Gene coding sites

	sites	Reads
chr1	DAB1*	20
chr1	ippoi_site	0
chr1	RYR2*	233
chr1	ippoi_site	46
chr3	ERC2*	323
chr3	ippoi_site	3
chr8	SLCO5A1*	396
chr8	ippoi_site	4807
chr11	INTS4*	904
chr11	ippoi_site	81

* Promoter site

Intergenic sites

	sites	Reads
chr2	ippoi_site	0
chr7	ippoi_site	0
chr20	ippoi_site	0
chrX	ippoi_site	3

rDNA sites

	sites	Reads
chr21	LOC100507412*	386
chr21	RNA28S5*	1072484
chr21	ippoi_site	60014
chr21	LOC100507412*	5061
chr21	ippoi_site	58569
chr21	RNA45S5*	1738784
chr21	RNA28S5*	1114280
chr21	ippoi_site	55222

Table S1. RNA-seq results at the I-PpoI sites of HEK293T cells.

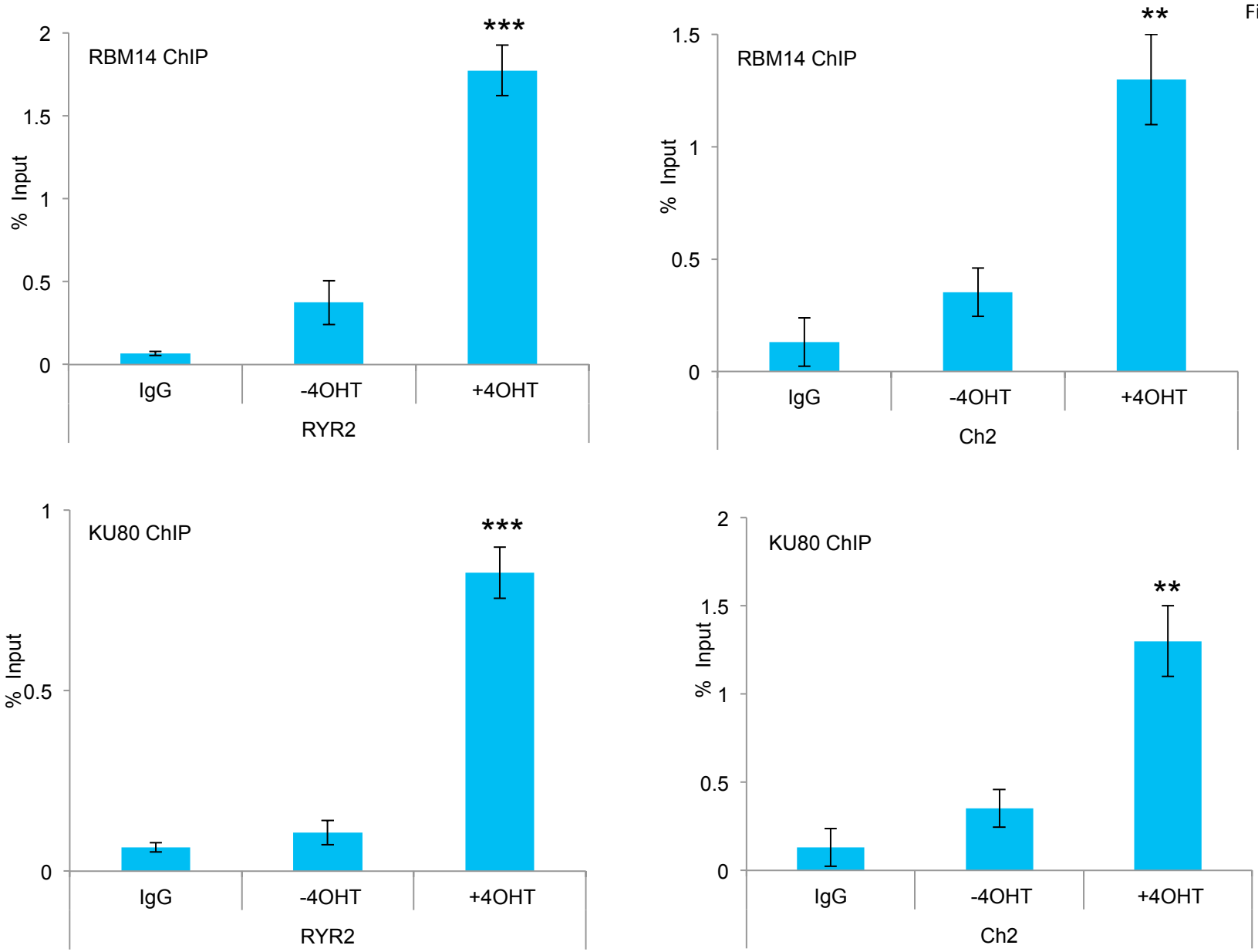


Figure S1. RBM14 and KU80 are recruited to I-PpoI sites in U2OS cells, related to Fig. 1. ChIP-qRT-PCR near RYR2 and the intergenic Chr 2 sites with anti-RBM14 and anti-KU80 antibodies before (-4OHT) and after (+4OHT) DSB induction in U2OS cells. Data shown are representative of three independent experiments. Each experiment was performed in triplicate. **p<0.01, ***p<0.001 by two-tailed t-test.

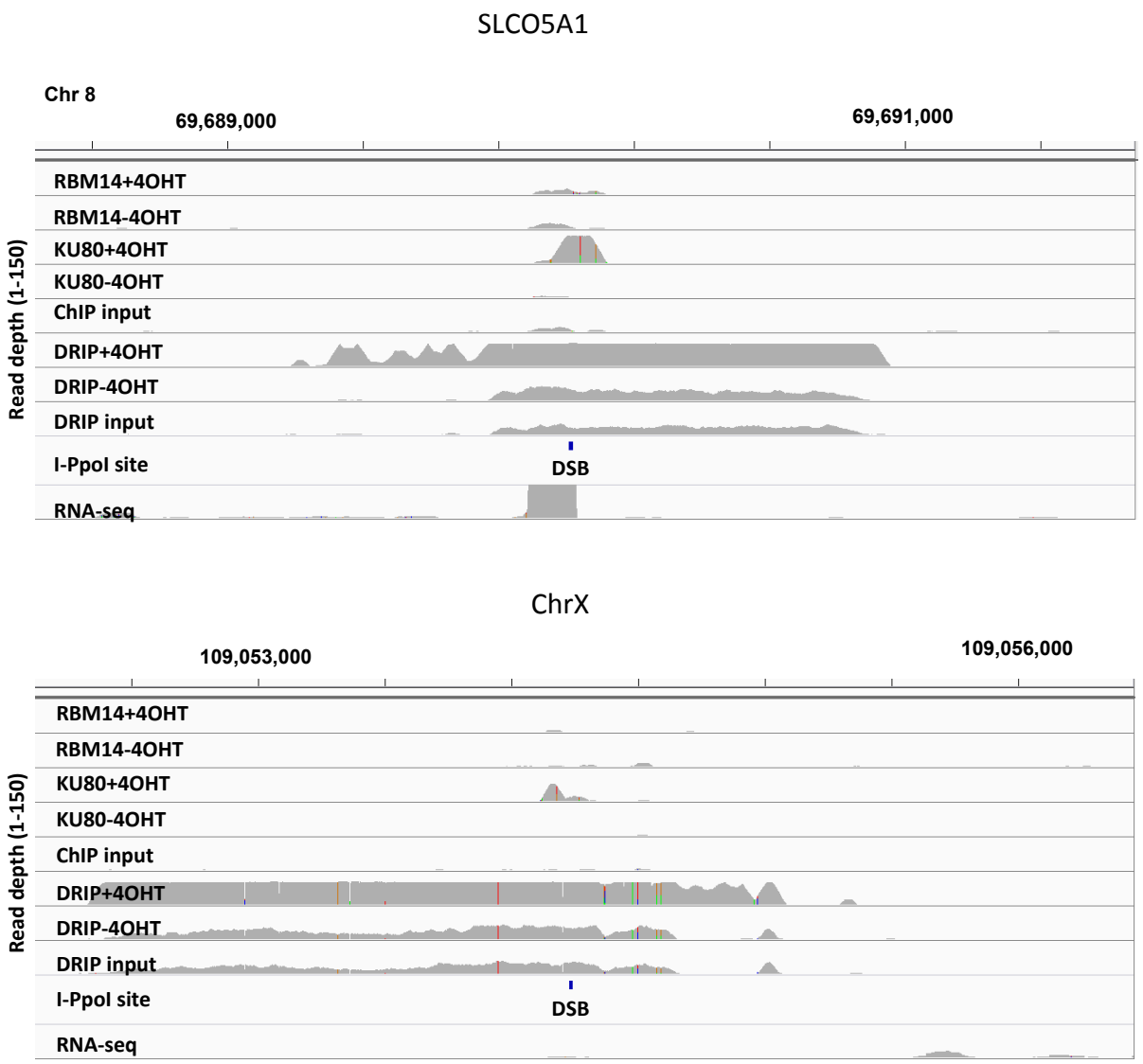


Figure S2. RBM14 is not recruited to SLCO5A1 and Chr X I-PpoI sites, related to Figure 1. Genome browser screen shots representing RBM14-ChIP-seq, KU80-ChIP-seq, and DRIP-seq read counts before I-PpoI activation (-4OHT) and after I-PpoI activation (+4OHT) at SLCO5A1 and ChrX sites. The I-PpoI site is indicated as DSB.

