

CLAIMS

What is claimed:

1. A targeted active gene editing (TAGE) agent comprising
(i) an extracellular cell membrane binding moiety that binds to an extracellular cell membrane-bound molecule, and
a site-directed modifying polypeptide that recognizes a nucleic acid sequence,
wherein the extracellular cell membrane binding moiety and the site-directed modifying polypeptide are stably associated such that the site-directed modifying polypeptide can be internalized into a cell displaying the extracellular cell membrane-bound molecule; and/or
(ii) a cell penetrating peptide (CPP) that facilitates uptake of a polypeptide into a cell, and
a site-directed modifying polypeptide that recognizes a nucleic acid sequence,
wherein the CPP and the site-directed modifying polypeptide are stably associated such that the site-directed modifying polypeptide can be internalized into a cell targeted via the CPP.
2. The TAGE agent of claim 1, wherein
the extracellular cell membrane binding moiety is an antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, a cell penetrating peptide (CPP), a ligand that binds to the extracellular cell membrane-bound molecule, or combinations thereof; and/or
the TAGE agent comprises at least two extracellular cell membrane binding moieties.
3. The TAGE agent of claim 2, wherein the antigen binding polypeptide is an antibody, an antigen-binding portion of an antibody, or an antibody mimetic.
- ~~4. The TAGE agent of any one of claims 1 to 3, wherein the TAGE agent comprises at least two extracellular cell membrane binding moieties.~~

54. The TAGE agent of claim 42, wherein the at least two extracellular cell membrane binding moieties of the TAGE agent are (i) a CPP and an antigen-binding polypeptide, or (ii) a CPP and a ligand.
- ~~6. The TAGE agent of claim 4, wherein the at least two extracellular cell membrane binding moieties of the TAGE agent are a CPP and a ligand.~~
75. The TAGE agent of any one of claims ~~1 to 6~~ 1 to 4, wherein the site-directed modifying polypeptide comprises a nuclease or a nickase.
86. The TAGE agent of claim 75, wherein the nuclease is a DNA endonuclease.
97. The TAGE agent of claim 86, wherein the DNA endonuclease is Cas9 or Cas12.
- ~~10. A targeted active gene editing (TAGE) agent comprising~~
~~————— a ligand that specifically binds to an extracellular cell membrane-bound molecule, and~~
~~a site-directed modifying polypeptide comprising an RNA-guided DNA endonuclease that recognizes a CRISPR sequence,~~
~~wherein the ligand and the site-directed modifying polypeptide are stably associated such that the site-directed modifying polypeptide can be internalized into a target cell via the ligand.~~
118. The TAGE agent of claim 2 ~~claim 10~~, wherein the ligand is a protein selected from IL-2, CCL2, CCL5, CCL7, CCL8, CCL13, CCL16, IGF2, IL7, IL15, IFN γ , CSF-1, CSF-2, CSF-3, TCR/DC4, or PD-L1.
- ~~12. A targeted active gene editing (TAGE) agent comprising~~
~~an antigen binding polypeptide which specifically binds to an extracellular cell membrane-bound molecule, and~~
~~a site-directed modifying polypeptide comprising an RNA-guided DNA endonuclease that recognizes a CRISPR sequence,~~
~~wherein the antigen binding polypeptide and the site-directed modifying polypeptide are stably associated such that the site-directed modifying~~

polypeptide can be internalized into a cell displaying the extracellular cell membrane bound molecule, and

~~wherein the antigen binding polypeptide is an antibody, an antigen-binding portion of an antibody, or an antibody mimetic.~~

~~13. The TAGE agent of any one of claims 10-12, wherein the RNA-guided DNA endonuclease is a Cas9 nuclease or a Cas12 nuclease.~~

9. The TAGE of agent of claim 1, wherein the site-directed modifying polypeptide comprises an RNA-guided DNA endonuclease that recognizes a CRISPR sequence.

