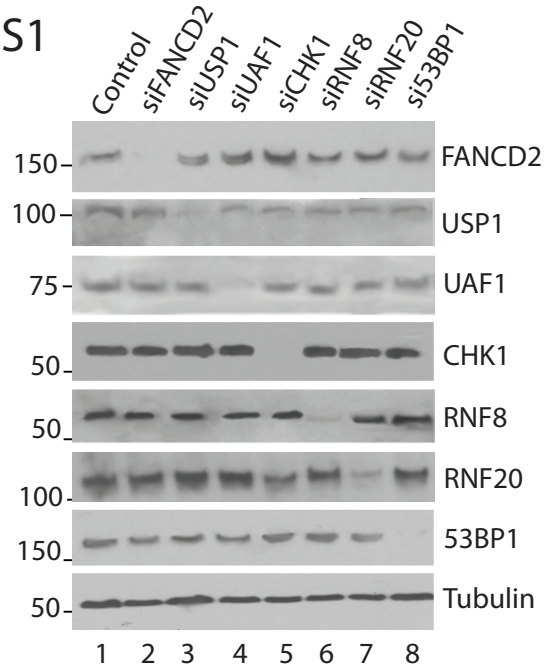
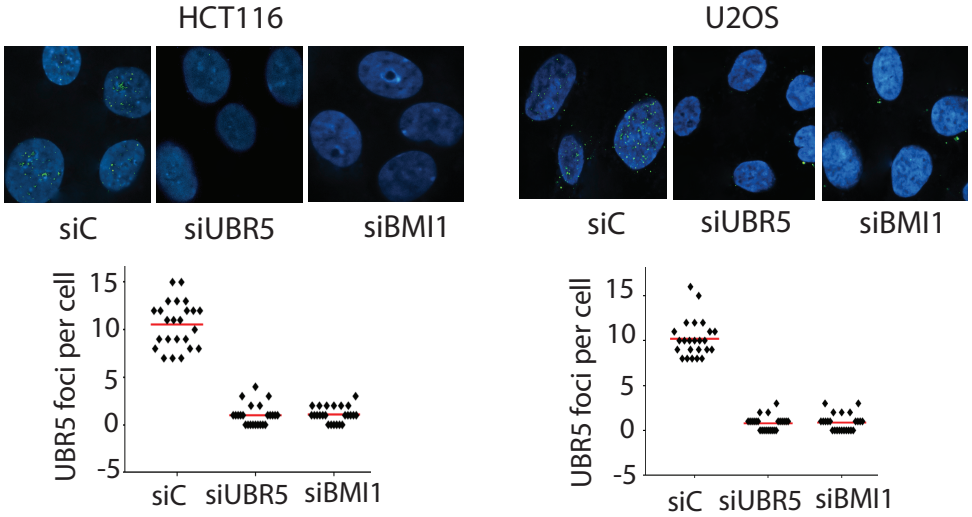
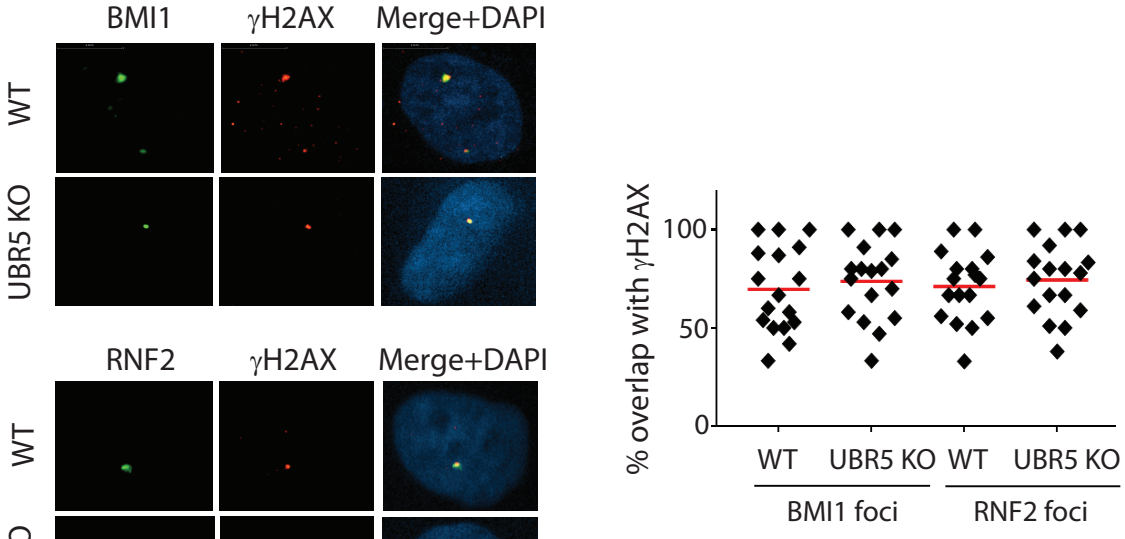
S1


Control
siFANCD2
siUSP1
siUAF1
siCHK1
siRNF8
siRNF20
si53BP1
150
100
75
50
50
100
150
50
FANCD2
USP1
UAF1
CHK1
RNF8
RNF20
53BP1
Tubulin
1 2 3 4 5 6 7 8

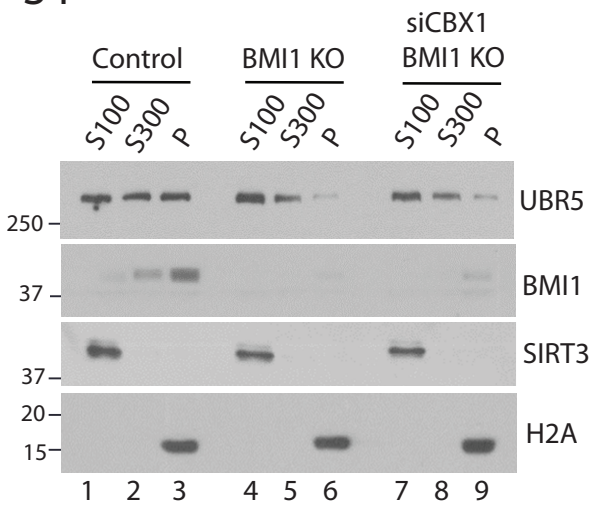
S2


HCT116
U2OS
siC
siUBR5
siBMI1
siC
siUBR5
siBMI1
UBR5 foci per cell
15
10
5
0
-5
siC siUBR5 siBMI1
UBR5 foci per cell
15
10
5
0
-5
siC siUBR5 siBMI1

