Supplementary Figure S1. Cell proliferation, germline transcripts and AID expression in activated Mre11/CtIP silenced WT and Lig4<sup>-/-</sup> cells

(A) The proliferation curves of WT and Lig4<sup>-/-</sup> cells transduced with shScramble or shMre11 lentivirus stimulated with α-CD40/IL-4/TGF-β. The cells were collected at 0, 24h, 48h and 72h after stimulation, stained with trypan blue and counted with hemacytometer. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(B) qRT-PCR analysis of spliced mature Iμ-Cμ and Ia-Ca germline transcripts in Mre11-silenced WT and Lig4<sup>-/-</sup> cells. Data were presented as mean ± SD from three independent experiments (Student’s t-test, n.s. (p>0.05) indicates non-significant differences).

(C) Western blot analysis of AID expression in Mre11-silenced WT and Lig4<sup>-/-</sup> cells.

(D) The proliferation curves of WT and Lig4<sup>-/-</sup> cells transduced with shScramble or shCtIP lentivirus stimulated with α-CD40/IL-4/TGF-β. The cells were collected at 0, 24h, 48h and 72h after stimulation were stained with trypan blue and counted with hemacytometer. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(E) qRT-PCR analysis of spliced mature Iμ-Cμ and Ia-Ca germline transcripts in CtIP-silenced WT and Lig4<sup>-/-</sup> cells. qRT-PCR analysis was performed at 16h after stimulation with α-CD40/IL-4/TGF-β. Data were presented as mean ± SD from three independent experiments (Student’s t-test, n.s. (p>0.05) indicates non-significant differences).

(F) Western blot analysis of AID expression in CtIP-silenced WT and Lig4<sup>-/-</sup> cells. AID expression was performed at 16h after stimulation with α-CD40/IL-4/TGF-β.
Supplementary Figure S2. Reduced IgA switching in Mre11/CtIP-silenced WT and Lig4−/− cells
(A) Representative flow cytometry analysis of switching to IgA in WT and Lig4−/− cells transduced with two different shRNAs to silence Mre11 expression.
(B) Representative flow cytometry analysis of switching to IgA in WT and Lig4−/− cells treated with Mre11 exonuclease inhibitor (Mirin) and Mre11 endonuclease inhibitor (PFM01).
(C) Representative flow cytometry analysis of switching to IgA in WT and Lig4−/− cells transduced with three different shRNAs to silence CtIP expression.
Supplementary Figure S3. Exd2 plays a mild role in A-EJ but not c-NHEJ-mediated CSR

(A) The scheme of deleting exon 3 of Exd2 with a pair of gRNAs.
(B) Genomic DNA PCR analysis of knockout of exon 3 with indicated 5’ primer and 3’ primer.
(C) Western blot analysis of CRISPR/Cas9-mediated knockout of Exd2 in WT and Lig4−/− cells. Two positive deletion clones were obtained in each background.
(D) Normalized IgA switching efficiency in Exd2 deleted WT and Lig4−/− cells measured by FACS. Data were presented as mean ± SD from four independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).
Supplementary Figure S4. HTGTS analysis of Mre11/CtIP-silenced WT and Lig4^/- cells
Linear distribution of pooled junctions recovered from CSR activated Mre11/CtIP-silenced WT and Lig4^/- cells with at least three experiment repeats each are shown in the forms of deletion or inversion along a 200kb region across IgH locus (Chr12: 114480001-114680000). Bin size is 20kb and 100 bins are presented in each plot. Numbers in the parenthesis represent total unique junctions in the indicated regions.
A

Exo1

5primer

5gRNA

3gRNA

Exon4/5

3primer

B

WT: 3436bp  Expected Mutants: 2107bp

C

Percentage CSR to IgA normalized to WT

**** p<0.0001

D

Flag

Tubulin

WT  Exo1−/−

Flag

β-actin

Lig4−/−  Lig4−/− Exo1−/−

120kDa  50kDa

120kDa  42kDa

E

WT  Exo1−/−  Lig4−/−  Lig4−/− Exo1−/−

IgA-PE

IgM-APC
Supplementary Figure S5. The nuclease of Exo1 is not required for AID-initiated and CRISPR/Cas9 induced CSR

