SUPPLEMENTAL MATERIAL

Pauklin et al., http://www.jem.org/cgi/content/full/jem.20080521/DC1

Cells and tissues.
For splenic B cells, red blood cells were removed according to manufacture's protocol (Lympholyte M; Cedarlane Laboratories Limited) and mouse splenic B cells were isolated with Mouse B cell Negative Isolation kit (Dynal Biotech) according to the manufacturer's instructions. For cell lines (except DT40), media was supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen), and cells were grown at 37°C with 5% CO_2.

qRT-PCR.
Total RNA was extracted from cells and mouse tissues using the RNeasy Mini kit (QIAGEN) according to the manufacturer's instructions. Genomic DNA was removed from RNA samples by using Turbo DNA-free kit (Ambion) before cDNA synthesis. cDNA was synthesized with Random hexamer (Promega) and/or oligo dT primer (Invitrogen) and Superscript III reverse transcription (Invitrogen), followed by qRT-PCR analysis for analyzing the mRNA expression and the presence of circle transcripts. Gene expression was normalized to the expression of GAPDH.

Promoter analysis.
Bioinformatic promoter analysis was performed with the AliBaba2.1 transcription factor response element-predicting program using the Transfac 4.0 transcription factor binding site database as a source for constructing matrices.

EMSA.
Complementary oligonucleotides (Table S3) were end labeled with [γ-32P]ATP by T4 polynucleotide kinase to a specific activity of ~300,000–500,000 cpm/ng. Nuclear extracts from Ramos HS13 cells were isolated with Nuclear Extraction kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Binding reactions were performed in 20 mM Hepes, pH 7.8, 50 mM KCl, 10% glycerol, 0.25 mM DTT (Invitrogen), 0.1 mM EDTA, 0.55 μg of poly-(dI-dC) (GE Healthcare) and 0.25 pmol of labeled oligonucleotides for 20 min at room temperature in a total volume of 12 μl. Competition assays included 1-, 3- and 10-fold mass excess of unlabeled oligonucleotides. Antibodies to NF-κB p65 (polyclonal; Santa Cruz biotechnology, Inc.), ERα (monoclonal; Abcam), or mouse λ (polyclonal; SouthernBiotech) were added 10 min after starting the reaction (0.2–1.8 μg/assay) and incubated for an additional 20 min at room temperature. Electrophoresis was performed at 4°C on a prerun, nondenaturing 4.5% poly-acrylamide gels (30:1) in low ionic strength TBE buffer (10 mM Tris-borate, pH 7.5; 0.025 mM EDTA) at 20 mA/gel for 120 min

ChIP.
SDS lysis buffer: 1% SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1; ChIP buffer: 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl; low-salt wash buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, and 150 mM NaCl; high-salt wash buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, and 500 mM NaCl; LiCl wash buffer: 0.25 M LiCl, 1% IGEPAL CA630, 1% deoxycholic acid, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.1; TE buffer: 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA; elution buffer: 1% SDS and 0.1 M NaHCO3, pH 7.5.

DNA isolation (proteinase K): Ten mM EDTA, pH 8.0, 40 mM Tris-HCl, pH 6.5, and 20 μg of proteinase K was added to the sample and incubated for 1 h at 45°C. DNA was then extracted with phenol/chloroform and precipitated with 130 mM NaOAc, pH 5.5, 30 μg glycogen (Roche), and 50% ethanol for 2 h at −20°C. The DNA pellet was washed with 70% ethanol and resuspended in 50 μl of water. CHIP1, 5′-GCGTGAGCTCTCTCTTGCCT-3′; CHIP2 5′-CACTGCTAATAAAGACATGCCTGAG-3′.

Endogenous tagging of AID.
The 3xFLAG-2xTEV-3xc-Myc tag was inserted into a pBluescript plasmid containing part of chicken AID gene (GenBank accession no. XP_416483) and a puromycin selection cassette between two loxP sites (Fig. S4, Maps + Sequence on request) creating a targeting construct for the AID locus (plasmid was sequence verified before transfection; Table S5). Linearized plasmid was transfected into DT40 cells by electroporation, clones were selected on puromycin, and target integration confirmed by Southern blotting (Buerstedde, J.-M., and S. Takeda. 2006. Reviews and Protocols in DT40 Research. Springer, New York. 1-9 pp.). Single clones from a transient transfection of Cre recombinase were then analyzed by Southern blotting (Fig. S4) to identify removal of the puromycin cassette. Expression of full-length AID-FM fusion protein was confirmed by Western blot from cell lysates. β-Tubulin was detected with an antibody from Abcam.

Mutation analysis.
Human CD95/Fas locus was PCR amplified and sequenced using nested PCR with outer forward primer SP3039 5′-ACCACCGGGGCTTTTCGTGA-3′ and outer reverse primer SP3041 5′-TATCTGTTCTGAAGGCTGCAG-3′, followed by amplification with inner forward primer SP3040 5′-TGAGCTCGTCTCTGATCTCG-3′ and inner reverse primer SP3042 5′-CGGAGCGGACCTTTGGCT-3′.

c-myc/IgH translocation.
MycIg1A, 5′-TGAGGACCAGAGAGGGATAAAAGAGAA-3′; MycIg1B, 5′ -GGGGAGGGGGTGTCAAATAATAAGA-3′; MycIg2A, 5′ -CACCCTGCTATTTCCTTGTTGCTAC-3′; MycIg2B, 5 -GACACCTCCCTTCTACACTCTAAACCG-3′. IgH probe, 5′-CCTGGTATACAGGACGAAACTGCAGCAG-3′; c-myc probe, 5′-GCAGCGATTCAGCACTGGGTGCAGG-3′.