1410. The TAGE agent of any one of claims 1-131-9, wherein the TAGE agent further comprises ~~comprising~~ a guide RNA that specifically hybridizes to a target region of the genome of the cell, wherein the guide RNA and the site-directed modifying polypeptide form a ribonucleoprotein.

1511. The TAGE agent of any one of claims 1 to 1410, wherein the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).

1612. The TAGE agent of any one of claims 2-4 to 1511, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the antigen binding polypeptide, ~~or~~ ligand, or CPP; or the site-directed modifying polypeptide and the antigen binding polypeptide ligand, or CPP are conjugated via a linker.

1713. The TAGE agent of claim 1612, wherein:
the conjugation moiety is a protein;
the linker is cleavable.

~~18. The TAGE agent of claim 17, wherein the protein is Protein A, SpyCatcher, or a Halo-Tag.~~

19. ~~The TAGE agent of any one of claims 1 to 18, wherein the site directed modifying polypeptide and the antigen binding polypeptide or ligand are conjugated via a linker.~~
20. ~~The TAGE agent of claim 19, wherein the linker is cleavable.~~
21. ~~The TAGE agent of claim 3 or 12, wherein:~~
- 21.14. The TAGE agent of claim 3 or 12, wherein:
- the antibody mimetic is an adnectin (i.e., fibronectin based binding molecules), an affilin, an affimer, an affitin, an alphabody, an affibody, a DARPIn, an anticalin, an avimer, a fynomer, a Kunitz domain peptide, a monobody, a nanoCLAMP, a unibody, a versabody, an aptamer, or a peptidic molecule;
 - the antigen-binding portion of the antibody is a nanobody, a single domain antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a minibody, a F(ab')₂, or an intrabody; or
 - the antibody is an intact antibody or a bispecific antibody.
22. ~~The TAGE agent of claim 3 or 12, wherein the antigen binding portion of the antibody is a nanobody, a domain antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a minibody, a F(ab')₂, or an intrabody.~~
23. ~~The TAGE agent of claim 3 or 12, wherein the antibody is an intact antibody or a bispecific antibody.~~
24. ~~A targeted active gene editing (TAGE) agent comprising~~
- ~~a ligand that specifically binds to an extracellular cell membrane bound molecule, and~~
 - ~~a site directed modifying polypeptide comprising a Cas9 nuclease,~~
 - ~~wherein the ligand and the site directed modifying polypeptide are stably associated via a conjugation moiety such that the site directed modifying polypeptide can be internalized into the cell targeted by the ligand.~~

25. ~~The TAGE agent of claim 24, wherein the ligand is a protein selected from IL-2, CCL2, CCL5, CCL7, CCL8, CCL13, CCL16, IGF2, IL7, IL15, IFN γ , CSF-1, TCR/DC4, or PD-L1.~~
26. ~~A targeted active gene editing (TAGE) agent comprising~~
~~an antibody, or an antigen-binding portion thereof, which specifically~~
~~binds to an extracellular cell membrane-bound protein, and~~
~~a site-directed modifying polypeptide comprising a Cas9 nuclease,~~
~~wherein the antibody, or antigen-binding portion thereof, and the site-~~
~~directed modifying polypeptide are stably associated via a conjugation moiety~~
~~such that the site-directed modifying polypeptide can be internalized into the cell~~
~~expressing the extracellular cell membrane-bound protein via the antibody, or the~~
~~antigen-binding portion thereof.~~
27. ~~The TAGE agent of any one of claims 24-25, wherein the site-directed modifying~~
~~polypeptide further comprises at least one nuclear localization signal (NLS).~~
2815. The TAGE agent of claim 11 ~~claim 27~~, wherein the at least one NLS comprises an SV40 NLS.
2916. The TAGE agent of claim 2815, wherein the SV40 NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 10).
30. ~~The TAGE agent of any one of claims 24 to 29, wherein the at least one NLS is~~
~~at the C-terminus, the N-terminus, or both of the site-directed modifying~~
~~polypeptide.~~
31. ~~The TAGE agent of any one of claims 24 to 30, comprising at least two NLSs.~~
32. ~~The TAGE agent of any one of claims 24 to 31, further comprising a guide RNA~~
~~that specifically hybridizes to a target region of the genome of a cell expressing~~
~~the extracellular cell membrane-bound molecule or protein, wherein the guide~~
~~RNA and the site-directed modifying polypeptide form a nucleoprotein.~~

