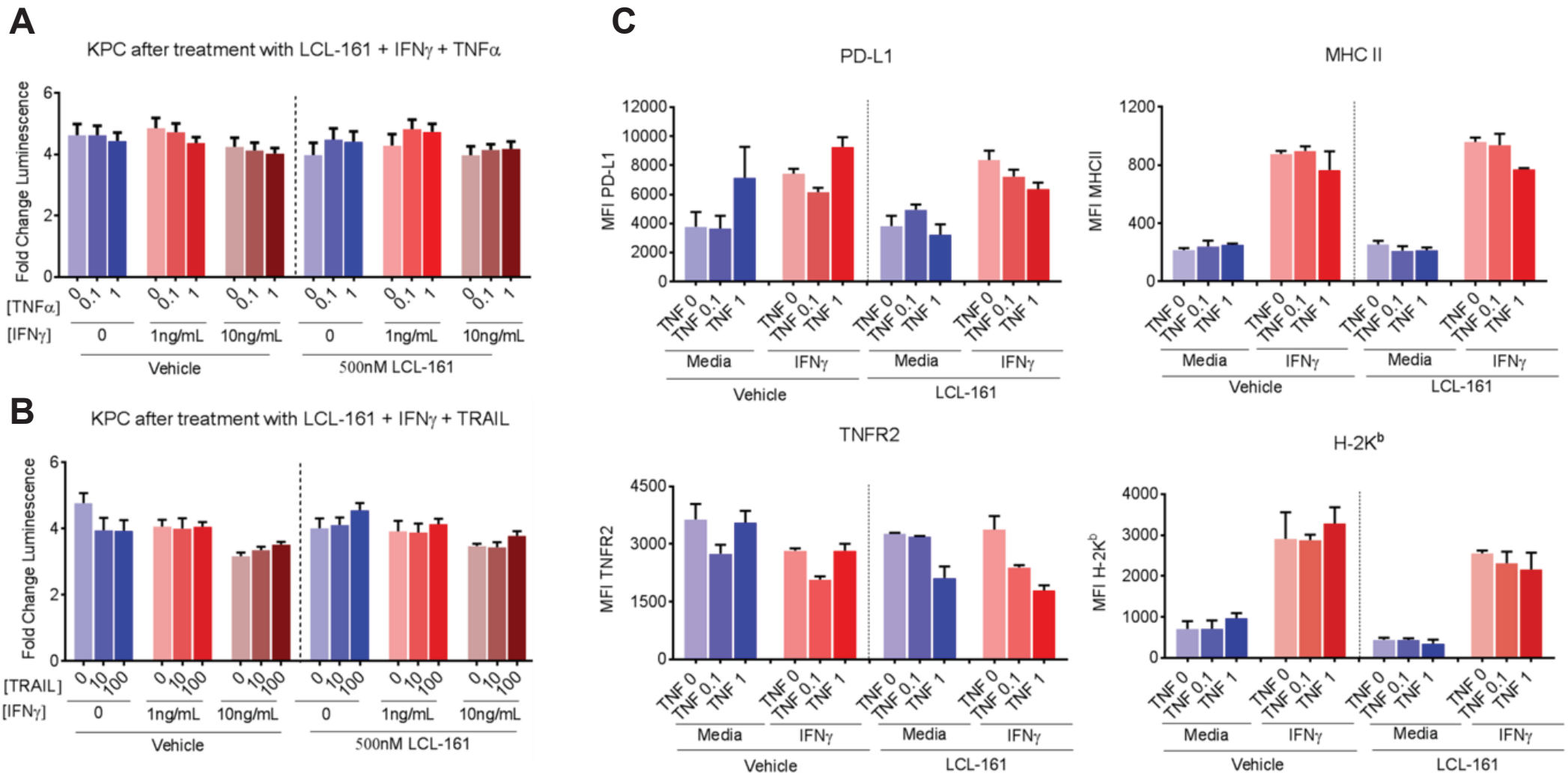
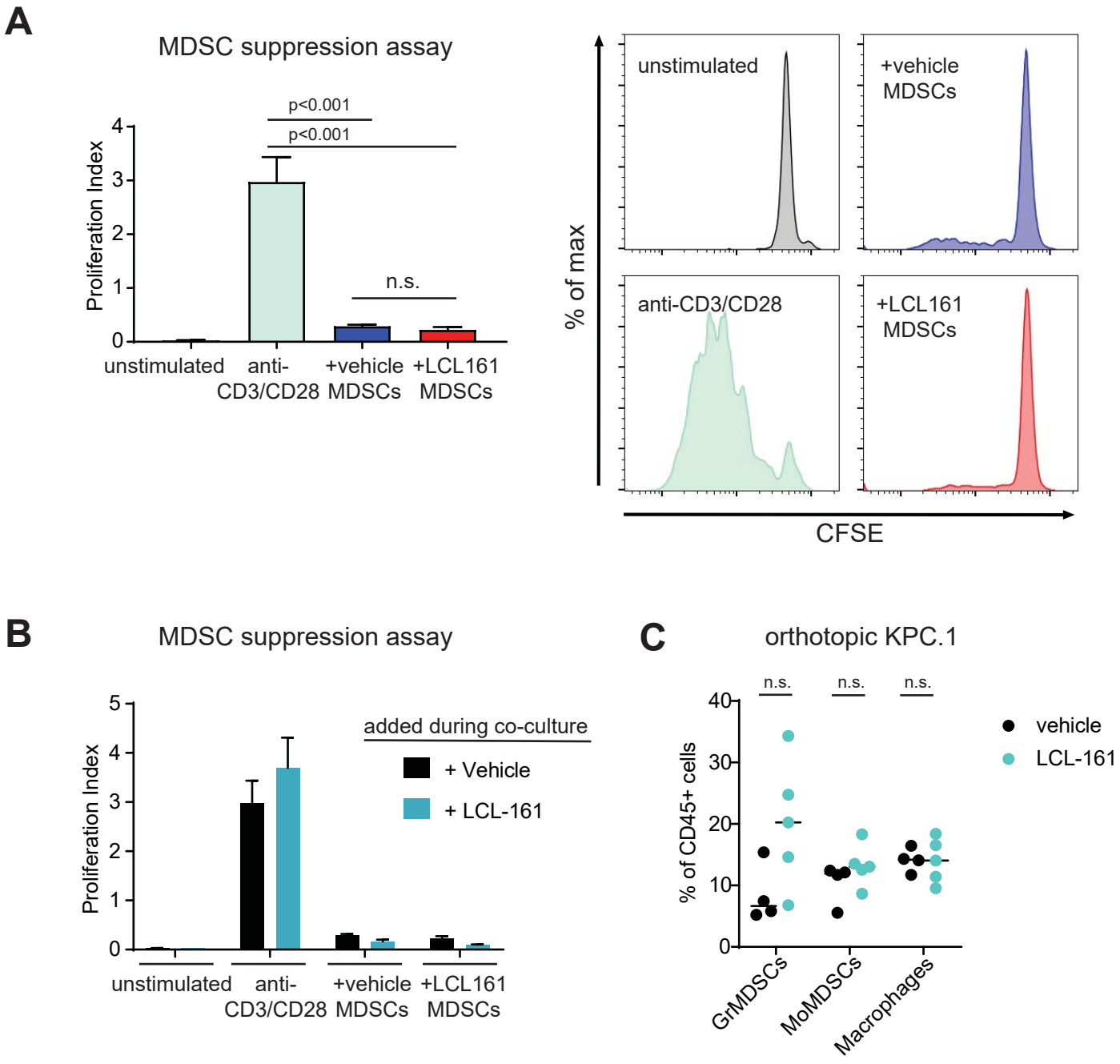
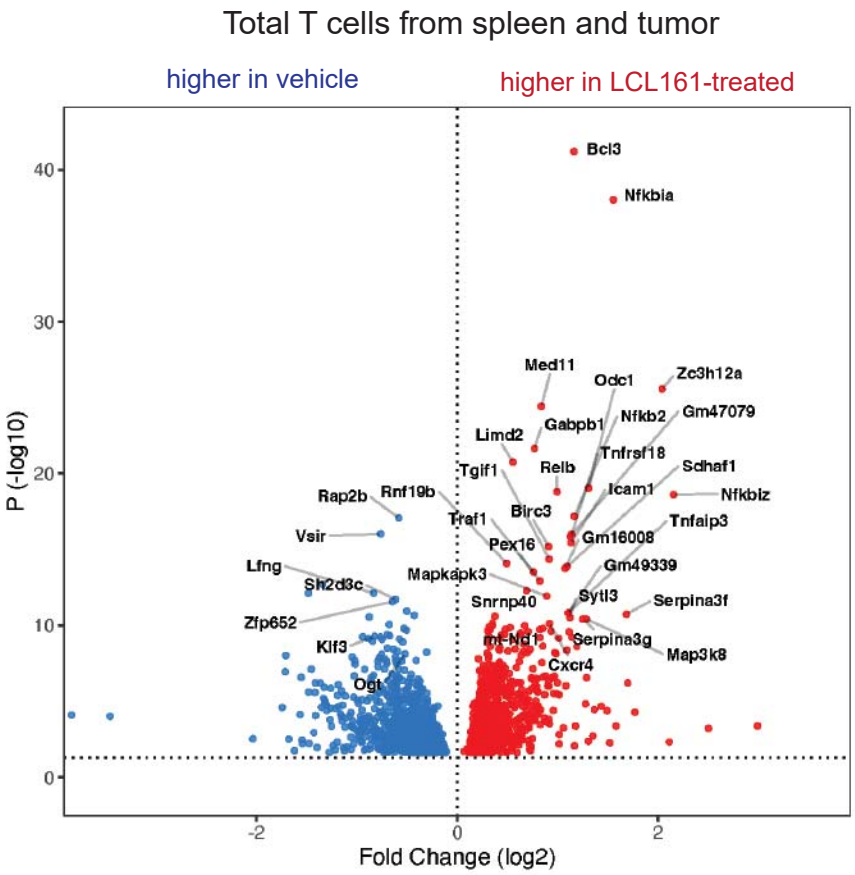


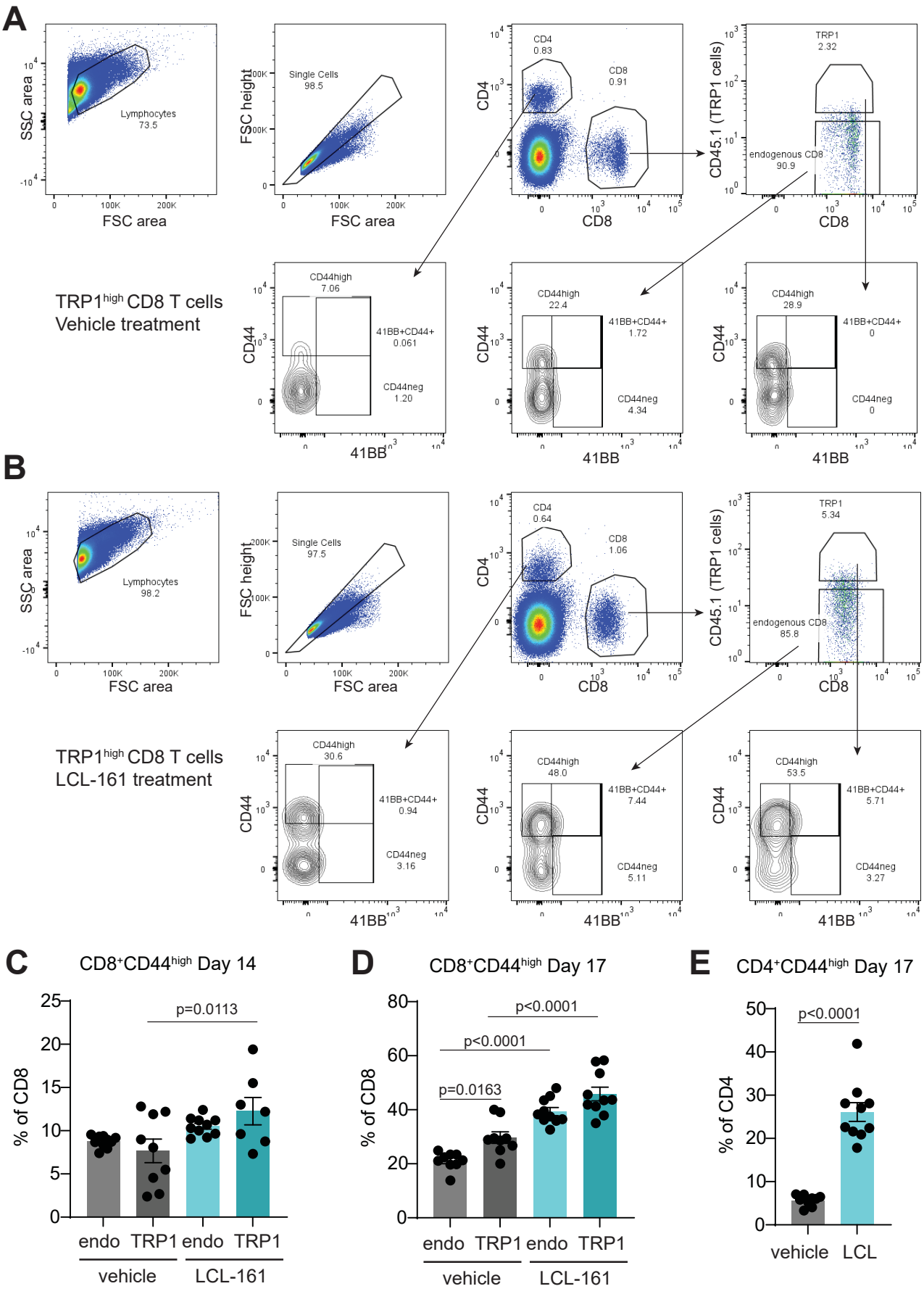
Supplemental Figure 1: Pancreatic tumor cells in vitro are not killed by LCL-161. A) KPC.1 cells were cultured in a 96 well plate with 500 nM LCL-161 and recombinant mouse TNFα and/or IFNγ at the indicated concentrations. 48 hr viability was measured by CellTiter Glo. B) KPC.1 cells were cultured in a 96 well plate with 500 nM LCL-161 and recombinant mouse TRAIL and/or IFNγ at the indicated concentrations. 48 hr viability was measured by CellTiter Glo C) Separately plated KPC.1 cells treated as in A were stained with antibodies to PD-L1, TNFR2, MHC class II, or H2-Kb and analyzed by flow cytometry.



