Appendix Information

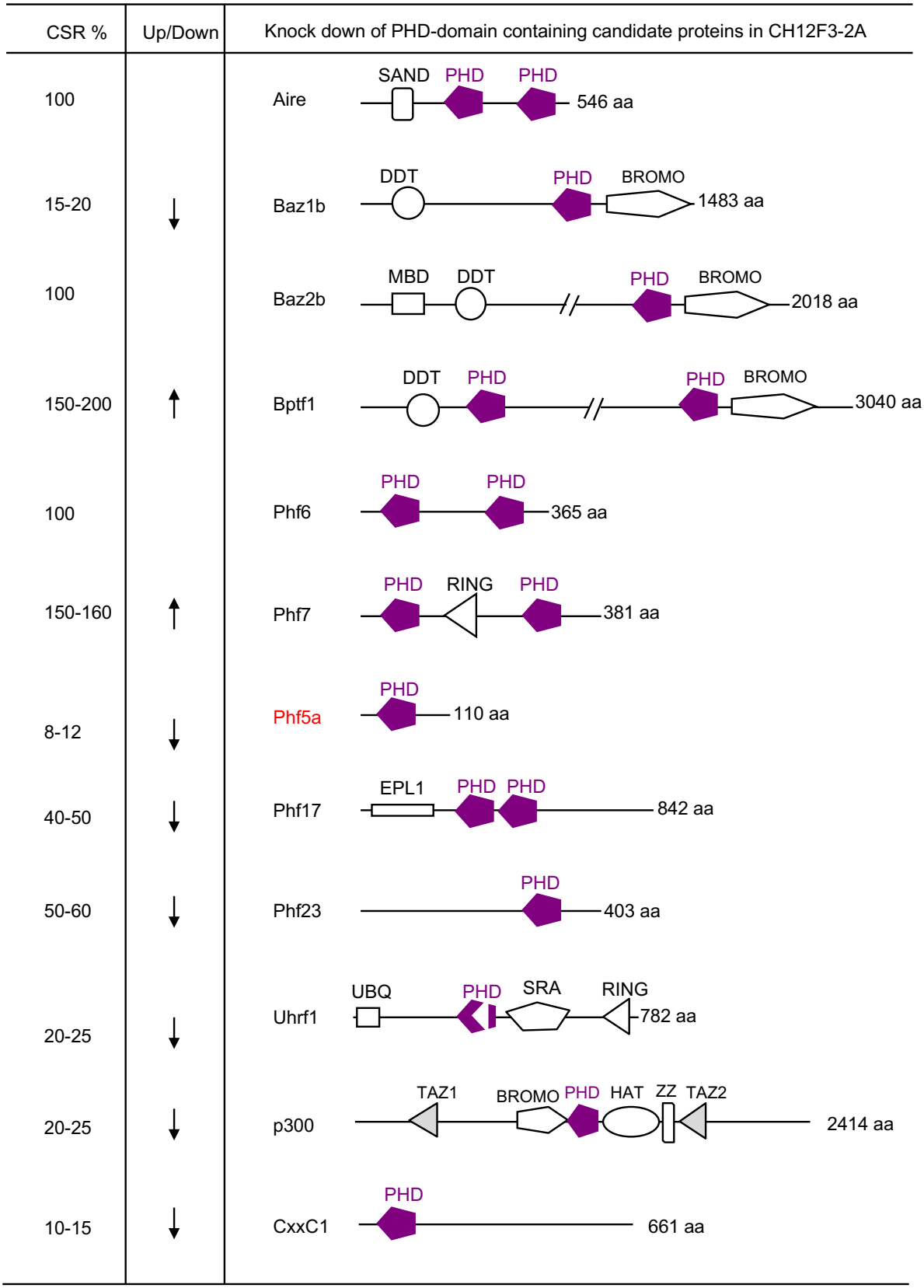
Non-canonical role of Phf5a in DNA repair and antibody class switch recombination relies on histone H2A variants regulation

Nasim A. Begum, Farazul Haque, Andre Stanlie, Afzal Husain, Samiran Mondal, Mikiyo Nakata, Takako Taniguchi, Hisaaki Taniguchi and Tasuku Honjo

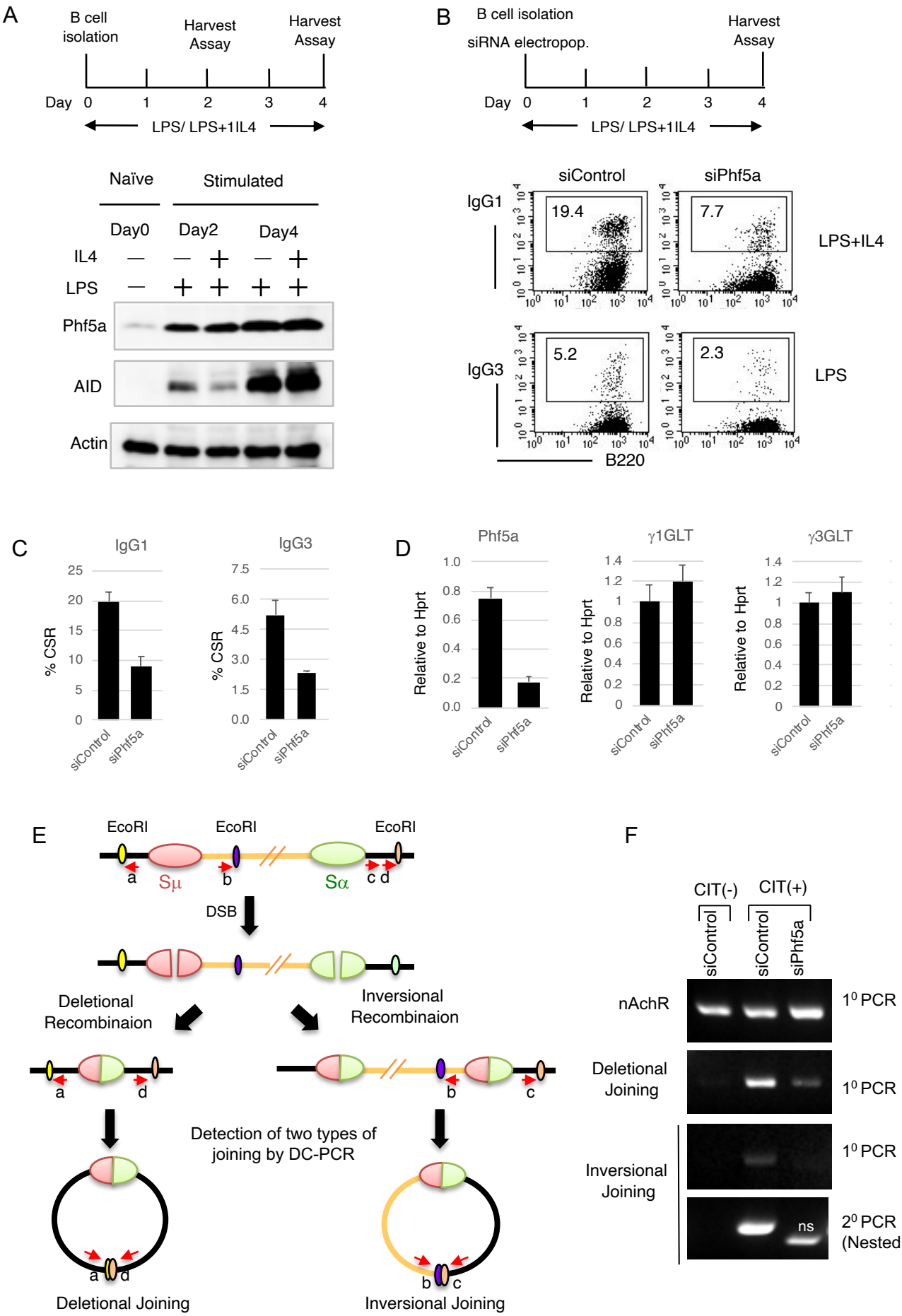
List of Contents

Appendix Figures

Appendix Tables



Appendix Figure S1. Screening of PHD domain-containing chromatin proteins for CSR. Schematic representation of the chromatin proteins selected for knock down in CH12F3-2A cells. The various functional domains in each protein are shown as different geometric shapes. The PHD (plant homeodomain) ring finger is depicted as a purple pentagon. The first column from the left represents the % of IgA switching, after KD of the indicated candidate genes, relative to that of siControl-transfected cells, which was set to 100. The positive or negative impact of the KD on CSR is indicated by an upward- or downward-pointing arrow, in the next column.



Appendix Figure S2

Appendix Figure S2 : Effect of Phf5a knockdown in Ig-CSR in primary B cells and CH12F3-2A cells. (A) Experimental design and the confirmation of of Phf5a expression in naïve and stimulated B cells purified from spleens of 6 weeks old mice. Cells were stimulated with LPS or LPS+IL4 and harvested at two different time points as depicted. **(B)** Experimental design to assay the effect of Phf5a KD on the CSR in primary B cells. Percentages of the IgG1 and IgG3 switching are indicated in the representative plots. **(C-D)** Compilation of CSR and qRT-PCR analysis of transcript (n=3, mean ± sd). **(E-F)** Analysis of the deletional and inversional recombination events during IgM to IgA class switching in CH12F3-2A cells. **(E)** Schematic illustration of the IgH locus undergoing deletional (productive) and inversional (non-productive) recombination after AID-induced DSB at Sμ (light pink oval) and Sα (light green oval). The position of the three *Eco*RI sites (3 small ellipses of different colors) relative to Sμ and Sα in the intact loci (top panel) will change after recombination (middle panel), which is detected in the DC-PCR assay. After *EcoR*I digestion and circularization of the isolated genomic DNA, two types of junctions were detected by PCR using a specific primer pair (deletional joining by primers a+d; inversional joining by primers b+c or b+d) (bottom panel). **(F)** A representative result of DC-PCR. CH12F3-2A cells were transfected with the indicated siRNAs, and 24 h later, the cells were stimulated by CIT for IgM to IgA switching. The genomic DNA was isolated and subjected to *Eco*RI digestion and the DC-PCR assay as described above and in the methods.

A

Condition	Total clones	Sequenced (bp)	Mutated clones	Total mutations	Mutation frequency
siControl OHT (-)	58	23664	1	2	$0.84x10^{-4}$
siControl OHT (+)	108	44064	26	42	$9.53x10^{-4}$
siPhf5a #20 OHT (+)	108	44064	22	38	$8.62x10^{-4}$
siPhf5a #28 OHT (+)	102	41616	20	37	$8.91x10^{-4}$

B

siControl OHT(+)

From \ To	A	G	C	T	Tot
A					0
G	15		6		21
C		3		16	19
T	1		1		2
					42

siPhf5a #20 OHT(+)

From \ To	A	G	C	T	Tot
A					0
G	17		3		20
C	1	4		13	18
T					0
					38

siPhf5a #28 OHT(+)

From \ To	A	G	C	T	Tot
A					0
G	13		5	2	20
C		2		14	16
T			1		1
					37

C

Condition	Total clones	Sequenced (bp)	Mutated clones	Total mutations	Mutation frequency
siControl	157	88705	49	96	$9.7x10^{-4}$
siPhf5a	150	84750	37	84	$9.9x10^{-4}$

D

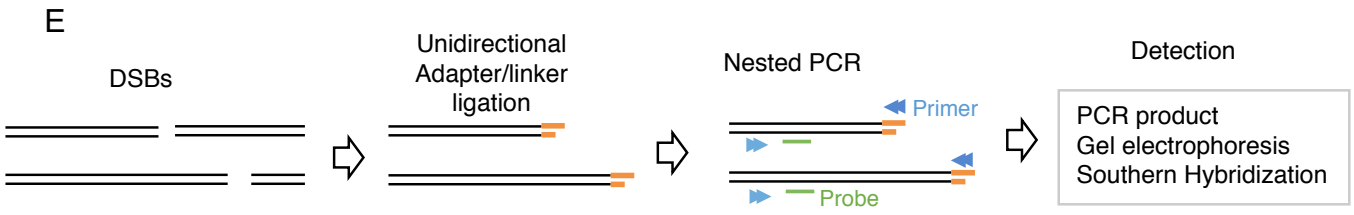
siControl CIT(+)

From \ To	A	G	C	T	Tot
A			1		1
G	25		2		27
C		1		67	68
T					
					96

siPhf5a CIT(+)

From \ To	A	G	C	T	Tot
A		2			2
G	19				19
C		1		62	63
T					
					84

E



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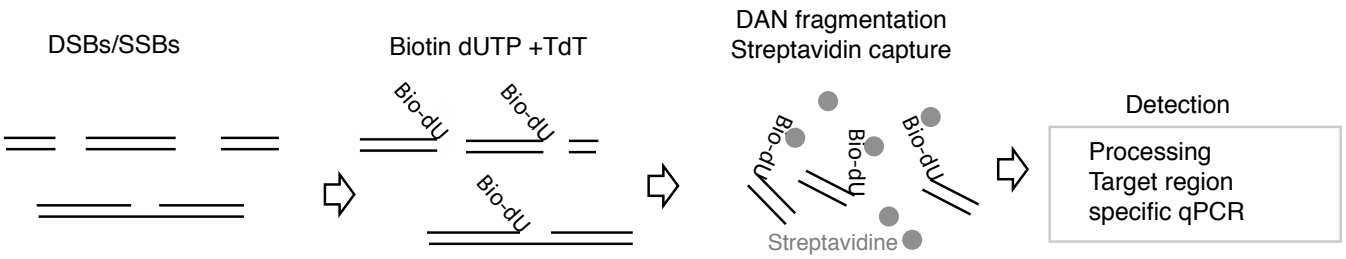
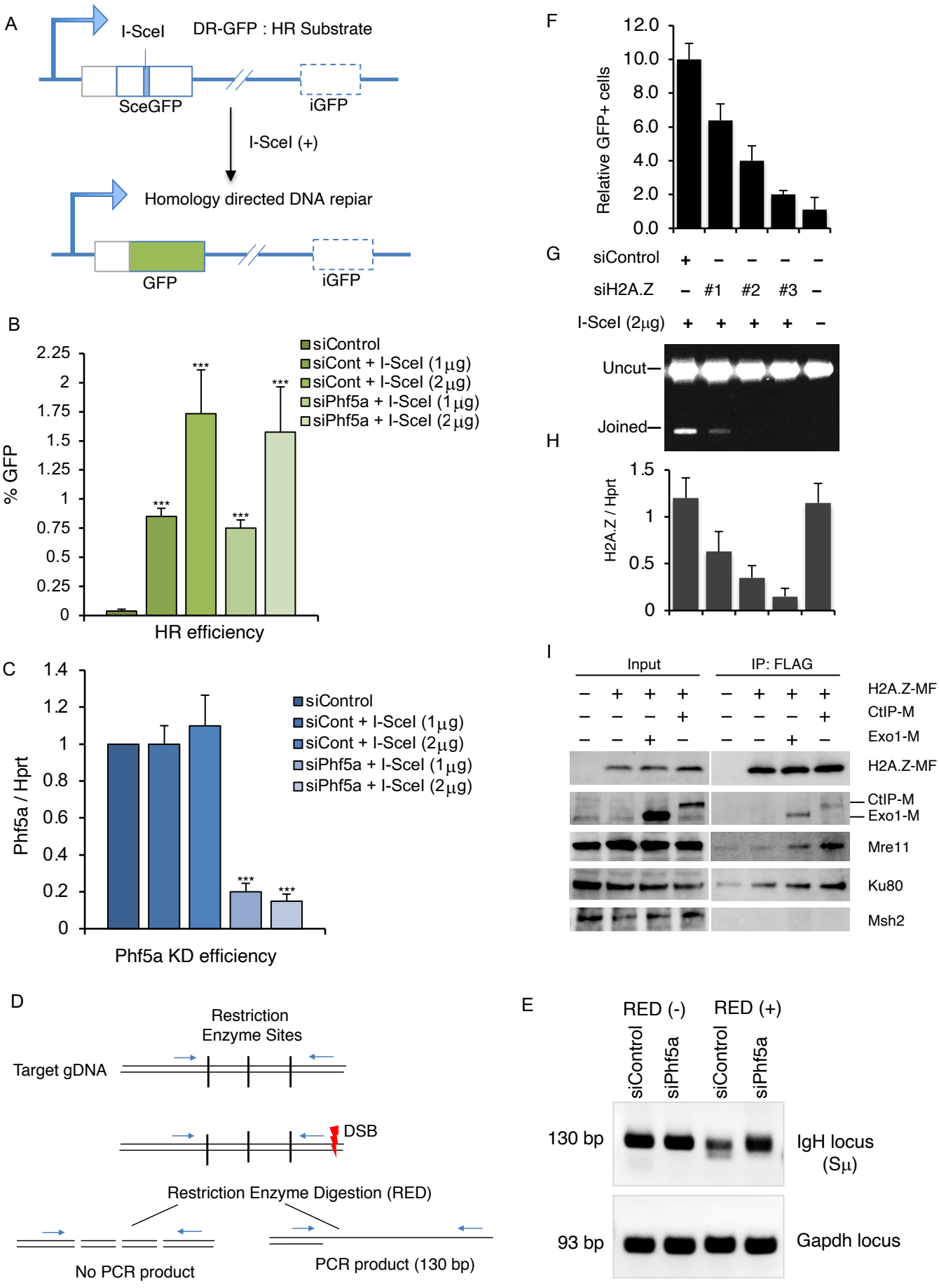


Appendix Figure S3

Appendix Figure S3 : Phf5a is dispensable for SHM and AID-induced DNA break. (A) A BL2 cell line expressing AID (JP8BdelER) was activated by OHT as depicted in Fig.3A, and described in the methods. The IgH V region was amplified by RT-PCR, cloned, and sequenced. The number of clones sequenced for mutation frequency analysis was tabulated. The data are compiled from two independent representative experiments. **(B)**. Nucleotide substitution profile summarized from the total number of mutations. **(C)** Somatic hypermutation analysis in the 5'Sμ in CH12F3-2A cells. Cells were transfected with indicated siRNAs and stimulated with CIT for 2 days. The target region was PCR amplified and mutations were analyzed by cloning and sequencing. Mutation frequency was calculated from two independent experiments, and the total numbers of mutations analyzed are as shown. **(D)** Comparable mutation spectra was evident between control and Phf5a KD. **(E)** LM PCR assay to detect DNA double strand breaks. **(F)** DNA break detection by Biotin-dUTP break end labeling assay.



