Supplemental Information

DNA Polymerase Eta Prevents Tumor Cell Cycle Arrest And Cell Death During Recovery from Replication Stress

Ryan P. Barnes, Wei-Chung Tsao, George-Lucian Moldovan, and Kristin A. Eckert

Supplemental Table 1. Statistical Summary of Figure 5. For each flow cytometry population, the 2-way ANOVA value is listed, as well as the results of the post-hoc test which define the time points between the two groups were the most different.

Supplemental Table 1

Parental 8988T vs POLH$^{-/-}$ C.1		
Phenotype After Release	**Significance of Cell Type (*2-Way ANOVA)***	**Significantly Different Time Points (Sidak *Post-Hoc)***
G1 Phase Population	0.0165*	0hr
G2/M Phase Population	<0.0001 ****	8h**
EdU Incorporation	<0.0001 ****	8h**
Sub G1 (Apoptosis)	<0.0001 ****	8h**, 12h**
>4C (Re-replication)	0.0002 ***	24h*
EdU- S Phase Cells (RS)	0.0030 **	24h*
Total pH3+ Cells (Mitotic)	0.0029 **	-

Supplemental Figures

Figure S1. **Supplemental data to support Figure 1.** (A) cDNA from treated BJ-5A cells was analyzed for POLH expression in comparison to 18S or Actin control genes. (B) HCT-116 cells were analyzed POLH expression following 24 hours of DMSO treatment, or no treatment (UT). Statistical significance is relative to wild-type cells.There was no statistical difference between the two. (C) Immunoblots of 8988T cells treated with the indicated doses of HU or Aph for the hours indicated. (D) Immunoblots of U2OS cells treated with the indicated doses of HU or Aph for the hours indicated. (E) U2OS cells were transfected with siRNA, and then treated with Aph for 24 hours before immunoblot analysis.

Figure S2. **Cell Cycle Analyses to Support Figure 1.** (A) Representative histograms of cells analyzed by PI staining and flow cytometry. Cells were treated as indicated for 24 hours. (B) Quantification of U2OS and 8988T cell cycle changes as a function of Aph treatment. Statistical significance is relative to DMSO for each line.

Figure S3. Supplemental Data to Support Figure 2. (A) 8988T cells were treated and analyzed for endogenous Pol Eta foci as in Figure 2A, B. (B) 8988T cells were fractionated following 24 hours 0.6 μM Aph as in Figure 2C. (C) Following 24 hours 250 μM HU, 8988T cells were separated into Triton X soluble (TS) or Triton X insoluble (TI) fractions and analyzed by immunoblot. (D) XPV and wild-type POLH complemented cells were treated with 0.6 μM Aph and analyzed as in (C). Arrows point to the mono-ubiquitinated form of PCNA.

Figure S4. **Supplemental data to support Figure 3.** (A) Quantification of XPV and wild-type complemented cell populations by flow cytometry in untreated or DMSO treated (24 hours). (B) XPV and wild-type POLH complemented cells were analyzed by PI staining and flow cytometry

after UV irradiation and 24 hour recovery. (C) Representative primary flow data for 8988T treated cell populations (Figure 3B). (C) 8988T cells were treated with HU for 24 hours, and then analyzed for pChk1 (Ser317) by immunoblot. (D) The expression of Pol η in XPV-complemented cells (Figure 3E) is quantified.

