Supporting Information

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SI Materials and Methods

Viruses and Infection. Sendai virus (Enders strain) and influenza virus A/HK-x31 (×31, H3N2) and A/PR/8 (PR8, H1N1) were grown, stored, and titered as described (1, 2). Mice were anesthetized by i.p. injection of 2,2,2-tribromoethanol (200 mg/kg) and infected intranasally with 250 (Sendai) or 300 (influenza) 50% egg infectious doses (EID_{50}) in a volume of 30 μL. Sham-infected animals received 30 μL PBS. For virus-challenge experiments, mice that had been infected with PR8 influenza 30–45d earlier were challenged with 30,000 EID_{50} ×31.

Mixed Bone Marrow Chimeras. CD45.1$^+$ C57BL/6 mice were lethally irradiated (950 rad) and reconstituted with 1×10^7 bone marrow cells, comprising equal parts CD45.1$^+$/CD45.2$^+$ WT Vert-X^{het} and CD45.2$^+$ *Il27ra*$^{-/-}$ Vert-X^{het} bone marrow. Chimeras were allowed to reconstitute for 6–8 wk before infection.

Flow Cytometry. Single cell suspensions were prepared from bronchoalveolar lavage, lung parenchyma, mediastinal lymph node, spleen, and peripheral blood as previously described (3, 4). Cells were incubated with Fc-block (24G2) on ice for 15 min before staining with PE- or APC-labeled tetramers specific for the Sendai virus nucleoprotein epitope (SenNP$_{324–332}$K^b) or influenza nucleoprotein epitope (FluNP$_{366–374}$D^b) for 1 h at room temperature. All tetramers were generated by the Trudeau Institute Molecular Biology Core. Some experiments instead used a Sendai-specific Pro5 Recombinant Murine MHC Pentamer (H-2K^b FAPGNYPAL) (Proimmune). Cells were stained with fluorochrome-labeled antibodies for CD8α (clone 53–6.7), CD44 (IM7), CD45.1 (A20), CD45.2 (104), CD62L (Mel-14), CD69 (H1.273), CD126 (D7715A7), or gp130 (125623). IL-27Rα expression was measured by pulsing cells with recombinant murine IL-27 on ice and staining with a polyclonal anti-IL-27 Ab (R&D Systems). Propidium iodide (0.5 μg/mL; Sigma) was used to exclude dead cells. Intracellular staining was performed using Cytofix/Cytoperm buffer and Cytoperm Permeabilization Buffer Plus (BD Biosciences) and antibodies against T-bet (clone 4B10; eBioscience) and GFP (chicken polyclonal anti-GFP from AbCam plus DyLight488-conjugated goat anti-chicken IgY from Jackson Immunoresearch). Data were acquired on a FACSCanto or LSRII cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar).

Analysis of Cell Proliferation. To compare IL-10 induction and cell division, naïve CD8+ cells were sorted from the spleen of Vert-X IL-10 reporter mice, using a FACSVantage cell sorter (BD Biosciences). Sorted cells were >98% CD8+ CD44lo CD62Lhi. Cells were labeled with a violet proliferation dye, VPD450 (BD Biosciences), by incubating 1×10^7/mL in 1μM VPD450 for 15 min at 37 °C. Cells were washed and cultured for 72 h at 5×10^6/mL with live APCs (TCRβδ$^{-/-}$ splenocytes, 1.5×10^6/mL), anti-CD3 (clone 145.2C11, 2 μg/mL, BD Biosciences), and anti-CD28 (37.51, 2 μg/mL, eBioscience) in the presence or absence of recombinant murine IL-27 (20 ng/mL; Peprotech). IL-10 induction was assessed by flow cytometry.

In Vitro Activation and Cytokine Quantification. For OTI activation in vitro, splenocytes from naive OTI animals were depleted of B cells using AffiniPure anti-mouse IgG (Jackson ImmunoResearch) and cultured at 5×10^5/mL with peptide-pulsed, irradiated APC [congenic splenocytes, 5×10^6/mL, incubated with SIINFEKL (2 μg/mL; New England Peptide), washed and irradiated at 2,000 rad] and IL-2 (100 U/mL; Peprotech). To compare cytokine production by naive and memory CD8+ cells, CD8+ CD44lo CD62Lhi and CD8+ CD44hi CD62Llo cells were sorted from the spleens of C57BL/6 mice infected with Sendai virus 30–45 d earlier. Sort purities were >98%. Cells were cultured for 72 h with live APC, anti-CD3, and anti-CD28 in the presence or absence of recombinant murine IL-27 or IL-6 (20 ng/mL; Peprotech), as above. Cytokines in culture supernatants were quantified with the Mouse Inflammation Cytometric Bead Array kit (BD Biosciences).