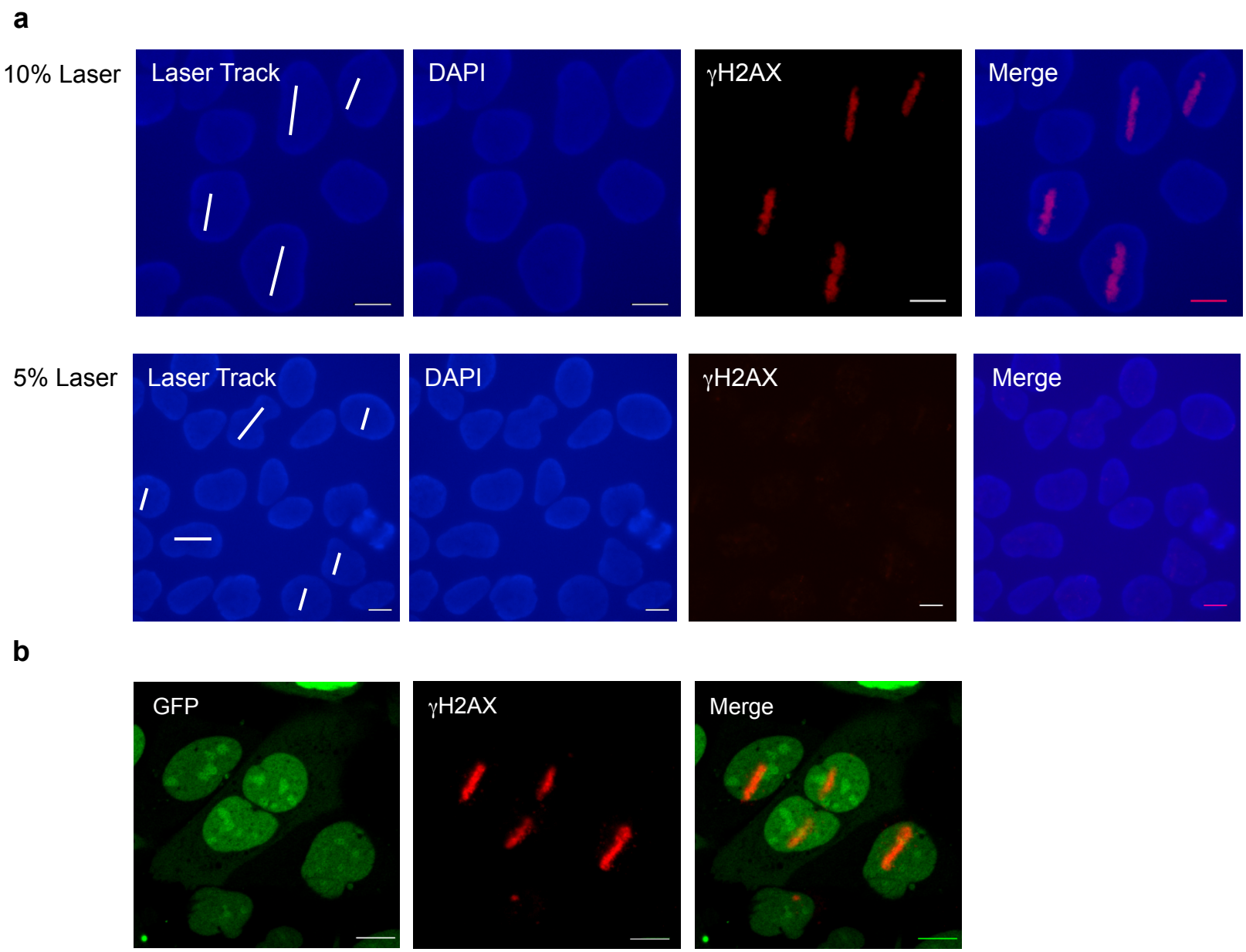


Figure S3. Induction of DSBs with micro-laser, related Figures. 1, 2 & 5. (a) 10% laser power with BrdU-induced DSBs, shown by γH2AX. (b) GFP is not recruited to microlaser-induced DNA damage sites.

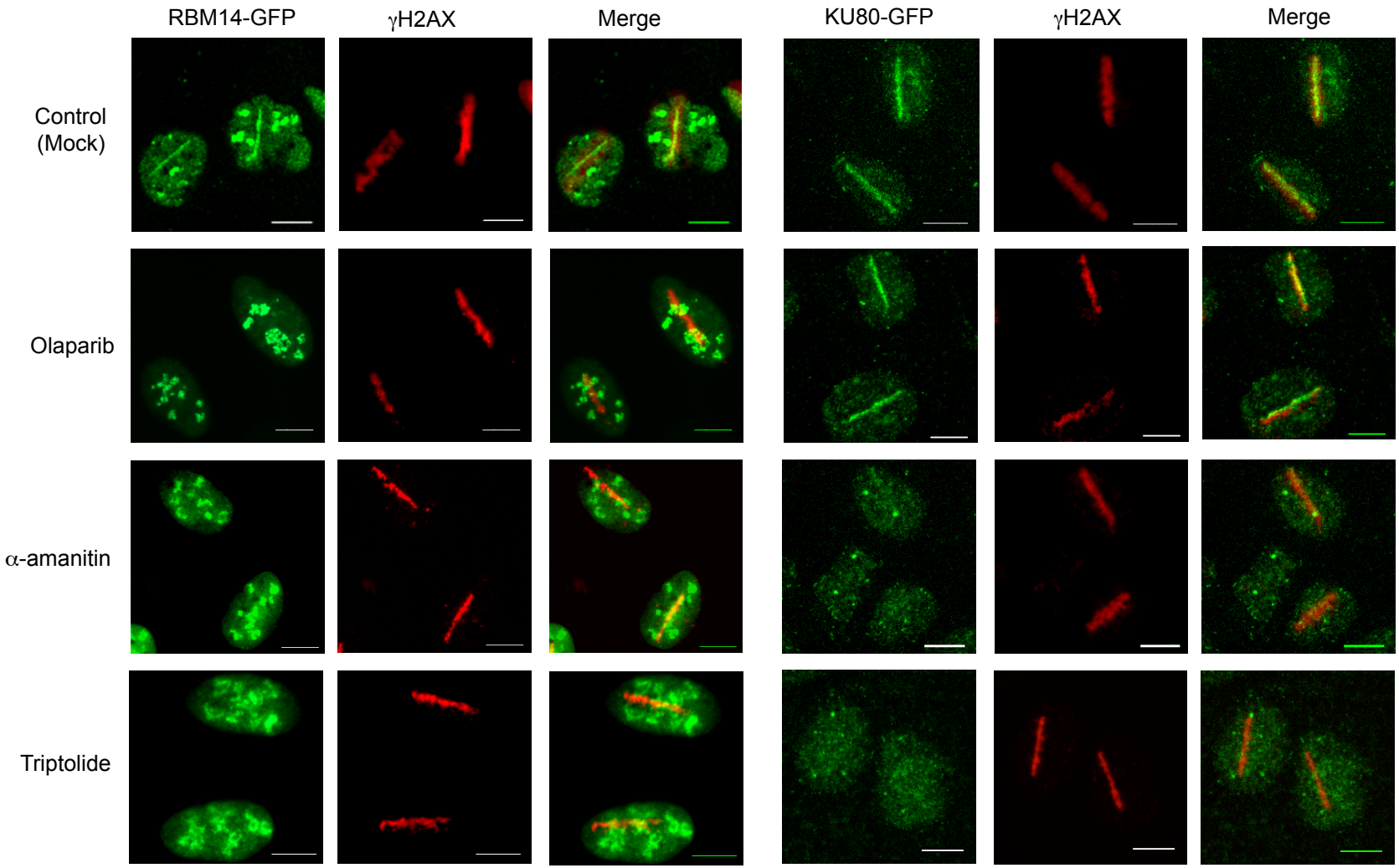


Figure S4. RBM14-GFP and KU80-GFP are recruited to microlaser-induced DNA damaged sites in RNAPII-dependent manner, related to Figures. 1 & 2. RBM14-GFP or KU80-GFP expressing U2OS cells were microlaser-irradiated, and stained for the DSB marker γH2AX. Scale bar represents 10 μm.

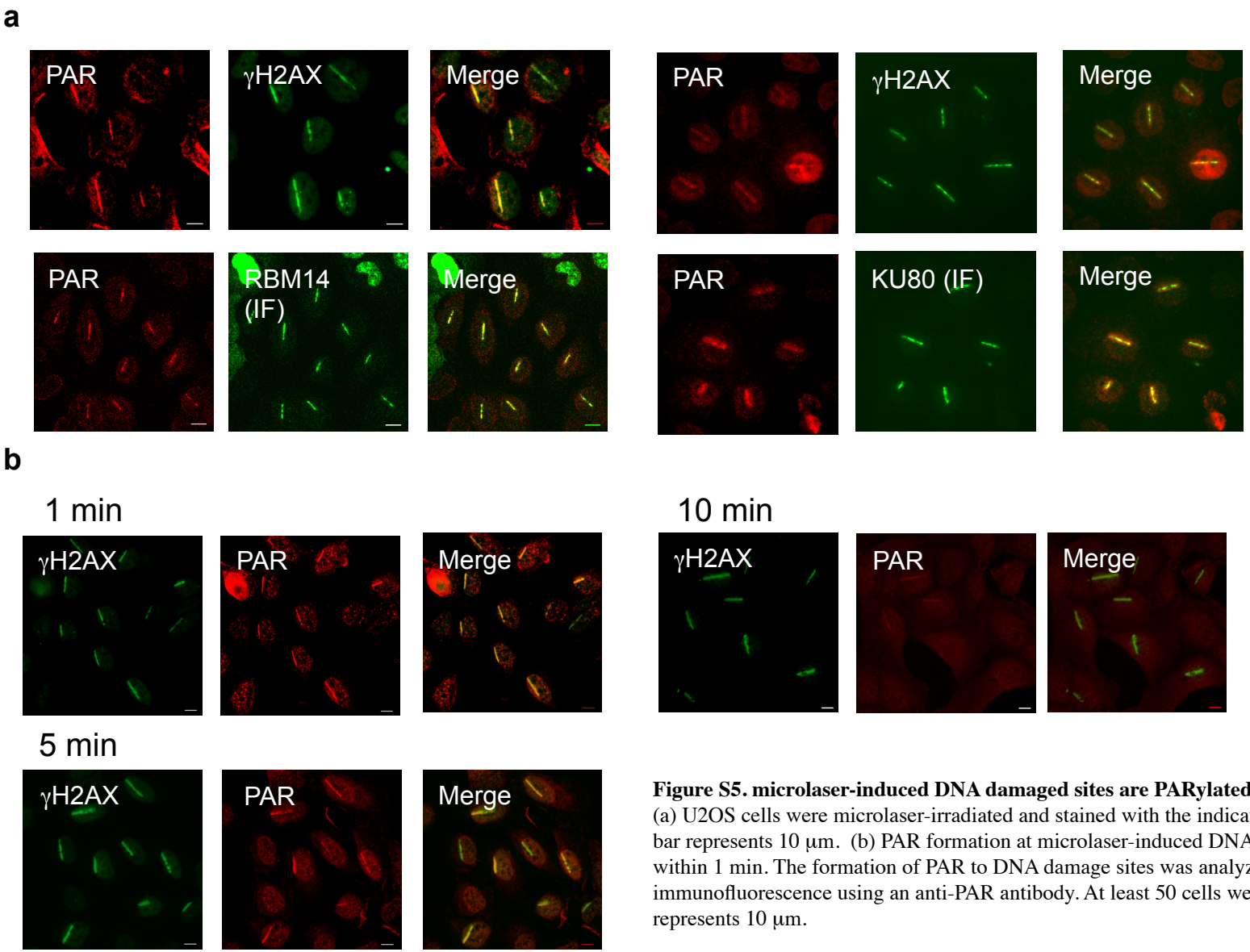


Figure S5. microlaser-induced DNA damaged sites are PARylated, related to Figure 1. (a) U2OS cells were microlaser-irradiated and stained with the indicated antibodies. Scale bar represents 10 μm. (b) PAR formation at microlaser-induced DNA damage sites occur within 1 min. The formation of PAR to DNA damage sites was analyzed by immunofluorescence using an anti-PAR antibody. At least 50 cells were tested. Scale bar represents 10 μm.

