

CLAIMS

What is claimed is:

- 5 1. A method of site-specific integration of a nucleic acid into a cell genome, the method comprising:
 - (a) incorporating an integration site at a desired location in the cell genome by introducing into a cell:
 - 10 i. a DNA binding nuclease linked to a reverse transcriptase domain, wherein the DNA binding nuclease comprises a nickase activity; and
 - ii. a guide RNA (gRNA) comprising a primer binding sequence linked to an integration sequence, wherein the gRNA interacts with the DNA binding nuclease and targets the desired location in the cell genome, wherein the DNA binding nuclease nicks a strand of the cell genome and the reverse
 - 15 transcriptase domain incorporates the integration sequence of the gRNA into the nicked site, thereby providing the integration site at the desired location of the cell genome; and
 - (b) integrating the nucleic acid into the cell genome by introducing into the cell:
 - 20 i. a DNA or RNA strand comprising the nucleic acid linked to a sequence that is complementary or associated to the integration site; and
 - ii. an integration enzyme, wherein the integration enzyme incorporates the nucleic acid into the cell genome at the integration site by integration, recombination, or reverse transcription of the sequence that is complementary or associated to the integration site, thereby introducing the nucleic acid into
 - 25 the desired location of the cell genome of the cell.
2. The method of claim 1, wherein the gRNA hybridizes to a complementary strand of the cell genome to the genomic strand that is nicked by the DNA binding nuclease.
3. The method of any one of the previous claims, wherein the integration enzyme is introduced as a peptide or a nucleic acid encoding the integration enzyme.
- 30 4. The method of any one of the previous claims, wherein the DNA binding nuclease is introduced as a peptide or a nucleic acid encoding the DNA binding nuclease.

5. The method of any one of the previous claims, wherein the DNA or RNA strand comprising the nucleic acid is introduced into the cell as a minicircle, a plasmid, mRNA or a linear DNA.
6. The method of any one of the previous claims, wherein the DNA or RNA strand comprising the nucleic acid is between 1000 bp and 36,000 bp.
7. The method of any one of the claims 1-5, wherein the DNA or RNA strand comprising the nucleic acid is more than 36,000 bp.
8. The method of any one of the claims 1-5, wherein the DNA or RNA strand comprising the nucleic acid is less than 1000 bp.
9. The method of any one of the previous claims, wherein the DNA comprising the nucleic acid is introduced into the cell as a minicircle.
10. The method of claim 9, wherein the minicircle does not comprise a sequence of a bacterial origin.
11. The method of any one of the previous claims, wherein the DNA binding nuclease linked to a reverse transcriptase domain and the integration enzyme are linked via a linker.
12. The method of claim 11, wherein the linker is cleavable.
13. The method of claim 11, wherein the linker is non-cleavable.
14. The method of claim 11, wherein the linker can be replaced by two associating binding domains of the DNA binding nuclease linked to a reverse transcriptase.
15. The method of any one of the previous claims, wherein the integration enzyme is selected from the group consisting of Cre, Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, R1, R2, R3, R4, R5, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia, Benedict, KSSJEB, PattyP, Doom, Scoy1, Lockley, Switzer, Bob3, Troube, Abrogate, Anglerfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV, retrotransposases encoded by R2, L1, Tol2 Tc1, Tc3, Mariner (Himar 1), Mariner (mos 1), and Minos, and any mutants thereof.
16. The method of any one of the previous claims, wherein the integration enzyme is Bxb1 or a mutant thereof.