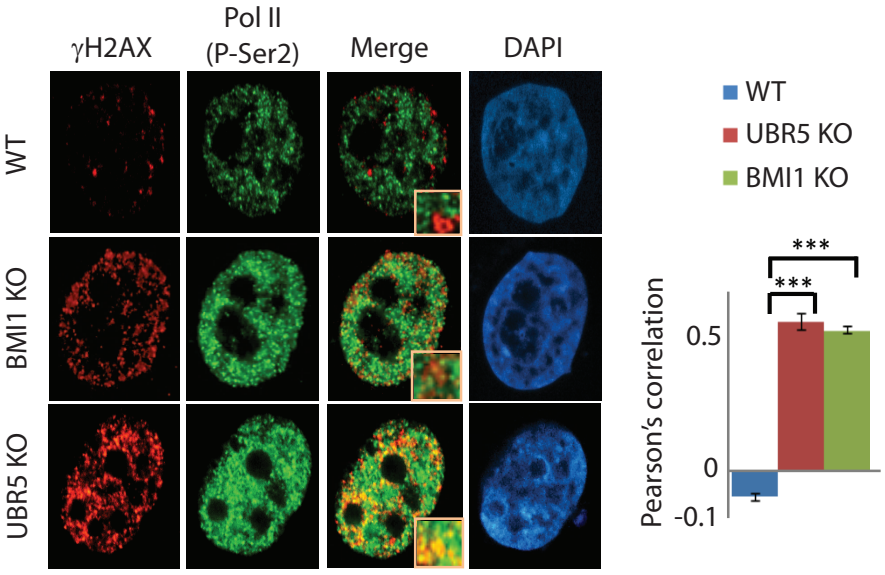
S3


BMI1
γH2AX
Merge+DAPI
WT
UBR5 KO
RNF2
γH2AX
Merge+DAPI
WT
UBR5 KO
% overlap with γH2AX
100
50
0
WT UBR5 KO WT UBR5 KO
BMI1 foci
RNF2 foci

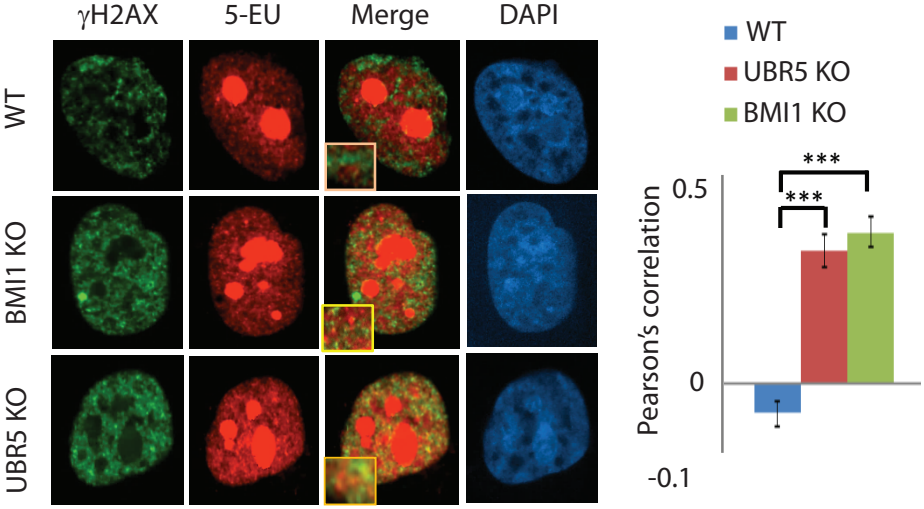
S4


Control
BMI1 KO
siCBX1
BMI1 KO
S100
S300
P
250
37
37
20
15
UBR5
BMI1
SIRT3
H2A
1 2 3 4 5 6 7 8 9

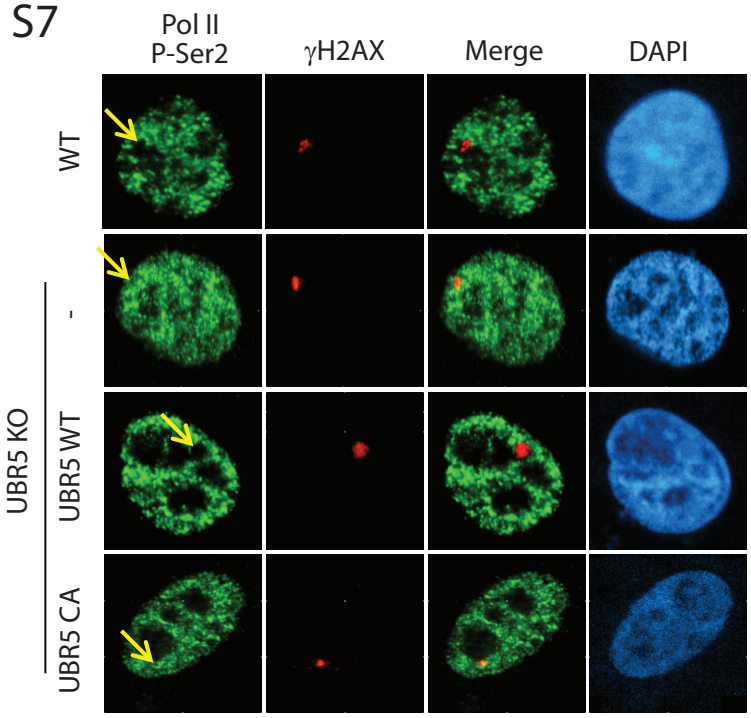
S5


γH2AX
Pol II
(P-Ser2)
Merge
DAPI
WT
BMI1 KO
UBR5 KO
WT
UBR5 KO
BMI1 KO
***
***
Pearson's correlation
0.5
0
-0.1

S6


γH2AX
5-EU
Merge
DAPI
WT
BMI1 KO
UBR5 KO
WT
UBR5 KO
BMI1 KO
***
***
Pearson's correlation
0.5
0
-0.1

S7



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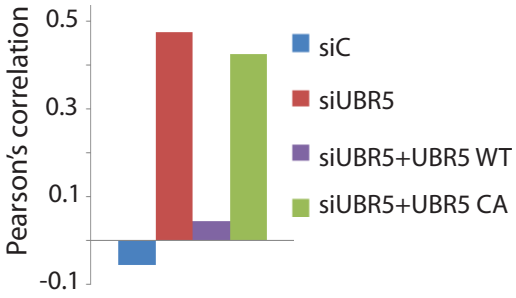