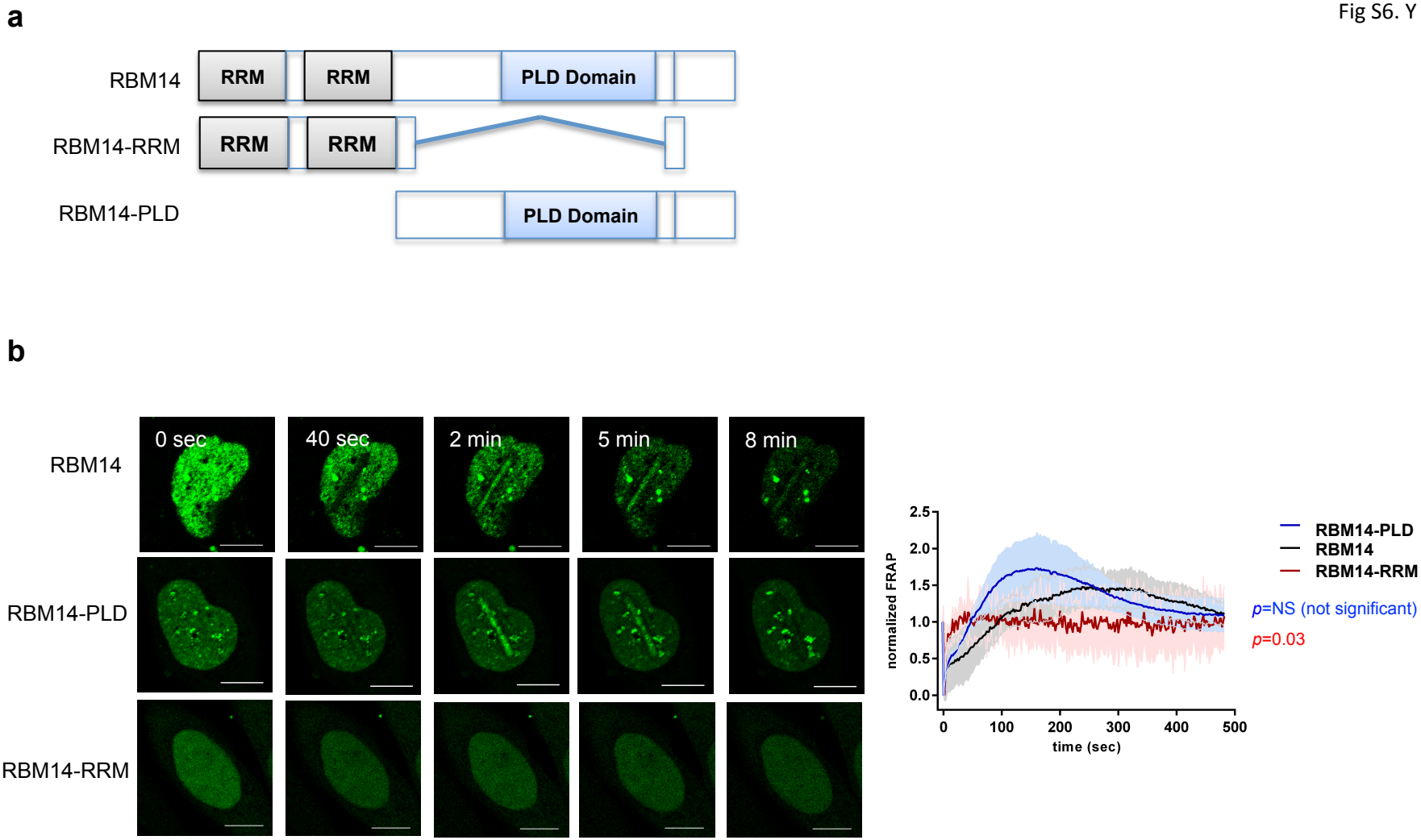
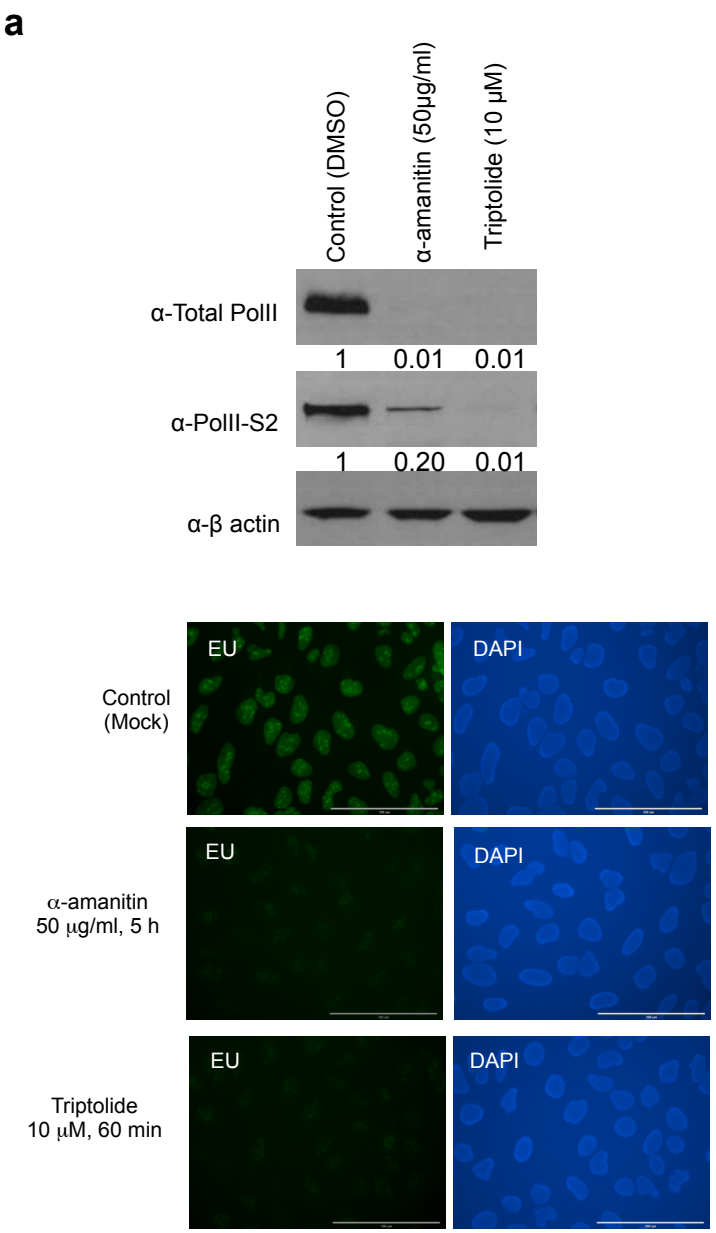


Figure S6. Prion-like domain (PLD) region of RBM14 is required for its recruitment to DNA damage sites, related to Figure 1. (a) Structure of RBM14 protein, which contains RNA recognition motif (RRM) and an unstructured domain called the prion-like domain (PLD). (b) Recruitment of GFP-tagged PLD or RRM of RBM14 to the sites of laser micro-irradiation. Quantification of the result is shown (right). Confocal images were recorded with a frame size of 512 x 512 pixels and a pixel dwell time 2.2 μs. Live cell imaging data from micro-irradiation of individual cells obtained in several independent experiments performed on different days were averaged, analyzed and displayed using Image J software. The colored shade indicates error bars. The p-values are at the 300 second time point. At least 50 cells were tested for each experiment. The Scale bar represents 10 μm.



