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Supplemental Information

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Suppl. Figure S1. Related to Figure 2

A

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAG
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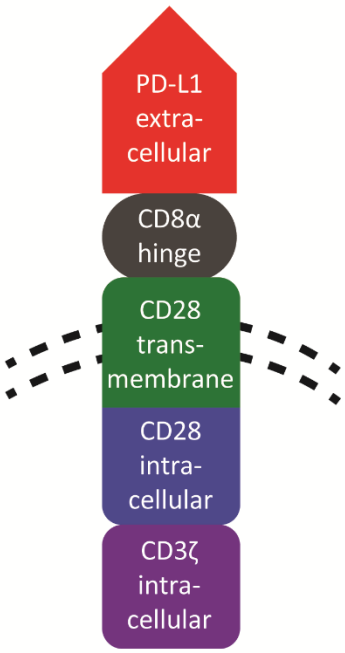
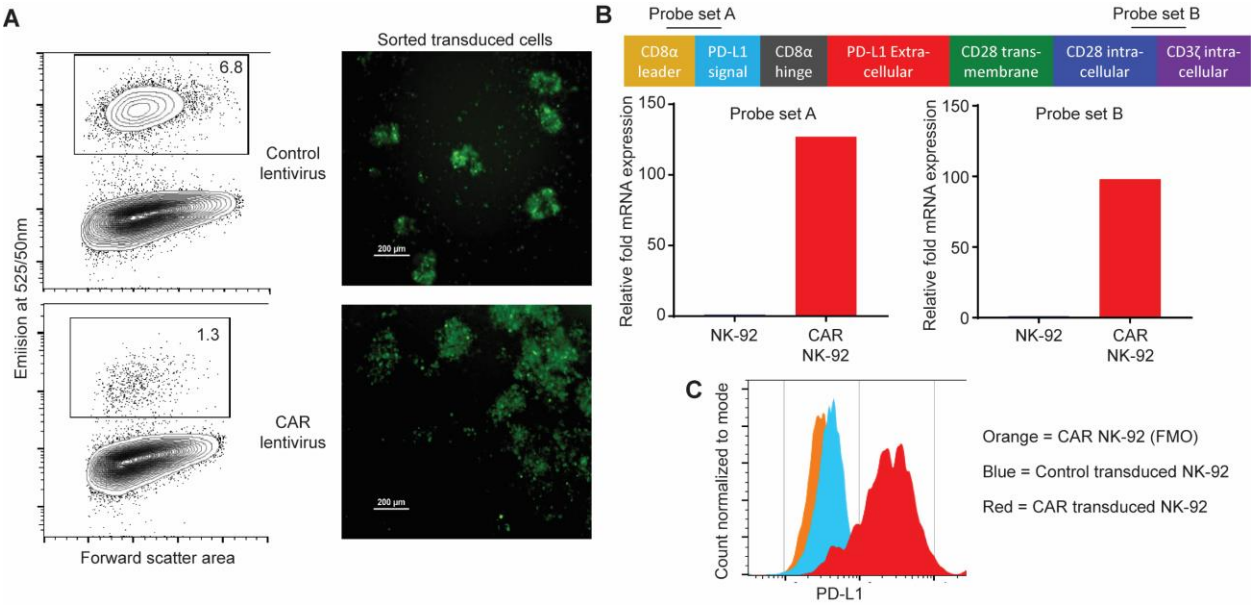
CD8a leader, PD-L1 signal sequence, PD-L1 extracellular domain, CD8a hinge, CD28 trans-membrane domain, CD28 intracellular domain, TCR-CD3ζ intracellular domain, Stop

