

CLAIMS

1. A gene repressor system comprising:
 - a) a catalytically-dead Class 2 CRISPR protein;
 - b) a transcription repressor domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57755, 57750, 57771, and 57779, or a sequence with 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; 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 fusion protein is capable of repressing expression of a reporter gene to a greater extent than a comparable fusion protein comprising SEQ ID NO: 59626 as the transcription repressor domain when assayed in an in vitro cellular assay.
3. The gene repressor system of claim 2, wherein the reporter gene is a beta-2-microglobulin (B2M) locus, 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%.
4. The gene repressor system of any one of claims 1-3, wherein the transcriptional repressor domain is linked at or near the C-terminus or at or near the N-terminus of the catalytically-dead Class 2 CRISPR protein by a linker peptide sequence.
5. A gene repressor system comprising:
 - a) a catalytically-dead Class 2 CRISPR protein;
 - b) a first transcription repressor domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57755, 57750, 57771, and 57779, or a sequence with 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 thereto;

- 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.
- 6.** The gene repressor system of claim 5, wherein the second and the third transcription repressor domains are each a DNA methyltransferase (DNMT) domain.
- 7.** The gene repressor system of claim 6, wherein the second transcription repressor domain is DNMT3A or a subdomain thereof.
- 8.** The gene repressor system of claim 7, wherein the second transcription repressor domain is a catalytic domain of DNMT3A (DNMT3A CD).
- 9.** The gene repressor system of claim 8, wherein the DNMT3A CD comprises a sequence of SEQ ID NO: 59450, or a sequence having 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 any one of claims 5-9, wherein the third transcription repressor domain is a DNMT3L interaction domain (DNMT3L ID).
- 11.** The gene repressor system of claim 10, wherein the DNMT3L ID comprises the sequence of SEQ ID NO: 59625, or a sequence having 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 8-11, wherein the fusion protein comprises an ATRX-DNMT3-DNMT3L (ADD) domain linked N-terminal to the DNMT3A catalytic domain.

13. The gene repressor system of claim 12, wherein the ADD domain comprises the sequence of SEQ ID NO: 59452, or a sequence having 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.
14. The gene repressor system of any one of claims 1-13, wherein the fusion protein comprises one or more linker peptide sequences.
15. The gene repressor system of any one of claims 1-14, wherein the catalytically-dead Class 2 CRISPR protein is selected from the group consisting of a catalytically-dead Type II, a catalytically-dead Type V, or a catalytically-dead Type VI protein.
16. The gene repressor system of claim 15, wherein the catalytically-dead Type II protein is a Cas9 protein.
17. The gene repressor system of claim 15, wherein the catalytically-dead Type V protein is 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.
18. The gene repressor system of claim 17, wherein the CRISPR protein is a catalytically-dead CasX protein (dCasX), wherein the dCasX comprises a sequence selected from the group consisting of SEQ ID NOS: 17-36 and 59353-59358, or a sequence having 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.
19. The gene repressor system of claim 18, wherein the dCasX comprises a sequence of SEQ ID NO: 18, or a sequence having 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.
20. The gene repressor system of any one of claims 1-19, wherein the fusion protein further comprises one or more nuclear localization signals (NLS).
21. The gene repressor system of claim 20, wherein
 - a. 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), KRKGDEV DGVDEVAKKSKK (SEQ ID NO: 33303), RKCLQAGMNLEARKTKK (SEQ ID NO: 33304), PRPRKIPR (SEQ ID NO: 33305), PPRKKRTVV (SEQ ID NO: 33306), NLSKKKKRKREK (SEQ ID NO: 33307), RRPSRPFKRP (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), PKTRRRPRRSQRKRPT (SEQ ID NO: 33315), SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 33316), KTRRRPRRSQRKRPT (SEQ ID NO: 33317), RRKKRRPRRKRR (SEQ ID NO: 33318), PKKKS RKP KKKSRK (SEQ ID NO: 33319), HKKKHPDASVNFSEFSK (SEQ ID NO: 33320), QRP GPYDRP QRP GPYDRP (SEQ ID NO: 33321), LSPSLSP LSPSLSP L (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), PLRKRPRRGSP L RKRPRR (SEQ ID NO: 33332), PAAKRVKLDGGKRTADGSEFESP K KKRKV (SEQ ID NO: 33333), PAAKRVKLDGGKRTADGSEFESP K KKRKVGIHGVPAA (SEQ ID NO: 33334), PAAKRVKLDGGKRTADGSEFESP K KKRKVAAEAAAKEAAAKEAAKA (SEQ ID NO: 33335), PAAKRVKLDGGKRTADGSEFESP K KKRKVPG (SEQ ID NO: 33336), KRKGSPERGERKRHW (SEQ ID NO: 33337), KRTADSQHSTPPKTKRKVEFEP K KKRKV (SEQ ID NO: 33338), and SEQ ID NOS: 37-112; and

b. the one or more linker peptides comprise sequences selected from of GGPSSGAPPPSGGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSE (Linker1, SEQ ID NO: (57621), SSGNSNANSRGPSFSSGLVPLSLRGSH (Linker 2, SEQ ID NO: 57623), GGSGGGS (Linker 3, SEQ ID NO: 57626), and GSGSGGG (Linker 4, SEQ ID NO: 57628).

22. The gene repressor system of claim 20 or claim 21, wherein the one or more NLS are linked at or near the C-terminus of the fusion protein, at or near the N-terminus of the fusion protein or at or near both the N-terminus and the C-terminus of the fusion protein.

