## SUPPORTING MATERIALS

### Materials and Methods

### Mouse and human switch region accession numbers

Accession numbers used for mouse and human switch regions are: mouse Sε, J00472 and NT_114985; mouse Sγ3, D78343; mouse Sγ2a, J00463-J00468; mouse Sγ2b, J00456-J00460; human Sμ, X54713; human Sα1, L19121; human Sα2, AF030305; human Sε, X56797; human Sγ1, U39737; human Sγ2, U39934; human Sγ3, U39935; human Sγ4, X56796. Note that human and mouse isotypes of the same nomenclature are not necessarily isologous [1]. For the human switch regions, some of the analyzed sequences are larger than the actual repeat regions.

### Analysis of DNA immunoprecipitated by ChIP

Primers used to amplify Sε and $S\gamma_3$ sequences were previously described [2]: Sε: CTGGGCTGAACTGAGATAAACTA and CAACTAAACCAAAAGACCTCTAA, Sγ3: GGGAGCTGGGGTAGGTTCAAGTATG and CTGCCCAGCCTGGTCCTCACAA. The additional S region primer sets are: Sγ2a: AGCTCGAGCTGGGCTGTTAG and GTTCCCTTCGTCTGTGTGTGG, Sγ2b: TGGCAGTTGTCGGAGTCTGG and AGCTCCCTCCTAGCTGTTAGG.

### Plasmid DNA constructions

pQE30LSF (from Q. Zhu) contains the human LSF cDNA-containing Bam HI/Kpn I fragment from pETLSF [3] in pQE30 (Qiagen). pQE30LBP-1a contains the human LBP-1a cDNA (amplified from pCXLBP1-a [4] with GATTCAGATCTATGGCCTGGGTGCTCAAG and ACCCTAAGCTTTCACTTCAAAATTATGTGGATGCC, and digested with Bgl II and Hind III) in pQE30. The parental pCMV was derived by insertion of AGCTTGATATCGAATTCGG-TTACCCGGGATCCTACGC plus TCGAGGCTAGGATCCCGGGTACCGAATTCGATATCA into Hind III/Xho I-igested pcDNA1 (Invitrogen). pCMV-LSFdn contains the LSFdn cDNA-containing Bam HI/Xho I fragment from pETLSFdn [3] in pCMV. MIGW-LSFdn contains the LSFdn cDNA-containing Bam HI/Xho I fragment from pCMV-LSFdn in MIGW.

### References


1. **Pan-Hammarström, Q., Zhao, Y. and Hammarström, L.,** Class switch recombination: a comparison between mouse and human. *Adv.Immunol.* 2007. **93**: 1-61.
2. **Nambu, Y., Sugai, M., Gonda, H., Lee, C.-G., Katakai, T., Agata, Y., Yokota, Y. *et al.*,** Transcription-coupled events associating with immunoglobulin switch region chromatin. *Science* 2003. **302**: 2137-2140.
3. **Shirra, M.K., Zhu, Q., Huang, H.-C., Pallas, D. and Hansen, U.,** One exon of the human LSF gene includes conserved regions involved in novel DNA-binding and dimerization motifs. *Mol Cell Biol* 1994. **14**: 5076-5087.
4. **Yoon, J.-B., Li, G. and Roeder, R.G.,** Characterization of a family of related cellular transcription factors which can modulate human immunodeficiency virus type 1 transcription in vitro. *Mol Cell Biol* 1994. **14**: 1776-1785.

