Figure 5: Immunofluorescent confocal microscopy (40X) staining for phospho-Histone H3 (red), a mitotic marker, and CENP-E (4). Panc-1 cells were left untreated (a-c) or were treated with 500nM UA62784 for 12 hrs. (d). Progression through mitosis is shown in a-c with CENP-E localizing diffusely in the cytoplasm during prophase (a), localizing to the DNA/kinetochores during prometaphase/metaphase (b), and localizing to the spindle midzone during anaphase (c). All cells in (a-c) are representative of one untreated sample. Note: only one cross-section of each confocal image is shown. These data are representative of 3 independent trials.

Figure 6: CENP-E microtubule-binding assay in the presence of UA62784. Purified taxol-stabilized microtubules were incubated with microtubule-associated protein extract (lanes 2-3), bovine serum albumin (4-5), CENP-E (6-7), or CENP-E and indicated concentrations of UA62784 (8-13) and ultracentrifuged to pellet microtubules. Any proteins that bind microtubules would be found primarily in the pellet (P) fraction whereas proteins that do not bind microtubules would be found primarily in the supernatant (S) fraction. Blots were visualized using IR secondaries (a) to indicate CENP-E and tubulin proteins only and Coomassie Blue staining (b) to indicate total protein. These data are representative of 4 independent experiments.

Figure S1: Induction of apoptosis in BxPC3 cells. Cells were incubated with UA62784 for 24 hours (a) or 48 hours (b). Cells were then stained with Annexin-V-FITC and propidium Iodide (PI) and analyzed by flow cytometry. Values indicate the percent of cells in the gated population. $Annexin^{+}/PI^{-}$ cells are considered apoptotic, whereas $Annexin^{-}/PI^{+}$ cells are considered necrotic, and $Annexin^{+}/PI^{+}$ cells are considered late-stage. Data shown is representative of 3 independent experiments.

Figure S2: Mitotic index in Panc-1 cells following UA62784 treatment visualized by confocal immunofluorescence microscopy (40X). Cells were incubated in media with 0.25% DMSO (a) or 300nM UA62784 for 12 hours (b). Samples were incubated with anti-phospho-Histone H3 (4) and anti-β-tubulin (red). The phospho-Histone-H3 stain correlates to a nuclear DAPI stain (not shown). Note the control cells shown in metaphase (a, arrowhead) and anaphase (a, arrow). Note: only one cross-section of each confocal image is shown.

Figure S3: IF microscopy staining for β-tubulin in Panc-1 cells treated with no drug (a), 50 nM UA62784 for 12 hrs (b), and 300nM UA62784 for 12 hrs. (c). A DAPI counterstain was also performed but could not be photographed. These data are representative of 8 independent experiments.

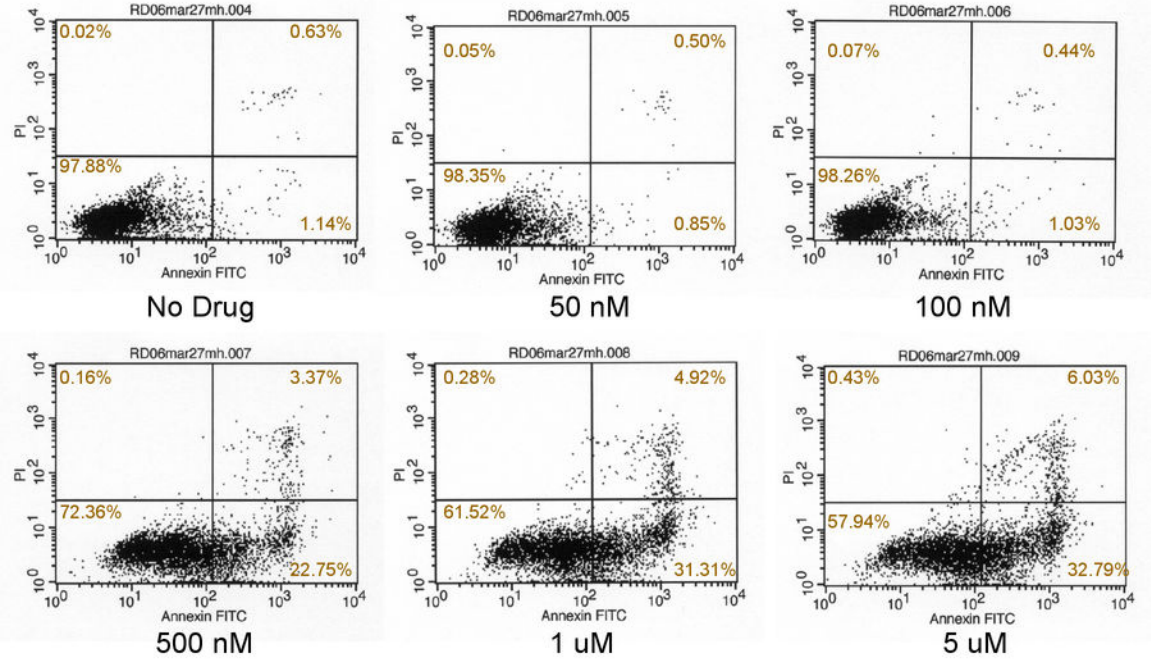
Figure S4: Cell-free tubulin polymerization assay. Increasing relative fluorescence units (RFU) is indicative of tubulin polymerization. 3 μM Paclitaxel (□) was used as a positive control to indicate hyperstabilized microtubules and 3 μM Vincristine (◊) was used to inhibit polymerization. Control (DMSO) samples (o) show expected polymerization curve which is largely unaltered in the presence of 50 μM UA62784 (Δ). These data are representative of 4 independent experiments.

