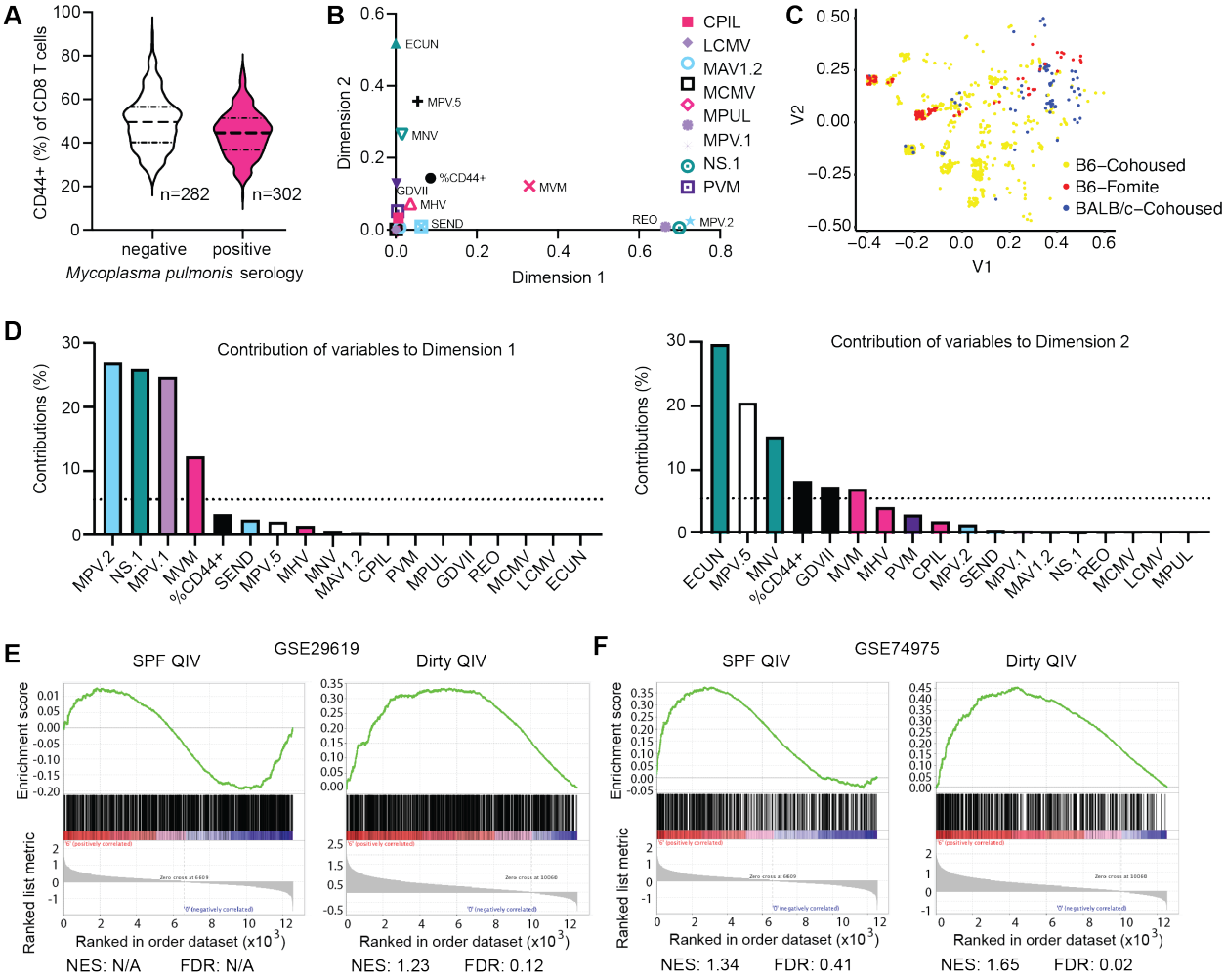

Figure S1: Wide variety of pathogen exposures results in similar immune experienced phenotype in cohoused mice, Related to Figure 1. (A to C) Mice from 3 different pet stores were cohoused with SPF C57BL/6J mice. After 60 days the expression of CD44 of blood $CD8^+$ T cells was determined and a panel of 18 pathogens was tested for by serology. (A). $CD44^+$ of $CD8^+$ T cells was plotted from *Mycoplasma pulmonis* negative (n=282) and positive (n=302) mice. (B) Correlation between variables (pathogen exposure and CD44 percentages) and the principal components calculated by factor analysis of mixed data (FAMD). Each point represents a pathogen in the serology panel or the measured CD44% of cohoused mice at 60 days post cohousing, n=719. Points located near an axis indicates the variable is correlated with the principal component, while points located near the origin indicates they are not. (C) Multidimensional scaling analysis from Figure 1C highlighting the three different housing conditions; cohousing C57Bl/6, cohousing BALB/c, fomite transfer to male C57BL/6. Distances