



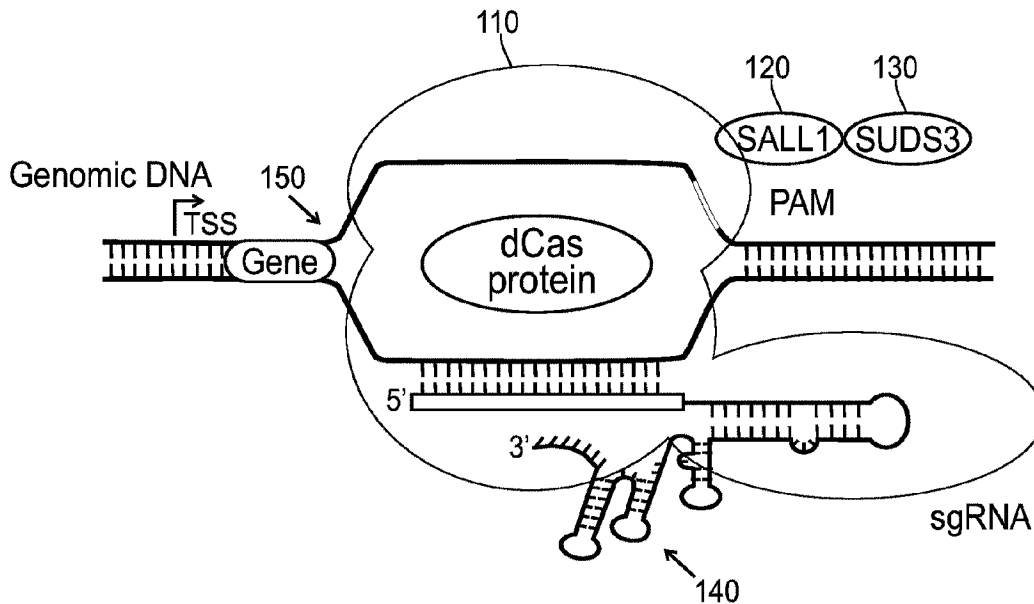
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(54) **Titre : PROTEINES DE FUSION POUR REPRESSION TRANSCRIPTIONNELLE BASEE SUR CRISPR**  
 (54) **Title: FUSION PROTEINS FOR CRISPR-BASED TRANSCRIPTIONAL REPRESSION**



**FIG. 1**

(57) **Abrégé/Abstract:**

The present disclosure provides compositions for modulating the expression of a nucleic acid and methods for using these compositions. The compositions comprise fusion proteins that contain the repressor domain for one or both of SALL1 and SUDS3. In some embodiments, the compositions are Cas fusion proteins that may be used in combination with a gRNA or other RNA. Additionally or alternatively, the compositions are RNA-repressor domain complexes.

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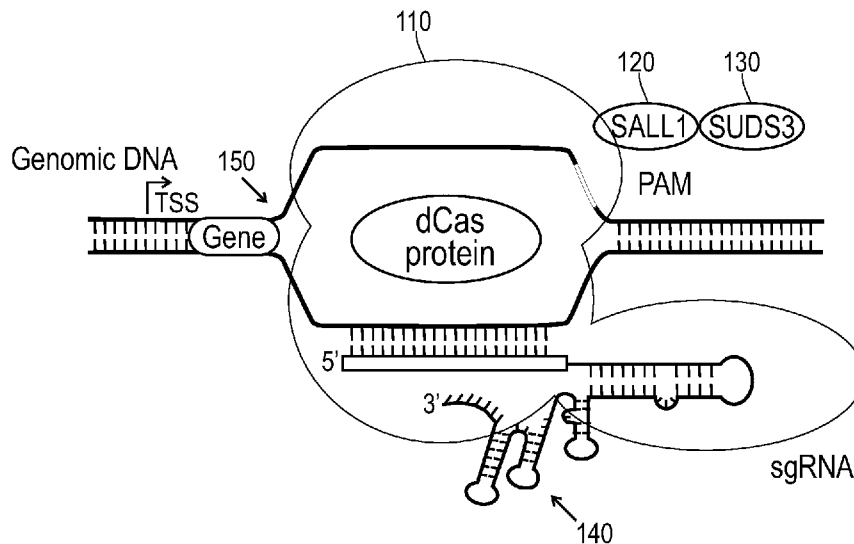
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(54) Title: FUSION PROTEINS FOR CRISPR-BASED TRANSCRIPTIONAL REPRESSION



**FIG. 1**

(57) Abstract: The present disclosure provides compositions for modulating the expression of a nucleic acid and methods for using these compositions. The compositions comprise fusion proteins that contain the repressor domain for one or both of SALL1 and SUDS3. In some embodiments, the compositions are Cas fusion proteins that may be used in combination with a gRNA or other RNA. Additionally or alternatively, the compositions are RNA-repressor domain complexes.



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## Fusion Proteins for CRISPR-based Transcriptional Repression

### [1] Cross-Reference to Related Application

[2] This application claims the benefit of the filing date of U.S. Provisional  
5 Application Serial No. 63/146,419, filed February 5, 2021, the entire disclosure of  
which is incorporated by reference as if set forth fully herein.

### [3] Field of the Invention

[4] The present invention relates to the field of CRISPR based transcriptional  
10 repression.

### [5] Background of the Invention

[6] The biotechnology community is now familiar with the CRISPR-Cas9  
system, which allows for specific targeting and editing of genes. This system was  
15 originally discovered within archaea and bacteria, but the great promise is for human  
applications.

[7] The basic CRISPR/Cas9 system comprises a Cas9 protein and a guide RNA  
("gRNA"). A spacer sequence (also referred to as a targeting sequence) within the  
gRNA leads the Cas9 protein to a genomic target site based on the complementarity  
20 between the spacer sequence and a sequence at the target site. After the Cas9 protein  
is brought to the target site it can cleave the target DNA and lead to DNA editing.  
Alternatively, a deactivated Cas9 ("dCas9") can be used for sequence-specific  
targeting and bringing other effectors with different functionalities.

[8] Because the CRISPR/Cas9 system is effective at locating and editing target  
25 sites, researchers have explored ways to piggyback on this system in order to  
introduce functions other than those that might be caused by the naturally occurring  
Cas9 protein's active sites. Further, researchers have begun to explore the use of  
other Cas proteins that rely on the specificity of gRNAs in order to bring those  
proteins to target sites.

[9] Although researchers originally discovered CRISPR-Cas9 systems in lower  
30 organisms, the systems have successfully been used for gene editing applications in  
mammalian cells, M. Jinek, *et al.*, "A programmable dual-RNA-guided DNA

endonuclease in adaptive bacterial immunity,” *Science*. 337: p. 816-821 (2012).

Further, researchers have been able to abolish the nuclease activity of the Cas protein by point mutations that are introduced into the catalytic residues (D10A and H840A in the case of the commonly used *Streptococcus pyogenes* Cas9 protein) yielding a deactivated Cas9 that maintains the ability to bind to target DNA when guided by sequence-specific guide RNAs. When the dCas9 is fused to transcriptional regulators and guided to gene promoter regions, it induces RNA-directed transcriptional regulation. CRISPR-based technologies for transcriptional regulation include CRISPR interference (CRISPRi) for transcriptional repression and CRISPR activation (CRISPRa) for transcriptional upregulation (Qi, L.S., *et al.*, “Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression,” *Cell*, 152(5): p. 1173-83 (2013); A.W. Cheng, *et al.*, “Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system,” *Cell Res.*, 23(10): p. 1163-71 (2013)).

[10] One known CRISPR-based approach for transcriptional repression utilizes the Krüppel associated box (KRAB) domain from zinc finger protein 10 (KRX1) as a transcriptional repressor, L.A.Gilbert, *et al.*, “CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes,” *Cell*, 154(2): p. 442-51 (2013); L. A. Gilbert *et al.*, “Genome-scale CRISPR-mediated control of gene repression and activation,” *Cell*, 159: p. 647–661 (2014). However, this approach has its limitations. Researchers have shown that it does not provide sufficient repression in all applications, and use of it can result in less robust repression of the target gene(s), L. Stojic *et al.*, “Specificity of RNAi, LNA and CRISPRi as loss-of-function methods in transcriptional analysis,” *Nucleic Acids Research*, 46(12): p. 5950–5966 (2018); Yeo, *et al.*, “An enhanced CRISPR repressor for targeted mammalian gene regulation,” *Nat. Methods*, 15(8): p. 611-616 (2018). Given the reported variability in performance of the CRISPR-KRAB fusion protein, which is the most commonly used fusion protein for transcriptional repression, there is a need for additional CRISPR-based approaches for transcriptional repression.

### [11] **Summary of the Invention**

[12] The present invention provides novel fusion proteins, nucleic acid sequences that encode those proteins, and methods of gene repression by using those proteins

and/or nucleic acids. Through the use of various embodiments of the present invention, one may efficiently and effectively regulate gene expression.

[13] According to a first embodiment, the present invention provides a Cas fusion protein comprising a Cas protein and one or both of a SALL1 repressor domain and a SUDS3 repressor domain. In some embodiments the Cas protein is deactivated, which also may be referred to as dead or attenuated.

[14] According to a second embodiment, the present invention provides a nucleic acid encoding a Cas fusion protein of the present invention.

[15] According to a third embodiment, the present invention provides an RNA-repressor domain complex. The RNA-repressor domain complex comprises: (a) a gRNA molecule, wherein the gRNA molecule contains 30 to 180 nucleotides; (b) a ligand binding moiety, wherein the ligand binding moiety is either (i) directly bound to the gRNA molecule, or (ii) bound through a ligand binding moiety linker to the gRNA molecule; (c) a ligand, wherein the ligand is capable of reversibly associating with the ligand binding moiety; and (d) a fusion protein, wherein the fusion protein comprises a SALL1 repressor domain and a SUDS3 repressor domain, and wherein the fusion protein is either (i) directly bound to the ligand, or (ii) bound through a linker to the ligand.

[16] According to a fourth embodiment, the present invention provides a method of modulating expression of a target nucleic comprising introducing a Cas fusion protein or an RNA-repressor domain complex of the present invention or a nucleic acid of the present invention into a cell such as a eukaryotic cell or an organism such as a mammal, *e.g.*, a human. In some embodiments, introduction is *in vivo*, *in vitro*, or *ex vivo*.

[17] According to a fifth embodiment, the present invention provides a kit comprising a Cas fusion protein of the present invention or a nucleic acid encoding a Cas fusion protein of the present invention and in some embodiments may further comprise either a gRNA or a nucleic acid that encodes for a gRNA.

[18] According to a sixth embodiment, the present invention provides a kit comprising an RNA-repressor domain complex, or a nucleic acid encoding, two molecules, an RNA-ligand binding domain and ligand-repressor of the present invention.

[19] According to a seventh embodiment, the present invention provides a protein that comprises, consists essentially of, or consists of a sequence at least 80% similar to SEQ ID NO: 10.

5 [20] **Brief Description of the Figures**

[21] **Figure 1** is a representation of Cas fusion protein of the present invention associated with a single guide RNA (“sgRNA”) and a target DNA.

[22] **Figure 2** is an example of an sgRNA that may be used in various embodiments of the present invention.

10 [23] **Figure 3A** is a graph that depicts gene knockdown in K562 cells nucleofected with either dCas9-KRAB or dCas9-SALL1-SUDS3 mRNA. **Figure 3B** is a graph that depicts gene knockdown in Jurkat cells nucleofected with either dCas9-KRAB or dCas9-SALL1-SUDS3 mRNA. **Figure 3C** is a graph that depicts gene knockdown in U2OS cells nucleofected with either dCas9-KRAB and dCas9-SALL1-SUDS3  
15 mRNA.

[24] **Figure 4** is a graph that compares repression of target genes when dCas9-SALL1-SUDS3 eGFP mRNA is introduced into HCT 116 cells to repression of target genes when dCas9-KRAB eGFP mRNA is introduced into HCT 116 cells. The genes are targeted with a pool of three synthetic sgRNAs delivered at 25 nM. Cells were  
20 sorted at 24 hours post-transfection into two categories: GFP negative (GFP Neg), and top 10% GFP expressing (Top 10%), and after 24 hours of recovery analyzed for transcriptional repression of the targeted genes.

[25] **Figures 5A – 5C** compare repression in systems that contain dCas9-KRAB versus systems that contain dCas9-SALL1-SUDS3 in different cell lines: U2OS  
25 (**figure 5A**); Jurkat (**figure 5B**); and hiPS stable hEF1 $\alpha$  (**figure 5C**).

[26] **Figure 6A** shows gene repression by dCas9-KRAB and dCas9-SALL1-SUDS3 against BRCA1, PSMD7, SEL1L, and ST3GAL4 in K562 cells. **Figure 6B** shows gene repression by dCas9-KRAB and dCas9-SALL1-SUDS3 against BRCA1, PSMD7, SEL1L, and ST3GAL4 in A375 cells.

30 [27] **Figures 7A-7D** compare the repression by dCas9-KRAB to repression by dCas9-SALL1-SUDS3 over a course of six days in U2OS cells for different gene targets: BRCA1 (**figure 7A**); CD46 (**figure 7B**); HBP1 (**figure 7C**); and SEL1L (**figure 7D**).

[28] **Figure 8A** shows repression using individual sgRNAs against PPIB, SEL1L, and RAB11A and pools of sgRNAs against these targets when introduced with Cas fusion proteins of the present invention. **Figure 8B** shows the repression of BRCA1, PSMD7, SEL1L, and ST3GAL4 by either individual sgRNAs or pools of sgRNAs  
5 against these targets when introduced with Cas fusion proteins of the present invention.

[29] **Figure 9** shows expression of the following genes: PPIB, RAB11A, and SEL1, in hiPSC cells in the presence of gRNAs and dCas9-SALL1-SUDS3 when multiplexing, *i.e.*, using sgRNAs against multiple genes.

10 [30] **Figure 10** is a graph that shows functional phenotype of the repression of PSMD3, PSMD8, and PSMD11 genes in U2OS-Ubi (G76V)-EGFP reporter cell line in the presence of gRNAs and dCas9 fused to KRAB or SALL1-SUDS3 at the N terminal amino acid of the dCas9 or the C terminal amino acid of dCas9.

[31] **Figure 11** is a graph of transcriptional repression in systems with a plasmid  
15 expressing gRNA and a plasmid expressing a fusion protein co-transfected in A375 cells.

[32] **Figure 12** is a graph of transcriptional repression in systems with a plasmid expressing gRNA and a plasmid expressing a fusion protein co-transfected in U2OS cells.

20 [33] **Figure 13** is a graph that shows the effect of combining SALL1 or SUDS3 each with an additional repressor domain.

[34] **Figure 14A** is a representation of repression by dMAD7-SALL1-SUDS3 as compared to dMAD7 in U2OS cells. **Figure 14B** is a representation of repression by dCasPhi8-SALL1-SUDS3 as compared to dCasPhi8 in U2OS cells.

25 [35]

[36] **Figure 15A** is a diagram of the effect of using sgRNAs of different crRNA-targeting sizes with Cas9 that is not deactivated for simultaneous repression and gene editing. **Figure 15B** is a graph that depicts the measurement of repression of MRE11a while LBR is simultaneously edited. **Figure 15C** is a graph that depicts the  
30 measurement of repression of MRE11a while PPIB is simultaneously edited. **Figure 15D** is a graph that depicts the measurement of repression of SEL1L while LBR is simultaneously edited. **Figure 15E** is a graph that depicts the measurement of repression of SEL1L while PPIB is simultaneously edited.

[37] **Figure 16** is a graph of repression effects of systems that contain single repressor dCas9 fusion proteins in the U2OS-Ubi (G76V)-EGFP reporter cell line.

[38] **Figure 17** is a graph that compares the transcriptional repression in U2OS cells stably expressing dCas9-KRAB, dCas9-KRAB MeCP2, or dCas9-SUDS3 that were transfected with synthetic guide RNAs.

[39] **Figure 18A** is a representation of the phenotypic effects of gene knockdown in U2OS Ubi[G76V]-EGFP reporter cells expressing either dCas9-KRAB or dCas9-SALL1-SUDS3 and transfected with synthetic guides targeting proteasome genes. **Figure 18B** depicts the corresponding transcriptional repression of the targeted proteasome genes.

[40] **Figure 19A** shows the transcriptional repression of PPIB and SEL1L in U2OS cells stably expressing either dCas9-SALL1-SUDS3 and a guide RNA from a single lentiviral vector or from two separate vectors. **Figure 19B** shows the transcriptional repression of PPIB and SEL1L in HCT 116 cells stably expressing either dCas9-SALL1-SUDS3 and a guide RNA from a single lentiviral vector or from two separate vectors.

[41] **Figure 20A** shows the transcriptional repression of BRCA1, PSMD7, SEL1L, and ST3GAL4 by either synthetic or plasmid sgRNAs in U2OS cells stably expressing dCas9-SALL1-SUDS3. **Figure 20B** shows the transcriptional repression of BRCA1, PSMD7, SEL1L, and ST3GAL4 by either synthetic or plasmid sgRNAs in A375 cells stably expressing dCas9-SALL1-SUDS3.

[42] **Figure 21** shows the transcriptional repression of CD151, SEL1L, SETD3, and TFRC by either synthetic sgRNAs or synthetic crRNA:tracrRNA complexes in U2OS cells stably expressing dCas9-SALL1-SUDS3.

[43] **Figure 22** shows the transcriptional repression of LBR, MRE11a, XRCC4, and SEL1L by synthetic sgRNAs with 5' truncated 14 mer targeting regions or full length 20 mer targeting regions in U2OS cells stably expressing dCas9-SALL1-SUDS3.

[44] **Figure 23A** is a representation of the phenotypic effects of gene knockdown of PSMD7 and PSMD11 by synthetic sgRNAs containing various combinations of two 2'-O-methyl and phosphorothioate linkages (2x MS) and two locked nucleic acid (LNA) modifications at the 5' and 3' end of the sgRNA in U2OS Ubi[G76V]-EGFP reporter cells expressing dCas9-SALL1-SUDS3. **Figure 23B** is a representation of the phenotypic effects of gene knockdown of PSMD7 and PSMD11 by synthetic sgRNAs

end stabilized with two 2'-O-methyl and phosphorothioate linkages (2x MS) and containing various locked nucleic acids (LNA) at different positions in the targeting region in U2OS Ubi[G76V]-EGFP reporter cells expressing dCas9-SALL1-SUDS3.

[45] **Figure 24A** shows the transcriptional repression of BRCA1, CD151, and SETD3 by synthetic crRNA:tracrRNA complexes in which the tracrRNA contains an MS2 stem loop at various positions (in stem loop 2 or 3' end of the tracrRNA) to recruit MCP-SALL1-SUDS3 to dCas9. **Figure 24B** shows the transcriptional repression of BRCA1, CD151, and SETD3 by synthetic crRNA:tracrRNA complexes in which the tracrRNA contains various MS2 stem loop sequences to recruit MCP-SALL1-SUDS3 to dCas9.

[46] **Figure 25A** is a graph that shows the transcriptional repression and protein level knockdown of CXCR3 in primary human CD4+ T cells nucleofected with dCas9-SALL1-SUDS3 and either a synthetic non-targeting control or a pool of three guides targeting the gene of interest one and three days post-nucleofection. **Figure 25B** provides representations of CXCR3 and CD4 protein expression in the aforementioned populations of T cells.

#### [47] **Detailed Description of the Invention**

[48] Reference will now be made in detail to various embodiments of the present invention, examples of which are illustrated in the accompanying figures. In the following description, numerous specific details are set forth in order to provide a thorough understanding of the present invention. However, unless otherwise indicated or implicit from context, the details are intended to be examples and should not be deemed to limit the scope of the invention in any way. Additionally, features described in connection with the various or specific embodiments are not to be construed as not appropriate for use in connection with other embodiments disclosed herein unless such exclusivity is explicitly stated or implicit from context.

[49] Headers are provided herein for the convenience of the reader and do not limit the scope of any of the embodiments disclosed herein.

30

#### [50] **Definitions**

[51] Unless otherwise stated or implicit from context the following terms and phrases have the meanings provided below.

[52] The phrase "2' modification" refers to a nucleotide unit having a sugar moiety that is modified at the 2' position of the sugar moiety. An example of a 2' modification is a 2'-O-alkyl modification that forms a 2'-O-alkyl modified nucleotide or a 2' halogen modification that forms a 2' halogen modified nucleotide.

5 [53] The phrase "2'-O-alkyl modified nucleotide" refers to a nucleotide unit having a sugar moiety, for example, a deoxyribosyl or ribosyl moiety that is modified at the 2' position such that an oxygen atom is attached both to the carbon atom located at the 2' position of the sugar and to an alkyl group. In various embodiments, the alkyl moiety consists of or consists essentially of carbon(s) and hydrogens. When the O  
10 moiety and the alkyl group to which it is attached are viewed as one group, they may be referred to as an O-alkyl group, *e.g.*, -O-methyl, -O-ethyl, -O-propyl, -O-isopropyl, -O-butyl, -O-isobutyl, -O-ethyl-O-methyl (-OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), and -O-ethyl-OH (-OCH<sub>2</sub>CH<sub>2</sub>OH). A 2'-O-alkyl modified nucleotide may be substituted or unsubstituted.

15 [54] The phrase "2' halogen modified nucleotide" refers to a nucleotide unit having a sugar moiety, for example, a deoxyribosyl moiety that is modified at the 2' position such that the carbon at that position is directly attached to a halogen species, *e.g.*, F, Cl, or Br.

[55] The term "complementarity" refers to the ability of a nucleic acid to form one  
20 or more hydrogen bonds with another nucleic acid sequence by either traditional Watson-Crick base-pairing or other non-traditional types of base pairs. A percent complementarity indicates the percentage of residues in a nucleic acid molecule that can form hydrogen bonds (*e.g.*, Watson-Crick base pairing) with a second nucleic acid sequence (*e.g.*, 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and  
25 100% complementary, respectively). "Perfect complementarity" means that all of the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Substantially complementary" as used herein refers to a degree of complementarity that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,  
30 at least 95%, at least 97%, at least 98%, or at least 99%, over a region of, for example, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more consecutive nucleotides, or refers to two nucleic acids that hybridize under stringent conditions.

[56] The term “encodes” refers to the ability of a nucleotide sequence or an amino acid sequence to provide information that describes the sequence of nucleotides or amino acids in another sequence or in a molecule. Thus, a nucleotide sequence encodes a molecule that contains the same nucleotides as in the nucleotide sequence that encodes it; that contains the complementary nucleotides according to Watson-Crick base pairing rules; that contains the RNA equivalent of the nucleotides that encode it; that contains the RNA equivalent of the complement of the nucleotides that encode it; that contains the amino acid sequence that can be generated based on the consecutive codons in the sequence; and that contains the amino acid sequence that can be generated based on the complement of the consecutive codons in the sequence.

[57] A “gRNA” is a guide RNA. A gRNA comprises, consists essentially of, or consists of a CRISPR RNA (crRNA) and in some embodiments, it may also comprise a trans-activating CRISPR RNA (tracrRNA). It may be created synthetically or enzymatically, and it may be in the form of a contiguous strand of nucleotides in which case it is a “sgRNA” or in some embodiments, formed by the hybridization of a crRNA and a tracrRNA that are not covalently linked together to form a contiguous chain of nucleotides. Additionally, each gRNA (or component thereof, *e.g.*, crRNA and tracrRNA if present) may independently be encoded by a plasmid, lentivirus, or AAV (adeno associated virus), a retrovirus, an adenovirus, a coronavirus, a Sendai virus or other vector. The gRNA introduces specificity into CRISPR/Cas systems. The specificity is dictated in part by base pairing between a target DNA and the sequence of a region of the gRNA that may be referred to as the spacer region or targeting region.

[58] Another factor affecting specificity to gRNAs binding to a target DNA sequence is the presence of a PAM (protospacer-adjacent motif) sequence (also referred to as a PAM site) in a target sequence. Each target sequence and its corresponding PAM site/sequence may collectively be referred to as a Cas-targeted site. For example, the Class 2 CRISPR system of *S. pyogenes* uses targeted sites having N12-20NGG, where NGG represents the PAM site from *S. pyogenes*, and N12-20 represents the 12-20 nucleotides directly 5' to the PAM site. Additional PAM site sequences from other species of bacteria include NGGNG, NNNNGATT, NNAGAA, NNAGAAW, and NAAAAC. See, *e.g.*, US 20140273233, WO 2013176772, Cong *et al.*, Science 339 (6121): 819–823 (2012), Jinek *et al.*, Science 337 (6096): p. 816–821 (2012), Mali *et al.*, Science 339 (6121): p. 823–826 (2013),

Gasiunas *et al.*, Proc Natl Acad. Sci. U S A, 109 (39): p. E2579–E2586 (2012), Cho *et al.*, Nature Biotechnology 31: p. 230–232 (2013), Hou *et al.*, Proc. Natl Acad. Sci. U S A. 110(39): p.15644-15649 (2013), Mojica *et al.*, Microbiology 155 (Pt 3): p. 733-740 (2009), and [www.addgene.org/CRISPR/](http://www.addgene.org/CRISPR/). The contents of these documents are  
5 incorporated herein by reference in their entireties.

[59] The terms “hybridization” and “hybridizing” refer to a process in which completely, substantially, or partially complementary nucleic acid strands come together under specified hybridization conditions to form a double-stranded structure or region in which the two constituent strands are joined by hydrogen bonds. Unless  
10 otherwise stated, the hybridization conditions are naturally occurring or lab-designed conditions. Although hydrogen bonds typically form between adenine and thymine or uracil (A and T or U) or between cytidine and guanine (C and G), other base pairs may form (see *e.g.*, Adams *et al.*, The Biochemistry of the Nucleic Acids, 11th ed., 1992).

[60] A “ligand binding moiety” refers to a moiety such as an aptamer *e.g.*, oligonucleotide or peptide or another compound that binds to a specific ligand and can reversibly or irreversibly be associated with that ligand. To be reversibly associated means that two molecules or complexes can retain association with each other by, for example, noncovalent forces such as hydrogen bonding, and be separated  
20 from each other without either molecule or complex losing the ability to associate with other molecules or complexes.

[61] The term “modified nucleotide” refers to a nucleotide having at least one modification in the chemical structure of the base, sugar and/or phosphate, including, but not limited to, 5-position pyrimidine modifications, 8-position purine  
25 modifications, modifications at cytosine exocyclic amines, and substitution of 5-bromo-uracil or 5-iodouracil; and 2'- modifications, including but not limited to, sugar-modified ribonucleotides in which the 2'-OH is replaced by a group such as an H, OR, R, halo, SH, SR, NH<sub>2</sub>, NHR, NR<sub>2</sub>, or CN.

[62] Modified bases refer to nucleotide bases such as, for example, adenine,  
30 guanine, cytosine, thymine, uracil, xanthine, inosine, and queuosine that have been modified by the replacement or addition of one or more atoms or groups. Some examples of these types of modifications include, but are not limited to, alkylated, halogenated, thiolated, aminated, amidated, or acetylated bases, alone and in various combinations. More specific modified bases include, for example, 5-propynyluridine,

5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N,-dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-amino)propyluridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 5 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methoxyuridine, deazanucleotides such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, 10 pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4,6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines 15 and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties 20 may be, or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4-thioribose, and other sugars, heterocycles, or carbocycles.

[63] The term “nucleotide” refers to a ribonucleotide or a deoxyribonucleotide or modified form thereof, as well as an analog thereof. Nucleotides include species that comprise purines, *e.g.*, adenine, hypoxanthine, guanine, and their derivatives and 25 analogs, as well as pyrimidines, *e.g.*, cytosine, uracil, thymine, and their derivatives and analogs. Preferably, a nucleotide comprises a cytosine, uracil, thymine, adenine, or guanine moiety. Further, the term nucleotide also includes those species that have a detectable label, such as for example a radioactive or fluorescent moiety, or mass label attached to the nucleotide. The term nucleotide also includes what are known in 30 the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine. Nucleotide analogs are, for example, meant to include nucleotides with bases such as inosine, queuosine, xanthine, sugars such as 2'-methyl ribose, and non-natural phosphodiester

internucleotide linkages such as methylphosphonates, phosphorothioates, phosphoroacetates and peptides.

[64] The term "repressor domain" refers to the amino acid sequence that form the domain of a repressor molecule that leads to inhibition of the expression of a gene.

5 [65] The terms "subject" and "patient" are used interchangeably herein to refer to an organism, *e.g.*, a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets such as dogs and cats. The tissues, cells and their progeny of an organism or other biological entity obtained *in vivo* or cultured *in vitro* are also  
10 encompassed within the terms subject and patient. Additionally, in some embodiments, a subject may be an invertebrate animal, for example, an insect or a nematode; while in others, a subject may be a plant or a fungus.

[66] A "terminal amino acid" is the last amino acid within a protein or within a region of a fusion protein. Within a fusion protein a terminal amino acid of a Cas  
15 protein may, for example, be bound not only to another amino acid within the Cas protein region of the fusion protein, but also to a repressor domain or to a linker. Similarly, within a fusion protein, a terminal amino acid of a repressor domain may, for example, be bound not only to another amino acid within the repressor domain, but also to another repressor domain or to a Cas protein region of a fusion protein or  
20 to a linker. A terminal amino acid may be a C terminal amino acid or an N terminal amino acid.

[67] As used herein, "treatment," "treating," "palliating," and "ameliorating" are used interchangeably. These terms refer to an approach for obtaining beneficial or  
25 desired results including, but not limited to, a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant any therapeutically relevant improvement in or effect on one or more diseases, conditions, or symptoms under treatment. For prophylactic benefit, the complexes of the present invention may be administered to a subject, or a subject's cells or tissues, or those of another subject extracorporeally before re-administration, at risk of developing a particular disease, condition, or  
30 symptom, or to a subject reporting one or more of the physiological symptoms of a disease, even though the disease, condition, or symptom might not have yet been manifested.

[68] The term "vector" refers to a molecule or complex that transports another molecule and includes but is not limited to a nucleic acid molecule capable of

transporting another nucleic acid molecule to which it has been linked, or that has been incorporated within the vector sequence. A vector can be introduced into cells and organisms to express RNA transcripts, proteins, and peptides, and may be termed an “expression vector.” Examples of vectors include, but are not limited to, plasmids, 5 lentiviruses, alphaviruses, adenoviruses, or adeno-associated viruses. The vector may be single stranded, double stranded or have at least one region that is single stranded and at least one region that is double stranded. Further, the nucleic acid may comprise, consist essentially of, or consist of RNA or DNA.

[69] As disclosed herein, a number of ranges of values are provided. It is 10 understood that each intervening value, to the tenth of the unit of the lower limit, unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these 15 smaller ranges may independently be included or excluded in the range, and each range where either, neither, or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[70] The term “about” generally refers to plus or minus 10% of the indicated 20 number. For example, “about 10%” may indicate a range of 9% to 11%, and “about 20” may mean from 18-22. Other meanings of “about” may be apparent from the context, such as rounding off; for example “about 1” may also mean from 0.5 to 1.4.

[71] Various embodiments of the present invention are directed to fusion proteins 25 and their uses. Fusion proteins are molecules that contain a portion or a complete amino sequence of each of two or more proteins. The components of fusion proteins may be fused directly to each other through, for example, covalent bonds or through linkers as described below. Fusion proteins may also be associated with moieties that 30 are do not contain amino acids such as nucleotides sequences.

[72] **Cas fusion proteins**

[73] According to a first embodiment, the present invention is directed to a Cas fusion protein. A Cas fusion protein comprises, consists essentially of, or consists of a

Cas protein and one or both of a SALL1 repressor domain and a SUDS3 repressor domain or a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% the same as one of the aforementioned repressor domains.

[74] The Cas protein may be any CRISPR associated protein that is naturally occurring  
 5 in for example, archaea or bacteria, or a modified version thereof such as a deactivated version, a truncated version thereof, or a derivative thereof. Amino acid sequences and nucleic acids sequences for numerous Cas proteins are available through publicly available sources such as the United States of America's National Institute of Health: <https://www.ncbi.nlm.nih.gov/> or Uniprot <https://www.uniprot.org/> the entire contents of  
 10 which are incorporated by reference herein.

[75] Examples of Cas proteins include but are not limited to: Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12f, Cas12h, Cas12i, Cas12j, Mad7, CasX, CasY, Cas 13a, Cas14, C2c1,  
 15 C2c2, C2c3, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Cse1, Cse2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cul966, and homologs or modified versions thereof. Unless otherwise stated or implicit from context the  
 20 recitation of a Cas protein includes all active and deactivated versions, as well as homologs and derivatives thereof.

[76] In some embodiments, the Cas protein is a Type II Cas protein such as Cas9 or a Type V Cas protein such as Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12f, Cas12h, Cas12i, Cas12j, and MAD7.

[77] Modified versions of Cas proteins that may be used in the present invention, include but are not limited to catalytically inactive versions such as dCas9 and dCas12 or versions that have modified attenuated catalytic activity to provide a nicking function such as the nickase nCas9. A nicking enzyme is an enzyme that cuts one strand of a double-stranded DNA at a specific recognition nucleotide sequence.  
 30 These enzymes cut only one strand of the DNA duplex, to produce DNA molecules that are "nicked," rather than cleaved. Examples of amino acid sequences of Cas proteins that may be of use in connection with the present invention are:

[78] Deactivated Cas9:

MDYKDDDDKMAPKKKRKVGIIHGVPAAADKKYSIGLAIGTNSVGWAVITDEY  
 KVPSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKN  
 RICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK  
 YPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKL  
 5 FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLF  
 GNLIASLGLTPNFKSNFDLAEDAKLQLSKDXYDDDLNLLAQIGDQYADLF  
 LAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL  
 PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNRE  
 DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYY  
 10 VGPLARGNSRFAMTRKSEETITPWNFEVVDKGGASAQSFIERMTNFDKNLP  
 NEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT  
 NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD  
 NEENEDILEDIVLTLTLFEDREMIERLKYAHLFDDKVMKQLKRRRYTGWG  
 RLSRKLINGIRDKQSGKTILDFLKSDFANRNFMQLIHDDSLTFKEDIQKAQVS  
 15 GQGDSLHEHIANLAGSPAIKKGILQTVKVDELVKVMGRHKPENIVIAMARE  
 NQTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYLQN  
 GRDMYVDQELDINRLSDYDVAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV  
 PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQL  
 VETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYK  
 20 VREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIAS  
 EQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD  
 FATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY  
 GGFDSPVAVSVLVVAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEA  
 KGYKEVKKDLIILPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL  
 25 YLASHYEKLGSPEDNEQKQLFVEQHKKHYLDEIIEQISEFSKRVILADANLKD  
 VLSAYNKHRDKPIREQAENIHLFTLNLGAPAAFKYFDTTIDRKRYSSTKEVL  
 DATLIHQSIITGLYETRIDLSQLGGDKRPAATKAGQAKKKK (SEQ ID NO:  
 182)

30 [79] Deactivated MAD7:

MVDGKPIPNPLLGLDSTPKKKRKNVNGTNNFQNFQFIGISSLQKTLRNALIPTETT  
 QQFIVKNGIIEKEDELGENRQILKDIMDDYYRGFISSETLSSIDDIDWTSLEKME  
 IQLKNGDNKDTLIKEQTEYRKAIHKKFANDDRFKNMFS AKLISDILPEFVIHNN  
 NYSASEKEEKTQVIKLSRFATSFKDYFKNRANCFSADDISSSSCHRIVNDNAE  
 35 IFFSNALVYRRIVKSLSNDDINKISGDMKDSLKEMSLEEIYSYEKYGEFITQEGI  
 SFYNDICGKVNFSMNLVCQKNKENKNLYKLQKLHKQILCIADTSYEVYPYKFE  
 SDEEVYQSVNGFLDNISSKHIVERLRKIGDNYNGYNLDKIYIVSKFYESVSQKT  
 YRDWETINTALEIHYNILPGNGKSKADKVKKAVKNDLQKSITEINELVSNYK  
 LCSDDNIKAETIHEISHILNNFEAQELKYNPEIHLVESELKASELKNVLDVIM  
 40 NAFHWCSVFMTEELVDKDNFYAELEEIYDEIYPVISLYNLVRNYVTQKPYST  
 KKIKLNFGIPTLADGWSKSKKEYSNNAIILMRDNLYYLGIFNAKNKPKKIIIEGN  
 TSENKGDYKMIYNLLPGPNKMIPKVFLLSSTGVTYKPSAYILEGYKQNKHI  
 KSSKDFDITFCHDLIDYFKNCIAIHPWKNFGFDFSDTSTYEDISGFYREVELQ  
 GYKIDWTYISEKDIDLLQEKGLYLFQIYNKDFSKKSTGNDNLHTMYLKNLFS  
 45 EENLKDIVLKLNGEAEIFFRKSIIKPIIHKKGSILVNRTYEAEEKDQFGNIQIV  
 RKNIPENIYQELYKYFNDKSDKELSDEAAKLNKVVGHHEAATNIVKDYRYTY  
 DKYFLHMPITINFKANKTGFINDRILQYIAKEKDLHVIGIARGERNLIYVSVIDT

CGNIVEQKSFNIVNGYDYQIKLKQQEGARQIARKEWKEIGKIKEIKEGYLSLVI  
 HEISKMVIKYNAILAMADLSYGFKKGRFKVERQVYQKFETMLINKLNYLVFK  
 DISITENGGLLKGYQLTYIPDKLKNVGHQCFCIFYVPAAYTSKIDPTTGFFVNIF  
 KFKDLTVDAKREFIKKFDSSIRYDSEKNLFCFTFDYNNFITQNTVMSKSSWSVY  
 5 TYGVRIKRRFVNGRFSNESDTIDITKDMEKTLEMMDINWRDGHDLRQDIIDYEI  
 VQHIFEIFRLTVQMRNSLSELEDRDYDRLISPVLNENNIFYDSAKAGDALPKD  
 AAANGAYCIALKGLYEIKQITENWKEDGKFSRDCLKISNKDWFDFIQNKRYL  
 KRPAATKKAGQAKKKK (SEQ ID NO: 39)

10 [80] Deactivated CasPhi8 (dCasPhi8):

MVDGSGPAAKRVKLDSSGGIKPTVSQFLTPGFKLIRNHSRTAGLKLKNEGEEA  
 CKKFFVRENEIPKDECPNFQGGPAIANIIAKSREFTEWEIYQSSLAIQEVIPTLPK  
 DKLPEPILKEEWRAQWLSEHGLDTPVYKEAAGLNLIKNAVNTYKGVQVKV  
 DNKNKNNLAKINRKNEIAKLNGEQEISFEEIKAFDDKGYLLQKPSPNKSIYCY  
 15 QSVSPKPFITSKYHNVNLPPEYIGYYRKSNEPIVSPYQFDRLRPIGEPGYVPKW  
 QYTFLSKKENRRRKLKRIKRVSPILGIIKIKDWCVFDMRGLLRTNHWKY  
 HKPTDSINDLFDYFTGDPVIDTKANVVRFRYKMEINGIVNYKPVREKKGKELL  
 ENICDQNGSCKLATVAVGQNNPVAIGLFELKKVNGELTKTLISRHPTPIDFCN  
 KITAYRERYDKLESSIKLDAIKQLTSEQKIEVDNYNNNFTPQNTKQIVCSKLN  
 20 NPNDLPWDKMISGTHFISEKAQVSNKSEIYFTSTDKGKTKDVMKSDYKWFQD  
 YKPKLSKEVRDALSDIEWRLRRESLEFNKLSKSREQDARQLANWISSMCDVIG  
 IENLVKKNFFGGSGKREPGWDFYKPKKENRWWINAIHKALTELSQNKGGK  
 RVILLPAMRTSITCPKCKYCDSKNRNGEKFNCLKCGIELNADIDVATENLATV  
 AITAQSMKPTCERSGDAKPKVRARKAKAPEFHDKLAPSYTVVLREAVKSPA  
 25 ATKKAGQAKKKK (SEQ ID NO: 40)

[81] The Cas proteins may be used with repressor domains. The repressor domain of SALL1 is:

30 MSRRKQAKPQHFQSDPEVASLPRRDGDTEKQPSRPTKSKDAHVCGRCCA  
 FELSDLLLHKKNCTKNQLVLIVNENPASPETFSPSPPPDNPDEQMNDTVNKT  
 DQVDCSDLSEHNGLDREESMEVEAPVANKSGSGTSSGSHSSTAPSSSSSSSSSS  
 GGGGSSSTGTSAITTSLPQLGDLT (SEQ ID NO: 1).

35 [82] The repressor domain of SUDS3 is:

MSAAGLLAPAPAQAGAPPAPEYYPEEDEELES AEDDERSCRGRESDEDTE  
 SETDLAKHDEEDYVEMKEQMYQDKLASLKRQLQQLQEGTLQEYQKRMKKL  
 DQQYKERIRNAELFLQLETEQVERNYIKEKKA AVKEFEDKKVELKENLIAELE  
 40 EKKKMIENEKLTMELTGDSMEVKPIMTRKLRRRPNDPVPIDKRRKPAPAQL  
 NYLLTDEQIMEDLRTLNLKSPKRPASPSSPEHLPATPAESPAQRFEARIEDGK  
 LYYDKRWYHKSQAIYLESKDNQKLSCVISSVGANEIWRKTS DSTKMRIYLG  
 QLQRGLFVIRRRSAA (SEQ ID NO: 2).