Retroviral Transduction. For OTI activation and transduction, full-length gp130 cDNA was cloned into the MIG retrovirus (5). Empty and gp130-expressing MIG viruses were produced in 293T cells (ATCC). Spleen and lymph node cells from naïve OTI animals were cultured at 2×10^6/mL in the presence of SIINFEKL peptide (0.1μM; New England Peptide) and IL-2 (100 U/mL; Peprotech) and transduced on days 1 and 2 by spinfection, in which 2 mL of retroviral supernatant containing Hepes (20 mM; Sigma) and Sequabrene (8μg/mL; Sigma) was mixed with ~5×10^6 activated cells in six-well plates and centrifuged at $1{,}200 \times g$ for 60 min at 30 °C. Cultures were washed on day 3, rested in IL-15 (10 ng/mL; R&D) and, 4 d later, restimulated with live APC, anti-CD3, and anti-CD28. Cytokines in culture supernatants were quantified with the Mouse Inflammation Cytometric Bead Array kit (BD Biosciences).

1. Daly K, Nguyen P, Woodland DL, Blackman MA (1995) Immunodominance of major histocompatibility complex class I-restricted influenza virus epitopes can be influenced by the T-cell receptor repertoire. *J Virol* 69(12):7416–7422.
2. Hou S, Doherty PC, Zijlstra M, Jaenisch R, Katz JM (1992) Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8+ T cells. *J Immunol* 149(4):1319–1325.
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5. Mullen AC, et al. (2001) Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. *Science* 292(5523):1907–1910.

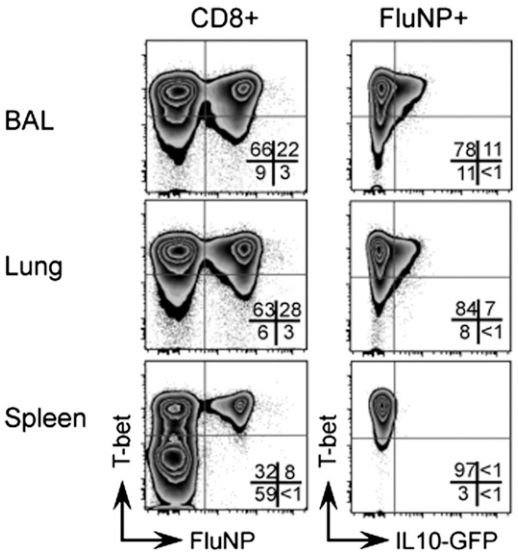

Fig. S1. CD8+ effector T cells in the lung and airways coexpress IL-10 and T-bet. Vert-X IL-10 reporter mice were infected intranasally with the influenza PR8 virus and, 10 d later, cells in the bronchoalveolar lavage (BAL), lung, and spleen analyzed by flow cytometry. Data shown are gated on either total CD8+ (left column) or FluNP-specific CD8+ lymphocytes (right column). Of note, influenza infection elicits little IL-10 expression in the spleen (Fig. 1). Numbers indicate the percentage of cells within each quadrant. Data are representative of two independent experiments using three mice per group.

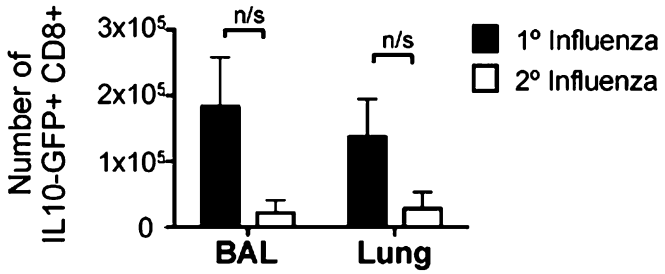

Fig. S2. Absolute numbers of IL-10+ CD8+ T cells in effector tissues during rechallenge infection. Vert-X mice were sham-infected (1°) or infected with PR8 influenza virus (2°) and, 30–35 d later, challenged with ×31 influenza virus. Eight days after challenge, the indicated tissues were harvested and cells were counted and analyzed by flow cytometry. Graphs indicate the mean ± SEM of individual mice and data are representative of four independent experiments with four or five mice per group.

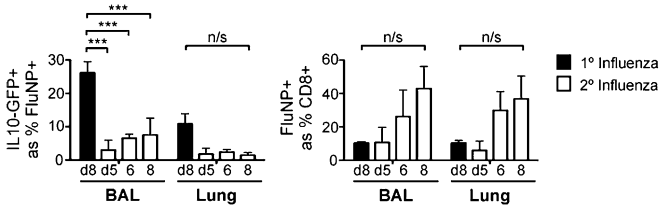

Fig. S3. CD8+ T-cell-derived IL-10 is absent at days 5, 6, and 8 after a rechallenge infection. Vert-X mice were sham-infected (1°) or infected with PR8 influenza virus (2°) and, 30–35 d later, challenged with ×31 influenza virus. At various times later, the indicated tissues were harvested and cells analyzed by flow cytometry. Data shown are for day 8 after primary infection (few antigen-specific CD8+ cells are detectable before this time) and days 5, 6, and 8 after rechallenge. Graphs indicate the mean ± SD of individual mice and data are representative of four independent experiments with four or five mice per group.

