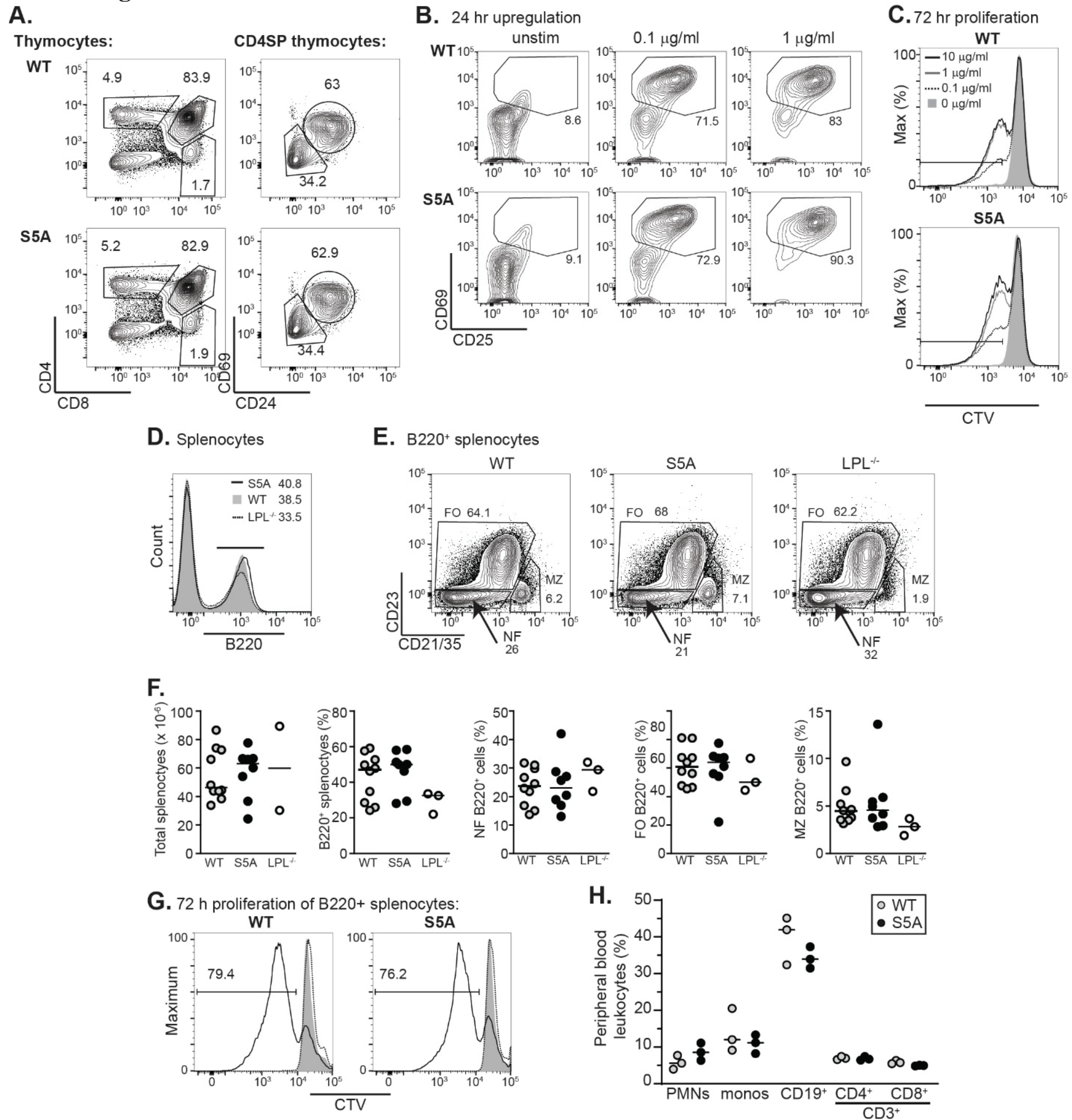
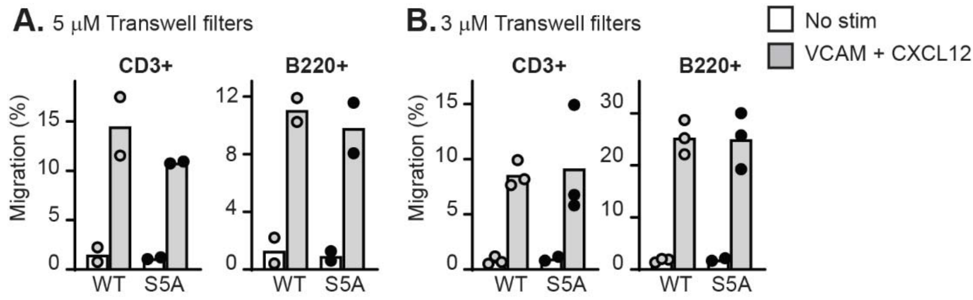
Supplemental Figure 1



