

CLAIMS

What is claimed:

1. A targeted active gene editing (TAGE) agent comprising
an antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, and
a site-directed modifying polypeptide that recognizes a nucleic acid 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.
2. The TAGE agent of claim 1, wherein the antigen binding polypeptide is an antibody, an antigen-binding portion of an antibody, or an antibody-mimetic.
3. The TAGE agent of claim 1 or 2, wherein the site-directed modifying polypeptide comprises a nuclease or a nickase.
4. The TAGE agent of claim 3, wherein the nuclease is a DNA endonuclease.
5. The TAGE agent of claim 1, wherein the site-directed modifying polypeptide comprises an RNA-guided DNA endonuclease that recognizes a CRISPR sequence.
- ~~56. The TAGE agent of claim 4, wherein the DNA endonuclease is Cas9 or Cas12.~~
- ~~6. The TAGE agent of claim 4, wherein the DNA endonuclease is Cas12.~~
7. The TAGE agent of any one of claims 1 to 6, 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; and/or

the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the antigen binding polypeptide; or the site-directed modifying polypeptide and the antigen binding polypeptide are conjugated via a linker; and/or the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).

8. ~~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.~~
9. ~~The TAGE agent of claim 8, 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.~~
10. ~~The TAGE agent of claim 8 or 9, wherein the RNA-guided DNA endonuclease is a Cas9 nuclease.~~
11. ~~The TAGE agent of any one of claims 8 to 10, wherein the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).~~
12. ~~The TAGE agent of any one of claims 1 to 11, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the antigen binding polypeptide.~~
138. The TAGE agent of claim 127, wherein:
the conjugation moiety is a protein; or

the linker is cleavable.

~~14. The TAGE agent of claim 13, wherein the protein is Protein A, SpyCatcher, or a Halo-Tag.~~

~~15. The TAGE agent of any one of claims 1 to 11, wherein the site-directed modifying polypeptide and the antigen-binding polypeptide are conjugated via a linker.~~

~~16. The TAGE agent of claim 15, wherein the linker is cleavable.~~

~~179. The TAGE agent of any one of claims 24 to 168, 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.~~

~~18. The TAGE agent of any one of claims 2 to 16, 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.~~

~~19. The TAGE agent of any one of claims 2 to 16, wherein the antibody is an intact antibody or a bispecific antibody.~~

~~20. A targeted active gene editing (TAGE) agent comprising~~

~~an antigen-binding polypeptide 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.~~

~~21. The TAGE agent of claim 20, wherein the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS).~~

~~2210. The TAGE agent of claim 7 ~~claim 21~~, wherein the at least one NLS comprises an SV40 NLS.~~

~~2311. The TAGE agent of claim 2210, wherein the SV40 NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 8).~~

~~24. The TAGE agent of any one of claims 20 to 23, wherein the at least one NLS is at the C terminus, the N terminus, or both of the site-directed modifying polypeptide.~~

~~25. The TAGE agent of any one of claims 20 to 24, comprising at least two NLSs.~~

~~26. The TAGE agent of any one of claims 20 to 25, further comprising a guide RNA that specifically hybridizes to a target region of the genome of a cell expressing the extracellular cell membrane bound protein, wherein the guide RNA and the site-directed modifying polypeptide form a nucleoprotein.~~

~~27. The TAGE agent of any one of claims 20 to 26, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that can bind to the antibody, or antigen-binding portion thereof.~~

~~28. The TAGE agent of claim 27, wherein the conjugation moiety is a protein.~~

~~29. The TAGE of claim 28, wherein the protein is Protein A, SpyCatcher, or a Halo-Tag.~~

~~30. The TAGE agent of any one of claims 20 to 29, wherein the Cas9 nuclease comprises the amino acid substitution C80A.~~

