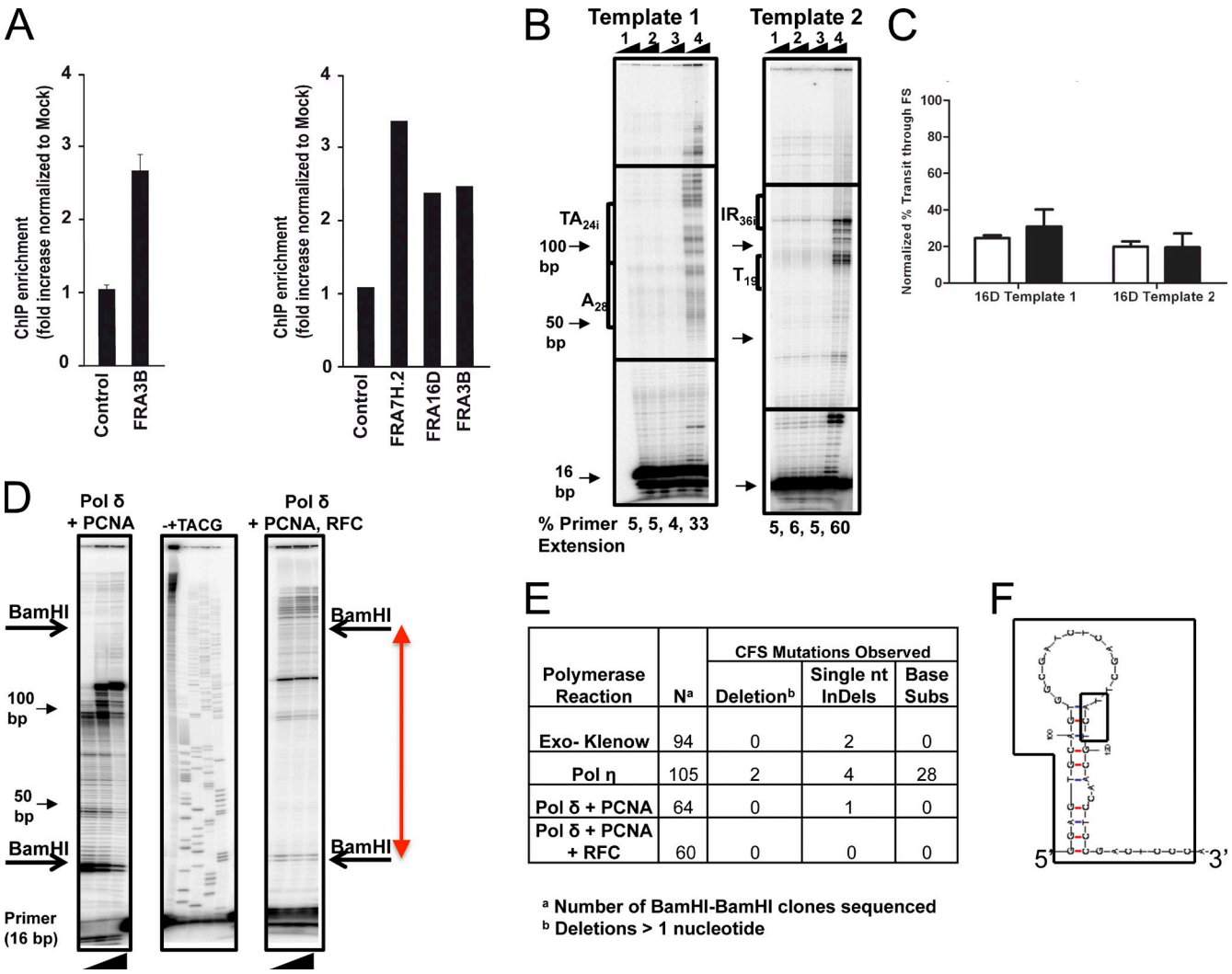
Bergoglio et al., http://www.jcb.org/cgi/content/full/jcb.201207066/DC1



Polymerase Reaction	N[a]	CFS Mutations Observed		
		Deletion[b]	Single nt InDels	Base Subs
Exo- Klenow	94	0	2	0
Pol η	105	2	4	28
Pol δ + PCNA	64	0	1	0
Pol δ + PCNA + RFC	60	0	0	0

[a] Number of BamHI-BamHI clones sequenced
[b] Deletions > 1 nucleotide

Figure S1. **Additional experiments showing Pol η recruitment on CFSs and in vitro DNA polymerization activity by purified DNA polymerases δ, η, and Klenow.** (A) ChIP analysis. ChIP was performed using anti-Pol η antibody or anti–rabbit IgG in U2OS cells stably expressing Dead Pol η exposed to 0.2 µM aphidicolin for 24 h. Pol η enrichment was assessed by real-time qPCR amplification. Fold increase of chromatin immunoprecipitation (ChIP) over input DNA was calculated as a ratio between Mock and Pol η for each replicate. Left panel represents Dead Pol η enrichment at the CFS FRA3B (this experiment was completed twice, mean ± SEM); right panel, which shows a representative experiment out of three repeats for FRA7H and FRA16D and two repeats for FRA3B, demonstrates that Dead Pol η binds to FRA7H, FRA16D, and FRA3B sequences. (B and C) Effect of replication accessory factors on Pol δ synthesis. Primer extension reactions were performed with 300 fmol human four-subunit Pol δ with or without 400 fmol human PCNA and 50 fmol yeast RFC, 5 mM ATP, 50 mM Tris-HCl, pH 7.0, 50 mM $MgCl_2$, 2 mM DTT, 0.2 mg/ml BSA, 2% glycerol, 50 mM NaCl, and 250 µM dNTPs. Products were separated by denaturing gel electrophoresis. (B) Representative gel for primer extension reactions on FRA16D templates 1 and 2. CFS template region is outlined by black bars. Black triangles represent 5-min and 15-min time points. Non-B DNA elements within CFS templates are outlined in brackets. Numbered lanes 1–4 represent reactions containing: (1) Pol δ only; (2) Pol δ and PCNA; (3) Pol δ and RFC; and (4) Pol δ with both PCNA and RFC. Percent