Figure S5: Colocalization of CENP-E and BubR1 as shown by immunofluorescence microscopy. Panc-1 cells were treated with either 500nM UA62784 (B) or an equal volume of DMSO (A) and incubated for 12 hours. Samples were stained with anti-BubR1 (4) and anti-CENP-E (red). A DAPI counterstained was also performed but could not be photographed. Scalebars shown indicate 10μm. These data are representative of two independent experiments.

Figure S6: Panc-1 cells stained with anti-phospho-histone-H3 (4) and anti-gamma-tubulin (red) visualized by immunofluorescence microscopy (40X). Panels show untreated cells in prophase (a) and metaphase (b). Panc-1 cells were treated with 300nM UA62784 for 12 hrs. and duplicated centrosomes are clearly visible (c). Arrows in (c) indicate examples of cells with duplicated centrosomes, arrowhead indicates a cell with duplicated and separated centrosomes. These data are representative of 3 independent experiments.

Figure S1

a.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b.

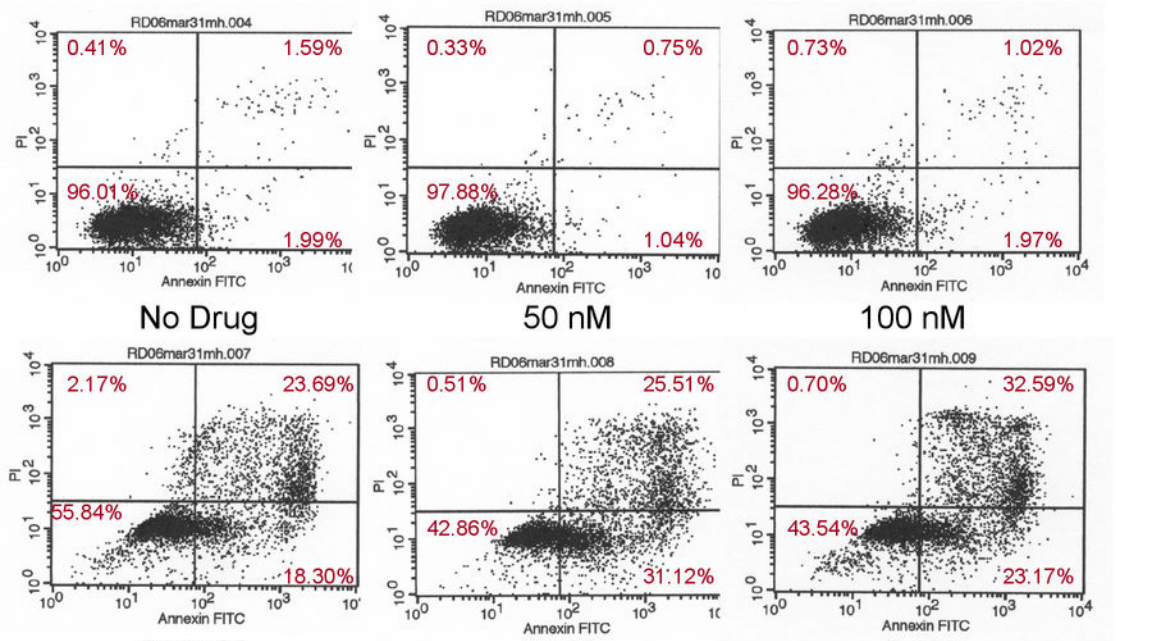
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Figure S2

