REV7 is required for processing AID initiated DNA lesions in activated B cells

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Activation-induced cytidine deaminase (AID) initiates both antibody class switch recombination (CSR) and somatic hypermutation (SHM) in antibody diversification. DNA double-strand break response (DSBR) factors promote rearrangement in CSR, while translesion synthesis (TLS) polymerases generate mutations in SHM. REV7, a component of TLS polymerase zeta, is also a downstream effector of 53BP1-RIF1 DSBR pathway. Here, we study the multi-functions of REV7 and find that REV7 is required for the B cell survival upon AID-deamination, which is independent of its roles in DSBR, G2/M transition or REV1-mediated TLS. The cell death in REV7-deficient activated B cells can be fully rescued by AID-deficiency in vivo. We further identify that REV7-dependent TLS across UNG-processed apurinic/apyrimidinic sites is required for cell survival upon AID/APOBEC deamination. This study dissects the multiple roles of Rev7 in antibody diversification, and discovers that TLS is not only required for sequence diversification but also B cell survival upon AID-initiated lesions.
Upon antigen stimulation, mature B cells can undergo antibody diversification processes including immunoglobulin heavy chain (IgH) class switch recombination (CSR) and variable (V) exon somatic hypermutation (SHM). Activation-induced cytidine deaminase (AID) initiates both CSR and SHM by specific targeting to immunoglobulin loci and converting cytosine (C) to uracil (U). Various DNA repair pathways function downstream and channel the deamination products into double-strand breaks (DSBs), mutations, or small insertions/deletions (indels). Related DNA repair factors are critical for immune diversity and their deficiency could lead to primary immunodeficiency in human patients.

During CSR, deamination products are processed by base excision repair (BER) and mismatch repair (MMR) factors to generate DSBs at upstream Switch (S) region and downstream S regions. The DSB activates DSB response (DSBR) factors including Ataxia telangiectasia mutated (ATM) and its substrates H2AX, 53BP1, etc. Eventually, non-homologous end joining (NHEJ) pathway juxtaposes the two S breaks and changes the antibody class from IgM to other isotypes. In DSBR, 53BP1 is the key factor to limit DSB end resection and promote NHEJ, and 53BP1 is recruited to DSB site through a dual H4K20me2 and H2AK15ub histone marks, and its N-terminal domain further recruits PTIP and Rif1 to inhibit end resection upon phosphorylation. Deletion of either factor in this pathway results in decreased CSR level in various mouse models.

SHM specifically happens at Ig V exons in germininal center (GC) B cells in vivo. U can be recognized by uracil DNA glycosylase (UNG) and processed into an apurinic/apyrimidinic (AP) site, which can also be cut into an single-stranded DNA nick by apurinic/apyrimidinic-endonuclease. The U-G mismatch can also be recognized by MMR factors and DNA strand containing mismatch will be cleaved to generate a single-nucleotide gap. Different from the canonical BER or MMR process, SHM utilizes error-prone translesion DNA synthesis (TLS) to fill in the gap/nick/AP site as a balance between error-free and error-prone DNA repair. In this context, many TLS polymerases are involved in SHM. REV7 can add C to the opposite position of AP site to produce a C-to-G conversion. DNA polymerase eta (POLH) and DNA polymerase zeta (POLZ complex, including REV7 and the catalytic subunit REV3L) can generate A/T mutations or tandem mutations, respectively. The uracil processing by UNG and MutS homolog (MSH) proteins are the key steps, as UDG or MSH3 is the only enzyme for the repair of U:G mismatches. AID is crucial for CSR and SHM, and functions in these processes through different pathways. REV7 promotes CSR via the recently identified 53BP1-RIF1-Shieldin pathway, whereas REV7-REV3L are required for B-cell survival upon AID-initiated DNA lesions.

