ENGINEERED CASX REPRESSOR SYSTEMS

CROSS-REFERENCE OF SEQUENCE LISTING

[0001] This application claims priority to U.S. provisional applications 63/246,543, filed on September 21, 2021, and 63/321,517, filed on March 18, 2022, the contents of each of which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (SCRB_034_02WO_SeqList_ST26.xml; Size: 63,394,386 bytes; and Date of Creation: September 16, 2022) is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Methods of modulating expression of a target gene in a cell are varied. In mammalian systems, cells use a system of chromatin regulators (CRs) and associated histone and DNA modifications to modulate gene expression and establish long-term epigenetic memory. This system is critical in development, aging, and disease, and may provide essential capabilities for incorporating regulation in synthetic biology. In experimental systems, methods such as RNA interference (RNAi) are useful for targeted-gene knockdown and have been widely used for large-scale library screens. RNAi, however, has several limitations. In particular, RNAi-based knockdown suffers from off-target effects, along with incomplete knockdown of the target (Jackson AL, et al. Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol. 21:635 (2003)); Sigoillot FD, et al., A bioinformatics method identifies prominent off-targeted transcripts in RNAi screens. Nat Methods. 19:9(4):363 (2012)). Tailored DNA binding proteins such as zinc finger proteins or transcription activator-like effectors (TALEs) linked to transcriptional repressor domains, while able to mediate selective gene suppression, are limited by the fact that each desired target gene necessitates the generation of a new protein.

[0004] The advent of CRISPR/Cas systems and the programmable nature of these systems has facilitated their use as a versatile technology for genomic manipulation and engineering. Particular CRISPR proteins are particularly well suited for such manipulation. For example, certain Class 2 CRISPR/Cas systems have a compact size, offering ease of delivery, and the nucleotide sequence encoding the protein is relatively short, an advantage for its incorporation into viral vectors for cellular delivery. However, in certain disease indications, gene silencing, or

repression, is preferable to gene editing. The ability to render CasX catalytically-inactive (dCasX) has been demonstrated (WO2020247882A1), which makes it an attractive platform for the generation of fusion proteins capable of gene silencing. Thus, there is a need in the art for additional gene repressor systems (e.g., a dCas protein plus repressor domain) that have been optimized and/or offer improvements over earlier generation gene repressor systems, such as those based on Cas9 for utilization in a variety of therapeutic, diagnostic, and research applications.

SUMMARY

[0005] Aspects of the present disclosure are directed to compositions and methods of modulating expression of a target nucleic acid in a cell.

[0006] The present disclosure provides compositions of a gene repressor system comprising catalytically-dead Class 2 CRISPR proteins, for example Class 2 Type V CRISPR proteins, linked with one or more transcription repressor domains as a fusion protein and guide ribonucleic acids (gRNA) comprising a targeting sequence complementary to a target nucleic acid sequence of a gene in a cell (dXR:gRNA system), nucleic acids encoding the fusion proteins, vectors encoding or comprising the components of the dXR:gRNA systems, and lipid nanoparticles encoding or encapsidating the components of the dXR:gRNA systems, and methods of making and using the dXR:gRNA systems. The dXR:gRNA systems of the disclosure have utility in methods of gene silencing, or gene repression, in diseases where repression of a gene product is useful to reverse the underlying cause of the disease or to ameliorate the signs or symptoms of the disease, which methods are also provided.

[0007] Further features and advantages of certain embodiments of the present disclosure will become more fully apparent in the following description of embodiments and drawings thereof, and from the claims.

INCORPORATION BY REFERENCE

[0008] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0010] FIG. 1 shows results of an assay evaluating targeting and non-targeting dXR molecules using non-targeting and targeting spacers (left and right bars, respectively), with percentage (%) loss of target mRNA as measured by qPCR in HEK293T cells, as described in Example 1. Data represent ΔΔCt values from biological duplicates.

[0011] FIG. 2 shows the dose response results of the diphtheria toxin titration for cells transduced with either catalytically active CasX editors with gRNAs targeting the gene encoding the Heparin Binding EGF-like Growth Factor (HBEGF), i.e., CasX-34.19 and CasX-34.21; a catalytically-dead CasX (dCasX) protein linked to a repressor domain as a fusion protein targeted to *HBEGF* (dXR fusion proteins, i.e., dXR1-34.28); or a non-targeting dXR molecule (CasX-NT or dXR-NT), as described in Example 2. Data represent the mean and standard deviation of two biological replicates.

[0012] FIG. 3 shows cell counts from arrayed testing of dXR and three spacers targeting the 5'UTR sequence of the *C9orf72* locus in a TK-GFP cell line after ganciclovir treatment, as described in Example 3. Data represent single point cell counts after treatment with ganciclovir. NT: non-targeting spacer.

[0013] FIG. 4 shows the schematics that depict the plasmids utilized in the creation of an XDP construct in which the dXR is encoded on a separate plasmid and the plasmid encoding Gag components also encodes an MS2 coat protein, with protease cleavage sequence sites indicated by arrows.

[0014] FIG. 5 shows the schematics that depict the plasmids utilized in the creation of an XDP construct in which the dXR is encoded on the plasmid encoding Gag components, with protease cleavage sequence sites indicated by arrows.

[0015] FIG. 6 is a bar graph showing the western blot quantification of PTBP1 protein levels in mouse astrocytes harvested 11 days post-transduction with lentiviral particles containing dXR with the indicated *PTBP1*-targeting spacer, as described in Example 5. Cells treated with XDPs containing CasX ribonuclear proteins (RNPs) using spacer 28.10 or lentiviral particles with the

NT spacer served as experimental controls. The ratio of PTBP1 protein over total protein was normalized to that determined for the NT control in the graph.

[0016] FIG. 7 illustrates the schematics of various configurations of the epigenetic long-term CasX repressor (ELXR) molecules tested for gene repression activity. D3A and D3L denote DNA methyltransferase 3 alpha (DNMT3A) and DNMT3A-like protein (DNMT3L), respectively, as described in Example 6. CD = catalytic domain, ID = interaction domain. L1-L4 are linkers. NLS is the nuclear localization signal. See Tables 24 and 25 for ELXR sequences.

[0017] FIG. 8A presents the results of a time-course experiment comparing beta-2-microglobulin (B2M) repression activities (represented as percentage of HLA-negative cells) of ELXR proteins Nos. 1-3, as described in Example 6. Data are presented as mean with standard deviation, N=3.

[0018] FIG. 8B presents the results of the same time-course experiment shown in FIG. 8A but illustrates the B2M repression activities of ELXR proteins Nos. 1-3 containing the ZIM3-KRAB domain, benchmarked against the same experimental controls, as described in Example 6. Data are presented as mean with standard deviation, N=3.

[0019] FIG. 9A presents the results of a time-course experiment comparing B2M silencing activities (represented as percentage of HLA-negative cells) of ELXR proteins #1, #4, and #5, as described in Example 6. Data are presented as mean with standard deviation, N=3.

[0020] FIG. 9B presents the results of the same time-course experiment shown in FIG. 9A but illustrates the B2M silencing activities of ELXR proteins #1, #4, and #5 containing the ZIM3-KRAB domain, benchmarked against the same experimental controls, as described in Example 6. Data are presented as mean with standard deviation, N=3.

[0021] FIG. 10 is a violin plot of percent CpG methylation for CpG sites around the transcription start site of the *B2M* locus for each indicated experimental condition as described in Example 6.

[0022] FIG. 11 is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 21) versus specificity (percentage of off-target CpG methylation at the *B2M* locus quantified at day 5) for ELXR proteins #1-3, benchmarked against catalytically-active CasX 491 and dCas9-ZNF10-DNMT3A/L, as described in Example 6.

[0023] FIG. 12 is a violin plot of percent CpG methylation for CpG sites downstream of the transcription start site of the *VEGFA* locus for each indicated experimental condition as described in Example 6.

[0024] FIG. 13A is a violin plot of percent CpG methylation for CpG sites around the transcription start site of the *VEGFA* locus for each indicated experimental condition assessing ELXR #1, 4, and 5 with the *B2M*-targeting spacer as described in Example 6.

[0025] FIG. 13B is a violin plot of percent CpG methylation for CpG sites around the transcription start site of the *VEGFA* locus for each indicated experimental condition assessing ELXR #1, 4, and 5 with the non-targeting spacer as described in Example 6.

[0026] FIG. 14 is a scatterplot showing the relative activity (average percentage of HLA-negative cells at day 21) versus specificity (median percentage of off-target CpG methylation at the *VEGFA* locus quantified at day 5) for ELXR proteins #1-5 harboring either the ZNF10- or ZIM-KRAB domain, and the ELXR proteins were benchmarked against catalytically-active CasX 491 and dCas9-ZNF10-DNMT3A/L, as described in Example 6.

[0027] FIG. 15 is a quantification of percent editing measured as indel rate detected by NGS at the human *B2M* locus for each of the indicated catalytically-dead CasX variant with either a *B2M*-targeting spacer or a non-targeting spacer, as described in Example 8. Catalytically-active CasX 491, catalytically-inactive CasX9 (dCas9), and a mock transfection served as experimental controls.

[0028] FIG. 16 provides violin plots showing the $\log_2$ (fold change) of sequences before and after selection for their ability to support dXR repression of the *HBEGF* locus, as described in Example 4. The plots show the results for the entire KRAB domain library, a negative control set of sequences, a positive control set of known KRAB repressors, the top 1597 KRAB domains tested with $\log_2$(fold change) > 2 and p-values <0.01, and the top 95 KRAB domains tested.

[0029] FIG. 17 shows *B2M* silencing activities (represented as percentage of HLA-negative cells) of dXR proteins with various KRAB domains, as described in Example 4. Data are presented as mean with standard deviation, N=3.

[0030] FIG. 18 shows *B2M* silencing activities (represented as percentage of HLA-negative cells) of dXR proteins with various KRAB domains, as described in Example 4. Data are presented as mean with standard deviation, N=3.

[0031] FIG. 19A provides the logo of KRAB domain motif 1, as described in Example 4.

[0032] FIG. 19B provides the logo of KRAB domain motif 2, as described in Example 4.

[0033] FIG. 19C provides the logo of KRAB domain motif 3 (SEQ ID NO: 59345), as described in Example 4.

[0034] FIG. 19D provides the logo of KRAB domain motif 4 (SEQ ID NO: 59346), as described in Example 4.

[0035] FIG. 19E provides the logo of KRAB domain motif 5 (SEQ ID NO: 59347), as described in Example 4.

[0036] FIG. 19F provides the logo of KRAB domain motif 6 (SEQ ID NO: 59348), as described in Example 4.

[0037] FIG. 19G provides the logo of KRAB domain motif 7 (SEQ ID NO: 59349), as described in Example 4.

[0038] FIG. 19H provides the logo of KRAB domain motif 8, as described in Example 4.

[0039] FIG. 19I provides the logo of KRAB domain motif 9, as described in Example 4.

[0040] FIG. 20A is a schematic illustrating the relative positions of the *CD151* sequences targeted by spacers for the assayed ELXR molecules and the dCas9-ZNF10-DNMT3A/L control, as described in Example 9. Positions targeted by gRNAs are indicated by light (paired with ELXRs) and dark gray (paired with dCas9-ZNF10-DNMT3A/L) bars.

[0041] FIG. 20B is a bar graph that illustrates the results of a time-course experiment comparing CD151 repression activities (represented as percentage of total cells with CD151 knockdown) of ELXR proteins #1, #4, and #5 containing the ZIM3-KRAB domain with the indicated targeting spacers, as described in Example 9. Data for each timepoint (day 6, day 15, and day 22) are superimposed and are presented as mean with standard deviation, N=3.

[0042] FIG. 21A is a schematic showing the positions of the various *B2M*-targeting gRNAs tiled across a ~1KB window at the promoter region of the *B2M* gene, as described in Example 10. The numbers correspond to a particular *B2M*-targeting spacer shown in FIG. 21B. Targeting gRNAs are indicated by gray bars.

[0043] FIG. 21B is a bar graph that illustrates the quantification of B2M repression, represented as the average percentage of HLA-negative cells, mediated by either dXR1 or ELXR #1 with the indicated *B2M*-targeting spacers, as described in Example 10. Data are presented as mean with standard deviation, N=3. NT = non-targeting spacer.

[0044] FIG. 22 presents the results of a time-course experiment comparing B2M repression activities (represented as percentage of HLA-negative cells) of the indicated ELXR5-ZIM3 and its variants with *B2M*-targeting gRNA using spacer 7.37, as described in Example 11. Data are presented as mean with standard deviation, N=3. CD = catalytic domain of DNMT3A.

[0045] FIG. 23 presents the results of the same time-course experiment shown in FIG. 22 but shows B2M repression activities of the indicated ELXR5-ZIM3 variants with *B2M*-targeting gRNA using spacer 7.160, as described in Example 11. Data are presented as mean with standard deviation, N=3.

[0046] FIG. 24 presents the results of the same time-course experiment shown in FIG. 22 but shows B2M repression activities of the indicated ELXR5-ZIM3 variants with *B2M*-targeting gRNA using spacer 7.165, as described in Example 11. Data are presented as mean with standard deviation, N=3.

[0047] FIG. 25 presents the results of the same time-course experiment shown in FIG. 22 but shows B2M repression activities of the indicated ELXR5-ZIM3 variants with a non-targeting gRNA, as described in Example 11. Data are presented as mean with standard deviation, N=3.

[0048] FIG. 26 is a violin plot of percent CpG methylation for CpG sites downstream of the transcription start site of the *VEGFA* locus for each indicated ELXR5-ZIM3 variant for the three *B2M*-targeting gRNA and non-targeting gRNA, as described in Example 11.

[0049] FIG. 27 is a scatterplot showing the relative activity (average percentage of HLA-negative cells at day 21 for spacer 7.160) versus specificity (percentage of off-target CpG methylation at the *VEGFA* locus quantified at day 7 for spacer 7.160) for the indicated ELXR5-ZIM3 variants, as described in Example 11.

[0050] FIG. 28 is a bar plot showing the percentage of mouse Hepa1-6 cells, treated with either dXR1 or ELXR1-ZIM3 mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at day 6, as described in Example 14. Spacer 6.7 targeting the human *PCSK9* locus served as a non-targeting control.

[0051] FIG. 29 is a time course plot showing the percentage of mouse Hepa1-6 cells, treated with dXR1 mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at 6, 13, and 25 days post-delivery, as described in Example 14. Spacer 6.7 targeting the human *PCSK9* locus served as a non-targeting control, and treatment with water served as a negative control.

[0052] FIG. 30 is a time course plot showing the percentage of mouse Hepa1-6 cells, treated with ELXR1-ZIM3 mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at 6, 13, and 25 days post-delivery, as described in Example 14. Spacer 6.7 targeting the human *PCSK9* locus served as a non-targeting control, and treatment with water served as a negative control.

[0053] FIG. 31 is a bar plot showing the percentage of mouse Hepa1-6 cells, treated with ELXR1-ZIM3, ELXR5-ZIM3, catalytically active CasX491, or dCas9-ZNF10-DNMT3A/3L mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at day 7, as described in Example 14. Spacer 6.7 targeting the human *PCSK9* locus served as a non-targeting control. Production of mRNA by in-house IVT or a third-party is indicated in parentheses.

[0054] FIG. 32 is a time course plot showing the percentage of mouse Hepa1-6 cells, treated with IVT-produced ELXR1-ZIM3 vs. ELXR5-ZIM5 mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at 7 and 14 days post-delivery, as described in Example 14.

[0055] FIG. 33 is a time course plot showing the percentage of mouse Hepa1-6 cells, treated with third-party-produced ELXR1-ZIM3 vs. dCas9-ZNF10-DNMT3A/3L mRNA paired with the indicated PCSK9-targeting gRNAs, that stained negative for intracellular PCSK9 at 7 and 14 days post-delivery, as described in Example 14.

[0056] FIG. 34 is a plot illustrating percentage of HEK293T cells, transfected with a plasmid encoding the indicated CasX or ELXR:gRNA construct, that expressed B2M six days post-treatment with the DNMT1 inhibitor 5-azadC at varying concentrations, as described in Example 12.

[0057] FIG. 35 is a plot that juxtaposes the quantification of B2M repression in HEK293T cells transfected with a plasmid encoding the indicated CasX or ELXR:gRNA construct and cultured for 58 days, with the quantification of B2M reactivation upon treatment of transfected cells with 5-azadC, as described in Example 12.

[0058] FIG. 36 illustrates the schematics of the various ELXR #5 architectures, where the additional DNMT3A domains were incorporated, as described in Example 11. The additional DNMT3A domains were the ADD domain of DNMT3A ("D3A ADD") and the PWWP domain of DNMT3A ("D3A PWWP"). "D3A endo" encodes for an endogenous sequence that occurs between DNMT3A PWWP and ADD domains. "D3A CD" and "D3L ID" denote the catalytic domain of DNMT3A and the interaction domain of DNMT3L respectively. "L1-L3" are linkers. "NLS" is the nuclear localization signal. See Table 33 for ELXR sequences.

[0059] FIG. 37 illustrates the schematics of the general architectures of the ELXR molecules with the ADD domain for ELXR configuration #1, #4, and #5 tested in Example 13. "D3A ADD", "D3A CD" and "D3L ID" denote the ADD domain of DNMT3A, the catalytic domain of

DNMT3A, and the interaction domain of DNMT3L respectively, as described in Example 13. "L1-L4" are linkers. "NLS" is the nuclear localization signal. See Table 35 for ELXR sequences.

[0060] FIG. 38 illustrates the schematic of a generic dXR configuration as described in Example 1. NLS is the nuclear localization signal, L3 is linker 3 (see Table 24 for AA sequence).

[0061] FIG. 39A presents the results of a time-course experiment comparing B2M repression activities (represented as percentage of HLA-negative cells) of ELXRs with the ZIM3-KRAB domain having configuration #1, #4, or #5 with or without the DNMT3A ADD domain when paired with the *B2M*-targeting gRNA with spacer 7.160, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0062] FIG. 39B is a plot showing the results of the same time-course experiment shown in FIG. 39A but illustrates B2M repression activities for ELXR #5 with the ZNF10 or ZIM3-KRAB domain, with or without the DNMT3A ADD domain, paired with the *B2M*-targeting gRNA with spacer 7.160, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0063] FIG. 39C is a plot showing the results of the same time-course experiment shown in FIG. 39A but illustrates B2M repression activities for ELXR5-ZIM3 with or without the DNMT3A ADD domain paired with a *B2M*-targeting gRNA with the indicated spacers, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0064] FIG. 40A is a plot illustrating the results of B2M repression activities on day 27 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #1 with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0065] FIG. 40B is a plot illustrating the results of B2M repression activities on day 27 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #4 with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0066] FIG. 40C is a plot illustrating the results of B2M repression activities on day 27 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #5

with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean with standard deviation, N=3. "NT" is a gRNA with a non-targeting spacer.

[0067] FIG. 41A is a plot illustrating the results of bisulfite sequencing used to determine off-target methylation at the *VEGFA* locus on day 5 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #1 with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean percentage of CpG methylation for CpG sites near the *VEGFA* locus; standard error of the mean is also presented; N=3. "NT" is a gRNA with a non-targeting spacer.

[0068] FIG. 41B is a plot illustrating the results of bisulfite sequencing used to determine off-target methylation at the *VEGFA* locus on day 5 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #4 with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean percentage of CpG methylation for CpG sites near the *VEGFA* locus; standard error of the mean is also presented; N=3. "NT" is a gRNA with a non-targeting spacer.

[0069] FIG. 41C is a plot illustrating the results of bisulfite sequencing used to determine off-target methylation at the *VEGFA* locus on day 5 post-transfection for ELXRs with either the ZNF10 or ZIM3-KRAB domain having configuration #5 with or without the DNMT3A ADD domain for the indicated gRNAs, as described in Example 13. Data are presented as mean percentage of CpG methylation for CpG sites near the *VEGFA* locus; standard error of the mean is also presented; N=3. "NT" is a gRNA with a non-targeting spacer.

[0070] FIG. 42A is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZIM3-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.160, as described in Example 13.

[0071] FIG. 42B is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZNF10-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.160, as described in Example 13.

[0072] FIG. 43A is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZIM3-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.37, as described in Example 13.

[0073] FIG. 43B is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZNF10-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.37, as described in Example 13.

[0074] FIG. 44A is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZIM3-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.165, as described in Example 13.

[0075] FIG. 44B is a dot plot showing the relative activity (average percentage of HLA-negative cells at day 27) versus specificity (percentage of off-target CpG methylation at the VEGFA locus quantified at day 5) for the ELXR molecules with the ZNF10-KRAB domain having configurations #1, #4, and #5, for *B2M*-targeting gRNA with spacer 7.165, as described in Example 13.

[0076] FIG. 45 illustrates the schematics of various configurations of ELXR molecules with the incorporation of the DNMT3A ADD. "D3A ADD", "D3A CD", and "D3L ID" denote the ADD domain of DNMT3A, the catalytic domain of DNMT3A, and the interaction domain of DNMT3L, respectively. L1-L3 are linkers. NLS is the nuclear localization signal.

DETAILED DESCRIPTION

[0077] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that

methods and structures within the scope of these claims and their equivalents be covered thereby.

[0078] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present embodiments, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention.

Definitions

[0079] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, terms "polynucleotide" and "nucleic acid" encompass single-stranded DNA; double-stranded DNA; multi-stranded DNA; single-stranded RNA; double-stranded RNA; multi-stranded RNA; genomic DNA; cDNA; DNA-RNA hybrids; and a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0080] "Hybridizable" or "complementary" are used interchangeably to mean that a nucleic acid (e.g., RNA, DNA) comprises a sequence of nucleotides that enables it to non-covalently bind, i.e., form Watson-Crick base pairs and/or G/U base pairs, "anneal", or "hybridize," to another nucleic acid in a sequence-specific, antiparallel, manner (i.e., a nucleic acid specifically binds to a complementary nucleic acid) under the appropriate in vitro and/or in vivo conditions of temperature and solution ionic strength. It is understood that the sequence of a polynucleotide need not be 100% complementary to that of its target nucleic acid sequence to be specifically hybridizable; it can have at least about 70%, at least about 80%, or at least about 90%, or at least about 95% sequence identity and still hybridize to the target nucleic acid sequence. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure, a 'bulge', 'bubble' and the like).

[0081] A “gene,” for the purposes of the present disclosure, includes a DNA region encoding a gene product (e.g., a protein, or RNA), as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory element sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene may include regulatory sequences including, but not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions. Coding sequences encode a gene product upon transcription or transcription and translation; the coding sequences of the disclosure may comprise fragments and need not contain a full-length open reading frame. A gene can include both the strand that is transcribed; e.g. the strand containing the coding sequence, as well as the complementary strand.

[0082] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0083] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5' side of a coding region or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[0084] The term “regulatory element” is used interchangeably herein with the term “regulatory sequence,” and is intended to include promoters, enhancers, and other expression regulatory elements (e.g., transcription termination signals, such as polyadenylation signals and poly-U sequences). Exemplary regulatory elements include a transcription promoter such as, but not limited to, CMV, CMV+intron A, SV40, RSV, HIV-Ltr, elongation factor 1 alpha (EF1α), MMLV-ltr, internal ribosome entry site (IRES) or P2A peptide to permit translation of multiple genes from a single transcript, metallothionein, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences. It will be understood that the choice of the appropriate regulatory element will depend on the encoded component to be expressed (e.g., protein or RNA) or whether the nucleic acid comprises multiple components that require different polymerases or are not intended to be expressed as a fusion protein.

[0085] The term "promoter" refers to a DNA sequence that contains an RNA polymerase binding site, transcription start site, TATA box, and/or B recognition element and assists or promotes the transcription and expression of an associated transcribable polynucleotide sequence and/or gene (or transgene). A promoter can be synthetically produced or can be derived from a known or naturally occurring promoter sequence or another promoter sequence. A promoter can be proximal or distal to the gene to be transcribed. A promoter can also include a chimeric promoter comprising a combination of two or more heterologous sequences to confer certain properties. A promoter of the present disclosure can include variants of promoter sequences that are similar in composition, but not identical to, other promoter sequence(s) known or provided herein. A promoter can be classified according to criteria relating to the pattern of expression of an associated coding or transcribable sequence or gene operably linked to the promoter, such as constitutive, developmental, tissue specific, inducible, etc.

[0086] The term “enhancer” refers to regulatory element DNA sequences that, when bound by specific proteins called transcription factors, regulate the expression of an associated gene. Enhancers may be located in the intron of the gene, or 5’ or 3’ of the coding sequence of the gene. Enhancers may be proximal to the gene (*i.e.*, within a few tens or hundreds of base pairs (bp) of the promoter), or may be located distal to the gene (*i.e.*, thousands of bp, hundreds of thousands of bp, or even millions of bp away from the promoter). A single gene may be regulated by more than one enhancer, all of which are envisaged as within the scope of the instant disclosure.

[0087] “Operably linked” means with reference to a juxtaposition of two or more components (such as sequence elements), in which the components are arranged such that both components function normally and allow the possibility that at least one of the components can mediate a function that is exerted upon at least one of the other components; e.g., a promoter and an encoding sequence.

[0088] “Repressor domain” refers to polypeptide factors that act as regulatory elements on DNA that inhibit, repress, or block transcription of DNA, resulting in repression of gene expression. A repressor domain can be a subunit of a repressor and individual domains can possess different functional properties. In the context of the present disclosure, the linking of a repressor domain to a catalytically inactive CRISPR protein that is paired as a ribonucleoprotein complex (RNP) with a guide RNA with binding affinity to certain regions of a target nucleic acid, can, when bound to the target nucleic acid, prevent transcription from a promoter or

otherwise inhibit the expression of a gene. Without wishing to be bound by theory, it is thought that transcriptional repressors can function by a variety of mechanisms, including physically blocking RNA polymerase passage by steric hindrance, altering the polymerase's post-translational modification state, modifying the epigenetic state of the nascent RNA, changing the epigenetic state of the DNA through methylation, changing the epigenetic state of the DNA through histone deacetylation or modulating nucleosome remodeling, or preventing enhancer-promoter interactions, thereby leading to gene silencing or a reduction in the level of gene expression.

[0089] As used herein a “catalytically-dead CRISPR protein” refers to a CRISPR protein that lacks endonuclease activity. The skilled artisan will appreciate that a CRISPR protein can be catalytically dead, and still able to carry out additional protein functions, such as DNA binding. Similarly, a "catalytically-dead CasX" refers to a CasX protein that lacks endonuclease activity but is still able to carry out additional protein functions, such as DNA binding.

[0090] "Recombinant," as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. Generally, DNA sequences encoding the structural coding sequence can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system. Such sequences can be provided in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA comprising the relevant sequences can also be used in the formation of a recombinant gene or transcriptional unit. Sequences of non-translated DNA may be present 5’ or 3’ from the open reading frame, where such sequences do not interfere with manipulation or expression of the coding regions, and may indeed act to modulate production of a desired product by various mechanisms (see "enhancers” and “promoters", above).

[0091] The term "recombinant polynucleotide" or "recombinant nucleic acid" refers to one which is not naturally occurring; e.g., is made by the artificial combination of two otherwise separated segments of sequence through human intervention. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids; e.g., by genetic engineering techniques. Such is usually done to

replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids; e.g., by genetic engineering techniques.

[0092] Similarly, the term "recombinant polypeptide" or "recombinant protein" refers to a polypeptide or protein which is not naturally occurring; e.g., is made by the artificial combination of two otherwise separated segments of amino sequence through human intervention. Thus; e.g., a protein that comprises a heterologous amino acid sequence is recombinant.

[0093] As used herein, the term "contacting" means establishing a physical connection between two or more entities. For example, contacting a target nucleic acid sequence with a guide nucleic acid means that the target nucleic acid sequence and the guide nucleic acid are made to share a physical connection; e.g., can hybridize if the sequences share sequence similarity.

[0094] "Dissociation constant", or "K_d", are used interchangeably and mean the affinity between a ligand "L" and a protein "P"; *i.e.*, how tightly a ligand binds to a particular protein. It can be calculated using the formula $K_d=[L]\ [P]/[LP]$, where [P], [L] and [LP] represent molar concentrations of the protein, ligand and complex, respectively.

[0095] As used herein, "homology-directed repair" (HDR) refers to the form of DNA repair that takes place during repair of double-strand breaks in cells. This process requires nucleotide sequence homology, and uses a donor template to repair or knock-out a target DNA, and leads to the transfer of genetic information from the donor (e.g., such as the donor template) to the target. Homology-directed repair can result in an alteration of the sequence of the target nucleic acid sequence by insertion, deletion, or mutation if the donor template differs from the target DNA sequence and part or all of the sequence of the donor template is incorporated into the target DNA.

[0096] As used herein, "non-homologous end joining" (NHEJ) refers to the repair of double-strand breaks in DNA by direct ligation of the break ends to one another without the need for a homologous template (in contrast to homology-directed repair, which requires a homologous

sequence to guide repair). NHEJ often results in the loss (deletion) of nucleotide sequence near the site of the double- strand break.

[0097] As used herein "micro-homology mediated end joining" (MMEJ) refers to a mutagenic DSB repair mechanism, which always associates with deletions flanking the break sites without the need for a homologous template (in contrast to homology-directed repair, which requires a homologous sequence to guide repair). MMEJ often results in the loss (deletion) of nucleotide sequence near the site of the double- strand break.

[0098] A polynucleotide or polypeptide (or protein) has a certain percent "sequence similarity" or "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence similarity (sometimes referred to as percent similarity, percent identity, or homology) can be determined in a number of different manners. To determine sequence similarity, sequences can be aligned using the methods and computer programs that are known in the art, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST. Percent complementarity between particular stretches of nucleic acid sequences within nucleic acids can be determined using any convenient method. Example methods include BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.); e.g., using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

[0099] The terms "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence.

[0100] A "vector" or "expression vector" is a replicon, such as plasmid, phage, virus, or cosmid, to which another DNA segment, i.e., an "insert", may be attached so as to bring about the replication or expression of the attached segment in a cell.

[0101] The term "naturally-occurring" or "unmodified" or "wild type" as used herein as applied to a nucleic acid, a polypeptide, a cell, or an organism, refers to a nucleic acid, polypeptide, cell, or organism that is found in nature.

[0102] As used herein, a "mutation" refers to an insertion, deletion, substitution, duplication, or inversion of one or more amino acids or nucleotides as compared to a wild-type or reference amino acid sequence or to a wild-type or reference nucleotide sequence.

[0103] As used herein the term "isolated" is meant to describe a polynucleotide, a polypeptide, or a cell that is in an environment different from that in which the polynucleotide, the polypeptide, or the cell naturally occurs. An isolated genetically modified host cell may be present in a mixed population of genetically modified host cells.

[0104] A "host cell," as used herein, denotes a eukaryotic cell, a prokaryotic cell, or a cell from a multicellular organism (e.g., in a cell line), cultured as a unicellular entity, which eukaryotic or prokaryotic cells are used as recipients for a nucleic acid (e.g., an expression vector), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. A "recombinant host cell" (also referred to as a "genetically modified host cell") is a host

[0105] The term “tropism” as used herein refers to preferential entry of the virus like particle (VLP or XDP) into certain cell or tissue type(s) and/or preferential interaction with the cell surface that facilitates entry into certain cell or tissue types, optionally and preferably followed by expression (e.g., transcription and, optionally, translation) of sequences carried by the VLP or XDP into the cell.

[0106] The terms “pseudotype” or “pseudotyping” as used herein, refers to viral envelope proteins that have been substituted with those of another virus possessing preferable characteristics. For example, HIV can be pseudotyped with vesicular stomatitis virus G-protein (VSV-G) envelope proteins (amongst others, described herein, below), which allows HIV to infect a wider range of cells because HIV envelope proteins target the virus mainly to CD4+ presenting cells.

[0107] The term “tropism factor” as used herein refers to components integrated into the surface of an XDP or VLP that provides tropism for a certain cell or tissue type. Non-limiting

examples of tropism factors include glycoproteins, antibody fragments (e.g., scFv, nanobodies, linear antibodies, etc.), receptors and ligands to target cell markers.

[0108] A "target cell marker" refers to a molecule expressed by a target cell including but not limited to cell-surface receptors, cytokine receptors, antigens, tumor-associated antigens, glycoproteins, oligonucleotides, enzymatic substrates, antigenic determinants, or binding sites that may be present in the on the surface of a target tissue or cell that may serve as ligands for a tropism factor.

[0109] The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of amino acids having amide-containing side chains consists of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

[0110] As used herein, "treatment" or "treating," are used interchangeably herein and refer to an approach for obtaining beneficial or desired results, including but not limited to a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder or disease being treated. A therapeutic benefit can also be achieved with the eradication or amelioration of one or more of the symptoms or an improvement in one or more clinical parameters associated with the underlying disease such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder.

[0111] The terms "therapeutically effective amount" and "therapeutically effective dose", as used herein, refer to an amount of a drug or a biologic, alone or as a part of a composition, that is capable of having any detectable, beneficial effect on any symptom, aspect, measured parameter or characteristics of a disease state or condition when administered in one or repeated doses to a subject such as a human or an experimental animal. Such effect need not be absolute to be beneficial.

[0112] As used herein, "administering" is meant a method of giving a dosage of a compound (e.g., a composition of the disclosure) or a composition (e.g., a pharmaceutical composition) to a subject.

[0113] A "subject" is a mammal. Mammals include, but are not limited to, domesticated animals, non-human primates, humans, rabbits, mice, rats and other rodents.

[0114] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

I. General Methods

[0115] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference.

[0116] Where a range of values is provided, it is understood that endpoints are included and 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 limit of that range and any other stated or intervening value in that stated range, is encompassed. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed, 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.

[0117] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0118] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

[0119] It will be appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. In other cases, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. It is intended that all combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

II. Repressor and Epigenetic Long-Term X-Repressor (ELXR) Systems

[0120] In a first aspect, the present disclosure provides gene repressor systems comprising a catalytically-dead CRISPR protein linked to one or more repressor domains, and one or more guide ribonucleic acids (gRNA) comprising a targeting sequence complementary to a target nucleic acid sequence of a gene targeted for repression, silencing, or downregulation, wherein the system is capable of binding to a target nucleic acid of the gene and repressing transcription of the gene.

[0121] In the context of the present disclosure and with respect to a gene, “repression”, "repressing", “inhibition of gene expression”, “downregulation”, and “silencing” are used interchangeably herein to refer to the inhibition or blocking of transcription of a gene or a portion thereof. A gene product capable of being repressed by the systems of the disclosure include mRNA, rRNA, tRNA, structural RNA or protein encoded by the mRNA. Accordingly, repression of a gene can result in a decrease in production of a gene product. Examples of gene repression processes which decrease transcription include, but are not limited to, those which inhibit formation of a transcription initiation complex, those which decrease transcription initiation rate, those which decrease transcription elongation rate, those which decrease processivity of transcription and those which antagonize transcriptional activation (by, for example, blocking the binding of a transcriptional activator). Gene repression can constitute, for example, prevention of activation as well as inhibition of expression below an existing level.

Transcriptional repression includes both reversible and irreversible inactivation of gene transcription. In some embodiments, repression by the systems of the disclosure comprises any detectable decrease in the production of a gene product in cells, preferably a decrease in production of a gene product by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%, or any integer there between, when compared to untreated cells or cells treated with a comparable system comprising a non-targeting spacer. Most preferably, gene repression results in complete inhibition of gene expression, such that no gene product is detectable. In some embodiments, the repression of transcription by the systems of the embodiments is sustained for at least about 8 hours, at least about 1 day, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 3 months, or at least about 6 months when assessed in an *in vitro* assay, including cell-based assays. In some embodiments, the repression of transcription by the gene repressor systems of the embodiments is sustained for at least about 1 day, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 3 months, or at least about 6 months when assessed in a subject that has been administered a therapeutically-effective dose of a system of the embodiments described herein. In some embodiments, gene repression by the system results in no or minimal detectable off-target methylation or off-target activity, when assessed in an *in vitro* assay. In other embodiments, gene repression by the system results in no or minimal detectable off-target methylation or off-target activity, when assessed in a subject that has been administered a therapeutically-effective dose of a system of the embodiments described herein.

[0122] In some embodiments, the present disclosure provides systems of catalytically-dead CRISPR proteins linked to one or more repressor domains as a fusion protein and one or more guide ribonucleic acids (gRNA) for use in repressing a target nucleic acid, inclusive of coding and non-coding regions.

[0123] In some embodiments, the present disclosure provides systems of catalytically-dead CasX (dCasX) proteins linked to one or more repressor domains as a fusion protein (dXR) and one or more guide ribonucleic acids (gRNA) for use in repressing a target nucleic acid, inclusive of coding and non-coding regions; collectively, a dXR:gRNA system. A gRNA variant and targeting sequence, and a dCasX variant protein and linked repressor domain(s) of any of the embodiments, can form a complex and bind via non-covalent interactions, referred to herein as a ribonucleoprotein (RNP) complex. In some embodiments, the use of a pre-complexed dXR:gRNA RNP confers advantages in the delivery of the system components to a cell or target

nucleic acid for repression of the target nucleic acid. In the RNP, the gRNA can provide target specificity to the RNP complex by including a targeting sequence (also referred to as a "spacer") having a nucleotide sequence that is complementary to a sequence of a target nucleic acid. In the RNP, the dCasX protein and linked repressor domain(s) of the pre-complexed dXR:gRNA provides the site-specific activity and is guided to a target site (and further stabilized at a target site) within a target nucleic acid sequence to be modified by virtue of its association with the gRNA. The dCasX protein and linked repressor domain(s) of the RNP complex provides the site-specific activities of the complex such as binding of the target sequence by the dCasX protein and the linked repressor domains provide the repression activity either directly or by the recruitment of other cellular factors.

[0124] Provided herein are compositions comprising or encoding the dCasX variant protein and linked repressor domains (dXR), gRNA variants, and dXR:gRNA gene repression pairs of any combination of dXR and gRNA, nucleic acids encoding the dXR and gRNA, as well as delivery modalities comprising the dXR:gRNA or encoding nucleic acids. Also provided herein are methods of making dCasX protein and linked repressor domain(s) and gRNA, as well as methods of using the CasX and gRNA, including methods of gene repression and methods of treatment. The dCasX protein and linked repressor domain(s) and gRNA components of the dXR:gRNA systems and their features, as well as the delivery modalities and the methods of using the compositions for the repression, down-regulation or silencing of a gene are described more fully, below.

III. Repressor Domain Fusion Proteins of the dXR:gRNA Systems

[0125] In one aspect, the disclosure relates to fusion proteins comprising one or more repressor domains operably linked to a catalytically dead CRISPR protein, e.g., a catalytically-dead Class 2 CRISPR protein. In some embodiments, the catalytically-dead Class 2 CRISPR protein is a catalytically-dead Class 2, Type V CRISPR protein. In some embodiments, the catalytically-dead CRISPR proteins include Class 2, Type II CRISPR/Cas nucleases such as Cas9. In other cases, the catalytically-dead CRISPR proteins include Class 2, Type V CRISPR/Cas nucleases such as a Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f, Cas12g, Cas12h, Cas12i, Cas12j, Cas12k, Cas12l, Cas14, and/or CasΦ. In some embodiments, the catalytically-dead Class 2, Type V CRISPR protein is a catalytically-dead CasX protein (dCasX) selected from the group of sequences of SEQ ID NOS:

17-36 and 59353-59358 as set forth in Table 4, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, linked to one or more repressor domains, resulting in a dXR fusion protein. In some embodiments, the catalytically-dead Class 2, Type V CRISPR protein is a catalytically-dead CasX protein (dCasX) selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4 linked to one or more repressor domains, resulting in a dXR fusion protein.

[0126] In some embodiments, the disclosure provides fusion proteins comprising a first repressor domain as a fusion protein wherein the first repressor domain is a Krüppel-associated box (KRAB) domain which can be fused to a catalytically dead CRISPR protein by linker peptides disclosed herein. In some embodiments, the disclosure provides dXR fusion proteins comprising a first repressor domain as a fusion protein wherein the first repressor domain is a Krüppel-associated box (KRAB) domain which can be fused to the dCasX by linker peptides disclosed herein, resulting in a dXR fusion protein.

[0127] Amongst repressor domains that have the ability to repress, or silence genes, the Krüppel-associated box (KRAB) repressor domain is amongst the most powerful in human genome systems (Alerasool, N., et al. An efficient KRAB domain for CRISPRi applications. Nat. Methods 17:1093 (2020)). KRAB domains are present in approximately 400 human zinc finger protein-based transcription factors that upon binding of the dXR to the target nucleic acid, is capable of recruiting additional repressor domains such as, but not limited to, Trim28 (also known as Kap1 or Tif1-beta) that, in turn, assembles a protein complex with chromatin regulators such as CBX5/HP1α and SETDB1 that induce repression of transcription of the gene. SETDB1 is a histone methyltransferase that deposit H3K9me3 marks on histones, which is a mark of heterochromatin (complexes which acetylate histones and deposit active H3K9ac marks are displaced). In some cases, DNA methyltransferases (the DNMT domains DNMT3A and DNMT3L) are subsequently recruited to deposit methylation marks on the DNA so that silencing of the gene will persist after the system complex is no longer bound to the target nucleic acid. The methylation of CpG dinucleotides (CpG) in mammalian cells is catalyzed by the DNA methyltransferases DNMT3a and 3b, which establish DNA methylation patterns, and DNMTL, which maintains the methylation pattern after DNA replication (Zhang, Y., et al. Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD

domain with the histone H3 tail. Nucleic Acids Research 38:4246 (2010)). Thus, SETDB1 and DNMT3's recruited by the KRAB domain act as co-repressors of the dXR fusion protein (Tatsumi, D., et al. DNMTs and SETDB1 function as co-repressors in MAX-mediated repression of germ cell–related genes in mouse embryonic stem cells. PLoS ONE 13(11): e0205969 (2018)).

[0128] Other repressor domains suitable for inclusion in the dXR of the disclosure include DNA methyltransferase 3 alpha (DNMT3A or subdomains thereof), DNMT3A-like protein (DNMT3L or subdomains thereof), DNA methyltransferase 3 beta (DNMT3B), DNA methyltransferase 1 (DNMT1), Friend of GATA-1 (FOG), Mad mSIN3 interaction domain (SID), enhanced SID (SID4X), nuclear receptor corepressor (NcoR), nuclear effector protein (NuE), KOX1 repression domain, the ERF repressor domain (ERD), the SRDX repression domain, histone lysine methyltransferases such as PR/SET domain containing protein (Pr-SET)7/8, lysine methyltransferase 5B (SUV4- 20H1), PR/SET domain 2 (RIZ1), histone lysine demethylases such as lysine demethylase 4A (JMJD2A/JHDM3A), lysine demethylase 4B (JMJD2B), lysine demethylase 4C (JMJD2C/GASC1), lysine demethylase 4D (JMJD2D), lysine demethylase 5A (JARID1A/RBP2), lysine demethylase 5B (JARID1B/PLU-1), lysine demethylase 5C (JARID 1C/SMCX), lysine demethylase 5D (JARID1D/SMCY), sirtuin 1 (SIRT1), SIRT2, DNA methylases such as HhaI DNA m5c-methyltransferase (M.HhaI), methyltransferase 1 (MET1), histone H3 lysine 9 methyltransferase G9a (G9a), S-adenosyl-L-methionine-dependent methyltransferases superfamily protein (DRM3), DNA cytosine methyltransferase MET2a (ZMET2), methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), GLP, chromomethylase 1 (CMT1), chromomethylase 2 (CMT2), heterochromatin protein 1 (HP1A), mixed lineage leukemia protein-5 (MLL5), histone-lysine N-methyltransferase SETDB1 (SETB1), Suppressor Of Variegation 3-9 Homolog 1 (SUV39H1), SUV39H2, euchromatic histone lysine methyltransferase 1 (EHMT1), histone-lysine N-methyltransferase EZH1 (EZH1), EZH2, nuclear receptor binding SET domain protein 1 (NSD1), NSD2, NSD3, ASH1 like histone lysine methyltransferase (ASH1L), tripartite motif containing 28 (TRIM28), Methyltransferase Like 3 (METTL3), METTL4, family with sequence similarity 208 member A (FAM208A), M-Phase Phosphoprotein 8 (MPHOSPH8), SET domain containing 2 (SETD2), histone deacetylase 1

(HDAC1), HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, Periphilin 1 (PPHLN1), and subdomains thereof.

[0129] Human genes encoding KRAB zinc-finger proteins include KOX1/ZNF10, KOX8/ZNF708, ZNF43, ZNF184, ZNF91, HPF4, HTF10, HTF34, and the sequences of SEQ ID NOS: 355-888. In some embodiments, the KRAB transcriptional repressor domain of the dXR:gRNA systems is selected from the group consisting of (in all cases, ZNF=zinc finger protein; KRBOX= KRAB box domain containing; ZKSCAN= zinc finger with KRAB and SCAN domains; SSX= SSX family member; KRBA= KRAB-A domain containing; ZFP=zinc finger protein) ZNF343, ZNF10, ZNF337, ZNF334, ZNF215, ZNF519, ZNF485, ZNF214, ZNF33B, ZNF287, ZNF705A, ZNF37A, KRBOX4, ZKSCAN3, ZKSCAN4, ZNF57, ZNF557, ZNF705B, ZNF662, ZNF77, ZNF500, ZNF558, ZNF620, ZNF713, ZNF823, ZNF440, ZNF441, ZNF136, small nuclear ribonucleoprotein polypeptides B and B1 (SNRPB), ZNF735, ZKSCAN2, ZNF619, ZNF627, ZNF333, ATP binding cassette subfamily A member 11 (ABCA11P), PLD5 pseudogene 1 (PLD5P1), ZNF25, ZNF727, ZNF595, ZNF14, ZNF33A, ZNF101, ZNF253, ZNF56, ZNF720, ZNF85, ZNF66, ZNF722P, ZNF486, ZNF682, ZNF626, ZNF100, ZNF93, ZKSCAN1, ZNF257, ZNF729, ZNF208, ZNF90, ZNF430, ZNF676, ZNF91, ZNF429, ZNF675, ZNF681, ZNF99, ZNF431, ZNF98, ZNF708, ZNF732, SSX family member 2 (SSX2), ZNF721, ZNF726, ZNF730, ZNF506, ZNF728, ZNF141, ZNF723, ZNF302, ZNF484, SSX2B, ZNF718, ZNF74, ZNF157, ZNF790, ZNF565, ZNF705G, vomeronasal 1 receptor 107 pseudogene (VN1R107P), solute carrier family 27 member 5 (SLC27A5), ZNF737, SSX4, ZNF850, ZNF717, ZNF155, ZNF283, ZNF404, ZNF114, ZNF716, ZNF230, ZNF45, ZNF222, ZNF286A, ZNF624, ZNF223, ZNF284, ZNF790-AS1, ZNF382, ZNF749, ZNF615, ZFP90, ZNF225, ZNF234, ZNF568, ZNF614, ZNF584, ZNF432, ZNF461, ZNF182, ZNF630, ZNF630-AS1, ZNF132, ZNF420, ZNF324B, ZNF616, ZNF471, ZNF227, ZNF324, ZNF860, ZFP28 zinc finger protein (ZFP28), ZNF470, ZNF586, ZNF235, ZNF274, ZNF446, ZFP1, ZIM3, ZNF212, ZNF766, ZNF264, ZNF480, ZNF667, ZNF805, ZNF610, ZNF783, ZNF621, ZNF8-DT, ZNF880, ZNF213-AS1, ZNF213, ZNF263, zinc finger and SCAN domain containing 32 (ZSCAN32), ZIM2, ZNF597, ZNF786, KRAB-A domain containing 1 (KRBA1), ZNF460, ZNF8, ZNF875, ZNF543, ZNF133, ZNF229, ZNF528, SSX1, ZNF81, ZNF578, ZNF862, ZNF777, ZNF425, ZNF548, ZNF746, ZNF282, ZNF398, ZNF599, ZNF251, ZNF195, ZNF181, RBAK-RBAKDN readthrough (RBAK-RBAKDN), ZFP37, RNA, 7SL, cytoplasmic 526, pseudogene (RN7SL526P), ZNF879, ZNF26, ZSCAN21, ZNF3, ZNF354C, ZNF10, ZNF75D,

ZNF426, ZNF561, ZNF562, ZNF846, ZNF782, ZNF552, ZNF587B, ZNF814, ZNF587, ZNF92, ZNF417, ZNF256, ZNF473, ZFP14, ZFP82, ZNF529, ZNF605, ZFP57, ZNF724, ZNF43, ZNF354A, ZNF547, SSX4B, ZNF585A, ZNF585B, ZNF792, ZNF789, ZNF394, ZNF655, ZFP92, ZNF41, ZNF674, ZNF546, ZNF780B, ZNF699, ZNF177, ZNF560, ZNF583, ZNF707, ZNF808, ZKSCAN5, ZNF137P, ZNF611, ZNF600, ZNF28, ZNF773, ZNF549, ZNF550, ZNF416, ZIK1, ZNF211, ZNF527, ZNF569, ZNF793, ZNF571-AS1, ZNF540, ZNF571, ZNF607, ZNF75A, ZNF205, ZNF175, ZNF268, ZNF354B, ZNF135, ZNF221, ZNF285, ZNF419, ZNF30, ZNF304, ZNF254, ZNF701, ZNF418, ZNF71, ZNF570, ZNF705E, KRBOX1, ZNF510, ZNF778, PR/SET domain 9 (PRDM9), ZNF248, ZNF845, ZNF525, ZNF765, ZNF813, ZNF747, ZNF764, ZNF785, ZNF689, ZNF311, ZNF169, ZNF483, ZNF493, ZNF189, ZNF658, ZNF564, ZNF490, ZNF791, ZNF678, ZNF454, ZNF34, ZNF7, ZNF250, ZNF705D, ZNF641, ZNF2, ZNF554, ZNF555, ZNF556, ZNF596, ZNF517, ZNF331, ZNF18, ZNF829, ZNF772, ZNF17, ZNF112, ZNF514, ZNF688, PRDM7, ZNF695, ZNF670-ZNF695, ZNF138, ZNF670, ZNF19, ZNF316, ZNF12, ZNF202, RBAK, ZNF83, ZNF468, ZNF479, ZNF679, ZNF736, ZNF680, ZNF273, ZNF107, ZNF267, ZKSCAN8, ZNF84, ZNF573, ZNF23, ZNF559, ZNF44, ZNF563, ZNF442, ZNF799, ZNF443, ZNF709, ZNF566, ZNF69, ZNF700, ZNF763, ZNF433-AS1, ZNF433, ZNF878, ZNF844, ZNF788P, ZNF20, ZNF625-ZNF20, ZNF625, ZNF606, ZNF530, ZNF577, ZNF649, ZNF613, ZNF350, ZNF317, ZNF300, ZNF180, ZNF415, vomeronasal 1 receptor 1 (VN1R1), ZNF266, ZNF738, ZNF445, ZNF852, ZKSCAN7, ZNF660, myosin phosphatase Rho interacting protein pseudogene 1 (MPRIPP1), ZNF197, ZNF567, ZNF582, ZNF439, ZFP30, ZNF559-ZNF177, ZNF226, ZNF841, ZNF544, ZNF233, ZNF534, ZNF836, ZNF320, KRBA2, ZNF761, ZNF383, ZNF224, ZNF551, ZNF154, ZNF671, ZNF776, ZNF780A, ZNF888, ZNF816-ZNF321P, ZNF321P, ZNF816, ZNF347, ZNF665, ZNF677, ZNF160, ZNF184, ZNF140, ZNF589, ZNF891, ZFP69B, ZNF436, pogo transposable element derived with KRAB domain (POGK), ZNF669, ZFP69, ZNF684, ZNF124, and ZNF496, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0130] In some embodiments, the gene repressor system comprises a single KRAB domain operably linked to the catalytically-dead CRISPR protein as a fusion protein, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-

33239, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the system comprises a single KRAB domain operably linked to the catalytically-dead CRISPR protein, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239. In some embodiments, the fusion protein of the systems comprises a single KRAB domain operably linked to the catalytically-dead CRISPR protein, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the fusion protein of the systems comprises a single KRAB domain operably linked to the catalytically-dead CRISPR protein, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the fusion protein of the systems comprises a single KRAB domain operably linked to the catalytically-dead CRISPR, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In a particular embodiment, the fusion protein of the systems comprises a single KRAB domain operably linked to a catalytically dead Cas9 protein, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0131] In some embodiments, the fusion proteins of the systems comprise a single KRAB domain operably linked by a peptide linker to the catalytically-dead CRISPR protein, wherein

the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342. In other embodiments, the fusion proteins of the systems comprise a single KRAB domain operably linked to the catalytically-dead CRISPR protein wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342. In other embodiments, the fusion proteins of

the systems comprise a single KRAB domain operably linked to the catalytically-dead CRISPR protein wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57840. In still other embodiments, the fusion proteins of the systems comprise a single KRAB domain operably linked to the catalytically-dead CRISPR protein wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755.

[0132] In some embodiments, the dXR:gRNA system comprises a single KRAB domain operably linked to a catalytically-dead Class 2, Type V CRISPR protein as a fusion protein, wherein the catalytically-dead Class 2, Type V CRISPR protein is a dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the system comprises a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is

selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239. In some embodiments, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In a particular embodiment, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX of SEQ ID NO: 18 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX of SEQ ID NO: 25, as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at

least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX of SEQ ID NO: 59357, as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR fusion protein of the systems comprises a single KRAB domain operably linked to the dCasX of SEQ ID NO: 59358, as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0133] In some embodiments, the dXR fusion proteins of the systems comprise a single KRAB domain operably linked by a peptide linker to a dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f)

$LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342. In other embodiments, the dXR fusion proteins of the systems comprise a single KRAB domain operably linked to the dCasX wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342. In other embodiments, the dXR fusion proteins of the systems comprise a single KRAB domain operably linked to the dCasX wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57840. In still other embodiments, the dXR fusion proteins of the systems comprise a single KRAB domain operably linked to the dCasX wherein the KRAB domain comprises a first domain comprising the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or

N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S, and a second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755. In a particular embodiment, the dXR fusion protein comprises a sequence selected from the group consisting of SEQ ID NOS: 59508-59567 and 59673-60012. In the foregoing embodiments of the paragraph, the dXR fusion proteins is capable of repressing expression of a reporter gene to a greater extent than a comparable fusion protein comprising a ZNF10 KRAB domain (SEQ ID NO: 59626) when assayed in an in vitro cellular assay, together with a gRNA targeting the reporter gene. In some embodiments, the reporter gene is a B2M locus of a eukaryotic cell such as, but not limited to, an HEK293 cell. In some embodiments, expression of reporter gene is repressed in the in vitro assay by at least about 75%, at least about 80%, at least about 85%, or at least about 90% at day 7 of the assay. Exemplary methods of measuring repression of a reporter gene are provided in the examples, for example, in Example 4.

[0134] In some embodiments, the dXR fusion protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA and, upon binding to the target nucleic acid of the cell in a cellular assay, the dXR:gRNA system is capable of repressing transcription of a gene encoded by the target nucleic acid by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%. In some embodiments, the dXR fusion protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA and, upon binding to the target nucleic acid of the cell in a cellular assay, the system is capable of repressing transcription of a gene encoded by the target nucleic acid, wherein the repression of transcription of the gene is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 2 months.

[0135] In some embodiments, the present disclosure provides systems comprising a first and a second repressor domain linked to a catalytically-dead CRISPR protein as a fusion protein, and one or more gRNA comprising a targeting sequence complementary to a target nucleic acid sequence of a gene targeted for silencing, wherein the system is capable of binding the target nucleic acid in a manner that leads to long-term epigenetic modification of the gene so that

repression persists even after the system is no longer present on the target nucleic acid. In some embodiments, the first and the second repressor domains are operably linked as a fusion protein, such as to a dCasX of the embodiments described herein. As used herein "epigenetic modification" means a modification to either DNA or histones associated with DNA, wherein the modification is either a direct modification by a component of the system or is indirect by the recruitment of one or more additional cellular components, but in which the DNA target nucleic acid sequence itself is not edited. For example, DNMT3A (or its catalytic domain) directly modifies the DNA by methylating it, whereas KRAB recruits KAP-1/TIF1β corepressor complexes that act as potent transcriptional repressors and can further recruit factors associated with DNA methylation and formation of repressive chromatin, such as heterochromatin protein 1 (HP1), histone deacetylases and histone methyltransferases (Ying, Y., et al. The Krüppel-associated box repressor domain induces reversible and irreversible regulation of endogenous mouse genes by mediating different chromatin states. Nucleic Acids Res. 43(3): 1549 (2015)). Together, the first and second repressor components of the systems work in synchrony to result in an additive or synergistic effect on transcriptional silencing of the targeted gene. In some embodiments, the present disclosure provides systems comprising a first and a second repressor domain operably linked to a dCasX, the first repressor is a KRAB domain of any of the foregoing embodiments, and the second repressor is selected from the group consisting of DNMT3A, DNMT3L, DNMT3B, DNMT1, FOG, SID4X, SID, NcoR, NuE, histone H3 lysine 9 methyltransferase G9a (G9a), methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), GLP, heterochromatin protein 1 (HP1A), mixed lineage leukemia protein-5 (MLL5), histone-lysine N-methyltransferase SETDB1 (SETB1), Suppressor Of Variegation 3-9 Homolog 1 (SUV39H1), SUV39H2, euchromatic histone lysine methyltransferase 1 (EHMT1), histone-lysine N-methyltransferase EZH1 (EZH1), EZH2, nuclear receptor binding SET domain protein 1 (NSD1), NSD2, NSD3, ASH1 like histone lysine methyltransferase (ASH1L), tripartite motif containing 28 (TRIM28), Methyltransferase Like 3 (METTL3), METTL4, family with sequence similarity 208 member A (FAM208A), M-Phase Phosphoprotein 8 (MPHOSPH8), SET domain containing 2 (SETD2), histone deacetylase 1 (HDAC1), HDAC2, HDAC3, Periphilin 1 (PPHLN1), and subdomains thereof (e.g., the DNMT3A catalytic domain and the ATRX-DNMT3-DNMT3L (ADD) domain

are subdomains of DNMT3A, and the DNMT3L interaction domain is a subdomain of DNMT3L).

[0136] In some embodiments, the present disclosure provides dXR:gRNA systems comprising a first and a second repressor domain operably linked to a dCasX. In some embodiments, the disclosure provides a dXR fusion protein comprising a dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first repressor is a KRAB domain selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the second repressor is a DNMT3A domain that lacks a regulatory subdomain and only maintains a catalytic domain selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, wherein the transcriptional repressor domains are linked by linker peptide sequences to the catalytically-dead CasX protein or to the other repressor domain. In some embodiments, the dXR comprising a DNMT3A catalytic domain effects methylation exclusively at CpG sequences. In a particular embodiment, the present disclosure provides systems comprising a first and a second repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first repressor is a KRAB domain selected from the group of sequences consisting of SEQ ID NOS: 57746-59342, or is selected from the group consisting of SEQ ID NOS: 57746-57840, or is selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence

having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the transcriptional repressor domains are linked by linker peptide sequences to the catalytically-dead CasX protein or to the other repressor domain. In a particular embodiment, the present disclosure provides systems comprising a first and a second repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, wherein the first repressor is a KRAB domain selected from the group of sequences consisting of SEQ ID NOS: 57746-59342, or is selected from the group consisting of SEQ ID NOS: 57746-57840, and the second repressor domain is a DNMT3A catalytic domain selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, wherein the transcriptional repressor domains are linked by linker peptide sequences to the catalytically-dead CasX protein or to the other repressor domain. In the foregoing embodiments, wherein the fusion protein comprises KRAB and the second transcriptional repressor domain comprises a DNMT3A catalytic domain, upon binding of the RNP of the fusion protein and the gRNA to the target nucleic acid, the system is capable of recruiting one or more of the additional repressor domains of the cell, including the repressor domains listed herein, in order to affect repression of transcription of a gene encoded by the target nucleic acid, such that upon binding of an RNP of the fusion protein and the gRNA to the target nucleic acid, transcription of the gene in the cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%, or anypercentage there between, when assayed in an in vitro assay, including cell-based assays. Most preferably, the epigenetic modification results in complete silencing of gene expression, such that no gene product is detectable. In some embodiments, the repression of transcription by the systems of the embodiments is sustained for at least about 1 day, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 3 months, or at least about 6 months when assessed in an *in vitro* assay. In some embodiments, the repression of transcription by the systems of the embodiments is sustained for at least about 1 day, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 3 months, at least about 6 months, or at least about 1 year when assessed in a subject that has been administered a therapeutically-effective dose of a system of the embodiments described herein. In some embodiments, use of the system

results in no or minimal detectable off-target methylation or off-target activity, when assessed in an *in vitro* assay. In some embodiments, use of the system results in off-target methylation or off-target activity that is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells. In other embodiments, use of the system results in no or minimal detectable off-target methylation or off-target activity, when assessed in a subject that has been administered a therapeutically-effective dose of a system of the embodiments described herein.

[0137] In other embodiments, the disclosure provides gene repressor systems wherein the fusion protein comprises a first, a second, and a third transcriptional repressor domain, wherein the third transcriptional repressor domain is different from the first and the second transcriptional repressor domains. In some embodiments, the present disclosure provides dXR:gRNA systems wherein the dXR comprises a KRAB domain of any of the embodiments described herein as the first repressor domain, a DNMT3A catalytic domain as the second repressor domain and a DNMT3L domain as the third repressor domain. It has been discovered that such dXR fusion proteins, when used in the dXR:gRNA systems, result in epigenetic long-term repression of transcription of target nucleic acid (and such fusion proteins are alternatively referred to herein as "ELXR"). In the foregoing, the DNMT3L helps maintains the methylation pattern after DNA replication. In an exemplary embodiment of the foregoing, the catalytically-dead Class 2 protein is a class 2 Type V CRISPR protein, for example a dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is a DNMT3A catalytic domain of DNMT3A, or a sequence variant thereof, including the sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least

about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor domain is a DNMT3L interaction domain is the sequence of SEQ ID NO: 59625, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In a particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first domain comprises a KRAB domain comprising one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or

V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In a particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first domain comprises a KRAB domain comprising one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346),

wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In a particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO:

18, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93%

at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In another particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 25, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K,

X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In another particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 59357, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and

X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In another particular embodiment, the present disclosure provides systems comprising a first, a second, and a third repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 59358, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least

about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the third repressor is a DNMT3L interaction domain

comprising the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In some embodiments of the system, the fusion protein components of the system are configured according to a configuration as schematically portrayed in FIG. 7. In some embodiments each of the repressor domains and the dCasX are operably linked, in some cases via a linker, as described herein. In some embodiments, the dXR fusion protein has a configuration of, N-terminal to C-terminal (with reference to the components of Table 45) of configuration 1 (NLS-Linker4-DNMT3A-Linker2-DNMT3L-Linker1-Linker3-dCasX-Linker3-KRAB-NLS), configuration 2 (NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-DNMT3A-Linker2-DNMT3L), configuration 3 (NLS-Linker3-dCasX-Linker1-DNMT3A-Linker2-DNMT3L-Linker3-KRAB-NLS), configuration 4 (NLS-KRAB-Linker3-DNMT3A-Linker2-DNMT3L-Linker1-dCasX-Linker3-NLS), or configuration 5 (NLS-DNMT3A-Linker2-DNMT3L-Linker3-KRAB-Linker1-dCasX-Linker3-NLS). In some embodiments, the dXR fusion protein comprises a sequence selected from the group consisting of SEQ ID NOS: 59508-59517, 59528-59537, 59548-59557, and 59673-59842, or a sequence having or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein is in configuration 1, 4 or 5.

[0138] In some embodiments, the dXR fusion protein comprises an ADD domain as a fourth domain, wherein the C-terminus of the ADD domain is operably to the N-terminus of the DNMT3A catalytic domain, representative configurations of which are schematically portrayed in FIG. 45. In some embodiments, the dXR comprises a dCasX and a first, second, third, and fourth repressor, and the dXR comprises a sequence selected from the group consisting of SEQ ID NOS: 59508-59567 and 59673-60012, or a sequence having or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion

protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In some embodiments of the system comprising a dCasX variant, a first, second, third repressor domain, including the constructs of configurations 1-5, upon binding of an RNP of the fusion protein and the gRNA to the target nucleic acid, the gene is epigenetically modified and transcription of the gene is repressed. In some embodiment, transcription of the gene is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%, when assayed in an in vitro assay, including cell-based assays. In some embodiments, the repression of transcription of the gene by the system compositions, including the constructs of configurations 1-5, is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 2 months, when assayed in an in vitro assay, including cell-based assays. In a particular embodiment, dXR configurations 4 and 5, when used in the dXR:gRNA system, result in less off-target methylation or off-target activity in an *in vitro* assay compared to configuration 1 (as shown in FIGS. 7 and 45). In some embodiments, use of the dXR configurations 4 and 5 (as shown in FIGS. 7 and 45), when used in the dXR:gRNA system, results in off-target methylation or off-target activity that is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells.

[0139] In still other embodiments, the present disclosure provides dXR:gRNA systems wherein the dXR comprises a dCasX and a first, second, third, and fourth repressor domain. In some embodiments, the dXR comprises a dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at

least about 99% identity thereto, the second repressor domain is DNMT3A catalytic domain selected from the group of sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor domain is a DNMT3L interaction domain having a sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth domain is a DNMT3A ADD domain of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In a particular embodiment, the dXR comprises a dCasX comprises a sequence of SEQ ID NO: 18 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is DNMT3A catalytic domain selected from the group of sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor domain is a DNMT3L interaction domain having a sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth domain is a DNMT3A ADD domain of SEQ ID NO: 59452, or a sequence variant

having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR comprises a dCasX comprises a sequence of SEQ ID NO: 25 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is DNMT3A catalytic domain selected from the group of sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor domain is a DNMT3L interaction domain having a sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth domain is a DNMT3A ADD domain of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR comprises a dCasX comprises a sequence of SEQ ID NO: 59357 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least

about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is DNMT3A catalytic domain selected from the group of sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor domain is a DNMT3L interaction domain having a sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth domain is a DNMT3A ADD domain of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In another particular embodiment, the dXR comprises a dCasX comprises a sequence of SEQ ID NO: 59358 as set forth in Table 4 or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the first repressor domain is a KRAB repressor domain selected from the group of sequences of SEQ ID NOS: 889-2100 and 2332-33239, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is DNMT3A catalytic domain selected from the group of sequences of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor domain is a DNMT3L interaction domain having a sequence of SEQ ID NO: 59625, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at

least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth domain is a DNMT3A ADD domain of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. The ADD domain is known to have two key functions: 1) it allosterically regulates the catalytic activity of DNMT3A by serving as a methyltransferase auto-inhibitory domain, and 2) it recognizes unmethylated H3K4 (H3K4me0). Without wishing to be bound by theory, it is thought that the interaction of the ADD domain with the H3K4me0 mark unveils the catalytic site of DNMT3A, thereby recruiting an active DNMT3A to chromatin to implement de novo methylation at these sites. In a surprising finding, it has been discovered that the addition of the DNMT3A ADD domain to the dXR constructs comprising the DNMT3A catalytic and DNMT3L interaction domains greatly enhances the repression of the target nucleic acid in comparison to dXR constructs lacking the ADD domain. Exemplary data for the improved repression are presented in the Examples.

[0140] In a particular embodiment, the present disclosure provides systems comprising a first, a second, a third, and a fourth repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is

A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the second repressor domain is a DNMT3A catalytic domain sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NO: 59625, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth repressor is an ADD domain comprising the sequence of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. The present disclosure provides systems comprising a first, a second, a third, and a fourth repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence

having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first repressor domain comprises a KRAB domain comprising one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at

least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor is a DNMT3L interaction domain comprising the amino acid sequence of SEQ ID NO: 59625, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth repressor is an ADD domain comprising the sequence of SEQ ID NO: 59452, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. The present disclosure provides systems comprising a first, a second, a third, and a fourth repressor domain operably linked to a dCasX comprising the sequence of SEQ ID NO: 18, or a sequence having at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, wherein the first repressor domain comprises a KRAB domain comprising one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V,

X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5$ $X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the second repressor domain is a DNMT3A catalytic domain comprising a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, the third repressor is a DNMT3L interaction domain comprising the sequence of SEQ ID NOS: 59625, or a sequence variant having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and the fourth repressor is an ADD domain comprising the sequence of SEQ ID NO: 59452, or sequence variants having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein comprises one or more linker peptides described herein, and wherein the fusion protein is capable of forming an RNP with a gRNA of the system that binds to the target nucleic acid. In some embodiments, the dXR fusion protein comprise an ADD domain and a DNMT3A catalytic domain, wherein the C terminus of the ADD domain is operably to the N terminus of the DNMT3A catalytic domain. In some embodiments each of the repressor domains and the dCasX are operably linked, in some cases via a linker, as described herein. In some embodiments, the dXR fusion protein has a configuration of, N-terminal to C-terminal of

configuration 1 (NLS-ADD-DNMT3A-Linker2-DNMT3A-Linker1-Linker3-dCasX-Linker3-KRAB-NLS), configuration 2 (NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-ADD-DNMT3A-Linker2-DNMT3L), configuration 3 (NLS-Linker3-dCasX-Linker1-ADD-DNMT3A-Linker2-DNMT3L-Linker3-KRAB-NLS), configuration 4 (NLS-KRAB-Linker3-ADD-DNMT3A-Linker2-DNMT3L-Linker1-dCasX-Linker3-NLS), or configuration 5 (NLS-ADD-DNMT3A-Linker2-DNMT3L-Linker3-KRAB-Linker1-dCasX-Linker3-NLS). In some embodiments of the system, the fusion protein components of the system are configured as schematically portrayed in FIG. 45. In some embodiments, the dXR fusion protein comprises a sequence selected from the group consisting of SEQ ID NOS: 59518-59526, 59538-59547, 59558-59567 and 59843-60012, or a sequence having or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto, and wherein the fusion protein is in configuration 1, 4 or 5.

[0141] In some embodiments of the system comprising a dCasX variant, a first, second, third, and fourth repressor domain, upon binding of an RNP of the fusion protein and the gRNA to the target nucleic acid, a gene encoded by the target nucleic acid is epigenetically-modified and transcription of the gene is repressed. In some embodiment, transcription of the gene is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%, when assayed in an in vitro assay, including cell-based assays. In some embodiments, the repression of transcription of the gene by the system compositions is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least 2 weeks, at least about 3 weeks, at least about 1 month, or at least about 2 months, when assayed in an in vitro assay, including cell-based assays. In a particular embodiment, dXR configurations 4 and 5, when used in the dXR:gRNA system, result in less off-target methylation or off-target activity in an *in vitro* assay compared to configuration 1. In some embodiments, use of the dXR configurations 4 and 5, when used in the dXR:gRNA system, results in off-target methylation or off-target activity that is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells.

[0142] In some embodiments, the transcriptional repressor domains are linked to each other, or to the catalytically-dead CRISPR protein or catalytically-dead Class 2, Type V CRISPR protein (e.g., dCasX) within the fusion protein by linker peptide sequences. In some cases, the one or more transcriptional repressor domains are linked at or near the C-terminus of the catalytically-dead Class 2, Type V CRISPR protein (e.g., dCasX) by linker peptide sequences. In other cases, the one or more transcriptional repressor domains are linked at or near the N-terminus of the catalytically-dead Class 2, Type V CRISPR protein (e.g., dCasX) by linker peptide sequences. In still other cases, a first transcriptional repressor domain is linked at or near the C-terminus of the catalytically-dead Class 2, Type V CRISPR protein (e.g., dCasX) by linker peptide sequences and a second, third, and, optionally, a fourth transcriptional repressor domain is linked at or near the N-terminus of the catalytically-dead Class 2, Type V CRISPR protein. Representative, but non-limiting configurations are schematically portrayed in FIG. 7, FIG. 38, and FIG. 45. In the foregoing, the linker peptide is selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GSGSGGG (SEQ ID NO: 57628), GGCGGTTCCGGCGGAGGAAGC (SEQ ID NO: 57624), GGCGGTTCCGGCGGAGGTTCC (SEQ ID NO: 57625), GGATCAGGCTCTGGAGGTGGA (SEQ ID NO: 57627), GGAGGGCCGAGCTCTGGCGCACCCCCACCAAGTGGAGGGTCTCCTGCCGGGTCCCCAACATCTACTGAAGAAGGCACCAGCGAATCCGCAACGCCCGAGTCAGGCCCTGGTACCTCCACAGAACCATCTGAAGGTAGTGCGCCTGGTTCCCCAGCTGGAAGCCCTACTTCCACCGAAGAAGGCACGTCAACCGAACCAAGTGAAGGATCTGCCCCTGGGACCAGCACTGAACCATCTGAG (SEQ ID NO: 57620), SSGNSNANSRGPSFSSGLVPLSLRGSH (SEQ ID NO: 57623), GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSE (SEQ ID NO: 57621), TCTAGCGGCAATAGTAACGCTAACAGCCGCGGGCCGAGCTTCAGCAGCGGCCTGGTGCCGTTAAGCTTGCGCGGCAGCCAT (SEQ ID NO: 57622), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ

ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO:33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

IV. Guide ribonucleic acids (gRNA) of the Systems

[0143] In another aspect, the disclosure provides guide ribonucleic acids (gRNAs) utilized in the gene repressor systems of the disclosure that have utility, with the other components of the gene repressor systems, in the repression of transcription of genes targeted by the design of the gRNA. The present disclosure provides specifically-designed gRNAs with targeting sequences (or "spacers") that are complementary to (and are therefore able to hybridize with) the target nucleic acid as a component of the gene repression systems, wherein the gRNA is capable of forming a ribonucleoprotein (RNP) complex with the catalytically-dead CRISPR protein (e.g., dCasX) of a fusion protein. In the case of a dCasX variant with linked repressor domains employed in the systems of the disclosure, the dCasX variant has specificity to a protospacer adjacent motif (PAM) sequence comprising a TC motif in the complementary non-target strand, and wherein the PAM sequence is located 1 nucleotide 5' of the sequence in the non-target strand that is complementary to the target nucleic acid sequence in the target strand of the target nucleic acid. The use of a pre-complexed RNP confers advantages in the delivery of the system components to a cell or target nucleic acid sequence for repression of transcription of the target nucleic acid sequence. The dCasX variant protein component of the RNP provides the site-specific activity that is guided to a target site (e.g., stabilized at a target site) within a target nucleic acid sequence by virtue of its association with the guide RNA comprising a targeting sequence complementary to the desired specific location of the target nucleic acid and proximal to the PAM sequence.

[0144] It is envisioned that in some embodiments, multiple gRNAs (e.g., multiple gRNAs) are delivered by the system for the repression at different regions of a gene, increasing the efficiency and/or duration of repression, as described more fully, below.

a. Reference gRNA and gRNA variants

[0145] In designing gRNA for incorporation into the gene repressor systems of the disclosure, comprehensive approaches termed Deep Mutational Evolution (DME), deep mutational scanning (DMS), error prone PCR, cassette mutagenesis, random mutagenesis, staggered extension PCR,

gene shuffling, or domain swapping, were utilized to, in a systematic way, introduce mutations and variations in the nucleic acid sequence of, first, naturally-occurring gRNA ("reference gRNA"), resulting in gRNA variants with improved properties, then re-applying the approaches to gRNA variants to further evolve and improve the resulting gRNA variants. gRNA variants also include variants comprising one or more chemical modifications. The activity of reference gRNAs may be used as a benchmark against which the activity of gRNA variants are compared, thereby measuring improvements in function or other characteristics of the gRNA variants. In other embodiments, a reference gRNA or gRNA variant may be subjected to one or more deliberate, targeted mutations in order to produce a gRNA variant, for example a rationally-designed variant.

[0146] The gRNAs of the disclosure comprise two segments; a targeting sequence and a protein-binding segment. The targeting segment of a gRNA includes a nucleotide sequence (referred to interchangeably as a guide sequence, a spacer, a targeter, or a targeting sequence) that is complementary to (and therefore hybridizes with) a specific sequence (a target site) within the target nucleic acid sequence (e.g., a target ssRNA, a target ssDNA, a strand of a double stranded target nucleic acid, etc.), described more fully below. The targeting sequence of a gRNA is capable of binding to a target nucleic acid sequence, including a coding sequence, a complement of a coding sequence, a non-coding sequence, and to regulatory elements. The protein-binding segment (or "activator" or "protein-binding sequence") interacts with (e.g., binds to) a dCasX protein as a complex, forming an RNP (described more fully, below). The protein-binding segment is alternatively referred to herein as a "scaffold", which is comprised of several regions, described more fully, below.

[0147] In the case of a dual guide RNA (dgRNA), the targeter and the activator portions each have a duplex-forming segment, where the duplex forming segment of the targeter and the duplex-forming segment of the activator have complementarity with one another and hybridize to one another to form a double stranded duplex (dsRNA duplex for a gRNA). The term "targeter" or "targeter RNA" is used herein to refer to a crRNA-like molecule (crRNA: "CRISPR RNA") of a CasX dual guide RNA (and therefore of a CasX single guide RNA when the "activator" and the "targeter" are linked together; e.g., by intervening nucleotides). The crRNA has a 5' region that anneals with the tracrRNA followed by the nucleotides of the targeting sequence. Thus, for example, a guide RNA (dgRNA or sgRNA) comprises a guide sequence and a duplex-forming segment of a crRNA, which can also be referred to as a crRNA

repeat. A corresponding tracrRNA-like molecule (activator) also comprises a duplex-forming stretch of nucleotides that forms the other half of the dsRNA duplex of the protein-binding segment of the guide RNA. Thus, a targeter and an activator, as a corresponding pair, hybridize to form a dual guide RNA, referred to herein as a "dual-molecule gRNA" or a "dgRNA". Site-specific binding of a target nucleic acid sequence (e.g., genomic DNA) by the dCasX protein and linked repressor domain(s) can occur at one or more locations (e.g., a sequence of a target nucleic acid) determined by base-pairing complementarity between the targeting sequence of the gRNA and the target nucleic acid sequence. Thus, for example, the gRNA of the disclosure have sequences complementarity to and therefore can hybridize with the target nucleic acid that is adjacent to a sequence complementary to a TC PAM motif or a PAM sequence, such as ATC, CTC, GTC, or TTC. Because the targeting sequence of a guide sequence hybridizes with a sequence of a target nucleic acid sequence, a targeting sequence can be modified by a user to hybridize with a specific target nucleic acid sequence, so long as the location of the PAM sequence is considered. In other embodiments, the activator and targeter of the gRNA are covalently linked to one another (rather than hybridizing to one another) and comprise a single molecule, referred to herein as a "single-molecule gRNA," "one-molecule guide RNA," "single guide RNA", "single guide RNA", a "single-molecule guide RNA," a "sgRNA", or a "one-molecule guide RNA".

[0148] Collectively, the assembled gRNAs of the disclosure comprise four distinct regions, or domains: the RNA triplex, the scaffold stem, the extended stem, and the targeting sequence that, in the embodiments of the disclosure is specific for a target nucleic acid and is located on the 3' end of the gRNA. The RNA triplex, the scaffold stem, and the extended stem, together, are referred to as the "scaffold" of the gRNA. The foregoing components of the gRNA are described in WO2020247882A1 and WO2022120095, incorporated by reference herein.

b. Targeting Sequence

[0149] In some embodiments of the gRNAs of the disclosure, the extended stem loop is followed by a region that forms part of the triplex, and then the targeting sequence (or "spacer") at the 3' end of the gRNA, with the scaffold being that region of the guide 5' relative to the targeting sequence. The targeting sequence targets the CasX ribonucleoprotein holo complex to a specific region of the target nucleic acid sequence of the gene to be repressed, 3' relative to the binding of the RNP. Thus, for example, gRNA targeting sequences of the disclosure have sequences complementarity to, and therefore can hybridize to, a portion of the gene in a nucleic

acid in a eukaryotic cell (e.g., a eukaryotic chromosome, chromosomal sequence, a eukaryotic RNA, etc.) as a component of the RNP when the TC PAM motif or any one of the PAM sequences TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to the non-target strand sequence complementary to the target sequence. The targeting sequence of a gRNA can be modified so that the gRNA can target a desired sequence of any desired target nucleic acid sequence, so long as the PAM sequence location is taken into consideration. In some embodiments, the PAM motif sequence recognized by the nuclease of the RNP is TC. In other embodiments, the PAM sequence recognized by the nuclease of the RNP is NTC. In other embodiments, the PAM sequence recognized by the nuclease of the RNP is TTC. In other embodiments, the PAM sequence recognized by the nuclease of the RNP is ATC. In other embodiments, the PAM sequence recognized by the nuclease of the RNP is CTC. In other embodiments, the PAM sequence recognized by the nuclease of the RNP is GTC.

[0150] The gene repressor systems of the present disclosure can be designed to target any region of, or proximal to, a gene or region of a gene for which repression of transcription is sought. When the entirety of the gene is to be repressed, designing a guide with a targeting sequence complementary to a sequence encompassing or proximal to the transcription start site (TSS) is contemplated by the disclosure. The TSS selection occurs at different positions within the promoter region, depending on promoter sequence and initiating-substrate concentration. The core promoter serves as a binding platform for the transcription machinery, which comprises Pol II and its associated general transcription factors (GTFs) (Haberle, V. et al. Eukaryotic core promoters and the functional basis of transcription initiation (Nat Rev Mol Cell Biol. 19(10):621 (2018)). Variability in TSS selection has been proposed to involve DNA 'scrunching' and 'anti-scrunching,' the hallmarks of which are: (i) forward and reverse movement of the RNA polymerase leading edge, but not trailing edge, relative to DNA, and (ii) expansion and contraction of the transcription bubble. In some embodiments, the target nucleic acid sequence bound by an RNP of the dXR:gRNA system is within 1 kb of a transcription start site (TSS) in the gene. In some embodiments, the target nucleic acid sequence bound by an RNP of the system is within 20 bp, 50 bp, 100 bp, 150 bp, 200 bp, 250 bp, 500 bps, or 1 kb upstream of a TSS of the gene. In some embodiments, the target nucleic acid sequence bound by an RNP of the system is within 20 bp, 50 bp, 100 bp, 150 bp, 200 bp, 250 bp, 500 bps or 1 kb downstream of a TSS of the gene. In some embodiments, the target nucleic acid sequence bound by an RNP of the system is within 500 bps upstream to 500 bps downstream, or 300 bps upstream to 300 bps downstream

of a TSS of the gene. In some embodiments, the target nucleic acid sequence bound by an RNP of the system is within 20 bp, 50 bp, 100 bp, 150 bp, 200 bp, 250 bp, 500 bps, or 1 kb of an enhancer of the gene. In some embodiments, the target nucleic acid sequence bound by an RNP of the system of the disclosure is within 1 kb 3' to a 5' untranslated region of the gene. In other embodiments, the target nucleic acid sequence bound by an RNP of the system is within the open reading frame of the gene, inclusive of introns (if any). In some embodiments, the targeting sequence of a gRNA of the system of the disclosure is designed to be specific for an exon of the gene of the target nucleic acid. In a particular embodiment, the targeting sequence of a gRNA of the system of the disclosure is designed to be specific for exon 1 of the gene of the target nucleic acid. In other embodiments, the targeting sequence of a gRNA of the system of the disclosure is designed to be specific for an intron of the gene of the target nucleic acid. In other embodiments, the targeting sequence of the gRNA of the system of the disclosure is designed to be specific for an intron-exon junction of the gene of the target nucleic acid. In other embodiments, the targeting sequence of the gRNA of the system of the disclosure is designed to be specific for a regulatory element of the gene of the target nucleic acid. In other embodiments, the targeting sequence of the gRNA of the system of the disclosure is designed to be complementary to a sequence of an intergenic region of the gene of the target nucleic acid. In other embodiments, the targeting sequence of a gRNA of the system of the disclosure is specific for a junction of the exon, an intron, and/or a regulatory element of the gene. In those cases where the targeting sequence is specific for a regulatory element, such regulatory elements include, but are not limited to promoter regions, enhancer regions, intergenic regions, 5' untranslated regions (5' UTR), 3' untranslated regions (3' UTR), conserved elements, and regions comprising cis-regulatory elements. The promoter region is intended to encompass nucleotides within 5 kb of the initiation point of the encoding sequence or, in the case of gene enhancer elements or conserved elements, can be thousands of bp, hundreds of thousands of bp, or even millions of bp away from the encoding sequence of the gene of the target nucleic acid. In the foregoing, the targets are those in which the encoding gene of the target is intended to be repressed such that the gene product is not expressed or is expressed at a lower level in a cell. In some embodiments, upon binding of the RNP of the system of the disclosure to the binding location of the target nucleic acid, the system is capable of repressing transcription of the gene 5’ to the binding location of the RNP. In other embodiments, upon binding of the RNP of the system to the binding location of the target nucleic acid, the system is capable of repressing transcription of

the gene 3' to the binding location of the RNP. In some embodiments, upon binding of the RNP of the system to the binding location of the target nucleic acid, the system is capable of repressing transcription of the gene by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% greater compared to an untreated gene, when assessed in an in vitro assay. In some embodiments, upon binding of the RNP of the system to the binding location of the target nucleic acid, the system is capable of repressing transcription of the gene for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, or at least about 6 months, or at least about 1 year.

[0151] In some embodiments, the targeting sequence of a gRNA of the system has between 14 and 20 consecutive nucleotides. In some embodiments, the targeting sequence has 14, 15, 16, 17, 18, 19, or 20 consecutive nucleotides. In some embodiments, the targeting sequence of the gRNA of the system consists of 20 consecutive nucleotides. In some embodiments, the targeting sequence consists of 19 consecutive nucleotides. In some embodiments, the targeting sequence consists of 18 consecutive nucleotides. In some embodiments, the targeting sequence consists of 17 consecutive nucleotides. In some embodiments, the targeting sequence consists of 16 consecutive nucleotides. In some embodiments, the targeting sequence consists of 15 consecutive nucleotides. In some embodiments, the targeting sequence has 14, 15, 16, 17, 18, 19, or 20 consecutive nucleotides and the targeting sequence can comprise 0 to 5, 0 to 4, 0 to 3, or 0 to 2 mismatches relative to the target nucleic acid sequence and retain sufficient binding specificity such that the RNP comprising the gRNA comprising the targeting sequence can form a complementary bond with respect to the target nucleic acid.

[0152] In some embodiments, dXR:gRNA a repressor system of the disclosure comprises a first gRNA and further comprises a second (and optionally a third, fourth, fifth, or more) gRNA, wherein the second gRNA or additional gRNA has a targeting sequence complementary to a different or overlapping portion of the target nucleic acid sequence compared to the targeting sequence of the first gRNA such that multiple points in the target nucleic acid are targeted, increasing the ability of the system to effectively repress transcription. It will be understood that in such cases, the second or additional gRNA is complexed with an additional copy of the dXR. By selection of the targeting sequences of the gRNA, defined regions of the target nucleic acid sequence can be repressed using the systems described herein.

c. gRNA scaffolds

[0153] With the exception of the targeting sequence region, the remaining regions of the gRNA are referred to herein as the scaffold. In some embodiments, the gRNA scaffolds are variants of reference gRNA wherein mutations, insertions, deletions or domain substitutions are introduced to confer desirable properties on the gRNA.

[0154] In some embodiments, a reference gRNA comprises a sequence isolated or derived from *Deltaproteobacteria*. In some embodiments, the sequence is a CasX tracrRNA sequence. Exemplary CasX reference tracrRNA sequences isolated or derived from *Deltaproteobacteria* may include:
ACAUCUGGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGUCGU AUGGACGAAGCGCUUAUUUAUCGGAGA (SEQ ID NO: 6) and
ACAUCUGGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGUCGU AUGGACGAAGCGCUUAUUUAUCGG (SEQ ID NO: 7). Exemplary crRNA sequences isolated or derived from *Deltaproteobacteria* may comprise a sequence of CCGAUAAGUAAAACGCAUCAAAG (SEQ ID NO: 33271).

[0155] In some embodiments, a reference guide RNA comprises a sequence isolated or derived from *Planctomycetes*. In some embodiments, the sequence is a CasX tracrRNA sequence. Exemplary reference tracrRNA sequences isolated or derived from *Planctomycetes* may include:
UACUGGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUA UGGGUAAAGCGCUUAUUUAUCGGAGA (SEQ ID NO: 8) and

[0156] UACUGGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGG (SEQ ID NO: 9). Exemplary crRNA sequences isolated or derived from *Planctomycetes* may comprise a sequence of UCUCCGAUAAAUAAGAAGCAUCAAAG (SEQ ID NO: 33272).

[0157] In some embodiments, a reference gRNA comprises a sequence isolated or derived from *Candidatus Sungbacteria*. In some embodiments, the sequence is a CasX tracrRNA sequence. Exemplary CasX reference tracrRNA sequences isolated or derived from *Candidatus Sungbacteria* may comprise sequences of: GUUUACACACUCCCUCUCAUAGGGU (SEQ ID NO: 10), GUUUACACACUCCCUCUCAUGAGGU (SEQ ID NO: 11), UUUUACAUACCCCCUCUCAUGGGAU (SEQ ID NO: 12) and GUUUACACACUCCCUCUCAUGGGGG (SEQ ID NO: 13). Table 1 provides the sequences

of reference gRNA tracr, cr and scaffold sequences that, in some embodiments, are modified to create the gRNA of the systems. In some embodiments, the disclosure provides gRNA variant sequences wherein the gRNA has a scaffold comprising a sequence having one or more nucleotide modifications relative to a reference gRNA sequence having a sequence of any one of SEQ ID NOS: 4-16 of Table 1. It will be understood that in those embodiments wherein a vector comprises a DNA encoding sequence for a gRNA, that thymine (T) bases can be substituted for the uracil (U) bases of any of the gRNA sequence embodiments described herein.

Table 1: Reference gRNA tracr, cr and scaffold sequences

SEQ ID NO.	Nucleotide Sequence
4	ACAUCUGGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGUCGUAUGGACGAAGC GCUUAUUUAUCGGAGAGAAACCGAUAAGUAAAACGCAUCAAAG
5	UACUGGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGGGUAAAGCG CUUAUUUAUCGGAGAGAAAUCCGAUAAAUAAGAAGCAUCAAAG
6	ACAUCUGGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGUCGUAUGGACGAAGC GCUUAUUUAUCGGAGA
7	ACAUCUGGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGUCGUAUGGACGAAGC GCUUAUUUAUCGG
8	UACUGGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGGGUAAAGCG CUUAUUUAUCGGAGA
9	UACUGGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGGGUAAAGCG CUUAUUUAUCGG
10	GUUUACACACUCCCUCUCAUAGGGU
11	GUUUACACACUCCCUCUCAUGAGGU
12	UUUUACAUACCCCCUCUCAUGGGAU
13	GUUUACACACUCCCUCUCAUGGGGG
14	CCAGCGACUAUGUCGUAUGG
15	GCGCUUAUUUAUCGGAGAGAAAUCCGAUAAAUAAGAAGC
16	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGGGUAAAGCGCUUA UUUAUCGGA

d. gRNA Variants

[0158] In another aspect, the disclosure relates to guide ribonucleic acid variants (referred to herein as "gRNA variant"), which comprise one or more modifications relative to a reference gRNA scaffold. As used herein, "scaffold" refers to all parts to the gRNA necessary for gRNA function with the exception of the spacer sequence.

[0159] In some embodiments, a gRNA variant comprises one or more nucleotide substitutions, insertions, deletions, or swapped or replaced regions relative to a reference gRNA sequence of

the disclosure. In some embodiments, a mutation can occur in any region of a reference gRNA scaffold to produce a gRNA variant. In some embodiments, the scaffold of the gRNA variant sequence has at least 50%, at least 60%, or at least 70%, at least 80%, at least 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

[0160] In some embodiments, a gRNA variant comprises one or more nucleotide changes within one or more regions of the reference gRNA scaffold that improve a characteristic of the reference gRNA. Exemplary regions include the RNA triplex, the pseudoknot, the scaffold stem loop, and the extended stem loop. In some cases, the variant scaffold stem further comprises a bubble. In other cases, the variant scaffold further comprises a triplex loop region. In still other cases, the variant scaffold further comprises a 5' unstructured region. In some embodiments, the gRNA variant scaffold comprises a scaffold stem loop having at least 60% sequence identity, at least 70% sequence identity, at least 80% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity to SEQ ID NO: 14. In some embodiments, the gRNA variant scaffold comprises a scaffold stem loop having at least 60% sequence identity to SEQ ID NO: 14. In other embodiments, the gRNA variant comprises a scaffold stem loop having the sequence of CCAGCGACUAUGUCGUAGUGG (SEQ ID NO: 33273). In other embodiments, the disclosure provides a gRNA scaffold comprising, relative to SEQ ID NO: 5, a C18G substitution, a G55 insertion, a U1 deletion, and a modified extended stem loop in which the original 6 nt loop and 13 most-loop-proximal base pairs (32 nucleotides total) are replaced by a Uvsx hairpin (4 nt loop and 5 loop-proximal base pairs; 14 nucleotides total) and the loop-distal base of the extended stem was converted to a fully base-paired stem contiguous with the new Uvsx hairpin by deletion of the A99 and substitution of G65U. In the foregoing embodiment, the gRNA scaffold comprises the sequence ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAG UGGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG (SEQ ID NO: 33274).

