Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/14/eaay2793/DC1

Supplementary Materials for

B cell Sirt1 deacetylates histone and non-histone proteins for epigenetic modulation of AID expression and the antibody response

Huoqun Gan, Tian Shen, Daniel P. Chupp, Julia R. Taylor, Helia N. Sanchez, Xin Li, Zhenming Xu, Hong Zan*, Paolo Casali*

*Corresponding author. Email: zan@uthscsa.edu (H.Z.); pcasali@uthscsa.edu (P.C.)

Published 1 April 2020, *Sci. Adv.* **6**, eaay2793 (2020)
DOI: 10.1126/sciadv.aay2793

This PDF file includes:

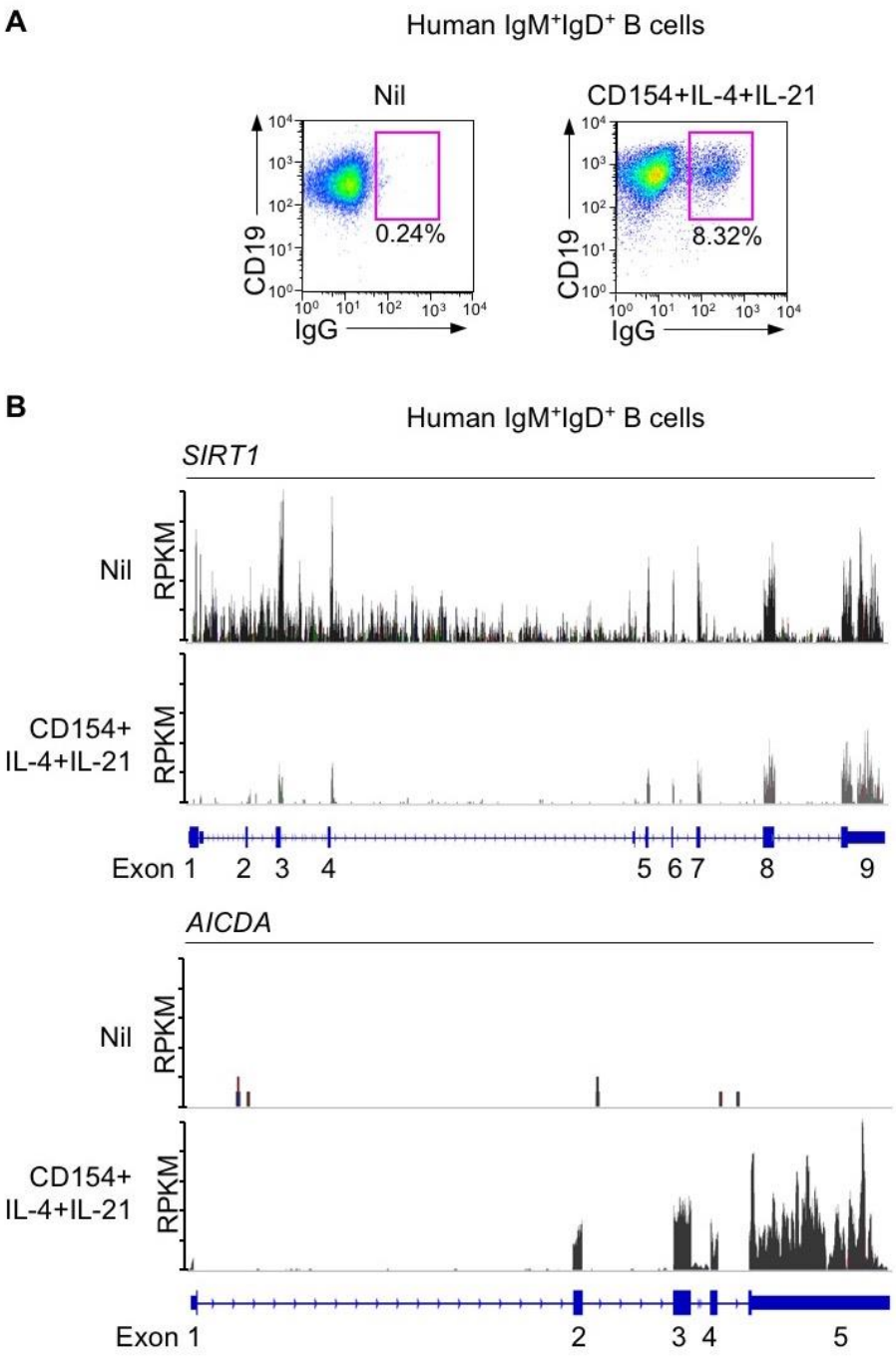

Fig. S1. Human B cells undergoing CSR downregulate *SIRT1* and upregulate *AICDA*. (**A**) Surface expression of CD19 and IgG on unstimulated human IgM$^+$IgD$^+$ naïve B cells (Nil) or similar B cells stimulated with CD154 plus IL-4 and IL-21 for 120 hours, as analyzed by flow cytometry. (**B**) Profile of RNA-Seq reads (RPKM) at the *SIRT1* and *AICDA* loci in unstimulated human IgM$^+$IgD$^+$ naïve B cells or similar B cells stimulated with CD154 plus IL-4 and IL-21 for 72 hours. Data are from one experiment.

