Supporting Information

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SI Materials and Methods

Cell Culture. Human Burkitt's lymphoma (BL)2 cells were cultured in RPMI medium 1640 (Invitrogen) supplemented with 10% (vol/vol) FBS and penicillin-streptomycin. The BL2 cell lines used in this study were established and maintained as described (1, 2). Splenocytes were isolated from the spleens of wild-type C57BL/6 mice and cultured for 2 d with lipopolysaccharide before being used for ChIP assays.

Expression Level Analysis by Quantitative RT-PCR. To analyze the mRNA expression levels of genes of interest, the total RNA was purified with TRIzol reagent (Invitrogen) and subjected to one-step quantitative RT-PCR analysis with the QuantiTect SYBR Green RT-PCR kit (Qiagen) and the Applied Biosystems 7500 Fast Real-Time PCR system. The relative expression level was calculated with the ΔΔCt method, using GAPDH mRNA expression as an internal control. The sequences of the primers are shown in Table S4.

Western Blot. Cells were lysed with RIPA buffer (10 mM Tris·HCl pH 7.9, 150 mM NaCl, 0.1% SDS, 0.1% deoxycholate, 1% TritonX-100, and 1 mM EDTA) and were subjected to Western blot following standard protocols. Information about the antibodies used is in Table S7.

Cell Cycle Analysis. Cells were fixed with 70% ethanol at 4 °C for 30 min followed by RNaseA treatment and 50 μg/mL propidium iodide staining and were analyzed by flow cytometry with Cell Quest software (BD).

Chromatin Immunoprecipitation Assay. The chromatin immunoprecipitation (ChIP) assay was performed as described previously (3). The primers and antibodies used for the ChIP assay are shown in Tables S6 and S7, respectively. Note that the lamin B2 (*Lmnb2*) intergenic primer set was designed by Tan et al. as a negative control for the structure specific recognition protein 1 (SSRP1) ChIP experiment (4).

1. Kobayashi M, et al. (2011) Decrease in topoisomerase I is responsible for activation-induced cytidine deaminase (AID)-dependent somatic hypermutation. *Proc Natl Acad Sci USA* 108(48):19305–19310.
2. Okazaki IM, et al. (2011) Histone chaperone Spt6 is required for class switch recombination but not somatic hypermutation. *Proc Natl Acad Sci USA* 108(19): 7920–7925.
3. Oshima S, et al. (2004) Interferon regulatory factor 1 (IRF-1) and IRF-2 distinctively up-regulate gene expression and production of interleukin-7 in human intestinal epithelial cells. *Mol Cell Biol* 24(14):6298–6310.
4. Tan BC, Chien CT, Hirose S, Lee SC (2006) Functional cooperation between FACT and MCM helicase facilitates initiation of chromatin DNA replication. *EMBO J* 25(17): 3975–3985.

