## Supplementary Figures

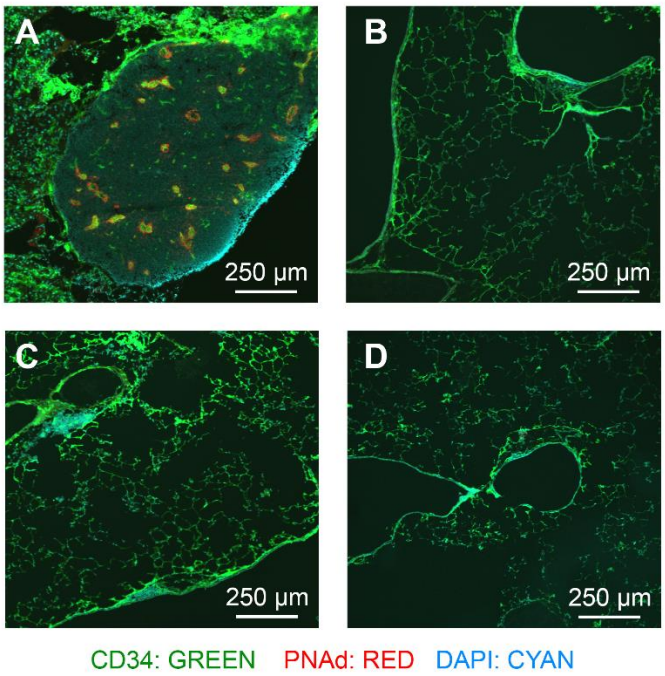


**Figure S1. Experienced mouse lungs do not contain HEVs.** Frozen sections of an experienced mouse mediastinal lymph node positive control (**A**) or of lungs from three experienced mice (**B-D**) were stained for nuclei (DAPI, blue), the blood vessel marker CD34 (green), and peripheral node addressin (PNAd, red) and visualized via confocal microscopy.

