## CLAIMS

We claim:

1. An expression repression system comprising:

a first expression repressor comprising a first DNA-targeting moiety and a first repressor domain, and

a second expression repressor comprising a second DNA-targeting moiety and a second repressor domain,

wherein the first DNA-targeting moiety specifically binds a first DNA sequence, and the second DNA-targeting moiety specifically binds a second DNA sequence different from the first DNA sequence, and

wherein the first repressor domain is different from the second repressor domain.

2. An expression repression system comprising:

a first expression repressor comprising a first DNA-targeting moiety and a first repressor domain, and

a second expression repressor comprising a second DNA-targeting moiety and a second repressor domain,

wherein the first or second repressor domain comprises an MQ1 domain or functional variant or fragment thereof,

wherein the first repressor domain is different from the second repressor domain.

3. The expression repression system of claim 2, wherein:

the first DNA-targeting moiety specifically binds a first DNA sequence, and the second DNA-targeting moiety specifically binds a second DNA sequence different from the first DNA sequence; or

the first DNA-targeting moiety and the second DNA-targeting moiety specifically bind the same DNA sequence.

4. The expression repression system of any of claims 1-3,

wherein the first DNA-targeting moiety comprises a first CRISPR/Cas molecule comprising a first CRISPR/Cas protein and first guide RNA, and the second DNA-targeting moiety comprises a second CRISPR/Cas molecule comprising a second CRISPR/Cas protein and a second guide RNA.

5. The expression repression system of any of claims 1-4:

wherein the first CRISPR/Cas protein comprises a different amino acid sequence than the second CRISPR/Cas protein.

6. The expression repression system of any of claims 1-5, wherein the first or second DNA-targeting moiety comprises a CRISPR/Cas molecule comprising a Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof.

7. The expression repression system of any of claims 1-6, wherein the first DNA-targeting moiety comprises a first CRISPR/Cas molecule comprising a Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof, and the second DNA-targeting moiety comprises a second CRISPR/Cas molecule comprising a different Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof.

8. The expression repression system of any of claims 1-7, wherein the first or second repressor domain comprises a histone methyltransferase activity (e.g., a protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, or a functional variant or fragment of any thereof, e.g., a SET domain of any thereof), a histone demethylase activity (e.g., a protein chosen from KDM1A (i.e., LSD1), KDM1B (i.e., LSD2), KDM2A, KDM2B, KDM5A, KDM5B, KDM5C, KDM5D, KDM4B, NO66, or a functional variant or fragment of any thereof), a histone deacetylase activity (e.g., a protein chosen from HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, or a functional variant or fragment of any thereof), a DNA methyltransferase activity (e.g., a protein chosen from MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, or a functional variant or fragment of any thereof), or a transcription repressor activity (e.g., a protein chosen from KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment of any thereof).

9. The expression repression system of any of claims 1-8, wherein:

the first repressor domain comprises a protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, KDM1A (i.e., LSD1), KDM1B (i.e., LSD2), KDM2A, KDM2B, KDM5A, KDM5B, KDM5C, KDM5D, KDM4B, NO66, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5,

DNMT3B6, DNMT3L, KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment thereof, and

the second repressor domain comprises a different protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, KDM1A (i.e., LSD1), KDM1B (i.e., LSD2), KDM2A, KDM2B, KDM5A, KDM5B, KDM5C, KDM5D, KDM4B, NO66, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment thereof.

10. The expression repression system of any of claims 1-9, wherein the first repressor domain comprises KRAB, and the second repressor domain comprises DNMT3A (e.g., human DNMT3A).

11. The expression repression system of any of claims 1-9, wherein the first repressor domain comprises KRAB, and the second repressor domain comprises bacterial MQ1.

12. The expression repression system of any of claims 1-11, wherein the first or second DNA-targeting moiety is selected from a TAL effector molecule, a CRISPR/Cas molecule, a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide.

13. The expression repression system of any of claims 1-12, wherein the first or second DNA-targeting moiety is or comprises a CRISPR/Cas molecule comprising a CRISPR/Cas protein (e.g., that is or comprises a Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof) and a guide RNA.

14. The expression repression system of any of claims 1-13, wherein:

the first DNA-targeting moiety is or comprises a CRISPR/Cas molecule comprising a CRISPR/Cas protein that is or comprises a Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof), and

the second DNA-targeting moiety is or comprises a CRISPR/Cas molecule comprising a different CRISPR/Cas protein that is or comprises a Cas protein or Cpf1 protein chosen from Table 1 or a variant (e.g., mutant) of any thereof).

