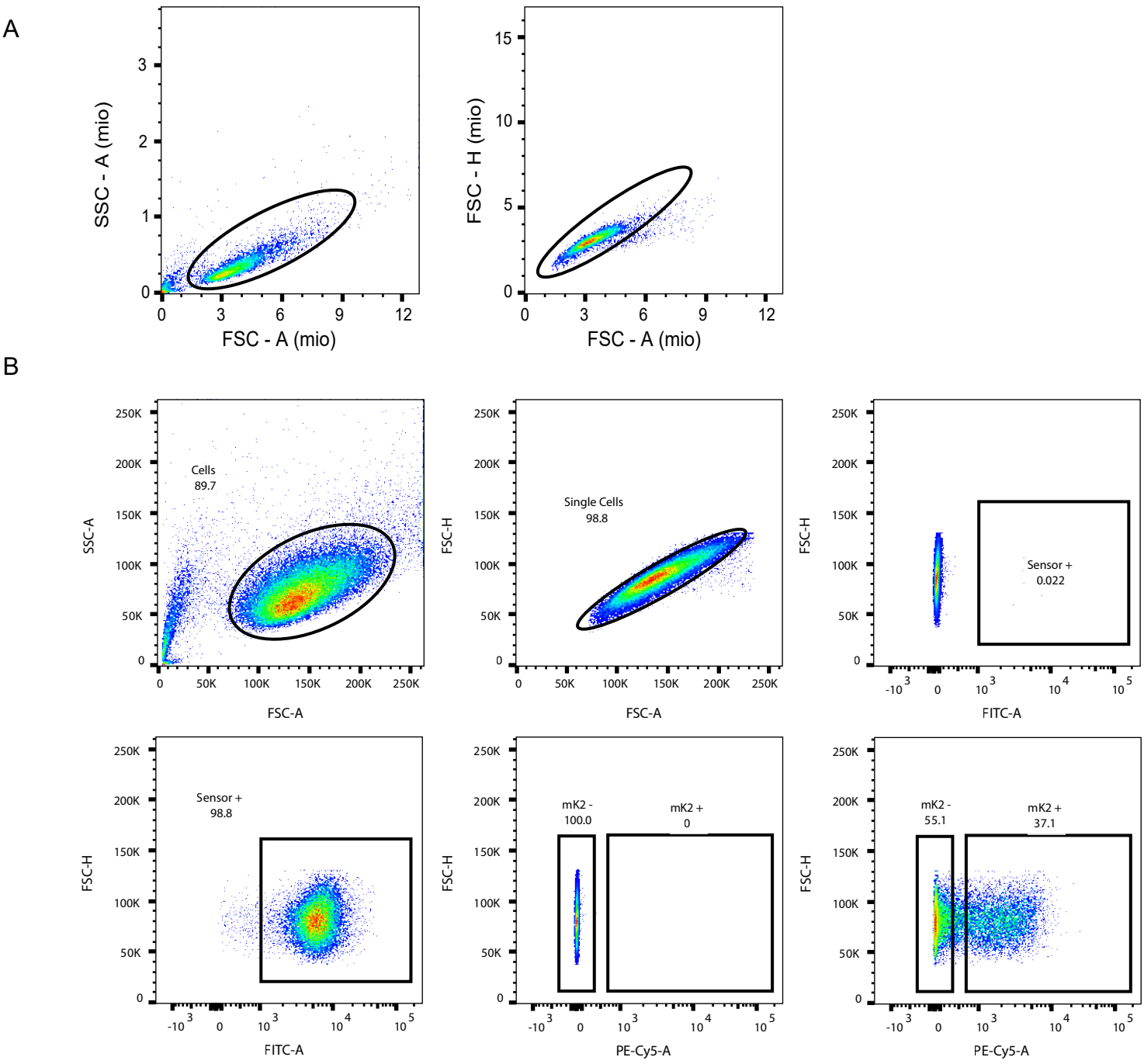
Gating strategy



A) Gating strategy for accurate cell counting (293 cell lines) Upon identification of the cell population (SSC-A/FSC-A), doublet exclusion was conducted (FSC-H/FSC-A).

B) Gating strategy for flow cytometric analysis for NAD biosensor experiments. Upon identification of the cell population (SSC-A/FSC-A), doublet exclusion was conducted (FSC-H/FSC-A). The sensor positve cell population was identified using parental HeLa S3 cells as a negatve control (FSC-H/FITC-A). These gates were then applied to HeLa NAD biosensor and cPVenus control cells. 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 (FSC-H/PE-Cy5-A)), which were then applied to HeLa NAD biosensor/cpVenus expressing cells transiently transfected with red PARP1cd constructs.