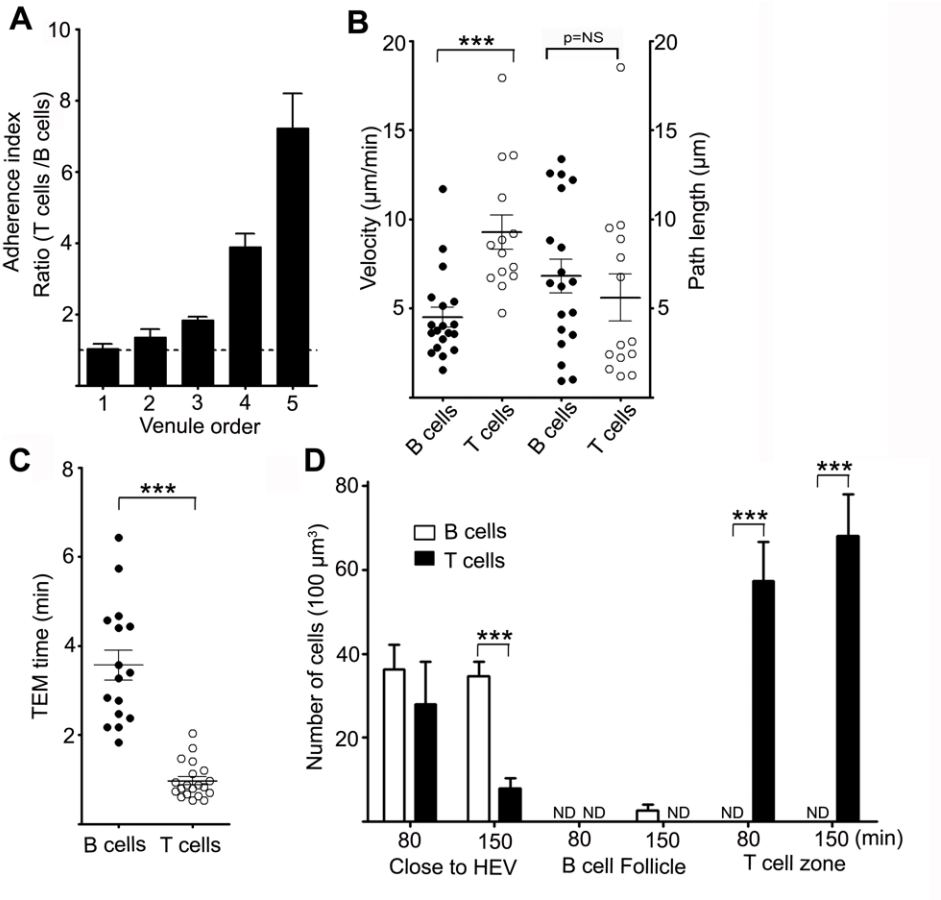

Figure S1. Comparison of B versus T cells in their initial trafficking into the inguinal LN
(A) Ratio of adherent T cells to B cells on HEVs fractionated by vessel diameter. Purified B and T cells were differentially labeled and transferred to recipient mice. Adherent cell numbers were measured using images 8-15 min post cell transfer. Venule order 1-5 are greater than 50 μm, 50-40 μm, 40-30 μm, 30-20 μm, and less than 20 μm, respectively. (B) The intraluminal velocity path length of individual B and T cells measured after firm adhesion until beginning transmigration. (C) Time for TEM of individual cells measured in the interval 5-65 minutes post transfer. (D) Transferred cell density at different sites in the LN and at various times post transfer. Cell densities in indicated areas were measured at 80 or 150 min post cell transfer (ND; non-detected). This result is representative of three separated experiment. (***, $p<0.001$)

