Table S1. List of primers for RT-PCR

Sequence	Sequence of sense and anti-sense primers
β-actin	5′-GATCATTGCTCCTCCTGAGC-3′
	5′-CGTCATACTCCTGCTTGCTG-3′
Lef1	5′-AATGAGAGCGAATGTCGTTGC-3′
	5′-GCTGTCTTTCTTTCCGTGCTA-3′
TCF7	5′-GAGGAGCAGGACGACAAGAG-3′
	5′-TTCACGAGCGACGACTTGAG-3′
PPARγ	5′-CCTATTGACCCAGAAAGCGATT-3′
	5′-CATTACGGAGAGATCCACGGA-3′
RXRα	5′-GGACTGCCTGATTGACAAGC-3′
	5′-TTCAGCCCCATGTTTGCCTC-3′
CD44	5′-CCTGGGATTGGTTTTCATGGT-3′
	5′-CCAGCCTGCTGAGATGGTATTT-3′
IL-10	5′-AAGCTGAGAACCAAGACCCAGACA-3′
	5′-AAAGGCATTCTTCACCTGCTCCAC-3′
IL-9	5′-AGGCCAGGGGTGTCCAACCT-3′
	5′-GCGTTGCCTGCCGTGGTTTC-3′
IL-13	5′-CACTGGGCCTCATGGCGCTT-3′
	5′-TGGCACTGCAGCCTGACACG-3′
VEGFα	5′-GATGCGGGGGCTGCTGCAAT-3′
	5′-CCGCTCTGAGCAAGGCCCAC-3′
IL-2	5′-AACTCCTGTCTTGCATTGCAC-3′
	5′-GCTCCAGTTGTAGCTGTGTTT-3′