Appendix Figure S4

Appendix Figure S4 : Phf5a and H2A.Z promote NHEJ and prevent DNA end resection. (A) Phf5a is dispensable for homologous recombination (HR). Schematic diagram of the DR-GFP construct, which expresses GFP only after *I-Sce*I-induced target site cleavage and DNA repair by HR. The *I-Sce*I cleavage site (light blue vertical rectangle) and the donor template for homology-mediated repair were located in SceGFP and iGFP (dotted rectangle), respectively. **(B)** Percentage of GFP-positive cells assessed by FACS analysis after co-transfection of the *I-Sce*I-expressing plasmid and the indicated siRNAs into GM7166VA cells (n=3, mean ± sd; two-tailed unpaired Student's t-test; *** $p \leq 0.001$). **(C)** Confirmation of Phf5a KD in the samples by qRT-PCR. **(D)** Schematic representation of DNA end resection assay. **(E)** Image of an agarose gel with PCR products obtained using genomic DNA isolated from wild type and Phf5a depleted CH12F3-2A cells. Prior to PCR, DNA was fragmented and left untreated or treated with target site cleaving enzyme (Restriction Endonuclease Digestion; RED-/+). Following purification of the digested DNA, Sμ (target) and Gapdh (loading control) locus specific PCR was performed. **(F-G)** Depletion of H2A.Z strongly impaired NHEJ as in the case of Phf5a depletion (Fig.4). NHEJ reporter line and assays are same as described in Fig.4B-D. **(H)** Confirmation of H2A.Z KD in the samples by qRT-PCR. (n=2, mean ± sd). **(I)** NHEJ repair factors interact with H2A.Z. Myc-FLAG (MF) epitope-tagged H2A.Z was co-transfected with the indicated plasmids. An anti-FLAG antibody was used for the detection and immunoprecipitation of H2A.Z-MF. An anti-Myc antibody was used to detect Myc epitope-tagged Exo-1 (Exo1-M) and CtIP (CtIP-M) in the Western blots. Interactions were also verified by reciprocal co-IP (not shown).

Ref seq	ATTCGAATTCGGATCTAGGGATAA TAAGCTTAAGCCTAGATCCC			CCCTAGGATCCTTCCGGT AATAGGGATCCTAGGAAGGCCA	Clone no.
siControl	ATTCGAATTCGGATCTAGGG	ATAAT		CCCTAGGATCCTTCCGGT	22
	ATTCGAATTCGGATCTAGGG	ATTAT		CCCTAGGATCCTTCCGGT	20
	ATTCGAATTCGGATCTAGGG	ATAT		CCCTAGGATCCTTCCGGT	4
	ATTCGAATTCGGATCTAGGG	AT		CCCTAGGATCCTTCCGGT	9
					55
	ATTCGAATTCGGATCTAGGG	In 6 bp		ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 4 bp	Δ1	ATTCGAATTCGGATCTAGGG	1
					2

Ref seq	ATTCGAATTCGGATCTAGGGATAA TAAGCTTAAGCCTAGATCCC			CCCTAGGATCCTTCCGGT AATAGGGATCCTAGGAAGGCCA	Clone no.
siPhf5a	ATTCGAATTCGGATCTAGGG	ATAAT		CCCTAGGATCCTTCCGGT	20
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	ATTCGAATTCGGATCTAGGG	ATAT		CCCTAGGATCCTTCCGGT	3
	ATTCGAATTCGGATCTAGGG	AT		CCCTAGGATCCTTCCGGT	2
					32
	ATTCGAATTCGGATCTAGGG	In 4 bp		ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In119 bp		ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 1 bp	Δ11	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 25 bp	Δ50	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 36 bp	Δ50	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 2 bp	Δ4	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 5 bp	Δ4	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 15 bp	Δ14	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 11 bp	Δ30	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 3 bp	Δ4	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 4 bp	Δ12	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 1 bp	Δ5	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 12 bp	Δ75	ATTCGAATTCGGATCTAGGG	1
					13

Ref seq	ATTCGAATTCGGATCTAGGGATAA TAAGCTTAAGCCTAGATCCC			CCCTAGGATCCTTCCGGT AATAGGGATCCTAGGAAGGCCA	Clone no.
siH2A.Z	ATTCGAATTCGGATCTAGGG	ATAAT		CCCTAGGATCCTTCCGGT	10
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	ATTCGAATTCGGATCTAGGG	AT		CCCTAGGATCCTTCCGGT	2
					34
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	ATTCGAATTCGGATCTAGGG	In 158 bp	~	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 32 bp	Δ76	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 36 bp	Δ352	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 127 bp	Δ353	ATTCGAATTCGGATCTAGGG	1
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	ATTCGAATTCGGATCTAGGG	In 8 bp	Δ10	ATTCGAATTCGGATCTAGGG	1
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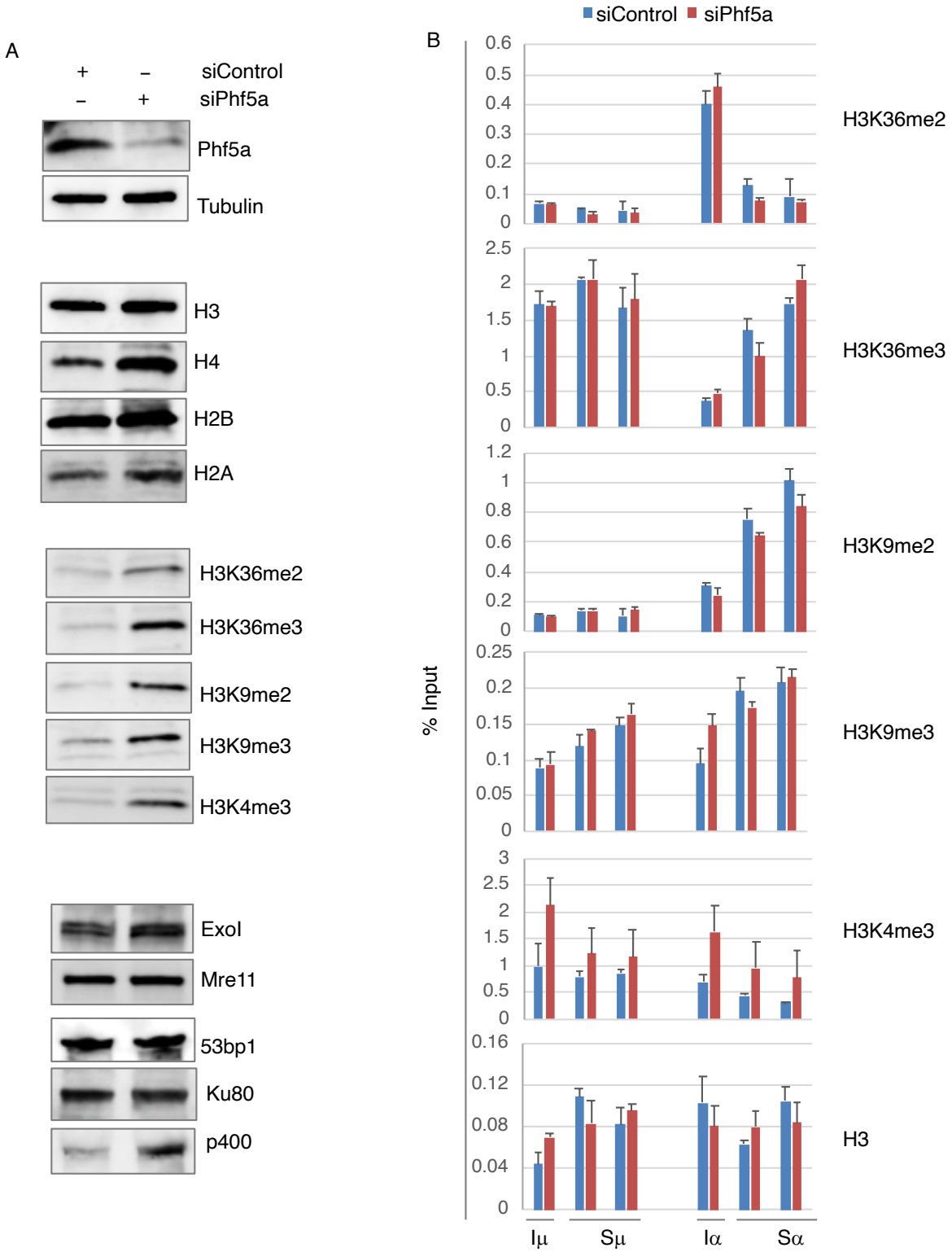
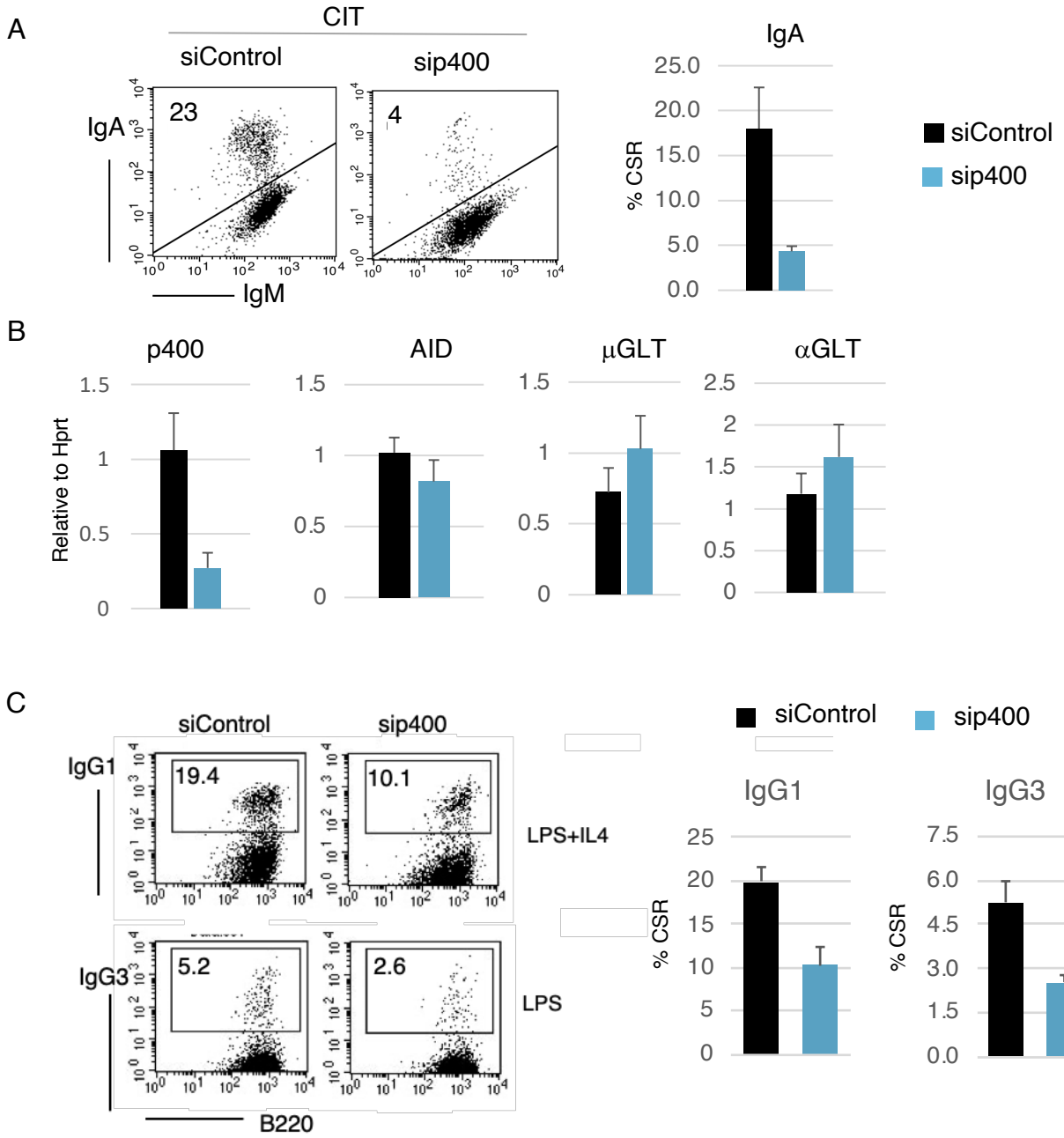
	ATTCGAATTCGGATCTAGGG	In 17 bp	Δ17	ATTCGAATTCGGATCTAGGG	1
	ATTCGAATTCGGATCTAGGG	In 3 bp	Δ4	ATTCGAATTCGGATCTAGGG	1
					13

