Zeman et al., http://www.jcb.org/cgi/content/full/jcb.201311063/DC1

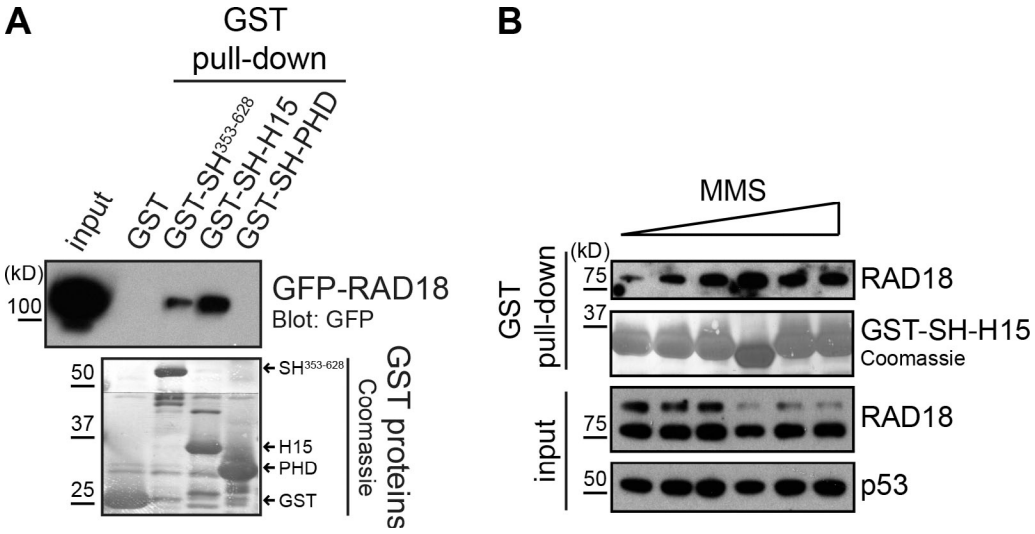

Figure S1. **The H15 domain of SHPRH binds to Rad18.** (A) The H15 domain of SHPRH is sufficient to bind Rad18. Purified GST-tagged fragments of SHPRH (SH; SHPRH$^{353–628}$; Fig. 1, A and B), the H15 domain (SHPRH$^{447–521}$), and the plant homeodomain (PHD) were incubated with GFP-Rad18 expressed in cell lysates and analyzed as in Fig. 1 C. (B) The H15 domain is sufficient to recapitulate damage-inducible Rad18 binding. The H15 domain was used to pull down endogenous Rad18 from cell lysates, which had been treated with increasing doses of MMS. Analyzed as in Fig. 1 C.

