Netterfield et al., Figure S1

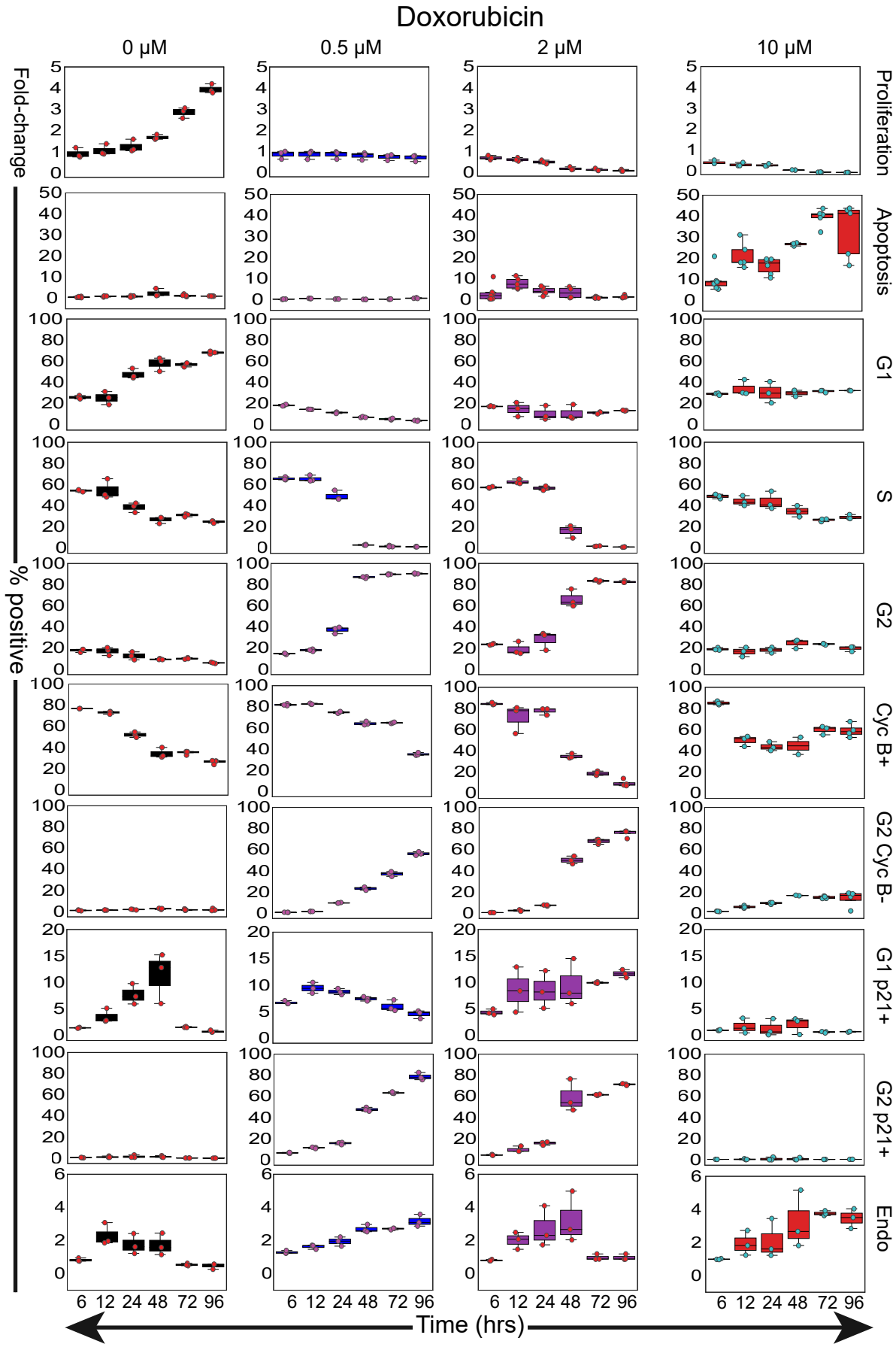


Figure S1: Boxplots of raw response data.

Boxplots of raw response data vs. time. Y-axis values represent fold-change for proliferation values, and percent positive values for responses measured by flow cytometry. Overlaid dots represent individual biological replicates.