- ~~33. The TAGE agent of any one of claims 24 to 32, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that can bind to the antibody, or antigen-binding portion thereof.~~
- ~~34. The TAGE agent of claim 33, wherein the conjugation moiety is a protein.~~
- ~~35. The TAGE of claim 34, wherein the protein is Protein A, SpyCatcher, or a Halo-Tag.~~
- ~~36. The TAGE agent of any one of claims 26 to 35, wherein the Cas9 nuclease comprises the amino acid substitution C80A.~~
- ~~37. The TAGE agent of any one of claims 26 to 35, wherein the Cas9 nuclease has an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to Cas9 described in the Sequence Table.~~
- ~~38. The TAGE agent of any one of claims 25 to 35, wherein the antigen-binding portion of the antibody is a nanobody, a domain antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a minibody, a F(ab')₂, or an intrabody.~~
- ~~39. The TAGE agent of any one of claims 25 to 35, wherein the antibody is an intact antibody or a bispecific antibody.~~
- ~~40~~17. The TAGE agent of any one of claims 1 to ~~39~~16, wherein the extracellular cell membrane-bound molecule or protein is HLA-DR, CD44, CD11a, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV.
- ~~41~~18. A site-directed modifying polypeptide comprising an RNA-guided DNA endonuclease that recognizes a CRISPR sequence and a conjugation moiety that binds to an antibody, an antigen-binding portion of an antibody, or an antibody mimetic that specifically binds to an extracellular cell membrane-bound

molecule; or a ligand that facilitates uptake of a polypeptide to which the ligand is conjugated.

~~42. A site directed modifying polypeptide comprising an RNA-guided DNA endonuclease that recognizes a CRISPR sequence and a conjugation moiety that binds to a ligand that facilitates uptake of a polypeptide to which the ligand is conjugated.~~

4319. The site-directed modifying polypeptide of claim 18 ~~claim 42~~, wherein the ligand is a protein selected from IL-2, CCL2, CCL5, CCL7, CCL8, CCL13, CCL16, IGF2, IL7, IL15, IFN γ , CSF-2, TCR/DC4, or PD-L1; or the extracellular cell membrane-bound molecule is a protein selected from the group consisting of HLA-DR, CD11a, CD44, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD74, CTLA4, EGFR, HER2, or EpCam.

~~44. The site directed modifying polypeptide of any one of claims 41 to 43, further comprising a guide RNA that specifically hybridizes to a target region of the genome of a cell.~~

~~45. The site directed modifying polypeptide of any one of claims 41 to 44, wherein the RNA-guided DNA endonuclease is a Cas9 nuclease.~~

~~46. The site directed modifying polypeptide of claim 45, wherein the Cas9 nuclease comprises the amino acid substitution C80A.~~

~~47. The site directed modifying polypeptide of claim 45, wherein the Cas9 nuclease comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 1 or SEQ ID NO: 55.~~

~~48. The site directed modifying polypeptide of any one of claims 41 to 47, wherein the RNA-guided DNA endonuclease is a Cas12 nuclease.~~