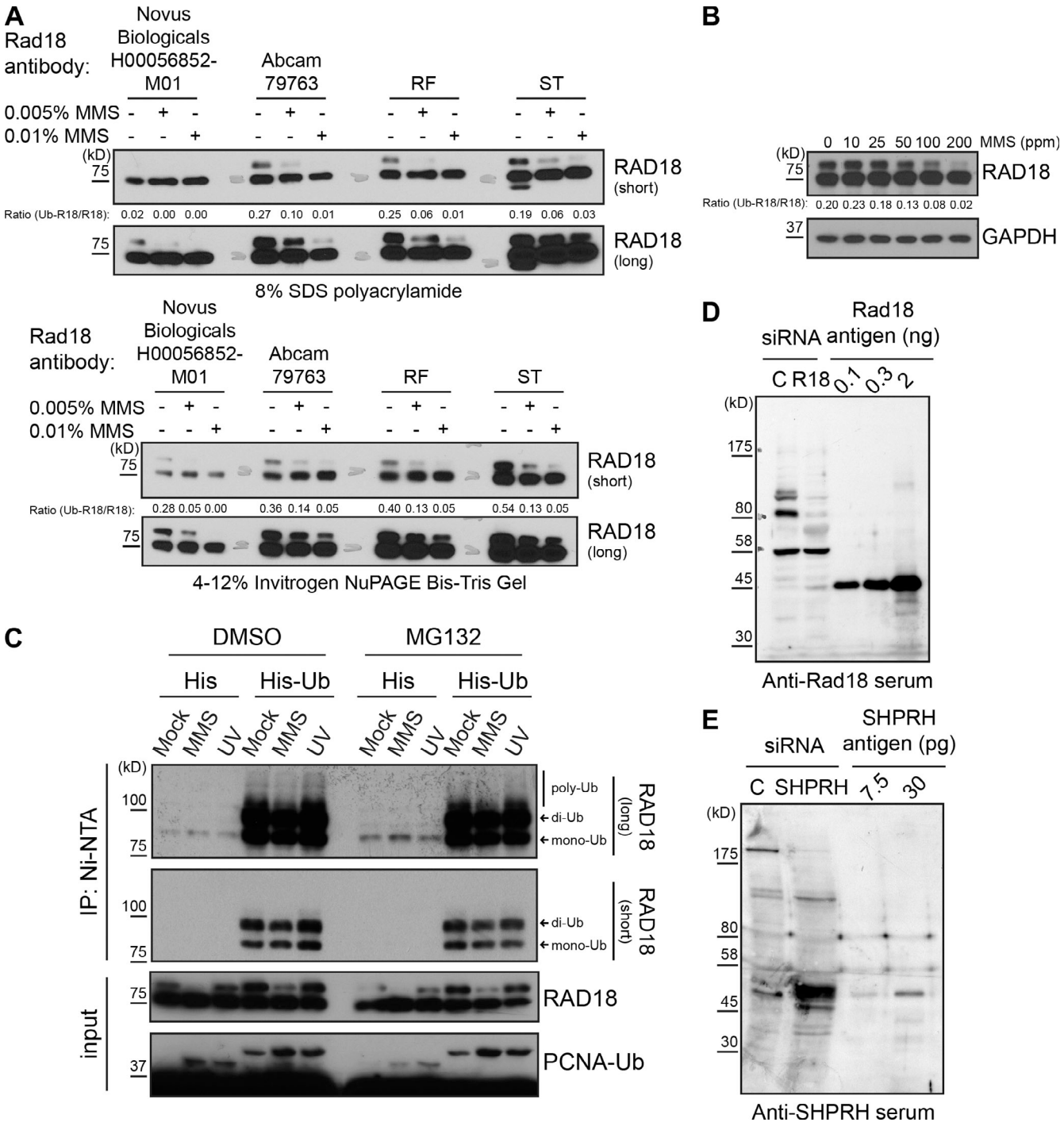

Figure S2. **Detection and characterization of Rad18 ubiquitination.** (A) The ratio of ubiquitinated to unmodified Rad18 depends on detection conditions. HEK 293T cells were treated with the indicated doses of MMS for 2 h before lysis. Lysates were loaded in quadruplicate on a poured or a precast (Invitrogen) gel and blotted for Rad18 (RF antibody obtained from R. Freire; ST antibody obtained from S. Tateishi). The Ub-Rad18/Rad18 ratio was quantified on an AlphaView FluorChem HD2. (B) Rad18 is also deubiquitinated in U2OS cells. U2OS cells were treated with the indicated doses of MMS for 2 h before being lysed and analyzed as in A, using the NuPAGE Bis-Tris gel and the RF Rad18 antibody. (C) Treatment with proteasome inhibitors does not promote polyubiquitination of Rad18. Cells transfected with or without His-tagged ubiquitin were treated with 50 µM MG132 in conjunction with 0.005% MMS or 50 J/m^2 UV for 4 h and processed and analyzed as in Fig. 3 C. NTA, nitrilotriacetic acid; Ub, ubiquitin. (D) Characterization of anti-Rad18 serum. Rabbits were immunized as described in Materials and methods, and crude serum was tested for specificity and sensitivity against the purified epitope or lysates treated with siRNA (C, control; R18, Rad18). (E) Characterization of anti-SHPRH serum. Rabbits were immunized as described in the Materials and methods, and crude serum was tested as in D.