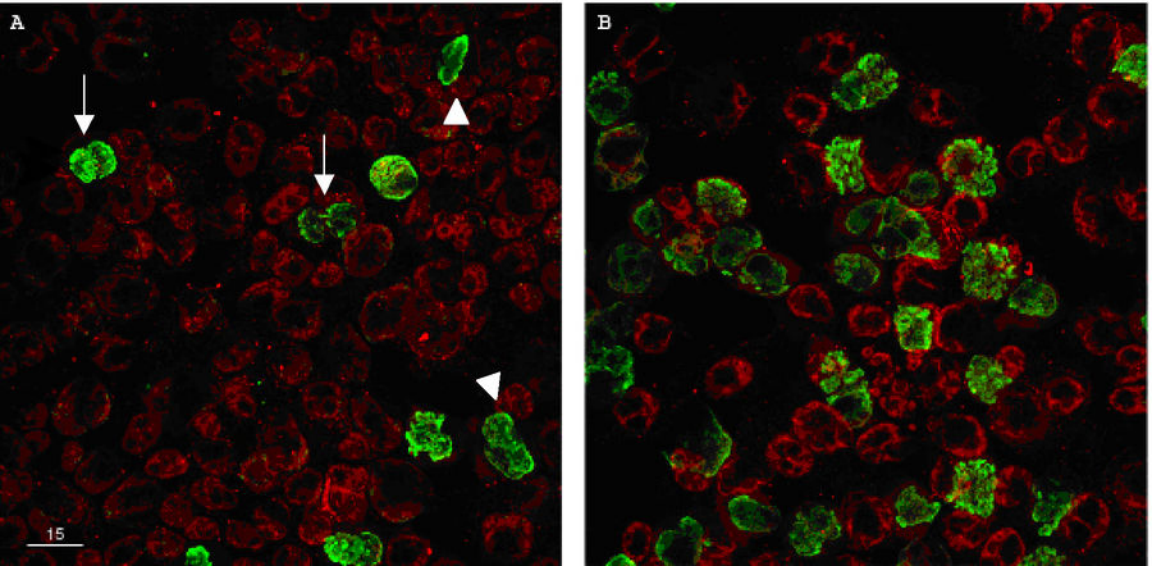
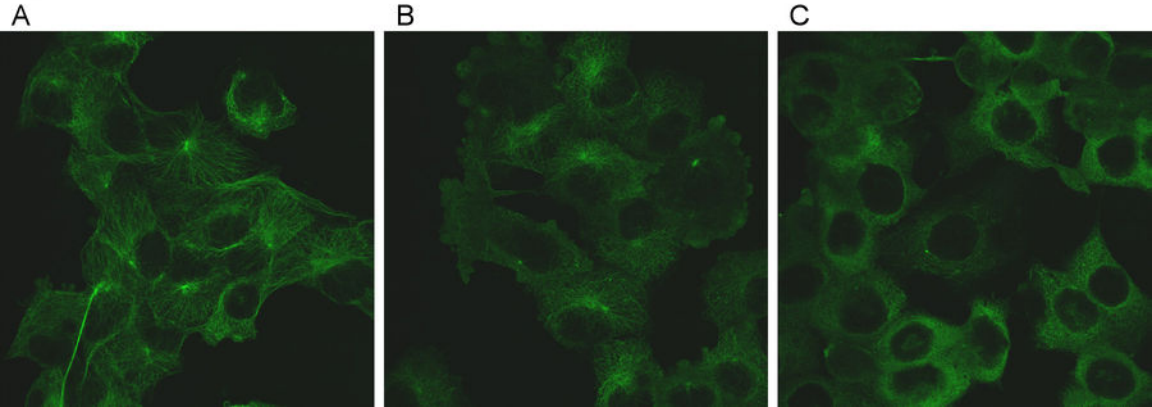


