(*EMBOJ-2011-78755*) Jones, et al.

SUPPLEMENTARY MATERIALS

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FACS analysis, PI staining and EdU pulse chase assay. U2OS cells were pulsed with 10 μM EdU for 30 mins and fixed in 70% ethanol or washed 2x in PBS, placed in fresh media and fixed 4, 8 and 18 hrs after the initial pulse. EdU incorporation was measured using Alexa fluor 647 and Propidium Iodine (PI) Click-iT EdU Flow Cytometry assay kit (Invitrogen). Flow cytometry data acquisition was performed on an LSR II and data analysis was performed using FACS Diva software (BD Pharmingen, San Diego, CA) or FlowJo software (Treestar, Ashland, OR).

Cellular fractionation. Whole-cell extracts were prepared in SDS sample buffer (0.1 M Tris pH 6.8, 2 % (w/v) SDS and 12% (v/v) β-mercaptoethanol). Soluble proteins were removed by extraction in cytoskeletal buffer (CSK) (10 mM Pipes pH 6.8, 300 mM sucrose, 100 mM NaCl, 3 mM $MgCl_2$, 1 mM EGTA and 0.5% Triton X-100) for 10 min at 4°C. Pellets were washed once with CSK buffer and lysed in SDS sample buffer.

SUPPLEMENTARY FIGURE LEGEND

Supplemental Figure 1. Completion of bulk DNA replication is not delayed in USP1-depleted cells. (A) U2OS cells were transfected with siRNAs as indicated and treated with cytochalasin-B for 24 hrs (with or without Aphidicolin, APH, 0.3 μM) prior to fixation

and assayed for micronucleation. Graph display percentage (%) of cells with micronuclei. Error bars represent standard deviation of experiment done in triplicate (n=300). (B) U2OS cells were transfected with Ctrl or USP1 siRNA and treated with 2 mM HU for the indicated time points and analyzed by Western blot. (C) U2OS cells were pulse-labeled with EdU for 30 minutes and harvested immediately or washed twice in PBS and placed into fresh media and harvested 4, 8 and 16 hrs after labeling. Cells labeled with EdU for the different samples were then analyzed by FACS. The PI panels represent the cell cycle distribution of the EdU labeled fraction of the population.

Supplemental Figure 2. USP1 knockdown enhances Polη foci formation in undamaged cells. Representative images of GFP-Polη in cells transfected with indicated siRNAs. Cells were immuno-stained with DAPI (gray), anti-GFP (green) and anti-PCNA (red) antibodies, with merged images displayed to show co-localization.

Supplemental Figure 3. Increased micronuclei formation caused by USP1 depletion with second USP1 (2) siRNA sequence is also Polκ-dependent. U2OS cells were transfected with the indicated siRNAs and assayed for micronucleation (n=600). Western blots were performed with cells from the same transfection to demonstrate efficiency of siRNA knockdown and probed with the indicated antibodies.

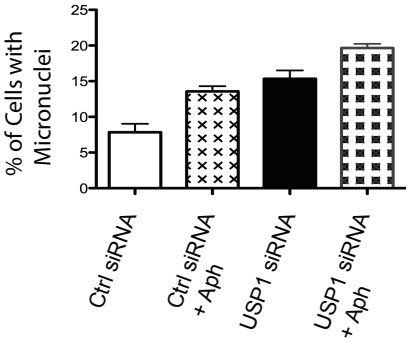
Supplemental Figure 4. USP1 depletion increases mitotic paired FANCD2 foci formation. (A) U2OS cells were transfected with the indicated siRNAs and fractionated into soluble and chromatin-bound fractions. Western blot shows cellular distribution of the indicated proteins. (B) U2OS cells were transfected with indicated siRNAs and fixed for immunofluorescence with anti-FANCD2 antibody. Metaphase cells were observed and scored for paired FANCD2 nuclear foci. A representative image of USP1

knockdown cell with FANCD2 mitotic foci is depicted. The graph on the left shows percent of cells containing paired FANCD2 mitotic foci. Experiments were done in triplicate. The graph on the right shows the total distribution of the number of FANCD2 mitotic foci per cell (n=65 for each condition). (C) U2OS cells were transfected with siRNAs as indicated and analyzed by Western blot and micronuclei formation. Graph display percentage (%) of cells with micronuclei. Error bars represent standard deviation of experiment done in triplicate (n=300).

