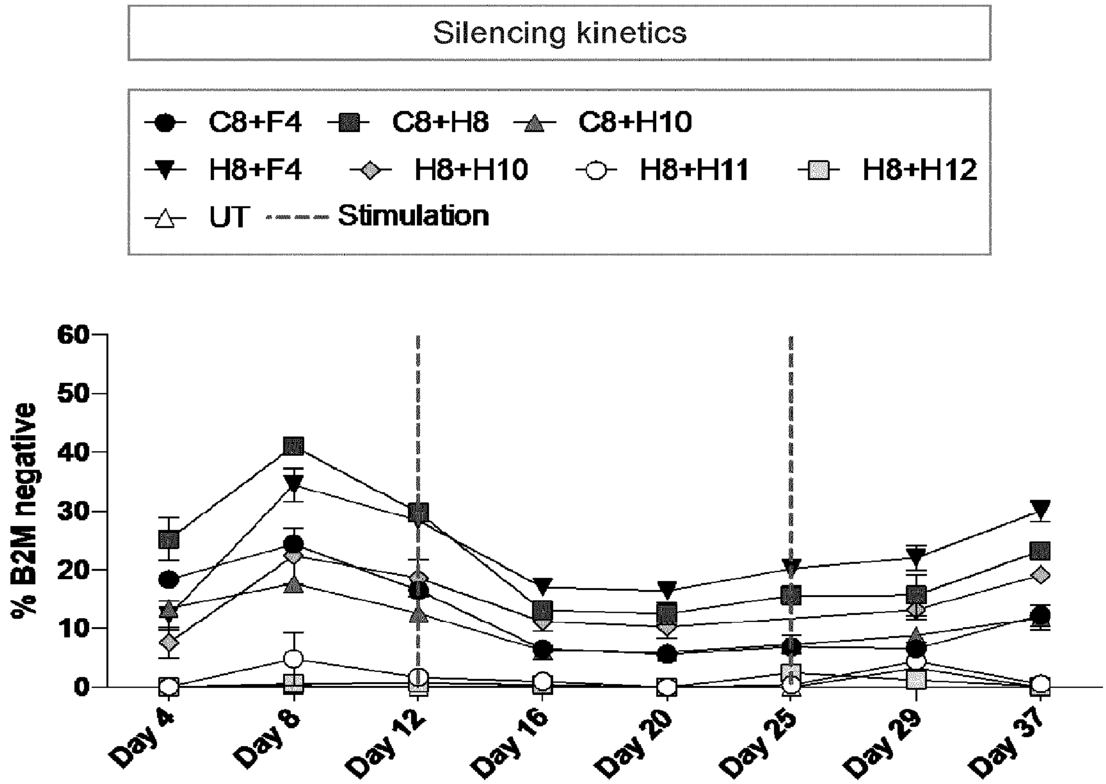


FIGURE 14

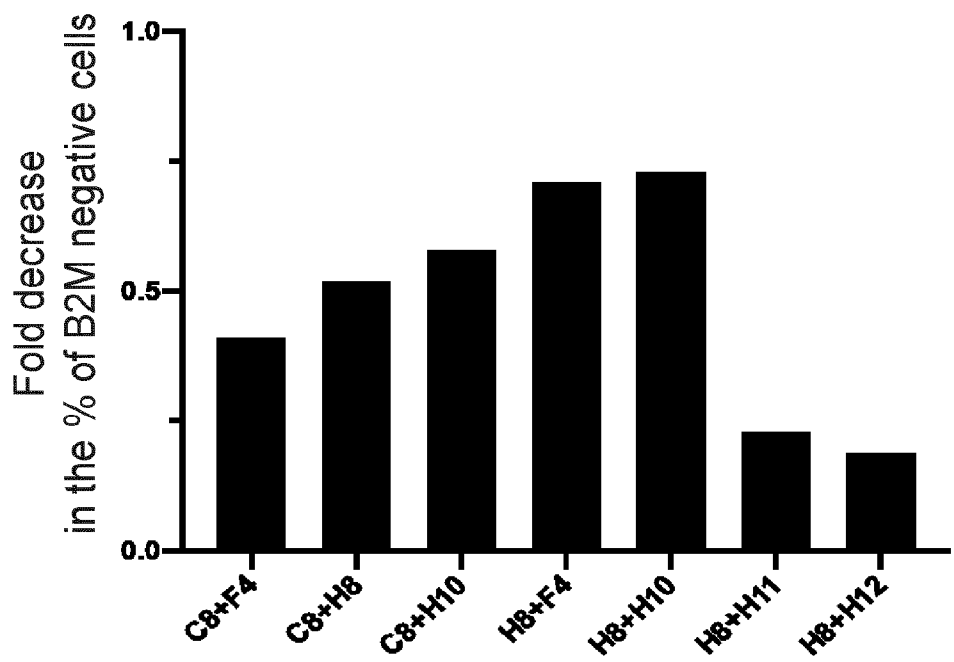


FIGURE 15

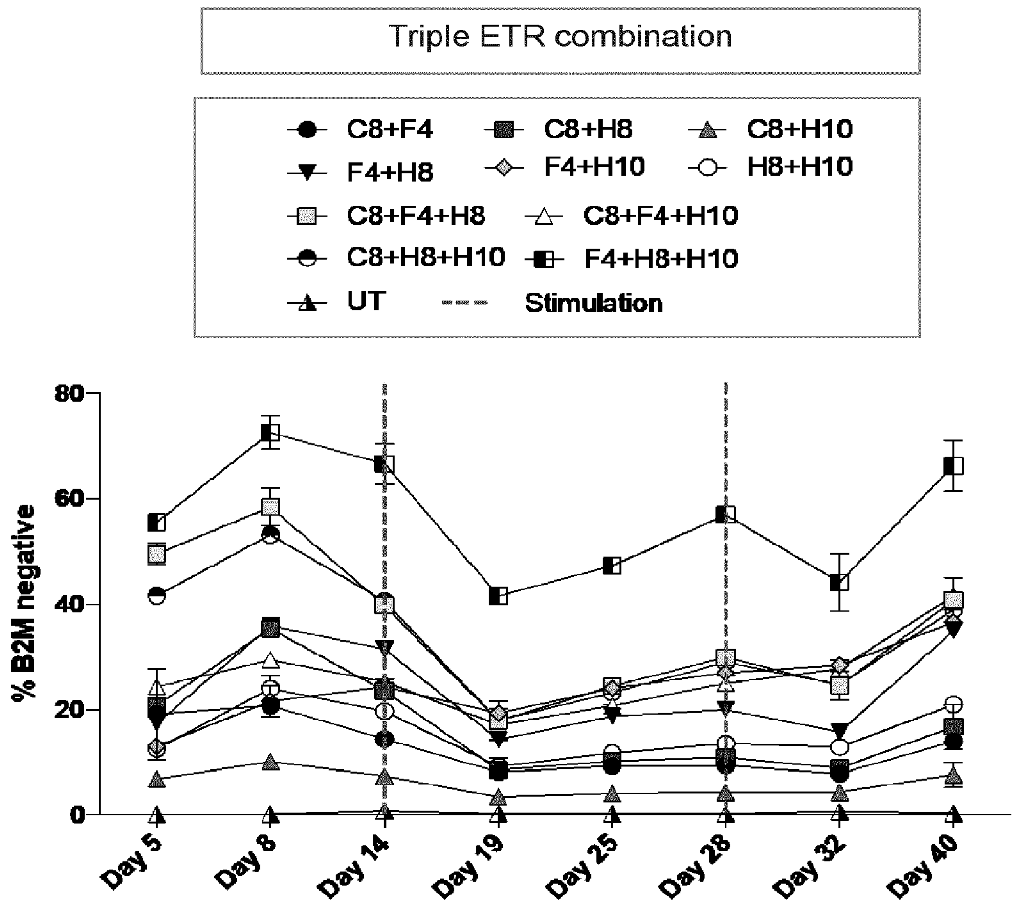

Triple ETR combination
C8+F4
C8+H8
C8+H10
F4+H8
F4+H10
H8+H10
C8+F4+H8
C8+F4+H10
C8+H8+H10
F4+H8+H10
UT
Stimulation
% B2M negative
80
60
40
20
0
Day 5
Day 8
Day 14
Day 19
Day 25
Day 28
Day 32
Day 40