represent similarities in past exposure to 18 pathogens. (D) The percentage of each variable from the FAMD variable plot contributing to the x- (left) or y- (right) axis. Dashed lined indicates the expected percent contribution if all variables contributed equally to the dimension. (E to F) SPF and dirty mice were vaccinated with 2019-2020 QIV. PBMCs were harvested on -3, 1 and 3 dpv. (E) GSEA plots comparing SPF or Dirty QIV mice with 35 subjects given the 2007-2008 or 2008-2009 seasonal TIV vaccine (GSE29619) or (F) with 8 pediatric subjects given the seasonal 2012-2013 TIV vaccine (GSE74975). CIPL - *Clostridium piliforme*, ECUN - *Encephalitozoon cuniculi,* GDVII – Theiler's encephalomyelitis virus, LCMV – lymphocytic choriomeningitis virus, MAV1.2 – mouse adenovirus 1/2, MHV – murine hepatitis virus, MNV – murine norovirus, MPUL- *Mycoplasma pulmonis,* MPV.1 – mouse parvovirus 1, MPV.2 – mouse parvovirus 2, MPV.5 – mouse parvovirus 5, MVM – minute virus of mice, NS.1 – pan parvovirus, PVM – pneumonia virus of mice, REO – reovirus, SEND – Sendai virus.

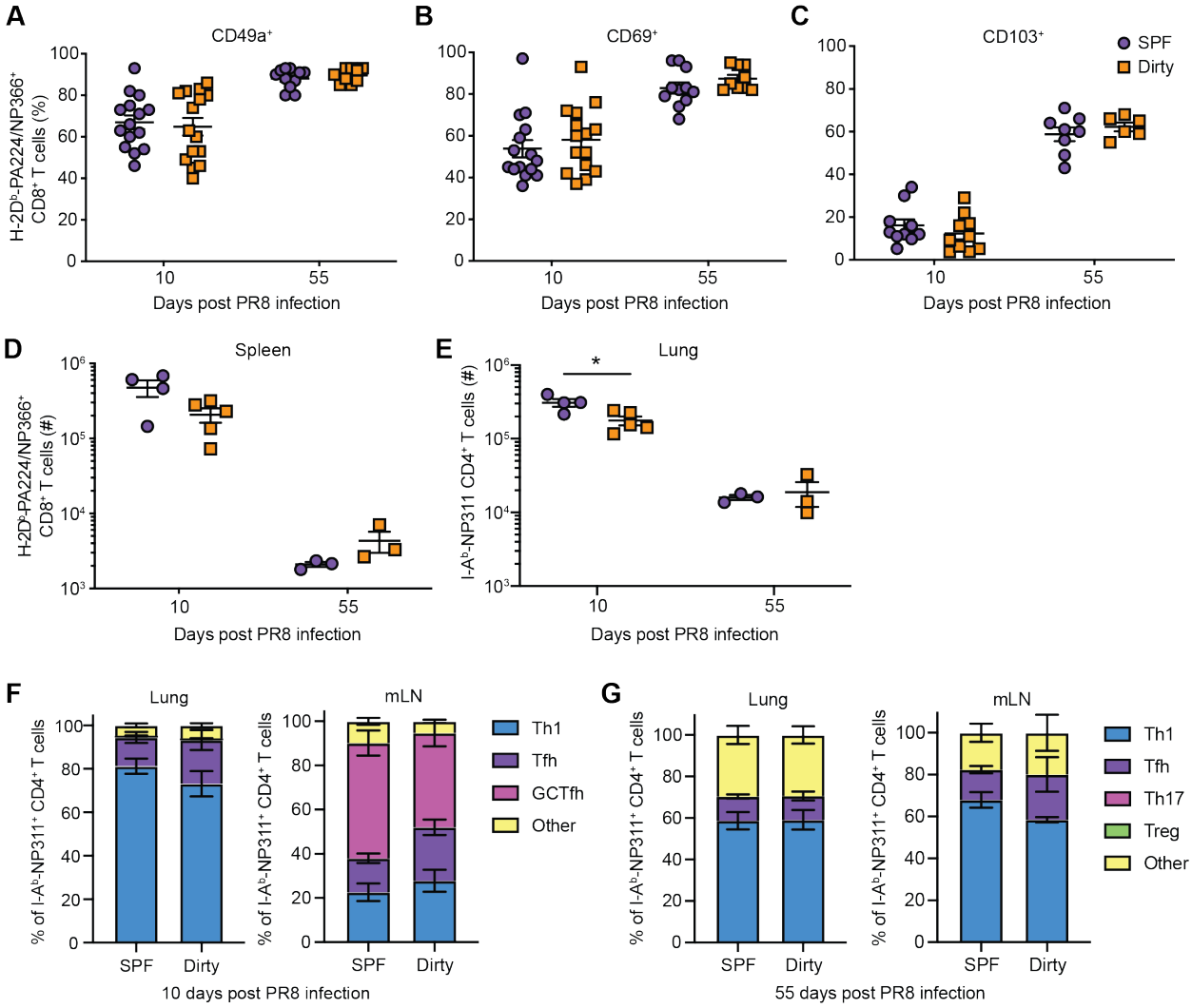

