SUPPLEMENTAL MATERIAL

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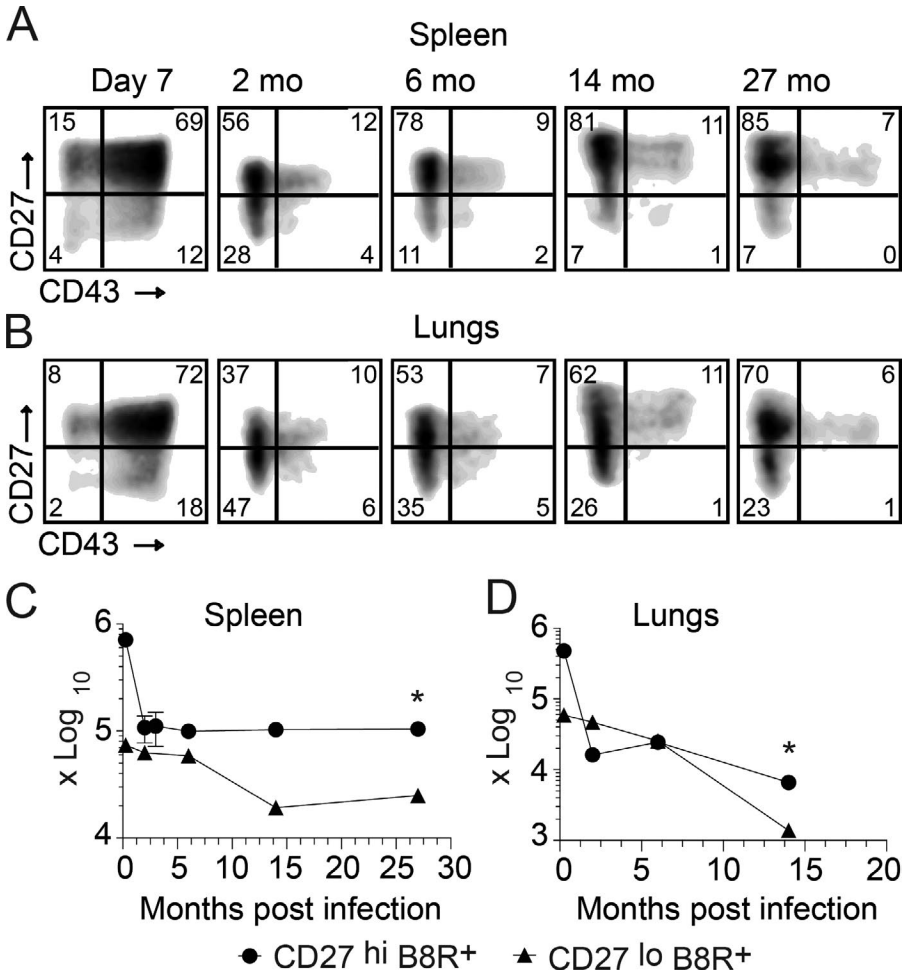

Figure S1. **Composition of splenic and lung memory CD8 T cell subsets after respiratory VACV-WR infection.** WT C57BL/6J mice were infected i.n. with a sublethal inoculum (1.5×10^4 PFU) of VACV-WR. At the indicated time points after infection, splenocytes (A) and lung cells (B) were harvested and stained for CD8, CD44, CD27, CD43, and VACV $B8R_{20-27}/K^b$-tetramer. (A and B) Representative FACS plots of CD27- and CD43-expressing cells after gating on $CD8^+CD44^{hi}$ $B8R_{20-27}/K^b$-$tetramer^+$ cells. Numbers indicate percentages of gated cells in each quadrant. Quadrant setting based on naive $CD44^{lo}$ cells in the same host. Total number of $CD8^+CD44^{hi}$ $CD27^{hi}$ or $CD27^{lo}$ cells per spleen (C) and lung (D) at the indicated time points after infection with VACV. Results are mean ± SEM from three to four mice per group (at least three experiments). *, $P < 0.05$ (two-tailed Student's *t* test).