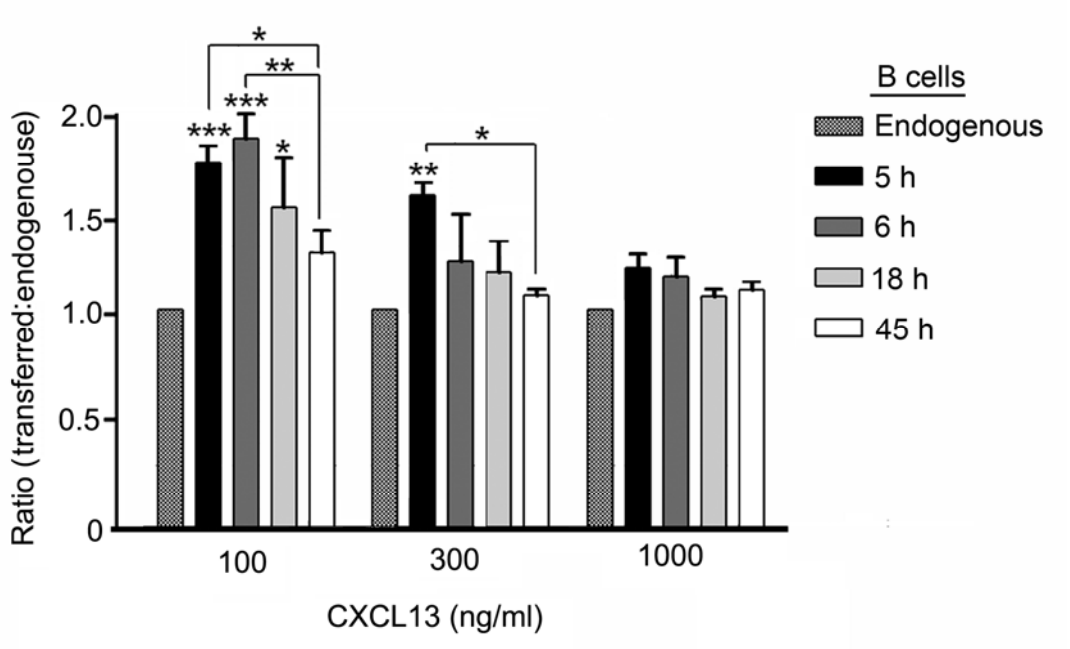

Figure S2. Recently arrived lymph node B cells respond better to low concentrations of CXCL13. CD45.2 B cells were adoptively transferred to CD45.1 mice. At various times after transfer lymph nodes were harvested and the cells immunostained for B220 and CD45.2 prior to chemotaxis assays using increasing concentrations of CXCL13. The amount of specific migration of the transferred cells shown is normalized to the specific migration of the endogenous B cells. Experiment performed three times with similar results. Data analyzed by 2 way ANOVA (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). Significance of the results of the transferred cells compared to the endogenous cells is indicated by asterisk directly over the error bar. The significance of the results comparing different times following transfer is indicated by an asterisk over the bracket.

