



### Supplementary Figure 1: Gating strategy for NADPH sensor experiments.

Top row indicates gating strategy for BY4741 strain complemented with pH,L,M and pU-mKate2-yeGFP NADH sensor plasmids. A threshold exclusion of 5000 was set on the cytometer, then FSC-A vs SSC-A was used to identify the main population. Doublet discrimination was performed using FSC-A vs FSC-H followed by SSC-A vs SSC-H. Then, cells that were double positive for mKate2 (Comp-610\_20-YE\_GR-A) and yeGFP (Comp-530\_30-BLUE-A) were gated. Bottom row shows representative overlay plots of final gated population treated with 0, 0.125 and 0.25 H<sub>2</sub>O<sub>2</sub> as indicated for SM alone and supplemented with L or D-lysine respectively (K or DK). Tables indicate the median fluorescence intensity (MFI) for each channel in the double positive population. Auxotrophic and prototrophic strains were gated by the same strategy. FSC: forward scatter, SSC: side scatter, A: area, H: height, Comp: compensated, YE\_GR: yellow-green laser, SM: synthetic minimal media.