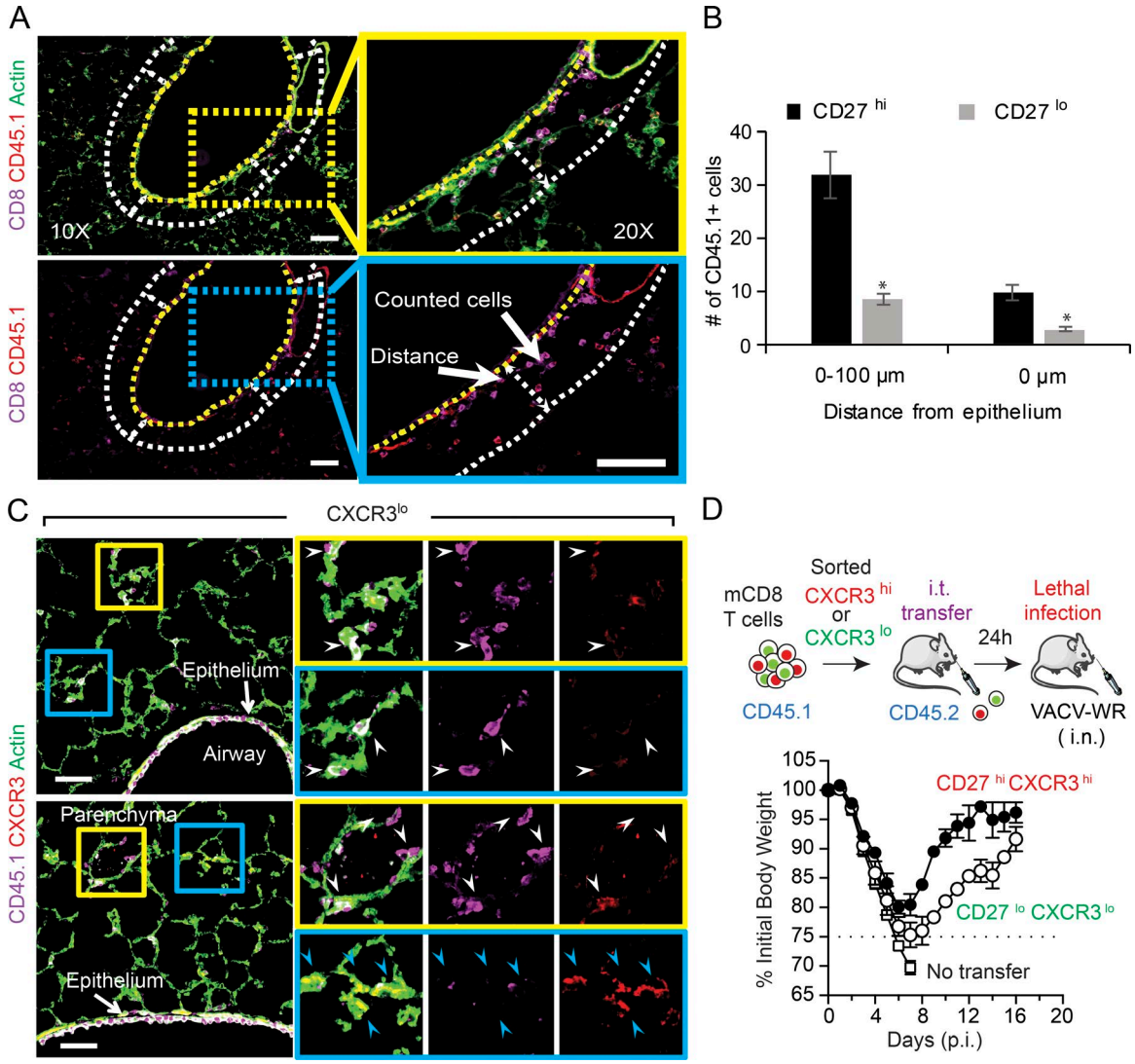

Figure S2. **CD27^{lo} (CXCR3^{lo}) memory CD8 T cells are visualized in the lung parenchyma but show reduced trafficking to airway epithelium and can protect against lethal challenge if localized to the initial sites of viral replication.** (A) IFA of lung sections from CD27^{hi} or CD27^{lo} recipient mice at day 7 after lethal infection are stained as in Fig. 3. The marked areas are shown in higher magnification to the right of each panel. (B) CD45.1^{+} donor cells, which are in direct contact (0 µm; yellow dotted lines) or within a distance of 100 µm from airway epithelium (white dotted line), are counted in 30–40 regions per section. Bars: 100 µm. (C) IFA of lung sections from CD27^{lo} (CXCR3^{lo}) recipient mice stained with CXCR3 (red), CD45.1 (magenta), and ActinGreen (green). Donor CXCR3^{lo} cells (magenta) are indicated with white arrows, and endogenous CXCR3^{hi} CD8^{+} cells (red) are delineated with blue arrows. Bars: 50 µm. (D) 30,000 CD44^{hi}B8R$_{20-27}$/K^{b}-tetramer^{+} CD27^{lo} (CXCR3^{lo}) or CD27^{hi} (CXCR3^{hi}) memory CD8 T cells were instilled into the lungs of naive mice via the trachea. 1 d later, recipient mice were lethally infected with VACV-WR and animals were weighed daily. Mean percent of initial body weight is shown. Data are from one representative experiment of at least three, with three to four mice per group. *, $P < 0.05$ (two-tailed Student's *t* test).

