Supporting Information

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SI Materials and Methods

List of Antibodies. Antibodies are listed: polyclonal anti-WWOX antibody (1:500; gift from Kay Huebner, Ohio State University, Columbus, Ohio); p-Histone H2A.X (S139; 20E3) rabbit mAb (9718S; Cell Signaling); P-Chk2 (T68) rabbit (2661S; Cell Signaling); ATM (pS1981) rabbit mAb (2152–1; Cell Signaling); Gout anti-ATM (A300-136A; Cell Signaling); P-Chk1 (S296) rabbit (2349S; Cell Signaling); Gout anti-Chk1 (A300-162A; Cell Signaling); Rb x-p-KAP-1 (S824; A300-767A; Bethyl); rabbit anti-KAP1 (A300-274A; Bethyl); P-Histone H3 (S10; D2C8; Alexa647) XP L rabbit mAb (Cell Signaling); anti-Ub, clone FK2 (that recognize both mono- and polyubiquitin; 04–263; Millipore); anti-Ub, clone FK1 (that mostly recognize polyubiquitin chains; 04–262; Millipore); anti-Ub, Lys63-specific, clone HWA4C4, mouse mAb (05–1313; Millipore); rabbit anti-P73 (A300-126A; Bethyl); anti-GAPDH mouse mAb (CB 100 1; CALBIOCHEM); anti-Lamin A/C (N-18; Santa Cruz Biotechnology); and anti-MYC HRB(9E10) (SC-40; Santa Cruz Biotechnology).

List of Primers Used for Quantitative RT-PCR.

Gene	Direction	Primer sequence
MAF	Forward	CCG TCC TCT CCC GAG TTT TTC
MAF	Reverse	ACA CTG GTA AGT ACA CGA TGC T
WWOX-7–8	Forward	TCC TCA GAG TCC CAT CGA TTT
WWOX-8–9	Reverse	CGG CAG TTG AAG TA
GAPDH	Forward	CAT GAG AAG TAT GAC AAC AGC CT
GAPDH	Reverse	AGT CCT TCC ACG ATA CCA AAG T
UBC	Forward	ATT TGG GTC GCG GTT CTT G
UBC	Reverse	TGC CTT GAC ATT CTC GAT GGT

Sequences of shRNAs Against the Human *WWOX* Gene. shRNA lentiviral plasmids (pLKO.1-puro; clone ID: NM_016373.1–720s1c1; Sigma) were used to deplete WWOX expression in MCF7 cells according to standard protocol.

Sequence: CCGGGCCAAGAATGTGCCTCTTCATCTCGAG-ATGAAGAGGCACATTCTTGGCTTTTTG.

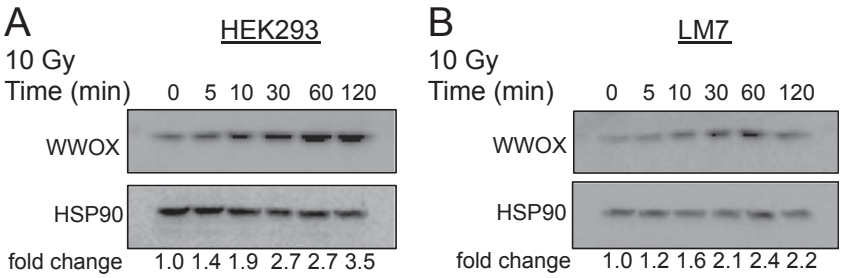

Fig. S1. Induction of WWOX expression early after DNA damage stimuli. Immunoblot blot analysis of WWOX levels in (*A*) HEK293 and (*B*) osteosarcoma LM7 cells after treatment with 10 Gy.