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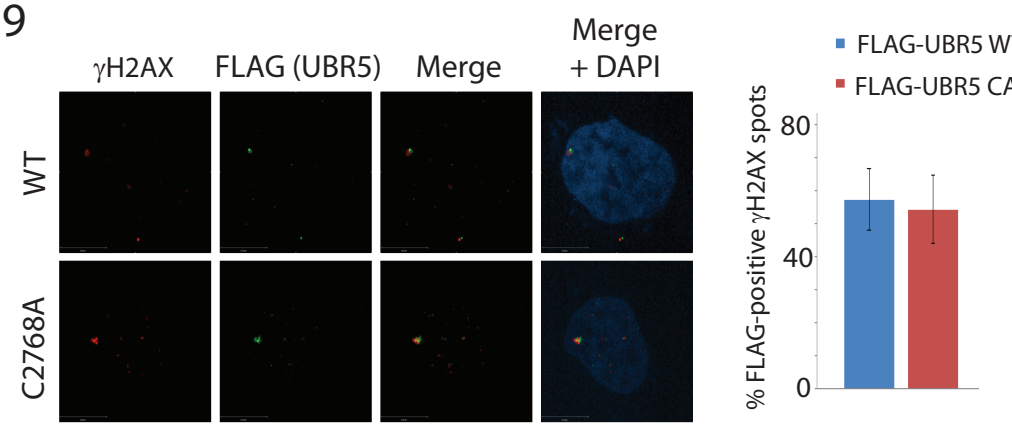

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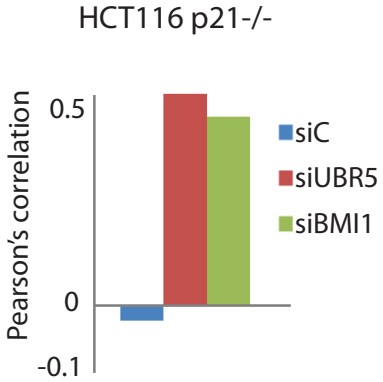

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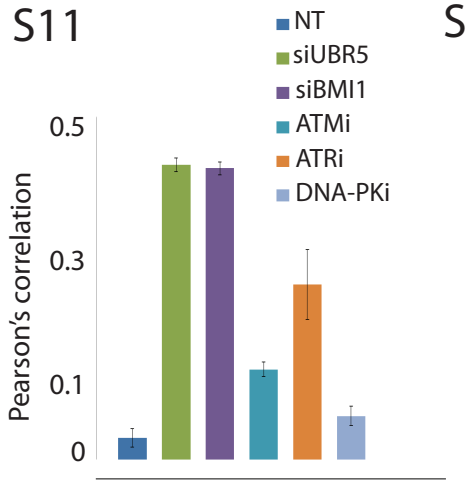
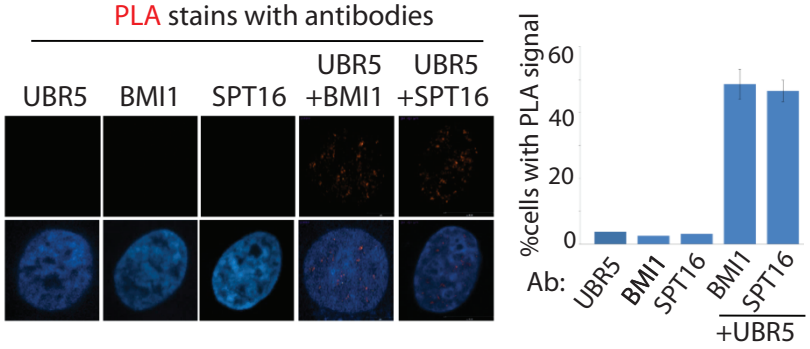


S12

	Exp.#1	Exp.#2	Exp.#3
UBR5	95	130	143
UBAP2L	7	8	7
SPT16	8	6	9
SSRP1	3	3	9
SUB1	7	2	0
SPT5	10	5	6
NONO	12	17	17
H2A	3	2	1
H2B	2	2	0

S13



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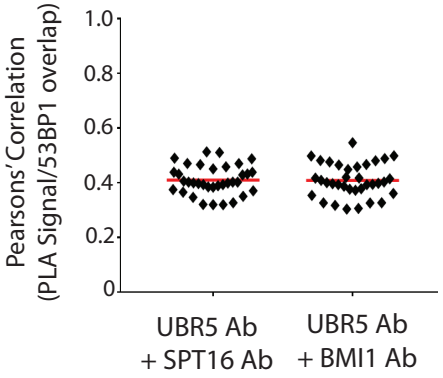

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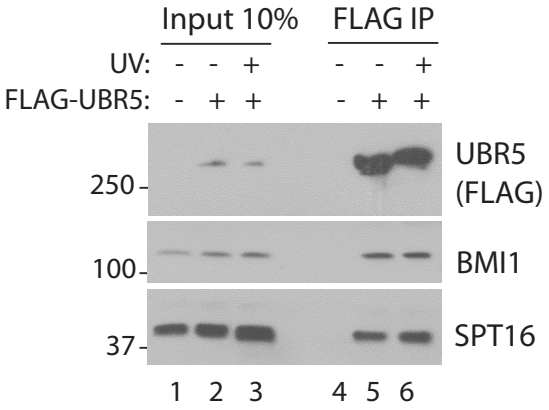

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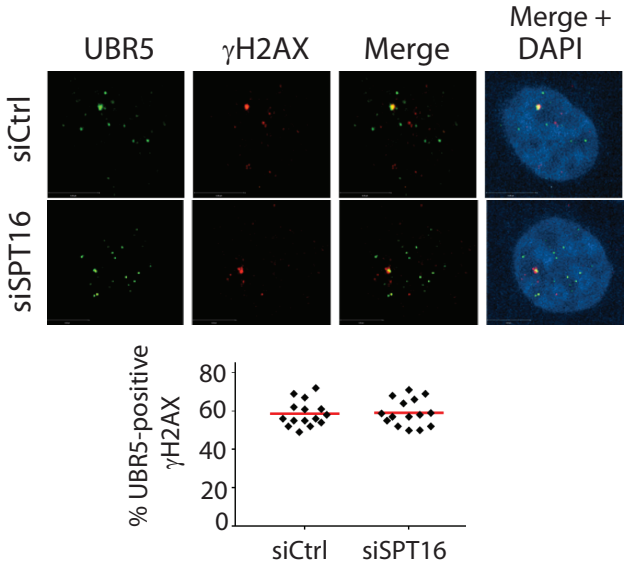

