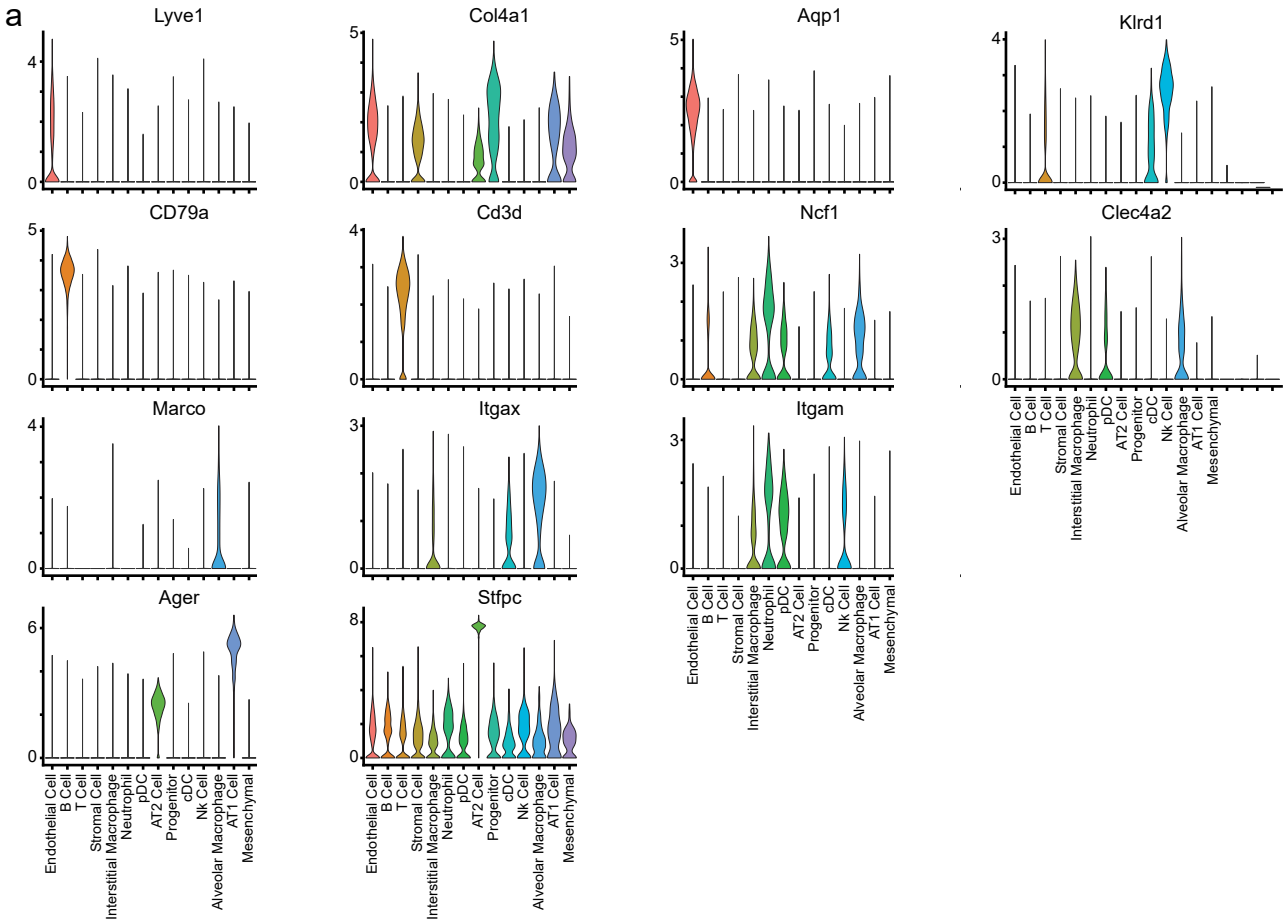
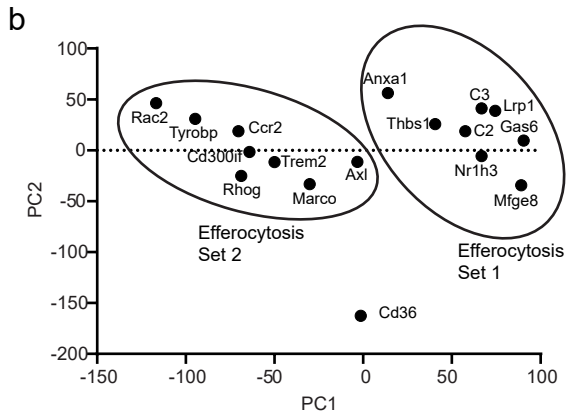
iScience, Volume *24*