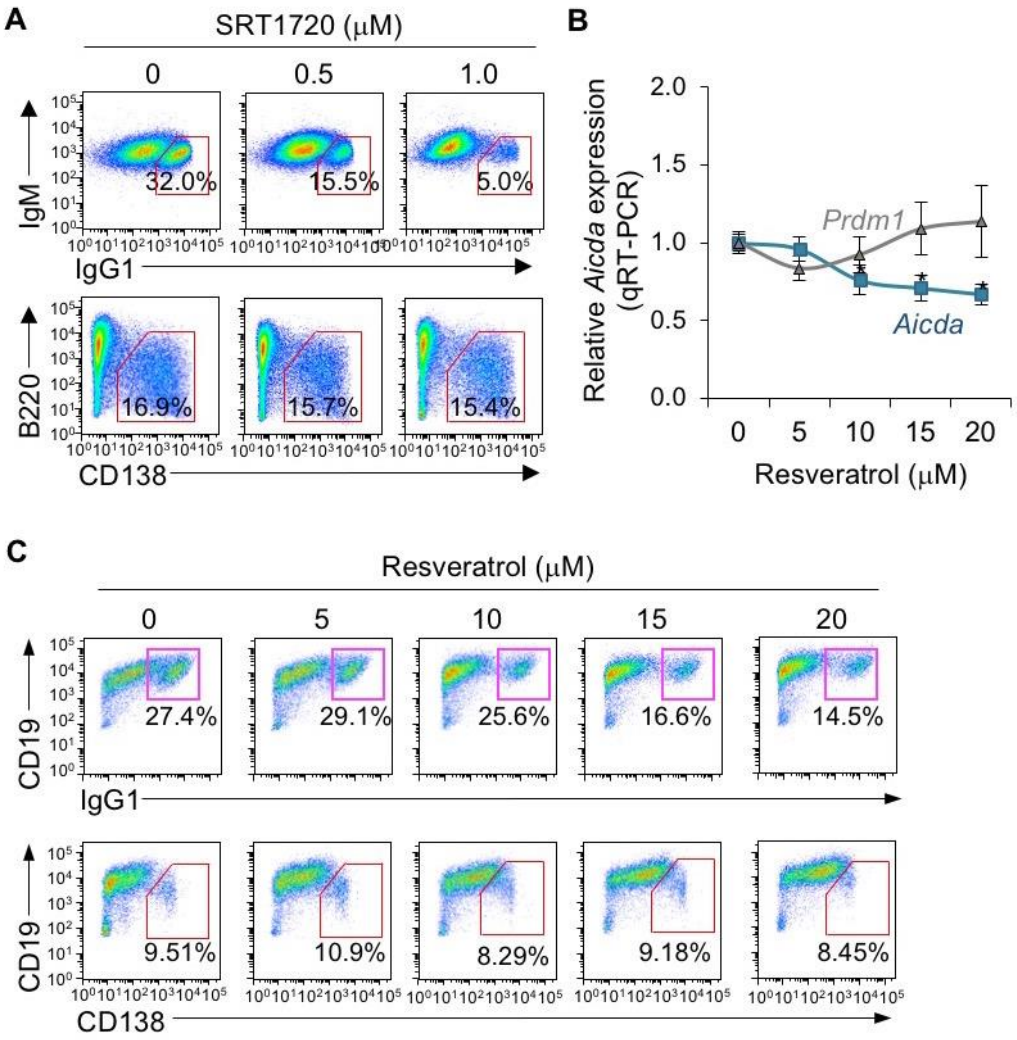

Fig. S2. Sirt1 activators dampen *Aicda* expression and CSR but not plasma cell differentiation. B cells isolated from WT C57BL/6 mice were stimulated with LPS plus IL-4 with nil or increasing doses of SRT1720 (**A**) or resveratrol (**B,C**). (**A**) Surface expression of IgM, IgG1, B220 and CD138 after 96-hour culture, as analyzed by flow cytometry. Data are representative of three independent experiments yielding comparable results. (**B**) Expression of *Aicda* and *Prdm1,* as analyzed by qRT-PCR and normalized to β-*Actin* expression after 72-hour culture. Data are means ± SEM of of three biological independent experiments, each consisting of triplicates. **P* < 0.05, paired two-tailed Student's *t*-test. (**C**) Surface expression of IgG1, CD19 and CD138 after 96-hour culture, as analyzed by flow cytometry. Data are representative of three independent experiments yielding comparable results.

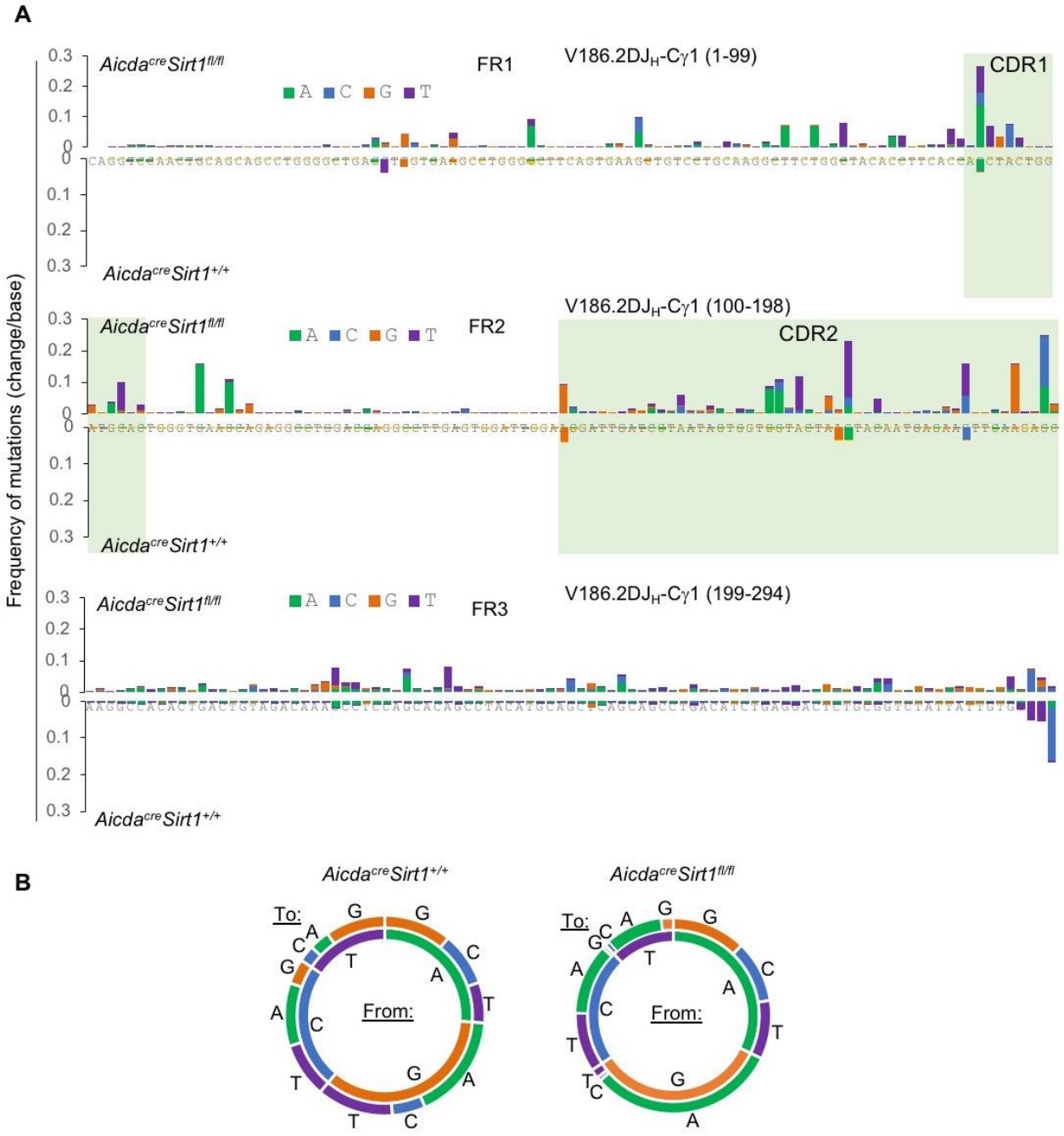

Fig. S3. Increased somatic mutations in *Aicda^{cre}Sirt1$^{fl/fl}$* mice. *Aicda^{cre}Sirt1$^{+/+}$* and *Aicda^{cre}Sirt1$^{fl/fl}$* mice were injected with NP_{16}-CGG (i.p.) at days 0 and 21. Somatic point mutations in the $V_{186.2}$ region of $V_{186.2}DJ_H$-Cγ1 transcripts amplified from spleen B cells 7 days after the second NP-CGG injection were analyzed by MiSeq amplicon sequencing. (**A**) Distribution of point-mutations in $V_{186.2}$ region. (**B**) Spectrum of point-mutations. Data were pooled from two *Aicda^{cre}Sirt1$^{+/+}$* and two *Aicda^{cre}Sirt1$^{fl/fl}$* mice, each including more than 5,000 sequences.

