Supplemental Figures

Figure S1. Imager based immunofluorescence phospho-Histone H3 assay. Shown is an example (U2OS cells) experiment using 12-well tissue culture plates of phospho-H3 immunostaining data collected on the Odyssey Imager. The data are quantified as described in the Methods (Section 2.4).

Figure S2. Mitotic arrest in primary human skin fibroblast (HSF) cells treated with PJ34 and the data plotted as a normalized mitotic index as in Fig. (1A) and corresponding p21 expression as measured by semi-quantitative PCR (lower panel). Error bars, SEM. *p=0.04, **p=0.005.

Figure S3. FACS plots for MCF7 and MCF7:PARP1KD cells in growth phase (Cont) or treated for 24 hours with either 10 or 50 μM PJ34. Data are from a single experiment and representative of multiple, separate experiments as plotted in Figure S4.

Figure S4. A graphic representation of the triplicate experiment used to produce the plots in Figure S3, plotted as in Figure 4. See the text Section 3.3 for details.

Figure S5. H1299 PARP knockdown cell lines. Western blot for PARP1 and semi-quantitative PCR for PARP2 demonstrated near compete knockdown of PARP1 and in this set of lines some cross disappearance of PARP1 by the PARP2 shRNA, which was not seen in other cell lines with these shRNAs (*e.g.,* HeLa [33]).

Figure S6. FACS plots time course shown for H1299 and H1299:PARP1KD cells treated with 50 μM PJ34, corresponding to the graphical data in Figure 4(B). By 6 hours the increase in the G2/M fraction is seen and by 24 hours there is a significant shift away from the G1/G0 fraction with both a G2/M and probable inter-S phase population owing to the inability to transit S-phase due to the absence of p53.

Figure S7. The absence of p53 failed to abrogate the caffeine attenuation of PJ34 mitotic arrest. MCF7:p53KD or p53-null H1299 cells were treated as in Figure 8. Similar to MCF7 wild type and MCF7:PARP1KD cells, caffeine

attenuated the PJ34 mitotic arrest while UCN01 did not, whereas both treatments attenuated a HU G1/S mitotic arrest. ***p<0.0001; **p=0.002, ns=not significant.

Figure S8. Inhibition of MMP2 failed to abrogate the PJ34 mitotic arrest. Since PJ34 was reported to interact with MMP2, we tested whether inhibition of MMP3 activity alone or in parallel with PJ34 affected mitotic arrest. MCF7 and MCF7:PARP1KD cells were treated as shown with PJ34 (50 μM), 3AB (2.5 mM), the MMP2 inhibitor Galardin (0.3 or 3 μM) or the MMP2 activator zinc (Zn^{2+}) for 24 hours and the normalized mitotic index examined. Galardin alone with or with out zinc did not result in mitotic arrest and failed to abrogate the PJ34 growth arrest in either MCF7 or MCF7:PARP1KD cells.