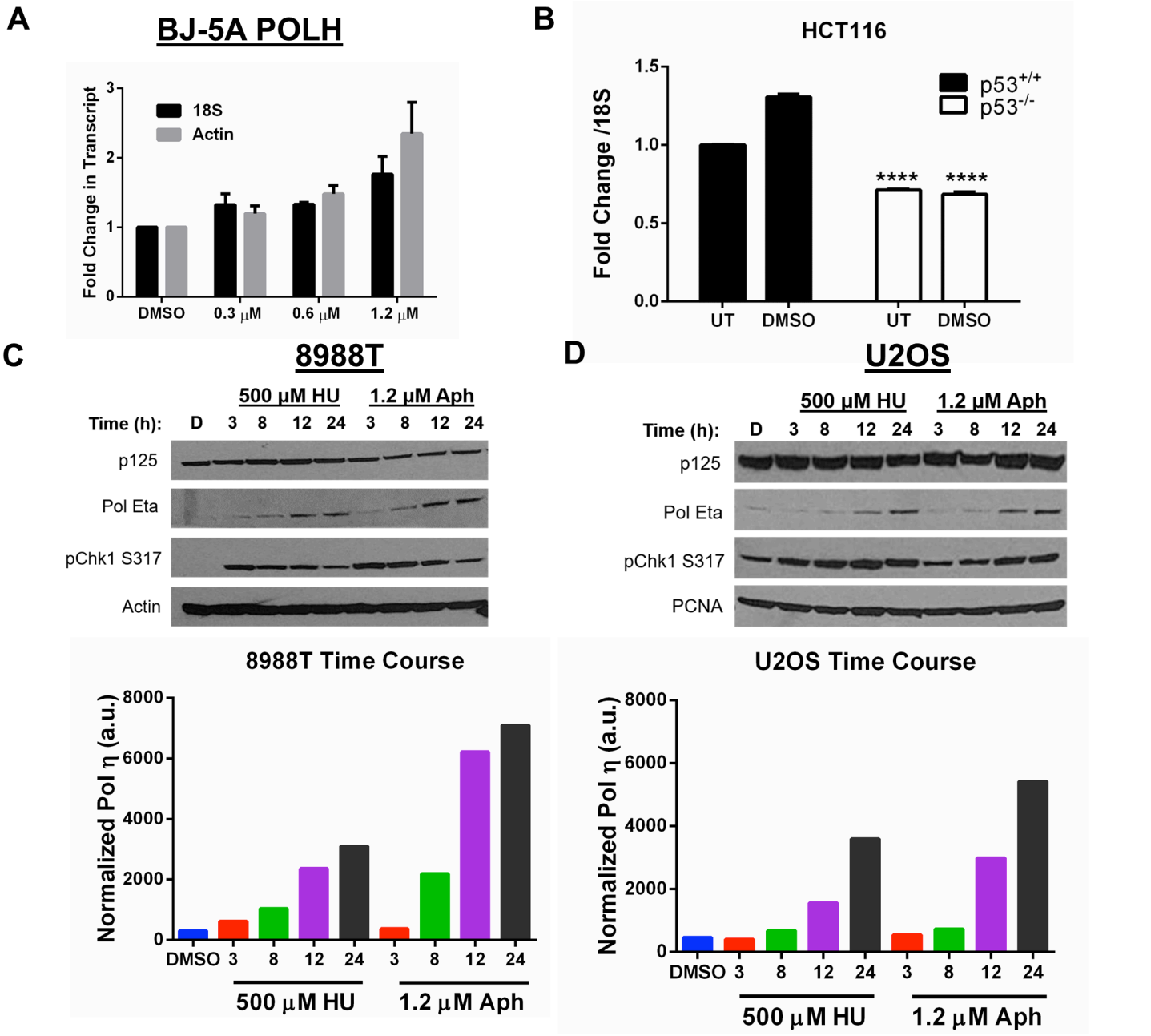
Figure S4. Supplemental data to support Figure 4. (A) Immunoblot showing no Pol η expression in U2OS POLH$^{-/-}$ C.2 even after Aph treatment. **(B)** Clonogenic survival of U2OS or 8988T cells following transfection with POLH siRNA, and Aph treatment. (C-D) Cell populations were analyzed on the third day aftr transfection for Pol η protein (C) or mRNA (D) expression.

Figure S5. Supplemental data to support Figure 5. (A) Representative scatterplots for the experiment performed in Figure 5 with a second POLH$^{-/-}$ clone. (B) 50,000 8988T cells were seeded in 6-well plates and allowed to grow for the indicated times before counting. Each time point was done in duplicate and counted at least twice.

