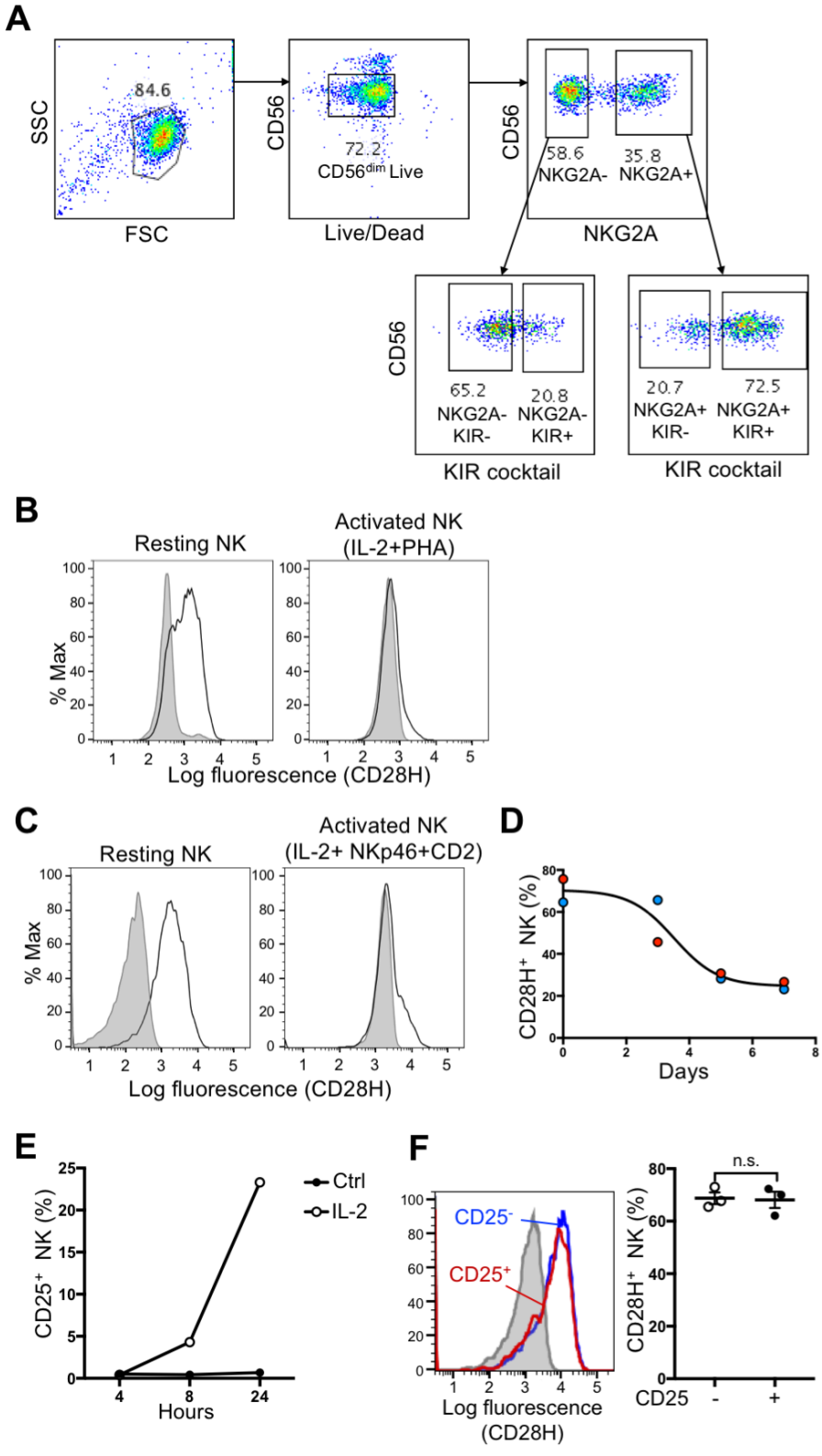
CD28 homolog is a strong activator of natural killer cells for lysis of B7H7-positive tumor cells