FIGURE 16

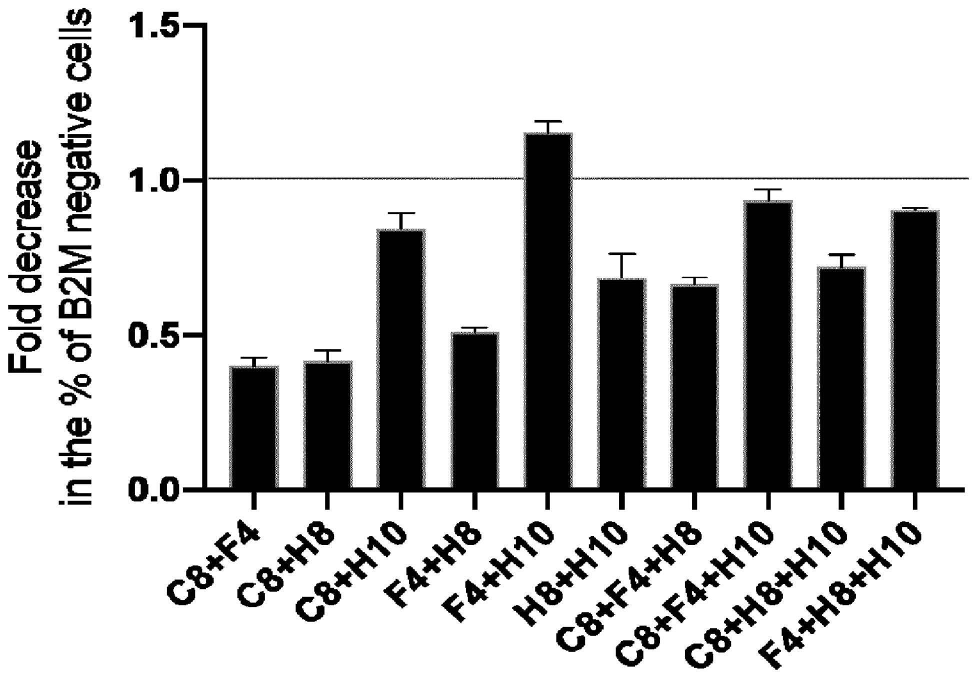


FIGURE 17

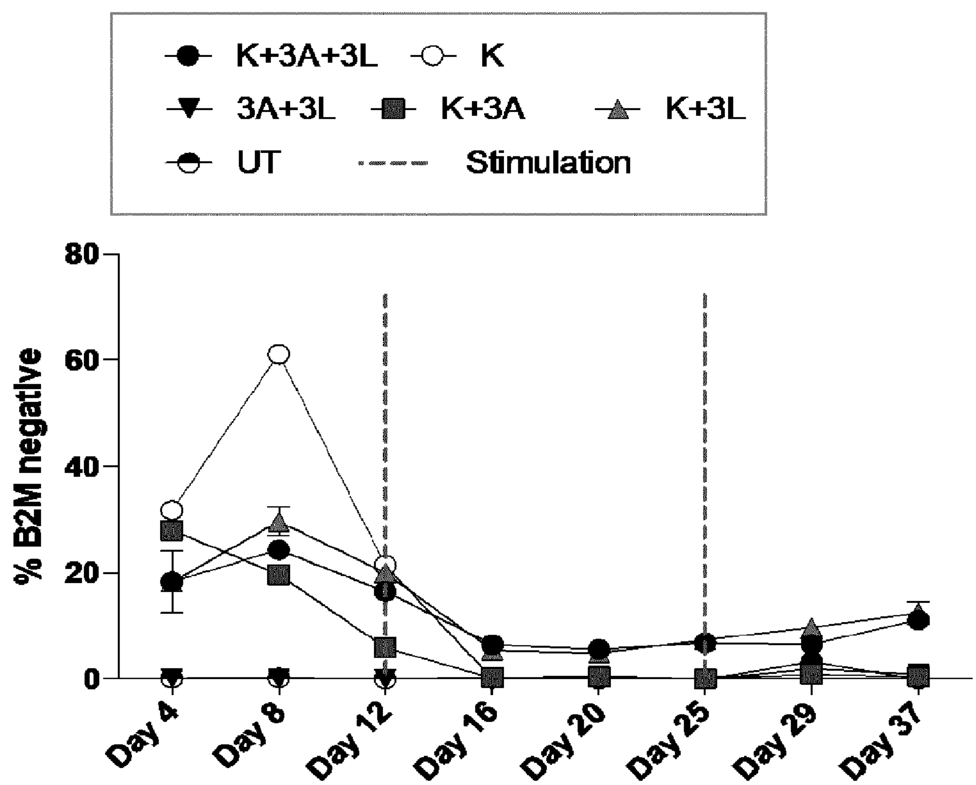


FIGURE 18A

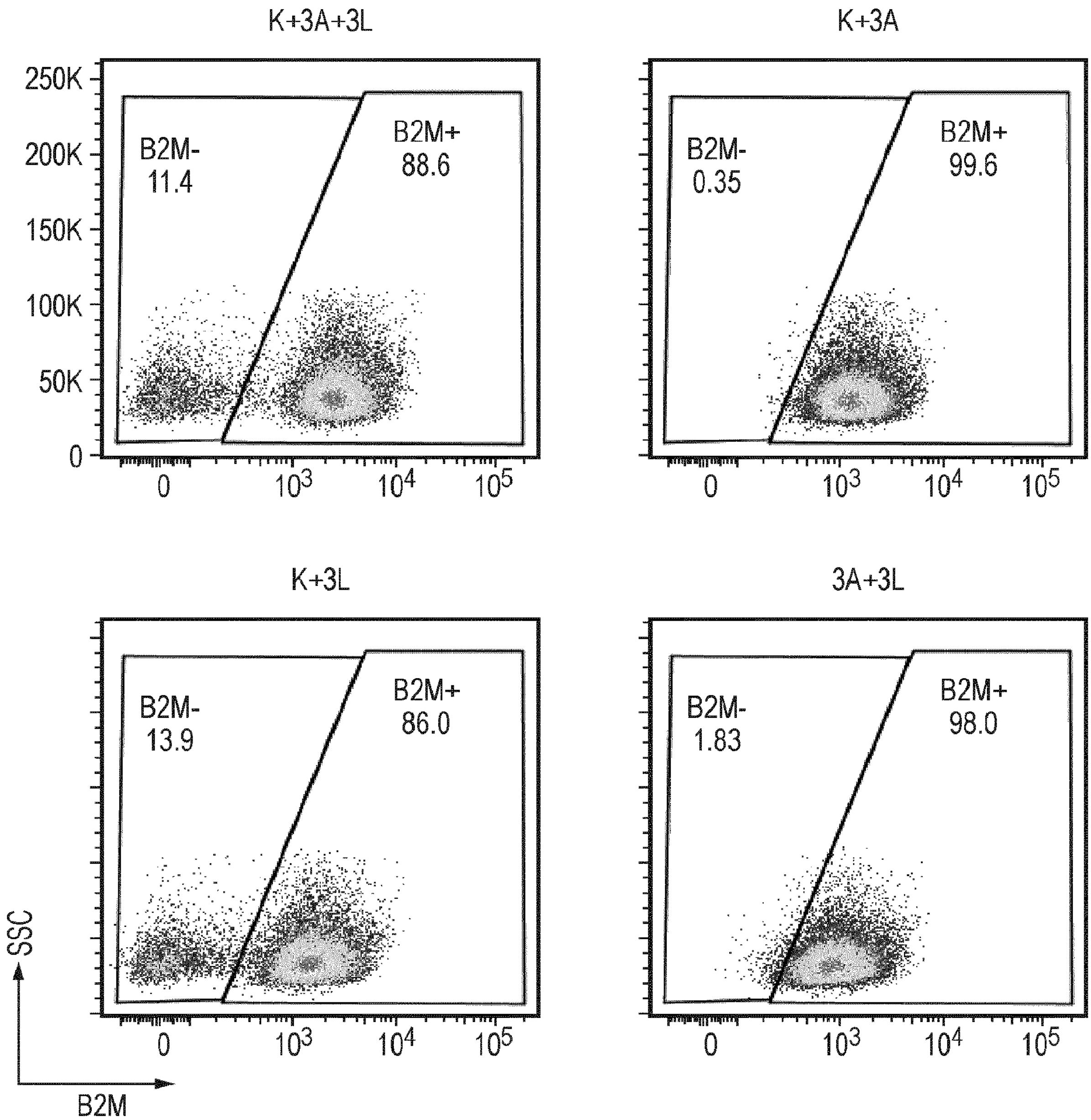

K+3A+3L
250K
200K
150K
100K
50K
0
B2M-
11.4
B2M+
88.6
K+3A
B2M-
0.35
B2M+
99.6
K+3L
B2M-
13.9
B2M+
86.0
3A+3L
B2M-
1.83
B2M+
98.0
0
10^3
10^4
10^5
SSC
B2M

FIGURE 18B

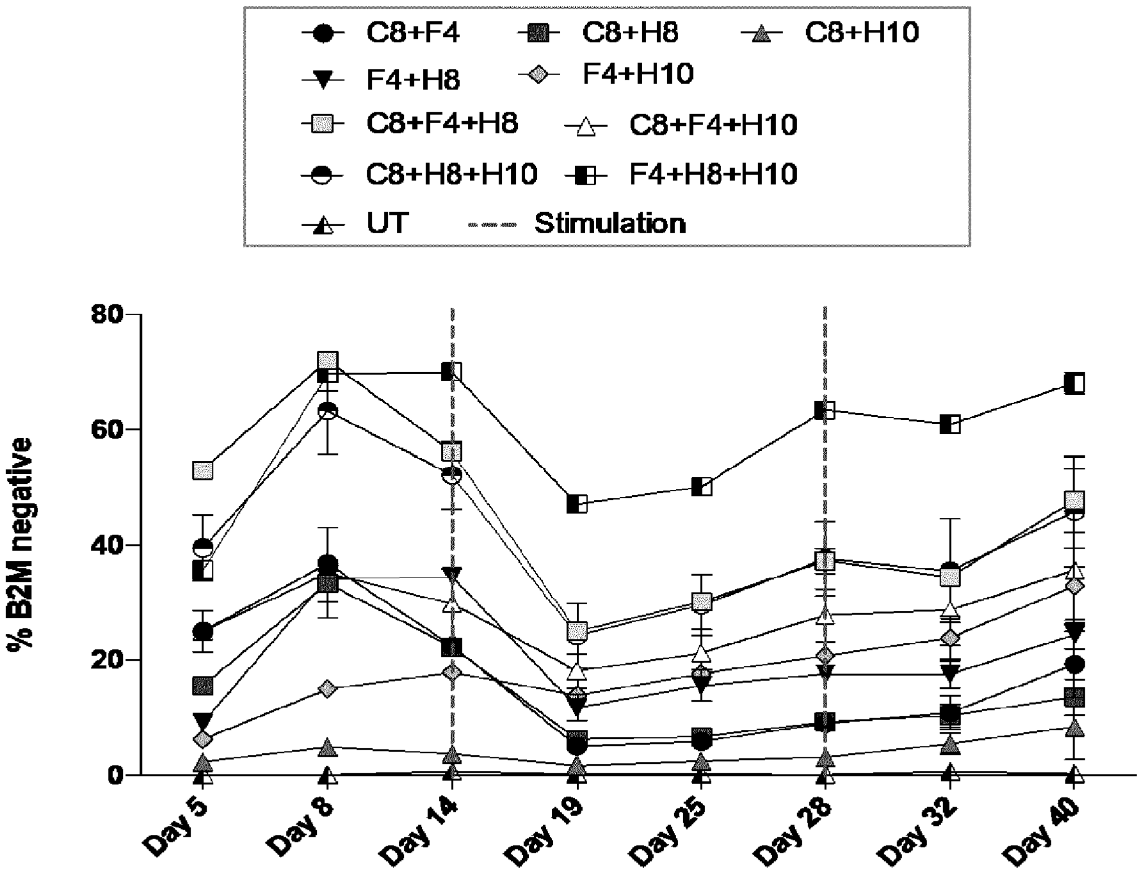
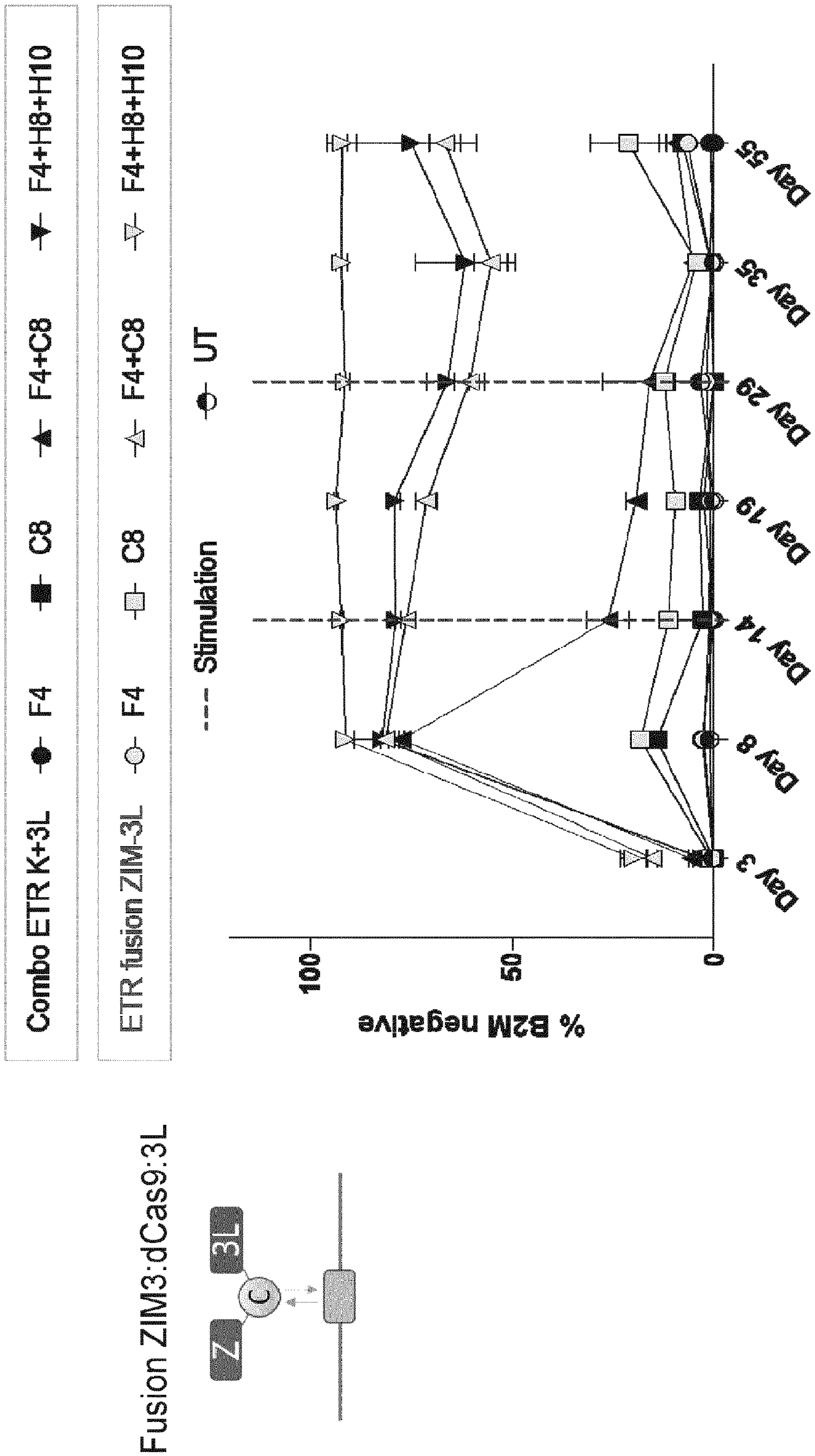
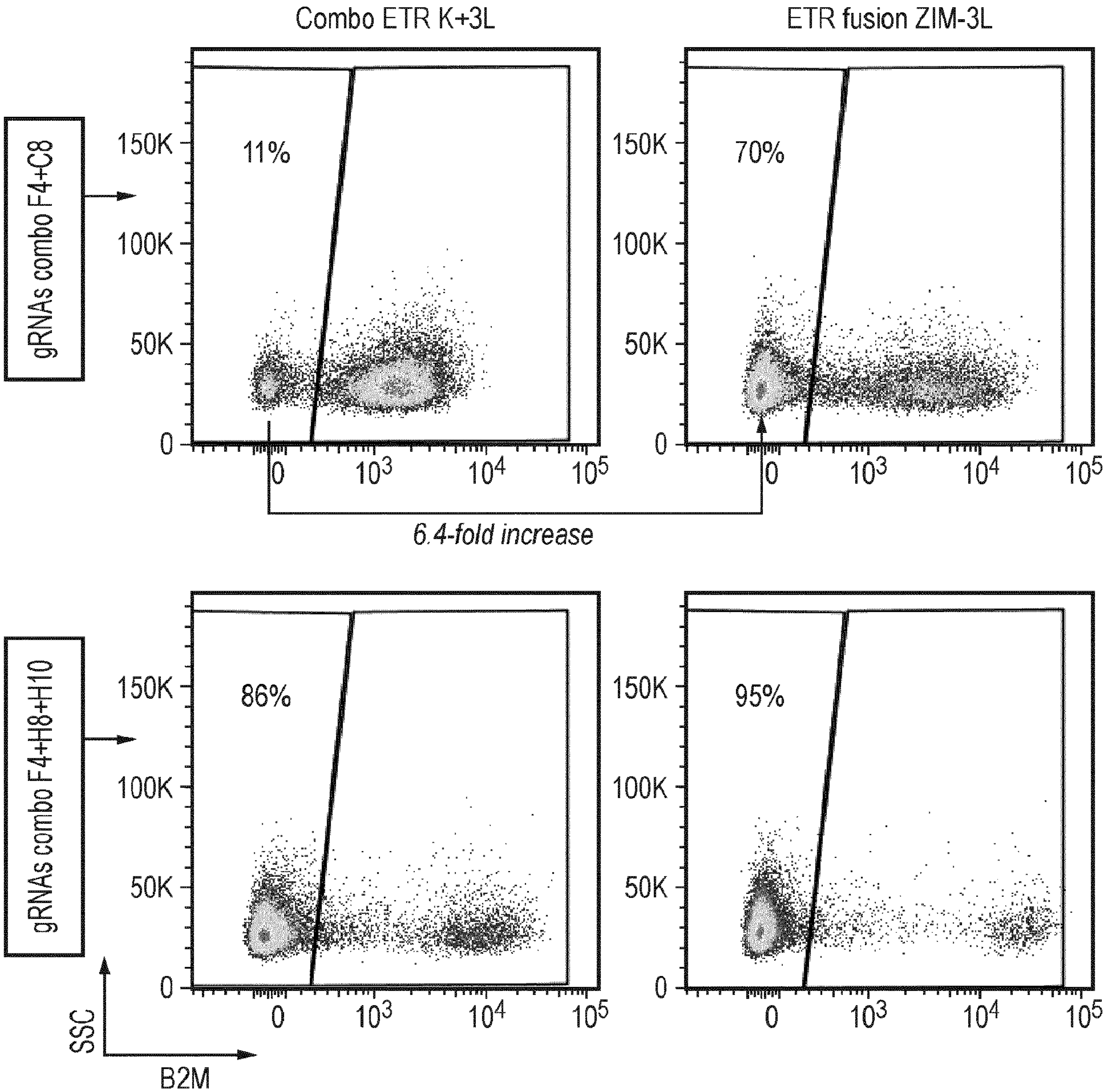

