Supplementary Methods

A. Animals, B cell Purification and Isotype Switch Assay

All animal experiments were approved by the Albert Einstein College of Medicine Animal Use Committee. Barrier facility housed 8-12 week old wildtype C57BL/6 or AID-deficient (backcrossed to C57BL/6) mice were used. Some experiments with AID-deficient mice were done with six month old mice and FACS analysis at 96h confirmed that littermate controls switched to the same degree as younger mice (data not shown). *Ex vivo* class switch recombination experiments were carried out as previously described (1). Briefly, mice were sacrificed and spleens were dissected out. Splenocytes were isolated using the balloon method and were subjected to hypotonic lysis and complement-mediated T cell depletion. B cells were routinely 85%-95% pure as checked by FACS analysis for CD19 or B220 expression. $5x10^5$ cells/ml were cultured in RPMI supplemented with 10% fetal bovine serum, 2% L-glutamine, 1% penicillin-streptomycin, 0.5% beta-mercaptoethanol, 0.1% gentamicin and 1% sodium pyruvate. Stimulants added were either LPS (50ug/ml, Sigma, L-2637, *E. coli* 055:B5) or LPS plus IL4 (25 ng/mL, R&D 404-ML) and mCD40L-CD8 at 1:50 dilution. Cultures were fed with warm fresh media without additional stimulants at 48h and 72h, and this increased the number of viable cells at later time points without changing the isotype switching efficiency or specificity.

B. Chromatin Immunoprecipitation

To prepare chromatin from resting and stimulated B cells, approximately 70-100 million cells in culture were directly cross-linked by addition of 11% formaldehyde (freshly diluted from 37% in cross-linking buffer) to a final concentration of 1% at room temperature for 5 min. Detailed composition of the cross-linking buffer and all other buffers are described below. The reaction was quenched with 2.5 M glycine on ice. Cells were washed once in cold PBS, incubated on ice for 20 min in 30 ml of lysis buffer and then pelleted by centrifugation at 300*g* for 10 min. The pellet was resuspended in 30 ml of rinse buffer and shaken at room temperature for 20 min. Chromatin was collected by centrifugation at 1,800*g* for 10 min and resuspended in 4 ml of sonication buffer. Sodium butyrate (10 mM) (Upstate Biotechnology) and Complete protease inhibitors (Roche) were freshly added to each buffer. Chromatin was sonicated (Fisher Dismembrator Model 500) to 200- to 500-bp fragments in a dry-ice-ethanol bath, and then spun at maximum speed to remove insoluble components. A mixture of Protein A and G Sepharose beads (Zymed), previously blocked with acetylated BSA (Sigma) and sheared salmon sperm DNA (Invitrogen), was used to preclear the chromatin.

The concentration of chromatin in samples from different treatments or time points was determined in two ways: measurement of OD at 260nm to get a rough estimate, and then followed by semi-quantitative PCR of reverse cross-linked DNA templates using primers that identify a region upstream of Iμ which does not undergo CSR. Equivalent aliquots of chromatin from different treatments or time points were immunoprecipitated with 3μg of each antibody overnight at 4°C with rotation. The IP reaction was in 0.8 ml of ChIP RIPA buffer supplemented with protease inhibitors and sodium butyrate. Then, 35-50 μl of a 50% protein A and G Sepharose slurry was added

and the IP reaction was continued at 4°C for 4 additional hours. The beads were recovered, washed with three different wash buffers followed by two washes with TE. The beads were transferred to a fresh tube, treated with RNase A (Sigma) at 55°C for 1 hr in TE containing 1% SDS, followed by Proteinase K (Roche) digestion at 55°C for 2hr. Formaldehyde cross-links were reversed by overnight incubation at 65°C and the ChIP DNA was purified using a Qiagen PCR purification kit.