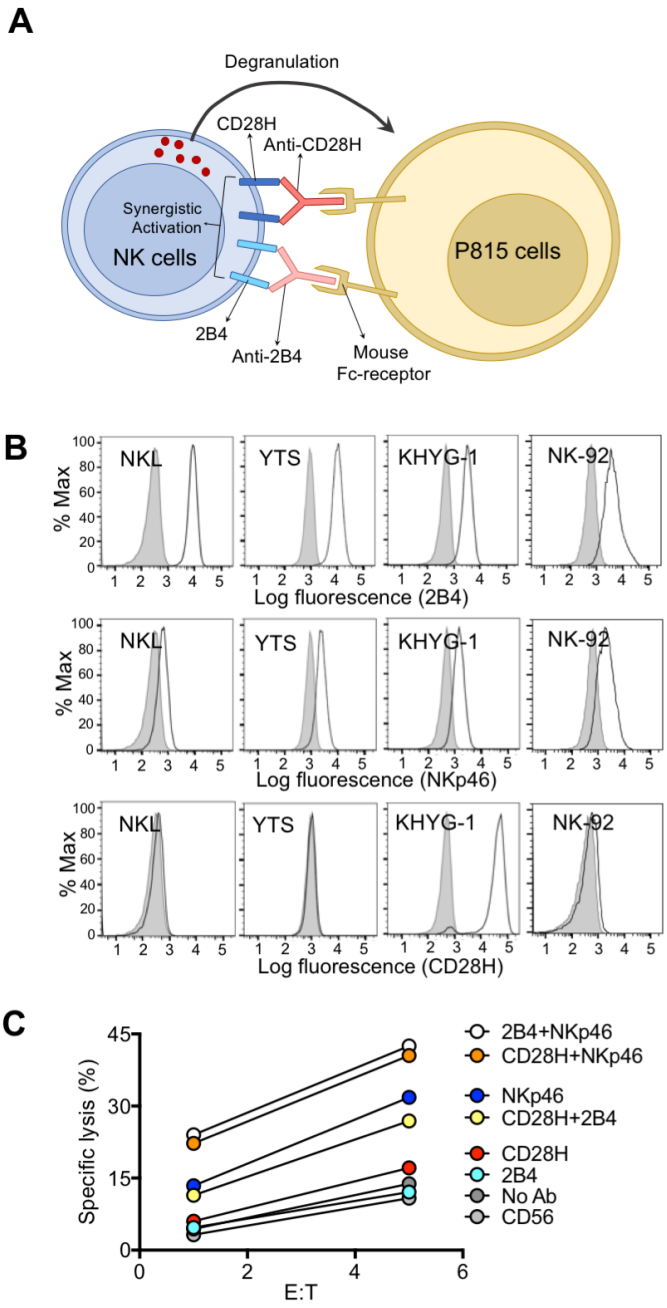
Supplemental Information

Supplemental Figures



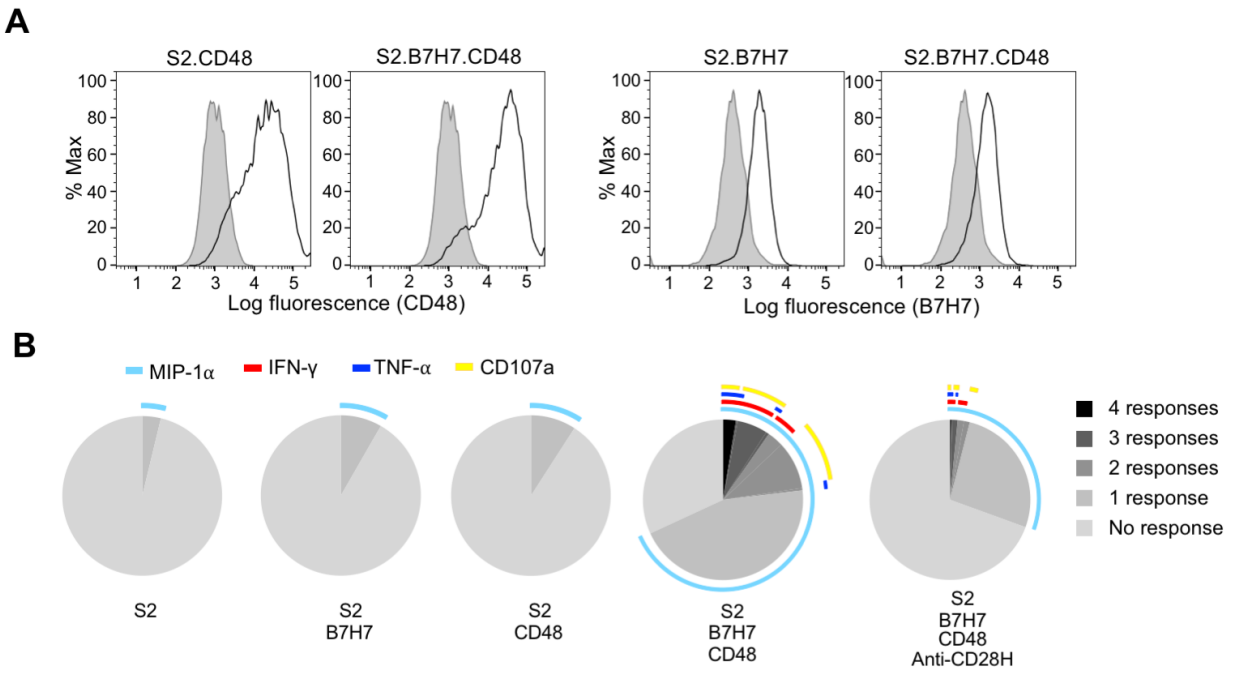
Supplemental Figure 1

(A) Gating strategy of different CD56dim NK cell populations based on NKG2A and KIR expression. KIR expression was determined by a cocktail of PE-conjugated antibodies (EB6, GL183 and DX9). (B) Representative histogram of immunostaining for CD28H in resting NK cells and NK cells expanded in IL-2 and PHA for 14 days. Freshly isolated or IL-2 cultured NK cells were stained with isotype control (shaded) or CD28H mAb (solid line), followed by incubation with PE-conjugated goat anti-mouse secondary antibody. (C) Representative histogram of immunostaining for CD28H in resting NK cells or NK cells activated by IL-2 and crosslinking of CD2 and NKp46. NK cells were activated by plate-bound mAbs to CD2 and NKp46 plus 100 U/ml IL-2 for 7 days. (D) Expression of CD28H at different time points after activation of NK cells from 2 donors as in (C). The percentage of NK cells that were CD28H+ was determined by staining for CD56 and CD28H. (E) CD25 expression on NK cells at different time points after IL-2 stimulation. (F) Resting NK cells were activated in IL-2 for 24 hours, followed by staining for CD56, CD25 and CD28H. Expression of CD28H in CD25- and CD25+ NK cells shown in histogram (right) and presented as data from 3 independent donors (left). mean ± SEM. n.s. not-significant (Mann-Whitney test, two-tailed).