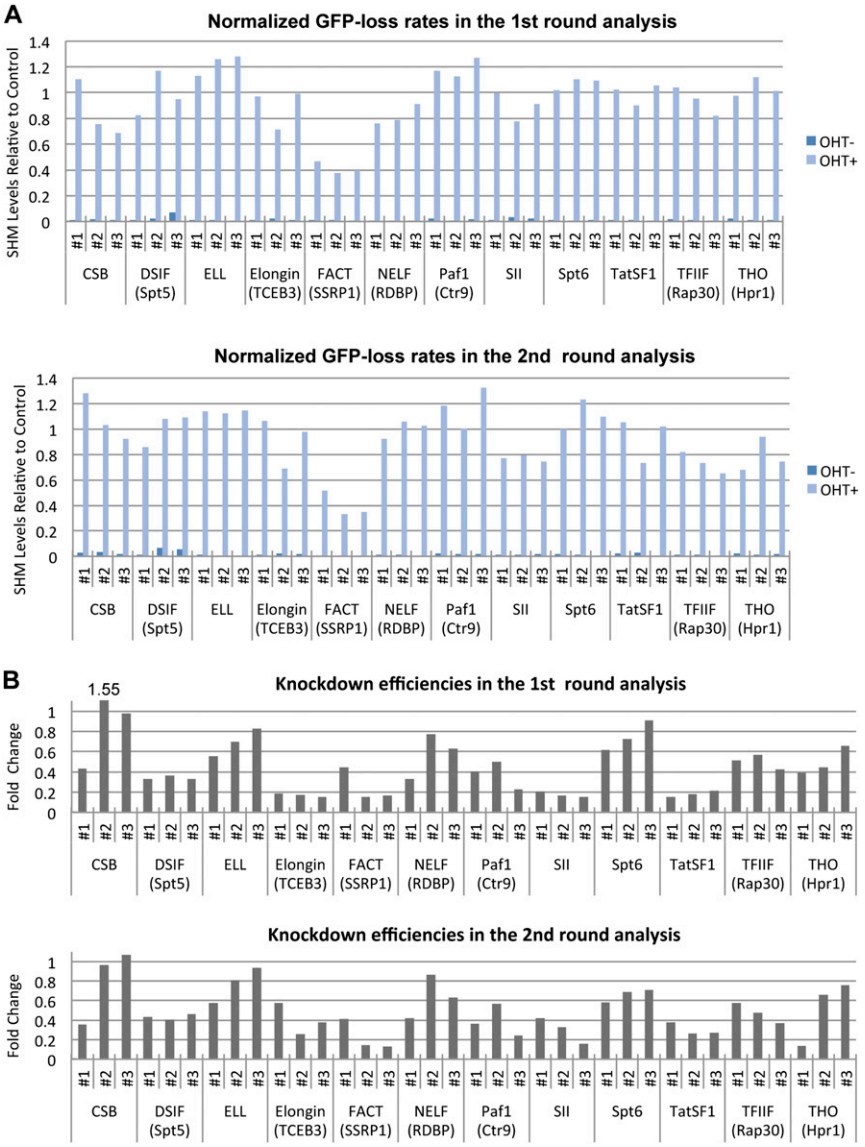
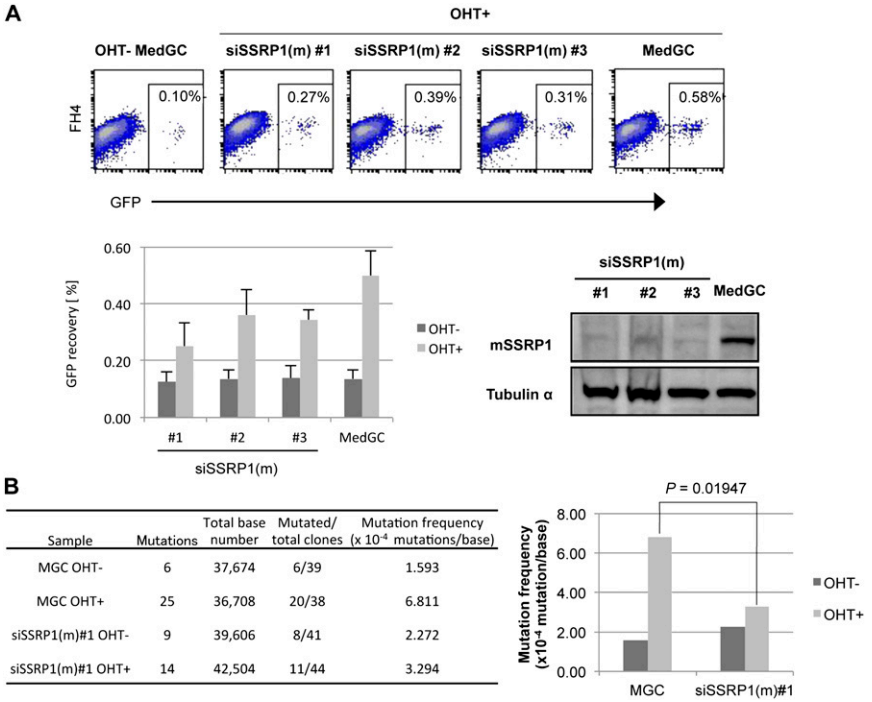


Fig. S1. Candidate-based screening for somatic hypermutation (SHM)-related transcription elongation factors. (*A*) Effects of RNAi oligos on the GFP fluorescence-negative cell population with or without 4-hydroxyl Tamoxifen (OHT) treatment (shown as OHT+ and OHT–, respectively). The GFP-negative cell population of each sample was normalized to that of OHT-treated control experiments in which cells were transfected with the control oligo medium GC (MedGC). Experiments were done in duplicate with three different oligos per candidate factor. The data of each round are shown. Details of the RNAi oligos are shown in Table S3. (*B*) Knockdown levels of each RNAi oligo. Total RNA was purified from each OHT– sample 48 h after the transfection. Target expression levels relative to those of MedGC control cells were then analyzed by qRT-PCR. Data for both rounds of experiments are shown.