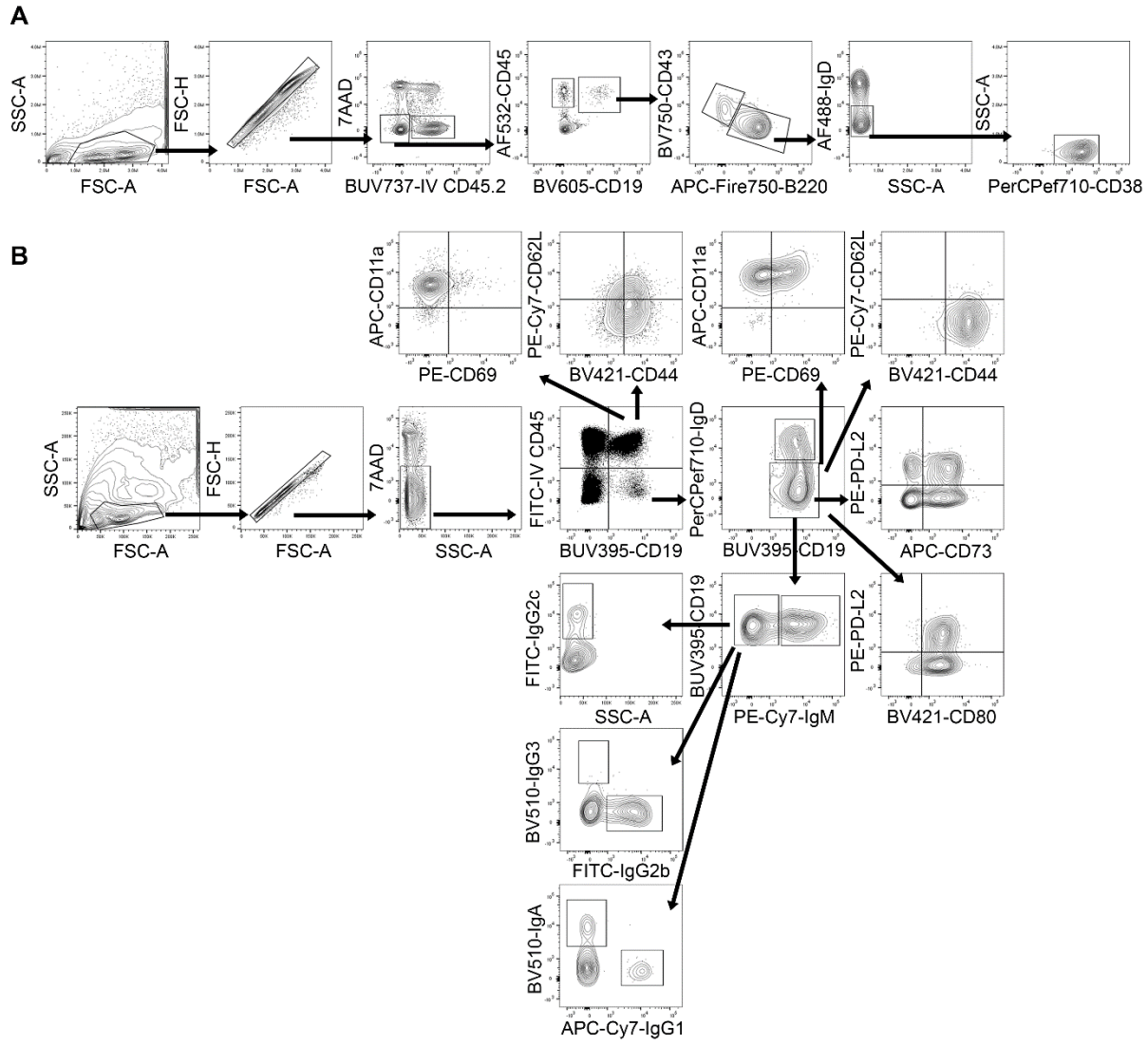


**Figure S2. Flow cytometry gating schemes used to analyze mouse lung B cells**. (**A**) Gating used in a subset of mice from Figure 2 I-J and all mice in Figure 2K, collected on the Cytek Aurora spectral cytometer. (**B**) Gating used for all other flow cytometry data, collected on the BD LSR II.

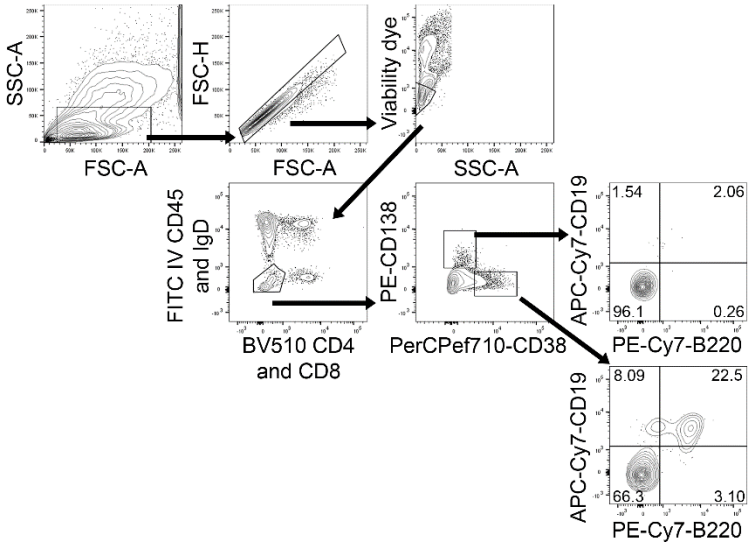


**Figure S3. Flow cytometry gating schemes used to analyze mouse lung plasma cells.** Gating used in all mice from Figure 3 E-G, collected on the BD LSR II.