Results

REV7 deficiency leads to B-cell death during CSR. To dissect REV7’s multiple roles in antibody diversification, we generated a Rev7 floxed mouse model (Supplementary Fig. 1a) and bred it with Cd19cre mice. Similar to a recent report, total splenic B-cell numbers were indistinguishable between REV7-deficient and control mice (Supplementary Fig. 1b). Splenic naive B cells were purified and stimulated with lipopolysaccharide (LPS) plus interleukin-4 (IL4) or LPS alone to induce CSR to IgG1 or IgG3 ex vivo (named as CSR-activated B cells). REV7 deficiency led to defective CSR (Fig. 1a and Supplementary Fig. 1c, d) as previously shown in B cells, without affecting AID protein level, germline transcription of Ig constant genes (Supplementary Fig. 1e, f).

However, when we counted the live cell numbers after cytokine stimulation, we noticed a growth defect in CSR-activated REV7-deficient B cells but not in 53BP1-deficiency. To dissect the multiple roles in antibody diversification, we generated a Rev7 floxed mouse model (Supplementary Fig. 1a) and bred it with Cd19cre mice. Similar to a recent report, total splenic B-cell numbers were indistinguishable between REV7-deficient and control mice (Supplementary Fig. 1b). Splenic naive B cells were purified and stimulated with lipopolysaccharide (LPS) plus interleukin-4 (IL4) or LPS alone to induce CSR to IgG1 or IgG3 ex vivo (named as CSR-activated B cells). REV7 deficiency led to defective CSR (Fig. 1a and Supplementary Fig. 1c, d) as previously shown in B cells, without affecting AID protein level, germline transcription of Ig constant genes (Supplementary Fig. 1e, f).

To identify the potential pathway ensuring B-cell survival upon CSR activation, we dissected REV7’s multiple roles (Fig. 1d) during CSR using different assays. First, we generated HTGTS assay to analyze AID-break resections at S regions (Supplementary Fig. 2a). REV7 deficiency resulted in expanded resection of...
comparisons test was performed for further deletion of Atm and Cd19cre
mutations was significantly decreased (Supplementary Fig. 4a).

Then, we analyzed mutations of the 5’ S′ region in CSR-activated B cell by high-throughput sequencing39, to access its role in TLS-mediated mutation. Although DSBs are the major outcomes of AID deamination in CSR, mutations at S regions are frequently observed42. Mutations in the ~200 bp 5′ S′ amplicon were analyzed via a SHM pipeline39. In DSBR deficiencies including 53bp1, Atm, and Rev7 deletion, fraction of reads with mutations was significantly decreased (Supplementary Fig. 4a). Further deletion of Atm in 53BP1 deficiency partially rescued expanded end resection but did not change the mutation frequency (Supplementary Fig. 4a–c) and deletion of DSBR genes in Ung−/− Msh2−/− CH12F3 cells had no effect on S region mutation frequency (Supplementary Fig. 4d–f), reflecting that many AID lesions were subjected to breakage and excluded from the amplicon-seq in DSBR deletion cells or some of these genes are required for converting the AID lesion into DSBs. In CSR, the 5′ S′ region DNA ends at a less severe level compared with that in 53BP1 deficiency (Fig. 1e and Supplementary Fig. 2b, c), suggesting DNA resection unlikely contributes to cell death in REV7 deficiency. Next, we examined cell cycle in the CSR-activated B cells (Supplementary Fig. 3) and found significant G2/M arrest in REV7 deficiency (Fig. 1f), which reflects REV7’s role in G2/M transition24,25.

The mechanisms of REV7 function in CSR was explored through 5′ S′ amplicon-seq. We previously reported43. In this context, we found that C > G transversion was significantly decreased in REV7 deficiency but not in 53BP1 deficiency (Fig. 1g), correlating with the REV1/REV7-dependent C > G during TLS15.