Figure S2: SPF and dirty mice exhibit similar T cell responses to influenza virus, Related to Figure 2. Dirty and SPF mice were infected with 40 PFU of PR8. At 10, and 55 dpi lungs and spleens were analyzed for expression of CD49a (A), CD69 (B) and CD103 (C) of H-2D^b-PA$_{224}$/NP$_{366}$$^+$ CD44$^+$ CD8$^+$ T cells in the lung. (D) Number of H-2D^b-PA$_{224}$/NP$_{366}$$^+$ CD44$^+$ CD8$^+$ T cells in the spleen. (E) Number of I-A^b-NP$_{311}$$^+$ CD44$^+$ CD4$^+$ T cells in the lung. (F-G) Frequency of I-A^b-NP$_{311}$$^+$ CD44$^+$ CD4$^+$ T cell phenotypes. (F) Th1 (PD-1$^-$ CXCR5$^-$), Tfh (PD-1$^-$, CXCR5^{int}), GCTfh (PD-1^{hi} CCXR5^{hi}). (G) Th1 (T-bet$^+$ Foxp3$^-$), Tfh (T-bet$^+$ Foxp3$^-$ CXCR5$^+$) Th17 (T-bet$^-$ FoxP3$^-$ Roryt+) or Treg (Foxp3$^+$). Data (A-C, F) are combined from 2-3 independent experiments PR8 with 6-15 mice per group. The data (D to E) are representative from 1 of 2 independent experiments with at least 3 mice per group. The data (G) are from 1 experiment with 3 mice per group. Significance was determined using student unpaired two-tailed *t*-test. Error bars indicate mean ± SEM. *$p < 0.05$.