49. ~~The site directed modifying polypeptide of any one of claims 41 to 48, further comprising at least one nuclear localization signal (NLS).~~
50. ~~The site directed modifying polypeptide of claim 49, wherein the at least one NLS comprises an SV40 NLS.~~
51. ~~The site directed modifying polypeptide of claim 50, wherein the SV40 NLS comprises PKKKRKV (SEQ ID NO: 10).~~
52. ~~The site directed modifying polypeptide of any one of claims 41 to 51, comprising at least two NLSs.~~
53. ~~The site directed modifying polypeptide of any one of claims 41 to 52, wherein the at least one NLS is at the C-terminus, the N-terminus, or both of the site-directed modifying polypeptide.~~
54. ~~The site directed modifying polypeptide of any one of claims 41 to 53, wherein the site directed modifying polypeptide further comprises a conjugation moiety that can bind to the ligand or antibody, antigen binding portion thereof, or antibody mimetic.~~
55. ~~The site directed modifying polypeptide of claim 54, wherein the conjugation moiety is a protein.~~
56. ~~The site directed modifying polypeptide claim 55, wherein the protein is Protein A, SpyCatcher, or a Halo-Tag.~~
57. ~~The site directed modifying polypeptide of any one of claims 41 to 56, wherein the extracellular cell membrane bound molecule is a protein selected from the group consisting of HLA-DR, CD11a, CD44, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD74, CTLA4, EGFR, HER2, or EpCam.~~

5820. A nucleoprotein comprising the site-directed modifying polypeptide of any one of claims 4418 to 5719 and a guide RNA, wherein the guide RNA specifically hybridizes to a target region of the genome of a cell displaying the extracellular cell membrane-bound protein.

~~59. A targeted active gene editing (TAGE) agent comprising~~
~~a cell penetrating peptide (CPP) that facilitates uptake of a polypeptide into a cell, and~~
~~a site directed modifying polypeptide that recognizes a nucleic acid sequence,~~
~~wherein the CPP and the site directed modifying polypeptide are stably associated such that the site directed modifying polypeptide can be internalized into a cell targeted via the CPP.~~

~~60. The TAGE agent of claim 59, wherein the site directed modifying polypeptide comprises a nuclease or a nickase.~~

~~61. The TAGE agent of claim 60, wherein the nuclease is a DNA endonuclease.~~

~~62. The TAGE agent of claim 61, wherein the DNA endonuclease is Cas9 or Cas12.~~

~~63. The TAGE agent of any one of claims 59 to 62, further comprising a guide RNA that specifically hybridizes to a target region of the genome of the cell, wherein the guide RNA and the site directed modifying polypeptide form a ribonucleoprotein.~~

~~64. A targeted active gene editing (TAGE) agent comprising~~
~~a cell penetrating peptide (CPP) that facilitates uptake of a polypeptide into a cell, and~~
~~a site directed modifying polypeptide comprising an RNA-guided DNA endonuclease that recognizes a CRISPR sequence,~~
~~wherein the CPP and the site directed modifying polypeptide are stably associated such that the site directed modifying polypeptide can be internalized into a target cell via the CPP.~~

- ~~65. The TAGE agent of claim 64, further comprising a guide RNA that specifically hybridizes to a target region of the genome of the cell, wherein the guide RNA and the site-directed modifying polypeptide form a ribonucleoprotein.~~
- ~~66. The TAGE agent of claim 65, wherein the RNA-guided DNA endonuclease is a Cas9 nuclease or a Cas12 nuclease.~~
- ~~67. The TAGE agent of any one of claims 64 to 66, wherein the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).~~
- ~~68. The TAGE agent of any one of claims 64 to 67, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the CPP.~~
- ~~69. The TAGE agent of claim 68, wherein the conjugation moiety is a protein.~~
- ~~70. The TAGE agent of claim 69, wherein the protein is SpyCatcher or a Halo-Tag.~~
- ~~71. The TAGE agent of any one of claims 64 to 67, wherein the site-directed modifying polypeptide and the CPP are conjugated via a linker.~~
- ~~72. The TAGE agent of claim 71, wherein the linker is cleavable.~~
- ~~73. A targeted active gene editing (TAGE) agent comprising
a cell penetrating peptide (CPP) that facilitates uptake of a polypeptide into a cell, and
a site-directed modifying polypeptide comprising a Cas9 nuclease,
wherein the CPP and the site-directed modifying polypeptide are stably associated via a conjugation moiety such that the site-directed modifying polypeptide can be internalized into the cell targeted by the CPP.~~