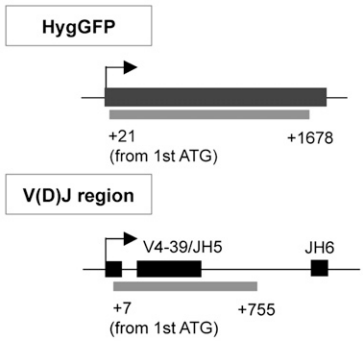


B

Sample	Mutations	Total base number	Mutated/ total clones	Mutation frequency (x 10^{-4} mutations/base)
MGC OHT-	6	37,674	6/39	1.593
MGC OHT+	25	36,708	20/38	6.811
siSSRP1(m)#1 OHT-	9	39,606	8/41	2.272
siSSRP1(m)#1 OHT+	14	42,504	11/44	3.294

Fig. S2. Reduction of SHM by SSRP1 knockdown in the GFP-recovery SHM. In this system, SHM by the activation induced-cytidine deaminase (AID) transgene fused with the estrogen-binding domain (AID-ER) can be monitored as the recovery of GFP fluorescence by a point mutation at a premature stop codon inserted in the middle of the GFP reporter gene. Twenty-four hours after transfecting siRNAs against mouse SSRP1 with Lipofectamine RNAiMAX (Invitrogen), SHM was induced by treating cells with 1 mM OHT followed by a 72-h incubation, before FACS analysis. (*A Upper*) Representative FACS results of SSRP1(m) (mouse SSRP1) siRNA- or MedGC-treated samples. The cell population surrounded by a rectangle represents the GFP-recovered cells. The percentage of this population is shown in each plot. SSRP1 knockdown was carried out with three different siRNA oligos (#1 to #3). (*Lower Left*) Summary of the assay results. The average GFP recovery rate in each sample was calculated in three independent experiments and is shown with SD. (*Lower Right*) Confirmation of mouse SSRP1 knockdown by Western blot. Whole-cell extract was prepared 48 h after siRNA transfection. (*B*) Mutation analysis by sequencing. The cells remaining in the MedGC and SSRP1(m)#1 samples after the FACS analysis in *A* were subjected to mutation analysis for the GFP reporter gene. The results of this analysis are shown in the table at *Left*, and the mutation frequencies are shown as a bar graph at *Right*. The *P* value for the significance of reduction by SSRP1 knockdown is shown in the graph.

A



B

Tested Region	Sample	Mutations	Total base number	Mutated/ total clones	Mutation frequency (x 10^{-4} mutations/base)
HygGFP	MedGC OHT-	0	58,030	0/35	<0.172
	MedGC OHT+	47	79,584	26/48	5.906
	siSSRP1 OHT-	0	56,372	0/34	<0.177
	siSSRP1 OHT+	13	74,610	11/45	1.742
V(D)J region	MedGC OHT-	3	50,932	3/68	0.5890
	MedGC OHT+	39	86,135	32/115	4.528
	siSSRP1 OHT-	3	35,203	3/47	0.8522
	siSSRP1 OHT+	20	92,127	18/123	2.171

C

HygGFP

MedGC OHT+

From \ To	A	T	G	C	Total (%)
A			2.1	2.1	4.2
T					0.0
G	42.6	6.4		6.4	55.4
C		38.3	2.1		40.4

siSSRP1 OHT+

From \ To	A	T	G	C	Total (%)
A		7.7			7.7
T					0.0
G	30.8			15.4	46.2
C		46.2			46.2

D

V(D)J region

MedGC OHT+

From \ To	A	T	G	C	Total (%)
A					0.0
T	2.6			5.1	7.7
G	33.3			5.1	38.4
C		41.0	12.8		53.8

siSSRP1 OHT+

From \ To	A	T	G	C	Total (%)
A					0.0
T					0.0
G	40.0	5.0		10.0	55.0
C		35.0	10.0		45.0