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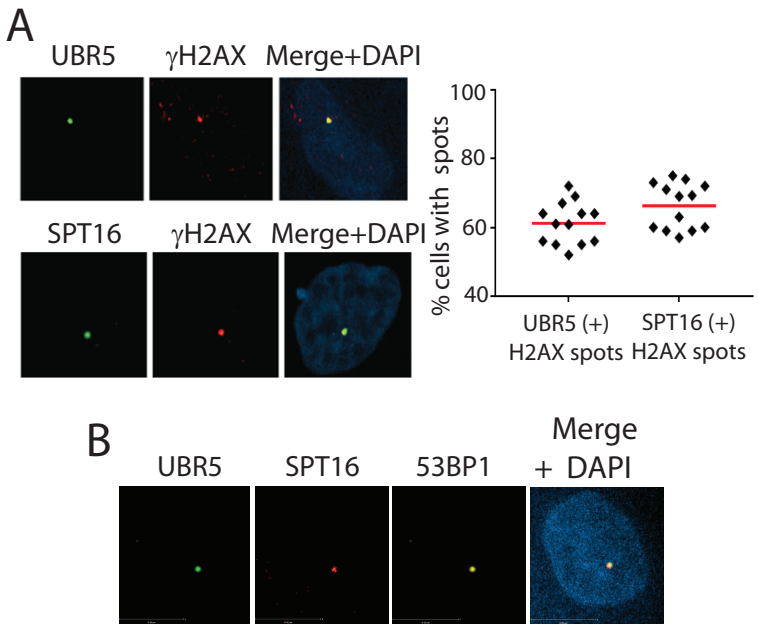

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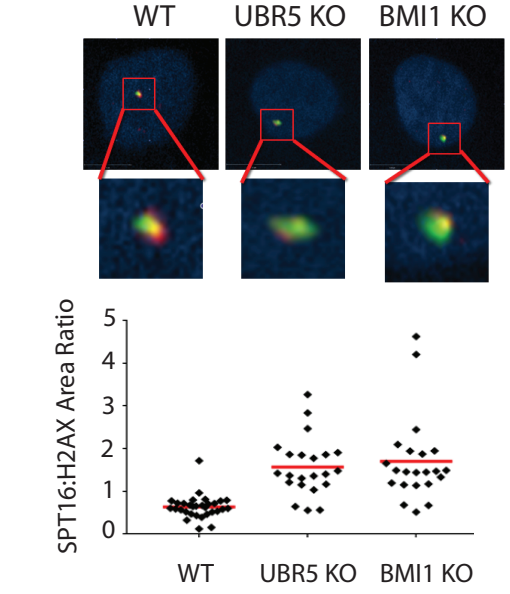

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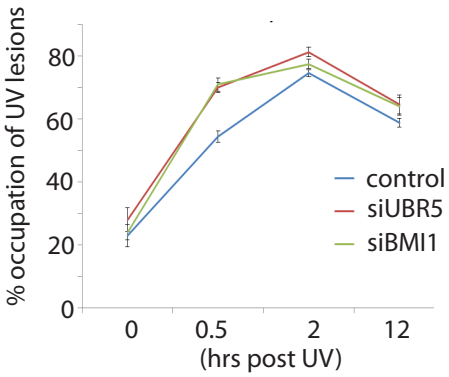

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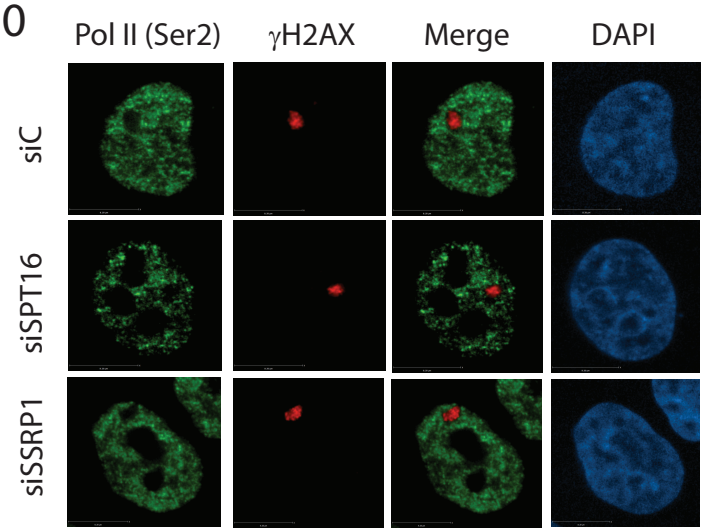

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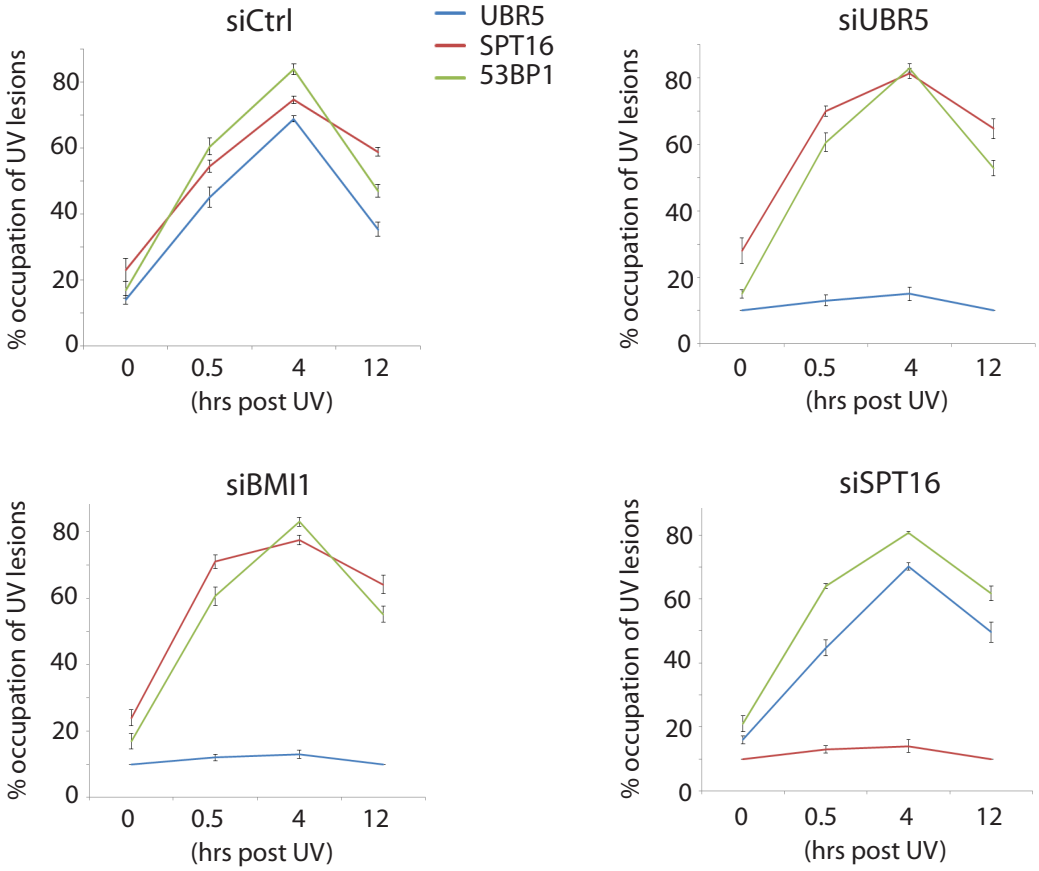