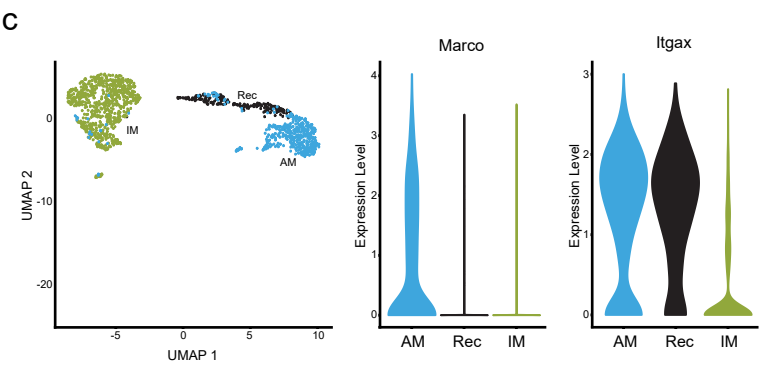
Supplemental information

Pulmonary infection induces persistent, pathogen-specific lipidomic changes influencing trained immunity

Lydia M. Roberts, Benjamin Schwarz, Emily Speranza, Ian Leighton, Tara Wehrly, Sonja Best, and Catharine M. Bosio







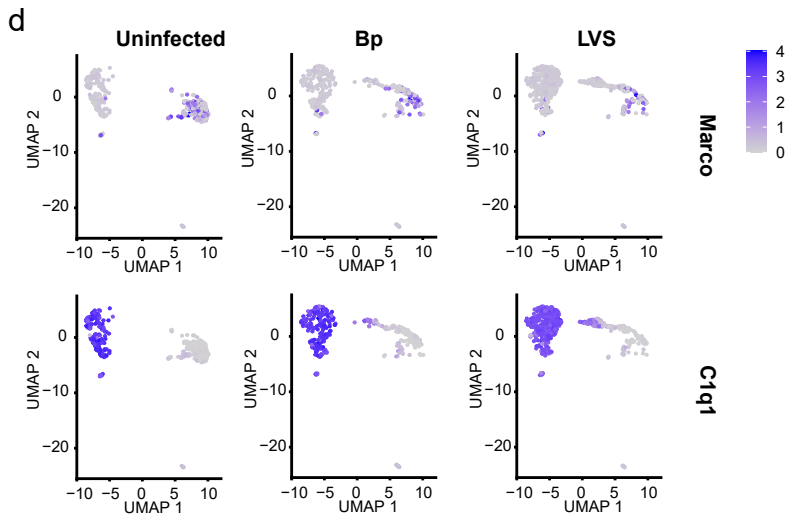


Figure S1. Validation of gene expression across the cell types and efferocytosis; related to Figure 3. a, Violin plots for marker gene expression across the various annotated cell types. Each graph shows a single gene. The x-axis is the various cell types colored to match annotations in figure 1. The annotation names are on the bottom graphs for each column and are in the same order for each graph. The y-axis shows the expression value for each gene. Individual cells are not shown for clarity. b, Principal component analysis of the efferocytosis genes across the different cells. Principal component 1 (PC1) is shown on the x-axis and PC2 is shown on the y-axis. Gene labels are shown next to their corresponding genes point. Circles are drawn to show the two clusters of genes present across the cells and labeled for efferocytosis gene set 1 and efferocytosis gene set 2. Cd36 was not present in either cluster. c, UMAP projection subset on macrophages colored by cell type with interstitial macrophages (IM; green), resident alveolar macrophages (resAM; blue), and recruited alveolar macrophages (recAMs; black). Violin plots for marker gene expression across the annotated cell types. Each graph shows a single gene. Individual points are not shown for clarity. d, UMAP project subset on macrophages for each group displaying expression levels of Marco (top) and C1q1 (bottom) for each individual cell.