17. The method of any one of the previous claims, wherein the integration site is an attB site, an attP site, an attL site, an attR site, a lox71 site a Vox site, or a FRT site.
18. The method of any one of the previous claims, wherein the DNA binding nuclease comprising a nickase activity is selected from Cas9-D10A, Cas9-H840A, and
5 Cas12a/b nickase.
19. The method of any one of the previous claims, wherein the reverse transcriptase domain is selected from the group consisting of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase domain, transcription xenopolymerase (RTX), avian myeloblastosis virus reverse transcriptase (AMV-RT), and Eubacterium rectale
10 maturase RT (MarathonRT).
20. The method of any one of the previous claims, wherein the reverse transcriptase domain comprises a mutation relative to the wild-type sequence.
21. The method of claim 19, wherein the M-MLV reverse transcriptase domain comprises one or more mutations selected from the group consisting of D200N, T306K, W313F,
15 T330P and L603W.
22. The method of any one of the previous claims, further comprising introducing a second nicking guide RNA (ngRNA).
23. The method of any one of the previous claims, wherein the gRNA, the nucleic acid encoding the DNA binding nuclease, the reverse transcriptase, the DNA comprising
20 nucleic acid linked to a complementary or associated integration site, the integration enzyme, and optionally the ngRNA, are introduced into a cell in a single reaction.
24. The method of any one of the previous claims, wherein the gRNA, the nucleic acid encoding the DNA binding nuclease, the reverse transcriptase, the DNA comprising
25 nucleic acid linked to a complementary integration site, the integration enzyme, and optionally the ngRNA, are introduced using a virus, a RNP, an mRNA, a lipid, or a polymeric nanoparticle.
25. The method of any one of the previous claims, wherein the nucleic acid is a reporter gene.
26. The method of claim 25, wherein the reporter gene is a fluorescent protein.
- 30 27. The method of any one of the previous claims, wherein the cell is a dividing cell.

28. The method of any one of the previous claims, wherein the cell is a non-dividing cell.
29. The method of any one of the previous claims, wherein the desired location in the cell genome is the locus of a mutated gene.
30. The method of any one of the previous claims 1, wherein the nucleic acid is a degradation tag for programmable knockdown of proteins in the presence of small molecules.
31. The method of claim 1, wherein the cell is a mammalian cell, a bacterial cell or a plant cell.
32. The method of claim 1, wherein the nucleic acid is a T-cell receptor (TCR), a chimeric antigen receptor (CAR), an interleukin, a cytokine, or an immune checkpoint gene for integration into a T-cell or natural killer (NK) cell.
33. The method of claim 32, wherein the TCR, the CAR, the interleukin, the cytokine, or the immune checkpoint gene is incorporated into the target site of the T-cell or NK cell genome using a minicircle DNA.
34. The method of claim 1, wherein the nucleic acid is a beta hemoglobin (HBB) gene and the cell is a hematopoietic stem cell (HSC).
35. The method of claim 34, wherein the HBB gene is incorporated into the target site in the HSC genome using a minicircle DNA.
36. The method of claim 34, wherein the nucleic acid is a gene responsible for beta thalassemia or sickle cell anemia.
37. The method of claim 1, wherein the nucleic acid is a metabolic gene.
38. The method of any one of claim 37, wherein the metabolic gene is involved in alpha-1 antitrypsin deficiency or ornithine transcarbamylase (OTC) deficiency.
39. The method of any one of claims 37-38, wherein the metabolic gene is a gene involved in an inherited disease.
40. The method of claim 1, wherein the nucleic acid is a gene involved in an inherited disease or an inherited syndrome.
41. The method of claim 40, wherein the inherited disease is cystic fibrosis, familial hypercholesterolemia, adenosine deaminase (ADA) deficiency, X-linked SCID (X-

SCID), Wiskott-Aldrich syndrome (WAS), hemochromatosis, Tay-Sachs, fragile X syndrome, Huntington's disease, Marfan syndrome, phenylketonuria, or muscular dystrophy.

42. A vector comprising a nucleic acid encoding a DNA binding nuclease comprising a nickase activity C-terminally linked to a reverse transcriptase linked to an integration enzyme via a linker.
43. The vector of claim 42, wherein the linker is cleavable.
44. The vector of claim 42, wherein the linker is non-cleavable.
45. The vector of claim 42, wherein the linker comprises two associating binding domains of the DNA binding nuclease linked to a reverse transcriptase.
46. The vector of claim 42, wherein the integration enzyme comprises a conditional activation domain or conditional expression domain.
47. The vector of claim 46, wherein the integration enzyme is fused to an estrogen receptor.
48. The vector of claim 42, wherein the DNA binding nuclease comprising a nickase activity is selected from the group consisting of Cas9-D10A, Cas9-H840A, and Cas12a/b.
49. The vector of claim 42, wherein the reverse transcriptase is a M-MLV reverse transcriptase, a AMV-RT, MarathonRT, or a RTX.
50. The vector of claim 49, wherein the reverse transcriptase is a modified M-MLV reverse transcriptase relative to the wildtype M-MLV reverse transcriptase.
51. The vector of claim 49, wherein the M-MLV reverse transcriptase domain comprises one or more of the mutations selected from the group consisting of D200N, T306K, W313E, T330P and L603W.
52. The vector of claim 42, wherein the integration enzyme is selected from the group consisting of Cre, Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, R1, R2, R3, R4, R5, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia, KSSJEB, PattyP, Doom, Scowl, Lockley, Switzer, Bob3, Troube, Abrogate, Anglerfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV, retrotransposases encoded

