SUPPLEMENTAL FIGURES

Supplemental Figure S1.

A: Doxorubicin treatment leads to the concentration-dependent induction of p53 and p21 in parental U2OS cells. U2OS cells were treated with doxorubicin at concentrations ranging from 0.05 μg/ml to 0.2 μg/ml for 24 hours and harvested. Cell extracts were analyzed by immunoblotting for expression of p53, p21, and β-actin (loading control).

B: Responses of control and p53-ablated cells to doxorubicin are not clonal in nature. U2OS derivatives expressing either a control shRNA (clone 1 and clone 4) or p53 shRNA (clone 7 and clone 8) were untreated or treated with 0.05 μg/ml doxorubicin for 1-6 days, and then analyzed by flow cytometry. The percentage of cells in each phase of the cell cycle or with a DNA content of less than 2N (<G1) is indicated in the upper right hand corner of each histogram.

C: U2OS derivatives expressing either a control shRNA (clone 1 and clone 4) or p53 shRNA (clone 7 and clone 8) were untreated or treated with 0.05 μg/ml doxorubicin for 1-6 days and then analyzed for p53, p21, and PARP levels by immunoblotting. Actin levels serve as a loading control.

Supplemental Figure S2. p53 status determines sensitivity to doxorubicin.

Cell lines wild-type for p53 including HCT116, HT-1080, U2OS, and G-361, or p53-null including HCT116 p53 -/-, Saos-2, and H1299, were either untreated or treated with doxorubicin for 1-6 days and then analyzed by flow cytometry. Doxorubicin levels were: 0.05 μg/ml (U2OS, HT-1080, Saos-2), 0.1 μg/ml (H1299, G-361), and 0.5 μg/ml (HCT116, HCT116 p53 -/-). The percentage of cells in each phase of the cell cycle or

with DNA contents of less than 2N (<G1) is indicated in the upper right corner of each histogram.

Supplemental Figure S3. Cells stably expressing an shRNA directed against p53 become multi-nucleated in response to treatment with a chemotherapeutic agent.

A-B: The U2OS derivatives, clone 1 (expressing control shRNA) or clone 7 (expressing p53 shRNA) cells were treated with 0.05 μg/ml doxorubicin for 6 days and examined by phase contrast microscopy. Representative fields of clone 1 (**A**) or clone 7 (**B**) cells at 6 days are shown. Arrows indicate multinucleated cells.

Supplemental Figure S4. p53-expressing cells show a decrease in the G2 markers, Cdc2, Cyclin B1, and Cyclin A, and an increase in the G1 marker, cyclin E, in response to treatment with a chemotherapeutic agent.