45 [83] In some embodiments, the Cas fusion protein comprises, consists essentially  
 of, or consists of a Cas protein and the SALL1 repressor domain or a repressor  
 domain that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ  
 ID NO: 1. In some embodiments, the SALL1 repressor domain or a repressor domain

that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 1 is attached to the N terminal amino acid of the Cas protein. In some embodiments, the SALL1 repressor domain or a repressor domain that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 1 is attached to the C terminal amino acid of the Cas protein.

[84] In some embodiments, the Cas fusion protein comprises, consists essentially of, or consists of a Cas protein and the SUDS3 repressor domain or a repressor domain that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 2. In some embodiments, the SUDS3 repressor domain or a repressor domain that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 2 is attached to the N terminal amino acid of the Cas protein. In some embodiments, the SUDS3 repressor domain or a repressor domain that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 2 is attached to the C terminal amino acid of the Cas protein.

[85] In some embodiments, the Cas fusion protein comprises, consists essentially of, or consists of a Cas protein and both the SALL1 repressor domain and the SUDS3 repressor domain. In some embodiments, this Cas fusion protein is organized in one of the following ways (written N terminus to C terminus):

[86] [Cas protein]-[SALL1 repressor domain]-[SUDS3 repressor domain]

[87] [Cas protein]-[SUDS3 repressor domain]-[SALL1 repressor domain]

[88] [SALL1 repressor domain]-[SUDS3 repressor domain]-[Cas protein]

[89] [SUDS3 repressor domain]-[SALL1 repressor domain]-[Cas protein]

[90] [SALL1 repressor domain]-[Cas protein]-[SUDS3 repressor domain]

[91] [SUDS3 repressor domain]-[Cas protein]-[SALL1 repressor domain]

[92] In some embodiments, the Cas fusion protein comprises a SALL1 repressor domain and a SUDS3 repressor domain, wherein the SALL1 repressor domain comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 1 and the SUDS3 repressor domain comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 2. In some embodiments, the Cas fusion protein comprises a SALL1 repressor domain and a SUDS3 repressor domain, wherein the SALL1 repressor domain comprises, consists essentially of, or consists of a sequence is the same as SEQ ID NO: 1 and the SUDS3 repressor domain comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 2.

[93] In some embodiments, the Cas fusion protein comprises, consists essentially of, or consists of a Cas protein and two or more copies of both the SALL1 repressor domain and the SUDS3 repressor domain. In some embodiments, this Cas fusion protein is organized in one of the following ways:

5 [94] [SALL1 repressor domain]-[SUDS3 repressor domain]-[Cas protein]-[SALL1 repressor domain]-[SUDS3 repressor domain]

[95] [SALL1 repressor domain]-[SUDS3 repressor domain]-[Cas protein]-[SUDS3 repressor domain]-[SALL1 repressor domain]

[96] [SUDS3 repressor domain]-[SALL1 repressor domain]-[Cas protein]-[SALL1  
10 repressor domain]-[SUDS3 repressor domain]

[97] [SUDS3 repressor domain]-[SALL1 repressor domain]-[Cas protein]-[SUDS3 repressor domain]-[SALL1 repressor domain]

[98] In some embodiments, the Cas fusion protein comprises a plurality of SALL1 repressor domains and a plurality of SUDS3 repressor domains, wherein each SALL1  
15 repressor domain comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 1 and each SUDS3 repressor domain comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 2. In some embodiments, the Cas fusion protein comprises a plurality of SALL1 repressor  
20 domains and a plurality of SUDS3 repressor domains, wherein each SALL1 repressor domain comprises, consists essentially of, or consists of a sequence is the same as SEQ ID NO: 1 and each SUDS3 repressor domain comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 2.

[99] In some embodiments, the Cas fusion protein also comprises a domain of an  
25 additional repressor protein: [R]. In some embodiments, [R] is selected from the group consisting of the NIPP1 repressor domain, the KRAB repressor domain, the DNMT3A repressor domain, the BCL6 repressor domain, the CbpA repressor domain, the H-NS repressor domain, the MBD3 repressor domain, and the KRAB-Me-CP2 repressor domain.

30 [100] The NIPP1 repressor domain, may be represented as follows:  
MVQTA VVPVKKKRVEGPGSLGLEESGSRMQNFAFSGGLYGGLPPTHSEAGSQP  
HGIHGTALIGGLPMPYPNLAPDVDLTPVVPSAVNMNPAPNPAVYNPEAVNEPKK  
KKYAKEAWPGKKPTPSLLI (SEQ ID NO: 34) or a sequence that is at least 80%, at  
least 85%, at least 90%, or at least 95% that same as SEQ ID NO: 34.

[101] The KRAB repressor domain, may be represented as follows:

MDAKSLTAWSTRLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNL  
VSLGYQLTKPDVILRLEKGEEPWLV (SEQ ID NO: 35) or a sequence that is at least  
80%, at least 85%, at least 90%, or at least 95% that same as SEQ ID NO: 35.

5 [102] The DNMT3A repressor domain, may be represented as follows:

PSRLQMFFANNHDQEFDPKVPYPPVPAEKRPKIRVLSLFDGIATGLLVKDLGI  
QVDRYIASEVCEDSITVGMVRHQGKIMYVGDVRSVTQKHIQEWGPFDLVIGG  
SPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFVWLFENVVA  
10 MGVS DKRDISRFLESNPVMIDAKEVSAHRARYFWGNLPGMNRPLASTVND  
KLELQECLEHGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKEDILWCTEM  
ERVFGFPVHYTDVSNMSRLARQRLGRSWSVPVIRHLFAPLKEYFACV

(SEQ ID NO: 36) or a sequence that is at least 80%, at least 85%, at least 90%, or at least  
15 95% that same as SEQ ID NO: 36.

[103] The BCL6 repressor domain may be represented as follows:

MASPADSCIQFTRHASDVLLNLRSLRSDILTDVVIVVSREQFRAHKTVLMAC  
SGLFYSIFTDQLKCNLSVINLDPEINPEGFCILLDFMYTSRLNLREGNIMAVMA  
20 TAMYLQMEHVVDTCRKFIAKASEAEM (SEQ ID: 173)  
or a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% that same as  
SEQ ID NO: 173.

[104] The CbpA repressor domain may be represented as follows:

MELKDYYAIMGVKPTDDLKTIKTAYRRLARKYHPDVSKEPDAEARFKEVAE  
25 AWEVLSDEQRRAEYDQMWHRNDPQFNRFHHGDGQSFNAEDFDDIFSSIF  
GQHARQSRQRPATRGHDIEIEVAVFLEETLTEHKRTISYNLPVYNAFGMIEQEI  
PKTLNVKIPAGVGNQRIKLGQGTGPGNGDLWLVIHAPHPLFDIVGQ  
DLEIVVPVSPWEAALGAKVTVP TLKESILLTIPPGSQAGQRLRVKGGKGLVSKK  
QTGDLAYVLKIVMPPKPDENTAALWQQLADAQSSFDPRKDWGKA  
30 (SEQ ID: 174) or a sequence that is at least 80%, at least 85%, at least 90%, or at least  
95% that same as SEQ ID NO: 174.

[105] The H-NS repressor domain may be represented as follows:

MSEALKILNNIRTLRAQARECTLETLEEMLEKLEVVVNERREEESAAAAEVEE  
35 RTRKLQYREMLIADGIDPNELLNSLAAVKSGTKAKRAQRPAKYSYVDENGE  
TKTWTGQGRTPAVIKKAMDEQKSLDDFLIKQ  
(SEQ ID: 175) or a sequence that is at least 80%, at least 85%, at least 90%, or at least  
95% that same as SEQ ID NO: 175.

[106] The MBD3 repressor domain may be represented as follows:

MERKRWECPALPQGWEREVPRRSGLSAGHRDVFYVSPSGKKFRSKPQLAR  
40 YLGGSMDLSTFDFTGKMLMSKMNKSRQVRVYDSSNQVKGKPDNLALPV  
RQTASIFKQPVTKITNHPNKNVKS DPQKAVDQPRQLFWKKLSGLNAFDIAEE  
LVKTMDLPKGLQGVGPGCTDETLLSAIASALHTSTMPITGQLSAAVEKNPGV

WLNTTQPLCKAFMVTDEDIRKQEELVQQVRKRLEEALMADMLAHVEELAR  
DGEAPLDKACAEDDDEEDEEEEEEPDPDPEMEHV

(SEQ ID: 176) or a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% that same as SEQ ID NO: 176.

5 [107] The KRAB-MeCP2 repressor domain may be represented as follows:

MDAKSLTAWSRITLVTFKDFVDFDTREEWKLLDTAQQIVYRNVMLENYKNL  
VSLGYQLTKPDVILRLEKGEPPWLVSGGGSGGGSSPKKKRKRKVEASVQVKRV  
LEKSPGKLLVKMPFQASPGGKGGGGATTSAQVMVIKRPGRKRKAEADPQAI  
PKKRGRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPKRRKTRETVSIEVK

10 EVVKPLLVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKE  
(SEQ ID: 177) or a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% that same as SEQ ID NO: 177.

[108] Examples of the orientation of these sequences may be represented as follows:

[109] [Cas protein]-[SALL1 repressor domain]-[R]

15 [110] [Cas protein]-[SUDS3 repressor domain]-[R]

[111] [R]-[SUDS3 repressor domain]-[Cas protein]

[112] [R]-[SALL1 repressor domain]-[Cas protein]

[113] [Cas protein]-[R]-[SUDS3 repressor domain]

[114] [Cas protein]-[R]-[SALL1 repressor domain]

20 [115] [SALL1 repressor domain]-[R] [Cas protein]

[116] [SUDS3 repressor domain]-[R]-[Cas protein]

[117] [R]-[Cas protein]-[SUDS3 repressor domain]

[118] [R]-[Cas protein]-[SALL1 repressor domain]

[119] [SALL1 repressor domain]-[Cas protein]-[R]

25 [120] [SUDS3 repressor domain]-[Cas protein]-[R]

[121] Further, in some embodiments, the Cas fusion protein comprises, consists essentially of, or consists of a Cas protein and each of the SALL1 repressor domain, the SUDS3 repressor domain, and the [R] repressor domain. When all three repressor domains are present, they may all be on the C terminal amino acid of the Cas protein, all be on the N terminal amino acid of the Cas protein, two be on the C terminal amino acid of the Cas protein and one be on the N terminal amino acid of the Cas protein, or two be on the N terminal amino acid of the Cas protein and one be on the C terminal amino acid of the Cas protein. Examples of the orientation of these sequences may be represented as follows:

35 [122] [Cas protein]-[SALL1 repressor domain]-[R]-[SUDS3 repressor domain]

[123] [Cas protein]-[SALL1 repressor domain]-[SUDS3 repressor domain]-[R]

- [124] [Cas protein]-[SUDDS3 repressor domain]-[SALL1 repressor domain]-[R]  
 [125] [Cas protein]-[SUDDS3 repressor domain]-[R]-[SALL1 repressor domain]  
 [126] [Cas protein]-[R]-[SUDDS3 repressor domain]-[SALL1 repressor domain]  
 [127] [Cas protein]-[R]-[SALL1 repressor domain]-[SUDDS3 repressor domain]  
 5 [128] [SALL1 repressor domain]-[R]-[SUDDS3 repressor domain]-[Cas protein]  
 [129] [SALL1 repressor domain]-[SUDDS3 repressor domain]-[R]-[Cas protein]  
 [130] [SUDDS3 repressor domain]-[SALL1 repressor domain]-[R]-[Cas protein]  
 [131] [SUDDS3 repressor domain]-[R]-[SALL1 repressor domain]-[Cas protein]  
 [132] [R]-[SUDDS3 repressor domain]-[SALL1 repressor domain]-[Cas protein]  
 10 [133] [R]-[SALL1 repressor domain]-[SUDDS3 repressor domain]-[Cas protein]  
 [134] [SALL1 repressor domain]-[Cas protein]-[R]-[SUDDS3 repressor domain]  
 [135] [SALL1 repressor domain]-[Cas protein]-[SUDDS3 repressor domain]-[R]  
 [136] [SUDDS3 repressor domain]-[Cas protein]-[SALL1 repressor domain]-[R]  
 [137] [SUDDS3 repressor domain]-[Cas protein]-[R]-[SALL1 repressor domain]  
 15 [138] [R]-[Cas protein]-[SUDDS3 repressor domain]-[SALL1 repressor domain]  
 [139] [R]-[Cas protein]-[SALL1 repressor domain]-[SUDDS3 repressor domain]  
 [140] [R]-[SUDDS3 repressor domain]-[Cas protein]-[SALL1 repressor domain]  
 [141] [SUDDS3 repressor domain]-[R]-[Cas protein]-[SALL1 repressor domain]  
 [142] [SALL1 repressor domain]-[R]-[Cas protein]-[SUDDS3 repressor domain]  
 20 [143] [R]-[SALL1 repressor domain]-[Cas protein]-[SUDDS3 repressor domain]  
 [144] [SUDDS3 repressor domain]-[SALL1 repressor domain]-[Cas protein]-[R]  
 [145] [SALL1 repressor domain]-[SUDDS3 repressor domain]-[Cas protein]-[R]  
 [146] By way of a non-limiting example, in some embodiments, in the Cas fusion  
 protein the Cas protein is dCas9 or dCas12 such as dCas12a and the Cas fusion protein  
 25 comprises, consists essentially of or consists of both the SALL1 repressor domain and the  
 SUDDS3 repressor domain.  
 [147] Examples of amino acid sequences of fusion constructs of the present  
 invention, include but are not limited to:  
 [148] MCP-SALL1-SUDDS3 amino acid sequence:  
 30 MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQ  
 SSAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMEITPIFATNSDCEL  
 IVKAMQGLLKDGNPIPSAIAANSIYSAGGGGSGGGGSGGGGSGPKKKRKVA  
 AAGSMSRRKQAKPQHFQSDPEVASLPRRDGDTEKGQPSRPTKSKDAHVCGR  
 CCAEFFELSDLLLHKKNCTKNQLVLIVNENPASPPETFSPSPPPDNPDEQMNDT  
 35 VNKTDQVDCSDLSEHNGLDREESMEVEAPVANKSGSGTSSGSHSSTAPSSSSS  
 SSSSSGGGSSSTGTSAITTSPLQLGDLTGSGGGGSGSGSMSAAGLLAPAPAQ

AGAPPAPEYYPEEDEELES AEDDERS CRG RESDED TED ASETDLAKHDEEDY  
 VEMKEQMYQDKLASLKRQLQQLQEGTLQEYQKRMKKLDQQYKERIRNAEL  
 FLQLETEQVERNIIKEKKA AVKEFEDKKVELKENLIAELEEKKKMIENEKLT  
 MELTGDSMEVKPIMTRKLRRRPNDPVPIPKRRKPAPAQLNYLLTDEQIMED  
 5 LRTLNLKSPKRPASPSSPEHLPATPAESPAQRFEARIEDGKLYYDKRWYHKS  
 QAIYLESKDNQKLSCVISSVGANEIWVRKTS DSTK MRIYLGQLQRGLFVIRRR  
 SAA (SEQ ID NO: 41)

[149] SEQ ID NO: 41 may, for example be coded by nucleic acid comprises,  
 consisting essentially of or consisting of SEQ ID NO: 170

10 [150] ATGGCTTCAAAC TTTACTCAGTTCGTGCTCGTGGACAATGGTGGGA  
 CAGGGGATGTGACAGTGGCTCCTTCTAATTTTCGCTAATGGGGTGGCAGAG  
 TGGATCAGCTCCAACTCACGGAGCCAGGCTACAAGGTGACATGCAGCGT  
 CAGGCAGTCTAGTGCCAGAAAGAGAAAGTATACCATCAAGGTGGAGGTCC  
 CCAAAGTGGCTACCCAGACAGTGGGCGGAGTCGAACTGCCTGTCGCCGCT  
 15 TGGAGGTCCTACCTGAACATGGAGCTCACTATCCCAATTTTCGCTACCAAT  
 TCTGACTGTGAACTCATCGTGAAGGCAATGCAGGGGCTCCTCAAAGACGG  
 TAATCCTATCCCTTCCGCCATCGCCGCTAACTCAGGTATCTACAGCGCTGG  
 AGGAGGTGGAAGCGGAGGAGGAGGAAGCGGAGGAGGAGGTAGCGGACC  
 TAAGAAAAAGAGGAAGGTGGCGGCCGCTGGATCCATGAGTAGGAGAAAA  
 20 CAAGCAAACACAGCACTTTCAAAGTGATCCTGAGGTAGCAAGCCTTCC  
 ACGGCGGGACGGTGACACGGAGAAGGGTCAACCAAGTCGACCCACGAAA  
 AGCAAAGATGCTCATGTATGTGGACGCTGTTGCGCAGAATTTTTTGAATTG  
 TCCGATCTTCTTCTTCAAAAAAGAACTGCACGAAGAATCAGTTGGTTTTG  
 ATAGTAAACGAAAATCCAGCTTCAACCCAGAAAATTTTTCCCGTCACCT  
 25 CCTCCAGATAATCCTGATGAACAAATGAATGACACCGTAAATAAAACCGA  
 CCAAGTAGACTGTTCTGATTTGAGCGAACACAACGGTTTGGATCGAGAAG  
 AGTCAATGGAAGTAGAGGCCCCAGTTGCCAATAAGTCAGGCAGCGGTACT  
 TCTTCCGGCTCCACAGTTCAACAGCTCCATCCTCAAGTAGTTCAAGCTCT  
 TCTAGTTCAGGAGGCGGGGGGAGTAGCTCTACCGGCACTTCTGCCATCAC  
 30 AACCTCACTTCTCAGCTTGGAGACTTGACAGGATCCGGTGGGGGATCTG  
 GGGGATCTGGCTCGATGTCTGCAGCTGGCCTTTTGGCTCCTGCCCCCGCAC  
 AAGCGGGAGCTCCTCCCGCACCGGAGTACTATCCAGAAGAGGATGAGGA  
 ACTGGAATCTGCCGAAGACGACGAGCGCAGTTGCCGGGGGAGGGAATCT  
 GACGAGGATACTGAGGATGCTTCTGAGACCGACCTCGCGAAACATGATGA  
 35 GGAAGACTACGTTGAAATGAAAGAGCAGATGTACCAAGACAACTTGCT  
 AGCCTCAAGAGACAGTTGCAGCAACTGCAAGAAGGCACGCTCCAGGAGT  
 ACCAGAAGAGAATGAAAAAACTCGACCAGCAGTACAAGGAACGAATTAG  
 AAACGCAGAGCTCTTCTTTCAGCTGGAGACTGAACAGGTTGAGCGCAATT  
 ATATTAAGGAAAAAAAAGCCGCTGTGAAGGAGTTCGAAGACAAGAAAGT  
 40 GGAACTTAAAGAAAACCTCATCGCCGAACTGGAGGAGAAGAAGAAGATG  
 ATAGAGAACGAAAAACTCACAATGGAAGTACGGGTGATTCCATGGAGG  
 TAAAACCGATTATGACCCGAAAGCTCCGCCGACGCCCAAACGATCCGGTA  
 CCGATCCCTGATAAGCGGCGCAAGCCCGCACCGGCTCAGCTCAATTACCT  
 GCTGACCGACGAACAAATAATGGAGGACCTGCGGACTCTTAATAAGCTGA  
 45 AGAGTCCTAAACGGCCAGCTTCCCCAGTTCCCCCGAACACCTGCCCGCT  
 ACTCCCGCGGAGAGCCCTGCTCAGCGCTTTGAGGCCCGAATCGAGGACGG  
 AAAATTGTAATGACAAACGCTGGTATCATAAGAGCCAGGCTATATACC  
 TGGAGTCAAAAGATAACCAAAAAGTTGTCATGTGTAATCTCCTCAGTCGGG  
 GCTAACGAAATATGGGTGCGGAAGACCTCTGATAGTACGAAGATGCGCAT

ATATCTGGGACAATTGCAAAGAGGACTTTTTGTTATAAGACGGAGAAGCG  
CTGCT

[151] SUDS3-SALL1-Active Cas9 amino acid sequence:

MSAAGLLAPAPAQAGAPPAPEYYPEEDEELES AEDDERSCRGRESD EDED  
5 SETDLAKHDEEDYVEMKEQMYQDKLASLKRQLQQLQEGTLQEYQKRMKKL  
DQYKERIRNAELFLQLETEQVERNYIKEKKA AVKEFEDKKVELKENLIAELE  
EKKKMIENEKLTMELTGDSMEV KPI MTRKLR RRPNDPVP I PDKRRKPAPAQL  
NYLLTDEQIMEDLR TLNKLKSPKRPASPSPEHL PATPAESPAQRFEARIEDGK  
10 LYYDKRWYHKSQAIYLESKDNQKLS CVISSVGANEIWVRKTS DSTKMRIYLG  
QLQRGLFVIRRRSAAGSGGSGGSGSMSRRKQAKPQH FQSDPEVASLPRRDG  
DTEKGQPSRPTKSKDAHVCGRCCA EFFELSDLLLHKKNCTKNQLVLIVNENP  
ASPPETFPSPPDNPDEQMNDTVNKTDQVDCSDLSEHNGLDREESMEVEAP  
VANKSGSGTSSGSHSSTAPSSSSSSSSSSSGGGSSSTG TSAITTSLPQLGDLTGS  
GGGSGGSGSM DYKDDDDKMAPKKR KVG I HG VPAADKKYSIGLDIGTNSVG  
15 WAVITDEYKVPSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARR  
RYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNVD  
EVAYHEKYPTIYHLRKKLVDSTDKADLR LIYLALAHMIKFRGHFLIEGDLNPD  
NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPG  
EKKNGLFGNLIALSLGLTPNFKSNFDLAEDA KLQLSKD TYDDDLDNLLAQIG  
20 DQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK  
ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEEL  
LVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKI  
LTFRIPIYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT  
NFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKA I  
25 VDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKII  
KDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRR  
RYTGWGRLSRKLINGIRDKQSGKTILDFL KSDGFANRNF MQLIHDDSLTFKED  
IQKAQVSGQGDSLHEHIANLAGSPA I KKGILQTVKVVDELVKVMGRHKPENI  
VIEMARENQTTQKGQKNSRERMKRIE EG I KELGSQILKEHPVENTQLQNEKLY  
30 LYYLQNGRDMYVDQELDINRLSDYDVHIVPQSFLKDDSIDNKVLTRSDKNR  
GKSDNPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAG  
FIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRK  
DFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVR  
KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV  
35 WDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD  
WDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN  
PIDFLEAKGYKEVKKDLI I KLPKYSLFELENGRKRMLASAGELQKGNELALPS  
KYVNFLYLASHYEKLGSPEDNEQKQLFVEQH KHYLDEIIEQISEFSKRVILAD  
ANLDKVL SAYNKHRDKPIREQAENIIHLFTL TNLGAPAAFKYFDTTIDRKRYT  
40 STKEVL DATLIHQ SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKK (SEQ  
ID NO: 171)

[152] SEQ ID NO: 171 may, for example be coded by nucleic acid comprises,  
consisting essentially of or consisting of SEQ ID NO: 172:

ATGTCTGCAGCTGGCCTTTTGGCTCCTGCCCCGCACAAGCGGGAGCTCCT  
45 CCCGCACCGGAGTACTATCCAGAAGAGGATGAGGAACTGGAATCTGCCGA  
AGACGACGAGCGCAGTTGCCGGGGGAGGGAATCTGACGAGGATACTGAG  
GATGCTTCTGAGACCGACCTCGCGAAACATGATGAGGAAGACTACGTTGA  
AATGAAAGAGCAGATGTACCAAGACAACTTGCTAGCCTCAAGAGACAG  
TTGCAGCAACTGCAAGAAGGCACGCTCCAGGAGTACCAGAAGAGAATGA  
50 AAAAACTCGACCAGCAGTACAAGGAACGAATTAGAAACGCAGAGCTCTTT

CTTCAGCTGGAGACTGAACAGGTTGAGCGCAATTATATTAAGGAAAAAA  
AGCCGCTGTGAAGGAGTTCGAAGACAAGAAAGTGGAACCTAAAGAAAAC  
CTCATCGCCGAACTGGAGGAGAAGAAGAAGATGATAGAGAACGAAAAAC  
TCACAATGGAAGTACGGGTGATTCCATGGAGGTAAAACCGATTATGACC  
5 CGAAAGCTCCGCCGACGCCAAACGATCCGGTACCGATCCCTGATAAGCG  
GCGCAAGCCCGCACCGGCTCAGCTCAATTACCTGCTGACCGACGAACAAA  
TAATGGAGGACCTGCGGACTCTTAATAAGCTGAAGAGTCCTAACGGCCA  
GCTTCCCCCAGTCCCCCGAACACCTGCCCGCTACTCCCGCGGAGAGCCCT  
GCTCAGCGCTTTGAGGCCCGAATCGAGGACGGAAAATTGTAATATGACAA  
10 ACGCTGGTATCATAAGAGCCAGGCTATATACCTGGAGTCAAAGATAACC  
AAAAGTTGTATGTGTAATCTCCTCAGTCGGGGCTAACGAAATATGGGTG  
CGGAAGACCTCTGATAGTACGAAGATGCGCATATATCTGGGACAATTGCA  
AAGAGGACTTTTTGTTATAAGACGGAGAAGCGCTGCTGGATCCGGTGGGG  
GATCTGGGGGATCTGGCTCGATGAGTAGGAGAAAACAAGCAAACCCACA  
15 GCACTTTCAAAGTGATCCTGAGGTAGCAAGCCTTCCACGGCGGGACGGTG  
ACACGGAGAAGGGTCAACCAAGTCGACCCACGAAAAGCAAAGATGCTCA  
TGTATGTGGACGCTGTTGCGCAGAATTTTTTGAATTGTCCGATCTTCTTCT  
CACAAAAGAAGTGCACGAAGAATCAGTTGGTTTTTGATAGTAAACGAAAA  
TCCAGCTTACCCCCAGAACTTTTTCCCGTACCTCCTCCAGATAATCC  
20 TGATGAACAAATGAATGACACCGTAAATAAAAACCGACCAAGTAGACTGTT  
CTGATTTGAGCGAACACAACGGTTTGGATCGAGAAGAGTCAATGGAAGTA  
GAGGCCCCAGTTGCCAATAAGTCAGGCAGCGGTACTTCTTCCGGCTCCCA  
CAGTTCAACAGCTCCATCCTCAAGTAGTTCAAGCTCTTCTAGTTCAGGAGG  
CGGGGGGAGTAGCTCTACCGGCACTTCTGCCATCACAACCTCACTTCCTCA  
25 GCTTGGAGACTTGACAGGATCCGGTGGGGGATCTGGGGGATCTGGCTCGA  
TGGATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGGAA  
GGTTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCATCGGCC  
TGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTAC  
AAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACA  
30 GCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTTCGACAGCGGCGAAACA  
GCCGAGGCCACCCGGCTGAAGAGAACC GCCAGAAGAAGATACACCAGAC  
GGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCC  
AAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCTTGGTGG  
AGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGAC  
35 GAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAGAAAGAA  
ACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCC  
TGGCCCACATGATCAAGTCCGGGGCCACTTCTGATCGAGGGCGACCTG  
AACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGTGCAGAC  
CTACAACCAGCTGTTTCGAGGAAAACCCATCAACGCCAGCGGCGTGGACG  
40 CCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAAT  
CTGATCGCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCGGCAACCT  
GATTGCCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACTTCGACC  
TGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGA  
CCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTC  
45 TGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGA  
GTGAACACCGAGATCACCAGGCCCCCCCTGAGCGCCTCTATGATCAAGAG  
ATACGACGAGCACCACAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGC  
AGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAAC  
GGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAA  
50 GTTCATCAAGCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCG

TGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAA  
CGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGC  
GGCGGCAGGAAGATTTTTACCCATTCTGAAGGACAACCGGGAAAAGATC  
GAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAG  
5 GGGAAACAGCAGATTTCGCTGGATGACCAGAAAGAGCGAGGAAACCATC  
ACCCCTGGAACCTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAG  
CTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGG  
TGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAG  
CTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCT  
10 GAGCGGCAGCAGAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAAC  
CGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCG  
AGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAAGATCGGTTCAACGCC  
TCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTT  
CCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCC  
15 TGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTAT  
GCCCACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGAT  
ACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGA  
CAAGCAGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTTCG  
CCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAA  
20 GAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACG  
AGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTG  
CAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACA  
AGCCCAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCA  
GAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGG  
25 CATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAAC  
ACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCG  
GGATATGTACGTGGACCAGGAACCTGGACATCAACCGGCTGTCCGACTACG  
ATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGAC  
AACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACG  
30 TGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAATACTGGCGGCAGCTG  
CTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGC  
CGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGA  
CAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGG  
ACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGA  
35 AGTGAAAGTGATCACCTGAAAGTCCAAGCTGGTGTCCGATTTCCGGAAGG  
ATTTCCAGTTTTACAAAGTGC GCGAGATCAACAATACTACCACCAGCCAC  
GACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCC  
TAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGC  
GGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAA  
40 GTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCT  
GGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAA  
ACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGC GGAA  
AGTGCTGAGCATGCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAG  
ACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAA  
45 GCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTC  
GACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAA  
GGGCAAGTCCAAGAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACC  
ATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGC  
CAAGGGCTACAAAGAAGTGA AAAAGGACCTGATCATCAAGCTGCCTAAG  
50 TACTCCCTGTTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGC

CGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGA  
 ACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAG  
 GATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGA  
 CGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCG  
 5 ACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAG  
 CCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAA  
 TCTGGGAGCCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGA  
 AGAGGTACACCAGCACCAAGAGGGTGCTGGACGCCACCCTGATCCACCAG  
 AGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGG  
 10 CGACAAGCGTCTGCTGCTACTAAGAAAGCTGGTCAAGCTAAGAAAAAGA  
 AA

[153] Within the scope of the present invention are proteins and polypeptide  
 sequences that are fragments of SEQ ID NO: 41 and 171 and derivatives of those  
 sequences that can be used to perform substantially similar functions. In some  
 15 embodiments, the proteins or polypeptides are at least 80%, at least 85%, at least  
 90%, at least 95% similar to either SEQ ID NO: 41 and 171. Additionally, within the  
 scope of the present invention are nucleic acid sequences comprises, consist  
 essentially of, or consist of SEQ ID NO: 170 or 172 or complement thereof, or  
 sequences that are at least 80%, at least 85%, at least 90%, at least 95% similar to or  
 20 complementary to either SEQ ID NO: 170 and 172.

[154] **Linkers**

[155] When a repressor domain is fused to a Cas protein the fusion may be by a  
 direct bond (*e.g.*, a covalent bond) between the N terminal amino acid of the  
 25 repressor protein and the C terminal amino acid of the Cas protein or the C terminal  
 amino acid of the repressor protein and the N terminal amino acid of the Cas protein.

[156] However, instead of directly linking or forming a bond between two  
 components, *i.e.*, two or more repressor domains or a repressor domain and a Cas  
 protein, one may use a linker. In some embodiments, the linker comprises, consists  
 30 essentially of, or consists of an amino acid sequence that is, *e.g.*, 1 to 100 amino acid  
 long or 3 to 90 amino acids long or 10 to 50 amino acids long. In some embodiments,  
 the linker comprises, consists essentially of, or consists of a sequence that is not an  
 amino acid sequence.

[157] When the linker is between a Cas protein and a repressor domain, the linker  
 35 may be referred to as a Cas linker. In some embodiments, the Cas protein has a C  
 terminal amino acid and the Cas fusion protein comprises a Cas linker, wherein the Cas  
 linker is covalently bound to the C terminal amino acid of the Cas protein. In some

embodiments, the Cas protein has an N terminal amino acid and the Cas fusion protein comprises a Cas linker, wherein the Cas linker is covalently bound to the N terminal amino acid of the Cas protein. In some embodiments, the Cas protein has a C terminal amino acid and an N terminal amino acid and the Cas fusion protein comprises two Cas linkers, wherein a first Cas linker is covalently bound to the C terminal amino acid of the Cas protein and a second Cas linker is covalently bound to the N terminal amino acid of the Cas protein. When there are a first Cas linker and a second Cas linker, the first Cas linker may be bound to a first repressor domain and the second Cas linker may be bound to a second repressor domain.

10 [158] In some embodiments, there is one Cas linker and the Cas linker comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 7: GSGGGSGGSGS. In some embodiments, the Cas linker comprises, consists essentially of, or consists of a sequence that is SEQ ID NO: 7. In some embodiments, there are two Cas linkers and each Cas linker comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 7: GSGGGSGGSGS. In some embodiments, each of two Cas linkers comprises, consists essentially of, or consists of a sequence that is SEQ ID NO: 7.

20 [159] In some embodiments, the Cas linker is covalently bound to a Cas protein and a repressor domain that comprises, consists essentially of or consists of a sequence that is at least 80%, at least 85%, at least 90%, at least 95% similar to SEQ ID NO: 1. In some embodiments, the Cas linker is covalently bound to a Cas protein and a repressor domain that comprises, consists essentially of or consists of a sequence that is SEQ ID NO: 1.

25 [160] In some embodiments, the Cas linker is covalently bound to a Cas protein and a repressor domain that comprises, consists essentially of or consists of a sequence that is at least 80%, at least 85%, at least 90%, at least 95% similar to SEQ ID NO: 2. In some embodiments, the Cas linker is covalently bound to a Cas protein and a repressor domain that comprises, consists essentially of or consists of a sequence that is SEQ ID NO: 2.

30 [161] When the Cas fusion protein comprises two or more repressor domains and two or more repressor domains are on the same side of the Cas protein, *i.e.*, on the N side or the C side, each pair of repressor domains may be directly, *e.g.*, covalently bound to each other, or they may be joined through a linker. A linker that joins two repressor domains may be referred to as a repressor linker.

[162] The repressor linker may be the same as or different from the Cas linker. In some embodiments, the repressor linker comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 7: GSGGGSGGSGS. In some embodiments, the repressor linker comprises,  
5 consists essentially of, or consists of a sequence that is SEQ ID NO: 7.

[163] By way of a non-limiting example, in a Cas fusion protein of the present invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein, wherein the Cas protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound  
10 to the C terminal amino acid of the Cas protein and to the N terminal amino acid of the SALL1 repressor domain and wherein the repressor linker is between the SALL1 repressor domain and the SUDS3 repressor domain.

[164] By way of another non-limiting example, in a Cas fusion protein of the present invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein,  
15 wherein the Cas protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to the C terminal amino acid of the Cas protein and to the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor domain and the SALL1 repressor domain.

[165] By way of another non-limiting example, in a Cas fusion protein of the present invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein, wherein the Cas protein has a N terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound  
20 to the N terminal amino acid of the Cas protein and to the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor domain and the SALL1 repressor domain.

[166] By way of another non-limiting example, in a Cas fusion protein of the present invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein, wherein the Cas protein has a N terminal amino acid and the Cas fusion protein further  
30 comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to the N terminal amino acid of the Cas protein and to the SALL1 repressor domain and wherein the repressor linker is bound to both the SALL1 repressor domain and the SUDS3 repressor domain.

[167] By way of another non-limiting example, in a Cas fusion protein of the present  
35 invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein,

wherein the Cas protein has a N terminal amino acid and a C terminal amino acid and the Cas fusion protein further comprises a first Cas linker and a second Cas linker, wherein the first Cas linker is covalently bound to the N terminal amino acid of the Cas protein and to the SUDS3 repressor domain and wherein the second Cas linker is bound to the C terminus of Cas protein and to the SALL1 repressor domain.

[168] By way of another non-limiting example, in a Cas fusion protein of the present invention, the Cas protein may be a dCas9 protein or dCas12 such as dCas12a protein, wherein the Cas protein has a N terminal amino acid and a C terminal amino acid and the Cas fusion protein further comprises a first Cas linker and a second Cas linker, wherein the first Cas linker is covalently bound to the C terminal amino acid of the Cas protein and to the SUDS3 repressor domain and wherein the second Cas linker is bound to the N terminal amino acid of Cas protein and to the SALL1 repressor domain.

[169] By way of another example, in some embodiments, the Cas fusion protein comprises a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% similar to SEQ ID NO: 10:

[170] GSGGGSGGGSGSMSRRKQAKPQHFQSDPEVASLPRRDGDTEKGQPSRP  
TKSKDAHVCGRCCAIEFFELSDLLHKKNCTKNQLVLIVNENPASPPETFSPSP  
PDNPDEQMNDTVNKTDQVDCSDLSEHNGLDREESMEVEAPVANKSGSGTSS  
GSHSSTAPSSSSSSSSSSSGGGGSSSTGTSAITTSLPQLGDLTGSGGGSGGGSGSMS  
AAGLLAPAPAQAGAPPAPEYYPEEDEELES AEDDERSCRGRESDEDTEASET  
DLAKHDEEDYVEMKEQMYQDKLASLKRQLQQLQEGTLQEYQKRMKKLDQ  
QYKERIRNAELFLQLETEQVERNVIKEKAAVKEFEDKKVELKENLIAELEEK  
KKMIENEKLTMELTGDSMEVKPIMTRKLRRRPNDPVIPIPKRRKPAPAQLNY  
LLTDEQIMEDLRTLNLKSPKRPASPSSPEHLPATPAESPAQRFEARIEDGKLY  
YDKRWYHKSQAIYLESKDNQKLSCVISSVGANEIWRKTS DSTKMRIYLGQL  
QRGLFVIRRRSAA.

[171] In some embodiments, the Cas fusion protein comprises a sequence that the same as SEQ ID NO: 10.

[172] In some embodiments, the Cas fusion protein is:

[173] [dCas9]-[Cas linker]-[SALL1 repressor domain]-[repressor linker]-[SUDS3 repressor domain].

[174] In some embodiments, the Cas fusion protein is:

[175] [dCas9]-[Cas linker]-[SUDS3 repressor domain]-[repressor linker]-[SALL1 repressor domain].

[176] In some embodiments, the Cas fusion protein is: [dMAD7]-[Cas linker]-[SALL1 repressor domain]-[repressor linker]-[SUDS3 repressor domain].

[177] In some embodiments, the Cas fusion protein is:

[178] [dMAD7]-[Cas linker]-[SUDDS3 repressor domain]-[repressor linker]-[SALL1 repressor domain].

[179] In some embodiments, the Cas fusion protein is:

[180] [SALL1 repressor domain]-[repressor linker]-[SUDDS3 repressor domain]-[Cas linker]-[dCas9].

[181] In some embodiments, the Cas fusion protein is:

[182] [SUDDS3 repressor domain]-[repressor linker]-[SALL1 repressor domain]-[Cas linker]-[dCas9].

[183] In some embodiments, the Cas fusion protein is:

[184] [SALL1 repressor domain]-[repressor linker]-[SUDDS3 repressor domain]-[Cas linker]-[dMAD7].

[185] In some embodiments, the Cas fusion protein is:

[186] [SUDDS3 repressor domain]-[repressor linker]-[SALL1 repressor domain]-[Cas linker]-[dMAD7].

15

[187] **gRNA**

[188] The Cas-fusion proteins of the present invention may be used in conjunction with gRNAs. In some embodiments, the gRNA contains 30 to 180 nucleotide or 45 to 135 nucleotides or 60 to 120 nucleotides. A gRNA may be chemically synthesized or enzymatically synthesized. When enzymatically synthesized, the synthesis may occur *in vitro*, *in vivo*, or *ex vivo*.

[189] The nucleotides of the gRNA may be exclusively modified ribonucleotides, exclusively unmodified ribonucleotides, or a combination of modified and unmodified ribonucleotides. In some embodiments, the gRNA contains one or more modification such as 2' modifications, *e.g.*, 2'-O-alkyl such as 2'-O-methyl or 2'-O-ethyl, or 2'-halogenmodifications such as 2' Fluoro. In some embodiments, the gRNA contains one or more modified internucleotide linkages such as phosphorothioate linkages.

[190] In some embodiments, the gRNA has the following modifications:

- 2'-O-methyl modifications on the first and second 5' most nucleotides,
- 2'-O-methyl modifications on the penultimate 3' nucleotide (second 3' most nucleotide) and the antepenultimate 3' nucleotide (third 3' most nucleotide)
- all other nucleotides are unmodified at their 2' positions,

- phosphorothioate linkages between the first and second 5' most nucleotides, the second and third 5' most nucleotides, the antepenultimate 3' nucleotide and the penultimate 3' nucleotide, and the penultimate 3' nucleotide and the 3' most nucleotide, and
- 5       • all other internucleotide linkages are phosphodiester linkages.

[191] In some embodiments, the gRNA has the following modifications:

- 2'-O-methyl modifications on the first and second 5' most nucleotides,
- all other nucleotides are unmodified at their 2' positions,
- phosphorothioate linkages between the first and second 5' most
- 10       nucleotides, the second and third 5' most nucleotides, and
- all other internucleotide linkages are phosphodiester linkages.