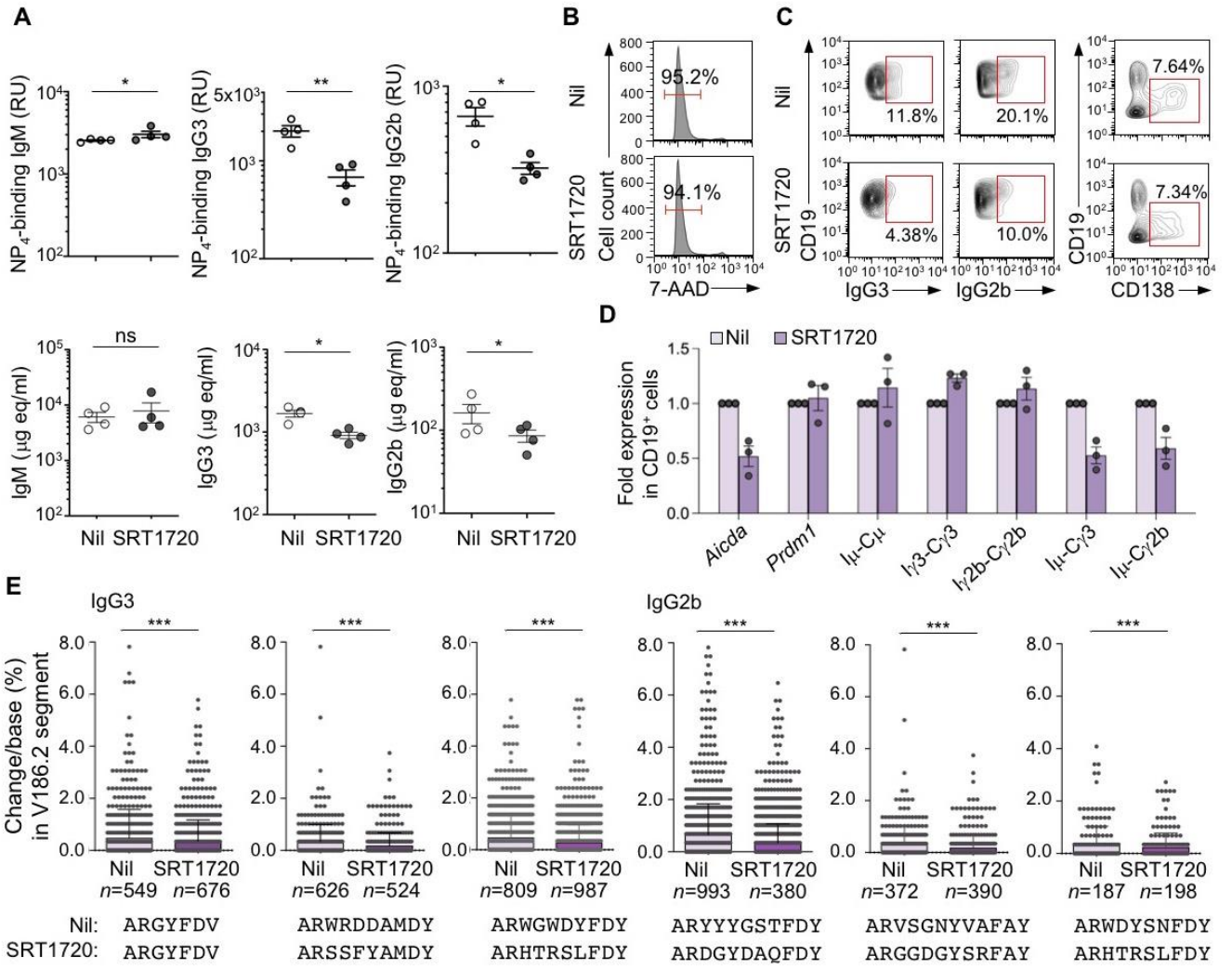

Fig. S4. Sirt1 activation dampens the class-switched/hypermutated T-independent antibody response in a B cell-intrinsic fashion. The NBSGW/B mice were injected (i.p.) with SRT1720 (0.3 mg in 200 ml PBS, *n* = 4) or PBS only (Nil, *n* = 4) once every two days. These mice were then i.p. injected with $NP_{0.5}$-LPS (50 μg in 200 ml PBS) at days 0, 2, 4 and 6, and sacrificed 7 d after the last $NP_{0.5}$-LPS injection. (**A**) Serum titers of total and NP_4-binding IgM, IgG3 and IgG2b as measured by ELISA (means ± SEM). (**B** and **C**) Viable (7-AAD^-) spleen $CD19^+$ B cells **(B)** as well as CSR and plasma cell differentiation (**C**) as analyzed by flow cytometry. Data are from mice representative of each group. (**D**) qRT-PCR analysis of gene expression, as indicated. Values were normalized to the expression of *Gapdh* and depicted as ratios to expression in nil-treated mice (means ± SEM of values from 4 mice, each consisting of triplicates). (**E**) Somatic point-mutations in the $V_{186.2}$ region of $V_{186.2}DJ_H$-Cγ2b and $V_{186.2}DJ_H$-Cγ3 cDNA clones from three pairs of mice treated with nil or SRT1720. The frequency of mutations in different NBSGW/B mice were compared by enumerating the point-mutations in the $V_{186.2}$ segment of three "comparable" $V_{186.2}DJ_H$-Cg3 and $V_{186.2}DJ_H$-Cg2b clone pairs (each pair consisting of a mouse treated with nil and one with SRT1720). Comparable pairs were defined as B cell clones expressing $V_{186.2}DJ_H$-Cg3 or $V_{186.2}DJ_H$-Cg2b with IgH CDR3s being identical in length (7 to 12 amino acids), identical in the first two amino acids and identical in the last three or four amino acids. One of the three $V_{186.2}DJ_H$-Cg3 clone pairs was in fact identical in the whole IgH CDR3 sequence (ARGYFDY). Each dot represents an individual sequence. The number of sequences analyzed from each clone is indicated below the dot plots. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, unpaired two-tailed Student's *t*-test.