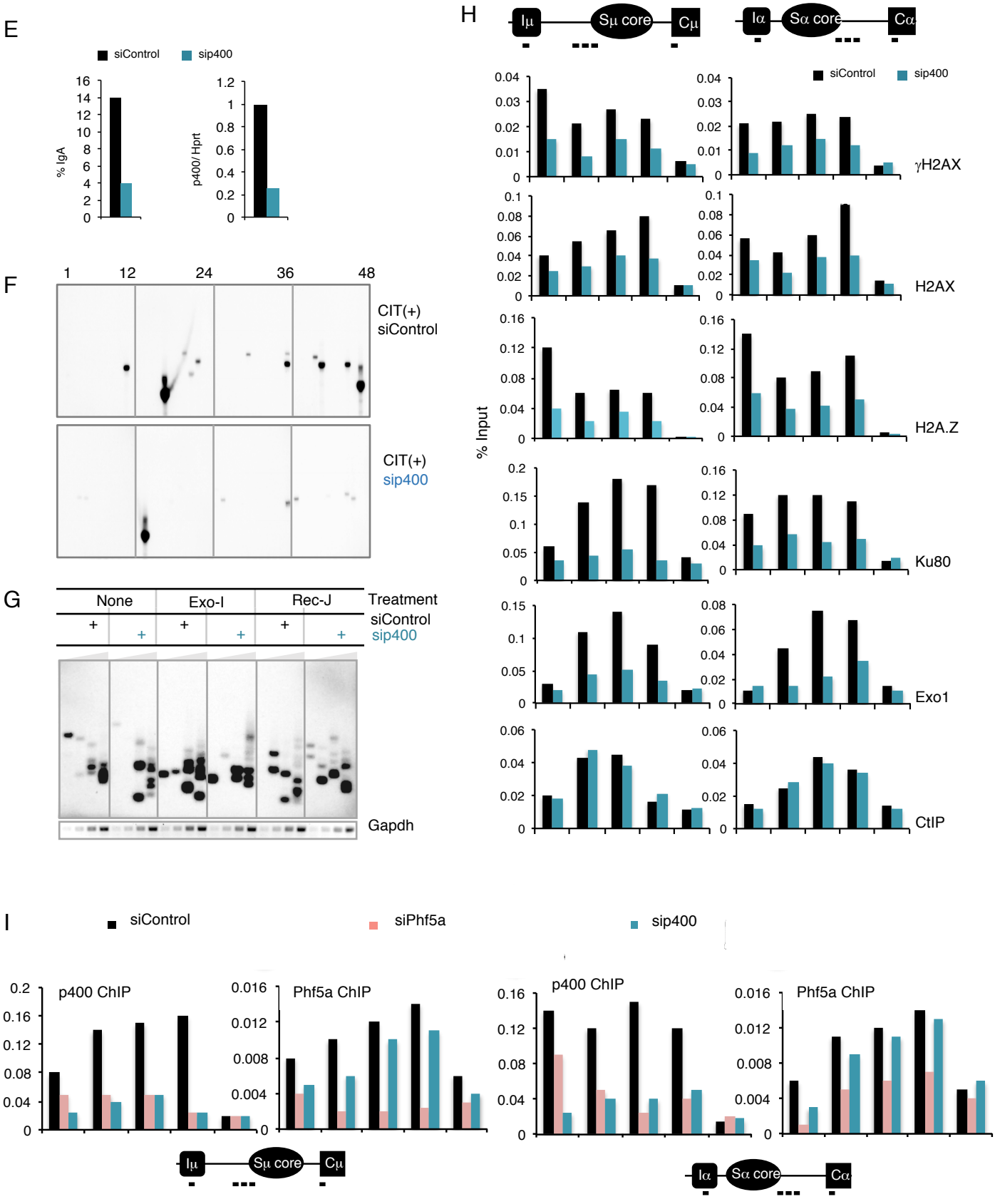
Appendix Figure S5 : Loss of Phf5a or H2A.Z similarly affected recombination junctions. Phf5a or H2A.Z KD increases the frequency of insertions and deletions at the repair junctions. Extended analysis of the NHEJ-mediated repair of *I-Sec*I-cleaved junctions. Genomic DNA was isolated from cells transfected with the indicated siRNA, and the repaired junctions were PCR amplified as described in Fig.4. Due to limited amounts of material, the PCR-amplified products were combined from multiple experiments prior to cloning. The total number of clones analyzed in the control, Phf5a KD, and H2A.Z KD samples was 57, 45, and 47, respectively.



Appendix Figure S6 : Expression of histones and histone-PTMs upon Phf5a depletion. (A) Western blots of whole-cell-extracts from stimulated CH12F3-2A cells transfected with siControl or siPhf5a. The antibodies used for immunoblotting are indicated next to the respective blot. **(B)** ChIP analyses of H3 and various H3K methylation for IgH locus using chromatin extract from stimulated CH12F3-2A cells as described in A. The data are the means ± sd from two experiments.

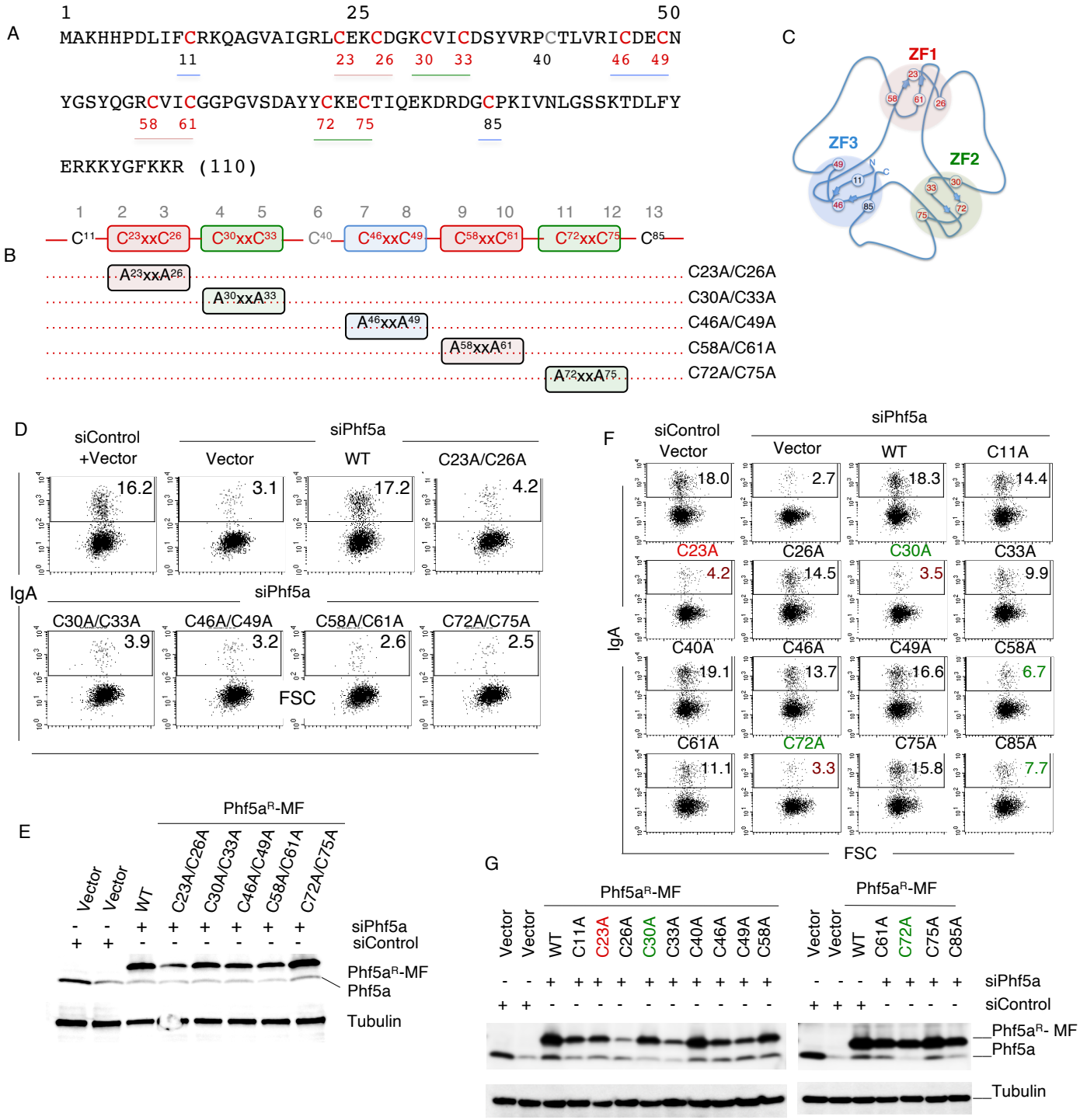


Appendix Figure S7

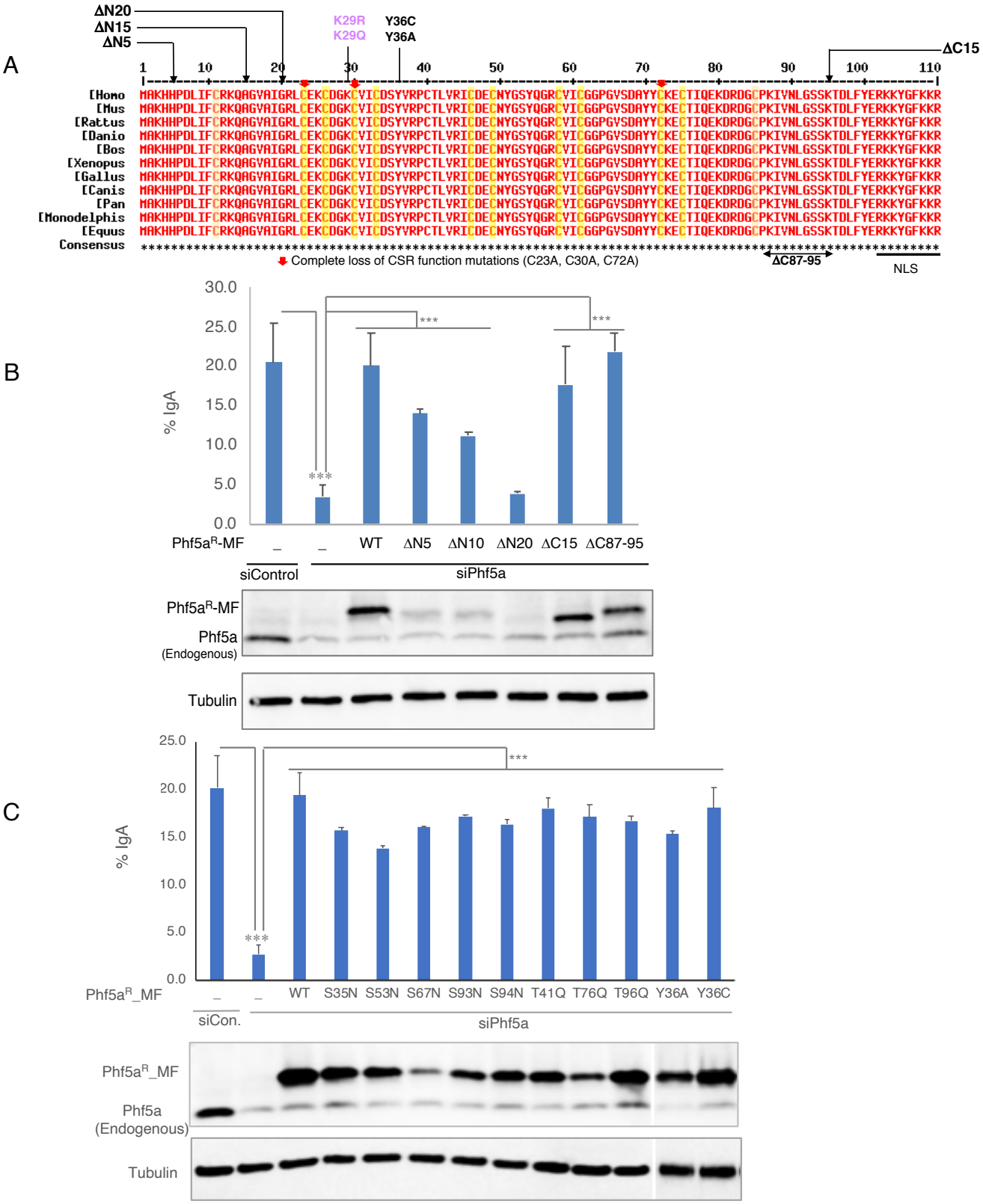


Appendix Figure S7

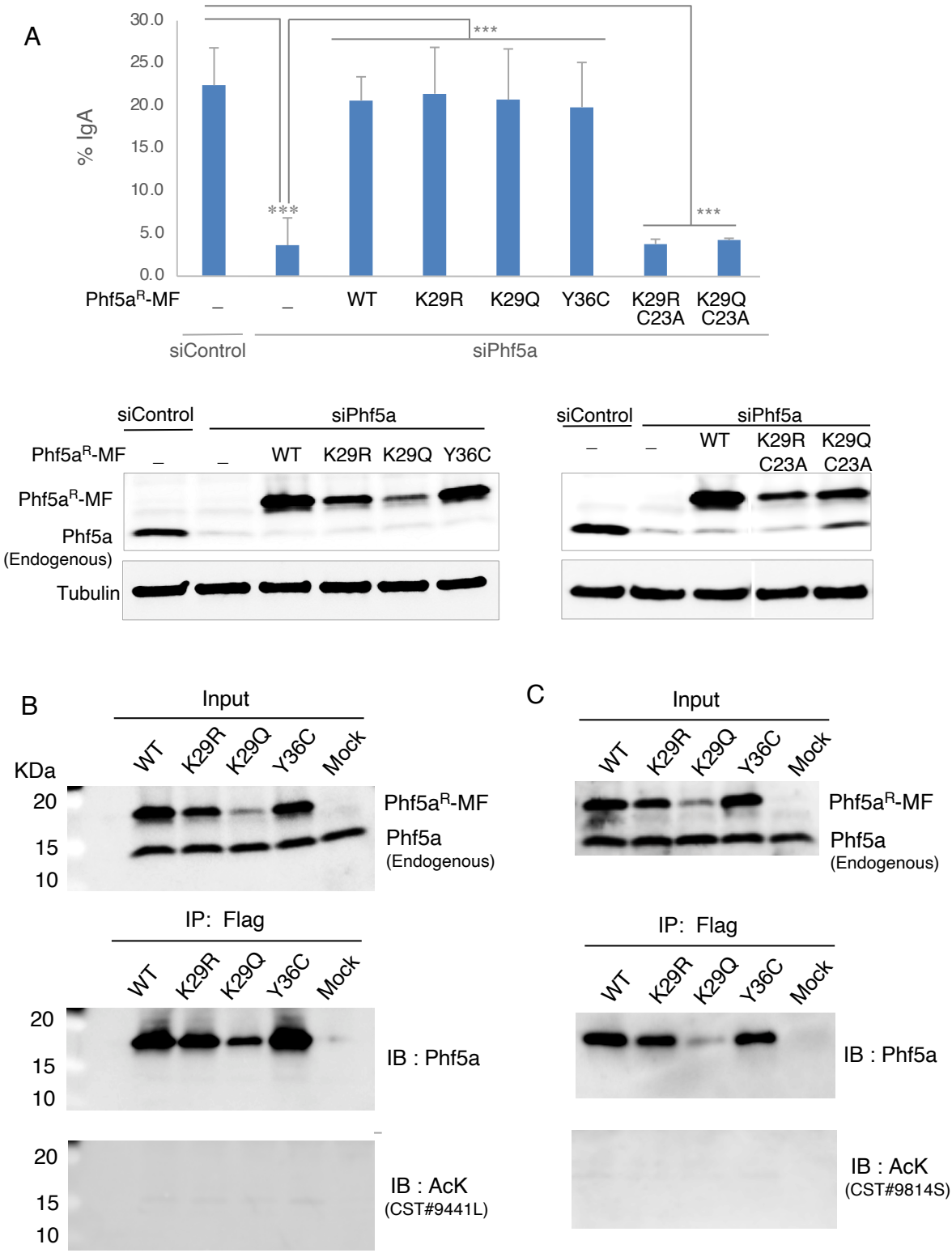
Appendix Figure S7: Knockdown of p400 affects AID-induced genomic instability similar to Phf5a KD. (**A-B)** Knockdown of p400 in CH12F3-2A cells impaired CSR. The cells were transfected with the indicated siRNAs, and IgA switching was examined 24h after CIT stimulation. Knockdown efficiency of p400, and transcription of AID and GLTs were examined by qRT-PCR (n=3, mean ± sd). **(C-D)** Knockdown of p400 in primary B cells, and CSR and transcript analyses. The experimental design and the siControl sample are the same as in Appendix Fig.S2B and C. Purified splenic B cells were transfected with siRNAs indicated and stimulated with LPS or LPS+IL4 for IgG3 and IgG1 switching, respectively. **(C)** A representative FACS profile as well as the summary of 3 independent experiments is shown (mean ± sd). **(D)** Quantitative RT-PCR analysis of p400, AID and GLTs. **(E-F)** CSR, Translocation, DNA break and ChIP analyses in control and p400 depleted CH23F3-2A cells.**(E)** Examination of CSR and KD efficiency in siControl and sip400 treated CH12F32A cells. **(F)** Representative Southern hybridization blots of the IgH/cMyc translocation assay using genomic DNA isolated from siControl- and sip400-treated cells. The data were combined from two independent experiments. **(G)** DNA DSB detection by LMPCR assay. Genomic DNA was isolated from siControl- and sip400-transfected cells stimulated 24 h with CIT. Prior to linker ligation, the DNA was either left untreated (None) or treated with ExoI or RecJ to repair any resected DNA ends. The linker-ligated DSBs were PCR amplified in combination with site-specific primer and a linker-primer, followed by hybridization with an Sμ–specific probe. **(H)** ChIP analysis of the indicated H2A family histones and DNA repair proteins before and after p400 depletion in CH12F3-2A cells. Locations of ChIP PCR products at the IgH locus are indicated as small horizontal bars in the scheme (Top). **(I)** ChIP analysis of p400 and Phf5a in Phf5a- and p400-depleted cells. Locations of ChIP PCR products in the IgH locus are indicated as small horizontal bars in the scheme (bottom). The data shown (D, E) are the mean of two independent experiments.



