Supplemental Methods

Generation of murine constructs and retroviral transduction

DNA constructs were ordered from Invitrogen or generated in-house by PCR. The constructs directionally TOPO-cloned into vector pENTR/D-TOPO were transferred to the retroviral vector (RV) pMP71-attR using Gateway® technology. The retroviral packaging cell line Plat-E (Cell-Bio Labs) was transduced with the RV using effectene transduction reagent (Qiagen). Viral supernatant was collected on days 2 and 3. One day prior to transfection, TCR_{gag} T cells were stimulated with anti-CD3/CD28 and 100 U/mL rhIL-2. Transduction of TCR_{gag} T cells was performed in 12 well plates in the presence of IL-2 and polybrene by spinfection for 90 minutes at 1000*g*. Transduced cells were restimulated 7 days post stimulation in the presence of irradiated splenocytes (5×10^6), irradiated FBL (3×10^6), and IL-2 (IU/mL).

T cell proliferation assay

T cells were stained with CellTrace Violet (CTV, Life Technologies) according to manufacturer's protocol. CTV-labeled T cells (10^5) were co-cultured with tumor cells in the absence of IL-2 and after 3 (mouse) or 6 days (human), CTV dilution of was assessed by flow cytometry and FlowJo analysis.

Intracellular cytokine production

Transduced cells were incubated for 4-6 hr with tumor cells in Golgiplug (BD Biosciences). Cells were then stained in the dark with antibodies to CD8 for 30 min on ice. Cells were washed twice and fixed for 20 min on ice with BD Biosciences Fix/Perm. Cells were washed with BD Biosciences Perm/Wash and incubated with antibodies to IFNγ, IL-2 and TNFα for 30 min on ice. All FACS data were acquired using a FACSCanto II.

Cytotoxicity assays

FBL and control EL4 tumors were incubated for 10 min at room temperature with 2.5 μM (hi) or 0.25 μM (lo) CFSE in PBS, respectively. Excess dye was removed by washing tumor cells in serum-containing media. A 1:1 mixture of EL4 and FBL tumor cells was incubated with titrated numbers of CD200R-CD28 or GFP transduced TCR_{gag} effector T cells for 4 h in 96-well, round-bottom plates at 37°C and 5% CO_2. Specific FBL lysis was determined by flow cytometric analyses of the % $CFSE_{hi}$ (FBL) of total CFSE positive cells (FBL+EL4) remaining in the well.

For human cytotoxicity assay, T cells were cultured with primary AML cells at the indicated E:T ratios for 24 hours. Surviving AML cells were quantified with Flow Count beads (Molecular Probes C36950) and flow cytometry. Percent survival was calculated by (Absolute number of targets in co-culture/ Absolute number of targets in target only control well)X100.

Supplemental Figure Legends

Supplemental Figures 1-3. Splenocytes from naive TCR_{gag} mice were stimulated *in vitro* with anti-CD3, anti-CD28, and recombinant human IL-2 (100 U/ml) and transduced with retroviral supernatant for 2 days. Cells were restimulated every 7 days with irradiated FBL and splenocytes, and cultured with rhIL-2 (50 U/mL) for up to three stimulations. T cells were used for assays 5-7 days after the last stimulation.

Supplemental Figure 1. CD28 signaling domain is required for costimulation

(A) Schematic representation of a truncated non-signaling version of a CD200R IFP with only the CD200Rec and CD28tm domains (trCD200R).
(B) Transgenic expression of trCD200R construct in transduced TCR_{gag} T cells as detected by anti-CD200R antibody.
(C) Proliferation of trCD200R- (blue lines) and GFP control-transduced (red lines) TCR_{gag} T cells as measured by CellTrace Violet dilution. T cells were stimulated with $CD200^+$ FBL for 3 days at the indicated E:T ratio.

Supplemental Figure 2. Restimulation with $CD200^+$ FBL results in a higher proportion of CD200R IFP-transduced T cells

A mixed population of transduced and non-transduced TCR_{gag} T cells were restimulated with irradiated $CD200^-$ (left panels) or $CD200^+$ (right panels) FBL, and splenocytes. The proportion of TCR_{gag} T cells transduced with CD200R-CD28-GFP (upper panels) or GFP only control (lower panels) was quantified by flow cytometry.

