

**Cell Reports Medicine, Volume 1**

**Supplemental Information**

**Therapeutic Targeting of Follicular**

**T Cells with Chimeric Antigen**

**Receptor-Expressing Natural Killer Cells**

**Seth D. Reighard, Stacey A. Cranert, Kelly M. Rangel, Ayad Ali, Ivayla E. Gyurova, Arthur T. de la Cruz-Lynch, Jasmine A. Tuazon, Marat V. Khodoun, Leah C. Kottyan, David F. Smith, Hermine I. Brunner, and Stephen N. Waggoner**

## **Supplemental Information**

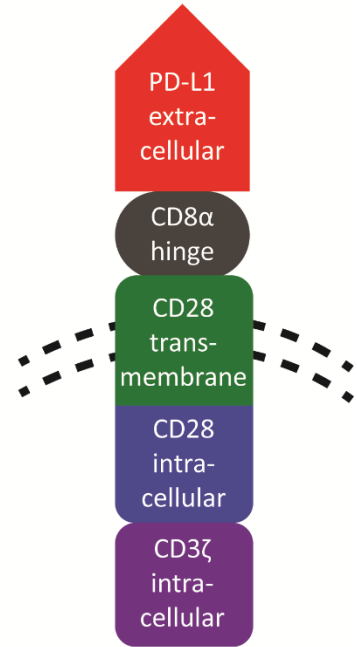
### **Therapeutic targeting of follicular T cells with chimeric antigen receptor-expressing natural killer cells**

Seth D. Reighard, Stacey A. Cranert, Kelly M. Rangel, Ayad Ali, Ivayla E. Gyurova, Arthur T. de la Cruz-Lynch, Jasmine A. Tuazon, Marat V. Khodoun, Leah C. Kottyan, David F. Smith, Hermine I. Brunner, Stephen N. Waggoner

**Suppl. Figure S1. Related to Figure 2**

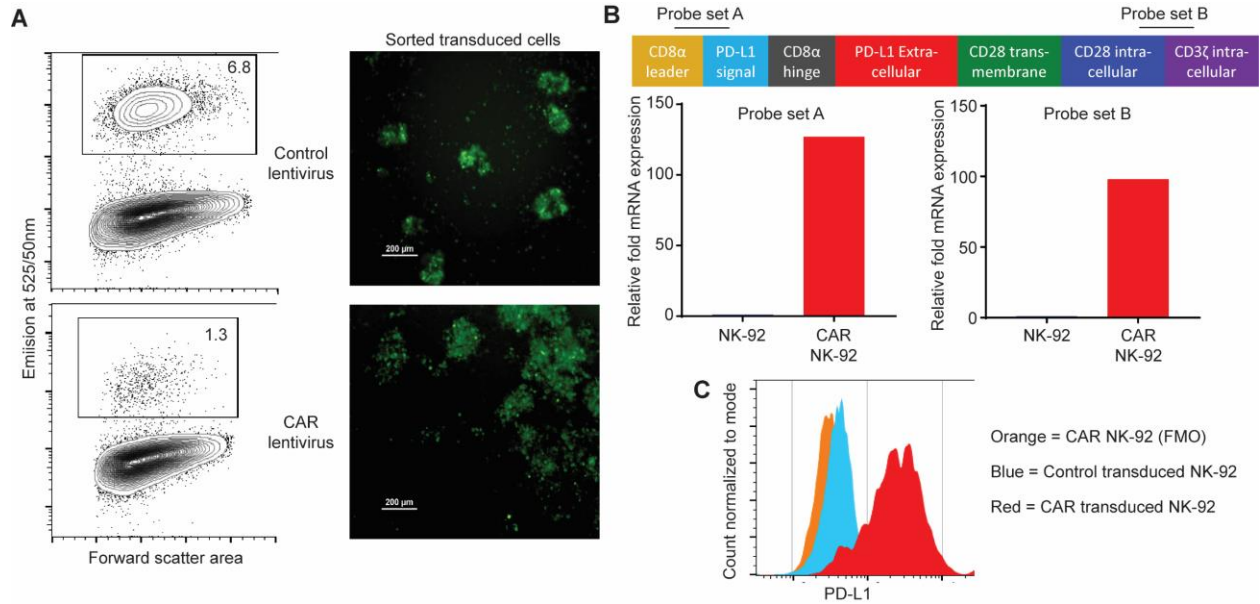
**A** ATGGCCTTACCAGTGACCGCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAG  
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 GGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTTACAGCAT  
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 GTACAGCCACCAAGGACACCTACGACGCCCTTACATGCAGGCCCTGCCCTCGCTAA

