Supporting Information

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SI Materials and Methods

CRISPR/Cas9-Mediated Gene Disruption. To design the guide RNAs (gRNAs) for hnRNP gene targeting, a software tool (crispr.genome-engineering.org) predicting unique target sites throughout the mouse genome was used. Three targeting sites were selected in each of the genes encoding hnRNP K and hnRNP L. Paired oligonucleotides designed for each target site (Table S1) were annealed and cloned into the linearized GeneArt CRISPR Nuclease CD4 Reporter Vector (Invitrogen). The three targeting constructs were mixed at a 1:1 ratio, a total of 3 μg was transfected into CH12F3-2A cells, and the $CD4^+$ cells were sorted 48 h later. The $CD4^+$ cells were subjected to serial dilutions and cultured for 7 d to obtain single clones. For each targeting experiment, 90 clones were selected for screening by genomic DNA analysis, protein expression, and CSR assay. The clones containing defects in the hnRNP K and hnRNP L genes were designated "K2-20" and "L11," respectively.

hnRNP K, hnRNP L, and AID Constructs. To generate hnRNP K/hnRNP L–cMyc–FLAG fusion constructs, mouse hnRNP K (NM_025279.1) and hnRNP L (NM_177301.5) were amplified by RT-PCR and cloned into the EcoRI and XhoI sites of the pCMV6-Entry vector (Origene). Additionally, the hnRNP K–cMyc–FLAG fragment was amplified and inserted into the NheI and KpnI sites of the pcDNA3.1-Zeo vector (Invitrogen). To generate siRNA-resistant constructs, the siRNA-targeting sequences in hnRNP K/L were modified (Table S1). We also generated C-terminal 3xFLAG fusion constructs of AID and its N/C-terminal mutants; the coding sequence of human AID (NM_020661.2) was amplified by RT-PCR and cloned into the EcoRI and ClaI sites of the 3xFLAG (C-terminal)-pCMV vector (Sigma).

CSR Assay and siRNA Oligonucleotide Transfection. CH12F3-2A cells and their derivatives, the K2-20 and L11 clones, were stimulated by CIT to induce class switching as previously described (1, 2). Electroporation (Amaxa) was used to introduce siRNA oligonucleotides (Invitrogen) (Table S2) into the cells. The transfected cells were cultured for 24 h before the addition of CIT and were subjected to FACS analysis after 24 h of CIT stimulation. FITC-conjugated anti-IgM (eBioscience) and phycoerythrin (PE)-conjugated anti-IgA (Southern Biotech) antibodies were used for surface IgM and IgA staining, respectively.

To perform CSR complementation assays for hnRNP K, siRNA-resistant, epitope (cMyc-FLAG)-tagged WT or mutant hnRNP K constructs were cotransfected with the hnRNP K siRNA into K2-20 or CH12F3-2A cells. IgA switching efficiency was monitored 24 h after CIT stimulation. Similar CSR rescue experiments were conducted by cotransfecting L11 cells with siRNA-resistant hnRNP L constructs and hnRNP L siRNA.

Cell Death and Proliferation Assay. Cells were stimulated with CIT 24 h after transfection with sihnRNP K or sihnRNP L. Twenty-four hours after stimulation, cell viability was determined by propidium iodide (PI) exclusion and FACS analysis. The cell proliferation assay was performed as described previously for CH12F3-2A cells (3, 4). Carboxyfluorescein succinimidyl ester (CFSE; Invitrogen), which labels long-lived intracellular molecules with the fluorescent dye, along with the standard cell counting, was used to monitor the cell proliferation status. Twenty-four hours after siRNA transfections, cells were labeled with CFSE (5 μM) for 15 min at 37 °C. Portions of cells were treated separately with Aphidicolin (2 μg/mL), a well-known inhibitor of cell-cycle progression, which served as a positive control for proliferation arrest. Cells then were stimulated with CIT, and FACS analysis and CFSE monitoring were done 48 h after stimulation.

SHM Analyses.

S region SHM. K2-20 or L11 cells were transfected with hnRNP K or L siRNA oligonucleotides 24 h before CIT stimulation for 48 h. The IgA^+ cells were sorted, followed by purification of genomic DNA. A 676-bp region located 5′ of the core Sμ region was PCR-amplified using high-fidelity PrimeSTAR DNA polymerase (Takara) with the following amplification conditions: 98 °C for 5 min, 30 cycles at 98 °C for 10 s, 58 °C for 8 s, and 72 °C for 1 min (5). The PCR product was cloned into a Zero Blunt vector (Invitrogen) for sequencing, and the subsequent mutational analysis was performed using Sequencher DNA software. For each set of reactions, 96 or more clones were sequenced bi-directionally, and only the unique mutations were counted.

