

Figure S1 (Figure 1 continued). USP7 Is Physically Associated with the MRN/MDC1 Complex. (A) Whole cell lysates from HeLa cells treated with vehicle or DNase I were immunoprecipitated with anti-USP7 and then immunoblotted with antibodies against the indicated proteins. (B) Fast protein liquid chromatography analysis of MDC1-containing protein complex. Nuclear extracts from HeLa cells were purified with FLAG affinity gel from FLAG-MDC1-expressing HeLa cells and eluted with FLAG peptide. The eluates were fractionated on Superose 6 size exclusion columns with high salt buffer. Chromatographic elution profiles (upper panel) and Western blotting analysis (lower panel) of the chromatographic fractions are shown. Equal volume from each fraction was analyzed and the elution positions of calibration proteins with known molecular masses (kDa) are indicated. (C) GFP-tagged deletion mutants of USP7 were transfected into HeLa cells followed by co-immunoprecipitation and immunoblotting analysis. MATH, the meprin and tumor necrosis factor-receptor associated factor (TRAF) homology domain; CD, catalytic domain; UBL, ubiquitin like domain. (D) FLAG-tagged deletion mutants of MDC1 were transfected into HeLa cells followed by co-immunoprecipitation and immunoblotting analysis. The asterisk indicates the FLAG-N3 mutant. (E) GST-pull down assays with bacterially expressed GST-fused PST or BRCT domain of MDC1 and His-tagged recombinant full length or deletion mutants of USP7 purified from Sf9 cells. GST affinity beads-precipitated proteins were examined by immunoblotting with His antibody. The asterisks indicate the recombinant proteins stained by Commassie Blue. (F) His-pull down assays with full length or deletion mutants of USP7 purified from Sf9 cells and in vitro transcribed/translated proteins as indicated. (G) GST-pull down assays with bacterially expressed GST-fused full length or deletion mutants of NBS1 and His-tagged recombinant full length or USP7/MATH purified from Sf9 cells. The asterisks indicate the recombinant proteins stained by Commassie Blue. (H) Illustration of the molecular interfaces required for the association of USP7 with the MRN/MDC1 Complex. FHA, fork head associated domain; C-ter, C-terminal region; PST, proline/serine/threonine rich repeats domain.

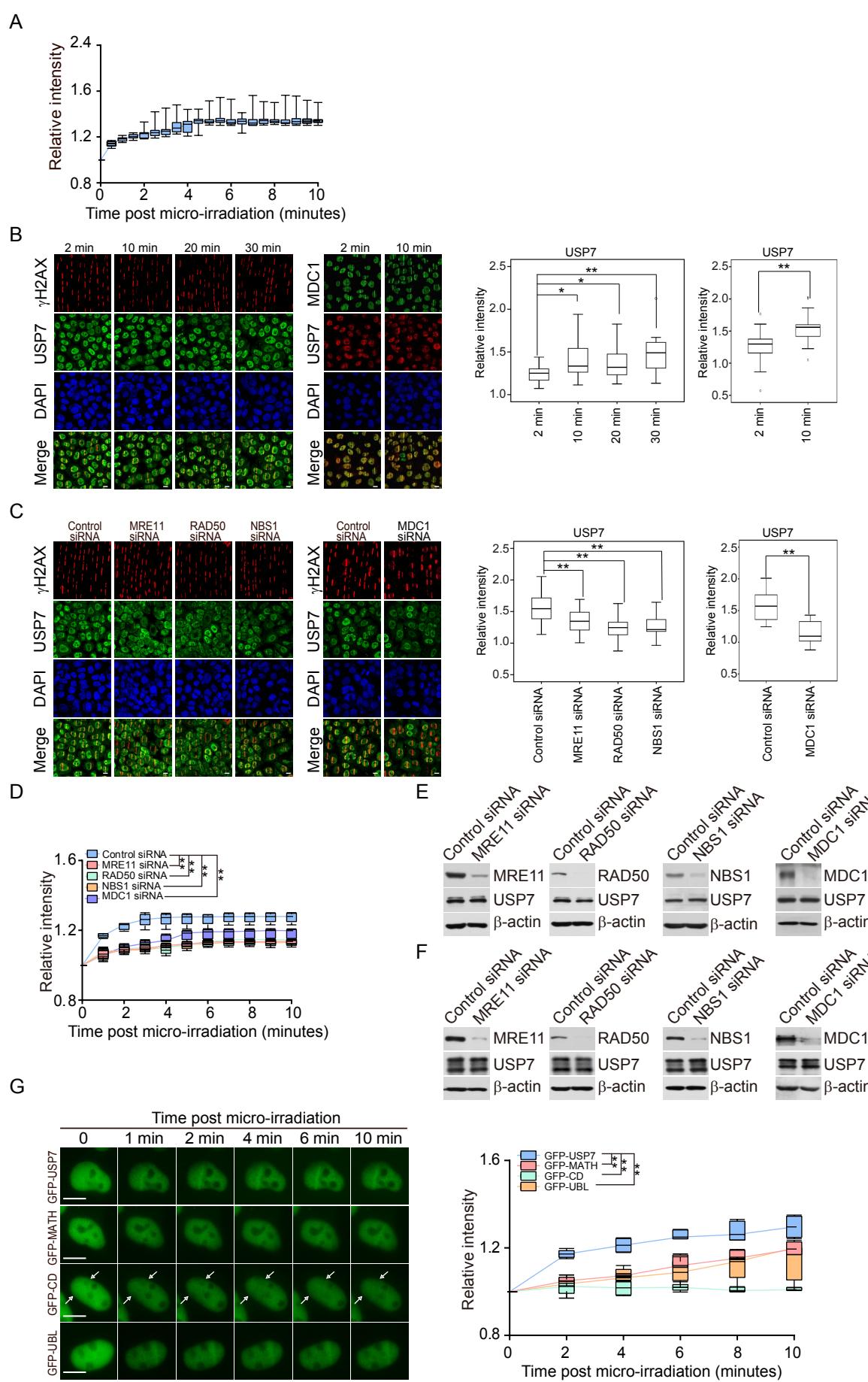
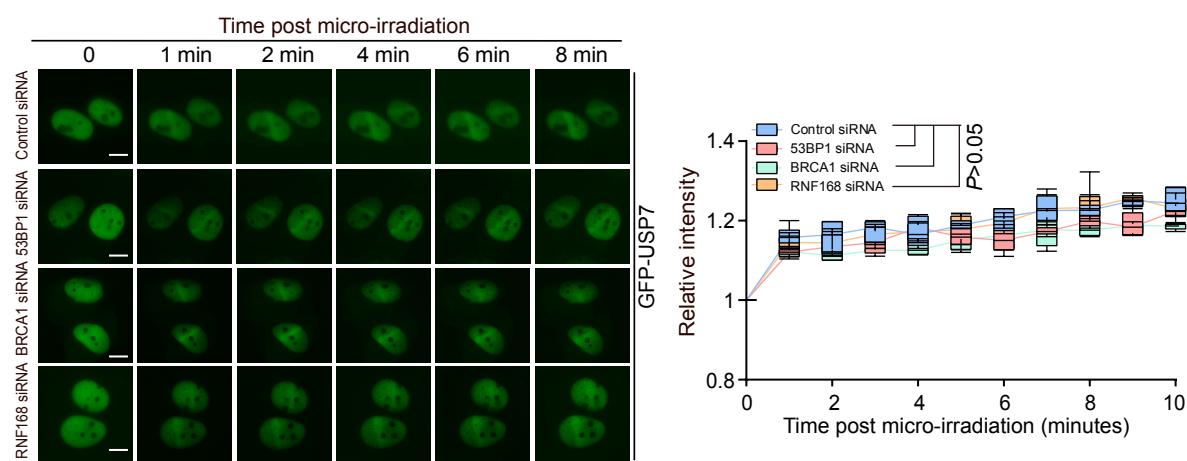
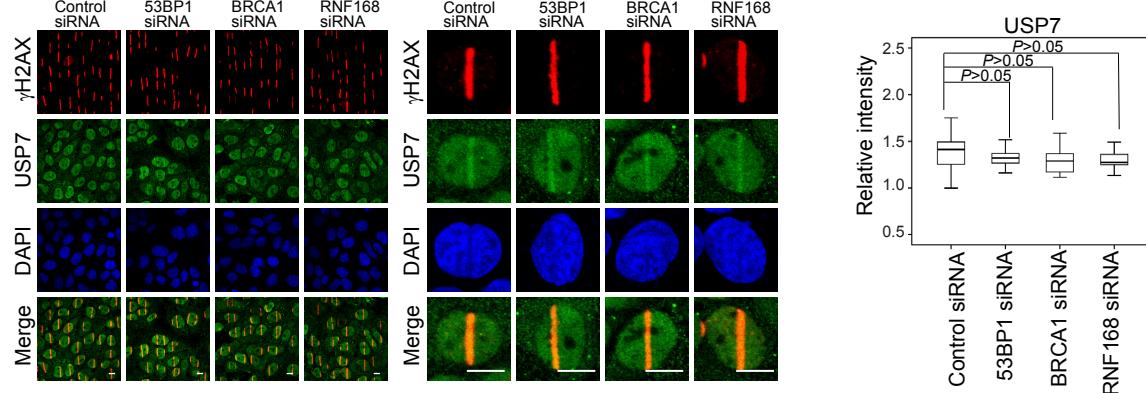


Figure S2 (Figure 2 continued). USP7 Is Recruited to DSB Sites. (A) Quantitation of the laser stripes intensity. HeLa cells stably expressing GFP-USP7 were subjected to laser micro-irradiation and live cell imaging at the indicated time points. The relative fluorescent intensities in micro-irradiated areas to the background signal of the undamaged regions (determined by Image J software) are plotted and shown. (B) HeLa cells subjected to UVA-laser microdissection were fixed and immunostained with antibodies against USP7 and γ H2AX, or USP7 and MDC1 followed by confocal microscopy analysis. Scale bar, 10 μ m. The relative fluorescent intensities are plotted as shown. (C) HeLa cells were transfected with the indicated siRNAs and subjected to UVA-laser microdissection. Then, cells were fixed and immunostained with antibodies against USP7 and γ H2AX followed by confocal microscopy analysis. Scale bar, 10 μ m. The relative fluorescent intensities are plotted as shown. (D) Quantitation of the laser stripes intensity. HeLa cells stably expressing GFP-USP7 were transfected with the indicated siRNAs, and cells were then subjected to laser micro-irradiation and live cell imaging analysis. (E) Western blotting analysis with cellular lysates from HeLa cells transfected with the indicated siRNAs. (F) Western blotting analysis with cellular lysates from GFP-USP7 stably expressing-HeLa cells with the indicated genes knockdown. For USP7 bands, the higher one with larger molecular weight represents GFP-tagged USP7, while the lower one indicates endogenous USP7. (G) HeLa cells stably expressing GFP-USP7 deletion mutants were subjected to laser micro-irradiation and live cell imaging analysis. Scale bar, 10 μ m. Quantitation of the laser stripes intensity is shown. The laser path is marked with arrows. In (A), (D) and (G), more than 20 nuclei were scored from biological triplicate experiments. ** P <0.01. Two-way ANOVA. In (B) and (C), more than 100 nuclei from biological triplicate experiments were scored. * P <0.05; ** P <0.01. One-way ANOVA.

