DLL3 BINDING PROTEINS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to US Provisional Patent Application Nos. 63/572,743, filed April 1, 2024, and 63/693,844, filed September 12, 2024, both of which are incorporated by reference herein in their entirety.

SEQUENCE LISTING

The application contains a Sequence Listing which has been submitted electronically in .XML format and is hereby incorporated by reference in its entirety. Said .XML copy, created on March 28, 2025, is named "01381-0001-00PCT.xml" and is 220,143 bytes in size. The sequence listing contained in this .XML file is part of the specification and is hereby incorporated by reference herein in its entirety.

FIELD

The present disclosure relates to molecules binding to delta-like ligand 3 (DLL3), such as anti-DLL3 chimeric antigen receptors (CARs), anti-DLL3 antibodies including antigen binding domains, bispecific antibodies, antibody conjugates, anti-DLL3 immune cell engagers, and other fusion proteins comprising anti-DLL3 antigen binding domains, and the like, polynucleotides and vectors encoding DLL3 binding molecules, and engineered cells expressing DLL3 binding proteins, and methods of making and using the DLL3 binding proteins and engineered cells expressing the DLL3 binding proteins.

BACKGROUND

Delta-like ligand 3 (DLL3) is a Notch inhibitory ligand, which is overexpressed on the surface of some cancer cells, such as small cell lung cancer (SCLC) cells, neuroendocrine carcinoma cells, and others. In contrast, DLL3 has limited expression or no expression on normal cells. (*See* Rudin et al., *J. Hematol. Oncol.* 16(article 66) (2023).) DLL3 is also expressed, for example, in pulmonary neuroendocrine carcinoma, large cell neuroendocrine carcinoma, gastric cancer, pancreatic cancer, such as gastroenteropancreatic cancer, bladder cancer, prostate cancer, and cervical cancer, such as gastric, pancreatic, gladder, prostate, and

cervical neuroendocrine carcinomas. (*See Id.*) Further, treatment options for cancers such as SCLC that express DLL3 have been limited, while responses to standard of care treatments such as chemotherapy have been poor, with initial responses followed by rapid recurrence in many subjects. (*See Id.*) Thus, new treatments for such cancers are needed, while targeting DLL3 on such cancer cells may provide an attractive means for treatment.

SUMMARY

The present disclosure relates to proteins that bind to delta-like ligand 3 (DLL3). Examples of such DLL3 binding proteins include anti-DLL3 chimeric antigen receptors, anti-DLL3 immune cell engagers, anti-DLL3 antibodies, anti-DLL3 antigen binding domains, and fusion proteins comprising anti-DLL3 antigen binding domains.

The present disclosure also encompasses exemplary embodiments, including but not limited to the following enumerated exemplary embodiments:

1. A delta-like ligand 3 (DLL3) binding protein that binds to the epidermal growth factor (EGF)-like repeat 6 (EGF6) domain of DLL3, optionally wherein the DLL3 binding protein is a fusion protein comprising an anti-DLL3 antigen binding domain, optionally wherein the fusion protein is an anti-DLL3 chimeric antigen receptor (CAR), an anti-DLL3 T cell receptor (TCR), or an anti-DLL3 immune cell engager.

2. An anti-DLL3 CAR, comprising an extracellular domain comprising an anti-DLL3 antigen binding domain.

3. The DLL3 binding protein or anti-DLL3 CAR of embodiment 1 or 2, wherein the anti-DLL3 antigen binding domain binds the epidermal growth factor (EGF)-like repeat 6 (EGF6) domain of DLL3.

4. The DLL3 binding protein or anti-DLL3 CAR of embodiment 1, 2 or 3, wherein the anti-DLL3 CAR comprises one or more of a linker sequence, a hinge domain, a transmembrane domain, one or more intracellular signaling domains, or a combination thereof.

5. The anti-DLL3 CAR of embodiment 2, 3, or 4, wherein the anti-DLL3 CAR comprises a linker sequence, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

6. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-5, wherein the DLL3 binding protein or the anti-DLL3 antigen binding domain is a single chain antigen binding domain.

7. The DLL3 binding protein or anti-DLL3 CAR of embodiment 6, wherein the single chain antigen binding domain is a single chain Fv (scFv).

8. The DLL3 binding protein or anti-DLL3 CAR of embodiment 6, wherein the single chain antigen binding domain is a camelid single-domain antibody, optionally wherein the camelid single-domain antibody is a VHH or nanobody.

9. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiment 1-8, wherein the anti-DLL3 antigen binding domain is a single domain antibody (sdAb), a fragment antigen binding moiety (Fab), a tandem scFv (bivalent and/or bispecific), tandem single domain antibody (sdAb), or a bi-specific Fab.

10. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-9, wherein the anti-DLL3 antigen binding domain comprises

a. a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6;

b. a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154; or

c. a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160.

11. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-10, wherein the anti-DLL3 antigen binding domain comprises a VH comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at

least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 7, and/or a VL comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 8 or 148.

12. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-11, wherein the anti-DLL3 antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 and/or a VL comprising the amino acid sequence of SEQ ID NO: 8 or 148.

13. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-12, wherein the anti-DLL3 antigen binding domain comprises a VH with an amino acid consisting of the sequence of SEQ ID NO: 7 and/or a VL with an amino acid sequence consisting of SEQ ID NO: 8 or 148.

14. The anti-DLL3 CAR of any one of embodiments 2-13, wherein the anti-DLL3 CAR is or comprises a single-chain CAR, a multi-chain CAR, a single-targeted CAR, a multi-targeted CAR, a bivalent tandem CAR, a bivalent loop CAR, a multicistronic CAR, a bicistronic CAR, a dimerizing agent regulated immune-receptor complex (DARIC), an antibody tethered orthogonal multiplexing compatible (ATOMIC), a T cell receptor fusion construct (TruC), an HLA-independent T cell (HIT) receptor, a synthetic T cell receptor and antigen receptor (STAR), a synNotch-CAR circuit, a synthetic intramembrane proteolysis receptor (SNIPR), a rapamycin inducible TCR, a rapamycin inducible Fc receptor, a constitutive TCR-like receptor, a multi-chain DAP-CAR, a TREM1/DAP12 CAR, or a DAP12/TREM1 CAR.

15. The anti-DLL3 CAR of any one of embodiments 2-13, wherein the CAR is a dimerizing agent regulated immune-receptor complex (DARIC), an antibody tethered orthogonal multiplexing compatible (ATOMIC), a synNotch-CAR circuit, a synthetic intramembrane proteolysis receptor (SNIPR), a rapamycin inducible Fc receptor, a multi-chain DAP-CAR, a TREM1/DAP12 CAR, or a DAP12/TREM1 CAR.

16. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 4-15, wherein the CAR comprises a linker sequence.

17. The DLL3 binding protein or anti-DLL3 CAR of embodiment 16, wherein the linker sequence is or comprises a Whitlow linker, a (G4S)n linker, or an SG4S linker.

18. The DLL3 binding protein or anti-DLL3 CAR of embodiment 17, wherein n = 0, 1, 2, 3, 4, 5, or 6.

19. The DLL3 binding protein or anti-DLL3 CAR of embodiment 17, wherein the linker sequence comprises the amino acid sequence of any one of SEQ ID NOs: 16, 38 or 40-60.

20. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 4-19, wherein the CAR comprises a hinge domain.

21. The DLL3 binding protein or anti-DLL3 CAR of embodiment 20, wherein the hinge domain is or comprises a CD8a hinge domain, a CD28 hinge domain, a IgG4 hinge domain, or a IgG4 hinge-CH2-CH3 domain.

22. The DLL3 binding protein or anti-DLL3 CAR of embodiment 20 or 21, wherein the hinge domain comprises the amino acid sequence of any one of SEQ ID NOs: 18 or 100-106.

23. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 4-22, wherein the CAR comprises a transmembrane domain.

24. The DLL3 binding protein or anti-DLL3 CAR of embodiment 23, wherein the transmembrane domain comprises a transmembrane region domain of TCR alpha chain, TCR beta chain, TCR zeta chain, TCRgamma, TCRdelta, CD3gamma, CD3delta, CD3epsilon, CD3zeta, CD4, CD5, CD8, CD8alpha, CD8beta, CD9, CD16, CD22, CD28, CD32, CD33, CD34, CD37, CD38, CD40, CD45, CD64, CD80, CD86, OX-40 (CD134), 4-1BB (CD137), CD154, Fc receptor for IgE (FcεRI), VEGFR2, FAS, or FGFR2B.

25. The DLL3 binding protein or anti-DLL3 CAR of embodiment 23, wherein the transmembrane domain comprises the amino acid sequence of any one of SEQ ID NOs: 20 or 107-109.

26. The DLL3 binding protein or anti-DLL3 CAR of embodiment 23, wherein the transmembrane domain comprises a CD8 transmembrane domain, such as comprising the amino acid sequence of SEQ ID NO: 20.

27. The anti-DLL3 CAR of any one of embodiments 2-26, wherein the intracellular signaling domain comprises an intracellular signaling domain of CD28, 41BB (CD137), OX-40 (CD134), CD2, CD7, CD27, CD30, CD40, PD-1, ICOS, LFA-1 (CDIIa/CD18), CD3gamma, CD3delta, CD3epsilon, CD3zeta, 1.xxCD3zeta, CD3zeta Q14K, CD247, CD276 (B7-H3), LIGHT, NKG2C, Ig alpha (CD79a), DAP-10, an Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, an integrin, a Signaling Lymphocytic Activation Molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, B7-H3, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CARD11, CD4, CD8alpha, CD8beta, IL-2Rbeta, IL-2R gamma, IL-7R alpha, ITGA4, VLAl, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 1d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 lb, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9

(CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a CD83 ligand, or any combination thereof.

28. The anti-DLL3 CAR of embodiment 27, wherein the intracellular signaling domain comprises an intracellular signaling domain of CD3zeta, 1xxCD3zeta, CD3zeta Q14K, CD28, 4-1BB, or OX-40, or any combination thereof.

29. The anti-DLL3 CAR of any one of embodiments 2-28, wherein the intracellular signaling domain comprises CD3zeta, such as comprising the amino acid sequence of SEQ ID NO: 24, 62, or 63, or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24, 62, or 63.

30. The anti-DLL3 CAR of any one of embodiments 2-28, wherein the intracellular signaling domain comprises CD3zeta, such as comprising the amino acid sequence of SEQ ID NO: 24 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24.

31. The anti-DLL3 CAR of any one of embodiments 2-30, wherein the intracellular signaling domain comprises an intracellular signaling domain of 4-1BB and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

32. The anti-DLL3 CAR of any one of embodiments 2-27 wherein the intracellular signaling domain comprises (a) an intracellular signaling domain of CD3zeta, and/or comprises the amino acid sequence of SEQ ID NO: 24, 62, or 63 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24, 62, or 63, and further comprises (b) an intracellular signaling domain of 41BB, and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

33. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-32, wherein the CAR further comprises a signal sequence.

34. The DLL3 binding protein or anti-DLL3 CAR of embodiment 33, wherein the signal sequence comprises a CD8alpha signal sequence, an IgK signal sequence, or a GMCSFR-alpha signal sequence.

35. The DLL3 binding protein or anti-DLL3 CAR of embodiment 33, wherein the signal sequence comprises a CD8alpha signal sequence.

36. The DLL3 binding protein or anti-DLL3 CAR of embodiment 33, wherein the signal sequence comprises the amino acid sequence of any one of SEQ ID NOs: 12, 74, or 75.

37. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-36, wherein the CAR further comprises a depletion tag, wherein the depletion tag comprises an epitope recognized by a monoclonal antibody.

38. The DLL3 binding protein or anti-DLL3 CAR of embodiment 37, wherein the epitope recognized by the monoclonal antibody comprises a CD20 epitope, a RSV protein F epitope, a EGF receptor epitope, a PD-1 epitope, a CD52 epitope, a CCR4 epitope, or a QBEND-10 epitope, or a combination thereof.

39. The DLL3 binding protein or anti-DLL3 CAR of embodiment 37 or 38, wherein the epitope recognized by the monoclonal antibody comprises any one of SEQ ID NOs: 64-73; or wherein the epitope recognized by the monoclonal antibody is a CCR4 epitope; or wherein the DLL3 binding protein or anti-DLL3 CAR comprises the amino acid sequence of SEQ ID NO: 162 or is encoded by the nucleotide sequence of SEQ ID NO:163.

40. The DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-39, wherein the binding protein or CAR is bispecific or multispecific and further comprises an antigen binding domain that binds a second target protein.

41. The anti-DLL3 CAR of any one of embodiments 2-39, wherein the CAR is a tandem CAR and further comprises an antigen binding domain that binds a second target protein.

42. The DLL3 binding protein or anti-DLL3 CAR of embodiment 40 or 41, wherein the second target protein is or comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR,

EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, or the extracellular portion of the APRIL protein.

43. The DLL3 binding protein or anti-DLL3 CAR of embodiment 40 or 41, wherein the second target protein is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB.

44. The DLL3 binding protein or anti-DLL3 CAR of embodiment 40 or 41, wherein the second target protein is or comprises TROP2, SSTR2, or GD2.

45. The DLL3 binding protein or anti-DLL3 CAR of embodiment 40 or 41, wherein the second target protein is or comprises DLL3.

46. The DLL3 binding protein or anti-DLL3 CAR of embodiment 40 or 41, wherein the second target protein is or comprises DLL3, and wherein the CAR binds to an epitope on DLL3 comprising the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

47. The anti-DLL3 CAR of any one of embodiments 2-46, wherein cells expressing the anti-DLL3 CAR (such as, for example, T cells) have one or more of the following properties:

a. IFNgamma and IL-2 expression when co-cultured with small cell lung carcinoma (SCLC) cells;

b. Higher IFNgamma and IL-2 expression when co-cultured with DMS273, NCI-H82, and/or SHP77 cells compared to cells expressing an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36;

c. Do not result in production of cytokines against DLL3 negative cells;

d. When administered to a SCLC murine tumor model, promote tumor regression and/or increased survival compared to an untransduced control and/or compared to a cell expressing a CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of SEQ ID NO: 36.

48. The anti-DLL3 CAR of any one of embodiments 2-47, wherein the CAR comprises:

(a) an anti-DLL3 antigen binding domain comprising:

(i) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6,

(ii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154, or

(iii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160;

(b) a CD8 hinge region;

(c) a CD8 transmembrane domain

(d) and a CD3zeta, CD3zeta Q14K, or 1xxCD3zeta intracellular signaling domain and a 41BB intracellular signaling domain.

49. The anti-DLL3 CAR of any one of embodiments 2-48, wherein the CAR comprises:

(a) an anti-DLL3 antigen binding domain comprising:

(i) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6,

(ii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154, or

(iii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160;

(b) a linker comprising the amino acid sequence of any one of SEQ ID NO: 16, 38, or 40-60;

(c) a hinge comprising the amino acid sequence of SEQ ID NO: 18;

(d) a transmembrane domain comprising the amino acid sequence of SEQ ID NO: 20;

(e) and an intracellular signaling domain comprising the amino acid sequence of any one of SEQ ID NOs: 24, 62 or 63, and further comprising the amino acid sequence of SEQ ID NO: 22.

50. The anti-DLL3 CAR of any one of embodiments 2-49, wherein the CAR comprises an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or identical to the sequence of SEQ ID NO: 9 or SEQ ID NO: 162, with or without a signal sequence.

51. A nucleic acid construct encoding the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50.

52. The nucleic acid construct of embodiment 51, wherein the construct comprises a polynucleotide sequence encoding the DLL3 binding protein or anti-DLL3 CAR operably linked to a promoter.

53. The nucleic acid construct of embodiment 52, wherein the promoter is a constitutive promoter or an inducible promoter.

54. The nucleic acid construct of embodiment 52 or 53, wherein the promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL-2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian

sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

55. The nucleic acid construct of embodiment 54, wherein the promoter is a constitutive promoter.

56. The nucleic acid construct of embodiment 55, wherein the constitutive promoter is or comprises a CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, or PIK3R3 promoter.

57. The nucleic acid construct of embodiment 52 or 53, wherein the promoter is or comprises an MND promoter or a short EF1a promoter.

58. The nucleic acid construct of embodiment 51, wherein the construct comprises a polynucleotide sequence encoding the DLL3 binding protein or anti-DLL3 CAR that is not operably linked to a promoter.

59. The nucleic acid construct of embodiments 51-58, wherein the nucleic acid construct further comprises a polynucleotide sequence encoding one or more cleavable linkers.

60. The nucleic acid construct of embodiment 59, wherein the one or more cleavable linkers comprise a P2A, E2A, F2A, or T2A self-cleaving peptide.

61. The nucleic acid construct of any one of embodiments 51-60, wherein the nucleic acid construct further comprises one or more nucleic acid element sequences.

62. The nucleic acid construct of embodiment 61, wherein the one or more nucleic acid element sequences is or comprises ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, a 70 bp poly(A) tract, a 100 bp poly(A) tract, a 172 bp poly(A) tract, a 200 bp poly(A) tract, a 300 bp poly(A) tract, a 325 bp poly(A) tract, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, cleavable linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or any combination thereof.

63. The nucleic acid construct of any one of embodiments 51-62, wherein the nucleic acid construct further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or one or more non-coding RNA.

64. The nucleic acid construct of embodiment 63, wherein the non-coding RNA comprises a shRNA or a microRNA.

65. The nucleic acid construct of embodiment 63, wherein the one or more additional polypeptides comprise a second chimeric antigen receptor (CAR), a recombinant T cell receptor (TCR), a potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, a safety switch, or any combination thereof.

66. The nucleic acid construct of embodiment 65, wherein the second CAR or recombinant TCR binds to a target antigen selected from CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A4, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17,

PAX3, androgen receptor, cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combinations thereof.

67. The nucleic acid construct of any one of embodiments 63-66, wherein the second CAR or recombinant TCR binds to TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB.

68. The nucleic acid construct of any one of embodiments 63-67, wherein the second CAR or recombinant TCR binds to TROP2, SSTR2, or GD2.

69. The nucleic acid construct of embodiments 63-68, wherein the second CAR or recombinant TCR binds to DLL3.

70. The nucleic acid construct of embodiment 69, wherein the second CAR or recombinant TCR binds to the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

71. The nucleic acid construct of embodiment 65, wherein the potency enhancement polypeptide is or comprises a patient derived CARD11-PIK3R3 fusion, an engineered CARD11-PIK3R3 fusion (such as comprising the amino acid sequence of any one of SEQ ID NOs: 114, 116, 118, or 120), a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (e.g., TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, CD8alpha/beta, a combination thereof, or variants thereof.

72. The nucleic acid construct of any one of embodiments 51-71, wherein the polynucleotide sequence encoding the DLL3 binding protein or anti-DLL3 CAR and the polynucleotide sequence encoding the one or more additional polypeptides are separated by a polynucleotide sequence encoding one or more cleavable linkers, and wherein all the polynucleotide sequences form a continuous open reading frame.

73. The nucleic acid construct of embodiment 72, wherein the one or more cleavable linkers comprise a P2A, E2A, F2A, or T2A self-cleaving peptide.

74. The nucleic acid construct of any one of embodiments 72-73, wherein the polynucleotide sequence encoding the DLL3 binding protein or anti-DLL3 CAR and the

polynucleotide sequence encoding the one or more additional polypeptides are separated by an internal ribozyme entry site (IRES).

75. The nucleic acid construct of any one of embodiments 51-74, wherein the nucleic acid construct is a DNA construct.

76. The nucleic acid construct of any one of embodiments 51-74, wherein the nucleic acid construct is an RNA construct.

77. The nucleic acid construct of embodiment 76, wherein the RNA construct is an mRNA construct.

78. The nucleic acid construct of any one of embodiments 51-77, wherein the nucleic acid construct comprises one or more modified nucleotides.

79. The nucleic acid construct of embodiment 78, wherein the one or more modified nucleotides are or comprise pseudouridine, N1-methylpseudouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl- pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or any combination thereof.

80. The nucleic acid construct of embodiment 79, wherein one or more modified nucleotides are selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, and a combination thereof.

81. The nucleic acid construct of embodiment 80, wherein one or more modified nucleotides comprise N1- methylpseudouridine.

82. The nucleic acid construct of any one of embodiments 51-81, comprising a first homology arm before the 5' end of the nucleic acid sequence encoding the recombinant polypeptide and a second homology arm after the 3' end of the nucleic acid sequence encoding the recombinant polypeptide; optionally, wherein the lengths of the two homology arms are between about 150 bp and about 1500 bp; optionally wherein the length of at least one of the first or second homology arms is about 200bp, 225 bp, about 250 bp, about 275 bp, about 300 bp, about 325 bp, about 350 bp, about 375 bp, about 400 bp, about 425 bp, about 450 bp, about 475 bp, or about 500 bp; optionally, wherein the lengths of both of the homology arms are about 225 bp, about 250 bp, about 275 bp, about 300 bp, about 325 bp, about 350 bp, about 375 bp, about 400 bp, about 425 bp, about 450 bp, about 475 bp, about 500 bp, about 525 bp, about 550 bp, about 575 bp, or about 600 bp; and optionally wherein the total length of the homology arms is between about 400 bp and about 500 bp, about 500 bp and about 600 bp, about 600 bp and

about 700 bp, about 700 bp and about 800 bp, about 800 bp and about 900 bp, about 900 bp and about 1000 bp, about 1000 bp and about 1100 bp, or about 1100 bp and about 1200 bp.

83. The nucleic acid construct of any one of embodiments 51-82, wherein the nucleic acid construct is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length, optionally wherein the nucleic acid construct is less than about 5.0 kb in length, and optionally wherein the nucleic acid construct is less than about 4.7 kb in length.

84. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter, and

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50.

85. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(3) a cleavable linker encoding sequence, and

(4) a polynucleotide encoding one or more additional polypeptides.

86. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence, and

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50.

87. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding one or more additional polypeptides,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

88. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

89. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50.

90. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding one or more additional polypeptides,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50.

91. A nucleic acid construct comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide encoding one or more additional polypeptides.

92. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50, and

(3) a cleavable linker encoding sequence.

93. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding one or more additional polypeptides, and

(5) a cleavable linker encoding sequence.

94. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50, and

(5) a cleavable linker encoding sequence.

95. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding one or more additional polypeptides,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(7) a cleavable linker encoding sequence.

96. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding one or more additional polypeptides,

(7) a cleavable linker encoding sequence.

97. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a

chemokine, or a growth factor,

(7) a cleavable linker encoding sequence.

98. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding one or more additional polypeptides,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(7) a cleavable linker encoding sequence.

99. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding one or more additional polypeptides,

(7) a cleavable linker encoding sequence.

100. A nucleic acid construct comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide encoding one or more additional polypeptides,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(7) a cleavable linker encoding sequence.

101. A nucleic acid construct comprising:

(1) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50, wherein the nucleic acid construct is an RNA construct.

102. A nucleic acid construct comprising:

(1) a first polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

and

(2) a second polynucleotide encoding one or more additional polypeptides,

optionally wherein the nucleic acid construct is an RNA construct.

103. A composition comprising:

(1) a first polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50, and

(2) a second polynucleotide encoding one or more additional polypeptides,

optionally wherein the first and the second polynucleotides are both RNA constructs.

104. A composition comprising:

(1) a first polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(2) a second polynucleotide sequence encoding one or more additional polypeptides, and

(3) a third polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

optionally wherein one or more of the polynucleotides is an RNA construct.

105. A composition comprising:

(1) a polynucleotide encoding the binding protein or CAR of any one of embodiments 1-50,

(2) a second nucleic acid construct encoding one or more additional polypeptides, and

(3) a third nucleic acid construct encoding a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and

optionally wherein the first, second, and/or third polynucleotides are RNA constructs.

106. A vector comprising the nucleic acid construct of any one of embodiments 51-102 or the composition of any one of embodiments 103-105.

107. The vector of embodiment 106, wherein the vector is a plasmid.

108. The vector of embodiment 106, wherein the vector is a synthetic DNA vector.

109. The vector of embodiment 106, wherein the vector is a linear DNA vector.

110. The vector of embodiment 106, wherein the vector is a closed linear DNA vector, or wherein the vector is a synthetic RNA vector.

111. The vector of embodiment 106, wherein the vector is a phagemid vector.

112. The vector of embodiment 106, wherein the vector is a viral vector.

113. The vector of embodiment 112, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is an AAV vector.

114. The vector of embodiment 113, wherein the retrovirus is a lentivirus.

115. The vector of embodiment 114, wherein the lentivirus is a VSV-G pseudotyped lentivirus.

116. The vector of embodiment 113, wherein the AAV vector is AAV6 vector.

117. The vector of embodiment 116, wherein the AAV vector is AAV9 vector.

118. The vector of embodiment 116, wherein the AAV vector is a split-intein dual AAV vector.

119. The vector of embodiment 106, wherein the vector is a redirected lentiviral vector.

120. The vector of embodiment 106, wherein the vector is a fusosome.

121. The vector of embodiment 106, wherein the vector is a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein.

122. The vector of embodiment 106, wherein the vector is an enveloped delivery vehicle.

123. The vector of embodiment 106, wherein the vector is self-replicating RNA virus.

124. The vector of embodiment 106, wherein the vector is mRNA-packaging virus-like particle.

125. The vector of embodiment 106, wherein the vector is RNP-packaging virus-like particle.

126. A particle comprising the nucleic acid construct of any one of embodiments 51-102 or the composition of any one of embodiments 103-105.

127. The particle of embodiment 126, wherein the particle is a lipid nanoparticle (LNP).

128. The particle of embodiment 126, wherein the particle is a selective organ targeting (SORT) LNP.

129. The particle of embodiment 126, wherein the particle is an antibody targeted LNP.

130. The LNP of any one of embodiments 127-129, wherein the LNP comprises: (i) an ionizable lipid (e.g., an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (e.g., a PEG-modified lipid).

131. The particle of embodiment 126, wherein the particle is a polymer nanoparticle.

132. The particle of embodiment 126, wherein the particle is a protein nanoparticle.

133. An engineered cell that expresses the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102,

wherein in the DLL3 binding protein or anti-DLL3 CAR or the nucleic acid construct is under the control of an endogenous promoter.

134. The engineered cell of embodiment 133, wherein the endogenous promoter is a TCRa promoter, a TCRb promoter, a CD3d promoter, a CD3g promoter, a CD3e promoter, a CD3z promoter, a CARD9 promoter, a CARD10 promoter, a CARD11 promoter, a CARD14 promoter, or a PIK3R3 promoter.

135. An engineered cell that expresses the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102, wherein in the DLL3 binding protein or anti-DLL3 CAR or the nucleic acid construct is under the control of an exogenous promoter.

136. The engineered cell of embodiment 135, wherein the exogenous promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL-2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2,

NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein- I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

137. The engineered cell of any one of embodiments 133-136, wherein the nucleic acid encoding the anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102 is inserted into a T-cell receptor (TCR) locus, a CD3 locus, a B2 microglobulin (B2M) locus, a class II transactivator (CIITA) locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, a PIK3R3 locus, or a safe harbor locus.

138. The engineered cell of embodiment 137, wherein the TCR locus is or comprises a TRAJ locus, a TRAC locus, a TRBC1 locus, or a TRBC2 locus, optionally wherein the TRAJ locus is or comprises a TRAJ intron splice acceptor locus, optionally wherein the cell does not express a functional gene product of an endogenous TRAJ locus.

139. The engineered cell of embodiment 138, wherein the cell does not express a functional gene product of an endogenous TRAC locus.

140. The engineered cell of embodiment 138, wherein the cell does not express a functional gene product of an endogenous TRBC1 locus.

141. The engineered cell of embodiment 138, wherein the cell does not express a functional gene product of an endogenous TRBC2 locus.

142. The engineered cell of embodiment 137, wherein the CD3 locus is or comprises: a CD3d locus, a CD3g locus, a CD3e locus, or CD3z locus.

143. The engineered cell of embodiment 142, wherein the cell does not express a gene product of an endogenous CD3d locus.

144. The engineered cell of embodiment 142, wherein the cell does not express a gene product of an endogenous CD3g locus.

145. The engineered cell of embodiment 142, wherein the cell does not express a gene product of an endogenous CD3e locus.

146. The engineered cell of embodiment 142, wherein the cell does not express a gene product of an endogenous CD3z locus.

147. The engineered cell of embodiment 137, wherein the safe harbor locus is or comprises an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

148. The engineered cell of any one of embodiments 133-147, wherein the nucleic acid encoding the anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102 is inserted into an exon, an intron, between an intron and an exon, or a regulatory region.

149. The engineered cell of embodiment any one of embodiments 133-148, wherein the cell is a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T (NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, or a progenitor cell of the lymphoid lineage.

150. The engineered cell of embodiment 149, wherein the T cell is a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, or an engineered T cell comprising a transcriptional receptor.

151. The engineered cell of any one of embodiments 133-150, wherein the cell is an autologous cell.

152. The engineered cell of any one of embodiments 133-150, wherein the cell is an allogeneic cell.

153. The engineered cell of any one of embodiments 133-152, wherein the cell is a primary cell.

154. The engineered cell of any one of embodiments 133-153, wherein the cell is derived from a stem cell.

155. The engineered cell of any one of embodiments 133-154, wherein the cell is genetically modified.

156. The engineered cell of any one of embodiments 133-155, wherein the cell has reduced or eliminated expression of an endogenous T cell receptor.

157. The engineered cell of any one of embodiments 133-156, wherein the cell further comprises:

(i) a second CAR that binds to a second target antigen; or

(ii) a TCR that binds to a second target antigen.

158. The engineered cell of embodiment 157, wherein the engineered cell comprises a nucleic acid construct encoding the second CAR or recombinant TCR; optionally, wherein the nucleic acid construct is an RNA construct.

159. The engineered cell of any one of embodiments 157-158, wherein the second target antigen is or comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2,

EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combinations thereof.

160. The engineered cell of any one of embodiments 157-159, wherein the second target antigen is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB.

161. The engineered cell of any one of embodiments 157-159, wherein the second target antigen is or comprises TROP2, SSTR2, or GD2.

162. The engineered cell of any one of embodiments 157-159, wherein the second target antigen is or comprises DLL3.

163. The engineered cell of embodiment 162, wherein the second target antigen is or comprises the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

164. The engineered cell of any one of embodiments 133-163, wherein the engineered cell expresses the anti-DLL3 CAR on the cell surface.

165. The engineered cell of any one of embodiments 133-164, wherein the cell further comprises a non-coding RNA.

166. The engineered cell of embodiment 165, wherein the non-coding RNA comprises a shRNA or a microRNA.

167. The engineered cell of any one of embodiments 133-166, wherein the cell further comprises a potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, a tolerogenic factor, depletion tag, a safety switch, or any combination thereof.

168. The engineered cell of embodiment 167, wherein the potency enhancement polypeptide is or comprises a patient derived CARD11-PIK3R3 fusion (such as comprising the amino acid sequence of any one of SEQ ID NOs: 114 or 116), an engineered CARD11-PIK3R3 fusion (such as comprising the amino acid sequence of any one of SEQ ID NOs: 118 or 120), a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (e.g., TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, CD8alpha/beta, or a combination thereof.

169. The engineered cell of embodiment 167, wherein the depletion tag comprises an epitope recognized by a monoclonal antibody, optionally wherein the epitope recognized by the monoclonal antibody comprises a CD20 epitope, a RSV protein F epitope, a EGF receptor

epitope, a PD-1 epitope, a CD52 epitope, a CCR4 epitope, or a QBEND-10 epitope, or a combination thereof.

170. The engineered cell of embodiment 169, wherein the epitope recognized by the monoclonal antibody comprises any one of SEQ ID NOs: 64-73, or wherein the epitope recognized by the monoclonal antibody is a CCR4 epitope.

171. The engineered cell of embodiment 167, wherein the safety switch is or comprises a herpesvirus thymidine kinase (HSV-tk) gene, an Escherichia coli cytosine deaminase (EC-CD) gene, or an inducible Caspase9 (iCasp9) protein.

172. The engineered cell of any one of embodiments 133-171, wherein the cell further comprises one or more modifications that inactivate or disrupt one or more alleles of TGFbeta, Regnase-1, FAS, PTPN2, NR4A3, CD52, PD-1, B2M, CIITA, SOCS1, CBLB, DGKalpha, and/or DGKζ.

173. The engineered cell of any one of embodiments 133-172, wherein the cell is a mammalian cell.

174. The engineered cell of embodiment 173, wherein the cell is a human cell, a mouse cell, and a canine cell.

175. The engineered cell of any one of embodiments 133-174, wherein the cell further comprises a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, an RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

176. The engineered cell of embodiment 175, wherein the cell further comprises Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, evoSpCas9 high-fidelity variants of SpCas9, SaCas9,

NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional fragment thereof.

177. The method of embodiment 175, wherein the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

178. The engineered cell of embodiment 175, wherein the recombinant nucleic acid sequence is integrated at exon 8 of an endogenous PIK3R3 locus.

179. The engineered cell of embodiment 175, wherein the recombinant nucleic acid sequence is integrated at intron 7 of an endogenous PIK3R3 locus.

180. The engineered cell of embodiment 175, wherein the recombinant nucleic acid sequence is integrated at exon 13 of an endogenous CARD11 locus.

181. The engineered cell of embodiment 175, wherein the recombinant nucleic acid sequence is integrated at intron 12 of an endogenous CARD11 locus.

182. A method of preparing the engineered cell of any one of embodiments 133-181, comprising contacting a cell with a vector comprising a nucleic acid encoding the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102 or the composition of any one of embodiments 103-105, or contacting the cell with a particle comprising the vector, such as the vector or particle of any one of embodiments 106-132.

183. The method of embodiment 182, wherein the method further comprises selecting a cell comprising the vector or the nucleic acid, nucleic acid construct, or composition.

184. A method of making the engineered immune cell of embodiment 182 or 183, comprising performing gene editing to insert a nucleic acid encoding the DLL3 binding protein or the anti-DLL3 CAR into the cellular genome.

185. The method of any one of embodiments 182-184, wherein the vector is a plasmid, a synthetic DNA vector, a linear DNA vector, a closed linear DNA vector, a phagemid vector, an RNA vector, an mRNA vector, or a viral vector.

186. The method of embodiment 185, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector.

187. The method of embodiment 186, wherein the retrovirus is a lentivirus, optionally a VSV-G pseudotyped lentivirus.

188. The method of embodiment 186, the AAV vector is an AAV6 vector or an AAV9 vector.

189. The method of embodiment 185, wherein the vector is a fusosome, a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein, or an enveloped delivery vehicle.

190. The method of embodiment 185, wherein the vector is a self-replicating RNA virus, an mRNA-packaging virus-like particle, or a ribonucleoprotein (RNP)-packaging virus-like particle.

191. The method of any one of embodiments 182-190, wherein the particle is a lipid nanoparticle (LNP), a selective organ targeting (SORT) LNP, or an antibody targeted LNP.

192. The method of embodiment 191, wherein the LNP comprises: (i) an ionizable lipid (e.g., an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (e.g., a PEG-modified lipid).

193. The method of any one of embodiments 182-192, wherein the particle is a polymer nanoparticle or a protein nanoparticle.

194. The method of any one of embodiments 182-193, wherein the cell is in vivo.

195. The method of any one of embodiments 182-193, wherein the engineered cell is prepared in vivo.

196. The method of any one of embodiments 194 or 195, wherein the cell is prepared in the body of a subject in need of treatment.

197. The method of any one of embodiments 182-193, wherein the cell is ex vivo.

198. The method of any one of embodiments 182-193, wherein the engineered cell is prepared ex vivo.

199. The method of any one of embodiments 182-193 or 197-198, wherein the cell is prepared outside of the body of a subject in need of treatment.

200. The method of embodiment 199, wherein the vector or particle is introduced into the cell by electroporation or transfection.

201. The method of embodiment 200, wherein the transfection is lipofection.

202. The method of any one of embodiments 182-201, wherein the method comprises introducing into the cell one or more nucleic acids encoding a sequence specific nuclease or a nucleic acid programmable DNA binding protein.

203. The method of any one of embodiments 182-202, wherein the method additionally comprises introducing into the cell one or more guide RNAs.

204. The method of embodiment 202, wherein the sequence-specific nuclease is an RNA guided nuclease.

205. The method of any one of embodiments 204, wherein the sequence-specific nuclease or nucleic acid programmable DNA binding domain is a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing

endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

206. The method of embodiment 205, wherein the Cas nuclease is Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional portion thereof.

207. The method of embodiment 204, wherein the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

208. The method of any one of embodiments 204-207, wherein the method comprises introducing into the cell a ribonucleoprotein (RNP) complex comprising the sequence-specific nuclease or nucleic acid programmable DNA binding.

209. The method of any one of embodiments 182-208, wherein the nucleic acid encoding the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50 or the nucleic acid construct of any one of embodiments 51-102 is inserted into an endogenous locus of the cell.

210. The method of embodiment 209, wherein the endogenous locus is or comprises a TRAJ gene locus, a TRAC gene locus, a TRBC1 gene locus, or a TRBC2 gene locus.

211. The method of embodiment 210, wherein the endogenous locus is or comprises a TRAC gene locus or a TRAJ gene locus, optionally wherein the TRAJ gene locus is a TRAJ intron splice acceptor locus.

212. The method of embodiment 210, wherein the endogenous locus is or comprises a safe harbor locus.

213. The method of embodiment 212, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

214. The method of any one of embodiments 182-213, wherein the insertion site of the nucleic acid encoding the anti-DLL3 CAR is an exon.

215. The method of any one of embodiments 182-213, wherein the insertion site of the nucleic acid encoding the anti-DLL3 CAR is an intron.

216. The method of any one of embodiments 182-213, wherein the insertion site of the nucleic acid encoding the anti-DLL3 CAR is between an intron and an exon.

217. The method of any one of embodiments 182-213, wherein the insertion site of the nucleic acid encoding the anti-DLL3 CAR is in a regulatory region.

218. The method of any one of embodiments 182-213, wherein the insertion site of the nucleic acid encoding the anti-DLL3 CAR is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is or comprises ngg, nag, ngrrt, ngrrn, nnnngatt, nnnnryac, nnagaaw, naaaac, tttv, ttn, attn, tttn, gttn, or yttn (see SEQ ID Nos: 121-131), wherein:

(i) r = a or g,

(ii) y = c or t,

(iii) w = a or t,

(iv) v = a or c or g, and

(v) n = a, c, t, or g.

219. The method of any one of embodiments 182-218, wherein the cell comprises a modified TRAC gene locus or a modified TRAJ gene locus, optionally wherein the TRAJ gene locus is a TRAJ intron splice acceptor locus, wherein the locus is modified by insertion of a nucleic acid encoding the DLL3 binding protein or anti-DLL3 CAR into the locus.

220. The method of any one of embodiments 182-218, wherein the cell does not express an endogenous TCR.

221. The method of any one of embodiments 182-220, wherein the method further comprises introducing a gene editing machinery into the cell.

222. The method of embodiment 221, wherein introducing the gene editing machinery comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery.

223. The method of embodiment 221 or 222, wherein the gene editing machinery comprises CRISPR/Cas9.

224. The method of any one of embodiments 221-223, wherein the gene editing machinery is introduced via electroporation.

225. The method of any one of embodiments 210-224, wherein the method thereby produces an engineered cell comprising: (i) the DLL3 binding protein or anti-DLL3 CAR; and (iii) a modified TRAC gene locus; or wherein the method thereby produces an engineered cell

comprising: (i) the anti-DLL3 CAR; and (iii) a modified TRAJ gene locus, optionally wherein the TRAJ gene locus is a TRAJ intron splice acceptor locus.

226. The method of any one of embodiments 182-225, wherein the engineered cell is for use in a cell therapy in a subject.

227. A pharmaceutical composition comprising the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50, the nucleic acid construct of any one of embodiments 51-102, the composition of any one of embodiments 103-105, the vector or particle of any one of embodiments 106-132, or the engineered cell of any one of embodiments 133-181.

228. The pharmaceutical composition of embodiment 227, further comprising a pharmaceutically acceptable excipient.

229. The pharmaceutical composition of embodiment 227 or 228, comprising engineered cells, wherein at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the engineered cells, or at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the total cells, in the pharmaceutical composition express the DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50.

230. The pharmaceutical composition of any one of embodiments 227-229, wherein the composition comprises CD4+ T cells and CD8+ T cells, and the percentage of CD4+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the percentage of CD8+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the ratio of CD4+ T cells to CD8+ T cells is from at or about 1:3 to at or about 3:1, optionally at or about 1:1.

231. A method of treating a subject in need thereof, comprising administering to the subject the engineered cell of any one of embodiments 133-181, or an engineered cell prepared by the method of any one of embodiments 182-226, or the pharmaceutical composition of any one of embodiments 227-230.

232. The method of embodiment 231, wherein the subject has cancer.

233. The method of embodiment 232, wherein the cancer is a solid tumor.

234. The method of embodiment 232, wherein the cancer is sarcoma, carcinoma, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer, kidney cancer, renal pelvis cancer, leukemia, lung cancer, small cell lung cancer, non-small cell lung cancer, melanoma, lymphoma, pancreatic cancer, prostate cancer, neuroendocrine prostate

cancer, ovarian cancer, fibrosarcoma, glioma, glioblastoma, glioblastoma multiforme, neuroblastoma, renal clear cell carcinoma, adrenocortical carcinoma, bladder urothelial carcinoma, diffuse large B-cell lymphoma, lung adenocarcinoma, pancreatic adenocarcinoma, renal cell cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, indolent B cell lymphoma, aggressive B cell lymphoma, T cell lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, myelodysplastic syndromes, myeloproliferative neoplasms, breast invasive carcinoma, triple-negative breast cancer, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, head and neck squamous cell carcinoma, kidney chromophobe, renal papillary cell carcinoma, lower grade glioma, hepatocellular carcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectal adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, or Wilms & rhabdoid tumors.

235. The method of embodiment 232 or 233, wherein the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate cancer.

236. The method of any one of embodiments 232-235, wherein the cancer expresses DLL3.

237. The method of any one of embodiments 232-236, wherein the cancer expresses TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, FN-EDB, or any combination thereof.

238. The method of any one of embodiments 237, wherein the cancer expresses TROP2, SSTR2, GD2, or any combination thereof.

239. The method of any one of embodiments 231-238, wherein the subject is not administered a lymphodepletive agent within 7 days prior to administration of the cell.

240. The method of embodiment 231-239, wherein the subject is not administered cyclophosphamide, fludarabine, or bendamustine within 7 days prior to administration of the cell.

241. The method of any one of embodiments 231-240, wherein the subject is not administered at least 600,000 IU/kg of IL-2 every 8 hours.

242. The method of any one of embodiments 231-241, wherein the method does not comprise a checkpoint therapy which blocks PD-1 or CTLA-4 signaling.

243. The method of any one of embodiments 231-242, wherein the engineered cell has reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, and/or increase intratumoral presence in vivo, enhanced IFNgamma production, enhanced IL-2 production, and/or promotes enhanced tumor regression, and/or wherein the engineered cell has one or more of the following properties:

a. IFNgamma and IL-2 expression when co-cultured with small cell lung carcinoma (SCLC) cells;

b. Higher IFNgamma and IL-2 expression when co-cultured with DMS273, NCI-H82, and/or SHP77 cells compared to cells expressing an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36;

c. Does not result in production of cytokines against DLL3 negative cells;

d. When administered to a SCLC murine tumor model, promotes tumor regression and/or increased survival compared to an untransduced control and/or compared to a cell expressing a CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of SEQ ID NO: 36.

244. The method of any one of embodiments 231-243, wherein the subject is administered one or more additional therapies.

245. The method of embodiment 244, wherein the one or more additional therapies comprise chemotherapy, immunotherapy, surgery, radiotherapy, anti-angiogenic agent, anti-DNA repair agent, anti-inflammatory agent, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, a hormonal agent, or any combination thereof, wherein for example, the one or more additional therapies may comprise an inhibitor, agonist, small molecule, peptide, protein, fusion protein, antibody, nucleic acid, virus, antibody drug conjugate, PROTAC (proteolysis targeting chimera), cell therapy, or natural substance.

246. The method of embodiment 244, wherein the one or more additional therapies comprises a second DLL3 binding protein, a second DLL3 CAR, an anti-DLL3 antibody, an anti-DLL3 immune cell engager, or tarlatamab.

247. Use of the engineered cell of any one of embodiments 133-181, or an engineered cell prepared by the method of any one of embodiments 182-226, or the pharmaceutical

composition of any one of embodiments 227-230, for the manufacture of a medicament for the treatment of a disease.

248. The use of embodiment 247, wherein the disease is cancer.

249. The use of embodiment 248, wherein the cancer is a solid tumor.

250. The use of embodiment 247, wherein the cancer is sarcoma, carcinoma, neuroendocrine neoplasm, neuroendocrine carcinoma, bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer, kidney cancer, renal pelvis cancer, leukemia, lung cancer, small cell lung cancer, non-small cell lung cancer, melanoma, lymphoma, pancreatic cancer, prostate cancer, neuroendocrine prostate cancer, ovarian cancer, fibrosarcoma, glioma, glioblastoma, glioblastoma multiforme, neuroblastoma, renal clear cell carcinoma, adrenocortical carcinoma, bladder urothelial carcinoma, diffuse large B-cell lymphoma, lung adenocarcinoma, pancreatic adenocarcinoma, renal cell cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, indolent B cell lymphoma, aggressive B cell lymphoma, T cell lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, myelodysplastic syndromes, myeloproliferative neoplasms, breast invasive carcinoma, triple-negative breast cancer, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, head and neck squamous cell carcinoma, kidney chromophobe, renal papillary cell carcinoma, lower grade glioma, hepatocellular carcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectal adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, or Wilms & rhabdoid tumors.

