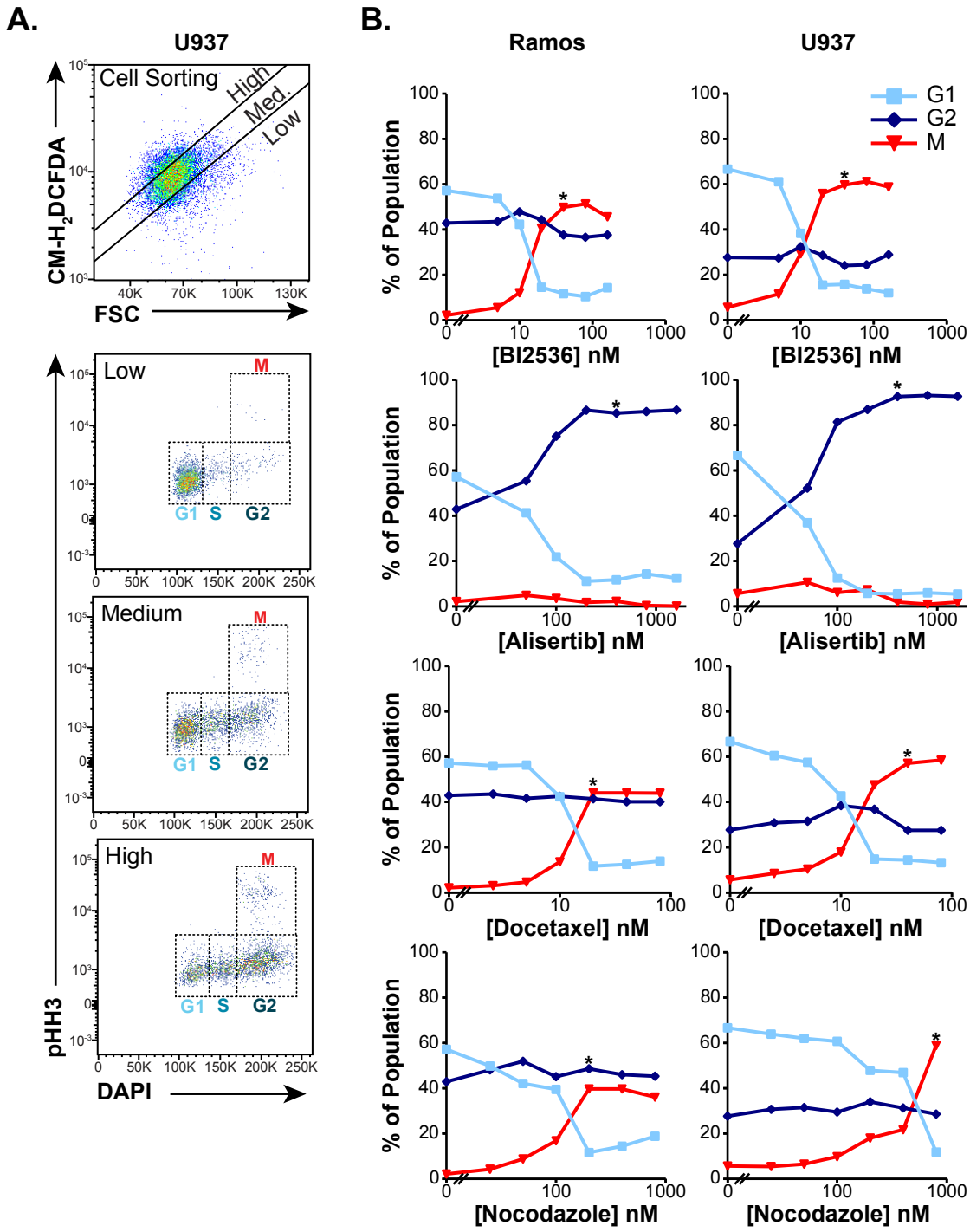
SUPPLEMENTAL INFORMATION

ROS and Oxidative Stress are Elevated in Mitosis During Asynchronous Cell Cycle Progression and are Exacerbated by Mitotic Arrest

Jesse C. Patterson*, Brian A. Joughin*, Bert van de Kooij, Daniel C. Lim, Douglas A. Lauffenburger, Michael B. Yaffe