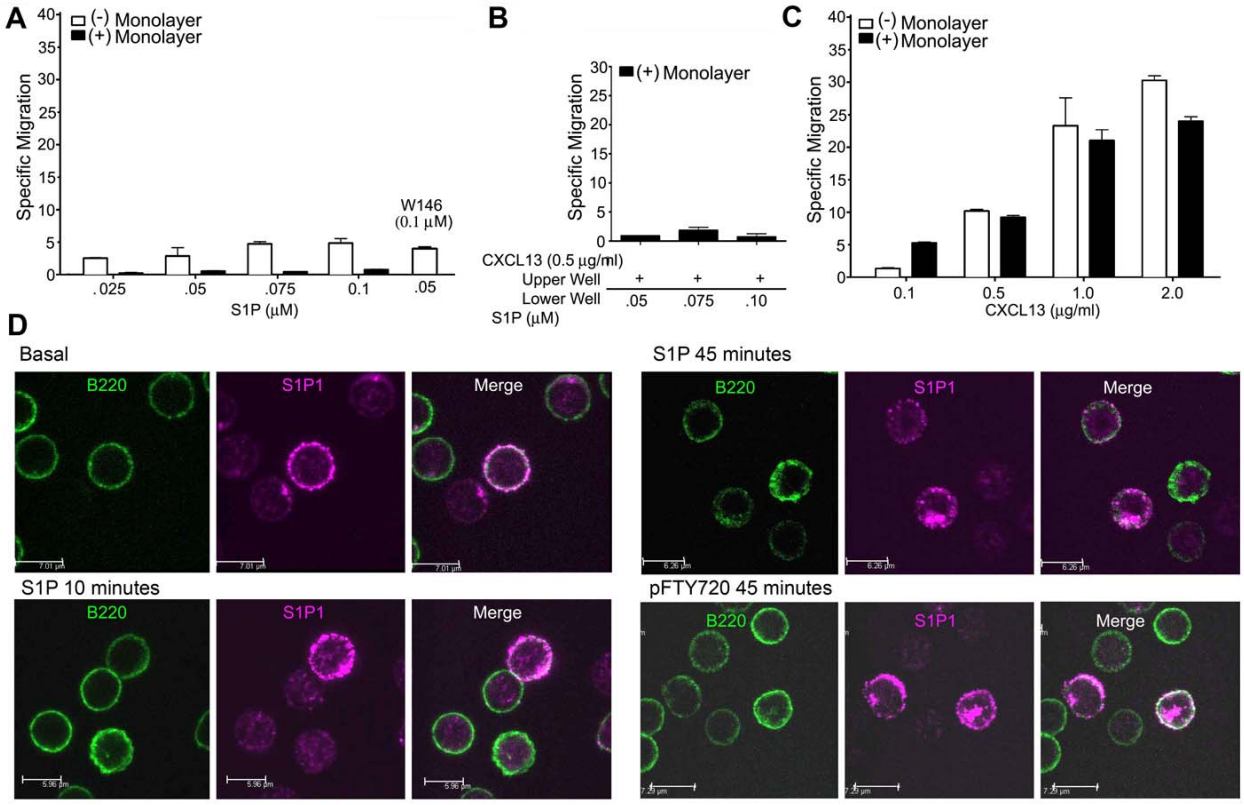

Figure S3. CXCL13, but not S1P, promotes LN B cell transmigration across a lymphatic endothelial cell monolayer. (A) S1P mediated chemotaxis and transmigration. Specific migration of LN B cells across bare inserts or iSVEC4-10 cells. In the result shown to the far right a S1P1 receptor antagonist (W146) was added to bottom well. LN cells were pooled from 4 mice. Experiment performed 3 times with similar results. (B) Adding CXCL13 to the upper well does not alter the S1P response. Specific migration of LN B cells across iSVEC4-10 cells in response to S1P with CXCL13 added to the upper well. (C) CXCL13 mediates B cell transmigration. Specific migration of B cells across bare inserts or iSVEC4-10 cells in response to CXCL13. Data are mean of 4 samples for each point. Experiments repeated twice with similar results. The cells were pre-incubated in media for 2 hours prior to the assay. (D) Treatment of B cells with S1P induces S1P1 receptor internalization. Following immunostaining for B220 splenic B cells were treated for 10 or 45 minutes with either S1P (50 nM) or pFTY720 (10 nM) or maintained in culture without ligand. Individual and merged confocal images are shown. Experiment performed three times with similar results.

