Mice

Congenic Ly5.1 mice (formerly Ly5.2 mice) and wild type transfer recipients were purchased from the animal production area of the National Cancer Institute (Frederick, MD). Rag2-/-OT-I mice (B6.129S6-*Rag2*^{tm1Fwa} Tg(TcraTcrb)1100Mjb) and wild-type littermate controls were purchased from Taconic Farms (Hudson, NY). All mice were 8-10 weeks of age and rested for 1 week prior to initiating experiments. Treatment protocols were consistent with regimens used in the original text.

Adoptive transfer studies

Splenocytes from congenic Ly5.1 mice were processed into single cell solutions and subjected to magnetic bead separation of untouched CD8+ T cells using the EasySep Negative CD8 T cell enrichment kit (Stem Cell Technologies, Vancouver, BC). Following magnetic bead separation, untouched CD8 T cells were stained with AF700-CD8 (Biolegend), PE-CD25 (BD Biosciences), Pacific blue CD44 (Biolegend) and PE-Cy7 NKG2D (eBiosciences). Cells were then sorted under sterile conditions using FACSAria II (Becton Dickinson). Immediately following sort, cells were requantified by hemacytometer and 10^6 cells were injected intravenously into wild-type recipients. Two days following adoptive transfer, mice were separated into two groups and the standard regimen of anti-CD40/IL-2 or rIgG/PBS for controls was administered. On day 11 following treatment, mice were sacrificed and speens and lymph nodes were harvested and analyzed for expression of NKG2D on congenic, adoptively transferred cells by flow cytometry.