V region SHM. BL2-JP8BdelER cells (6, 7) were stimulated with OHT for 24 h after siRNA introduction and were incubated for an additional 48 h in the absence of OHT. The genomic DNA was purified by phenol/chloroform extraction, and PCR was performed using PrimeSTAR DNA polymerase (TaKaRa) with the following amplification conditions: 98 °C for 5 min, 30 cycles at 98 °C for 10 s, 58 °C for 8 s, and 72 °C for 1 min. The purified PCR fragments were cloned and sequenced as described above. Only the unique mutations were counted, and the mutation frequency was calculated from the number of mutations identified per total bases analyzed. The relevant PCR primers are listed in Table S1.

ChIP Assay. The ChIP assay was performed using the ChIP-IT Express Kit (Active Motif) according to the manufacturer's instructions. In brief, 5×10^6 cells were fixed in the presence of 1% formaldehyde for 5 min at room temperature. The reaction was stopped by the addition of 0.125 M glycine. A soluble chromatin fraction containing fragmented DNA of 500–2,000 bp was obtained after cell lysis and sonication. ChIP was performed by incubating the cleared lysate with 2–3 μg of anti-γH2AX antibody. The immunoprecipitated DNA was analyzed by real-time PCR, and the data were normalized first to the amount of input and then to the maximum value in each dataset, as described previously (2).

DNA Break Assays.

Biotin-dUTP end labeling of DNA break ends. K2-20 or L11 cells were transfected with gene-specific siRNAs, incubated for 24 h, and then stimulated with CIT. After 24 h the switching efficiency was monitored by surface IgA staining. The live cells were collected by Percoll gradient and fixed at room temperature, and then the nuclei were permeabilized and subjected to biotin-16-dUTP incorporation at the DSBs with T4 polymerase DNA polymerase (Takara). The genomic DNA was isolated by phenol/chloroform extraction and subjected to HindIII digestion overnight. The biotinylated fragments were captured with streptavidin magnetic beads and analyzed by PCR as described previously (8). The primers for amplifying Sμ, Sγ1, and β2M loci are listed in Table S1.

LM-PCR. The cells were stimulated for CSR as described above, and the live cells were embedded in low-melt agarose plugs and processed for linker ligation as described previously (9). The samples were treated with T4 polymerase (Takara) before linker ligation, and the ligated DNA was subjected to GAPDH DNA

PCR analysis to adjust DNA input before LM-PCR. Threefold dilutions of input DNA were amplified by KOD-FX-Neo polymerase (Toyobo). The PCR products were electrophoresed on 1% agarose gels and validated by Southern blot using a 5′ Sμ probe (9, 10). The primers and probe sequences are shown in Table S1.

3C Assay. The 3C assay using CH12F3-2A cells was described previously (11) and was adopted from the procedure described by Wuerffel et al. (12). In brief, 7×10^6 cells were washed with PBS and subjected to 1% formaldehyde cross-linking for 5 min at room temperature. The nuclear lysate was prepared using 500 μL of lysis buffer following the instructions in the Active Motif ChIP Kit manual. The cross-linked chromatin was digested with HindIII overnight and then ligated with T4 polymerase DNA ligase (Takara). The ligated chromatin was treated with proteinase K and reverse cross-linked, and then the DNA was purified by phenol/chloroform extraction. PCRs were performed as described previously (9). The PCR primers are listed in Table S1.

RT-PCR Analysis. Total RNA was extracted from CH12F3–2A cells expressing Bcl2 or BL2 cells using TRIzol (Gibco BRL). The cDNA was synthesized using SuperScript II and an oligo d(T) primer, followed by real-time PCR using the SYBR Green Master Mix (Applied Biosystems) and mRNA-specific primers (Table S1).

AID Immunoprecipitation. The 293T cells (1.5×10^6) were transiently transfected with 8 μg of the 3xFLAG-tagged human AID construct and were incubated for 34 h in medium supplemented with 100 mM 4-SU. The live cells were irradiated with 365 nm UV light 14 h later (13) and then were lysed in 200 μL of RNA-binding protein immunoprecipitation (RIP) lysis buffer (Millipore), followed by treatment with RNase A and T1 (Ambion). The FLAG-tagged proteins in 50 μL of lysate were immunoprecipitated with 5 μg of anti-FLAG antibody (Sigma) bound to protein G Dynabeads. The beads were washed and resuspended in RIP wash buffer (Millipore), and the protein–RNA complexes were eluted with a 0.2-M glycine solution.

