Han et al., http://www.jcb.org/cgi/content/full/jcb.201311007/DC1

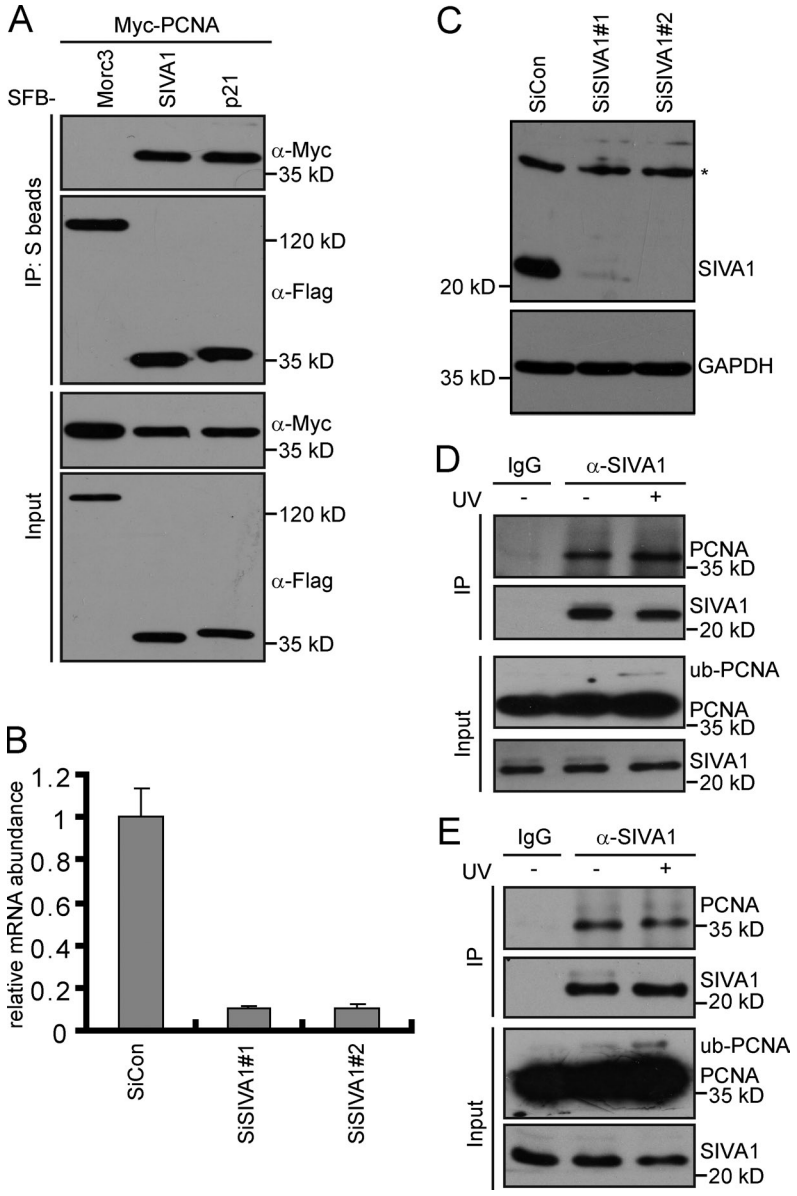

Figure S1. **SIVA1 interacts with PCNA.** (A) HEK293T cells were transiently transfected with plasmids encoding Myc-tagged PCNA together with plasmids encoding SFB-tagged SIVA1, p21, or Morc3. Cell lysates were immunoprecipitated with S beads, and Western blot analysis was performed with anti-Flag and anti-Myc antibodies. (B) Quantitative RT-PCR showing SIVA1 mRNA levels are down-regulated by siRNAs. Bars represent the mean of three experiments, and error bars are SDs. (C) Representative immunoblotting of cells transfected with the indicated siRNAs. The asterisk indicates a nonspecific band. (D and E) SIVA1–PCNA interaction was UV damage independent. HeLa (D) or HEK293T (E) cells were treated with 50 J/m^2 UV for 1 h or left untreated and then lysed with NETN buffer containing Benzonase. Cell lysates were then incubated with protein A agarose beads conjugated with anti-SIVA1 antibody, and Western blot analysis was performed according to standard procedures. IP, immunoprecipitation; SiCon, control siRNA; ub, ubiquitin.