- ~~31. The TAGE agent of any one of claims 20 to 29, wherein the Cas9 nuclease has an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to Cas9 as described in the Sequence Table.~~
- ~~32. The TAGE agent of any one of claims 20 to 29, wherein the antigen binding portion of the antibody is a nanobody, a doman antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a minibody, a F(ab')₂, or an intrabody.~~
- ~~33. The TAGE agent of any one of claims 20 to 29, wherein the antibody is an intact antibody or a bispecific antibody.~~
- ~~34~~12. The TAGE agent of any one of claims 1 to ~~33~~11, 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, CD73, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV.
- ~~35~~13. The TAGE agent of any one of claims 1 to ~~33~~11, wherein
the extracellular cell membrane-bound molecule or protein is CD11a and
the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding
fragment thereof; or
the extracellular cell membrane-bound molecule or protein is CD25 and the
antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment
thereof.
- ~~36. The TAGE agent of claim 35, wherein the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding fragment thereof.~~
- ~~37~~14. The TAGE agent of claim ~~36~~13, wherein
the anti-CD11a antibody is efalizumab; or
the anti-CD25 antibody is daclizumab.

~~38. The TAGE agent of any one of claims 1 to 33, wherein the extracellular cell membrane bound molecule or protein is CD25.~~

~~39. The TAGE agent of claim 38, wherein the antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment thereof.~~

~~40. The TAGE agent of claim 39, wherein the anti-CD25 antibody is daclizumab.~~

4115. 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.

4216. The site-directed modifying polypeptide of claim 4115, wherein
the site-directed modifying polypeptide further comprises ~~comprising~~ a guide RNA that specifically hybridizes to a target region of the genome of a cell; and/or

the RNA-guided DNA endonuclease is a Cas9 nuclease or a Cas12 nuclease; and/or

the site-directed modifying polypeptide further comprises at least one nuclear localization signal (NLS); and/or

the site-directed modifying polypeptide further comprises a conjugation moiety that can bind to the antibody, antigen-binding portion thereof, or antibody mimetic; and/or

the extracellular cell membrane-bound molecule is a protein selected from the group consisting of HLA-DR, CD44, CD11a, CD22, CD3, CD20, CD33, CD32, CD44, CD47, CD59, CD54, CD25, AchR, CD70, CD73, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV.

~~43. The site directed modifying polypeptide of claim 41 or 42, wherein the RNA-guided DNA endonuclease is a Cas9 nuclease.~~

- ~~44. The site directed modifying polypeptide of claim 43, wherein the Cas9 nuclease comprises the amino acid substitution C80A.~~
- ~~45. The site directed modifying polypeptide of claim 43, wherein the Cas9 nuclease comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to Cas9 as described in the Sequence Table.~~
- ~~46. The site directed modifying polypeptide of claim 41 or 42, wherein the RNA-guided DNA endonuclease is a Cas12 nuclease.~~
- ~~47. The site directed modifying polypeptide of any one of claims 41 to 46, further comprising at least one nuclear localization signal (NLS).~~
- ~~48~~17. The site-directed modifying polypeptide of claim ~~46~~16, wherein the at least one NLS comprises an SV40 NLS.
- ~~49~~18. The site-directed modifying polypeptide of claim ~~47~~16, wherein the SV40 NLS comprises PKKKRKV (SEQ ID NO: 8).
- ~~50. The site directed modifying polypeptide of any one of claims 41 to 49, comprising at least two NLSs.~~
- ~~51. The site directed modifying polypeptide of any one of claims 41 to 50, wherein the at least one NLS is at the C terminus, the N terminus, or both of the site directed modifying polypeptide.~~
- ~~52. The site directed modifying polypeptide of any one of claims 41 to 51, wherein the site directed modifying polypeptide further comprises a conjugation moiety that can bind to the antibody, antigen binding portion thereof, or antibody mimetic.~~
- ~~53~~19. The site-directed modifying polypeptide of claim ~~52~~16, wherein the conjugation moiety is a protein.