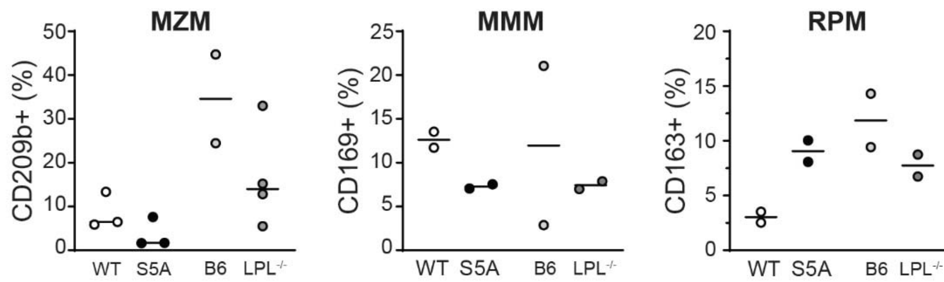
Supplemental Figure 1. No change in T or B cell development or activation noted in S5A mice. A. Flow cytometric analysis of thymocytes from WT and S5A mice revealed equivalent populations of CD4 single positive (CD4SP), CD8 single positive, and CD4/CD8 double positive thymocytes. There was no appreciable difference between populations of mature CD4SP, indicated by $CD24^{low}CD69^{low}$ CD4SP, in S5A and WT thymocytes. **B**. Activation of mature T cells from WT and S5A mice, as assessed by upregulation of CD69 and CD25, 24 h following anti-CD3 (at the indicated concentration) and anti-CD28 (1 μg/ml) stimulation. **C**. Activation of mature T cells from WT and S5A mice, as assessed by CTV dilution, following anti-CD3 (at the indicated concentration) and anti-CD28 (1 μg/ml) stimulation. D. Flow cytometric analysis of total $B220^{+}$ population from splenocytes derived from S5A (solid line), WT (gray shaded histogram) and $LPL^{-/-}$ (dotted line) mice. **E.** Flow cytometric analysis of splenic B cell subsets (CD21/35 and CD23 markers) from splenocytes derived from WT, S5A and $LPL^{-/-}$ mice. NF = newly-forming B cells; FO = follicular B cells, MZ = marginal zone B cells. F. Quantification from two independent analyses showing B cell splenic subsets from WT, S5A and $LPL^{-/-}$ mice. Each symbol indicates data from an individual mouse, line at median. **G.** Activation of B cells from WT and S5A, as assessed by CTV dilution, 72 h following stimulation with anti-IgM Fab (10 μg/ml) and IL-4 (10 ng/ml). **H.** Proportions of the indicated leukocyte populations in peripheral blood derived from WT (gray circles) and S5A (filled circles) mice. Each symbol represents the value from a single mouse, with line at median value. Data shown are from one representative experiment of three independent experiments performed.

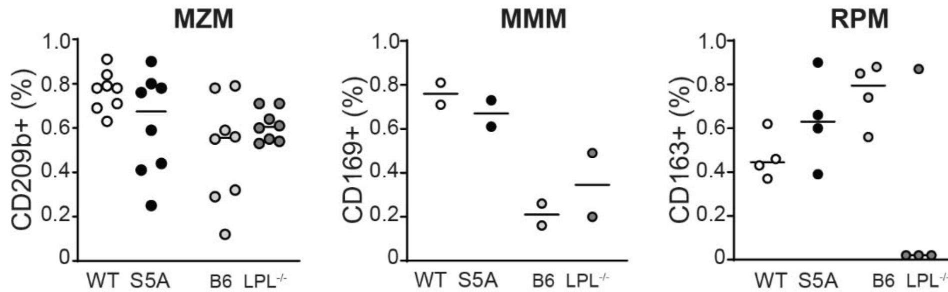
Supplemental Figure 2



Supplemental Figure 2. No effect on T or B cell motility by expression of S5A LPL. *In vitro* migration of mature T and B splenocytes across (**A**) 5 μM or (**B**) 3 μM Transwell filters coated with VCAM (1ug/mL) and stimulated with CXCL12 (100 ng/ml). Each symbol represents the average of duplicate samples within each experiment. The bar represents the average of independent experiments.