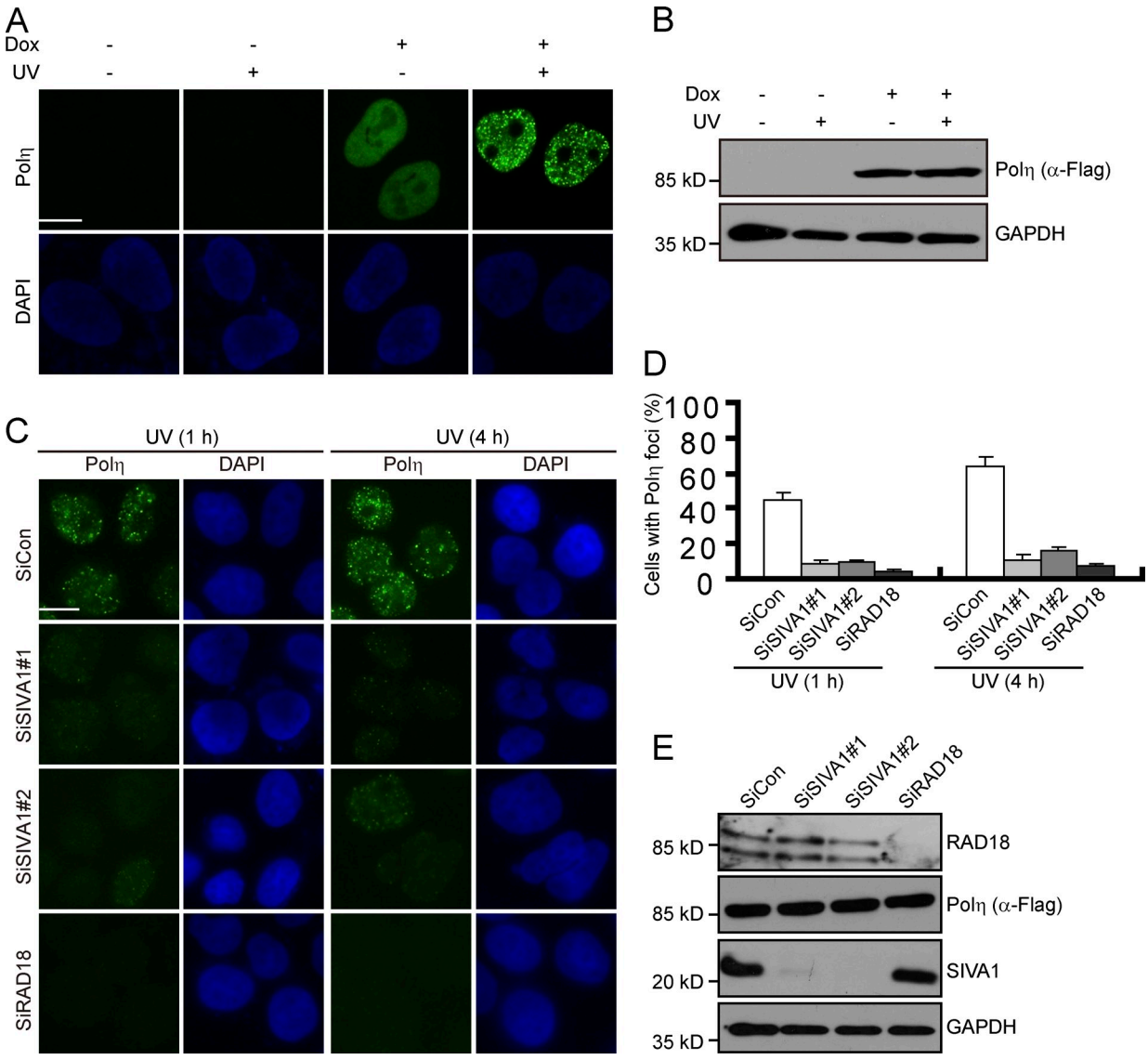

Figure S2. **SIVA1 is required for Polη foci formation.** (A and B) A XP30RO cell line to express SFB-tagged Polη (XP30RO-Polη) under the control of a tetracycline-inducible promoter was generated. The resulting cell line was mock induced or induced by 1 μg/ml doxycycline (Dox) addition for 24 h before 50 J/m^2 UV treatment. 4 h later, cells were fixed and processed for Polη immunofluorescence. (A) Representative Polη foci were shown. (B) The exogenous Polη expression was confirmed by immunoblotting using the anti-Flag antibody. (C–E) A HeLa-derivative cell line stably expressing HA-Flag–tagged Polη was generated. The resulting cell line transfected twice with indicated siRNAs was treated with 50 J/m^2 UV for the indicated times. Cells were then fixed and processed for Polη immunofluorescence. (C) Representative Polη foci were shown. (D) Quantification results were the mean of three independent experiments and were presented as means ± SD. More than 100 cells were counted in each experiment. (E) Knockdown efficiency was confirmed by immunoblotting. SiCon, control siRNA. Bars, 10 μm.

