Supporting Information

Cheng *et al.* 10.1073/pnas.0812304106

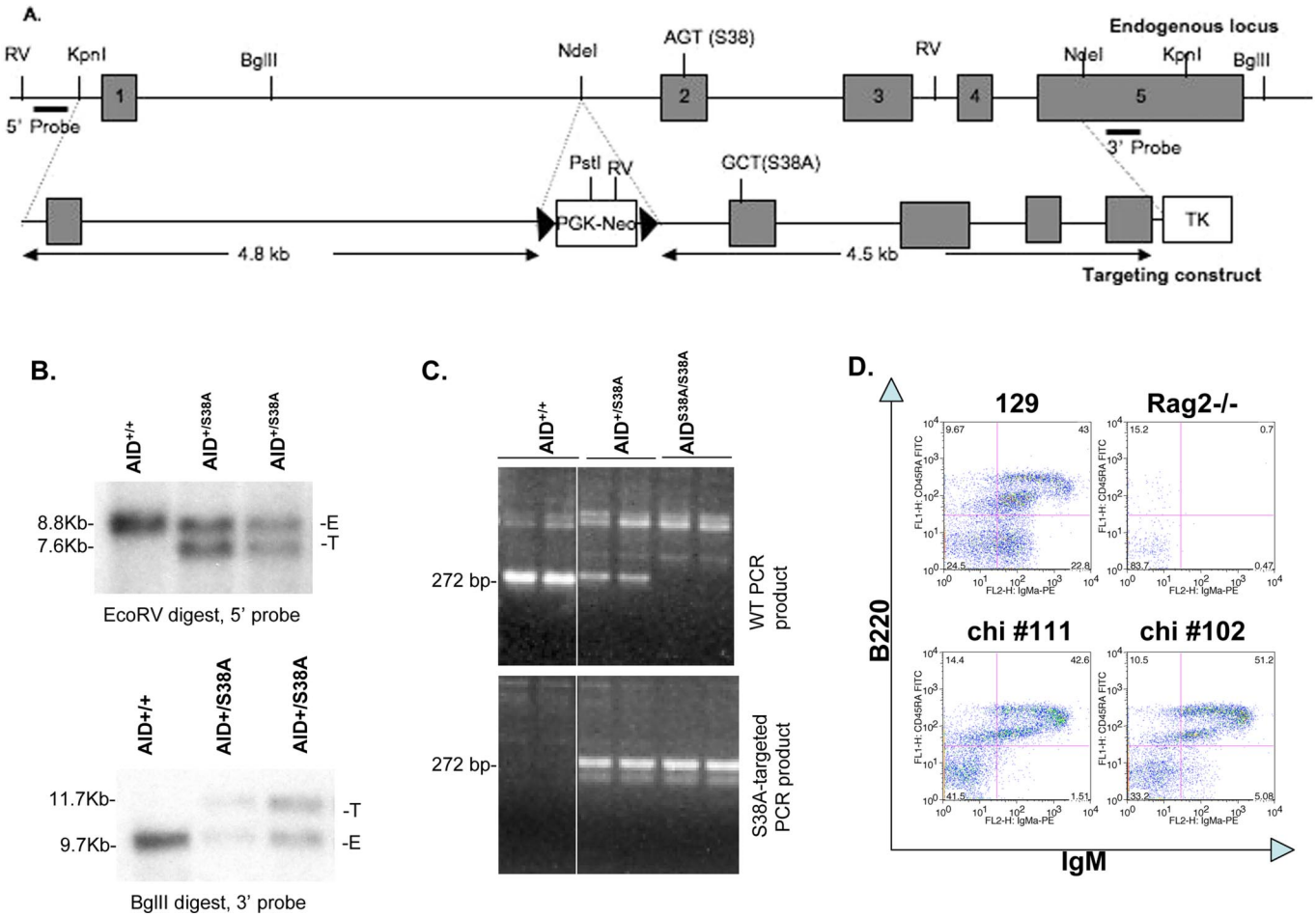

Fig. S1. Generation of AID^{S38A} mutant ES cells. (*A*) Targeting construct. The 4.8-kb and 4.5-kb 5′ and 3′ homology arms of the targeting construct are shown. The AGT in exon 2 of the endogenous locus encodes S38, and the GCT in the 3′ homology arm encodes alanine. The exons are represented as gray rectangles, and the *loxP* sites are represented as triangles. (*B*) Southern blot of 2 correctly targeted ES cell clones. Genomic DNA from ES cells was digested with EcoRV or BglII and probed with either a 5′ (*Upper*) or 3′ (*Lower*) probe, respectively. E, endogenous band; T, targeted band. (*C*) PCR-based detection of AID^{S38A} mutation in targeted clones. Genomic DNA samples from correctly targeted clones were subject to PCR analysis using primers that are specific to the AGT WT (*Upper*) or GCT mutant (*Lower*) sequence. (*D*) Normal development of B cells. Splenic B cells were stained with anti-IgM and anti-B220 antibodies and analyzed by FACS.