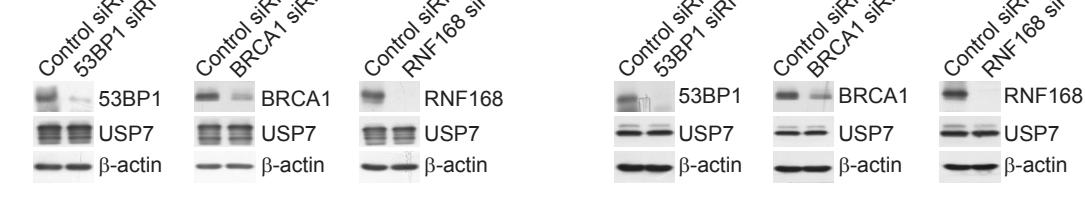
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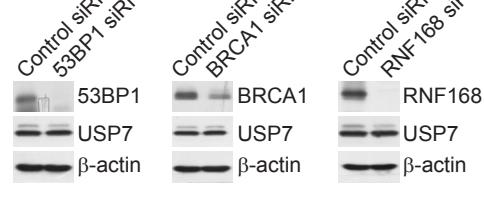


Figure S3 (Figure 2 continued). USP7 Acts Upstream of 53BP1, BRCA1, and RNF168. (A) HeLa cells stably expressing GFP-USP7 were transfected with the indicated siRNAs, then cells were subjected to laser micro-irradiation and live cell imaging analysis (left panel). Scale bar, 10 μ m. The relative fluorescent intensities in micro-irradiated areas to the background signal of the undamaged regions (determined by Image J software) are shown from biological triplicate experiments with more than 20 nuclei (right panel). P values were determined by two-way ANOVA. (B) HeLa cells were transfected with the indicated siRNAs and subjected to UVA-laser microdissection. Then, cells were fixed and immunostained with antibodies against γ H2AX and USP7 followed by confocal microscopy analysis (left panel). Scale bar, 10 μ m. The relative intensities of laser stripes to uncut regions are presented with box plots from biological triplicate experiments with more than 100 nuclei (right panel). P values were determined by one-way ANOVA. (C) Western blotting analysis with cellular lysates from GFP-USP7 stably expressing HeLa cells transfected with the indicated siRNAs. For USP7 bands, the higher one with larger molecular weight represents GFP-tagged USP7, while the lower one indicates endogenous USP7. (D) Western blotting analysis with cellular lysates from HeLa cells transfected with the indicated siRNAs.

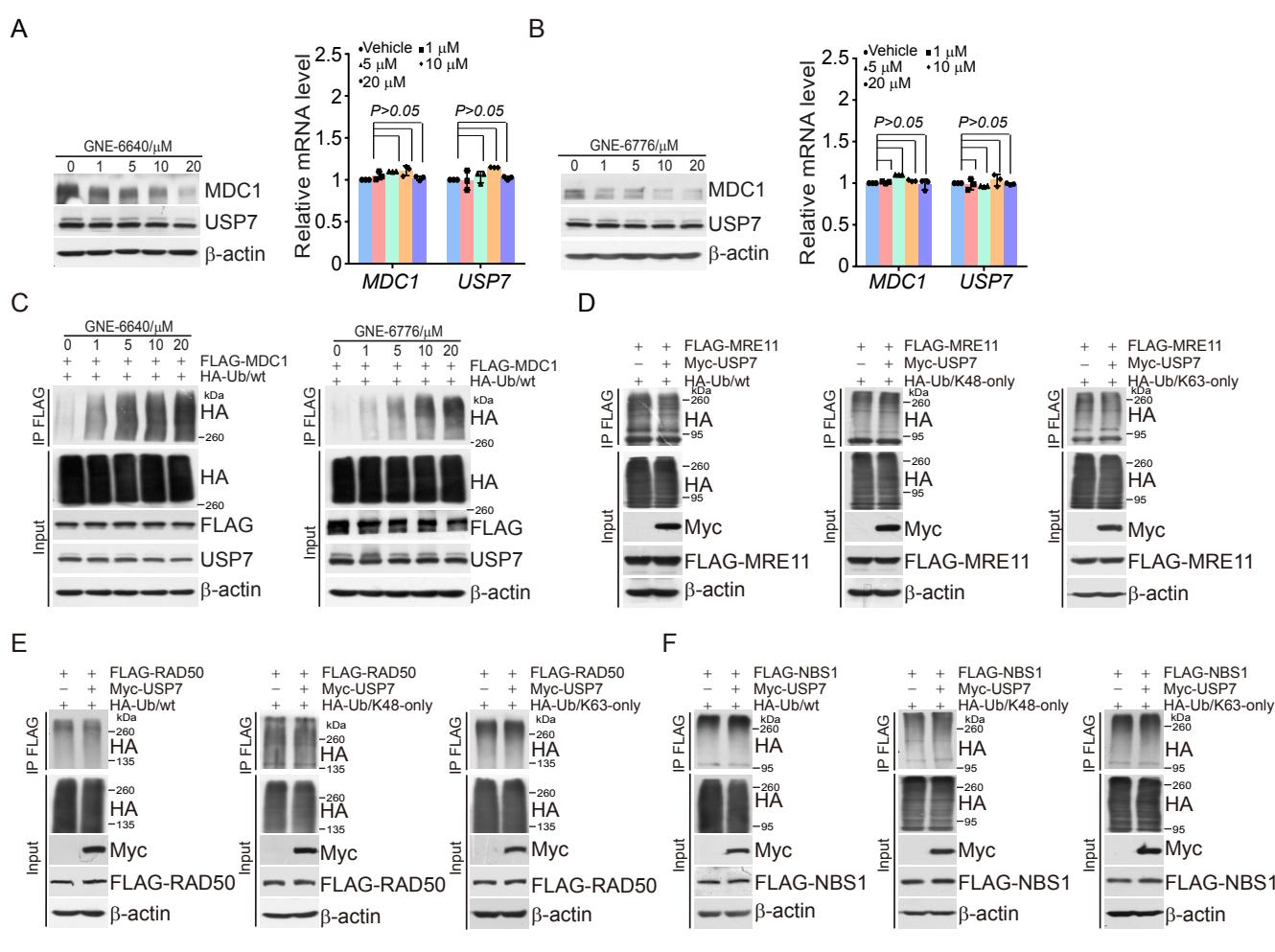
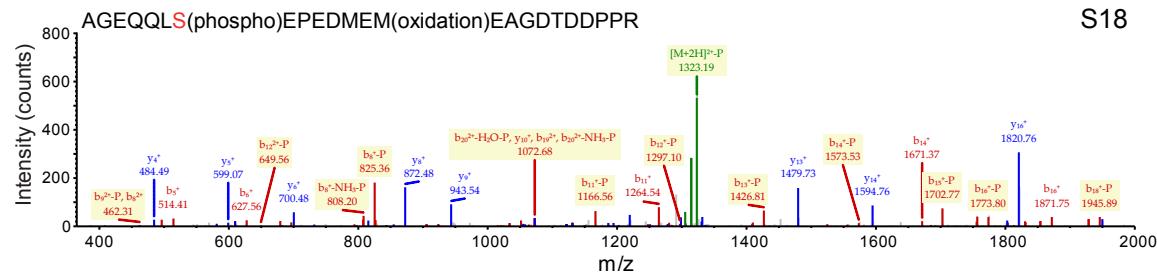
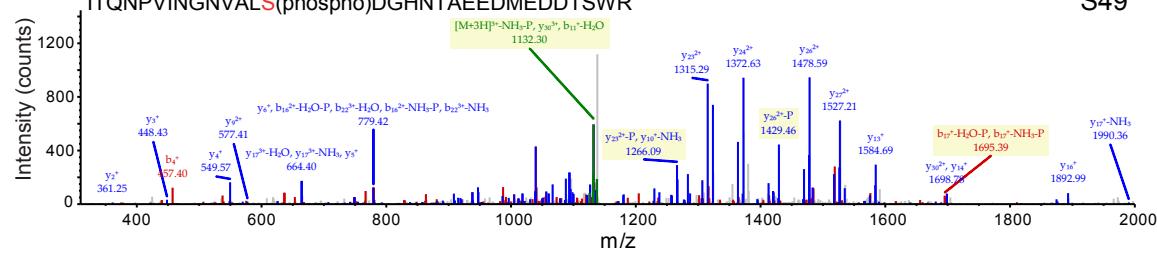


Figure S4 (Figure 4 continued). USP7 Deubiquitinates MDC1 but Not the MRN Complex. (A) HeLa cells were cultured in the absence or presence of increasing amounts of GNE-6640 for 24 hours as indicated. Cellular extracts and total RNAs were collected for Western blotting (left panel) and qRT-PCR (right panel) analysis, respectively. Each bar represents the mean \pm S.D. from biological triplicate experiments. P values were calculated by one-way ANOVA. (B) Analogous to (A) for GNE-6776. Each bar represents the mean \pm S.D. from biological triplicate experiments. P values were calculated by one-way ANOVA. (C) HeLa cells stably expressing FLAG-MDC1 were transfected with HA-Ub/wt and cultured in the presence or absence of GNE-6640 or GNE-6776. Cellular extracts were immunoprecipitated with anti-FLAG followed by IB with anti-HA. (D) HeLa cells stably expressing control vector or Myc-USP7 were co-transfected with FLAG-MRE11 and HA-Ub/wt, HA-Ub/K48-only or HA-Ub/K63-only as indicated. Cellular extracts were immunoprecipitated with anti-FLAG followed by IB with anti-HA. (E) Analogous to (D) for RAD50. (F) Analogous to (D) for NBS1.

