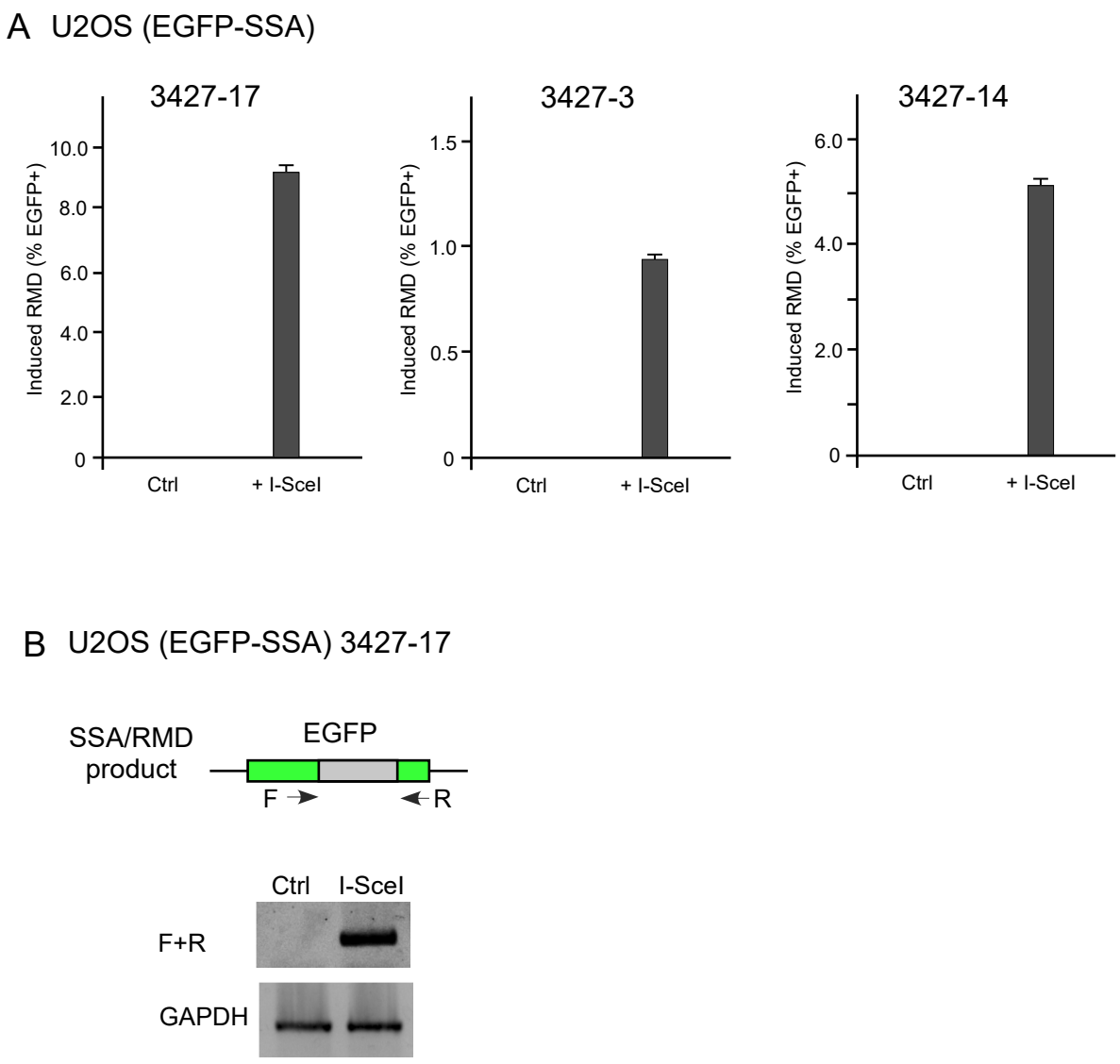
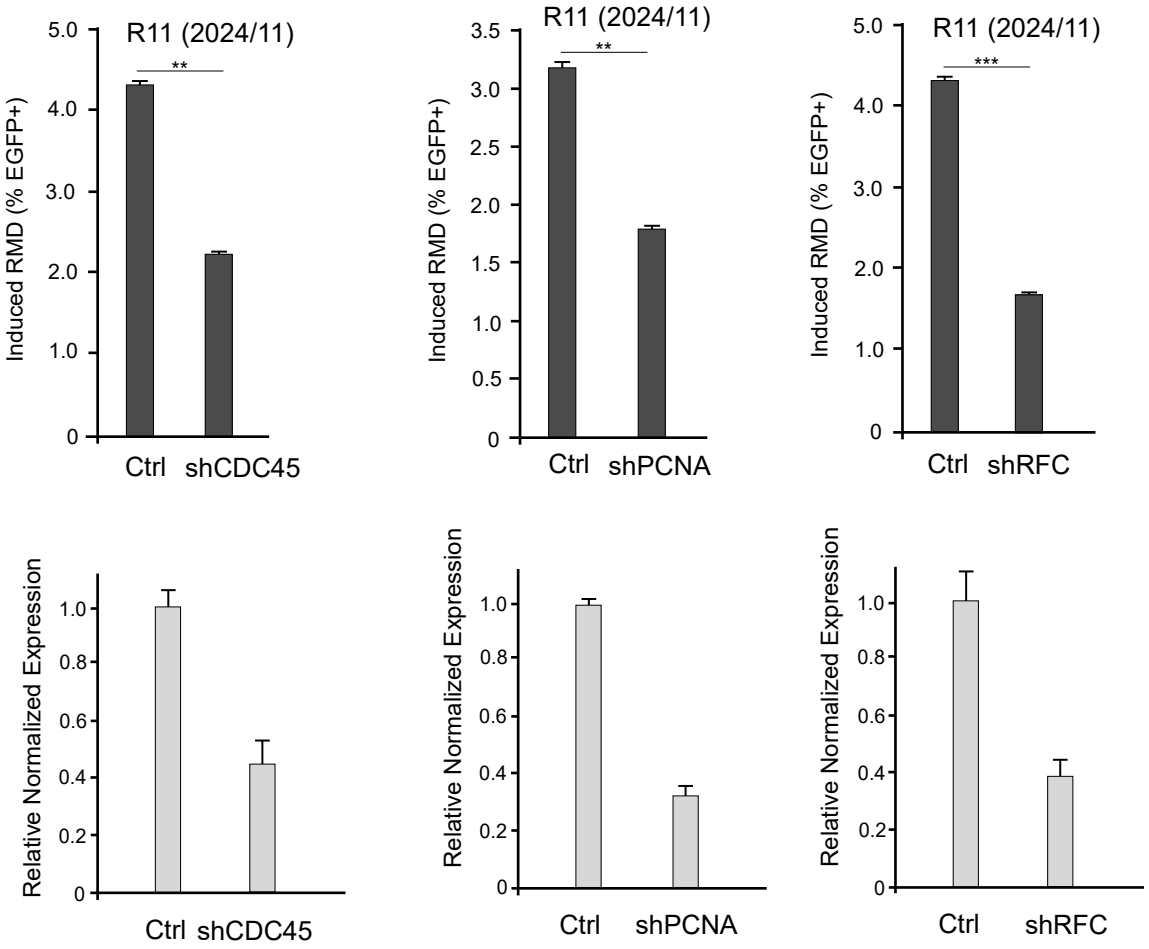
**Appendix:**