C8+F4
C8+H8
C8+H10
F4+H8
F4+H10
C8+F4+H8
C8+F4+H10
C8+H8+H10
F4+H8+H10
UT
Stimulation
% B2M negative
80
60
40
20
0
Day 5
Day 8
Day 14
Day 19
Day 25
Day 28
Day 32
Day 40

FIGURE 19



Combo ETR K+3L
F4
C8
F4+C8
F4+H8+H10
ETR fusion ZIM-3L
F4
C8
F4+C8
F4+H8+H10
Stimulation
UT
% B2M negative
100
50
0
Day 3
Day 8
Day 14
Day 19
Day 29
Day 35
Day 55
Fusion ZIM3:dCas9:3L
Z
C
3L

FIGURE 20A



Combo ETR K+3L
ETR fusion ZIM-3L
gRNAs combo F4+C8
gRNAs combo F4+H8+H10
11%
70%
86%
95%
6.4-fold increase
150K
100K
50K
0
0
10^3
10^4
10^5
SSC
B2M

FIGURE 20B

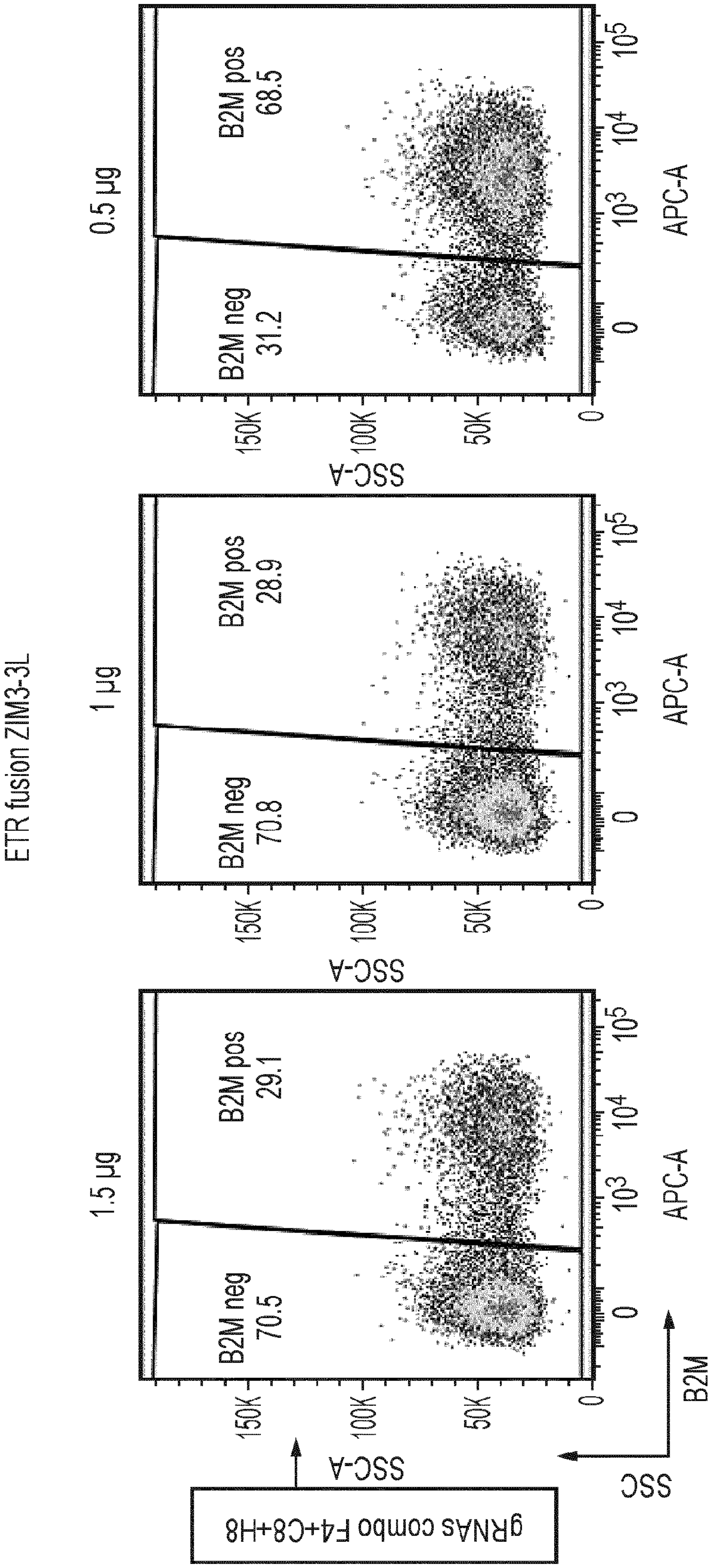

gRNAs combo F4+C8+H8
ETR fusion ZIM3-3L
1.5 µg
1 µg
0.5 µg
B2M neg
70.5
B2M pos
29.1
B2M neg
70.8
B2M pos
28.9
B2M neg
31.2
B2M pos
68.5
SSC-A
APC-A
SSC
B2M

FIGURE 21

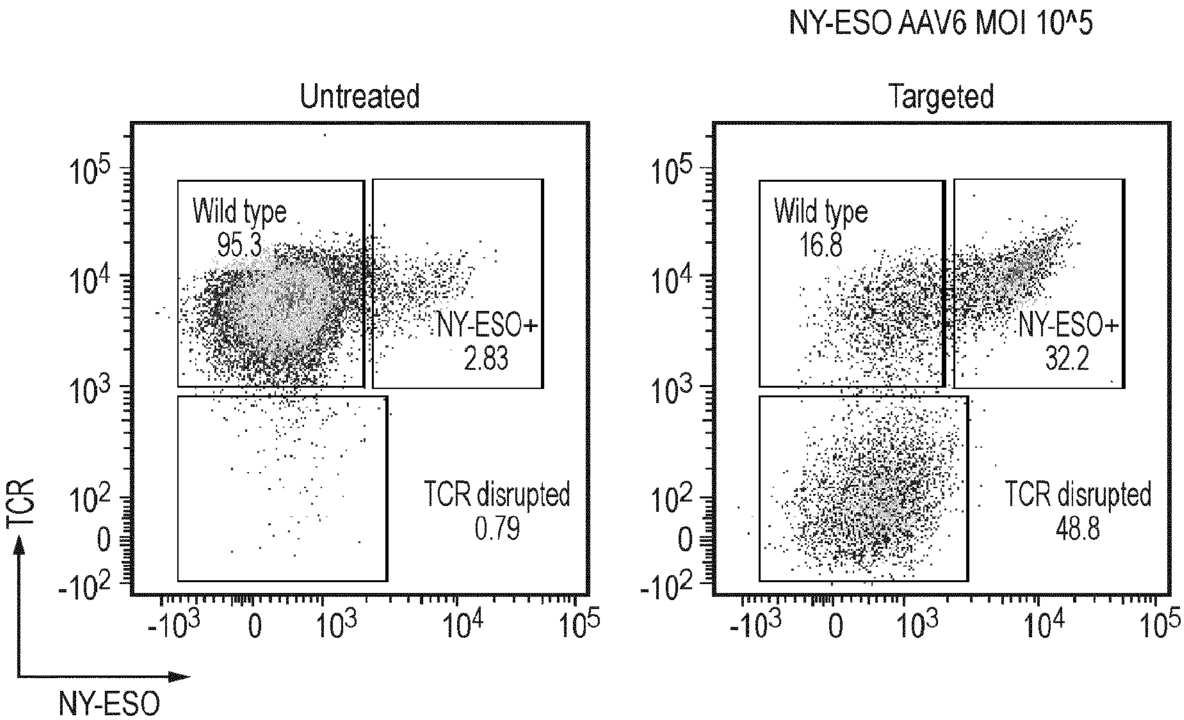

NY-ESO AAV6 MOI 10^5
Untreated
Targeted
Wild type
95.3
NY-ESO+
2.83
TCR disrupted
0.79
Wild type
16.8
NY-ESO+
32.2
TCR disrupted
48.8
TCR
NY-ESO