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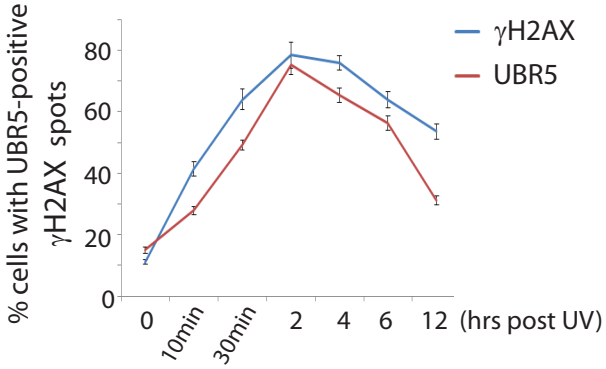

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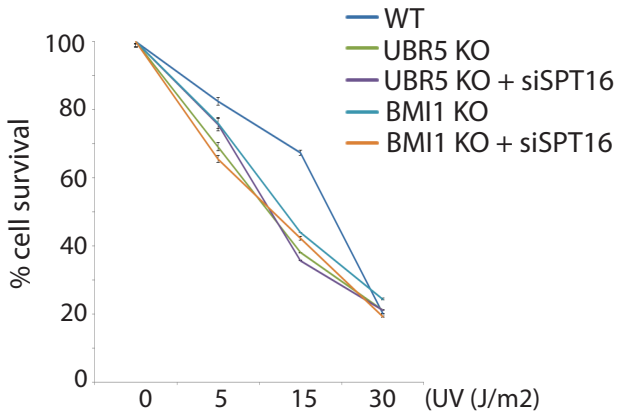
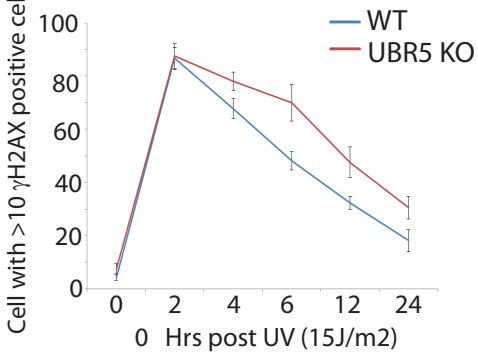


S24

A



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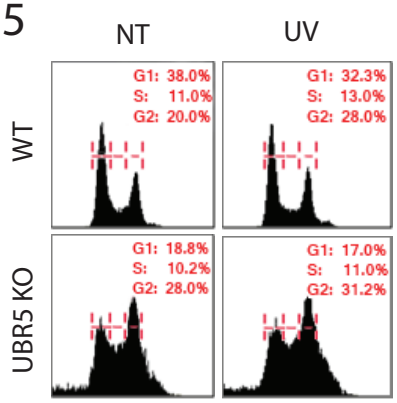
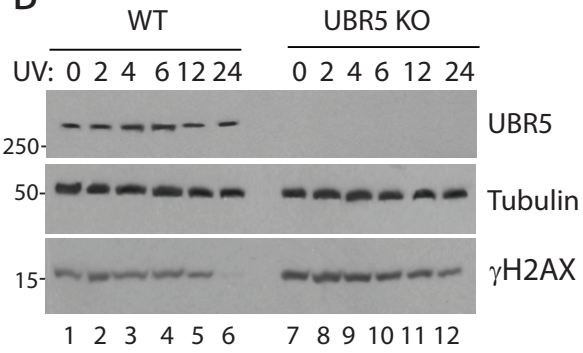
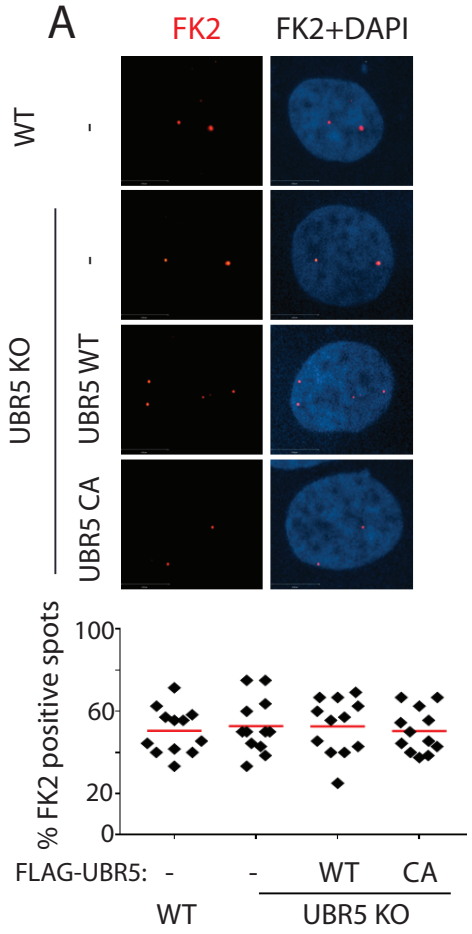


B



S26

A



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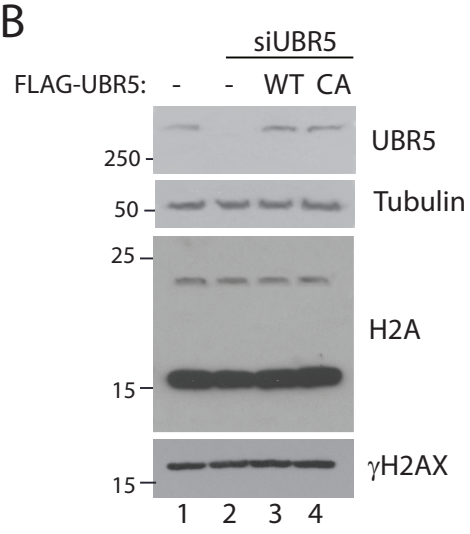

Supplementary Information

Materials and Methods

Cell line, plasmids, and chemicals

HeLa, 293T, and U2OS cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Bovine serum and L-glutamine. HCT116 cells (WT and p21-/-; gift from Dr. Bert Vogelstein) were grown in RPMI, or McCoy's Medium supplemented with 10% Bovine serum and L-glutamine. The Crispr-mediated knockout of UBR5 and BMI1 gene used the guide-RNA synthesized from Sanracruzebiotechnology. The KO HeLa cells were generated following the manufacturer's protocol. Subclones that are depleted of the corresponding proteins were isolated and confirmed by western blottings. All these cells were mycoplasma tested. All cells were grown in 37°C in 5% CO_2. HeLa S3 cells were cultured with Minimum Essential Media Eagle with Joklik modification (Sigma) adjusted to pH 7.4 and grown in spinner culture flasks. UBR5 plasmids (pCMV-Tag2-UBR5 WT and C2768A) were purchased from Addgene, and BMI1 cDNA was cloned to the pOZ-N retroviral vector for expression studies. Etoposide and the inhibitors for ATM, ATR, PARP, and DNA-PK were purchased from Selleck Chemical. Mitomycin C was purchased from Sigma Aldrich. 4-OHT was purchased from Sigma and Shield-1 was purchased from Chemipharma.