Chromatin Extraction

1. Cross-linking Buffer: 50mM HEPES (pH 8.0), 100 mM NaCl, 1mM EDTA and 0.5mM EGTA.
2. Lysis buffer (modified from Takahashi protocol): 50 mM HEPES-KOH (pH 8.0), 140mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.25% Triton X-100 and 10% glycerol
3. Rinse Buffer: 10 mM Tris (pH 8.0), 200mM NaCl,1 mM EDTA, and 0.5 mM EGTA.
4. Sonication Buffer: 40mM Tris (pH 8.0), 300mM NaCl,1% Triton X-100, and 4mM EDTA

Chromatin IP

1. ChIP RIPA buffer: 20 mM Tris (pH 8.0), 300mM NaCl, 0.01% SDS, 1% Triton X-100, and 2 mM EDTA.
2. Wash Buffer I: 20mM Tris-HCl (pH8.0), 150mM NaCl, 0.1% SDS, 1% Triton X-100 and 2mM EDTA
3. Wash Buffer II: Identical to Wash Buffer I except NaCl is 500mM
4. Wash Buffer III: 10mM Tris-HCl (pH 8.0), 0.25M LiCl, 1% NP40, 1% Sodium deoxycholate and 1mM EDTA

C. ChIP Antibodies

Histone Modification Recognized	**Source(s)**
H3 tri-methyl K9	Abcam ab8898, Upstate #07-442
H3 di-methyl K9	Abcam ab7312, Upstate #07-212
H3 mono-methyl K9	Abcam ab9045
H3 tri-methyl K27	Upstate #07-449
H3 tri-methyl K4	Abcam ab8580
H3 di-methyl K4	Upstate #07-030
H3 mono-methyl K4	Abcam ab8895
H3 tri-methyl K36	Abcam ab9050
H3 tri-methyl K79	Abcam ab2621
H3 acetyl K9	Upstate #06-942
H3 acetyl K14	Upstate #06-911
H3 citrullinated	Abcam ab5103
Mitotic H3	Upstate #06-570
H3.3	Abcam ab4263
H3 phosphorylated T11	Upstate #07-492
H2A Ubiquitylated	Upstate #05-678
H4 di-methyl K20	Upstate #07-367
Total H3	Abcam ab1791

D. Primers

SR ChIP primers were described in (1): Sμ 5'-AACTAGGCTGGCTTAACCGAGATG-3' and 5'-GTCCAGTGTAGGCAGTAGAGTTTA- 3'; Sγ1 5'-GGAGGTCCAGTTGAGTGTC-TTTAG-3' and 5'-TTGTTATCCCCCATCCTGTCACCT-3'; Sγ3 5'-CAGGCTGGGAAAC-TCTTG- 3' and 5'-GTCCCCACATCCTCACTTAT-3'.

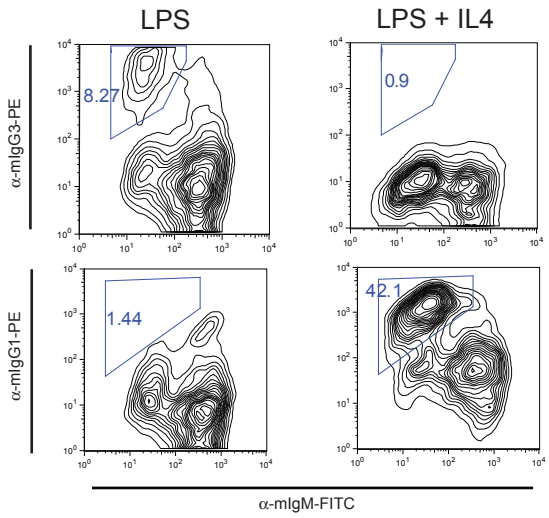
The remaining primers were designed in this study except those indicated with * which were designed by (2). Primers were designed using AJ851868 (annotated 129/Sv mIgH) and confirmed by aligning with AC160982 (C57 mIgH).