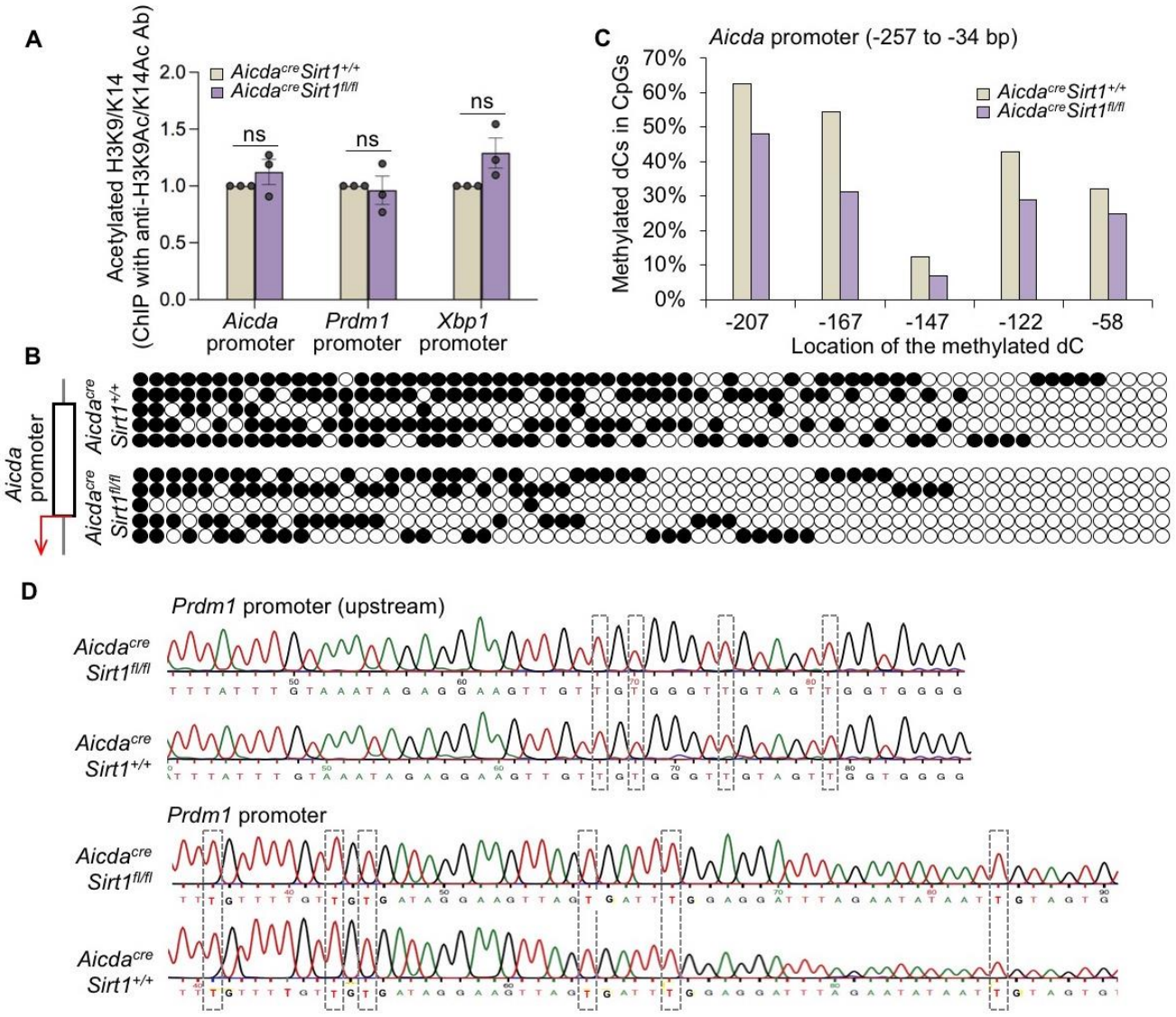

Fig. S5. *Aicda^{cre}Sirt1$^{fl/fl}$* B cells display altered DNA methylation in *Aicda* promoter but not DNA methylation in *Prdm1* promoter and Acetylated H3K9/K14 in *Aicda, Prdm1* or *Xbp1* promoter. *Aidca^{cre}Sirt1$^{+/+}$* and *Aicda^{cre}Sirt1$^{fl/fl}$* B cells stimulated with LPS plus IL-4 for 72 hours. (**A**) Acetylated H3K9/K14 in *Aicda, Prdm1* or *Xbp1* promoter were analyzed by ChIP using a rabbit anti-H3K9Ac/K14Ac Ab followed by qPCR. Data are means ± SEM of three biological independent experiments, each consisting of triplicates. ns, not significant, paired two-tailed Student's *t*-test. (**B**) CpG DNA methylation of *Aicda* promoter DNA as analyzed by bisulfite sequencing. Upon treatment with bisulfite, unmethylated dC nucleotides are converted to dU (read as dT in DNA sequence), whereas methylated dC are not. Methylation patterns at each dCs within CpG motifs are indicated by circles: ●, methylated dCs; ○, unmethylated dCs. Each row is a unique sequence, and each dC is represented by a column of circles. (**C**) Proportion of methylated dC nucleotides within CpG motifs. Pooled data from B cells derived from two *Aicda^{cre}Sirt1$^{+/+}$* and two *Aicda^{cre}Sirt1$^{fl/fl}$* mice; each data cluster included more than 5,000 sequences. (**D**) CpG DNA methylation of the *Prdm1* promoter, as analyzed by bisulfite sequencing. Depicted are DNA sequences of PCR products of bisulfite-treated genomic DNA. The sequence signal from dCs in CpG motifs is outlined. Because unmethylated dC nucleotides can be converted to dU (read as dT in DNA sequence), whereas methylated dC cannot, the ratio of the dC (blue)/dT (red) signal provides and index of dC methylation at any given positions. Data are representative of three independent experiments yielding comparable results.

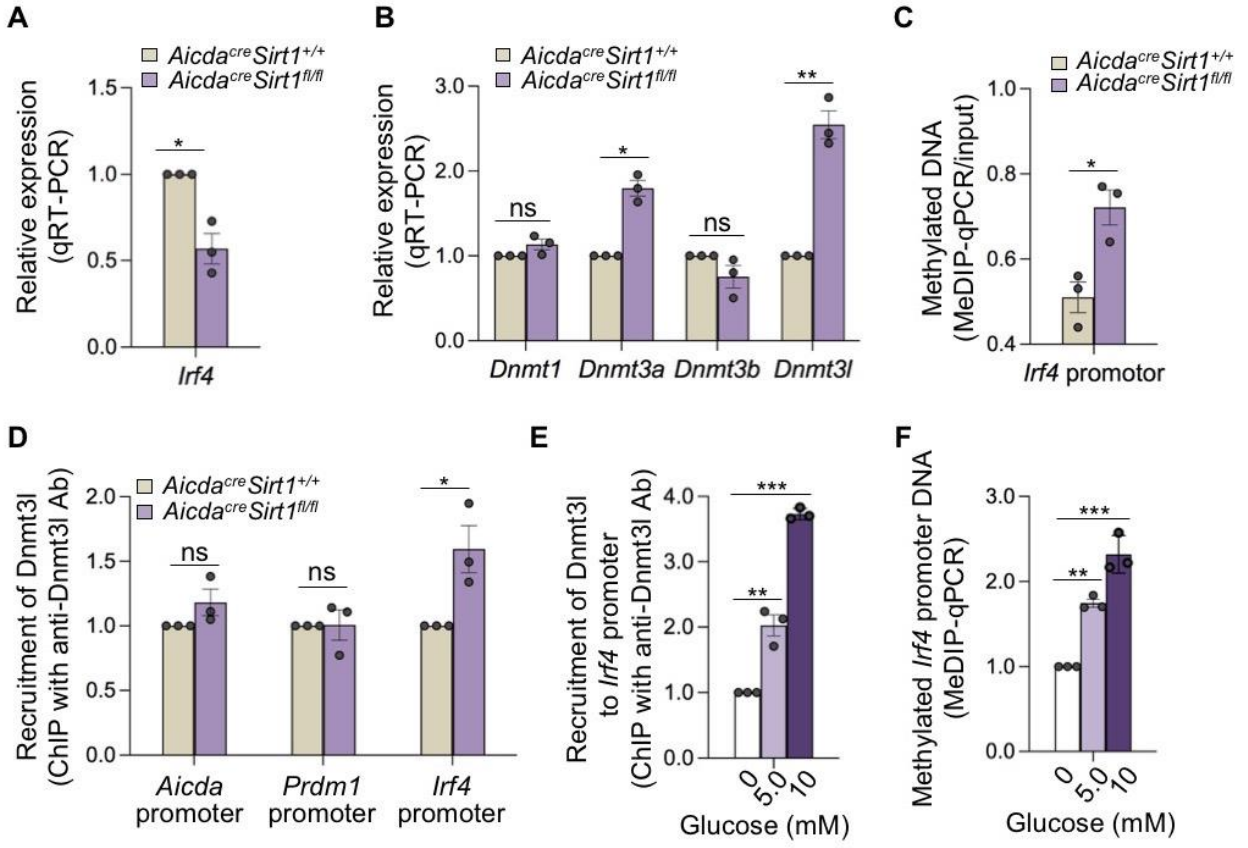

Fig. S6. *Aicda^{cre}Sirt1$^{fl/fl}$* B cells stimulated to undergo CSR and plasma cell differentiation increase *Dnmt3l* expression and Dnmt3l recruitment to the *Irf4* promoter. (**A**) Expression of *Irf4 in Aidca^{cre}Sirt1$^{+/+}$* and *Aicda^{cre}Sirt1$^{fl/fl}$* B cells stimulated with LPS plus IL-4 for 72 hours. (**B**) Expression of *Dnmt1, Dnmt3a, Dnmt3b* and *Dnmt3l* transcripts were analyzed by qRT-PCR and normalized to β-*Actin* expression. (**C**) Increased DNA methylation of *Irf4* promoter in and *Aicda^{cre}Sirt1$^{fl/fl}$* B cells. (**D**) Recruitment of Dnmt3l to the *Aicda, Prdm1* or *Irf4* promoter, as detected by ChIP assays using anti-Dnmt3l,followed by qPCR. (**E** and **F**) C57BL/6 mouse B cells were cultured in glucose-free medium supplied with 0, 5.0, or 10 mM of glucose and stimulated with LPS plus IL-4 for 72 hours (**E**). DNA methylation of the *Irf4* promoter, as analyzed by MeDIP-qPCR (**F**). Data in **A** to **F** are means ± SEM of three biological independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns, not significant, paired two-tailed Student's *t*-test.

