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Supplemental information

Polθ promotes the repair of 5′-DNA-protein crosslinks by microhomology-mediated end-joining

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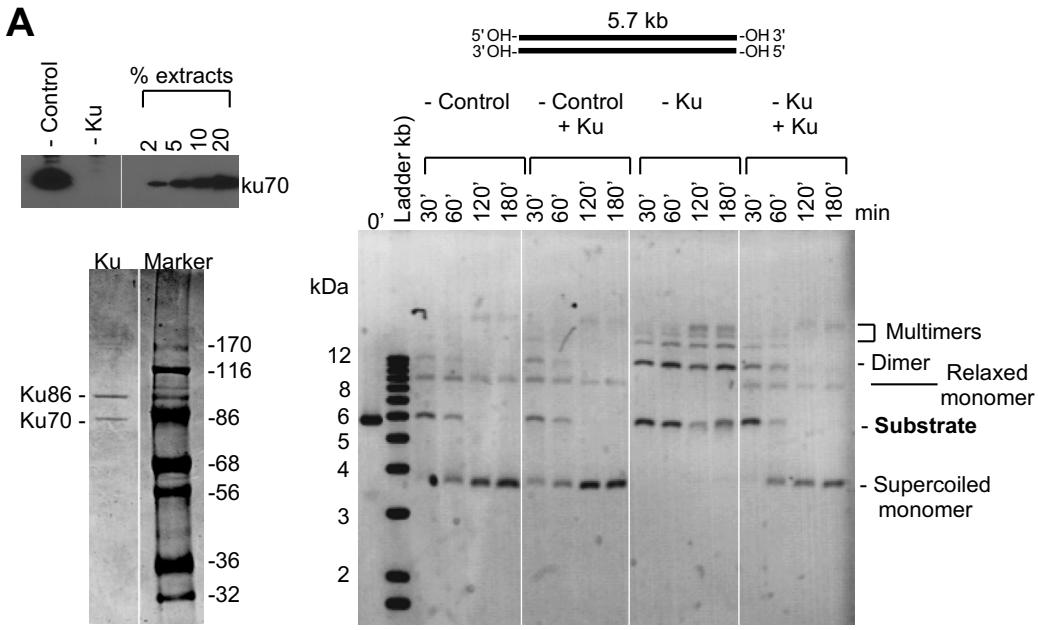
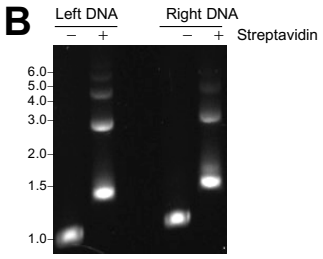
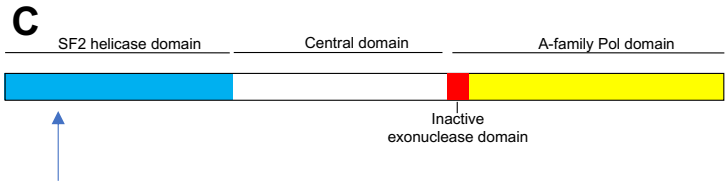
Figure S1







gRNA Sequence GAGGCGATTGATCAGACCATTGG IN EXON 4

CTAACAGATCCATCTTATTTTCCTCTATGAGGCGATTGATCAGACCATTGGCTCTCT
CAATTGTGCAGACTGCAATATCCAATGAAGAGAAATGCCTTGATGGAGA

HEK 293T POLQ -/- CLONE # 4: -1/-1 bp, 1 bp deletion in both alleles:
CAGTCTGCACAATTGAGAGAGCCAATG–TCTGATCAATCGCCTCATAGAGGAAA

Figure S1. Controls for testing the structural and genetic requirements for MMEJ of DSBs.