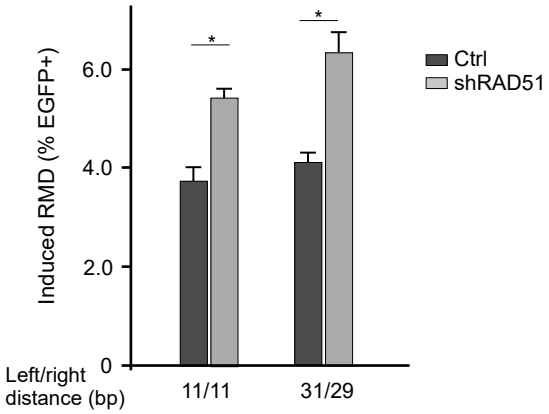

**Appendix Figure S1.** RMD analysis of independent U2OS (EGFP-SSA-3427-17) reporter cell lines. (A) RMD frequency was determined 3 days after I-SceI expression in U2OS (EGFP-SSA) reporter cell lines 3427-17, 3427-3 and 3427-14.
(B) A diagram of the RMD product from the EGFP-SSA reporter is shown on the top. PCR results of genomic DNA purified from parental U2OS (EGFP-SSA)-3427-17 cells (Ctrl) and sorted GFP positive cells (I-SceI) using indicated primers F (ACGACGGCAACTACAAGACC) and R (CCAGCAGGACCATGTGATCG) are shown on the bottom, with GAPDH gene as a positive control.

U2OS (EGFP-SSA)

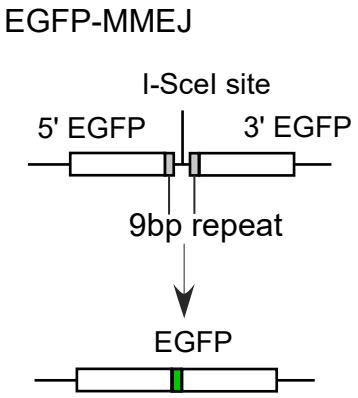



**Appendix Figure S2.** CDC45, PCNA and RFC are needed for BIR/RMD. U2OS (EGFP-SSA-3427-17) cells expressing shRNAs for CDC45, PCNA and RFC or vector (Ctrl) were transfected with gRNA/Cas9 to cleave at R11 on the EGFP-SSA reporter, and RMD frequency was determined (top). qPCR was used to determine the knock-down efficiency of CDC45, PCNA and RFC after shRNA expression (bottom).

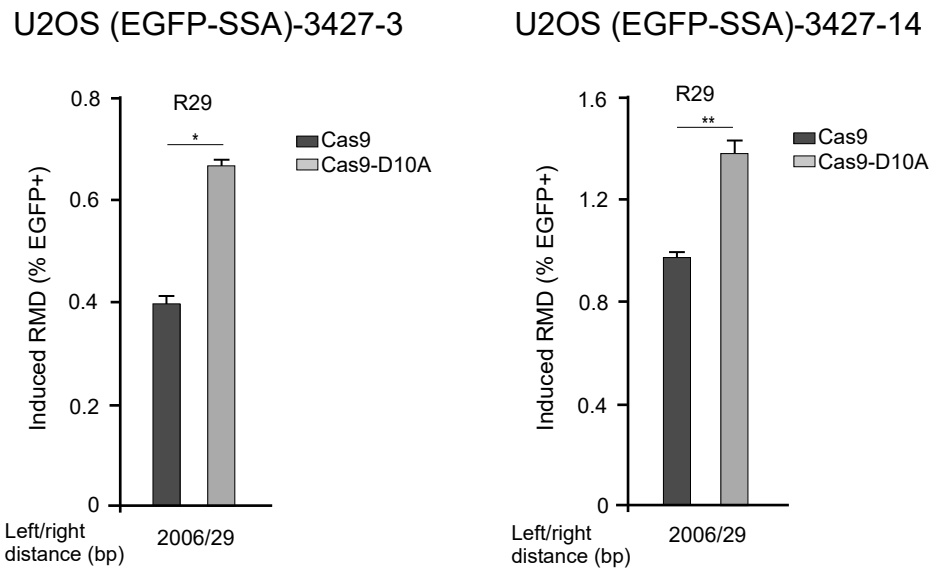
U2OS (EGFP-SSA)




**Appendix Figure S3.** RAD51 inhibits SSA/RMD. U2OS (EGFP-SSA-3427-17) cells expressing shRNAs for RAD51 or vector (Ctrl) were transfected with gRNAs/Cas9 to cleave at L11 and R11 or L31 and R29 on the EGFP-SSA reporter, and RMD frequency was determined.



