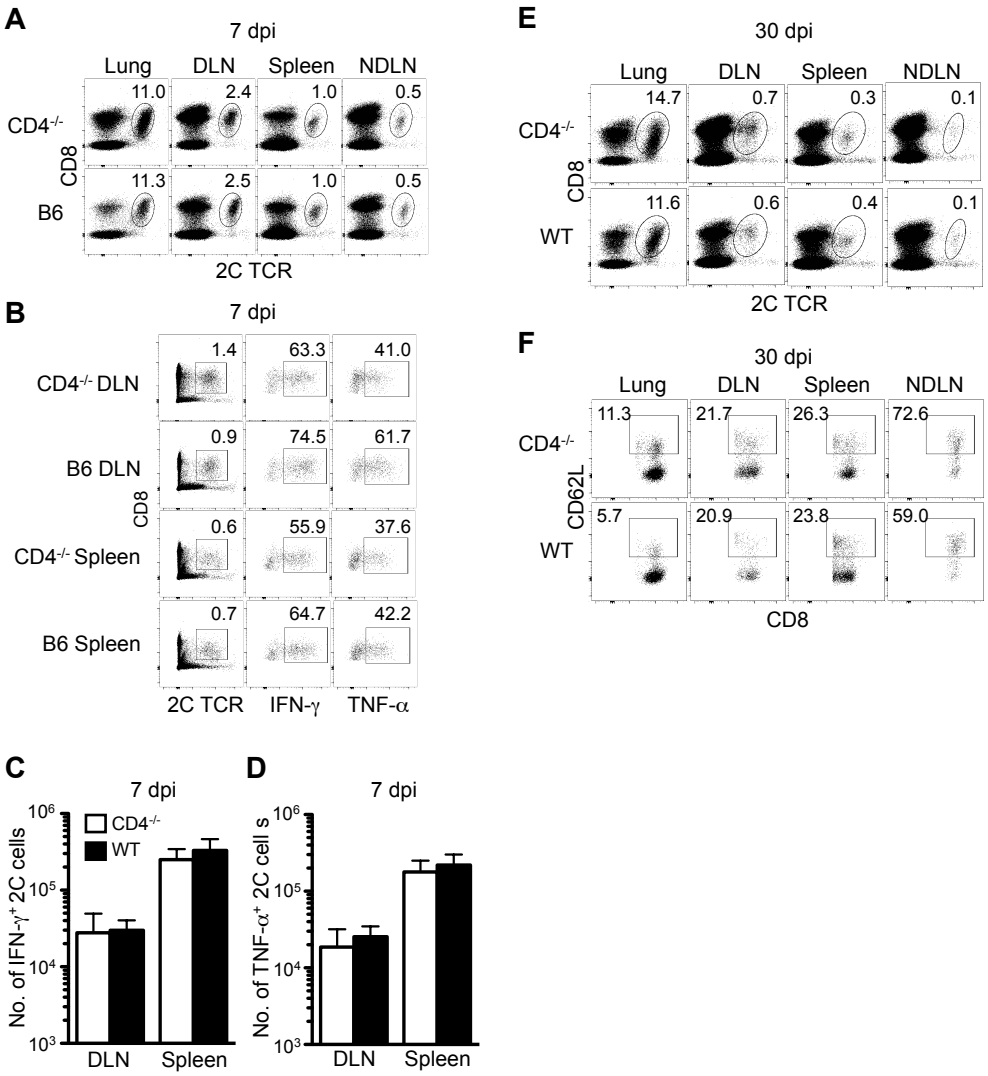

Supplementary Figure 1. Comparison of primary 2C T cell response in different organs in WT and CD4$^{-/-}$ mice at 7 and 30 dpi. The experiment was carried out as in Fig. 1A. Mice were sacrificed 7 dpi or 30 dpi and single cell suspensions were prepared from the lung, DLN, spleen and NDLN. (**A**) Cells were stained for CD8 and the 2C TCR and analyzed by flow cytometer. Shown are representative CD8 vs. 2C TCR staining profiles of cells from the lung, DLN, spleen and NDLN of WT and CD4$^{-/-}$ mice gating on live cells at 7 dpi. (**B**) Cells from DLN and spleen at 7 dpi were stimulated with SIY peptides for 4 hours in the presence of Golgi-plug and then stained for CD8 and 2C TCR plus intracellular IFN-γ or TNF-α. Shown are representative CD8 vs. 2C TCR staining profiles of total cells (left panel), CD8 vs. IFN-γ (middle panel) and CD8 vs. TNF-α (right panel) staining profiles gating on CD8$^+$ 2C TCR$^+$ cells. (**C**-**D**) Comparison of the number of IFN-γ$^+$ (**C**) and TNF-α$^+$ (**D**) 2C cells in DLN and spleen between WT and CD4$^{-/-}$ mice (n=4 per group) at 7 dpi. (**E**) Representative flow cytometry staining profiles of CD8 vs. 2C TCR gating on live cells at 30 dpi. (**F**) Representative flow cytometry staining profiles of CD62L vs. CD8 gating on 2C TCR$^+$ cells at 30 dpi. The numbers indicate the percentages of cells in the gated regions. Error bars: SEM. The numbers in A, B, E and F indicate the percentages of cells in the gated regions.