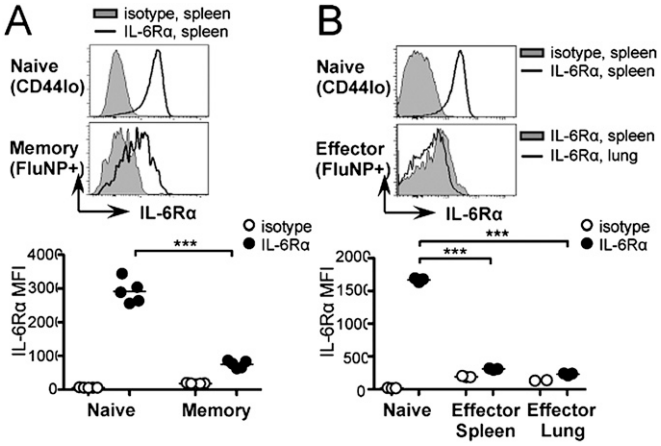

Fig. S4. IL-6 receptor expression during respiratory viral infection. (*A*) C57BL/6 mice were infected with PR8 influenza virus and, 35 d later, splenocytes were harvested, stained with an IL-6Rα (CD126) mAb or an isotype-matched control (rat IgG2b), and analyzed by flow cytometry. (*B*) C57BL/6 mice were infected with PR8 influenza virus and, 10 d later, cells were harvested from the lungs and spleen and analyzed as in *A*. In all panels, data are gated on naïve (CD44lo) and influenza-specific memory or effector (CD44hi FluNP+) CD8+ lymphocytes from the same mouse. Graphs depict mean fluorescence intensity (MFI) and each data point represents an individual mouse. All panels are representative of three to five mice per group in at least two independent experiments.

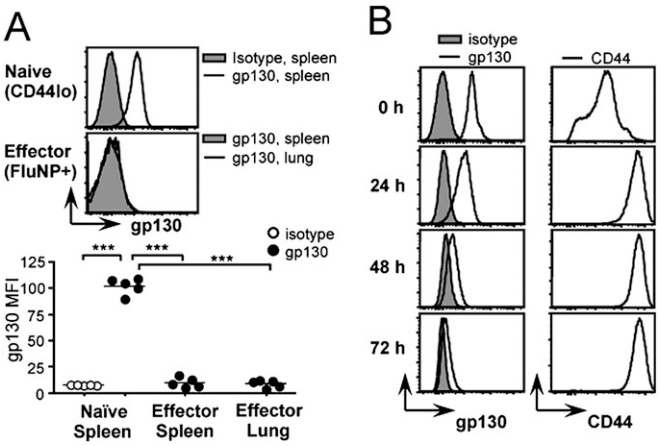

Fig. S5. Down-regulation of gp130 after antigen-driven activation. (*A*) C57BL/6 mice were infected with PR8 influenza virus and 10 d later cells were harvested from the spleen and lungs and stained with a gp130 mAb or an isotype-matched control (rat IgG2a). Data are gated on naïve (CD44lo) or influenza-specific effector (CD44hi FluNP+) CD8+ lymphocytes from the same mouse. Graphs depict mean fluorescence intensity (MFI) and each data point represents an individual mouse. (*B*) CD8+ OTI cells were cultured with peptide-pulsed, irradiated APC, and their expression of gp130 and CD44 was analyzed by flow cytometry at the indicated times. All data are representative of three or four independent experiments.

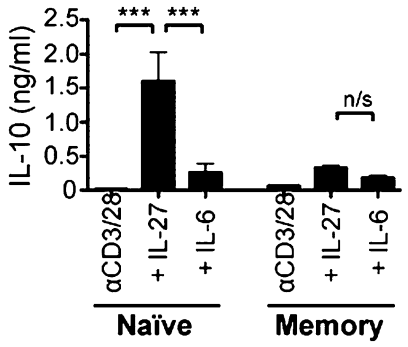

Fig. S6. IL-6 elicits little IL-10 production from either naïve or memory CD8+ T cells. Naïve (CD44lo CD62Lhi) and memory (CD44hi CD62Llo) CD8+ lymphocyte populations were purified from the spleens of C57BL/6 mice infected with PR8 influenza virus 30–45 d earlier, and cultured with anti-CD3 and anti-CD28 in the presence or absence of recombinant IL-6 or IL-27. Culture supernatants were harvested after 72 h and analyzed by cytometric bead array. Graphs depict the mean ± SD of triplicate cultures using cells pooled from three to five mice. Data are representative of two independent experiments.