~~74. The TAGE agent of claim 77, wherein the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).~~

~~75. The TAGE agent of claim 74, wherein the at least one NLS comprises an SV40 NLS.~~

~~76. The TAGE agent of claim 75, wherein the SV40 NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 10).~~

~~77~~21. The TAGE agent of claim 1 or 2 ~~any one of claims 59 to 76~~, wherein the CPP is Tat, Tat-NLS, His-Tat-NLS (HTN), Tat-HA, S19-Tat, CM18, CM18-Tat, hPH1, L17E, IMT-P8, IMT-P8 (C14S), TDP, TDP-KDEL (SEQ ID NO: 17), penetratin, polyR, Aurein, LAH4-L1, LMWP, Pardaxin, S10, S18, S19, S85, Vectofusin1, or ZF5.3.

~~78~~22. An isolated nucleic acid encoding the site-directed modifying polypeptide of any one of claims 4117 to 5719.

~~79~~23. A vector comprising the nucleic acid of claim ~~78~~22.

~~80~~24. A cell comprising the site-directed modifying polypeptide of any one of claims ~~4117 to 5719~~.

~~81~~25. A method of modifying the genome of a target cell, the method comprising contacting the target cell with a targeted active gene editing (TAGE) agent of any one of claims 1 to ~~4017 and 59 to 77~~.

~~82~~26. The method of claim ~~81~~25, wherein the target cell is a eukaryotic cell.

~~83~~27. The method of claim ~~82~~26, wherein the eukaryotic cell is a mammalian cell.

~~84~~28. The method of claim ~~83~~27, wherein the mammalian cell is a mouse cell, a non-human primate cell, or a human cell.

8529. The method of any one of claims 8125 to 8428, wherein the site-directed modifying polypeptide produces a cleavage site at the target region of the genome, thereby modifying the genome.

8630. The method of any one of claims 8125 to 8529, wherein the target region of the genome is a target gene.

8731. The method of claim 8630, wherein said method is effective to modify expression of the target gene.

~~88. The method of claim 87, wherein said method is effective to increase expression of the target gene relative to a reference level.~~

~~89. The method of claim 88, wherein said method is effective to decrease expression of the target gene relative to a reference level.~~

9032. A method of modifying a nucleic acid sequence within a target cell in a mammalian subject, the method comprising:

contacting the target cell in the subject with a targeted active gene editing (TAGE) agent comprising an extracellular cell membrane binding moiety, and a site-directed modifying polypeptide that recognizes the nucleic acid sequence within the target cell, such that the nucleic acid sequence of the target cell is modified; or

locally administering to the subject a targeted active gene editing (TAGE) agent comprising an extracellular cell membrane binding moiety, and a site-directed modifying polypeptide that recognizes the nucleic acid sequence within the target cell, such that the nucleic acid sequence of the target cell is modified.

~~91. A method of modifying a nucleic acid sequence within a target cell in a mammalian subject, the method comprising locally administering to the subject a targeted active gene editing (TAGE) agent comprising an extracellular cell membrane binding moiety, and a site-directed modifying polypeptide that recognizes the nucleic acid sequence within the target cell, such that the nucleic acid sequence of the target cell is modified.~~

9233. The method of claim ~~90 or 91~~32, wherein the method comprises locally administering the TAGE agent to the subject by intramuscular injection, intraosseous injection, intraocular injection, intratumoral injection, intrathecal delivery, or intradermal injection; and/or
the method is effective to increase the number of genetically modified target cells in the subject following administration of the TAGE agent; and/or
the mammalian subject is a human subject; and/or
the subject has a disease selected from an eye disease, a stem cell disorder, and a cancer, and wherein the method is effective to treat the disease.

