SUPPLEMENTAL MATERIAL

McGill et al., http://www.jem.org/cgi/content/full/jem.20091711/DC1

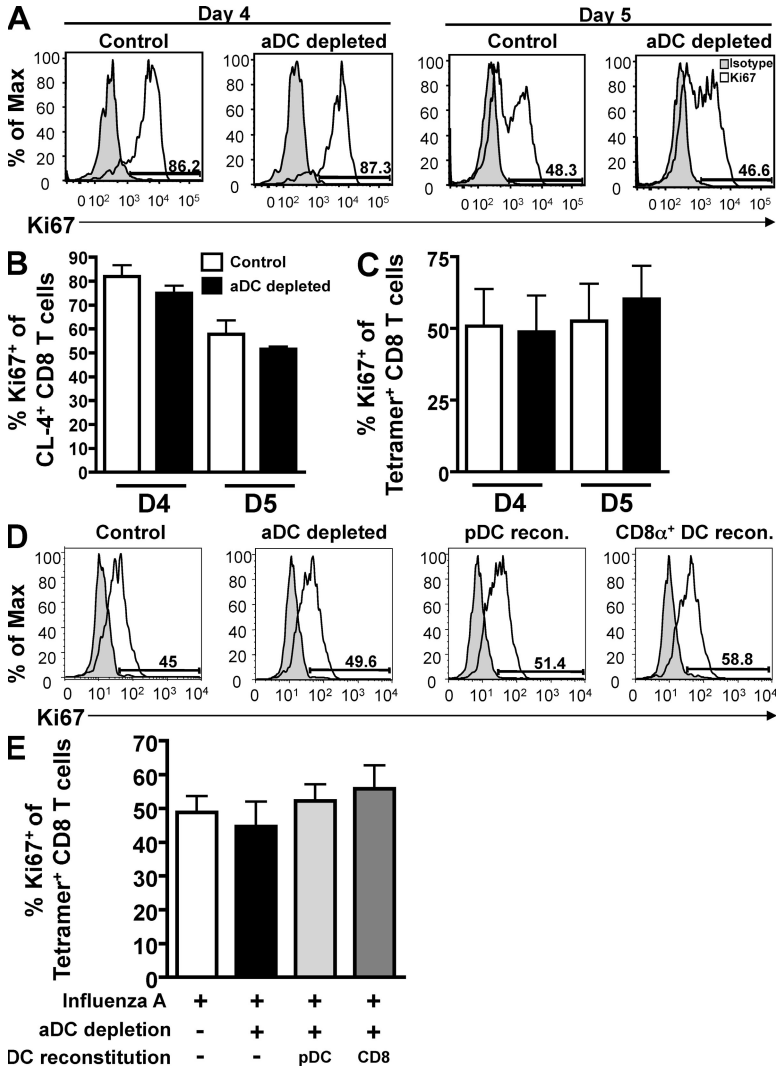


**Figure S1. aDC depletion at 48 h p.i. does not alter IAV-specific CD8 T cell proliferation in the lungs.** (A and B) Influenza-specific, CD90.2+ CL-4 T cells were adoptively transferred to groups of CD90.1+ BALB/c mice as in Fig. 1. 24 h later, mice were infected with a sublethal dose of IAV with or without aDC depletion. On days 4 and 5 p.i., mice were sacrificed and proliferation, as measured by Ki67 expression, of adoptively transferred CD90.2+ CL-4 T cells was assessed by flow cytometry. (A) Shown are representative isotype (shaded) or Ki67 (continuous line) staining for control, undepleted, and aDC-depleted mice on days 4 and 5 p.i. Data in A and B are representative of three experiments and represent means ± SEM ($n$ = 3–4 mice/group). (C) Groups of BALB/c mice were IAV infected with or without aDC depletion as in Fig. S1. On days 4 and 5 p.i., mice were sacrificed and proliferation, as measured by Ki67 expression, of tetramer+ CD8 T cells was assessed by flow cytometry. Data in C are pooled from two separate experiments and represent means ± SEM ($n$ = 6 mice/group). (D and E) Mice were infected and aDC depleted as in C; however, groups of aDC-depleted mice were reconstituted with purified pulmonary pDCs (light gray bars) or CD8α+ DCs (dark gray bars) on day 3 p.i. (i.e., 24 h after depletion). Mice were sacrificed on day 5 p.i. and assessed for IAV-specific CD8 T cell proliferation as in C. (D) Shown are representative isotype (shaded) or Ki67 staining (continuous line) for control undepleted, aDC-depleted, pDC-reconstituted, and CD8α+ DC–reconstituted groups. Data are gated on CD3+CD8+tetramer+ T cells. Data in E are pooled from two separate experiments and represent means ± SEM ($n$ = 6 mice/group).