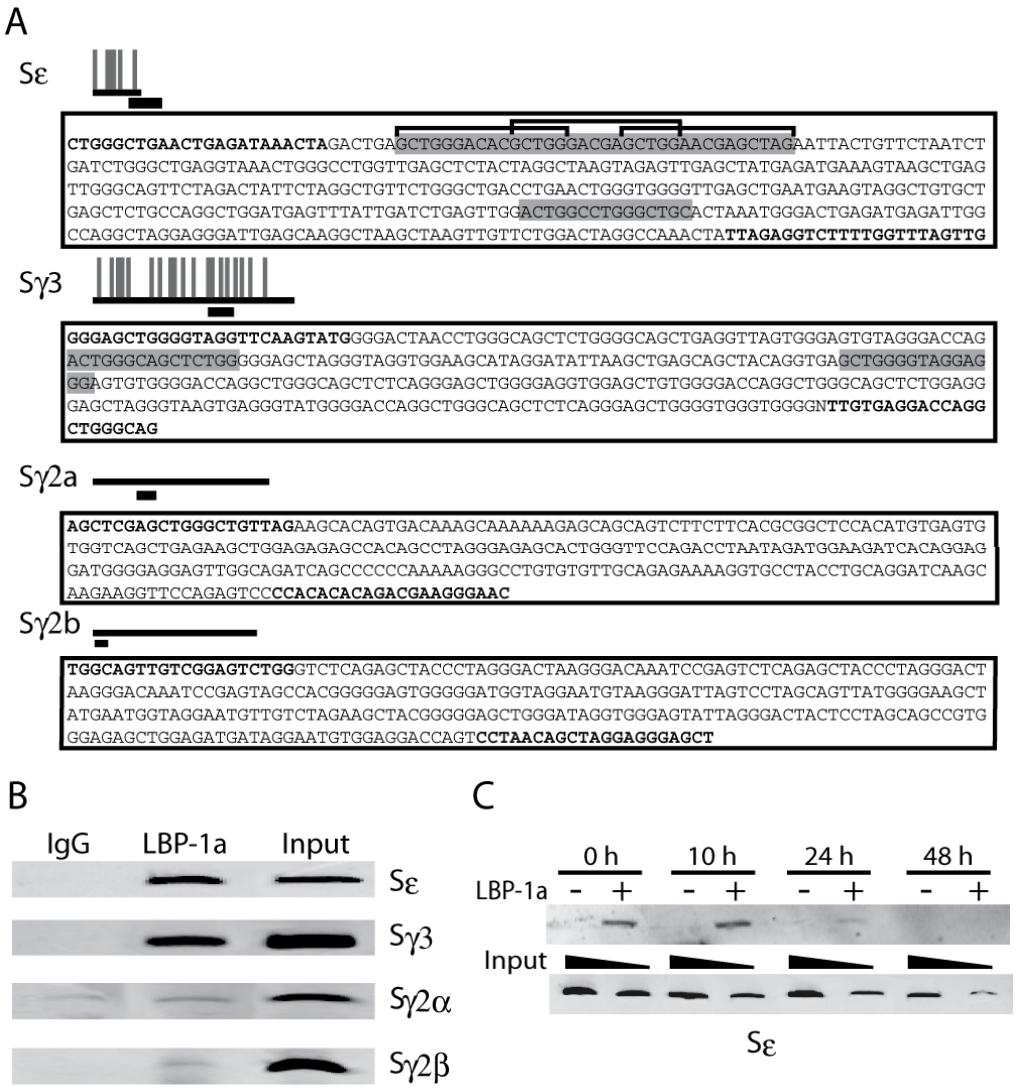


**Figure S1**. LBP-1a binds specifically to Sε and Sγ3 in resting B cells. A) Schematic of mouse immunoglobulin S region sequences with predicted LSF/LBP-1a binding sites. Long horizontal lines, to scale, represent each S region sequence; intersecting vertical lines represent predicted LSF/LBP-1a binding sites. Shorter horizontal lines below each S region represent regions amplified in ChIP assays. Amplicon sequences are in boxes below each S region schematic; PCR primers are in bold. Predicted LSF/LBP-1a binding sites within each amplicon are in shaded boxes. Brackets indicate overlapping sites. B) Isolated primary splenic murine B-lymphocytes were analyzed by ChIP with LBP-1a antiserum. As a control, samples were immunoprecipitated with non-specific rabbit IgG (IgG). Input represents 1.25% of ChIP starting material. DNAs were amplified with the primers in text of supporting material. Data are