CLAIMS

1. A method for producing a modified immune cell, the method comprising expressing or introducing in an immune cell a nucleobase editor polypeptide and contacting the cell with two or more guide RNAs that target the nucleobase editor polypeptide to effect an alteration in a nucleic acid molecule encoding at least one polypeptide selected from the group consisting of a T Cell Receptor Alpha Constant (TRAC), beta-2 microgloblulin (B2M), programmed cell death 1 (PD1), Cluster of Differentiation 7 (CD7), Cluster of Differentiation 5 (CD5), Cluster of Differentiation 33 (CD33), Cluster of Differentiation 123 (CD123), Cbl Proto-Oncogene B (CBLB), and Class II Major Histocompatibility Complex Transactivator (CIITA) polypeptide, wherein the nucleobase editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and at least one base editor domain comprising an adenosine deaminase variant domain comprising an alteration at amino acid position 82 and/or 166 of the following amino acid sequence:
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGG
LVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

2. The method of claim 1, wherein the adenosine deaminase variant domain comprises alterations at amino acid position 82 and 166.

3. The method of claim 1 or claim 2, wherein the adenosine deaminase variant domain comprises a V82S alteration and/or a T166R alteration.

4. The method of any one of claims 1 to 3, wherein the adenosine deaminase variant domain further comprises an alteration selected from the group consisting of Y147T, Y147R, Q154S, Y123H, and Q154R.

5. The method of any one of claims 1 to 4, wherein the adenosine deaminase variant domain comprises a combination of alterations selected from the group consisting of:
Y147T and Q154R;
Y147T and Q154S;

Y147R and Q154S;
V82S, Q154S; V82S, and Y147R;
V82S, Q154R; V82S, and Y123H;
I76Y, V82S; V82S, Y123H, and Y147T;
V82S, Y123H, and Y147R;
V82S, Y123H, and Q154R;
Y147R, Q154R, and Y123H;
Y147R, Q154R, and I76Y;
Y147R, Q154R, and T166R;
Y123H, Y147R, Q154R, and I76Y;
V82S, Y123H, Y147R, and Q154R; and
I76Y, V82S, Y123H, Y147R, and Q154R.

6. The method of any one of claims 1 to 5, wherein the adenosine deaminase variant domain comprises a TadA*8.

7. The method of claim 6, wherein the TadA*8 is selected from the group consisting of TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24.

8. The method of any one of claims 1-7, wherein the nucleobase editor polypeptide is selected from the group consisting of ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, and ABE8.24-m

9. The method of any one of claims 1 to 8, wherein the base editor domain is an adenosine deaminase variant heterodimer comprising a wild-type adenosine deaminase domain and the adenosine deaminase variant domain.

10. The method of any one of claims 1 to 9, wherein the napDNAbp comprises the following sequence:

CP5 (with MSP “NGC” PID and “D10A” nickase)

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNI
VKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEKGKSKKLKSVK
ELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAKFLQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYN
KHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDGGSGGSGGSGGSGGSGGSGGMDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRH
SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVE
EDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLN
PDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI
ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVN
TEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFI
KPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKI
LTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD
DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKA
QVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELD
KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREIN
NYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKK
RKV.

11. The method of any one of claims 1 to 10, wherein the napDNAbp is selected from the group consisting of Staphylococcus aureus Cas9 (SaCas9), Streptococcus thermophilus 1 Cas9 (St1Cas9), a Streptococcus pyogenes Cas9 (SpCas9), and variants thereof.

12. The method of any one of claims 1 to 11, wherein the nucleobase editor polypeptide further comprises a linker between the napDNAbp and the adenosine deaminase variant domain.

13. The method of any one of claims 1 to 12, wherein the nucleobase editor polypeptide further comprises or more nuclear localization signals (NLS).

14. The method of any one of claims 1 to 13, wherein the immune cell is a T cell.

15. The method of any one of claims 1 to 14, wherein the nucleobase editor polypeptide further comprises one or more uracil glycosylase inhibitors.

16. The method of any one of claims 1 to 15, further comprising expressing a chimeric antigen receptor (CAR) in the modified immune cell.

17. A modified immune cell produced according to the method of any one of claims 1 to 16.

18. The modified immune cell of claim 17, wherein the cell comprises one or more mutations in polynucleotides encoding at least one polypeptide selected from the group consisting of:

beta-2 microglobulin (B2M), Cluster of Differentiation 7 (CD7), Class II Major Histocompatibility Complex Transactivator (CIITA), programmed cell death 1 (PD1), Cbl Proto-Oncogene B (CBLB), and T Cell Receptor Alpha Constant (TRAC);

T Cell Immunoreceptor With Ig and ITIM Domains (TIGIT), Transforming Growth Factor Beta Receptor II (TGFBR2), zeta chain of T cell receptor associated protein kinase 70 (ZAP70), nuclear factor of activated T cells 1 (NFATc1), and Tet Methylcytosine Dioxygenase (TET2); or

V-Set Immunoregulatory Receptor (VISTA), T Cell Immunoglobulin Mucin 3 (Tim-3), T Cell Immunoreceptor With Ig and ITIM Domains (TIGIT), Transforming Growth Factor Beta Receptor II (TGFBRII), Regulatory Factor X Associated Ankyrin Containing Protein (RFXANK), PVR Related Immunoglobulin Domain Containing (PVRIG), Lymphocyte-Activation Gene 3 (Lag3), Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), Chitinase 3 Like 1 (Chi3l1), Cluster of Differentiation 96 (CD96), B and T Lymphocyte Associated (BTLA), Tet Methylcytosine Dioxygenase 2 (TET2), Sprouty RTK Signaling Antagonist 1 (Spry1), Sprouty