by R2, L1, Tol2 Tc1, Tc3, Mariner (Himar 1), Mariner (mos 1), Minos, and mutants thereof.

53. The vector of claim 52, wherein the recombinase or integrase is Bxb1 or a mutant thereof.

5 54. A cell comprising:

(a) a vector comprising a nucleic acid encoding a DNA binding nuclease comprising a nickase activity that is C-terminally linked to a reverse transcriptase that is linked to an integration enzyme via a linker;

10 (b) a gRNA comprising a primer binding sequence, an integration sequence, and a guide sequence, wherein the gRNA can interact with the encoded nuclease comprising a nickase activity;

(c) a DNA minicircle comprising a nucleic acid and a sequence recognized by the encoded integrase, recombinase, or reverse transcriptase; and

15 (d) a nicking guide RNA (ngRNA) capable of binding the encoded nuclease comprising a nickase activity, wherein the ngRNA targets a sequence away from the gRNA.

55. The cell of claim 54, wherein the minicircle does not comprise a sequence of bacterial origin.

56. The cell of claim 54, wherein the integration enzyme is selected from the group consisting of Cre, Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, R1, R2, R3, R4, R5, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia, KSSJEB, PattyP, Doom, Scowl, Lockley, Switzer, Bob3, Troube, Abrogate, Anglerfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV, retrotransposases encoded by R2, L1, Tol2 Tc1, Tc3, Mariner (Himar 1), Mariner (mos 1), Minos, and any mutants thereof.

57. The cell of claim 54, wherein the integration enzyme is Bxb1 or a mutant thereof.

58. The cell of claim 54, wherein the DNA binding nuclease comprising a nickase activity is selected from the group consisting of Cas9-D10A, Cas9-H840A and Cas12a.

59. The cell of claim 54, wherein the reverse transcriptase is a M-MLV reverse transcriptase.

60. The cell of claim 59, wherein the reverse transcriptase is a modified M-MLV reverse transcriptase.
61. The cell of claim 59, wherein the amino acid sequence of the M-MLV reverse transcriptase comprises one or more mutations selected from the group consisting of D200N, T306K, W313F, T330P, and L603W.
62. The cell of any one of the claims 54-61, further comprising ngRNA.
63. A polypeptide comprising a DNA binding nuclease comprising a nickase activity C-terminally linked to a reverse transcriptase linked to an integration enzyme via a linker.
64. The polypeptide of claim 63, wherein the linker is cleavable.
65. The polypeptide of claim 63, wherein the linker is non-cleavable.
66. The polypeptide of claim 63, wherein the integration enzyme is fused to an estrogen receptor.
67. The polypeptide of claim 63, wherein the DNA binding nuclease comprising a nickase activity is selected from the group consisting of Cas9-D10A, Cas9-H840A, and Cas12a/b/c/d/e/f/g/h/i/j.
68. The polypeptide of claim 63, wherein the reverse transcriptase is a M-MLV reverse transcriptase, a AMV-RT, a MarathonRT, or a XRT.
69. The polypeptide of claim 68, wherein the reverse transcriptase is a modified M-MLV relative to a wild-type M-MLV reverse transcriptase.
70. The polypeptide of claim 69, wherein the M-MLV reverse transcriptase domain comprises one or more of mutations selected from the group consisting of D200N, T306K, W313F, T330P, and L603W.
71. The polypeptide of claim 63, wherein the integration enzyme is selected from group consisting of Cre, Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, R1, R2, R3, R4, R5, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia, KSSJEB, PattyP, Doom, Scowl, Lockley, Switzer, Bob3, Troube, Abrogate, Anglerfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV, retrotransposases encoded

by R2, L1, Tol2 Tc1, Tc3, Mariner (Himar 1), Mariner (mos 1), Minos, and any mutants thereof.