primer utilization for the 15-min time point of each reaction is displayed below the gels, and was calculated as: [amount of reaction past the primer region] / [amount of reaction past the primer region + within the primer region] × 100. (C) Quantification of Pol δ synthesis past the CFS template. Percent transit was calculated as [amount of product past the CFS template] / [amount of product past the CFS template + within the CFS template] × 100. Percent transit for FRA16D templates 1 and 2 was then normalized to that on the control template, under identical reaction conditions. Although primer utilization by Pol δ was greatly increased in the presence of both RFC and PCNA, consistent with RFC loading of PCNA onto the DNA primer template, the Pol δ percent transit past the CFS region remained dramatically decreased relative to the control template, and pausing at specific DNA elements was unchanged. (D–F) Polymerase fidelity through FRA16D template 2. (D) Representative denaturing gel electrophoresis showing Pol δ synthesis products within FRA16D template 2, using the two reaction conditions of the fidelity experiment (Pol δ with PCNA, and Pol δ with PCNA and RFC). Triangles indicate increasing reaction time: 15, 30, and 60 min. −, no polymerase control. +, hybridization control. TACG, sequencing ladder. Arrows indicate positions of the BamHI restriction enzyme sites that were used to isolate DNA products synthesized through the FRA16D sequence. Note that DNA products terminated within the FRA16D sequence will not be analyzed by this approach. (E) Observed mutations within exonuclease-deficient Klenow polymerase, human Pol η, and human four-subunit Pol δ (with and without RFC-loaded PCNA) reaction products. Values indicate the number of each type of mutational event identified within sequenced clones. (F) Mfold predicted hairpin structure formed within the FRA16D template 2 inverted repeat sequence. Boxes indicate the regions deleted in two sequenced Pol η clones.