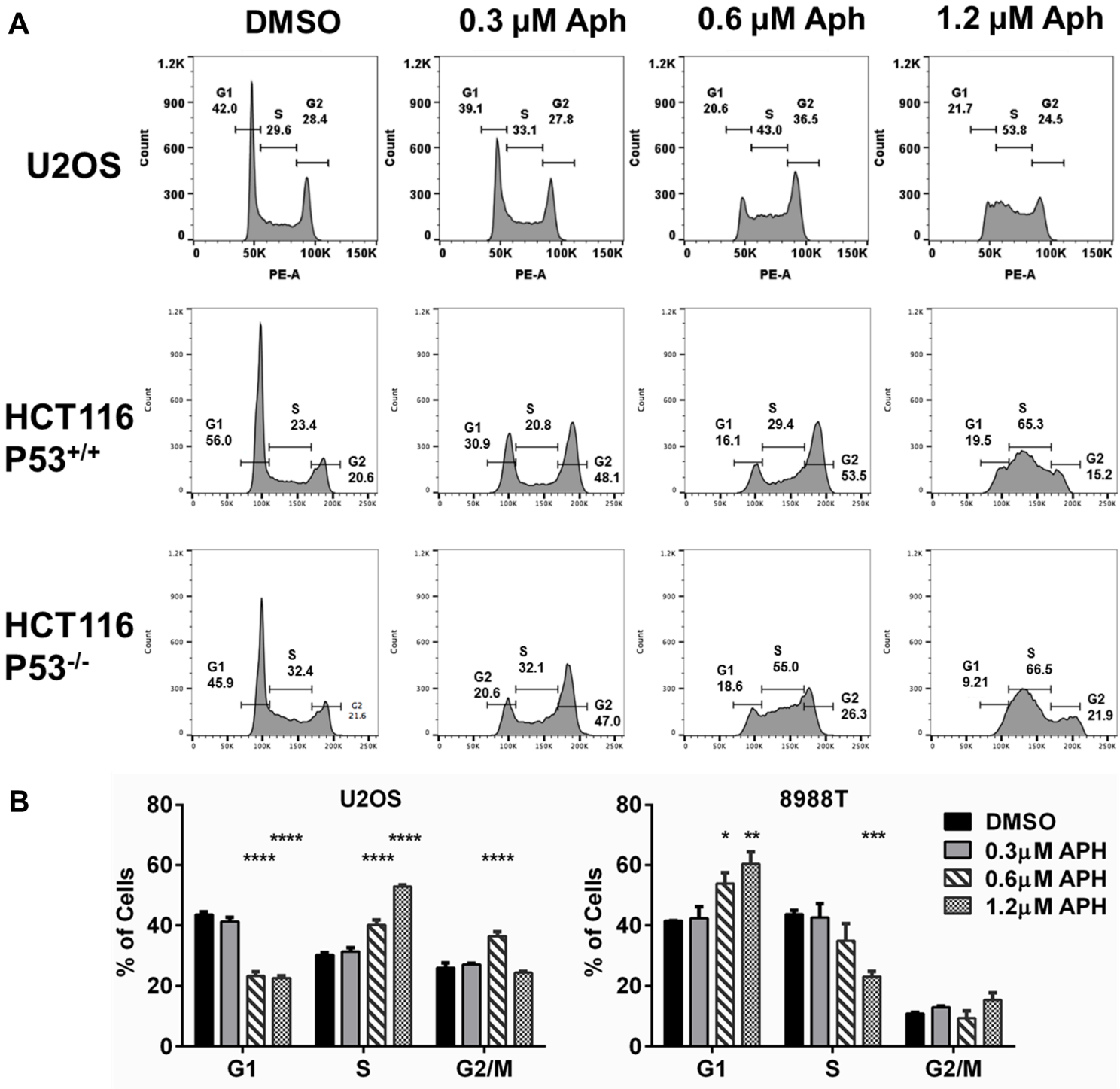
Figure S6. Model for Polη requirement in the replication stress response. (A). In cells with functional Pol η, replicative polymerases stall at difficult-to-replicate sequences, such as CFSs, and this stalling is elevated during replication stress. Pol η is induced and recruited to nuclear foci where it extends stalled and abandoned replication intermediates via both its ability to efficiently replicate repetitive DNA and its Aph-resistant synthesis activity. Rad18 is dispensable for this process. By generating optimal DNA templates for replicative polymerases, Pol η has an essential role in traversing the mitotic checkpoint and ensuring that cells properly enter the next cell cycle after replication stress. (B). In the absence of Pol η, cells cannot complete duplication of genomic regions where replicative polymerases are inhibited, creating genome gaps. The

number of gaps is increased by replication stress, causing increased ATR checkpoint activation. As a consequence, Pol η-deficient cells display elevated apoptosis as well as mitotic DNA synthesis, leading to overall reduced cell survival.