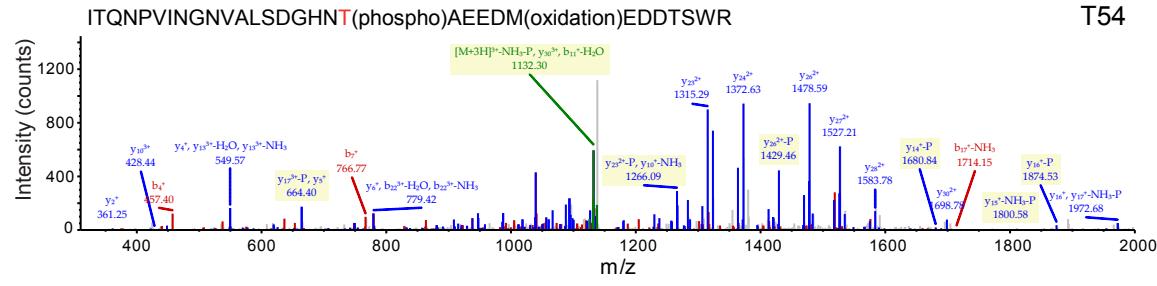
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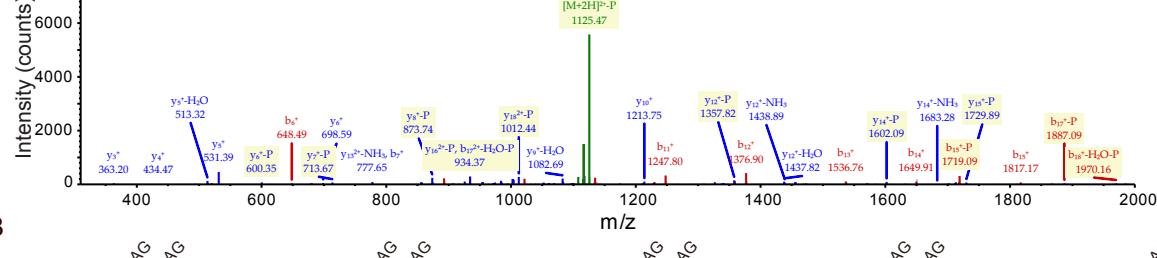
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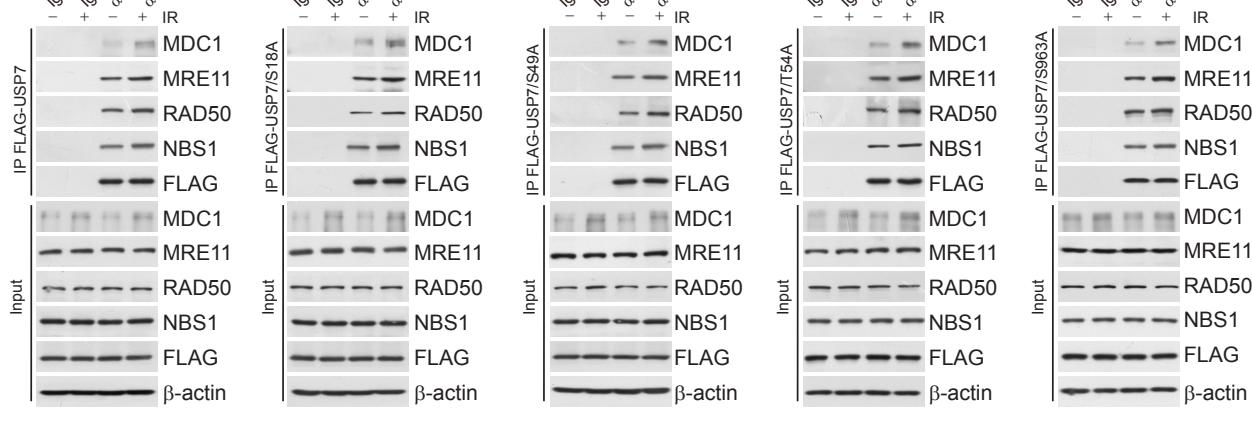
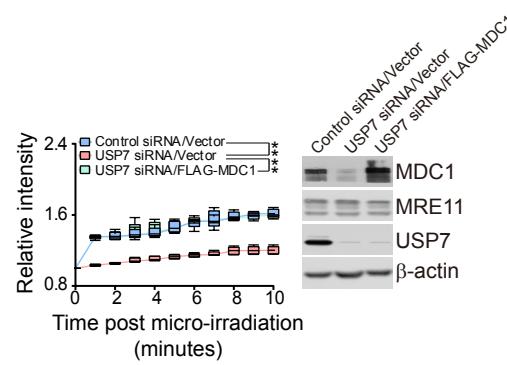
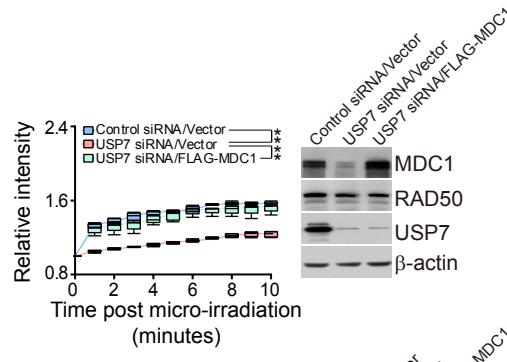


Figure S5 (Figure 5 continued). USP7-promoted MDC1 Stabilization Is Potentiated by DNA Damage. (A) Mass spectrometry analysis of USP7 phosphorylation sites under DNA damage. HeLa cells stably expressing FLAG-USP7 were exposed to IR (10 Gy) and cellular extracts were prepared 1 hour later with high salt buffer followed by FLAG affinity gel purification in the presence of phosphatase inhibitors. After trypsinization, the retrieved peptides were subjected to mass spectrometry analysis. Fragmentation spectra of the identified USP7 peptides with phosphorylation are shown. (B) Co-immunoprecipitation analysis with cellular extracts from HeLa cells expressing the indicated USP7 mutants. HeLa cells were transfected with USP7 mutants carrying S18A, S49A, T54A or S963A and whole cell lysates from these cells were immunoprecipitated with anti-FLAG and then immunoblotted with antibodies against the indicated proteins.

A



B



C

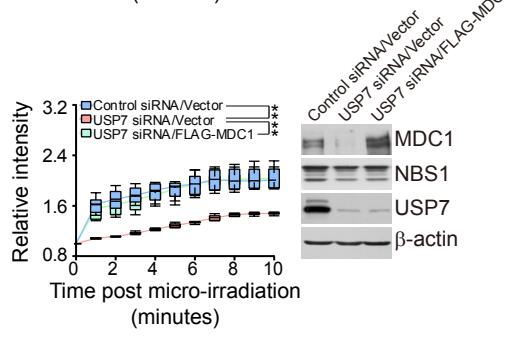


Figure S6 (Figure 6 continued). MDC1 Overexpression Is Able to Restore the Recruitment of the MRN Complex in USP7 Deficient Cells. (A) HeLa cells stably expressing control vector or FLAG-MDC1 were co-transfected with the indicated siRNAs and GFP-MRE11. Then, cells were subjected to UV-laser micro-irradiation and live cell imaging analysis at the indicated time points. Relative fluorescent intensities in micro-irradiated areas to the background signal of the undamaged regions were determined and shown. More than 20 nuclei were scored from biological triplicate experiments. ** $P<0.01$, two-way ANOVA. The expression of the indicated proteins was examined by Western blotting. For MRE11 bands the higher one with larger molecular weight represents GFP-tagged MRE11, while the lower one indicates endogenous MRE11. (B) Analogous to (A) for GFP-RAD50. ** $P<0.01$, two-way ANOVA. (C) Analogous to (A) for GFP-NBS1. ** $P<0.01$, two-way ANOVA.

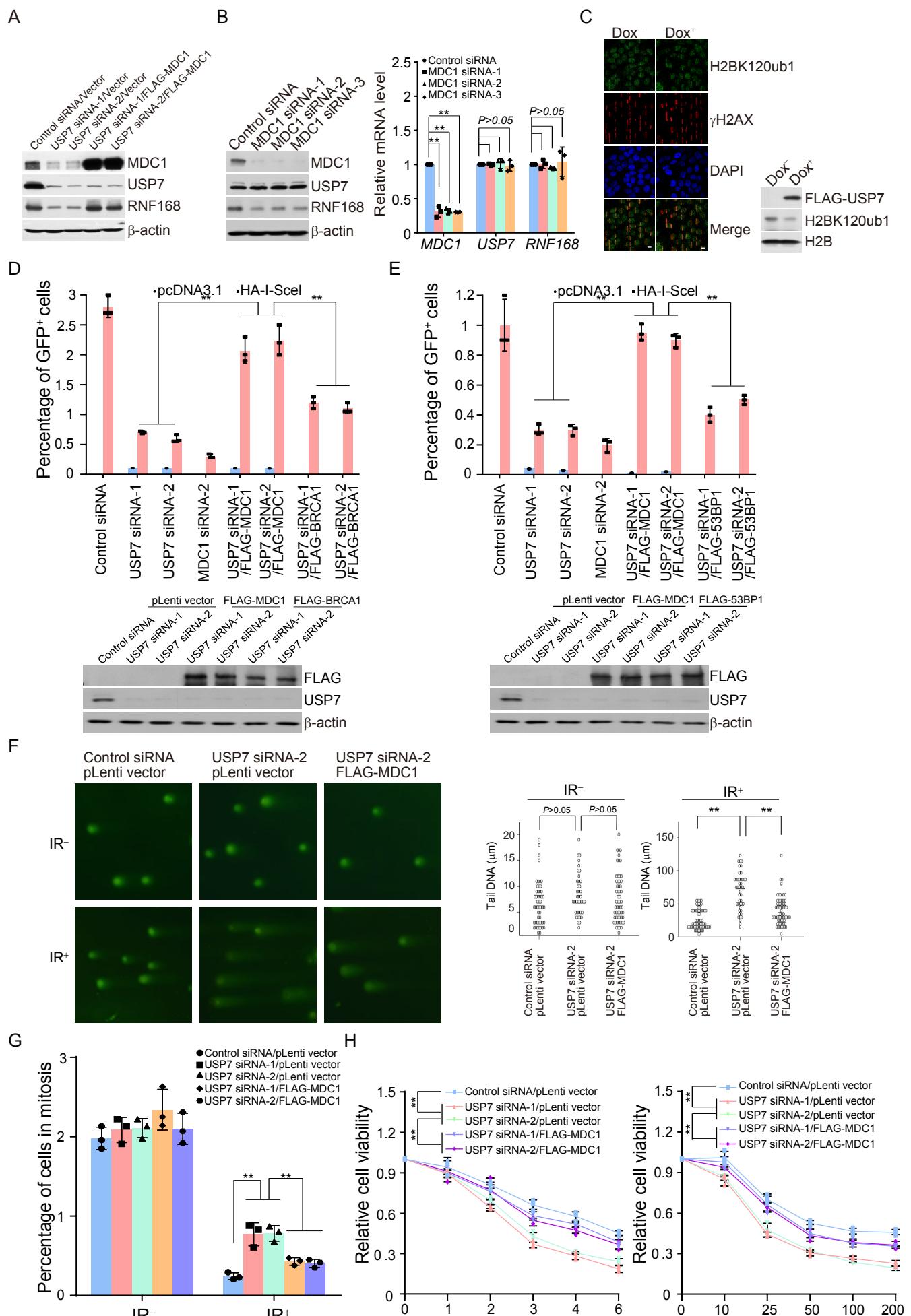


Figure S7 (Figure 6 continued). MDC1 Is an Essential Mediator in USP7-regulated DNA Damage Response. (A) HeLa cells stably expressing control vector or FLAG-MDC1 were transfected with the indicated siRNAs. Cellular extracts were prepared and analyzed by Western blotting. (B) HeLa cells were transfected with control siRNA or different sets of MDC1 siRNAs. Cellular extracts and total RNAs were prepared and analyzed by Western blotting (left panel) and qRT-PCR analysis (right panel), respectively. (C) HeLa cells with Dox-inducible expression of FLAG-USP7 in the absence or presence of Dox were subjected to UVA-laser microdissection, fixed and immunostained with antibodies against H2BK120ub1 and γH2AX followed by confocal microscopy analysis. Scale bar, 10 μm. The expression of the indicated proteins and the level of H2BK120ub1 were examined by Western blotting. (D) HR efficiency was determined by FACS in DR-GFP U2OS cells with depletion or overexpression of genes as indicated (upper panel). The knockdown or/and overexpression efficiency was determined by Western blotting (lower panel). (E) NHEJ efficiency was determined by FACS in EJ5-U2OS cells with depletion or overexpression of genes as indicated (upper panel). The knockdown or/and overexpression efficiency was determined by Western blotting (lower panel). (F) Efficiency of DSB repair analyzed by neutral comet assay after IR (6 Gy) in U2OS cells with depletion or overexpression of genes as indicated. Representative images (left panel) and quantitative data (right panel) around 50 cells in each case are shown. (G) Analysis of the G₂/M checkpoint. U2OS cells with depletion or overexpression of genes as indicated were untreated or X ray-irradiated (4 Gy), then incubated for 4 h before fixation. Mitotic cells were determined by phospho-histone H3 staining and FACS analysis. (H) Viability analysis was performed with U2OS cells expressing the indicated siRNAs or genes under different doses of IR or CPT treatment. In (B), (D), (E), (G), and (H), each bar represents the mean ± S.D. from biological triplicate experiments. **P<0.01. One-way ANOVA for (B), (D), (E), (F) and (G); two-way ANOVA for (H).