Thus, many aspects of REV7 functions can be visualized during CSR (Fig. 1d), which offers an experimental model to dissect its pathway in CSR.
REV7 and REV3L protect activated CH12 cells from cell death. To study the molecular basis for the cell death in CSR-activated REV7-deficient B cells, we made a panel of knockouts using CRISPR/Cas9 in B-lineaged CH12F3 cells (Supplementary Fig. 5a), which can undergo CSR to IgA upon anti-CD40/IL4/TGF-β (CIT) stimulation44. The gene knockouts were genotyped by PCR from genomic DNA and western blotting with whole-cell lysate (Supplementary Fig. 5a), except for Shil3 whose antibody is not commercially available so far, and the knockout was verified by using reverse transcription quantitative real-time PCR (RT-qPCR) (Supplementary Fig. 5a). The knockout clones were further confirmed by their known functions in repairing γ-IR-, cisplatin-, and ultraviolet (UVC, wavelength 254 nm)-caused DNA damages (Fig. 2a). In this context, 53BP1-RIF1-Shieldin pathway and Rev3l gene knockouts were sensitive to IR-generated DSBs (Fig. 2a, upper), whereas Rev1, Rev3l, and Rev7 knockouts were sensitive to UV-generated crosslinks and cisplatin-caused DNA interstrand crosslinks (Fig. 2a). As REV1 protein can function as a scaffold for other TLS polymerases besides its catalytic activity23,45, we also generated a Rev1βγ cells expressing a REV1 protein without catalytic motif (in-frame Exon 9 deletion) along with Rev1−/− cells (out-frame Exon 10 deletion with no protein detected) (Supplementary Fig. 5a), and the corresponding cells showed similar sensitivity to DNA damages as previously reported26–28,45.

Multiple independent knockout clones of each genotype were verified and stimulated with CIT for CSR. Consistent with previous reports, deletion of 53BP1-RIF1-Shieldin pathway genes led to decreased CSR and double knockouts of Rev7 with 53BP1-RIF1-Shieldin pathway genes resulted in similar decreased CSR levels as those in single knockouts (Fig. 2b and Supplementary Fig. 5b). In Rev1−/− or Rev1−/− cells, CSR level was not affected (Fig. 2b), similar to that reported in Rev1-knockout or catalytically inactivate Rev1 mutant mouse models45,46 but different from another report47, whereas in Rev3l deletion cells, CSR was decreased (Fig. 2b) as previously reported18,48.

We then examined end resection of S region breaks with HTGTS method in all mutant cell lines. Rearrangement was cloned from a CRISPR/Cas9-generated bait break at the 1y3 region to avoid the interference of the nonproductive IgH allele in CH12 cells, which already undergoes IgM-IgA switching37,38, and rearrangements between 1y3CaS and SβqAID were analyzed (Supplementary Fig. 6a). Expanded S region resection was observed in all 53BP1-RIF1-Shieldin-deficient cell lines but not in Rev1 or REV3L deficiency (Fig. 2c and Supplementary Fig. 6b). However, significantly decreased C > G transversion of 5′ Sβ mutations was observed in Rev1, REV3L, and REV7 deficiencies (Fig. 2d and Supplementary Fig. 6c). We also examined the cell cycle of these knockout CH12 cell lines. Similar to previous observation49, RIF1 deficiency results in a significant G2/M arrest (Supplementary Fig. 7). Similar G2/M arrest phenotype was observed in both REV7 and REV3L deficiencies regardless of CIT stimulation or not (Supplementary Fig. 7). The panel of CH12F3 knockouts helps to dissect the multiple roles of REV7 in DSBR and TLS, in which context end resection is counteracted by 53BP1-RIF1-Shieldin and C > G transversion is mediated by REV3L/TLS polymerases.

We then examined cell growth in these genotypes and found that growth defect was only observed in Rev7 or Rev3l-knockout cells upon CIT stimulation (Fig. 2e). In apoptosis assay, Rev7 or Rev3l-knockout cells showed increased apoptotic population in either CIT stimulation conditions and a slight but significant increasing in non-CIT condition (Fig. 2f). Combining the results of end resection, C > G transversion, G2/M arrest, and cell viability in these gene knockout cell lines, we found the REV7 is required for cell survival upon AID lesions in CH12 cells, for which the phenotype is also observed in REV3L deficiency.