~~93. The method of any one of claims 90-92, wherein the method is effective to increase the number of genetically modified target cells in the subject following administration of the TAGE agent.~~

~~94. The method of any one of claims 90-92, wherein the mammalian subject is a human subject.~~

~~95. The method of any one of claims 90-94, wherein the subject has a disease selected from an eye disease, a stem cell disorder, and a cancer, and wherein the method is effective to treat the disease.~~

9634. A method of modifying a nucleic acid sequence within a target mammalian cell, the method comprising contacting the target mammalian cell with a targeted active gene editing (TAGE) agent under conditions in which the TAGE agent is internalized into the target cell, such that the nucleic acid sequence is modified,
wherein the TAGE agent comprises an extracellular cell membrane binding moiety, and a site-directed modifying polypeptide that recognizes the nucleic acid sequence within the target cell,
wherein the internalization of the TAGE agent is not dependent on electroporation.

9735. The method of any one of claims 96-32 to 34, wherein

the target mammalian cell is (i) a hematopoietic cell (HSC), a neutrophil, a T cell, a B cell, a dendritic cell, a macrophage, or a fibroblast; or (ii) a hematopoietic stem cell (HSC) or a bone marrow cell that is not an HSC; and/or
the target mammalian cell is contacted with the TAGE by co-incubation ex vivo; or the target mammalian cell is contacted with the TAGE in situ by injection into a tissue of a subject; and/or
the nucleic acid is a gene in the genome of the cell, wherein the expression of said gene is altered following said modification; and/or
the target mammalian cell is a mouse cell, a non-human primate cell, or a human cell.

~~98. The method of claim 96, wherein the target mammalian cell is a hematopoietic stem cell (HSC) or a bone marrow cell that is not an HSC.~~

9936. The method of claim ~~98~~35, wherein the extracellular cell membrane binding moiety specifically binds an extracellular cell membrane-bound molecule on a human HSC.

~~100~~37. The method of claim ~~99~~36, wherein the extracellular cell membrane-bound molecule on the HSC is CD34, EMCN, CD59, CD90, ckit, CD45, or CD49F.

~~101. The method of any one of claims 96 to 100, wherein the target mammalian cell is contacted with the TAGE by co-incubation ex vivo.~~

~~102~~38. The method of claim ~~96~~34 to ~~101~~37, wherein the method provides a genetically-modified target cell which is administered to a subject in need thereof.

~~103. The method of any one of claims 96 to 100, wherein the target mammalian cell is contacted with the TAGE in situ by injection into a tissue of a subject.~~

~~104~~39. The method of any one of claims ~~96~~34 to ~~100~~37, wherein the TAGE is administered to the subject by intramuscular injection, intraosseous injection, intraocular injection, intratumoral injection, or intradermal injection.

~~105. The method of any one of claims 90 to 104, wherein the nucleic acid is a gene in the genome of the cell, wherein the expression of said gene is altered following said modification.~~

~~106. The method of any one of claims 90 to 105, wherein the target mammalian cell is a mouse cell, a non-human primate cell, or a human cell.~~

~~107~~40. The method of any one of claims ~~90~~32 to ~~106~~39, wherein

the extracellular cell membrane binding moiety of the TAGE agent is a cell penetrating peptide, a ligand, an antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, or combinations thereof, and/or

the extracellular cell membrane binding moiety of the TAGE agent comprises one or more cell penetrating peptides (CPPs); and/or

the extracellular cell membrane binding moiety of the TAGE agent comprises an antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule; and/or

the extracellular cell membrane binding moiety of the TAGE agent comprises a ligand that specifically binds to an extracellular cell membrane-bound molecule; and/or

the TAGE agent further comprises at least one nuclear localization signal (NLS); and/or

the TAGE agent comprises at least two extracellular cell membrane binding moieties.