representative of at least 3 independent experiments. C) Resting B-cells were stimulated with 50 μg/ml LPS for the indicated times; LBP-1a occupancy at Sε was analyzed as in (B). Upper panel: ChIP analysis with nonspecific rabbit IgG (-) or LBP-1a antiserum (+) at the indicated hours post-LPS stimulation. Lower panel: Input samples for each time point representing 0.125% (odd lanes) or 0.0125% (even lanes) of ChIP starting material. Data are representative of 4 independent experiments; error bars represent standard errors of the mean.

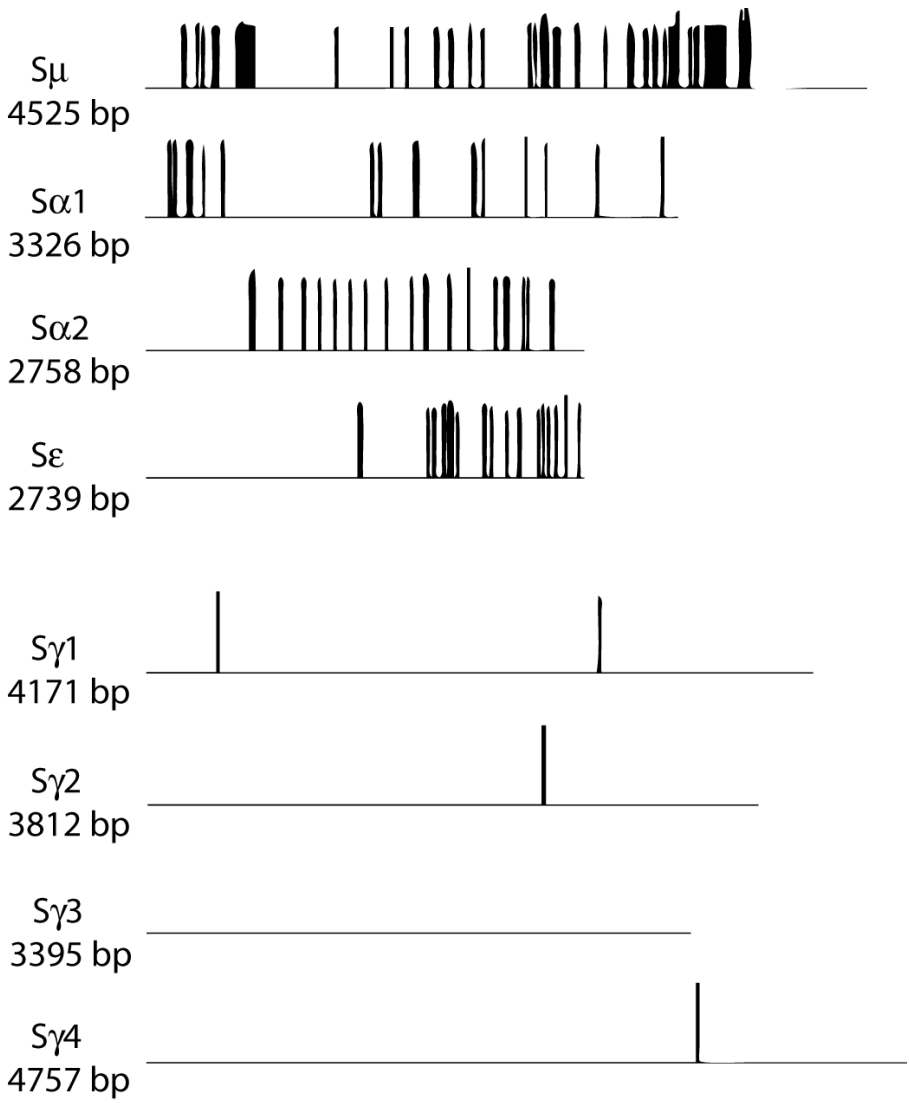


**Figure S2.** LBP-1a is predicted to bind only to specific human switch regions, including Sμ, Sα1, Sα2, and Sε. We note that human and mouse isotypes of the same nomenclature are not necessarily isologous [1]. Shown are predictions of LBP-1a binding sites in the human switch regions. Predicted LBP-1a binding sites are schematically diagrammed, as described in Fig. S1 (A).