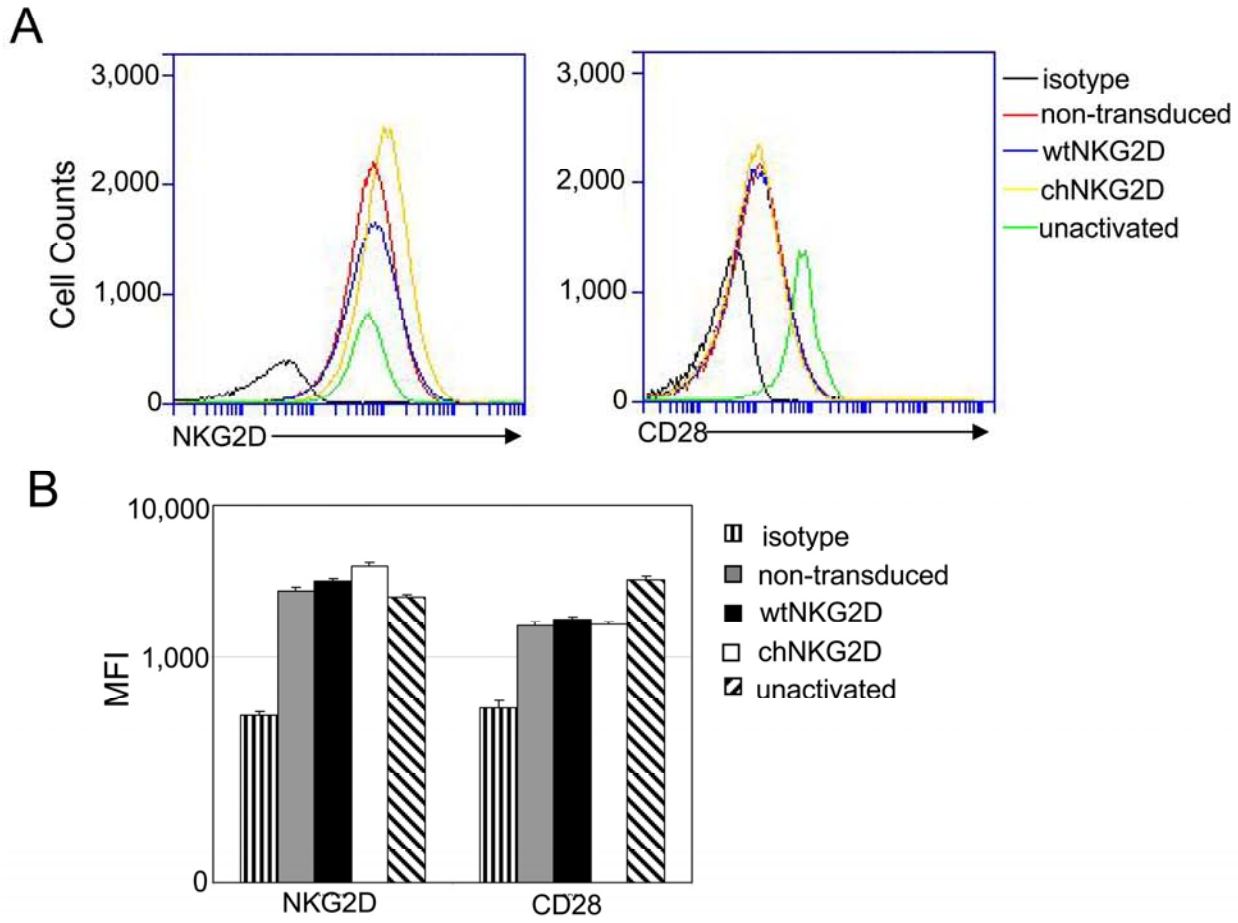

Figure S1. NKG2D and CD28 expression on effector CD8$^+$ T cells
(A) Representative histograms or (b) MFI of CD8$^+$ T cells transduced with wtNKG2D or chNKG2D receptors, activated and non-transduced, or unactivated (gated on CD3$^+$CD8$^+$ cells) were stained with antibodies specific for NKG2D, CD28, or isotype controls. Data shown in (B) are an average of three donors + s.d.