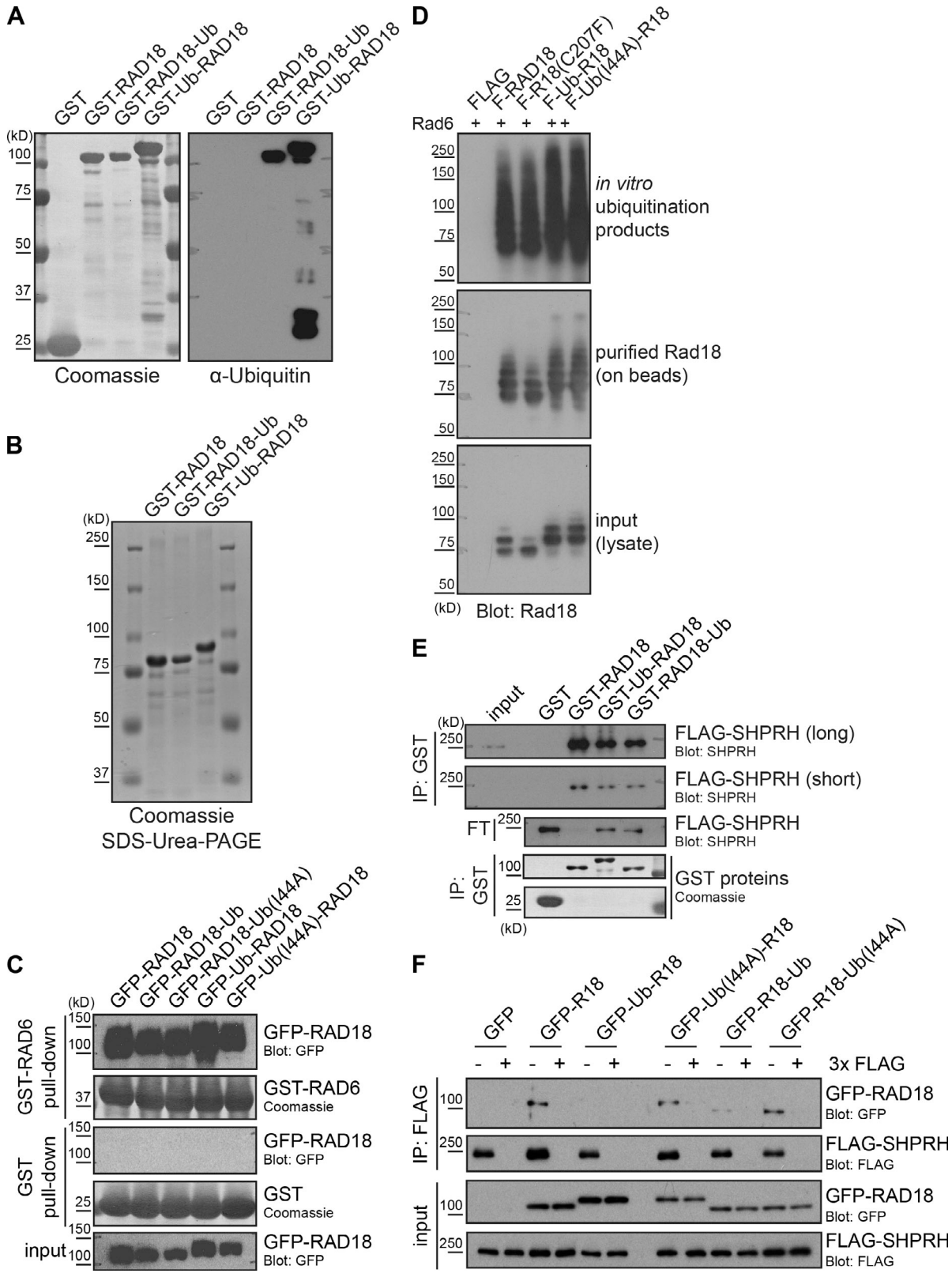

Figure S3. **Characterization of Rad18-Ub chimeras.** (A) Characterization of GST constructs as ubiquitin fusions. Purified GST-tagged proteins were run on SDS-PAGE and then blotted with anti-ubiquitin antibodies. F, FLAG. (B) Characterization of GST constructs as ubiquitin fusions. Purified GST-tagged proteins were run on SDS-Urea-PAGE to look for changes in migration after harsh denaturation. Proteins are visualized with Coomassie stain. (C) GFP–Rad18-Ub chimeras can still interact with Rad6. Purified GST-tagged Rad6 was used to pull-down GFP-Rad18 constructs from transiently transfected cell lysates. Bound proteins were analyzed via Western blot as in Fig. 1 C. (D) Ubiquitin does not inhibit Rad18 ligase activity in vitro. FLAG-tagged Rad18-Ub fusions co-expressed with untagged Rad6 were purified on beads from mammalian cells. The bound protein was then autoubiquitinated in vitro, and final products were analyzed by Western blotting. (E) Rad18-Ub chimeras have decreased SHPRH binding in vitro. GST-tagged Rad18-Ub fusions were incubated with FLAG-tagged SHPRH purified from mammalian cells. Pull-downs and flow through were analyzed by Western blotting. Differences in SHPRH binding can be observed by increased levels in flow through. (F) GFP–Rad18-Ub chimeras interact poorly with SHPRH in cells. FLAG-tagged SHPRH was cotransfected with the different GFP-Rad18 constructs and processed as in Fig. 1 B. 3x-FLAG peptide was used as a negative control. R18, Rad18.

