SUPPLEMENTAL MATERIAL

Uchimura et al., http://www.jem.org/cgi/content/full/jem.20110856/DC1

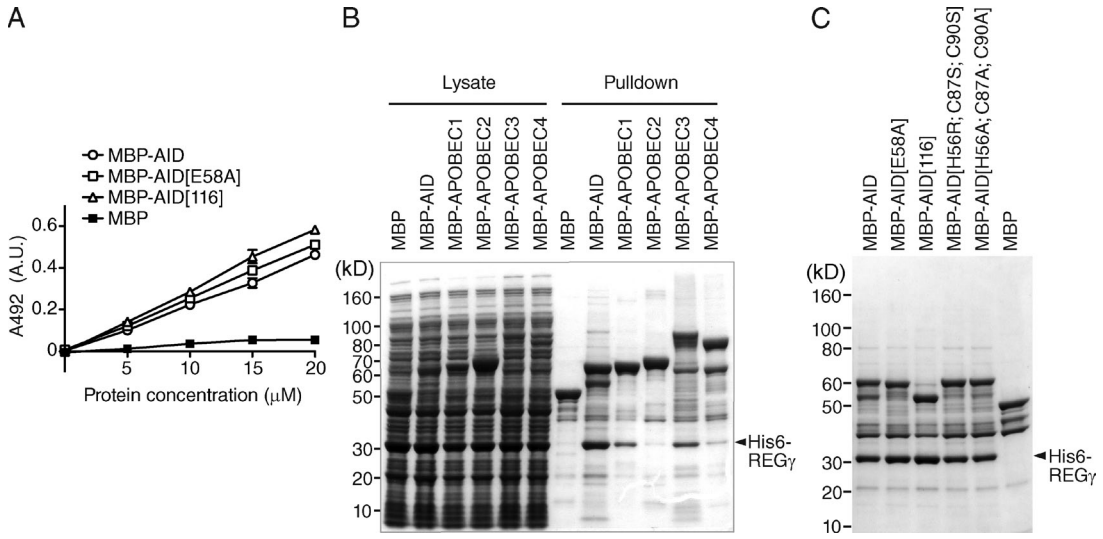

Figure S1. Zn and REG-γ binding by MBP-AID and MBP-APOBEC proteins. (A) The Zn binding by recombinant MBP-AID, MBP-AID[E58A], MBP-AID[116], and MBP proteins that had been purified on amylose resin from the extracts of BL21(DE3) *E. coli* transformants was measured by incubating different quantities of the purified proteins (as specified on the x-axis) in 20 mM Hepes, pH 7.9, 200 mM NaCl, and 1 mM DTT with 50 µg/ml proteinase K (56°C, 30 min) and then adding 4-(2-pyridylazo) resorcinol (Sigma-Aldrich) to 1 mM and measuring the absorbance at 492 nm. Error bars indicate ± SEM. (B) Lysates of *E. coli* cells expressing of His6–REG-γ together with MBP, MBP-AID, or various MBP-APOBEC fusion proteins were analyzed by SDS-PAGE before (left six lanes) and after pulldown on amylose resin and elution with maltose (right six lanes). (C) MBP-AID mutants carrying amino acid substitutions in the catalytic site still bind REG-γ in bacterial coexpression assays. Lysates of *E. coli* cells coexpressing His6–REG-γ together with MBP, MBP-AID, and MBP-AID variants carrying the indicated substitution mutations were analyzed by SDS-PAGE after purification of MBPs by binding onto amylose resin and elution with maltose.