Supplementary Figure 5. Tethering Polκ to PCNA can increase the severity of genomic instability. (A) Representative images of U2OS cells transiently expressing GFP-Polκ p21 PIP WT. Cells were fixed in ice cold 100% methanol (No pre-extraction) and co-stained for PCNA (red) and DAPI (gray). GFP positive cells only formed foci during S-phase (as indicated by the diffuse G1 staining pattern observed for PCNA in the GFP positive cells that were negative for foci. GFP expressing cells that were positive for foci all colocalized with PCNA foci, which is indicative of S phase).
(B) U2OS cells were transfected with GFP vector, GFP-Polκ WT, GFP-Polκ p21 PIP WT and GFP-Polκ p21 PIP ΔUBZ constructs and scored for the number of micronuclei per binucleate cell (n=300). Graph displays percentage (%) of cells with greater than one (>1) micronucleus per cell. Error bars represent standard deviation of experiment done in triplicate. (C) Representative images of a U2OS cell transiently expressing GFP-Polκ p21 PIP CAT. Cells were co-stained for PCNA (red) and DAPI (gray). GFP expressing cells were still capable of forming foci without the catalytic activity of Polκ.

Supplemental Figure 1

A



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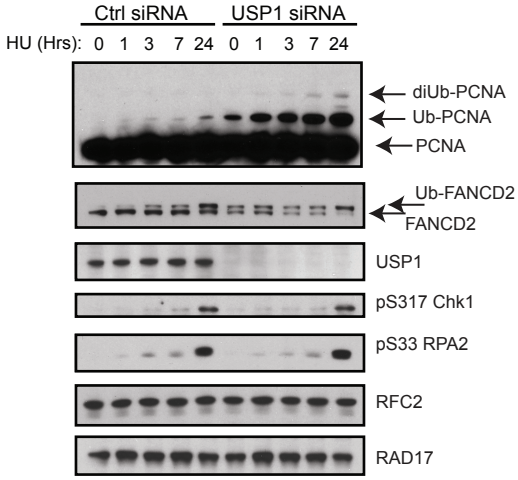