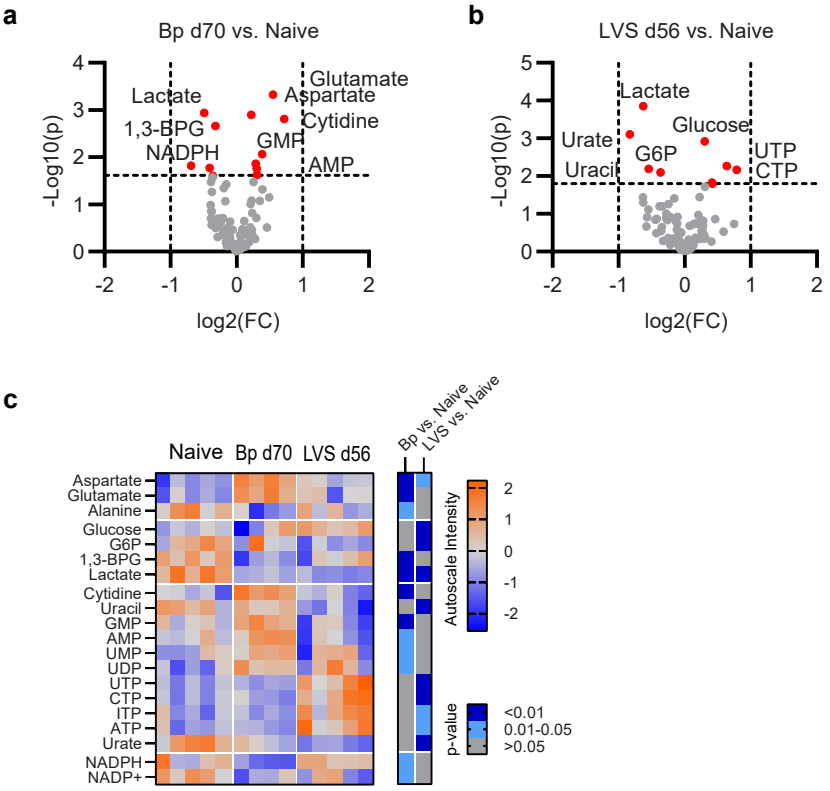


Figure S2. Persistent differences in polar metabolites in the post infection lung; related to Figure 2. Targeted metabolomics of experienced lungs are displayed with a, Bordetella pertussis (Bp; day 70 post-infection) or b, Francisella tularensis LVS (LVS; day 56 post-infection) as compared to naïve animals. Features above a 10 % FDR cut-off line, as calculated using Benjamini-Hochberg correction, are shown in red (Bp p = 0.044, LVS p = 0.058). Vertical lines reflect a fold change of 2 and –2. c, Heatmap of the autoscaled value of metabolites of interest from each biological replicate are displayed with the accompanying p-value from the binary comparison of each experienced group to naïve. All heatmap features listed pass the 10% FDR cut-off for either Bp vs. naïve or LVS vs. naïve. G6P: glucose 6-phosphate, 1,3-BPG: 1,3,-bisphosphoglycerate. Data are representative of 2 independent experiments with n=4-5/group.