FIGURE 22

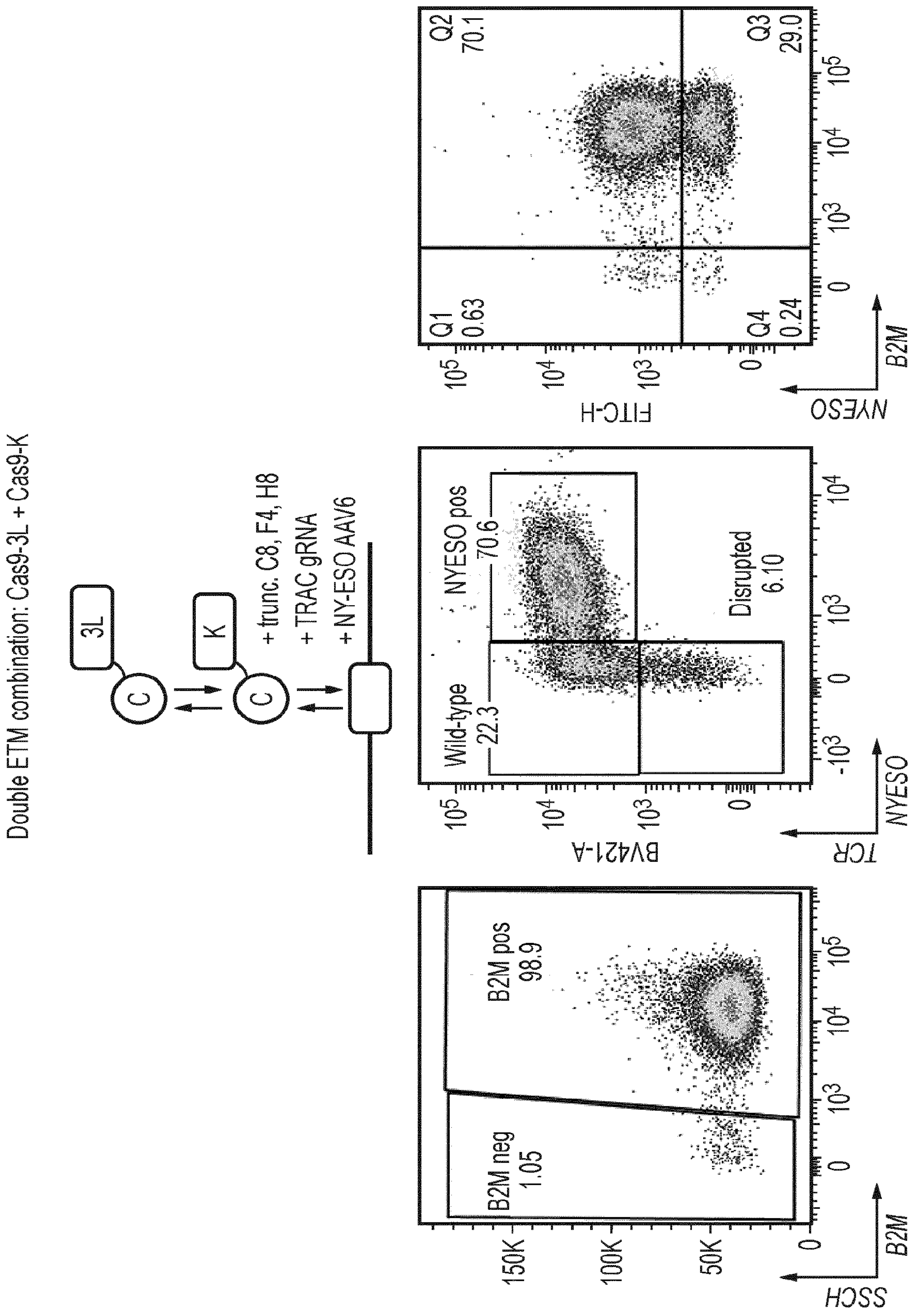

Double ETM combination: Cas9-3L + Cas9-K
3L
C
K
C
+ trunc. C8, F4, H8
+ TRAC gRNA
+ NY-ESO AAV6
B2M neg
1.05
B2M pos
98.9
SSCH
150K
100K
50K
0
0
10^3
10^4
10^5
SSCH
B2M
Wild-type
22.3
NYESO pos
70.6
Disrupted
6.10
BV421-A
10^5
10^4
10^3
0
-10^3
0
10^3
10^4
TCR
NYESO
Q1
0.63
Q2
70.1
Q3
29.0
Q4
0.24
FITC-H
10^5
10^4
10^3
0
0
10^3
10^4
10^5
NYESO
B2M

FIGURE 23

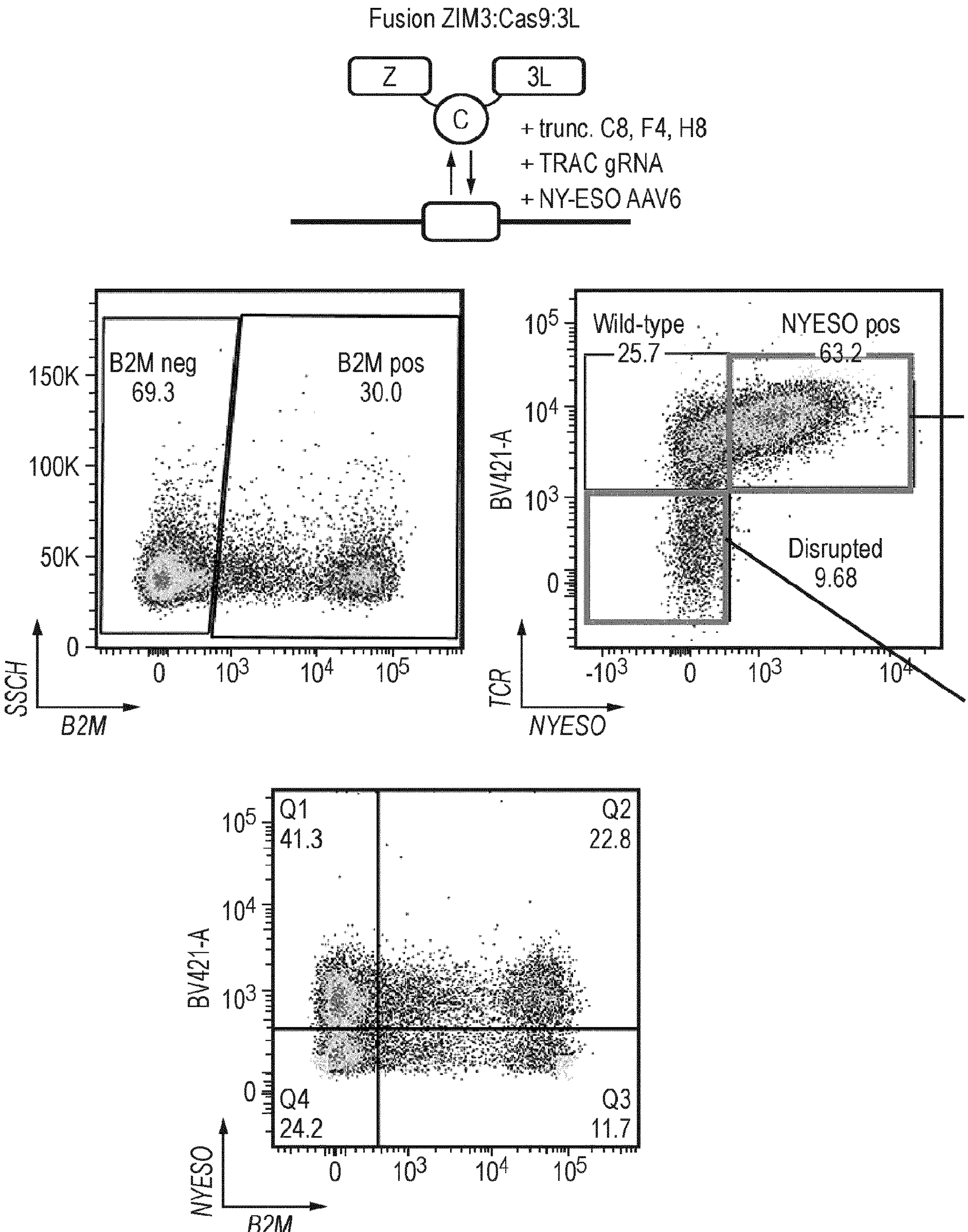

Fusion ZIM3:Cas9:3L
Z
3L
C
+ trunc. C8, F4, H8
+ TRAC gRNA
+ NY-ESO AAV6
B2M neg
69.3
B2M pos
30.0
150K
100K
50K
0
0
10^3
10^4
10^5
SSCH
B2M
Wild-type
25.7
NYESO pos
63.2
Disrupted
9.68
BV421-A
-10^3
TCR
NYESO
Q1
41.3
Q2
22.8
Q4
24.2
Q3
11.7
BV421-A
NYESO
B2M

FIGURE 24

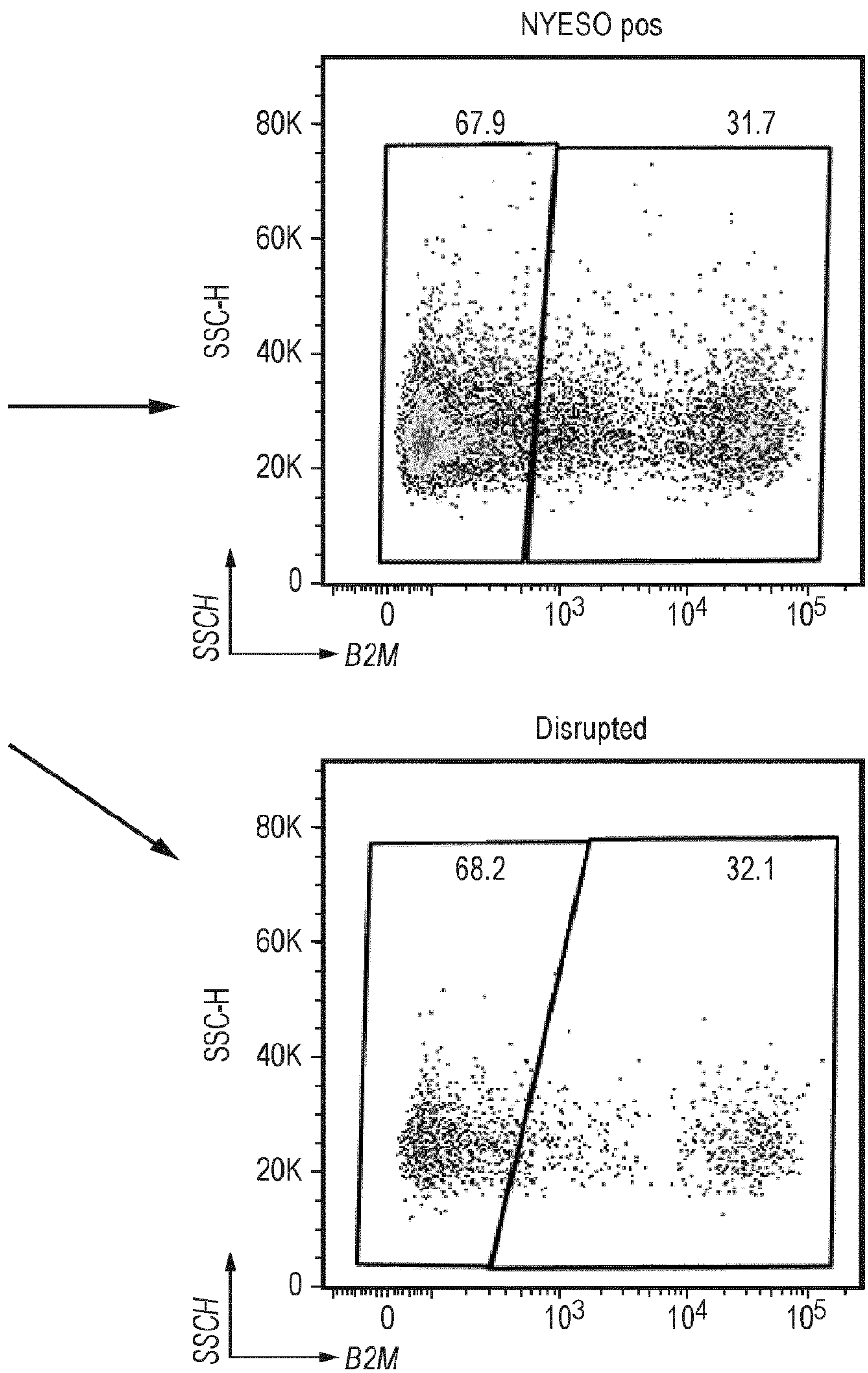


FIGURE 24 (Continued)