72. The polypeptide of claim 63, wherein the integration enzyme is Bxb1 or a mutant thereof.
- 5 73. A gRNA that specifically binds to a DNA binding nuclease comprising nickase activity, the gRNA comprising:
- (a) a primer binding site, which hybridizes to a nicked DNA strand;
 - (b) a recognition site for an integration enzyme; and
 - (c) a target recognition sequence recognizing a target site in a cell genome and
- 10 hybridizing to a genomic strand complementary to the strand that is nicked by the DNA binding nuclease.
74. The gRNA of claim 73, wherein the DNA binding nuclease comprising a nickase activity is selected from the group consisting of Cas9-D10A, Cas9-H840A, and Cas12a/b/c/d/e/f/g/h/i/j.
- 15 75. The gRNA of claim 73, wherein the primer binding site hybridizes to the 3' end of the nicked DNA strand.
76. The gRNA of claim 73, wherein the recognition site for the integration enzyme is selected from an attB site, an attP site, an attL site, an attR site, a lox71 site, and a FRT site.
- 20 77. The gRNA of claim 73, wherein the recognition site for the integration enzyme is a Bxb1 site.
78. A method of site-specific integration of two or more nucleic acids into a cell genome, the method comprising:
- (a) incorporating two integration sites at desired locations in the cell genome by
- 25 introducing into the cell:
- i. a DNA binding nuclease linked to a reverse transcriptase domain, wherein the DNA binding nuclease comprises a nickase activity; and
 - ii. two guide RNAs (gRNAs), each comprising, a primer binding sequence, and
- 30 is linked to a unique integration sequence, wherein the gRNA interacts with the DNA binding nuclease and targets the desired locations in the cell genome, wherein the DNA binding nuclease nicks a strand of the cell genome and the

reverse transcriptase domain incorporates each of the integration sequence of the gRNA into the nicked site, thereby providing the integration site at the desired locations of the cell genome; and

(b) integrating the nucleic acid by introducing into the cell:

- 5 i. two or more DNA or RNA comprising the nucleic acids, wherein each DNA is flanked by orthogonal integration sites; and
- ii. an integration enzyme, wherein the integration enzyme incorporates the nucleic acids into the cell genome at the integration sites by integrase, recombinase, or reverse transcriptase of the sequence that is complementary or
- 10 associated to the integration site, thereby introducing the nucleic acids into the desired locations of the cell genome of the cell.

79. The method of claim 78, wherein each of the two different integration sites inserted into the cell genome are attB sequences comprising different palindromic or non-palindromic central dinucleotide.

15 80. The method of claim 78, wherein each of the two different integration sites inserted into the cell genome are attP sequences comprising different palindromic or non-palindromic central dinucleotide.

81. The method of claim 78, wherein the integration enzyme enables each of the two or more DNA or RNA comprising the nucleic acids to directionally enable integration of

20 the nucleic acids into a genome via recombination of a pair of orthogonal attB site sequence and an attP site sequence.

82. The method of claim 78, wherein the integration enzyme is selected from the group consisting of Cre, Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia,

25 KSSJEB, PattyP, Doom, Scowl, Lockley, Switzer, Bob3, Troube, Abrogate, Anglenfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV, retrotransposases encoded by R1, R2, R3, R4, R5, L1, Tol2 Tc1, Tc3, Mariner (Himar 1), Mariner (mos 1), Minos, and any mutants thereof.

30 83. The method of claim 78, wherein the integration enzyme is Bxb1 or a mutant thereof.