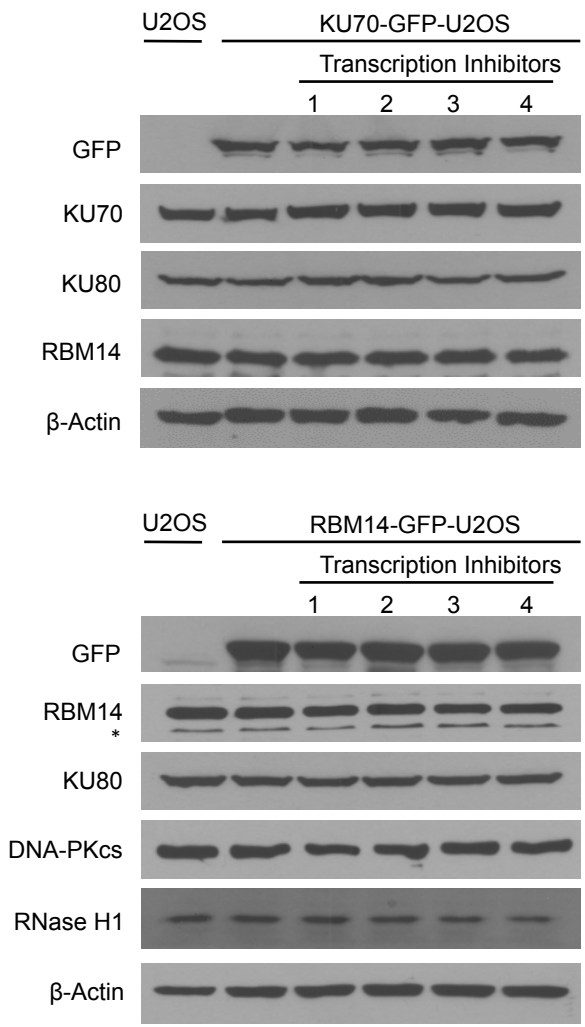
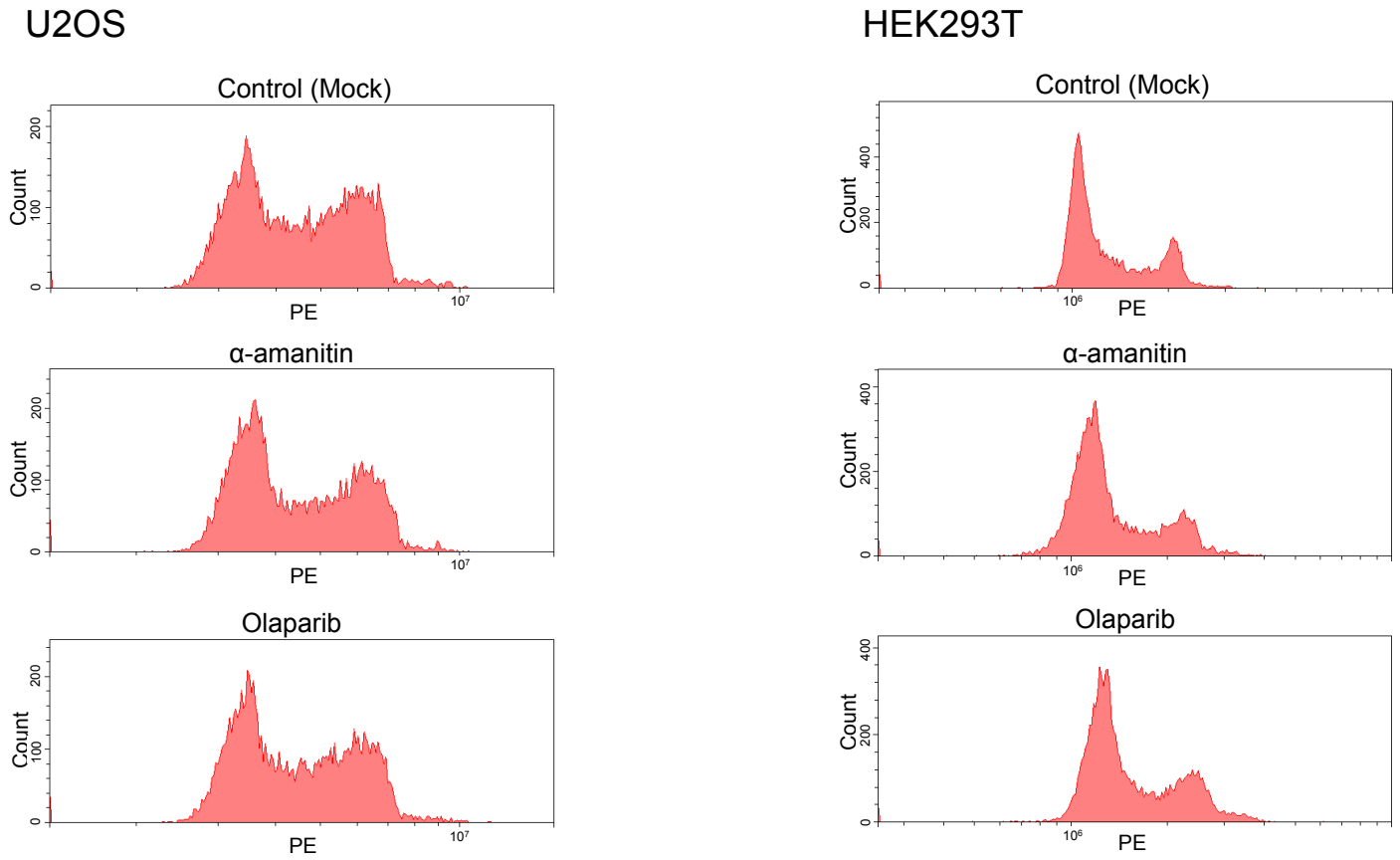


Figure S7. Effects of transcription inhibitors, related to Figures 2. (a) Human U2OS cells were treated with α-amanitin (50 μg/ml, 5 h), triptolide (10 μM, 4 h), and the effects on RNAPII (total, phospho S2) were detected by western blot (top panel). The transcription inhibitors inhibited EU incorporation in cells (bottom panel). The expression levels of β-actin were used as a loading control. (b) The protein levels of RBM14 and KU are not affected by transcription inhibition. 1. α-Amanitin 50 μg/ml for 5 hours, 2. DRB 100 μM for 2 hours, 3. Actinomycin D 2 μM for 40 min, 4. Triptolide 10 μM for 4 hours.



Cell population	% of Gated cells		
	control	α-amanitin	olaparib
G0/G1	45.46	46.65	44.14
S	16.07	17.21	17.29
G2/M	34.84	33.53	35.26

Cell population	% of Gated cells		
	control	α-amanitin	olaparib
G0/G1	63.43	63.97	61.32
S	15.18	12.6	10.82
G2/M	17.45	17.25	21.46

Figure S8. The cell cycle status upon transcription inhibition and olaparib treatment, related to Figure 1 & 2. FACS analyses were performed to examine the status of U2OS (left) and HEK293T (right) after α-amanitin (50 μg/ml, 5 h) and olaparib (10μM, 1 hour) treatment.

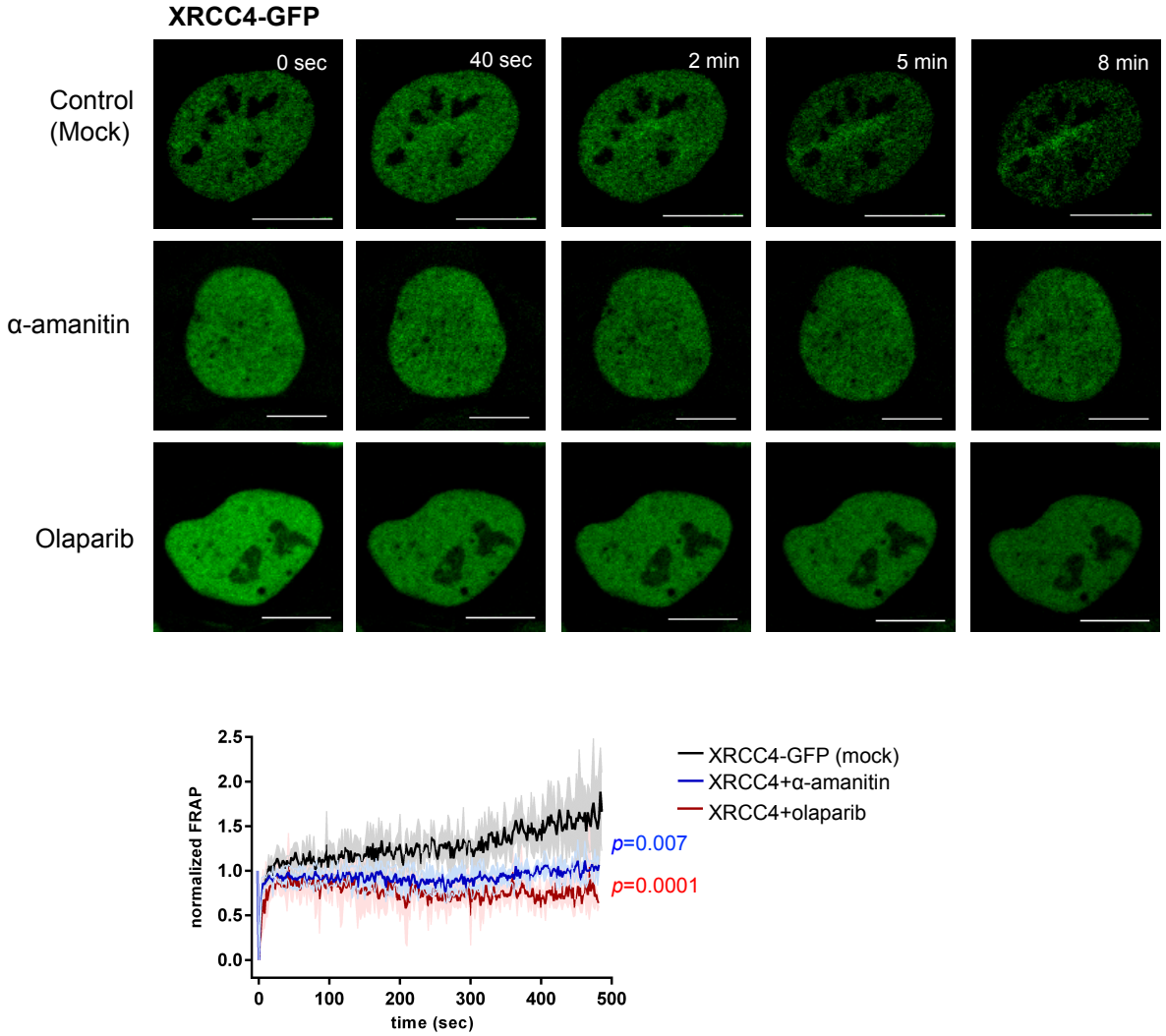


Figure S9, related to Figure 1 &2. XRCC4 recruitment to DNA damage sites is inhibited by transcription inhibition and PARP inhibition. The effects of transcription and PARP inhibition on the recruitment of XRCC4 were analyzed by laser micro-irradiation. Representative images of cells expressing GFP-XRCC4 with either α-amanitin or olaparib after laser micro-irradiation were shown (to panel). Quantification of the recruitment is shown (bottom panel). Confocal images were recorded with a frame size of 512 x 512 pixels and a pixel dwell time 2.2 μs. Live cell imaging data from micro-irradiation of individual cells obtained in several independent experiments performed on different days were averaged, analyzed and displayed using Image J software. The colored shade indicates error bars. The p-values are at the 300 second time point. At least 50 cells were tested for each experiment. The Scale bar represents 10 μm.