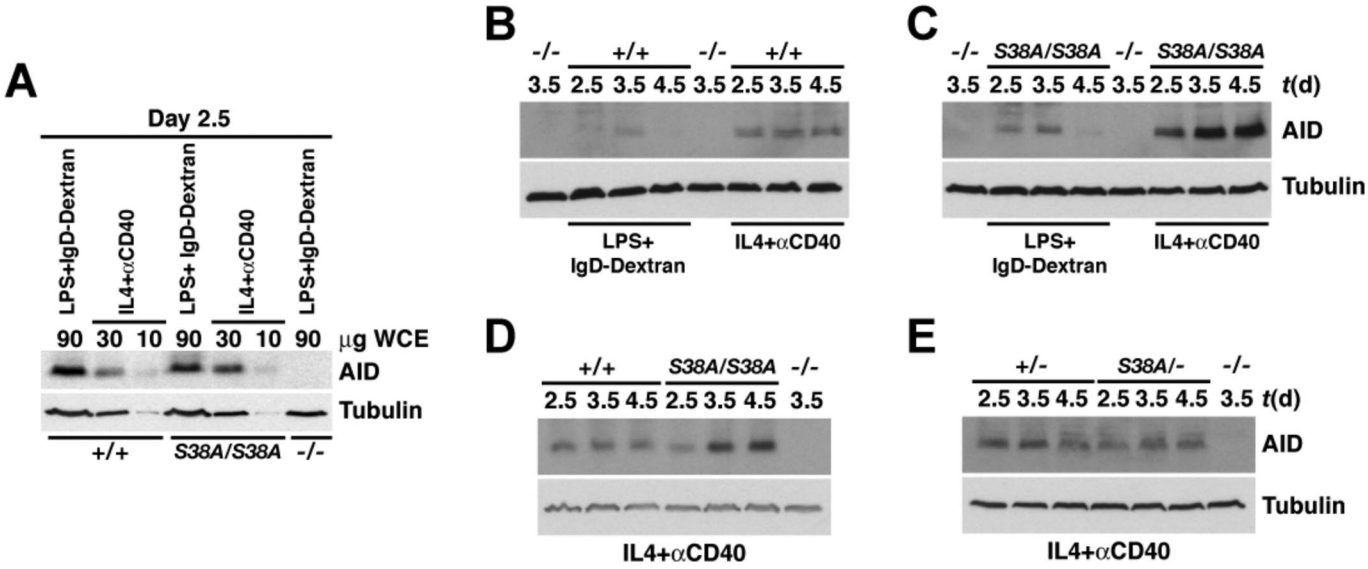

Fig. S2. Expression of AID^{WT} and AID^{S38A} proteins. (*A*) Comparison of AID^{WT} or AID^{S38A} level in response to LPS plus anti-δ-dextran or anti-CD40 plus IL-4 stimulation at day 2.5. The indicated amounts of whole cell extracts (WCE) were analyzed by Western blotting using polyclonal anti-AID antibodies or antitubulin antibodies as loading control. (*B* and *C*) Time course of AID expression under different stimulation conditions. Splenic B cells were stimulated with LPS plus anti-δ-dextran or anti-CD40 plus IL-4 stimulation; whole cell extracts were prepared at the time points indicated, and 60 μg was analyzed on Western blotting using anti-AID and antitubulin antibodies. Extracts from LPS plus anti-δ-dextran or anti-CD40 plus IL-4–stimulated B cells were analyzed on the same gel to indicate the relative levels of AID expressed under these conditions. (*D* and *E*) AID^{WT} or AID^{S38A} protein levels in response to anti-CD40 plus IL-4 stimulation over time. Sixty micrograms of whole cell extract from splenic B cells of the indicated genotypes was analyzed by Western blotting.