Residues in HORMA domain are crucial for B-cell survival. Key residues on REV7 protein responsible for its interactions to cofactors were clearly mapped (Fig. 3a). Thus, we used these mutants to dissect REV7’s roles in CSR. REV7 mutants were overexpressed in REV7-deficient B cells with a retroviral vector with wild-type (WT) REV7 and empty vector (EV) as controls. All cellular defects in REV7-deficient B cells can be fully rescued by overexpressing WT REV7 (Fig. 3b, c and Supplementary Fig. 8a).

Decreased CSR and increased end resection were observed in mutants losing interaction of other Shieldin subunits, including Y63A (abolished interaction with SHLD335) and K129A (abolished interaction with SHLD235) (Fig. 3b, c and Supplementary Fig. 8b, c). The L186A mutant, which fails to interact with REV150, fully rescued the CSR defect and expanded S region resection (Fig. 3b, c and Supplementary Fig. 8b, c), but showed increased C > G transversion frequency (Fig. 3d and Supplementary Fig. 9), suggesting an interaction between REV1 and REV7 is required for proper C > G transversion. In the HORMA domain, both Y63 and W171 contribute to REV7–REV3L interaction50, whereas only Y63 is the major contributor for in-vivo REV7–SHLD3 interaction35,51. The separation-of-function mutant W171A showed normal end-resection level and increased C > G mutation (Fig. 3c, d, and Supplementary Figs. 8b, c and 9), correlating with the observation that REV7–REV3L interaction affects REV1 function through the REV7–REV3L–REV1 complex50. In this context, the Rev7-Y63A/W171A mutation50 completely abolished its functions in CSR similar to the EV control (Fig. 3b and Supplementary Fig. 8a).

Complementation in Rev7-deleted CH12F3 cells yield same results as in CSR-activated primary B cells (Fig. 3e). The cell growth and viability were examined in mutated CH12F3 cells and only Y63A/W171A and V85E showed growth defect and increased cell death upon CIT stimulation (Fig. 3f, g). The W171A mutant did not affect cell death (Fig. 3f, g), probably resulting from its remaining interaction with REV3L50. Combining the cellular outputs of various REV7 mutants (Fig. 3), we conclude that the intact of REV7 HORMA domain is required for its function in promoting B-cell survival, potentially by its interaction with REV3L via the safety-belt model.

AID-initiated lesions cause cell death in REV7 deficiency. The much severe cell death observed upon CIT stimulation in REV7-deficient CH12F3 cells (Fig. 2f) indicated the B-cell activation could be the cause of cell death. The increased cell death at Day 4 vs. Day 3 after stimulation (Fig. 1b) prompted us to examine the Cre efficiency at the two time points (Fig. 4a). We designed a quantitative PCR assay to access the knockout efficiency. At Day 4 after stimulation, the Rev7 floxed allele was significantly increased indicating a counter-selection of deletion alleles of Cd19creRev7fl/flo mice (Fig. 4a). Thus, the Cd19creRev7fl/flo mice were bred with Aicda−/− mice to generate REV7 and AID double-deficient B cells. In the absence of AID, CSR was completely abolished (Supplementary Fig. 10a). Surprisingly, the growth defect and cell death of REV7 deficiency were fully rescued (Fig. 4b, c and Supplementary Fig. 10b). Accordingly, no significant change of deletion/floxed ratio was observed between.
Day 3 and 4 after stimulation (Fig. 4d). However, the G2/M arrest in REV7 deficiency could not be rescued by Aicda deletion (Fig. 4e and Supplementary Fig. 10c). The G2/M fractions of CSR-activated REV7 single-deficient and REV7/AID double-deficient B cells were at a comparable level (Figs. 1f and 4e), suggesting the G2/M phase arrest is not a major contributor of the higher apoptosis rate in REV7-deficient cells. We conclude that AID-initiated DNA lesions are the cause of cell death in REV7 deficiency.