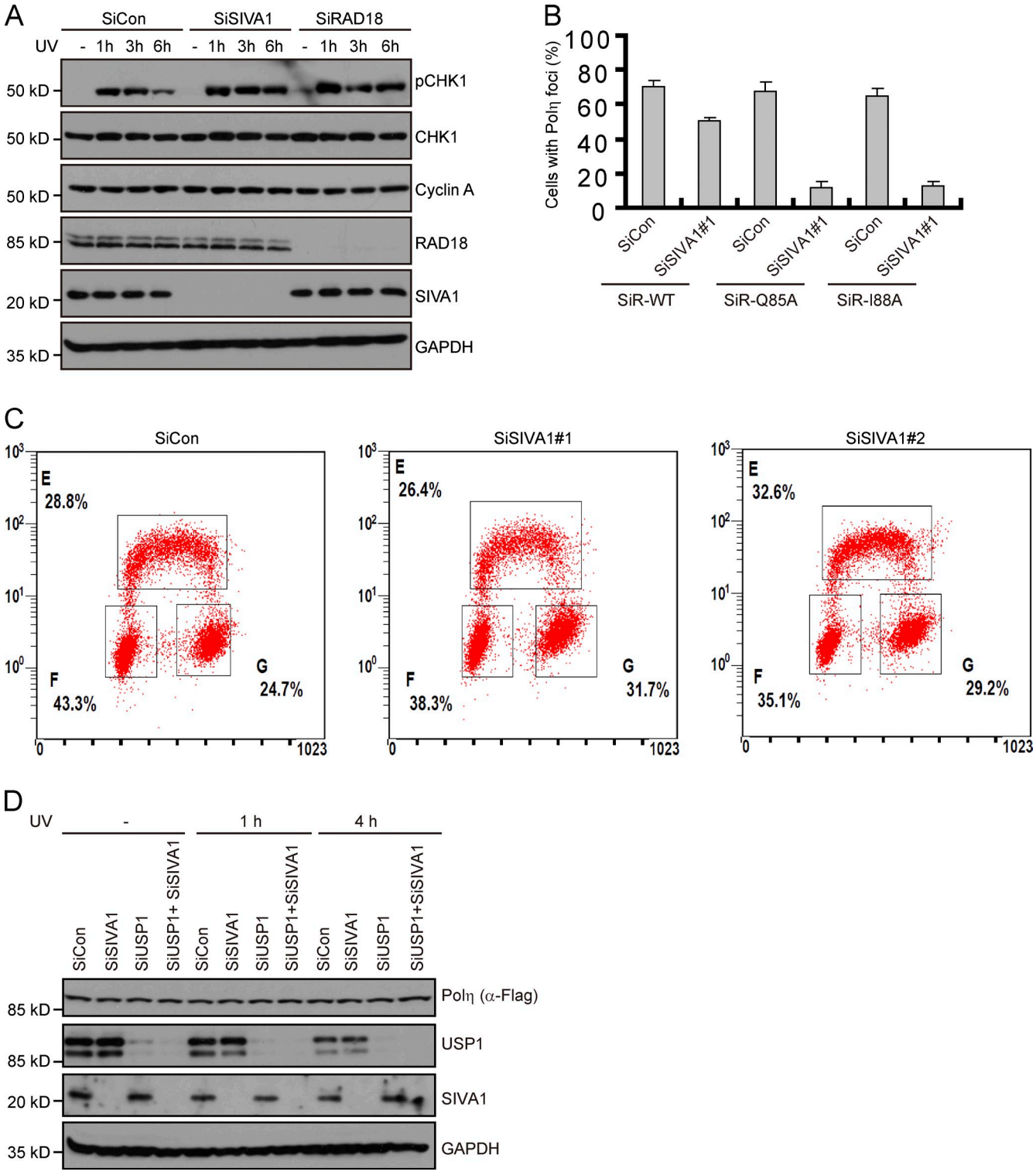

Figure S3. **SIVA1 depletion does not markedly affect cell proliferation.** (A) SIVA1 depletion results in prolonged CHK1 phosphorylation. HeLa cells transfected with the indicated siRNAs were untreated or treated with 50 J/m^2 UV and recovered for the indicated times. Cell lysates were then analyzed by immunoblotting with indicated antibodies. (B) PIP box mutants of SIVA1 could not rescue Polη foci formation in SIVA1-depleted cells. XP30RO-Polη–derivative cell lines stably expressing siRNA#1-resistant wild-type SIVA1 or two PIP box mutants (Q85A and I88A) were generated. The resulting cell line was transfected twice with the indicated siRNAs and was induced by 1 μg/ml doxycycline addition for 24 h before 50 J/m^2 UV treatment. 4 h later, cells were fixed and processed for Polη immunofluorescence. Quantification results were the mean of three independent experiments and were presented as means ± SD. More than 100 cells were counted in each experiment. (C) SIVA1 depletion does not markedly affect cell proliferation. HeLa cells were transfected twice with control siRNA or siRNAs specific for SIVA1. 48 h after the second transfection, BrdU incorporation assays were performed as described in the Materials and methods section. E refers to S phase, F refers to G1 phase, G refers to G2/M phase, and the corresponding numbers represent the percentage of each phase. (D) A HeLa cell line to stably express HA-Flag–tagged Polη was generated. The resulting cell line was transfected twice with the indicated siRNAs. 48 h after transfection, cells were untreated or treated with 50 J/m^2 UV for the indicated times, and knockdown efficiency was confirmed by immunoblotting. SiR, siRNA resistant; SiCon, control siRNA; WT, wild type.