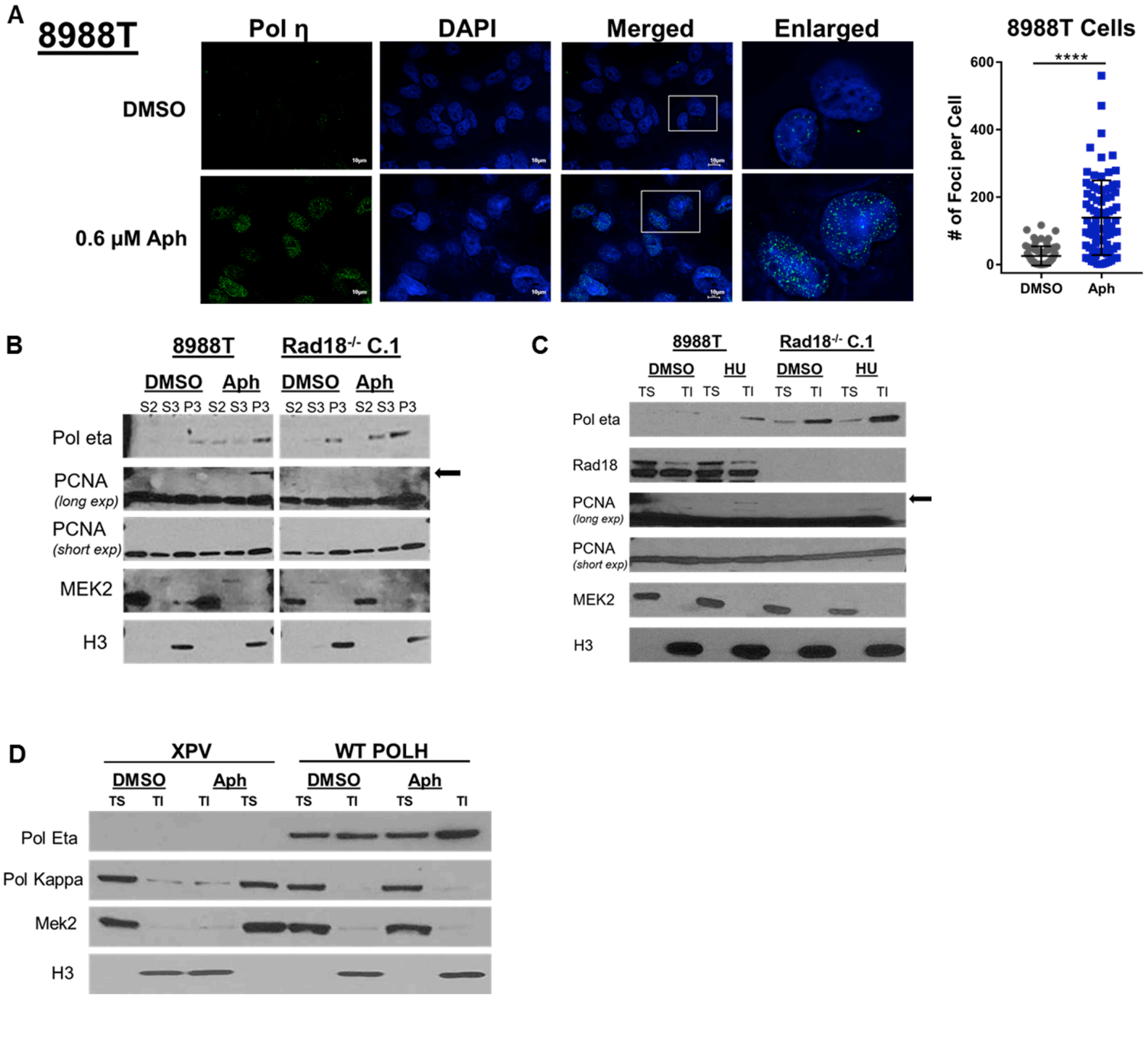
Supplemental Figure 1



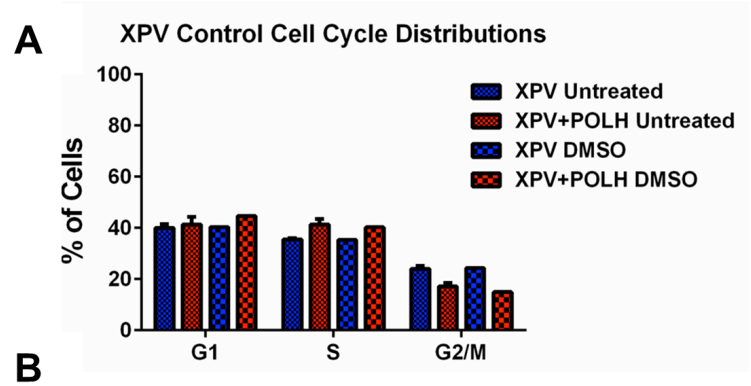
Supplemental Figure 2

