

SUMMARY

In one aspectexample, the present disclosure provides a chimeric antigen receptor (CAR) targeting DLL3 (anti-DLL3 CAR). The anti-DLL3 CAR comprises a DLL3 binding domain, wherein the DLL3 binding domain comprises or is derived from a single domain antibody (sdAb) or a single chain variable fragment (scFv).

In some embodimentexamples, the sdAb comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some embodimentexamples, the sdAb comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243, or a variant of the polypeptide comprising up to about 3 amino acid substitutions in the CDR1, CDR2 and CDR3.

In some embodimentexamples, the sdAb comprises a polypeptide comprising any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 168, or a variant thereof comprising up to about 3 amino acid substitutions;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about

3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 amino acid substitutions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201, or a variant thereof comprising up to about 3 amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 3 amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 170, or a variant thereof comprising up to about 3 amino acid substitutions; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 171, or a variant thereof comprising up to about 3 amino acid substitutions.

In some embodiment examples, the sdAb comprises a polypeptide comprising any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 168;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 196;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 201;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88; and a CDR 3 comprising the

amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171.

In one aspect, the present disclosure provides an antibody or antigen-binding fragment that specifically binds to DLL3, comprising:

i. a single domain antibody (sdAb) moiety comprising:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 168;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 196;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 201;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171; or

ii. an antigen-binding fragment comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein

(1) the VH comprises a CDR1 set forth in SEQ ID NO: 498, a CDR2 set forth in SEQ ID NO: 499, and a CDR3 set forth in SEQ ID NO: 500, and the VL comprises a CDR1 set forth in SEQ ID NO: 495, a CDR2 set forth in SEQ ID NO: 496, and a CDR3 set forth in SEQ ID NO: 497; or

(2) the VH comprises a CDR1 set forth in SEQ ID NO: 504, a CDR2 set forth in SEQ ID NO: 505, and a CDR3 set forth in SEQ ID NO: 506, and the VL comprises a CDR1 set forth in SEQ ID NO: 501, a CDR2 set forth in SEQ ID NO: 502, and a CDR3 set forth in SEQ ID NO: 503.

In some embodimentexamples, the sdAb is a camel sdAb raised against human or rhesus DLL3.

In some embodimentexamples, the sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 274-354.

In some embodimentexamples, the sdAb is humanized through CDR grafting.

In some embodimentexamples, the humanized sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 355-367.

In some embodimentexamples, the scFv comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 498 or 504 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 499 or 505 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 500 or 506 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 495 or 501 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 496 or 502 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 497 or 503 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some embodimentexamples, the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 498, a CDR2 set forth in SEQ ID NO: 499, and a CDR3 set forth in SEQ ID NO: 500, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 495, a CDR2 set forth in SEQ ID NO: 496, and a CDR3 set forth in SEQ ID NO: 497; or the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 504, a CDR2 set forth in SEQ ID NO: 505, and a CDR3 set forth in SEQ ID NO: 506, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 501, a CDR2 set forth in SEQ ID NO: 502, and a CDR3 set forth in SEQ ID NO: 503.

In some embodimentexamples, the VH domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 508, and the VL domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 507; or the VH domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 510, and the VL domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set

forth in SEQ ID NO: 509.

In another aspect, the present disclosure provides a chimeric antigen receptor (CAR) comprising a DLL3 binding domain, wherein the DLL3 binding domain comprises or is derived from the antibody or antigen-binding fragment as described herein.

In some embodimentexamples, the scFv is obtained from a synthetic human Fab phage library.

In some embodimentexamples, the DLL3 is human or rhesus DLL3.

In some embodimentexamples, the anti-DLL3 CAR comprises, from N-terminus to C-terminus, a signal peptide, the DLL3 binding domain, a hinge domain, a transmembrane domain, and an intracellular signaling domain.

In some embodimentexamples, the intracellular signaling domain is derived from CD3 ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, or CD66d.

In some embodimentexamples, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence.

In some embodimentexamples, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof.

In some embodimentexamples, the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 476-484, SEQ ID NOs: 485-494 or SEQ ID NOs: 515-516.

In some embodimentexamples, the DLL3 binding domain comprises two sdAbs linked to each other.

In some embodimentexamples, each of the sdAbs independently comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:356 or SEQ ID NO:366.