AID-initiated lesions lead to dysfunctional GC in REV7 deficiency. SHM of Ig V exons is required for antibody affinity maturation and also initiated by AID in GC B cells. Cell death caused by AID-initiated DNA lesions in REV7 or REV3L deficiency during CSR indicated similar mechanism could happen in vivo in SHM. The GC B-cell numbers in the spleen or Peyer's patch of SRBC (sheep red blood cell)-immunized REV7-deficient mice were significantly decreased (Fig. 5a, b). The size of GC decreased in REV7-deficient spleens (Fig. 5c) and the floxed allele

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**Fig. 2 REV7 and REV3L protect activated CH12 cells from cell death.**

**a** Survival curves of indicated CH12F3 cell lines upon γ-irradiation (IR), UVC, and cisplatin treatment are plotted as means ± SD at left. Area-under-curve (AUC) are calculated and means ± SEM are compared at the right. In IR treatment, n = 13 for parental CH12F3 cells; n = 12 for Rev7−/−; n = 4 for Rev1−/− and Rev49/49; n = 9 for the other genotypes. In UVC treatment, n = 4 for parental CH12F3 cells and Rev7−/−; n = 6 for Rev7−/− and Rev3′−/−; n = 3 for the other genotypes. In cisplatin treatment, n = 9 for parental CH12F3 cells; n = 9 for Rev7−/−; n = 3 for the other genotypes. CRS rate from IgM to IgA (b), end resection level in S phase (c), and percentage of C > G transversion in S phase region (d) are shown for the indicated genotypes. Cell numbers (e) and percentage of apoptotic population (f) with/without cytokine stimulation (CIT, w.o.CIT) at Day 3 are showed. Colored points indicate individual knockout clones. In b, n = 5 for parental CH12F3 cells; n = 4 for Rev7−/−, 53bp1−/−, Rev7−/−Rif1−/−, Rev1−/−, and Rev49/49, n = 3 for the other genotypes. In c, n = 7 for parental CH12F3 cells; n = 5 for Rev7−/−; n = 4 for 53bp1−/− and Rev1−/−; n = 3 for the other genotypes. In d, n = 8 for parental CH12F3 cells; n = 4 for Rev7−/− and Rev3′−/−; n = 6 for Rev1−/−; n = 5 for Rev49/49, n = 3 for the other genotypes. In e, n = 5 for parental CH12F3 cells; n = 3 for the other genotypes. In f, n = 8 for parental CH12F3 cells; n = 6 for Rev7−/−; n = 4 for Rev1−/−, Rev49/49, and Rev3′−/−; n = 3 for 53bp1−/−, Rif1−/−, and Shld3−/−. Three or more independent clones for each genotype were assayed and n represents independent experiments. Data are represented as mean ± SD; one-way ANOVA followed by Dunnett’s multiple comparisons test was performed for all panels. Data in knockouts are compared with those in parental CH12F3 cells for all panels. In d, an extra comparison is shown by using Rev7−/− as the reference group to highlight the difference between DSBR deficiencies and TLS deficiencies. ***p < 0.0001, **p < 0.001, *p < 0.01, †p < 0.05, ns: p > 0.05. Source data are provided as a Source Data file.
significantly increased in the remaining GC B cells (Fig. 5d), indicating the counter-selection of Rev7-knockout B cells in GC. The counter-selection of REV7-knockout GC B cells suggested that AID-initiated DNA lesions are very toxic in the absence of REV7 in vivo and most survived GC B cells are either REV7-proficient or with low AID expression.

We thus examined mutation profiles of J54 intron and Jκ5 intron in the REV7-deficient GC B cells. We found the mutation frequency was significantly decreased in the Cd19creRev7fl/fl GC B cells (Supplementary Fig. 11a, b). Consistent with a previous report, 53BP1 deficiency does not affect SHM frequency in the Ig V exon regions (Supplementary Fig. 11a, b), suggesting the decreased mutation frequency in REV7 deficiency is unlikely caused by its role in DSBR. We then checked the mutation spectrum of J54 and Jκ5 introns, and found that the overall mutation spectrum in REV7 deficiency was similar to the spectrum in WT (Supplementary Fig. 11c–f). The counter-selection of REV7 deficiency and differential DNA repair pathways may contribute to the mild effect of mutation spectrum in GC B cells.