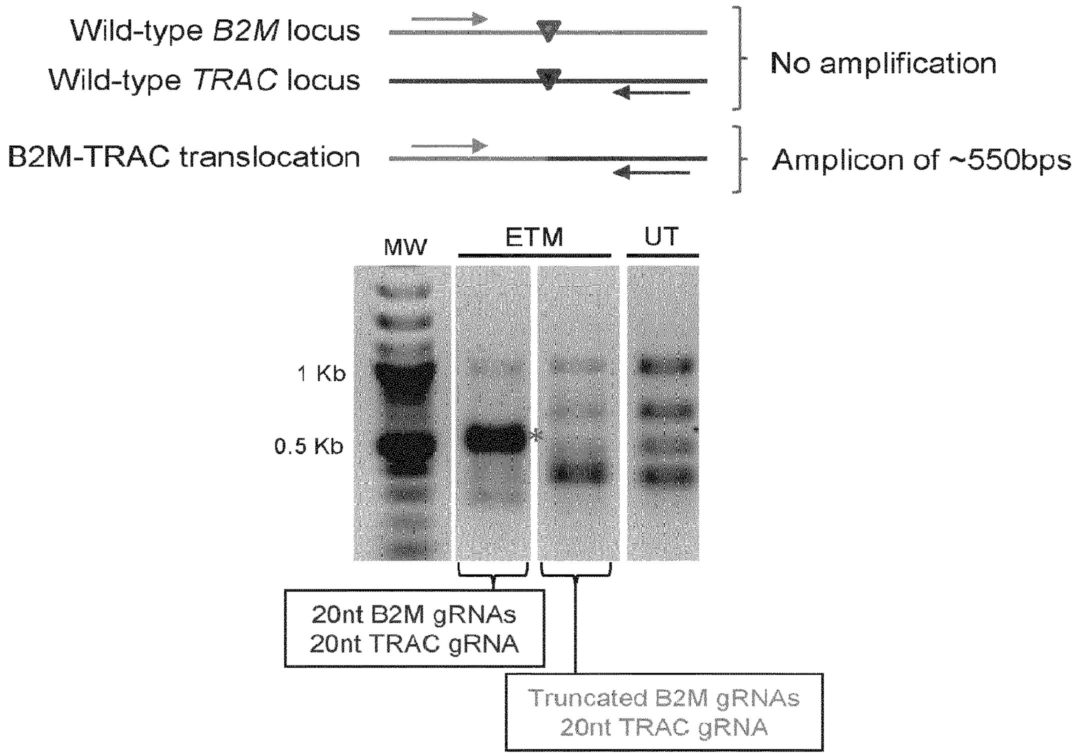


FIGURE 25

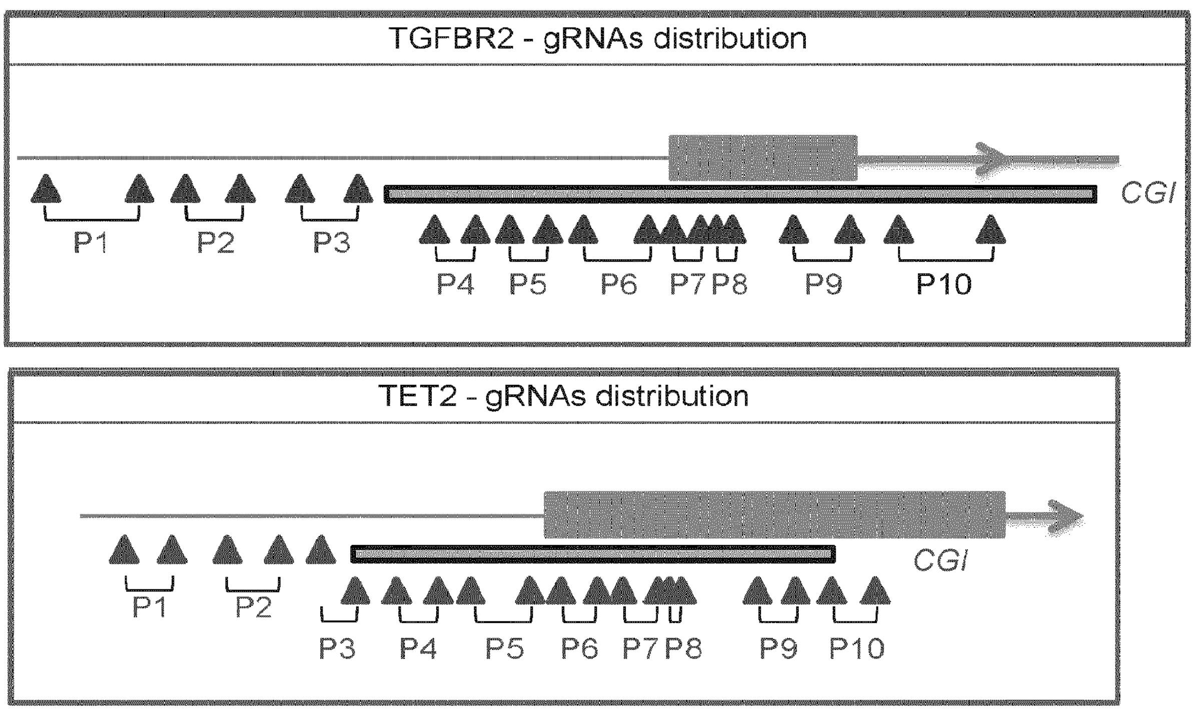


FIGURE 26

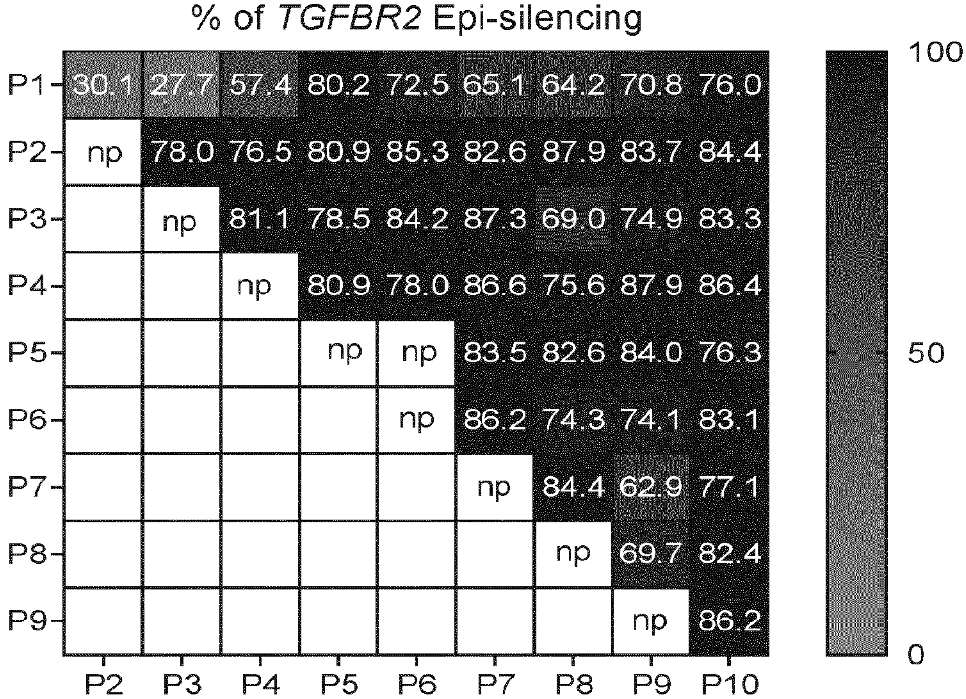


FIGURE 27

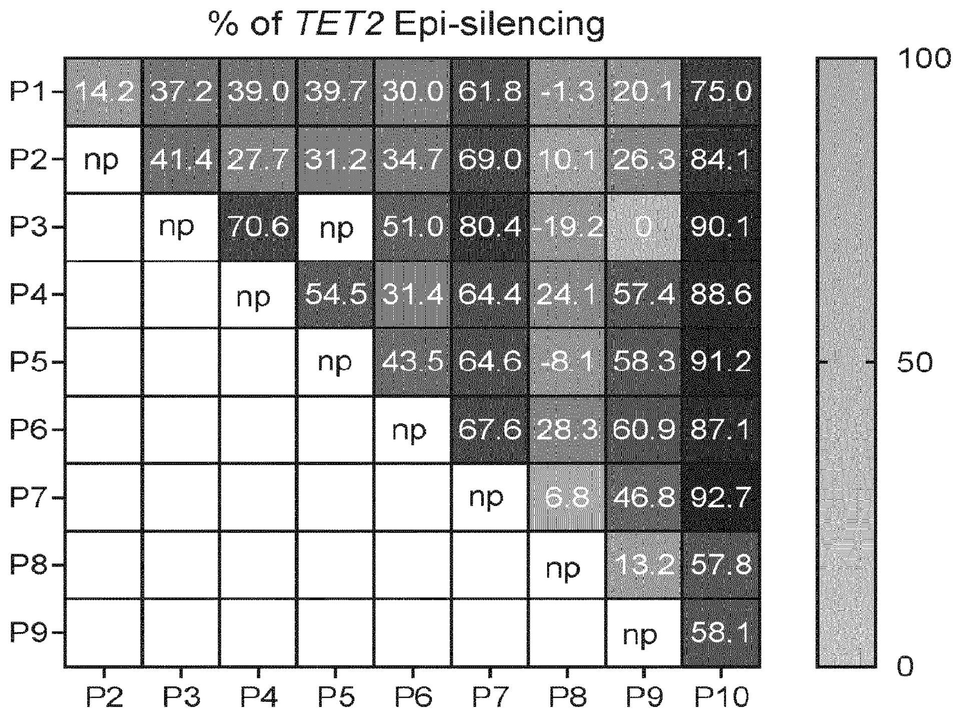


FIGURE 28

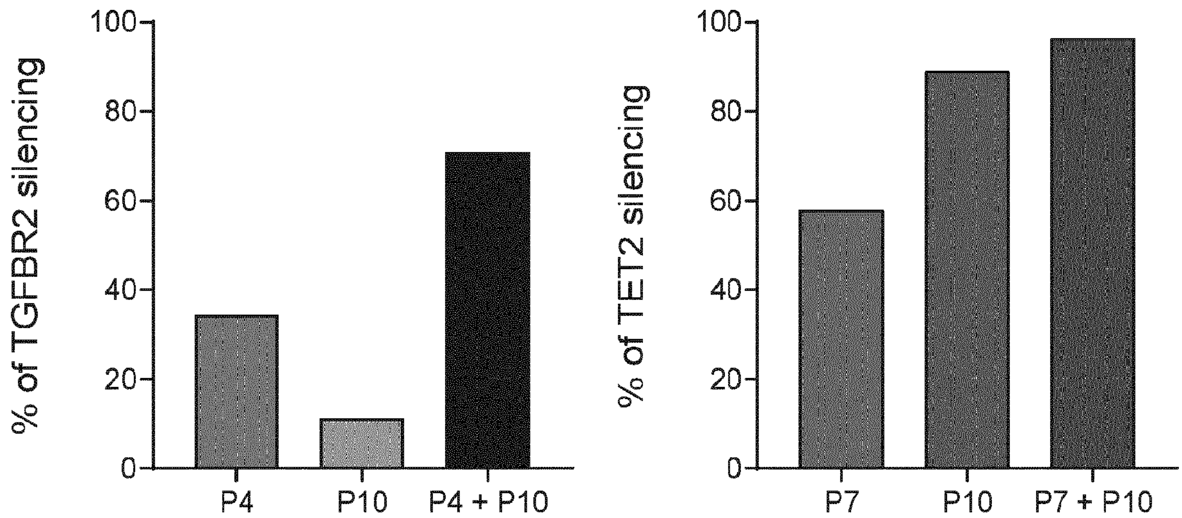


FIGURE 29

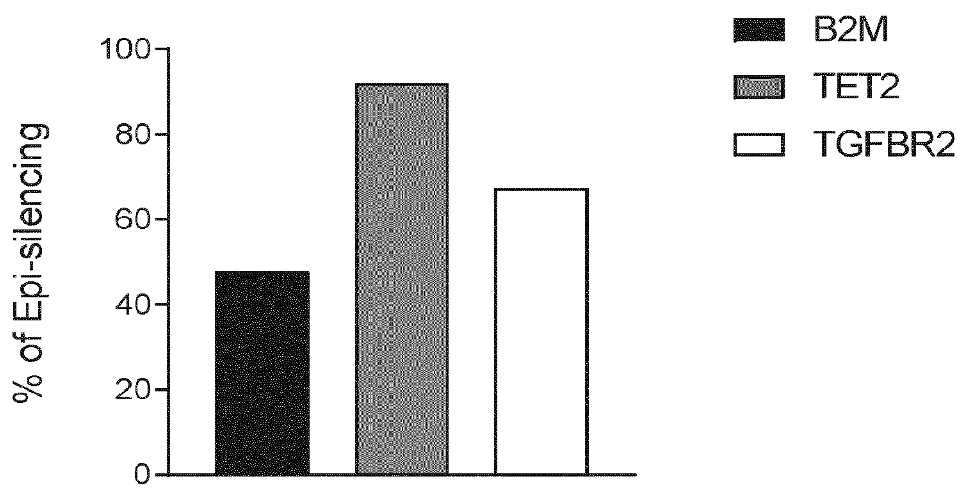


FIGURE 30

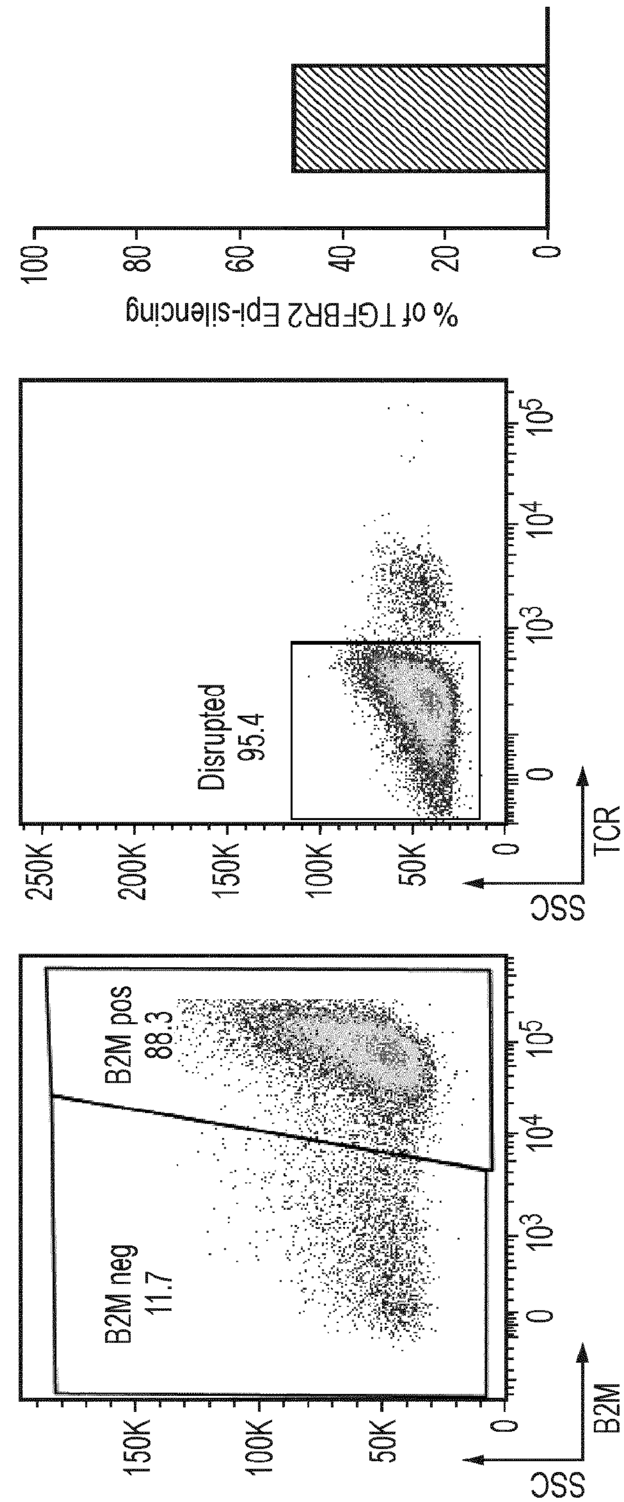

% of TGFBR2 Epi-silencing
100
80
60
40
20
0
SSC
TCR
250K
200K
150K
100K
50K
0
Disrupted
95.4
10^3
10^4
10^5
B2M
150K
100K
50K
B2M neg
11.7
B2M pos
88.3

FIGURE 31

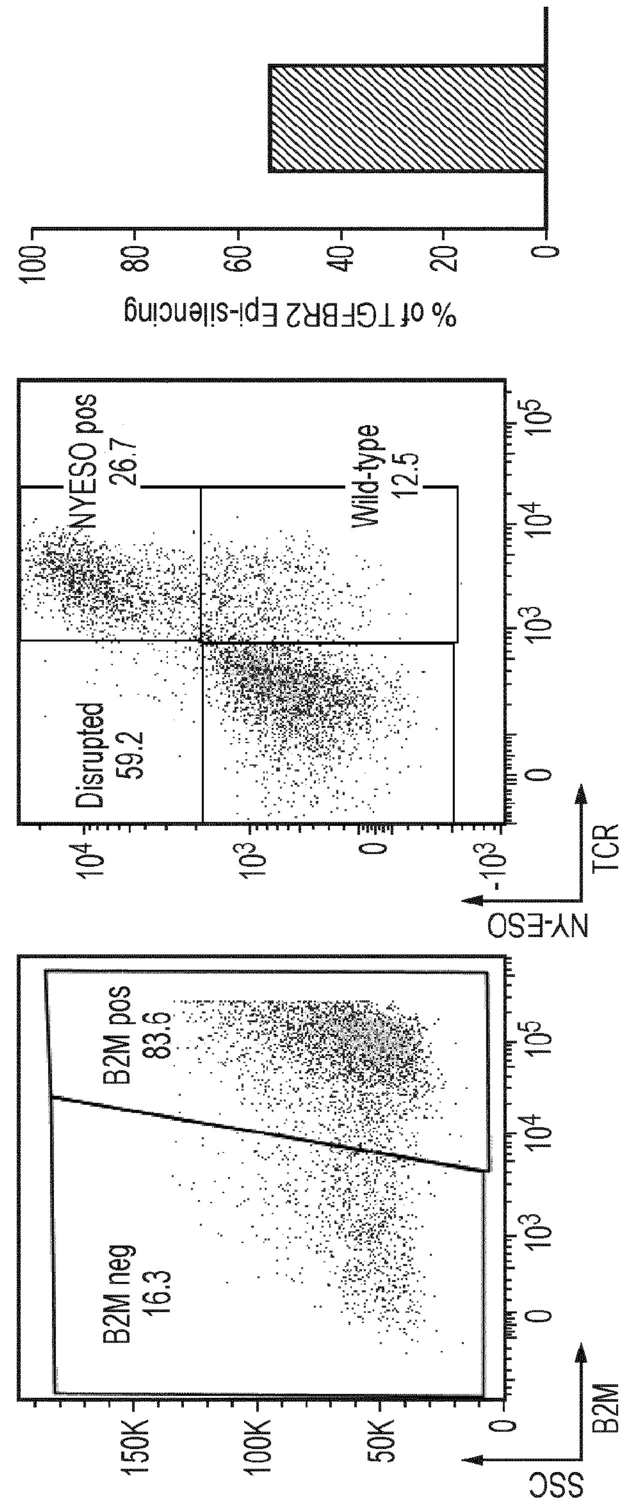

SSC
B2M
150K
100K
50K
0
B2M pos
83.6
B2M neg
16.3
NY-ESO
TCR
Disrupted