CD8a leader, PD-L1 signal sequence, PD-L1 extracellular domain, CD8a hinge, CD28 trans-membrane domain, CD28 intracellular domain, TCR-CD3 $\zeta$  intracellular domain, Stop



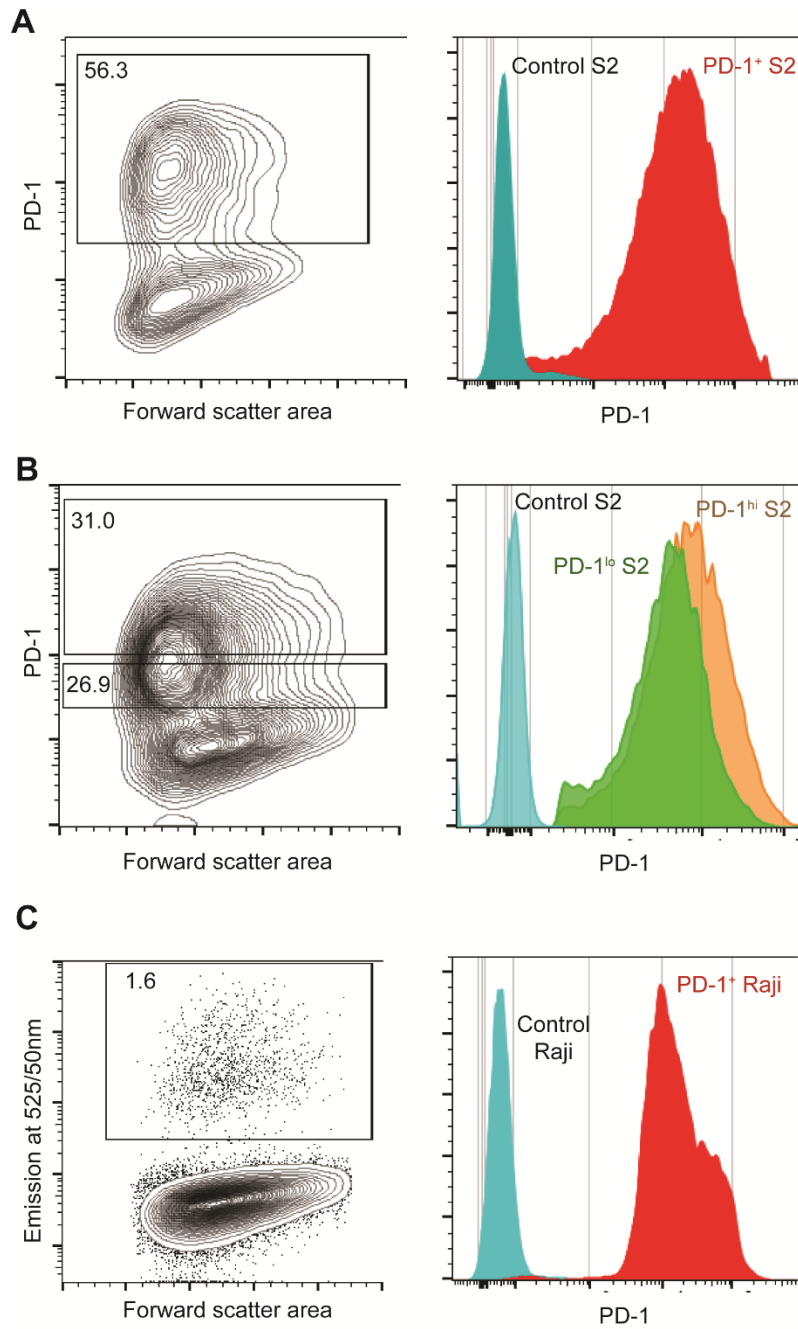
**Supplemental Figure S1. PD-L1 CAR design.** (A) Coding sequence for CAR construct, color-coded by domain. (B) Graphical depiction of CAR protein expressed on cell surface highlighting specific domains (dashed line = plasma membrane).