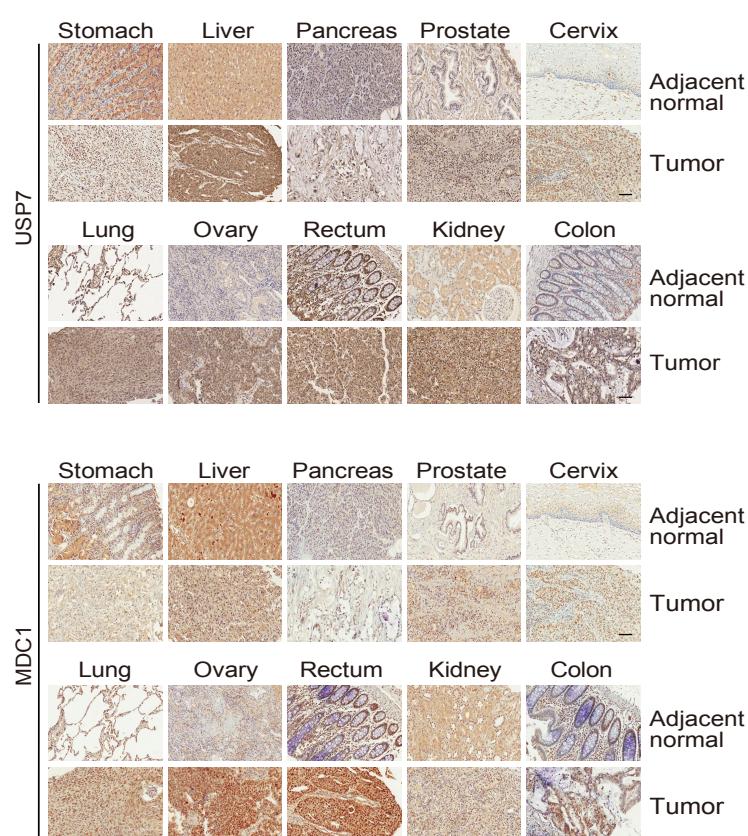


Figure S8. IHC Analysis of the Expression of USP7 and MDC1 in Multiple Tumor Samples. Human tissue arrays including series of paired normal and tumor samples as indicated were used to examine the expression profiles of USP7 and MDC1. Representative images (200 X magnification) from three paired samples in each case are shown. Scale bar, 50 μm .

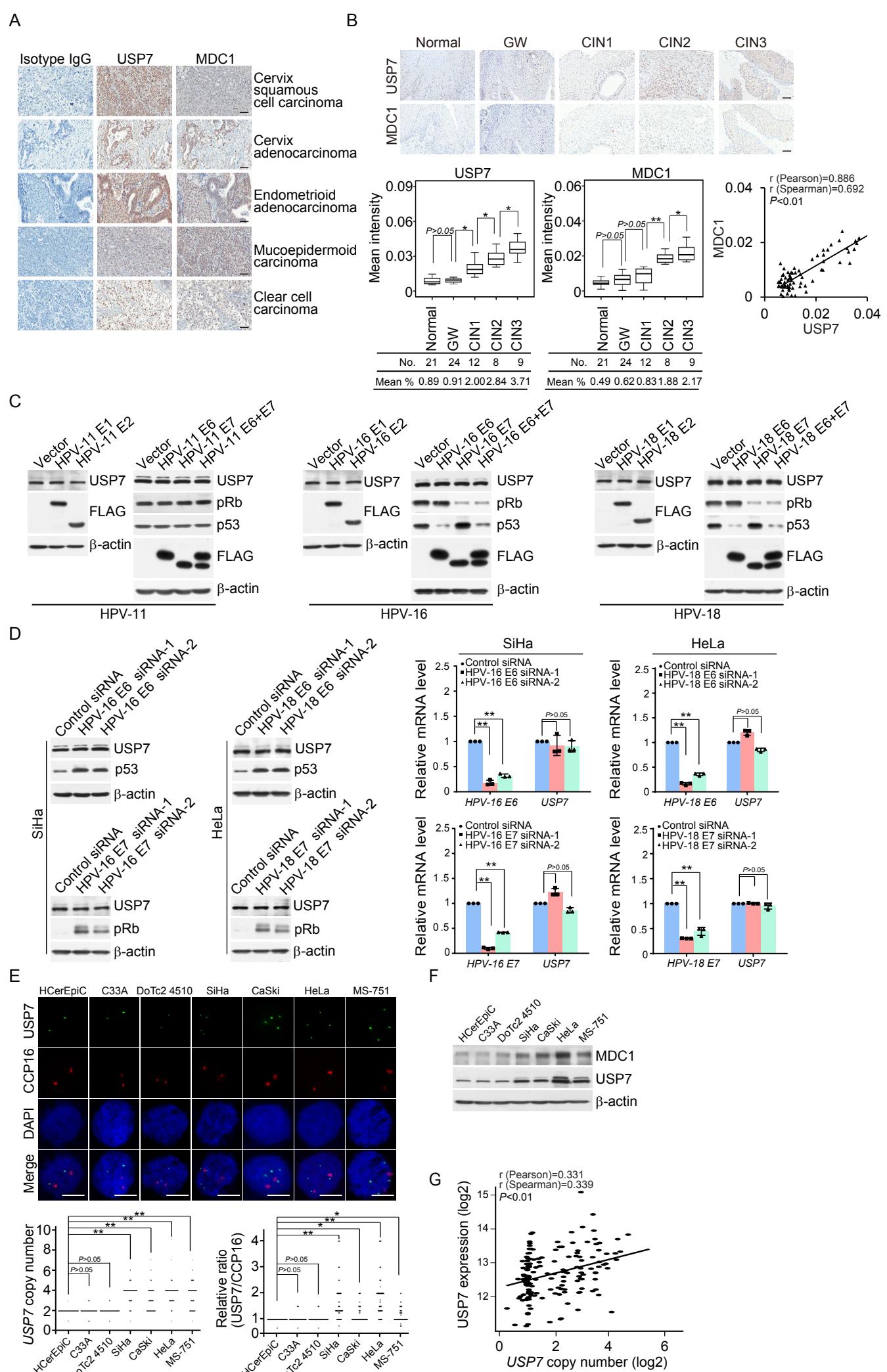


Figure S9 (Figure 7 continued). USP7 Is Implicated in Cervical Carcinogenesis and Patient Survival. (A) Uterine tissue samples derived from the same patient were immunostained with isotype IgG or corresponding antibodies against the indicated proteins. Representative images are shown. Scale bar, 50 μ m. (B) Human tissues containing genital warts (GW), CIN lesions and normal cervical samples were analyzed by immunohistochemical stainings. Representative images are shown (upper panel). Scale bar, 50 μ m. The values of the stainings were determined and presented with box plots. * $P<0.05$; ** $P<0.01$. One-way ANOVA. The correlation coefficient and P values were analyzed as indicated (lower panel). (C) FLAG-tagged E1, E2, E6, E7 or bicistronic E6/E7 (E6+E7) from HPV-11, HPV-16 or HPV-18, was transfected into HPV negative C33A cervical cancer cells, and cellular lysates were collected and analyzed by Western blotting. (D) Different sets of siRNAs targeting E6 or E7 of HPV-16, or E6 or E7 of HPV-18 were transfected into SiHa or HeLa cells, respectively, and cellular lysates were collected and analyzed by Western blotting (left panel) or q(RT)-PCR (right panel). Each bar represents the mean \pm S.D. from biological triplicate experiments. ** $P<0.01$, one-way ANOVA. (E) DNA fluorescence in situ hybridization (FISH) assays with USP7 FISH probe (16p13.2, green) and chromosome 16 centromeric probe (CCP16, red) to examine USP7 copy number in human normal cervical cells and cervical cancer cells followed by confocal microscopy analysis. Cells were synchronized at G₁ phase by double-thymidine block before hybridization. Scale bar, 10 μ m. Representative images (upper panel) and the distribution of copy numbers in each type of cells with around 100 cells (lower panel) are shown. * $P<0.05$; ** $P<0.01$. One-way ANOVA. (F) Cellular extracts of cells used in (E) were collected and analyzed by Western blotting. (G) Coefficient analysis of USP7 copy number and its mRNA expression level in cervical carcinoma samples with TCGA dataset (<https://portal.gdc.cancer.gov/projects/TCGA-CESC>).

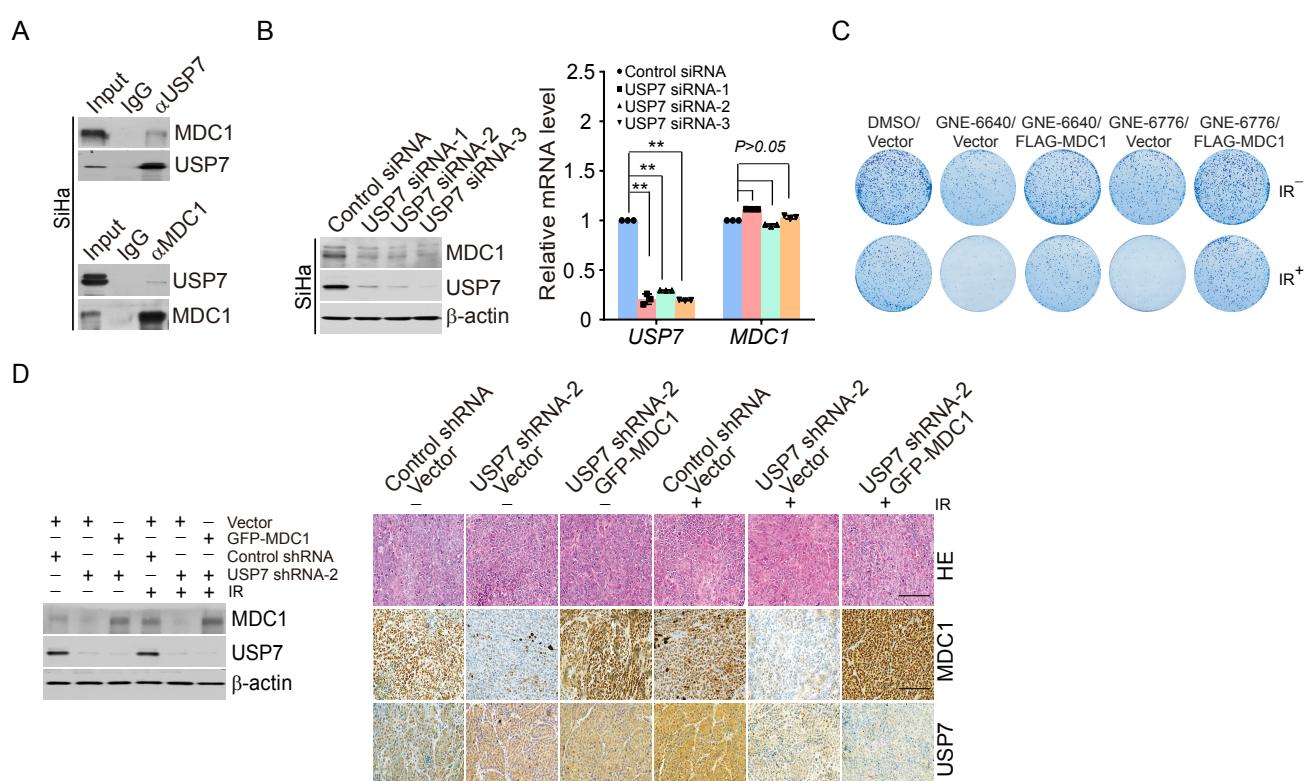


Figure S10 (Figure 8 continued). USP7-promoted MDC1 Stabilization Is Required for the Growth of Cervical Cancer Cells. (A) Whole cell lysates from SiHa cells were immunoprecipitated and then immunoblotted with antibodies against the indicated proteins. (B) SiHa cells were transfected with control siRNA or different sets of USP7 siRNAs. Cellular extracts and total RNAs were prepared and analyzed by Western blotting (left panel) and qRT-PCR (right panel), respectively. ** $P<0.01$, one-way ANOVA. (C) SiHa Cells stably expressing control vector or FLAG-MDC1 were cultured in the presence or absence of GNE-6640 (7.5 μ M) or GNE-6776 (10 μ M) followed by X-ray irradiation (2 Gy). Colony formation assays were conducted and representative images from biological triplicate experiments are shown. (D) The expression levels of the indicated proteins in SiHa tumors were examined by Western blotting and immunohistochemical stainings. Scale bar, 50 μ m.