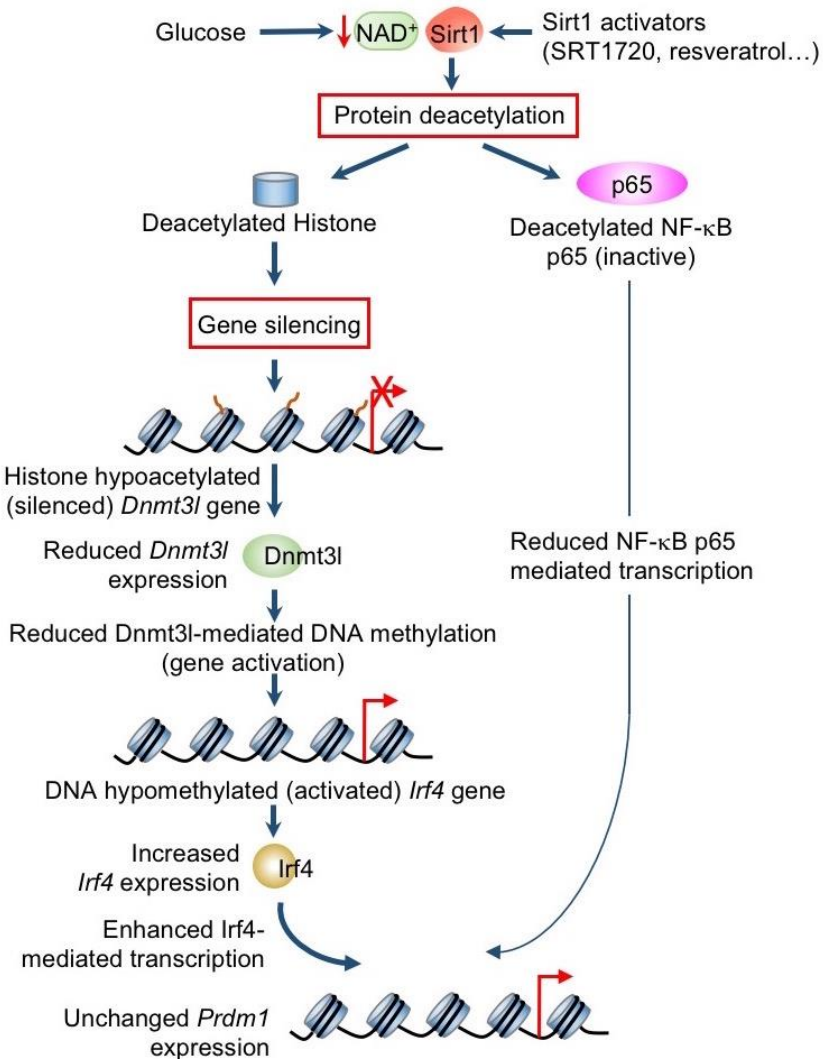

Fig. S7. Sirt1 increases expression of *Irf4,* a positive regulator of *Prdm1*, by reducing *Dnmt3l* expression and Dnmt3l-mediated DNA methylation of the *Irf4* gene, thereby nullified a potential *Prdm1* upregulation brought about by increased p65 acetylation. As shown in **Fig. 4I,** in *Aicda*^{cre}*Sirt1*$^{fl/fl}$ B cells stimulated by LPS or CD154 plus IL-4, increased NF-κB p65 acetylation resulted in increased recruitment of p65-Ac to the *Aicda* promoter (by up to 8.7 folds, as shown by ChIP with an anti-p65-Ac mAb), further increasing *Aicda* expression. p65 acetylation also resulted in some recruitment of p65-Ac to the *Prdm1* promoter (by less than two folds). This was not, however, associated with upregulated *Prdm1* expression **(Fig. 2M).** This was possibly due to decreased *Irf4,* which positively regulates *Prdm1* expression by targeting *Prdm1* regulatory region (Sciammas et al., *Immunity,* 25:225–236 , 2006). *Irf4* expression is negatively controlled by DNA methylation of its promoter (Ortmann et al., *Nucleic Acids Res.* 33:6895-6905,2005), which, as suggested by our experiments, can be effected by the DNA methytransferase Dnmt3l (**fig. S6**). Sirt1 can downregulate the expression of Dnmt3l by histone deacetylation of the *Dnmt3l* promoter (Heo et al., *Cell Rep*., 18:1930-1945, 2017). Dnmt3l can be recruited to the promoter of *Irf4* and modulate *Irf4* DNA methylation and expression. B cell-specific *Sirt1* deletion or metabolic Sirt1 inactivation by higher glucose concentration resulting inincreased *Dnmt3l* expression and Dnmt3l recruitment to the *Irf4* promoter, which resulted in increased DNA methylation in the *Irf4* promoter that led to reduced *Irf4* expression (**fig. S6**). This would have nullified a potential *Prdm1* upregulation brought about by increased p65 acetylation and p65-Ac recruitment to the *Prdm1* promoter, thereby leading to virtually unchanged *Prdm1* expression in B cells in which *Sirt1* was downregulated, deleted or Sirt1 metabolically inactivated.