Netterfield et al., Figure S2

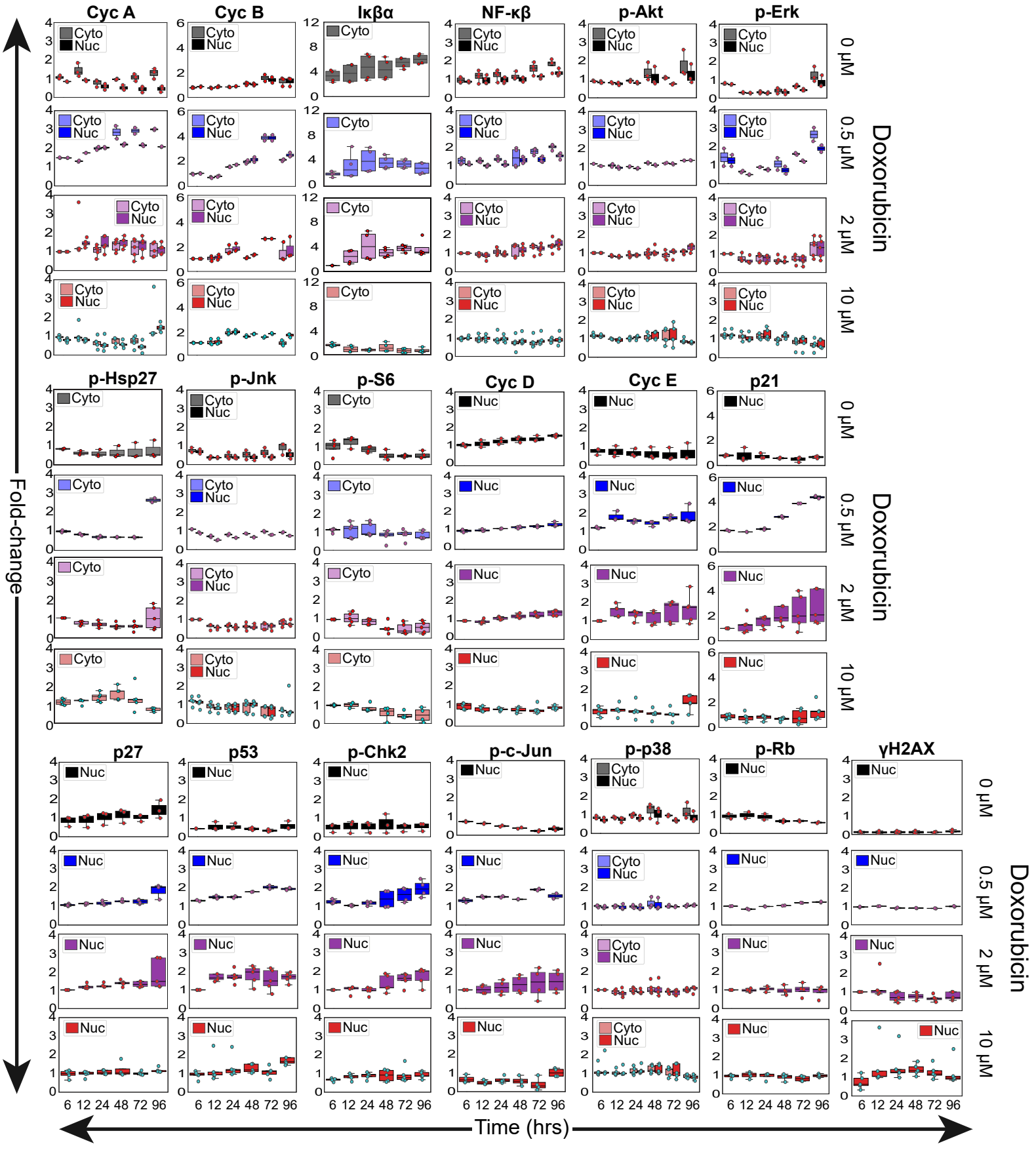


Figure S2: Boxplots of raw signals data.

Boxplots of raw signals data vs. time. Y-axis values represent fold-change of the mean intensity normalized to the 2 µM dose at the 6 hour timepoint for both the nuclear (Nuc) and cytoplasmic (Cyto) compartments. Overlaid dots represent individual biological replicates.