[192] In some embodiments, the gRNA comprises, consists essentially of or consists of a crRNA. In some embodiments, the gRNA comprises, consists essentially of or consists of a crRNA sequence and a tracrRNA sequence. When the gRNA comprises,

15       consists essentially of or consists of a crRNA sequence and a tracrRNA sequence, the crRNA and the tracrRNA may be part of a sgRNA or they each may be on a separate strand of nucleotides and form a crRNA molecule and a tracrRNA molecule, each of which is a polynucleotide. When they are part of two separate nucleotides, one of the tracrRNA molecule and the crRNA molecule may be referred to as a first RNA

20       molecule and the other of the other tracrRNA molecule and the crRNA molecule may be referred to as a second RNA molecule. When there is a separate tracrRNA molecule and crRNA molecule, the total number of nucleotides in those two molecules combined may, for example, be the same as in the sgRNA described in various embodiments of the present invention. Further, any chemical modifications to

25       nucleotides of sgRNAs may be present in either or both of the tracrRNA molecule and crRNA molecule, and any internucleotide modifications of sgRNAs may be present in either or both of the tracrRNA molecule and crRNA molecule. Additionally, any moieties described as being present on the 5' end or 3' end of a gRNA may in the case of a sgRNA be present on the 5' end or 3' end of the sgRNA, and in the case of

30       separate tracrRNA molecules and crRNA molecules, each of which has a 5' end or 3' end, be present on the 5' end or 3' end of the tracrRNA molecule or crRNA molecule.

[193] The crRNA comprises, consists essentially of or consists of a Cas association region and a spacer region (also referred to as a targeting region). The targeting

region is sufficiently complementary to and capable of hybridizing to a pre-selected target site of interest. In various embodiments, the target specifying component of the guide sequence can comprise from about 10 nucleotides to more than about 25 nucleotides, for example up to 36 nucleotides. In some embodiments, the region of base pairing between the guide sequence and the corresponding target site sequence is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In some embodiments, the targeting region is 12 to 30 nucleotides long, or 14 to 25 nucleotides long or about 17 to 20 nucleotides long or about 14 nucleotides long or about 20 nucleotides long. The targeting region may be at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% complementary to a region of the target dsDNA over at least 14 contiguous nucleotides, at least 15 contiguous nucleotides, at least 16 contiguous nucleotides, at least 17 contiguous nucleotides, at least 18 contiguous nucleotides, at least 19 contiguous nucleotides, at least 20 contiguous nucleotides, or 14 to 20 contiguous nucleotides.

[194] When the targeting region is about 20 nucleotides long and used with an active Cas protein that is capable of cleaving both DNA strands, a double-strand break will be generated on the targeted DNA that can lead to insertions and/or deletions (indel) in the genome. If one wishes to cause repression without creating indels, one may either use an inactive Cas protein, such as a deactivated Cas9 protein. If using an active Cas protein that is generally capable of cleaving both DNA strands or a Cas nickase variant that is generally capable of cleaving one strand of the targeted DNA, one may use a gRNA that has a shorter targeting region, such as about 14 nucleotides long for gene repression. Guides with a 20nt targeting region can lead the active Cas9-repressor to another genomic site for DNA cleaving and subsequent editing.

[195] The Cas association region, which may for example, be about 18 – 36 nucleotides long is the portion of the crRNA that allows the crRNA (and thus the gRNA to retain association with the Cas protein). In some embodiments, association with the Cas protein is possible in the absence of a tracrRNA. In other embodiments, association requires the presence of a tracrRNA.

[196] When a crRNA requires a tracrRNA to be present for association with the Cas protein, the Cas association region hybridizes with an anti-repeat region within the tracrRNA. The tracrRNA may also contain a distal region that is 3' of the anti-repeat region and is not complementary to any region of the crRNA.

[197] When there is hybridization between the Cas association region, which is also referred to as a repeat region and the anti-repeat region, the repeat: anti-repeat region of the gRNA scaffold can be split into 3 parts: the lower stem, bulge, and upper stem. The lower stem is 6 base pairs in length and forms through both Watson-Crick and no  
 5 Watson-Crick base pairing; this is followed by a bulge structure of 6 nucleotides. Finally there is an upper stem that consists of a 4 base pair structure.

[198] When the gRNA is a sgRNA, in some embodiments, the single strand may contain regions that are complementary and that when the complementary regions hybridize allow association with a Cas protein such as Type II Cas enzymes,  
 10 including but not limited to Cas9 in active or deactivated form, and Type V Cas enzymes such as Cas12c, Cas12d, Cas12e, and Cas12f in active or deactivated form. In other embodiments, when the gRNA is a sgRNA, there are no regions that are complementary, but the sgRNA is capable of association with a Cas enzyme, such as certain Type V Cas enzymes such as Cas12a, MAD7 (an engineered variant of  
 15 ErCas12a), Cas12h, Cas12i, and Cas12j (Cas $\phi$ ) in active or deactivated form.

[199] A non-limiting example of an sgRNA is shown in **figure 2**. The sgRNA of **figure 2** has the following sequence: (SEQ ID NO: 11):

20 5'mN\*mN\*NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAG  
 CAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC  
 ACCGAGUCGGUGCUmU\*mU\*U3'

m signifies a 2'O-methyl group;

\* signifies a phosphorothioate linkage; and

N signifies any of A, C, G, or U.

[200] As shown in **figure 2**, the crRNA region and the tracrRNA regions are joined  
 25 by a tetra loop and the tracrRNA region has three stem loop regions. This example of an sgRNA has 100 nucleotides. In some embodiments, the sgRNA is 60 to 120 nucleotides long or 90 to 110 nucleotides long.

[201] The N region as shown is 20 nucleotides long. In some embodiments, the N region is 10 to 36 nucleotides long or 14 to 26 nucleotides long or 18 to 22  
 30 nucleotides long.

[202] Various tracrRNA sequences are known in the art and examples include SEQ ID Nos: 27-34, as well as active portions thereof.

GGAACCAUUCAAAACAGCAUAGCAAGUUAAAUAAGGCUAGUCCGUUA  
 UCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 28);  
 35 UAGCAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA

CCGAGUCGGUGC (SEQ ID NO: 29);

AGCAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU  
GGCACCGAGUCGGUGC (SEQ ID NO: 30);

5 CAAAACAGCAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCAACUUGA  
AAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 31);  
UAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG  
(SEQ ID NO: 32); UAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCA (SEQ ID  
NO: 33); and UAGCAAGUUA AAAUAAGGCUAGUCCG (SEQ ID NO: 34).

As used herein, an active portion of a tracrRNA retains the ability to form a complex  
10 with a Cas protein, such as Cas9 or dCas9 or nCas9.

[203] By way of a non-limiting example, the gRNA can be a hybrid RNA molecule  
where the above-described crRNA comprises a programmable gRNA fused to a  
tracrRNA to mimic the natural crRNA:tracrRNA duplex. An example of this type of  
hybrid is crRNA:tracrRNA, gRNA sequence: 5'-(20 nt guide)-

15 GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUA AAAUAAGGCUAG  
UCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC UUUUUUU-3'  
(SEQ ID NO: 27).

[204] Methods for generating crRNA-tracrRNA hybrid RNAs (also known as  
sgRNAs) are known in the art. In one embodiment in which the crRNA and  
20 tracrRNA are provided as a sgRNA, the two components are linked together via a  
tetra stem loop. In some embodiments, the repeat anti-repeat region is extended.  
There may, for example, be an extension of 2, 3, 4, 5, 6, 7 bases or more than 7 bases  
at either side of the repeat: anti-repeat region. In another embodiment, the repeat: anti-  
repeat region has an extension of 7 nucleotides at either side of the stem. The  
25 extension of 7 bases at either side results in a region that is 14 base pairs longer. In  
other embodiments, the extension may be more than 7 bases. See *e.g.*,  
WO2014099750, US 20140179006, and US 20140273226 for additional disclosure of  
tracrRNAs. The contents of these documents are incorporated herein by reference in  
their entireties.

30 [205] In some embodiments the tracrRNA is from or derived from *S. pyogenes*.

[206] In some embodiments, the target site resides on DNA. Within the DNA, the  
target nucleic acid strand can be either of the two strands and *e.g.*, be in genomic  
DNA within a host cell. Examples of such genomic dsDNA include, but are not

necessarily limited to, a host cell chromosome, mitochondrial DNA and a stably maintained plasmid. However, it is to be understood that the present method can be practiced on other dsDNA present in a host cell, such as non-stable plasmid DNA, viral DNA, and phagemid DNA, as long as there is Cas-targeted site.

5 [207] In some embodiments, rather than using fusion proteins in combination with gRNA, one uses the fusion proteins of the present invention in combination with scoutRNA and the applicable crRNA. For example, the fusion proteins of the present invention may be used in a system or as part of a complex that has: (a) a crRNA, wherein the crRNA is 30 to 60 nucleotides long and the crRNA comprises a Cas  
10 association region and a targeting region, wherein the Cas association region is 15 to 30 nucleotides long and the targeting region is 15 to 30 nucleotides long; (b) a scoutRNA, wherein the scoutRNA is 20 to 100 nucleotides long and wherein the scoutRNA comprises an anti-repeat region, wherein the anti-repeat region is 3 to 10 nucleotides long, and the anti-repeat region is complementary to at least 3 consecutive  
15 nucleotides within the Cas association region, and the anti-repeat region is capable of hybridizing with said at least 3 consecutive nucleotides within the Cas association region to form a hybridization region, wherein when the crRNA and scoutRNA form the hybridization region, and the crRNA and the scoutRNA are capable of retaining association with an RNA binding domain of a Type V Cas protein.

20 [208] **RNA-Repressor Domain Complexes**

[209] In some embodiments, the present invention is directed to the use of an RNA-repressor domain complex. An RNA-repressor domain complex comprises, consists essentially of, or consists of: a gRNA such as a gRNA described above or a  
25 scoutRNA and/or a crRNA capable of associating with a scoutRNA as described above, a ligand binding moiety, a ligand, and one or more repressor domains. The RNA-repressor domain complexes may be used in conjunction with the Cas-fusion proteins of the present invention or with other Cas proteins that are not fusion proteins.

30 [210] The gRNA or scoutRNA or crRNA capable of associating with a scoutRNA may be fused directly to a ligand binding moiety or associated with a ligand binding moiety through a ligand binding moiety linker. The ligand binding moiety is capable of reversibly associating with a ligand. The ligand is directly or through a ligand

linker fused to a repressor domain. The repressor domain may be any effector. Each of the ligand binding moiety linker and the ligand linker if either or both are present may comprise, consist essentially of or consist of nucleotide(s), amino acids and other organic and inorganic moieties and combinations thereof.

- 5 [211] A non-exhaustive list of examples of ligand binding moiety-ligand pairs that may be used in various embodiments of the present invention is provided in Table 1. Both unmodified and chemically modified versions of the ligand binding moieties and ligands are within the scope of the present invention.

10 [212] **Table 1.**

<b>Ligand Binding Moieties</b>	<b>Ligands</b>
Telomerase Ku binding motif	Ku
Telomerase Sm7 binding motif	Sm7
MS2 phage operator stem-loop	MS2 Coat Protein (MCP)
PP7 phage operator stem-loop	PP7 coat protein (PCP)
Qbeta phage operator stem-loop	<u>Qbeta coat protein [Q65H]</u>
SfMu phage Com stem-loop	Com RNA binding protein
Non-natural RNA aptamer	Corresponding aptamer ligand
Biotin	Streptavidin
Oligosaccharide	Lectin
Benzylguanine or benzylcytosine	SNAP/CLIP tag
6x-His binding motif	6x-His tag
PDGFbeta chain binding motif	PDGF B-chain
GST binding motif	GST protein
Tat binding motif	BIV Tat protein
Tat binding motif	HIV Tat protein
Pumilio binding motif	PUM-HD domain
BoxB binding motif	Lambda N22plus
Csy4 binding motif	Csy4[H29A]

1. **Telomerase Ku binding motif / Ku heterodimer**

a. **Ku binding hairpin 5'-**

15 UUCUUGUCGUACUUAUAGAUCGCUACGUUAUUUCAAUUUU  
GAAAUCUGAGUCCUGGGAGUGCGGA-3' (SEQ ID No: 12)

b. **heterodimer**

20 MSGWESYYKTEGDEEAEEEQEENLEASGDYKYSGRDSLIFLVD  
ASKAMFESQSEDELTPFDMSIQCIQS VYISKIISDRDLLAVVFY  
GTEKDKNSVNFKNIIYVLQELDNPGAKRILELDQFKGQQGQKRF  
QDMMGHGSDYSLSEVLWVCANLFSVDVQFKMSHKRIMLFTNED  
NPHGNDSAKASRARTKAGDLRDTGIFLDMHLKKPGGFDISLF  
YRDIISIAEDEDLRVHFEESKLEDLLRKVRACKETRKRALSRLKL  
25 KLNKDIVISVGIYNLVQKALKPPPIKLYRETNEPVKTKTRTFNTS  
TGLLLPSDTKRSQIYGSRQIILEKEETEELKRFDDPGLMLMGF  
KPLVLLKKHHYLRPSLFFVPEESLVIGSSTLFSALLIKCLEKEVA

ALCRYTPRRNIPPYFVALVPQEEELDDQKIQVTPPGFQLVFLPFA  
 DDKRKMPFTEKIMATPEQVGKMKAIVEKLRFTYRSDSFENPVL  
 QQHFRNLEALALDLMEPEQAVDLTLPKVEAMNKRLGSLVDEF  
 KELVYPPDYNPEGKVTKRKHDNEGSGSKRPKVEYSEEELKTHI  
 SKGTLGKFTVPMLKEACRAYGLKSGLKKQELLEALTKHFQD  
 (SEQ ID No: 13)

MVRSGNKA AAVVLCMDVGFTMSNSIPGIESPFEQAKKVITMFVQ  
 RQVFAENKDEIALVLFQTDGTDNPLSGGDQYQNITVHRHMLP  
 DFDLLEDIESKIQPGSQADFLDALIVSMDVIQHETIGKKFEKRH  
 IEIFTDLSSRFSSQLDIIIHSLKCKDISERHSIHWPCRLTIGSNLSI  
 RIAAYKSILQERVKKTWTVVDAKTLKKEDIQKETVYCLNDDDE  
 TEVLKEDIQGFYRYSDIVPFSKVDEEQMKYKSEGKCFSVLGFC  
 KSSQVQRRFFMGNQVLKVFAARDDEAAVALSSLIHALDDLD  
 MVAIVRYAYDKRANPQVGVAFPHIKHNYECLVYVQLPFMEDL  
 RQYMFSSLKNSKKYAPTEAQLNAVDALIDSMMLAKKDEKTD  
 LEDLFPTTKIPNPRFQRLFQCLLHRALHPREPLPIQQHIWNMLN  
 PPAEVTTKSQIPLSKIKTLFPLIEAKKKDQVTAQEIQDNHEDGP  
 TAK (SEQ ID No: 14)

**2. Telomerase Sm7 binding motif / Sm7 homoheptamer**

**c. Sm consensus site (single stranded)**

5'-AAUUUUUGGA-3' (SEQ ID No: 15)

**d. Monomeric Sm – like protein (archaea)**

GSVIDVSSQRVNVQRPLDALGNSLNSPVIKLLKGDREFRGLKS  
 FDLHMNLVLNDAEELEDGEVTRRLGTVLIRGDNIVYISP (SEQ  
 ID No: 16)

**3. MS2 phage operator stem loop / MS2 coat protein**

**a. MS2 phage operator stem loop 5'-**

GCACAUGAGGAUCACCCAUGUGC -3' (SEQ ID No:17)

**b. MS2 coat protein**

MASNFTQFVLVDNNGGTGDVTVAPSNFANGIAEWISSNSRSQ  
 AYKVTCSVRQSSAQNRKYTIKVEVPKGAWRSYLNMEITPI  
 FATNSDCELIVKAMQGLLKDGNIPIPSAIAANSIY (SEQ ID  
 No: 18)

**4. PP7 phage operator stem loop / PP7 coat protein**

**a. PP7 phage operator stem loop**

5'-AUAAGGAGUUUAUAUGGAAACCCUUA -3' (SEQ ID No: 19)

- 5           **b. PP7 coat protein (PCP)**  
 MSKTIVLSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASL  
 RQNGAKTAYRVNLKLDQADVDCSTSVCGELPKVRYTQVWS  
 HDVTIVANSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR.  
 (SEQ ID No: 20)
- 10           **5. SfMu Com stem loop / SfMu Com binding protein**
- a. SfMu Com stem loop**  
 5'-CUGAAUGCCUGCGAGCAUC-3' (SEQ ID No: 21)
- b. SfMu Com binding protein**  
 MKSIRCKNCNKLLFKADSFHDHIEIRCPRCKRHIIMLNACEHPTEK  
 HCGKREKITHSDETVRY (SEQ ID No: 22)
- 15           **6. BoxB aptamer/lambda N22plus**
- e. BoxB aptamer**  
 5'- GCCCUGAAGAAGGGC-3' (SEQ ID No: 23)
- f. Lambda N22plus protein**  
 MNARTRRRERRAEKQAQWKAAN (SEQ ID No: 24)
- 20           **7. Csy4 binding stem loop/Csy4[H29A]**
- a. Csy4 binding motif**  
 5'- CUGCCGUAUAGGCAGC-3' (SEQ ID No: 25)
- b. Csy4[H29A]**
- 25           MDHYLDIRLRPDPEFPPAQLMSVLFKLAQALVAQGGDRIGVS  
 FPDLDESRSRLGERLRIHASADDLRALLARPWLEGLRDHLQFGE  
 PAVVPHPTPYRQVSRVQAKSNPERLRRRLMRRHDLSEEEARKR  
 IPDTVARALDLPFVTLRSQSTGQHFRLLFIRHGPLQVTAEEGGFTC  
 YGLSKGGFVPWF (SEQ ID No: 26)
- 30           **8. Qbeta binding stem loop [Q65H]**
- a. Qbeta phage operator stem loop**  
 35           5'- ATGCTGTCTAAGACAGCAT -3'(SEQ ID No: 180)
- b. Qbeta coat protein [Q65H]**  
 MAKLETVTLGNIGKDQKQTLVLNPRGVNPTNGVASLSQAGAVPALEKRVTV  
 SVSQPSRNRKKNYKVHVKIQNPTACTANGSCDPSVTRQAYADVTFSTQYSTD  
 EERAFVRTELAALLASPLLIDAIDQLNPAY (SEQ ID No: 181)

[213] In each of the aforementioned sequences, one may, for example, use the identical sequence or sequences that have one or more insertions, deletions or substitutions in one or both sequences of a binding pair. By way of a non-limiting  
5 example, for either or both members of a binding pair one may use a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% the same as an aforementioned sequence.

[214] In some embodiments, a complex is formed that comprises, consists essentially of, or consists of a Cas-fusion protein of the present invention and RNA-repressor domain complex of the present invention. Thus, if the Cas-fusion protein  
10 comprises a Cas protein fused to the repressor domain SUDS3, the ligand may be fused to SALL1 or to any other repressor domain that is now known or that comes to be known. Similarly, if the Cas-fusion protein comprises a Cas protein fused to the repressor domain SALL1, the ligand may be fused to SUDS3 or to any other repressor  
15 domain that is now known or that comes to be known. Further, in some embodiments, the Cas-fusion protein comprises, consists essentially of, or consists of a Cas protein, a SALL1 repressor domain and a SUDS3 repressor domain, and the RNA-repressor domain complex comprises a gRNA, a ligand binding moiety, a ligand and one or more repressor domains other than SALL1 or SUDS3. By way of  
20 non-limiting examples, the one or more repressor domains may be selected from the group consisting of NIPP1, KRAB and DNMT3A.

[215] Alternatively, one can use the RNA-repressor domain complexes with Cas enzymes that are not part of Cas-fusion protein complexes. For example, the RNA-repressor domain complex may comprise a gRNA, a ligand-binding moiety and one  
25 or both of the SUDS3 repressor domain and the SALL1 repressor domain as defined above. A repressor linker as defined above may be present between the SUDS3 repressor domain and the SALL1 repressor domain, and the ligand may be attached directly or through a ligand linker to either one of the SALL1 repressor domain and the SUDS3 repressor domain.

30 **[216] Nucleic Acids that Encode Cas fusion proteins**

[217] In some embodiments, the present invention provides a nucleic acid that encodes for a fusion protein of the present invention. The nucleic acid may be single stranded, double stranded or have at least one region that is single stranded and at

least one region that is double stranded. Further, the nucleic acid may comprise, consist essentially of, or consist of RNA or DNA.

[218] In some embodiments, the nucleic acid that encodes the fusion protein only contains nucleotides for the fusion protein and any linkers that are present. In other  
5 embodiments, the nucleic acid that encodes the fusion protein is part of a larger nucleic acid or a vector.

[219] In some embodiments, the present invention is directed to a vector that comprises a nucleic acid that encodes a fusion protein of the present invention. In some embodiments, the vector is a plasmid or a viral vector. When the vector is a  
10 viral vector, in some embodiments, the viral vector is a lentiviral vector. In some embodiments, rather than a vector that comprises a polynucleotide sequence that encodes a Cas fusion protein, the present invention is directed to an mRNA that encodes a Cas fusion protein of the present invention.

[220] In some embodiments, the nucleic acid comprises a sequence that encodes a  
15 Cas protein and at least one repressor domain such as SUDS3 or SALL1. In some embodiments, the nucleic acid comprises a sequence that encodes a Cas protein and at least two repressor domains, such as SUDS3 and SALL1. In some embodiments, the nucleic acid comprises a sequence that encodes a Cas protein and at least three repressor domains such as SUDS3, SALL1, and one or more of NIPP1, KRAB, and  
20 DNMT3A.

[221] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% the same as or complementary to SEQ ID NO: 4, which encodes the SALL1 repressor domain:

25 ATG AGT AGG AGA AAA CAA GCA AAA CCA CAG CAC TTT CAA AGT  
GAT CCT GAG GTA GCA AGC CTT CCA CGG GAC GGT GAC ACG GAG  
AAG GGT CAA CCA AGT CGA CCC ACG AAA AGC AAA GAT GCT CAT  
GTA TGT GGA CGC TGT TGC GCA GAA TTT TTT GAA TTG TCC GAT CTT  
30 CTT CTT CAC AAA AAG AAC TGC ACG AAG AAT CAG TTG GTT TTG ATA  
GTA AAC GAA AAT CCA GCT TCA CCC CCA GAA ACT TTT TCC CCG TCA  
CCT CCT CCA GAT AAT CCT GAT GAA CAA ATG AAT GAC ACC GTA AAT  
AAA ACC GAC CAA GTA GAC TGT TCT GAT TTG AGC GAA CAC AAC GGT  
TTG GAT CGA GAA GAG TCA ATG GAA GTA GAG GCC CCA GTT GCC AAT  
AAG TCA GGC AGC GGT ACT TCT TCC GGC TCC CAC AGT TCA ACA GCT  
35 CCA TCC TCA AGT AGT TCA AGC TCT TCT AGT TCA GGA GGC GGG GGG  
AGT AGC TCT ACC GGC ACT TCT GCC ATC ACA ACC TCA CTT CCT CAG  
CTT GGA GAC TTG ACA.

[222] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 4.

[223] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence is at least 80%, at least 85%, at least 90%, or  
5 at least 95% the same as or complementary to SEQ ID NO: 5, which encode the SUDS3 repressor domain:

ATG TCT GCA GCT GGC CTT TTG GCT CCT GCC CCC GCA CAA GCG GGA  
GCT CCT CCC GCA CCG GAG TAC TAT CCA GAA GAG GAT GAG GAA CTG  
GAA TCT GCC GAA GAC GAC GAG CGC AGT TGC CGG GGG AGG GAA  
10 TCT GAC GAG GAT ACT GAG GAT GCT TCT GAG ACC GAC CTC GCG AAA  
CAT GAT GAG GAA GAC TAC GTT GAA ATG AAA GAG CAG ATG TAC  
CAA GAC AAA CTT GCT AGC CTC AAG AGA CAG TTG CAG CAA CTG CAA  
GAA GGC ACG CTC CAG GAG TAC CAG AAG AGA ATG AAA AAA CTC  
GAC CAG CAG TAC AAG GAA CGA ATT AGA AAC GCA GAG CTC TTT CTT  
15 CAG CTG GAG ACT GAA CAG GTT GAG CGC AAT TAT ATT AAG GAA  
AAA AAA GCC GCT GTG AAG GAG TTC GAA GAC AAG AAA GTG GAA  
CTT AAA GAA AAC CTC ATC GCC GAA CTG GAG GAG AAG AAG AAG  
ATG ATA GAG AAC GAA AAA CTC ACA ATG GAA CTG ACG GGT GAT  
TCC ATG GAG GTA AAA CCG ATT ATG ACC CGA AAG CTC CGC CGA CGC  
20 CCA AAC GAT CCG GTA CCG ATC CCT GAT AAG CGG CGC AAG CCC GCA  
CCG GCT CAG CTC AAT TAC CTG CTG ACC GAC GAA CAA ATA ATG GAG  
GAC CTG CGG ACT CTT AAT AAG CTG AAG AGT CCT AAA CGG CCA GCT  
TCC CCC AGT TCC CCC GAA CAC CTG CCC GCT ACT CCC GCG GAG AGC  
CCT GCT CAG CGC TTT GAG GCC CGA ATC GAG GAC GGA AAA TTG TAC  
25 TAT GAC AAA CGC TGG TAT CAT AAG AGC CAG GCT ATA TAC CTG GAG  
TCA AAA GAT AAC CAA AAG TTG TCA TGT GTA ATC TCC TCA GTC GGG  
GCT AAC GAA ATA TGG GTG CGG AAG ACC TCT GAT AGT ACG AAG ATG  
CGC ATA TAT CTG GGA CAA TTG CAA AGA GGA CTT TTT GTT ATA AGA  
CGG AGA AGC GCT GCT.

30 [224] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 5.

[225] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%,  
35 or at least 95% the same as or complementary to SEQ ID NO: 6, which encodes the NIPP1 repressor domain:

ATGGTGCAAACCTGCAGTGGTCCCAGTCAAGAAGAAGCGTGTGGAGGGCCC  
TGGCTCCCTGGGCCTGGAGGAATCAGGGAGCAGGCGCATGCAGAACTTTG  
CCTTCAGCGGAGGACTCTACGGGGGCTGCCCCACACACAGTGAAGCA  
40 GGCTCCCAGCCACATGGCATCCATGGGACAGCACTCATCGGTGGCTTGCC  
CATGCCATACCCAAACCTTGCCCCTGATGTGGACTTGACTCCTGTTGTGCC  
GTCAGCAGTGAACATGAACCCTGCACCAAACCCTGCAGTCTATAACCCTG  
AAGCTGTAAATGAACCAAGAAGAAGAAATATGCAAAAGAGGCTTGCC  
AGGCAAGAAGCCCACACCTTCCTTGCTGATT.

[226] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 6.

[227] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%,  
5 or at least 95% the same as or complementary to SEQ ID NO: 37, which encodes the KRAB repressor domain:

ATGGACGCGAAATCACTTACGGCATGGTCGAGAACACTGGTTACGTTCAA  
GGACGTGTTTGTGGACTTTACACGTGAGGAGTGGAAATTGCTGGATACTG  
CGCAACAAATTGTGTATCGAAATGTCATGCTTGAGAATTACAAGAACCTC  
10 GTCAGTCTCGGATACCAGTTGACGAAACCGGATGTGATCCTTAGGCTCGA  
AAAGGGGGAAGAACCTTGGCTGGTA.

[228] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 37.

[229] In some embodiments, the nucleic acid sequence comprises, consists  
15 essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% the same as or complementary to SEQ ID NO: 38, which encodes the DNMT3A repressor domain:

CCCTCCCGGCTCCAGATGTTCTTCGCTAATAACCACGACCAGGAATTTGAC  
CCTCCAAAGGTTTACCCACCTGTCCCAGCTGAGAAGAGGAAGCCCATCCG  
20 GGTGCTGTCTCTCTTTGATGGAATCGCTACAGGGCTCCTGGTGCTGAAGGA  
CTTGGGCATTTCAGGTGGACCGCTACATTGCCTCGGAGGTGTGTGAGGACT  
CCATCACGGTGGGCATGGTGCGGCACCAGGGGAAGATCATGTACGTCGGG  
GACGTCCGCAGCGTCACACAGAAGCATATCCAGGAGTGGGGCCCATTCGA  
TCTGGTGATTGGGGGCAGTCCCTGCAATGACCTCTCCATCGTCAACCCTGC  
25 TCGCAAGGGCCTCTACGAGGGCACTGGCCGGCTCTTCTTTGAGTTCTACCG  
CCTCCTGCATGATGCGCGGCCCAAGGAGGGAGATGATCGCCCCTTCTTCT  
GGCTCTTTGAGAATGTGGTGGCCATGGGCGTTAGTGACAAGAGGGACATC  
TCGCGATTTCTCGAGTCCAACCCTGTGATGATTGATGCCAAAGAAGTGTCA  
GCTGCACACAGGGCCCGCTACTTCTGGGGTAACCTTCCCGGTATGAACAG  
30 GCCGTTGGCATCCACTGTGAATGATAAGCTGGAGCTGCAGGAGTGTCTGG  
AGCATGGCAGGATAGCCAAGTTCAGCAAAGTGAGGACCATTACTACGAG  
GTCAAACCTCATAAAGCAGGGCAAAGACCAGCATTTTCTCCTGTCTTCATGA  
ATGAGAAAGAGGACATCTTATGGTGCCTGAAATGGAAAGGGTATTTGGT  
TTCCAGTCCACTATACTGACGTCTCCAACATGAGCCGCTTGGCGAGGCA  
35 GAGACTGCTGGGCCGGTCATGGAGCGTGCCAGTCATCCGCCACCTCTTCG  
CTCCGCTGAAGGAGTATTTTTCGTGTGTG.

[230] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 38.

40 [231] In some embodiments, the nucleic acid sequence comprises a sequence that encodes at least one a linker sequence and is at least 80%, at least 85%, at least 90%,

or at least 95% the same as or complementary to SEQ ID NO: 8:

GGATCCGGTGGGGGATCTGGGGGATCTGGCTCG.

[232] In some embodiments, the nucleic acid sequence comprises a sequence that is the same as SEQ ID NO: 8.

- 5 [233] In some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% the same as or complementary to SEQ ID NO: 184, which encodes for both the SALL1 and SUDS3 repressor domains:

10 ATGAGTAGGAGAAAACAA GCAAACCACAGCACTTTCAAA GTGAT CCT  
 GAGGTAGCAAGCCTTCCA CGGCGGGACGGTGACACGGAGAAGGGTCA  
 ACCAAGTCGACCCACGAAAAGCAAAGATGCTCATGTATGTGGACGCTGTT  
 GCGCAGAATTTTTTGAATTGTCCGATCTTCTTCTTCACAAAAAGAACTGCA  
 CGAAGAATCAGTTGGTTTTGATAGTAAACGAAAATCCAGCTTCACCCCCA  
 GAAACTTTTTCCCCGTCACCTCCTCCAGATAATCCTGATGAACAAATGAAT  
 15 GACACCGTAAATAAAACCGACCAAGTAGACTGTTCTGATTTGAGCGAACA  
 CAACGGTTTGGATCGAGAAGAGTCAATGGAAGTAGAGGCCCCAGTTGCCA  
 ATAAGTCAGGCAGCGGTACTTCTCCGGCTCCACAGTTCAACAGCTCCAT  
 CCTCAAGTAGTTCAAGCTCTTCTAGTTTCAGGAGGCGGGGGGAGTAGCTCT  
 ACCGGCACTTCTGCCATCACAACTCACTTCCTCAGCTTGGAGACTTGACA  
 20 GGATCCGGTGGGGGATCTGGGGGATCTGGCTCGATGTCTGCAGCTGGCCT  
 TTTGGCTCCTGCCCCCGCACAAAGCGGGAGCTCCTCCCGCACCGGAGTACT  
 ATCCAGAAGAGGATGAGGAAGTGAATCTGCCGAAGACGACGAGCGCAG  
 TTGCCGGGGGAGGGAATCTGACGAGGATACTGAGGATGCTTCTGAGACCG  
 ACCTCGCGAAACATGATGAGGAAGACTACGTTGAAATGAAAGAGCAGAT  
 25 GTACCAAGACAAACTTGCTAGCCTCAAGAGACAGTTGCAGCAACTGCAAG  
 AAGGCACGCTCCAGGAGTACCAGAAGAGAATGAAAAAACTCGACCAGCA  
 GTACAAGGAACGAATTAGAAACGCAGAGCTCTTTCTTCAGCTGGAGACTG  
 AACAGGTTGAGCGCAATTATATTAAGGAAAAAAAAGCCGCTGTGAAGGA  
 GTTCGAAGACAAGAAAGTGGAACCTTAAAGAAAACCTCATCGCCGAACTG  
 30 GAGGAGAAGAAGAAGATGATAGAGAACGAAAAAATCACAATGGAAGTGA  
 CGGGTGATTCCATGGAGGTAAAACCGATTATGACCCGAAAGCTCCGCCGA  
 CGCCCAAACGATCCGGTACCGATCCCTGATAAAGCGGCGCAAGCCCGCACC  
 GGCTCAGCTCAATTACCTGCTGACCGACGAACAAATAATGGAGGACCTGC  
 GGACTCTTAATAAGCTGAAGAGTCCTAAACGGCCAGCTTCCCCCAGTTCC  
 35 CCCGAACACCTGCCCGCTACTCCCGCGGAGAGCCCTGCTCAGCGCTTTGA  
 GGCCCGAATCGAGGACGGAAAATTGTAATATGACAAACGCTGGTATCATA  
 AGAGCCAGGCTATATACCTGGAGTCAAAGATAACCAAAAAGTTGTCATGT  
 GTAATCTCCTCAGTCGGGGCTAACGAAATATGGGTGCGGAAGACCTCTGA  
 TAGTACGAAGATGCGCATATATCTGGGACAATTGCAAAGAGGACTTTTTG  
 40 TTATAAGACGGAGAAGCGCTGCT.

[234] Additionally or alternatively, in some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ ID NO: 183, which encodes deactivated Cas9 (dCas9):

[235] ATGGATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAG  
CGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCA  
TCGGCCTGGCCATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGAC  
GAGTACAAGGTGCCAGCAAGAAATCAAGGTGCTGGGCAACACCGACC  
5 GGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTTCGACAGCGGC  
GAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACA  
CCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAG  
ATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCT  
GGTGGAAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATC  
10 GTGGACGAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAG  
AAAGAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATC  
TGGCCCTGGCCCACATGATCAAGTTCGGGGCCACTTCCTGATCGAGGGC  
GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGT  
GCAGACCTACAACCAGCTGTTTCGAGGAAAACCCCATCAACGCCAGCGGCG  
15 TGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTG  
GAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCG  
CAACCTGATTGCCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACT  
TCGACCTGGCCGAGGATGCCAACTGCAGCTGAGCAAGGACACCTACGAC  
GACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAGTACGCCGACCT  
20 GTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCT  
GAGAGTGAACACCGAGATCACCAAGGCCCCCTGAGCGCCTCTATGATCA  
AGAGATACGACGAGCACCACCAGGACCTGACCTGCTGAAAGCTCTCGTG  
CGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAA  
GAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCT  
25 ACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTG  
CTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGA  
CAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTC  
TGCGGCGGCAGGAAGATTTTTACCCATTCTGAAGGACAACCGGGAAAAG  
ATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCC  
30 AGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCA  
TACCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCAG  
AGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCAACGAGAA  
GGTGTGCCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACG  
AGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTT  
35 CCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCA  
ACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAAT  
CGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACG  
CCTCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGAC  
TTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGAC  
40 CCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCT  
ATGCCACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAG  
ATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGG  
GACAAGCAGTCCGGCAAGACAATCCTGGATTTCTGAAAGTCCGACGGCTT  
CGCCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTA  
45 AAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCA  
CGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCC  
TGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAGTGATGGGCCGGCA  
CAAGCCCAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACC  
CAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAG  
50 GGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAA

ACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGG  
 CGGGATATGTACGTGGACCAGGAAGTGGACATCAACCGGCTGTCCGACTA  
 CGATGTGGACGCTATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCG  
 ACAACAAGGTGCTGACCAGAAGCGACAAGAACCAGGGGCAAGAGCGACAA  
 5 CGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAAGTACTGGCGGCAG  
 CTGCTGAACGCCAAGCTGATTACCCAGAGAAAAGTTCGACAATCTGACCAA  
 GGCCGAGAGAGGCCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAG  
 AGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCC  
 TGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGG  
 10 GAAGTGAAAGTGATCACCCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAA  
 GGATTTCCAGTTTACAAAGTGC GCGAGATCAACA ACTACCACCACGCC  
 ACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTAC  
 CCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGT  
 GCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCC  
 15 AAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACC  
 CTGGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCG  
 AAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGG  
 AAAGTGCTGAGCATGCCCAAGTGAATATCGTGAAAAAGACCGAGGTGC  
 AGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGAT  
 20 AAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCCT  
 TCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAA  
 AAGGGCAAGTCCAAGAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCA  
 CCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAA  
 GCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTA  
 25 AGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCT  
 GCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGT  
 GAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCG  
 AGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAAGCACTACCTG  
 GACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGC  
 30 CGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATA  
 AGCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACC  
 AATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCG  
 GAAGAGGTACACCAGCACCAAGAGGTGCTGGACGCCACCCTGATCCACC  
 AGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGA  
 35 GCGACAAGCGTCTTGCTGCTACTAAGAAAGCTGGTCAAGCTAAGAAAAA  
 GAAA

[236] Additionally or alternatively, in some embodiments, the nucleic acid sequence comprises, consists essentially of, or consists of a sequence that is the same as SEQ

ID NO: 178, which encodes deactivated MAD7 (dMAD7):

40 [237] ATGGTCGACGGTAAGCCTATCCCTAACCCCTCCTCGGTCTCGATTC  
 TACGCCAAAAAAGAAGAGAAAGGTCAATAACGGAACATAA ACTTCCAA  
 AACTTCATCGGGATCAGTTCCTTGCAGAAA ACTCTCCGGAATGCTCTCATC  
 CCAACTGAGACTACTCAGCAGTTCATTGTTAAGAATGGAATCATAAAAAGA  
 GGACGAGCTTAGGGGGGAAAATAGGCAAATCCTCAAGGATATCATGGAT  
 45 GACTATTATAGGGGCTTTATATCCGAGACACTGAGCAGCATTGATGATAT  
 AGACTGGACCTCTCTTTTCGAAAAGATGGAAATACA ACTTAAAAATGGAG  
 ATAACAAGGACACCCTGATAAAGGAACAGACCGAATATAGGAAGGCAAT  
 TCATAAAAAGTTTGCTAACGATGATAGGTTTAAAAACATGTTCTCAGCAA  
 AACTCATTTTCAGATATACTGCCCCGAATTCGTTATCCACAACAACA ACTACT

CCGCTAGCGAAAAAGAGGAAAAGACCCAAGTCATAAAGCTGTTCTCTCGA  
 TTCGCGACGAGTTTTAAAGATTATTTCAAGAATCGCGCAAACCTGTTTCTCA  
 GCTGATGATATCAGCAGCTCATCCTGTCATCGGATCGTTAACGATAATGCT  
 GAAATCTTCTTCTCCAATGCACTTGTTTATAGGCGCATTGTTAAATCTCTCT  
 5 CAAACGATGATATCAATAAGATTTCCGGCGATATGAAGGACAGTCTTAAG  
 GAGATGAGCCTCGAAGAGATATACTCATAACGAGAAATATGGCGAATTTAT  
 CACCCAGGAAGGGATTTCCCTTCTATAATGACATTTGCGGCAAAGTCAATTC  
 CTTTCATGAACCTGTATTGCCAAAAAATAAAGAAAACAAGAACCTCTATA  
 AGCTGCAAAAAGTTGCATAAGCAAATACTTTGTATCGCGGATAACAAGCTAT  
 10 GAAGTTCCCTACAAGTTCGAGAGTGATGAGGAGGTGTATCAATCTGTCAA  
 TGGTTTCCTTGATAATATTTCTTCTAAGCATATTGTTGAACGACTCCGAAA  
 GATAGGAGACAACATAATGGATACAATTTGGATAAAAATCTACATCGTGT  
 CTAATTTTACGAGAGTGTGTCACAAAAACATATAGAGACTGGGAGACA  
 ATTAATACCGCCCTGGAGATACATTACAACAATATACTTCCCGGGAACGG  
 15 GAAGTCTAAGGCAGACAAGGTGAAGAAAGCCGTGAAGAACGACTTGCAA  
 AAGTCAATTACCGAAATCAATGAGCTTGTTTCAAACATAAACTTTGTTCA  
 GATGACAATATTAAGCCGAAACCTATATTCATGAAATCTCTCATATTCTG  
 AATAACTTTGAGGCGCAAGAAGTAAATATAACCCAGAAATACACCTCGT  
 TGAGTCCGAACTGAAAGCAAGCGAACTGAAAAATGTTTTGGACGTGATAA  
 20 TGAACGCTTTTCATTGGTGCTCAGTCTTTATGACAGAGGAGCTTGTTGACA  
 AGGATAACAATTTCTATGCGGAACTGGAAGAGATTTACGACGAAATCTAT  
 CCGGTCATATCCCTGTATAACCTGGTTCGCAACTATGTCACGCAAAAACCA  
 TACAGCACGAAGAAGATTAACCTGAACTTTGGTATTCCGACGCTGGCCGA  
 TGGATGGTCAAAATCTAAGGAATACTCAAACAATGCCATAATCCTGATGC  
 25 GAGATAACCTCTACTACCTTGGAACTTTAATGCTAAAAATAAACCCGAT  
 AAAAAAATTATCGAAGGGAACACGAGTGAAAACAAAGGTGATTATAAAA  
 AAATGATATATAATCTGCTTCCAGGACCAAATAAGATGATACCCAAAGTT  
 TTCCTTTCTTCAAAGACCGGCGTCGAGACATATAAACCATCCGCGTACATA  
 CTTGAAGGCTACAAACAAAATAAACATATCAAATCATCTAAGGATTTTGA  
 30 CATTACGTTCTGTCATGATTTGATTGACTATTTCAAAAATTGCATAGCCAT  
 TCATCCAGAGTGGAAAACTTTGGGTTTACTTCTCTGATACCAGTACATA  
 TGAAGACATAAGTGGATTTTACCGAGAAGTAGAGCTCCAAGGTTATAAAA  
 TAGACTGGACCTATATATCTGAAAAGGATATAGACCTTTTGCAAGAGAAG  
 GGACAGCTTTATCTTTTCAAATCTACAACAAAGACTTCAGTAAGAAAAG  
 35 TACCGGGAATGACAATCTTCATACCATGTATCTGAAGAACCTGTTCTCCGA  
 AGAAAATCTGAAGGACATAGTCCTGAAGCTTAATGGCGAAGCGGAAATTT  
 TTTCCGAAAGAGCTCTATTAAGAACCCATAATACATAAGAAGGGGAAGC  
 ATTCTCGTTAATCGAACGTATGAGGCCGAAGAGAAAGATCAATTTGGGAA  
 TATCCAAATCGTTCGAAAGAACATAACAGAAAATATTTACCAAGAATTGT  
 40 ACAAATATTTTAACGATAAAAAGCGACAAAGAAGTGTCTGATGAAGCTGCT  
 AAGCTGAAAAACGTCGTCGGCCATCATGAGGCCGCGACGAATATAGTCAA  
 GGATTACCGATATACATACGATAAGTATTTCCCTGCATATGCCCATCACTAT  
 CAACTTTAAGGCAAATAAGACTGGATTCATTAATGACAGAATACTGCAAT  
 ACATAGCTAAAGAAAAAGATTTGCATGTTATTGGCATTGCCAGGGGTGAG  
 45 CGCAATCTTATCTATGTAAGCGTCATTGATACTTGCGGGAATATCGTAGAG  
 CAGAAGTCATTTAATATTGTAATGGGTACGATTACCAAATCAAGTTGAA  
 GCAGCAAGAGGGAGCACGACAGATTGCCCGCAAGGAGTGGAAAGAGATC  
 GGAAAGATAAAGGAGATCAAGGAGGGGTATTTGTCCCTTGTTATACACGA  
 AATTTCCAAGATGGTAATCAAGTACAACGCTATAATTGCTATGGCGGATC  
 50 TCTCCTATGGATTTAAAAAGGGAAGATTTAAAGTCGAGCGGCAGGTATAT

CAGAAATTTGAAACAATGCTTATTAATAAACTTAATTATCTCGTTTTCAA  
 GACATTAGTATCACCGAAAACGGTGGGCTGTTGAAGGGCTATCAACTTAC  
 GTACATACCAGATAAGCTTAAGAATGTGGGTCACCAATGCGGATGCATAT  
 TCTACGTGCCCGCAGCTTATAACAAGCAAAATCGACCCAACAACGGGTTTC  
 5 GTAAACATATTTAAGTTCAAGGATCTCACCGTGGATGCCAAGCGAGAGTT  
 CATAAAAAAATTTGACTCAATCAGATATGACTCAGAAAAGAATCTTTTTT  
 GTTTTACCTTCGACTACAATAATTTATTACACAAAATACGGTTATGAGCA  
 AGTCATCCTGGTCCGTATATACGTATGGAGTGCGCATAAAGCGGAGATTC  
 GTTAACGGGCGATTTTCTAATGAGTCCGATACAATCGATATAACAAAGGA  
 10 TATGGAAAAACTCTGGAAATGACTGATATAAATTGGAGGGACGGTCATG  
 ACCTCAGGCAAGACATTATCGATTATGAGATCGTGCAACATATTTTTGAG  
 ATCTTTCGGTTGACTGTCCAAATGAGGAACTCTCTGTCTGAATTGGAAGAT  
 AGGGACTACGATCGCCTGATAAGCCCCGTGTTGAACGAGAATAACATATT  
 CTACGATTCCGCGAAAGCCGGGGATGCGCTCCCTAAGGACGCCGCTGCAA  
 15 ATGGGGCCTATTGTATTGCTTTGAAAGGGCTGTACGAAATCAAACAGATC  
 ACCGAAAACCTGGAAAGAAGACGGGAAGTTTAGTCGGGATAAACTGAAGA  
 TATCCAACAAGGACTGGTTTGACTTTATCCAAAATAAGCGATATTTGAAG  
 CGTCCTGCTGCTACTAAGAAAGCTGGTCAAGCTAAGAAAAAGAAA

20 [238] In some embodiments, the nucleic acid sequence comprises, consists  
 essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%,  
 or at least 95% the same as or complementary to SEQ ID NO: 178.