**Figure S3.** Primary flow cytometric analyses of the degree of surface IgA (panel A) or IgG1 (panel B) expression on splenic B lymphocytes, isolated from mice following bone marrow transplantation. In this example experiment, B cells were stimulated with LPS alone to undergo CSR in vitro. Procedures were as described in the Materials and Methods of the main text. Mice were transplanted with bone marrow transduced either with the parental retrovirus (control) or with the retrovirus expressing LSFdn (LSFdn), as indicated. After stimulation, cells were analyzed for Ig expression by fluorescence activated flow cytometry, using either an isotype control antibody (A and B, top panels) or antibody recognizing either IgA (A, bottom panels) or IgG1 (B, bottom panels), as described in Materials and Methods. The degree of specific cell surface Ig expression is indicated on the y-axis. The degree of GFP expression is indicated in the x-axis. The data were analyzed in four quandrants: GFP-Ig-, GFP-Ig+, GFP+Ig-, and GFP+Ig+, as indicated. The percentage of total cells in each of these groups are presented by the numbers in each corner (or to the left of the plot, when necessary). Directly above each plot are indicated the percentages of GFP-negative and GFP-positive cells that underwent CSR to the indicated isotype. For further analysis, the percentages of cells expressing IgA or IgG1 in the isotype controls were taken as background, and subtracted from the percentages of cells expressing IgA or IgG1 in the experimental samples. In the occasional instance where the percentages of Ig+ cells in the isotype control samples were high relative to the experimental samples (e.g. IgG1 expression in the LSFdn-expressing B cells, panel B), the results were excluded from further analyses and compilations. Note that lymphocytes transduced with the parental, control retrovirus express higher levels of GFP than do those in which LSFdn is also expressed (lower GFP levels are due to expression solely from the IRES in the latter construct).

