

SUMMARY OF THE INVENTION

[0008] The present ~~invention~~disclosure 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~~disclosure 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~~disclosure 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~~disclosure 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.

[0012a] In one aspect, the present disclosure provides 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.

[0012b] In another aspect, the present disclosure 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 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.

[0012c] In another aspect, the present disclosure provides a pharmaceutical composition comprising the combination of the disclosure.

[0012d] In another aspect, the present disclosure provides a cell comprising the combination of the disclosure, or a progeny of the cell.

[0012e] In another aspect, the present disclosure provides a method of modifying transcription, expression and/or activity of one or more gene in a human T cell, comprising introducing the combination of the disclosure into the cell *in vitro* or *ex vivo*.

[0012f] In another aspect, the present disclosure provides a cell obtained by the method of the disclosure, or a progeny of the cell.

[0012g] In another aspect, the present disclosure provides a cell of the disclosure for use in therapy.

[0012h] In another aspect, the present disclosure provides use of the cell of the disclosure in the manufacture of medicament for modifying transcription, expression and/or activity of one or more gene in a human T cell in a human in need thereof.

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

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

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

[0016] In some ~~embodiment~~examples, 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 ~~embodiment~~examples, the epigenetic effector domain is a transcriptional repressor domain (e.g., a Krüppel-associated box (KRAB) domain).

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

[0018] In some embodiment example, 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, the ETM may comprise as epigenetic effector domains KRAB and DNMT3A; KRAB and DNMT3L; or KRAB, DNMT3A, and

DNMT3L. In some [embodimentexamples](#), the ETM (e.g., ETR) comprises a transcriptional repressor domain (e.g., a Krüppel-associated box (KRAB) domain) and a DNMT3L domain. In some [embodimentexamples](#), 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 [embodimentexamples](#), the endonuclease comprises an RNA binding domain.

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

[0021] In some [embodimentexamples](#), the endonuclease is a Cas endonuclease.

[0022] In certain [embodimentexample](#), the endonuclease is a Cas9 endonuclease. In certain [embodimentexamples](#), the endonuclease is a SpCas9 endonuclease

[0023] In some [embodimentexamples](#), 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 [embodimentexamples](#), the ETM (e.g., ETR) is a bi- or tri-partite fusion protein.

[0025] In another [aspectexample](#), the present [inventiondisclosure](#) 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 [aspectexample](#), the present [inventiondisclosure](#) 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 [aspectexample](#), 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 [aspectexample](#), 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 [aspectexample](#), 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 [aspectexample](#), 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 [aspectexample](#), the present [inventiondisclosure](#) provides a combination (e.g., a system) comprising an ETM (e.g., ETR) according to the present [inventiondisclosure](#), and at least one gRNA. The gRNA(s) may target the ETM (e.g., ETR) to one or more target gene(s). In another [aspectexample](#), the present [inventiondisclosure](#) provides a combination (e.g., a system) comprising an ETM (e.g., ETR) according to the present [inventiondisclosure](#), 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 [inventiondisclosure](#), such as one, two or three ETMs (e.g., ETRs), or polynucleotides encoding therefor.

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

[0030] In another [aspectexample](#), the present [inventiondisclosure](#) 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the spacer sequence is less than or equal to 16 nucleotides in length. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the spacer sequence is less than or equal to 17 nucleotides in length. In some [embodimentexamples](#), 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 [embodimentexamples](#), the one or more gRNAs in (B) has a spacer sequence of less than or equal to 17 nucleotides. In some [embodimentexamples](#), 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 [embodimentexamples](#), the spacer sequence is 18 or more nucleotides in length, such as 19 or more, or 20 or more nucleotides in length. In some [embodimentexamples](#), the spacer sequence is 18 to 30 nucleotides in length, such as 19 to 30 or 20 to 30 nucleotides in length. In some [embodimentexamples](#), the spacer sequence is 18 to 25 nucleotides in length, such as 19 to 25 or 20 to 25 nucleotides in length. In some [embodimentexamples](#), the spacer sequence is 18 to 21 nucleotides in length, such as 19 to 21 or 20 to 21 nucleotides in length. In some [embodimentexamples](#), the spacer

sequence is 18 to 20 nucleotides in length, such as 19 to 20 nucleotides in length. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the at least two gRNAs comprise spacer sequences of different lengths.

[0041] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, 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 embodiment examples, 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 [embodimentexamples](#), 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 [embodimentexamples](#), the target genes may comprise, e.g., *B2M* and *TRAC*. In some [embodimentexample](#), the target genes may comprise, e.g., *B2M*, *TRAC*, *TET2*, and *TGFBR2*. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 embodimentexamples, 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 embodimentexamples, the combination, further comprises a donor DNA comprising 5' and 3' arms that are homologous to sequences in the second gene.

[0061] In some embodimentexamples, 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 embodimentexamples, the agent is a CAR or transgenic TCR. In some embodimentexamples, the agent is FIX.

[0062] In another aspectexample the inventiondisclosure 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

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 [embodimentexamples](#), the combination comprises at least one gRNA according to the present [inventiondisclosure](#). In some [embodimentexamples](#), the combination comprises one or more gRNAs comprising one or more gRNA sequences shown in Table 8. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 embodiment examples, 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 embodiment examples, 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 embodimentexamples, 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 embodimentexamples, 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 embodimentexamples, 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 embodimentexamples, 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 embodimentexamples, 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 embodimentexample, 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 embodimentexample, 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 embodimentexamples, 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 aspectexample, the present inventiondisclosure provides a polynucleotide encoding at least one ETM (e.g., ETR) according to the present inventiondisclosure.

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

[0083] In some embodimentexamples, 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 aspectexample, the present inventiondisclosure provides a vector comprising a polynucleotide according to the present inventiondisclosure or a nucleic acid construct according to the present inventiondisclosure.