[239] Additionally or alternatively, in some embodiments, the nucleic acid sequence  
 comprises, consists essentially of, or consists of a sequence that is the same as SEQ  
 25 ID NO: 179, which encodes deactivated CasPhi8 (dCasPhi8):

ATGGTCGACGGGAGCGGGCCGGCAGCTAAACGGGTGAAGTTGGACAGTG  
 GTGGAATTAACCTACAGTTTCTCAGTTTCTTACCCCTGGTTTTAAGCTGA  
 TAAGAAACCATAGTCGGACGGCTGGACTTAAGCTGAAGAATGAGGGCGA  
 30 AGAGGCATGCAAGAAGTTCGTACGGGAGAACGAAATCCCAAAGATGAA  
 TGTCCAAACTTTCAAGGTGGACCCGCAATCGCGAACATTATAGCCAAGAG  
 TCGCGAATTTACCGAGTGGGAAATATATCAAAGTTCACTGGCGATCCAAG  
 AGGTGATTTTACCTTGCCGAAGGATAAGCTGCCCGAGCCTATACTCAAG  
 GAAGAATGGCGCGCCCAATGGTTGAGCGAACACGGCCTCGATACGGTGCC  
 TTACAAGGAAGCTGCCGACTTAATTTGATAATTAAGAACGCGGTCAACA  
 35 CTTACAAAGGGGTCCAGGTGAAAGTCGATAATAAGAATAAGAACAACCT  
 GGCCAAAATCAACCGCAAGAACGAAATCGCGAAATTGAACGGCGAACAA  
 GAAATCAGCTTCGAAGAGATCAAAGCCTTCGATGATAAAGGATATCTCCT  
 GCAAAGCCAAGTCCGAATAAGAGCATATATTGCTACCAAAGCGTGTCTC  
 CAAAGCCATTCATAACCTCTAAATACCATAACGTGAATCTGCCCGAAGAA  
 40 TATATCGGCTACTACCGCAAGTCAAACGAGCCCATCGTTAGTCCCTATCAA  
 TTCGATAGATTGCCAATCCCAATTGGCGAACCCGGATATGTACCAAATG  
 GCAGTATACCTTTCTGTCTAAGAAAGAGAATAAGCGGAGAAAGCTCTCCA  
 AGCGGATTAAGAATGTTAGTCCTATTCTTGGGATAATATGCATTAAGAAA  
 GACTGGTGCATTCGATATGAGGGGCTGCTCAGAACGAACCACTGGAA  
 45 GAAATACCATAAACCGACAGATTCTATCAATGACCTCTTCGATTATTTAC  
 TGGAGACCCTGTAATCGACACGAAAGCGAACGTCGTCCGATTCAGATATA  
 AAATGGAAAATGGCATTGTTAATTACAAGCCGGTGCGCGAAAAGAAAG

CAAGGAACTTTTGGAAAACATATGTGATCAAAATGGGAGCTGTAAGTTGG  
 CCACTGTGGCCGTTGGTCAAAACAACCCAGTGGCAATTGGACTGTTTGAA  
 CTTAAGAAAGTAAATGGTGAACCTTACCAAAACCTTGATTTACGGCATCC  
 TACTCCGATCGACTTTTGTAAATAAAATTACGGCTTACAGGGAGCGGTATG  
 5 ATAAGCTCGAATCCAGCATCAAGTTGGATGCCATAAAGCAATTGACATCT  
 GAGCAAAAGATCGAAGTTGATAACTATAACAATAATTTTACCCCTCAAAA  
 CACTAAGCAGATAGTGTGCAGCAAGCTCAATATCAATCCAAACGACCTTC  
 CTTGGGATAAAATGATTTCTGGGACTCATTTTCATTAGCGAGAAAGCCCAA  
 GTCAGTAATAAAATCAGAAATATACTTCACATCTACCGATAAGGGGAAAAC  
 10 TAAGGACGTAATGAAGAGCGACTACAAGTGGTTTCAAGACTATAAACCAA  
 AACTGTCAAAGGAAGTAAGGGACGCACTCAGCGATATTGAATGGCGGCTT  
 AGGAGAGAAAGTCTTGAATTTAACAAATTGAGTAAATCACGGGAACAAG  
 ATGCACGGCAACTGGCCAATTGGATCTCTTCCATGTGTGATGTTATCGGAA  
 TAGAGAACCCTGGTGAAGAAGAACAATTTCTTTGGTGGAAAGCGGCAAGAG  
 15 GGAACCGGGGTGGGACAACCTTCTATAAACCGAAGAAGGAGAATCGATGG  
 TGGATCAACGCAATTCATAAAGCTCTCACAGAACTCTCTCAAAACAAAGG  
 GAAAAGAGTGATTCTCTTGCCAGCAATGAGAACATCTATCACATGCCCTA  
 AATGTAAGTACTGTGACAGCAAGAACCGGAACGGCGAGAAGTTCAATTGT  
 CTGAAGTGTGGCATAGAACTCAACGCAGACATTGATGTTGCTACCGAAAA  
 20 TCTCGCGACCGTTGCTATTACCGCGCAAAGTATGCCTAAACCCACCTGTGA  
 GAGGAGTGGTGTATGCCAAGAAGCCCGTACGTGCACGAAAGGCAAAGGCG  
 CCAGAATTTTCATGACAAACTCGCGCCCTCATACACAGTTGTCTTGCGCGAA  
 GCTGTAAAGCGTCCTGCTGCTACTAAGAAAGCTGGTCAAGCTAAGAAAAA  
 GAAA

25 [240] In some embodiments, the nucleic acid sequence comprises, consists  
 essentially of, or consists of a sequence that is at least 80%, at least 85%, at least 90%,  
 or at least 95% the same as or complementary to SEQ ID NO: 179.

[241] In some embodiments, the fusion protein of the present invention may be  
 linked to nuclear localization signals (NLS), epitope tags, or reporter gene sequences.

30 Examples of nuclear localization signals include, but are not limited to, those of the  
 SV40 Large T-antigen, nucleoplasmin, EGL-13, and TUS-protein. Examples of  
 epitope tags include, but are not limited to, FLAG tags, V5 tags, histidine (His) tags,  
 and influenza hemagglutinin (HA) tags. Examples of reporter genes include, but are  
 not limited to, green fluorescent protein (GFP), red fluorescent protein (RFP), small  
 35 ubiquitin-like modifier (SUMO), ubiquitin, glutathione-S-transferase (GST),  
 horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), and  
 luciferase.

[242] In some embodiments, the nucleic acid or vector that encodes the fusion  
 proteins of present invention will also encode various regulatory elements or selection  
 40 markers. Regulatory elements include, but are not limited to promoters such as the  
 cytomegalovirus (CMV) promoter or human EFl $\alpha$  promoter, enhancers such as the  
 woodchuck hepatitis post-transcriptional regulatory element (WPRE) or HIV-1 Rev

response element (RRE), polyadenylation signals, self-cleaving peptides such as T2A, and internal ribosomal entry sites (IRES). Examples of selection markers include, but are not limited to, green fluorescent protein (GFP), red fluorescent protein (RFP), puromycin *N*-acetyl-transferase (PAC) conferring resistance to puromycin, the  
5 hygromycin resistance gene, and blasticidin-S deaminase (BSD).

[243] **Methods of Modulating Expression**

[244] In some embodiments, the present invention is directed to a method of modulating expression of a target nucleic acid in a eukaryotic cell. The method  
10 comprises providing to the cell a gRNA and a Cas fusion protein of any of the embodiments of the present invention. When associated with a gRNA as shown in **figure 1**, the Cas protein (shown as a dCas protein) **110** that is fused to SALL1 **120**, which is fused to SUDS3 **130** may act upon a target region of genomic DNA **150**.

[245] In some embodiments, the method comprises introduce a plurality of gRNAs  
15 with the Cas fusion protein. The plurality of gRNAs may be two or more, *e.g.*, 2 - 10 or 4 - 8 gRNAs.

[246] Two or more or all of the gRNAs may target the same gene or the same locus within a gene. If two or more gRNAs target the same locus, they may have the same or overlapping spacer sequences or non-overlapping sequences. In some  
20 embodiments, two or more or all of the gRNAs may target the different genes or the different loci within a gene.

[247] In some embodiments, one or more gRNAs are provided to a cell by introducing to the cell a nucleic acid encoding the gRNA, and the Cas fusion protein is provided to the cell by introducing to the cell a nucleic acid encoding the Cas fusion  
25 protein. The cell may be placed under conditions in which the cell expresses the gRNA and the Cas fusion protein.

[248] In some embodiments, the present invention is directed to a method of modulating expression of a target nucleic acid in a eukaryotic cell by introduce a Cas fusion protein and an RNA-repressor domain complex. In some embodiments, the  
30 present invention is directed to a method of modulating expression of a target nucleic acid in a eukaryotic cell by introduce a Cas protein that is not a fusion protein and an RNA-repressor domain complex.

[249] In some embodiments, the eukaryotic cell is a yeast cell, a plant cell or a mammalian cell such as a human or murine cell. In some embodiments, the cell is part of a cell line, *e.g.*, HEK293, K562, Jurkat, or US2OS.

[250] When a fusion protein is introduced, the fusion protein may be synthesized  
5 outside of a cell or an organism. Alternatively, one may introduce an mRNA that encodes the fusion protein. In some embodiments, a gRNA is synthetically made outside of the cell and a Cas fusion protein is provided to the cell by introducing to the cell a nucleic acid encoding the Cas fusion protein.

[251] The Cas fusion proteins, RNA-repressor domain complexes and/or gRNAs  
10 may be delivered to target cells and organisms via other various methods and various formats (DNA, RNA or protein) or combination of these different formats. For example, different components may be delivered as: (a) DNA polynucleotides that encode the relevant sequence for the Cas fusion protein or the gRNAs; (b) RNA encoding the sequence for the Cas fusion protein (messenger RNA) and synthetic  
15 gRNAs; (c) purified protein for the Cas fusion protein; (d) RNA that encode gRNA; and (e) purified RNA-repressor domain complexes.

[252] When delivering a Cas fusion protein in a protein format, the Cas protein can be assembled with the applicable gRNA to form a ribonucleoprotein complex (RNP) for delivery into target cells, organisms and subjects. For example, the components or  
20 complexes ([Cas fusion protein]-[gRNA]) as assembled may be delivered together or separately by electroporation, by nucleofection, by transfection, via nanoparticles, via viral mediated RNA delivery, via non-viral mediated delivery, via extracellular vesicles (for example, exosome and microvesicles), via eukaryotic cell transfer (for example, by recombinant yeast) and other methods that can package molecules such  
25 that they can be delivered to a target viable cell without changes to the genomic landscape. Other methods include, but are not limited to, non-integrative transient transfer of DNA polynucleotides that include the relevant sequence for the protein recruitment so that the molecule can be transcribed into the desired RNA molecule. This includes, without limitation, DNA-only vehicles (for example, plasmids,  
30 MiniCircles, MiniVectors, MiniStrings, Protelomerase generated DNA molecules (for example, Doggybones), artificial chromosome (for example HAC), and cosmids), via DNA vehicles by nanoparticles, extracellular vesicles (for example, exosome and microvesicles), via eukaryotic cell transfer (for example, by recombinant yeast), transient viral transfer by AAV, non-integrating viral particles (for example, lentivirus

and retrovirus based systems), cell penetrating peptides and other technology that can mediate the introduction of DNA into a cell without direct integration into the genomic landscape.

[253] Another method for the introduction of the RNA components include the use of integrative gene transfer technology for stable introduction of the machinery for RNA transcription into the genome of the target cells. These methods can be controlled via constitutive or promoter inducible systems to attenuate the RNA expression and this can also be designed so that the system can be removed after the utility has been met (for example, introducing a Cre-Lox recombination system), such technology for stable gene transfer includes, but is not limited to, integrating viral particles (for example lentivirus, adenovirus and retrovirus based systems), transposase mediate transfer (for example, Sleeping Beauty and Piggybac), exploitation of the non-homologous repair pathways introduced by DNA breaks (for example, utilizing CRISPR and TALEN) technology and a surrogate DNA molecule, and other technology that encourages integration of the target DNA into a cell of interest.

[254] The various components of the complexes of the present invention, if not synthesized enzymatically within a cell or solution, may be created chemically or, if naturally occurring, isolated and purified from naturally occurring sources. Methods for chemically and enzymatically synthesizing the various embodiments of the present invention are well known to persons of ordinary skill in the art. Similarly, methods for ligating or introducing covalent bonds between components of the present invention are also well known to persons of ordinary skill in the art.

#### [255] **Kits**

[256] In some embodiments, the present invention is directed to a kit that comprises, consists essentially of, or consists of a Cas fusion protein of the present invention or a polynucleotide with a nucleic acid sequence that encodes a protein of the present invention. In some embodiments, the kit further comprises a gRNA or a nucleic acid that encodes a gRNA or a plurality of gRNAs or a library of gRNAs, and optionally reagents for transfection and/or other delivery into a cell or to a subject. In some embodiments, the kit comprises a nucleic acid that is capable of expressing both a gRNA and a Cas fusion protein of the present invention. In some embodiments, the kit comprises a cell

line that has been engineered to express a Cas fusion protein of the present invention and optionally further comprises a gRNA or a nucleic acid that encodes a gRNA.

[257] In some embodiments, the present invention is directed to a kit that comprises, consists essentially of, or consists of an RNA-repressor domain complex of the present invention. In some embodiments, the kit further comprises a Cas protein or a Cas fusion protein or a nucleic acid that encodes a Cas protein or a Cas fusion protein, and optionally reagents for transfection and/or other delivery into a cell or to a subject.

[258] In one embodiment, the present invention provides a kit, wherein the kit comprises: (1) a lentiviral particle, wherein the lentiviral particle comprises a first polynucleotide that encodes a Cas fusion protein of the present invention, such as dCas9-SALL1-SUDS3; and (2) a second polynucleotide, wherein the second polynucleotide is an sgRNA.

[259] In another embodiment, the present invention provides a kit, wherein the kit comprises: (1) a first lentiviral particle, wherein the first lentiviral particle comprises a first polynucleotide that encodes a Cas fusion protein of the present invention, such as dCas9-SALL1-SUDS3; and (2) a second lentiviral particle, wherein the second lentiviral particle comprises a second polynucleotide, wherein the second polynucleotide codes for an sgRNA.

[260] In another embodiment, the present invention provides a kit, wherein the kit comprises: (1) a lentiviral particle, wherein the lentiviral particle comprises a first polynucleotide that encodes a Cas fusion protein of the present invention, such as dCas9-SALL1-SUDS3; and (2) a second polynucleotide, wherein the second polynucleotide is a plasmid, wherein the plasmid encodes a second polynucleotide and the second polynucleotide is an sgRNA.

[261] In another embodiment, the present invention provides a kit, wherein the kit comprises: (1) a first polynucleotide, wherein the first polynucleotide is an mRNA that encodes a Cas fusion protein of the present invention, such as dCas9-SALL1-SUDS3; and (2) a second polynucleotide, wherein the second polynucleotide is an sgRNA.

[262] The sgRNAs in the kits may be designed to associate with the Cas fusion protein that is encoded by the polynucleotides described above. Optionally, within the kits may be one or more of the following: target cells, and one or more a selection chemicals and/or media (*e.g.*, blasticidin, puromycin).

[263] **Applications**

[264] In another embodiment the present invention provides method for simultaneous repression of multiple genes. In some of these methods one may deliver the same dCas9-repressor Cas fusion protein with different gRNAs that target different gene promoters or different transcriptional start sites of the same gene. In another embodiment, the present invention provides a method for simultaneous repression and gene editing. In these methods one may deliver the Cas9-repressor Cas fusion protein with regular gRNAs (20 nucleotide targeting region) to cause gene editing and truncated gRNAs (14 nucleotide targeting region) to cause gene repression. These methods may be used to repress an inflammatory response such as the myeloid differentiation primary response 88 (MyD88) while performing gene editing, or to repress various genes involved in non-homologous end-joining thereby increasing the likelihood of a homology-directed DNA repair event (HDR) or to modulate host genes that are involved in the regulation of repair of double-stranded DNA breaks, leading to different outcomes. These methods may be used to effect synthetic lethality whereby a gene target can be edited and a secondary gene target can be repressed to cause a cytotoxic response not present in cells containing only one of the genomic perturbations.

[265] **Figure 15** illustrates the effect of using different sized crRNA regions with an active Cas9 that is fused to SALL1 and SUDS3. When a 14-mer crRNA targeting region is used, there is transcriptional repression of the target (left y-axis of figure). Whereas, when a 20-mer crRNA targeting region is used, there is gene-editing of the target (right y-axis of figure).

[266] The various embodiments of the present invention may also be used in arrayed screening applications. For example, one may use a library of arrayed gRNAs for systematic loss-of-function studies. In some embodiments 2-5 synthetic guide RNAs can be pooled for arrayed screening applications.

[267] The various embodiments of the present invention may also be used in pooled lentiviral screening applications. For example, one may use a pooled library of lentiviral sgRNA constructs targeting a set of gene targets or the whole genome for systematic loss-of-function studies. These gRNAs can be delivered in cells expressing the Cas fusion constructs of the present invention, or via a lentiviral construct that expresses both the Cas fusion protein and a gRNA.

[268] Additionally, in other embodiments, one may combine different CRISPR Cas systems with different effectors in the same cells to cause transcriptional repression with one system and another effect (activation, gene editing, base editing, or epigenetic modification) with the other Cas system.

5 [269] These methods may, for example, be used to cause specific gene repression of an immune cell selected from a T cell (including a primary T cell), Natural Killer (NK cell), B cell, or CD34+ hematopoietic stem progenitor cell (HSPC). The immune cell may be an engineered immune cell, such as T-cell comprising a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). The methods herein may  
10 thus be applied to further modulate gene expression of a cell that has already been modified to include a CAR and/or TCR that is useful in therapy. By way of further example, primary immune cells, either naturally occurring within a host animal or patient, or derived from a stem cell or an induced pluripotent stem cell (iPSC) may be used for specific gene repression using the methods and complexes provided herein.

15 Suitable stem cells include, but are not limited to, mammalian stem cells such as human stem cells, including, but not limited to, hematopoietic, neural, embryonic, iPSC, mesenchymal, mesodermal, liver, pancreatic, muscle, and retinal stem cells. Other stem cells include, but are not limited to, mammalian stem cells such as mouse stem cells, *e.g.*, mouse embryonic stem cells.

20 [270] Also provided herein are methods for genome engineering (*e.g.*, altering or manipulating the expression of one or more genes or one or more gene products) in prokaryotic or eukaryotic cells, *in vitro*, *in vivo*, or *ex vivo*. In particular, the methods provided herein may be useful for targeted gene expression modulation in mammalian cells including primary human T cells, NK cells, CD34+ HSPCs, such as HSPCs  
25 isolated from umbilical cord blood or bone marrow and cells differentiated from them.

[271] Also provided herein are genetically engineered cells arising from haematopoietic stem cells, such as T cells, that have been modified according to the methods described herein.

30 [272] By way of a non-limiting example, the various embodiments of the present invention may be used for the following applications, base editing, genome editing, genome screening, generation of therapeutic cells, genome tagging, epigenome editing, karyotype engineering, chromatin imaging, transcriptome and metabolic pathway engineering, genetic circuits engineering, cell signaling sensing, cellular

events recording, lineage information reconstruction, gene drive, DNA genotyping, miRNA quantification, in vivo cloning, site-directed mutagenesis, genomic diversification, and proteomic analysis in situ. In some embodiments, a cell or a population of cells are exposed to a fusion protein of the present invention and the cell  
5 or cells are introduced to a subject by infusion.

[273] Applications also include research of human diseases such as cancer immunotherapy, antiviral therapy, bacteriophage therapy, cancer diagnosis, pathogen screening, microbiota remodeling, stem-cell reprogramming, immunogenomic engineering, vaccine development, and antibody production.

10 [274] In some embodiments, one or more molecules or complexes described herein, including a Cas fusion protein, a fusion protein, a Cas protein, a gRNA, and a nucleic acid that encodes any of the foregoing is introduced to a subject. Introduction may, for example, be in the form of a medicament.

15 [275] **Examples**

[276] In the examples below, applicable protocols from the following methods and materials were used:

[277] **Stable cell line generation**

20 [278] U2OS Ubi[G76V]-EGFP (BioImage, discontinued), U2OS (ATCC, cat # HTB-96), A375 (ATCC, cat # CRL-161), K-562 (ATCC, cat # CCL-243), Jurkat (ATCC, cat #TIB-152), HCT 116 (ATCC, cat # CCL-247), and WTC-11 hiPS (Coriell institute, cat # GM25256) cells were transduced at a multiplicity of infection (MOI) of 0.3 with lentiviral particles co-expressing the blasticidin resistance gene and  
25 various Cas-based effector proteins designed for CRISPRi. Cells were subsequently cultured in cell-line-specific medium containing 5-10 µg/mL blasticidin for a minimum of ten days to select cell populations that stably expressed the CRISPRi effector proteins. U2OS cells stably expressing dCas9 were subsequently transduced at an MOI of 0.3 with lentiviral particles that co-expressed MCP-SALL1-SUDS3 and  
30 the hygromycin resistance gene. The cells were cultured for 14 days in medium containing 200 µg/ mL hygromycin to select for a population that stably expressed both MCP-SALL1-SUDS3 and dCas9.

**[279] Synthesis of guide RNAs**

[280] All sgRNA, crRNA and tracrRNA were synthesized at Horizon Discovery (formerly Dharmacon). sgRNA and crRNA molecules were designed based on the CRISPRi version 2.1 (v2.1) guide RNA prediction algorithm developed in 2016, M. A. Horlbeck *et al.*, “Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation,” *eLife*. 5, e19760 (2016). Unless otherwise stated, experiments utilized modified sgRNAs delivered as an equimolar pool of the top three algorithmically ranked sgRNAs, labeled g1-g3 in **table 2** below. The same targeting sequences were used for the sgRNA, crRNA, and expressed sgRNA with the exception that the first base in the expressed sgRNAs is always G.

**[281] Lipid transfections with synthetic guide RNAs**

[282] U2OS or A375 cells were seeded in 96-well plates at 10,000 or 20,000 cells per well, respectively, one day prior to transfection. Cells were transfected with synthetic guide RNAs targeting specific genes at a final concentration of 25 nM. Synthetic guide RNAs were complexed with DharmaFECT 4 Transfection Reagent (Horizon Discovery, cat # T-2005-01) for each experiment in serum-free medium (GE Healthcare HyClone, cat #SH30564.01) for 20 minutes. Medium on the plated cells was removed and replaced with the transfection mixture. The cells were incubated at 37° C with 5% CO<sub>2</sub> for 24-144 hours until the assays were performed.

**[283] Co-transfections with dCas9 mRNA and synthetic sgRNA**

[284] U2OS cells were seeded at 10,000 cells per well in clear 96-well plates one day prior to transfection; HCT 116 cells were seeded at 200,000 cells per well in clear 6-well plates one day prior to transfection. U2OS cells were co-transfected with 0.2 µg/well of dCas9-SALL1-SUDS3 or dCas9-KRAB mRNA and 25 nM synthetic sgRNA; HCT 116 cells were co-transfected with 2.5 µg/well of dCas9-SALL1-SUDS3 or dCas9-KRAB mRNA and 25 nM synthetic sgRNA. dCas9 mRNA and sgRNAs were complexed with DharmaFECT Duo Transfection Reagent (Horizon Discovery, cat #T-2010) in serum-free medium (GE Healthcare HyClone, cat #SH30564.01) for 20 minutes. Medium on the plated cells was removed and replaced with the transfection mixture. The cells were incubated at 37° C with 5% CO<sub>2</sub>.

[285] **Nucleofection**

[286] K562, Jurkat, WTC-11 human induced pluripotent stem cells (hiPS cells), and primary human CD4+ T cells were electroporated per well using the Amaxa 96-well Shuttle System. 200,000 K562 cells per replicate were resuspended in SF buffer (Lonza, cat #V4SC-2096) and nucleofected using the FF-120 program; 200,000 Jurkat cells were resuspended in SE buffer (Lonza, cat #V4SC-1960) and nucleofected using program Cl-120; 80,000 hiPS cells were resuspended in P3 buffer (Lonza, cat #V4SP-3096) and nucleofected using program DC-100; 250,000 primary human CD4+ T cells were resuspended in P3 buffer and nucleofected using program E0-115.

10 Synthetic guide RNAs were delivered at cell-line-dependent final concentrations between 2.5 and 9  $\mu$ M. In cases where the cells were not stably expressing a dCas9 CRISPRi construct, dCas9-SALL1-SUDS3 or dCas9-KRAB mRNA was delivered at cell-line-dependent concentrations ranging from 1-2.5  $\mu$ g per nucleofection.

15 [287] **Transfections with plasmid sgRNA**

[288] U2OS and A375 cells were seeded in 96-well plates at 10,000 or 20,000 cells per well one day prior to transfection with CRISPRi sgRNA plasmids. Plasmids were complexed with DharmaFECT kb Transfection Reagent (Horizon Discovery, Cat #T-2006) in serum-free medium (GE Healthcare HyClone, #SH30564.01) for 10 minutes.

20 Medium on the plated cells was removed and replaced with the transfection mixture. The cells were incubated at 37° C with 5% CO<sub>2</sub> for 72 hours until the assays were performed.

[289] **Lentiviral transduction**

25 [290] U2OS and HCT 116 cells were seeded at 10,000 cells per well and transduced with CRISPRi sgRNA lentiviral particles at a multiplicity of infection (MOI) of 0.3 to obtain cells with a single integrant. Cells were selected with 2.5  $\mu$ g/mL puromycin for 7 days with passaging every 3-4 days prior to RT-qPCR analysis.

30 [291] **RT-qPCR**

[292] Total RNA was isolated, reverse-transcribed using Maxima First Strand cDNA Synthesis Kit for RT-qPCR, with dsDNase (ThermoFisher Scientific, cat #K1672) and assessed with qPCR using TaqMan Gene Expression Master Mix and TaqMan Gene Expression Assays. The relative expression of each gene was calculated with

the  $\Delta\Delta Cq$  method using GAPDH or ACTB as the housekeeping gene and normalized to a non-targeting control (NTC).

[293] **Proteasome assay – a functional reporter assay for proteasome gene**

5 **inhibition**

[294] The proteasome assay utilizes a recombinant U2OS cell line that stably expresses a mutant Ubiquitin fused to enhanced green fluorescent protein (Ubi[G76V]-EGFP). At the experimental endpoint, cell media was replaced with Dulbecco's Phosphate Buffered Saline (Cytivia, cat # SH30028.02) and EGFP

10 fluorescence was measured using an EnVision® plate reader. Fluorescent values of cell populations transfected with guide RNAs targeting critical proteasome genes were normalized to fluorescent values of the untreated cell populations.

[295] **Sanger sequencing gene editing analysis**

15 [296] Cells were lysed in 100  $\mu$ L of a buffer containing proteinase K (Thermo Scientific, #FEREO0492), RNase A (Thermo Scientific, #FEREN0531), and Phusion HF buffer (Thermo Scientific, #F-518L) for 30 min at 56 °C, followed by a 5 minute heat inactivation at 95 °C. This cell lysate was used to generate 400-600 nucleotide PCR amplicons spanning the region containing the gene editing site(s). Unpurified  
20 PCR amplicons were subjected to Sanger sequencing. Gene editing efficiencies were calculated from AB1 files using TIDE analysis, Brinkman *et al.*, “Easy quantitative assessment of genome editing by sequence trace decomposition,” *Nucleic acids research*, 42(22) (2014). . TIDE quantifies the frequency and types of small insertions and deletions (indels) at a target locus using quantitative sequence trace data from a  
25 targeted sample that is normalized to sequence trace data of a control sample.

[297] **FACS analysis**

[298] 24 and 72 hours post-nucleofection, functional knockdown of *CXCR3* was assessed as a percent of cells expressing the target gene by FACS analysis. Cells were  
30 resuspended in a 1:50 solution of Fc block (BD Biosciences, cat # 564220) and stained for *CD4* as a positive expression control using an Alexa Fluor 488 conjugated antibody (Biolegend, cat # 50166932) and *CXCR3* using an APC conjugated primary antibody (Biolegend, cat # 353707). Unstained cells were used to gate for *CD4* and

*CXCR3* positive cells. The percent *CXCR3* positive cells in the targeted populations was normalized to that in the control populations to determine functional knockdown.

[299] **Example 1: Comparison of silencing of dCas9-KRAB and dCas9-SALL1-SUDS3 delivered as mRNA**

5

[300] A comparison of silencing by dCas9-KRAB and dCas9-SALL1-SUDS3 was undertaken against each of the following targets: BRCA1, PPIB, CD46, PSMD7, SEL1L, and ST3GAL4. K562 cells were nucleofected with either dCas9-KRAB or dCas9-SALL1-SUDS3 mRNA and gene knockdown was then measured. In each

10 case, the cells were supplied with a 5  $\mu$ M mixture of a pool of three pooled synthetic sgRNAs targeting the respective gene.

[301] Forty-eight hours after nucleofection, gene-expression was measured relative to a non-targeting control. The results appear in **figure 3A**. As the bar graph shows, in each instance, silencing of gene expression by dCas9-SALL1-SUDS3 was

15 comparable to, if not better than, silencing by dCas9-KRAB.

[302] **Figure 3B** shows the results of a similar study, except that the target cells were Jurkat cells and the expression was measured 72 hours after nucleofection. In **figure 3B**, one again sees that in each instance, silencing of gene expression by dCas9-SALL1-SUDS3 was comparable to, if not better than, silencing by dCas9-

20 KRAB.

[303] **Figure 3C** shows the results of another similar study, except that the target cells were U2OS cells, reagents were delivered via lipid transfection using a 25 nM mixture of a pool of three pooled synthetic sgRNAs targeting the respective gene, and the expression was measured 72 hours after transfection. In **figure 3C**, one again

25 sees that in each instance, silencing of gene expression by dCas9-SALL1-SUDS3 was comparable to, if not better than, silencing by dCas9-KRAB.

[304] **Example 2: Comparison of Effectiveness of Cas fusion protein Repressor to dCas9-KRAB**

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[305] HCT116 cells were plated at 400,000 cells per well. Twenty-four hours later, the cells were co-transfected with dCas9-SALL1-SUDS3 eGFP mRNA or dCas9-KRAB eGFP mRNA and a 25 nM mixture of a pool of three synthetic sgRNAs targeting each of the following genes: PPIB, PSMD7, and SEL1L, as well as a

35 nontargeting control (NTC), using DharmaFECT® Duo Transfection reagent. At 24 hours post-transfection, cells were trypsinized, and FACS was performed. Cells were

sorted into two categories: Negative, and Top 10%, then plated in 6-well dishes and allowed to recover. After 24 hours of recovery (48 hours total), the total amount of RNA was isolated and relative gene expression was measured using RT-qPCR. The relative expression of each gene was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control.

[306] As **figure 4** shows, dCas9-SALL1-SUDS3 eGFP mRNA can be used for FACS enrichment and provides greater repression of target genes than dCas9-KRAB eGFP mRNA in both selected and unselected populations.

10 [307] **Example 3: Comparison repression of dCas9-KRAB to dCas9-SALL1-SUDS3 in Different Cell Lines**

[308] U2OS, Jurkat, and hiPS cells stably expressing dCas9-SALL1-SUDS3 or dCas9-KRAB were transfected or nucleofected with pools of three synthetic sgRNAs targeting the listed genes, as well as NTCs. Cells were harvested 72 hours later. In each cell line dCas9-KRAB or dCas9-SALL1-SUDS3 were under control of the hEF1 $\alpha$  promoter. The total RNA was isolated and relative gene expression was measured using RT-qPCR. The relative expression of each gene was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control.

[309] As **figure 5A** shows, in the U2OS stable hEF1 $\alpha$  cell line, dCas9-SALL1-SUDS3 demonstrated greater gene repression against BRCA1, PSMD7, SEL1L, and ST3GAL4. As **figure 5B** shows, in the Jurkat stable hEF1 $\alpha$  cell line, dCas9-SALL1-SUDS3 also demonstrated greater gene repression against BRCA1, PSMD7, SEL1L, and ST3GAL4. As **figure 5C** shows, in the USOS stable hEF1 $\alpha$  cell line, dCas9-SALL1-SUDS3 demonstrated greater or similar gene repression against RAB11A, PPB, and SEL1L. As **figure 6A** shows, in the K562 stable hEF1 $\alpha$  cell line, dCas9-SALL1-SUDS3 also demonstrated greater gene repression against BRCA1, PSMD7, SEL1L, and ST3GAL4. As **figure 6B** shows, in the A375 stable hEF1 $\alpha$  cell line, dCas9-SALL1-SUDS3 demonstrated greater or similar gene repression against BRCA1, PSMD7, SEL1L, and ST3GAL4.

35 [310] **Example 4: dCas9-KRAB versus dCas9-SALL1-SUDS3 over course of 6 days**

[311] U2OS cell lines stably expressing dCas9-SALL1-SUDS3 or dCas9-KRAB under the control of the hEF1 $\alpha$  promoter were transfected with the pools of three synthetic sgRNAs targeting each of the following genes : BRCA1, CD46, HBP1, and SEL1L. Repression was measured over six days with samples harvested every 24  
5 hours post-transfection. Total RNA was isolated, and gene expression was assessed via RT-qPCR. The relative expression of each gene was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control.

[312] **Figure 7A** shows that dCas9-SALL1-SUDS3 caused greater repression than  
10 dCas9-KRAB did against BRCA1 at all timepoints. **Figure 7B** shows that dCas9-SALL1-SUDS3 caused greater repression than dCas9-KRAB did against CD46 at all timepoints. **Figure 7C** shows that dCas9-SALL1-SUDS3 caused greater repression than dCas9-KRAB did against HBP1 at all timepoints. **Figure 7D** shows that dCas9-SALL1-SUDS3 caused greater repression than dCas9-KRAB did against SEL1L at all  
15 timepoints. Note that in each example there was a more rapid onset of the repression mediated by dCas9-SALL1-SUDS3 than that mediated by dCas9-KRAB, and that the repression mediated by dCas9-SALL1-SUDS3 persisted at close to maximal levels for longer than the repression mediated by dCas9-KRAB.

20 [313] **Example 5: Pooling sgRNAs**

[314] WTC-11 hiPSCs stably expressing dCas9-SALL1-SUDS3, and U2OS cells stably expressing dCas9-SALL1-SUDS3 were nucleofected or transfected with individual or a pool of three synthetic sgRNAs targeting PPIB (3  $\mu$ M), SEL1L (3  
25  $\mu$ M), RAB11A (3  $\mu$ M) - 3  $\mu$ M of each sgRNA electroporated, BRCA1 (25 nM), PSDM7 (25 nM), SEL1L (25 nM), and ST3GAL4 (25 nM) delivered via lipid transfection. Cells were harvested 72 hours later. The total RNA was isolated and relative gene expression was measured using RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control.

30 [315] As **figure 8A** shows, in the WTC-11 hiPSCs, the pooling was comparable to or better than the use of each individual sgRNA. Similarly, as **figure 8B** shows, in the US2OS hEF1 $\alpha$  dCas9-SALL1-SUDS3, the pooling was comparable to or better than the use of each individual sgRNA.

[316] **Example 6: Multiplexing of gRNAs for simultaneous repression of multiple genes**

[317] hiPSCs stably expressing dCas9-SALL1-SUDS3 were nucleofected with individual sgRNAs and pools of up to 6 sgRNAs targeting unique genes. Cells were  
5 harvested 72 hours later. The total RNA was isolated and the relative gene expression was measured using RT-qPCR. The relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control

[318] As **figure 9** shows, when up to six genes were targeted for simultaneous  
10 repression in human iPS cells, the levels of target gene repression was comparable to when only one of the genes was targeted.

[319] **Example 7: Fusion to N-terminal amino acid and to C-terminal amino acid of Cas protein**

15 [320] The structures of three Cas fusion proteins are represented at the bottom of **figure 10**: dCas9-KRAB; dCas9-SALL1-SUDS3, and SUDS3-SALL1-dCas9. The Cas fusion proteins were expressed under the control of the human EF1 $\alpha$  promoter.

[321] U2OS Ubi[G76V]-EGFP cell lines were generated that stably expressed various bipartite dCas9 fusion proteins based, along with a cell line stably expressing  
20 dCas9-KRAB. Cells were transfected with 25 nM synthetic sgRNAs targeting genes known to be critical to proteasome function, as well as non-targeting controls. The fluorescence of each transfection condition was determined at 72 hours post-transfection with an EnVision<sup>®</sup> plate reader and values were normalized to that those of the untreated cell line.

