Supplemental Experimental Procedures

CD8 T cells from naïve OT-I TCR-Tg mice were enriched by negative selection. $CD44^{hi}$ cells were depleted by incubation with PE-conjugated anti-mouse CD44 (clone IM7, eBioscience, San Diego, CA) diluted 1:200, followed by labeling with anti-PE coated magnetic beads (Miltenyi Biotec, Auburn, CA) according to manufacturer's instructions. Cells were separated into 2 populations by serial passage over 2 manual drip LS columns. Cells retained on the column were $CD44^{int\text{-}hi}$, and cells collected in the flow through were $CD44^{lo}$.

Supplemental Figure 1. – *Magnitude of naïve TCR-Tg T cell expansion in vivo is not influenced by $CD44^{hi}$ TCR-Tg T cells.* **(A)** Enriched OT-I Thy1.1 cells from naive mice (Pre-separation) were separated into $CD44^{low}$ (Post-Flow Thru) and $CD44^{int\text{-}hi}$ (Post-Retained) and transferred (100 or 1,000 cells/mouse) into naïve C57Bl/6 mice before infection with Att LM-Ova ($5x10^6$ CFU/mouse). Numbers represent either frequency of OT-I ($V\beta5^+/CD8^+$) T cells (upper row) or frequency of $CD44^+$ OT-I T cells before or after selection (bottom row). **(B)** Detection of OT-I Thy1.1 cells in the blood of representative mice on day 6 p.i. Numbers represent the frequency of OT-I cells among PBL. **(C)** Percent (mean + SD for 2-3 mice per group) of OT-I cells detected in the blood at day 6 p.i. in the various groups of mice.

JT Harty - Supplemental Figure 1.