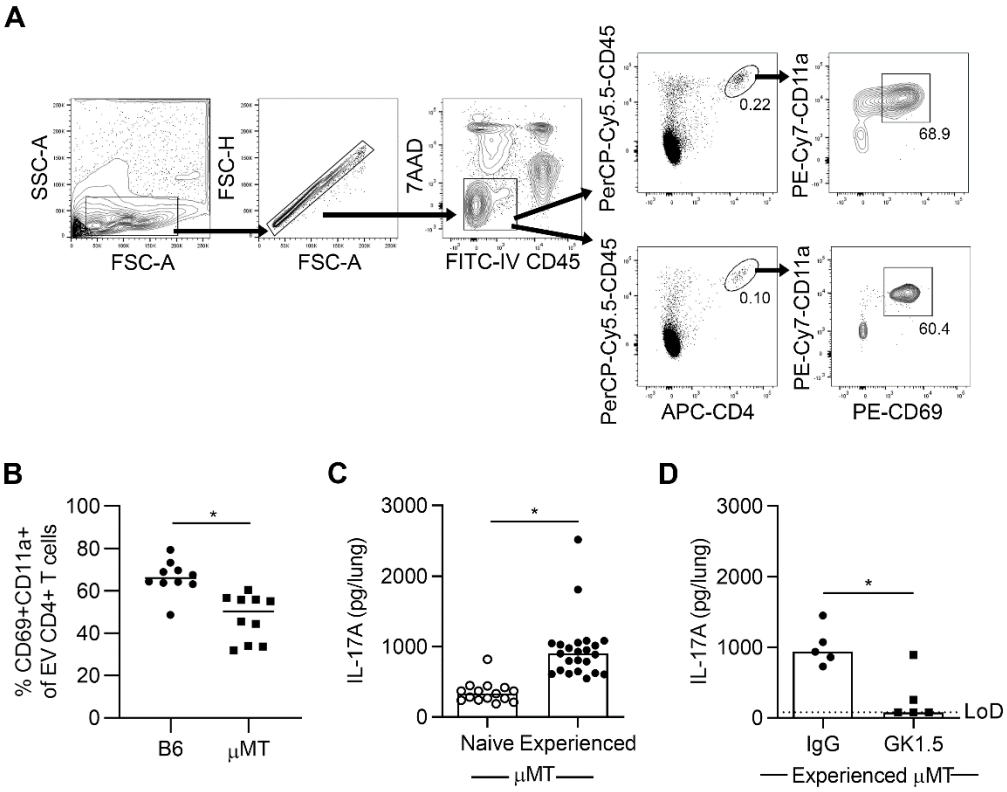


**Figure S4. Experienced μMT mice possess lung TRM cells comparable to those of B6 mice.** (**A**) Flow cytometry gating scheme used to assess lungs of experienced B6 and μMT mice for EV T cells bearing a resident memory phenotype (CD69+CD11a$^{hi}$). (**C**) Results obtained from gating in (**A**), showing the percent of EV lung CD4+ T cells that were CD69+CD11a$^{hi}$ in lungs of experienced B6 and μMT mice (Mann-Whitney test, *P=0.0002). (**C**) IL-17A levels determined by ELISA in whole lung homogenates of naïve and experienced μMT mice after 24 h of Sp3 pneumonia (Mann-Whitney test, *P<0.0001). (**D**) IL-17A levels determined by ELISA in whole-lung homogenates of of experienced μMT mice treated with either an IgG isotype control or a CD4+ T cell depleting antibody (GK1.5) prior to 24 h of Sp3 challenge (Mann-Whitney test, *P=0.032).

