Supporting Information

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SI Materials and Methods

Cell Culture and Reagents. *Ape1*-null CH12F3-2A cells were obtained from Kefei Yu (Michigan State University, East Lansing, MI) (1). *Ape1*-null CH12F3-2A cells and CH12F3-2A–Bcl2 cells were cultured and stimulated for the indicated time as described (2). MRE11 inhibitor Mirin was purchased from Calbiochem (catalog no. 475954). Proteasome inhibitor Bortezomib was purchase from Janssen Pharmaceutical.

Construction of Vectors and Generation of Cell Lines. To make APE1 constructs and its mutants, the mouse APE1 gene was amplified by PCR and cloned into the SgfI/MluI sites of the pCMV6-Entry vector (Life Technologies). Mutant constructs were made by amplifying WT construct with the indicated primers (Table S3). For making stable cell lines, WT and APE1 mutants DNA were amplified from the pCMV6-Entry construct and cloned into the EcoRI/BamHI sites of the pEF1α-IRES-ZsGreen vector (Clontech). Constructs were linearized with AseI (New England Biolabs) and introduced into *Ape1*-null CH12F3-2A cells by nucleofection (Lonza). After selection with G418 (Nacalai Tesque) for 1 wk, GFP-positive cells were sorted and used without limited dilution as stable lines expressing WT or mutant of APE1.

Western Blot. Cells were lysed with radioimmunoprecipitation assay buffer (10 mM Tris·HCl, pH 7.9, 150 mM NaCl, 0.1% SDS, 0.1% deoxycholate, 1% Triton X-100, and 1 mM EDTA), and the cell lysates were subjected to Western blot following the standard protocols with antibodies listed in Table S4.

5′ Sμ Mutation Analysis. Mutation analysis was performed as described (3) with minor modifications. Genomic DNA was prepared from cells stimulated with CIT for 72 h. A 625-bp region located 5′ of core Sμ was amplified with primers listed in Table S3 by using PrimeSTAR DNA polymerase (TaKaRa). The PCR product was cloned into pGEM-T easy vector (Promega) vector and sequenced with T7 primer by using an ABI 3130xl Genetic Analyzer (Applied Biosystems). The 565-bp sequence between the two primers was analyzed with Sequencher software (Gene Codes) for mutation.

IgH/c-myc Translocation Analysis. Genomic DNA was prepared from cells stimulated with CIT for 72 h, and IgH/c-myc translocation of derivative chromosome 15 was performed as described (4).

ChIP. Chromatin was prepared with the truChIP Chromatin Shearing Reagent Kit according to the manufacturer's instructions (Covaris; PN520127). In brief, 1×10^7 cells in fixing buffer were fixed for 5 min in the presence of fresh 1% formaldehyde at room temperature, and then the reaction was stopped by quenching buffer for 5 min. Fixed cells were washed with cold PBS and lysed in lysis buffer for 10 min. After washing, nuclei pellet was suspended in shearing buffer, and sonication was performed with the S2 Focused-ultrasonicator (Covaris). Immunoprecipitation was performed overnight by incubating 35 μg of chromatin with 3 μg of antibody in the presence of protease inhibitors and phosphatase inhibitors as well as protein-G–conjugated magnetic beads (Life Technologies; 10004D). The pulled-down DNA was subjected to detection by real-time PCR and normalized to the amount of input. Primers and antibodies used are shown in Tables S3 and S4, respectively.

Sμ–Sα Junction Analysis. Genomic DNA was prepared from cells stimulated with CIT for 72 h. Sμ–Sα junction DNA was amplified by nested PCR using Sμ and Sα primers as listed in Table S2. A total of 1 μL of first-round PCR product (25 cycles) was used for the second round of PCR (38 cycles). The PCR products, which spanned from 0.5 to 2 kb, were gel-extracted, cloned into pGEM-Teasy (Promega), and sequenced with T7 primer.

The 3C Assay. The 3C assay was performed as described (3, 5).

siRNA Oligonucleotide Transfection. Various Stealth RNAi oligonucleotides (Invitrogen) were introduced into CH12F3-2A cells by the Nucleofector 96-well electroporation system (Lonza) with the CM-150 program. As a negative control (siCont.), Stealth siRNA Negative Control (catalog no. 12935-300) was used. The Stealth siRNAs used in this study are listed in Table S3.

1. Masani S, Han L, Yu K (2013) Apurinic/apyrimidinic endonuclease 1 is the essential nuclease during immunoglobulin class switch recombination. *Mol Cell Biol* 33(7): 1468–1473.
2. Nakamura M, et al. (1996) High frequency class switching of an IgM+ B lymphoma clone CH12F3 to IgA+ cells. *Int Immunol* 8(2):193–201.
3. Yousif AS, Stanlie A, Mondal S, Honjo T, Begum NA (2014) Differential regulation of S-region hypermutation and class-switch recombination by noncanonical functions of uracil DNA glycosylase. *Proc Natl Acad Sci USA* 111(11):E1016–E1024.
4. Ramiro AR, et al. (2004) AID is required for c-myc/IgH chromosome translocations in vivo. *Cell* 118(4):431–438.
5. Sabouri S, et al. (2014) C-terminal region of activation-induced cytidine deaminase (AID) is required for efficient class switch recombination and gene conversion. *Proc Natl Acad Sci USA* 111(6):2253–2258.