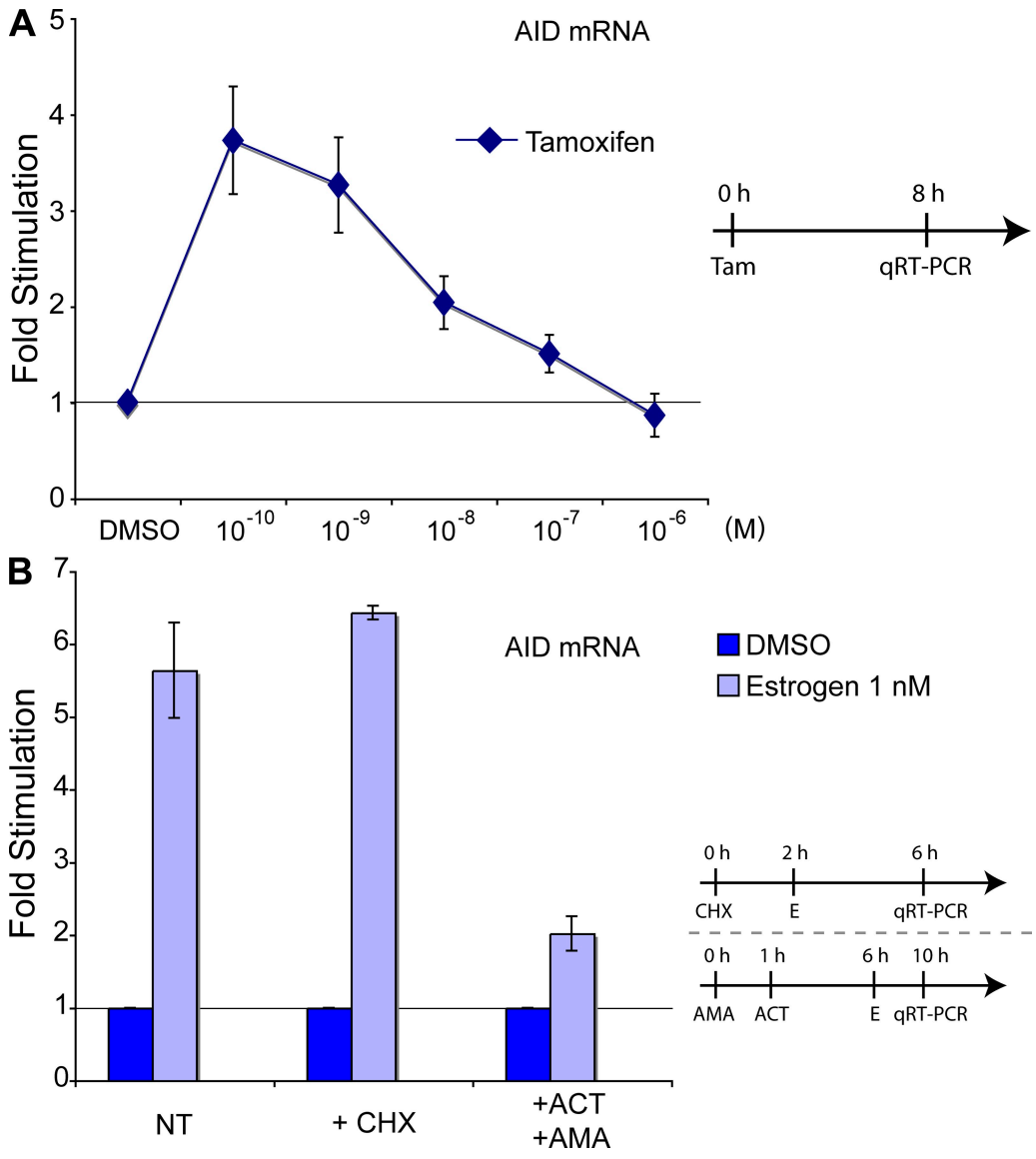


Figure S1. The effect of tamoxifen on AID mRNA in mouse splenic B cells. (A) Isolated mouse splenic B cells were treated with different concentrations of Tamoxifen for 8 h, and AID mRNA was analyzed by qRT-PCR. Data are representative of three independent experiments, and error bars indicate standard deviations from the average. Time line of cell treatment is indicated beside the graph. (B) Estrogen affects AID transcription directly. Isolated mouse splenic B cells were treated with cycloheximide (CHX) for 2 h or with actinomycin D for 6 h and α-amanitin for 5 h, followed by 1 nM estrogen treatment for 4 h; AID mRNA was analyzed by qRT-PCR. Gene expression is normalized to the control treatments with DMSO. Data are representative of three independent experiments and error bars indicate standard deviations from the average. Time lines of cell treatments are indicated next to the graphs. NT, not treated.

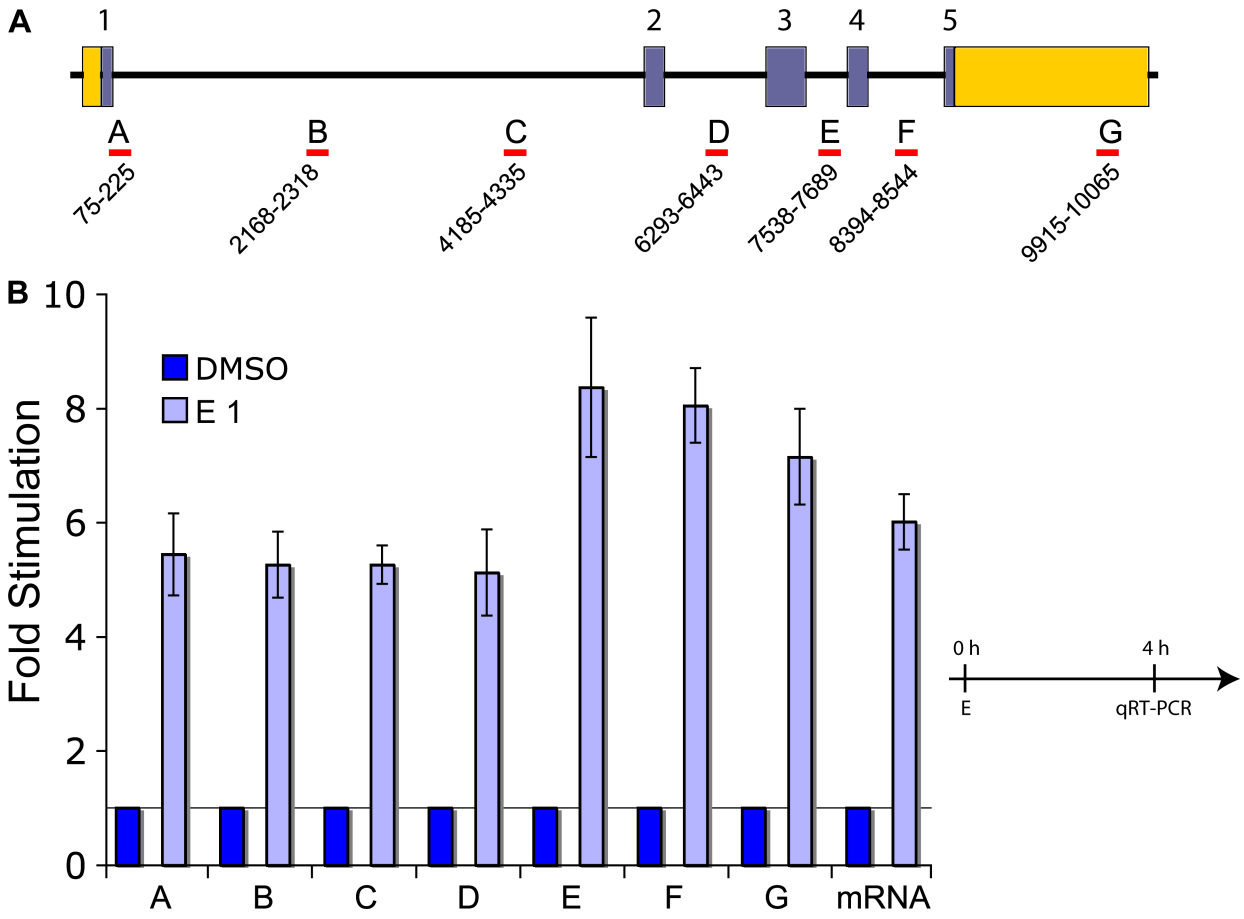


Figure S2. Estrogen does not significantly alter AID pre-mRNA stability or splicing in mouse splenic B cells. (A) Schematic depiction of the AID locus. Exons (blue) with corresponding numbers and UTRs (yellow) are indicated as boxes. Red lines with capital letters mark the relative positions of the PCR products (A–G) along the AID locus. (B) The effects of estrogen on AID pre-mRNA. Unstimulated mouse spleen B cells were treated with 1 nM (E1) for 4 h, cDNA was synthesized, followed by analysis of AID pre-mRNA by qRT-PCR (Vandenbroucke, I.I., J. Vandesompele, A.D. Paepe, and L. Messiaen. 2001. *Nucleic Acids Res.* 29:E68). Results are normalized to DMSO-treated cells for each PCR product. Data represents results from three independent experiments, and error bars indicate standard deviations from the average. Time lines of cell treatments are indicated next to the graphs.