Analysis of hnRNP K- and hnRNP L-Expression–Defective Clones. We used the CRISPR/Cas9 system to generate hnRNP K- and hnRNP L-null CH12F3-2A cell lines. As depicted in Fig. S2*A*, exons 4–6 (arrowheads) of the hnRNP K gene were targeted simultaneously. The genomic DNA, which was extracted from several targeted clones, was subjected to PCR-based screening to identify clones with the designed deletions. PCR analysis of clone K-2, using primers G1 and G2, resulted in 1.4- and 1.2-kb products, representing the WT and disrupted alleles, respectively (Fig. S2 *A* and *B*). Sequencing of the smaller product confirmed that a 168-bp sequence encompassing the initiation codon was deleted in exon 4. The K-2 clone was subjected to another round of CRISPR/Cas9-mediated gene deletion to obtain CH12F3-2A cell lines with a homozygous hnRNP K knockout. Among the 82 candidate clones obtained, a single clone, K2-20, which lost the 1.4-kb WT band, was identified. Genomic DNA sequencing of K2-20 showed a 290-bp deletion in exon 4 in the other allele (Fig. S2*B*, *Lower*).

Western blot analysis showed no detectable hnRNP K expression in clone K2-20, compared with the parental K2 clone or WT cells (Fig. S2*B*, *Right*). However, the anti-hnRNP K antibody used in this experiment was specific to the N terminus of hnRNP K and thus may not have detected N-terminally truncated products expressed from the disrupted allele. Therefore we examined hnRNP K transcripts from clone K2-20 by RT-PCR using the M1–M5 primers and detected a C-terminal–specific product (169 bp) with the M4–M5 primer pair (Fig. S2*C*). As expected, the M1–M3 primer pair did not yield a 240-bp RT-PCR product specific to the N terminus in the K2-20 clone, although both the 169-bp and 240-bp products were easily detectable in WT cells (Fig. S2*C*). Thus, we concluded that the K2-20 clone expresses an N-terminal–truncated form of hnRNP K from one allele, which resulted in a compromised level of CSR (Fig. S2*D*). To deplete the remaining hnRNP K transcripts, we introduced an siRNA targeted to the KH3 region (located at the C terminus) of the hnRNP K gene into K2-20 cells and thereby reduced CSR drastically.

The hnRNP L alleles were disrupted using a strategy similar to the one described above (Fig. S2*E*). The first round of CRISPR/Cas9-mediated gene disruption produced clone L11 that contained a 2,834-bp deletion spanning exons 2–3 in one hnRNP L allele (Fig. S2*F*). We therefore performed a second round of CRISPR/Cas9-mediated disruption, targeting the other hnRNP allele in L11 cells; however, all the isolated clones expressed levels of hnRNP L protein similar to that of the parental clone. We thus introduced an siRNA targeted to hnRNP L into the L11 cells and observed almost complete depletion of the hnRNP L protein (Fig. S2*F*). Because treatment of K2-20 and L11 cells with hnRNP K and L siRNAs, respectively, resulted in drastic inhibition of CSR (Fig. S2 *D* and *G*), this combined blocking system was used in most of the experiments. We speculated that hnRNP K and L may be essential for cell viability. Consistent with this theory, the embryonic lethality of A1CF-KO mice has been reported (14).

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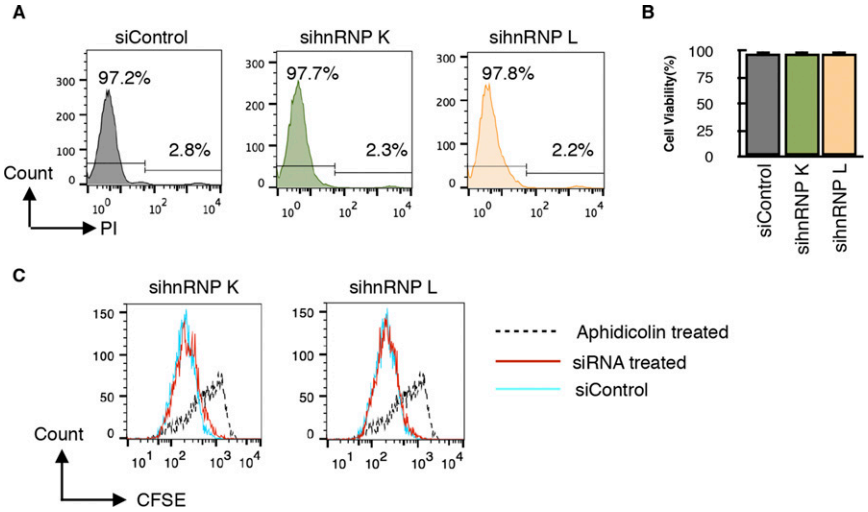

Fig. S1. Cell viability and proliferation are not affected by transient knockdown of hnRNP K or hnRNP L. (*A*) FACS profile of PI-stained cells 48 h after transfection. Percentages of PI^+ and PI^- cells are indicated. (*B*) Histograms show triplicate experiments using PI^- cells derived from various siRNA samples as indicated. (*C*) Cell proliferation after siRNA transfection was measured by FACS analysis of CFSE dilution at 48 h after CIT stimulation. The profile of Aphidicolin-treated cells represents the cell proliferation arrest status (the positive control for the CFSE assay). These results are representative of three independent experiments.