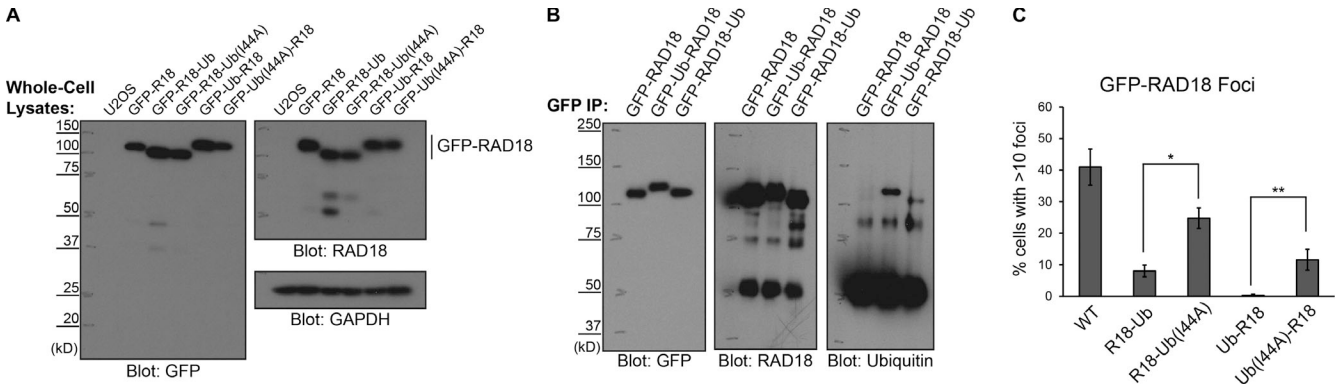

Figure S4. **Characterization of GFP-Rad18 stable cell clones.** (A) Characterization of stable clones. To ensure equal expression of Rad18 fusion proteins and confirm the absence of free GFP (~25 kD) or excessive breakdown of the GFP-Rad18 fusion proteins, stable cell clones were lysed and analyzed by Western blotting for GFP and Rad18. (B) Characterization of stable clones. To ensure that Rad18 fusion proteins were still ubiquitinated in stable cell clones, cells were lysed under high-salt conditions, and the GFP constructs were enriched by anti-GFP before being detected with Rad18 and ubiquitin antibodies. (C) The Rad18 C-terminal and N-terminal ubiquitin fusion proteins behave similarly after exposure to MMS. Stable clones were treated and analyzed as in Fig. 7 (B and C). At least 100 cells were counted per condition. Data represent mean percentages of cells with >10 foci and SEMs (n = 3). *, $P < 0.05$; **, $P < 0.01$ using Student's t test. R18, Rad18; WT, wild type.