~~54. The site directed modifying polypeptide claim 53, wherein the protein is Protein A, SpyCatcher, or a Halo Tag.~~

~~55. The site directed modifying polypeptide of any one of claims 41 to 54, wherein the extracellular cell membrane bound molecule is a protein selected from the group consisting of 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.~~

~~56~~20. The site-directed modifying polypeptide of any one of claims ~~41 to 55~~15 to 19, wherein

~~the extracellular cell membrane-bound molecule or protein is CD11a and the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding fragment thereof; or~~

~~the extracellular cell membrane-bound molecule or protein is CD25 and the antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment thereof.~~

~~57. The site directed modifying polypeptide of claim 56, wherein the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding fragment thereof.~~

~~58~~21. The site-directed modifying polypeptide of claim ~~57~~20, wherein

~~the anti-CD11a antibody is efalizumab; or~~

~~the anti-CD25 antibody is daclizumab.~~

~~59. The site directed modifying polypeptide of any one of claims 41 to 55, wherein the extracellular cell membrane bound molecule or protein is CD25.~~

~~60. The site directed modifying polypeptide of claim 59, wherein the antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment thereof.~~

~~61. The site directed modifying polypeptide of claim 60, wherein the anti-CD25 antibody is daclizumab.~~

- ~~6222.~~ A nucleoprotein comprising the site-directed modifying polypeptide of any one of claims ~~41 to 61~~ 15 to 21 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.
- ~~6323.~~ An isolated nucleic acid encoding the site-directed modifying polypeptide of any one of claims ~~41 to 61~~ 15 to 21.
- ~~6424.~~ A vector comprising the nucleic acid of claim ~~6323~~.
- ~~6525.~~ A cell comprising the site-directed modifying polypeptide of any one of claims ~~41 to 61~~ 15 to 21.
- ~~6626.~~ 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 ~~40~~ 15.
- ~~6727.~~ The method of claim ~~6626~~, wherein
the target cell is a eukaryotic cell; and/or
wherein the site-directed modifying polypeptide produces a cleavage site at the target region of the genome, thereby modifying the genome; and/or
wherein the target region of the genome is a target gene.
- ~~6828.~~ The method of claim ~~6727~~, wherein the eukaryotic cell is a mammalian cell.
- ~~6929.~~ The method of claim ~~6828~~, wherein the mammalian cell is a mouse cell, a non-human primate cell, or a human cell.
- ~~70.~~ ~~The method of any one of claims 66 to 69, wherein the site-directed modifying polypeptide produces a cleavage site at the target region of the genome, thereby modifying the genome.~~
- ~~71.~~ ~~The method of any one of claims 66 to 70, wherein the target region of the genome is a target gene.~~

~~72~~30. The method of claim ~~71~~27, wherein said method is effective to modify expression of the target gene.

~~73.~~ ~~The method of claim 72, wherein said method is effective to increase expression of the target gene relative to a reference level.~~

~~74.~~ ~~The method of claim 60, wherein said method is effective to decrease expression of the target gene relative to a reference level.~~

~~75~~31. 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 antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, 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 antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, 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.

~~76.~~ ~~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 antigen binding polypeptide that specifically binds to an extracellular cell membrane bound molecule, 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.~~

~~77~~32. The method of claim ~~75~~ or ~~76~~31, wherein

the method comprises locally administering the TAGE agent to the subject by intramuscular injection, intraosseous injection, intraocular injection, intratumoral injection, 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.

~~78. The method of any one of claims 75 to 77, wherein the method is effective to increase the number of genetically modified target cells in the subject following administration of the TAGE agent.~~

~~79. The method of any one of claims 75 to 77, wherein the mammalian subject is a human subject.~~

~~80. The method of any one of claims 75 to 79, 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.~~

~~81~~33. 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 antigen binding polypeptide that specifically binds to an extracellular cell membrane-bound molecule, 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.

