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 each individually target the nucleobase editor polypeptide to effect an alteration in a nucleic acid molecule encoding a different 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 amino acid sequence having at least 90% sequence identity to the following amino acid sequence and further comprising an alteration at amino acid position 82 and/or an arginine (R) at position 166 compared to the following amino acid sequence:
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGG
LVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTD
thereby altering the nucleic acid molecules encoding the polypeptides.

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

3. The method of claim 1 or claim 2, wherein the adenosine deaminase variant domain comprises a V82S alteration and 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:
V82S and Q154S;
V82S and Y147R;
V82S and Q154R;
V82S and Y123H;
I76Y and V82S;
V82S, Y123H, and Y147T;
V82S, Y123H, and Y147R;
V82S, Y123H, and Q154R;
Y147R, Q154R, and T166R;
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 nucleobase editor polypeptide comprises a single adenosine deaminase variant domain.

7. The method of any one of claims 1 to 6, wherein the base editor domain is an adenosine deaminase variant heterodimer comprising a wild-type adenosine deaminase domain and the adenosine deaminase variant domain.

8. The method of any one of claims 1 to 7, 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.

9. The method of any one of claims 1 to 8, wherein the napDNAbp is selected from the group consisting of Staphylococcus aureus Cas9 (SaCas9), Streptococcus thermophilus 1 Cas9 (St1Cas9), and a Streptococcus pyogenes Cas9 (SpCas9).

10. The method of any one of claims 1 to 9, wherein the nucleobase editor polypeptide further comprises a linker between the napDNAbp and the adenosine deaminase variant domain.

11. The method of any one of claims 1 to 10, wherein the nucleobase editor polypeptide further comprises or more nuclear localization signals (NLS).

12. The method of any one of claims 1 to 11, wherein the immune cell is a T cell.

13. The method of any one of claims 1 to 12, wherein the nucleobase editor polypeptide further comprises one or more uracil glycosylase inhibitors.

14. The method of any one of claims 1 to 13, further comprising expressing a chimeric antigen receptor (CAR) in the modified immune cell.

15. A modified immune cell produced according to the method of any one of claims 1 to 14.

16. The modified immune cell of claim 15, wherein the immune cell expresses a chimeric antigen receptor.

17. The modified immune cell of claim 16, wherein the chimeric antigen receptor confers specificity onto the modified immune cell for a marker associated with a neoplasia.

18. The modified immune cell of claim 17, wherein the neoplasia is selected from the group consisting of a B cell cancer, a lymphoma, a leukemia, and multiple myeloma.

19. Use of an effective amount of a modified immune cell of any one of claims 15 to 18 in the preparation of a medicament for 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.

20. A pharmaceutical composition or kit comprising an effective amount of the modified immune cell of any one of claims 15 to 18 in a pharmaceutically acceptable excipient.

21. 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 each individually capable of targeting a nucleic acid molecule encoding a different 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), wherein the adenosine deaminase variant domain comprising an amino acid sequence having at least 90% sequence identity to the following amino acid sequence and further comprising an alteration at amino acid position 82 and/or an arginine (R) at position 166 compared to the following amino acid sequence:
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGG
LVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTD;

thereby altering the nucleic acid molecules encoding the polypeptides.

22. The method of claim 21, 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 following Cas9 polypeptide reference sequence
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRT
ARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPT
IYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPI
NASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSK
DTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLL
KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ
RTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKS
EETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRK
PAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD
KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGI
LQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT
QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVP
SEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSR
MNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLE
SEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV

AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFEL
ENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK
EVLDATLIHQSITGLYETRIDLSQLGGD;

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

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

23. The method of claim 22, wherein the adenosine deaminase variant is inserted within a flexible loop of the Cas9 polypeptide.

24. A modified immune cell produced according to the method of any one of claims 21 to 23.

25. 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 amino acid sequence having at least 90% sequence identity to the following amino acid sequence and further comprising an alteration at amino acid position 82 and/or an arginine (R) at position 166 compared to 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.

26. The base editor system of claim 25 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.

27. The base editor system of claim 25 or claim 26, wherein the 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.

28. A cell comprising the base editor system of any one of claims 25 to 27.