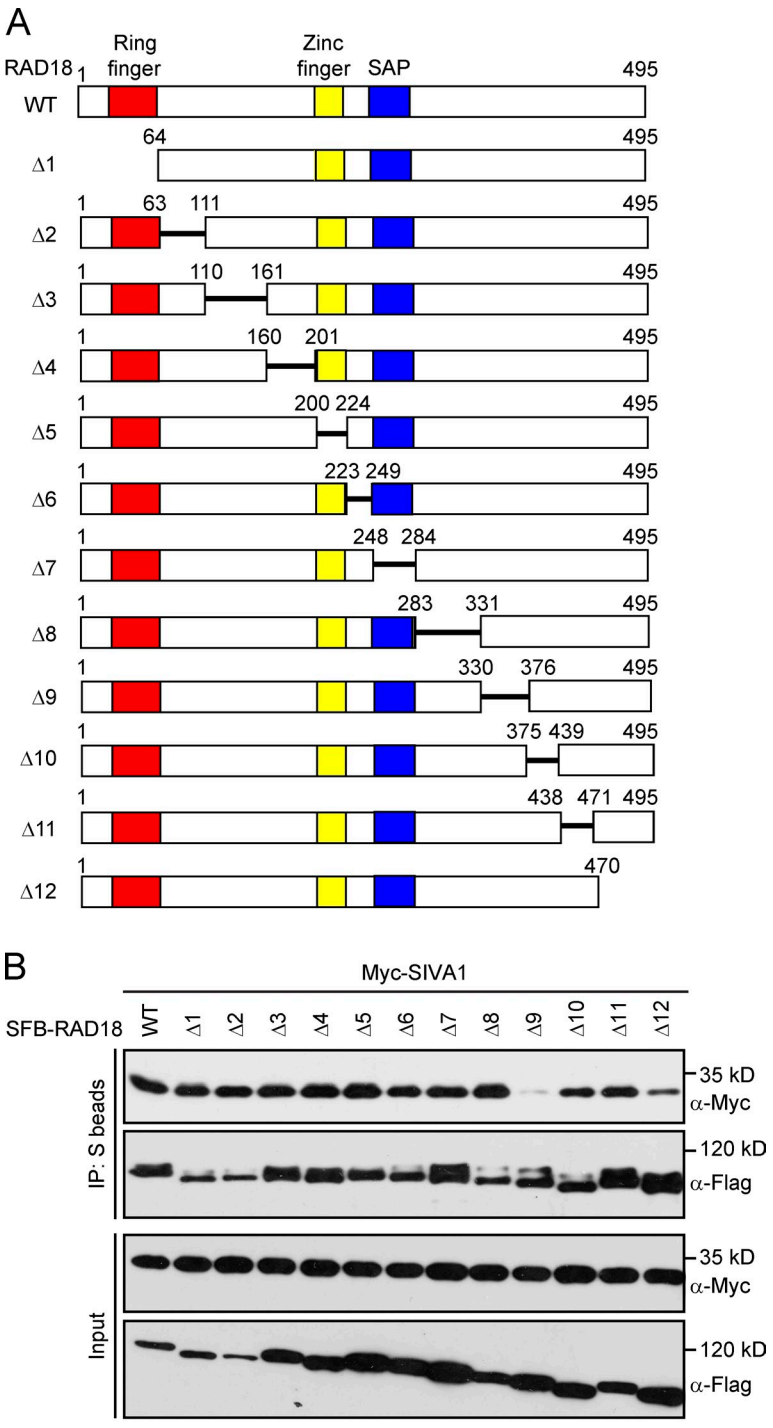

Figure S4. **Residues 331–375 of RAD18 are responsible for SIVA1 binding.** (A) Schematic representation of wild-type and deletion mutants of RAD18 used in this study. (B) Residues 331–375 of RAD18 are responsible for SIVA1 binding. HEK293T cells were transiently transfected with plasmids encoding tagged RAD18 and SIVA1. 24 h after transfection, cells were treated with 50 J/m^2 UV for 1 h. Cell lysates were then immunoprecipitated with S beads, and Western blot analysis was performed with anti-Flag and anti-Myc antibodies. IP, immunoprecipitation; WT, wild type.

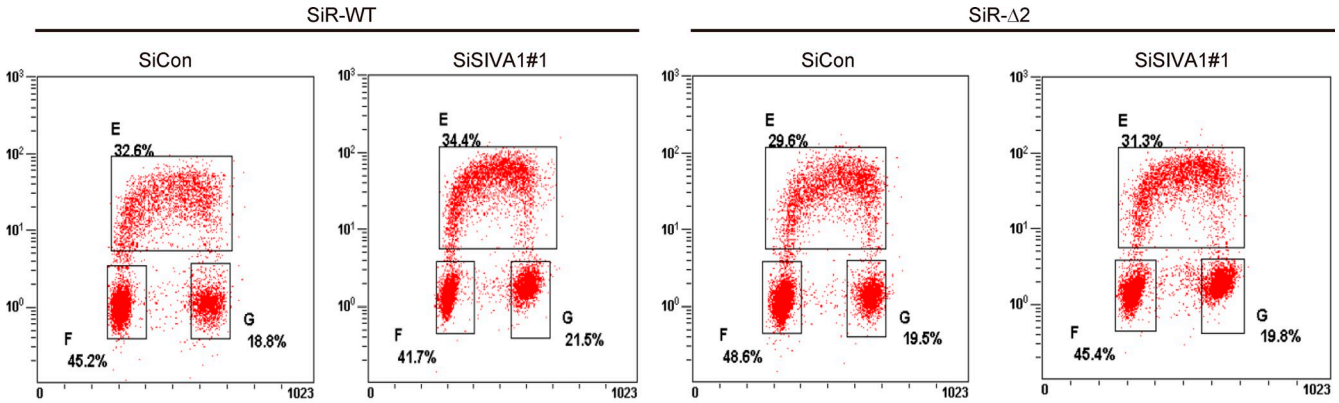

Figure S5. **SIVA1-depleted cells complemented with wild-type or Δ2 mutant of SIVA1 have comparable cell cycle profiles.** A HeLa cell line stably expressing siRNA#1-resistant wild-type SIVA1 (SiR-WT) or its deletion mutant defective in RAD18 binding (SiR-Δ2) was generated. The resulting cell lines were transfected twice with control siRNA or SIVA1 siRNA#1. 48 h after the second transfection, BrdU incorporation assays were performed as described in the Materials and methods section. E refers to S phase, F refers to G1 phase, G refers to G2/M phase, and the corresponding numbers represent the percentage of each phase.