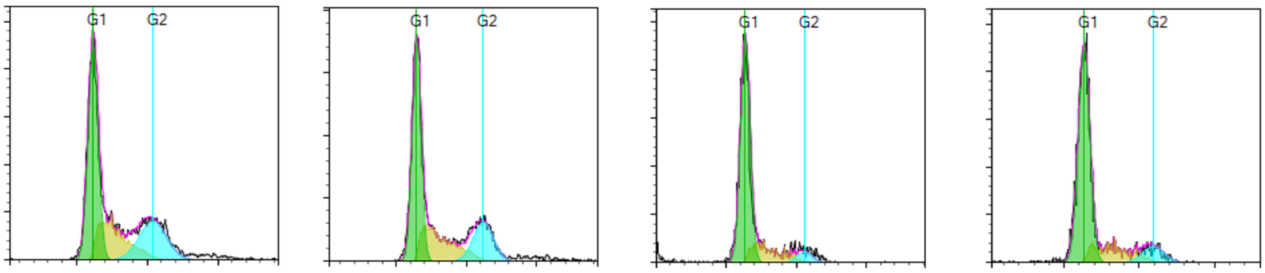

**Appendix Figure S4.** A schematic drawing of the EGFP-MMEJ reporter.




**Appendix Figure S5.** RMD is stimulated when Cas9-D10A is used to create a nick near one repeat. RMD frequencies were determined in U2OS (EGFP-SSA-3427-3) and U2OS (EGFP-SSA-3427-14) cell lines after cleavage with indicated sgRNAs/Cas9 and sgRNAs/Cas9-D10A.

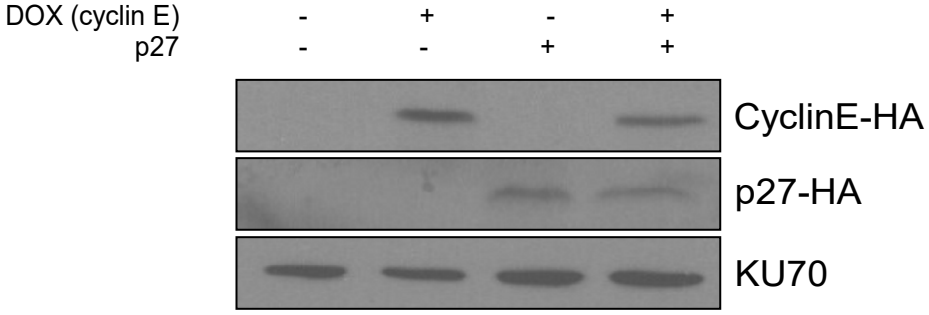
A

U2OS (EGFP-SSA)



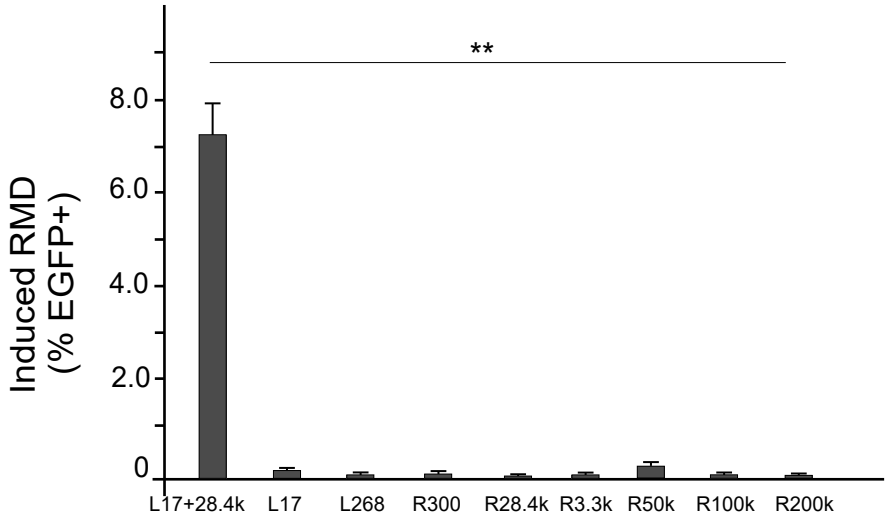

| | | | | |
|---|---|---|---|---|
| DOX (cyclinE) | - | + | - | + |
| p27 | - | - | + | + |
| G0/G1 | 48.7% | 50.0% | 74.8% | 71.3% |
| S | 24.2% | 25.7% | 16.7% | 17.4% |
| G2/M | 23.9% | 20.4% | 5.6% | 9.9% |