25 [322] The U2OS cell line stably expressing a mutant Ubiquitin fused to enhanced green fluorescent protein (Ubi[G76V]-EGFP). In untreated cells, the expressed ubiquitin EGFP protein is constitutively degraded, leaving only background fluorescence, whereas cells with inhibited proteasome function display an accumulation of EGFP. Repression of target genes therefore results in increased  
30 fluorescence.

[323] As **figure 10** shows, the Cas fusion proteins containing dCas9, SUDS3, and SALL1 showed substantially more repression than dCas9-KRAB regardless of whether the fusion occurred at the N-terminal amino acid or the C-terminal amino acid of the Cas protein. (A higher mean GFP expression correlates to greater  
35 repression.)

[324] **Example 8: Plasmid:Plasmid Co-Transfection in A375 & U2OS cells**

[325] Plasmid repressors: (1) hEF1 $\alpha$ -dCas9 KRAB; or (2) dCas9-SALL1-SUDS3 were co-transfected with guides (total = 100 ng) using 0.6  $\mu$ L/well of DharmaFECT<sup>®</sup> 5 kb. **Figure 11** shows the results when measuring gene expression by RT-qPCR at three days post-plasmid co-transfection of repressor and gene targets in A375 cells. **Figure 12** shows the results when measuring gene expression by RT-qPCR at three days post-plasmid co-transfection of repressor and gene targets in U2OS cells. Both figures consistently show greater repression in systems that contained the plasmid for 10 dCas9-SALL1-SUDS3.

[326] **Example 9: Additional Repressors**

[327] U2OS Ubi[G76V]-EGFP cell lines were generated that stably expressed various bipartite dCas9 fusion proteins based, along with a cell line stably expressing 15 dCas9-KRAB. Cells were transfected with 25 nM synthetic sgRNAs targeting genes known to be critical to proteasome function, as well with non-targeting controls. The fluorescence of each transfection condition was determined at 72 hours post-transfection with an Envision<sup>®</sup> plate reader. The values were normalized to those of the untreated cell line.

20 [328] As **figure 13** shows, a Cas fusion protein containing: (i) dCas9; (ii) either SUDS3 or SALL1; and (iii) KRAB or NIPP1 shows greater than or comparable repression as dCas9. (A taller bar indicates greater repression. The system was designed in the same manner as the system in example 8.)

25 [329] **Example 10: Type V Cas protein-SALL1-SUDS3 fusion constructs**

[330] A deactivated MAD7 (an engineered Cas12a protein)-SALL1-SUDS3 fusion construct was cloned (dMAD7-SALL1-SUDS3), and U2OS cells were generated that stably expressed it under control of the minimal CMV (mCMV) promoter. A deactivated CasPhi8 (a Cas12J protein)-SALL1-SUDS3 fusion construct was cloned 30 (dCasPhi8-SALL1-SUDS3), and U2OS cells were generated that stably expressed it under control of the mCMV promoter. These cells, along with U2OS cells stably expressing dMAD7 or dCasPhi8, were lipid transfected with synthetic guides designed for the respective Cas proteins, in each case delivered at 25 nM. Transcriptional repression was assessed 48 hours post-transfection.

[331] **Figure 14A** shows CRISPRi induced transcriptional repression in U2OS cells stably expressing either dMAD7 or dMAD7-SALL1-SUDS3 for two individual synthetic guide RNAs against each of BRCA1 and PPIB, as well as for a pool of synthetic guide RNA, and an NTC. The figure shows significantly greater repression  
5 effected by dMAD7-SALL1-SUDS3 as compared to dMAD7.

[332] **Figure 14B** shows CRISPRi induced transcriptional repression in U2OS cells stably expressing either dCasPhi8 or dCasPhi8-SALL1-SUDS3 for three iterations of individual synthetic guide RNAs targeting the same site in BRCA1, and basal BRCA1 expression in untreated U2OS cells. The figure shows significantly greater repression  
10 effected by dCasPhi8-SALL1-SUDS3 as compared to dCasPhi8.

[333] These figures demonstrate that SALL1 and SUDS3 can be fused to various Type V Cas proteins and programmed with synthetic guide RNA to effect significant target gene repression.

15 [334] **Example 11: Simultaneous editing and repression with active Cas9 fusion proteins**

[335] U2OS cells stably expressing SUDS3-SALL1-WtCas9 under the control of the hEF1 $\alpha$  promoter were transfected with 25 nM pools of guide RNAs designed for both CRISPRi and CRISPR editing. Guides designed for CRISPRi contained a truncated  
20 14-mer targeting region. Guides designed for CRISPR editing contained the full 20-mer targeting region. Cells were harvested 72 hours later post-transfection. The total RNA was isolated and the relative gene expression was measured using RT-qPCR. The relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control. Genomic DNA was  
25 isolated, target regions were amplified using PCR and Sanger sequenced, and indel formation was analyzed using TIDE.

[336] **Figure 15B** shows MRE11A can be repressed while LBR is simultaneously edited. **Figure 15C** shows MRE11A can be repressed while PPIB is simultaneously edited. **Figure 15D** shows SEL1L can be repressed while LBR is simultaneously  
30 edited. **Figure 15E** shows SEL1L can be repressed while PPIB is simultaneously edited.

[337] **Example 12: Comparison of single repressor domains as dCas9-fusion Protein**

[338] U2OS Ubi[G76V]-EGFP cell lines were generated that stably expressed various single repressor dCas9 fusion proteins (BCL6, CbpA, H-NS, MBD3, NIPP1, SALL1, and SUDS3), along with a cell line stably expressing dCas9-KRAB, all under the control of the human EF1 $\alpha$  promoter. Cells were transfected with 25 nM synthetic sgRNAs targeting genes known to be critical to proteasome function, as well as non-targeting controls.

[339] The fluorescence of each transfection condition was determined at 72 hours post-transfection, with an EnVision® plate reader and values were normalized to those of the untreated cell line. The U2OS cell line stably expressed a mutant Ubiquitin fused to enhanced green fluorescent protein (Ubi[G76V]-EGFP). In untreated cells, the expressed ubiquitin EGFP is constitutively degraded, leaving only background fluorescence, whereas cells with inhibited proteasome function display an accumulation of EGFP. Repression of target genes therefore results in increased fluorescence. As **figure 16** shows, the dCas fusion proteins containing NIPP1, SALL1, and SUDS3, showed substantially more repression than dCas9-KRAB. (A higher mean GFP expression correlates to greater repression.)

[340] **Example 13: Comparison of dCas9-SUDS3 repressor to dCa9-KRAB and dCas9-KRAB-MeCP2 systems**

[341] U2OS Ubi[G76V]-EGFP cell lines were generated that stably expressed either dCas9-KRAB, dCas9-KRAB MeCP2, or dCas9-SUDS3 under the control of the human EF1 $\alpha$  promoter. Cells were transfected with 25 nM synthetic sgRNAs targeting genes known to be critical to proteasome function, as well as non-targeting controls. Cells were harvested 72 hours post-transfection, total RNA was isolated, and expression of the target genes was assessed via RT-qPCR. Relative expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control. **Figure 17** shows that for each gene target dCas9-SUDS3 effected substantially more transcriptional repression than either dCas9-KRAB or dCas9-KRAB-MeCP2.

[342] **Example 14: Proteasome Functional Reporter assay and Transcriptional Repression**

[343] U2OS Ubi[G76V]-EGFP cell lines were generated that stably expressed either dCas9-KRAB or dCas9-SALL1-SUDS3 under the control of the human EF1 $\alpha$

promoter. Cells were transfected with 25 nM synthetic sgRNAs targeting genes known to be critical to proteasome function, as well as non-targeting controls. The fluorescence of each transfection condition was determined at 72 hours post-transfection, with an EnVision® plate reader and values were normalized to those of the untreated cell line. The U2OS cell line stably expressed a mutant Ubiquitin fused to enhanced green fluorescent protein (Ubi[G76V]-EGFP). In untreated cells, the expressed ubiquitin EGFP is constitutively degraded, leaving only background fluorescence, whereas cells with inhibited proteasome function display an accumulation of EGFP. Repression of target genes therefore results in increased fluorescence. Total RNA was also isolated and expression of the target genes was assessed via RT-qPCR. Relative expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control. **Figure 18A** shows dCas9-SALL1-SUDS3 effected significantly more phenotypic knockdown than dCas9-KRAB. (A higher mean GFP expression correlates to greater repression.) **Figure 18B** shows that the more pronounced phenotype observed with dCas9-SALL1-SUDS3 correlated with increased transcriptional repression of the targeted proteasome genes.

#### [344] **Example 15: Lentiviral Delivery**

[345] **Figure 19A** shows the transcriptional repression of PPIB and SEL1L in U2OS cells stably expressing either dCas9-SALL1-SUDS3 and a guide RNA from a single lentiviral vector or from two separate vectors. **Figure 19B** shows the transcriptional repression of PPIB and SEL1L in HCT 116 cells stably expressing either dCas9-SALL1-SUDS3 and a guide RNA from a single lentiviral vector or from two separate vectors.

[346] Lentiviral vectors were used to generate U2OS and HCT 116 cells that stably expressed dCas9-SALL1-SUDS3 under the control of the human EF1 $\alpha$  promoter (hEF1 $\alpha$ ) or mouse CMV promoter (mCMV) respectively. These cells were subsequently transduced with lentiviral particles containing vectors that expressed individual guide RNAs from the human U6 promoter and targeted PPIB, SEL1L, or contained a non-targeting control sequence. Parental U2OS and HCT 116 cells were transduced with lentiviral particles containing a single vector that expressed dCas9-SALL1-SUDS3 under the control of the hEF1 $\alpha$  (U2OS) or mCMV (HCT 116) promoters, and an individual guide RNA from the human U6 promoter. These single

vector systems also targeted PPIB or SEL1L, or contained a non-targeting control sequence. Twenty-four hours post-transduction, media containing 2.5  $\mu\text{g}/\text{mL}$  puromycin was added to enrich for transduced cells. Cells were cultured in this media for 7 days and passaged every 3 to 4 days. Eight days post-transduction cells were  
5 harvested, total RNA was isolated, and the relative expression of the target genes was determined by RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta\text{Cq}$  method using GAPDH as the housekeeping gene and normalized to the non-targeting control.

[347] **Figure 19A** shows that either a single lentiviral or dual lentiviral vector  
10 system can be used to express dCas9-SALL1-SUDS3 and a guide RNA to robustly repress a target gene in U2OS cells. **Figure 19B** shows that either a single lentiviral or dual lentiviral system can be used to express dCas9-SALL1-SUDS3 and a guide RNA to robustly repress a target gene in HCT 116 cells.

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[348] **Example 16: Plasmid sgRNAs vs. Synthetic sgRNAs**

[349] U2OS and A375 cells stably expressing dCas9-SALL1-SUDS3 under the control of the hEF1 $\alpha$  promoter were transfected with individual, matched 25 nM synthetic sgRNAs or 100 ng of plasmid sgRNA targeting BRCA1, PSMD7, SEL1L,  
20 and ST3GAL4. Cells were harvested 72 hours post-transfection, total RNA was isolated, and the relative gene expression of each target genes was assessed using RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta\text{Cq}$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control. **Figure 20A** demonstrates that dCas9-SALL1-SUDS3 mediates substantially greater target gene  
25 expression when delivered with synthetic sgRNAs than when delivered with plasmid sgRNAs in U2OS cells. **Figure 20B** demonstrates that dCas9-SALL1-SUDS3 mediates substantially more target gene expression when delivered with synthetic sgRNAs than when delivered with plasmid sgRNAs in A375 cells.

30 [350] **Example 17: Synthetic sgRNA vs crRNA:tracrRNA**

[351] U2OS cells stably expressing dCas9-SALL1-SUDS3 under the control of the hEF1 $\alpha$  promoter were transfected with pooled 25 nM synthetic sgRNAs or synthetic crRNA:tracrRNA complexes. Cells were harvested 72 hours post-transfection, total RNA was isolated, and the relative gene expression of each target genes was

measured using RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control. **Figure 21** demonstrates that while repression is markedly more pronounced with pooled synthetic sgRNAs, both synthetic sgRNA and synthetic crRNA:tracrRNA complexes can be delivered with dCas9-SALL1-SUDS3 to cause target gene repression.

[352] **Example 18: 5' Truncated Spacer**

[353] U2OS cells stably expressing dCas9-SALL1-SUDS3 under the control of the hEF1 $\alpha$  promoter were transfected with 25 nM pools of guide RNAs containing either truncated 14-mer targeting regions or full length 20-mer targeting regions. Cells were harvested 72 hours post-transfection. Total RNA was isolated and the relative gene expression of the target genes was measured using RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeted control. **Figure 22** shows that the targeting region of a guide RNA can be shortened at the 5' end by at least 6-mer and still effect transcriptional repression when delivered with dCas9-SALL1-SUDS3.

[354] **Example 19: LNA modified sgRNAs**

[355] U2OS Ubi[G76V]-EGFP cells stably expressing dCas9-SALL1-SUDS3 under the control of the human EF1 $\alpha$  promoter were transfected with 25 nM synthetic sgRNAs targeting two genes known to be critical to proteasome function, as well as non-targeting controls. The guides contained various combinations of 2'-O-methyl and phosphorothioate linkages and locked nucleic acids at the ends of the sgRNA molecule, and in the 20-mer targeting region, position 1 to position 20 from the 5' end. The fluorescence of each transfection condition was determined 144 hours post-transfection with an EnVision® plate reader and values were normalized to those of the untreated cell line. The U2OS cell line stably expressed a mutant Ubiquitin fused to enhanced green fluorescent protein (Ubi[G76V]-EGFP). In untreated cells, the expressed ubiquitin EGFP is constitutively degraded, leaving only background fluorescence, whereas cells with inhibited proteasome function display an accumulation of EGFP. Repression of target genes therefore results in increased fluorescence.

[356] **Figure 23A** shows the effects on dCas9-SALL1-SUDS3 mediated functional knockdown of various chemical end modifications to the sgRNA molecule. The incorporation of locked nucleic acids at the 5' and 3' end of the sgRNA molecule can be used to stabilize the gRNA. The incorporation of two locked nucleic acid at the 3' end of the sgRNAs targeting PSMD7 and PSMD11 further improves target gene repression. (A higher mean GFP expression correlates to greater repression.) **Figure 23B** shows the impact of the incorporation of locked nucleic acid positions into the sgRNA targeting region on dCas9-SALL1-SUDS3 mediated functional knockdown. Locked nucleic acids can be incorporated at some positions of the sgRNA targeting region to improve target gene repression.

[357] **Example 20: RNA -repressor complex recruitment**

[358] U2OS cells stably expressing dCas9 and SALL1-SUDS3 fused to the MS2 Coat protein ligand (MCP-SALL1-SUDS3), each under the control of the human EF1 $\alpha$  promoter, were generated through sequential transduction of the respective lentiviral expression vector. The cells were then transfected with 25 nM synthetic crRNA:tracrRNA complexes targeting BRCA1, CD151, and SETD3, along with NTCs. Several tracrRNA designs containing different MS2 ligand binding moiety sequences and positions were tested against each gene target and compared to complexes containing a tracrRNA without an MS2 ligand binding moiety, labeled crRNA:tracrRNA w/out MS2. Cells were harvested 72 hours post-transfection, total RNA was isolated, and the relative gene expression of each target genes was measured using RT-qPCR. Relative gene expression was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control.

[359] **Figure 24A** demonstrates that MCP-SALL1-SUDS3 can be recruited to dCas9 through the C-5 MS2 sequence positioned at the either sgRNA stem loop 2 or at the 3' terminus of the tracrRNA molecule. The recruitment of MCP-SALL1-SUDS3 can enhance the repressive effect of dCas9 binding, represented here as crRNA:tracrRNA w/out MS2. **Figure 24B** shows that MCP-SALL1-SUDS3 can be recruited to dCas9 through both the C-5 MS2 sequence and the F-5 MS2 sequence containing a 2dAP chemical mod to significantly improve the repressive effect of dCas9 binding.

[360] **Example 21: Knockdown in T-cells**

- [361] Primary human CD4+ T cells were nucleofected with dCas9-SALL1-SUDS3 mRNA and pooled synthetic sgRNA via a Lonza 96-well Shuttle system. 24 and 72 hours post-nucleofection, functional knockdown of *CXCR3* was assessed as a percent of cells expressing the target gene by FACS analysis. Cells were stained for *CD4* as a positive expression control using an Alexa Fluor 488 conjugated antibody and compared to *CXCR3* using APC conjugated primary antibodies. Total RNA was isolated at each timepoint and mRNA expression of *CXCR3* was assessed via RT-qPCR. The relative expression of *CXCR3* was calculated with the  $\Delta\Delta Cq$  method using GAPDH as the housekeeping gene and normalized to a non-targeting control.
- 5 [362] **Figure 25A** is a graph that shows the transcriptional repression and protein level knockdown of *CXCR3* in primary human CD4+ T cells nucleofected with dCas9-SALL1-SUDS3 and either a synthetic non-targeting control or a pool of 3 guides targeting the gene of interest 1 and 3 days post-nucleofection.
- [363] **Figures 25B** shows that the onset of knockdown with dCas9-SALL1-SUDS3 was rapid and persisted for several days in this clinically relevant primary cell type, comparing protein expression in the non-transfected control system on day 1, protein expression in the non-transfected control system on day 3, protein expression in the *CXCR3* pool system on day 1 and protein expression in the *CXCR3* pool system on day 3.
- 15 [364] **Table 2: synthetic sgRNAs (Sp Cas9)**
- [365] Target region is bolded, chemical modifications are italicized.
- 20

Target	Guide Name	SEQUENCE
NTC	NTC sgRNA	<i>(mG)*(mU)*AACGCGAACUACGCGGGUGUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGUCU(mU)*(mU)*U</i> (SEQ ID: 43)
BRCA 1	BRCA1_g1	<i>(mC)*(mU)*CGCUGAGACUCCUGGACGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGUCU(mU)*(mU)*U</i> (SEQ ID: 44)
	BRCA1_g2	<i>(mU)*(mG)*AAGGCCUCCUGAGCGCAGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGUCU(mU)*(mU)*U</i> (SEQ ID: 45)
	BRCA1_g3	<i>(mC)*(mC)*ACAGCCUGUCCCCCGUCCGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGUCU(mU)*(mU)*U</i> (SEQ ID: 46)
CD46	CD46_g1	<i>(mU)*(mC)*CCUUCUGGGUCCAGAUUGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGUCU(mU)*(mU)*U</i> (SEQ ID: 47)

Target	Guide Name	SEQUENCE
	CD46_g 2	<i>(mG)*(mG)*AUUGUUGC</i> GUCCCAUAUCGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 48)
	CD46_g 3	<i>(mG)*(mA)*CUAGAGCUCUCCUCAGUC</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 49)
<b>PPIB</b>	PPIB_g 1	<i>(mC)*(mG)*GAGAGGCGCAGCAUCCAC</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 50)
	PPIB_g 2	<i>(mA)*(mG)*AGGCGCAGCAUCCACAGG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 51)
	PPIB_g 3	<i>(mG)*(mG)*ACCCGCGAUGAGGGCGG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 52)
<b>PSMD 7</b>	PSMD7 _g1	<i>(mA)*(mA)*CUGGGCCUGAAAGGGUAC</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 53)
	PSMD7 _g2	<i>(mG)*(mC)*GCCGCCGGCCAGCUAUA</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 54)
	PSMD7 _g3	<i>(mU)*(mC)*CCUGCCACACGCAAACAC</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 55)
<b>SEL1 L</b>	SEL1L_ g1	<i>(mA)*(mG)*GGGGCGGAUACUGACCCG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 56)
	SEL1L_ g2	<i>(mA)*(mU)*ACUGACCCGAGGACGCCG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 57)
	SEL1L_ g3	<i>(mG)*(mG)*UGGUGGCUGAGUCCGUGG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 58)
<b>ST3G AL4</b>	ST3GA L4_g1	<i>(mC)*(mC)*GCUAGGCGCACCGACCGG</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 59)
	ST3GA L4_g2	<i>(mG)*(mA)*UCCGCUAGGCGCACCGAC</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 60)
	ST3GA L4_g3	<i>(mG)*(mC)*UGGCGCGACGGCUCGAC</i> UGUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 61)

Target	Guide Name	SEQUENCE
RAB1 1A	RAB11 A_g1	<i>(mU)*(mG)*CGCGGCCGAGGAGCGAAA</i> GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 62)
	RAB11 A_g2	<i>(mG)*(mC)*GGCCGAGGAGCGAAA</i> GGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 63)
	RAB11 A_g3	<i>(mG)*(mG)*AGCAGCAGUGGUAUCUGUGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 64)
HBP1	HBP1_ g1	<i>(mA)*(mA)*GCUUGAAAGACUUGGUAAGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 65)
	HBP1_ g2	<i>(mU)*(mU)*GAGGAGUAAGAGCUGCCGGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 66)
	HBP1_ g3	<i>(mU)*(mG)*GCGACGGUUUGGUAAGUGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 67)
PSMD 3	PSMD3 _g1	<i>(mC)*(mA)*CGAGCGCGAGAUAGCGUCGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 68)
	PSMD3 _g2	<i>(mA)*(mG)*CGUCGGGCCGCACGAUGAGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 69)
	PSMD3 _g3	<i>(mG)*(mC)*UCGUGUGCAGGCCCGGCUGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 70)
PSMD 8	PSMD8 _g1	<i>(mG)*(mG)*CGGCCGCGCGGUGAACGGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 71)
	PSMD8 _g2	<i>(mU)*(mG)*CCGCAUCACGCAAGAUGGGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 72)
	PSMD8 _g3	<i>(mC)*(mG)*GCGCUGCCGUAAAUCAGGGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 73)
PSMD 11	PSMD1 1_g1	<i>(mA)*(mC)*GGUGUGAGAGCGGUAAGAGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 74)
	PSMD1 1_g2	<i>(mG)*(mG)*CCGGGGACGGUGUGAGAGGUUUU</i> AGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU( <i>mU)*(mU)*U</i> ) (SEQ ID: 75)

Target	Guide Name	SEQUENCE
	PSMD1 1_g3	<i>(mG)*(mU)*GUGAGAGCGGUAAGAUGGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 76)
VCP	VCP_g 1	<i>(mG)*(mG)*CUCCGGAGUUUAUCCUCCGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 77)
	VCP_g 2	<i>(mG)*(mA)*GAAGGAGCAAGAAGUGUCGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 78)
	VCP_g 3	<i>(mC)*(mC)*GCGAGGUGGAGUGGAGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 79)
MRE1 1A	MRE11 A_g1	<i>(mC)*(mU)*GAAUCCGCGGGAGAGAAGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 80)
	MRE11 A_g2	<i>(mU)*(mC)*CGUGAAAAGAAAACAACAGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 81)
	MRE11 A_g3	<i>(mG)*(mG)*CCGUAAACCUGAAUCCGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 82)
PSMA 2	PSMA2 _g1	<i>(mG)*(mG)*GUAAGAUGGCGGAGCGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 83)
	PSMA2 _g2	<i>(mG)*(mC)*UUUCGCUGACUACAUCGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 84)
	PSMA2 _g3	<i>(mG)*(mA)*CUACGCUGAAGACCUCGAGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 85)
CD151	CD151_ g1	<i>(mC)*(mC)*CGGACUCGGACGCGUGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 86)
	CD151_ g2	<i>(mG)*(mC)*GGCCCGGAGCCUACGAGGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 87)
	CD151_ g3	<i>(mA)*(mG)*GGCCCGGACUCGGACGCGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 88)
SETD 3	SETD3 _g1	<i>(mA)*(mA)*CCAACCCAGGCGGUGGUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCU(mU)*(mU)*U</i> (SEQ ID: 89)

Target	Guide Name	SEQUENCE
	SETD3_g2	<i>(mC)*(mU)*CAACCAACCCCCAGGCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 90)
	SETD3_g3	<i>(mC)*(mC)*UCGCAGAGCUCGGAGACGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 91)
TFRC	TFRC_g1	<i>(mG)*(mC)*AGCCAUAGGGAGCCGCACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 92)
	TFRC_g2	<i>(mG)*(mG)*AUGGCGGCCCCUAACCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 93)
	TFRC_g3	<i>(mC)*(mA)*GAGCGUCGGGAUAUCGGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 94)
LBR	LBR_g1	<i>(mA)*(mU)*AGUCGCACAGCAACCCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 95)
	LBR_g2	<i>(mA)*(mG)*AAUAGUCGCACAGCAACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 96)
	LBR_g3	<i>(mG)*(mG)*UCCGGCGGUGACACGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 97)
XRCC4	XRCC4_g1	<i>(mC)*(mC)*GGAAGUAGAGUCACGGAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 98)
	XRCC4_g2	<i>(mA)*(mG)*AGGUAGGAUCCGGAAGUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 99)
	XRCC4_g3	<i>(mA)*(mG)*AUACCGGAAGUAGAGUCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 100)
CXCR3	CXCR3_g1	<i>(mU)*(mU)*ACCUCAAGGACCAUGGCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 101)
	CXCR3_g2	<i>(mG)*(mG)*GCAGCAGCACUUACCUCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 102)
	CXCR3_g3	<i>(mC)*(mC)*ACAAGCACAAAGCAGAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA</i> CCGAGUCGGUGCU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 103)

Target	Guide Name	SEQUENCE
LBR	20 nt LBR_g 2	<b>(mG)*(mC)*CGAUGGUGAAGUGGUAAG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 104)
PPIB	20 nt PPIB_g 2	<b>(mG)*(mU)*GUAUUUUGACCUACGAAUGUUU</b> AGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 105)

[366] **Table 3: 5' truncated 14 mer targeting region synthetic sgRNAs (Sp Cas9)**

[367] Target region is **bolded**, chemical modifications are *italicized*.

Target	Guide Name	SEQUENCE
NTC	14 nt NTC	<b>(mC) * (mG) *AACUACGCGGGU</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 106)
LBR	14 nt LBR_g 1	<b>(mG) * (mC) *ACAGCAACCCGG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 107)
	14nt LBR_g 2	<b>(mG) * (mU) *CGCACAGCAACC</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 108)
	14nt LBR_g 3	<b>(mG) * (mG) *CGGUGACACGGA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 109)
MRE1 1A	14 nt MRE11 A_g1	<b>(mU) * (mC) *CGCGGGAGAGAA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 110)
	14 nt MRE11 A_g2	<b>(mA) * (mA) *AAGAAAACAACA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 111)
	14 nt MRE11 A_g3	<b>(mA) * (mA) *ACCUGAAUCCG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 112)
SEL1 L	14 nt SEL1L _g1	<b>(mC) * (mG) *GAUACUGACCCG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 113)
	14 nt SEL1L _g2	<b>(mA) * (mC) *CCGAGGACGCCG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 114)
	14 nt SEL1L _g3	<b>(mG) * (mG) *CUGAGUCCGUGG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 115)
XRCC 4	14 nt XRCC4 _g1	<b>(mG) * (mU) *AGAGUCACGGAG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 116)

14 nt XRCC4 _g2	<b>(mA) * (mG) *GAUCCGGAAGUG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 117)
14 nt XRCC4 _g3	<b>(mC) * (mG) *GAAGUAGAGUCA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 118)
14 nt LBR_g 2	<b>(mG) * (mG) *UGAAGUGGUAAG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 119)
14 nt PPIB_ g2	<b>(mU) * (mU) *UGACCUACGAAU</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 120)

[368] **Table 4: LNA modified synthetic sgRNAs (Sp Cas9)**[369] Target region is **bolded**, chemical modifications are *italicized*.

Target	Guide Name	SEQUENCE
PSMD 7	PSMD7 2x 5' LNA, 2x O'me 3' sgRNA	<b>(G-LNA) (5mC- LNA) GCCGCCGGCCAGCUAUA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU) * (mU) *U (SEQ ID: 121)
	PSMD7 2x 5' O'me, 2x 3' LNA sgRNA	<b>(mG) * (mC) *GCCGCCGGCCAGCUAUA</b> GUUUUAGAGCUAGAAAUAGCA AGUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGU CGGUGC (T-LNA) (T-LNA) U (SEQ ID: 122)
	PSMD7 2x LNA 5' and 3' sgRNA	<b>(G-LNA) (5mC- LNA) GCCGCCGGCCAGCUAUA</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (T-LNA) (T-LNA) U (SEQ ID: 123)
PSMD 11	PSMD1 1 2x 5' LNA, 2x O'me 3' sgRNA	<b>(G-LNA) (T- LNA) GUGAGAGCGGUAAGAUGG</b> GUUUUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGUCGGUGC U (mU*) (mU*) U (SEQ ID: 124)
	PSMD1 1 2x 5' O'me, 2x 3' LNA sgRNA	<b>(mG) * (mU) *GUGAGAGCGGUAAGAUGG</b> GUUUUAGAGCUAGAAAUAGCA AGUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCCGAGU CGGUGC (T-LNA) (T-LNA) U (SEQ ID: 125)

	PSMD1 1 2x LNA 5' and 3' sgRNA	<b>(G-LNA) (T-LNA) GUGAGAGCGGUAAGAUGG</b> GUUUUAGAGCUAGAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U (T-LNA) (T-LNA) U (SEQ ID: 126)
PSMD 7	PSMD7 LNA posit ion 6	<b>(mG) * (mC) *GCC (G-LNA) CCGGCCAGCUAUA</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU ) * (mU) *U (SEQ ID: 127)
	PSMD7 LNA posit ion 10	<b>(mG) * (mC) *GCCGCCG (G-LNA) CCCAGCUAUA</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGC UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU) * (m U) *U (SEQ ID: 128)
	PSMD7 LNA posit ion 12	<b>(mG) * (mC) *GCCGCCGC (5mC-LNA) CAGCUAUA</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU) * (mU) *U (SEQ ID: 129)
PSMD 11	PSMD1 1 LNA posit ion 6	<b>(mG) * (mU) *GUG (A-LNA) GAGCGGUAAGAUGG</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU *) (mU*) U (SEQ ID: 130)
	PSMD1 1 LNA posit ion 10	<b>(mG) * (mU) *GUGAGAG (5mC-LNA) GGUAAGAUGG</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGC UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU*) (m U*) U (SEQ ID: 131)
	PSMD1 1 LNA posit ion 12	<b>(mG) * (mU) *GUGAGAGCG (G-LNA) UAAGAUGG</b> GUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (mU*) (mU*) ) U (SEQ ID: 132)

[370] **Table 5: synthetic crRNAs (Sp Cas9)**

[371] Target region is **bolded**, chemical modifications are *italicized*.

Target	Guide Name	SEQUENCE
NTC	NTC (crRNA )	<i>(mG)*(mU)*AACGCGAACUACGCGGGUGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 133)
	CD151_ cr1	<i>(mC)*(mC)*CGGACUCGGACGCGUGGUGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 134)
	CD151_ cr2	<i>(mG)*(mC)*GGCCCGGAGCCUACGAGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 135)
CD151	CD151_ cr3	<i>(mA)*(mG)*GGCCCGGACUCGGACGCGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 136)
	SEL1L_ cr1	<i>(mA)*(mG)*GGGGCGGAUACUGACCCGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 137)
	SEL1L_ cr2	<i>(mA)*(mU)*ACUGACCCGAGGACGCCGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 138)
SEL1L	SEL1L_ cr3	<i>(mG)*(mG)*UGGUGGCUGAGUCCGUGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 139)
	SETD3_ _cr1	<i>(mA)*(mA)*CCAACCCCAAGGCGGUGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 140)
	SETD3_ _cr2	<i>(mC)*(mU)*CAACCAACCCCAAGGCGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 141)
SETD3	SETD3_ _cr3	<i>(mC)*(mC)*UCGCAGAGCUCGGAGACGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 142)
	TFRC_ cr1	<i>(mG)*(mC)*AGCCAUAGGGAGCCGCACGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 143)
	TFRC_ cr2	<i>(mG)*(mG)*AUGGCGGCCCCUAACCGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 144)
TFRC	TFRC_ cr3	<i>(mC)*(mA)*GAGCGUCGGGAUAUCGGGGUUUUAGAGCUAUGCUGUU</i> UUG (SEQ ID: 145)

[372] **Table 6: synthetic tracrRNAs (Sp Cas9)**

[373] MS2 aptamer region is **bolded**, chemical modifications are *italicized*.

Tracr Name	SEQUENCE
tracrRNA w/ out MS2	AACAGCAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCUUUU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 146)
stem loop 2 C-5 MS2 tracrRNA	AACAGCAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUG <b>G</b> <b>CCAACAUGAGGAUCACCCAUGUCUGCAGGGCCAAGUGGCACCGA</b> GUCGGUGCUUUU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 147)
3' C-5 MS2 tracrRNA	AACAGCAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCGCGCACAUGAGGAUCACCCAUGU <b>G</b> CUUUU( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 148)
3' chemically enhanced 3' F-5 MS2 tracrRNA	AACAGCAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCGCG <b>GGCCCGG-(2AdP)-</b> <b>GGAUCACCACGGGCCUUUU</b> ( <i>mU</i> )*( <i>mU</i> )*U (SEQ ID: 149)

[374] **Table 7: synthetic Class V Cas crRNAs**[375] Target region is **bolded**, chemical modifications are *italicized*.

Target	Guide Name	SEQUENCE
NTC	NTC (dMAD7)	UUAUUUCUACUCUUGUAGAUAGAGUGCCUAGAAAGAUGACA (SEQ ID: 150)
BRCA1	dMAD7 BRCA1 _g2	UUAUUUCUACUCUUGUAGAUUCUGGACGGGGGACAGGCUGUG (SEQ ID: 151)
	dMAD7 BRCA1 _g4	UUAUUUCUACUCUUGUAGAUUCAGAUAAACUGGGCCCCUGCG (SEQ ID: 152)
PPIB	dMAD7 PPIB_g 2	UUAUUUCUACUCUUGUAGAUCCCCUCCGGCUCGGCGCCGG (SEQ ID: 153)
	dMAD7 PPIB_g 3	UUAUUUCUACUCUUGUAGAUGCCUCCGCCUGUGGAUGCUGC (SEQ ID: 154)
NTC	NTC (dCas Phi8)	<i>(mC)*(mU)*UUCAAGACUAAUAGAUUGCUCUUACGAGGAGACAGAG UGCCUAGAAAG(mA)*(mU)*G</i> (SEQ ID: 155)
BRCA1	pre- crRNA w/ 18 nt spacer	<i>(mC)*(mU)*UUCAAGACUAAUAGAUUGCUCUUACGAGGAGACCUGG ACGGGGGACAG(mG)*(mC)*U</i> (SEQ ID: 156)
	crRNA only w/18 nt spacer	<i>(mA)*(mA)*UAGAUUGCUCUUACGAGGAGACCUGGACGGGGGACAG (mG)*(mC)*U</i> (SEQ ID: 157)
	crRNA only w/ 14 nt spacer	<i>(mA)*(mA)*UAGAUUGCUCUUACGAGGAGACCUGGACGGGGG(mA)* (mC)*A</i> (SEQ ID: 158)

[376] **Table 8: Lentiviral guide RNAs (Sp Cas9) delivered via particles or as plasmids**

[377] Target region is **bolded**

Target	Guide Name	SEQUENCE
NTC	LV NTC	<b>GTAACGCGAACTACGCGGGT</b> GTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 159)
CD46	LV CD46 g2	<b>GGATTGTTGCGTCCCATAT</b> CGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 160)
	LV CD46 g4	<b>GCTCAGTCGGGCAAGAGT</b> CGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 161)
PSMD7	LV PSMD7 g1	<b>GACTGGGCCTGAAAGGGT</b> ACGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 162)
	LV PSMD7 g2	<b>GCGCCGCCGGCCAGCT</b> ATAGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 163)
	LV PSMD7 g4	<b>GCGGCAGCAGTAGCGGT</b> CACGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 164)
SEL1L	LV SEL1L g1	<b>GGGGGGCGGATACTG</b> ACCCGGTTTAAGAGCTATGCTGGAAACAGC ATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCGAGTCGGTGCTTTTTTT (SEQ ID: 165)
	LV SEL1L g3	<b>GGTGGTGGCTGAGT</b> CCGTGGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 166)
PPIB	LV PPIB g1	<b>GGGAGAGGCGCAGC</b> ATCCACGTTTAAGAGCTATGCTGGAAACAGC ATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCGAGTCGGTGCTTTTTTT (SEQ ID: 167)
BRCA1	LV BRCA1 g3	<b>GCACAGCCTGTCCCC</b> CGTCCGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 168)
ST3GAL4	LV ST3GAL4 g2	<b>GATCCGCTAGGCGCAC</b> CGACGTTTAAGAGCTATGCTGGAAACAGCA TAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTT (SEQ ID: 169)

**Claims**

We claim:

1. A Cas fusion protein comprising a Cas protein and one or both of a SALL1 repressor domain and a SUDS3 repressor domain.  
5
2. The Cas fusion protein of claim 1, wherein the Cas fusion protein comprises the SALL1 repressor domain.
3. The Cas fusion protein of claim 1, wherein the Cas fusion protein comprises the  
10 SUDS3 repressor domain.
4. The Cas fusion protein of claim 1, wherein the Cas fusion protein comprises both the SALL1 repressor domain and the SUDS3 repressor domain.
- 15 5. The Cas fusion protein of any of claims 2 to 4 further comprising an additional repressor domain, wherein the additional repressor domain is a repressor domain other than the SALL1 repressor domain or the SUDS3 repressor domain.
6. The Cas fusion protein of claim 5, wherein the additional repressor domain is a  
20 NIPP1 repressor domain.
7. The Cas fusion protein of any of claims 1 to 4, wherein the Cas protein is catalytically inactive.
- 25 8. The Cas fusion protein of any of claims 1 to 4, wherein the Cas protein is a nickase.
9. The Cas fusion protein of any of any of claims 1 to 4, wherein the Cas protein is Cas9 in active or deactivated form.
- 30 10. The Cas fusion protein of any of claims 1 to 4, wherein the Cas protein is a TYPE V Cas protein.
11. The Cas fusion protein of claim 10, wherein the Cas protein is selected from the  
35 group consisting of Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12f, Cas12h, Cas12i and Cas12j.

12. The Cas fusion protein of claim 11, wherein the Cas protein is Cas12a.
13. The Cas fusion protein of any of claims 1 to 4, wherein the Cas fusion protein  
5 further comprises a Cas linker.
14. The Cas fusion protein of claim 13, wherein the Cas linker is an amino acid  
sequence that comprises a sequence that is at least 80% similar to SEQ ID NO: 7.
- 10 15. The Cas fusion protein of claim 14, wherein the Cas linker comprises SEQ ID  
NO: 7.
16. The Cas fusion protein of claim 15, wherein the Cas linker is SEQ ID NO: 7.
- 15 17. The Cas fusion protein of claim 13, wherein the Cas protein has a N terminal  
amino acid and the Cas fusion protein comprises the SUDS3 repressor domain and the  
Cas linker is bound to both the SUDS3 repressor domain and the N terminal amino acid  
of the Cas fusion protein.
- 20 18. The Cas fusion protein of claim 13, wherein the Cas protein has a N terminal  
amino acid and the Cas fusion protein comprises the SALL1 repressor domain and the  
Cas linker is bound to both the SALL1 repressor domain and the N terminal amino acid  
of the Cas fusion protein.
- 25 19. The Cas fusion protein of claim 13, wherein the Cas protein has a C terminal  
amino acid and the Cas fusion protein comprises the SUDS3 repressor domain and the  
Cas linker is bound to both the SUDS3 repressor domain and the C terminal amino acid  
of the Cas fusion protein.
- 30 20. The Cas fusion protein of claim 13, wherein the Cas protein has a C terminal  
amino acid and the Cas fusion protein comprises the SALL1 repressor domain and the  
Cas linker is bound both to the SALL1 repressor domain and the C terminal amino acid of  
the Cas fusion protein.
- 35 21. The Cas fusion protein of claim 2 or claim 4, wherein the SALL1 repressor  
domain comprises a sequence that is at least 80% similar to SEQ ID NO: 1.

22. The Cas fusion protein of claim 2 or claim 4, wherein the SALL1 repressor domain comprises SEQ ID NO: 1.
23. The Cas fusion protein of claim 3 or claim 4, wherein the SUDS3 repressor domain comprises a sequence that is at least 80% similar to SEQ ID NO: 2.
24. The Cas fusion protein of claim 3 or claim 4, wherein the SUDS3 repressor domain comprises SEQ ID NO: 2.
25. The Cas fusion protein of claim 4, wherein the SALL1 repressor domain comprises a sequence that is at least 80% similar to SEQ ID NO: 1 and the SUDS3 repressor domain comprises a sequence that is at least 80% similar to SEQ ID NO: 2.
26. The Cas fusion protein of claim 25, wherein the SALL1 repressor domain comprises SEQ ID NO: 1 and the SUDS3 repressor domain comprises SEQ ID NO: 2.
27. The Cas fusion protein of claim 25, wherein the SALL1 repressor domain is SEQ ID NO: 1 and the SUDS3 repressor domain is SEQ ID NO: 2.
28. The Cas fusion protein of claim 4, wherein the Cas protein is a dCas9 protein, wherein the dCas9 protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to both the C terminal amino acid of the dCas9 protein and the N-terminal amino acid of the SALL1 repressor domain and wherein the repressor linker is bound to both the C-terminal amino acid of the SALL1 repressor and the N-terminal amino acid of the SUDS3 repressor domain.
29. The Cas fusion protein of claim 4, wherein the Cas protein is a dCas9 protein, wherein the dCas9 protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to both the C terminal amino acid of the dCas9 protein and the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor and the SALL1 repressor domain.
30. The Cas fusion protein of claim 4, wherein the Cas protein is a dCas9 protein, wherein the dCas9 protein has a N terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound

to both the N terminal amino acid of the dCas9 protein and the SALL1 repressor domain and wherein the repressor linker is bound to both the SALL1 repressor and the SUDS3 repressor domain.