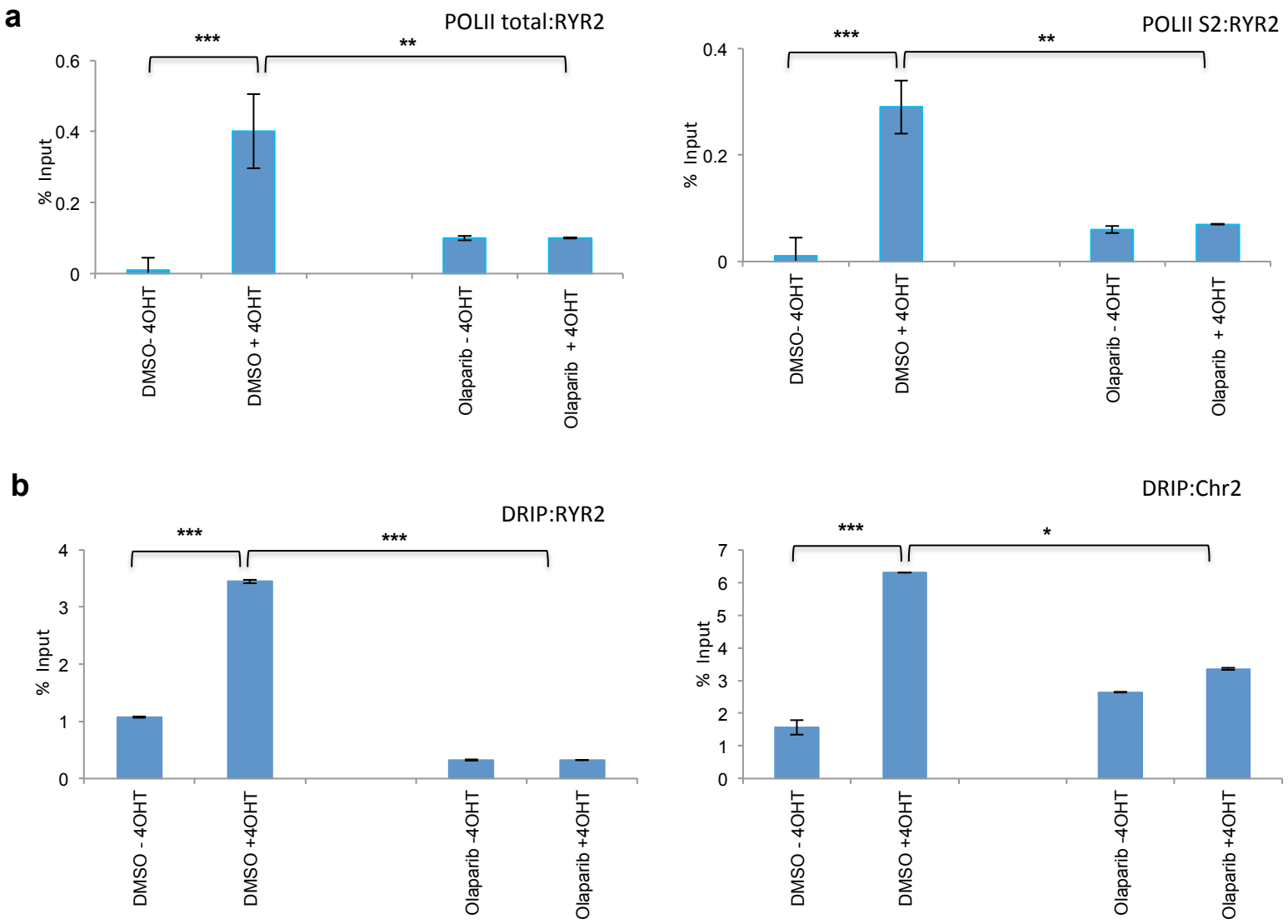


Figure S10. RNAPII is recruited to I-PpoI sites in a PARP-dependent manner, related to Figure 3 & 4. (a) ChIP-qRT-PCR near RYR2 with anti-RNAPII (total) and RNAPII-S2 antibodies before (-4OHT) and after (+4OHT) DSB induction with and without olaparib treatement. (b) DRIP-qRT-PCR near RYR2 and intergenic Chr2 sites with and without olaparib treatment. Data shown are representative of three independent experiments. Each experiment was performed in triplicate. *p<0.05, **p<0.01, ***p<0.001 by two-tailed t-test.

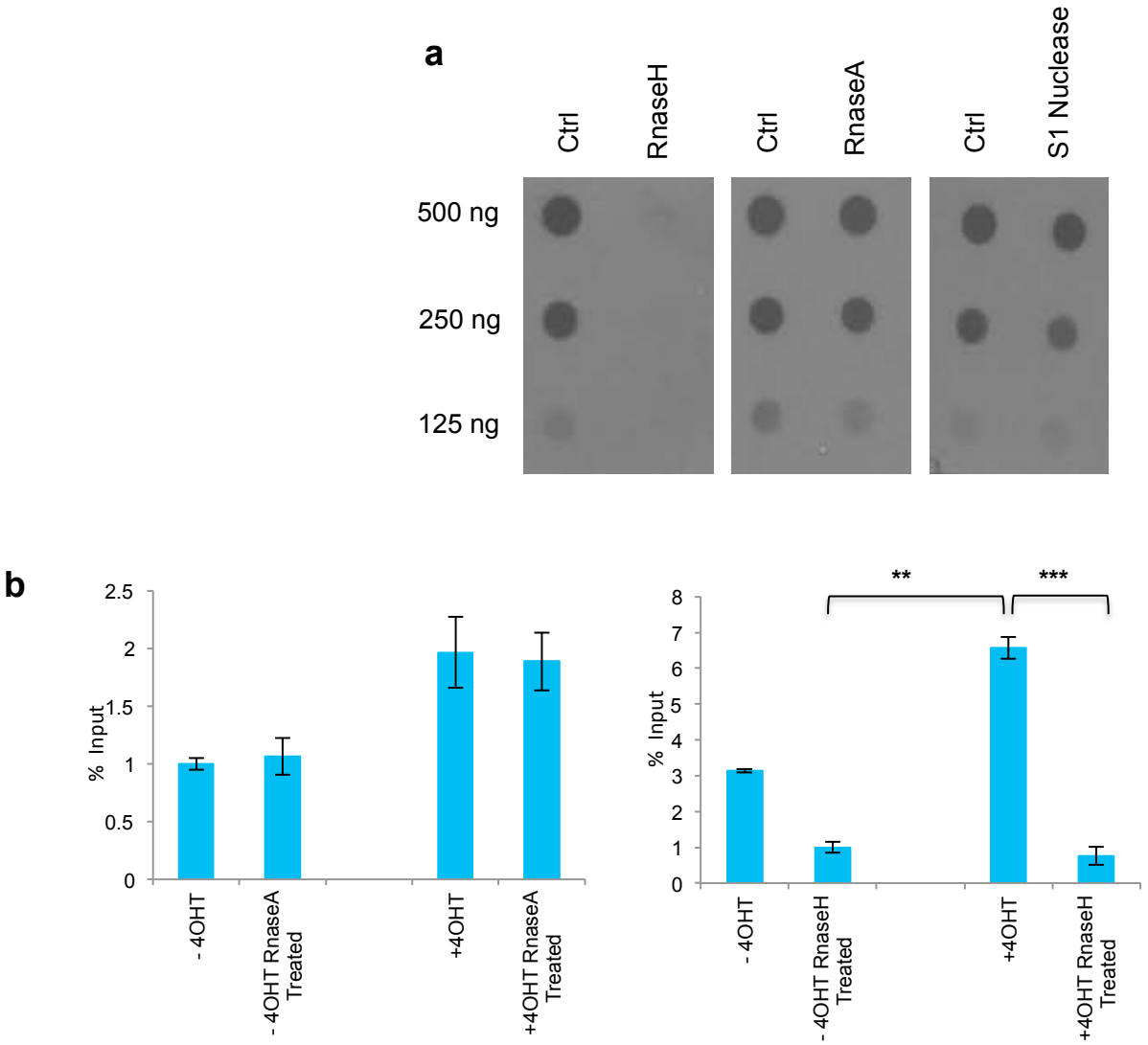


Figure S11. Verification of anti-S9.6 antibody, related to Figures 3 & 4. (a) Genomic DNA/RNA samples were treated with RNaseH1, RNaseA, and S1 nuclease prior to dot-blotting with anti-S9.6 antibody. (b) DRIP-qRT-PCR. The cell extracts were treated with RNaseA (left), and with RNaseH (right) prior to anti-S9.6 antibody pull down. **p<0.01, ***p<0.001 by two-tailed t-test.

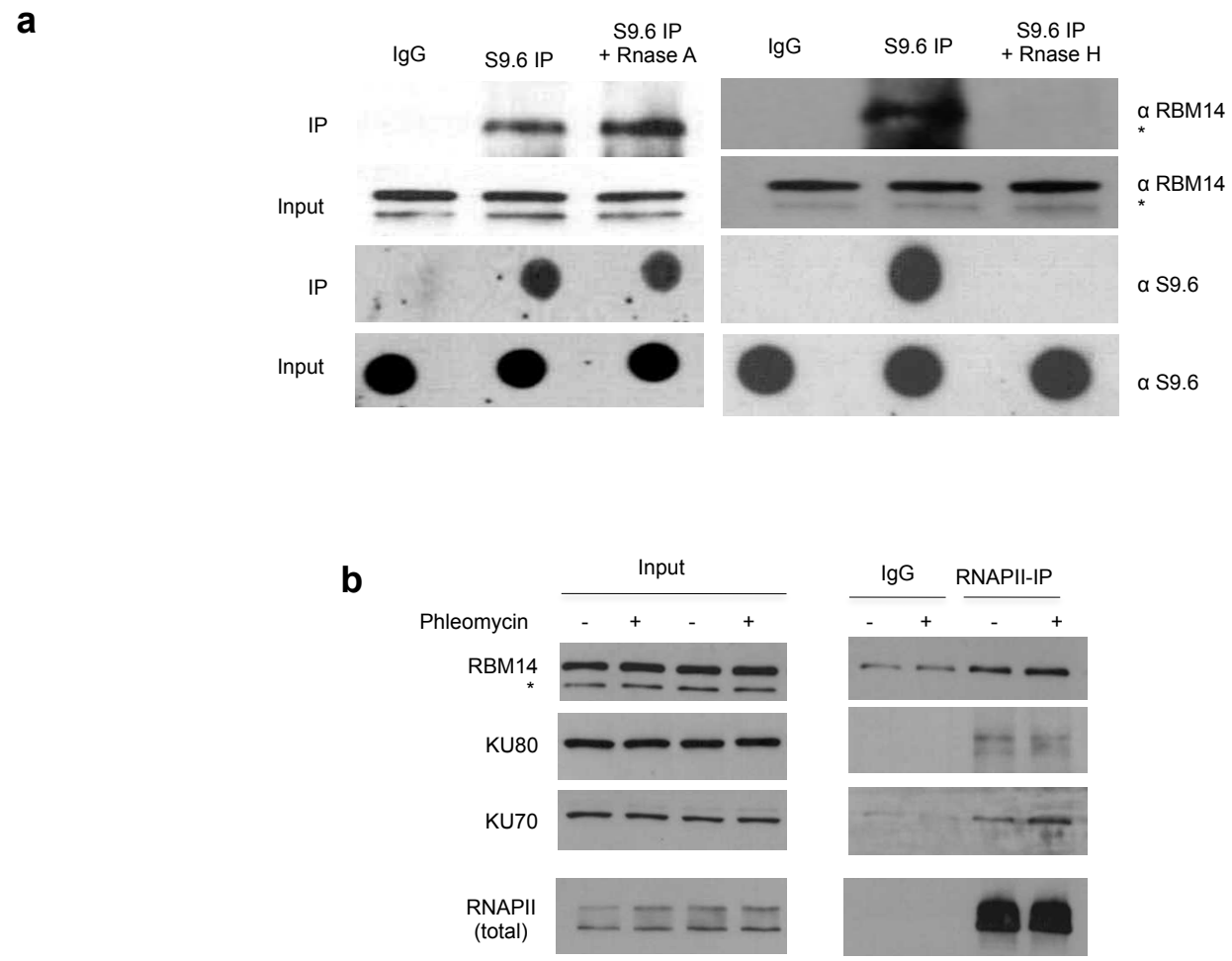


Figure S12. RBM14 interacts with RNAPII and RNA:DNA hybrids, related to Figures 3 & 4. (a) RNA:DNA hybrids were immunoprecipitated, and western-blotting was performed with anti-RBM14 and anti-S9.6. (b) Immunoprecipitation analysis in DNA damage-induced human HEK293T cells. The RNAPII protein was immunoprecipitated with anti-RNAP II (total) after either mock or phleomycin treatment, and RBM14 and KU80 were detected by western-blotting with anti-RBM14, anti-KU80 and anti-KU70 antibodies.