[0161] All gRNA variants that have one or more improved characteristics, or add one or more new functions when the variant gRNA is compared to a reference gRNA described herein, are envisaged as within the scope of the disclosure. A representative example of such a gRNA variant appropriate for the gene repressor systems is gRNA variant 174 (SEQ ID NO: 2238). Another representative example of such a gRNA variant appropriate for the gene repressor systems is gRNA variant 235 (SEQ ID NO: 2292). In some embodiments, the gRNA variant

adds a new function to the RNP comprising the gRNA variant. In some embodiments, the gRNA variant has an improved characteristic selected from: improved stability; improved solubility; improved transcription of the gRNA; improved resistance to nuclease activity; increased folding rate of the gRNA; decreased side product formation during folding; increased productive folding; improved binding affinity to a dXR fusion protein and linked repressor domain(s); improved binding affinity to a target nucleic acid when complexed with a dXR fusion protein and linked repressor domain(s); and improved ability to utilize a greater spectrum of one or more PAM sequences, including ATC, CTC, GTC, or TTC, in the binding of target nucleic acid when complexed with a dXR fusion protein, and any combination thereof. In some cases, the one or more of the improved characteristics of the gRNA variant is at least about 1.1 to about 100,000-fold improved relative to the reference gRNA of SEQ ID NO: 4 or SEQ ID NO: 5. In other cases, the one or more improved characteristics of the gRNA variant is at least about 1.1, at least about 10, at least about 100, at least about 1000, at least about 10,000, at least about 100,000-fold or more improved relative to the reference gRNA of SEQ ID NO: 4 or SEQ ID NO: 5. In other cases, the one or more of the improved characteristics of the gRNA variant is about 1.1 to 100,00-fold, about 1.1 to 10,00-fold, about 1.1 to 1,000-fold, about 1.1 to 500-fold, about 1.1 to 100-fold, about 1.1 to 50-fold, about 1.1 to 20-fold, about 10 to 100,00-fold, about 10 to 10,00-fold, about 10 to 1,000-fold, about 10 to 500-fold, about 10 to 100-fold, about 10 to 50-fold, about 10 to 20-fold, about 2 to 70-fold, about 2 to 50-fold, about 2 to 30-fold, about 2 to 20-fold, about 2 to 10-fold, about 5 to 50-fold, about 5 to 30-fold, about 5 to 10-fold, about 100 to 100,00-fold, about 100 to 10,00-fold, about 100 to 1,000-fold, about 100 to 500-fold, about 500 to 100,00-fold, about 500 to 10,00-fold, about 500 to 1,000-fold, about 500 to 750-fold, about 1,000 to 100,00-fold, about 10,000 to 100,00-fold, about 20 to 500-fold, about 20 to 250-fold, about 20 to 200-fold, about 20 to 100-fold, about 20 to 50-fold, about 50 to 10,000-fold, about 50 to 1,000-fold, about 50 to 500-fold, about 50 to 200-fold, or about 50 to 100-fold, improved relative to the reference gRNA of SEQ ID NO: 4 or SEQ ID NO: 5. In other cases, the one or more improved characteristics of the gRNA variant is about 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, 200-fold, 210-fold, 220-fold, 230-fold, 240-fold, 250-fold, 260-fold, 270-fold, 280-fold, 290-

fold, 300-fold, 310-fold, 320-fold, 330-fold, 340-fold, 350-fold, 360-fold, 370-fold, 380-fold, 390-fold, 400-fold, 425-fold, 450-fold, 475-fold, or 500-fold improved relative to the reference gRNA of SEQ ID NO: 4 or SEQ ID NO: 5.

[0162] In some embodiments, a gRNA variant can be created by subjecting a reference gRNA to a one or more mutagenesis methods, such as the mutagenesis methods described herein, below, which may include Deep Mutational Evolution (DME), deep mutational scanning (DMS), error prone PCR, cassette mutagenesis, random mutagenesis, staggered extension PCR, gene shuffling, or domain swapping, in order to generate the gRNA variants of the disclosure. The activity of reference gRNAs may be used as a benchmark against which the activity of gRNA variants are compared, thereby measuring improvements in function of gRNA variants. In other embodiments, a reference gRNA may be subjected to one or more deliberate, targeted mutations, substitutions, or domain swaps in order to produce a gRNA variant, for example a rationally designed variant. Exemplary gRNA variants produced by such methods are presented in Table 2.

[0163] In some embodiments, the gRNA variant comprises one or more modifications compared to a reference guide ribonucleic acid scaffold sequence, wherein the one or more modification is selected from: at least one nucleotide substitution in a region of the reference gRNA; at least one nucleotide deletion in a region of the reference gRNA; at least one nucleotide insertion in a region of the reference gRNA; a substitution of all or a portion of a region of the reference gRNA; a deletion of all or a portion of a region of the reference gRNA; or any combination of the foregoing. In some cases, the modification is a substitution of 1 to 15 consecutive or non-consecutive nucleotides in the reference gRNA in one or more regions. In other cases, the modification is a deletion of 1 to 10 consecutive or non-consecutive nucleotides in the reference gRNA in one or more regions. In other cases, the modification is an insertion of 1 to 10 consecutive or non-consecutive nucleotides in the reference gRNA in one or more regions. In other cases, the modification is a substitution of the scaffold stem loop or the extended stem loop with an RNA stem loop sequence from a heterologous RNA source with proximal 5' and 3' ends. In some cases, a gRNA variant of the disclosure comprises two or more modifications in one region relative to a reference gRNA. In other cases, a gRNA variant of the disclosure comprises modifications in two or more regions. In other cases, a gRNA variant comprises any combination of the foregoing modifications described in this paragraph.

[0164] In some embodiments, a 5' G is added to a gRNA variant sequence, relative to a reference gRNA, for expression *in vivo*, as transcription from a U6 promoter is more efficient and more consistent with regard to the start site when the +1 nucleotide is a G. In other embodiments, two 5' Gs are added to generate a gRNA variant sequence for *in vitro* transcription to increase production efficiency, as T7 polymerase strongly prefers a G in the +1 position and a purine in the +2 position. In some cases, the 5' G bases are added to the reference scaffolds of Table 1. In other cases, the 5' G bases are added to the variant scaffolds of Table 2.

[0165] Table 2 provides exemplary gRNA variant scaffold sequences. In some embodiments, the gRNA variant scaffold comprises any one of the sequences listed in Table 2, or a sequence having at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto. It will be understood that in those embodiments wherein a vector comprises a DNA encoding sequence for a gRNA, that thymine (T) bases can be substituted for the uracil (U) bases of any of the gRNA sequence embodiments described herein.

Table 2: Exemplary gRNA Variant Scaffold Sequences

SEQ ID NO.	Guide No.	Sequence
2238	174	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2239	175	ACUGGCGCCUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2240	176	GCUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2241	177	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2242	181	ACUGGCGCCUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2243	182	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2244	183	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2245	184	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2246	185	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUUG GGUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
2247	186	ACUGGCGCCUUUAUCAUCAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUG GGUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2248	187	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCGCCCUCUUCGGAGGGAAGCAUCAAAG
2249	188	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUCACCCAUGUGAGCAUCAAAG
2250	189	ACUGGCACUUUUACCUGAUUACUUUGAGAGCCAACACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2251	190	ACUGGCACUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2252	191	ACUGGCCCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2253	192	ACUGGCGCUUUUACCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2254	193	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAACACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2255	195	ACUGGCACCUUUACCUGAUUACUUUGAGAGCCAACACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2256	196	ACUGGCACCUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2257	197	ACUGGCCCCUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2258	198	ACUGGCGCCUUUAUCUGAUUACUUUGAGAGCCAACACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2259	199	GCUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2260	200	GACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2261	201	ACUGGCGCCUUUAUCUGAUUACUUUGGAGAGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2262	202	ACUGGCGCAUUUAUCUGAUUACUUUGUGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2263	203	ACUGGCGCCUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2264	204	ACUGGCGCUUUUAUCUGAUUACUUUGGAGAGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2265	205	ACUGGCGCAUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2266	206	ACUGGCGCUUUUAUCUGAUUACUUUGUGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG
2267	207	ACUGGCGCUUUUAUUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
2268	208	ACGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUGG GUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2269	209	ACUGGCGCUUUUAUAUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2270	210	ACUGGCGCUUUUAUCUUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2271	211	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAGCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2272	212	ACUGGCGCUGUUAUCUGAUUACUUCGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCGAAG
2273	213	ACUGGCGCUCUUAUCUGAUUACUUCGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCGAAG
2274	214	ACUGGCGCUUGUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAGAG
2275	215	ACUGGCGCUUCUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAGAG
2276	216	ACUGGCGCUUUGAUCUGAUUACCUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAGG
2277	217	ACUGGCGCUUUCAUCUGAUUACCUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAGG
2278	218	ACUGGCGCUGUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2279	219	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCGAAG
2280	220	ACUGGCGCUUUUAUCUGAUUACUUCGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAAAG
2281	221	ACUGGCACUUCUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2282	222	ACUGGCACUUCUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAGAG
2283	223	ACUGGCACCUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAAAG
2284	224	ACUGGCACUUGUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2285	225	ACUGGCACUUGUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUCUUCGGAGGGAGCAUCAGAG
2286	229	ACUGGCACUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAAAG
2287	230	ACUGGCACUUCUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCUUACGGACUUCGGUCCGUAAGAAGCAUCAGAG
2288	231	ACUGGCGCUUCUAUCUGAUUACUCUGAGAGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG

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2289	232	ACUGGCACUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2290	233	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2291	234	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAUGG GUAAAGCGCCUUACGGACUUCGGUCCGUAAGGAGCAUCAGAG
2292	235	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2293	236	ACGGGACUUUCUAUCUGAUUACUCUGAAGUCCCUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2294	237	ACCUGUAGUUCUAUCUGAUUACUCUGACUACAGUCACCAGCGACUAUGUCGUAUGG GUAAAGCCGCUUACGGACUUCGGUCCGUAAGAGGCAUCAGAG
2295	238	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGGGCGCAGCUUCGGCUGACGGUACACCGUGCAGCAUCAAA G
2296	239	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGCAGCU UCGGCUGACGGUACACCGUGCAGCAUCAAAG
2297	240	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGCAGCU UCGGCUGACGGUACACCGGUGGGCGCAGCUUCGGCUGACGGUACACCGUGCAGCAU CAAAG
2298	241	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGCAGCU UCGGCUGACGGUACACCGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGC AGCUUCGGCUGACGGUACACCGUGCAGCAUCAAAG
2299	242	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGCAGCU UCGGCUGACGGUACACCGGUGGGCGCAGCUUCGGCUGACGGUACACCGGUGGGCGC AGCUUCGGCUGACGGUACACCGGUGGGCGCAGCUUCGGCUGACGGUACACCGUGCA GCAUCAAAG
2300	243	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACCUAGCGGAGGCUAGGUGCAGCAUCAAAG
2301	244	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACCUCGGCUUGCUGAAGCGCGCACGGCAAGAGGCGAGGUGCAGCA UCAAAG
2302	245	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACCUCUCUCGACGCAGGACUCGGCUUGCUGAAGCGCGCACGGCAA GAGGCGAGGGGCGGCGACUGGUGAGUACGCCAAAAAUUUUGACUAGCGGAGGCUAG AAGGAGAGAGGUGCAGCAUCAAAG
2303	246	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGUGCCCGUCUGUUGUGUCGAGAGACGCCAAAAAUUUUGACUA GCGGAGGCUAGAAGGAGAGAGAUGGGUGCCGUGCAGCAUCAAAG
2304	247	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACAUGGAGAGGAGAUGUGCAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
2305	248	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACAUGGAGAUGUGCAGCAUCAAAG
2306	249	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUUGGGCGCAGCGUCAAUGACGCUGACGGUACAAGCAUCAAAG
2307	250	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCACAUGA GGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
2308	251	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCUCAUGAGGAUCACCCAUGAGCUGACGGUACA GGCCACAUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
2309	252	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCACAUGG CAGUCGUAACGACGCGGGUGGUAUAGUGCAGCAUCAAAG
2310	253	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCAAACAUGGCAGUCCUAAGGACGCGGGUUUUG CUGACGGUACAGGCCACAUGGCAGUCGUAACGACGCGGGUGGUAUAGUGCAGCAUC AAAG
2311	254	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGACAUGGCAGUCGUAACGACGCGGGUCUGACGG UACAGGCCACAUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
2312	255	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAAGGAGUUUAUAUGGAAACCCUUAGUGCAGCAUCAAAG
2313	256	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCAGGAAGCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCC AGACAAUUAUUGUCUGGUAUAGUGCAGCAGCAGAACAAUUUGCUGAGGGCUAUUGA GGCGCAACAGCAUCUGUUGCAACUCACAGUCUGGGGCAUCAAGCAGCUCCAGGCAA GAAUCCUGAGCAUCAAAG
2314	257	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGCCCUGAAGAAGGGCGUGCAGCAUCAAAG
2315	258	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGCUCGUGUAGCUCAUUAGCUCCGAGCCGUGCAGCAUCAAAG
2316	259	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACCCGUGUGCAUCCGCAGUGUCGGAUCCACGGGUGCAGCAUCAAA G
2317	260	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACGGAAUCCAUUGCACUCCGGAUUUCACUAGGUGCAGCAUCAAAG
2318	261	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACAUGCAUGUCUAAGACAGCAUGUGCAGCAUCAAAG
2319	262	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACAAAACAUAAGGAAAACCUAUGUUGUGCAGCAUCAAAG
2320	263	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCC AGACAAUUAUUGUCUGGUAUAGUCCGUAAGAGGCAUCAGAG
2321	264	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGGUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCAGA CAAUUAUUGUCUGGUACCCGUAAGAGGCAUCAGAG

SEQ ID NO.	Guide No.	Sequence
2322	265	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCAC AUGAGGAUCACCCAUGUGGUAUACCGUAAGAGGCAUCAGAG
2323	266	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCACAUGAG GAUCACCCAUGUGGUAUAGGGAGCAUCAAAG
2324	267	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGUAUGGGCGCAGCUCAUGAGGAUCACCCAUGAGCUGACGG UACAGGCCACAUGAGGAUCACCCAUGUGGUAUACCGUAAGAGGCAUCAGAG
2325	268	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUAUGGGCGCAGCUCAUGAGGAUCACCCAUGAGCUGACGGUACAG GCCACAUGAGGAUCACCCAUGUGGUAUAGGGAGCAUCAAAG
2326	269	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCAC AUGGCAGUCGUAACGACGCGGGUGGUAUACCGUAAGAGGCAUCAGAG
2327	270	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCACAUGGC AGUCGUAACGACGCGGGUGGUAUAGGGAGCAUCAAAG
2328	271	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGUAUGGGCGCAGCAAACAUGGCAGUCCUAAGGACGCGGGU UUUGCUGACGGUACAGGCCACAUGGCAGUCGUAACGACGCGGGUGGUAUACCGUAA GAGGCAUCAGAG
2329	272	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUAUGGGCGCAGCAAACAUGGCAGUCCUAAGGACGCGGGUUUUGC UGACGGUACAGGCCACAUGGCAGUCGUAACGACGCGGGUGGUAUAGGGAGCAUCAA AG
2330	273	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCCGCUUACGGUAUGGGCGCAGACAUGGCAGUCGUAACGACGCGGGUCUG ACGGUACAGGCCACAUGAGGAUCACCCAUGUGGUAUACCGUAAGAGGCAUCAGAG
2331	274	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUAUGGGCGCAGACAUGGCAGUCGUAACGACGCGGGUCUGACGGU ACAGGCCACAUGAGGAUCACCCAUGUGGUAUAGGGAGCAUCAAAG
57544	275	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACCUGAGGAUCACCCAGGUGCUGACGGUACA GGCCACCUGAGGAUCACCCAGGUGGUAUAGUGCAGCAUCAAAG
57545	276	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGCAUGAGGAUCACCCAUGCGCUGACGGUACA GGCCGCAUGAGGAUCACCCAUGCGGUAUAGUGCAGCAUCAAAG
57546	277	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGCCUGAGGAUCACCCAGGCGCUGACGGUACA GGCCGCCUGAGGAUCACCCAGGCGGUAUAGUGCAGCAUCAAAG
57547	278	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGCCUGAGCAUCAGCCAGGCGCUGACGGUACA GGCCGCCUGAGCAUCAGCCAGGCGGUAUAGUGCAGCAUCAAAG
57548	279	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGCAUCAGCCAUGUGCUGACGGUACA GGCCACAUGAGCAUCAGCCAUGUGGUAUAGUGCAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
57549	280	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGUAUCAACCAUGUGCUGACGGUACA GGCCACAUGAGUAUCAACCAUGUGGUAUAGUGCAGCAUCAAAG
57550	281	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGAAUCAGCCAUGUGCUGACGGUACA GGCCACAUGAGAAUCAGCCAUGUGGUAUAGUGCAGCAUCAAAG
57551	282	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCCCUUGAGGAUCACCCAUGUGCUGACGGUACA GGCCCCUUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
57552	283	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACUUGAGGAUCACCCAUGUGCUGACGGUACA GGCCACUUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
57553	284	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACCUGAGGAUCACCCAUGUGCUGACGGUACA GGCCACCUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
57554	285	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGAUCACCUAUGUGCUGACGGUACA GGCCACAUGAGGAUCACCUAUGUGGUAUAGUGCAGCAUCAAAG
57555	286	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUUAGGAUCACCAAUGUGCUGACGGUACA GGCCACAUUAGGAUCACCAAUGUGGUAUAGUGCAGCAUCAAAG
57556	287	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUUAGGAUCACCGAUGUGCUGACGGUACA GGCCACAUUAGGAUCACCGAUGUGGUAUAGUGCAGCAUCAAAG
57557	288	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUUAGGAUCACCUAUGUGCUGACGGUACA GGCCACAUUAGGAUCACCUAUGUGGUAUAGUGCAGCAUCAAAG
57558	289	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGAUUACCCAUGUGCUGACGGUACA GGCCACAUGAGGAUUACCCAUGUGGUAUAGUGCAGCAUCAAAG
57559	290	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGAUAACCCAUGUGCUGACGGUACA GGCCACAUGAGGAUAACCCAUGUGGUAUAGUGCAGCAUCAAAG
57560	291	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGAUGACCCAUGUGCUGACGGUACA GGCCACAUGAGGAUGACCCAUGUGGUAUAGUGCAGCAUCAAAG
57561	292	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGACCACCCAUGUGCUGACGGUACA GGCCACAUGAGGACCACCCAUGUGGUAUAGUGCAGCAUCAAAG
57562	293	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCAGAUGAGGAUCACCCAUGGGCUGACGGUACA GGCCAGAUGAGGAUCACCCAUGGGGUAUAGUGCAGCAUCAAAG
57563	294	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGGGGAUCACCCAUGUGCUGACGGUACA GGCCACAUGGGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
57564	295	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCACAUGAGGAUCACCCAUGUGCUGACGGUACA GGCCACAUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAAAG
57565	296	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACCUGAGGAUCACCCAGGUGAGCAUCAAAG
57566	297	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCGCAUGAGGAUCACCCAUGCGAGCAUCAAAG
57567	298	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCGCCUGAGGAUCACCCAGGCGAGCAUCAAAG
57568	299	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCGCCUGAGCAUCAGCCAGGCGAGCAUCAAAG
57569	300	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGCAUCAGCCAUGUGAGCAUCAAAG
57570	301	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGUAUCAACCAUGUGAGCAUCAAAG
57571	302	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGAAUCAGCCAUGUGAGCAUCAAAG
57572	303	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCCUUGAGGAUCACCCAUGUGAGCAUCAAAG
57573	304	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACUUGAGGAUCACCCAUGUGAGCAUCAAAG
57574	305	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACCUGAGGAUCACCCAUGUGAGCAUCAAAG
57575	306	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUCACCUAUGUGAGCAUCAAAG
57576	307	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUUAGGAUCACCAAUGUGAGCAUCAAAG
57577	308	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUUAGGAUCACCGAUGUGAGCAUCAAAG
57578	309	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUUAGGAUCACCUAUGUGAGCAUCAAAG
57579	310	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUUACCCAUGUGAGCAUCAAAG
57580	311	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUAACCCAUGUGAGCAUCAAAG
57581	312	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUGACCCAUGUGAGCAUCAAAG
57582	313	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGACCACCCAUGUGAGCAUCAAAG
57583	314	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCAGAUGAGGAUCACCCAUGGGAGCAUCAAAG
57584	315	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGGGGAUCACCCAUGUGAGCAUCAAAG

SEQ ID NO.	Guide No.	Sequence
59352	316	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCCCUCUUCGGAGGGAGCAUCAGAG
57585	317	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUCACAUGAGGAUCACCCAUGUGAGCAUCAGAG
57586	318	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCACAUGA GGAUCACCCAUGUGGUAUAGUGCAGCAUCAGAG
57587	319	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGCUCAUGAGGAUCACCCAUGAGCUGACGGUACA GGCCACAUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAGAG
57588	320	ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGCGCAGACAUGGCAGUCGUAACGACGCGGGUCUGACGG UACAGGCCACAUGAGGAUCACCCAUGUGGUAUAGUGCAGCAUCAGAG
57589	321	ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUG GGUAAAGCUGCACUAUGGGGCCACAUGAGGAUCACCCAUGUGGUGUACAGCGCAGC GUCAAUGACGCUGACGAUAGUGCAGCAUCAAAG

[0166] In some embodiments, a gRNA variant of the gene repressor systems comprises a sequence of any one of SEQ ID NOs: 2238-2331, 57544-57589, and 59352, set forth in Table 2. In some embodiments, a gRNA variant comprises a sequence of any one of SEQ ID NOS: 2238, 2241, 2244, 2248, 2249, or 2259-2280. In some embodiments, a gRNA variant comprises a sequence of any one of SEQ ID NOS: 2238, 2241, 2244, 2248, 2249, or 2259-2280. In some embodiments, a gRNA variant comprises a sequence of any one of SEQ ID NOS: 2281-2331. In some embodiments, a gRNA variant comprises a sequence of any one of SEQ ID NOS: 57544-57589 and 59352. In some embodiments, a gRNA variant comprises one or more chemical modifications to the sequence.

[0167] Additional representative gRNA variant scaffold sequences for use with the gene repressor systems of the instant disclosure are included as SEQ ID NOS: 2101-2237.

e. gRNA 316

[0168] Guide scaffolds can be made by several methods, including recombinantly or by solid-phase RNA synthesis. However, the length of the scaffold can affect the manufacturability when using solid-phase RNA synthesis, with longer lengths resulting in increased manufacturing costs, decreased purity and yield, and higher rates of synthesis failures. For use in lipid nanoparticle (LNP) formulations, solid-phase RNA synthesis of the scaffold is preferred in order to generate the quantities needed for commercial development. While previous experiments had identified gRNA scaffold 235 (SEQ ID NO: 2292) as having enhanced properties relative to gRNA

scaffold 174 (SEQ ID NO: 2238) its increased length rendered its use for LNP formulations problematic. Accordingly, alternative sequences were sought. In some embodiments, the disclosure provides gRNA wherein the gRNA and linked targeting sequence has a sequence less than about 120 nucleotides, less than about 110 nucleotides, or less than about 100 nucleotides.

[0169] In one embodiment, a scaffold was designed wherein the scaffold 235 sequence was modified by a domain swap in which the extended stem loop of scaffold 174 replaced the extended stem loop of the 235 scaffold, resulting in the chimeric RNA scaffold 316 having the sequence ACUGGCGCUUCUAUCUGAUUACUCUGAGCGCCAUCACCAGCGACUAUGUCGUAGU GGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAGAG (SEQ ID NO: 59352), having 89 nucleotides, compared with the 99 nucleotides of gRNA scaffold 235. In addition to improvements in manufacturability, the 316 scaffold was determined to perform comparably or more favorably than gRNA scaffold 174 in editing assays, as described in the Examples. The resulting 316 scaffold had the further advantage in that the extended stem loop did not contain CpG motifs; an enhanced property described more fully, below.

f. Chemically-modified Scaffolds

[0170] In another aspect, the present disclosure relates to gRNAs having chemical modifications. In some embodiments, the chemical modification is addition of a 2'O-methyl group to one or more nucleotides of the sequence. In some embodiments, the chemical modification is substitution of a phosphorothioate bond between two or more nucleotides of the sequence.

g. Stem Loop Modifications

[0171] In some embodiments, the gRNA variant of the gene repressor systems comprises an exogenous extended stem loop, with such differences from a reference gRNA described as follows. In some embodiments, an exogenous extended stem loop has little or no identity to the reference stem loop regions disclosed herein (e.g., SEQ ID NO: 15). In some embodiments, an exogenous stem loop is at least 10 bp, at least 20 bp, at least 30 bp, at least 40 bp, at least 50 bp, at least 60 bp, at least 70 bp, at least 80 bp, at least 90 bp, at least 100 bp, at least 200 bp, at least 300 bp, at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, or at least 1,000 bp. In some embodiments, the gRNA variant comprises an extended stem loop region comprising at least 10, at least 100, at least 500, or at least 1000 nucleotides. In some embodiments, the heterologous stem loop increases the stability of the gRNA. In some

embodiments, the heterologous RNA stem loop is capable of binding a protein, an RNA structure, a DNA sequence, or a small molecule. In some embodiments, an exogenous stem loop region comprises one or more RNA stem loops or hairpins, for example a thermostable RNA such as MS2 binding (or tagging) sequence (ACAUGAGGAUCACCCAUGU (SEQ ID NO: 33276), Qβ hairpin (AUGCAUGUCUAAGACAGCAU (SEQ ID NO: 33277)), U1 hairpin II (GGAAUCCAUUGCACUCCGGAUUUCACUAG (SEQ ID NO: 33278)), Uvsx (CCUCUUCGGAGG (SEQ ID NO: 33279)), PP7 (AAGGAGUUUAUAUGGAAACCCUU (SEQ ID NO: 33280)), Phage replication loop (AGGUGGGACGACCUCUCGGUCGUCCUAUCU (SEQ ID NO: 33281)), Kissing loop. a (UGCUCGCUCCGUUCGAGCA (SEQ ID NO: 33282)), Kissing loop_b1 (UGCUCGACGCGUCCUCGAGCA (SEQ ID NO: 33283)), Kissing loop_b2 (UGCUCGUUUGCGGCUACGAGCA (SEQ ID NO: 33284)), G quadriplex M3q (AGGGAGGGAGGGAGAGG (SEQ ID NO: 33285)), G quadriplex telomere basket (GGUUAGGGUUAGGGUUAGG (SEQ ID NO: 33286)), Sarcin-ricin loop (CUGCUCAGUACGAGAGGAACCGCAG (SEQ ID NO: 33287)), Pseudoknots (UACACUGGGAUCGCUGAAUUAGAGAUCGGCGUCCUUUCAUUCUAUAUACUUUGG AGUUUUAAAAUGUCUCUAAGUACA (SEQ ID NO: 33288)), transactivation response element (TAR) (GGCUCGUGUAGCUCAUUAGCUCCGAGCC (SEQ ID NO: 57741)), iron responsive element (IRE) CCGUGUGCAUCCGCAGUGUCGGAUCCACGG (SEQ ID NO: 57742)), transactivation response element (TAR) GGCUCGUGUAGCUCAUUAGCUCCGAGCC (SEQ ID NO: 57743)), phage GA hairpin (AAAACAUAAGGAAAACCUAUGUU (SEQ ID NO: 57744)), phage ΛN hairpin (GCCCUGAAGAAGGGC (SEQ ID NO: 57745)), or sequence variants thereof. In some embodiments, one of the foregoing hairpin sequences is incorporated into the stem loop to help traffic the incorporation of the gRNA (and an associated CasX in an RNP complex) into a budding XDP (described more fully, below).

[0172] In some embodiments, a sgRNA variant of the gene repressor systems of the disclosure comprises one or more additional changes to a previously generated variant, the previously generated variant itself serving as the reference sequence. In some embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 2238, SEQ ID NO: 2239, SEQ ID NO: 2240, SEQ ID NO: 2241, SEQ ID NO: 2241, SEQ ID NO: 2274, SEQ ID NO: 2275, SEQ ID NO: 2279, or SEQ ID NO: 59352.

[0173] In exemplary embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 2238 (Variant Scaffold 174, referencing Table 2).

[0174] In exemplary embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 2239 (Variant Scaffold 175, referencing Table 2).

[0175] In exemplary embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 2275 (Variant Scaffold 215, referencing Table 2).

[0176] In exemplary embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 2292 (Variant Scaffold 235, referencing Table 2).

[0177] In exemplary embodiments, a sgRNA variant comprises one or more additional changes to a sequence of SEQ ID NO: 59352 (Variant Scaffold 316, referencing Table 2).

h. Complex Formation with dCasX Protein

[0178] In some embodiments, a gRNA variant of the disclosure has an improved affinity for a dCasX and linked repressor domain(s) when compared to a reference gRNA, thereby improving its ability to form a ribonucleoprotein (RNP) complex with the dCasX protein and linked repressor domain(s). Improving ribonucleoprotein complex formation may, in some embodiments, improve the efficiency with which functional RNPs are assembled. In some embodiments, greater than 90%, greater than 93%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% of RNPs comprising a gRNA variant and a spacer are competent for binding to a target nucleic acid.

[0179] Exemplary nucleotide changes that can improve the ability of gRNA variants to form a complex with dXR may, in some embodiments, include replacing the scaffold stem with a thermostable stem loop. Without wishing to be bound by any theory, replacing the scaffold stem with a thermostable stem loop could increase the overall binding stability of the gRNA variant with the dXR. Alternatively, or in addition, removing a large section of the stem loop could change the gRNA variant folding kinetics and make a functional folded gRNA easier and quicker to structurally-assemble, for example by lessening the degree to which the gRNA variant can get "tangled" in itself. In some embodiments, choice of scaffold stem loop sequence could change with different spacers that are utilized for the gRNA. In some embodiments, scaffold sequence can be tailored to the spacer and therefore the target sequence. Biochemical assays can be used to evaluate the binding affinity of dXR for the gRNA variant to form the RNP, including the assays of the Examples. For example, a person of ordinary skill can measure changes in the amount of a fluorescently tagged gRNA that is bound to an immobilized dXR, as a response to

increasing concentrations of an additional unlabeled "cold competitor" gRNA. Alternatively, or in addition, fluorescence signal can be monitored to or seeing how it changes as different amounts of fluorescently-labeled gRNA are flowed over immobilized dXR. Alternatively, the ability to form an RNP can be assessed using *in vitro* assays against a defined target nucleic acid sequence.

i. Adding or Changing gRNA Function

[0180] In some embodiments, gRNA variants of the system can comprise larger structural changes that change the topology of the gRNA variant with respect to the reference gRNA, thereby allowing for different gRNA functionality. For example, in some embodiments a gRNA variant has swapped an endogenous stem loop of the reference gRNA scaffold with a previously identified stable RNA structure or a stem loop that can interact with a protein or RNA binding partner to recruit additional moieties to the dCasX variant or to recruit dCasX variant to a specific location, such as the inside of a XDP capsid, that has the binding partner to the said RNA structure. The RNA binding domain can be a retroviral Psi packaging element inserted into the gRNA or is a stem loop or hairpin (e.g., MS2 hairpin, Qβ hairpin, U1 hairpin II, Uvsx, or PP7 hairpin) with affinity to a protein selected from the group consisting of MS2 coat protein, PP7 coat protein, Qß coat protein, U1A protein, or phage R-loop, which can facilitate the binding of gRNA to the dCasX variant. Similar RNA components with affinity to protein structures incorporated into the dCasX variant include kissing loop, a, kissing loop_b1, kissing loop_b2, G quadriplex M3q, G quadriplex telomere basket, sarcin-ricin loop, and pseudoknots. In some embodiments, the gRNA variants of the disclosure comprise multiple components of the foregoing, or multiple copies of the same component.

V. CRISPR Proteins of the Gene Repressor Systems

[0181] Provided herein are gene repressor systems comprising fusion proteins comprising catalytically dead CRISPR proteins. In some embodiments, the catalytically-dead CRISPR protein is a catalytically-dead class 2 CRISPR protein. Class 2 systems are distinguished from Class 1 systems in that they have a single multi-domain effector protein and are further divided into a Type II, Type V, or Type VI system, described in Makarova, et al. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. *Nature Rev. Microbiol.* 18:67 (2020), incorporated herein by reference. In some embodiments, the catalytically-dead CRISPR protein is a Class 2, Type II CRISPR/Cas nucleases such as Cas9. In

other cases, the catalytically-dead CRISPR is a Class 2, Type V CRISPR/Cas nucleases such as a Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f, Cas12g, Cas12h, Cas12i, Cas12j, Cas12k, Cas12l, Cas14, and/or CasΦ.

[0182] The nucleases of Type V systems differ from Type II effectors (e.g., Cas9), which contain two nuclear domains that are each responsible for the cleavage of one strand of the target DNA, with the HNH nuclease inserted inside the Ruv-C like nuclease domain sequence. The Type V nucleases possess a single RNA-guided RuvC domain-containing effector but no HNH domain, and they recognize a T-rich protospacer adjacent motif (PAM) 5' upstream to the target region on the non-targeted strand, which is different from Cas9 systems which rely on G-rich PAM at 3'side of target sequences. Type V nucleases generate staggered double-stranded breaks distal to the PAM sequence, unlike Cas9, which generates a blunt end in the proximal site close to the PAM. In addition, Type V nucleases degrade ssDNA in trans when activated by target dsDNA or ssDNA binding in cis. In some embodiments, the Type V nucleases utilized in the XDP embodiments recognize a 5' TC PAM motif and produce staggered ends cleaved by the RuvC domain. The Type V systems (e.g., Cas12) only contain a RuvC-like nuclease domain that cleaves both strands. Type VI (Cas13) are unrelated to the effectors of Type II and V systems and contain two HEPN domains and target RNA.

[0183] The term "CasX protein", as used herein, refers to a family of proteins, and encompasses all naturally occurring CasX proteins ("reference CasX"), as well as CasX variants possessing one or more improved characteristics relative to a naturally-occurring reference CasX protein. In the context of the present disclosure, catalytically-dead CasX variants are prepared from reference CasX and CasX variant proteins, and exemplary dCasX variant sequences are presented in SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4. The CasX and dCasX proteins of the disclosure comprise at least one of the following domains: a non-target strand binding (NTSB) domain, a target strand loading (TSL) domain, a helical I domain, a helical II domain, an oligonucleotide binding domain (OBD), and a RuvC domain (the last of which may be modified or deleted to create the catalytically dead CasX variant), described more fully, below.

a. Reference CasX Proteins

[0184] The disclosure provides reference CasX proteins that are naturally-occurring and that were the starting material for the aforementioned protocols for introducing sequence modifications for generation of the dCasX variants. For example, reference CasX proteins can be

isolated from naturally occurring prokaryotes, such as *Deltaproteobacteria*, *Planctomycetes*, or *Candidatus Sungbacteria* species. A reference CasX protein (sometimes referred to herein as a reference CasX polypeptide) is a type II CRISPR/Cas endonuclease belonging to the CasX (sometimes referred to as Cas12e) family of proteins that is capable of interacting with a guide RNA to form a ribonucleoprotein (RNP) complex.

[0185] In some cases, a reference CasX protein is isolated or derived from *Deltaproteobacteria* having a sequence of:

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   1 MEKRINKIRK KLSADNATKP VSRSGPMKTL LVRVMTDDLK KRLEKRRKKP EVMPQVISNN
  61 AANNLRMLLD DYTKMKEAIL QVYWQEFKDD HVGLMCKFAQ PASKKIDQNK LKPEMDEKGN
 121 LTTAGFACSQ CGQPLFVYKL EQVSEKGKAY TNYFGRCNVA EHEKLILLAQ LKPEKDSDEA
 181 VTYSLGKFGQ RALDFYSIHV TKESTHPVKP LAQIAGNRYA SGPVGKALSD ACMGTIASFL
 241 SKYQDIIIEH QKVVKGNQKR LESLRELAGK ENLEYPSVTL PPQPHTKEGV DAYNEVIARV
 301 RMWVNLNLWQ KLKLSRDDAK PLLRLKGFPS FPVVERRENE VDWWNTINEV KKLIDAKRDM
 361 GRVFWSGVTA EKRNTILEGY NYLPNENDHK KREGSLENPK KPAKRQFGDL LLYLEKKYAG
 421 DWGKVFDEAW ERIDKKIAGL TSHIEREEAR NAEDAQSKAV LTDWLRAKAS FVLERLKEMD
 481 EKEFYACEIQ LQKWYGDLRG NPFAVEAENR VVDISGFSIG SDGHSIQYRN LLAWKYLENG
 541 KREFYLLMNY GKKGRIRFTD GTDIKKSGKW QGLLYGGGKA KVIDLTFDPD DEQLIILPLA
 601 FGTRQGREFI WNDLLSLETG LIKLANGRVI EKTIYNKKIG RDEPALFVAL TFERREVVDP
 661 SNIKPVNLIG VDRGENIPAV IALTDPEGCP LPEFKDSSGG PTDILRIGEG YKEKQRAIQA
 721 AKEVEQRRAG GYSRKFASKS RNLADDMVRN SARDLFYHAV THDAVLVFEN LSRGFGRQGK
 781 RTFMTERQYT KMEDWLTAKL AYEGLTSKTY LSKTLAQYTS KTCSNCGFTI TTADYDGMLV
 841 RLKKTSDGWA TTLNNKELKA EGQITYYNRY KRQTVEKELS AELDRLSEES GNNDISKWTK
 901 GRRDEALFLL KKRFSHRPVQ EQFVCLDCGH EVHADEQAAL NIARSWLFLN SNSTEFKSYK
 961 SGKQPFVGAW QAFYKRRLKE VWKPNA (SEQ ID NO: 1).
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[0186] In some cases, a reference CasX protein is isolated or derived from *Planctomycetes* having a sequence of:

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   1 MQEIKRINKI RRRLVKDSNT KKAGKTGPMK TLLVRVMTPD LRERLENLRK KPENIPQPIS
  61 NTSRANLNKL LTDYTEMKKA ILHVYWEEFQ KDPVGLMSRV AQPAPKNIDQ RKLIPVKDGN
 121 ERLTSSGFAC SQCCQPLYVY KLEQVNDKGK PHTNYFGRCN VSEHERLILL SPHKPEANDE
 181 LVTYSLGKFG QRALDFYSIH VTRESNHPVK PLEQIGGNSC ASGPVGKALS DACMGAVASF
 241 LTKYQDIILE HQKVIKKNEK RLANLKDIAS ANGLAFPKIT LPPQPHTKEG IEAYNNVVAQ
 301 IVIWVNLNLW QKLKIGRDEA KPLQRLKGFP SFPLVERQAN EVDWWDMVCN VKKLINEKKE
 361 DGKVFWQNLA GYKRQEALLP YLSSEEDRKK GKKFARYQFG DLLLHLEKKH GEDWGKVYDE
 421 AWERIDKKVE GLSKHIKLEE ERRSEDAQSK AALTDWLRAK ASFVIEGLKE ADKDEFCRCE
 481 LKLQKWYGDL RGKPFAIEAE NSILDISGFS KQYNCAFIWQ KDGVKKLNLY LIINYFKGGK
 541 LRFKKIKPEA FEANRFYTVI NKKSGEIVPM EVNFNFDDPN LIILPLAFGK RQGREFIWND
 601 LLSLETGSLK LANGRVIEKT LYNRRTRQDE PALFVALTFE RREVLDSSNI KPMNLIGIDR
 661 GENIPAVIAL TDPEGCPLSR FKDSLGNPTH ILRIGESYKE KQRTIQAAKE VEQRRAGGYS
 721 RKYASKAKNL ADDMVRNTAR DLLYYAVTQD AMLIFENLSR GFGRQGKRTF MAERQYTRME
 781 DWLTAKLAYE GLPSKTYLSK TLAQYTSKTC SNCGFTITSA DYDRVLEKLK KTATGWMTTI
 841 NGKELKVEGQ ITYYNRYKRQ NVVKDLSVEL DRLSEESVNN DISSWTKGRS GEALSLLKKR
 901 FSHRPVQEKF VCLNCGFETH ADEQAALNIA RSWLFLRSQE YKKYQTNKTT GNTDKRAFVE
 961 TWQSFYRKKL KEVWKPAV (SEQ ID NO: 2).
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[0187] In some cases, a reference CasX protein is isolated or derived from *Candidatus Sungbacteria* having a sequence of

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   1 MDNANKPSTK SLVNTTRISD HFGVTPGQVT RVFSFGIIPT KRQYAIIERW FAAVEAARER
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 61 LYGMLYAHFQ ENPPAYLKEK FSYETFFKGR PVLNGLRDID PTIMTSAVFT ALRHKAEGAM
121 AAFHTNHRRL FEEARKKMRE YAECLKANEA LLRGAADIDW DKIVNALRTR LNTCLAPEYD
181 AVIADFGALC AFRALIAETN ALKGAYNHAL NQMLPALVKV DEPEEAEESP RLRFFNGRIN
241 DLPKFPVAER ETPPDTETII RQLEDMARVI PDTAEILGYI HRIRHKAARR KPGSAVPLPQ
301 RVALYCAIRM ERNPEEDPST VAGHFLGEID RVCEKRRQGL VRTPFDSQIR ARYMDIISFR
361 ATLAHPDRWT EIQFLRSNAA SRRVRAETIS APFEGFSWTS NRTNPAPQYG MALAKDANAP
421 ADAPELCICL SPSSAAFSVR EKGGDLIYMR PTGGRRGKDN PGKEITWVPG SFDEYPASGV
481 ALKLRLYFGR SQARRMLTNK TWGLLSDNPR VFAANAELVG KKRNPQDRWK LFFHMVISGP
541 PPVEYLDFSS DVRSRARTVI GINRGEVNPL AYAVVSVEDG QVLEEGLLGK KEYIDQLIET
601 RRRISEYQSR EQTPPRDLRQ RVRHLQDTVL GSARAKIHSL IAFWKGILAI ERLDDQFHGR
661 EQKIIPKKTY LANKTGFMNA LSFSGAVRVD KKGNPWGGMI EIYPGGISRT CTQCGTVWLA
721 RRPKNPGHRD AMVVIPDIVD DAAATGFDNV DCDAGTVDYG ELFTLSREWV RLTPRYSRVM
781 RGTLGDLERA IRQGDDRKSR QMLELALEPQ PQWGQFFCHR CGFNGQSDVL AATNLARRAI
841 SLIRRLPDTD TPPTP (SEQ ID NO: 3).
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b. Catalytically-dead CasX Variant Proteins (dCasX variant)

[0188] In the gene repressor systems, the CasX protein is catalytically dead (dCasX) but retains the ability to bind a target nucleic acid. The present disclosure provides catalytically-dead variants (interchangeably referred to herein as "dCasX variant" or "dCasX variant protein"), wherein the catalytically-dead CasX variants comprise at least one modification in at least one domain relative to the catalytically-dead versions of sequences of SEQ ID NOS:1-3 (described, *supra*). An exemplary catalytically dead CasX protein comprises one or more mutations in the active site of the RuvC domain of the CasX protein. In some embodiments, a catalytically dead reference CasX protein comprises substitutions at residues 672, 769 and/or 935 with reference to SEQ ID NO: 1. In one embodiment, a catalytically-dead reference CasX protein comprises substitutions of D672A, E769A and/or D935A with reference to SEQ ID NO: 1. In other embodiments, a catalytically-dead reference CasX protein comprises substitutions at amino acids 659, 756 and/or 922 with reference to SEQ ID NO: 2. In some embodiments, a catalytically-dead reference CasX protein comprises D659A, E756A and/or D922A substitutions with reference to of SEQ ID NO: 2. An exemplary RuvC domain of the dCasX of the disclosure comprises amino acids 661-824 and 935-986 of SEQ ID NO: 1, or amino acids 648-812 and 922-978 of SEQ ID NO: 2, with one or more amino acid modifications relative to said RuvC cleavage domain sequence, wherein the dCasX variant exhibits one or more improved characteristics compared to the reference dCasX. In further embodiments, a catalytically-dead CasX variant protein comprises deletions of all or part of the RuvC domain of the reference CasX protein. It will be understood that the same foregoing substitutions or deletions can similarly be introduced into any of the CasX variants of SEQ ID NOS: 33352-33624 or 57647-57735 of the disclosure,

relative to the corresponding positions (allowing for any insertions or deletions) of the starting variant, resulting in a dCasX variant (*see*, e.g., Table 4 for exemplary sequences).

[0189] In some embodiments, the dCasX variant with linked repressor domain exhibits at least one improved characteristic compared to the reference dCasX protein with linked repressor domain configured in a comparable fashion, *e.g.* a catalytically dead version of a CasX variant of any one of SEQ ID NOS: 33352-33624 or 57647-57735. All variants that improve one or more functions or characteristics of the dCasX variant protein when with linked repressor domain compared to a reference dCasX protein with linked repressor domain described herein are envisaged as being within the scope of the disclosure. In some embodiments, the modification is a mutation in one or more amino acids of the reference dCasX. In some embodiments, the modification is a mutation in one or more amino acids of a dCasX variant that has been subjected to additional mutations or alterations in the sequence. In other embodiments, the modification is a substitution of one or more domains of the reference dCasX with one or more domains from a different CasX. In some embodiments, insertion includes the insertion of a part or all of a domain from a different CasX protein. Mutations can occur in any one or more domains of the reference dCasX protein or dCasX variant, and may include, for example, deletion of part or all of one or more domains, or one or more amino acid substitutions, deletions, or insertions in any domain. The domains of CasX proteins include the non-target strand binding (NTSB) domain, the target strand loading (TSL) domain, the helical I domain, the helical II domain, the oligonucleotide binding domain (OBD), and the RuvC DNA cleavage domain, which can further comprise subdomains, described below. Any change in amino acid sequence of a reference dCasX protein that leads to an improved characteristic of the protein is considered a dCasX variant protein of the disclosure. For example, dCasX variants can comprise one or more amino acid substitutions, insertions, deletions, or swapped domains, or any combinations thereof, relative to a reference dCasX protein sequence.

[0190] Suitable mutagenesis methods for generating dCasX variant proteins of the disclosure may include, for example, Deep Mutational Evolution (DME), deep mutational scanning (DMS), error prone PCR, cassette mutagenesis, random mutagenesis, staggered extension PCR, gene shuffling, or domain swapping. In some embodiments, the dCasX variants are designed, for example by selecting one or more desired mutations in a reference dCasX. In certain embodiments, the activity of a reference dCasX protein is used as a benchmark against which the

activity of one or more dCasX variants are compared, thereby measuring improvements in function of the dCasX variants.

[0191] In some embodiments of the dCasX variants described herein, the at least one modification comprises: (a) a substitution of 1 to 100 consecutive or non-consecutive amino acids in the dCasX variant; (b) a deletion of 1 to 100 consecutive or non-consecutive amino acids in the dCasX variant; (c) an insertion of 1 to 100 consecutive or non-consecutive amino acids in the dCasX; or (d) any combination of (a)-(c). In some embodiments, the at least one modification comprises: (a) a substitution of 5-10 consecutive or non-consecutive amino acids in the dCasX variant; (b) a deletion of 1-5 consecutive or non-consecutive amino acids in the dCasX variant; (c) an insertion of 1-5 consecutive or non-consecutive amino acids in the dCasX; or (d) any combination of (a)-(c).

[0192] Any amino acid can be substituted for any other amino acid in the substitutions described herein. The substitution can be a conservative substitution (e.g., a basic amino acid is substituted for another basic amino acid). The substitution can be a non-conservative substitution (e.g., a basic amino acid is substituted for an acidic amino acid or vice versa). For example, a proline in a reference dCasX protein can be substituted for any of arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine or valine to generate a dCasX variant protein of the disclosure.

[0193] Any permutation of the substitution, insertion and deletion embodiments described herein can be combined to generate a dCasX variant protein of the disclosure. For example, a dCasX variant protein can comprise at least one substitution and at least one deletion relative to a reference dCasX protein sequence, at least one substitution and at least one insertion relative to a reference dCasX protein sequence, at least one insertion and at least one deletion relative to a reference dCasX protein sequence, or at least one substitution, one insertion and one deletion relative to a reference dCasX protein sequence.

[0194] In some embodiments, the dCasX variant protein comprises between 700 and 1200 amino acids, between 800 and 1100 amino acids or between 900 and 1000 amino acids.

[0195] The dCasX and linked repressor domains of the disclosure have an enhanced ability to efficiently bind target nucleic acid, when complexed with a gRNA as an RNP, utilizing PAM TC motif, including PAM sequences selected from TTC, ATC, GTC, or CTC, compared to an RNP of a reference dCasX protein and reference gRNA. In the foregoing, the PAM sequence is

located at least 1 nucleotide 5' to the non-target strand of the protospacer having identity with the targeting sequence of the gRNA in an assay system compared to the binding of an RNP comprising a reference dCasX protein and reference gRNA in a comparable assay system.

[0196] In some embodiments, an RNP comprising the dCasX variant protein with linked repressor domains and a gRNA of the disclosure, at a concentration of 20 pM or less, is capable of binding a double stranded DNA target with an efficiency of at least 70%, at least 80%, at least 85%, at least 90% or at least 95%. In one embodiment, an RNP of a dCasX variant with linked repressor domains and a gRNA variant exhibits greater binding of a target sequence in the target nucleic acid compared to an RNP comprising a reference dCasX protein with linked repressor domains and a reference gRNA in a comparable assay system, wherein the PAM sequence of the target nucleic acid is TTC. In another embodiment, an RNP of a dCasX variant with linked repressor domains and gRNA variant exhibits greater binding affinity of a target sequence in the target nucleic acid compared to an RNP comprising a reference dCasX protein with linked repressor domains and a reference gRNA in a comparable assay system, wherein the PAM sequence of the target nucleic acid is ATC. In another embodiment, an RNP of a dCasX variant with linked repressor domains and gRNA variant exhibits greater binding affinity of a target sequence in the target nucleic acid compared to an RNP comprising a reference dCasX protein with linked repressor domains and a reference gRNA in a comparable assay system, wherein the PAM sequence of the target nucleic acid is CTC. In another embodiment, an RNP of a dCasX variant with linked repressor domains and gRNA variant exhibits greater binding affinity of a target sequence in the target nucleic acid compared to an RNP comprising a reference dCasX protein with linked repressor domains and a reference gRNA in a comparable assay system, wherein the PAM sequence of the target nucleic acid is GTC. In the foregoing embodiments, the increased binding affinity for the one or more PAM sequences is at least 1.5-fold greater or more compared to the binding affinity of an RNP of any one of the reference dCasX proteins (modified from SEQ ID NOS:1-3) with linked repressor domains and the gRNA of Table 1 for the PAM sequences.

c. dCasX Variant Proteins with Domains from Multiple Source Proteins

[0197] In certain embodiments, the disclosure provides a chimeric dCasX variant protein for use in the dXR systems comprising protein domains from two or more different CasX proteins, such as two or more naturally occurring CasX proteins, or two or more CasX variant protein sequences as described herein. As used herein, a "chimeric dCasX protein" refers to a

catalytically-dead CasX containing at least two domains isolated or derived from different sources, such as two naturally occurring proteins, which may, in some embodiments, be isolated from different species. For example, in some embodiments, a chimeric dCasX variant protein comprises a first domain from a first CasX protein and a second domain from a second, different CasX protein. In some embodiments, the first domain can be selected from the group consisting of the NTSB, TSL, helical I-I, helical I-II, helical II, OBD-I, OBD-II, RuvC-I and RuvC-II domains. In some embodiments, the second domain is selected from the group consisting of the NTSB, TSL, helical I-I, helical I-II, helical II, OBD-I, OBD-II, RuvC-I and RuvC-II domains with the second domain being different from the foregoing first domain. A chimeric dCasX variant protein may comprise an NTSB, TSL, helical I-I, helical I-II, helical II, OBD-I, and OBD-II domains from a CasX protein of SEQ ID NO: 2, and a RuvC-I and/or RuvC-II domain from a CasX protein of SEQ ID NO: 1, or vice versa, in which mutations or other sequence alterations are introduced to create the catalytically dead variant with improved properties of the variant, relative to the reference dCasX protein. As an example of the foregoing, the chimeric RuvC domain comprises amino acids 661 to 824 of SEQ ID NO: 1 and amino acids 922 to 978 of SEQ ID NO: 2. As an alternative example of the foregoing, a chimeric RuvC domain comprises amino acids 648 to 812 of SEQ ID NO: 2 and amino acids 935 to 986 of SEQ ID NO: 1. In a particular embodiment, a dCasX for use in the dXR comprises an NTSB domain and helical I-II domain from SEQ ID NO: 1 and a helical I-I domain from SEQ ID NO:2; the latter being a chimeric domain. Coordinates of CasX domains in the reference CasX proteins of SEQ ID NO: 1 and SEQ ID NO: 2 are provided in Table 3 below.

Table 3: Domain coordinates in Reference CasX proteins

Domain Name	**Coordinates in SEQ ID NO: 1**	**Coordinates in SEQ ID NO: 2**
OBD-I	1-55	1-57
helical I-I	56-99	58-101
NTSB	100-190	102-191
helical I-II	191-331	192-332
helical II	332-508	333-500
OBD-II	509-659	501-646
RuvC-I	660-823	647-810
TSL	824-933	811-920

Domain Name	Coordinates in SEQ ID NO: 1	Coordinates in SEQ ID NO: 2
RuvC-II	934-986	921-978

*OBD I and II, helical I-I and I-II, and RuvC I and II are also sometimes referred to as OBD a and b, helical I a and b, and RuvC a and b.

[0198] In some embodiments, an improved characteristic of the dCasX variant is at least about 1.1 to about 100,000-fold improved relative to the reference dCasX protein. In some embodiments, an improved characteristic of the CasX variant is at least about 1.1 to about 10,000-fold improved, at least about 1.1 to about 1,000-fold improved, at least about 1.1 to about 500-fold improved, at least about 1.1 to about 400-fold improved, at least about 1.1 to about 300-fold improved, at least about 1.1 to about 200-fold improved, at least about 1.1 to about 100-fold improved, at least about 1.1 to about 50-fold improved, at least about 1.1 to about 40-fold improved, at least about 1.1 to about 30-fold improved, at least about 1.1 to about 20-fold improved, at least about 1.1 to about 10-fold improved, at least about 1.1 to about 9-fold improved, at least about 1.1 to about 8-fold improved, at least about 1.1 to about 7-fold improved, at least about 1.1 to about 6-fold improved, at least about 1.1 to about 5-fold improved, at least about 1.1 to about 4-fold improved, at least about 1.1 to about 3-fold improved, at least about 1.1 to about 2-fold improved, at least about 1.1 to about 1.5-fold improved, at least about 1.5 to about 3-fold improved, at least about 1.5 to about 4-fold improved, at least about 1.5 to about 5-fold improved, at least about 1.5 to about 10-fold improved, at least about 5 to about 10-fold improved, at least about 10 to about 20-fold improved, at least 10 to about 30-fold improved, at least 10 to about 50-fold improved or at least 10 to about 100-fold improved than the reference CasX protein. In some embodiments, an improved characteristic of the dCasX variant is at least about 10 to about 1000-fold improved relative to the reference dCasX protein.

[0199] In some embodiments, a dCasX variant protein utilized in the gene repressor systems of the disclosure comprises a sequence of SEQ ID NOS: 33352-33624 or 57647-57735 and one or more insertions, substitutions or deletions thereto as described *supra* that inactivate the catalytic domain of the CasX variant to produce a dCasX variant. In some embodiments, a dCasX variant protein utilized in the gene repressor systems of the disclosure comprises a sequence of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4. In some embodiments, a dCasX variant protein consists of a sequence of SEQ ID NOS: 17-36 and

59353-59358 as set forth in Table 4. In other embodiments, a dCasX variant protein comprises a sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical to a sequence of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4.

Table 4: dCasX Variant Sequences

SEQ ID NO	dCasX	Amino Acid Sequence
17	dCasX533	QEIKRINKIRRRLVKDSNTKKAGKTRGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEK DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASYPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
18	dCasX491	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV

SEQ ID NO	dCasX	Amino Acid Sequence
19	dCasX532	QEIKRINKIRRRLVKDSNTKKAGKTRGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEK DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
20	dCasX529	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASNPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
21	dCasX531	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGYGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
22	dCasX530	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD

SEQ ID NO	dCasX	Amino Acid Sequence
		SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGWGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
23	dCasX528	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASYPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
24	dCasX527	QEIKRINKIRRRLVKDSNTKKAGKTRGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEK DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
25	dCasX515	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL

SEQ ID NO	dCasX	Amino Acid Sequence
		INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
26	dCasX514	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTIHTSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
27	dCasX516	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNHNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
28	dCasX517	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGAPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY

SEQ ID NO	dCasX	Amino Acid Sequence
		LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
29	dCasX518	RQEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEK DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
30	dCasX519	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHIQLRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
31	dCasX520	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTTQAK

SEQ ID NO	dCasX	Amino Acid Sequence
		KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
32	dCasX522	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKRSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
33	dCasX523	QEIKRINKIRRRLVKDSNTKKAGKTYPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
34	dCasX524	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTIHSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT

SEQ ID NO	dCasX	Amino Acid Sequence
		KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
35	dCasX525	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAATQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
36	dCasX526	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAA KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLE KLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWT KGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
59353	dCasX535	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASSPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV

SEQ ID NO	dCasX	Amino Acid Sequence
59354	dCasX593	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRWWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
59355	dCasX668	QEIKRINKIRRRLVKDSNTKKAGKTRGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEK DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASSPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
59356	dCasX672	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLIKLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASSPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
59357	dCasX676	QEIKRINKIRRRLVKDSNTKKAGKTRGPMKTLLVRVMTPDLRERLENLRKKPENIPQPI SNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMD EKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLIKLAQLKPEK

SEQ ID NO	dCasX	Amino Acid Sequence
		DSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASSPVGKALSDACMG TIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVDWWDMVCNVKK LINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGE DWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA DKDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLY LIINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFG KRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSS NIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQA KKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQG KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRV LEKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISS WTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKK YQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV
59358	dCasX812	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQPIS NTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKPEMDE KGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPEKD SDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGT IASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYN EVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKKFPSFPLVERQANEVDWWDMVCNVKKL INEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGKKFARYQLGDLLLHLEKKHGED WGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEAD KDEFCRCELKLQKWYGDLRGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYL IINYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAFGK RQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSN IKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAK KEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGK RTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVL EKLKKTATGWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSW TKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKY QTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV

d. Affinity for the gRNA

[0200] In some embodiments, a dCasX with linked repressor domains has improved affinity for the gRNA relative to a reference dCasX protein, leading to the formation of the ribonucleoprotein complex. Increased affinity of the dXR for the gRNA may, for example, result in a lower K_d for the generation of a RNP complex, which can, in some cases, result in a more stable ribonucleoprotein complex formation. In some embodiments, the K_d of a dXR for a gRNA is increased relative to a reference dCasX protein by a factor of at least about 1.1, at least about 1.2, at least about 1.3, at least about 1.4, at least about 1.5, at least about 1.6, at least about 1.7, at least about 1.8, at least about 1.9, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 60, at least about 70, at least about 80,

at least about 90, or at least about 100. In some embodiments, the dCasX variant has about 1.1 to about 10-fold increased binding affinity to the gRNA compared to the catalytically-dead variant of reference CasX protein of SEQ ID NO: 2.

[0201] In some embodiments, increased affinity of the dCasX with linked repressor domains for the gRNA results in increased stability of the ribonucleoprotein complex when delivered to mammalian cells, including *in vivo* delivery to a subject. This increased stability can affect the function and utility of the complex in the cells of a subject, as well as result in improved pharmacokinetic properties in blood, when delivered to a subject. In some embodiments, increased affinity of the dXR, and the resulting increased stability of the ribonucleoprotein complex, allows for a lower dose of the dXR to be delivered to the subject or cells while still having the desired activity; for example *in vivo* or *in vitro* gene repression. The increased ability to form RNP and keep them in stable form can be assessed using in vitro assays known in the art.

[0202] In some embodiments, a higher affinity (tighter binding) of a dCasX variant protein and linked repressor domain to a gRNA allows for a greater amount of repression events when both the dCasX variant protein and the gRNA remain in an RNP complex. Increased repression events can be assessed using repression assays described herein.

[0203] Methods of measuring dXR fusion protein binding affinity for a gRNA include *in vitro* methods using purified dXR fusion protein and gRNA. The binding affinity for reference dXR can be measured by fluorescence polarization if the gRNA or dXR fusion protein is tagged with a fluorophore. Alternatively, or in addition, binding affinity can be measured by biolayer interferometry, electrophoretic mobility shift assays (EMSAs), or filter binding. Additional standard techniques to quantify absolute affinities of RNA binding proteins such as the reference dCasX and variant proteins of the disclosure for specific gRNAs such as reference gRNAs and variants thereof include, but are not limited to, isothermal calorimetry (ITC), and surface plasmon resonance (SPR), as well as the methods of the Examples.

e. Improved Specificity for a Target Site

[0204] In some embodiments, a dCasX variant protein with linked repressor domains has improved specificity for a target nucleic acid sequence relative to a reference dCasX protein with linked repressor domains. As used herein, "specificity," sometimes referred to as "target specificity," refers to the degree to which a CRISPR/Cas system ribonucleoprotein complex binds off-target sequences that are similar, but not identical to the target nucleic acid sequence;

e.g., a dXR RNP with a higher degree of specificity would exhibit reduced off-target methylation of sequences relative to a reference dXR protein. The specificity, and the reduction of potentially deleterious off-target effects, of CRISPR/Cas system proteins can be vitally important in order to achieve an acceptable therapeutic index for use in mammalian subjects.

[0205] In some embodiments, a dCasX variant protein with linked repressor domains has improved specificity for a target site within the target sequence that is complementary to the targeting sequence of the gRNA. Without wishing to be bound by theory, it is possible that amino acid changes in the helical I and II domains that increase the specificity of the dXR for the target nucleic acid strand can increase the specificity of the dXR for the target nucleic acid overall. In some embodiments, amino acid changes that increase specificity of dXRs for target nucleic acid may also result in decreased affinity of dXRs for DNA.

f. Protospacer and PAM Sequences

[0206] Herein, the protospacer is defined as the DNA sequence complementary to the targeting sequence of the guide RNA and the DNA complementary to that sequence, referred to as the target strand and non-target strand, respectively. As used herein, the PAM is a nucleotide sequence proximal to the protospacer that, in conjunction with the targeting sequence of the gRNA, helps the orientation and positioning of the CasX on the DNA strand.

[0207] PAM sequences may be degenerate, and specific RNP constructs may have different preferred and tolerated PAM sequences that support different efficiencies of binding and, in the case of catalytically-active nucleases, cleavage. Following convention, unless stated otherwise, the disclosure refers to both the PAM and the protospacer sequence and their directionality according to the orientation of the non-target strand. This does not imply that the PAM sequence of the non-target strand, rather than the target strand, is determinative of cleavage or mechanistically involved in target recognition. For example, when reference is to a TTC PAM, it may in fact be the complementary GAA sequence that is required for target binding, or it may be some combination of nucleotides from both strands. In the case of the CasX proteins disclosed herein, the PAM is located 5' of the protospacer with a single nucleotide separating the PAM from the first nucleotide of the protospacer. Thus, in the case of reference CasX, a TTC PAM should be understood to mean a sequence following the formula 5'-…NN**TTC**N(protospacer)NNNNNN…3' where 'N' is any DNA nucleotide and '(protospacer)' is a DNA sequence having identity with the targeting sequence of the guide RNA. In the case of a CasX variant with expanded PAM recognition, a TTC, CTC, GTC, or ATC PAM should be

understood to mean a sequence following the formulae: 5'-...NNTTCN(protospacer)NNNNNN...3'; 5'-...NNCTCN(protospacer)NNNNNN...3'; 5'-...NNGTCN(protospacer)NNNNNN...3'; or 5'-...NNATCN(protospacer)NNNNNN...3'. Alternatively, a TC PAM should be understood to mean a sequence following the formula 5'-...NNNTCN(protospacer)NNNNNN...3'.

[0208] In some embodiments, a dCasX variant exhibits greater repression efficiency and/or binding of a target sequence in the target nucleic acid when any one of the PAM sequences TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to the non-target strand of the protospacer having identity with the targeting sequence of the gRNA in a cellular assay system compared to the repression efficiency and/or binding of an RNP comprising a reference dCasX protein in a comparable assay system. In some embodiments, the PAM sequence is TTC. In some embodiments, the PAM sequence is ATC. In some embodiments, the PAM sequence is CTC. In some embodiments, the PAM sequence is GTC.

g. dCasX Fusion Proteins

[0209] In some embodiments, the disclosure provides dXR fusion proteins comprising a heterologous protein.

[0210] In some cases, a heterologous polypeptide (a fusion partner) for use with a dXR provides for subcellular localization, i.e., the heterologous polypeptide contains a subcellular localization sequence (e.g., a nuclear localization signal (NLS) for targeting to the nucleus, a sequence to keep the fusion protein out of the nucleus, e.g., a nuclear export sequence (NES), a sequence to keep the fusion protein retained in the cytoplasm, a mitochondrial localization signal for targeting to the mitochondria, a chloroplast localization signal for targeting to a chloroplast, an ER retention signal, and the like).

[0211] In some cases, a dXR fusion protein includes (is fused to) a nuclear localization signal (NLS). In some cases, a dXR fusion protein is fused to 2 or more, 3 or more, 4 or more, or 5 or more 6 or more, 7 or more, 8 or more NLSs. In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the N-terminus and/or the C-terminus of the dXR fusion protein. In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the N-terminus of the dXR fusion protein. In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the C-terminus of the dXR fusion protein. In some cases, one or more NLSs (3

or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) both the N-terminus and the C-terminus of the dXR fusion protein. In some cases, an NLS is positioned at the N-terminus and an NLS is positioned at the C-terminus of the dXR fusion protein. Representative configurations of dXR with NLS are shown in FIGS. 7, 38, and 45.

[0212] In some cases, non-limiting examples of NLSs suitable for use with a dXR include sequences having at least about 80%, at least about 90%, or at least about 95% identity or are identical to sequences derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO: 33289); the NLS from nucleoplasmin (e.g., the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK (SEQ ID NO: 33290); the c-myc NLS having the amino acid sequence PAAKRVKLD (SEQ ID NO: 33291) or RQRRNELKRSP (SEQ ID NO: 33292); the hRNPAl M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 33293); the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 33294) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO: 33295) and PPKKARED (SEQ ID NO: 33296) of the myoma T protein; the sequence PQPKKKPL (SEQ ID NO: 33297) of human p53; the sequence SALIKKKKKMAP (SEQ ID NO: 33298) of mouse c-abl IV; the sequences DRLRR (SEQ ID NO: 33299) and PKQKKRK (SEQ ID NO: 33300) of the influenza virus NS1; the sequence RKLKKKIKKL (SEQ ID NO: 33301) of the Hepatitis virus delta antigen; the sequence REKKKFLKRR (SEQ ID NO: 33302) of the mouse Mxl protein; the sequence KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 33303) of the human poly(ADP-ribose) polymerase; the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: 33304) of the steroid hormone receptors (human) glucocorticoid; the sequence PRPRKIPR (SEQ ID NO: 33305) of Borna disease virus P protein (BDV-P1); the sequence PPRKKRTVV (SEQ ID NO: 33306) of hepatitis C virus nonstructural protein (HCV-NS5A);the sequence NLSKKKKRKREK (SEQ ID NO: 33307) of LEF1; the sequence RRPSRPFRKP (SEQ ID NO: 33308) of ORF57 simirae; the sequence KRPRSPSS (SEQ ID NO: 33309) of EBV LANA; the sequence KRGINDRNFWRGENERKTR (SEQ ID NO: 33310) of Influenza A protein; the sequence PRPPKMARYDN (SEQ ID NO: 33311) of human RNA helicase A (RHA); the sequence KRSFSKAF (SEQ ID NO: 33312) of nucleolar RNA helicase II; the sequence KLKIKRPVK (SEQ ID NO: 33313) of TUS-protein; the sequence PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33314) associated with importin-alpha; the sequence PKTRRRPRRSQRKRPPT (SEQ ID NO: 33315) from the Rex protein in HTLV-1; the

sequence SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 33316) from the EGL-13 protein of Caenorhabditis elegans; and the sequences KTRRRPRRSQRKRPPT (SEQ ID NO: 33317), RRKKRRPRRKKRR (SEQ ID NO: 33318), PKKKSRKPKKKSRK (SEQ ID NO: 33319), HKKKHPDASVNFSEFSK (SEQ ID NO: 33320), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 33321), LSPSLSPLLSPSLSPL (SEQ ID NO: 33322), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 33323), PKRGRGRPKRGRGR (SEQ ID NO: 33324), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33325), PKKKRKVPPPPKKKRKV (SEQ ID NO: 33326), PAKRARRGYKC (SEQ ID NO: 33327), KLGPRKATGRW (SEQ ID NO: 33328), PRRKREE (SEQ ID NO: 33329), PYRGRKE (SEQ ID NO: 33330), PLRKRPRR (SEQ ID NO: 33331), PLRKRPRRGSPLRKRPRR (SEQ ID NO: 33332), PAAKRVKLDGGKRTADGSEFESPKKKRKV (SEQ ID NO: 33333), PAAKRVKLDGGKRTADGSEFESPKKKRKVGIHGVPAA (SEQ ID NO: 33334), PAAKRVKLDGGKRTADGSEFESPKKKRKVAEAAAKEAAAKEAAAKA (SEQ ID NO: 33335), PAAKRVKLDGGKRTADGSEFESPKKKRKVPG (SEQ ID NO: 33336), KRKGSPERGERKRHW (SEQ ID NO: 33337), KRTADSQHSTPPKTKRKVEFEPKKKRKV (SEQ ID NO: 33338), and PKKKRKVGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV (SEQ ID NO: 33339). In some embodiments, the one or more NLS are linked to the dXR or to adjacent NLS with a linker peptide wherein the linker peptide is selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GSGSGGG (SEQ ID NO: 57628), GGCGGTTCCGGCGGAGGAAGC (SEQ ID NO: 57624), GGCGGTTCCGGCGGAGGTTCC (SEQ ID NO: 57625), GGATCAGGCTCTGGAGGTGGA (SEQ ID NO: 57627), GGAGGGCCGAGCTCTGGCGCACCCCCACCAAGTGGAGGGTCTCCTGCCGGGTCCCCAACATCTACTGAAGAAGGCACCAGCGAATCCGCAACGCCCGAGTCAGGCCCTGGTACCTCCACAGAACCATCTGAAGGTAGTGCGCCTGGTTCCCCAGCTGGAAGCCCTACTTCCACCGAAGAAGGCACGTCAACCGAACCAAGTGAAGGATCTGCCCCTGGGACCAGCACTGAACCATCTGAG (SEQ ID NO: 57620), SSGNSNANSRGPSFSSGLVPLSLRGSH (SEQ ID NO: 57623),

GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGT STEPSEGSAPGTSTEPSE (SEQ ID NO: 57621), TCTAGCGGCAATAGTAACGCTAACAGCCGCGGGCCGAGCTTCAGCAGCGGCCTGGT GCCGTTAAGCTTGCGCGGCAGCCAT (SEQ ID NO: 57622), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO:33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

[0213] In general, NLS (or multiple NLSs) are of sufficient strength to drive accumulation of a reference or dCasX variant fusion protein in the nucleus of a eukaryotic cell. Detection of accumulation in the nucleus may be performed by any suitable technique. For example, a detectable marker may be fused to a reference or dCasX variant fusion protein such that location within a cell may be visualized. Cell nuclei may also be isolated from cells, the contents of which may then be analyzed by any suitable process for detecting protein, such as immunohistochemistry, Western blot, or enzyme activity assay. Accumulation in the nucleus may also be determined indirectly.

[0214] In some embodiments, a dXR comprising an N-terminal NLS comprises a sequence of any one of SEQ ID NOS: 37-112 as set forth in Tables 5 and 6 and SEQ ID NOS: 59359-59432 as set forth in Table 7.

Table 5: N-terminal NLS sequences

NLS Amino Acid Sequence*	NLS ID	SEQ ID NO
PKKKRKVSR	1	37
PKKKRKVGGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**SR	2	38
PKKKRKVGGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**SR	3	39
PAAKRVKLDSR	4	40
PAAKRVKLDGGS**PAAKRVKLD**SR	5	41
PAAKRVKLDGGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**SR	6	42
PAAKRVKLDGGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**SR	7	43
KRPAATKKAGQAKKKKSR	8	44
KRPAATKKAGQAKKKKGGS**KRPAATKKAGQAKKKK**SR	9	45

NLS Amino Acid Sequence*	NLS ID	SEQ ID NO
PAAKRVKLDGGS**PKKKRKV**SR	10	46
PAAKKKKLDGGS**PKKKRKV**SR	11	47
PAAKKKKLDSR	12	48
PAAKKKKLDGGS**PAAKKKKLD**GGS**PAAKKKKLD**SR	13	49
PAAKKKKLDGGS**PAAKKKKLD**GGS**PAAKKKKLD**GGS**PAAKKKKLD**SR	14	50
PAKRARRGYKCSR	15	51
PAKRARRGYKCGS**PAKRARRGYKC**SR	16	52
PRRKREESR	17	53
PYRGRKESR	18	54
PLRKRPRRSR	19	55
PLRKRPRRGS**PLRKRPRR**SR	20	56
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**GGS	21	57
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**PPPPG	22	58
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**GIHGVPAAPG	23	59
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**GGGSGGGSPG	24	60
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**PGGGSGGGSPG	25	61
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**AEAAAKEAAAKEAAAKAPG	26	62
PAAKRVKLDGG**KRTADGSEFESPKKKRKV**PG	27	63
PAAKRVKLDGGS**PKKKRKV**GGS	28	64
PAAKRVKLDPPP**PKKKRKV**PG	29	65
PAAKRVKLDPG	30	66
PAAKRVKLDGGGSGGGSGGGS	31	67
PAAKRVKLDPPP	32	68
PAAKRVKLDGGGSGGGSGGGSPPP	33	69
PKKKRKVPPP	34	70
PKKKRKVGGS	35	71

* Sequences in bold are NLS, while unbolded sequences are linkers.

Table 6: C-terminal NLS sequences

NLS Amino Acid Sequence	NLS ID	SEQ ID NO
GS**PKKKRKV**	1	72
GS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**	2	73
GS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**GGS**PKKKRKV**	3	74
GS**PAAKRVKLD**	4	75
GS**PAAKRVKLD**GGS**PAAKRVKLD**	5	76
GS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**	6	77
GS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**GGS**PAAKRVKLD**	7	78
GS**KRPAATKKAGQAKKKK**	8	79
KRPAATKKAGQAKKKKGGS**KRPAATKKAGQAKKKK**	9	80
GSPAAKRVKLGGSPAAKRVKLGGS**PKKKRKV**GGS**PKKKRKV**	10	81
GS**KLGPRKATGRW**GS	11	82
GS**KRKGSPERGERKRHW**GS	12	83
GS**PKKKRKV**GSGS**KRPAATKKAGQAKKKK**LE	13	84
GP**KRTADSQHSTPPKTKRKV**EFE**PKKKRKV**	14	85
GGGSGGGS**KRTADSQHSTPPKTKRKV**EFE**PKKKRKV**	15	86
AEAAAKEAAAKEAAAKA**KRTADSQHSTPPKTKRKV**EFE**PKKKRKV**	16	87
GPPKKKRKVGGS**KRTADSQHSTPPKTKRKV**EFE**PKKKRKV**	17	88
GPAEAAAKEAAAKEAAAKA**PAAKRVKLD**	18	89
GPGGGSGGGSGGGS**PAAKRVKLD**	19	90
GP**PKKKRKV**PPP**PAAKRVKLD**	20	91
GP**PAAKRVKLD**	21	92
VGS**KRPAATKKAGQAKKKK**	24	95
TGGGPGGGAAAGSGS**PKKKRKV**GSGS**KRPAATKKAGQAKKKK**LE	25	96
TGGGPGGGAAAGSGS**PKKKRKV**GSGS	27	98
PPP**PKKKRKV**PPP	28	99
GGS**PKKKRKV**PPP	29	100
PPP**PKKKRKV**	30	101
GGS**PKKKRKV**	31	102

NLS Amino Acid Sequence	NLS ID	SEQ ID NO
GGS**PKKKRKV**GGSGGSGGS	32	103
GGS**PKKKRKV**GGSPKKKRKV	33	104
GGSGGSGGS**PKKKRKV**GGS**PKKKRKV**	34	105
VGGGSGGGSGGGS**PAAKRVKLD**	35	106
VPPP**PAAKRVKLD**	36	107
VPPPGGGSGGGSGGGS**PAAKRVKLD**	37	108
VGS**PAAKRVKLD**	41	112

* Sequences in bold are NLS, while unbolded sequences are linkers.

Table 7: Additional NLS sequences

N-terminal NLS Sequences	SEQ ID NO
PKKKRKVGGSPKKKRKVSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59359
PKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59360
PKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59361
PAAKRVKLDGGSPAAKRVKLDSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59362
PAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59363
PAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59364
KRPAATKKAGQAKKKKSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59365

C-terminal NLS Sequences	SEQ ID NO
TLESPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDTLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKK	59396
TLESKRPAATKKAGQAKKKKTLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKK	59397
TLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKTLESPKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKV	59398
TLEGGSPKKKRKVTLESPKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKV	59399
TLEGGSPKKKRKVTLESPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLD	59400
TLEGGSPKKKRKVTLESPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLD	59401
TLEGGSPKKKRKVTLESKRPAATKKAGQAKKKK	59402

N-terminal NLS Sequences	SEQ ID NO
KRPAATKKAGQAKKKKSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59366
KRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59367
KRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59368
KRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59369
PKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59370
PAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59371
PAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59372
PAAKRVKLDGGKRTADGSEFESPKKKRKVGGSSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59373
PAAKRVKLDGGKRTADGSEFESPKKKRKVPPPPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59374
PAAKRVKLDGGKRTADGSEFESPKKKRKVGIHGVPAAPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59375
PAAKRVKLDGGKRTADGSEFESPKKKRKVGGGSGGGSPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59376
PAAKRVKLDGGKRTADGSEFESPKKKRKVPGGGSGGGSPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59377
PAAKRVKLDGGKRTADGSEFESPKKKRKVAEAAAKEAAAKEAAAKAPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59378

C-terminal NLS Sequences	SEQ ID NO
TLEGGSPKKKRKVTLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKK	59403
TLEGGSPKKKRKVTLEGGSPKKKRKV	59404
TLEVGPKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEGGSPKKKRKV	59405
TLEVGGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEGGSPKKKRKV	59406
TLEVAEAAAKEAAAKEAAAKAKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEGGSPKKKRKV	59407
TLEVGPPKKKRKVGGSKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEGGSPKKKRKV	59408
TLEVGPAEAAAKEAAAKEAAAKAPAAKRVKLDTLEGGSPKKKRKV	59409
TLEVGPGGGSGGGSGGGSPAAKRVKLDTLEVGPKRTADSQHSTPPKTKRKVEFEPKKKRKV	59410
TLEVGPPKKKRKVPPPPAAKRVKLDTLEVGGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV	59411
TLEVGPPAAKRVKLDTLEVAEAAAKEAAAKEAAAKAKRTADSQHSTPPKTKRKVEFEPKKKRKV	59412
TLEVGPKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEVGPPKKKRKVGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV	59413
TLEVGGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKVTLEVGPAEAAAKEAAAKEAAAKAPAAKRVKLD	59414
GSKRPAATKKAGQAKKKKTLEVGPGGGSGGGSGGGSPAAKRVKLD	59415

N-terminal NLS Sequences	SEQ ID NO
PAAKRVKLDGGKRTADGSEFESPKKKRKVPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59379
PAAKRVKLDGGSPKKKRKVGGSSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59380
PAAKRVKLDPPPPKKKRKVPGSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59381
PAAKRVKLDPGRSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59382
PKKKRKVSRDISRQEIKRINKIRRRLVKDSNTKKAGKTGP	59383
PKKKRKVSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59384
PAAKRVKLDSRQEIKRINKIRRRLVKDSNTKKAGKTGP	59385
TSPKKKRKVALEYPYDVPDYA	59386
TLESKRPAATKKAGQAKKKKAPGEYPYDVPDYA	59387
GSKRPAATKKAGQAKKKKYPYDVPDYA	59388
TLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKAPGEYPYDVPDYATSPKKKRKVALEYPYDVPDYA	59389
TLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKTSPKKKRKVALEYPYDVPDYA	59390
TLESKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKTSPKKKRKVALEYPYDVPDYA	59391
TLESPKKKRKVGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVTLESKRPAATKKAGQAKKKKAPGEYPYDVPDYA	59392
APAAKRVKLDSR	59393
MAPKKKRKVSR	59394

C-terminal NLS Sequences	SEQ ID NO
GSKRPAATKKAGQAKKKKTLEVGPPKKKRKVPPPPAAKRVKLD	59416
GSKRPAATKKAGQAKKKKTLEVGPPAAKRVKLD	59417
GSPKKKRKVTLEVGPKRTADSQHSTPPKTKRKVEFEPKKKRKV	59418
GSKRPAATKKAGQAKKKKTLEVGGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV	59419
GSKRPAATKKAGQAKKKKGSKRPAATKKAGQAKKKK	59420
GSPKKKRKVGSPKKKRKV	59421
GGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKVGSKRPAATKKAGQAKKKK	59422
GPPKKKRKVGGSKRTADSQHSTPPKTKRKVEFEPKKKRKVGSKRPAATKKAGQAKKKK	59423
TGGGPGGGAAAGSGSPKKKRKVGSGSGSKRPAATKKAGQAKKKK	59424
GPKRTADSQHSTPPKTKRKVEFEPKKKRKVGSKRPAATKKAGQAKKKK	59425
AEAAAKEAAAKEAAAKAKRTADSQHSTPPKTKRKVEFEPKKKRKVGSPKKKRKV	59426
GPPKKKRKVPPPPAAKRVKLDGGGSGGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV	59427
GSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGGSPAAKRVKLDGPPKKKRKVGGSKRTADSQHSTPPKTKRKVEFEPKKKRKV	59428
GSPAAKRVKLGGSPAAKRVKLGGSPKKKRKVGGSPKKKRKVTGGGPGGGAAAGSGSPKKKRKVGSGS	59429
GSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKGPKRTADSQHSTPPKTKRKVEFEPKKKRKV	59430
GSKRPAATKKAGQAKKKKGGSKRPAATKKAGQAKKKKAEAAAKEAAAKEAAAKAKRTADSQHSTPPKTKRKVEFEPKKKRKV	59431

N-terminal NLS Sequences	SEQ ID NO
GSPKKKRKV	59395

C-terminal NLS Sequences	SEQ ID NO
GGGSPAAKRVKLD	59432

[0215] In some cases, a dXR fusion protein includes a "Protein Transduction Domain" or PTD (also known as a CPP - cell penetrating peptide), which refers to a protein, polynucleotide, carbohydrate, or organic or inorganic compound that facilitates traversing a lipid bilayer, micelle, cell membrane, organelle membrane, or vesicle membrane. A PTD attached to another molecule, which can range from a small polar molecule to a large macromolecule and/or a nanoparticle, facilitates the molecule traversing a membrane, for example going from an extracellular space to an intracellular space, or from the cytosol to within an organelle. In some embodiments, a PTD is covalently linked to the amino terminus of a dXR fusion protein. In some embodiments, a PTD is covalently linked to the carboxyl terminus of a dXR fusion protein. Examples of PTDs include but are not limited to peptide transduction domain of HIV TAT comprising YGRKKRRQRRR (SEQ ID NO: 33340), RKKRRQRR (SEQ ID NO: 33341); YARAAARQARA (SEQ ID NO: 33342); THRLPRRRRRR (SEQ ID NO: 33343); and GGRRARRRRRR (SEQ ID NO: 33344); a polyarginine sequence comprising a number of arginines sufficient to direct entry into a cell (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines (SEQ ID NO: 33345)); a VP22 domain (Zender et al. (2002) Cancer Gene Ther. 9(6):489-96); an Drosophila Antennapedia protein transduction domain (Noguchi et al. (2003) Diabetes 52(7): 1732-1737); a truncated human calcitonin peptide (Trehin et al. (2004) Pharm. Research 21 :1248-1256); polylysine (Wender et al. (2000) Proc. Natl. Acad. Sci. USA 97: 13003-13008); RRQRRTSKLMKR (SEQ ID NO: 33346); Transportan GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO: 33347); KALAWEAKLAKALAKALAKHLAKALAKALKCEA (SEQ ID NO: 33348); and RQIKIWFQNRRMKWKK (SEQ ID NO: 33349).

[0216] In some embodiments, the individual components of the dXR may be linked via a linker polypeptide (e.g., one or more linker polypeptides). The linker polypeptide may have any of a variety of amino acid sequences. Proteins can be joined by a spacer peptide, generally of a flexible nature, although other chemical linkages are not excluded. Suitable linkers include polypeptides of between 4 amino acids and 40 amino acids in length, or between 4 amino acids and 25 amino acids in length. These linkers are generally produced by using synthetic, linker-

encoding oligonucleotides to couple the proteins. Peptide linkers with a degree of flexibility can be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that the preferred linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine, serine, proline and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art. A variety of different linkers are commercially available and are considered suitable for use. Example linker polypeptides include one or more linkers selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO: 33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), TPPKTKRKVEFE (SEQ ID NO: 33263), GSGSGGG (SEQ ID NO: 57628), GGCGGTTCCGGCGGAGGAAGC (SEQ ID NO: 57624), GGCGGTTCCGGCGGAGGTTCC (SEQ ID NO: 57625), GGATCAGGCTCTGGAGGTGGA (SEQ ID NO: 57627), GGAGGGCCGAGCTCTGGCGCACCCCCACCAAGTGGAGGGTCTCCTGCCGGGTCCCC AACATCTACTGAAGAAGGCACCAGCGAATCCGCAACGCCCGAGTCAGGCCCTGGTA CCTCCACAGAACCATCTGAAGGTAGTGCGCCTGGTTCCCCAGCTGGAAGCCCTACTT CCACCGAAGAAGGCACGTCAACCGAACCAAGTGAAGGATCTGCCCCTGGGACCAGC ACTGAACCATCTGAG (SEQ ID NO: 57620), SSGNSNANSRGPSFSSGLVPLSLRGSH (SEQ ID NO: 57623), GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGT STEPSEGSAPGTSTEPSE (SEQ ID NO: 57621), and TCTAGCGGCAATAGTAACGCTAACAGCCGCGGGCCGAGCTTCAGCAGCGGCCTGGT GCCGTTAAGCTTGCGCGGCAGCCAT (SEQ ID NO: 57622), wherein n is an integer of 1 to 5. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

VI. gRNA and dCRISPR Protein-repressor domain Gene Repression Pairs

[0217] In another aspect, provided herein are compositions comprising a gene repression pair, the gene repression pair comprising a catalytically-dead CRISPR protein with one or more linked repressor domains and a guide RNA. In some embodiments, the gene repressor pair comprises a catalytically-dead Class 2 CRISPR-Cas with one or more linked repressor domains. In some embodiments, the gene repressor pair comprises a catalytically-dead Class 2, Type II, Type V, or Type VI CRISPR protein. In some embodiments, the gene repression pair includes Class 2, Type II CRISPR/Cas proteins such as a catalytically-dead Cas9. In other cases, the gene repression pair include Class 2, Type V CRISPR/Cas nucleases such as catalytically-dead Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f, Cas12g, Cas12h, Cas12i, Cas12j, Cas12k, Cas12l, Cas14, and/or CasΦ proteins.

[0218] In certain embodiments, the gene repression pair comprises a dCasX variant protein as described herein (e.g., any one of the sequences set forth in Table 4) linked to one or more repressor domains (e.g., any one of the sequences of SEQ ID NOS: 889-2100, 2332-33239, 33625-57543, and 59450, while the guide RNA is a gRNA variant as described herein (e.g., SEQ ID NOS: 2238-2331, 57544-57589 and 59352 or a sequence as set forth in Table 2), or sequence variants having at least 60%, or at least 70%, at least about 80%, or at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity thereto, wherein the gRNA comprises a targeting sequence complementary to the target nucleic acid. In some embodiments, the gene repression pair comprises a dCasX selected from any one of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, one or more repressor domains linked to the dCasX selected from any one of the sequences of SEQ ID NOS: 889-2100, 2332-33239, 33625-57543 and 59450, and a gRNA selected from any one of SEQ ID NOS: 2238, 2239, and 2292. In some embodiments, the gene repression pair comprises a dCasX selected from any one of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, one or more repressor domains linked to the dCasX selected from any one of the sequences of SEQ ID NOS: 355-888, 33625-57543, and 59450, and a gRNA selected from any one of SEQ ID NOS: 2238, 2239, and 2292, wherein the gRNA comprises a targeting sequence complementary to the target nucleic acid. In some embodiments, the gene repression pair comprises a dCasX selected from any one of SEQ ID NOS: 17-36 and 59353-59358, one or more repressor domains linked to the dCasX selected from any one of the

sequences of SEQ ID NOS: 355-888, 33625-57543, and 59450, and a gRNA selected from any one of SEQ ID NOS: 2238-2331, 57544-57589 and 59352, wherein the gRNA comprises a targeting sequence complementary to the target nucleic acid.

[0219] In some embodiments, the gene repression pair comprises a dXR comprising a dCasX of SEQ ID NO:18, a KRAB domain sequence of SEQ ID NOS: 57746-57755, a DNMT3A catalytic domain of SEQ ID NOS: 33625-57543 and 59450, a DNMT3L interaction domain of SEQ ID NO: 59625, and an ADD domain of SEQ ID NO: 59452, wherein the dXR has the configuration of configurations 1, 4 or 5 of FIG. 45, and a gRNA of SEQ ID NOS: 2292 or 59352, wherein the gRNA comprises a targeting sequence complementary to the target nucleic acid.

[0220] In other embodiments, a gene repression pair comprises the dCasX protein selected from any one of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4 and one or more repressor domains linked to the dCasX, a first gRNA (a gRNA variant as described herein (e.g., SEQ ID NOS: 2238-2331, 57544-57589 and 59352, or a sequence as set forth in Table 2) with a targeting sequence, and a second gRNA variant and dXR, wherein the second gRNA variant has a targeting sequence complementary to a different or overlapping portion of the target nucleic acid compared to the targeting sequence of the first gRNA.

[0221] In some embodiments, wherein the gene repression pair comprises both a dCasX variant protein and the linked repressor domain and a gRNA variant as described herein, the one or more characteristics of the gene repression pair is improved beyond what can be achieved by varying the dCasX protein or the gRNA alone. In some embodiments, the dCasX variant protein and the gRNA variant act additively to improve one or more characteristics of the gene repression pair. In some embodiments, the dCasX variant protein and the gRNA variant act synergistically to improve one or more characteristics of the gene repression pair. In the foregoing embodiments, the improvement is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 50-fold, at least about 100-fold, at least about 500-fold, at least about 1000-fold, at least about 5000-fold, at least about 10,000-fold, or at least about 100,000-fold compared to the characteristic of a reference dCasX protein and reference gRNA pair.

VII. Vectors

[0222] In some embodiments, provided herein are vectors comprising polynucleotides encoding the catalytically-dead CRISPR protein and linked repressor domains and gRNA

variants described herein. In some cases, the vectors are utilized for the expression and recovery of the catalytically-dead CRISPR protein (e.g., dXR) and the gRNA components of the gene repression pair or the RNP. In other cases, the vectors are utilized for the delivery of the encoding polynucleotides to target cells for the repression of the target nucleic acid, as described more fully, below.

[0223] In some embodiments, provided herein are polynucleotides encoding the gRNA variants described herein. In some embodiments, said polynucleotides are DNA. In other embodiments, said polynucleotides are RNA. In other embodiments, said polynucleotides are mRNA. In some embodiments, provided herein are vectors comprising the polynucleotides sequences encoding the gRNA variants described herein. In some embodiments, the vectors comprising the polynucleotides include bacterial plasmids, viral vectors, and the like. In some embodiments, a dXR and a gRNA variant are encoded on the same vector. In some embodiments, a dXR and a gRNA variant are encoded on different vectors.

[0224] In some embodiments, the disclosure provides a vector comprising a nucleotide sequence encoding the components of the dXR:gRNA system. For example, in some embodiments provided herein is a recombinant expression vector comprising a) a nucleotide sequence encoding a dXR fusion protein; and b) a nucleotide sequence encoding a gRNA variant described herein. In some cases, the nucleotide sequence encoding the dXR fusion protein and/or the nucleotide sequence encoding the gRNA variant are operably linked to a promoter that is operable in a cell type of choice (e.g., a prokaryotic cell, a eukaryotic cell, a plant cell, an animal cell, a mammalian cell, a primate cell, a rodent cell, a human cell). Suitable promoters for inclusion in the vectors are described herein, below.

[0225] In some embodiments, the nucleotide sequence encoding the dXR fusion protein is codon optimized. This type of optimization can entail a mutation of a dCasX-encoding nucleotide sequence to mimic the codon preferences of the intended host organism or cell while encoding the same protein. Thus, the codons can be changed, but the encoded protein remains unchanged. For example, if the intended target cell was a human cell, a human codon-optimized dCasX variant-encoding nucleotide sequence could be used. As another non-limiting example, if the intended host cell were a mouse cell, then a mouse codon-optimized dCasX variant-encoding nucleotide sequence could be generated. As another non-limiting example, if the intended host cell were a bacterial cell, then a bacterial codon-optimized dXR fusion protein-encoding nucleotide sequence could be generated.

[0226] In some embodiments, a nucleotide sequence encoding a dXR fusion protein is mRNA, designed for incorporation into an LNP. In some embodiments, an mRNA encoding a dXR fusion protein of the disclosure is chemically modified, wherein the chemical modification is substitution of N1-methyl-pseudouridine for one or more uridine nucleotides of the sequence. In some embodiments, an mRNA encoding a dXR fusion protein of the disclosure is codon optimized. In some embodiments, an mRNA encoding a dXR fusion protein of the disclosure comprises one or more sequences selected from the group consisting of SEQ ID NOS: 59584, 59585, 59610, 59611, 59622 and 59623. In some embodiments, an mRNA encoding a dXR fusion protein of the disclosure comprises one or more sequences encoded by a sequence selected from the group consisting of 59444-59449, 59455-59456, 59488-59497, 59568-59583, 59595-59609, and 59612-59621.

[0227] In some embodiments, provided herein are one or more recombinant expression vectors such as (i) a nucleotide sequence that encodes a gRNA as described herein (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell); and (ii) a nucleotide sequence encoding a dXR fusion protein (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell). In some embodiments, the sequences encoding the gRNA and dXR fusion proteins are in different recombinant expression vectors, and in other embodiments the gRNA and dXR fusion proteins are in the same recombinant expression vector. In some embodiments, either the gRNA in the recombinant expression vector, the dXR fusion protein encoded by the recombinant expression vector, or both, are variants of a reference dCasX protein or gRNAs as described herein. In the case of the nucleotide sequence encoding the gRNA, the recombinant expression vector can be transcribed *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase in order to produce the gRNA, which can then be recovered by conventional methods; e.g., purification via gel electrophoresis. Once synthesized, the gRNA may be utilized in the gene repression pair to directly contact a target nucleic acid or may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc.).

[0228] Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector.

[0229] In some embodiments, a nucleotide sequence encoding a dXR and/or gRNA is operably linked to a control element; e.g., a transcriptional control element, such as a promoter. In some embodiments, a nucleotide sequence encoding a dXR fusion protein is operably linked to a control element; e.g., a transcriptional control element, such as a promoter. In some cases, the promoter is a constitutively active promoter. In some cases, the promoter is a regulatable promoter. In some cases, the promoter is an inducible promoter. In some cases, the promoter is a tissue-specific promoter. In some cases, the promoter is a cell type-specific promoter. In some cases, the transcriptional control element (e.g., the promoter) is functional in a targeted cell type or targeted cell population. For example, in some cases, the transcriptional control element can be functional in eukaryotic cells, e.g., hematopoietic stem cells (e.g., mobilized peripheral blood (mPB) CD34(+) cell, bone marrow (BM) CD34(+) cell, etc.). By transcriptional activation, it is intended that transcription will be increased above basal levels in the target cell by 10-fold, by 100-fold, more usually by 1000-fold.