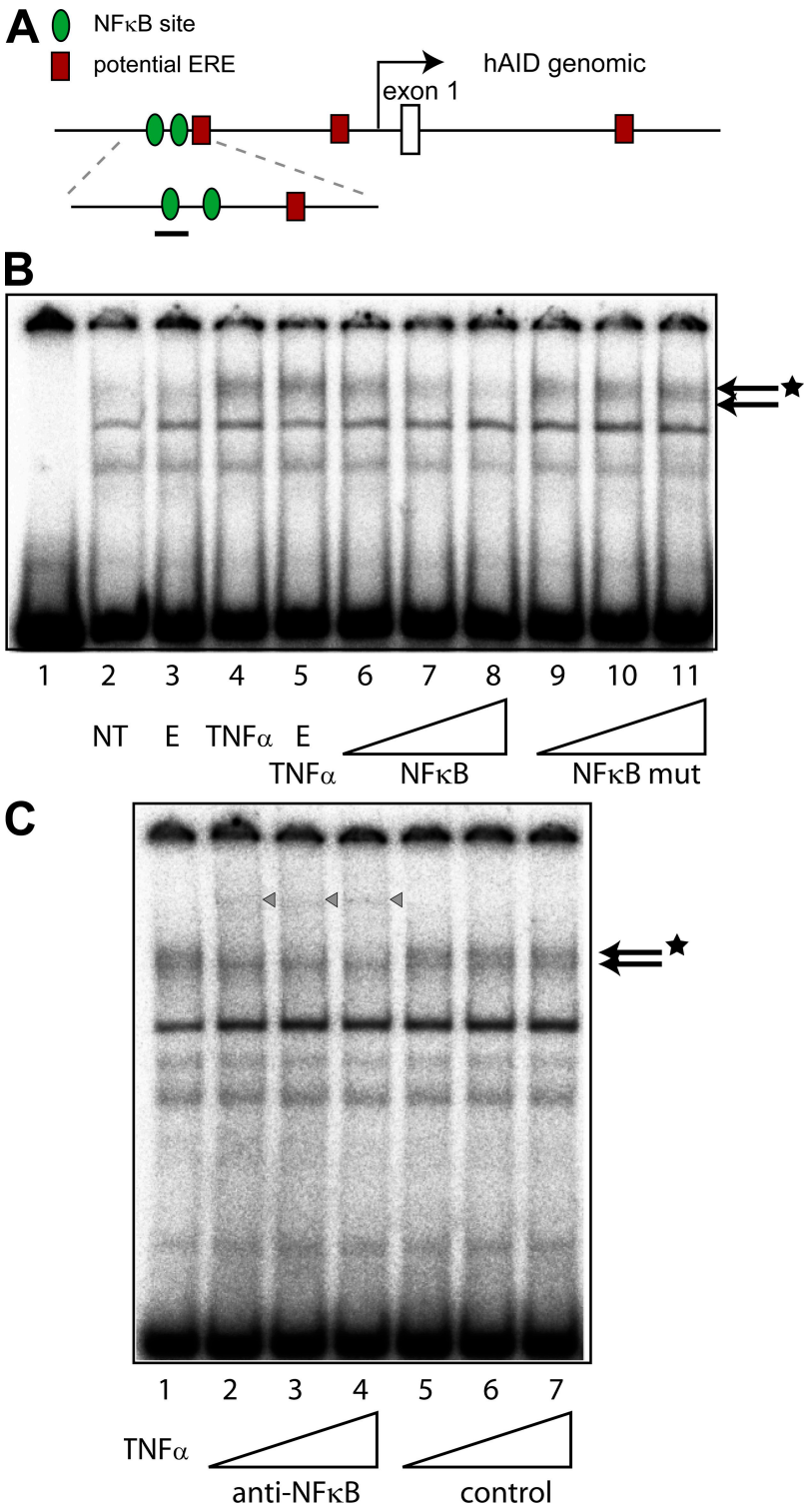


Figure S3. Estrogen treatment does not affect NF-κB binding to its response element in human AID promoter. (A) Schematic representation of human AID promoter region and the positions of potential EREs and NF-κB sites. The position of the oligonucleotide used for EMSA containing the distal NF-κB response element is marked as a black line. (B) The appearance of two oligonucleotide–protein complexes (marked with arrows) upon cell treatment with TNFα. Ramos cells were treated as in Fig. 3. Different concentrations of cold competitors (NF-κB, lanes 6–8) and mutated (NF-κBmut, lanes 9–11) competitors were used to assess the specificity of these bands for NF-κB binding. (C) EMSA with anti–NF-κB antibodies. Cells were treated as in Fig. 3 using anti–NF-κB antibodies. The disappearing band and a super-shifted band, which appears upon anti–NF-κB p65 antibody addition, are marked with a black arrow/star and triangles, respectively, indicating that one of the shifted bands contained NF-κB p65. NT, not treated.