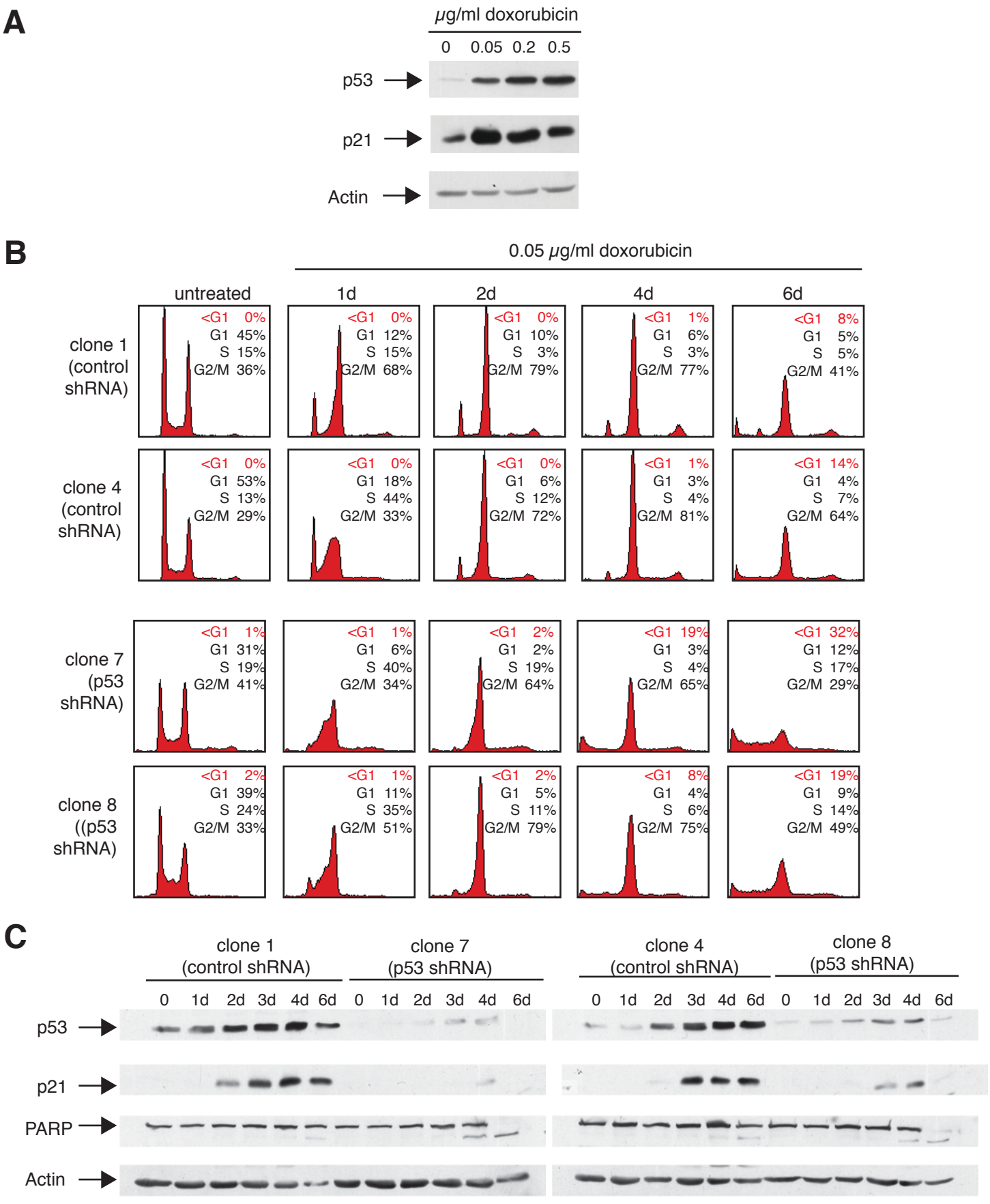
U2OS derivatives expressing either a control shRNA (clone 1) or p53 shRNA (clone 7 and clone 8) were untreated or treated with 0.05 μg/ml doxorubicin for 1-6 days and then analyzed for p53, p21, Cyclin E, and PARP levels by immunoblotting. HU denotes cells arrested at the G1/S border with 2 mM hydroxyurea. Actin levels serve as a loading control.

Supplemental Figure S5. Cells stably expressing an shRNA directed against p53 become multi-nucleated in response to transient treatment with a chemotherapeutic agent.