Region	5' primer	3' primer
Iμ (-600)	5'-TTGTTGGAAGGAGAGCTGTCTTAGTGAT-3'	5'-AACCCTCTCACATTTTAAAGTCACAGTATT-3'
Iμ-Exon	5'-GGTCTCCATTCAATTCTTTTCCAATACC-3'	5'-ACCAACCAGCATGTTCAACCGAA-3'
Up-Sμ	5'-TAAAATGCGCTAAACTGAGGTGATTACT-3'	5'-CATCTCAGCTCAGAACAGTCCAGTG-3'
Down-Sμ	5'-CTGTGGTGAGGGGAGCTGGATT-3'	5'-CCATGCTAGCTCAGCCTCACATAAT-3'
Cμ-H1H2	5'-TACCTGGTATGCAAAATCCACTACG-3'	5'-CCCGTGGTGGGACGAACAC-3'
Up-Iγ3*	5'-GGCCCCTTGAACCAAACCT-3'	5'-TCAGTCTCACCTGCATGGTCAT-3'
Iγ3-P*	5'-CACTCACTCTTGCCTTCTCCTACAT-3'	5'-GGGGGAAAACTGGTACAGCAGTGTG-3'
Iγ3-Exon*	5'-AGATCCCAAAGCTAAGCTCCTG-3'	5'-CAGAGAGACCCCCTCCACAGT-3'
Sγ3*	5'-GGACCAGGCTGGGAAACTCT-3'	5'-AGTCTGGTCCCTACACTCCCACTAACC-3'
Cγ3-CH3-S*	5'-CCATACCCCCACCTCGTGAACA-3'	5'-GGTTCTTCTGTGTGTGGTGGTTATGG-3'
Cγ3-D*	5'-ATGGTGACCACGAGGGAGAG-3'	5'-CACCTTGGGAAGGCAGTGAGA-3'
Iγ1-P	5'-CACTGGTTCCACAACTGTAAATCATCA-3'	5'-GGCTATATCTCCCTTTACCTGTATCTTGTTG-3'
Iγ1-Exon	5'-CCCACTGTCAATCCTGTTCTTAGTCAA-3'	5'-CTCCCGTGATAGGATGACTCAAAGAT-3'
Up-Sγ1	5'-GGGATAGCAGGGCTAAGAACACG-3'	5'-ACTTTCTCTTTCCCTGCAGGCTTC-3'
Mid-Sγ1	5'-CTAGTATGAAGGTGGAGGTCCAGTTGAG-3'	5'-GACTGCTAAGTCTGGAATTCTGCATTACT-3'
Down-Sγ1	5'-TTCATAGGGAGTAGGTATGGAGGCTAATCTA-3'	5'-AGTCCCTTCCTGTCTTTGCCCAC-3'
Cγ1-CH2	5'-GCACACAGCTCAGACGCAACC-3'	5'-TGGTCAGCACAGAGGTCACGGA-3'
Sγ2b	5'-AGCAGGTGTAGTTAACAGGGGTCC-3'	5'-CACAGTATCTTTACAGATGCTCAGCTTG-3'
Cγ2b-CH3	5'-GGGGCTGGGATGGGCATAAGAATA-3'	5'-GCTGGTGGCGGCAAGAT-3'

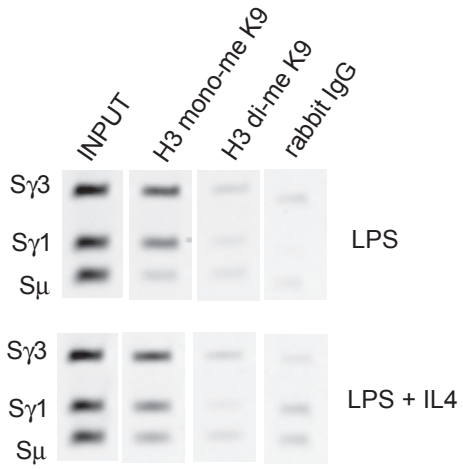
E. PCR

ChIP DNA samples were analyzed by endpoint PCR using AmpliTaq Gold LD polymerase (Applied Biosystems). PCR products were resolved on 7% acrylamide gels, which were subsequently stained with ethidium bromide and visualized using a ChemImager 4000 under various exposures. Densitometry was performed using ImageQuant on non-saturating images with background subtracted using the local average method. Relative Input was determined using background subtracted band intensities in the following manner: (Specific IP – rIgG / INPUT). Quantitative real time PCR was performed using SYBR Green (ABI) on an AB 7900 system at the AECOM Genomics Facility. Data was analyzed according to ABI User Bulletin 2. Absolute copy numbers were determined using standard curves generated with each set of primers and chromatin derived DNAs. These data are plotted as normalized to INPUT and isotype control values are depicted alongside specific IP values.