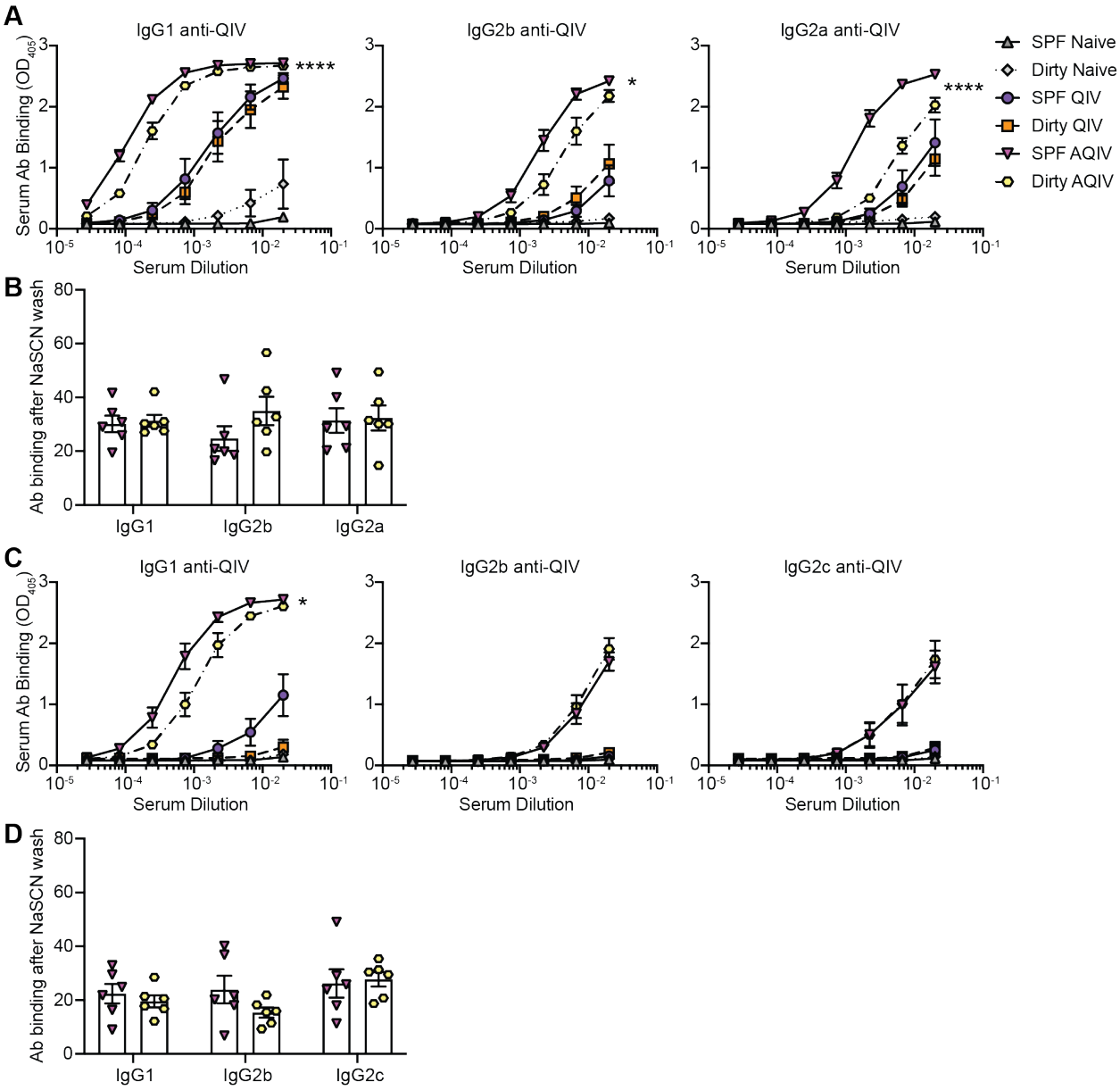

Figure S3: Response to split killed seasonal vaccine with and without adjuvant in cohoused BALB/c mice and male B6 mice exposed to pet store fomites, Related to Figure 4. SPF and dirty BALB/c mice were untreated or vaccinated with 2019-2020 QIV with or without AddaVax i.m. (A) Serum analyzed at 30 dpv for anti-QIV antibodies (B) Antibody avidity from (A) measured with exposure to chaotropic NaSCN. (C) SPF and B6 mice exposed to fomites from pet store mice were untreated or vaccinated with 2019-2020 QIV with or without AddaVax i.m. (C) Serum analyzed at 30 dpv for anti-QIV antibodies. (D) Antibody avidity from (C) measured with exposure to chaotropic NaSCN. The data (A-D) are from 1 experiment with at least 5 mice per group. Significance (A and C) was determined using AUC and one-way ANOVA. Error bars indicate mean ± SEM. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

