

CLAIMS

What is claimed is:

1. A construct of Formula I:

$5'-(A_{m1}-B_{m2})_{n1}-\text{CasN}-(A_{m3}-B_{m4})_{n2}-\text{CasC}-T_p-E-3'$ (I),

wherein:

one of A and B is a polynucleotide encoding DNMT3A or a portion thereof; and the other of A and B is a polynucleotide encoding DNMT3L or a portion thereof;

CasN is a polynucleotide encoding a N-terminal portion of dCas9;

CasC is a polynucleotide encoding a C-terminal portion of dCas9;

E is $5'-(A_{m5}-B_{m6})_{n3}-K_r-D_q-3'$ or $5'-K_r-D_q-(A_{m5}-B_{m6})_{n3}-3'$;

K is a polynucleotide encoding KRAB or a portion thereof;

D is a polynucleotide encoding a modulator of gene expression;

T comprises a polynucleotide encoding (i) an epitope capable of binding to an antibody or an antigen binding fragment thereof or (ii) a polypeptide sequence capable of binding to a nucleic acid structural element;

m_1, m_2, m_3, m_4, m_5 , and m_6 are each independently an integer selected from 0 to 3;

n_1, n_2 , and n_3 are each independently an integer selected from 0 to 2;

p is an integer selected from 0 to 20;

q is an integer selected from 0 to 5;

r is an integer selected from 0 to 5; and

wherein when p is 0, at least one of m_1, m_2, m_3 , and m_4 is not 0, at least one of n_1 and n_2 is not 0, and at least one of q and r is not 0.

2. The construct of claim 1, of Formula II:

$5'-\text{CasN}-(A_{m3}-B_{m4})_{n2}-\text{CasC}-K_r-D_q-3'$ (II),

wherein:

n_2 is an integer selected from 1 and 2;

r is an integer selected from 1 to 5;

q is an integer selected from 0 to 5; and

at least one of m_3 and m_4 is not 0.

3. The construct of claim 2, of Formula IIa:

5'-CasN-(A-B)-CasC-K_r-D_q-3' (IIa).

4. The construct of claim 1, of Formula III:

5'-(A_{m1}-B_{m2})_{n1}-CasN-(A_{m3}-B_{m4})_{n2}-CasC-T_p-E-3' (III),

wherein p is an integer selected from 1 to 20.

5. The construct of claim 4, of Formula IIIb-1:

5'-(A-B)-CasN-CasC-T_p-K_r-D_q-3' (IIIb-1),

wherein:

r is an integer selected from 1 to 5; and

q is an integer selected from 0 to 5.

6. The constructs of claim 1, of Formula IV:

5'-(A-B)-CasN-CasC-K_r-D_q-3',

wherein:

r is an integer selected from 0 to 5;

q is an integer selected from 0 to 5; and

at least one of q and r is not 0.

7. The construct of claim 1, wherein the DNMT3A comprises the amino acid sequence of SEQ ID NO: 69.

8. The construct of claim 1, wherein the DNMT3L comprises the amino acid sequence of any one of SEQ ID NOS: 74-82.

9. The construct of claim 1, wherein the KRAB comprises the amino acid sequence of SEQ ID NO: 51, 53, or 230-241.

10. The construct of claim 1, wherein the modulator of gene expression comprises the amino acid sequence of SEQ ID NO: 51, 53, 55, 57, 59, 61, 63, 65, or 67.
11. The construct of claim 1, wherein the dCas9 comprises the amino acid sequence of any one of SEQ ID NOs: 1 and 106-122.
12. The construct of claim 1, wherein
 - 1) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 2 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 3; or
 - 2) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 4 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 5; or
 - 3) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 6 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 7; or
 - 4) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 8 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 9; or
 - 5) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 10 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 11; or
 - 6) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 12 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 13; or
 - 7) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 14 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 15; or
 - 8) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 16 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 17; or
 - 9) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 18 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 19; or
 - 10) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 20 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 21; or
 - 11) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 22 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 23; or
 - 12) the N-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 24 and the C-terminal portion of dCas9 comprises the amino acid sequence of SEQ ID NO: 25.

13. The construct of claim 1, wherein the epitope capable of binding to an antibody or an antigen binding fragment thereof is selected from a group consisting of GCN4, T2A, 10x GCN4, and 10XGFP-11.
14. The construct of claim 13, wherein the antigen binding fragment is a scFv.
15. The construct of claim 1, comprising the nucleic acid sequence of any one of SEQ ID NOs: 162-189.
16. A polypeptide expressed by the construct of claim 1.
17. Use of the construct of claim 1 or a polypeptide(s) expressed by the construct, in the manufacture of a reagent for modifying the expression of a gene product and minimizing off-target modifications in a population of cells, wherein the modifying and minimizing are via the steps comprising introducing into the population of cells:
 - i) the construct or a polypeptide(s) expressed by the construct; and
 - ii) at least one sgRNA,wherein the KRAB and/or modulator of gene expression provides a modification of at least one nucleotide near the gene and/or within a regulatory element of the gene, thereby modifying the expression of the gene product.