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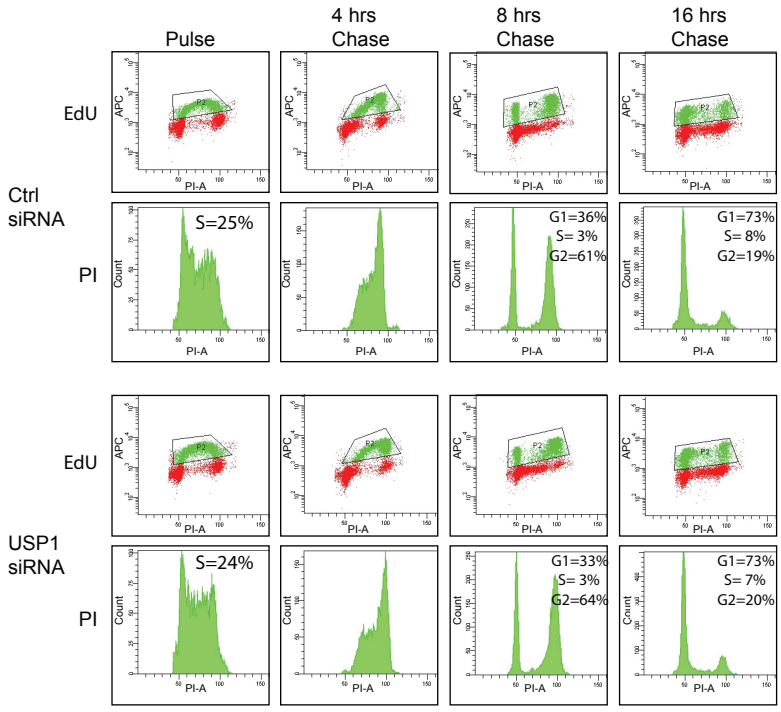
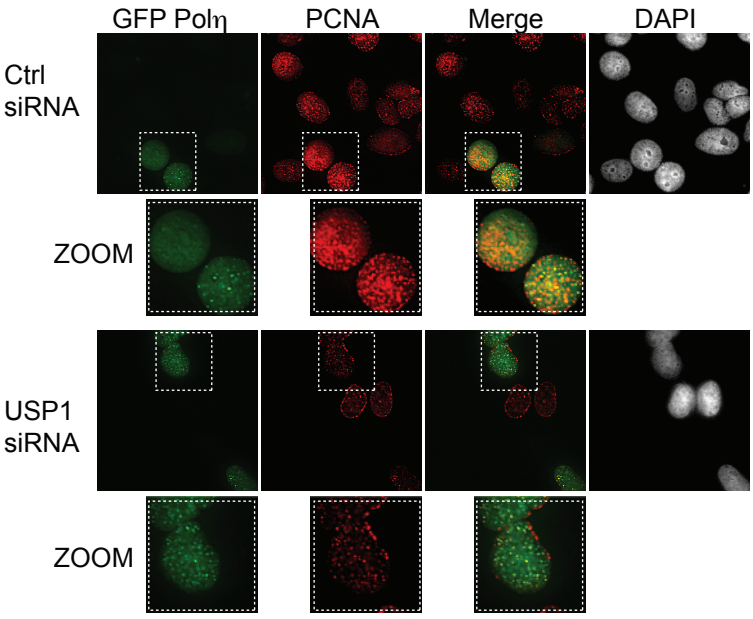
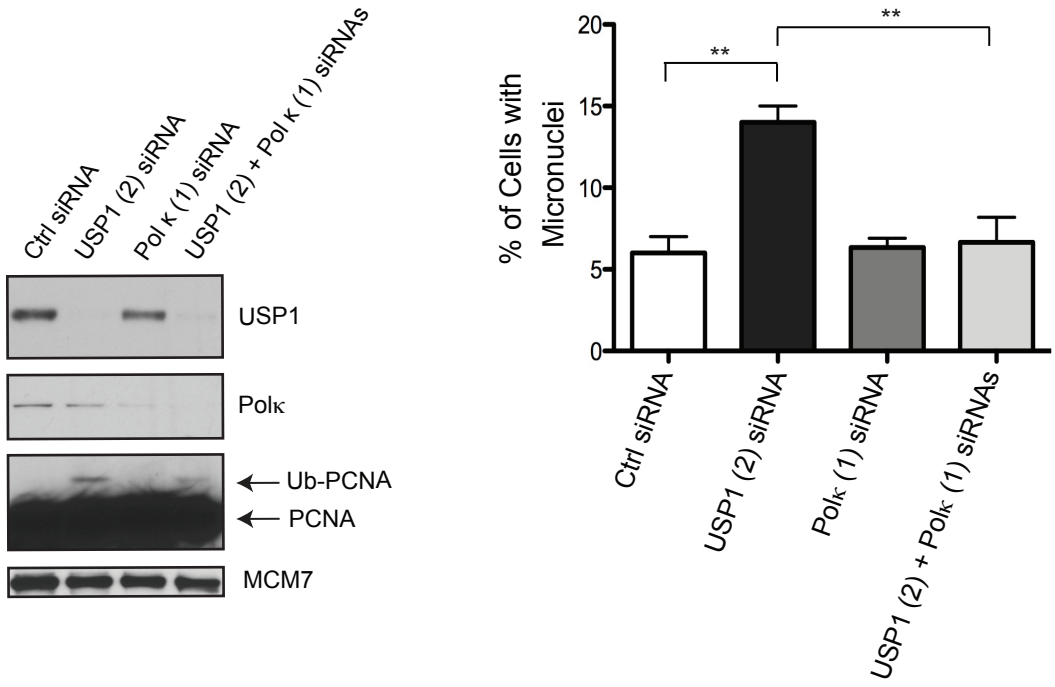