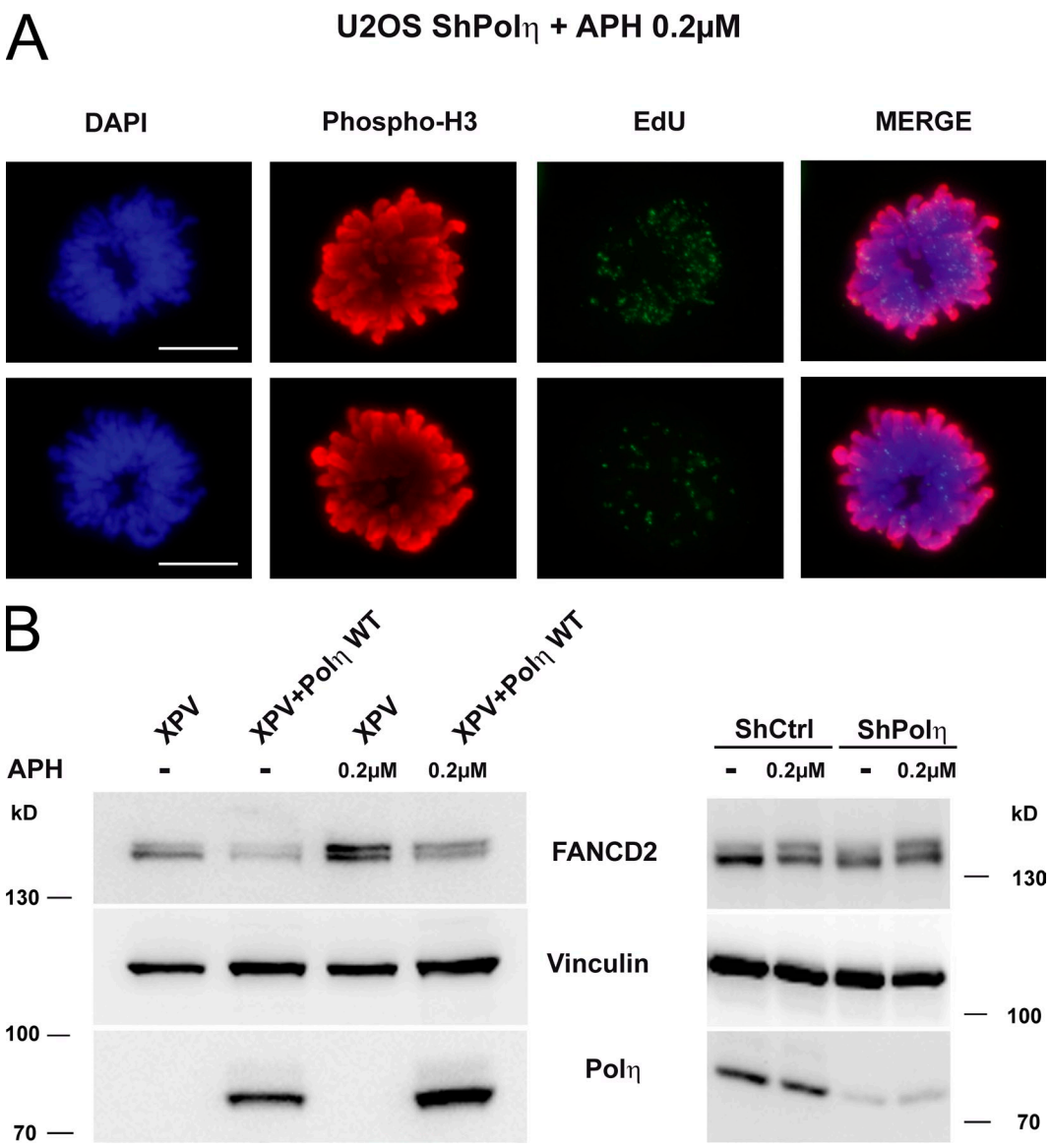


Figure S2. **EdU incorporation in mitotic cells and analysis of FANCD2 monoubiquitination.** (A) EdU incorporation in mitotic cells. Asynchronously growing cells treated with 0.2 μM APH for 24 h were labeled with EdU for 45 min, fixed, and stained with a phospho-H3 (Ser10)–specific antibody. Representative images are presented showing the presence of EdU signals on p-H3–positive mitotic chromosomes. Bar, 10 μM. (B) FANCD2 monoubiquitination in aphidicolin-treated Pol η–deficient cells. Western blot experiments of untreated and APH-treated mock-depleted (ShCtrl) or Pol η–depleted (ShPolη) U2OS cells and XP30RO cell (XPV) and XP30RO cells stably complemented with the WT Pol η (XPV + Pol η), showing normal FANCD2 monoubiquitination after APH treatment in Pol η–deficient cells. Vinculin serves as a loading control.

