SUPPLEMENTARY DATA

Supplementary Figure 1

Labeling with propidium iodide (PI) does not alter cell motility. Invasive breast cancer MDA-MB-231 cells were cultured to 95% confluence. Mechanical scratching of the cell monolayer was then made together with subsequent addition of 0.5 µg/ml of PI. Following cell culture was carried out for up to 72 hours to assess directional cell motility using would-closure assay. Similar results were obtained with remaining cell impermeant probes such as SYTOX Green, SYTOX Red, PO-PRO 1 and YO-PRO 1. Results are representatives of three independent experiments.

Supplementary Figure 2

Multiplexed exposure to viability stains has no influence on microenvironmental conditions.

A) Human adherent U2OS cells were exposed to a mixture of PO-PRO 1 (250 nM), YO-PRO 1 (250 nM) and SYTOX Red (250 nM) probes for up 5 days. In a reference chip cells were exposed to 1 µM of Hoechst 33342 (positive control). The number of viable cells was assessed using dye exclusion assay during the 5-day study. Note lack of any cell viability changes following continuous exposure to a mixture of three cell impermeant DNA stains. The results represent mean of at least three independent experiments; Normalized SD values were lower than ±7.

B) Human adherent U2OS cells were exposed to a mixture of cyanine probes like in A). The number of viable cells was assessed using dye exclusion assay during the 5-day study. Note lack of any cell growth retardation following continuous exposure to a mixture of three cell impermeant DNA stains. The

results represent mean of at least three independent experiments; Normalized SD values were lower than ±7.

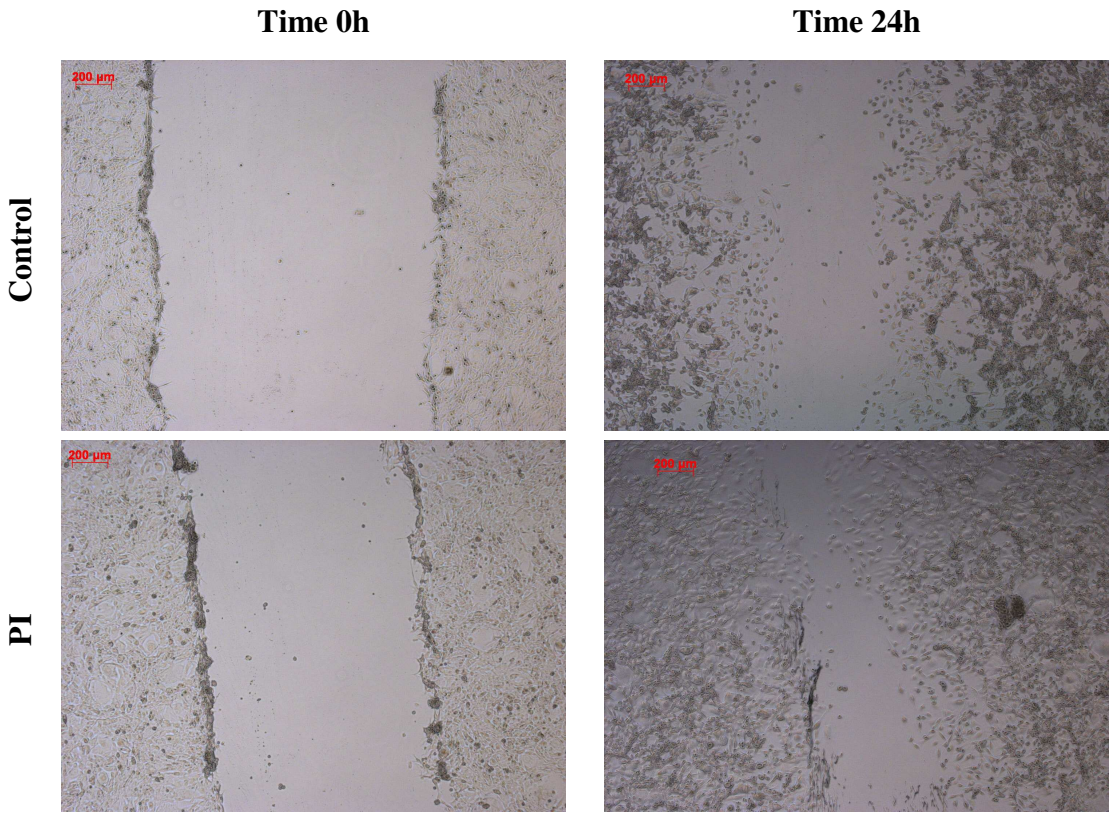
Supplementary Figure 3

Prolonged exposure to cell impermeable DNA stains has no influence on mitochondrial function based on measurements of mitochondrial transmembrane potential ($\Delta\Psi_m$). Human promyelocytic leukemia HL60 cells were exposed to selected concentrations of propidium iodide (PI; 1 μg/ml), SYTOX Green (SY-G; 250 nM), SYTOX Red (250 nM) and YO-PRO 1 (250 nM) for up 72 hours. Mitochondrial function was assessed by flow cytometry using the $\Delta\Psi_m$ sensitive probe TMRM. Note lack of $\Delta\Psi_m$ loss during the duration of experiments. Results are representative of three independent experiments. Similar results were also obtained on other cell lines (not shown).