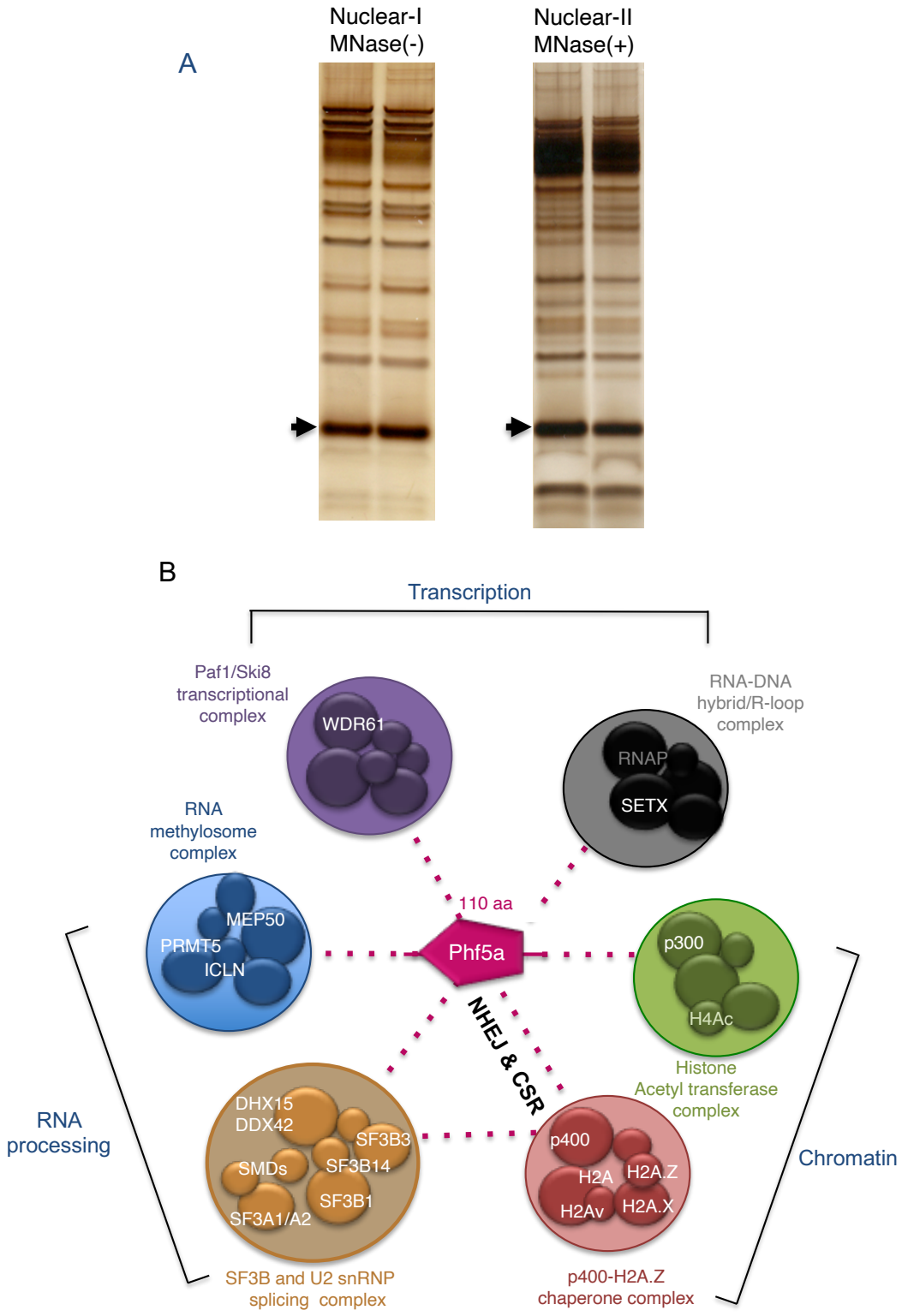
Appendix Figure S8 : Identification of loss-of-CSR function Phf5a mutant by alanine scanning muta genesis. (A) Amino acid sequence of mouse Phf5a with color coded Cys residues involved in zinc finger formation as shown in Fig.7A. **(B)** The mutagenesis [Cys(C) to Ala(A)] scheme of the five CxxC motifs, which are boxed and color-coded as underlined in A. Each dotted line with AxxA represents the respective CxxC motif mutant. The superscript indicates the number of each Cys residue in the amino acid sequence. **(C)** Diagram of the predicted triquetra knot structure of Phf5a involving the three ZFs. The illustration was adapted from the structure of the yeast Phf5a homologue, Rds3. **(D)**Analysis of CSR complementation efficiency of the CxxC mutants. Representative FACS profile of IgA switching in CH12F3-2A cells transfected with the indicated siRNAs along with an empty vector or a Phf5A expression plasmid (Phf5a^R-MF). **(E)** Confirmation of Phf5a depletion and expression of various CxxC motif mutants. Phf5a and Phf5a^R-MF indicated the expression of endogenous and exogenous Phf5a, respectively. **(F)** Analysis of CSR complementation efficiency of the single Cys mutants [Cys(C) to Ala(A)]. Representative FACS profile of IgA switching in CH12F3-2A cells transfected with the indicated siRNAs along with an empty vector or a Phf5A expression plasmid (Phf5a^R-MF). **(G)** Confirmation of the Phf5a depletion and expression of wild-type and mutant Phf5a^R-MF.



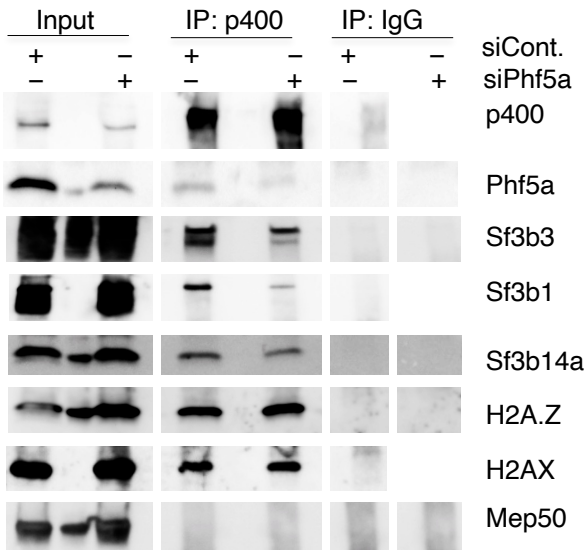
Appendix Figure S9. Phf5a mutants that do not impair CSR. (A) Multiple alignments of Phf5a proteins from different species. Position of various deletions generated in DN and DC mutants are indicated at the N- and C-terminus, respectively. Point mutations generated at K29 or at Y36 are also indicated. The three read arrowheads show the three critical Cys residues essential for CSR. **(B)** CSR complementation assay in CH12F3-2A cells, using indicated deletion mutants. Immunoblotting below the plots show the Phf5a KD efficiency and the expression profile of individual Phf5a mutants. **(C)** CSR complementation assay in CH12F3-2A cells using point mutants generated at the indicated S/T/Y residue. Immunoblotting below the plots show the Phf5a KD efficiency and the expression profile of individual Phf5a mutants.



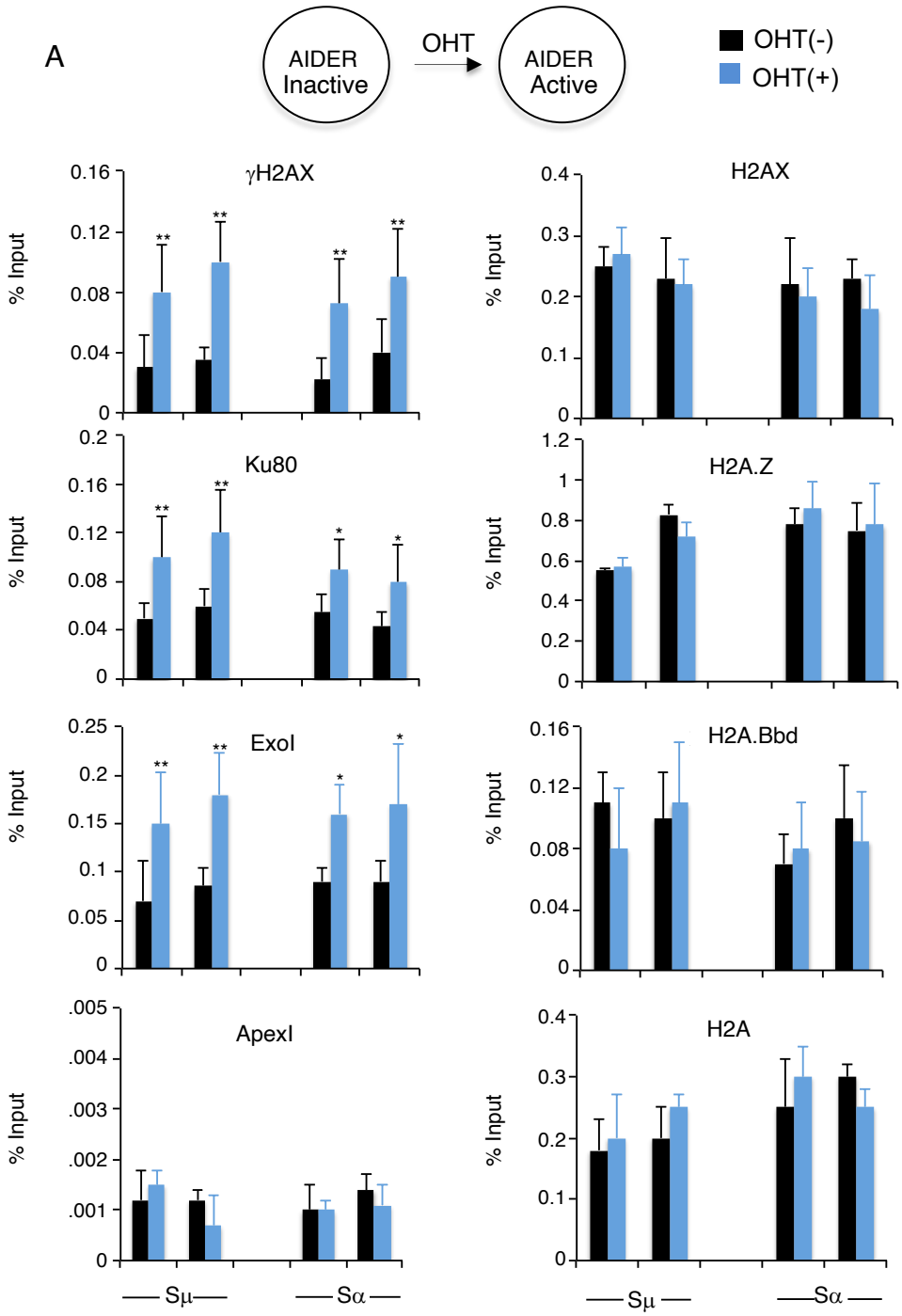
Appendix Figure S10 : Phf5a K29 acetylation is dispensable for CSR. (A) CSR complementation assay in CH12F3-2A cells using indicated point mutants. K29R/Q and Y36C mutants were described (mean ± sd; two-tailed unpaired Student's t-test; *** $p \leq 0.001$). Immunoblotting below the plots show the Phf5a KD efficiency and the expression of the WT and Phf5a mutants transfected. **(B-C)** Anti-Flag IP of WT and Mutant Phf5a, followed western blot with indicated antibodies to detect Phf5a acetylation.



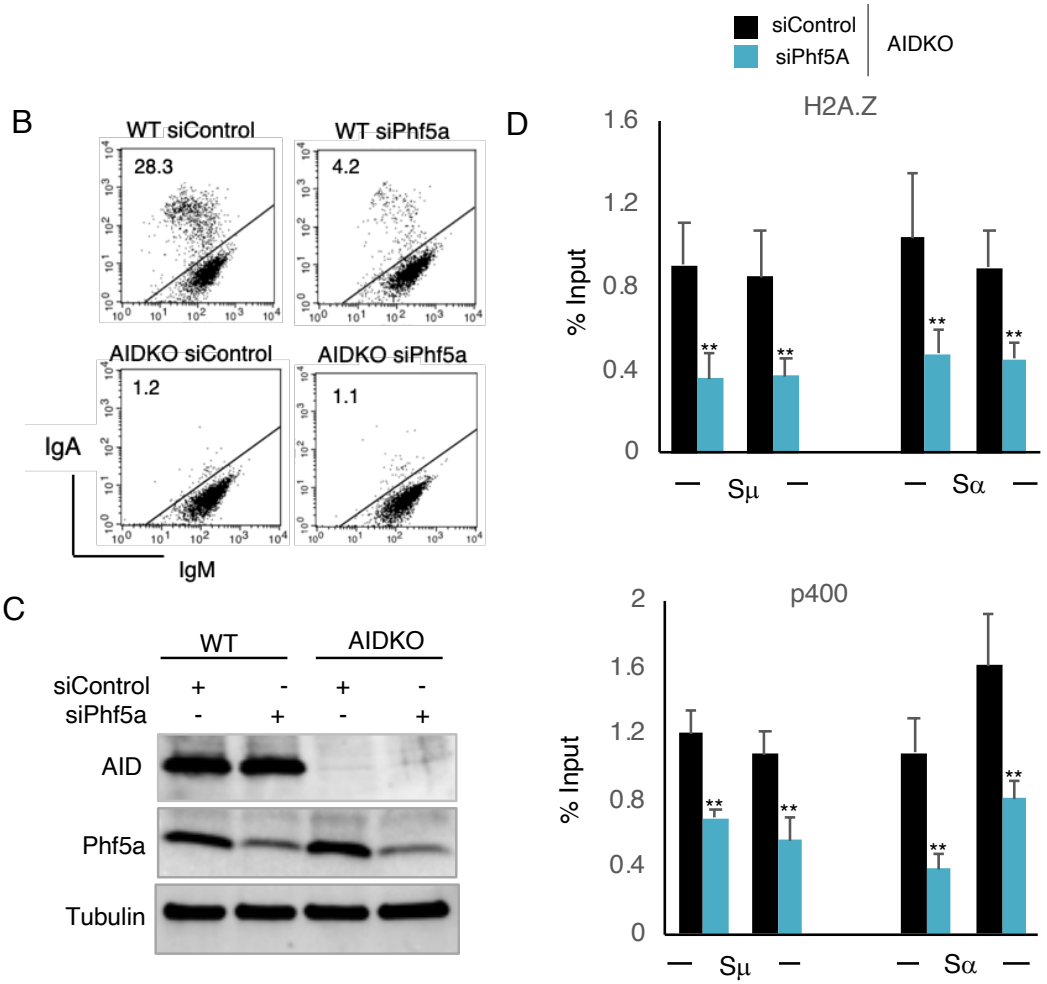
Appendix Figure S11 : Identification of Phf5a-interacting proteins from CH12F3-2A cells. (A) Phf5a-MF was expressed in CH12F3-2A cells, and the Phf5a-MF was IPed by anti-Flag. Silver stained gel shows the Phf5a-associated proteins co-immunoprecipitated from the nuclear extracts with or without micrococcal nuclease (MNase) treatment. The arrowhead indicates the bait protein, Phf5a-MF. Proteins identified by MS analysis were shortlisted in Table S1. **(B)** Interaction of Phf5a with with various proteins were grouped to show its interaction with multi-protein chromatin complexes. This study, for the first time, shows that Phf5a is required for NHEJ and CSR (bold), which is directly linked to H2A variant regulation through p400 chaperone complex. The interaction/crosstalk among Phf5a, p400 and U2SnRNP are indicated by three dotted lines.