15. The expression repression system of any of claims 1-14, wherein the first or the second DNA-targeting moiety specifically binds to:

a promoter sequence or to DNA proximal to a promoter sequence, e.g., a promoter sequence operably linked to a target gene;

an enhancer sequence or to DNA proximal to an enhancer sequence, e.g., an enhancer sequence that affects expression of and/or is operably linked to a target gene;

a target gene, e.g., sequence comprised between the transcription start site and stop codon of a target gene;

the transcription start site of a target gene;

an intron, e.g., a splice site associated with an intron, of a target gene; or

an exon of a target gene.

16. The expression repression system of any of claims 1-15, wherein the first DNA sequence is a first site within or proximal to a promoter operably linked to a target gene and the second DNA sequence is a second site within or proximal to the promoter operably linked to the target gene.

17. The expression repression system of any of claims 1-16, wherein the distance between the first DNA sequence and the second DNA sequence is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 base pairs or any size therebetween, e.g., 20-500 base pairs.

18. The expression repression system of any of claims 1-17, wherein the distance between the first DNA sequence and the second DNA sequence is no more than 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 base pairs or any size therebetween, e.g., 20-500 base pairs.

19. The expression repression system of any of claims 1-18, wherein the target gene is β-2-microglobulin, MYC, HSPA1B, GATA1, APOB, FOXP3, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL6, CXCL7, and/or CXCL8.

20. The expression repression system of any of claims 1-19, wherein the first DNA-targeting moiety does not appreciably bind to the second DNA sequence and the second DNA-targeting moiety does not appreciably bind to the first DNA sequence, e.g., wherein the first DNA-targeting moiety binds the second DNA sequence with a $K_D$ of at least 500, 600, 700, 800, 900, 1000, 2000, 5000, 10,000, or 100,000 nM, and the second DNA-targeting moiety binds the first DNA sequence with binds with a $K_D$ of at least 500, 600, 700, 800, 900, 1000, 2000, 5000, 10,000, or 100,000 nM.

21. The expression repression system of any of claims 1-20, wherein binding of the first expression repressor to the first DNA sequence and/or binding of the second expression repressor to the second DNA sequence decreases expression of a target gene, e.g., a target gene operably linked to the first and/or second DNA sequence, in a cell, e.g., by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% compared to expression in the absence of the first and/or second expression repressor.

22. The expression repression system of any of claims 1-21, wherein binding of the first and/or second expression repressor to the first and/or second DNA sequence appreciably decreases expression of a target gene, e.g., a target gene operably linked to the first and/or second DNA sequence, for a time period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell divisions, e.g., as measured by ELISA or as described in Examples 2-4.

23. The expression repression system of either of claims 21 or 22, wherein expression is decreased by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% compared to expression in the absence of the first and second expression repressors, e.g., as measured by ELISA or as described in Examples 2-4.

24. The expression repression system of any of claims 21-23, wherein binding of the first expression repressor to the first DNA sequence and the second expression repressor to the second DNA sequence appreciably decreases expression of a target gene, e.g., a target gene operably linked to the first DNA sequence, for a time period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 hours, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell divisions, e.g., as measured by ELISA or as described in Examples 2-4.

25. The expression repression system of any of claims 21-24, wherein the decrease in expression resulting from the binding of the first expression repressor to the first DNA sequence and the second expression repressor to the second DNA sequence is greater than the decrease in expression resulting

from the binding of the first expression repressor to the first DNA sequence or the binding of the second expression repressor to the second DNA sequence individually.

26. The expression repression system of claim 25, wherein the binding of the first expression repressor to the first DNA sequence and the second expression repressor to the second DNA sequence decreases expression 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.6x, 1.7x, 1.8x, 1.9x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 50x, or 100x more than either the binding of the first expression repressor to the first DNA sequence or the binding of the second expression repressor to the second DNA sequence individually, e.g., as measured by ELISA or as described in Examples 2-4.

27. The expression repression system of any of claim 21-26, wherein the decrease in expression resulting from the binding of the first expression repressor to the first DNA sequence and the second expression repressor to the second DNA sequence persists for a longer time (e.g., more hours, days, or cell divisions) than the decrease in expression resulting from the binding of the first expression repressor to the first DNA sequence or the binding of the second expression repressor to the second DNA sequence individually.

28. The expression repression system of claim 27, wherein the binding of the first expression repressor to the first DNA sequence and the second expression repressor to the second DNA sequence decreases expression 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.6x, 1.7x, 1.8x, 1.9x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 50x, or 100x longer (e.g., as measured in hours, days, or cell divisions) than either the binding of the first expression repressor to the first DNA sequence or the binding of the second expression repressor to the second DNA sequence individually, e.g., as measured by ELISA or as described in Examples 2-4.