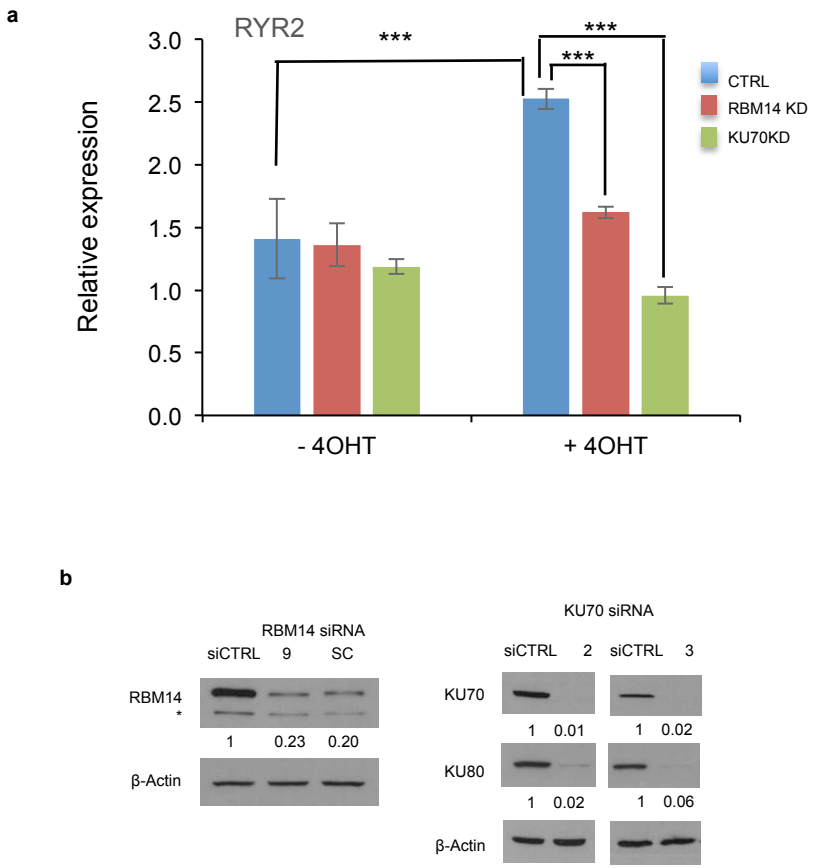


Figure S13. Nascent RNA is expressed at DNA damage sites in a PARP, transcription, RBM14, and KU dependent manner, related to Figure 4 (a) qRT-PCR analyses of nascent RNA expression at the RYR2 site. Cells were transfected with I-PpoI and RBM14 &KU80 siRNA. Cells were collected, RNA was extracted, and qRT-PCR was performed at Chr 2 site. Nascent RNA was quantified using ΔΔ Ct method. Cyclophilin B was used as a reference gene to calculate the Δ Ct value for each sample then each time point was normalized to 0 h time point using ΔΔ Ct formula. To calculate the fold change in gene expression we took the 2 to the power of negative ΔΔ Ct. Data shown are representative of three independent experiments. Each experiment was performed in triplicate (b) Knockdown levels of RBM14 and KU70 are shown. The values indicated under the blot are the mean fold protein expression relative to control taken as 1 after normalization by β-actin (Image J quantification). siCTRL: control siRNA. * non-specific band.

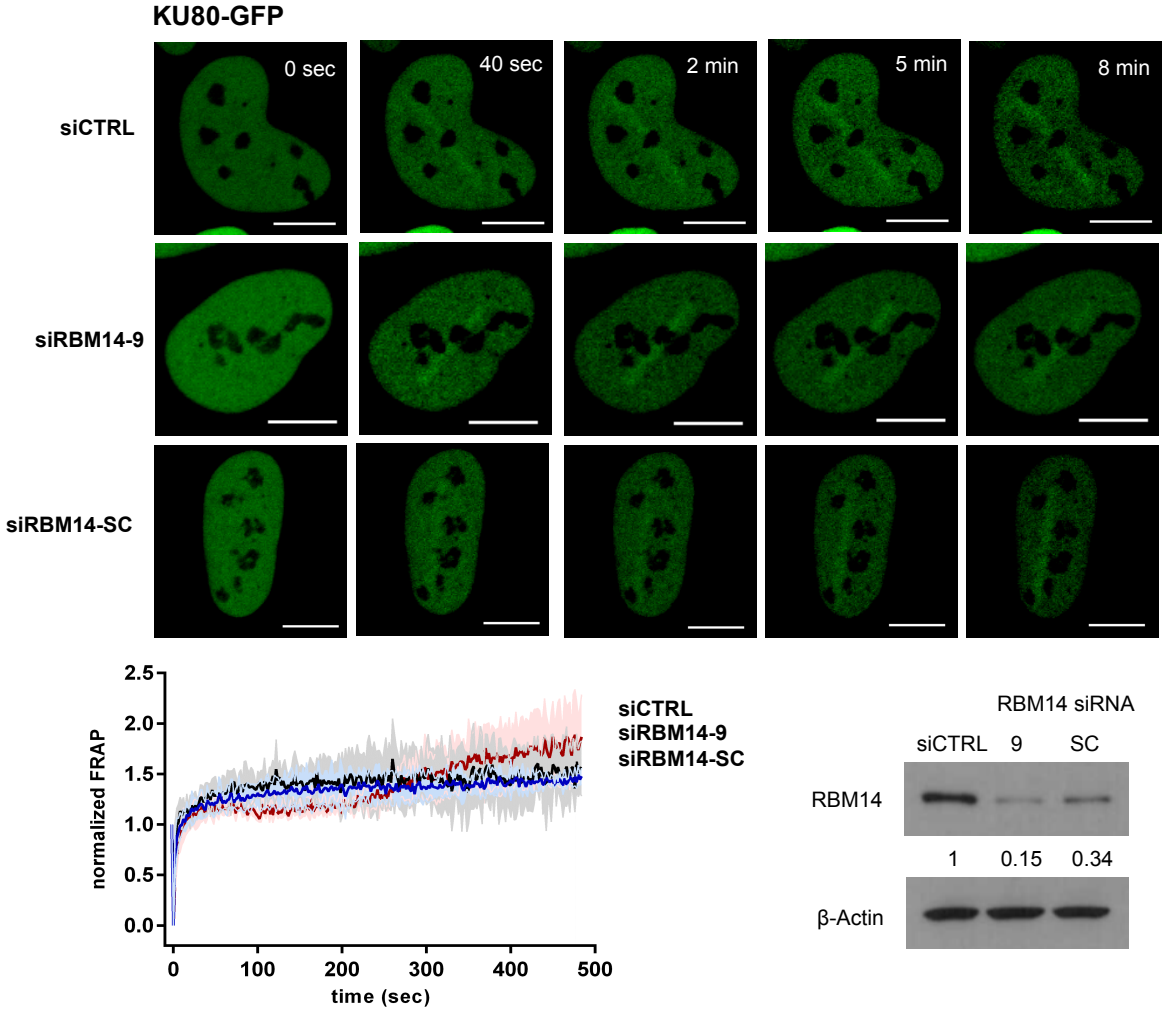


Figure S14, related to Figure 5. KU80 recruitment to DNA damage sites does not require RBM14. The effects of RBM14 knockdown on the recruitment of KU80 were analyzed by laser micro-irradiation. Representative images of cells expressing GFP-KU80 with either negative control siRNA- or RBM14 siRNAs-transfection after laser micro-irradiation were shown (to panel). Quantification of the recruitment is shown (bottom left). Confocal images were recorded with a frame size of 512 x 512 pixels and a pixel dwell time 2.2 μs. Live cell imaging data from micro-irradiation of individual cells obtained in several independent experiments performed on different days were averaged, analyzed and displayed using Image J software. The colored shade indicates error bars. The p-values are at 300 second time point. Scale bar represents 10 μm.
The knockdown levels of RBM14 were confirmed by western blot (bottom right). At least 50 cells were tested for each experiment. Scale bar represents 10 μm.