251. The use of embodiment 248 or 249, wherein the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate cancer.

252. The use of embodiment any one of embodiments 247-251, wherein the cancer expresses DLL3.

253. The use of embodiment any one of embodiments 247-252, wherein the cancer expresses TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, and FN-EDB or any combination thereof.

254. The use of embodiment 253, wherein the cancer expresses TROP2, SSTR2, GD2, or any combination thereof.

255. The use of any one of embodiments 247-254, wherein the medicament is not for administration within 7 days after administration of a lymphodepletive agent.

256. The use of embodiment 247-255, wherein the medicament is not for administration within 7 days after administration of cyclophosphamide, fludarabine, or bendamustine.

257. The use of any one of embodiments 247-256, wherein the medicament is not for administration with at least 600,000 IU/kg of IL-2 every 8 hours.

258. The use of any one of embodiments 247-257, wherein the medicament is not for administration with a checkpoint therapy which blocks PD-1 or CTLA-4 signaling.

259. The use of any one of embodiments 247-258, wherein the engineered cell has reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, and/or increase intratumoral presence in vivo, enhanced IFNgamma production, enhanced IL-2 production, and/or promotes enhanced tumor regression and/or wherein the engineered cell has one or more of the following properties:

a. IFNgamma and IL-2 expression when co-cultured with small cell lung carcinoma (SCLC) cells;

b. Higher IFNgamma and IL-2 expression when co-cultured with DMS273, NCI-H82, and/or SHP77 cells compared to cells expressing an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36;

c. Does not result in production of cytokines against DLL3 negative cells;

d. When administered to a SCLC murine tumor model, promotes tumor regression and/or increased survival compared to an untransduced control and/or compared to a cell expressing a CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of SEQ ID NO: 36.

260. The use of any one of embodiments 247-259, wherein the medicament is for administration with one or more additional therapies.

261. The use of embodiment 260, wherein the one or more additional therapies comprise chemotherapy, immunotherapy, surgery, radiotherapy, anti-angiogenic agent, anti-DNA repair agent, anti-inflammatory agent, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, a hormonal agent, or any combination thereof, wherein for example, the one or

more additional therapies may comprise an inhibitor, agonist, small molecule, peptide, protein, fusion protein, antibody, nucleic acid, virus, antibody drug conjugate, PROTAC (proteolysis targeting chimera), cell therapy, or natural substance.

262. The use of embodiment 260, wherein the one or more additional therapies comprise a second DLL3 binding protein, a second DLL3 CAR, an anti-DLL3 antibody, an anti-DLL3 immune cell engager, or tarlatamab.

263. An anti-DLL3 antibody, wherein the anti-DLL3 antibody comprises: (i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and/or wherein the anti-DLL3 antibody comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6; (ii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and/or wherein the anti-DLL3 antibody comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154; or (iii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and/or wherein the anti-DLL3 antibody comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160.

264. The anti-DLL3 antibody of embodiment 263, wherein the anti-DLL3 antibody comprises a VH comprising an amino acid sequence at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 7, and/or a VL comprising an amino acid sequence at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 8 or 148.

265. The anti-DLL3 antibody of embodiment 263 or 264, wherein the anti-DLL3 antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 and/or a VL comprising the amino acid sequence of SEQ ID NO: 8 or 148.

266. The anti-DLL3 antibody of any one of embodiments 263-265, wherein the anti-DLL3 antibody comprises a VH with an amino acid consisting of the sequence of SEQ ID NO: 7 and/or a VL with an amino acid sequence consisting of SEQ ID NO: 8 or 148.

267. The anti-DLL3 antibody of any one of embodiments 263-266, wherein the antibody is an antigen binding domain, such as a Fab, F(ab)2, Fv, or scFv.

268. The anti-DLL3 antibody of any one of embodiments 263-266, wherein the antibody is an IgG, IgA, or IgM antibody.

269. The anti-DLL3 antibody of embodiment 268, wherein the antibody is an IgG antibody such as a human IgG1, IgG2, IgG3, or IgG4 antibody.

270. The anti-DLL3 antibody of embodiment 269, wherein the antibody is a full length antibody, optionally comprising a heavy chain constant region comprising or lacking a C-terminal Lys or Gly-Lys residue.

271. The anti-DLL3 antibody of embodiment 269 or 270, wherein the antibody lacks effector function.

272. The anti-DLL3 antibody of embodiment 269 or 270, wherein the antibody has enhanced effector function.

273. The anti-DLL3 antibody of any one of embodiments 263-272, wherein the antibody is a bispecific or multispecific antibody.

274. The anti-DLL3 antibody of embodiment 273, wherein the antibody is a bispecific T cell engager antibody (BiTE), simultaneous multiple interaction T cell engager antibody (SMITE), or trispecific killer engager (TRiKE).

275. The anti-DLL3 antibody of any one of embodiments 263-274, wherein the antibody is attached directly or indirectly to a drug or label.

276. The anti-DLL3 antibody of any one of embodiments 263-274, which is an antibody-drug conjugate (ADC)

277. A nucleic acid construct encoding the anti-DLL3 antibody of any one of embodiments 263-276.

278. A vector comprising the nucleic acid construct of embodiment 277.

279. The vector of embodiment 278, optionally wherein the vector is a plasmid, synthetic DNA vector, linear DNA vector, closed linear DNA vector, phagemid vector, viral vector, AAV vector such as AAV6 or AAV9 or split-intein dual AAV vector, lentiviral vector such as a redirected lentiviral vector or lentiviral particle engineered with anti-CD3 Cocal glycoprotein, adenoviral vector, retroviral vector, enveloped delivery vehicle, self-replicating RNA virus, mRNA, mRNA-packaging virus-like particle (VLP), RNP-packaging VLP,

optionally wherein the vector is enclosed within a lipid nanoparticle (LNP) or selective organ targeting LNP, or antibody targeted LNP, polymer nanoparticle, or protein nanoparticle, optionally wherein, if the vector is enclosed within an LNP, the LNP comprises (i) an ionizable lipid (e.g., an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (e.g., a PEG-modified lipid).

280. A host cell that expresses the anti-DLL3 antibody of any one of embodiments 263-276.

281. A host cell comprising the vector of embodiment 278 or 279 or the nucleic acid construct of embodiment 277.

282. An engineered cell that expresses the anti-DLL3 antibody of any one of embodiments 263-276.

283. A method of making the antibody of any one of embodiments 263-276, comprising incubating the host cell of embodiment 280 or 281 so as to express the antibody, and optionally isolating the antibody.

284. A pharmaceutical composition comprising the anti-DLL3 antibody of any one of embodiments 263-276, the polynucleotide of embodiment 277, the vector of embodiment 278, or the engineered cell of embodiment 282, and optionally further comprising a pharmaceutically acceptable carrier.

285. Use of the anti-DLL3 antibody of any one of embodiments 263-276 or the pharmaceutical composition of embodiment 284, in the preparation of a medicament for treating cancer in a subject.

286. A method of treating a subject in need thereof, comprising administering to the subject the anti-DLL3 antibody of any one of embodiments 263-276 or the pharmaceutical composition of embodiment 284.

287. The use or method of embodiment 285 or 286, wherein the subject has cancer.

288. The use or method of embodiment 287, wherein the cancer is a solid tumor.

289. The use or method of any one of embodiments 287 or 288, wherein the cancer is sarcoma, carcinoma, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer, kidney cancer, renal pelvis cancer, leukemia, lung cancer, small cell lung cancer, non-small cell lung cancer, melanoma, lymphoma, pancreatic cancer, prostate cancer, neuroendocrine prostate cancer, ovarian cancer, fibrosarcoma, glioma, glioblastoma, glioblastoma multiforme, neuroblastoma, renal clear cell carcinoma, adrenocortical carcinoma, bladder urothelial carcinoma, diffuse large B-cell lymphoma, lung adenocarcinoma, pancreatic

adenocarcinoma, renal cell cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, indolent B cell lymphoma, aggressive B cell lymphoma, T cell lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, myelodysplastic syndromes, myeloproliferative neoplasms, breast invasive carcinoma, triple-negative breast cancer, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, head and neck squamous cell carcinoma, kidney chromophobe, renal papillary cell carcinoma, lower grade glioma, hepatocellular carcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectal adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, or Wilms & rhabdoid tumors.

290. The use or method of embodiment 287 or 288, wherein the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate cancer.

291. The use or method any one of embodiments 287-290, wherein the cancer expresses DLL3.

292. The use or method of any one of embodiments 287-291, wherein the cancer expresses TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, and FN-EDB or any combination thereof.

293. The use or method of embodiment 292, wherein the cancer expresses TROP2, SSTR2, GD2, or any combination thereof.

294. The use or method of any one of embodiments 285-293, wherein the medicament is not for administration within 7 days after administration of a lymphodepletive agent.

295. The use or method of any one of embodiments 285-294, wherein the medicament is not for administration within 7 days after administration of cyclophosphamide, fludarabine, or bendamustine.

296. The use or method of any one of embodiments 285-295, wherein the medicament is not for administration with at least 600,000 IU/kg of IL-2 every 8 hours.

297. The use or method of any one of embodiments 285-296, wherein the medicament is not for administration with a checkpoint therapy which blocks PD-1 or CTLA-4 signaling.

298. The use or method of any one of embodiments 285-297, wherein the medicament is for administration with one or more additional therapies.

299. The use or method of embodiment 298, wherein the one or more additional therapies comprise chemotherapy, immunotherapy, surgery, radiotherapy, anti-angiogenic agent, anti-DNA repair agent, anti-inflammatory agent, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, a hormonal agent, or any combination thereof, wherein for example, the one or more additional therapies may comprise an inhibitor, agonist, small molecule, peptide, protein, fusion protein, antibody, nucleic acid, virus, antibody drug conjugate, PROTAC (proteolysis targeting chimera), cell therapy, or natural substance.

300. The use or method of embodiment 298, wherein the one or more additional therapies comprise a second DLL3 binding protein, a second DLL3 CAR, an anti-DLL3 antibody, an anti-DLL3 immune cell engager, or tarlatamab.

301. A DLL3 binding protein or anti-DLL3 CAR of any one of embodiments 1-50 or an anti-DLL3 antibody of any one of embodiments 263-276, wherein the binding protein, CAR, or antibody binds to cynomolgus DLL3 and does not bind to human DLL1 or human DLL4.

Further description of these and other exemplary embodiments of the disclosure are provided elsewhere herein. References cited herein are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows affinity and cross-reactivity data for a humanized rabbit monoclonal antibody (RabmAb), and shows that the antibody binds to the EGF6 domain of human DLL3, and that the antibody also binds to the cynomolgus DLL3, has some cross-reactivity with murine DLL3, and does not bind to human DLL1 or to human DLL4.

Figs. 2A-2C: Fig. 2A shows the architecture of the ML73 CAR. Figs. 2B-2C show that the ML73 CAR and comparative constructs showed robust expression in primary T cells as measured by percent CAR positivity (Fig. 2B) and CAR MFI (Fig. 2C).

Figs. 3A-3B show that the CAR T cells expressing ML73 produced significantly more cytokines IFN-gamma (Fig. 3A) or IL-2 (Fig. 3B) in response to antigen compared to comparator CARs or CARs made with alternative rabbit monoclonal antibodies.

Figs. 4A-4B show CAR positivity (Fig. 4A) and CAR MFI (Fig. 4B) for ML73 compared to that of five comparator CARs transduced into T cells from healthy donors.

Figs. 5A-5B show IFN-gamma (Fig. 5A) and IL-2 (Fig. 5B) secretion levels following co-culturing of CAR T cells expressing ML73 or comparator CARs with A549 lung carcinoma cells.

Figs. 6A-6F show IL-2 (Figs. 6A-6C) and IFN-gamma (Figs. 6D-6F) secretion levels from CAR T cells co-cultured with a cell line expressing a low level of DLL3 (Fig. 6A and 6D), a middle level of DLL3 (Fig. 6B and 6E), and a high level of DLL3 (Fig. 6C and 6F), in each case showing that CAR T cells expressing ML73 CAR produced significantly higher levels of IL-2 and IFN-gamma.

Figs. 7A-7B show tumor progression (Fig. 7A) and percent survival (Fig. 7B) following administration of ML73 CAR T cells to mice engrafted with small cell lung cancer (SCLC) tumor cells.

Figs. 8A-8D show results from delivery of ML73 CAR to the TRAJ intron splice acceptor locus of primary human T cells via an AAV6 delivery mechanism. Fig. 8A shows CAR positivity. Fig 8B shows CAR MFI, comparing the AAV delivery mechanism to a that of a lentivirus mechanism and an untransduced control, comparing the AAV delivery mechanism to a that of a lentivirus mechanism and an untransduced control. Figs. 8C and 8D show IFN-gamma and IL-2 secretion, respectively, comparing the AAV delivery mechanism to a that of a lentivirus mechanism and an untransduced control, and when T cells are co-cultured with three different cell lines, A375, SHP-77, and DMS-273, the latter two being DLL3 antigen positive tumor cell lines.

Figs. 9A-9C show results from an *in vivo* experiment assessing the potency of ML73 CAR inserted into the TRAJ intron splice acceptor locus of primary human T cells via an AAV6 delivery mechanism. Fig. 9A shows tumor growth for mice administered Untransduced T cells population, while Fig. 9B and Fig. 9C show tumor growth for mice administered 1e6 CAR+T cells and 0.2e6 CAR+ T cells respectively.

DETAILED DESCRIPTION

I. DEFINITIONS

Unless otherwise defined, all terms of art, notations, and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this application pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures

described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art. All publications, patent applications, patents, GenBank or other accession numbers and other references mentioned herein are incorporated by reference in their entirety for all purposes.

The singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells, including mixtures thereof. "A and/or B" is used herein to include all of the following alternatives: "A", "B", "A or B", and "A and B."

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value.

As will be understood by one having ordinary skill in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least,"

"greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

It is understood that aspects and embodiments of the disclosure described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments. As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any elements, steps, or ingredients not specified in the claimed composition or method. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claimed composition or method. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of steps of a method, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or step.

The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acids linked by peptide bonds.

The term "delta-like ligand 3" or "DLL3" refers to human DLL3 protein unless specifically noted otherwise (i.e., murine DLL3 or cynomolgus DLL3 or the like). An exemplary sequence of DLL3 comprises that of SEQ ID NO: 26. An exemplary sequence of DLL3 also comprises the NCBI accession number NP_058637.1.

A "DLL3 binding protein" or similar term herein indicates a protein that specifically binds to DLL3, such as an anti-DLL3 antibody or anti-DLL3 chimeric antigen receptor, or the like. The term "anti-DLL3" refers to a molecule that binds specifically to DLL3.

The terms "domain" or "region" of a protein refer to a portion of a protein, that may in some instances have a particular function. Examples of domains include, for example, portions of a protein that resemble specific proteins or protein domains, such as a growth factor-like (EGF-like) domain or an antibody constant region domain, or the like.

A "signal peptide" or "signal sequence" or "leader peptide" or "leader sequence" herein refers to an N-terminal sequence present in a protein that directs the protein following translation in the cell to a molecular membrane, either for secretion or for placement as a transmembrane protein.

An "isolated" protein herein is one that has been separated from a component of its natural environment.

In some aspects, a protein is also "purified" to some extent to remove one or more contaminants. In some cases, a protein can be purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For a review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

In this disclosure, "binds" or "binding" or "specific binding" and similar terms, when referring to a protein and its ligand or a binding protein or antibody and its antigen target, for example, means that the binding affinity is sufficiently strong that the interaction between the members of the binding pair cannot be due to random molecular associations (i.e. "nonspecific binding"). A molecule that "is selective for" a particular target antigen similarly indicates that the molecule "binds" to that target antigen.

The term "antibody" herein refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies, diabodies, etc., as well as Immune cell engagers such as bispecific T cell engager antibodies (BiTE), simultaneous multiple interaction T cell engager antibodies (SMITE), or trispecific killer engagers (TRiKE) and the like), full length antibodies, single-chain antibodies, antibody conjugates, and antibody fragments, so long as they exhibit the desired binding activity.

An "antigen" refers to the target of an antibody, i.e., the molecule to which the antibody specifically binds. The term "epitope" denotes the site on an antigen, either proteinaceous or non-proteinaceous, to which an antibody binds. Epitopes on a protein can be formed both from contiguous amino acid stretches (linear epitope) or comprise non-contiguous amino acids (conformational epitope), e.g., coming in spatial proximity due to the folding of the antigen, i.e. by the tertiary folding of a proteinaceous antigen. Linear epitopes are typically still bound by an antibody after exposure of the proteinaceous antigen to denaturing agents, whereas conformational epitopes are typically destroyed upon treatment with denaturing agents.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity

which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein.

The term "heavy chain" refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term "full-length heavy chain" refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence.

The term "light chain" refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term "full-length light chain" refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.

The term "complementary determining region" or "CDR" or "hypervariable region" or "HVR" refers to each of the regions of an antibody variable region which are hypervariable in sequence and which determine antigen binding specificity. Generally, antibodies comprise six CDRs: three in the VH (CDR-H1 or heavy chain CDR1, CDR-H2, CDR-H3), and three in the VL (CDR-L1, CDR-L2, CDR-L3). The precise amino acid sequence boundaries of a given CDR can be determined by one of a number of well-known schemes. For example, "Kabat CDRs" occur at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)); "Chothia CDRs" occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)); and "McCallum CDRs" (also called "Contact CDRs") occur at amino acid residues 30-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)). Other schemes include the IMGT scheme (LeFranc et al., *Dev. Comp. Immunol.* 27(1): 55-77 (2003), the "Aho" numbering scheme (Honegger and Pliickthun, *J. Mol. Biol.* 309(3): 657-70 (2001), the "AbM" numbering scheme (Martin et al., *PNAS* 86(23): 9268-9272 (1989), and the Martin "enhanced Chothia" numbering scheme (Abhinandan and Martin, *Mol. Immunol.* 45(14): 3832-9 (2008)). As another alternative, structural modeling applications (e.g., AlphaFold or the like) can be used to identify the minimal set of amino acids comprising the putative antigen binding interface. For example, AbM CDRs occur at amino acid residues 24-34 (L1, 50-56 (L2, 89-97

(L3), 26-35b (H1), 50-58 (H2), and 95-102 (H3). Accordingly, a "CDR" or individual, specified CDR (e.g., CDR H1, or CDR L2 or the like) should be understood to encompass a (or the specific) CDR as defined by any of the above schemes, or other known numbering schemes. Exemplary Kabat CDR sequences for an anti-DLL3 antibody are included in the sequence table herein (e.g., SEQ ID NOs: 1-6), exemplary Martin "enhanced Chothia" CDR sequences for an anti-DLL3 antibody are included in the sequence table herein (e.g., SEQ ID NOs: 149-154), and exemplary structural modeling minimal CDR sequences for an anti-DLL3 antibody are included in the sequence table herein (e.g., SEQ ID NOs: 155-160). However, it is understood that CDRs of such an antibody may be defined by those sequences, or by sequences identified using any of the above schemes, or other known schemes.

"Framework" or "FR" refers to the residues of the variable region residues that are not part of the complementary determining regions (CDRs). The FR of a variable region generally consists of four FRs: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in VH (or VL): FR1-CDR-H1(CDR-L1)-FR2- CDR-H2(CDR-L2)-FR3- CDR-H3(CDR-L3)-FR4. An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some aspects, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some aspects, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

The term "variable region" or "variable domain" interchangeably refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementary determining regions (CDRs). See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007). A variable domain may comprise heavy chain (HC) CDR1-FR2-CDR2-FR3-CDR3 with or without all or a portion of FR1 and/or FR4; and light chain (LC) CDR1-FR2-CDR2-FR3-CDR3 with or without all or a portion of FR1 and/or FR4. That is, a variable domain may lack a portion of FR1 and/or FR4 so long as it retains antigen-binding activity. A single VH or VL domain may be sufficient to

confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The light chain and heavy chain "constant regions" of an antibody refer to additional sequence portions outside of the FRs and CDRs and variable regions. Certain antibody fragments may lack all or some of the constant regions. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant heavy domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a light chain variable region, followed by a constant light (CL) domain.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one aspect, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore, an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain. This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to EU index). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (Lys447), of the Fc region may or may not be present. Thus, a "full-length IgG1" for example, includes an IgG1 with Gly446 and Lys447, or without Lys447, or without both Gly446 and Lys447. Amino acid sequences of heavy chains including an Fc region are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise. In one aspect, a heavy chain including an Fc region as specified herein, comprised in an antibody according to the invention, may comprise Gly446 and Lys447 (numbering according to EU index). In one aspect, a heavy chain including an Fc region as specified herein, comprised in an antibody according to the invention, may comprise Gly446 (numbering according to EU index). Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain aspects, the antibody is of the human IgG_1 IgG_2, IgG_3, or IgG_4 isotype. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

An "antibody fragment" or "antigen binding domain" herein refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen (i.e. DLL3 or a domain of DLL3) to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv, and scFab); single domain antibodies (dAbs); tandem single domain antibodies (sdAbs); and multispecific antibodies formed from antibody fragments. For a review of certain antibody fragments, see Holliger and Hudson, Nature Biotechnology 23:1126-1136 (2005).

The terms "full length antibody", "intact antibody", and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or, in the case of an IgG antibody, having heavy chains that contain an Fc region as defined herein.

The term "multispecific" herein refers to a molecule that can bind to more than one different target or antigen, such as to two or three or more different targets or antigens. The term "bispecific" herein refers to a molecule such as a binding protein or antibody that is able to specifically bind to two different targets or antigens. A "multispecific" or "bispecific" antibody herein may include the appropriate full length heavy and light chains for binding to two different antigens, or it may include appropriate antibody fragments for binding to two different antigens. There are a variety of different platforms for creating DLL3 binding proteins that are compatible with this disclosure.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In certain aspects, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "conjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a drug such as a cytotoxic agent or a label or another protein domain. In some cases, the antibody portion of a "conjugate" is an antigen binding domain.

A "fusion protein" or a "chimeric protein" herein, such as an anti-DLL3 fusion protein refers to a protein that is composed of full length proteins or protein domains from two different proteins that are not normally fused in nature. For example, in some embodiments an anti-DLL3 fusion protein may comprise an anti-DLL3 antigen binding domain or anti-DLL3 antibody and another polypeptide sequence, such as a transmembrane domain, signaling domain, or a second binding domain.

A "cell surface receptor" or "receptor" generally refers to a protein molecule that is expressed on or associated with the surface of a cell and that can bind or be recognized by molecules external to the cell, such as in the extracellular space or on the surface of other cells.

A "chimeric antigen receptor" or "CAR" refers to a fusion protein comprising an extracellular domain comprising an antigen binding domain of an antibody. In some embodiments a CAR comprises an extracellular domain comprising an antigen binding domain of an antibody as well as a transmembrane domain. Thus, in some embodiments, a CAR is intended for expression on the cell surface, for example, to act as a cell surface receptor. In some embodiments the CAR further comprises one or more intracellular signaling domains. In some embodiments, the CAR comprises a hinge between the antigen binding domain and the transmembrane domain. In some embodiments, the CAR comprises a signal peptide, which may be cleaved to form a mature protein. In some cases, a CAR comprise two or more intracellular signaling domains, such as CD3ζ (CD3zeta) (including 1XX CD3zeta and CD3zeta with a Q to K modification at position 14), as well as one or more co-stimulatory domains such as 41BB, OX-40, and/or CD28. In some cases, the antigen binding domain is a single chain antigen binding domain such as an scFv. In some embodiments, the CAR can be combined with a safety switch. In some embodiments, the safety switch comprises a depletion tag (or epitope tag) that is bound by a monoclonal antibody. In some embodiments, a CAR herein may also comprise a variety of architectures, such as bispecific or multispecific antigen binding domains, which may have a variety of configurations. In some cases, a CAR may also comprise further domains in addition to those noted above, for example, for further control of its stability, activity, toxicity, signaling functions, or binding specificity. Further exemplary CAR domains and structures are described elsewhere herein.

A "T cell receptor" or "TCR" is a type of cell surface receptor found on the surface of T cells, which recognizes antigens bound to major histocompatibility complex (MHC) molecules. Certain chimeric antigen receptors or CARs described herein are structurally based on TCRs or may comprise domains found in TCRs, and thus are termed as types of artificial TCRs. For example, an anti-DLL3 antigen binding domain may be fused with one or more components of a

TCR. Thus, as used herein, in some embodiments an anti-DLL3 CAR may encompass an anti-DLL3 TCR.

An "immune cell engager" refers to a fusion protein, such as an anti-DLL3 fusion protein, that is intended to direct immune cells to tumor cells, for example, to aid in killing of tumor cells. For instance, in certain examples, an immune cell engager may comprise antibodies or antigen binding domains that bind to molecules on the surface of both a tumor cell and an immune cell, thus linking the two types of cells. In some embodiments, an immune cell engager may be soluble, i.e., is secreted from the cell that expresses it.

A "hinge" refers to a relatively flexible polypeptide sequence that may be placed between two domains of a protein to allow for greater flexibility.

A "transmembrane domain" refers to a polypeptide domain that traverses a bipolar cellular membrane or intracellular membrane.

A "signaling domain" refers to a polypeptide domain that functions by directly or indirectly influencing the activity or structure of other proteins.

A "safety switch" herein refers to a small molecule or polypeptide that is intended to modulate a CAR, such as to reduce toxicity in vivo, such as, by controlling the activation of the CAR in T cells or other cells expressing the CAR or by controlling downstream effects of the activity of the CAR in T cells or other cells expressing the CAR, such as cytokine release.

A "depletion tag" herein refers to a substance or protein, such as in some cases a membrane-expressed protein or fragment thereof, which allows for cell depletion after administration of an antibody that recognizes that substance or protein.

A "potency enhancing element" or "potency enhancing polypeptide" herein refers to a substance or polypeptide that is capable of enhancing the in vivo persistence of T cells in tumors or the function or activity of T cells in tumors.

The term "nucleic acid molecule" or "polynucleotide" or "nucleic acid construct" herein includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g., complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these

molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples of non-naturally occurring nucleotides include modified nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention *in vitro* and/or *in vivo*, e.g., in a host or patient. Such DNA (e.g., cDNA) or RNA (e.g., mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody *in vivo* (see e.g., Stadler et al, Nature Medicine 2017, published online 12 June 2017, doi:10.1038/nm.4356 or EP 2 101 823 B1).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding" a protein refers to one or more nucleic acid molecules encoding a protein or protein complex, including one or more antibody heavy and light chains of chimeric antigen receptors or antibodies herein (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "vector", as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In some cases a "vector" is comprised within a "particle" herein, which particle may contain in some embodiments non-nucleic acid elements such as lipids or polymers. Examples of particles include, for instance, lipid nanoparticles (LNPs) or virus-like particles (VLPs) and the like, among others described herein.

As used herein, the term "viral vector" refers either to a nucleic acid molecule (e.g., a transfer plasmid) that includes virus-derived nucleic acid elements that generally facilitate

transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will generally include various viral components and sometimes also host cell components in addition to nucleic acid(s). The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. Viral vectors and transfer plasmids contain structural and/or functional genetic elements that are primarily derived from a virus. Viral vectors that can be used in the disclosure include, for example, retrovirus vectors, adenovirus vectors, and adeno-associated virus vectors, lentivirus vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors (see, for example, Gluzman (Ed.), Eukaryotic Viral Vectors, CSH Laboratory Press, Cold Spring Harbor, N.Y.). For example, a recombinant polypeptide as disclosed herein can be produced in a eukaryotic host, such as a mammalian cells (e.g., COS cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, VA). In selecting an expression system, care should be taken to ensure that the components are compatible with one another. Artisans or ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult P. Jones, "Vectors: Cloning Applications", John Wiley and Sons, New York, N.Y., 2009).

As used herein, the term "retroviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. The retroviral vector can be a lentiviral vector. As used herein, the term "lentiviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus, which is a genus of retrovirus. Lentiviral vectors offer several attractive properties as gene-delivery vehicles, including: (i) sustained gene delivery through stable vector integration into host genome; (ii) the capability of infecting both dividing and non-dividing cells; (iii) broad tissue tropisms, including important gene- and cell-therapy-target cell types; (iv) no expression of viral proteins after vector transduction; (v) the ability to deliver complex genetic elements, such as polycistronic or intron-containing sequences; (vi) a potentially safer integration site profile; and (vii) a relatively easy system for vector manipulation and production.

The term "an engineered cell" herein generally refers to a cell into which an exogenous nucleic acid has been introduced, including the progeny of such cells. The terms "host cell", "host cell line", and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells, and which are used to express the product of the introduced nucleic acid, such as a nucleic acid or protein

product. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. References to "a cell" herein include reference to a population of such cells, including their progeny.

The term "percent identity," as used herein in the context of two or more nucleic acids or proteins, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acids that are the same (e.g., about 60% sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. See e.g., the NCBI web site at ncbi.nlm.nih.gov/BLAST. Such sequences are then the to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. This definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Generally, sequence identity can exist over a region that is at least about 20 amino acids or nucleotides in length, or over a region that is 10-100 amino acids or nucleotides in length, or over the entire length of a given sequence

If necessary, sequence identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux et al, Nucleic Acids Res. 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul et al., J. Molecular Biol. 215:403, 1990). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof.

As used herein, the term "mutation" refers to a point mutation, a gene fusion, a substitution, a gain-of-function mutation, a stop-gain mutation, an insertion mutation, a deletion mutation, a duplication mutation and/or a translocation. The mutation may be in one or more genes. The mutation may be naturally occurring. Alternatively, the mutation may be induced or engineered. As used herein, the term "vector" refers to a recombinant polynucleotide construct designed for transfer between host cells, and that may be used for the purpose of transformation, e.g., the introduction of heterologous DNA into a host cell. As such, in some embodiments, the vector can be a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. In some embodiments, the expression vector can be an integrating vector.

By "reduce" or "inhibit" is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. In some embodiments, reduce or inhibit can refer to a relative reduction compared to a reference (*e.g.*, reference level of biological activity or binding). Similarly, by "increase" is meant the ability to cause an overall increase of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. In some embodiments, increase can refer to a relative reduction compared to a reference (*e.g.*, reference level of biological activity or binding). In some embodiments, reduce or increase may refer to reduction of the "level" (i.e. the amount or concentration) of a cell surface protein on a cell, for example.

The term "pharmaceutical composition" or "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the pharmaceutical composition would be administered.

As used herein, the term "pharmaceutically acceptable carrier" means any suitable carriers, diluents or excipients. These include all aqueous and non-aqueous isotonic sterile injection solutions, which may contain anti-oxidants, buffers and solutes, which render the composition isotonic with the blood of the intended recipient; aqueous and non-aqueous sterile suspensions, which may include suspending agents and thickening agents, dispersion media, antifungal and antibacterial agents, isotonic and absorption agents and the like. It will be understood that compositions of the present disclosure may also include other supplementary physiologically active agents. The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the subject.

As used herein, the terms "administration" and "administering" refer to the delivery of a bioactive composition or formulation by an administration route including, but not limited to, oral, intravenous, intra-arterial, intramuscular, intraperitoneal, subcutaneous, intramuscular, and topical administration, or combinations thereof. The term includes, but is not limited to, administering by a medical professional and self-administering. As used herein, the term "injection" includes intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion.

The term "cancer" generally refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features.

Cancer cells can be in the form of a tumor, but such cells can exist alone within an animal subject, or can be a non-tumorigenic cancer cell, such as a leukemia cell. These terms include a solid tumor, a soft tissue tumor, or a metastatic lesion. As used herein, the term "cancer" includes premalignant, as well as malignant cancers. In some embodiments, the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

As used herein, and unless otherwise specified, a "therapeutically effective" or "pharmaceutically effective" amount or number of a subject construct, nucleic acid, cell, or composition of the disclosure generally refer to an amount or number sufficient for a construct, nucleic acid, cell, or composition to accomplish a stated purpose relative to the absence of the composition, e.g., to provide a therapeutic benefit in the treatment or management of the cancer, or to delay or minimize one or more symptoms associated with the cancer. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapeutic agents, which provides a therapeutic benefit in the treatment or management of the cancer. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of the cancer, or enhances the therapeutic efficacy of another therapeutic agent. An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The exact amount of a composition including a "therapeutically effective amount" will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Pickar, Dosage Calculations (1999); and Remington: The Science and Practice of Pharmacy, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins)

An "individual" or "subject" or "patient" herein is a human unless otherwise specified. In some embodiments, a "subject" or "individual" is a patient under the care of a physician. Thus, the subject can be a human patient or an individual who has, is at risk of having, or is suspected of having a disease of interest (e.g., cancer) and/or one or more symptoms of the disease. The subject can also be an individual who is diagnosed with a risk of the condition of interest at the time of diagnosis or later. In some cases, where specified, an "individual" or "subject" is a non-human mammal or includes non-human mammals (e.g. "a mammalian subject" or a "non-human mammal subject"). Mammals include, but are not limited to, domesticated animals (e.g., cows,

sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats).

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some aspects, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

An "effective amount" of an agent, e.g., a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

Headings herein, e.g., (a), (b), (i) etc., are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented. It is appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub- combination was individually and explicitly disclosed herein.

Further definitions are provided in the sections that follow.

II. EXEMPLARY DLL3 BINDING PROTEINS

In one aspect, the disclosure herein concerns proteins binding to delta-like ligand 3 (DLL3). In certain aspects, the molecule may be an antigen binding domain of an antibody. In some embodiments, the DLL3 binding protein binds to the epidermal growth factor (EGF)-like repeat 6 (EGF6) domain of DLL3. In some cases, the DLL3 binding protein is a fusion protein

comprising such an antigen binding domain. For example, in some cases, the fusion protein may comprise one or more of a linker sequence, transmembrane domain, hinge domain, and one or more intracellular signaling domains. In some cases, the DLL3 binding protein is a chimeric antigen receptor (CAR), or the like, such as a T cell receptor (TCR). In some cases, the DLL3 binding protein is an immune cell engager. Exemplary and nonlimiting CAR, TCR, and immune cell engager architectures are discussed below.

A. Exemplary Anti-DLL3 Antigen Binding Domains

Embodiments herein include, for example, a DlL3 binding protein, which comprises an anti-DLL3 antigen binding domain. In some embodiments, the anti-DLL3 antigen binding domain binds to the EGF6 domain of DLL3. In some embodiments, the anti-DLL3 antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6. The above SEQ ID NOs: 1-6 represent Kabat CDR sequences, for example. In some embodiments, the anti-DLL3 antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154. CDR sequences of SEQ ID NO: 149-154 represent Martin "enhanced Chothia" CDR sequences. In some embodiments, the anti-DLL3 antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a

CDRL3 comprising the amino acid sequence of SEQ ID NO: 160. CDR sequences of SEQ ID NOs: 155-160 represent CDR sequences identified by structural modeling. In some embodiments, the anti-DLL3 antigen binding domain comprises a VH comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 7, and/or a VL comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 8 or 148. In some embodiments, the anti-DLL3 antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 and/or a VL comprising the amino acid sequence of SEQ ID NO: 8 or 148. In some embodiments, the anti-DLL3 antigen binding domain comprises a VH with an amino acid consisting of the sequence of SEQ ID NO: 7 and/or a VL with an amino acid sequence consisting of SEQ ID NO: 8 or 148. In some cases, the anti-DLL3 binding domain comprises a signal sequence. In other cases, it does not comprise a signal sequence.

In certain aspects, the antigen binding domain is a single domain antibody (sdAb), a fragment antigen binding moiety (Fab), an Fv, a single chain Fv (scFv), a tandem scFv (bivalent and/or bispecific), tandem single domain antibody (sdAb), or a bi-specific Fab. In certain aspects, a single chain antigen binding domain is a camelid single-domain antibody, such as a VHH or nanobody. In some embodiments, the single chain antigen binding domain is an scFv

Single chain antibodies such as scFv, for example in some cases, may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (VL and VH). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, Prot. Eng. 10:423; Kortt et al., 2001, Biomol. Eng. 18:95-108). By combining different VL- and VH-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, Biomol. Eng. 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879; Ward et al., 1989, Nature 334:544, and de Graaf et al., 2002, Methods Mol. Biol. 178:379-87. In some instances, scFvs can be prepared according to methods described in, for example, Bird et al, (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883).

In some embodiments, an anti-DLL3 antigen binding domain may be comprised within a chimeric antigen receptor (CAR).

B. Anti-DLL3 Chimeric Antigen Receptors (CARs)

In some embodiments, the present disclosure encompasses CARs comprising an extracellular domain comprising an anti-DLL3 antigen binding domain, such as an anti-DLL3 antigen binding domain as described above. In some embodiments, the CAR comprises an anti-DLL3 antibody fused to a CAR scaffold. In some embodiments, the anti-DLL3 CAR binds to the EGF6 domain of DLL3. In some embodiments, the anti-DLL3 CAR comprises an antigen binding domain as described above, and further comprises one or more of a linker sequence, e.g., as described above, a hinge domain, a transmembrane domain, one or more intracellular signaling domains, or a combination thereof. In some embodiments, the anti-DLL3 CAR comprises each of an antigen binding domain as described above, and further comprises one or more of a linker sequence, e.g., as described above, a hinge domain, a transmembrane domain, one or more intracellular signaling domains. In some embodiments, the CAR may also comprise a signal sequence.

The disclosed anti-DLL3 CARs further comprise a linker sequence or a functional fragment thereof. In some embodiments, the linker sequence comprises a Whitlow linker, a (G4S)n linker, or SG4S.

The disclosed anti-DLL3 CARs further comprise a hinge domain, or a functional fragment thereof. In some embodiments, the hinge domain comprises a CD8a hinge domain, a CD28 hinge domain, an IgG4 hinge domain, an IgG4 hinge-CH2-CH3 domain, or a variant thereof.

The disclosed anti-DLL3 CARs further comprise a transmembrane domain, or a functional fragment thereof. In some embodiments, the transmembrane domain is a CD8, CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRγ, TCRδ, TCRζ, CD32, CD64, CD5, CD9, CD22, CD33, CD37, CD38, CD40, CD45, CD64, CD80, CD86, CD137, CD154, LFA-1 T cell co-receptor, CD2 T cell co-receptor/adhesion molecule, CD4OL/CD154, VEGFR2, FAS, FcεRI, or FGFR2B transmembrane domain, or a variant thereof. Thus, in some embodiments, an anti-DLL3 CAR may be intended to be expressed on the cell surface, and thus, for instance, to act as a cell surface receptor.

The disclosed anti-DLL3 CARs may further comprise an intracellular domain comprising one or more of an activation domain and/or a costimulatory signaling domain (or a costimulatory domain). In some embodiments, the intracellular domain comprises a sequence encoding an activation domain. In some embodiments, the intracellular domain comprises a costimulatory

signaling domain. In some embodiments, the intracellular domain comprises an activation domain and a costimulatory signaling domain. In some embodiments, the intracellular domain comprises an intracellular signaling domain of CD28, 41BB (CD137), OX-40 (CD134), CD2, CD7, CD27, CD30, CD40, PD-1, ICOS, LFA-1 (CDIla/CD18), CD3gamma, CD3delta, CD3epsilon, CD3zeta, 1.xxCD3zeta, CD247, CD276 (B7-H3), LIGHT, NKG2C, Ig alpha (CD79a), DAP-10, an Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, an integrin, a Signaling Lymphocytic Activation Molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, B7-H3, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CARD11, CD4, CD8alpha, CD8beta, IL-2Rbeta, IL-2R gamma, IL-7R alpha, ITGA4, VLAl, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 1d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 lb, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGLl, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, or a CD83 ligand costimulatory domain, or any combination thereof.

1. Linker sequences

In some embodiments, the anti-DLL3 CAR comprises a linker sequence. For instance, single chain antibody binding domains such as scFvs can, in some cases, be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker to assist with interchain folding, to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, e.g., Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference. In some cases, an scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions.

In some embodiments, the linker sequence comprises any naturally occurring amino acid. In some embodiments, the linker sequence comprises a Whitlow linker, a (G4S)n linker, where n = 0, 1, 2, 3, 4, 5, or 6, or SG4S linker. Certain exemplary linker sequences that may be included in a CAR herein are provided at SEQ ID Nos: 16, 38, or 40-60. In some embodiments, a linker is included between VH and VL domains of an scFv. In some cases, a linker is included between a transmembrane domain and an intracellular domain, as described below. In some

cases, the linker comprises a G4S linker with 3 repeats (G4S)3. In some cases, the linker comprises the sequence of SEQ ID NO: 16.

2. Hinge domains

In some embodiments, the anti-DLL3 CAR described herein further comprises a hinge domain. The hinge domain in some cases is located between the antigen-binding region and the transmembrane domain, if present, or between the antigen binding domain and a subsequent domain. A hinge domain in some embodiments permits the movement of one or both of the antigen-binding region and transmembrane domain (or alternatively a subsequent domain) relative to each other. In some embodiments, the hinge domain comprises from about 10 to about 100 amino acids, e.g., from about 15 to about 75 amino acids, from about 20 to about 50 amino acids, or from about 30 to about 60 amino acids. In some embodiments, the hinge domain is a hinge domain of a naturally occurring protein.

In some embodiments, the hinge domain may comprise a CD8a hinge domain, a CD28 hinge domain, an IgG4 hinge domain, an IgG4 hinge-CH2-CH3 domain, including a variant thereof. In some embodiments, the hinge domain comprises an amino acid sequence of any one of SEQ ID Nos: 18 or 101-106. In some embodiments, the hinge domain is a CD8a hinge domain. In some embodiments, the hinge domain comprises the amino acid sequence of SEQ ID NO: 18.

3. Transmembrane domains

In some embodiments, the anti-DLL3 CAR described herein further comprises a transmembrane domain. In some embodiments, the transmembrane domain is located after the antigen binding domain, in some cases wherein a hinge domain is located between the antigen binding domain and the transmembrane domain. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane section, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane domain was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR e.g., in one embodiment, the transmembrane domain may be from the same protein that a hinge domain, an intracellular domain, and/or a costimulatory domain, for example. In another aspect, the transmembrane domain is not derived from the same protein that any other domain of the CAR. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to

avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the cell surface of a CAR-expressing cell. In a different aspect, the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell. In some cases, the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein, such as a human protein. The transmembrane domain may be derived, for example in some embodiments, from CD8, CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRγ, TCRδ, TCRζ, CD32, CD64, CD5, CD9, CD22, CD33, CD37, CD38, CD40, CD45, CD64, CD80, CD86, CD137, CD154, LFA-1 T cell co-receptor, CD2 T cell co-receptor/adhesion molecule, CD4OL/CD154, VEGFR2, FAS, FcεRI, or FGFR2B, including variants thereof. In some embodiments, the transmembrane domain used in a CAR comprises a CD8 transmembrane domain. In some embodiments, the transmembrane domain comprises the amino acid sequence of any one of SEQ ID NO: 20 or 107-109. In some embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the transmembrane domain may be recombinant, in which case it can in some embodiments comprise predominantly hydrophobic residues such as leucine and valine. In one aspect, a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

Optionally, a further linker sequence may be found between the intracellular portion of the transmembrane domain and an intracellular domain. In some embodiments, the linker sequence comprises a Whitlow linker, a (G4S)n linker, where n = 0, 1, 2, 3, 4, 5, or 6, or SG4S linker. Certain exemplary linker sequences that may be included in a CAR herein are provided at SEQ ID Nos: 16, 38, or 40-60.

4. Intracellular domains

In some embodiments, the anti-DLL3 CARs described herein further comprise one or more intracellular domains. In some cases, the intracellular domain comprises an intracellular signaling domain. In some cases, the intracellular signaling domain is capable of signaling when the CAR is bound to a target. In some embodiments of the present disclosure having an

intracellular signaling domain, such a domain can comprise, e.g., one or more of an activation domain and/or a costimulatory domain. In some embodiments, the intracellular signaling domain comprises a sequence encoding an activation domain. In some embodiments, the intracellular signaling domain comprises a costimulatory domain. In some embodiments, the intracellular signaling domain comprises an activation domain and a costimulatory domain.

As noted above, optionally, a further linker sequence may be found between the intracellular portion of the transmembrane domain and the start of the intracellular domain. In some embodiments, the linker sequence comprises a Whitlow linker, a (G4S)n linker, where n = 0, 1, 2, 3, 4, 5, or 6, or SG4S linker. Certain exemplary linker sequences that may be included in a CAR herein are provided at SEQ ID Nos: 16, 38, or 40-60. In some cases, a further linker, or a further hinge domain, such as described above, may also be found in between two intracellular domains of a CAR.

In some embodiments, the intracellular domain comprises an intracellular signaling domain of CD28, 41BB (CD137), OX-40 (CD134), CD2, CD7, CD27, CD30, CD40, PD-1, ICOS, LFA-1 (CDlla/CD18), CD3gamma, CD3delta, CD3epsilon, CD3zeta, 1.xxCD3zeta, CD247, CD276 (B7-H3), LIGHT, NKG2C, Ig alpha (CD79a), DAP-10, an Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, an integrin, a Signaling Lymphocytic Activation Molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, B7-H3, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CARD11, CD4, CD8alpha, CD8beta, IL-2Rbeta, IL-2R gamma, IL-7R alpha, ITGA4, VLAl, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 1d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 lb, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGLl, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a CD83 ligand, or any combination thereof, including variants thereof. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain of CD3zeta, 1xxCD3zeta, CD3zeta Q14K, CD28, 4-1BB, or OX-40, or any combination thereof, including variants thereof. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain of CD3zeta, such as comprising the amino acid sequence of SEQ ID NO: 24, 62, or 63, or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of

SEQ ID NO: 24, 62, or 6. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain of 4-1BB and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the intracellular signaling domain comprises (a) an intracellular signaling domain of CD3zeta, and/or comprises the amino acid sequence of SEQ ID NO: 24, 62, or 63 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24, 62, or 63, and further comprises (b) an intracellular signaling domain of 41BB, and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

Activation domains

In some embodiments, the anti-DLL3 CARs comprise an intracellular signaling domain, which is an activation domain. The activation domain is generally responsible for activation of at least one of the normal effector functions of a cell. The term "effector function" describes a specialized function of a cell. For example, the effector function of a T-cell or an NK cell includes a cytolytic activity or helper activity. "Activation domain" describes the portion of a protein which transduces the effector function signal and directs the cell to perform its specialized function. While the entire activation domain can be employed, in many cases it is not necessary to use an entire chain or domain. To the extent that a truncated portion of the activation domain is used, such truncated portion may be used in place of the intact domain as long as it transduces the effector function signal.