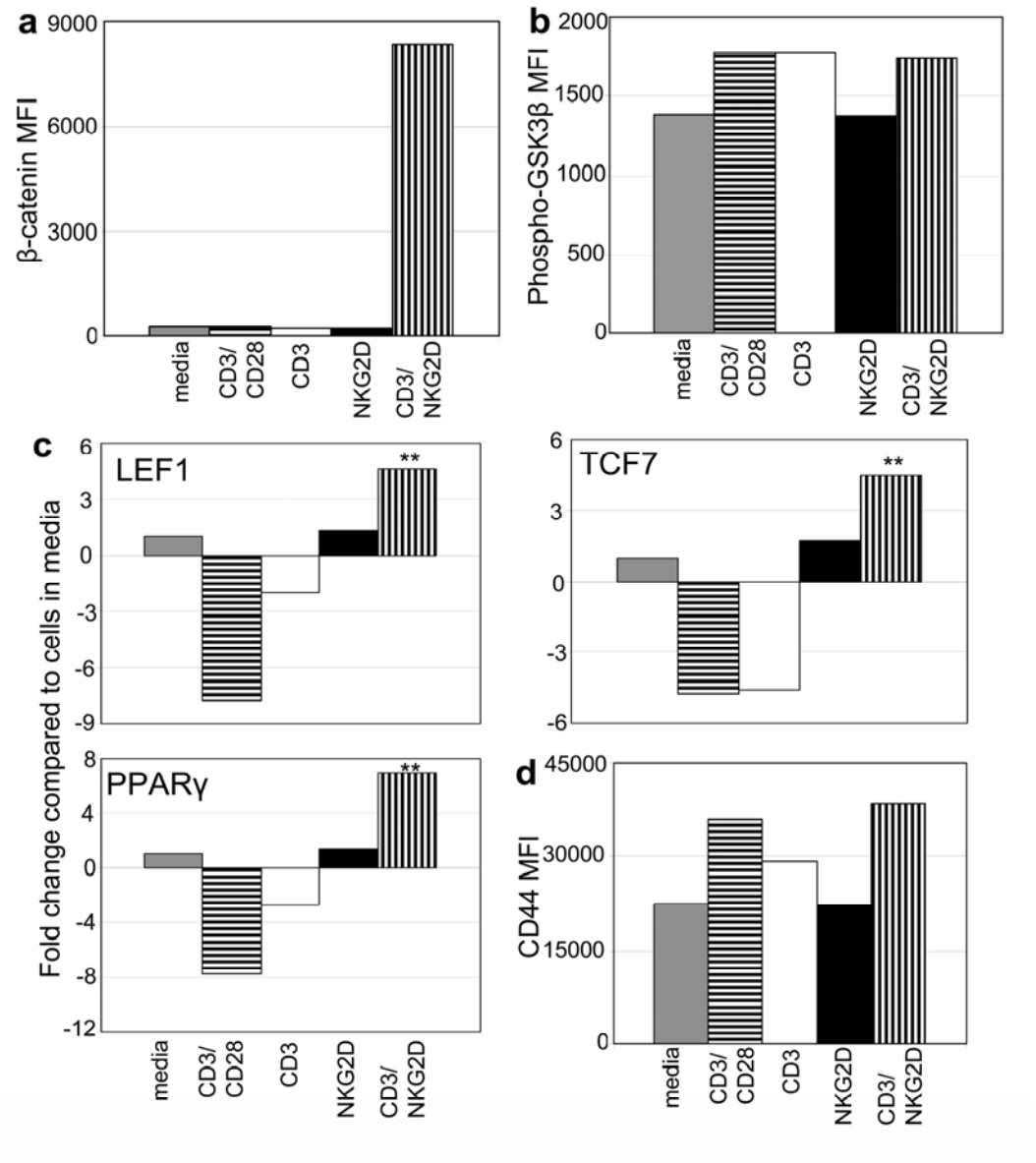

Figure S2. Stimulation of activated T cells through NKG2D and CD3 activates β-catenin
Activated, non-transduced T cells were stimulated with media, anti-CD3/CD28, anti-CD3-, anti-NKG2D-, or anti-CD3 and anti-NKG2D antibody coated beads. (A) β-catenin was measured with anti- β-catenin antibodies 5 hours after stimulation. (B) Phosphorylated (Ser9) GSK3β-expression was measured 30 minutes after stimulation. (C) Eight hours after stimulation, gene expression was determined by RT-PCR. Data are shown as the fold change in gene expression compared to T cells cultured in media. Stimulation of T cells through CD3/NKG2D significantly changed gene expression compared to CD3 stimulation (**-$p<0.05$). (D) CD44 cell surface expression was determined by FACS 24 hours after stimulation. Data shown were obtained from one donor and are representative of data from three donors.

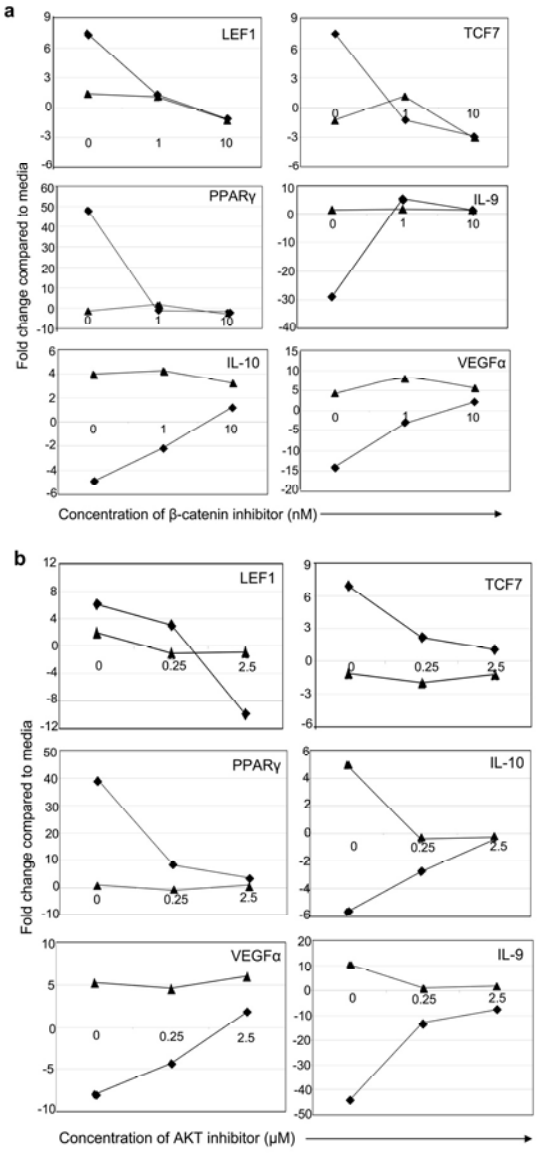
a
LEF1
TCF7
PPARγ
IL-9
IL-10
VEGFα
Fold change compared to media
Concentration of β-catenin inhibitor (nM)
b
LEF1
TCF7
PPARγ
IL-10
VEGFα
IL-9
Fold change compared to media
Concentration of AKT inhibitor (μM)
c
β-catenin inhibitor
IFNγ pg/ml
Inhibitor (nM): 0 1 10 0 1 10 0 1 10
Stimulation: media anti-CD3 anti-NKG2D
AKT inhibitor
IFNγ pg/ml
Inhibitor (μM): 0 0.25 2.5 0 0.25 2.5 0 0.25 2.5
Stimulation: media anti-CD3 anti-NKG2D

