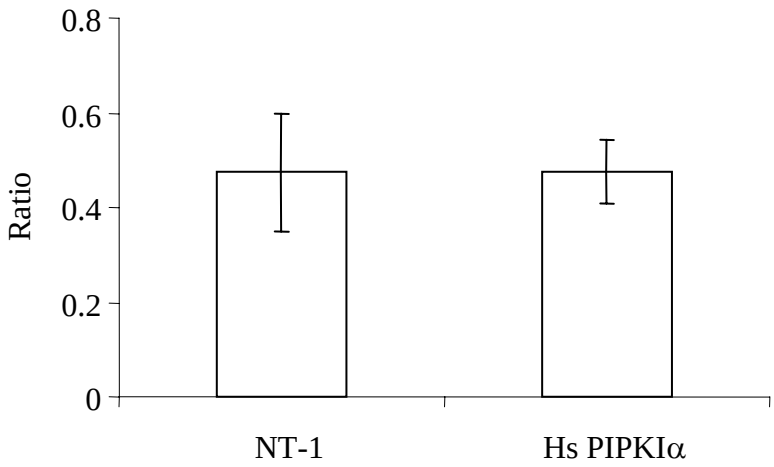
Supplemental Figure 3



No significant differences in cytosolic free calcium in whole cells using Indo-1. Ratiometric imaging of cytosolic free calcium was carried out on a confocal laser scanning microscope using the Ca^{2+} indicator, Indo-1. Three day old cells (0.5 g fresh wt) were incubated overnight in 5 mL conditioned medium with 20 µM Indo-1. Indo-1 loaded cells were excited with an argon uv laser (351 nm and 364 nm) and emission was collected at 400 - 445 nm (E1) and 460 -500 nm (E2). For ratiometric analysis the fluorescence emission intensity of cytoplasmic regions was measured at E1 and E2 and displayed as the ratio E1/E2. Data are the averages $\pm$ SD from 8 different cells from each cell lines. According to the calibration curve with calcium buffer standards the ratios correspond to roughly 150 nM free calcium (the ratio for the calibration buffer with 150nm free calcium is about 0.487).