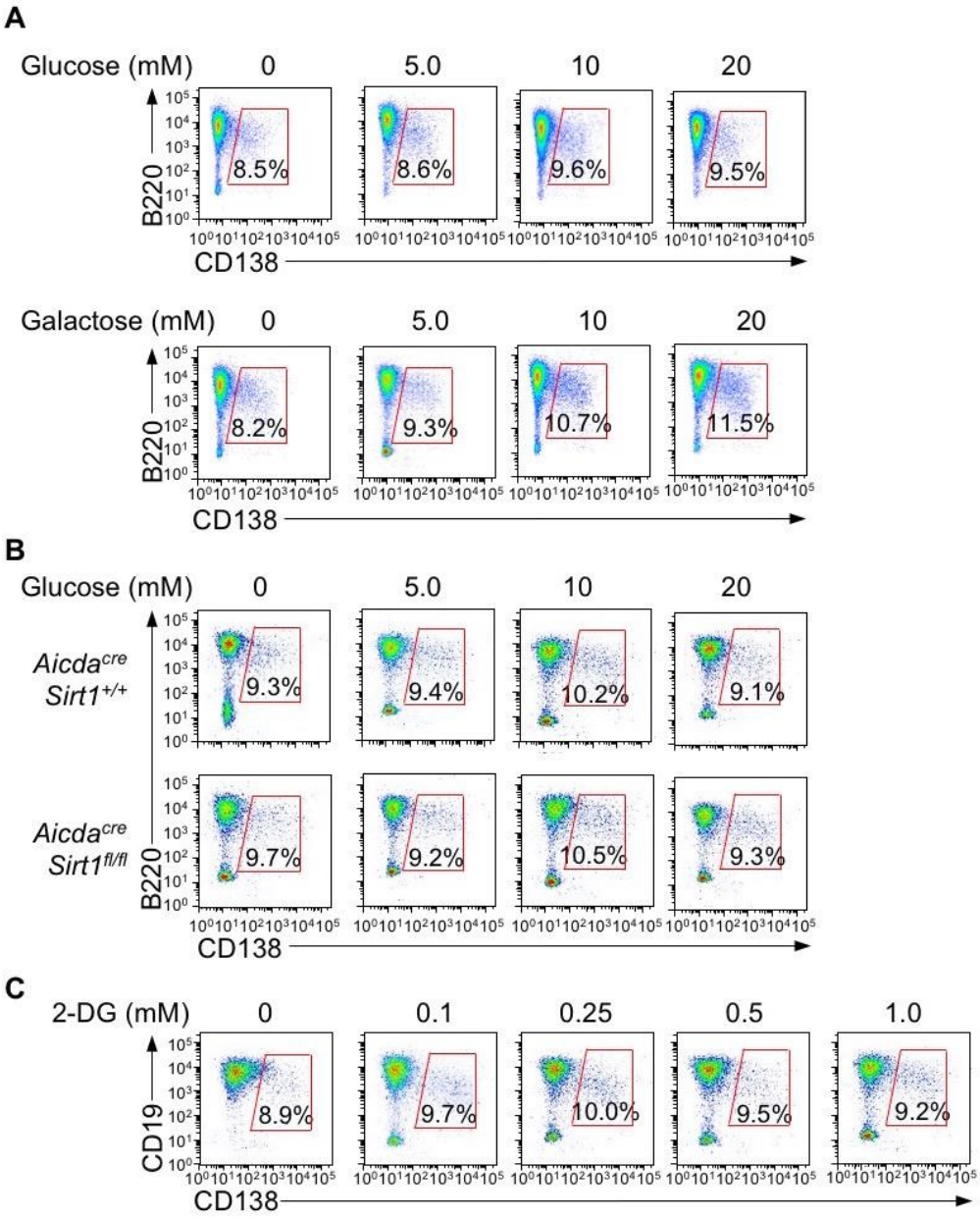

Fig. S8. Plasma cell differentiation is not significantly altered by increased concentration of glucose. (**A**) Murine C57BL/6 B cells were cultured in glucose-free medium supplied with 0 to 20 mM of glucose or galactose and stimulated with LPS plus IL-4 for 96 hours. Surface expression of B220 and CD138, as analyzed by flow cytometry. (**B**) *Aicda*^{cre}*Sirt1*$^{fl/fl}$ and *Aicda*^{cre}*Sirt1*$^{+/+}$ B cells were cultured in glucose-free medium supplied with 0 to 20 mM of glucose and stimulated with LPS plus IL-4 for 96 hours. (**C**) Surface expression of B220 and CD138 were analyzed by flow cytometry. Data are representative of three independent experiments yielding comparable results.

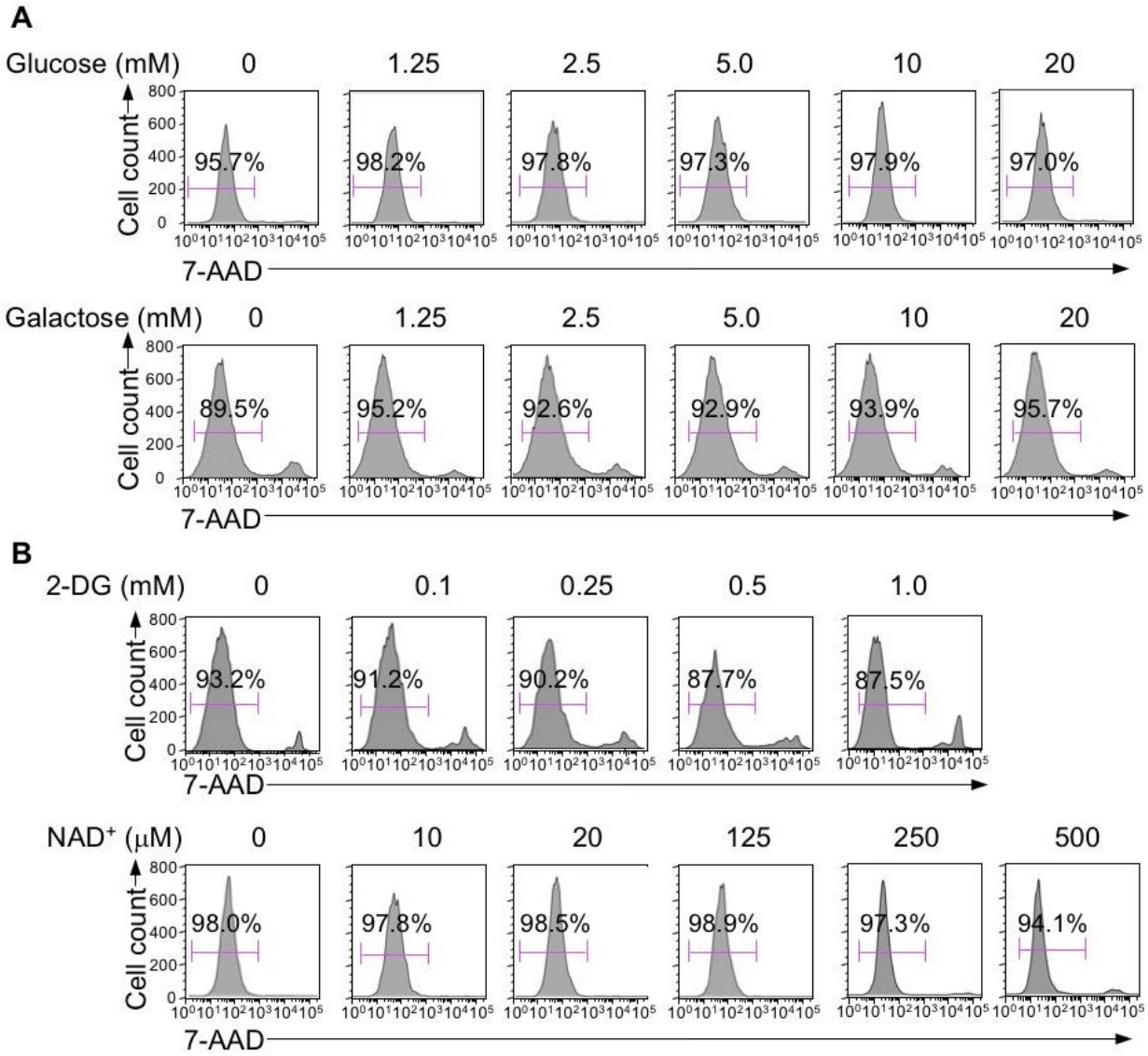

Fig. S9. Impact of glucose, galactose, 2-Deoxyglucose (2-DG) and NAD^+ on B cell viability. B cells were cultured in glucose-free medium supplied with increased concentration of glucose or galactose (**A**), or in complete RPMI-1640 medium in the presence of increased concentration of 2-DG or NAD^+ (**B**), and stimulated with LPS plus IL-4. Live ($7AAD^-$) B cells were analyzed by flow cytometry after 96-hour culture. Data are representative of three independent experiments yielding comparable results.