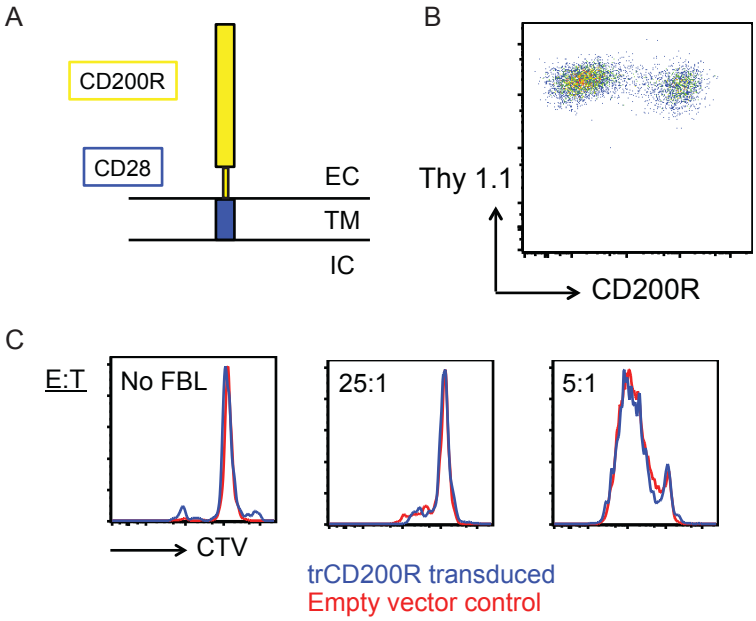
Supplemental Figure 3. Phenotype of IFP- and GFP-control-transduced T cells is similar

Expression of surface markers on TCR_{gag} T cells transduced with CD200R-CD28 (red lines) or nontransduced (blue lines) 5 days after stimulation *in vitro*. Both T cell populations were generated *in vitro* and identically expanded, and appeared phenotypically similar 5 days after the third stimulation.

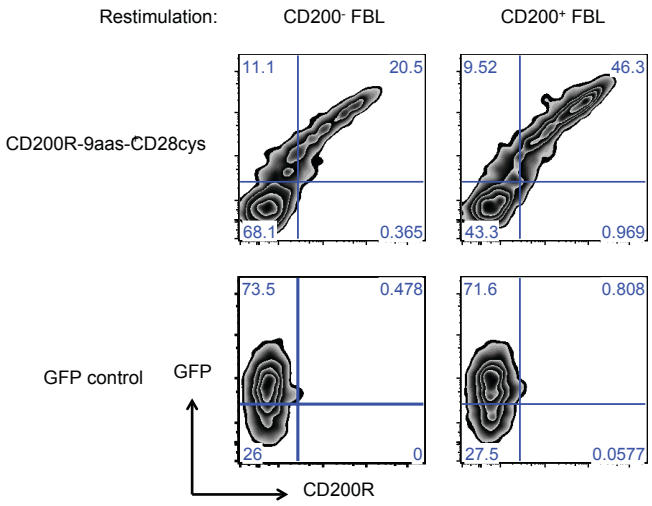
Supplemental Figure 4. Expression of CD200 on human cell lines

Expression of CD200 as detected by anti-CD200 antibody of the T2 lymphoblastoid cell line in comparison with $CD200^-$ K562 chronic myelogenous leukemia (CML) cell line.

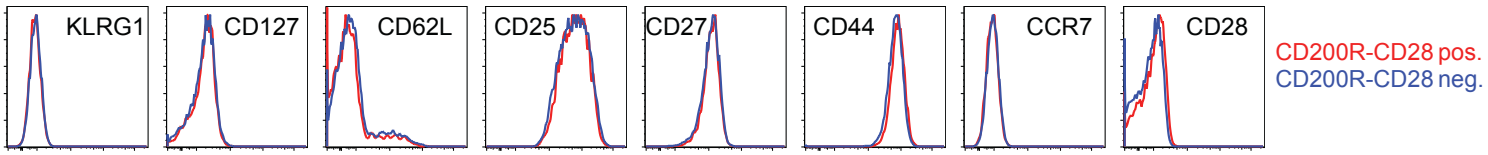
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4