B

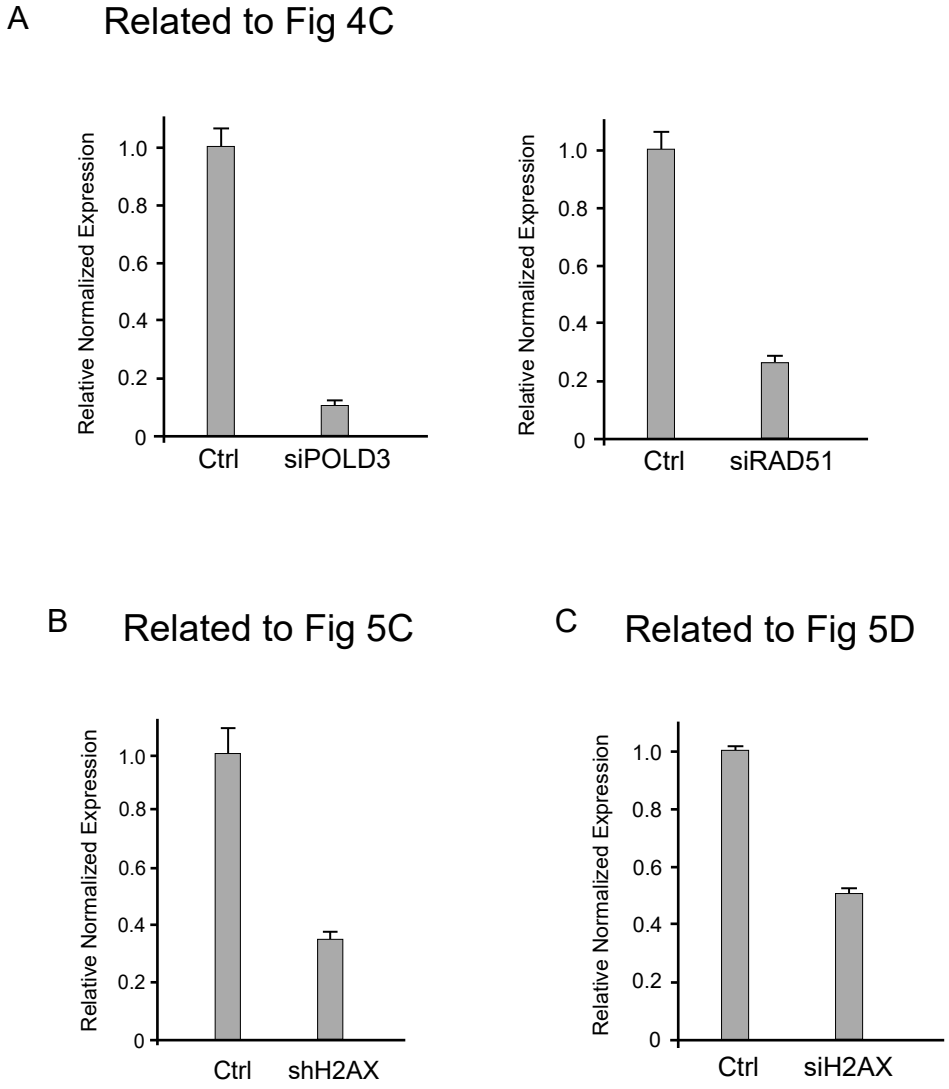



**Appendix Figure S6.** Cell cycle profiles with or without expression of cyclin E and p27. (A) Cell cycle profiles of U2OS (EGFP-SSA-3427-3) cells expressing p27 or control vector with or without DOX-induced expression of cyclin E are shown. (B) Expression of cyclin E and p27 was determined by Western blot analysis using KU70 as the loading control.

mES (RMD-GFP)

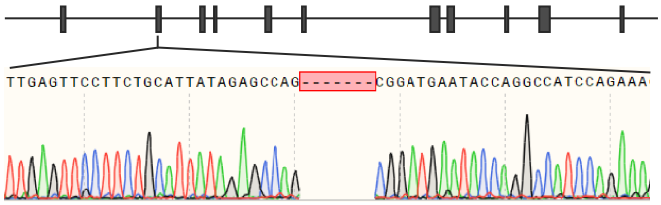



**Appendix Figure S7.** RMD was determined in mES (RMD-GFP) cells. RMD was determined after cleavage with double gRNA/Cas9 at L17 and R28.4k, and single cleavage at L17, L268, R300, R3.3k, R28.4k, R50k, R100k and R200k.

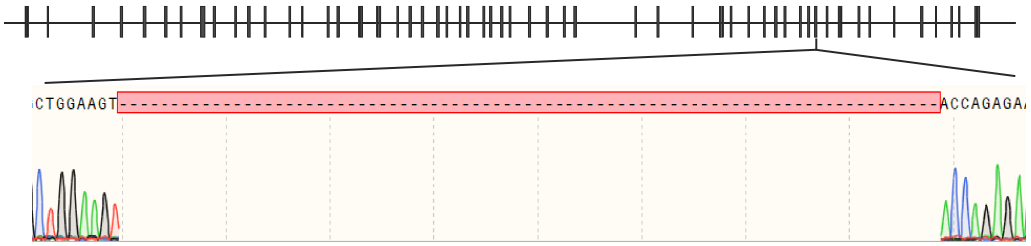



**Appendix Figure S8.** qPCR analysis to show knockdown efficiency. (A) Knockdown of POLD3 and RAD51 by siRNA in mES (GFP-RMD) cells for the analysis shown in Fig 4C. (B) Knockdown of H2AX by shRNAs in U2OS (EGFP-SSA-3427-17) cells for the analysis shown in Fig 5C. (C) Knockdown of H2AX by siRNA in mES (GFP-RMD) cells for the analysis shown in Fig 5D.

A *RAD52*KO in mESC (RMD-GFP): 7 bp deletion

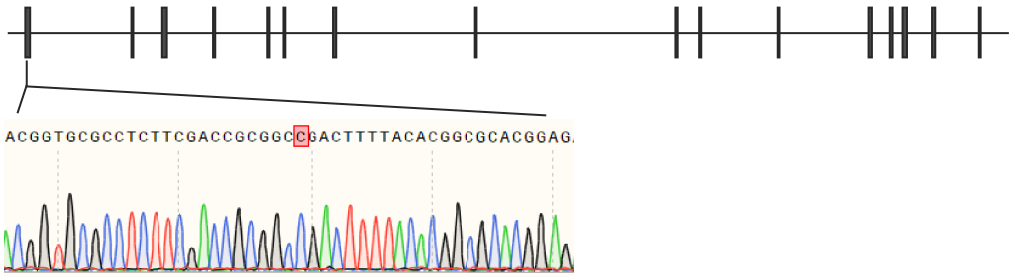



B *ATM*KO in mESC (RMD-GFP): 79 bp deletion




C *MSH2*KO in mESC:

1%-RMD reporter 1 bp Insertion




3%-RMD reporter Insertion-Deletion

**CCACGGTGCGCCTCTTC-**Insetion**-**Deletion**-GCCGGAAACCTGGGCGCATG**

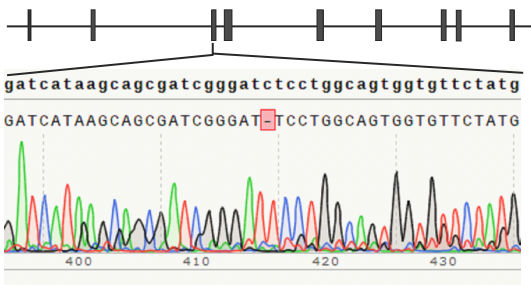
Insetion 770 bp

ctagccaaatggatggacctggagagcatcatcctgagtgaggtaacacaatcacaaaggaactcacacaatatgtactcactgataagtggatactag
cccaaaacctaggatacccacgatataagatacaatttcctaaacacatgaaactcaagaaaaatgaagactgaagtgtggacactatgcccctcctta
gaagtgggaacaaaacacccatggaaggagttacagaaacaaagtatggagctgagatgaaaggatggaccatgtagagactgccatatccagggat
ccaccccataatcagcttccaaatgctgacaccattgcatacactagcaagattttactgaaaggacccagatgtagctgtctcttgtgagactatgccggg
gcctagcaaacacagaagtggatgctcacagtcagctaatggatggatcacagggctcccaatggaggagctagagaaagtacccaaggagctaaag
ggatcttcaaccctataggtggaacaacattatgaactaaccagtaccctgagctcttgactctagctgcatatgtatcaaagatggcctagtcggccatc
actggaaagagaggcccattggacacgcagactttgtgtgccccggtacaggggacgccagggccaaaggggggagtgggtgggtaggggagtgg
gggtgggtggggtaaaggggacttttggtatagcattggaaatgtaaatgagctaaatacctaataaaaatggaaaa

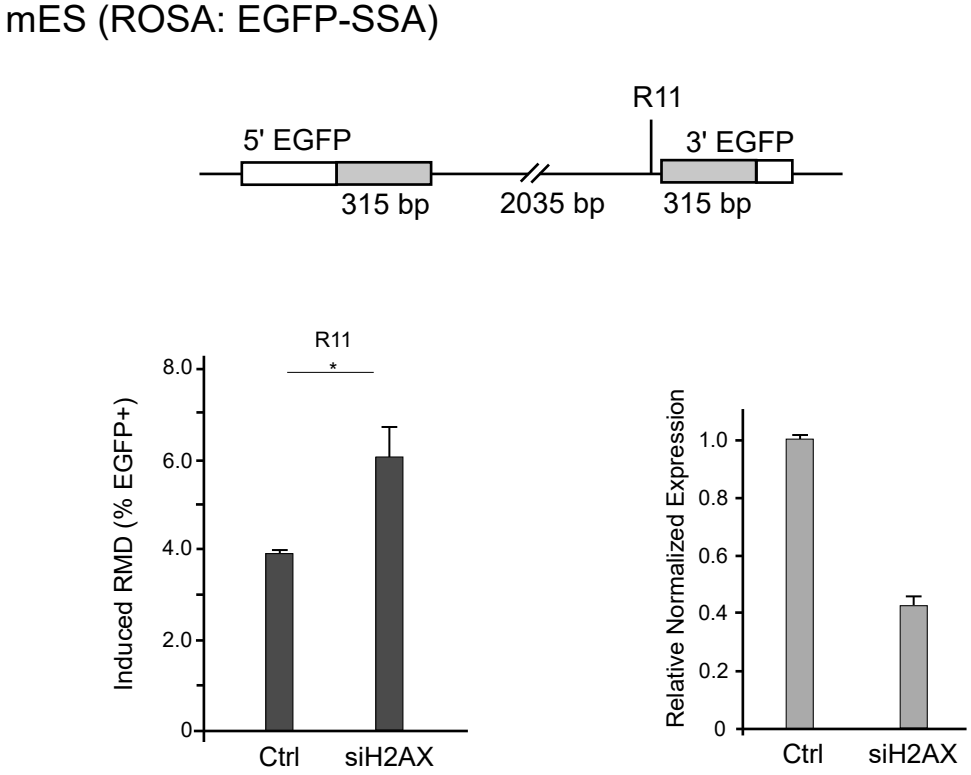
Deletion 148 bp

gaccgcggcgacttttacacggcgcacggagaggacgcgctgctggcggcccgcgaggtgttcaagacccagggcgtgatcaagtacatggggccggcaggtgagggca
gccgcggcggcggggcccgggccggctcggggccgcgtt

D *KU70*KO in mESC (RMD-GFP): 1 bp deletion



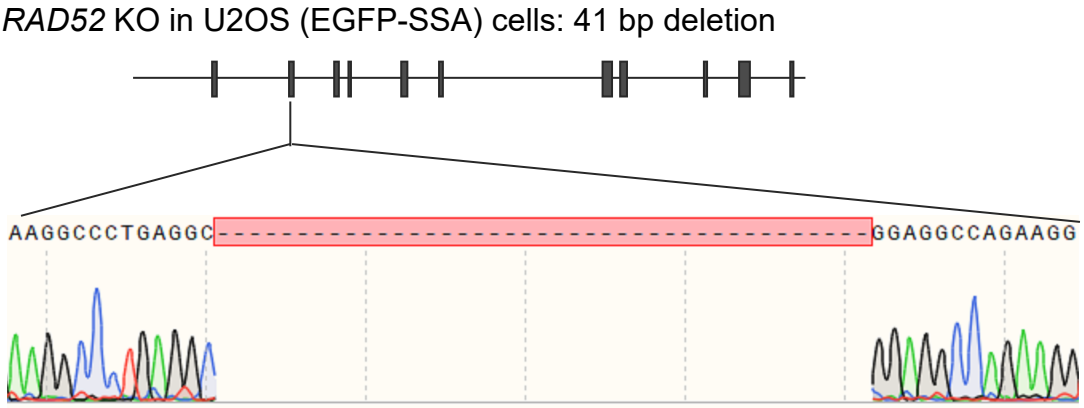

**Appendix Figure S9.** Generation of knockout of *RAD52*, *ATM*, *MSH2* and *KU70* in mES (RMD-GFP) cells by sgRNA/Cas9. Genomic DNA sequencing results at sgRNA targeting sites in KO clones are shown with indicated sequences of deletions and insertions.




**Appendix Figure S10.** H2AX suppresses short-range RMD. The EGFP-SSA reporter was inserted into the ROSA locus in mES cells and RMD frequencies were determined after transfecting cells with H2AX siRNA or control (left). qPCR was performed to determine H2AX knockdown efficiency by siRNA (right).