Figure S1. NKG2D is upregulated on memory CD8+ T cells after immunotherapy

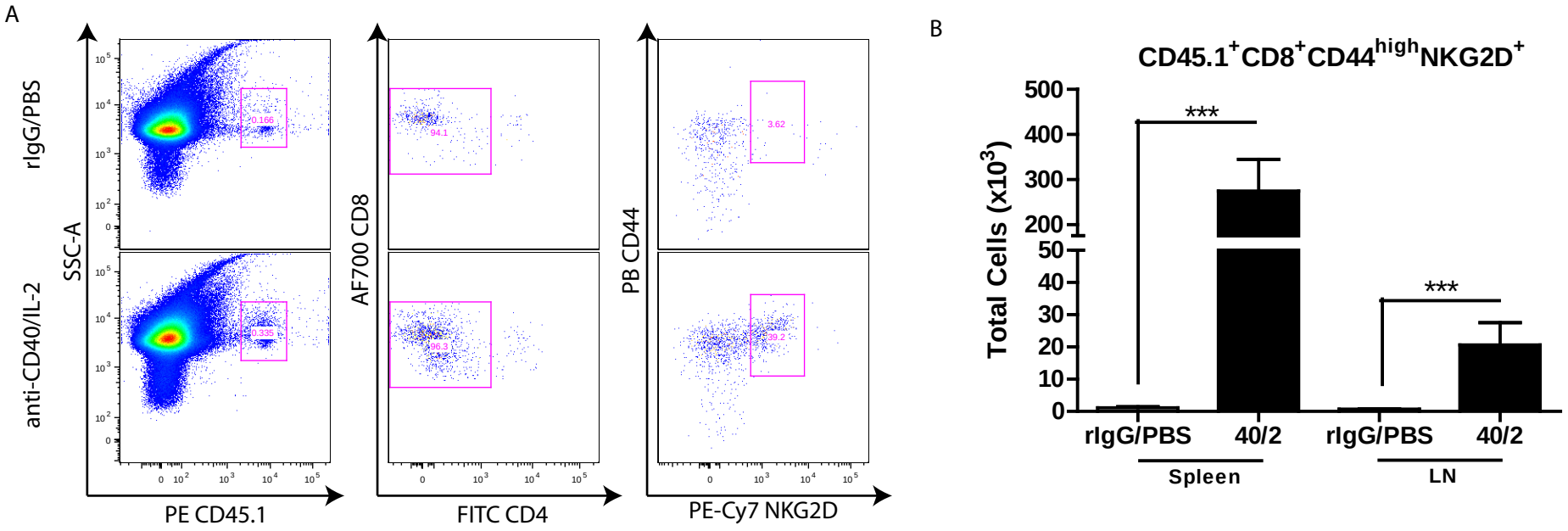

***Figure S1*. NKG2D is upregulated on memory CD8 T cells after immunotherapy.** CD25^{-} CD8^{+}CD44^{high}NKG2D^{neg} T cells were sorted from splenocytes from congenic Ly5.1 mice and 10^{6} sorted cells were adoptively transferred into WT (Ly5.2) C57BL/6 mice. Two days following transfer, mice were separated into two groups and treated with the standard anti-CD40/IL-2 regimen or rIgG/PBS as controls. On day 11 following the initiation of immunotherapy, splenocytes and lymph nodes were processed and analyzed by flow cytometry for NKG2D expression of adoptively transferred congenic cells. A) Representative dot plots depicting gating schema from both control and anti-CD40/IL-2 treated mice. B) Absolute numbers of CD45.1^{+}CD8^{+}CD44^{high}NKG2D^{+} cells in both the lymph nodes and spleens. Statistical analysis was performed using Two-way ANOVA with Bonferoni's post test; ***p<0.001.

Figure S2. Expression of NKG2DL on Renca cell lines

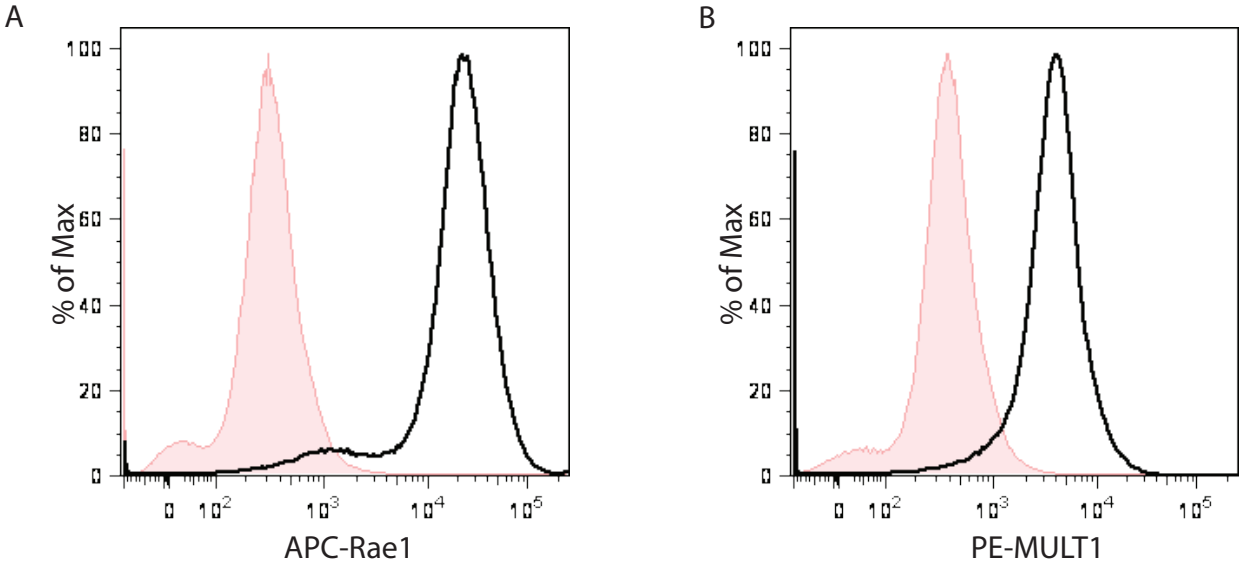

Figure S2. **Expression of NKG2DL on Renca cell lines.** Renca cells were grown in complete media and stained with either A) Pan-Rae1 or isotype or B) MULT1 or isotype. Shaded represents isotype controls vs black represents stain.