(A) Controls showing Ku depletion has no effect on intermolecular DSB repair in Xenopus egg extracts. Western blot showing depletion of Ku (-Ku) and positive control for anti-body and presence of Ku via Western blot of control extracts (Control; % extracts)(top left). SDS protein gel showing purified recombinant Ku proteins (bottom left). Gel showing a time course of DNA end-joining products formed in *Xenopus* egg extracts from the ^{32}P-internally labeled DNA substrate indicated at top (right). Supercoiled monomer and relaxed monomer products require the presence of Ku (compare right two panels). Multimers which are due to Polq-dependent MMEJ occur more efficiently in the absence of Ku (second panel from right), indicating competition between NHEJ and MMEJ. -control, mock depleted extracts; -control +Ku, mock depleted extracts with recombinant Ku proteins added; -Ku, Ku depleted extracts; -Ku +Ku, Ku depleted extracts with recombinant Ku added back.

(B) Non-denaturing gel showing left and right DNA MMEJ reporter constructs with and without streptavidin conjugation. Slower migration of DNA demonstrates streptavidin conjugation.

(C) gRNA sequence used to generate *POLQ-/-* HEK293T cells via CRISPR-Cas9 engineering. Schematic representation of human Polq with protein domains indicated. Approximate location of the gRNA sequence (red) designed from Exon 4 is indicated. The genome sequence flanking the gRNA sequence (red) is shown in grey. *POLQ-/-* clone #4 was generated by CRISPR-Cas9 engineering and carries a 1 bp deletion in both alleles. Sequence of the region harboring the 1 bp deletion is indicated in blue.