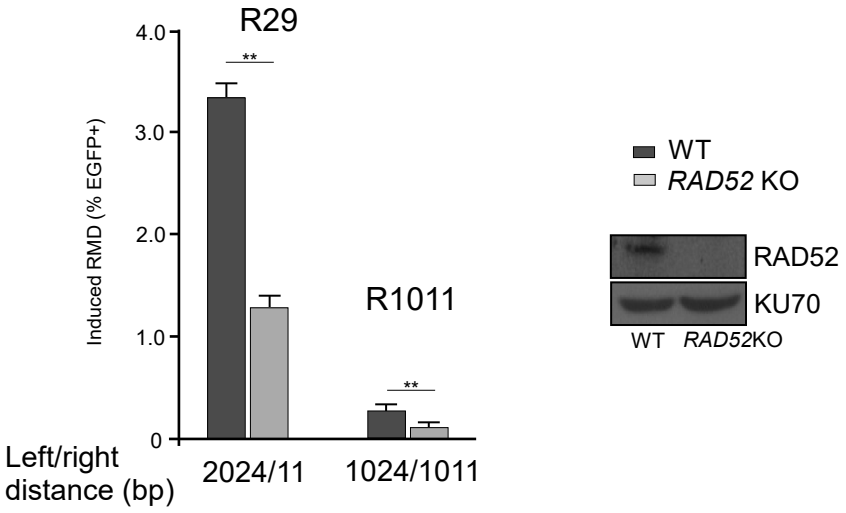
A

**RAD52 KO**

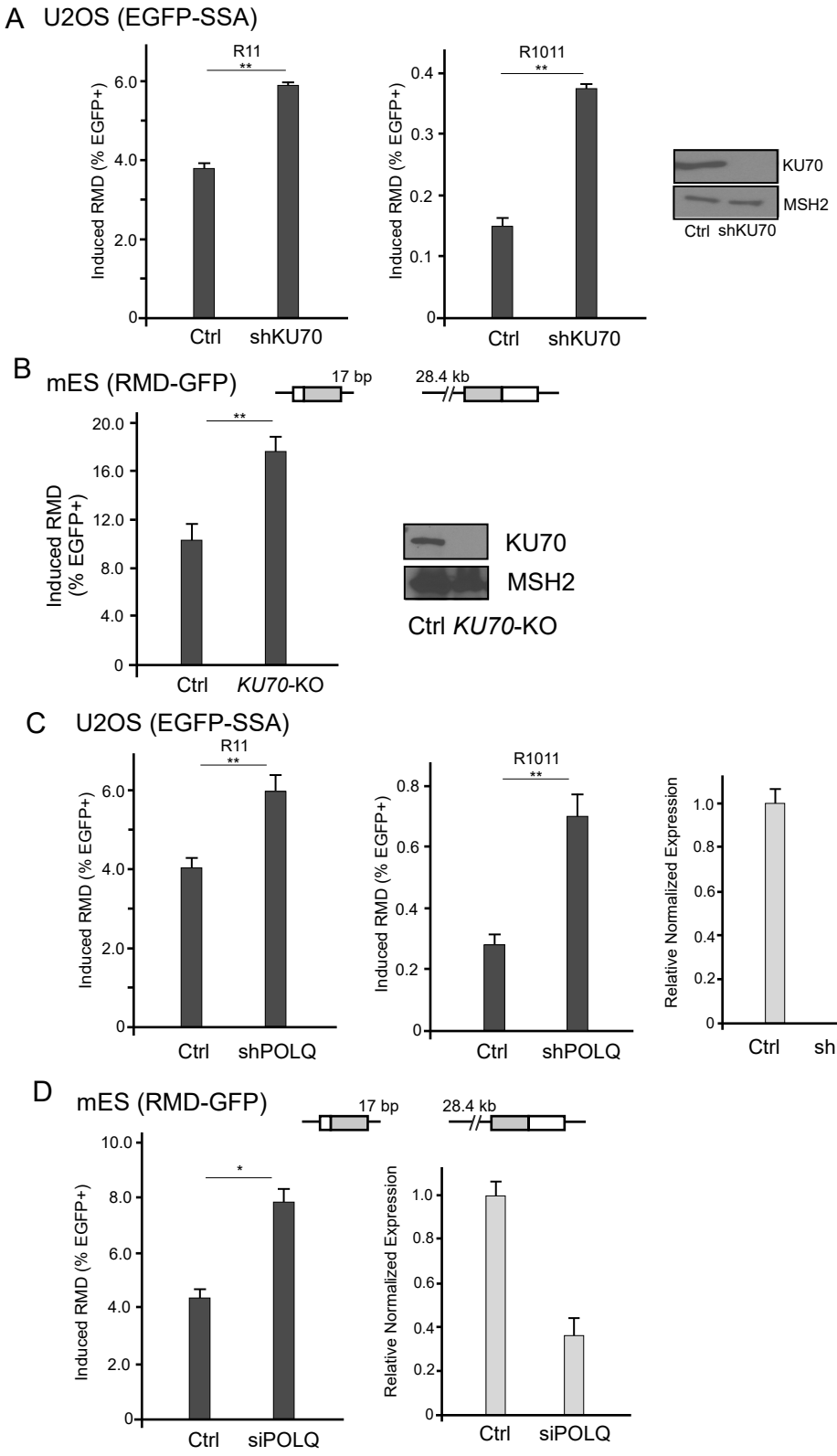



B

U2OS (EGFP-SSA)

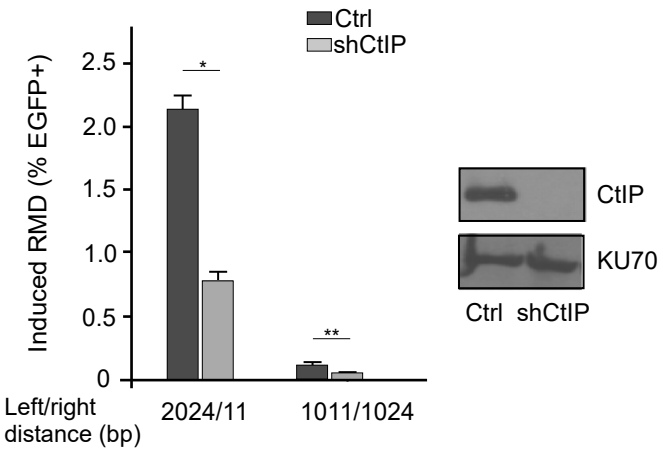



**Appendix Figure S11.** Generation of *RAD52* knockout (KO) in U2OS (EGFP-SSA-3427-17) cells by sgRNA/Cas9. (A) Genomic DNA sequencing results at sgRNA targeting sites in *RAD52* KO clones are shown with indicated deletions. (B) Determination of RMD frequency in WT and *RAD52* KO U2OS (EGFP-SSA-3427-17) cells after cleavage with indicated sgRNA/Cas9.