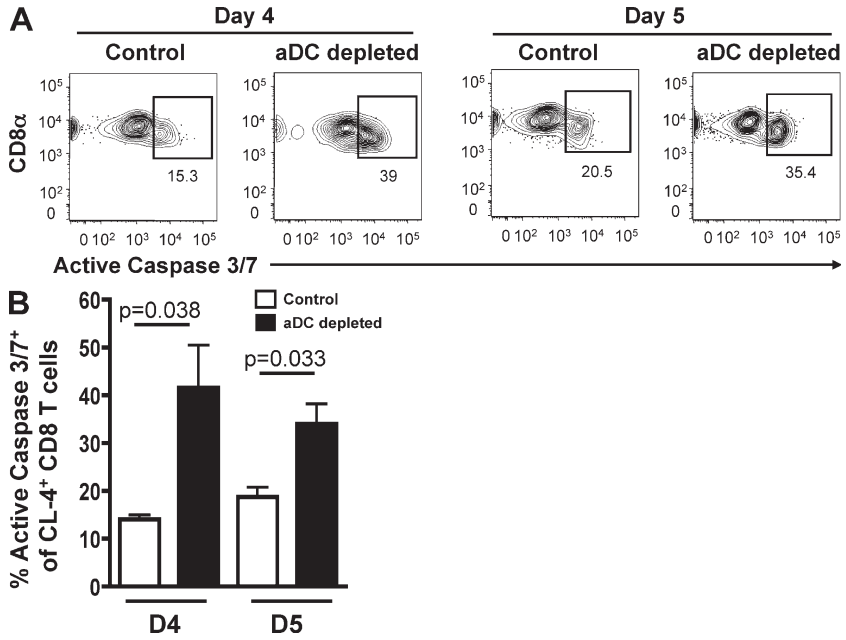


**Figure S2. aDC depletion at 48 h p.i. results in increased apoptosis by virus-specific CD8 T cells.** Influenza-specific, CD90.2$^+$ CL-4 T cells were adoptively transferred to groups of CD90.2$^+$ BALB/c mice as described in Materials and methods. 24 h later, mice were infected with a sublethal dose of IAV with or without aDC depletion as outlined in Fig. S1. On days 4 and 5 p.i., mice were sacrificed and apoptosis, as measured by the presence of active caspase 3/7, of CD90.2$^+$ CL-4 T cells was measured by flow cytometry. (A) Representative FACS plots from day 4 and 5 p.i. are gated on CD3$^+$CD8$^+$ T cells. Data are representative of three separate experiments ($n$ = 3–4 mice/group). Means ± SEM are shown.

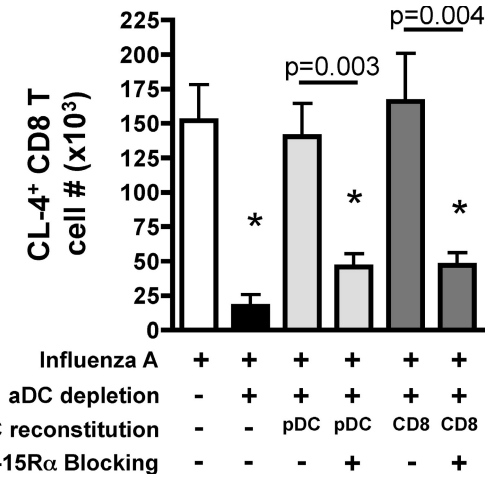


**Figure S3. Pulmonary pDC– and CD8α$^+$ DC–mediated rescue of IAV-specific CD8 T cell responses from aDC-depleted mice requires IL-15 trans-presentation.** Influenza-specific, CD90.1$^+$ CL-4 T cells were adoptively transferred to groups of CD90.2$^+$ BALB/c mice as described in Materials and methods. 24 h later, mice were infected with a sublethal dose of IAV with or without aDC depletion as outlined in Fig. 1. On day 3 p.i., groups of aDC-depleted mice were reconstituted i.n. with 2.5 × 10$^4$ purified pulmonary pDCs (light gray bars) or CD8α$^+$ DCs (dark gray bars) that were left untreated or blocked in vitro with anti–IL-15Rα antibody. On day 6 p.i., the number of pulmonary adoptively transferred, CD90.1$^+$ CL-4 CD8 T cells was enumerated by flow cytometry. Data are pooled from two separate experiments and represent means ± SEM ($n$ = 7–8 mice/group). *, P < 0.05 compared to influenza-only control mice.