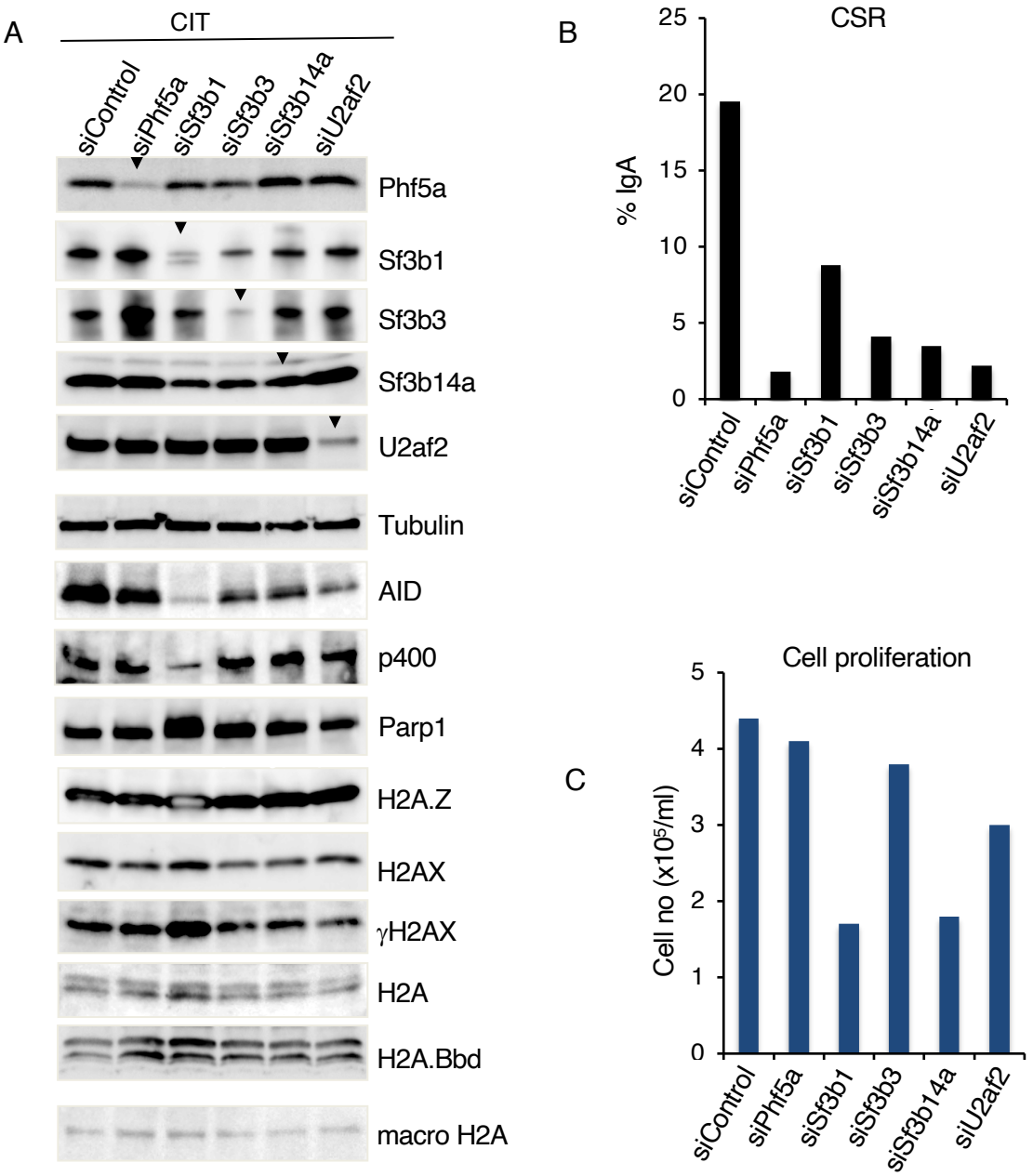
Appendix Figure S12 : Phf5a KD weakens the interaction of p400 with Sf3b subunits but not with H2A.Z or H2AX. Representative western blot analysis of the interacting proteins co-immunoprecipitated with p400. Nuclear extract was prepared from CH12F3-2A cells transfected with the indicated siRNAs and stimulated with CIT for 24 h. Anti-p400 and IgG IP were performed, followed by immunoblot analysis of the IPed products



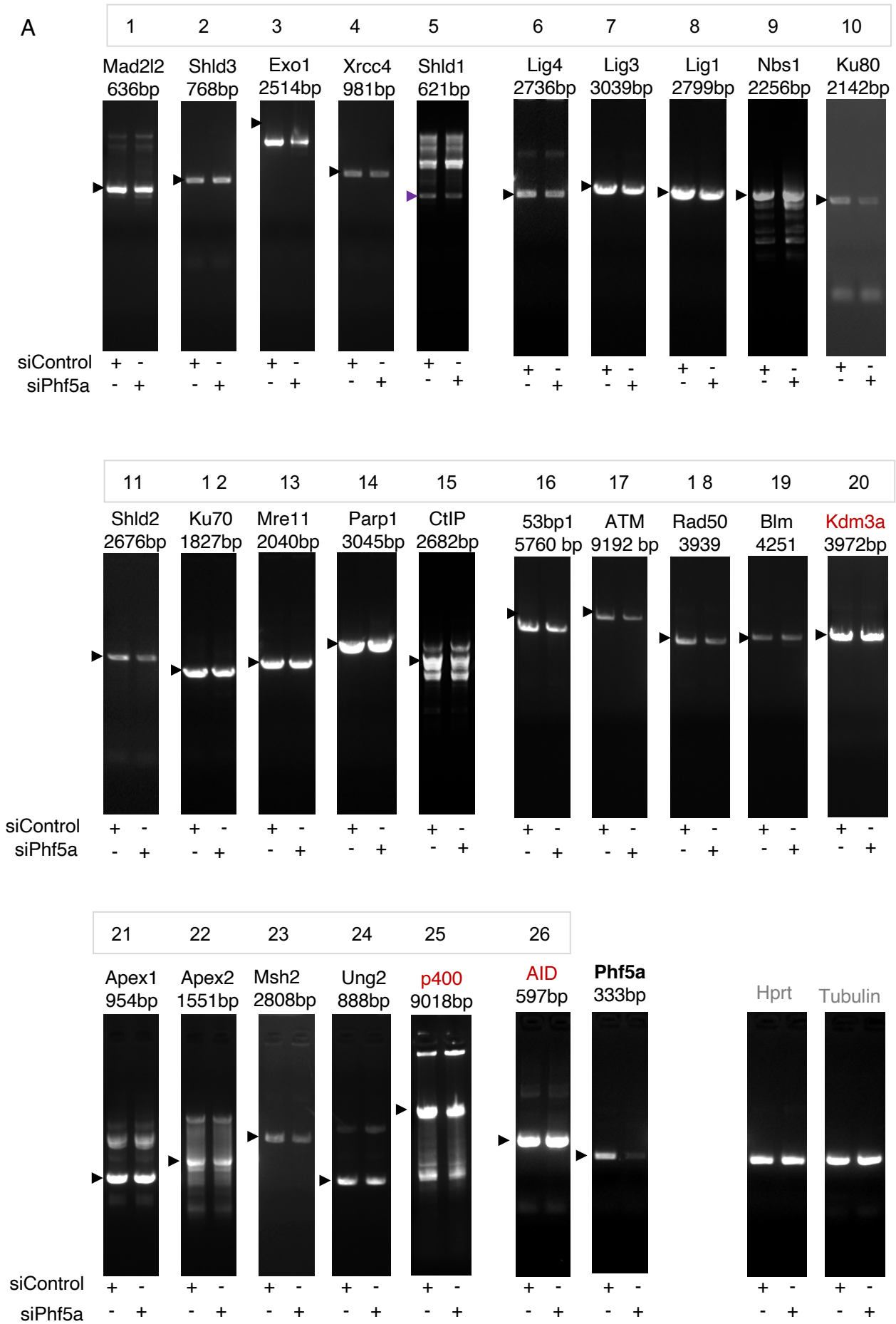
Appendix Figure S13



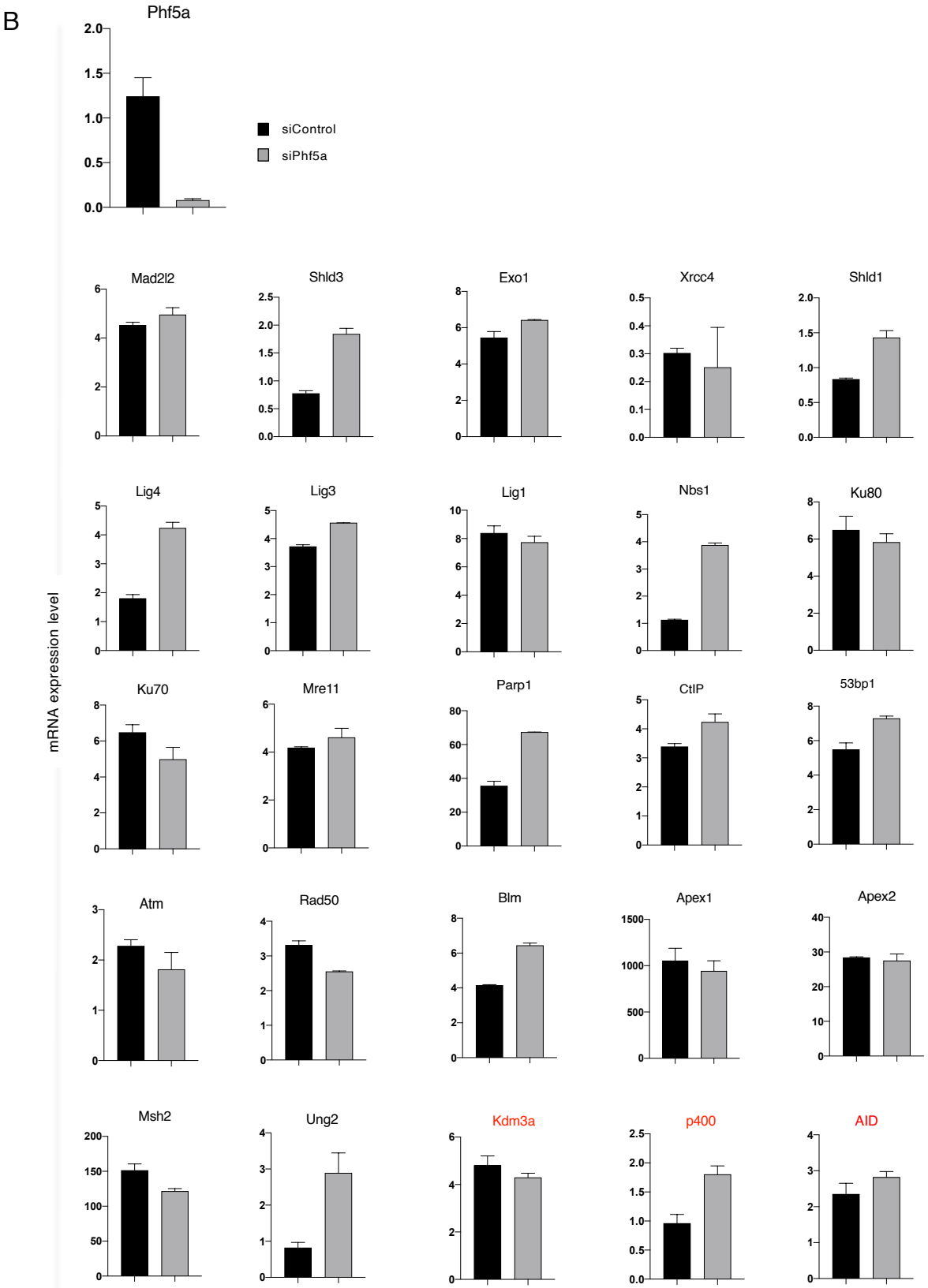
Appendix Figure S13 : AID expression does not impact IgH locus H2A.Z regulation. (A) Activation of AID induced accumulation of γH2AX, Ku80, and Exo1 but not of H2AX, H2A.Z, H2A.Bbd, or H2A at the IgH locus. CH12F3-2A cells expressing AIDER (AID fused with ER) were stimulated with tamoxifen (OHT) to activate AID for 24 h and then subjected to IgH locus-specific ChIP analysis for the indicated proteins. Two pairs of PCR primers were used to examine the Sμ– and Sα–specific ChIP enrichment (n=2; mean ± sd) . **(B)** Representative FACS profiles of IgM to IgA switching in WT ($AID^{+/+}$) and AIDKO ($AID^{-/-}$) CH12F3-2A cells transfected with siRNAs as indicated. Numbers inside the plots indicate % IgA 24 h after CIT stimulation. **(C)** Western blots of AID, Phf5a and Tubulin. **(D)** Switch region ChIP analysis with indicated antibodies in AIDKO transfected with siControl or siPhf5a. Data presented were complied from 3 experiments (mean ± sd; two-tailed unpaired Student's t-test; $^{**}p \leq 0.01$).



Appendix Figure S14 : Knockdown of U2snRNP subunits reduced AID expression and CSR. **(A)** Confirmation of KD of the indicated spliceosomal subunits in CH12F3-2A cells by Western blot analyses. The expression of several other proteins, including AID, H2A family members, and DNA repair-associated proteins were examined. Except Phf5a, KD of all the other spliceosomal subunits reduced the expression of AID. Sf3b1 KD had the strongest inhibitory effect on the AID and p400 expression. **(B)** IgA switching of CH12F3-2A cells transfected with the indicated siRNA and stimulated by CIT for 24 h. **(C)** KD of Sf3b1 and Sf3b14a impaired cell proliferation. The data plotted are mean of 2 independent experiments.