B



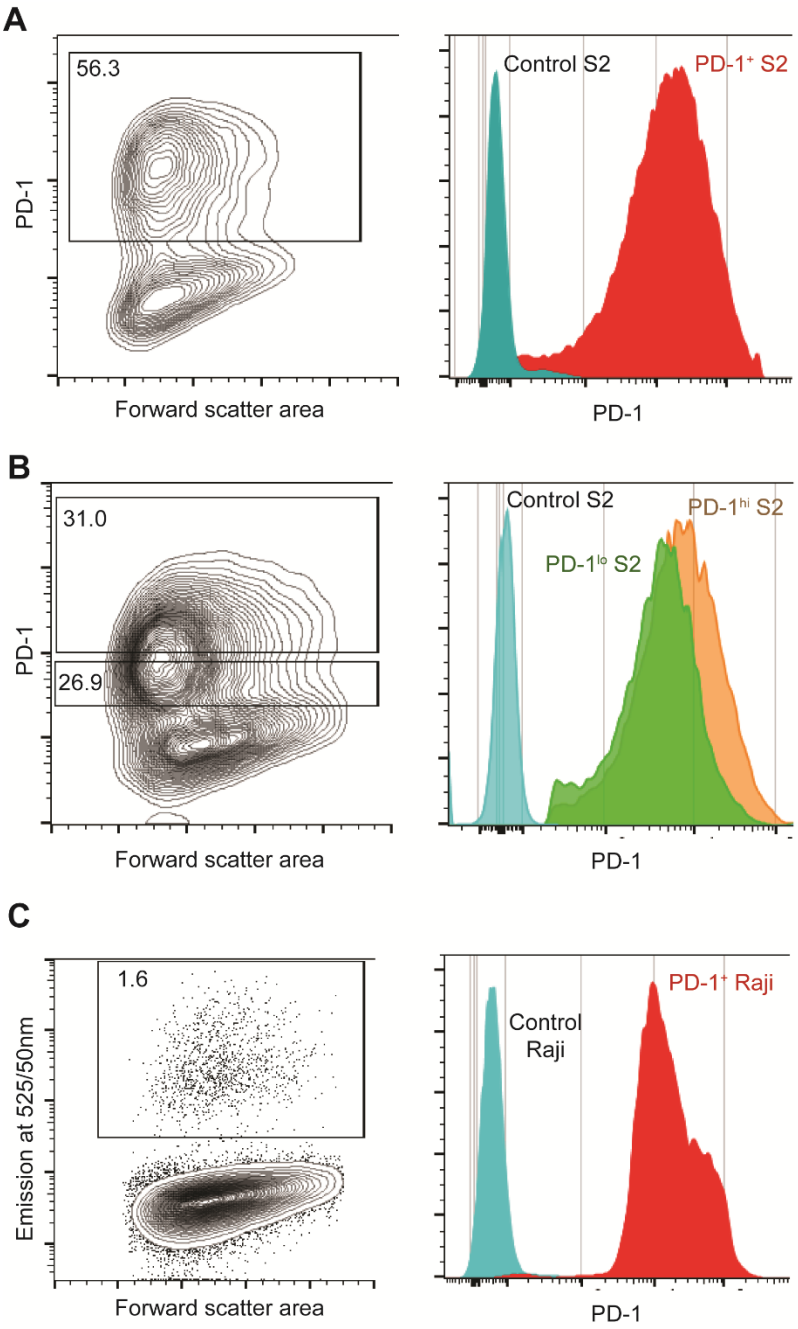
Supplemental Figure S1. PD-L1 CAR design. (**A**) Coding sequence for CAR construct, color-coded by domain. (**B**) Graphical depiction of CAR protein expressed on cell surface highlighting specific domains (dashed line = plasma membrane).

Suppl. Figure S2. Related to Figure 2



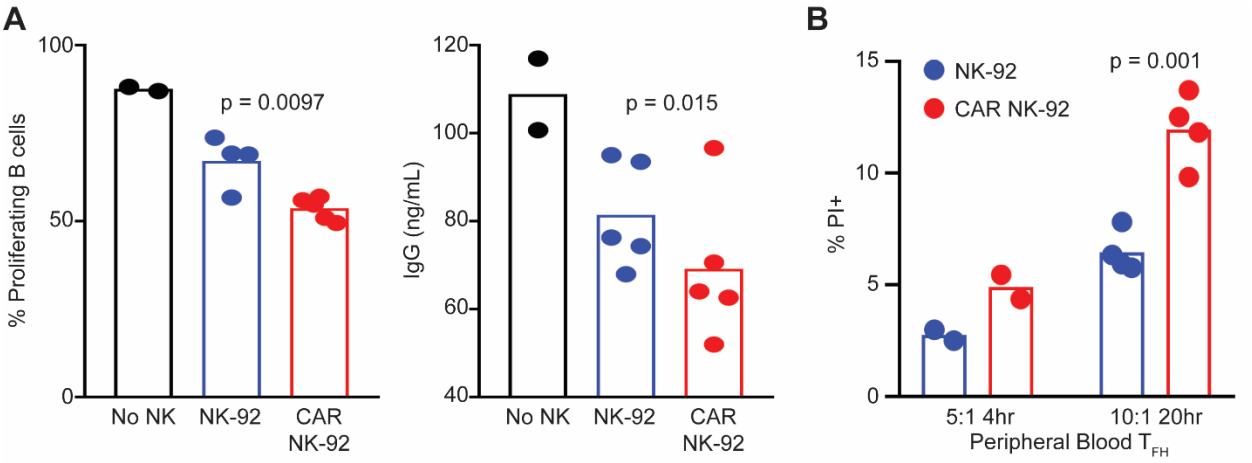
Supplemental Figure 2. Expression of CAR in NK-92 via lentiviral transduction. (A) Percent transduction efficiency of NK-92 with empty lentiviral vector (top-left) and CAR-containing lentiviral vector (bottom-left) as measured by reporter fluorescence. Images of the corresponding sorted fluorescent NK-92 (right, top and bottom) following one week of culture (growing in characteristic clumps). **(B)** Graphical depiction of RNA alignment of CAR-specific qPCR primer/probe sets (top) and the corresponding fold CAR mRNA expression (bottom) in control (NK-92) and CAR NK-92. CAR mRNA expression normalized to GAPDH. **(C)** PD-L1 expression of empty-vector-transduced NK-92 and CAR-lentivirus-transduced NK-92, including PD-L1 fluorescence-minus-one (FMO) control.