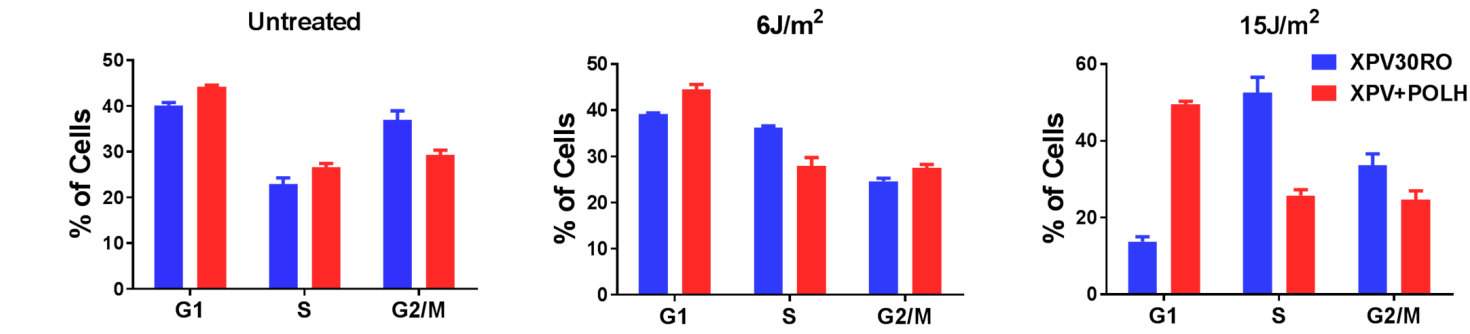


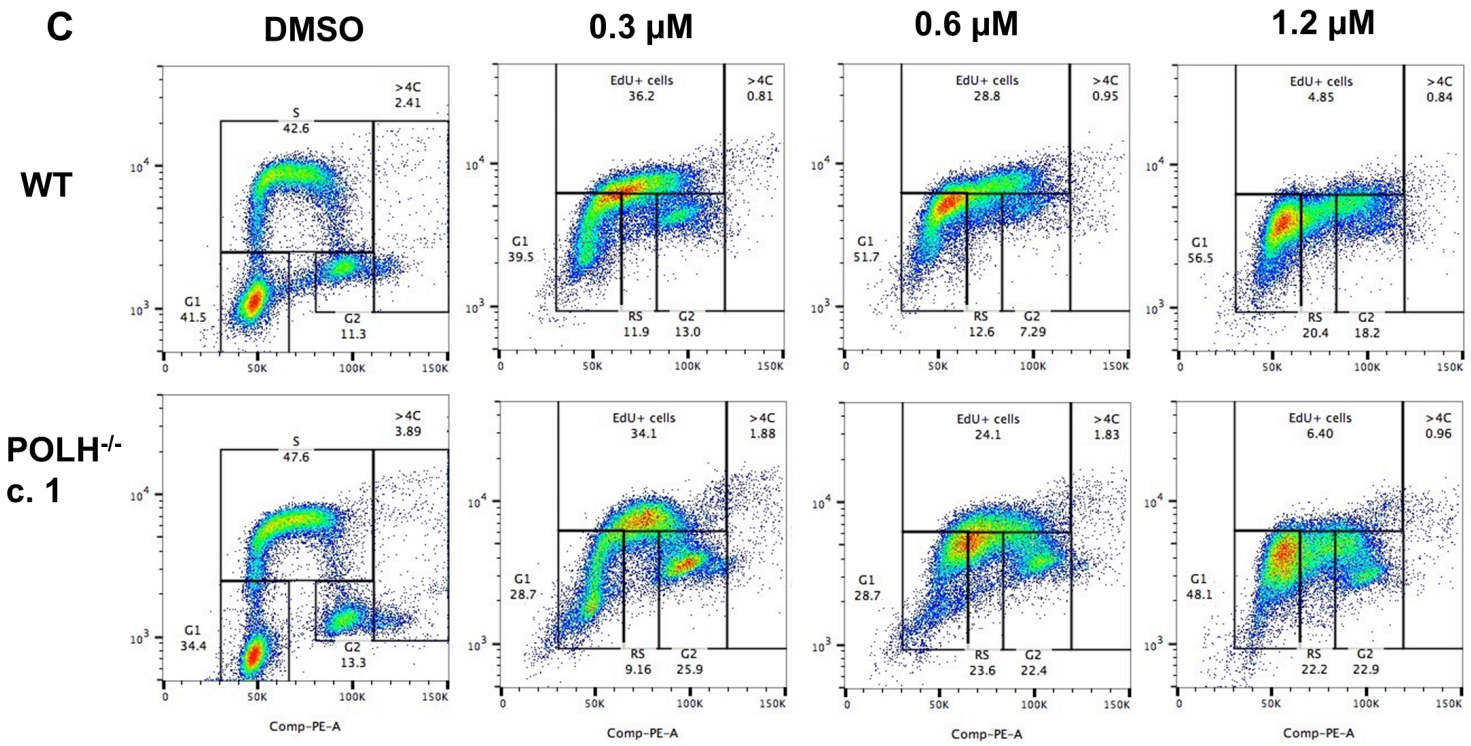
Supplemental Figure 3

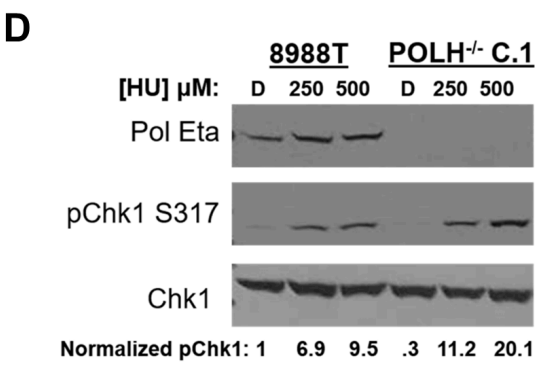