SUPPLEMENTAL METHODS

Antibodies and Reagents

The sources of antibodies against the following proteins were as follows: HA (sc-805) from Santa Cruz Biotechnology; β -actin (A1978) and FLAG (F3165) from Sigma; γ H2AX (05-636, for IF and ChIP) and USP7 (05-1946, for WB, IF, IP and ChIP) from Millipore; Ubiquityl-Histone H2B (K120) (#5546, for IF), BRCA1 (#9010S, for WB and IF), 53BP1 (#4837S, for WB and IF) from Cell Signaling Technology; MRE11 (ab214, for WB, IF and IP), RAD50 (ab89, for WB, IF and IP), NBS1 (ab32074, for WB, IF and IP), γ H2AX (ab2893, for WB and IF), H2B (ab52484) and Histone H3 (ab1791) from Abcam; USP7 (A300-033A, IP, IF and IHC), MDC1 (A300-503A, IP and IF) from Bethyl Lab; RNF168 (21393-1-AP), MDC1 (24721-1-AP, WB and IHC) and His (66005-1-Ig) from Proteintech; and Myc (M047-3) from MBL; anti-HA affinity gel (E6779), anti-FLAG M2 affinity gel (A2220), 3 \times FLAG peptide (F4799), MG132 (SML1135), 4-OHT (H7904), blasticidin (15205), puromycin (P8833) and doxycycline (D9891) were purchased from Sigma. Ni-NTA Purification System (K950-01) was purchased from Invitrogen. CHX and HBX 41,108 were purchased from TOCRIS. DNase I (GD201-01) was purchased from Transgen Biotech. Mitomycin C ((M4287) was purchased from sigma. GNE-6640 and GNE-6776 were purchased from Glixx Laboratories.

Plasmids

The FLAG- or Myc-tagged USP7/wt was amplified from USP7 cDNA kindly provided by Dr. Yang Shi (Harvard Medical School, Boston) and Dr. Ruaidhri J. Carmody (University of Glasgow, Scotland, UK) and integrated into pLVX-Tight-Puro or pLenti-hygro vector, while the FLAG-tagged USP7/C223S carried by pLVX-Tight-Puro or pLenti-hygro vector was generated by quick change point mutation assay. The GFP-tagged deletion mutants of USP7 carried by pLenti-NLS (nuclear localization signal)-GFP-Puro vector were generated by PCR and standard cloning procedure. His-tagged USP7/wt, USP7/C223S, and deletion mutants of USP7 were carried by pFastBac-HTA vector. The deletion mutants of MDC1 including FLAG-tagged N1, N2, N3, C1 and C2 were gifts from Pro. Xingzhi Xu (Shenzhen University, Shenzhen, China) and Dr. Yue Zhao (China Medical University, Shenyang, China). The FLAG-tagged PST and BRCT carried by pcDNA3.1 vector or GST-tagged versions in pGEX4T-3 vector were amplified from FLAG-MDC1 kindly provided by Pro. Junjie Chen (MD Anderson Cancer Center, The University of Texas, Texas), while GST-PST was a gift from Dr. Manuel Stucki (University of Zürich, Zürich, Switzerland) and GST-BRCT was a gift from Dr. Michal Goldberg (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem, Israel). GFP-MDC1 (Addgene plasmid #26285) in pLenti vector was a gift from Dr. Eric Campeau (Zenith Epigenetics Ltd, Calgary, Canada) and FLAG-tagged MDC1 carried by pLenti-hygro vector was generated by PCR and standard cloning procedure. The MRE11 construct was a gift from Dr. Xiaohua Wu

(Burnham Institute for Medical Research, La Jolla, CA), while NBS1 and RAD50 were gifts from Pro. Junjie Chen (MD Anderson Cancer Center, The University of Texas, Texas). The FLAG-tagged MRE11, RAD50 and NBS1 used in vitro transcribed/translated proteins were carried by pcDNA3.1 vector, and the GFP- or FLAG-tagged MRE11, RAD50 and NBS1 were carried by pLVX-GFP-Puro or pLVX-puro vector. GST-NBS1 was a gift from Dr. Weiguo Zhu (Department of Biochemistry and Molecular Biology, Shenzhen University, Shenzhen, China) and GST-tagged deletion mutants were further generated. The Myc-RNF168 was a gift from Dr. Michael Shing-Yan Huen (The University of Hong Kong, Hong Kong Special Administrative Region, China). The HA-AsiSI in pLVX-puro vector was derived from pBabe HA-AsiSI kindly provided by Dr. Gaelle Legube (Paul Sabatier University, Toulouse, France). CRISPR/Cas9 constructs lentiCas9-Blast (Addgene plasmid # 52962) and lentiGuide-Puro (Addgene plasmid # 52963) were gifts from Dr. Feng Zhang (Broad Institute, Cambridge), and HA-tagged ubiquitin K48-only (Plasmid #17605) and K63-only (Plasmid #17606) were gifts from Dr. Ted Dawson (Johns Hopkins University School of Medicine, Baltimore). FLAG-tagged E1, E2, E6, E7, or bicistronic E6/E7 of HPV-11, HPV-16 or HPV-18, was chemically synthesized and integrated into pLVX-puro vector by Youbio (Hunan, China).

Cell Culture

U2OS, MCF-7, HEK293T, SiHa, HeLa, C33A, DoTc2 4510, CaSki, MS-751 and Sf9

cells were got from the American Type Culture Collection (Manassas, VA) and cultured under the manufacturer's instructions. HCerEpiC was got from ScienCell Research Laboratories (Carlsbad, CA) and cultured under the manufacturer's instructions. Cells that allow protein expression under doxycycline treatment were created in two steps. First, cells were infected with lentivirus carrying rtTA and subjected to Neomycin selection. Subsequently, the established rtTA cells were infected with virus carrying pLenti-Tight-Puro vector that encodes USP7/wt or USP7/C223S followed by puromycin selection. All of the cells integrated with rtTA were cultured in Tet Approved FBS and medium from Clontech. All of the Cells were authenticated by examination of morphology and growth characteristics and confirmed to be mycoplasma free.

USP7 Knockout Cell Generation

USP7 knockout HeLa cells were generated by co-transfection of plasmid encoding FLAG-Cas9 (lentiCas9-Blast) and sgRNA plasmid (lentiGuide-Puro) targeting USP7 (AATCAGATTCAAGCATTGCAC). Forty-eight hours after transfection, cells were selected by blasticidin (5 µg/ml) and puromycin (1 µg/ml) for 2 days.

Immunopurification and Silver Staining

Lysates from HeLa cells stably expressing FLAG-MDC1, FLAG-NBS1 or FLAG-MRE11 were prepared by incubating the cells in lysis buffer containing protease inhibitor cocktail (Roche). Anti-FLAG immunoaffinity columns were prepared using

anti-FLAG M2 affinity gel (Sigma) following the manufacturer's suggestions. Cell lysates were obtained from about 5×10^7 cells and applied to an equilibrated FLAG column of 1 ml bed volume to allow for adsorption of the protein complex to the column resin. After binding, the column was washed with cold PBS plus 0.2% Nonidet P-40. FLAG peptide (Sigma) was applied to the column to elute the FLAG protein complex as described by the vendor. The elutes were collected and visualized on NuPAGE 4-12% Bis-Tris gel (Invitrogen) followed by silver staining with silver staining kit (Pierce). The distinct protein bands were retrieved and analyzed by LC-MS/MS.

Nano-HPLC-MS/MS analysis

LC-MS/MS analysis was performed using a Thermo Finnigan LTQ linear ion trap mass spectrometer in line with a Thermo Finnigan Surveyor MS Pump Plus HPLC system. Tryptic peptides generated were loaded onto a trap column (300SB-C18, 5 X 0.3mm, 5 μ m particle) (Agilent Technologies, Santa Clara CA) which was connected through a zero dead volume union to the self-packed analytical column (C18, 100 μ m i.d X 100mm, 3 μ m particle) (SunChrom, Germany). The peptides were then eluted over a gradient (0-45% B in 55 minutes, 45-100% B in 10 minutes, where B = 80% Acetonitrile, 0.1% formic acid) at a flow rate of 500 nL/min and introduced online into the linear ion trap mass spectrometer (Thermo Fisher Corporation, San Jose, CA) using nano electrospray ionization (ESI). Data dependent scanning was incorporated to select the 5 most abundant ions (one microscan per spectra; precursor isolation width 1.0 m/z, 35% collision energy,

30 ms ion activation, exclusion duration: 90s; repeat count: 1) from a full-scan mass spectrum for fragmentation by collision induced dissociation (CID). MS data were analysed using SEQUEST (v. 28) against NCBI human protein database (Dec, 14, 2011 downloaded, 33,256 entries), and results were filtered, sorted, and displayed using the Bioworks 3.2. Peptides (individual spectra) with Preliminary Score (Sp) ≥ 500 ; Rank of Sp (RSp) ≤ 5 ; and peptides with +1, +2, or +3 charge states were accepted if they were fully enzymatic and had a cross correlation (Xcorr) of 1.90, >2.75, and >3.50, respectively. At least two distinct peptides were assigned to each identified protein. The following residue modifications were allowed in the search: carbamidomethylation on cysteine as fix modification and oxidation on methionine as variable modification. Peptide sequences were searched using trypsin specificity and allowing a maximum of two missed cleavages. Sequest was searched with a peptide tolerance of 3 Da and a fragment ion tolerance of 1.0 Da.