Netterfield et al., Figure S3

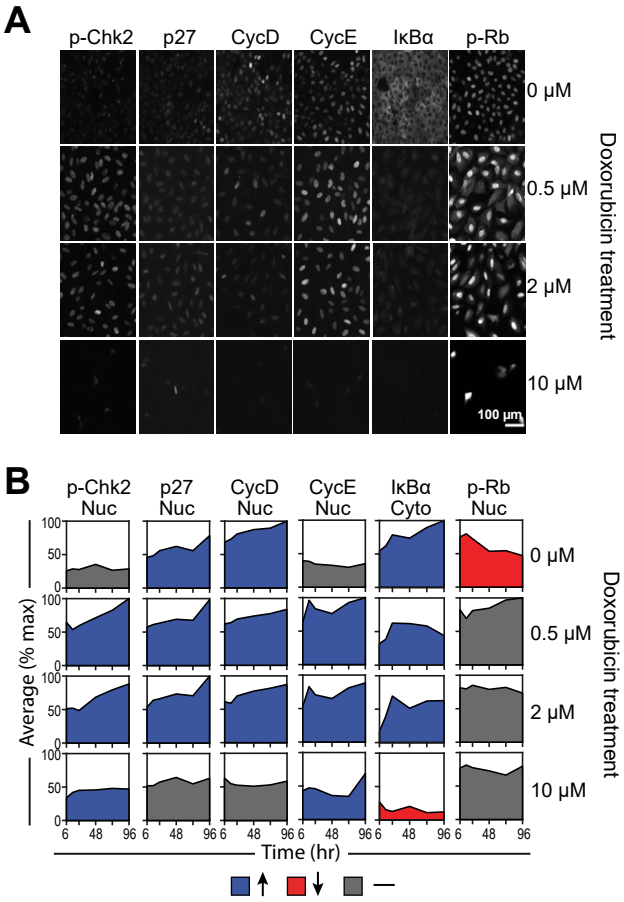


Figure S3: Quantification of remaining signaling measurements.

A) Representative immunofluorescence images of remaining signals not shown in figure 3.

B) Quantification of the mean fluorescence intensity (normalized to the maximum value across time and drug treatments) over time in either the nuclear or cytoplasmic compartment, depending on the given protein measured. Tick marks on the x-axis represent 6, 24, 48, 72, and 96 hrs. Images are representative of 250-6,000 individual cells per biological replicate, depending on the dose of doxorubicin.

Netterfield et al., Figure S4

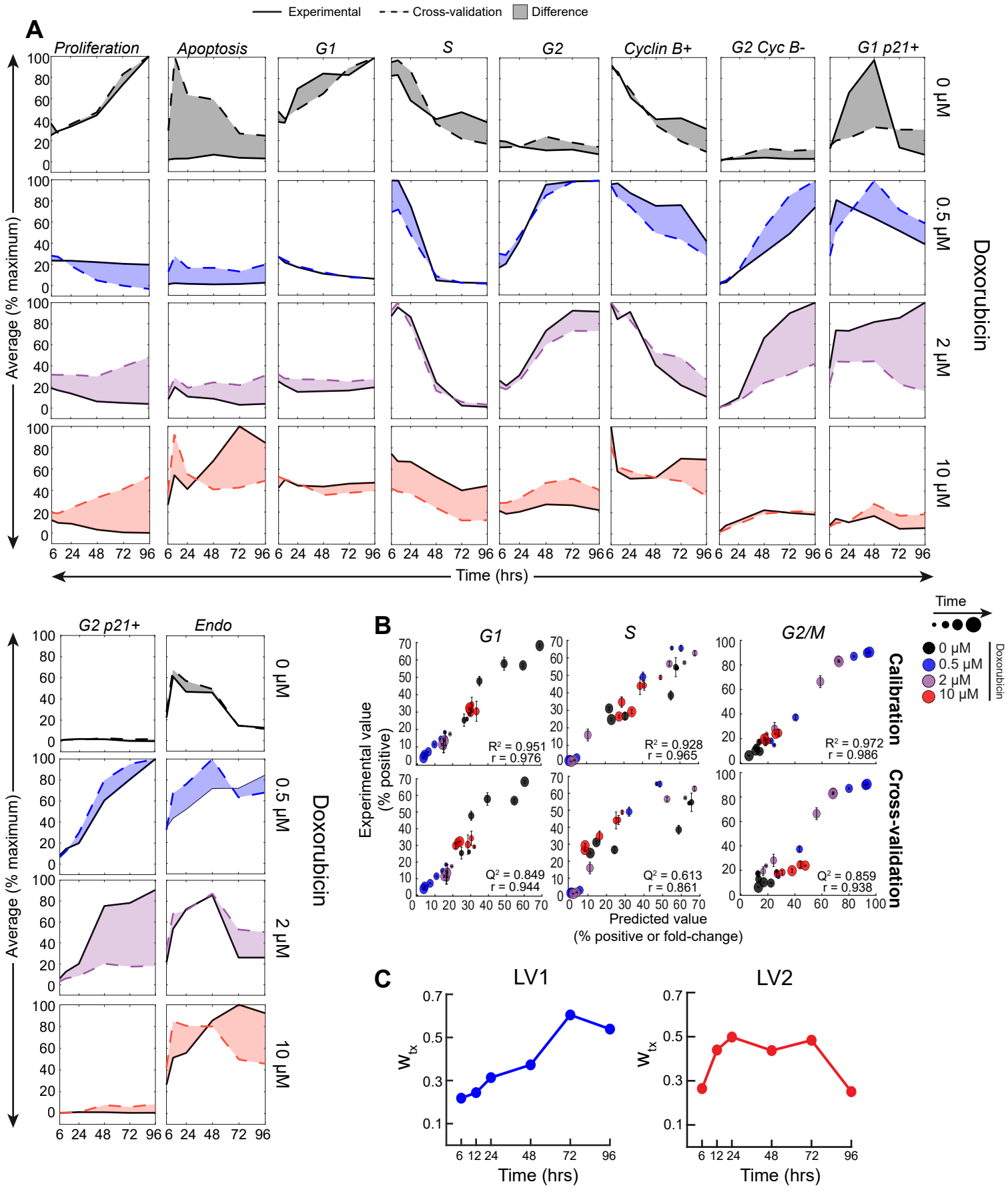


Figure S4: Constructed t-PLSR model can most accurately predict cell cycle distributions, and time weights reveal which timepoints are most heavily weighted on constructed LVs.