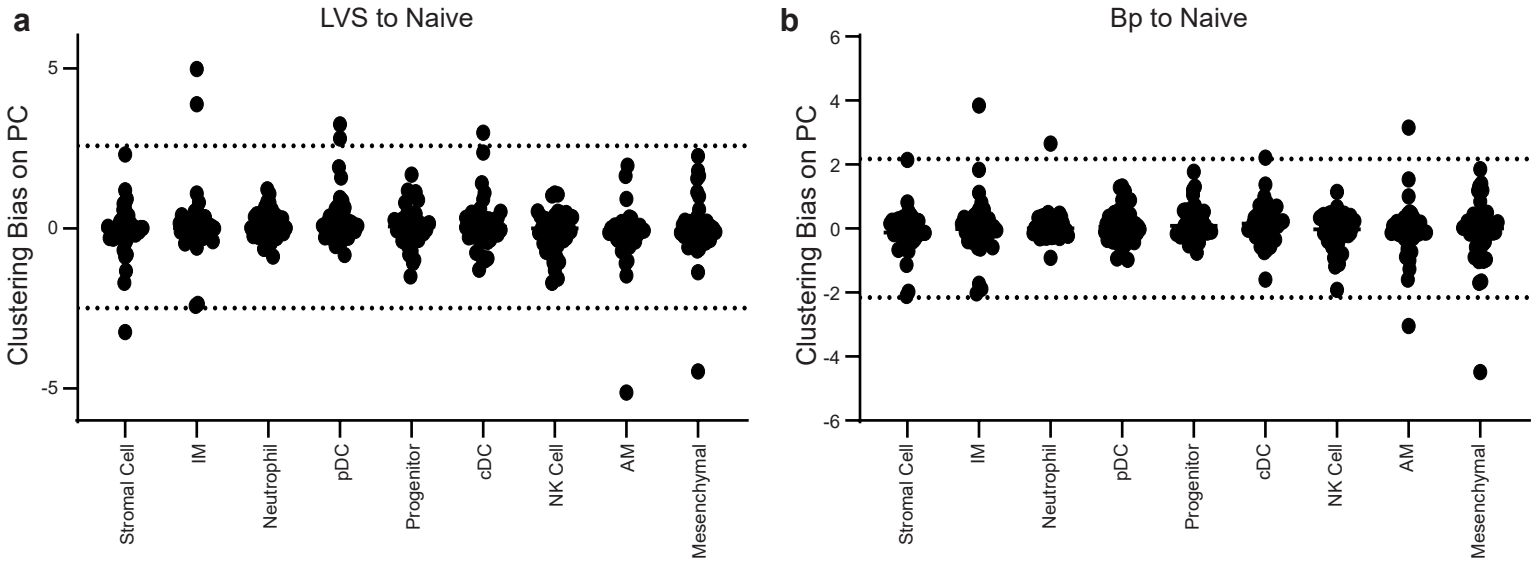


Figure S3. Clustering bias between recovered animals and naive animals; related to Figure 3. a, *Francisella tularensis* LVS (LVS; day 28 post-infection) recovered animals and b, *Bordetella pertussis* (Bp; day 42 post-infection) recovered animals compared to naive. The x-axis represents individual cell types and the y-axis represents the clustering bias determined by the mean of the experimental sample along a single PC minus the mean of the control. Each point is a principal component for the specific cell type. Dashed lines are drawn at 3 standard deviations from the mean of all the points for comparison across samples.

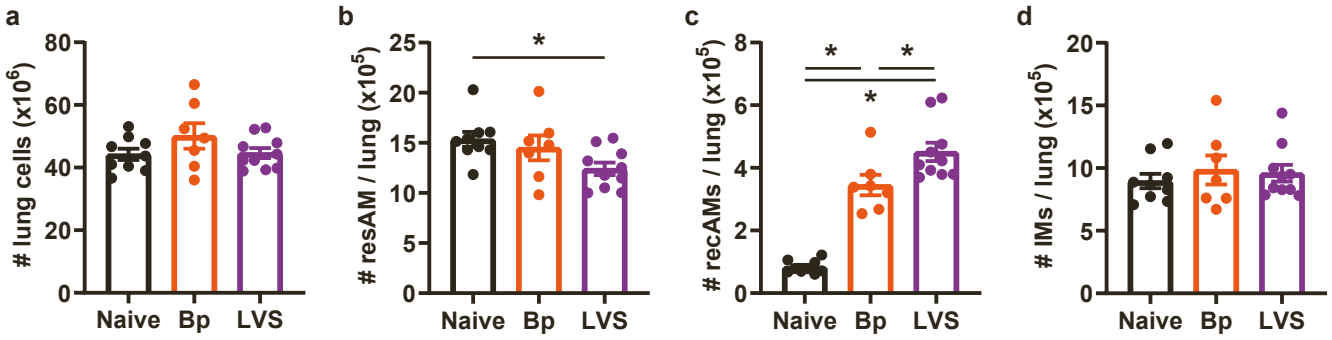


Figure S4. Persistent changes in alveolar macrophage populations following clearance of bacterial infection; related to Figure 4. a, The number of total lung cells was determined from naïve, *Bordetella pertussis* (Bp; day 70 post-infection), and *Francisella tularensis* LVS (LVS; day 56 post-infection) experienced lungs. The number of b, resAMs c, recAMs and d, IMs were also determined at the same time points post-infection. Statistical significance was determined by a one-way ANOVA with a correction for multiple comparisons; * indicate $p < 0.05$. Only statistically significant differences are indicated. Data are combined from two independent experiments with n=8-10 mice/group total.