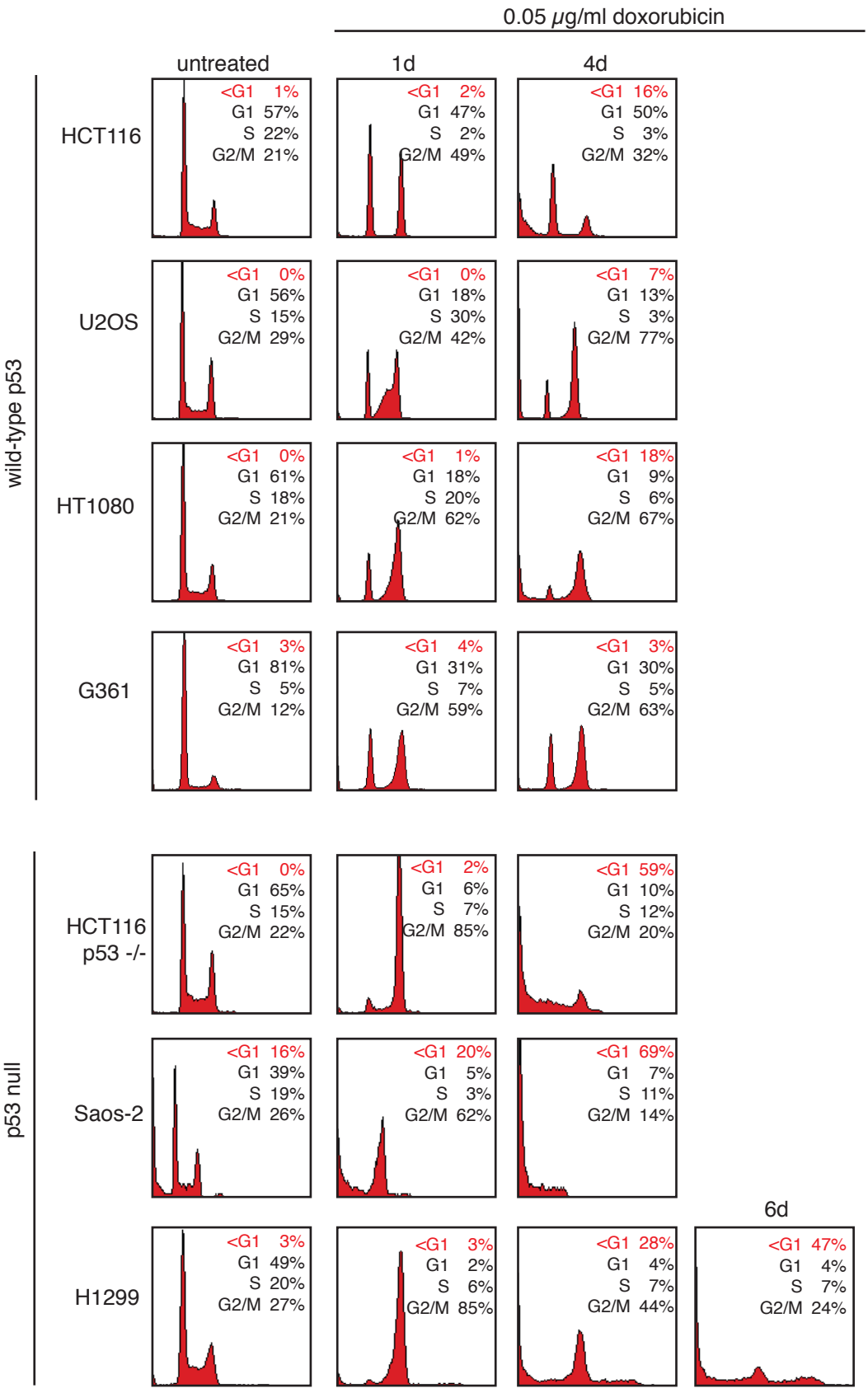
The U2OS derivatives, clone 1 (expressing control shRNA) or clone 7 (expressing p53 shRNA) cells were untreated or treated continuously for 2-6 days with 0.05 μg/ml doxorubicin, or treated for 6 hours with doxorubicin, washed, and incubated in the absence of drug for 2-6 days. Cells were then examined by phase contrast microscopy, and the number of multinucleated cells was quantitated.

Supplemental Figure S6. p53-expressing resume proliferation following cell cycle arrest induced with transient doxorubicin treatment.

U2OS-derived cells stably expressing control shRNA were treated with 0.05 μg/ml doxorubicin for either 6 or 48 hours. Drug was washed out and cells were harvested in two or seven days and the percentage of cells in S phase was quantitated by BrdU incorporation assays.