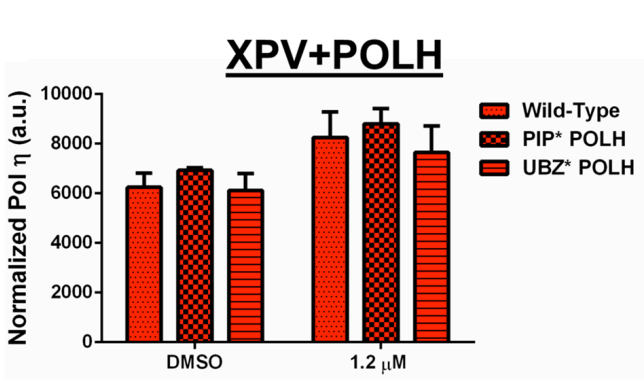
Supplemental Figure 4



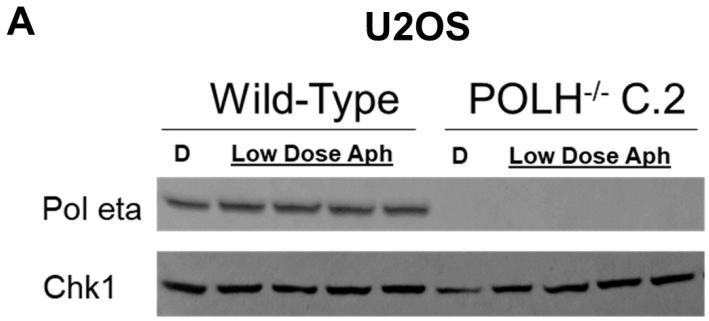