[0230] Non-limiting examples of Pol II promoters include, but are not limited to EF-1alpha, EF-1alpha core promoter, Jens Tornoe (JeT), promoters from cytomegalovirus (CMV), CMV immediate early (CMVIE), CMV enhancer, herpes simplex virus (HSV) thymidine kinase, early and late simian virus 40 (SV40), the SV40 enhancer, long terminal repeats (LTRs) from retrovirus, mouse metallothionein-I, adenovirus major late promoter (Ad MLP), CMV promoter full-length promoter, the minimal CMV promoter, the chicken β-actin promoter (CBA), CBA hybrid (CBh), chicken β-actin promoter with cytomegalovirus enhancer (CB7), chicken beta-Actin promoter and rabbit beta-Globin splice acceptor site fusion (CAG), the rous sarcoma virus (RSV) promoter, the HIV-Ltr promoter, the hPGK promoter, the HSV TK promoter, a 7SK promoter, the Mini-TK promoter, the human synapsin I (SYN) promoter which confers neuron-specific expression, beta-actin promoter, super core promoter 1 (SCP1), the Mecp2 promoter for selective expression in neurons, the minimal IL-2 promoter, the Rous sarcoma virus enhancer/promoter (single), the spleen focus-forming virus long terminal repeat (LTR) promoter, the TBG promoter, promoter from the human thyroxine-binding globulin gene (Liver specific), , the PGK promoter, the human ubiquitin C promoter (UBC), the UCOE promoter (Promoter of HNRPA2B1-CBX3), the synthetic CAG promoter, the Histone H2 promoter, the Histone H3 promoter, the U1a1 small nuclear RNA promoter (226 nt), the U1a1 small nuclear RNA promoter (226 nt), the U1b2 small nuclear RNA promoter (246 nt) 26, the GUSB promoter, the CBh promoter, rhodopsin (Rho) promoter, silencing-prone spleen focus forming virus (SFFV)

promoter, a human H1 promoter (H1), a POL1 promoter, the TTR minimal enhancer/promoter, the b-kinesin promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter, the human eukaryotic initiation factor 4A (EIF4A1) promoter, the ROSA26 promoter, the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, tRNA promoters, and truncated versions and sequence variants of the foregoing. In a particular embodiment, the Pol II promoter is EF-1alpha, wherein the promoter enhances transfection efficiency, the transgene transcription or expression of the CRISPR nuclease, the proportion of expression-positive clones and the copy number of the episomal vector in long-term culture. Non-limiting examples of Pol III promoters include, but are not limited to U6, mini U6, U6 truncated promoters, BiH1 (Bidirectional H1 promoter), BiU6, Bi7SK, BiH1 (Bidirectional U6, 7SK, and H1 promoters), gorilla U6, rhesus U6, human 7SK, human H1 promoter, and truncated versions and sequence variants thereof. In the foregoing embodiment, the Pol III promoter enhances the transcription of the gRNA.

[0231] Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression. The expression vector may also include nucleotide sequences encoding protein tags (e.g., 6xHis tag, hemagglutinin tag, fluorescent protein, etc.) that can be fused to the dXR fusion protein, thus resulting in a chimeric CasX variant polypeptide.

[0232] Recombinant expression vectors of the disclosure can also comprise elements that facilitate robust expression of dXR and/or variant gRNAs of the disclosure. For example, recombinant expression vectors can include one or more of a polyadenylation signal (poly(A), an intronic sequence or a post-transcriptional regulatory element such as a woodchuck hepatitis post-transcriptional regulatory element (WPRE). Exemplary poly(A) sequences include hGH poly(A) signal (short), HSV TK poly(A) signal, synthetic polyadenylation signals, SV40 poly(A) signal, β-globin poly(A) signal and the like. In addition, vectors used for providing a nucleic acid encoding a gRNA and/or a dXR protein to a cell may include nucleic acid sequences that encode for selectable markers in the target cells, so as to identify cells that have taken up the gRNA and/or dXR protein. A person of ordinary skill in the art will be able to select suitable elements to include in the recombinant expression vectors described herein.

[0233] A recombinant expression vector sequence can be packaged into a virus or virus-like particle (also referred to herein as a “particle” or “virion”) for subsequent infection and

transformation of a cell, ex vivo, *in vitro* or *in vivo*. Such particles or virions will typically include proteins that encapsidate or package the vector genome. In some embodiments, a recombinant expression vector of the present disclosure is a recombinant adeno-associated virus (AAV) vector. In some embodiments, a recombinant expression vector of the present disclosure is a recombinant lentivirus vector. In some embodiments, a recombinant expression vector of the present disclosure is a recombinant retroviral vector.

a. Recombinant AAV for delivery of dXR:rRNA

[0234] Adeno-associated virus (AAV) is a small (20 nm), nonpathogenic virus that is useful in treating human diseases in situations that employ a viral vector for delivery to a cell such as a eukaryotic cell, either *in vivo* or *ex vivo* for cells to be prepared for administering to a subject. A construct is generated, for example a construct encoding a fusion protein and gRNA embodiments as described herein, and is flanked with AAV inverted terminal repeat (ITR) sequences, thereby enabling packaging of the AAV vector into an AAV viral particle, with the assistance of the AAV cap coding region sequences, described below.

[0235] An "AAV" vector may refer to the naturally occurring wild-type virus itself or derivatives thereof. The term covers all subtypes, serotypes and pseudotypes, and both naturally occurring and recombinant forms, except where required otherwise. As used herein, the term "serotype" refers to an AAV which is identified by and distinguished from other AAVs based on capsid protein reactivity with defined antisera, e.g., there are many known serotypes of primate AAVs. In some embodiments, the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV 10, AAV11, AAV12, AAV 44.9, AAV 9.45, AAV 9.61, AAV-Rh74 (Rhesus macaque-derived AAV), and AAVRh10, and modified capsids of these serotypes. For example, serotype AAV-2 is used to refer to an AAV which contains capsid proteins encoded from the cap gene of AAV-2 and a genome containing 5′ and 3′ ITR sequences from the same AAV-2 serotype. Pseudotyped AAV refers to an AAV that contains capsid proteins from one serotype and a viral genome including 5′-3′ ITRs of a second serotype. Pseudotyped rAAV would be expected to have cell surface binding properties of the capsid serotype and genetic properties consistent with the ITR serotype. Pseudotyped recombinant AAV (rAAV) are produced using standard techniques described in the art. As used herein, for example, rAAV1 may be used to refer an AAV having both capsid proteins and 5′-3′ ITRs from the same serotype or it may refer to an AAV having capsid proteins from serotype 1 and 5′-3′ ITRs from a different AAV serotype, e.g., AAV serotype 2. For each example illustrated herein

the description of the vector design and production describes the serotype of the capsid and 5′-3′ ITR sequences.

[0236] An "AAV virus" or "AAV viral particle" refers to a viral particle composed of at least one AAV capsid protein (preferably by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide. If the particle additionally comprises a heterologous polynucleotide (i.e., a polynucleotide other than a wild-type AAV genome to be delivered to a mammalian cell, termed a "transgene"), it is typically referred to as "rAAV". An exemplary heterologous polynucleotide is a polynucleotide comprising a dXR protein and/or sgRNA of any of the embodiments described herein. Being naturally replication-defective and capable of transducing nearly every cell type in the human body, AAV represents a suitable vector for therapeutic use in gene therapy or vaccine delivery. Typically, when producing a recombinant AAV vector, the sequence between the two ITRs is replaced with one or more sequences of interest (e.g., a transgene), and the Rep and Cap sequences are provided in trans, making the ITRs the only viral DNA that remains in the vector. The resulting recombinant AAV vector genome construct comprises two cis-acting 130 to 145-nucleotide ITRs flanking an expression cassette encoding the transgene sequences of interest, providing at least 4.7 kb or more for packaging of foreign DNA that can include a transgene, one or more promoters and accessory elements, such that the total size of the vector is below 5 to 5.2 kb, which is compatible with packaging within the AAV capsid (it being understood that as the size of the construct exceeds this threshold, the packaging efficiency of the vector decreases). The transgene may be used, in the context of the present disclosure to repress transcription of a defective gene in the cells of a subject. In the context of CRISPR-mediated gene repression, however, the size limitation of the expression cassette is a challenge for most CRISPR systems (e.g., Cas9), given the large size of the nucleases. It has been discovered, however, that the small size of the dCasX and gRNA permits the creation of "all in one" constructs that can deliver dXR:gRNA capable of gene repression in cells.

[0237] By "adeno-associated virus inverted terminal repeats" or "AAV ITRs" is meant the art recognized regions found at each end of the AAV genome which function together in cis as origins of DNA replication and as packaging signals for the virus. AAV ITRs, together with the AAV rep coding region, provide for the efficient excision and rescue from, and integration of a nucleotide sequence interposed between two flanking ITRs into a mammalian cell genome. The nucleotide sequences of AAV ITR regions are known. See, for example Kotin, R.M. (1994)

Human Gene Therapy 5:793-801; Berns, K. I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.). As used herein, an AAV ITR need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, and AAVRh10, and modified capsids of these serotypes. Furthermore, 5′ and 3′ ITRs which flank a selected nucleotide sequence in an AAV vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the heterologous sequence into the recipient cell genome when AAV Rep gene products are present in the cell. Use of AAV serotypes for integration of heterologous sequences into a host cell is known in the art (*see*, e.g., WO2018195555A1 and US20180258424A1, incorporated by reference herein.). In one particular embodiment, the ITRs are derived from serotype AAV1. In another particular embodiment of the AAV of the disclosure, the ITRs are derived from serotype AAV2, the 5' ITR having sequence CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGAC CTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACT CCATCACTAGGGGTTCCT (SEQ ID NO: 33350) and the 3' ITR having sequence AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG AGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTG AGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO: 33351).

[0238] By "AAV rep coding region" is meant the region of the AAV genome which encodes the replication proteins Rep 78, Rep 68, Rep 52 and Rep 40. These Rep expression products have been shown to possess many functions, including recognition, binding and nicking of the AAV origin of DNA replication, DNA helicase activity and modulation of transcription from AAV (or other heterologous) promoters. The Rep expression products are collectively required for replicating the AAV genome.

[0239] By "AAV cap coding region" is meant the region of the AAV genome which encodes the capsid proteins VP1, VP2, and VP3, or functional homologues thereof. These Cap expression products supply the packaging functions which are collectively required for packaging the viral genome.

[0240] In some embodiments, AAV capsids utilized for delivery of a transgene comprising the encoding sequences for the dXR and gRNA of the disclosure to a host cell can be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 44.9, AAV-Rh74 (Rhesus macaque-derived AAV), and AAVRh10, and the AAV ITRs are derived from AAV serotype 1 or serotype 2.

[0241] In order to produce rAAV viral particles, an AAV expression vector is introduced into a suitable host cell using known techniques, such as by transfection. Packaging cells are typically used to form virus particles; such cells include HEK293 cells (and other cells known in the art), which package adenovirus. A number of transfection techniques are generally known in the art; see, e.g., Sambrook et al. (1989) Molecular Cloning, a laboratory manual, Cold Spring Harbor Laboratories, New York. Particularly suitable transfection methods include calcium phosphate co-precipitation, direct microinjection into cultured cells, electroporation, liposome mediated gene transfer, lipid-mediated transduction, and nucleic acid delivery using high-velocity microprojectiles.

[0242] In some embodiments, host cells transfected with the above-described AAV expression vectors are rendered capable of providing AAV helper functions in order to replicate and encapsidate the nucleotide sequences flanked by the AAV ITRs to produce rAAV viral particles. AAV helper functions are generally AAV-derived coding sequences which can be expressed to provide AAV gene products that, in turn, function in trans for productive AAV replication. AAV helper functions are used herein to complement necessary AAV functions that are missing from the AAV expression vectors. Thus, AAV helper functions include one, or both of the major AAV ORFs (open reading frames), encoding the rep and cap coding regions, or functional homologues thereof. Accessory functions can be introduced into and then expressed in host cells using methods known to those of skill in the art. Commonly, accessory functions are provided by infection of the host cells with an unrelated helper virus. In some embodiments, accessory functions are provided using an accessory function vector. Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc., may be used in the expression vector.

[0243] The present disclosure provides AAV comprising a transgene encoding a dXR and a gRNA, wherein the dXR comprises a dCasX and a KRAB domain as the single repressor, given

the size limitations of the transgene. In some embodiments, the transgene encodes a dXR fusion protein of the systems comprising a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the transgene encodes a dXR fusion protein of the systems comprising a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments, the transgene encodes a dXR fusion protein of the systems comprising a single KRAB domain operably linked to the dCasX selected from the group of sequences of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In a particular embodiment, the transgene encodes a dXR fusion protein of the systems comprising a single KRAB domain operably linked to the dCasX of SEQ ID NOS: 18 as set forth in Table 4, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. The transgene of the foregoing embodiments further encodes a gRNA having a scaffold comprising a sequence of SEQ ID NO: 2292 or 59352, or a sequence having at least about 70%, at least about 80%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto, wherein the gRNA comprises a targeting

sequence complementary to a target nucleic acid sequence of a gene targeted for repression. In the foregoing embodiments, the dXR and gRNA are each operably linked to a promoter, embodiments of which are described herein.

b. VLP and XDP for delivery of dXR:gRNA

[0244] In other embodiments, retroviruses, for example, lentiviruses, may be suitable for use as vectors for delivery of the encoding nucleic acids of the gene repressor systems of the present disclosure. Commonly used retroviral vectors are "defective"; e.g. unable to produce viral proteins required for productive infection, and may be referred to a virus-like particles (VLP) or as a delivery particle (XDP), depending on the components utilized. Rather, replication of the vector requires growth in a packaging cell line. To generate viral particles comprising nucleic acids of interest, the retroviral nucleic acids comprising the nucleic acid are packaged into VLP or XDP capsids by a packaging cell line. Different packaging cell lines provide a different envelope protein (ecotropic, amphotropic or xenotropic) to be incorporated into the capsid, this envelope protein determining the specificity of the viral particle for the cells (ecotropic for murine and rat; amphotropic for most mammalian cell types including human, dog and mouse; and xenotropic for most mammalian cell types except murine cells). The appropriate packaging cell line may be used to ensure that the cells are targeted by the packaged viral particles. Methods of introducing subject vector expression vectors into packaging cell lines and of collecting the viral particles that are generated by the packaging lines are well known in the art.

[0245] In some embodiments, the disclosure provides vectors encoding or comprising a gene repressor system comprising a dXR fusion protein, wherein the dXR fusion protein comprises a first transcriptional repressor domain, and wherein the dXR comprises a catalytically-dead CasX of any of the embodiments described herein linked to a KRAB domain of any of the embodiments described herein as the first repressor domain.

[0246] In other embodiments, the disclosure provides vectors encoding or comprising a gene repressor system comprising a fusion protein, wherein the fusion protein comprises a catalytically-dead CasX of any of the embodiments described herein linked to a first, a second, and a third transcriptional repressor domain, wherein first transcriptional repressor domain is a KRAB domain of any of the embodiments described herein, the second domain is a DNMT3A catalytic domain of any of the embodiments described herein, the third transcriptional repressor domain is a DNMT3L interaction domain, and the fusion protein comprises one or more NLS and linker peptides. In some embodiments, the fusion protein is configured, from N-terminus to

C-terminus: NLS-Linker4-DNMT3A CD-Linker2- DNMT3L ID-Linker 1-Linker3-dCasX-Linker3-KRAB-NLS; NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-DNMT3A CD-Linker2-DNMT3L ID; NLS-Linker3-dCasX-Linker1-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-NLS; NLS-KRAB-Linker3-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS, or NLS-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-Linker1-dCasX-Linker3-NLS.

[0247] In other embodiments, the disclosure provides vectors encoding or comprising a gene repressor system comprising a fusion protein, wherein the fusion protein comprises a catalytically-dead CasX of any of the embodiments described herein linked to a first, a second, a third, and a fourth transcriptional repressor domain, wherein first transcriptional repressor domain is a KRAB domain of any of the embodiments described herein, the second domain is a DNMT3A catalytic domain of any of the embodiments described herein, the third transcriptional repressor domain a DNMT3L interaction domain, and the fourth transcriptional repressor domain is a ATRX-DNMT3-DNMT3L (ADD) domain linked N-terminal to the DNMT3A catalytic domain and the fusion protein comprises one or more NLS and linker peptides. In some embodiments, the fusion protein is configured, from N-terminus to C-terminus: NLS-Linker4-ADD-DNMT3A CD-Linker2- DNMT3L ID-Linker 1-Linker3-dCasX-Linker3-KRAB-NLS; NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-ADD-DNMT3A CD-Linker2-DNMT3L ID; NLS-Linker3-dCasX-Linker1-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-NLS; NLS-KRAB-Linker3- ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS, or NLS- ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-Linker1-dCasX-Linker3-NLS.

[0248] In some embodiments, the present disclosure provides XDP comprising components selected from all or a portion of a retroviral gag polyprotein, a gag-poly polyprotein, dXR:gRNA RNPs, RNA trafficking components, and one or more tropism factors having binding affinity for a cell surface marker of a target cell to facilitates entry of the XDP into the target cell.

[0249] In some embodiments, the retroviral components of the XDP system are derived from a *Orthretrovirinae* virus or a *Spumaretrovirinae* virus wherein the *Orthretrovirinae* virus is selected from the group consisting of *Alpharetrovirus, Betaretrovirus, Deltaretrovirus, Epsilonretrovirus, Gammaretrovirus*, and *Lentivirus,* and the *Spumaretrovirinae* virus is selected from the group consisting of *Bovispumavirus, Equispumavirus, Felispumavirus, Prosimiispumavirus, Simiispumavirus,* and *Spumavirus.*

[0250] XDP for use with the dXR:gRNA system can be constructed in different configurations based on the components utilized. In some embodiments, XDP comprise one or more retroviral components selected from a Gag polyprotein, a Gag-transframe region-pol protease polyprotein (Gag-TFR-PR), matrix protein (MA), a nucleocapsid protein (NC), a capsid protein (CA), a p1 peptide, a p6 peptide, a p2A peptide, a p2B peptide, a p10 peptide, a p12 peptide, a p21/24 peptide, a p12/p3/p8 peptide, a p20 peptide, a protease cleavage site, and a protease capable of cleaving the protease cleavage sites, which can be encoded on one or more nucleic acids for the production of the XDP in the packaging cell. The remaining components, such as the encapsidated payload of dXR and the gRNA (complexed as RNPs), RNA trafficking components (described below) used to increase the incorporation of RNP into the XDP, and the tropism factor, can be incorporated into the nucleic acid encoding the retroviral components or can be encoded on separate nucleic acids. In some embodiments, the components of the XDP system are encoded on a single nucleic acid, on two nucleic acids, on three nucleic acids, on four nucleic acids, or on five nucleic acids which, in turn, are incorporated into plasmids used in the transfection to create the XDP in packaging cells. Representative, non-limiting configurations of plasmids used to make XDP in the packaging cells are presented in FIGS. 4 and 5. In a particular embodiment of the configuration of FIG. 4, the Gag polyprotein of plasmid 1 and the Gag-TFR-PR polyprotein of plasmid 2 are derived from Lentivirus (with an HIV-1 protease), the encoded MS2 of plasmid 1 comprises the sequence of SEQ ID NO: 33276, the encoded dXR fusion protein of plasmid 3 comprises any of the dXR embodiments described herein, the VSV-G plasmid encodes the VSV-G sequence of SEQ ID NO: 113, and the gRNA plasmid encodes a scaffold of SEQ ID NO: 2292 or 59352. In some embodiments, the components of the XDP system are capable of self-assembling into an XDP with the incorporated RNP of the dXR:gRNA when the one or more nucleic acids are introduced into a eukaryotic host cell and are expressed. In the foregoing embodiment, the dXR:gRNA RNP is encapsidated within the XDP upon self-assembly of the XDP. In a particular embodiment, the tropism factor is incorporated on the XDP surface upon self-assembly of the XDP. XDP compositions and methods of making XDP are described in WO2021113772A1 and PCT/US22/32579, incorporated by reference herein.

[0251] The polynucleotides encoding the Gag, dXR and gRNA of any of the embodiments described herein can further comprise paired components designed to assist the trafficking of the components out of the nucleus of the host cell and facilitate recruitment of the complexed

CasX:gRNA into the budding XDP. Non-limiting examples of such non-covalent trafficking components include hairpin RNA or loops such as MS2 hairpin, PP7 hairpin, Qβ hairpin, boxB, transactivation response element (TAR), Rev response element, phage GA hairpin, and U1 hairpin II that have binding affinity for MS2 coat protein, PP7 coat protein, Qβ coat protein, protein N, protein Tat, Rev, phage GA coat protein, and U1A signal recognition particle, respectively, that are fused to the Gag polyprotein. It has been discovered that the incorporation of the binding partner inserted into the guide RNA and the packaging recruiter into the nucleic acid comprising the Gag polypeptide facilitates the packaging of the XDP particle due, in part, to the affinity of the CasX for the gRNA, resulting in an RNP, such that both the gRNA and CasX are associated with Gag during the encapsidation process of the XDP, increasing the proportion of XDP comprising RNP compared to a construct lacking the binding partner and packaging recruiter. In other embodiments, the gRNA can comprise Rev response element (RRE) or portions thereof that have binding affinity to Rev, which can be linked to the Gag polyprotein. In other embodiments, the gRNA can comprise one or more RRE and one or more MS2 hairpin sequences. The RRE can be selected from the group consisting of Stem IIB of Rev response element (RRE), Stem II-V of RRE, Stem II of RRE, Rev-binding element (RBE) of Stem IIB, and full-length RRE. In the foregoing embodiment, the components include sequences of UGGGCGCAGCGUCAAUGACGCUGACGGUACA (Stem IIB, SEQ ID NO: 57736), GCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCAGACAAUUAUUGU CUGGUAUAGUGC (Stem II, SEQ ID NO: 57737), CAGGAAGCACUAUGGGCGCAGCGUCAAUGACGCUGACGGUACAGGCCAGACAAU UAUUGUCUGGUAUAGUGCAGCAGCAGAACAAUUUGCUGAGGGCUAUUGAGGCGC AACAGCAUCUGUUGCAACUCACAGUCUGGGGCAUCAAGCAGCUCCAGGCAAGAA UCCUG (Stem II-V, SEQ ID NO: 57738), GCUGACGGUACAGGC (RBE, SEQ ID NO: 57739), and AGGAGCUUUGUUCCUUGGGUUCUUGGGAGCAGCAGGAAGCACUAUGGGCGCAGC GUCAAUGACGCUGACGGUACAGGCCAGACAAUUAUUGUCUGGUAUAGUGCAGCA GCAGAACAAUUUGCUGAGGGCUAUUGAGGCGCAACAGCAUCUGUUGCAACUCAC AGUCUGGGGCAUCAAGCAGCUCCAGGCAAGAAUCCUGGCUGUGGAAAGAUACCU AAAGGAUCAACAGCUCCU (full-length RRE, SEQ ID NO: 57740). In other embodiments, the gRNA can comprise one or more RRE and one or more MS2 hairpin sequences. In a

particular embodiment, the gRNA comprises an MS2 hairpin variant that is optimized to increase the binding affinity to the MS2 coat protein, thereby enhancing the incorporation of the gRNA and associated CasX into the budding XDP.

[0252] In some embodiments, the tropism factor incorporated on the XDP surface is selected from the group consisting of a glycoprotein, an antibody fragment, a receptor, and a ligand to a target cell marker. In one embodiment of the foregoing, the tropism factor is a glycoprotein having a sequence selected from the group consisting of the sequences set forth in Table 8, or a sequence having at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto. In a particular embodiment, the glycoprotein is VSV-G.

Table 8: Glycoproteins for XDP

SEQ ID NO	Virus	Plasmid
113	Vesicular Stomatitis Virus	pGP2
114	Human Immunodeficiency Virus	pGP3
115	Avian leukosis virus	pGP4
116	Rous Sarcoma Virus	pGP5
117	Mouse mammary tumor virus	pGP6
118	Human T-lymphotropic virus 1	pGP7
119	RD114 Endogenous Feline Retrovirus	pGP8
120	Gibbon ape leukemia virus	pGP9
121	Moloney Murine leukemia virus	pGP10
122	Baboon Endogenous Virus	pGP11
123	Human Foamy Virus	pGP12
124	Pseudorabies virus	pGP13.1
125	Pseudorabies virus	pGP13.2
126	Pseudorabies virus	pGP13.3
127	Pseudorabies virus	pGP13.4
128	Herpes simplex virus 1 (HHV1)	pGP14.1
129	Herpes simplex virus 1 (HHV1)	pGP14.2
130	Herpes simplex virus 1 (HHV1)	pGP14.3
131	Herpes simplex virus 1 (HHV1)	pGP14.4
132	Hepatitis C Virus	pGP23
133	Rabies Virus	pGP29
134	Mokola Virus	pGP30

SEQ ID NO	Virus	Plasmid
135	Measles Virus	pGP32.1
136	Measles Virus	pGP32.2
137	Ebola Zaire Virus	pGP41
138	Dengue	pGP25
139	Zika virus	pGP26
140	West Nile Virus	pGP27
141	Japanese Encephalitis Virus	pGP28
142	Hepatitis G Virus	pGP24
143	Mumps Virus F	pGP31.1
144	Mumps Virus HN	pGP31.2
145	Sendai Virus F	pGP33.1
146	Sendai Virus HN	pGP33.2
147	AcMNPV gp64	pGP59
148	Ross River Virus	pGP54
149	Codon optimized rabies virus	pGP29.2
150	Rabies virus (strain Nishigahara RCEH) (RABV)	pGP29.3
151	Rabies virus (strain India) (RABV)	pGP29.4
152	Rabies virus (strain CVS-11) (RABV)	pGP29.5
153	Rabies virus (strain ERA) (RABV)	pGP29.6
154	Rabies virus (strain SAD B19) (RABV)	pGP29.7
155	Rabies virus (strain Vnukovo-32) (RABV)	pGP29.8
156	Rabies virus (strain Pasteur vaccins / PV) (RABV)	pGP29.9
157	Rabies virus (strain PM1503/AVO1) (RABV)	pGP29.1
158	Rabies virus (strain China/DRV) (RABV)	pGP29.11
159	Rabies virus (strain China/MRV) (RABV)	pGP29.12
160	Rabies virus (isolate Human/Algeria/1991) (RABV)	pGP29.13
161	Rabies virus (strain HEP-Flury) (RABV)	pGP29.14
162	Rabies virus (strain silver-haired bat-associated) (RABV) (SHBRV)	pGP29.15
163	HSV2 gB	pGP15.1
164	HSV2 gD	pGP15.2
165	HSV2 gH	pGP15.3
166	HSV2 gL	pGP15.4
167	Varicella gB	pGP16.1
168	Varicella gK	pGP16.2
169	Varicella gH	pGP16.3
170	Varicella gL	pGP16.4
171	Hepatitis B gL	pGP22.1

SEQ ID NO	Virus	Plasmid
172	Hepatitis B gM	pGP22.2
173	Hepatitis B gS	pGP22.3
174	Eastern equine encephalitis virus (EEEV)	pGP65
175	Venezuelan equine encephalitis viruses (VEEV)	pGP66
176	Western equine encephalitis virus (WEEV)	pGP67
177	Semliki Forest virus	pGP68
178	Sindbis virus	pGP69
179	Chikungunya virus (CHIKV)	pGP70
180	Bornavirus BoDV-1	pGP58
181	Tick-borne encephalitis virus (TBEV)	pGP71
182	Usutu virus	pGP72
183	St. Louis encephalitis virus	pGP73
184	Yellow fever virus	pGP74
185	Dengue virus 2	pGP75
186	Dengue virus 3	pGP76
187	Dengue virus 4	pGP77
188	Murray Valley encephalitis virus (MVEV)	pGP78
189	Powassan virus	pGP79
190	H5 Hemagglutinin	pGP80
191	H7 Hemagglutinin	pGP81
192	N1 Neuraminidase	pGP82
193	Canine Distemper Virus	pGP83
194	VSAV	pGP92
195	ABVV	pGP99
196	CARV	pGP98
197	CHPV	pGP97
198	COCV	pGP100
199	VSIV	pGP91
200	ISFV	pGP90
201	JURV	pGP87
202	MSPV	pGP89
203	MARV	pGP88
204	MORV	pGP101
205	VSNJV	pGP84
206	PERV	pGP85
207	PIRYV	pGP94
208	RADV	pGP96
209	YBV	pGP86

SEQ ID NO	Virus	Plasmid
210	VSV CEN AM - 94GUB	pGP93
211	VSV South America 85CLB	pGP95
212	Nipah Virus	pGP34.1
213	Nipah Virus	pGP34.2
214	Hendra Virus	pGP35.1
215	Hendra Virus	pGP35.2
216	Newcastle disease virus	pGP37.1
217	Newcastle disease virus	pGP37.2
218	RSV f0	pGP55.1
219	RSV G	pGP55.2
220	Bovine respiratory syncytial virus (strain Rb94) (BRS)	pGP102
221	Murine pneumonia virus (strain 15) (MPV)	pGP103
222	Measles virus (strain Edmonston) (MeV) (Subacute sclerose panencephalitis virus)	pGP104
223	Measles virus (strain Edmonston B) (MeV) (Subacute sclerose panencephalitis virus)	pGP105
224	Human respiratory syncytial virus B (strain B1)	pGP106
225	Rinderpest virus (strain RBOK) (RDV)	pGP107
226	Simian virus 41 (SV41)	pGP108
227	Mumps virus (strain Miyahara vaccine) (MuV)	pGP109
228	Canine distemper virus (strain Onderstepoort) (CDV)	pGP110
229	Human respiratory syncytial virus A (strain Long)	pGP111
230	Sendai virus (strain Fushimi) (SeV)	pGP112
231	Human respiratory syncytial virus A (strain RSS-2)	pGP113
232	Rinderpest virus (strain RBT1) (RDV)	pGP114
233	Measles virus (strain Leningrad-16) (MeV) (Subacute sclerose panencephalitis virus)	pGP115
234	Human parainfluenza 2 virus (HPIV-2)	pGP116
235	Avian metapneumovirus (isolate Canada goose/Minnesota/15a/2001) (AMPV)	pGP117
236	Phocine distemper virus (PDV)	pGP118
237	Sendai virus (strain Harris) (SeV)	pGP119
238	Bovine parainfluenza 3 virus (BPIV-3)	pGP120
239	Measles virus (strain Ichinose-B95a) (MeV) (Subacute sclerose panencephalitis virus)	pGP121
240	Human parainfluenza 2 virus (strain Toshiba) (HPIV-2)	pGP122
241	Newcastle disease virus (strain B1-Hitchner/47) (NDV)	pGP123
242	Measles virus (strain Yamagata-1) (MeV) (Subacute sclerose panencephalitis virus)	pGP124

SEQ ID NO	Virus	Plasmid
243	Measles virus (strain IP-3-Ca) (MeV) (Subacute sclerose panencephalitis virus)	pGP125
244	Measles virus (strain Edmonston-AIK-C vaccine) (MeV) (Subacute sclerose panencephalitis virus)	pGP126
245	Turkey rhinotracheitis virus (TRTV)	pGP127
246	Human parainfluenza 2 virus (strain Greer) (HPIV-2)	pGP128
247	Hendra virus (isolate Horse/Autralia/Hendra/1994)	pGP129
248	Human metapneumovirus (strain CAN97-83) (HMPV)	pGP130
249	Bovine respiratory syncytial virus (strain Copenhagen) (BRS)	pGP131
250	Sendai virus (strain Z) (SeV) (Sendai virus (strain HVJ))	pGP132
251	Human parainfluenza 3 virus (strain Wash/47885/57) (HPIV-3) (Human parainfluenza 3 virus (strain NIH 47885))	pGP133
252	Mumps virus (strain SBL-1) (MuV)	pGP134
253	Measles virus (strain Edmonston-Zagreb vaccine) (MeV) (Subacute sclerose panencephalitis virus)	pGP135
254	Human parainfluenza 1 virus (strain C39) (HPIV-1)	pGP136
255	Sendai virus (strain Hamamatsu) (SeV)	pGP137
256	Mumps virus (strain RW) (MuV)	pGP138
257	Infectious hematopoietic necrosis virus (strain Oregon69) (IHNV)	pGP139
258	Drosophila melanogaster sigma virus (isolate Drosophila/USA/AP30/2005) (DMelSV)	pGP140
259	Hirame rhabdovirus (strain Korea/CA 9703/1997) (HIRRV)	pGP141
260	Sonchus yellow net virus (SYNV)	pGP142
261	European bat lyssavirus 1 (strain Bat/Germany/RV9/1968) (EBLV1)	pGP143
262	Lagos bat virus (LBV)	pGP144
263	Duvenhage virus (DUVV)	pGP145
264	West Caucasian bat virus (WCBV)	pGP146
265	European bat lyssavirus 2 (strain Human/Scotland/RV1333/2002) (EBLV2)	pGP147
266	Irkut virus (IRKV)	pGP148
267	Tupaia virus (isolate Tupaia/Thailand/-/1986) (TUPV)	pGP149
268	Rabies virus (strain ERA) (RABV)	pGP150
269	Ovine respiratory syncytial virus (strain WSU 83-1578) (ORSV)	pGP151
270	Human respiratory syncytial virus A (strain rsb5857)	pGP152
271	Piry virus (PIRYV)	pGP153
272	Human respiratory syncytial virus A (strain rsb6190)	pGP154
273	Rabies virus (strain SAD B19) (RABV)	pGP155

SEQ ID NO	Virus	Plasmid
274	Australian bat lyssavirus (isolate Human/AUS/1998) (ABLV)	pGP156
275	Rabies virus (strain Vnukovo-32) (RABV)	pGP157
276	Aravan virus (ARAV)	pGP158
277	Sigma virus	pGP159
278	Viral hemorrhagic septicemia virus (strain 07-71) (VHSV)	pGP160
279	Rabies virus (strain Pasteur vaccins / PV) (RABV)	pGP161
280	Bovine respiratory syncytial virus (strain Rb94) (BRS)	pGP162
281	Tibrogargan virus (strain CS132) (TIBV)	pGP163
282	Infectious hematopoietic necrosis virus (strain Round Butte) (IHNV)	pGP164
283	Human respiratory syncytial virus B (strain 18537)	pGP165
284	Adelaide River virus (ARV)	pGP166
285	Australian bat lyssavirus (isolate Bat/AUS/1996) (ABLV)	pGP167
286	Bovine ephemeral fever virus (strain BB7721) (BEFV)	pGP168
287	Isfahan virus (ISFV)	pGP169
288	Rabies virus (strain silver-haired bat-associated) (RABV) (SHBRV)	pGP170
289	Snakehead rhabdovirus (SHRV)	pGP171
290	Infectious hematopoietic necrosis virus (strain WRAC) (IHNV)	pGP172
291	Zaire ebolavirus (strain Kikwit-95) (ZEBOV) (Zaire Ebola virus)	pGP173
292	Sudan ebolavirus (strain Maleo-79) (SEBOV) (Sudan Ebola virus)	pGP174
293	Tai Forest ebolavirus (strain Cote d'Ivoire-94) (TAFV) (Cote d'Ivoire Ebola virus)	pGP175
294	Reston ebolavirus (strain Philippines-96) (REBOV) (Reston Ebola virus)	pGP176
295	Lake Victoria marburgvirus (strain Angola/2005) (MARV)	pGP177
296	Zaire ebolavirus (strain Eckron-76) (ZEBOV) (Zaire Ebola virus)	pGP178
297	Reston ebolavirus (strain Reston-89) (REBOV) (Reston Ebola virus)	pGP179
298	Tai Forest ebolavirus (strain Cote d'Ivoire-94) (TAFV) (Cote d'Ivoire Ebola virus)	pGP180
299	Lake Victoria marburgvirus (strain Ozolin-75) (MARV) (Marburg virus (strain South Africa/Ozolin/1975))	pGP181
300	Zaire ebolavirus (strain Mayinga-76) (ZEBOV) (Zaire Ebola virus)	pGP182
301	Lake Victoria marburgvirus (strain Popp-67) (MARV) (Marburg virus (strain West Germany/Popp/1967))	pGP183

SEQ ID NO	Virus	Plasmid
302	Sudan ebolavirus (strain Boniface-76) (SEBOV) (Sudan Ebola virus)	pGP184
303	Reston ebolavirus (strain Reston-89) (REBOV) (Reston Ebola virus)	pGP185
304	Sudan ebolavirus (strain Human/Uganda/Gulu/2000) (SEBOV) (Sudan Ebola virus)	pGP186
305	Zaire ebolavirus (strain Gabon-94) (ZEBOV) (Zaire Ebola virus)	pGP187
306	Reston ebolavirus (strain Reston-89) (REBOV) (Reston Ebola virus)	pGP188
307	Simian virus 41 (SV41)	pGP189
308	Newcastle disease virus (strain D26/76) (NDV)	pGP190
309	Xenotropic MuLV-related virus (isolate VP42) (XMRV)	pGP191
310	Xenotropic MuLV-related virus (isolate VP62) (XMRV)	pGP192
311	Simian immunodeficiency virus (isolate F236/smH4) (SIV-sm) (Simian immunodeficiency virus sooty mangabey monkey)	pGP193
312	Simian immunodeficiency virus (isolate Mm251) (SIV-mac) (Simian immunodeficiency virus rhesus monkey)	pGP194
313	Simian immunodeficiency virus (isolate GB1) (SIV-mnd) (Simian immunodeficiency virus mandrill)	pGP195
314	Simian immunodeficiency virus (isolate Mm142-83) (SIV-mac) (Simian immunodeficiency virus rhesus monkey)	pGP196
315	Simian immunodeficiency virus (isolate MB66) (SIV-cpz) (Chimpanzee immunodeficiency virus)	pGP197
316	Simian immunodeficiency virus (isolate EK505) (SIV-cpz) (Chimpanzee immunodeficiency virus)	pGP198
317	Feline immunodeficiency virus (strain UK2) (FIV)	pGP199
318	Feline immunodeficiency virus (strain San Diego) (FIV)	pGP200
319	Feline immunodeficiency virus (isolate Wo) (FIV)	pGP201
320	Feline immunodeficiency virus (isolate Petaluma) (FIV)	pGP202
321	Feline immunodeficiency virus (strain UK8) (FIV)	pGP203
322	Feline immunodeficiency virus (strain UT-113) (FIV)	pGP204
323	Mayoro Virus	pGP205
324	Barmah Forest Virus	pGP206
325	Aura virus	pGP207
326	Bebaru Virus	pGP208
327	Middleburg virus	pGP209
328	Mucambo virus	pGP210
329	Ndumu Virus	pGP211
330	O'nyong-nyong virus	pGP212

SEQ ID NO	Virus	Plasmid
331	Pixuna virus	pGP213
332	Tonate Virus	pGP214
333	Trocara virus	pGP215
334	Whataroa virus	pGP216
335	Bussuquara virus	pGP217
336	Jugra virus	pGP218

[0253] In some embodiments, the protease encoded in the nucleic acids utilized in the XDP system is selected from the group consisting of HIV-1 protease, tobacco etch virus protease (TEV), potyvirus HC protease, potyvirus P1 protease, PreScission (HRV3C protease), b virus NIa protease, B virus RNA-2-encoded protease, aphthovirus L protease, enterovirus 2A protease, rhinovirus 2A protease, picorna 3C protease, comovirus 24K protease, nepovirus 24K protease, RTSV (rice tungro spherical virus) 3C-like protease, parsnip yellow fleck virus protease, 3C-like protease, heparin, cathepsin, thrombin, factor Xa, metalloproteinase, and enterokinase.

[0254] In some embodiments, the present disclosure provides eukaryotic cells transfected with the plasmids encoding the XDP system of any one of the foregoing embodiments, wherein the cell is a packaging cell capable of facilitating the expression of the encoded dXR:gRNA and XDP components and the assembly of the XDP particles that encapsidate RNP of the dXR and gRNA. In some embodiments, the eukaryotic cell is selected from the group consisting of HEK293 cells, HEK293T cells, Lenti-X 293T cells, BHK cells, HepG2, Saos-2, HuH7, NS0 cells, SP2/0 cells, YO myeloma cells, A549 cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells, hybridoma cells, VERO, NIH3T3 cells, COS, WI38, MRC5, A549, HeLa cells, CHO cells, and HT1080 cells. In some embodiments, the packaging host cell can be modified to reduce or eliminate cell surface markers or receptors that would otherwise be incorporated into the XDP, thereby reducing an immune response to the cell surface markers or receptors by the subject receiving an administration of the XDP. Such markers can include receptors or proteins capable of being bound by MHC receptors or that would otherwise trigger an immune response in a subject. In some embodiments, the packaging host cell is modified to reduce or eliminate the expression of a cell surface marker selected from the group consisting of B2M, CIITA, PD1, and HLA-E KI, wherein the incorporation of the marker is reduced on the surface of the XDP. In some embodiments, the packaging host cell is modified to express one or more cell surface

markers selected from the group consisting of CD46, CD47, CD55, CD59, CD24, CD58, SLAMF4, and SLAMF3 (serving as "don't eat me" signals), wherein the cell surface marker is incorporated onto the surface of the XDP, wherein said incorporation disables XDP engulfment and phagocytosis by host surveillance cells such as macrophages and monocytes.

[0255] For non-viral delivery, vectors can also be delivered wherein the vector or vectors encoding and/or comprising the dXR and gRNA are formulated in nanoparticles, wherein the nanoparticles contemplated include, but are not limited to nanospheres, liposomes, quantum dots, polyethylene glycol particles, hydrogels, and micelles. As described more fully, below, lipid nanoparticles are generally composed of an ionizable cationic lipid and three or more additional components, such as cholesterol, DOPE, polylactic acid-co-glycolic acid, and a polyethylene glycol (PEG) containing lipid. In some embodiments, mRNA encoding the dXR variants of the embodiments disclosed herein are formulated in a lipid nanoparticle. In some embodiments, the nanoparticle comprises the gRNA of the embodiments disclosed herein. In some embodiments, the nanoparticle comprises mRNA encoding the dXR and the gRNA. In some embodiments, the components of the dXR:gRNA system are formulated in separate nanoparticles for delivery to cells or for administration to a subject in need thereof.

c. Lipid Nanoparticles (LNP)

[0256] In another aspect, the present disclosure provides lipid nanoparticles (LNP) for delivery of a gRNA and an mRNA encoding a fusion protein of any of the system embodiments disclosed herein. In certain embodiments, a composition described herein comprises LNP encapsidating a gene repressor system of the disclosure (i.e., an mRNA encoding a fusion protein (e.g., a dXR) and a gRNA with a targeting sequence to the target nucleic acid) which represses transcription of a target gene.

[0257] In some embodiments, the LNP of the disclosure are tissue- or organ-specific, have excellent biocompatibility, and can deliver the systems comprising mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid with high efficiency, and thus can be usefully used for the repression or silencing of the target nucleic acid of a gene in cells of a subject having a disease or disorder.

[0258] In their native forms, nucleic acid polymers are unstable in biological fluids and cannot penetrate the membrane of target cells to be delivered to the cytoplasm, thus requiring delivery systems capable of entering a cell. Lipid nanoparticles (LNP) have proven useful for both the protection and delivery of nucleic acids to tissues and cells. Furthermore, the use of mRNA in

LNP to encode the CRISPR nuclease eliminates the possibility of undesirable genome integration compared to DNA vectors. Moreover, mRNA efficiently transfects both mitotic and non-mitotic cells, as it does not require entry into the nucleus since it exerts its function in the cytoplasmic compartment. LNP as a delivery platform offers the additional advantage of being able to co-formulate both the mRNA encoding the CRISPR nuclease and the gRNA into single LNP particles.

[0259] Accordingly, in various embodiments, the disclosure encompasses LNP and compositions that may be used for a variety of purposes, including the delivery of encapsulated dXR:gRNA systems to cells, both in vitro and in vivo. In some embodiments, the gRNA for use in the LNP is the sequence of SEQ ID NO: 59352. In some embodiments, the gRNA for use in the LNP comprises one or more chemical modifications to the sequence. In some embodiments, the mRNA for incorporation into the LNP of the disclosure encode any of the dXR embodiments described herein. In some embodiments, the mRNA for incorporation into the LNP of the disclosure are codon optimized. In some embodiments, an mRNA encoding a dXR fusion protein of the disclosure is chemically modified, wherein the chemical modification is substitution of N1-methyl-pseudouridine for one or more uridine nucleotides of the sequence. In some embodiments, an mRNA for incorporation into the LNP of the disclosure comprises one or more sequences selected from the group consisting of SEQ ID NOS: 59584-59585, 59610, 59611, 59622 and 59623. In some embodiments, In some embodiments, an mRNA for incorporation into the LNP of the disclosure comprises one or more sequences encoded by a sequence selected from the group consisting of 59444-59449, 59455-59456, 59488-59497, 59568-59583, 59595-59609, and 59612-59621.

[0260] In some embodiments, the disclosure encompasses LNP encapsidating a gRNA and an mRNA encoding a fusion protein of a dCasX linked to a first repressor domain, wherein the repressor domain is a KRAB domain of any of the embodiments described herein. In some embodiments, the disclosure encompasses LNP encapsidating a gRNA and an mRNA encoding a fusion protein of a dCasX linked to a first and a second repressor domain, wherein the first repressor domain is a KRAB domain and the second repressor domain is a DNMT3A catalytic domain. In some embodiments, the disclosure encompasses LNP encapsidating a gRNA and an mRNA encoding a fusion protein of a dCasX linked to a first, a second, and a third repressor domain, wherein the first repressor domain is a KRAB domain, the second repressor domain is a DNMT3A catalytic domain, and the third domain is a DNMT3L interaction domain. In some

embodiments, the disclosure encompasses LNP encapsidating a gRNA and an mRNA encoding a fusion protein of a dCasX linked to a first, a second, a third, and a fourth repressor domain, wherein the first repressor domain is a KRAB domain, the second repressor domain is a DNMT3A catalytic domain, the third domain is a DNMT3L interaction domain, and the fourth domain is a DNMT3A ADD domain. In the foregoing embodiments, the components of the fusion protein can be arrayed in alternate configurations, as portrayed in FIG. 7 and FIG. 45. In certain embodiments, the disclosure encompasses methods of treating or preventing diseases or disorders in a subject in need thereof by contacting the subject with an LNP that encapsulates the dXR:gRNA systems of the embodiments described herein, wherein the dXR is an encoding mRNA and the gRNA comprises a targeting sequence complementary to a target nucleic acid in cells of the subject.

[0261] In some embodiments, the present disclosure provides LNP in which the gRNA and mRNA encoding the dXR are incorporated into single LNP particles. In certain embodiments, the LNP composition includes a ratio of gRNA to dXR mRNA of the embodiments described herein from about 25:1 to about 1:25, as measured by weight. In certain embodiments, the LNP formulation includes a ratio of gRNA to dXR mRNA, such as dXR mRNA from about 10: 1 to about 1:10. In certain embodiments, the LNP formulation includes a ratio of gRNA to dXR mRNA from about 8:1 to about 1:8. In some embodiments, the LNP formulation includes a ratio of gRNA to dXR mRNA, from about 5:1 to about 1:5. In some embodiments, ratio range is about 3:1 to 1:3, about 2:1 to 1:2, about 5:1 to 1:2, about 5:1 to 1:1, about 3:1 to 1:2, about 3:1 to 1:1, about 3:1, about 2:1 to 1:1. In some embodiments, the gRNA to mRNA ratio is about 3:1 or about 2:1. In some embodiments the ratio of gRNA to dXR mRNA is about 1:1. The ratio may be about 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25.

[0262] In other embodiments, the present disclosure provides LNP in which the gRNA and mRNA encoding the dXR are incorporated into separate LNP particles, which can be formulated together in varying ratios for administration.

[0263] In some embodiments, the optimized mRNA of the disclosure encoding the CasX protein may be provided in a solution to be mixed with a lipid solution such that the mRNA may be encapsulated in the LNP. A suitable mRNA solution may be any aqueous solution containing mRNA to be encapsulated at various concentrations. For example, a suitable mRNA solution may contain an mRNA at a concentration of or greater than about 0.01 mg/ml, 0.05 mg/ml, 0.06 mg/ml, 0.07 mg/ml, 0.08 mg/ml, 0.09 mg/ml, 0.1 mg/ml, 0.15 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4

mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.25 mg/ml, 1.5 mg/ml, 1.75 mg/ml, or 2.0 mg/ml. In some embodiments, a suitable mRNA solution may contain an mRNA at a concentration ranging from about 0.01-2.0 mg/ml, 0.01-1.5 mg/ml, 0.01-1.25 mg/ml, 0.01-1.0 mg/ml, 0.01-0.9 mg/ml, 0.01-0.8 mg/ml, 0.01-0.7 mg/ml, 0.01-0.6 mg/ml, 0.01-0.5 mg/ml, 0.01-0.4 mg/ml, 0.01-0.3 mg/ml, 0.01-0.2 mg/ml, 0.01-0.1 mg/ml, 0.05-1.0 mg/ml, 0.05-0.9 mg/ml, 0.05-0.8 mg/ml, 0.05-0.7 mg/ml, 0.05-0.6 mg/ml, 0.05-0.5 mg/ml, 0.05-0.4 mg/ml, 0.05-0.3 mg/ml, 0.05-0.2 mg/ml, 0.05-0.1 mg/ml, 0.1-1.0 mg/ml, 0.2-0.9 mg/ml, 0.3-0.8 mg/ml, 0.4-0.7 mg/ml, or 0.5-0.6 mg/ml. In some embodiments, a suitable mRNA solution may contain an mRNA at a concentration up to about 5.0 mg/ml, 4.0 mg/ml, 3.0 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.02 mg/ml, 0.01 mg/ml, or 0.05 mg/ml.

[0264] In some embodiments, the gRNA of the disclosure may be provided in a solution to be mixed with a lipid solution such that the gRNA may be encapsulated in the LNP. A suitable gRNA solution may be any aqueous solution containing gRNA to be encapsulated at various concentrations. For example, a suitable gRNA solution may contain a gRNA at a concentration of or greater than about 0.01 mg/ml, 0.05 mg/ml, 0.06 mg/ml, 0.07 mg/ml, 0.08 mg/ml, 0.09 mg/ml, 0.1 mg/ml, 0.15 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.25 mg/ml, 1.5 mg/ml, 1.75 mg/ml, or 2.0 mg/ml. In some embodiments, a suitable gRNA solution may contain an gRNA at a concentration ranging from about 0.01-2.0 mg/ml, 0.01-1.5 mg/ml, 0.01-1.25 mg/ml, 0.01-1.0 mg/ml, 0.01-0.9 mg/ml, 0.01-0.8 mg/ml, 0.01-0.7 mg/ml, 0.01-0.6 mg/ml, 0.01-0.5 mg/ml, 0.01-0.4 mg/ml, 0.01-0.3 mg/ml, 0.01-0.2 mg/ml, 0.01-0.1 mg/ml, 0.05-1.0 mg/ml, 0.05-0.9 mg/ml, 0.05-0.8 mg/ml, 0.05-0.7 mg/ml, 0.05-0.6 mg/ml, 0.05-0.5 mg/ml, 0.05-0.4 mg/ml, 0.05-0.3 mg/ml, 0.05-0.2 mg/ml, 0.05-0.1 mg/ml, 0.1-1.0 mg/ml, 0.2-0.9 mg/ml, 0.3-0.8 mg/ml, 0.4-0.7 mg/ml, or 0.5-0.6 mg/ml. In some embodiments, a suitable gRNA solution may contain a gRNA at a concentration up to about 5.0 mg/ml, 4.0 mg/ml, 3.0 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.02 mg/ml, 0.01 mg/ml,or 0.05 mg/ml.

[0265] Early formulations of LNP utilizing permanently cationic lipids resulted in LNPs with positive surface charge that proved toxic in vivo, plus were rapidly cleared by phagocytic cells. By changing to ionizable cationic lipids bearing tertiary or quaternary amines, especially those

with pKa < 7, resulting LNP achieve efficient encapsulation of nucleic acid polymers at low pH by interacting electrostatically with the negative charges of the phosphate backbone of mRNA or gRNA, that also result in largely neutral systems at physiological pH values, thus alleviating problems associated with permanently-charged cationic lipids. Herein, "ionizable lipid" means an amine-containing lipid which can be easily protonated, and for example, it may be a lipid of which charge state changes depending on the surrounding pH. The ionizable lipid may be protonated (positively charged) at a pH below the pKa of a cationic lipid, and it may be substantially neutral at a pH over the pKa. In one example, the LNP may comprise a protonated ionizable lipid and/or an ionizable lipid showing neutrality. In some embodiments, the LNP has a pKa of 5 to 8, 5.5 to 7.5, 6 to 7, or 6.5 to 7. The pKa of the LNP is important for in vivo stability and release of the nucleic acid payload of the LNP. In some embodiments, the LNP having the foregoing pKa ranges may be safely delivered to a target organ (for example, the liver, lung, heart, spleen, as well as to tumors) and/or target cell (hepatocyte, LSEC, cardiac cell, cancer cell, etc.) in vivo, and after endocytosis, exhibit a positive charge to release the encapsulated payload through electrostatic interaction with an anionic protein of the endosome membrane.

[0266] The ionizable lipid is an ionizable compound having characteristics similar to lipids generally, and through electrostatic interaction with a nucleic acid (for example, an mRNA or gRNA of the disclosure), may play a role of encapsulating the nucleic acid within the LNP with high efficiency.

[0267] According to the type of the amine comprised in the ionizable lipid, (i) the nucleic acid encapsulation efficiency, (ii) PDI (polydispersity index) and/or (iii) the nucleic acid delivery efficiency to tissue and/or cells constituting an organ (for example, hepatocytes or liver sinusoidal endothelial cells in the liver) of the LNP may be different. In certain embodiments, the ionizable cationic lipid comprises from about 46 mol % to about 66 mol % of the total lipid present in the particle.

[0268] The LNP comprising an ionizable lipid comprising an amine may have one or more kinds of the following characteristics: (1) encapsulating a drug or biologic with high efficiency; (2) uniform size of prepared particles (or having a low PDI value); and/or (3) superior nucleic acid delivery efficiency to organs such as liver, lung, heart, spleen, as well as to tumors, and/or cells constituting such organs (for example, hepatocytes, LSEC, cardiac cells, cancer cells, etc.).

[0269] The lipid composition of lipid nanoparticles usually consists of an ionizable amino lipid, a helper lipid (usually a phospholipid), cholesterol, and a polyethylene glycol-lipid conjugate (PEG-lipid) to improve the colloidal stability in biological environments by reducing a specific absorption of plasma proteins and forming a hydration layer over the nanoparticles, and are formulated at typical mole ratios of 50:10:37-39:1.5-2.5, with variations made to adjust individual properties. As the PEG-lipid forms the surface lipid, the size of the LNP can be readily varied by varying the proportion of surface (PEG) lipid to the core (ionizable cationic) lipids. In some embodiments, the PEG-lipid can be varied from ~1 to 5 mol% to modify particle properties such as size, stability, and circulation time. In particular, the cationic lipid form plays a crucial role both in nucleic acid encapsulation through electrostatic interactions and intracellular release by disrupting endosomal membranes. The mRNA and gRNA (with targeting sequences) are encapsulated within the LNP by the ionic interactions they form with the positively charged cationic (or ionizable) lipid. Non-limiting examples of ionizable cationic lipid components utilized in the LNP of the disclosure are selected from DLin-MC3-DMA (heptatriaconta-6,9,28,31-tetraen-19-yl4-(dimethylamino)butanoate), DLin- KC2-DMA (2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane), and TNT (1,3,5-triazinane-2,4,6-trione) and TT (N1,N3,N5-tris(2-aminoethyl)benzene-1,3,5-tricarboxamide). Non-limiting examples of helper lipids utilized in the LNP of the disclosure are selected from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), POPC (2-Oleoyl-1- palmitoyl-sn-glycero-3-phosphocholine) and DOPE (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine). Cholesterol and PEG-DMG ((R)-2,3-bis(octadecyloxy)propyl-1-(methoxy polyethylene glycol 2000) carbamate) or PEG-DSG (1,2-Distearoyl-rac-glycero-3-methylpolyoxyethylene glycol 2000) are components utilized for the stability, circulation, and size of the LNP.

[0270] In other embodiments, the ionizable cationic lipid in the nucleic acid-lipid particles of the disclosure may comprise, for example, one or more ionizable cationic lipids wherein the ionizable cationic lipid is a dialkyl lipid. In another embodiment, the ionizable cationic lipid is a trialkyl lipid. In one particular embodiment, the ionizable cationic lipid is selected from the group consisting of 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-di-.gamma.-linolenyloxy-N,N-dimethylaminopropane (gamma.-DLinDMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-K-C2-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), dilinoleylmethyl-3-dimethylaminopropionate (DLin-M-C2-DMA), or salts thereof and

mixtures thereof. In a particular embodiment, the ionizable cationic lipid is selected from the group consisting of 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-di-.gamma.-linolenyloxy-N,N-dimethylaminopropane (.gamma.-DLenDMA; a salt thereof, or a mixture thereof. In some embodiments, the N/P ratio (nitrogen from the cationic/ionizable lipid and phosphate from the nucleic acid) is in the range of is about 3:1 to 7:1, or about 4:1 to 6:1, or is 3:1, or is 4:1, or is 5:1, or is 6:1, or is 7:1.

[0271] The phospholipid of the elements of the LNP according to one example plays a role of covering and protecting a core formed by interaction of the ionizable lipid and nucleic acid in the LNP, and may facilitate cell membrane permeation and endosomal escape during intracellular delivery of the nucleic acid by binding to the phospholipid bilayer of a target cell.

[0272] For the phospholipid, a phospholipid which can promote fusion of the LNP according to one example may be used without limitation, and for example, it may be one or more kinds selected from the group consisting of dioleoylphosphatidylethanolamine (DOPE), distearoylphosphatidylcholine (DSPC), palmitoyloleoylphosphatidylcholine (POPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), distearoylphosphatidylethanolamine (DSPE), phosphatidylethanolamine (PE), dipalmitoylphosphatidylethanolamine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine(POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine(POPC), 1,2-dioleoyl-sn-glycero-3-[phospho-L-serine](DOPS), 1,2-dioleoyl-sn-glycero-3-[phospho-L-serine] and the like. In one example, the LNP comprising DOPE may be effective in mRNA delivery (excellent delivery efficacy).

[0273] The cholesterol of the elements of the LNP according to one example may provide morphological rigidity to lipid filling in the LNP and be dispersed in the core and surface of the nanoparticle to improve the stability of the nanoparticle.

[0274] Herein, "lipid-PEG (polyethyleneglycol) conjugate", "lipid-PEG", "PEG-lipid", "PEG-lipid", or "lipid-PEG" refers to a form in which lipid and PEG are conjugated, and means a lipid in which a polyethylene glycol (PEG) polymer which is a hydrophilic polymer is bound to one end. The lipid-PEG conjugate contributes to the particle stability in serum of the nanoparticle within the LNP, and plays a role of preventing aggregation between nanoparticles. In addition, the lipid-PEG conjugate may protect nucleic acids from degrading enzyme during in vivo

delivery of the nucleic acids and enhance the stability of nucleic acids in vivo and increase the half-life of the drug or biologic encapsulated in the nanoparticle. Examples of PEG-lipid conjugates include, but are not limited to, PEG-DAG conjugates, PEG-DAA conjugates, and mixtures thereof. In certain embodiments, the PEG-lipid conjugate is selected from the group consisting of a PEG-diacylglycerol (PEG-DAG) conjugate, a PEG-dialkyloxypropyl (PEG-DAA) conjugate, a PEG-phospholipid conjugate, a PEG-ceramide (PEG-Cer) conjugate, and a mixture thereof. In certain embodiments, the PEG-lipid conjugate is a PEG-DAA conjugate. In certain embodiments, the PEG-DAA conjugate in the lipid particle may comprise a PEG-didecyloxypropyl (C_{10}) conjugate, a PEG-dilauryloxypropyl (C_{12}) conjugate, a PEG-dimyristyloxypropyl (C_{14}) conjugate, a PEG-dipalmityloxypropyl (C_{16}) conjugate, a PEG-distearyloxypropyl (C_{18}) conjugate, or mixtures thereof. In certain embodiments, wherein the PEG-DAA conjugate is a PEG-dimyristyloxypropyl (C_{14}) conjugate. In other embodiments, the lipid-PEG conjugate may be PEG bound to phospholipid such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramide (PEG-CER, ceramide-PEG conjugate, ceramide-PEG, cholesterol or PEG conjugated to derivative thereof, PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE(DSPE-PEG), and a mixture thereof, and for example, may be C16-PEG2000 ceramide (N-palmitoyl-sphingosine-1-{succinyl[methoxy(polyethylene glycol)2000]}), DMG-PEG 2000, 14:0 PEG2000 PE.

[0275] In certain embodiments, the conjugated lipid that inhibits aggregation of particles comprises from about 0.5 mol % to about 3 mol % of the total lipid present in the particle.

[0276] In one example, the average molecular weight of the lipid-PEG conjugate may be 100 daltons to 10,000 daltons, 200 daltons to 8,000 daltons, 500 daltons to 5,000 daltons, 1,000 daltons to 3,000 daltons, 1,000 daltons to 2,600 daltons, 1,500 daltons to 2,600 daltons, 1,500 daltons to 2,500 daltons, 2,000 daltons to 2,600 daltons, 2,000 daltons to 2,500 daltons, or 2,000 daltons.

[0277] For the lipid in the lipid-PEG conjugate, any lipid capable of binding to polyethyleneglycol may be used without limitation, and the phospholipid and/or cholesterol which are other elements of the LNP may be also used. Specifically, the lipid in the lipid-PEG conjugate may be ceramide, dimyristoylglycerol (DMG), succinoyl-diacylglycerol (s-DAG), distearoylphosphatidylcholine (DSPC), distearoylphosphatidylethanolamine (DSPE), or cholesterol, but not limited thereto.

[0278] In the lipid-PEG conjugate, the PEG may be directly conjugated to the lipid or linked to the lipid via a linker moiety. Any linker moiety suitable for binding PEG to the lipid may be used, and for example, includes an ester-free linker moiety and an ester-containing linker moiety. The ester-free linker moiety includes not only amido (-C(O)NH-), amino (-NR-), carbonyl (-C(O)-), carbamate (-NHC(O)O-), urea (-NHC(O)NH-), disulfide (-S-S-), ether (-O-), succinyl (-(O)CCH2CH2C(O)-), succinamidyl (-NHC(O)CH2CH2C(O)NH-), ether, disulfide but also combinations thereof (for example, a linker containing both a carbamate linker moiety and an amido linker moiety), but not limited thereto. The ester-containing linker moiety includes for example, carbonate (-OC(O)O-), succinoyl, phosphate ester (-O-(O)POH-O-), sulfonate ester, and combinations thereof, but not limited thereto.

[0279] In certain embodiments, the nucleic acid-lipid particle has a total lipid:gRNA mass ratio of from about 5:1 to about 15:1. In some embodiments, the weight ratio of the ionizable lipid and nucleic acid comprised in the LNP may be 1 to 20:1, 1 to 15:1, 1 to 10:1, 5 to 20:1, 5 to 15:1, 5 to 10:1, 7.5 to 20:1, 7.5 to 15:1, or 7.5 to 10:1.

[0280] In some embodiments, the LNP may comprise the ionizable lipid of 20 to 50 parts by weight, phospholipid of 10 to 30 parts by weight, cholesterol of 20 to 60 parts by weight (or 20 to 60 parts by weight), and lipid-PEG conjugate of 0.1 to 10 parts by weight (or 0.25 to 10 parts by weight, 0.5 to 5 parts by weight). The LNP may comprise the ionizable lipid of 20 to 50 % by weight, phospholipid of 10 to 30 % by weight, cholesterol of 20 to 60 % by weight (or 30 to 60 % by weight), and lipid-PEG conjugate of 0.1 to 10 % by weight (or 0.25 to 10 % by weight, 0.5 to 5 % by weight) based on the total nanoparticle weight. In other example, the LNP may comprise the ionizable lipid of 25 to 50 % by weight, phospholipid of 10 to 20 % by weight, cholesterol of 35 to 55 % by weight, and lipid-PEG conjugate of 0.1 to 10 % by weight (or 0.25 to 10 % by weight, 0.5 to 5 % by weight), based on the total nanoparticle weight.

[0281] In some embodiments, the approach to formulating the LNP of the disclosure (described more fully in the examples) is to dissolve lipids in an organic solvent such as ethanol, which is then mixed through a micromixer with the nucleic acid dissolved in an acidic buffer (usually pH 4). At this pH the ionizable cationic lipid is positively charged and interacts with the negatively-charged nucleic acid polymers. The resulting nanostructures containing the nucleic acids are then converted to neutral LNP when dialyzed against a neutral buffer during the ethanol removal step. The LNP formed by this have a distinct electron-dense nanostructured core

where the ionizable cationic lipids are organized into inverted micelles around the encapsulated mRNA molecules, as opposed to the traditional bilayer liposomal structures.

[0282] In some embodiments, the LNP may have an average diameter of 20nm to 200nm, 20nm to 180nm, 20nm to 170nm, 20nm to 150nm, 20nm to 120nm, 20nm to 100nm, 20nm to 90nm, 30nm to 200nm, 30 to 180nm, 30nm to 170nm, 30nm to 150nm, 30nm to 120nm, 30nm to 100nm, 30nm to 90nm, 40nm to 200nm, 40 to 180nm, 40nm to 170nm, 40nm to 150nm, 40nm to 120nm, 40nm to 100nm, 40nm to 90nm, 40nm to 80nm, 40nm to 70nm, 50nm to 200nm, 50 to 180nm, 50nm to 170nm, 50nm to 150nm, 50nm to 120nm, 50nm to 100nm, 50nm to 90nm, 60nm to 200nm, 60 to 180nm, 60nm to 170nm, 60nm to 150nm, 60nm to 120nm, 60nm to 100nm, 60nm to 90nm, 70nm to 200nm, 70 to 180nm, 70nm to 170nm, 70nm to 150nm, 70nm to 120nm, 70nm to 100nm, 70nm to 90nm, 80nm to 200nm, 80 to 180nm, 80nm to 170nm, 80nm to 150nm, 80nm to 120nm, 80nm to 100nm, 80nm to 90nm, 90nm to 200nm, 90 to 180nm, 90nm to 170nm, 90nm to 150nm, 90nm to 120nm, or 90nm to 100nm. The LNP may be sized for easy introduction into organs or tissues, including but not limited to liver, lung, heart, spleen, as well as to tumors. When the size of the LNP is smaller than the above range, it is difficult to maintain stability as the surface area of the LNP is excessively increased, and thus delivery to the target tissue and/or therapeutic effect may be reduced. The LNP may specifically target liver tissue. The LNP may imitate metabolic behaviors of natural lipoproteins very similarly, and may be usefully applied for the lipid metabolism process by the liver and therapeutic mechanism through this. During the drug or biologic delivery to hepatocytes or and/or LSEC (liver sinusoidal endothelial cells), the diameter of the fenestrae leading from the sinusoidal lumen to the hepatocytes and LSEC is about 140 nm in mammals and about 100 nm in humans, so LNPs having a diameter in the above ranges may have superior delivery efficiency to hepatocytes and LSEC compared to LNP having the diameter outside the above range.

[0283] According to some embodiments, the LNP comprised in the composition for nucleic acid delivery into target cells may comprise the ionizable lipid : phospholipid : cholesterol : lipid-PEG conjugate in the range described above or at a molar ratio of 20 to 50:10 to 30:30 to 60:0.5 to 5, at a molar ratio of 25 to 45:10 to 25:40 to 50:0.5 to 3, at a molar ratio of 25 to 45:10 to 20:40 to 55:0.5 to 3, or at a molar ratio of 25 to 45:10 to 20:40 to 55:1.0 to 1.5. The LNP comprising components at a molar ratio in the above range may have excellent delivery efficiency specific to cells of target organs.

[0284] The LNP according to some embodiments exhibits a positive charge under the acidic pH condition by showing a pKa of 5 to 8, 5.5 to 7.5, 6 to 7, or 6.5 to 7, and may encapsulate a nucleic acid with high efficiency by easily forming a complex with a nucleic acid through electrostatic interaction with a therapeutic agent such as a nucleic acid showing a negative charge, and it may be usefully used as a composition for intracellular or in vivo delivery of a drug or biologic (for example, nucleic acid or protein). Herein, "encapsulation" refers to encapsulating a delivery substance for surrounding and embedding it in vivo efficiently, and the encapsulation efficiency (encapsulation efficiency) mean the content of the drug or biologic encapsulated in the LNP for the total drug or biologic content used for preparation.

[0285] The encapsulation efficiency of the nucleic acids of the composition in the LNP may be 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 94% or more, or 95% or more. In other embodiments, the encapsulation efficiency of the nucleic acids of the composition in the LNP is over 80% to 99% or less, over 80% to 97% or less, over 80% to 95% or less, 85% or more to 95% or less, 87% or more to 95% or less, 90% or more to 95% or less, 91% or more to 95% or less, 91% or more to 94% or less, over 91% to 95% or less, 92% or more to 99% or less, 92% or more to 97% or less, or 92% or more to 95% or less. As used herein, "encapsulation efficiency" means the percentage of LNP particles containing the nucleic acids to be incorporated within the LNP. In some embodiments, the mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid of the disclosure are fully encapsulated in the nucleic acid-lipid particle.

[0286] The target organs to which a nucleic acid is delivered by the LNP include, but are not limited to the liver, lung, heart, spleen, as well as to tumors. The LNP according to one embodiment is liver tissue-specific and has excellent biocompatibility and can deliver the nucleic acids of a dXR:gRNA system composition with high efficiency, and thus it can be usefully used in related technical fields such as lipid nanoparticle-mediated gene therapy. In a particular embodiment, the target cell to which the nucleic acids of the dXR:gRNA system are delivered by the LNP according to one example may be a hepatocyte and/or LSEC *in vivo*. In other embodiments, the disclosure provides LNP formulated for delivery of the nucleic acids of the embodiments to cells *ex vivo*.

[0287] Accordingly, in certain embodiments, the disclosure encompasses gRNA molecules that target the expression of one or more target nucleic acids, nucleic acid-lipid particles comprising an mRNA encoding a dXR fusion protein of the disclosure and one or more of the

gRNAs that target the expression of one or more target nucleic acids, nucleic acid-lipid particles comprising one or more (e.g., a cocktail) of the gRNAs, and methods of delivering and/or administering the nucleic acid-lipid particles. The gRNA molecules may be delivered concurrently with or sequentially with a mRNA molecule that encodes the dXR fusion protein, thereby delivering components to utilize the system to treat disease in a human in need of such treatment, for example, a human in need of treatment or prevention of a disorder. In certain embodiments the mRNA that encodes the dXR fusion protein and gRNA may be present in the same nucleic acid-lipid particle, or they may be present in different nucleic acid-lipid particles.

[0288] The disclosure also provides a pharmaceutical composition comprising one or more (e.g., a cocktail) of the gRNA targeting different sequences, together with one or more of the dXR described herein, and a pharmaceutically acceptable carrier. With respect to formulations comprising an dXR:gRNA cocktail, the different types of gRNA species present in the cocktail (e.g., gRNA with different targeting sequences) may be co-encapsulated in the same particle, or each type of gRNA species present in the cocktail may be encapsulated in a separate particle. The LNP cocktail may be formulated in the particles described herein using a mixture of two, three or more individual gRNA (each having a unique targeting sequence) at identical, similar, or different concentrations or molar ratios.

[0289] In one embodiment, a cocktail of mRNA encoding the fusion protein and two or more gRNA with different targeting sequences to the target nucleic acid is formulated using identical, similar, or different concentrations or molar ratios of each gRNA species, and the different types of gRNA are co-encapsulated in the same particle. In another embodiment, each type of gRNA species present in the cocktail is encapsulated in different particles at identical, similar, or different gRNA concentrations or molar ratios, and the particles thus formed (each containing a different gRNA payload) are administered separately (e.g., at different times in accordance with a therapeutic regimen), or are combined and administered together as a single unit dose (e.g., with a pharmaceutically acceptable carrier). The particles described herein are serum-stable, are resistant to nuclease degradation, and are substantially non-toxic to mammals such as humans.

[0290] In certain embodiments, the nucleic acid-lipid particle has an electron dense core.

[0291] In some embodiments, the disclosure provides nucleic acid-lipid particles comprising: (a) one or more (e.g., a cocktail) of mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) one or more ionizable cationic lipids or salts thereof comprising from about 50 mol % to about 85 mol % of the total lipid present in the

particle; (c) one or more non-cationic lipids comprising from about 13 mol % to about 49.5 mol % of the total lipid present in the particle; and (d) one or more conjugated lipids that inhibit aggregation of particles comprising from about 0.5 mol % to about 2 mol % of the total lipid present in the particle.

[0292] In one embodiment, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 52 mol % to about 62 mol % of the total lipid present in the particle; (c) a mixture of a phospholipid and cholesterol or a derivative thereof comprising from about 36 mol % to about 47 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 1 mol % to about 2 mol % of the total lipid present in the particle. In one particular embodiment, the formulation is a four-component system comprising about 1.4 mol % PEG-lipid conjugate (e.g., PEG2000-C-DMA), about 57.1 mol % ionizable cationic lipid (e.g., DLin-K-C2-DMA) or a salt thereof, about 7.1 mol % DPPC (or DSPC), and about 34.3 mol % cholesterol (or derivative thereof).

[0293] In another embodiment, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 46.5 mol % to about 66.5 mol % of the total lipid present in the particle; (c) cholesterol or a derivative thereof comprising from about 31.5 mol % to about 42.5 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 1 mol % to about 2 mol % of the total lipid present in the particle. In one particular embodiment, the formulation is a three-component system which is phospholipid-free and comprises about 1.5 mol % PEG-lipid conjugate (e.g., PEG2000-C-DMA), about 61.5 mol % ionizable cationic lipid (e.g., DLin-K-C2-DMA) or a salt thereof, and about 36.9 mol % cholesterol (or derivative thereof).

[0294] Additional formulations are described in PCT Publication No. WO 09/127060 and published US patent application publication numbers US 2011/0071208 A1 and US 2011/0076335 A1, the disclosures of which are herein incorporated by reference in their entirety.

[0295] In other embodiments, the present disclosure provides nucleic acid-lipid particles comprising: (a) one or more (e.g., a cocktail) gRNA molecules described herein; (b) one or more ionizable lipids or salts thereof comprising from about 2 mol % to about 50 mol % of the total lipid present in the particle; (c) one or more non-cationic lipids comprising from about 5 mol %

to about 90 mol % of the total lipid present in the particle; and (d) one or more conjugated lipids that inhibit aggregation of particles comprising from about 0.5 mol % to about 20 mol % of the total lipid present in the particle.

[0296] In one aspect of this embodiment, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 30 mol % to about 50 mol % of the total lipid present in the particle; (c) a mixture of a phospholipid and cholesterol or a derivative thereof comprising from about 47 mol % to about 69 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 1 mol % to about 3 mol % of the total lipid present in the particle. In one particular embodiment, the formulation is a four-component system which comprises about 2 mol % PEG-lipid conjugate (e.g., PEG2000-C-DMA), about 40 mol % ionizable cationic lipid (e.g., DLin-K-C2-DMA) or a salt thereof, about 10 mol % DPPC (or DSPC), and about 48 mol % cholesterol (or derivative thereof).

[0297] In further embodiments, the present disclosure provides nucleic acid-lipid particles comprising: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) one or more ionizable cationic lipids or salts thereof comprising from about 50 mol % to about 65 mol % of the total lipid present in the particle; (c) one or more non-cationic lipids comprising from about 25 mol % to about 45 mol % of the total lipid present in the particle; and (d) one or more conjugated lipids that inhibit aggregation of particles comprising from about 5 mol % to about 10 mol % of the total lipid present in the particle.

[0298] In another embodiment, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 50 mol % to about 60 mol % of the total lipid present in the particle; (c) a mixture of a phospholipid and cholesterol or a derivative thereof comprising from about 35 mol % to about 45 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 5 mol % to about 10 mol % of the total lipid present in the particle.

[0299] In certain embodiments, the non-cationic lipid mixture in the formulation comprises: (i) a phospholipid of from about 5 mol % to about 10 mol % of the total lipid present in the particle; and (ii) cholesterol or a derivative thereof of from about 25 mol % to about 35 mol % of

the total lipid present in the particle. In one particular embodiment, the formulation is a four-component system which comprises about 7 mol % PEG-lipid conjugate (e.g., PEG750-C-DMA), about 54 mol % ionizable cationic lipid (e.g., DLin-K-C2-DMA) or a salt thereof, about 7 mol % DPPC (or DSPC), and about 32 mol % cholesterol (or derivative thereof).

[0300] In another embodiment, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 55 mol % to about 65 mol % of the total lipid present in the particle; (c) cholesterol or a derivative thereof comprising from about 30 mol % to about 40 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 5 mol % to about 10 mol % of the total lipid present in the particle. In one particular embodiment, the formulation is a three-component system which is phospholipid-free and comprises about 7 mol % PEG-lipid conjugate (e.g., PEG750-C-DMA), about 58 mol % ionizable cationic lipid (e.g., DLin-K-C2-DMA) or a salt thereof, and about 35 mol % cholesterol (or derivative thereof).

[0301] In certain embodiments of the disclosure, the nucleic acid-lipid particle comprises: (a) one or more (e.g., a cocktail) mRNA encoding the dXR and a gRNA with a targeting sequence to the target nucleic acid described herein; (b) a ionizable cationic lipid or a salt thereof comprising from about 48 mol % to about 62 mol % of the total lipid present in the particle; (c) a mixture of a phospholipid and cholesterol or a derivative thereof, wherein the phospholipid comprises about 7 mol % to about 17 mol % of the total lipid present in the particle, and wherein the cholesterol or derivative thereof comprises about 25 mol % to about 40 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 0.5 mol % to about 3.0 mol % of the total lipid present in the particle.

VIII. Applications

[0302] The fusion proteins, gRNA, nucleic acids encoding the fusion proteins and variants thereof provided herein, as well as vectors encoding such components, particle systems for the delivery of the gene repressor systems, or LNP comprising nucleic acids are useful for various applications, including therapeutics, diagnostics, and research.

[0303] Provided herein are methods of repression of transcription of a target gene encoded by a target nucleic acid in a cell, comprising contacting the target nucleic acid with a dXR and a

gRNA with a targeting sequence that is complementary to the target nucleic acid. In some embodiments of the method, the repressor system is provided to the cells as a dXR:gRNA RNP complex, embodiments of which have been described *supra*, wherein the contacting results in repression or silencing of transcription. In other embodiments of the method, the repressor system is provided to the cells as a nucleic acid or a vector comprising the nucleic acids encoding the dXR and gRNA, or as a lipid nanoparticle (LNP) comprising mRNA encoding the dXR and gRNA components, wherein the contacting results in repression or silencing of transcription of the target nucleic acid upon expression of the dXR and gRNA and binding of the resulting RNP complex to the target nucleic acid. In some embodiments, the vector is an AAV encoding the dXR and gRNA components. In other embodiments of the method, the vector is a virus-like particle, an XDP comprising multiple dXR:gRNA RNPs, wherein the contacting of the target nucleic acid results in repression or silencing of transcription of the gene proximal to the binding location of the RNP of the target nucleic acid.

[0304] In some embodiments of the method of repressing expression of a target nucleic acid in a cell, the repressor system is provided to the cells encapsidated in a population of lipid nanoparticles (LNP), described more fully, above. An LNP represents a particle made from lipids, wherein the nucleic acids of the system are fully encapsulated within the lipid. In certain instances, LNP are extremely useful for systemic applications, as they can exhibit extended circulation lifetimes following intravenous (i.v.) injection, they can accumulate at distal sites within the subject, and when used to encapsidate the dXR:gRNA systems of the embodiments, they can mediate repression or silencing of target gene expression at these distal sites. Preferably, these LNP compositions would encapsulate the nucleic acids of the system with high-efficiency, have high drug:lipid ratios, protect the encapsulated nucleic acid from degradation and clearance in serum, be suitable for systemic delivery, and provide intracellular delivery of the encapsulated nucleic acid. In some embodiments of the method, the repressor system is provided to the cells as a first and a second lipid nanoparticle (LNP) wherein the first LNP encapsidates mRNA encoding the dXR fusion protein of any of the embodiments described herein and the second LNP encapsidates the gRNA of any of the embodiments described herein, wherein the contacting of the cell and uptake of the LNP results in expression of the dXR fusion protein and complexing of the dXR and gRNA as an RNP, wherein upon binding of the resulting RNP complex to the target nucleic acid, repression or silencing of transcription of the target nucleic acid occurs. In other embodiments, the repressor system is provided to the cells as a

population of LNPs wherein the LNP encapsidates both the mRNA encoding the dXR fusion protein of any of the embodiments described herein and a gRNA of any of the embodiments described herein, wherein the contacting of the cells and the uptake of the LNP results in expression of the dXR fusion protein and complexing of the RNP repression, wherein upon binding of the resulting RNP complex to the target nucleic acid, repression or silencing of transcription of the target nucleic occurs.

[0305] In some embodiments of the method, upon binding of the dXR:gRNA RNP to the target nucleic acid, transcription of the gene in the population of cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% greater compared to the repression effected by an RNP comprising a comparable guide RNA and a catalytically dead CasX variant without a repressor domain, when assessed in an *in vitro* assay. In other embodiments, transcription of the gene in the population of cells is repressed by at least about 10% to about 90%, or at least 20% to about 80%, or at least about 30% to about 60% compared to the repression effected by an RNP comprising a comparable guide RNA and a catalytically dead CasX variant without a repressor domain, when assessed in an *in vitro* assay. In some embodiments of the method, the repression of transcription in the populations of cells is sustained for at least about 8 hours, at least about 1 day, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, or at least about 6 months or longer. Exemplary assays to measure repression are described herein, including the Examples, below.

[0306] In some cases, off-target methylationor off-target transcription repression by the dXR:gRNA RNP is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells genome-wide.

[0307] In some embodiments of the method of repressing a target nucleic acid in a cell, the gRNA scaffold utilized in the dXR:gRNA systems of the disclosure is selected from the group of sequences consisting of SEQ ID NOS: 2238-2331, 57544-57589, and 59352, set forth in Table 2, or a sequence having at least about 80%, at least about 90%, at least about 95%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto, and the gRNA further comprises a targeting sequence that is complementary to

the target nucleic acid to be repressed. In some embodiments of the method, the gRNA scaffold utilized in the dXR:gRNA systems of the disclosure comprises one or more chemical modifications. In some embodiments of the method, the dCasX variant is a sequence of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4 , or a sequence having at least about 80%, at least about 90%, at least about 95%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto, and is linked to a first repressor domain, a first and a second repressor domain, a first, second and third repressor domain, or a first, second, third, and fourth repressor domains. In some embodiments of the method, the first domain linked to the dCasX as a fusion protein (or encoded to be expressed as a fusion protein) is a KRAB domain of any of the embodiments described herein. In some embodiments of the method, the first domain linked to the dCasX as a fusion protein is a KRAB domain sequence and the second repressor domain is a DNMT3A catalytic domain sequence. In some embodiments of the method, the first domain linked to the dCasX as a fusion protein is a KRAB domain sequence, the second repressor domain is a DNMT3A catalytic domain sequence, and the third repressor is a DNMT3L interaction domain sequence. In some embodiments of the method, the first domain linked to the dCasX as a fusion protein is a KRAB domain sequence, the second repressor domain is a DNMT3A catalytic domain sequence, the third repressor is a DNMT3L interaction domain sequence, and the fourth domain in a DNMT3A ADD domain. In some embodiments of the foregoing, KRAB domain is selected from the group consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 80%, at least about 90%, at least about 95%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto. In some embodiments of the method of repressing a target nucleic acid in a cell, the KRAB domain comprises one or more motifs selected from the group consisting of a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein X_1 is A, D, E, or N, X_2 is L or V, X_3 is I or V, X_4 is S, T, or F, X_5 is H, K, L, Q, R or W, X_6 is L or M, and X_7 is G, K, Q, or R; b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein X_1 is L or V, X_2 is A, G, L, T or V, X_3 is A, F, or S, X_4 is L or V, X_5 is C, F, H, I, L or Y, X_6 is A, C, P, Q, or S, X_7 is A, F, G, I, S, or V, X_8 is A, P, S, or T, and X_9 is K or R; c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein X_1 is K or R, X_2 is A, D, E, G, N, S, or T, X_3 is D, E, or S, and X_4 is L or R; d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein X_1 is A, L, P, or S, X_2 is L or V, X_3 is S or T, X_4 is A, E, G, K, or R, X_5 is A or T, X_6 is I or V, X_7 is D, E, N, or Y, X_8 is S or T, X_9 is E, P, Q, R, or W, X_{10} is E or N, and X_{11} is E or Q; e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$,

wherein X_1 is E, G, or R, X_2 is E or K, X_3 is A, D, or E, X_4 is C or W, X_5 is I, K, L, M, T, or V, X_6 is I, L, P, or V, X_7 is D, E, K, or V, X_8 is E, G, K, P, or R, X_9 is A, D, R, G, K, Q, or V, and X_{10} is D, E, G, I, L, R, S, or V; f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein X_1 is K or R, X_2 is D or E, X_3 is L, Q, or R, X_4 is N or T, X_5 is F or Y, X_6 is A, E, G, Q, R, or S, X_7 is H, L, or N, X_8 is L or V, X_9 is A, G, I, L, T, or V, and X_{10} is A, F, or S; g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein X_1 is A, E, G, K, or R, X_2 is A, S, or T, X_3 is I or V, X_4 is D, E, N, or Y, X_5 is S or T, X_6 is E, L, P, Q, R, or W, X_7 is D or E, and X_8 is A, E, G, Q, or R; h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is K or R, X_2 is A, D, E, or N, X_3 is I, L, M, or V, X_4 is I or V, X_5 is F, S, or T, X_6 is H, K, L, Q, R, or W, X_7 is K, Q, or R, X_8 is E, G, or R, X_9 is D, E, or K, X_{10} is A, D, or E, X_{11} is L or P, and X_{12} is C or W; or i) $X_1LX_2X_3X_4QX_5X_6$, wherein X_1 is C, H, L, Q, or W, X_2 is D, G, N, R, or S, X_3 is L, P, S, or T, X_4 is A, S, or T, X_5 is K or R, and X_6 is A, D, E, K, N, S, or T, and the KRAB domain comprises a sequence selected from the group consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments of the method, the DNMT3A catalytic domain comprises a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments of the method, the DNMT3L interaction domain comprises a sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto. In some embodiments of the method, the DNMT3A ADD domain comprises a sequence of SEQ ID NO: 59452, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90% at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0308] In some embodiments of the method of repressing a target nucleic acid in a cell, the method further comprises inclusion of a second gRNA, or a nucleic acid encoding the second

gNA, wherein the second gNA has a targeting sequence complementary to a different portion of the target nucleic acid sequence and is capable of forming a ribonuclear protein complex (RNP) with the dXR fusion protein.

[0309] In some embodiments of the method of repressing a target nucleic acid in a cell, the repression occurs in vitro, outside of a cell, in a cell-free system. In some embodiments, the repression occurs in vitro, inside of a cell, for example in a cell culture system. In some embodiments, the repression occurs in vivo inside of a cell, for example in a cell in an organism. In some embodiments, the cell is a eukaryotic cell. Exemplary eukaryotic cells may include a mammalian cell, a rodent cell, a mouse cell, a rat cell, a pig cell, a dog cell, a primate cell, and a non-human primate cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is an embryonic stem cell, an induced pluripotent stem cell, a germ cell, a fibroblast, an oligodendrocyte, a glial cell, a hematopoietic stem cell, a neuron progenitor cell, a neuron, an astrocyte, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, a retinal cell, a cancer cell, a T-cell, a B-cell, an NK cell, a fetal cardiomyocyte, a myofibroblast, a mesenchymal stem cell, an autotransplanted expanded cardiomyocyte, an adipocyte, a totipotent cell, a pluripotent cell, a blood stem cell, a myoblast, a bone marrow cell, a mesenchymal cell, a parenchymal cell, an epithelial cell, an endothelial cell, a mesothelial cell, fibroblasts, osteoblasts, chondrocytes, a hematopoietic stem cell, a bone-marrow derived progenitor cell, a myocardial cell, a skeletal cell, a fetal cell, an undifferentiated cell, a multi-potent progenitor cell, a unipotent progenitor cell, a monocyte, a cardiac myoblast, a skeletal myoblast, a macrophage, a capillary endothelial cell, a xenogeneic cell, an allogenic cell, or a post-natal stem cell. The cell can be in a subject. In some embodiments, repression occurs in the subject having a mutation in an allele of a gene wherein the mutation causes a disease or disorder in the subject. In some embodiments, repression reduces or silence transcription of an allele of a gene causing a disease or disorder in the subject, wherein the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human. In some embodiments, repression occurs *in vitro* inside of the cell prior to introducing the cell into a subject. In some embodiments, the cell is autologous or allogeneic with respect to the subject.

[0310] Methods of introducing a nucleic acid (e.g., nucleic acids encoding a dXR:gRNA system, or variants thereof as described herein) into a cell in vitro are known in the art, and any convenient method can be used to introduce a nucleic acid into a cell. Suitable methods include viral infection, transfection, lipofection, electroporation, calcium phosphate precipitation,

polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, nucleofection, electroporation, LNP transfection, direct addition by cell penetrating dXR proteins that are fused to or recruit donor DNA, cell squeezing, calcium phosphate precipitation, direct microinjection, nanoparticle -mediated nucleic acid delivery, and the like. Nucleic acids may be provided to the cells using well-developed transfection techniques, and the commercially available TransMessenger® reagents from Qiagen, Stemfect™ RNA Transfection Kit from Stemgent, and TransIT®-mRNA Transfection Kit from Mirus Bio LLC, Lonza nucleofection, Maxagen electroporation and the like. In some embodiments, vectors may be provided directly to a target host cell such that the vectors are taken up by the cells. Introducing recombinant expression vectors into cells can occur in any suitable culture media and under any suitable culture conditions that promote the survival of the cells.

[0311] A dXR protein or an mRNA encoding the dXR of the disclosure may be prepared by *in vitro* synthesis, using conventional methods as known in the art. Various commercial synthetic apparatuses are available, for example, automated synthesizers by Applied Biosystems, Inc., Beckman, etc. By using synthesizers, naturally occurring amino acids or nucleotides (as applicable) may be substituted with unnatural amino acids or nucleotides. The particular sequence and the manner of preparation will be determined by convenience, economics, purity required, and the like.

[0312] The dXR fusion protein may also be prepared by recombinantly producing a polynucleotide sequence coding for the dXR of any of the embodiments described herein and incorporating the encoding gene into an expression vector appropriate for a host cell. For production of the encoded dXR of any of the embodiments described herein, the methods include transforming an appropriate host cell with an expression vector comprising the encoding polynucleotide, and culturing the host cell under conditions causing or permitting the resulting dXR of any of the embodiments described herein to be expressed or transcribed in the transformed host cell, thereby producing the dXR, which are recovered by methods described herein or by standard purification methods known in the art or as described in the Examples. Standard recombinant techniques in molecular biology are used to make the polynucleotides and expression vectors of the present disclosure.

[0313] A dXR protein of the disclosure may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host

and the lysate purified using high performance liquid chromatography (HPLC), exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. For the most part, the compositions which are used will comprise 50% or more by weight of the desired product, more usually 75% or more by weight, preferably 95% or more by weight, and for therapeutic purposes, usually 99.5% or more by weight, in relation to contaminants related to the method of preparation of the product and its purification. Usually, the percentages will be based upon total protein. Thus, in some cases, a dXR polypeptide, or a dXR fusion polypeptide, of the present disclosure is at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure (e.g., free of contaminants, non-dXR proteins or other macromolecules, etc.).

[0314] In some embodiments, to induce repression of transcription of a target nucleic acid (e.g., genomic DNA) in an *in vitro* cell, the dXR and gRNA of the present disclosure, whether they be introduced as nucleic acids (including encapsidated within an LNP or within an AAV) or an RNP, are provided to the cells for about 30 minutes to about 24 hours, e.g., 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 18 hours, 20 hours, or any other period from about 30 minutes to about 24 hours, which may be repeated with a frequency of about every day to about every 7 days, e.g., every 1.5 days, every 2 days, every 3 days, or any other frequency from about every day to about every 7days. In some embodiments, to induce repression of transcription of a target nucleic acid in a subject, the dXR and gRNA of the present disclosure may be provided to the subject cells one or more times; e.g., one time, twice, three times, or more than three times, and the cells allowed to incubate with the agent(s) for some amount of time following each contacting event; e.g., 16-24 hours, after which time the media is replaced with fresh media and the cells are cultured further.

[0315] In some embodiments, the present disclosure provides a method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective dose of: (i) an AAV vector encoding the dXR:gRNA systems of any of the embodiments described herein, (ii) an XDP comprising RNP of the dXR:gRNA systems of any of the embodiments described herein, (iii) LNP comprising gRNA and mRNA encoding the dXR (which may be a single LNP, or are formulated as a first and second LNP encapsidating the mRNA encoding the dXR fusion protein and gRNA, respectively), or (iv) combinations of (i)-(iii), wherein upon binding of the RNP of the gene repressor system to the target nucleic acid of a gene in cells of the subject represses transcription of the gene proximal to the binding location

of the RNP. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within 1 kb of a transcription start site (TSS) in the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within 500 bps upstream to 500 bps downstream of a TSS of the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within 300 bps upstream to 300 bps downstream of a TSS of the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within 1 kb of an enhancer of the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within the 3' untranslated region of the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within an exon of the gene, wherein upon binding of the RNP transcription is repressed. In some embodiments, the gRNA target nucleic acid sequence complementary to the targeting sequence is within exon 1 of the gene, wherein upon binding of the RNP transcription is repressed.

[0316] In some embodiments of the methods of treating a subject with a therapeutically-effective dose of the dXR:gRNA systems, transcription of the targeted gene in the cells of the subject is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%. In some embodiments of the methods of treating a subject with the dXR:gRNA systems with a therapeutically-effective dose of the foregoing dXR systems, the repression of transcription of the gene in the targeted cells of the subject is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, or at least about 6 months or longer.