A) The mean fluorescence intensity (normalized to the maximum value across time and drug treatments) vs. time is plotted for each of the predicted responses, with the solid lines representing the experimental values, the dotted lines representing the cross-validation predictions, and the shaded area highlighting the difference between the two curves.

B) Experimental vs. predicted values of the G1, S, and G2 responses from the calibration and cross-validation model. R^2, Q^2, and Pearson correlation values are also shown. Dot sizes increase with time.

C) Calibration model time weights (w_{tx}) for LV1 (blue curve, left panel) and LV2 (red curve, right panel).

Netterfield et al., Figure S5

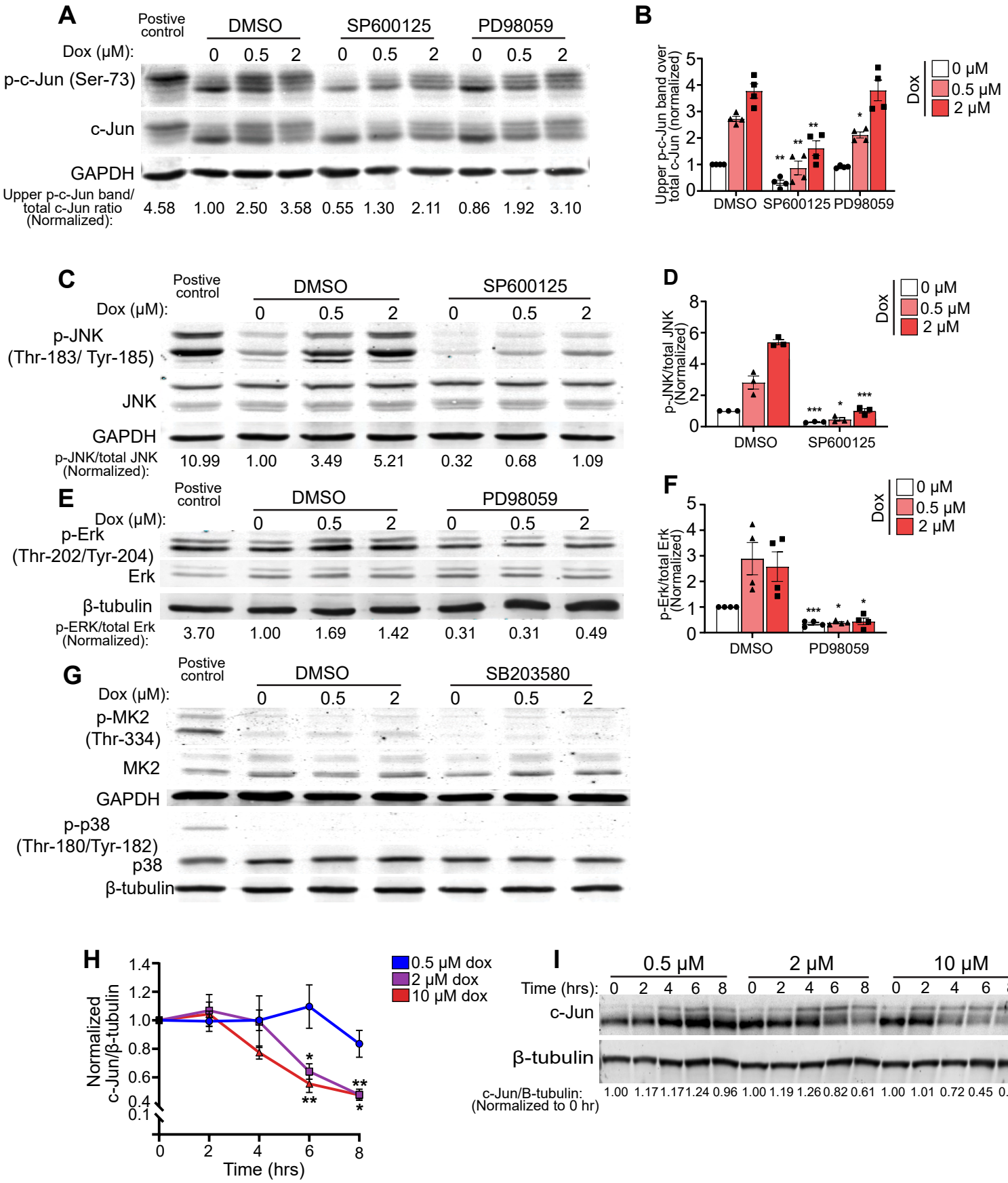


Figure S5: Western blotting data of components of the JNK, Erk, and p38MAPK pathways with or without SP600125, PD98059, or SB203580.