(A) The scheme of deleting exons 4 and 5 of Exo1 with a pair of gRNAs in WT and Lig4−/− cells.
(B) Genomic DNA PCR analysis of knockout of exons 4-5 with indicated 5’ primer and 3’ primer, two positive knockout clones in each background were obtained.
(C) Normalized switching efficiency to IgA in Exo1 deleted WT and Lig4−/− cells. Data were presented as mean ± SD from four independent experiments (Student’s t-test, ****p < 0.0001, n.s. (p>0.05) indicates non-significant differences).
(D) Western blot analysis of Exo1-deficient WT and Lig4−/− cells reconstituted with retrovirus expressing indicated constructs.
(E) Representative flow cytometry analysis of switching to IgA in Exo1−/− and Lig4−/− EXO1−/− cells transduced with retrovirus expression vector expressing Exo1-Flag or Exo1EK-Flag.
(F) The joining assay with CRISPR/gRNAs targeting Sμ and Sγ1 locus multiple times.
(G) Representative flow cytometry analysis result of Sμ-CRISPR/Cas9 and Sγ1-CRISPR/Cas9-mediated switching to IgG1 in Exo1-deficient WT and Lig4−/− cells measured 72h after transfection.

(H) GFP positive ratio measure 24h, 48h and 72h post CRISPR/Cas9 plasmids nucleofection in Exo1-deficient WT and Lig4−/− cells.
**A**

DNA2 mRNA relative expression normalized to WT.

**B**

Cell number (×10^9) over time.

**C**

Diagram of gRNA and primer locations.

**D**

Genotype and allele deletion comparisons for BLAΔHelicase.

**E**

Percentage CSR to lgA normalized to WT.

**F**

Diagram of gRNA and primer locations for BLMΔHRDC.

**G**

Genotype and allele deletion comparisons for BLMΔHRDC.
Supplementary Figure S6. DNA2 and helicase activity of BLM in A-EJ-mediated CSR

(A) qRT-PCR analysis of DNA2 mRNA expression in WT and Lig4−/− cells transduced with indicated shDNA2 lentivirus. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n.s. (p>0.05) indicates non-significant differences).

(B) The proliferation curves of WT and Lig4−/− cells transduced with Scramble or shDNA2 lentivirus stimulated with αCD40/IL-4/TGF-β. The cells were collected at 0, 24h, 48h and 72h after stimulation, stained with trypan blue and counted with hemacytometer. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, **p < 0.01, ***p < 0.001, n.s. (p>0.05) indicates non-significant differences).

(C) The scheme of deleting BLM helicase domain with a pair of gRNAs flanking exon 8.

(D) Genomic DNA PCR analysis of Blm helicase domain deleted with indicated 5’ primer and 3’ primer and the results of T-A cloning and sequencing analysis of junctions by deleting exon 8 of Blm with a pair of CRISPR-Cas9 gRNAs.

(E) Normalized IgA switching efficiency in Blm helicase domain deleted WT and Lig4−/− cells. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(F) The scheme of deleting HRDC domain of BLM with a pair of gRNAs flanking exon 19.

(G) Genomic DNA PCR analysis of HRDC domain deletion with indicated 5’ primer and 3’ primer (left). The results Sanger sequencing of PCR products of exon19 deletion junctions by Cas9 with indicated primers are shown (right).

(H) Representative flow cytometry analysis result of scramble and shDNA2 lenti-virus transduction in WT and Lig4−/− background cells after 72h CIT stimulation.

(I) Representative flow cytometry analysis result of BLM ΔHRDC in WT and Lig4−/− background cells after 72h CIT stimulation.
A

WT

B

WT

C

**p=0.0018

D

% Resection

% total

MH (bp)
Supplementary Figure S7. HTGTS profile of HRDC domain of BLM-deleted WT and Lig4−/− cells

(A) Linear distribution of pooled junctions recovered from CSR activated WT and Lig4−/− cells with HRDC domain of BLM deleted were shown in the forms of deletion or inversion along a 200kb region across IgH locus.