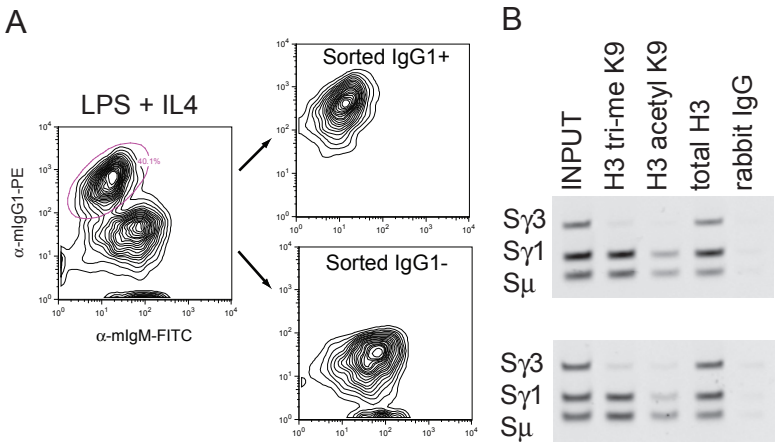
1. Li Z, Luo Z, & Scharff MD (2004) Differential regulation of histone acetylation and generation of mutations in switch regions is associated with Ig class switching. *Proc Natl Acad Sci U S A* 101**:**15428-15433.
2. Wang L, Whang N, Wuerffel R, & Kenter AL (2006) AID-dependent histone acetylation is detected in immunoglobulin S regions. *J Exp Med* 203**:**215-226.



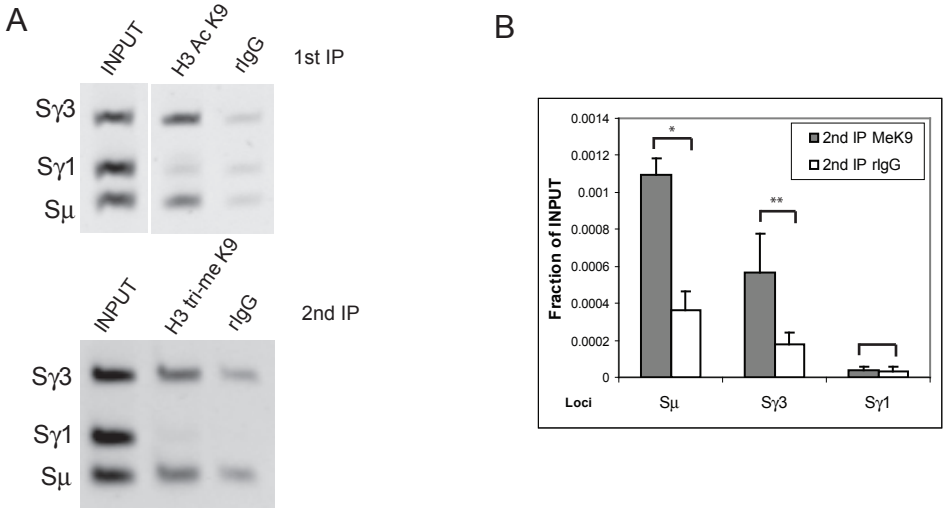
Supplementary Figure 1
Splenic B cells treated with either LPS (left) or LPS plus IL4 (right) for 96 hours, and double-stained with anti-mIgM-FITC and either anti-mIgG3-PE (top) or anti-mIgG1-PE (bottom). FACS plots display cells gated as live based on forward and side scatter.



Supplementary Figure 2
Chromatin IP performed on chromatin derived from splenic B cells treated with LPS (top) or LPS plus IL4 (bottom) for 72 hours, using rabbit antibodies against indicated modified histones or control rabbit IgG. PCR was performed using primers that identify Sμ, Sγ1 and Sγ3. These gels are representative of two independent experiments.



Supplementary Figure 3 Isotype Switched Cells contain Both Histone Modifications Splenic B cells treated with LPS plus IL4 for 96 hours to isotype switch to IgG1 were double stained for surface IgM and surface IgG1 and then FACS sorted into IgG1+ and IgG1- cell populations (A). Chromatin was prepared from each population and subject to ChIP as described earlier (B). These data are representative of three independent experiments.



Supplementary Figure 4 H3 acetyl K9 and H3 tri-methyl K9 co-associate on SR DNAs
Sequential ChIP was performed on chromatin from B cells treated for 72 hours with LPS using anti-H3 acetyl K9 antibodies, followed by anti-H3 tri-methyl K9 antibodies or rabbit IgG. (A) PCR was performed on first and second IP reactions using primers that identify SRs. (B) qPCR was performed in triplicate on second IP reactions using primers that identify SRs. These data are representative of three independent experiments. Wilcoxon rank sum statistical analysis was performed on all qPCR data.
* indicates p-value < 0.01, ** indicates p-value < 0.05.