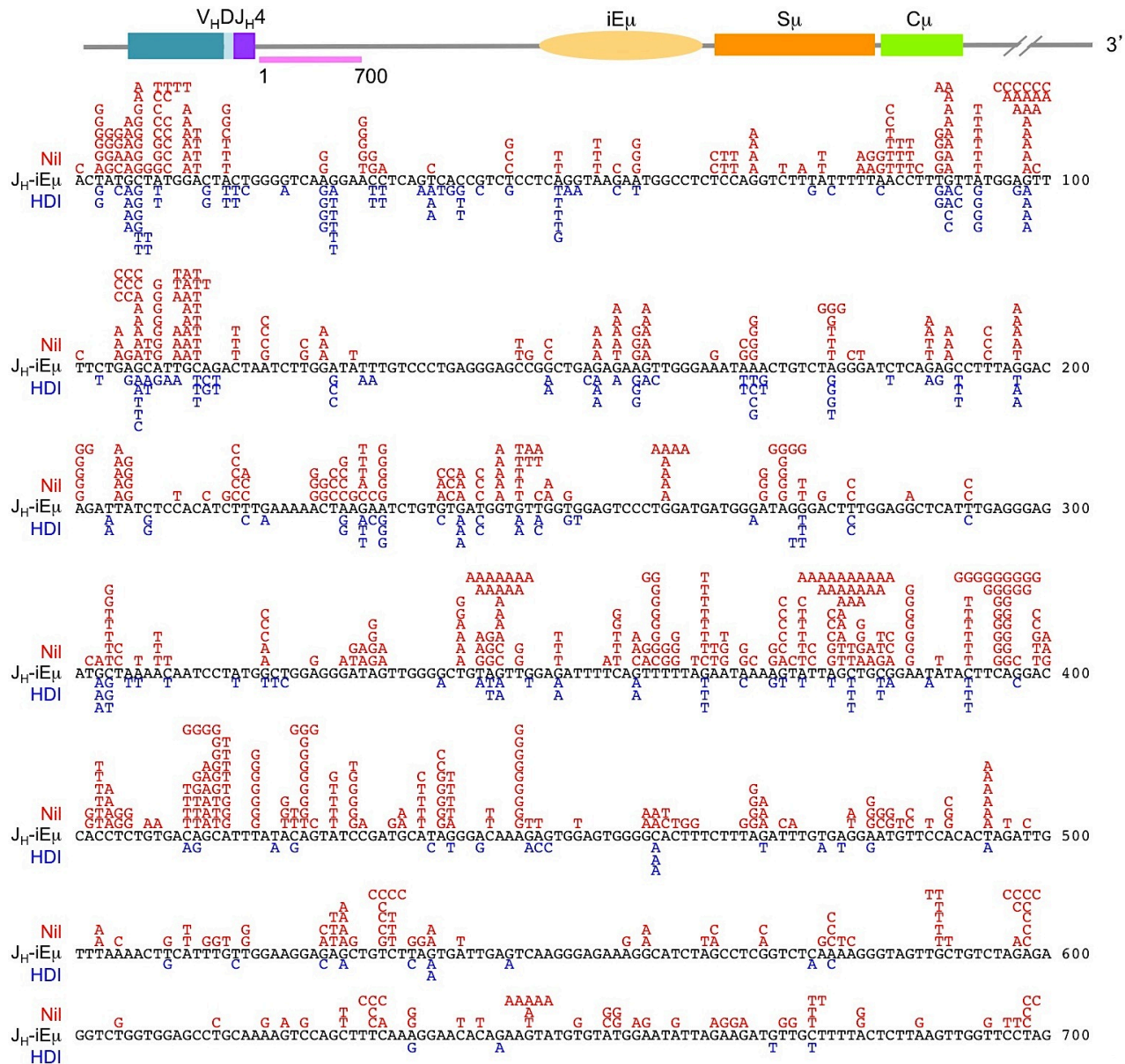


**Supplemental Figure 1.** HDI inhibit CSR and *Aicda* expression but do not alter the spectrum of somatic point-mutations as well as viability and apoptosis of B cells and plasma cells *in vivo* and *in vitro*. **(A)** Spectrum of somatic mutations in the V<sub>186.2</sub> region of V<sub>186.2</sub>DJ<sub>H</sub>-C $\gamma$ 1 transcripts from mice that were on HDI-water or untreated water, as in Figure 1E. Values are expressed as the actual numbers of different nucleotide substitutions (top panels) or as the percentage of total point-mutations (bottom panels). **(B)** Dose-dependent inhibition of CSR and *Aicda* expression by HDI TSA in B cells. Mouse naïve B cells were stimulated with LPS plus IL-4 in the presence of 0, 10 or 20 nM (0, 3 or 6 ng/ml) TSA. IgG1<sup>+</sup> B cells were analyzed 4 days after the stimulation. *Aicda* transcripts were measured by qRT-PCR and normalized to *Cd79b* expression, 60 hours after the stimulation. Values in B cells cultured in the presence of TSA are depicted as relative to *Aicda* transcript level in B cells cultured in the absence of TSA, set as 1. **(C-F)** Mouse naïve B cells were stimulated for 4 days with LPS plus IL-4 in the presence of nil or increased doses of HDI (VPA) to assess viability and apoptosis. Proportions of **(C)** viable (7-AAD<sup>-</sup>) or **(D)** apoptotic (Annexin V<sup>+</sup>) B220<sup>+</sup> B cells are indicated. **(E)** Proportions of Annexin V<sup>+</sup> B220<sup>lo</sup>CD138<sup>+</sup> plasma cells are indicated. **(F)** Expression of anti-apoptotic genes *Bcl2*, *Mcl1* and *Bcl2l1* measured by qRT-PCR and normalized to *Gapdh* expression. Values in cells treated with VPA are depicted as relative to the expression of each transcript in cells treated with nil, set as 1. Data are presented as mean and SEM from three independent experiments. **(G-I)** C57BL/6 mice on HDI-water or untreated water were injected with NP<sub>16</sub>-CGG 10 days before analysis. Proportions of **(G)** 7-AAD<sup>-</sup> viable cells or Annexin V<sup>+</sup> apoptotic cells among spleen B220<sup>lo</sup>CD138<sup>+</sup> plasma cells are indicated. Expression of *Bcl2*, *Mcl1* and *Bcl2l1* transcripts in **(H)** CD19<sup>+</sup>CD138<sup>-</sup> B cells and **(I)** CD19<sup>lo</sup>CD138<sup>+</sup> plasma cells were measured by qRT-PCR and normalized to *Gapdh* expression. Values in cells treated with HDI or cells isolated from mice that were on HDI-water are depicted as relative to the expression of each transcript in cells cultured in the absence of HDI, or cells isolated from mice that were on untreated water, respectively, set as 1. Data are presented as mean  $\pm$  SEM from three independent experiments.