Figure S2

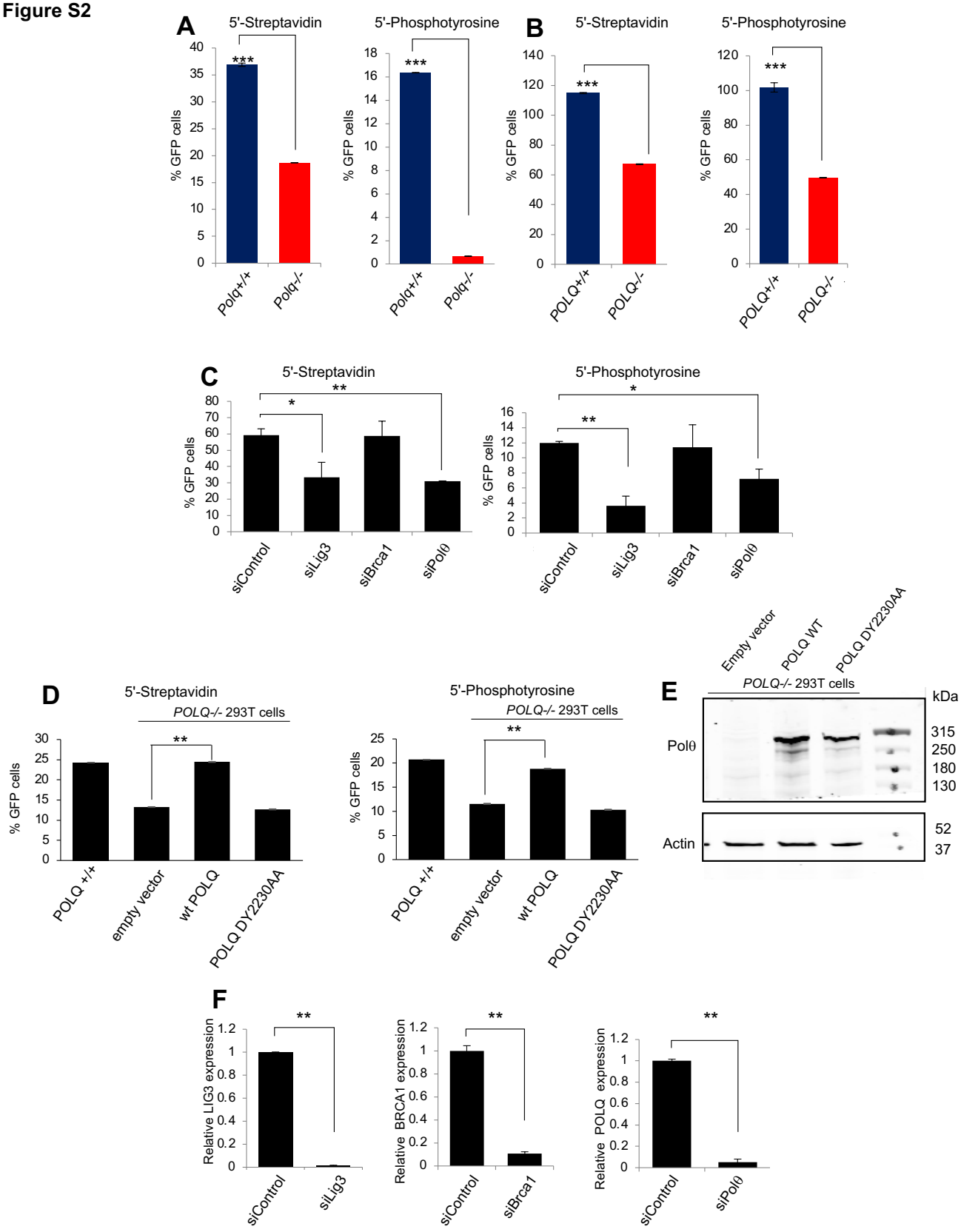

A
5'-Streptavidin
5'-Phosphotyrosine
% GFP cells
***
Polq+/+
Polq-/-
Polq+/+
Polq-/-
B
5'-Streptavidin
5'-Phosphotyrosine
% GFP cells
***
POLQ+/+
POLQ-/-
POLQ+/+
POLQ-/-
C
5'-Streptavidin
5'-Phosphotyrosine
% GFP cells
*
**
siControl
siLig3
siBrca1
siPolθ
D
5'-Streptavidin
5'-Phosphotyrosine
POLQ-/- 293T cells
% GFP cells
**
POLQ +/+
empty vector
wt POLQ
POLQ DY2230AA
E
Empty vector
POLQ WT
POLQ DY2230AA
POLQ-/- 293T cells
kDa
Polθ
315
250
180
130
Actin
52
37
F
Relative LIG3 expression
Relative BRCA1 expression
Relative POLQ expression
**
siControl
siLig3
siBrca1
siPolθ

Figure S2. Controls for Pol□-dependent MMEJ repair of 5' DPCs in cells.

(A) Bar plot showing relative GFP following co-transfection of left and right MMEJ reporter DNA constructs conjugated with streptavidin (left) and following co-transfection of left and right MMEJ reporter DNA constructs conjugated with phosphotyrosine (right) in *Polq+/+* and *Polq-/-* mESCs. GFP+ frequencies are normalized to transfection efficiency. Raw data pooled from two separate experiments performed in triplicate for each condition. +/-s.e.m. * P < 0.05, **P<0.01, ***P<0.001. * = statistical significance from two sample *t*-test between *Polq+/+* vs *Polq-/-*. P = 0.0003 (left). P = 0.001 (right).

(B) Same in *POLQ+/+* and *POLQ-/-* HEK293T cells. GFP+ frequencies are normalized to transfection efficiency. Raw data pooled from two separate experiments performed in triplicate for each condition. +/-s.e.m. * P < 0.05, **P<0.01, ***P<0.001.* = statistical significance from two sample *t*-test between *POLQ+/+* vs *POLQ-/-.* P = 0.00006 (left). P = 0.0001 (right)

(C) Bar plot showing relative GFP following co-transfection of left and right MMEJ reporter DNA constructs conjugated with streptavidin (left), following co-transfection of left and right MMEJ reporter DNA constructs conjugated with phosphotyrosine (right) in *Polq+/+* mESCs and following co-transfection of siRNA control, siRNA against LIG3, siRNA against BRCA1 and siRNA against Pol□ in *Polq+/+* mESCs. GFP+ frequencies are normalized to transfection efficiency. Raw data pooled from two separate experiments performed in triplicate for each condition. +/-s.e.m. * P < 0.05, **P<0.01, ***P<0.001.* = statistical significance from two sample *t*-test between si control vs si Lig3, P = 0.048; si control vs siPolq, P = 0.009 (left), between si control vs si Lig3, P = 0.01; si control vs siPolq, P = 0.02 (right).

