Supporting Information

Liu et al. 10.1073/pnas.1301022110

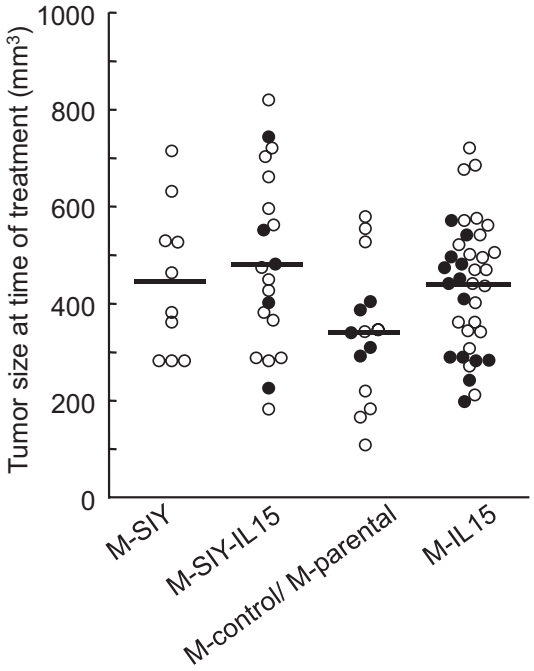

Fig. S1. Similar sizes of tumors at time of treatment. Sizes of mesenchymal fibrosarcoma tumors (MC57) reported in this study at the time of treatment with SIY-K^b specific 2C (white) or OVA-K^b specific OT-1 (black) T cells (day 13–15). Lines denote averages for each cell line.

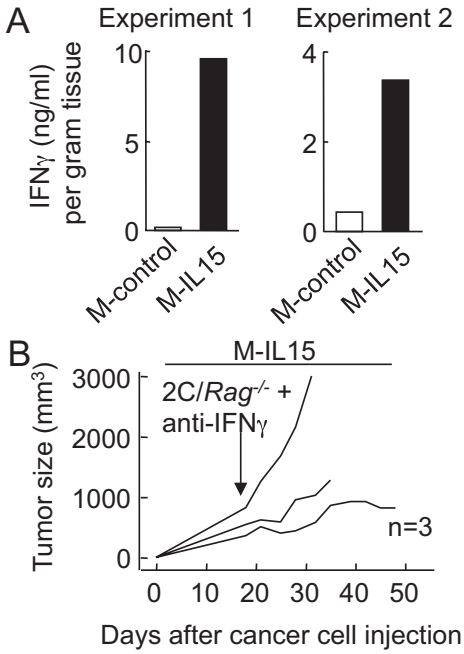

Fig. S2. IFN-γ is required for tumor eradication, and IFN-γ production in tumor tissue increases in the presence of IL-15. (*A*) $Rag^{-/-}\gamma C^{-/-}$ mice were injected s.c. with MC57 cells expressing the empty ECFP vector (M-control; white bars) or MC57 cells expressing IL-15 (M-IL15; black bars). IFN-γ production in homogenized tissue of M-control or M-IL15 tumors at 10 d after $2C/Rag^{-/-}$ T-cell treatment was determined by ELISA. Two individual experiments using single mice are shown. (*B*) $Rag^{-/-}\gamma C^{-/-}$ mice were injected s.c. with M-IL15 cells, followed by $2C/Rag^{-/-}$ splenocytes i.v. IFN-γ was blocked by administration of anti–IFN-γ antibody. Failed rejection was significant compared with treatment of M-IL15 with $2C/Rag^{-/-}$ ($P = 0.018$) (Fig. 3).