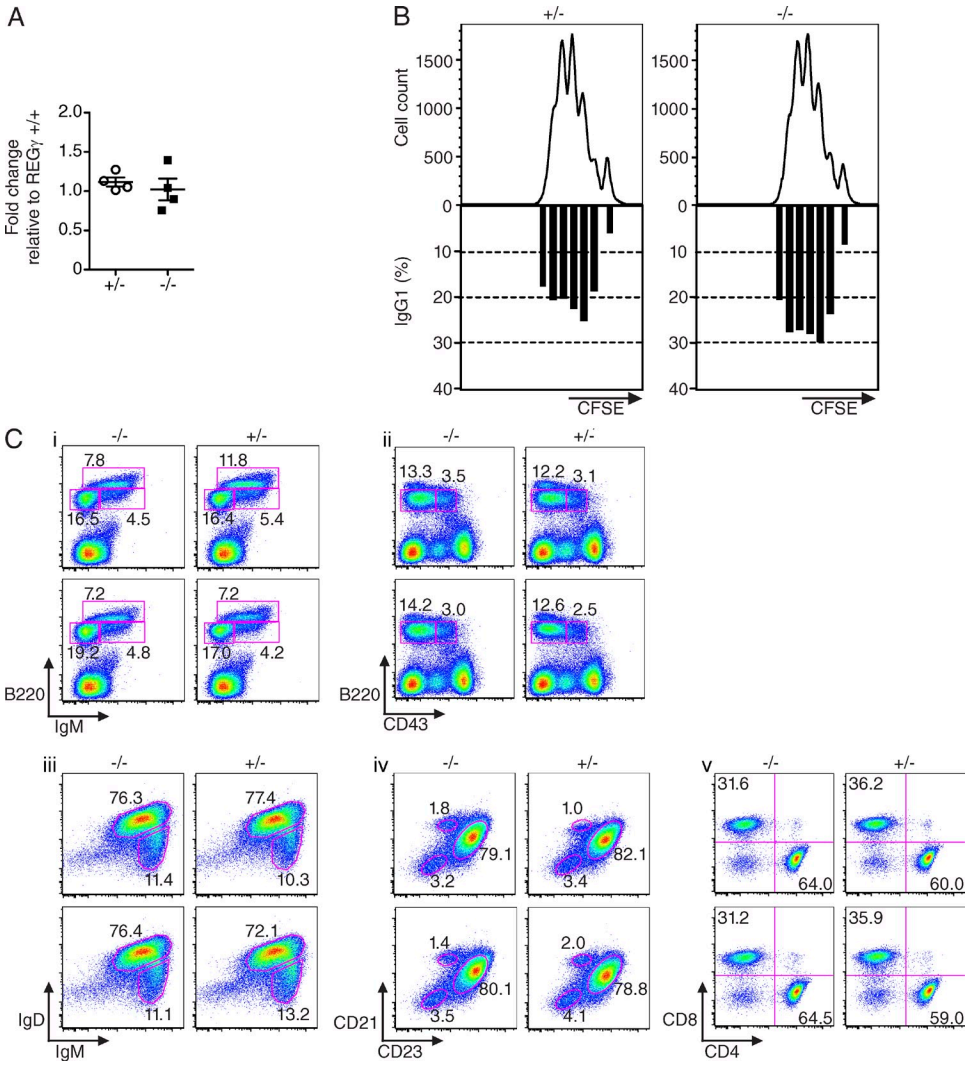

Figure S2. Class switching and lymphoid subpopulations in REG-γ–deficient mice. (A) Real-time RT-PCR of γ1-sterile transcripts in (IL-4+LPS)-activated splenic B cells from REG-$\gamma^{+/-}$ and REG-$\gamma^{-/-}$ mice were compared with REG-$\gamma^{+/+}$ mice at day 3 of culture. Each symbol corresponds to B cells isolated from an individual mouse with bars indicating the mean ± SEM. For RT-PCR, RNA was extracted with TRIzol (Invitrogen) and converted to cDNA with the high capacity cDNA reverse transcription kit (Applied Biosystems). Synthesized cDNA was mixed with SYBR GreenER qPCR SuperMix Universal (Invitrogen) and the primers for γ1-sterile (5′-TCGAGAAGCCTGAGGAATGTG-3′ and 5′-ATGGAGTTAGTTTGGGCAGCA-3′) and GAPDH (5′-TGAAGCAG-GCATCTGAGGG-3′ and 5′-CGAAGGTGGAAGAGTGGGAG-3′; Reina-San-Martin et al., 2003). Reaction mixtures are amplified with 7900HT Fast real-time PCR system (Applied Biosystems) and each sample was normalized to GAPDH expression. Error bars indicate ± SEM. (B) Representative examples of cell division analysis of (IL-4+LPS)-stimulated switching to IgG1 of the experiment summarized in Fig. 4 B. The graph shows the number of cells (top) and percentage of IgG1-positive cells (bottom) on the y-axis plotted against the number of cell divisions undergone (x-axis) in a representative pair of REG-$\gamma^{+/-}$ and REG-$\gamma^{-/-}$ littermates. (C) Flow cytometric analysis of lymphoid subpopulations in REG-$\gamma^{+/-}$ and REG-$\gamma^{-/-}$ siblings. Representative flow cytometric analyses (of two independent sets of comparisons) of cells from bone marrow (i and ii) and spleens (iii, iv, and v) from 8-wk-old REG-$\gamma^{+/-}$ and REG-$\gamma^{-/-}$ mice. The bone marrow cells analyzed were gated on being $CD43^-$ (i) or IgM^- (ii). The spleen cells analyzed were gated on being $B220^+$ (iii), on being both $B220^+$ and $CD19^+$ (iv), and on being $CD3^+$ $B220^-$ (v). All antibodies used for flow cytometry were from BD except APC-conjugated anti-B220 (Invitrogen) and APC-conjugated anti-IgM and PECY7-conjugated anti-CD3 (eBioscience).

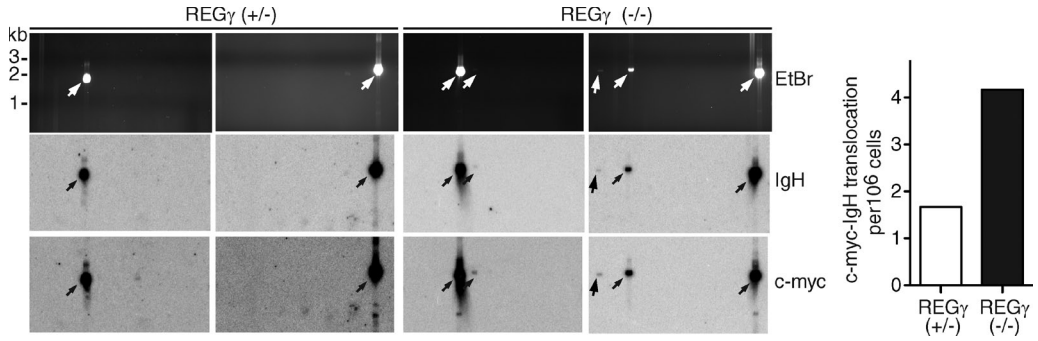

Figure S3. c-*myc*-IgH translocations in B cells from REG-γ$^{+/-}$ and REG-γ$^{-/-}$ mice. Chromosomal translocations between c-myc and IgH in in vitro cultured REG-γ–proficient and REG-γ–deficient splenic B cells. Splenic B cells were transduced with pMX-AID-IRES-GFP for 3 d and GFP^{+} cells were sorted by flow cytometry. Specifically PCR-amplified fragments were analyzed by Southern blotting as described by Ramiro et al. (2006). The experiment shown here is based on analyses of 48 pools of 25,000 cells for each set but with a similar low frequency of translocations (around two to four translocations per 10^6 cells) observed in two independent experiments.

REFERENCES

Ramiro, A.R., M. Jankovic, E. Callen, S. Difilippantonio, H.T. Chen, K.M. McBride, T.R. Eisenreich, J. Chen, R.A. Dickins, S.W. Lowe, et al. 2006. Role of genomic instability and p53 in AID-induced c-myc-Igh translocations. *Nature*. 440:105–109. http://dx.doi.org/10.1038/nature04495

Reina-San-Martin, B., S. Difilippantonio, L. Hanitsch, R.F. Masilamani, A. Nussenzweig, and M.C. Nussenzweig. 2003. H2AX is required for recombination between immunoglobulin switch regions but not for intra-switch region recombination or somatic hypermutation. *J. Exp. Med.* 197:1767–1778. http://dx.doi.org/10.1084/jem.20030569