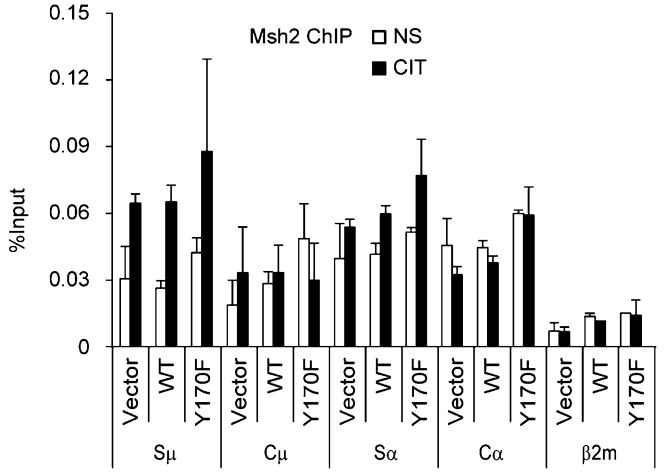

Fig. S1. APE1 deficiency does not affect the recruitment of MMR protein Msh2. ChIP and quantitative PCR analysis for Msh2 in cells stimulated (or not) with CIT for 24 h. Data are represented as mean ± SD.

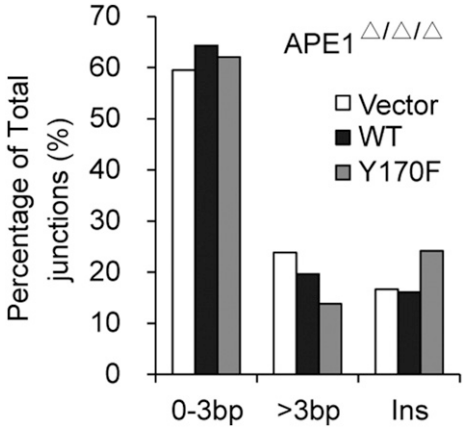

Fig. S2. APE1 deficiency does not affect the microhomology used at the Sμ–Sα junction. Analysis of Sμ–Sα junctions of DNA isolated from three cell lines stimulated with CIT for 72 h.

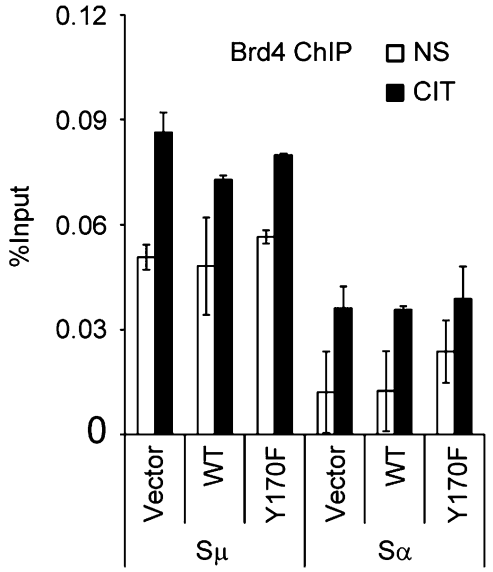

Fig. S3. Recruitment of Brd4 to S regions is independent of APE1. ChIP and quantitative PCR analysis for Brd4 in cells stimulated (or not) with CIT for 24 h. Data are represented as mean ± SD.

Table S1. Mutation analysis for 5′Sμ region

APE1-△/△/△	Mutated/clones	Mutated/total bases	Mut. freq., $\times 10^{-4}$	Del/ins
Vector	35/186	28/105090	2.66	14
WT	26/177	24/100005	2.40	2
Y170F	43/183	56/103395	5.42	10

Del, deletion; Ins, insertion; mut. freq., mutation frequency.

Table S2. Mutation bases for 5′ Sμ region

		To					
Transfectants		G	C	A	T	Total	%
Vector	G		5	11	1	17	92.9
	C	2		1	6	9	
	A	1	0		0	1	7.1
	T	0	1	0		1	
	Total					28	
WT	G		3	10	0	13	95.8
	C	1		1	8	10	
	A	0	0		0	0	4.2
	T	0	1	0		1	
	Total					24	
Y170F	G		8	19	6	33	96.4
	C	5		2	14	21	
	A	0	0		0	0	3.6
	T	1	1	0		2	
	Total					56	

