Supplemental Figure 1. Mice were infected with a 0.5LD$_{50}$ inoculum of IAV and the indicated cytokines and chemokines in lung homogenate supernatants were quantified by ELISA on the indicated day post-infection. Data are pooled from two separate experiments, error bars represent SEM, n=7-10 per group.
Supplemental Figure 2. (A-F) Mice were infected with a 0.5 LD<sub>50</sub> inoculum of IAV, then T cells were enumerated by flow cytometry. Spleen (A, B) and lung draining LN (C, D) were harvested at the indicated times post-infection, and lungs (E, F) at seven days post-infection. Data are pooled from two (E, F, n=3-5 per group) or three (A-D, n=8-16 per group) separate experiments, error bars represent SEM. **p<0.01, two-tailed Student’s t-test.
Supplemental Figure 3. (A-E) Mice were infected with a 0.5LD_{50} IAV, and the indicated cell populations from lung draining lymph nodes (dLN) were quantified by flow cytometry. (A, B) Mice were administered CFSE intranasally prior to IAV infection to stain cells in the lung. CFSE^+ cells in the lung dLN were quantified at 24 hrs post-infection. (C-H) DCs from lung dLN were analyzed by flow cytometry at the indicated times post-infection. Numbers in (C) are per LN. Data are pooled from two (A, B, n=9-10) or three (C-H, n=12-16) separate experiments, error bars represent SEM.
Supplemental Figure 4. (A-C) Mice were infected with a 0.5LD_{50} inoculum of IAV and spleens and lungs were harvested at the indicated time post-infection (B), or lungs harvested at day seven post-infection (A, C). (A) Representative gating strategy is shown. (B, C) FasL{\textsuperscript{+}} DCs were enumerated by flow cytometry; frequencies correspond to numbers shown in Figure 4. Data are from one (B, day 5) or three (B, day 7, and C, n=10-14 per group) independent experiments, error bars represent SEM. ****p<0.0001, two-tailed Student’s t-test.
Supplemental Figure 5. (A-D) Mice were infected with a \(0.5\text{LD}_{50}\) inoculum of IAV and lungs were harvested at day seven post-infection. (A) FasL\(^+\) DCs were enumerated by flow cytometry; frequencies correspond to numbers shown in Figure 4. (B) Fas\(^+\) T cells were enumerated in the lungs by flow cytometry. (C) Bulk splenic DCs (CD11c\(^+\)) of the indicated genotype were incubated with pooled WT+\(Nlrc4\)–/– lung CD4 T cells. The number of live CD4 T cells (Annexin V'Viability Dye') after incubation for 12 hrs were normalized to lung CD4 T cells cultured alone; (D) CD4 T cells of the indicated genotype and origin were incubated without DCs for 12 hrs with (+Fas-Fc) or without (No trx) 2.5\(\mu\)g/mL Fas-Fc, and live CD4 T cells (Annexin V'Viability Dye') were enumerated by flow cytometry. Data are from one (A, \(n=4-5\) per group), or two (B-C, \(n=6-10\) per group) independent experiments, error bars represent SEM. **\(<0.01\), ***\(<0.001\), two-tailed Student's t-test.
Supplemental Figure 6. (A) FasL$^+$ expression on BMDC from WT and Nlrc4$^{-/-}$ mice was assessed by flow cytometry. (B-E) WT mice were infected with a 0.5LD$_{50}$ IAV; at 5 days post-infection, WT mice received 5x10$^5$ WT or Nlrc4$^{-/-}$ BMDC intranasally. Pulmonary CD4 and CD8 T cells were quantified at 7 days post-infection. Frequencies correspond to numbers shown in Figure 5D-G. (F) Fasl mRNA expression was assessed in WT and Nlrc4$^{-/-}$ (independently generated line) BMDC by qPCR and normalized to $\beta$-actin. (G) FasL$^+$ expression on BMDC from WT and Nlrc4$^{-/-}$ (independently generated line) mice was assessed by flow cytometry. (H) Fasl mRNA expression was assessed in WT, Asc$^{-/-}$, and Casp1/11$^{-/-}$ BMDC by qPCR and normalized to $\beta$-actin. (I) FasL$^+$ expression on BMDC from WT, Asc$^{-/-}$, and Casp1/11$^{-/-}$ mice was assessed by flow cytometry. Data are from one (G, n=4 per group) or two (A, n=4 per group, B-E, n=10 per group, I, n=3-7 per group) separate experiments. Data are pooled from three independent experiments (F) or representative of two independent experiments (H). **p<0.01, ***p<0.001, ****p<0.0001, two-tailed Student’s t-test.