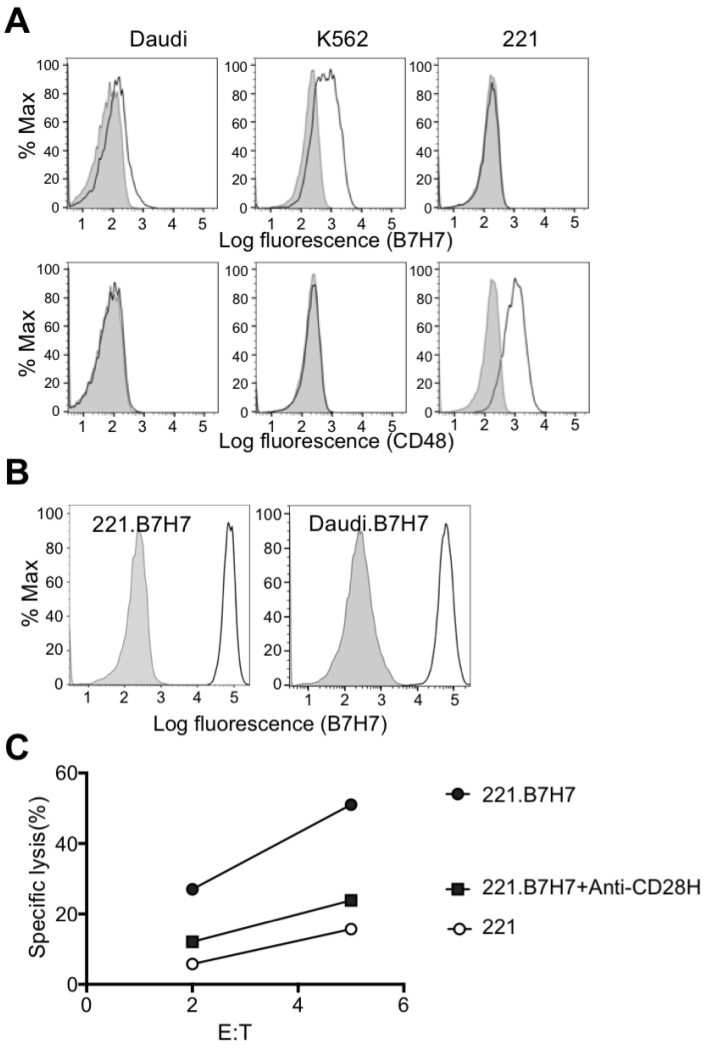


Supplemental Figure 2.

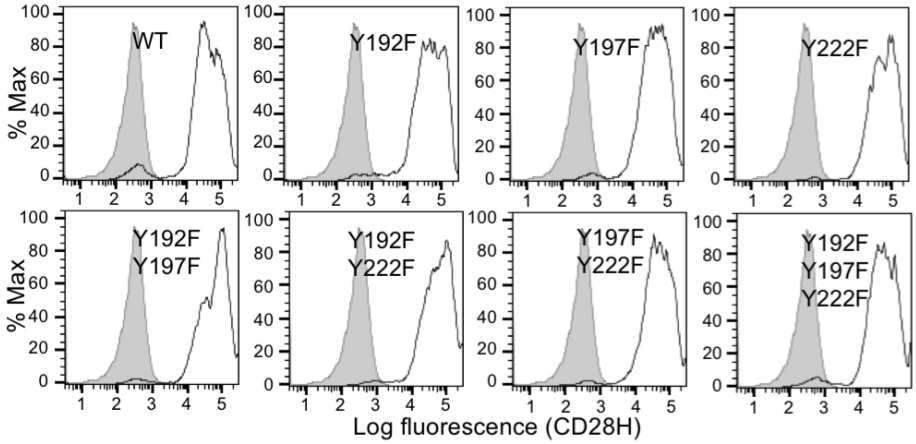
(A) Schematics of redirected cytotoxicity assay. In the assay, the F(ab')$_2$ portions of the mAbs specifically recognize NK cell receptors, while the Fc fragments bind to Fc receptor on mouse P815 cells. NK cells are synergistically activated by the crosslinked NK cell activation receptors. (B) Surface expression of 2B4, NKp46 and CD28H in the NKL, YTS, KHYG-1, and NK-92 cell lines determined by immunostaining with fluorophore-conjugated mAbs. Shaded histograms represent staining with isotype controls. (C) Lysis of P815 cells by KHYG-1 cells in redirected cytotoxicity assays. KHYG-1 cells were rested without IL-2 for 24 hours before incubation with P815 cells and the indicated mAbs for 6 hours at E to T ratios of 1 and 5.



Supplemental Figure 3. NK cell degranulation and cytokine production induced by B7H7 and CD48 on *Drosophila* S2 cells. (A) Expression of CD48 and B7H7 on S2 cells transfected with CD48, B7H7, or both. Shaded histogram indicates staining of untransfected S2 cells. (B) Pie charts represent the frequency of NK cells positive for the indicated number of responses. Arcs represent the proportion of NK cells positive for MIP-1α, IFN-γ, TNF-α and CD107a. Values represent mean of 3 donors.