**Suppl. Figure S2. Related to Figure 2**



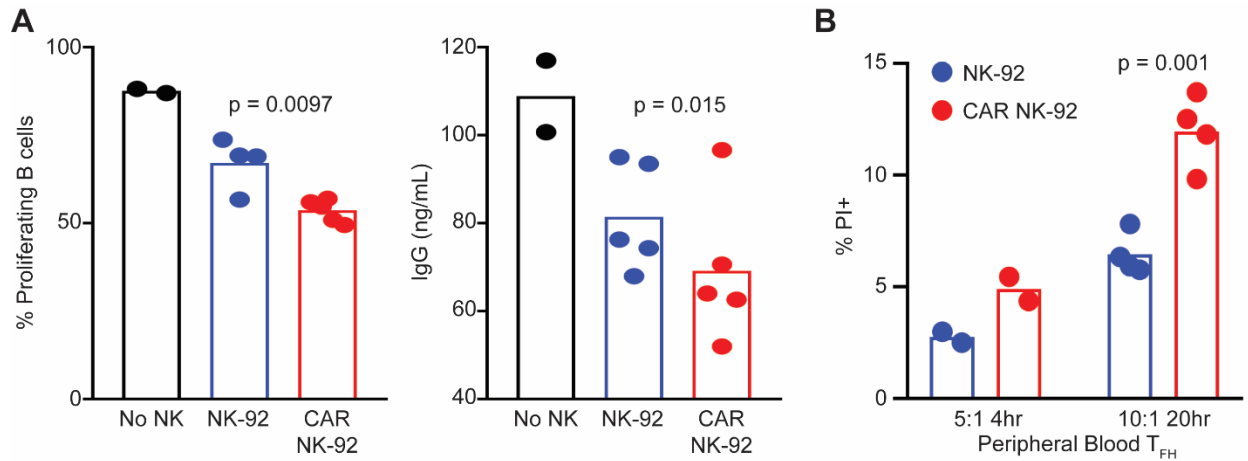
**Supplemental Figure 2. Expression of CAR in NK-92 via lentiviral transduction. (A)** Percent transduction efficiency of NK-92 with empty lentiviral vector (top-left) and CAR-containing lentiviral vector (bottom-left) as measured by reporter fluorescence. Images of the corresponding sorted fluorescent 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<sup>+</sup> 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<sup>Hi</sup> and PD-1<sup>Lo</sup> S2 cells, and histogram (right) of PD-1 expression on sorted PD-1<sup>Hi</sup> and PD-1<sup>Lo</sup> 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<sup>+</sup> Raji cells.

**Suppl. Figure S4. Related to Figure 4**



**Supplemental Figure 4. PD-L1 CAR NK cells kill peripheral T<sub>FH</sub> cells and suppress T-dependent B-cell responses. (A)** Frequency of proliferating (CTV<sup>-</sup>) CD27<sup>+</sup> B cells following 4-day co-culture with SEB-stimulated tonsillar T<sub>FH</sub> 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<sup>+</sup> CXCR5<sup>+</sup> 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)