~~108. The method of any one of claims 90 to 107, wherein the extracellular cell membrane binding moiety of the TAGE agent comprises one or more cell penetrating peptides (CPPs).~~

~~109~~41. The method of claim ~~108~~40, wherein

the one or more CPPs is NLS, Tat, Tat-NLS, His-Tat-NLS (HTN), Tat-HA, S19-Tat, CM18, CM18-Tat, hPH1, L17E, IMT-P8, IMT-P8 (C14S), TDP, TDP-KDEL (SEQ ID NO: 17), penetratin, polyR, Aurein, LAH4-L1, LMWP, Pardaxin, S10, S18, S19, S85, Vectofusin1, ZF5.3, or a combination thereof; or

the extracellular cell membrane-bound molecule bound by the antigen binding polypeptide is HLA-DR, CD44, CD11a, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV; or

the ligand is selected from IL-2, CCL2, CCL5, CCL7, CCL8, CCL13, CCL16, IGF2, IL7, IL15, IFN γ , CSF-1, TCR/DC4, or PD-L1.

~~110. The method of claim 108, wherein the one or more CPPs comprises a TAT peptide.~~

~~111. The method of claim 108, wherein the one or more CPPs comprises a His-Tat-NLS (HTN) peptide.~~

~~112. The method of any one of claims 90 to 111, wherein the extracellular cell membrane binding moiety of the TAGE agent comprises an antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule.~~

~~113~~42. The method of claim ~~112~~40, wherein the antigen binding polypeptide is an antibody, an antigen-binding portion of an antibody, or an antibody-mimetic.

~~114~~43. The method of claim ~~113~~42, wherein:

the antibody mimetic is an adnectin (i.e., fibronectin based binding molecules), an affilin, an affimer, an affitin, an alphabody, an affibody, a DARPIn, an anticalin, an avimer, a fynomer, a Kunitz domain peptide, a monobody, a nanoCLAMP, a unibody, a versabody, an aptamer, or a peptidic molecule;

the antigen-binding portion of the antibody is a nanobody, a domain antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a mnibody, a F(ab')₂, or an intrabody; or

the antibody is an intact antibody or a bispecific antibody.

~~115. The method of claim 113, wherein the antigen-binding portion of the antibody is a nanobody, a domain antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a mnibody, a F(ab')₂, or an intrabody.~~

~~116. The method of claim 113, wherein the antibody is an intact antibody or a bispecific antibody.~~

~~117. The method of any one of claims 112-116, wherein the extracellular cell membrane-bound molecule bound by the antigen-binding polypeptide is HLA-DR, CD44, CD11a, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV.~~

~~118. The method of any one of claims 90 to 117, wherein the extracellular cell membrane-binding moiety of the TAGE agent comprises a ligand that specifically binds to an extracellular cell membrane-bound molecule.~~

~~119. The method of claim 118, wherein the ligand is selected from, IL-2, CCL2, CCL5, CCL7, CCL8, CCL13, CCL16, IGF2, IL7, IL15, IFN γ , CSF-1, TCR/DC4, or PD-L1.~~

~~120. The method of any one of claims 90 to 119, wherein the extracellular cell membrane-binding moiety of the TAGE agent further comprises at least one nuclear localization signal (NLS).~~

~~121. The method of any one of claims 90 to 120, wherein the extracellular cell membrane-binding moiety of the TAGE agent comprises at least two nuclear localization signals (NLSs).~~

~~122~~44. The method of any one of claims ~~120-121~~40-43, wherein the NLS comprises an SV40 NLS.

~~123~~45. The method of claim ~~122~~44, wherein the SV40 NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 10).

~~124. The method of any one of claims 90 to 123, wherein the TAGE agent comprises at least two extracellular cell membrane binding moieties.~~

~~125~~46. The method of claim ~~124~~45, wherein the at least two extracellular cell membrane binding moieties of the TAGE agent are (i) a CPP and an antigen-binding polypeptide; or (ii) a CPP and a ligand.