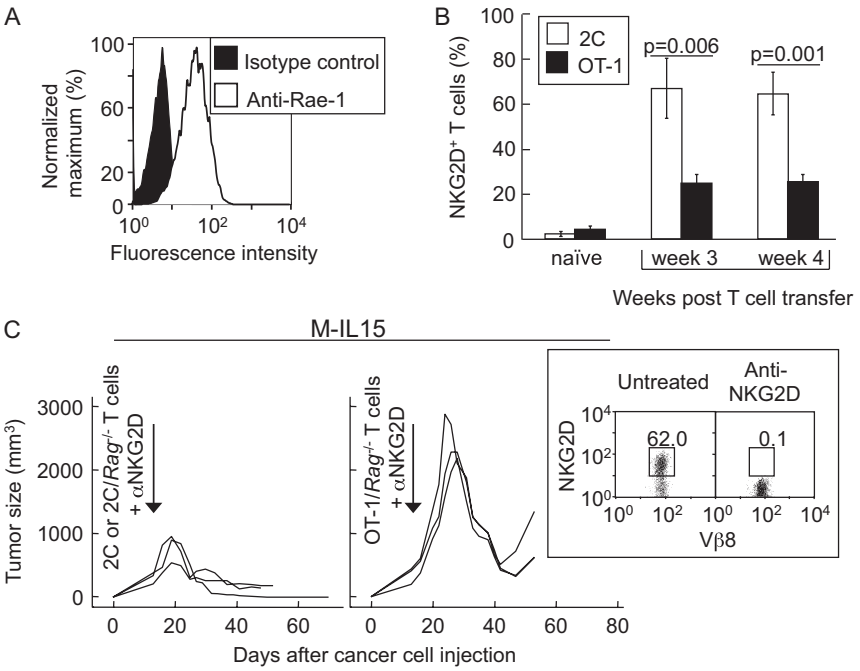

Fig. S3. Despite high NKG2D expression on 2C T cells, NKG2D is not required for rejection of M-IL15 tumors. (*A*) MC57 cells were analyzed by flow cytometry for RNA export 1 (Rae-1) expression. (*B*) Peripheral blood specimens were obtained at various time points from M-IL15–bearing animals, and T cells were analyzed for the natural killer group 2D receptor (NKG2D) expression. Mean ± SEM percentages of NKG2D$^+$ cells among CD8$^+$ cells are shown. (*C*) *Rag*$^{-/-}\gamma C^{-/-}$ mice were injected s.c. with M-IL15 cells and received 2C or OT-1 splenocytes i.v. after 2 wk, along with anti-NKG2D antibody treatment. (*Inset*) FACS analysis showing NKG2D blockade with antibody.

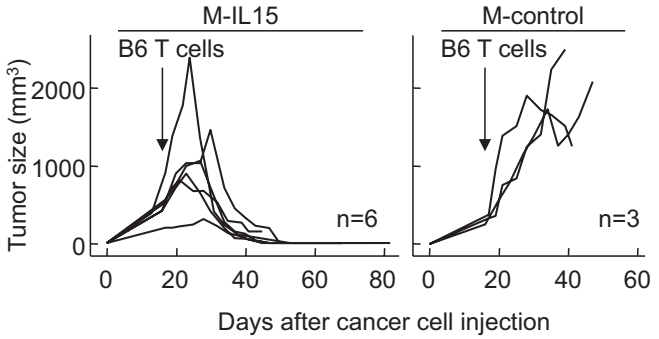

Fig. S4. Rapid IL-15–dependent eradication is also achieved by T cells with a WT T-cell receptor repertoire. *Rag*$^{-/-}\gamma C^{-/-}$ mice were injected s.c. with M-IL15 or M-control cells, followed 2 wk later by naïve B6 WT splenocytes i.v. Lines represent tumors of individual mice compiled from three individual experiments. Eradication of M-IL15 tumors (five of six mice) was statistically significant compared with continued growth of M-control (three of three mice; $P < 0.05$).