Suppl. Figure S3. Related to Figure 2



Supplemental Figure 3. Generation of target cell lines expressing human PD-1. (A) Contour plots (left) showing PD-1 expression in pAc5/V5-His-PD1-transfected S2 cells and histogram (right) of PD-1 expression on sorted PD-1^{+} S2 or sorted control vector transduced S2 cells after one week of culture. **(B)** Contour plots (left) showing electronic gates for sorting of PD-1^{Hi} and PD-1^{Lo} S2 cells, and histogram (right) of PD-1 expression on sorted PD-1^{Hi} and PD-1^{Lo} and control S2 cells after one week of culture. **(C)** Contour plot (left) showing percent PiggyBac transposition efficiency in Raji cells, and histogram (right) of PD-1 expressed on control and PD-1^{+} Raji cells.

Suppl. Figure S4. Related to Figure 4



Supplemental Figure 4. PD-L1 CAR NK cells kill peripheral T_{FH} cells and suppress T-dependent B-cell responses. (A) Frequency of proliferating (CTV^-) $CD27^+$ B cells following 4-day co-culture with SEB-stimulated tonsillar T_{FH} to which control, CAR, or no NK-92 cells were added for 24 hours (day 3) at an NK:T:B cell ratio of 5:1:2 (n=2-5). Total supernatant IgG (right) at day 4 in the co-culture assay. Data analyzed via 1-way ANOVA with multiple comparisons (comparing each group to every other group). One of two similar and independent experiments is shown. **(B)** PI uptake in sorted, SEB-stimulated $CD4^+$ $CXCR5^+$ peripheral blood T cells co-cultured with either control or CAR NK-92 at a 5:1 E:T ratio for 4 hours (n=2), or a 10:1 ratio for 20 hours (n=4). Later analyzed via unpaired Student's t-test.

Suppl. Table S1. Primer/Probe sequences. Related to Figure 2.

Table S1

qPCR primer/probe sets for verification of CAR expression:

PD-L1 CAR Set A:

Probe: 5'-/56-FAM/ATCGCTCCA/ZEN/GAGTGAAGTTCAGCA/3IABkFQ/-3'
Primer 1: 5'-GCAAGCATTACCAGCCCTAT-3'
Primer 2: 5'-TTCTGGCCCTGCTGGTA-3'

PD-L1 CAR Set B:

Probe: 5'-/56-FAM/CCAGGCCGA/ZEN/TGAGGATATTTGCTGT/3IABkFQ/-3'
Primer 1: 5'-CTTACCAGTGACCGCCTTG-3'
Primer 2: 5'-CTTGGGAACCGTGACAGTAAA-3'

Sequencing primers to verify PD-L1 CAR insertion into pLVX-IRES-ZsGreen plasmid:

5'-GCACACCGGCCTTATTCCAA-3' (Rev 1)
5'-CATTCAACAGACCTTGCATTCC-3' (Rev 2)
5'-CTACTAGAGGATCTATTTCCGG-3' (Fwd)

Sequencing primers to verify PD-1 insertion into pAc/V5-His plasmid:

5'-TAGAAGGCACAGTCGAGG-3' (Fwd)
5'-ACACAAAGCCGCTCCATCAG-3' (Rev)

Sequencing primers to verify PD-1 insertion into PB513 plasmid:

5'-AGAGCTCGTTTAGTGAACCGTC-3' (Fwd)
5'-AACTCCTCGGGGACTGTG-3' (Rev)