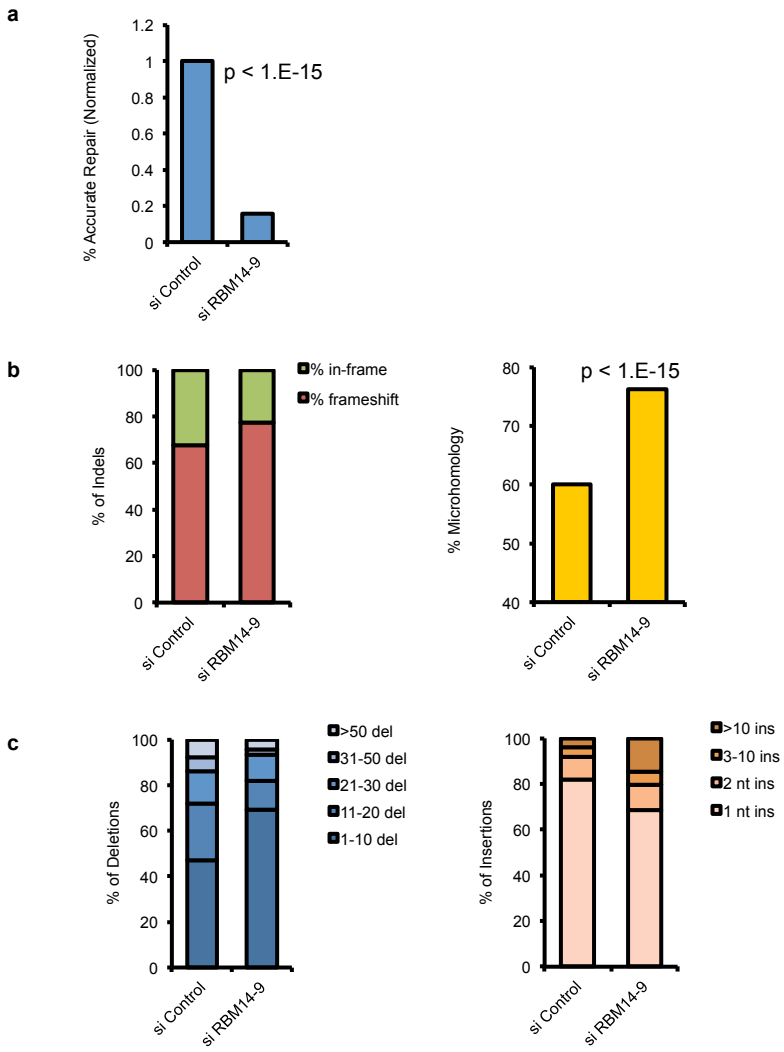


Figure S15, related to Figure 6 . RBM14 knockdown impairs the fidelity of DSB repair via the cNHEJ pathway and increases microhomology utilization in HEK293T cells. (a) Histogram depicting the fraction of sequence reads lacking indels and mapping to the DSB repair sites within the Cas9-targeted reporter in control and RBM14 knockdown cells. (b) Histogram showing the percentage of sequence reads with frame-shift mutations in control and RBM14-knockdown cells (left). Histograms depicting the frequency of microhomology flanking the deletions at the repair junctions within the DSB-integrated reporter in control and RBM14-knockdown cells (right). (c) Graph depicting the distribution of deletion sizes (left) and insertions of various sizes (right) within the repair junctions in control and RBM14-knockdown cells (left).

Supplemental Methods

Laser microirradiation

U2OS cells were plated on 35-mm glass-bottom culture dishes (MatTek) in phenol red-free medium and pre-sensitized with 10 μM BrdU (Invitrogen) for 24 h. Nikon A1+ confocal laser microscope equipped with a heating chamber set at 37°C in 5% CO_2 atmosphere was used for all micro-irradiation experiments. Nuclei micro-irradiation was performed at 405 nm laser at 10% power for 1 sec (BrdU), and confocal images were recorded with a frame size of 512 x 512 pixels and a pixel dwell time 2.2 μs. For the quantitative evaluation of the fluorescence recovery, the fluorescence signal measures in a region of interest was normalized to the change in the background intensity, FRAP raw intensity, and reference intensity. Data from micro-irradiation of individual cells obtained in at least four independent experiments performed on different days were averaged, analyzed and displayed using PRISM software.

Inducible DSB system

Induction of DSBs by the I-PpoI nuclease was performed as described in (1). Briefly, cells were transiently transfected or infected with DD-HA-ER-I-PpoI from addgene (plasmid no. 49052). To stabilize the expression of the dd I-PpoI fusion protein, cells were treated with 1 μM. Shield-1 (Cheminpharma cat. no. AS1-0001) for 3 hrs followed by 2 μM 4OHT (Sigma cat. no. H7904) treatment to translocate the I-PpoI to the nucleus. The transcription inhibitor α-amanitin (Sigma cat. no.A2263) was used at 50 μg /ml for 5 hrs.

Chromatin immunoprecipitation & ChIP-seq

For ChIP, cells were counted and cross-linked for 10 min in 1% (v/v) paraformaldehyde (Sigma, cat. no. F1635), washed three times with 1X PBS and then incubated in Cell lysis buffer I (10 mM HEPES (pH 6.5), 10 mM EDTA, 0.5 mM EGTA and 0.25% Triton X-100) on ice for 10 minutes then centrifuged at 16,000*g* for 5 min at 4˚C. Pellet was incubated for another 10 minutes at 4 ˚C in Cell lysis buffer II (10 mM HEPES (pH 6.5), 1 mM EDTA, 0.5 mM EGTA and 200 mM NaCl) then centrifuged at 1,700*g* for 5 min at 4 C. The nuclear pellet was resuspended in nuclei lysis buffer (50 mM Tris-Cl (pH 8.1), 10 mM EDTA and 0.5% SDS) sonicated to fragment the chromatin to 100-700bp. After sonication, lysed chromatin was centrifuged at 16,000*g* for 15 min at 4˚C, then samples were diluted 1:5 with IP dilution buffer (1% Triton X-100, 2 mM EDTA, 20 mM Tris-Cl (pH 8.1) and 150 mM NaCl). Immunoprecipitation was done using 5 μg antibody and 50 μl of appropriate dynabeads at 4˚C overnight. The beads were washed one time for 5 min with low-salt buffer (20 mM Tris-HCl, pH 8, 150 mM NaCl, 2 mM EDTA, and 1% (v/v) Triton X-100), once for 5 min with high-salt buffer (20 mM Tris-HCl, pH 8, 500 mM NaCl, 2 mM EDTA, and 1% (v/v) Triton X-100) and once for 5 min with LiCl buffer (10 mM Tris-HCl, pH 8, 250 mM LiCl, 1 mM EDTA, 1% (v/v) NP-40 and 1% (w/v) sodium deoxycholate) and two times for 5 min each with TE buffer (10 mM Tris-Cl (pH 8) and 1 mM EDTA). The precipitated protein –DNA complex was eluted in 0.1 M $NaHCO_3$ in 1% SDS at 65˚C for 1 hr, and cross-linking was reversed by adding 20 µl of 5 M NaCl and incubation at 65˚C for 6 h to overnight. The DNA was phenol-chloroform extracted after proteinase K and RNaseA treatment and was resuspended in 50 µl of TE buffer. The signal in each experiment represents the DNA concentration

relative to the standard curve of the input. The antibodies and primers used are described in SI Appendix, Table S2. The antibodies and primers used are described in SI Appendix, Table S2. Libraries were constructed according to the protocol provided by Illumina's TruSeq Nano DNA Sample Preparation Kit, beginning with end repair and adenlyation of blunt end fragments. Adaptor ligation with indexing adaptors was followed by PCR amplification for 15 cycles and assessment of the yield and size distribution on the Agilent 2100 Bioanalyzer using the High Sensitivity Chip. Quantitation of the libraries was performed by qPCR using the KAPA SYBER FAST qPCR Kit supplied by Kapa Biosystems. Multiplex libraries were pooled to a final concentration of 2nM for paired end sequencing on an Illumina HiSeq 2500. Illumina's CASAVA 1.8.2 was used to convert BCL files to FASTQ files, and bowtie 2.2.1 was used to align reads to the hg38 reference, plus a custom rDNA sequence. MACS 2.1.1 was used to call peaks.

Quantitative PCR and quantitative RT-PCR analysis

Total RNA was purified from HEK293T cells expressing I-PpoI using the RNeasy total RNA purification kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized with the RT-PCR kit (Qiagen) according to the manufacturer's instructions. To isolate premature transcripts, RNA was isolated using Trizol (Thermofisher cat. no. 15596026) according to the manufacturer's instructions. 5-µg of Total and premature RNA were treated with DNase I (Ambion cat. no. AM2222) for 5 h and extracted with phenol-chloroform. 500 ng of treated RNA were reverse transcriped using the first-strand cDNA synthesis kit (invitrogen) using oligo-dt for mRNA and random hexamers for premature RNA. Expression was determined by RT-

qPCR using using SsoAdvanced universal SYBR green supermix (Biorad) according to the manufacturer's instructions. Transcripts enrichment was determined by the ΔΔCt method after normalization to the control gene U6 snRNA unless indicated.

DNA-RNA hybrid Co-immunoprecipitation (CoIP)

Native chromatin was isolated according to (1). Cells were lysed in lysis buffer (50-mM Hepes pH 7.5, 150-mM NaCl, 1-mM EDTA, and 0.05% NP-40) on ice for 5 minutes then centrifuged for 5 min at 1000 g. The supernatant was removed and the pellet was resuspended in the same buffer and centrifuged as above. The supernatant was removed and the pellet was resuspended in 0.5% NP-40 lyses buffer, and incubated on ice for 45 minutes, then centrifuged at 16,000 g for 15 minutes. The supernatant was removed and the pellet (chromatin fraction) was resuspended in TGN lyses buffer containing 1% Triton, sonicated, then centrifuged at 16,000 g for 10 minutes. Chromatin was incubated overnight at 4˚C with S9.6 antibody or mouse IgG. Immunoprecipitation was performed using Protein A Dynabeads for 4 hours at 4 ˚C then the co-IP proteins were eluted at 95˚C for 5 minutes then analyzed by western blot.

RNA-seq

Nuclear RNA was extracted using TRI reagent and recovered by phenol-chloroform extraction and isopropanol precipitation. After DNaseI treatment, both quality and size of RNA was assessed by Agilent Bioanayzer. 200-250 ng of RNA were used for ribosomal RNA depletion and construction of the whole transcription library was done according to the Illumina's Trueseq standard Total RNA Sample Preparation Guide. Sequencing was done using Illumina NextSeq 500 (paired-end, 150 bp) at the JHU core facility. Paired end reads were mapped to the reference genome (human build hg38) and expression

levels of all known gene isoforms annotated in RefSeq, Ensembl, and UCSC gene annotations were derived using the STAR aligner (PMID: 23104886) along with the RSEM 1.3 package (reference PMID: 21816040), which uses an expectation maximization algorithm to derive the abundance of each gene isoform after taking into account the read mapping uncertainty with a statistical model. Differential expression between groups was determined using the RSEM output with the EBSeq software (PMID: 23428641).