Supplemental Figure 2: MDSC suppressive function is not affected by LCL-161. A) MDSCs were differentiated from C57BL/6 bone marrow by culturing in G-CSF, GM-CSF and IL-6. During the final 24 hours of culture, 500nM LCL-161 was added to the media. MDSCs were washed and replated with CFSE-labeled CD8 T cells and anti-CD3/CD28 beads. T cell proliferation was measured by flow cytometry 72 hours later. N=3 per group. Representative of 3 independent experiments. B) MDSC suppression assay was performed as in A, except that vehicle or 500nM LCL-161 was added to the culture media with the T cells and present throughout the 72-hour coculture. C) KPC.1 cells were implanted orthotopically into C57BL/6 mice that were treated with vehicle or LCL-161 (75mg/kg) every 3 days by oral gavage starting on day 4. Tumors were harvested at day 12 and analyzed by flow cytometry.



Supplemental Figure 3: Common transcriptional signature of cIAP1/2 antagonism in T cells. TCRα-/- mice were implanted orthotopically with M8 live passage organoids and treated with vehicle or LCL-161 (75mg/kg) by oral gavage every 3 days starting on day 4. Tumors and spleens were harvested on day 12 after 3 doses of LCL-161 and single cell suspensions prepared. CD4 and CD8 T cells were isolated by FACS and subjected to limited bulk transcriptional profiling. N=3 per group. Differential gene expression analysis was performed by combining CD4 and CD8 T cell groups across both tumor and spleen to identify LCL-161 induced changes common to tumor and spleen that were significantly differentially expressed in combined CD4 and CD8 T cells.



Supplemental Figure 4: cIAP1/2 antagonism induces CD44 expression on CD4 and CD8 peripheral blood T cells. A) Peripheral blood from mice in Figure 7C at 17 days after tumor inoculation and treatment with adoptive transfer of TRP1 T cells and vehicle control. Representative flow plots and gating strategy are shown. Expression of 4-1BB on CD4 T cells was used to set negative gates. B) Peripheral blood from mice in Figure 7C at 17 days after tumor inoculation and treatment with adoptive transfer of TRP1 T cells and LCL-161 75 mg/kg by oral gavage. Representative flow plots and gating strategy are shown. C) Peripheral blood at day 14 was analyzed by flow cytometry and gated on CD8+ CD45.1+ (TRP1) transferred cells or CD8+CD45.1- (endo) endogenous cells. Fraction of cells in each group that are CD44high is shown. D) Peripheral blood at day 17 was analyzed by flow cytometry and gated on CD8+ CD45.1+ (TRP1) transferred cells or CD8+CD45.1- (endo) endogenous cells. E) Peripheral blood at day 17 was analyzed by flow cytometry and gated on CD4+ cells.