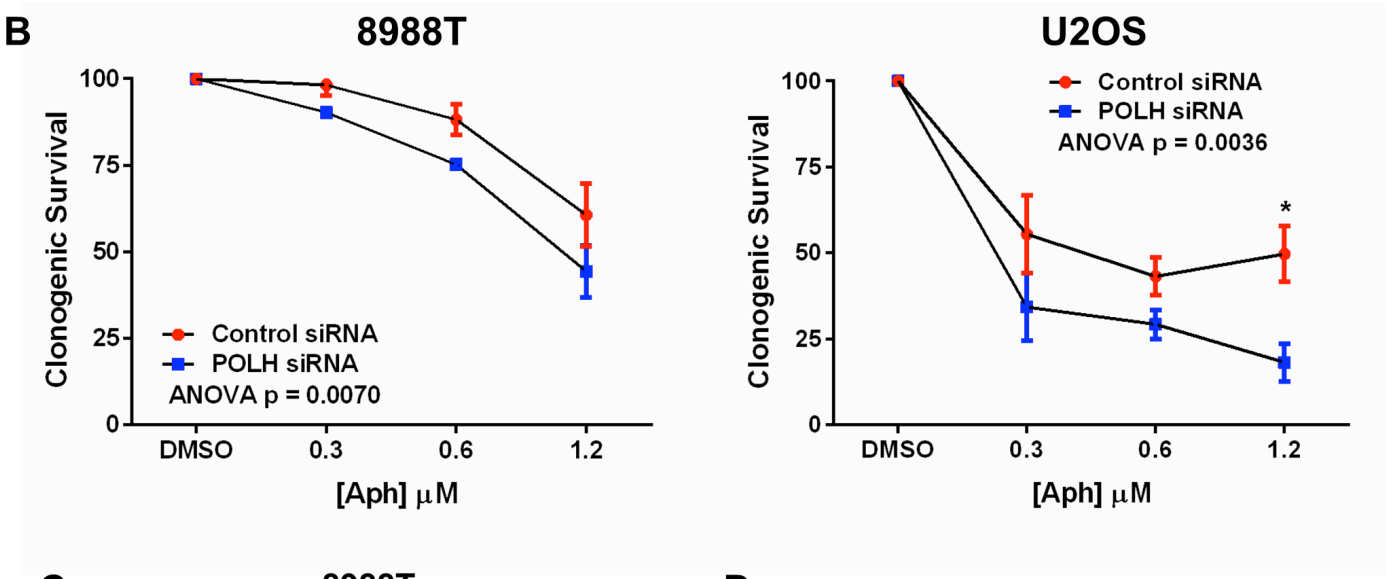


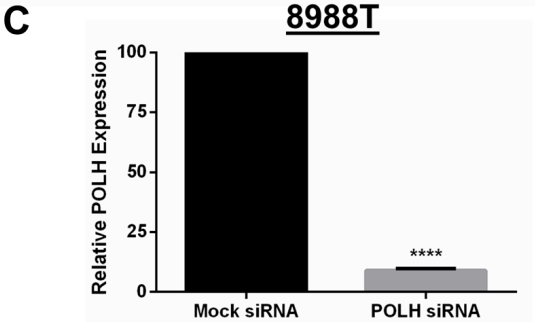


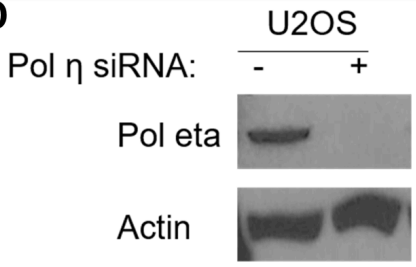


Supplemental Figure 5