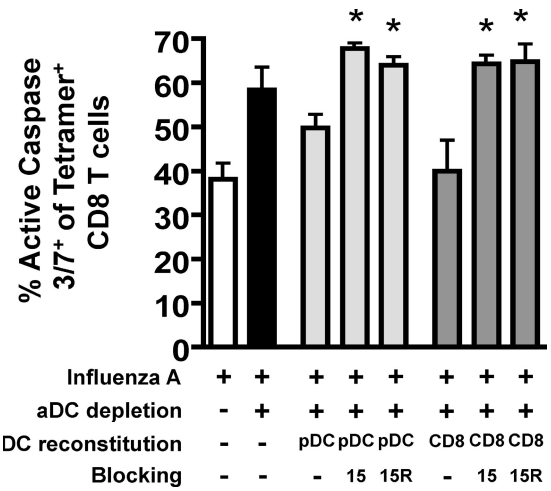


**Figure S4. Blocking IL-15 or IL-15Rα on the surface of pulmonary DC subsets before adoptive transfer ablates pulmonary DC–mediated rescue of IAV-specific CD8 T cell apoptosis in the lungs of aDC-depleted mice.** Groups of BALB/c mice were infected with a sublethal dose of IAV with or without aDC depletion as in Fig. 1. On day 3 p.i., groups of aDC-depleted mice were then reconstituted i.n. with $2.5 \times 10^4$ purified pulmonary pDCs (light gray bars) or CD8α+ DCs (dark gray bars) that were blocked in vitro with anti–IL-15 (15) or anti–IL-15Rα (15R) antibody before adoptive transfer. On day 5 p.i., the lungs were analyzed for the frequency of apoptosis of tetramer+ CD8 T cells. Data are representative of two separate experiments and represent means ± SEM ($n$ = 3–4 mice/group). *, $P \leq 0.05$ relative to influenza virus infection–only controls.

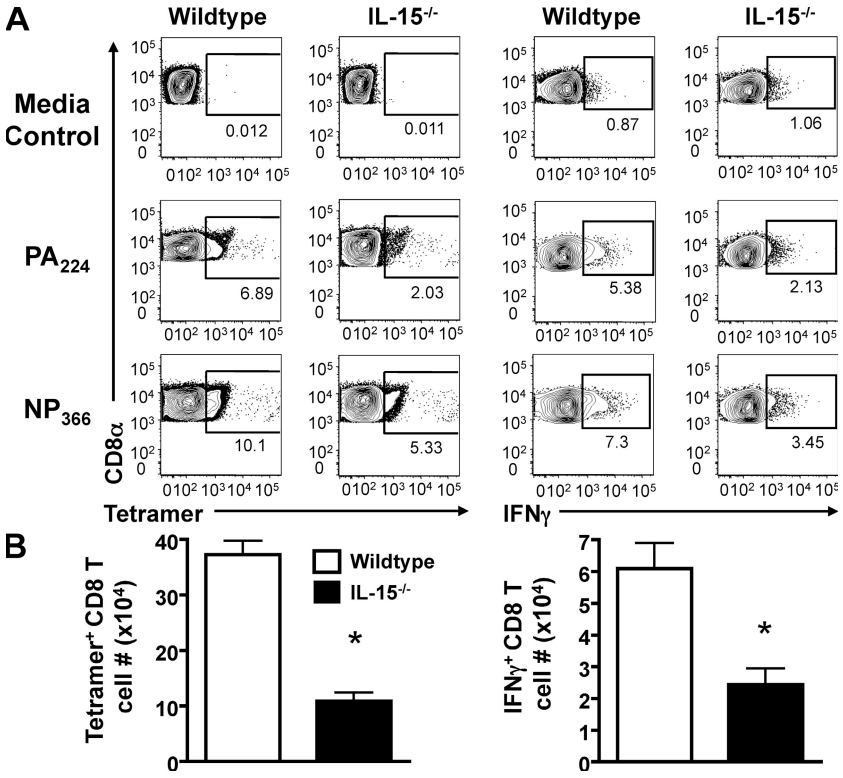


**Figure S5. IL-15$^{-/-}$ mice exhibit reduced IAV-specific CD8 T cell numbers in the lungs after IAV infection.** Groups of IL-15$^{-/-}$ and wild-type control IL-15$^{+/+}$ C57BL/6 mice were infected with a sublethal dose of IAV. On day 8 p.i., the (A) frequency and (B) number of virus-specific CD8 T cells in the lungs were measured by tetramer (left) or ICS for IFN-γ (right). Representative FACS plots in A are gated on CD3+CD8+ T cells. Numbers of tetramer+ or IFN-γ+ CD8 T cells in the lungs were determined by subtracting background staining using the media control (A, top). Data are representative of two separate experiments and represent means ± SEM ($n$ = 4–5 mice/group). *, $P \leq 0.05$ relative to influenza virus infection–only controls.