**Supplemental Figure 2.** (A-D) HDI-mediated inhibition of CSR as indicated by reduced mature  $V_HDJ_H-C_H$  and post-recombination  $I\mu-C_H$  transcripts in HDI-treated B cells. (A) Purified spleen B cells were labelled with CFSE and stimulated with LPS for 4 days in the presence of nil or HDI (VPA, 500  $\mu$ M). The percentage of  $B220^+IgG3^+$  B cells among total  $B220^+$  B cells that had completed the same number of divisions when treated with nil or HDI is depicted in the scatter plot. Data are representative of three independent experiments. (B) Germline  $I\gamma3-C\gamma3$  transcripts in B cells stimulated for 60 hours with LPS in the presence of nil or increasing doses of VPA, measured by qRT-PCR and normalized to *Cd79b* transcripts (all other germline  $I_H-C_H$  transcripts were also normal, not shown). (C and D) B cells were stimulated with LPS plus IL-4 (for IgG1 and IgE), CD154 plus IL-4 (for IgE), LPS (for IgG3), or LPS or CD154 plus TGF- $\beta$ , IL-4, IL-5 and anti-IgD dextran (for IgA) for 60 hours in the presence of nil or increasing doses of VPA. (C) Mature  $V_HDJ_H-C\gamma1$ ,  $V_HDJ_H-C\gamma3$ ,  $V_HDJ_H-C\alpha$  and  $V_HDJ_H-C\epsilon$  transcripts and (D) post-recombination  $I\mu-C\gamma1$ ,  $I\mu-C\gamma3$ ,  $I\mu-C\alpha$  and  $I\mu-C\epsilon$  transcripts, which are both hallmarks of completed CSR were analyzed by qRT-PCR and normalized to *Cd79b* transcripts. Data are from three independent experiments (mean and SEM). Values in B cells cultured in medium containing HDI are depicted as relative to the expression of each transcript in B cells cultured in the absence of HDI, set as 1. Data are presented as mean and SEM from three independent experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , unpaired *t*-test. (E) Alignment of unmutated and mut *Aicda* and *Prdm1* mRNA 3'UTR sequences used in luciferase reporter assays and the miRNAs that target them. Unmutated and mut 3'UTRs of the *Aicda* and *Prdm1* mRNA 3'UTRs that were cloned into luciferase vectors for experiments in Figure 7E and 7F are diagramed. Base pairing of miRNAs that target the *Aicda* and *Prdm1* mRNA 3'UTRs is represented by vertical lines. Grey boxes indicate the seed sequence of miRNAs. Mutations to predicted and known miRNA target sites were designed to disrupt miRNA binding to mRNA 3'UTRs and are shown in red. The latter three miRNA target sites in mut *Prdm1* mRNA 3'UTR were deleted (rather than mutated).



**Supplemental Figure 3.