Göhler et al., http://www.jcb.org/cgi/content/full/jcb.201008076/DC1

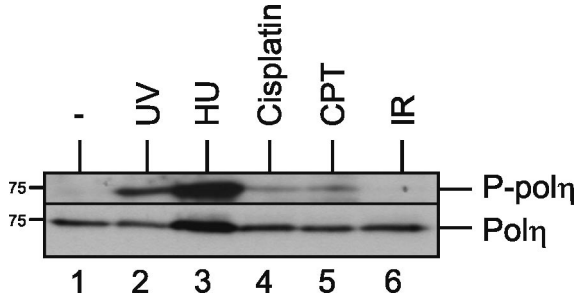

Figure S1. **Phosphorylation of polη in response to different DNA-damaging agents.** MRC5 cells were treated at 50–80% confluency as follows: UV, 20 J/m^2; HU, 1 mM for 24 h; Cisplatin 50 µg/ml for 1 h; Camptothecin (CPT), 1 µM; ionizing radiation (IR), 2 Gy. With the exception of HU treatment, cells were incubated for 6 h after treatment with damaging agent before lysis.

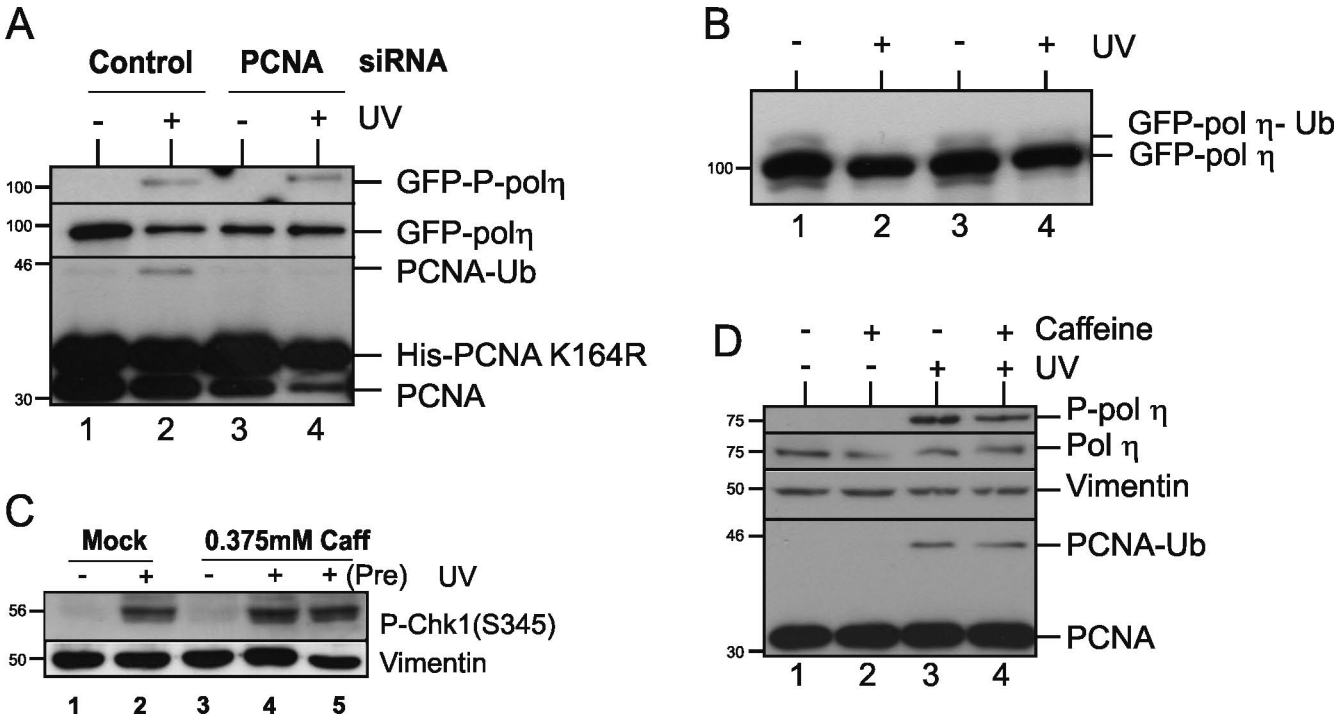

Figure S2. **Phosphorylation of polη is not dependent on PCNA or polη ubiquitination and is resistant to low concentrations of caffeine.** (A) PCNA ubiquitination: MRC5 cells expressing wild-type EGFP-polη and His-PCNA K164R were transfected with control siRNA (lanes 1 and 2) and PCNA siRNA (lanes 3 and 4). Cells were either unirradiated or UV irradiated (20 J/m^2) and incubated for 6 h. (B) Polη ubiquitination: XP30RO cells expressing polη, either wild-type (lanes 1 and 2) or S601A mutant (lanes 3 and 4) were either unirradiated or UV irradiated (20 J/m^2) and incubated for 6 h. (C) Chk1 phosphorylation: MRC5 cells were either unirradiated or UV irradiated (8 J/m^2) and then incubated with or without 0.375 mM caffeine for 3 h. The sample in lane 5 was in addition preincubated with caffeine for 30 min before irradiation. (D) Caffeine: MRC5 cells were either unirradiated or UV irradiated (20 J/m^2) and incubated for 6 h with or without caffeine, as indicated.

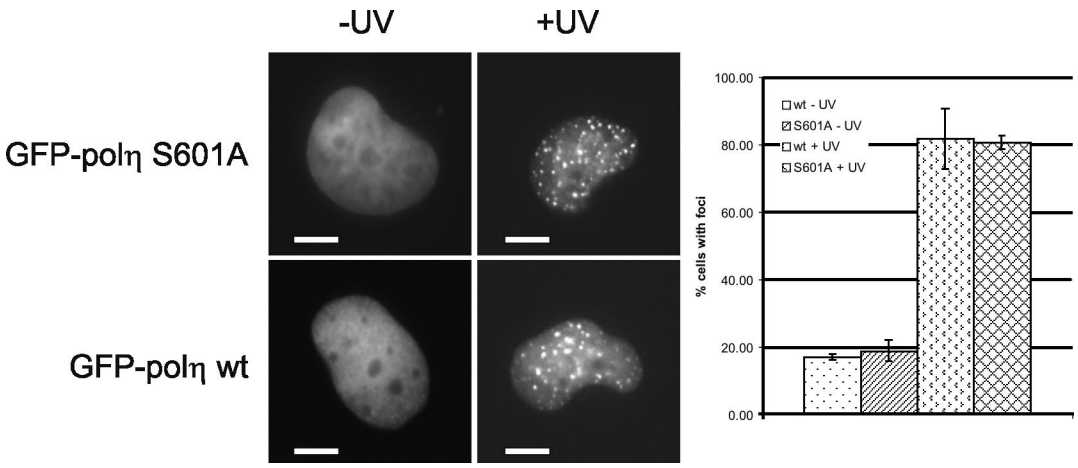

Figure S3. **Foci formation of GFP-polη wild-type and GFP-polη S601A.** MRC5 cells were transfected with GFP-polη wild-type and GFP-polη S601A expression plasmids. Cells were either unirradiated or UV irradiated after 24 h and fixed 6 h after irradiation. Slides were analyzed at room temperature using a 40x objective on a fluorescence microscope (Axioplan2; Carl Zeiss, Inc.) equipped with an ORCA-ER camera (Hamamatsu Photonics). Images were captured using SimplePCI software. Bar, 5 μm.