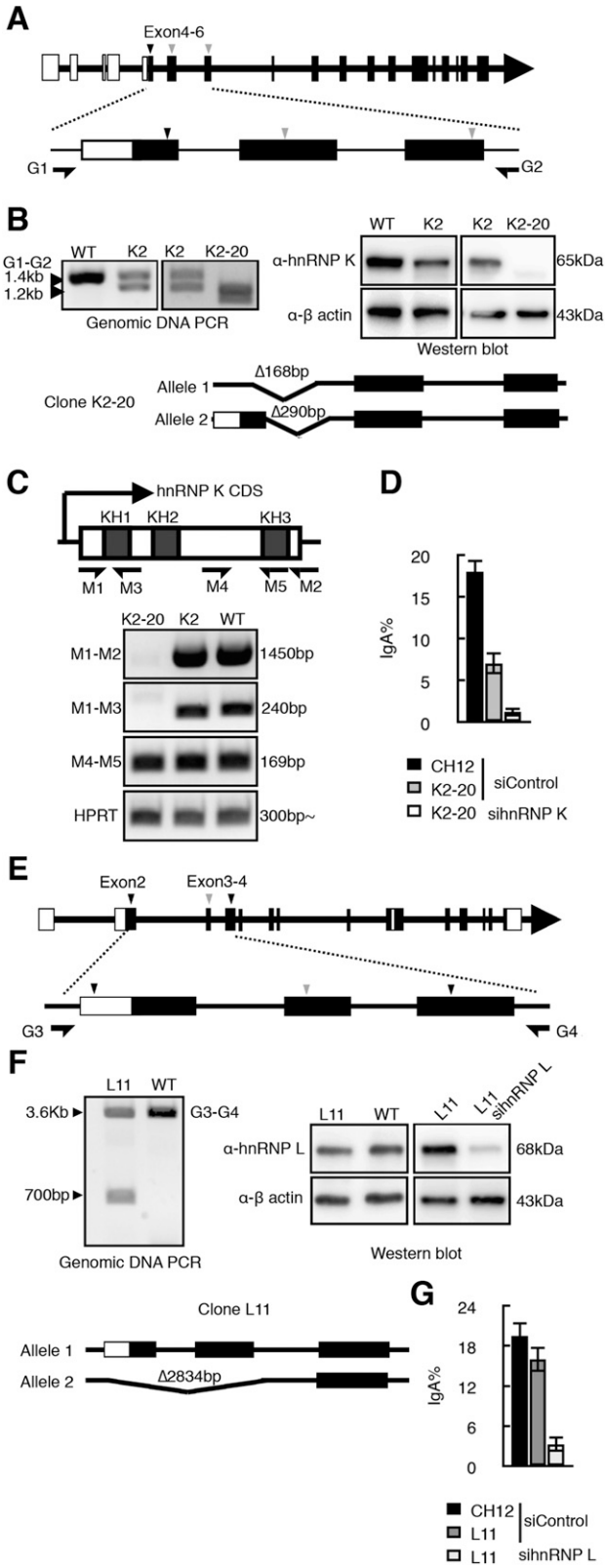

Fig. S2. Generation of hnRNP K- and hnRNP L-defective CH12F3-2A cells by CRISPR/Cas9-mediated gene disruption. (*A*) Schematic of Cas9/gRNA-targeting sites in the hnRNP K genomic locus. Black and white rectangles represent coding and noncoding exons, respectively. The black and white arrowheads above the exons indicate the positions of Cas9/gRNA targeting; the black arrowhead indicates the exon most effectively targeted. The arrows below the scheme show the positions of the PCR primers G1 and G2 used in *B*. (*B*, *Upper*) PCR and Western blot analyses of K2 and K2-20 clones. (*Lower*) Schematic representation of the deleted regions in the hnRNP K alleles in the K2-20 clone. (*C*, *Lower*) RT-PCR analysis of hnRNP K mRNA expression in WT cells and hnRNP K-defective clones. (*Upper*) Arrows below the scheme of the hnRNP K gene indicate the positions of PCR primers (M1–M5). (*D*) IgA switching efficiency of clone K2-20 after

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the introduction of control and hnRNP K siRNAs. (*E*) Structure of the mouse hnRNP L locus showing the Cas9/gRNA-targeted regions; black arrowheads indicate the most effectively targeted exons. PCR primers G3 and G4 are shown below. (*F*, *Upper*) PCR and Western blot analyses of the hnRNP L-targeted clone, L11, with or without sihnRNP L knockdown. (*Lower*) Schematic representation of the deleted region in one of the hnRNP L alleles in L11. (*G*) IgA switching efficiency in L11 cells treated with hnRNP L siRNA.