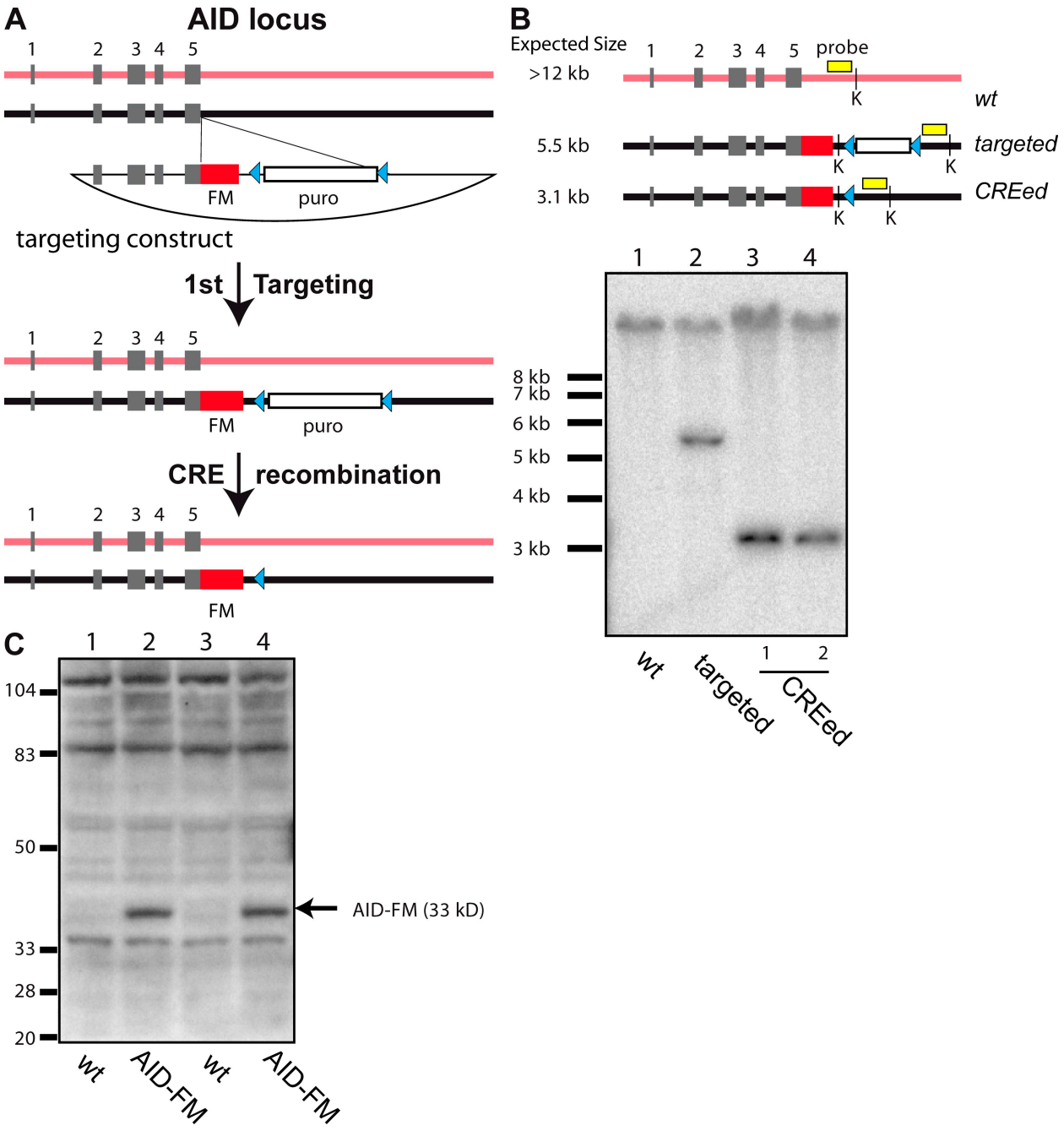


Figure S4. Endogenous tagging of AID with FLAG and Myc epitopes in DT40. See Supplemental materials and methods for details. (A) Schematic representation of the targeting of chicken AID locus with the 3xFLAG-2xTEV-3xMyc–tagged AID construct (AID-FM). A targeting construct that contained part of a chicken AID genomic locus, an in-frame FM tag (red rectangle), and a puromycin selection cassette (white rectangle) between two loxP sites (blue triangles) was targeted into chicken AID allele by homologous recombination. After confirming the targeting of the allele by Southern blotting, puromycin cassette was removed by Cre recombinase. (B) Verification of targeting the AID locus and the excision of puromycin selection cassette. A schematic depiction of the relative positioning of the radioactive probe (yellow) and Kpn I restriction sites (marked with K), which were utilized for identifying the proper excision of the puromycin cassette by Cre recombinase. The genomic DNA from the clones was treated with Kpn I and analyzed by Southern blot. Nontargeted allele (red line) results in the appearance of a >12-kb band, the targeted allele indicates a 5.5-kb band, and, upon successful excision of the puromycin cassette (CREed), a 3.1-kb band appears along with the disappearance of the 5.5-kb band. The Cre excision for two DT40 clones is shown on the Southern blot. (C) Anti-Flag (M2) Western blot confirming the expression of full-length AID–FM fusion protein. A specific band (arrow) can be detected only in the targeted DT40 cell lines.

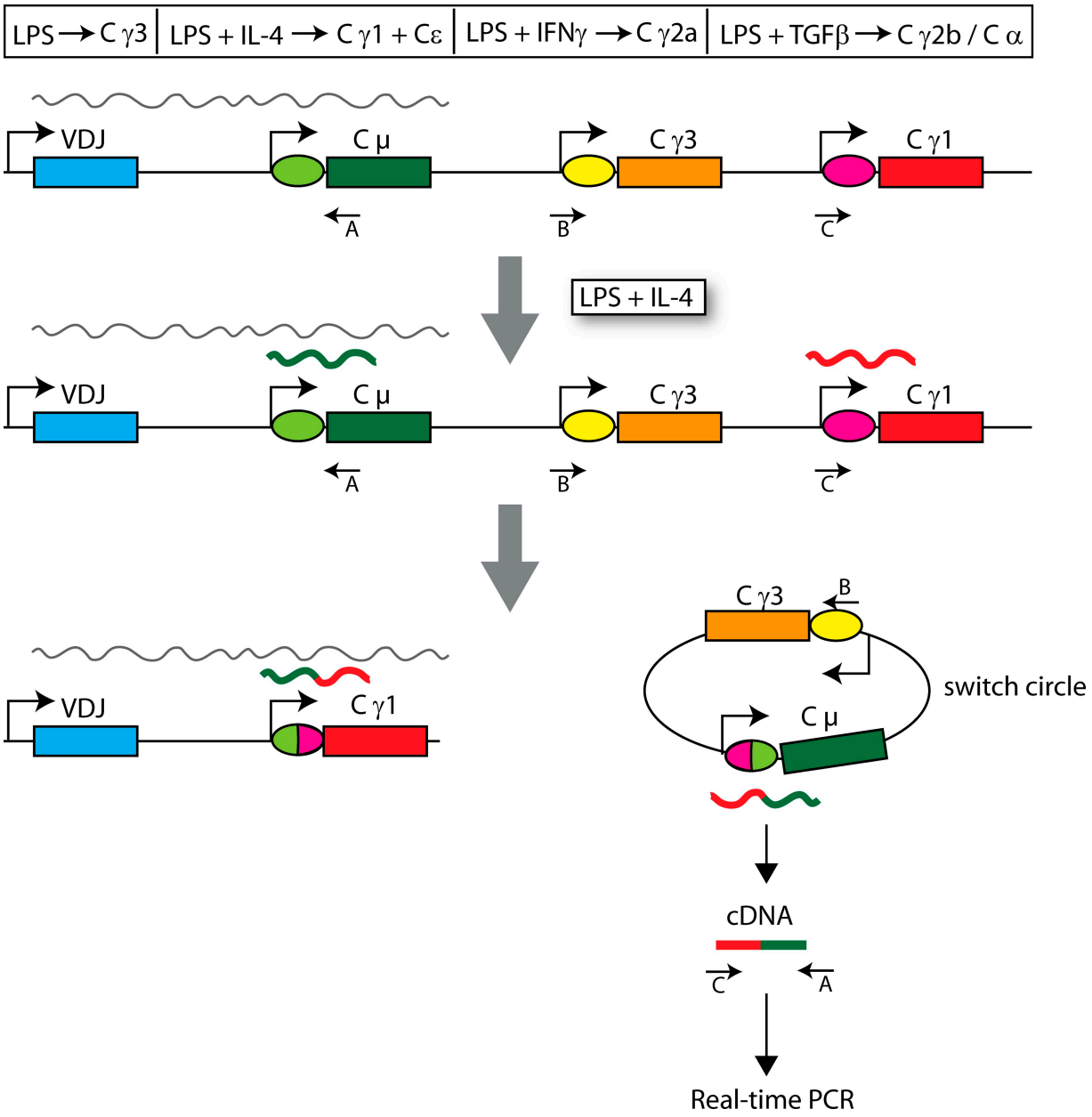


Figure S5. Schematic representation of class switching, the formation of switch circles, and production of "circle" transcripts. The combinations of cytokines and LPS that lead to the switching of Ig isotypes are indicated at the top. Ovals indicate switch regions, arrows mark the promoters, and rectangles represent different constant region exons (Cμ, Cγ3, Cγ1). Class switching can result in the formation of circular DNA (switch circle), from which a hybrid transcript (switch circle transcript, wavy line) is expressed (Kinoshita, K., M. Harigai, S. Fagarasan, M. Muramatsu, and T. Honjo. 2001. *Proc. Natl. Acad. Sci. USA.* 98:12620–12623). These transcripts can be reverse transcribed for cDNA, and using specific primers (e.g., arrows marked with C and A for detecting the switching to Cγ1 isotype), qRT-PCR can quantitate the amount of switch circle formation.