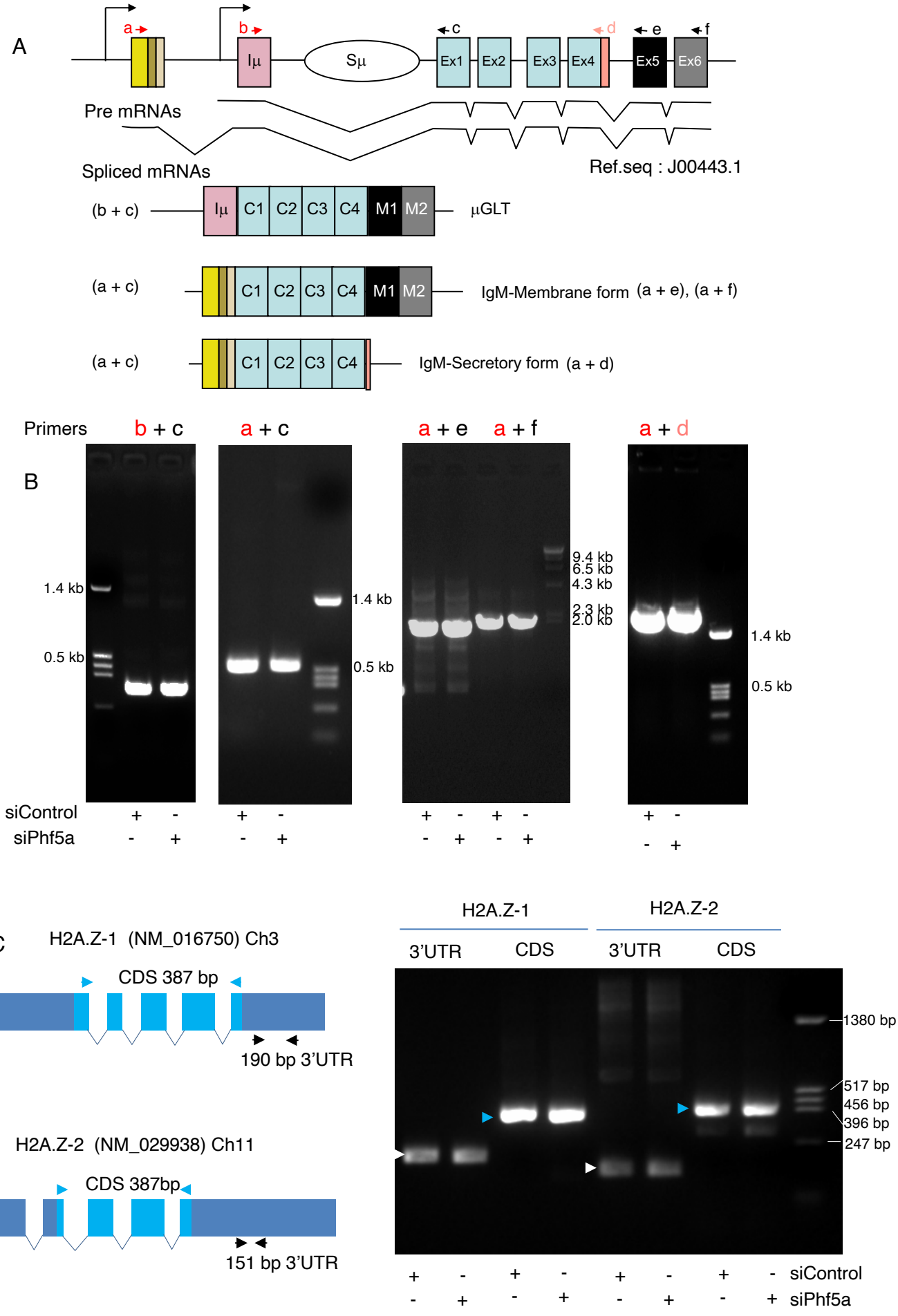


Appendix Figure S15

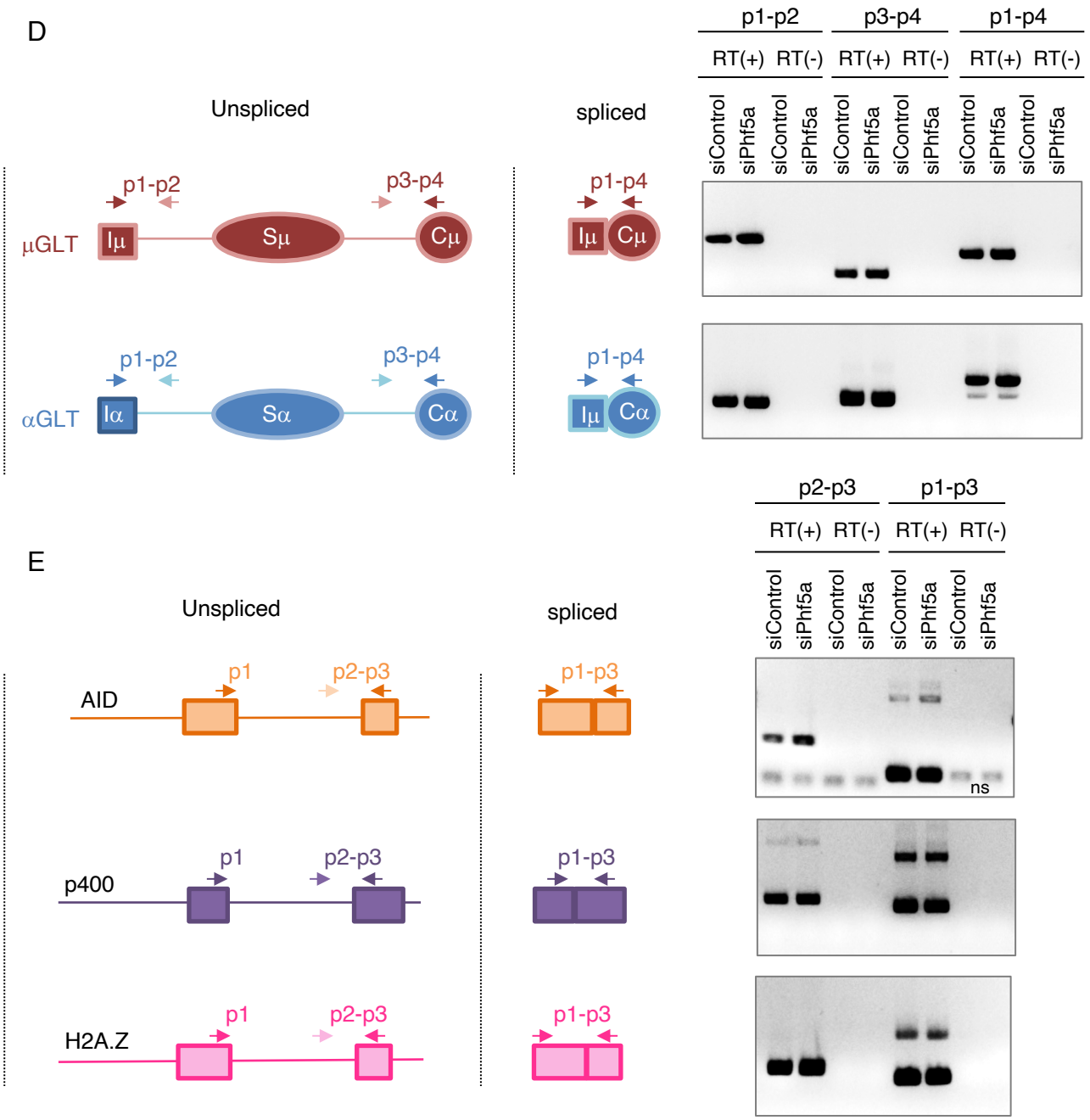


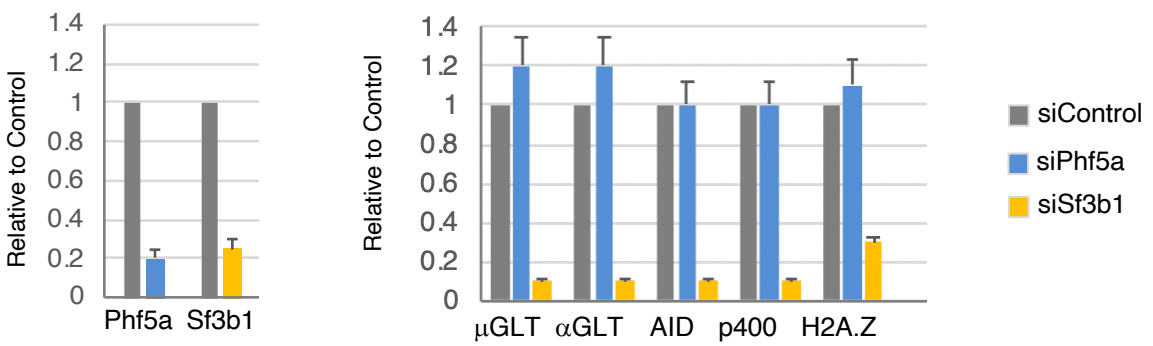
Appendix Figure S15 continued

Appendix Figure S15 : Phf5a depletion did no inhibit expression of genes important for CSR. (A) RT-PCR of full-length coding region of 26 genes including CSR associated DNA repair genes. The RNA was isolated from siControl and siPhf5a treated CH12F3-2A cells stimulated for CSR. The three genes, Kdm3a, p400, and AID are color coded to separate them from the DNA repair genes. Knockdown of Phf5a (bold text) was confirmed, which was shown along with two housekeeping genes (Gray text) in the lower panel. PCR condition was described in the Method. **(B)** Quantitative RT-PCR analysis of selected DNA repair genes including Kdm3a, p400 and AID. The data was generated using RNA isolated from stimulated CH12F3-2A cells transfected with siControl or siPhf5a; knockdown efficiency of Phf5a was shown on the top. A representative data was shown with the means of technical replicates ± sem.



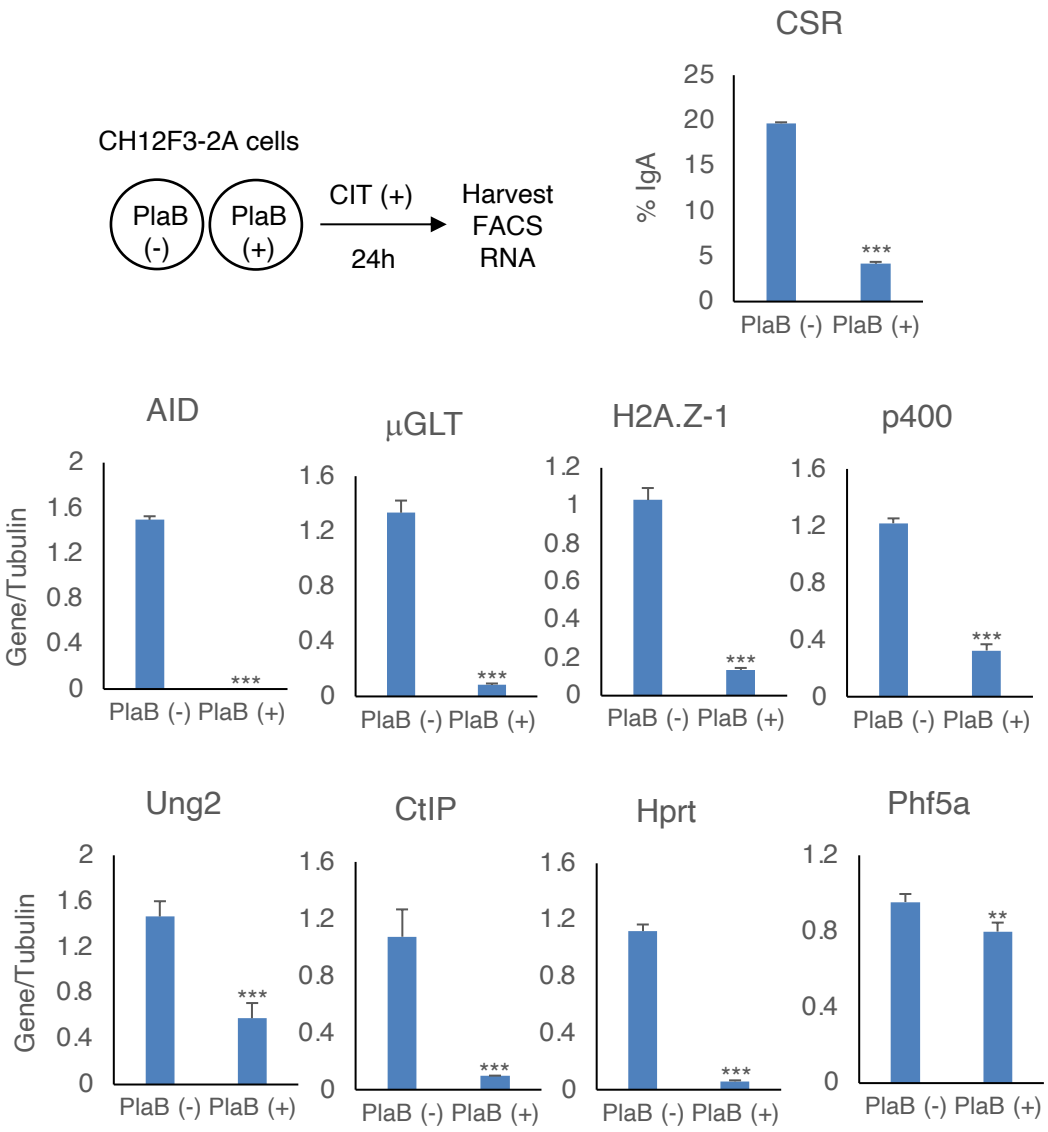
Appendix Figure S16





Appendix Figure S16 continued

Appendix Figure S16 : Phf5a depletion did not perturb splicing of IgH and some other transcripts. (**A**) Schematics of IgM locus along with exon-intron organization. Primers (a-f) are shown on the top of the illustration, and the direction was indicated by the arrows. Unspliced as well as various spliced transcripts (μGLT, IgM-M, IgM-s) produced from the locus are shown just below the genomic organization. (**B**) RTPCR primer combination b+c was used to verify proper splicing out of Iμ and Sμ, which is important to produce IgM transcript having the variable region. Similarly, combination a+c are used to confirm the Sμ is appropriately spliced out from the μGLT. Expected size RTPCR products were obtained from both siControl and siPhf5a treated samples (knockdown of Phf5a was confirmed, see Fig.15A, bottom panel). Cloning and sequencing of the PCR products (b+c/a+c) confirmed the correct splicing site in both samples (data not shown). Primer combination a+e and a+f were used to examine splicing of IgM-M form, and a+d was used for the IgM-S form. (**C**) Analysis of H2A.Z transcript isoforms and their splicing by RTPCR. Schematic representation of two H2A.Z isoforms that differs at 3'UTR. Since coding region are appropriately spliced out, expected size PCR products are evident in both siControl and siPhf5a treated sample. (**D**) RTPCR analysis Sμ and Sα intron splicing from μGLT and αGLT, respectively. Position and directions of the primers (p1-p4) are shown in the diagram. Gel images with the PCR products are shown next to the illustration. RNA was extracted from siControl and siPhf5a treated cells stimulated with CIT for 24h. (**E**) RTPCR analysis of site specific splicing in AID, p400 and H2A.Z transcripts. Similar primer designing strategy was applied and schematically shown their location on the top of respective gene. Gel images of the PCR products are shown on the right. Primer pair p1-p3 can detect both spliced (shorter, dominant) and unspliced (longer, weaker) transcripts. (**F**) Quantitative RTPCR showing KD efficiency of Phf5a and Sf3b1 in CH12F3-2A cells. RNA isolated from siPhf5a and siSf3b1 treated samples were subjected to qR-PCR. Effect of Phf5a and Sf3b1 depletion on splicing was examined for a set of CSR linked genes (n=2, mean ± sd).



Appendix Figure S17: Effect of a splicing inhibitor on CSR and associated transcripts. Splicing inhibitor PladienolideB (PlaB) strongly inhibited CSR and the transcription of several genes, including the AID and µGLT that are indispensable for CSR. The qRT-PCR data were compiled from three experiments (mean ± sd; two-tailed unpaired Student's t-test; **p ≤ 0.01; *** p ≤ 0.001).

Appendix Table S1. Proteins identified in Phf5a-MF IP by MS analysis

Symbol	Protein Name	Mass	Score	Nuclear Extract
SF3B1	Splicing factor 3B subunit 1	145,724	10,189	Nu-I
SF3B3	Splicing factor 3B subunit 3	135,465	8,724	Nu-I
SF3A1	Splicing factor 3A subunit 1	88,489	2,722	Nu-I
PHF5A (Bait)	PHD finger-like domain-containing protein 5A	12,397	2,470	Nu-I
WDR61	WD repeat-containing protein 61	33,752	1,666	Nu-I
TMOD3	Tropomodulin-3	39,478	1,410	Nu-I
ANM5 (PRMT5)	Protein arginine N-methyltransferase 5	72,634	1,409	Nu-I
DHX15	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	90,949	1,218	Nu-I
RU2A	U2 small nuclear ribonucleoprotein A	28,340	1,199	Nu-I
TTC33	Tetratricopeptide repeat protein 33	29,353	1,096	Nu-I
GRP78	78 kDa glucose-regulated protein	72,377	999	Nu-I
SF3B4	Splicing factor 3B subunit 4	44,327	821	Nu-I
PM14/Sf3b14	Pre-mRNA branch site protein p14	14,576	790	Nu-I
SMD3	Small nuclear ribonucleoprotein Sm D3	13,907	759	Nu-I
RUXE	Small nuclear ribonucleoprotein	10,797	728	Nu-I
MEP50	Methylosome protein 50	36,919	697	Nu-I
SF3A2	Splicing factor 3A subunit 2	49,880	667	Nu-I
SMD2	Small nuclear ribonucleoprotein Sm D2	13,518	655	Nu-I
C1TM	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	105,662	585	Nu-I
STK38	Serine/threonine-protein kinase 38	54,139	451	Nu-I
RSMB	Small nuclear ribonucleoprotein-associated protein B	23,640	443	Nu-I
RU2B	U2 small nuclear ribonucleoprotein B	25,307	439	Nu-I
SMD1	Small nuclear ribonucleoprotein Sm D1	13,273	429	Nu-I
SETX	Helicase senataxin	297,401	413	Nu-I
SR140	U2 snRNP-associated SURP motif-containing protein	118,188	325	Nu-I
SF3B5	Splicing factor 3B subunit 5	10,113	293	Nu-I
RUXF	Small nuclear ribonucleoprotein F	9,719	260	Nu-I
HNRH1	Heterogeneous nuclear ribonucleoprotein H	49,168	254	Nu-I
SPF45	Splicing factor 45	45,276	217	Nu-I
ICLN	Methylosome subunit pICln	26,005	154	Nu-I
PPM1B	Protein phosphatase 1B	42,768	140	Nu-I
HNRPC	Heterogeneous nuclear ribonucleoproteins C1/C2	34,364	109	Nu-I
H2A1F	Histone H2A type 1-F	14,153	39	Nu-I
H2B1A	Histone H2B type 1-A OS=Mus musculus GN=Hist1h2ba PE=2 SV=3	14,228	36	Nu-I
MRE11	Double-strand break repair protein MRE11A	80,173	35	Nu-I
RBM5	RNA-binding protein 5	92,254	33	Nu-I
H2AX	Histone H2A.X	15,133	39	Nu-I
H2AZ	Histone H2A.Z	13,545	39	Nu-I
NU214	Nuclear pore complex protein Nup214	212,847	32	Nu-I