[0317] In some embodiments, the present disclosure provides a method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective amount of an AAV vector of any of the embodiments described herein, wherein upon the contacting of the targeted cell, the dXR:gRNA is expressed and complexes as an RNP, and upon binding of the RNP to the target nucleic acid in cells of the subject, transcription of the

gene proximal to the binding location of the RNP is repressed wherein the treatment results in improvement in at least one clinically-relevant endpoint associated with the disorder. In some embodiments of the method, the AAV vector is administered at a dose of at least about 1 x 10^5 viral genomes (vg)/kg, at least about 1 x 10^6 vg/kg, at least about 1 x 10^7 vg/kg, at least about 1 x 10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 1 x 10^{14} vg/kg, at least about 1 x 10^{15} vg/kg, at least about 1 x 10^6 vg/kg. In other embodiments, the AAV vector is administered to the subject at a dose of at least about 1 x 10^5 vg/kg to about 1 x 10^{16} vg/kg, at least about 1 x 10^6 vg/kg to about 1 x 10^{15} vg/kg, or at least about 1 x 10^7 vg/kg to about 1 x 10^{14} vg/kg. In one embodiment of the foregoing, transcription of the gene in the targeted cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%. In another embodiment of the foregoing, transcription of the gene in the cells is repressed by at least about 10% to about 99%, or at least 20% to about 90%, at least about 30% to about 80%, or at least about 40% to about 60%.

[0318] In some embodiments, the present disclosure provides a method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective amount of an XDP of any of the embodiments described herein, wherein upon the contacting of the targeted cell and the binding of the RNP of the XDP to the target nucleic acid in cells of the subject, transcription of the gene proximal to the binding location of the RNP is repressed wherein the treatment results in improvement in at least one clinically-relevant endpoint associated with the disorder. In some embodiments of the method, the XDP is administered at a dose of at least about 1 x 10^5 particles/kg, at least about 1 x 10^6 particles/kg, at least about 1 x 10^7 particles/kg, at least about 1 x 10^8 particles/kg, at least about 1 x 10^9 particles/kg, at least about 1 x 10^{10} particles/kg, at least about 1 x 10^{11} particles/kg, at least about 1 x 10^{12} particles/kg, at least about 1 x 10^{13} particles/kg, at least about 1 x 10^{14} particles/kg, at least about 1 x 10^{15} particles/kg, at least about 1 x 10^6 particles/kg. In other embodiments, the XDP is administered to the subject at a dose of at least about 1 x 10^5 particles/kg to about 1 x 10^{16} particles/kg, or at least about 1 x 10^6 particles/kg to about 1 x 10^{15} particles/kg, or at least about 1 x 10^7 particles/kg to about 1 x 10^{14} particles/kg. In one embodiment of the foregoing, transcription of the gene in the targeted cells is repressed by at least about 10%, at least about

20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%. In another embodiment of the foregoing, transcription of the gene in the cells is repressed by at least about 10% to about 99%, or at least 20% to about 90%, at least about 30% to about 80%, or at least about 40% to about 60%.

[0319] In some embodiments, the present disclosure provides a method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective amount of an LNP comprising mRNA encoding the dXR fusion protein and a gRNA (which may be a single LNP, or are formulated as a first and second LNP encapsidating the mRNA encoding the dXR fusion protein and gRNA, respectively), of any of the embodiments described herein, wherein upon the contacting of the targeted cell the dXR fusion protein is expressed and complexed with the gRNA to form an RNP, and upon the binding of the RNP to the target nucleic acid in cells of the subject, transcription of the gene proximal to the binding location of the RNP is repressed wherein the treatment results in improvement in at least one clinically-relevant endpoint associated with the disorder. In some embodiments of the method, the LNP are administered at a dose of at least about 1 x 10^5 particles/kg, at least about 1 x 10^6 particles/kg, at least about 1 x 10^7 particles/kg, at least about 1 x 10^8 particles/kg, at least about 1 x 10^9 particles/kg, at least about 1 x 10^{10} particles/kg, at least about 1 x 10^{11} particles/kg, at least about 1 x 10^{12} particles/kg, at least about 1 x 10^{13} particles/kg, at least about 1 x 10^{14} particles/kg, at least about 1 x 10^{15} particles/kg, at least about 1 x 10^6 particles/kg. In other embodiments, the LNP are administered to the subject at a dose of at least about 1 x 10^5 particles/kg to about 1 x 10^{16} particles/kg, or at least about 1 x 10^6 particles/kg to about 1 x 10^{15} particles/kg, or at least about 1 x 10^7 particles/kg to about 1 x 10^{14} particles/kg. In one embodiment of the foregoing, transcription of the gene in the targeted cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%. In another embodiment of the foregoing, transcription of the gene in the cells is repressed by at least about 10% to about 99%, or at least 20% to about 90%, at least about 30% to about 80%, or at least about 40% to about 60%.

[0320] In the embodiments of the method of treatment, the AAV vector, the XDP, or the LNP is administered to the subject by a route of administration selected from subcutaneous,

intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatical, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation, or combinations thereof. In some embodiments, the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human.

[0321] A number of therapeutic strategies have been used to design the compositions for use in the methods of treatment of a subject with a disease. In some embodiments, the invention provides a method of treatment of a subject having a disease, the method comprising administering to the subject a dXR:gRNA composition, an AAV vector, an XDP, of an LNP of any of the embodiments disclosed herein according to a treatment regimen comprising one or more consecutive doses using a therapeutically effective dose. In some embodiments of the treatment regimen, the therapeutically effective dose of the composition or vector is administered as a single dose. In other embodiments of the treatment regimen, the therapeutically effective dose is administered to the subject as two or more doses over a period of at least two weeks, or at least one month, or at least two months, or at least three months, or at least four months, or at least five months, or at least six months. In some embodiments of the treatment regimen, the effective doses are administered by a route selected from the group consisting of subcutaneous, intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatical, intravitreal, subretinal, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation. In some embodiments of the treatment regimen, the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human.

[0322] In some embodiments, the administering of the therapeutically effective amount of a dXR:gRNA modality, including a vector or an LNP comprising a polynucleotide encoding a dXR protein and a guide ribonucleic acid composition disclosed herein, to repress expression of a gene product to a subject with a disease leads to the prevention or amelioration of the underlying disease such that an improvement is observed in at least one clinically-relevant endpoint associated with the disease, notwithstanding that the subject may still be afflicted with the underlying disease. In some embodiments, the administration of the therapeutically effective amount of the dXR:gRNA modality leads to an improvement in at least two clinically-relevant parameters associated with the disease.

[0323] In embodiments in which two or more different targeting complexes are provided to the cell (e.g., two dXR:gRNA comprising two or more different targeting sequences that are complementary to different sequences within the same or different target nucleic acid), the complexes may be provided simultaneously or they may be provided consecutively; e.g. the first dXR:gRNA targeted complex being provided first, followed by the second targeted complex.

[0324] To improve the delivery of a DNA vector into a target cell, the DNA can be protected from damage and its entry into the cell facilitated, for example, by using lipoplexes and polyplexes. Thus, in some cases, a nucleic acid of the present disclosure (e.g., a recombinant expression vector of the present disclosure) can be covered with lipids in an organized structure like a micelle, a liposome, or a lipid nanoparticle, embodiments of which have been described more fully, above. There are four types of lipids, anionic (negatively-charged), neutral, cationic (positively-charged), or ionizable cationic employed in LNP. Cationic lipids (or ionizable lipids at the appropriate pH) of LNP, due to their positive charge, naturally complex with the negatively charged DNA. Also, as a result of their charge, they interact with the cell membrane. Endocytosis of the LNP then occurs, and the DNA is released into the cytoplasm. The cationic lipids also protect against degradation of the DNA by the cell.

[0325] In another aspect, the present disclosure provides compositions of gene repressor systems of any of the embodiments described herein for use as a medicament in the treatment of a disease in a subject. In some embodiments, the subject the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human.

IX. Kits and Articles of Manufacture

[0326] In another aspect, provided herein are kits comprising a fusion protein and one or a plurality of gRNA of any of the embodiments of the disclosure formulated in a pharmaceutically acceptable excipient and contained in a suitable container (for example a tube, vial or plate). In some embodiments, the kit comprises a gRNA variant of the disclosure. Exemplary gRNA variants that can be included comprise a sequence of any one of SEQ ID NOS: 2238-2331, 57544-57589, and 59352, or a sequence of Table 2, together with a targeting sequence appropriate for the gene to be repressed linked to the 3' end of the scaffold. In some embodiments, the kit comprises a dCasX variant protein of the disclosure (e.g., a sequence of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4) linked to one or more repressor

domains of the embodiments described herein; e.g,, DNMT3A catalytic domain, DNMT3L interaction domain, and DNMT3A ADD domain.

[0327] In some embodiments, the kit comprises a vector encoding a dXR:gRNA of any of the embodiments described herein, formulated in a pharmaceutically acceptable excipient and contained in a suitable container.

[0328] In certain embodiments, provided herein are kits comprising an LNP comprising an mRNA encoding a dXR as described herein, formulated in a pharmaceutically acceptable excipient and contained in a suitable container.

[0329] In some embodiments, the kit further comprises a buffer, a nuclease inhibitor, a protease inhibitor, a liposome, a therapeutic agent, a label, instructions for use, a label visualization reagent, or any combination of the foregoing.

[0330] The present description sets forth numerous exemplary configurations, methods, parameters, and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure, but is instead provided as a description of exemplary embodiments. Embodiments of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting embodiments of the disclosure are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered embodiments may be used or combined with any of the preceding or following individually numbered embodiments. This is intended to provide support for all such combinations of embodiments and is not limited to combinations of embodiments explicitly provided below:

[0331] The following Examples are merely illustrative and are not meant to limit any aspects of the present disclosure in any way.

ENUMERATED EMBODIMENTS

[0332] The disclosure can be understood with respect to the following illustrated, enumerated embodiments:

SET 1.

[0333] 1. A gene repressor system comprising:

(a) a catalytically-dead Class 2, Type V CRISPR protein;

(b) one or more transcription repressor domains; and

(c) a guide ribonucleic acid (gRNA)

wherein:

i) the one or more transcription repressor domains are linked to the catalytically-dead Class 2, Type V CRISPR protein as a fusion protein;

ii) the gRNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene; and

iii) the fusion protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA.

[0334] 2. The gene repressor system of embodiment 1, wherein the gene encodes mRNA, rRNA, tRNA, structural RNA, or protein.

[0335] 3. The gene repressor system of embodiment 1, wherein the one or more transcription repressor domains are selected from the group consisting of Krüppel-associated box (KRAB), methyl-CpG (mCpG) binding domain 2 (MeCP2), DNMT3A, DNMT3L, FOG, EZH2, SID4X, SID, NcoR, NuE, methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), and heterochromatin protein 1 (HP1A).

[0336] 4. The gene repressor system of embodiment 3, wherein the KRAB transcriptional repressor domain is selected from the group consisting of ZNF343, ZNF337, ZNF334, ZNF215, ZNF519, ZNF485, ZNF214, ZNF33B, ZNF287, ZNF705A, ZNF37A, KRBOX4, ZKSCAN3, ZKSCAN4, ZNF57, ZNF557, ZNF705B, ZNF662, ZNF77, ZNF500, ZNF558, ZNF620, ZNF713, ZNF823, ZNF440, ZNF441, ZNF136, SNRPB, ZNF735, ZKSCAN2, ZNF619, ZNF627, ZNF333, ABCA11P, PLD5P1, ZNF25, ZNF727, ZNF595, ZNF14, ZNF33A, ZNF101, ZNF253, ZNF56, ZNF720, ZNF85, ZNF66, ZNF722P, ZNF486, ZNF682, ZNF626, ZNF100, ZNF93, ZKSCAN1, ZNF257, ZNF729, ZNF208, ZNF90, ZNF430, ZNF676, ZNF91, ZNF429, ZNF675, ZNF681, ZNF99, ZNF431, ZNF98, ZNF708, ZNF732, SSX2, ZNF721, ZNF726, ZNF730, ZNF506, ZNF728, ZNF141, ZNF723, ZNF302, ZNF484, LINC00960, SSX2B, ZNF718, ZNF74, ZNF157, ZNF790, ZNF565, ZNF705G, VN1R107P, SLC27A5, ZNF737, SSX4, ZNF850, ZNF717, ZNF155, ZNF283, ZNF404, ZNF114, ZNF716, ZNF230, ZNF45, ZNF222, ZNF286A, ZNF624, ZNF223, ZNF284, ZNF790-AS1, ZNF382, ZNF749, ZNF615, ZFP90, ZNF225, ZNF234, ZNF568, ZNF614, ZNF584, ZNF432, ZNF461, ZNF182, ZNF630,

ZNF630-AS1, ZNF132, ZNF420, ZNF324B, ZNF616, ZNF471, ZNF227, ZNF324, ZNF860, ZFP28, ZNF470, ZNF586, ZNF235, ZNF274, ZNF446, ZFP1, ZIM3, ZNF212, ZNF766, ZNF264, ZNF480, ZNF667, ZNF805, ZNF610, ZNF783, ZNF621, ZNF8-DT, ZNF880, ZNF213-AS1, ZNF213, ZNF263, ZSCAN32, ZIM2, ZNF597, ZNF786, KRBA1, ZNF460, ZNF8, ZNF875, ZNF543, ZNF133, ZNF229, ZNF528, SSX1, ZNF81, ZNF578, ZNF862, ZNF777, ZNF425, ZNF548, ZNF746, ZNF282, ZNF398, ZNF599, ZNF251, ZNF195, ZNF181, RBAK-RBAKDN, ZFP37, RN7SL526P, ZNF879, ZNF26, ZSCAN21, ZNF3, ZNF354C, ZNF10, ZNF75D, ZNF426, ZNF561, ZNF562, ZNF846, ZNF782, ZNF552, ZNF587B, ZNF814, ZNF587, ZNF92, ZNF417, ZNF256, ZNF473, ZFP14, ZFP82, ZNF529, ZNF605, ZFP57, ZNF724, ZNF43, ZNF354A, ZNF547, SSX4B, ZNF585A, ZNF585B, ZNF792, ZNF789, ZNF394, ZNF655, ZFP92, ZNF41, ZNF674, ZNF546, ZNF780B, ZNF699, ZNF177, ZNF560, ZNF583, ZNF707, ZNF808, ZKSCAN5, ZNF137P, ZNF611, ZNF600, ZNF28, ZNF773, ZNF549, ZNF550, ZNF416, ZIK1, ZNF211, ZNF527, ZNF569, ZNF793, ZNF571-AS1, ZNF540, ZNF571, ZNF607, ZNF75A, ZNF205, ZNF175, ZNF268, ZNF354B, ZNF135, ZNF221, ZNF285, ZNF419, ZNF30, ZNF304, ZNF254, ZNF701, ZNF418, ZNF71, ZNF570, ZNF705E, KRBOX1, ZNF510, ZNF778, PRDM9, ZNF248, ZNF845, ZNF525, ZNF765, ZNF813, ZNF747, ZNF764, ZNF785, ZNF689, ZNF311, ZNF169, ZNF483, ZNF493, ZNF189, ZNF658, ZNF564, ZNF490, ZNF791, ZNF678, ZNF454, ZNF34, ZNF7, ZNF250, ZNF705D, ZNF641, ZNF2, ZNF554, ZNF555, ZNF556, ZNF596, ZNF517, ZNF331, ZNF18, ZNF829, ZNF772, ZNF17, ZNF112, ZNF514, ZNF688, PRDM7, ZNF695, ZNF670-ZNF695, ZNF138, ZNF670, ZNF19, ZNF316, ZNF12, ZNF202, RBAK, ZNF83, ZNF468, ZNF479, ZNF679, ZNF736, ZNF680, ZNF273, ZNF107, ZNF267, ZKSCAN8, ZNF84, ZNF573, ZNF23, ZNF559, ZNF44, ZNF563, ZNF442, ZNF799, ZNF443, ZNF709, ZNF566, ZNF69, ZNF700, ZNF763, ZNF433-AS1, ZNF433, ZNF878, ZNF844, ZNF788P, ZNF20, ZNF625-ZNF20, ZNF625, ZNF606, ZNF530, ZNF577, ZNF649, ZNF613, ZNF350, ZNF317, ZNF300, ZNF180, ZNF415, VN1R1, ZNF266, ZNF738, ZNF445, ZNF852, ZKSCAN7, ZNF660, MPRIPP1, ZNF197, ZNF567, ZNF582, ZNF439, ZFP30, ZNF559-ZNF177, ZNF226, ZNF841, ZNF544, ZNF233, ZNF534, ZNF836, ZNF320, KRBA2, ZNF761, ZNF383, ZNF224, ZNF551, ZNF154, ZNF671, ZNF776, ZNF780A, ZNF888, ZNF816-ZNF321P, ZNF321P, ZNF816, ZNF347, ZNF665, ZNF677, ZNF160, ZNF184, ZNF140, ZNF589, ZNF891, ZFP69B, ZNF436, POGK, ZNF669, ZFP69, ZNF684, ZNF124, and ZNF496.

[0337] 5. The gene repressor system of embodiment 1, wherein the one or more transcription repressor domains are selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0338] 6. The gene repressor system of embodiment 1, wherein the one or more transcription repressor domains are selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239.

[0339] 7. The gene repressor complex of any one of the preceding embodiments, wherein the fusion protein comprises two transcriptional repressor domains, wherein the first transcriptional repressor domain is different from the second transcriptional repressor domain.

[0340] 8. The gene repressor complex of embodiment 7, wherein the first transcriptional repressor domain is KRAB and the second transcriptional repressor domain is selected from the group consisting of methyl-CpG (mCpG) binding domain 2 (MeCP2), DNMT3A, DNMT3L, FOG, EZH2, SID4X, SID, NcoR, NuE, methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), heterochromatin protein 1 (HP1A), mixed lineage leukemia protein-1 (MLL1), MLL2, MLL3, MLL4, MLL5, SET Domain Containing 1A (SETD1A), SETD1B, SETD2, Suppressor Of Variegation 3-9 Homolog 1 (SUV39H1), SUV39H2, euchromatic histone lysine methyltransferase 1 (EHMT1), histone-lysine N-methyltransferase EZH1 (EZH1), nuclear receptor binding SET domain protein 1 (NSD1), NSD2, NSD3, ASH1 like histone lysine methyltransferase (ASH1L), tripartite motif containing 28 (TRIM28), Methyltransferase Like 3 (METTL3), METTL4, family with sequence similarity 208 member A (FAM208A), M-Phase Phosphoprotein 8 (MPHOSPH8), and Periphilin 1 (PPHLN1).

[0341] 9. The gene repressor complex of embodiment 7 or embodiment 8, wherein the fusion protein comprises a third transcriptional repressor domain, wherein the third transcriptional repressor domain is different from the first and the second transcriptional repressor domains.

[0342] 10. The gene repressor complex of embodiment 9, wherein the first transcriptional repressor domain is KRAB and the second and third transcriptional repressor domains are selected from the group consisting of methyl-CpG (mCpG) binding domain 2 (MeCP2),

DNMT3A, DNMT3L, FOG, EZH2, SID4X, SID, NcoR, NuE, methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), heterochromatin protein 1 (HP1A), mixed lineage leukemia protein-1 (MLL1), MLL2, MLL3, MLL4, MLL5, SET Domain Containing 1A (SETD1A), SETD1B, SETD2, Suppressor Of Variegation 3-9 Homolog 1 (SUV39H1), SUV39H2, euchromatic histone lysine methyltransferase 1 (EHMT1), histone-lysine N-methyltransferase EZH1 (EZH1), nuclear receptor binding SET domain protein 1 (NSD1), NSD2, NSD3, ASH1 like histone lysine methyltransferase (ASH1L), tripartite motif containing 28 (TRIM28), Methyltransferase Like 3 (METTL3), METTL4, family with sequence similarity 208 member A (FAM208A), M-Phase Phosphoprotein 8 (MPHOSPH8), and Periphilin 1 (PPHLN1)..

[0343] 11. The gene repressor complex of any one of embodiments 7-10, wherein the transcriptional repressor domains are linked by linker peptide sequences.

[0344] 12. The gene repressor complex of any one of the preceding embodiments, wherein the one or more transcriptional repressor domains are linked at or near the C-terminus of the catalytically-dead Class 2, Type V CRISPR protein by linker peptide sequences.

[0345] 13. The gene repressor complex of embodiments 1-11, wherein the one or more transcriptional repressor domains are linked at or near the N-terminus of the catalytically-dead Class 2, Type V CRISPR protein by linker peptide sequences.

[0346] 14. The gene repressor complex of any one of embodiments 11-13, wherein the linker peptide is selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO:33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

[0347] 15. The gene repressor system of any one of the preceding embodiments, wherein the catalytically-dead Class 2, Type V CRISPR protein comprises a catalytically-dead CasX variant protein (dCasX) comprising a sequence selected from the group consisting of SEQ ID NOS: 17-36 as set forth in Table 4, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

[0348] 16. The gene repressor system of any one of embodiments 1-14, wherein the catalytically-dead Class 2, Type V CRISPR protein comprises a catalytically-dead CasX variant protein (dCasX) comprising a sequence selected from the group consisting of the sequences SEQ ID NOS: 17-36 as set forth in Table 4.

[0349] 17. The gene repressor system of embodiment 15 or embodiment 16, comprising a sequence selected from the group consisting of the sequences as set forth in SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

[0350] 18. The gene repressor system of embodiment 15 or embodiment 16, comprising a sequence selected from the group consisting of the sequences as set forth in SEQ ID NOS: 889-2100 and 2332-33239.

[0351] 19. The gene repressor system of any one of embodiments 15-18, wherein the fusion protein further comprises one or more nuclear localization signals (NLS).

[0352] 20. The gene repressor system of embodiment 19, wherein the one or more NLS are selected from the group of sequences consisting of PKKKRKV (SEQ ID NO: 33289), KRPAATKKAGQAKKKK (SEQ ID NO: 33290), PAAKRVKLD (SEQ ID NO: 33291), RQRRNELKRSP (SEQ ID NO: 33292), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 33293), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 33294), VSRKRPRP (SEQ ID NO: 33295), PPKKARED (SEQ ID NO: 33296), PQPKKKPL (SEQ ID NO: 166), SALIKKKKKMAP (SEQ ID NO: 33298), DRLRR (SEQ ID NO: 33299), PKQKKRK (SEQ ID NO: 33300), RKLKKKIKKL (SEQ ID NO: 33301), REKKKFLKRR (SEQ ID NO: 33302), KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 33303), RKCLQAGMNLEARKTKK (SEQ ID NO: 33304), PRPRKIPR (SEQ ID NO: 33305), PPRKKRTVV (SEQ ID NO: 33306), NLSKKKKRKREK (SEQ ID NO: 33307),

RRPSRPFRKP (SEQ ID NO: 33308), KRPRSPSS (SEQ ID NO: 33309), KRGINDRNFWRGENERKTR (SEQ ID NO: 33310), PRPPKMARYDN (SEQ ID NO: 33311), KRSFSKAF (SEQ ID NO: 33312), KLKIKRPVK (SEQ ID NO: 33313), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33314), PKTRRRPRRSQRKRPPT (SEQ ID NO: 33315), SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 33316), KTRRRPRRSQRKRPPT (SEQ ID NO: 33317), RRKKRRPRRKKRR (SEQ ID NO: 33318), PKKKSRKPKKKSRK (SEQ ID NO: 33319), HKKKHPDASVNFSEFSK (SEQ ID NO: 33320), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 33321), LSPSLSPLLSPSLSPL (SEQ ID NO: 33322), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 33323), PKRGRGRPKRGRGR (SEQ ID NO: 33324), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33325), PKKKRKVPPPPKKKRKV (SEQ ID NO: 33326), PAKRARRGYKC (SEQ ID NO: 33327), KLGPRKATGRW (SEQ ID NO: 33328), PRRKREE (SEQ ID NO: 33329), PYRGRKE (SEQ ID NO: 33330), PLRKRPRR (SEQ ID NO: 33331), PLRKRPRRGSPLRKRPRR (SEQ ID NO: 33332), PAAKRVKLDGGKRTADGSEFESPKKKRKV (SEQ ID NO: 33333), PAAKRVKLDGGKRTADGSEFESPKKKRKVGIHGVPAA (SEQ ID NO: 33334), PAAKRVKLDGGKRTADGSEFESPKKKRKVAEAAAKEAAAKEAAAKA (SEQ ID NO: 33335), PAAKRVKLDGGKRTADGSEFESPKKKRKVPG (SEQ ID NO: 33336), KRKGSPERGERKRHW (SEQ ID NO: 33337), KRTADSQHSTPPKTKRKVEFEPKKKRKV (SEQ ID NO: 33338).

[0353] 21. The gene repressor system of embodiment 19 or embodiment 20, wherein the one or more NLS are linked at or near the C-terminus of the dCasX or the repressor domain.

[0354] 22. The gene repressor system of embodiment 19 or embodiment 20, wherein the one or more NLS are linked at or near the N-terminus of the dCasX or the repressor domain.

[0355] 23. The gene repressor system of embodiment 19 or embodiment 20, wherein the one or more NLS are linked at or near both the N-terminus and the C-terminus of the dCasX or the repressor domain.

[0356] 24. The gene repressor system of embodiment 19, wherein the one or more NLS are selected from the group of sequences as set forth in Table 5 and are linked at or near the N-terminus of the dCasX or the repressor domain.

[0357] 25. The gene repressor system of embodiment 19, wherein the one or more NLS are selected from the group of SEQ ID NOS: 72-112 as set forth in Table 6 and are linked at or near the C-terminus of the dCasX or the repressor domain.

[0358] 26. The gene repressor system of embodiment 19, wherein one or more NLS are selected from the group of SEQ ID NOS: 37-71 as set forth in Table 5 and are linked at or near the N-terminus of the dCasX or the repressor domain and one or more NLS are selected from the group of sequences as set forth in Table 6 and are linked at or near the C-terminus of the dCasX or the repressor domain.

[0359] 27. The gene repressor system of any one of embodiments 19-26, wherein the one or more NLS are linked to the dCasX variant protein, the repressor domain, or to adjacent NLS with one or more linker peptides wherein the linker peptides are selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO: 33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

[0360] 28. The gene repressor system of any one of the preceding embodiments, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2101-2331 as set forth in Table 2, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

[0361] 29. The gene repressor system of any one of embodiments 1-28, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2101-2331, as set forth in Table 2.

[0362] 30. The gene repressor system of any one of the preceding embodiments, wherein the gRNA comprises a targeting sequence having 15, 16, 17, 18, 19, 20, or 21 nucleotides.

[0363] 31. The gene repressor system of embodiment 30, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of a transcription start site (TSS) in the gene.

[0364] 32. The gene repressor system of embodiment 30, wherein the target nucleic acid sequence complementary to the targeting sequence is within 500 bps upstream to 500 bps downstream of a TSS of the gene.

[0365] 33. The gene repressor system of embodiment 30, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb 3' or 5' to an untranslated region of the gene.

[0366] 34. The gene repressor system of embodiment 30, wherein the target nucleic acid sequence complementary to the targeting sequence is within the open reading frame of the gene.

[0367] 35. The gene repressor system of embodiment 30, wherein the target nucleic acid sequence complementary to the targeting sequence is within an exon of the gene.

[0368] 36. The gene repressor system of embodiment 35, wherein the target nucleic acid sequence complementary to the targeting sequence is within exon 1 of the gene.

[0369] 37. The gene repressor system of any one of the preceding embodiments, wherein the RNP is capable of binding the target nucleic acid but is not capable of cleaving the target nucleic acid.

[0370] 38. A nucleic acid encoding the fusion protein of the gene repressor system of any one of the preceding embodiments.

[0371] 39. A nucleic acid encoding the gRNA of any one of the preceding embodiments.

[0372] 40. The nucleic acid of embodiment 38 or embodiment 39, wherein the nucleic acid sequence is codon optimized for expression in a eukaryotic cell.

[0373] 41. A vector comprising the nucleic acids of embodiments 38-40.

[0374] 42. The vector of embodiment 41, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP) vector, a delivery particle system (XDP) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

[0375] 43. The vector of embodiment 42, wherein the vector is an AAV vector.

[0376] 44. The vector of embodiment 43, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 44.9, AAV-Rh74, or AAVRh10.

[0377] 45. The vector of embodiment 43 or embodiment 44, wherein the nucleic acid encoding the fusion protein and the gRNA are incorporated as a transgene between a 5' and a 3' inverted terminal repeat (ITR) sequence within the AAV.

[0378] 46. The vector of embodiment 42, wherein the vector is a XDP vector comprising a nucleic acid encoding one or more components of a retroviral gag polyprotein or a gag-pol polyprotein.

[0379] 47. The vector of embodiment 46, wherein the nucleic acid encodes one or more components are selected from the group consisting of a gag-transframe region-pol protease polyprotein (gag-TFR-PR), a matrix protein (MA), a nucleocapsid protein (NC), a capsid protein (CA), a p1 peptide, a p6 peptide, a P2A peptide, a P2B peptide, a P10 peptide, a p12 peptide, a PP21/24 peptide, a P12/P3/P8 peptide, a P20 peptide, an MS2 coat protein, a protease, and a protease cleavage site.

[0380] 48. The vector of embodiment 46 or embodiment 47, wherein the nucleic acid further encodes the fusion protein of embodiment 38.

[0381] 49. The vector of embodiment 46 or embodiment 47, wherein the vector comprises a first nucleic acid encoding the fusion protein and a second nucleic acid encoding the one or more components of the gag polyprotein.

[0382] 50. The vector of embodiment 48 or embodiment 49, further comprising a nucleic acid encoding a pseudotyping viral envelope glycoprotein or antibody fragment that provides for binding and fusion of the XDP to a target cell.

[0383] 51. The vector of any one of embodiments 47-50, wherein the encoded gRNA further comprises an MS2 hairpin sequence.

[0384] 52. The vector of any one of embodiments 47-51, further comprising a nucleic acid encoding a Gag-transframe region-Pol protease polyprotein (Gag-TFR-PR) and intervening protease cleavage sites between each component of the Gag-TFR-PR.

[0385] 53. The vector of embodiment 52, wherein the nucleic acids are configured as depicted in FIG. 4 or FIG. 5.

[0386] 54. A host cell comprising the vector of any one of embodiments 41-53.

[0387] 55. The host cell of embodiment 54, wherein the host cell is selected from the group consisting of BHK, HEK293, HEK293T, NS0, SP2/0, YO myeloma cells, P3X63 mouse myeloma cells, PER, PER.C6, NIH3T3, COS, HeLa, CHO, and yeast cells.

[0388] 56. An XDP comprising:

(a) one or more components of selected from the group consisting of a matrix protein (MA), a nucleocapsid protein (NC), a capsid protein (CA), a p1 peptide, a p6 peptide, a P2A peptide, a P2B peptide, a P10 peptide, a p12 peptide, a PP21/24 peptide, a P12/P3/P8 peptide, a P20 peptide, an MS2 coat protein, a protease, and a protease cleavage site;

(b) an RNP comprising the gene repressor system of any one of embodiments 1-37 wherein the RNP is encapsidated within the XDP upon self-assembly of the XDP;

(c) a pseudotyping viral envelope glycoprotein or antibody fragment incorporated on the XDP capsid surface that provides for binding and fusion of the XDP to a target cell.

[0389] 57. A method of repressing transcription of a target nucleic acid sequence in a population of cells, the method comprising introducing into cells:

(a) RNP comprising the gene repressor system of any one of embodiments 1-37;

(b) the nucleic acid of any one of embodiments 38-40;

(c) the vector as in any one of embodiments 41-52;

(e) the XDP of embodiment 56; or

(f) combinations thereof,

wherein upon binding of the RNP to the target nucleic acid, transcription of the gene proximal to the binding location of the RNP is repressed in the cells.

[0390] 58. The method of embodiment 57, wherein transcription of the gene in the population of cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% greater compared to the repression effected by an RNP comprising a comparable guide RNA and a catalytically dead CasX variant without a repressor domain, when assessed in an *in vitro* assay.

[0391] 59. The method of embodiment 57 or embodiment 58, wherein off-target binding or off-target transcription repression is less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells.

[0392] 60. The method of any one of embodiments 57-59, wherein the repression of transcription in the cells is sustained for at least about 8 hours, at least about 1 day, at least about 1 week, or at least about 1 month.

[0393] 61. The method of any one of embodiments 57-60, further comprising a second gRNA or a nucleic acid encoding the second gNA, wherein the second gNA has a targeting sequence complementary to a different portion of the target nucleic acid sequence and is capable of

forming a ribonuclear protein complex (RNP) with the catalytically-dead Class 2, Type V CRISPR protein.

[0394] 62. A method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective amount of:

(a) the AAV vector of embodiment 43 or embodiment 44; or

(b) the XDP of embodiment 56,

wherein upon binding of the RNP to the target nucleic acid in cells of the subject contacted by the AAV vector or XDP, transcription of the gene proximal to the binding location of the RNP is repressed.

[0395] 63. The method of embodiment 62, wherein transcription of the gene in the targeted cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%.

[0396] 64. The method of embodiment 62 or embodiment 63, wherein the treating results in improvement in at least one clinically-relevant endpoint associated with the disease or disorder.

[0397] 65. The method of any one of embodiments 62-64, wherein the AAV vector or XDP is administered to the subject by a route of administration selected from subcutaneous, intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatical, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation, or combinations thereof.

[0398] 66. The method of embodiment 65, wherein the XDP is administered at a dose of at least about 1×10^5 particles/kg, or at least about 1×10^6 particles/kg, or at least about 1×10^7 particles/kg, or at least about 1×10^8 particles/kg, or at least about 1×10^9 particles/kg, or at least about 1×10^{10} particles/kg, or at least about 1×10^{11} particles/kg, or at least about 1×10^{12} particles/kg, or at least about 1×10^{13} particles/kg, or at least about 1×10^{14} particles/kg, or at least about 1×10^{15} particles/kg, or at least about 1×10^{16} particles/kg.

[0399] 67. The method of embodiment 65, wherein the XDP is administered to the subject at a dose of at least about 1×10^5 particles/kg to about 1×10^{16} particles/kg, or at least about 1×10^6 particles/kg to about 1×10^{15} particles/kg, or at least about 1×10^7 particles/kg to about 1×10^{14} particles/kg.

[0400] 68. The method of embodiment 65, wherein the AAV vector is administered to the subject at a dose of at least about 1×10^8 vector genomes (vg), at least about 1×10^5 vector

genomes/kg (vg/kg), at least about 1 x 10^6 vg/kg, at least about 1 x 10^7 vg/kg, at least about 1 x 10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 1 x 10^{14} vg/kg, at least about 1 x 10^{15} vg/kg, or at least about 1 x 10^{16} vg/kg.

[0401] 69. The method of embodiment 65, wherein the AAV vector is administered to the subject at a dose of at least about 1 x 10^5 vg/kg to about 1 x 10^{16} vg/kg, at least about 1 x 10^6 vg/kg to about 1 x 10^{15} vg/kg, or at least about 1 x 10^7 vg/kg to about 1 x 10^{14} vg/kg.

[0402] 70. The method of any one of embodiments 62-69, wherein the XDP or AAV vector is administered to the subject according to a treatment regimen comprising one or more consecutive doses of the XDP or AAV.

[0403] 71. The method of embodiment 70, wherein the therapeutically effective dose is administered to the subject as two or more doses over a period of at least two weeks, or at least one month, or at least two months, or at least three months, or at least four months, or at least five months, or at least six months, or once a year.

[0404] 72. The method of any one of embodiments 62-71, wherein the subject is selected from the group consisting of mouse, rat, pig, and non-human primate.

[0405] 73. The method of any one of embodiments 62-71, wherein the subject is human.

[0406] 74. A pharmaceutical composition comprising the gene repressor system of any one of embodiments 1-37 and a pharmaceutically acceptable excipient.

[0407] 75. The gene repressor system of any one of embodiments 1-37 for use as a medicament in the treatment of a subject a disorder caused by a genetic mutation.

[0408] 76. The gene repressor system of any one of embodiments 1-37, wherein the targeting sequence of the gRNA is complementary to a non-target strand sequence located 1 nucleotide 3' of a protospacer adjacent motif (PAM) sequence.

[0409] 77. The composition of embodiment 76, wherein the PAM sequence comprises a TC motif.

[0410] 78. The composition of embodiment 77, wherein the PAM sequence comprises ATC, GTC, CTC or TTC.

SET 2.

[0411] 1. A gene repressor system comprising:

(a) a catalytically-dead Class 2, Type V CRISPR protein;

(b) one or more transcription repressor domains; and

(c) a guide ribonucleic acid (gRNA)

wherein:

i) the one or more transcription repressor domains are linked to the catalytically-dead Class 2, Type V CRISPR protein as a fusion protein;

ii) the gRNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene targeted for repression, silencing, or downregulation;

iii) the fusion protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA; and

iv) the RNP is capable of binding to the target nucleic acid.

[0412] 2. The gene repressor system of embodiment 1, wherein the gene encodes mRNA, rRNA, tRNA, or structural RNA.

[0413] 3. The gene repressor system of embodiment 1, wherein the one or more transcription repressor domains are selected from the group consisting of a Krüppel-associated box (KRAB), DNA methyltransferase 3 alpha (DNMT3A), DNMT3A-like protein (DNMT3L), DNA methyltransferase 3 beta (DNMT3B), DNA methyltransferase 1 (DNMT1), Friend of GATA-1 (FOG), Mad mSIN3 interaction domain (SID), enhanced SID (SID4X), nuclear receptor corepressor (NcoR), nuclear effector protein (NuE), KOX1 repression domain, the ERF repressor domain (ERD), the SRDX repression domain, histone lysine methyltransferases such as PR/SET domain containing protein (Pr-SET)7/8, lysine methyltransferase 5B (SUV4- 20H1), PR/SET domain 2 (RIZ1), histone lysine demethylases such as lysine demethylase 4A (JMJD2A/JHDM3A), lysine demethylase 4B (JMJD2B), lysine demethylase 4C (JMJD2C/GASC1), lysine demethylase 4D (JMJD2D), lysine demethylase 5A (JARID1A/RBP2), lysine demethylase 5B (JARID1B/PLU-1), lysine demethylase 5C (JARID 1C/SMCX), lysine demethylase 5D (JARID1D/SMCY), sirtuin 1 (SIRT1), SIRT2, DNA methylases such as HhaI DNA m5c-methyltransferase (M.HhaI), methyltransferase 1 (MET1), histone H3 lysine 9 methyltransferase G9a (G9a), S-adenosyl-L-methionine-dependent methyltransferases superfamily protein (DRM3), DNA cytosine methyltransferase MET2a (ZMET2), methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase-1 (NIPP1), GLP, chromomethylase 1 (CMT1), chromomethylase 2 (CMT2),

heterochromatin protein 1 (HP1A), mixed lineage leukemia protein-5 (MLL5), histone-lysine N-methyltransferase SETDB1 (SETB1), Suppressor Of Variegation 3-9 Homolog 1 (SUV39H1), SUV39H2, euchromatic histone lysine methyltransferase 1 (EHMT1), histone-lysine N-methyltransferase EZH1 (EZH1), EZH2, nuclear receptor binding SET domain protein 1 (NSD1), NSD2, NSD3, ASH1 like histone lysine methyltransferase (ASH1L), tripartite motif containing 28 (TRIM28), Methyltransferase Like 3 (METTL3), METTL4, family with sequence similarity 208 member A (FAM208A), M-Phase Phosphoprotein 8 (MPHOSPH8), SET domain containing 2 (SETD2), histone deacetylase 1 (HDAC1), HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, and Periphilin 1 (PPHLN1) domain.

[0414] 4. The gene repressor system of embodiment 3, wherein the transcription repressor domain is a KRAB domain.

[0415] 5. The gene repressor system of embodiment 4, wherein the KRAB transcriptional repressor domain is selected from the group consisting of ZNF343, ZNF337, ZNF334, ZNF215, ZNF519, ZNF485, ZNF214, ZNF33B, ZNF287, ZNF705A, ZNF37A, KRBOX4, ZKSCAN3, ZKSCAN4, ZNF57, ZNF557, ZNF705B, ZNF662, ZNF77, ZNF500, ZNF558, ZNF620, ZNF713, ZNF823, ZNF440, ZNF441, ZNF136, SNRPB, ZNF735, ZKSCAN2, ZNF619, ZNF627, ZNF333, ABCA11P, PLD5P1, ZNF25, ZNF727, ZNF595, ZNF14, ZNF33A, ZNF101, ZNF253, ZNF56, ZNF720, ZNF85, ZNF66, ZNF722P, ZNF486, ZNF682, ZNF626, ZNF100, ZNF93, ZKSCAN1, ZNF257, ZNF729, ZNF208, ZNF90, ZNF430, ZNF676, ZNF91, ZNF429, ZNF675, ZNF681, ZNF99, ZNF431, ZNF98, ZNF708, ZNF732, SSX2, ZNF721, ZNF726, ZNF730, ZNF506, ZNF728, ZNF141, ZNF723, ZNF302, ZNF484, LINC00960, SSX2B, ZNF718, ZNF74, ZNF157, ZNF790, ZNF565, ZNF705G, VN1R107P, SLC27A5, ZNF737, SSX4, ZNF850, ZNF717, ZNF155, ZNF283, ZNF404, ZNF114, ZNF716, ZNF230, ZNF45, ZNF222, ZNF286A, ZNF624, ZNF223, ZNF284, ZNF790-AS1, ZNF382, ZNF749, ZNF615, ZFP90, ZNF225, ZNF234, ZNF568, ZNF614, ZNF584, ZNF432, ZNF461, ZNF182, ZNF630, ZNF630-AS1, ZNF132, ZNF420, ZNF324B, ZNF616, ZNF471, ZNF227, ZNF324, ZNF860, ZFP28, ZNF470, ZNF586, ZNF235, ZNF274, ZNF446, ZFP1, ZIM3, ZNF212, ZNF766, ZNF264, ZNF480, ZNF667, ZNF805, ZNF610, ZNF783, ZNF621, ZNF8-DT, ZNF880, ZNF213-AS1, ZNF213, ZNF263, ZSCAN32, ZIM2, ZNF597, ZNF786, KRBA1, ZNF460, ZNF8, ZNF875, ZNF543, ZNF133, ZNF229, ZNF528, SSX1, ZNF81, ZNF578, ZNF862, ZNF777, ZNF425, ZNF548, ZNF746, ZNF282, ZNF398, ZNF599, ZNF251, ZNF195, ZNF181, RBAK-RBAKDN, ZFP37, RN7SL526P, ZNF879, ZNF26, ZSCAN21, ZNF3, ZNF354C,

ZNF10, ZNF75D, ZNF426, ZNF561, ZNF562, ZNF846, ZNF782, ZNF552, ZNF587B, ZNF814, ZNF587, ZNF92, ZNF417, ZNF256, ZNF473, ZFP14, ZFP82, ZNF529, ZNF605, ZFP57, ZNF724, ZNF43, ZNF354A, ZNF547, SSX4B, ZNF585A, ZNF585B, ZNF792, ZNF789, ZNF394, ZNF655, ZFP92, ZNF41, ZNF674, ZNF546, ZNF780B, ZNF699, ZNF177, ZNF560, ZNF583, ZNF707, ZNF808, ZKSCAN5, ZNF137P, ZNF611, ZNF600, ZNF28, ZNF773, ZNF549, ZNF550, ZNF416, ZIK1, ZNF211, ZNF527, ZNF569, ZNF793, ZNF571-AS1, ZNF540, ZNF571, ZNF607, ZNF75A, ZNF205, ZNF175, ZNF268, ZNF354B, ZNF135, ZNF221, ZNF285, ZNF419, ZNF30, ZNF304, ZNF254, ZNF701, ZNF418, ZNF71, ZNF570, ZNF705E, KRBOX1, ZNF510, ZNF778, PRDM9, ZNF248, ZNF845, ZNF525, ZNF765, ZNF813, ZNF747, ZNF764, ZNF785, ZNF689, ZNF311, ZNF169, ZNF483, ZNF493, ZNF189, ZNF658, ZNF564, ZNF490, ZNF791, ZNF678, ZNF454, ZNF34, ZNF7, ZNF250, ZNF705D, ZNF641, ZNF2, ZNF554, ZNF555, ZNF556, ZNF596, ZNF517, ZNF331, ZNF18, ZNF829, ZNF772, ZNF17, ZNF112, ZNF514, ZNF688, PRDM7, ZNF695, ZNF670-ZNF695, ZNF138, ZNF670, ZNF19, ZNF316, ZNF12, ZNF202, RBAK, ZNF83, ZNF468, ZNF479, ZNF679, ZNF736, ZNF680, ZNF273, ZNF107, ZNF267, ZKSCAN8, ZNF84, ZNF573, ZNF23, ZNF559, ZNF44, ZNF563, ZNF442, ZNF799, ZNF443, ZNF709, ZNF566, ZNF69, ZNF700, ZNF763, ZNF433-AS1, ZNF433, ZNF878, ZNF844, ZNF788P, ZNF20, ZNF625-ZNF20, ZNF625, ZNF606, ZNF530, ZNF577, ZNF649, ZNF613, ZNF350, ZNF317, ZNF300, ZNF180, ZNF415, VN1R1, ZNF266, ZNF738, ZNF445, ZNF852, ZKSCAN7, ZNF660, MPRIPP1, ZNF197, ZNF567, ZNF582, ZNF439, ZFP30, ZNF559-ZNF177, ZNF226, ZNF841, ZNF544, ZNF233, ZNF534, ZNF836, ZNF320, KRBA2, ZNF761, ZNF383, ZNF224, ZNF551, ZNF154, ZNF671, ZNF776, ZNF780A, ZNF888, ZNF816-ZNF321P, ZNF321P, ZNF816, ZNF347, ZNF665, ZNF677, ZNF160, ZNF184, ZNF140, ZNF589, ZNF891, ZFP69B, ZNF436, POGK, ZNF669, ZFP69, ZNF684, ZNF124, ZNF496, and sequence variants thereof.

[0416] 6. The gene repressor system of embodiment 4 or embodiment 5, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93% at least about 94% at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity thereto.

[0417] 7. The gene repressor system of embodiment 4 or embodiment 5, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239.

[0418] 8. The gene repressor complex of any one of embodiments 1-7, wherein the one or more transcriptional repressor domains are linked at or near the C-terminus of the catalytically-dead Class 2, Type V CRISPR protein by linker peptide sequences.

[0419] 9. The gene repressor complex of embodiments 1-7, wherein the one or more transcriptional repressor domains are linked at or near the N-terminus of the catalytically-dead Class 2, Type V CRISPR protein by linker peptide sequences.

[0420] 10. The gene repressor complex of any one of embodiments 1-9, wherein the fusion protein comprises two transcriptional repressor domains, wherein the first transcriptional repressor domain is different from the second transcriptional repressor domain.

[0421] 11. The gene repressor complex of embodiment 10, wherein the first transcriptional repressor domain is KRAB and the second transcriptional repressor domain is selected from the group consisting of DNMT3A, DNMT3L, DNMT3B, DNMT1, FOG, SID, SID4X, NcoR, NuE, KOX1, ERD, Pr-SET 7/8, SUV4- 20H1, RIZ1, JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, JARID1A/RBP2, JARID1B/PLU-1, JARID 1C/SMCX, JARID1D/SMCY, SIRT1, SIRT2, M.HhaI, MET1, G9a, DRM3, ZMET2, meCP2, SIN3A, HDT1, MBD2B, NIPP1, GLP, CMT1, CMT2, HP1A, MLL5, SETB1, SUV39H1, SUV39H2, EHMT1, EZH1, EZH2, NSD1, NSD2, NSD3, ASH1L, TRIM28, METTL3, METTL4, FAM208A, MPHOSPH8, SETD2, HDAC1, HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, and PPHLN1.

[0422] 12. The gene repressor complex of embodiment 11, wherein the second transcriptional repressor domain is a DNMT3A domain, or a sequence variant thereof.

[0423] 13. The gene repressor complex of embodiment 12, wherein the DNMT3A domain is selected from the group consisting of SEQ ID NOS: 33625-57543.

[0424] 14. The gene repressor complex of any one of embodiments 10-13, wherein the fusion protein comprises a third transcriptional repressor domain, wherein the third transcriptional repressor domain is different from the first and the second transcriptional repressor domains.

[0425] 15. The gene repressor complex of embodiment 14, wherein the third transcriptional repressor domain is selected from the group consisting of DNMT3L, DNMT3B, DNMT1, FOG, SID, SID4X, NcoR, NuE, KOX1, ERD, Pr-SET 7/8, SUV4- 20H1, RIZ1, JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, JARID1A/RBP2, JARID1B/PLU-1, JARID 1C/SMCX, JARID1D/SMCY, SIRT1, SIRT2, M.HhaI, MET1, G9a, DRM3, ZMET2, meCP2, SIN3A, HDT1, MBD2B, NIPP1, GLP, CMT1, CMT2, HP1A, MLL5, SETB1, SUV39H1, SUV39H2, EHMT1, EZH1, EZH2, NSD1, NSD2, NSD3, ASH1L, TRIM28, METTL3, METTL4,

FAM208A, MPHOSPH8, SETD2, HDAC1, HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, and PPHLN1.

[0426] 16. The gene repressor complex of embodiment 14 or embodiment 15, wherein the third transcriptional repressor domain is DMNT3L, or a sequence variant thereof.

[0427] 17. The gene repressor complex of any one of embodiments 1-16, wherein the second and/or third transcriptional repressor domains are linked to the catalytically-dead Class 2, Type V CRISPR protein or to a transcriptional repressor domain by a linker peptide sequence.

[0428] 18. The gene repressor complex of any one of embodiments 8-17, wherein the linker peptide is selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GSGSGGG (SEQ ID NO: 57628), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO:33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

[0429] 19. The gene repressor system of any one of embodiments 1-18, wherein the catalytically-dead Class 2, Type V CRISPR protein comprises a catalytically-dead CasX variant protein (dCasX) comprising a sequence selected from the group consisting of SEQ ID NOS: 17-36 as set forth in Table 4, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

[0430] 20. The gene repressor system of any one of embodiments 1-18, wherein the catalytically-dead Class 2, Type V CRISPR protein comprises a catalytically-dead CasX variant protein (dCasX) comprising a sequence selected from the group consisting of the sequences SEQ ID NOS: 17-36 as set forth in Table 4.

[0431] 21. The gene repressor system of any one of embodiments 1-20, wherein the fusion protein further comprises one or more nuclear localization signals (NLS).

[0432] 22. The gene repressor system of embodiment 21, wherein the one or more NLS are selected from the group of sequences consisting of PKKKRKV (SEQ ID NO: 33289), KRPAATKKAGQAKKKK (SEQ ID NO: 33290), PAAKRVKLD (SEQ ID NO: 33291), RQRRNELKRSP (SEQ ID NO: 33292), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 33293), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 33294), VSRKRPRP (SEQ ID NO: 33295), PPKKARED (SEQ ID NO: 33296), PQPKKKPL (SEQ ID NO: 166), SALIKKKKKMAP (SEQ ID NO: 33298), DRLRR (SEQ ID NO: 33299), PKQKKRK (SEQ ID NO: 33300), RKLKKKIKKL (SEQ ID NO: 33301), REKKKFLKRR (SEQ ID NO: 33302), KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 33303), RKCLQAGMNLEARKTKK (SEQ ID NO: 33304), PRPRKIPR (SEQ ID NO: 33305), PPRKKRTVV (SEQ ID NO: 33306), NLSKKKKRKREK (SEQ ID NO: 33307), RRPSRPFRKP (SEQ ID NO: 33308), KRPRSPSS (SEQ ID NO: 33309), KRGINDRNFWRGENERKTR (SEQ ID NO: 33310), PRPPKMARYDN (SEQ ID NO: 33311), KRSFSKAF (SEQ ID NO: 33312), KLKIKRPVK (SEQ ID NO: 33313), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33314), PKTRRRPRRSQRKRPPT (SEQ ID NO: 33315), SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 33316), KTRRRPRRSQRKRPPT (SEQ ID NO: 33317), RRKKRRPRRKKRR (SEQ ID NO: 33318), PKKKSRKPKKKSRK (SEQ ID NO: 33319), HKKKHPDASVNFSEFSK (SEQ ID NO: 33320), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 33321), LSPSLSPLLSPSLSPL (SEQ ID NO: 33322), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 33323), PKRGRGRPKRGRGR (SEQ ID NO: 33324), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33325), PKKKRKVPPPPKKKRKV (SEQ ID NO: 33326), PAKRARRGYKC (SEQ ID NO: 33327), KLGPRKATGRW (SEQ ID NO: 33328), PRRKREE (SEQ ID NO: 33329), PYRGRKE (SEQ ID NO: 33330), PLRKRPRR (SEQ ID NO: 33331), PLRKRPRRGSPLRKRPRR (SEQ ID NO: 33332), PAAKRVKLDGGKRTADGSEFESPKKKRKV (SEQ ID NO: 33333), PAAKRVKLDGGKRTADGSEFESPKKKRKVGIHGVPAA (SEQ ID NO: 33334), PAAKRVKLDGGKRTADGSEFESPKKKRKVAEAAAKEAAAKEAAAKA (SEQ ID NO: 33335), PAAKRVKLDGGKRTADGSEFESPKKKRKVPG (SEQ ID NO: 33336), KRKGSPERGERKRHW (SEQ ID NO: 33337), KRTADSQHSTPPKTKRKVEFEPKKKRKV (SEQ ID NO: 33338), and SEQ ID NOS: 37-112.

[0433] 23. The gene repressor system of embodiment 21 or embodiment 22, wherein the one or more NLS are linked at or near the C-terminus of the dCasX or the repressor domain.

[0434] 24. The gene repressor system of embodiment 21 or embodiment 22, wherein the one or more NLS are linked at or near the N-terminus of the dCasX or the repressor domain.

[0435] 25. The gene repressor system of embodiment 21 or embodiment 22, wherein the one or more NLS are linked at or near both the N-terminus and the C-terminus of the dCasX or the repressor domain.

[0436] 26. The gene repressor system of embodiment 21, wherein the one or more NLS are selected from the group of SEQ ID NOS: 37-71 as set forth in Table 5 and are linked at or near the N-terminus of the dCasX or the repressor domain.

[0437] 27. The gene repressor system of embodiment 21, wherein the one or more NLS are selected from the group of SEQ ID NOS: 72-112 as set forth in Table 6 and are linked at or near the C-terminus of the dCasX or the repressor domain.

[0438] 28. The gene repressor system of embodiment 21, wherein one or more NLS comprise an NLS selected from the group consisting of SEQ ID NOS: 37-71 as set forth in Table 5 and are linked at or near the N-terminus of the dCasX or the repressor domain, and an NLS selected from the group consisting of SEQ ID NOS: 72-112 as set forth in Table 6 and are linked at or near the C-terminus of the dCasX or the repressor domain.

[0439] 29. The gene repressor system of any one of embodiments 21-28, wherein the one or more NLS are linked to the dCasX variant protein, the repressor domain, or to adjacent NLS with one or more linker peptides wherein the linker peptides are selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO: 33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO: 33262), and TPPKTKRKVEFE (SEQ ID NO: 33263), wherein n is an integer of 1 to 5.

[0440] 30. The gene repressor complex of any one of embodiments 21-29, wherein the fusion protein is configured according to a configuration as portrayed in FIG. 7.

[0441] 31. The gene repressor system of any one of embodiments 1-30, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2238-2331 and 57544-57589 as set forth in Table 2, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

[0442] 32. The gene repressor system of any one of embodiments 1-31, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2238-2331 and 57544-57589, as set forth in Table 2.

[0443] 33. The gene repressor system of any one of embodiments 1-32, wherein the gRNA comprises a targeting sequence having 15, 16, 17, 18, 19, 20, or 21 nucleotides.

[0444] 34. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of a transcription start site (TSS) in the gene.

[0445] 35. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within 500 bps upstream to 500 bps downstream of a TSS of the gene.

[0446] 36. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within 300 bps upstream to 300 bps downstream of a TSS of the gene.

[0447] 37. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of an enhancer of the gene.

[0448] 38. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within the 3' untranslated region of the gene.

[0449] 39. The gene repressor system of embodiment 33, wherein the target nucleic acid sequence complementary to the targeting sequence is within an exon of the gene.

[0450] 40. The gene repressor system of embodiment 39, wherein the target nucleic acid sequence complementary to the targeting sequence is within exon 1 of the gene.

[0451] 41. The gene repressor system of any one of embodiments 1-40, wherein the RNP is capable of binding to the target nucleic acid but is not capable of cleaving the target nucleic acid.

[0452] 42. A nucleic acid encoding the fusion protein of the gene repressor system of any one of embodiments 1-41.

[0453] 43. A nucleic acid encoding the gRNA of the gene repressor system of any one of embodiments 1-41.

[0454] 44. The nucleic acid of embodiment 42, wherein the nucleic acid sequence is codon optimized for expression in a eukaryotic cell.

[0455] 45. A lipid nanoparticle comprising the nucleic acid of embodiment 42.

[0456] 46. A lipid nanoparticle comprising the nucleic acid of embodiment 43.

[0457] 47. A lipid nanoparticle comprising a first nucleic acid encoding the fusion protein and a second nucleic acid comprising the gRNA of the repressor system of any one of embodiments 1-41.

[0458] 48. A lipid nanoparticle composition comprising a first population of lipid nanoparticles and a second population of lipid nanoparticles, and nucleic acids encoding the gene repressor system of any one of embodiments 1-41, wherein the first population comprises lipid nanoparticles that encapsidate a first nucleic acid encoding the fusion protein and the second population of lipid nanoparticles comprises nanoparticles that encapsidate a second nucleic acid encoding the gRNA or that comprises the gRNA.

[0459] 49. A vector comprising the nucleic acid of any one of embodiments 42-44.

[0460] 50. The vector of embodiment 49, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

[0461] 51. The vector of embodiment 50, wherein the vector is an AAV vector.

[0462] 52. The vector of embodiment 51, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 44.9, AAV-Rh74, or AAVRh10.

[0463] 53. The vector of embodiment 51 or embodiment 52, wherein the nucleic acid encoding the fusion protein and the gRNA are incorporated as a transgene between a 5' and a 3' inverted terminal repeat (ITR) sequence within the AAV.

[0464] 54. A delivery particle system (XDP) comprising:

(a) one or more components of selected from the group consisting of a matrix protein (MA), a nucleocapsid protein (NC), a capsid protein (CA), a p1 peptide, a p6 peptide, a p2A

peptide, a p2B peptide, a p10 peptide, a p12 peptide, a pp21/24 peptide, a p12/p3/p8 peptide, a p20 peptide, an MS2 coat protein, PP7 coat protein, Q coat protein, U1A signal recognition particle, phage R-loop, Rev protein, and Psi packaging element;

(b) an RNP comprising the gene repressor system of any one of embodiments 1-41 wherein the RNP is encapsidated within the XDP;

(c) a tropism factor incorporated on the XDP surface that provides for binding and fusion of the XDP to a target cell.

[0465] 55. The XDP of embodiment 54, wherein the tropism factor is selected from the group consisting of a pseudotyping viral envelope glycoprotein, an antibody fragment, or a cell receptor fragment.

[0466] 56. A method of repressing transcription of a target nucleic acid sequence of a gene in a population of cells, the method comprising introducing into the cells:

(a) an RNP comprising the gene repressor system of any one of embodiments 1-41;

(b) the nucleic acid of any one of embodiments 42-44;

(c) the vector of any one of embodiments 49-53;

(d) the XDP of embodiment 54 or 55;

(e) the lipid nanoparticle of any one of embodiments 45-47; or

(f) the lipid nanoparticle composition of embodiment 48,

wherein upon binding of the RNP of the gene repressor system to the target nucleic acid, transcription of the gene proximal to the binding location of the RNP is repressed in the cells.

[0467] 57. The method of embodiment 56, wherein the binding location of the RNP is selected from the group consisting of:

(a) a sequence within 300 to 1,000 base pairs 5' to a transcription start site (TSS) in the gene;

(b) a sequence within 300 to 1,000 base pairs 3' to a TSS in the gene;

(c) a sequence within 300 to 1,000 base pairs to an enhancer of the gene;

(d) a sequence within the open reading frame of the gene;

(e) a sequence within an exon of the gene; or

(f) a sequence in the 3' untranslated region (UTR) of the gene.

[0468] 58. The method of embodiment 56 or embodiment 57, wherein transcription of the gene is repressed 5' to the binding location of the RNP.

[0469] 59. The method of embodiment 56 or embodiment 57, wherein transcription of the gene is repressed 3' to the binding location of the RNP.

[0470] 60. The method of any one of embodiments 56-59, wherein transcription of the gene in the population of cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% greater compared to untreated cells, when assessed in an *in vitro* assay.

[0471] 61. The method of any one of embodiments 56-60, wherein off-target methylation or off-target transcription repression is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells, when assessed in an *in vitro* assay.

[0472] 62. The method of any one of embodiments 56-61, wherein the repression of transcription in the cells is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 1 month, or at least about 2 months.

[0473] 63. The method of any one of embodiments 56-62, further comprising a second gRNA or a nucleic acid encoding the second gNA, wherein the second gNA has a targeting sequence complementary to a different portion of the target nucleic acid sequence and is capable of forming a ribonuclear protein complex (RNP) with the fusion protein comprising the catalytically-dead Class 2, Type V CRISPR protein and the one or more transcription repressor domains.

[0474] 64. The method of any one of embodiments 56-63, wherein the method mediates a heritable epigenetic change in the gene of the cells.

[0475] 65. A method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective dose of:

(a) the AAV vector of any one of embodiments 51-53;

(b) the XDP of embodiment 54 or embodiment 55;

(c) the lipid nanoparticle of any one of embodiments 45-47; or

(d) the lipid nanoparticle composition of embodiment 48;

wherein upon binding of the RNP of the gene repressor system to the target nucleic acid of a gene in cells of the subject transcription of the gene proximal to the binding location of the RNP is repressed.

[0476] 66. The method of embodiment 65, wherein transcription of the gene is repressed 5' to the binding location of the RNP.

[0477] 67. The method of embodiment 65, wherein transcription of the gene is repressed 3' to the binding location of the RNP.

[0478] 68. The method of any one of embodiments 65, wherein transcription of the gene in the cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%.

[0479] 69. The method of any one of embodiments 65, wherein the repression of transcription of the gene in the cells is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 1 month, or at least about 2 months.

[0480] 70. The method of any one of embodiments 65-69, wherein the method mediates a heritable epigenetic change in the gene of the cells of the subject.

[0481] 71. The method of any one of embodiments 65-70, wherein the AAV vector, XDP, or the lipid nanoparticles are administered to the subject by a route of administration selected from subcutaneous, intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatical, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation, or combinations thereof.

[0482] 72. The method of embodiment 71, wherein the XDP or the lipid nanoparticles are administered at a dose of at least about 1×10^5 particles/kg, or at least about 1×10^6 particles/kg, or at least about 1×10^7 particles/kg, or at least about 1×10^8 particles/kg, or at least about 1×10^9 particles/kg, or at least about 1×10^{10} particles/kg, or at least about 1×10^{11} particles/kg, or at least about 1×10^{12} particles/kg, or at least about 1×10^{13} particles/kg, or at least about 1×10^{14} particles/kg, or at least about 1×10^{15} particles/kg, or at least about 1×10^{16} particles/kg.

[0483] 73. The method of embodiment 71, wherein the XDP or the lipid nanoparticles are administered to the subject at a dose of at least about 1×10^5 particles/kg to about 1×10^{16} particles/kg, or at least about 1×10^6 particles/kg to about 1×10^{15} particles/kg, or at least about 1×10^7 particles/kg to about 1×10^{14} particles/kg.

[0484] 74. The method of embodiment 71, wherein the AAV vector is administered to the subject at a dose of at least about 1×10^8 vector genomes (vg), at least about 1×10^5 vector genomes/kg (vg/kg), at least about 1×10^6 vg/kg, at least about 1×10^7 vg/kg, at least about $1 \times$

10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 1 x 10^{14} vg/kg, at least about 1 x 10^{15} vg/kg, or at least about 1 x 10^{16} vg/kg.

[0485] 75. The method of embodiment 71, wherein the AAV vector is administered to the subject at a dose of at least about 1 x 10^5 vg/kg to about 1 x 10^{16} vg/kg, at least about 1 x 10^6 vg/kg to about 1 x 10^{15} vg/kg, or at least about 1 x 10^7 vg/kg to about 1 x 10^{14} vg/kg.

[0486] 76. The method of embodiment 71, wherein the first and second lipid nanoparticles are each administered at a dose of at least about 1 x 10^5 particles/kg, or at least about 1 x 10^6 particles/kg, or at least about 1 x 10^7 particles/kg, or at least about 1 x 10^8 particles/kg, or at least about 1 x 10^9 particles/kg, or at least about 1 x 10^{10} particles/kg, or at least about 1 x 10^{11} particles/kg, or at least about 1 x 10^{12} particles/kg, or at least about 1 x 10^{13} particles/kg, or at least about 1 x 10^{14} particles/kg, or at least about 1 x 10^{15} particles/kg, or at least about 1 x 10^{16} particles/kg.

[0487] 77. The method of embodiment 71, wherein the first and the second lipid nanoparticles are each administered to the subject at a dose of at least about 1 x 10^5 particles/kg to about 1 x 10^{16} particles/kg, or at least about 1 x 10^6 particles/kg to about 1 x 10^{15} particles/kg, or at least about 1 x 10^7 particles/kg to about 1 x 10^{14} particles/kg.

[0488] 78. The method of any one of embodiments 65-77, wherein the XDP, the AAV vector, or the first and second lipid nanoparticles are administered to the subject according to a treatment regimen comprising one or more consecutive doses.

[0489] 79. The method of any one of embodiments 65-78, wherein the therapeutically effective dose is administered to the subject as two or more doses over a period of at least two weeks, or at least one month, or at least two months, or at least three months, or at least four months, or at least five months, or at least six months, or once a year.

[0490] 80. The method of any one of embodiments 65-79, wherein the treating results in improvement in at least one clinically-relevant endpoint associated with the disorder in the subject.

[0491] 81. The method of any one of embodiments 65-79, wherein the subject is selected from the group consisting of mouse, rat, pig, and non-human primate.

[0492] 82. The method of any one of embodiments 65-79, wherein the subject is human.

[0493] 83. A pharmaceutical composition comprising the gene repressor system of any one of embodiments 1-41 and a pharmaceutically acceptable excipient.

[0494] 84. The gene repressor system of any one of embodiments 1-41 for use as a medicament in the treatment of a subject a disorder caused by a genetic mutation.

[0495] 85. The gene repressor system of any one of embodiments 1-41, wherein the targeting sequence of the gRNA is complementary to a non-target strand sequence located 1 nucleotide 3' of a protospacer adjacent motif (PAM) sequence.

[0496] 86. The composition of embodiment 85, wherein the PAM sequence comprises a TC motif.

[0497] 87. The composition of embodiment 85 or embodiment 86, wherein the PAM sequence comprises ATC, GTC, CTC or TTC.

EXAMPLES

Example 1: Demonstration of a catalytically-dead CasX repressor (dXR) system on repression of B2M at RNA and protein levels

[0498] Experiments were performed to determine if various catalytically-dead CasX repressor (dXR) constructs can act as transcriptional repressors in mammalian cells.

Materials and Methods:

[0499] dXR variant plasmids encoding constructs having the configuration of U6-gRNA + Ef1α -NLS-GGS-dCasX491-GGS-KRAB variant-NLS (dCasX491 refers to catalytically-dead CasX 491), were transiently transfected into HEK293T cells in an arrayed 96-well format. These constructs also contained a 2x FLAG sequence, as well as sequences encoding either a gRNA scaffold 174 (SEQ ID NO: 2238) having a spacer (spacer 7.37) targeting the endogenous *B2M* (beta-2-microglobulin) gene or a non-targeting control (spacer 0.0), which were all cloned upstream of a P2A-puromycin element on the plasmid. Four different effector domains were tested in addition to the "naked" dCasX491 (KRAB variant domains listed in Table 9; spacer sequences listed in Table 10; sequences of additional elements listed in Table 11). The sequences encoding the full dXR molecule are listed in Table 12. The corresponding protein sequences of the dXR molecule are listed in Table 13, and the generic configuration of the dXR molecule is illustrated in FIG. 38. Positive and negative controls based on a catalytically-dead Cas9 nuclease (with or without a ZNF10 repressor) with a *B2M*-targeting gRNA (spacer 7.14) or a non-targeting gRNA control (spacer 0.0) were included, along with a catalytically-active CasX 491 and gRNA with the same 7.37 and 0.0 spacers. Two days after transfection, total RNA was harvested, and reverse transcribed to generate a cDNA library. Changes in gene expression were calculated by performing qPCR on the targeted gene and a housekeeping gene as reference. Relative gene expression represents the amount of target-specific RNA relative to a reference gene normalized to the non-targeting guide condition for two biological replicates. In addition to the wells used for RNA measurements, a separate set of wells was harvested seven days post-transfection and analyzed for B2M protein expression. Expression of B2M protein was determined by using an antibody that detects the B2M-dependent HLA protein complex on the cell surface. Cells that expressed B2M (B2M+) were measured using flow cytometry, and the relevant data are shown in Table 14.

Table 9: Sequences of KRAB domains tested fused to CasX.

Domain Name	KRAB domain sequence	Construct name	SEQ ID NO
ZIM3	MNNSQGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVG QGETTKPDVILRLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDV KESL	dXR1	337
ZNF10	MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYK NLVSLGYQLTKPDVILRLEKGEEP	dXR2	338
ZNF10-MeCP2	MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYK NLVSLGYQLTKPDVILRLEKGEEPWLVSGGGSGGSGSSPKKKRKVEAS VQVKRVLEKSPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRK RKAEADPQAIPKKRGRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLP IKKRKTRETVSIEVKEVVKPLLVSTLGEKSGKGLKTCKSPGRKSKESS PKGRSSSASSPPKKEHHHHHHHAESPKAPMPLLPPPPPPEPQSSEDPI SPPEPQDLSSSICKEEKMPRAGSLESDGCPKEPAKTQP	dXR3	339
ZNF334	KMKKFQIPVSFQDLTVNFTQEEWQQLDPAQRLLYRDVMLENYSNLVSV GYHVSKPDVIFKLEQGEEPWIVEEFSNQNYPD	dXR4	340

Table 10: Sequences of spacers tested.

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO
7.37	GGCCGAGATGTCTCGCTCCG	341	GGCCGAGAUGUCUCGCUCCG	59628
7.148	CGCGAGCACAGCTAAGGCCA	342	CGCGAGCACAGCUAAGGCCA	59629
0.0	CGAGACGTAATTACGTCTCG	343	CGAGACGUAAUUACGUCUCG	59630

Table 11: Sequences of additional key dXR elements to generate the dXR construct having the configuration illustrated in FIG. 38. Note that buffer sequences are not listed.

Key component	SEQ ID NO (DNA)	SEQ ID NO (Protein)
dCasX491	57618	57619
Linker 3A	57624	57626
Linker 3B	57625	
NLS A	57629	57631
NLS B	57630	

Table 12: DNA sequences of dXR constructs.

dXR ID	KRAB domain	SEQ ID NO (DNA sequence of dXR encoding construct)
dXR1	ZIM3	59434
dXR2	ZNF10	59435

dXR ID	KRAB domain	SEQ ID NO (DNA sequence of dXR encoding construct)
dXR3	ZNF10-MeCP2	59436
dXR4	ZNF334	59437

Table 13: Protein sequences of the dXR molecules.

dXR ID	KRAB domain	SEQ ID NO (Amino Acid Sequence of dXR Molecule)
dXR1	ZIM3	59438
dXR2	ZNF10	59439
dXR3	ZNF10-MeCP2	59440
dXR4	ZNF334	59441

Results:

[0500] All conditions with a guide RNA targeting the gene resulted in repression, although the strength of repression varied by the choice of domain (FIG. 1). Catalytically-dead CasX molecules with effector domains depleted most of the targeted RNA in 48 hours (~81% of the RNA is depleted on average) comparable to dCas9-KRAB (~82% of RNA depleted). On the protein level, dCasX confers slight repression on its own (~10% of cells negative at the protein level), but addition of any KRAB domain considerably contributed to further repression (a range of 80-89% of cells were negative for the B2M protein (Table 14). Furthermore, most CasX constructs compared favorably in depleting protein compared to the dCas9 controls (22% of cells negative for dCas9 and 81% of cells negative for dCas9-KRAB) (Table 14).

Table 14: Repression of B2M protein levels by CasX and Cas9 molecules and repressor constructs. Data represent biological triplicates.

Molecule	Spacer	% cells expressing B2M protein*	std deviation
dCas9	0.0	97.34	0.16
dCas9-KRAB	0.0	98.54	0.19
CasX	0.0	98.62	0.66
dCasX	0.0	95.90	0.50
dXR1	0.0	98.09	0.18
dXR2	0.0	98.01	0.11
dXR3	0.0	97.51	0.28
dXR4	0.0	98.23	0.11

Molecule	Spacer	% cells expressing B2M protein*	std deviation
dCas9	7.14	77.87	0.15
dCas9-KRAB	7.14	18.83	0.45
CasX	7.37	21.60	0.56
dCasX	7.37	85.70	0.00
dXR1	7.37	13.50	0.66
dXR2	7.37	16.90	0.96
dXR3	7.37	10.20	0.46
dXR4	7.37	19.80	1.64

*Data represent % of cells counted that were positive

[0501] In Table 14, dCasX refers to catalytically-dead CasX 491, dXR1-4 refer to dCasX491 fused to the KRAB domains indicated in Table 9, in the following orientation: U6-gRNA + Ef1α-NLS-GGS-dCasX-GGS-KRAB variant-NLS, and CasX refers to catalytically active CasX 491. dCas9-KRAB refers to dCas9 fused to a ZNF10-KRAB domain.

[0502] The results demonstrate that dXR can transcriptionally repress an endogenous locus (*B2M*) resulting in loss of target protein. Furthermore, the addition and choice of transcriptional effector domains affects the overall potency of the molecule.

Example 2: Demonstration of dXR effectiveness on HBEGF for high-throughput screening

[0503] Experiments were performed to determine the feasibility of using dXR constructs for high-throughput screening of molecules in mammalian cells.

Materials and Methods:

[0504] HEK293T cells were seeded in a 6-well plate at 300,000 cells/well and lipofected with 1 µg of plasmid encoding either a CasX molecule (491), a catalytically-dead CasX 491 with the ZNF10-KRAB repressor domain (dXR) and a guide scaffold 174 (SEQ ID NO: 2238) with a spacer targeting the *HBEGF* gene or a non-targeting spacer. Five combinations of CasX-based molecules and gRNAs with the indicated spacers (Table 15) were transfected into five separate wells. HBEGF is the receptor that mediates entry of diphtheria toxin that, when added to the cells, inhibits translation and leads to cell death. Targeting of the *HBEGF* gene with a CasX or dXR molecule and targeting gRNA should prevent toxin entry and allow survival of the cells,

whereas cells treated with CasX and dXR molecules and a non-targeting gRNA should not survive. One day post-transfection, cells in each transfected well were split into 12 different wells in a 96-well plate and selected with puromycin. Over three days, cells were treated with six different concentrations of diphtheria toxin (0, 0.2, 2, 20, 200, and 2000 ng/mL), and biological duplicates were performed. After another two days, cells were split into fresh media, and total cell counts were measured on an ImageXpress Pico Automated Cell Imaging System.

Table 15: Sequences of spacers tested.

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO	Molecule
34.19	ACTGGGAGGCTCAGCCCATG	344	ACUGGGAGGCUCAGCCCAUG	59631	CasX
34.21	TGTTCTGTCTTGAACTAGCT	345	UGUUCUGUCUUGAACUAGCU	59632	CasX
34.28	TGAGTGTCTTGTCTTGCTCA	346	UGAGUGUCUUGUCUUGCUCA	59633	dXR
0.0	CGAGACGTAATTACGTCTCG	343	CGAGACGUAAUUACGUCUCG	59630	CasX & dXR

Results:

[0505] The results of the diphtheria toxin assay are illustrated in the plot in FIG. 2. dXR-mediated repression of the *HBEGF* gene resulted in survival of cells, but only at low doses of toxin (0.2 - 20 ng/mL). However, those same doses led to complete cell death in the control cells treated with non-targeting constructs. High doses (> 20 ng/mL) of toxin led to cell death in both the dXR and control samples, suggesting that the basal level of transcription permitted by dXR allows sufficient toxin to enter and trigger cell death. The results show that CasX-edited cells remained protected as editing of the locus leads to complete loss of functional protein. The non-targeting controls died at all doses, demonstrating the efficacy of the toxin when HBEGF is not repressed or edited.

[0506] The results show that dXR protects at low doses of toxin, demonstrating that this molecule can be screened in a range of 0.2-20 ng/mL diphtheria toxin, with highest fold-enrichment between dXR and control observed at 0.2 ng/mL. Note that while CasX protects at all doses, repression by dXR still induces low basal expression of the target that leads to toxicity of the cells at high doses of the toxin.

Example 3: Demonstration of the ability of catalytically dead CasX-based repressor (dXR) to repress C9orf72

[0507] Experiments were performed to determine if dCasX-based repressors can induce transcriptional silencing of a reporter constructed with the 5'UTR of the C9orf72 gene. This system will allow studying the efficacy of dXR-gRNA combinations in cell types in which C9orf72 is not endogenously expressed and, furthermore, allow high-throughput screening of additional dXR molecules using a gRNA with spacers known to be active in editing systems.

Materials and Methods:

[0508] A clonal reporter cell line was constructed by nucleofecting K562 (a human myelogenous leukemia cell line) cells with a plasmid reporter containing the CMV promoter, the C9orf72 complete 5'UTR (Exon1a-Exon1b-Exon2 with all potential ATG start codons mutated and two artificial PAMs added at the 5' and 3' ends), and a coding sequence of TurboGFP-PEST-p2A-HSV_TK. The CMV promoter allows constitutive expression of the reporter, the C9orf72 5'UTR provides a sequence to target with dCasX constructs, and the GFP and TK (Herpes Simplex Virus-1 Thymidine Kinase) proteins provide markers for selection and counter-selection. Specifically, TK metabolizes the typically inert pro-drug ganciclovir into a toxic thymidine analog that leads to cell death. The nucleofected cells were selected in hygromycin for 1 month, sorted to single cells and characterized for ganciclovir sensitivity. A single clone (GFP-TK-c10) was selected that displayed complete cell death within 5 days at a ganciclovir concentration of 5 µg/mL.

[0509] GFP-TK-c10 cells were transduced (250,000 cells; 6-well format) with lentiviruses encoding dXR molecule containing the ZNF10-KRAB domain and gRNA with scaffold 174 (SEQ ID NO: 2238) and spacers targeting the 5'UTR sequence of the *C9orf72* locus present in the GFP-TK reporter (Table 16). Transductions were carried out in an arrayed fashion in which one lentivirus was applied to one well of cells. 48 hours after transduction, cells were treated with 5 µg/mL ganciclovir for 5 days and then stained with trypan blue and counted on an automated cell counter.

Table 16: Spacers tested in arrayed transductions.

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO
29.2000	CGTAACCTACGGTGTCCCGC	347	CGUAACCUACGGUGUCCCGC	59670

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO
29.168	TAGCGGGACACCGTAGGTTA	348	UAGCGGGACACCGUAGGUUA	59671
29.163	CTTTTGGGGGCGGGGTCTAG	349	CUUUUGGGGGCGGGGUCUAG	59672
0.0	CGAGACGTAATTACGTCTCG	343	CGAGACGUAAUUACGUCUCG	59630

[0510] Separately, cells were transduced (250,000 cells; 6-well format) with multiple virus combinations at defined ratios (Table 17). 48 hours post-transduction, half of the cells in each well were harvested and frozen as cell pellets, and the other half were selected in the same manner (5 days; 5 µg/mL ganciclovir). After ganciclovir selection the remaining cells were harvested and gDNA was extracted from both pre- and post-ganciclovir treatment samples. Primers flanking the region containing the spacer sequence in the lentivirus constructs were used to generate amplicons for next generation sequencing analysis in which the ratios of the spacers in each well were compared pre- and post-selection. These ratios were used to calculate spacer fitness scores for each competition by taking the log2 of the fold change in the spacer frequency from pre-selection to post-selection. Fitness was determined by the following equation:

$$Fitness = log_2 (spacer\ frequency\ post\text{-}selection / spacer\ frequency\ pre\text{-}selection)$$

Table 17: Matrix of competition experiments (each virus present at equal ratio).

Experiment	29.2000 (1)	29.168 (2)	29.163 (3)	0.0 (NT)
1	+	-	-	+
2	-	+	-	+
3	-	-	+	+
4	+	+	-	+
5	+	-	+	+
6	-	+	+	+
7	+	+	+	+

Results:

[0511] Treatment with dXR containing the ZNF10-KRAB domain and guide 174 with Spacers 1 (29.2000) and 2 (29.168) permitted cell survival (FIG. 3), while mock, NT (0.0) and Spacer 3 (29.163) conditions all resulted in cell death. The results of constructs utilizing Spacers 1 and 2 demonstrate that the combination of a dXR molecule and a C9orf72-targeting spacer can induce potent transcriptional repression, establishing this system as a platform by which to measure dXR and spacer potency at a therapeutically-relevant locus.

[0512] Furthermore, measurements of spacer fitness in Table 18 demonstrate the quantitative and reproducible nature of this assay as constructs utilizing Spacers 1 and 2 both permitted cell survival, with Spacer 2 measurably more potent than Spacer 1 in all competitions. Furthermore, constructs with Spacer 3 were ineffective in almost all competitions, demonstrating the utility of this system in screening for effective spacers.

[0513] The results demonstrate that dXR molecules can transcriptionally repress therapeutically-relevant sequences and distinguish between functional and non-functional spacers.

Table 18: Spacer fitness calculated from lentivirus competition experiments.

Experiment	Spacer	Fitness*
1	1	0.65
1	NT	-3.38
2	2	0.88
2	NT	-3.10
3	3	0.12
3	NT	-0.38
4	1	-0.09
4	2	0.83
5	1	0.90
5	2	0.98
5	3	-4.40
5	NT	-3.44

*Data represent the log2 fold change in frequency of spacer counts as measured by next generation sequencing; a positive score indicates a spacer is more fit than the other spacers present in the competition.

Example 4: Development of a selection to identify improved repressors for inclusion in dXR compositions

[0514] To develop better dXR molecules, a library of transcriptional effector domains from many species was tested in a selection assay. As KRAB domains are one of the largest and most rapidly-evolved domains in vertebrates, domains from species not previously evaluated were anticipated to provide improved strength and permanence of repression.

Materials and Methods:

Identification of candidate KRAB domains:

[0515] KRAB domains were identified by downloading all sequences annotated with Prosite accession ps50805 (the accession number for KRAB domains). All domains were extended by 100 amino acids (with the annotation centered in the middle) to include potential unannotated functional sequence. In addition, HMMER, a tool to identify domains, was run on a set of high-quality primate annotations from recently completed alignments of long-read primate genome assemblies described (Warren, WC, et al. Sequence diversity analyses of an improved rhesus macaque genome enhance its biomedical utility. Science 370, Issue 6523, eabc6617in (2020); Fiddes, IT, et al. Comparative Annotation Toolkit (CAT)-simultaneous clade and personal genome annotation. Genome Res. 28(7):1029 (2018); Mao, Y, et al. A high-quality bonobo genome refines the analysis of hominid evolution. Nature 594:77 (2021)), to identify KRAB domains in these assemblies most of which were not present in UniProt. The search resulted in 32,120 unique sequences from 159 different organisms that will be tested for their potency in repression. The complete list of sequences is listed as SEQ ID NOS: 355-2100 and 2332-33239. Additionally, 580 random amino acid sequence 80 residues in length were included in the library as negative controls, and 304 human KRAB domains were included based on work by Tycko, J. et al. (*Cell.* 2020 Dec 23;183(7):2020-2035).

Screening methods:

[0516] The KRAB domains described above were synthesized as DNA oligos, amplified, and cloned into a dCasX491 C-terminal GS linker lentiviral construct along with guide scaffold 174 (SEQ ID NO: 2238) with either Spacer 34.28 or Spacer 29.168, both of which repress their

respective targets (i.e., *HBEGF* and GFP-TK) and confer survival in the assays described in the above Examples. For each KRAB domain, the C-terminal GS linker was synonymously substituted to produce unique DNA barcodes that could be differentiated by NGS allowing internal technical replicates to be assessed in each pooled experiment. These plasmids were used to generate the lentiviral constructs of the library. The lentiviral library with 29,168 plasmids were used to transduce GFP-TK cells, which were treated with 1 µg/mL puromycin to remove untransduced cells, then 5 µg/mL ganciclovir for 5 days. After selection, gDNA was extracted, and gDNA containing the KRAB domain in the surviving cells was amplified and sequenced.

[0517] An analogous assay was performed with the lentiviral library with spacer 34.28 targeting *HBEGF*. HEK293T cells were transduced, treated with 1 µg/mL puromycin to remove untransduced cells, and selection was carried out at 2 ng/mL diphtheria toxin for 48 hours. gDNA was extracted, amplified, and sequenced as described above. gDNA samples were also extracted, amplified, and sequenced from the cells before selection with ganciclovir or diphtheria toxin, as a control. Two independent replicates were performed for both the diphtheria toxin and GFP-TK selections.

Assessment of B2M repression:

[0518] Representative KRAB domains were cloned into a dCasX491 C-terminal GS linker lentiviral construct along with guide scaffold 316 (SEQ ID NO: 59352) with spacer 7.15 (GGAAUGCCCGCCAGCGCGAC; SEQ ID NO: 59634), targeting the *B2M* locus. Separately, representative KRAB domains were cloned into a dCasX491 C-terminal GS linker lentiviral construct along with guide scaffold 174 (SEQ ID NO: 2238) with spacer 7.37 (SEQ ID NO: 57644), targeting the *B2M* locus. The lentiviral plasmid constructs encoding dXRs with various KRAB domains were generated using standard molecular cloning techniques. These constructs included sequences encoding dCasX491, and a KRAB domain from ZNF10, ZIM3, or one of the KRAB domains tested in the library. Cloned and sequence- validated constructs were midi-prepped and subjected to quality assessment prior to transfection in HEK293T cells.

[0519] HEK293T cells were seeded at a density of 30,000 cells in each well of a 96-well plate. The next day, each well was transiently transfected using lipofectamine with 100 ng of dXR plasmids, each containing a dXR construct with a different KRAB domain and a gRNA having a targeting spacer to the B2M locus. Experimental controls included dXR constructs with KRAB domains from ZNF10 or ZIM3, KRAB domains that were in the library but not in the top 95 or 1597 KRAB domains, or dCas9-ZNF10, each with a corresponding B2M-targeting gRNA. Each

construct was tested in triplicate. 24 hours post-transfection, cells were selected with 1μg/mL puromycin for two days. Seven or ten days after transfection, cells were harvested for editing repression analysis by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry. B2M expression was determined by using an antibody that would detect the B2M-dependent HLA protein expressed on the cell surface. HLA+ cells were measured using the Attune™ NxT flow cytometer.

Data analysis:

[0520] To understand the diversity of protein sequences in the tested KRAB library, an evolutionary scale modeling (ESM) transformer (ESM-1b) was applied to the initial library of 32,120 KRAB domain amino acid sequences to generate a high dimensional representation of the sequences (Rives, A. et al. *Proc Natl Acad Sci USA*. 2021 Apr 13;118(15)). Next, Uniform Manifold Approximation and Projection (UMAP) was applied to reduce the data set to a two-dimensional representation of the sequence diversity (McInnes, L., Healy, J., *ArXiv e-prints* 1802.03426, 2018). Using this technique, 75 clusters of KRAB domain sequences were identified.

[0521] Protein sequence motifs were generated using the STREME algorithm (Bailey, T., *Bioinformatics*. 2021 Mar 24;37(18):2834-2840) to identify motifs enriched in strong repressors.

Results:

[0522] Selections were performed to identify the KRAB domains out of a library of 32,120 unique sequences that were the most potent transcriptional repressors. The diphtheria toxin selections produced higher quality NGS libraries and were therefore selected for further analysis. The fold change in the abundance of each KRAB domain in the library before and after selection was calculated for each barcode-KRAB pair such that together the two independent replicates of the experiment represent 12 measurements of each KRAB domain's fitness.

[0523] FIG. 16 shows the range of $\log_2$(fold change) values for the entire library, the randomized sequences that served as negative controls, a positive control set of KRAB domains that were shown to have a $\log_2$(fold change) greater than 1 on day 5 of the HT-recruit experiment performed by Tycko et al. (*Cell*. 2020 Dec 23;183(7):2020-2035). As shown in FIG. 16, the diphtheria toxin selection successfully enriched for KRAB domains that were more potent repressors. The negative control sequences were de-enriched from the library following selection.

[0524] To identify the KRAB domains that were reproducibly enriched in the post-selection library, a p-value threshold of less than 0.01 and a $\log_2$(fold change) threshold of greater than 2 was set. 1597 KRAB domains met these criteria. P-values were calculated via the MAGeCK algorithm which uses a permutation test and false discovery rate adjustment for multiple testing (Wei, L. et al. *Genome Biol.* 2014;15(12):554). The $\log_2$(fold change) values of these top 1597 KRAB domains are shown in FIG. 16, and the amino acid sequences, p-values, and $\log_2$(fold change) values are provided in Table 19, below. In contrast, Zim3 had a $\log_2$(fold change) of 1.7787, standard Znf10 had a $\log_2$(fold change) of 1.3637, and an alternate Znf10 corresponding to the Znf10 KRAB domain used in Tycko, J. et al. (*Cell.* 2020 Dec 23;183(7):2020-2035) had a $\log_2$(fold change) of 1.6182. Therefore, the 1597 top KRAB domains were substantially superior repressors to Znf10 and Zim3. Many of these top KRAB repressors contained amino acids with residues that are predicted to stabilize interactions with the Trim28 protein when compared to Zim3 and Znf10 (Stoll, G.A. et al., *bioRxiv* 2022.03.17.484746)

[0525] To further narrow down the list of KRAB domains while maintaining a breadth of amino acid sequence diversity, a set of 95 lead domains was chosen from within the 1597 by selecting the best domains from each cluster, as well as the top 25 best repressors of the 1597. These top 95 KRAB domains were further narrowed to a top 10 based on by choosing the top domains by $\log_2$(fold change), p-value, and performance in independent repression assays, as described below. The top 10 KRAB domains identified were DOMAIN_737, DOMAIN_10331, DOMAIN_10948, DOMAIN_11029, DOMAIN_17358, DOMAIN_17759, DOMAIN_18258, DOMAIN_19804, DOMAIN_20505, and DOMAIN_26749.

Table 19: List of 1,597 KRAB domain candidates identified from the high throughput screen assessing dXR repression of the *HBEGF* gene and subsequent application of the following criteria: p-value < 0.01 and $\log_2$(fold change) > 2.