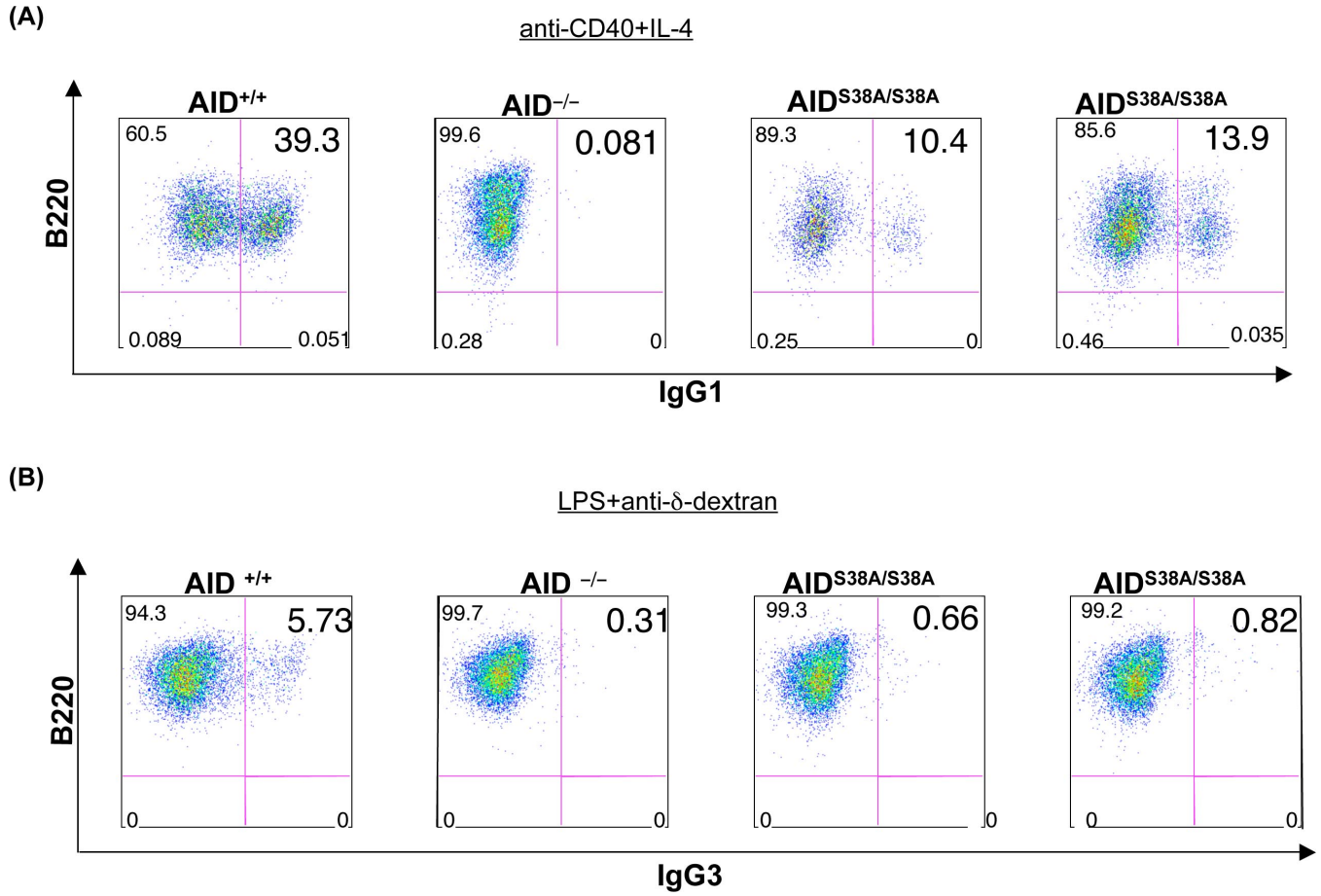

Fig. S3. CSR assays in AID$^{S38A/S38A}$ RAG chimeras. Splenic B cells of the indicated genotype were stimulated in culture for 3.5 days with anti-CD40 plus IL-4 (*A*) or LPS plus anti-δ-dextran (*B*) and assayed for, respectively, CSR to IgG1 or IgG3 by flow cytometry.

