Supplementary Information

Balanced responsiveness to chemoattractants emanating from adjacent microenvironments determines B cell position

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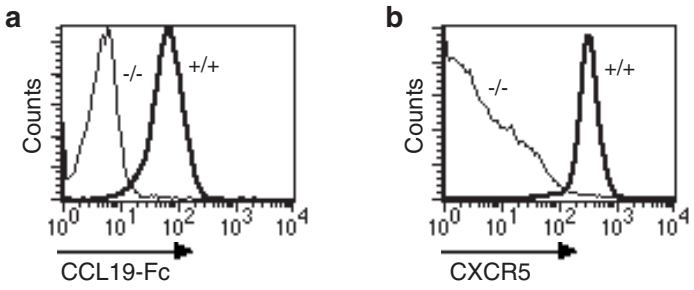

Supplementary Figure 1 Specificity controls for flow cytometric staining of B cells with CCL19-Fc and anti-CXCR5. **a**, CCL19-Fc staining of gated $B220^+IgM^{a+}$ B cells isolated from Ig^{HEL}-transgenic mice (+/+, thick histogram) or CCR7-deficient Ig^{HEL}-transgenic mice (-/-, thin histogram). **b**, CXCR5 staining of gated $B220^+$ B cells isolated from Ig^{HEL}-transgenic mice (+/+, thick histogram) or CXCR5-deficient Ig^{HEL}-transgenic mice (-/-, thin histogram).

METHODS. Cells were incubated with rat anti-CD16/CD32 (BD PharMingen) to block Fc receptors. CCL19-Fc or rabbit anti-CXCR5 was then added, followed by biotin-conjugated goat anti-human Fc-gamma (Jackson ImmunoResearch Laboratories) or goat anti-rabbit Ig (BD PharMingen) preincubated for 30-60 min with 4% normal mouse serum, 2% normal rat serum and 2% normal goat serum (Sigma-Aldrich). Rat anti-B220-PerCP (BD PharMingen), mouse anti-IgM^a-PE (BD PharMingen), and streptavidin-APC (Molecular Probes) were added to the cells stained with chemokine receptor reagents.