**Appendix Figure S12.** NHEJ and MMEJ suppress BIR/RMD and SSA/RMD. (A) and (C) U2OS (EGFP-SSA-3427-17) cells expressing shRNAs for KU70 (A) or POLQ (B) were assayed for RMD after cleavage at R11 or R1011. (B) and (D) mES (RMD-GFP) WT and *KU70*-KO cells (B) or WT and POLQ knockdown cells (D) were assayed for RMD after cleavage at L17/R28.4k. Depletion of KU70 is shown by Western blot analysis using MSH2 as a loading control. Depletion levels of POLQ were determined by qPCR.

U2OS (EGFP-SSA)




**Appendix Figure S13.** CtIP is important for BIR/RMD and SSA/RMD. U2OS (EGFP-SSA-3427-17) cells expressing shRNAs for CtIP or vector (Ctrl) were transfected with gRNAs/Cas9 to cleave at R11 or R1024 on the EGFP-SSA reporter, and RMD frequency was determined. Depletion of CtIP is shown by Western blot with KU70 as a loading control.

A

| Name | Guide Sequence(5'to3') | PAM | ICE(Indel percentage)* | R Squared(quality of analysis)# |
|---|---|---|---|---|
| EGFP-SSA-R1011 | CTCATCGAGAGCCTGCGCGA | ACG | 43 | 0.98 |
| EGFP-SSA-R634 | GGCGCAGCTATTTACCCGC | AGG | 30 | 0.98 |
| EGFP-SSA-R316 | CAATGACAAGACGCTGGGCG | GGG | 32 | 0.99 |
| EGFP-SSA-R101 | TCTCCAGCAGCCGCACGCGG | TGG | 32 | 0.96 |
| EGFP-SSA-R51 | CGCGCTGGGCTACGTCTTGC | TGG | 31 | 0.98 |
| EGFP-SSA-R29 | CGTTCGGCGAGTCGACCTGC | AGG | 32 | 0.99 |
| EGFP-SSA-R11 | GCAGGCATGCAGGGATAAC | AGG | 29 | 0.97 |
| EGFP-SSA-L11 | ACGAGTAAGGATCCTCTAG | AGG | 39 | 0.94 |

B

| Name | Guide Sequence(5'to3') | PAM | ICE(Indel percentage)* | R Squared(quality of analysis)# |
|---|---|---|---|---|
| mES-RMD-L17 | GCTGCAGGGGCCAGCTCCGG | AGG | 36 | 0.98 |
| mES-RMD-L38 | TGTCGCTGTCTTGCACTCTG | AGG | 29 | 0.98 |
| mES-RMD-L50 | CTTGTCGCTGTCTTGCACTC | TGG | 31 | 0.98 |
| mES-RMD-L268 | CCATAGGCGTGGGACCTCGT | GGG | 35 | 0.94 |
| mES-RMD-R300 | GGATTGGAGCTACGGGGGT | GGG | 35 | 0.98 |
| mES-RMD-R28.4K | CGAAGCTAACGACACTAACG | GGG | 46 | 0.98 |
| mES-RMD-R3.3K | GCGCCGCACCGACACCCTGG | AGG | 36 | 0.97 |
| mES-RMD-50K | CAAGCCAGTCACATGCGTGG | AGG | 42 | 0.99 |
| mES-RMD-100K | CTAGTATGCCTCTCAGTCGA | GGG | 34 | 0.99 |
| mES-RMD-150K | CAGACTCTACAGATAGGCGG | TGG | 42 | 0.98 |
| mES-RMD-200K | GCAGGCCAACTCCACGGGGT | TGG | 35 | 0.98 |

* Indel percentage
# How well the proposed Indel distribution fits the Sanger sequence data of the edited sample

**Appendix Figure S14.** The targeting efficiency of indicated sgRNAs in U2OS (EGFP-SSA-3427-17) and mES (RMD-GFP) reporter cell lines. sgRNA targeting efficiency was determined by mutagenic end joining frequency, analyzed by Inference of CRISPR Edits (ICE). Website: https://ice.synthego.com/.

Appendix Table S1. sgRNAs for assays using the RMD reporters

| Name | Guide Sequence (5’ to 3’) |
|---|---|
| EGFP-SSA-3427-L31 | GGCACGCCAGAAATCCGCG |
| EGFP-SSA-3427-L651 | CGACAGTCCCGGCTCCGGAT |
| EGFP-SSA-3427-R1011 | CTCATCGAGAGCCTGCGCGA |
| EGFP-SSA-3427-R634 | GGCGCAGCTATTTACCCGC |
| EGFP-SSA-3427-R316 | CAATGACAAGACGCTGGGCG |
| EGFP-SSA-3427-R101 | TCTCCAGCAGCCGCACGCGG |
| EGFP-SSA-3427-R51 | CGCGCTGGGCTACGTCTTGC |
| EGFP-SSA-3427-R29 | CGTTCGCGAGTCGACCTGC |
| EGFP-SSA-3427-R11 | GCAGGCATGCTAGGGATAAC |
| mESC-RMD-L17 | GCTGCAGGGGCCAGCTCCGG |
| mESC-RMD-L38 | TGTCGCTGTCTTGCACTCTG |
| mESC-RMD-L50 | CTTGTCGCTGTCTTGCACTC |
| mESC-RMD-L268 | CCATAGGCGTGGGACCTCGT |
| mESC-RMD-R300 | AGGATTGGAGCTACGGGGGT |
| mESC-RMD-R3.3K | GCGCCGCACCGACACCCTGG |
| mESC-RMD-R9.1K | TGGCCATACAATCCAAACAT |
| mESC-RMD-R19K | GTCCTGCCATAGCCTTGATG |
| mESC-RMD-R28.4K | CGAAGCTAACGACACTAACG |
| mESC-RMD-R50K | CAAGCCAGTCACATGCGTGG |
| mESC-RMD-R100K | CTAGTATGCCTCTCAGTCGA |
| mESC-RMD-R150K | CAGACTCTACAGATAGGCGG |
| mESC-RMD-R200K | GCAGGCCAACTCCACGGGGT |
| mESC-RMD-R-D10K | GCTCAGCAATGGCGGGTTAA |