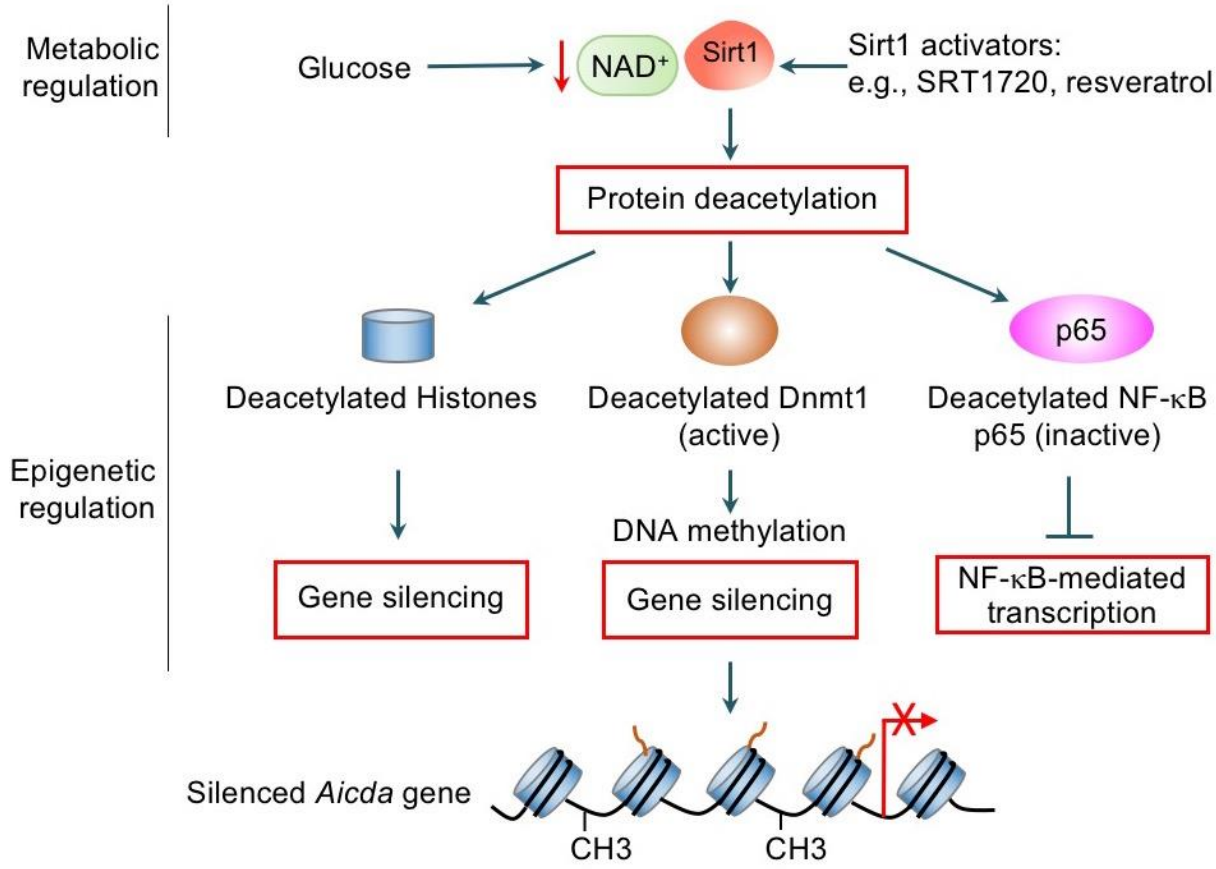

Fig. S10. Three-prong Sirt1-mediated modulation of *Aicda* expression, through deacetylation of B cell histone and non-histone proteins. By deacetylating histone and non-histone proteins, Sirt1 exerts a three-prong repression of *Aicda*: (i) By deacetylating histones in the *Aicda* promoter, Sirt1 directly represses *Aicda* expression; (ii) By deacetylating p65, Sirt1 reduces the activity of NF-κB, which is an important activator of the *Aicda* promoter; (iii) By deacetylating Dnmt1, Sirt1 enhances the DNA methylation activity of this methyltransferase, which leads to an increased DNA methylation of the *Aicda* promoter, thereby further repressing *Aicda* expression.

Table S1A. Antibodies used for this study.

Antibody to	Type	Company	Cat. No. (mAb clone)	Assays
Human/Mouse B220, PE-conjugated	Rat mAb	BioLegend	103208 (RA3-6B2)	FCM
Human/Mouse B220, Pacific Blue-conjugated	Rat mAb	BioLegend	103227 (RA3-6B2)	FCM
Mouse B220, FITC-conjugated	Rat mAb	BioLegend	103206 (RA3-6B2)	FCM
Mouse CD19, Pacific Blue-conjugated	Rat mAb	BioLegend	115523 (6D5)	FCM
Mouse IgM, PE-conjugated	Rat mAb	BioLegend	406508 (RMM-1)	FCM, IF
Mouse IgM, FITC-conjugated	Rat mAb	BioLegend	406506 (RMM-1)	FCM
Mouse IgG1, FITC-conjugated	Rat mAb	BD Biosciences	553443 (A85-1)	FCM, ANA, IC
Mouse IgG1, APC-conjugated	Rat mAb	BD Biosciences	550874 (X56)	FCM
Mouse IgG2a, FITC-conjugated	Rat mAb	BD Biosciences	553390 (R19-15)	FCM, ANA, IC
Mouse IgD, FITC-conjugated	Rat mAb	BioLegend	405713 (11-26C.2a)	FCM
Mouse IgA, FITC-conjugated	Rat mAb	BD Biosciences	559354 (C10-3)	FCM
Mouse CD138, PE-Cy7-conjugated	Rat mAb	BioLegend	142514 (281-2)	FCM
Mouse CD38	Rat, mAb	BioLegend	102718 (90)	FCM
Mouse CD3, biotin-conjugated	Rat mAb	BioLegend	100244 (17A2)	Cell isolation
Mouse CD3, FITC conjugated	Rat mAb	BioLegend	100204 (17A2)	FCM
Human/Mouse Sirt1	Rabbit pAb	ABclonal	A11267	FCM, IB, ChIP
Mouse AID, FITC-conjugated	Rabbit pAb	Bioss	bs-7855R-FITC	FCM
Mouse IgA, unconjugated	Rabbit mAb	Thermo Fisher	PA-1-30826	IF
Rabbit-IgG (H+L), Alexa Fluor 488-conjugated	Goat pAb	Cell Signaling	4412	IF
Mouse IgE, FITC-conjugated	Rat mAb	eBioscience	50-995-0 (23G3)	FCM
GL7, PE-conjugated	Rat mAb	BioLegend	144607 (GL7)	FCM, IF
Human CD19, PE-conjugated	Mouse mAb	BioLegend	302208 (HIB19)	FCM
Human IgG, allophycocyanin	Mouse mAb	BD Biosciences	562025 (G18-145)	FCM
Human CD19, Biotin-conjugated	Mouse mAb	BioLegend	302204 (HIB19)	Cell isolation
Mouse Ig, unconjugated	Goat pAb	Southern Biotech	1010-08	ELISA, ELISPOT
Mouse IgG, unconjugated	Goat pAb	Southern Biotech	1030-08	ELISA, ELISPOT
Mouse IgM, unconjugated	Goat pAb	Southern Biotech	1020-01	ELISA, ELISPOT
Mouse IgA, unconjugated	Goat pAb	Southern Biotech	1040-01	ELISA, ELISPOT