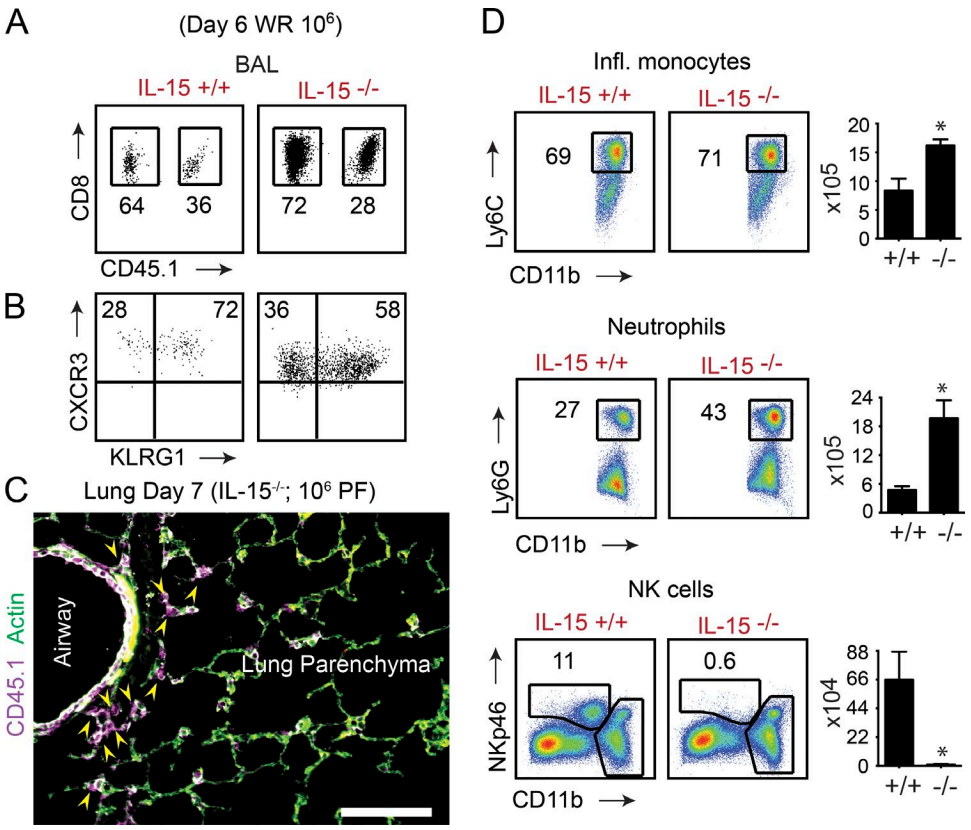

Figure S3. **IL-15$^{-/-}$ recipient mice have increased accumulation of donor CXCR3^{hi} memory cells in the airways and in proximity of airway epithelium, as well as enhanced local innate immune response in the absence of NK cells after lethal respiratory VACV-WR infection.** Mice that received CD27^{hi}/CXCR3^{hi} CD8 memory cells in Fig. 5 H were analyzed at day 6 (A and B) or day 7 (C and D) after lethal infection. (A) Representative FACS plots showing total donor (CD45.1^{+}) and host (CD45.1^{-}) cells. (B) Representative FACS plots showing CXCR3 and KLRG1 expression on gated donor (CD45.1^{+}) cells. Numbers indicate percentages of positive cells within the gated population. Quadrant setting based on naive CD44^{lo} cells in the same host. (C) IFA of lung sections from IL-15$^{-/-}$ recipient mice stained with CD45.1 (magenta) and ActinGreen (green). Bar: 100 µm. (D) Lung mononuclear cells were stained for CD3, CD11b, Siglec-F, Ly6G, Ly6C, and NKp46. Dead cells, doublets, and T cells are excluded. Representative FACS dot plots for NK cells (CD11b^{+}NKp46^{+}), Neutrophils (CD11b^{+}Ly6G^{+}) and inflammatory monocytes (CD11b^{+}Ly6C^{hi}), are shown. Total cell numbers in lungs are presented as the mean results ± SEM from 3–4 mice per group (two to three experiments). *, $P < 0.05$ (two-tailed Student's *t* test).