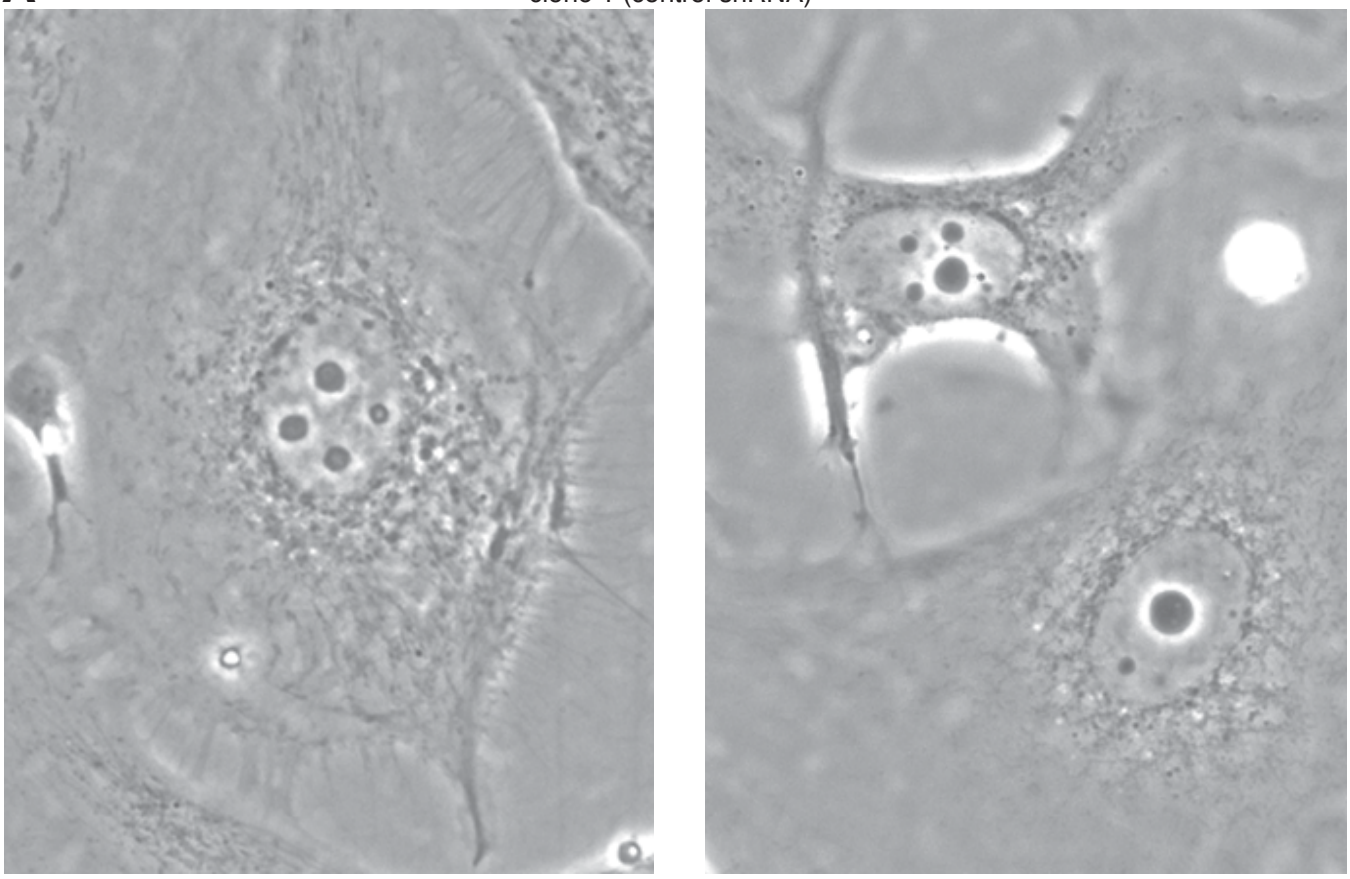
Lukin_Suppl. Fig. S1



Lukin_Suppl. Fig. S2