A) Representative western blot probed for p-c-Jun (Ser-73), c-Jun, and GAPDH in U2OS cells that were treated with doxorubicin for four hours, and cells were either co-treated with vehicle (DMSO), or with either 10 μM SP600125 (SP, JNKi) or 10 μM PD98059 (PD, Meki). Cells were lysed 6 hours after doxorubicin treatment for western blotting. Samples were run with a positive control of U2OS cells treated with 25 μg/mL anisomycin for 15 minutes.

B) Quantification of the ratio of the upper p-c-Jun(Ser-73) band to total c-Jun. Bars represent the mean ± SEM of four biological replicates, and bars represent SEM

C) Representative western blot of p-JNK (Thr-183/Tyr-185), JNK, and GAPDH in U2OS cells that were treated with either vehicle (0 μM) or doxorubicin (0.5 μM and 2 μM) for four hours, and cells were either co-treated with vehicle (DMSO), SP600125 (SP, JNKi) or PD98059 (PD, Meki. Samples were run with a positive control of U2OS cells treated with 25 μg/mL anisomycin for 15 minutes.

D) Quantification of p-JNK to total JNK ratio, normalized to DMSO, 0 μM condition. Bars represent mean ± SEM of three biological replicates.

E) Representative western blot of p-Erk (Thr-202/Tyr-204), Erk1/2, and β-tubulin in U2OS samples in the same treatment conditions as subpanel A. Samples were run with a positive control of 100 nM PMA treatment for 30 minutes.

F) Quantification of p-Erk to total Erk1/2 ratio, normalized to DMSO, 0 μM condition. Bars represent mean ± SEM of four biological replicates. Western blots are representative of two biological replicates. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ with a two-tailed t-test with Bonferroni correction tested vs. the respective doxorubicin dose in the vehicle inhibitor control in subpanels B, D, and F.

G) Representative western blots of p-MK2 (Thr-334), MK2, p-p38 (Thr180/Tyr-182), p38, GAPDH, and β-tubulin in in U2OS cells that were treated with either vehicle (0 μM) or doxorubicin (0.5 μM and 2 μM) for four hours, and cells were either co-treated with vehicle (DMSO), or SB203580 (SB, p38i). Samples were run with a positive control of U2OS cells treated with 25 μg/mL anisomycin for 15 minutes.

H) Quantification of total c-Jun over β-tubulin after treatment with either 0.5 μM, 2 μM, or 10 μM doxorubicin normalized to 0 hour timepoint. Media was replaced with drug free 1% FBS media at

the 4 hour timepoint. Points represent mean ± SEM of four biological replicates of three or four biological replicates. **: $p < 0.01$, *: $p < 0.05$ vs. the 0.5 μM dose with a one-way ANOVA and post-hoc Tukey's post-hoc test.

I) Representative western blot of total c-Jun and β-tubulin after treatment with either 0.5 μM, 2 μM, or 10 μM doxorubicin normalized to 0 hour timepoint.

