Supplementary Figure 6

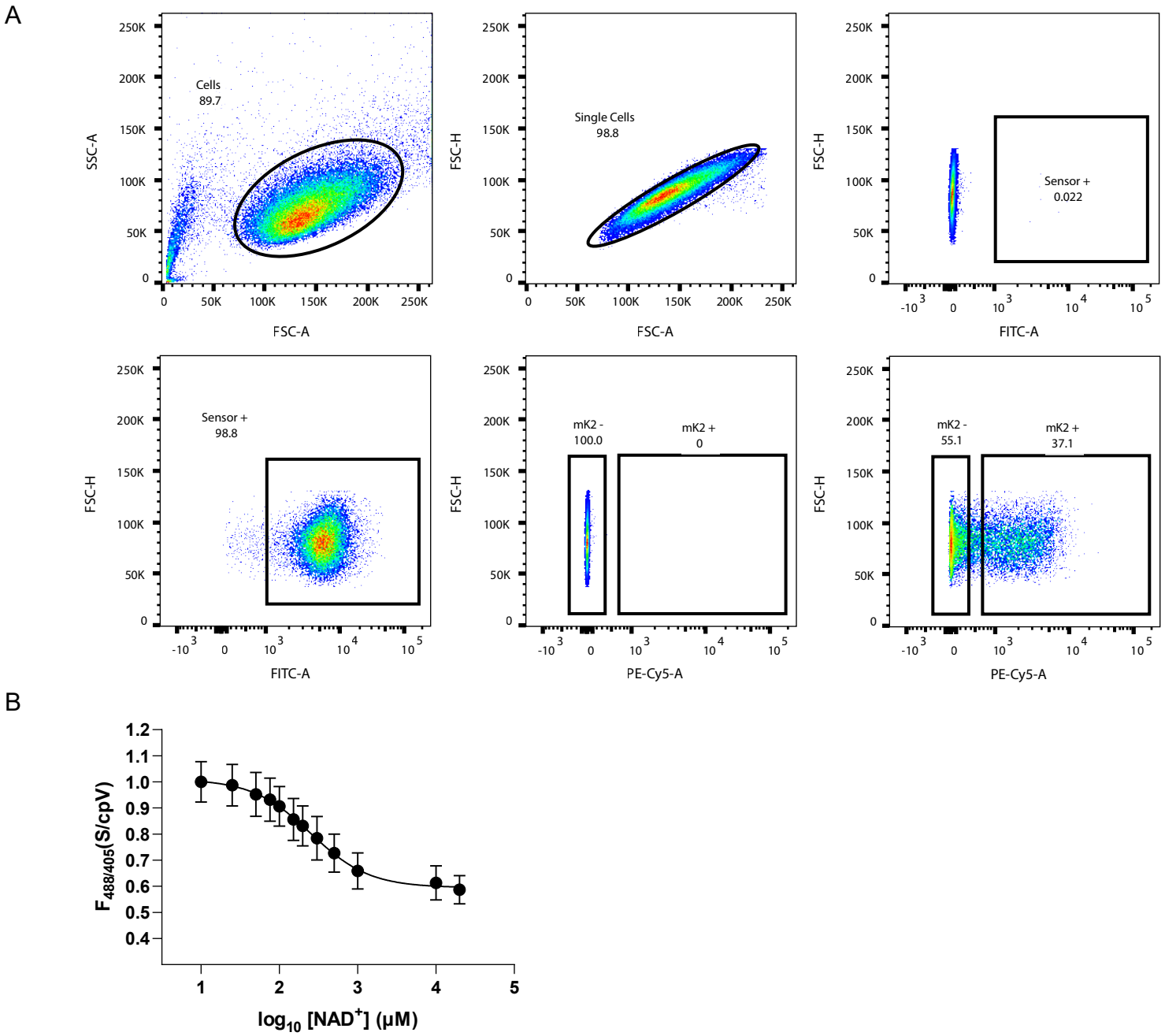

Figure S6

A) Gating strategy for flow cytometric analysis for NAD biosensor experiments. Upon identification of the cell population (panel 1), and doublet exclusion (panel 2), the sensor positive (sensor +) cell population was identified using parental HeLa S3 cells as a negative control (panel 3). These gates were then applied to HeLa NAD biosensor and cPVenus control cells (panel 4). HeLa NAD biosensor cells that have not been transiently transfected with red PARP1cd constructs (MTS-mKate2-PARP1cd-myc or mKate2-PARP1cd-SKL) were then used to define the PARP1cd positive (mK2+) and PARP1cd negative (mk2 -) gates (panel 5), which were then applied to HeLa NAD biosensor/cpVenus expressing cells transiently transfected with red PARP1cd constructs (panel 6).

B) Dose-response curve of the NAD biosensor upon permeabilization by Digitonin. HeLa cells stably expressing the NAD biosensor or the cpVenus control in the cytosol were permeabilized with digitonin and exposed to varying concentrations of NAD_+. The fluorescence ratio (488/405 nm) of the NAD biosensor, as measured by flow cytometry, was normalized to the fluorescence ratio (488/405 nm) of the corresponding cpVenus control and the values were plotted relative to 10 µM NAD_+. Each point represents the mean ± SD, n > 3.