~~126. The method of claim 124, wherein the at least two extracellular cell membrane binding moieties of the TAGE agent are a CPP and a ligand.~~

~~127~~47. The method of any one of claims ~~90~~32 to ~~126~~46, wherein

- the target mammalian cell is a population of target mammalian cells, optionally wherein the method is effective to increase the number of genetically modified target mammalian cells; and/or
- the site-directed modifying polypeptide of the TAGE has increased cellular internalization in the target mammalian cell; and/or
- the site-directed modifying polypeptide of the TAGE has increased nuclear internalization in the target mammalian cell; and/or
- the site-directed modifying polypeptide comprises a nuclease or a nickase; and/or
- the site-directed modifying polypeptide is a nucleic acid-guided nuclease, and the TAGE agent further comprises a guide nucleic acid that specifically hybridizes to a target region of the nucleic acid sequence of the target mammalian cell, wherein the guide nucleic acid and the nucleic acid-guided nuclease form a nucleoprotein; and/or
- the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the extracellular cell membrane binding moiety or a complementary binding moiety attached thereto and/or
- the site-directed modifying polypeptide and the extracellular cell membrane binding moiety are conjugated via a linker; and/or
- the TAGE agent further comprises an endosomal escape agent.

~~128. The method of claim 127, wherein the method is effective to increase the number of genetically modified target mammalian cells.~~

~~129. The method of any one of claims 90 to 128, wherein the site-directed modifying polypeptide of the TAGE has increased cellular internalization in the target mammalian cell.~~

~~130. The method of any one of claims 90 to 129, wherein the site-directed modifying polypeptide of the TAGE has increased nuclear internalization in the target mammalian cell.~~

~~131. The method of any one of claims 90 to 130, wherein the site-directed modifying polypeptide comprises a nuclease or a nickase.~~

~~132. The method of any one of claims 90 to 131, wherein the site-directed modifying polypeptide is a nucleic acid-guided nuclease, and the TAGE agent further comprises a guide nucleic acid that specifically hybridizes to a target region of the nucleic acid sequence of the target mammalian cell, wherein the guide nucleic acid and the nucleic acid-guided nuclease form a nucleoprotein.~~

~~133~~48. The method of claim ~~132~~47, wherein the site-directed modifying polypeptide is a RNA-guided nuclease, and the TAGE agent further comprises a guide RNA that specifically hybridizes to a target region of the nucleic acid sequence of the target mammalian cell, wherein the guide RNA and the RNA-guided nuclease form a ribonucleoprotein.

~~134~~49. The method of claim ~~133~~48, wherein the guide RNA is a single guide RNA (sgRNA) or a cr:trRNA.

~~135~~50. The method of claim ~~133~~48, wherein the RNA-guided nuclease is a Class 2 Cas polypeptide.

~~136~~51. The method of claim ~~135~~50, wherein the Class 2 Cas polypeptide is a Type II Cas polypeptide or a Type V Cas polypeptide.

~~137~~52. The method of claim ~~136~~51, wherein the Type II Cas polypeptide is Cas9 or the Type V Cas polypeptide is Cas12.

~~138.~~ The method of claim 135, wherein the Class 2 Cas polypeptide is a Type V Cas polypeptide.

~~139.~~ The method of claim 138, wherein the Type V Cas polypeptide is Cas12.

~~140.~~ The method of any one of claims 90 to 139, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the extracellular cell membrane binding moiety or a complementary binding moiety attached thereto.

~~141~~53. The method of claim ~~140~~47, wherein:
the conjugation moiety is a protein;
the linker is a cleavable linker;
the endosomal escape agent is TDP or TDP-KDEL.

~~142.~~ The method of claim 141, wherein the protein is SpyCatcher or a Halo Tag.

~~143.~~ The method of any one of claims 90 to 142, wherein the site-directed modifying polypeptide and the extracellular cell membrane binding moiety are conjugated via a linker.

~~144.~~ The method of claim 143, wherein the linker is a cleavable linker.

~~145.~~ The method of any one of claims 90 to 144, wherein the TAGE agent further comprises an endosomal escape agent.

~~146.~~ The method of claim 145, wherein the endosomal escape agent is TDP or TDP-KDEL.