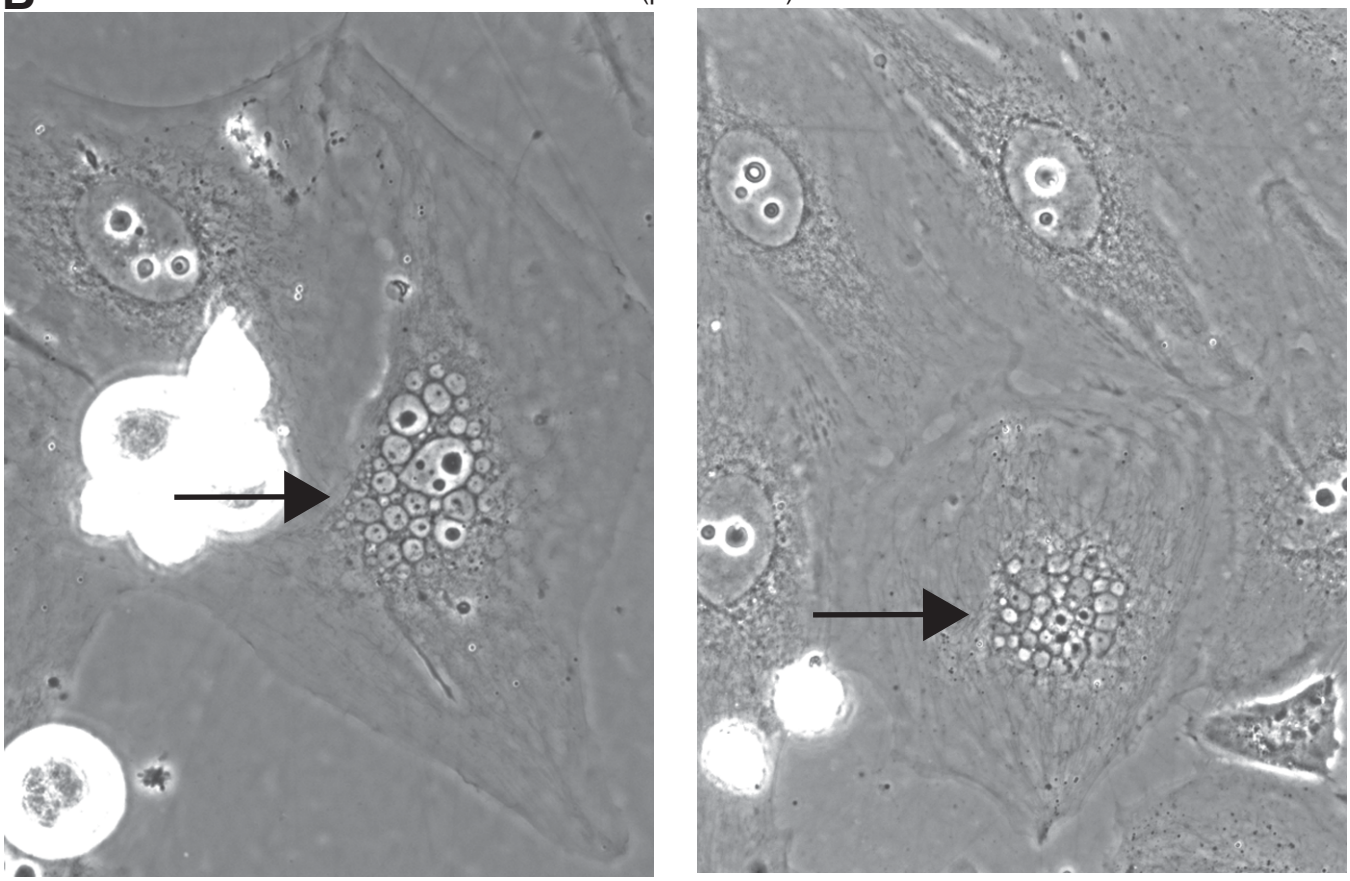
A

clone 1 (control shRNA)