Netterfield et al., Figure S6

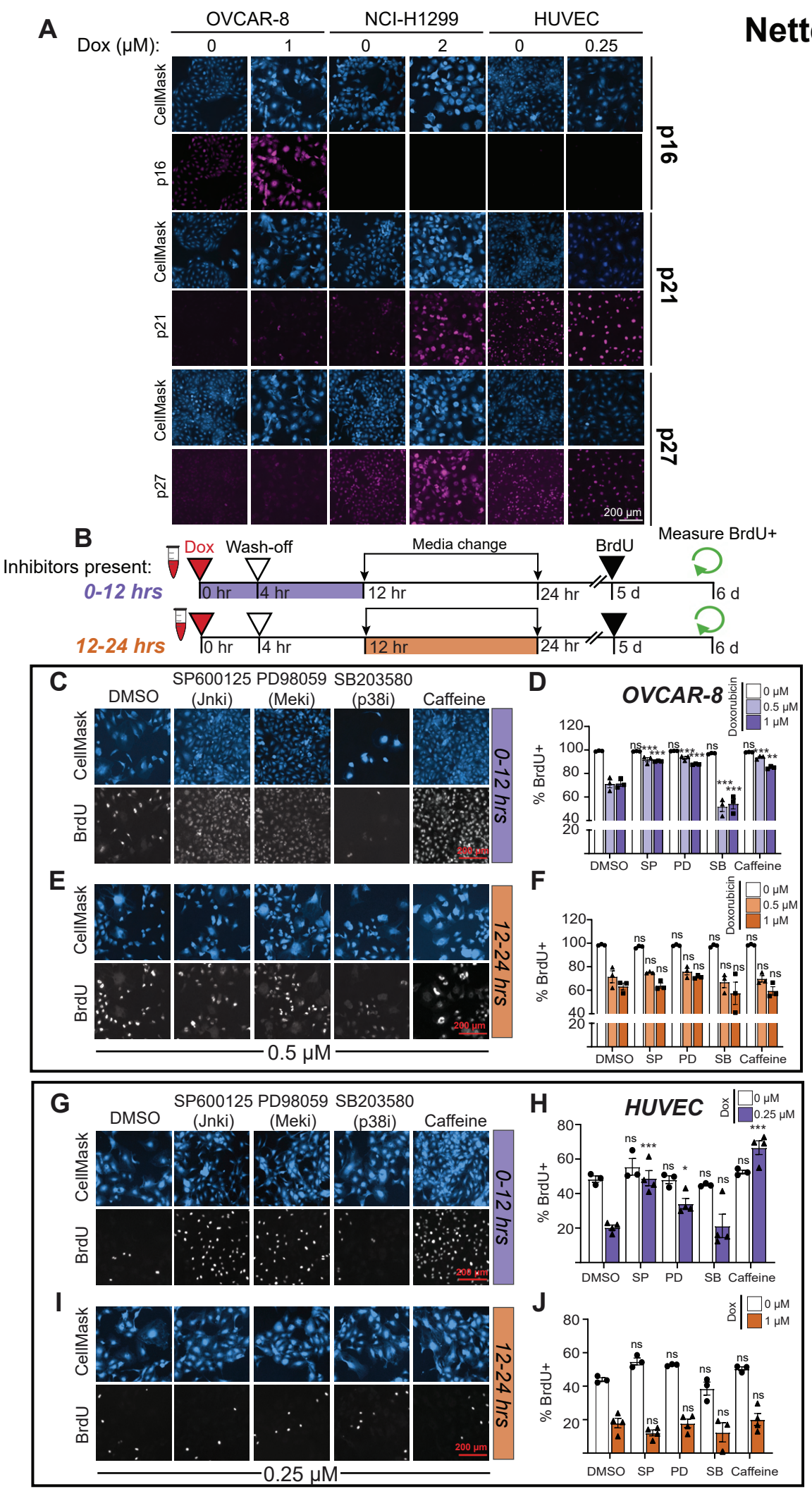


Figure S6: OVCAR-8 and HUVEC cells make the early decision to senesce through the JNK and Erk pathways, and overexpress CDK inhibitors p16, p21, or p27.

A) Representative immunofluorescence for p16, p21, and p27 (magenta) in OVCAR-8, NCIH1299, and HUVEC cells four days after a 4 hour pulse of vehicle of doxorubicin. Cells were also stained for CellMask blue to visualize cell morphology (blue). Scale bar represents 200 μm. Images are representative of 100-300 individual cells, depending on the dose of doxorubicin.
B) Schematic of inhibitor pulse experiment, as seen in figure 6A.
C) Representative immunofluorescence (IF) of BrdU incorporation into DNA in OVCAR-8 cells treated with inhibitors (10 μM for SP600125, PD98059, and SB203580 drugs, and 1 mM of caffeine) for 0-12 hrs and co-stained with HCS CellMask Blue. Scale bar represents 200 μm. Images are representative of about 1,500-7,500 individual cells per biological replicate, depending on condition.
D) Quantification of the percent of nuclear BrdU+ in OVCAR-8 cells after the 0-12 hour inhibitor condition.
E) Representative IF of BrdU incorporation into DNA in OVCAR-8 cells treated with inhibitors for 12-24 hrs and co-stained with HCS CellMask Blue. Scale bar represents 200 μm. Images are representative of 1,500-7,500 individual cells per biological replicate, depending on condition.
F) Quantification of the percent of nuclear BrdU+ in OVCAR-8 cells after the 12-24 hour inhibitor condition.
G) Representative immunofluorescence (IF) of BrdU incorporation into DNA in HUVEC cells treated with inhibitors (10 μM for SP600125, and SB203580 drugs, 5 μM for PD98059, and 1 mM of caffeine) for 0-12 hrs and co-stained with HCS CellMask Blue. Scale bar represents 200 μm. Images are representative of 7,000-18,000 individual cells per biological replicate, depending on condition.
H) Quantification of the percent of nuclear BrdU+ in HUVEC cells after the 0-12 hour inhibitor condition.
I) Representative IF of BrdU incorporation into DNA in HUVEC cells treated with inhibitors for 12-24 hrs and co-stained with HCS CellMask Blue. Images are representative of 7,000-18,000 individual cells per biological replicate, depending on condition.
J) Quantification of the percent of nuclear BrdU+ in HUVEC cells after the 0-12 hour inhibitor condition. For all bar graphs, bars represent mean ± SEM of three or four biological replicates.