In some embodimentexamples, the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 518-520.

In some embodimentexamples, the CAR comprises an amino acid sequence of SEQ ID NO: 520.

In another aspectexample, the present disclosure provides a DLL3 binding protein comprising a single domain antibody (sdAb) moiety that specifically binds to DLL3, wherein the sdAb moiety comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some embodimentexamples, the sdAb moiety comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243, or a variant of the polypeptide comprising up to about 3 amino acid substitutions in the

CDR1, CDR2 and CDR3.

In some embodiment examples, the sdAb moiety comprises a polypeptide comprising any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 87, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 168, or a variant thereof comprising up to about 3 amino acid substitutions;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 amino acid substitutions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 201, or a variant thereof comprising up to about 3 amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 3 amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 89, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 170, or a variant thereof comprising up to about 3 amino acid substitutions; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9, or a variant

thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171, or a variant thereof comprising up to about 3 amino acid substitutions.

In some embodimentexamples, the sdAb moiety comprises a polypeptide comprising any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 168;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 102; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 183;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 186;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 171.

In some embodimentexamples, the sdAb moiety is a camel sdAb raised against human or rhesus DLL3.

In some embodimentexamples, the sdAb moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 274-354.

In some embodimentexamples, the sdAb moiety is humanized through CDR grafting.

In some embodimentexamples, the humanized sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 355-367.

In some embodimentexamples, the DLL3 is human or rhesus DLL3.

In another aspectexample, the present disclosure provides a DLL3 binding protein

comprising a single chain variable fragment (scFv) moiety that specifically binds to DLL3, wherein the scFv moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 498 or 504 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 499 or 505 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 500 or 506 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3, and the VL domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 495 or 501 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 496 or 502 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 497 or 503 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some embodimentexamples, the scFv moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 498, a CDR2 set forth in SEQ ID NO: 499, and a CDR3 set forth in SEQ ID NO: 500, and the VL domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 495, a CDR2 set forth in SEQ ID NO: 496, and a CDR3 set forth in SEQ ID NO: 497; or the VH domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 504, a CDR2 set forth in SEQ ID NO: 505, and a CDR3 set forth in SEQ ID NO: 506, and the VL domain of the scFv moiety comprises a CDR1 set forth in SEQ ID NO: 501, a CDR2 set forth in SEQ ID NO: 502, and a CDR3 set forth in SEQ ID NO: 503.

In some embodimentexamples, the VH domain of the scFv moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 508, and the VL domain of the scFv moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 507; or the VH domain of the scFv moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 510, and the VL domain of the scFv moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 509.

In some embodimentexample, the scFv moiety is obtained from a synthetic human Fab phage library.

In some embodimentexamples, the DLL3 is human or rhesus DLL3.

In one aspect, the present disclosure provides a nucleic acid molecule encoding a CAR as described herein or the antibody or antigen-binding fragment as described herein. In another aspectexample, the present disclosure provides an isolated nucleic acid molecule encoding an anti-DLL3 CAR or a DLL3 binding protein as described above.

In some embodimentexamples, the isolated nucleic acid molecule comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 368-448 which encodes a camel single domain antibody (sdAb).

In some embodimentexamples, the isolated nucleic acid molecule comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 449-461 which encodes a humanized camel sdAb.

In some [embodimentexamples](#), the isolated nucleic acid molecule comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 511-514 which encodes a VL or VH domain of a human scFv.

In some [embodimentexamples](#), the nucleic acid molecule further comprises a polynucleotide sequence encoding a Chimeric Switch Receptor (CSR) or a Dominant Negative Receptor (DNR).

In some [embodimentexample](#), the nucleic acid molecule further comprises a polynucleotide sequence encoding a PD-1 Dominant Negative Receptor (PD-1 DNR), a PD-1 Chimeric Switch Receptor (PD-1 CSR), or a TGF- β Dominant Negative Receptor (TGF- β DNR).

In some [embodimentexamples](#), the PD-1 DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 523.

In some [embodimentexamples](#), the PD-1 CSR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 524.

In some [embodimentexamples](#), the TGF- β DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 529.

In some [embodimentexamples](#), the polynucleotide sequence encoding the PD-1 DNR, the PD-1 CSR or the TGF- β DNR is linked to the polynucleotide sequence encoding the CAR through a polynucleotide sequence encoding a 2A self-cleaving peptide.

