

SUPPLEMENTARY FIG. S1. HyPerCyto detects mainly  $H_2O_2$  imported through the plasma membrane. Addition of extracellular catalase (CAT, 5000 U/ml) after  $H_2O_2$  exposure causes the rapid reduction of *HyPerCyto* by cytosolic antioxidant systems, indicating that continuous entry of exogenous  $H_2O_2$  is needed to sustain reoxidation of the sensor. These data also indicate that most if not all *HyPer*oxidation depends on exogenously added  $H_2O_2$ . The data represent the mean fold changes of the 488/405 nm ratio measured by confocal laser scanning, plotted against time  $\pm$  SEMs.