***: $p < 0.001$, **: $p < 0.01$ *: $p < 0.05$ with a two-way ANOVA and post-hoc Dunnett's test for subpanels D, F, H, and J. All comparisons were vs. the DMSO treatment at the same dose of doxorubicin.

Netterfield et al., Figure S7

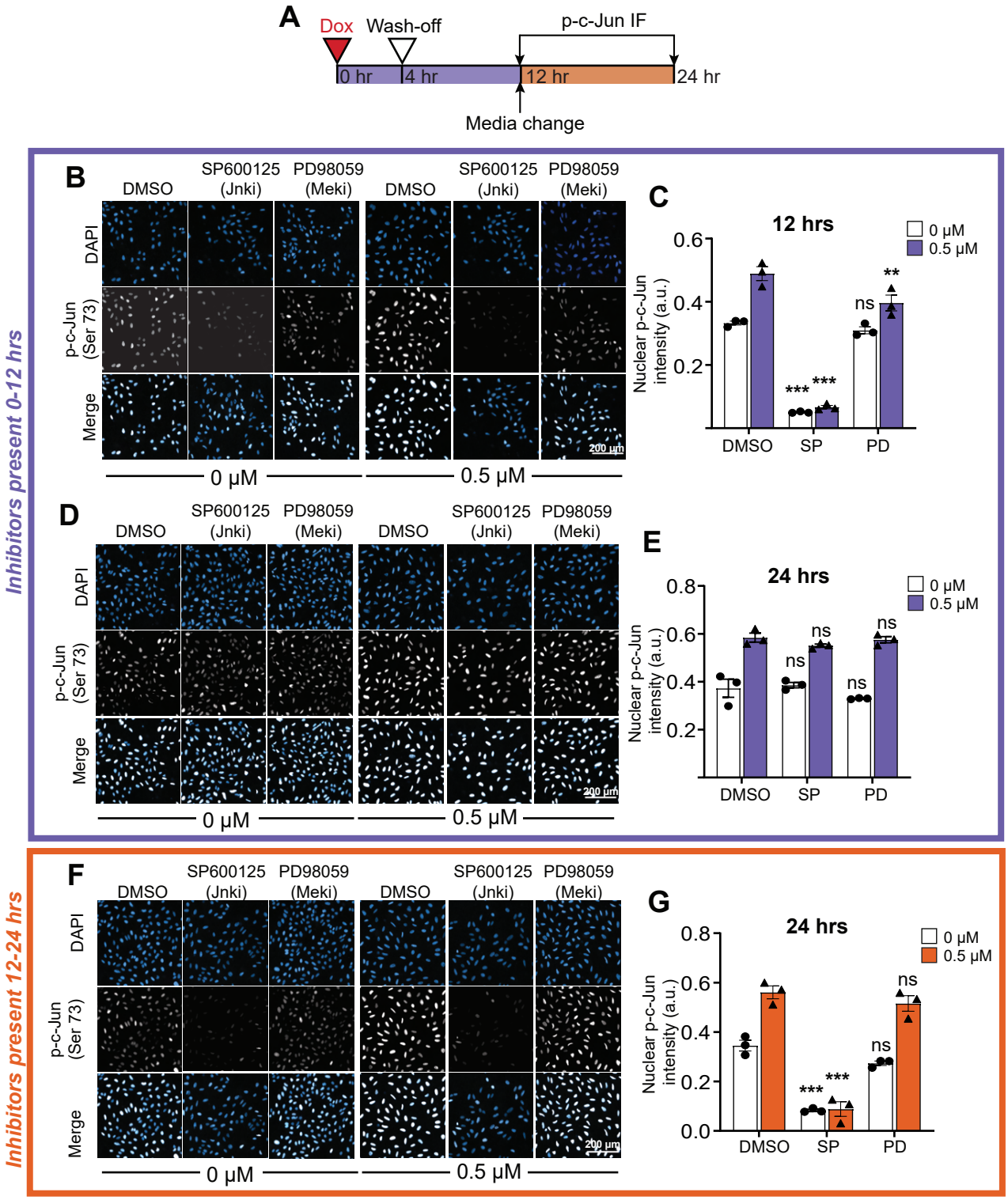


Figure S7: Time course of p-c-Jun(Ser 73) show inhibitor-dependent decreases in phosphorylation levels in the first 12 hours after doxorubicin treatment in U2OS cells.

A) Schematic of the experiment performed.

B) Representative images of cells immuno-stained for p-c-Jun(Ser-73) and DAPI co-treated with inhibitors the first 12 hours of the experiment, and fixed at the 12 hour timepoint. Images are representative of 1,500-2,500 individual cells per biological replicate, depending on the condition.

C) Quantification of the nuclear mean p-c-Jun(Ser-73) intensity 12 hour timepoint.