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
Top 10 KRAB domains				
DOMAIN_737	Bonobo	57746	4.544	1.53E-07
DOMAIN_10331	Colobus angolensis palliatus	57747	3.6796	1.53E-07
DOMAIN_10948	Colobus angolensis palliatus	57748	3.2959	2.30E-06
DOMAIN_11029	Mandrillus leucophaeus	57749	3.5748	1.53E-07

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_17358	Bos indicus x Bos taurus	57750	4.9878	1.53E-07
DOMAIN_17759	Felis catus	57751	3.3159	1.38E-06
DOMAIN_18258	Physeter macrocephalus	57752	3.75	3.42E-04
DOMAIN_19804	Callorhinus ursinus	57753	3.8217	1.53E-07
DOMAIN_20505	Chlorocebus sabaeus	57754	3.4989	2.91E-06
DOMAIN_26749	Ophiophagus hannah	57755	5.4323	1.53E-07
Remaining KRAB domains in the top 95 KRAB domains				
DOMAIN_221	Bonobo	57756	3.5533	3.06E-06
DOMAIN_881	Bonobo	57757	4.3546	4.59E-07
DOMAIN_2380	Orangutan	57758	3.2024	1.74E-04
DOMAIN_2942	Gibbon	57759	3.3658	1.38E-06
DOMAIN_4687	Marmoset	57760	5.2288	3.22E-06
DOMAIN_4806	Marmoset	57761	3.3896	1.58E-04
DOMAIN_4968	Marmoset	57762	3.0315	0.0022262
DOMAIN_5066	Marmoset	57763	2.9062	0.0067409
DOMAIN_5290	Owl Monkey	57764	3.0993	5.16E-05
DOMAIN_5463	Owl Monkey	57765	3.2102	0.0022788
DOMAIN_6248	Saimiri boliviensis boliviensis	57766	2.4415	0.0056883
DOMAIN_6445	Alligator sinensis	57767	3.1151	4.51E-04
DOMAIN_6802	Pantherophis guttatus	57768	3.0403	5.18E-04
DOMAIN_6807	Xenopus laevis	57769	3.1615	5.16E-05
DOMAIN_7255	Microcaecilia unicolor	57770	4.5265	1.38E-06
DOMAIN_7694	Columba livia	57771	3.7111	1.13E-04
DOMAIN_8503	Mus caroli	57772	2.8193	0.003503
DOMAIN_8790	Marmota monax	57773	2.7436	2.06E-04
DOMAIN_8853	Mesocricetus auratus	57774	4.6199	1.53E-07
DOMAIN_9114	Peromyscus maniculatus bairdii	57775	2.2058	0.0048423
DOMAIN_9331	Peromyscus maniculatus bairdii	57776	4.1063	4.59E-07
DOMAIN_9538	Mus musculus	57777	3.5443	1.20E-04
DOMAIN_9960	Octodon degus	57778	3.4751	1.07E-06
DOMAIN_10123	Rattus norvegicus	57779	3.6356	8.11E-06
DOMAIN_10277	Dipodomys ordii	57780	2.8257	4.16E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_10577	Colobus angolensis palliatus	57781	4.1248	1.53E-07
DOMAIN_11348	Chlorocebus sabaeus	57782	3.3651	2.95E-05
DOMAIN_11386	Capra hircus	57783	3.7637	4.75E-06
DOMAIN_11486	Bos mutus	57784	4.8326	1.53E-07
DOMAIN_11683	Nomascus leucogenys	57785	2.9249	0.0015672
DOMAIN_12292	Sus scrofa	57786	4.3194	1.53E-07
DOMAIN_12452	Neophocaena asiaeorientalis asiaeorientalis	57787	3.8774	5.05E-06
DOMAIN_12631	Macaca fascicularis	57788	3.6926	1.53E-07
DOMAIN_13331	Macaca fascicularis	57789	3.5154	2.15E-04
DOMAIN_13468	Phascolarctos cinereus	57790	4.1548	1.38E-06
DOMAIN_13539	Gorilla	57791	3.4924	1.79E-05
DOMAIN_14659	Acinonyx jubatus	57792	4.0495	1.06E-05
DOMAIN_14755	Cebus imitator	57793	3.1667	1.88E-04
DOMAIN_15126	Callithrix jacchus	57794	2.9781	4.08E-04
DOMAIN_15507	Cebus imitator	57795	3.8531	1.53E-07
DOMAIN_16444	Acinonyx jubatus	57796	3.2246	2.30E-06
DOMAIN_16688	Lipotes vexillifer	57797	3.5601	4.26E-05
DOMAIN_16806	Sapajus apella	57798	3.9386	1.53E-07
DOMAIN_17317	Otolemur garnettii	57799	3.4551	1.81E-04
DOMAIN_17432	Otolemur garnettii	57800	3.11	1.36E-05
DOMAIN_17905	Chimp	57801	2.5038	5.60E-04
DOMAIN_18137	Monodelphis domestica	57802	3.292	3.51E-05
DOMAIN_18216	Physeter macrocephalus	57803	3.0602	9.40E-04
DOMAIN_18563	OwlMonkey	57804	3.0406	0.0034849
DOMAIN_19229	Enhydra lutris kenyoni	57805	4.0294	5.01E-05
DOMAIN_19460	Monodelphis domestica	57806	3.995	1.97E-05
DOMAIN_19476	OwlMonkey	57807	4.1343	1.53E-07
DOMAIN_19821	Rhinopithecus roxellana	57808	3.583	1.53E-07
DOMAIN_19892	Ursus maritimus	57809	3.1396	5.21E-04
DOMAIN_19896	Ovis aries	57810	2.2228	1.58E-04
DOMAIN_19949	Callorhinus ursinus	57811	3.2903	2.62E-04
DOMAIN_21247	Neovison vison	57812	2.741	0.0043129

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_21317	Pteropus vampyrus	57813	4.0893	1.18E-05
DOMAIN_21336	Equus caballus	57814	2.738	0.005135
DOMAIN_21603	Lipotes vexillifer	57815	2.8535	4.35E-04
DOMAIN_21755	Equus caballus	57816	3.1889	0.0028238
DOMAIN_22153	Zalophus californianus	57817	3.6967	3.52E-06
DOMAIN_22270	Bonobo	57818	2.3813	0.0030391
DOMAIN_23394	Vicugna pacos	57819	4.0769	3.06E-07
DOMAIN_23723	Carlito syrichta	57820	3.5301	8.71E-05
DOMAIN_24125	Saimiri boliviensis boliviensis	57821	3.9692	1.53E-07
DOMAIN_24458	Lynx pardinus	57822	3.4012	9.66E-05
DOMAIN_24663	Myotis brandtii	57823	2.9806	1.49E-04
DOMAIN_25289	Ursus maritimus	57824	3.4113	7.70E-05
DOMAIN_25379	Sapajus apella	57825	3.5892	1.53E-07
DOMAIN_25405	Desmodus rotundus	57826	3.8846	3.20E-05
DOMAIN_26070	Geotrypetes seraphini	57827	3.7958	1.53E-07
DOMAIN_26322	Geotrypetes seraphini	57828	2.9265	7.13E-04
DOMAIN_26732	Meleagris gallopavo	57829	2.7548	0.0057183
DOMAIN_27060	Gopherus agassizii	57830	2.7943	0.0029172
DOMAIN_27385	Octodon degus	57831	4.1339	2.77E-05
DOMAIN_27506	Bos mutus	57832	3.8121	4.29E-06
DOMAIN_27604	Ailuropoda melanoleuca	57833	2.8198	6.05E-05
DOMAIN_27811	Callithrix jacchus	57834	2.9728	8.34E-05
DOMAIN_28640	Colinus virginianus	57835	3.624	4.13E-06
DOMAIN_28803	Monodelphis domestica	57836	3.0697	2.07E-05
DOMAIN_29304	Peromyscus maniculatus bairdii	57837	4.0496	1.53E-07
DOMAIN_30173	Phyllostomus discolor	57838	2.2538	5.41E-04
DOMAIN_30661	Physeter macrocephalus	57839	2.15	4.76E-05
DOMAIN_31643	Micrurus lemniscatus lemniscatus	57840	3.8782	3.57E-04
Remaining KRAB domains in the top 1597 KRAB domains				
DOMAIN_10870	Vicugna pacos	57841	2.5964	0.004315
DOMAIN_10918	Odobenus rosmarus divergens	57842	3.2079	9.21E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_92	Bonobo	57843	2.1475	0.0021413
DOMAIN_98	Bonobo	57844	2.7848	0.0055875
DOMAIN_134	Bonobo	57845	2.9322	0.004676
DOMAIN_143	Bonobo	57846	3.63	3.17E-05
DOMAIN_145	Bonobo	57847	3.1497	4.09E-05
DOMAIN_214	Bonobo	57848	2.1073	0.00941
DOMAIN_225	Bonobo	57849	2.259	0.0013991
DOMAIN_226	Bonobo	57850	3.0188	2.76E-04
DOMAIN_235	Bonobo	57851	2.9615	0.0016622
DOMAIN_302	Bonobo	57852	2.5092	0.0033327
DOMAIN_313	Bonobo	57853	2.4558	0.0049862
DOMAIN_344	Bonobo	57854	2.4948	0.0087725
DOMAIN_362	Bonobo	57855	3.6736	2.38E-04
DOMAIN_382	Bonobo	57856	3.1625	0.0019781
DOMAIN_389	Bonobo	57857	3.011	3.42E-04
DOMAIN_407	Bonobo	57858	3.8312	1.59E-04
DOMAIN_418	Bonobo	57859	3.2429	1.37E-04
DOMAIN_419	Bonobo	57860	3.5913	5.13E-05
DOMAIN_421	Bonobo	57861	3.2969	1.06E-05
DOMAIN_451	Bonobo	57862	3.0774	0.0018269
DOMAIN_504	Bonobo	57863	3.2187	4.17E-04
DOMAIN_516	Bonobo	57864	2.0448	0.0018554
DOMAIN_621	Bonobo	57865	2.1025	0.0034678
DOMAIN_623	Bonobo	57866	3.3299	6.50E-04
DOMAIN_624	Bonobo	57867	2.8281	0.0031625
DOMAIN_629	Bonobo	57868	3.6318	1.09E-05
DOMAIN_668	Bonobo	57869	2.9256	6.60E-04
DOMAIN_718	Bonobo	57870	3.9	8.73E-06
DOMAIN_731	Bonobo	57871	2.1318	0.0058273
DOMAIN_749	Bonobo	57872	3.1162	0.0060655
DOMAIN_759	Bonobo	57873	3.3019	0.0046077
DOMAIN_761	Bonobo	57874	3.181	9.64E-04
DOMAIN_784	Bonobo	57875	2.4886	0.0083818
DOMAIN_801	Bonobo	57876	2.4863	0.0040602

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_802	Bonobo	57877	2.6563	5.66E-04
DOMAIN_811	Bonobo	57878	2.4706	0.0035997
DOMAIN_812	Bonobo	57879	2.8201	0.0013526
DOMAIN_888	Bonobo	57880	2.8951	0.0033756
DOMAIN_893	Bonobo	57881	2.7511	5.41E-04
DOMAIN_938	Bonobo	57882	2.2926	0.0040367
DOMAIN_966	Chimp	57883	3.3535	5.49E-04
DOMAIN_972	Chimp	57884	3.7627	5.59E-05
DOMAIN_980	Chimp	57885	2.9297	0.0011707
DOMAIN_987	Chimp	57886	2.6881	5.48E-04
DOMAIN_999	Chimp	57887	2.7361	0.0038248
DOMAIN_1006	Chimp	57888	3.2119	1.28E-04
DOMAIN_1079	Chimp	57889	3.7915	3.90E-05
DOMAIN_1137	Chimp	57890	3.1719	4.58E-04
DOMAIN_1153	Chimp	57891	3.7928	5.16E-04
DOMAIN_1184	Chimp	57892	3.2772	5.47E-04
DOMAIN_1237	Chimp	57893	2.1795	0.0059151
DOMAIN_1242	Chimp	57894	2.7144	0.0037672
DOMAIN_1247	Chimp	57895	2.9622	4.18E-04
DOMAIN_1378	Gorilla	57896	3.2279	0.0022191
DOMAIN_1381	Gorilla	57897	4.1424	3.35E-05
DOMAIN_1382	Gorilla	57898	3.0579	1.91E-04
DOMAIN_1457	Gorilla	57899	2.6896	0.0026956
DOMAIN_1523	Gorilla	57900	2.8607	0.0042127
DOMAIN_1539	Gorilla	57901	2.9337	0.0028055
DOMAIN_1561	Gorilla	57902	2.8783	0.0011557
DOMAIN_1565	Gorilla	57903	2.771	3.04E-04
DOMAIN_1578	Gorilla	57904	3.4875	5.97E-04
DOMAIN_1621	Gorilla	57905	3.3004	1.20E-04
DOMAIN_1790	Gorilla	57906	3.0669	0.0038707
DOMAIN_1816	Gorilla	57907	3.108	0.0011178
DOMAIN_1818	Gorilla	57908	3.2866	6.15E-04
DOMAIN_1822	Gorilla	57909	2.4697	1.04E-04
DOMAIN_1870	Gorilla	57910	2.215	0.0044522

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_1875	Gorilla	57911	2.5576	0.0043383
DOMAIN_1893	Gorilla	57912	2.3898	0.0043422
DOMAIN_1946	Orangutan	57913	3.1449	9.41E-04
DOMAIN_1952	Orangutan	57914	3.0762	5.53E-04
DOMAIN_1964	Orangutan	57915	2.3009	0.0099771
DOMAIN_1978	Orangutan	57916	3.2215	0.0029968
DOMAIN_2014	Orangutan	57917	2.7323	3.95E-04
DOMAIN_2034	Orangutan	57918	3.7415	1.38E-06
DOMAIN_2119	Orangutan	57919	2.2117	0.0054271
DOMAIN_2208	Orangutan	57920	2.3044	0.009903
DOMAIN_2223	Orangutan	57921	2.6106	0.0087315
DOMAIN_2229	Orangutan	57922	2.9337	0.0032308
DOMAIN_2245	Orangutan	57923	3.2712	0.0012727
DOMAIN_2255	Orangutan	57924	3.1952	0.002815
DOMAIN_2295	Orangutan	57925	3.2816	6.61E-04
DOMAIN_2299	Orangutan	57926	2.5125	0.0042678
DOMAIN_2376	Orangutan	57927	2.1539	9.52E-04
DOMAIN_2391	Orangutan	57928	2.4608	0.0045936
DOMAIN_2398	Orangutan	57929	3.3125	3.44E-04
DOMAIN_2470	Orangutan	57930	2.3815	0.0031273
DOMAIN_2499	Orangutan	57931	3.114	0.0050479
DOMAIN_2563	Orangutan	57932	2.8105	0.003781
DOMAIN_2576	Orangutan	57933	3.1733	2.56E-04
DOMAIN_2590	Orangutan	57934	2.8348	0.0091663
DOMAIN_2629	Orangutan	57935	3.092	0.0015715
DOMAIN_2652	Orangutan	57936	4.3981	4.59E-07
DOMAIN_2744	Gibbon	57937	2.863	0.003897
DOMAIN_2754	Gibbon	57938	3.7601	1.17E-04
DOMAIN_2786	Gibbon	57939	2.5449	0.0037666
DOMAIN_2806	Gibbon	57940	3.1649	0.0083733
DOMAIN_2808	Gibbon	57941	2.6227	0.0079231
DOMAIN_2813	Gibbon	57942	2.9522	4.12E-04
DOMAIN_2851	Gibbon	57943	3.3945	3.80E-04
DOMAIN_2867	Gibbon	57944	3.0591	4.79E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_2888	Gibbon	57945	2.4267	0.0043214
DOMAIN_2891	Gibbon	57946	2.7489	0.0082897
DOMAIN_2896	Gibbon	57947	2.7253	0.0094587
DOMAIN_2904	Gibbon	57948	2.8035	0.0019408
DOMAIN_2908	Gibbon	57949	2.6452	0.0062379
DOMAIN_2943	Gibbon	57950	2.9574	9.75E-04
DOMAIN_2962	Gibbon	57951	2.1784	6.34E-04
DOMAIN_2992	Gibbon	57952	2.6341	0.0045667
DOMAIN_2994	Gibbon	57953	3.1921	0.0022412
DOMAIN_2997	Gibbon	57954	2.9911	0.0016588
DOMAIN_3000	Gibbon	57955	2.9522	5.36E-04
DOMAIN_3062	Gibbon	57956	2.6076	0.0035414
DOMAIN_3087	Gibbon	57957	2.7999	5.44E-04
DOMAIN_3092	Gibbon	57958	3.1954	2.80E-05
DOMAIN_3094	Gibbon	57959	3.7195	2.83E-05
DOMAIN_3096	Gibbon	57960	3.3962	2.16E-04
DOMAIN_3123	Gibbon	57961	3.1293	1.88E-05
DOMAIN_3137	Gibbon	57962	2.8303	0.0038836
DOMAIN_3300	Gibbon	57963	3.0127	2.76E-04
DOMAIN_3328	Gibbon	57964	2.3718	0.0015893
DOMAIN_3332	Gibbon	57965	2.8786	0.0036582
DOMAIN_3335	Gibbon	57966	4.0001	4.75E-06
DOMAIN_3336	Gibbon	57967	3.5946	4.75E-06
DOMAIN_3337	Gibbon	57968	2.9398	0.0053162
DOMAIN_3344	Gibbon	57969	3.2218	4.60E-04
DOMAIN_3373	Gibbon	57970	3.0768	0.0030033
DOMAIN_3434	Gibbon	57971	2.4767	0.0035835
DOMAIN_3463	Gibbon	57972	3.5462	5.96E-04
DOMAIN_3557	Rhesus	57973	2.4416	0.0024889
DOMAIN_3575	Rhesus	57974	3.7842	1.53E-07
DOMAIN_3585	Rhesus	57975	2.4981	0.0036466
DOMAIN_3586	Rhesus	57976	2.365	0.0033728
DOMAIN_3602	Rhesus	57977	2.0444	0.0061662
DOMAIN_3661	Rhesus	57978	2.4083	0.0088114

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_3691	Rhesus	57979	2.8393	0.0018244
DOMAIN_3759	Rhesus	57980	2.5324	0.004454
DOMAIN_3760	Rhesus	57981	2.7025	0.0017399
DOMAIN_3781	Rhesus	57982	2.9317	0.0024892
DOMAIN_3782	Rhesus	57983	2.3058	0.0048669
DOMAIN_3803	Rhesus	57984	3.0165	0.0083941
DOMAIN_3832	Rhesus	57985	2.7334	0.0026058
DOMAIN_4030	Rhesus	57986	2.5274	0.0038526
DOMAIN_4036	Rhesus	57987	2.7725	0.001577
DOMAIN_4046	Rhesus	57988	2.7847	0.0088564
DOMAIN_4120	Rhesus	57989	3.3237	4.55E-05
DOMAIN_4121	Rhesus	57990	3.3195	1.53E-07
DOMAIN_4126	Rhesus	57991	3.529	1.65E-04
DOMAIN_4129	Rhesus	57992	3.7382	9.33E-04
DOMAIN_4184	Rhesus	57993	3.2397	9.40E-04
DOMAIN_4185	Rhesus	57994	2.9116	0.0032623
DOMAIN_4199	Rhesus	57995	2.6844	0.0058444
DOMAIN_4239	Rhesus	57996	4.4187	9.19E-07
DOMAIN_4394	Marmoset	57997	3.8103	4.09E-05
DOMAIN_4425	Marmoset	57998	2.9741	0.0087646
DOMAIN_4461	Marmoset	57999	3.0094	0.0076595
DOMAIN_4463	Marmoset	58000	2.9717	0.008252
DOMAIN_4515	Marmoset	58001	4.2166	1.21E-05
DOMAIN_4516	Marmoset	58002	2.7603	0.0027577
DOMAIN_4534	Marmoset	58003	2.6242	0.0034292
DOMAIN_4574	Marmoset	58004	2.7135	9.16E-04
DOMAIN_4580	Marmoset	58005	2.9618	3.22E-06
DOMAIN_4589	Marmoset	58006	2.507	0.0070104
DOMAIN_4665	Marmoset	58007	3.2985	0.0011116
DOMAIN_4705	Marmoset	58008	3.5232	5.02E-04
DOMAIN_4722	Marmoset	58009	4.8639	1.53E-07
DOMAIN_4748	Marmoset	58010	3.0477	5.73E-04
DOMAIN_4749	Marmoset	58011	3.5545	2.83E-05
DOMAIN_4751	Marmoset	58012	3.238	4.91E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_4774	Marmoset	58013	2.8894	0.0029528
DOMAIN_4823	Marmoset	58014	2.7527	0.0083334
DOMAIN_4913	Marmoset	58015	2.8878	0.0028098
DOMAIN_4921	Marmoset	58016	3.5291	4.44E-06
DOMAIN_4922	Marmoset	58017	4.0258	1.82E-05
DOMAIN_4978	Marmoset	58018	2.7787	0.0025526
DOMAIN_5005	Marmoset	58019	2.8406	0.00183
DOMAIN_5006	Marmoset	58020	3.8614	1.38E-06
DOMAIN_5029	Marmoset	58021	2.2642	0.0022609
DOMAIN_5031	Marmoset	58022	2.8605	0.0025559
DOMAIN_5060	Marmoset	58023	2.6043	8.74E-04
DOMAIN_5096	Marmoset	58024	2.456	0.008963
DOMAIN_5099	Marmoset	58025	3.1407	0.0021138
DOMAIN_5102	Marmoset	58026	2.7241	0.0024099
DOMAIN_5103	Marmoset	58027	2.1016	0.0093552
DOMAIN_5125	Marmoset	58028	2.911	0.0015369
DOMAIN_5188	OwlMonkey	58029	2.1842	0.0046295
DOMAIN_5201	OwlMonkey	58030	3.3658	1.53E-07
DOMAIN_5217	OwlMonkey	58031	2.4689	0.0031316
DOMAIN_5235	OwlMonkey	58032	3.437	4.62E-04
DOMAIN_5246	OwlMonkey	58033	2.7473	0.0042075
DOMAIN_5248	OwlMonkey	58034	4.1052	1.53E-07
DOMAIN_5267	OwlMonkey	58035	3.1247	0.0016383
DOMAIN_5273	OwlMonkey	58036	2.4023	0.0069063
DOMAIN_5299	OwlMonkey	58037	2.7399	0.0093892
DOMAIN_5337	OwlMonkey	58038	3.7616	4.52E-05
DOMAIN_5370	OwlMonkey	58039	3.0452	0.0088803
DOMAIN_5440	OwlMonkey	58040	2.7871	0.0048658
DOMAIN_5485	OwlMonkey	58041	2.7826	0.0080202
DOMAIN_5489	OwlMonkey	58042	2.6774	0.0021808
DOMAIN_5518	OwlMonkey	58043	2.8542	0.0030235
DOMAIN_5527	OwlMonkey	58044	3.1092	0.0016793
DOMAIN_5603	OwlMonkey	58045	3.2806	0.0015418
DOMAIN_5716	OwlMonkey	58046	3.0606	5.36E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_5742	Homo sapiens	58047	2.8617	0.0029913
DOMAIN_5765	Rattus norvegicus	58048	4.2973	1.53E-07
DOMAIN_5774	Homo sapiens	58049	2.9608	3.75E-05
DOMAIN_5782	Homo sapiens	58050	2.9086	4.56E-04
DOMAIN_5791	Homo sapiens	58051	2.6823	0.0051494
DOMAIN_5792	Homo sapiens	58052	3.0218	8.56E-04
DOMAIN_5806	Homo sapiens	58053	2.866	0.0037801
DOMAIN_5822	Homo sapiens	58054	2.9335	0.0074467
DOMAIN_5843	Homo sapiens	58055	3.1821	2.83E-05
DOMAIN_5866	Homo sapiens	58056	2.6362	0.0080677
DOMAIN_5883	Homo sapiens	58057	3.0097	5.52E-04
DOMAIN_5896	Bos taurus	58058	2.9429	0.0023166
DOMAIN_5901	Homo sapiens	58059	3.2935	0.0012981
DOMAIN_5914	Homo sapiens	58060	2.5527	0.0029099
DOMAIN_5921	Homo sapiens	58061	2.4715	0.00101
DOMAIN_5943	Mus musculus	58062	2.501	0.0027917
DOMAIN_5946	Homo sapiens	58063	3.2998	1.38E-06
DOMAIN_5968	Bos taurus	58064	3.2856	3.86E-04
DOMAIN_5984	Homo sapiens	58065	2.9852	2.37E-04
DOMAIN_5989	Mus musculus	58066	3.6632	9.30E-04
DOMAIN_5994	Orangutan	58067	2.9214	5.04E-04
DOMAIN_6038	Homo sapiens	58068	3.3315	2.59E-04
DOMAIN_6053	Orangutan	58069	3.2566	1.21E-04
DOMAIN_6063	Homo sapiens	58070	3.5653	0.0019059
DOMAIN_6078	Homo sapiens	58071	2.6246	0.0075453
DOMAIN_6134	Homo sapiens	58072	2.7081	0.0034203
DOMAIN_6169	Homo sapiens	58073	3.3909	1.68E-06
DOMAIN_6172	Homo sapiens	58074	3.883	1.07E-06
DOMAIN_6249	Saimiri boliviensis boliviensis	58075	3.5469	4.44E-06
DOMAIN_6293	Rattus norvegicus	58076	2.6707	0.0034812
DOMAIN_6354	Terrapene carolina triunguis	58077	2.4812	0.0095055
DOMAIN_6356	Terrapene carolina triunguis	58078	2.9197	0.0031965

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_6382	Gopherus agassizii	58079	3.2875	1.66E-04
DOMAIN_6398	Gopherus agassizii	58080	2.8238	0.0059966
DOMAIN_6410	Podarcis muralis	58081	2.7633	0.0034243
DOMAIN_6433	Podarcis muralis	58082	3.0313	1.16E-04
DOMAIN_6458	Gopherus agassizii	58083	2.8973	0.0048435
DOMAIN_6472	Alligator sinensis	58084	2.9259	0.0052565
DOMAIN_6482	Paroedura picta	58085	3.3106	0.0019705
DOMAIN_6501	Paroedura picta	58086	3.4172	0.0010204
DOMAIN_6539	Paroedura picta	58087	3.2371	0.0025654
DOMAIN_6555	Paroedura picta	58088	3.534	4.92E-04
DOMAIN_6577	Terrapene carolina triunguis	58089	3.3168	3.95E-04
DOMAIN_6595	Terrapene carolina triunguis	58090	2.2407	0.0027133
DOMAIN_6599	Terrapene carolina triunguis	58091	3.3653	4.49E-05
DOMAIN_6697	Podarcis muralis	58092	2.6712	7.35E-04
DOMAIN_6737	Microcaecilia unicolor	58093	2.4861	0.0065704
DOMAIN_6738	Microcaecilia unicolor	58094	2.9275	7.79E-04
DOMAIN_6741	Microcaecilia unicolor	58095	3.5726	2.50E-04
DOMAIN_6866	Alligator mississippiensis	58096	3.5825	1.02E-04
DOMAIN_6936	Callipepla squamata	58097	3.5294	9.07E-04
DOMAIN_6938	Alligator mississippiensis	58098	2.6093	0.0020584
DOMAIN_6952	Alligator mississippiensis	58099	2.3403	0.0084774
DOMAIN_6970	Phasianus colchicus	58100	3.343	3.02E-04
DOMAIN_7000	Phasianus colchicus	58101	2.8279	0.0039843
DOMAIN_7098	Microcaecilia unicolor	58102	2.7074	0.0030553
DOMAIN_7109	Microcaecilia unicolor	58103	2.9932	0.0077318
DOMAIN_7123	Microcaecilia unicolor	58104	2.9074	0.0043723
DOMAIN_7166	Microcaecilia unicolor	58105	3.1419	5.72E-04
DOMAIN_7183	Microcaecilia unicolor	58106	2.4918	1.27E-04
DOMAIN_7184	Microcaecilia unicolor	58107	2.2019	0.0099168
DOMAIN_7328	Terrapene carolina triunguis	58108	3.1808	5.04E-05
DOMAIN_7353	Microcaecilia unicolor	58109	2.6649	0.0042219

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_7365	Microcaecilia unicolor	58110	2.597	0.0042403
DOMAIN_7480	Gopherus agassizii	58111	3.1707	5.44E-04
DOMAIN_7510	Gopherus agassizii	58112	3.0452	6.73E-04
DOMAIN_7534	Gopherus agassizii	58113	3.4086	2.50E-04
DOMAIN_7553	Gopherus agassizii	58114	2.9036	0.0088341
DOMAIN_7605	Alligator sinensis	58115	2.8444	0.0018789
DOMAIN_7607	Alligator sinensis	58116	2.7102	0.0018612
DOMAIN_7641	Gallus gallus	58117	3.6727	4.51E-04
DOMAIN_7653	Gallus gallus	58118	3.3772	0.0028364
DOMAIN_7678	Chelonia mydas	58119	2.7348	0.0039197
DOMAIN_7711	Columba livia	58120	3.7965	1.67E-05
DOMAIN_7716	Pogona vitticeps	58121	3.1171	0.0011931
DOMAIN_7745	Meleagris gallopavo	58122	3.4946	0.0016126
DOMAIN_7750	Columba livia	58123	2.8111	0.0012249
DOMAIN_7774	Pogona vitticeps	58124	3.427	8.09E-04
DOMAIN_7796	Chelonia mydas	58125	2.9513	1.04E-04
DOMAIN_7813	Columba livia	58126	3.4645	7.95E-04
DOMAIN_7824	Columba livia	58127	2.9383	5.45E-04
DOMAIN_7850	Terrapene carolina triunguis	58128	3.124	5.15E-04
DOMAIN_7895	Patagioenas fasciata monilis	58129	3.2254	0.0013863
DOMAIN_7925	Gallus gallus	58130	3.3919	0.0025195
DOMAIN_8012	Callipepla squamata	58131	3.2046	0.0023734
DOMAIN_8013	Callipepla squamata	58132	3.9783	2.13E-05
DOMAIN_8014	Callipepla squamata	58133	3.7425	6.23E-05
DOMAIN_8036	Alligator mississippiensis	58134	2.3504	0.0094483
DOMAIN_8041	Dipodomys ordii	58135	3.6568	3.47E-04
DOMAIN_8054	Cavia porcellus	58136	3.5889	4.15E-05
DOMAIN_8148	Cricetulus griseus	58137	3.6904	4.82E-05
DOMAIN_8151	Cricetulus griseus	58138	3.1527	0.0034782
DOMAIN_8154	Cricetulus griseus	58139	2.8774	0.0027807
DOMAIN_8167	Mus musculus	58140	3.9362	1.04E-04
DOMAIN_8179	Mesocricetus auratus	58141	3.0623	0.0026242

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_8182	Mus caroli	58142	2.2411	0.0018051
DOMAIN_8216	Cricetulus griseus	58143	3.1747	9.05E-05
DOMAIN_8226	Rattus norvegicus	58144	2.4602	0.0090772
DOMAIN_8235	Mus caroli	58145	2.8965	0.0012522
DOMAIN_8282	Peromyscus maniculatus bairdii	58146	3.9882	1.07E-06
DOMAIN_8289	Peromyscus maniculatus bairdii	58147	3.3026	2.94E-04
DOMAIN_8301	Mesocricetus auratus	58148	3.1084	0.0017647
DOMAIN_8303	Ictidomys tridecemlineatus	58149	3.6843	1.34E-04
DOMAIN_8305	Ictidomys tridecemlineatus	58150	2.5554	0.0084633
DOMAIN_8308	Marmota monax	58151	2.6564	3.69E-04
DOMAIN_8317	Mus caroli	58152	3.3091	2.40E-05
DOMAIN_8340	Peromyscus maniculatus bairdii	58153	2.2764	0.0086378
DOMAIN_8353	Peromyscus maniculatus bairdii	58154	2.7989	4.14E-04
DOMAIN_8370	Cavia porcellus	58155	3.5737	2.58E-04
DOMAIN_8412	Mus musculus	58156	2.4486	0.0077639
DOMAIN_8418	Cricetulus griseus	58157	2.4014	0.001307
DOMAIN_8424	Peromyscus maniculatus bairdii	58158	2.7945	0.0019818
DOMAIN_8425	Peromyscus maniculatus bairdii	58159	2.8391	0.004804
DOMAIN_8460	Peromyscus maniculatus bairdii	58160	3.1352	6.66E-05
DOMAIN_8467	Mesocricetus auratus	58161	3.8156	7.15E-05
DOMAIN_8489	Mus caroli	58162	2.8336	0.0042299
DOMAIN_8492	Mus musculus	58163	3.3107	0.0032374
DOMAIN_8502	Cricetulus griseus	58164	2.1429	4.22E-04
DOMAIN_8545	Rattus norvegicus	58165	3.1044	0.0011282
DOMAIN_8546	Mus musculus	58166	2.9439	0.0033958
DOMAIN_8547	Mus caroli	58167	3.3997	0.0022286
DOMAIN_8549	Mus caroli	58168	2.8508	0.0052033
DOMAIN_8555	Cricetulus griseus	58169	3.2852	5.62E-05
DOMAIN_8618	Mesocricetus auratus	58170	2.6363	0.008293
DOMAIN_8688	Mus musculus	58171	2.4409	2.00E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_8689	Mus musculus	58172	2.8548	6.62E-04
DOMAIN_8712	Mesocricetus auratus	58173	2.7776	0.0028768
DOMAIN_8742	Peromyscus maniculatus bairdii	58174	2.3354	0.002149
DOMAIN_8746	Mesocricetus auratus	58175	3.317	1.64E-04
DOMAIN_8789	Marmota monax	58176	3.1756	0.0021937
DOMAIN_8793	Mus caroli	58177	2.6774	9.60E-05
DOMAIN_8816	Peromyscus maniculatus bairdii	58178	2.4156	2.32E-04
DOMAIN_8830	Cavia porcellus	58179	3.0644	0.0025588
DOMAIN_8839	Peromyscus maniculatus bairdii	58180	3.0637	0.0036542
DOMAIN_8844	Peromyscus maniculatus bairdii	58181	4.1629	7.81E-06
DOMAIN_8850	Peromyscus maniculatus bairdii	58182	2.695	0.0040575
DOMAIN_8862	Marmota monax	58183	2.3521	0.0061537
DOMAIN_8881	Cricetulus griseus	58184	3.743	1.49E-05
DOMAIN_8886	Cricetulus griseus	58185	3.5727	1.94E-05
DOMAIN_8899	Mesocricetus auratus	58186	3.2182	9.45E-05
DOMAIN_8931	Cricetulus griseus	58187	2.9497	8.73E-04
DOMAIN_8936	Cricetulus griseus	58188	4.3486	1.07E-06
DOMAIN_8953	Mus caroli	58189	2.5941	0.0032969
DOMAIN_8982	Mesocricetus auratus	58190	3.1585	3.54E-05
DOMAIN_8989	Marmota monax	58191	2.2309	0.0094553
DOMAIN_9012	Mus musculus	58192	2.3905	0.0070058
DOMAIN_9042	Mus caroli	58193	2.5894	0.0033885
DOMAIN_9060	Cricetulus griseus	58194	2.5974	0.0027286
DOMAIN_9119	Mesocricetus auratus	58195	2.2985	0.0052412
DOMAIN_9141	Mus caroli	58196	3.035	2.62E-05
DOMAIN_9159	Dipodomys ordii	58197	3.0141	0.0023052
DOMAIN_9174	Peromyscus maniculatus bairdii	58198	2.5194	0.0035749
DOMAIN_9175	Peromyscus maniculatus bairdii	58199	2.4231	0.0042293
DOMAIN_9189	Heterocephalus glaber	58200	3.3801	1.76E-04
DOMAIN_9192	Mus caroli	58201	2.7981	0.008526

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_9217	Mesocricetus auratus	58202	3.8919	5.43E-05
DOMAIN_9235	Mus musculus	58203	2.7307	0.0035899
DOMAIN_9250	Marmota monax	58204	3.466	0.0012007
DOMAIN_9265	Mus musculus	58205	2.1221	0.0021172
DOMAIN_9290	Peromyscus maniculatus bairdii	58206	4.256	1.07E-06
DOMAIN_9303	Marmota monax	58207	2.5344	0.0051732
DOMAIN_9313	Mus musculus	58208	2.7692	0.0061916
DOMAIN_9324	Peromyscus maniculatus bairdii	58209	3.1782	0.0020198
DOMAIN_9329	Peromyscus maniculatus bairdii	58210	4.263	7.81E-06
DOMAIN_9332	Peromyscus maniculatus bairdii	58211	3.9002	1.38E-06
DOMAIN_9356	Ictidomys tridecemlineatus	58212	2.9297	0.0037302
DOMAIN_9389	Marmota monax	58213	3.1785	2.65E-05
DOMAIN_9424	Dipodomys ordii	58214	3.771	1.53E-07
DOMAIN_9435	Fukomys damarensis	58215	3.1672	3.01E-04
DOMAIN_9446	Marmota monax	58216	2.8722	3.80E-04
DOMAIN_9489	Dipodomys ordii	58217	3.0215	0.0074336
DOMAIN_9503	Ictidomys tridecemlineatus	58218	2.9864	0.0021536
DOMAIN_9526	Mesocricetus auratus	58219	2.9435	0.0042492
DOMAIN_9530	Mesocricetus auratus	58220	2.7003	0.0026178
DOMAIN_9541	Dipodomys ordii	58221	2.8442	0.0028404
DOMAIN_9542	Octodon degus	58222	2.6734	0.0036809
DOMAIN_9544	Octodon degus	58223	2.9143	0.0054966
DOMAIN_9559	Mus caroli	58224	3.327	0.001653
DOMAIN_9563	Mus musculus	58225	3.7261	3.81E-05
DOMAIN_9576	Octodon degus	58226	2.1952	0.0094564
DOMAIN_9617	Mesocricetus auratus	58227	2.4034	0.0040152
DOMAIN_9643	Dipodomys ordii	58228	3.4306	0.0023603
DOMAIN_9697	Octodon degus	58229	2.7566	0.0063579
DOMAIN_9704	Dipodomys ordii	58230	3.1674	0.0013462
DOMAIN_9706	Octodon degus	58231	2.821	0.0041809
DOMAIN_9713	Cricetulus griseus	58232	3.0323	0.002243

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_9716	Mus caroli	58233	2.9009	0.0040762
DOMAIN_9723	Mus caroli	58234	2.1903	0.0058971
DOMAIN_9725	Mus caroli	58235	2.9654	0.0028095
DOMAIN_9776	Marmota monax	58236	2.6258	0.0084697
DOMAIN_9787	Mus caroli	58237	3.2962	8.37E-05
DOMAIN_9789	Mus musculus	58238	2.5801	0.0012534
DOMAIN_9822	Ictidomys tridecemlineatus	58239	2.9382	0.0065879
DOMAIN_9824	Heterocephalus glaber	58240	3.1306	8.34E-05
DOMAIN_9827	Mus caroli	58241	2.1904	0.0077554
DOMAIN_9843	Mus musculus	58242	2.3385	0.0035982
DOMAIN_9846	Cricetulus griseus	58243	2.7865	0.0025033
DOMAIN_9857	Mesocricetus auratus	58244	3.3666	8.92E-04
DOMAIN_9858	Mesocricetus auratus	58245	3.0047	1.33E-04
DOMAIN_9878	Marmota monax	58246	3.7349	2.61E-04
DOMAIN_9891	Mus caroli	58247	2.8116	3.13E-04
DOMAIN_9915	Mus caroli	58248	3.4011	3.45E-04
DOMAIN_9962	Rattus norvegicus	58249	2.7249	0.004063
DOMAIN_9993	Rattus norvegicus	58250	2.7601	0.0035973
DOMAIN_10018	Octodon degus	58251	3.3372	4.27E-04
DOMAIN_10041	Mus caroli	58252	2.8662	0.0062437
DOMAIN_10044	Mus musculus	58253	2.826	0.0043095
DOMAIN_10050	Octodon degus	58254	3.3147	0.0020066
DOMAIN_10057	Mus musculus	58255	2.2961	0.0026799
DOMAIN_10091	Fukomys damarensis	58256	2.1679	4.36E-04
DOMAIN_10127	Peromyscus maniculatus bairdii	58257	3.6912	3.83E-06
DOMAIN_10160	Ictidomys tridecemlineatus	58258	2.9333	4.23E-04
DOMAIN_10184	Mus caroli	58259	4.2854	1.53E-07
DOMAIN_10241	Octodon degus	58260	3.5766	8.19E-05
DOMAIN_10257	Octodon degus	58261	3.1757	5.20E-04
DOMAIN_10294	Mus musculus	58262	2.689	0.0067073
DOMAIN_10334	Mustela putorius furo	58263	3.3529	5.07E-05
DOMAIN_10351	Delphinapterus leucas	58264	3.3309	3.78E-04
DOMAIN_10359	Delphinapterus leucas	58265	2.9199	0.0036842

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_10381	Vicugna pacos	58266	2.215	0.0057838
DOMAIN_10386	Odobenus rosmarus divergens	58267	2.8337	0.0028753
DOMAIN_10403	Vicugna pacos	58268	3.3993	0.0016441
DOMAIN_10420	Odobenus rosmarus divergens	58269	3.7185	1.01E-04
DOMAIN_10425	Delphinapterus leucas	58270	2.8616	0.0041775
DOMAIN_10427	Carlito syrichta	58271	2.3719	0.0078328
DOMAIN_10491	Vicugna pacos	58272	3.7199	0.0012761
DOMAIN_10495	Delphinapterus leucas	58273	3.4705	5.27E-04
DOMAIN_10526	Delphinapterus leucas	58274	2.4499	0.0033355
DOMAIN_10573	Cervus elaphus hippelaphus	58275	2.4077	5.02E-04
DOMAIN_10612	Vicugna pacos	58276	2.4997	0.0035134
DOMAIN_10613	Odobenus rosmarus divergens	58277	2.9148	5.62E-05
DOMAIN_10623	Carlito syrichta	58278	3.2233	0.0018333
DOMAIN_10646	Delphinapterus leucas	58279	2.9354	0.0036496
DOMAIN_10647	Delphinapterus leucas	58280	2.9514	7.60E-04
DOMAIN_10675	Ornithorhynchus anatinus	58281	3.2777	5.13E-05
DOMAIN_10684	Odobenus rosmarus divergens	58282	4.531	1.64E-05
DOMAIN_10704	Colobus angolensis palliatus	58283	3.1582	0.004292
DOMAIN_10705	Colobus angolensis palliatus	58284	3.6392	4.09E-05
DOMAIN_10733	Odobenus rosmarus divergens	58285	3.315	0.0028523
DOMAIN_10762	Erinaceus europaeus	58286	3.9254	4.55E-05
DOMAIN_10763	Mustela putorius furo	58287	2.5924	0.0073193
DOMAIN_10765	Mustela putorius furo	58288	2.5661	0.0076445
DOMAIN_10807	Erinaceus europaeus	58289	3.5237	1.54E-04
DOMAIN_10882	Vicugna pacos	58290	3.6289	2.93E-04
DOMAIN_10902	Vicugna pacos	58291	3.1052	0.0096752
DOMAIN_10917	Odobenus rosmarus divergens	58292	3.7871	1.53E-07
DOMAIN_10943	Cervus elaphus hippelaphus	58293	2.5554	0.0037715
DOMAIN_10974	Chelonia mydas	58294	2.6444	0.0091318

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_11006	Loxodonta africana	58295	2.6669	6.71E-04
DOMAIN_11024	Suricata suricatta	58296	3.2397	2.77E-04
DOMAIN_11031	Mandrillus leucophaeus	58297	2.5516	0.005857
DOMAIN_11034	Mandrillus leucophaeus	58298	2.2541	0.0042161
DOMAIN_11040	Sus scrofa	58299	3.5161	3.39E-04
DOMAIN_11049	Neophocaena asiaeorientalis asiaeorientalis	58300	2.7072	0.0015299
DOMAIN_11053	Nomascus leucogenys	58301	3.677	4.44E-06
DOMAIN_11069	Capra hircus	58302	3.2745	0.0036948
DOMAIN_11071	Chrysochloris asiatica	58303	3.1268	0.0012421
DOMAIN_11097	Mandrillus leucophaeus	58304	3.239	0.0011508
DOMAIN_11110	Sus scrofa	58305	3.6632	4.76E-04
DOMAIN_11129	Nomascus leucogenys	58306	2.3864	1.88E-04
DOMAIN_11130	Nomascus leucogenys	58307	2.3487	6.64E-04
DOMAIN_11132	Bos indicus	58308	3.5671	3.08E-05
DOMAIN_11157	Suricata suricatta	58309	3.6671	8.22E-05
DOMAIN_11158	Chrysochloris asiatica	58310	2.6889	0.0035388
DOMAIN_11162	Mandrillus leucophaeus	58311	3.2804	2.65E-04
DOMAIN_11178	Sus scrofa	58312	2.4845	0.0043413
DOMAIN_11192	Neophocaena asiaeorientalis asiaeorientalis	58313	2.8798	2.10E-04
DOMAIN_11202	Nomascus leucogenys	58314	3.5851	4.18E-05
DOMAIN_11204	Nomascus leucogenys	58315	3.5793	5.22E-05
DOMAIN_11225	Capra hircus	58316	3.606	0.0011566
DOMAIN_11227	Capra hircus	58317	2.7556	0.0032733
DOMAIN_11264	Sus scrofa	58318	3.5019	5.64E-04
DOMAIN_11265	Sus scrofa	58319	4.2521	1.53E-07
DOMAIN_11282	Suricata suricatta	58320	3.536	1.53E-07
DOMAIN_11289	Suricata suricatta	58321	2.69	2.48E-04
DOMAIN_11291	Suricata suricatta	58322	4.0373	4.59E-07
DOMAIN_11307	Mandrillus leucophaeus	58323	3.6383	1.07E-06
DOMAIN_11312	Sus scrofa	58324	3.8532	9.26E-05
DOMAIN_11314	Sus scrofa	58325	2.9575	0.0015357

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_11321	Nomascus leucogenys	58326	2.9718	0.0086853
DOMAIN_11331	Capra hircus	58327	3.0611	4.37E-04
DOMAIN_11332	Capra hircus	58328	3.0468	2.19E-04
DOMAIN_11356	Sus scrofa	58329	2.6549	0.0027629
DOMAIN_11359	Sus scrofa	58330	3.1036	0.0092232
DOMAIN_11381	Nomascus leucogenys	58331	3.1705	4.83E-04
DOMAIN_11393	Suricata suricatta	58332	3.4256	1.65E-04
DOMAIN_11401	Suricata suricatta	58333	2.6345	0.0077459
DOMAIN_11403	Suricata suricatta	58334	3.4222	2.27E-04
DOMAIN_11413	Sus scrofa	58335	2.1814	0.0084919
DOMAIN_11433	Neophocaena asiaeorientalis asiaeorientalis	58336	3.3986	1.91E-05
DOMAIN_11446	Nomascus leucogenys	58337	2.6971	3.26E-04
DOMAIN_11461	Equus caballus	58338	2.508	0.0090515
DOMAIN_11466	Suricata suricatta	58339	3.4716	0.0027896
DOMAIN_11470	Mandrillus leucophaeus	58340	3.1038	0.0012895
DOMAIN_11502	Trichechus manatus latirostris	58341	3.601	4.21E-05
DOMAIN_11505	Trichechus manatus latirostris	58342	3.0969	9.19E-07
DOMAIN_11534	Sus scrofa	58343	3.8118	1.91E-05
DOMAIN_11554	Nomascus leucogenys	58344	3.0498	4.11E-04
DOMAIN_11567	Zalophus californianus	58345	3.4239	0.0010611
DOMAIN_11581	Equus caballus	58346	3.1882	4.10E-04
DOMAIN_11612	Loxodonta africana	58347	3.3006	0.0040119
DOMAIN_11621	Chrysochloris asiatica	58348	3.2074	5.42E-04
DOMAIN_11643	Nomascus leucogenys	58349	2.3544	0.0020207
DOMAIN_11662	Capra hircus	58350	3.7889	2.36E-04
DOMAIN_11672	Suricata suricatta	58351	3.318	0.0022931
DOMAIN_11701	Capra hircus	58352	2.5282	0.0084694
DOMAIN_11726	Sus scrofa	58353	3.4183	1.09E-05
DOMAIN_11749	Chlorocebus sabaeus	58354	3.2721	0.0023817
DOMAIN_11753	Mandrillus leucophaeus	58355	2.6119	0.0062269

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_11760	Neophocaena asiaeorientalis asiaeorientalis	58356	2.8102	0.0039794
DOMAIN_11796	Sus scrofa	58357	2.2811	0.0010219
DOMAIN_11813	Canis lupus familiaris	58358	3.5195	7.62E-04
DOMAIN_11825	Mandrillus leucophaeus	58359	3.9893	1.53E-07
DOMAIN_11851	Nomascus leucogenys	58360	3.0241	1.32E-04
DOMAIN_11858	Canis lupus familiaris	58361	3.6419	1.53E-07
DOMAIN_11862	Canis lupus familiaris	58362	2.8817	0.0032412
DOMAIN_11865	Muntiacus muntjak	58363	3.0474	0.0026931
DOMAIN_11868	Mandrillus leucophaeus	58364	3.5158	4.44E-06
DOMAIN_11908	Canis lupus familiaris	58365	2.894	0.0035529
DOMAIN_11923	Sus scrofa	58366	3.2271	0.0018734
DOMAIN_11925	Mandrillus leucophaeus	58367	3.5582	3.04E-04
DOMAIN_11928	Neophocaena asiaeorientalis asiaeorientalis	58368	3.751	7.59E-04
DOMAIN_11933	Neophocaena asiaeorientalis asiaeorientalis	58369	4.1135	1.52E-05
DOMAIN_11944	Bos indicus	58370	3.2762	0.0022727
DOMAIN_11950	Canis lupus familiaris	58371	4.3869	2.91E-06
DOMAIN_11988	Muntiacus muntjak	58372	3.5916	3.83E-06
DOMAIN_11996	Canis lupus familiaris	58373	3.0831	0.0015161
DOMAIN_11999	Canis lupus familiaris	58374	3.7891	5.04E-05
DOMAIN_12001	Mandrillus leucophaeus	58375	2.4384	0.0057376
DOMAIN_12021	Canis lupus familiaris	58376	2.4637	0.0018489
DOMAIN_12051	Muntiacus muntjak	58377	2.7925	0.0039375
DOMAIN_12057	Muntiacus muntjak	58378	2.0631	0.0086017
DOMAIN_12079	Muntiacus muntjak	58379	2.4029	0.0095567
DOMAIN_12092	Bos mutus	58380	3.1752	1.82E-05
DOMAIN_12114	Neophocaena asiaeorientalis asiaeorientalis	58381	3.3227	6.62E-04
DOMAIN_12133	Canis lupus familiaris	58382	3.0204	0.0034751
DOMAIN_12139	Canis lupus familiaris	58383	2.8097	0.0066678

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_12147	Neophocaena asiaeorientalis asiaeorientalis	58384	2.6974	9.14E-04
DOMAIN_12158	Nomascus leucogenys	58385	3.0332	0.006631
DOMAIN_12187	Canis lupus familiaris	58386	3.6477	5.13E-05
DOMAIN_12191	Muntiacus muntjak	58387	3.6138	8.18E-04
DOMAIN_12195	Canis lupus familiaris	58388	2.9023	1.11E-04
DOMAIN_12206	Bos mutus	58389	2.9101	5.13E-04
DOMAIN_12210	Bos indicus	58390	3.6136	0.0018284
DOMAIN_12214	Muntiacus muntjak	58391	2.613	9.76E-04
DOMAIN_12231	Nomascus leucogenys	58392	2.6703	0.00421
DOMAIN_12261	Neophocaena asiaeorientalis asiaeorientalis	58393	2.7989	0.0029785
DOMAIN_12285	Gorilla	58394	2.2573	0.0091023
DOMAIN_12313	Bos indicus	58395	2.6903	0.0012684
DOMAIN_12320	Muntiacus muntjak	58396	2.5075	0.0023021
DOMAIN_12365	Nomascus leucogenys	58397	3.5626	7.78E-04
DOMAIN_12395	Ailuropoda melanoleuca	58398	3.1504	3.56E-04
DOMAIN_12459	Bos indicus	58399	4.0425	3.06E-06
DOMAIN_12463	Ailuropoda melanoleuca	58400	3.2567	0.009339
DOMAIN_12467	Gorilla	58401	2.9575	4.85E-04
DOMAIN_12498	Muntiacus muntjak	58402	2.8947	0.0075569
DOMAIN_12499	Muntiacus muntjak	58403	2.2932	0.0064341
DOMAIN_12508	Gorilla	58404	3.0173	0.0024497
DOMAIN_12511	Gorilla	58405	3.0694	0.0023557
DOMAIN_12517	Lynx canadensis	58406	2.6983	0.0017522
DOMAIN_12544	Gorilla	58407	3.306	4.83E-04
DOMAIN_12550	Ailuropoda melanoleuca	58408	3.0229	2.37E-04
DOMAIN_12576	Gorilla	58409	3.04	0.0044151
DOMAIN_12590	Bos indicus	58410	2.5531	0.0020023
DOMAIN_12591	Bos indicus	58411	3.4169	0.0011553
DOMAIN_12598	Muntiacus muntjak	58412	3.3709	4.18E-05
DOMAIN_12599	Muntiacus muntjak	58413	2.2098	0.007064
DOMAIN_12630	Macaca fascicularis	58414	3.6424	4.03E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_12646	Myotis lucifugus	58415	3.487	0.0014708
DOMAIN_12686	Phascolarctos cinereus	58416	2.76	0.0032103
DOMAIN_12698	Phascolarctos cinereus	58417	2.8029	0.0066675
DOMAIN_12704	Myotis lucifugus	58418	2.9127	0.0034078
DOMAIN_12712	Puma concolor	58419	2.1195	0.008023
DOMAIN_12728	Lynx canadensis	58420	3.1999	9.49E-04
DOMAIN_12734	Phyllostomus discolor	58421	3.5207	1.38E-06
DOMAIN_12755	Oryctolagus cuniculus	58422	2.8082	0.0061475
DOMAIN_12764	Desmodus rotundus	58423	3.9505	1.53E-07
DOMAIN_12769	Macaca fascicularis	58424	2.0555	0.0080928
DOMAIN_12777	Phascolarctos cinereus	58425	2.1778	0.0057731
DOMAIN_12780	Phascolarctos cinereus	58426	3.2671	1.01E-04
DOMAIN_12801	Sapajus apella	58427	2.0238	0.006988
DOMAIN_12811	Macaca fascicularis	58428	2.4278	0.0068959
DOMAIN_12815	Macaca fascicularis	58429	2.7296	0.0029445
DOMAIN_12818	Macaca fascicularis	58430	3.6211	9.69E-05
DOMAIN_12829	Phascolarctos cinereus	58431	3.3994	3.20E-04
DOMAIN_12831	Phascolarctos cinereus	58432	2.9845	0.0029084
DOMAIN_12839	Oryctolagus cuniculus	58433	3.4039	3.03E-04
DOMAIN_12849	Muntiacus muntjak	58434	4.1042	1.53E-07
DOMAIN_12896	Macaca fascicularis	58435	2.0413	0.0010397
DOMAIN_12901	Macaca fascicularis	58436	3.5686	4.75E-06
DOMAIN_12902	Macaca fascicularis	58437	3.3489	0.0016432
DOMAIN_12912	Puma concolor	58438	2.7422	4.78E-04
DOMAIN_12941	Phyllostomus discolor	58439	2.4012	0.0062382
DOMAIN_12985	Phascolarctos cinereus	58440	3.7331	3.05E-05
DOMAIN_13004	Macaca fascicularis	58441	3.2216	1.37E-04
DOMAIN_13022	Phascolarctos cinereus	58442	3.0468	0.003082
DOMAIN_13029	Myotis lucifugus	58443	3.1708	3.58E-04
DOMAIN_13062	Ursus maritimus	58444	2.9752	2.10E-04
DOMAIN_13068	Ailuropoda melanoleuca	58445	3.6132	2.43E-05
DOMAIN_13089	Sapajus apella	58446	2.8761	0.0065934
DOMAIN_13111	Ailuropoda melanoleuca	58447	2.6151	0.0090675
DOMAIN_13121	Macaca fascicularis	58448	3.353	3.98E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_13125	Macaca fascicularis	58449	3.2101	3.31E-04
DOMAIN_13171	Phascolarctos cinereus	58450	3.0052	0.0061932
DOMAIN_13193	Sapajus apella	58451	3.8948	1.53E-07
DOMAIN_13227	Oryctolagus cuniculus	58452	2.3234	0.0034855
DOMAIN_13269	Desmodus rotundus	58453	2.7236	0.0010081
DOMAIN_13277	Macaca fascicularis	58454	2.9151	4.66E-04
DOMAIN_13282	Phascolarctos cinereus	58455	3.5504	8.75E-04
DOMAIN_13284	Phascolarctos cinereus	58456	3.0903	0.0057642
DOMAIN_13293	Myotis lucifugus	58457	2.5884	6.56E-04
DOMAIN_13325	Macaca fascicularis	58458	2.4051	0.0085787
DOMAIN_13332	Phascolarctos cinereus	58459	2.685	0.0052498
DOMAIN_13333	Phascolarctos cinereus	58460	2.9787	0.0079948
DOMAIN_13339	Puma concolor	58461	3.2731	5.64E-04
DOMAIN_13346	Oryctolagus cuniculus	58462	2.9551	0.0031649
DOMAIN_13363	Phyllostomus discolor	58463	2.2178	0.0041619
DOMAIN_13364	Macaca fascicularis	58464	3.5606	2.40E-05
DOMAIN_13379	Phascolarctos cinereus	58465	3.2967	0.0018734
DOMAIN_13380	Myotis lucifugus	58466	3.6615	1.09E-05
DOMAIN_13387	Sapajus apella	58467	2.8731	0.001777
DOMAIN_13417	Ailuropoda melanoleuca	58468	3.7056	1.17E-04
DOMAIN_13439	Sapajus apella	58469	2.5091	0.0050786
DOMAIN_13470	Phascolarctos cinereus	58470	3.7598	2.40E-05
DOMAIN_13486	Puma concolor	58471	3.4895	7.93E-04
DOMAIN_13501	Macaca fascicularis	58472	2.8162	0.0083892
DOMAIN_13509	Phascolarctos cinereus	58473	2.8053	0.00351
DOMAIN_13516	Phascolarctos cinereus	58474	2.4421	0.0034809
DOMAIN_13536	Gorilla	58475	3.3269	0.0064418
DOMAIN_13537	Ailuropoda melanoleuca	58476	3.3265	8.83E-05
DOMAIN_13562	Phascolarctos cinereus	58477	3.7608	4.71E-04
DOMAIN_13565	Phascolarctos cinereus	58478	2.994	0.0032926
DOMAIN_13574	Puma concolor	58479	3.1114	6.89E-04
DOMAIN_13591	Lynx canadensis	58480	3.215	5.12E-04
DOMAIN_13601	Macaca fascicularis	58481	2.4865	0.0065955
DOMAIN_13609	Phascolarctos cinereus	58482	3.1787	0.002393

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_13610	Phascolarctos cinereus	58483	3.1925	0.0018707
DOMAIN_13644	Phascolarctos cinereus	58484	3.2677	0.001927
DOMAIN_13648	Oryctolagus cuniculus	58485	3.1393	0.0014022
DOMAIN_13650	Ailuropoda melanoleuca	58486	3.8556	4.44E-06
DOMAIN_13664	Macaca fascicularis	58487	2.7443	0.002582
DOMAIN_13670	Phascolarctos cinereus	58488	3.154	4.21E-04
DOMAIN_13690	Sapajus apella	58489	3.2587	0.0017001
DOMAIN_13691	Sapajus apella	58490	2.6205	0.0033052
DOMAIN_13703	Lynx canadensis	58491	3.7947	2.43E-05
DOMAIN_13705	Phyllostomus discolor	58492	2.496	0.009207
DOMAIN_13722	Phascolarctos cinereus	58493	2.4814	0.0058557
DOMAIN_13723	Phascolarctos cinereus	58494	2.9677	0.0026349
DOMAIN_13733	Sapajus apella	58495	3.3285	1.82E-04
DOMAIN_13783	Macaca fascicularis	58496	2.5821	0.0093056
DOMAIN_13805	Lynx canadensis	58497	3.1769	0.0088613
DOMAIN_13823	Macaca fascicularis	58498	4.219	1.53E-07
DOMAIN_13830	Phascolarctos cinereus	58499	2.6435	0.0033465
DOMAIN_13832	Phascolarctos cinereus	58500	2.9705	0.0077505
DOMAIN_13843	Phascolarctos cinereus	58501	3.6119	1.81E-04
DOMAIN_13851	Canis lupus familiaris	58502	2.6472	0.0033845
DOMAIN_13859	Macaca fascicularis	58503	2.2006	0.0086366
DOMAIN_13878	Ailuropoda melanoleuca	58504	4.3232	4.75E-06
DOMAIN_13880	Lynx canadensis	58505	3.0991	0.0013743
DOMAIN_13907	Phascolarctos cinereus	58506	2.4263	0.0084749
DOMAIN_13910	Bos mutus	58507	2.9556	0.0048664
DOMAIN_13915	Muntiacus muntjak	58508	2.8554	0.0080147
DOMAIN_13958	Phascolarctos cinereus	58509	3.2926	9.78E-05
DOMAIN_13970	Lynx canadensis	58510	2.89	0.0058701
DOMAIN_13979	Macaca fascicularis	58511	2.6188	0.0016793
DOMAIN_13981	Phascolarctos cinereus	58512	2.8041	0.0024451
DOMAIN_13984	Phascolarctos cinereus	58513	2.8513	0.0029797
DOMAIN_13987	Myotis lucifugus	58514	3.0633	4.59E-04
DOMAIN_13997	Puma concolor	58515	2.984	2.51E-04
DOMAIN_14009	Ailuropoda melanoleuca	58516	2.9207	5.05E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_14013	Ailuropoda melanoleuca	58517	2.4619	0.0082352
DOMAIN_14031	Phyllostomus discolor	58518	3.0963	0.0045422
DOMAIN_14040	Phascolarctos cinereus	58519	3.0933	0.0065673
DOMAIN_14041	Phascolarctos cinereus	58520	2.9069	0.0077333
DOMAIN_14049	Phascolarctos cinereus	58521	2.7761	0.0052936
DOMAIN_14069	Lynx canadensis	58522	2.9182	0.0020008
DOMAIN_14082	Phyllostomus discolor	58523	3.2495	2.19E-04
DOMAIN_14083	Phyllostomus discolor	58524	2.7465	0.0042213
DOMAIN_14108	Canis lupus familiaris	58525	3.0621	0.004127
DOMAIN_14129	Lynx canadensis	58526	2.8195	0.0026925
DOMAIN_14135	Bos mutus	58527	2.426	0.0033513
DOMAIN_14147	Canis lupus familiaris	58528	3.3683	2.59E-04
DOMAIN_14153	Muntiacus muntjak	58529	2.883	0.0011637
DOMAIN_14197	Muntiacus muntjak	58530	2.9589	0.0041555
DOMAIN_14219	Ailuropoda melanoleuca	58531	2.6653	0.0035657
DOMAIN_14226	Lynx canadensis	58532	3.1176	0.0020645
DOMAIN_14228	Lynx canadensis	58533	3.3445	7.54E-04
DOMAIN_14256	Lynx canadensis	58534	2.4946	0.0066852
DOMAIN_14287	Bos indicus	58535	3.6232	1.66E-04
DOMAIN_14295	Muntiacus muntjak	58536	3.4018	7.22E-04
DOMAIN_14322	Desmodus rotundus	58537	3.3716	1.94E-04
DOMAIN_14337	Muntiacus muntjak	58538	3.2753	2.86E-05
DOMAIN_14338	Ailuropoda melanoleuca	58539	3.1071	0.0022421
DOMAIN_14358	Lynx canadensis	58540	2.7094	8.85E-04
DOMAIN_14365	Desmodus rotundus	58541	3.0706	1.39E-04
DOMAIN_14373	Macaca fascicularis	58542	2.5861	0.0069375
DOMAIN_14382	Phascolarctos cinereus	58543	4.0523	7.52E-05
DOMAIN_14444	Phyllostomus discolor	58544	2.4641	0.0037357
DOMAIN_14487	Ailuropoda melanoleuca	58545	2.7981	0.0050538
DOMAIN_14526	Ailuropoda melanoleuca	58546	3.2232	0.003818
DOMAIN_14532	Lynx canadensis	58547	3.2071	2.43E-04
DOMAIN_14534	Lynx canadensis	58548	2.8122	0.0039834
DOMAIN_14546	Muntiacus muntjak	58549	3.5039	5.01E-05
DOMAIN_14551	Ailuropoda melanoleuca	58550	3.6894	2.30E-06

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_14557	Lynx canadensis	58551	2.9876	2.85E-04
DOMAIN_14574	Gorilla	58552	3.3356	7.83E-04
DOMAIN_14576	Ailuropoda melanoleuca	58553	3.2158	0.0028459
DOMAIN_14602	Gorilla	58554	3.2145	0.0037718
DOMAIN_14627	Acinonyx jubatus	58555	2.9501	0.0033732
DOMAIN_14639	Rhesus	58556	2.7046	0.0033915
DOMAIN_14714	Odocoileus virginianus texanus	58557	3.2752	2.48E-04
DOMAIN_14746	Odocoileus virginianus texanus	58558	2.605	0.0084645
DOMAIN_14773	Sapajus apella	58559	3.5997	1.45E-05
DOMAIN_14794	Acinonyx jubatus	58560	3.4295	4.09E-04
DOMAIN_14795	Rhinopithecus roxellana	58561	2.8119	0.0024062
DOMAIN_14800	Rhinopithecus roxellana	58562	2.274	0.0012494
DOMAIN_14815	Cebus imitator	58563	3.3826	0.0075808
DOMAIN_14820	Callithrix jacchus	58564	2.8836	0.0021743
DOMAIN_14829	Rhinopithecus roxellana	58565	2.7188	4.08E-04
DOMAIN_14845	Cebus imitator	58566	2.7224	0.0041993
DOMAIN_14849	Cebus imitator	58567	2.3659	0.0093133
DOMAIN_14862	Callithrix jacchus	58568	2.8116	0.0079314
DOMAIN_14864	Rhesus	58569	3.3492	2.46E-04
DOMAIN_14885	Cebus imitator	58570	3.5373	4.09E-05
DOMAIN_14901	Bos taurus	58571	2.9774	0.0085175
DOMAIN_14905	Rhinopithecus roxellana	58572	3.372	0.0034794
DOMAIN_14928	Callithrix jacchus	58573	3.1547	2.58E-04
DOMAIN_14939	Callorhinus ursinus	58574	2.3884	0.0071338
DOMAIN_14946	Acinonyx jubatus	58575	3.2842	7.46E-04
DOMAIN_14948	Acinonyx jubatus	58576	3.3727	1.73E-04
DOMAIN_14974	Sapajus apella	58577	2.9963	0.0091608
DOMAIN_14977	Sapajus apella	58578	3.0085	5.11E-04
DOMAIN_14978	Acinonyx jubatus	58579	3.0358	0.0017363
DOMAIN_14983	Rhinopithecus roxellana	58580	3.704	1.53E-07
DOMAIN_14994	Bison bison bison	58581	2.4997	0.0054874
DOMAIN_14995	Cebus imitator	58582	3.5057	4.13E-06

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_15042	Ovis aries	58583	3.0774	0.0045881
DOMAIN_15070	Callithrix jacchus	58584	4.0108	2.60E-04
DOMAIN_15083	Ovis aries	58585	2.7541	7.89E-04
DOMAIN_15086	Ovis aries	58586	3.5994	2.56E-04
DOMAIN_15089	Vulpes vulpes	58587	2.3585	0.0076298
DOMAIN_15102	Acinonyx jubatus	58588	3.0929	0.0033921
DOMAIN_15103	Bison bison bison	58589	2.652	0.0021839
DOMAIN_15119	Callithrix jacchus	58590	3.3838	2.60E-06
DOMAIN_15137	Ovis aries	58591	2.7071	0.0022528
DOMAIN_15138	Vulpes vulpes	58592	3.1771	6.85E-04
DOMAIN_15159	Ovis aries	58593	3.2135	0.0012084
DOMAIN_15171	Vulpes vulpes	58594	3.2837	2.40E-05
DOMAIN_15174	Vulpes vulpes	58595	3.1387	0.0033116
DOMAIN_15184	Acinonyx jubatus	58596	3.0092	0.0021588
DOMAIN_15197	Acinonyx jubatus	58597	3.0957	0.0012736
DOMAIN_15227	Rhinopithecus roxellana	58598	3.5532	4.75E-06
DOMAIN_15233	Rhinopithecus roxellana	58599	2.788	0.0046622
DOMAIN_15234	Acinonyx jubatus	58600	3.546	0.0019916
DOMAIN_15241	Odocoileus virginianus texanus	58601	3.3955	3.85E-04
DOMAIN_15251	Callithrix jacchus	58602	2.2209	9.47E-04
DOMAIN_15254	Callithrix jacchus	58603	3.5159	2.32E-04
DOMAIN_15267	Ovis aries	58604	2.8528	0.0020149
DOMAIN_15269	Ovis aries	58605	2.0839	0.0057336
DOMAIN_15278	Callithrix jacchus	58606	3.2523	0.0089241
DOMAIN_15279	Callithrix jacchus	58607	3.8574	6.87E-05
DOMAIN_15352	Cebus imitator	58608	3.0832	0.0079363
DOMAIN_15354	Tursiops truncatus	58609	3.5099	5.16E-05
DOMAIN_15356	Acinonyx jubatus	58610	3.5466	0.0019099
DOMAIN_15360	Neophocaena asiaeorientalis asiaeorientalis	58611	3.2575	3.95E-04
DOMAIN_15363	Orangutan	58612	4.3121	1.53E-07
DOMAIN_15391	Leptonychotes weddellii	58613	3.9053	1.53E-07
DOMAIN_15406	Chimp	58614	3.4616	1.53E-07

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_15419	Rhinopithecus roxellana	58615	2.6943	0.0012439
DOMAIN_15426	Odocoileus virginianus texanus	58616	2.9673	0.0024959
DOMAIN_15447	Rhinopithecus roxellana	58617	3.1112	0.0031907
DOMAIN_15451	Bison bison bison	58618	3.2905	0.0024601
DOMAIN_15527	Balaenoptera acutorostrata scammoni	58619	3.0354	0.0023685
DOMAIN_15536	Cebus imitator	58620	2.4515	0.0048713
DOMAIN_15540	Callithrix jacchus	58621	3.124	0.0020464
DOMAIN_15575	Callithrix jacchus	58622	2.594	0.0095671
DOMAIN_15577	Callithrix jacchus	58623	2.4456	0.0010642
DOMAIN_15581	Callorhinus ursinus	58624	3.2465	0.0031873
DOMAIN_15586	Callorhinus ursinus	58625	2.6157	0.002815
DOMAIN_15603	Cebus imitator	58626	3.5111	0.0027084
DOMAIN_15605	Cebus imitator	58627	3.8196	2.50E-04
DOMAIN_15634	Delphinapterus leucas	58628	3.3574	0.0025587
DOMAIN_15636	Chimp	58629	2.2086	0.0062339
DOMAIN_15638	Sapajus apella	58630	3.4277	1.53E-07
DOMAIN_15669	Callorhinus ursinus	58631	2.8865	0.0027889
DOMAIN_15687	Cebus imitator	58632	2.5362	0.0063187
DOMAIN_15688	Cebus imitator	58633	3.2098	6.98E-04
DOMAIN_15693	Rhesus	58634	3.8571	9.95E-06
DOMAIN_15699	Bos taurus	58635	3.5255	7.09E-04
DOMAIN_15753	Ovis aries	58636	3.1699	0.0035272
DOMAIN_15759	Ovis aries	58637	3.1884	0.0011061
DOMAIN_15764	Otolemur garnettii	58638	3.107	3.97E-04
DOMAIN_15800	Otolemur garnettii	58639	3.4462	1.80E-04
DOMAIN_15814	Rhesus	58640	3.9503	3.26E-05
DOMAIN_15823	Ovis aries	58641	2.8458	0.0034405
DOMAIN_15834	Otolemur garnettii	58642	3.7629	1.30E-05
DOMAIN_15839	Callithrix jacchus	58643	2.3399	0.0090833
DOMAIN_15863	Vulpes vulpes	58644	2.7434	0.0042734
DOMAIN_15931	Ovis aries	58645	3.0861	0.0028731
DOMAIN_15940	Enhydra lutris kenyoni	58646	2.8684	0.007571

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_15956	Bos taurus	58647	3.2271	4.36E-04
DOMAIN_15972	Enhydra lutris kenyoni	58648	2.3299	1.05E-04
DOMAIN_16009	Zalophus californianus	58649	3.2738	0.0020718
DOMAIN_16011	Delphinapterus leucas	58650	4.3363	1.53E-07
DOMAIN_16017	Ovis aries	58651	2.6715	0.0041604
DOMAIN_16023	Rhinopithecus bieti	58652	2.2831	0.0064672
DOMAIN_16050	Ovis aries	58653	2.7105	0.0086883
DOMAIN_16063	Rhesus	58654	2.1603	0.0054023
DOMAIN_16084	Enhydra lutris kenyoni	58655	3.0131	0.0022672
DOMAIN_16115	Bos taurus	58656	2.9023	0.0027605
DOMAIN_16123	Ovis aries	58657	2.3799	0.0079176
DOMAIN_16147	Orangutan	58658	2.5699	4.83E-04
DOMAIN_16184	Ovis aries	58659	3.7743	4.44E-06
DOMAIN_16188	Otolemur garnettii	58660	2.5145	0.0014414
DOMAIN_16238	Orangutan	58661	3.8734	2.87E-04
DOMAIN_16246	Rhesus	58662	2.3971	3.89E-04
DOMAIN_16253	Ovis aries	58663	4.488	1.53E-07
DOMAIN_16266	Otolemur garnettii	58664	3.075	0.0019834
DOMAIN_16274	Otolemur garnettii	58665	2.7655	0.0014904
DOMAIN_16312	Vicugna pacos	58666	2.4302	0.0024702
DOMAIN_16323	Trichechus manatus latirostris	58667	4.0053	2.15E-04
DOMAIN_16340	Ovis aries	58668	2.778	0.0034068
DOMAIN_16372	Odocoileus virginianus texanus	58669	4.2664	1.53E-07
DOMAIN_16378	Callithrix jacchus	58670	2.9868	0.0037718
DOMAIN_16399	Rhinopithecus roxellana	58671	4.0639	1.53E-07
DOMAIN_16408	Cebus imitator	58672	2.0194	0.009233
DOMAIN_16461	Cebus imitator	58673	3.1155	0.0020676
DOMAIN_16471	Acinonyx jubatus	58674	3.3465	0.0021006
DOMAIN_16478	Rhinopithecus roxellana	58675	2.8285	0.0023275
DOMAIN_16516	Rhesus	58676	3.8473	1.94E-05
DOMAIN_16517	Callithrix jacchus	58677	3.3189	2.60E-06
DOMAIN_16534	Acinonyx jubatus	58678	2.7531	0.0057425

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_16556	Rhinopithecus roxellana	58679	2.4217	0.0084734
DOMAIN_16566	Odocoileus virginianus texanus	58680	3.3903	7.82E-04
DOMAIN_16576	Chimp	58681	2.6949	0.0021998
DOMAIN_16597	Cebus imitator	58682	2.9869	0.0023416
DOMAIN_16611	Papio anubis	58683	3.4786	1.53E-07
DOMAIN_16618	Ursus maritimus	58684	3.1184	0.0015351
DOMAIN_16629	Cebus imitator	58685	3.7569	1.57E-04
DOMAIN_16630	Cebus imitator	58686	3.2435	1.36E-04
DOMAIN_16638	Macaca nemestrina	58687	3.3871	0.0011337
DOMAIN_16648	Physeter macrocephalus	58688	3.629	1.88E-05
DOMAIN_16651	Delphinapterus leucas	58689	2.0926	0.0074143
DOMAIN_16659	Leptonychotes weddellii	58690	3.8913	2.37E-05
DOMAIN_16664	Leptonychotes weddellii	58691	3.4502	1.76E-05
DOMAIN_16673	Phascolarctos cinereus	58692	3.0938	0.0039727
DOMAIN_16677	Orangutan	58693	3.1577	0.0023254
DOMAIN_16694	Callorhinus ursinus	58694	2.0979	0.0094743
DOMAIN_16695	Callorhinus ursinus	58695	3.965	3.06E-07
DOMAIN_16696	Tursiops truncatus	58696	3.0806	0.002705
DOMAIN_16703	Phascolarctos cinereus	58697	3.3969	2.19E-04
DOMAIN_16731	Ursus arctos horribilis	58698	2.849	1.30E-05
DOMAIN_16734	Leptonychotes weddellii	58699	3.4791	2.57E-04
DOMAIN_16738	Chimp	58700	3.5957	8.11E-06
DOMAIN_16744	Enhydra lutris kenyoni	58701	3.637	6.38E-05
DOMAIN_16763	Monodelphis domestica	58702	2.9244	0.0053031
DOMAIN_16771	Saimiri boliviensis boliviensis	58703	3.3025	0.0027295
DOMAIN_16773	Balaenoptera acutorostrata scammoni	58704	4.5309	1.38E-06
DOMAIN_16776	Callorhinus ursinus	58705	3.0877	0.0024757
DOMAIN_16809	Delphinapterus leucas	58706	2.4357	0.0068567
DOMAIN_16811	Balaenoptera acutorostrata scammoni	58707	3.5141	3.08E-04
DOMAIN_16856	Ursus maritimus	58708	2.7613	0.0040844
DOMAIN_16865	Papio anubis	58709	3.9619	1.53E-07

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_16876	Callorhinus ursinus	58710	3.2183	4.66E-04
DOMAIN_16877	Rhinolophus ferrumequinum	58711	3.3745	3.78E-05
DOMAIN_16936	Rhinopithecus roxellana	58712	2.9808	0.0044295
DOMAIN_16953	Callorhinus ursinus	58713	3.3286	1.62E-04
DOMAIN_16973	Delphinapterus leucas	58714	3.0187	0.0041062
DOMAIN_16994	Odocoileus virginianus texanus	58715	3.0575	0.0025431
DOMAIN_17001	Rhinolophus ferrumequinum	58716	3.045	0.003661
DOMAIN_17023	Sapajus apella	58717	2.5472	0.0041588
DOMAIN_17027	Balaenoptera acutorostrata scammoni	58718	3.131	0.0028042
DOMAIN_17041	Rhinopithecus roxellana	58719	2.7589	0.0074146
DOMAIN_17062	Rhinopithecus roxellana	58720	3.2594	7.33E-05
DOMAIN_17105	Rhesus	58721	2.637	0.0054256
DOMAIN_17108	Phyllostomus discolor	58722	2.4499	0.0018315
DOMAIN_17134	Panthera pardus	58723	3.2502	0.0016926
DOMAIN_17139	Ursus arctos horribilis	58724	4.0326	2.13E-05
DOMAIN_17153	Ursus arctos horribilis	58725	2.1759	0.0043459
DOMAIN_17167	Ursus maritimus	58726	4.1644	1.52E-05
DOMAIN_17177	Physeter macrocephalus	58727	3.2446	0.002928
DOMAIN_17180	Zalophus californianus	58728	2.945	0.0082198
DOMAIN_17195	Ursus maritimus	58729	3.0566	0.0037464
DOMAIN_17202	Ursus arctos horribilis	58730	2.6589	0.0072284
DOMAIN_17206	Pteropus vampyrus	58731	3.7092	5.05E-06
DOMAIN_17234	Delphinapterus leucas	58732	2.0152	0.0059669
DOMAIN_17236	Rhinolophus ferrumequinum	58733	2.7166	0.0039056
DOMAIN_17241	Muntiacus muntjak	58734	2.2217	0.003544
DOMAIN_17264	Vicugna pacos	58735	3.0866	0.0021294
DOMAIN_17278	Tursiops truncatus	58736	3.4898	4.12E-05
DOMAIN_17279	Bison bison bison	58737	3.591	8.11E-06
DOMAIN_17333	Camelus dromedarius	58738	2.8765	0.003642
DOMAIN_17340	Leptonychotes weddellii	58739	3.1536	5.34E-05
DOMAIN_17382	Leptonychotes weddellii	58740	3.075	0.0035284

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_17383	Leptonychotes weddellii	58741	2.953	0.0032519
DOMAIN_17412	Ovis aries	58742	4.9319	1.53E-07
DOMAIN_17421	Vulpes vulpes	58743	3.3129	2.83E-05
DOMAIN_17474	Monodelphis domestica	58744	2.683	0.0036059
DOMAIN_17483	Cercocebus atys	58745	3.5742	3.44E-05
DOMAIN_17495	Neomonachus schauinslandi	58746	3.1828	5.59E-05
DOMAIN_17497	Monodelphis domestica	58747	2.8088	5.07E-05
DOMAIN_17509	Physeter macrocephalus	58748	3.438	8.07E-04
DOMAIN_17516	Monodelphis domestica	58749	3.1523	4.18E-04
DOMAIN_17525	Myotis davidii	58750	3.4986	7.28E-04
DOMAIN_17534	Cercocebus atys	58751	2.9374	0.0033612
DOMAIN_17547	Neomonachus schauinslandi	58752	3.2455	5.64E-04
DOMAIN_17548	Neomonachus schauinslandi	58753	2.8002	5.08E-04
DOMAIN_17574	Cercocebus atys	58754	3.4893	2.80E-05
DOMAIN_17632	Monodelphis domestica	58755	3.3689	2.06E-04
DOMAIN_17658	Monodelphis domestica	58756	3.8781	1.99E-06
DOMAIN_17662	Monodelphis domestica	58757	2.7612	0.0040459
DOMAIN_17666	Monodelphis domestica	58758	2.6895	0.002059
DOMAIN_17671	Monodelphis domestica	58759	3.0937	0.008519
DOMAIN_17689	Cercocebus atys	58760	3.6469	1.53E-07
DOMAIN_17704	Neomonachus schauinslandi	58761	3.1047	0.0028404
DOMAIN_17714	Monodelphis domestica	58762	2.2724	0.0043612
DOMAIN_17717	Physeter macrocephalus	58763	2.9442	7.54E-04
DOMAIN_17748	Leptonychotes weddellii	58764	3.0918	2.44E-04
DOMAIN_17752	Leptonychotes weddellii	58765	3.2541	4.59E-04
DOMAIN_17775	Camelus dromedarius	58766	2.6595	0.0033885
DOMAIN_17798	Orangutan	58767	3.3458	5.16E-05
DOMAIN_17801	Orangutan	58768	2.9733	0.0022819
DOMAIN_17871	Leptonychotes weddellii	58769	3.1894	1.49E-05
DOMAIN_17873	Leptonychotes weddellii	58770	3.4076	3.00E-04
DOMAIN_17890	Cercocebus atys	58771	4.2356	2.80E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_17898	Enhydra lutris kenyoni	58772	3.2117	0.0034476
DOMAIN_17903	Orangutan	58773	2.4683	0.0030976
DOMAIN_17925	Otolemur garnettii	58774	2.7982	0.0042639
DOMAIN_18048	OwlMonkey	58775	2.5186	0.0087422
DOMAIN_18083	Papio anubis	58776	2.9283	4.79E-04
DOMAIN_18100	Neomonachus schauinslandi	58777	2.3606	0.0061598
DOMAIN_18103	Monodelphis domestica	58778	2.7334	0.0056181
DOMAIN_18136	Monodelphis domestica	58779	2.7288	6.75E-04
DOMAIN_18155	Sarcophilus harrisii	58780	2.7528	0.0052222
DOMAIN_18161	Cercocebus atys	58781	2.6663	0.0060803
DOMAIN_18181	Physeter macrocephalus	58782	4.696	4.59E-07
DOMAIN_18203	Monodelphis domestica	58783	3.7912	4.81E-04
DOMAIN_18206	Monodelphis domestica	58784	2.3929	0.0046062
DOMAIN_18214	Physeter macrocephalus	58785	2.6389	0.0094737
DOMAIN_18227	OwlMonkey	58786	3.5267	5.66E-06
DOMAIN_18241	Leptonychotes weddellii	58787	3.8187	9.60E-05
DOMAIN_18243	Felis catus	58788	3.5331	6.96E-04
DOMAIN_18244	Leptonychotes weddellii	58789	3.1726	0.0050817
DOMAIN_18272	Neomonachus schauinslandi	58790	2.9141	0.0085916
DOMAIN_18303	Monodelphis domestica	58791	2.9174	0.0018489
DOMAIN_18312	Monodelphis domestica	58792	2.8473	8.20E-04
DOMAIN_18323	Monodelphis domestica	58793	2.3956	0.0040336
DOMAIN_18325	Monodelphis domestica	58794	2.7636	0.0038297
DOMAIN_18332	Monodelphis domestica	58795	3.4328	4.68E-04
DOMAIN_18345	Monodelphis domestica	58796	3.349	4.43E-04
DOMAIN_18356	Monodelphis domestica	58797	3.1967	4.67E-04
DOMAIN_18385	Neomonachus schauinslandi	58798	2.1472	0.0044932
DOMAIN_18415	Neomonachus schauinslandi	58799	2.9768	4.55E-04
DOMAIN_18424	Physeter macrocephalus	58800	3.7744	3.31E-04
DOMAIN_18426	Physeter macrocephalus	58801	2.8011	0.0079672
DOMAIN_18428	Physeter macrocephalus	58802	2.5903	0.0095383

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_18433	OwlMonkey	58803	3.4614	0.0022427
DOMAIN_18441	Felis catus	58804	3.7534	1.77E-04
DOMAIN_18458	Monodelphis domestica	58805	3.1061	0.0018603
DOMAIN_18459	Monodelphis domestica	58806	3.1352	2.38E-04
DOMAIN_18483	Monodelphis domestica	58807	2.8259	5.19E-04
DOMAIN_18485	Monodelphis domestica	58808	2.8817	0.0011922
DOMAIN_18498	OwlMonkey	58809	2.7354	0.0021141
DOMAIN_18502	Myotis davidii	58810	3.4127	1.93E-04
DOMAIN_18504	Cercocebus atys	58811	3.2213	5.38E-04
DOMAIN_18536	Camelus dromedarius	58812	3.2028	0.0011217
DOMAIN_18580	Cercocebus atys	58813	4.4477	3.22E-06
DOMAIN_18589	Neomonachus schauinslandi	58814	3.039	0.0025063
DOMAIN_18594	Monodelphis domestica	58815	3.2119	0.0036607
DOMAIN_18618	Physeter macrocephalus	58816	2.6489	0.0072165
DOMAIN_18646	Monodelphis domestica	58817	2.4678	0.007646
DOMAIN_18670	Neomonachus schauinslandi	58818	3.1792	3.80E-04
DOMAIN_18677	Monodelphis domestica	58819	2.2686	0.0068996
DOMAIN_18693	Camelus dromedarius	58820	3.0179	0.0013759
DOMAIN_18698	Felis catus	58821	3.3067	0.0093304
DOMAIN_18711	Vulpes vulpes	58822	2.2749	0.0063986
DOMAIN_18724	Chimp	58823	3.2062	5.16E-04
DOMAIN_18726	Myotis davidii	58824	2.9362	0.0025771
DOMAIN_18734	Monodelphis domestica	58825	2.8813	0.0092612
DOMAIN_18752	Monodelphis domestica	58826	3.5544	4.85E-05
DOMAIN_18753	Monodelphis domestica	58827	2.6101	3.54E-04
DOMAIN_18760	Chimp	58828	3.1806	7.49E-05
DOMAIN_18785	Leptonychotes weddellii	58829	2.9139	0.0019203
DOMAIN_18817	Monodelphis domestica	58830	2.2496	0.0091589
DOMAIN_18830	Monodelphis domestica	58831	3.2719	0.0032764
DOMAIN_18835	Camelus dromedarius	58832	2.4878	8.56E-05
DOMAIN_18873	Camelus dromedarius	58833	3.262	0.0049846
DOMAIN_18891	Orangutan	58834	3.6429	1.38E-06

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_18923	Callithrix jacchus	58835	2.2053	0.0054504
DOMAIN_18935	Ovis aries	58836	3.4507	3.14E-05
DOMAIN_18947	Enhydra lutris kenyoni	58837	3.3167	7.58E-04
DOMAIN_18971	Enhydra lutris kenyoni	58838	3.3941	5.05E-06
DOMAIN_18977	Orangutan	58839	3.6262	9.03E-06
DOMAIN_18979	Orangutan	58840	2.0034	0.0071822
DOMAIN_19005	Enhydra lutris kenyoni	58841	3.4092	4.57E-04
DOMAIN_19028	Orangutan	58842	2.3618	0.0022277
DOMAIN_19056	Bos indicus x Bos taurus	58843	3.0542	0.001874
DOMAIN_19072	Vulpes vulpes	58844	2.8133	0.0016331
DOMAIN_19079	Otolemur garnettii	58845	4.0159	4.88E-05
DOMAIN_19125	Otolemur garnettii	58846	2.9892	8.36E-04
DOMAIN_19207	Enhydra lutris kenyoni	58847	2.655	0.0091617
DOMAIN_19220	Camelus dromedarius	58848	3.1947	0.0088687
DOMAIN_19221	Camelus dromedarius	58849	3.1733	4.21E-04
DOMAIN_19299	Myotis davidii	58850	2.8882	0.0043533
DOMAIN_19351	Orangutan	58851	3.1988	2.17E-04
DOMAIN_19385	Monodelphis domestica	58852	2.9198	0.008105
DOMAIN_19387	Monodelphis domestica	58853	3.4706	1.85E-04
DOMAIN_19388	Physeter macrocephalus	58854	3.2831	7.71E-04
DOMAIN_19404	Monodelphis domestica	58855	2.0125	0.0031965
DOMAIN_19423	Monodelphis domestica	58856	3.49	0.002544
DOMAIN_19424	Monodelphis domestica	58857	2.5838	0.0041846
DOMAIN_19437	OwlMonkey	58858	2.826	0.001773
DOMAIN_19445	Monodelphis domestica	58859	2.1105	0.0078325
DOMAIN_19447	Monodelphis domestica	58860	3.4492	1.40E-04
DOMAIN_19487	Monodelphis domestica	58861	3.4312	6.00E-04
DOMAIN_19497	Monodelphis domestica	58862	3.466	2.80E-05
DOMAIN_19517	Monodelphis domestica	58863	3.3361	1.04E-04
DOMAIN_19533	Papio anubis	58864	2.5831	4.67E-04
DOMAIN_19563	Papio anubis	58865	2.5522	0.0089134
DOMAIN_19580	Monodelphis domestica	58866	3.5716	3.29E-05
DOMAIN_19585	Monodelphis domestica	58867	3.0031	0.0032403
DOMAIN_19596	Monodelphis domestica	58868	3.8583	8.18E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_19597	Monodelphis domestica	58869	3.5081	4.46E-04
DOMAIN_19600	Monodelphis domestica	58870	2.5854	0.0042185
DOMAIN_19602	Physeter macrocephalus	58871	2.7219	0.0058524
DOMAIN_19611	Lipotes vexillifer	58872	3.3901	4.24E-04
DOMAIN_19629	Monodelphis domestica	58873	3.0535	0.0017954
DOMAIN_19699	Otolemur garnettii	58874	2.8474	3.15E-04
DOMAIN_19708	Bos indicus x Bos taurus	58875	3.6339	8.02E-04
DOMAIN_19713	Chimp	58876	3.845	2.95E-05
DOMAIN_19721	Otolemur garnettii	58877	2.6913	0.0089069
DOMAIN_19776	Enhydra lutris kenyoni	58878	2.617	0.0093497
DOMAIN_19777	Orangutan	58879	3.2427	0.0075444
DOMAIN_19780	Orangutan	58880	3.0867	1.72E-04
DOMAIN_19786	Chimp	58881	2.9155	5.94E-04
DOMAIN_19788	Enhydra lutris kenyoni	58882	3.3393	4.71E-04
DOMAIN_19800	Zalophus californianus	58883	2.368	0.009162
DOMAIN_19805	Rhinolophus ferrumequinum	58884	2.6527	0.0030997
DOMAIN_19818	Rhinopithecus roxellana	58885	2.3477	0.0022161
DOMAIN_19883	Zalophus californianus	58886	3.5504	3.42E-04
DOMAIN_19886	Panthera pardus	58887	2.8642	4.04E-05
DOMAIN_19889	Vicugna pacos	58888	3.1963	4.15E-05
DOMAIN_19891	Zalophus californianus	58889	3.2135	0.0010023
DOMAIN_19921	Callorhinus ursinus	58890	2.0083	0.0055679
DOMAIN_19944	Zalophus californianus	58891	3.8559	8.71E-05
DOMAIN_19947	Bonobo	58892	2.2608	0.00818
DOMAIN_19967	Tursiops truncatus	58893	2.9548	0.0027997
DOMAIN_19968	Tursiops truncatus	58894	2.8089	0.004093
DOMAIN_19990	Panthera pardus	58895	3.5329	0.0018768
DOMAIN_19993	Tursiops truncatus	58896	3.4227	0.0047476
DOMAIN_20012	Leptonychotes weddellii	58897	3.8253	3.41E-05
DOMAIN_20023	Physeter macrocephalus	58898	3.6893	5.78E-04
DOMAIN_20025	Carlito syrichta	58899	2.2451	0.002157
DOMAIN_20030	Tursiops truncatus	58900	4.1273	3.22E-06
DOMAIN_20089	Panthera pardus	58901	4.2275	8.99E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_20095	Phascolarctos cinereus	58902	3.7141	1.55E-05
DOMAIN_20115	Physeter macrocephalus	58903	3.1154	0.0030089
DOMAIN_20134	Acinonyx jubatus	58904	3.2457	3.20E-04
DOMAIN_20136	Sus scrofa	58905	3.3856	2.94E-04
DOMAIN_20147	Odocoileus virginianus texanus	58906	3.7467	1.53E-07
DOMAIN_20171	Trichechus manatus latirostris	58907	3.951	1.03E-05
DOMAIN_20208	Pteropus vampyrus	58908	2.4805	0.0041634
DOMAIN_20249	Vicugna pacos	58909	2.7041	0.0043741
DOMAIN_20250	Phascolarctos cinereus	58910	3.5525	1.37E-04
DOMAIN_20287	Cercocebus atys	58911	3.4486	5.29E-04
DOMAIN_20318	Callithrix jacchus	58912	3.5311	3.52E-06
DOMAIN_20332	Callithrix jacchus	58913	3.2855	0.0011689
DOMAIN_20336	Panthera pardus	58914	2.3293	0.0076785
DOMAIN_20345	Cebus imitator	58915	3.8132	1.53E-07
DOMAIN_20352	Vicugna pacos	58916	2.9839	9.79E-04
DOMAIN_20359	Pteropus vampyrus	58917	3.9594	4.06E-05
DOMAIN_20371	Ursus arctos horribilis	58918	2.8418	0.0061393
DOMAIN_20381	Saimiri boliviensis boliviensis	58919	2.0412	0.0013486
DOMAIN_20398	Physeter macrocephalus	58920	3.1266	0.0039215
DOMAIN_20436	Sus scrofa	58921	2.724	0.0058616
DOMAIN_20455	Nomascus leucogenys	58922	3.112	2.94E-04
DOMAIN_20462	Trichechus manatus latirostris	58923	5.4429	1.53E-07
DOMAIN_20469	Equus caballus	58924	2.7506	0.0077201
DOMAIN_20487	Mandrillus leucophaeus	58925	2.8325	0.0020982
DOMAIN_20524	Nomascus leucogenys	58926	3.2893	0.0024993
DOMAIN_20537	Chlorocebus sabaeus	58927	3.2762	0.0027249
DOMAIN_20540	Mandrillus leucophaeus	58928	2.8477	0.0021931
DOMAIN_20545	Sus scrofa	58929	2.711	0.0086718
DOMAIN_20561	Chrysochloris asiatica	58930	3.8309	3.52E-05
DOMAIN_20565	Suricata suricatta	58931	3.148	2.90E-04
DOMAIN_20601	Sus scrofa	58932	2.9097	0.0037911

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_20652	Neophocaena asiaeorientalis asiaeorientalis	58933	2.7283	0.0038931
DOMAIN_20667	Suricata suricatta	58934	3.7485	1.38E-06
DOMAIN_20674	Mandrillus leucophaeus	58935	3.3115	1.53E-07
DOMAIN_20716	Suricata suricatta	58936	3.6174	3.02E-05
DOMAIN_20729	Mandrillus leucophaeus	58937	2.5535	0.0090894
DOMAIN_20746	Chrysochloris asiatica	58938	3.4727	4.79E-04
DOMAIN_20767	Sus scrofa	58939	3.1224	3.16E-04
DOMAIN_20835	Suricata suricatta	58940	3.0025	0.0031432
DOMAIN_20915	Mandrillus leucophaeus	58941	2.4373	0.0054586
DOMAIN_20998	Bonobo	58942	2.6659	0.0044767
DOMAIN_21010	Equus caballus	58943	2.2253	0.0040982
DOMAIN_21023	Sarcophilus harrisii	58944	3.1196	0.0023342
DOMAIN_21067	Zalophus californianus	58945	3.0246	0.0010917
DOMAIN_21082	Loxodonta africana	58946	3.2032	0.0040056
DOMAIN_21086	Pteropus vampyrus	58947	2.1339	0.0079029
DOMAIN_21095	Trichechus manatus latirostris	58948	2.5003	0.0091721
DOMAIN_21110	Neovison vison	58949	2.499	0.0065113
DOMAIN_21123	Callorhinus ursinus	58950	3.237	4.13E-04
DOMAIN_21133	Suricata suricatta	58951	3.1021	4.18E-04
DOMAIN_21161	Sarcophilus harrisii	58952	3.2208	5.87E-04
DOMAIN_21162	Sarcophilus harrisii	58953	2.885	6.85E-04
DOMAIN_21175	Callorhinus ursinus	58954	3.3334	2.29E-04
DOMAIN_21197	Tursiops truncatus	58955	2.214	0.0073288
DOMAIN_21226	Sarcophilus harrisii	58956	2.6942	0.0033484
DOMAIN_21260	Pteropus vampyrus	58957	3.1806	0.0039855
DOMAIN_21276	Mandrillus leucophaeus	58958	3.0178	0.0029699
DOMAIN_21277	OwlMonkey	58959	2.7115	0.0075352
DOMAIN_21312	Lipotes vexillifer	58960	3.5287	4.75E-06
DOMAIN_21333	Zalophus californianus	58961	3.5801	3.57E-05
DOMAIN_21334	Equus caballus	58962	2.9508	8.67E-04
DOMAIN_21335	Equus caballus	58963	2.518	0.0034809
DOMAIN_21367	Equus caballus	58964	2.9921	0.0091001