Symbol	Protein Name	Mass	Score	Nuclear Extract
SF3B3	Splicing factor 3B subunit 3	135,465	6,982	Nu-II
SF3B1	Splicing factor 3B subunit 1	145,724	2,648	Nu-II
H4	Histone H4 (Ac)	11,360	2,547	Nu-II
DDX42	ATP-dependent RNA helicase DDX42	101,902	930	Nu-II
PHF5A (Bait)	PHD finger-like domain-containing protein 5A	12,397	910	Nu-II
PM14	Pre-mRNA branch site protein p14	14,576	543	Nu-II
SF3B4	Splicing factor 3B subunit 4	44,327	404	Nu-II
SMD3	Small nuclear ribonucleoprotein Sm D3	13,907	273	Nu-II
WDR61	WD repeat-containing protein 61	33,752	264	Nu-II
SF3A1	Splicing factor 3A subunit 1	88,489	231	Nu-II
SMD1	Small nuclear ribonucleoprotein Sm D1	13,273	228	Nu-II
H2A1	Histone H2A type 1	14,127	211	Nu-II
GRP78	78 kDa glucose-regulated protein	72,377	205	Nu-II
C1TM	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	105,662	161	Nu-II
SMD2	Small nuclear ribonucleoprotein Sm D2	13,518	156	Nu-II
H2AV	Histone H2A.V	13,501	137	Nu-II
H2AZ	Histone H2A.Z	13,545	137	Nu-II
DHX15	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	90,949	84	Nu-II
NH2L1	NHP2-like protein 1	14,165	74	Nu-II
TTC33	Tetratricopeptide repeat protein 33	29,353	72	Nu-II
SF3B5	Splicing factor 3B subunit 5	10,113	70	Nu-II
ANM5(PRMT5)	Protein arginine N-methyltransferase 5	72,634	65	Nu-II
H2AX	Histone H2A.X	15,133	60	Nu-II
H2B1A	Histone H2B type 1-A	14,228	40	Nu-II

Appendix Table S2. List of antibodies, reagents and construts used

Reagent	Source	Catalog #	Use/Assay
Antibodies			
Anti-IgM,FITC	eBioscience	1-5890-85	FACS
Anti-IgA,PE	Southern Biotech	1040-09	FACS
Biotin Anti Mouse IgG1	BD Pharmingen	553441	FACS
Biotin Anti mouse IgG3	BD Pharmingen	553401	FACS
APC Conjugated Streptavidine	eBioscience	17-4317-82	FACS
Rabbit IgG	Millipore	PP64B	ChIP, IP
Anti-PHF5A	Proteintech Group	15554-1-AP	ChIP, WB
Anti-p400	Abcam	ab70163	ChIP, WB, IP
Anti-H2A	Abcam	ab13923	ChIP,WB
Anti-H2B	Abcam	ab1790	WB
Anti-H3	Abcam	ab1791	ChIP, WB
Anti-H4	Active Motif	61300	WB
Anti-H3K4me3	Millipore	07-473	ChIP, WB
Anti-H3K9me2	Active Motif	39041	ChIP, WB
Anti-H3K9me3	Active Motif	39062	ChIP, WB
Anti-H3K36me2	Abcam	ab9049	ChIP, WB
Anti-H3K36me3	Abcam	ab9050	ChIP, WB
Anti-KU80	Santa Cruz	sc-1485	ChIP, WB
Anti-EXO1	Novus	NBP1-19709	ChIP, WB
Anti MRE11	Santa Cruz	sc-135992	ChIP, WB
Anti-CtIP	Abcam	ab70163	ChIP
Anti-APE1	Abcam	ab194	ChIP
Anti-MSH2	Santa Cruz	sc-494	WB
Anti-53BP1	Novus	NB100-304	WB
Anti-PARP1	abcam	ab6079	WB
Anti-AID	Cell Signaling	4949s	WB
Anti-SF3B1	Abcam	ab135946	WB
Anti-SF3B3	Proteintech Group	14577-1-AP	WB
Anti-SF3B14a	Thermo	PA5-24736	WB
Anti-U2AF65/U2F2	abcam	ab37483	WB
Anti-WDR61	Proteintech Group	22536-1-AP	WB
Anti-MEP50	Cell Signaling	2018S	WB
Anti-FLAG M2	Sigma	F-3165	WB, IP
Anti-cMyc	Santa Cruz	sc-40	WB
Anti-AcK	Cell Signaling	9441L,9814S	WB
Anti-mouse-HRP	Rockland	18-8817-33	WB
Anti-rabbit-HRP	Rockland	18-8816-33	WB
Anti-Tubulin	Calbiochem	CP06	WB
Anti-βActin	Sigma	A1978	WB
Chemicals, Enzymes, Kits			
mCD40L supernatant	Dr. Tarsuko Honjo's Lab	Nakamura *et al*, 1996	CSR
TGF-β	R&D Systems	240-B-010	CSR
IL-4	WAKO	090-06621	CSR
LPS	Sigma	L7261 or 7136	CSR
OHT (Tamoxifen)	Sigma	H7904	AIDER
Pladienolide B (PlaB)	Cayman Chemical	16538	Splicing Inhibitor
QuickChange Site-Directed Mutagenesis Kit	Agilent Technologies	200518	PCR,Cloning
Expand Long Template PCR System	Roche	11 681 842 001	IgH/c-Myc Translocation Assay
PrimeSTAR HS DNA Polymerase	TaKaRa	R010A	PCR,Cloning
LaTaq DNA Polymerase	TaKaRa	PR002B	αCD PCR
Pyrobest DNA Polymerase	TaKaRa	R005B	NHEJ PCR
KOD FX Neo DNA Polymerase	TOYOBO	KFX-201	RT-PCR
Superscript III Reverse Transcripatse	Thermo Fisher Sc.	18080044	RT
PowerUp SYBR Green Master Mix	Applied Biosystems	A25742	qPCR
Expand Long Template PCR System	Roche	11681842001	LMPCR
T4 DNA polymerase	TaKaRa	2040A	LMPCR
Exonuclease I	NEB	M0293S	LMPCR
RecJF	NEB	M0264S	LMPCR
BD IMag Anti-PE Magnetic Particles DM	BD Biosciences	557899	IgA(+) cells isolation
Mouse B Lymphocyte Enrichment Set-DM	BD Biosciences	557792	Splenic B cell isolation
Lipofectamine 2000	Thermo Fisher Sc.	11668027	Transfection
SF Cell line 96-well Nucleofector Kit	Lonza	V4SC-2096	Transfection (CH12 and BL2 cells)
P4 Primary Cell 96-well Nucleofector Kit	Lonza	V4SP-4096	Transfection (Primary B cells)
ChIP-IT Express Kit	Active Motif	53008	ChIP
Dynabeads M-280 Streptavidin	Thermo Fisher Sc.	11205D	IP
Subcellular Protein Fractionation Kit	Thermo Fisher Sc.	78840	Phf5a-MF IP
Silver Quest Staining Kit	Thermo Fisher Sc.	LC6070	Phf5a-MF IP
Stealth siRNA			
		MSS229122	Gene knockdown
Mouse Phf5a		MSS229123	CH12F3-2A cells
	Thermo Fisher Sc.	MSS290234	Primary B cells
		HSS189220	Gene knockdown
Human Phf5a		HSS131327	BL2-JP8bdelER cell line
	Thermo Fisher Sc.	HSS131328	NHEJ and HR assay cell lines

		MSS233138	Gene knockdown
Mouse p400		MSS233139	CH12F3-2A cells
	Thermo Fisher Sc.	MSS233140	Primary B cells
		MSS234656	Gene knockdown
Mouse Sf3b1		MSS234658	CH12F3-2A cells
	Thermo Fisher Sc.	MSS294473	
		MSS200205	Gene knockdown
Mouse Sf3b3		MSS200206	CH12F3-2A cells
	Thermo Fisher Sc.	MSS272152	
		MSS227228,	Gene knockdown
Mouse Sf3b14a		MSS227229,	CH12F3-2A cells
	Thermo Fisher Sc.	MSS227230	
		MSS212201	Gene knockdown
Mouse U2af2		MSS212202	CH12F3-2A cells
	Thermo Fisher Sc.	MSS212203	
		HSS142376	Gene knockdown
Human H2A.Z		HSS142377	CH12F3-2A cells
	Thermo Fisher Sc.	HSS179165	NHEJ assay cell line

Construts used			
Constructs in pCMV6-Entry vector			
H2A.Z_MF (Myc,Flag)	This paper	N/A	Co-IP, WB
CtIP_MF	This paper	N/A	Co-IP, WB
Exo1_MF	This paper	N/A	Co-IP, WB
H2A.Z_M	This paper	N/A	Co-IP, WB
CtIP_M	This paper	N/A	Co-IP, WB
Exo1_M	This paper	N/A	Co-IP, WB
Phf5a^{R}_MF_WT	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_C11A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C23A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C26A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C30A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C33A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C40A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C46A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C49A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C58A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C61A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C72A	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_C75A	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_C85A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C23A/C26A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C30A/C33A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C46A/C49A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C58A/C61A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_C72A/C75A	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_K29Q	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_S35N	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_S53N	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_S67N	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_S93N	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_S94N	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_T41Q	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_T76Q	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_T96Q	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_Y36A	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_Y36C	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_K29R	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_K29Q	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_K29R/C23A	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_K29Q/C23A	This paper	N/A	CSR rescue, WB, IP
Phf5a^{R}_MF_del N5	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_del N10	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_del N20	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_del C15	This paper	N/A	CSR rescue, WB
Phf5a^{R}_MF_delC87-95	This paper	N/A	CSR rescue, WB

Appendix Table S3. List of assay specific oligonucleotides

Use/Assay	Forward/Reverse	Oligo/Primer Sequences
Stealth siPhf5a #3 sequence	Forward	5'-CACCAUUCAGGAGAAGGAUAGAGAU
siPhf5a #3 Resistant Mutagenesis	Forward	5'-GTCGCGGTCTTTTTGTTGGATCGTACACTCTTTACAGTAGTA
RT-PCR		
μGLT	Forward	5'-CTCTGGCCCTGCTTATTGTTG
	Reverse	5'-AATGGTGCTGGGCAGGAAGT
αGLT	Forward	5'-CCTGGCTGTTCCCCTATGAA
	Reverse	5'-GAGCTGGTGGGAGTGTCAGTG
γ1GLT	Forward	5′-GGCCCTTCCAGATCTTTGAG
	Reverse	5'-GGATCCAGAGTTCCAGGTCACT
γ3GLT	Forward	5'-TGGGCAAGTGGATCTGAACA
	Reverse	5'-CTCAGGGAAGTAGCCTTTGACA
AID	Forward	5'-ATGGACAGCCTTCTGATGAAGCAAAAG
	Reverse	5'-TCAAAATCCCAACATACGAAATGCATCTCG
Phf5a	Forward	5'-ATGGCTAAACATCATCCAGAT
	Reverse	5'-CCTCTTCTTGAAGCCGTA
p400	Forward	5′-CTCCACAGACGGTGACGCTCACACA
	Reverse	5′-CTACTGGCATGGAGGTTTGGTGGGCGT
Hprt	Forward	5'-CTCGAAGTGTTGGATACAGG
	Reverse	5'-TGGCCTATAGGCTCATAGTG
Tubulin	Forward	5'-CAACGTCAAGACGGCCGTGTG
	Reverse	5'-GACAGAGGCAAACTGAGCACC
αCircle DNA	Forward	5'-CCAGGCATGGTTGAGATAGAGATAG
	Reverse	5'-AATGGTGCTGGGCAGGAAGT
Gapdh	Forward	5'-ATCCTGTAGGCCAGGTGATG
	Reverse	5'-CTGGGTCAGTGCGAGGCTGTGA
IgH/c-Myc Translocation	Forward (Sμ) 1st PCR	5'-ACTATGCTATGGACTACTGGGGTCAAG
	Reverse (cMyc) 1st PCR	5'-GTGAAAACCGACTGTGGCCCTGGAA
	Forward (Sμ) 2nd PCR	5'-CCTCAGTCACCGTCTCCTCAGGTA
	Reverse (cMyc) 2nd PCR	5'-GTGGAGGTGTATGGGGTGTAGAC
	c-Myc Probe	5'DIG-GGACTGCGCAGGGAGACCTACAGGGG
SHM		
BL2-IgH V	Forward	5'-CTATAACCATGGTTCATGAAACACCTGTGGTTC
	Reverse	5'-TGCATGCATTCTAGAAAGGGTTGGGGCGGATGCACTCC
CH12-IgH 5'Sμ	Forward	5′-AATGGATACCTCAGTGGTTTTTAATGGTGG
	Reverse	5′-GCGGCCCGGCTCATTCCAGTTCATTACAG
DNA break assay (Biotin-dUTP)	Sμ-F	5'-GCTTCTAAAATGCGCTAAACTGAGGTGATT
	Sμ-R	5'-GTTTAGCTCTATTCAACCTAG
	Sα-F	5'-TCACCCAGAAGACCATCGACC
	Sα−R	5'-CGCTGACATTGGTGGGTTTACC
β2m	β2m-F	5'-GGTGACGACCTCCGGATCTG
	β2m-R	5'-GCCGAGTAGCAGCCACTGAAA
DNA break assay (LMPCR)	Linker-long	5'-GCGGTGACCCGGGAGATCTGAATTCAC
	Linker-short	5'-GTGAATTCAGATC
	Forward (Sμ)	5'-GCAGAAAATTTAGATAAAATGGATACCTCAGTGG
	Reverse (Linker)	5'-GCGGTGACCCGGGAGATCTGAATTC
Gapdh	Forward	5'-ATCCTGTAGGCCAGGTGATG
	Reverse	5'-GCTCAAGGGCTTTTAAGGCT
Sμ-Sα switch junction PCR	Forward (Sμ)1st PCR	5'-ATTCCACACAAAGACTCTGGACC
	Reverse (Sα)1st PCR	5'-AGCGCTCCAGATTTCTAAGCCCCACTCCTG
	Forward (Sμ) 2nd PCR	5'-GTAAGGAGGGACCCAGGCTAAG
	Reverse (Sμ) 2nd PCR	5'-TTTGGGCAGTGGATAGAGCTATGTTCTCAG
NHEJ substrate PCR	NHEJ-F	5'-GTACGGTGGGAGGTCTATATAAG
	NHEJ-R	5'-TTCATCTTGTTGGTCATGCGG
DC PCR		
Direct Joining (Sμ-Sα)	Forward	5'-GGCCCGTCGACGGAGACCAATAATCAGAGGGAAG
	Reverse	5'-CAGGCCAGAAGGAGCTGAGGCCTCAGAACA
Inverse Joining (Sμ-Sα)	Forward (1st)	5′-CCATAGCAGTTGGTCAATCCTTGTCTCC
	Reverse (1st)	5′-CCATATATCCCTCTGAGGCACACCCTCACA
	Forward (2nd)	5′-CCATAGCAGTTGGTCAATCCTTGTCTCC
	Reverse (2nd)	5'-CAGGCCAGAAGGAGCTGAGGCCTCAGAACA
nAchRe-R	Forward	5′-GCGCCATCGATGGACTGCTGTGGGTTTCACCCAG
	Reverse	5′-GGCCGGTCGACAGGCGCGCACTGACACCACTAAG
DNA End Ressection Assay (5'Sμ)	Forward	5'-CTACTGCCTACACTGGACTGTTCT
	Reverse	5'-CTCACCCCATCTCACCCCATCT
Gapdh	Forward	5'-ATCCTGTAGGCCAGGTGATG
	Reverse	5'-GCTCAAGGGCTTTTAAGGCT