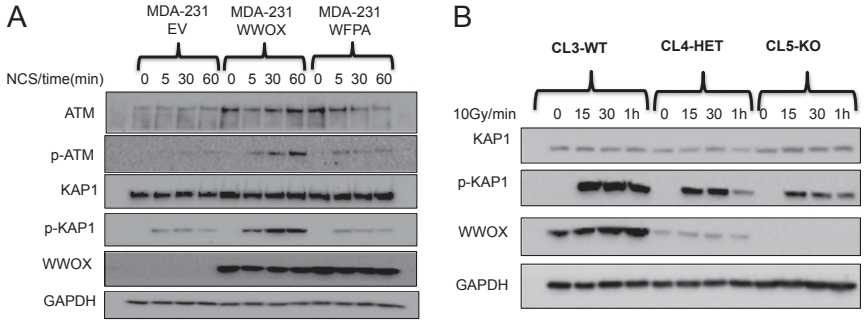

Fig. S2. Checkpoint activation in WWOX-manipulated cells after DSBs. (*A*) MDA-MB231 cells were transduced with Lenti-EV, Lenti-WWOX, or Lenti-WWOX-WFPA. Cells were selected, and clones were treated with NCS (200 ng/mL) for the indicated time points. Whole-cell lysates were blotted with antibodies against ATM, p-ATM, KAP1, p-KAP1, WWOX, and GAPDH. (*B*) Early-passage MEFs isolated from embryonic day 13.5 embryos were γ-irradiated for the indicated time points. Cell lysates were then probed with antibodies against KAP1, p-KAP1, WWOX, and GAPDH.

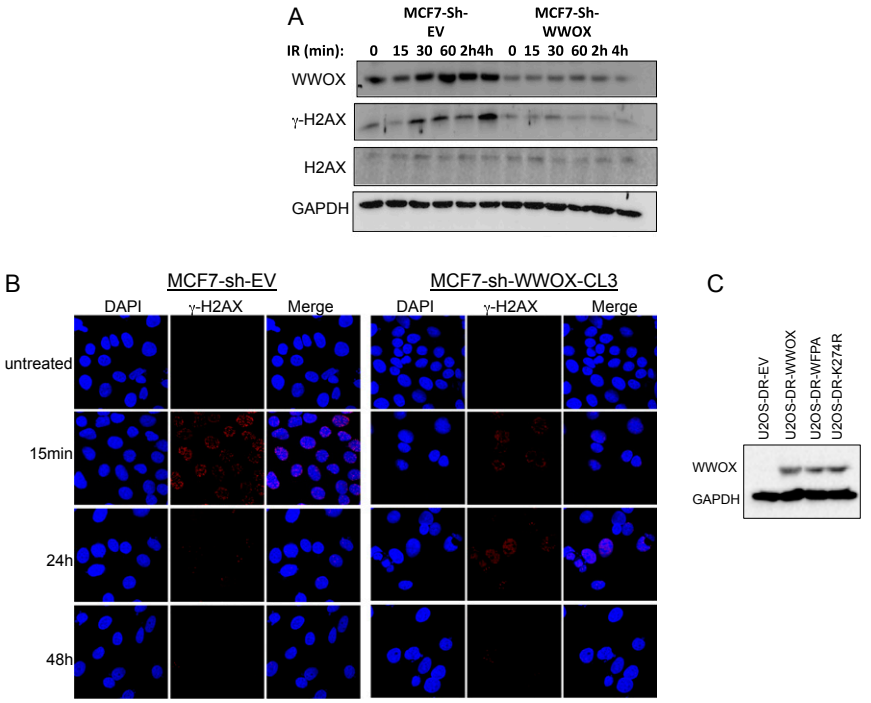

Fig. S3. (*A* and *B*) Impaired phosphorylation of H2AX in WWOX-depleted MCF7 cells. (*A*) Immunoblot analysis of control and WWOX-depleted MCF7 cells after ionizing radiation treatment for the indicated times. Whole-cell lysates were propped with antibodies against WWOX, Ser139-H2AX, total H2AX, and GAPDH. (*B*) Representative confocal microscopy images of γ-H2AX foci (red) in control MCF7-Sh-EV and WWOX-depleted (MCF7-Sh-WWOX) cells after treatment of ionizing radiation at different time points. DAPI staining (blue) was used to stain nuclei. Foci were quantified and are presented in Fig. 3*C*. (*C*) Overexpression of WWOX in U2OS-DR-GFP cells. Cells were transduced with the indicated Lenti-viral constructs (EV, WWOX, WWOX-WFPA, or WWOX-K274R), and stable clones were established. Immunoblot analysis showing WWOX expression in the different clones. GAPDH was used as loading control. IR, ionizing radiation.