23. The gene repressor system of any one of claims 20-22, 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-first repressor domain-NLS;
- b) NLS-Linker3-dCasX-Linker3-first repressor domain-NLS-Linker1-DNMT3A CD-Linker2-DNMT3L ID;
- c) NLS-Linker3-dCasX-Linker1-DNMT3A CD-Linker2-DNMT3L ID-Linker3-first repressor domain-NLS;
- d) NLS-first repressor domain-Linker3-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS;
- e) NLS-DNMT3A CD-Linker2-DNMT3L ID-Linker3-first repressor domain-Linker1-dCasX-Linker3-NLS;
- f) NLS -ADD-DNMT3A CD-Linker 2-DNMT3L ID-Linker1-Linker3-dCasX-Linker3-first repressor domain-NLS;
- g) NLS-Linker3-dCasX-Linker3-first repressor domain-NLS-Linker1-ADD-DNMT3A CD-Linker2-DNMT3L ID;
- h) NLS-Linker3-dCasX-Linker1-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker3-first repressor domain-NLS;
- i) NLS-first repressor domain-Linker3-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker1-dCasX-Linker3-NLS; or
- j) NLS-ADD-DNMT3A CD-Linker2-DNMT3L ID-Linker3-first repressor domain-Linker1-dCasX-Linker3-NLS.

24. The gene repressor system of any one of claims 1-23, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2238-2331, 57544-57589 and 59352, or a sequence having 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.

25. The gene repressor system of claim 24, wherein the gRNA scaffold comprises one or more chemical modifications to the sequence.

26. The gene repressor system of claim 25, wherein the chemical modification is addition of a 2'O-methyl group to one or more nucleotides of the sequence, and/or the chemical modification is substitution of a phosphorothioate bond between two or more nucleotides of the sequence.

27. The gene repressor system of any one of claims 1-26, wherein the gRNA comprises a targeting sequence having 15, 16, 17, 18, 19, 20 or 21 nucleotides.
28. The gene repressor system of claim 27, wherein:
- (i) the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of a transcription start site (TSS) in the gene;
 - (ii) the target nucleic acid sequence complementary to the targeting sequence is within 1 kb of an enhancer of the gene;
 - (iii) the target nucleic acid sequence complementary to the targeting sequence is within the 3' untranslated region of the gene; or
 - (iv) the target nucleic acid sequence complementary to the targeting sequence is within an exon of the gene.
29. The gene repressor system of any one of claims 1-28, wherein the RNP is capable of binding to the target nucleic acid but is not capable of cleaving the target nucleic acid, wherein upon binding to the target nucleic acid, the gene is epigenetically modified, and wherein upon epigenetic modification, transcription of the gene is repressed.
30. The gene repressor system of claim 29, 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 about 99% compared to an untreated gene, when assessed in an *in vitro* assay.
31. The gene repressor system of claim 29, 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.
32. A nucleic acid encoding the fusion protein of the gene repressor system of any one of claims 1-31.
33. The nucleic acid of claim 32, wherein the nucleic acid sequence is mRNA.
34. The nucleic acid of claim 33, wherein the mRNA is chemically modified, wherein
- a. the chemical modification is substitution of N1-methyl-pseudouridine for one or more uridine nucleotides of the sequence; or
 - b. the mRNA comprises a sequence selected from the group consisting of SEQ ID NOS: 59584-59585, 59610, 59611, 59622 and 59623.

35. A nucleic acid encoding the gRNA of the gene repressor system of any one of claims 1-31.

36. A lipid nanoparticle (LNP) comprising the nucleic acid of any one of claims 32-34, wherein the LNP comprises one or more components selected from the group consisting of an ionizable lipid, a helper phospholipid, a polyethylene glycol (PEG)-modified lipid, and cholesterol.

37. A lipid nanoparticle (LNP) 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 1-31, wherein the LNP comprises one or more components selected from the group consisting of an ionizable lipid, a helper phospholipid, a polyethylene glycol (PEG)-modified lipid, and cholesterol.

38. A lipid nanoparticle (LNP) composition comprising a first population of lipid nanoparticles and a second population of lipid nanoparticles encapsidating the repressor system of any one of claims 1-31, 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 the gRNA, wherein the LNP comprises one or more components selected from the group consisting of an ionizable lipid, a helper phospholipid, a polyethylene glycol (PEG)-modified lipid, and cholesterol.

39. A vector comprising the nucleic acid of any one of claims 32-35.

40. The vector of claim 39, 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 plasmid, a minicircle, a nanoplasmid, and an RNA vector.

41. A method of repressing transcription of a target nucleic acid sequence of a gene in a population of cells *in vitro* or *ex vivo*, the method comprising introducing into the cells:

- a) an RNP comprising the gene repressor system of any one of claims 1-31;
- b) the nucleic acid of any one of claims 32-35;
- c) the vector of claim 39 or 40;
- d) the lipid nanoparticle of claim 36 or 37; or
- e) the lipid nanoparticle composition of claim 38,

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.

- 42.** The method of claim 41, wherein the method mediates a heritable epigenetic change in the gene of the cells.
- 43.** A composition for use as a medicament in the treatment of a subject with a disorder, the composition comprising:
- a) an RNP comprising the gene repressor system of any one of claims 1-31;
 - b) the nucleic acid of any one of claims 32-35;
 - c) the vector of claim 39 or 40;
 - d) the lipid nanoparticle of claim 36 or 37; or
 - e) the lipid nanoparticle composition of claim 38.
- 44.** A pharmaceutical composition comprising the gene repressor system, the nucleic acid, the vector, or the LNP of any one of claims 1-40 and a pharmaceutically acceptable excipient.