



**Supporting Figure S1.** Cell plate lipid order analysis with the membrane order-sensitive probe PY3174.

(a) Color-coded GP images from ratiometric fluorescence live imaging analyses of membrane lipid order in cytokinetic cells of Col-0 five-day-old seedling roots labeled with PY3174. Red, high lipid order; blue, low lipid order.

(b) Quantitative analysis of mean GP-value distributions from cell plates (CP) and plasma membranes (PM) obtained from multiple Col-0 cells ( $n = 66$ ) collected in 3 imaging experiments. Horizontal lines, means of non-normal distributions. p-values obtained from non-parametric, two-tailed Mann-Whitney test indicate differences between indicated distributions as highly significant \*\*\*  $p < 0.001$  with a significance threshold level at  $p < 0.05$ . For exact p-values see Table S2.