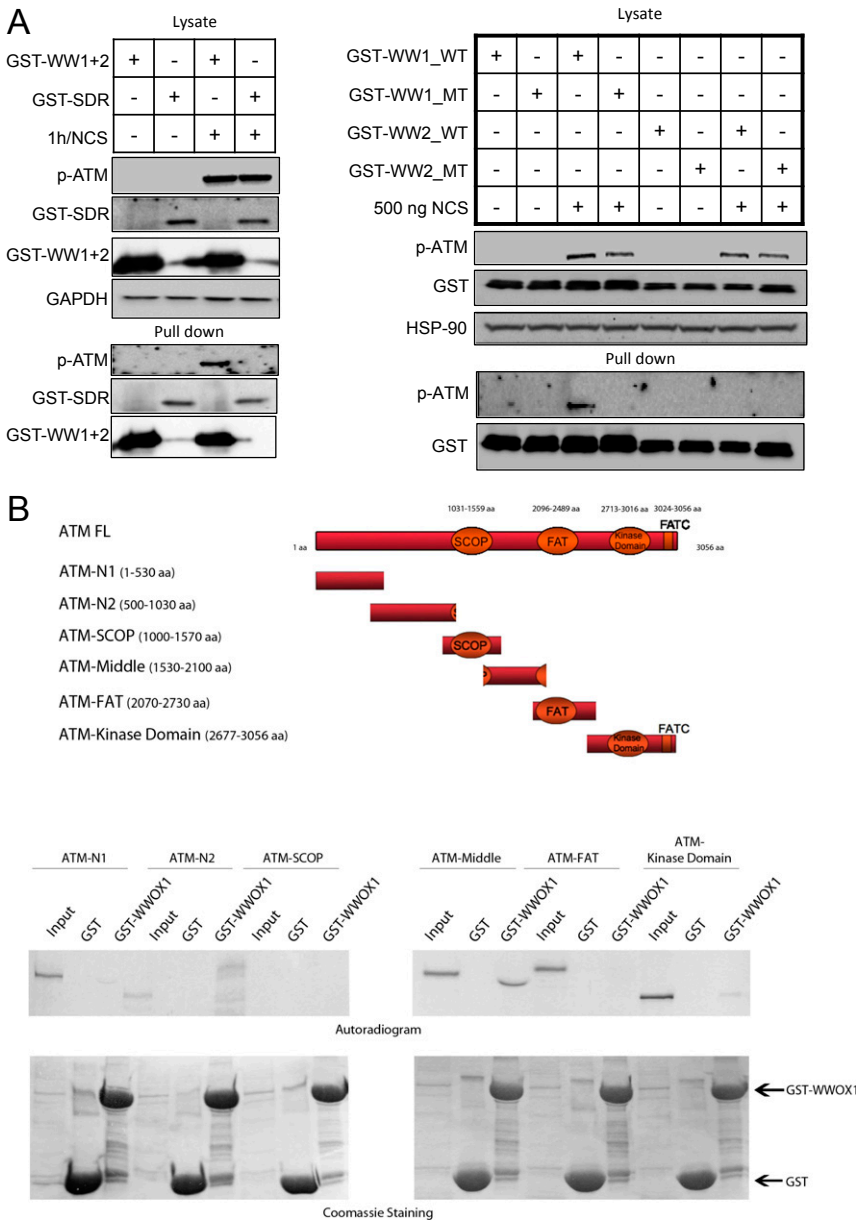

Fig. S4. (*A*) WW1 domain of WWOX physically associates with ATM. HEK293 cells were transfected with (*Left*) GST-WW1+2 or GST-SDR or (*Right*) GST-WW1, mutant GST-WW1-WFPA (WW1_MT), GST-WW2, or GST-WW2-YFPA (GST-WW2_MT). At 24 h, cells were treated with NCS (200 ng/mL) for an additional 1 h. GST pull down was performed for 2 h, and complexes were analyzed by immunoblotting using antibodies against GST, p-ATM, and GAPDH or HSP90. (*B*) GST-WWOX interacts with ATM (amino acids1,530–2,100) in vitro. (*Top*) Schematic representation of ATM deletion constructs used in GST pull down as in vitro-transcribed, translated, and 35S-labeled fragments. The 35S-labeled fragments were incubated with bacterial extracts expressing GST-WWOX followed by washing and electrophoresis. GST alone was used as the negative control. *Middle* shows the autoradiogram of 35S-labeled ATM fragments pulled down with GST alone or GST-WWOX. *Bottom* shows the Coomassie-stained loading control of inputs and pulled fragments.