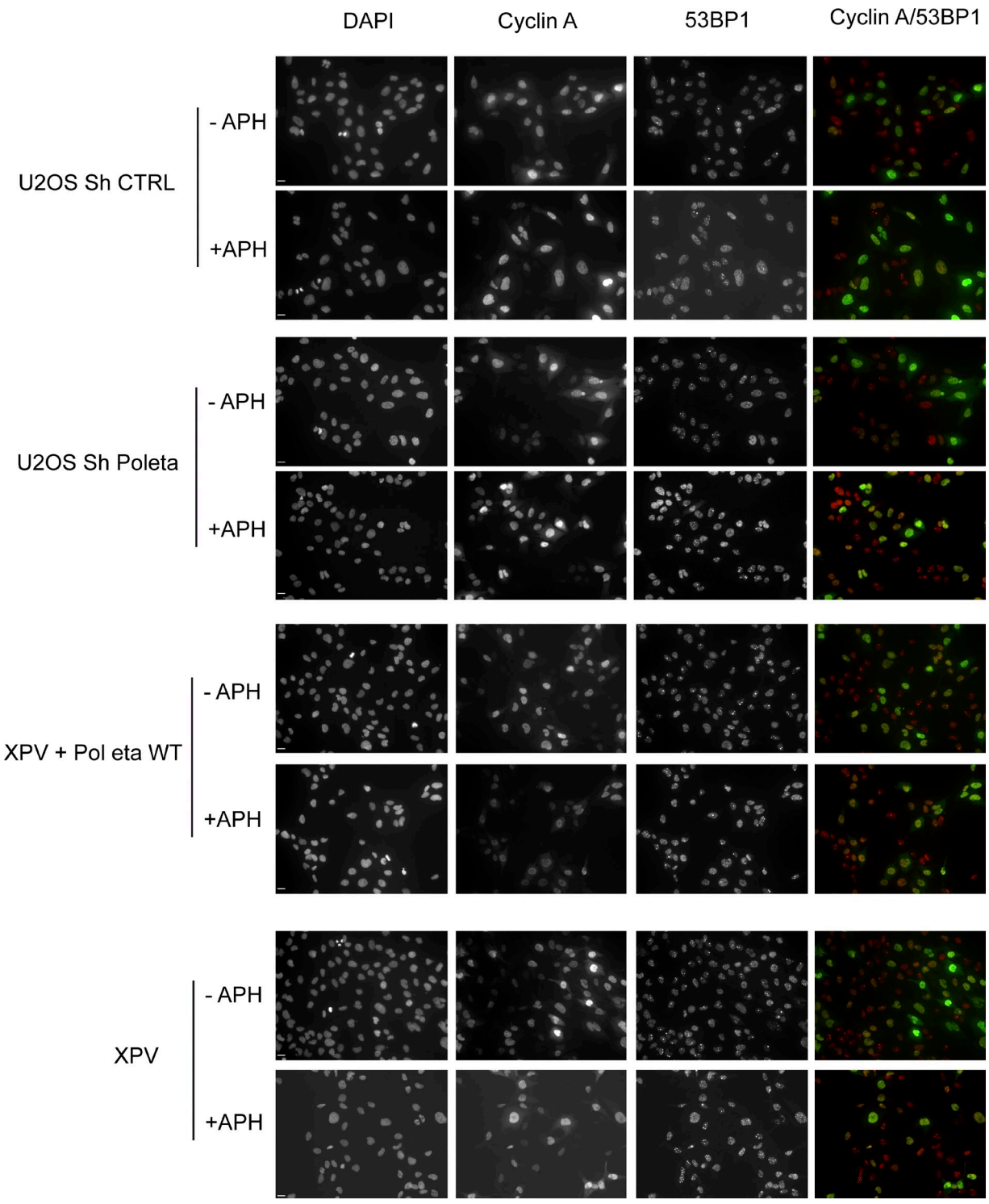


Figure S3. **Examples of wide-field microscopy images of the 53BP1 bodies, cyclin A and DAPI staining from the indicated cell lines, untreated or treated with 0.2 μM aphidicolin.** Image acquisition of multiple random fields were performed on a wide-field microscope (20× objective, model DMLA; Leica). Bar, 10 μM.

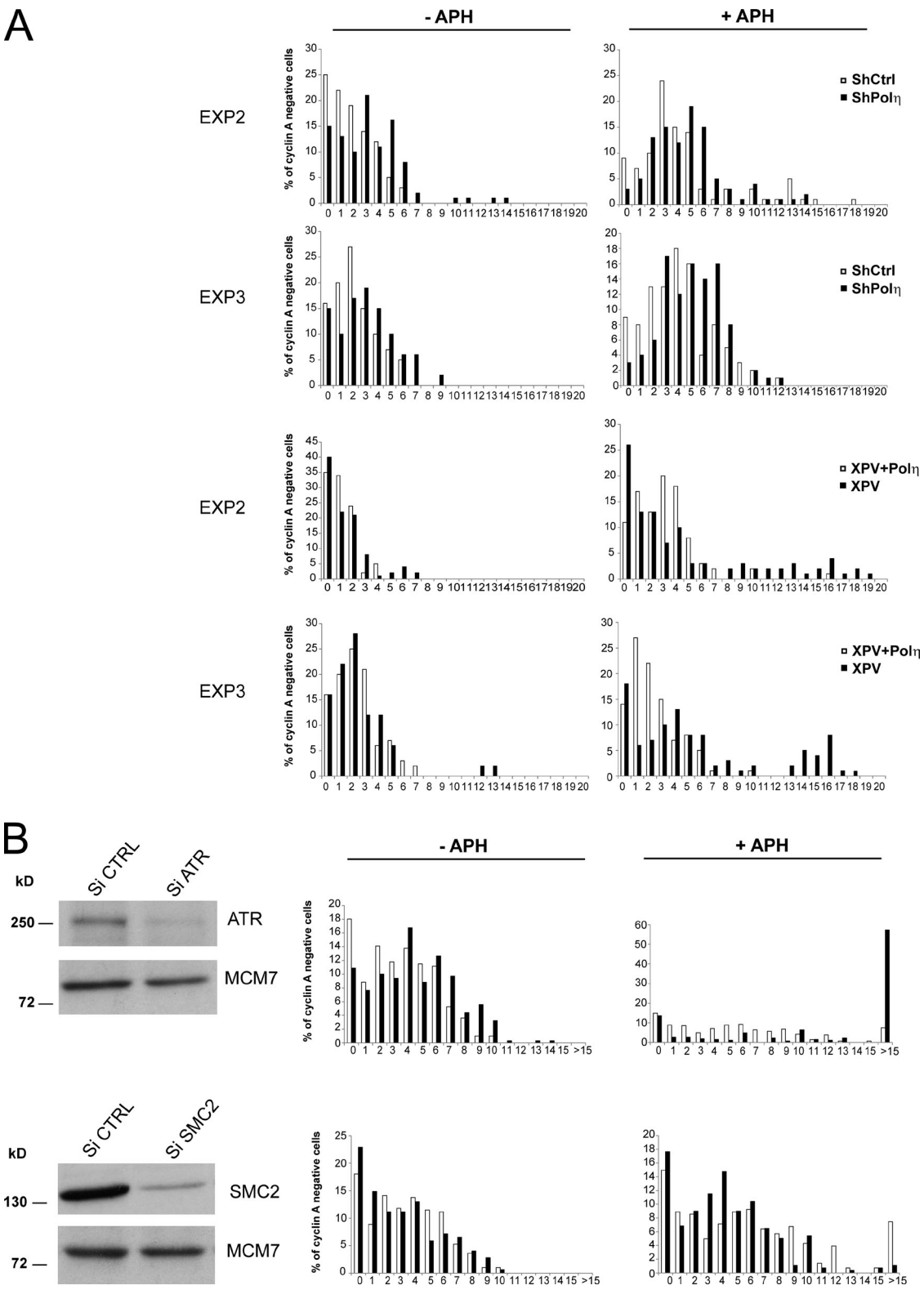


Figure S4. **Quantification of 53BP1 bodies in G1 nuclei from the indicated cell lines.** (A) Effect of Pol η depletion: replica of the experiments shown in Fig. 3 c. The data shown are from a single representative experiment out of three repeats (n = 100). (B) Extracts from U2OS