29. The expression repression system of any of claims 1-28, wherein

the first expression repressor comprises a first DNA-targeting moiety comprising a CRISPR/Cas molecule comprising an *S. pyogenes* CRISPR/Cas protein selected from Table 1 or variant (e.g., mutant) thereof, e.g., an *S. pyogenes* dCas9, and a first repressor domain comprising KRAB or a functional variant or fragment thereof; and

the second expression repressor comprises a second DNA-targeting moiety comprising a CRISPR/Cas molecule comprising a *S. aureus* CRISPR/Cas protein selected from Table 1 or variant (e.g., mutant) thereof, e.g., an *S. aureus* dCas9, and a second repressor domain comprising bacterial MQ1 or a functional variant or fragment thereof.

30. A polypeptide comprising:

a DNA-targeting moiety; and

a repressor domain comprising bacterial MQ1 or a functional variant or fragment thereof.

31. A nucleic acid encoding:

the first expression repressor,

the second expression repressor,

the expression repression system (e.g., the first expression repressor and the second expression repressor), or the polypeptide of any of claims 1-30.

32. A vector comprising the nucleic acid of claim 31.

33. A cell comprising the expression repression system, nucleic acid, vector, or polypeptide of any of claims 1-32.

34. A pharmaceutical composition comprising the expression repression system, nucleic acid, polypeptide, or vector of any of claims 1-32, and at least one pharmaceutically acceptable excipient or carrier.

35. The expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 for use in a method of decreasing expression of a target genomic sequence in a cell, comprising:

providing the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector; and

contacting the cell with the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector,

thereby decreasing expression of the target genomic sequence.

36. Use of the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 in the manufacture of a medicament for use in a method of decreasing expression of a target genomic sequence in a cell, comprising:

providing the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector; and

contacting the cell with the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector,

thereby decreasing expression of the target genomic sequence.

37. The expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 for use in a method of epigenetically modifying a target genomic sequence in a cell, comprising:

providing the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector; and

contacting the cell with the expression repression system, nucleic acid, vector, polypeptide, or pharmaceutical composition,

thereby epigenetically modifying the target genomic sequence.

38. Use of the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 in the manufacture of a medicament for use in a method of epigenetically modifying a target genomic sequence in a cell, comprising:

providing the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector; and

contacting the cell with the expression repression system, nucleic acid, vector, polypeptide, or pharmaceutical composition,

thereby epigenetically modifying the target genomic sequence.

39. The use of any one of claims 35-38, wherein the target genomic sequence is:

a target gene (e.g., a site within a target gene, e.g., an exon, intron, or splice site), e.g., the gene encoding β-2-microglobulin (β2M),

a transcription control element operably linked to a target gene, or

an anchor sequence proximal to a target gene or associated with an anchor sequence-mediated conjunction operably linked to the target gene.

40. The use of any of claims 35-39, wherein expression is:

decreased by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% compared to expression in an otherwise similar cell in the absence of the expression repression system; and/or

decreased for a time period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell divisions, e.g., as measured by ELISA or as described in Examples 2-4.

41. The use of any of claims 35-40, wherein the decrease in expression comprises a decrease in:

the level of RNA, e.g., mRNA, encoded by the target gene; and/or

the level of a protein encoded by the target gene.

42. The use of any of claims 35-41, wherein providing comprises:

contacting the cell with a nucleic acid, e.g., vector, encoding the expression repression system to the cell comprising the target gene; or

contacting the cell with a lipid nanoparticle (LNP) comprising the expression repression system or a nucleic acid, e.g., vector, encoding the expression repression system.

43. The expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 for use in a method of treating a condition associated with over-expression or mis-regulation of a target gene in a subject, comprising:

administering the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector to the subject,

thereby treating a condition associated with over-expression of a target gene in a subject.

44. Use of the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector of any of claims 1-32 or 34 in the manufacture of a medicament for use in a method of treating a condition associated with over-expression or mis-regulation of a target gene in a subject, comprising:

administering the expression repression system, nucleic acid, polypeptide, pharmaceutical composition, or vector to the subject,

thereby treating a condition associated with over-expression of a target gene in a subject.

45. A method of making a cell comprising an expression repression system, the method comprising:

providing the expression repression system, nucleic acid, pharmaceutical composition, or vector of any of claims 1-29, 31, 32 or 34; and

contacting the cell with the expression repression system, nucleic acid, vector, or pharmaceutical composition,

thereby making a cell comprising an expression repression system.