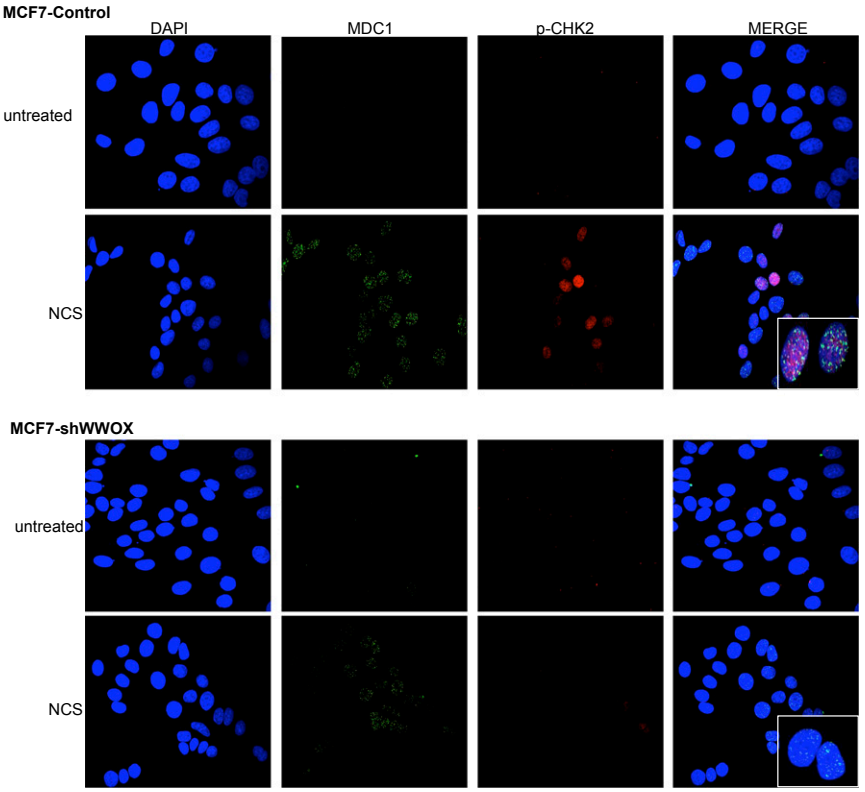

Fig. S5. Impaired activation and recruitment of MDC1 and p-CHK-2 in WWOX-depleted MCF7 cells. MCF7 and MCF7-ShWWOX cells were treated or untreated with NCS (200 ng/mL) for 30 min. Cells were then fixed and stained with anti-MDC1 (green) and anti–p-CHK2 (red). Nuclei were counterstained with DAPI (blue). Cells were visualized with confocal microscopy.