Supplementary Figure 4

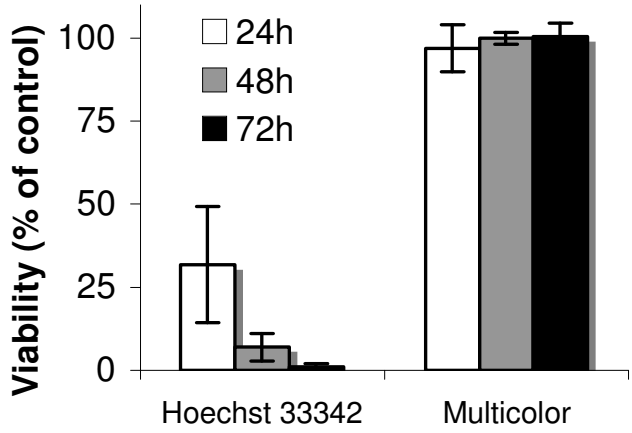
Intracellular uptake of propidum iodide by live human carcinomic alveolar cells (A549). A549 cells were incubated in the presence of 5 μg/ml of PI for 24 hours. Microscopic examination was performed following 24 hours of incubation. Note weak nucleolar fluorescence resulting most likely from biding to RNA and characteristic diffusive cytoplasmic staining that might be related to PI uptake by energized mitochondria, lysosomes or endosomes.

Supplementary Figure 1



Supplementary Figure 2

A)



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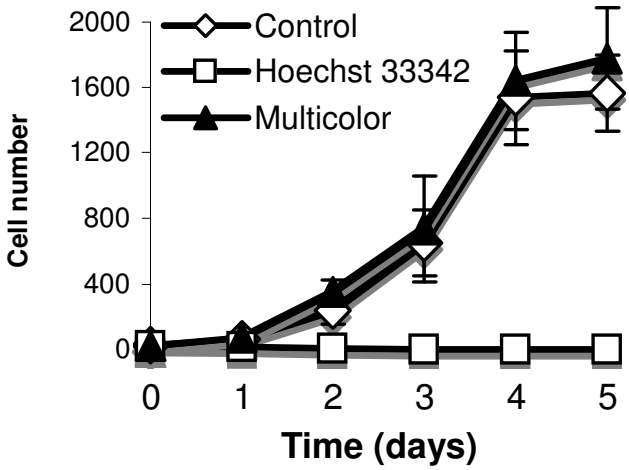
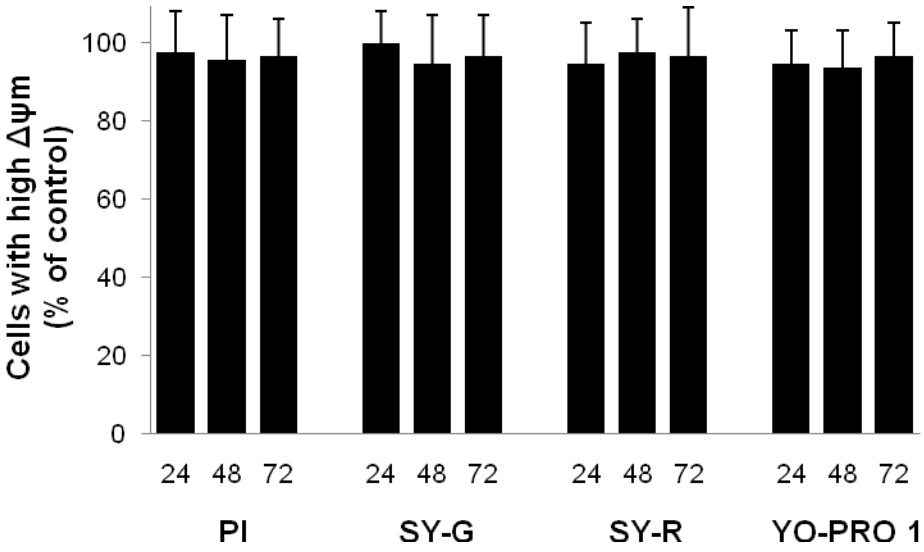


Supplementary Figure 3



Supplementary Figure 4