(D) Bar plot showing relative GFP following overexpression of indicated plasmids and co-transfection of left and right MMEJ reporter DNA constructs conjugated with streptavidin (left) and following co-transfection of left and right MMEJ reporter DNA constructs conjugated with phosphotyrosine (right) in HEK293T cells. GFP+ frequencies are normalized to transfection efficiency. Raw data pooled from one experiment performed in triplicate for each condition. +/-s.e.m. * P < 0.05, **P<0.01, ***P<0.001. * = statistical significance from two sample *t*-test between Empty vector vs wtPOLQ. P = 0.002 (left), P = 0.001 (right)

(E) Immunoblot with whole cell extracts of *POLQ-/-* HEK293T cells that were overexpressed with indicated plasmids and used for assay in S2C. Immunoblotting was performed against POLQ antibody (top) and actin (bottom, loading control)

(F) RT qPCR analysis of Lig3, Brca1 and Polq expression. mRNA levels were corrected with internal control for GAPDH in siRNA-treated cells used in Figures 4F and S2C as well as normalized to non-targeting siRNA (siControl = 1). Data represent mean. n = 3 +/-s.e.m. * P < 0.05, **P<0.01, ***P<0.001. Statistical significance was determined from two sample *t*-test. P values are as follows: siControl vs siLig3 = 0.005; siControl vs siBrca1 = 0.002; siControl vs siPolq = 0.003

Figure S3

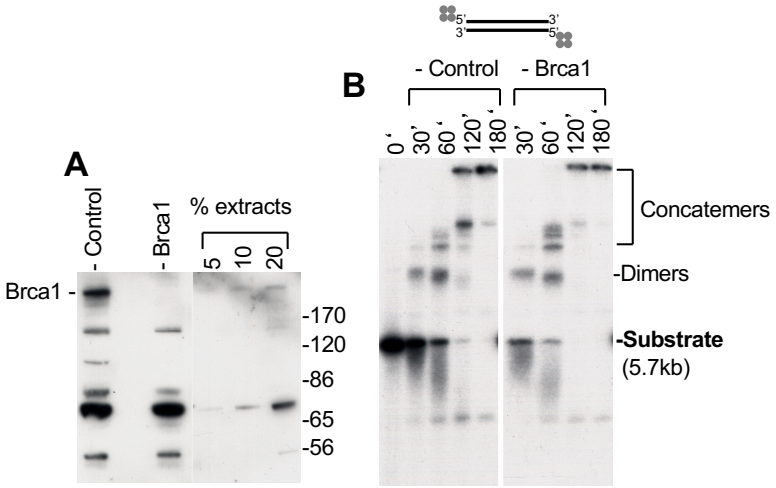


Figure S3 MMEJ repair of 5' DPCs occurs in the absence of BRCA1
(A) Western blots showing the presence and absence of BRCA1 in mock depleted (control) and BRCA1 depleted *Xenopus* egg extracts. % extracts loaded indicated at right.
(B) Non-denaturing gels showing a time course of DSB repair of the indicated DNA substrate in mock depleted (control; left) and BRCA1 depleted (right) *Xenopus* egg extracts.

Table S1

Oligo name	Sequence (5'-3')
RP 500B	/5Biosg/GCTAGCCAGTCAGTGGGCCCGC
RP 503B	/5Biosg/TGATTACGCCAAGTTAATTAAGGACGTCCTCCTGCTGG
RP506B	/5Biosg/AAAAAAAAAATCGGGCATGGCGGACTTGAAGAAGTCG
RP507B	/5Biosg/AAAAAAAAAAGCCCGAAGGCTACGTCCAGGAGCG
RP506PT	(5'-ptyr)AAAAAAAAAATCGGGCATGGCGGACTTGAAGAAGTCG
RP507PT	(5'-ptyr)AAAAAAAAAAGCCCGAAGGCTACGTCCAGGAGCG