mock-depleted (SiCtrl), ATR-depleted (SiATR), or SMC2-depleted (SiSMC2) cells were analyzed by immunoblotting for the detection of ATR and SMC2 as well as MCM7 as loading control. The data shown on histograms correspond to a single representative experiment out of three repeats (n = 300).

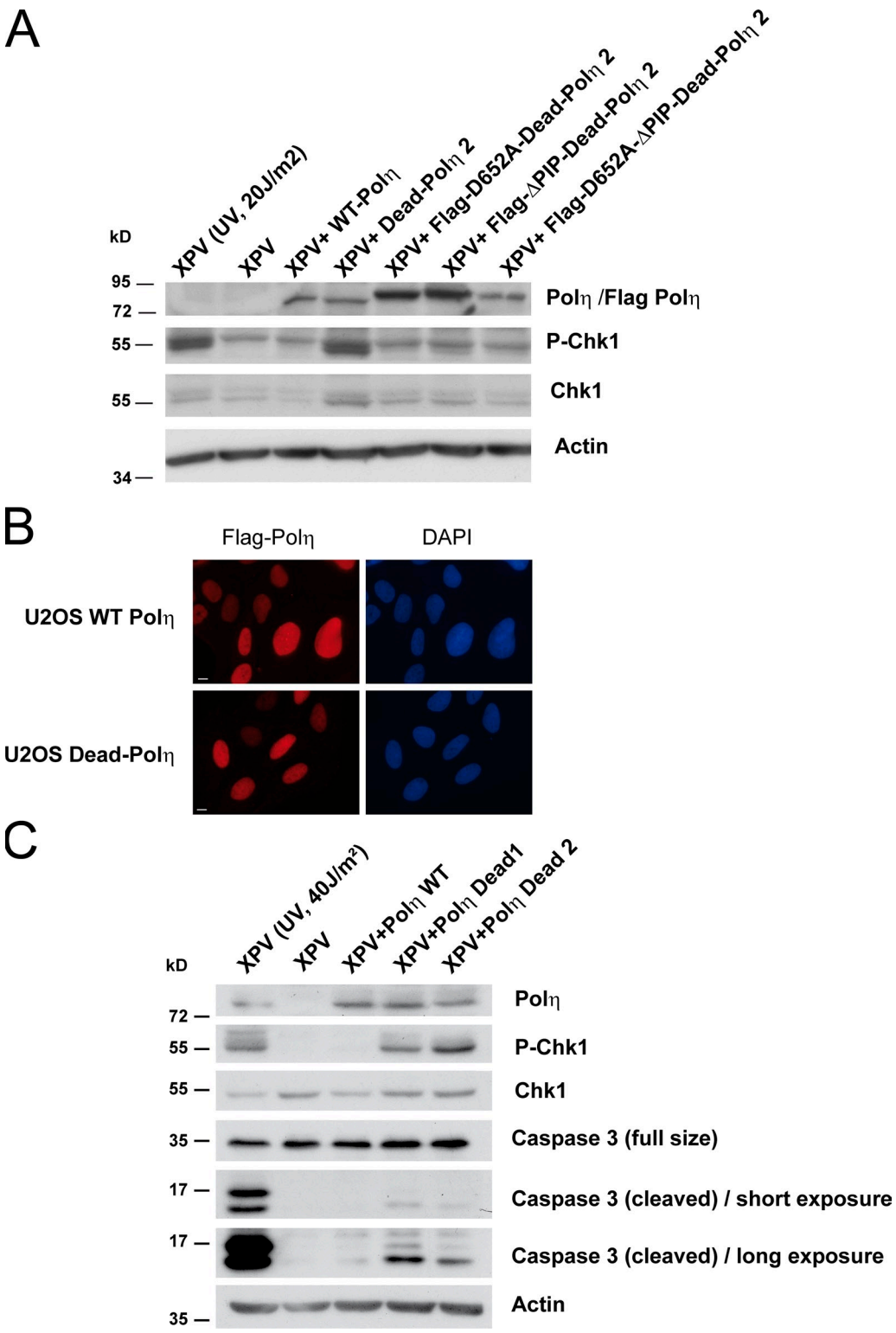


Figure S5. **Effects of Dead Pol η expression.** (A) Additional experiments performed with independent cellular clones stably expressing Dead Pol η (XPV+Dead Pol η), D652A-Dead Pol η (XPV+ Flag-D652A-Dead Pol η), ΔPIP-Dead Pol η (XPV+Flag-ΔPIP-Dead Pol η), and the D652A-ΔPIP-Dead Pol η (XPV+Flag-D652A-ΔPIP-Dead Pol η) to demonstrate the requirement