Appendix Table S2. sgRNAs for generating knockout cell lines

| Name | Sequence (5’ to 3’) |
|---|---|
| mESC-KU70-KO-sgRNA1 | AAGCAGCGATCGGGATCTCC |
| mESC-RAD52-KO-sgRNA1 | GTATACAGCGGATGAATACC |
| mESC-ATM-KO-sgRNA1 | AAGGTTGCTGGAAGTTACGA |
| mESC-ATM-KO-sgRNA2 | AGGCCTTTCTCTCATTGGCA |
| mESC-MSH2-KO-sgRNA1 | CGGCGACTTTTACACGGCGC |
| Human-RAD52-sgRNA1 | TCCAGAAGGCCCTGAGGCAG |
| Human-RAD52-sgRNA2 | AGTAGCCGCATGGCTGGCGG |

Appendix Table S3. PCR primers for screening knockout cell lines

| Name | Sequence (5’ to 3’) |
|---|---|
| mESC-KU70-KO-PCR-F | ATGCAGCTGGTATGGCAAGT |
| mESC-KU70-KO-PCR-R | CAAAGCTGCTTCCTCCTCCA |

| | |
|---|---|
| mESC-RAD52-KO-PCR-F | TGGATGGATCCTCAGCCTCA |
| mESC-RAD52-KO-PCR-R | AGCCCCTCACCTTTAACTGC |
| mESC-H2AX-KO-PCR-F | GGCGTGTCCAGGGAGTTTAT |
| mESC-H2AX-KO-PCR-R | AAGGGCCTTTGTGGAGGTG |
| mESC-ATM-KO-PCR-F | AATGTCGGGCTGGAGAGATG |
| mESC-ATM-KO-PCR-R | GGCAGTTTTCACGTCAGCAG |
| mESC-MSH2-KO-PCR-F | CTGTGCTCTAGTGGTGTTGGT |
| mESC-MSH2-KO-PCR-R | TCTCAAAACGCCCTCCACTC |
| Human-RAD52-KO-PCR-F | CTAATGGCATGCATGCCT |
| Human-RAD52-KO-PCR-R | CAGCAGACTTCCGAATCCC |

Appendix Table S4. shRNAs sequences for knockdown

| Name | Sequence (5’ to 3’) |
|---|---|
| human-RAD52-shRNA | GAUGUUGGUUAUGGUGUUAGU |
| human-H2AX-shRNA | GGGACGAAGCACUUGGUAACA |
| human-RAD51-shRNA | AAGCUAUGUUCGCCAUUAAUG |
| human-POLQ-shRNA | ACAACAACCCUUAUCGUAAAG |
| human-POLD3-shRNA | CAAUUAGUGGUUAGGGAAA |
| human-ATR-shRNA | CGAGACUUCUGCGGAUUGCAG |
| human-CDC45-shRNA | UCAAUGUCGUCAAUGUAUACA |
| human-RFC-shRNA | GAAAGGCGGCCUCUAAAUCAAA |
| human-KU70-shRNA | ACAGAGAUAUCAUCAGCAUAG |
| human-PCNA-shRNA | GUGGAGAACUUGGAAAUGGAA |

Appendix Table S5. qPCR primers for determining knockdown efficiency

| Name | Sequence (5’ to 3’) |
|---|---|
| MOUSE-GAPDH-qPCR-F | TCAACAGCAACTCCCACTCTTCCA |
| MOUSE-GAPDH-qPCR-R | ACCACCCTGTTGCTGTAGCCGTAT |
| MOUSE-POLD3-qPCR-F | TCTGTCACTGAACCAAAGCTG |
| MOUSE-POLD3-qPCR-R | GTGTTCGGTTTCCTTTGCC |
| MOUSE-POLQ-qPCR-F | TTGGTCACGTCTTGGAAGG |
| MOUSE-POLQ-qPCR-F | TTAGCCACAGACACAAAGGG |
| MOUSE-H2AX-qPCR-F | AGGCCTCTCAGGAGTACTG |
| MOUSE-H2AX-qPCR-F | TGGCTCAGCTCTTTCTGTG |
| MOUSE-RAD51-qPCR-F | ACAGCCTATTTCACGGTTAGAG |
| MOUSE-RAD51-qPCR-F | GCTTTGGCTTCACTAATTCCC |
| Human-CDC45-qPCR-F | GGTTCAAGCACAAGTTTCTGG |
| Human-CDC45-qPCR-R | GTACAGCTTGTCCAGGTTACTC |
| Human-RFC-qPCR-F | GGTTCAAGCACAAGTTTCTGG |
| Human-RFC-qPCR-R | GTACAGCTTGTCCAGGTTACTC |

| | |
|---|---|
| Human-PCNA-qPCR-F | CTAGCCTGACAAATGCTTGC |
| Human-PCNA-qPCR-R | AGGAAAGTCTAGCTGGTTTCG |
| Human-POLQ-qPCR-F | GCCAGGGTTCTCTATGCTTC |
| Human-POLQ-qPCR-R | TCTTCAACTGCTTCCTCTTCC |
| Human-Actin-qPCR-F | CAAGGCCAACCGCGAGAAGATGAC |
| Human-Actin-qPCR-R | GCCAGAGGCGTACAGGGATAGCACA |
| Human-H2AX-qPCR-F | TCTGTTCTAGTGTTTGAGCCG |
| Human-H2AX-qPCR-R | CACTGGGAACTGGAGGC |
| Human-GAPDH-qPCR-F | ATCACTGCCACCCAGAAGAC |
| Human-GAPDH-qPCR-R | AGAAGATGAAAAGAGTTGTCAGGGC |