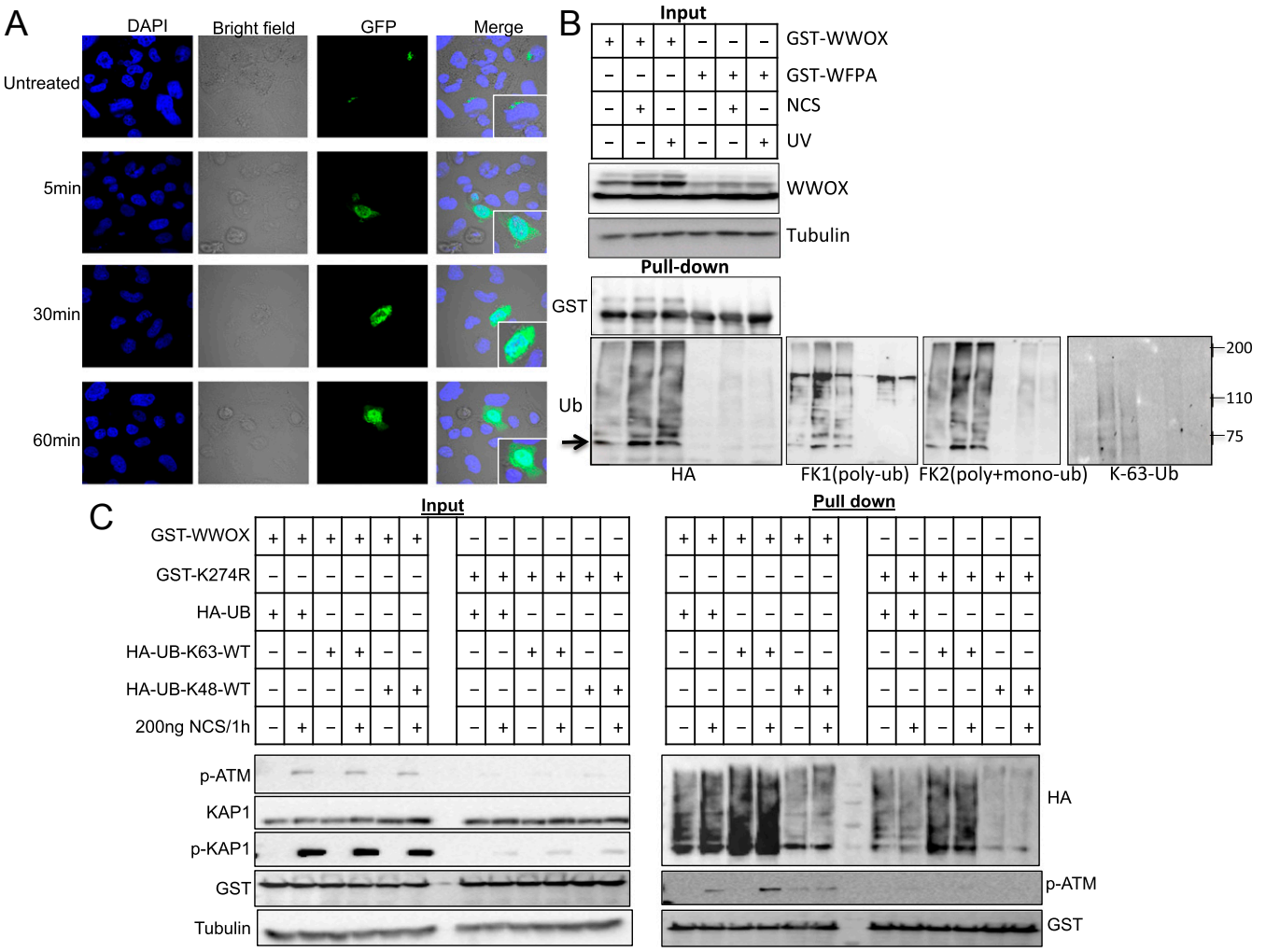

Fig. S6. (*A*) Nuclear localization of WWOX after NCS treatment. MCF7 or HeLa cells were transfected with GFP-WWOX. Twenty-four hours later, cells were untreated or treated with NCS (200 ng/mL). At the indicated times after NCS treatment, the cells were fixed and visualized with confocal microscopy using 60× objective lens. Nuclei were counterstained with DAPI (blue); bright field is shown in gray. Nuclear GFP-WWOX was observed in 50–70% of cells. (*B* and *C*) WWOX ubiquitination after DNA damage. (*B*) HEK293 cells transfected with HA-Ub and *pEBG-WWOX* or *pEBG-WWOX-WFPA* plasmids. At 24 h, cells were treated with UV (100 J/m^2) or NCS (200 ng/mL) for an additional 1 h. Cells were then subjected to GST pull down, and lysates were blotted against GST (WWOX) and tubulin. Pulled down complexes were blotted with anti-GST (WWOX), anti-HA (Ub), anti-FK1 (recognizing polyubiquitin), anti-FK2 (recognizing both mono- and polyubiquitin), and anti-K63 polyubiquitin antibodies. (*C*) HEK293 cells were transfected with vectors encoding GST-WWOX or GST-K274R and HA-Ub, HA-Ub-K63-WT, or HA-Ub-K48-WT (ubiquitin constructs are either K63 only or K48 only, whereas all other lysines are mutated to arginines). At 24 h, cells were treated with NCS for an additional 1 h; (*Right*) lysates were then subjected to GST pull down, and precipitates were blotted as indicated.