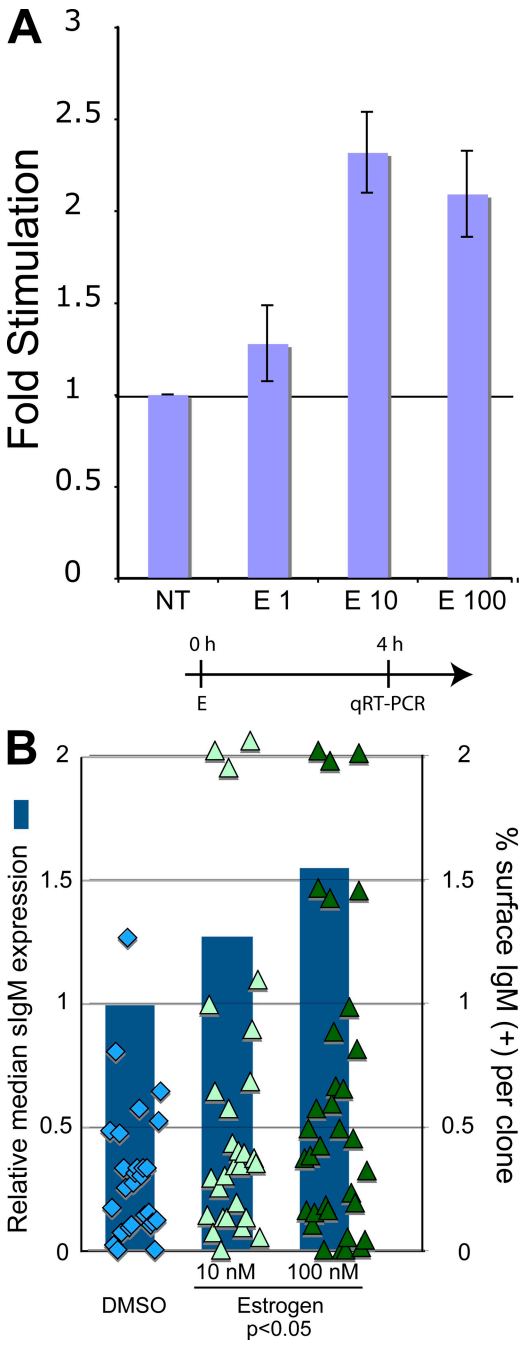
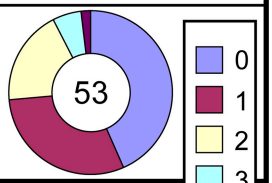
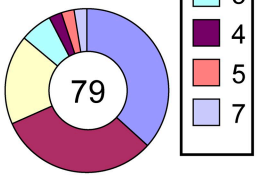


Figure S6. The effect of estrogen on AID mRNA in Ramos HS13 cells. (A) Cells were treated for 72 h in hormone-depleted serum before treatment with 1 nM estrogen (marked as E1), 10 nM estrogen (E10), or 100 nM estrogen (E100) for 4 h. Data are representative of three independent experiments and error bars indicate standard deviations from the average. Time line of cell treatment is indicated below the graph. NT, not treated. (B) Hormonal effects on sIgM expression in Ramos. Sorted individual sIgM-negative cells were grown in the presence of indicated amounts of estrogen (higher physiological concentrations were chosen to ensure maximal continuous stimulation) for ~20 cell doublings. Clones were analyzed for sIgM expression by flow cytometry. Each colored dot represents the relative proportion of sIgM-positive cells per clone (y axis on right), with the relative median of at least 26 individual clones per treatment shown as a bar (y axis on left). Paralleling the effect on AID mRNA, surface IgM expression was increased with estrogen. To monitor a change in mutation frequency, we sequenced the V region of the mutated clones, and detected an increase in mutations within the VH (Fig. 5 C [Ramos VH] and Fig. S7 A).

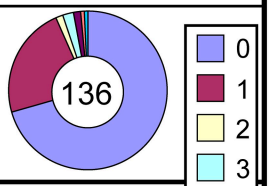
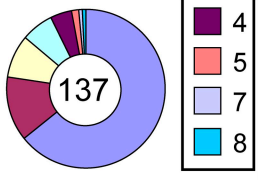
A VH Ramos

Treatment	Seq	BP	Mut	Mut/bp	% C:G	% Ts	Mutations/seq
DMSO	53	18468	50	2.7×10^{-3}	83.7	41.5	
Estrogen 100 nM	79	27360	100	3.7×10^{-3}	91.2	48.2	

B CD95/Fas Ramos

Treatment	Seq	BP	Mut	Mut/bp
DMSO	78	54600	6	1.1×10^{-4}
Estrogen 100 nM	92	63700	12	1.9×10^{-4}

C Sg3 mouse splenic B cells

Treatment	Seq	BP	Mut	Mut/bp	% C:G	% Ts	Mutations/seq
DMSO	136	192694	73	3.8×10^{-4}	60.5	35.8	
Estrogen 10 nM	137	194051	126	6.5×10^{-4} *	72.3	41.6	

*$p < 0.02$

Figure S7. Estrogen increases the mutation frequency in Ig and non-Ig loci. (A and B) Human Ramos cell lines were grown in the presence of indicated amounts of estrogen for 20 doublings, followed by cloning and sequencing of individual human VH (A) or human CD95/Fas (B) loci. The number of sequences analyzed (Seq), total base pairs (BP), number of mutations (Mut), mutation frequency per base pair (Mut/bp), overall percentage of mutations at C:G base pairs (% C:G), percentage of transitions (% Ts), and pie charts (mutations per sequence) are indicated for each of the treatments. As the overall mutations and percentage of transitions are already intrinsically very high in Ramos, estrogen treatment only modestly increases these percentages. (B) From single-cell sorted clones in A, genomic DNA was isolated and ~750 bp of the 5′ CD95/Fas locus was sequenced. The number of mutations in CD95/Fas locus in Ramos and does not allow for meaningful statistical analysis of mutations at C:G base pairs (% C:G) or percentage of transitions (% Ts). (C) Mouse splenic B cells from $AID^{+/-}$ were isolated and stimulated ex vivo with LPS and co-treated with DMSO or 10 nM estrogen for 72 h. Genomic DNA was amplified and 750 bp of the γ3 switch region sequenced (Xue, K., C. Rada, and M.S. Neuberger. 2006. *J. Exp. Med.* 203:2085–2094). The table was generated as indicated in A. The difference between the two samples' mutation per base pair was significant to $P < 0.02$ (two-tailed unpaired T-test).