Figure S3



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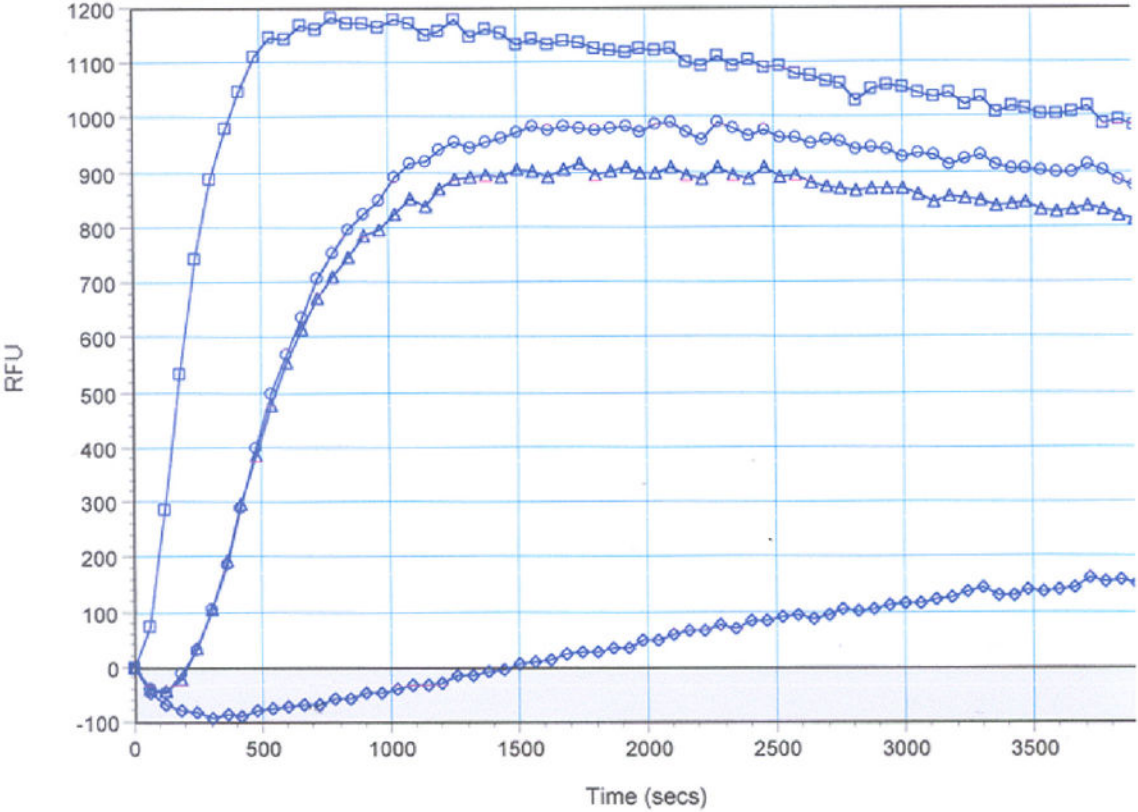
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Figure S5

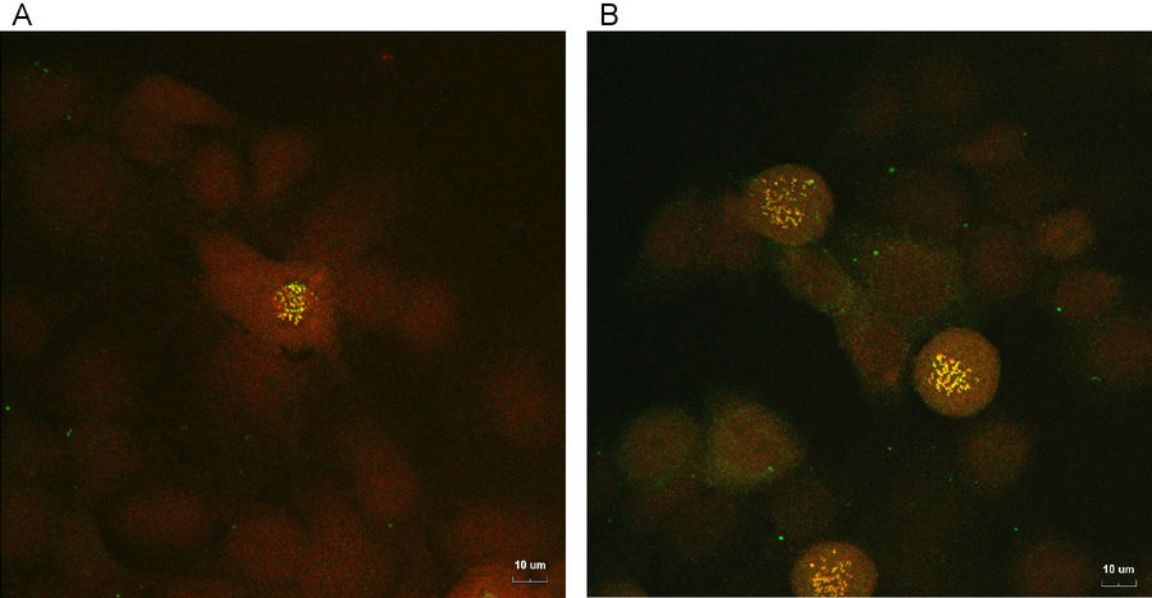

Figure S6