(B) Linear distribution of pooled Sμ-Sα junctions recovered from HTGTS libraries with CSR activated BLM HRDC domain deleted WT cells.

(C) Percentage of long resection junctions recovered from HTGTS libraries with CSR activated BlmΔHRDC cells. Data were presented as mean ± SEM. (Student’s t-test, **p < 0.01, n.s. (p>0.05) indicates non-significant differences).

(D) The MH pattern of Sμ-Sα junctions in CSR activated BlmΔHRDC cells. Data were presented as mean ± SEM. (Student’s t-test).
**Supplementary Figure S8. HTGTS profile of Atm-deleted WT and Lig4<sup>-/-</sup> cells**

(A) Western blot analysis of ATM expression in WT and Lig4<sup>-/-</sup> cells with CRISPR/Cas9-mediated deletion of exons 59-60 of Atm.

(B) Western blot analysis of phospho-KAP1 expression in knockout of ATM WT and Lig4<sup>-/-</sup> cells irradiated with 0 or 10 Gy IR. The cell lysates were collected 2h after IR for western blot analysis.

(C-D) qRT-PCR analysis of spliced mature 1μ-μ and 1α-α germline transcripts in WT and Lig4<sup>-/-</sup> cells pretreated with DMSO or 100nM AZD1390 without (-) or with (+) αCD40/IL-4/TGF-β stimulation for 16h. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(E) qRT-PCR analysis of AID mRNA levels in WT and Lig4<sup>-/-</sup> cells pretreated with DMSO or 100nM AZD1390 without (-) or with (+) αCD40/IL-4/TGF-β stimulation for 16h. Data were presented as mean ± SD from three independent experiments (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(F) Linear distribution of pooled junctions recovered from CSR activated Atm-deleted or AZD1390-treated WT and Lig4<sup>-/-</sup> cells with three experiment replicates each are shown in the forms of deletion or inversion along a 200kb region across IgH locus (Chr12: 114480001-114680000). Bin size is 20kb and 100 bins are presented in each plot. Numbers in the parenthesis represent total unique junctions in the indicated regions.

(G) Distribution of junctions within IgH locus in CSR activated ATM deleted or inhibited WT and Lig4<sup>-/-</sup> cells. The percentage of Sμ-Sμ and Sμ-Sα joining in either orientation in indicated backgrounds were shown. Data were presented as mean ± SEM. (Student’s t-test, n.s. (p>0.05) indicates non-significant differences).

(H) The ratio of deletion versus inversion for Sα junctions in CSR activated Atm knockout and kinase inhibitor treated WT and Lig4<sup>-/-</sup> cells. Data were presented as mean ± SEM. (Student’s t-test, ***p < 0.001, n.s. (p>0.05) indicates non-significant differences).
**Supplementary Figure S9. DNA-PKcs is required for only c-NHEJ mediated CSR**

(A) The scheme of deleting exons 82 and 83 of Prkdc gene with a pair of CRISPR-Cas9 gRNAs.

(B) Genomic DNA PCR analysis of knockout of exons 82 and 83 of Prkdc with indicated 5’ and 3’ primers.

(C) Sequencing analysis of junctions by deleting exons 82 and 83 of Prkdc with a pair of CRISPR-Cas9 gRNA. The genomic DNA surrounding Prkdc exons 82 and 83 were amplified by PCR with indicated 5’ primer and 3’ primer. PCR products were subjected to T-A clone and Sanger sequencing for confirmation.

(D) Western blot analysis of DNA-PKcs expression in WT and Lig4−/− cells with CRISPR/Cas9-mediated deletion of Prkdc.

(E) Normalized IgA switching in Prkdc exons 82-83 deleted WT and Lig4−/− cells measured by FACS at 72h post stimulation with αCD40/IL-4/TGF-β. Data were presented as mean ± SD from six independent experiments (Student’s t-test, *p < 0.05, ***p < 0.001, n.s. (p>0.05) indicates non-significant differences).
Supplementary Figure S10. knockdown of Mre11/CtIP in ATM-deficient B cells

(A) Western blot analysis of Mre11/CtIP expression in Atm<sup>−/−</sup> cells transduced with lentivirus expressing the indicated shRNAs.

