Poulsen et al., http://www.jcb.org/cgi/content/full/jcb.201212075/DC1

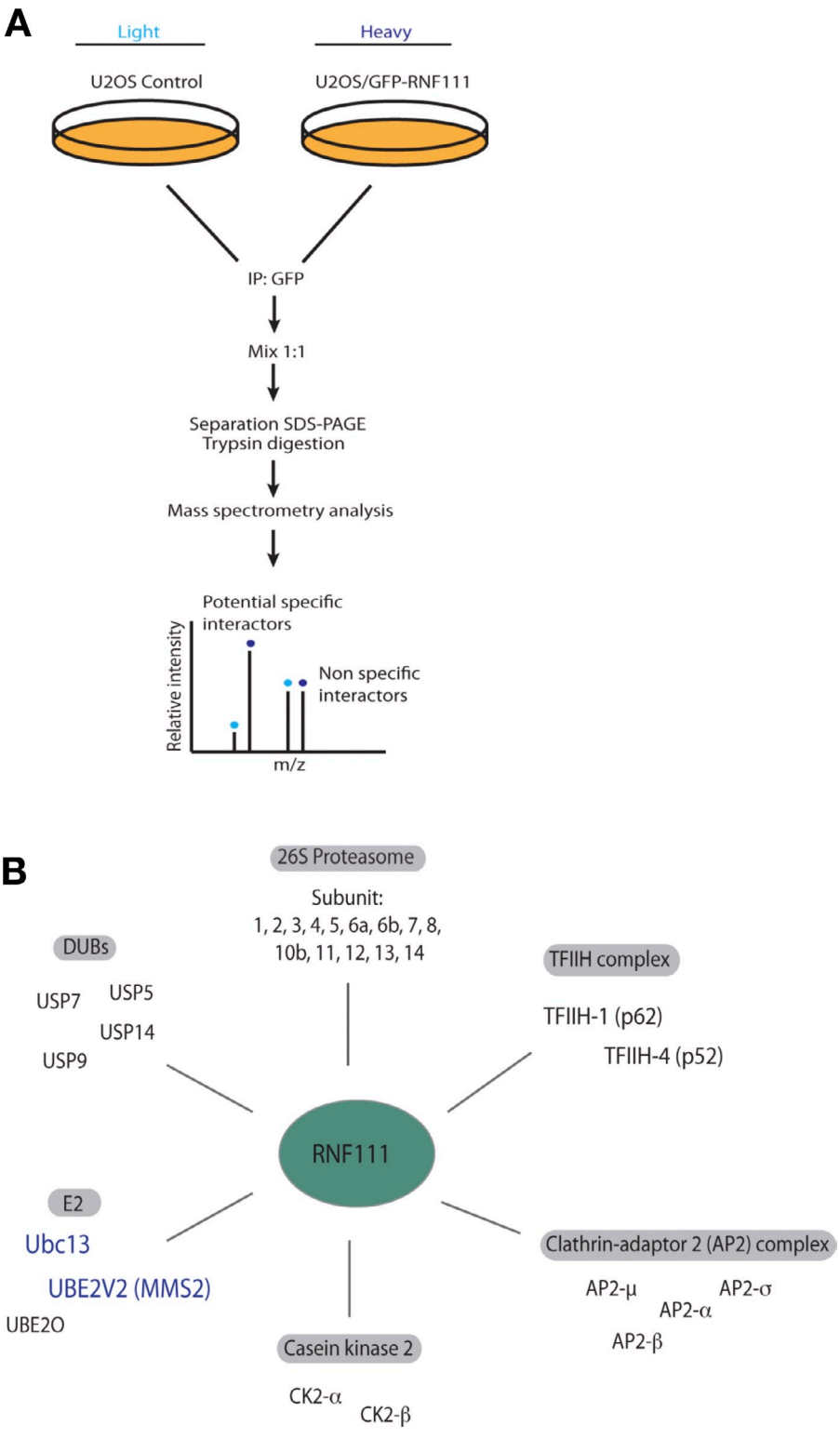

Figure S1. **Analysis of cellular RNF111-interacting proteins.** (A) MS-based analysis of RNF111-interacting proteins. U2OS or U2OS/GFP-RNF111 cells grown in light or heavy SILAC medium, respectively, were lysed and subjected to GFP IP. Subsequently, samples were combined, resolved by SDS-PAGE, and analyzed by MS. SILAC (heavy/light) ratios for individual proteins were determined. m/z, mass per charge. (B) Overview of selected proteins with high SILAC (heavy/light) ratios identified by the experimental approach outlined in A. DUB, deubiquitylating enzyme.