5 31. The Cas fusion protein of claim 4, wherein the Cas protein is a dCas9 protein, wherein the dCas9 protein has a N terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to both the N terminal amino acid of the dCas9 protein and the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor and the SALL1  
10 repressor domain.

32. The Cas fusion protein of any of claims 28 to 31, wherein each of the Cas linker and the repressor linker comprises an amino acid sequence that is 3 to 90 amino acids  
15 long.

33. The Cas fusion protein of claim 32, wherein each of the Cas linker and the repressor linker comprises SEQ ID NO: 7.

34. The Cas fusion protein of claim 33, wherein each of the Cas linker and the repressor linker is SEQ ID NO: 7.  
20

35. The Cas fusion protein of claim 4, wherein the Cas protein is a Cas12a protein, wherein the Cas12a protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently  
25 bound to the C terminal amino acid of the Cas12a protein and to the SALL1 repressor domain and wherein the repressor linker is bound to both the SALL1 repressor and the SUDS3 repressor domain.

36. The Cas fusion protein of claim 4, wherein the Cas protein is a Cas12a protein, wherein the Cas12a protein has a C terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently  
30 bound to both the C terminal amino acid of the Cas12a protein and the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor and the SALL1 repressor domain.

37. The Cas fusion protein of claim 4, wherein the Cas protein is a Cas12a protein, wherein the Cas12a protein has a N terminal amino acid and the Cas fusion protein  
35

further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to both the N terminal amino acid of the Cas12a protein and the SALL1 repressor domain and wherein the repressor linker is bound to both the SALL1 repressor and the SUDS3 repressor domain.

5

38. The Cas fusion protein of claim 4, wherein the Cas protein is a Cas12a protein, wherein the Cas12a protein has a N terminal amino acid and the Cas fusion protein further comprises a Cas linker and a repressor linker, wherein the Cas linker is covalently bound to both the N terminal amino acid of the Cas12a protein and to the SUDS3 repressor domain and wherein the repressor linker is bound to both the SUDS3 repressor and the SALL1 repressor domain.

10

39. The Cas fusion protein of any of claims 35 - 38, wherein each of the Cas linker and the repressor linker comprises SEQ ID NO: 7.

15

40. The Cas fusion protein of claim 39, wherein each of the Cas linker and the repressor linker is SEQ ID NO: 7.

20

41. The Cas fusion protein of claim 40, wherein each of the Cas linker and the repressor linker comprises SEQ ID NO: 7.

42. The Cas fusion protein of claim 41, wherein each of the Cas linker and the repressor linker is SEQ ID NO: 7.

25

43. The Cas fusion protein of claim 4, wherein the Cas fusion protein comprises a sequence that is at least 80% similar to SEQ ID NO: 10.

44. The Cas fusion protein of claim 43, wherein the Cas fusion protein comprises a sequence that is SEQ ID NO: 10.

30

45. A nucleic acid encoding the Cas fusion protein of any of claims 1 to 44.

46. The nucleic acid of claim 45, wherein the nucleic acid is present in a vector.

35

47. The nucleic acid of claim 46, wherein the vector is a plasmid or a viral vector.

48. The nucleic acid of claim 47, wherein the nucleic acid is present in a viral vector selected from the group consisting of a lentivirus, a retrovirus, an adenovirus, an adeno-associated virus, a coronavirus, and a Sendai virus.
- 5 49. A method of modulating expression of a target nucleic acid in a eukaryotic cell comprising providing to the cell a gRNA and a Cas fusion protein of any of claims 1 to 44.
50. The method according to claim 49, wherein the gRNA is made synthetically  
10 outside of the cell and wherein the Cas fusion protein is provided to the cell by introducing to the cell an mRNA encoding the Cas fusion protein.
51. The method of claim 50, wherein the eukaryotic cell is a yeast cell, a plant cell or a mammalian cell.
- 15 52. The method of claim 51, wherein the eukaryotic cell is the mammalian cell.
53. The method of claim 52, wherein the mammalian cell is a human cell.
- 20 54. The method according to claim 49, wherein said providing comprises obtaining the gRNA and the Cas fusion protein from a vector, wherein the vector encodes the gRNA and the Cas fusion protein.
55. The method according to claim 49 further comprising synthesizing the gRNA  
25 outside of the cell and synthesizing the Cas fusion outside of the cell.
56. A method of modulating expression of a target nucleic acid in a eukaryotic cell, said method comprising providing to the cell a Cas fusion protein of any of claims 1 to 44 and an RNA-repressor domain complex, wherein the RNA-repressor  
30 domain complex comprises:
- (a) a gRNA molecule, wherein the gRNA molecule contains 30 to 180 nucleotides;
  - (b) a ligand binding moiety, wherein the ligand binding moiety is either (i) directly bound to the gRNA molecule, or (ii) bound through a ligand binding  
35 moiety linker to the gRNA molecule;

- (c) a ligand, wherein the ligand is capable of reversibly associating with the ligand binding moiety; and
- (d) a repressor domain, wherein the repressor domain is either (i) directly bound to the ligand, or (ii) bound through a ligand linker to the ligand.

5

57. The method or claim 56, wherein the eukaryotic cell is a human cell.

58. A kit comprising a Cas fusion protein of claims 1 to 44 and an RNA-repressor domain complex, wherein the RNA-repressor domain complex comprises:

10

- (a) a gRNA molecule, wherein the gRNA molecule contains 30 to 180 nucleotides;
- (b) a ligand binding moiety, wherein the ligand binding moiety is either (i) directly bound to the gRNA molecule, or (ii) bound through a ligand binding moiety linker to the gRNA molecule;

15

- (c) a ligand, wherein the ligand is capable of reversibly associating with the ligand binding moiety; and
- (d) a repressor domain and wherein the repressor domain is either (i) directly bound to the ligand, or (ii) bound through a ligand linker to the ligand.

20

59. A RNA-repressor domain complex, wherein the RNA-repressor domain complex comprises:

- (a) a gRNA molecule, wherein the gRNA molecule contains 30 to 180 nucleotides;
- (b) a ligand binding moiety, wherein the ligand binding moiety is either (i) directly bound to the gRNA molecule, or (ii) bound through a ligand binding moiety linker to the gRNA molecule;
- (c) a ligand, wherein the ligand is capable of reversibly associating with the ligand binding moiety; and

25

- (d) a fusion protein, wherein the fusion protein comprises a SALL1 repressor domain and a SUDS3 repressor domain, and wherein the fusion protein is either (i) directly bound to the ligand, or (ii) bound through a ligand linker to the ligand.

30

60. The RNA-repressor domain complex of claim 59, wherein the gRNA molecule comprises a crRNA sequence.
61. The RNA-repressor domain complex of claim 60, wherein the crRNA sequence  
5 comprises a targeting sequence, wherein the targeting sequence is 12 to 30 nucleotides long.
62. The RNA-repressor domain complex of claim 60, wherein the gRNA molecule further comprises a tracrRNA sequence.
- 10 63. The RNA-repressor domain complex of claim 59, wherein the gRNA molecule comprises a tracrRNA sequence.
64. The RNA-repressor domain complex of claim 59 comprising the ligand linker,  
15 wherein the ligand linker is an amino acid sequence.
65. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA is an sgRNA molecule and the sgRNA molecule has a 3' end and the ligand binding moiety is attached to the gRNA molecule at said 3' end.
- 20 66. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA molecule is an sgRNA molecule and the sgRNA molecule has a 5' end and the ligand binding moiety is attached to the gRNA molecule at said 5' end.
- 25 67. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA molecule is an sgRNA molecule and the sgRNA molecule forms at least one stem-loop structure and the ligand binding moiety is attached to the gRNA molecule at one of the at least one stem-loop structures.
- 30 68. The RNA-repressor domain complex of claim 67, wherein at least one of the stem-loop structures is 4 to 40 nucleotides long.
69. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA is an sgRNA molecule and the sgRNA molecule comprises at least one 2'  
35 modified nucleotide.

70. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA molecule is and sgRNA molecule and the sgRNA molecule comprises at least one phosphorothioate linkage.
- 5
71. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA molecule is a sgRNA and the sgRNA comprises a tracrRNA sequence and a crRNA sequence.
- 10
72. The RNA-repressor domain complex of any of claims 59-64, wherein the gRNA molecule comprises a first RNA molecule and a second RNA molecule, wherein the first RNA molecule comprises a tracrRNA sequence, the second RNA molecule comprises a crRNA sequence, the tracrRNA sequence comprises an anti-repeat region, and the crRNA sequence comprises a Cas association region,
- 15
- wherein the anti-repeat region and the Cas association region are at least 80% complementary over a span of at least 18 nucleotides.
73. The RNA-repressor domain complex of claim 72, wherein each of the first RNA molecule and the second RNA molecule has a 3' end and the ligand binding
- 20
- moiety is attached to either the 3' end of the first RNA molecule or to the 3' end of the second RNA molecule.
74. The RNA-repressor domain complex of claim 72, wherein each of the first RNA molecule and the second RNA molecule has a 5' end and the ligand binding
- 25
- moiety is attached to either the 5' end of the first RNA molecule or to the 5' end of the second RNA molecule.
75. The RNA-repressor domain complex of claim 72, wherein either or both of the first RNA molecule and the second RNA molecule comprises at least one 2'
- 30
- modified nucleotide.
76. The RNA-repressor domain complex of claim 72, wherein either or both of the first RNA molecule and the second RNA molecule comprises at least one phosphorothioate linkage.
- 35

77. The RNA-repressor domain complex of claim 59 further comprising a Cas protein.
78. The RNA-repressor domain complex of claim 77, wherein the Cas protein is a  
5 Type II Cas protein.
79. The RNA-repressor domain complex of claim 78, wherein the Cas protein is deactivated.
- 10 80. The RNA-repressor domain complex of claim 78, wherein the Cas protein is dCas9.
81. The RNA-repressor domain of claim 77, wherein the Cas protein is a Type V  
15 Cas protein.
82. The RNA-repressor domain complex of claim 81, wherein the Cas protein is deactivated.
83. The RNA-repressor domain complex of claim 82, wherein the Cas protein is  
20 dCas12a.
84. The RNA-repressor domain complex of claim 59, wherein the ligand is selected from the group consisting of: MS2, Ku, PP7, SfMu, Sm7, Tat, Glutathione S-transferase (GST), CSY4, Qbeta, COM, pumilio, lambda N22, and PDGF beta-chain.  
25
85. The RNA-repressor domain complex of claim 84, wherein the ligand is MS2.
86. The RNA-repressor domain complex of any of claim 59, wherein the fusion  
30 protein comprises a sequence that is at least 80% similar to SEQ ID NO: 10.
87. The RNA-repressor domain complex of claim 86, wherein the Cas fusion protein comprises a sequence that is SEQ ID NO: 10.
- 35 88. A method for transcriptional repression comprising exposing the RNA-repressor domain complex of any of claims 59-87 to double-stranded DNA.

89. The method of claim 88, wherein said method is conducted *in vitro*.
90. The method of claim 88, wherein said method is conducted *in vivo*.
- 5 91. The method of claim 88, wherein said method is conducted *ex vivo*.
92. A kit comprising the RNA-repressor domain complex of any of claims 59-87.
- 10 93. A method of treating a subject, said method comprising administering a Cas fusion protein of any of claims 1 to 44 to the subject.
94. The method of claim 93 further comprising administering a gRNA.
- 15 95. The method of claim 94 further comprising administering an mRNA that encodes the gRNA.
96. A method of treating a subject, said method comprising administering a repressor domain complex of any of claims 59 to 76 to the subject.
- 20 97. The method of claim 96 further comprising administering a Cas protein.
98. The method of claim 97 further comprising administering an mRNA that encodes the Cas protein.
- 25 99. The RNA-repressor domain complex of any of claims 59-63, wherein the gRNA has
- (a) 2'-O-methyl modifications on the first and second 5' most nucleotides,
- (b) 2'-O-methyl modifications on the penultimate 3' nucleotide and the
- 30 antepenultimate 3' nucleotide, and
- (c) phosphorothioate linkages between the first and second 5' most nucleotides, between the second and third 5' most nucleotides, between the antepenultimate 3' nucleotide and the penultimate 3' nucleotide, and between the penultimate 3' nucleotide and the 3' most nucleotide,
- 35 and
- wherein all other internucleotide linkages are phosphodiester linkages and all other nucleotides are unmodified at their 2' positions.

100. The RNA-repressor domain complex of any of claims 59-63, wherein the gRNA has:
- (a) 2'-O-methyl modifications on the first and second 5' most nucleotides, and all other nucleotides are unmodified at their 2' positions, and
  - (b) phosphorothioate linkages between the first and second 5' most nucleotides and between the second and third 5' most nucleotides, and all other internucleotide linkages are phosphodiester linkages.
101. A method of modulating expression of a target nucleic acid in a cell comprising providing to the cell a sgRNA and a Cas fusion protein of claim 1.
102. The method of claim 101, wherein the sgRNA is chemically modified.
103. The method of claim 101 or claim 102, wherein the sgRNA has a spacer region that is 12 to 30 nucleotides long.
104. A method of modulating expression of a target nucleic acid in a cell comprising providing to the cell a crRNA molecule, a tracrRNA molecule and a Cas fusion protein of claim 1.
105. The method of claim 104, wherein one or both of the crRNA molecule and the tracrRNA molecule is chemically modified.
106. The method of claim 104 or claim 105, wherein the crRNA molecule has a spacer region that is 12 to 30 nucleotides long.



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K562 cells

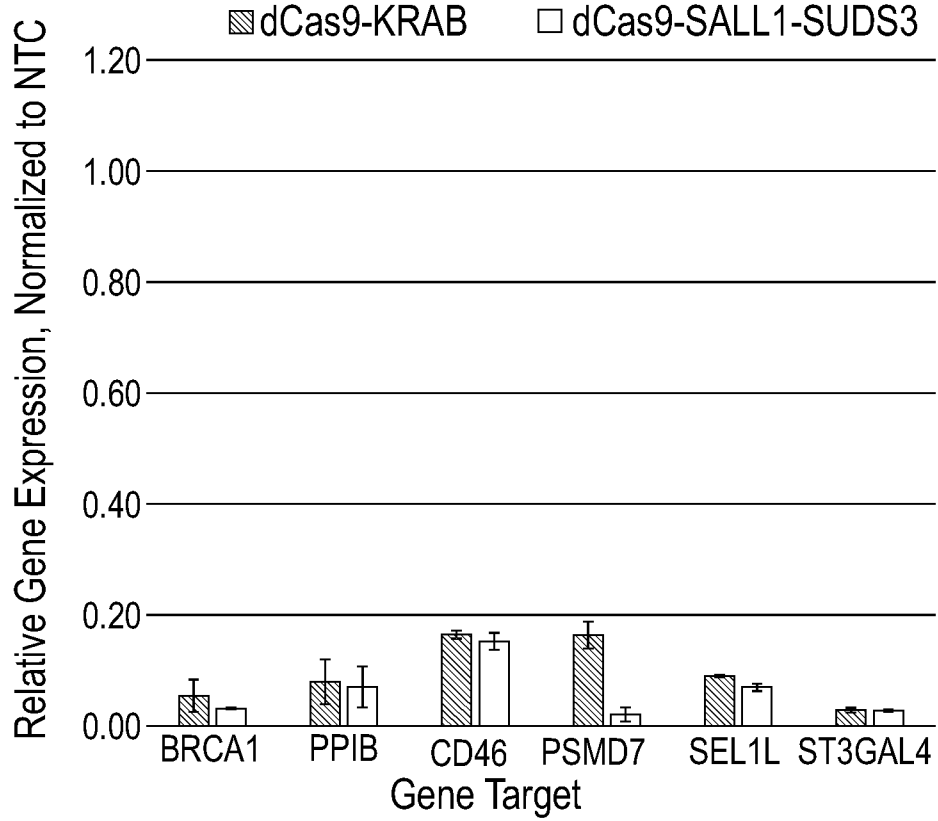


FIG. 3A

Jurkat cells

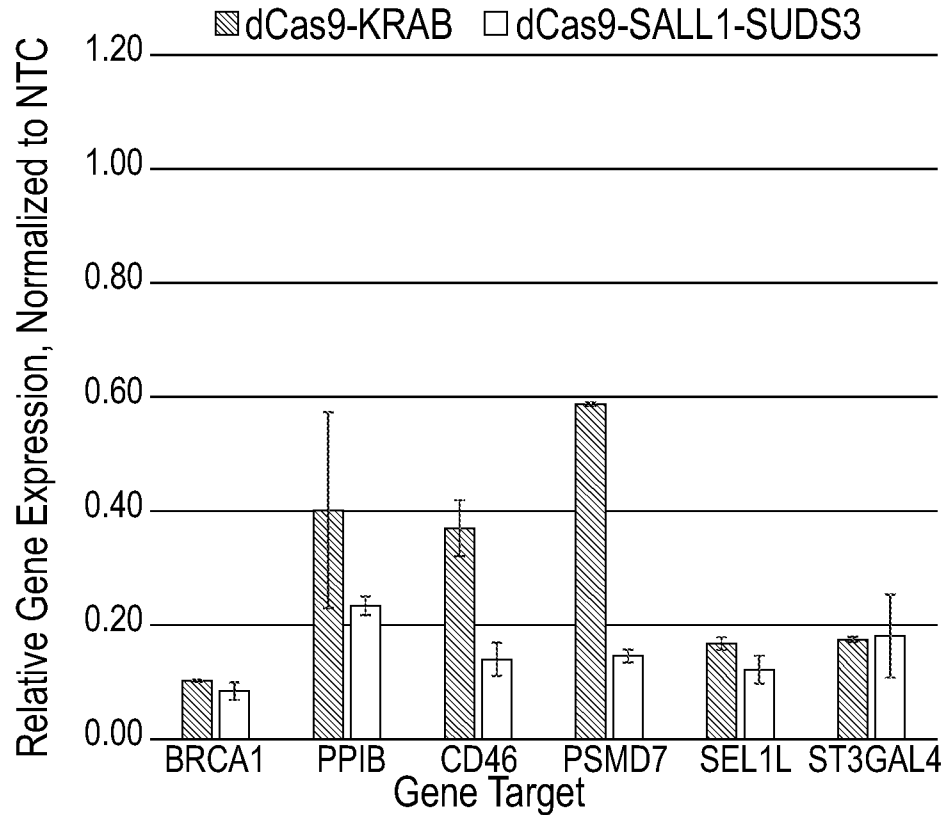


FIG. 3B

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FIG. 3C

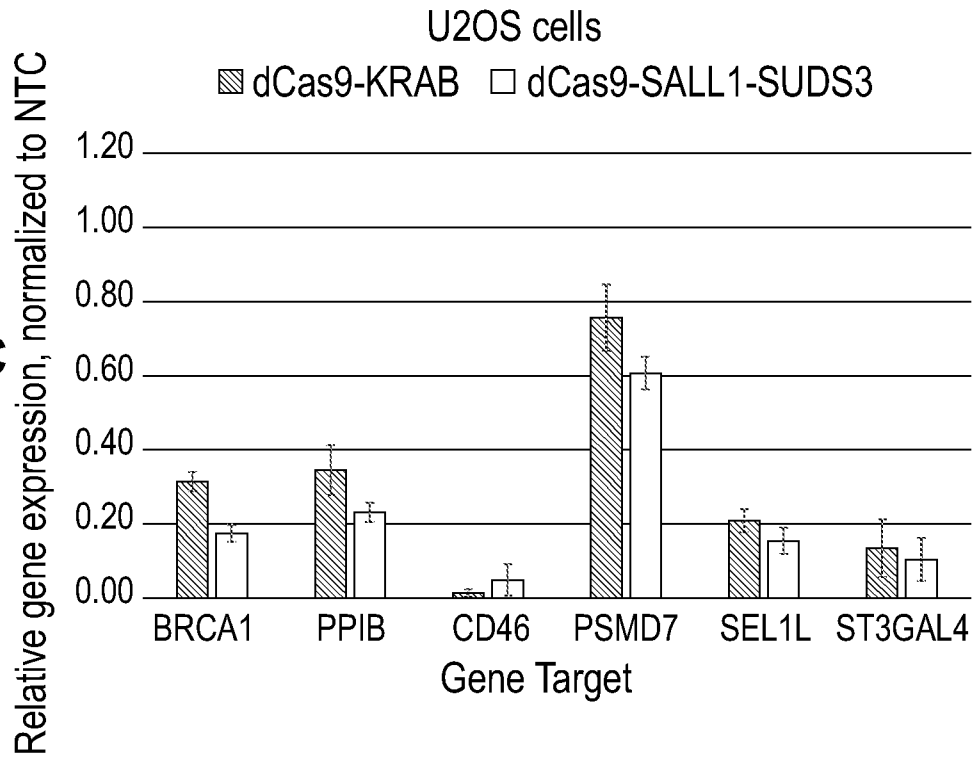
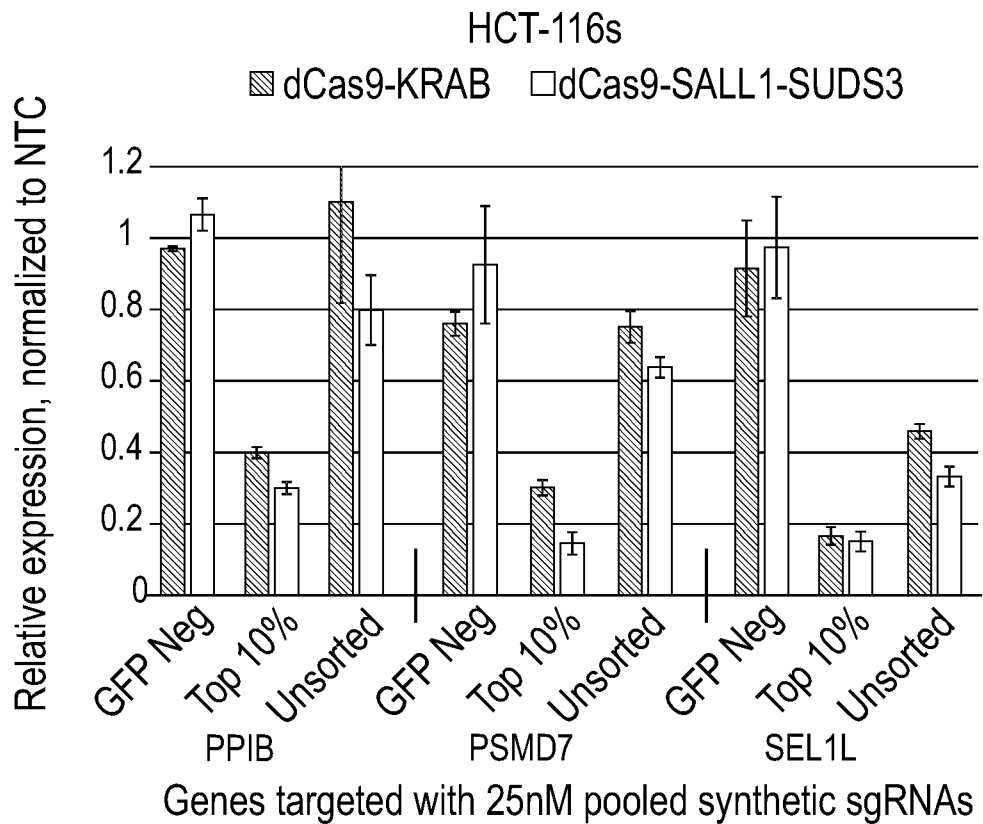


FIG. 4



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FIG. 5A

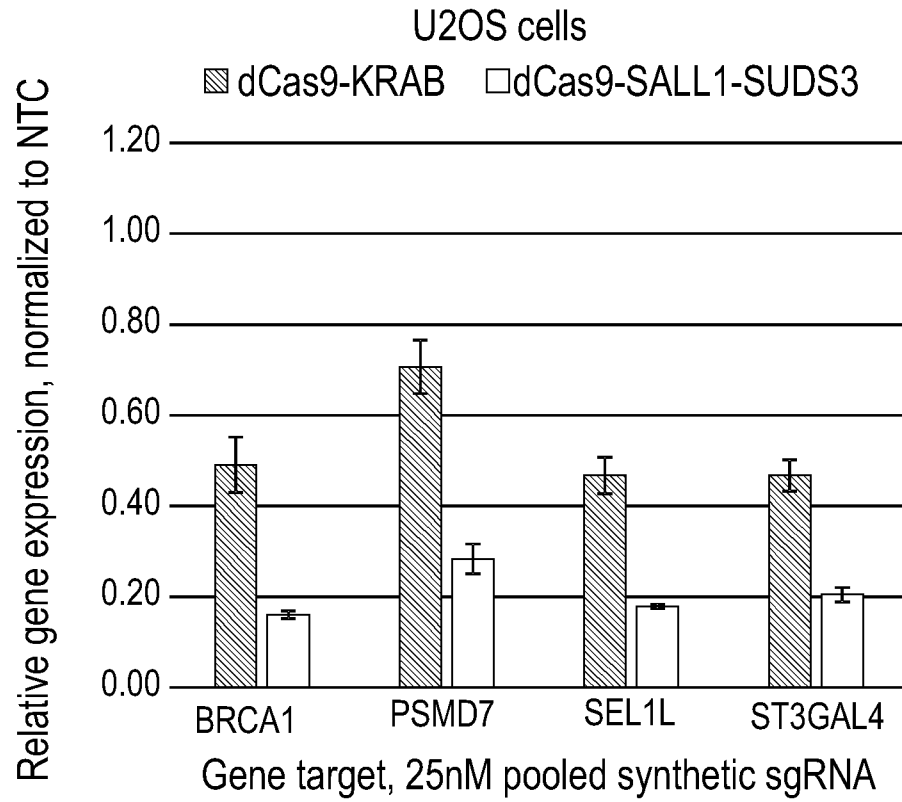
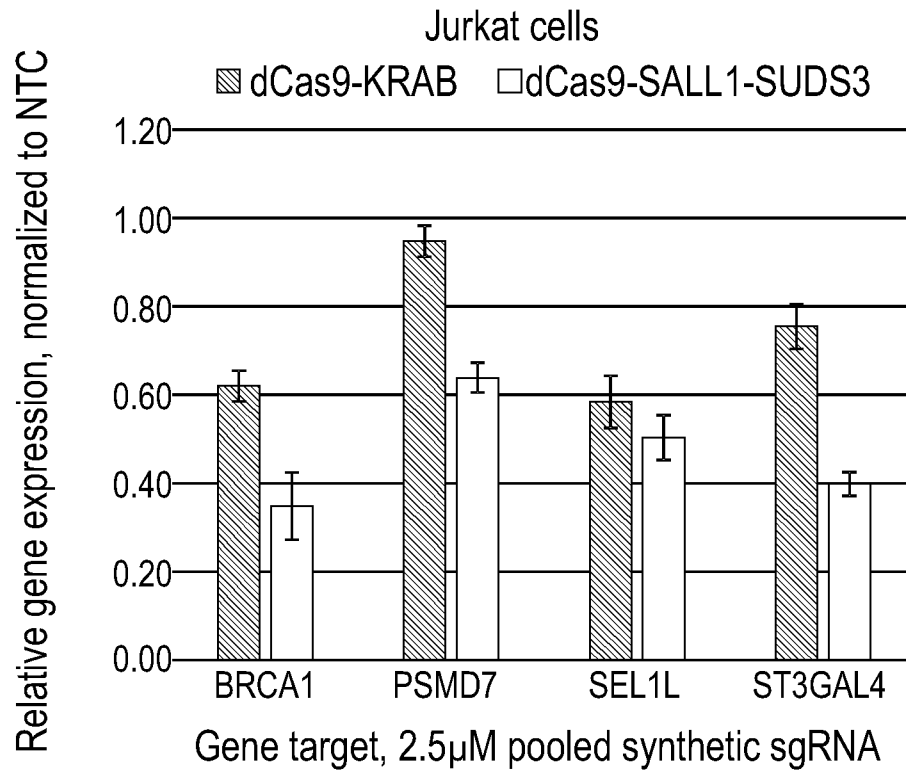
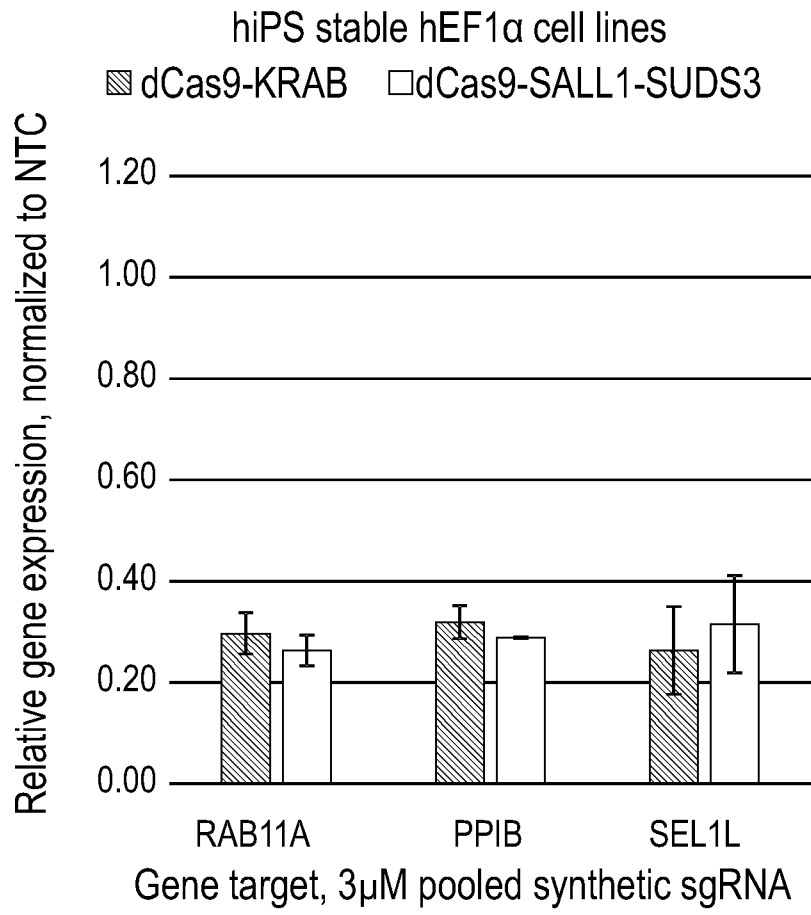


FIG. 5B



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FIG. 5C



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FIG. 6A

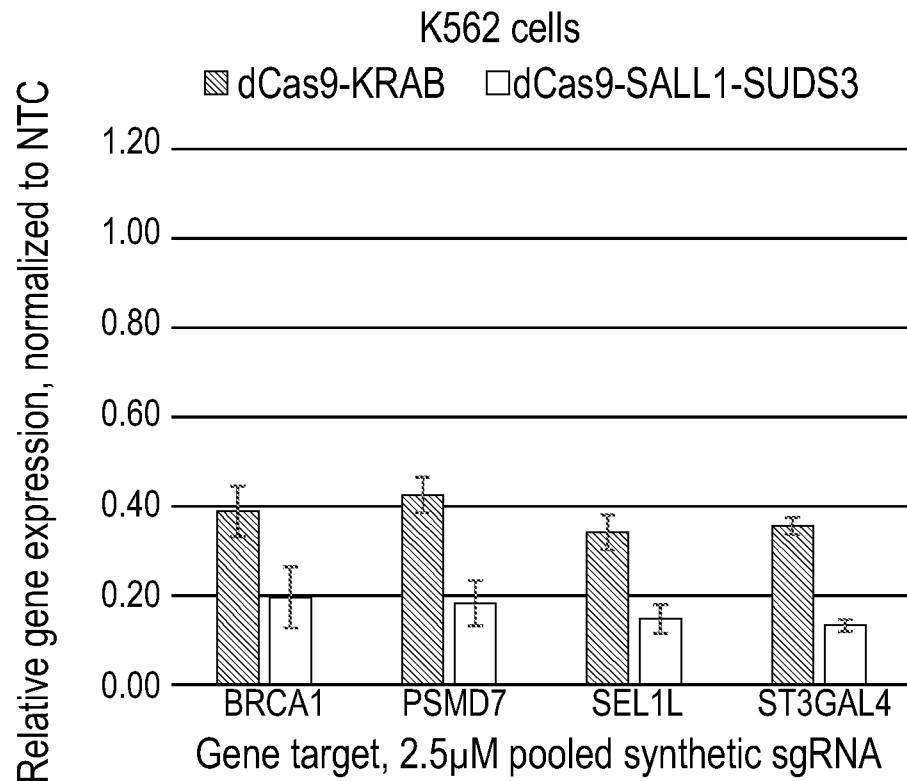
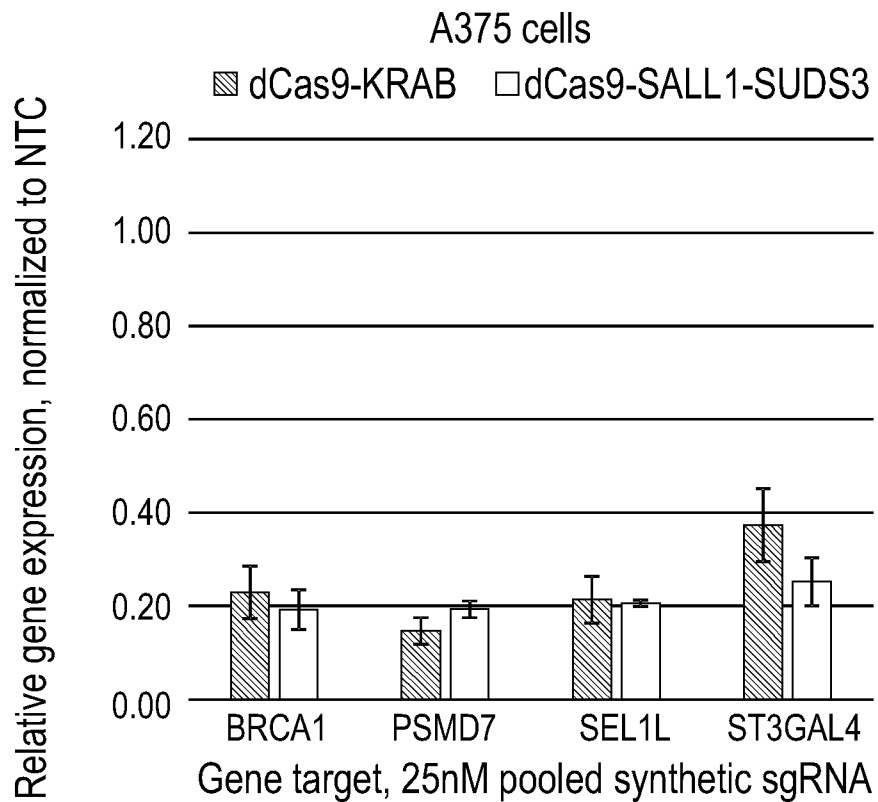


FIG. 6B



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FIG. 7A

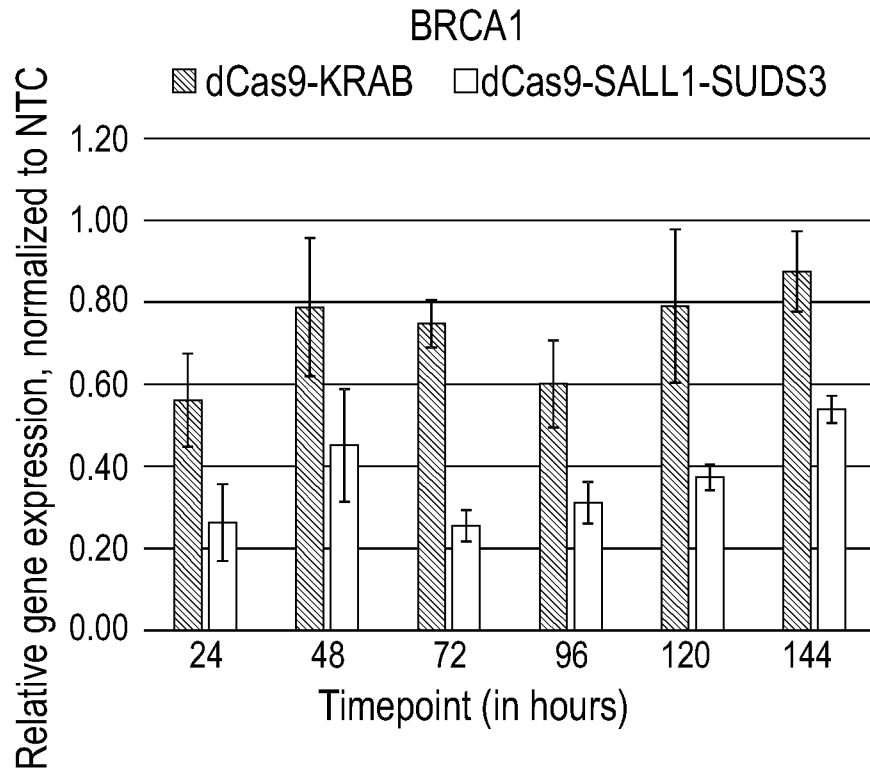
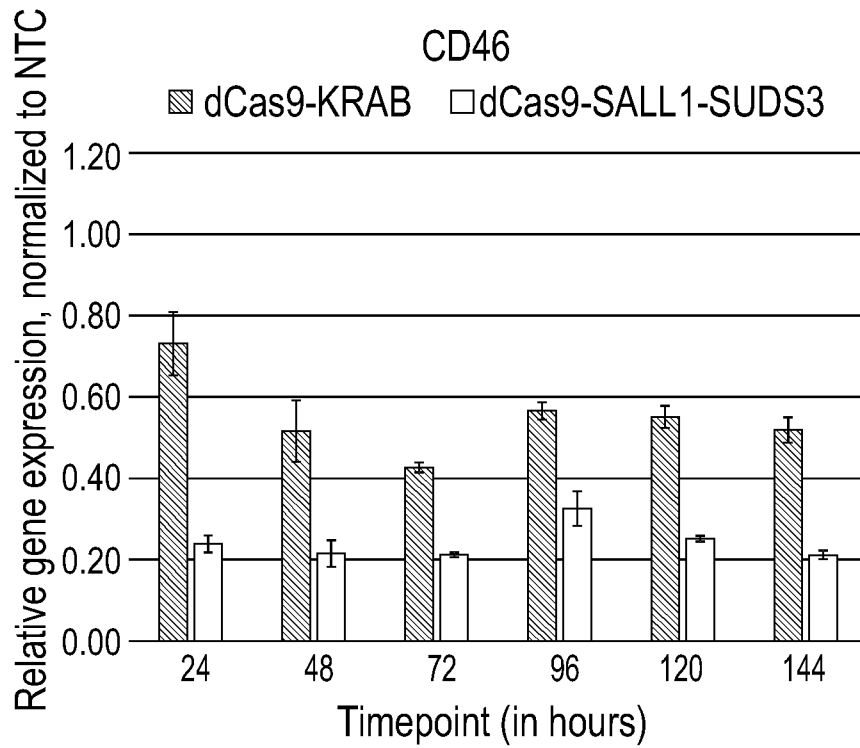