84. The method of claim 78, wherein the DNA comprising genes are genes involved in a cell maintenance pathway, cell-division, or a signal transduction pathway.
85. The method of claim 78, wherein the reverse transcriptase domain comprises Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase domain,
 5 transcription xenopolymerase (RTX), or avian myeloblastosis virus reverse transcriptase (AMV-RT).
86. The method of claim 78, wherein the DNA binding nuclease comprising a nickase activity is selected from the group consisting of Cas9-D10A, Cas9-H840A, and Cas12a/b.
- 10 87. The method of claim 78, wherein the pair of an attB site sequence and an attP site sequence are selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID
 15 NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, and SEQ ID NO: 35 and SEQ ID NO: 36.
88. A cell comprising:
- 20 (a) a vector comprising a nucleic acid encoding a DNA binding nuclease comprising a nickase activity, wherein the DNA binding nuclease is C-terminally linked to a reverse transcriptase, wherein the reverse transcriptase is linked to a recombinase or integrase via a linker;
- (b) two guide RNAs (gRNAs) comprising a primer binding sequence, an integration
 25 sequence and a guide sequence, wherein the gRNA can interact with the encoded DNA binding nuclease comprising a nickase activity;
- (c) two or more DNA or RNA strands comprising a nucleic acid and a pair of flanking attB site sequence and an attP site sequence recognized by the encoded integrase or recombinase; and optionally
- 30 (d) a nicking guide RNA (ngRNA) capable of binding the encoded nuclease comprising a nickase activity, and wherein the ngRNA targets a sequence away from the gRNA.

89. A cell comprising a modified genome, wherein the modification comprises incorporation of two orthogonal integration sites within a cell genome by introducing into the cell:
- 5 (a) a vector comprising a nucleic acid encoding a DNA binding nuclease comprising a nickase activity, wherein the DNA binding nuclease is C-terminally linked to a reverse transcriptase;
- (b) two guide RNAs (gRNAs), each comprising a primer binding sequence, a genomic integration sequence, and a guide sequence, wherein the gRNA can interact with the encoded nuclease comprising a nickase activity; and optionally,
- 10 (c) a nicking guide RNA (ngRNA) capable of binding the encoded nuclease comprising a nickase activity, wherein the ngRNA targets a sequence away from the gRNA.
90. A method of integrating two or more nucleic acids into the cell genome of cell of claim 90, the method comprising introducing into the cell:
- 15 (a) two or more DNA, each comprising a nucleic acid and a pair of flanking orthogonal integration site sequences;
- (b) an integration enzyme that can recognize the integration site sequence enabling directional linking of the two or more DNA comprising nucleic acid; and
- (c) enabling incorporation of the nucleic acids into the cell genome by integrating the 5' orthogonal integration sequence of a first DNA with the first genomic integration sequence and 3' orthogonal integration sequence of a last DNA with the last genomic integration sequence, thereby incorporating the two or more nucleic acids into the cell genome.
- 20
91. A cell comprising a modified genome, wherein a modification of the modified genome comprises incorporation of two orthogonal integration sites within the cell genome by introducing into the cell:
- 25 (a) a vector comprising a nucleic acid encoding a DNA binding nuclease comprising a nickase activity, wherein the DNA binding nuclease is C-terminally linked to a reverse transcriptase;
- (b) two guide RNAs (gRNAs), each comprising a primer binding sequence, a genomic integration sequence, and a guide sequence, wherein the gRNA can interact with the encoded nuclease comprising a nickase activity; and optionally
- 30

- (c) a nicking guide RNA (ngRNA) capable of binding the encoded nuclease comprising a nickase activity, and wherein the ngRNA targets a sequence away from the gRNA;
- (d) two or more DNA or RNA comprising the nucleic acids, wherein each DNA is flanked by orthogonal integration sites; and
- 5 (e) an integration enzyme, wherein the integration enzyme incorporates the nucleic acids into the cell genome at the integration sites.