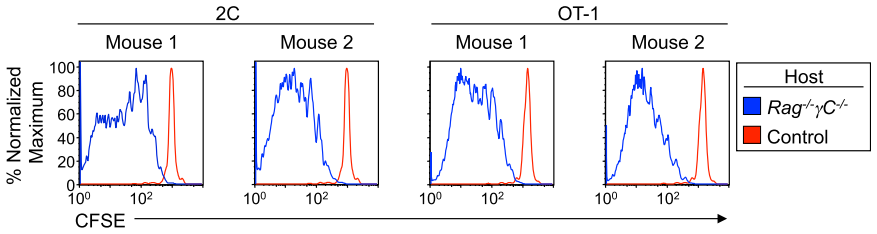

Fig. S5. 2C and OT-1 T cells proliferate similarly within 4 d after transfer into *Rag*$^{-/-}\gamma C^{-/-}$ hosts. Here 1×10^7 splenocytes from 2C/*Rag*$^{-/-}$ and OT-1/*Rag*$^{-/-}$ mice were labeled with carboxyfluorescein succinimidyl ester (CFSE), mixed, and injected into two *Rag*$^{-/-}\gamma C^{-/-}$ recipients (blue; shown separately). Four days later, splenocytes from the recipient mice were stained with anti-CD8 and anti-Vα2 and evaluated by flow cytometry. 2C cells (*Left*) were gated on CD8$^+$Vα2$^-$ cells, and OT-1 cells (*Right*) were gated on CD8$^+$Vα2$^+$ cells. The labeled cells were also transferred into a control immunocompetent recipient (red); its carboxyfluorescein succinimidyl ester (CFSE)-positive cells are shown on each plot for comparison.

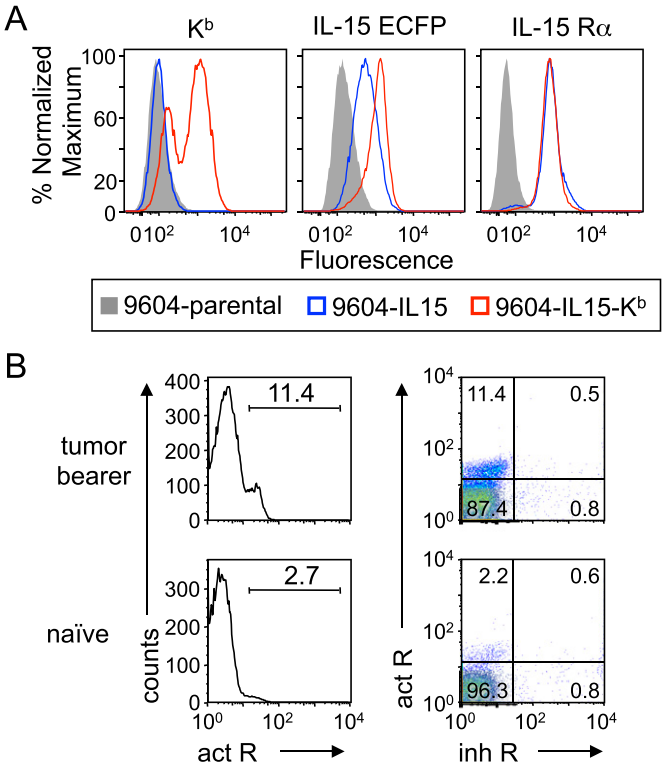

Fig. S6. IL-15 up-regulates activating NK receptors on T cells. (*A*) The 9604-parental, 9604-IL15, and 9604-IL15-K^b cancer cell lines were analyzed for expression of the MHC class I molecule K^b, IL-15 (enhanced cyan fluorescent protein, ECFP), and IL-15 receptor alpha (IL-15Rα) by flow cytometry. (*B*) Flow cytometry analysis of blood ($CD8^+$ T cells) from a $K^{b-/-}D^{b-/-}$ mouse growing contralateral 9604-IL15 and 9604-IL15-K^b tumors under administration of anti-NK1.1 antibody and treated with OT-1 splenocytes (tumor-bearer) and a naïve OT-1 mouse (naïve). T cells were analyzed for expression of activating natural killer (NK) receptors (act R: NKG2D, Ly-49D, and CD94) and inhibitory receptors (inh R: NKG2A, Ly-49A, and F). Plots of the tumor-bearer are representative of four individual mice.

Table S1. MC57 and 9604 cell lines used in the present study

Designation	Transgene 1	Transgene 2	Transgene 3
M-SIY-IL15	IL-15-ECFP	SIY-EGFP	–
M-SIY	ECFP	SIY-EGFP	–
M-IL15	IL-15-ECFP	–	–
M-control	ECFP	–	–
M-parental	–	–	–
9604-IL15-K^b	IL-15-ECFP	IL-15-Rα	K^b
9604-IL15	IL-15-ECFP	IL-15-Rα	–
9604-parental	–	–	–