An activation domain can, in some embodiments, promote the activation of a T cell receptor (TCR) complex. Such activation domains may comprise immunoreceptor tyrosine-based activation motifs (ITAMs). Activation domains containing ITAMs, for example, include the intracellular signaling domains of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. In some embodiments, an activation domain comprises the intracellular signaling domain of CD3zeta, 1xxCD3zeta, CD3zeta Q14K, or CD28.

Costimulatory domains

In some embodiments, the disclosed anti-DLL3 CARs comprise a costimulatory domain. Examples of costimulatory domains for use in the chimeric receptors are cytoplasmic signaling domains of costimulatory proteins such as B7/CD28 family (B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7, BTLA/CD272, CD28, CTLA-4,

Gi24/VISTA/B7-H5, ICOS/CD278, PD-1, PD-L2/B7-DC, and PDCD6); members of the TNF superfamily (4-1BB/TNFSF9/CD137, 4-1BB ligand/TNFSF9, BAFF/BlyS/TNFSF13B, BAFF R/TNFRSF13C, CD27/TNFRSF7, CD27 ligand/TNFSF7, CD30/TNFRSF8, CD30 ligand/TNFSF8, CD40/TNFRSF5, CD40/TNFSF5, CD40 ligand/TNFSF5, DR3/TNFRSF25, GITR/TNFRSF18, GITR ligand/TNFSF18, HVEM/TNFRSF14, LIGHT/TNFSF14, lymphotoxin-alpha/TNF-beta, OX40/TNFRSF4, OX40 ligand/TNFSF4, RELT/TNFRSF19L, TACI/TNFRSF13B, TL1A/TNFSF15, TNF-α, and TNF RII/TNFRSF1B); members of the interleukin-1 receptor/toll-like receptor (TLR) superfamily (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and TLR10); members of the SLAM family (2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, and SLAM/CD150); CD2, CD7, CD53, CD82/Kai-1, CD90/Thy1, CD96, CD160, CD200, CD300a/LMIR1, HLA Class I, HLA-DR, ikaros, integrin alpha 4/CD49d, integrin alpha 4 beta 1, integrin alpha 4 beta 7/LPAM-1, LAG-3, TCL1A, TCL1B, CRTAM, DAP10, DAP12, MYD88, TRIF, TIRAP, TRAF, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, TIM-1/KIM-1/HAVCR, TIM-4, TSLP, TSLP R, lymphocyte function associated antigen-1 (LFA-1), or NKG2C. In some embodiments, the costimulatory domain comprises an intracellular signaling domain of an activating receptor protein such as α4β1 integrin, β2 integrins (CD11a-CD18, CD11b-CD18, CD11b-CD18), CD226, CRTAM, CD27, NKp46, CD16, NKp30, NKp44, NKp80, CARD11, NKG2D, KIR-S, CD100, CD94/NKG2C, CD94/NKG2E, NKG2D, PEN5, CEACAM1, BY55, CRACC, Ly9, CD84, NTBA, 2B4, SAP, DAP10, DAP12, EAT2, FcRγ, CD3ζ, or ERT. In some embodiments, the costimulatory domain comprises an intracellular signaling domain of an inhibitory receptor protein such as KIR-L, LILRB1, CD94/NKG2A, KLRG-1, NKR-P1A, TIGIT, CEACAM, SIGLEC 3, SIGLEC 7, SIGLEC9, or LAIR-1. In some embodiments, the costimulatory domain comprises an intracellular signaling domain of CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83. In some embodiments, the costimulatory domain comprises an intracellular signaling domain of 41BB (CD137).

5. Signal Sequences

In some embodiments, the anti-DLL3 CAR comprises a signal sequence, for example, to direct the protein to the cellular membrane. In some embodiments, the signal sequence comprises a CD8, CD8α, CD28, CD16, an IgK signal sequence, or a GMCSFR-alpha signal sequence, including variants thereof. In some embodiments, the signal sequence comprises a CD8alpha signal sequence, an IgK signal sequence, or a GMCSFR-alpha signal sequence. In

some embodiments, the signal sequence comprises the amino acid sequence of any one of SEQ ID Nos: 12, 74, or 75. In some embodiments, the signal sequence comprises a CD8α signal sequence, such as that comprising SEQ ID NO: 12.

6. Depletion Tags

In some embodiments, the anti-DLL3 CAR may further comprise a depletion tag. In some embodiments, the depletion tag comprises an epitope recognized by an antibody. In some embodiments, the depletion tag comprises an epitope recognized by a monoclonal antibody. In some embodiments, the epitope recognized by the monoclonal antibody comprises a CD20 epitope, an RSV protein F epitope, an EGF receptor epitope, a PD-1 epitope, a CD52 epitope, a CCR4 epitope, or a QBEND-10 epitope, a combination thereof, including variants thereof. In some embodiments, the epitope recognized by the monoclonal antibody comprises any one of SEQ ID Nos: 64-73. In some embodiments, the anti-DLL3 CAR comprising an epitope recognized by a monoclonal antibody is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 162, or is encoded by a nucleic acid sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 163.

C. Exemplary CAR Architectures

In some embodiments, the anti-DLL3 CAR may have any one of a number of different CAR architectures, such as a single-chain CAR, a multi-chain CAR, a single-targeted CAR, a multi-targeted CAR, a bivalent tandem CAR, a bivalent loop CAR, a multicistronic CAR, a bicistronic CAR, a synNotch-CAR circuit, a synthetic intramembrane proteolysis receptor (SNIPR), a multi-chain DAP-CAR, a TREM1/DAP12 CAR, a DAP12/TREM1 CAR, or a dimerizing agent regulated immune-receptor complex (DARIC). An anti-DLL3 CAR may also have one of a number of different T cell receptor (TCR) architectures or other cell surface receptor architectures, such as a T cell receptor fusion construct (TruC), an HLA-independent T cell (HIT) receptor, a synthetic T cell receptor and antigen receptor (STAR), a rapamycin inducible TCR, a rapamycin inducible Fc receptor, or a constitutive TCR-like receptor. In some cases, the CAR is a dimerizing agent regulated immune-receptor complex (DARIC), an antibody tethered orthogonal multiplexing compatible (ATOMIC), a synNotch-CAR circuit, a synthetic intramembrane proteolysis receptor (SNIPR), a rapamycin inducible Fc receptor, a multi-chain DAP-CAR, a TREM1/DAP12 CAR, or a DAP12/TREM1 CAR. Some of these exemplary architectures are described below, and in later sections of the disclosure referring to engineered cells.

Bispecific (Tandem) CARs

In some embodiments, the CAR is a bispecific CAR or tandem CAR. In some embodiments, the CAR is a bispecific CAR or tandem CAR comprising an antigen binding domain that binds a second target protein.

In some embodiments, a bispecific CAR may comprise, from N-terminus to C-terminus, a signal sequence, a first antigen binding domain (*e.g.,* an scFv), a linker, a second antigen binding domain (*e.g.,* an scFv), a hinge domain, a transmembrane domain, a costimulatory domain, and an activation domain. In some embodiments, the first antigen binding domain is an anti-DLL3 antigen binding domain. In some embodiments, the second antigen binding domain is an anti-DLL3 antigen binding domain.

In some embodiments, the second target protein is or comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17,

PAX3, androgen receptor, cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, or the extracellular portion of the APRIL protein, including any variants thereof. In some cases, the second target protein is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB. In some cases, the second target protein is or comprises TROP2, SSTR2, or GD2.

In other cases, the second target protein is DLL3, and thus, the anti-DLL3 CAR has two binding domains that bind to DLL3, such as different epitopes of DLL3. In some cases, the second target protein is or comprises DLL3, and the CAR binds to a second epitope on DLL3 comprising the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

Multi-Chain CARs

In some embodiments, the CAR is a multi-chain CAR, in which extracellular ligand binding and intracellular signaling domains are provided on different transmembrane polypeptides. The different transmembrane polypeptides comprising a multi-chain CAR, once assembled together, can, in some embodiments, specifically bind to one or several ligand(s) in a target and induce activation of engineered cells in which they are expressed and, for instance, provoke an immune response. In some embodiments, the multi-chain CAR comprises at least one transmembrane polypeptide comprising at least one extracellular antigen binding domain (e.g., anti-DLL3), and one transmembrane polypeptide comprising at least one intracellular signaling domain.

Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell more specific to targets, a multi-chain CAR can, in some cases, comprise several extracellular antigen binding domains, to simultaneously bind different target antigens. In some embodiments, the extracellular antigen binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In other embodiments, the different extracellular antigen binding domains can be placed on different transmembrane polypeptides.

In some embodiments, wherein the multi-chain CAR comprises several extracellular antigen binding domains, the multi-chain CAR comprises an anti-DLL3 antigen binding domain and at least one further antigen binding domain binding a second target protein, such as CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a,

CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4l, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, or the extracellular portion of the APRIL protein, including variants thereof. In some cases, the second target protein is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB. In some cases, the second target protein is or comprises TROP2, SSTR2, or GD2.

In other cases, the second target protein is DLL3, and thus, the anti-DLL3 CAR has two binding domains that bind to DLL3, such as different epitopes of DLL3. In some cases, the second target protein is or comprises DLL3, and the CAR binds to a second epitope on DLL3

comprising the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

Dimerizing Agent Regulated Immunoreceptor Complexes (DARICs)

In some embodiments, the anti-DLL3 CAR is a dimerizing agent regulated immunoreceptor complex (DARIC). A DARIC construct, for example, in some cases may comprise an anti-DLL3 CAR comprising an anti-DLL3 antigen binding domain, and optionally a linker sequence, hinge domain, and/or transmembrane domain, wherein interaction with an intracellular signaling domain occurs via a dimerization or multimerization domain.

Bridging factors in some embodiments mediate or promote the association of an anti-DLL3 CAR with a signaling domain through multimerization domains in the respective CAR and signaling domains. A bridging factor associates with and is disposed between the multimerization domains to promote association of the anti-DLL3 CAR and signaling domains. Thus, in the presence of a bridging factor, the anti-DLL3 CAR and the signaling domains are linked, and the CAR is thus active. For example, in the presence of the bridging factor, the DARIC may initiate immune effector cell activity against a target cell when the anti-DLL3 antigen binding domain binds DLL3 expressed on the target cell. In the absence of a bridging factor, the anti-DLL3 DARIC is inactive.

In particular embodiments, an anti-DLL3 CAR component of the DAIRC and an intracellular signaling domain component each comprise a cognate pair of multimerization domains. In some embodiments, the multimerization domains comparise FKBP and FKBP12-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYRl-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1). In certain embodiments, the multimerization domains associate with a bridging factor selected from rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

Properties of Anti-DLL3 CARs

In some embodiments, when expressed in engineered cells, such as T cells, an anti-DLL3 CAR herein has one or more properties described in the Examples herein. For example, in some embodiments, engineered cells, such as T cells, expressing an anti-DLL3 CAR herein, when co-cultured with small cell lung carcinoma (SCLC) cells, cause the engineered cell to express both IFNgamma and IL-2. In some cases, when the engineered cells, such as T cells, are co-cultured with DMS273, NCI-H82, and/or SHP77 cells, expression of IFNgamma and IL-2 is higher than

similar experiments performed with an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in the amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36 (Comp 1, 2, 3, 4, or 5 of the Examples). In some cases, the engineered cells, such as T cells, when co-cultured with DLL3 negative cells, do not produce cytokines. In some embodiments, engineered cells expressing the anti-DLL3 CAR, such as T cells, when administered to a SCLC murine tumor model, promote tumor regression and/or increased survival compared to an untransduced control and/or compared to administration of engineered cells expressing a CAR comprising the anti-DLL3 binding sequences present in the amino acid sequence of SEQ ID NO: 36 (Comp 2).

D. Exemplary Immune Cell Receptor or T Cell Receptor Architectures

In some embodiments, an anti-DLL3 CAR has an architecture derived from immune cell receptors, such as T cell receptors, and thus is a type of TCR or similar cell surface receptor. Certain examples of such architectures include, for instance, HIT and STAR, among others.

HIT

In some embodiments, an anti-DLL3 CAR is an HLA independent T cell receptor (HIT). For instance, in one method of generating an HIT molecule, the heavy chain variable region (VH) of the anti-DLL3 CAR antigen binding domain may be fused to a TCR beta domain constant region (Cβ) to form a VH-Cβ polypeptide. Similarly, the light chain variable region of the anti-DLL3 CAR antigen binding domain may be fused to a TCR alpha domain constant region (Cα) to form a VL-Cα polypeptide. In some embodiments, this engineered VH-Cβ and VL-Cα polypeptide construct can be expressed as part of a TCR, for example, by inserting a nucleic acid construct encoding it under the control of an endogenous TCRα promoter and poly-A element. For example, the nucleic acid construct, in some embodiments, may be inserted into a TRAJ locus in a cell or a TRAC locus in a cell. (*See, e.g.* J. Mansilla-Soto et al., *Nature Medicine*, 28(2): 345-351 (2022).)

STAR

In some embodiments, an anti-DLL3 CAR is a synthetic TCR and antigen receptor (STAR) molecule. A STAR, for example, also comprises antigen binding VH and VL domains of an antibody, e.g., an anti-DLL3 antibody, and TCR alpha and beta chain constant regions, which are then fused to a co-stimulatory domain, such as those provided herein in the sub-section above. (*See* J. Wang et al., *Am. J. Hematol.* 97(8): 992-1004 (2022).) Like other TCR-based CARs a STAR construct interacts with CD3 subunits to form a TCR-like complex similar to a native TCR complex. In some embodiments, a co-stimulatory domain may be included, for example a co-stimulatory domain as described in the section above. For instance, such a domain

may be fused to the C-terminal portion of the STAR, for instance, with an intervening transmembrane domain and/or linker.

I. Exemplary Anti-DLL3 Immune Cell Engager Molecules

In another aspect, the present disclosure pertains to soluble DLL3 binding proteins, such as soluble anti-DLL3 fusion proteins. In some embodiments, the anti-DLL3 binding protein is an immune cell engager. In some embodiments, the anti-DLL3 binding protein is a T cell engager. In some embodiments, the anti-DLL3 binding protein is a natural killer (NK) cell engager. In some embodiments, the anti-DLL3 immune cell engager molecule comprises an anti-DLL3 antigen binding domain as described above, and optionally comprises a linker domain.

Bispecific T Cell Engagers (BITEs) and Simultaneous Multiple Interaction T Cell Engagers (SMITEs)

In some embodiments, the anti-DLL3 immune cell engager molecule is a bispecific T-cell engager (BITE). BITEs, as described herein, are artificial bispecific monoclonal antibodies that, in some embodiments can be used to bridge cancer cells, e.g., cancer cells expressing DLL3, with T cells.

In some embodiments, a BITE comprises tandem scFv molecules fused by a linker sequence (see e.g. Nagorsen and Bauerle, Exp Cell Res 317, 1255-1260 (2011). In some embodiments, the first scFv molecule may comprise an anti-DLL3 binding domain, e.g., an scFv targeting DLL3. In some embodiments, the second scFv molecule may bind a surface molecule on a T cell. In some embodiments, the surface molecule is a component of the T cell receptor (TCR) complex. In some embodiments, the surface molecule is CD3 or CD28.

In some embodiments, a first BITE targeting a first antigen expressed by a cancer cell (e.g., DLL3) and a first surface molecule on a T cell (e.g., CD3 or CD28) may be provided along with a second BITE targeting a second antigen expressed by the cancer cell and a second surface molecule on the T cell. This approach, termed simultaneous multiple interaction T cell engaging (SMITE), in some embodiments provides for co-activation of T cells. In some embodiments, the first and second BITE may be provided at concentrations where one or both BITEs are inactive when provided separately. In some embodiments, the first or second antigen is DLL-3. In some embodiments, the first or second surface molecule is CD3 or CD28.

Trispecific Killer Engagers (TRIKEs)

In some embodiments, the anti-DLL3 immune cell engager molecule is a trispecific killer engager (TRIKE). TRIKEs, as described herein, include one or more NK cell engager domains, one or more targeting domains, and one or more NK activating domains. In some embodiments, the NK engager domain comprises an antibody or ligand that selectively binds to an NK receptor. In some embodiments, the NK receptor is the cell cytotoxicity receptor 2B4, the low

affinity Fc receptor CD16, the killer immunoglobulin-like receptor (KIR), CD2, NKG2A, TIGIT, NKG2C, LIR-1, or DNAM-1. In some embodiments, the targeting domain comprises an anti-DLL3 antigen binding domain. In some embodiments, the NK activating domain can include an amino acid sequence that activates NK cells, promotes sustaining NK cells, or otherwise promotes NK cell activity. The NK activating domain can be, or can be derived from, one or more cytokines that can activate and/or sustain NK cells. In embodiments that include more than one NK activating domain, the NK activating domains may be provided in series or in any other combination. Additionally, each cytokine-based NK activating domain can include either the full amino acid sequence of the cytokine or may be an amino acid fragment, independent of the nature of other NK activating domains included in the TRIKE molecule. Exemplary cytokines on which an NK activating domain may be based include, for example, IL-15, IL-18, IL-12, and IL-21.

II. Exemplary Nucleic Acid Constructs and Vectors

In another aspect, the present disclosure pertains to a nucleic acid construct encoding a DLL3 binding protein herein, such as an anti-DLL3 antibody or antigen binding domain, an anti-DLL3 CAR, or an anti-DLL3 immune cell engager. In some cases, the nucleic acid construct comprises a polynucleotide sequence encoding the recombinant polypeptide operably linked to a promoter. In some cases, the promoter is a constitutive promoter or an inducible promoter. In some cases, the promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL-2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3

(TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter. In some cases, the promotr is a constitutive promoter, such as a CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, or PIK3R3 promoter. In some cases, the promoter is or comprises an MND promoter or a short EF1a promoter.

In other cases, the construct comprises a polynucleotide sequence encoding the recombinant polypeptide that is not operably linked to a promoter.

In some examples, the polynucleotide contains two, three, or more promoters operatively linked to control expression of the polynucleotide encoding the DLL3 binding protein. In some embodiments, polynucleotide can contain regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the polynucleotide is to be introduced, as appropriate and taking into consideration whether the polynucleotide is DNA- or RNA-based. In some embodiments, the polynucleotide can contain regulatory/control elements, such as a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, internal ribosome entry sites (IRES), a 2A sequence, and splice acceptor or donor. In some embodiments, the polynucleotide can contain a non-native promoter operably linked to the nucleotide sequence encoding the polynucleotide encoding the DLL3 binding protein and/or one or more additional

polypeptide(s). In some embodiments, the promoter is selected from among an RNA pol I, pol II or pol III promoter. In some embodiments, the promoter is recognized by RNA polymerase II (e.g., a CMV, SV40 early region or adenovirus major late promoter). In another embodiment, the promoter is recognized by RNA polymerase III (e.g., a U6 or Hl promoter). In some embodiments, the promoter can be a non-viral promoter or a viral promoter, such as a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus. Other known promoters also are contemplated.

In some embodiments, the promoter is or comprises a constitutive promoter. Exemplary constitutive promoters include, MND promoter, EF1a promoter, sEF1 promoter, gamma retroviral LTR promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, or a CD3z promoter. In some embodiments, the promoter is a minimal TATA promoter, a pGK, actin promoter, CD25 promoter, IL-2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter,CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NF-κB promoter, AP-1 promoter, TNF-alpha promoter, and CD130 promoter, NR4A1 promoter, NR4A2, or NR4A3 promoter. Exemplary constitutive promoters include, e.g., simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter

(CMV), human Ubiquitin C promoter (UBC), human elongation factor la promoter (EFla), mouse phosphoglycerate kinase 1 promoter (PGK), and chicken P-Actin promoter coupled with CMV early enhancer (CAG). In some embodiments, the constitutive promoter is a synthetic or modified promoter. In some embodiments, the promoter is or comprises an MND promoter, a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer (see Challita et al. (1995) J. Virol. 69(2):748-755). In some embodiments, the promoter is a tissue-specific promoter. In another embodiment, the promoter is a viral promoter. In another embodiment, the promoter is a non-viral promoter. In some embodiments, exemplary promoters can include, but are not limited to, human elongation factor 1 alpha (EFla) promoter or a modified form thereof or the MND promoter.

In another embodiment, the promoter is a regulated promoter (e.g., inducible promoter). In some embodiments, the promoter is an inducible promoter or a repressible promoter. In some embodiments, the promoter comprises a Lac operator sequence, a tetracycline operator sequence, a galactose operator sequence or a doxycycline operator sequence, or is an analog thereof or is capable of being bound by or recognized by a Lac repressor or a tetracycline repressor, or an analog thereof. In some embodiments, the polynucleotide does not include a regulatory element, e.g. promoter.

In some cases, the nucleic acid sequence encoding the polynucleotide encoding the DLL3 binding protein contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide, such as the exemplary signal peptide of a GMCSFR alpha chain, CD8, or IgK.

In some cases, the nucleic acid construct is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length. In some cases, the nucleic acid construct is less than about 4.7 kb in length.

In some embodiments, the nucleic acid construct is a DNA construct. In other embodiments, the nucleic acid construct is an RNA construct, such as an mRNA construct. In some cases, the nucleic acid construct comprises one or more modified nucleotides, such as one or more of pseudouridine, N1-methylpseudouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl- pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or any combination thereof. In some cases, the one or more modified nucleotides

are selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, and a combination thereof. In some cases, the one or more modified nucleotides comprise N1- methylpseudouridine.

In some embodiments, the nucleic acid construct comprises a nucleic acid sequence encoding one or more additional polypeptides, e.g., one or more marker(s) and/or one or more effector molecules. In some embodiments, the one or more marker(s) includes a transduction marker, a surrogate marker and/or a selection marker. Among additional nucleic acid sequences introduced, e.g., encoding for one or more additional polypeptide(s), include nucleic acid sequences that encode a second chimeric antigen receptor or heterologous TCR, nucleic acid sequences that can improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; nucleic acid sequences to provide a genetic marker for selection and/or evaluation of the cells, such as to assess in vivo survival or localization; nucleic acid sequences to improve safety, for example, by making the cell susceptible to negative selection in vivo as described by Lupton S. D. et al., Mol. and Cell Biol., 11:6 (1991); and Riddell et al., Human Gene Therapy 3:319-338 (1992); see also WO 1992008796 and WO 1994028143 describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker, and US Patent No. 6,040,177.

In some embodiments, polynucleotides can be engineered as a bicistronic unit containing an IRES, which allows coexpression of gene products (e.g. encoding the recombinant receptor and the additional polypeptide) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (e.g. encoding the marker and encoding the recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (e.g., 2A sequences) or a protease recognition site (e.g., furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A) or after translation, is processed into the individual proteins. In some cases, the peptide, such as a T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (see, e.g., de Felipe, Genetic Vaccines and Ther. 2:13 (2004) and de Felipe et al. Traffic 5:616-626 (2004)). Various 2A elements are known. Examples of 2A sequences that can be used in the methods and system disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), Thosea asigna virus (T2A), and porcine teschovirus-1 (P2A) as described in U.S. Patent Pub. No. 20070116690.

In some cases, the nucleic acid construct further comprises one or more nucleic acid elements, which may also be referred to as nucleic acid control sequences. In some cases, the one or more nucleic acid elements is or comprises ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, a 70 bp poly(A) tract, a 100 bp poly(A) tract, a 172 bp poly(A) tract, a 200 bp poly(A) tract, a 300 bp poly(A) tract, a 325 bp poly(A) tract, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, cleavable linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or any combination thereof.

In some cases, the nucleic acid construct further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or one or more non-coding RNA. In some cases, the non-coding RNA comprises a shRNA or a microRNA. In some such cases, the nucleic acid construct further comprises a polynucleotide sequence encoding one or more cleavable linkers, such as a P2A, E2A, F2A, or T2A self-cleaving peptide. For example, a cleavable linker may allow the translated polypeptide species to separate after being produced from one long open reading frame. In some cases, the polynucleotide sequence encoding the recombinant polypeptide and the polynucleotide sequence encoding the one or more additional polypeptides are separated by an internal ribozyme entry site (IRES).

In some cases, the one or more additional polypeptides comprise a second chimeric antigen receptor (CAR), a recombinant T cell receptor (TCR), a potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, a safety switch, or any combination thereof. In some cases, the second CAR or recombinant TCR binds to a target antigen selected from CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3,

LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combinations thereof. In some cases, the second CAR or recombinant TCR binds to TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB. In some cases, the second CAR or recombinant TCR binds to TROP2, SSTR2, or GD2. In some cases, the second CAR or recombinant TCR binds to DLL3. In some such cases, the second CAR or recombinant TCR binds to the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

In some cases, wherein the second polypeptide encoded by the nucleic acid construct is a potency enhancement polypeptide, the potency enhancement polypeptide is or comprises a patient derived CARD11-PIK3R3 fusion, an engineered CARD11-PIK3R3 fusion (e.g., comprising the amino acid sequence of any one of SEQ ID NO: 114, 116, 118, or 120), a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (e.g., TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch

receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, CD8alpha/beta, a combination thereof, or variants thereof.

In some embodiments, a nucleic acid construct may be organized from 5' to 3' according to one of the following designs:

(1) a promoter, and (2) a polynucleotide encoding the DLL3 binding protein (i.e., an anti-DLL3 antigen binding domain or antibody, an anti-DLL3 CAR, or an anti-DLL3 T cell enhancer);

(1) a promoter, (2) a polynucleotide encoding the DLL3 binding protein, (3) a cleavable linker encoding sequence, and (4) a polynucleotide encoding one or more additional polypeptides;

(1) a promoter, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, and (4) a polynucleotide encoding the DLL3 binding protein;

(1) a promoter, (2) a polynucleotide encoding the DLL3 binding protein, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding one or more additional polypeptides, (5) a cleavable linker encoding sequence, and (6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor;

(1) a promoter, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding the DLL3 binding protein, (5) a cleavable linker encoding sequence, and (6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor;

(1) a promoter, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (5) a cleavable linker encoding sequence, and (6) a polynucleotide encoding the DLL3 binding protein;

(1) a promoter, (2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding one or more additional polypeptides (5) a cleavable linker encoding sequence, and (6) a polynucleotide encoding the DLL3 binding protein:

(1) a promoter, (2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding the DLL3 binding protein, (5) a cleavable linker

encoding sequence, and (6) a polynucleotide encoding one or more additional polypeptides:

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding the DLL3 binding protein, and (3) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding the DLL3 binding protein, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding one or more additional polypeptides, and (5) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding the DLL3 binding protein, and (5) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding the DLL3 binding protein, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding one or more additional polypeptides, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (7) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding the DLL3 binding protein, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding one or more additional polypeptides, and (7) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding the DLL3 binding protein, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and (7) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding one or more additional polypeptides, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding the DLL3 binding protein, and (7) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding the DLL3 binding protein, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding one or more additional polypeptides, and (7) a cleavable linker encoding sequence;

(1) a cleavable linker encoding sequence, (2) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide encoding one or more additional polypeptides, (5) a cleavable linker encoding sequence, (6) a polynucleotide encoding the DLL3 binding protein, and (7) a cleavable linker encoding sequence.

In some cases, multiple nucleic acid constructs may be used to express different polypeptides or nucleic acids. Examples include compositions comprising: (1) a first polynucleotide encoding the DLL3 binding protein, and (2) a second polynucleotide encoding one or more additional polypeptides, optionally wherein the first and the second polynucleotides are both RNA constructs;

(1) a first polynucleotide encoding the DLL3 binding protein, (2) a second polynucleotide sequence encoding one or more additional polypeptides, and (3) a third polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, optionally wherein one or more of the polynucleotides is an RNA construct; or

(1) a polynucleotide encoding the DLL3 binding protein, (2) a second nucleic acid construct encoding one or more additional polypeptides, and (3) a third nucleic acid construct encoding a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and optionally wherein the first, second, and/or third polynucleotides are RNA constructs.

In some embodiments, one or more nucleic acid constructs herein may be comprised within one or more vectors. In some cases, a vector may further be comprised within a particle.

The vector can include, but is not limited to, viral vectors, plasmid DNA, and closed-end DNA. Viral vectors can include, but are not limited to, adenoviral vectors, lentiviral vectors, retroviral vectors, adeno-associated viral vectors, vaccinia viruses, poxviruses, and herpes simplex viruses, or any of the viruses described elsewhere herein. Commonly, expression vectors contain selection markers such as ampicillin-resistance, hygromycin-resi stance, tetracycline resistance, kanamycin resistance, or neomycin resistance to permit detection of those cells transformed with the desired DNA sequences. Suitable vectors, promoter, and enhancer elements are known in the art; many are commercially available for generating subject recombinant constructs. In some embodiments, the vector is a polycistronic vector. In some embodiments, the vector is a bicistronic vector or a tricistronic vector. Bicistronic or multicistronic expression vectors may include (1) multiple promoters fused to each of the open reading frames; (2) insertion of splicing signals between genes; (3) fusion of genes whose expressions are driven by

a single promoter; and (4) insertion of proteolytic cleavage sites between genes (self-cleavage peptide) or insertion of internal ribosomal entry sites (IRESs) between genes.

A polycistronic vector is used to co-express multiple genes in the same cell. Two strategies are most commonly used to construct a multi cistronic vector. First, an Internal Ribosome Entry Site (IRES) element is typically used for bi-cistronic vectors. The IRES element, acting as another ribosome recruitment site, allows initiation of translation from an internal region of the mRNA. Thus, two proteins are translated from one mRNA. IRES elements are quite large (usually 500-600 bp) (Pelletier et al., 1988; Jang et al., 1988).

In some embodiments, the polycistronic construct comprises two expression cassettes, i.e., is bicistronic. In some embodiments, the polycistronic construct comprises three expression cassettes, i.e., is tricistronic. In some embodiments, the polycistronic construct comprises four expression cassettes, i.e., is quadcistronic. In some embodiments, the polycistronic construct comprises more than four expression cassettes. In any of these embodiments, each of the expression cassettes comprises a nucleotide sequence encoding a protein of interest (e.g., a DLL3 binding protein and/or an anti-DLL3 CAR or T cell engager). In certain embodiments, the two or more genes being expressed are under the control of a single promoter and are separated from one another by one or more cleavage sites to achieve co-expression of the proteins of interest from one transcript. In other embodiments, the two or more genes may be under the control of separate promoters. As the name suggests, a polycistronic construct allows simultaneous expression of two or more separate proteins from one mRNA transcript in a host cell. Cleavage sites can be used in the design of a polycistronic construct to achieve such co-expression of multiple genes.

In some embodiments, the one or more cleavage sites comprise one or more self-cleaving sites. The second strategy relies on “self-cleaving” 2A peptides. These peptides, first discovered in picomaviruses, are short (about 20 amino acids) and produce equimolar levels of multiple genes from the same mRNA. The term "self-cleaving" is not entirely accurate, as these peptides are thought to function by making the ribosome skip the synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (Kim et al., 2011). The "cleavage" occurs between the glycine and proline residues found on the C-terminus. Thus, the upstream cistron will have a few additional residues added to the end, while the downstream cistron will start with the proline. There are four 2A peptides commonly employed in molecular biology, T2A, P2A, E2A, and F2A, the sequences of which are summarized below. A glycine-serine-glycine (GSG) linker is optionally added to the

N-terminal of a 2A peptide to increase cleavage efficiency. The use of "()" around a sequence in the present disclosure means that the enclosed sequence is optional.

Sequences of 2A Peptides

(GSG) EGRGSLLTCGDVEENPGP – T2A – SEQ ID NO: 79 (with GSG) or 80 (without GSG)

(GSG) ATNFSLLKQAGDVEENPGP – P2A – SEQ ID NO: 76 (with GSG) or 77 (without GSG)

(GSG) QCTNYALLKLAGDVESNPGP – E2A – SEQ ID NO: 82 (with GSG) or 83 (without GSG)

(GSG) VKQTLNFDLLKLAGDVESNPGP – F2A – SEQ ID NO: 85 (with GSG) or 86 (without GSG)

In some embodiments, the one or more cleavage sites additionally comprise one or more protease sites. The one or more protease sites can either precede or follow the self-cleavage sites (e.g., 2 A sites) in the 5' to 3' order. The protease site may be cleaved by a protease after translation of the full transcript or after translation of each expression cassette such that the first expression product is released prior to translation of the next expression cassette. In these embodiments, having a protease site in addition to the 2A site, especially preceding the 2A site in the 5' to 3' order, may reduce the number of extra amino acid residues attached to the expressed proteins of interest. In some embodiments, the protease site comprises a furin site, also known as a Paired basic Amino acid Cleaving Enzyme (PACE) site. There are at least three furin cleavage sequences, FC1, FC2, and FC3, the amino acid sequences of which are summarized below. Similar to the 2A sites, one or more optional glycine-serine-glycine (GSG) sequences can be included for cleavage efficiency.

Sequences of Furin Sites

RRRR (GSG) – FC1 – SEQ ID NO: 140 (with GSG) or 141 (without GSG)

RKRR (GSG) – FC2 – SEQ ID NO: 142 (with GSG) or 143 (without GSG)

RKRR (GSG) TPDPW (GSG) – FC3 – SEQ ID NOs: 144-147 (with or without one or both GSG sequences)

In some embodiments, the one or more cleavage sites comprise one or more self-cleaving sites, one or more protease sites, and/or any combination thereof. For example, the cleavage site can include a 2A site alone. For another example, the cleavage site can include a FC2 or FC3 site, followed by a 2A site. In these embodiments, the one or more self-cleaving sites may be the same or different. Similarly, the one or more protease sites may be the same or different.

In some embodiments, the polycistronic construct may be in the form of a vector. Any type of vector suitable for introduction of nucleotide sequences into a host cell can be used, including,

for example, plasmids, adenoviral vectors, adenoviral-associated vectors, retroviral vectors, lentiviral vectors, phages, and homology-directed repair (HDR)-based donor vectors.

In some embodiments, the vector herein is a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule, including into the cell or into the genome of a cell. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. In some embodiments, the vector herein is a plasmid. In some embodiments, the vector herein is a DNA vector. In some embodiments, the vector herein is a closed linear DNA vector. In some embodiments, the vector herein is a phagemid vector. In some embodiments, the vector herein is a synthetic DNA vector. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, e.g., replication defective retroviruses and lentiviruses. Non-viral vectors may require a delivery vehicle to facilitate entry of the nucleic acid molecule into a cell.

A viral vector can comprise a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). A viral vector can comprise, e.g., a virus or viral particle capable of transferring a nucleic acid into a cell, or to the transferred nucleic acid (e.g., as naked DNA). Viral vectors and transfer plasmids can comprise structural and/or functional genetic elements that are primarily derived from a virus. A retroviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus.

In some embodiments, the vector is a viral vector, such as a retroviral vector. In some embodiments, the polynucleotide encoding the DLL3 binding protein are introduced into the cell via retroviral or lentiviral vectors, or via transposons (see, e.g., Baum et al. (2006) Molecular Therapy: The Journal of the American Society of Gene Therapy. 13:1050-1063; Frecha et al. (2010) Molecular Therapy 18:1748-1757; and Hackett et al. (2010) Molecular Therapy 18:674-683).

In some vectors described herein, at least part of one or more protein coding regions that contribute to or are essential for replication may be absent compared to the corresponding wild-type virus. This makes the viral vector replication-defective. In some embodiments, the vector is

capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

In some embodiments, the vector is a retroviral vector. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector is a VSV-G pseudotyped lentivirus. In some embodiments, the vector is an AAV vector. In some embodiments, the vector is an AAV6 vector. In some embodiments, the vector is an AAV9 vector. In some embodiments, the vector is a split-intein dual AAV vector. In some embodiments, the vector is a redirected lentiviral vector. In some embodiments, the vector is a fusosome. In some embodiments, the vector is a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein. In some embodiments, the vector is an enveloped delivery vehicle. In some embodiments, the vector is a self-replicating RNA virus. In some embodiments, the vector is an mRNA-packaging virus-like particle. In some embodiments, the vector is RNP-packaging virus-like particle. In some embodiments, the vectors used in the context of this disclosure are the vectors described in WO2023193015A1; WO2022164935A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." Cell (2022); Hamilton, Jennifer R., et al. "In vivo human T cell engineering with enveloped delivery vehicles." Nature Biotechnology (2024): 1-9; Lundstrom, Kenneth. "Self-replicating RNA viruses for RNA therapeutics." Molecules 23.12 (2018): 3310.

In some embodiments, the retroviral nucleic acid comprises one or more of (e.g., all of): a 5' promoter (e.g., to control expression of the entire packaged RNA), a 5' LTR (e.g., that includes R (polyadenylation tail signal) and/or U5 which includes a primer activation signal), a primer binding site, a psi packaging signal, a RRE element for nuclear export, a promoter directly upstream of the transgene to control transgene expression, a transgene (or other exogenous agent element), a polypurine tract, and a 3' LTR (e.g., that includes a mutated U3, a R, and U5). In some embodiments, the retroviral nucleic acid further comprises one or more of a cPPT, a WPRE, and/or an insulator element (e.g., as described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," Biomedicines, 4(1):4 (2016)).

A retrovirus typically replicates by reverse transcription of its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. The structure of a wild-type retrovirus genome often comprises a 5' long terminal repeat (LTR) and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome and gag, pol and env genes encoding the packaging components which promote the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated

provirus from the nucleus to the cytoplasm of an infected target cell. In the provirus, the viral genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are involved in proviral integration and transcription. LTRs also serve as enhancer/promoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

The LTRs themselves are typically similar (e.g., identical) sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For the viral genome, the site of transcription initiation is typically at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses comprise any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: tot, rev, tax and rex.

With regard to the structural genes gag, pol and env themselves, gag encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The pol gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The env gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction promotes infection, e.g., by fusion of the viral membrane with the cell membrane.

In a replication-defective retroviral vector genome gag, pol and env may be absent or not functional. The R regions at both ends of the RNA are typically repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively. Retroviruses may also contain additional genes which code for proteins other than gag, pol and env. Examples of additional genes include (in HIV), one or more of vif, vpr, vpx, vpu, tat, rev and nef. EIAV has (amongst others) the additional gene S2.

Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV),

gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV) and human immunodeficiency virus (HIV).

In some embodiments the retrovirus is a Gammretrovirus. In some embodiments the retrovirus is an Epsilonretrovirus. In some embodiments the retrovirus is an Alpharetrovirus. In some embodiments the retrovirus is a Betaretrovirus. In some embodiments the retrovirus is a Deltaretrovirus. In some embodiments the retrovirus is a Spumaretrovirus. In some embodiments the retrovirus is an endogenous retrovirus. In some embodiments the retrovirus is a lentivirus.

In some embodiments, a retroviral or lentivirus vector further comprises one or more insulator elements, e.g., an insulator element described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," Biomedicines, 4(1):4 (2016). In various embodiments, the vectors comprise a promoter operably linked to a polynucleotide encoding an exogenous agent. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one of more accessory elements to increase transduction efficiency (e.g., a cPPT/FLAP), viral packaging (e.g., a Psi (Y) packaging signal, RRE), and/or other elements that increase exogenous gene expression (e.g., poly (A) sequences), and may optionally comprise a WPRE or HPRE. In some embodiments, a lentiviral nucleic acid comprises one or more of, e.g., all of, e.g., from 5' to 3', a promoter (e.g., CMV), an R sequence (e.g., comprising TAR), a U5 sequence (e.g., for integration), a PBS sequence (e.g., for reverse transcription), a DIS sequence (e.g., for genome dimerization), a psi packaging signal, a partial gag sequence, an RRE sequence (e.g., for nuclear export), a cPPT sequence (e.g., for nuclear import), a promoter to drive expression of the exogenous agent, a gene encoding the exogenous agent, a WPRE sequence (e.g., for efficient transgene expression), a PPT sequence (e.g., for reverse transcription), an R sequence (e.g., for polyadenylation and termination), and a U5 signal (e.g., for integration).

Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In some embodiments, HIV based vector backbones (i.e., HIV cis-acting sequence elements) are used. A lentivirus vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus.

In embodiments, a lentivirus vector (e.g., lentiviral expression vector) may comprise a lentiviral transfer plasmid (e.g., as naked DNA) or an infectious lentiviral particle. With respect to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, etc., it is to be understood that the sequences of these elements can be present in RNA form in lentiviral particles and can be present in DNA form in DNA plasmids.

In embodiments, a lentivirus vector is a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell can comprise reverse transcription and integration into the target cell genome. The recombinant lentivirus vector (RLV) typically carries non-viral coding sequences which are to be delivered by the vector to the target cell. In embodiments, an RLV is incapable of independent replication to produce infectious retroviral particles within the target cell. Usually the RLV lacks a functional gag-pol and/or env gene and/or other genes involved in replication. The vector may be configured as a split-intron vector, e.g., as described in PCT patent application WO 99/15683, which is herein incorporated by reference in its entirety.

In some embodiments, the lentivirus vector comprises a minimal viral genome, e.g., the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell, e.g., as described in WO 98/17815, which is herein incorporated by reference in its entirety.

A minimal lentiviral genome may comprise, e.g., (5')R-U5-one or more first nucleotide sequences-U3-R(3'). However, the plasmid vector used to produce the lentiviral genome within a source cell can also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a source cell. These regulatory sequences may comprise the natural sequences associated with the transcribed retroviral sequence, e.g., the 5' U3 region, or they may comprise a heterologous promoter such as another viral promoter, for example the CMV promoter. Some lentiviral genomes comprise additional sequences to promote efficient virus production. For example, in the case of HIV, rev and RRE sequences may be included.

In some embodiments, the vector can be a vector of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), or the pEX series (Clontech, Palo Alto, Calif.). In some cases, bacteriophage vectors, such as ZG I O. XGT11, XZapII (Stratagene), XEMBL4, and ZNM I 149, also can be used. In some embodiments, plant

expression vectors can be used and include pBIOl, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). In some embodiments, animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech).

In some embodiments, the vector (e.g., a viral vector) is a double stranded DNA vector. In some embodiments, the DNA vector is a closed-end vector. In some embodiments, the closed-end vector is Doggybone™ DNA (dbDNA). Closed-end DNA vectors, including dbDNA, are known in the art and have been described in e.g., Karda et al., Gene Ther (2019) 26:86-92.

In some cases, a vector may be used that does not require that the cells, e.g., T cells, are activated. In some such instances, the cells may be selected and/or transduced prior to activation. Thus, the cells may be engineered prior to, or subsequent to culturing of the cells, and in some cases at the same time as or during at least a portion of the culturing.

In some embodiments, the cells are activated prior to introduction of the polynucleotide or vector. In some embodiments, the T cells are incubated with e.g., anti-CD3/anti-CD28 antibodies. In some embodiments, the T cells are incubated with apololipoprotein E (ApoE) prior to, during, and/or subsequent to introduction of the polynucleotide or vector. For example, in some instances, the T cells are incubated with ApoE (e.g. ApoE4) prior to incubation with LNPs containing the polynucleotide or a composition thereof. In some embodiments, the T cells are incubated with about 1 pg/mL ApoE4 prior to incubation with LNPs containing the polynucleotide or a composition thereof.

III. DELIVERY OF NUCLEIC ACIDS

Introduction of the nucleic acid molecules or amino acid molecules of the disclosure into cells can be achieved by methods known to those skilled in the art such as, for example, viral infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, nucleofection, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro-injection, nanoparticle-mediated nucleic acid delivery, naked nucleic acid delivery, as nucleic acid complexed with materials such as a liposome, nanoparticle or poloxamer, or can be delivered by viruses (e.g., adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)), and the like. Introduction of the nucleic acid molecules or amino acid molecules of the disclosure into cells can be ex vivo or in vivo.

In some embodiments, introducing the polynucleotides encoding the engineered polypeptides described herein into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate, lipid-mediated transfection, electroporation,

nucleofection, fusogens, transposons (e.g., a Sleeping Beauty system), and transduction or infection using a viral vector, as discussed herein. In some embodiments, the polynucleotides are introduced into a cell via viral transduction (e.g., AAV transduction, lentiviral transduction) or otherwise delivered on a viral vector (e.g., fusogen-mediated delivery). In some embodiments, the polynucleotides are introduced into a cell via a fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mosl transposons, and conditional or inducible Tol2 transposons.

The process of introducing the nucleic acid molecule into the cell can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, and transduction or infection using a viral vector. In some embodiments, the nucleic acid molecule comprises DNA. In some embodiments, the nucleic acid molecule comprises a modified DNA. In some embodiments, the nucleic acid molecule comprises RNA. In some embodiments, the nucleic acid molecule comprises mRNA. In some embodiments, the nucleic acid molecule comprises a modified mRNA.

In some embodiments, the nucleic acid molecules can be delivered by conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (2012, supra) and other standard molecular biology laboratory manuals, such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, nucleoporation, hydrodynamic shock, and infection.