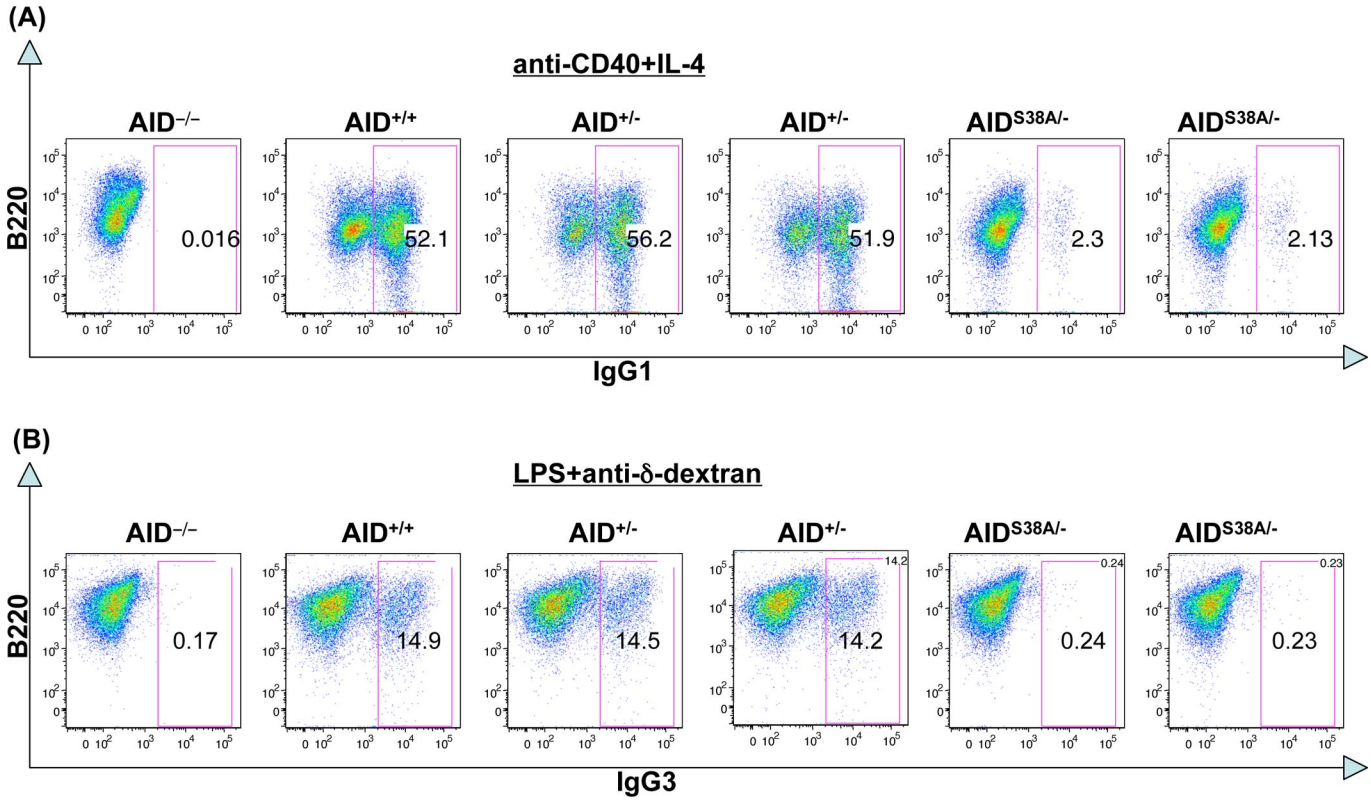

Fig. S4. CSR assays for haploinsufficient germline mice. Splenic B cells of the indicated genotype were stimulated in culture for 4.5 days with anti-CD40 plus IL-4 (*A*) or LPS plus anti-δ-dextran (*B*) and assayed for, respectively, CSR to IgG1 or IgG3 by flow cytometry.

Table S1. Splenic B cells from mice of various genotypes were stimulated in culture for indicated time points and stimulation conditions, and CSR to IgG1 or IgG3 was measured by FACS

Assay	AID$^{-/-}$	wt/wt	wt/−	S38A/−	S38A/S38A	Duration of stimulation
LPS + IgD-dextran						
CSR 1	0.18	11	ND	ND	0.43	Day 3.5
					0.38	
CSR 2	0.31	5.73	ND	ND	0.66	Day 3.5
					0.82	
	0.78	10.5	ND	ND	1.34	Day 4.5
					1.47	
CSR 3	0.3	8.98	7.7	0.24	ND	Day 3.5
			6.99	0.21	ND	
	0.17	14.9	14.5	0.24	ND	Day 4.5
			14.2	0.23	ND	
CSR 4	0.18	5.4	4.06	0.18	0.22	Day 3
	0.11	17	7.68	0.13	0.46	Day 3.5
	0.29	18.7	11.9	0.3	0.66	Day 4.5
CSR 5	0.2	6.88	2.61	0.24	0.27	Day 3
	0.17	14.8	8.7	0.16	0.49	Day 3.5
	0.48	27.5	13.3	0.68	1.09	Day 4.5
CSR 8	0.29	4.47	2.4	0.3	0.41	Day 2.5