Fig. S3. Detailed information for the sequencing analysis in Fig. 2. (*A*) Schematics showing the positions of the sequenced regions the Hygromycin-GFP fusion reporter gene (HygGFP) (*Upper*) and the V(D)J region of the immunoglobulin heavy chain (Igh) locus (*Lower*). Numbers are the distance from the first ATG of each ORF. Note that the primer sequences are not included in the tested regions. (*B*) Summary table of the analysis for the HygGFP and V(D)J regions. (*C* and *D*) Tables for the mutation patterns. The mutation patterns of the OHT-treated cells transfected with the MedGC control (MedGC) or the SSRP1#3 siRNA oligonucleotide (siSSRP1) in the HygGFP transgene and the V(D)J region are shown in *C* and *D*, respectively. The numbers indicate the percentage of each substitution.

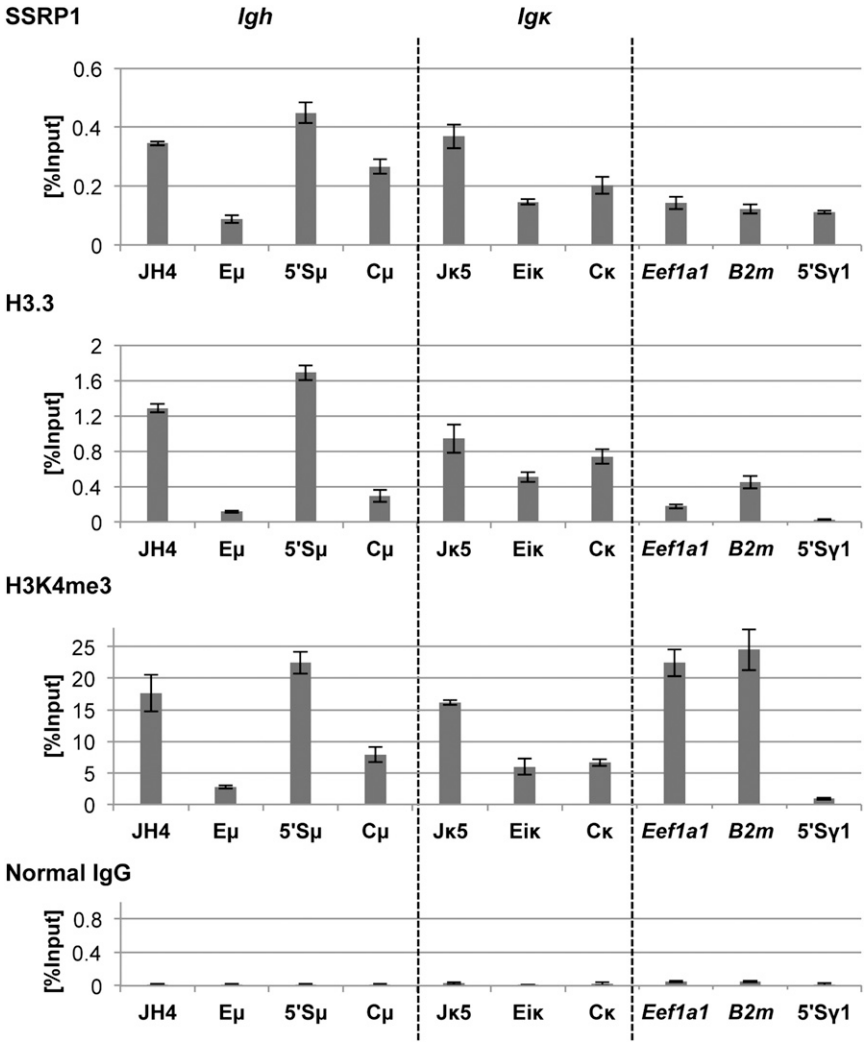


Fig. S4. The "facilitates chromatin transcription" (FACT) complex and histone H3.3 enrichment on the heavy and light chain genes in mouse primary B cells. Splenocytes were isolated from wild-type C57BL/6 mice and subjected to ChIP assays after being incubated with LPS for 2 d to ensure that the B cells became the predominant population. The chromatin occupancies of SSRP1, H3.3, and the trimethylation on histone H3K4 (H3K4me3) in the indicated regions are shown in individual graphs. Normal IgG was used to evaluate the background signal level. Data are the mean and SD of three independent experiments. Note that the heavy chain JH4 and the kappa light chain Jκ5 segments are the closest genomic regions to the rearranged variable regions in *Igh* and the immunoglobulin kappa light chain gene (*Igκ*), respectively. We chose the *Igκ* locus for light chain gene analysis because it is expressed in ~95% of the B cells in C57BL/6 mice (1).