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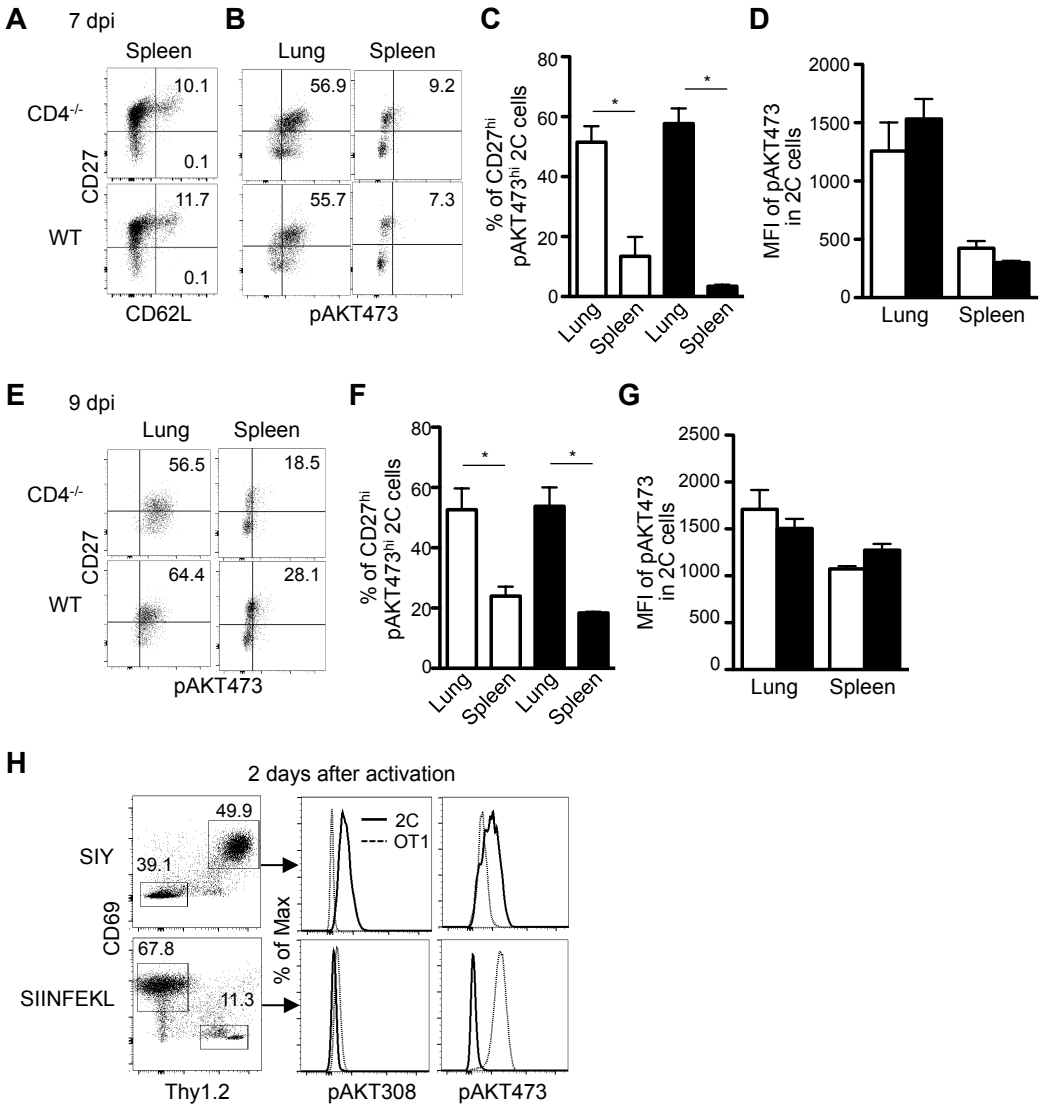

Supplementary Figure 2. Antigen activates AKT pathway in the absence of CD4 T cells. (**A-H**) Comparison of AKT473 phosphorylation in WT and CD4$^{-/-}$ mice. Naive 2C T cells were adoptively transferred into WT and CD4$^{-/-}$ mice followed by intranasal infection with 100 pfu WSN-SIY. At 7 and 9 dpi, cells from the lung and spleen were stained for CD8, 2C TCR, CD27, CD62L, pAKT308, and pAKT473. Shown are CD27 vs. CD62L staining profiles gating on CD8$^+$ 2C TCR$^+$ cells in spleen at 7 dpi (**A**). Shown are CD27 vs. pAKT473 staining profiles gating on CD8$^+$ 2C TCR$^+$ cells at 7 dpi (**B**) and 9 dpi (**E**), percentages of CD27^{hi} pAKT473$^+$ 2C cells at 7 dpi (**C**) and 9 dpi (**F**), and MFI of pAKT473 of CD27^{hi} 2C cells at 7 dpi (**D**) and 9 dpi (**G**). (**H**) Naïve OTI and 2C cells were isolated from OTI rag1$^{-/-}$ Thy1.1$^+$ and 2C rag1$^{-/-}$ Thy1.2$^+$ mice, respectively. The cells were mixed at the 1 to 1 ratio and stimulated with either SIY or SIINFEKL peptide. Two days later, cells were stained for Thy1.1, Thy1.2, CD8 plus CD69 or pAKT308 or pAKT473. Dot plots show CD69 vs. Thy1.2 staining profiles gating on CD8$^+$ cells (left panel). Histograms compare pAKT308 (middle panel) or pAKT473 (right panel) of the gated CD8$^+$ Thy1.2$^+$ 2C cells (black line) vs. CD8$^+$ Thy1.1$^+$ OT1 cells (dotted line). Error bars: SEM from 4 mice per group in one of two experiments. * P<0.05

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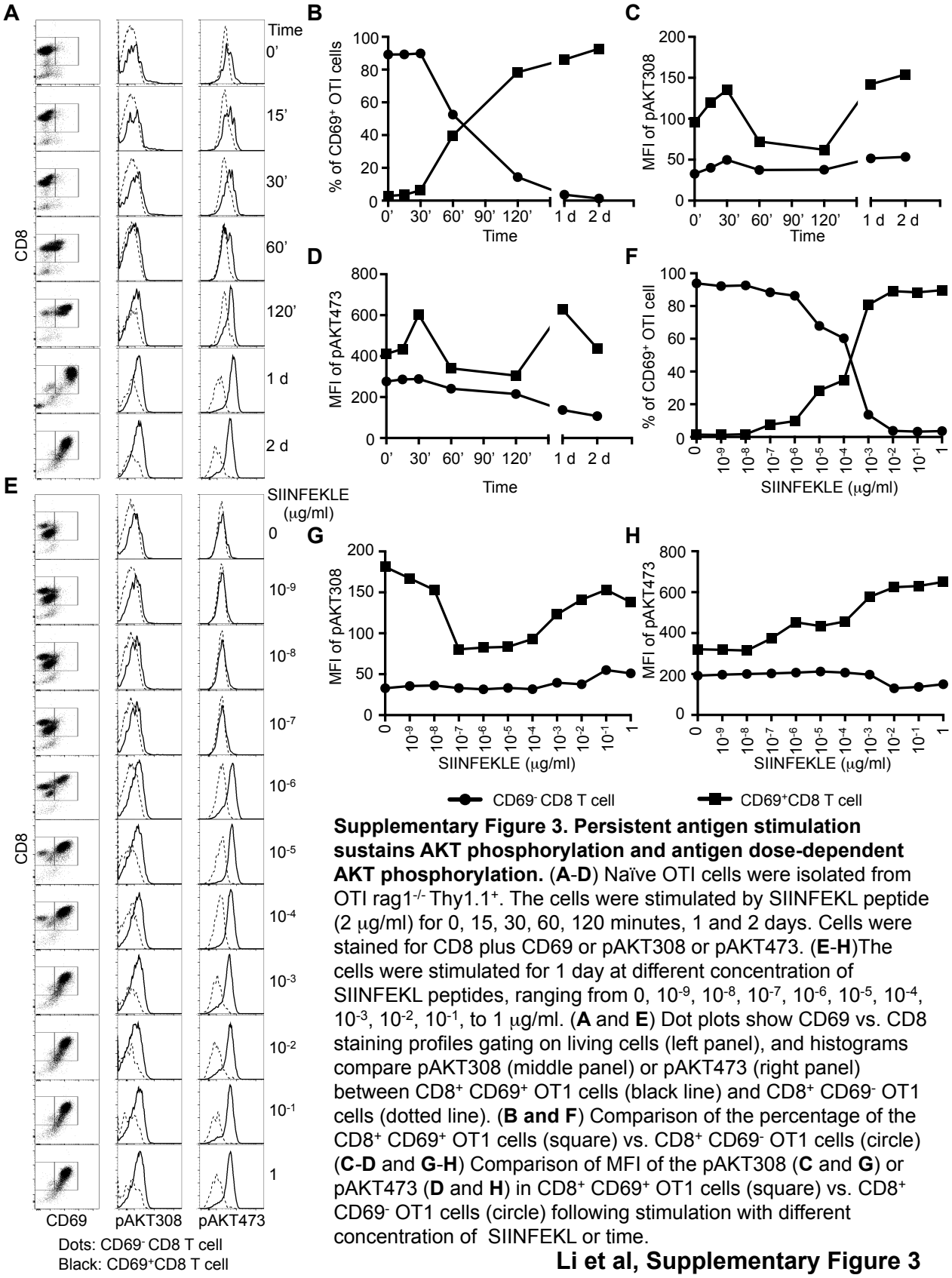

Supplementary Figure 3. Persistent antigen stimulation sustains AKT phosphorylation and antigen dose-dependent AKT phosphorylation. (**A**-**D**) Naïve OTI cells were isolated from OTI rag1$^{-/-}$ Thy1.1^{+}. The cells were stimulated by SIINFEKL peptide (2 μg/ml) for 0, 15, 30, 60, 120 minutes, 1 and 2 days. Cells were stained for CD8 plus CD69 or pAKT308 or pAKT473. (**E**-**H**)The cells were stimulated for 1 day at different concentration of SIINFEKL peptides, ranging from 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, to 1 μg/ml. (**A** and **E**) Dot plots show CD69 vs. CD8 staining profiles gating on living cells (left panel), and histograms compare pAKT308 (middle panel) or pAKT473 (right panel) between CD8^{+} CD69^{+} OT1 cells (black line) and CD8^{+} CD69^{-} OT1 cells (dotted line). (**B and F**) Comparison of the percentage of the CD8^{+} CD69^{+} OT1 cells (square) vs. CD8^{+} CD69^{-} OT1 cells (circle) (**C**-**D** and **G**-**H**) Comparison of MFI of the pAKT308 (**C** and **G**) or pAKT473 (**D** and **H**) in CD8^{+} CD69^{+} OT1 cells (square) vs. CD8^{+} CD69^{-} OT1 cells (circle) following stimulation with different concentration of SIINFEKL or time.