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_21369	Equus caballus	58965	2.7947	0.0011824
DOMAIN_21371	Physeter macrocephalus	58966	3.8804	4.44E-06
DOMAIN_21421	Pteropus vampyrus	58967	2.7713	7.52E-05
DOMAIN_21481	Bonobo	58968	2.7056	0.0012415
DOMAIN_21494	Tursiops truncatus	58969	3.783	1.36E-04
DOMAIN_21583	Sarcophilus harrisii	58970	3.1529	0.0026931
DOMAIN_21588	Callorhinus ursinus	58971	3.4914	5.39E-04
DOMAIN_21612	OwlMonkey	58972	3.2931	4.09E-05
DOMAIN_21626	Monodelphis domestica	58973	3.5419	1.57E-04
DOMAIN_21632	Monodelphis domestica	58974	2.6551	0.0071923
DOMAIN_21658	Monodelphis domestica	58975	3.1325	2.50E-04
DOMAIN_21786	Trichechus manatus latirostris	58976	3.2249	2.76E-04
DOMAIN_21822	Equus caballus	58977	3.5647	3.22E-06
DOMAIN_21823	Equus caballus	58978	3.2474	0.0072446
DOMAIN_21844	OwlMonkey	58979	3.467	4.44E-06
DOMAIN_21862	Chlorocebus sabaeus	58980	2.3797	0.0032299
DOMAIN_21889	Equus caballus	58981	3.6563	4.18E-04
DOMAIN_21896	Lipotes vexillifer	58982	2.8718	0.0093653
DOMAIN_21900	Equus caballus	58983	2.7606	0.0041711
DOMAIN_21909	Suricata suricatta	58984	3.2301	3.40E-04
DOMAIN_21928	Callorhinus ursinus	58985	3.758	1.67E-05
DOMAIN_21947	Trichechus manatus latirostris	58986	3.1204	0.003623
DOMAIN_21951	Equus caballus	58987	2.8972	3.24E-04
DOMAIN_21985	Suricata suricatta	58988	3.6273	1.99E-06
DOMAIN_21988	Sarcophilus harrisii	58989	3.3393	0.0011817
DOMAIN_21993	Lipotes vexillifer	58990	2.5494	0.0039206
DOMAIN_22022	Tursiops truncatus	58991	3.9558	4.44E-06
DOMAIN_22079	Trichechus manatus latirostris	58992	3.4511	6.43E-04
DOMAIN_22117	Sarcophilus harrisii	58993	2.5969	0.0040801
DOMAIN_22143	Pteropus vampyrus	58994	2.6595	9.36E-04
DOMAIN_22151	Trichechus manatus latirostris	58995	3.1615	5.26E-04

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_22158	Lipotes vexillifer	58996	2.0562	0.0010562
DOMAIN_22166	Trichechus manatus latirostris	58997	4.2024	2.53E-05
DOMAIN_22192	Trichechus manatus latirostris	58998	2.8134	0.0083622
DOMAIN_22220	Bonobo	58999	2.8922	0.0013379
DOMAIN_22268	Lipotes vexillifer	59000	2.6534	0.0053876
DOMAIN_22278	Pteropus vampyrus	59001	3.3575	0.0037798
DOMAIN_22280	Pteropus vampyrus	59002	3.1521	0.0017347
DOMAIN_22285	Trichechus manatus latirostris	59003	3.0261	6.83E-04
DOMAIN_22297	Sarcophilus harrisii	59004	2.4261	0.0066953
DOMAIN_22311	Monodelphis domestica	59005	2.9903	0.0017115
DOMAIN_22322	Tursiops truncatus	59006	3.4452	3.85E-04
DOMAIN_22366	OwlMonkey	59007	4.848	3.06E-07
DOMAIN_22375	Tursiops truncatus	59008	2.5484	0.0090894
DOMAIN_22381	Tursiops truncatus	59009	3.8641	2.63E-04
DOMAIN_22383	Pteropus vampyrus	59010	3.4752	2.48E-04
DOMAIN_22407	OwlMonkey	59011	2.5308	0.0081831
DOMAIN_22425	OwlMonkey	59012	3.0333	0.0032208
DOMAIN_22430	Callorhinus ursinus	59013	2.982	0.0064761
DOMAIN_22454	Monodelphis domestica	59014	2.6042	0.0022491
DOMAIN_22458	Monodelphis domestica	59015	3.0003	0.0025373
DOMAIN_22459	Monodelphis domestica	59016	2.9261	0.0013171
DOMAIN_22462	Monodelphis domestica	59017	3.5597	2.34E-05
DOMAIN_22471	Papio anubis	59018	3.6293	1.68E-06
DOMAIN_22479	OwlMonkey	59019	3.9668	4.18E-05
DOMAIN_22483	OwlMonkey	59020	2.1702	0.0013107
DOMAIN_22495	Callorhinus ursinus	59021	2.2623	0.0043918
DOMAIN_22512	OwlMonkey	59022	2.93	0.003255
DOMAIN_22518	Lipotes vexillifer	59023	2.8869	0.0024472
DOMAIN_22520	Callorhinus ursinus	59024	3.3586	2.83E-05
DOMAIN_22527	Tursiops truncatus	59025	2.989	9.71E-04
DOMAIN_22566	Papio anubis	59026	3.5278	6.63E-05
DOMAIN_22586	Nomascus leucogenys	59027	2.1811	0.0021723

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_22615	Homo sapiens	59028	3.0957	4.43E-04
DOMAIN_22654	Ursus arctos horribilis	59029	3.248	5.59E-05
DOMAIN_22667	Saimiri boliviensis boliviensis	59030	3.4947	0.0037256
DOMAIN_22669	Balaenoptera acutorostrata scammoni	59031	3.583	4.34E-04
DOMAIN_22692	Propithecus coquereli	59032	3.2791	3.52E-04
DOMAIN_22710	Propithecus coquereli	59033	3.4387	0.0032081
DOMAIN_22740	Panthera pardus	59034	2.692	0.0027611
DOMAIN_22742	Panthera pardus	59035	2.9133	0.0027938
DOMAIN_22768	Ursus maritimus	59036	4.0609	7.81E-06
DOMAIN_22771	Ursus americanus	59037	3.3498	2.83E-05
DOMAIN_22776	Propithecus coquereli	59038	2.7757	2.88E-04
DOMAIN_22778	Saimiri boliviensis boliviensis	59039	3.1251	4.93E-04
DOMAIN_22782	Vombatus ursinus	59040	3.1663	4.24E-04
DOMAIN_22917	Cervus elaphus hippelaphus	59041	3.8061	2.77E-05
DOMAIN_22919	Colobus angolensis palliatus	59042	2.8609	0.003796
DOMAIN_22928	Tupaia chinensis	59043	3.0141	0.0015348
DOMAIN_22937	Ursus arctos horribilis	59044	3.0779	0.0032951
DOMAIN_22939	Muntiacus reevesi	59045	3.6187	1.78E-04
DOMAIN_22944	Muntiacus reevesi	59046	3.3908	5.28E-04
DOMAIN_23007	Lynx pardinus	59047	3.7329	1.09E-04
DOMAIN_23009	Saimiri boliviensis boliviensis	59048	3.1269	0.0062706
DOMAIN_23011	Cervus elaphus hippelaphus	59049	3.6236	3.51E-05
DOMAIN_23012	Cervus elaphus hippelaphus	59050	3.6131	2.50E-04
DOMAIN_23013	Cervus elaphus hippelaphus	59051	3.4615	4.85E-04
DOMAIN_23018	Colobus angolensis palliatus	59052	3.4177	2.30E-04
DOMAIN_23039	Saimiri boliviensis boliviensis	59053	2.8829	5.70E-04
DOMAIN_23040	Saimiri boliviensis boliviensis	59054	2.5742	0.0056531
DOMAIN_23041	Vombatus ursinus	59055	3.6194	1.92E-04
DOMAIN_23050	Balaenoptera acutorostrata scammoni	59056	2.9754	0.003318

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_23082	Mustela putorius furo	59057	3.9481	5.17E-05
DOMAIN_23093	Propithecus coquereli	59058	3.2165	5.48E-04
DOMAIN_23109	Mustela putorius furo	59059	2.9639	0.0019589
DOMAIN_23113	Camelus ferus	59060	3.4612	3.52E-04
DOMAIN_23136	Vicugna pacos	59061	3.285	2.16E-04
DOMAIN_23181	Colobus angolensis palliatus	59062	2.7665	0.0021609
DOMAIN_23196	Odobenus rosmarus divergens	59063	4.3363	3.22E-06
DOMAIN_23200	Ursus americanus	59064	3.755	1.84E-06
DOMAIN_23215	Vombatus ursinus	59065	3.0212	0.0035725
DOMAIN_23217	Vombatus ursinus	59066	4.1674	2.76E-06
DOMAIN_23239	Vicugna pacos	59067	3.0945	0.0090937
DOMAIN_23250	Delphinapterus leucas	59068	2.71	3.14E-04
DOMAIN_23260	Tupaia chinensis	59069	2.7567	0.0029622
DOMAIN_23281	Colobus angolensis palliatus	59070	2.5048	0.0036625
DOMAIN_23286	Mustela putorius furo	59071	3.3651	1.66E-04
DOMAIN_23301	Gulo gulo	59072	2.6839	0.0035226
DOMAIN_23323	Erinaceus europaeus	59073	3.2619	0.0031362
DOMAIN_23331	Carlito syrichta	59074	2.8995	5.23E-04
DOMAIN_23336	Carlito syrichta	59075	2.239	0.0065533
DOMAIN_23341	Carlito syrichta	59076	2.656	0.0058992
DOMAIN_23375	Vicugna pacos	59077	3.266	7.64E-04
DOMAIN_23378	Odobenus rosmarus divergens	59078	3.0623	0.0016508
DOMAIN_23419	Gulo gulo	59079	3.5213	7.41E-04
DOMAIN_23453	Carlito syrichta	59080	2.2331	0.006161
DOMAIN_23454	Carlito syrichta	59081	3.0632	7.96E-04
DOMAIN_23458	Vicugna pacos	59082	2.4232	0.0045857
DOMAIN_23480	Odobenus rosmarus divergens	59083	3.4432	3.38E-04
DOMAIN_23494	Mustela putorius furo	59084	3.847	5.05E-06
DOMAIN_23508	Mustela putorius furo	59085	2.3582	0.0047712
DOMAIN_23513	Tupaia chinensis	59086	3.2927	5.31E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_23514	Odobenus rosmarus divergens	59087	3.0166	4.77E-04
DOMAIN_23561	Colobus angolensis palliatus	59088	3.2392	0.0021906
DOMAIN_23574	Gulo gulo	59089	2.939	0.0083249
DOMAIN_23575	Erinaceus europaeus	59090	3.4624	0.001589
DOMAIN_23576	Erinaceus europaeus	59091	3.8014	2.89E-05
DOMAIN_23590	Odobenus rosmarus divergens	59092	2.8653	0.0052881
DOMAIN_23604	Vicugna pacos	59093	2.6984	0.0046123
DOMAIN_23641	Carlito syrichta	59094	2.6942	0.0081075
DOMAIN_23642	Delphinapterus leucas	59095	3.8829	2.28E-04
DOMAIN_23654	Carlito syrichta	59096	2.337	0.0083622
DOMAIN_23679	Tupaia chinensis	59097	3.7951	5.10E-05
DOMAIN_23680	Vicugna pacos	59098	2.712	0.0034785
DOMAIN_23709	Carlito syrichta	59099	4.545	1.53E-07
DOMAIN_23711	Gulo gulo	59100	2.658	0.0016432
DOMAIN_23721	Carlito syrichta	59101	2.972	0.0022972
DOMAIN_23731	Colobus angolensis palliatus	59102	3.1609	2.35E-04
DOMAIN_23745	Myotis brandtii	59103	3.4544	3.54E-04
DOMAIN_23793	Odobenus rosmarus divergens	59104	2.7573	0.0081197
DOMAIN_23804	Colobus angolensis palliatus	59105	2.3403	0.0086366
DOMAIN_23827	Odobenus rosmarus divergens	59106	2.3013	0.009767
DOMAIN_23854	Gulo gulo	59107	3.838	7.18E-05
DOMAIN_23856	Erinaceus europaeus	59108	3.1072	0.0035694
DOMAIN_23863	Mustela putorius furo	59109	2.8758	0.0085493
DOMAIN_23885	Colobus angolensis palliatus	59110	3.033	0.0034316
DOMAIN_23895	Mustela putorius furo	59111	2.6148	0.003318
DOMAIN_23898	Mustela putorius furo	59112	2.7383	0.0035921
DOMAIN_23916	Odobenus rosmarus divergens	59113	3.3232	1.63E-04
DOMAIN_23931	Gulo gulo	59114	3.8077	1.49E-05
DOMAIN_23940	Homo sapiens	59115	2.5087	0.0010424

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_23953	Muntiacus reevesi	59116	2.4156	0.0075055
DOMAIN_23979	Balaenoptera acutorostrata scammoni	59117	4.0461	5.77E-05
DOMAIN_24020	Rhinolophus ferrumequinum	59118	3.1125	1.66E-04
DOMAIN_24028	Ursus arctos horribilis	59119	3.8797	1.53E-07
DOMAIN_24035	Propithecus coquereli	59120	3.2225	0.0017975
DOMAIN_24042	Propithecus coquereli	59121	3.3038	4.75E-06
DOMAIN_24083	Myotis brandtii	59122	3.9804	2.77E-05
DOMAIN_24113	Propithecus coquereli	59123	3.3264	2.89E-04
DOMAIN_24152	Vombatus ursinus	59124	3.3664	0.0022672
DOMAIN_24204	Propithecus coquereli	59125	3.0779	4.60E-04
DOMAIN_24212	Pteropus alecto	59126	2.498	0.0034998
DOMAIN_24230	Muntiacus reevesi	59127	3.1832	1.53E-07
DOMAIN_24256	Ursus arctos horribilis	59128	2.7933	0.0018808
DOMAIN_24282	Muntiacus reevesi	59129	2.694	0.0052575
DOMAIN_24306	Propithecus coquereli	59130	3.2084	0.0023952
DOMAIN_24317	Myotis brandtii	59131	3.9767	3.17E-05
DOMAIN_24379	Macaca nemestrina	59132	2.4643	0.0086804
DOMAIN_24393	Propithecus coquereli	59133	3.8008	2.45E-06
DOMAIN_24446	Propithecus coquereli	59134	3.6312	7.27E-05
DOMAIN_24463	Balaenoptera acutorostrata scammoni	59135	2.5362	0.007147
DOMAIN_24496	Ursus americanus	59136	3.6403	4.24E-04
DOMAIN_24515	Balaenoptera acutorostrata scammoni	59137	3.7358	5.28E-05
DOMAIN_24518	Balaenoptera acutorostrata scammoni	59138	3.4135	3.05E-05
DOMAIN_24546	Ursus americanus	59139	3.4262	8.42E-06
DOMAIN_24570	Saimiri boliviensis boliviensis	59140	3.6773	1.45E-05
DOMAIN_24571	Balaenoptera acutorostrata scammoni	59141	2.6912	0.0038376
DOMAIN_24600	Ursus americanus	59142	3.156	0.0012483
DOMAIN_24614	Cervus elaphus hippelaphus	59143	2.6295	0.0046463
DOMAIN_24615	Colobus angolensis palliatus	59144	2.4075	0.0069247

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_24653	Cervus elaphus hippelaphus	59145	2.9883	0.0083016
DOMAIN_24677	Lynx pardinus	59146	2.3115	0.0094713
DOMAIN_24719	Muntiacus reevesi	59147	2.7499	0.005142
DOMAIN_24725	Ursus arctos horribilis	59148	3.4496	4.09E-05
DOMAIN_24771	Myotis brandtii	59149	3.3701	0.0025351
DOMAIN_24786	Vombatus ursinus	59150	2.9237	0.0078001
DOMAIN_24788	Vombatus ursinus	59151	2.7694	0.0021557
DOMAIN_24838	Pteropus alecto	59152	2.3323	0.0042954
DOMAIN_24867	Nomascus leucogenys	59153	3.469	2.97E-04
DOMAIN_24903	Ailuropoda melanoleuca	59154	3.0377	0.0030054
DOMAIN_24939	Phascolarctos cinereus	59155	3.3066	6.08E-04
DOMAIN_24947	Ursus maritimus	59156	2.9491	0.0055208
DOMAIN_24975	Muntiacus muntjak	59157	3.2737	0.0069767
DOMAIN_24993	Oryctolagus cuniculus	59158	3.3817	5.00E-04
DOMAIN_25016	Oryctolagus cuniculus	59159	2.9822	0.0034776
DOMAIN_25052	Pteropus alecto	59160	2.3634	0.0072024
DOMAIN_25060	Ailuropoda melanoleuca	59161	3.6002	4.82E-04
DOMAIN_25063	Phascolarctos cinereus	59162	2.9436	0.0042752
DOMAIN_25070	Sapajus apella	59163	2.9649	0.0043634
DOMAIN_25091	Phascolarctos cinereus	59164	2.9006	0.0039332
DOMAIN_25094	Phascolarctos cinereus	59165	3.0413	0.0026876
DOMAIN_25106	Canis lupus familiaris	59166	2.8622	0.0075508
DOMAIN_25126	Puma concolor	59167	2.1478	0.005514
DOMAIN_25128	Sapajus apella	59168	2.588	0.0029475
DOMAIN_25131	Sapajus apella	59169	2.592	0.0051895
DOMAIN_25146	Macaca nemestrina	59170	3.629	1.68E-06
DOMAIN_25150	Muntiacus reevesi	59171	3.147	0.0018391
DOMAIN_25157	Myotis brandtii	59172	3.0902	0.0012442
DOMAIN_25194	Macaca nemestrina	59173	2.4613	0.003597
DOMAIN_25204	Panthera pardus	59174	2.7595	0.0027917
DOMAIN_25234	Saimiri boliviensis boliviensis	59175	2.743	0.0042296
DOMAIN_25235	Oryctolagus cuniculus	59176	3.6965	1.76E-05
DOMAIN_25334	Phascolarctos cinereus	59177	2.7501	0.0096299

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_25384	Rhinolophus ferrumequinum	59178	3.5139	8.10E-05
DOMAIN_25389	Ursus maritimus	59179	3.0814	6.54E-04
DOMAIN_25400	Lynx canadensis	59180	2.2285	3.10E-04
DOMAIN_25410	Puma concolor	59181	2.8699	0.0022843
DOMAIN_25443	Muntiacus reevesi	59182	3.2531	0.0016615
DOMAIN_25534	Ursus maritimus	59183	2.2698	0.0054246
DOMAIN_25554	Panthera pardus	59184	3.0101	0.003898
DOMAIN_25564	Muntiacus reevesi	59185	3.4378	6.04E-04
DOMAIN_25565	Muntiacus reevesi	59186	2.6133	0.0011572
DOMAIN_25623	Ursus maritimus	59187	3.4886	2.91E-06
DOMAIN_25628	Rhinopithecus bieti	59188	2.8332	0.0022213
DOMAIN_25649	Ursus arctos horribilis	59189	3.6884	5.62E-05
DOMAIN_25654	Pteropus alecto	59190	2.2996	0.0031144
DOMAIN_25671	Muntiacus reevesi	59191	3.5244	1.53E-07
DOMAIN_25682	Rhinopithecus bieti	59192	2.5621	0.002108
DOMAIN_25686	Panthera pardus	59193	2.8635	0.0031882
DOMAIN_25726	Pteropus alecto	59194	2.8203	0.0039506
DOMAIN_25741	Sapajus apella	59195	3.7244	1.32E-04
DOMAIN_25780	Rhinopithecus bieti	59196	2.8383	0.0018385
DOMAIN_25807	Puma concolor	59197	3.6511	0.0018679
DOMAIN_25842	Rhinolophus ferrumequinum	59198	3.0942	2.44E-04
DOMAIN_25844	Ursus maritimus	59199	2.5635	0.0037997
DOMAIN_25857	Balaenoptera acutorostrata scammoni	59200	2.898	0.0026959
DOMAIN_25865	Vombatus ursinus	59201	3.1027	0.0066133
DOMAIN_25869	Vombatus ursinus	59202	2.3538	0.006932
DOMAIN_25972	Geotrypetes seraphini	59203	3.2178	0.0036689
DOMAIN_25973	Geotrypetes seraphini	59204	2.7804	0.001766
DOMAIN_25996	Geotrypetes seraphini	59205	3.984	1.24E-05
DOMAIN_26010	Geotrypetes seraphini	59206	2.1911	0.008383
DOMAIN_26012	Geotrypetes seraphini	59207	2.3532	9.70E-04
DOMAIN_26044	Geotrypetes seraphini	59208	2.8874	0.0068616
DOMAIN_26103	Geotrypetes seraphini	59209	2.5308	0.0033422

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_26127	Geotrypetes seraphini	59210	2.5183	0.00586
DOMAIN_26131	Geotrypetes seraphini	59211	2.4087	0.0068533
DOMAIN_26134	Geotrypetes seraphini	59212	2.4433	0.0072939
DOMAIN_26163	Geotrypetes seraphini	59213	2.4527	0.0041806
DOMAIN_26177	Geotrypetes seraphini	59214	3.4467	1.27E-05
DOMAIN_26180	Geotrypetes seraphini	59215	3.4522	1.35E-04
DOMAIN_26194	Geotrypetes seraphini	59216	2.8857	0.0031518
DOMAIN_26211	Pelodiscus sinensis	59217	2.6058	0.0064871
DOMAIN_26233	Colinus virginianus	59218	3.6739	1.77E-04
DOMAIN_26236	Pelodiscus sinensis	59219	2.7094	0.003991
DOMAIN_26265	Geotrypetes seraphini	59220	2.5922	3.31E-04
DOMAIN_26268	Geotrypetes seraphini	59221	2.1404	0.0020397
DOMAIN_26292	Geotrypetes seraphini	59222	2.4722	0.0074388
DOMAIN_26299	Geotrypetes seraphini	59223	2.3704	0.0058481
DOMAIN_26305	Geotrypetes seraphini	59224	3.0107	0.0084216
DOMAIN_26306	Geotrypetes seraphini	59225	2.6178	0.0051922
DOMAIN_26335	Colinus virginianus	59226	4.0965	3.41E-04
DOMAIN_26340	Pelodiscus sinensis	59227	3.1704	0.003352
DOMAIN_26353	Pelodiscus sinensis	59228	3.5785	1.16E-04
DOMAIN_26373	Pseudonaja textilis	59229	3.3204	5.13E-04
DOMAIN_26407	Colinus virginianus	59230	2.9778	0.0049206
DOMAIN_26414	Pelodiscus sinensis	59231	2.9544	0.0089308
DOMAIN_26415	Pelodiscus sinensis	59232	2.5032	0.0035489
DOMAIN_26416	Pelodiscus sinensis	59233	3.6321	4.36E-05
DOMAIN_26417	Pelodiscus sinensis	59234	4.1057	4.46E-05
DOMAIN_26423	Pelodiscus sinensis	59235	3.0169	0.0025697
DOMAIN_26430	Pelodiscus sinensis	59236	2.6946	0.0051824
DOMAIN_26439	Pelodiscus sinensis	59237	3.2468	0.0010568
DOMAIN_26463	Pelodiscus sinensis	59238	2.8812	0.003427
DOMAIN_26469	Pelodiscus sinensis	59239	3.021	5.08E-04
DOMAIN_26496	Geotrypetes seraphini	59240	2.7991	0.0040994
DOMAIN_26501	Geotrypetes seraphini	59241	2.6513	0.0041882
DOMAIN_26518	Geotrypetes seraphini	59242	2.397	0.0087878
DOMAIN_26577	Geotrypetes seraphini	59243	2.4722	0.0035247

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_26634	Gopherus agassizii	59244	2.8182	0.0079972
DOMAIN_26636	Gopherus agassizii	59245	2.6934	0.0090052
DOMAIN_26660	Phasianus colchicus	59246	3.201	4.90E-04
DOMAIN_26679	Paroedura picta	59247	2.6033	0.001326
DOMAIN_26780	Meleagris gallopavo	59248	3.1696	0.0031591
DOMAIN_26783	Meleagris gallopavo	59249	3.2848	0.0020241
DOMAIN_26795	Meleagris gallopavo	59250	3.3538	0.001228
DOMAIN_26800	Meleagris gallopavo	59251	3.8197	1.62E-04
DOMAIN_26803	Aquila chrysaetos chrysaetos	59252	3.4265	0.001246
DOMAIN_26852	Mus musculus	59253	2.8783	0.0025253
DOMAIN_26853	Mus musculus	59254	3.6235	7.59E-04
DOMAIN_26886	Homo sapiens	59255	3.3209	0.0016312
DOMAIN_26925	Alligator sinensis	59256	3.2248	0.0036928
DOMAIN_26999	Xenopus laevis	59257	3.4317	4.75E-06
DOMAIN_27032	Alligator mississippiensis	59258	3.4805	0.0019423
DOMAIN_27285	Peromyscus maniculatus bairdii	59259	3.092	5.16E-04
DOMAIN_27498	Sus scrofa	59260	2.9278	0.0029754
DOMAIN_27521	Suricata suricatta	59261	2.7447	0.0010703
DOMAIN_27563	Muntiacus muntjak	59262	3.6292	6.63E-05
DOMAIN_27566	Muntiacus muntjak	59263	2.7825	0.0020795
DOMAIN_27579	Muntiacus muntjak	59264	3.8878	7.50E-06
DOMAIN_27581	Canis lupus familiaris	59265	2.4582	0.0090172
DOMAIN_27639	Macaca fascicularis	59266	2.452	0.0032574
DOMAIN_27642	Puma concolor	59267	2.8615	0.0015287
DOMAIN_27690	Myotis lucifugus	59268	3.1465	0.0012118
DOMAIN_27705	Phascolarctos cinereus	59269	2.5921	0.0030483
DOMAIN_27759	Bos taurus	59270	2.2124	0.0070756
DOMAIN_27767	Callithrix jacchus	59271	2.2153	0.0023952
DOMAIN_27777	Odocoileus virginianus texanus	59272	2.6766	0.0067364
DOMAIN_27784	Ovis aries	59273	2.1631	0.0040915
DOMAIN_27809	Cebus imitator	59274	2.8715	0.0025161
DOMAIN_27827	Vulpes vulpes	59275	3.1318	2.13E-05

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_27833	Callithrix jacchus	59276	3.0164	4.27E-04
DOMAIN_27866	Orangutan	59277	2.9226	0.0029981
DOMAIN_27886	Bison bison bison	59278	2.735	0.0036356
DOMAIN_27902	Vulpes vulpes	59279	2.9068	0.0039341
DOMAIN_27988	Camelus dromedarius	59280	2.5381	0.0015476
DOMAIN_28051	Neomonachus schauinslandi	59281	2.4353	0.0018581
DOMAIN_28071	Enhydra lutris kenyoni	59282	3.2938	2.61E-04
DOMAIN_28085	Enhydra lutris kenyoni	59283	2.2962	0.0029074
DOMAIN_28103	Physeter macrocephalus	59284	2.4116	0.009594
DOMAIN_28118	OwlMonkey	59285	3.1049	0.0027807
DOMAIN_28158	Odocoileus virginianus texanus	59286	3.0762	0.0016156
DOMAIN_28164	Callithrix jacchus	59287	2.7356	0.0064115
DOMAIN_28299	Capra hircus	59288	3.5584	6.41E-05
DOMAIN_28309	Pteropus vampyrus	59289	3.5338	3.28E-04
DOMAIN_28335	Bonobo	59290	3.3013	2.50E-04
DOMAIN_28341	Homo sapiens	59291	2.7008	5.14E-04
DOMAIN_28417	Gulo gulo	59292	2.5366	5.02E-04
DOMAIN_28421	Erinaceus europaeus	59293	3.0763	0.0038713
DOMAIN_28507	Muntiacus reevesi	59294	3.2874	8.76E-04
DOMAIN_28513	Propithecus coquereli	59295	2.3747	0.0050076
DOMAIN_28533	Propithecus coquereli	59296	2.7575	0.0031303
DOMAIN_28588	Rhinolophus ferrumequinum	59297	2.6131	0.0030648
DOMAIN_28619	Rhinolophus ferrumequinum	59298	2.6504	0.0027237
DOMAIN_28823	Microcaecilia unicolor	59299	2.331	0.0078575
DOMAIN_28845	Camelus ferus	59300	3.0175	0.0017733
DOMAIN_28929	Mus musculus	59301	3.1025	6.70E-04
DOMAIN_29066	Xenopus tropicalis	59302	2.6393	3.67E-04
DOMAIN_29164	Chelonia mydas	59303	2.1345	0.0029635
DOMAIN_29260	Peromyscus maniculatus bairdii	59304	2.5127	0.0074146
DOMAIN_29339	Mesocricetus auratus	59305	2.9581	0.0028165
DOMAIN_29377	Mesocricetus auratus	59306	2.672	0.0070692

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_29426	Mus caroli	59307	2.0491	6.64E-04
DOMAIN_29434	Mus caroli	59308	2.2707	0.005184
DOMAIN_29467	Mus caroli	59309	3.4689	5.79E-05
DOMAIN_29471	Cricetulus griseus	59310	3.1911	4.18E-05
DOMAIN_29511	Peromyscus maniculatus bairdii	59311	3.4739	7.00E-05
DOMAIN_29614	Peromyscus maniculatus bairdii	59312	3.4528	1.82E-04
DOMAIN_29616	Mesocricetus auratus	59313	2.2807	0.0035376
DOMAIN_29765	Erinaceus europaeus	59314	3.3088	9.79E-04
DOMAIN_29900	Nomascus leucogenys	59315	2.1583	0.0098463
DOMAIN_30185	Rhinopithecus roxellana	59316	3.0766	5.83E-05
DOMAIN_30211	Bison bison bison	59317	2.3322	0.0023122
DOMAIN_30236	Callithrix jacchus	59318	2.7293	0.0021744
DOMAIN_30329	Rhesus	59319	2.1216	0.0099018
DOMAIN_30783	Chimp	59320	2.952	0.001698
DOMAIN_31235	Vicugna pacos	59321	2.2828	0.0067045
DOMAIN_31340	Homo sapiens	59322	2.8261	0.0021028
DOMAIN_31383	Propithecus coquereli	59323	2.1919	0.0087058
DOMAIN_31638	Balaenoptera acutorostrata scammoni	59324	2.0254	0.0036297
DOMAIN_31798	Notechis scutatus	59325	4.8007	7.82E-04
DOMAIN_31935	Rhinolophus ferrumequinum	59326	3.5544	0.0084786
DOMAIN_32127	Human	59327	3.7547	2.62E-05
DOMAIN_32145	Human	59328	3.1866	1.67E-05
DOMAIN_32146	Human	59329	2.7628	0.0016129
DOMAIN_32159	Human	59330	2.7874	0.0021753
DOMAIN_32215	Human	59331	3.2653	0.001461
DOMAIN_32223	Human	59332	2.8836	0.0068873
DOMAIN_32255	Human	59333	3.8237	1.39E-05
DOMAIN_32279	Human	59334	2.4917	0.0060199
DOMAIN_32286	Human	59335	2.8921	0.0070992
DOMAIN_32312	Human	59336	2.9151	0.0030308
DOMAIN_32321	Human	59337	3.0441	0.0040854

Domain ID	Species	SEQ ID NO	Log2 (fold change)	P-value
DOMAIN_32327	Human	59338	3.1024	0.0044212
DOMAIN_32334	Human	59339	2.8117	0.0015241
DOMAIN_32351	Human	59340	2.0727	0.0036362
DOMAIN_32386	Human	59341	3.5521	3.87E-04
DOMAIN_32390	Human	59342	3.757	4.30E-05

[0526] The KRAB domain with the highest $\log_2$(fold change) was derived from the king cobra, *Ophiophagus hannah* (DOMAIN_26749; SEQ ID NO: 57755). Surprisingly, this sequence was highly divergent from human KRAB domains (with only 41% sequence identity) and was grouped in a sequence cluster of poor repressor domains.

[0527] To verify that the KRAB domains identified in the selection supported transcriptional repression in an independent assay, representative members of the top 95 and 1597 KRAB domains were used to generate dXR constructs, and their ability to repress transcription of the *B2M* locus was tested. As shown in FIG. 17, seven days after transduction, dXRs with all but one of the representative top 95 or 1597 KRAB domains tested repressed *B2M* to a greater extent than did the dXR with ZNF10. As shown in FIG. 18, ten days after transduction, the majority of the dXRs with representative top 95 or 1597 KRAB domains tested repressed *B2M* to a greater extent than did ZNF10 or ZIM3. dXR repression of a target locus tends to deteriorate over time, and ten days following transduction is believed to be a relatively late timepoint for measuring dXR repression. Therefore, it is particularly notable that many of the dXR constructs with KRAB domains in the top 95 and 1597 were able to repress *B2M* to a greater extent than dXR with KRAB domains derived from ZNF10 or ZIM3 as late as ten days following transduction.

[0528] To further understand the basis of the superior ability of the identified KRAB domains to repress transcription, protein sequence motifs were generated for the top 1597 KRAB domains using the STREME algorithm. Specifically, five motifs (motifs 1-5) were generated by comparing the amino acid sequences of the top 1597 KRAB domains to a negative training set of 1506 KRAB domains with p-values less than 0.01, and $\log_2$(fold change) values less than 0. Logos of motifs 1-5 are provided in FIGS. 19A, 19B, 19C, 19D, and 19E. In addition, four motifs (motifs 6-9) were generated by comparing the top 1597 KRAB domains to shuffled

sequences derived from the 1597 sequences. Logos of motifs 6-9 are provided in FIGS. 19F, 19G, 19H, and 19I.

[0529] Table 20, below, provides the p-value, E-value (a measure of statistical significance), and number and percentage of sequences matching the motif in the top 1597 KRAB domains for each of the nine motifs, as calculated by STREME. Table 21 provides the sequences of each motif, showing the amino acid residues present at each position within the motifs (from N- to C-terminus).

Table 20: Characteristics of protein sequence motifs of top 1597 KRAB domains.

Motif ID	**P-value**	**E-value**	**Number and percentage of sites matching motif in top 1597 KRAB domains**
Motifs generated compared to a negative training set			
1	3.7e-014	7.1e-013	1158 (72.5%)
2	3.4e-012	6.4e-011	978 (61.2%)
3	7.5e-010	1.4e-008	1017 (63.7%)
4	7.0e-008	1.3e-006	987 (61.8%)
5	1.7e-007	3.3e-006	678 (42.5%)
Motifs generated compared to shuffled sequences			
6	1.2e-048	1.5e-047	1597 (100.0%)
7	1.2e-048	1.5e-047	1597 (100.0%)
8	1.3e-042	1.6e-041	1377 (86.2%)
9	2.1e-040	2.7e-039	1483 (92.9%)

Table 21: Sequences of protein sequence motifs of top 1597 KRAB domains.

Motif ID	**Position in motif**	**Amino acid residues with >5% representation in motif**
Motifs generated compared to a negative training set		
1	1	P
	2	A, D, E, N
	3	L, V
	4	I, V

Motif ID	**Position in motif**	**Amino acid residues with >5% representation in motif**
Motifs generated compared to shuffled sequences		
6	1	L
	2	Y
	3	K, R
	4	D, E

Motif ID	Position in motif	Amino acid residues with >5% representation in motif
	5	S, T, F
	6	H, K, L, Q, R, W
	7	L, M
	8	E
	9	G, K, Q, R
2	1	L, V
	2	A, G, L, T, V
	3	A, F, S
	4	L, V
	5	G
	6	C, F, H, I, L, Y
	7	A, C, P, Q, S
	8	A, F, G, I, S, V
	9	A, P, S, T
	10	K, R
3	1	Q
	2	K, R
	3	A, D, E, G, N, S, T
	4	L
	5	Y
	6	R
	7	D, E, S
	8	V
	9	M
	10	L, R
4	1	A, L, P, S
	2	L, V
	3	S, T
	4	F
	5	A, E, G, K, R

Motif ID	Position in motif	Amino acid residues with >5% representation in motif
	5	V
	6	M
	7	L, Q, R
	8	E
	9	N, T
	10	F, Y
	11	A, E, G, Q, R, S
	12	H, L, N
	13	L, V
	14	A, G, I, L, T, V
	15	A, F, S
7	1	F
	2	A, E, G, K, R
	3	D
	4	V
	5	A, S, T
	6	I, V
	7	D, E, N, Y
	8	F
	9	S, T
	10	E, L, P, Q, R, W
	11	D, E
	12	E
	13	W
	14	A, E, G, Q, R
8	1	K, R
	2	P
	3	A, D, E, N
	4	I, L, M, V
	5	I, V

Motif ID	Position in motif	Amino acid residues with >5% representation in motif
	6	D
	7	V
	8	A, T
	9	I, V
	10	D, E, N, Y
	11	F
	12	S, T
	13	E, P, Q, R, W
	14	E, N
	15	E, Q
5	1	E, G, R
	2	E, K
	3	A, D, E
	4	P
	5	C, W
	6	I, K, L, M, T, V
	7	I, L, P, V
	8	D, E, K, V
	9	E, G, K, P, R
	10	A, D, R, G, K, Q, V
	11	D, E, G, I, L, R, S, V

Motif ID	Position in motif	Amino acid residues with >5% representation in motif
	6	F, S, T
	7	H, K, L, Q, R, W
	8	L
	9	E
	10	K, Q, R
	11	E, G, R
	12	D, E, K
	13	A, D, E
	14	L, P
	15	C, W
9	1	C, H, L, Q, W
	2	L
	3	D, G, N, R, S
	4	L, P, S, T
	5	A, S, T
	6	Q
	7	K, R
	8	A, D, E, K, N, S, T

[0530] Notably, motifs 6 and 7 were present in 100% of the top 1597 KRAB domains. Many of the highly conserved positions in motif 6 (e.g., amino acid residues L1, Y2, V5, M6, and E8) are known to form an interface with Trim28 (also known as Kap1), which is responsible for recruiting transcriptional repressive machinery to a locus. Similarly, residues in motif 7 (D3, V4, E11, E12) all contribute to Trim28 recruitment. It is believed that many of the amino acid residues identified as enriched in the top KRAB domains strengthen Trim28 recruitment. Notably, some of these residues are lacking in commonly used KRAB domains. Specifically, in the site in ZNF10 that matches motif 6, the residue at the first position is a valine instead of a

leucine. In the site in ZIM3 that matches motif 7, the residue at position 11 is a glycine instead of a glutamic acid. Many of the other motifs described above that are not present in all KRAB domains may represent additional and novel mechanisms of repression that are specific to sequence clusters of KRABs.

[0531] Taken together, the experiments described herein have identified a suite of KRAB domains that are effective for promoting transcriptional repression in the context of a dXR molecule. These KRAB domains repressed transcription to a greater extent than ZNF10 and ZIM3. Finally, protein sequence motifs were identified that are associated with the KRAB domains that are the strongest transcriptional repressors.

Example 5: Demonstration of a catalytically-dead CasX repressor (dXR) system on repression of PTBP1 at the protein level

[0532] Experiments were performed to demonstrate that various dXR constructs can act to repress the expression of the PTBP1 (Polypyrimidine Tract Binding Protein 1) protein in primary midbrain astrocyte cultures.

Materials and Methods:

Lentiviral plasmid cloning:

[0533] Lentiviral plasmid constructs coding for a dXR molecule were built using standard molecular cloning techniques. These constructs comprised of sequences coding for catalytically-dead CasX protein 491 (dCasX491; SEQ ID NO: 18) linked to the ZNF10 KRAB domain, along with guide RNA scaffold variant 174 (SEQ ID NO: 2238) and spacers targeting the *PTBP1* locus (Table 23) or a non-targeting (NT; spacer 0.0) spacer. These spacers targeted either exon 1, 2, or 3 of the murine *PTBP1* gene. Cloned and sequence-validated constructs were midi-prepped and subjected to quality assessment prior to transfection in HEK293T cells for production of lentiviral particles, which was performed using standard methods.

XDP (a CasX delivery particle) construct cloning and production:

[0534] XDP plasmid constructs comprising sequences coding for CasX protein variant 491, guide scaffold 174, and a spacer targeting *PTBP1* were cloned following standard methods and verified through Sanger sequencing.

[0535] XDPs containing ribonucleoproteins (RNPs) of CasX protein variant 491 and gRNA using scaffold 174 and a *PTBP1*-targeting spacer were produced using either suspension-adapted or adherent HEK293T Lenti-X cells. The methods to produce XDPs are described in

WO2021113772A1, incorporated by reference in its entirety. Exemplary plasmids used to create these particles (and their configurations) are shown in FIGS. 4 and 5.

Transduction of primary midbrain mouse astrocytes and western blotting:

[0536] Primary midbrain mouse astrocytes were seeded at 150,000 cells per well in a 6-well plate format in NbAstro glial culture medium. Two days post-plating, cells were transduced with lentivirus-packaged dXR2 constructs encoding dCasX491 linked to the ZNF10 KRAB domain and guide scaffold 174 (SEQ ID NO: 2238) with spacers targeting *PTBP1* (Table 22) or a non-targeting spacer. As a positive control, cells were transduced with XDP-28.10 containing RNPs of a catalytically-active CasX 491 and guide 174 with *PTBP1*-targeting spacer 28.10) in a separate well. 11 days post-transduction cells were harvested, pelleted, and lysed with RIPA buffer containing protease inhibitor for western blotting, which was performed following standard methods. Briefly, denatured protein samples were resolved by SDS-PAGE and transferred from gel onto PVDF membrane, which was immunoblotted for the PTBP1 protein. Protein quantification based on the western blot was quantified by densitometry using the Image Lab software. The ratio of PTBP1 protein/total protein for each experimental condition was normalized dXR relative to the ratio determined for the condition using dXR with the NT spacer, and the results were shown in FIG. 6 and Table 23.

Table 22: Sequences of mouse *PTBP1*-targeting spacers tested with dXR molecules in arrayed transductions.

Spacer ID	Spacer DNA sequence	SEQ ID NO	Spacer RNA sequence	SEQ ID NO
28.5	CGCTGCGGTCTGTGGGCGTG	350	CGCUGCGGUCUGUGGGCGUG	59635
28.9	GTGTGCCATGGACGGGTAAG	351	GUGUGCCAUGGACGGGUAAG	59636
28.10	CAGCGGGGATCCGACGAGCT	352	CAGCGGGGAUCCGACGAGCU	59637
28.11	CCACGTGTGTCAGCAACGGC	353	CCACGUGUGUCAGCAACGGC	59638
28.16	ACAGCATCGTCCCAGACATA	354	ACAGCAUCGUCCCAGACAUA	59639

Results:

[0537] Of the various dXR constructs with different *PTBP1*-targeting spacers delivered via lentiviral particles, treatment with the dXR and gRNA with spacer 28.16 construct showed reduced PTBP1 protein levels, while dXR constructs with guides having spacers 28.5, 28.9, 28.10 or 28.11 did not show any change in protein levels relative to protein levels determined in

the NT spacer (dXR 0.0) condition (FIG. 6; Table 23). Specifically, use of spacer 28.16 resulted in nearly a 50% decrease in PTBP1 levels relative to the NT control (FIG. 6; Table 23). As expected, treatment with XDPs containing the catalytically-active CasX RNP showed the strongest decrease (>70%) in PTBP1 protein levels compared relative to the NT control (FIG. 6; Table 23). These data show that a dXR molecule and a guide having a *PTBP1*-targeting spacer can induce transcriptional repression, which results in decreased PTBP1 protein levels.

[0538] The results from these experiments demonstrate that dXR molecules with gRNAs targeting the *PTBP1* locus were able to transcriptionally repress the therapeutically-relevant PTBP1 target efficiently in vitro, and the assay was able to distinguish between functional and non-functional spacers in the CasX repressor system.

Table 23: Ratio of PTBP1 protein over total protein determined for each experimental condition and normalized relative to the ratio determined for the NT (dXR 0.0) condition.

Experimental condition	Ratio of PTBP1 protein / total protein
dXR 0.0	1
XDP 28.10	0.285
dXR 28.5	0.939
dXR 28.9	0.945
dXR 28.10	0.945
dXR 28.11	0.933
dXR 28.16	0.464

Example 6: Use of a catalytically-dead CasX repressor (dXR) system fused with additional domains from DNMT3A and DNMT3L to induce durable silencing of the *B2M* locus

[0539] Experiments were performed to determine whether rationally-designed epigenetic long-term CasX repressor (ELXR) molecules, with three repressor domains composed of a KRAB domain, the catalytic domain from DNMT3A and the interaction domain from DNMT3L fused to catalytically-dead CasX 491, would induce durable long-term repression of the endogenous *B2M* locus *in vitro*. In addition, multiple configurations of the ELXR molecules, which contain varying placements of the epigenetic domains relative to dCasX, were designed to assess how

their arrangement would affect the duration of silencing of the *B2M* locus, as well as the specificity of their on-target methylation activity.

Materials and Methods:

Generation of ELXR constructs and lentiviral plasmid cloning:

[0540] Lentiviral plasmid constructs coding for an ELXR molecule were built using standard molecular cloning techniques. These constructs comprised of sequences coding for catalytically-dead CasX protein 491 (dCasX491), KRAB domain from ZNF10 or ZIM3, and the catalytic domain and interaction domain from DNMT3A (D3A) and DNMT3L (D3L) respectively. Briefly, constructs were ordered as oligonucleotides and assembled by overlap extension PCR followed by isothermal assembly. The resulting plasmids (sequences of key ELXR elements listed in Table 24 and select plasmid constructs in Table 25) contained constructs positioned in varying configurations to generate an ELXR molecule. The protein sequences for the ELXR molecules are listed in Table 26, and the ELXR configurations are illustrated in FIG. 7. Sequences encoding the ELXR molecules also contained a 2x FLAG tag. Plasmids also harbored sequences encoding gRNA scaffold variant 174 having either a spacer targeting the endogenous B2M locus or a non-targeting control (spacer sequences listed in Table 27). These constructs were all cloned upstream of a P2A-puromycin element on the lentiviral plasmid. Cloned and sequence-validated constructs were midi-prepped and subjected to quality assessment prior to transfection in HEK293T cells.

Table 24: Sequences of key ELXR elements (e.g., additional domains fused to CasX) to generate ELXR variant plasmids illustrated in FIG. 7.

Key component	DNA SEQ ID NO	Protein sequence	Protein SEQ ID NO
ZNF10 KRAB domain	57610	MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIV YRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEP	57611
ZIM3 KRAB domain	57612	MNNSQGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVML ENYSNLVSVGQGETTKPDVILRLEQGKEPWLEEEEVLG SGRAEKNGDIGGQIWKPKDVKESL	57613
DNMT3A catalytic domain	57614	MNHDQEFDPPKVYPPVPAEKRKPIRVLSLFDGIATGLL VLKDLGIQVDRYIASEVCEDSITVGMVRHQGKIMYVGD VRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGLY EGTGRLFFEFYRLLHDARPKEGDDRPFFWLFENVVAMG VSDKRDISRFLESNPVMIDAKEVSAAHRARYFWGNLPG MNRPLASTVNDKLELQECLEHGRIAKFSKVRTITTRSN	57615

Key component	DNA SEQ ID NO	Protein sequence	Protein SEQ ID NO
		SIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTD VSNMSRLARQRLLGRSWSVPVIRHLFAPLKEYFACV	
DNMT3L interaction domain	57616	MGPMEIYKTVSAWKRQPVRVLSLFRNIDKVLKSLGFLE SGSGSGGGTLKYVEDVTNVVRRDVEKWGPFDLVYGSTQ PLGSSCDRCPGWYMFQFHRILQYALPRQESQRPFFWIF MDNLLLTEDDQETTTRFLQTEAVTLQDVRGRDYQNAMR VWSNIPGLKSKHAPLTPKEEEYLQAQVRSRSKLDAPKV DLLVKNCLLPLREYFKYFSQNSLPL	57617
dCasX491	57618	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTP DLRERLENLRKKPENIPQPISNTSRANLNKLLTDYTEM KKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKLKP EMDEKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYT NYFGRCNVAEHEKLILLAQLKPEKDSDEAVTYSLGKFG QRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKA LSDACMGTIASFLSKYQDIIIEHQKVVKGNQKRLESLR ELAGKENLEYPSVTLPPQPHTKEGVDAYNEVIARVRMW VNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEV DWWDMVCNVKKLINEKKEDGKVFWQNLAGYKRQEALRP YLSSEEDRKKGKKFARYQLGDLLLHLEKKHGEDWGKVY DEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDW LRAKASFVIEGLKEADKDEFCRCELKLQKWYGDLRGKP FAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYLI INYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPM EVNFNFDDPNLIILPLAFGKRQGREFIWNDLLSLETGS LKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLD SSNIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDS LGNPTHILRIGESYKEKQRTIQAKKEVEQRRAGGYSRK YASKAKNLADDMVRNTARDLLYYAVTQDAMLIFANLSR GFGRQGKRTFMAERQYTRMEDWLTAKLAYEGLSKTYLS KTLAQYTSKTCSNCGFTITSADYDRVLEKLKKTATGWM TTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSE ESVNNDISSWTKGRSGEALSLLKKRFSHRPVQEKFVCL NCGFETHAAEQAALNIARSWLFLRSQEYKKYQTNKTTG NTDKRAFVETWQSFYRKKLKEVWKPAV	57619
Linker 1	57620	GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGT STEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTST EPSE	57621
Linker 2	57622	SSGNSNANSRGPSFSSGLVPLSLRGSH	57623
Linker 3A	57624	GGSGGGS	57626
Linker 3B	57625		
Linker 4	57627	GSGSGGG	57628
NLS A	57629	PKKKRKV	57631
NLS B	57630		

Table 25: DNA sequences of ELXR constructs*.

ELXR ID	DNA sequence of ELXR molecule with the 2x FLAG (SEQ ID NO)
1.A	59477
1.B	59478
2.A	59479
2.B	59480
3.A	59481
3.B	59482
4.A	59483
4.B	59484
5.A	59485
5.B	59486

* See Table 28 and 29 for construct ID.

Table 26: Protein sequences of ELXR molecules*.

ELXR ID	Protein sequence of ELXR molecule (SEQ ID NO)
1.A	59467
1.B	59468
2.A	59469
2.B	59470
3.A	59471
3.B	59472
4.A	59473
4.B	59474
5.A	59475
5.B	59476

*See Tables 28 and 29 for ELXR construct ID.

Table 27: Sequences of spacers used in constructs.

Spacer ID	Target gene	PAM	Sequence	SEQ ID NO
7.37	B2M	TTC	GGCCGAGAUGUCUCGCUCCG	57644
7.148	B2M	NGG	CGCGAGCACAGCUAAGGCCA	57645
0.0	Non-target	N/A	CGAGACGUAAUUACGUCUCG	57646

Transfection of HEK293T cells:

[0541] HEK293T cells were seeded at a density of 30,000 cells in each well of a 96-well plate. The next day, each well was transiently transfected using lipofectamine with 100 ng of ELXR variant plasmids, each containing a dCasX:gRNA construct encoding for a differently configured ELXR protein (FIG. 7), with the gRNA having either non-targeting spacer 0.0 or targeting spacer 7.37 to the B2M locus. Specifically, for one experiment, HEK293T cells were transfected with plasmids encoding ELXR proteins #1-3, and in a second experiment, cells were lipofected with plasmids encoding for ELXR protein #1, 4, and 5 (see Table 25 for sequences). In both experiments, ELXR molecules harbored a KRAB domain either from ZNF10 or ZIM3. Experimental controls included dCasX491 (with or without the ZNF10 repressor domain), catalytically-active CasX 491, and a catalytically-dead Cas9 fused to both the ZNF10-KRAB domain and DNMT3A/L domains, each with the same B2M-targeting or non-targeting gRNA. Each construct was tested in triplicate. 24 hours post-transfection, cells were selected with 1μg/mL puromycin for two days. Six days after transfection, cells were harvested for repression analysis every 2-3 days by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry. B2M expression was determined by using an antibody that would detect the B2M-dependent HLA protein expressed on the cell surface. HLA+ cells were measured using the Attune™ NxT flow cytometer. In addition, in a separate experiment, HEK293T cells transiently transfected with ELXR variant plasmids and the *B2M*-targeting gRNA or non-targeting gRNA were harvested at five days post-lipofection for genomic DNA (gDNA) extraction for bisulfite sequencing.

Bisulfite sequencing to assess ELXR specificity measured by off-target methylation levels at target locus:

[0542] To determine off-target methylation levels at the B2M locus, gDNA from harvested cells was extracted using the Zymo Quick-DNA Miniprep Plus kit following the manufacturer's instructions. The extracted gDNA was then subjected to bisulfite conversion using the EZ DNA Methylation™ Kit (Zymo) following the manufacturer's protocol, converting any non-methylated cytosine into uracil. The resulting bisulfite-treated DNA was subsequently sequenced using next-generation sequencing (NGS) to determine the levels of off-target methylation at the *B2M* and *VEGFA* loci.

NGS processing and analysis:

[0543] Target amplicons were amplified from 100 ng bisulfite-treated DNA via PCR with a set of primers specific to the bisulfite-converted target locations of interest (human *B2M* and *VEGFA* loci). These gene-specific primers contained an additional sequence at the 5′ end to introduce an Illumina™ adapter. Amplified DNA products were purified with the Cytiva Sera-Mag Select DNA cleanup kit. Quality and quantification of the amplicon were assessed using a Fragment Analyzer DNA Analysis kit (Agilent, dsDNA 35-1500 bp). Amplicons were sequenced on the Illumina™ Miseq™ according to the manufacturer's instructions. Raw fastq files from sequencing were processed using Bismark Bisulfite Read Mapper and Methylation caller. PCR amplification of the bisulfite-treated DNA would convert all uracil nucleotides into thymine, and sequencing of the PCR product would determine the rate of cytosine-to-thymine conversion as a readout of the level of potential off-target methylation at the *B2M* and *VEGFA* loci mediated by each ELXR molecule.

Results:

[0544] ELXR variant plasmids encoding for differently configured ELXR proteins (FIG. 7) were transiently transfected into HEK293T cells to determine whether the rationally-designed ELXR molecules could heritably silence gene expression of the target B2M locus *in vitro*. FIGS. 8A and 8B depict the results of a time-course experiment assessing B2M protein repression mediated by ELXR proteins #1-3, each of which harbored a KRAB domain from ZNF10 (FIG. 8A) or ZIM3 (FIG. 8B). Table 28 shows the average percentage of cells characterized as HLA-negative (indicative of depleted B2M expression) for each condition at 50 days post-transfection. The results illustrate that all ELXR molecules with a gRNA targeting the B2M locus were able to demonstrate sustained B2M repression for 50 days *in vitro*, although the potency of repression varied by the choice of KRAB domain and ELXR configuration. For instance, harboring a ZIM3-KRAB domain rendered the ELXR protein a more efficacious repressor than harboring a ZNF10-KRAB, and this effect was most prominently observed for ELXR #2 (compare FIG. 8A to FIG. 8B). Furthermore, positioning the DNMT3A/L domains at the N-terminus of dCasX491 (ELXR #1) resulted in more stable silencing of B2M expression compared to effects mediated by ELXRs with DNMT3A/L domains at the C-terminus of dCasX491 (ELXR #2 and #3; FIGS. 8A and 8B). These results also revealed that the relative positioning of the two types of repressor domains (i.e., dCasX491-KRAB-DNMT3A/L for ELXR #2 vs. dCasX491-DNMT3A/L-KRAB

for ELXR #3) could also influence the overall potency of the ELXR molecule, despite both configurations being C-terminal fusions of dCasX491 (ELXR #2 and #3; FIGS. 8A and 8B).

[0545] In a second time-course experiment, durable B2M repression was assessed for ELXR proteins #1, #4, and #5, where both the DNMT3A/L and KRAB domains were positioned at the N-terminus of dCasX491 for ELXR #4 and #5 (FIG. 7). Table 29 shows the average percentage of HLA-negative cells for each condition at 73 days post-lipofection. As similarly seen in the first time-course, all ELXR conditions with a B2M-targeting gRNA maintained durable silencing of the B2M locus (FIGS. 9A and 9B; Table 29). In fact, the results in this experiment demonstrate that ELXR #5 was able to achieve and sustain the highest level of B2M repression compared to that achieved by ELXR #1 or ELXR #4 for 73 days *in vitro* (FIGS. 9A and 9B). Furthermore, ELXR #4 containing the ZIM3-KRAB also appeared to outperform its ELXR #1 counterpart (FIG. 9B). For both time-course experiments discussed above, CasX 491-mediated editing resulted in durable silencing of the B2M expression, while an XR construct fusing only the KRAB domain to dCasx491 (dCasX491-ZNF10) only resulted in transient B2M knockdown.

Table 28: Levels of B2M repression mediated by CasX and Cas9 molecules and ELXR constructs #1-3 quantified at 50 days post-transfection.

Molecule	Spacer	% HLA-negative cells (mean)	Standard deviation
CasX 491	0.0	0.29	0.09
dCasX491	0.0	N/A	N/A
dCasX491-ZNF10	0.0	0.40	0.18
dCas9-ZNF10-D3A/L	0.0	1.05	0.63
ELXR1-ZNF10	0.0	0.99	0.35
ELXR2-ZNF10	0.0	0.61	0.11
ELXR3-ZNF10	0.0	0.79	0.29
ELXR1-ZIM3	0.0	0.99	0.22
ELXR2-ZIM3	0.0	0.78	0.27
ELXR3-ZIM3	0.0	0.71	0.53
CasX 491	7.37	76.57	11.03
dCasX491	7.37	0.49	0.10
dCasX491-ZNF10	7.148	0.89	0.19
dCas9-ZNF10-D3A/L	7.148	57.30	17.36
ELXR1-ZNF10 (ELXR #1.B)	7.37	69.97	7.89

Molecule	Spacer	% HLA-negative cells (mean)	Standard deviation
ELXR2-ZNF10 (ELXR #2.B)	7.37	36.87	8.31
ELXR3-ZNF10 (ELXR #3.B)	7.37	17.07	3.50
ELXR1-ZIM3 (ELXR #1.A)	7.37	73.70	9.28
ELXR2-ZIM3 (ELXR #2.A)	7.37	58.83	0.87
ELXR3-ZIM3 (ELXR #3.A)	7.37	17.50	4.30

Table 29: Levels of B2M repression mediated by CasX and Cas9 molecules and ELXR constructs #1, #4, and #5 quantified at 73 days post-transfection.

Molecule	Spacer	% HLA-negative cells (mean)	Standard deviation
CasX 491	0.0	0.71	0.05
dCasX491	0.0	N/A	N/A
dCasX491-ZNF10	0.0	0.76	0.12
dCas9-ZNF10-D3A/L	0.0	0.83	0.08
ELXR1-ZNF10	0.0	1.04	0.44
ELXR4-ZNF10	0.0	1.17	0.52
ELXR5-ZNF10	0.0	1.94	1.27
ELXR1-ZIM3	0.0	1.83	0.76
ELXR4-ZIM3	0.0	N/A	N/A
ELXR5-ZIM3	0.0	1.15	0.26
CasX 491	7.37	73.30	8.43
dCasX491	7.37	0.83	0.16
dCasX491-ZNF10	7.148	1.37	0.37
dCas9-ZNF10-D3A/L	7.148	68.97	5.21
ELXR1-ZNF10 (ELXR #1.B)	7.37	48.27	3.66
ELXR4-ZNF10 (ELXR #4.B)	7.37	55.17	4.83
ELXR5-ZNF10 (ELXR #5.B)	7.37	60.77	8.12
ELXR1-ZIM3 (ELXR #1.A)	7.37	58.90	2.69
ELXR4-ZIM3 (ELXR #4.A)	7.37	69.00	6.58

Molecule	Spacer	% HLA-negative cells (mean)	Standard deviation
ELXR5-ZIM3 (ELXR #5.A)	7.37	74.90	10.61

[0546] To evaluate the degree of off-target CpG methylation at the *B2M* locus mediated by the DNMT3A/L domains within the ELXR molecules, bisulfite sequencing was performed using genomic DNA extracted from HEK293T cells treated with ELXR proteins #1-3 containing the ZIM3-KRAB domain and harvested at five days post-lipofection. FIG. 10 illustrates the findings from bisulfite sequencing, specifically showing the distribution of the number of CpG sites around the transcription start site of the *B2M* locus that harbored a certain level of CpG methylation for each experimental condition. The results revealed that while ELXR #1 demonstrated the strongest on-target CpG-methylating activity (ELXR1-ZIM3 7.37), it induced the highest level of off-target CpG methylation (ELXR1-ZIM3 NT). ELXR #2 and ELXR #3 displayed weaker on-target CpG-methylating activity but relatively lower off-target methylation (FIG. 10). FIG. 11 is a scatterplot mapping the activity-specificity profiles for ELXR proteins #1-3 benchmarked against CasX 491 and dCas9-ZNF10-DNMT3A/L, where activity was measured as the average percentage of HLA-negative cells at day 21, and specificity was represented by the percentage of off-target CpG methylation at the *B2M* locus quantified at day 5.

[0547] The degree of off-target CpG methylation mediated by the DNMT3A/L domain was further evaluated by assessing the level of CpG methylation at a different locus, i.e., *VEGFA*, by performing bisulfite sequencing using the same extracted gDNA as was used previously for FIG. 10. The violin plot in FIG. 12 illustrates the bisulfite sequencing results showing the distribution of CpG sites with CpG methylation at the *VEGFA* locus in cells treated with ELXR proteins #1-3 containing the ZIM3-KRAB domain and a *B2M*-targeting gRNA. The findings further demonstrate that use of ELXR #1 resulted in the highest level of off-target CpG methylation, supporting the data shown earlier in FIG. 10. In comparison, use of either ELXR #2 or ELXR #3 resulted in substantially lower off-target methylation at the *-3* locus (FIG. 12).

[0548] The extent of off-target CpG methylation at the *VEGFA* locus for ELXR molecules #1, #4, and #5 was also analyzed. The plots in FIGS. 13A-13B illustrate bisulfite sequencing results showing the distribution of CpG-methylated sites at the *VEGFA* locus in cells treated with

ELXR #1, 4, and 5 containing a ZNF10 or ZIM3-KRAB domain and either a non-targeting gRNA (FIG. 13B) or a *B2M*-targeting gRNA (FIG. 13A). The data in FIG. 13B show that use of ELXR4-ZNF10, ELXR5-ZFN10, or ELXR5-ZIM3 resulted in markedly lower off-target CpG methylation at the *VEGFA* locus in comparison to use of ELXR1-ZNF10 or ELXR1-ZIM3. Similarly, the data in FIG. 13A show that use of ELXR #4 or ELXR #5 with either KRAB domain resulted in substantially lower levels of off-target CpG methylated sites compared to use with ELXR1-ZNF10. As exhibited in both FIGS. 13A and 13B, the level of non-specific CpG methylation demonstrated by ELXR #1 is comparable to that achieved by the dCas9-ZNF10-DNMT3A/L benchmark.

[0549] FIG. 14 is a scatterplot mapping the activity-specificity profiles for ELXR molecules #1-5, containing either ZNF10- or ZIM3-KRAB domain, benchmarked against CasX 491 and dCas9-ZNF10-DNMT3A/L, where activity was measured as the average percentage of HLA-negative cells at day 21, and specificity was represented by the median percentage of off-target CpG methylation at the *VEGFA* locus detected at day 5. The data show that of the five ELXR molecules assessed, use of ELXR #5 resulted in the highest level of repressive activity, while use of ELXR #4 resulted in the strongest level of specificity.

[0550] The experiments demonstrate that the rationally-engineered ELXR molecules were able to transcriptionally and heritably repress the endogenous *B2M* locus, resulting in sustained depletion of the target protein. The findings also show that the choice of KRAB domain and position and relative configuration of the DNMT3A/L domains could affect the overall potency and specificity of the ELXR molecule in durably silencing the target locus.

Example 7: Development of functional screens to assess the activity and specificity of rationally-engineered improved ELXR variants

[0551] To engineer ELXR variants with improved repression activity and target methylation specificity, a pooled screening assay will be developed. Briefly, systematic mutagenesis of the DNMT3A catalytic domain is performed to generate a library of DNMT3A variants (SEQ ID NOS: 33625-57543) that will be tested in an ELXR molecule to screen for improved ELXR variants using various functional assays.

Materials and Methods:

Generation of a library of DNMT3A catalytic domain variants:

[0552] The following methods will be used to construct a DME library of the DNMT3A catalytic domain variants. A staging vector will be created to harbor the DNMT3A sequence flanked by restriction sites compatible with the destination vectors used for screening. The DNMT3A catalytic domain sequence will be divided into five ~200bp fragments, and each fragment will be synthesized as an oligonucleotide pool. Each oligonucleotide pool will be constructed to contain three different types of modification libraries. First, a substitution oligonucleotide library that will result in each codon of the DNMT3A catalytic domain fragment being replaced with one of the 19 possible alternative codons coding for the 19 possible amino acid mutations. Second, a deletion oligonucleotide library will be prepared that will result in each codon of the fragment being systematically removed to delete that amino acid. Third, an insertion oligonucleotide library will be prepared that will insert one of the 20 possible codons at every position of the DNMT3A catalytic domain fragment. These oligonucleotide pools will be amplified and cloned into the staging vector using Golden Gate reactions and PCR-generated backbones. The pooled DNMT3A catalytic domain DME libraries will then be transferred into the lentiviral ELXR constructs coding for the ELXR molecule as described in Example 6 via restriction enzyme digestion and ligation prior to library amplification. To determine adequate library coverage, each fragment of the DNMT3A catalytic domain DME will be PCR amplified separately with gene specific primers, followed by NGS on the Illumina™ Miseq™ using overlapping paired end sequencing.

[0553] High-throughput screening of ELXR variants generated using DNMT3A catalytic domain DME libraries:

[0554] After following standard protocols for lentivirus production and titering, the resulting lentiviral library of ELXR variants will be subjected to different high-throughput functional screens. These functional screens are briefly described below.

[0555] A specificity-focused screen aims to identify DNMT3A catalytic domain variants that will yield ELXR molecules with decreased off-target methylation. For instance, an *in vitro* dropout assay could be used to identify DNMT3A catalytic domain variants that would not induce deleterious nonspecific methylation. Overexpression of DNMT3A leads to extraneous methylation which adversely affects cell growth, likely due to increased repression of genes critical for cell survival and proliferation. In this assay, HEK293T cells will be transduced with

the lentiviral ELXR library at a low multiplicity of infection (MOI), and an initial population of transduced cells will be harvested prior to selection with puromycin for five days. After selection, multiple time point populations will be harvested at days 5, 7, 10 and 14, and gDNA will be extracted from all populations and subjected to PCR amplification and NGS sequencing of target amplicons containing the DNMT3A catalytic domain variants. Comparing the library composition readout between the initial and terminal populations will yield non-deleterious DNMT3A catalytic domain variants that confer cell survivability and growth. In parallel, methylation-sensitive promoters coupled to GFP have been developed in which overexpression of untargeted ELXR molecules lead to GFP repression due to off-target global methylation. An orthogonal screen will therefore be performed in which the DNMT3A catalytic domain DME libraries will be transduced in cell lines harboring these methylation-sensitive reporters, and quantification of GFP levels would allow assessment and identification of ELXR variants that cause off-target methylation over time.

[0556] An activity-focused screen aims to identify DNMT3A catalytic domain variants that will reveal ELXR molecules with increased on-target methylating activity. Here, the approach can leverage the spreading of DNA methylation to potentially repress the activity of a nearby promoter to identify ELXR-specific spacers and evaluate ELXR molecule activity at earlier time points. Briefly, HEK293T suspension cells will be transduced with the lentiviral ELXR library with the spacer targeting the B2M locus and selected with puromycin for five days. After selection, B2M protein expression will be measured by immunostaining, and cells that exhibit B2M repression (indicated by HLA-negative cells) will be sorted by FACS. Genomic DNA will be extracted from sorted HLA-negative cells for NGS analysis. Enrichment scores for each variant can be calculated by comparing the frequency of mutations in the sorted population relative to the naive cells to identify the DNMT3A catalytic domain variants that more potently repress B2M expression.

[0557] In addition to screening the library of DNMT3A catalytic domain variants, screening the library of KRAB repressor domains in parallel, which is described in Example 4 above, will help identify ELXR variants with improved activity and specificity profiles.

[0558] The experiments described in this example are expected to identify additional ELXR leads with improved durable repression activity and specificity. These improved ELXR molecules will be tested in various cell types against a therapeutic target of interest to further characterize and identify lead candidates for development.

Example 8: Demonstration that catalytically-dead CasX does not edit at the endogenous *B2M* locus in vitro

[0559] Experiments were performed to demonstrate that catalytically-dead CasX is unable to edit the endogenous *B2M* gene in an in vitro assay.

Materials and Methods:

Generation of catalytically-dead CasX (dCasX) constructs and cloning:

[0560] CasX variants 491, 527, 668 and 676 with gRNA scaffold variant 174 were used in these experiments. To generate catalytically-dead CasX 491 (dCasX491; SEQ ID NO: 18) and catalytically-dead CasX 527 (dCasX527; SEQ ID NO: 24), the D659, E756, D921 catalytic residues of the RuvC domain of CasX variant 491, and D660, E757, and the D922 catalytic residue of the RuvC domain of CasX variant 527 were mutated to alanine to abolish the endonuclease activity. Similarly, D660, E757, D923-to-alanine mutations at catalytic residues within the RuvC domain of CasX variants 668 and 676 were designed to generate catalytically-dead CasX 668 (dCasX668; SEQ ID NO: 59355) and catalytically-dead CasX 676 (dCasX676; SEQ ID NO: 59357). The resulting plasmids contained constructs with the following configuration: Ef1α-SV40NLS-dCasX variant-SV40NLS. Plasmids also contained sequences encoding a gRNA scaffold variant 174 having a *B2M*-targeting spacer (spacer. 7.37; GGCCGAGAUGUCUCGCUCCG, SEQ ID NO: 59628) or a non-targeting spacer control (spacer 0.0; CGAGACGUAAUUACGUCUCG; SEQ ID NO: 59630).

[0561] Plasmids encoding for the catalytically-dead CasX variants (dCasX491, dCasX527, dCasX668, and dCasX676) were generated using standard molecular cloning methods and validated using Sanger-sequencing. Sequence-validated constructs were midi-prepped for subsequent transfection into HEK293T cells.

Plasmid transfection into HEK293T cells:

[0562] ~30,000 HEK293T cells were seeded in each well of a 96-well plate; the next day, cells were transiently transfected with a plasmid containing a dCasX:gRNA construct encoding for dCasX491, dCasX527, dCasX668, or dCasX676 (sequences in Table 4), with the gRNA having either non-targeting spacer 0.0 or targeting spacer 7.37 to the *B2M* locus. Each construct was tested in triplicate. 24 hours post-transfection, cells were selected with puromycin, and six days after transfection, cells were harvested for editing analysis at the *B2M* locus by NGS. The

following experimental controls were also included in this experiment: 1) catalytically-active CasX 491 with a *B2M*-targeting gRNA or a non-targeting gRNA; 2) catalytically-dead variant of Cas9 (dCas9) with the appropriate gRNAs; and 3) mock (no plasmid) transfection.

NGS processing and analysis:

[0563] Using the Zymo Quick-DNA Miniprep Plus kit following the manufacturer's instructions, gDNA was extracted from harvested cells. Target amplicons were amplified from extracted gDNA with a set of primers specific to the human *B2M* locus. These gene-specific primers contained an additional sequence at the 5′ end to introduce an Illumina™ adapter and a 16-nucleotide unique molecule identifier. Amplified DNA products were purified with the Ampure XP DNA cleanup kit. Quality and quantification of the amplicon were assessed using a Fragment Analyzer DNA Analysis kit (Agilent, dsDNA 35-1500bp). Amplicons were sequenced on the Illumina™ Miseq™ according to the manufacturer's instructions. Raw fastq files from sequencing were quality-controlled and processed using cutadapt v2.1, flash2 v2.2.00, and CRISPResso2 v2.0.29. Each sequence was quantified for containing an insertion or deletion (indel) relative to the reference sequence, in a window around the 3′ end of the spacer (30 bp window centered at –3 bp from 3′ end of spacer). CasX activity was quantified as the total percent of reads that contain insertions, substitutions, and/or deletions anywhere within this window for each sample.

Results:

[0564] The plot in FIG. 15 shows the results of the editing analysis, specifically the percent editing at the *B2M* locus measured as indel rate detected by NGS for each of the indicated treatment conditions. The data demonstrate that >80% editing was achieved at the *B2M* locus mediated by catalytically-active CasX 491. On the other hand, dCasX491, dCasX527, dCasX668, and dCasX676 did not exhibit editing at the *B2M* locus with the *B2M*-targeting spacer.

[0565] The results of this experiment demonstrate that catalytically-dead CasX does not edit at an endogenous target locus in vitro.

Example 9: Demonstration that use of ELXR molecules can induce durable silencing of the endogenous *CD151* gene

[0566] Experiments were performed to demonstrate that ELXR molecules can induce long-term repression of an alternative endogenous locus, i.e., the *CD151* gene, in a cell-based assay.

Materials and Methods:

[0567] ELXR molecules #1, #4, and #5 containing the ZIM3-KRAB domain (see FIG. 7 for specific configurations and Table 25 for encoding sequences) were assessed in this experiment.

Transfection of HEK293T cells:

[0568] Seeded HEK293T cells were transiently transfected with 100 ng of ELXR variant plasmids, each containing an ELXR:gRNA construct encoding for ELXR molecule #1, #4, or #5, with four different gRNAs targeting the *CD151* gene that encodes for an endogenous cell surface receptor (spacer sequences listed in Table 30). The next day, cells were selected with puromycin for four days. Cells were harvested for repression analysis at day 6, day 15, and day 22 after transfection. Repression analysis was performed by quantifying the level of CD151 protein expression via CD151 immunolabeling followed by flow cytometry using the Attune™ NxT flow cytometer. As experimental controls, HEK293T cells were also transfected with dCas9-ZNF10-DNMT3A/L with the appropriate *CD151*-targeting gRNAs (with targeting spacers 1-3 listed in Table 30). FIG. 20A is a schematic illustrating the relative positions of the targeting spacers listed in Table 30.

Table 30: Sequences of human *CD151*-targeting spacers used in constructs.

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO
39.1	CAGCGCTGGGAGCCGCCGCC	59640	CAGCGCUGGGAGCCGCCGCC	59647
39.2	GCCCAGGGGTCCCGGGACGC	59641	GCCCAGGGGUCCCGGGACGC	59648
39.3	CTCCGCCCGCAGCAGCCCCC	59642	CUCCGCCCGCAGCAGCCCCC	59649
39.4	GACCTGCCGAGCGCCCGCCG	59643	GACCUGCCGAGCGCCCGCCG	59650
dCas9 spacer 1	ACCACGCGTCCGAGTCCGG	59644	ACCACGCGUCCGAGUCCGG	59651
dCas9 spacer 2	TGCTCATTGTCCCTGGACA	59645	UGCUCAUUGUCCCUGGACA	59652
dCas9 spacer 3	GGACACCCTGCTCATTGTC	59646	GGACACCCUGCUCAUUGUC	59653

Results:

[0569] ELXR variant plasmids encoding for ELXR #1, #4, and #5 harboring the ZIM3-KRAB domain were transiently transfected into HEK293T cells to determine whether these ELXR

molecules could durably silence expression of the target *CD151* gene in a cell-based assay. Quantification of the resulting *CD151* knockdown by ELXRs is illustrated in FIG. 20B. The data demonstrate that use of three of the four tested targeting spacers resulted in durable silencing of the *CD151* locus through 22 days post-transfection, albeit to varying levels of knockdown. Specifically, use of ELXR #1, #4, or #5 with targeting spacer 39.1 resulted in the strongest durable *CD151* knockdown compared to that achieved when using other targeting spacers (FIG. 20B). The findings also show that use of ELXR #5 resulted in the strongest repressive activity, observable at Day 15 and Day 22 post-transfection across the tested spacers (FIG. 20B). Transfections with ELXR #5 and spacer pool or dCas9-ZNF10-DNMT3A/L and the appropriate gRNAs similarly resulted in durable silencing of the *CD151* locus.

[0570] The results of this experiment demonstrate that ELXR molecules can induce heritable silencing of an alternative endogenous locus in vitro. Furthermore, the findings show that use of the ELXR #5 molecule resulted in the highest repression activity among the various ELXR configurations tested, indicating that position and relative arrangement of the DNMT3A/L domains affect overall activity of the ELXR molecule at the target locus.

Example 10: Demonstration that ELXRs have a broader targeting window compared to dXRs

[0571] Experiments were performed to determine the targeting window of ELXR molecules at a gene promoter and to demonstrate that ELXRs have a wider targeting window compared to that of dXR molecules. As described in earlier examples, dXR is dCasX fused with a KRAB repressor domain, while ELXR is dCasX fused with a KRAB domain, DNMT3A catalytic domain, and a DNMT3L interaction domain.

Materials and Methods:

[0572] ELXR #1 containing the ZIM3-KRAB domain, as described in Example 6, and dXR1, as described in Example 1, were assessed in this experiment. Various gRNAs with scaffold 174 containing a *B2M*-targeting spacer were used in this experiment.

Transfection of HEK293T cells:

[0573] Seeded HEK293T cells were lipofected with 100 ng of a plasmid containing a CasX:gRNA construct encoding for either XR1 or an ELXR #1 containing the ZIM3-KRAB domain, with nine different targeting gRNAs that tiled across ~1KB region of the *B2M* promoter (spacer sequences listed in Table 31). The next day, cells were selected with puromycin for four

additional days. Cells were harvested at six days after lipofection to determine B2M protein expression by flow cytometry as described in Example 6. HEK293T cells transfected with either ELXR #1 or dXR1 with a non-targeting gRNA was included as an experimental control. FIG. 21A is a schematic illustrating the tiling of the various *B2M*-targeting gRNAs (spacers listed in Table 31) within a ~1KB window of the *B2M* promoter.

Table 31: Sequences of human *B2M*-targeting spacers used in constructs in this experiment.

Spacer ID	DNA sequence	SEQ ID NO	RNA sequence	SEQ ID NO
7.37	GGCCGAGATGTCTCGCTCCG	341	GGCCGAGAUGUCUCGCUCCG	59628
7.160	TAAACATCACGAGACTCTAA	59654	UAAACAUCACGAGACUCUAA	59662
7.161	AGGACTTCAGGCTGGAGGCA	59655	AGGACUUCAGGCUGGAGGCA	59663
7.162	CGAATGAAAAATGCAGGTCC	59656	CGAAUGAAAAAUGCAGGUCC	59664
7.163	GTTTATAACTACAGCTTGGG	59657	GUUUAUAACUACAGCUUGGG	59665
7.164	CTGAGCTGTCCTCAGGATGC	59658	CUGAGCUGUCCUCAGGAUGC	59666
7.165	TCCCTATGTCCTTGCTGTTT	59659	UCCCUAUGUCCUUGCUGUUU	59667
7.166	AGCGCCCTCTAGGTACATCA	59660	AGCGCCCUCUAGGUACAUCA	59668
7.167	GTTTACTGAGTACCTACTAT	59661	GUUUACUGAGUACCUACUAU	59669

Results:

[0574] To determine and compare the targeting window of ELXR molecules with that of dXR molecules, HEK293T cells were transfected with a plasmid encoding for either ELXR #1 or dXR1 with the various *B2M*-targeting gRNAs tiled across a ~1KB region of the *B2M* promoter (Table 31). FIG. 21B is a plot depicting the results of the experiment assessing B2M protein repression (indicated by average percentage of cells characterized as HLA-negative) mediated by ELXR #1 compared with that mediated by dXR1 for the various *B2M*-targeting spacers. The data demonstrate that ELXR #1 was able to induce substantial B2M repression with more targeting spacers compared to that observed with dXR1 (FIG. 21B). Specifically, unlike the effects seen with dXR1, ELXR #1 was able to achieve meaningful B2M repression with spacers 7.160, 7.163, 7.164, and 7.165, suggesting that these four spacers are ELXR-specific spacers at the *B2M* locus. As anticipated, both ELXR #1 and dXR1 were able to induce a marked decrease

in B2M protein expression with spacer 7.37 and a negligible decrease with a non-targeting spacer (FIG. 21B).

[0575] The results of this experiment demonstrate that ELXR molecules have a broader targeting window at the target locus compared to that of dXR molecules, and that ELXRs can function at longer distances from the gene promoter to induce repression of the target gene.

Example 11: Demonstration that inclusion of the ADD domain from DNMT3A enhances activity and specificity of ELXR molecules

[0576] In addition to its C-terminal methyltransferase domain, DNMT3A contains two N-terminal domains that regulate its function and recruitment to chromatin: the ADD domain and the PWWP domain. The PWWP domain reportedly interacts with methylated histone tails, including H3K36me3. The ADD domain is known to have two key functions: 1) it allosterically regulates the catalytic activity of DNMT3A by serving as a methyltransferase auto-inhibitory domain, and 2) it recognizes unmethylated H3K4 (H3K4me0). The interaction of the ADD domain with the H3K4me0 mark unveils the catalytic site of DNMT3A, thereby recruiting an active DNMT3A to chromatin to implement de novo methylation at these sites.

[0577] Given these functions of the ADD domain, it is possible that including the ADD domain could enhance the activity and specificity of ELXR molecules. Here, experiments were performed to assess whether the incorporation of the ADD domain into the ELXR #5 molecule, described previously in Example 6, would result in improved long-term repression of the target locus and reduced off-target methylation. The effect of incorporating the PWWP domain along with the ADD domain on ELXR activity and specificity was also assessed.

Materials and Methods:

Generation of ELXR constructs and plasmid cloning:

[0578] Plasmid constructs encoding for variants of the ELXR #5 construct with the ZIM3-KRAB domain (ELXR #5.A; see FIG. 7 for ELXR #5 configuration) were built using standard molecular cloning techniques. The resulting constructs comprised of sequences encoding for one of the following four alternative variations of ELXR5-ZIM3, where the additional DNMT3A domains were incorporated: 1) ELXR5-ZIM3 + ADD; 2) ELXR5-ZIM3 + ADD + PWWP; 3) ELXR5-ZIM3 + ADD without the DNMT3A catalytic domain; and 4) ELXR5-ZIM3 + ADD + PWWP without the DNMT3A catalytic domain. The sequences of key elements within the ELXR5-ZIM3 molecule and its variants are listed in Table 32, with the full encoding sequences

for each ELXR5-ZIM3 and its variants listed in Table 33. FIG. 36 is a schematic that illustrates the various ELXR #5 architectures assayed in this example. Sequences encoding the ELXR molecules also contained a 2x FLAG tag. Plasmids also harbored constructs encoding for the gRNA scaffold variant 174 having either a spacer targeting the endogenous *B2M* locus or a non-targeting control (spacer sequences listed in Table 34).

Table 32: Sequences of key ELXR elements (e.g., additional domains fused to dCasX) to generate ELXR5 variant plasmids illustrated in FIG. 36.

Key component	DNA Sequence (SEQ ID NO)	Protein sequence	SEQ ID NO
ZIM3 KRAB domain	57612	MNNSQGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQ GETTKPDVILRLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKE SL	57613
DNMT3A catalytic domain (CD)	59444	NHDQEFDPPKVYPPVPAEKRKPIRVLSLFDGIATGLLVLKDLGIQVDRY IASEVCEDSITVGMVRHQGKIMYVGDVRSVTQKHIQEWGPFDLVIGGSP CNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFENV VAMGVSDKRDISRFLESNPVMIDAKEVSAAHRARYFWGNLPGMNRPLAS TVNDKLELQECLEHGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKE DILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGRSWSVPVIRHLFAPL KEYFACV	59450
DNMT3L interaction domain	59445	MGPMEIYKTVSAWKRQPVRVLSLFRNIDKVLKSLGFLESGSGSGGGTLK YVEDVTNVVRRDVEKWGPFDLVYGSTQPLGSSCDRCPGWYMFQFHRILQ YALPRQESQRPFFWIFMDNLLLTEDDQETTTRFLQTEAVTLQDVRGRDY QNAMRVWSNIPGLKSKHAPLTPKEEEYLQAQVRSRSKLDAPKVDLLVKN CLLPLREYFKYFSQNSLPL	57617
dCasX491	57618	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRK KPENIPQPISNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSR VAQPASKKIDQNKLKPEMDEKGNLTTAGFACSQCGQPLFVYKLEQVSEK GKAYTNYFGRCNVAEHEKLILLAQLKPEKDSDEAVTYSLGKFGQRALDF YSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQ DIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAY NEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVERQANEVD WWDMVCNVKKLINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGK KFARYQLGDLLLHLEKKHGEDWGKVYDEAWERIDKKVEGLSKHIKLEEE RRSEDAQSKAALTDWLRAKASFVIEGLKEADKDEFCRCELKLQKWYGDL RGKPFAIEAENSILDISGFSKQYNCAFIWQKDGVKKLNLYLIINYFKGG KLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNLIILPLAF GKRQGREFIWNDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVAL TFERREVLDSSNIKPMNLIGVARGENIPAVIALTDPEGCPLSRFKDSLG NPTHILRIGESYKEKQRTIQAKKEVEQRRAGGYSRKYASKAKNLADDMV RNTARDLLYYAVTQDAMLIFANLSRGFGRQGKRTFMAERQYTRMEDWLT AKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLEKLKKTAT GWMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDIS SWTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARS WLFLRSQEYKKYQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV	57619

Key component	DNA Sequence (SEQ ID NO)	Protein sequence	SEQ ID NO
Linker 1	57620	GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPG SPAGSPTSTEEGTSTEPSEGSAPGTSTEPSE	57621
Linker 2	57622	SSGNSNANSRGPSFSSGLVPLSLRGSH	57623
Linker 3A'	59446	GGSGGG	59451
Linker 3B	57625	GGSGGGS	57626
Linker 4	57627	GSGSGGG	57628
NLS A	57629	PKKKRKV	57631
NLS B	57630		
DNMT3A ADD domain	59447	ERLVYEVRQKCRNIEDICISCGSLNVTLEHPLFIGGMCQNCKNCFLECA YQYDDDGYQSYCTICCGGREVLMCGNNNCCRCFCVECVDLLVGPGAAQA AIKEDPWNCYMCGHKGTYGLLRRREDWPSRLQMFFAN	59452
DNMT3A PWWP domain	59448	TKAADDEPEYEDGRGFGIGELVWGKLRGFSWWPGRIVSWWMTGRSRAAE GTRWVMWFGDGKFSVVCVEKLMPLSSFCSAFHQATYNKQPMYRKAIYEV LQVASSRAGKLFPACHDSDESDSGKAVEVQNKQMIEWALGGFQPSGPKG LEPPEEEKNPYKEV	59453
Endogenous sequence between DNMT3A PWWP and ADD domains (endo)	59449	YTDMWVEPEAAAYAPPPPAKKPRKSTTEKPKVKEIIDERTR	59454

Table 33: DNA sequences of constructs encoding ELXR5 variants assayed in this example, and protein sequences of ELXR5 variants.

ELXR ID	DNA Sequence (SEQ ID NO)	Protein SEQ ID NO
ELXR5-ZIM3	59455	59460
ELXR5-ZIM3 + ADD	59456	59461
ELXR5-ZIM3 + ADD + PWWP	59457	59462
ELXR5-ZIM3 + ADD - CD	59458	59463
ELXR5-ZIM3 + ADD + PWWP - CD	59459	59464

Table 34: Sequences of spacers used in constructs.

Spacer ID	Target gene	Sequence	SEQ ID NO
0.0	Non-target	CGAGACGUAAUUACGUCUCG	57646
7.37	B2M	GGCCGAGAUGUCUCGCUCCG	57644
7.160	B2M	UAAACAUCACGAGACUCUAA	59662

Spacer ID	Target gene	Sequence	SEQ ID NO
7.165	B2M	UCCCUAUGUCCUUGCUGUUU	59667

Transfection of HEK293T cells:

[0579] Seeded HEK293T cells were transiently transfected with 100 ng of ELXR5 variant plasmids, each containing an ELXR:gRNA construct encoding for ELXR5-ZIM3 or one of its alternative variations (FIG. 36; Table 33 for sequences), with the gRNA having either non-targeting spacer 0.0 or a *B2M*-targeting spacer (Table 34 for spacer sequences). The results in Example 10 identified spacers 7.160 and 7.165 to be ELXR-specific spacers. Each construct was tested in triplicate. 24 hours post-transfection, cells were selected with 1μg/mL puromycin for three days. Cells were harvested for repression analysis at day 5, day 12, day 21, and day 51 post-transfection. Briefly, repression analysis was conducted by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry, as described in Example 6. In addition, HEK293T cells transiently transfected with ELXR5 variant plasmids and a *B2M*-targeting gRNA or non-targeting gRNA were harvested at seven days post-transfection for gDNA extraction for bisulfite sequencing to assess off-target methylation at the *VEGFA* locus, which was performed as described in Example 6.

Results:

[0580] The effects of incorporating the ADD domain with or without the PWWP domain into the ELXR5 molecule on increasing long-term repression of the target *B2M* locus and reducing off-target methylation were assessed. Variations of the ELXR5-ZIM3 molecule were evaluated with either a *B2M*-targeting gRNA (with spacer 7.37 and ELXR-specific spacers 7.160 and 7.165) or a non-targeting gRNA, and the results are depicted in the plots in FIGS. 22-25. FIG. 22 shows that use of spacer 7.37 resulted in saturating levels of repression activity when paired with ELXR5-ZIM3, ELXR5-ZIM3 + ADD, and ELXR5-ZIM3 + ADD + PWWP, rendering it more challenging to assess activity differences among the ELXR5 variants. However, the differences in repression activity among the ELXR5 variants were more pronounced when using spacers 7.160 and 7.165 (FIGS. 23 and 24). The data demonstrate that incorporation of the ADD domain resulted in a significant increase in long-term repression when paired with the two ELXR-specific spacers compared to the repression levels achieved with the other ELXR5-ZIM3 molecules. Meanwhile, incorporation of both ADD and PWWP domains did not result in improved repression of the *B2M* locus, especially compared to the baseline ELXR5-ZIM3

molecule. As anticipated, the two ELXR5 variants without the DNMT3A catalytic domain exhibited poor long-term repression. Furthermore, FIG. 25 indicates that addition of the ADD domain appeared to result in increased specificity, given the lower percentage of HLA-negative cells observed, relative to the baseline ELXR5-ZIM3 molecule.

[0581] Off-target CpG methylation at the *VEGFA* locus potentially mediated by the ELXR5 variants was assessed using bisulfite sequencing. FIG. 26 depicts the results from bisulfite sequencing, specifically showing the percentage of CpG methylation around the *VEGFA* locus. The results demonstrate that for all the *B2M*-targeting gRNAs, as well as the non-targeting gRNA, incorporation of the ADD domain into the ELXR5-ZIM3 molecule dramatically reduced the level of off-target methylation at the *VEGFA* locus (FIG. 26). FIG. 27 is a scatterplot mapping the activity-specificity profiles for the ELXR5-ZIM3 variants investigated in this example, where activity was measured as the average percentage of HLA-negative cells at day 21 when paired with spacer 7.160, and specificity was represented by the percentage of off-target CpG methylation at the *VEGFA* locus quantified at day 7 when paired with spacer 7.160. The scatterplot clearly shows that addition of the ADD domain significantly increases activity of the ELXR5 molecule relative to the baseline ELX5 molecule without the ADD domain (FIG. 27).

[0582] The experiments demonstrate that inclusion of the DNMT3A ADD domain, but not inclusion of both the ADD and PWWP domains, improves repression activity and specificity of ELXR molecules. This enhancement of activity and specificity is observed with multiple gRNAs, demonstrating the significance of the incorporation of the ADD domain into ELXRs.

Example 12: Demonstration that silencing of a target locus mediated by ELXR molecules is reversible using a DNMT1 inhibitor

[0583] Experiments were performed to demonstrate that durable repression of a target locus mediated by ELXR molecules is reversible, such that treatment with a DNMT1 inhibitor would remove methyl marks to reactivate expression of the target gene.

Materials and Methods:

[0584] ELXR #5 containing the ZIM3-KRAB domain, which was generated as described in Example 6, and CasX variant 491 were used in this experiment. A *B2M*-targeting gRNA with

scaffold 174 containing spacer 7.37 (SEQ ID NO: 57644) or a non-targeting gRNA containing spacer 0.0 (SEQ ID NO: 57646) were used in this experiment.

Transfection of HEK293T cells:

[0585] HEK293T cells were transfected with 100 ng of a plasmid containing a construct encoding for either CasX 491 or ELXR #5 containing the ZIM3-KRAB domain with a B2M-targeting gRNA or non-targeting gRNA and cultured for 58 days. These transfected HEK293T cells were subsequently re-seeded at ~30,000 cells well of a 96-well plate and were treated with 5-aza-2′-deoxycytidine (5-azadC), a DNMT1 inhibitor, at concentrations ranging from 0μM to 20μM. Six days post-treatment with 5-azadC, cells were harvested for B2M silencing analysis at day 5, day 12, and day 21 post-transfection. Briefly, repression analysis was conducted by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry, as described in Example 6. Treatments for each dose of 5-azadC for each experimental condition were performed in triplicates.

Results:

[0586] The plot in FIG. 34 shows the percentage of transfected HEK293T cells treated with the indicated concentrations of 5-azadC that expressed the B2M protein. The data demonstrate that 5-azadC treatment of cells transfected with a plasmid encoding ELXR5-ZIM3 with the *B2M*-targeting gRNA resulted in a reactivation of the *B2M* gene (FIG. 34). Specifically, ~75% of treated cells exhibited B2M expression with 20μM 5-azadC, compared to the 25% of cells with B2M expression at 0μM concentration (FIG. 34). Furthermore, 5-azadC treatment of cells transfected with a plasmid encoding CasX 491 with the B2M-targeting gRNA did not exhibit reactivation of the *B2M* gene. FIG. 35 is a plot that juxtaposes B2M repression activity with gene reactivation upon 5-azadC treatment. The data show B2M repression post-transfection with either CasX 491 or ELXR5-ZIM3 with the *B2M*-targeting gRNA, resulting in ~75% repression of B2M expression by day 58; however, B2M expression is increased upon 5-azadC treatment (FIG. 35). As anticipated, 5-azadC treatment of cells transfected with either CasX 491 or ELXR5-ZIM3 with the non-targeting gRNA did not demonstrate repression or reactivation (FIGS. 34-35).