RNAi

Cells were cultured in medium without antibiotics and transfected once with 20nM siRNA using the RNAiMAX (Invitrogen) reagent following the manufacturer's protocol.
The sequences for siRNAs used are as following (5' to 3'): UBR5#1: CAGGUAUGCUUGAGAAAUAAU, UBR5#2: GAAUGUAUUGGAACAGGCUACUAUU, BMI1#1: AUGGGUCAUCAGCAACUUCUU, BMI1#2: CAAGACCAGACCACUACUGAA, RNF1: CAGGGUCAGAUCAGACCACAA, RNF2#1: UGGGCUAGAGCUUGAUAAUAA, RNF2#2: AACGCCACUGUUGAUCACUUA, H2AX: AAGCACCUAGAUACCAGCACA, MDC1: CAGGGUCAGCCAUUAUACAUA, RNF8: CAGCCUGUGAUAGGCAUUCAA, UAF1: AAUCAGCACAAGCAAGAUCCAUAUA, USP1: AGCUUCUGAAUAUAGAGCAUCUGAA, CHK1: UUGGAAUAACUCACAGGGAUA, BRCA1: CAGCAGUUUAUUACUCACUAA, UBC13: ACCUUGTCUCUUGCAGUUUAA, UBC9: UUUCCUCCAUGCUUUCCUCUCCUGG, RNF20: ACGGGUGAAUUCCAAAGGUUA, FANCD2: GAGCCUGACAGAAGAUGCCUCCAAA, 53BP1: CAGGACAGUCUUUCCACGAAU, NBS1: UCGGAUAUAUGUAUUGAGAUA, SPT16: ACCGGAGUAAUCCGAAACUGA, SSRP1: UUCGUUGACUCUGAACAUGAA, RAD18: CCCGAGGUUAAUGUAGUUGUU, CBX1: AAGGGAAGGAGTTCTACTTGT

Western blots and antibodies

Cell extracts were run on an SDS-PAGE gel and then transferred to a PVDF membrane (Bio-Rad, Hercules, CA). Membranes were probed with primary antibodies overnight at 4°C. The membranes were then washed and incubated with either mouse or rabbit secondary antibody linked with horseradish peroxidase (Cell Signaling Technologies) and washed. The bound antibodies were viewed via Pierce ECL Western Blotting Substrate (Thermo Scientific). The following primary antibodies were used: α-SPT16, α-BMI1, α-UBR5, α-Ring1a (RNF1), α-Ring1b (RNF2), α-H2A, α-SSRP1, α-CDK9, α-P21, α-MED12, α-Rpb1 rabbit polyclonal antibodies from Cell Signaling Technologies; α-UBAP2L, α-53BP1, rabbit polyclonal antibodies from Bethyl Laboratory; α-FK2, α-H2AX, α-Tubulin mouse monoclonal antibodies from Millipore; α-FLAG mouse monoclonal antibody from Sigma Aldrich, α-BRCA1, α-RNF8 mouse monoclonal antibodies from Santa Cruz Biotechnology.

Immunoprecipitation and mass spectrometry analysis

293T cells were grown to ~70% confluence, at this time tagged constructs were transfected using Lipofectamin 2000 or PEI transfection reagents in a 3:1 (volume to mass) ratio; the cells were harvested ~36 hours later by scraping. The harvested cells were lysed with a lysis buffer (25mM Tris pH 7.4, 0.5%

NP40, 100mM NaCl, 0.1mM EDTA, supplemented with protease inhibitor mix solution (ThermoFisher)) for 10 minutes while rotating at 4C followed. Occasionally, the lysis was enhanced by ~10 pulses of sonication. The lysates were cleared by centrifuging for 30 minutes at 14,000RPM, 10% of the supernatant was collected for input samples while the remaining volume was incubated for 10 hours with the anti-FLAG M2 agarose (Sigma Aldrich) at 4C while rotating. The M2 beads were washed 3 times with lysis buffer, and the bound proteins before eluted with the addition of 2X Laemmli buffer followed by boiling. For the mass spec sample, the bound proteins before eluted with the addition of 4% SDS. The eluate containing total protein was processed using the FASP method, digested with trypsin-LysC and desalted using HYPERSEP C18 columns as previously described. Peptides were then concentrated by vacuum centrifugation and resuspended in 0.1% formic acid. Peptides were separated on an Acclaim PepMap C18 (75 µm x 50 cm) UPLC column (Thermo) using an EASY-nLC 1000 with gradient times of 60-90 min (2-40% acetonitrile in 0.1% formic acid). Mass spectrometric analysis was performed by a hybrid quadrupole-Orbitrap (Q Exactive Plus, Thermo) or hybrid linear ion trap-Orbitrap (Orbitrap XL) using a top 10 data-dependent acquisition method. For LC-MS/MS analysis using the Q Exactive, full scan and MS/MS resolution was 70,000 and 17,500, respectively. For LC-MS/MS analysis using the Orbitrap XL, full scan mass resolution was 60,000 (Orbitrap detection) with parallel MS/MS acquisition performed in the linear ion trap. Protein identifications were assigned through MaxQuant (version 1.5.0.30) using the UniProt Homo sapiens database. Carbamidomethyl (C) was set as a fixed modification and acetyl (protein N-terminus) and oxidation (M) were set as variable modifications. Trypsin/P was designated as the digestion enzyme with the possibility of two missed cleavages. A mass tolerance of 20 ppm (first search)/4.5 ppm (recalibrated second search) was used for precursor ions while fragment ion mass tolerance was 20 ppm and 0.6 Da for Q Exactive and Orbitrap XL data, respectively. All proteins were identified at a false discovery rate of <1% at the protein and peptide level.