59.2
NYESO pos
26.7
Wild-type
12.5
% of TGFBR2 Epi-silencing
100
80
60
40
20
0

FIGURE 32

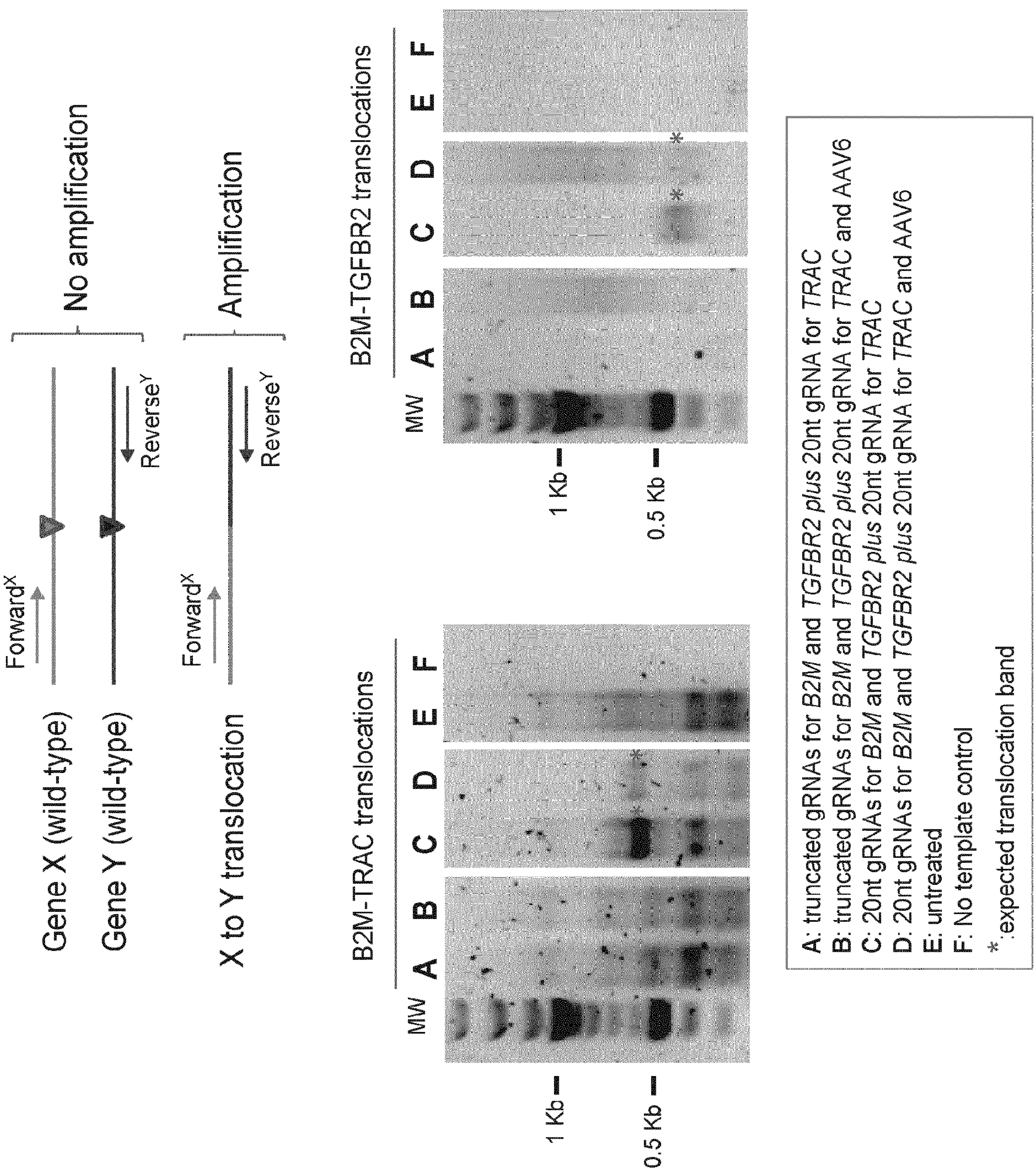

Gene X (wild-type)
Gene Y (wild-type)
X to Y translocation
Forward^X
Reverse^Y
No amplification
Amplification
B2M-TRAC translocations
B2M-TGFBR2 translocations
MW
A B C D E F
1 Kb
0.5 Kb
*
A: truncated gRNAs for B2M and TGFBR2 plus 20nt gRNA for TRAC
B: truncated gRNAs for B2M and TGFBR2 plus 20nt gRNA for TRAC and AAV6
C: 20nt gRNAs for B2M and TGFBR2 plus 20nt gRNA for TRAC
D: 20nt gRNAs for B2M and TGFBR2 plus 20nt gRNA for TRAC and AAV6
E: untreated
F: No template control
*:expected translocation band