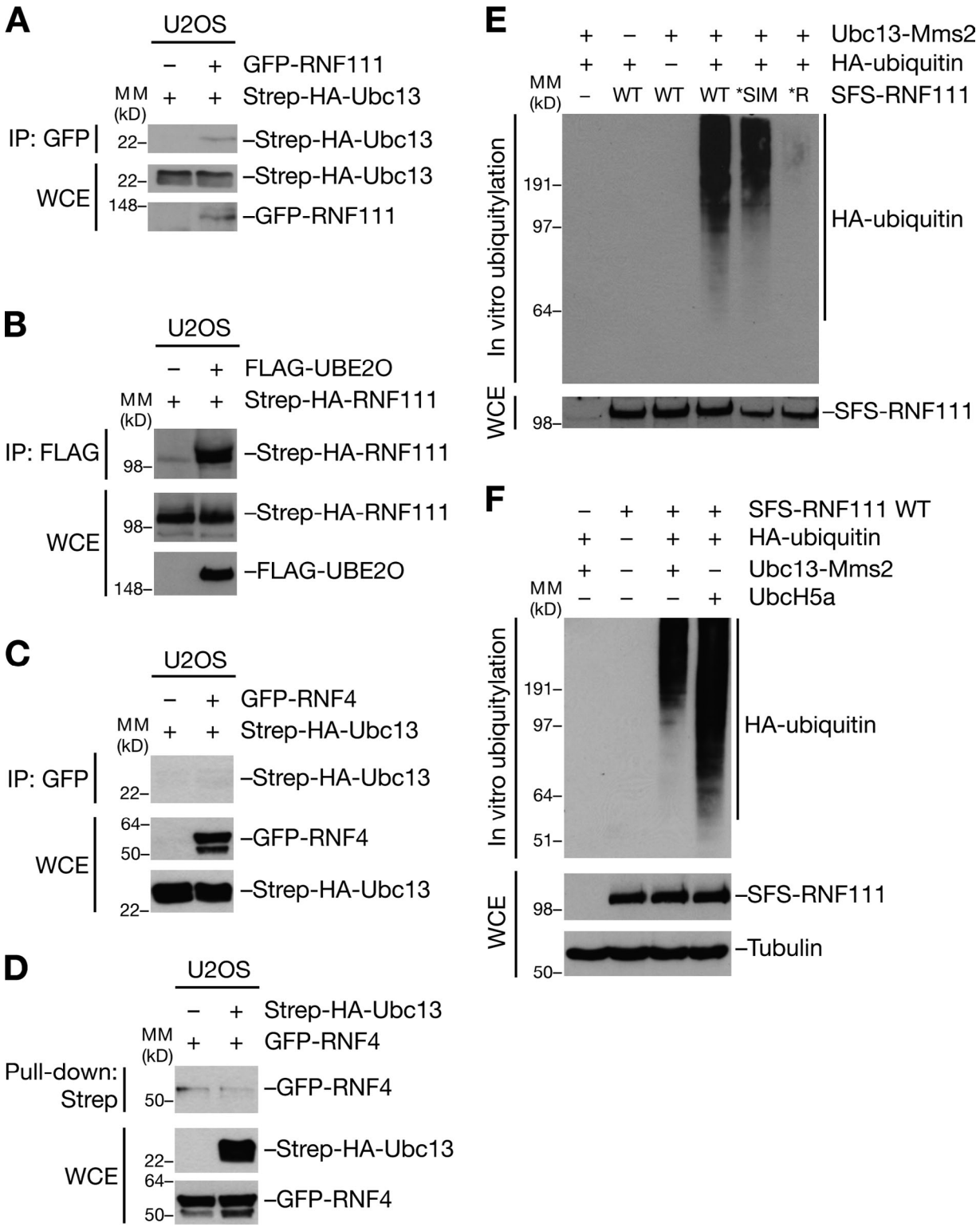

Figure S2. **RNF111 promotes Ubc13–Mms2-dependent ubiquitylation.** (A) U2OS cells were cotransfected with indicated combinations of GFP-RNF111 and Strep-HA-Ubc13 plasmids. Whole-cell extracts (WCE) were subjected to GFP IP followed by immunoblotting with HA antibody. (B) U2OS cells were cotransfected with the indicated combinations of FLAG-UBE2O and Strep-HA-RNF111 plasmids. Whole-cell extracts were subjected to FLAG IP followed by immunoblotting with HA antibody. (C) As in A, except that cells were transfected with combinations of GFP-RNF4 and Strep-HA-Ubc13 antibodies. (D) As in C, except that extracts were subjected to Strep-Tactin pull-down followed by immunoblotting with the GFP antibody. (E) Extracts of U2OS cells sequentially transfected with RNF111 siRNA and S-FLAG-Strep–tagged RNF111 (SFS-RNF111) plasmids as indicated were subjected to Strep-Tactin pull-down. Bound complexes were incubated with ubiquitylation reaction mixture containing E1, Ubc13–Mms2 complex, and HA-ubiquitin as indicated and washed extensively, and RNF111 autoubiquitylation activity was analyzed by immunoblotting with HA antibody. (F) As in E, except that UbcH5a was used as an E2 enzyme instead of Ubc13–Mms2 where indicated. MM, molecular mass.