1. Durdik J, Moore MW, Selsing E (1984) Novel kappa light-chain gene rearrangements in mouse lambda light chain-producing B lymphocytes. *Nature* 307(5953):749–752.

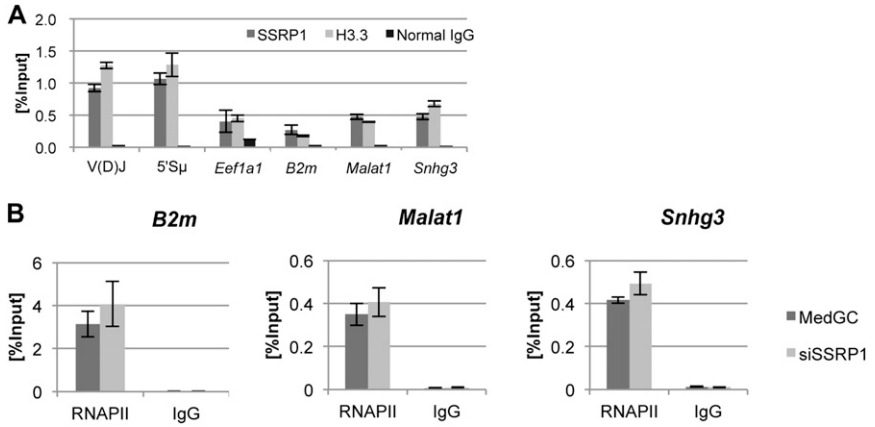


Fig. S5. Supporting ChIP results for Fig. 5. (*A*) FACT and H3.3 occupancies in AID-ER BL2 cells. (*B*) RNAPII occupancy on the non-Ig genes used for mutation analysis. Immunoprecipitated DNA by an anti-RNA polymerase II (RNAPII) antibody and normal IgG prepared in the experiment shown in Fig. 3*B* were used for this test. All data in this figure are the mean ± SD from three independent analyses.

Table S1. Data for the SHM sequencing analysis shown in Fig. 5

Analyzed region	AID type	Samples	Mutations	Total base no.	Mutated/total clones	Mutation frequency ($\times 10^{-4}$ mutations per base)
VDJ region	JP8Bdel-ER	MedGC OHT−	3	50,932	3/68	0.5890
		MedGC OHT+	39	86,135	32/115	4.528
		siSSRP1 OHT+	20	92,127	18/123	2.171
	AID-ER	MedGC OHT−	0	44,191	0/59	<2.263
		MedGC OHT+	9	77,896	9/104	1.155
		siSSRP1 OHT+	2	77,896	2/104	0.2568
Sμ region	JP8Bdel-ER	MedGC OHT−	5	36,834	4/42	1.357
		MedGC OHT+	68	60,513	36/69	11.24
		siSSRP1 OHT+	51	64,898	34/74	7.858
	AID-ER	MedGC OHT−	0	35,957	0/41	<2.781
		MedGC OHT+	39	69,283	26/79	5.629
		siSSRP1 OHT+	9	64,021	8/73	1.406
Eef1a1	JP8Bdel-ER	MedGC OHT−	1	39,117	1/39	0.2556
		MedGC OHT+	14	44,132	9/44	3.172
		siSSRP1 OHT+	1	41,123	1/41	0.2432
	AID-ER	MedGC OHT−	2	52,156	2/52	0.3834
		MedGC OHT+	3	53,159	3/53	0.5643
		siSSRP1 OHT+	0	59,177	0/59	<0.1690
B2m	JP8Bdel-ER	MedGC OHT−	0	40,432	0/38	<0.2473
		MedGC OHT+	0	40,432	0/38	<0.2473
		siSSRP1 OHT+	1	39,368	1/37	0.2540
	AID-ER	MedGC OHT−	1	41,496	1/39	0.2410
		MedGC OHT+	1	46,816	1/44	0.2136
		siSSRP1 OHT+	0	44,688	0/42	<0.2238
Malat1	JP8Bdel-ER	MedGC OHT−	3	25,850	3/47	1.161
		MedGC OHT+	20	25,300	13/46	7.905
		siSSRP1 OHT+	8	25,300	7/46	3.162
	AID-ER	MedGC OHT−	0	23,650	0/43	<0.4228
		MedGC OHT+	1	25,850	1/47	0.3868
		siSSRP1 OHT+	1	25,300	1/46	0.3953
Snhg3	JP8Bdel-ER	MedGC OHT−	0	23,364	0/44	<0.4280
		MedGC OHT+	18	28,674	15/54	6.277
		siSSRP1 OHT+	7	29,205	7/55	2.397
	AID-ER	MedGC OHT−	0	20,709	0/39	<0.4829
		MedGC OHT+	1	22,833	1/43	0.4380
		siSSRP1 OHT+	0	24,426	0/46	<0.4094

