Supplemental Figure 1. Gating strategy to identify lung and BAL T cell populations. Mice were infected with RSV. Cells were obtained from airways by bronchoalveolar lavage and from lung tissue by collagenase digestion and stained for the indicated cell markers. (a) After gating on non-debris, singlets, live and CD45+ cells, the gating strategy as shown was used to identify CD8+ and CD4+ T cells. (b) After gating on non-debris, singlets, live and CD45+ cells, the gating strategy as shown was used to identify effector molecules produced by CD8+ T cells using intracellular staining for GzmB and IFN-γ. To identify RSV-specific CD8+ T cells, cells were stained with tetrarmers containing the immunodominant peptide M187-195.
Supplemental Figure 2. Frequency and total number of multifunctional (Granzyme B (GzmB) and IFN-γ producing) CD8+ T cells in (a) lung tissue and (b) airways on day 8 p.i. in RSV-infected wt and Mavs-/- mice, was determined by flow cytometry. Data are represented as mean ± SEM and are pooled from two independent experiments, n=5-10.
Supplemental Figure 3. C57BL/6 mice were re-infected with RSV (RSV/RSV) or mock-infected (RSV/PBS) 21 days after primary RSV infection. At day 4 post re-infection mice were culled 10 minutes after intravenous injection with anti-CD45 antibody conjugated with BUV395 for discerning intravenous cells (BUV395- and BV605+) from lung resident cells BV605+. (a) Gating strategy for
identification of CD4⁺ and CD8⁺ T cells in the parenchyma or vasculature by flow cytometry. (b) Representative dot plots for CD4⁺ and CD8⁺ T cells harvested from the lung. (c) Frequency and total number of CD4⁺ or CD8⁺ T cells in the vasculature or lung parenchyma. (d) Representative dot plots for CD4⁺ and CD8⁺ T cells recovered from BAL. (e) Frequency and total number of CD4⁺ or CD8⁺ T cells in the airways. Data are presented as mean ± SEM of five mice per group and are representative of two independent experiments. Statistical significances of differences between groups were determined by one-way ANOVA with Tukey’s post hoc test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Supplemental Figure 4. Fewer GzmB and IFN-γ/GzmB producing T cells in the lung of Mavs⁻/⁻ mice during secondary RSV infection. Total CD8⁺ T cells expressing GzmB and/or IFN-γ were quantified in (a) the lung tissue or (b) airways of wt and Mavs⁻/⁻ mice re-challenged with RSV (RSV/RSV). Data are represented as mean ± SEM and pooled from two independent experiments, n=5-10. Statistical significances of differences between groups were determined by unpaired student’s t test.