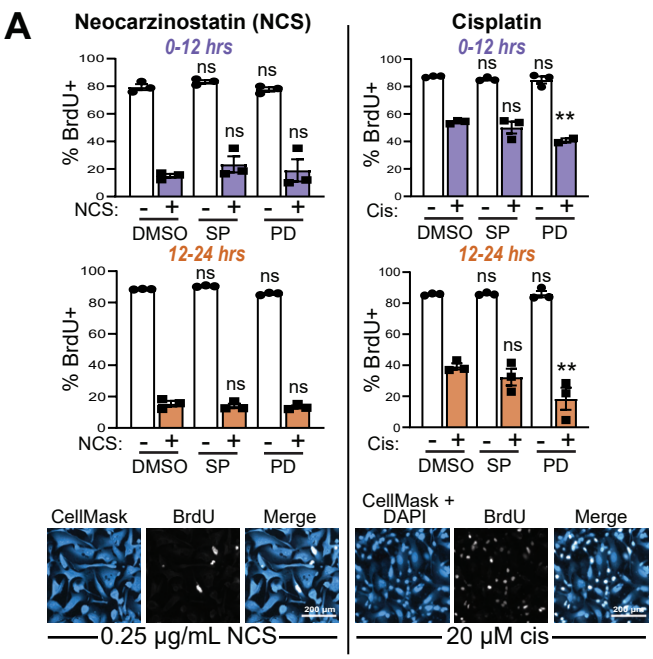
D) Representative images of cells immune-stained for p-c-Jun(Ser-73) and DAPI co-treated with inhibitors the first 12 hours of the experiment, and fixed at the 24 hour timepoint. Images are representative of 1,500-4,0000 individual cells per biological replicate, depending on the condition.

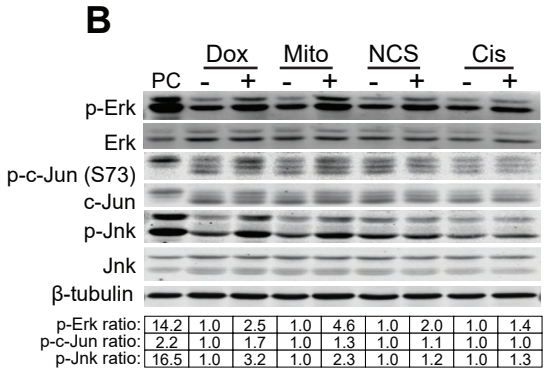
E) Quantification of the nuclear mean p-c-Jun(Ser-73) intensity for the 24 hour timepoint after early inhibitor.

F) Representative images of cells immune-stained for p-c-Jun(Ser-73) and DAPI co-treated with inhibitors the 12-24 hour window of the experiment, and fixed at the 24 hour timepoint. Images are representative of 1,500-4,000 individual cells per biological replicate, depending on the condition.

G) Quantification of the nuclear mean p-c-Jun(Ser-73) intensity for the 24 hour timepoint after late inhibitor. For subpanels C, E, and G, bars represent the mean ± SEM of three biological replicates. ***: $p < 0.001$ **: $p < 0.01$ with a two-way ANOVA and post-hoc Dunnett's test when compared to the DMSO vehicle control at the same doxorubicin dose.

Netterfield et al., Figure S8





p-Erk ratio:	14.2	1.0	2.5	1.0	4.6	1.0	2.0	1.0	1.4
p-c-Jun ratio:	2.2	1.0	1.7	1.0	1.3	1.0	1.1	1.0	1.0
p-Jnk ratio:	16.5	1.0	3.2	1.0	2.3	1.0	1.2	1.0	1.3

Figure S8: Neocarzinostatin and cisplatin treatment do not respond to SP600125 or PD98059, induce replications stress, or activation of the JNK and Erk pathways

A) Immunofluorescence images and quantification of SP600125 and PD98059 inhibitor pulse experiments (similar experimental details as in fig 6A-I) with either 4 hour pulse of 0.25 µg/mL neocarzinostatin (NCS), 20 µM cisplatin (+) or vehicle (-). U2OS cells were stained with anti-BrdU and HCS CellMask Blue six days after mitoxantrone treatment, with quantification of nuclear BrdU positivity six days after drug treatments. Bars represent the mean ± SEM of three biological replicates. CellMask blue was visualized with gamma=0.6 in order to better visualize the nuclear and cytoplasmic compartments. Images are representative of 5,500-31,000 individual cells per biological replicate, depending on the condition.

B) Representative western blots for phospho and total JNK, Erk, and c-Jun 6 hours after the addition of drug. The positive controls for phospho- JNK and c-Jun was treatment with 25 µg/mL anisomycin for 15 minutes, and the positive control for phosphor-Erk was 100 nM PMA for 30 minutes. Blots are representative of four experiments. See figure 7J for quantification across biological replicates.