LC-MS/MS Analysis of USP7 Phosphorylation Sites

Cells stably expressing FLAG-USP7 were exposed to IR (10 Gy) and cellular extracts were prepared with high salt buffer followed by FLAG affinity gel purification in the presence of phosphatase inhibitors. LC-MS/MS analysis was performed using a nanoLC-LTQ-Orbitrap XL mass spectrometer (Thermo, San Jose, CA) in line with a Eksigent nano LC 1D plus HPLC system. Tryptic peptides generated above were loaded onto a self-packed trap column (ReproSil-Pur C18-AQ, 150 μ m i.d. X 2mm, 5 μ m particle)

(Dr. Maisch GmbH, Ammerbuch) which was connected to the self-packed analytical column (ReproSil-Pur C18-AQ, 75 μ m i.d X 200mm, 3 μ m particle). The peptides were then eluted over a gradient (0-36% B in 78 minutes, 36-80% B in 12 minutes, where B = 100% Acetonitrile, 0.5% formic acid) at a flow rate of 300nL/min and introduced online into the linear ion trap mass spectrometer using nano electrospray ionization (ESI). Data dependent scanning was incorporated to select the 10 most abundant ions (one microscan per spectra; precursor isolation width \pm 1.0m/z, 35% collision energy, 30ms ion activation, exclusion duration: 120s; repeat count: 1) from a full-scan mass spectrum (300 to 1800 m/z) for fragmentation by collision induced dissociation (CID). Lock mass option was enabled for the 462.14658 m/z. MS data were analyzed using Proteome Discoverer (version 1.4.0.288, Thermo Scientific). MS2 spectra were searched with SEQUEST engine against uniprot human protein database. Peptides with and above +2 charge states were accepted if they were fully enzymatic. The following residue modifications were allowed in the search: carbamidomethylation on Cysteine (Static modification), oxidation on Methionine, phosphorylation on Serine, Threonine and Tyrosine (Dynamic modification). Sequest was searched with a peptide tolerance of 20 ppm and a fragment ion tolerance of 0.6 Da. Peptide spectral matches (PSM) were validated by a targeted decoy database search at 1% false discovery rate (FDR). Peptide identifications were grouped into proteins according to the law of parsimony.

FPLC Chromatography

HeLa nuclear extracts, FLAG-USP7- or FLAG-MDC1-containing protein complexes were applied to a Superose 6 size exclusion column (GE healthcare) that had been equilibrated with dithiothreitol-containing buffer and calibrated with protein standards (Amersham Biosciences). The column was eluted at a flow rate of 0.5 ml/min and fractions were collected.

Immunoprecipitation

Cell lysates were prepared by incubating the cells in NETN buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.2% Nonidet P-40, 2 mM EDTA) in the presence of protease inhibitor Cocktails (Roche) for 20 min at 4 °C. This was followed by centrifugation at 14,000 g for 15 min at 4 °C. For immunoprecipitation, about 500 µg of protein was incubated with control or specific antibodies (1-2 µg) for 12 hours at 4 °C with constant rotation; 50 µl of 50% protein G magnetic beads (Invitrogen) was then added and the incubation was continued for an additional 2 hours. Beads were then washed five times using the lysis buffer. Between washes, the beads were collected by magnetic stand (Invitrogen) at 4 °C. The precipitated proteins were eluted from the beads by re-suspending the beads in 2 × SDS-PAGE loading buffer and boiling for 5 min. The boiled immune complexes were subjected to SDS-PAGE followed by immunoblotting with appropriate antibodies.

Lentiviral Production

The shRNAs targeting USP7 (Sigma) in pLKO.5 vector or vectors encoding rtTA, USP7, MDC1 carrying by pLenti vectors, as well as three assistant vectors: pMDLg/pRRE, pRSV-REV, and pVSVG, were transiently transfected into HEK293T cells. Viral supernatants were collected 48 hours later, clarified by filtration, and concentrated by ultracentrifugation.

In Vivo Deubiquitination Assay

Cells with different treatments were lysed in RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.1% SDS and protease inhibitor at 4 °C for 30 min with rotation, and centrifuged at 20,000 g for 15 min. About 0.5-1.5 mg of cellular extracts were immunoprecipitated with anti-FLAG agarose affinity gel for 2 hours. The beads were then washed five times with RIPA buffer, boiled in SDS loading buffer and subjected to SDS-PAGE followed by immunoblotting.

In Vitro Deubiquitination Assay

HeLa cells expressing full-length MDC1 and HA-ubiquitin were collected and then lysed in RIPA Buffer (300 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, and 50 mM Tris-HCl, pH 8.0). The resulting lysate was purified with anti-FLAG affinity gel, eluted with 3 × FLAG peptide, and then subjected to HA affinity gel to enrich HA-Ub conjugated MDC1 (MDC1-Ub). His-tagged USP7/wt or USP7/C223S was affinity purified using Nickel-chelating resin from extracts of baculovirus-infected insect

cells. Recombinant MDC1-Ub and USP7/wt or USP7/C223S were then incubated in DUB buffer (50 mM Tris-HCl, pH 8.0; 50 mM NaCl; 1 mM EDTA; 10 mM DTT and 5% glycerol) at 37 °C for 2 hours. The reactions were stopped by boiling for 5 min in 5 × SDS-PAGE loading buffer, and the boiled protein complexes were subjected to SDS-PAGE followed by immunoblotting with appropriate antibodies.

Recombinant Protein Purification and Pull-down Assays

Recombinant baculovirus carrying full-length USP7/wt, USP7/C223S, or deletion mutants of USP7 was generated with the Bac-to-Bac System (Invitrogen). Infected Sf9 cells were grown in spinner culture for 48 to 96 hours at 27 °C and His-tagged protein purified using Ni²⁺-NTA agarose (Invitrogen) according to standard procedures. For His pull-down assay, His-tagged protein were incubated with recombinant MRE11, RAD50 or NBS1 that was in vitro transcribed and translated according to the manufacturer's procedures (TNT T7 Quick Coupled Transcription/Translation Kit; Promega, Leiden, The Netherlands) at 4 °C overnight. GST-fusion proteins were purified from *Escherichia coli* by glutathione-Sepharose 4B beads (GE Health Care) and then washed with high salt buffer (20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, and 300 mM NaCl). For GST pull-down assay, GST-fusion proteins were incubated with His-tagged proteins or in vitro transcribed and translated proteins at 4 °C overnight. The beads were washed 3 times, then boiled in SDS loading buffer and subjected to SDS-PAGE followed by immunoblotting.

RNA Interference

All siRNA transfections were performed using Lipofectamine RNAi MAX (Invitrogen) following the manufacturer's recommendations. The final concentration of the siRNA molecules is 10 nM and cells were harvested 72 or 96 hours later according to the purposes of the experiments. Control siRNA (ON-TARGETplus Non-Targeting Pool, D-001810-10), USP7 siRNA (ON-TARGETplus, L-006097-00-0005) and MDC1 siRNA (siGENOME, M-003506-04-0005) were got from Dharmacon in a smart pool manner, while the individual siRNAs against USP7, MRN complex and MDC1 were chemically synthesized by Sigma (Shanghai, China).

qRT-PCR

Total cellular RNA was isolated with TRIzol reagent (Invitrogen) and used for first strand cDNA synthesis with the Reverse Transcription System (Roche). Quantitation of all gene transcripts was done by qPCR using a Power SYBR Green PCR Master Mix (Roche) and an ABI PRISM 7500 sequence detection system (Applied Biosystems) with the expression of *GAPDH* as the internal control.

Chromatin Immunoprecipitation

About 10 million cells were crosslinked with 1% formaldehyde for 10 min at room temperature and quenched by the addition of glycine to a final concentration of 125 mM for 5 min. The fixed cells were resuspended in SDS lysis buffer (1% SDS, 5 mM EDTA,

50 mM Tris-HCl pH 8.1) in the presence of protease inhibitors and subjected to 3 x 10 cycles (30 seconds on and 30 seconds off) of sonication (Bioruptor, Diagenode) to generate chromatin fragments of ~300 bp in length. Lysates were diluted in buffer containing 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl. For immunoprecipitation, the diluted chromatin was incubated with control or specific antibodies (2 µg) for 12 hours at 4 °C with constant rotation; 50 µl of 50% protein G magnetic beads was then added and the incubation was continued for an additional 2 hours. Beads were then washed with the following buffers: TSE I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), buffer III (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1), and Tris-EDTA buffer. Between washes, the beads were collected by magnetic stand at 4 °C. Then the pulled down chromatin complex together with input were de-crosslinked at 70 °C for two hours in elution buffer (1% SDS, 5 mM EDTA, 20 mM Tris-HCl, pH 8.1, 50 mM NaCl, 0.1 mg/ml Proteinase K). Eluted DNA was purified with PCR purification kit (Qiagen) and analyzed by qPCR.

Colony Formation Assay

HeLa or SiHa cells stably expressing shRNAs and GFP or GFP-MDC1/FLAG-MDC1 were cultured in the presence of vehicle or USP7 inhibitors for 48 hours followed by X-ray irradiation. Cells were then maintained for 14 days, fixed with methanol and

stained by crystal violet.

HR and NHEJ Reporter Assay

HR efficiency was examined with DR-GFP U2OS cells, in which two incomplete copies of GFP genes are integrated into chromosomal DNA and cleavage of the I-SceI sites leads to the restoration of GFP gene through HR, while NHEJ efficiency was determined with EJ5-U2OS cells, in which excision of the two I-SceI sites followed by NHEJ eliminates the translation start codon of the otherwise non-sense transcript and enables the reading frame shift and subsequently expression of the GFP gene. Percentage of GFP positive cells was counted by FACS analysis with Accuri C6 (BD Biosciences). For each treatment, a minimum of 10,000 cells were analyzed by FACS. Data analysis was done using Flowjo software.

Cell Viability Assay

Cells plated on 96-well dishes were cultured in the presence of compound for 72 hours or transfected with siRNAs for 48 hours. Then cells were exposed to IR and cultured for another 72 hours before assaying viability. Specifically, Cell Titer Aqueous One Solution Reagent (G3582, Promega) was added to each well according to the manufacturer's instructions and cell viability was determined after 1 hour incubation by measuring the absorbance at 490 nm using a 550 BioRad plate-reader (Bio-Rad, Hertfordshire, UK).

Comet Assay

Cells were exposed to IR (6 Gy) followed by 1 h recovery in culture medium. Neutral comet assays were conducted with the Comet Assay kit (Trevigen) using GelBond films (Lonza) to support agarose gels. Samples stained with SYBR–Green I were observed under an epifluorescence microscope. Images were analyzed with Casplab-Comet Assay Software by scoring around 50 cells in each case.

G₂/M Checkpoint Assay

U2OS cells stably expressing control vector or FLAG-MDC1 were transfected with siRNAs. Cells were untreated or X ray-irradiated (4 Gy), then incubated for 4 h at 37 °C before fixation. Mitotic cells were determined by phospho-histone H3 staining and FACS analysis.

FISH Analysis

The slide samples from tumor or normal cells were hybridized by 2-color FISH with a *USP7*-specific probe (CT-LSP447-10-G, CytoTest, Rockville, MD) and a chromosome 16-specific centromeric probe (CT-CCP016-10-O, CytoTest, Rockville, MD). The *USP7* and centromere 16 probes were hybridized with nuclei from cells synchronized in G₁ phase of the cell cycle by double-thymidine block. We counterstained slides with DAPI and used the Olympus confocal microscope to capture FISH images. Results were expressed as a ratio of the number of copies of the *USP7* gene to the number of

chromosome 16-centromeric markers. A ratio of greater than 2 indicated gene amplification.