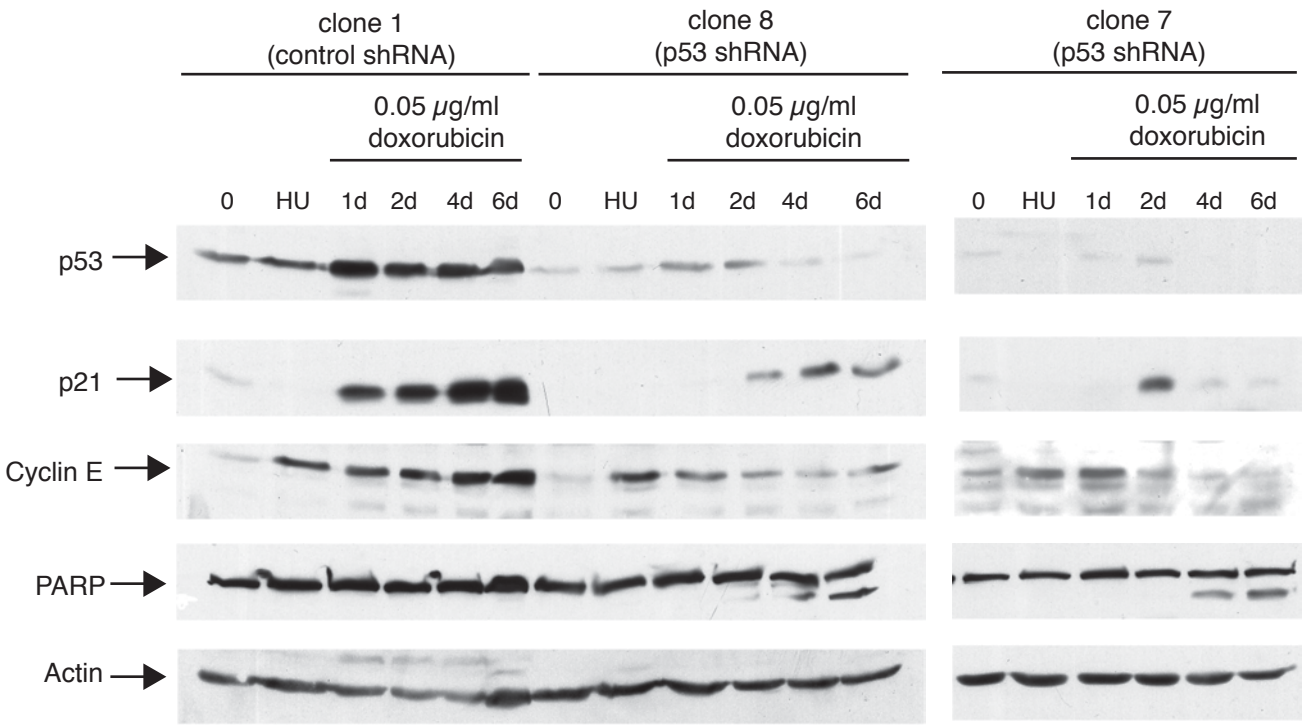


B

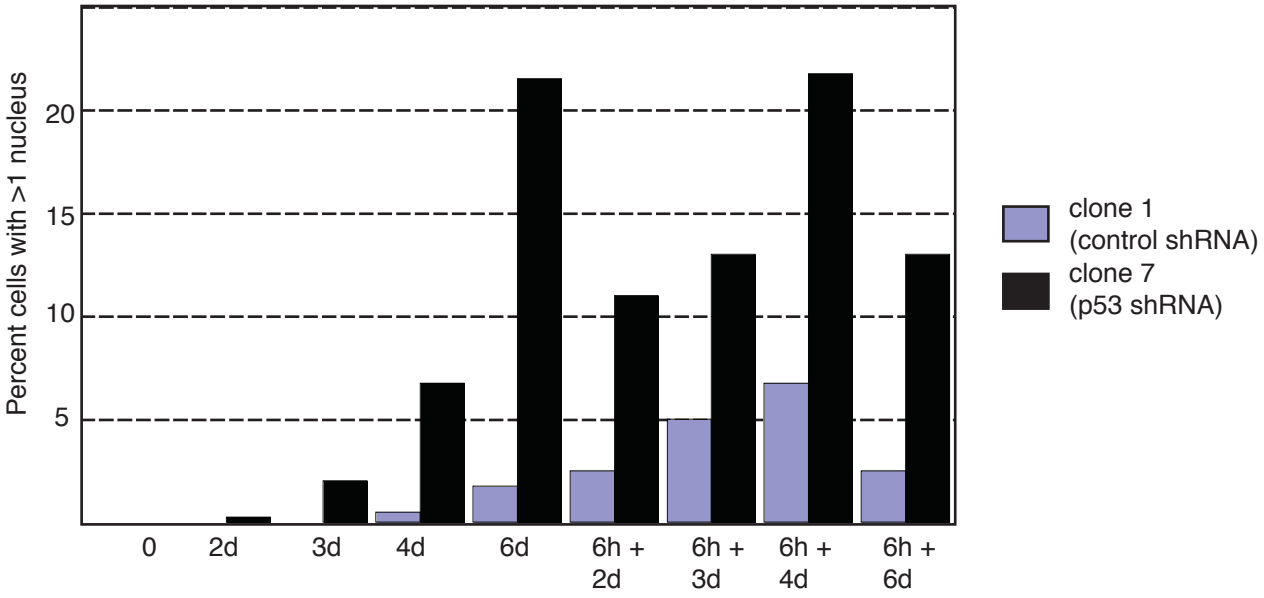
clone 7 (p53 shRNA)



Lukin_Suppl. Fig. S3