In some embodiments, one or more polynucleotide(s) are introduced into a cell using electroporation (see, e.g., Chicaybam et al, (2013) PLoS ONE 8(3): e60298 and Van Tedeloo et al. (2000) Gene Therapy 7(16): 1431-1437). For examples, in some embodiments, one or more RNA molecules is introduce into a T cell using electroporation. Methods and systems for electroporation are known in the art, including e.g. Nucleofection® Technology (Lonza). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (see, e.g., Manuri et al. (2010) Hum Gene Ther 21(4): 427-437; Sharma et al. (2013) Molec Ther Nucl Acids 2, e74; and Huang et al. (2009) Methods Mol Biol 506: 115-126). Other methods of introducing and expressing genetic material, e.g., polynucleotides and/or vectors, into immune cells include calcium phosphate transfection (e.g., as described in Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.), protoplast fusion, cationic liposome-mediated

transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., Mol. Cell Biol., 7: 2031-2034 (1987) and other approaches described in, e.g., International Pat. App. Pub. No. WO 2014055668, and U.S. Patent No. 7,446,190.

In some embodiments, the nucleic acid molecules can be delivered by viral or non-viral delivery vehicles known in the art. For example, the nucleic acid molecule can be stably integrated in the host genome, or can be episomally replicating, or present in the recombinant host cell as a mini-circle expression vector for transient expression. Accordingly, in some embodiments, the nucleic acid molecule is maintained and replicated in the recombinant host cell as an episomal unit. In some embodiments, the nucleic acid molecule is stably integrated into the genome of the recombinant cell. Stable integration can be achieved using classical random genomic recombination techniques or with more precise techniques such as guide RNA-directed CRISPR/Cas9 genome editing, or DNA-guided endonuclease genome editing with NgAgo (Natronobacterium gregoryi Argonaute), or TALENs genome editing (transcription activator-like effector nucleases). In some embodiments, the nucleic acid molecule is present in the recombinant host cell as a mini-circle expression vector for transient expression.

In some embodiments, the nucleic acid molecules can be encapsulated in a viral capsid or a lipid nanoparticle, or can be delivered by viral or non-viral delivery means and methods known in the art, such as electroporation. For example, introduction of nucleic acids into cells may be achieved by viral transduction. In a non-limiting example, adeno-associated virus (AAV) is engineered to deliver nucleic acids to target cells via viral transduction. Several AAV serotypes have been described, and all of the known serotypes can infect cells from multiple diverse tissue types. AAV is capable of transducing a wide range of species and tissues *in vivo* with no evidence of toxicity, and it generates relatively mild innate and adaptive immune responses.

In some embodiments, the DLL3 binding protein is delivered using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self- inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide.

In some embodiments, a lentiviral vector can be used for delivery. Lentiviral-derived vector systems are useful for nucleic acid delivery and gene therapy via viral transduction. Lentiviral vectors offer several attractive properties as gene-delivery vehicles, including: (i) sustained gene delivery through stable vector integration into host genome; (ii) the capability of infecting both dividing and non-dividing cells; (iii) broad tissue tropisms, including important

gene- and cell-therapy-target cell types; (iv) no expression of viral proteins after vector transduction; (v) the ability to deliver complex genetic elements, such as polycistronic or intron-containing sequences; (vi) a potentially safer integration site profile; and (vii) a relatively easy system for vector manipulation and production.

Methods of lentiviral transduction are known. Exemplary methods are described in, e.g., Wang et al. (2012) J. Immunother. 35(9): 689-701; Cooper et al. (2003) Blood. 101:1637-1644; Verhoeyen et al. (2009) Methods Mol Biol. 506: 97-114; and Cavalieri et al. (2003) Blood. 102(2): 497-505. In some embodiments, the polynucleotide encoding the recombinant receptor and/or one or more additional polypeptide(s), is introduced into a population containing cultured cells, such as by retroviral transduction, transfection, or transformation.

In some embodiments, a recombinant adeno-associated virus (AAV) vector can be used for delivery. Techniques to produce rAAV particles, in which an AAV genome to be packaged that includes the polynucleotide to be delivered, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (e.g., not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes can be from any AAV serotype for which recombinant virus can be derived, and can be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, international patent application publication number WO 01/83692.

In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a retrovirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a VSV-G pseudotyped lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV6. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV9. I In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a split-intein dual AAV. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is

delivered with a redirected lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a fusosome. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a enveloped delivery vehicle. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a self-replicating RNA virus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an mRNA-packaging virus-like particle. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an RNP-packaging virus-like particle. In some embodiments, the delivery used in the context of this disclosure are described in WO2023193015A1; WO2022164935A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." *Cell* (2022); Hamilton, Jennifer R., et al. "In vivo human T cell engineering with enveloped delivery vehicles." *Nature Biotechnology* (2024): 1-9; Lundstrom, Kenneth. "Self-replicating RNA viruses for RNA therapeutics." *Molecules* 23.12 (2018): 3310.

In some embodiments, the nucleic acid is or comprises mRNA encoding the engineered protein of the disclosure. The mRNA may be delivered to the cell using any appropriate technique, such as electroporation or using a lipid nanoparticle (LNP). In some embodiments, the nucleic acid is delivered with a lipid nanoparticle (LNP). In some embodiments, the nucleic acid is delivered with a selective organ targeting (SORT) LNP). In some embodiments, the nucleic acid is delivered with an antibody targeted LNP. In some embodiments, the LNP comprises: (i) an ionizable lipid (e.g., an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (e.g., a PEG-modified lipid). In some embodiments, the nucleic acid is delivered with a polymer nanoparticle. In some embodiments, the nucleic acid is delivered with a protein nanoparticle. In some embodiments, the delivery used in the context of this disclosure are described in WO2024006960A1; WO2019152557A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." *Cell* (2022); Rurik, Joel G., et al. "CAR T cells produced in vivo to treat cardiac injury." *Science* 375.6576 (2022): 91-96.

In some embodiments, the polynucleotides disclosed herein can be delivered or introduced using one or more agent(s), capable of inducing a genetic disruption (e.g., CRISPR/Cas and/or gRNA components), to a cell, using any of a number of known delivery methods or vehicles for introduction or transfer to cells, for example, using viral delivery vectors, or any of the known

methods or vehicles for delivering CRISPR/Cas molecules and gRNAs. Exemplary methods are described in, e.g., Wang et al. (2012) J. Immunother. 35(9): 689-701; Cooper et al. (2003) Blood. 101:1637-1644; Verhoeyen et al. (2009) Methods Mol Biol. 506: 97-114; and Cavalieri et al. (2003) Blood. 102(2): 497- 505. In some embodiments, nucleic acid sequences encoding one or more components of one or more agent(s) capable of inducing a genetic disruption is introduced into the cells, e.g., by any methods for introducing nucleic acids into a cell described herein or known. In some embodiments, a vector encoding components of one or more agent(s) capable of inducing a genetic disruption such as a CRISPR guide RNA and/or a Cas enzyme can be delivered into the cell.

In some embodiments, the one or more agent(s) capable of inducing a genetic disruption (e.g., one or more agent(s) that is a CRISPR/Cas/gRNA), is introduced into the cell as a ribonucleoprotein (RNP) complex. RNP complexes include a sequence of ribonucleotides, such as an RNA or a gRNA molecule, and a protein, such as a Cas9 protein or variant thereof. For example, the Cas9 protein is delivered as RNP complex that comprises a Cas9 protein and a gRNA molecule targeting the target sequence, e.g., using electroporation or other physical delivery method. In some embodiments, the RNP is delivered into the cell via electroporation or other physical means, e.g., particle gun, Calcium Phosphate transfection, cell compression or squeezing. In some embodiments, the RNP can cross the plasma membrane of a cell without the need for additional delivery agents (e.g., small molecule agents, lipids, etc.). In some embodiments, delivery of the one or more agent(s) capable of inducing genetic disruption, e.g., CRISPR/Cas9, as an RNP offers an advantage that the targeted disruption occurs transiently, e.g., in cells to which the RNP is introduced, without propagation of the agent to cell progenies. For example, delivery by RNP minimizes the agent from being inherited to its progenies, thereby reducing the chance of off-target genetic disruption in the progenies. In such cases, the genetic disruption and the integration of transgene can be inherited by the progeny cells, but without the agent itself, which may further introduce off-target genetic disruptions, being passed on to the progeny cells.

Agent(s) and components capable of inducing a genetic disruption (e.g., a CRISPR/Cas molecule and gRNA molecule), can be introduced into target cells in a variety of forms using a variety of delivery methods and formulations, as set forth in Tables 8 and 10, or methods described in, e.g., WO 2015/161276; US 2015/0056705, US 2016/0272999, US 2017/0211075; or US 2017/0016027. As described further herein, the delivery methods and formulations can be used to deliver template polynucleotides and/or other agents to the cell (such as those required for engineering the cells) in prior or subsequent steps of the methods described herein. When a

CRISPR/Cas nuclease or gRNA component is encoded as DNA for delivery, the DNA may typically but not necessarily include a control region, e.g., comprising a promoter, to effect expression. Exemplary promoters for CRISPR/Cas nuclease sequences include, e.g., CMV, EFla, EFS, MSCV, PGK, or CAG promoters. Useful promoters for gRNAs include, e.g., Hl, EF-la, tRNA or U6 promoters. Promoters with similar or dissimilar strengths can be selected to tune the expression of components. Sequences encoding a CRISPR/Cas nuclease molecule may comprise a nuclear localization signal (NLS), e.g., an SV40 NLS. In some embodiments a promoter for a CRISPR/Cas nuclease molecule or a gRNA molecule may be, independently, inducible, tissue specific, or cell specific. In some embodiments, an agent capable of inducing a genetic disruption is introduced RNP complexes.

Table 1: Exemplary Delivery Methods

Elements		**Comments**
Cas9 Molecule(s)	**gRNA molecule(s)**	
DNA	DNA	In this embodiment, a Cas9 molecule and a gRNA are transcribed from DNA. In this embodiment, they are encoded on separate molecules.
DNA		In this embodiment, a Cas9 molecule and a gRNA are transcribed from DNA, here from a single molecule.
DNA	RNA	In this embodiment, a Cas9 molecule is transcribed from DNA, and a gRNA is provided as in vitro transcribed or synthesized RNA
mRNA	RNA	In this embodiment, a Cas9 molecule is translated from in vitro transcribed mRNA, and a gRNA is provided as in vitro transcribed or synthesized RNA.
mRNA	DNA	In this embodiment, a Cas9 molecule is translated from in vitro transcribed mRNA, and a gRNA is transcribed from DNA.
Protein	DNA	In this embodiment, a Cas9 molecule is provided as a protein, and a gRNA is transcribed from DNA.
Protein	RNA	In this embodiment, a Cas9 molecule is provided as a protein, and a gRNA is provided as transcribed or synthesized RNA.

Table 2: Comparison of Exemplary Delivery Methods

Delivery Vector/Mode		**Delivery into Non-Dividing Cells**	**Duration of Expression**	**Genome Integration**	**Type of Molecule Delivered**
Physical (e.g., electroporation, particle gun, Calcium Phosphate transfection, cell compression or squeezing)		YES	Transient	NO	Nucleic Acids and Proteins
Viral	**Retrovirus**	NO	Stable	YES	RNA
	Lentivirus	YES	Stable	YES/NO with modifications	RNA
	Adenovirus	YES	Transient	NO	DNA
	Adeno-Associated Virus (AAV)	YES	Stable	NO	DNA
	Vaccinia Virus	YES	Very Transient	NO	DNA
	Herpes Simplex Virus	YES	Stable	NO	DNA
Non-Viral	**Cationic Liposomes**	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
	Polymeric Nanoparticles	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
Biological Non-Viral Delivery Vehicles	**Attenuated Bacteria**	YES	Transient	NO	Nucleic Acids
	Engineered Bacteriophages	YES	Transient	NO	Nucleic Acids
	Mammalian Virus-like Particles	YES	Transient	NO	Nucleic Acids
	Biological liposomes: Erythrocyte Ghosts and Exosomes	YES	Transient	NO	Nucleic Acids

In some embodiments, the polynucleotide encoding the engineered proteins of the disclosure can be delivered into cells by known methods or as described herein. For example, by vectors (e.g., viral or non-viral vectors), non-vector based methods (e.g., using naked DNA or DNA complexes), or a combination thereof. In some embodiments, the polynucleotide containing the agent(s) and/or components thereof is delivered by a vector (e.g., viral vector/virus or plasmid). The vector may be any described herein.

In some embodiments, the polynucleotide encoding the engineered proteins of the disclosure is delivered by a non-vector based method (e.g., using naked DNA or DNA

complexes). For example, the DNA or RNA or proteins or combination thereof, e.g., ribonucleoprotein (RNP) complexes, can be delivered, e.g., by organically modified silica or silicate (Ormosil), electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Lett 12: 6322-27, Kollmannsperger et al (2016) Nat Comm 7, 10372), gene gun, sonoporation, magnetofection, lipid-mediated transfection, dendrimers, inorganic nanoparticles, calcium phosphates, or a combination thereof.

In some embodiments, delivery via electroporation comprises mixing the cells with the polynucleotide encoding the engineered proteins of the disclosure or RNP complex in a cartridge, chamber or cuvette and applying one or more electrical impulses of defined duration and amplitude. In some embodiments, delivery via electroporation is performed using a system in which cells are mixed with the DNA in a vessel connected to a device (e.g., a pump) which feeds the mixture into a cartridge, chamber or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel.

In some embodiments, the delivery vehicle is a non- viral vector. In some embodiments, the non-viral vector is an inorganic nanoparticle. Exemplary inorganic nanoparticles include, e.g., magnetic nanoparticles (e.g., FesMnCh) and silica. The outer surface of the nanoparticle can be conjugated with a positively charged polymer (e.g., polyethylenimine, polylysine, polyserine) which allows for attachment (e.g., conjugation or entrapment) of payload. In some embodiments, the non-viral vector is an organic nanoparticle. Exemplary organic nanoparticles include, e.g., SNALP liposomes that contain cationic lipids together with neutral helper lipids which are coated with polyethylene glycol (PEG), and protamine-nucleic acid complexes coated with lipid. Exemplary lipids and polymers for gene transfer include those described in, for example, WO 2019/195492 and WO 2020/223535.

In some embodiments, the vehicle has targeting modifications to increase target cell update of nanoparticles and liposomes, e.g., cell specific antigens, monoclonal antibodies, single chain antibodies, aptamers, polymers, sugars, and cell penetrating peptides. In some embodiments, the vehicle uses fusogenic and endosome-destabilizing peptides/polymers. In some embodiments, the vehicle undergoes acid-triggered conformational changes (e.g., to accelerate endosomal escape of the cargo). In some embodiments, a stimulus-cleavable polymer is used, e.g., for release in a cellular compartment. For example, disulfide-based cationic polymers that are cleaved in the reducing cellular environment can be used.

In some embodiments, the delivery vehicle is a biological non-viral delivery vehicle. In some embodiments, the vehicle is an attenuated bacterium (e.g., naturally or artificially engineered to be invasive but attenuated to prevent pathogenesis and expressing the transgene

(e.g., Listeria monocytogenes, certain Salmonella strains, Bifidobacterium longum, and modified Escherichia coli), bacteria having nutritional and tissue- specific tropism to target specific cells, bacteria having modified surface proteins to alter target cell specificity). In some embodiments, the vehicle is a genetically modified bacteriophage (e.g., engineered phages having large packaging capacity, less immunogenicity, containing mammalian plasmid maintenance sequences and having incorporated targeting ligands). In some embodiments, the vehicle is a mammalian virus-like particle. For example, modified viral particles can be generated (e.g., by purification of the "empty" particles followed by ex vivo assembly of the virus with the desired cargo). The vehicle can also be engineered to incorporate targeting ligands to alter target tissue specificity. In some embodiments, the vehicle is a biological liposome. For example, the biological liposome is a phospholipid-based particle derived from human cells (e.g., erythrocyte ghosts, which are red blood cells broken down into spherical structures derived from the subject (e.g., tissue targeting can be achieved by attachment of various tissue or cell-specific ligands), or secretory exosomes -subject-derived membrane-bound nanovescicles (30 -100 nm) of endocytic origin (e.g., can be produced from various cell types and can therefore be taken up by cells without the need for targeting ligands).

In some embodiments, an RNA polynucleotide encoding the engineered proteins of the disclosure can be delivered into cells, e.g., target cells described herein, by known methods or as described herein. For example, RNA can be delivered, e.g., by microinjection, electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Let 12: 6322-27), lipid-mediated transfection, peptide-mediated delivery, e.g., cell-penetrating peptides, or a combination thereof.

In some embodiments, delivery via electroporation comprises mixing the cells with the RNA in a cartridge, chamber or cuvette and applying one or more electrical impulses of defined duration and amplitude. In some embodiments, delivery via electroporation is performed using a system in which cells are mixed with the RNA encoding CRISPR/Cas nuclease molecules and/or gRNA molecules in a vessel connected to a device (e.g., a pump) which feeds the mixture into a cartridge, chamber or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel.

In some embodiments, engineered polypeptide molecules can be delivered into cells by known methods or as described herein. For example, protein molecules can be delivered, e.g., by microinjection, electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Let 12: 6322-27), lipid-mediated transfection, peptide-mediated delivery,

or a combination thereof. Delivery can be accompanied by DNA encoding a polypeptide (e.g., an engineered polypeptide, gRNA, CAR, TCR, etc.).

In some embodiments, the exogenous Cas protein can be introduced into the cell in polypeptide form. In certain embodiments, the Cas proteins can be conjugated to or fused to a cell-penetrating polypeptide or cell-penetrating peptide. As used herein, "cell-penetrating polypeptide" and "cell-penetrating peptide" refers to a polypeptide or peptide, respectively, which facilitates the uptake of molecule into a cell. The cell-penetrating polypeptides can contain a detectable label.

In many embodiments, Cas proteins can be conjugated to or fused to a charged protein (e.g., that carries a positive, negative or overall neutral electric charge). Such linkage may be covalent. In some embodiments, the Cas protein can be fused to a superpositively charged GFP to significantly increase the ability of the Cas protein to penetrate a cell (Cronican et al. ACS Chem Biol. 2010; 5(8):747-52). In certain embodiments, the Cas protein can be fused to a protein transduction domain (PTD) to facilitate its entry into a cell. Exemplary PTDs include Tat, oligoarginine, and penetratin. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a cell-penetrating peptide. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a PTD. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a tat domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to an oligoarginine domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a penetratin domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a superpositively charged GFP. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a cell-penetrating peptide. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a PTD. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a tat domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to an oligoarginine domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a penetratin domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a superpositively charged GFP.

In some embodiments, the Cas protein can be introduced into a cell containing the target polynucleotide sequence in the form of a nucleic acid encoding the Cas protein. The process of introducing the nucleic acids into cells can be achieved by any suitable technique. Suitable techniques include those described herein.

IV. Engineered Cells

The disclosure also encompasses an engineered cell that expresses a DLL3 binding protein, such as an anti-DLL3 CAR, anti-DLL3 antigen binding domain or antibody, or anti-DLL3 immune cell engager protein, optionally under the control of an endogenous promoter or optionally under the control of an exogenous promoter. In some embodiments, the engineered cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the cell is *in vivo*. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vitro*. In some embodiments, the engineered cell is a eukaryotic cell. In some embodiments, the engineered cell is an animal cell. In some embodiments, the animal cell is a mammalian cell. In some embodiments, the animal cell is a human cell. In some embodiments, the cell is a non-human primate cell. In some embodiments, the cell is a canine cell. In some embodiments, the cell is a non-natural cell or has been genetically engineered (i.e., an engineered cell). In some embodiments, the cell is a host cell. In some embodiments, the cell is an immune cell. In some embodiments, the polynucleotide is an exogenous nucleic acid. In some embodiments, the mammalian cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell.

In some embodiments, the cell is a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T (NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, and a progenitor cell of the lymphoid lineage. In some embodiments, the cell is selected from the group consisting of a T cell, a macrophage, a monocyte, and a natural killer (NK) cell. In some embodiments, the cell is a T cell. In some embodiments, the T cell is selected from the group consisting of a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, or an engineered T cell comprising a transcriptional receptor.

Sources for the T cells include, but are not limited to, peripheral blood, bone marrow, or other sources of hematopoietic cells. T cells can be isolated by methods well known in the art, including commercially available isolation methods (see, for example, Rowland-Jones et al.,

Lymphocytes: A Practical Approach, Oxford University Press, New York (1999), Su et al., Methods Mol. Biol. 806:287-299 (2012); Bluestone et al., Sci. Transl. Med. 7(315) (doi: 10.1126/scitranslmed.aad4134)(2015); Miyara et al., Nat. Rev. Rheumatol. 10:543-551 (2014); Liu et al., J. Exp. Med. 203:1701-1711 (2006); Seddiki et al., J. Exp. Med. 203:1693-1700 (2006); Ukena et al., Exp. Hematol. 39:1152-1160 (2011); Chen et al., J. Immunol. 183:4094-4102 (2009); Putnam et al., Diabetes 58:652-662 (2009); Putnam et al., Am. Tranplant. 13:3010-3020 (2013); Lee et al., Cancer Res. 71:2871-2881 (2011); MacDonald et al., J Clin. Invest. 126:1413-1424 (2016)).

Various known techniques can be employed to isolate or enrich for desired immune cells such as T cells. If a particular type of T cell is to be isolated, various cell surface markers or combinations of markers, including but not limited to, CD3, CD4, CD8, CD34 (for hematopoietic stem and progenitor cells) and the like, can be used to separate the cells, as is well known in the art (see Kearse, T Cell Protocols: Development and Activation, Humana Press, Totowa N.J. (2000); De Libero, T Cell Protocols, Vol. 514 of Methods in Molecular Biology, Humana Press, Totowa N.J. (2009)) Negative selection methods can be used to remove cells that are not the desired immune cells. Additionally, positive selection methods can be used to isolate or enrich for desired T cells. In some instances, a combination of both positive and negative selection methods can be used.

In some embodiments, the provided modified cells are modified such that they are able to evade immune recognition and responses when administered to a patient (e.g., recipient subject). The cells can evade killing by immune cells in vitro and in vivo. In some embodiments, the cells evade killing by macrophages and NK cells. In some embodiments, the cells are ignored by immune cells or a subject's immune system. In other words, the cells administered in accordance with the methods described herein are not detectable by immune cells of the immune system. In some embodiments, the cells are cloaked and therefore avoid immune rejection.

Methods of determining whether a modified cell provided herein evades immune recognition include, but are not limited to, IFN-y Elispot assays, microglia killing assays, cell engraftment animal models, cytokine release assays, ELISAs, killing assays using bioluminescence imaging or chromium release assay or Xcelligence analysis, mixed-lymphocyte reactions, immunofluorescence analysis, etc. In some embodiments, the immunogenicity of the cells is evaluated in a complement-dependent cytotoxicity (CDC) assay. CDC can be assayed in vitro by incubating cells with IgG or IgM antibodies targeting an HLA-independent antigen expressed on the cell surface in the presence of serum containing complement and analyzing cell killing. In some embodiments, CDC can be assayed by incubating cells with ABO blood type

incompatible serum, wherein the cells comprise A antigens or B antigens, and the serum comprises antibodies against the A antigens and/or B antigens of the cells.

The cell may be a vertebrate cell, for example, a mammalian cell, such as a human cell, a canine cell, or a mouse cell. The cell may also be a vertebrate stem cell, for example, a mammalian stem cell, such as a human stem cell (or a cell derived from such a stem cell), a canine stem cell, or a mouse stem cell (or a cell derived from such a stem cell). In embodiments, the cell or stem cell or a cell derived from such a stem cell is amenable to modification.

In some embodiments, the cells that are engineered as provided herein are cells from a subject that is known or suspected of having a particular disease or condition to be treated.

In some embodiments, the cells that are engineered as provided herein are cells from a healthy subject, such as a subject that is not known or suspected of having a particular disease or condition to be treated. For instance, if T cells are isolated or obtained from a donor subject, such as for treating a cancer, the donor subject is a healthy subject not known or suspected of suffering from cancer or another disease or condition.

The polynucleotide encoding the DLL3 binding protein of the present disclosure can be introduced into a target cell, such as, for example, a human T lymphocyte, to produce a recombinant or engineered cell containing the nucleic acid molecule.

Among the cells expressing the DLL3 binding protein disclosed herein are engineered cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into a composition containing the cells, such as by retroviral transduction, transfection, or transformation.

In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, e.g., myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some

embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, and re introducing them into the same subject, before or after cryopreservation.

In other embodiments, the methods include isolating cells from a donor, preparing, processing, culturing, and/or engineering them, and introducing them into a subject who is not the donor, before or after cryopreservation.

In some embodiments, the cells of the present disclosure include a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T (NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, and a progenitor cell of the lymphoid lineage.

Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naive T (TN) cells, effector T cells (TEFF), memory T cells and sub-types thereof, such as stem cell memory T (TSCM), central memory T (TCM), effector memory T (TEM), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, e.g., myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

Among the sub-types and subpopulations of NK cells are CD56+ bright and CD56+ dim NK cells. CD56+ bright NK cells, known for their high expression of CD56, play a pivotal role in immune regulation and cytokine production, particularly in response to interleukin-12 (IL-12), IL-15, and IL-18. These cells are potent producers of cytokines like interferon-gamma (IFN-γ) but have limited cytotoxic capabilities. In contrast, CD56+ dim NK cells, which express CD56 at lower levels, are highly cytotoxic and specialize in directly killing virus-infected cells and tumor cells. Without wishing to be bound by theory, CD56+ dim NK cells achieve this through the release of granules containing perforin and granzymes. Additionally, there are other NK subsets based on the expression of receptors like NKG2A, NKG2C, and KIRs (Killer-cell Immunoglobulin-like Receptors), which modulate NK cell activity and specificity. These NK subsets demonstrate variations in response to viral infections, tumor surveillance, and interaction with other immune cells.

In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

In some embodiments, the cells are isolated from a biological sample from a patient or donor. In some embodiments, the sample include tissue, fluid, and other samples taken directly from the subject or donor, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g., transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone

marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, dog, and pig.

Primary Cells

In some embodiments, the cell is a primary cell. Primary cells are isolated directly from human or animal tissue using enzymatic or mechanical methods. Once isolated, they are placed in an artificial environment in plastic or glass containers supported with specialized medium containing essential nutrients and growth factors to support proliferation. Primary cells could be of two types: adherent or suspension. Adherent cells require attachment for growth and are said to be anchorage-dependent cells. Adherent cells are usually derived from tissues of organs. Suspension cells do not require attachment for growth and are said to be anchorage independent cells. Most suspension cells are isolated from the blood system, but some tissue- derived cells can also be used in suspension, such as hepatocytes or intestinal cells. Although primary cells usually have a limited lifespan, they offer a number of advantages compared to cell lines. Primary cell culture enables researchers to study donors and not just cells. Several factors such as age, medical history, race, and sex can be considered when building an experimental model. With a growing trend towards personalized medicine, such donor variability and tissue complexity can be achieved with use of primary cells, but are difficult to replicate with cell lines that are more systematic and uniform in nature and do not capture the true diversity of a living tissue.

In some embodiments, the starting material is a primary cell collected from a subject (e.g., a patient). In some embodiments, the starting material is a primary blood cell collected from a subject (e.g., a patient), e.g., via a leukopak. For example, in some embodiments, the starting material are unmodified T cells obtained from a subject (e.g., a patient). In some embodiments, the starting material is a primary cell collected from a donor. In some embodiments, the starting material is a primary blood cell collected from a donor, e.g., via a leukopak. For example, in some embodiments, the starting material are unmodified T cells obtained from a donor. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes.

In some embodiment the cells that are engineered as provided herein comprise cells derived from primary cells obtained or isolated from one or more individual subjects or donors. In some embodiments, the cells are derived from a pool of isolated primary cells obtained from a subject (e.g., the patient administered the cells). In some embodiments, the cells are derived from a pool of isolated primary cells obtained from one or more (e.g., two or more, three or more, four or more, five or more, ten or more, twenty or more, fifty or more, or one hundred or more) different donor subjects. In some embodiments, the primary cells isolated or obtained from the plurality of different donor subjects (e.g., two or more, three or more, four or more, five or more, ten or more, twenty or more, fifty or more, or one hundred or more) are pooled together in a batch and are engineered in accord with the provided methods.

Pluripotent Stem Cells and Stem Cell Derived Cells

In some embodiments, the cell is a stem cell. In some embodiments, the cell is a pluripotent stem cell. In some embodiments, the cell is derived from a pluripotent stem cell. Pluripotent stem cells are cells that have the capacity to self-renew by dividing and to develop into the three primary germ cell layers of the early embryo and therefore into all cells of the adult body, but not extra-embryonic tissues such as the placenta. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are pluripotent stem cells. In some embodiments, the cell is an ESC. ESCs are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. In some embodiments, the cell is an iPSC. iPSCs are derived from adult somatic cells that have been genetically reprogrammed back into a pluripotent state that enables the development of an unlimited source of any type of cell needed for therapeutic purposes.

"Pluripotent stem cells" as used herein have the potential to differentiate into any of the three germ layers: endoderm (e.g., the stomach lining, gastrointestinal tract, lungs, etc.), mesoderm (e.g., muscle, bone, blood, urogenital tissue, etc.) or ectoderm (e.g., epidermal tissues and nervous system tissues). The term "pluripotent stem cells," as used herein, also encompasses ESCs and iPSCs (or iPS cells). In some embodiments, a pluripotent stem cell is produced or generated from a cell that is not a pluripotent cell. In other words, pluripotent stem cells can be direct or indirect progeny of a non-pluripotent cell. Examples of parent cells include somatic cells that have been reprogrammed to induce a pluripotent, undifferentiated phenotype by various means. Such "iPS" or "iPSC" cells can be created by inducing the expression of certain regulatory genes or by the exogenous application of certain proteins. Methods for the induction of iPS cells are known in the art and are further described below. (See, e.g., Zhou et al., Stem Cells 27 (11): 2667-74 (2009); Huangfu et al., Nature Biotechnol. 26 (7): 795 (2008); Woltjen et

al., Nature 458 (7239): 766-770 (2009); and Zhou et al., Cell Stem Cell 8:381-384 (2009); each of which is incorporated by reference herein in their entirety.) As used herein, "hiPSCs" are human induced pluripotent stem cells. In some embodiments, the cell is a mesenchymal stem cell (MSCs).

In some embodiments, the cell is a differentiated cell. In some embodiments, the cell is differentiated from a pluripotent stem cell. Differentiated cells are cells that have undergone differentiation. They are mature cells that perform a specialized function. Some examples of differentiated cells are T cells, NK cells, etc. Generally, these cells have a unique morphology, metabolic activity, membrane potential, and responsiveness to signals facilitating their function in a body tissue or organ.

In some embodiments, the engineered cells described are derived from an ESC or iPSC or a progeny thereof. As used herein, the term "derived from an ESC or iPSC or a progeny thereof" encompasses the initial ESC or iPSC that is generated and any subsequent progeny thereof. As used herein, the term "progeny" encompasses, e.g., a first-generation progeny, i.e., the progeny is directly derived from, obtained from, obtainable from or derivable from the initial ESC or iPSC by, e.g., traditional propagation methods. The term "progeny" also encompasses further generations such as second, third, fourth, fifth, sixth, seventh, or more generations, i.e., generations of cells which are derived from, obtained from, obtainable from or derivable from the former generation by, e.g., traditional propagation methods. The term "progeny" also encompasses modified cells that result from the modification or alteration of the initial ESC or iPSC or a progeny thereof.

In embodiments, the cell or stem cell, or a cell derived from such a stem cell, has or is believed to have therapeutic value, such that the cell or stem cell or a cell derived or differentiated from such stem cell may be used to treat a disease, disorder, defect or injury in a subject in need of treatment for same.

In some embodiments, the cell is a stem cell or progenitor cell (e.g., iPSC, ESC, hematopoietic stem cell, mesenchymal stem cell, endothelial stem cell, epithelial stem cell, adipose stem or progenitor cells, germline stem cells, lung stem or progenitor cells, mammary stem cells, olfactory adult stem cells, hair follicle stem cells, multipotent stem cells, amniotic stem cells, cord blood stem cells, or neural stem or progenitor cells). In some embodiments, the stem cells are adult stem cells (e.g., somatic stem cells or tissue specific stem cells). In some embodiments, the stem or progenitor cell is capable of being differentiated (e.g., the stem cell is totipotent, pluripotent, or multipotent). In some embodiments, the cell is isolated from embryonic or neonatal tissue. In some embodiments, the cell is a fibroblast, monocytic precursor,

B cell, exocrine cell, pancreatic progenitor, endocrine progenitor, hepatoblast, myoblast, preadipocyte, progenitor cell, hepatocyte, chondrocyte, smooth muscle cell, K562 human erythroid leukemia cell line, bone cell, synovial cell, tendon cell, ligament cell, meniscus cell, adipose cell, dendritic cells, or natural killer cell. In some embodiments, the cell is manipulated (e.g., converted or differentiated) into a muscle cell, erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell, endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or brown adipocyte. In some embodiments, the cell is a muscle cell (e.g., skeletal, smooth, or cardiac muscle cell), erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell (e.g., red blood cell, white blood cell, or platelet), endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or white or brown adipocyte. In some embodiments, the cell is a hormone-secreting cell (e.g., a cell that secretes insulin, oxytocin, endorphin, vasopressin, serotonin, somatostatin, gastrin, secretin, glucagon, thyroid hormone, bombesin, cholecystokinin, testosterone, estrogen, or progesterone, renin, ghrelin, amylin, or pancreatic polypeptide), an epidermal keratinocyte, an epithelial cell (e.g., an exocrine secretory epithelial cell, a thyroid epithelial cell, a keratinizing epithelial cell, a gall bladder epithelial cell, or a surface epithelial cell of the cornea, tongue, oral cavity, esophagus, anal canal, distal urethra, or vagina), a kidney cell, a germ cell, a skeletal joint synovium cell, a periosteum cell, a bone cell (e.g., osteoclast or osteoblast), a perichondrium cell (e.g., a chondroblast or chondrocyte), a cartilage cell (e.g., chondrocyte), a fibroblast, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, or a serosal cell (e.g., a serosal cell lining body cavities).

In some embodiments, the cell is a T cell or NK cell that has been differentiated from an engineered ESC or iPSC. In some embodiments, the cell is an engineered cell that has been modified from a primary cell. In some embodiments, the cell comprises increased expression of the DLL3 binding protein disclosed herein.

As will be appreciated by those in the art, there are a variety of different methods for the generation of ESCs and iPCSs. The original induction of iPSCs was done from mouse embryonic or adult fibroblasts using the viral introduction of four transcription factors, Oct3/4, Sox2, c-Myc and Klf4; see Takahashi and Yamanaka Cell 126:663-676 (2006), hereby incorporated by reference in its entirety and specifically for the techniques outlined therein. Since then, a number of methods have been developed; see Seki et al, World J. Stem Cells 7(1): 116-125 (2015) for a

review, and Lakshmipathy and Vermuri, editors, Methods in Molecular Biology: Pluripotent Stem Cells, Methods and Protocols, Springer 2013, both of which are hereby expressly incorporated by reference in their entirety, and in particular for the methods for generating hiPSCs (see for example Chapter 3 of the latter reference).

Generally, iPSCs are generated by the transient expression of one or more reprogramming factors" in the host cell, usually introduced using episomal vectors. Under these conditions, small amounts of the cells are induced to become iPSCs (in general, the efficiency of this step is low, as no selection markers are used). Once the cells are "reprogrammed", and become pluripotent, they lose the episomal vector(s) and produce the factors using the endogeneous genes.

Any of the pluripotent stem cells described herein can be differentiated into any cells of an organism and tissue. In an aspect, provided herein are engineered cells that are differentiated into different cell types from ESCs or iPSCs for subsequent transplantation into recipient subjects. Differentiation can be assayed as is known in the art, generally by evaluating the presence of cell-specific markers. As will be appreciated by those in the art, the differentiated engineered pluripotent cell derivatives can be transplanted using techniques known in the art that depends on both the cell type and the ultimate use of these cells. In some embodiments, the iPSCs are differentiated into cell types selected from T cells, NK cells.

T Cells

In some embodiments, the cell is a T cell. In some embodiments, the T cell comprises a polynucleotide encoding the DLL3 binding protein of the present disclosure. T cells of the disclosure or precursors thereof can be immune cells of the lymphoid lineage. Relevant information concerning T cells as referred to in the context of the present disclosure is known in the art, including certain information regarding desired features of T cells when used for cell therapy. It will be understood that embodiments concerning T cells described herein may be readily and appropriately combined. It will be understood that embodiments concerning T cells described herein may be readily and appropriately combined with other embodiments described herein (for example, but not limited to, the DLL3 binding protein).

The T cells described herein may be used to treat or prevent a disease in a subject.

A T cell is a type of lymphocyte. T cells are one of the white blood cells of the immune system and play a central role in the adaptive immune response. T cells can be derived from T cells in a patient's own blood (autologous) or derived from the T cells of another healthy donor (allogeneic). T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain

embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, e.g., FICOLL™ separation, antibody-conjugated bead-based methods such as MACS™ separation (Miltenyi).

In some embodiments, the T cells can be CD4+ or CD8+ and can include, but are not limited to, regulatory T cells, cytotoxic T cells, T helper cells (CD4+), and memory T cells, including central memory T cells (TCM), stem memory T cells (TSCM), stem-cell-like memory T cells (or stem-like memory T cells), and effector memory T cells such as, for example, TEM cells and TEMRA (CD45RA+) cells, effector T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, Th22 cells, Tfh (follicular helper) cells, T regulatory cells, natural killer T cells, mucosal associated invariant T cells (MAIT), and γδ T cells. Major T cell subtypes include TSCM (stem cell memory), TCM (central memory), TTM (Transitional Memory), TEM (Effector memory), TTE (Terminal Effector) and TN (naive). In some embodiments, the T cells express CCR7, CD27, CD28, and CD45RA. In some embodiments, the central T cells express CCR7, CD27, CD28, and CD45RO. In other embodiments, the effector memory T cells express PD-1, CD27, CD28, and CD45RO. In other embodiments, the effector memory RA T cells express PD-1, CD57, and CD45RA.

In some embodiments, the T cells of the disclosure can be hematopoietic stem and/or progenitor cells of the lymphoid lineage that can differentiate into T cells. Hematopoietic stem and/or progenitor cells can be derived from bone marrow, umbilical cord blood, and adult peripheral blood.

In some embodiments, the T cells as provided herein are primary T cells. In some embodiments, the primary T cells are isolated or obtained from and individual subject or one or more donors, such as one or more healthy donors (e.g., a subject that is not known or suspected of, e.g., not exhibiting clinical signs of, a disease or infection). In some instances, the T cells are populations of primary T cells. In some instances, the T cells are subpopulations or sub-types or subsets of primary T cells. Subpopulations and sub-types and subsets of primary T cells are herein described in further detail below. As will be appreciated by those in the art, methods of isolating or obtaining T lymphocytes from an individual can be achieved using known techniques. Provided herein are engineered primary T lymphocytes that contain modifications (e.g., genetic modifications) described herein for subsequent transplantation or engraftment into subjects (e.g., recipients).

In some embodiments, primary T cells are obtained (e.g., harvested, extracted, removed, or taken) from a subject or an individual. In some embodiments, primary T cells are produced from a pool of T cells such that the T cells are from one or more subjects (e.g., one or more human

including one or more healthy humans). In some embodiments, the pool of primary T cells is from 1-100, 1-50, 1-20, 1-10, 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, or 100 or more subjects. In some embodiments, the donor subject is different from the patient (e.g., the recipient that is administered the therapeutic cells). In some embodiments, the pool of T cells does not include cells from the patient. In some embodiments, one or more of the donor subjects from which the pool of T cells is obtained are different from the patient.

In some embodiments, primary T cells are primary T cells of a T cell subset or a sub-type or subpopulation. Such T cell subsets may be found in US2018/0319862A1 and/or WO20 16/090190A1, the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, the T cell subset, such as a CD62L+ T cell subset, that is increased in subjects upon administration of the genetically engineered cells are or include or share phenotypic characteristics with memory T cells or particular subsets thereof, such as long-lived memory T cells. In some embodiments, such memory T cells are central memory T cells (Tcm) or T memory stem cells (Tsc) cells. In some embodiments, the memory T cells are Tsc cells. Tsc cells may be described as having one or more phenotypic differences or functional features compared to other memory T cell subsets or compared to naive T cells, such as being less differentiated or more naive (see e.g., Ahlers and Belyakov (2010) Blood, 115: 1678); Cieri et al (2015) Blood, 125:2865; Flynn et al. (2014) Clinical & Translational Immunology, 3, e20; Gattinoni et al. (2012) Nat. Med., 17: 1290-1297; Gattinoni et al. (2012) Nat .Reviews, 12:671; Li et al. (2013) PLOS ONE, 8:e67401; and published PCT Appl. No. W02014/039044). In some cases, Tsc cells are thought to be the only memory T cells able to generate effector T cells and all three subsets of memory T cells (Tsc, Tcm, and Tern). In some aspects, Tsc cells have the highest survival and proliferation response to antigenic or homeostatic stimuli of all the memory T cell subsets, and the least attrition absent cognate antigen. In some embodiments, the less - differentiated Tsc cells may exhibit greater expansion, long-term viability, and target cell destruction following adoptive transfer than other memory T cells, and thus may be able to mediate more effective treatment with fewer transferred cells than would be possible for either Tcm or Tern cells.

In some embodiments, the cells as provided herein are T lymphocytes differentiated from engineered pluripotent cells that contain modifications (e.g., genetic modifications) described herein and that are differentiated into T lymphocyte. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. In some

embodiments, the cells differentiated into a T lymphocyte may be used for subsequent transplantation or engraftment into subjects (e.g., recipients).

Methods for generating T cells from pluripotent stem cells (e.g., iPSC) are described, for example, in Iriguchi et al., Nature Communications 12, 430 (2021); Themeli et al. 16(4):357-366 (2015); Themeli et al., Nature Biotechnology 31 :928-933 (2013). In some embodiments, the cell is an iPSC-derived T cell that is engineered to contain modifications (e.g., genetic modifications) described herein. In some embodiments, the cell is a primary T cell that is engineered to contain modifications (e.g., genetic modifications) described herein. In some embodiments, the cell comprises overexpression or increased expression of one or more complement inhibitor. In some embodiments, the T cell can be engineered with a chimeric antigen receptor (CAR), including any as described herein. In some embodiments, the engineered T cell can be used to treat a variety of indications with allogenic cell therapy, including any as described herein, e.g., Section IV. In some embodiments, the engineered T cell can be used to treat cancer.

Cells disclosed herein may be T cells, for example a population of T cells. It will be understood that any reference to "a cell" e.g. "a T cell" below also applies to "a population of cells" e.g. "a population of T cells" as described in the present application.

NK Cells

In some embodiments, the cell is a natural killer (NK) cell. In some embodiments, the NK cell comprises a polynucleotide encoding the DLL3 binding protein of the present disclosure. Relevant information concerning NK cells as referred to in the context of the present disclosure is known in the art, including certain information regarding desired features of NK cells when used for cell therapy and for example may be found from WO2017214569A1, WO 2011/068896A1, the contents of which are herein incorporated by reference. It will be understood that embodiments concerning NK cells described herein may be readily and appropriately combined with other embodiments described herein (for example, but not limited to, the DLL3 binding protein).

The NK cells described herein may be used to treat or prevent a disease in a subject.

NK cells, which arise through the lymphoid lineage and are part of the innate immune system, may be used in anti-cancer therapy as they have been found to detect and kill certain types of tumor cells. Natural killer (NK) cells are cytotoxic lymphocytes capable of human immune surveillance. WO2017214569A1 (the contents of which are incorporated herein by reference in their entirety) describes genome-edited primary NK cells, methods of making those cells, and methods of administering those cells.

In some embodiments, the cells that are engineered or modified as provided herein are Natural Killer (NK) cells. In some embodiments, the NK cells are isolated or obtained from an individual subject or one or more donors, such as one or more healthy donor (e.g., a donor that is not known or suspected of, e.g., not exhibiting clinical signs of, a disease or infection). As will be appreciated by those in the art, methods of isolating or obtaining NK cells from an individual can be achieved using known techniques. Provided herein are engineered primary NK cells that contain modifications (e.g., genetic modifications) described herein for subsequent transplantation or engraftment into subjects (e.g., recipients. For instance, the engineered NK cells are administered to a subject (e.g., recipient, such as a patient), by infusion of the engineered NK cells into the subject.

In some embodiments, the NK cells are primary NK cells. In some embodiments, NK cells, including primary NK cells isolated from an individual subject or one or more donors (e.g., healthy donors) or NK cells differentiated from iPSCs derived from one or more donors (e.g., healthy donors) express CD56 (e.g., CDSb^111 or CD56bright) and lack CD3 (e.g., CD3neg). In some embodiments, NK cells as described herein may also express the low-affinity Fey receptor CD16, which, without wishing to be bound by theory, mediate ADCC. In some embodiments, the NK cells also express one or more natural killer cell receptors NKG2A and NKG2D or one or more natural cytotoxicity receptors NKp46, NKp44, NKp30. For example, for the case of primary NK cells, in specific cases, the primary cells may be isolated from a starting source of NK cells, such as a sample containing peripheral blood mononuclear cells (PBMCs), by depletion of cells positive for CD3, CD14, and/or CD19. For instance, the cells may be subject to depletion using immunomagnetic beads having attached thereto antibodies to CD3, CD 14, and/or CD19, respectively), thereby producing an enriched population of NK cells. In other cases, primary NK cells may be isolated from a starting source that is a mixed population (e.g., PBMCs) by selecting cells for the presence of one or more markers on the NK cells, such as CD56, CD16, NKp46, and/or NKG2D.