RTK Signaling Antagonist 2 (Spry2), Class II Major Histocompatibility Complex Transactivator (CIITA), Cluster of Differentiation 7 (CD7), Cluster of Differentiation 33 (CD33), Cluster of Differentiation 52 (CD52), Cluster of Differentiation 123 (CD123), T Cell Receptor Beta Constant 1 (TRBC1), T Cell Receptor Beta Constant 2 (TRBC2), Cytokine Inducible SH2 Containing Protein (CISH), Acetyl-CoA Acetyltransferase 1 (ACAT1), Cytochrome P450 Family 11 Subfamily A Member 1 (Cyp11a1), GATA Binding Protein 3 (GATA3), Nuclear Receptor Subfamily 4 Group A Member 1 (NR4A1), Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2), Nuclear Receptor Subfamily 4 Group A Member 3 (NR4A3), Methylation-Controlled J Protein (MCJ), Fas Cell Surface Death Receptor (FAS), and Selectin P Ligand/P-Selectin Glycoprotein Ligand-1 (SELPG/PSGL1).

19. The modified immune cell of claim 17 or claim 18, wherein the immune cell expresses a chimeric antigen receptor.

20. The modified immune cell of claim 19, wherein the chimeric antigen receptor comprises an extracellular domain having an affinity for a marker associated with a neoplasia.

21. The modified immune cell of claim 20, wherein the neoplasia is selected from the group consisting of a B cell cancer, a lymphoma, a leukemia, and multiple myeloma.

22. Use of an effective amount of a modified immune cell of any one of claims 17 to 21 in the preparation of a medicament for modulating an immune response, treating a neoplasia in a subject, or treating graft-versus-host disease (GVHD) in a subject having or having a propensity to develop graft-versus-host disease, wherein the medicament is to be administered to the subject.

23. A pharmaceutical composition or kit comprising an effective amount of the modified immune cell of any one of claims 17 to 21 in a pharmaceutically acceptable excipient.

24. A method for producing a modified immune cell, the method comprising expressing or introducing in an immune cell a nucleobase editor polypeptide and contacting the cell with two or more guide RNAs capable of targeting a nucleic acid molecule encoding at least one polypeptide

selected from the group consisting of a T Cell Receptor Alpha Constant (TRAC), beta-2 microgloblulin (B2M), programmed cell death 1 (PD1), Cluster of Differentiation 7 (CD7), Cluster of Differentiation 5 (CD5), Cluster of Differentiation 33 (CD33), Cluster of Differentiation 123 (CD123), Cbl Proto-Oncogene B (CBLB), and Class II Major Histocompatibility Complex Transactivator (CIITA) polypeptide, wherein the nucleobase editor polypeptide comprises at least one base adenosine deaminase variant domain inserted within a nucleic acid programmable DNA binding protein (napDNAbp).

25. The method of claim 24, wherein:

the nucleic acid programmable DNA binding protein (napDNAbp) is a Cas9 polypeptide that comprises a deletion of amino acids 1017-1069 as numbered in the Cas9 polypeptide reference sequence, or corresponding amino acids thereof;

the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in the Cas9 polypeptide reference sequence, or corresponding amino acids thereof; or

the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in the Cas9 polypeptide reference sequence, or corresponding amino acids thereof.

26. The method of claim 25, wherein the adenosine deaminase variant is inserted within a flexible loop of the Cas9 polypeptide.

27. A modified immune cell produced according to the method of any one of claims 24 to 26.

28. A base editor system comprising a polynucleotide programmable DNA binding domain and at least one base editor domain that comprises an adenosine deaminase variant comprising an alteration at amino acid position 82 or 166 of the following amino acid sequence:
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI
MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD)
and two or more guide RNAs that target the nucleobase editor polypeptide to effect an alteration in a nucleic acid molecule encoding at least one polypeptide selected from the group consisting of a T

Cell Receptor Alpha Constant (TRAC), beta-2 microgloblulin (B2M), programmed cell death 1 (PD1), Cluster of Differentiation 7 (CD7), Cluster of Differentiation 5 (CD5), Cluster of Differentiation 33 (CD33), Cluster of Differentiation 123 (CD123), Cbl Proto-Oncogene B (CBLB), and Class II Major Histocompatibility Complex Transactivator (CIITA) polypeptide.

29. The base editor system of claim 28 wherein the adenosine deaminase variant comprises a V82S alteration and/or a T166R alteration; and, optionally, one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, and Q154R.

30. The base editor system of claim 28 or claim 29, comprising two or more guide RNAs and a fusion protein comprising a polynucleotide programmable DNA binding domain comprising the following sequence:
CP5 (with MSP "NGC" PID and "D10A" nickase)
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKV
EKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
MLASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEY
RSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSGGSGGSGGSGGSGGSGGMDKKYSIGL
AIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARR
RYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKY
PTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ
LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKI
LTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPN
EKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFA
NRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKL
YLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVP
SEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA
VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV,
and at least one base editor domain comprising an adenosine deaminase variant comprising an alteration at amino acid position 82 and/or 166 of the following amino acid sequence:
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI
MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSST,
and wherein the two or more guide RNAs target the nucleobase editor polypeptide to effect an alteration in a nucleic acid molecule encoding at least one polypeptide selected from the group consisting of a T Cell Receptor Alpha Constant (TRAC), beta-2 microgloblulin (B2M), programmed cell death 1 (PD1), Cluster of Differentiation 7 (CD7), Cluster of Differentiation 5 (CD5), Cluster of Differentiation 33 (CD33), Cluster of Differentiation 123 (CD123), Cbl Proto-Oncogene B (CBLB), and Class II Major Histocompatibility Complex Transactivator (CIITA) polypeptide.

31. A cell comprising the base editor system of any one of claims 28 to 30.