Similar to the phenotype observed in CSR-activated B cells (Fig. 4b), the decreased GC B-cell numbers can be fully rescued by the Aicda deletion in the spleen or Peyer’s patch (Fig. 5e, f). Thus, REV7 is required for GC B-cell survival upon AID-initiated DNA lesions.

**Unrepaired AP site leads to B-cell death.** AID-initiated deamination products U’s are processed through BER or MMR factors into AP site or nicks, which can be further channeled into DSB during CSR. The REV7/REV3L-dependent and DSBR-
in independent cell survival in activated B cells indicated inter-
mediate products but not DSBs are the cause of cell death. Thus,
we examined the cell growth in UNG and MSH2 double defi-
ciency, in which background the majority of UNG is processed by
DNA replication machinery to generate C > T transition muta-
tion. Deletion of POLH function in those B cells, although mild changes
in mutation spectrum change could be contributed by the
counter-selection of AID expression REV7-deficient GC B cells,
relative lower mutation rates in the Ig V introns, intrinsic sequence difference, or different downstream processing path-
ways. For example, in mutation spectrum analysis of 5′Sμ in CSR-
activated B cells, no AT-spreading is observed, implicating a lack
of POLH function in those B cells. Although the mild changes
of C > G transition could be masked by other error-prone poly-
merases, e.g., POLH during SHM in GC B cells. This hypothesis is
supported by the previous observation by Saribasak et al. that
POLZ’s function in SHM is masked by POLH. Mammalian S
regions contain long repetitive GC-rich sequences and the
sequence intrinsic feature might also contribute to the different
mutation profiles of V sequence and S sequence. In this context,
we checked the mutation profile of VDJ exon in REV7-deficient
CH12F3 cells (Supplementary Fig. 12). REV7 deficiency lead to a
similar mutation profile, i.e., decreased mutation frequency
(Supplementary Fig. 12a, b) and no significant change of muta-
tion spectrum (Supplementary Fig. 12c).

Besides the well-characterized AID outcomes, we discovered an
unexpected role of TLS in maintaining B-cell proliferation upon
AID-initiated AP sites. The AID-dependent cell death of activated
REV7-deficient B cells is independent of its role in G2/M arrest,
as AID deficiency fully rescues the cell proliferation but not G2/M
arrest. In mammalian cells, AP sites are replicated by TLS poly-
merases, e.g., POLH during SHM in GC B cells. This hypothesis is
supported by the previous observation by Saribasak et al. that
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tion spectrum (Supplementary Fig. 12c).

Discussion
Here we report that multiple roles of REV7 are required to
process AID-initiated DNA lesions in CSR-activated B cells
ex vivo and GC B cells in vivo. In AID-initiated antibody
diversification processes, REV7-REV3L replicates across the AP
sites to ensure the cell proliferation, REV1-REV7-REV3L gen-
erates C > G transversion and REV7-SHLD1/2/3 functions
downstream of 53BP1-RIF1 to inhibit resection of DSB ends.

The differential outcomes of AID activity in REV7-deficient
cells can be dissected with our panels of gene knockout cell lines
and REV7 mutants. During CSR, we found the DSBs at S regions
undergo expanded end resection in REV7-deficient CSR-activated
B cells, consistent with its role in the Shieldin complex. By
examining the cell cycle, we found G2/M arrest in REV7-deficient
cells resulted from REV7’s role in G2/M transition. We also
noticed a decreased C > G transversion in the remnant 5′Sμ sequence,
which is mainly contributed by the defective TLS of
REV7 deficiency. During SHM, the mutation frequency at profiles
of J μ4 and Jκ5 introns was decreased with no significant change
in mutation spectrum in REV7-deficient GC B cells. The absence
of mutation spectrum change could be contributed by the
counter-selection of AID expression REV7-deficient GC B cells,
relative lower mutation rates in the Ig V introns, intrinsic sequence difference, or different downstream processing path-
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similar mutation profile, i.e., decreased mutation frequency
(Supplementary Fig. 12a, b) and no significant change of muta-
tion spectrum (Supplementary Fig. 12c).
REV7 deficiency could happen quickly, as assessment of cell proliferation using CellTrace dye cannot reflect the defect as described in previous reported Rev7 conditional knockout (Mbi1cre) mouse model. This uncovered function of REV7-REV3L also helped to understand the previous revealed CSR/SHM phenotype in Rev3l mutant mouse models. REV3L is responsible for generation of tandem mutations demonstrated by using Polh−/−Rev3l−/− or Rev3l-hypermutant mouse models. We did not observe changes of tandem mutation frequency in our conditional Rev7-knockout mouse model, probably because the counter-selection of Rev7-knockout GC B cells, the redundant role of POLH, or separation of subunit functions within the POLZ complex.