In some embodiments, the NK cells can be immature NK cells and can be CD56+ and CD16-. In some embodiments, the NK cells can be mature NK cells and can be CD56- and CD16+, or CD561o and CD16+ (as described in WO 2011/068896A1 (the contents of which are incorporated herein by reference in their entirety)).

A primary NK cell may express CD16 and/or CD56. In some embodiments, an NK cell does not express CD3. In some embodiments, an "NK cell" is preferably defined as a cell that is CD56+ and CD3-. In some embodiments, an "NK cell" is defined as a cell that is CD16+ and CD3-. NK cells are lymphocytes of the innate immune system that kill virally infected or

transformed cells. Like T cells, NK cells are cytotoxic lymphocytes. Unlike T cells, NK cells do not require antigen recognition, and require integration of signals from many activating and inhibitory receptors to perform their function. Despite their similarities to T cells, NK cells behave differently under stimulation conditions and do not tolerate electroporation in the same way as T cells (as described in WO2017214569A1 (the contents of which are incorporated herein by reference in their entirety)).

A primary NK cell may be isolated from, for example, peripheral blood, umbilical cord cells, ascites, and/or a solid tumor (as described in WO2017214569A1, the contents of which are incorporated herein by reference in their entirety). In some embodiments, a "primary NK cell" is an NK cell that is freshly isolated. In some embodiments, a "primary NK cell" is an NK cell that has undergone up to 5 replications or divisions after being isolated, up to 10 replications or divisions after being isolated, up to 15 replications or divisions after being isolated, up to 20 replications or divisions after being isolated, up to 25 replications or divisions after being isolated, up to 30 replications or divisions after being isolated, up to 35 replications or divisions after being isolated, or up to 40 replications or divisions after being isolated. In some embodiments, the primary NK cell is a non-clonal cell. In some embodiments, primary NK cell is a proliferating cell. In some embodiments, primary NK cell is an expanded cell.

Some desired features of NK cells when used for cell therapy are described herein. NK cells may be distinguished using a number of assays, for example as set out in WO 2011/068896A1 (the contents of which is hereby incorporated by reference in its entirety). For example, IL12-p70 secretion may be measured: IL12p70 is secreted by mature DCs in order to elicit a Thl-directed response from CD4+ T cells. Human BM-derived DCs can produce >500pg/ml of IL12p70 yet blast-derived DCs did not produce any detectable IL12p70 upon maturation. Mixed lymphocyte reaction (MLR) assay: The MLR assay determines the ability of DCs to stimulate proliferation of allogenic T cells. Cord blood mononuclear cells (CBMCs, which include T cells) were used as responders, fluorescently labeled, and their proliferation was measured after 4-5 days coculture with immature or mature blast- derived DCs. Preliminary results show that the responder cells proliferate in response to mature (m)DCs. Hemangioblast-derived natural killer cells: Cell surface markers CD45, CD7, CD94, CD56, CD16, and NKG2D were evaluated in blast-derived and human bone marrow-derived NK cells.

Methods of generating natural killer (NK) cells are known in the art, for example methods described in WO 2011/068896A1 (the contents of which are incorporated by reference herein in their entirety) comprising: providing hemangioblasts; culturing the hemangioblasts on methylcellulose and a first cytokine mixture comprising IL2, IL3, IL6, IL7, IL15, SCF and FL;

harvesting the cultured cells; and culturing the harvested cells in liquid media comprising human serum, and a second cytokine mixture comprising IL7, IL15, SCF and FL to generate NK cells.

In some embodiments, the cells as provided herein are NK cells differentiated from engineered pluripotent cells that contain modifications (e.g., genetic modifications) described herein and that are differentiated into NK cells. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. In some embodiments, the cells differentiated into NK cells may be used for subsequent administration to a subject (e.g., recipient, such as a patient), such as by infusion of the differentiated NK cells into the subject. Methods for generating NK cells from pluripotent stem cells (e.g., iPSC) are described, for example, in U.S. Patent No. 10626373; Shankar et al. Stem Cell Res Ther. 2020; 11 : 234; Euchner et al. Frontiers in Immunology, 2021; 12, Article 640672. doi=10.3389/fimmu.2021.640672;. Methods of differentiating NK cells are known in the art, for example as described in WO 2011/068896A1 (the contents of which are hereby incorporated by reference in their entirety). An exemplary method as described in WO 2011/068896A1. In one embodiment, the initial differentiation procedure for both cell types may be the same and may involve a 4 day culture of hESCs in Stemline II (Sigma) plus cytokines in order to generate embryoid bodies (EBs). The cytokines, VEGF and BMP4 may be used throughout the EB culture while bFGF may be added after the first 2 days. After 4 days total, the resulting EBs may be disaggregated with 0.05% trypsin and then the trypsin may be inactivated with serum-containing media. Individual cells may be subsequently filtered through a 40 pM cell strainer, counted, and seeded into H4436 or H4536 methylcellulose (Stem Cell Technologies) containing additional cytokines, such as TPO (50pg/ml), VEGF(50pg/ml), FL (50pg/ml) and bFGF (20-50pg/ml). For NK differentiation, the cytokines IL-2 (l-10pg/ml), IL7 (l-20pg/ml), and/or IL15 (l-10pg/ml) may also be added at this stage. Methylcellulose cultures may be plated at a concentration of 50,000 to 150,000 cells per ml for the production and expansion of a hemangioblastic population. Blast-like cells may be harvested from methylcellulose between day 6 and 10 and further differentiated by one of the following procedures.

Cells disclosed herein may be natural killer (NK) cells, for example a population of NK cells. It will be understood that any reference to "a cell" e.g. "a NK cell" below also applies to "a population of cells" e.g. "a population of NK cells" as described in the present application.

V. METHODS OF GENOME MODIFICATION

In some embodiments, a polynucleotide encoding the DLL3 binding protein of the present disclosure may be inserted into the genome of a cell. The insertion may be non-targeted (i.e., insertion into a random genomic locus of the cell) or targeted (i.e., insertion into a specific

genomic locus of the cell), using any of the non-targeted or site-directed (targeted) insertion methods described herein.

Non-Targeted Insertion

In some embodiments, t a polynucleotide encoding the DLL3 binding protein of the present disclosure can be inserted into a random genomic locus of a host cell. As known to a person skilled in the art, viral vectors, including, for example, retroviral vectors, lentiviral vectors, adenoviral vectors, and adeno-associated viral vectors, are commonly used to deliver genetic material into host cells and randomly insert the foreign or exogenous nucleic acid into the genome of the cell to facilitate stable expression and replication of the foreign or exogenous nucleic acid.

HDR

In some embodiments, homology-directed repair (HDR) can be utilized for targeted integration or insertion of one or more nucleic acid sequences, e.g., transgene sequences, at one or more target site(s) in the genome. In some embodiments, the nuclease-induced HDR can be used to alter a target sequence, integrate a transgene at a particular target location, and/or to edit or repair a mutation in a particular target gene.

Alteration of nucleic acid sequences at the target site can occur by HDR with an exogenously provided polynucleotide (also referred to as donor polynucleotide or template sequence). For example, the template polynucleotide provides for alteration of the target sequence, such as insertion of the transgene contained within the template polynucleotide. In some embodiments, a plasmid or a vector can be used as a template for homologous recombination. In some embodiments, a linear DNA fragment can be used as a template for homologous recombination. In some embodiments, a single stranded template polynucleotide can be used as a template for alteration of the target sequence by alternate methods of homology directed repair (e.g., single strand annealing) between the target sequence and the template polynucleotide. Template polynucleotide-effected alteration of a target sequence depends on cleavage by a nuclease, e.g., a targeted nuclease such as CRISPR/Cas9. Cleavage by the nuclease can comprise a double strand break or two single strand breaks.

In some embodiments, a template polynucleotide comprises the following components: [5' homology arm] -[transgene] -[3' homology arm]. The homology arms provide for recombination into the chromosome, thus insertion of the transgene into the DNA at or near the cleavage site, e.g., target site(s). In some embodiments, the homology arms flank the most distal target site(s).

In some embodiments, "recombination" refers to a process of exchange of genetic information between two polynucleotides. In some embodiments, "homologous recombination

(HR)" refers to the specialized form of such exchange that takes place, for example, during repair of double-strand breaks in cells via homology-directed repair mechanisms. This process requires nucleotide sequence homology, uses a template polynucleotide to template repair of a target DNA (i.e., the one that experienced the double-strand break, e.g., target site in the endogenous gene), and is variously known as "non-crossover gene conversion" or "short tract gene conversion," because it leads to the transfer of genetic information from the template polynucleotide to the target. In some embodiments, such transfer can involve mismatch correction of heteroduplex DNA that forms between the broken target and the template polynucleotide, and/or "synthesis-dependent strand annealing," in which the template polynucleotide is used to resynthesize genetic information that will become part of the target, and/or related processes. Such specialized HR often results in an alteration of the sequence of the target molecule such that part or all of the sequence of the template polynucleotide is incorporated into the target polynucleotide. As described herein, the genetic disruption of the target site or target position can be created by any mechanisms, such as ZFNs, TALENs, CRISPR/Cas9 system, or TtAgo nucleases.

In some cases, a template polynucleotide comprises a first homology arm before the 5' end of the nucleic acid sequence encoding the recombinant polypeptide and a second homology arm after the 3' end of the nucleic acid sequence encoding the recombinant polypeptide. In some embodiments, the lengths of the two homology arms are between about 150 bp and about 1500 bp; optionally wherein the length of at least one of the first or second homology arms is about 200bp, 225 bp, about 250 bp, about 275 bp, about 300 bp, about 325 bp, about 350 bp, about 375 bp, about 400 bp, about 425 bp, about 450 bp, about 475 bp, or about 500 bp. In some embodiments, the lengths of both of the homology arms are about 225 bp, about 250 bp, about 275 bp, about 300 bp, about 325 bp, about 350 bp, about 375 bp, about 400 bp, about 425 bp, about 450 bp, about 475 bp, about 500 bp, about 525 bp, about 550 bp, about 575 bp, or about 600 bp. In some embodiments, the total length of the homology arms is between about 400 bp and about 500 bp, about 500 bp and about 600 bp, about 600 bp and about 700 bp, about 700 bp and about 800 bp, about 800 bp and about 900 bp, about 900 bp and about 1000 bp, about 1000 bp and about 1100 bp, or about 1100 bp and about 1200 bp.

In some embodiments, double strand cleavage is effected by a nuclease, e.g., a Cas9 molecule having cleavage activity associated with an HNH-like domain and cleavage activity associated with a RuvC-like domain, e.g., an N-terminal RuvC-like domain, e.g., a wild type Cas9.

In some embodiments, DNA repair mechanisms can be induced by a nuclease after (1) a single double-strand break, (2) two single strand breaks, (3) two double stranded breaks with a break occurring on each side of the target site, (4) one double stranded break and two single strand breaks with the double strand break and two single strand breaks occurring on each side of the target site (5) four single stranded breaks with a pair of single stranded breaks occurring on each side of the target site, or (6) one single stranded break. In some embodiments, a single-stranded template polynucleotide is used and the target site can be altered by alternative HDR.

Template polynucleotide-effected alteration of a target site depends on cleavage by a nuclease molecule. Cleavage by the nuclease can comprise a nick, a double strand break, or two single strand breaks, e.g., one on each strand of the DNA at the target site. After introduction of the breaks on the target site, resection occurs at the break ends resulting in single stranded overhanging DNA regions.

In canonical HDR, a double- stranded template polynucleotide is introduced, comprising homologous sequence to the target site that will either be directly incorporated into the target site or used as a template to insert the transgene or correct the sequence of the target site. After resection at the break, repair can progress by different pathways, e.g., by the double Holliday junction model (or double strand break repair, DSBR, pathway) or the synthesis-dependent strand annealing (SDSA) pathway.

In some embodiments, other DNA repair pathways such as single strand annealing (SSA), single- stranded break repair (SSBR), mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), intrastrand cross-link (ICL), translesion synthesis (TLS), error-free post-replication repair (PRR) can be employed by the cell to repair a double-stranded or single-stranded break created by the nucleases.

Site-Directed Insertion

In some embodiments, the methods for genetically modifying cells to knock out, knock down, or otherwise modify one or more genes comprise using a site-directed nuclease, including, for example, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, transposases, and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems, as well as nickase systems, base editing systems, prime editing systems, and any other gene editing systems known in the art.

ZFN

ZFNs are fusion proteins comprising an array of site-specific DNA binding domains adapted from zinc finger-containing transcription factors attached to the endonuclease domain of the bacterial FokI restriction enzyme. A ZFN may have one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, or more) DNA binding domains or zinc finger domains. See, e.g., Carroll et al., Genetics Society of America (2011) 188:773-782; Kim et al., Proc. Natl. Acad. Sci. USA (1996) 93: 1156-1160. The individual DNA binding domains are typically referred to as "fingers" or "zinc fingers." Each zinc finger domain is a small protein structural motif stabilized by one or more zinc ions and usually recognizes a 3- to 4-bp DNA sequence. Tandem domains can thus potentially bind to an extended nucleotide sequence that is unique within a cell's genome. Each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A DNA binding domain binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid, zinc-chelating, DNA-binding subdomain. Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues coordinated with zinc along with the two cysteine residues of a single beta turn (see, e.g., Berg & Shi, Science 271: 1081-1085 (1996)).

Various zinc fingers of known specificity can be combined to produce multifinger polypeptides which recognize about 6, 9, 12, 15, or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells. Zinc fingers can be engineered to bind a predetermined nucleic acid sequence. Criteria to engineer a zinc finger to bind to a predetermined nucleic acid sequence are known in the art. See, e.g., Sera et al., Biochemistry (2002) 41 :7074-7081; Liu et al., Bioinformatics (2008) 24: 1850-1857.

ZFNs containing FokI nuclease domains or other dimeric nuclease domains function as a dimer. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. See Bitinaite < / al., Proc. Natl. Acad. Sci. USA (1998) 95: 10570-10575. To cleave a specific site in the genome, a pair of ZFNs are designed to recognize two sequences flanking the site, one on the forward strand and the other on the reverse strand. Upon binding of the ZFNs on either side of the site, the nuclease domains dimerize and cleave the DNA at the site, generating a DSB with 5' overhangs. HDR can then be utilized to introduce a specific mutation, with the help of a repair template containing the desired mutation flanked by homology arms. The repair template is usually an exogenous double-stranded DNA vector introduced to the cell. See Miller et al., Nat. Biotechnol. (2011) 29: 143-148; Hockemeyer et al., Nat. Biotechnol. (2011) 29:731-734.

TALENS

TALENs are another example of an artificial nuclease which can be used to edit a target gene. A "TALE-nuclease" (TALEN) is a fusion protein consisting of a nucleic acidbinding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, for instance I-TevI, ColE7, NucA and Fok-I. In numerous embodiments, the TALE domain can be fused to a meganuclease, for instance LCrel and I-Onul or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE- Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927.

Transcription Activator like Effector (TALE) are proteins from the bacterial species Xanthomonas and comprise a plurality of repeated sequences. Each repeat is 33 to 35 amino acids in length, with two adjacent amino acids (termed the repeat-variable di-residue, or RVD) in position 12 and 13 that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALEN kits are sold commercially.

TALENs are produced artificially by fusing one or more TALE DNA binding domains (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) to a nuclease domain, for example, a FokI endonuclease domain. See Zhang, Nature Biotech. (2011) 29: 149-153. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. See Cermak et al., Nucl. Acids Res. (2011) 39:e82; Miller et al., Nature Biotech. (2011) 29: 143-148; Hockemeyer et al., Nature Biotech. (2011) 29:731-734; Wood et al., Science (2011) 333 :307; Doyon et al., Nature Methods (2010) 8:74-79; Szczepek et al., Nature Biotech (2007) 25:786-

793; Guo et al., J. Mol. Biol. (2010) 200:96. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI nuclease domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al., Nature Biotech. (2011) 29: 143-148.

By combining engineered TALE repeats with a nuclease domain, a site-specific nuclease can be produced specific to any desired DNA sequence. Similar to ZFNs, TALENs can be introduced into a cell to generate DSBs at a desired target site in the genome, and so can be used to knock out genes or knock in mutations in similar, HDR-mediated pathways. See Boch, Nature Biotech. (2011) 29: 135-136; Boch et al., Science (2009) 326: 1509-1512; Moscou et al., Science (2009) 326:3501.

Meganucleases

Meganucleases are sequence-specific enzymes in the endonuclease family which are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs). (Chevalier, B. S. and B. L. Stoddard, Nucleic Acids Res., 2001, 29, 3757-3774). They can cleave unique sites in living cells, thereby enhancing gene targeting by 1000-fold or more in the vicinity of the cleavage site (Puchta et al., Nucleic Acids Res., 1993, 21, 5034-5040; Rouet et al., Mol. Cell. Biol., 1994, 14, 8096-8106; Choulika et al., Mol. Cell. Biol., 1995, 15, 1968-1973; Puchta et al., Proc. Natl. Acad. Sci. USA, 1996, 93, 5055-5060; Sargent et al., Mol. Cell. Biol., 1997, 17, 267-77; Donoho et al., Mol. Cell. Biol, 1998, 18, 4070-4078; Elliott et al., Mol. Cell. Biol., 1998, 18, 93-101; Cohen-Tannoudji et al., Mol. Cell. Biol., 1998, 18, 1444- 1448).

Meganucleases are grouped into families based on their structural motifs which affect nuclease activity and/or DNA recognition. The most widespread and best known meganucleases are the proteins in the LAGLID ADG family, which owe their name to a conserved amino acid sequence. See Chevalier et al., Nucleic Acids Res. (2001) 29(18): 3757- 3774. On the other hand, the GIY-YIG family members have a GIY-YIG module, which is 70- 100 residues long and includes four or five conserved sequence motifs with four invariant residues, two of which are required for activity. See Van Roey et al., Nature Struct. Biol. (2002) 9:806-811. The His-Cys family meganucleases are characterized by a highly conserved series of histidines and cysteines over a region encompassing several hundred amino acid residues. See Chevalier et al., Nucleic Acids Res. (2001) 29(18):3757-3774. Members of the NHN family are defined by motifs containing two pairs of conserved histidines surrounded by asparagine residues. See Chevalier et al, Nucleic Acids Res. (2001) 29(18):3757-3774.

Because the chance of identifying a natural meganuclease for a particular target DNA sequence is low due to the high specificity requirement, various methods including mutagenesis and high throughput screening methods have been used to create meganuclease variants that recognize unique sequences. Strategies for engineering a meganuclease with altered DNA-binding specificity, e.g., to bind to a predetermined nucleic acid sequence are known in the art. See, e.g., Chevalier et al., Mol. Cell. (2002) 10:895-905; Epinat et al., Nucleic Acids Res (2003) 31 :2952-2962; Silva et al., J Mol. Biol. (2006) 361 :744-754; Seligman et al., Nucleic Acids Res (2002) 30:3870-3879; Sussman et al., J Mol Biol (2004) 342:31-41; Doyon et al., J Am Chem Soc (2006) 128:2477-2484; Chen et al, Protein Eng Des Sei (2009) 22:249-256; ArnovAd et al., JMol Biol. (2006) 355:443-458; Smith et al., Nucleic Acids Res. (2006) 363(2):283-294.

Like ZFNs and TALENs, Meganucleases can create DSBs in the genomic DNA, which can create a frame-shift mutation if improperly repaired, e.g., via NHEJ, leading to a decrease in the expression of a target gene in a cell. Alternatively, foreign DNA can be introduced into the cell along with the meganuclease. Depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to modify the target gene. See Silva et al., Current Gene Therapy (2011) 11: 11-27.

Transposases

Transposases are enzymes that bind to the end of a transposon and catalyze its movement to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. By linking transposases to other systems such as the CRISPER/Cas system, new gene editing tools can be developed to enable site specific insertions or manipulations of the genomic DNA. There are two known DNA integration methods using transposons which use a catalytically inactive Cas effector protein and Tn7-like transposons. The transposase-dependent DNA integration does not provoke DSBs in the genome, which may guarantee safer and more specific DNA integration.

CRISPR/Cas

The CRISPR system was originally discovered in prokaryotic organisms (e.g., bacteria and archaea) as a system involved in defense against invading phages and plasmids that provides a form of acquired immunity. Now it has been adapted and used as a popular gene editing tool in research and clinical applications.

CRISPR/Cas systems generally comprise at least two components: one or more guide RNAs (gRNAs) and a Cas protein. The Cas protein is a nuclease that introduces a DSB into the target site. CRISPR-Cas systems fall into two major classes: class 1 systems use a complex of multiple Cas proteins to degrade nucleic acids; class 2 systems use a single large Cas protein for

the same purpose. Class 1 is divided into types I, III, and IV; class 2 is divided into types II, V, and VI. Different Cas proteins adapted for gene editing applications include, but are not limited to, Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpf 1), Casl2b (C2cl), Casl2c (C2c3), Casl2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Casl2g, Casl2h, Casl2i, Casl2k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmr5, Csel, Cse2, Csfl, Csm2, Csn2, CsxlO, Csxl l, Csyl, Csy2, Csy3, Mad7, LEG14. The most widely used Cas9 is described herein as illustrative. These Cas proteins may be originated from different source species. For example, Cas9 can be derived from S. pyogenes or S. aureus.

In the original microbial genome, the type II CRISPR system incorporates sequences from invading DNA between CRISPR repeat sequences encoded as arrays within the host genome. Transcripts from the CRISPR repeat arrays are processed into CRISPR RNAs (crRNAs) each harboring a variable sequence transcribed from the invading DNA, known as the "protospacer" sequence, as well as part of the CRISPR repeat. Each crRNA hybridizes with a second transactivating CRISPR RNA (tracrRNA), and these two RNAs form a complex with the Cas9 nuclease. The protospacer-encoded portion of the crRNA directs the Cas9 complex to cleave complementary target DNA sequences, provided that they are adjacent to short sequences known as "protospacer adjacent motifs" (PAMs).

Since its discovery, the CRISPR system has been adapted for inducing sequence specific DSBs and targeted genome editing in a wide range of cells and organisms spanning from bacteria to eukaryotic cells including human cells. In its use in gene editing applications, artificially designed, synthetic gRNAs have replaced the original crRNA:tracrRNA complex. For example, the gRNAs can be single guide RNAs (sgRNAs) composed of a crRNA, a tetraloop, and a tracrRNA. The crRNA usually comprises a complementary region (also called a spacer, usually about 20 nucleotides in length) that is user-designed to recognize a target DNA of interest. The tracrRNA sequence comprises a scaffold region for Cas nuclease binding. The crRNA sequence and the tracrRNA sequence are linked by the tetraloop and each have a short repeat sequence for hybridization with each other, thus generating a chimeric sgRNA. One can change the genomic target of the Cas nuclease by simply changing the spacer or complementary region sequence present in the gRNA. The complementary region will direct the Cas nuclease to the target DNA site through standard RNA-DNA complementary base pairing rules.

In order for the Cas nuclease to function, there must be a PAM immediately downstream of the target sequence in the genomic DNA. Recognition of the PAM by the Cas protein is thought to destabilize the adjacent genomic sequence, allowing interrogation of the sequence by the gRNA and resulting in gRNA-DNA pairing when a matching sequence is present. The specific

sequence of PAM varies depending on the species of the Cas gene. For example, the most commonly used Cas9 nuclease derived from S. pyogenes recognizes a PAM sequence of 5'-NGG-3' or, at less efficient rates, 5'-NAG-3', where "N" can be any nucleotide. Other Cas nuclease variants with alternative PAMs have also been characterized and successfully used for genome editing, which are summarized below, and are also provided as SEQ ID Nos: 121-131 and in the Sequence Table.

Table 3: Exemplary Cas nuclease variants and their PAM sequences

CRISPR Nuclease	Source Organism	PAM Sequence (5'→3')
SpCas9	*Streptococcus pyogenes*	NGG or NAG
SaCas9	*Staphylococcus aureus*	NGRRT or NGRRN

CRISPR Nuclease	Source Organism	PAM Sequence (5'→3')
NmeCas9	*Neisseria meningitidis*	NNNNGATT
CjCas9	*Campylobacter jejuni*	NNNNRYAC
StCas9	*Streptococcus thermophilus*	NNAGAAW
TdCas9	*Treponema denticola*	NAAAAC
LbCas12a (Cpf1)	*Lachnospiraceae bacterium*	TTTV
AsCas12a (Cpf1)	*Acidaminococcus sp.*	TTTV
AacCas12b	*Alicyclobacillus acidiphilus*	TTN
BhCas12b v4	*Bacillus hisashii*	ATTN, TTTN, or GTTN

R = A or G; Y = C or T; W = A or T; V = A or C or G; N = any base

In some embodiments, Cas nucleases may comprise one or more mutations to alter their activity, specificity, recognition, and/or other characteristics. For example, the Cas nuclease may have one or more mutations that alter its fidelity to mitigate off- target effects (e.g., eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high- fidelity variants of SpCas9). For another example, the Cas nuclease may have one or more mutations that alter its PAM specificity.

Nuclease domains of the Cas, in particular the Cas9, nuclease can be mutated independently to generate enzymes referred to as DNA "nickases". Nickases are capable of introducing a single-strand cut with the same specificity as a regular CRISPR/Cas nuclease system, including for example CRISPR/Cas9. Nickases can be employed to generate double strand breaks which can find use in gene editing systems (Mali et al., Nat Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957-963 (2013); Mali et al, Science, 339(6121):823-826 (2013)). In some instances, when two Cas nickases are used, long overhangs are produced on each of the cleaved ends instead of blunt ends which allows for additional control over precise gene integration and insertion (Mali et al., Nat Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957-963 (2013); Mali et al., Science, 339(6121):823-826 (2013)). As both nicking Cas enzymes must effectively nick their target DNA, paired nickases can have lower off-target

effects compared to the double-strand-cleaving Cas-based systems (Ran et al., Cell, 155(2):479-480(2013); Mali et al, Nat Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957-963 (2013); Mali et al., Science, 339(6121):823-826 (2013)).

The molecular machinery of such Cas proteins that allows the CRISPR/Cas system to alter target polynucleotide sequences in cells include RNA binding proteins, endo- and exo-nucleases, helicases, and polymerases. In some embodiments, the CRISPR/Cas system includes a Cas protein and at least one to two ribonucleic acids that are capable of directing the Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. As used herein, "protein" and "polypeptide" are used interchangeably to refer to a series of amino acid residues joined by peptide bonds (i.e., a polymer of amino acids) and include modified amino acids (e.g., phosphorylated, glycated, glycosylated, etc.) and amino acid analogs. Exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, paralogs, fragments and other equivalents, variants, and analogs of the above.

In some embodiments, a Cas protein comprises one or more amino acid substitutions or modifications. In some embodiments, the one or more amino acid substitutions comprises a conservative amino acid substitution. In some instances, substitutions and/or modifications can prevent or reduce proteolytic degradation and/or extend the half-life of the polypeptide in a cell. In some embodiments, the Cas protein can comprise a peptide bond replacement (e.g., urea, thiourea, carbamate, sulfonyl urea, etc.). In some embodiments, the Cas protein can comprise a naturally occurring amino acid. In some embodiments, the Cas protein can comprise an alternative amino acid (e.g., D-amino acids, beta-amino acids, homocysteine, phosphoserine, etc.). In some embodiments, a Cas protein can comprise a modification to include a moiety (e.g., PEGylation, glycosylation, lipidation, acetylation, end-capping, etc.).

In some embodiments, a Cas protein comprises a core Cas protein, isoform thereof, or any Cas-like protein with similar function or activity of any Cas protein or isoform thereof. In some embodiments, a Cas protein comprises a core Cas protein. Exemplary Cas core proteins include, but are not limited to Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 and Cas9. In some embodiments, a Cas protein comprises type V Cas protein. In some embodiments, a Cas protein comprises a Cas protein of an E. coli subtype (also known as CASS2). Exemplary Cas proteins of the E. Coli subtype include, but are not limited to Csel, Cse2, Cse3, Cse4, and Cas5e. In some embodiments, a Cas protein comprises a Cas protein of the Ypest subtype (also known as CASS3). Exemplary Cas proteins of the Ypest subtype include, but are not limited to Csyl, Csy2, Csy3, and Csy4. In some embodiments, a Cas protein comprises a Cas protein of the Nmeni subtype (also known as CASS4). Exemplary Cas proteins of the Nmeni subtype include, but are

not limited to Csnl and Csn2. In some embodiments, a Cas protein comprises a Cas protein of the Dvulg subtype (also known as CASS1). Exemplary Cas proteins of the Dvulg subtype include Csdl, Csd2, and Cas5d. In some embodiments, a Cas protein comprises a Cas protein of the Tneap subtype (also known as CASS7). Exemplary Cas proteins of the Tneap subtype include, but are not limited to, Cstl, Cst2, Cas5t. In some embodiments, a Cas protein comprises a Cas protein of the Hmari subtype. Exemplary Cas proteins of the Hmari subtype include, but are not limited to Cshl, Csh2, and Cas5h. In some embodiments, a Cas protein comprises a Cas protein of the Apem subtype (also known as CASS5). Exemplary Cas proteins of the Apem subtype include, but are not limited to Csal, Csa2, Csa3, Csa4, Csa5, and Cas5a. In some embodiments, a Cas protein comprises a Cas protein of the Mtube subtype (also known as CASS6). Exemplary Cas proteins of the Mtube subtype include, but are not limited to Csml, Csm2, Csm3, Csm4, and Csm5. In some embodiments, a Cas protein comprises a RAMP module Cas protein. Exemplary RAMP module Cas proteins include, but are not limited to, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, and Cmr6. See, e.g., Klompe et al., Nature 571, 219-225 (2019); Strecker et al., Science 365, 48-53 (2019). Examples of Cas proteins include, but are not limited to: Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and/or GSU0054. In some embodiments, a Cas protein comprises Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and/or GSU0054. Examples of Cas proteins include, but are not limited to: Cas9, Csn2, and/or Cas4. In some embodiments, a Cas protein comprises Cas9, Csn2, and/or Cas4. In some embodiments, Examples of Cas proteins include, but are not limited to: CaslO, Csm2, Cmr5, CaslO, Csxl 1, and/or CsxlO. In some embodiments, a Cas protein comprises a CaslO, Csm2, Cmr5, CaslO, Csxl 1, and/or CsxlO. In some embodiments, examples of Cas proteins include, but are not limited to: Csfl. In some embodiments, a Cas protein comprises Csfl.In some embodiments, examples of Cas proteins include, but are not limited to: Casl2a, Casl2b, Casl2c, C2c4, C2c8, C2c5, C2cl0, and C2c9; as well as CasX (Casl2e) and CasY (Casl2d). Also see, e.g., Koonin et al., Curr Opin Microbiol. 2017; 37:67-78: "Diversity, classification and evolution of CRISPR- Cas systems." In some embodiments, a Cas protein comprises Casl2a, Casl2b, Casl2c, Casl2d, Casl2e, Casl2d, and/or Casl2e. In some embodiments, a Cas protein comprises Casl3, Casl3a, C2c2, Casl3b, Casl3c, and/or Casl3d. In some embodiments, the CRISPR/Cas system comprises a Cas effector protein selected from the group consisting of: a) Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and GSU0054; b) Cas9, Csn2, and Cas4; c) CaslO, Csm2, Cmr5, CaslO, Csxl l, and CsxlO; d) Csfl; e) Casl2a, Casl2b, Casl2c, C2c4, C2c8, C2c5, C2cl0, C2c9, CasX (Casl2e), and CasY (Casl2d); and f) Casl3, Casl3a, C2c2, Casl3b, Casl3c, and Casl3d.

In some embodiments, a Cas protein comprises any one of the Cas proteins described herein or a functional portion thereof. As used herein, "functional portion" refers to a portion of a peptide which retains its ability to complex with at least one ribonucleic acid (e.g., guide RNA (gRNA)) and cleave a target polynucleotide sequence. In some embodiments, the functional portion comprises a combination of operably linked Cas9 protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional portion comprises a combination of operably linked Casl2a (also known as Cpf1) protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional domains form a complex. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of a RuvC-like domain. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of the HNH nuclease domain. In some embodiments, a functional portion of the Casl2a protein comprises a functional portion of a RuvC-like domain.

In some embodiments, the Cas protein is complexed with one to two ribonucleic acids. In some embodiments, the Cas protein is complexed with two ribonucleic acids. In some embodiments, the Cas protein is complexed with one ribonucleic acid. In some embodiments, the Cas protein is encoded by a modified nucleic acid, as described herein (e.g., a synthetic, modified mRNA).

The methods of the present disclosure contemplate the use of any ribonucleic acid that is capable of directing a Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. In some embodiments, at least one of the ribonucleic acids comprises tracrRNA. In some embodiments, at least one of the ribonucleic acids comprises CRISPR RNA (crRNA). In some embodiments, a single ribonucleic acid comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, at least one of the ribonucleic acids comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, both of the one to two ribonucleic acids comprise a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. The ribonucleic acids of the present disclosure can be selected to hybridize to a variety of different target motifs, depending on the particular CRISPR/Cas system employed, and the sequence of the target polynucleotide, as will be appreciated by those skilled in the art. The one to two ribonucleic acids can also be selected to minimize hybridization with nucleic acid

sequences other than the target polynucleotide sequence. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least two mismatches when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least one mismatch when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids are designed to hybridize to a target motif immediately adjacent to a deoxyribonucleic acid motif recognized by the Cas protein. In some embodiments, each of the one to two ribonucleic acids are designed to hybridize to target motifs immediately adjacent to deoxyribonucleic acid motifs recognized by the Cas protein which flank a mutant allele located between the target motifs.

In some embodiments, each of the one to two ribonucleic acids comprises guide RNAs that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell.

In some embodiments, one or two ribonucleic acids (e.g., guide RNAs) are complementary to and/or hybridize to sequences on the same strand of a target polynucleotide sequence. In some embodiments, one or two ribonucleic acids (e.g., guide RNAs) are complementary to and/or hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (e.g., guide RNAs) are not complementary to and/or do not hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (e.g., guide RNAs) are complementary to and/or hybridize to overlapping target motifs of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (e.g, guide RNAs) are complementary to and/or hybridize to offset target motifs of a target polynucleotide sequence.

In some embodiments, CRISPR systems of the present disclosure comprise TnpB polypeptides. In some embodiments, TnpB polypeptides may comprise a Ruv-C-like domain. The RuvC domain may be a split RuvC domain comprising RuvC-I, RuvC-II, and RuvC-III subdomains. In some embodiments, a TnpB may further comprise one or more of a HTH domain, a bridge helix domain and a zinc finger domain. TnpB polypeptides do not comprise an HNH domain. In some embodiments, a TnpB protein comprises, starting at the N-terminus: a HTH domain, a RuvC-I subdomain, a bridge helix domain, a RuvC-II sub-domain, a zinger finger domain, and a RuvC-III sub-domain. In some embodiments, a RuvC-III sub-domain forms the C- terminus of a TnpB polypeptide. In some embodiments, a TnpB polypeptide is from Epsilonproteobacteria bacterium, Actinoplanes lobatus strain DSM 43150, Actinomadura celluolosilytica strain DSM 45823, Actinomadura namibiensis strain DSM 44197,

Alicyclobacillus macrosprangiidus strain DSM 17980, Lipingzhangella hal ophila strain DSM 102030, or Ktedonobacter recemifer. In some embodiments, a TnpB polypeptide is from Ktedonobacter racemifer, or comprises a conserved RNA region with similarity to the 5' ITR of K. racemifer TnpB loci. In some embodiments, a TnpB may comprise a Fanzor protein, a TnpB homolog found in eukaryotic genomes. In some embodiments, a CRISPR system comprising a TnpB polypeptide binds a target adjacent motif (TAM) sequence 5' of a target polynucleotide. In some embodiments, a TAM is a transposon-associated motif. In some embodiments, a TAM sequence comprises TCA. In some embodiments, a TAM sequence comprises TTCAN. In some embodiments, a TAM sequence comprises TTGAT. In some embodiments, a TAM sequence comprises ATAAA.

In certain embodiments, the first and/or the second transgene may function as a DNA repair template to be integrated into the target site through HDR in associated with a gene editing system (e.g., the CRISPR/Cas system) as described. Generally, the transgene to be inserted would comprise at least the expression cassette encoding the protein of interest (e.g., the tolerogenic factor or CAR) and would optionally also include one or more regulatory elements (e.g., promoters, insulators, enhancers). In certain of these embodiments, the transgene to be inserted would be flanked by homologous sequence immediately upstream and downstream of the target, i.e., left homology arm (LHA) and right homology arm (RHA), specifically designed for the target genomic locus to serve as template for HDR. The length of each homology arm is generally dependent on the size of the insert being introduced, with larger insertions requiring longer homology arms.

In some embodiments, target-primed reverse transcription (TPRT) or prime editing may be used to engineer exogenous genes into specific loci. In some embodiments, prime editing mediates targeted insertions, deletions, all 12 possible base-to-base conversions, and combinations thereof in human cells without requiring DSBs or donor DNA templates.

Prime editing is a genome editing method that directly writes new genetic information into a specified DNA site using a nucleic acid programmable DNA binding protein ("napDNAbp") working in association with a polymerase (i.e., in the form of a fusion protein or otherwise provided in trans with the napDNAbp), wherein the prime editing system is programmed with a prime editing (PE) guide RNA ("PEgRNA") that both specifies the target site and templates the synthesis of the desired edit in the form of a replacement DNA strand by way of an extension (either DNA or RNA) engineered onto a guide RNA (e.g., at the 5' or 3' end, or at an internal portion of a guide RNA). The replacement strand containing the desired edit (e.g., a single nucleobase substitution) shares the same sequence as the endogenous strand of the target site to

be edited (with the exception that it includes the desired edit). Through DNA repair and/or replication machinery, the endogenous strand of the target site is replaced by the newly synthesized replacement strand containing the desired edit. In some cases, prime editing may be thought of as a "search-and- replace" genome editing technology since the prime editors search and locate the desired target site to be edited, and encode a replacement strand containing a desired edit which is installed in place of the corresponding target site endogenous DNA strand at the same time. For example, prime editing can be adapted for conducting precision CRISPR/Cas-based genome editing in order to bypass double stranded breaks. In some embodiments, a homologous protein is or encodes for a Cas protein-reverse transcriptase fusions or related systems to target a specific DNA sequence with a guide RNA, generate a single strand nick at the target site, and use the nicked DNA as a primer for reverse transcription of an engineered reverse transcriptase template that is integrated with the guide RNA. In some embodiments, a prime editor protein is paired with two prime editing guide RNAs (pegRNAs) that template the synthesis of complementary DNA flaps on opposing strands of genomic DNA, resulting in the replacement of endogenous DNA sequence between the PE-induced nick sites with pegRNA-encoded sequences.

In some embodiments, a gene editing technology is associated with a prime editor that is a reverse transcriptase, or any DNA polymerase known in the art. Thus, in one aspect, a prime editor may comprise Cas9 (or an equivalent napDNAbp) which is programmed to target a DNA sequence by associating it with a specialized guide RNA (i.e., PEgRNA) containing a spacer sequence that anneals to a complementary protospacer in the target DNA. Such methods include any disclosed in Anzalone et al., (doi.org/10.1038/s41586-019-1711-4), or in PCT publication Nos. WO2020191248, WO2021226558, or W02022067130, which are hereby incorporated in their entirety.

In some embodiments, the base editing technology may be used to introduce single nucleotide variants (SNVs) into DNA or RNA in living cells. Base editing is a CRISPR-Cas9-based genome editing technology that allows the introduction of point mutations in RNAs or DNAs without generating DSBs. Base editors (BEs) are typically fusions of a Cas ("CRISPR-associated") domain and a nucleobase modification domain (e.g., a natural or evolved deaminase, such as a cytidine deaminase that include AP0BEC1 ("apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1"), CD A ("cytidine deaminase"), and AID ("activation-induced cytidine deaminase")) domains. In some embodiments, base editors may also include proteins or domains that alter cellular DNA repair processes to increase the efficiency and/or stability of the resulting single-nucleotide change. Two major classes of base editors have been

developed: cytidine base editors (CBEs) (e.g., BE4) that allow C:G to T:A conversions and adenine base editors (ABEs) (e.g., ABE7.10) that allow A:T to G:C conversions. Base editors are composed by a catalytically dead Cas9 (dCas9) or a nickase Cas9 (nCas9) fused to a deaminase and guided by a sgRNA to the locus of interest. The d/nCas9 recognizes a specific PAM sequence and the DNA unwinds thanks to the complementarity between the sgRNA and the DNA sequence usually located upstream of the PAM (also called protospacer). Then, the opposite DNA strand is accessible to the deaminase that converts the bases located in a specific DNA stretch of the protospacer. Compared to HDR- based strategies, base editing is a promising tool to precisely correct genetic mutations as it avoids gene disruption by NHEJ associated with failed HDR-mediated gene correction. Rat deaminase AP0BEC1 (rAPOBECl) fused to deactivated Cas9 (dCas9) has been used to successfully convert cytidines to thymidines upstream of the PAM of the sgRNA. In some embodiments, this first BE system was optimized by changing the dCas9 to a "nickase" Cas9 D10A, which nicks the strand opposite the deaminated cytidine. Without being bound by theory, this is expected to initiate long- patch base excision repair (BER), where the deaminated strand is preferentially used to template the repair to produce a U:A base pair, which is then converted to T:A during DNA replication.

In some embodiments, a base editor is a nucleobase editor containing a first DNA binding protein domain that is catalytically inactive, a domain having base editing activity, and a second DNA binding protein domain having nickase activity, where the DNA binding protein domains are expressed on a single fusion protein or are expressed separately (e.g., on separate expression vectors). In some embodiments, a base editor is a fusion protein comprising a domain having base editing activity (e.g., cytidine deaminase or adenosine deaminase), and two nucleic acid programmable DNA binding protein domains (napDNAbp), a first comprising nickase activity and a second napDNAbp that is catalytically inactive, wherein at least the two napDNAbp are joined by a linker. In some embodiments, a base editor is a fusion protein that comprises a DNA domain of a CRISPR-Cas (e.g., Cas9) having nickase activity (nCas; nCas9), a catalytically inactive domain of a CRISPR-Cas protein (e.g., Cas9) having nucleic acid programmable DNA binding activity (dCas; e.g., dCas9), and a deaminase domain, wherein the dCas is joined to the nCas by a linker, and the dCas is immediately adjacent to the deaminase domain. In some embodiments, a base editor is an adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor. Exemplary base editor and base editor systems include any as described in patent publication Nos. US20220127622, US20210079366, US20200248169, US20210093667, US20210071163, W02020181202, WO2021158921, WO2019126709,

W02020181178, W02020181195, WO2020214842, W02020181193, which are hereby incorporated in their entirety.

In some embodiments, a gene editing technology is Programmable Addition via Site-specific Targeting Elements (PASTE). In some aspects, PASTE is platform in which genomic insertion is directed via a CRISPR-Cas9 nickase fused to both a reverse transcriptase and serine integrase. As described in Ioannidi et al. (doi.org/10.1101/2021.11.01.466786), PASTE does not generate double stranded breaks, but allows for integration of sequences as large as ~36 kb. In some embodiments, a serine integrase can be any known in the art. In some embodiments, a serine integrase has sufficient orthogonality such that PASTE can be used for multiplexed gene integration, simultaneously integrating at least two different genes at at least two genomic loci. In some embodiments, PASTE has editing efficiencies comparable to or better than those of homology directed repair or non-homologous end joining based integration, with activity in non-dividing cells and fewer detectable off-target events.

Genomic Loci for Insertion

The specific site for insertion within a gene locus may be located within any suitable region of the gene, including but not limited to a gene coding region (also known as a coding sequence or "CDS"), an exon, an intron, a sequence spanning a portion of an exon and a portion of an adjacent intron, or a regulatory region (e.g., promoter, enhancer). In some embodiments, the insertion occurs in one allele of the specific genomic locus. In some embodiments, the insertion occurs in both alleles of the specific genomic locus. In either of these embodiments, the orientation of the transgene inserted into the target genomic locus can be either the same or the reverse of the direction of the endogenous gene in that locus.

In some embodiments, polynucleotides of the present disclosure are inserted into a T cell receptor alpha chain constant region (TRAC) locus. The TRAC gene encodes the TCR alpha chain constant region. The human TRAC gene resides on chromosome 14 at 22,547,506-22,552,156, forward strand. The TRAC genomic sequence is set forth in Ensembl ID ENSG00000277734.

In some embodiments, polynucleotides of the present disclosure are inserted into a T cell receptor beta chain (TRBC) locus. The TRBC genes encode the TCR beta chain constant region. TRBC1 and TRBC2 are analogs of the same gene, and T cells mutually exclusively express either TRBC1 or TRBC2. The human TRBC1 gene resides on chromosome 7 at 142,791,694-142,793,368, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000211751. The human TRBC2 gene resides on chromosome 7 at 142,801,041-

142,802,748, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000211772.

In some embodiments, polynucleotides of the present disclosure are inserted into a TRAJ (T cell receptor alpha joining) splice acceptor site locus. The human TRAJ gene resides on chromosome 14 at 22,545,037-22,545,098, forward strand. The TRAJ genomic sequence is set forth in Ensembl ID ENSG00000211888.

In some embodiments, polynucleotides of the present disclosure are inserted into a CD247 (also known as the CD3 zeta chain) locus. The CD247 gene encodes the zeta chain of the T-cell receptor. The CD247 gene resides on chromosome 1: 167,425,027-167,518,640, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000198821.

In some embodiments, polynucleotides of the present disclosure are inserted into a beta-2-microglobulin (B2M) locus. The B2M gene encodes the B2M component of class I major histocompatibility complex (MHC I). The B2M gene resides on chromosome 15: 45,003,675-45,011,075, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000166710.