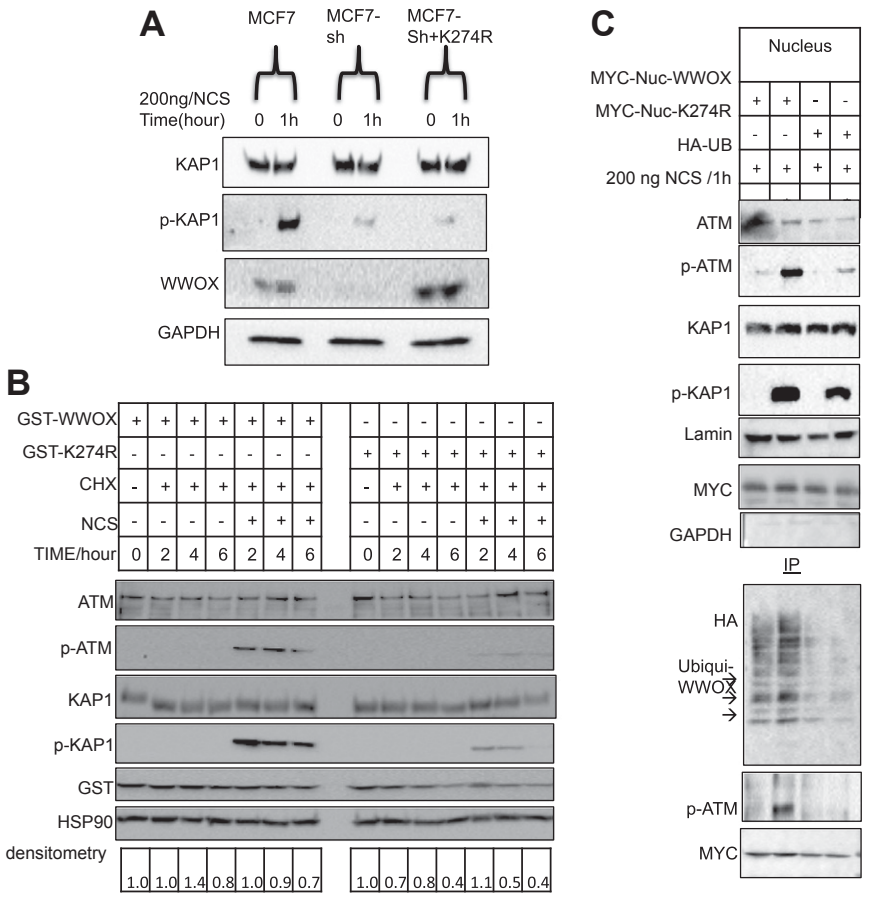

Fig. S7. (*A*) WWOX-depleted MCF7 cells were transduced with lentiviral vectors expressing EV or WWOX-K274R mutant and selected with neomycin. The different cells were untreated or treated with NCS (200 ng/mL). Whole-cell lysates were prepared at the indicated time points, and immunoblot analysis was performed using specific antibodies against KAP1, p-KAP1, WWOX, and GAPDH. (*B*) HEK293 cells were transfected with vectors encoding GST-WWOX or GST-K274R. At 24 h posttransfection, cells were treated with cyclohexamide (CHX; 100 μg/mL) in the presence or absence of NCS (200 ng/mL) for 0, 2, 4, or 6 h. Whole-cell lysates were blotted with antibodies as indicated. Densitometry shows levels of GST-WWOX or GST-WWOXK274R relative to HSP90 levels. (*C*) HEK293 cells were transfected with Myc-Nuc-WWOX or Myc-Nuc-WWOX-K274R. At 24 h, cells were treated with NCS for an additional 1 h. Cells were then lysed and immunoprecipitated with anti-Myc antibody. (*Lower*) Precipitates were blotted with anti-HA, anti–p-ATM, and anti-Myc. (*Upper*) Input lysates were blotted as indicated. IP, immunoprecipitation.