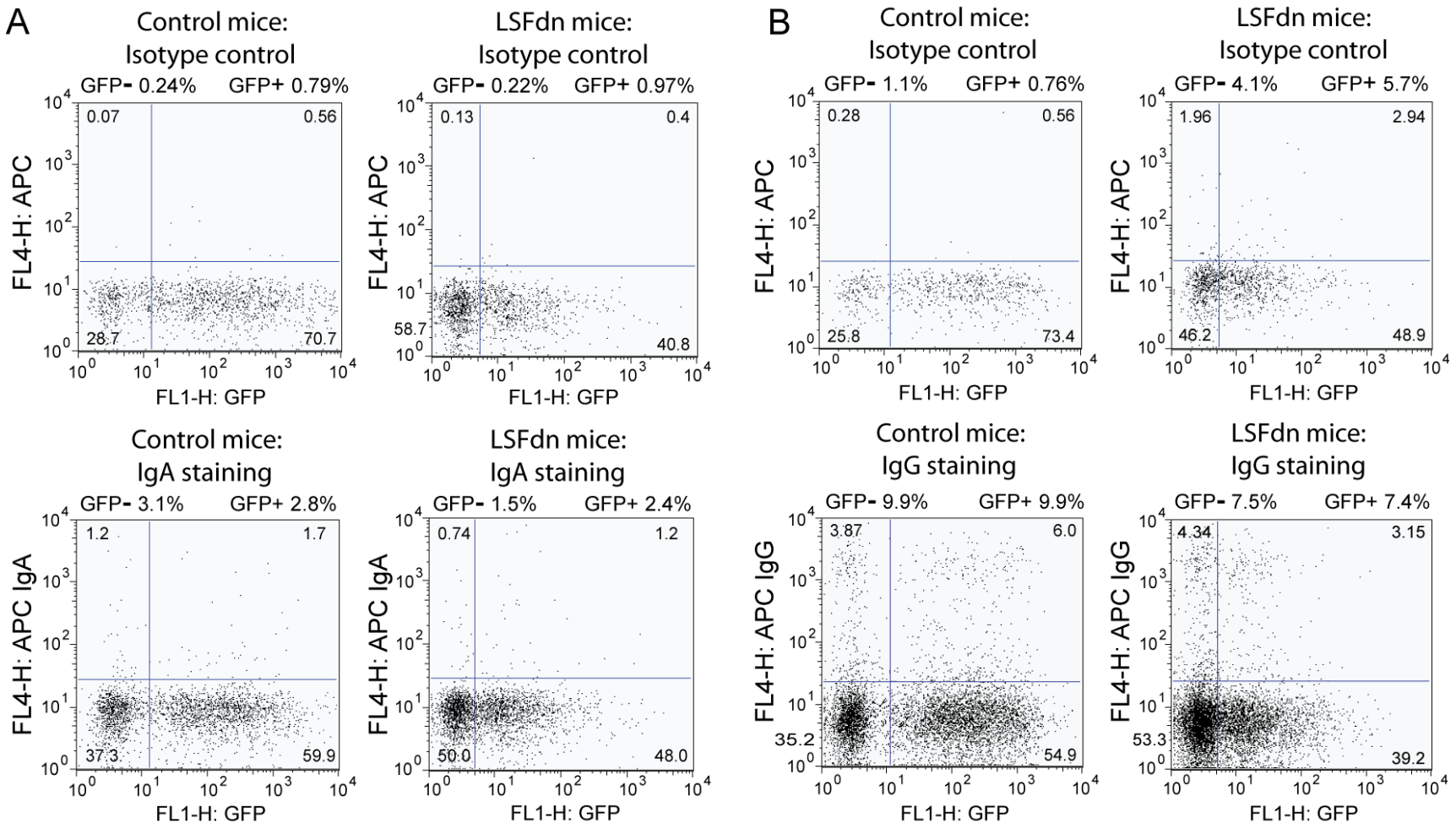
A
Control mice:
Isotype control
GFP- 0.24%
GFP+ 0.79%
0.07
0.56
28.7
70.7
FL4-H: APC
FL1-H: GFP
LSFdn mice:
Isotype control
GFP- 0.22%
GFP+ 0.97%
0.13
0.4
58.7
40.8
FL4-H: APC
FL1-H: GFP
Control mice:
IgA staining
GFP- 3.1%
GFP+ 2.8%
1.2
1.7
37.3
59.9
FL4-H: APC IgA
FL1-H: GFP
LSFdn mice:
IgA staining
GFP- 1.5%
GFP+ 2.4%
0.74
1.2
50.0
48.0
FL4-H: APC IgA
FL1-H: GFP
B
Control mice:
Isotype control
GFP- 1.1%
GFP+ 0.76%
0.28
0.56
25.8
73.4
FL4-H: APC
FL1-H: GFP
LSFdn mice:
Isotype control
GFP- 4.1%
GFP+ 5.7%
1.96
2.94
46.2
48.9
FL4-H: APC
FL1-H: GFP
Control mice:
IgG staining
GFP- 9.9%
GFP+ 9.9%
3.87
6.0
35.2
54.9
FL4-H: APC IgG
FL1-H: GFP
LSFdn mice:
IgG staining
GFP- 7.5%
GFP+ 7.4%
4.34
3.15
53.3
39.2
FL4-H: APC IgG
FL1-H: GFP

**FIGURE S3**