JP8Bdel-ER, a C-terminally truncated AID mutant (JP8Bdel) fused with the estrogen-binding domain; *B2m*, beta-2 microglobulin; *Eef1a1*, eukaryotic translation elongation factor 1 alpha 1; *Malat1*, metastasis associated lung adenocarcinoma transcript 1; *Snhg3*, small nucleolar RNA host gene 3; Sμ, the Sμ switch region.

Table S2. Spt5-depletion blocked SHM in the HygGFP gene in the sequencing analysis

Sample	Mutations	Total base no.	Mutated/ total clones	Mutation frequency ($\times 10^{-4}$ mutations per base)
MedGC OHT−	0	48,960	0/30	<0.204
MedGC OHT+	45	48,960	20/30	9.19
siSpt5#2 OHT−	1	40,800	0/25	0.245
siSpt5#2 OHT+	16	48,960	12/30	3.27

The effect of Spt5-depletion on mutation frequency in HygGFP was tested as the same to the SSRP1-depletion in Fig. 2. The *P* value for the significance of reduction by Spt5 knockdown is $P = 0.00013$ (Fisher's exact test). Knockdown efficiency of the siSpt5#2 oligonucleotide is shown in Fig. S1.

Table S3. Stealth siRNA oligonucleotides

siRNA name	Stealth siRNA official ID
CSB#1	HSS103357
CSB#2	HSS103358
CSB#3	HSS103359
Spt5#1	HSS110371
Spt5#2	HSS110372
Spt5#3	HSS110373
ELL#1	HSS112024
ELL#2	HSS112025
ELL#3	HSS112026
TCEB3#1	HSS110530
TCEB3#2	HSS110531
TCEB3#3	HSS110532
SSRP1#1	HSS110245
SSRP1#2	HSS110246
SSRP1#3	HSS110247
RDBP#1	HSS111862
RDBP#2	HSS111863
RDBP#3	HSS111864
Ctr9#1	HSS114421
Ctr9#2	HSS114422
Ctr9#3	HSS114423
SII#1	HSS110525
SII#2	HSS110526
SII#3	HSS186223
Spt6#1	HSS110374
Spt6#2	HSS110375
Spt6#3	HSS110376
Spt16#1	HSS117400
Spt16#2	HSS117401
Spt16#3	HSS117402
TafSF1#1	HSS120649
TafSF1#2	HSS120650
TafSF1#3	HSS120651
Rap30#1	HSS104572
Rap30#2	HSS104573
Rap30#3	HSS104574
Hpr1#1	HSS115144
Hpr1#2	HSS115145
Hpr1#3	HSS115146
Histone H3.3A	NM_002107_stealth_76
Histone H3.3B	NM_005324_stealth_817
SSRP1(m)#1	MSS209558
SSRP1(m)#2	MSS209559
SSRP1(m)#3	MSS277353

Invitrogen's official ID number for each oligo is shown. Note that siRNAs for histone H3.3A and -B were designed at their unique untranslated regions to avoid cross-reaction to the normal histone H3 genes.