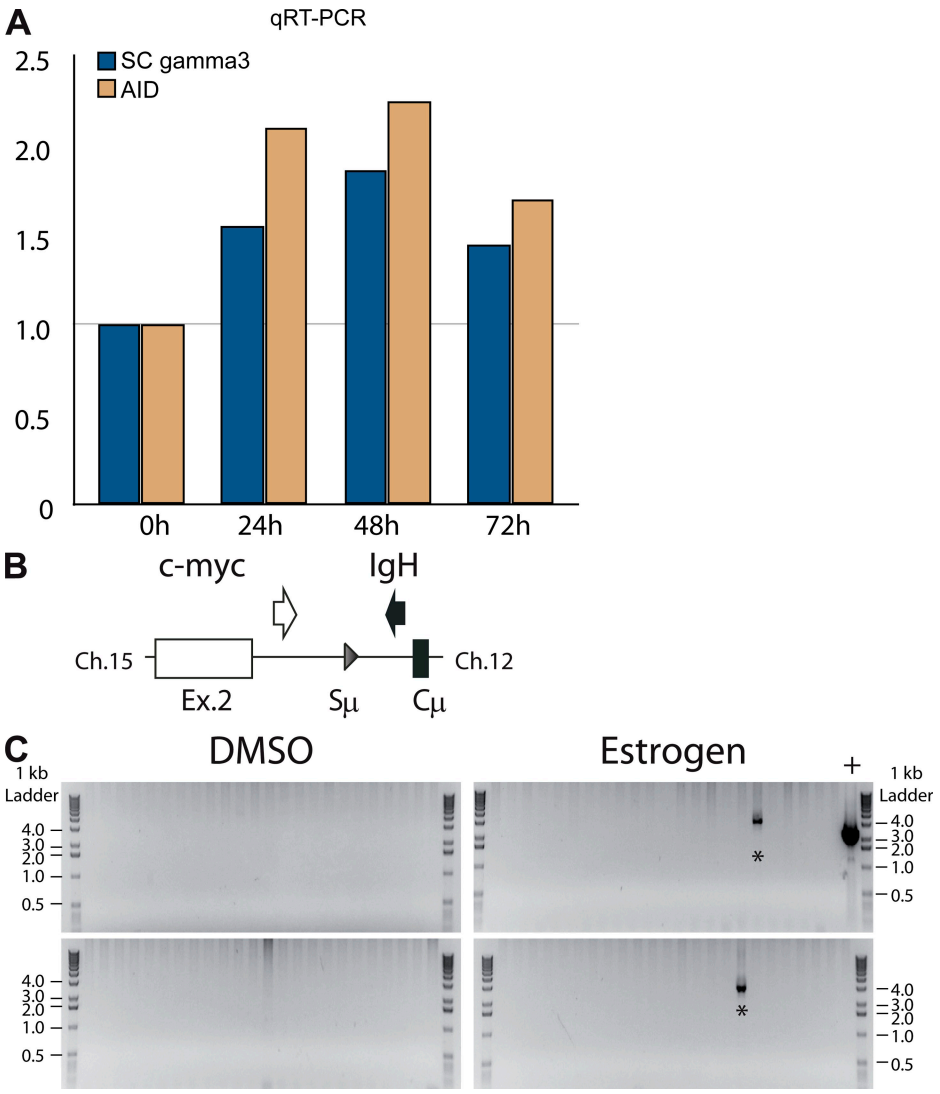


Figure S8. Estrogen enhances c-myc/IgH translocations. (A) Isolated splenic B cells ($p53^{+/-}$) were ex vivo treated with 50 nM estrogen for up to 72 h. qRT-PCR of AID and Sγ3 mRNA was performed as in Figs. 1 and 5, respectively. (B) Schematic representation of a c-myc/IgH translocation and the PCR assay used for translocation detection. C-myc exon 2 and Cμ exon 1 are represented as white and black boxes, respectively. Sμ region is shown as a grey triangle. Priming sites of the oligonucleotides used in the PCR reaction are represented as arrows. (C) Representative amplification products analysed in ethidium bromide-stained gels. DNA was isolated from estrogen-treated spleen B cells (72 h) and PCR-amplified as described in Materials and methods. DNA from IL6tg lymph nodes was used as a positive control (+). *, amplification products detected (and Southern blot confirmed) in the presence of estrogen.

	Jurkat			JAR			JAMA2			MCF7			HeLa			PC3			HepG2			T47D		
	T cell			Placenta			Ovarian			Breast			Cervix			Prostate			Liver			Breast		
	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50	E 1	E 10	T 50
AID	10.1	3.19	2.19	5.81	1.54	2.58	1.36	4.78	2.92	7.64	2.80	1.69	0.97	1.35	1.24	6.92	21.8	4.93	1.66	0.76	1.62	4.42	2.10	1.00
	1.93	0.71	0.54	0.94	0.15	0.96	0.17	0.81	0.36	0.63	1.8	0.13	0.34	0.48	0.42	1.43	5.35	0.96	0.4	0.9	0.32	0.7	0.63	0.3
APOBEC2	0.96	1.26	0.99	1.30	1.13	1.00	1.30	1.15	0.77	1.39	1.60	0.88	1.19	1.50	0.94	1.21	1.24	1.40	1.16	1.26	0.90	1.15	1.28	1.00
	0.15	0.17	0.14	0.11	0.09	0.09	0.49	0.38	0.16	0.25	0.22	0.14	0.33	0.33	0.25	0.4	0.28	0.19	0.32	0.17	0.14	0.38	0.2	0.2
APOBEC3A	2.30	2.36	1.38	1.79	2.05	1.46	1.05	0.87	1.14	1.52	1.85	0.08	1.3	1.99	1.25	1.53	1.95	1.18	0.78	0.79	0.99	1.25	1.28	1.09
	0.88	0.61	0.37	0.11	0.11	0.38	0.08	0.10	0.32	1.18	0.63	0.35	0.36	0.41	0.34	0.23	0.20	0.33	0.03	0.06	0.23	0.27	0.06	0.24
APOBEC3B	2.46	3.90	1.69	1.92	2.63	1.31	1.75	2.42	1.47	1.26	1.83	1.50	2.31	4.89	1.53	1.39	1.31	0.87	1.26	1.45	1.24	1.18	1.41	1.35
	0.35	0.76	0.39	0.26	0.38	0.16	0.22	0.61	0.29	0.13	0.44	0.15	0.29	0.94	0.14	0.46	0.27	0.19	0.3	0.33	0.12	0.27	0.35	0.7
APOBEC3C	1.75	1.89	1.36	1.91	3.28	1.48	1.22	1.07	0.94	1.33	1.32	1.01	0.88	1.29	1.1	1.54	1.5	1.34	0.94	0.85	0.85	1.23	1.71	1.28
	0.33	0.39	0.28	0.17	0.36	0.28	0.20	0.21	0.34	0.17	0.11	0.37	0.06	0.19	0.27	0.04	0.30	0.15	0.26	0.19	0.07	0.09	0.44	0.32
APOBEC3D	1.10	1.31	0.97	1.24	1.56	0.95	1.1	1.41	1.48	1.55	1.54	0.87	1.21	1.44	1.08	1.95	3.61	2.15	1.39	0.98	0.81	1.31	3.51	1.48
	0.16	0.22	0.23	0.35	0.28	0.02	0.47	0.22	0.18	0.18	0.20	0.11	0.29	0.25	0.02	0.32	0.35	0.35	0.18	0.16	0.12	0.30	0.08	0.37
APOBEC3F	1.94	4.35	0.97	2.20	4.46	1.96	1.62	4.20	1.98	1.35	1.89	0.96	1.77	6.56	2.35	1.46	0.90	1.00	1.19	1.38	1.10	0.99	1.97	1.80
	0.13	0.7	0.2	0.51	0.8	0.57	0.63	1.55	0.81	0.15	0.65	0.19	0.45	0.87	0.33	0.3	0.13	0.19	0.5	0.45	0.16	0.7	0.62	0.23
APOBEC3G	2.57	3.74	1.46	2.20	3.14	1.45	1.58	2.22	1.68	1.55	1.60	0.98	2.80	5.19	2.30	1.58	1.47	1.20	1.60	1.35	1.23	1.27	1.97	1.10
	0.23	1.5	0.21	0.31	0.31	0.09	0.39	0.78	0.22	0.57	0.05	0.06	0.38	1.27	0.32	0.29	0.44	0.17	0.29	0.43	0.06	0.05	0.62	0.22
APOBEC3H	1.98	2.92	1.85	1.83	2.56	1.27	1.16	0.96	1.62	1.4	1.57	1.42	0.98	1.32	1.42	1.42	1.55	1.18	1.34	1.56	0.68	1.12	1.69	1.02
	0.24	0.33	0.22	0.11	0.30	0.18	0.11	0.08	0.35	0.20	0.11	0.27	0.11	0.08	0.14	0.10	0.22	0.09	0.27	0.20	0.10	0.06	0.28	0.13

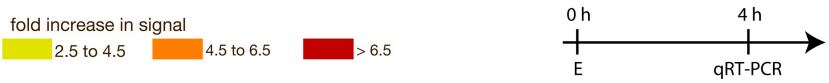


Figure S9. Estrogen induces the transcription of AID and several APOBEC3 family members in various hormone-responsive human cell lines. The indicated human cell lines were treated with 1 nM (E1), 10 nM estrogen (E10), or 50 nM tamoxifen (T 50) for 4 h, followed by gene expression analysis by qRT-PCR. Results are normalized to control-treated cells. Small numbers below the fold induction indicate standard deviations for three independent experiments. Yellow, orange, and red rectangles indicate the extent of fold induction in response to these treatments. Time line of cell treatments are indicated below the table.