CLAIMS

1. A method of site-specifically integrating an exogenous nucleic acid sequence into a mammalian cell genome or intracellular target nucleic acid, the method comprising:

(a) incorporating at least one integration sequence at a specific target site in the cell genome or intracellular target nucleic acid by introducing *ex vivo* into a mammalian cell:

(i) an expressible polynucleotide construct encoding an editing polypeptide, wherein the editing polypeptide comprises a DNA binding nuclease domain linked via a linker to a reverse transcriptase domain, wherein the DNA binding nuclease domain comprises a nickase activity; and

(ii) a guide RNA (gRNA) comprising a targeting sequence, a primer binding sequence, and a complement of the at least one integration sequence,

wherein the gRNA interacts with the expressed editing polypeptide to direct the editing polypeptide to the specific target site of the cell genome or intracellular target nucleic acid,

wherein the DNA binding nuclease domain nicks a strand of the cell genome or intracellular target nucleic acid to form a nicked site, and

wherein the reverse transcriptase domain reverse transcribes the complement of the at least one integration sequence within the gRNA and thereby incorporates the at least one integration sequence into the nicked site, thereby incorporating the at least one integration sequence at the specific target site of the cell genome or intracellular target nucleic acid; and

(b) integrating an exogenous nucleic acid sequence into the cell genome or intracellular target nucleic acid by introducing into the cell:

(i) the exogenous nucleic acid sequence linked to a sequence that is an integration cognate to the site-specifically incorporated-integration sequence; and

(ii) an expressible polynucleotide construct encoding an integration enzyme, wherein the integration enzyme integrates the exogenous nucleic acid sequence into the cell genome or the intracellular target nucleic acid at the at least one site-specifically incorporated integration sequence, thereby site-specifically integrating the exogenous nucleic acid sequence into the cell genome or the intracellular target nucleic acid,

wherein the expressible polynucleotide encoding the editing polypeptide, the gRNA, the expressible polynucleotide construct encoding the integration enzyme, and the exogenous nucleic acid sequence are introduced into the mammalian cell concurrently.

- 5 2. The method of claim 1, wherein the gRNA, the expressible polynucleotide construct encoding the editing polypeptide, and the expressible polynucleotide construct encoding the integration enzyme are introduced into the mammalian cell using a virus, a RNP, an mRNA, a lipid, or a polymeric nanoparticle.
- 10 3. The method of claim 1, wherein the gRNA hybridizes to a strand of the mammalian cell genome.
4. The method of claim 1, wherein the exogenous nucleic acid is introduced into the mammalian cell as an adeno-associated virus (AAV) or an adenovirus (AdV).
- 15 5. The method of claim 1, wherein the exogenous nucleic acid is introduced into the mammalian cell as a minicircle, a plasmid, mRNA₂ or a linear DNA.
6. The method of claim 5, wherein the minicircle does not comprise a sequence of a bacterial origin.
- 20 7. The method of claim 1, wherein the linker is cleavable.
8. The method of claim 1, wherein the linker is non-cleavable.
9. The method of claim 1, wherein the linker is two associating binding domains of the DNA binding
25 nuclease linked to a reverse transcriptase.
10. The method of claim 1, wherein the integration enzyme is selected from the group consisting of Dre, Vika, Bxb1, ϕ C31, RDF, FLP, ϕ BT1, R1, R2, R3, R4, R5, TP901-1, A118, ϕ FC1, ϕ C1, MR11, TG1, ϕ 370.1, W β , BL3, SPBc, K38, Peaches, Veracruz, Rebeuca, Theia, Benedict, KSSJEB, PattyP, 30 Doom, Scowl, Lockley, Switzer, Bob3, Troube, Abrogate, Anglerfish, Sarfire, SkiPole, ConceptII, Museum, Severus, Airmid, Benedict, Hinder, ICleared, Sheen, Mundrea, BxZ2, ϕ RV,

retrotransposases encoded by R2, L1, Tol2 Tc1, Tc3, Mariner Himar 1, Mariner mos-1, and Minos, and any mutants thereof.

11. The method of claim 1, wherein the integration sequence is an attB sequence, an attP sequence,
5 an attL sequence, an attR sequence, a lox71 sequence, a Vox sequence, or a FRT sequence.

12. The method of claim 1, wherein the DNA binding nuclease domain comprising a nickase activity is selected from Cas9-D10A, Cas9-H840A, and Cas12a/b nickase.

10 13. The method of claim 1, wherein the reverse transcriptase domain comprises a mutation relative to a wild-type sequence.

14. The method of claim 1, wherein the reverse transcriptase domain is selected from the group consisting of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase domain, transcription
15 xenopolymerase (RTX), avian myeloblastosis virus reverse transcriptase (AMV-RT), and Eubacterium rectale maturase RT.