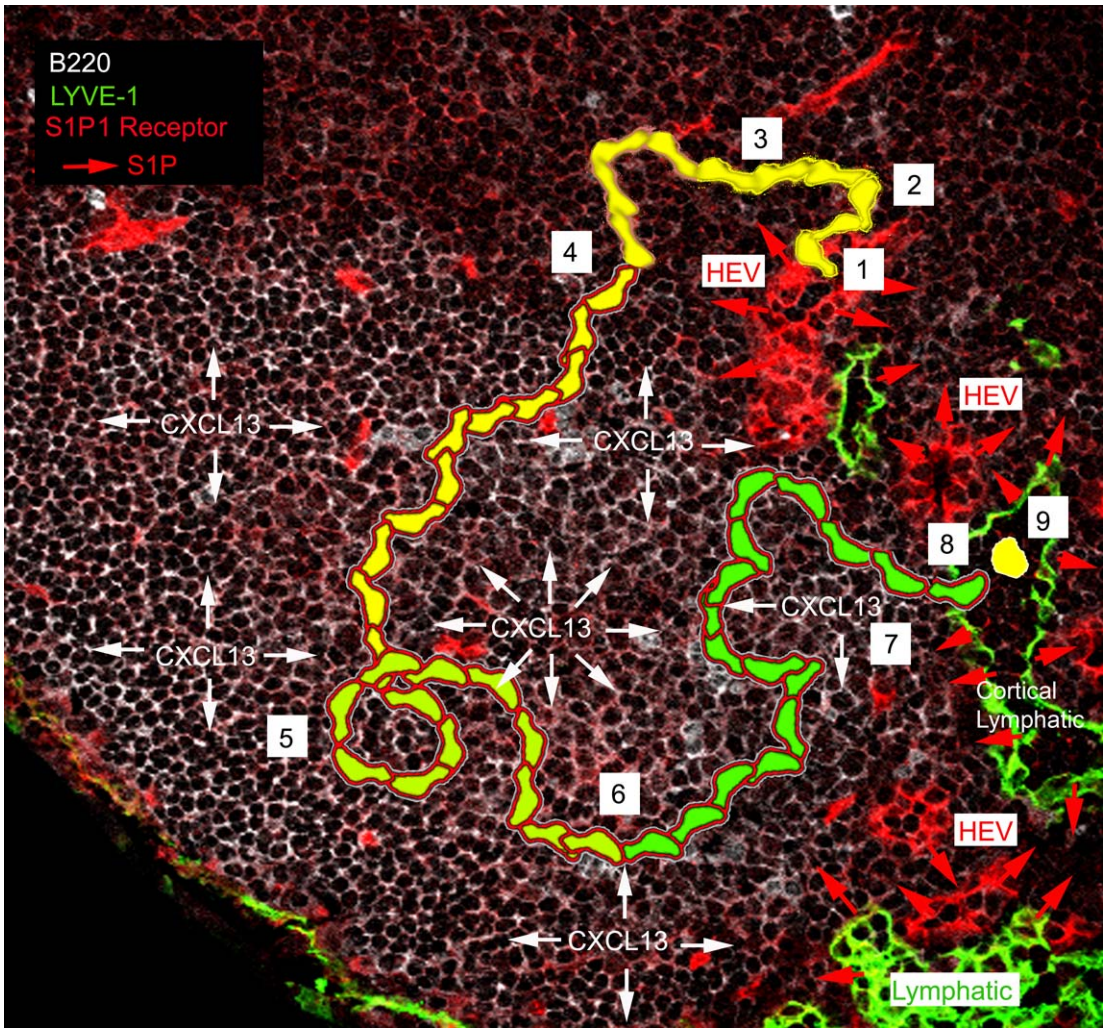

Figure S4. Model of B-cell LN trafficking. The various steps in the trafficking of B cells through a LN are illustrated using an inguinal LN section immunostained for S1P1 receptor (red), LYVE-1 (green), and B220 (white). S1P from the lymphatics and HEVs is shown with red arrows and CXCL13 in the follicle is indicated with white arrows. The path of B cell from the HEV to the lymphatic is shown. Step 1) B cells firmly adhere to the HEV endothelium predominantly by using CCR7 signaling. Once a permissive TEM site is located the cells rapidly cross the endothelium adopting a more polarized morphology. Step 2) the cell is now located in the perivenule space and must cross the basement membrane and overlying pericytes before entering the surrounding paracortical corridor. Step 3) having exited the HEV the B cells remain localized close to the HEVs avoiding the LN follicle perhaps mediated by GPR183 signaling. The cells are refractory to any S1P-mediated exit signal because of desensitized receptors. Step 4) the cells exit the PCC and enter the basal portion of the LN follicle using CXCR5 signaling during which time the cells re-acquire their S1P receptors. Step 5) the B cells tend towards the center of the follicle the richest source of CXCL13 further upregulating their S1P1 receptors. Step 6) the B cells gradually desensitize their chemoattractant receptors likely mediated by receptor uncoupling and induction of RGS proteins. Step 7) the loss of the strong

LN follicle localizing signal allows the B cells to return to the efferent lymphatics at the edge of the follicle or to re-enter the PCCs. Step 8) the cells are now sensitive to an S1P-mediated egress signal and are competent to cross into adjacent efferent lymphatics. Cells that fail to exit may desensitize their S1P receptors, resensitize their CXCR5 receptors, and reenter the follicle repeating the cycle. Step 9) once in the lymphatics the B cells rapidly depolarize adopting a spherical morphology. The cells downregulate their S1P receptors and begin to upregulate their chemokine receptors. Within several hours their CXCR4, CCR7, and CXCR5 receptors are highly sensitive to chemokine.