A. Mutation analysis of 5' core Sμ after CIT stimulation in hRNP K depletion cells.

Sample	Mut.clone/Total	Total Seq.	Mutation	Mut. Freq.
CH12F3-2A+siControl	35/85	57460	71	1.24E-03
K2-20+sihnRNP K	20/79	53404	35	6.55E-04

B. Mutation analysis of 5' core Sμ after CIT stimulation in hRNP L depletion cells.

Sample	Mut.clone/Total	Total Seq.	Mutation	Mut. Freq.
CH12F3-2A+siControl	36/91	63700	91	1.48E-03
L11+sihnRNP L	33/87	60900	89	1.51E-03

C. Analysis of SHM of the V4-39/JH5 region in BL2-JP8Bdel-ER cells treated with indicated siRNAs after AID activation.

Sample	Mut.clone/Total	Total Seq.	Mutation	Mut. Freq.
BL2+siControl	23/61	45750	35	7.65E-04
BL2+sihnRNP K	15/56	42000	19	4.52E-04
BL2+sihnRNP L	30/53	39750	56	1.41E-03

Fig. S3. Summary of SHM analysis of the 5′ Su region in CH12F3-2A cells (*A* and *B*) and the rearranged V region in BL2-JP8BdelER cells (*C*). (See Fig. 2.)

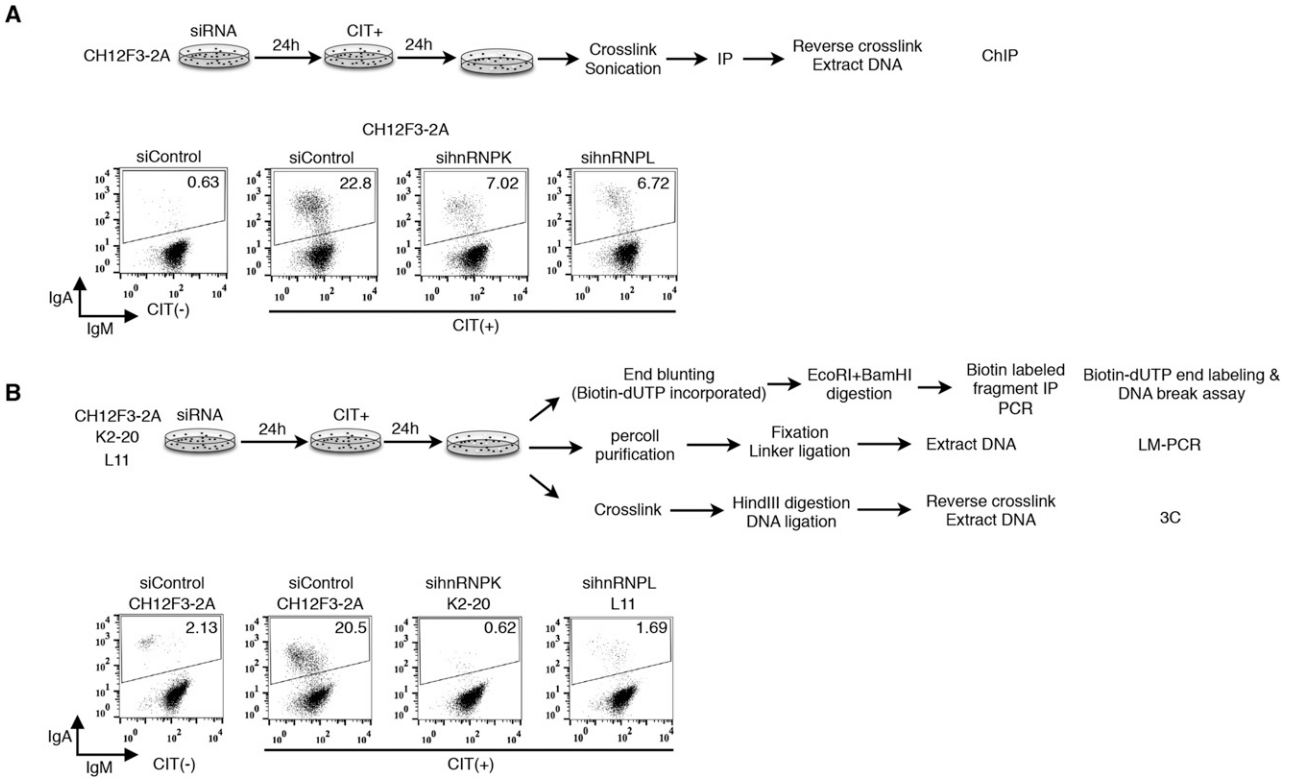

Fig. S4. Experimental designs of the various DNA break assays and the 3C assay. Representative FACS profiles of the cells used in the CSR, DNA break, or 3C assay. (See Fig. 3.)