** Spectrum and distribution of point-mutations in the intronic  $J_H4$ -iE $\mu$  region of MRL/*Fas*<sup>lpr/lpr</sup> mice is not altered by HDI. A 700 bp sequences (outlined by pink line) of intronic  $J_H4$ -iE $\mu$  DNA in CD19<sup>+</sup>PNA<sup>hi</sup> GC B cells from Peyer's patches of 12-week-old lupus-prone MRL/*Fas*<sup>lpr/lpr</sup> mice that were on HDI-water or untreated water (starting at 6 weeks of age, as in Figure 9) were analyzed for nature and distribution of mutations. Red letters above the black germline sequence depict point-mutations in sequences from MRL/*Fas*<sup>lpr/lpr</sup> mice that were on untreated water, blue letters below the germline sequence show point-mutations in sequences from mice that were on HDI-water.

**Supplemental Table 1. Primers for qRT-PCR, detection of mature miRNA transcripts and bisulfite PCR.**

	Forward primer	Reverse primer
<u>Mouse genes</u>		
<i>Aicda</i>	5'-AGAAAGTCACGCTGGAGACC-3'	5'-CTCCTCTTACCACGTAGCA-3'
<i>Ung</i>	5'-TAATCAAGCTCACGGCTCT-3'	5'-TTGAGGAGGAGGACACCTTG-3'
<i>IL-6</i>	5'-GCTACCAAACCTGGATATAATCAGGA-3'	5'-CCAGGTAGCTATGGTACTCCAGAA-3'
<i>Prdm1</i>	5'-GCTGCTGGGCTGCCTTTGGA-3'	5'-GGAGAGGAGGCCGTTCCCCA-3'
<i>Xbp1</i>	5'-AAGCCCGGATGAGCGAGCTG-3'	5'-ACCCGGCCACCAGCCTTACT-3'
<i>Irf4</i>	5'-CCCCAAAGCCCTCAGTCGTTGT-3'	5'-CAGCCACTCAGGCACCGCAC-3'
<i>Bach2</i>	5'-AGGGCTAGAGCCAATGGTA-3'	5'-CTTCCCCATTAAGCAGCCCA-3'
<i>Pax5</i>	5'-CACCAACAAACGCAAGAGGG-3'	5'-CTGTGTGAACAGGTCTCCCC-3'
<i>Bcl6</i>	5'-TGTCACAGCAACATCTACTCGCC-3'	5'-CAATCTCATCCTCCGAAGAAGGTC-3'
<i>Rev1</i>	5'-TGGTTTATTGTCTCCGCTCTCTG-3'	5'-AATCTGGGATGGTGATGGGACC-3'
<i>Gapdh</i>	5'-TTCACCACCATGGAGAAGGC-3'	5'-GGCATGGACTGTGGTCATGA-3'
<i>Cd79b</i>	5'-CCACACTGGTGTCTTCC-3'	5'-GGGCTTCCTTGGAAATTCAG-3'
<i>Bcl2</i>	5'-ACCAATCTGGAAACCTCCTG-3'	5'-CATAAGGCAACCACCATCG-3'
<i>Mcl1</i>	5'-GCTCCGGAACTGGACATTA-3'	5'-CCCAGTTTGTTACGCCATCT-3'
<i>Bcl2l1</i>	5'-TTCGGGATGGAGTAACTGG-3'	5'-TGCAATCCGACTCACCAATA-3'
<u>Pri-miR transcripts</u>		
pri-miR-155	5'-TATCCCTTATCCTCTGGCTGCTGG-3'	5'-ACTCTTGGACTTGTTCATCCTCCC-3'
pri-miR-181b	5'-GCCCCGCTTTTTGCTGTCGC-3'	5'-AGCAGGAAGCCGTGCCAACC-3'
<u>Germline transcripts</u>		
I $\gamma$ 3-C $\gamma$ 3	5'-AACTACTGCTACCACCACCACCAG-3'	5'-ACCAAGGGATAGACAGATGGGG-3'
<u>Post-recombination transcripts</u>		
I $\mu$ -C $\gamma$ 1	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-ATGGAGTTAGTTTGGGCAGCA-3'
I $\mu$ -C $\gamma$ 3	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-AGCCAGGGACCAAGGGATAGAC-3'
I $\mu$ -C $\alpha$	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-TAATCGTGAATCAGGCAG-3'
I $\mu$ -C $\epsilon$	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-ACAGGGCTTCAAGGGGTAGA-3'
<u>Mature V<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub> transcripts</u>		