[0587] The experiments demonstrate reversibility of ELXR-mediated repression of a target locus. By using a DNMT1 inhibitor to remove methyl marks implemented by ELXR molecules, the silenced target gene was reactivated to induce expression of the target protein.

Example 13: Demonstration that inclusion of the ADD domain from DNMT3A into ELXRs enhances on-target activity and decreases off-target methylation

[0588] Experiments were performed to assess the effects of incorporating the ADD domain into ELXR molecules having configurations #1, #4, and #5, described previously in Example 6, on long-term repression of the target locus and off-target methylation.

Materials and Methods:

Generation of ELXR constructs and plasmid cloning:

[0589] Plasmid constructs encoding for ELXR molecules having configurations #1, #4, and #5 with the ZNF10-KRAB or ZIM3-KRAB domain and the DNMT3A ADD domain were built using standard molecular cloning techniques. Sequences of the resulting ELXR molecules are listed in Table 35, which also shows the abbreviated construct names for a particular ELXR molecule (e.g., ELXR #1.A, #1.B). FIG. 37 is a schematic that illustrates the general architectures of ELXR molecules with the ADD domain incorporated for ELXR configuration #1, #4, and #5. Sequences encoding the ELXR molecules also contained a 2x FLAG tag. Plasmids also harbored sequences encoding gRNA scaffold 174 having either a spacer targeting the endogenous *B2M* locus or a non-targeting control (spacer sequences listed in Table 34).

Table 35: DNA and protein sequences of the various ELXR #1, #4, and #5 variants assayed in this example.

ELXR #	Domains	DNA SEQ ID NO	Protein SEQ ID NO
ELXR #1	ZNF10-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #1.D)	59488	59498
	ZIM3-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #1.C)	59489	59499
	ZNF10-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #1.B)	59490	59500
	ZIM3-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #1.A)	59491	59501
ELXR #4	ZNF10-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #4.D)	59492	59502

ELXR #	Domains	DNA SEQ ID NO	Protein SEQ ID NO
	ZIM3-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #4.C)	59493	59503
	ZNF10-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #4.B)	59494	59504
	ZIM3-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #4.A)	59495	59507
ELXR #5	ZNF10-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #5.D)	59496	59505
	ZIM3-KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction (ELXR #5.C)	59456	59461
	ZNF10-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #5.B)	59497	59509
	ZIM3-KRAB, DNMT3A CD, DNMT3L Interaction (ELXR #5.A)	59455	59460

Transfection of HEK293T cells:

[0590] Seeded HEK293T cells were transiently transfected with 100 ng of ELXR variant plasmids, each containing an ELXR:gRNA construct encoding for an ELXR molecule (Table 35; FIG. 37), with the gRNA having either non-targeting spacer 0.0 or a *B2M*-targeting spacer (Table 34). Each construct was tested in triplicate. 24 hours post-transfection, cells were selected with 1μg/mL puromycin for 3 days. Cells were harvested for repression analysis at day 8, day 13, day 20, and day 27 post-transfection. Briefly, repression analysis was conducted by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry, as described in Example 6. In addition, cells were also harvested on day 5 post-transfection for gDNA extraction for bisulfite sequencing to assess off-target methylation at the non-targeted *VEGFA* locus, which was performed using similar methods as described in Example 6.

Results:

[0591] The effects of incorporating the ADD domain into the ELXR molecules having configurations #1, #4, or #5, with either a ZNF10 or ZIM-KRAB, on long-term repression of the

B2M locus and off-target methylation were evaluated. ELXR molecules were tested with either a *B2M*-targeting gRNA or a non-targeting gRNA, and the results are depicted in the plots in FIGS. 39A-42B. The data demonstrate that incorporation of the ADD domain into the ELXR molecules clearly resulted in a substantial increase in B2M repression across all the time points for all ELXR orientations containing the ZIM3-KRAB when using spacer 7.160 (FIG. 39A), and similar findings were observed when using spacers 7.165 and 7.37 (data not shown). FIG. 39B shows the resulting B2M repression upon use of ELXR #5 containing either the ZNF10 or ZIM3-KRAB when paired with a gRNA with spacer 7.160; the data demonstrate that including the ADD domain increased durable B2M repression overall, with ELXR5-ZIM3 + ADD having a higher activity compared with that of ELXR5-ZNF10 + ADD. Similar time course findings were observed for ELXR #1 and ELXR #4 and the other two spacers (data not shown). FIG. 39C shows the resulting B2M repression upon use of ELXR #5 containing the ZIM3-KRAB when paired with any of the three *B2M*-targeting gRNAs, and the data demonstrate that inclusion of the ADD domain resulted in higher B2M repression overall. Similar time course findings were also observed for ELXR #1 and ELXR #4 (data not shown).

[0592] FIGS. 40A-40C shows the resulting B2M repression at the day 27 time point for all the ELXR configurations and gRNAs tested. The results show that the increase in B2M repression activity is more prominent with use of the sub-optimal spacers 7.160 and 7.165 compared to use of spacer 7.37. Furthermore, use of ELXR #1 and ELXR #5, which contained the DNMT3A and DNMT3L domains on the N-terminus of the molecule, resulted in the highest increase in B2M repression upon addition of the DNMT3A ADD domain (FIGS. 40A-40C). Use of ELXR #4, which harbored the DNMT3A/3L domains 3' to the KRAB domain and 5' to the dCasX, resulted in lower activity gains, which may be attributable to a decreased ability of the ADD domain to interact with chromatin properly.

[0593] The specificity of ELXR molecules was determined by profiling the level of CpG methylation at the *VEGFA* gene, an off-target locus, using bisulfite sequencing, and the data are illustrated in FIGS. 41A-44B. The data demonstrate that inclusion of the DNMT3A ADD domain resulted in a substantial decrease in off-target methylation of the *VEGFA* locus across all conditions tested (FIGS. 41A-41C). Notably, the increased specificity mediated by the inclusion of the ADD domain was most prominent with the ELXR #1 and ELXR #5 configurations, both of which harbored the DNMT3A/3L domains on the N-terminal end of the molecule. Interestingly, ELXR molecules containing the ZIM3-KRAB domain led to stronger off-target

methylation of the *VEGFA* locus. Furthermore, use of ELXR #4 and #5 configurations, even in the absence of an ADD domain, resulted in higher specificity compared to use of the ELXR #1 configuration. Compared to ELXR1-ZIM3 and ELXR4-ZIM3 configurations, inclusion of the ADD domain into ELXR5-ZIM3 resulted in the lowest off-target methylation.

[0594] FIGS. 42A-44B are a series of scatterplots mapping the activity-specificity profiles for the various ELXR molecules, where activity was measured as the average percentage of HLA-negative cells at day 27, and specificity was determined by the percentage of off-target CpG methylation at the *VEGFA* locus at day 5. The data demonstrate that across all three *B2M*-targeting spacers tested, inclusion of the ADD domain resulted in increased on-target B2M repression and decreased off-target methylation at the *VEGFA* locus. ELXR molecules having #1 and #5 configurations exhibited the greatest increases in activity and specificity at each spacer tested.

[0595] The results of the experiments discussed in this example support the findings in Example 11, in that the data demonstrate that inclusion of the DNMT3A ADD domain enhances both the strength of repression at early timepoints and the heritability of silencing across cell divisions, as well as decreases the off-target methylation incurred by the DNMT3A catalytic domain in the ELXR molecules. The data also confirm that different ELXR orientations have intrinsic differences in specificity, which can be exacerbated by use of a more potent KRAB domain. This decrease in specificity can be mitigated by inclusion of the DNMT3A ADD domain, which also can lead to greater on-target repression overall. The gains in repression activity are believed to be mediated by the function of the DNMT3A ADD domain to recognize H3K4me0 and subsequent recruitment to chromatin. The gains in specificity are believed to be mediated via the function of the DNMT3A ADD domain to induce allosteric inhibition of the catalytic domain of DNMT3A in the absence of binding to H3K4me0. The results also highlight that positioning of the ADD domain in the different configurations tested is important to achieve the strongest gains in both specificity and activity of ELXR molecules.

Example 14: Demonstration that use of ELXRs can induce silencing of an endogenous locus in mouse Hepa 1-6 cells

[0596] Experiments were performed to demonstrate the ability of ELXRs to induce durable repression of an alternative endogenous locus in mouse Hepa 1-6 liver cells, when delivered as mRNA co-transfected with a targeting gRNA.

Materials and Methods:

Experiment #1: dXR1 vs. ELXR #1 in Hepa1-6 cells when delivered as mRNA

Generation of dXR1 and ELXR #1 mRNA:

[0597] mRNA encoding dXR1 or ELXR #1 containing the ZIM3-KRAB domain was generated by in vitro transcription (IVT). Briefly, constructs encoding for a 5'UTR region, dXR1 or ELXR #1 harboring the ZIM3-KRAB domain with flanking SV40 NLSes, and a 3'UTR region were generated and cloned into a plasmid containing a T7 promoter and 80-nucleotide poly(A) tail. These constructs also contained a 2x FLAG sequence. Sequences encoding the dXR1 and ELXR #1 molecules were codon-optimized using a codon utilization table based on ribosomal protein codon usage, in addition to using a variety of publicly available codon optimization tools and adjusting parameters such as GC content as needed. The resulting plasmid was linearized prior to use for IVT reactions, which were carried out with CleanCap® AG and N1-methyl-pseudouridine. IVT reactions were then subjected to DNase digestion and oligodT purification on-column. For experiment #1, the DNA sequences encoding the dXR1 and ELXR #1 molecules are listed in Table 36. The corresponding mRNA sequences encoding the dXR1 and ELXR#1 mRNAs are listed in Table 37. The protein sequences of the dXR1 and ELXR#1 are shown in Table 38.

Table 36: Encoding sequences of the dXR1 and ELXR #1 containing the ZIM3-KRAB domain mRNA molecules assessed in experiment #1 of this example*.

XR or ELXR ID	Component	DNA SEQ ID NO
dXR1 (codon-optimized)	5'UTR	59568
	START codon + NLS + linker	59569
	dCasX491	59570
	Linker + buffer sequence	59571
	ZIM3-KRAB	59572
	Buffer sequence + NLS	59573
	Tag	59574
	STOP codon + buffer sequence	59575

XR or ELXR ID	Component	DNA SEQ ID NO
	3'UTR	59576
	Buffer sequence	59577
	Poly(A) tail	59578
ELXR #1 (codon-optimized)	5'UTR	59568
	START codon + NLS + buffer sequence + linker	59579
	START codon + DNMT3A catalytic domain	59580
	Linker	59581
	DNMT3L interaction domain	59582
	Linker	59583
	dCasX491	59570
	Linker	59571
	ZIM3-KRAB	59572
	Buffer sequence + NLS	59573
	Tag	59574
	STOP codons + buffer sequence	59575
	3'UTR	59576
	Buffer sequence	59577
	Poly(A) tail	59578

*Components are listed in a 5' to 3' order within the constructs

Table 37: Full-length RNA sequences of dXR1 and ELXR #1 containing the ZIM3-KRAB domain mRNA molecules assessed in experiment #1 of this example. Modification 'mψ' = N1-methyl-pseudouridine.

XR or ELXR ID	RNA sequence	SEQ ID NO
dXR1	AAAmψAAGAGAGAAAAGAAGAGmψAAGAAGAAAmψAmψAAGAGCCACCAmψGGCC CCmψAAGAAGAAGCGmψAAAGmψGAGCCGGGGCGGCAGCGGCGGCGGCAGCGCCC AGGAGAmψmψAAACGGAmψCAACAAGAmψCAGAAGAAGACmψmψGmψGAAAGACA GCAACACCAAGAAGGCCGGCAAGACAGGCCCCAmψGAAAACCCmψGCmψGGmψmψ AGAGmψGAmψGACACCCGAmψCmψGAGAGAGCGGCmψGGAAAACCmψGAGAAAGA AGCCmψGAAAAmψAmψCCCCCAGCCCAmψCAGCAAmψACAmψCmψAGAGCCAACC mψGAAmψAAGCmψGCmψGACCGAmψmψACACCGAAAmψGAAGAAGGCGAmψCCmψ GCAmψGmψGmψACmψGGGAAGAGmψmψCCAGAAGGACCCmψGmψGGGCCmψGAmψ GAGCCGGGmψGGCCCAGCCmψGCCAGCAAGAAGAmψCGAmψCAGAACAAGCmψGA AACCmψGAGAmψGGACGAGAAGGGCAACCmψGACCACCGCCGGCmψmψmψGCCmψ GCmψCmψCAGmψGmψGGCCAGCCCCmψGmψmψCGmψGmψACAAGCmψGGAGCAGG mψGmψCmψGAGAAGGGCAAGGCmψmψACACCAACmψACmψmψCGGACGGmψGCAA mψGmψGGCCGAGCACGAAAAGCmψGAmψCCmψGCmψGGCCCAGCmψGAAGCCCGA GAAGGAmψAGCGACGAAGCCGmψGACAmψAmψAGCCmψGGGAAAGmψmψmψGGGC AGAGGGCCCmψGGAmψmψmψCmψACAGCAmψmψCAmψGmψGACCAAGGAGmψCCA CCCACCCCGmψGAAGCCCCmψGGCCCAGAmψCGCCGGAAACAGAmψACGCCmψCC	59584

XR or ELXR ID	RNA sequence	SEQ ID NO
	GGACCmψGmψGGGAAAGGCCCmψGAGCGACGCAmψGmψAmψGGGCACAAmψCGCC mψCCmψmψCCmψGmψCmψAAGmψACCAGGACAmψCAmψCAmψCGAACACCAGAAG GmψGGmψGAAGGGCAACCAGAAGAGACmψGGAGAGCCmψGCGGGAGCmψGGCCGG CAAGGAAAACCmψGGAAmψACCCmψAGCGmψGACCCmψGCCACCmψCAGCCmψCA CACCAAGGAGGGCGmψmψGAmψGCCmψACAACGAAGmψGAmψCGCCCGGGmψGCG AAmψGmψGGGmψGAACCmψGAACCmψGmψGGCAGAAGCmψGAAGCmψAAGCAGAG AmψGAmψGCCAAGCCmψCmψGCmψGAGACmψGAAGGGAmψmψCCCmψmψCCmψmψ mψCCmψCmψGGmψCGAGAGACAGGCCAACGAAGmψGGACmψGGmψGGGACAmψGG mψGmψGmψAACGmψGAAGAAGCmψGAmψCAACGAGAAAAAGGAGGAmψGGCAAGG mψGmψmψmψmψGGCAGAAmψCmψGGCmψGGCmψACAAGAGACAGGAAGCCCmψGA GACCAmψACCmψGAGCAGCGAGGAAGAmψCGGAAGAAGGGAAAGAAAmψmψCGCm ψCGGmψACCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAAAAGAAGCACGGC GAGGACmψGGGGAAAGGmψGmψACGACGAGGCCmψGGGAGCGGAmψmψGACAAGA AAGmψGGAAGGCCmψGAGCAAGCACAmψCAAGCmψGGAAGAGGAACGGAGAAGCG AGGACGCCCAGAGCAAGGCCGCCCmψGACCGACmψGGCmψGCGGGCmψAAGGCCA GCmψmψCGmψGAmψCGAGGGCCmψGAAGGAGGCCGACAAGGACGAGmψmψCmψGC AGAmψGCGAGCmψGAAGCmψGCAGAAGmψGGmψACGGGGACCmψGCGGGGAAAGC CCmψmψCGCCAmψCGAAGCCGAGAACAGCAmψCCmψGGACAmψCAGCGGCmψmψC AGCAAGCAGmψACAACmψGmψGCCmψmψCAmψCmψGGCAGAAGGACGGCGmψGAA GAAGCmψGAACCmψGmψACCmψGAmψCAmψCAACmψACmψmψCAAGGGCGGCAAG CmψGCGGmψmψCAAGAAGAmψCAAACCmψGAAGCCmψmψCGAAGCCAACAGAmψm ψCmψACACCGmψGAmψCAACAAAAAGAGCGGCGAGAmψCGmψGCCCAmψGGAGGm ψGAACmψmψCAACmψmψCGACGACCCCAACCmψGAmψCAmψCCmψGCCmψCmψGG CCmψmψmψGGCAAGAGACAGGGCAGAGAAmψmψCAmψCmψGGAACGACCmψGCmψ GmψCCCmψGGAAACCGGCAGCCmψGAAGCmψGGCCAACGGAAGAGmψGAmψCGAG AAGACACmψGmψACAACAGAAGAACCCGGCAGGAmψGAGCCmψGCCCmψGmψmψC GmψGGCCCmψGACCmψmψCGAGCGGCGGGAGGmψCCmψGGACmψCCmψCCAAmψA mψCAAACCAAmψGAACCmψGAmψCGGCGmψGGCAAGAGGCGAAAACAmψCCCCGC CGmψGAmψCGCCCmψGACCGACCCCGAGGGCmψGCCCACmψGAGCCGGmψmψmψA AGGAmψAGCCmψGGGAAACCCAACCCACAmψCCmψGAGAAmψCGGCGAGAGCmψA mψAAGGAGAAGCAGCGGACCAmψCCAGGCCAAGAAGGAGGmψGGAGCAGCGGAGA GCCGGCGGCmψACAGCCGGAAGmψACGCCAGCAAAGCCAAGAAmψCmψGGCAGAC GAmψAmψGGmψGAGAAACACCGCmψAGAGAmψCmψGCmψGmψACmψACGCCGmψG ACCCAGGAmψGCCAmψGCmψGAmψCmψmψCGCCAACCmψGAGCCGGGGCmψmψCG GCCGGCAGGGCAAGCGGACCmψmψCAmψGGCCGAGAGACAGmψACACACGGAmψG GAGGACmψGGCmψGACCGCCAAGCmψGGCCmψACGAGGGCCmψGAGCAAGACCmψ ACCmψGmψCCAAGACACmψGGCCCAGmψACACCmψCCAAGACAmψGCAGCAACmψ GmψGGGmψmψmψACCAmψCACCAGCGCCGACmψACGACAGGGmψGCmψGGAGAAG CmψGAAGAAGACAGCAACAGGCmψGGAmψGACCACAAmψmψAACGGCAAGGAGCm ψGAAGGmψGGAGGGCCAGAmψmψACCmψACmψACAACAGAmψACAAGAGACAGAA CGmψAGmψCAAGGACCmψGmψCCGmψCGAGCmψGGAmψAGACmψGAGCGAAGAAm ψCmψGmψGAACAACGACAmψCmψCCmψCCmψGGACAAAGGGCAGAAGCGGAGAAG CmψCmψGAGCCmψCCmψGAAGAAAAGAmψmψCmψCCCAmψAGACCCGmψGCAGGA GAAGmψmψCGmψGmψGCCmψGAACmψGCGGCmψmψCGAGACACACGCAGCCGAGC AAGCCGCCCmψGAACAmψCGCCAGAmψCCmψGGCmψGmψmψCCmψGCGGAGCCAG GAGmψACAAGAAAmψACCAGACAAACAAGACAACCGGCAACACCGAmψAAGAGAG CCmψmψCGmψCGAGACCmψGGCAGmψCCmψmψmψmψACCGGAAGAAGCmψmψAAG GAGGmψGmψGGAAACCmψGCCGmψGCGGmψCmψGGCGGAmψCmψGGCGGAGGCmψ CCACAAGCAmψGAACAACmψCCCAGGGCAGAGmψGACCmψmψCGAGGACGmψGAC CGmψGAAmψmψmψmψACACAGGGAGAGmψGGCAGAGACmψGAACCCCGAGCAGAG AAACCmψGmψACCGGGAmψGmψGAmψGCmψGGAAAACmψACAGCAAmψCmψGGmψ GmψCCGmψGGGCCAGGGCGAGACCACAAAGCCmψGACGmψGAmψCCmψGCGmψCm ψGGAGCAGGGCAAGGAACCCmψGGCmψGGAGGAGGAGGAGGmψGCmψGGGAAGCG GACGGGCCGAGAAGAACGGCGACAmψCGGCGGACAGAmψCmψGGAAGCCmψAAGG	

XR or ELXR ID	RNA sequence	SEQ ID NO
	ACGmψGAAAGAAAGCCmψGACCAGCCCCAAGAAAAAGAGAAAAGmψCGACmψACA AGGAmψGACGAmψGACAAGGACmψACAAGGAmψGACGACGACAAGmψAAmψAGAm ψAAGCGGCCGCmψmψAAmψmψAAGCmψGCCmψmψCmψGCGGGGCmψmψGCCmψmψ CmψGGCCAmψGCCCmψmψCmψmψCmψCmψCCCmψmψGCACCmψGmψACCmψCmψm ψGGmψCmψmψmψGAAmψAAAGCCmψGAGmψAGGAAGmψcmψagaaaaaaaaaaaa aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa aaaaaaaaaaaaa	
ELXR #1	AAAmψAAGAGAGAAAAGAAGAGmψAAGAAGAAAmψAmψAAGAGCCACCAmψGGCC CCmψAAGAAGAAGCGmψAAAGmψGAGCCGGGmψGAACGGCAGCGGCAGCGGCGGC GGCAmψGAACCACGACCAGGAGmψmψCGACCCCCCmψAAGGmψGmψACCCmψCCC GmψCCCCGCCGAGAAGAGAAAGCCCAmψCCGGGmψCCmψGAGCCmψGmψmψCGAm ψGGCAmψCGCCACCGGmψCmψGCmψGGmψGCmψGAAGGACCmψGGGCAmψCCAGG mψGGAmψAGGmψACAmψmψGCCmψCCGAGGmψGmψGCGAGGACmψCCAmψCACCG mψGGGAAmψGGmψGCGmψCAmψCAGGGCAAGAmψCAmψGmψACGmψGGGCGACGm ψGCGGAGCGmψGACACAGAAGCAmψAmψCCAGGAGmψGGGCCCmψmψmψCGACC mψGGmψGAmψCGGCGGCAGCCCmψmψGCAAmψGACCmψGAGCAmψCGmψGAACCC AGCCCGGAAGGGCCmψGmψACGAGGGAACCGGCAGACmψGmψmψCmψmψCGAGmψ mψmψmψACAGACmψGCmψGCACGACGCCCGGCCmψAAGGAAGGCGACGACCGGCC CmψmψCmψmψmψmψGGCmψGmψmψCGAGAAmψGmψGGmψGGCCAmψGGGAGmψCA GCGACAAGCGGGAmψAmψmψAGCCGGmψmψCCmψGGAGAGCAACCCCGmψGAmψG AmψCGAmψGCCAAGGAAGmψGAGCGCCGCCCACCGGGCCAGAmψACmψmψCmψGG GGCAAmψCmψGCCmψGGCAmψGAACAGACCCCmψGGCCAGCACCGmψGAACGACA AGCmψGGAGCmψGCAGGAGmψGCCmψGGAGCACGGCCGGAmψCGCCAAGmψmψCA GCAAGGmψGAGAACCAmψCACCACCCGAAGCAACAGCAmψCAAACAAGGCAAGGA CCAGCACmψmψmψCCmψGmψGmψmψCAmψGAACGAGAAGGAGGACAmψCCmψGmψ GGmψGmψACCGAGAmψGGAGAGAGmψGmψmψCGGGmψmψCCCAGmψCCACmψACA CAGAmψGmψCAGCAACAmψGmψCmψAGACmψGGCCAGACAGAGACmψGCmψGGGA AGAAGCmψGGmψCCGmψCCCmψGmψGAmψCAGACACCmψGmψmψCGCCCCmψCmψ GAAGGAGmψACmψmψCGCCmψGCGmψGAGCAGCGGCAACAGCAACGCCAACAGCC GGGGCCCCAGCmψmψCmψCmψAGCGGCCmψGGmψGCCACmψGmψCCCmψGAGAGG GAGCCACAmψGGGCCCCAmψGGAGAmψCmψACAAAACCGmψGAGCGCCmψGGAAG CGGCAGCCmψGmψGCGCGmψGCmψGAGCCmψGmψmψmψCGGAAmψAmψCGAmψAA AGmψCCmψGAAAAGCCmψGGGAmψmψCCmψGGAGAGCGGCmψCmψGGCmψCCGGC GGmψGGCACCCmψGAAGmψACGmψGGAGGAmψGmψGACAAACGmψGGmψCAGACG GGAmψGmψGGAGAAGmψGGGGCCCCCmψmψCGAmψCmψGGmψGmψACGGCAGCACC CAACCCCmψGGGCAGCmψCmψmψGmψGACCGGmψGCCCmψGGCmψGGmψACAmψG mψmψmψCAGmψmψCCACCGGAmψCCmψGCAGmψACGCCCmψGCCGAGACAGGAGm ψCCCAGCGGCCAmψmψCmψmψmψmψGGAmψmψmψmψCAmψGGACAACmψmψGCmψ GCmψGACCGAGGAmψGACCAGGAAACmψACCACmψCGGmψmψCCmψGCAGACCGA AGCCGmψGACCCmψGCAGGACGmψGAGAGGCCGGGACmψACCAGAACGCCAmψGC GGGmψGmψGGmψCCAACAmψCCCmψGGACmψGAAAAGCAAGCACGCACCmψCmψG ACCCCmψAAAGAAGAGGAGmψACCmψGCAGGCCCAGGmψGCGGAGCAGAAGCAAG CmψGGACGCCCCmψAAGGmψGGAmψCmψGCmψGGmψGAAGAAmψmψGCCmψCCmψ GCCCCmψGAGAGAGmψACmψmψCAAGmψAmψmψmψCAGCCAGAAmψAGmψCmψGC CCCmψGGGCGGCCCAAGCAGCGGCGCCCCmψCCmψCCCAGCGGCGGCAGCCCAGC CGGCmψCCCCAACCmψCmψACCGAGGAGGGCACCmψCmψGAGmψCCGCCACCCCC GAGAGCGGCCCmψGGCACCmψCCACCGAGCCCAGCGAGGGCAGCGCACCCGGCAG CCCmψGCCGGCAGCCCCACCmψCCACAGAGGAGGGAACCAGCACCGAGCCCAGCG AAGGCAGCGCCCCAGGCACCAGCACCGAGCCmψAGmψGAGGCGGCmψCmψGGCG GCGGCAGCGCCCAGGAGAmψmψAAACGGAmψCAACAAGAmψCAGAAGAAGACmψm ψGmψGAAAGACAGCAACACCAAGAAGGCCGGCAAGACAGGCCCCAmψGAAAACCC mψGCmψGGmψmψAGAGmψGAmψGACACCCGAmψCmψGAGAGAGCGGCmψGGAAAA CCmψGAGAAAGAAGCCmψGAAAAmψAmψCCCCCAGCCCAmψCAGCAAmψACAmψC mψAGAGCCAACCmψGAAmψAAGCmψGCmψGACCGAmψmψACACCGAAAmψGAAGA	59585

XR or ELXR ID	RNA sequence	SEQ ID NO
	AGGCGAmψCCmψGCAmψGmψGmψACmψGGGAAGAGmψmψCCAGAAGGACCCmψGm ψGGGCCmψGAmψGAGCCGGGmψGGCCCAGCCmψGCCAGCAAGAAGAmψCGAmψCA GAACAAGCmψGAAACCmψGAGAmψGGACGAGAAGGGCAACCmψGACCACCGCCGG CmψmψmψGCCmψGCmψCmψCAGmψGmψGGCCAGCCCCmψGmψmψCGmψGmψACAA GCmψGGAGCAGGmψGmψCmψGAGAAGGGCAAGGCmψmψACACCAACmψACmψmψC GGACGGmψGCAAmψGmψGGCCGAGCACGAAAAGCmψGAmψCCmψGCmψGGCCCAG CmψGAAGCCCGAGAAGGAmψAGCGACGAAGCCGmψGACAmψAmψAGCCmψGGGAA AGmψmψmψGGGCAGAGGGCCCmψGGAmψmψmψCmψACAGCAmψmψCAmψGmψGAC CAAGGAGmψCCACCCACCCCGmψGAAGCCCCmψGGCCCAGAmψCGCCGGAAACAG AmψACGCCmψCCGGACCmψGmψGGGAAAGGCCCmψGAGCGACGCAmψGmψAmψGG GCACAAmψCGCCmψCCmψmψCCmψGmψCmψAAGmψACCAGGACAmψCAmψCAmψC GAACACCAGAAGGmψGGmψGAAGGGCAACCAGAAGAGACmψGGAGAGCCmψGCGG GAGCmψGGCCGGCAAGGAAAACCmψGGAAmψACCCmψAGCGmψGACCCmψGCCAC CmψCAGCCmψCACACCAAGGAGGGCGmψmψGAmψGCCmψACAACGAAGmψGAmψC GCCCGGGmψGCGAAmψGmψGGGmψGAACCmψGAACCmψGmψGGCAGAAGCmψGAA GCmψAAGCAGAGAmψGAmψGCCAAGCCmψCmψGCmψGAGACmψGAAGGGAmψmψC CCmψmψCCmψmψmψCCmψCmψGGmψCGAGAGACAGGCCAACGAAGmψGGACmψGG mψGGGACAmψGGmψGmψGmψAACGmψGAAGAAGCmψGAmψCAACGAGAAAAAGGA GGAmψGGCAAGGmψGmψmψmψmψGGCAGAAmψCmψGGCmψGGCmψACAAGAGACA GGAAGCCCmψGAGACCAmψACCmψGAGCAGCGAGGAAGAmψCGGAAGAAGGGAAA GAAAmψmψCGCmψCGGmψACCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAA AAGAAGCACGGCGAGGACmψGGGGAAAGGmψGmψACGACGAGGCCmψGGGAGCGG AmψmψGACAAGAAAGmψGGAAGGCCmψGAGCAAGCACAmψCAAGCmψGGAAGAGG AACGGAGAAGCGAGGACGCCCAGAGCAAGGCCGCCCmψGACCGACmψGGCmψGCG GGCmψAAGGCCAGCmψmψCGmψGAmψCGAGGGCCmψGAAGGAGGCCGACAAGGAC GAGmψmψCmψGCAGAmψGCGAGCmψGAAGCmψGCAGAAGmψGGmψACGGGGACCm ψGCGGGGAAAGCCCmψmψCGCCAmψCGAAGCCGAGAACAGCAmψCCmψGGACAmψ CAGCGGCmψmψCAGCAAGCAGmψACAACmψGmψGCCmψmψCAmψCmψGGCAGAAG GACGGCGmψGAAGAAGCmψGAACCmψGmψACCmψGAmψCAmψCAACmψACmψmψC AAGGGCGGCAAGCmψGCGGmψmψCAAGAAGAmψCAAACCmψGAAGCCmψmψCGAA GCCAACAGAmψmψCmψACACCGmψGAmψCAACAAAAAGAGCGGCGAGAmψCGmψG CCCAmψGGAGGmψGAACmψmψCAACmψmψCGACGACCCCAACCmψGAmψCAmψCC mψGCCmψCmψGGCCmψmψmψGGCAAGAGACAGGGCAGAGAAmψmψCAmψCmψGGA ACGACCmψGCmψGmψCCCmψGGAAACCGGCAGCCmψGAAGCmψGGCCAACGGAAG AGmψGAmψCGAGAAGACACmψGmψACAACAGAAGAACCCGGCAGGAmψGAGCCmψ GCCCmψGmψmψCGmψGGCCCmψGACCmψmψCGAGCGGCGGGAGGmψCCmψGGACm ψCCmψCCAAmψAmψCAAACCAAmψGAACCmψGAmψCGGCGmψGGCAAGAGGCGAA AACAmψCCCCGCCGmψGAmψCGCCCmψGACCGACCCCGAGGGCmψGCCCACmψGA GCCGGmψmψmψAAGGAmψAGCCmψGGGAAACCCAACCCACAmψCCmψGAGAAmψC GGCGAGAGCmψAmψAAGGAGAAGCAGCGGACCAmψCCAGGCCAAGAAGGAGGmψG GAGCAGCGGAGAGCCGGCGGCmψACAGCCGGAAGmψACGCCAGCAAAGCCAAGAA mψCmψGGCAGACGAmψAmψGGmψGAGAAACACCGCmψAGAGAmψCmψGCmψGmψA CmψACGCCGmψGACCCAGGAmψGCCAmψGCmψGAmψCmψmψCGCCAACCmψGAGC CGGGGCmψmψCGGCCGGCAGGGCAAGCGGACCmψmψCAmψGGCCGAGAGACAGmψ ACACACGGAmψGGAGGACmψGGCmψGACCGCCAAGCmψGGCCmψACGAGGGCCmψ GAGCAAGACCmψACCmψGmψCCAAGACACmψGGCCCAGmψACACCmψCCAAGACA mψGCAGCAACmψGmψGGGmψmψmψACCAmψCACCAGCGCCGACmψACGACAGGGm ψGCmψGGAGAAGCmψGAAGAAGACAGCAACAGGCmψGGAmψGACCACAAmψmψAA CGGCAAGGAGCmψGAAGGmψGGAGGGCCAGAmψmψACCmψACmψACAACAGAmψA CAAGAGACAGAACGmψAGmψCAAGGACCmψGmψCCGmψCGAGCmψGGAmψAGACm ψGAGCGAAGAAmψCmψGmψGAACAACGACAmψCmψCCmψCCmψGGACAAAGGGCA GAAGCGGAGAAGCmψCmψGAGCCmψCCmψGAAGAAAAGAmψmψCmψCCCAmψAGA CCCGmψGCAGGAGAAGmψmψCGmψGmψGCCmψGAACmψGCGGCmψmψCGAGACAC ACGCAGCCGAGCAAGCCGCCCmψGAACAmψCGCCAGAmψCCmψGGCmψGmψmψCC	

XR or ELXR ID	RNA sequence	SEQ ID NO
	mψGCGGAGCCAGGAGmψACAAGAAAmψACCAGACAAACAAGACAACCGGCAACAC CGAmψAAGAGAGCCmψmψCGmψCGAGACCmψGGCAGmψCCmψmψmψmψACCGGAA GAAGCmψmψAAGGAGGmψGmψGGAAACCmψGCCGmψGCGGmψCmψGGCGGAmψCm ψGGCGGAGGCmψCCACAAGCAmψGAACAACmψCCCAGGGCAGAGmψGACCmψmψC GAGGACGmψGACCGmψGAAmψmψmψmψACACAGGGAGAGmψGGCAGAGACmψGAA CCCCGAGCAGAGAAACCmψGmψACCGGGAmψGmψGAmψGCmψGGAAAACmψACAG CAAmψCmψGGmψGmψCCGmψGGGCCAGGGCGAGACCACAAAGCCmψGACGmψGAm ψCCmψGCGmψCmψGGAGCAGGGCAAGGAACCCmψGGCmψGGAGGAGGAGGAGGmψ GCmψGGGAAGCGGACGGGCCGAGAAGAACGGCGACAmψCGGCGGACAGAmψCmψG GAAGCCmψAAGGACGmψGAAAGAAAGCCmψGACCAGCCCCAAGAAAAAGAGAAAA GmψCGACmψACAAGGAmψGACGAmψGACAAGGACmψACAAGGAmψGACGACGACA AGmψAAmψAGAmψAAGCGGCCGCmψmψAAmψmψAAGCmψGCCmψmψCmψGCGGGG CmψmψGCCmψmψCmψGGCCAmψGCCCmψmψCmψmψCmψCmψCCCmψmψGCACCmψ GmψACCmψCmψmψGGmψCmψmψmψGAAmψAAAGCCmψGAGmψAGGAAGmψcmψag aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa aaaaaaaaaaaaaaaaaaaaaaaa	

Table 38: Full-length protein sequences of dXR1 and ELXR #1 containing the ZIM3-KRAB domain molecules assessed in experiment #1 of this example. Modification 'mψ' = N1-methyl-pseudouridine.

XR or ELXR ID	Protein SEQ ID NO
dXR1	59586
ELXR #1	59467

Synthesis of gRNAs:

[0598] In this experiment #1, gRNAs targeting the *PCSK9* locus were designed using gRNA scaffold 174 and chemically synthesized. The sequences of the *PCSK9*-targeting spacers are listed in Table 39.

Table 39: Sequences of spacers targeting the *PCSK9* locus used in this example.

gRNA ID (scaffold-variant spacer)	Target	Targeting spacer sequence (RNA)	SEQ ID NO:
174-6.7	human *PCSK9*	UCCUGGCUUCCUGGUGAAGA	59587
174-27.1	mouse *PCSK9*	GCCUCGCCCUCCCCAGACAG	59588
174-27.88	mouse *PCSK9*	CGCUACCUGCCUAAACUUUG	59589

gRNA ID (scaffold-variant spacer)	Target	Targeting spacer sequence (RNA)	SEQ ID NO:
174-27.92	mouse *PCSK9*	CCCUCCAACAAUAUUAACUA	59590
174-27.93	mouse *PCSK9*	GGGGUCUCCCAGCCACCCCU	59591
174-27.94	mouse *PCSK9*	CCCCUCUUAAUCCCCACUCC	59592
174-27.100	mouse *PCSK9*	CUCUCUCUUUCUGAGGCUAG	59593
174-27.103	mouse *PCSK9*	UAAUCUCCAUCCUCGUCCUG	59594

Transfection of mRNA and gRNA into Hepa1-6 cells and intracellular PCSK9 staining:

[0599] Seeded Hepa1-6 cells treated with the NATE™ inhibitor were lipofected with 300 ng of mRNA encoding dXR1 or ELXR #1 with a ZIM3-KRAB domain (Table 37) and 150 ng of a *PCSK9*-targeting gRNA (Table 39). Seven different gRNAs spanning the promoter region of the mouse *PCSK9* locus were tested, in addition to a non-targeting sequence complementary to the human *PCSK9* gene (Table 39). Cells were harvested at 6, 13, and 25 days after transfection to measure intracellular levels of the PCSK9 protein using an intracellular flow cytometry staining protocol. Briefly, cells were fixed using 4% paraformaldehyde in PBS, permeabilized, and stained using a mouse anti-PCSK9 primary antibody (R&D Systems), followed by a fluorescent goat anti-mouse IgG secondary antibody (Thermo Fisher). Fluorescence levels were measured using the Attune™ NxT flow cytometer, and data were analyzed using the FlowJo™ software. Cell populations were gated using the non-targeting gRNA as a negative control.

Experiment #2: ELXR #1 vs. ELXR #5 in Hepa1-6 cells when delivered as mRNA

Generation of mRNA:

[0600] mRNA encoding ELXR #1 or ELXR #5 containing the ZIM3-KRAB domain was generated by IVT in-house using PCR templates. Briefly, PCR was performed on plasmids encoding ELXR #1 or ELXR #5 harboring the ZIM3-KRAB domain with flanking NLSes with a forward primer containing a T7 promoter and reverse primer encoding a 120-nucleotide poly(A) tail. These constructs also contained a 2x FLAG sequence. DNA sequences encoding these molecules are listed in Table 40. The resulting PCR templates were used for IVT reactions, which were carried out with CleanCap® AG and N1-methyl-pseudouridine. IVT reactions were

then subjected to DNase digestion and on-column oligo dT purification. Full-length RNA sequences encoding the ELXR mRNAs are listed in Table 41.

[0601] As experimental controls, mRNA encoding catalytically-active CasX 491 was also similarly generated by IVT using a PCR template as described. Generation of mRNAs encoding ELXR #1 containing the ZIM3-KRAB domain and dCas9-ZNF10-DNMT3A/3L (described in Example 6) by IVT by a third-party was performed as described above for experiment #1.

Table 40: Encoding sequences of the ELXR #1 and ELXR #5 containing the ZIM3-KRAB domain mRNA molecules assessed in experiment #2 of this example*.

ELXR ID	Component	DNA SEQ ID NO
ELXR #1 - ZIM3-KRAB	5'UTR	59595
	START codon + NLS + linker	59596
	START codon + DNMT3A catalytic domain	59597
	Linker	59598
	DNMT3L interaction domain	59445
	Linker	59599
	Linker + buffer	59600
	dCasX491	59601
	Linker + buffer	59602
	ZIM3-KRAB	59603
	Buffer + NLS	59604
	Tag	59605
	Buffer	59606
	Poly(A) tail	59607
ELXR #5 - ZIM3-KRAB	5'UTR	59595
	START codon + NLS + buffer	59608
	START codon + DNMT3A catalytic domain	59597
	Linker	59598
	DNMT3L interaction domain	59445
	Linker	59446
	ZIM3-KRAB	59603
	Linker	59599
	dCasX491	59601
	Linker + buffer	59602
	NLS	59609
	Tag	59605
	Buffer	59606
	Poly(A) tail	59607

*Components are listed in a 5' to 3' order within the constructs

Table 41: Full-length RNA sequences of ELXR #1 and ELXR #5 containing the ZIM3-KRAB domain mRNA molecules assessed in experiment #2 of this example. Modification 'mψ' = N1-methyl-pseudouridine.

ELXR ID	RNA sequence	SEQ ID NO
ELXR #1 - ZIM3-KRAB	GACCGGCCGCCACCAmψGGCCCCAAAGAAGAAGCGGAAGGmψCmψCmψAGAGmψmψAACGGAmψ CAGGCmψCmψGGAGGmψGGAAmψGAACCAmψGACCAGGAAmψmψmψGACCCCCCAAAGGmψmψm ψACCCACCmψGmψGCCAGCmψGAGAAGAGGAAGCCCAmψCCGCGmψGCmψGmψCmψCmψmψ mψGAmψGGGAmψmψGCmψACAGGGCmψCCmψGGmψGCmψGAAGGACCmψGGGCAmψCCAAGmψG GACCGCmψACAmψCGCCmψCCGAGGmψGmψGmψGAGGACmψCCAmψCACGGmψGGGCAmψGGmψ GCGGCACCAGGGAAAGAmψCAmψGmψACGmψCGGGGACGmψCCGCAGCGmψCACACAGAAGCAm ψAmψCCAGGAGmψGGGGCCCAmψmψCGACCmψGGmψGAmψmψGGAGGCAGmψCCCmψGCAACGA CCmψCmψCCAmψmψGmψCAACCCmψGCCCGCAAGGGACmψmψmψAmψGAGGGmψACmψGGCCGC CmψCmψmψCmψmψmψGAGmψmψCmψACCGCCmψCCmψGCAmψGAmψGCGCGGCCCAAGGAGGGA GAmψGAmψCGCCCCmψmψCmψmψCmψGGCmψCmψmψmψGAGAAmψGmψGGmψGGCCAmψGGGCG mψmψAGmψGACAAGAGGGACAmψCmψCGCGAmψmψmψCmψmψGAGmψCmψAACCCCGmψGAmψG AmψmψGACGCCAAAGAAGmψGmψCmψGCmψGCACACAGGGCCCGmψmψACmψmψCmψGGGGmψA ACCmψmψCCmψGGCAmψGAACAGGCCmψmψmψGGCAmψCCACmψGmψGAAmψGAmψAAGCmψGG AGCmψGCAAGAGmψGmψCmψGGAGCACGGCAGAAmψAGCCAAGmψmψCAGCAAAGmψGAGGACC AmψmψACCACCAGGmψCAAACmψCmψAmψAAAGCAGGGCAAAGACCAGCAmψmψmψCCCCGmψC mψmψCAmψGAACGAGAAGGAGGACAmψCCmψGmψGGmψGCACmψGAAAmψGGAAAGGGmψGmψm ψmψGGCmψmψCCCCGmψCCACmψACACAGACGmψGmψCCAACAmψGAGCCGCmψmψGGCGAGGC AGAGACmψGCmψGGGCCGGmψCGmψGGAGCGmψGCCGGmψCAmψCCGCCACCmψCmψmψCGCmψ CCGCmψGAAGGAAmψAmψmψmψmψGCmψmψGmψGmψGmψCmψAGCGGCAAmψAGmψAACGCmψA ACAGCCGCGGGCCGAGCmψmψCAGCAGCGGCCmψGGmψGCCGmψmψAAGCmψmψGCGCGGCAGC CAmψAmψGGGCCCmψAmψGGAGAmψAmψACAAGACAGmψGmψCmψGCAmψGGAAGAGACAGCCA GmψGCGGGmψACmψGAGCCmψCmψmψCAGAAACAmψCGACAAGGmψACmψAAAGAGmψmψmψGG GCmψmψCmψmψGGAAAGCGGmψmψCmψGGmψmψCmψGGGGGAGGAACGCmψGAAGmψACGmψGG AAGAmψGmψCACAAAmψGmψCGmψGAGGAGGGACGmψGGAGAAAmψGGGGCCCCmψmψmψGACC mψGGmψGmψACGGCmψCGACGCAGCCCCmψAGGCAGCmψCmψmψGmψGAmψCGCmψGmψCCCGG CmψGGmψACAmψGmψmψCCAGmψmψCCACCGGAmψCCmψGCAGmψAmψGCGCmψGCCmψCGCCA GGAGAGmψCAGCGGCCCmψmψCmψmψCmψGGAmψAmψmψCAmψGGACAAmψCmψGCmψGCmψGA CmψGAGGAmψGACCAAGAGACAACmψACCCGCmψmψCCmψmψCAGACAGAGGCmψGmψGACCCm ψCCAGGAmψGmψCCGmψGGCAGAGACmψACCAGAAmψGCmψAmψGCGGGmψGmψGGAGCAACAm ψmψCCAGGGCmψGAAGAGCAAGCAmψGCGCCCCmψGACCCCAAAGGAAGAAGAGmψAmψCmψGC AAGCCCAAGmψCAGAAGCAGGAGCAAGCmψGGACGCCCCGAAAGmψmψGACCmψCCmψGGmψGA AGAACmψGCCmψmψCmψCCCGCmψGAGAGAGmψACmψmψCAAGmψAmψmψmψmψmψCmψCAAAA CmψCACmψmψCCmψCmψmψGGAGGGCCGAGCmψCmψGGCGCACCCCCACCAAGmψGGAGGGmψC mψCCmψGCCGGGmψCCCCAACAmψCmψACmψGAAGAAGGCACCAGCGAAmψCCGCAACGCCCGA GmψCAGGCCCmψGGmψACCmψCCACAGAACCAmψCmψGAAGGmψAGmψGCGCCmψGGmψmψCCC CAGCmψGGAAGCCCmψACmψmψCCACCGAAGAAGGCACGmψCAACCGAACCAAGmψGAAGGAmψ CmψGCCCCmψGGGACCAGCACmψGAACCAmψCmψGAGGGCGGmψmψCCGGCGGAGGAAGCGCmψ CAAGAGAmψCAAGAGAAmψCAACAAGAmψCAGAAGGAGACmψGGmψCAAGGACAGCAACACAAA GAAGGCCGGCAAGACAGGCCCCAmψGAAAACCCmψGCmψCGmψCAGAGmψGAmψGACCCCmψGA CCmψGAGAGAGCGGCmψGGAAAACCmψGAGAAAGAAGCCCGAGAACAmψCCCmψCAGCCmψAmψ CAGCAACACCAGCAGGGCCAACCmψGAACAAGCmψGCmψGACCGACmψACACCGAGAmψGAAGA AAGCCAmψCCmψGCACGmψGmψACmψGGGAAGAGmψmψCCAGAAAGACCCCGmψGGGCCmψGAm ψGAGCAGAGmψmψGCmψCAGCCmψGCCAGCAAGAAGAmψCGACCAGAACAAGCmψGAAGCCCGA GAmψGGACGAGAAGGGCAAmψCmψGACCACAGCCGGCmψmψmψGCCmψGCmψCmψCAGmψGmψG GCCAGCCmψCmψGmψmψCGmψGmψACAAGCmψGGAACAGGmψGmψCCGAGAAAGGCAAGGCCmψ ACACCAACmψACmψmψCGGCAGAmψGmψAACGmψGGCCGAGCACGAGAAGCmψGAmψmψCmψGC mψGGCCCAGCmψGAAACCmψGAGAAGGACmψCmψGAmψGAGGCCGmψGACCmψACAGCCmψGGG CAAGmψmψmψGGACAGAGAGCCCmψGGACmψmψCmψACAGCAmψCCACGmψGACCAAAGAAAGC ACACACCCCGmψGAAGCCCCmψGGCmψCAGAmψCGCCGGCAAmψAGAmψACGCCmψCmψGGACC mψGmψGGGCAAAGCCCmψGmψCCGAmψGCCmψGCAmψGGGAACAAmψCGCCAGCmψmψCCmψGA	59610

ELXR ID	RNA sequence	SEQ ID NO
	GCAAGmψACCAGGACAmψCAmψCAmψCGAGCACCAGAAGGmψGGmψCAAGGGCAACCAGAAGAG ACmψGGAAAGCCmψGAGGGAGCmψGGCCGGCAAAGAGAACCmψGGAAmψACCCCAGCGmψGACC CmψGCCmψCCmψCAGCCmψCACACAAAAGAAGGCGmψGGACGCCmψACAACGAAGmψGAmψCGC CAGAGmψGAGAAmψGmψGGGmψCAACCmψGAACCmψGmψGGCAGAAGCmψGAAACmψGmψCCAG GGACGACGCCAAGCCmψCmψGCmψGAGACmψGAAGGGCmψmψCCCmψAGCmψmψCCCmψCmψGG mψGGAAAGACAGGCCAAmψGAAGmψGGAmψmψGGmψGGGACAmψGGmψCmψGCAACGmψGAAGA AGCmψGAmψCAACGAGAAGAAAGAGGAmψGGCAAGGmψmψmψmψCmψGGCAGAACCmψGGCCGG CmψACAAGAGACAAGAAGCCCmψGAGGCCmψmψACCmψGAGCAGCGAAGAGGACCGGAAGAAGG GCAAGAAGmψmψCGCCAGAmψACCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAAAAGAAG CACGGCGAGGACmψGGGGCAAAGmψGmψACGAmψGAGGCCmψGGGAGAGAAmψCGACAAGAAGG mψGGAAGGCCmψGAGCAAGCACAmψmψAAGCmψGGAAGAGGAAAGAAGGAGCGAGGACGCCCAA mψCmψAAAGCCGCmψCmψGACCGAmψmψGGCmψGAGAGCCAAGGCCAGCmψmψmψGmψGAmψCG AGGGCCmψGAAAGAGGCCGACAAGGACGAGmψmψCmψGCAGAmψGCGAGCmψGAAGCmψGCAGA AGmψGGmψACGGCGAmψCmψGAGAGGCAAGCCCmψmψCGCCAmψmψGAGGCCGAGAACAGCAmψ CCmψGGACAmψCAGCGGCmψmψCAGCAAGCAGmψACAACmψGCGCCmψmψCAmψmψmψGGCAGA AAGACGGCGmψCAAGAAACmψGAACCmψGmψACCmψGAmψCAmψCAAmψmψACmψmψCAAAGGC GGCAAGCmψGCGGmψmψCAAGAAGAmψCAAACCCGAGGCCmψmψCGAGGCmψAACAGAmψmψCm ψACACCGmψGAmψCAACAAAAAGmψCCGGCGAGAmψCGmψGCCCAmψGGAAGmψGAACmψmψCA ACmψmψCGACGACCCCAACCmψGAmψmψAmψCCmψGCCmψCmψGGCCmψmψCGGCAAGAGACAG GGCAGAGAGmψmψCAmψCmψGGAACGAmψCmψGCmψGAGCCmψGGAAACCGGCmψCmψCmψGAA GCmψGGCCAAmψGGCAGAGmψGAmψCGAGAAAACCCmψGmψACAACAGGAGAACCAGACAGGAC GAGCCmψGCmψCmψGmψmψmψGmψGGCCCmψGACCmψmψCGAGAGAAGAGAGGmψGCmψGGACA GCAGCAACAmψCAAGCCCAmψGAACCmψGAmψCGGCGmψGGCCCGGGGCGAGAAmψAmψCCCmψ GCmψGmψGAmψCGCCCmψGACAGACCCmψGAAGGAmψGCCCACmψGAGCAGAmψmψCAAGGACm ψCCCmψGGGCAACCCmψACACACAmψCCmψGAGAAmψCGGCGAGAGCmψACAAAGAGAAGCAGA GGACAAmψCCAGGCCAAGAAAGAGGmψGGAACAGAGAAGAGCCGGCGGAmψACmψCmψAGGAAG mψACGCCAGCAAGGCCAAGAAmψCmψGGCCGACGACAmψGGmψCCGAAACACCGCCAGAGAmψC mψGCmψGmψACmψACGCCGmψGACACAGGACGCCAmψGCmψGAmψCmψmψCGCGAAmψCmψGAG CAGAGGCmψmψCGGCCGGCAGGGCAAGAGAACCmψmψmψAmψGGCCGAGAGGCAGmψACACCAG AAmψGGAAGAmψmψGGCmψCACAGCmψAAACmψGGCCmψACGAGGGACmψGAGCAAGACCmψAC CmψGmψCCAAAACACmψGGCCCAGmψAmψACCmψCCAAGACCmψGCAGCAAmψmψGCGGCmψmψ CACCAmψCACCAGCGCCGACmψACGACAGAGmψGCmψGGAAAAGCmψCAAGAAAACCGCCACCG GCmψGGAmψGACCACCAmψCAACGGCAAAGAGCmψGAAGGmψmψGAGGGCCAGAmψCACCmψAC mψACAACAGGmψACAAGAGGCAGAACGmψCGmψGAAGGAmψCmψGAGCGmψGGAACmψGGACAG ACmψGAGCGAAGAGAGCGmψGAACAACGACAmψCAGCAGCmψGGACAAAGGGCAGAmψCAGGCG AGGCmψCmψGAGCCmψGCmψGAAGAAGAGGmψmψmψAGCCACAGACCmψGmψGCAAGAGAAGmψ mψCGmψGmψGCCmψGAACmψGCGGCmψmψCGAGACACACGCCGCmψGAACAGGCmψGCCCmψGA ACAmψmψGCCAGAAGCmψGGCmψGmψmψCCmψGAGAAGCCAAGAGmψACAAGAAGmψACCAGAC CAACAAGACCACCGGCAACACCGACAAGAGGGCCmψmψmψGmψGGAAACCmψGGCAGAGCmψmψ CmψACAGAAAAAAGCmψGAAAGAAGmψCmψGGAAGCCCGCCGmψGCGAmψCGGGCGGmψmψCCG GCGGAGGmψmψCCACmψAGmψAmψGAACAAmψmψCCCAGGGAAGAGmψGACCmψmψCGAGGAmψ GmψCACmψGmψGAACmψmψCACCCAGGGGGAGmψGGCAGCGGCmψGAAmψCCCGAACAGAGAAA CmψmψGmψACAGGGAmψGmψGAmψGCmψGGAGAAmψmψACAGCAACCmψmψGmψCmψCmψGmψG GGACAAGGGGAAACCACCAAACCCGAmψGmψGAmψCmψmψGAGGmψmψGGAACAAGGAAAGGAG CCAmψGGmψmψGGAGGAAGAGGAAGmψGCmψGGGAAGmψGGCCGmψGCAGAAAAAAAmψGGGGA CAmψmψGGAGGGCAGAmψmψmψGGAAGCCAAAGGAmψGmψGAAAGAGAGmψCmψCACmψAGmψC CAAAAAAGAAGAGAAAGGmψAGAmψmψACAAAGAmψGACGAmψGACAAAGACmψACAAGGAmψG AmψGAmψGAmψAAGGGAmψCCGGCmψGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAA	

ELXR ID	RNA sequence	SEQ ID NO
ELXR #5 - ZIM3-KRAB	GACCGGCCGCCACCAmψGGCCCCAAAGAAGAAGCGGAAGGmψCmψCmψAGAAmψGAACCAmψGA CCAGGAAmψmψmψGACCCCCCAAAGGmψmψmψACCCACCmψGmψGCCAGCmψGAGAAGAGGAAG CCCAmψCCGCGmψGCmψGmψCmψCmψCmψmψmψGAmψGGGAmψmψGCmψACAGGGCmψCCmψGG mψGCmψGAAGGACCmψGGGCAmψCCAAGmψGGACCGCmψACAmψCGCCmψCCGAGGmψGmψGmψ GAGGACmψCCAmψCACGGmψGGGCAmψGGmψGCGGCACCAGGGAAAGAmψCAmψGmψACGmψCG GGGACGmψCCGCAGCGmψCACACAGAAGCAmψAmψCCAGGAGmψGGGGCCCAmψmψCGACCmψG GmψGAmψmψGGAGGCAGmψCCCmψGCAACGACCmψCmψCCAmψmψGmψCAACCCmψGCCCGCAA GGGACmψmψmψAmψGAGGGmψACmψGGCCGCCmψCmψmψCmψmψmψGAGmψmψCmψACCGCCmψ CCmψGCAmψGAmψGCGCGGCCCAAGGAGGGAGAmψGAmψCGCCCCmψmψCmψmψCmψGGCmψCm ψmψmψGAGAAmψGmψGGmψGGCCAmψGGGCGmψmψAGmψGACAAGAGGGACAmψCmψCGCGAmψ mψmψCmψmψGAGmψCmψAACCCCGmψGAmψGAmψmψGACGCCAAAGAAGmψGmψCmψGCmψGCA CACAGGGCCCGmψmψACmψmψCmψGGGGmψAACCmψmψCCmψGGCAmψGAACAGGCCmψmψmψG GCAmψCCACmψGmψGAAmψGAmψAAGCmψGGAGCmψGCAAGAGmψGmψCmψGGAGCACGGCAGA AmψAGCCAAGmψmψCAGCAAAGmψGAGGACCAmψmψACCACCAGGmψCAAACmψCmψAmψAAAG CAGGGCAAAGACCAGCAmψmψmψCCCCGmψCmψmψCAmψGAACGAGAAGGAGGACAmψCCmψGm ψGGmψGCACmψGAAAmψGGAAAGGGmψGmψmψmψGGCmψmψCCCCGmψCCACmψACACAGACGm ψGmψCCAACAmψGAGCCGCmψmψGGCGAGGCAGAGACmψGCmψGGGCCGGmψCGmψGGAGCGmψ GCCGGmψCAmψCCGCCACCmψCmψmψCGCmψCCGCmψGAAGGAAmψAmψmψmψGCmψmψGmψ GmψGmψCmψAGCGGCAAmψAGmψAACGCmψAACAGCCGCGGGCCGAGCmψmψCAGCAGCGGCCm ψGGmψGCCGmψmψAAGCmψmψGCGCGGCAGCCAmψAmψGGGCCCmψAmψGGAGAmψAmψACAAG ACAGmψGmψCmψGCAmψGGAAGAGACAGCCAGmψGCGGGmψACmψGAGCCmψCmψmψCAGAAAC AmψCGACAAGGmψACmψAAAGAGmψmψmψGGGCmψmψCmψmψGGAAAGCGGmψmψCmψGGmψmψ CmψGGGGGAGGAACGCmψGAAGmψACGmψGGAAGAmψGmψCACAAAmψGmψCGmψGAGGAGGGA CGmψGGAGAAAmψGGGGCCCCmψmψmψGACCmψGGmψGmψACGGCmψCGACGCAGCCCCmψAGG CAGCmψCmψmψGmψGAmψCGCmψGmψCCCGGCmψGGmψACAmψGmψmψCCAGmψmψCCACCGGA mψCCmψGCAGmψAmψGCGCmψGCCmψCGCCAGGAGAGmψCAGCGGCCCmψmψCmψmψCmψGGAm ψAmψmψCAmψGGACAAmψCmψGCmψGCmψGACmψGAGGAmψGACCAAGAGACAACmψACCCGCm ψmψCCmψmψCAGACAGAGGCmψGmψGACCCmψCCAGGAmψGmψCCGmψGGCAGAGACmψACCAG AAmψGCmψAmψGCGGGmψGmψGGAGCAACAmψmψCCAGGGCmψGAAGAGCAAGCAmψGCGCCCC mψGACCCCAAAGGAAGAAGAGmψAmψCmψGCAAGCCCAAGmψCAGAAGCAGGAGCAAGCmψGGA CGCCCCGAAAGmψmψGACCmψCCmψGGmψGAAGAACmψGCCmψmψCmψCCCGCmψGAGAGAGmψ ACmψmψCAAGmψAmψmψmψmψmψCmψCAAAACmψCACmψmψCCmψCmψmψGGCGGmψmψCCGGC GGAGGAAmψGAACAAmψmψCCCAGGGAAGAGmψGACCmψmψCGAGGAmψGmψCACmψGmψGAAC mψmψCACCCAGGGGGAGmψGGCAGCGGCmψGAAmψCCCGAACAGAGAAACmψmψGmψACAGGGA mψGmψGAmψGCmψGGAGAAmψmψACAGCAACCmψmψGmψCmψCmψGmψGGGACAAGGGGAAACC ACCAAACCCGAmψGmψGAmψCmψmψGAGGmψmψGGAACAAGGAAAGGAGCCAmψGGmψmψGGAG GAAGAGGAAGmψGCmψGGGAAGmψGGCCGmψGCAGAAAAAAAmψGGGGACAmψmψGGAGGGCAG Amψmψmψ GGAAGCCAAAGGAmψGmψGAAAGAGAGmψCmψCGGAGGGCCGAGCmψCmψGGCGCAC CCCCACCAAGmψGGAGGGmψCmψCCmψGCCGGGmψCCCCAACAmψCmψACmψGAAGAAGGCACC AGCGAAmψCCGCAACGCCCGAGmψCAGGCCCmψGGmψACCmψCCACAGAACCAmψCmψGAAGGm ψAGmψGCGCCmψGGmψmψCCCCAGCmψGGAAGCCCmψACmψmψCCACCGAAGAAGGCACGmψCA ACCGAACCAAGmψGAAGGAmψCmψGCCCCmψGGGACCAGCACmψGAACCAmψCmψGAGCAAGAG AmψCAAGAGAAmψCAACAAGAmψCAGAAGGAGACmψGGmψCAAGGACAGCAACACAAAGAAGGC CGGCAAGACAGGCCCCAmψGAAAACCCmψGCmψCGmψCAGAGmψGAmψGACCCCmψGACCmψGA GAGAGCGGCmψGGAAAACCmψGAGAAAGAAGCCCGAGAACAmψCCCmψCAGCCmψAmψCAGCAA CACCAGCAGGGCCAACCmψGAACAAGCmψGCmψGACCGACmψACACCGAGAmψGAAGAAAGCCA mψCCmψGCACGmψGmψACmψGGGAAGAGmψmψCCAGAAAGACCCCGmψGGGCCmψGAmψGAGCA GAGmψmψGCmψCAGCCmψGCCAGCAAGAAGAmψCGACCAGAACAAGCmψGAAGCCCGAGAmψGG ACGAGAAGGGCAAmψCmψGACCACAGCCGGCmψmψmψGCCmψGCmψCmψCAGmψGmψGGCCAGC CmψCmψGmψmψCGmψGmψACAAGCmψGGAACAGGmψGmψCCGAGAAAGGCAAGGCCmψACACCA ACmψACmψmψCGGCAGAmψGmψAACGmψGGCCGAGCACGAGAAGCmψGAmψmψCmψGCmψGGCC CAGCmψGAAACCmψGAGAAGGACmψCmψGAmψGAGGCCGmψGACCmψACAGCCmψGGGCAAGmψ mψmψGGACAGAGAGCCCmψGGACmψmψCmψACAGCAmψCCACGmψGACCAAAGAAAGCACACAC CCCGmψGAAGCCCCmψGGCmψCAGAmψCGCCGGCAAmψAGAmψACGCCmψCmψGGACCmψGmψG GGCAAAGCCCmψGmψCCGAmψGCCmψGCAmψGGGAACAAmψCGCCAGCmψmψCCmψGAGCAAGm	59611

ELXR ID	RNA sequence	SEQ ID NO
	ψACCAGGACAmψCAmψCAmψCGAGCACCAGAAGGmψGGmψCAAGGGCAACCAGAAGAGACmψGG AAAGCCmψGAGGGAGCmψGGCCGGCAAAGAGAACCmψGGAAmψACCCCAGCGmψGACCCmψGCC mψCCmψCAGCCmψCACACAAAAGAAGGCGmψGGACGCCmψACAACGAAGmψGAmψCGCCAGAGm ψGAGAAmψGmψGGGmψCAACCmψGAACCmψGmψGGCAGAAGCmψGAAACmψGmψCCAGGGACGA CGCCAAGCCmψCmψGCmψGAGACmψGAAGGGCmψmψCCCmψAGCmψmψCCCmψCmψGGmψGGAA AGACAGGCCAAmψGAAGmψGGAmψmψGGmψGGGACAmψGGmψCmψGCAACGmψGAAGAAGCmψG AmψCAACGAGAAGAAAGAGGAmψGGCAAGGmψmψmψmψCmψGGCAGAACCmψGGCCGGCmψACA AGAGACAAGAAGCCCmψGAGGCCmψmψACCmψGAGCAGCGAAGAGGACCGGAAGAAGGGCAAGA AGmψmψCGCCAGAmψACCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAAAAGAAGCACGGC GAGGACmψGGGGCAAAGmψGmψACGAmψGAGGCCmψGGGAGAGAAmψCGACAAGAAGGmψGGAA GGCCmψGAGCAAGCACAmψmψAAGCmψGGAAGAGGAAAGAAGGAGCGAGGACGCCCAAmψCmψA AAGCCGCmψCmψGACCGAmψmψGGCmψGAGAGCCAAGGCCAGCmψmψmψGmψGAmψCGAGGGCC mψGAAAGAGGCCGACAAGGACGAGmψmψCmψGCAGAmψGCGAGCmψGAAGCmψGCAGAAGmψGG mψACGGCGAmψCmψGAGAGGCAAGCCCmψmψCGCCAmψmψGAGGCCGAGAACAGCAmψCCmψGG ACAmψCAGCGGCmψmψCAGCAAGCAGmψACAACmψGCGCCmψmψCAmψmψmψGGCAGAAAGACG GCGmψCAAGAAACmψGAACCmψGmψACCmψGAmψCAmψCAAmψmψACmψmψCAAAGGCGGCAAG CmψGCGGmψmψCAAGAAGAmψCAAACCCGAGGCCmψmψCGAGGCmψAACAGAmψmψCmψACACC GmψGAmψCAACAAAAAGmψCCGGCGAGAmψCGmψGCCCAmψGGAAGmψGAACmψmψCAACmψmψ CGACGACCCCAACCmψGAmψmψAmψCCmψGCCmψCmψGGCCmψmψCGGCAAGAGACAGGGCAGA GAGmψmψCAmψCmψGGAACGAmψCmψGCmψGAGCCmψGGAAACCGGCmψCmψCmψGAAGCmψGG CCAAmψGGCAGAGmψGAmψCGAGAAAACCCmψGmψACAACAGGAGAACCAGACAGGACGAGCCm ψGCmψCmψGmψmψmψGmψGGCCCmψGACCmψmψCGAGAGAAGAGAGGmψGCmψGGACAGCAGCA ACAmψCAAGCCCAmψGAACCmψGAmψCGGCGmψGGCCCGGGGCGAGAAmψAmψCCCmψGCmψGm ψGAmψCGCCCmψGACAGACCCmψGAAGGAmψGCCCACmψGAGCAGAmψmψCAAGGACmψCCCmψ GGGCAACCCmψACACACAmψCCmψGAGAAmψCGGCGAGAGCmψACAAAGAGAAGCAGAGGACAA mψCCAGGCCAAGAAAGAGGmψGGAACAGAGAAGAGCCGGCGGAmψACmψCmψAGGAAGmψACGC CAGCAAGGCCAAGAAmψCmψGGCCGACGACAmψGGmψCCGAAACACCGCCAGAGAmψCmψGCmψ GmψACmψACGCCGmψGACACAGGACGCCAmψGCmψGAmψCmψmψCGCGAAmψCmψGAGCAGAGG CmψmψCGGCCGGCAGGGCAAGAGAACCmψmψmψAmψGGCCGAGAGGCAGmψACACCAGAAmψGG AAGAmψmψGGCmψCACAGCmψAAACmψGGCCmψACGAGGGACmψGAGCAAGACCmψACCmψGmψ CCAAAACACmψGGCCCAGmψAmψACCmψCCAAGACCmψGCAGCAAmψmψGCGGCmψmψCACCAm ψCACCAGCGCCGACmψACGACAGAGmψGCmψGGAAAAGCmψCAAGAAAACCGCCACCGGCmψGG AmψGACCACCAmψCAACGGCAAAGAGCmψGAAGGmψmψGAGGGCCAGAmψCACCmψACmψACAA CAGGmψACAAGAGGCAGAACGmψCGmψGAAGGAmψCmψGAGCGmψGGAACmψGGACAGACmψGA GCGAAGAGAGCGmψGAACAACGACAmψCAGCAGCmψGGACAAAGGGCAGAmψCAGGCGAGGCmψ CmψGAGCCmψGCmψGAAGAAGAGGmψmψmψAGCCACAGACCmψGmψGCAAGAGAAGmψmψCGmψ GmψGCCmψGAACmψGCGGCmψmψCGAGACACACGCCGCmψGAACAGGCmψGCCCmψGAACAmψm ψGCCAGAAGCmψGGCmψGmψmψCCmψGAGAAGCCAAGAGmψACAAGAAGmψACCAGACCAACAA GACCACCGGCAACACCGACAAGAGGGCCmψmψmψGmψGGAAACCmψGGCAGAGCmψmψCmψACA GAAAAAAGCmψGAAAGAAGmψCmψGGAAGCCCGCCGmψGCGAmψCGGGCGGmψmψCCGGCGGAG GmψmψCCACmψAGmψCCAAAAAAGAAGAGAAAGGmψAGAmψmψACAAAGAmψGACGAmψGACAA AGACmψACAAGGAmψGAmψGAmψGAmψAAGGGAmψCCGGCmψGAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	

[0602] For experiment #2, synthesis of *PCSK9*-targeting gRNAs was performed as described above for experiment #1, and the sequences of the targeting spacers are listed in Table 39. For pairing with dCas9-ZNF10-DNMT3A/3L, targeting spacers were as follows: 1) 7.148 (*B2M*, as non-targeting control; SEQ ID NO: 57645), 27.126 (*PCSK9*; CACGCCACCCCGAGCCCCAU;

SEQ ID NO: 60013), and 27.128 (*PCSK9*; CAGCCUGCGCGUCCACGUGA; SEQ ID NO: 60014).

Transfection of mRNA and gRNA into Hepa1-6 cells and intracellular PCSK9 staining:

[0603] Seeded Hepa1-6 cells treated with the NATE™ inhibitor were lipofected with 300 ng of mRNA encoding ELXR #1 with the ZIM3-KRAB, ELXR #5 with the ZIM3-KRAB, catalytically-active CasX 491, or dCas9-ZNF10-DNMT3A/3L, and 150 ng of *PCSK9*-targeting gRNA (Table 39). Intracellular levels of PCSK9 protein were measured at day 7 and day 14 post-transfection using an intracellular staining protocol as described earlier for experiment #1.

Results:

[0604] In experiment #1, mRNAs encoding dXR1 or ELXR #1 containing the ZIM3-KRAB domain were co-transfected with a *PCSK9*-targeting gRNA into mouse Hepa1-6 cells to assess their ability to induce PCSK9 knockdown by silencing the mouse *PCSK9* locus. The quantification of the resulting PCSK9 knockdown is shown in FIGS. 28-30. The data demonstrate that at day 6, use of six out of seven gRNAs targeting the mouse *PCSK9* locus with ELXR #1 mRNA resulted in >50% knockdown of intracellular PCSK9, with the top spacer 27.94 achieving >80% repression level (FIG. 28). A similar trend was observed with use of dXR1 mRNA at day 6, although the degree of repression was less substantial when paired with certain spacers, such as spacer 27.92 and 27.100 (FIG. 28). The results also demonstrate that use of ELXR #1 mRNA led to sustained repression of the *PCSK9* locus through at least 25 days, with use of the top spacers 27.94 and 27.88 showing the strongest permanence in silencing *PCSK9* (FIG. 30). However, the PCSK9 repression mediated by dXR1 that was observed at day 6 reverted to similar levels of PCSK9 as detected with the non-targeting control (spacer 6.7) by day 13; such transient repression was noticeable for all gRNAs assayed that targeted the mouse *PCSK9* gene (FIG. 29).

[0605] In experiment #2, mRNAs encoding ELXR #1 or ELXR #5 containing the ZIM3-KRAB domain, dCas9-ZNF10-DNMT3A/3L, or catalytically active CasX491 were co-transfected with a *PCSK9*-targeting gRNA into mouse Hepa1-6 cells to assess their ability to induce PCSK9 knockdown by silencing the mouse *PCSK9* locus. The quantification of the resulting PCSK9 repression is shown in FIGS. 31-33. The data demonstrate that delivery of IVT-produced ELXR #1 or ELXR #5 mRNA resulted in comparable levels of sustained PCSK9 knockdown when paired with a targeting gRNA with the top spacer 27.94 (>70%), while use of gRNA with spacer 27.88 resulted in slightly higher repression with ELXR #1 than with ELXR

#5 (FIGS. 31-33). Furthermore, third-party-produced mRNA encoding ELXR #1 and dCas9-ZNF10-DNMT3A/3L led to similar levels (>70%) of durable PCSK9 knockdown when paired with gRNAs containing the top spacers (FIGS. 31-33).

[0606] These experiments demonstrate that ELXR molecules, having different configurations, can induce heritable silencing of an endogenous locus in a mouse liver cell line. Meanwhile, as anticipated, use of dXR constructs result in efficient repression of the target locus at early timepoints, but their use does not lead to durable silencing. These findings also show that dXR and ELXR molecules (of different configurations) can be delivered as mRNA and co-transfected with a targeting gRNA to cells, indicating that the transient nature of this delivery modality is still sufficient to induce silencing.

Example 15: ELXR mRNA and targeting gRNA can be delivered via LNPs to achieve repression of target locus in vitro

[0607] Experiments will be performed to demonstrate that delivery of lipid nanoparticles (LNPs) encapsulating ELXR mRNA and targeting gRNA will induce durable repression of a target endogenous locus in a cell-based assay.

Materials and Methods:

Generation of ELXR mRNAs:

[0608] mRNA encoding an ELXR molecule will be generated by IVT, as described earlier in Example 14. Sequences encoding the ELXR molecule will be codon-optimized as briefly described in Example 14. Examples of DNA sequences encoding ELXR mRNA are listed in Table 36 and Table 40, with the corresponding mRNA sequences listed in Table 37 and Table 41. Additional examples of DNA sequences encoding ELXR mRNA are presented in Table 42 below, with their corresponding mRNA sequences shown in Table 43.

Table 42: Encoding sequences of additional ELXR mRNA molecules that may be assessed*.

ELXR ID	Component	DNA SEQ ID NO
ELXR1-ZIM3_vs2	5'UTR	59568
	START codon + NLS + linker	59612
	START codon + DNMT3A catalytic domain	59580
	Linker	59581
	DNMT3L interaction domain	59582

ELXR ID	Component	DNA SEQ ID NO
	Linker	59583
	dCasX491	59570
	Linker	59571
	ZIM3-KRAB	59572
	Buffer sequence + NLS	59613
	STOP codons + buffer sequence	59575
	3'UTR	59576
	Buffer sequence	59577
	Poly(A) tail	59578
ELXR5-ZIM3	5'UTR	59568
	START codon + NLS + linker	59614
	START codon + DNMT3A catalytic domain	59580
	Linker	59581
	DNMT3L interaction domain	59582
	Linker	59615
	ZIM3-KRAB	59572
	Linker	59616
	dCasX491	59570
	Buffer + linker	59617
	NLS + STOP codon + buffer sequence	59618
	3'UTR	59576
	Buffer sequence	59577
	Poly(A) tail	59618
ELXR5-ZIM3 + ADD	5'UTR	59568
	START codon + NLS + linker	59614
	START codon + DNMT3A ADD domain	59620
	DNMT3A catalytic domain	59621
	Linker	59581
	DNMT3L interaction domain	59582
	Linker	59615
	ZIM3-KRAB	59572
	Linker	59616
	dCasX491	59570
	Buffer + linker	59617
	NLS + STOP codon + buffer sequence	59618
	3'UTR	59576
	Buffer sequence	59577
	Poly(A) tail	59619

*Components are listed in a 5' to 3' order within the constructs

Table 43: Full-length RNA sequences of additional ELXR mRNA molecules in Table 42 for assessment. Modification 'mψ' = N1-methyl-pseudouridine.

ELXR ID	RNA sequence	SEQ ID NO
ELXR1-ZIM3_vs2	AAAmψAAGAGAGAAAAGAAGAGmψAAGAAGAAAmψAmψAAGAGCCACCAmψGGCCCCCGCCGCC AAGAGAGmψGAAGCmψGGAmψmψCCCGGGmψGAAmψGGCAGCGGCAGCGGGGGCGGCAmψGAAC CACGACCAGGAGmψmψCGACCCCCCmψAAGGmψGmψACCCmψCCCGmψCCCCGCCGAGAAGAGA AAGCCCAmψCCGGGmψCCmψGAGCCmψGmψmψCGAmψGGCAmψCGCCACCGGmψCmψGCmψGGm ψGCmψGAAGGACCmψGGGCAmψCCAGGmψGGAmψAGGmψACAmψmψGCCmψCCGAGGmψGmψGC GAGGACmψCCAmψCACCGmψGGGAAmψGGmψGCGmψCAmψCAGGGCAAGAmψCAmψGmψACGmψ GGGCGACGmψGCGGAGCGmψGACACAGAAGCAmψAmψCCAGGAGmψGGGGCCCmψmψmψCGACC mψGGmψGAmψCGGCGGCAGCCCmψmψGCAAmψGACCmψGAGCAmψCGmψGAACCCAGCCCGGAA GGGCCmψGmψACGAGGGAACCGGCAGACmψGmψmψCmψmψCGAGmψmψmψmψACAGACmψGCmψ GCACGACGCCCGGCCmψAAGGAAGGCGACGACCGGCCCmψmψCmψmψmψGGCmψGmψmψCGA GAAmψGmψGGmψGGCCAmψGGGAGmψCAGCGACAAGCGGGAmψAmψmψAGCCGGmψmψCCmψGG AGAGCAACCCCGmψGAmψGAmψCGAmψGCCAAGGAAGmψGAGCGCCGCCCACCGGGCCAGAmψA CmψmψCmψGGGGCAAmψCmψGCCmψGGCAmψGAACAGACCCCmψGGCCAGCACCGmψGAACGAC AAGCmψGGAGCmψGCAGGAGmψGCCmψGGAGCACGGCCGGAmψCGCCAAGmψmψCAGCAAGGmψ GAGAACCAmψCACCACCCGAAGCAACAGCAmψCAAACAAGGCAAGGACCAGCACmψmψmψCCmψ GmψGmψmψCAmψGAACGAGAAGGAGGACAmψCCmψGmψGGmψGmψACCGAGAmψGGAGAGAGmψ GmψmψCGGGmψmψCCCAGmψCCACmψACACAGAmψGmψCAGCAACAmψGmψCmψAGACmψGGCC AGACAGAGACmψGCmψGGGAAGAAGCmψGGmψCCGmψCCCmψGmψGAmψCAGACACCmψGmψmψ CGCCCCmψCmψGAAGGAGmψACmψmψCGCCmψGCGmψGAGCAGCGGCAACAGCAACGCCAACAG CCGGGGCCCCAGCmψmψCmψCmψAGCGGCCmψGGmψGCCACmψGmψCCCmψGAGAGGGAGCCCAC AmψGGGCCCCAmψGGAGAmψCmψACAAAACCGmψGAGCGCCmψGGAAGCGGCAGCCmψGmψGCG CGmψGCmψGAGCCmψGmψmψmψCGGAAmψAmψCGAmψAAAGmψCCmψGAAAAGCCmψGGGAmψm ψCCmψGGAGAGCGGCmψCmψGGCmψCCGGCGGmψGGCACCCmψGAAGmψACGmψGGAGGAmψGm ψGACAAACGmψGGmψCAGACGGGAmψGmψGGAGAAGmψGGGGCCCCmψmψCGAmψCmψGGmψGm ψACGGCAGCACCCAACCCCmψGGGCAGCmψCmψmψGmψGACCGGmψGCCCmψGGCmψGGmψACA mψGmψmψmψCAGmψmψCCACCGGAmψCCmψGCAGmψACGCCCmψGCCGAGACAGGAGmψCCCAG CGGCCAmψmψCmψmψmψmψGGAmψmψmψmψCAmψGGACAACmψmψGCmψGCmψGACCGAGGAmψ GACCAGGAAACmψACCACmψCGGmψmψCCmψGCAGACCGAAGCCGmψGACCCmψGCAGGACGmψ GAGAGGCCGGGACmψACCAGAACGCCAmψGCGGGmψGmψGGmψCCAACAmψCCCmψGGACmψGA AAAGCAAGCACGCACCmψCmψGACCCCmψAAAGAAGAGGAGmψACCmψGCAGGCCCAGGmψGCG GAGCAGAAGCAAGCmψGGACGCCCCmψAAGGmψGGAmψCmψGCmψGGmψGAAGAAmψmψGCCmψ CCmψGCCCCmψGAGAGAGmψACmψmψCAAGmψAmψmψmψCAGCCAGAAmψAGmψCmψGCCCCmψ GGGCGGCCCAAGCAGCGGCGCCCCmψCCmψCCCAGCGGCGGCAGCCCAGCCGGCmψCCCCAACC mψCmψACCGAGGAGGGCACCmψCmψGAGmψCCGCCACCCCCGAGAGCGGCCCmψGGCACCmψCC ACCGAGCCCAGCGAGGGCAGCGCACCCGGCAGCCCmψGCCGGCAGCCCCACCmψCCACAGAGGA GGGAACCAGCACCGAGCCCAGCGAAGGCAGCGCCCCAGGCACCAGCACCGAGCCmψAGmψGAGG GCGGCmψCmψGGCGGCGGCAGCGCCCAGGAGAmψmψAAACGGAmψCAACAAGAmψCAGAAGAAG ACmψmψGmψGAAAGACAGCAACACCAAGAAGGCCGGCAAGACAGGCCCCAmψGAAAACCCmψGC mψGGmψmψAGAGmψGAmψGACACCCGAmψCmψGAGAGAGCGGCmψGGAAAACCmψGAGAAAGAA GCCmψGAAAAmψAmψCCCCCAGCCCAmψCAGCAAmψACAmψCmψAGAGCCAACCmψGAAmψAAG CmψGCmψGACCGAmψmψACACCGAAAmψGAAGAAGGCGAmψCCmψGCAmψGmψGmψACmψGGGA AGAGmψmψCCAGAAGGACCCmψGmψGGGCCmψGAmψGAGCCGGGmψGGCCCAGCCmψGCCAGCA AGAAGAmψCGAmψCAGAACAAGCmψGAAACCmψGAGAmψGGACGAGAAGGGCAACCmψGACCAC CGCCGGCmψmψmψGCCmψGCmψCmψCAGmψGmψGGCCAGCCCCmψGmψmψCGmψGmψACAAGCm ψGGAGCAGGmψGmψCmψGAGAAGGGCAAGGCmψmψACACCAACmψACmψmψCGGACGGmψGCAA mψGmψGGCCGAGCACGAAAAGCmψGAmψCCmψGCmψGGCCCAGCmψGAAGCCCGAGAAGGAmψA GCGACGAAGCCGmψGACAmψAmψAGCCmψGGGAAAGmψmψmψGGGCAGAGGGCCCmψGGAmψmψ mψCmψACAGCAmψmψCAmψGmψGACCAAGGAGmψCCACCCACCCCGmψGAAGCCCCmψGGCCCA GAmψCGCCGGAAACAGAmψACGCCmψCCGGACCmψGmψGGGAAAGGCCCmψGAGCGACGCAmψG mψAmψGGGCACAAmψCGCCmψCCmψmψCCmψGmψCmψAAGmψACCAGGACAmψCAmψCAmψCGA	59622

ELXR ID	RNA sequence	SEQ ID NO
	ACACCAGAAGGmψGGmψGAAGGGCAACCAGAAGAGACmψGGAGAGCCmψGCGGGAGCmψGGCCG GCAAGGAAAACCmψGGAAmψACCCmψAGCGmψGACCCmψGCCACCmψCAGCCmψCACACCAAGG AGGGCGmψmψGAmψGCCmψACAACGAAGmψGAmψCGCCCGGGmψGCGAAmψGmψGGGmψGAACC mψGAACCmψGmψGGCAGAAGCmψGAAGCmψAAGCAGAGAmψGAmψGCCAAGCCmψCmψGCmψGA GACmψGAAGGGAmψmψCCCmψmψCCmψmψmψCCmψCmψGGmψCGAGAGACAGGCCAACGAAGmψ GGACmψGGmψGGGACAmψGGmψGmψGmψAACGmψGAAGAAGCmψGAmψCAACGAGAAAAAGGAG GAmψGGCAAGGmψGmψmψmψmψGGCAGAAmψCmψGGCmψGGCmψACAAGAGACAGGAAGCCCmψ GAGACCAmψACCmψGAGCAGCGAGGAAGAmψCGGAAGAAGGGAAAGAAAmψmψCGCmψCGGmψA CCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAAAAGAAGCACGGCGAGGACmψGGGGAAAG GmψGmψACGACGAGGCCmψGGGAGCGGAmψmψGACAAGAAAGmψGGAAGGCCmψGAGCAAGCAC AmψCAAGCmψGGAAGAGGAACGGAGAAGCGAGGACGCCCAGAGCAAGGCCGCCCmψGACCGACm ψGGCmψGCGGGCmψAAGGCCAGCmψmψCGmψGAmψCGAGGGCCmψGAAGGAGGCCGACAAGGAC GAGmψmψCmψGCAGAmψGCGAGCmψGAAGCmψGCAGAAGmψGGmψACGGGGACCmψGCGGGGAA AGCCCmψmψCGCCAmψCGAAGCCGAGAACAGCAmψCCmψGGACAmψCAGCGGCmψmψCAGCAAG CAGmψACAACmψGmψGCCmψmψCAmψCmψGGCAGAAGGACGGCGmψGAAGAAGCmψGAACCmψG mψACCmψGAmψCAmψCAACmψACmψmψCAAGGGCGGCAAGCmψGCGGmψmψCAAGAAGAmψCAA ACCmψGAAGCCmψmψCGAAGCCAACAGAmψmψCmψACACCGmψGAmψCAACAAAAAGAGCGGCG AGAmψCGmψGCCCAmψGGAGGmψGAACmψmψCAACmψmψCGACGACCCCAACCmψGAmψCAmψC CmψGCCmψCmψGGCCmψmψmψGGCAAGAGACAGGGCAGAGAAmψmψCAmψCmψGGAACGACCmψ GCmψGmψCCCmψGGAAACCGGCAGCCmψGAAGCmψGGCCAACGGAAGAGmψGAmψCGAGAAGAC ACmψGmψACAACAGAAGAACCCGGCAGGAmψGAGCCmψGCCCmψGmψmψCGmψGGCCCmψGACC mψmψCGAGCGGCGGGAGGmψCCmψGGACmψCCmψCCAAmψAmψCAAACCAAmψGAACCmψGAmψ CGGCGmψGGCAAGAGGCGAAAACAmψCCCCGCCGmψGAmψCGCCCmψGACCGACCCCGAGGGCm ψGCCCACmψGAGCCGGmψmψmψAAGGAmψAGCCmψGGGAAACCCAACCCACAmψCCmψGAGAAm ψCGGCGAGAGCmψAmψAAGGAGAAGCAGCGGACCAmψCCAGGCCAAGAAGGAGGmψGGAGCAGC GGAGAGCCGGCGGCmψACAGCCGGAAGmψACGCCAGCAAAGCCAAGAAmψCmψGGCAGACGAmψ AmψGGmψGAGAAACACCGCmψAGAGAmψCmψGCmψGmψACmψACGCCGmψGACCCAGGAmψGCC AmψGCmψGAmψCmψmψCGCCAACCmψGAGCCGGGGCmψmψCGGCCGGCAGGGCAAGCGGACCmψ mψCAmψGGCCGAGAGACAGmψACACACGGAmψGGAGGACmψGGCmψGACCGCCAAGCmψGGCCm ψACGAGGGCCmψGAGCAAGACCmψACCmψGmψCCAAGACACmψGGCCCAGmψACACCmψCCAAG ACAmψGCAGCAACmψGmψGGGmψmψmψACCAmψCACCAGCGCCGACmψACGACAGGGmψGCmψG GAGAAGCmψGAAGAAGACAGCAACAGGCmψGGAmψGACCACAAmψmψAACGGCAAGGAGCmψGA AGGmψGGAGGGCCAGAmψmψACCmψACmψACAACAGAmψACAAGAGACAGAACGmψAGmψCAAG GACCmψGmψCCGmψCGAGCmψGGAmψAGACmψGAGCGAAGAAmψCmψGmψGAACAACGACAmψC mψCCmψCCmψGGACAAAGGGCAGAAGCGGAGAAGCmψCmψGAGCCmψCCmψGAAGAAAAGAmψm ψCmψCCCAmψAGACCCGmψGCAGGAGAAGmψmψCGmψGmψGCCmψGAACmψGCGGCmψmψCGAG ACACACGCAGCCGAGCAAGCCGCCCmψGAACAmψCGCCAGAmψCCmψGGCmψGmψmψCCmψGCG GAGCCAGGAGmψACAAGAAAmψACCAGACAAACAAGACAACCGGCAACACCGAmψAAGAGAGCC mψmψCGmψCGAGACCmψGGCAGmψCCmψmψmψmψACCGGAAGAAGCmψmψAAGGAGGmψGmψGG AAACCmψGCCGmψGCGGmψCmψGGCGGAmψCmψGGCGGAGGCmψCCACAAGCAmψGAACAACmψ CCCAGGGCAGAGmψGACCmψmψCGAGGACGmψGACCGmψGAAmψmψmψmψACACAGGGAGAGmψ GGCAGAGACmψGAACCCCGAGCAGAGAAACCmψGmψACCGGGAmψGmψGAmψGCmψGGAAAACm ψACAGCAAmψCmψGGmψGmψCCGmψGGGCCAGGGCGAGACCACAAAGCCmψGACGmψGAmψCCm ψGCGmψCmψGGAGCAGGGCAAGGAACCCmψGGCmψGGAGGAGGAGGAGGmψGCmψGGGAAGCGG ACGGGCCGAGAAGAACGGCGACAmψCGGCGGACAGAmψCmψGGAAGCCmψAAGGACGmψGAAAG AAAGCCmψGGGCmψCmψCCCGCCGCCAAGAGAGmψGAAGCmψGGACmψAAmψAGAmψAAGCGGC CGCmψmψAAmψmψAAGCmψGCCmψmψCmψGCGGGGCmψmψGCCmψmψCmψGGCCAmψGCCCmψm ψCmψmψCmψCmψCCCmψmψGCACCmψGmψACCmψCmψmψGGmψCmψmψmψGAAmψAAAGCCmψG AGmψAGGAAGmψCmψAGAAAAA