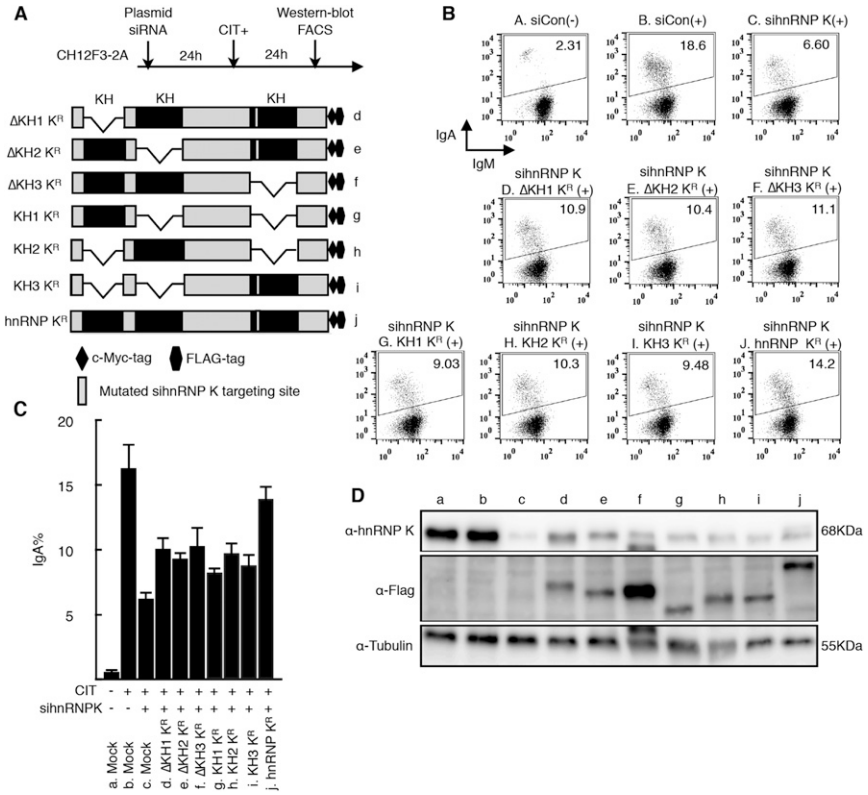

Fig. S5. The KH domains of hnRNP K are required for efficient CSR. (*A*–*C*) WT and mutant hnRNP K constructs (*A*) used for CSR rescue in CH12F3-2A cells transfected with hnRNP K siRNA (*B* and *C*). The cells were treated with (+) or without (–) CIT for 24 h after cotransfection with hnRNP K siRNA and the indicated constructs. SD values in *C* were calculated from three independent experiments. (*D*) Western blot analysis of the epitope-tagged hnRNP K constructs used. (See Fig. 4.)

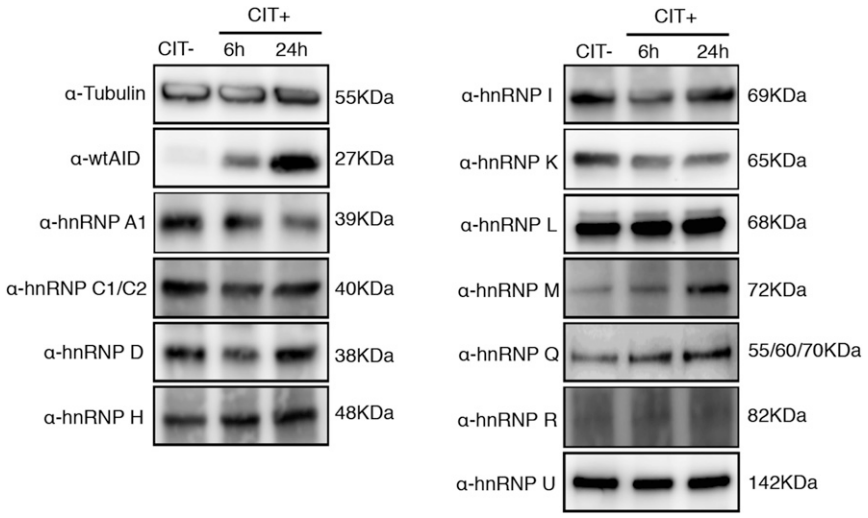

Fig. S6. Expression analysis of hnRNP proteins in CH12F3-2A cells treated with or without CIT stimulation. (See Fig. 6.)