Appendix Table S4. List of PCR primers used in Appendix Figure 15A

No.	Primer ID	Primer Sequence	Gene	Refseq
1	ORF-163F	5'-ATGACCACCCTCACGCGCCAAGA	Mad2l2	NM_001305420.1
	ORF-135R	5'-TCAGCTGTTCTTATGCGCTCGCTCTTCA		
2	ORF-164F	5'-ATGGATAGCTGCAGAATGACCAC	Shld3	NM_001365338.1
	ORF-136R	5'-TTACATACTAAAAATAACACCATATTTGTGTACAAA		
3	ORF-173F	5'-TGGGGATTCAAGGGTTACTTCAGTTCA	Exo1	NM_012012.4
	ORF-143R	5'-AATCCATTTAAAAAATGACATTGAGACTAATACATCTCTCC		
4	ORF-180F	5'-ATGGAAAGGAAAGTAAGCAGAATCTATCTTGCT	Xrcc4	NM_028012.4
	ORF-150R	5'-TCTTTCAAGGTCTGGGGTAGTGAAGAGGCAA		
5	ORF-166F	5'-ATGGAATCCCAGGCAGCCACACC	Shld1	NM_001358260.1
	ORF-138R	5'-CTAGTGGGTAAAAGCGTGTGTCCCTGG		
6	ORF-181F	5'-ATGGCTTCCTCACAAACTTCACAAACT	Lig4	NM_176953.3
	ORF-151R	5'-AGCAAGCTCTAAAGCAAATACTGGTTTTCCT		
7	ORF-182F	5'-ATGACTTTGGCTTTCAAGATCCTCTTCCCGA	Lig3	NM_001291245.1
	ORF-152R	5'-CAAAGTCCTAGCAGGGAGCTATCAGCCT		
8	ORF-183F	5'-ATGAGAAAAAAAGAGCAAGAGAGGAAAGGAG	Lig1	NM_001083188.1
	ORF-153R	5'-GGAGGTTAATAGTCTTCAACGTCGGAGTC		
9	ORF-176F	5'-ATGTGGAAGCTGCTCCCGGC	Nbs1	NM_013752.3
	ORF-146R	5'-GCTTCCTTTACAAGGTTCAATTATCTTCTTTTTACATTAGGAT		
10	ORF-169F	5'-ATGGGTAACTCCTTTCCTGGTGAAGAA	Ku80	NM_001357519.1
	ORF-141R	5'-CTATATCATGTCCAGTAAATCATCCACATCACC		
11	ORF-165F	5'-ATGAGTCAAGGATCACAAGTTCACATTTTTTTGGGTGC	Shld2	NM_001360074.1
	ORF-137R	5'-TCACTCCTTCCCAGGAATGCCTCCTG		
12	ORF-168F	5'-ATGTCAGAGTGGGAGTCCTACTACA	Ku70	NM_010247.2
	ORF-140R	5'-CTTTAGTCAGTTCTTCTCCAAGTGTCTGATAAG		
13	ORF-174F	5'-ATGAGCCCCACAGATCCACTTGAC	Mre11	NM_001310728.1
	ORF-144R	5'-ACATTATCTTCGGTTTCTTCTTGGGCAACTAC		
14	ORF-179F	5'-ATGGCGGAGGCCTCGGAGAG	Parp1	NM_007415.3
	ORF-149R	5'-CACTTTACCACAGGGATGTCTTAAAATTGAACTT		
15	ORF-171F	5'-ATGAGCATTTCAGGAAGCGGCTGTGG	CtIP/Rbp8	NM_001081223
	ORF-154R	5'-TCACGCCAGTGTCTAGGTCCTCTGT		
16	ORF_162F	5'-ATGCCAGGGGAGCAGATGGACCC	53bp1	NM_001290830.1
	ORF_134R	5'-TTAGTGAGAAACATAATCATGTTTATATTTTGGATGCTG		
17	ORF-167F	5'-ATGAGTCTAGCACTCAATGATCTGCTCATTTGCT	ATM	NM_007499.3
	ORF-139R	5'-AAGGTCACACCCAAGCTTTCCATCCT		
18	ORF-175F	5'-ATGTCCCGGATCGAAAAGATGAGCATTCT	Rad50	NM_009012.2
	ORF-145R	5'-AAGACAAGTTAGTGAACATAAGAACCCAGGGAG		
19	ORF-177F	5'-ATGGCTGCTGTTCCTCTGAACAATCTACAAGAA	DNA2_Blm	NM_001042527.2
	ORF-147R	5'-AACAAGATCTATGCGCAGAGAACTGTTAGGAGA		
20	ORF_885F1	5'-ATGGTGCTCACGCTCGGAGAAAGTTG	Kdm3a	NM_173001
	ORF_885R	5'-TTAAGGTTTGCCCAAACTGGATTCACTGG		
21	ORF_904F	5'-CGTCACAGCGATGCCAAAGCGGGGA	Apex 1	NM_009687
	ORF_904R	5'-TCACAGTGCTAGGTAAAGGGTGATGG		
22	ORF_903F	5'-ATGCTGCGCGTGGTAAGCTGGAAC	Apex2	NM_029943
	ORF_903R	5'-CAGTCTGTTCAGCTGGGCCTGCTCC		
23	ORF_902F	5'-GAAATGGCGGTGCAGCCTAAGGAG	Msh2	NM_008628
	ORF_902R	5'-TCACGGAGCCGGAGCCTTTATCCGT		
24	ORF_901F	5'-TGCTCGGCTGGACCATGGGCGTCTT	Ung2	NM_011677
	ORF_901R	5'-GGGTCACAGCTCCTTCCAGTTGATG		
25	ORF_917F	5'-ATGCACCATGGCAGTGGTCCTCAGA	p400	NM_029337
	ORF_917R	5'-CTACTGGCATGGAGGTTTGGTGGGCGT		
26	ORF_AID_F	5'-ATGGACAGCCTTCTGATGAAGCAA	AID/AICDA	NM_009645
	ORF_AID_R	5'-CGCGGATCCAAATCCCAACATACGAAATGCATC		
27	ORF_Phf5a_F	5'-GGCGGATCCCGCTAAACATCATCCAGATTTG	Phf5a	NM_026737
	ORF_Ph5a_R	5'-ATCGAATTCTTACCTCTTCTTGAAGCCGTATTT		

Appendix Table S5. List of PCR primers used in Appendix Figure 15B

No	Primer ID	Primer Sequence	Gene
1	Mad2l2_F	5'-TGCACACAAGAGAAGCTGCTACTCGAAAC	Mad2l2
	Mad2l2_R	5'-TCAGCTGTTCTTATGCGCTCGCTCTTCA	
2	Shlld3_F	5'-ACATTTGGACAAAACTCAATCGC	Shld3
	Shld3_R	5'-TTACATACTAAAAATAACACCATATTTGTGTACAAA	
3	Exo1_F	5'-GGGTTTAAAAAAGATTCTGAAAAGCTTCCTT	Exo1
	Exo1_R	5'-AATCCATTTAAAAAATGACATTGAGACTAATACATCTCTCC	
4	Xrcc4_F	5'-GCTGCAGAAACTCTTCATAAGGATGATTCCATATT	Xrcc4
	Xrcc4_R	5'-TCTTTCAAGGTCTGGGGTAGTGAAGAGGCAA	
5	Shld1_F	5'-ATTCTATGAAGCATTTGCCCATCCACTGC	Shld1
	Shld1_R	5'-CTAGTGGGTAAAAGCGTGTGTCCCTGG	
6	Lig4_F	5'-GGACTTGTATGCTGTTATTAATGACTTGAGTTCCAG	Lig4
	Lig4_R	5'-AGCAAGCTCTAAAGCAAATACTGGTTTTCCT	
7	Lig3_F	5'-CGACGGGGACCTGGTACAGGAATTTGA	Lig3
	Lig3_R	5'-CAAAGTCCTAGCAGGGAGCTATCAGCCT	
8	Lig1_F	5'-GGTTCATTCGTGTCCGTAAAGACAAGCAG	Lig1
	Lig_1R	5'-GGAGGTTAATAGTCTTCAACGTCGGAGTC	
9	Nbs1_F	5'-ATGTGTAAATGAATGTGGTCCACTGAAGAATTTCA	Nbs1
	Nbs1_R	5'-GCTTCCTTTACAAGGTTCAATTATCTTCTTTTTACATTAGGAT	
10	Ku80_F	5'-AAAAGTATGGACTGCATCAAAGCTTTCC	Ku80
	Ku80_R	5'-CTATATCATGTCCAGTAAATCATCCACATCACC	
11	Ku70_F	5'-ATGAGTTTAAAGAACTTGTCTATCCTCCAGGTTAT	Ku70
	Ku70_R	5'-CTTTAGTCAGTTCTTCTCCAAGTGTCTGATAAG	
12	Mre11_F	5'-TAGACGCTTTCAGATCTACCCGACAAC	Mre1
	Mre11_R	5'-ACATTATCTTCGGTTTCTTCTTGGGCAACTAC	
13	Parp1_F	5'-CACAGTGTCAAAGGTTTGGGAAAAACCA	Parp1
	Parp1_R	5'-CACTTTACCACAGGGATGTCTTAAAATTGAACTT	
14	CtIP_F	5'-GCAGATTTGCCAGCAGAAGAAAGAG	CtIP
	CtIP_R	5'-TCACGCCAGTGTCTAGGTCCTCTGT	
15	Tp53bp1_F	5'-AAAACCCTTTCCAGAATCTGAAGGTCCTC	T53bp1
	Tp53bp1_R	5'-TTAGTGAGAAACATAATCATGTTTATATTTTGGATGCTG	
18	Atm_F	5'-AATGCAGATGATCAAGAATGCAAACAAAGTCTTAG	Atm
	Atm_R	5'-AAGGTCACACCCAAGCTTTCCATCCT	
17	Rad50_F	5'-AAACCTTCTGTCTGAACTGTGGCATCCTT	Rad50
	Rad50_R	5'-AAGACAAGTTAGTGAACATAAGAACCCAGGGAG	
18	Blm_F	5'-ATGAAAGAAAGAGAAAGAAAATGTCAGCCACCC	Blm
	Blm_R	5'-AACAAGATCTATGCGCAGAGAACTGTTAGGAGA	
19	Apex1_F	5'-TGCTGGCTTTACTCCCCAGGAGCGC	Apex1
	Apex1_R	5'-TCACAGTGCTAGGTAAAGGGTGATGG	
20	Apex2_F	5'-GAAGTCTATGCTGAGTGGGCCCTCA	Apex2
	Apex2_R	5'-CAGTCTGTTCAGCTGGGCCTGCTCC	
21	Msh2_F	5'-CTCGCTGGGATGTGACGAAGCCGA	Msh2
	Msh2_R	5'-TCACGGAGCCGGAGCCTTTATCCGT	
22	Ung2_F	5'-TGCTCGGCTGGACCATGGGCGTCTT	Ung2
	Ung2_R	5'-AGCGCCGCGGCCTTGTTCCTCTGG	
23	Kdm3a_F	5'-GTGGCTGAAGACTTTGTGTCTCCAGA	Kdm3a
	Kdm3a_R	5'-TTAAGGTTTGCCCAAACTGGATTCACTGG	
24	p400_F	5'-CTCCACAGACGGTGACGCTCACACA	p400
	p400_R	5'-CTACTGGCATGGAGGTTTGGTGGGCGT	
25	Aid_F	5'-ATGGACAGCCTTCTGATGAAGCAA	Aid
	Aid_R	5'-CGCGGATCCAAATCCCAACATACGAAATGCATC	
26	Phf5a_F	5'-GGCGGATCCCGCTAAACATCATCCAGATTTG	Phf5a
	Phf5a_R	5'-ATCGAATTCTTACCTCTTCTTGAAGCCGTATTT	
27	Tubulin_F	5'-CAACGTCAAGACGGCCGTGTG	Tubulin
	Tubulin_R	5'-GACAGAGGCAAACTGAGCACC	
28	Hprt_F	5'-CTCGAAGTGTTGGATACAGG	Hprt
	Hprt_R	5'-TGGCCTATAGGCTCATAGTG	

Appendix Table S6. List of PCR primers used in Appendix Figures 16 and 17

Appendix Figure	Primer ID	Primer Sequence
S16B	a_IgHμ_F	5′-CTCACCATGGGATGGAGCTGTATCA
	b_IgHμ_F	5′-CTCTGGCCCTGCTTATTGTTG
	c_IgHμ_R	5′-GAAGGAAATGGTGCTGGGCAGGAAGTCCCG
	d_IgHμ_R	5'-TCAATAGCAGGTGCCGCCTGTGTCAGACAT
	e_IgHμ_R	5′-CTTGAACAGGGTGACGGTGGTGCTG
	f_IgHμ_R	5′-GCTCAGCTGTCTGTGGGCCAGACAT
S16C	H2A.Z-1cds_F	5′-TCGGAGCTTCAGCACGGTCCGAGATG
	H2A.Z-1cds_R	5′-CCCGATCAGCGATTTGTGGATGTGT
	H2A.Z-1utr3'_F	5′-CCAACCAACCAAATTTCTGC
	H2A.Z-1utr3'_R	5′-CCACCAGAGTGGAAACAATG
	H2A.Z-2cds_F	5′-GTCGCGCGGCCGAGACCATG
	H2A.Z-2cds_R	5′-CACGTCCTAAGCAGTTTTCTGCTGC
	H2A.Z-2utr3'_F	5′-GGTCTGTAACAGGGCAGAGG
	H2A.Z-2utr3'_R	5′-CTGGCCAATCAACACATGAC
S16D	p1_μGLT_F	5′-CTCTGGCCCTGCTTATTGTTG
	p2_μGLT_R	5′-CCATGAGCTCTATGATTATTGGTTAACAGGCAAC
	p3_μGLT_F	5'-CAAGGAAATAGCAGGGTGTAGAGGAATCTC
	p4_μGLT_R	5′-GAAGGAAATGGTGCTGGGCAGGAAGTCCCG
	p1_αGLT_F	5'-CCTGGCTGTTCCCCTATGAA
	p2_αGLT_R	5'-TCTAGCCTGGGAGTCTCCTG
	p3_αGLT_F	5'-GGGGCTTAGAAATCTGGAGCGCTAGACTGC
	p4_αGLT_R	5'-GAGCTGGTGGGAGTGTCAGTG
S16E	p1_AID_F	5′-CCGGCTAACCAGACAACTTCGGCGC
	p2_AID_F	5′-CAGGTGGGGTTCCTTCCAGGGCAGA
	p3_AID_R	5'-TCAAAATCCCAACATACGAAATGCATCTCG
	p1_p400_F	5′-GCCCAAATCCTTCGCTGCGTTTCTG
	p2_p400_F	5′-TGTGGCCAGTATACAACAAGTTGCA
	p3_p400_R	5′-CTACTGGCATGGAGGTTTGGTGGGCGT
	p1_H2A.Z_F	5′-GTACTTGAGTTGGCAGGAAATGCGT
	p2_H2A.Z_F	5′-TCAGTACAGGGTGCTTTCATGGGGT
	p3_H2A.Z_R	5′-CCCGATCAGCGATTTGTGGATGTGT
S16F, S17	Phf5a_F	5'-GGCGGATCCCGCTAAACATCATCCAGATTTG
	Phf5a_R	5'-ATCGAATTCTTACCTCTTCTTGAAGCCGTATTT
	Sf3b1_F	5'-AGGGCCTGAGGGTTGCTATTGGACC
	Sf3b1_R	5'-TATGACCAGCTCTACACTGATCGGC
	μGLT	p1_μGLT_F / p4_μGLT_R
	αGLT	p1_αGLT_F / p4_αGLT_R
	AID	p1_AID_F/ p3_AID_R
	p400	p1_p400_F/ p3_p400_R
	H2A.Z	p1_H2A.Z_F/ p3_H2A.Z_R
	Ung2_F	5′-TGCTCGGCTGGACCATGGGCGTCTT
	Ung2_R	5′-AGCGCCGCGGCCTTGTTCCTCTGG
	CtIP_F	5′-GCAGATTTGCCAGCAGAAGAAAGAG
	CtIP_R	5′-TCACGCCAGTGTCTAGGTCCTCTGT
	Hprt_F	5′-CTCGAAGTGTTGGATACAGG
	Hprt_R	5′-TGGCCTATAGGCTCATAGTG
	Tubulin_F	5'-CAACGTCAAGACGGCCGTGTG
	Tubulin_R	5'-GACAGAGGCAAACTGAGCACC