ELXR ID	RNA sequence	SEQ ID NO
ELXR 5-ZIM3	AAAmψAAGAGAGAAAAGAAGAGmψAAGAAGAAAmψAmψAAGAGCCACCAmψGGCCCCmψAAGAA GAAGCGmψAAAGmψGAGCCGGAmψGAACCACGACCAGGAGmψmψCGACCCCCCmψAAGGmψGmψ ACCCmψCCCGmψCCCCGCCGAGAAGAGAAAGCCCAmψCCGGGmψCCmψGAGCCmψGmψmψCGAm ψGGCAmψCGCCACCGGmψCmψGCmψGGmψGCmψGAAGGACCmψGGGCAmψCCAGGmψGGAmψAG GmψACAmψmψGCCmψCCGAGGmψGmψGCGAGGACmψCCAmψCACCGmψGGGAAmψGGmψGCGmψ CAmψCAGGGCAAGAmψCAmψGmψACGmψGGGCGACGmψGCGGAGCGmψGACACAGAAGCAmψAm ψCCAGGAGmψGGGGCCCmψmψmψCGACCmψGGmψGAmψCGGCGGCAGCCCmψmψGCAAmψGACC mψGAGCAmψCGmψGAACCCAGCCCGGAAGGGCCmψGmψACGAGGGAACCGGCAGACmψGmψmψC mψmψCGAGmψmψmψmψACAGACmψGCmψGCACGACGCCCGGCCmψAAGGAAGGCGACGACCGGC CCmψmψCmψmψmψmψGGCmψGmψmψCGAGAAmψGmψGGmψGGCCAmψGGGAGmψCAGCGACAAG CGGGAmψAmψmψAGCCGGmψmψCCmψGGAGAGCAACCCCGmψGAmψGAmψCGAmψGCCAAGGAA GmψGAGCGCCGCCCACCGGGCCAGAmψACmψmψCmψGGGGCAAmψCmψGCCmψGGCAmψGAACA GACCCCmψGGCCAGCACCGmψGAACGACAAGCmψGGAGCmψGCAGGAGmψGCCmψGGAGCACGG CCGGAmψCGCCAAGmψmψCAGCAAGGmψGAGAACCAmψCACCACCCGAAGCAACAGCAmψCAAA CAAGGCAAGGACCAGCACmψmψmψCCmψGmψGmψmψCAmψGAACGAGAAGGAGGACAmψCCmψG mψGGmψGmψACCGAGAmψGGAGAGAGmψGmψmψCGGGmψmψCCCAGmψCCACmψACACAGAmψG mψCAGCAACAmψGmψCmψAGACmψGGCCAGACAGAGACmψGCmψGGGAAGAAGCmψGGmψCCGm ψCCCmψGmψGAmψCAGACACCmψGmψmψCGCCCCmψCmψGAAGGAGmψACmψmψCGCCmψGCGm ψGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCCAGCmψmψCmψCmψAGCGGCCmψGGmψ GCCACmψGmψCCCmψGAGAGGGAGCCACAmψGGGCCCCAmψGGAGAmψCmψACAAAACCGmψGA GCGCCmψGGAAGCGGCAGCCmψGmψGCGCGmψGCmψGAGCCmψGmψmψmψCGGAAmψAmψCGAm ψAAAGmψCCmψGAAAAGCCmψGGGAmψmψCCmψGGAGAGCGGCmψCmψGGCmψCCGGCGGmψGG CACCCmψGAAGmψACGmψGGAGGAmψGmψGACAAACGmψGGmψCAGACGGGAmψGmψGGAGAAG mψGGGGCCCCmψmψCGAmψCmψGGmψGmψACGGCAGCACCCAACCCCmψGGGCAGCmψCmψmψG mψGACCGGmψGCCCmψGGCmψGGmψACAmψGmψmψmψCAGmψmψCCACCGGAmψCCmψGCAGmψ ACGCCCmψGCCGAGACAGGAGmψCCCAGCGGCCAmψmψCmψmψmψmψGGAmψmψmψmψCAmψGG ACAACmψmψGCmψGCmψGACCGAGGAmψGACCAGGAAACmψACCACmψCGGmψmψCCmψGCAGA CCGAAGCCGmψGACCCmψGCAGGACGmψGAGAGGCCGGGACmψACCAGAACGCCAmψGCGGGmψ GmψGGmψCCAACAmψCCCmψGGACmψGAAAAGCAAGCACGCACCmψCmψGACCCCmψAAAGAAG AGGAGmψACCmψGCAGGCCCAGGmψGCGGAGCAGAAGCAAGCmψGGACGCCCCmψAAGGmψGGA mψCmψGCmψGGmψGAAGAAmψmψGCCmψCCmψGCCCCmψGAGAGAGmψACmψmψCAAGmψAmψm ψmψCAGCCAGAAmψAGmψCmψGCCCCmψGGGAGGCAGCGGCGGCGGCAmψGAACAACmψCCCAG GGCAGAGmψGACCmψmψCGAGGACGmψGACCGmψGAAmψmψmψmψACACAGGGAGAGmψGGCAG AGACmψGAACCCCGAGCAGAGAAACCmψGmψACCGGGAmψGmψGAmψGCmψGGAAAACmψACAG CAAmψCmψGGmψGmψCCGmψGGGCCAGGGCGAGACCACAAAGCCmψGACGmψGAmψCCmψGCGm ψCmψGGAGCAGGGCAAGGAACCCmψGGCmψGGAGGAGGAGGAGGmψGCmψGGGAAGCGGACGGG CCGAGAAGAACGGCGACAmψCGGCGGACAGAmψCmψGGAAGCCmψAAGGACGmψGAAAGAAAGC CmψGGGCGGCCCAAGCAGCGGCGCCCCmψCCmψCCCAGCGGCGGCAGCCCAGCCGGCmψCCCCA ACCmψCmψACCGAGGAGGGCACCmψCmψGAGmψCCGCCACCCCCGAGAGCGGCCCmψGGCACCm ψCCACCGAGCCCAGCGAGGGCAGCGCACCCGGCAGCCCmψGCCGGCAGCCCCACCmψCCACAGA GGAGGGAACCAGCACCGAGCCCAGCGAAGGCAGCGCCCCAGGCACCAGCACCGAGCCmψAGmψG AGCAGGAGAmψmψAAACGGAmψCAACAAGAmψCAGAAGAAGACmψmψGmψGAAAGACAGCAACA CCAAGAAGGCCGGCAAGACAGGCCCCAmψGAAAACCCmψGCmψGGmψmψAGAGmψGAmψGACAC CCGAmψCmψGAGAGAGCGGCmψGGAAAACCmψGAGAAAGAAGCCmψGAAAAmψAmψCCCCCAGC CCAmψCAGCAAmψACAmψCmψAGAGCCAACCmψGAAmψAAGCmψGCmψGACCGAmψmψACACCG AAAmψGAAGAAGGCGAmψCCmψGCAmψGmψGmψACmψGGGAAGAGmψmψCCAGAAGGACCCmψG mψGGGCCmψGAmψGAGCCGGGmψGGCCCAGCCmψGCCAGCAAGAAGAmψCGAmψCAGAACAAGC mψGAAACCmψGAGAmψGGACGAGAAGGGCAACCmψGACCACCGCCGGCmψmψmψGCCmψGCmψC mψCAGmψGmψGGCCAGCCCCmψGmψmψCGmψGmψACAAGCmψGGAGCAGGmψGmψCmψGAGAAG GGCAAGGCmψmψACACCAACmψACmψmψCGGACGGmψGCAAmψGmψGGCCGAGCACGAAAAGCm ψGAmψCCmψGCmψGGCCCAGCmψGAAGCCCGAGAAGGAmψAGCGACGAAGCCGmψGACAmψAmψ AGCCmψGGGAAAGmψmψmψGGGCAGAGGGCCCmψGGAmψmψmψCmψACAGCAmψmψCAmψGmψG ACCAAGGAGmψCCACCCACCCCGmψGAAGCCCCmψGGCCCAGAmψCGCCGGAAACAGAmψACGC CmψCCGGACCmψGmψGGGAAAGGCCCmψGAGCGACGCAmψGmψAmψGGGCACAAmψCGCCmψCC	59623

ELXR ID	RNA sequence	SEQ ID NO
	mψmψCCmψGmψCmψAAGmψACCAGGACAmψCAmψCAmψCGAACACCAGAAGGmψGGmψGAAGGG CAACCAGAAGAGACmψGGAGAGCCmψGCGGGAGCmψGGCCGGCAAGGAAAACCmψGGAAmψACC CmψAGCGmψGACCCmψGCCACCmψCAGCCmψCACACCAAGGAGGGCGmψmψGAmψGCCmψACAA CGAAGmψGAmψCGCCCGGGmψGCGAAmψGmψGGGmψGAACCmψGAACCmψGmψGGCAGAAGCmψ GAAGCmψAAGCAGAGAmψGAmψGCCAAGCCmψCmψGCmψGAGACmψGAAGGGAmψmψCCCmψmψ CCmψmψmψCCmψCmψGGmψCGAGAGACAGGCCAACGAAGmψGGACmψGGmψGGGACAmψGGmψG mψGmψAACGmψGAAGAAGCmψGAmψCAACGAGAAAAAGGAGGAmψGGCAAGGmψGmψmψmψmψG GCAGAAmψCmψGGCmψGGCmψACAAGAGACAGGAAGCCCmψGAGACCCAmψACCmψGAGCAGCGA GGAAGAmψCGGAAGAAGGGAAAGAAAmψmψCGCmψCGGmψACCAGCmψGGGCGACCmψGCmψGC mψGCACCmψGGAAAAGAAGCACGGCGAGGACmψGGGGAAAGGmψGmψACGACGAGGCCmψGGGA GCGGAmψmψGACAAGAAAGmψGGAAGGCCmψGAGCAAGCACAmψCAAGCmψGGAAGAGGAACGG AGAAGCGAGGACGCCCAGAGCAAGGCCGCCCmψGACCGACmψGGCmψGCGGGCmψAAGGCCAGC mψmψCGmψGAmψCGAGGGCCmψGAAGGAGGCCGACAAGGACGAGmψmψCmψGCAGAmψGCGAGC mψGAAGCmψGCAGAAGmψGGmψACGGGGACCmψGCGGGGAAAGCCCmψmψCGCCAmψCGAAGCC GAGAACAGCAmψCCmψGGACAmψCAGCGGCmψmψCAGCAAGCAGmψACAACmψGmψGCCmψmψC AmψCmψGGCAGAAGGACGGCGmψGAAGAAGCmψGAACCmψGmψACCmψGAmψCAmψCAACmψAC mψmψCAAGGGCGGCAAGCmψGCGGmψmψCAAGAAGAmψCAAACCmψGAAGCCmψmψCGAAGCCA ACAGAmψmψCmψACACCGmψGAmψCAACAAAAAGAGCGGCGAGAmψCGmψGCCCAmψGGAGGmψ GAACmψmψCAACmψmψCGACGACCCCAACCmψGAmψCAmψCCmψGCCmψCmψGGCCmψmψmψGG CAAGAGACAGGGCAGAGAAmψmψCAmψCmψGGAACGACCmψGCmψGmψCCCmψGGAAACCGGCA GCCmψGAAGCmψGGCCAACGGAAGAGmψGAmψCGAGAAGACACmψGmψACAACAGAAGAACCCG GCAGGAmψGAGCCmψGCCCmψGmψmψCGmψGGCCCmψGACCmψmψCGAGCGGCGGGAGGmψCCm ψGGACmψCCmψCCAAmψAmψCAAACCAAmψGAACCmψGAmψCGGCGmψGGCAAGAGGCGAAAAC AmψCCCCGCCGmψGAmψCGCCCmψGACCGACCCCGAGGGCmψGCCCACmψGAGCCGGmψmψmψA AGGAmψAGCCmψGGGAAACCCAACCCACAmψCCmψGAGAAmψCGGCGAGAGCmψAmψAAGGAGA AGCAGCGGACCAmψCCAGGCCAAGAAGGAGGmψGGAGCAGCGGAGAGCCGGCGGCmψACAGCCG GAAGmψACGCCAGCAAAGCCAAGAAmψCmψGGCAGACGAmψAmψGGmψGAGAAACACCGCmψAG AGAmψCmψGCmψGmψACmψACGCCGmψGACCCAGGAmψGCCAmψGCmψGAmψCmψmψCGCCAAC CmψGAGCCGGGGCmψmψCGGCCGGCAGGGCAAGCGGACCmψmψCAmψGGCCGAGAGACAGmψAC ACACGGAmψGGAGGACmψGGCmψGACCGCCAAGCmψGGCCmψACGAGGGCCmψGAGCAAGACCm ψACCmψGmψCCAAGACACmψGGCCCAGmψACACCmψCCAAGACAmψGCAGCAACmψGmψGGGmψ mψmψACCAmψCACCAGCGCCGACmψACGACAGGGmψGCmψGGAGAAGCmψGAAGAAGACAGCAA CAGGCmψGGAmψGACCACAAmψmψAACGGCAAGGAGCmψGAAGGmψGGAGGGCCAGAmψmψACC mψACmψACAACAGAmψACAAGAGACAGAACGmψAGmψCAAGGACCmψGmψCCGmψCGAGCmψGG AmψAGACmψGAGCGAAGAAmψCmψGmψGAACAACGACAmψCmψCCmψCCmψGGACAAAGGGCAG AAGCGGAGAAGCmψCmψGAGCCmψCCmψGAAGAAAAGAmψmψCmψCCCAmψAGACCCGmψGCAG GAGAAGmψmψCGmψGmψGCCmψGAACmψGCGGCmψmψCGAGACACACGCAGCCGAGCAAGCCGC CCmψGAACAmψCGCCAGAmψCCmψGGCmψGmψmψCCmψGCGGAGCCAGGAGmψACAAGAAAmψA CCAGACAAACAAGACAACCGGCAACACCGAmψAAGAGAGCCmψmψCGmψCGAGACCmψGGCAGm ψCCmψmψmψmψACCGGAAGAAGCmψmψAAGGAGGmψGmψGGAAACCmψGCCGmψGCGGmψCmψG GCGGAmψCmψGGCGGAGGCmψCCACCAGCCCCAAGAAAAAGAGAAAAGmψCmψAAmψAGAmψAA GCmψGCCmψmψCmψGCGGGGCmψmψGCCmψmψCmψGGCCAmψGCCCmψmψCmψmψCmψCmψCCC mψmψGCACCmψGmψACCmψCmψmψGGmψCmψmψmψGAAmψAAAGCCmψGAGmψAGGAAGmψCmψ AGAAAAA

ELXR ID	RNA sequence	SEQ ID NO
ELXR 5-ZIM3 + ADD	AAAmψAAGAGAGAAAAGAAGAGmψAAGAAGAAAmψAmψAAGAGCCACCAmψGGCCCCmψAAGAA GAAGCGmψAAAGmψGAGCCGGAmψGGAACGCCmψCGmψCmψACGAGGmψGCGGCAGAAGmψGCA GAAACAmψCGAGGACAmψCmψGCAmψCmψCCmψGCGGAmψCmψCmψGAACGmψGACCCmψGGAG CACCCACmψGmψmψCAmψCGGCGGCAmψGmψGCCAGAACmψGmψAAAAACmψGmψmψmψmψCmψ GGAGmψGmψGCCmψAmψCAAmψACGACGAmψGACGGCmψACCAGAGCmψACmψGCACCAmψCmψ GmψmψGCGGCGGAAGAGAGGmψGCmψGAmψGmψGmψGGAAAmψAACAACmψGCmψGCCGGmψGC mψmψCmψGCGmψGGAAmψGCGmψGGACCmψGCmψGGmψGGGCCCCGGCGCCGCCCAGGCCGCmψ AmψmψAAGGAAGAmψCCmψmψGGAACmψGCmψACAmψGmψGCGGCCACAAGGGCACAmψACGGC CmψGCmψGAGACGGAGAGAGGACmψGGCCmψAGCAGACmψGCAGAmψGmψmψCmψmψCGCCAAm ψAACCACGACCAGGAGmψmψCGACCCCCCmψAAGGmψGmψACCCmψCCCGmψCCCCGCCGAGAA GAGAAAGCCCAmψCCGGGmψCCmψGAGCCmψGmψmψCGAmψGGCAmψCGCCACCGGmψCmψGCm ψGGmψGCmψGAAGGACCmψGGGCAmψCCAGGmψGGAmψAGGmψACAmψmψGCCmψCCGAGGmψG mψGCGAGGACmψCCAmψCACCGmψGGGAAmψGGmψGCGmψCAmψCAGGGCAAGAmψCAmψGmψA CGmψGGGCGACGmψGCGGAGCGmψGACACAGAAGCAmψAmψCCAGGAGmψGGGGCCCmψmψmψC GACCmψGGmψGAmψCGGCGGCAGCCCmψmψGCAAmψGACCmψGAGCAmψCGmψGAACCCAGCCC GGAAGGGCCmψGmψACGAGGGAACCGGCAGACmψGmψmψCmψmψCGAGmψmψmψmψACAGACmψ GCmψGCACGACGCCCGGCCmψAAGGAAGGCGACGACCGGCCCmψmψCmψmψmψmψGGCmψGmψm ψCGAGAAmψGmψGGmψGGCCAmψGGGAGmψCAGCGACAAGCGGGAmψAmψmψAGCCGGmψmψCC mψGGAGAGCAACCCCGmψGAmψGAmψCGAmψGCCAAGGAAGmψGAGCGCCGCCCACCGGGCCAG AmψACmψmψCmψGGGGCAAmψCmψGCCmψGGCAmψGAACAGACCCCmψGGCCAGCACCGmψGAA CGACAAGCmψGGAGCmψGCAGGAGmψGCCmψGGAGCACGGCCGGAmψCGCCAAGmψmψCAGCAA GGmψGAGAACCAmψCACCACCCGAAGCAACAGCAmψCAAACAAGGCAAGGACCAGCACCmψmψmψ CCmψGmψGmψmψCAmψGAACGAGAAGGAGGACAmψCCmψGmψGGmψGmψACCGAGAmψGGAGAG AGmψGmψmψCGGGmψmψCCCAGmψCCACmψACACAGAmψGmψCAGCAACAmψGmψCmψAGACmψ GGCCAGACAGAGACmψGCmψGGGAAGAAGCmψGGmψCCGmψCCCmψGmψGAmψCAGACACCmψG mψmψCGCCCCmψCmψGAAGGAGmψACmψmψCGCCmψGCGmψGAGCAGCGGCAACAGCAACGCCA ACAGCCGGGGCCCCAGCmψmψCmψCmψAGCGGCCmψGGmψGCCACmψGmψCCCmψGAGAGGGAG CCACAmψGGGCCCCAmψGGAGAmψCmψACAAAACCGmψGAGCGCCmψGGAAGCGGCAGCCmψGm ψGCGCGmψGCmψGAGCCmψGmψmψmψCGGAAmψAmψCGAmψAAAGmψCCmψGAAAAGCCmψGGG Amψmψ CCmψGGAGAGCGGCmψCmψGGCmψCCGGCGGmψGGCACCCmψGAAGmψACGmψGGAGGA mψGmψGACAAACGmψGGmψCAGACGGGAmψGmψGGAGAAGmψGGGGCCCCmψmψCGAmψCmψGG mψGmψACGGCAGCACCCAACCCCmψGGGCAGCmψCmψmψGmψGACCGGmψGCCCmψGGCmψGGm ψACAmψGmψmψmψCAGmψmψCCACCGGAmψCCmψGCAGmψACGCCCmψGCCGAGACAGGAGmψC CCAGCGGCCAmψmψCmψmψmψmψGGAmψmψmψmψCAmψGGACAACmψmψGCmψGCmψGACCGAG GAmψGACCAGGAAACmψACCACmψCGGmψmψCCmψGCAGACCGAAGCCGmψGACCCmψGCAGGA CGmψGAGAGGCCGGGACmψACCAGAACGCCAmψGCGGGmψGmψGGmψCCAACAmψCCCmψGGAC mψGAAAAGCAAGCACGCACCmψCmψGACCCCmψAAAGAAGAGGAGmψACCmψGCAGGCCCAGGm ψGCGGAGCAGAAGCAAGCmψGGACGCCCCmψAAGGmψGGAmψCmψGCmψGGmψGAAGAAmψmψG CCmψCCmψGCCCCmψGAGAGAGmψACmψmψCAAGmψAmψmψmψCAGCCAGAAmψAGmψCmψGCC CCmψGGGAGGCAGCGGCGGCGGCAmψGAACAACmψCCCAGGGCAGAGmψGACCmψmψCGAGGAC GmψGACCGmψGAAmψmψmψmψACACAGGGAGAGmψGGCAGAGACmψGAACCCCGAGCAGAGAAA CCmψGmψACCGGGAmψGmψGAmψGCmψGGAAAACmψACAGCAAmψCmψGGmψGmψCCGmψGGGC CAGGGCGAGACCACAAAGCCmψGACGmψGAmψCCmψGCGmψCmψGGAGCAGGGCAAGGAACCCm ψGGCmψGGAGGAGGAGGAGGmψGCmψGGGAAGCGGACGGGCCGAGAAGAACGGCGACAmψCGGC GGACAGAmψCmψGGAAGCCmψAAGGACGmψGAAAGAAAGCCmψGGGCGGCCCAAGCAGCGGCGC CCCmψCCmψCCCAGCGGCGGCAGCCCAGCCGGCmψCCCCAACCmψCmψACCGAGGAGGGCACCm ψCmψGAGmψCCGCCACCCCCGAGAGCGGCCCmψGGCACCmψCCACCGAGCCCAGCGAGGGCAGC GCACCCGGCAGCCCmψGCCGGCAGCCCCACCmψCCACAGAGGAGGGAACCAGCACCGAGCCCAG CGAAGGCAGCGCCCCAGGCACCAGCACCGAGCCmψAGmψGAGCAGGAGAmψmψAAACGGAmψCA ACAAGAmψCAGAAGAAGACmψmψGmψGAAAGACAGCAACACCAAGAAGGCCGGCAAGACAGGCC CCAmψGAAAACCCmψGCmψGGmψmψAGAGmψGAmψGACACCCGAmψCmψGAGAGAGCGGCmψGG AAAACCmψGAGAAAGAAGCCmψGAAAAmψAmψCCCCCAGCCCAmψCAGCAAmψACAmψCmψAGA GCCAACCmψGAAmψAAGCmψGCmψGACCGAmψmψACACCGAAAmψGAAGAAGGCGAmψCCmψGC AmψGmψGmψACmψGGGAAGAGmψmψCCAGAAGGACCCmψGmψGGGCCmψGAmψGAGCCGGGmψG	59624

ELXR ID	RNA sequence	SEQ ID NO
	GCCCAGCCCmψGCCAGCAAGAAGAmψCGAmψCAGAACAAGCmψGAAACCmψGAGAmψGGACGAGA AGGGCAACCmψGACCACCGCCGGCmψmψmψGCCmψGCmψCmψCAGmψGmψGGCCAGCCCCmψGm ψmψCGmψGmψACAAGCmψGGAGCAGGmψGmψCmψGAGAAGGGCAAGGCmψmψACACCAACmψAC mψmψCGGACGGmψGCAAmψGmψGGCCGAGCACGAAAAGCmψGAmψCCmψGCmψGGCCCAGCmψG AAGCCCGAGAAGGAmψAGCGACGAAGCCGmψGACAmψAmψAGCCmψGGGAAAGmψmψmψGGGCA GAGGGCCCmψGGAmψmψmψCmψACAGCAmψmψCAmψGmψGACCAAGGAGmψCCACCCACCCCGm ψGAAGCCCCmψGGCCCAGAmψCGCCGGAAACAGAmψACGCCmψCCGGACCmψGmψGGGAAAGGC CCmψGAGCGACGCAmψGmψAmψGGGCACAAmψCGCCmψCCmψmψCCmψGmψCmψAAGmψACCAG GACAmψCAmψCAmψCGAACACCAGAAGGmψGGmψGAAGGGCAACCAGAAGAGACmψGGAGAGCC mψGCGGGAGCmψGGCCGGCAAGGAAAACCmψGGAAmψACCCmψAGCGmψGACCCmψGCCACCmψ CAGCCmψCACACCAAGGAGGGCGmψmψGAmψGCCmψACAACGAAGmψGAmψCGCCCGGGmψGCG AAmψGmψGGGmψGAACCmψGAACCmψGmψGGCAGAAGCmψGAAGCmψAAGCAGAGAmψGAmψGC CAAGCCmψCmψGCmψGAGACmψGAAGGGAmψmψCCCmψmψCCmψmψmψCCmψCmψGGmψCGAGA GACAGGCCAACGAAGmψGGACmψGGmψGGGACAmψGGmψGmψGmψAACGmψGAAGAAGCmψGAm ψCAACGAGAAAAAGGAGGAmψGGCAAGGmψGmψmψmψmψGGCAGAAmψCmψGGCmψGGCmψACA AGAGACAGGAAGCCCmψGAGACCAmψACCmψGAGCAGCGAGGAAGAmψCGGAAGAAGGGAAAGA AAmψmψCGCmψCGGmψACCAGCmψGGGCGACCmψGCmψGCmψGCACCmψGGAAAAGAAGCACGG CGAGGACmψGGGGAAAGGmψGmψACGACGAGGCCmψGGGAGCGGAmψmψGACAAGAAAGmψGGA AGGCCmψGAGCAAGCACAmψCAAGCmψGGAAGAGGAACGGAGAAGCGAGGACGCCCAGAGCAAG GCCGCCCmψGACCGACmψGGCmψGCGGGCmψAAGGCCAGCmψmψCGmψGAmψCGAGGGCCmψGA AGGAGGCCGACAAGGACGAGmψmψCmψGCAGAmψGCGAGCmψGAAGCmψGCAGAAGmψGGmψAC GGGGACCmψGCGGGGAAAGCCCmψmψCGCCAmψCGAAGCCGAGAACAGCAmψCCmψGGACAmψC AGCGGCmψmψCAGCAAGCAGmψACAACmψGmψGCCmψmψCAmψCmψGGCAGAAGGACGGCGmψG AAGAAGCmψGAACCmψGmψACCmψGAmψCAmψCAACmψACmψmψCAAGGGCGGCAAGCmψGCGG mψmψCAAGAAGAmψCAAACCmψGAAGCCmψmψCGAAGCCAACAGAmψmψCmψACACCGmψGAmψ CAACAAAAAGAGCGGCGAGAmψCGmψGCCCAmψGGAGGmψGAACmψmψCAACmψmψCGACGACC CCAACCmψGAmψCAmψCCmψGCCmψCmψGGCCmψmψmψGGCAAGAGACAGGGCAGAGAAmψmψC AmψCmψGGAACGACCmψGCmψGmψCCCmψGGAAACCGGCAGCCmψGAAGCmψGGCCAACGGAAG AGmψGAmψCGAGAAGACACmψGmψACAACAGAAGAACCCGGCAGGAmψGAGCCmψGCCCmψGmψ mψCGmψGGCCCmψGACCmψmψCGAGCGGCGGGAGGmψCCmψGGACmψCCmψCCAAmψAmψCAAA CCAAmψGAACCmψGAmψCGGCGmψGGCAAGAGGCGAAAACAmψCCCCGCCGmψGAmψCGCCCmψ GACCGACCCCGAGGGCmψGCCCACmψGAGCCGGmψmψmψAAGGAmψAGCCmψGGGAAACCCAAC CCACAmψCCmψGAGAAmψCGGCGAGAGCmψAmψAAGGAGAAGCAGCGGACCAmψCCAGGCCAAG AAGGAGGmψGGAGCAGCGGAGAGCCGGCGGCmψACAGCCGGAAGmψACGCCAGCAAAGCCAAGA AmψCmψGGCAGACGAmψAmψGGmψGAGAAACACCGCmψAGAGAmψCmψGCmψGmψACmψACGCC GmψGACCCAGGAmψGCCAmψGCmψGAmψCmψmψCGCCAACCmψGAGCCGGGGCmψmψCGGCCGG CAGGGCAAGCGGACCmψmψCAmψGGCCGAGAGACAGmψACACACGGAmψGGAGGACmψGGCmψG ACCGCCAAGCmψGGCCmψACGAGGGCCmψGAGCAAGACCmψACCmψGmψCCAAGACACmψGGCC CAGmψACACCmψCCAAGACAmψGCAGCAACmψGmψGGGmψmψmψACCAmψCACCAGCGCCGACm ψACGACAGGGmψGCmψGGAGAAGCmψGAAGAAGACAGCAACAGGCmψGGAmψGACCACAAmψmψ AACGGCAAGGAGCmψGAAGGmψGGAGGGCCAGAmψmψACCmψACmψACAACAGAmψACAAGAGA CAGAACGmψAGmψCAAGGACCmψGmψCCGmψCGAGCmψGGAmψAGACmψGAGCGAAGAAmψCmψ GmψGAACAACGACAmψCmψCCmψCCmψGGACAAAGGGCAGAAGCGGAGAAGCmψCmψGAGCCmψ CCmψGAAGAAAAGAmψmψCmψCCCAmψAGACCCGmψGCAGGAGAAGmψmψCGmψGmψGCCmψGA ACmψGCGGCmψmψCGAGACACACGCAGCCGAGCAAGCCGCCCmψGAACAmψCGCCAGAmψCCmψ GGCmψGmψmψCCmψGCGGAGCCAGGAGmψACAAGAAAmψACCAGACAAACAAGACAACCGGCAA CACCGAmψAAGAGAGCCmψmψCGmψCGAGACCmψGGCAGmψCCmψmψmψmψACCGGAAGAAGCm ψmψAAGGAGGmψGmψGGAAACCmψGCCGmψGCGGmψCmψGGCGGAmψCmψGGCGGAGGCmψCCA CCAGCCCCAAGAAAAAGAGAAAAGmψCmψAAmψAGAmψAAGCmψGCCmψmψCmψGCGGGGCmψm ψGCCmψmψCmψGGCCAmψGCCCmψmψCmψmψCmψCmψCCCmψmψGCACCmψGmψACCmψCmψmψ GGmψCmψmψmψGAAmψAAAGCCmψGAGmψAGGAAGmψCmψAGAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	

[0609] Synthesis of targeting gRNAs (e.g., targeting the endogenous *B2M* locus) will be performed as described above in Example 14.

[0610] LNP formulations will be performed as described in Example 16.

[0611] Delivery of LNPs encapsulating ELXR mRNA and targeting gRNAs into mouse liver Hepa1-6 cells:

[0612] Hepa1-6 cells will be seeded in a 96-well plate. The next day, seeded cells will be treated with varying concentrations of LNPs, which will be prepared in six 2-fold serial dilutions starting at 250 ng. These LNPs will be formulated to encapsulate an ELXR mRNA and a *B2M*-targeting gRNA. Media will be changed 24 hours after LNP treatment, and cells will be cultured before being harvested at multiple timepoints (e.g., 7, 14, 21, 28, and 56 days post-treatment) for gDNA extraction for editing assessment at the *B2M* locus by NGS and for bisulfite sequencing to assess off-target methylation at the *VEGFA* locus as described in Example 6.

[0613] The results from this experiment are expected to show that ELXR mRNA and targeting gRNA can be co-encapsulated within LNPs to be delivered to target cells to induce heritable silencing of a target endogenous locus.

Example 16: Formulation of lipid nanoparticles (LNPs) to deliver XR or ELXR mRNA and gRNA payloads to target cells and tissue

[0614] Experiments will be performed to encapsulate XR or ELXR mRNA and gRNA into LNPs for delivery to target cells and tissue. Here, XR or ELXR mRNA and gRNA will be encapsulated into LNPs using GenVoy-ILM™ lipids using the Precision NanoSystems Inc. (PNI) Ignite™ Benchtop System, following the manufacturer's guidelines. GenVoy-ILM™ lipids are a composition of ionizable lipid:DSPC:cholesterol:stabilizer at 50:10:37.5:2.5 mol%. Briefly, to formulate LNPs, equal mass ratios of XR or ELXR mRNA and gRNA will be diluted in PNI Formulation Buffer, pH 4.0. GenVoy-ILM™ lipids will be diluted 1:1 in anhydrous ethanol. mRNA/gRNA co-formulations will be performed using a predetermined N/P ratio. The RNA and lipids will be run through a PNI laminar flow cartridge at a predetermined flow rate ratio on the PNI Ignite™ Benchtop System. After formulation, the LNPs will be diluted in PBS, pH 7.4, to decrease the ethanol concentration and increase the pH, which increases the stability of the particles. Buffer exchange of the mRNA/sgRNA-LNPs will be achieved by overnight

dialysis into PBS, pH 7.4, at 4°C using 10k Slide-A-Lyzer™ Dialysis Cassettes (Thermo Scientific™). Following dialysis, the mRNA/gRNA-LNPs will be concentrated to > 0.5 mg/mL using 100 kDa Amicon®-Ultra Centrifugal Filters (Millipore) and then filter-sterilized. Formulated LNPs will be analyzed on a Stunner (Unchained Labs) to determine their diameter and polydispersity index (PDI). Encapsulation efficiency and RNA concentration will be determined by RiboGreen™ assay using Invitrogen's Quant-iT™ RiboGreen™ RNA assay kit. LNPs will be used in various experiments to deliver XR or ELXR mRNA and gRNA to target cells and tissue.

Example 17: Members of the top 95 KRAB domains increase ELXR5 activity

[0615] As described in Example 4, KRAB domains were identified that were superior repressors in the context of dXR constructs. As described herein, experiments were performed to test whether the KRAB domains identified in Example 4 were also superior transcriptional repressors in Example 4 in the context of ELXR5.

Materials and Methods:

[0616] Representative KRAB domains identified in Example 4 and determined to be members of the top 95 performing repressors were cloned into an ELXR5 construct (see FIG. 7 for ELXR #5 configuration). The ELXR5 constructs were constructed as described in Example 6 (Table 25 and Table 26), except that an SV40 NLS was present downstream of the KRAB domains. An ELXR5 molecule with a KRAB domain derived from ZIM3 was used as a control. A separate plasmid was used to encode guide scaffold 316 (SEQ ID NO: 59352) with spacer 7.165 (UCCCUAUGUCCUUGCUGUUU; SEQ ID NO: 59667) targeting the *B2M* locus. Additional controls included a dXR molecule with a KRAB domain derived from ZIM3 with the same guide and spacer, and ELXR5 and dXR molecules with KRAB domains derived from ZIM3 and non-targeting 0.0 spacers (SEQ ID NO: 57646). Notably, spacer 7.165 was chosen because it is known to be a relatively inefficient spacer which would therefore increase the dynamic range of the assay for discerning differences between the various ELXR molecules tested.

[0617] HEK293T cells were transfected as described in Example 11, except that the cells were transfected with 50 ng each of a plasmid encoding the ELXR construct and a plasmid encoding the sgRNA. Repression analysis was conducted by analyzing B2M protein expression via HLA immunostaining followed by flow cytometry seven days following transfection, as described in Example 6.

Results:

[0618] The results of the B2M assay are provided in Table 44, below.

Table 44: Levels of *B2M* repression mediated by XR and ELXR constructs with various KRAB domains quantified at seven days post-transfection.

Repressor construct	KRAB domain	Spacer	Mean % HLA negative cells	Standard deviation	Sample size
ELXR5	ZIM3	0.0	6.703333	1.169031	3
XR	ZIM3	7.165	7.36	1.626346	2
XR	ZIM3	0.0	7.786667	0.721757	3
ELXR5	DOMAIN_27811	7.165	22.63333	0.64291	3
ELXR5	DOMAIN_17317	7.165	25.93333	0.585947	3
ELXR5	DOMAIN_17358	7.165	27.76667	3.06159	3
ELXR5	DOMAIN_18258	7.165	29.13333	0.776745	3
ELXR5	DOMAIN_8503	7.165	29.7	0.888819	3
ELXR5	DOMAIN_4968	7.165	30.13333	2.804164	3
ELXR5	DOMAIN_15126	7.165	30.33333	0.305505	3
ELXR5	DOMAIN_28803	7.165	30.36667	0.90185	3
ELXR5	DOMAIN_19949	7.165	31.96667	2.510644	3
ELXR5	DOMAIN_22270	7.165	32.5	1.1	3
ELXR5	DOMAIN_5463	7.165	32.53333	0.404145	3
ELXR5	DOMAIN_24125	7.165	32.66667	1.289703	3
ELXR5	ZIM3	7.165	32.9	0.43589	3
ELXR5	DOMAIN_23723	7.165	33.4	2.170253	3
ELXR5	DOMAIN_11029	7.165	33.46667	1.289703	3
ELXR5	DOMAIN_19229	7.165	33.96667	0.321455	3
ELXR5	DOMAIN_21603	7.165	34.36667	0.404145	3
ELXR5	DOMAIN_8790	7.165	34.9	0.608276	3
ELXR5	DOMAIN_11386	7.165	35.63333	1.677299	3
ELXR5	DOMAIN_16806	7.165	35.66667	1.450287	3
ELXR5	DOMAIN_6248	7.165	36	2.351595	3
ELXR5	DOMAIN_16444	7.165	36.36667	1.703917	3
ELXR5	DOMAIN_11486	7.165	36.66667	1.320353	3
ELXR5	DOMAIN_4806	7.165	36.76667	1.747379	3

Repressor construct	KRAB domain	Spacer	Mean % HLA negative cells	Standard deviation	Sample size
ELXR5	DOMAIN_17905	7.165	36.93333	1.446836	3
ELXR5	DOMAIN_14755	7.165	37.35	0.070711	2
ELXR5	DOMAIN_5066	7.165	37.83333	1.02632	3
ELXR5	DOMAIN_21247	7.165	37.86667	2.218859	3
ELXR5	DOMAIN_14659	7.165	37.93333	1.767295	3
ELXR5	DOMAIN_10331	7.165	38.3	1.30767	3
ELXR5	DOMAIN_11348	7.165	38.43333	1.28582	3
ELXR5	DOMAIN_25289	7.165	38.53333	0.945163	3
ELXR5	DOMAIN_21755	7.165	38.66667	1.497776	3
ELXR5	DOMAIN_13331	7.165	38.7	2.163331	3
ELXR5	DOMAIN_24663	7.165	39.43333	6.047589	3
ELXR5	DOMAIN_27506	7.165	39.46667	1.504438	3
ELXR5	DOMAIN_6807	7.165	39.5	0.43589	3
ELXR5	DOMAIN_28640	7.165	39.9	1.276715	3
ELXR5	DOMAIN_11683	7.165	40.26667	0.152753	3
ELXR5	DOMAIN_12631	7.165	40.3	0.6245	3
ELXR5	DOMAIN_23394	7.165	40.73333	2.285461	3
ELXR5	DOMAIN_13539	7.165	40.8	2.306513	3
ELXR5	DOMAIN_2380	7.165	41.1	1.276715	3
ELXR5	DOMAIN_16643	7.165	41.13333	1.205543	3
ELXR5	DOMAIN_18216	7.165	41.4	0.818535	3
ELXR5	DOMAIN_737	7.165	41.46667	3.257811	3
ELXR5	DOMAIN_16688	7.165	41.8	0.264575	3
ELXR5	DOMAIN_19804	7.165	42.06667	1.913984	3
ELXR5	DOMAIN_10948	7.165	42.73333	0.92376	3
ELXR5	DOMAIN_26322	7.165	42.76667	4.66083	3
ELXR5	DOMAIN_17759	7.165	43.23333	0.92376	3
ELXR5	DOMAIN_9114	7.165	43.26667	1.501111	3
ELXR5	DOMAIN_5290	7.165	43.4	1.135782	3
ELXR5	DOMAIN_221	7.165	43.43333	0.750555	3
ELXR5	DOMAIN_881	7.165	43.53333	1.858315	3
ELXR5	DOMAIN_7255	7.165	43.56667	0.450925	3

Repressor construct	KRAB domain	Spacer	Mean % HLA negative cells	Standard deviation	Sample size
ELXR5	DOMAIN_24458	7.165	43.56667	1.331666	3
ELXR5	DOMAIN_19896	7.165	43.6	0.6245	3
ELXR5	DOMAIN_13468	7.165	43.7	1.571623	3
ELXR5	DOMAIN_9960	7.165	43.96667	2.362908	3
ELXR5	DOMAIN_17432	7.165	43.96667	0.907377	3
ELXR5	DOMAIN_18137	7.165	44.03333	0.404145	3
ELXR5	DOMAIN_15507	7.165	44.06667	0.907377	3
ELXR5	DOMAIN_20505	7.165	45.36667	0.568624	3
ELXR5	DOMAIN_6445	7.165	45.66667	2.730079	3
ELXR5	DOMAIN_6802	7.165	45.76667	1.887679	3
ELXR5	DOMAIN_25379	7.165	46.46667	3.868247	3
ELXR5	DOMAIN_22153	7.165	46.83333	0.64291	3
ELXR5	DOMAIN_10123	7.165	47.83333	0.665833	3
ELXR5	DOMAIN_8853	7.165	48.1	4.457578	3
ELXR5	DOMAIN_29304	7.165	51.7	1.4	3
ELXR5	DOMAIN_7694	7.165	52.4	0.43589	3
ELXR5	DOMAIN_30173	7.165	53.9	0.1	3

[0619] As shown in Table 44, constructs with many of the KRAB domains in the top 95 KRAB domains produced higher levels of *B2M* repression in the context of an ELXR5 molecule with spacer 7.165 compared to an ELXR5 construct with a KRAB domain derived from ZIM3. The highest level of repression was achieved by an ELXR5 molecule with KRAB domain ID 30173, which produced a 35% stronger repression than ELXR5 with a KRAB domain derived from ZIM3. Later timepoints will be assessed to measure the durability of the repression.

[0620] Accordingly, the experiments described herein demonstrate that the KRAB domains identified in Example 4 support improved levels of transcriptional repression both in the context of a dXR construct and an ELXR construct.

Example 18: Exemplary sequences of dXR and ELXR constructs

[0621] Table 45 provides exemplary amino acid sequences of components of dXR and ELXR constructs. In Table 45, the protein domains are shown without starting methionines.

Table 45: Exemplary protein sequences of components of dXR and ELXR constructs.

Key component	Protein sequence	SEQ ID NO
ZNF10 KRAB domain	DAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQL TKPDVILRLEKGEEP	59626
ZIM3 KRAB domain	NNSQGRVTFEDVTVNFTQGEWQRLNPEQRNLYRDVMLENYSNLVSVGQGETTKPDV ILRLEQGKEPWLEEEEVLGSGRAEKNGDIGGQIWKPKDVKESL	59627
DNMT3A catalytic domain (CD)	NHDQEFDPPKVYPPVPAEKRKPIRVLSLFDGIATGLLVLKDLGIQVDRYIASEVCE DSITVGMVRHQGKIMYVGDVRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGL YEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFENVVAMGVSDKRDISRFLESNPVM IDAKEVSAAHRARYFWGNLPGMNRPLASTVNDKLELQECLEHGRIAKFSKVRTITT RSNSIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGR SWSVPVIRHLFAPLKEYFACV	59450
DNMT3L interaction domain	GPMEIYKTVSAWKRQPVRVLSLFRNIDKVLKSLGFLESGSGSGGGTLKYVEDVTNV VRRDVEKWGPFDLVYGSTQPLGSSCDRCPGWYMFQFHRILQYALPRQESQRPFFWI FMDNLLLTEDDQETTTRFLQTEAVTLQDVRGRDYQNAMRVWSNIPGLKSKHAPLTP KEEEYLQAQVRSRSKLDAPKVDLLVKNCLLPLREYFKYFSQNSLPL	59625
dCasX491	QEIKRINKIRRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLRKKPENIPQ PISNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPASKKIDQNKL KPEMDEKGNLTTAGFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLIL LAQLKPEKDSDEAVTYSLGKFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGP VGKALSDACMGTIASFLSKYQDIIIEHQKVVKGNQKRLESLRELAGKENLEYPSVT LPPQPHTKEGVDAYNEVIARVRMWVNLNLWQKLKLSRDDAKPLLRLKGFPSFPLVE RQANEVDWWDMVCNVKKLINEKKEDGKVFWQNLAGYKRQEALRPYLSSEEDRKKGK KFARYQLGDLLLHLEKKHGEDWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQ SKAALTDWLRAKASFVIEGLKEADKDEFCRCELKLQKWYGDLRGKPFAIEAENSIL DISGFSKQYNCAFIWQKDGVKKLNLYLIINYFKGGKLRFKKIKPEAFEANRFYTVI NKKSGEIVPMEVNFNFDDPNLIILPLAFGKRQGREFIWNDLLSLETGSLKLANGRV IEKTLYNRRTRQDEPALFVALTFERREVLDSSNIKPMNLIGVARGENIPAVIALTD PEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAKKEVEQRRAGGYSRKYASKAK NLADDMVRNTARDLLYYAVTQDAMLIFANLSRGFGRQGKRTFMAERQYTRMEDWLT AKLAYEGLSKTYLSKTLAQYTSKTCSNCGFTITSADYDRVLEKLKKTATGWMTTIN GKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWTKGRSGEALSLL KKRFSHRPVQEKFVCLNCGFETHAAEQAALNIARSWLFLRSQEYKKYQTNKTTGNT DKRAFVETWQSFYRKKLKEVWKPAV	18
Linker 1	GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPT STEEGTSTEPSEGSAPGTSTEPSE	57621
Linker 2	SSGNSNANSRGPSFSSGLVPLSLRGSH	57623
Linker 3A'	GGSGGG	59451
Linker 3B	GGSGGGS	57626
Linker 4	GSGSGGG	57628
SV40NLS	PKKKRKV	57631
cMYC NLS	PAAKRVKLD	33291
DNMT3A ADD domain	ERLVYEVRQKCRNIEDICISCGSLNVTLEHPLFIGGMCQNCKNCFLECAYQYDDDG YQSYCTICCGGREVLMCGNNNCCRCFCVECVDLLVGPGAAQAAIKEDPWNCYMCGH KGTYGLLRRREDWPSRLQMFFAN	59452

[0622] Table 46 provides exemplary full-length ELXR constructs (including dCaX, NLS, linkers, and repressor domains) in configurations 1, 4, or 5, with or without the ADD domain, with each of the top ten KRAB domains: DOMAIN_737, DOMAIN_10331, DOMAIN_10948, DOMAIN_11029, DOMAIN_17358, DOMAIN_17759, DOMAIN_18258, DOMAIN_19804, DOMAIN_20505, and DOMAIN_26749. Further exemplary full-length ELXR sequences are provided in SEQ ID NOs: 59673-60012.

Table 46: Exemplary protein sequences of ELXR molecules containing the top ten KRAB domains with or without the ADD domain and having the #1, #4, or #5 configurations.

ELXR #	Domains	KRAB domain ID	SEQ ID NO
ELXR #1	KRAB, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59508
		DOMAIN_10331	59509
		DOMAIN_10948	59510
		DOMAIN_11029	59511
		DOMAIN_17358	59512
		DOMAIN_17759	59513
		DOMAIN_18258	59514
		DOMAIN_19804	59515
		DOMAIN_20505	59516
		DOMAIN_26749	59517
	KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59518
		DOMAIN_10331	59519
		DOMAIN_10948	59520
		DOMAIN_11029	59521
		DOMAIN_17358	59522
		DOMAIN_17759	59523
		DOMAIN_18258	59524
		DOMAIN_19804	59525
		DOMAIN_20505	59526
		DOMAIN_26749	59527
ELXR #4	KRAB, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59528
		DOMAIN_10331	59529
		DOMAIN_10948	59530
		DOMAIN_11029	59531
		DOMAIN_17358	59532
		DOMAIN_17759	59533
		DOMAIN_18258	59534
		DOMAIN_19804	59535
		DOMAIN_20505	59536
		DOMAIN_26749	59537

ELXR #	Domains	KRAB domain ID	SEQ ID NO
	KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59538
		DOMAIN_10331	59539
		DOMAIN_10948	59540
		DOMAIN_11029	59541
		DOMAIN_17358	59542
		DOMAIN_17759	59543
		DOMAIN_18258	59544
		DOMAIN_19804	59545
		DOMAIN_20505	59546
		DOMAIN_26749	59547
ELXR #5	KRAB, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59548
		DOMAIN_10331	59549
		DOMAIN_10948	59550
		DOMAIN_11029	59551
		DOMAIN_17358	59552
		DOMAIN_17759	59553
		DOMAIN_18258	59554
		DOMAIN_19804	59555
		DOMAIN_20505	59556
		DOMAIN_26749	59557
	KRAB, DNMT3A ADD, DNMT3A CD, DNMT3L Interaction	DOMAIN_737	59558
		DOMAIN_10331	59559
		DOMAIN_10948	59560
		DOMAIN_11029	59561
		DOMAIN_17358	59562
		DOMAIN_17759	59563
		DOMAIN_18258	59564
		DOMAIN_19804	59565
		DOMAIN_20505	59566
		DOMAIN_26749	59567

CLAIMS

What is claimed is:

1. A gene repressor system comprising:

a) a catalytically-dead Class 2 CRISPR protein;

b) a transcription repressor domain; and

c) a guide ribonucleic acid (gRNA)

wherein:

i) the transcription repressor domain is linked to the catalytically-dead Class 2 CRISPR protein as a fusion protein;

ii) the gRNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene targeted for repression, silencing, or downregulation;

iii) the fusion protein is capable of forming a ribonucleoprotein (RNP) with the gRNA; and

iv) the RNP is capable of binding to the target nucleic acid.

2. The gene repressor system of claim 1, wherein the gene encodes a messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), or structural RNA.

3. The gene repressor system of claim 1 or claim 2, wherein the transcription repressor domain is a Krüppel-associated box (KRAB) domain.

4. The gene repressor system of claim 3, wherein the KRAB domain is selected from the group consisting of ZNF343, ZNF337, ZNF334, ZNF215, ZNF519, ZNF485, ZNF214, ZNF33B, ZNF287, ZNF705A, ZNF37A, KRBOX4, ZKSCAN3, ZKSCAN4, ZNF57, ZNF557, ZNF705B, ZNF662, ZNF77, ZNF500, ZNF558, ZNF620, ZNF713, ZNF823, ZNF440, ZNF441, ZNF136, SNRPB, ZNF735, ZKSCAN2, ZNF619, ZNF627, ZNF333, ABCA11P, PLD5P1, ZNF25, ZNF727, ZNF595, ZNF14, ZNF33A, ZNF101, ZNF253, ZNF56, ZNF720, ZNF85, ZNF66, ZNF722P, ZNF486, ZNF682, ZNF626, ZNF100, ZNF93, ZKSCAN1, ZNF257, ZNF729, ZNF208, ZNF90, ZNF430, ZNF676, ZNF91, ZNF429, ZNF675, ZNF681, ZNF99, ZNF431, ZNF98, ZNF708, ZNF732, SSX2, ZNF721, ZNF726, ZNF730, ZNF506, ZNF728, ZNF141, ZNF723, ZNF302, ZNF484, LINC00960, SSX2B, ZNF718, ZNF74, ZNF157, ZNF790, ZNF565, ZNF705G, VN1R107P, SLC27A5, ZNF737, SSX4, ZNF850, ZNF717, ZNF155, ZNF283, ZNF404, ZNF114, ZNF716, ZNF230, ZNF45, ZNF222, ZNF286A, ZNF624, ZNF223, ZNF284, ZNF790-AS1, ZNF382, ZNF749, ZNF615, ZFP90, ZNF225, ZNF234, ZNF568, ZNF614, ZNF584, ZNF432, ZNF461, ZNF182, ZNF630, ZNF630-AS1, ZNF132,

ZNF420, ZNF324B, ZNF616, ZNF471, ZNF227, ZNF324, ZNF860, ZFP28, ZNF470, ZNF586, ZNF235, ZNF274, ZNF446, ZFP1, ZIM3, ZNF212, ZNF766, ZNF264, ZNF480, ZNF667, ZNF805, ZNF610, ZNF783, ZNF621, ZNF8-DT, ZNF880, ZNF213-AS1, ZNF213, ZNF263, ZSCAN32, ZIM2, ZNF597, ZNF786, KRBA1, ZNF460, ZNF8, ZNF875, ZNF543, ZNF133, ZNF229, ZNF528, SSX1, ZNF81, ZNF578, ZNF862, ZNF777, ZNF425, ZNF548, ZNF746, ZNF282, ZNF398, ZNF599, ZNF251, ZNF195, ZNF181, RBAK-RBAKDN, ZFP37, RN7SL526P, ZNF879, ZNF26, ZSCAN21, ZNF3, ZNF354C, ZNF10, ZNF75D, ZNF426, ZNF561, ZNF562, ZNF846, ZNF782, ZNF552, ZNF587B, ZNF814, ZNF587, ZNF92, ZNF417, ZNF256, ZNF473, ZFP14, ZFP82, ZNF529, ZNF605, ZFP57, ZNF724, ZNF43, ZNF354A, ZNF547, SSX4B, ZNF585A, ZNF585B, ZNF792, ZNF789, ZNF394, ZNF655, ZFP92, ZNF41, ZNF674, ZNF546, ZNF780B, ZNF699, ZNF177, ZNF560, ZNF583, ZNF707, ZNF808, ZKSCAN5, ZNF137P, ZNF611, ZNF600, ZNF28, ZNF773, ZNF549, ZNF550, ZNF416, ZIK1, ZNF211, ZNF527, ZNF569, ZNF793, ZNF571-AS1, ZNF540, ZNF571, ZNF607, ZNF75A, ZNF205, ZNF175, ZNF268, ZNF354B, ZNF135, ZNF221, ZNF285, ZNF419, ZNF30, ZNF304, ZNF254, ZNF701, ZNF418, ZNF71, ZNF570, ZNF705E, KRBOX1, ZNF510, ZNF778, PRDM9, ZNF248, ZNF845, ZNF525, ZNF765, ZNF813, ZNF747, ZNF764, ZNF785, ZNF689, ZNF311, ZNF169, ZNF483, ZNF493, ZNF189, ZNF658, ZNF564, ZNF490, ZNF791, ZNF678, ZNF454, ZNF34, ZNF7, ZNF250, ZNF705D, ZNF641, ZNF2, ZNF554, ZNF555, ZNF556, ZNF596, ZNF517, ZNF331, ZNF18, ZNF829, ZNF772, ZNF17, ZNF112, ZNF514, ZNF688, PRDM7, ZNF695, ZNF670-ZNF695, ZNF138, ZNF670, ZNF19, ZNF316, ZNF12, ZNF202, RBAK, ZNF83, ZNF468, ZNF479, ZNF679, ZNF736, ZNF680, ZNF273, ZNF107, ZNF267, ZKSCAN8, ZNF84, ZNF573, ZNF23, ZNF559, ZNF44, ZNF563, ZNF442, ZNF799, ZNF443, ZNF709, ZNF566, ZNF69, ZNF700, ZNF763, ZNF433-AS1, ZNF433, ZNF878, ZNF844, ZNF788P, ZNF20, ZNF625-ZNF20, ZNF625, ZNF606, ZNF530, ZNF577, ZNF649, ZNF613, ZNF350, ZNF317, ZNF300, ZNF180, ZNF415, VN1R1, ZNF266, ZNF738, ZNF445, ZNF852, ZKSCAN7, ZNF660, MPRIPP1, ZNF197, ZNF567, ZNF582, ZNF439, ZFP30, ZNF559-ZNF177, ZNF226, ZNF841, ZNF544, ZNF233, ZNF534, ZNF836, ZNF320, KRBA2, ZNF761, ZNF383, ZNF224, ZNF551, ZNF154, ZNF671, ZNF776, ZNF780A, ZNF888, ZNF816-ZNF321P, ZNF321P, ZNF816, ZNF347, ZNF665, ZNF677, ZNF160, ZNF184, ZNF140, ZNF589, ZNF891, ZFP69B, ZNF436, POGK, ZNF669, ZFP69, ZNF684, ZNF124, ZNF496, and sequence variants thereof.

5. The gene repressor system of claim 3, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

6. The gene repressor system of claim 3, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239.

7. The gene repressor system of claim 5 or claim 6, wherein the KRAB domain comprises one or more sequence motifs selected from the group consisting of:

a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein
   i) X_1 is A, D, E, or N,
   ii) X_2 is L or V,
   iii) X_3 is I or V,
   iv) X_4 is S, T, or F,
   v) X_5 is H, K, L, Q, R or W,
   vi) X_6 is L or M, and
   vii) X_7 is G, K, Q, or R;

b) $X_1X_2X_3X_4\mathbf{G}X_5X_6X_7X_8X_9$, wherein
   i) X_1 is L or V,
   ii) X_2 is A, G, L, T or V,
   iii) X_3 is A, F, or S,
   iv) X_4 is L or V,
   v) X_5 is C, F, H, I, L or Y,
   vi) X_6 is A, C, P, Q, or S,
   vii) X_7 is A, F, G, I, S, or V,
   viii) X_8 is A, P, S, or T, and
   ix) X_9 is K or R;

c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein
   i) X_1 is K or R,
   ii) X_2 is A, D, E, G, N, S, or T,
   iii) X_3 is D, E, or S, and
   iv) X_4 is L or R;

d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein

i) X_1 is A, L, P, or S,

ii) X_2 is L or V,

iii) X_3 is S or T,

iv) X_4 is A, E, G, K, or R,

v) X_5 is A or T,

vi) X_6 is I or V,

vii) X_7 is D, E, N, or Y,

viii) X_8 is S or T,

ix) X_9 is E, P, Q, R, or W,

x) X_{10} is E or N, and

xi) X_{11} is E or Q;

e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein

i) X_1 is E, G, or R,

ii) X_2 is E or K,

iii) X_3 is A, D, or E,

iv) X_4 is C or W,

v) X_5 is I, K, L, M, T, or V,

vi) X_6 is I, L, P, or V,

vii) X_7 is D, E, K, or V,

viii) X_8 is E, G, K, P, or R,

ix) X_9 is A, D, R, G, K, Q, or V, and

x) X_{10} is D, E, G, I, L, R, S, or V;

f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein

i) X_1 is K or R,

ii) X_2 is D or E,

iii) X_3 is L, Q, or R,

iv) X_4 is N or T,

v) X_5 is F or Y,

vi) X_6 is A, E, G, Q, R, or S,

vii) X_7 is H, L, or N,

viii) X_8 is L or V,

ix) X_9 is A, G, I, L, T, or V, and

x) X_{10} is A, F, or S,

g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein

i) X_1 is A, E, G, K, or R,

ii) X_2 is A, S, or T,

iii) X_3 is I or V,

iv) X_4 is D, E, N, or Y,

v) X_5 is S or T,

vi) X_6 is E, L, P, Q, R, or W,

vii) X_7 is D or E, and

viii) X_8 is A, E, G, Q, or R;

h) $X_1PX_2X_3X_4X_5$ $X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein

i) X_1 is K or R,

ii) X_2 is A, D, E, or N,

iii) X_3 is I, L, M, or V,

iv) X_4 is I or V,

v) X_5 is F, S, or T,

vi) X_6 is H, K, L, Q, R, or W,

vii) X_7 is K, Q, or R,

viii) X_8 is E, G, or R,

ix) X_9 is D, E, or K,

x) X_{10} is A, D, or E,

xi) X_{11} is L or P, and

xii) X_{12} is C or W; or

i) $X_1LX_2X_3X_4QX_5X_6$, wherein

i) X_1 is C, H, L, Q, or W,

ii) X_2 is D, G, N, R, or S,

iii) X_3 is L, P, S, or T,

iv) X_4 is A, S, or T,

v) X_5 is K or R, and

vi) X_6 is A, D, E, K, N, S, or T.

8. The gene repressor system of claim 7, wherein the KRAB domain comprises a first and a second sequence motif wherein:

a) the first sequence motif comprises the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein

i) X_1 is K or R,

ii) X_2 is D or E,

iii) X_3 is L, Q, or R,

iv) X_4 is N or T,

v) X_5 is F or Y,

vi) X_6 is A, E, G, Q, R, or S,

vii) X_7 is H, L, or N,

viii) X_8 is L or V,

ix) X_9 is A, G, I, L, T, or V, and

x) X_{10} is A, F, or S; and

b) the second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein

i) X_1 is A, E, G, K, or R,

ii) X_2 is A, S, or T,

iii) X_3 is I or V,

iv) X_4 is D, E, N, or Y,

v) X_5 is S or T,

vi) X_6 is E, L, P, Q, R, or W,

vii) X_7 is D or E, and

viii) X_8 is A, E, G, Q, or R.

9. The gene repressor system of claim 7 or claim 8, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

10. The gene repressor system of claim 9, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least

about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

11. The gene repressor system of claim 9, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

12. The gene repressor system of any one of claims 7-11, wherein the fusion protein is capable of repressing expression of a reporter gene to a greater extent than a comparable fusion protein comprising a ZNF10 KRAB domain (SEQ ID NO: 59626) when assayed in an in vitro cellular assay.

13. The gene repressor system of claim 12, wherein the reporter gene is a B2M locus in HEK293 cells, and wherein expression of B2M is repressed by at least about 75%, at least about 80%, at least about 85%, or at least about 90% at day 7 of the cellular assay.

14. The gene repressor system of any one of claims 1-13, wherein the KRAB domain is linked at or near the C-terminus of the catalytically-dead Class 2 CRISPR protein by a linker peptide sequence.

15. The gene repressor system of any one of claims 1-13, wherein the KRAB domain is linked at or near the N-terminus of the catalytically-dead Class 2 CRISPR protein by a linker peptide sequence.

16. A gene repressor system comprising:

a) a catalytically-dead Class 2 CRISPR protein;

b) a first transcription repressor domain;

c) a second transcription repressor domain;

d) a third transcription repressor domain; and

e) a guide ribonucleic acid (gRNA)

wherein:

i) the catalytically-dead Class 2 CRISPR protein, the first transcription repressor domain, the second transcription repressor domain, and the third transcription repressor domain are linked as a fusion protein;

ii) the gRNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene targeted for repression, silencing, or downregulation;

iii) the fusion protein is capable of forming a ribonucleoprotein (RNP) with the gRNA; and

iv) the RNP is capable of binding to the target nucleic acid of the gene.

17. The gene repressor system of claim 16, wherein the gene encodes an mRNA, rRNA, tRNA, or structural RNA.

18. The gene repressor system of claim 16 or claim 17, wherein the first transcription repressor is a Krüppel-associated box (KRAB) domain.

19. The gene repressor system of claim 18, wherein the KRAB domain is selected from the group consisting of ZNF343, ZNF337, ZNF334, ZNF215, ZNF519, ZNF485, ZNF214, ZNF33B, ZNF287, ZNF705A, ZNF37A, KRBOX4, ZKSCAN3, ZKSCAN4, ZNF57, ZNF557, ZNF705B, ZNF662, ZNF77, ZNF500, ZNF558, ZNF620, ZNF713, ZNF823, ZNF440, ZNF441, ZNF136, SNRPB, ZNF735, ZKSCAN2, ZNF619, ZNF627, ZNF333, ABCA11P, PLD5P1, ZNF25, ZNF727, ZNF595, ZNF14, ZNF33A, ZNF101, ZNF253, ZNF56, ZNF720, ZNF85, ZNF66, ZNF722P, ZNF486, ZNF682, ZNF626, ZNF100, ZNF93, ZKSCAN1, ZNF257, ZNF729, ZNF208, ZNF90, ZNF430, ZNF676, ZNF91, ZNF429, ZNF675, ZNF681, ZNF99, ZNF431, ZNF98, ZNF708, ZNF732, SSX2, ZNF721, ZNF726, ZNF730, ZNF506, ZNF728, ZNF141, ZNF723, ZNF302, ZNF484, LINC00960, SSX2B, ZNF718, ZNF74, ZNF157, ZNF790, ZNF565, ZNF705G, VN1R107P, SLC27A5, ZNF737, SSX4, ZNF850, ZNF717, ZNF155, ZNF283, ZNF404, ZNF114, ZNF716, ZNF230, ZNF45, ZNF222, ZNF286A, ZNF624, ZNF223, ZNF284, ZNF790-AS1, ZNF382, ZNF749, ZNF615, ZFP90, ZNF225, ZNF234, ZNF568, ZNF614, ZNF584, ZNF432, ZNF461, ZNF182, ZNF630, ZNF630-AS1, ZNF132, ZNF420, ZNF324B, ZNF616, ZNF471, ZNF227, ZNF324, ZNF860, ZFP28, ZNF470, ZNF586, ZNF235, ZNF274, ZNF446, ZFP1, ZIM3, ZNF212, ZNF766, ZNF264, ZNF480, ZNF667, ZNF805, ZNF610, ZNF783, ZNF621, ZNF8-DT, ZNF880, ZNF213-AS1, ZNF213, ZNF263, ZSCAN32, ZIM2, ZNF597, ZNF786, KRBA1, ZNF460, ZNF8, ZNF875, ZNF543, ZNF133, ZNF229, ZNF528, SSX1, ZNF81, ZNF578, ZNF862, ZNF777, ZNF425, ZNF548, ZNF746, ZNF282, ZNF398, ZNF599, ZNF251, ZNF195, ZNF181, RBAK-RBAKDN, ZFP37, RN7SL526P, ZNF879, ZNF26, ZSCAN21, ZNF3, ZNF354C, ZNF10, ZNF75D, ZNF426, ZNF561, ZNF562, ZNF846, ZNF782, ZNF552, ZNF587B, ZNF814, ZNF587, ZNF92, ZNF417, ZNF256, ZNF473, ZFP14, ZFP82, ZNF529, ZNF605, ZFP57, ZNF724, ZNF43, ZNF354A, ZNF547, SSX4B, ZNF585A, ZNF585B, ZNF792, ZNF789, ZNF394, ZNF655, ZFP92, ZNF41, ZNF674, ZNF546, ZNF780B, ZNF699, ZNF177, ZNF560, ZNF583, ZNF707, ZNF808,

ZKSCAN5, ZNF137P, ZNF611, ZNF600, ZNF28, ZNF773, ZNF549, ZNF550, ZNF416, ZIK1, ZNF211, ZNF527, ZNF569, ZNF793, ZNF571-AS1, ZNF540, ZNF571, ZNF607, ZNF75A, ZNF205, ZNF175, ZNF268, ZNF354B, ZNF135, ZNF221, ZNF285, ZNF419, ZNF30, ZNF304, ZNF254, ZNF701, ZNF418, ZNF71, ZNF570, ZNF705E, KRBOX1, ZNF510, ZNF778, PRDM9, ZNF248, ZNF845, ZNF525, ZNF765, ZNF813, ZNF747, ZNF764, ZNF785, ZNF689, ZNF311, ZNF169, ZNF483, ZNF493, ZNF189, ZNF658, ZNF564, ZNF490, ZNF791, ZNF678, ZNF454, ZNF34, ZNF7, ZNF250, ZNF705D, ZNF641, ZNF2, ZNF554, ZNF555, ZNF556, ZNF596, ZNF517, ZNF331, ZNF18, ZNF829, ZNF772, ZNF17, ZNF112, ZNF514, ZNF688, PRDM7, ZNF695, ZNF670-ZNF695, ZNF138, ZNF670, ZNF19, ZNF316, ZNF12, ZNF202, RBAK, ZNF83, ZNF468, ZNF479, ZNF679, ZNF736, ZNF680, ZNF273, ZNF107, ZNF267, ZKSCAN8, ZNF84, ZNF573, ZNF23, ZNF559, ZNF44, ZNF563, ZNF442, ZNF799, ZNF443, ZNF709, ZNF566, ZNF69, ZNF700, ZNF763, ZNF433-AS1, ZNF433, ZNF878, ZNF844, ZNF788P, ZNF20, ZNF625-ZNF20, ZNF625, ZNF606, ZNF530, ZNF577, ZNF649, ZNF613, ZNF350, ZNF317, ZNF300, ZNF180, ZNF415, VN1R1, ZNF266, ZNF738, ZNF445, ZNF852, ZKSCAN7, ZNF660, MPRIPP1, ZNF197, ZNF567, ZNF582, ZNF439, ZFP30, ZNF559-ZNF177, ZNF226, ZNF841, ZNF544, ZNF233, ZNF534, ZNF836, ZNF320, KRBA2, ZNF761, ZNF383, ZNF224, ZNF551, ZNF154, ZNF671, ZNF776, ZNF780A, ZNF888, ZNF816-ZNF321P, ZNF321P, ZNF816, ZNF347, ZNF665, ZNF677, ZNF160, ZNF184, ZNF140, ZNF589, ZNF891, ZFP69B, ZNF436, POGK, ZNF669, ZFP69, ZNF684, ZNF124, ZNF496, and sequence variants thereof.

20. The gene repressor system of claim 19, wherein the KRAB domain is selected from ZNF10 or ZIM3.

21. The gene repressor system of claim 18, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

22. The gene repressor system of claim 18, wherein the KRAB domain is selected from the group of sequences consisting of SEQ ID NOS: 889-2100 and 2332-33239.

23. The gene repressor system of claim 21 or claim 22, wherein the KRAB domain comprises one or more sequence motifs selected from the group consisting of:

a) $PX_1X_2X_3X_4X_5X_6EX_7$, wherein

i) X_1 is A, D, E, or N,

ii) X_2 is L or V,

iii) X_3 is I or V,

iv) X_4 is S, T, or F,

v) X_5 is H, K, L, Q, R or W,

vi) X_6 is L or M, and

vii) X_7 is G, K, Q, or R;

b) $X_1X_2X_3X_4GX_5X_6X_7X_8X_9$, wherein

i) X_1 is L or V,

ii) X_2 is A, G, L, T or V,

iii) X_3 is A, F, or S,

iv) X_4 is L or V,

v) X_5 is C, F, H, I, L or Y,

vi) X_6 is A, C, P, Q, or S,

vii) X_7 is A, F, G, I, S, or V,

viii) X_8 is A, P, S, or T, and

ix) X_9 is K or R;

c) $QX_1X_2LYRX_3VMX_4$ (SEQ ID NO: 59345), wherein

i) X_1 is K or R,

ii) X_2 is A, D, E, G, N, S, or T,

iii) X_3 is D, E, or S, and

iv) X_4 is L or R;

d) $X_1X_2X_3FX_4DVX_5X_6X_7FX_8X_9X_{10}X_{11}$ (SEQ ID NO: 59346), wherein

i) X_1 is A, L, P, or S,

ii) X_2 is L or V,

iii) X_3 is S or T,

iv) X_4 is A, E, G, K, or R,

v) X_5 is A or T,

vi) X_6 is I or V,

vii) X_7 is D, E, N, or Y,

viii) X_8 is S or T,

ix) X_9 is E, P, Q, R, or W,

x) X_{10} is E or N, and

xi) X_{11} is E or Q;

e) $X_1X_2X_3PX_4X_5X_6X_7X_8X_9X_{10}$, wherein

i) X_1 is E, G, or R,

ii) X_2 is E or K,

iii) X_3 is A, D, or E,

iv) X_4 is C or W,

v) X_5 is I, K, L, M, T, or V,

vi) X_6 is I, L, P, or V,

vii) X_7 is D, E, K, or V,

viii) X_8 is E, G, K, P, or R,

ix) X_9 is A, D, R, G, K, Q, or V, and

x) X_{10} is D, E, G, I, L, R, S, or V;

f) $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein

i) X_1 is K or R,

ii) X_2 is D or E,

iii) X_3 is L, Q, or R,

iv) X_4 is N or T,

v) X_5 is F or Y,

vi) X_6 is A, E, G, Q, R, or S,

vii) X_7 is H, L, or N,

viii) X_8 is L or V,

ix) X_9 is A, G, I, L, T, or V, and

x) X_{10} is A, F, or S;

g) $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein

i) X_1 is A, E, G, K, or R,

ii) X_2 is A, S, or T,

iii) X_3 is I or V,

iv) X_4 is D, E, N, or Y,

v) X_5 is S or T,

vi) X_6 is E, L, P, Q, R, or W,

vii) X_7 is D or E, and

viii) X_8 is A, E, G, Q, or R;

h) $X_1PX_2X_3X_4X_5X_6LEX_7X_8X_9X_{10}X_{11}X_{12}$, wherein

i) X_1 is K or R,

ii) X_2 is A, D, E, or N,

iii) X_3 is I, L, M, or V,

iv) X_4 is I or V,

v) X_5 is F, S, or T,

vi) X_6 is H, K, L, Q, R, or W,

vii) X_7 is K, Q, or R,

viii) X_8 is E, G, or R,

ix) X_9 is D, E, or K,

x) X_{10} is A, D, or E,

xi) X_{11} is L or P, and

xii) X_{12} is C or W; or

i) $X_1LX_2X_3X_4QX_5X_6$, wherein

i) X_1 is C, H, L, Q, or W,

ii) X_2 is D, G, N, R, or S,

iii) X_3 is L, P, S, or T,

iv) X_4 is A, S, or T,

v) X_5 is K or R, and

vi) X_6 is A, D, E, K, N, S, or T.

24. The gene repressor system of claim 22 or claim 23, wherein the KRAB domain comprises a first and a second sequence motif wherein:

a) the first sequence motif comprises the sequence $LYX_1X_2VMX_3EX_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 59348), wherein

i) X_1 is K or R,

ii) X_2 is D or E,

iii) X_3 is L, Q, or R,

iv) X_4 is N or T,

v) X_5 is F or Y,

vi) X_6 is A, E, G, Q, R, or S,

vii) X_7 is H, L, or N,

viii) X_8 is L or V,

ix) X_9 is A, G, I, L, T, or V, and

x) X_{10} is A, F, or S; and

b) the second sequence motif comprises the sequence $FX_1DVX_2X_3X_4FX_5X_6X_7EWX_8$ (SEQ ID NO: 59349), wherein

i) X_1 is A, E, G, K, or R,

ii) X_2 is A, S, or T,

iii) X_3 is I or V,

iv) X_4 is D, E, N, or Y,

v) X_5 is S or T,

vi) X_6 is E, L, P, Q, R, or W,

vii) X_7 is D or E, and

viii) X_8 is A, E, G, Q, or R.

25. The gene repressor system of claim 24, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-59342, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

26. The gene repressor system of claim 25, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-57840, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

27. The gene repressor system of claim 25, wherein the KRAB domain sequence is selected from the group consisting of SEQ ID NOS: 57746-57755, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

28. The gene repressor system of any one of claims 16-27, wherein the second and the third transcription repressor domains are each a DNA methyltransferase (DNMT) domain.

29. The gene repressor system of claim 28, wherein the second transcription repressor domain is DNMT3A or a subdomain thereof.

30. The gene repressor system of claim 29, wherein the second transcription repressor domain is a catalytic domain of DNMT3A (DNMT3A CD).

31. The gene repressor system of claim 30, wherein the DNMT3A CD comprises a sequence selected from the group consisting of SEQ ID NOS: 33625-57543 and 59450, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

32. The gene repressor system of claim 26, wherein the third transcription repressor domain is a DNMT3L interaction domain (DNMT3L ID).

33. The gene repressor system of claim 32, wherein the DNMT3L ID comprises the sequence of SEQ ID NO: 59625, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

34. The gene repressor system of claim 30 or claim 31, wherein the fusion protein comprises an ATRX-DNMT3-DNMT3L (ADD) domain linked N-terminal to the DNMT3A catalytic domain.

35. The gene repressor system of claim 34, wherein the ADD domain comprises the sequence of SEQ ID NO: 59452, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

36. The gene repressor system of any one of claims 1-35, wherein the fusion protein comprises one or more linker peptide sequences.

37. The gene repressor system of claim 36, wherein the linker peptide sequence is selected from the group consisting of RS, (G)n (SEQ ID NO: 33240), (GS)n (SEQ ID NO: 33241), (GGS)n (SEQ ID NO: 33242), (GSGGS)n (SEQ ID NO: 33243), (GGSGGS)n (SEQ ID NO: 33244), (GGGS)n (SEQ ID NO: 33245), GGSG (SEQ ID NO: 33246), GGSGG (SEQ ID NO: 33247), GSGSG (SEQ ID NO: 33248), GSGGG (SEQ ID NO: 33249), GGGSG (SEQ ID NO: 33250), GSSSG (SEQ ID NO: 33251), (GP)n (SEQ ID NO: 33252), GPGP (SEQ ID NO: 33253), GGSGGGS (SEQ ID NO: 33254), GGP, PPP, PPAPPA (SEQ ID NO: 33255), PPPGPPP (SEQ ID NO: 33256), PPPG (SEQ ID NO: 33257), PPP(GGGS)n (SEQ ID NO: 33258), (GGGS)nPPP (SEQ ID NO: 33259), AEAAAKEAAAKEAAAKA (SEQ ID NO:33260), AEAAAKEAAAKA (SEQ ID NO: 33261), SGSETPGTSESATPES (SEQ ID NO:

33262), TPPKTKRKVEFE (SEQ ID NO: 33263), GSGSGGG (SEQ ID NO: 57628), GGCGGTTCCGGCGGAGGAAGC (SEQ ID NO: 57624), GGCGGTTCCGGCGGAGGTTCC (SEQ ID NO: 57625), GGATCAGGCTCTGGAGGTGGA (SEQ ID NO: 57627), GGAGGGCCGAGCTCTGGCGCACCCCCACCAAGTGGAGGGTCTCCTGCCGGGTCCCC AACATCTACTGAAGAAGGCACCAGCGAATCCGCAACGCCCGAGTCAGGCCCTGGTA CCTCCACAGAACCATCTGAAGGTAGTGCGCCTGGTTCCCCAGCTGGAAGCCCTACTT CCACCGAAGAAGGCACGTCAACCGAACCAAGTGAAGGATCTGCCCCTGGGACCAGC ACTGAACCATCTGAG (SEQ ID NO: 57620), SSGNSNANSRGPSFSSGLVPLSLRGSH (SEQ ID NO: 57623), GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGT STEPSEGSAPGTSTEPSE (SEQ ID NO: 57621), and TCTAGCGGCAATAGTAACGCTAACAGCCGCGGGCCGAGCTTCAGCAGCGGCCTGGT GCCGTTAAGCTTGCGCGGCAGCCAT (SEQ ID NO: 57622), wherein n is an integer of 1 to 5.

38. The gene repressor system of any one of claims 1-37, wherein the CRISPR protein is a catalytically-dead Class 2 CRISPR protein selected from the group consisting of a catalytically-dead Type II, a catalytically-dead Type V, or a catalytically-dead Type VI protein.

39. The gene repressor system of claim 38, wherein the catalytically-dead Type II protein is a Cas9 protein.

40. The gene repressor system of claim 38, wherein the catalytically-dead Type V protein selected from the group consisting of catalytically-dead Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f, Cas12g, Cas12h, Cas12i, Cas12j, Cas12k, Cas14, and CasΦ proteins.

41. The gene repressor system of claim 40, wherein the CRISPR protein is a catalytically-dead CasX protein (dCasX).

42. The gene repressor system of claim 41, wherein the dCasX comprises a sequence selected from the group consisting of SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

43. The gene repressor system of claim 41, wherein the dCasX comprises a sequence selected from the group consisting of the sequences SEQ ID NOS: 17-36 and 59353-59358 as set forth in Table 4.

44. The gene repressor system of claim 43, wherein the dCasX comprises the sequence of SEQ ID NO: 18, or a sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

45. The gene repressor system of any one of claims 1-44, wherein the fusion protein further comprises one or more nuclear localization signals (NLS).

46. The gene repressor system of claim 45, wherein the one or more NLS are selected from the group of sequences consisting of PKKKRKV (SEQ ID NO: 33289), KRPAATKKAGQAKKKK (SEQ ID NO: 33290), PAAKRVKLD (SEQ ID NO: 33291), RQRRNELKRSP (SEQ ID NO: 33292), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 33293), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 33294), VSRKRPRP (SEQ ID NO: 33295), PPKKARED (SEQ ID NO: 33296), PQPKKKPL (SEQ ID NO: 166), SALIKKKKKMAP (SEQ ID NO: 33298), DRLRR (SEQ ID NO: 33299), PKQKKRK (SEQ ID NO: 33300), RKLKKKIKKL (SEQ ID NO: 33301), REKKKFLKRR (SEQ ID NO: 33302), KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 33303), RKCLQAGMNLEARKTKK (SEQ ID NO: 33304), PRPRKIPR (SEQ ID NO: 33305), PPRKKRTVV (SEQ ID NO: 33306), NLSKKKKRKREK (SEQ ID NO: 33307), RRPSRPFRKP (SEQ ID NO: 33308), KRPRSPSS (SEQ ID NO: 33309), KRGINDRNFWRGENERKTR (SEQ ID NO: 33310), PRPPKMARYDN (SEQ ID NO: 33311), KRSFSKAF (SEQ ID NO: 33312), KLKIKRPVK (SEQ ID NO: 33313), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33314), PKTRRRPRRSQRKRPPT (SEQ ID NO: 33315), SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 33316), KTRRRPRRSQRKRPPT (SEQ ID NO: 33317), RRKKRRPRRKKRR (SEQ ID NO: 33318), PKKKSRKPKKKSRK (SEQ ID NO: 33319), HKKKHPDASVNFSEFSK (SEQ ID NO: 33320), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 33321), LSPSLSPLLSPSLSPL (SEQ ID NO: 33322), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 33323), PKRGRGRPKRGRGR (SEQ ID NO: 33324), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 33325), PKKKRKVPPPPKKKRKV (SEQ ID NO: 33326), PAKRARRGYKC (SEQ ID NO: 33327),

KLGPRKATGRW (SEQ ID NO: 33328), PRRKREE (SEQ ID NO: 33329), PYRGRKE (SEQ ID NO: 33330), PLRKRPRR (SEQ ID NO: 33331), PLRKRPRRGSPLRKRPRR (SEQ ID NO: 33332), PAAKRVKLDGGKRTADGSEFESPKKKRKV (SEQ ID NO: 33333), PAAKRVKLDGGKRTADGSEFESPKKKRKVGIHGVPAA (SEQ ID NO: 33334), PAAKRVKLDGGKRTADGSEFESPKKKRKVAEAAAKEAAAKEAAAKA (SEQ ID NO: 33335), PAAKRVKLDGGKRTADGSEFESPKKKRKVPG (SEQ ID NO: 33336), KRKGSPERGERKRHW (SEQ ID NO: 33337), KRTADSQHSTPPKTKRKVEFEPKKKRKV (SEQ ID NO: 33338), and SEQ ID NOS: 37-112.

47. The gene repressor system of claim 45 or claim 46, wherein the one or more NLS are linked at or near the C-terminus of the fusion protein.

48. The gene repressor system of claim 45 or claim 46, wherein the one or more NLS are linked at or near the N-terminus of the fusion protein.

49. The gene repressor system of claim 45 or claim 46, wherein the one or more NLS are linked at or near both the N-terminus and the C-terminus of the fusion protein.

50. The gene repressor system of claim 45, wherein the one or more NLS are selected from the group of SEQ ID NOS: 37-71 as set forth in Table 5 and are linked at or near the N-terminus of the fusion protein.

51. The gene repressor system of claim 45, wherein the one or more NLS are selected from the group of SEQ ID NOS: 72-112 as set forth in Table 6 and are linked at or near the C-terminus of the fusion protein.

52. The gene repressor system of claim 45, wherein one or more NLS comprise an NLS selected from the group consisting of SEQ ID NOS: 37-71 as set forth in Table 5 and are linked at or near the N-terminus of the fusion protein, and an NLS selected from the group consisting of SEQ ID NOS: 72-112 as set forth in Table 6 and are linked at or near the C-terminus of the fusion protein.

53. The gene repressor system of any one of claims 45-52, wherein the fusion protein is configured, from N-terminus to C-terminus:

a) NLS-Linker4-DNMT3A CD-Linker2- DNMT3L ID-Linker 1-Linker3-dCasX-Linker3-KRAB-NLS;

b) NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-DNMT3A CD-Linker2-DNMT3L ID;

c) NLS-Linker3-dCasX-Linker1-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-NLS;

d) NLS-KRAB-Linker3-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS, or

e) NLS-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-Linker1-dCasX-Linker3-NLS.

54. The gene repressor system of any one of claims 45-52, wherein the fusion protein is configured according to a configuration as portrayed in FIG. 7.

55. The gene repressor system of any one of claims 45-52, wherein the fusion protein is configured, from N-terminus to C-terminus:

a) NLS -ADD-DNMT3A CD-Linker 2-DNMT3L ID-Linker1-Linker3-dCasX-Linker3-KRAB-NLS;

b) NLS-Linker3-dCasX-Linker3-KRAB-NLS-Linker1-ADD-DNMT3A CD-Linker2-DNMT3L ID;

c) NLS-Linker3-dCasX-Linker1-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-NLS;

d) NLS-KRAB-Linker3-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS; or

e) NLS-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker3-KRAB-Linker1-dCasX-Linker3-NLS.

56. The gene repressor system of any one of claims 45-52, wherein the fusion protein is configured according to a configuration as portrayed in FIG. 45.

57. The gene repressor system of claim 55 or claim 56, wherein the fusion protein comprises a sequence selected from the group consisting of SEQ ID NOS: 59508-59567 and 59673-60012, or a sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

58. The gene repressor system of claim 55 or claim 56, wherein the fusion protein comprises a sequence selected from the group consisting of SEQ ID NOS: 59508-59567 and 59673-60012.

59. The gene repressor system of any one of claims 1-58, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2238-2331, 57544-57589 and 59352 as set forth in Table 2, or a sequence having at least about 85%, at least about

90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

60. The gene repressor system of any one of claims 1-58, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2238-2331, 57544-57589 and 59352, as set forth in Table 2.

61. The gene repressor system of any one of claims 1-60, wherein the gRNA has a scaffold comprising a sequence of SEQ ID NO: 2292, or a sequence having at least about 70%, at least about 80%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

62. The gene repressor system of any one of claims 1-60, wherein the gRNA has a scaffold comprising a sequence of SEQ ID NO: 59352, or a sequence having at least about 70%, at least about 80%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto.

63. The gene repressor system of claim 61 or claim 62, wherein the gRNA scaffold comprises one or more chemical modifications to the sequence.

64. The gene repressor system of claim 63, wherein the chemical modification is addition of a 2'O-methyl group to one or more nucleotides of the sequence.

65. The gene repressor system of claim 63 or claim 64, wherein the chemical modification is substitution of a phosphorothioate bond between two or more nucleotides of the sequence.

66. The gene repressor system of any one of claims 1-65, wherein the gRNA comprises a targeting sequence having 15, 16, 17, 18, 19, or 20 nucleotides.

67. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of a transcription start site (TSS) in the gene.

68. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within 500 bps upstream to 500 bps downstream of a TSS of the gene.

69. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within 300 bps upstream to 300 bps downstream of a TSS of the gene.

70. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of an enhancer of the gene.

71. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within the 3' untranslated region of the gene.

72. The gene repressor system of claim 66, wherein the target nucleic acid sequence complementary to the targeting sequence is within an exon of the gene.

73. The gene repressor system of claim 72, wherein the target nucleic acid sequence complementary to the targeting sequence is within exon 1 of the gene.

74. The gene repressor system of any one of claims 1-73, wherein the RNP is capable of binding to the target nucleic acid but is not capable of cleaving the target nucleic acid.

75. The gene repressor system of claim 74, wherein upon binding to the target nucleic acid, the gene is epigenetically modified.

76. The gene repressor system of claim 75, wherein upon epigenetic modification, transcription of the gene is repressed.

77. The gene repressor system of claim 76, wherein transcription of the gene is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% compared to an untreated gene, when assessed in an *in vitro* assay.

78. The gene repressor system of claim 76, wherein the repression of transcription of the gene is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 1 month, or at least about 2 months.

79. A nucleic acid encoding the fusion protein of the gene repressor system of any one of claims 1-78.

80. The nucleic acid of claim 79, wherein the nucleic acid sequence is mRNA.

81. The nucleic acid of claim 80, wherein the mRNA is chemically modified.

82. A nucleic acid encoding the gRNA of the gene repressor system of any one of claims 1-78.

83. The nucleic acid of any one of claims 79-81, wherein the nucleic acid sequence is codon optimized for expression in a eukaryotic cell.

84. A lipid nanoparticle comprising the nucleic acid of any one of claims 79-81.

85. A lipid nanoparticle comprising the nucleic acid of claim 82.

86. A lipid nanoparticle comprising a first nucleic acid encoding the fusion protein and a second nucleic acid comprising the gRNA of the gene repressor system of any one of claims 1-78.

87. A lipid nanoparticle composition comprising a first population of lipid nanoparticles and a second population of lipid nanoparticles encapsidating the repressor system of any one of claims 1-78, wherein the first population comprises lipid nanoparticles that encapsidate a first nucleic acid encoding the fusion protein and the second population of lipid nanoparticles comprises nanoparticles that encapsidate a second nucleic acid encoding the gRNA or that comprises the gRNA.

88. A vector comprising the nucleic acid of any one of claims 79-83.

89. The vector of claim 88, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

90. The vector of claim 89, wherein the vector is an AAV vector.

91. The vector of claim 90, wherein the AAV vector has a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 44.9, AAV 9.45, AAV 9.61, AAV-Rh74, and AAVRh10.

92. The vector of claim 90 or claim 91, comprising a nucleic acid encoding the fusion protein and the gRNA incorporated as a transgene between 5' and a 3' inverted terminal repeat (ITR) sequences within the AAV.

93. A delivery particle system (XDP) comprising:

a) one or more components of selected from the group consisting of a matrix protein (MA), a nucleocapsid protein (NC), a capsid protein (CA), a p1 peptide, a p6 peptide, a p2A peptide, a p2B peptide, a p10 peptide, a p12 peptide, a pp21/24 peptide, a p12/p3/p8 peptide, a p20 peptide, an MS2 coat protein, PP7 coat protein, Q coat protein, U1A signal recognition particle, phage R-loop, Rev protein, and Psi packaging element;

b) an RNP comprising the gene repressor system of any one of claims 1-74 wherein the RNP is encapsidated within the XDP;

c) a tropism factor incorporated on the XDP surface that provides for binding and fusion of the XDP to a target cell.

94. The XDP of claim 93, wherein the tropism factor is selected from the group consisting of a pseudotyping viral envelope glycoprotein, an antibody fragment, or a cell receptor fragment.

95. A method of repressing transcription of a target nucleic acid sequence of a gene in a population of cells, the method comprising introducing into the cells:

a) an RNP comprising the gene repressor system of any one of claims 1-78

b) the nucleic acid of any one of claims 79-83;

c) the vector of any one of claims 88-92;

d) the XDP of claim 93 or 94;

e) the lipid nanoparticle of any one of claims 84-86; or

f) the lipid nanoparticle composition of claim 87,

wherein upon binding of the introduced or expressed RNP of the gene repressor system to the target nucleic acid, transcription of the gene is repressed in the cells.

96. The method of claim 95, wherein the cells are selected from the group consisting of an embryonic stem cell, an induced pluripotent stem cell, a germ cell, a fibroblast, an oligodendrocyte, a glial cell, a hematopoietic stem cell, a neuron progenitor cell, a neuron, an astrocyte, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, a retinal cell, a cancer cell, a T-cell, a B-cell, an NK cell, a fetal cardiomyocyte, a myofibroblast, a mesenchymal stem cell, an autotransplanted expanded cardiomyocyte, an adipocyte, a totipotent cell, a pluripotent cell, a blood stem cell, a myoblast, a bone marrow cell, a mesenchymal cell, a parenchymal cell, an epithelial cell, an endothelial cell, a mesothelial cell, fibroblasts, osteoblasts, chondrocytes, a hematopoietic stem cell, a bone-marrow derived progenitor cell, a myocardial cell, a skeletal cell, a fetal cell, an undifferentiated cell, a multi-potent progenitor cell, a unipotent progenitor cell, a monocyte, a cardiac myoblast, a skeletal myoblast, a macrophage, a capillary endothelial cell, a xenogeneic cell, an allogenic cell, and a post-natal stem cell.

97. The method of claim 95 or claim 96, wherein the binding location of the RNP is selected from the group consisting of:

a) a sequence within 300 to 1,000 base pairs 5’ to a transcription start site (TSS) in the gene;

b) a sequence within 300 to 1,000 base pairs 3’ to a TSS in the gene;

c) a sequence within 300 to 1,000 base pairs to an enhancer of the gene;

d) a sequence within the open reading frame of the gene;

e) a sequence within an exon of the gene; or

f) a sequence in the 3' untranslated region (UTR) of the gene.

98. The method of any one of claims 95-97, wherein transcription of the gene in the population of cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99% greater compared to untreated cells, when assessed in an *in vitro* assay.

99. The method of any one of claims 95-98, wherein off-target methylation or off-target transcription repression is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% in the cells, when assessed in an *in vitro* assay.

100. The method of any one of claims 95-99, wherein the repression of transcription in the cells is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, or at least about 6 months.

101. The method of any one of claims 95-100, further comprising a second gRNA or a nucleic acid encoding the second gNA, wherein the second gNA has a targeting sequence complementary to a different portion of the target nucleic acid sequence and is capable of forming a ribonucleoprotein (RNP) with the fusion protein comprising the catalytically-dead Class 2 CRISPR protein and the one or more transcription repressor domains.

102. The method of any one of claims 95-101, wherein the method mediates a heritable epigenetic change in the cells.

103. A method of treating a subject with a disorder caused by a genetic mutation, comprising administering a therapeutically-effective dose of:

a) an RNP comprising the gene repressor system of any one of claims 1-78

b) the nucleic acid of any one of claims 79-83;

c) the vector of any one of claims 88-92;

d) the XDP of claim 93 or 94;

e) the lipid nanoparticle of any one of claims 84-86; or

f) the lipid nanoparticle composition of claim 87,

wherein upon binding of the administered or expressed RNP of the gene repressor system to the target nucleic acid of a gene in cells of the subject transcription of the gene is repressed.

104. The method of any one of claims 103, wherein transcription of the gene in the cells is repressed by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least 99%.

105. The method of any one of claims 103, wherein the repression of transcription of the gene in the cells is sustained for at least about 8 hours, at least about 1 day, at least about 7 days, at least about 1 month, or at least about 2 months.

106. The method of any one of claims 103-105, wherein the method mediates a heritable epigenetic change in the cells of the subject.

107. The method of any one of claims 103-106, wherein the RNP, nucleic acid, AAV vector, XDP, or the lipid nanoparticles are administered to the subject by a route of administration selected from subcutaneous, intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatical, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation, or combinations thereof.

108. The method of claim 107, wherein the XDP or the lipid nanoparticles are administered at a dose of at least about 1 x 10^5 particles/kg, or at least about 1 x 10^6 particles/kg, or at least about 1 x 10^7 particles/kg, or at least about 1 x 10^8 particles/kg, or at least about 1 x 10^9 particles/kg, or at least about 1 x 10^{10} particles/kg, or at least about 1 x 10^{11} particles/kg, or at least about 1 x 10^{12} particles/kg, or at least about 1 x 10^{13} particles/kg, or at least about 1 x 10^{14} particles/kg, or at least about 1 x 10^{15} particles/kg, or at least about 1 x 10^{16} particles/kg.

109. The method of claim 107, wherein the XDP or the lipid nanoparticles are administered to the subject at a dose of at least about 1 x 10^5 particles/kg to about 1 x 10^{16} particles/kg, or at least about 1 x 10^6 particles/kg to about 1 x 10^{15} particles/kg, or at least about 1 x 10^7 particles/kg to about 1 x 10^{14} particles/kg.

110. The method of claim 107, wherein the AAV vector is administered to the subject at a dose of at least about 1 x 10^8 vector genomes (vg), at least about 1 x 10^5 vector genomes/kg (vg/kg), at least about 1 x 10^6 vg/kg, at least about 1 x 10^7 vg/kg, at least about 1 x 10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 1 x 10^{14} vg/kg, at least about 1 x 10^{15} vg/kg, or at least about 1 x 10^{16} vg/kg.

111. The method of claim 107, wherein the AAV vector is administered to the subject at a dose of at least about 1 x 10^5 vg/kg to about 1 x 10^{16} vg/kg, at least about 1 x 10^6 vg/kg to about 1 x 10^{15} vg/kg, or at least about 1 x 10^7 vg/kg to about 1 x 10^{14} vg/kg.

112. The method of claim 107, wherein the first and second lipid nanoparticles are each administered at a dose of at least about 1 x 10^5 particles/kg, or at least about 1 x 10^6 particles/kg, or at least about 1 x 10^7 particles/kg, or at least about 1 x 10^8 particles/kg, or at least about 1 x 10^9 particles/kg, or at least about 1 x 10^{10} particles/kg, or at least about 1 x 10^{11} particles/kg, or at least about 1 x 10^{12} particles/kg, or at least about 1 x 10^{13} particles/kg, or at least about 1 x 10^{14} particles/kg, or at least about 1 x 10^{15} particles/kg, or at least about 1 x 10^{16} particles/kg.

113. The method of claim 107, wherein the first and the second lipid nanoparticles are each administered to the subject at a dose of at least about 1 x 10^5 particles/kg to about 1 x 10^{16} particles/kg, or at least about 1 x 10^6 particles/kg to about 1 x 10^{15} particles/kg, or at least about 1 x 10^7 particles/kg to about 1 x 10^{14} particles/kg.

114. The method of any one of claims 103-113, wherein the XDP, the AAV vector, the lipid nanoparticles, or the first and second lipid nanoparticles are administered to the subject according to a treatment regimen comprising one or more consecutive doses.

115. The method of any one of claims 103-114, wherein the therapeutically effective dose is administered to the subject as two or more doses over a period of at least two weeks, or at least one month, or at least two months, or at least three months, or at least four months, or at least five months, or at least six months, or once a year.

116. The method of any one of claims 103-115, wherein the treating results in improvement in at least one clinically-relevant endpoint associated with the disorder in the subject.

117. The method of any one of claims 103-115, wherein the subject is selected from the group consisting of mouse, rat, pig, and non-human primate.

118. The method of any one of claims 103-115, wherein the subject is human.

119. A pharmaceutical composition comprising the gene repressor system, the nucleic acid, the vector, the XDP, or the LNP of any one of claims 1-92and a pharmaceutically acceptable excipient.

120. The gene repressor system of any one of claims 1-78 for use as a medicament in the treatment of a subject with a disorder caused by a genetic mutation.

121. The gene repressor system of any one of claims 1-78, wherein the targeting sequence of the gRNA is complementary to a non-target strand sequence located 1 nucleotide 3' of a protospacer adjacent motif (PAM) sequence.

122. The composition of claim 121, wherein the PAM sequence comprises a TC motif.

123. The composition of claim 121 or claim 122, wherein the PAM sequence comprises ATC, GTC, CTC or TTC.

124. The gene repressor system of any one of claims 1-78 for use in the manufacture of a medicament in the treatment of a subject with a disorder caused by a genetic mutation.