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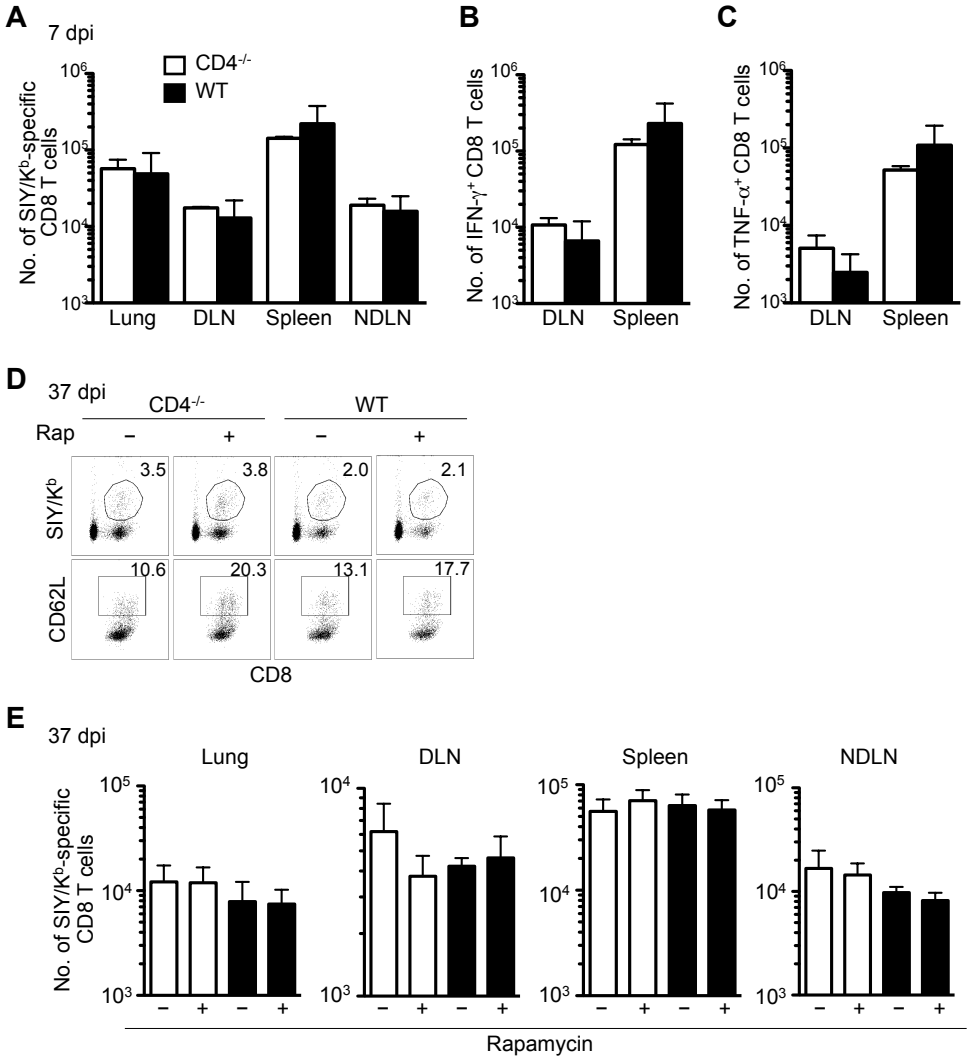

Supplementary Figure 4. Primary responses of endogenous CD8 T cells at 7 dpi and Comparison of SIY/K^b-specific CD8 T cells of various tissues at 37 dpi. CD4$^{-/-}$ and WT mice were infected i.n. with 100 pfu of WSN-SIY virus. (**A**-**C**) Seven dpi, cells were harvested from lung, DLN, spleen, and NDLN, then SIY/K^b-specific CD8 T cells were quantified by SIY/K^b-dimer staining or intracellular IFN-γ and TNF-α staining. (**A**) SIY/K^b-specific CD8 T cells in the indicated organs in WT and CD4$^{-/-}$ mice. (**B** and **C**) Comparison of the percentage of IFN-γ$^+$ (**B**) and TNF-α$^+$ (**C**) CD8 T cells in DLN and spleen between WT and CD4$^{-/-}$ mice. (**D**-**E**) Thirty-seven dpi, the cells were harvested from the lungs; then SIY/K^b-specific CD8 T cells were quantified by SIY/K^b-dimer, CD62L, and CD8 staining. (**D**) Representative flow cytometry staining profiles. Shown are representative staining profiles of SIY/K^b-dimer vs. CD8 gating on live cells (upper panel) and CD62L vs. CD8 gating on SIY/K^b-specific CD8 T cells in the lung. (**E**) The comparison of SIY/K^b-specific memory CD8 T cells in the lung, DLN, spleen, NDLN in CD4$^{-/-}$ and WT mice with or without rapamycin treatment at 37 dpi. Error bars: SEM from3 (A-C) and 5 mice (D-E) per group from one of two independent experiments.