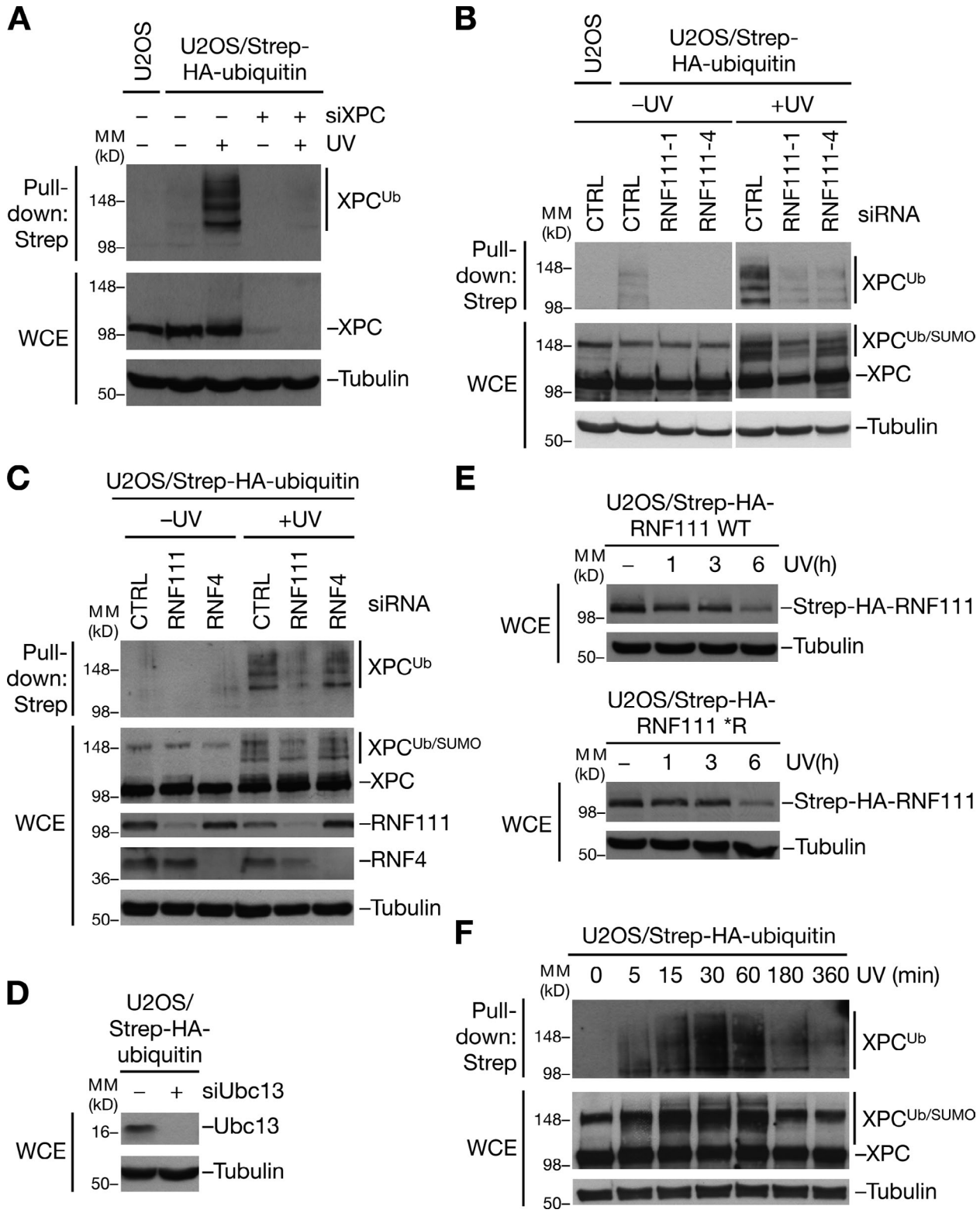

Figure S3. **RNF111 promotes UV-induced ubiquitylation of XPC.** (A) U2OS or U2OS/Strep-HA-ubiquitin cells transfected with control (−) or XPC siRNAs were exposed or not exposed to UV as indicated and collected 1 h later, and XPC ubiquitylation was analyzed by immunoblotting Strep-Tactin pull-downs of whole-cell extracts (WCE) with XPC antibody. (B) As in A, except that cells were transfected with indicated control (CTRL) or RNF111 siRNAs. (C) As in A, except that cells were transfected with indicated control, RNF111, or RNF4 siRNAs. (D) Knockdown efficiency of Ubc13 siRNA. U2OS/Strep-HA-ubiquitin cells were transfected with control (−) or Ubc13 siRNAs, collected 72 h later, and analyzed by immunoblotting with the Ubc13 antibody. (E) U2OS cell lines stably expressing Strep-HA–tagged RNF111 WT or *R mutant were collected at the indicated times after exposure to UV and analyzed by immunoblotting with HA antibody. (F) Time course analysis of UV-induced XPC ubiquitylation. U2OS/Strep-HA-ubiquitin cells were collected at the indicated times after exposure to UV, and XPC ubiquitylation was analyzed as in A. MM, molecular mass.