Figure S3. Immunotherapy results in nonspecific lytic activity in both WT and Rag2-/-OT-I mice

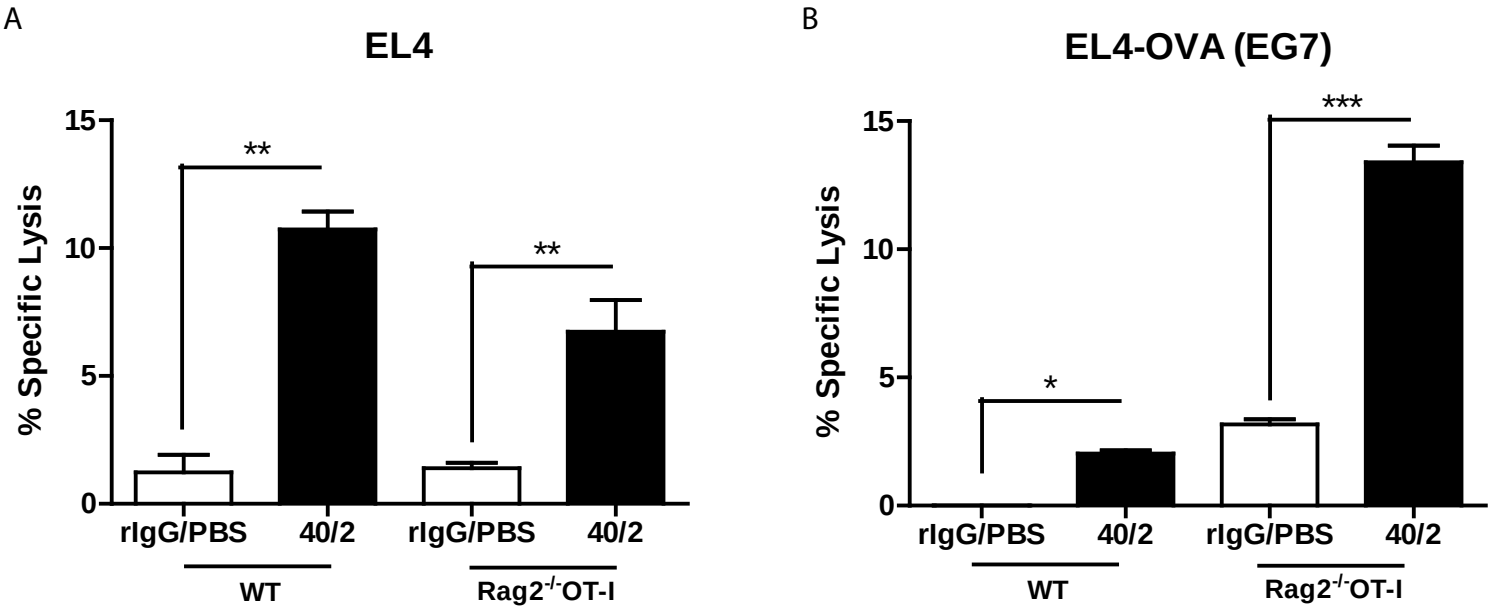

Figure S3. **Immunotherapy results in nonspecific lytic activity in both WT and Rag2$^{-/-}$OT-I mice.** Wild type C57BL/6 and Rag2$^{-/-}$OT-I mice were treated with anti-CD40/IL-2 or rIgG/PBS as control. Spleens were harvested on day 5 following the initiation of treatment and subjected to A) EL-4 and B) EG-7 (EL-4 expressing OVA) killing assays. Statistical analysis was performed using One-way ANOVA with Tukey's post test; *$p<0.05$, **$p<0.01$, ***$p<0.001$.