Table S1. Primers and siRNAs

Purpose	Nucleotide sequence
Gene-specific primers	
Mouse	
hnRNP K-F	GCAAATGGCTTATGAACCACA
hnRNP K-R	TTGTTTAATCCGCTGACCAC
hnRNP L-F	CGCGCCAAGGCCTCACTCAA
hnRNP L-R	GGGGCCCGTAGCCCTCATCA
hnRNP M-F	TTGAGCCATATTCCAACCCA
hnRNP M-R	GACTTTCCTTCAGCGTCCA
hnRNP Q-F	GATCCTGAAGTTATGGCAAA
hnRNP Q-R	TTCATTTCTTCCATAGCCTT
Transcript analysis	
μGLT-F	CTCTGGCCCTGCTTATTGTTG
μGLT-R	AATGGTGCTGGGCAGGAAGT
Knockdown efficiency check (Figs. 1 and 2)	
αGLT-F	CCAGGCATGGTTGAGATAGAGATAG
αGLT-R	GAGCTGGTGGGAGTGTCAGTG
mAID-F	CGTGGTGAAGAGGAGAGATAGTG
mAID-R	CAGTCTGAGATGTAGCGTAGGAA
18s-F	TAGAGTGTTCAAAGCAGGCCC
18s-R	CCAACAAAATAGAACCGCGGT
HPRT-F	CTCGAAGTGTTGGATACAGG
HPRT-R	TGGCCTATAGGCTCATAGTG
Human	
hnRNP K-F	GCAAATGGCTTATGAACCACA
hnRNP K-R	TTGTTTAATCCGCTGACCAC
hnRNP L-F	CGGGCCAAGGCCTCTCTCAA
hnRNP L-R	GGGGCCCGTAGCCCTCATCA
18s-F	TAGAGTGTTCAAAGCAGGCCC
18s-R	CCAACAAAATAGAACCGCGGT
hnRNP-K–targeting oligo pairs	
K_exon2_F	CTTCTCACCAAATTCACCATGTTTT
K_exon2_R	ATGGTGAATTTGGTGAGAAGCGGTG
K_exon3_F	GCGCATTTTGCTTCAGAGCAGTTTT
K_exon3_R	TGCTCTGAAGCAAAATGCGCCGGTG
K_exon4_F	GTTTAATACTTACGTCTGTAGTTTT
K_exon4_R	TACAGACGTAAGTATTAAACCGGTG
hnRNP-L–targeting oligo pairs	
L_exon1_F	GGGCAGCAGCCTCCGCGACAGTTTT
L_exon1_R	TGTCGCGGAGGCTGCTGCCCCGGTG
CRISPR/Cas9 targeting in CH12 F3-2A (Fig. 4 and Fig. S2)	
L_exon2_F	CTTCCACTACTCCGTCAATCGTTTT
L_exon2_R	GATTGACGGAGTAGTGGAAGCGGTG
L_exon3_F	AAGCACGCTGTTTACGCTCCGTTTT
L_exon3_R	GGAGCGTAAACAGCGTGCTTCGGTG
Targeted allele and transcript analysis	
G1	GCTGGAAGCAGAATCCTTTTT
G2	TGGTTTGTAGCTAGTCAGTGATT
G3	CGGAGGCCCACTTCCATTTA
G4	ACAGATGGTGTAAAGAACATCCT
M1	GCGGCCTATTGGTGGATCCA
M2	CTTCAGTTCTTCACTAGTCTTAG
M3	GAGCCTTAATATTCTTGCCTCC
M4	GCAAATGGCTTATGAACCACA
M5	TTGTTTAATCCGCTGACCAC
5′ Sμ sequencing in CH12F3-2A in mouse B-cell line [SHM analysis (Fig. 2)]	
Sμ-F	AATGGATACCTCAGTGGTTTTTAATGGTGG
Sμ-R	GAACAGTCCAGTGTAGGCAGT
V region sequencing in BL2 human B-cell line	
BL2-V-F	ATCTCATGTGCAAGAAAATGAA
BL2-V-R	AGTCCCACCACGCAATCAT
γH2AX ChIP	
Iμ-F	AAGGGCTTCTAAGCCAGTCC

Table S1. Cont.