Table S3. List of primers and RNAi oligonucleotides used in this study

Primers	Sequence, 5′–3′
APE1 mutagenesis	
mAPE1-WT-SgfI-F	ATAGCGATCGCATGCCAAAGCGGGGAAAGAAAG
mAPE1-WT-MluI-R	CGCACGCGTCAGTGCTAGGTAAAGGGTGATG
mAPE1-K6R-K7R-F	CGAAGAGCGGCGGCCGACGACG
mAPE1-K6R-K7R-R	TCCCCGCTTTGGCATGCGATCG
mAPE1-C64S-F	TCCTCCTGGAATGTGGATGGGCTTC
mAPE1-C64S-R	TATCTTGAGTGTGGCAGATTTGCCACTGG
mAPE1-Y170F-F	TTTGTTCCCAATGCAGGCAGGGGTC
mAPE1-Y170F-R	GGCTGTTACCAGGACAAAGGACTC
ZsGreen-mAPE1-EcoRI-F	TATGAATTCATGCCAAAGCGGGGAAAGAAA
ZsGreen-mAPE1-BamHI-R	CGCGGATCCTTAAACCTTATCGTCGTCATC
5′ Sm mutation	
5′Sμ-seq-625bp-F	AATGGATACCTCAGTGGTTTTTAATGGTGG
5′Sμ-seq-625bp-R	AGCGGCCCGGCTCATTCCAGTTCATTACAG
IgH/c-myc translocation	
Sμ5′1a (first)	ACTATGCTATGGACTACTGGGGTCAAG
c-myc5′1a (first)	GTGAAAACCGACTGTGGCCCTGGAA
Sμ5′1b (second)	CCTCAGTCACCGTCTCCTCAGGTA
c-myc5′1b (second)	GTGGAGGTGTATGGGGTGTAGAC
c-myc probe	GGACTGCGCAGGGAGACCTACAGGGG
Bio-dUTP break assay	
5′ Sμ-F (γH2AX ChIP)	AAGCTTGCTGAGCAAAATTAAGGGAACAAGG
5′ Sμ-R (γH2AX ChIP)	GTCTACTTCATTTTGGCTCAAGCCCAGCTTT
3′ Sμ-F (γH2AX ChIP)	TGGCCAGAACCAGAATCAATT
3′ Sμ-R (γH2AX ChIP)	GCCTCACATAATCCATGTCAGCTA
Sγ1-F	GCAATGCTTCTGTCTGTAGACAAGA
Sγ1-R	CCCGGATTCGCCTGAGGACAGAGGCT
β2m-F (ChIP)	GGTGACGACCTCCGGATCTG
β2m-R (ChIP)	GCCGAGTAGCAGCCACTGAAA
ChIP assay	
Sμ-F	TAGTAAGCGAGGCTCTAAAAAGCAC
Sμ-R	ACTCAGAGAAGCCCACCCAT
Cμ-F	CTGAACCTGAGGGAGTCAGC
Cμ-R	GCCACTGCACACTGATGTCT
Sα-F	CTTGGCTAGGCTACAATGGATTGAGC
Sα-R	GTGCAACTCTATCTAGGTCTGCCCGGT
Cα-F	GCCAGCTGTCGAGTGCCCAG
Cα-R	CACCCCCAGCTGACCCCCTA
LM-PCR assay	
LMPCR.1	GCGGTGACCCGGGAGATCTGAATTC
LMPCR.2	GAATTCAGATC
5′ Sμ-F	GCAGAAAATTTAGATAAAATGGATACCTCAGTGG
5′ Sμ-probe	DIG-AGGGACCCAGGCTAAGAAGGCAAT
Sμ-Sα junction	
SμJxnF (first)	GTATCAAAGGACAGTGCTTAGATCCGAGGT
SαJxnR (first)	GATACTAGGTTGCATGGCTCCATTCACACA
SμJxnF (second)	TTGAGAGCCCTAGTAAGCGAGGCTCTA
SαJxnR (second)	GAACTGTGAATAAGTCCAGTCATGCTAAT
3C assay	
Sμ	GCTGACATGGATTATGTGAGG
Sα	GCCTAGCCCAGACCATGCCA
GAPDH-F	CAGTAGACTCCACGACATAC
GAPDH-R	AGTAGTGCGTTCTGTAGATTCC
Stealth RNAi oligo	
siTDP1 (ID: MSS200510)	UGGAGUUGUACUUUGCCUUAAUCCC
siMRE11 (ID: MSS206767)	AAGUGAUUCACAAACCCAGCACAGC
siCtIP (ID: MSS212779)	UGUCCGAUCAAACAUAUCUUCUAGG

Table S4. List of antibodies used in this study

Antibody	Catalog no.	Supplier
APE1 (13B8E5C2)	ab194	Abcam
Flag (M2)	F-3165	Sigma
phospho-H2A.X (Ser139)	05-636	Millipore
Ku80 (M-20)	sc-1484	Santa Cruz
Msh2 (*N*-20)	sc-494	Santa Cruz
Mouse IgG	sc-2025	Santa Cruz
MRE11 (18)	sc-135992	Santa Cruz
CtIP	ab70163	Abcam
TDP1	NB100-81642	Novus Biologicals
Tubulin (DM1A)	CP06	Calbiochem
Brd4	A301-985A100	Bethyl Laboratories