Table S4. Sequences of the primers used for qRT-PCR analysis

Primer set name	Forward	Reverse
CSB	GGGCACCATTGAAGAAAAGA	TGTTTCAGTGCTCTGGGATG
DSIF (Spt5)	CATCGGTGTGGTGAAAGATG	GTCCTCCCATAGGTCGAGGT
ELL	ACCCCAGGTTTAAACGGAAC	TGTACTCGGCATTGAAGTCG
Elongin (TCEB3)	AAGCTGAGAAAGGTGCCTGA	TCTTCCTGGGGTGAAGAGAA
FACT (SSRP1)	GAAGAAAGAGGAGTGGGATCG	GCGTGGATTTCTTTTCCATC
NELF (RDBP)	ACACAGCCACAGCAACAGAG	CTGGAAAGTGGGGACTGGT
Paf (Ctr9)	CCAATGGCATAGGAGCTGTT	GCGCTGATGTACTGCTTTTG
SII	CGGCAATGTAAGCAACAGAA	GTTCGAAGAGCTGCAGCAAG
Spt6	GCCAGAAGAACGAGTGAAGG	TAGGTGTCTTTGGGCAGCTT
TatSF1	CCATGAGCGAGTTGTCATCA	ACACCATCTGGGTGCCTATC
TFIIF (Rap30)	TGGAAAACCAGCTTCAGTCA	GTCGGCATTCAGCTCTTTGT
THO (Hpr1)	AAGGTTGCCCAAGTTTTGTG	TCAGGGCAAAGATTCCAAAG
Histone H3.3A	TAAAGCACCCAGGAAGCAAC	GGGAAGTTTGCGAATCAGAA
Histone H3.3B	AGGAAAAGCGCTCCCTCTAC	ATCTCCCTCACCAACCTCTG
Spt16	TGCCATCACACTGCTAATACG	GGCAGTCATTCCAGCTCTTC
Igh in BL2 cells	CTAATTACTACTTGAGTTGGATCCG	TCAGTTTCAGGGAGAATTGGTT
HygGFP	GATGTAGGAGGGCGTGGATA	ATAGGTCAGGCTCTCGCTGA
Gapdh	ATCCTGGGCTACACTGAGCA	GGTGGTCCAGGGGTCTTACT

All sequences are from 5′ end to 3′ end. CSB, Cockayne syndrome B protein; DSIF, 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity-inducing factor; Spt5, suppressor of Ty 5 homolog; ELL, eleven-nineteen lysine-rich leukemia protein; TCEB3, transcription elongation factor B polypeptide 3; NELF, negative elongation factor; RDBP, RD RNA binding protein; Paf, polymerase associated factor; Ctr9, Cln three-requiring protein 9; SII, transcription elongation factor TFIIS; Spt6, suppressor of Ty 6 homolog; TatSF1, Tat specific factor 1; TFIIF, general transcription factor IIF; Rap30, RNA polymerase II associated protein 30; THO, suppressor of the transcriptional defect of Hpr1 by overexpression; Spt16, suppressor of Ty 16 homolog; Gapdh, glyceraldehyde-3-phosphate dehydrogenase.

Table S5. Sequences of the primers used for the mutation analysis by sequencing

Target region	Forward	Reverse
HygGFP	ATGTGTCGACACCATGAAAAAGCCTGAACTCAC	GCGGATCCTTACTTGTACAGCTCGTCCA
	Position: +21 to +1678 from first ATG	
BL2 Igh VDJ	ATCTCATGTGCAAGAAAATGAAA	AGTCCCACCACGCAATCAT
	Position: +7 to +755 from first ATG	
5′Sμ	GCTGCTGCATTTGCTTCTC	GCCCAGTTCAGCCTTGTTTA
	Position: 877 bp intronic region between Iμ exon and Sμ repeat	
Eef1a1	CCGCCAGAACACAGGTAAGT	CCCGAATCTACGTGTCCAAT
	Position: +40 to +1042 from TSS	
Snhg3	GCCCAGGAGTGACCTATACTCAAA	GGTATCCACGTTGGAATGCTCA
	Position: Described in Kato et al. (1).	
Malat1	GGCAGAAGGCTTTTGGAAGA	CAACATATTGCCGACCTCACGGAT
	Described in Kato et al. (1).	
B2m	CTGTGCTCGCGCTACTCTCT	GGAAACAACCAGGCAAAGAG
	Position: +113 to +1176 from TSS	
GFP in NTZ (Fig. S2)	TGACCTCCATAGAAGACACCG	TTATGTTTCAGGTTCAGGGGG
	Position: Described in Yoshikawa et al. (2).	