Purpose	Nucleotide sequence
Iμ-R	CACAACCATACATTCCCAGGT
Sμ-F	CAATGTGGTTTAATGAATTTGAAGTTGCCA
Sμ-R	TCTCACACTCACCTTGGATCTAAGCACTGT
Sα-F	TGAAAAGACTTTGGATGAAATGTGAACCAA
Sα-R	GATACTAGGTTGCATGGCTCCATTCACACA
Biotin-dUTP end labeling	
Sμ-F	AAGCTTGCTGAGCAAAATTAAGGGAACAAGG
Sμ-R	GTCTACTTCATTTTGGCTCAAGCCCAGCTTT
Sγ1-F	AGTGTGGGAACCCAGTCAAA
Sγ1-R	GTACTCTCACCGGGATCAGC
DNA break assays (Fig. 3)	
β2M-F	GGTGACGACCTCCGGATCTG
β2M-R	GCCGAGTAGCAGCCACTGAAA
LM-PCR	
LMPCR.1	GCGGTGACCCGGGAGATCTGAATTC
LMPCR.2	GAATTCAGATC
5′ Sμ-F	GCAGAAAATTTAGATAAAATGGATACCTCAGTGG
5′ Sμ-R	LMPCR.1
5′ Sμ-probe	DIG–AGGGACCCAGGCTAAGAAGGCAAT
GAPDH (forward)	ATCCTGTAGGCCAGGTGATG
GAPDH (reverse)	AGGCTCAAGGGCTTTTAAGG
3C assay	
Eμ	GGAACAATTCCACACAAAGACTC
Eα	CAAGGTGTTAAGGAAAACTTGCTC
Synapsis by 3C	
Sμ	GCTGACATGGATTATGTGAGG
Sγ1 (Fig. 3)	CGACACTGGGCAGTTCATTTTG
Sα	GAGCTAGGCTAGACTTACTAAGC
GAPDH3c (forward)	AGTAGTGCGTTCTGTAGATTCC
GAPDH3c (reverse)	CAGTAGACTCCACGACATAC
hnRNP K NM_025279.1, bases 1334–1357	
WT	TCCCAAAGATTTGGCTGGATCTAT
Modified	CCCGAAGGACCTAGCGGGCTCCAT
siRNA target site modification hnRNP L NM_177301.5, bases 939–963	
WT	CCTGGGAGATCATCCCGCAGAATAT
Modified	GCTAGGTGACCACCCTGCGGAGTAC

Table S2. SiRNA oligos

siRNA oligo	Catalog no.	Nucleotide sequence
Human		
hnRNP K	HSS179311	UCCCAAAGAUUUGGCUGGAUCUAUU
hnRNP L	HSS104917	GGAGCGUGAACAGUGUGCUUCUCUU
Mouse		
hnRNP K	MSS205172	UCCCAAAGAUUUGGCUGGAUCUAUU
hnRNP L	MSS236779	CCUGGGAGAUCAUCCCGCAGAAUAU
hnRNP M	MSS233822	GGCAGUCACUUAAAGACCUGGUUAA
HnRNP Q	MSS226121	GCAAGCAGCAAAGAAUCAAAUGUAU

Table S3. Antibodies

Antibody	Company	Catalog no.
hnRNP K(D-6)	Santa Cruz	sc-28380
hnRNP L(D-5)	Santa Cruz	sc-48391
hnRNP M1-4(1D8)	Santa Cruz	sc-20002
c-Myc(9E10)	Santa Cruz	sc-40
CD4-FITC(RPA-T4)	eBioscience	11-0049
IgM-FITC	eBioscience	E00715-1631
IgA-PE	Southern Biotech	C2904-W100
FLAG	Sigma	F1804
mAID	eBioscience	K10211
Tubulin	Calbiochem	CP06
phospho-H2A.X(Ser139)	Millipore	05-636

Table S4. Constructs

Construct name	Description
pcDNA3.1-hnRNP K-cMyc-FLAG (wt-K^R)	WT mouse hnRNP K
pcDNA3.1-ΔKH3^R-cMyc-FLAG	KH3 deletion mutants
pCMV6-hnRNP K-cMyc-FLAGg	WT mouse hnRNP K
pCMV6-ΔKH1^R-cMyc-FLAG	KH1 deletion mutants
pCMV6-ΔKH2^R-cMycFLAG	KH2 deletion mutants
pCMV6-ΔKH3^R-cMyc-FLAG	KH3 deletion mutants
pCMV6-KH1^R-cMyc-FLAG	KH2 and KH3 deletion mutants
pCMV6-KH2^R-cMyc-FLAG	KH1 and KH3 deletion mutants
pCMV6-KH3^R-cMyc-FLAG	KH1 and KH2 deletion mutants
pCMV6-hnRNP L-cMyc-FLAG (wt-L^R)	WT mouse hnRNP L
pCMV6-Δ12-L^R-cMyc-FLAG	Atypical RRM1 and aRRM2 deletion mutants
pCMV6-Δ34-L^R-cMyc-FLAG	Atypical RRM3 and aRRM4 deletion mutants
pCMV6-Δ234-L^R-cMyc-FLAG	Atypical RRM2, 3 and 4 deletion mutants
pCMV6-Δ123-L^R-cMyc-FLAG	Atypical RRM1, 2 and 3 deletion mutants
pCMV6-Δall-L^R-cMyc-FLAG	All atypical RRMs deletion mutants
pCMV-wtAID-3xFLAG	WT human AID
pCMV-AID-ΔN20-3xFLAG	20-aa N-terminal deletion mutant
pCMV-AID-ΔN50-3xFLAG	50-aa N-terminal deletion mutant
pCMV-P20-3xFLAG	34-aa C-terminal insertion mutant (natural mutant)
pCMV-JP8Bdel-3xFLAG	16-aa C-terminal deletion mutant (natural mutant)