Immunofluorescence and image quantification

Cells were seeded in 12 well plates onto coverslips, indicated siRNA and damage treatments were applied. For UV irradiation, cells were irradiated with 15~100J/m^2 UVC (UV stratalinker 2400), depending on the type of experiments. ATM inhibitor (1uM), ATR inhibitor (100nM), PARP inhibitor (5uM), DNA-PK inhibitor (1uM) were treated for 12hrs prior to fixing. Media was removed from the wells, coverslips were washed twice with ice cold PBS and fixed for 10 minutes in the dark with cold 4% paraformaldehyde. The coverslips were washed twice with cold PBS and permeabilized for 5 minutes via incubation with 0.25% Trition and washed twice with cold PBS. Primary antibodies were diluted in PBS (1:300-1:500) and 30ul was applied to each coverslip before incubating for 1 hour in the dark, coverslips were washed twice with cold PBS. Secondary antibodies were diluted 1:1000 in PBS and 35ul was applied to each coverslip before incubating for 1 hour in the dark, coverslips were washed twice in PBS and placed onto glass slides. Vectashield mounting medium for fluorescence with DAPI (Vector Laboratories Inc) was used to stain cellular nuclei. Images were collected by a Zeiss Axiovert 200 microscope equipped with a Perkin Elmer ERS spinning disk confocal imager and a 63x/1.45NA oil objective using Volocity software (Perkin Elmer). All fluorescence quantification was performed using ImageJ. To measure relative fluorescence intensity (RFI) single cells were manually selected, the integrated density was measured and corrected to account for background in the image. The density measurements were normalized with a value of 10 corresponding to the brightest reading. Pearson's overlap correlations were obtained with the use of the "Colocalization finder" plugin for ImageJ. Full color images were imported into ImageJ and the channels were split into blue, red, and green; the red and green channels were analyzed and the degree of colocalization was determined. All Pearson's correlation graphs are representative of at least three independent experiments, error bars represent standard error. For measuring the SPT16/ϒH2AX foci area (Figure 5B), the pixel areas were automatically selected using the 'wand tracing tool' in the Image J. Vector Quantification (Figure 3A, C) was done using the ImageJ: a vector of 150 pixels was drawn across γH2AX spots at the widest possible point. The fluorescence intensity for each pixel (RNA Polymerase II staining) in the vector was measured and the average fluorescence intensity for each pixel was calculated from 10 independent nuclei. The average RNA

polymerase II relative fluorescence intensity (RFI) for each pixel was graphed for WT, UBR5 knockdown and BMI1 knockdown conditions.5-EU stain was also quantified in the manner.

The PTuner 263 cells (provided by Dr. Greenberg) were seeded on coverslips in 12 well plates at ~30% confluence; indicated siRNAs were treated at 20uM for 48hrs. Stabilization of the Fok1-mCherry fusion protein was induced with the addition of 4-hydroxytamoxifin (1uM) and Shield-1 (1uM) to the cell growth medium 3hrs prior to fixing. Transcription of YFP-MS2 was induced with the addition of tetracycline (1ug/ml) to the growth medium 3hrs prior to fixing. For the Fok1-UBR5 colocalization study, fixing and staining was performed as described above.

In Vitro Ubiquitination

293T cells were grown in 10cm plates and transfected with pCMV-EDD WT or CA constructs as previously described, cells were harvested by scraping when confluence >95% and FLAG constructs were immunoprecipitated as previously described. A 30ul reaction mix containing 1mM Tris (pH 7.5), 1mM NaCl, 0.1mM $MgCl_2$, 0.01mM DTT is added to each reaction sample; a control IP (with no reaction mix) is resuspended with 30ul of PBS. Purified E1 is added to the reactions at a final concentration of 5nM, and the E2 UbcH5b is added at a final concentration of 100nM. The reaction is left at room temperature for 1 hour before quenching with the addition of 2xLaemmli buffer, and the reaction products are analyzed via western blotting.

Proximity ligation assay

Proximity ligation assays were preformed using the Duolink kit from Sigma Aldrich; cells were grown in a 12 well format on coverslips. Cells were fixed and permeabilized according to the standard immunofluorescence protocol (previously described), primary antibodies were added at a 1:500 dilution in PBS and incubated for 1 hour at room temperature. PLA minus and plus probes were diluted 1:5 in the provided dilution buffer, 30ul of the probe reaction was added to each coverslip and incubated for 1hr at 37C; the coverslips were washed twice with buffer A. The provided ligation buffer was diluted 1:5 in water, the ligase was added at a 1:30 dilution; the ligation reaction was left at 37C for 30minutes before washing twice with wash buffer A. The provided amplification buffer was diluted 1:5 in water before adding the provided polymerase at a 1:80 ratio, the amplification reaction was left at 37C for 100 minutes, the reaction was quenched by washing twice with buffer B. The coverslips were mounted on slides with DAPI containing mounting medium.

Recovery of RNA Synthesis assay

Cells were seeded in a 12 well format on coverslips and grown under the specified growth conditions (siRNA), cells were irradiated with 30J/m^2 UVC (UV stratalinker 2400), followed by addition of 5-EU at concentration of 500uM. Cells were fixed at different time points post UV irradiation.Coverslips were fixed permeabilized according to the standard immunofluorescence protocol and the incorporation of 5-EU was detected using the Click-IT reaction kit; the provided 10X reaction buffer was diluted in water and the reaction components were added in accordance with the Click-IT protocol. The Click-IT reaction was left at room temperature in the dark for 1 hour and washed with the provided Click-IT rinse buffer before mounting on slides with DAPI containing mounting medium.

Cell fractionation assay

HeLa cells treated with the indicated siRNAs and Damaging agents were harvested from 6cm plates at >95% confluence. Cells were lysed for 10minutes on ice with buffer containing .5%NP40 and 100mM NaCl, the supernatant was cleared by centrifuging for 5minutes at 3,000RPM. The supernatant was collected and 4X Laemmli buffer was added (S100 fraction). The remaining pellet was lysed for 10minutes on ice with buffer containing 1% NP40 and 300mM NaCl for 10 minutes on ice, the supernatant was cleared by centrifuging for 5 minutes at 3,000RPM. The supernatant was collected and 2X Laemmli buffer was added (S300 fraction), the remaining pellet was resuspended with PBS and 2X Laemmli buffer (P fraction). For “S – P” fractionation the harvested cells were lysed with 0.5%NP40

buffer (100mM NaCl) for 10 minutes on ice, the supernatant was cleared by centrifuging for 5 minutes at 3,000RPM. The supernatant was removed and 2X laemmli buffer was added (S fraction), the remaining pellet was resuspended in PBS and 2X Laemmli buffer (P fraction). The S300 fraction generally represents proteins associated loosely to chromatin in our methods.