	0.31	10.7	7.32	0.36	0.26	Day 3
	0.2	18.2	13	0.23	0.53	Day 3.5
	0.46	29.7	21	0.52	1.31	Day 4.5
CSR 9 (germline)	0.11	16.9	ND	ND	0.83	Day 3.5
	0.24	26.5	ND	ND	1.41	Day 4.5
IL-4 + αCD40						
CSR 1	0.011	59.5	ND	ND	15.2	Day 3.5
					15.3	
	0.07	54.7	ND	ND	26.1	Day 4.5
					24.9	
CSR 2	0.081	39.3	ND	ND	10.4	Day 3.5
					13.9	
	0.05	41.8	ND	ND	20.2	Day 4.5
					23.6	
CSR 3	0.056	52.2	56.6	1.21	ND	Day 3.5
			54.8	0.97	ND	
	0.016	52.1	56.2	2.3	ND	Day 4.5
			51.9	2.13	ND	
CSR 4	0.076	30.2	35.2	0.55	3.29	Day 3
	0	52.5	51.5	1.68	9.78	Day 4.5
CSR 5	0.049	45.8	33.6	0.46	2.26	Day 3
	0.031	57.4	55.7	1.55	7.96	Day 3.5
	0.075	51.2	58.5	2.46	16.5	Day 4.5
CSR 6	0.51	60.4	43.5	1.21	5.82	Day 3.5
CSR 7	0.13	40.1	36.6	0.39	2.01	Day 3
	0.064	59.4	60	1.16	7.55	Day 3.5
CSR 8	0.1	31.3	21.2	0.17	1.1	Day 2.5
	0.085	47.4	40.4	0.47	1.95	Day 3
	0.03	66.5	57.2	0.91	5.83	Day 3.5
	0.057	50	53.2	1.36	10.7	Day 4.5
CSR 9 (germline)	0.13	62.3	ND	ND	11.5	Day 3.5

The values represent percentage of cells that had switch to either IgG1 or IgG3. ND, not determined.