Figure S3. β-catenin is required for altered expression of anti-inflammatory cytokines but not required for secretion of IFNγ

chNKG2D T cells were stimulated with anti–CD3- or anti–NKG2D-coated beads in the presence of (A,C) β-catenin inhibitor XAV939 (1nM, 10nM) or DMSO vehicle control (0nM) or (B,C) AKT inhibitor API-2 (0.25μM, 2.5 μM) or DMSO vehicle control (0 μM). (a,b) After 8 hours, gene expression was determined by RT-PCR. Data are shown as the fold change in gene expression compared to T cells cultured in media at each concentration of inhibitor. (C) After 24 hours, secretion of IFNγ was determined in cell-free supernatants by ELISA. Data are shown as mean + s.d., were obtained from one donor, and are representative of data at least two donors. Inhibiting AKT significantly reduced IFNγ secretion when chNKG2D T cells were stimulated through CD3 or NKG2D (*- $p<0.05$).

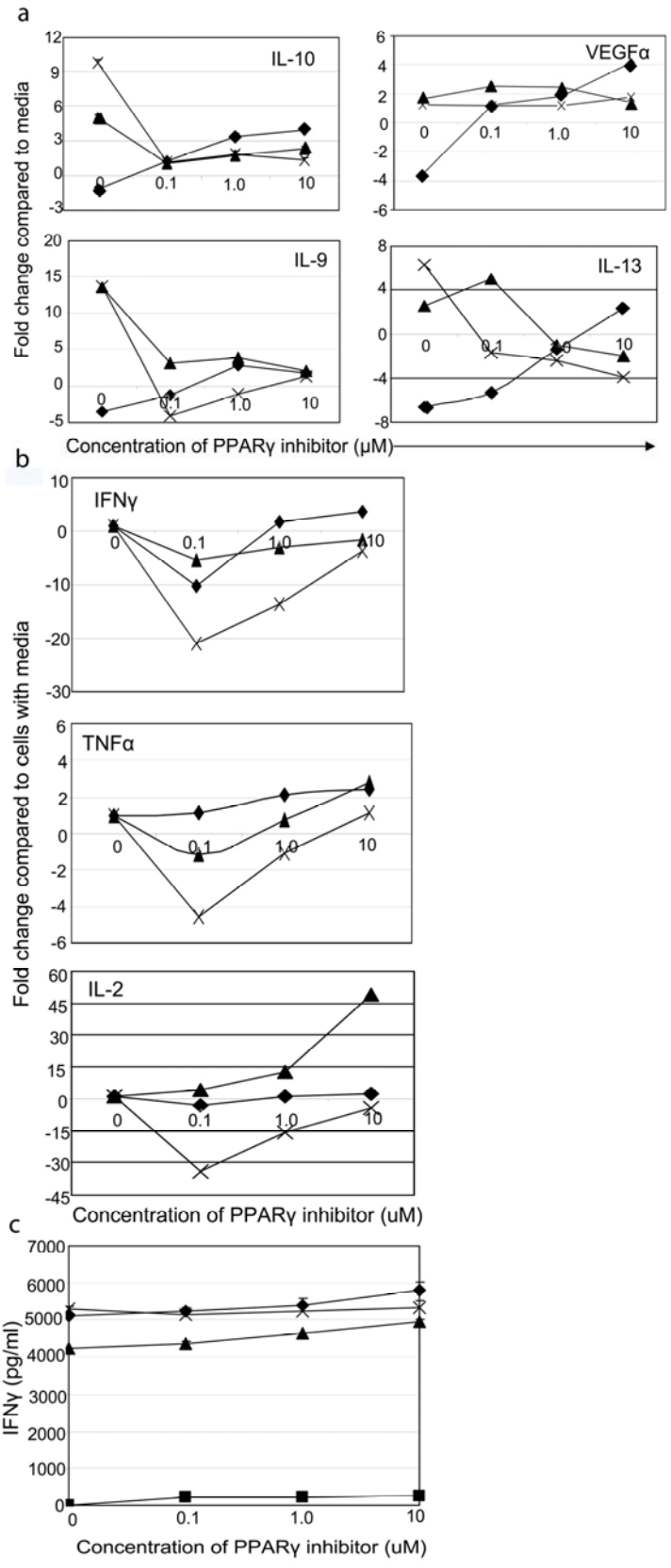
a
IL-10
VEGFα
IL-9
IL-13
Fold change compared to media
Concentration of PPARγ inhibitor (μM)
b
IFNγ
TNFα
IL-2
Fold change compared to cells with media
Concentration of PPARγ inhibitor (uM)
c
IFNγ (pg/ml)
Concentration of PPARγ inhibitor (uM)