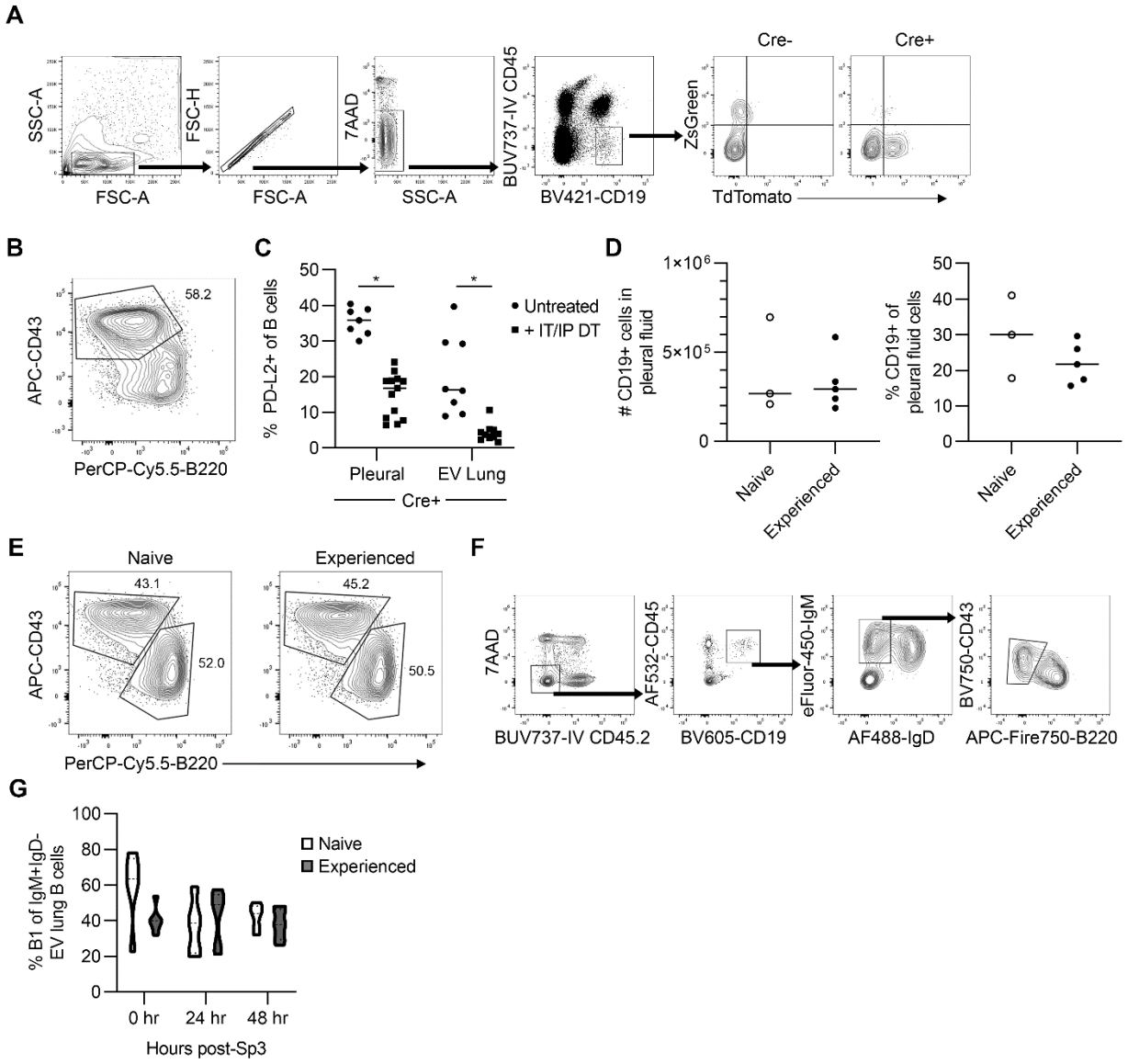


**Figure S5. Although they are depleted by DT in PZTD mice, pleural fluid and B1 B cells are unlikely to contribute to lung protection after prior pneumococcal experience.** (**A**) Flow cytometry gating scheme used in PZTD mouse lungs. (**B**) Representative flow plot gated on pleural fluid CD19+TdTomato+ cells from a Cre+ PZTD mouse, showing that the majority of PD-L2+ B cells in the pleural fluid have a B1 B cell phenotype ($CD43^{hi}B220^{lo}$). **(C**) The percent of pleural fluid and total EV lung B cells that are PD-L2+ in Cre+ PZTD mice with or without DT treatment (2-way ANOVA, *P<0.0001). (**D**) The number (left) and percent (right) of CD19+ cells in the pleural fluid of naïve and experienced C57BL6/J mice as determined by flow cytometry. (**E**) Representative flow cytometry plots showing the similar distributions of B1 and B2 cells in the pleural fluid of naïve and experienced C57BL6/J mice. (**F**) Gating scheme used to assess lung B1 B cells in (**G**). Initial gates for lymphocytes and single cells done as in previous experiments. (**G**) The percent of EV lung IgM+ B cells with a B1 phenotype at baseline and after one to two days of Sp3 pneumonia in naïve and experienced C57BL6/J mice. N=6 mice per group (no significant differences by 2-way ANOVA).