All sequences are from 5′ end to 3′ end. NTZ, NIH3T3-NTZ cells.

1. Kato L, et al. (2012) Nonimmunoglobulin target loci of activation-induced cytidine deaminase (AID) share unique features with immunoglobulin genes. *Proc Natl Acad Sci USA* 109(7): 2479–2484.
2. Yoshikawa K, et al. (2002) AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. *Science* 296(5575):2033–2036.

Table S6. Sequences of the primers used for ChIP assays

Primer set name	Forward	Reverse
Primers for BL2 (human) ChIP experiments		
V(D)J	CACTGTGGGTTTTTCTGTTCA	CGGATCCAACTCAAGTAGTAATTAG
Intron#1	CTCAGGTGAGTCCTCACCAC	AGTCCCACCACGCAATCAT
Intron#2	GTTTCCTGAGCATTGCAGGT	TACAGACACCGCTCCTGAGA
Eμ	GGTCACCGCGAGAGTCTATT	GAAACGCAAATTGTCCAGGT
5′Sμ	CCAGGTAGTGGAGGGTGGTA	CAGCTAAAGCCATCTCATTGC
3′Sμ	TGGGCCAATCTTCATGATCT	CCAGCTCAGTCACACTCCAG
Cμ	GCAAGTCCAAGCTCATCTGC	TGGTCACCTTGTAGGTCGTG
Eef1a1	CCGCCAGAACACAGGTAAGT	ACTCTCCCACCCACTTCCA
B2m	CTGTGCTCGCGCTACTCTCT	CTTGGAGAAGGGAAGTCACG
Odf4	TGGGGCCTTCTCTTGGAT	CTGTCTCCCCTTTCCCTCAC
Lmnb2 intergenic	AAACGTGACCTCAGACAGAGC	CTGGCAGGTCTGGGACTATG
Malat1 hotspot	TGACCCAGGTGCTACACAGA	GGCTCCTGGACTCTTTTCCT
Snhg3 hotspot	GGAGCCCAGGATGACCTAT	CCTTAACAAATCCTGCAAAACA
Primers for mouse spleen ChIP experiments		
JH4	AGAATGGCCTCTCCAGGTCT	AAGGCTCTGAGATCCCTAGACA
Eμ	AGGTCATGTGGCAAGGCTAT	TTACCCAGGTGGTGTTTTGC
5′Sμ	GCCCTAGTAAGCGAGGCTCT	CCCAGCTCATTCCAGTTCAT
Cμ	CAGCACCATTTCCTTCACCT	ACCTTCAAGGATGCTCTTGG
Jκ	GAGAAAATGGAGAGGGCTCA	TCCAATCTCTTGGATGGTGA
iEκ	TCCCTAGCCAAAGGCAACTA	AGAATTATGAGCAGCCTTTCC
Cκ	TATCCATCTTCCCACCATCC	ACTGTTCAGGACGCCATTTT
5′Sg1	TAAGAACATGGGGAGCAGGA	TACTCCCCTGGTCCCTTACC
Eef1a1	AGTCGCCTTGGACGTTCTT	GGGAATGCTCGCAGCTAAT
B2m	TGGTGCTTGTCTCACTGACC	CCACTCCTTTCCCGAGAGAC

All sequences are from 5′ end to 3′ end. Odf4, outer dense fiber of sperm tails 4; iEκ, intronic enhancer of the kappa light chain gene; 5′Sg1, 5′ flanking sequence of the S gamma 1 switch region.

Table S7. Antibody information

Antigen	Host	Company	Product no.
SSRP1 (10D1)	Mouse	BioLegend	609702
Spt16	Goat	Sigma-Aldrich	SAB2500377
H3.3	Rabbit	Abcam	ab62642
H3K4me3	Rabbit	ActiveMotif	39159
H3	Rabbit	ActiveMotif	39163
RNAPII (H-224)	Rabbit	Santa Cruz	sc9001
Spt5	Rabbit	Gift from H. Handa	Tokyo Institute of Technology, Yokohama, Japan
Normal mouse IgG	Mouse	Santa Cruz	sc2025