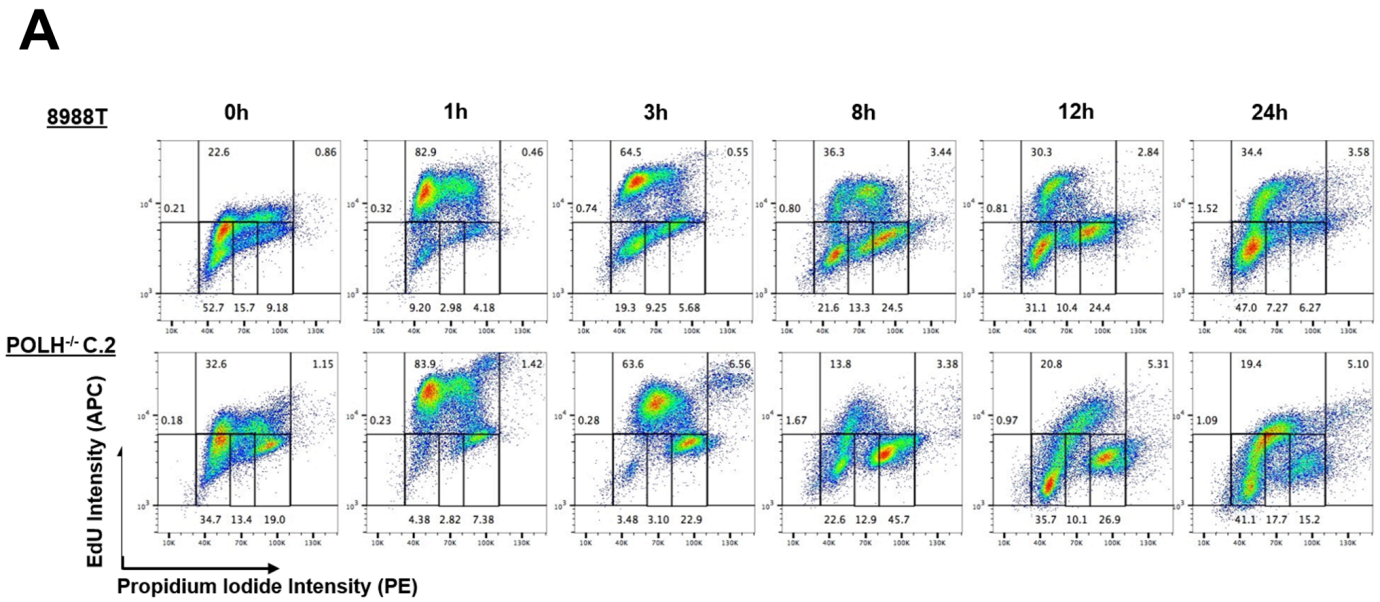


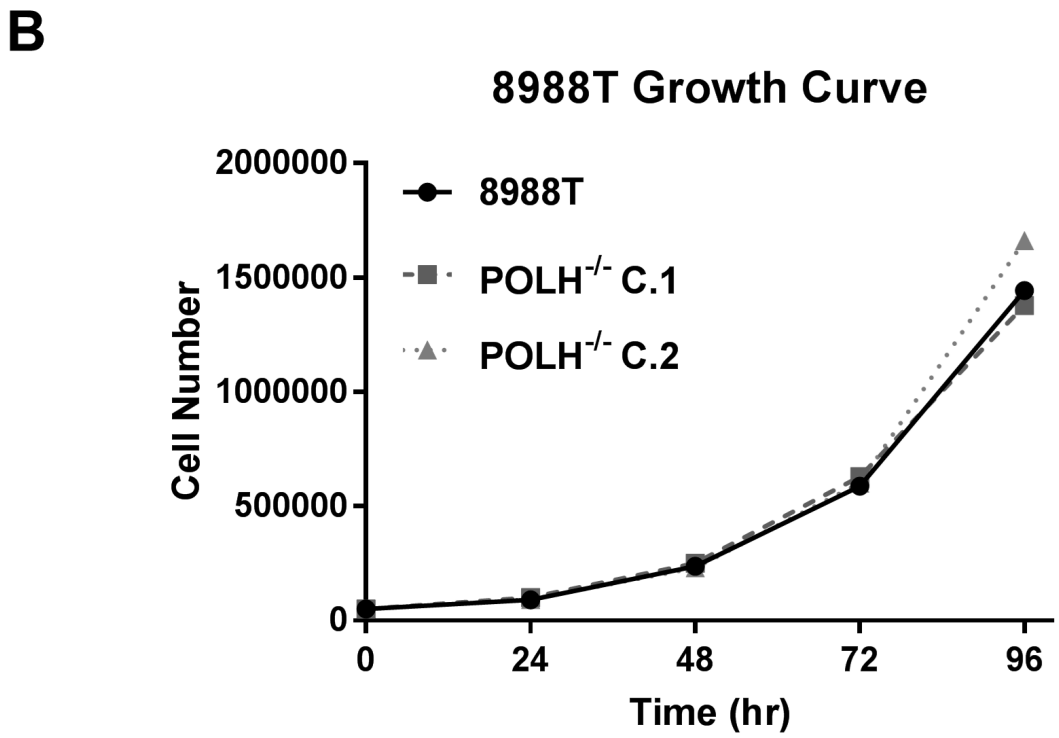






Supplemental Figure 6





Supplemental Figure 7