# Supplementary Methods

| Antibody (target-fluorophore) | Clone | Supplier | Product Number |
|---|---|---|---|
| *Mouse flow cytometry* | | | |
| Blimp1-APC | 150007 | Biolegend | 150007 |
| B220-APC Fire 750 | RA3-6B2 | Biolegend | 103259 |
| B220- PerCp Cy5.5 | RA3-6B2 | Biolegend | 103235 |
| B220-PECy7 | RA3-6B2 | Biolegend | 103221 |
| CD4-APC | GK1.5 | Biolegend | 100411 |
| CD4-BV510 | GK1.5 | Biolegend | 100449 |
| CD8-BV510 | 53-6.7 | Biolegend | 100751 |
| CD11a-APC | M17/4 | Biolegend | 101119 |
| CD11a-PECy7 | M17/4 | Biolegend | 101121 |
| CD19-BUV395 | 1D3 | BD Biosciences | 563557 |
| CD19-BV605 | 6D5 | Biolegend | 115539 |
| CD19-BV421 | 6D5 | Biolegend | 115537 |
| CD19-APCCy7 | 6D5 | Biolegend | 115529 |
| CD20-APC | SA275A11 | Biolegend | 150411 |
| CD38-PerCp ef710 | 90 | Thermo Fisher | 46-0381-80 |
| CD43-BV750 | S7 | BD Biosciences | 747277 |
| CD43-APC | S11 | Biolegend | 143207 |
| CD44-BV421 | IM7 | BD Biosciences | 563970 |
| CD45-FITC | 30-F11 | Biolegend | 103108 |
| CD45-AF532 | 30-F11 | Thermo Fisher | 58-0451-82 |
| CD45-PerCp Cy5.5 | 30-F11 | Biolegend | 103131 |
| CD45-BUV737 | 30-F11 | BD Biosciences | 748371 |
| CD45.2-BUV737 | 104 | BD Biosciences | 612778 |
| CD62L-PECy7 | MEL-14 | Biolegend | 104418 |
| CD69-PE | H1.2F3 | Biolegend | 104508 |
| CD73-APC | TY/11.8 | Biolegend | 127209 |
| CD80-BV421 | 16-10A1 | BD Biosciences | 562611 |
| CD138-PE | 281-2 | Biolegend | 142503 |
| IgA-Biotin | RMA-1 | Biolegend | 407003 |
| IgD-PerCp ef710 | 11-26c | Thermo Fisher | 46-5993-82 |
| IgD-AF488 | 11-26c | Biolegend | 405717 |
| IgG1-FITC | RMG1-1 | Biolegend | 406606 |
| IgG2b-FITC | RMG2b-1 | Biolegend | 406705 |
| IgG2c-FITC | polyclonal | Southern Biotech | 1079-02 |
| IgG3-Biotin | RMG3-1 | Biolegend | 406803 |
| IgM-PECy7 | RMM-1 | Biolegend | 406514 |
| IgM-eF450 | eB121-15F9 | Thermo Fisher | 48-5890-82 |
| PD-L2-PE | TY25 | Biolegend | 107205 |
| *Human flow cytometry* | | | |
| B220-BUV737 | RA3-6B2 | BD Biosciences | 564449 |
| CD4-PECy7 | RPA-T4 | BD Biosciences | 560649 |
| CD19-FITC | HIB19 | Biolegend | 302206 |
| CD27-BV421 | M-T271 | BD Biosciences | 562513 |
| CD38-APCCy7 | HB-7 | Biolegend | 356616 |
| CD69-PE | FN50 | Biolegend | 310906 |
| CD83-APC | HB15e | Biolegend | 305311 |
| IgD-PerCP Cy5.5 | 1A6-2 | BD Biosciences | 561315 |
| IgM-BV510 | G20-127 | BD Biosciences | 563113 |

**Table S1. Antibodies used in flow cytometry of mouse and human tissues**

| Multiplex fluorescent immunohistochemical optimization parameters | | | | | | |
|---|---|---|---|---|---|---|
| **Sequence** | **Species and Clone** | **Antigen** | **Catalog Number** | **Primary Antibody Dilution** | **Fluorophore (Akoya Biosciences)** | **Fluorophore Dilution** |
| 1 | Rb D7D2Z | CD4 | CST 25229 | 1:50 | Opal 480-FP1500001KT | 1:40 |
| 2 | Rb D4V4B | CD19 | CST 90176 | 1:600 | Opal 620-FP1495001KT | 1:50 |

**Table S2. Multiplex fluorescent immunohistochemical optimization parameters**