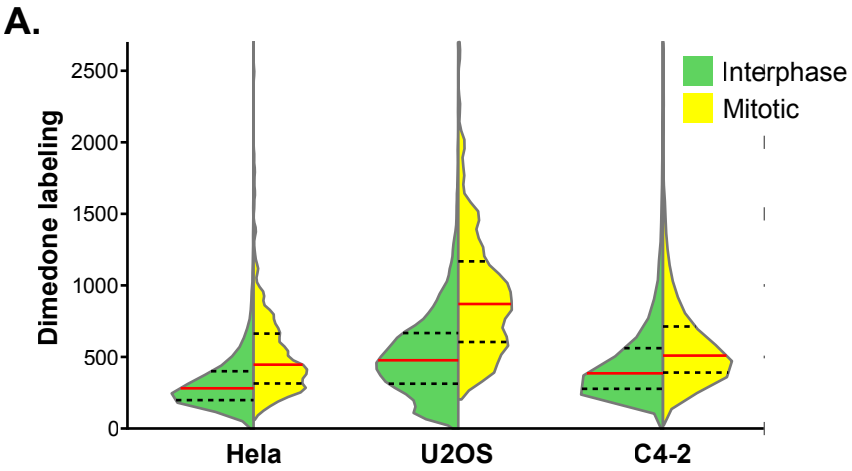


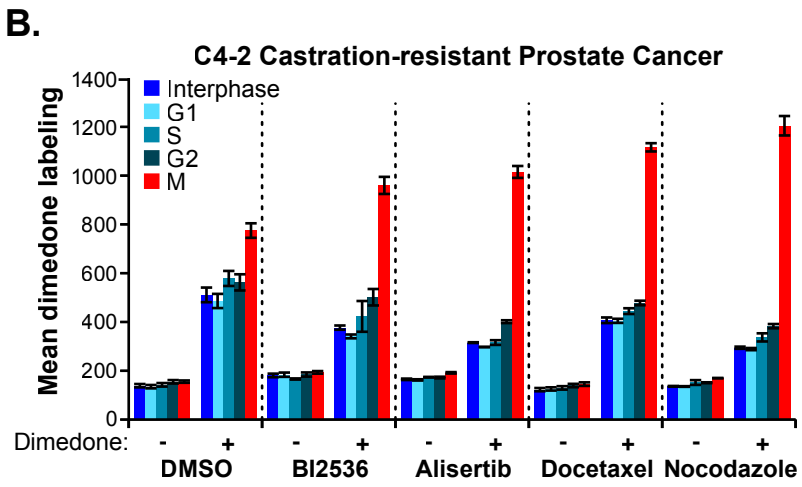
SUPPLEMENTARY FIGURE 1

Figure S1. Related to Figure 1. ROS increases in mitotic cancer cells.

A, U937 acute myeloid leukemia cells were loaded with the ROS-responsive dye CM-H_2DCFDA, then physically separated into populations with a low, medium, or high CM-H_2DCFDA fluorescence relative to forward scatter (FSC), comprising approximately 15%, 70% and 15% of the total population respectively, using fluorescence-activated cell sorting (top panel). These cell populations were immediately fixed, stained for pHH3 and DNA content (DAPI) and analyzed by flow cytometry to determine the relative proportion of cells in various stages of the cell cycle (bottom three panels).

B, Ramos or U937 cells, as indicated, were treated with multiple doses of the antimitotic drugs BI2536, alisertib, docetaxel, or nocodazole for 24 hours, and then fixed, stained for pHH3 and DNA content (DAPI), and analyzed by flow cytometry to determine the relative proportion of cells in various stages of the cell cycle. Black stars indicate the lowest doses at which maximal M-phase arrest (or G2/M-phase in the case of alisertib) is achieved, which were the doses used to assess CM-H_2DCFDA oxidation during antimitotic treatment (Figure 1C)





SUPPLEMENTARY FIGURE 2

Figure S2. Related to Figure 2. Oxidative damage of biomolecules increases in mitotic cancer cells.

A, Mitotic cells display an increase in the distribution of dimedone labeling (yellow halves of the violin plots) relative to the distribution of dimedone labeling measured in interphase cells (green halves of the violin plots). C4-2 CPRC, U2OS osteosarcoma and HeLa cervical carcinoma cells were treated with dimedone or vehicle, then stained with anti-dimedone sulfenic acid antibody, anti-pHH3 and DAPI. Gating was performed to separate interphase from mitotic cells. Red lines indicate median and dotted black lines indicate the boundaries for the upper and lower quartiles.

B, A wide variety of antimitotic drugs increase dimedone labeling of mitotically-arrested C4-2 CPRC cells. Cells were treated with minimum effective doses of antimitotic drugs (10 nM BI2536, 100 nM alisertib, 2.5 nM docetaxel or 50 nM nocodazole) and assessed for dimedone labeling and cell cycle stage. "Mean dimedone labeling" was defined as the mean dimedone channel signal normalized to FSC on an individual cell basis. Gating was performed to isolate G1, S, G2, all interphase, and M phase cells. Mean ± SEM of three independent experiments.