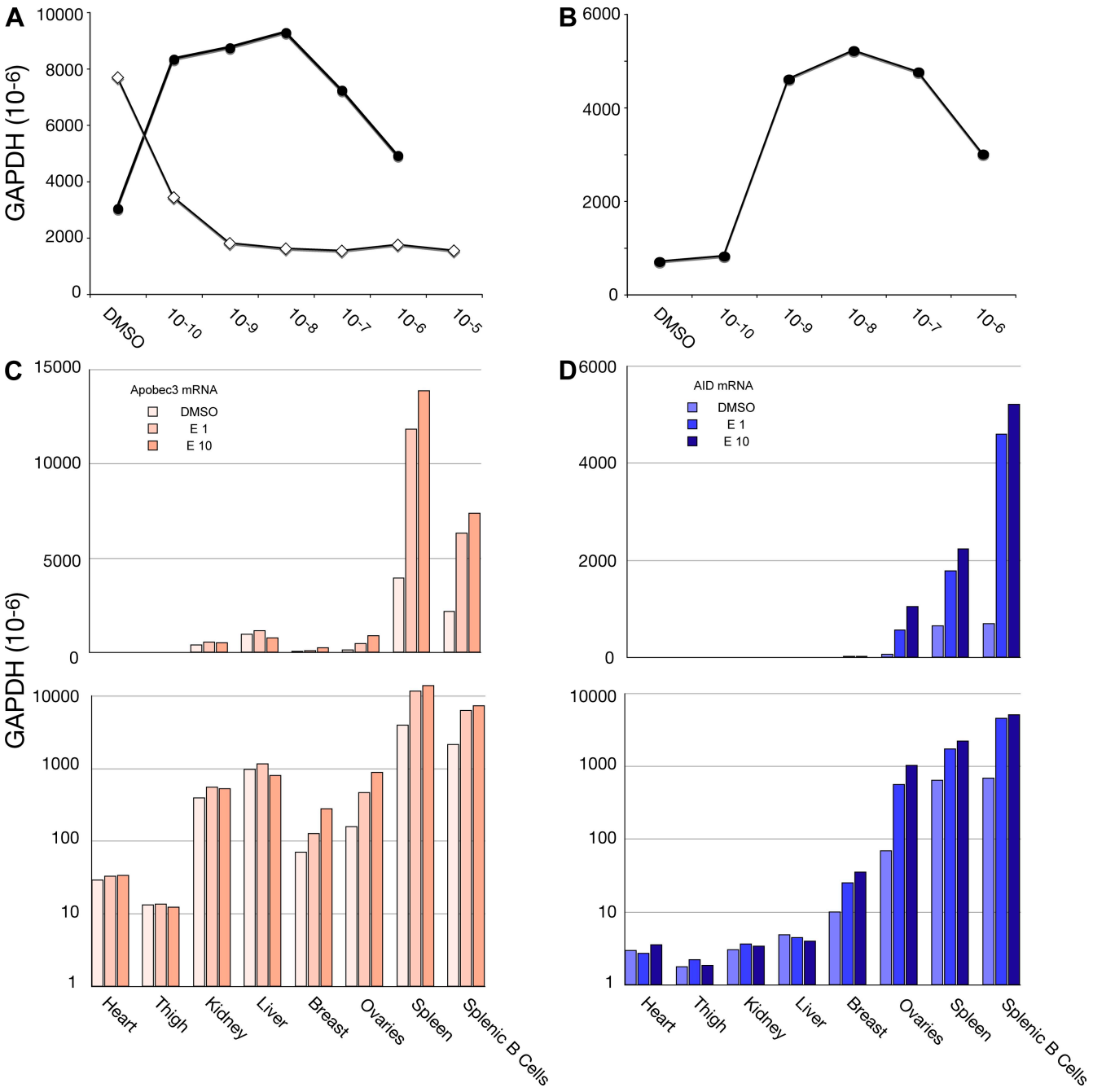


Figure S10. Absolute qRT-PCR values. Absolute qRT-PCR values as compared to GAPDH expression from Fig. 1 A (A), Fig. 1 B (B), Apobec3 data of Fig. 6 (C), and AID data of Fig. 6 (D). (C and D) Values are plotted on a linear scale in the top graphs; in the bottom graphs, values are plotted on a logarithmic scale.