15. The method of claim 14, wherein the M-MLV reverse transcriptase domain comprises one or more mutations selected from the group consisting of D200N, T306K, W313F, T330P, and L603W.

20 16. The method of claim 1, further comprising introducing a nicking guide RNA (ngRNA).

17. The method claim 1, wherein:

the exogenous nucleic acid is a reporter gene;

25 the exogenous nucleic acid is a degradation tag for programmable knockdown of proteins in the presence of small molecules;

the exogenous nucleic acid is a T-cell receptor (TCR), a chimeric antigen receptor (CAR), an interleukin, a cytokine, or an immune checkpoint gene and the mammalian cell is a T-cell or natural killer (NK) cell, the exogenous nucleic acid is a beta hemoglobin (HBB) gene and the cell is a
30 hematopoietic stem cell (HSC)

the exogenous nucleic acid is a metabolic gene; or

the exogenous nucleic acid is a gene involved in an inherited disease or an inherited syndrome.

18. The method of claim 17, wherein the reporter gene is a fluorescent protein.

19. The method of claim 1, wherein the mammalian cell is a dividing cell or a non-dividing cell.

20. The method of claim 1, wherein:

the exogenous nucleic acid is between 1000 bp and 36,000 bp;

the exogenous nucleic acid is more than 36,000 bp; [[and/]] or

the exogenous nucleic acid is less than 1000 bp.

21. The method of claim 17 wherein the inherited disease is cystic fibrosis, familial hypercholesterolemia, adenosine deaminase (ADA) deficiency, X-linked SCID (X-SCID), Wiskott-Aldrich syndrome (WAS), hemochromatosis, Tay-Sachs, fragile X syndrome, Huntington's disease, Marfan syndrome, phenylketonuria, or muscular dystrophy.

22. A method of site-specifically integrating an exogenous nucleic acid sequence into a mammalian cell genome or intracellular target nucleic acid, the method comprising:

(a) incorporating at least one integration sequence at a specific target site in the cell genome or intracellular target nucleic acid by introducing *ex vivo* into a mammalian cell:

(i) an expressible polynucleotide construct encoding an editing polypeptide, wherein the editing polypeptide comprises a DNA-binding nuclease domain linked via a linker to a reverse transcriptase domain, wherein the DNA-binding nuclease domain comprises a nickase activity; and

(ii) an expressible polynucleotide construct encoding a guide RNA (gRNA) comprising a targeting sequence, a primer binding sequence, and a complement of the at least one integration sequence,

wherein the gRNA interacts with the expressed editing polypeptide to direct the editing polypeptide to the specific target site of the cell genome or intracellular target nucleic acid, wherein the DNA-binding nuclease domain nicks a strand of the cell genome or intracellular target nucleic acid to form a nicked site, and

wherein the reverse transcriptase domain reverse transcribes the complement of the at least one integration sequence and thereby incorporates the at least one integration sequence into the nicked site, thereby incorporating the at least one integration sequence at the specific target site of the cell genome or intracellular target nucleic acid; and

5 (b) integrating the exogenous nucleic acid sequence into the cell genome or intracellular target nucleic acid by introducing into the mammalian cell:

(i) an exogenous nucleic acid sequence linked to a sequence that is an integration cognate to the site-specifically incorporated integration sequence; and

10 (ii) an expressible polynucleotide construct encoding an integration enzyme, wherein the integration enzyme integrates the exogenous nucleic acid into the cell genome or intracellular target nucleic acid at the at least one site-specifically incorporated integration sequence, thereby site-specifically integrating the exogenous nucleic acid into the cell genome or the intracellular target nucleic acid,

15 wherein the integration sequence is an attB sequence, an attP sequence, an attL sequence, an attR sequence, a lox71 sequence, a Vox sequence, or a FRT sequence,

wherein the integration sequence is longer than 38 basepairs, and

20 wherein the expressible polynucleotide constructs encoding the editing polypeptide, the gRNA, and the integration enzyme, and the exogenous nucleic acid, are introduced into the mammalian cell concurrently.

23. The method of claim 22, wherein the integration sequence is 40, 42, 44, or 46 base pairs.