~~82~~34. The method of any one of claims 81 ~~claims 81~~ claims 31 to 33, 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; and/or

the TAGE agent is administered to the subject by intramuscular injection, intraosseous injection, intraocular injection, intratumoral injection, or intradermal injection; and/or

the TAGE agent further comprises at least one nuclear localization signal (NLS).

~~83. The method of claim 81, wherein the target mammalian cell is a hematopoietic stem cell (HSC) or a bone marrow cell that is not an HSC.~~

8435. The method of claim 8334, wherein the antigen binding polypeptide specifically binds an extracellular cell membrane-bound molecule on a human HSC.

8536. The method of claim 8435, wherein the extracellular cell membrane-bound molecule on the HSC is CD34, EMCN, CD59, CD90, ckit, CD45, or CD49F.

~~86. The method of any one of claims 81 to 85, wherein the target mammalian cell is contacted with the TAGE agent by co-incubation *ex vivo*.~~

8737. The method of claim 81 to 8633 to 36, wherein the method provides a genetically-modified target cell which is administered to a subject in need thereof.

~~88. The method of any one of claims 81 to 85, wherein the target mammalian cell is contacted with the TAGE agent *in situ* by injection into a tissue of a subject.~~

~~89. The method of claim 88, wherein the TAGE agent is administered to the subject by intramuscular injection, intraosseous injection, intraocular injection, intratumoral injection, or intradermal injection.~~

~~90. The method of any one of claims 75 to 89, wherein the nucleic acid is a gene in the genome of the target cell, wherein the expression of said gene is altered following said modification.~~

~~91. The method of any one of claims 75 to 90, wherein the target mammalian cell is a mouse cell, a non-human primate cell, or a human cell.~~

9238. The method of any one of claims ~~75 to 91~~31 to 37, wherein the antigen binding polypeptide is

an antibody, an antigen-binding portion of an antibody, or an antibody-mimetic.

9339. The method of claim ~~92~~38, wherein:

the antibody mimetic is an adnectin (i.e., fibronectin based binding molecules), an affilin, an affimer, an affitin, an alphabody, an aptamer, 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; or

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

the antibody is an intact antibody or a bispecific antibody.

~~94. The method of claim 92, wherein the antigen binding portion of the antibody is a nanobody, a doman antibody, an scFv, a Fab, a diabody, a BiTE, a diabody, a DART, a minibody, a F(ab')₂, or an intrabody.~~

~~95. The method of claim 92, wherein the antibody is an intact antibody or a bispecific antibody.~~

- ~~9640.~~ The method of any one of claims ~~75 to 95~~31 to 39, 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, CD73, CD74, CTLA4, EGFR, HER2, EpCam, OX40, PD-1, PD-L1, GITR, CD52, CD34, CD27, CD30, ICOS, or RSV.
- ~~9741.~~ The method of any one of claims ~~75 to 95~~31 to 39, wherein
the extracellular cell membrane-bound molecule or protein is CD11a and
the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding
fragment thereof; or
the extracellular cell membrane-bound molecule or protein is CD25 and the
antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment
thereof.
- ~~98.~~ ~~The method of claim 97, wherein the antigen binding polypeptide is an anti-CD11a antibody, or antigen binding fragment thereof.~~
- ~~9942.~~ The method of claim ~~98~~41, wherein the anti-CD11a antibody is efalizumab or the
anti-CD25 antibody is daclizumab.
- ~~100.~~ ~~The method of any one of claims 75 to 95, wherein the extracellular cell membrane-bound molecule or protein is CD25.~~
- ~~101.~~ ~~The method of claim 100, wherein the antigen binding polypeptide is an anti-CD25 antibody, or antigen binding fragment thereof.~~
- ~~102.~~ ~~The method of claim 101, wherein the anti-CD25 antibody is daclizumab.~~
- ~~10343.~~ The method of any one of ~~claims 75 to 102~~claims 31 to 42, wherein the TAGE agent further comprises at least one nuclear localization signal (NLS).
- ~~10444.~~ The method of any one of ~~claims 75 to 103~~claims 31 to 43, wherein the TAGE agent comprises at least two, at least four, at least six, at least seven, or at least eight nuclear localization signals (NLSs).