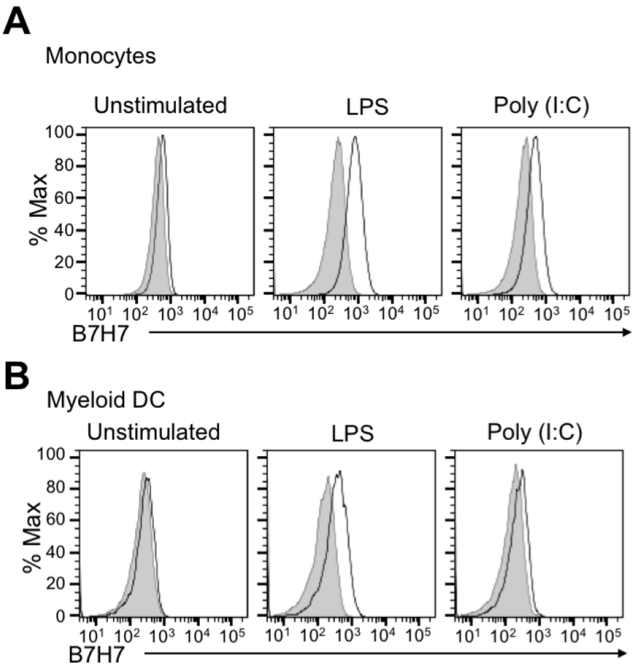


Supplemental Figure 4. B7H7 on 221 cells promotes CD28H-dependent lysis by NK cells. (A) Staining for B7H7 and CD48 on Daudi, K562, and 221 cells. Shaded histograms represent staining with isotype controls. (B) Expression of B7H7 on transfected 221 and Daudi cells. Shaded histograms represent staining of untransfected cells. (C) Lysis of 221 and 221.B7H7 cells by resting NK cells at the indicated E to T ratios after 6 hours. A mAb to CD28H was added at 10 μg/ml to block the CD28H–B7H7 interaction (square symbols).



Supplemental Figure 5.

CD28H Expression on NKL cells transfected with the indicated CD28H mutants. Shaded histograms represent staining of untransfected cells.



Supplemental Figure 6. B7H7 expression on circulating monocytes and myeloid dendritic cells. (A) Either untreated or LPS- and poly (I:C)-stimulated human PBMC were gated on the $CD14^+$ population and tested for B7H7 expression with fluorophore-conjugated B7H7 mAb. (B) Expression of B7H7 in circulating myeloid dendritic cells. Human PBMC treated as in (A) were gated on lineage-negative ($CD3^-CD19^-CD14^-NKp46^-$) $CD11c^+$ cells, and tested for expression of B7H7 by staining with B7H7 mAb. Shaded histograms represent staining with isotype control.

A

CD28H-TCRζ

MGSPGMVLGLLVQIWALQEASSLSVQQGPNLLQVRQGSQATLVCQVDQATAWERLRVKWTKDGAILCQPYITNGSLSL
GVCGPQGRLSWQAPSHLTLQLDPVSLNHSGAYVCWAAVEIPELEEAEGNITRLFVDPDDPTQNRNRIASFPGFLFVLLG
VGSMGVAAIVWGAWFWGRRSCQQRDSGNSPGNAFYSNVLYRPRGAPKKSEDCSGEGKDQRGQSIYSTSFPQPAPR
QPHLASRPCPSPRPCPSPRPGHPVSMVRVSPRPSPTQQPRPKGFPKVGEERVKFSRSADAPAYQQGQNQLYNELNL
GRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY
DALHMQALPPR

B

CD28H(ΔCD)-TCRζ

MGSPGMVLGLLVQIWALQEASSLSVQQGPNLLQVRQGSQATLVCQVDQATAWERLRVKWTKDGAILCQPYITNGSLSL
GVCGPQGRLSWQAPSHLTLQLDPVSLNHSGAYVCWAAVEIPELEEAEGNITRLFVDPDDPTQNRNRIASFPGFLFVLLG
VGSMGVAAIVWGAWRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Supplemental Figure 7.

Amino acid sequences of CD28H-TCRζ (A) and CD28H(ΔCD)-TCRζ (B).