Tissue Specimens

The tissue samples were obtained from surgical specimens from patients with genital warts, CIN lesions and cervical cancer. Samples were frozen in liquid nitrogen immediately after surgical removal and maintained at minus 80 °C until protein extraction. Human cervical tissues were prepared, incubated with antibodies against USP7 or MDC1 and processed for immunohistochemistry with standard DAB staining protocols. Images for normal (21), genital warts (24), CIN1 (12), CIN2 (8) and CIN3 (9); tumor adjacent normal (26) and cervical carcinoma (Grade I, 19; Grade II, 54; Grade III, 20) samples were collected under microscopy with 200 X magnification. The image quality was evaluated and the background with uneven illumination was corrected with Image-Pro Plus software. Then, the cervical cells or cervical carcinoma cells were selected as region of interest (ROI) according to morphology features of the tissue or cells. The scores of the stained sections were determined by evaluating the mean intensity and nuclear staining extent of immunopositivity following the instructions of Image-Pro Plus software. All studies were approved by the Ethics Committee of the Tianjin Medical University, and informed consent was obtained from all patients.

Tumor Xenografts

SiHa cells were plated and infected in vitro with mock or lentiviruses carrying control shRNA, USP7 shRNA together with pLenti vector or GFP-tagged MDC1. Forty-eight hours after infection, 3×10^6 viable SiHa cells in 100 μl PBS were injected into the 6- to 8-week-old female athymic mice (BALB/c; Charles River, Beijing, China). After one week of inoculation, tumors were received 10 Gy of IR exposure for half mice in each group, then tumor growth and mice weight were monitored over another 3 weeks. Six animals per group were used in each experiment. Tumors were measured weekly using a vernier calliper and the volume was calculated according to the formula: $\pi/6 \times \text{length} \times \text{width}^2$. The measurement and data processing were done with blinding. All studies were approved by the Animal Care Committee of Tianjin Medical University.

wLQDNLTTLR	5	3	1	Q92878	N-Term(Acetyl)	0	0	0.0000256	2.71	2	1200.63664	-0.46	21.65	0
NIDQGSEIVK	7	3	1	Q92878	C5(Carbamidomethyl)	0	0	0.00002701	2.71	2	1205.58183	-1.06	15.53	0
GYEEEIEHFK	6	3	1	Q92878		0	0	0.00002838	2.48	2	1264.6198	-0.85	22.18	0
EAQLTSEKIVK	2	5	1	Q92878		0	0	0.00002299	2.38	2	1332.73377	-0.91	11.49	1
EQEGIPLTK	4	3	1	Q92878	N-Term(Acetyl)	0	0	0.00002611	2.32	3	1575.56352	-0.06	15.53	2
DIEENYQPK	2	3	1	Q92878		0	0	0.00002245	2.17	2	1301.61529	-0.19	21.36	0
ykTCDFPPTK	5	5	1	Q92878	N-Term(Acetyl); C3(Carbamidomethyl)	0	0	0.00001786	2.36	3	1397.64031	-0.23	26.70	0
LEENDDNIK	2	3	1	Q92878		0	0	0.00002448	2.17	2	1087.56267	-0.33	16.07	0
qKFDEISATR	6	6	1	Q92878	N-Term(Acetyl)	0	0	0.00001665	3.06	2	1383.69231	1.4	23.27	1
DEMGLGVPMR	11	3	1	Q92878		0	0	0.00001783	2.89	2	1160.57952	-0.69	25.67	0
EMQMQLVQLQM	8	3	1	Q92878	N-Term(Acetyl)	0	0	0.00000659	2.81	2	1322.62505	-1.18	21.8	0
IAQDKLNIDK	1	3	1	Q92878	N-Term(Acetyl)	0	0	0.00001297	2.4	2	1199.65662	0.45	16.41	1
AMIGEVQSK	5	5	1	Q92878	N-Term(Acetyl)	0	0	0.00002163	2.67	2	1602.66645	-0.53	29.57	0
IQSTINQLQR	9	3	1	Q92878	N-Term(Acetyl)	0	0	0.00001355	2.39	2	1114.62604	-1.34	22.20	0
IENEDNDIK	4	3	1	Q92878	N-Term(Acetyl)	0	0	0.00001999	2.74	2	1129.57907	-2.32	20.5	0
VKEYQMEKL	8	5	1	Q92878		0	0	0.00001649	2.6	3	1167.60743	-0.38	15.22	1
DEmGLGVPMR	5	3	1	Q92878	M3(Oxidation)	0	0	0.00001843	2.44	2	1176.57402	-1.02	23.37	0
dEMGLGVPMR	3	3	1	Q92878	N-Term(Acetyl)	0	0	0.00001868	2.5	2	1202.59099	0.09	28.67	0
IQMMLMLTK	3	5	1	Q92878	N-Term(Acetyl)	0	0	0.00002373	2.28	2	1023.48369	-1.27	21.73	0
LQNMMNDIQK	1	3	1	Q92878		0	0	0.0000279	2.77	3	1255.61362	-0.12	10.18	1
TTIIHLK	9	5	1	Q92878	N-Term(Acetyl); C6(Carbamidomethyl)	0	0	0.00002799	2.52	2	1058.56365	0.26	19.74	0
RDEgALGLPmN	6	5	1	Q92878	M4(Oxidation); M10(Oxidation)	0	0	0.00002484	2.42	2	977.53374	0.11	20.90	0
vAQETDVR	2	5	1	Q92878	N-Term(Acetyl)	0	0	0.00004666	3.24	3	1348.67246	0.89	15.82	1
EVQSVLETTLEK	15	3	1	Q92878	M2(Oxidation)	0	0	0.00007435	3.02	2	1373.71477	-0.83	21.67	0
QRGVYEELHK	8	3	1	Q92878	M5(Oxidation)	0	0	0.00004815	2.89	3	1520.77448	0.06	18.32	1
LMRQDQDTDQK	3	3	1	Q92878	N-Term(Acetyl)	0	0	0.00005055	2.43	3	1247.61582	0.41	20.12	1
qEDELLFR	1	3	1	Q92878		0	0	0.00005132	1.94	2	1073.62102	0.81	14.59	1
QSHPLKEK	2	3	1	Q92878	M2(Oxidation); M5(Oxidation); M10(Oxidation)	0	0	0.00006002	2.45	2	1370.6082	-2.3	10.67	0
EnGQmQNLQmK	4	5	1	Q92878	N-Term(Acetyl); C6(Carbamidomethyl)	0	0	0.0000661	2.25	2	1019.54314	-1.04	22.05	0
TLDQDAIK	7	4	1	Q92878	M1(Oxidation)	0	0	0.00017154	2.26	2	919.49138	-0.44	17.2	0
mEVLSLQNEK	5	4	1	Q92878	M4(Oxidation); M10(Oxidation)	0	0	0.0007388	2.99	2	1206.60076	-2.3	16.42	0
QnEKDNENSELLEKMEK	1	5	1	Q92878	M5(Oxidation)	0	0	0.0008013	2.99	3	1883.83808	-1.14	11.1	2
YERBLTQVIR	3	3	1	Q92878	N-Term(Acetyl)	0	0	0.000322	2.53	3	1351.67580	0.86	16.91	1
SEVVELEYFR	1	3	1	Q92878	M2(Oxidation)	0	0	0.000882	2.53	3	1220.59997	1.01	16.36	1
VLASLILR	38	4	1	Q92878	N-Term(Acetyl)	0	0	0.0008814	2.66	2	884.59184	-1.09	24.56	0
vKEYQMEKL	1	5	1	Q92878	M4(Oxidation)	0	0	0.0009006	1.91	2	1209.61833	-0.08	17.97	1
FHSnKMEEINK	1	4	1	Q92878	N-Term(Acetyl); M2(Oxidation)	0	0	0.0001007	2.6	3	1409.65738	1.51	10.37	1
IAQDKLNIDK	11	3	1	Q92878	N-Term(Acetyl); C6(Carbamidomethyl)	0	0	0.0001033	2.94	3	1157.65198	-0.47	11.74	1
gDQEYIEHR	6	4	1	Q92878	M7(Oxidation)	0	0	0.0001074	2.42	2	1277.63054	-5.26	22.3	0
TLQDAIK	1	4	1	Q92878	N-Term(Acetyl)	0	0	0.00011469	1.98	2	935.88589	-0.86	15.6	0
asNLVTLK	6	4	1	Q92878	M1(Oxidation)	0	0	0.0001232	2.35	2	940.61485	-0.23	12.40	0
KGKEPLK	6	3	1	Q92878	M5(Oxidation)	0	0	0.0001332	2.41	3	1303.65379	-0.25	14.22	0
QKEPEHESATR	19	6	1	Q92878	N-Term(Acetyl)	0	0	0.0001387	3.03	3	1341.6803	0.37	21.14	1
cNMIVMR	5	3	1	Q92878	N-Term(Acetyl)	0	0	0.0001484	2.16	2	951.44536	-0.79	26.38	0
LQGDILDLR	7	3	1	Q92878		0	0	0.0001616	2.45	2	929.50517	0.05	17.93	0
SELLVEQQR	12	5	1	Q92878	M4(Oxidation)	0	0	0.000167	2.65	2	1030.55242	-0.37	15.54	0
fGHEDDK	4	5	1	Q92878	N-Term(Acetyl); M2(Oxidation)	0	0	0.00016806	2.67	2	1080.5219	0.98	19.91	1
GQDFK	8	4	1	Q92878	M5(Oxidation); M10(Oxidation)	0	0	0.000178	2.68	3	1335.70602	-1.24	18.87	0
EMQmQNLQmK	3	3	1	Q92878	M2(Oxidation)	0	0	0.0002052	2.47	2	1246.61645	0.05	13.95	0
EcGQmQNLQmK	18	3	1	Q92878	N-Term(Acetyl)	0	0	0.0003357	2.47	2	1338.6209	-0.47	19.26	0
iLEGITR	6	5	1	Q92878	M1(Oxidation)	0	0	0.0001917	2.48	2	930.26248	1.03	24.23	0
IMKLNDIJK	5	5	1	Q92878	N-Term(Acetyl)	0	0	0.0001986	2.59	3	1103.61124	-1.57	14.7	1
IEKMSLGLVR	4	4	1	Q92878		0	0	0.0002134	3.28	3	1145.67176	0.52	18.73	1
vLASLILR	4	4	1	Q92878	N-Term(Acetyl)	0	0	0.0002327	2.21	2	926.60301	-0.39	28.52	0
LQSTKQLQKR	15	3	1	Q92878	M4(Oxidation)	0	0	0.0002598	2.42	2	1072.60979	-1.1	18.01	0
RDEdLQLPmN	9	3	1	Q92878		0	0	0.000296	2.44	3	1324.63592	-0.29	16.78	2
iaKLNDIJK	6	2	1	Q92878	M5(Oxidation)	0	0	0.000567	2.43	2	1013.56224	-0.39	13.45	1
EnSLGSGVSK	2	5	1	Q92878	M2(Oxidation)	0	0	0.000852	2.46	2	1030.55022	0.03	17.76	0
TQMEMLT	10	5	1	Q92878	N-Term(Acetyl); M2(Oxidation)	0	0	0.0003442	2.03	2	981.47447	0.05	15.98	0
TEGVTR	3	5	1	Q92878	N-Term(Acetyl)	0	0	0.0004481	2.17	2	888.51457	-0.42	16.59	0
TQQLAD	2	5	1	Q92878	N-Term(Acetyl)	0	0	0.0004646	2.58	2	971.51866	0.17	22.79	0
EVQMV	1	5	1	Q92878	N-Term(Acetyl)	0	0	0.0004736	2.35	3	1553.74692	1.24	12.44	2
ELRPFLQDAAEK	1	3	1	Q92878		0	0	0.000505	2.35	3	1074.43535	-0.22	18.95	0
GDALDMR	6	4	1	Q92878	N-Term(Acetyl)	0	0	0.001263	2.16	2	878.02084	-0.87	14.12	0
IEKEEIKR	2	7	1	Q92878	N-Term(Acetyl)	0	0	0.001353	2.05	2	930.51939	-0.35	14.33	0
EKEIPELRL	6	3	1	Q92878	M1(Oxidation)	0	0	0.001434	2.04	2	1013.56224	-0.39	13.45	1
eKEIPELRL	2	3	1	Q92878	N-Term(Acetyl)	0	0	0.001435	2.06	2	1055.57561	2.28	17.13	1
mSILG	7	5	1	Q92878	N-Term(Acetyl)	0	0	0.001882	1.91	2	817.45959	-0.92	24.3	0
MSILG	6	3	1	Q92878	M1(Oxidation)	0	0	0.00216	2.1	2	816.82353	-0.29	18.11	0
asDGLD	7	3	1	Q92878	M5(Oxidation)	0	0	0.002450	2.41	2	703.51878	-0.07	15.50	0
IDNEIK	1	5	1	Q92878	N-Term(Acetyl)	0	0	0.00256	2.17	2	773.40392	-0.1	12.94	0
MSILGVR	11	5	1	Q92878	N-Term(Acetyl)	0	0	0.002984	2.28	2	775.44908	-0.56	20.29	0
FOMELK	1	3	1	Q92878		0	0	0.003255	1.94	2	795.40715	0.21	17.89	0
mEEINK	1	4	1	Q92878	N-Term(Acetyl)	0	0	0.004985	1.95	2	805.75747	-0.75	13.37	0
sGHEDK	1	5	1	Q92878	M1(Oxidation)	0	0	0.005733	2.13	2	837.39799	-1.17	19.33	0
iEDMRL	1	3	1	Q92878	N-Term(Acetyl)	0	0	0.0057989	1.92	2	819.36622	-0.46	13.38	0
vYDNPQLQK	1	4	1	Q92878	M5(Oxidation); M12(Oxidation)	0	0	0.006216	2.08	2	1480.74626	-2.46	15.06	1
YGDfDQLTmDmR	1	4	1	Q92878		0	0	0.00624	2.74	2	754.84949	-0.57	15.28	0
NBS1	51,46	4	41	41	403	754	849	6,9	2.1	2	1247.69927	-0.16	27.76	0
Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	ΔCn	q-value	Number of Cleavages	xCorr	Charge	RT [min]	RT [min] # Missed Cleavages		
NEKQHESVPPVDNDSNNLFDTDLK	2	1	1	P27694	N-Term(Acetyl)	0	0	4.66E-16	5.05	3	1217.42148	-0.95</td		