AID/APOBEC cytidine deaminases are widely used in base editors. Similar to AID-initiated antibody diversification, cytidine base editors could generate AP site that is crucial for cell survival in REV7 deficiency. In this context, AID or APOBEC3A (A3A) was ectopically expressed in B cells (Supplementary Fig. 13a), which was much more toxic to REV7-deficient cells (Supplementary Fig. 13b). Although UNG inhibitor UGI is usually included in the BE tools, the incomplete inhibition could lead to death of edited cells. In this context, base-editing tools generate a burden of DNA damages in certain genetic background, suggesting that extra caution should be taken when choosing genome editing tool in gene therapy of TLS mutant patients. On the other hand, more sophisticated base-editing tools can be designed based on the revealed downstream repair mechanism.

**Methods**

**Mice.** Rev7 floxed mouse line was constructed by this study. Cd19cre, Pnai1cre−/−, Atm−/−, and Aicda−/− mouse lines have been described previously. All mice were co-housed under specific pathogen-free conditions in sterile isolated cages at the animal core facility of Shanghai Institute of Biochemistry and Cell Biology. Mice were kept at maximum of 5 mice/cage at ambient temperature of 20–22°C, 40–60% humidity under 8 am to 6 pm light cycle, 6 pm to 8 am dark cycle. All animal experiments were performed under protocols approved by the
Plasmids. The pX330-U6-Chimeric_BB-CBh-hSpCas9 plasmid was obtained from Addgene (#42230). The coding sequence of Rev7, Aicda, and APOBEC3A was in vitro synthesized and Rev7 mutants were obtained with site-directed mutagenesis, which were cloned into a retrovirus vector.

Antibodies. Antibodies for ATM (2873S; Cell Signaling; 1:1000), 53BP1 (NB100-304; NOVUSBio; 1:1000), RFI1 (ab1213422; Abcam; 1:500), REV7 (A9861; Abclonal; 1:1000), REV1 (sc-393022; Santa Cruz; 1:1000), REV3L (GTX17515; ProgennoTech; 1:1000), RIF1 (ab1213422; Abcam; 1:500), REV7 (A9861; Abclonal; 1:1000), MSH2 (ab227941; Abcam; 1:1000), β-actin (AC028; Abclonal; 1:10,000), FLAG (F1804, Sigma; 1:1000), β-actin (AC028; Abclonal; 1:10,000), FLAG (F1804, Sigma; 1:1000), β-Tubulin (A01030HRP; Abbkine; 1:10,000), glyceraldehyde 3-phosphate dehydrogenase (AB2000; Abways; 1:20,000), and Rabbit TrueBlot (18-8816, ApeXBio). Cell cycle was monitored with an EdU cell proliferation detection kit (C10338-3; Ribobio). Cell apoptosis was monitored with a cell apoptosis assay kit (V13245; Invitrogen).

CH12F3 cell line CSR assay and drug sensitivity assay. CH12F3 cell lines were stimulated with anti-CD40 (16-0402-86; Ebioscience; 1 μg ml⁻¹), TGF-β (CA59; Novoprotein; 0.5 ng ml⁻¹), and IL4 (CK15; Novoprotein; 5 ng ml⁻¹) for the indicated times. For testing drug sensitivity, cells were plated at a concentration of 6 × 10⁴ cells ml⁻¹ with indicated chemicals or different doses of treatments and viability was tested with a Cell Counting Kit-8 assay (K1018; ApeXBio). Cell cycle was monitored with an EdU cell proliferation detection kit (C10338-3; Ribobio). Cell apoptosis was monitored with a cell apoptosis assay kit (V13245; Invitrogen).