(B) Western blot analysis of Mre11/CtIP expression in Lig4<sup>−/−</sup> Atm<sup>−/−</sup> cells transduced with lentivirus expressing the indicated shRNAs.
Supplementary Figure S11. Effect of resection factors on A-EJ mediated CSR in 53bp1\(^{-/-}\) cells

(A) Western blot analysis of Mre11/CtIP expression in 53bp1\(^{-/-}\) cells transduced with lentivirus expressing the indicated shRNAs.

(B) Normalized IgA switching efficiency in 53bp1\(^{-/-}\) cells treated with AZD1390 at concentrations ranging from 0\(\mu\)M, 0.001\(\mu\)M, 0.01\(\mu\)M, 0.05\(\mu\)M, 0.1\(\mu\)M, 0.5\(\mu\)M, 1\(\mu\)M to 5\(\mu\)M. Data were presented as mean \(\pm\) SD from three independent experiments (Student’s t-test).

(C) Western blot of ATM expression in CRISPR/Cas9-mediated deletion of Atm in 53bp1\(^{-/-}\) cells.

(D) Western blot analysis of phospho-KAP1 expression in 53bp1\(^{-/-}\) Atm\(^{-/-}\) cells irradiated with 0 or 10 gy IR. The cell lysates were collected 2h after IR.

(E) Western blot analysis of Exd2 expression in 53bp1\(^{-/-}\) cells with CRISPR/Cas9 mediated deletion of Exd2.

(F) Normalized switching to IgA in 53bp1\(^{-/-}\) Exd2\(^{-/-}\) cells examined by FACS with \(\alpha\)-CD40/IL-4/TGF-\(\beta\). Data were presented as mean \(\pm\) SD from six independent experiments (Student’s t-test, *\(p < 0.05\), n.s. (p>0.05) indicates non-significant differences).

(G) Percentage of CSR switching to IgA in 53bp1\(^{-/-}\) Exo1\(^{-/-}\) cell lines. Data were presented as mean \(\pm\) SD from four independent experiments (Student’s t-test, ****\(p < 0.0001\), n.s. (p>0.05) indicates non-significant differences).

(H) Western blot analysis of flag-tagged proten expression in 53bp1 and Exo1 double-deficient B cell reconstituted with retrovirus expressing indicated constructs.

(I) Percentage of CSR switching to IgA in 53bp1\(^{-/-}\) Exo1\(^{-/-}\) cell lines reconstituted with retrovirus expressing indicated constructs. Data were presented as mean \(\pm\) SD from six independent experiments (Student’s t-test, *\(p < 0.05\), ****\(p < 0.0001\), n.s. (p>0.05) indicates non-significant differences).
Supplementary Figure S12. HTGTS analysis of Mre11/CtIP/ATM ablated 53bp1−/− cells

(A) Linear distribution of pooled junctions recovered from CSR activated shMre11 or shCtIP transduced or Atm deleted 53bp1−/− cells with three experiment repeats each are shown in the forms of deletion or inversion along a 200kb region across IgH locus (Chr12: 114480001-114680000). Bin size is 20kb and 100 bins are presented in each plot. Numbers in the parenthesis represent total unique junctions in the indicated regions.

(B) Junctions distribution within IgH locus in CSR activated Mre11 or CtIP-silenced and Atm-deleted 53bp1−/− cells. The percentage of Sμ-Sμ and Sμ-Sα joining in either orientation in indicated backgrounds were shown. Data were presented as mean ± SEM. (Student’s t-test).

(C) The ratio of deletion versus inversion for Sα junctions in CSR activated Mre11/CtIP silenced and Atm-deleted 53bp1−/− cells. Data were presented as mean ± SEM. (Student’s t-test, *p < 0.05, n.s. (p>0.05) indicates non-significant differences).

(D) MH pattern in Sμ-Sα junctions recovered from HTGTS libraries with CSR activated Mre11/CtIP-silenced and ATM deficient 53bp1−/− cells. Data were presented as mean ± SEM. (Student’s t-test)