of PIP and UBZ domains of Dead Pol η for replication checkpoint activation. The detection of Pol η was performed with Pol η antibodies (Abcam) in extracts from XPV+ WT Polη, XPV+Dead Pol η, and XPV+Flag-D652A-Dead Pol η, whereas the XPV+Flag-ΔPIP-Dead Pol η and the XPV+Flag-D652A-ΔPIP-Dead extracts were blotted with the FlagM2 antibody because the ΔPIP mutants are not recognized by the Pol η antibody (Abcam). Extracts from XPV cells treated with UV (20 J/m^2, 6 h) serves as positive control for Chk1 phosphorylation. Actin: loading control. (B) U2OS cells stably expressing FLAG-tagged wild-type or Dead Pol η used for the FISH experiments shown in Fig. 5 c were analyzed by immunofluorescence to show that the expression levels of both proteins are similar (more than 90% of cells were positive). Images were obtained with a microscope (63× objective, model DMLA; Leica). Bar, 10 μM. (C) Apoptosis induction after Dead Pol η expression. Extracts from XPV cells, XPV cells stably complemented with Pol η WT (XPV+ WT Polη), or the Dead form of Pol η (XPV + Polη Dead1 and XPV+Polη Dead2) were fractionated and the soluble fractions were analyzed by immunoblotting for the detection of Pol η, P-Chk1(ser 345), Chk1, actin (as loading control), and caspase-3. Activation of caspase-3 requires proteolytic processing into two activated p17 and p12 fragments.