FIGURE 33

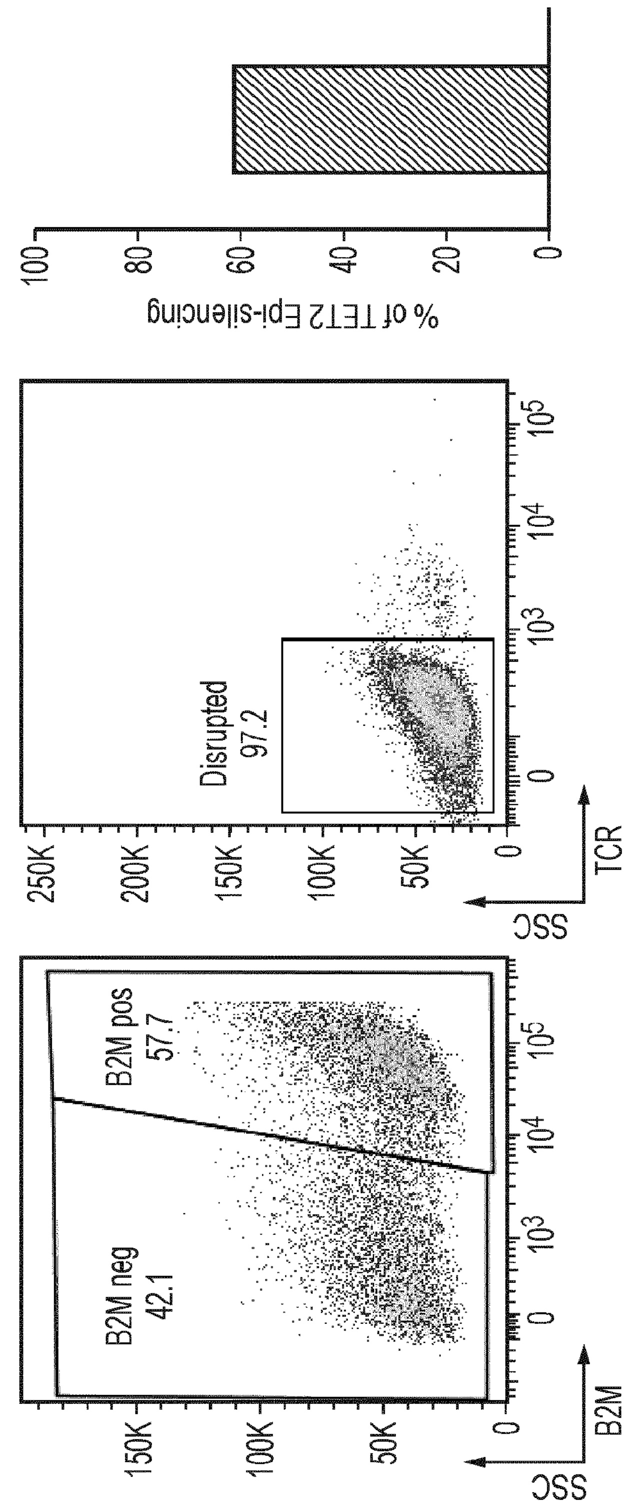

% of TET2 Epi-silencing
100
80
60
40
20
0
Disrupted
97.2
250K
200K
150K
100K
50K
0
SSC
TCR
10^3
10^4
10^5
B2M neg
42.1
B2M pos
57.7
150K
100K
50K
0
SSC
B2M

FIGURE 34

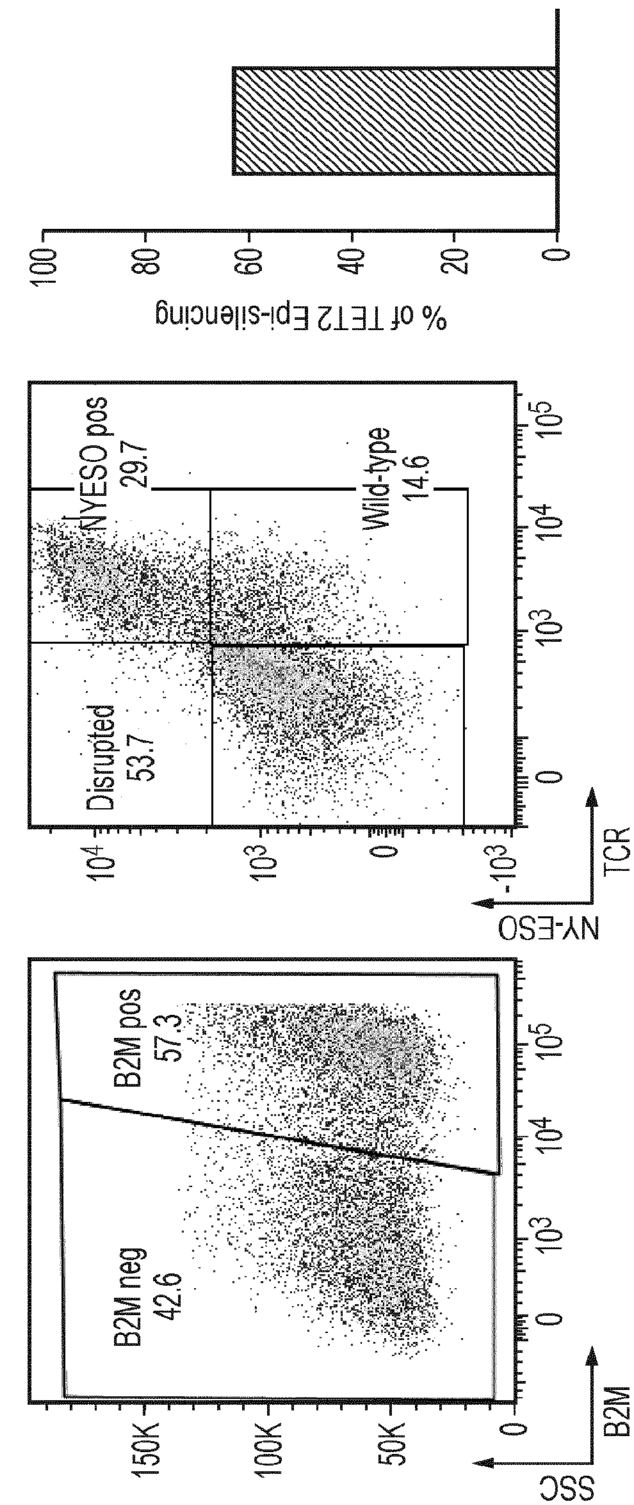
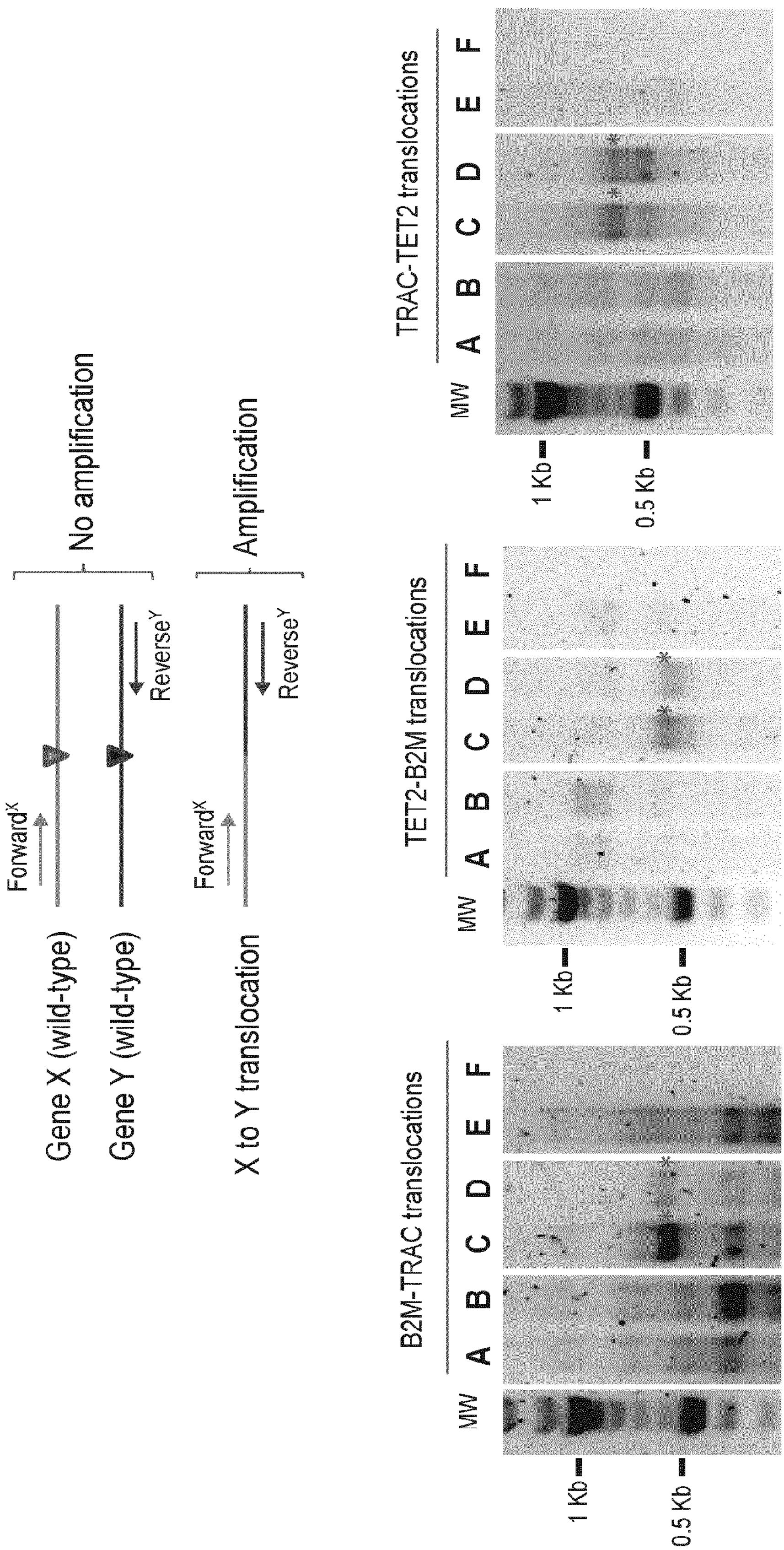

% of TET2 Epi-silencing
100
80
60
40
20
0
NYESO pos
29.7
Wild-type
14.6
Disrupted
53.7
NY-ESO
TCR
B2M pos
57.3
B2M neg
42.6
SSC
B2M
150K
100K
50K

