

**Supplemental Figure S1. Equivalent engraftment in uninfected mice and in vitro proliferation of WT and LAG-3**<sup>-/-</sup> **P14 cells.** As illustrated in (**A**), 10<sup>6</sup> Thy1.2+Ly5a+ WT P14 cells and 10<sup>6</sup> Thy1.2+Ly5a- LAG-3<sup>-/-</sup> P14 cells were co-adoptively transferred to Thy1.1+ B6.PL mice. (**B**) An example of staining for Ly5a on the gated CD8+Thy1.2+ transferred P14 cells in the spleen. (**C**) The total number of splenic WT and LAG-3<sup>-/-</sup> P14 cells in the spleens of uninfected mice 45 days post-transfer. These data represent 4 mice from 4 independent experiments. (**D**) WT and LAG3<sup>-/-</sup> were CFSE labeled and stimulated in vitro with WT splenic APCs in the presence or absence of LCMV GP<sub>33-41</sub> peptide. The amount of CFSE dilution was measured at 72 hours post stimulation. One experiment, representative of 3, is shown.



Supplemental Figure S2 . Direct LAG-3 signaling does not affect memory and effector CD8+ T cell differentiation following acute infection.  $2x10^3$  Thy1.2+Ly5a+ WT P14 cells and  $2x10^3$  Thy1.2+Ly5a- LAG-3<sup>-/-</sup> P14 cells mice were adoptively transferred into Thy1.1+ B6.PL mice; 4 days later the mice were infected with LCMV-Armstrong. The frequency of splenic WT and LAG-3<sup>-/-</sup> P14 cells that were positive for CD44, CD62L, IL-7R $\alpha$ , and KLRG-1 at day 8 (A) and day 45 (B) after infection. The data in panel A represent 5 mice from 2 independent experiments. The data in panel B represent 9 mice from 3 independent experiments.