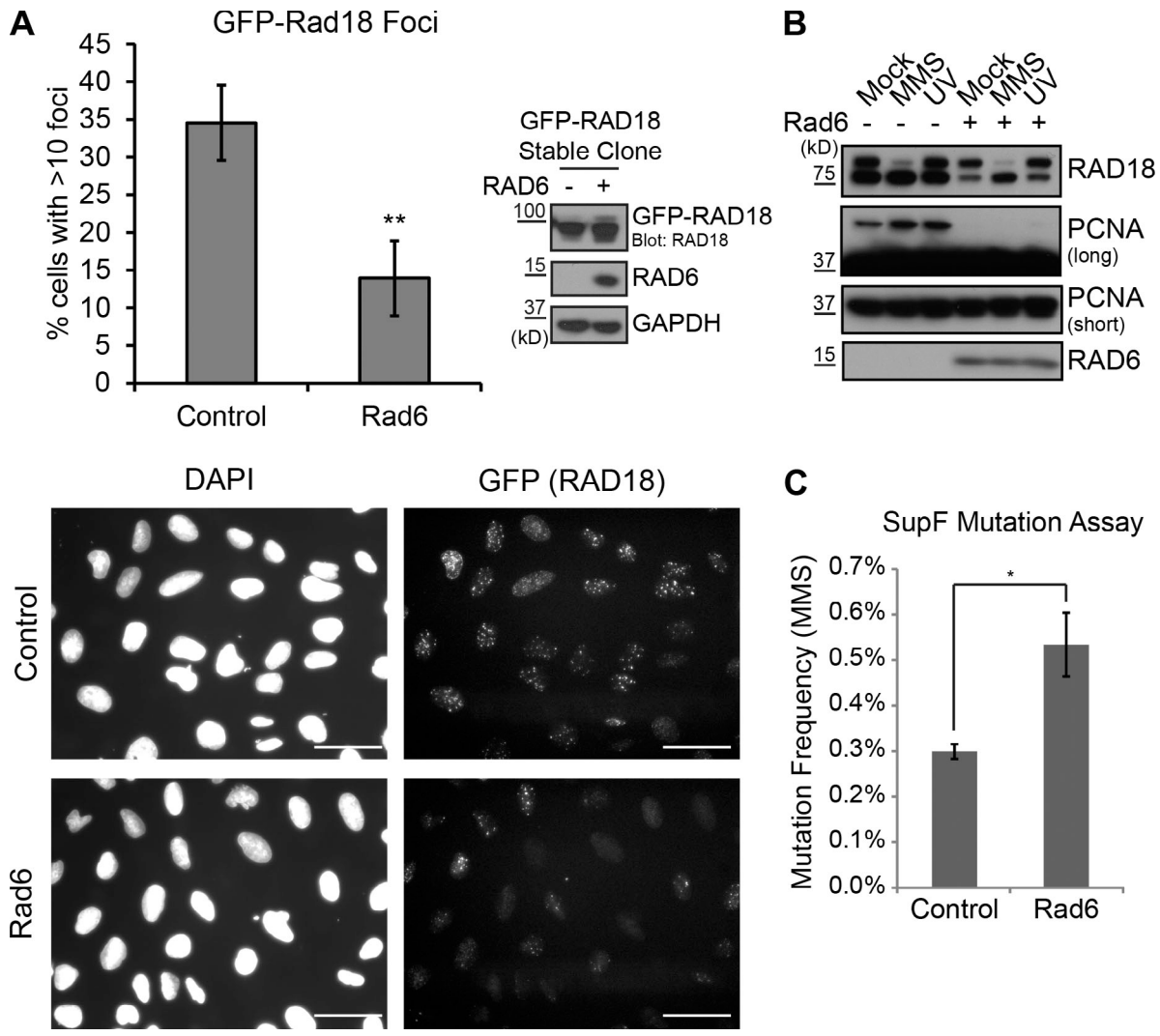

Figure S5. **Overexpression of Rad6 recapitulates Rad18-Ub functional defects.** (A) Overexpression of Rad6 reduces GFP-Rad18 foci after MMS treatment. Stable clones were treated and analyzed as in Fig. 7 (B and C). At least 100 cells were counted per condition. Data represent mean percentages of cells with >10 foci and SEMs ($n = 3$). **, $P < 0.01$ using Student's t test. Western blot confirms overexpression of Rad6 under these conditions. Selected immunofluorescence images are representative of the three experiments. Bars, 50 µm. (B) Rad6 overexpression decreases PCNA monoubiquitination. HEK 293T cells were transiently transfected with untagged Rad6 and damaged with 0.005% MMS or 100 J/m^2 UV for 4 h before being analyzed as in Fig. 2 C. (C) Rad6 overexpression increases mutation frequency on MMS-damaged plasmids. SupF reporter plasmid (0.5% MMS) was recovered from HEK 293T cells 48 h after cotransfection with Rad6 or a control plasmid. Data represents means and SEMs from four independent experiments; at least 2,000 colonies were analyzed per condition. *, $P < 0.05$.

Word file 1 contains an ImageJ macro script used to identify cells in a microscopic image using the DAPI channel.

Word file 2 contains a macro script for counting Rad18 foci within previously defined nuclear masks.