FIG. 7B



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FIG. 7C

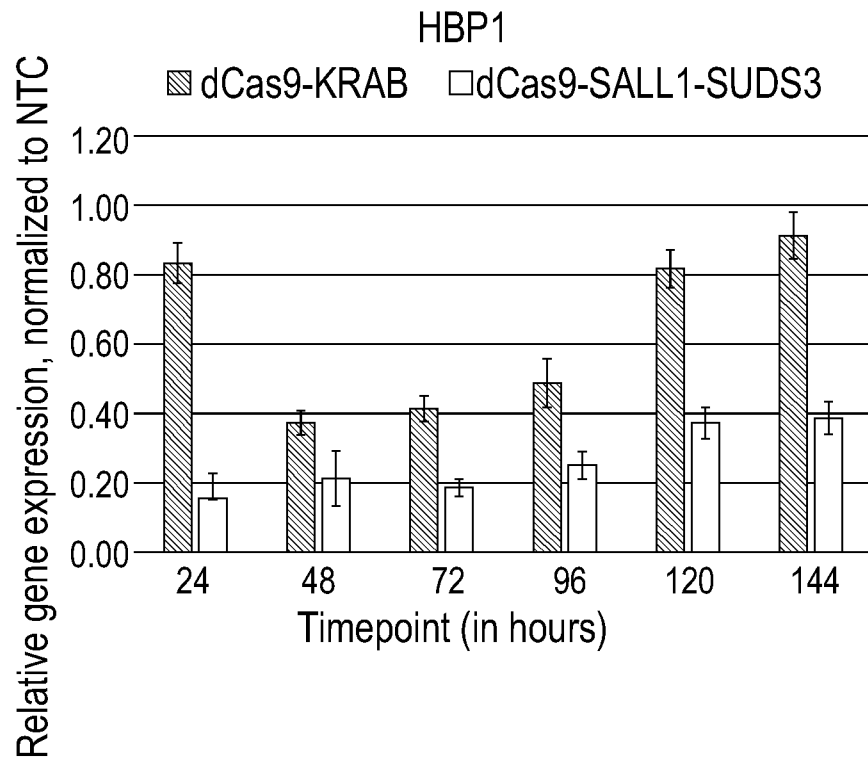
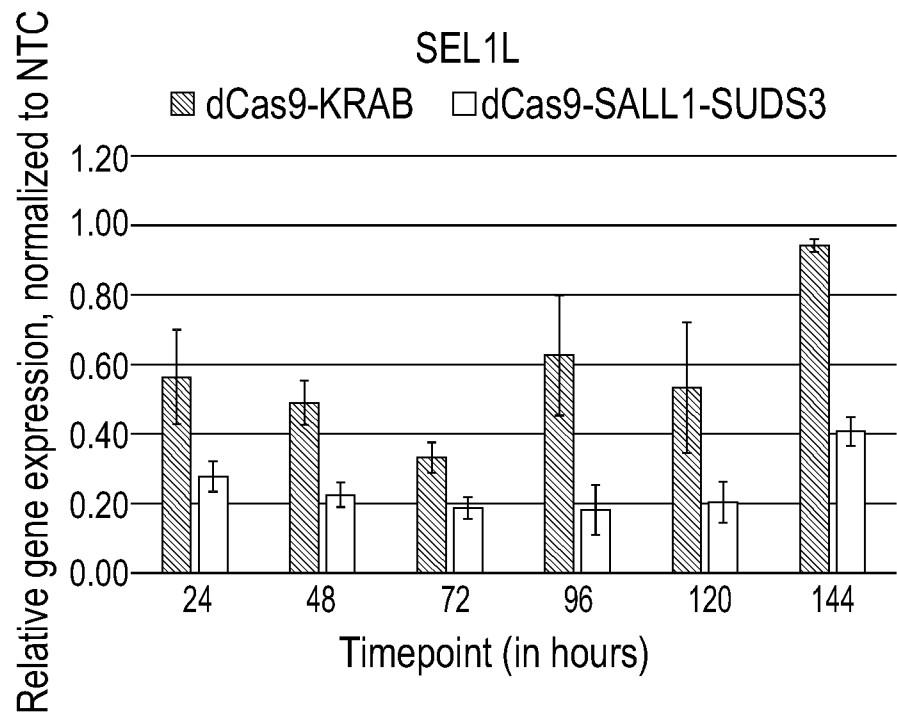


FIG. 7D



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WTC-11 hiPSCs

FIG. 8A

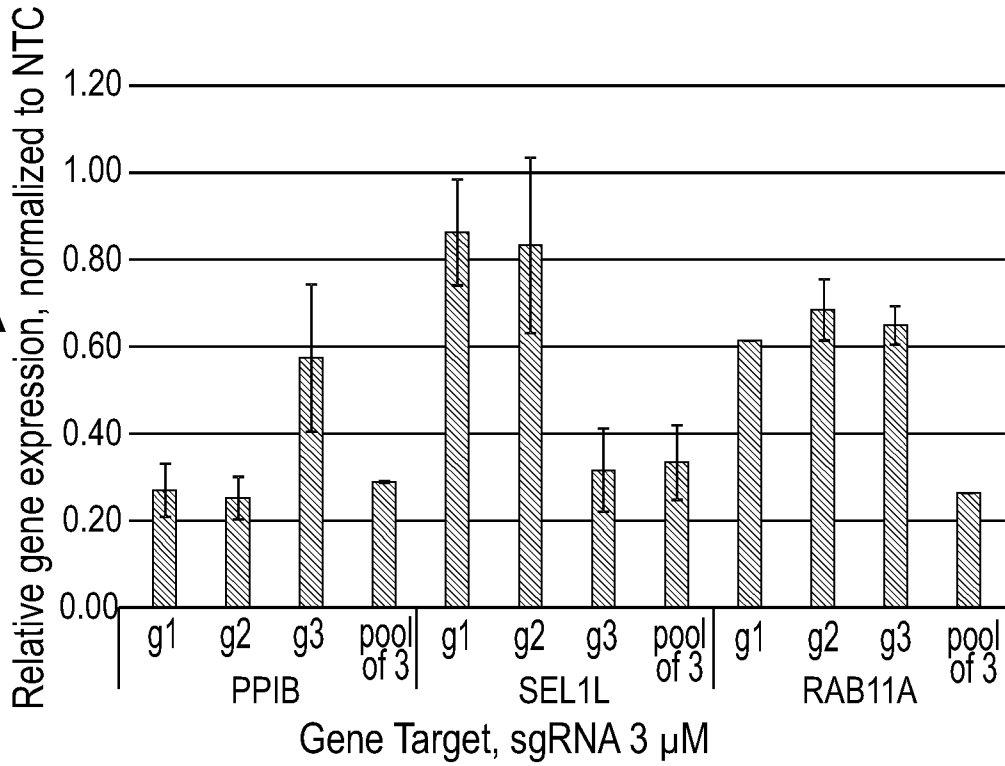
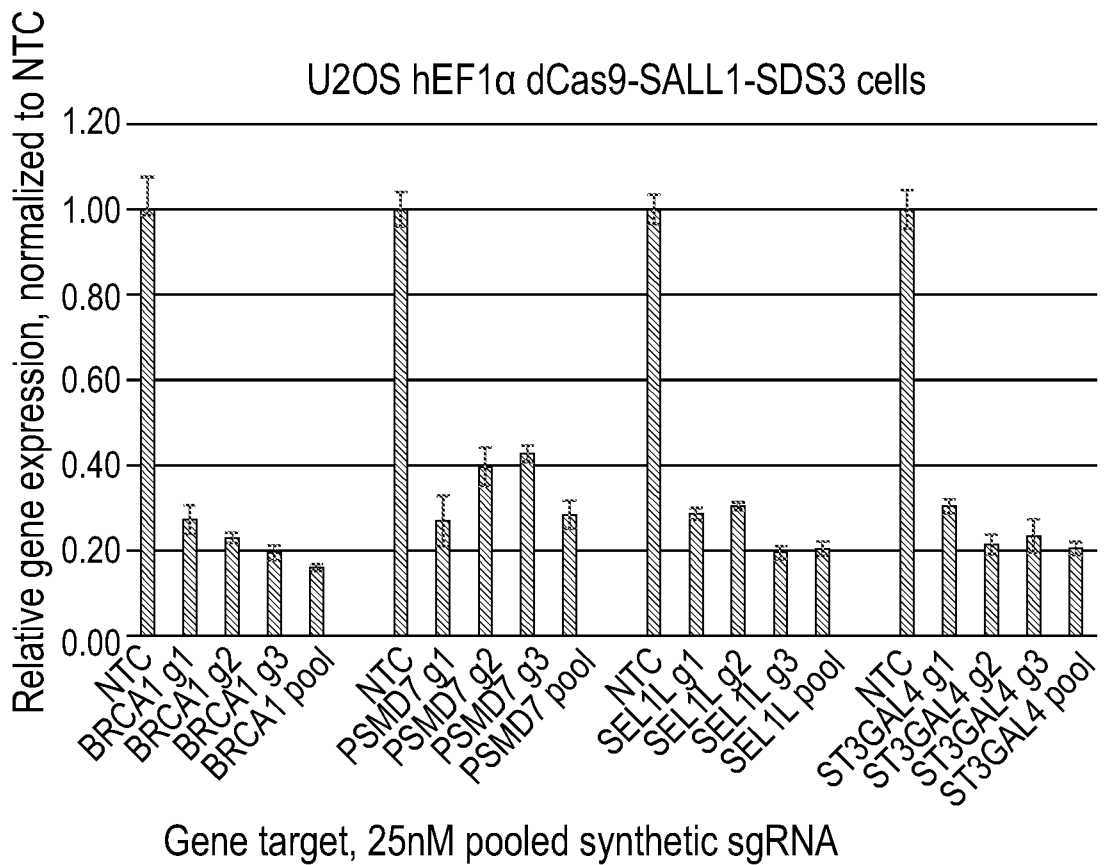
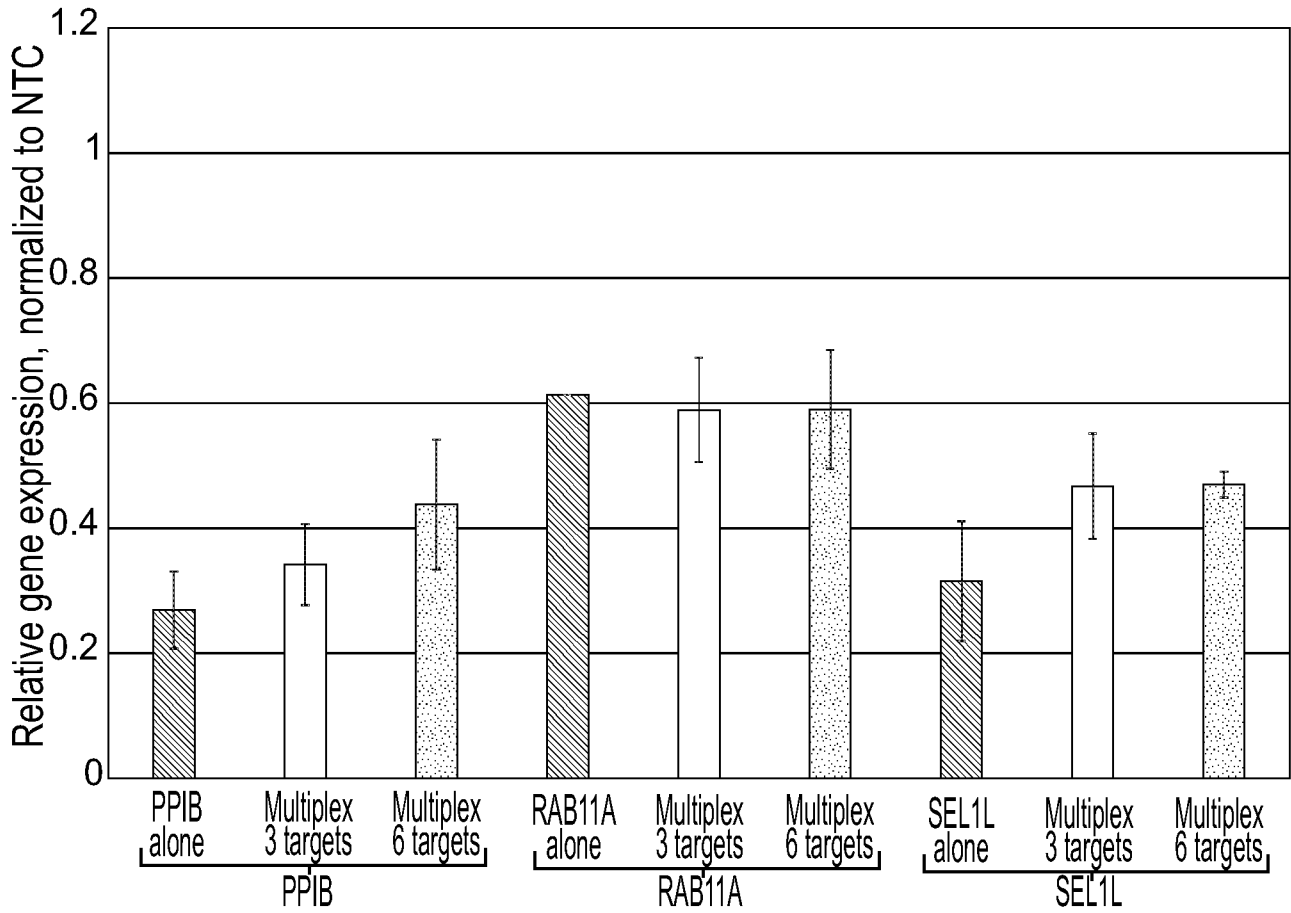


FIG. 8B



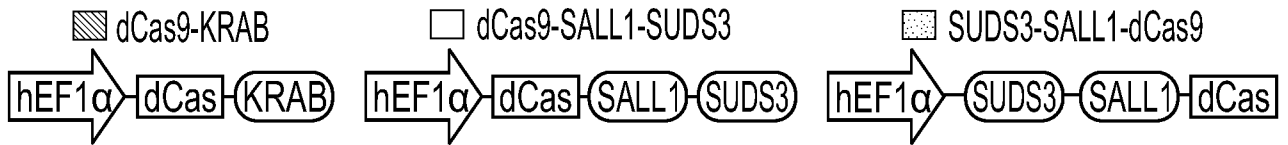
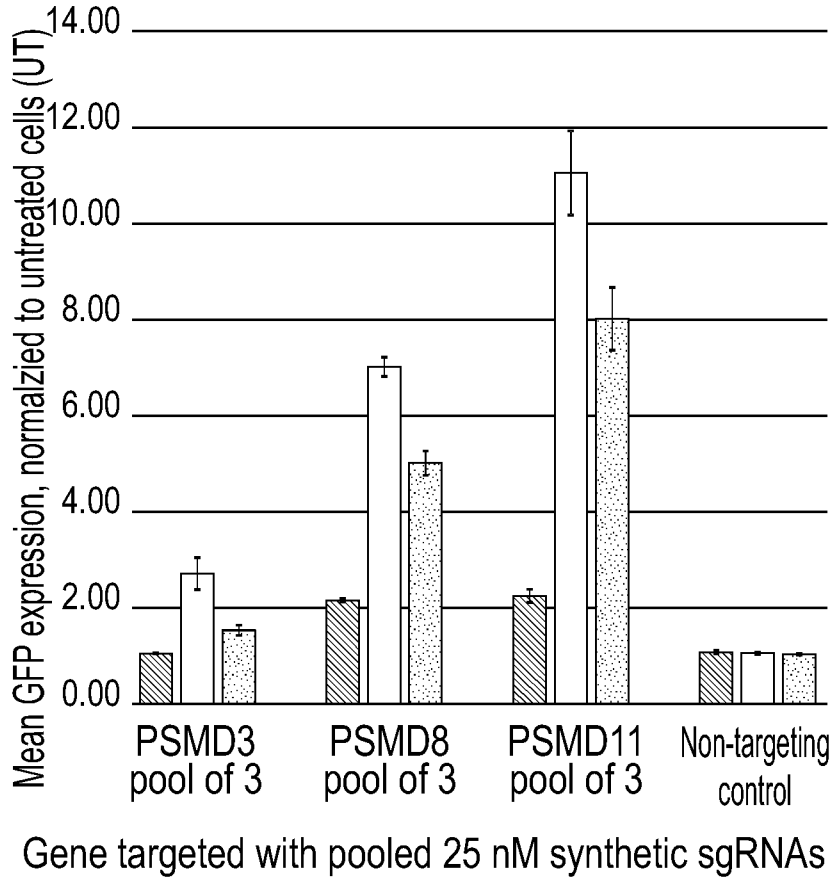
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hEF1 $\alpha$  dCas9-SALL1-SDS3 hiPSCs

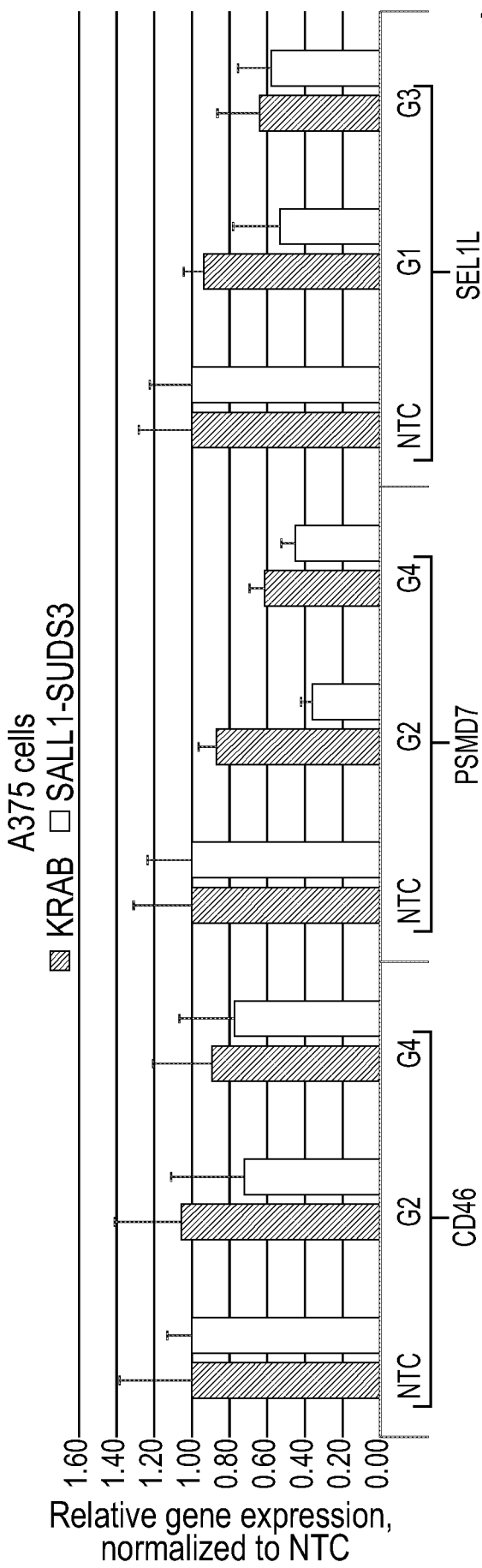
Gene target, sgRNAs multiplexed, 3  $\mu$ M/sgRNA  
 Genes in Multiplex of 3: PPIB, RAB11A, SEL1L  
 Genes in Multiplex of 6: PPIB, RAB11A, SEL1L, TFRC, PPP1R12C, CSNK1

FIG. 9

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 U2O2 Ubi[G76V] - EGFP  
 stable cell lines

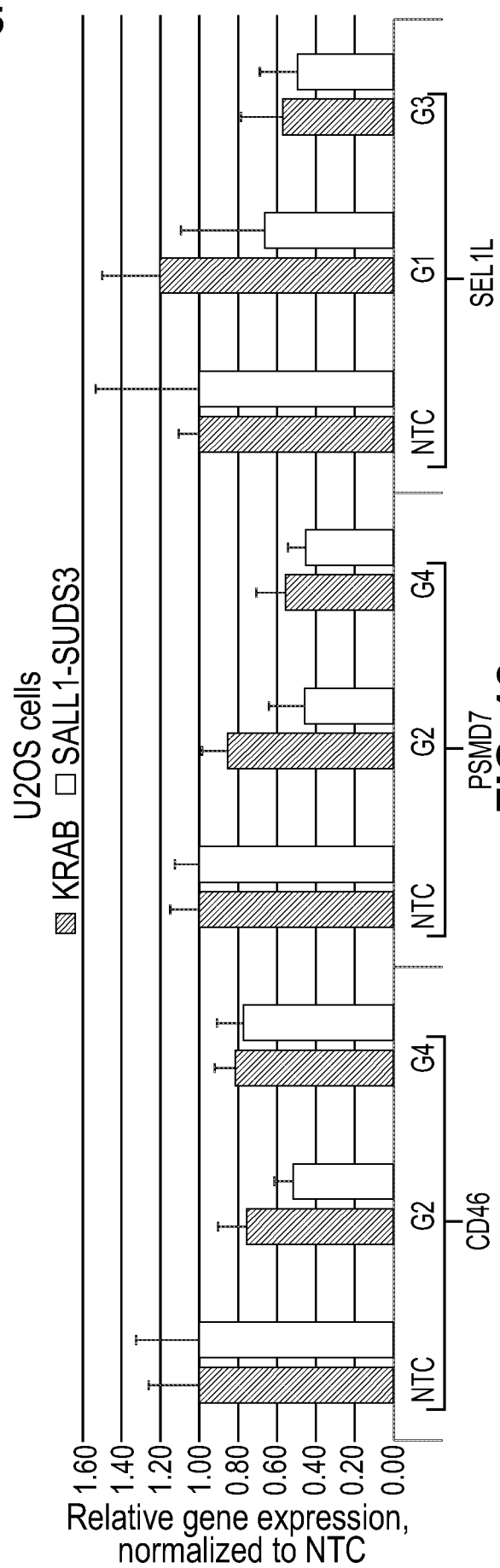


**FIG. 10**



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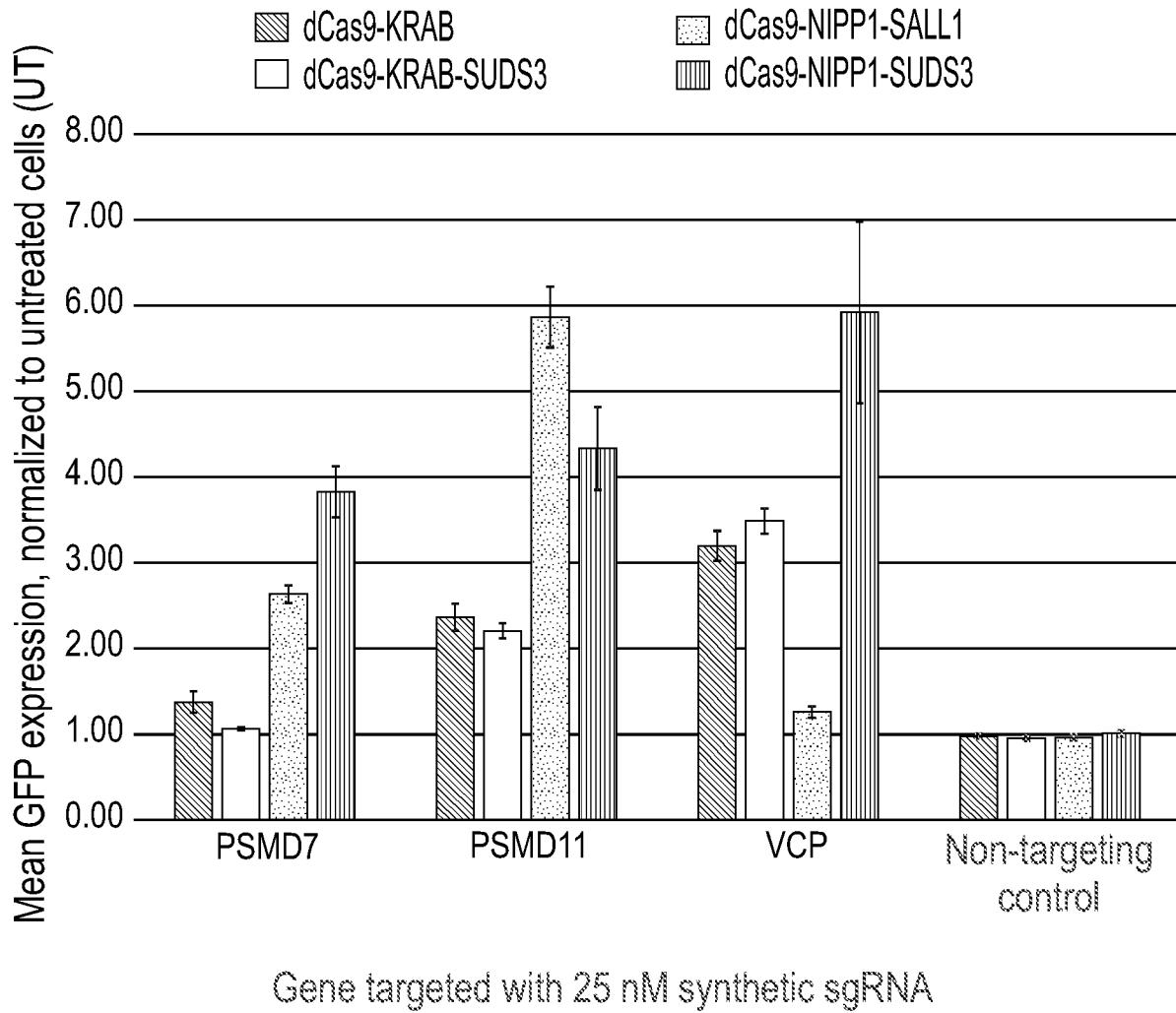
**FIG. 11**



**FIG. 12**

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U2OS Ubi[G76V] EGFP cells stably expressing bipartite Cas repressors

**FIG. 13**

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FIG. 14A

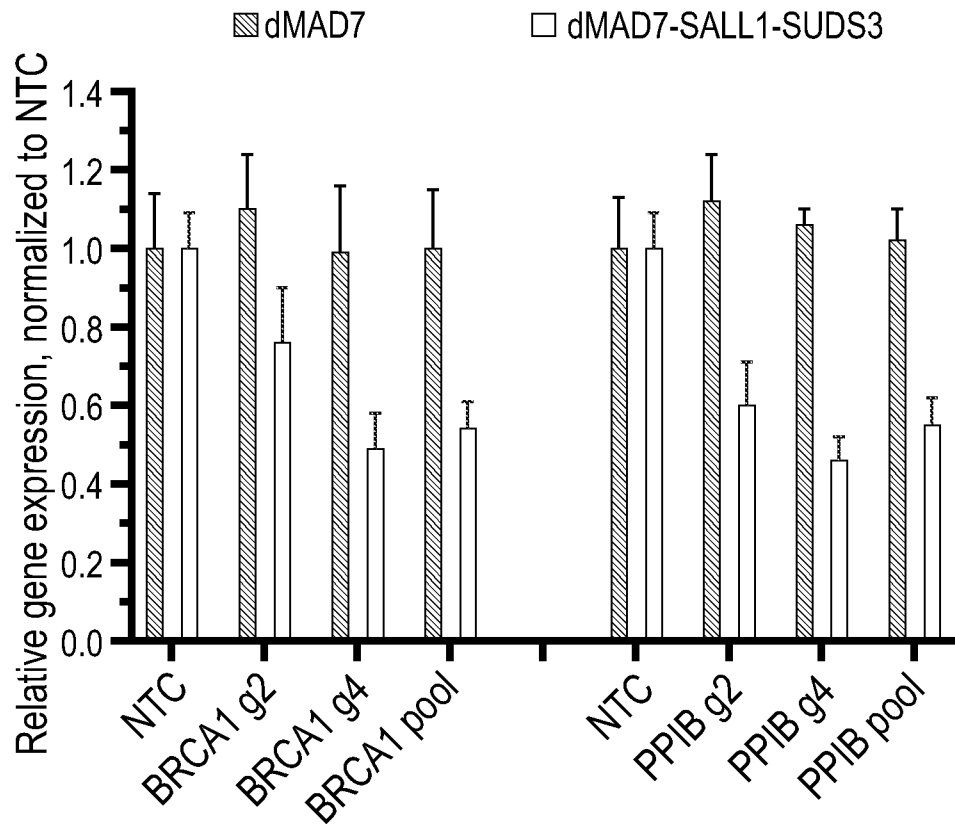
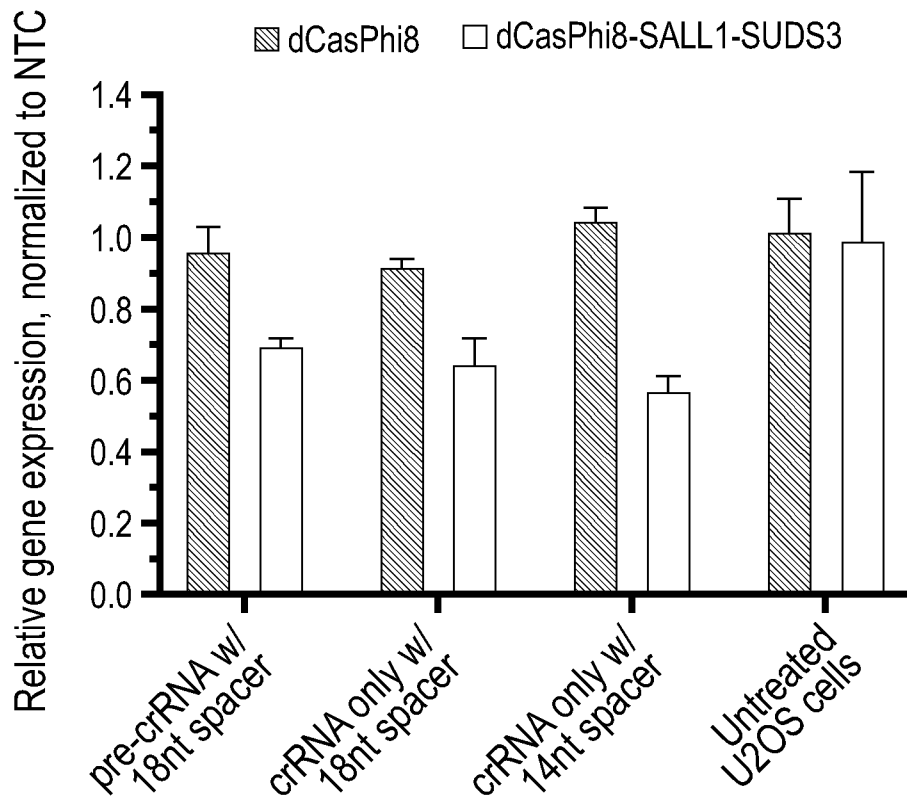


FIG. 14B



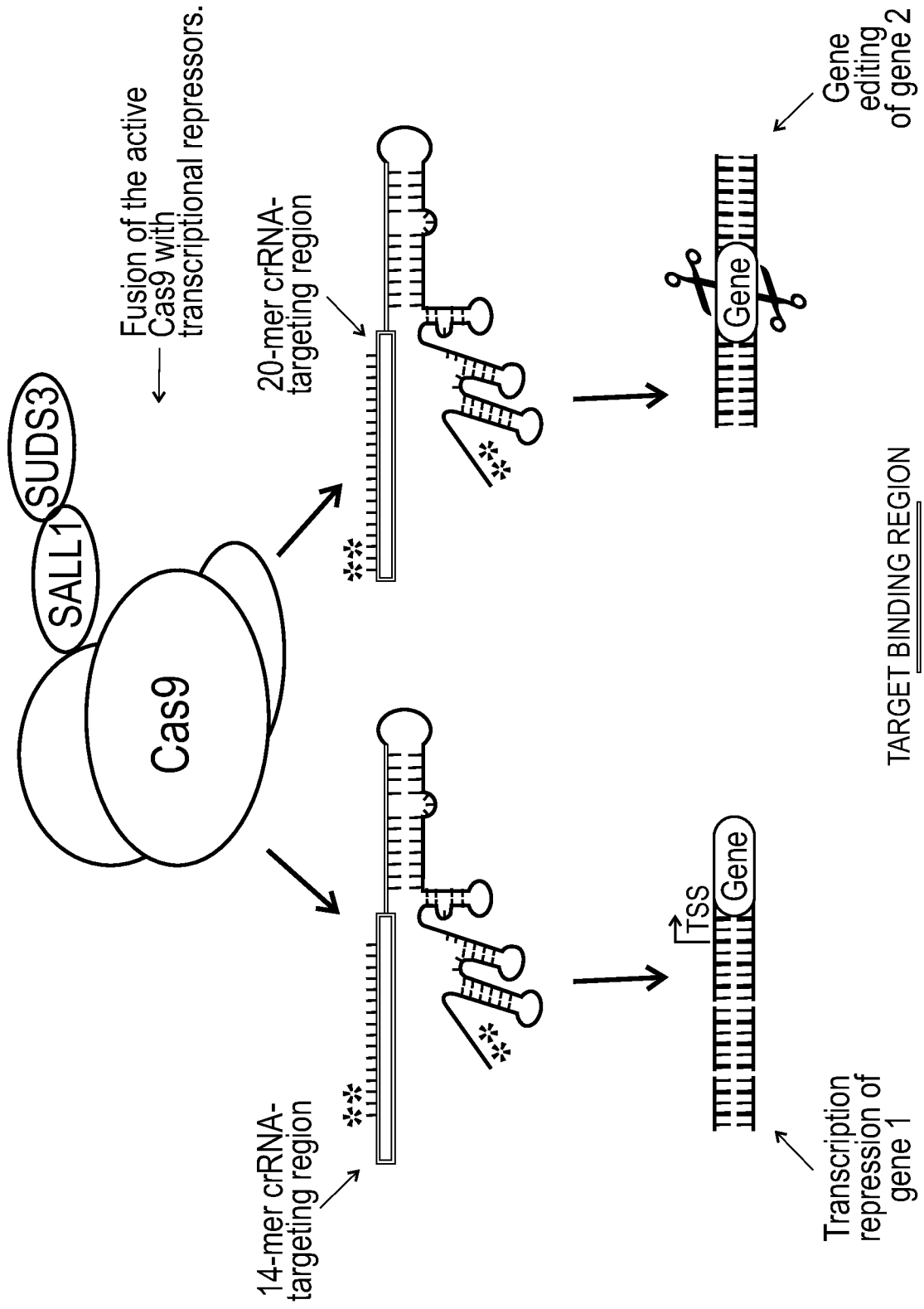


FIG. 15A

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FIG. 15B

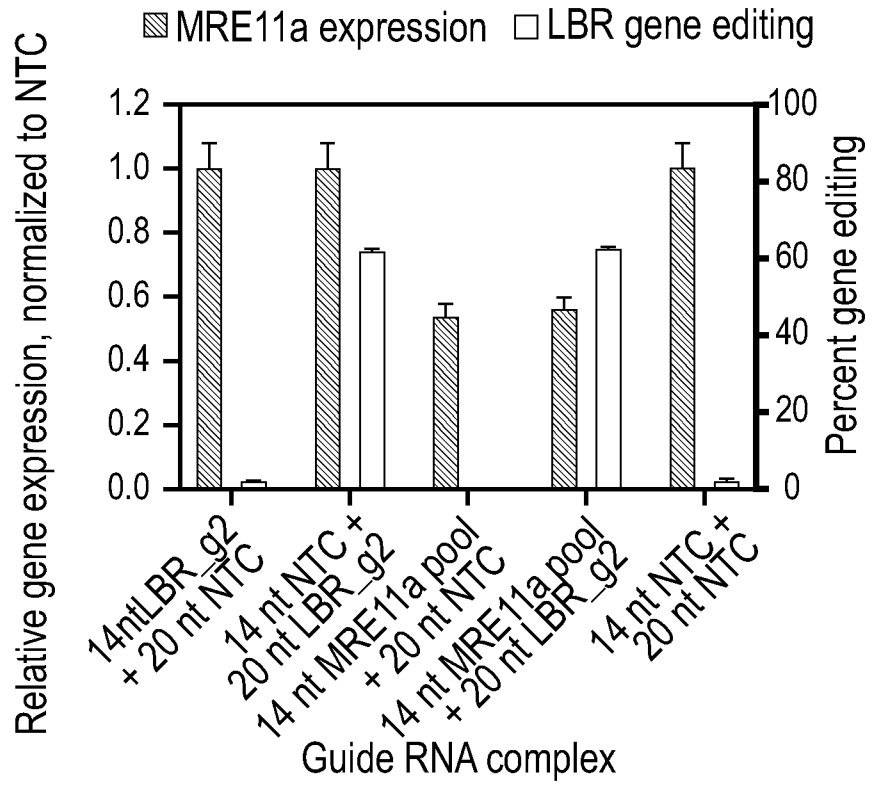
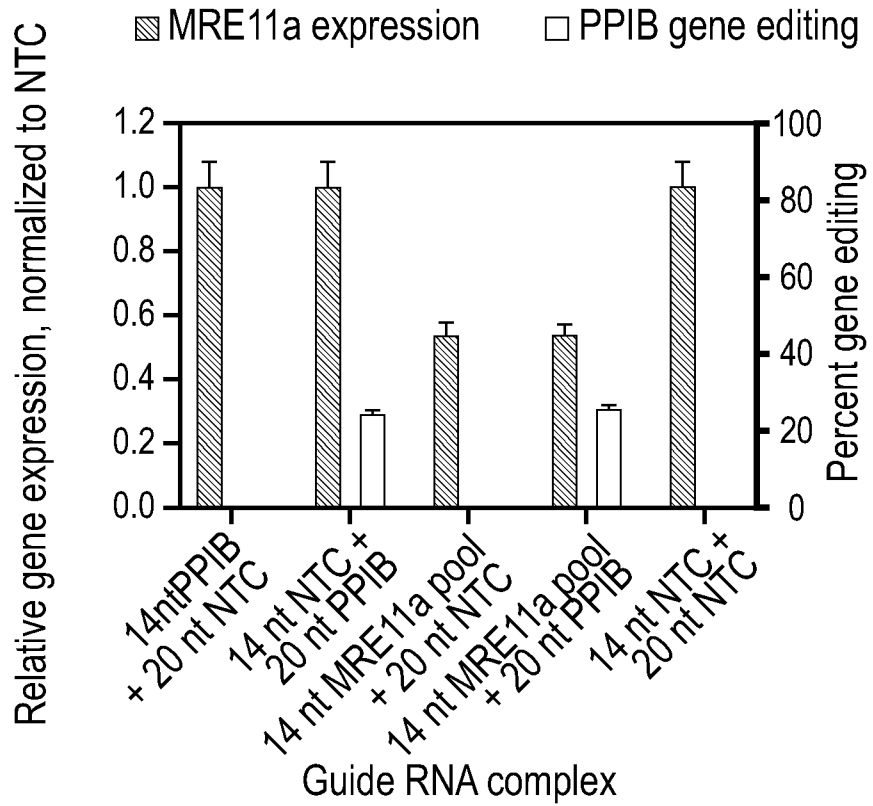
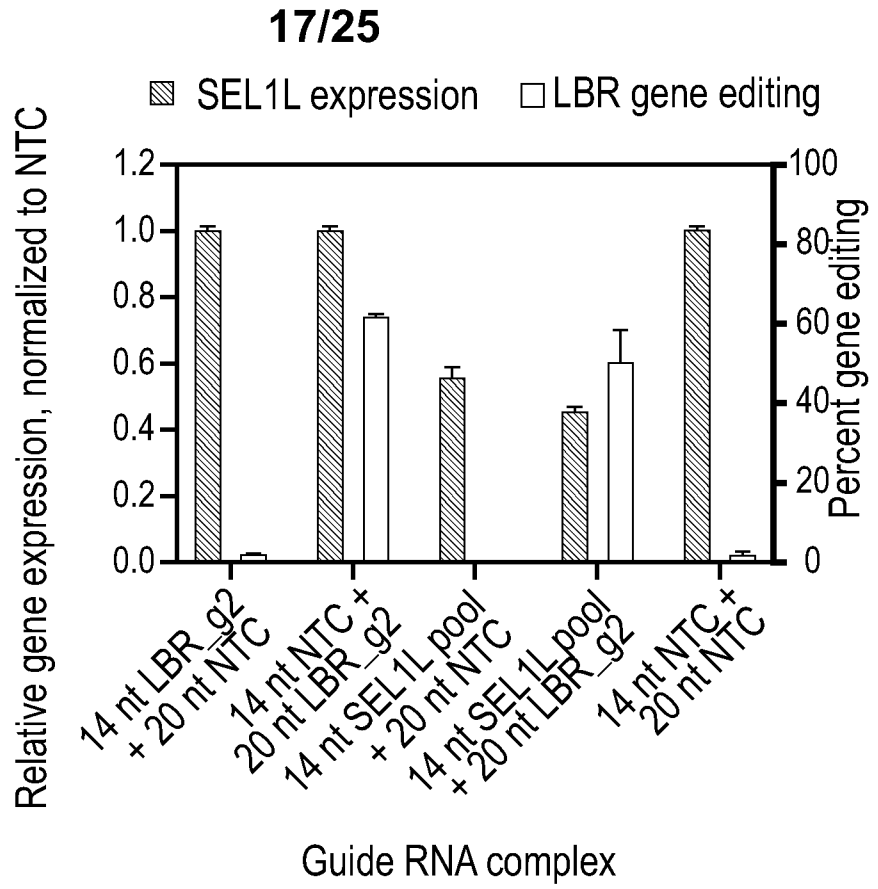