FIGURE 35



A: truncated gRNAs for *B2M* and *TET2 plus* 20nt gRNA for *TRAC*
B: truncated gRNAs for *B2M* and *TET2 plus* 20nt gRNA for *TRAC* and AAV6
C: 20nt gRNAs for *B2M* and *TET2 plus* 20nt gRNA for *TRAC*
D: 20nt gRNAs for *B2M* and *TET2 plus* 20nt gRNA for *TRAC* and AAV6
E: untreated
F: No template control
*:expected translocation band

FIGURE 36

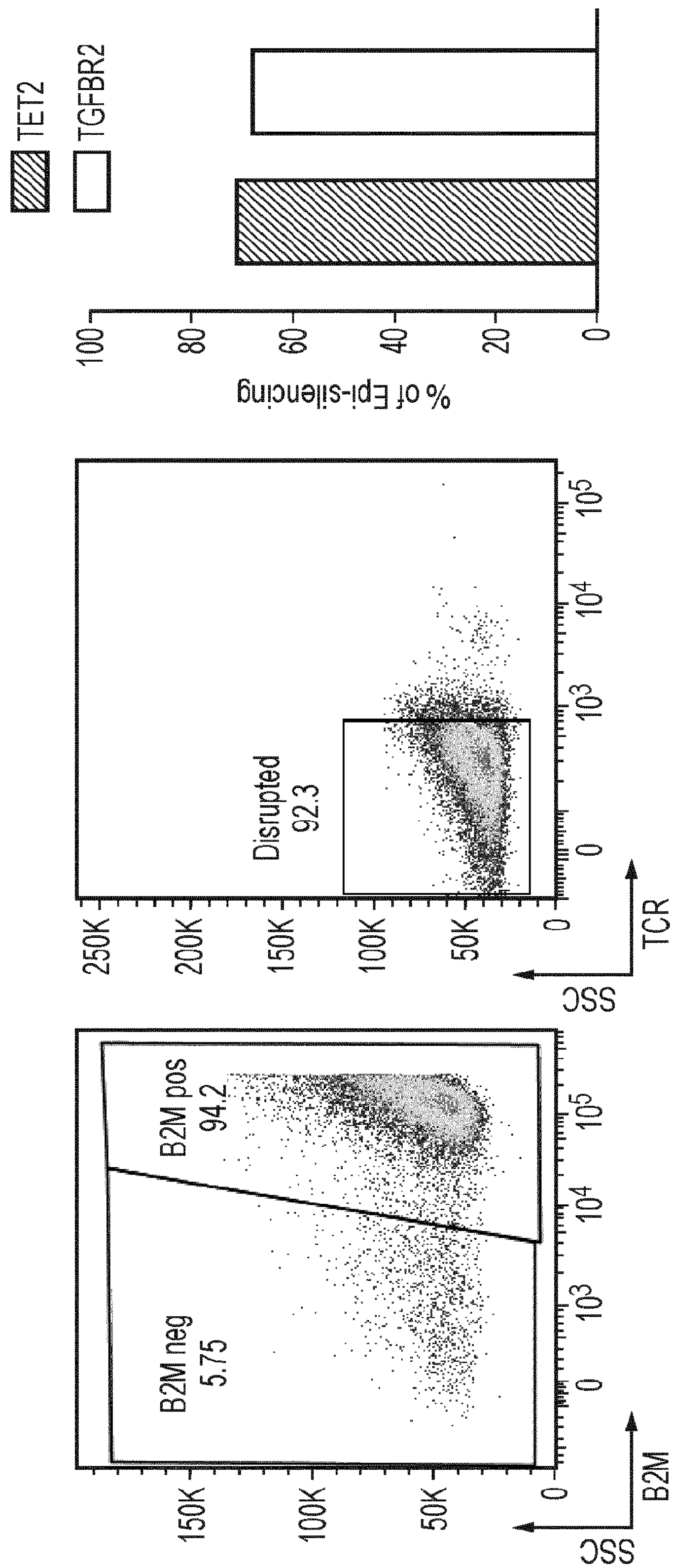

TET2
TGFBR2
% of Epi-silencing
100
80
60
40
20
0
Disrupted
92.3
250K
200K
150K
100K
50K
0
SSC
TCR
B2M pos
94.2
B2M neg
5.75
150K
100K
50K
0
SSC
B2M

FIGURE 37

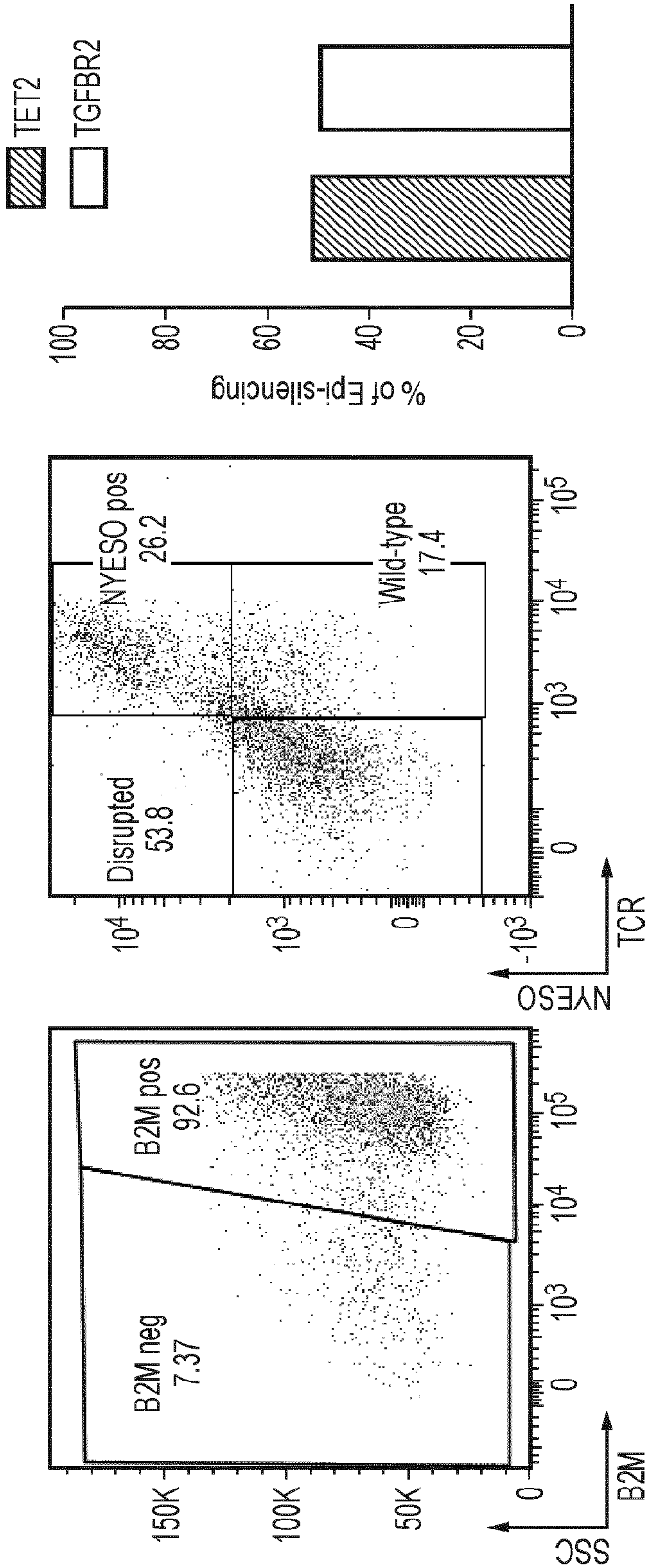
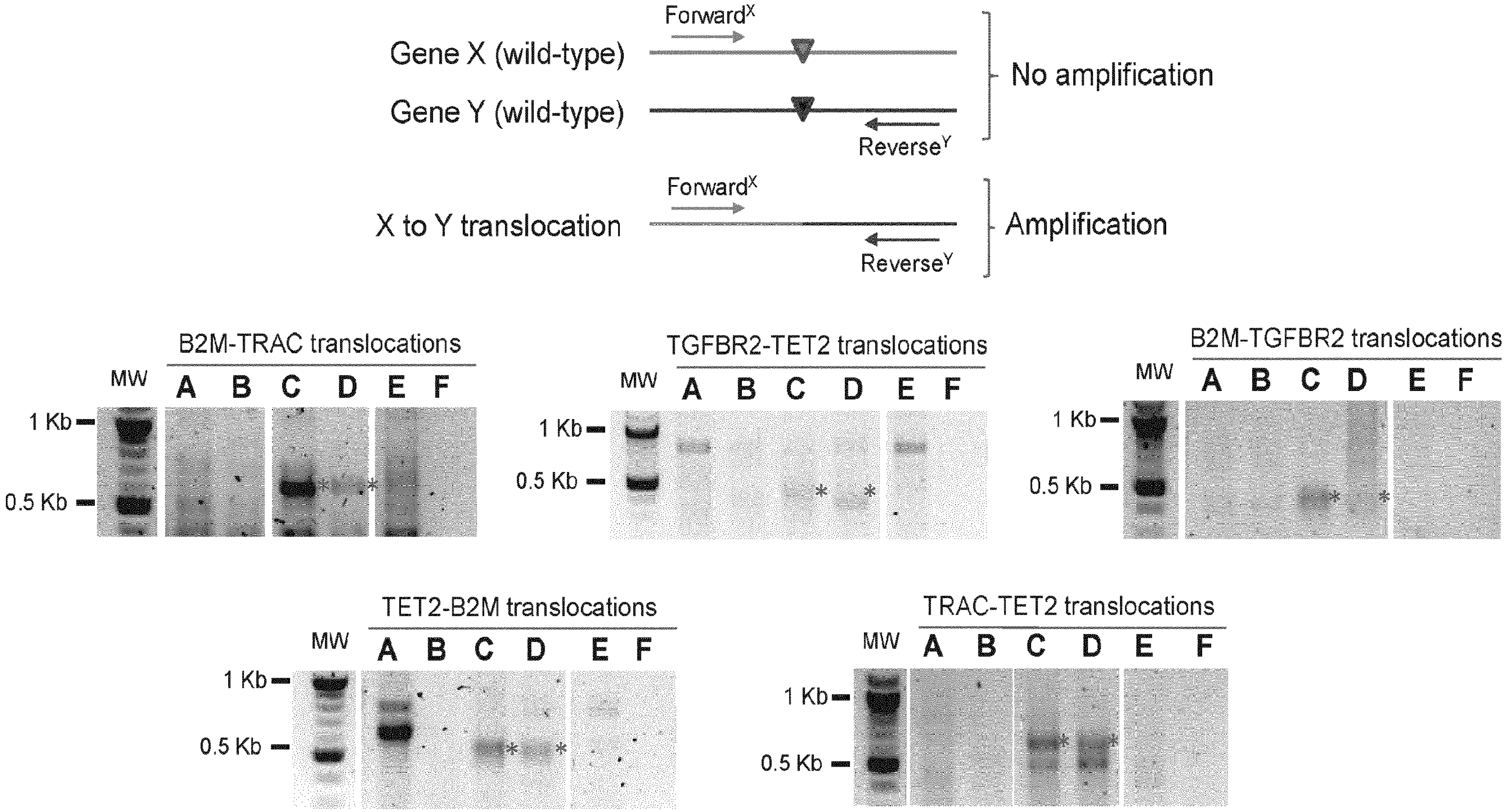

TET2
TGFBR2
% of Epi-silencing
100
80
60
40
20
0
NYESO pos
26.2
Wild-type
17.4
Disrupted
53.8
NYESO
TCR
B2M pos
92.6
B2M neg
7.37
SSC
B2M
150K
100K
50K

FIGURE 38



A: truncated gRNAs for *B2M, TGFBR2* and *TET2 plus* 20nt gRNA for *TRAC*
B: truncated gRNAs for *B2M, TGFBR2* and *TET2 plus* 20nt gRNA for *TRAC* and AAV6
C: 20nt gRNAs for *B2M, TGFBR2* and *TET2 plus* 20nt gRNA for *TRAC*
D: 20nt gRNAs for *B2M, TGFBR2* and *TET2 plus* 20nt gRNA for *TRAC* and AAV6
E: untreated
F: No template control
*:expected translocation band

FIGURE 39