Mouse IgM, biotin-conjugated	Goat pAb	Southern Biotech	1022-08	ELISA, ELISPOT
Mouse IgG1, biotin-conjugated	Goat pAb	Southern Biotech	1070-08	ELISA, ELISPOT
Mouse IgG2a, biotin-conjugated	Goat pAb	Southern Biotech	1080-08	ELISA
Mouse IgG2b, biotin-conjugated	Rat mAb	BioLegend	406704 (RMG2b-1)	ELISA
Mouse IgG3, biotin-conjugated	Goat pAb	Southern Biotech	1100-08	ELISA
Mouse IgA, biotin-conjugated	Goat pAb	Southern Biotech	1040-08	ELISA
Mouse AID	Mouse mAb	Invitrogen	39-2500 (ZA001)	IB
acetyl-histone H3	Rabbit Ab	Millipore	17-615	ChIP
Mouse β-Actin	Mouse mAb	Sigma	A5441 (AC-15)	IB
NF-kB p65	Rabbit mAb	Cell Signaling	8242 (D14E12)	IB
Acetyl-NF-kB p65 (Lys310)	Rabbit mAb	Cell Signaling	(D2S3J)	IB, ChIP
Dnmt1	Rabbit pAb	Epigentek	A-1700	IB, ChIP
Acetyl-Dnmt1	Rabbit pAb	ABclonal	A11267	IB

Abbreviations: mAb, monoclonal antibody; pAb, polyclonal antibody; ANA, anti-nuclear antibody analysis; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunosorbent spot; FCM, flow cytometry; IB, immunoblotting; IC, immunocomplex analysis.

Table S1B. Primers used for this study.

	Forward primer	Reverse primer
Mouse genes		
Aicda	5'-AGAAAGTCACGCTGGAGACC-3'	5'-CTCCTCTTCACCACGTAGCA-3'
Prdm1	5'-GCTGCTGGGCTGCCTTTGGA-3'	5'-GGAGAGGAGGCCGTTCCCCA-3'
Sirt1	5'-TGTCTCCTGTGGGATTCCTGA-3'	5'-CTTGAGGGTCTGGGAGGTCT-3'
Xbp1	5'-AAGCCCGGATGAGCGAGCTG-3'	5'-ACCCGGCCACCAGCCTTACT-3'
Irf4	5'-AAAGAGCTGACCACGACGAG-3'	5'-AAAGCCCATCTGGAGCCATC-3'
Dnmt1	5'- GTGTCCTAACTTGGCGGTGA-3'	5'- TCTTCATAGGCTGCCCAAGC-3'
Dnmt3a	5'- AATAGAGACCCTCGGAGGCAG-3'	5'- GTGTCTGGTGTGGTGTTCTCT-3'
Dnmt3b	5'- CCTGGAGAGTCACTGGAGGA-3'	5'- TGGTTGTGCGTCTTCGACTT-3'
Dnmt3l	5'- TGCGGGTACTGAGCCTTTTT-3'	5'- ACGTACTTCAGCGTTCCTCC-3'
β-Actin	5'-CTAAGGCCAACCGTGAAAG-3'	5'-ACCAGAGGCATACAGGGACA-3'
Gapdh	5′-TTCACCACCATGGAGAAGGC-3′	5′-GGCATGGACTGTGGTCATGA-3′
Human genes		
SIRT1	5'-ACAGGTTGCGGGAATCCAAA-3'	5'-GTTCATCAGCTGGGCACCTA-3'
β-ACTIN	5'-AGAGCTACGAGCTGCCTGAC-3'	5'-AGCACTGTGTTGGCGTACAG-3'
Germline transcripts		
Iμ-Cμ	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-GCAGGCAGGGCTAGATATGG-3'
Iγ1-Cγ1	5′-TCGAGAAGCCTGAGGAATGTG-3′	5′-ATGGAGTTAGTTTGGGCAGCA-3′
Iγ3-Cγ3	5'-AACTACTGCTACCACCACCACCAG-3'	5'-AGCCAGGGACCAAGGGATAGAC-3'
Iγ2b-Cγ2b	5'GATGGGGAGGAGTTGGCAGAT-3'	5'-CGGAGGAACCAGTTGTATC-3'
Iα-Cα	5'-GCTTCCTGGAAAGCAGCAAC-3'	5'-TAATCGTGAATCAGGCAG-3'
Iε-Cε	5'-CCCCACTTTTAGCTGAGGGC-3'	5'-ACAGGGCTTCAAGGGGTAGA-3'
Post-recombination transcripts		
Iμ-Cγ1	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5′-ATGGAGTTAGTTTGGGCAGCA-3′
Iμ-Cγ3	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-AGCCAGGGACCAAGGGATAGAC-3'
Iμ-Cγ2b	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-CGGAGGAACCAGTTGTATC-3'
Iμ-Cα	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-TAATCGTGAATCAGGCAG-3'
Iμ-Cε	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-ACAGGGCTTCAAGGGGTAGA-3'
Somatic mutations		
$V_{186.2}DJ_H$-Cγ1	5'-TCGTCGGCAGCGTCAGATGTGTATAAG GACAGCAGGTCCAACTGCAGCAG-3'	5'-GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAGTGCTCAGCTTGGATCTCTGC-3'
$V_{186.2}DJ_H$-Cγ3	5'-TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCAGGTCCAACTGCAGCAG-3'	5'-GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAGACCAAGGGATAGACAGATGGGG-3'
$V_{186.2}DJ_H$-Cγ2b	5'-TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCAGGTCCAACTGCAGCAG-3'	5'-GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAGCGGAGGAACCAGTTGTATC-3'
ChIP and MeDIP		
Aicda promoter	5'-GGAGGCAGATGTTGGATACC-3'	5'-ATATCGGTCTCCAGCGTGAC-3'
Prdm1 promoter	5'-ACTCCAGGACTACACAGCGA-3'	5'-GGATCGCTAGCTTCCTGTCG-3'
Prdm1 intron	5'-CTCTGACTCTGGTCTGAAGT-3'	5'-GTCTCCTGCTTCGTGTTATC-3'
Xbp1 promoter	5'-CCTAAGCCGGATATGCCACC-3'	5'-CCCCATTTTAATGTCCGGCG-3'
Irf4 promoter	5'- CTCTGACAATGGAAAACTAATTG-3'	5'- GAAAACTGGTGCAGAGATGC-3'
Bisulfite PCR		
Aicda promoter	5'-TGATTTTTGTTATTTGTGGTATTTG-3'	5'-TACTCTTATAAACTCCTCCCCCAC-3'

Prdm1 promoter	5’-TGTTGAAGGTAAAAGATTATTGAAGG-3’	5’-ATTATCCCTACCTCTCATACCCAAA-3’
Prdm1 reg. region	5’-GGGATATGGTGTTTATTTTGATTGA-3’	5’-TCCAAAAATAACCTAAATTTCCTCA-3’
Xbp1 promoter	5’-TGTAGTTTAGGTTGTTTTTAAATTGG-3’	5’-TTTAATTTTTGTTGTATTGGGAAGTG-3’
Bisulfite-Seq		
Aicda promoter	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGTGATTTTTGTTATTTGTGGTATTTG-3’	5’-GTCTCGTGGGCTCGGAGATGTGTATAAGA GACAGTACTCTTATAAACTCCTCCCCCAC-3’
Prdm1 promoter	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGTTTTTTGAGGAGGAGTTTTTTATTT-3’	5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAGAAATCTTCTTACTTCCCTTTACAAAC-3’
Prdm1 reg. region	[TCGTCGGCAGCGTCAGATGTGTATAAG AGACAG]GGGATATGGTGTTTATTTTGATTGA	5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAGTCCAAAAATAACCTAAATTTCCTCA-3’
Xbp1 promoter	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGTGTAGTTTAGGTTGTTTTTAAATTGG-3’	5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAGTTTAATTTTTGTTGTATTGGGAAGTG-3’