Table S1. Primers used for gene expression analysis by real-time PCR

Gene	Species	Oligo	Direction	Sequence
Aid	mouse	SP3029	forward	5′-AACCCAATTTTCAGATCGCG-3′
Aid	mouse	SP3030	reverse	5′-AGCGGTTCCTGGCTATGATAAC-3′
Gapdh	mouse	SP3033	forward	5′-GCACAGTCAAGGCCGAGAAT-3′
Gapdh	mouse	SP3034	reverse	5′-GCCTTCTCCATGGTGGTGAA-3′
Greb1	mouse	SP3065	forward	5′-TCCGAGTTCAGAGGTCGGC-3′
Greb1	mouse	SP3066	reverse	5′-GTCCTACCTGTTGAGCTCCCACT-3′
Aid A	mouse	SP3069	forward	5′-GTCACGCTGGAGACCGATATG-3′
Aid A	mouse	SP3070	reverse	5′-AAAGACCTGAGCAGAGGGTGG-3′
Aid B	mouse	SP3071	forward	5′-TTCCCATCTAGGTAACACAGGAAGT-3′
Aid B	mouse	SP3072	reverse	5′-TCACCACCACAGTACAGGTAAACTC-3′
Aid C	mouse	SP3073	forward	5′-AATAAAATCAACAAAACTGACCCAGC-3′
Aid C	mouse	SP3074	reverse	5′-CGAGGGATCAAACTCAAGACATC-3′
Aid D	mouse	SP3075	forward	5′-GAGGCCAATGACCGACCAC-3′
Aid D	mouse	SP3076	reverse	5′-CCATGGAAGCCAATCTGCA-3′
Aid E	mouse	SP3077	forward	5′-GCCACTCAGAGTGAGTGTCAGC-3′
Aid E	mouse	SP3078	reverse	5′-GAGGTCGGAGAATTGCAAGTTG-3′
Aid F	mouse	SP3087	forward	5′-TCTTGCCTCCTCTTCGCTCA-3′
Aid F	mouse	SP3088	reverse	5′-GTCTAAACGAAGTGGGTGGCTC-3′
Aid G	mouse	SP3079	forward	5′-GTAAGAGGGTGGCAAAATAGGGA-3′
Aid G	mouse	SP3080	reverse	5′-TTTACCAGAACCCAATTCTGGCT-3′
Apobec3	mouse	SP3153	forward	5′-TTCCACTGGAAGAGGCCCTT-3′
Apobec3	mouse	SP3154	reverse	5′-TCTCCAATCCTTTCCATGGC-3′
AID	human	SP3055	forward	5′-TCGGCGTGAGACCTACCTGT-3′
AID	human	SP3056	reverse	5′-GCCAGGGTCTAGGTCCCAGT-3′
GAPDH	human	SP3063	forward	5′-TCACCACCATGGAGAAGGCT-3′
GAPDH	human	SP3064	reverse	5′-CAGGAGGCATTGCTGATGATC-3′
Apobec2	human	SP3225	forward	5′-TGTGGAGCAAGAAGAGGGTGA-3′
Apobec2	human	SP3226	reverse	5′-GCTGTCTCTTGGTGGCAGC-3′
Apobec3A	human	SP3136	forward	5′-ACACACGTGAGACTGCGCAT-3′
Apobec3A	human	SP3137	reverse	5′-GGTCCACAAAGGTGTCCCAG-3′
Apobec3B	human	SP3138	forward	5′-GGAGCGGATGTATCGAGACAC-3′
Apobec3B	human	SP3139	reverse	5′-CACCTGGCCTCGAAAGACC-3′
Apobec3C	human	SP3140	forward	5′-CCAACGATCGGAACGAAACT-3′
Apobec3C	human	SP3141	reverse	5′-TCGCAGAACCAAGAGAGGAAG-3′
Apobec3D	human	SP3142	forward	5′-TGGCACTGATTGCAACTGACA-3′
Apobec3D	human	SP3143	reverse	5′-GGCATGAATGGCTGACCTTC-3′
Apobec3F	human	SP3144	forward	5′-ATTCATGCCTTGGTACAAATTCG-3′
Apobec3F	human	SP3145	reverse	5′-GCTTTCGTTCCGACCATAGG-3′
Apobec3G	human	SP3146	forward	5′-AAGTGGAGGAAGCTGCATCG-3′
Apobec3G	human	SP3147	reverse	5′-AGTAGTAGAGGCGGGCAACG-3′
Apobec3H	human	SP3148	forward	5′-CCCGCCTGTACTACCACTGG-3′
Apobec3H	human	SP3149	reverse	5′-GGGTTGAAGGAAAGCGGTTT-3′
GAPDH	chicken	SP3083	forward	5′-GCACTGTCAAGGCTGAGAACG-3′
GAPDH	chicken	SP3084	reverse	5′-GCCTTCTCCATGGTGGTGAA-3′
AID	chicken	SP3081	forward	5′-TATGTTGTGAAGCGCCGTGA-3′
AID	chicken	SP3082	reverse	5′- ACCATGTGATGCGGTAGCAG-3′

Table S2. Primer sequences for hAID promoter analysis

Oligo name	Approximate position relative to transcription start	Direction	Sequence
SP3089	0 bp	forward	5′-CTCGAGGCCAATGCACTGTCAGACTA-3′
SP3090	0 bp	reverse	5′-CAGCTGGAAAAATCTCACTTCAATTAATGATGGTTC-3′
SP3092	−2,000 bp	forward	5′-CTCGAGGATGGTGTAAGCCACAACCA -3′
SP3093	−1,500 bp	forward	5′-CTCGAGCAAGAAGAGTAGGTAAGGCAG-3′
SP3094	−1,000 bp	forward	5′-CTCGAGATTTGAAAATCATCAAGGTATAGATG-3′
SP3095	−500 bp	forward	5′-CTCGAGACTGAGTTCATTTGCTTAACTGCA-3′
SP3096	500 bp	forward	5′-CAGCTGTTACAAAATTATTACGAAAATTAGCACTACC-3′
SP3099	2,000 bp	reverse	5′-CAGCTGTCTCTTTGAGGCCCAGTG-3′
SP3108	1500bp	forward	5′-CAGCTGTTTCAGGCTTGCAGGCTGACAG-3′
SP3101	pE1BLuc	forward	5′-ACATATTGTCGTTAGAACGCGGCTAC-3′
SP3102	pE1BLuc	reverse	5′-CCAACAGTACCGGAATGCCAAG-3′

Table S3. Primer sequences for hAID promoter analysis

Oligo name	Direction	Sequence
NF-κB	forward	5′-GGGGGTGGGTCTTTCCCATGC-3′
NF-κB	reverse	5′-GCATGGGAAAGACCCACCCCC-3′
NF-κB Mut	forward	5′-GGGGGTTAACTTACCCCATGC-3′
NF-κB Mut	reverse	5′-GCATGGGGTAAGTTAACCCCC-3′
ER	forward	5′-CCCCAGCCATGTGGAACTGTGAGTCAACTAAACC-3′
ER	reverse	5′-GGTTTAGTTGACTCACAGTTCCACATGGCTGGGG-3′
ER Mut	forward	5′-CCCCAGCCATGTGTAACCGTGAGTCAACTAAACC-3′
ER Mut	reverse	5′-GGTTTAGTTGACTCACGGTTACACATGGCTGGGG-3′

Table S4. Primers used for detecting circle transcripts

Subclass	Orientation	Sequence
IgG1	forward	5′-TCGAGAAGCCTGAGGAATGTG-3′
IgG1	reverse	5′-GAAGACATTTGGGAAGGACTGACT-3′
IgG3	forward	5′-TGGGCAAGTGGATCTGAACA-3′
IgG3	reverse	5′-AATGGTGCTGGGCAGGAAGT-3′
IgA	forward	5′-CCAGGCATGGTTGAGATAGAGATAG-3′
IgA	reverse	5′-AATGGTGCTGGGCAGGAAGT-3′
IgE	forward	5′-TTGGACTACTGGGGTCAAGG-3′
IgE	reverse	5′-CAGTGCCTTTACAGGGCTTC-3′

Table S5. Primers for sequencing tagged AID constructs.

Name	Sequence
S1	5′-ACCGTTACCTTAAAATACTGC-3′
S2	5′-GTCCCTGCTGCTTTAAC-3′
S3	5′-GGATATGATGATTTAGTGAGC-3′
S4	5′-GATGCCTTTAAAACTCTGG-3′
S5	5′-AATTCAGAGGAAGTCATCAG-3′
S6	5′-TACAAATGTGGTATGGCTGA-3′
S7	5′-ATCTCGGCGAACACC-3′
S8	5′-TCACACGCCAGAAGC-3′
S9	5′-TGCTCAGCAACTCGG-3′
S10	5′-GCGCGCTTCGCTTTT-3′
S11	5′-CTATGACAGGTTGAAACTAG-3′
S12	5′-TTGGGGTTATGTGAGTTC-3′
S13	5′-AAGGGACCCAATCATATCT-3′
S14	5′-ACTGTCCACAAAACCAGATA-3′
S15	5′-TCAAAAGAACTCACATAACCC-3′
S16	5′-GTCTAGTTTCAACCTGTCA-3′
S17	5′-TTTCCTTTTATGGCGAGG-3′
S18	5′-TCCCCGAGTTGCTGA-3′
S19	5′-TTCTCCCTCTCCAGC-3′
S20	5′-TGTTCGCCGAGATCG-3′
S21	5′-GGATCATAATCAGCCATAC-3′
S22	5′-TATTGCTGATGACTTCCTC-3′
S23	5′-CTAGTAAGTCCCAGAGTTT-3′
S24	5′-TCTCTTCTAGGCTCACTAA-3′
S25	5′-CTGAGGTACTGTTAAAGCA-3′
S26	5′-GCAGTATTTTAAGGTAACGG-3′
m13F	5′-GTTTTCCCAGTCACGAC-3′
m13R	5′-GGAAACAGCTATGACCATG-3′