V <sub>H</sub> DJ <sub>H</sub> -C $\gamma$ 1	5'-CATCTGAGGACTCTGCNGTCTAT-3'	5'-ATGGAGTTAGTTTGGGCAGCA-3'
V <sub>H</sub> DJ <sub>H</sub> -C $\gamma$ 3	5'-CATCTGAGGACTCTGCNGTCTAT-3'	5'-ACCAAGGGATAGACAGATGGGG-3'
V <sub>H</sub> DJ <sub>H</sub> -C $\alpha$	5'-CATCTGAGGACTCTGCNGTCTAT-3'	5'-TAATCGTGAATCAGGCAG-3'
V <sub>H</sub> DJ <sub>H</sub> -C $\epsilon$	5'-CATCTGAGGACTCTGCNGTCTAT-3'	5'-ACAGGGCTTCAAGGGGTAGA-3'
<u>Mature miRNA and sn/snoRNA forward primers (used with Qiagen miScript Universal Primer as reverse primer)</u>		
mmu-miR-155-5p	5'-TTAATGCTAATTGTGATAGG-3'	
hsa-miR-155-5p	5'-TTAATGCTAATCGTGATAGGGG-3'	
miR-181b-5p	5'-AACATTCATTGCTGTCGGTG-3'	
miR-361-5p	5'-TTATCAGAATCTCCAGGGGTAC-3'	
miR-23b-3p	5'-ATCACATTGCCAGGGATTACC-3'	
miR-30a-5p	5'-TGTAACATCCTCGACTGG-3'	

miR-125b-5p	5'-TCCCTGAGACCCTAACTTGTGA-3'	
miR-19a/b-3p	5'-TGTGCAAATCTATGCAAAACTG-3'	
miR-20a-5p	5'-AAAGTGCTTATAGTGCAGGTAG-3'	
miR-25-3p	5'-CATTGCACTTGTCTCGGTCTG-3'	
Rnu6/RNU6-1/2	5'-GCTTCGGCAGCACATATACTAAAAT-3'	
Snord61/SNORD61	5'-CCACTGATCTTCCGACATGA-3'	
Snord68/SNORD68	5'-GCTGTACTGACTTGATGA-3'	
Snord70/SNORD70	5'-TTTTGGAAGTGAATCTAAGTGATTT-3'	
<u>Human genes</u>		
V <sub>H</sub> DJ <sub>H</sub> FR3-C $\gamma$ 1	5'-GACACGGCYGTRTATTACTGTGCG-3'	5'-AGTAGTCCTTGACCAGGCAGCC-3'
V <sub>H</sub> DJ <sub>H</sub> FR3-C $\epsilon$	5'-GACACGGCYGTRTATTACTGTGCG-3'	5'-CGGAGGTGGCATTGGAGG-3'
V <sub>H</sub> DJ <sub>H</sub> FR3-C $\alpha$	5'-GACACGGCYGTRTATTACTGTGCG-3'	5'-GTGGGAAGTTTCTGGCGGT-3'
<i>AICDA</i>	5'-CATCTCGGACTGGGACCTAGA-3'	5'-GGTCCCTCGCAGAAAGTCG-3'
<i>PRDM1</i>	5'-AGCCCTGGGAATACGGTGT-3'	5'-CGTTGTACGAGGGGATGAAAG-3'
<i>XBP1</i>	5'-GCAGGCCAGTTGTCACCCC-3'	5'-TGGCAGGCTCTGGGGAAGGG-3'
<i>HPRT1</i>	5'-TGCTCGAGATGTGATGAAGG-3'	5'-TCCCCTGTTGACTGGTCATT-3'
<u>Somatic mutations</u>		
V <sub>186.2</sub> -C $\gamma$ 1	5'-CATGCTCTTCTTGGCAGCAACAGC-3'	5'-GTGCACACCGCTGGACAGGGATCC-3'
JX13F-JX4R	5'-AGCCTGACATCTGAGGAC-3'	5'-TCTGATCGGCCATCTTGACTC-3'
JX15F-JX5R	5'-CATCTGAGGACTCTGCNGTCTAT-3'	5'-CCTCACTCCCATTCTCGGTTAAA-3'
<u>miRNA host gene (HG) promoter ChIP</u>		
miR-155 HG	5'-AAGGTCATGAGTTCAAGGCCAGC-3'	5'-TGTGCATGTGTGCATGAGTGCCT-3'
miR-181b HG	5'-GGAGTTGAATTTCAAGCAGTAGGCA-3'	5'-ACTGCAAGGACGCATGTAGGTCA-3'
miR-361 HG	5'-ACATGCCTTGGTTTGCAGAG-3'	5'-GGAGGTGACAGTTATGGAGGC-3'
miR-23b HG	5'-AGCTGTACCTGCTTCACACC-3'	5'-AAGCCACATGATGAGCCACA-3'
miR-30a HG	5'-TGCAAAAGACTAGAGTGGTGCT-3'	5'-GTGAAGGGTCTCCTAGTTGCC-3'
miR-125b HG-1	5'-TCCTCTCCGAGTCAATCGTGCT-3'	5'-ACAGCCCTGTATATGCAACACACAC-3'
miR-125b HG-2	5'-CGTGTTCGCTCCCCTCAGT-3'	5'-ATGCAAAGGCACGACCCGCA-3'
<u>Bisulfite PCR</u>		
<i>Aicda</i> promoter	5'-TGATTTTTGTTATTTGTTGTTATTTG-3'	5'-TACTCTTATAAACTCCTCCCCAC-3'

---