In some embodiments, polynucleotides of the present disclosure are inserted into a class II, major histocompatibility complex, transactivator (CIITA) locus. The CIITA gene encodes the CIITA protein that is a positive regulator of class II major histocompatibility complex (MHC II) gene transcription. The CIITA gene resides on chromosome 16: 10,866,222-10,943,021, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000179583.

In some embodiments, polynucleotides of the present disclosure are inserted into a caspase recruitment domain family member 11 (CARD11) locus. In some embodiments, polynucleotides encoding the PIK3R3 protein, or a portion thereof, of the present disclosure are inserted into a CARD11 locus. The CARD11 gene encodes the CARD11 protein which plays a role in various immune responses including, among others, lymphocyte activation, proliferation, and differentiation, and plays a role as a positive regulator of NF-kB activation. The CARD11 gene resides on chromosome 7: 2,906,142-3,044,228, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000198286.

In some embodiments, polynucleotides of the present disclosure are inserted into a phosphoinositide-3-kinase regulatory subunit 3 (PIK3R3) locus. In some embodiments, polynucleotides encoding the CARD11 protein, or a portion thereof, of the present disclosure are inserted into a PIK3R3 locus. The PIK3R3 gene encodes the PIK3R3 protein which plays a role in inhibiting cell senescence and enhancing cell proliferation. The PIK3R3 gene resides on

chromosome 1: 46,040,140-46,133,036, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000117461.

In some embodiments, polynucleotides of the present disclosure are inserted into a safe harbor locus. Non-limiting examples of safe harbor loci include, but are not limited to, an AAVS1 (also known as PPP1R12C; Ensembl ID: ENSG00000125503), ABO (Ensembl ID: ENSG00000175164), CCR5 (Ensembl ID: ENSG00000160791), CLYBL (Ensembl ID: ENSG00000125246), CXCR4 (Ensembl ID: ENSG00000121966), F3 (also known as CD142; Ensembl ID: ENSG00000117525), FUT1 (Ensembl ID: ENSG00000174951), HMGB1 (Ensembl ID: ENSG00000189403), KDM5D (Ensembl ID: ENSG00000012817), LRP1 (also known as CD91; Ensembl ID: ENSG00000123384), MICA (Ensembl ID: ENSG00000204520), MICB (Ensembl ID: ENSG00000206449), RHD (Ensembl ID: ENSG00000187010), human ROSA26 (GRCh37/hg19, chr3: 9,415,082–9,414,043), and SHS 231 (GRCh37/hg19, chr4: 58,976,613–58,976,632).

gRNAs for Site-Directed Insertion

In some embodiments, provided are gRNAs for use in site-directed insertion of a polynucleotides according to various embodiments provided herein, especially in association with the CRISPR/Cas system. The gRNAs comprise a crRNA sequence, which in turn comprises a complementary region (also called a spacer) that recognizes and binds a complementary target DNA of interest. The length of the spacer or complementary region is generally between 15 and 30 nucleotides, usually about 20 nucleotides in length, although will vary based on the requirements of the specific CRISPR/Cas system. In certain embodiments, the spacer or complementary region is fully complementary to the target DNA sequence. In other embodiments, the spacer is partially complementary to the target DNA sequence, for example at least 80%, 85%, 90%, 95%, 98%, or 99% complementary.

In certain embodiments, the gRNAs provided herein further comprise a tracrRNA sequence, which comprises a scaffold region for binding to a nuclease. The length and/or sequence of the tracrRNA may vary depending on the specific nuclease being used for editing. In certain embodiments, nuclease binding by the gRNA does not require a tracrRNA sequence. In those embodiments where the gRNA comprises a tracrRNA, the crRNA sequence may further comprise a repeat region for hybridization with complementary sequences of the tracrRNA.

In some embodiments, the gRNAs provided herein comprise two or more gRNA molecules, for example, a crRNA and a tracrRNA, as two separate molecules. In other embodiments, the gRNAs are single guide RNAs (sgRNAs), including sgRNAs comprising a

crRNA and a tracrRNA on a single RNA molecule. In certain of these embodiments, the crRNA and tracrRNA are linked by an intervening tetraloop.

In some embodiments, one gRNA can be used in association with a site-directed nuclease for targeted editing of a gene locus of interest. In other embodiments, two or more gRNAs targeting the same gene locus of interest can be used in association with a site-directed nuclease.

In some embodiments, exemplary gRNAs (e.g., sgRNAs) for use with various common Cas nucleases that require both a crRNA and tracrRNA, including Cas9 are provided in Table 2 below. See, e.g., Jinek et al., Science (2012) 337 (6096):816-821; Dang et al., Genome Biology (2015) 16:280; Ran et al., Nature (2015) 520: 186-191; Strecker et al., Nature Comm. (2019) 10:212. For each exemplary gRNA, sequences for different portions of the gRNA, including the complementary region or spacer, crRNA repeat region, tetraloop, and tracrRNA, are shown. The sequences of the table below include each of SEQ ID NOs: 132-139, as further provided in the Sequence Table below.

Table 4: Exemplary gRNA Structure and Sequences for CRISPR/Cas

Sequence (5' to 3')	Description
nnnnnnnnnnnnnnnnnnn	Exemplary spCas9 1 complementary region (spacer)
guuuuagagcua	Exemplary spCas9 1 crRNA repeat region
gaaa	Exemplary spCas9 1 crRNA tetraloop
uagcaaguuaaaauaaggcuaguccguuaucaacuu gaaaaaguggcaccgagucggugcuuuuuu	Exemplary spCas9 1 crRNA tracrRNA
nnnnnnnnnnnnnnnnnnn	Exemplary spCas9 2 complementary region (spacer)
guuusagagcuaugcug	Exemplary spCas9 2 crRNA repeat region
gaaa	Exemplary spCas9 2 crRNA tetraloop
cagcauagcaaguusaaauaaggcuaguccguuauc aacuugaaaaaguggcaccgagucggugcuuuuuu	Exemplary spCas9 2 crRNA tracrRNA
nnnnnnnnnnnnnnnnnnn	Exemplary saCas9 complementary region (spacer)
guuuuaguacucug	Exemplary saCas9 crRNA repeat region
gaaa	Exemplary saCas9 tetraloop
cagaaucuacuaaaacaaggcaaaaugccguguuua ucucgucaacuuguuggcgagauuuuuu	Exemplary saCas9 tracrRNA

s = c or g; n = any base

In some embodiments, the gRNA comprises a complementary region specific to a target gene locus of interest, for example, a TRAJ locus, a TRAC locus, a TRBC1 locus, a TRBC2

locus, a CD3zeta locus, a B2M locus, a CIITA locus, a CARD11 locus, a PIK3R3 locus, or a safe harbor locus selected from the group consisting of an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a MICA locus, a MICB locus, a RHD locus, a ROSA26, locus and a SHS231 locus. The complementary region may bind a sequence in any region of the target gene locus, including for example, a CDS, an exon, an intron, a sequence spanning a portion of an exon and a portion of an adjacent intron, or a regulatory region (e.g., promoter, enhancer). Where the target sequence is a CDS, exon, intron, or sequence spanning portions of an exon and intron, the CDS, exon, intron, or exon/intron boundary may be defined according to any splice variant of the target gene. In some embodiments, the genomic locus targeted by the gRNA is located within 4000 bp, within 3500 bp, within 3000 bp, within 2500 bp, within 2000 bp, within 1500 bp, within 1000 bp, or within 500 bp of any of the loci or regions thereof as described. Further provided herein are compositions comprising one or more gRNAs provided herein and a Cas protein or a nucleotide sequence encoding a Cas protein. In certain of these embodiments, the one or more gRNAs and a nucleotide sequence encoding a Cas protein are comprised within a vector, for example, a viral vector.

In some embodiments, provided are methods of identifying new loci and/or gRNA sequences for use in the site-directed genomic insertion approaches as described. For example, for CRISPR/Cas systems, when an existing gRNA for a particular locus (e.g., within an endogenous TCR gene locus) is known, an "inch worming" approach can be used to identify additional loci for targeted insertion of transgenes by scanning the flanking regions on either side of the locus for PAM sequences, which usually occurs about every 100 base pairs (bp) across the genome. The PAM sequence will depend on the particular Cas nuclease used because different nucleases usually have different corresponding PAM sequences. The flanking regions on either side of the locus can be between about 500 to 4000 bp long, for example, about 500 bp, about 1000 bp, about 1500 bp, about 2000 bp, about 2500 bp, about 3000 bp, about 3500 bp, or about 4000 bp long. When a PAM sequence is identified within the search range, a new guide can be designed according to the sequence of that locus for use in site-directed insertion of transgenes. Although the CRISPR/Cas system is described as illustrative, any gene editing approaches as described can be used in this method of identifying new loci, including those using ZFNs, TALENs, meganucleases, and transposases.

In some embodiments, the activity, stability, and/or other characteristics of gRNAs can be altered through the incorporation of chemical and/or sequential modifications. As one example, transiently expressed or delivered nucleic acids can be prone to degradation by, e.g., cellular

nucleases. Accordingly, the gRNAs described herein can contain one or more modified nucleosides or nucleotides which introduce stability toward nucleases. While not being bound by a particular theory, it is believed that certain modified gRNAs described herein can exhibit a reduced innate immune response when introduced into a population of cells, particularly the cells of the present technology. As used herein, the term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, e.g., the interferons, and cell death. Other common chemical modifications of gRNAs to improve stabilities, increase nuclease resistance, and/or reduce immune response include 2'-O-methyl modification, 2'-fluoro modification, 2'-O-methyl phosphorothioate linkage modification, and 2'- O-methyl 3' thioPACE modification.

One common 3' end modification is the addition of a poly(A) tract comprising one or more (and typically 5-200) adenine (A) residues. The poly(A) tract can be contained in the nucleic acid sequence encoding the gRNA or can be added to the gRNA during chemical synthesis or following in vitro transcription using a polyadenosine polymerase (e.g., E. coll poly(A) polymerase). In vivo, poly(A) tracts can be added to sequences transcribed from DNA vectors through the use of polyadenylation signals. Examples of such signals are provided in Maeder. Other suitable gRNA modifications include, without limitations, those described in U.S. Patent Application No. US 2017/0073674 Al and International Publication No. WO 2017/165862 Al, the entire contents of each of which are incorporated by reference herein.

In some embodiments, a tool for designing a gRNA as disclosed herein comprises: Benchling, Broad Institute GPP, CasOFFinder, CHOPCHOP, CRISPick, CRISPOR, Deskgen, E- CRISP, Geneious, Guides, Horizon Discovery, IDT, Off-Spotter, Synthego, or TrueDesign (ThermoFisher). One of ordinary skill in the art would understand that a tool that predicts both activity and specificity (e.g., to limit off-target modification) would be useful for designing a gRNA in certain instances as disclosed herein.

VI. Additional Cell Modifications

In some embodiments, an engineered cell expressing a DLL3 binding protein, such as an anti-DLL3 CAR, anti-DLL3 immune cell engager, or anti-DLL3 antibody or antigen binding domain, or the like, may further comprise one or more additional modifications. For example, in some cases, the cell may be further engineered to express one or more additional polypeptides, or one or more RNAs. In some cases, the one or more RNAs comprise a non-coding RNA, an shRNA, or a microRNA. In some cases, the cell further comprises or expresses potency

enhancement polypeptide, a cytokine, a chemokine, a growth factor, a tolerogenic factor, a safety switch, or any combination thereof.

Second target antigens

In some cases, the engineered cell comprises a second binding protein, such as a CAR or TCR that binds to a second target antigen. In some cases, the second target antigen is or comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, or the extracellular portion of the APRIL protein, or any combinations thereof, including variants thereof. In some cases, the

second target antigen is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB, or any combination thereof. In some cases, the second target antigen is or comprises TROP2, SSTR2, or GD2, or any combination thereof.

In some cases, the second target antigen is or comprises DLL3, such as the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

Dual CAR

In some embodiments, a cell comprising an anti-DLL3 binding protein may further comprise a second CAR.

In some embodiments, the second CAR has a specificity for a target antigen selected from CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN,

RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or combinations thereof, including variants thereof.

In some cases, the target antigen is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB, or any combination thereof. In some cases, the second target antigen is or comprises TROP2, SSTR2, or GD2, or any combination thereof.

In some cases, the target antigen is or comprises DLL3, such as the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3.

Potency enhancement polypeptides

In some embodiments, the cell may further comprise a potency enhancement polypeptide. In some embodiments, the potency enhancement polypeptide is or comprises a patient derived CARD11-PIK3R3 fusion, an engineered CARD11-PIK3R3 fusion, a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (e.g., TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, CD8alpha/beta, or a combination thereof, including variants thereof. Examples of CARD11-PIK3R3 fusions include those comprising the amino acid sequence of any one of SEQ ID NOs: 114, 116, 118, or 120, or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOs: 114, 116, 118, or 120.

In some embodiments, the engineered cell may comprise one or more polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids that can alter the *in vivo* persistence of T cells in tumors or the other functions or activities of T cells. In some embodiments, the recombinant nucleic constructs and/or recombinant nucleic acids of the disclosure, the polypeptides can include more than one mutation. For example, one or two or three or four or five or more mutations. In some embodiments, the mutations are a combination of different types of mutations. The different types of mutations can be point mutations, gene fusions, substitutions, gain-of-function mutations, stop-gain mutations, insertion mutations, deletion mutations, duplication mutations or translocations. The mutation can be a T cell

lymphoma mutation or a mutation in a clonally expanded population of T cells. In some embodiments, the mutation is a point mutation or substitution.

In some embodiments, the mutation is a gene fusion. In some embodiments, the gene fusion comprises a polypeptide comprising a caspase-associated recruitment domain (CARD). In some embodiments, the gene fusion comprises a polypeptide comprising a CARD containing protein or a functional fragment thereof. A functional fragment of a CARD containing protein can be, for example, a fragment that provides NF-κB transcriptional activity, AP-1 transcriptional activity, and/or MALT1 proteolytic activity. In some embodiments, the gene fusion comprises a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a phosphorylated tyrosine (pTyr). In some embodiments, the gene fusion comprises a CARD containing protein and a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a pTyr.

In some embodiments, the domain is capable of binding to a substrate indirectly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. In some embodiments, the domain is capable of binding to a substrate directly localized to the intracellular side of the plasma membrane. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to the intracellular side of the plasma membrane itself. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with the intracellular side of the plasma membrane itself.

In some embodiments, the polypeptides, recombinant nucleic acid constructs and/or recombinant nucleic acids can alter T cell signaling in various ways. In some embodiments, the altering of the T cell signaling can be by enhancing, promoting, improving, reducing, regulating, or modulating the signaling pathway within a T cell. In some embodiments, the altering of the T cell signaling can be by activating, increasing, suppressing, inhibiting, or other means of changing the signaling.

In some embodiments, the polypeptides, recombinant nucleic acid constructs and/or recombinant nucleic acids can alter T cell signaling through one or more T cell signaling pathways. In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter T cell signaling by increasing one or more pathways and/or reducing one or more pathways, or by a combination of enhancing and/or reducing various pathways. In some embodiments, the mutation can alter cytokine production. The cytokine can be, without limitation, IL-2, IL-4, IL-5, TNF alpha, IFN- gamma, IL-13 and/or other cytokines. In some embodiments, the cytokine is IL-2. In some embodiments, the cytokine is IL-2, and the production of IL-2 is increased.

In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids can alter T cell signaling through one or more T cell signaling pathways. Signaling pathways can be, without limitation, the NFAT pathway, NF-κB pathway, AP-1 pathway, JAK/STAT pathway, RAS/MEK/ERK, and/or phospholipase gamma signaling, or described elsewhere herein.

In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids can alter CARD11-BCL10-MALT1 complex signaling, co-stimulatory molecule signaling, cytokine production and/or transcription factor activity in T cells.

In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids may have one or more mutations that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increase intratumoral presence of therapeutic T cells. T cell fitness can refer to the ability of a T cell to generate an immune response. The ability of a T cell to perform T cell functions such as signaling, cytokine production, survival, and persistence in a tumor, may contribute to the fitness of a T cell. T cell exhaustion may contribute to reducing its fitness. The *in vivo* persistence of therapeutic T cells can refer to the length of time that the therapeutic T cells exist within the tumor of the host after infusion. In some embodiments, *in vivo* persistence of therapeutic T cells is enhanced. Enhanced *in vivo* persistence of therapeutic T cells can include at least a positive log2 fold change in therapeutic T cells as compared to the input total.

In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids can alter the *in vivo* persistence of T cells in tumors or the other functions or activities of T cells described herein, through one or more of the following genes: BCL6, BCOR, BRAF, CARD11, CARMIL2, CCND3, CD28, CD3E, CSNK1A1, CSNK2B,

ECSIT, EIFS1, FYN, GATA, GNAQ, IRF4, ITGB2, JAK1, JAK3, JUNB, KCNQ1, LATS1, MSC, MYCN, NFKB1, NFKB2, NRAS, PDCD1, PLCG1, PRKCB1 , RARA, RASGRP1, RHOA, SMARCB1, STAT3, STAT5, TBL1XR1, TNFRSF1B, TP53 and VAV1.

In some embodiments, the genes include caspase recruitment domain family member 11 (CARD11). In some embodiments, the genes include capping protein regulator and myosin 1 linker 2 (CARMIL2), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), B-cell lymphoma 6 (BCL6), B-cell lymphoma 10 (BCL10), and MYCN. In some embodiments, the gene can be a STAT3, STAT5B, JAK1, JAK2, or JAK3 gene. In some embodiments, the gene can be BRAF, or RASGRP1. In some embodiments, the gene can be phospholipase C gamma 1 (PLCG1) gene. In some embodiments, the gene can be NFKB1, NFKB2, or JUNB gene. In some embodiments, the gene can be TNFRSF1B.

In some embodiments, the further polypeptide, recombinant nucleic acid construct and/or recombinant nucleic acid relates to a CARD11-PIK3R3 fusion polypeptide, such as including a CARD domain (i.e., containing protein or functional fragment thereof), a CARD inhibitory domain (ID), a coiled-coil domain, and an SH2 domain from PIK3R3. In some embodiments, the CARD11-PIK3R3 fusion polypeptide includes a functional fragment of a CARD containing protein derived from CARD11domain, a coiled-coil domain, and an SH2 domain from PIK3R3.

CARD11-PIK3R3 Fusion Proteins

In some embodiments, a cell may comprise or express a CARD11-PIK3R3 fusion protein, for instance, as a potency enhancement polypeptide.

CARD11

CARD11, also known as Caspase Recruitment Domain Family Member 11, CARMA1, Carma 1, BIMP3, Caspase Recruitment Domain-Containing Protein 11, BCL10-Interacting Maguk Protein 3, CARD-Containing MAGUK Protein 1, Card-Maguk Protein 1, IMD11A, BENTA, IMD11, or PPBL, is encoded by the human CARD11 gene. CARD11 is a membrane-associated guanylate kinase (MAGUK) family protein, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. CARD11 is also a member of the CARD protein family, defined by the presence of a caspase-associated recruitment domain (CARD). CARD11 plays a role in various immune responses including, among others, lymphocyte activation, proliferation, and differentiation, and plays a role as a positive regulator of NF-kB activation.

CARD11 is ~130 kDa and exhibits a modular structure comprising a CARD domain, a LATCH domain, a coiled-coil domain, an inhibitory domain, and a MAGUK domain (which itself is made up of a PDZ domain, SH3 domain, and GUK domain) CARD11 is expressed in

immune cells, with variations in expression levels observed across different cell types and states (Lu et al. The CBM-opathies-A Rapidly Expanding Spectrum of Human Inborn Errors of Immunity Caused by Mutations in the CARD11-BCL10-MALT1 Complex. *Front Immunol.* 2018 Sep 19;9:2078).

CARD11 is a member of the CARD11-BCL10-MALT1 (CBM) signalosome, which is essential for T cell activation and function upon antigen stimulation. The CBM complex is known to have three important outputs: NF-kB transcriptional activity, AP-1 transcriptional activity, and MALT1 proteolytic activity. In the absence of signaling from the TCR, the CARD11 inhibitory domain blocks CARD11 from forming a complex with BCL10 and MALT1. However, after TCR signaling, the inhibitory domain is phosphorylated and the CARD11 protein is able to oligomerize and recruit BCL10 and MALT1 to form the CBM complex.

Human CARD11 (isoform 203) has a CARD domain, a LATCH domain, a coiled-coil domain, an inhibitory domain, and a MAGUK domain. The MAGUK domain is made up of a PDZ domain, SH3 domain, and GUK domain.

As used herein, a human CARD11 CARD domain refers to the CARD domain near the N-terminus of the human CARD11 protein.

As used herein, a human CARD11 LATCH domain refers to the LATCH domain between the N-terminal CARD domain and the C-terminal coiled-coil domain of the human CARD11 protein.

As used herein, a human CARD11 coiled-coil domain refers to the coiled-coil domain between the N-terminal LATCH domain and the C-terminal inhibitory domain of the human CARD11 protein.

As used herein, a human CARD11 inhibitory domain refers to the inhibitory domain (ID) between the N-terminal coiled-coil domain and the C-terminal MAGUK domain of the human CARD11 protein.

As used herein, a human CARD11 MAGUK domain refers to the MAGUK domain near the C-terminus of the human CARD11 protein.

The human CARD11 gene has eight transcripts, three of which encode a protein isoform of CARD11 (Ensembl, Gene: CARD11). The eight transcripts are named CARD11-201, CARD11-202, CARD11-203, CARD11-204, CARD11-205, CARD11-206, CARD11-201, and CARD11-208 (Ensembl, Gene: CARD11, ENSG00000198286).

Transcript CARD11-203 (Ensembl Transcript ID: ENST00000396946.9; NCBI Reference Sequence: NM_001324281.3 and NM_032415.7) encodes isoform CARD11-203 (Uniprot Accession No. Q9BXL7; NCBI Reference Sequence: NP_001311210.1), which has 1154 amino

acids. CARD11-203 is the longest transcript of the human CARD11 gene. It is designated as the representative transcript in the Ensembl database. In identifying the representative transcript, Ensembl aims to identity the transcript that, on balance, has the highest coverage of conserved exons, highest expression, longest coding sequence and is represented in other key resources, such as NCBI and UniProt. All splice junctions of the CARD11-203 transcript are supported by at least one non-suspect mRNA.

Transcript CARD11-201 (Ensembl Transcript ID: ENST00000355508.3; NCBI Reference Sequence:) encodes isoform CARD11-201 (Uniprot Accession No. H7BY05), which has 271 amino acids.

Transcript CARD11-202 (Ensembl Transcript ID: ENST00000356408.3; NCBI Reference Sequence:) encodes isoform CARD11-202 (Uniprot Accession No. E2QRC0), which has 73 amino acids. The amino acid sequences of the three isoforms are listed in Table 5 below. The amino acids corresponding to the various domains in the human CARD11 protein are also identified in Table 5. "Beginning Sequence" refers to the region N-terminal to the CARD domain. "End Sequence" refers to the region C-terminal to the MAGUK domain.

Table 5. Amino Acid SEQ ID NOs for CARD11 Isoforms

	CARD11-201	CARD11-202	CARD11-203
Beginning Sequence	N/A	1-10	1-10
CARD Domain	N/A	11-73	11-103
LATCH Domain	N/A	N/A	104-122
Coiled-Coil Domain	N/A	N/A	123-442
Inhibitory Domain	1-49	N/A	443-577
First Connection	50-136	N/A	578-666
MAGUK Domain	137-271	N/A	667-1140
End Sequence	N/A	N/A	1141-1154

As used herein, a human CARD11 PDZ domain refers to the PDZ domain near the N-terminus of the human CARD11 MAGUK domain.

As used herein, a human CARD11 SH3 domain refers to the SH3 domain between the N-terminal PDZ domain and the C-terminal GUK domain of the human CARD11 MAGUK domain.

As used herein, a human CARD11 GUK domain refers to the GUK domain near the C-terminus of the human CARD11 MAGUK domain.

The amino acid sequences of the CARD11-203 PDZ, SH3, and GUK domains in Table 6. "PDZ to SH3 Connection" refers to the region connecting the PDZ domain and the SH3 domain. "SH3 to GUK Connection" refers to the region connecting the SH3 domain and the GUK domain.

Table 6. Amino Acid SEQ ID NOs for CARD11 Domains

	CARD11-203
PDZ Domain	667-755
PDZ to SH3 Connection	756-772
SH3 Domain	773-806
SH3 to GUK Connection	807-972
GUK Domain	973-1140

PIK3R3

PIK3R3, also known as phosphatidylinositol 3-kinase regulatory subunit gamma (UniProt Q92569), is encoded by the human PIK3R3 gene (NCBI gene ID 8503).

PIK3R3, also known as phosphatidylinositol 3-kinase regulatory subunit gamma, p55, p55-GAMMA, p55PIK, phosphoinositide-3-kinase regulatory subunit 3, is encoded by the human PIK3R3 gene. PIK3R3 is a regulatory subunit of PI3K and phosphorylates phosphatidylinositol (and similar compounds).

PIK3R3 is ~62kDa and comprises both a catalytic and regulatory subunit including. PIK3R3 comprises two SH2 domains through which it binds activated (phosphorylated) protein-tyrosine kinases to regulate their activity.

The human PIK3R3 gene has seven transcripts, five of which encode a protein isoform of PIK3R3 (Ensembl, Gene: PIK3R3, ENSG00000117461). The seven transcripts are named PIK3R3-201, PIK3R3-202, PIK3R3-203, PIK3R3-204, PIK3R3-205, PIK3R3-206, and PIK3R3-207 (Ensembl, Gene: PIK3R3, ENSG00000117461).

CARD11-PIK3R3 Fusion Protein

A CARD11-PIK3R3 fusion has previously been derived from a patient with CD4+ T cell lymphoma (Wang et al., Genomic profiling of Sézary syndrome identifies alterations of key T cell signaling and differentiation genes. *Nature Genetics* 47, 1426-1434 (2015). Therapeutic T cells expressing this CARD11-PIK3R3 fusion protein demonstrate augmented CARD11–BCL10–MALT1 complex signaling and anti-tumour efficacy in several immunotherapy-refractory models in an antigen-dependent manner (Garcia et al., Naturally occurring T cell

mutations enhance engineered T cell therapies. *Nature*. 626, 626-634 (2024)). Therapeutic T cells expressing the CARD11-PIK3R3 fusion protein showed no evidence of malignant transformation up to 418 days after T-cell transfer, underscoring the safety of such an approach. These references are herein incorporated by reference in their entirety.

The present disclosure provides recombinant nucleic acids encoding a modified peptide epitope or recombinant polypeptide as described herein, wherein the recombinant nucleic acid additionally encodes a CARD-containing protein (e.g., a CARD11-PIK3R3 fusion protein) or a functional fragment thereof, for example those described in PCT/US2023/075738, which is herein incorporated by reference in its entirety.

Engineered CARD11-PIK3R3 Fusion Proteins

In some embodiments, a cell may comprise or express an engineered CARD11-PIK3R3 protein that has fewer amino acids than the patient derived full-length human CARD11-PIK3R3 fusion. In some embodiments, the engineered CARD11-PIK3R3 protein is a recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof, wherein the recombinant polypeptide comprises no more than 724 amino acids. In some embodiments, the recombinant polypeptide does not comprise a full length CARD containing protein. In some embodiments, the recombinant polypeptide exhibits decreased signaling and/or elicits decreased IFNg and/or IL-2 production compared to an immune cell expressing a control polypeptide consisting of SEQ ID NOs: 114 or 116. In some embodiments, the recombinant polypeptide exhibits equivalent or enhanced signaling and/or elicits equivalent or enhanced IFNg and/or IL-2 production compared to an immune cell expressing a control polypeptide consisting of SEQ ID NOs: 114 or 116. In some embodiments, the recombinant polypeptide affords delivery via integrating vectors.

In some embodiments, the cell comprises at least one copy, or at least two copies, of endogenous nucleic acid sequence encoding a CARD11 protein, or a protein comprising a CARD domain from CARD11 without any SH2 domain. In some embodiments, the recombinant nucleic acid of the cell is located at the endogenous CARD11-encoding gene locus, or comprises at least a portion of the endogenous CARD11-encoding gene, of the cell.

In some embodiments, the cell comprises at least one copy, or at least two copies, of endogenous nucleic acid sequence encoding a PIK3R3 protein, or a protein comprising an SH2 domain from PIK3R3 without any CARD domain. In some embodiments, the recombinant nucleic acid of the cell is located at the endogenous PIK3R3-encoding gene locus, or comprises at least a portion of the endogenous PIK3R3-encoding gene, of the cell.

Biological Activity of Engineered CARD11-PIK3R3 Fusion Proteins

In some embodiments the engineered CARD11-PIK3R3 fusion protein is functionally equivalent to a patient derived full length CARD11-PIK3R3 fusion protein. As used herein, "functionally equivalent" refers to having at least one type of biological activity of a patient-derived human CARD11-PIK3R3 fusion protein.

In some embodiments an engineered cell comprising the engineered CARD11-PIK3R3 fusion protein is functionally equivalent to an engineered cell comprising a patient derived full length CARD11-PIK3R3 fusion protein. As used herein, "functionally equivalent" refers to having at least one type of biological activity of an engineered cell comprising a patient-derived human CARD11-PIK3R3 fusion protein.

Safety Switches

In some embodiments, the cell further comprises or expresses a safety switch. In some cases, the safety switch is or comprises a protease. In some cases, the protease is a kinase, such as a herpesvirus thymidine kinase (HSV-tk) gene. In some cases, the safety switch is a deaminase, such as an Escherichia coli cytosine deaminase (EC-CD). In some cases, the safety switch is a nuclease, such as an inducible Caspase9 (iCasp9) protein. Additional nucleases are discussed below.

Nucleases

In some embodiments, the cell may further comprise or express a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, an RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

In some embodiments, the cell may further comprise or express any one or more of Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, evoSpCas9 high-fidelity variants of SpCas9, SaCas9,

NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional fragment thereof.

In some embodiments, the cell may further comprise or express a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

Cytokines and T Cells Redirected for Universal Cytokine-Mediated Killing (TRUCKs)

In some embodiments, the cell may be engineered to further express transgenic proteins such as cytokines, chemokines, growth factors, or tolerogenic factors. For instance, in some such embodiments, the cell is a T cell. Such T cells are in some cases termed T cells redirected for universal cytokine-mediated killing (TRUCKs).

Following antigen binding, CARs initiate a signaling cascade that culminates in the activation of transcription factors such as NFAT. In some embodiments, CAR activation may result in expression of a cytokine, for example. Such a cytokine, in some cases, thereby promotes tumor killing though one or more potentially synergistic mechanisms, including exocytosis (perforin, granzyme) and death ligand–death receptor (Fas–FasL, TRAIL) systems.

In some embodiments, the transgenic protein is a cytokine, e.g., IL-2, IL-7, IL-12, IL-15, IL-18, IL-23, IL-27.

Synthetic Intramembrane Proteolysis Receptors (SNIPRs)

In some embodiments, it may be desirable to restrict anti-DLL3 CAR or DLL3 binding protein expression and activity to a specific environment, e.g., a tumor microenvironment. In some embodiments, it may be desirable to selectively induce CAR expression and activity in the context of a specific tissue or environment, e.g., a tumor microenvironment.

Engineered T cells comprising synthetic intramemebrane proteolysis receptors (SNIPRs) display on their surface receptors that drive anti-DLL3 CAR expression upon binding with an antigen. SNIPRs are designed to express an antigen binding domain. Binding of an antigen to the SNIPR antigen binding domain triggers cleavage of the SNIPR and release of a transcriptional activation domain, e.g., a transcription factor.

The effector action of the SNIPR, for example, occurs through the transcriptional activation of the CAR. The one or more transcription factors (such as, for example, Gal4-VP64, a synthetic zinc finger, HNF1A, ZF10, and Pax6) can be specifically designed to activate transcription of T cells where the response genes can be T cell effector molecules including, but not limited to an anti DLL3 CAR. The transcriptional activation can occur through the use of native or designer transcription factors (such as, for example, Gal4-VP64, a synthetic zinc finger, HNF1A, ZF10, and Pax6). It is contemplated herein that the transcription factor (such as, for example, Gal4-VP64, a synthetic zinc finger, HNF1A, ZF10, and Pax6) can be specific for a transcriptional response element (such as, for example, Gal4-VP64, a synthetic zinc finger,

HNF1A, ZF10, and Pax6) on a vector or transgene, thereby allowing for unique non native interaction and expression of one or more response genes (such as, for example, an anti-DLL3 CAR). One or more response genes can be encoded on the vector or transgene along with the transcription response element and a promoter which drives the expression of the effector molecule. Thus, in some cases, engineered cells herein may comprise a SNIPR, further comprising a vector or transgene comprising with a transcriptional response element operatively linked to a promoter driving expression of one or more response genes (such as, for example, an anti-DLL3 CAR); wherein the transcriptional response element is specific for one or more of the transcription factors on the SNIPR.

In some embodiments, the SNIPR comprises, from N-terminus to C-terminus, an antigen binding domain, an extracellular regulatory domain comprising a protease cleavage site, a transmembrane domain, an intracellular juxtamembrane domain, and a transcriptional activation domain, e.g., a transcription factor.

In some embodiments, the SNIPR binds to DLL3, such as the EGF1 domain of DLL3, the EGF2 domain of DLL3, the EGF3 domain of DLL3, the EGF4 domain of DLL3, the EGF5 domain of DLL3, or the EGF6 domain of DLL3. Thus, in some cases, the anti-DLL3 CAR is an anti-DLL3 SNIPR.

In some cases, the SNIPR binds to a different target antigen. For example, in some cases, the SNIPR binds to a target antigen selected from CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX,

LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or combinations thereof, including variants thereof. In some cases, the target antigen is or comprises TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, or FN-EDB, or any combination thereof. In some cases, the target antigen is or comprises TROP2, SSTR2, or GD2, or any combination thereof.

Synthetic Notch (SynNotch) Receptors

In some embodiments, it may be desirable to restrict anti-DLL3 CAR expression and activity to a specific environment, e.g., a tumor microenvironment. In some embodiments, it may be desirable to selectively induce CAR expression and activity in the context of a specific tissue or environment, e.g., a tumor microenvironment.

Engineered T cells comprising synthetic notch (synNotch) secrete or display on their surface receptors that drive CAR expression upon binding with an antigen. SynNotch receptors are designed to express antibody or an antibody fragment rather than the native notch receptor. Binding of an antigen to the antibody or antibody fragment of a synNotch triggers cleavage of the notch receptor and release of a transcriptional activation domain, e.g., a transcription factor. SynNotch receptors can be further modified to incorporate specific or unique transcription elements to trigger expression of a desired gene such as an anti-DLL3 CAR or DLL3 binding protein.

The effector action of the synNotch receptor, in some embodiments, can occur through the transcriptional activation of the anti-DLL3 CAR. The one or more transcription factors (such as, for example, Gal4-VP64, Gal4-VPl6, TetR-VP64, LacI-VP64, and the like) can be specifically designed to activate transcription of T cell where the response genes can be T cell effector molecules including, but not limited to an anti-DLL3 CAR or DLL3 binding protein. The

transcriptional activation can occur through the use of native or designer transcription factors (Crispr/Cas9, TALEN, or zinc finger). The transcription factor (such as, for example, Gal4-VP64, Gal4-VPl6, TetR-VP64, LacI-VP64, and the like) can be specific for a transcriptional response element (such as, for example, Gal4-VP64, Gal4-VP16, TetR-VP64, LacI-VP64, and the like) on a vector or transgene, thereby allowing for unique non native interaction and expression of one or more response genes (such as, for example, an anti-DLL3 CAR or DLL3 binding protein). One or more response genes can be encoded on the vector or transgene along with the transcriptions response element and a promoter which drives the expression of the effector molecule. Thus, in some embodiments, engineered cells may comprise a synNotch receptor, further comprising a vector or transgene comprising a transcriptional response element operatively linked to a promoter driving expression of one or more response genes (such as, for example, an anti-DLL3 CAR or DLL3 binding protein); wherein the transcriptional response element is specific for one or more of the transcription factors on the synNotch receptor.

In some embodiments, the synNotch receptor comprises, from N-terminus to C-terminus, an antigen binding domain, a Notch core comprising a protease cleavage site, and a transcriptional activation domain, e.g., a transcription factor.

VII. Therapeutic Methods and Routes of Administration

A. Pharmaceutical Compositions

Compositions including the CAR T cells of the present disclosure may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are known in the art (see, for example, Rosenberg et al., New Engl J Med, (1988) 319:1676). The optimal dosage and treatment regimen for a particular subject can be determined by one skilled in the art by monitoring the subject for signs of disease and adjusting the treatment accordingly.

In some embodiments, administration of any of the compositions embodied herein, for the treatment of, for example, an autoimmune or inflammatory disease, can be combined with other cell-based therapies, for example, stem cells, antigen presenting cells, pancreatic islets etc.

The composition of the present disclosure may be prepared in a manner known in the art and in a manner suitable for parenteral administration to mammals, particularly humans, including a therapeutically effective amount of the composition alone, with one or more pharmaceutically acceptable carriers.

In some embodiments, the composition is suitable for parenteral administration. In another embodiment, the composition is suitable for intravenous administration.

Compositions suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes, which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

B. Methods for Treating a Subject

The disclosure also provides methods of treating a subject in need of cell therapy, comprising administering to the subject a cell as described herein or a T-cell prepared by any of the methods described herein. The disclosure also provides methods for enhancing the in vivo persistence of a T cell (e.g., a therapeutic T cell) in a subject in need thereof, by administering to the subject a therapeutically effective amount of the T cells of the disclosure. The T cells can include any of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure.

This administering step can be accomplished using any method of implantation delivery in the art. For example, the engineered cells of the disclosure can be infused directly in the individual's bloodstream or otherwise administered to the individual.

In some embodiments, the methods disclosed herein include administering which term is used interchangeably with the terms "introducing," implanting," and "transplanting," engineered cells into an individual, by a method or route that results in at least partial localization of the introduced cells at a desired site such that a desired effect(s) is/are produced. The engineered cells or their differentiated progeny can be administered by any appropriate route that results in delivery to a desired location in the individual where at least a portion of the administered cells or components of the cells remain viable. The period of viability of the cells after administration to an individual can be as short as a few hours, e.g., twenty-four hours, to a few days, to as long as several years, or even the lifetime of the individual, i.e., long-term engraftment.

When provided prophylactically, the engineered cells described herein can be administered to an individual in advance of any symptom of a disease or condition to be treated. Accordingly, in some embodiments the prophylactic administration of a engineered cell population prevents the occurrence of symptoms of the disease or condition.

When provided therapeutically in some embodiments, engineered cells are provided at (or after) the onset of a symptom or indication of a disease or condition, e.g., upon the onset of disease or condition.

For use in the various embodiments described herein, an effective amount of engineered cells as disclosed herein, can be at least 10^2 cells, at least 5×10^2 cells, at least 10^3 cells, at least

5×10^3 cells, at least 10^4 cells, at least 5×10^4 cells, at least 10^5 cells, at least 2×10^5 cells, at least 3×10^5 cells, at least 4×10^5 cells, at least 5×10^5 cells, at least 6×10^5 cells, at least 7×10^5 cells, at least 8×10^5 cells, at least 9×10^5 cells, at least 1×10^6 cells, at least 2×10^6 cells, at least 3×10^6 cells, at least 4×10^6 cells, at least 5×10^6 cells, at least 6×10^6 cells, at least 7×10^6 cells, at least 8×10^6 cells, at least 9×10^6 cells, or multiples thereof. The engineered cells can be derived from one or more donors or can be obtained from an autologous source. In some embodiments, the engineered cells are expanded in culture prior to administration to an individual in need thereof.

In some embodiments, the delivery of an engineered cell composition (e.g., a composition including a plurality of engineered cells according to any of the cells described herein) into an individual by a method or route results in at least partial localization of the cell composition at a desired site. A composition including engineered cells can be administered by any appropriate route that results in effective treatment in the individual, e.g., administration results in delivery to a desired location in the individual where at least a portion of the composition delivered, e.g., at least 1 × 104 cells, is delivered to the desired site for a period of time. Modes of administration include injection, infusion, and instillation. "Injection" includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion. In some embodiments, the route is intravenous. For the delivery of cells, delivery by injection or infusion is a preferred mode of administration.

In some embodiments, the engineered cells are administered systemically, e.g., via infusion or injection. For example, a population of engineered cells are administered other than directly into a target site, tissue, or organ, such that it enters the individual's circulatory system and, thus, is subject to metabolism and other similar biological processes.

The efficacy of a treatment including any of the compositions provided herein for the treatment of a disease or condition can be determined by a skilled clinician. However, one skilled in the art will appreciate that a treatment is considered effective if any one or all of the signs or symptoms or markers of disease are improved or ameliorated. Efficacy can also be measured by failure of an individual to worsen as assessed by decreased hospitalization or need for medical interventions (e.g., progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the disease, e.g., arresting, or slowing the

progression of symptoms; or (2) relieving the disease, e.g., causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of symptoms.

As discussed above, a therapeutically effective amount includes an amount of a therapeutic composition that is sufficient to promote a particular beneficial effect when administered to an individual, such as one who has, is suspected of having, or is at risk for a disease. In some embodiments, an effective amount includes an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate effective amount can be determined by one of ordinary skill in the art using routine experimentation.

In some embodiments of the disclosed methods, the individual is a mammal. In some embodiments, the mammal is a human. In some embodiments, the individual has or is suspected of having a disease associated with inhibition of cell signaling mediated by a cell surface ligand or antigen. The diseases suitable for being treated by the compositions and methods of the disclosure include, but are not limited to, cancers, autoimmune diseases, inflammatory diseases, and infectious diseases. In some embodiments, the disease is cancer or a chronic infection.

Methods for CAR design, delivery and expression in T cells, and the manufacturing of clinical-grade CAR-T cell populations are known in the art. See, for example, Lee et al., Clin Cancer Res (2012) 18(10):2780-90, hereby incorporated by reference in its entirety. For example, the engineered CARs may be introduced into T cells using retroviruses, which efficiently and stably integrate a nucleic acid sequence encoding the chimeric antigen receptor into the target cell genome.

Other methods known in the art include, but are not limited to, lentiviral transduction, transposon-based systems, direct RNA transfection, and CRISPR/Cas systems (e.g., type I, type II, or type III systems using a suitable Cas protein such Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12a (Cpf1), Cas13a (C2c2), Cas13b, Cas13d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), CasX, CasY, Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, etc.).

In some embodiments, a recombinant adeno-associated virus (AAV) vector can be used for delivery. Techniques to produce rAAV particles, in which an AAV genome to be packaged that includes the polynucleotide to be delivered, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV requires that the following

components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (e.g., not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes can be from any AAV serotype for which recombinant virus can be derived, and can be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, international patent application publication number WO 01/83692.

The CAR-T cells, once they have been expanded ex vivo in response to, for example, an autoimmune disease antigen, can be reinfused into the subject in a therapeutically effective amount.

The precise amount of CAR T cells to be administered can be determined by a physician with consideration of individual differences in age, weight, extent of disease and condition of the subject.

Administration of T cell therapies may be defined by number of total cells per infusion or number of cells per kilogram of body weight, especially for pediatric subjects (e.g., patients). As T cells replicate and expand after transfer, the administered cell dose may not resemble the final steady-state number of cells. In some embodiments, a pharmaceutical composition including the CAR T cells of the present disclosure may be administered at a dosage of 104 to 1010 total cells. In another embodiment, a pharmaceutical composition including the CAR T cells of the present disclosure may be administered at a dosage of 103 to 108 cells/kg body weight, including all integer values within those ranges.

C. Subjects for Treatment

In some embodiments, the subject has cancer or an autoimmune disease. In some embodiments, the cancer can be a solid tumor. In some embodiments, the cancer can be a hematological cancer. In some embodiments, the cancer expresses DLL3. In some cases, the cancer expresses TROP2, SSTR2, GD2, EGFR, CEA, CEACAMs, B7H3, PSMA, CA9, EPCAM, FN-EDB, or any combination thereof. In some cases, the cancer expresses TROP2, SSTR2, GD2, or any combination thereof.

Exemplary cancers comprise sarcoma, carcinoma, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer, kidney cancer, renal pelvis cancer, leukemia, lung cancer, small cell lung cancer, non-small cell lung cancer, melanoma, lymphoma, pancreatic cancer, prostate cancer, neuroendocrine prostate cancer, ovarian cancer, fibrosarcoma,

glioma, glioblastoma, glioblastoma multiforme, neuroblastoma, renal clear cell carcinoma, adrenocortical carcinoma, bladder urothelial carcinoma, diffuse large B-cell lymphoma, lung adenocarcinoma, pancreatic adenocarcinoma, renal cell cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, indolent B cell lymphoma, aggressive B cell lymphoma, T cell lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, myelodysplastic syndromes, myeloproliferative neoplasms, breast invasive carcinoma, triple-negative breast cancer, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, head and neck squamous cell carcinoma, kidney chromophobe, renal papillary cell carcinoma, lower grade glioma, hepatocellular carcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectal adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, or Wilms & rhabdoid tumors. In some cases, the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), neuroendocrine carcinoma (NEC), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate cancer. In some cases, the cancer is pulmonary neuroendocrine carcinoma, large cell neuroendocrine carcinoma, gastric cancer, pancreatic cancer, such as gastroenteropancreatic cancer, bladder cancer, prostate cancer, cervical cancer, or a gastric, pancreatic, gladder, prostate, or cervical neuroendocrine carcinoma. In some cases, the cancer is small cell lung cancer (SCLC).