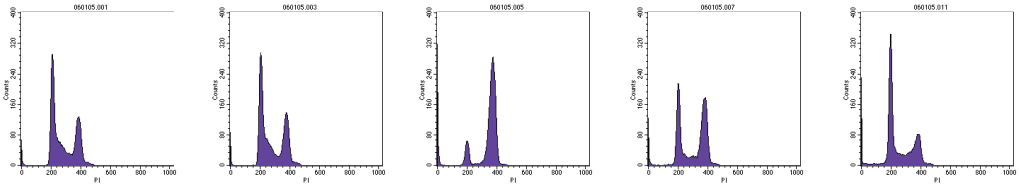


Lukin_Suppl. Fig. S4

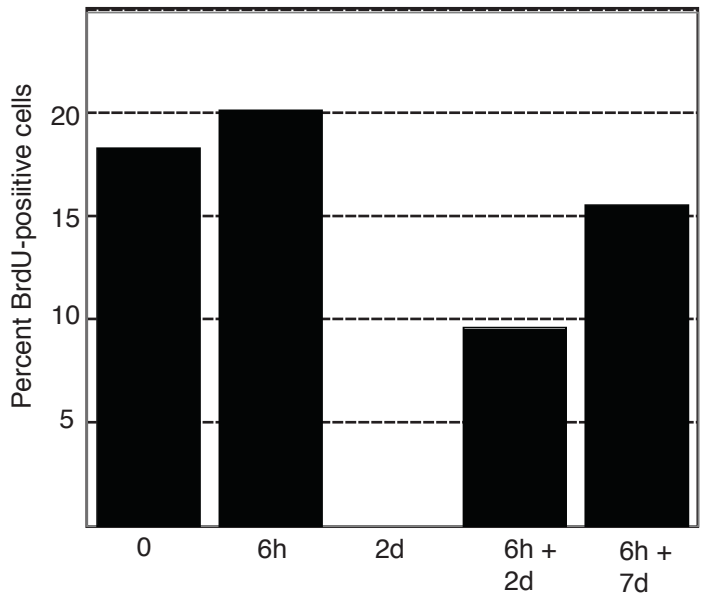


Lukin_Suppl. Fig. S5

A



B



Lukin_Suppl. Fig. S6