Clonogenic survival assay

Cells were seeded into 24 well plates (100 cells per well) and treated with indicated siRNA for 48hours. UV irradiation (254nm) was applied using the Stratalinker UV crosslinker, 2400, then the cells were allowed to grow for 10~14 days. The cells were fixed with a 10% methanol, 10% Acetic acid solution for 15minutes at room temperature, followed by staining with crystal violet. Dried colonies were dissolved and resuspended with Sorensen buffer (0.1M sodium citrate, 50% ethanol), then the colorimetric intensity of each solution was quantified using Gen5 software on a Synergy 2 (BioTek, Winooksi, VT) plate reader (OD at 595nm). Error bars are representative of 3 independent experiments.

Supplementary Figure legends

Figure S1. Knockdown efficiencies of siRNAs used in Figure 2A are shown.

Figure S2. Damage-inducible UBR5 foci are eliminated by siRNAs against UBR5 and BMI1 in HC116 (left) and U2OS (right) cells. The number of cells with positive UBR5 foci (>5) were counted and plotted on the graphs below.

Figure S3. UV-inducible foci formation of BMI1 or RNF2 is not affected by UBR5 depletion. Wild type **and** UBR5 Crispr KO HeLa cells were irradiated with UV (100J/m^2) through 0.3uM micropore filters. BMI1 and RNF2 staining at H2AX spots was manually counted, N= 25 for each condition.

Figure S4. Additional depletion of CBX1, a component of the canonical PRC1 complex, does not affect the UBR5 chromatin localization in BMI1 KO cells. The corresponding cells were treated with UV (**70J/m^2**) then subjected to fractionation into S100, S300, and P fractions (see Methods).

Figure S5. BMI1 and UBR5 repress Pol II elongation at damaged chromatin. The HeLa WT, BMI1 KO, UBR5 KO cells were globally irradiated with UV (50J/m^2), then the cells were fixed 1 hour later. Cells were co-stained with antibodies to RPB1 (P-Ser2) and γH2AX. Pearson's correlation was generated using the quantification information obtained from Image J software, and plotted on the right (***P<0.0005, n=75)

Figure S6. BMI1 and UBR5 repress nascent RNA synthesis at the UV-induced lesions. The CRISPR-Cas9 KO HeLa cells were globally irradiated with UV (50J/m^2), then stained with γH2AX and 5-EU. Pearson's correlation was generated on the right (***P<0.0005, n=75).

Figure S7. The catalytic activity of UBR5 is required for the repression of Pol II elongation at the UV lesions. Shown are the representative images for the Figure 3G.

Figure S8. The catalytic activity of UBR5 is required for the repression of Pol II elongation at the UV lesions. Shown is the quantification of the rescue experiments in the UBR5 and BMI1 knockdown cells. (n=50)

Figure S9. Both FLAG-UBR5 WT and C2768A mutants are localized to damage sites. HeLa cells were transfected with either FLAG-UBR5 WT or C2768A plasmids, then 24 hours later the cells were irradiated with UV (100J/m^2) through 0.3uM micropore filter. 1 hour later the cells were fixed and stained. On the right is the quantification from three independent experiments (n=35)

Figure S10. The P-Ser2 and ϒH2AX overlap occurs in the p21 knockout cells. The HCT116 p21-/- cells were treated with UV (50J/m^2), and co-stained with P-Ser2 and ϒH2AX. Pearson's correlation for the overlap is provided (n=75).

Figure S11. ATM, ATR inhibition partially relieves the transcriptional repression at UV lesions. HeLa cells were treated overnight with ATMi (KU-60019, 1uM), ATRi (AZ-20, 100nM), or DNA PK inhibitor (KU-57788, 1uM), before irradiated with UV (50J/m^2). After 1 hour, cells were fixed and co-stained with p-Ser2 and ϒH2AX. Pearson's Correlation for the overlap is provided (n=100).

Figure S12. Mass spec analysis (unique peptides #) of three independent FLAG-UBR5 IP experiments are shown. Shown is the proteins identified in all three times but not in control IPs.

Figure S13 The Proximity Ligation Assay (PLA) was performed using the indicated primary antibodies (n= 50). On the right, % cells with specific PLA signals were counted manually.

Figure S14. The PLA spots are partially overlapped with 53BP1 spots. Pearson's correlation for the UBR5-SPT16 and UBR5-BMI1 PLA signals with 53BP1 were quantified using ImageJ. (n=35)

F**igure S15. FLAG-UBR5 associate with BMI1 and SPT16 in the present and absence of UV damage.** 293T cells transfected with the FLAG-UBR5 WT plasmids were irradiated with UV (50J/m^2), then the cells were harvested for anti-FLAG IP analysis.

Figure S16. SPT16 depletion does not affect the UBR5 chromatin recruitment. HeLa cells transfected with either control or SPT16 siRNAs were irradiated with UV (100J/m^2) through 0.4uM micropore filters then stained with UBR5 and ϒH2AX antibodies. On the right is the quantification (n=20).

Figure S17. UBR5 and SPT16 co-localize to damaged sites. A. HeLa cells were irradiated with UV (100J/m^2) through 0.4uM micropore filters, then co-stained as indicated antibodies. On the right is the quantification (n=20). B. The cells were stained with three antibodies (UBR5, SPT16, 53BP1).

Figure S18. SPT16 foci size increases in UBR5 KO and BMI1 KO cells. The same experiment in Figure 5B was performed using the CRISPR KO cells. (n=35)

Figure S19. SPT16 foci formation kinetics at the UV lesions is altered in the UBR5 and BMI1 knockdown cells.

Figure S20. Pol II elongation does not occur through the UV lesions in the WT cells depleted of siSPT16 or siSSRP1.

Figure S21. Measurements of UBR5, SPT16, 53BP1 foci during the RRS assay shown in Figure 6A.

Figure S22. UBR5 foci formation kinetics in relation to the H2AX foci kinetics.

Figure S23. Depletion of SPT16 does not rescue the UV sensitivity of UBR5 KO or BMI1 KO cells. (see methods)

Figure S24. UBR5 KO HeLa cells display increased ϒH2AX that are not resolved over time, compared to the WT HeLa cells. The cells were irradiated with UV (30J/m^2), then fixed and stained with anti-ϒH2AX antibody at indicated times. (A. immunofluorescence. B. western blottings)

Figure S25. UBR5 KO cells display increased G2/M peak with or without UV damage. Cells were harvested 1 hour post-UV (30J/m^2).

Figure S26. Expression of UBR5 WT or C2768A mutant in UBR5 KO cells does not alter the overall ubiquitin signals at the damaged sites and the H2A-Ub. A. The indicated cells were irradiated with UV (50J/m^2), then stained with anti-ubiquitin (FK2) antibodies. The percentage of FK2 positive cells was manually counted. B. Western blots show that the H2A-Ub level does not change.