In some embodiments, the method of treatment does not comprise administration of lymphodepletive agents within 7 days prior to administration of the T cell therapy. Non-limiting examples of lymphodepletive agents include cyclophosphamide, fludarabine, and/or bendamustine within 7 days prior to administration of the T cell therapy. In some embodiments, the method of treatment does not comprise administration of at least 600,000 IU/kg of IL-2 every 8 hours. In some embodiments, the method of treatment does not comprise a checkpoint therapy which blocks PD-1 or CTLA-4 signaling.

In some cases, the subject also receives an additional therapy, such as one or more of chemotherapy, immunotherapy, surgery, radiotherapy, anti-angiogenic agent, anti-DNA repair agent, anti-inflammatory agent, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a hormonal agent. Additional drug therapies may be administered as inhibitors, agonists, small molecules, proteins, recombinant proteins, fusion proteins, antibodies, antibody

drug complexes, CARs, TCRs, Immune cell engagers, PROTACs, natural products, cell therapy, gene therapy, nucleic acids, vaccines, and the like.

Methods suitable for genetically engineering cells for recombinant expression are well known in the art.

All publications and patent applications mentioned in this disclosure are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors/disclosers reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

VIII. EXAMPLES

The following examples are nonlimiting and are meant to assist in illustrating certain embodiments or properties of the disclosure. It is understood that various other embodiments may be practiced, given the general descriptions provided above.

Example 1: Generation of Anti-DLL3 Rabbit Monoclonal Antibody

This Example describes the generation of anti-DLL3 rabbit monoclonal antibodies (rabmAbs). Specifically, the anti-DLL3 rabmAbs used in accordance with the invention were generated via immunization of two rabbits with recombinant DLL3 ECD-HIS6 protein. Single B cells were isolated from splenocytes and cultured in individual wells. Then, B cell supernatants were screened by ELISA against DLL3 protein. Next, B cells were sequenced and paired VH/VL sequences were humanized. Humanized rabmAbs that demonstrated binding to soluble DLL3 were selected for further development. Domain mapping identified a humanized rabmAb that binds to the membrane proximal domain of DLL3, EGF6 (**FIG. 1**). Further, the humanized rabmAb demonstrated single digit nM affinity or below determined by BLI (**FIG. 1**). Cross-reactivity to cyno DLL3, mouse DLL3, human DLL1 and human DLL4 were assessed for the binder (**FIG. 1**). Finally, the ability of the humanized rabmAb to bind cells that express DLL3

was confirmed by expressing full length DLL3 in HEK293T cells and staining with the humanized rabmAb (data not shown).

Example 2: Generation and Characterization of anti-DLL3 Chimeric Antigen Receptor

This Example describes the generation and characterization of an anti-DLL3 chimeric antigen receptor (CAR). Specifically, this example describes the generation and characterization of a highly potent anti-DLL3 CAR targeting a membrane proximal epitope of DLL3 (EGF6) that demonstrates optimal potency and specificity compared to existing anti-DLL3 CARs.

Methods:

Cloning:

For non-targeted insertion, nucleic acid sequences encoding the DLL3 CARs comprising an scFv, hinge domain, transmembrane domain, and intracellular signaling domains were synthesized and cloned into lentiviral vectors containing an MND promoter. For targeted insertion into the TRAJ intron splice acceptor locus (and expression controlled by the TRAC promoter), nucleic acid sequences encoding the DLL3 CARs comprising an scFv, hinge domain, transmembrane domain, and intracellular signaling domains were synthesized and cloned into AAV6 viral vectors for use as a homology directed repair (HDR) template for knock-in to the TRAJ intron splice acceptor locus.

T cell manufacturing

Pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells with the GOI transgene expression vector and viral packaging plasmids using Mirus TransIT-Lenti. Viral supernatants were harvested at 48 hours post transfection, filtered, and mixed with activated T cells. Primary CD3+ T cells were sourced from donors (AllCells). After thawing, T cells were cultured in human T cell medium consisting of X-VIVO 15, 5% Human AB serum, 10mM HEPEs, and 1x Glutamax, supplemented with 10ng/mL IL-7 and 5ng/mL IL-15. T cells were activated with TransACT (CD3 and CD28 activation) for 24 hours, before being transduced with lentivirus. Virus and TransACT were removed 24 hours post transduction, and T cells were seeded into G-Rex plates to expand for 3-5 days before harvest. At harvest T cells were used immediately for co-culture assays or frozen in CryoStor10 and stored in liquid nitrogen for thaw and use later.

For experiments including TRAJ intron splice acceptor knock-in, T cells were electroporated with RNPs consisting of Cas9 protein and a single guide RNA targeting the TRAJ intron splice acceptor locus (sgRNA: CAGGGTTCTGGATATCTGT; SEQ ID NO: 161). The cells were cultured in human T cell medium without serum (X-VIVO 15, 5% Human AB serum, 10mM HEPEs, and 1x Glutamax, supplemented with 10ng/mL IL-7 and 5ng/mL IL-15) and

transduced with an AAV6 vector at an MOI of 2.5e4 providing the HDR template (HDRT). AAV6 transduced T cell cultures were supplemented with the NHEJ inhibitor M3814 at a concentration of 1uM and rested overnight, then washed, and transferred to a G-Rex plate for expansion.

In vitro co-culture assays:

For *in vitro* co-culture assays, transduced primary T cells from healthy donors were normalized by CAR expression, then co-cultured with target tumor cells. Supernatants were collected at 18-24 hours post co-culture set up and assessed for IFNy and IL-2 via MSD using manufacturer protocols (see www.mesoscale.com /~/media/files/ product%20inserts/ proinflammatory%20panel%201%20human%20insert.pdf).

Flow cytometry

For all *in vitro* primary T cell assays, cells were washed with PBS 2% FBS, stained with surface staining markers at room temperature for 20 minutes, washed once, and resuspended in PBS 2% FBS before analysis on an Attune Flow Cytometer. The following antibody was used for CAR detection: FITC conjugated human DLL3 protein.

Cell Lines

The cancer cell lines used were the lung carcinoma line A549 (ATCC CCL-185), the small cell lung carcinoma lines DMS 273 (Millipore Sigma 95062830-1VL), NCI-H82 (ATCC HTB-175) and SHP-77 (ATCC CRL-2195).

In vivo efficacy studies

5e6 SHP77 cells were delivered via subcutaneous injection into 8-10 week old female NSG mice. Mice were randomized into treatments groups by tumor volume. At day 21 post tumor injection, mice were treated with untransduced T cells or 5e6 CAR+ T cells via RO injection. Untransduced T cells were matched to the highest total T cells infused for the CAR T groups. Tumors were measured by caliper 2x/week and followed for survival. N=5 animals per group.

Results:

The humanized rabmAb binder targeting the membrane proximal domain of DLL3, EGF6, described in Example 1 was reformatted into an scFv and cloned into a CAR construct (**FIG. 2A**), ML73, comprising (from 5' to 3') an MND promoter, a CD8 signal sequence, the humanized rabmAb scFv (with a 3xG4S linker between the VL and VH), a CD8 hinge, a CD8 transmembrane domain, a 4-1BB co-stimulatory domain, and a CD3z co-stimulatory domain. Then, the entire CAR construct was inserted into a lentiviral vector. Primary human T cells from healthy donors were transduced with the ML73 CAR vector, alternative rabmAb CAR constructs, or a comparator CAR and surface expression was detected with an Fc-DLL3 reagent

and measured by flow cytometry. Untransduced T cells were included as a negative control. All CAR constructs demonstrated robust expression in primary T cells as measured by percent CAR positivity **(FIG. 2B)** and CAR MFI **(FIG. 2C),** with the ML73 binder demonstrating a higher average CAR MFI.

CAR T cells were then subjected to various assays to characterize *in vitro* functionality. First, CAR T cells were co-cultured with SHP-77 cells, which express high levels of DLL3, and IFNgamma and IL-2 were measured by MSD (**FIGS. 3A-3B**). While all CAR T cells secreted IFNgamma and IL-2, the CAR T expressing ML73 produced significantly more cytokines in response to antigen compared to the comparator CAR or CARs made with alternative rabmAbs (**FIGS. 3A-3B**).

Subsequent experiments focus on comparing ML73 to preclinical and clinical comparator CARs. To this end, lentiviral vectors encoding ML73 and five comparator anti-DLL3 CARs (comprising both a 4-1BB and CD3z co-stimulatory domain) were transduced into three healthy primary T cell donors. ML73 demonstrated a similar percent CAR positivity (**FIG. 4A**) and CAR MFI (**FIG. 4B**) relative to all comparator CARs screened**.** CAR T cells were then co-cultured with the DLL3 negative, A549 lung carcinoma cell line, and IFNgamma and IL-2 cytokine secretion was assessed. Notably, ML73 and comparator CARs 2, 3 and 4 demonstrated antigen specificity because they did not secrete IFNgamma (**FIG. 5A**) or IL-2 (**FIG. 5B**) when co-cultured with the DLL3 negative A549 target cells. Comparator CARs 1 and 4 demonstrated low levels of cytokine secretion in the presence of these antigen negative target cells, suggesting a potential off-target interaction of these binders (**FIGS. 5A and 5B**). Next, CAR T cells were co-cultured with a cell line that expresses a low level of DLL3, DMS273, a cell line that expresses a middle level of DLL3, NCI-H82, and a cell line that expresses a high amount of DLL3, SHP-77**.** CAR T cells expressing the ML73 CAR produced significantly greater levels of IL-2 when co-cultured with the low, medium, and high DLL3 expressing cell lines compared to all five comparators (**FIGS. 6A-6C**). Furthermore, CAR T cells expressing the ML73 CAR also produced significantly greater IFNgamma when co-cultured with all three cell lines (**FIGS. 6D-6F**).

To test the *in vivo* potency of the ML73 CAR NSG mice were engrafted with SHP77 SCLC cells via subcutaneous injection. Day 21 post tumor injection, 5e6 CAR+T cells or untransduced T cells were delivered via RO injection. CAR T cells expressing the ML73 CAR demonstrated complete tumor regression, whereas, by comparison, CAR T cells expressing a comparator CAR demonstrated only modest delays in tumor progression relative to the untransduced T cell group (**FIG. 7A**). Furthermore, CAR T cells expressing the ML73 CAR

significantly improved the overall survival of the mice, with 100% of mice surviving in the ML73 group at the end of study, compared to only 20% who received CAR T cells expressing the comparator CAR and 0% in the untransduced T cell group (**FIG. 7B**).

Furthermore, experiments to assess the function of the ML73 CAR delivered via alternative gene delivery mechanisms were performed. To this end, the ML73 CAR was reformatted as an HDR template, delivered via AAV6, and knocked-in to the TRAJ intron splice acceptor locus. Further, in these experiments, expression of the ML73 CAR was controlled by the endogenous TRAC promoter. TRAJ intron splice acceptor knock-in of the ML73 CAR into primary human T cells demonstrates good expression across three donors, albeit with lower percent CAR positivity (**FIG. 8A**) and lower MFI (**FIG. 8B**) compared to the CAR delivered by LVV under an MND promoter, which is consistent with AAV HDRt expression driven by the endogenous TRAC promoter. Furthermore, the CAR delivered via AAV TRAJ intron splice acceptor KI maintains reactivity to DLL3+ tumor cells as measured by IFNgamma secretion (**FIG. 8C**) and IL-2 secretion (**FIG. 8D**) when co-cultured with antigen positive SHP77 and DMS-273 tumor cell lines. Importantly the ML73 CAR delivered by either AAV or LVV did not demonstrate reactivity to the antigen negative cell line, A375 (**FIGS. 8C and 8D**). This data demonstrates that the ML73 CAR is a potent and specific CAR that can be delivered to T cells via multiple methods. Further, this data demonstrates that ML73 is a membrane proximal DLL3 binder with superior *in vitro* and *in vivo* potency compared to numerous preclinical and clinical development.

Example 3: In Vivo Assessment of CAR T Cells with an anti-DLL3 Chimeric Antigen Receptor Inserted into the TRAJ Intron Splice Acceptor Locus

This Example describes the in vivo assessment of CAR T cells with an anti-DLL3 chimeric antigen receptor (CAR) inserted into the TRAJ intron splice acceptor locus.

Methods:

Cloning:

For targeted insertion into the TRAJ intron splice acceptor locus (and expression controlled by the TRAC promoter), nucleic acid sequences encoding the DLL3 CARs comprising an scFv, hinge domain, transmembrane domain, and intracellular signaling domains were synthesized and cloned into AAV6 viral vectors for use as a homology directed repair (HDR) template for knock-in to the TRAJ intron splice acceptor locus.

T cell manufacturing

T cells were electroporated with RNPs consisting of Cas9 protein and a single guide RNA targeting the TRAJ intron splice acceptor locus (sgRNA: CAGGGTTCTGGATATCTGT; SEQ

ID NO: 161). The cells were cultured in human T cell medium without serum (X-VIVO 15, 5% Human AB serum, 10mM HEPEs, and 1x Glutamax®, supplemented with 10ng/mL IL-7 and 5ng/mL IL-15) and transduced with an AAV6 vector at an MOI of 2.5e4 providing the HDR template (HDRT). AAV6 transduced T cell cultures were supplemented with the NHEJ inhibitor M3814 at a concentration of 1uM and rested overnight, then washed, and transferred to a G-Rex plate for expansion.

Cell Lines

The cancer cell lines used were the small cell lung carcinoma line SHP-77 (ATCC CRL-2195).

In vivo efficacy studies

5e6 SHP77 cells were delivered via subcutaneous injection into 8-10 week old female NSG mice. Mice were randomized into treatments groups by tumor volume. At day 17 post tumor injection, mice were treated with untransduced T cells, 1e6 CAR+ T cells, or 0.2e6 CAR+ T cells via RO injection. Untransduced T cells were matched to the highest total T cells infused for the CAR T groups. Tumors were measured by caliper 2x/week and followed for survival. N=10 animals per group.

Results:

Experiments to test the *in vivo* function of the ML73 CAR delivered via alternative gene delivery mechanisms were performed. To this end, the ML73 CAR was reformatted as an HDR template, delivered via AAV6, and knocked-in to the TRAJ intron splice acceptor locus. Further, in these experiments, expression of the ML73 CAR was controlled by the endogenous TRAC promoter. Specifically, NSG mice were engrafted with SHP77 SCLC cells via subcutaneous injection and on day 17 post tumor injection, 1e6 CAR+T cells, 0.2e6 CAR+ T cells, or untransduced T cells were delivered via RO injection. CAR T cells with the ML73 CAR inserted into the TRAJ intron splice acceptor locus demonstrated complete tumor regression with 10/10 complete responses at the 1e6 dose (**FIG. 9B**) and 6/10 complete responses at the 0.2e6 dose (**FIG. 9C**).

IX. SEQUENCE TABLE

The table below provides exemplary sequences referred to in this disclosure.

SEQ ID NO	DESCRIPTION	SEQUENCE
1	Anti-DLL3 heavy chain CDR1 (CDRH1) Kabat	GIDFSSYYYMC
2	Anti-DLL3 CDRH2 Kabat	CVNMESGLAAYATWAKG
3	Anti-DLL3 CDRH3 Kabat	ARYESSSGYMDL
4	Anti-DLL3 light chain CDR1 (CDRL1) Kabat	QASQSIYSFLA
5	Anti-DLL3 CDRL2 Kabat	ETSKLAS
6	Anti-DLL3 CDRL3 Kabat	HQAYSYANIDNV
7	Anti-DLL3 heavy chain variable region (VH)	EVQLVESGGGLVQPGGSLRLSCTASGIDFSSYYYMCWVRQAPGKGLEWVGC VNMESGLAAYATWAKGRFTISRDTSKNTVYLQMNSLRAEDTAVYYCARYES SSGYMDLWGQGTLVTVSS
8	Anti-DLL3 light chain variable region (VL) (C-terminal LTVL)	EIVMTQSPSTLSASVGDRVIITCQASQSIYSFLAWYQQKPGKAPKLLIYET SKLASGVPSRFSGSGSGAEFTLTISSLQPDDFATYYCHQAYSYANIDNVFG QGTKLTVL
9	Anti-DLL3 chimeric antigen receptor (CAR) amino acid sequence (including signal sequence)	MALPVTALLLPLALLLHAARPEIVMTQSPSTLSASVGDRVIITCQASQSIY SFLAWYQQKPGKAPKLLIYETSKLASGVPSRFSGSGSGAEFTLTISSLQPD DFATYYCHQAYSYANIDNVFGQGTKVEIKGGGGSGGGGSGGGGSEVQLVES GGGLVQPGGSLRLSCTASGIDFSSYYYMCWVRQAPGKGLEWVGCVNMESGL AAYATWAKGRFTISRDTSKNTVYLQMNSLRAEDTAVYYCARYESSSGYMDL WGQGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT QEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR
10	Anti-DLL3 chimeric antigen receptor (CAR) amino acid sequence mature form(excluding signal sequence)	EIVMTQSPSTLSASVGDRVIITCQASQSIYSFLAWYQQKPGKAPKLLIYET SKLASGVPSRFSGSGSGAEFTLTISSLQPDDFATYYCHQAYSYANIDNVFG QGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTASGID FSSYYYMCWVRQAPGKGLEWVGCVNMESGLAAYATWAKGRFTISRDTSKNT VYLQMNSLRAEDTAVYYCARYESSSGYMDLWGQGTLVTVSSTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR
11	Anti-DLL3 CAR polynucleotide sequence	atggccctgccagtgacagccctgctgttgccactggccttgctgctccac gctgccagaccagaaatcgtcatgactcagagcccgctctacactcagtgcc tctgtcggagacagagtcatcataacgtgccaagcttcccagagcatttat agcttcctggcttggtatcagcagaagcctggaaaggctccgaagctgctc atctacgaaacctctaagctcgcttccggcgtgccatctcgcttcagcggc tctggaagcggcgctgagttcaccctgaccattagcctctccaacctgac gacttcgccacatactattgccaccaagcttacagctacgccaacatcgat aacgtcttcggacaaggcaccaaagtcgaaatcaagggcggtggaggatct ggaggtggcggaagtggaggaggtggttctgaggtccaactggtcgaaagc ggtggcggtctggtacagccaggaggctccctgcgactgagctgtaccgct tctggcattgacttctcttcctactactacatgtgctgggtcagacaggct cctggcaagggtctggagtgggtcggatgcgtcaacatggagagcggactg gctgcctacgctacttgggccaagggtcgcttcacaatcagtagagacaca tctaagaacaccgtgtacctccaaatgaactctctcagagctgaggacacc

SEQ ID NO	DESCRIPTION	SEQUENCE
		gctgtgtactattgcgccagatacgagtccagttccggctacatggacctg tggggacaaggaacactcgttacagtcagcagcaccacaacacctgctcca aggccacccacaccagctcccaccatcgccagtcagccactgagcctgaga cctgaagcctgtagaccagccgcaggaggagccgtgcataccagaggactg gacttcgcctgcgacatctacatctgggctcccttggctggaacttgtgga gtgctgctcctttctcttgtcattacactgtactgcaagagaggcaggaag aaactcctgtacatcttcaaacagcccttcatgagaccagtgcagaccact caggaggaagatggctgtagctgcagattcccagaagaggaggagggtggc tgtgagctgagagtgaagttcagccggagcgcagacgcacctgcctaccag caaggacagaaccagctgtacaacgagctgaacctgggaagaagggaagag tacgacgtgctggataagaggagaggaagggatcctgagatgggaggcaag ccaaggagaaagaaccctcaggaaggcctgtacaatgaactccagaaggac aagatggccgaagcctactctgagattggcatgaaaggcgagagacggaga ggcaaaggccacgatggactgtaccagggactgagtactgctaccaaagac acctacgatgccctgcacatgcaagcactgccacccagatga
12	CD8 signal sequence	MALPVTALLLPLALLLHAARP
13	Polynucleotide encoding CD8 signal sequence	atggccctgccagtgacagccctgctgttgccactggccttgctgctccac gctgccagacca
14	Polynucleotide encoding anti-DLL VH	gaggtccaactggtcgaaagcggtggcggtctggtacagccaggaggctcc ctgcgactgagctgtaccgcttctggcattgacttcgcttcctactactac atgtgctgggtcagacaggctcctggcaagggtctggagtgggtcggatgc gtcaacatggagagcggactggctgcctacgctactcgggccaagggtcgc ttcacaatcagtagagacacatctaagaacaccgtgcacctccaaatgaac tctctcagagctgaggacaccgctgtgtactattgcgccagatacgagtcc agttccggctacatggacctgtggggacaaggaacactcgttacagtcagc agc
15	Polynucleotide encoding anti-DLL VL	gaaatcgtcatgactcagagcccgtctacactcagtgcctctgtcggagac agagtcatcataacgtgccaagcttcccagagcattcatagcttcctggct tggtaccagcagaagcctggaaaggctccgaagctgctcatctacgaaacc tctaagctcgcttccggcgtgccatctcgcttcagcggctctggaagcggc gctgagttcaccctgaccattagctctctccaacctgacgacttcgccaca tactattgccaccaagcttacagctacgccaacatcgataacgtcttcgga caaggcaccaaagtcgaaatcaag
16	Glycine serine linker $(GGGGS)_3$ or G4Sx3	GGGGSGGGGSGGGGS
17	Polynuceotide encoding glycine serine linker	ggcggtggaggatctggaggtggcggaagtggaggaggtggttct
18	CD8 hinge domain	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
19	Polynucleotide encoding CD8 hinge	accacaacacctgctccaaggccacccacaccagctcccaccatcgccagt cagccactgagcctgagacctgaagcctgtagaccagccgcaggaggagcc gtgcataccagaggactggactttgcctgcgac
20	CD8 transmembrane domain (TM)	IYIWAPLAGTCGVLLLSLVITLYC
21	Polynucleotide encoding CD8 transmembrane domain	atctacatctgggctcccttggctggaacttgtggagtgctgctcctttct cttgtcattacactgtactgc
22	41BB signaling domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
23	Polynucleotide encoding 41BB	aagagaggcaggaagaaactcctgtacatcttcaaacagcccttcatgaga ccagtgcagaccactcaggaggaagatggctgtagctgcagattcccagaa gaggaggagggtggctgtgagctg

SEQ ID NO	DESCRIPTION	SEQUENCE
	signaling domain	
24	CD3zeta signaling domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
25	Polynucleotide encoding CD3zeta signaling domain	agagtgaagttcagccggagcgcagacgcacctgcctaccagcaaggacag aaccagctgtacaacgagctgaacctgggaagaagggaagagtacgatgtg ctggataagaggagaggaagggatcctgagatgggaggcaagccaaggaga aagaaccctcaggaaggcctgtacaatgaactccagaaggacaagatggcc gaagcctactctgagattggcatgaaaggcgagagacggagaggcaaaggc cacgatggactgtaccagggactgagtactgctaccaaagacacctacgat gccctgcacatgcaagcactgccacccagatga
26	Human DLL3	MVSPRMSGLLSQTVILALIFLPQTRPAGVFELQIHSFGPGPGPGAPRSPCS ARLPCRLFFRVCLKPGLSEEAAESPCALGAALSARGPVYTEQPGAPAPDLP LPDGLLQVPFRDAWPGTFSFIIETWREELGDQIGGPAWSLLARVAGRRRLA AGGPWARDIQRAGAWELRFSYRARCEPPAVGTACTRLCRPRSAPSRCGPGL RPCAPLEDECEAPLVCRAGCSPEHGFCEQPGECRCLEGWTGPLCTVPVSTS SCLSPRGPSSATTGCLVPGPGPCDGNPCANGGSCSETPRSFECTCPRGFYG LRCEVSGVTCADGPCFNGGLCVGGADPDSAYICHCPPGFQGSNCEKRVDRC SLQPCRNGGLCLDLGHALRCRCRAGFAGPRCEHDLDDCAGRACANGGTCVE GGGAHRCSCALGFGGRDCRERADPCAARPCAHGGRCYAHFSGLVCACAPGY MGARCEFPVHPDGASALPAAPPGLRPGDPQRYLLPPALGLLVAAGVAGAAL LLVHVRRRGHSQDAGSRLLAGTPEPSVHALPDALNNLRTQEGSGDGPSSSV DWNRPEDVDPQGIYVISAPSIYAREVATPLFPPLHTGRAGQRQHLLFPYPS SILSVK
27	EGF6 domain of human DLL3	RADPCAARPCAHGGRCYAHFSGLVCACAPGYMGARCE
28	Comp 4 CAR (including CD8 signal sequence)	MALPVTALLLPLALLLHAARPDIVLTQSPASLAVSLGQRATISCKASQSVD YDGDSYLTWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHP VEEEDAATYYCQQSNEDPYTFGGGTKLEIKRGGGGSGGGGSGGGGSEVQLQ QSGPDLVKPGASVKISCKASGYSFTGYYMHWVKQSHGKSLEWIGRVNPNNG GTSYNQKFKGKAILTADKSSSTAYMELRSLTSEDSAVYYCARGSYDYAEGW GQGTLVTVSATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQ EEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR
29	Comp 4 CAR DNA	ATGGCCCTGCCAGTGACAGCCCTGCTGTTGCCACTGGCCTTGCTGCTCCAC GCTGCCAGACCAGACATTGTGCTGACCCAGAGCCCAGCCTCCCTCGCTGTG TCCCTGGGACAAAGAGCCACCATCAGTTGCAAAGCAAGCCAGAGTGTGGAC TACGACGGAGATTCTTACCTGACCTGGTATCAGCAGAAACCAGGACAACCT CCCAAGCTGCTGATCTACGCTGCCTCCAACCTGGAGTCTGGCATCCCTGCT AGATTCTCTGGGAGCGGCTCTGGAACCGACTTCACCCTGAACATCCATCCT GTGGAGGAAGAAGACGCAGCCACCTACTATTGCCAACAGAGCAACGAGGAT CCATACACCTTTGGCGGTGGCACCAAGCTGGAAATCAAAAGAGGCGGTGGA GGATCTGGAGGTGGCGGAAGTGGAGGAGGTGGTTCTGAAGTGCAACTGCAG CAGTCTGGACCTGATCTGGTGAAACCCGGAGCCAGCGTGAAGATCTCCTGC AAGGCCTCTGGCTACAGCTTTACAGGCTACTACATGCACTGGGTCAAACAG TCCCACGGCAAGTCTCTGGAGTGGATTGGCAGAGTGAATCCCAACAACGGC GGAACAAGCTACAATCAGAAGTTCAAAGGCAAAGCCATCCTGACTGCTGAC AAGTCTAGCAGCACAGCCTACATGGAGCTTAGAAGCCTCACCTCTGAGGAC AGTGCCGTGTATTACTGCGCTAGAGGCAGCTACGACTACGCTGAGGGTTGG GGACAAGGAACACTGGTGACCGTGTCCGCTACCACAACACCTGCTCCAAGG CCACCCACACCAGCTCCCACCATCGCCAGTCAGCCACTGAGCCTGAGACCT GAAGCCTGTAGACCAGCCGCAGGAGGAGCCGTGCATACCAGAGGACTGGAC TTTGCCTGCGACATCTACATCTGGGCTCCCTTGGCTGGAACTTGTGGAGTG CTGCTCCTTTCTCTTGTCATTACACTGTACTGCAAGAGAGGCAGGAAGAAA CTCCTGTACATCTTCAAACAGCCCTTCATGAGACCAGTGCAGACCACTCAG GAGGAAGATGGCTGTAGCTGCAGATTCCCAGAAGAGGAGGAGGGTGGCTGT

SEQ ID NO	DESCRIPTION	SEQUENCE
		GAGCTGAGAGTGAAGTTCAGCCGGAGCGCAGACGCACCTGCCTACCAGCAA GGACAGAACCAGCTGTACAACGAGCTGAACCTGGGAAGAAGGGAAGAGTAC GATGTGCTGGATAAGAGGAGAGGAAGGGATCCTGAGATGGGAGGCAAGCCA AGGAGAAAGAACCCTCAGGAAGGCCTGTACAATGAACTCCAGAAGGACAAG ATGGCCGAAGCCTACTCTGAGATTGGCATGAAAGGCGAGAGACGGAGAGGC AAAGGCCACGATGGACTGTACCAGGGACTGAGTACTGCTACCAAAGACACC TACGATGCCCTGCACATGCAAGCACTGCCACCCAGATGA
30	Comp 1 CAR (including CD8 signal sequence)	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPASISCRSSQSLV YRDGNTYLNWFQQRPGQSPRRLIYKVSNRDSGVPDRFRGSGSGTDFTLKIS RVEAEDVGVYYCMQGTHWPPTFGQGTKVEIKGGGGSGGGGSGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGIIDPSDG STNYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREYNYYGL DVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPR
31	Comp 1 CAR DNA	ATGGCCCTGCCAGTGACAGCCCTGCTGTTGCCACTGGCCTTGCTGCTCCAC GCTGCCAGACCAGACGTGGTGATGACCCAGAGCCCACTGTCCCTGCCTGTG ACCCTCGGACAGCCTGCCAGCATCAGTTGCAGATCCAGCCAGAGCCTGGTG TACAGAGACGGCAACACCTACCTGAACTGGTTCCAGCAGAGACCAGGACAA TCTCCAAGGAGGCTGATCTACAAAGTCTCTAACAGAGACTCTGGCGTGCCA GACAGATTCAGAGGCAGCGGCTCTGGAACCGACTTCACCCTCAAGATCAGC AGAGTGGAAGCCGAGGACGTGGGAGTGTACTACTGCATGCAAGGCACCCAT TGGCCACCTACATTTGGCCAAGGAACCAAGGTGGAAATCAAGGGCGGTGGA GGATCTGGAGGTGGCGGAAGTGGAGGAGGTGGTTCTCAAGTCCAGCTGGTG CAGTCTGGAGCTGAAGTGAAGAAGCCTGGAGCCAGCGTGAAAGTGTCCTGC AAGGCCTCTGGCTACACCTTTACATCCTACTACATCCACTGGGTCAGACAG GCTCCCGGACAAGGCCTGGAGTGGATGGGCATCATCGATCCCAGCGACGGT AGCACCAACTACGCCCAGAAGTTCCAAGGACGGGTGACAATGACCAGAGAC ACATCTACCAGCACCGTGTACATGGAGCTGAGCAGCCTCCGGCTCTGAGGAC ACAGCCGTGTATTACTGCGCCAGAGATAGAGAATACAACTACTACGGCTTG GACGTGTGGGGACAAGGCACAACGGTGACCGTGTCCTCTACCACAACACCT GCTCCAAGGCCACCCACACCAGCTCCCACCATCGCCAGTCAGCCACTGAGC CTGAGACCTGAAGCCTGTAGACCAGCCGCAGGAGGAGCCGTGCATACCAGA GGACTGGACTTTGCCTGCGACATCTACATCTGGGCTCCCTTGGCTGGAACT TGTGGAGTGCTGCTCCTTTCTCTTGTCATTACACTGTACTGCAAGAGAGGC AGGAAGAAACTCCTGTACATCTTCAAACAGCCCTTCATGAGACCAGTGCAG ACCACTCAGGAGGAAGATGGCTGTAGCTGCAGATTCCCAGAAGAGGAGGAG GGTGGCTGTGAGCTGAGAGTGAAGTTCAGCCGGAGCGCAGACGCACCTGCC TACCAGCAAGGACAGAACCAGCTGTACAACGAGCTGAACCTGGGAAGAAGG GAAGAGTACGATGTGCTGGATAAGAGGAGAGGAAGGGATCCTGAGATGGGA GGCAAGCCAAGGAGAAAGAACCCTCAGGAAGGCCTGTACAATGAACTCCAG AAGGACAAGATGGCCGAAGCCTACTCTGAGATTGGCATGAAAGGCGAGAGA CGGAGAGGCAAAGGCCACGATGGACTGTACCAGGGACTGAGTACTGCTACC AAAGACACCTACGATGCCCTGCACATGCAAGCACTGCCACCCAGATGA
32	Comp 3 CAR	MALPVTALLLPLALLLHAARPQAQLQQSGAELVRPGTSVKVSCKASGYAFT NYLIEWVKQRPGQGLEWIGVINPGTGGTNYNENFKGKATLTADKSSSTAYM QLSSLTSDDSAVYFCARSPYDYHEGAMDYWGQGTSVTVSSGGGGSGGGGSG GGGSEIQMTQSPSSMSASLGDRITITCQATQDIVKNLNWYQQKPGKPPSFL IYYAIELAEGVPSRFSGSGSGSDYSLTISNLESEDFADYYCLQFYEFPFTF GAGTKLELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
33	Comp 3 CAR DNA	ATGGCCCTGCCAGTGACAGCCCTGCTGTTGCCACTGGCCTTGCTGCTCCAC GCTGCCAGACCACAGGCCCAGCTGCAGCAGTCTGGAGCTGAGCTCGTTAGA CCCGGAACCTCTGTGAAAGTCAGCTGCAAGGCCTCTGGCTACGCCTTCACC

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		AACTACCTGATCGAGTGGGTCAAGCAGAGACCTGGCCAAGGCCTGGAGTGG ATCGGCGTGATCAATCCTGGCACCGGAGGCACTAACTACAACGAGAACTTC AAGGGCAAAGCCACCCTGACTGCCGACAAGAGCTCCAGCACTGCTTACATG CAACTGAGCTCCCTGACCTCTGACGATTCTGCCGTGTACTTCTGTGCTCGG TCTCCCTACGACTACCACGAAGGAGCCATGGACTACTGGGGACAAGGCACC TCCGTGACCGTGTCCTCCGGCGGTGGAGGATCTGGAGGTGGCGGAAGTGGA GGAGGTGGTTCTGAAATCCAGATGACCCAGAGCCCATCCAGCATGAGCGCC TCTCTGGGCGACAGAATCACCATCACCTGTCAAGCCACCCAGGACATCGTG AAGAACCTGAACTGGTATCAACAGAAGCCTGGCAAGCCACCTAGCTTCCTG ATCTACTACGCCATCGAGCTGGCCGAAGGCGTGCCTAGCAGGTTTAGCGGC AGCGGAAGCGGCTCTGACTACAGCCTGACCATCAGCAACCTGGAGTCCGAG GACTTCGCTGACTATTACTGCCTGCAGTTCTACGAGTTTCCATTCACCTTC GGTGCCGGAACCAAACTCGAGCTGAAGACCACAACACCTGCTCCAAGGCCA CCCACACCAGCTCCCACCATCGCCAGTCAGCCACTGAGCCTGAGACCTGAA GCCTGTAGACCAGCCGCAGGAGGAGCCGTGCATACCAGAGGACTGGACTTT GCCTGCGACATCTACATCTGGGCTCCCTTGGCTGGAACTTGTGGAGTGCTG CTCCTTTCTCTTGTCATTACACTGTACTGCAAGAGAGGCAGGAAGAAACTC CTGTACATCTTCAAACAGCCCTTCATGAGACCAGTGCAGACCACTCAGGAG GAAGATGGCTGTAGCTGCAGATTCCCAGAAGAGGAGGAGGGTGGCTGTGAG CTGAGAGTGAAGTTCAGCCGGAGCGCAGACGCACCTGCCTACCAGCAAGGA CAGAACCAGCTGTACAACGAGCTGAACCTGGGAAGAAGGGAAGAGTACGAT GTGCTGGATAAGAGGAGAGGAAGGGATCCTGAGATGGGAGGCAAGCCAAGG AGAAAGAACCCTCAGGAAGGCCTGTACAATGAACTCCAGAAGGACAAGATG GCCGAAGCCTACTCTGAGATTGGCATGAAAGGCGAGAGACGGAGAGGCAAA GGCCACGATGGACTGTACCAGGGACTGAGTACTGCTACCAAAGACACCTAC GATGCCCTGCACATGCAAGCACTGCCACCCAGATGA
34	Comp 5 CAR	MALPVTALLLPLALLLHAARPEIVLTQSPGTLSLSPGERVTLSCRASQRVN NNYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYDRSPLTFGGGTKLEIKGGGGSGGGGSGGGGSQVQLQESGP GLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYVYYSGTINYN PSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCASIAVTGFYFDYWGQG TLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
35	Comp 5 CAR DNA	ATGGCCCTGCCAGTGACAGCCCTGCTGTTGCCACTGGCCTTGCTGCTCCAC GCTGCCAGACCAGAGATTGTGCTGACCCAGAGCCCTGGAACACTCAGCCTG TCTCCTGGAGAAAGAGTCACCCTCTCCTGCCGTGCCAGCCAAAGAGTGAAC AATAACTACCTTGCTTGGTATCAACAAAGACCTGGGCAAGCTCCTAGACTC CTGATCTATGGTGCATCTTCTAGAGCCACTGGAATCCCAGATAGATTCTCT GGGAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCAGACTGGAACCT GAGGACTTCGCTGTTTACTATTGCCAACAGTACGACAGATCTCCACTCACC TTTGGCGGTGGTACCAAGCTGGAAATCAAGGGCGGTGGAGGATCTGGAGGT GGCGGAAGTGGAGGAGGTGGTTCTCAAGTCCAGCTGCAAGAGTCTGGACCT GGACTGGTGAAGCCTTCCGAGACCCTTAGCCTGACCTGCACCGTGAGCGGA GGTTCCATCTCTTCCTACTACTGGTCCTGGATCAGACAGCCTCCCGGAAAG GGACTGGAGTGGATCGGCTACGTCTATTACAGCGGAACCACAAACTACAAC CCTAGCCTCAAGAGCAGAGTGACCATCTCCGTGGATACATCCAAGAACCAA TTCAGCCTGAAGCTGAGCTCTGTGACAGCTGCCGATACAGCCGTGTATTAC TGTGCCAGCATCGCCGTCACTGGGTTCTACTTCGACTACTGGGGACAAGGA ACACTGGTGACCGTGTCCTCTACCACAACACCTGCTCCAAGGCCACCCACA CCAGCTCCCACCATCGCCAGTCAGCCACTGAGCCTGAGACCTGAAGCCTGT AGACCAGCCGCAGGAGGAGCCGTGCATACCAGAGGACTGGACTTTGCCTGC GACATCTACATCTGGGCTCCCTTGGCTGGAACTTGTGGAGTGCTGCTCCTT TCTCTTGTCATTACACTGTACTGCAAGAGAGGCAGGAAGAAACTCCTGTAC ATCTTCAAACAGCCCTTCATGAGACCAGTGCAGACCACTCAGGAGGAAGAT GGCTGTAGCTGCAGATTCCCAGAAGAGGAGGAGGGTGGCTGTGAGCTGAGA GTGAAGTTCAGCCGGAGCGCAGACGCACCTGCCTACCAGCAAGGACAGAAC

SEQ ID NO	DESCRIPTION	SEQUENCE
		CAGCTGTACAACGAGCTGAACCTGGGAAGAAGGGAAGAGTACGATGTGCTG GATAAGAGGAGAGGAAGGGATCCTGAGATGGGAGGCAAGCCAAGGAGAAAG AACCCTCAGGAAGGCCTGTACAATGAACTCCAGAAGGACAAGATGGCCGAA GCCTACTCTGAGATTGGCATGAAAGGCGAGAGACGGAGAGGCAAAGGCCAC GATGGACTGTACCAGGGACTGAGTACTGCTACCAAAGACACCTACGATGCC CTGCACATGCAAGCACTGCCACCCAGATGA
36	Comp 2 CAR	MALPVTALLLPLALLLHAARPQVQLVESGGGVVQPGGSLRLSCAASFSGYG VSTMAWFRQAPGKGLEGVAAITVGSGNTYYADSVKGRFTISRDNSKNTVYL QMNSLRAEDTAMYYCAVGYLSGGSWDVPGRYNYWGQGTLVTVSSGGGGSGG GGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGNTYSSNYMGWFRQAPGK GLEEVAVIYTRGGHTYYVDSVRGRFTISQDNAKNSLYLQMNSLRAEDTAVY YCAASSRHRLGLNNPRDYDYWGQGTLVTVSSTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
37	Comp 2 CAR DNA	ATGGCCCTGCCAGTGACAGCCCTGCTGTTGCCACTGGCCTTGCTGCTCCAC GCTGCCAGACCACAGGTCCAGCTGGTCGAGAGCGGAGGTGGAGTTGTGCAG CCTGGCGGATCACTGAGACTCAGTTGCGCTGCAAGCTTCAGTGGCTACGGA GTGTCTACCATGGCCTGGTTTAGGCAGGCACCAGGAAAAGGCCTGGAGGGA GTCGCTGCCATCACCGTCGGTTCCGGAAACACCTACTACGCCGATTCCGTC AAGGGTAGATTCACTATCAGCAGGGATAACTCAAAGAACACCGTCTACCTC CAAATGAATTCTCTGAGAGCTGAAGACACAGCCATGTACTATTGCGCAGTG GGCTATCTGTCTGGTGGCAGCTGGGACGTTCCAGGCAGATACAACTACTGG GGACAAGGCACACTTGTGACAGTCAGTTCCGGCGGTGGAGGACTCTGGAGGT GGCGGAAGTGGAGGAGGTGGTTCTGAAGTGCAACTGGTCGAGTCCGGAGGC GGTCTGGTGCAACCCGGAGGCAGCCTGAGGCTCTCTTGTGCTGCATCTGGC AACACCTATTCTAGCAACTACATGGGTTGGTTTAGACAGGCTCCTGGCAAA GGACTGGAGGAAGTTGCCGTCATCTACACCCGTGGTGGCCACACATACTAC GTTGACAGCGTCAGAGGCAGATTCACCATCTCTCAAGACAACGCTAAGAAC TCTCTGTATCTTCAAATGAATAGCCTCAGAGCTGAGGACACTGCCGTGTAT TACTGCGCTGCATCATCTAGACACAGACTGGGACTCAACAATCCCAGAGAC TACGACTATTGGGGTCAAGGAACACTCGTGACCGTGTCCTCTACCACAACA CCTGCTCCAAGGCCACCCACACCAGCTCCCACCATCGCCAGTCAGCCACTG AGCCTGAGACCTGAAGCCTGTAGACCAGCCGCAGGAGGAGCCGTGCATACC AGAGGACTGGACTTTGCCTGCGACATCTACATCTGGGCTCCCTTGGCTGGA ACTTGTGGAGTGCTGCTCCTTTCTCTTGTCATTACACTGTACTGCAAGAGA GGCAGGAAGAAACTCCTGTACATCTTCAAACAGCCCTTCATGAGACCAGTG CAGACCACTCAGGAGGAAGATGGCTGTAGCTGCAGATTCCCAGAAGAGGAG GAGGGTGGCTGTGAGCTGAGAGTGAAGTTCAGCCGGAGCGCAGACGCACCT GCCTACCAGCAAGGACAGAACCAGCTGTACAACGAGCTGAACCTGGGAAGA AGGGAAGAGTACGATGTGCTGGATAAGAGGAGAGGAAGGGATCCTGAGATG GGAGGCAAGCCAAGGAGAAAGAACCCTCAGGAAGGCCTGTACAATGAACTC CAGAAGGACAAGATGGCCGAAGCCTACTCTGAGATTGGCATGAAAGGCGAG AGACGGAGAGGCAAAGGCCACGATGGACTGTACCAGGGACTGAGTACTGCT ACCAAAGACACCTACGATGCCCTGCACATGCAAGCACTGCCACCCAGATGA
38	Single G4S linker	GGGS
39	Single G4S linker DNA	GGTGGAGGCGGTTCT
40	G4Sx2 Linker	GGGGSGGGGS
41	G4Sx4 Linker	GGGGSGGGGSGGGGSGGGGS
42	G4Sx5 Linker	GGGGSGGGGSGGGGSGGGGSGGGGS
43	SG4S linker	SGGGGS
44	SG4S linker DNA	AGCGGAGGCGGAGGCTCT
45	Whitlow Linker	GSTSGSGKPGSGEGSTKG
46	Linker	DGGGS
47	Linker	TGEKP
48	Linker	GGRR

SEQ ID NO	DESCRIPTION	SEQUENCE
49	Linker	EGKSSGSGSESKVD
50	Linker	KESGSVSSEQLAQFRSLD
51	Linker	GGRRGGGS
52	Linker	LRQRDGERP
53	Linker	LRQKDGGGSGGGSERP
54	Linker	GSTSGSGKPGSGEGSTKG
55	Linker	GSTSGSGKSSEGSGSTKG
56	Linker	GSTSGSGKSSEGKG
57	Linker	GSTSGSGKPGSGEGS
58	Linker	GGGS
59	yuTCR Linker (uLNK)	LEKT
60	uLNK + G4S Linker	LEKTGGGGS
61	CD28 co-stimulatory domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
62	CD3zeta (Q to K mutation at position 14)	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
63	CD3zeta (1XX)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFD ALHMQALPPR
64	Rituximab epitope for depletion tag	CPYSNPSLC
65	Palivizumab epitope for depletion tag	SELLSLINDMPITNDQKKLMSNN
66	Cetuximab epitope 1 for depletion tag	CQFDLSTRRLKC
67	Cetuximab epitope 2 for depletion tag	CQYNLSSRALKC
68	Cetuximab epitope 3 for depletion tag	CVWQRWQKSYVC
69	Cetuximab epitope 4 for depletion tag	CMWDRFSRWYKC
70	Nivolumab epitope 1 for depletion tag	SFVLNWYRMSPSNQTDKLAAFPEDR
71	Nivolumab epitope 2 for depletion tag	SGTYLCGAISLAPKAQIKE
72	Qbend-10 epitope for depletion tag	ELPTQGTFSNVSTNVSPAKPTTTA
73	Alemtuzumab epitope for depletion tag	GQNDTSQTSSPS
74	IgK Signal Peptide	METDTLLLWVLLLWVPGSTG
75	GMCSFR-alpha (CSF2RA) signal peptide	MLLLVTSLLLCELPHPAFLLIP