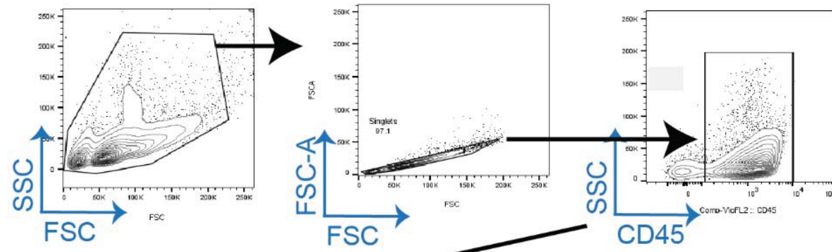
Supplemental Figure 3

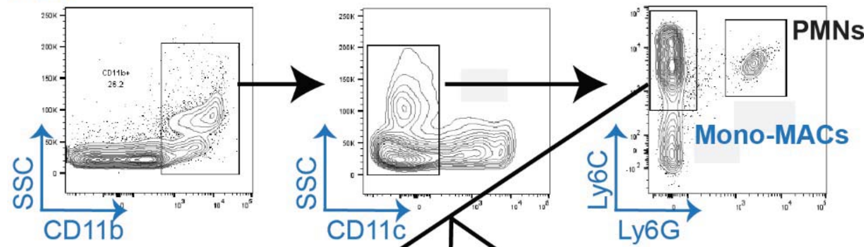


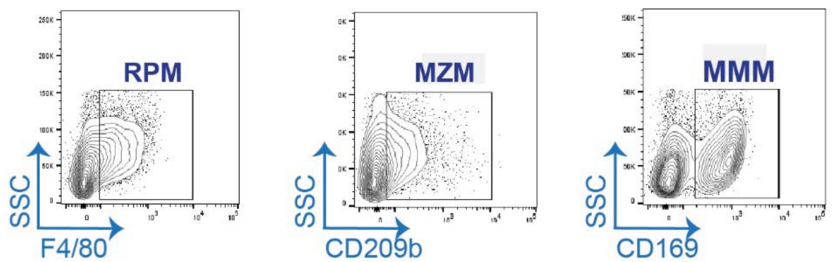


Supplemental Figure 3. Quantification of macrophage lineages in Fig. 4 (A) and of pneumococcal phagocytosis in Fig. 6 (B). A. The area of cells staining positive for the indicated markers within each splenic section from WT (open circles), S5A (filled circles), B6 (light gray circles) or LPL$^{-/-}$ (dark gray circles) mice, as shown in Fig. 4, along with the values derived from additional randomly selected fields imaged within the same experiment. Each symbol represents the value of a single, randomly selected field, line at median. Values from one representative experiment shown. Two independent experiments were performed. B. The degree of colocalization between macrophage lineages (red) and pneumococci (green) was quantified in each field using Pearson's Coefficient. A value of "0" indicates no overlap, while "1" represents perfect correspondence between green and red signals. Each symbol represents the value for arease contained pneumococcus from high-powered fields obtained from randomly selected splenic sections, derived from WT (open circles), S5A (filled circles), B6 (light gray circles) or LPL$^{-/-}$ (dark gray circles) mice, as shown in Fig. 6, along with the values derived from additional randomly selected fields imaged within the same experiment. Line at median. Values from one representative experiment shown. Two independent experiments were performed.

Supplemental Figure 4







Supp. Fig. 4. Gating strategy for identification of splenic macrophage lineages in Fig. 5. All events were gated SSC x FSC, FSC-A x FSC, SSC x CD45 to isolate singlet hematopoietic ($CD45^{+}$) cells. $CD45^{+}$ cells were then gated by SSC x CD11b, SSC x CD11c, Ly6C x Ly6G to define $CD11b^{+}/CD11c^{neg}/Ly6C^{+}/Ly6G^{neg}$ (Mono-MACs) and $CD11b^{+}/CD11c^{neg}/Ly6C^{+}/Ly6G^{+}$ (PMNs) populations. Finally, Mono-MACs were gated SSC x F4/80, SSC x CD209b and SSC x CD169, to define RPM, MZM and MMM, respectively, as shown in Fig. 5A. Percentages shown in Fig. 5B are calculated as percent of total $CD45^{+}$ population.