~~105. The method of claim 104, wherein the TAGE agent comprises four nuclear localization signals (NLSs).~~

~~106. The method of claim 104, wherein the TAGE agent comprises six nuclear localization signals (NLSs).~~

~~107. The method of claim 104, wherein the TAGE agent comprises seven nuclear localization signals (NLSs).~~

~~108. The method of claim 104, wherein the TAGE agent comprises eight nuclear localization signals (NLSs).~~

~~109~~45. The method of any one of claims ~~103 to 108~~43 to 44, wherein the NLS comprises an SV40 NLS.

~~110~~46. The method of claim ~~109~~45, wherein the SV40 NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 8).

~~111~~47. The method of any one of claims ~~75 to 110~~31 to 46, wherein
the target mammalian cell is a population of 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 antigen binding polypeptide or a complementary binding moiety attached thereto; and/or

the site-directed modifying polypeptide and the antigen binding polypeptide are conjugated via a linker; and/or

the TAGE agent further comprises an endosomal escape agent.

11248. The method of claim 11147, wherein the method is effective to increase the number of genetically modified target mammalian cells.

~~113. The method of any one of claims 75 to 112, wherein the site-directed modifying polypeptide of the TAGE agent has increased cellular internalization in the target mammalian cell.~~

~~114. The method of any one of claims 75 to 113, wherein the site-directed modifying polypeptide of the TAGE agent has increased nuclear internalization in the target mammalian cell.~~

~~115. The method of any one of claims 75 to 114, wherein the site-directed modifying polypeptide comprises a nuclease or a nickase.~~

~~116. The method of any one of claims 75 to 115, 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.~~

11749. The method of claim 11647, 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.

~~118~~50. The method of claim ~~117~~49, wherein the guide RNA is a single guide RNA (sgRNA) or a cr:trRNA.

~~119~~51. The method of claim ~~117~~49, wherein the RNA-guided nuclease is a Class 2 Cas polypeptide.

~~120~~52. The method of claim ~~119~~51, wherein the Class 2 Cas polypeptide is a Type II Cas polypeptide or a Type V Cas Polypeptide.

~~121~~53. The method of claim ~~120~~52, wherein the Type II Cas polypeptide is Cas9 or the Type V Cas polypeptide is Cas12.

~~122~~. The method of claim ~~119~~, wherein the Class 2 Cas polypeptide is a Type V Cas polypeptide.

~~123~~. The method of claim ~~122~~, wherein the Type V Cas polypeptide is Cas12.

~~124~~. The method of any one of claims ~~75~~ to ~~123~~, wherein the site-directed modifying polypeptide further comprises a conjugation moiety that binds to the antigen binding polypeptide or a complementary binding moiety attached thereto.

~~125~~54. The method of claim ~~124~~47, wherein:
the conjugation moiety is a protein;
the linker is a cleavable linker; or
the endosomal escape agent is TDP or TDP-KDEL (SEQ ID NO: 123).

~~126~~. The method of claim ~~125~~, wherein the protein is SpyCatcher or a Halo Tag.

~~127~~. The method of any one of claims ~~75~~ to ~~126~~, wherein the site-directed modifying polypeptide and the antigen binding polypeptide are conjugated via a linker.

~~128~~. The method of claim ~~127~~, wherein the linker is a cleavable linker.

~~129. The method of any one of claims 75 to 128, wherein the TAGE agent further comprises an endosomal escape agent.~~

~~130. The method of claim 129, wherein the endosomal escape agent is TDP or TDP-KDEL (SEQ ID NO: 123).~~