SEQ ID NO	DESCRIPTION	SEQUENCE
76	2A Peptide	GSGATNFSLLKQAGDVEENPGP
77	2A Peptide	ATNFSLLKQAGDVEENPGP
78	2A Peptide	LLKQAGDVEENPGP
79	2A Peptide	GSGEGRGSLLTCGDVEENPGP
80	2A Peptide	EGRGSLLTCGDVEENPGP
81	2A Peptide	LLTCGDVEENPGP
82	2A Peptide	GSGQCTNYALLKLAGDVESNPGP
83	2A Peptide	QCTNYALLKLAGDVESNPGP
84	2A Peptide	LLKLAGDVESNPGP
85	2A Peptide	GSGVKQTLNFDLLKLAGDVESNPGP
86	2A Peptide	VKQTLNFDLLKLAGDVESNPGP
87	2A Peptide	LLKLAGDVESNPGP
88	2A Peptide	LLNFDLLKLAGDVESNPGP
89	2A Peptide	TLNFDLLKLAGDVESNPGP
90	2A Peptide	LLKLAGDVESNPGP
91	2A Peptide	NFDLLKLAGDVESNPGP
92	2A Peptide	QLLNFDLLKLAGDVESNPGP
93	2A Peptide	APVKQTLNFDLLKLAGDVESNPGP
94	2A Peptide	VTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVKQT
95	2A Peptide	LNFDLLKLAGDVESNPGP
96	2A Peptide	LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
97	2A Peptide	EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
98	Spacer Sequence	SGSG
99	Self-Cleaving Sequence	RAKR
100	Minimal CD4 Hinge	SNIKVLPTWSTPVQP
101	CD28 Hinge Domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP
102	CD28 Hinge Domain	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP
103	Minimal CD28 Hinge	KGKHLCPSPLFPGPSKP
104	IgG4 Hinge Domain	ESKYGPPCPPCP
105	IgG4 Hinge CH2-CH3 Domain	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLSLGK
106	Hinge Linker	GGR
107	CD28 Transmembrane Domain	FWVLVVVGGVLACYSLLVTVAFIIFWV
108	CD28 Transmembrane Domain	MFWVLVVVGGVLACYSLLVTVAFIIFWV
109	CD4 Transmembrane Domain	MALIVLGGVAGLLFIGLGIFF
110	CD28 Costimulatory Domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
111	CD4 Coreceptor Domain	CVRCRHRRRQAERMSQIKRLLSEKKTCQCPHRFQKTCSPI
112	CD8 Coreceptor Domain	LYCNHRNRRRVCKCPRPWKSGDKPSLSARYV

SEQ ID NO	DESCRIPTION	SEQUENCE
113	Patient-derived CARD11-PIK3R3	ATGGACGACTACATGGAGACCCTGAAAGACGAGGAGGACGCCCTGTGGGAG AACGTGGAGTGCAACAGGCACATGCTGAGCAGATACATCAACCCTGCCAAG CTGACACCCTACCTGAGACAGTGCAAGGTGATCGACGAGCAGGACGAGGAC GAAGTGCTGAACGCTCCCATGCTGCCCTCTAAAATCAACAGAGCCGGCAGA CTGCTGGACATCCTGCACACCAAGGGCCAGAGAGGCTACGTGGTGTTCCTG GAGAGCCTGGAGTTCTACTATCCCGAGCTCTACAAGCTCGTGACCGGCAAG GAGCCCACCAGAAGATTCAGCACCATTGTGGTGGAGGAGGGACACGAAGGC CTGACCCACTTCCTGATGAACGAGGTGATCAAGCTGCAACAGCAAATGAAG GCCAAAGACCTGCAACGGTGCGAGCTGCTGGCCAGACTGAGGCAGCTGGAG GACGAGAAGAAGCAGATGACCCTGACCAGAGTGGAACTGCTGACCTTCCAA GAGAGATACTACAAGATGAAAGAGGAGAGAGATAGCTACAACGACGAACTT GTCAAAGTGAAGGACGACAACTACAACCTGGCCATGAGATACGCCCAGCTG AGCGAAGAGAAGAACATGGCCGTGATGAGGAGCAGGGATCTGCAACTGGAG ATCGACCAGCTGAAGCACAGGCTGAACAAGATGGAAGAGGAGAGTGTAAGCTG GAAAGGAACCAGAGCCTGAAGCTGAAGAACGACATCGAGAACAGACCCAAG AAGGAGCAAGTGCTGGAGCTGGAGCGGGAAAACGAGATGCTCAAGACCAAG AATCAGCAGCTGCAGAGCATCATCCAAGCCGGAAAGAGAAGCCTGCCCGAC AGCGACAAGGCCATCCTGGATATTCTGGAGCACGATAGGAAGGAGGCCCTG GAAGACAGACAGGAACTGGTGAACAGAATCTACAATCTGCAAGAGGAAGCC AGACAGGCCGAGGAGCTGAGAGACAAATACCTGGAGGAAAAGGAGGACCTG GAACTGAAGTGCTCCACCCTGGGAAAGGACTGCGAGATGTACAAGCACAGA ATGAACACCGTGATGCTGCAGCTGGAAGAGGTCGAGAGGGAGAGAGACCAG GCCTTCCACAGCAGGGACGAAGCCCAGACACAGTACAGCCAGTGCCTGATC GAGAAGGACAAGTACAGGAAGCAGATCAGAGAACTGGAGGAGAAGAACGAC GAGATGAGGATCGAGATGGTCAGAAGGGAGGCCTGCATCGTGAACCTGGAA AGCAAGCTGAGAAGGCTGTCCAAGGACAGCAACAATCTGGATCAGAGTCTG CCCAGAAACCTGCCCGTGACCATCATCAGCCAGGACTTCGGCGACGCCAGC CCAAGGACCAACGGACAGGAGGCCGACGACAGCTCTACCAGCGAAGAGTCT CCCGAGGACTCTAAATACTTCCTGCCCTACCATCCTCCACAAAGAAGGATG AACCTGAAGGGCATTCAACTCCAGAGAGCCAAGAGCCCAATCAGTCTGAAG AGAACCAGCGACTTCCAAGCCAAAGGCCACGAGGAAGAAGGCACAGACGCC TCTCCAAGCTCCTGCGGCTCTCTGCCCATCACCAACTCCTTCACCAAGATG CAGCCACCCAGGAGTAGAAGCAGCATCATGTCCATCACAGCCGAGCCACCT GGCAACGACTCCATCGTGAGAAGATACAAGGAAGACGCTCCACATAGGAGG TGGCTGAACCACAAGGGAGTGAGACAGAAGAGACTGAACGTGTGGCTGGGC ATCAAGAACGAAGACGCTGCCGAGAACTACTTCATCAACGAAGAAGACGAG AACCTGCCTCACTACGACGAAAAGACTTGGTTCGTGGAGGACATCAATAGG GTGCAAGCCGAGGATCTGCTGTACGGCAAGCCCGACGGAGCCTTTCTGATT CGGGAGAGCAGCAAGAAGGGCTGCTACGCCTGCTCTGTGGTGGCCGACGGC GAAGTGAAGCACTGCGTGATCTACAGCACAGCCAGAGGATACGGCTTCGCC GAGCCATACAACCTGTACAGCAGCCTCAAAGAGCTGGTGCTGCACTACCAG CAGACCAGCCTGGTGCAGCACAACGATTCCCTCAACGTGAGACTGGCCTAC CCTGTGCACGCCCAGATGCCCAGTCTGTGCAGATGA
114	Patient-derived CARD11-PIK3R3	MDDYMETLKDEEDALWENVECNRHMLSRYINPAKLTPYLRQCKVIDEQDED EVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVTGK EPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLRQLE DEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLAMRYAQL SEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLKNDIENRPK KEQVLELERENEMLKTKNQELQSIIQAGKRSLPDSDKAILDILEHDRKEAL EDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGKDCEMYKHR MNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRKQIRELEEKND EMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTIISQDFGDAS PRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQRAKSPISLK RTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSIMSITAEPP GNDSIVRRYKEDAPHRRWLNHKGVRQKRLNVWLGIKNEDAAENYFINEEDE NLPHYDEKTWFVEDINRVQAEDLLYGKPDGAFLIRESSKKGCYACSVVADG EVKHCVIYSTARGYGFAEPYNLYSSLKELVLHYQQTSLVQHNDSLNVRLAY PVHAQMPSLCR

SEQ ID NO	DESCRIPTION	SEQUENCE
115	Patient-derived CARD11-PIK3R3	ATGGACGACTACATGGAGACCCTGAAAGACGAGGAGGACGCCCTGTGGGAG AACGTGGAGTGCAACAGGCACATGCTGAGCAGATACATCAACCCTGCCAAG CTGACACCCTACCTGAGACAGTGCAAGGTGATCGACGAGCAGGACGAGGAC GAAGTGCTGAACGCTCCCATGCTGCCCTCTAAAATCAACAGAGCCGGCAGA CTGCTGGACATCCTGCACACCAAGGGCCAGAGGGGCTACGTGGTGTTCCTG GAGAGCCTGGAGTTCTACTATCCCGAGCTCTACAAGCTCGTGACCGGCAAG GAGCCCACCAGAAGATTCAGCACCATTGTGGTGGAGGAGGGACACGAAGGC CTGACCCACTTCCTGATGAACGAGGTGATCAAGCTGCAACAGCAAATGAAG GCCAAAGACCTGCAACGGTGCGAGCTGCTGGCCAGACTGAGGCAGCTGGAG GACGAGAAGAAGCAGATGACCCTGACCAGAGTGGAACTTCTGACCTTCCAA GAGAGATACTACAAGATGAAAGAGGAACGCGATAGCTACAACGACGAACTT GTCAAAGTGAAGGACGACAACTACAACCTGGCCATGAGATACGCCCAGCTG AGCGAAGAGAAGAACATGGCCGTGATGAGGAGCAGGGATCTGCAACTGGAG ATCGACCAGCTGAAGCACAGGCTGAACAAGATGGAAGAGGAGTGTAAGCTG GAACGGAACCAGAGCCTGAAGCTTAAGAACGACATCGAGAACAGACCCAAG AAGGAGCAAGTGCTGGAGCTGGAGCGCGAAAACGAGATGCTCAAGACCAAG AATCAGCAGTTGCAGAGCATCATCCAAGCCGGAAAGAGAAGCCTGCCCGAC AGCGACAAGGCCATCCTGGATATTCTGGAGCACGATAGGAAGGAGGCCCTG GAAGACAGACAAGAACTGGTGAACAGAATCTACAATCTTCAAGAGGAAGCC AGACAGGCAGAAGAGCTCAGGGACAAATACCTTGAGGAAAAGGAGGACCTG GAACTGAAGTGCTCCACCCTGGGAAAGGACTGCGAGATGTACAAGCACAGA ATGAACACCGTGATGCTGCAGCTGGAAGAGGTCGAGAGGGAGCGCGACCAG GCCTTCCACAGCAGGGACGAAGCCCAGACACAGTACAGCCAGTGCCTGATC GAGAAGGACAAGTACAGGAAGCAGATCAGAGAACTGGAGGAGAAGAATGAC GAGATGAGGATCGAGATGGTCAGAAGGGAGGCCTGCATCGTGAACCTGGAA AGCAAACTGAGAAGGCTTTCCAAGGACAGCAACAATCTGGATCAGAGTCTG CCCAGAAACCTGCCCGTGACCATCATTAGCCAGGACTTCGGCGACGCCAGT CCAAGGACCAACGGACAGGAGGCCGACGACAGCTCTACCAGCGAAGAGTCT CCCGAGGACTCTAAATACTTTCTGCCCTACCATCCTCCACAAAGAAGGATG AACCTGAAGGGCATTCAACTCCAAAGAGCCAAGAGCCCAATCAGTCTGAAG AGAACCAGCGACTTCCAAGCCAAAGGCCACGAGGAAGAAGGAACAGACGCC TCTCCAAGCTCCTGCGGCTCTCTGCCCATCACCAACTCCTTCACCAAGATG CAGCCACCCAGGAGTAGAAGCAGCATCATGTCCATCACAGCCGAACCACCT GGCAACGACTCCATCGTGAGAAGATACAAGGAAGACGCTCCACATAGGAGG TGGCTGAACCACAAGGGAGTGAGACAGAAGAGACTGAACGTGTGGCTTGGC ATCAAGAACGAAGACGCCGACGAGAACTACTTCATCAACGAAGAAGACGAG AACCTGCCACACTACGACGAAAAGACTTGGTTCGTGGAGGACATCAATAGG GTTCAAGCTGAGGATCTGCTGTACGGCAAGCCCGACGGAGCCTTTCTGATT CGGGAGAGCAGCAAGAAGGGCTGCTACGCCTGCTCTGTGGTGGCCGACGGC GAAGTGAAGCACTGCGTCATCTACAGCACAGCTAGAGGATACGGCTTCGCC GAGCCATACAACCTGTACAGCAGCCTCAAAGAGCTGGTTCTTCACTACCAG CAGACCAGCCTGGTGCAGCACAACGATTCCCTCAACGTGAGACTGGCCTAC CCTGTGCACGCCCAGATGCCCAGTCTGTGCAGATGA
116	Patient-derived CARD11-PIK3R3	MDDYMETLKDEEDALWENVECNRHMLSRYINPAKLTPYLRQCKVIDEQDED EVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVTGK EPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLRQLE DEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLAMRYAQL SEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLKNDIENRPK KEQVLELERENEMLKTKNQELQSIIQAGKRSLPDSDKAILDILEHDRKEAL EDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGKDCEMYKHR MNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRKQIRELEEKND EMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTIISQDFGDAS PRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQRAKSPISLK RTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSIMSITAEPP GNDSIVRRYKEDAPHRRWLNHKGVRQKRLNVWLGIKNEDADENYFINEEDE NLPHYDEKTWFVEDINRVQAEDLLYGKPDGAFLIRESSKKGCYACSVVADG EVKHCVIYSTARGYGFAEPYNLYSSLKELVLHYQQTSLVQHNDSLNVRLAY PVHAQMPSLCR

SEQ ID NO	DESCRIPTION	SEQUENCE
117	Truncated CARD11-PIK3R3	atggacgactacatggagaccctgaaagacgaggaggacgccctgtgggag aacgtggagtgcaacaggcacatgctgagcagatacatcaaccctgccaag ctgacaccctacctgagacagtgcaaggtgatcgacgagcaggacgaggac gaagtgctgaacgctcccatgctgccctctaaaatcaacagagccggcaga ctgctggacatcctgcacaccaagggccagaggggcxacgtggtgttcctg gagagcctggagttctactatcccgagctctacaagctcgtgaccggcaag gagcccaccagaagattcagcaccattgtggtggaggagggacacgaaggc ctgacccacttcctgatgaacgaggtgatcaagctgcaacagcaaatgaag gccaaagacctgcaacggtgcgagctgctggccagactgaggcagctggag gacgagaagaagcagatgaccctgaccagagtggaacttctgaccttccaa gagagatactacaagatgaaagaggaacgcgatagcxacaacgacgaactt gtcaaagtgaaggacgacaactacaacctggccatgagatacgcccagctg agcgaagagaagaacatggccgtgatgaggagcagggatctgcaactggag atcgaccagctgaagcacaggctgaacaagatggaagaggagtgtaagctg gaacggaaccagagcctgaagcttaagaacgacatcgagaacagacccaag aaggagcaagtgctggagctggagcgcgaaaacgagatgctcaagaccaag aatcaggagttgcagagcatcatccaagccggaaagagaagcctgcccgac agcgacaaggccatcctggatattctggagcacgataggaaggaggccctg gaagacagacaagaactggtgaacagaatctacaatcttcaagaggaagcc agacaggcagaagagctcagggacaaataccttgaggaaaaggaggacctg gaactgaagtgctccaccctgggaaaggactgcgagatgtacaagcacaga atgaacaccgtgatgctgcagctggaagaggtcgagagggagcgcgaccag gccttccacagcagggacgaagcccagacacagtacagccagtgcctgatc gagaaggacaagtacaggaagcagatcagagaactggaggagaagaatgac gagatgaggatcgagatggtcagaagggaggcctgcatcgtgaacctggaa agcaaactgagaaggctttccaaggacagcaacaatctggatcagagtctg cccagaaacctgcccgtgaccatcattagccaggacxtcggcgacgccagt ccaaggaccaacggacaggaggccgacgacagctctaccagcgaagagtct cccgaggactctaaatactttctgccctaccatcctccacaaagaaggatg aacctgaagggcattcaactccaaagagccaagagcccaatcagtctgaag agaaccagcgacttccaagccaaaggccacgaggaagaaggaacagacgcc tctccaagctcctgcggctctctgcccatcaccaacxccttcaccaagatg cagccacccaggagtagaagcagcatcatgtccatcacagccgaaccacct ggcaacgactccatcgtgagaagatacaaggaagacgctccacataggtcc accgtggaagaagacgagaacctgccacactacgacgaaaagacttggttc gtggaggacatcaatagggttcaagctgaggatctgctgtacggcaagccc gacggagcctttctgattcgggagagcagcaagaagggctgctacgcctgc tctgtggtggccgacggcgaagtgaagcactgcgtcatctacagcacagct agaggatacggcttcgccgagccatacaacctgtacagcagcctcaaagag ctggtxcttcactaccagcagaccagcctggtgcagcacaacgattccctc aacgtgagactggcctaccctgtgcacgcccagatgcccagtctgtgcaga tga
118	Truncated CARD11-PIK3R3	MDDYMETLKDEEDALWENVXCNRHMLSRYINPAKLTPYLRQCKVIDEQDED EVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVTGK EPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLRQLX DEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLAMRYAQL SEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLKNDIENRPK KEQVLELERENEMLKTKNQXLQSIIQAGKRSLPDSDKAILDILEHDRKEAL EDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGKDCEMYKHR MNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRKQXRELEEKND EMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTIXSQDFGDAS PRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQRAKSPISLK RTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSXMSITAEPP GNDSIVRRYKEDAPHRSTVXEDENLPHYDEKTWFVEDINRVQAEDLLYGKP DGAFLIRESSKKGCYACSVVADGEVKHCVIYSTARGYGFAEPYNLYSSLKX LVLHYQQTSLVQHNDSLNVRLAYPVHAQMPSLCR
119	Truncated CARD11-PIK3R3	atggacgactacatggagaccctgaaagacgaggaggacgccctgtgggag aacgtggagtgcaacaggcacatgctgagcagatacatcaaccctgccaag ctgacaccctacctgagacagtgcaaggtgatcgacgagcaggacgaggac

SEQ ID NO	DESCRIPTION	SEQUENCE
		gaagtgctgaacgctcccatgctgccctctaaaatcaacagagccggcaga ctgctggacatcctgcacaccaagggccagaggggcxacgtggtgttcctg gagagcctggagttctactatcccgagctctacaagctcgtgaccggcaag gagcccaccagaagattcagcaccattgtggtggaggagggacacgaaggc ctgacccacttcctgatgaacgaggtgatcaagctgcaacagcaaatgaag gccaaagacctgcaacggtgcgagctgctggccagactgaggcagctggag gacgagaagaagcagatgaccctgaccagagtggaacttctgaccttccaa gagagatactacaagatgaaagaggaacgcgatagcxacaacgacgaactt gtcaaagtgaaggacgacaactacaacctggccatgagatacgcccagctg agcgaagagaagaacatggccgtgatgaggagcagggatctgcaactggag atcgaccagctgaagcacaggctgaacaagatggaagaggagtgtaagctg gaacggaaccagagcctgaagcttaagaacgacatcgagaacagacccaag aaggagcaagtgctggagctggagcgcgaaaacgagatgctcaagaccaag aatcaggagttgcagagcatcatccaagccggaaagagaagcctgcccgac agcgacaaggccatcctggatattctggagcacgataggaaggaggccctg gaagacagacaagaactggtgaacagaatctacaatcttcaagaggaagcc agacaggcagaagagctcagggacaaataccttgaggaaaaggaggacctg gaactgaagtgctccaccctgggaaaggactgcgagatgtacaagcacaga atgaacaccgtgatgctgcagctggaagaggtcgagagggagcgcgaccag gccttccacagcagggacgaagcccagacacagtacagccagtgcctgatc gagaaggacaagtacaggaagcagatcagagaactggaggagaagaatgac gagatgaggatcgagatggtcagaagggaggcctgcatcgtgaacctggaa agcaaactgagaaggctttccaaggacagcaacaatctggatcagagtctg cccagaaacctgcccgtgaccatcattagccaggacxtcggcgacgccagt ccaaggaccaacggacaggaggccgacgacagctctaccagcgaagagtct cccgaggactctaaatactttctgccctaccatcctccacaaagaaggatg aacctgaagggcattcaactccaaagagccaagagcccaatcagtctgaag agaaccagcgacttccaagccaaaggccacgaggaagaaggaacagacgcc tctccaagctcctgcggctctctgcccatcaccaacxccttcaccaagatg cagccacccaggagtagaagcagcatcatgtccatcacagccgaaccacct ggcaacgactccatcgtgagaagatacaaggaagacgctgacgagaactac ttcatcaacgaagaagacgagaacctgccacactacgacgaaaagacttgg ttcgtggaggacatcaatagggttcaagctgaggatctgctgtacggcaag cccgacggagcctttctgattcgggagagcagcaagaagggctgctacgcc tgctcxgtggtggccgacggcgaagtgaagcactgcgtcatctacagcaca gctagaggatacggcttcgccgagccatacaacctgxacagcagcctcaaa gagctggttcttcactaccagcagaccagcctggtgcagcacaacgattcc ctcaacgtgagactggcctaccctgtgcacgcccagatgcccagtctgtgc agatga
120	Truncated CARD11-PIK3R3	MDDYMETLKDEEDALWENVXCNRHMLSRYINPAKLTPYLRQCKVIDEQDED EVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVTGK EPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLRQLX DEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLAMRYAQL SEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLKNDIENRPK KEQVLELERENEMLKTKNQXLQSIIQAGKRSLPDSDKAILDILEHDRKEAL EDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGKDCEMYKHR MNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRKQIRELEEKND EMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTIISQDFGDAS PRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQRAKSPISLK RTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSIMSITAEPP GNDSIVRRYKEDADENYFINEEDENLPHYDEKTWFVEDINRVQAEDLLYGK PDGAFLIRESSKKGCYACSVVADGEVKHCVIYSTARGYGFAEPYNLYSSLK ELVLHYQQTSLVQHNDSLNVRLAYPVHAQMPSLCR
121	Protospacer adjacent motif (PAM) DNA sequence example, wherein	ngrrt

SEQ ID NO	DESCRIPTION	SEQUENCE
	(i) r = a or g, (ii) y = c or t, (iii) w = a or t, (iv) v = a or c or g, and (v) n = a, c, t, or g	
122	PAM sequence example (see above)	ngrrn
123	PAM sequence example (see above)	nnnngatt
124	PAM sequence example (see above)	nnnnryac
125	PAM sequence example (see above)	nnagaaw
126	PAM sequence example (see above)	naaaac
127	PAM sequence example (see above)	tttv
128	PAM sequence example (see above)	attn
129	PAM sequence example (see above)	tttn
130	PAM sequence example (see above)	gttn
131	PAM sequence example (see above)	yttn
132	Exemplary spCas9 1, spCas9 2, or saCas9 complementary region RNA (spacer); n = any RNA base	nnnnnnnnnnnnnnnnnnnn
133	Exemplary spCas9 1 crRNA repeat region; s = c or g and n = any RNA base	guuuuagagcua
134	Exemplary RNA tetraloop sequence for spCas9 1, spCas9 2, or saCas9	gaaa
135	Exemplary spCas9 1 crRNA	uagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccg agucggugcuuuuuu

SEQ ID NO	DESCRIPTION	SEQUENCE
	tracrRNA; s = c or g and n = any RNA base	
136	Exemplary spCas9 2 crRNA repeat region; s = c or g and n = any RNA base	guuusagagcuaugcug
137	Exemplary spCas9 2 crRNA tracrRNA; s = c or g and n = any RNA base	cagcauagcaaguusaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcuuuuuu
138	Exemplary saCas9 crRNA repeat region; s = c or g and n = any RNA base	guuuuaguacucug
139	Exemplary saCas9 tracrRNA; s = c or g and n = any RNA base	cagaaucuacuaaaacaaggcaaaaugccguguuuaucucgucaacuuguu ggcgagauuuuuu
140	FC1 furin cleavage sequence	RRRRGSG
141	FC1 furin cleavage sequence	RRRR
142	FC2 furin cleavage sequence	RKRRGSG
143	FC2 furin cleavage sequence	RKRR
144	FC3 furin cleavage sequence	RKRRTPDPW
145	FC3 furin cleavage sequence	RKRRGSGTPDPW
146	FC3 furin cleavage sequence	RKRRGSGTPDPWGSG
147	FC3 furin cleavage sequence	RKRRTPDPWGSG
148	Anti-DLL3 light chain variable region (VL) (C-terminal VEIK)	EIVMTQSPSTLSASVGDRVIITCQASQSIYSFLAWYQQKPGKAPKLLIYET SKLASGVPSRFSGSGSGAEFTLTISSLQPDDFATYYCHQAYSYANIDNVFG QGTKVEIK
149	Anti-DLL3 heavy chain CDR1 (CDRH1) Martin ("enhanced Chothia")	GIDFSSYYYMC

SEQ ID NO	DESCRIPTION	SEQUENCE
150	Anti-DLL3 CDRH2 Martin ("enhanced Chothia")	CVNMESGLAAYATWAKG
151	Anti-DLL3 CDRH3 Martin ("enhanced Chothia")	YESSSGYMDL
152	Anti-DLL3 light chain CDR1 (CDRL1) Martin ("enhanced Chothia")	QASQSIYSFLA
153	Anti-DLL3 CDRL2 Martin ("enhanced Chothia")	ETSKLAS
154	Anti-DLL3 CDRL3 Martin ("enhanced Chothia")	HQAYSYANIDNV
155	Anti-DLL3 heavy chain CDR1 (CDRH1) structural modeling	GIDFSSYYY
156	Anti-DLL3 CDRH2 structural modeling	NMESGLAA
157	Anti-DLL3 CDRH3 structural modeling	YESSGY
158	Anti-DLL3 light chain CDR1 (CDRL1) structural modeling	ASQSIYSF
159	Anti-DLL3 CDRL2 structural modeling	YETSKLAS
160	Anti-DLL3 CDRL3 structural modeling	AYSYANIDN
161	TRAJ intron splice acceptor locus	CAGGGTTCTGGATATCTGT
162	Anti-DLL3 CAR amino acid sequence (including signal sequence and CCR4 epitope tag in the linker)	MALPVTALLLPLALLLHAARPEIVMTQSPSTLSASVGDRVIITCQASQSIY SFLAWYQQKPGKAPKLLIYETSKLASGVPSRFSGSGSGAEFTLTISSLQPD DFATYYCHQAYSYANIDNVFGQGTKVEIKGGSDESIYSNYYLYESIPKPSG GSEVQLVESGGGLVQPGGSLRLSCTASGIDFSSYYYMCWVRQAPGKGLEWV GCVNMESGLAAYATWAKGRFTISRDTSKNTVYLQMNSLRAEDTAVYYCARY ESSSGYMDLWGQGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVH

SEQ ID NO	DESCRIPTION	SEQUENCE
163	Anti-DLL3 CAR nucleic acid sequence (including signal sequence and CCR4 epitope tag in the linker)	atggccctgccagtgacagccctgctgttgccactggccttgctgctccac gctgccagaccagaaatcgtcatgactcagagcccgctacactcagtgcc tctgtcggagacagagtcatcataacgtgccaagcttcccagagcatttat agcttcctggcttggtatcagcagaagcctggaaaggctccgaagctgctc atctacgaaacctctaagctcgcttccggcgtgccatctcgcttcagcggc tctggaagcggcgctgagttcaccctgaccattagcctctccaacctgac gacttcgccacatactattgccaccaagcttacagctacgccaacatcgat aacgtcttcggacaaggcaccaaagtcgaaatcaagggcggttc

WHAT IS CLAIMED IS:

1. An anti-delta-like ligand 3 (DLL3) chimeric antigen receptor (CAR), comprising an extracellular domain comprising an anti-DLL3 antigen binding domain.

2. The anti-DLL3 CAR of claim 1, wherein the anti-DLL3 antigen binding domain binds the epidermal growth factor (EGF)-like repeat 6 (EGF6) domain of DLL3.

3. The anti-DLL3 CAR of any one of claims 1-2, wherein the DLL3 binding protein or the anti-DLL3 antigen binding domain is a single chain antigen binding domain.

4. The anti-DLL3 CAR of claim 3, wherein the single chain antigen binding domain is a single chain Fv (scFv).

5. The anti-DLL3 CAR of any one of claims 1-4, wherein the anti-DLL3 antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6,

(ii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154, or

(iii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region 1 (CDRH1) comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and/or wherein the anti-DLL3 antigen binding domain further comprises a light chain variable region (VL) comprising a light chain CDR1 (CDRL1) comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160.

6. The anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-5, wherein the anti-DLL3 binding protein or the anti-DLL3 antigen binding domain comprises a VH comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 7, and/or a VL comprising an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 8 or 148.

7. The anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-5, wherein the anti-DLL3 binding protein or anti-DLL3 antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 and/or a VL comprising the amino acid sequence of SEQ ID NO: 148.

8. The anti-DLL3 CAR of any one of claims 1-7, wherein the anti-DLL3 CAR is or comprises a single-chain CAR, a multi-chain CAR, a single-targeted CAR, a multi-targeted CAR, a bivalent tandem CAR, a bivalent loop CAR, a multicistronic CAR, a bicistronic CAR, a dimerizing agent regulated immune-receptor complex (DARIC), an antibody tethered orthogonal multiplexing compatible (ATOMIC), a T cell receptor fusion construct (TruC), an HLA-independent T cell (HIT) receptor, a synthetic T cell receptor and antigen receptor (STAR), a synNotch-CAR circuit, a synthetic intramembrane proteolysis receptor (SNIPR), a rapamycin inducible TCR, a rapamycin inducible Fc receptor, a constitutive TCR-like receptor, a multi-chain DAP-CAR, a TREM1/DAP12 CAR, or a DAP12/TREM1 CAR.

9. The anti-DLL3 CAR of any one of claims 1-8, wherein the CAR comprises a linker sequence.

10. The anti-DLL3 CAR of claim 9, wherein the linker sequence is or comprises a Whitlow linker, a (G4S)n linker, or an SG4S linker.

11. The anti-DLL3 CAR of claim 10, wherein n = 0, 1, 2, 3, 4, 5, or 6.

12. The anti-DLL3 CAR of claim 10, wherein the linker sequence comprises the amino acid sequence of any one of SEQ ID NOs: 16, 38 or 40-60.

13. The anti-DLL3 CAR of any one of claims 1-12, wherein the CAR comprises a hinge domain.

14. The anti-DLL3 CAR of claim 13, wherein the hinge domain is or comprises a CD8a hinge domain, a CD28 hinge domain, a IgG4 hinge domain, or a IgG4 hinge-CH2-CH3 domain.

15. The anti-DLL3 CAR of claim 13 or 14, wherein the hinge domain comprises the amino acid sequence of any one of SEQ ID NOs: 18 or 100-106.

16. The anti-DLL3 CAR of any one of claims 1-15, wherein the CAR comprises a transmembrane domain.

17. The anti-DLL3 CAR of claim 16, wherein the transmembrane domain comprises a transmembrane region domain of TCR alpha chain, TCR beta chain, TCR zeta chain, TCRgamma, TCRdelta, CD3gamma, CD3delta, CD3epsilon, CD3zeta, CD4, CD5, CD8, CD8alpha, CD8beta, CD9, CD16, CD22, CD28, CD32, CD33, CD34, CD37, CD38, CD40, CD45, CD64, CD80, CD86, OX-40 (CD134), 4-1BB (CD137), CD154, Fc receptor for IgE (FcεRI), VEGFR2, FAS, or FGFR2B.

18. The anti-DLL3 CAR of claim 16, wherein the transmembrane domain comprises the amino acid sequence of any one of SEQ ID NOs: 20 or 107-109.

19. The anti-DLL3 CAR of claim 16, wherein the transmembrane domain comprises a CD8 transmembrane domain, such as comprising the amino acid sequence of SEQ ID NO: 20.

20. The anti-DLL3 CAR of any one of claims 1-19, wherein the CAR comprises an intracellular signaling domain of: CD28, 41BB (CD137), OX-40 (CD134), CD2, CD7, CD27, CD30, CD40, PD-1, ICOS, LFA-1 (CDIIa/CD18), CD3gamma, CD3delta, CD3epsilon, CD3zeta, CD3zeta Q14K, 1.xxCD3zeta, CD247, CD276 (B7-H3), LIGHT, NKG2C, Ig alpha (CD79a), DAP-10, an Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, an integrin, a Signaling Lymphocytic Activation Molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, B7-H3, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CARD11, CD4, CD8alpha, CD8beta, IL-2Rbeta, IL-2R gamma, IL-7R alpha, ITGA4, VLAl, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 1d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 lb, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGLl, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a CD83 ligand, or any combination thereof.

21. The anti-DLL3 CAR of any one of claims 1-19, wherein the CAR comprises an intracellular signaling domain of: CD3zeta, 1xxCD3zeta, CD3zeta Q14K, CD28, 4-1BB, or OX-40, or any combination thereof.

22. The anti-DLL3 CAR of any one of claims 1-19, wherein the CAR comprises an intracellular signaling domain of CD3zeta, such as comprising the amino acid sequence of SEQ ID NO: 24, 62, or 63, or an amino acid sequence at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24, 62, or 63.

23. The anti-DLL3 CAR of any one of claims 1-19, wherein the CAR comprises an intracellular signaling domain of 4-1BB and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

24. The anti-DLL3 CAR of any one of claims 1-19, wherein the CAR comprises (a) an intracellular signaling domain of CD3zeta, and/or comprises the amino acid sequence of SEQ ID NO: 24, 62, or 63 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24, 62, or 63, and further comprises (b) an intracellular signaling domain of 41BB, and/or comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

25. The anti-DLL3 CAR of any one of claims 1-24, wherein the CAR further comprises a signal sequence.

26. The anti-DLL3 CAR of claim 25, wherein the signal sequence comprises a CD8alpha signal sequence, an IgK signal sequence, or a GMCSFR-alpha signal sequence.

27. The anti-DLL3 CAR of claim 25, wherein the signal sequence comprises the amino acid sequence of any one of SEQ ID NOs: 12, 74, or 75.

28. The anti-DLL3 CAR of any one of claims 1-27, wherein the anti-DLL3 CAR comprises each of: a linker sequence, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

29. The anti-DLL3 CAR of any one of claims 1-28, wherein engineered cells expressing the anti-DLL3 CAR have one or more of the following properties:

a. IFNgamma and IL-2 expression when co-cultured with small cell lung carcinoma (SCLC) cells;

b. Higher IFNgamma and IL-2 expression when co-cultured with DMS273, NCI-H82, and/or SHP77 cells compared to cells expressing an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36;

c. Do not result in production of cytokines against DLL3 negative cells;

d. When administered to a SCLC murine tumor model, promote tumor regression and/or increased survival compared to an untransduced control and/or compared to a cell expressing a CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of SEQ ID NO: 36.

30. The anti-DLL3 CAR of any one of claims 1-29, wherein the CAR comprises:
(a) an anti-DLL3 antigen binding domain comprising (i) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6, (ii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154, or (iii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160;
(b) a CD8 hinge region;
(c) a CD8 transmembrane domain
(d) and a CD3zeta, CD3zeta Q14K, or 1xxCD3zeta intracellular signaling domain and a 41BB intracellular signaling domain.

31. The anti-DLL3 CAR of any one of claims 1-29, wherein the CAR comprises:
(a) an anti-DLL3 antigen binding domain comprising: (i) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6, (ii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 149, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 151; and a VL comprising a

CDRL1 comprising the amino acid sequence of SEQ ID NO: 152, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 154, or (iii) a VH comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 155, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 156, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 157; and a VL comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 158, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 159, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 160;

(b) a linker comprising the amino acid sequence of any one of SEQ ID NO: 16, 38, or 40-60;

(c) a hinge comprising the amino acid sequence of SEQ ID NO: 18;

(d) a transmembrane domain comprising the amino acid sequence of SEQ ID NO: 20;

(e) and an intracellular signaling domain comprising the amino acid sequence of any one of SEQ ID NOs: 24, 62 or 63, and further comprising the amino acid sequence of SEQ ID NO: 22.

32. The anti-DLL3 CAR of any one of claims 1-31, wherein the CAR comprises an amino acid sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or identical to the sequence of SEQ ID NO: 9 or SEQ ID NO: 162, with or without a signal sequence.

33. A nucleic acid construct encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32.

34. The nucleic acid construct of claim 33, wherein the nucleic acid construct further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or one or more non-coding RNA.

35. The nucleic acid construct of claim 34, wherein the polynucleotide sequence encoding the anti-DLL3 CAR or anti-DLL3 binding protein and a polynucleotide sequence encoding the one or more additional polypeptides and/or one or more non-coding RNA are separated by a polynucleotide sequence encoding one or more cleavable linkers, and wherein all the polynucleotide sequences form a continuous open reading frame.

36. The nucleic acid construct of any one of claims 33-35, wherein the nucleic acid construct comprises one or more modified nucleotides.

37. The nucleic acid construct of any one of claims 33-36, wherein the nucleic acid construct is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length.

38. The nucleic acid construct of claim 37, wherein the nucleic acid construct is less than 5 kb in length.

39. The nucleic acid construct of claim 37, wherein the nucleic acid construct is less than 4.7 kb in length.

40. A vector comprising the nucleic acid construct of any one of claims 33-39.

41. The vector of claim 40, wherein the vector is a viral vector, optionally wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector, wherein the AAV vector is optionally AAV6 vector.

42. An engineered cell that expresses the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or the nucleic acid construct of any one of claims 33-39, or that comprises the vector of claim 40 or 41.

43. An engineered cell that expresses the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or the nucleic acid construct of any one of claims 33-39, or that comprises the vector of claim 40 or 41, wherein the anti-DLL3 binding protein or anti-DLL3 CAR is expressed under the control of an endogenous promoter.

44. The engineered cell of claim 43, wherein the endogenous promoter is a TCRa promoter, a TCRb promoter, a CD3d promoter, a CD3g promoter, a CD3e promoter, a CD3z promoter, a CARD9 promoter, a CARD10 promoter, a CARD11 promoter, a CARD14 promoter, or a PIK3R3 promoter.

45. The engineered cell of any one of claims 42-44, wherein the cell comprises a nucleic acid encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or the nucleic acid construct of any one of claims 33-39 that is inserted into a T-cell receptor (TCR) locus, a CD3 locus, a B2 microglobulin (B2M) locus, a class II transactivator (CIITA) locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, a PIK3R3 locus, or a safe harbor locus.

46. The engineered cell of claim 45, wherein the TCR locus is or comprises a TRAJ locus, a TRAC locus, a TRBC1 locus, or a TRBC2 locus.

47. The engineered cell of any one of claims 42-46, wherein a nucleic acid encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or the nucleic acid construct of any one of claims 33-39 is inserted into an exon, an intron, between an intron and an exon, or a regulatory region.

48. The engineered cell of claim 47, wherein the cell is a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, or an engineered T cell comprising a transcriptional receptor.

49. The engineered cell of any one of claims 42-48, wherein the cell is an autologous cell, an allogeneic cell, a primary cell, or a cell derived from a stem cell.

50. The engineered cell of any one of claims 42-49, wherein the cell is genetically modified, optionally wherein the genetic modification reduces or eliminates expression of an endogenous T cell receptor.

51. The engineered cell of any one of claims 42-50, wherein the cell further comprises a potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, a tolerogenic factor, a depletion tag, a safety switch, or any combination thereof.

52. The engineered cell of claim 51, wherein the potency enhancement polypeptide is or comprises a patient derived CARD11-PIK3R3 fusion (such as comprising the amino acid sequence of any one of SEQ ID NOs: 114 or 116), an engineered CARD11-PIK3R3 fusion (such as comprising the amino acid sequence of any one of SEQ ID NOs: 118 or 120), a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (e.g., TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, CD8alpha/beta co-expression, or a combination thereof.

53. A method of preparing the engineered cell of any one of claims 42-52, comprising contacting a cell with a vector comprising a nucleic acid encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or with the nucleic acid construct of any one of claims 33-39, or with the vector of claim 40 or 41, or with a particle comprising the vector or nucleic acid.

54. The method of claim 53, wherein the method further comprises selecting a cell comprising the vector or the nucleic acid or the nucleic acid construct.

55. A method of making the engineered immune cell of claim 53 or 54, comprising performing gene editing to insert a nucleic acid encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 into the cellular genome.

56. The method of any one of claims 53-55, wherein the vector is a plasmid, a synthetic DNA vector, a linear DNA vector, a closed linear DNA vector, a phagemid vector, an RNA vector, an mRNA vector, or a viral vector, optionally wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector, optionally an AAV6 vector or an AAV9 vector.

57. The method of any one of claims 53-56, wherein the engineered cell is prepared ex vivo.

58. The method of any one of claims 53-57, wherein the method comprises introducing into the cell one or more nucleic acids encoding a sequence specific nuclease or a nucleic acid programmable DNA binding protein, optionally wherein the sequence-specific nuclease is an RNA guided nuclease.

59. The method of any one of claims 53-58, wherein the method additionally comprises introducing into the cell one or more guide RNAs.

60. The method of claim 59, wherein the Cas nuclease is Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional portion thereof.

61. The method of any one of claims 58-60, wherein the method comprises introducing into the cell a ribonucleoprotein (RNP) complex comprising the sequence-specific nuclease or nucleic acid programmable DNA binding.

62. The method of any one of claims 53-61, wherein the nucleic acid encoding the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32 or the nucleic acid construct of any one of claims 33-39 is inserted into an endogenous locus of the cell.

63. The method of claim 62, wherein the endogenous locus is or comprises a TRAJ gene locus, a TRAC gene locus, a TRBC1 gene locus, or a TRBC2 gene locus, optionally wherein the TRAJ gene locus is a TRAJ intron splice acceptor locus.

64. The method of any one of claims 53-63, wherein the method further comprises introducing a gene editing machinery into the cell.

65. The method of claim 64, wherein introducing the gene editing machinery comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery.

66. The method of claim 64 or 65, wherein the gene editing machinery comprises CRISPR/Cas9.

67. The method of any one of claims 64-66, wherein the gene editing machinery is introduced via electroporation.

68. The method of any one of claims 53-67, wherein the method thereby produces an engineered cell comprising: (i) an anti-DLL3 CAR; and (iii) a modified TRAC gene locus.

69. The method of any one of claims 53-67, wherein the method thereby produces an engineered cell comprising: (i) an anti-DLL3 CAR; and (iii) a modified TRAJ gene locus, optionally wherein the TRAJ gene locus is a TRAJ intron splice acceptor locus.

70. An engineered cell produced by the method of any one of claims 53-69 for use in a cell therapy in a subject.

71. A pharmaceutical composition comprising the anti-DLL3 binding protein or anti-DLL3 CAR of any one of claims 1-32, the nucleic acid construct of any one of claims 33-39, the vector or a particle comprising the vector of any one of claims 41-42, or the engineered cell of any one of claims 42-52.

72. A method of treating a subject in need thereof, comprising administering to the subject the engineered cell of any one of claims 42-52, or an engineered cell prepared by the method of any one of claims 53-69, or the pharmaceutical composition of claim 71.

73. The method of claim 72, wherein the subject has cancer.

74. The method of claim 73, wherein the cancer is a solid tumor.

75. The method of claim 73 or 74, wherein the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate.

76. The method of any one of claims 73-75, wherein the cancer expresses DLL3.

77. Use of the engineered cell of any one of claims 42-52, or an engineered cell prepared by the method of any one of claims 53-69, or the pharmaceutical composition of claim 71, for the manufacture of a medicament for the treatment of a disease.

78. The use of claim 77, wherein the disease is cancer.

79. The use of claim 78, wherein the cancer is a solid tumor.

80. The use of claim 78 or 79, wherein the cancer is small cell lung cancer, neuroendocrine neoplasm (NEN), glioma, glioblastoma, neuroblastoma, melanoma, Wilms & rhabdoid tumors, or neuroendocrine prostate.

81. The use of any one of claims 78-80, wherein the cancer expresses DLL3.

82. The engineered cell of claim 70, or the method of any one of claims 72-76, or the use of any one of claims 77-81, wherein the engineered cell has reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, increased intratumoral presence in

vivo, enhanced IFNgamma production, enhanced IL-2 production, and/or promotes enhanced tumor regression, and/or wherein the engineered cell has one or more of the following properties:

a. IFNgamma and IL-2 expression when co-cultured with small cell lung carcinoma (SCLC) cells;

b. Higher IFNgamma and IL-2 expression when co-cultured with DMS273, NCI-H82, and/or SHP77 cells compared to cells expressing an anti-DLL3 CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of any one or more of SEQ ID NOs: 28, 30, 32, 34, or 36;

c. Does not result in production of cytokines against DLL3 negative cells;

d. When administered to a SCLC murine tumor model, promotes tumor regression and/or increased survival compared to an untransduced control and/or compared to a cell expressing a CAR comprising the anti-DLL3 binding sequences present in amino acid sequence of SEQ ID NO: 36.

83. The method or use of any one of claims 72-82, wherein the subject is administered one or more additional therapies or wherein the medicament is for administration with one or more additional therapies.

84. The method or use of claim 83, wherein the one or more additional therapies comprise chemotherapy, immunotherapy, surgery, radiotherapy, anti-angiogenic agent, anti-DNA repair agent, anti-inflammatory agent, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, a hormonal agent, or any combination thereof.