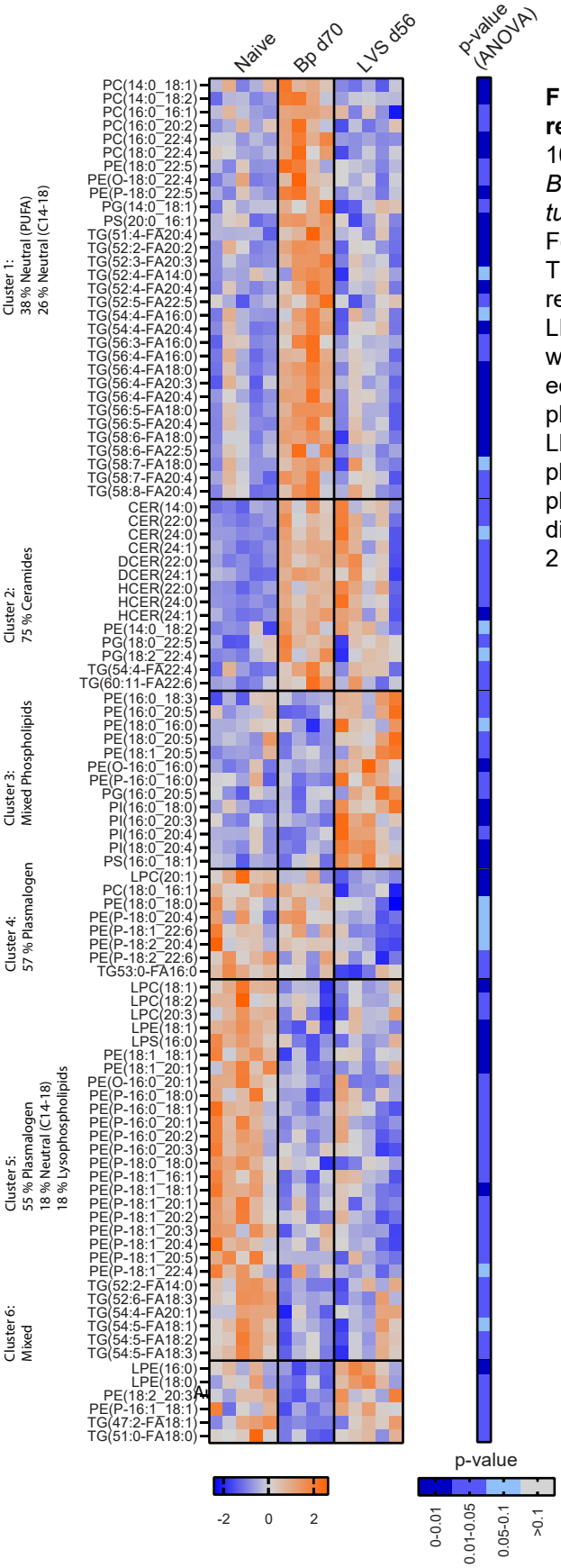


Figure S5. Persistent lipidome alterations in experienced lungs; related to Figure 6. Heatmap of the autoscaled intensity of the top 100 lipids from one-way ANOVA analysis of lipid features naïve, *Bordetella pertussis* (Bp; day 70 post-infection), and *Francisella tularensis* LVS (LVS; day 56 post-infection) experienced lungs. Features are clustered broadly by their behavior between groups. The displayed percentage indicates the percentage within the cluster reflecting that identity. Individual lipid species were labeled with LIPID MAPS abbreviations. Poly-unsaturated fatty acids (PUFA) were defined as a length and degree of unsaturation greater than or equal to 18:3. The ANOVA p-value is displayed to the right. PE: phosphatidylethanolamine, LPE: lyso-PE, PC: phosphatidylcholine, LPC: lyso-PC, PS: phosphatidylserine,; LPS: Lyso-PS, PE(O): plasmanyl PE plasmalogen, PE(P): plasmenyl PE plasmalogen, PG: phosphatidylglycerol, TG: triacylglycerol, Cer: ceramide, DCer: dihydroceramide, HCer: hexosylceramide. Data are representative of 2 independent experiments with n=4-5/group.