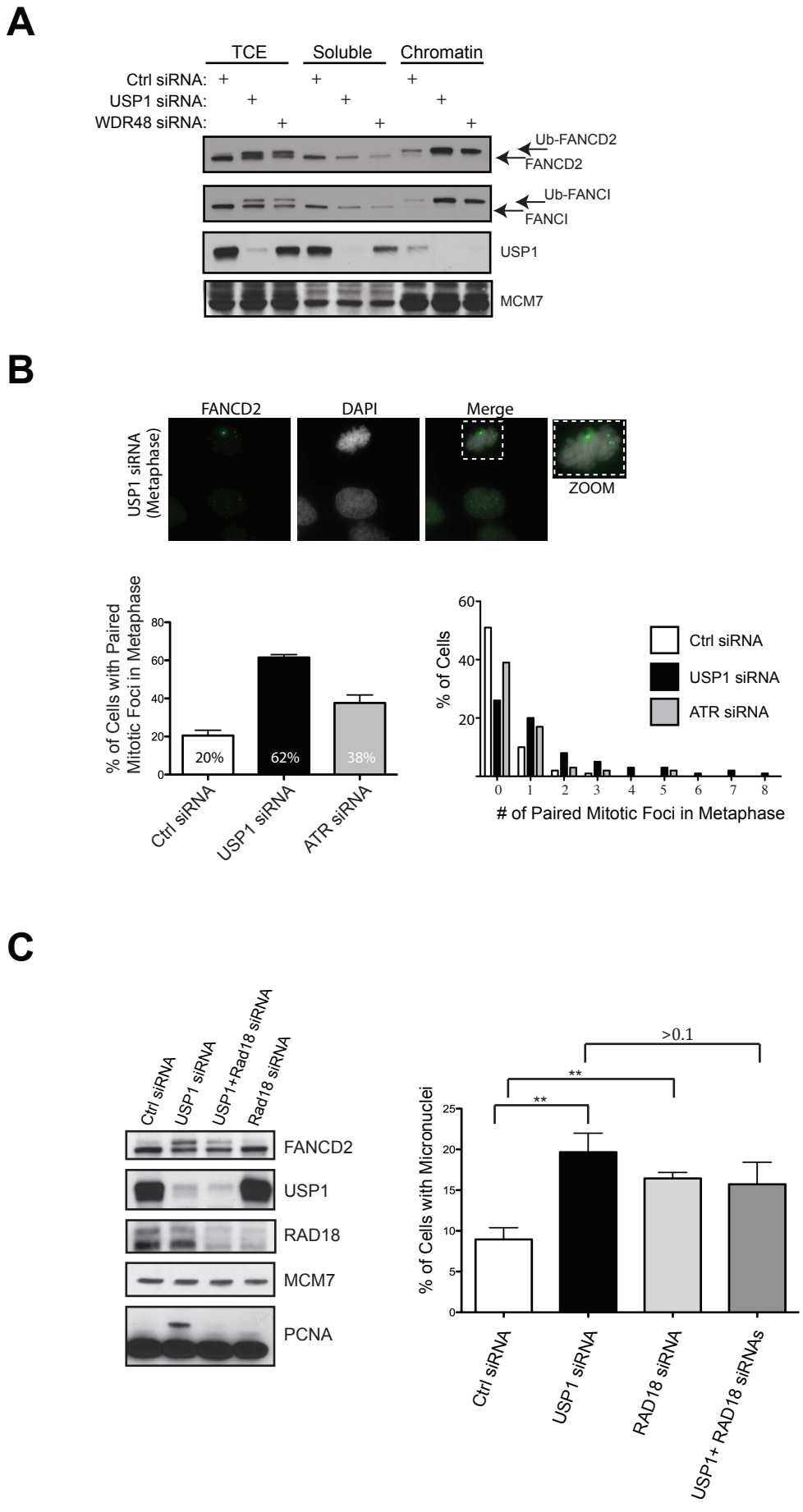
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5