HTGTS and data analysis. HTGTS was performed according to a published protocol. In brief, genomic DNA was extracted from cells. Ten micrograms of DNA was fragmented via sonication by using a Bioruptor UCD-300 and the fragmented DNA (100–2000 bp) was used as templates for linear PCR amplification with a biotin primer (Supplementary Table 1). Single-stranded PCR products were purified using Dynabeads MyOne C1 streptavidin beads (65001, Invitrogen) and ligated to the adaptor (Supplementary Table 1). Adaptor-ligated products were PCR amplified. The PCR product was further tagged with illumine P5 and P7 index primers, size-selected via gel extraction (300–800 bp) and subjected to sequencing. In primary B cells, S region rearrangements were cloned from endogenous AID-initiated Sμ breaks with 5′-RED-Sμ primer as described previously. In CH12F3 cells, one IgH allele (nonproductive DJ allele) already underwent CSR (5′-DJ-Sµ/Sµ-3′), whereas on the other allele (VDJ allele) IgH C region genes are in WT configuration. Thus, the μα primer used in CSR-activated B cells cannot be used in CH12F3 cells. Thus, a CRISPR/Cas9 break at 3′Y region was introduced, which can help to capture AID-initiated breaks on the productive VDJ allele during CSR from IgM to IgA. Designer endonucleases, including I-Sce1 and Cas9, are the most frequently used tools to generate bait breaks in HTGTS technology. The sgRNA sequence and the HTGTS cloning primers are listed in Supplementary Table 1. The data were analyzed with a previously published pipeline, in which the
raw reads were first aligned to the mouse genome (mm9) and the prey sequence was extracted. Translocation junctions were identified with the alignment files and a BED file was generated. To estimate the region resection ratio, we calculated the junction numbers at both the S region (s) and a 4 kb downstream region (ds), and calculated the resection ratio as \(\frac{s}{s+d}\).

### Mutation analysis of S' region in CH12F3 cells and CSR-activated B cells.

The S'-region of S' was PCR-amplified from gDNA of CH12F3 cells and CSR-activated B cells with indicated primers in Supplementary Table 1. The PCR product was further tagged with illumina P5 and P7 index primers and subjected to illumina HiSeq or MiSeq. Demultiplexed PE150/PE250 reads were processed with SHM pipeline as described[26]. The raw reads were first aligned to the reference amplicon sequence, and substitutions/deletions/insertions were called with the indicated primers and the ~1.2 kb JH4 fragment[53] were gel-purified. The PCR products were further tagged with illumina P5 and P7 index primers and subjected to illumina HiSeq. Data were analyzed as similar as performed with S region mutations.

### Statistical analysis.

The data in the figures are presented as the mean ± SD, unless otherwise indicated. Statistical analyses were performed using R (Version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org). GraphPad Prism 7 software, or Microsoft Excel (v16.16.21). The number of replicates and statistical test procedures are indicated in the figure legends. Two-tailed paired or unpaired t-test, or one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test was performed if not specified. A P-value < 0.05 was considered significant and denoted as single asterisk, and P < 0.01, P < 0.001, P < 0.0001 were denoted as two, three and four asterisks. Statistics including sample sizes (n), P-values, F-values and degrees of freedom for ANOVA, and t-values and degrees of freedom for t-tests for each panel are listed in Supplementary Table 2.

### Reporting summary.

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

### Data availability

HTGS and SHM sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) with the BioProject accession code: PRJNA590097. The source data underlying Figs. 1a–c, e, 2a–f, 3a–g, 4a–e, 5a–f, and 6a, b, and Supplementary Figs. 1a, b, d–i, 2c, 3a–f, 4a–f, 5a–b, 6b, 7b, 9a, b, 11a–f, 12a–c, and 13a, b are provided as a Source Data file. All other relevant data are available in the Article, Supplementary Information, or from the corresponding author upon reasonable request. Source data are provided with this paper.

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