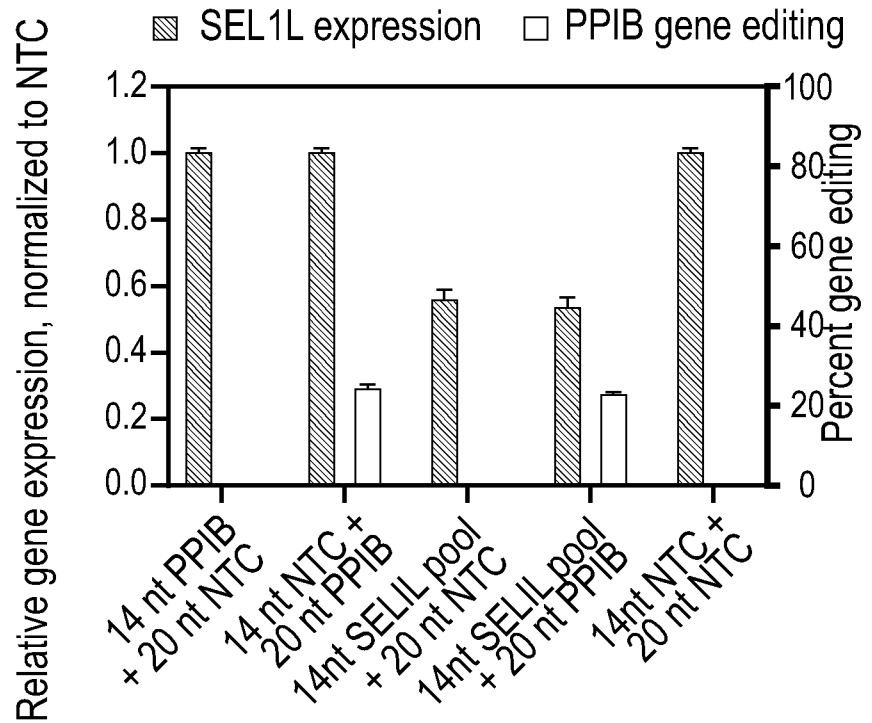
FIG. 15C



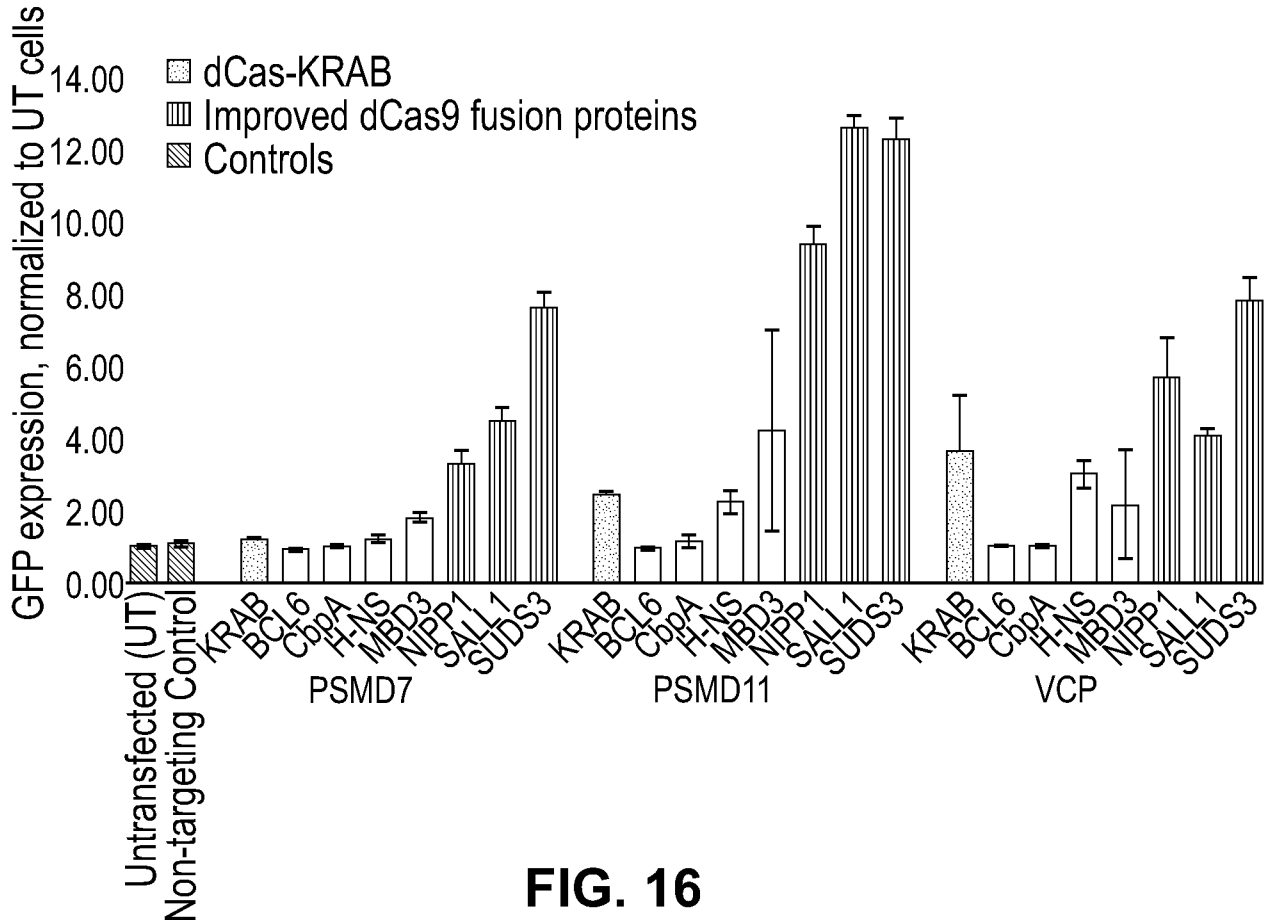
**FIG. 15D**



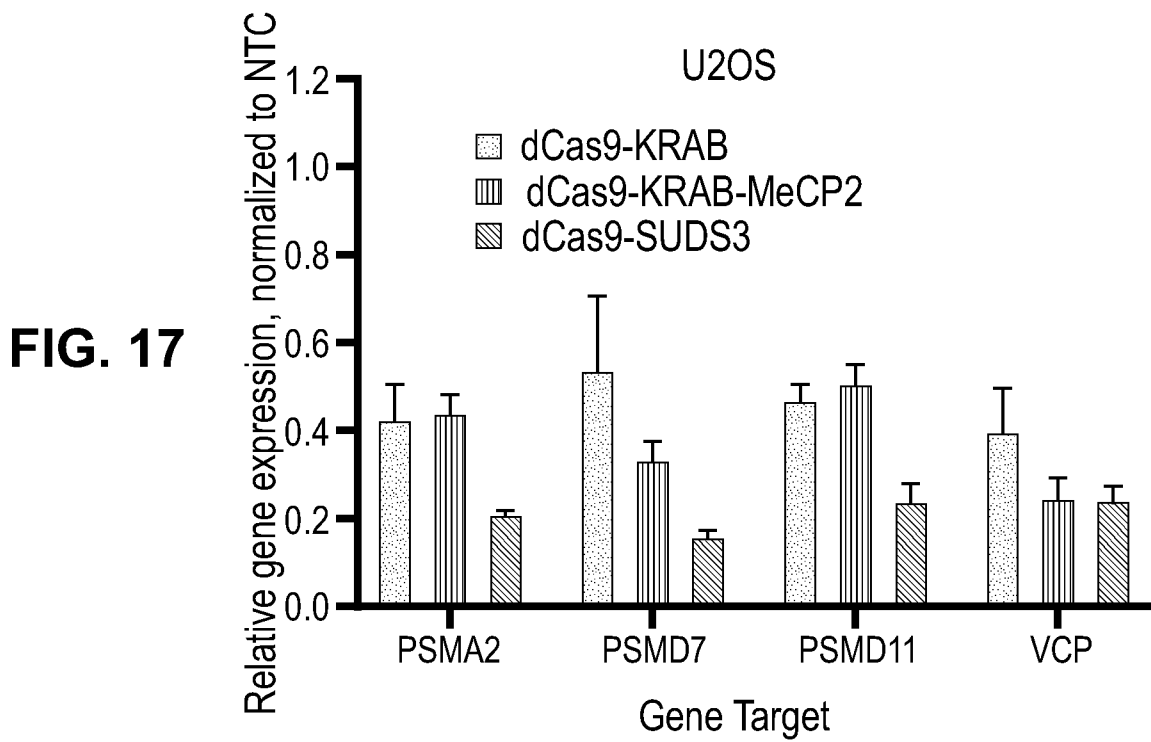
**FIG. 15E**



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**FIG. 16**



**FIG. 17**

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FIG. 18A

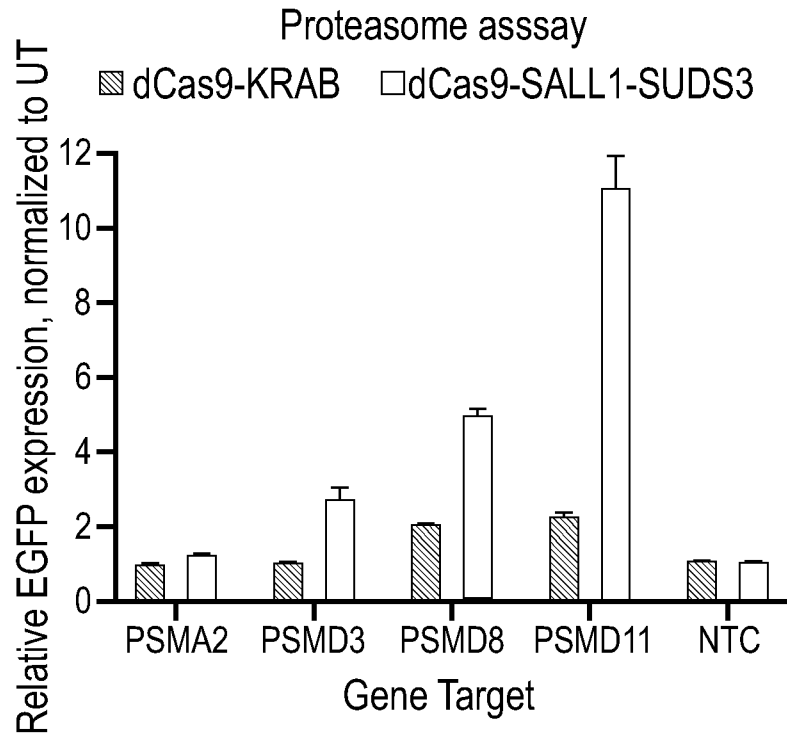
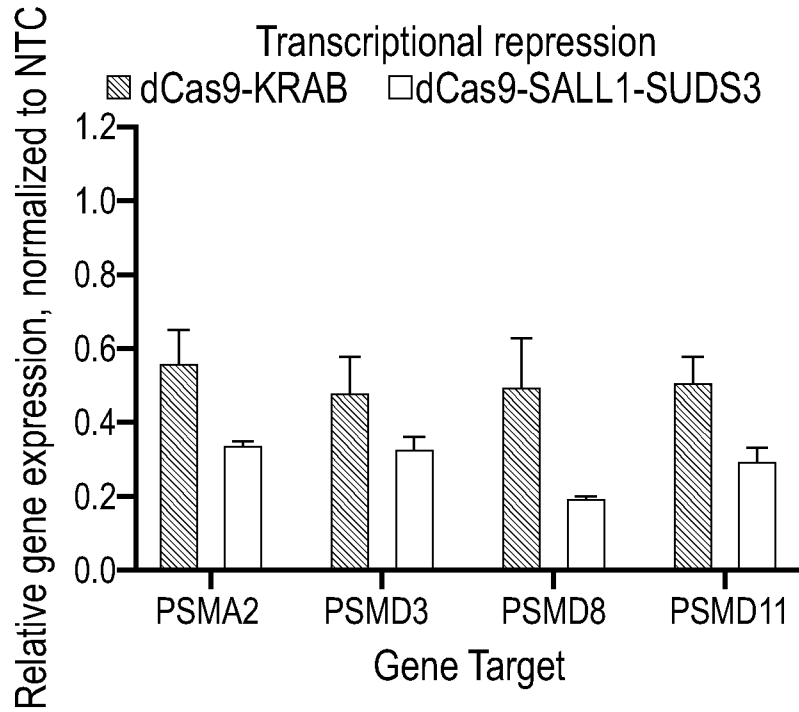


FIG. 18B



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FIG. 19A

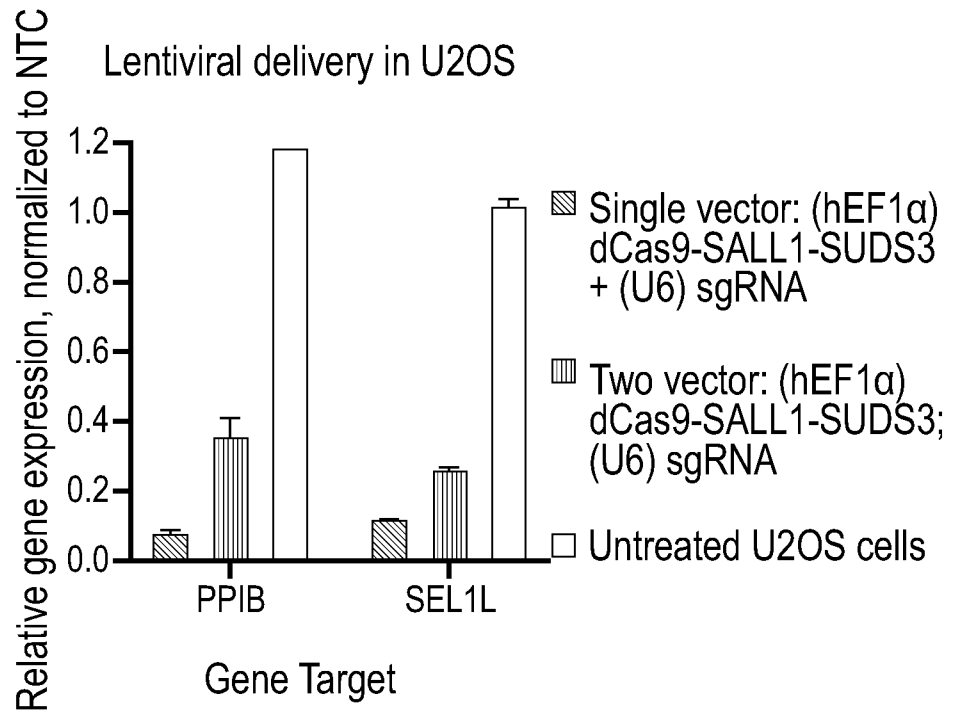
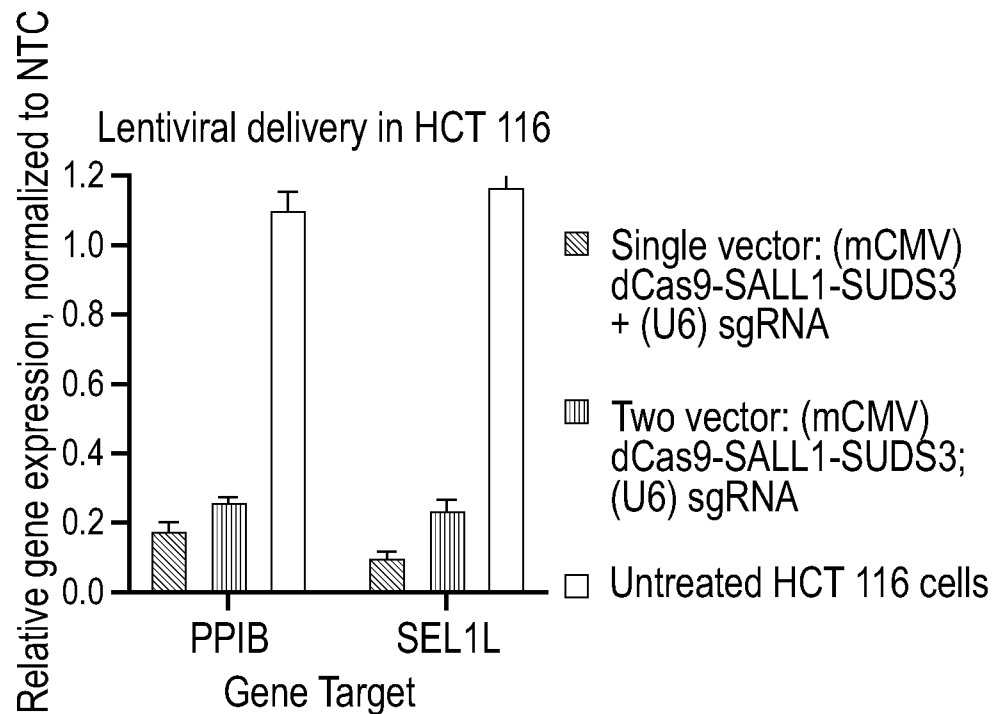


FIG. 19B



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FIG. 20A

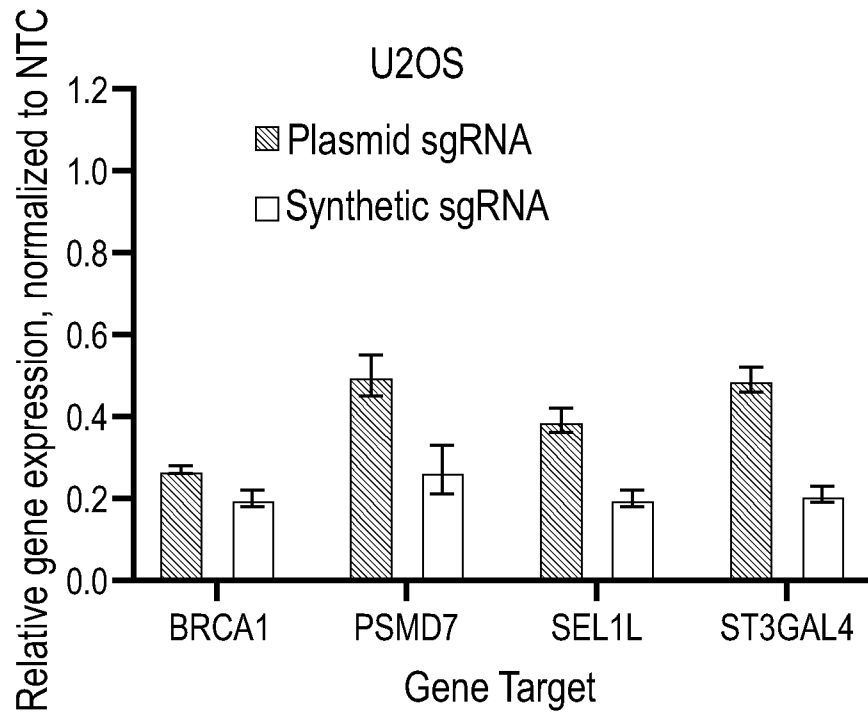
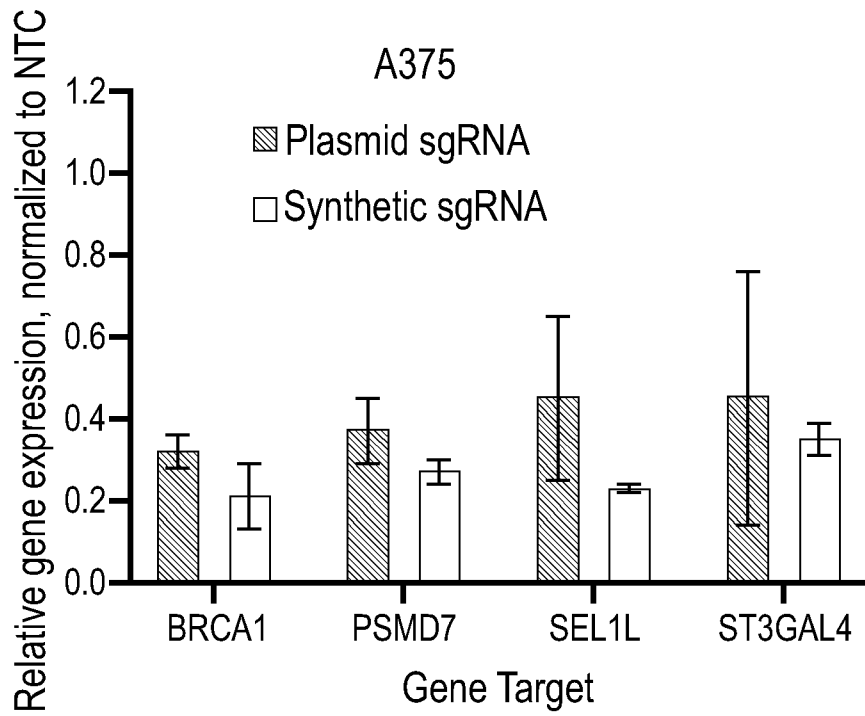


FIG. 20B



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FIG. 21

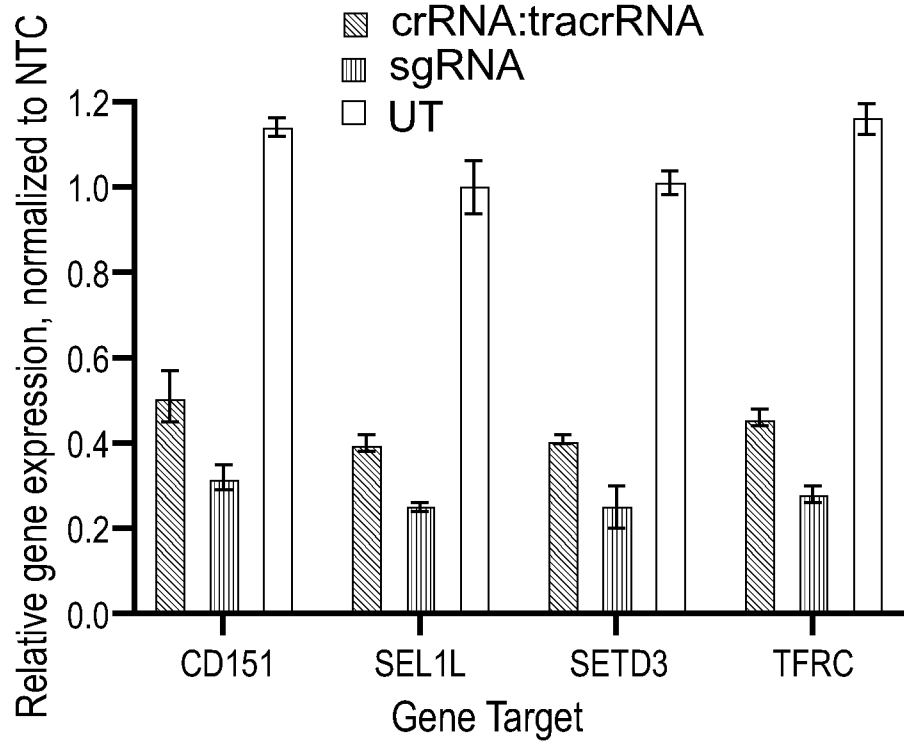
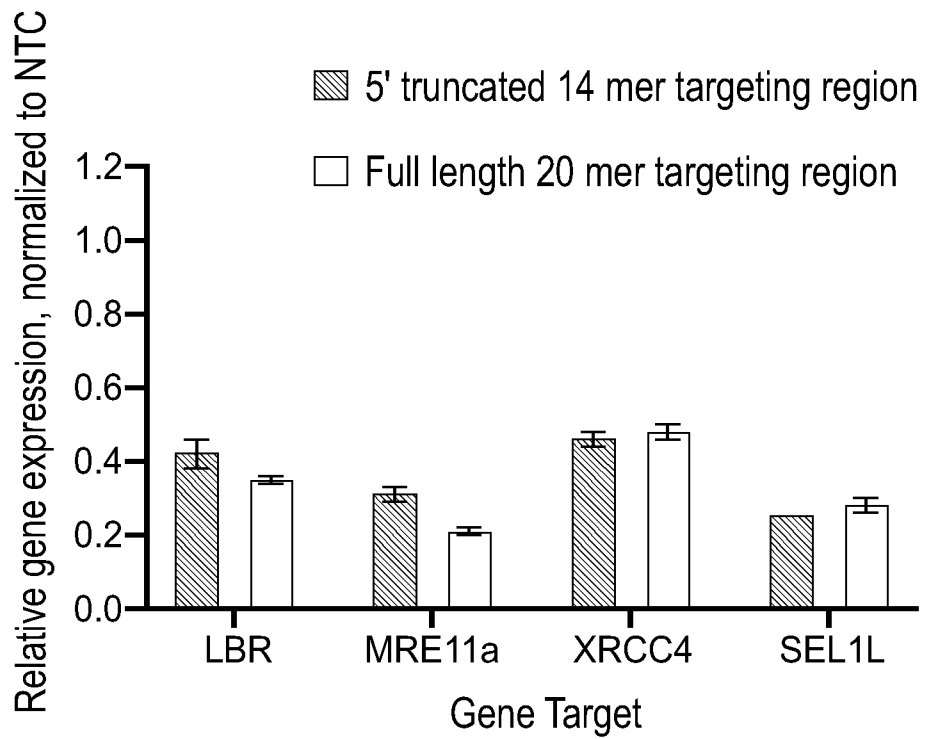
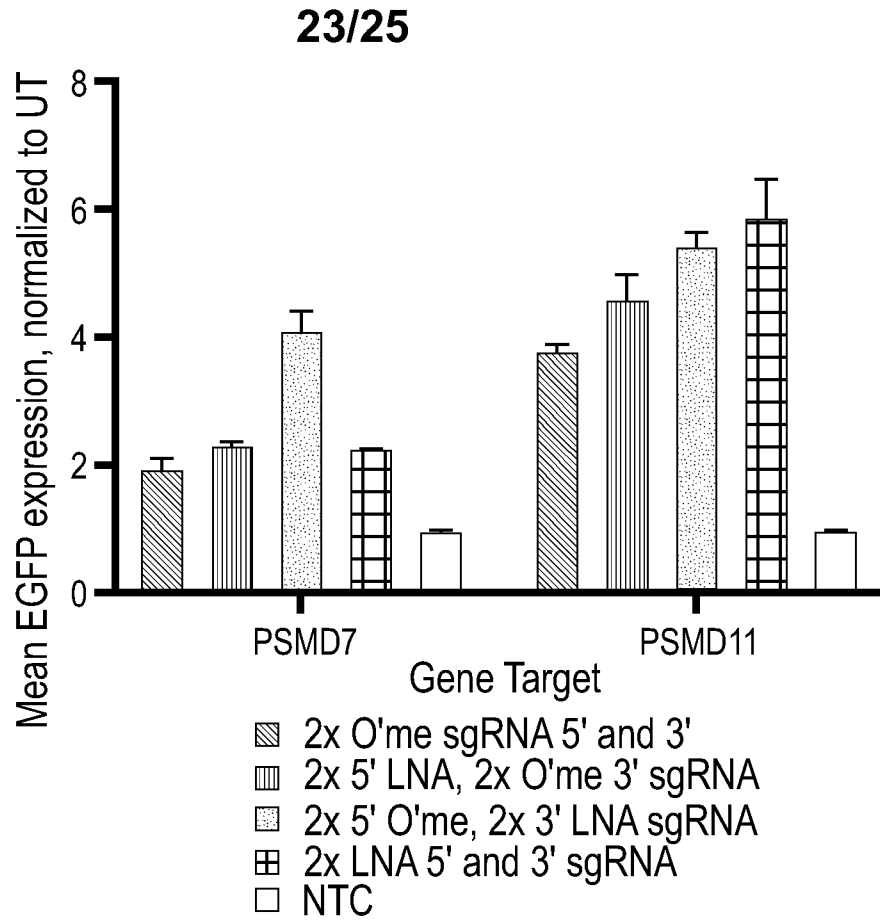


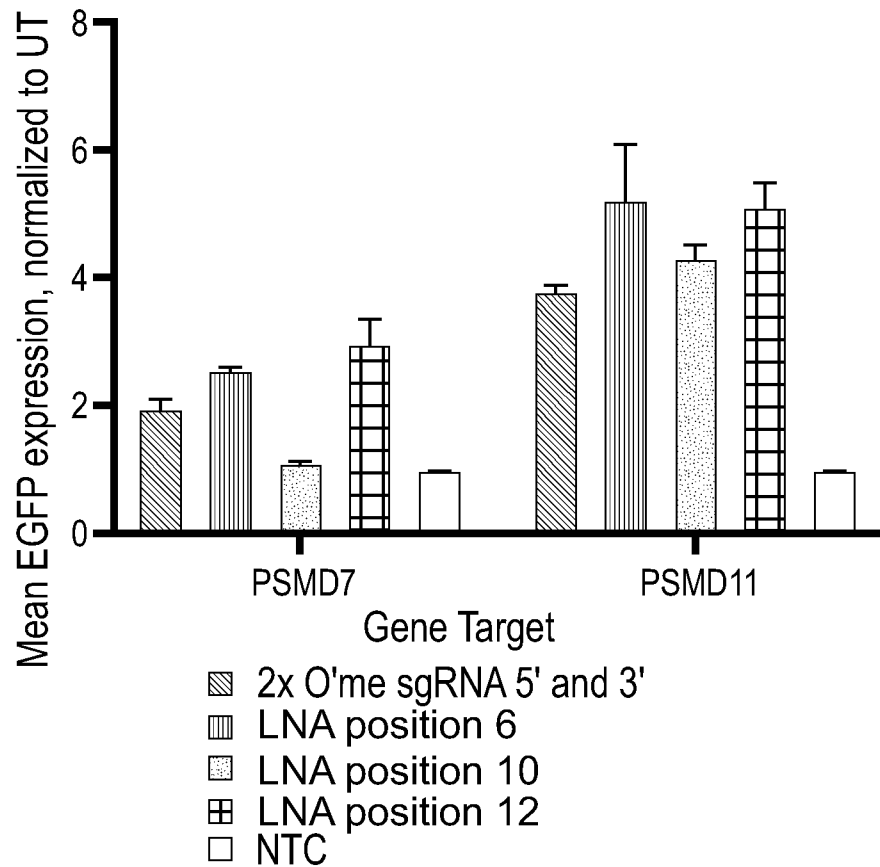
FIG. 22



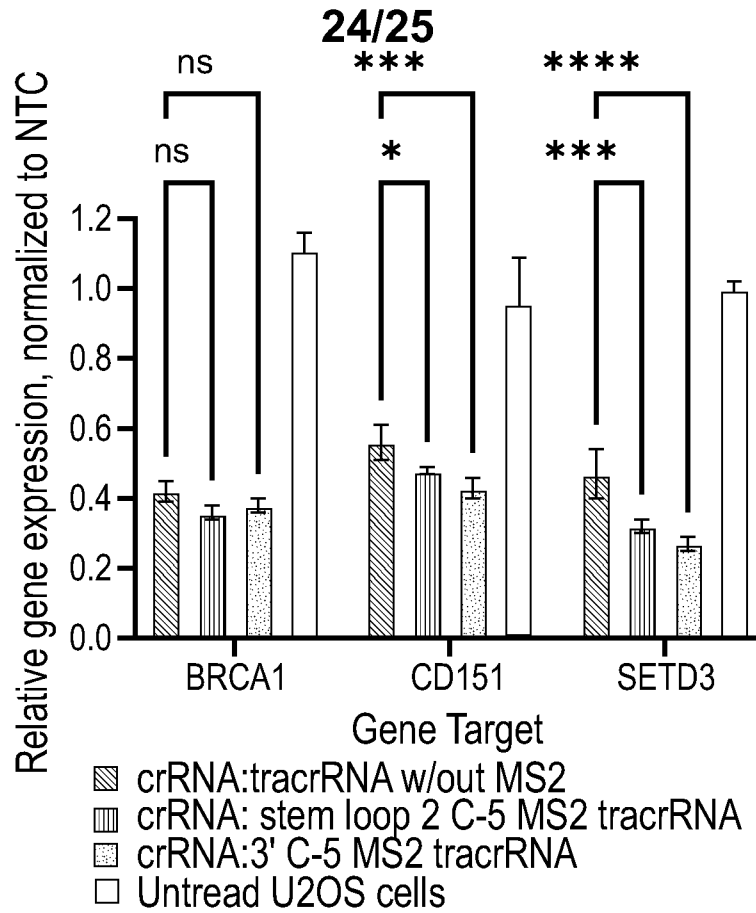
**FIG. 23A**



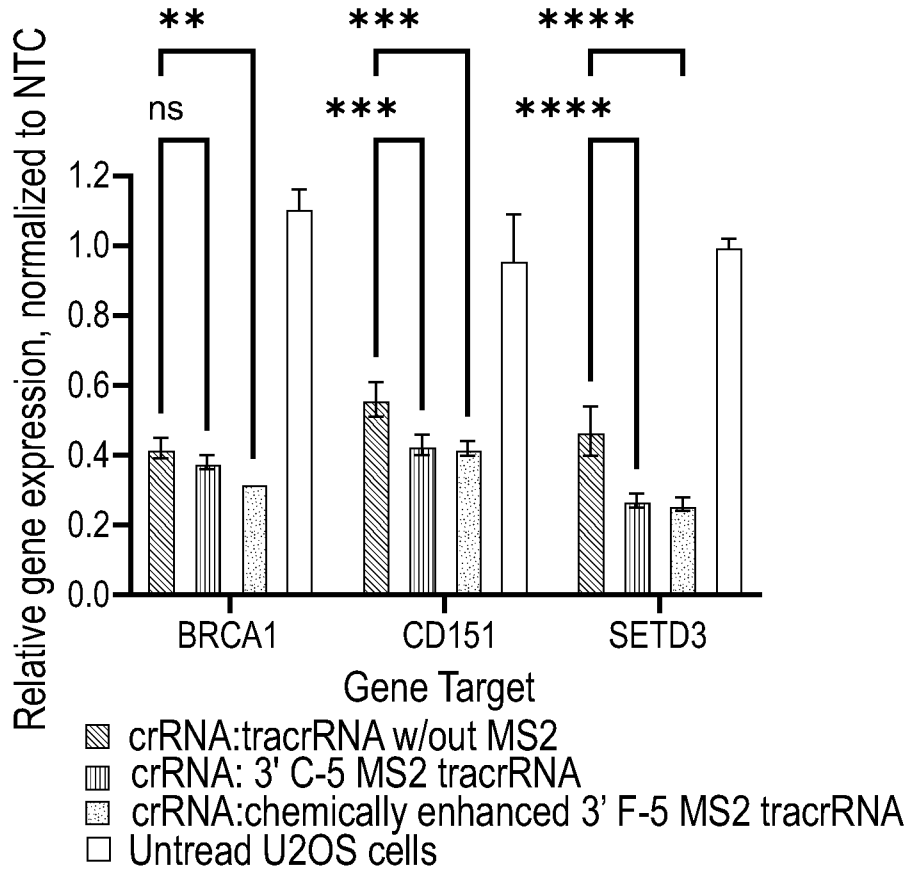
**FIG. 23B**



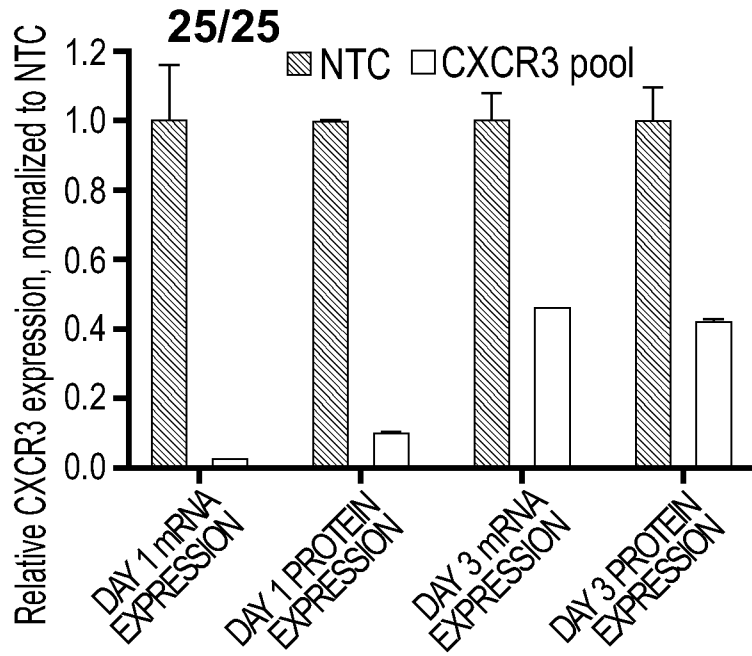
**FIG. 24A**



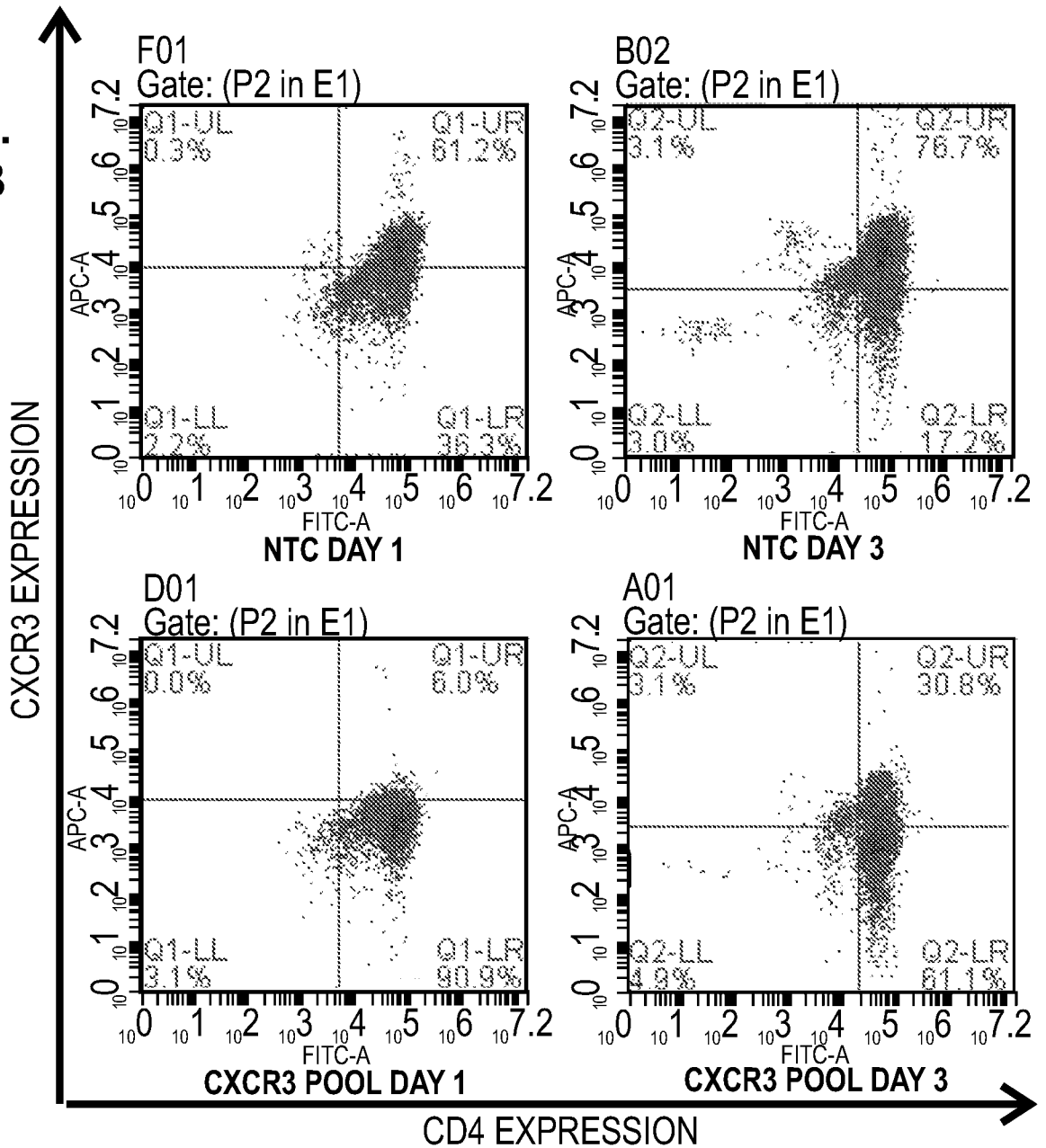
**FIG. 24B**

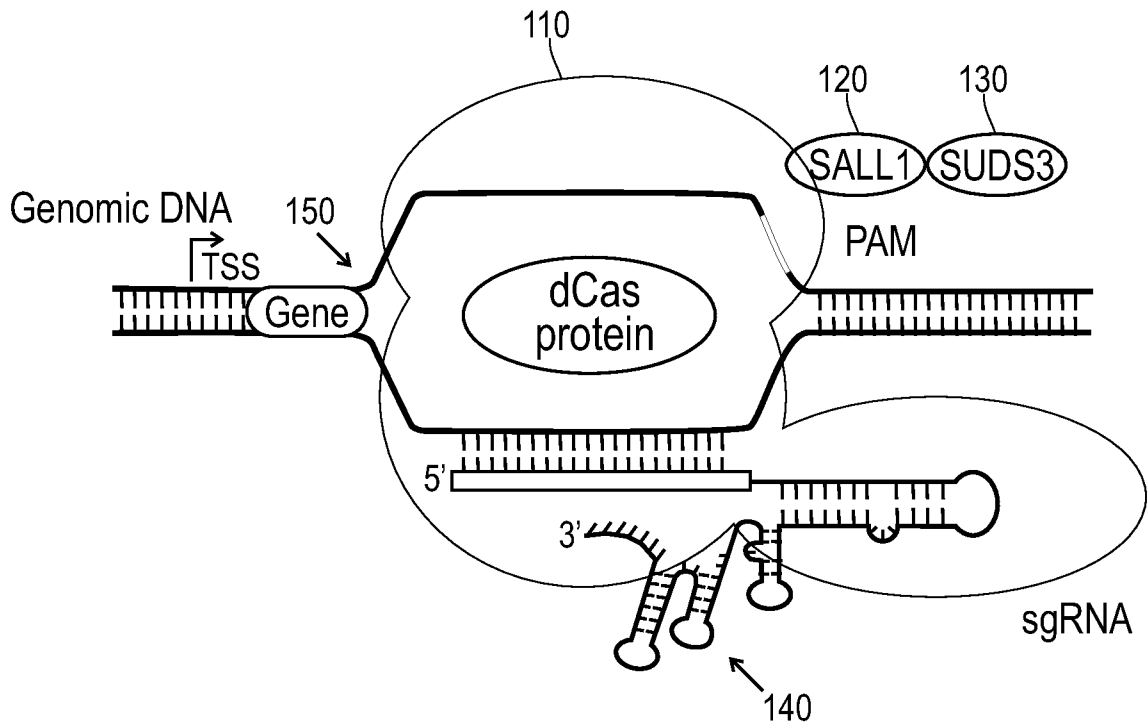


**FIG. 25A**



**FIG. 25B**





**FIG. 1**