Figure S4. PPARγ is required for altered expression of anti-inflammatory cytokines but not required for the expression of IFNγ, TNFα, or IL-2

ChNKG2D T cells were stimulated with anti-CD3/CD28- (X), anti–CD3- (triangles) or anti-NKG2D- (diamonds) coated beads or media (squares) in the presence of PPARγ inhibitor GW9662 (0.1μM, 1.0 μM, 10 μM) or DMSO vehicle control (0 μM). (A) After 8 hours, gene expression was determined by RT-PCR. Data are shown as the fold change in gene expression compared to T cells cultured in media at each concentration of inhibitor. (B) After 24 hours, secretion of IFNγ was determined in cell-free supernatants by ELISA. Data are shown as mean + s.d. Data shown were obtained from one donor and are representative of data from at least two donors.

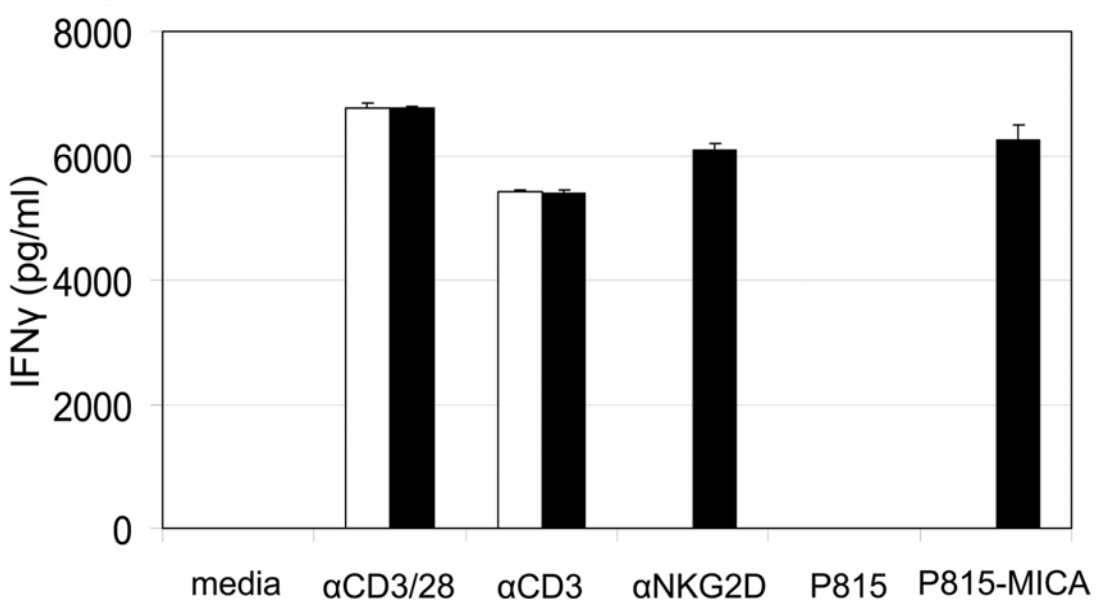

Figure S5. chNKG2D T cells produce similar amounts of IFNγ when activated with antibody-coated beads or NKG2D ligand-positive tumor cells
wtNKG2D (open bars) or chNKG2D (closed bars) T cells were stimulated with media, beads coated with anti-CD3/CD28, anti-CD3, or anti-NKG2D antibodies, or P815 or P815-MICA tumor cells for 24 hours. Secretion of IFNγ was determined in cell-free supernatants by ELISA. Data are shown as mean + s.d. and were obtained from at least three donors.