Supplementary methods

Isolation of human and mouse GC B cells. Human GC B cells were purified as previously described (Klein *et al*, 2003). Mouse splenic B cells were isolated from wild-type and $S1P_2^{-/-}$ animals ten days after immunization with sheep red blood cells, using the MACS B cell isolation kit (Miltenyi Biotec). For purification of the GC B cell subpopulation, single cell suspensions were stained with fluorochrome-conjugated PNA, anti-B220 and anti-CD95/FAS antibodies (BD-Pharmingen), and the triple positive cells were sorted.

Mutation analysis of the S1P2 gene in normal B cell subpopulations. Genomic DNA was extracted from naive (IgD^+CD27^+) and GC ($CD38^+CD77^+$) B cell populations purified from human tonsils of healthy individuals as described in Ref.40, and used for amplification of *S1P2* (1252 bp), *BCL6* (781 bp) and the rearranged IgV_H genes using the Pfu Turbo polymerase (Stratagene). The IMR91 fibroblast cell line was included as a control for background error rate. After 30 cycles (35 for IgV_H), PCR products were purified, incubated with 200 mM dATP and Taq polymerase (GIBCO BRL) for 15 min at 72ºC, and cloned into the pGEM-T vector (Promega) as recommended by the manufacturer. Seventy-five to 122 clones were analyzed from each S1P2 amplicon.

References

1. Klein U, Tu Y, Stolovitzky GA, et al. Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci USA. 2003;100(5):2639-44.