SMGGAAIAPPRLSLVEKDKEKPR	2	5	1	Q96125		0	0	1.40E-14	5.66	4	2267.20083	-2.52	20.34	2
HEQQLSTALSLVEK	2	3	1	Q96125		0	0	1.59E-13	4.37	2	1398.71965	-1.96	16.41	0
IIVGDATEKDKASK	2	1	1	Q96125		0	0	1.65E-08	4.35	3	1346.7136	-1.98	11.2	1
<P>PFPAPDLEAVR	1	1	1	Q96125	C1(Carbamidomethyl)	0	0	1.73E-13	3.21	2	1374.7136	-1.14	27.33	0
IIVGDATEKDKASKK	1	1	1	Q96125		0	0	5.94E-11	3.09	3	1474.81034	-0.61	10.0	2
KSDSNPTEHLK	1	1	1	Q96125		0	0	5.00001376	2.98	2	1344.73393	-2.27	21.9	1
AAIPPPVYEEQQRPR	1	4	1	Q96125		0	0	0.000004831	2.88	3	1737.88895	-1.72	17.41	0
QSTVLAPVDELK	1	4	1	Q96125		0	0	0.004584	2.72	2	1283.75603	-0.75	24.63	0
QSTVLAPVDELKRR	1	4	1	Q96125		0	0	0.000004831	2.51	3	1439.85624	-1.3	20.92	1
DPPFVPSRSPR	1	5	1	Q96125		0	0	3.95E-10	2.42	3	1398.71364	-2.02	17.02	0
IIVGDATEKDKASK	1	2	1	Q96125		0	0	0.0000095	2.22	2	945.52344	-0.84	12.0	0
SMGGAAIAPPRLSLVEK	1	5	1	Q96125		0	0	1.05E-09	1.94	2	1528.79949	-2.99	21.85	0
LLQSQLOVK	1	3	1	Q96125		0	0	0.00008363	1.91	2	1056.64067	-0.51	16.99	0
IPLFEPR	1	7	2	Q96125;O43663-3	M2(Oxidation)	0	0.001	0.01031	1.9	2	953.50835	-0.83	24.58	0
SaGGAAIAPPRLSLVEK	1	5	1	Q96125		0	0.002	0.01872	2.48	2	1544.80175	1.8	20.16	0
AVDPLNLGR	1	1	1	Q96125		0	0.003	0.04115	1.93	2	843.46727	-1.27	13.68	0
PRC1	23.8					525	61.3	7.83						
Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	ΔC_n	q Value	PEP	XCorr	Charge	MH+ [Da]	ΔM [ppm]	RT [min]	# Missed Cleavages
ALQLEVDRLEELK	1	5	1	O43663-3		0	0	3.40E-08	3.18	2	1555.87126	1.4	24.64	1
LIPSGSKPVAAStSGK	4	8	1	O43663-3	C14(Carbamidomethyl)	0	0	9.97E-13	3.12	3	1643.8436	0.84	10.61	0
SGNQVSEGLR	1	5	1	O43663-3	C7(Carbamidomethyl)	0	0	0.000015352	2.87	2	1262.57805	-1.1	12.95	0
LLEIFVME	1	7	2	Q96125;O43663-3		0	0.001	0.01031	1.9	2	953.50835	-0.83	24.58	0
TOVELMR	2	6	1	O43663-3		0	0.002	0.03031	2.17	2	876.45922	-1.82	14.53	0

EVFGITGIPPLLR	9	1	Q93009		0.0000	0	0.00003521	2.67	2	1495.83257	1.15	84.67	0
VFLALVQDKEKQGATMGPQQR	1	2	Q93009_HUBND8		0.0000	0	0.00000201	4.37	3	2925.90162	1.12	82.46	1
IEPDLQVDEENQNMIVYADHRRKVGVTHGIPPLLR	1	1	Q93009		0.0000	0	0.000252	2.24	4	181.70000	1.81	83.83	2
NOGATVNNSLQLOTLFFTNQLR	2	2	Q93009_HUBND8	C6(Carbamidomethyl)	0.0000	0	0.004065	2.86	3	2620.20999	2.00	84.76	0
IQDYDVKLKADELMDGDRNQ	1	2	Q93009_HUBND8	M2(Oxidation)	0.0000	0	4.264E-07	4.88	3	2883.43540	0.71	84.99	1
RPAHSDVQVWVQVQVQVPPVTELYDPPELAASGATLPK	1	2	Q93009_HUBND8	M4(Oxidation); M12(Oxidation)	0.0000	0	0.00014503	5.97	4	2620.20999	0.71	83.83	2
TMEHSIDNENPWTIETTPPELAASGATLPK	2	2	Q93009_HUBND8	M2(Oxidation)	0.0000	0	0.0001503	5.33	3	3513.71987	1.44	85.58	0
TMHEISDNENPWTIETTPPELAASGATLPK	1	2	Q93009_HUBND8		0.0000	0	4.659E-09	4.82	3	3502.72702	0.84	85.66	0

Supplementary File 3

siRNA sequences

siRNAs	Sequences
USP7-1	GACGUUUCGAAUAGAGGAA
USP7-2	GCACUAUAGCUUACAUGUU
USP7-3	GACUUUGAGAACAGGGCAA
MDC1-1	GUCUCCCAGAACAGACAGUGA
MDC1-2	GAAGAUCCUCCAUGGAGUA
MRE11	GAGCAUAACUCCAUAAAGUA
RAD50	GGAGAAGGAAAUACCAGAA
NBS1-1	GUACCUUUGUUAUAGAGGA
NBS1-2	GGAAGAACGUGAACUCAA
USP7 5'UTR-1	CUCACCUCGUCAGCCACUA
USP7 5'UTR-2	CAAGUCUUGUGUUUAGGCU
RNF168	GACACUUUCUCCACAGAU
53BP1	GAAGGACGGAGUACUAAUA
BRCA1	GGAACCUGUCUCCACAAAG
HPV-16 E6-1	GAAUGUGUGUACUGCAAGC
HPV-16 E6-2	GCUGCAAACAACUAAUACAU
HPV-16 E7-1	AGGAGGAUGAAAAGAUGG
HPV-16 E7-2	CAGAGCCAUUACAAUAAU
HPV-18 E6-1	CACUUCACUGCAAGACAU
HPV-18 E6-2	GGUGCAGAACCGUUGAA
HPV-18 E7-1	CCACAACGUCACACAAUGU
HPV-18 E7-2	GCAAGACAUUGUAUUGCAU

Lentiviral shRNA sequences

shRNAs	Sequences
Control	CCGG GATATGGGCTGAATA ACAAACTCGAG TTTGTATT CAGCCATATCTTTTG
USP7-1	CCGG CGTGGTGTCAAGGTGT ACTAACTCGAG TTAGTACAC CTTGACACCACGTTTTG
USP7-2	CCGG CCTGGATTGTGGTTACGTTA CTCGAG TAACGTAACC ACAAATCCAGGTTTTG
USP7-3	CCGG CCTGGATTGTGGTTACGTTA CTCGAG TAACGTAACC ACAAATCCAGGTTTTG

Note: Red color indicates the targeting sequence against the corresponding genes.

qRT-PCR primers

Genes	Sequences
<i>GAPDH</i>	F: GAAGGTGAAGGTCGGAGTC R: GAAGATGGTATGGGATTTC
<i>USP7</i>	F: ATTCCTAACATTGCCACCAG R: ATTTACACCATTGCCATCC
<i>MDC1</i>	F: GTAGGCCGAATGCCTGACTG R: CGGAGGATAGGTGCCTTGTC
<i>NBS1</i>	F: AGGTGGGAAAGCTAGGTTGAT R: CACCGCCAATCCAATTCTGC
<i>RNF168</i>	F: TCAACGTGAACTGTGGACG R: CAGGTTTACTGAGCAGACGAAC
<i>HPV-16 E6</i>	F: GAGCAATTAAATGACAGCTCAGA R: GGGCACACAATTCTAGTG
<i>HPV-16 E7</i>	F: TGCAACCAGAGACAAC TGAT R: CAATTCTAGTGCCCATTAAAC
<i>HPV-18-E6</i>	F: GTGCCAGAAACCGTTGAATC R: GAATGGCACTGGCCTCTAT
<i>HPV-18-E7</i>	F: GAACCACAAACGTCACACAATG R: CGTCTGCTGAGCTTCTACTAC

qChIP primers

Genes	Sequences
chr22 proximal	F: CCTTCTTCCCAGTGGTTCA R: GTGGTCTGACCCAGAGTGGT
chr22 distal	F: CCCATCTCAACCTCCACACT R: CTTGTCCAGATTGCTGTGA