[0085] In another aspectexample, the present inventiondisclosure provides a kit of polynucleotides comprising:

- a) at least one polynucleotide encoding at least one ETM (e.g., ETR) according to the present inventiondisclosure; 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 aspectexample, the present inventiondisclosure provides a cell (such as an engineered cell) comprising an ETM (e.g., ETR) according to the present inventiondisclosure, at least one gRNA according to the present inventiondisclosure, a combination according to the present inventiondisclosure, a polynucleotide according to the present inventiondisclosure, a nucleic acid construct according to the present inventiondisclosure, a vector according to the present inventiondisclosure or a kit of polynucleotides according to the present inventiondisclosure. In another aspectexample, the inventiondisclosure provides a progeny of the cell.

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

[0088] In some [embodimentexamples](#), 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 [aspectexample](#), the present [inventiondisclosure](#) provides a composition comprising an ETM (e.g., ETR) according to the present [inventiondisclosure](#), at least one gRNA according to the present [inventiondisclosure](#), a combination according to the present [inventiondisclosure](#), a polynucleotide according to the present [inventiondisclosure](#), a nucleic acid construct according to the present [inventiondisclosure](#), a vector according to the present [inventiondisclosure](#), a kit of polynucleotides according to the present [inventiondisclosure](#) or a cell according to the present [inventiondisclosure](#).

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

[0091] In a further [aspectexample](#), the present [inventiondisclosure](#) provides the use of an ETM (e.g., ETR) according to the present [inventiondisclosure](#), at least one gRNA according to the present [inventiondisclosure](#), a combination according to the present [inventiondisclosure](#), a polynucleotide according to the present [inventiondisclosure](#), a nucleic acid construct according to the present [inventiondisclosure](#), a vector according to the present [inventiondisclosure](#), a kit of polynucleotides according to the present [inventiondisclosure](#) or a cell according to the present [inventiondisclosure](#) 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 [aspectexample](#), the present [inventiondisclosure](#) 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 [inventiondisclosure](#), at least one gRNA according to the present [inventiondisclosure](#), a combination according to the present [inventiondisclosure](#), a polynucleotide according to the present [inventiondisclosure](#), a nucleic acid construct according to the present [inventiondisclosure](#), a vector according to the present [inventiondisclosure](#) or a kit of polynucleotides according to the present [inventiondisclosure](#) to a cell. The cell may be, for example, a T cell.

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

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

[0095] In some [embodimentexamples](#), 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 [aspectexample](#), an ETM (e.g., ETR) according to the present [inventionanddisclosure](#), at least one gRNA according to the present [inventionanddisclosure](#), a combination according to the present [inventionanddisclosure](#), a polynucleotide according to the present [inventionanddisclosure](#), a nucleic acid construct according to the present [inventionanddisclosure](#), a vector according to the present [inventionanddisclosure](#), a kit of polynucleotides according to the present [inventionanddisclosure](#), a cell according to the present [inventionanddisclosure](#) or a pharmaceutical composition according to the present [inventionanddisclosure](#) may be for use in therapy.

[0098] In another [aspectexample](#) the [inventionanddisclosure](#) provides use of an ETM (e.g., ETR) according to the present [inventionanddisclosure](#), at least one gRNA according to the present [inventionanddisclosure](#), a combination according to the present [inventionanddisclosure](#), a polynucleotide according to the present [inventionanddisclosure](#), a nucleic acid construct according to the present [inventionanddisclosure](#), a vector according to the present [inventionanddisclosure](#), a kit of polynucleotides according to the present [inventionanddisclosure](#), a cell according to the present [inventionanddisclosure](#) or a pharmaceutical composition according to the present [inventionanddisclosure](#) 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 [aspectexample](#), the present [inventionanddisclosure](#) 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 [inventionanddisclosure](#), at least one gRNA according to the present [inventionanddisclosure](#), a combination according to the present [inventionanddisclosure](#), a polynucleotide according to the present [inventionanddisclosure](#), a nucleic acid construct according to the present [inventionanddisclosure](#), a vector according to the present [inventionanddisclosure](#), a kit of polynucleotides

according to the present inventiondisclosure, a cell according to the present inventiondisclosure or a pharmaceutical composition according to the present inventiondisclosure 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 aspectexample, the present inventiondisclosure 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 inventiondisclosure, at least one gRNA according to the present inventiondisclosure, a polynucleotide according to the present inventiondisclosure, a nucleic acid construct according to the present inventiondisclosure, a vector according to the present inventiondisclosure or a kit of polynucleotides according to the present inventiondisclosure 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 embodimentexamples, the cell is autologous. In some embodimentexamples, the cell is allogeneic.

[0105] It is understood that an ETM (e.g., ETR) according to the present inventiondisclosure, at least one gRNA according to the present inventiondisclosure, a combination according to the present inventiondisclosure, a polynucleotide according to the present inventiondisclosure, a nucleic acid construct according to the present inventiondisclosure, a vector according to the present inventiondisclosure, a kit of polynucleotides according to the present inventiondisclosure, a cell according to the present inventiondisclosure or a pharmaceutical composition according to the present inventiondisclosure 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 inventiondisclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodimentexamples and aspectexamples of the inventiondisclosure, is given by way of illustration only, not limitation. Various changes and modification

within the scope of the ~~invention~~disclosure 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.).

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 *TGFBP2* 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 *TGFBP2* 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.

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~~therapy.

33. Use of the cell of claim 28, 30, or 31 in the manufacture of medicament for modifying transcription, expression and/or activity of one or more gene in a human T cell in ~~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.~~34. The cell for use according to claim 32, or the use of claim 33, ~~or the method of claim 34~~, wherein the cell is allogeneic or autologous to the human.