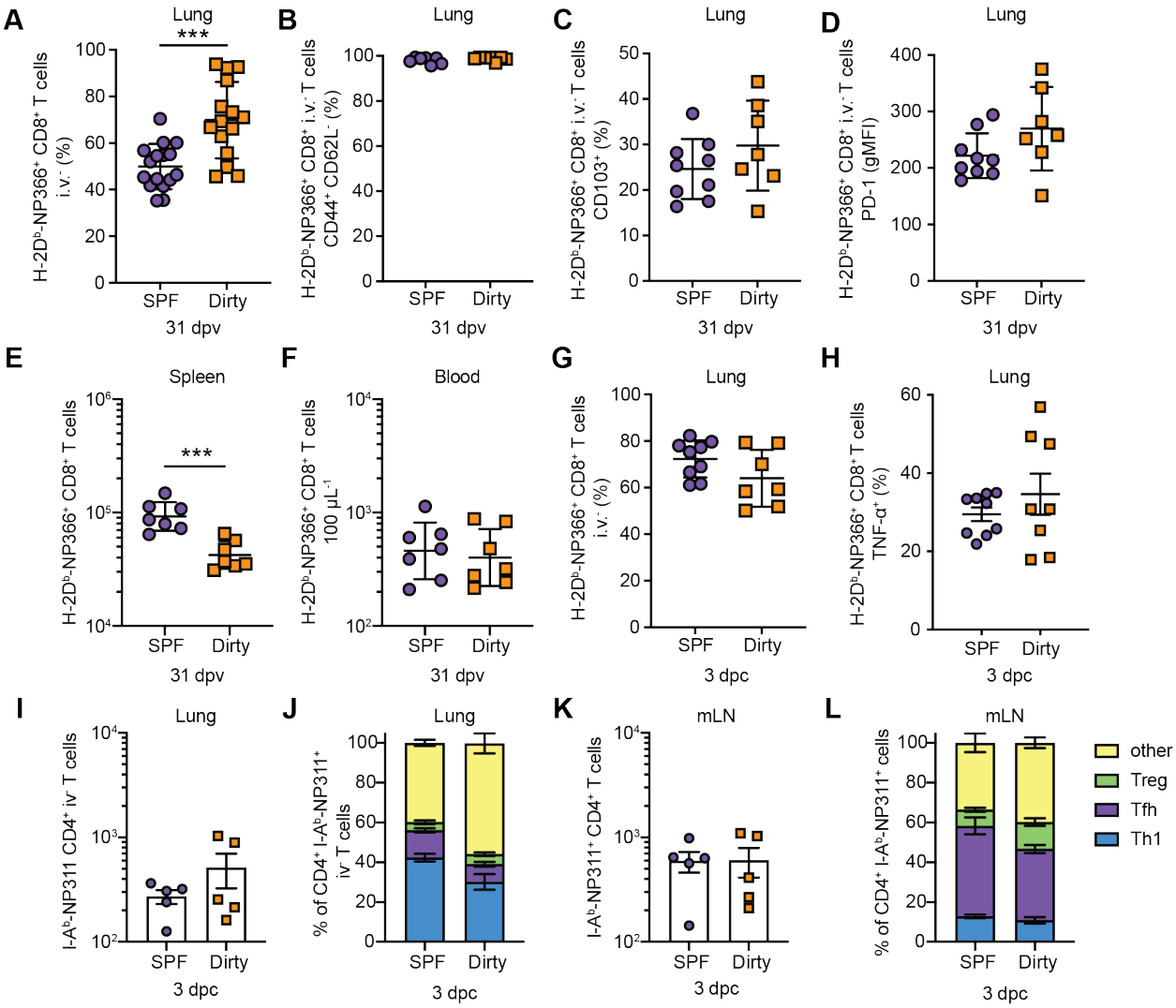

Figure S4: T cell responses to X31 vaccination and challenge are similar in SPF and dirty mice, Related to Figure 5. SPF and dirty mice were vaccinated with 1000 PFU X31. (A) Frequency of H-2D^b-NP$_{366}^+$ CD8$^+$ i.v.$^-$ T cells in the lung at 31 dpv. (B to D) Surface expression of CD44 and (B) CD62L or (C) CD103, or (D) gMFI of PD-1 of H-2D^b-NP$_{366}^+$ CD8$^+$ i.v.$^-$ T cells in the lung at 31 dpv. (E to F) Number of H-2D^b-NP$_{366}^+$ CD8$^+$ i.v.$^-$ T cells in the (E) spleen or (F) blood at 31 dpv. (G to H) Vaccinated mice were challenged after 30 days with 1000 PFU PR8. (G) Frequency of H-2D^b-NP$_{366}^+$ CD8$^+$ i.v.$^-$ T cells in the lung at 3 dpc. (H) Surface expression of TNF-α on H-2D^b-NP$_{366}^+$ CD8$^+$ T cells in the lung at 31 dpv. (I, K) Number of I-A^b-NP$_{311}^+$ CD4$^+$ T cells in the lungs (I) or mLN (K) of X31 primed mice 3 days post challenge with PR8. (J, L) Frequency of cells in (I or K, respectively) Th1 (T-bet$^+$ Foxp3$^-$), Tfh (T-bet$^+$ Foxp3$^-$ CXCR5$^+$) Th17 (T-bet$^-$ FoxP3$^-$ Roryt+) or Treg (Foxp3$^+$). The (A-H) data are combined from 2-3 independent experiments with at least 5 animals per group. The (I-L) data are from 1 experiment with 5 animals per group. Significance was determined using student unpaired two-tailed *t*-test. Error bars indicate mean ± SD (A-G) or ± SEM (H-L). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

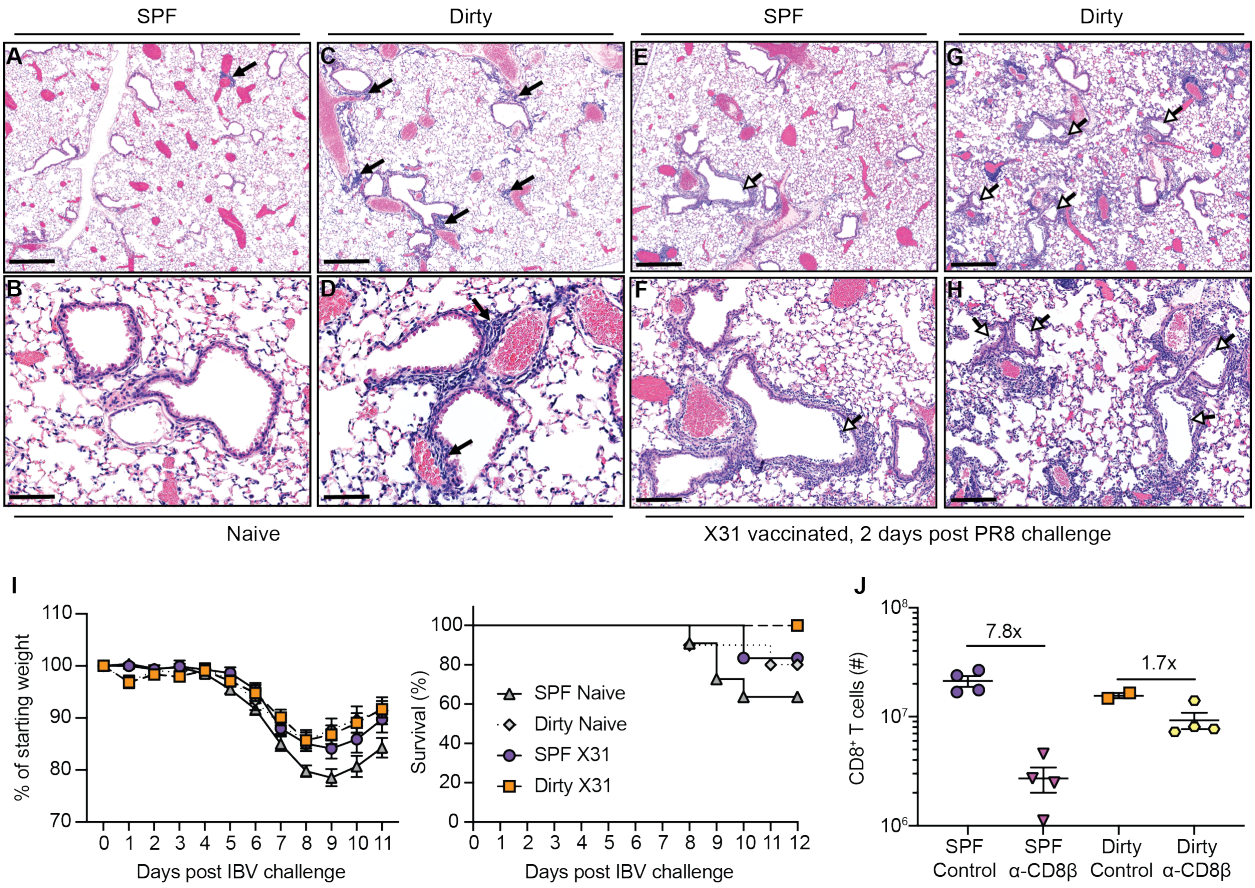

Figure S5: Increased lung damage after heterologous challenge in dirty mice and successful control of antigen irrelevant secondary infection, Related to Figure 5. SPF and dirty mice were untreated or vaccinated with 1000 PFU X31 and were challenged after 30 days with 1000 PFU PR8. Lungs were sectioned and stained with H&E. (A-D) Representative lung histopathology from naïve SPF (A-B) and dirty (C-D) mice. (E-H) Representative lung histopathology from X31 vaccinated SPF (A-B) and dirty (C-D) mice on 2 days post PR8 challenge. Bar = 246 μm (A, C, E, and G), Bar = 79 μm (B, D, F and H). Closed arrows indicate perivascular/peribronchiolar lymphoid aggregates. Open arrows indicate influenza-associated necropurulent bronchiolitis. (I) SPF and dirty mice were vaccinated with 1000 PFU X31 and 30 days later challenged with 1000 PFU of antigenically distinct IBV. Animals were monitored for morbidity and mortality. (J) SPF and dirty mice were treated with anti-CD8β depletion or control IgG antibody on days -6, -5, -3, -2 and total $CD8^+$ T cells numbers evaluated. Data are representative of 3 independent experiments.