Protein Coimmunoprecipitation

HEK-293T cells were washed with PBS and lysed on ice in TGN buffer [50 mM Tris (pH 7.5), 200 mM NaCl, 50 mM β-glycerophosphate, 1% Tween-20 , 0.2% NP-40, protease inhibitors], DNase I (100 unites/ml), RNaseA (10μg/ml) for 1 hr at 4 ˚C. Samples were centrifuged at 16,000 g for 15 minutes, and incubated with the indicated antibodies or with normal rabbit/ mouse IgG and Protein A/ Protein G agarose beads overnight at 4 ˚C. Beads were recovered and washed extensively with TGN buffer, resuspended in 50 µL of sample loading buffer, and analyzed by immunoblot. The used antibodies are described in SI Appendix, Table S2.

DNA-RNA Hybrid Immunoprecipitation (DRIP)-seq

For DRIP, HEK-293T cells were fixed with 1% paraformaldehyde for 10 min, then quenched with 500 mM glycine for 5 min at room temperature. Cells were lysed in suitable volume of 2× lysis buffer (1% SDS, 20 mM Tris-HCl pH 7.5, 40 mM EDTA pH 8, 100 mM NaCl, ddH_2O) , and diluted with TE buffer (100 mM Tris-HCl pH 8, 10 mM EDTA pH 8) to 1X SDS lysis buffer. Cell lysis was performed at 37°C overnight, then lysate was sonicated to get a 500 bp fragmented DNA. After sonication, the whole cell

nucleic acids were extracted by phrenol-choloroform extraction. DNA-RNA hybrid was immunoprecipitated with S9.6 antibody that is prebound to Dynabeads Protein A (Thermo Fisher Scientific) in IP buffer (50 mM Hepes/KOH at pH 7.5; 0.14 M NaCl; 5 mM EDTA; 1% Triton X100; 0.1% Na-Deoxycholate, ddH_2O) at 4°C for 4 h with rotation. For IP, 2 μg of S9.6 antibody were used per μg of DNA, and the mixture rotated at 4°C overnight. Beads were washed successively with 1 mL low salt buffer (50 mM Hepes/ KOH pH 7.5, 0.14 M NaCl, 5 mM EDTA pH 8, 1% Triton X-100, 0.1% Na-Deoxycholate), 1 ml high salt buffer (50 mM Hepes/KOH pH 7.5, 0.5 M NaCl, 5 mM EDTA pH 8, 1% Triton X100, 0.1% Na-Deoxycholate), 1 mL LiCL buffer (10 mM Tris-HCl pH 8, 0.25 M LiCl, 0.5% NP-40, 0.5% Na-Deoxycholate, 1 mM EDTA pH 8), and 1 ml TE (100 mM Tris-HCl pH 8, 10 mM EDTA pH 8) at 4 μC, two times. Elution was performed in 500 μL of elution buffer (1% SDS, 0.1M $NaHCO_3$) for 1 h at 65°C, and the eluted DNA was purified by phenol-cholorform extraction and dissolved in 25 μL of elution buffer (5 mM Tris-HCl pH 8.5). Next-generation genome-wide sequencing of the DRIP samples were performed as described in ChIP-seq section above. The DNA was then analyzed by quantitative real-time PCR (qPCR), and the DNA-RNA hybrid enrichment represents the IP/Input ratio after normalization to the SNRPN negative control site.

S9.6 Dot blot

Similar to the DRIP, HEK-293T cells were fixed, lysed, sonicated and isolated DNA was treated with 1 μl of RNase A (10 mg/ml; Thermofisher cat. no. EN0531) in high salt concentration (500 mM Nacl) at 37 ˚C for 1hr. RNase H (5000 U/ml; NEB) treatment was done using 8 μl at 37 ˚C overnight. For S1 nuclease digestion, we used 1U/μg of

DNA in 1x S1 buffer at room temperature for 30 minutes. Samples were blotted on nitrocellulose membrane (Biorad) in triplicates and the membrane was cross-linked using UV. The membrane was blocked with 5% milk in 1× PBS/0.1% Tween-20 for 30 minutes then incubated with S9.6 primary antibody (1 μg/ml) at 4 °C overnight. Then the membrane was washed and incubated with the secondary antibody (goat anti-mouse HRP from thermofisher), and the signal was developed using the Clarity Western ECL Substrate (Bio-Rad) and autoradiography film.

DSB repair assays

DSB repair assays were performed in 293T cells with a single copy of an integrated DSB repair reporter that constitutively express mCherry from and EF1α-driven promoter (2). Following transfection with Cas9 and sgRNA targeting the reporter, repair via mutagenic NHEJ results in the loss of mCherry expression, which is quantified by flow cytometry (FACS) analyses. Cas9-mediated DSBs targeting the indicated genes using RNAiMAX according to the manufacture's protocol (Thermofisher). Parallel samples were prepared at that time points for immunoblotting with the relevant targeted proteins. Cells were propagated in culture for one week following the expression of Cas9/sg-RNAs prior to FACS analyses.

Cell cycle analysis

Cells were plated on 60-mm culture dishes and allowed to adhere overnight. The cells were treated with α-amanitin (50 μg/ml, 5 h) or olaparib (10 μM, 1 h) and harvested, followed by fixation with cold 70% ethanol for overnight at 4 °C. After washing in PBS twice, cells were resuspended in PBS with 1 μL of RNaseA (10 mg/mL) for 30 min at room temperature, then added 2 uL of propidium iodide (5 mg/mL) and incubated in the

dark for 30 min. Cell cycle analyses were performed by flow cytometry using the Beckman Coulter CytoFlex system.

1. Pankotai T, Bonhomme C, Chen D, & Soutoglou E (2012) DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks. *Nat Struct Mol Biol* 19(3):276-282.
2. Yang CS, *et al.* (2017) Ubiquitin Modification by the E3 Ligase/ADP-Ribosyltransferase Dtx3L/Parp9. *Mol Cell* 66(4):503-516 e505.

ChIP-QPCR Primers

RYR2 -0.3Kb For	TGAAGAAGGGGATTGAGCAT
RYR2 -0.3Kb Rev	TCAAATTATTGACACCTCATTGCT
ERC2 -0.3kb For	TGGAGGAAGGGAAACATCC
ERC2 -0.3kb Rev	TGTTCCTGTGATTAGGTCTCCA
RYR -1Kb For	ATGTTCCCCAAGCAGTGTTC
RYR -1Kb Rev	CAGAAGGAGCCCAGAGATTTT
Ch2 -1Kb For	CTGCTGTATCCTTCCGCCT
Ch2 -1Kb Rev	GACATGTGCTGGTTGAGGA
ChX -1Kb For	TCTTCCCTCCTTCACCAAGC
ChX -1Kb Rev	GCAGCAACATGGATTTAACTGG
APOE For	CCGGTGAGAAGCGCAGTCGG
APOE Rev	CCCAAGCCCGACCCCGAGTA
RPL13AF	GCTTCCAGCACAGGACAGGTAT
RPL13AR	CACCCACTACCCGAGTTCAAG
EGR1F	TTCGGATTCCCGCAGTGT
EGR1R	TCACTTTCCCCCCTTTATCCA
BTBD19F	CCCCAAAGGGTGGTGACTT
BTBD19R	TTCACATTACCCAGACCAGACTGT
SNRPNF	TGCCAGGAAGCCAAATGAGT
SNRPNR	TCCCTCTTGGCAACATCCA

RT-PCR primers

RYR2 For	AAGCCCTCTCGTCTGAAACA
RYR2 Rev	CCACCCAGACATTAGCAGGT
ERC2 For	TTTCAAATATTCATGTGTTTCC
ERC2 Rev	GAGCTGAGAGCTTGCCACTT
Ch2 For	CTGCTGTATCCTTCCGCCT
Ch2 Rev	GACATGTGCTGGTTGAGGA
U6 For	CGCTTCACGAATTTGCGTGTCA
U6 Rev	GCTTCGGCAGCACATATACTAAAAT

Used Antibodies	**Cat. No.**	**Application**
Anti-RNA polymerase II CTD repeat YSPTSPS	ab817	ChIP, IP, and WB
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5)	ab5408	ChIP, IP, and WB
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2)	ab5095	ChIP
Anti-Pol II S2p Antibody (Monoclonal)	CI1134	IP, and WB
Anti-PAR antibody	ALX-804-220- R100	IF
Anti-DNA-RNA Hybrid [S9.6] Antibody, 100ug	MABE1095	DRIP, and Co-IP
Anti-RBM14 antibody 100 uL	ab70636	ChIP, and WB
Anti-Ku80 Antibody, A302-627A	A302-627A	ChIP
Anti-phospho-Histone H2A.X (Ser139) Antibody	05-636	ChIP

NGS Primers

Primer Name	Sequence (5'-3')
R1-Fwd-1	CTTTCCCTACACGACGCTCTTCCGATCTtCTACCCCAGCGGCTACGAGAA
R1-Fwd-2	CTTTCCCTACACGACGCTCTTCCGATCTatCTACCCCAGCGGCTACGAGAA
R1-Fwd-3	CTTTCCCTACACGACGCTCTTCCGATCTgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-4	CTTTCCCTACACGACGCTCTTCCGATCTcgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-5	CTTTCCCTACACGACGCTCTTCCGATCTtcgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-6	CTTTCCCTACACGACGCTCTTCCGATCTatcgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-7	CTTTCCCTACACGACGCTCTTCCGATCTgatcgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-8	CTTTCCCTACACGACGCTCTTCCGATCTcgatcgatCTACCCCAGCGGCTACGAGAA
R1-Fwd-9	CTTTCCCTACACGACGCTCTTCCGATCTacgatcgatCTACCCCAGCGGCTACGAGAA
R1-Rev-mCherry	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTATCCTCCTCGCCCTTGCTCAC
R2-Fwd-1	AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGAC GCTCTTCC
R2-Fwd-2	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGAC GCTCTTCC
R2-Fwd-3	AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGACG CTCTTCC
R2-Rev-1	CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGACGTGTGCTC TTC
R2-Rev-2	CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGTGCTC TTC
R2-Rev-3	CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGTGCT CTTC

Paired-end sequencing was performed on the MiSeq NGS Platform (University of Virginia) using the 500-cycle MiSeq Kit V2 (Illumina); Read 1 = 250 cycles, Read 2 = 250 cycles, Index 1 = 8 cycles, Index 2 = 8 cycles. Trimming and quality filtering of reads was performed using Cutadapt. Analysis of the indels surrounding the Cas9 cleavage site was performed using CRISPResso2 to categorize insertions, and deletions, and substitutions[1]. Statistical differences were calculated using chi-squared tests with Yates' continuity correction.

1 Concordet, J. P. & Haeussler, M. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Res* **46**, W242-W245, doi:10.1093/nar/gky354 (2018).