In some [embodimentexample](#), the 2A self-cleaving peptide is a T2A peptide or a P2A peptide.

In some [embodimentexamples](#), the nucleic acid molecule comprises, in the 5' to 3' direction, a polynucleotide sequence encoding the CAR, a polynucleotide sequence encoding the 2A self-cleaving peptide, and a polynucleotide sequence encoding the PD-1 DNR, the PD-1 CSR or the TGF- β DNR.

In some [embodimentexamples](#), the nucleic acid molecule encoding a peptide having at least about 95% sequence identity to SEQ ID NO: 521 or 522, or the nucleic acid molecule encoding a peptide having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 525-528.

In one aspect, the present disclosure provides an expression vector comprising the nucleic acid molecule as described herein. In another [aspectexample](#), the present disclosure provides an expression vector comprising an isolated nucleic acid molecule as described above.

In one aspect, the present disclosure provides an engineered immune cell comprising the nucleic acid molecule as described herein or an expression vector as described herein. In another [aspectexample](#), the present disclosure provides an engineered immune cell comprising an isolated nucleic acid molecule as described above.

In some [embodimentexamples](#), the engineered immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, a $\gamma\delta$ T cell, a NKT cell and a Nature Killer cell.

In another [aspectexample](#), the present disclosure provides an engineered immune cell expressing an anti-DLL3 CAR as described above.

In some [embodimentexample](#), the engineered immune cell also expresses a CSR or a DNR.

In some [embodimentexamples](#), the CSR is a PD-1 CSR, the DNR is a PD-1 DNR or a TGF- β DNR.

In some [embodimentexamples](#), the PD-1 DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 523.

In some embodimentexamples, the PD-1 CSR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 524.

In some embodimentexamples, the TGF- β DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 529.

In some embodimentexamples, the CAR and the CSR, or the CAR and the DNR, are co-expressed through a 2A self-cleaving peptide.

In some embodimentexamples, the 2A self-cleaving peptide is a T2A peptide or a P2A peptide.

In some embodimentexamples, the engineered immune cell expresses the CAR and the PD-1 DNR, and is stimulated by a cell expressing DLL3 and PD-L1.

In some embodimentexamples, the engineered immune cell expresses the CAR and the PD-1 CSR, and is stimulated by a cell expressing DLL3 and PD-L1.

In some embodimentexamples, the engineered immune cell expresses the CAR and the TGF- β DNR, and is stimulated by a cell expressing DLL3 in the present of TGF- β .

In some embodimentexamples, the engineered immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, a $\gamma\delta$ T cell, a NKT cell and a Nature Killer cell.

In one aspect, the present disclosure provides a pharmaceutical composition comprising the engineered immune cell as described herein, and a physiologically acceptable excipient. In another aspectexample, the present disclosure provides a pharmaceutical composition comprising an anti-DLL3 CAR, an isolated DLL3 binding protein, an expression vector, or an engineered immune cell as described above, and a physiologically acceptable excipient.

In another aspectexample, the present disclosure provides a method for treating a DLL3 associated disorder in a subject, comprising administering to the subject a therapeutically effective amount of engineered immune cells as described above or a therapeutically effective amount of a pharmaceutical composition as described above.

In one aspect, the present disclosure provides use of an effective amount of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a DLL3 associated disorder in a subject, preferably the DLL3 associated disorder is cancer, more preferably the cancer is selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.

In another aspectexample, the present disclosure provides the use of an anti-DLL3 CAR, an isolated DLL3 binding protein, an expression vector, or an engineered immune cell as described above for the preparation of a medicament for treating a DLL3 associated disorder.

In another aspectexample, the present disclosure provides a medicament for use in treating a DLL3 associated disorder comprising an anti-DLL3 CAR, a DLL3 binding protein, an expression vector, or an engineered immune cell as described above.

In some embodimentexamples, the DLL3 associated disorder is a cancer selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.

In some embodimentexamples, the cancer expresses DLL3 and PD-L1.

In some embodimentexamples, the cancer has a higher expression level of TGF- β compared to corresponding normal tissue.

In some embodimentexamples, the DLL3 associated disorder is small cell lung cancer.

BRIEF DESCRIPTION OF THE DRAWINGS