Both asynchronous and hydroxyurea-arrested (G1/S arrested) mid-log cultures were prepared for each strain. Briefly, cultures were grown to saturation and then diluted back 1:200. Back-diluted cultures were then grown to an OD of 0.4-0.6. For each strain, one ml mid-log culture was transferred into 200 μl 1M hydroxyurea and incubated on a room temperature roller drum for approximately half the time the respective strain took to grow from back dilution to an OD of 0.4-0.6. This ranged between 3 and 12 hours. At the same time, one ml of asynchronous mid-log culture was harvested for fixation. All samples were fixed in 70% ethanol overnight, treated with RNase and Proteinase K, and finally stained with Sytox Green (Molecular Probes). Stained cell suspensions were sonicated before flow cytometry. Flourescence was measured with a BL1 laser (488 nm) on an Attune NxT (Invitrogen) flow cytometer at the lowest available flow rate. To accomodate for extremes in ploidy and cell size, voltage was adjusted to 250 for BL1 and FSC. All samples were run at the same voltage.

Flow cytometry data files were processed in FlowJo v 10.4.2. Samples were first gated on SSC and FSC to remove debris. Doublets were then removed by gating on BL1-A and FSC-A. A histogram of BL1-A values were then generated for remaining cells. Hydroxyurea peaks were identified and gated manually. Asynchronous G1 and G2 peaks were identified by applying a Watson (Pragmatic) Cell Cycle model and identifying G1 and G2 means. Resulting data were analyzed and plotted in R.

A subset of strains were used for microscopy analysis of cell-size. Each strain was spotted from frozen stock onto YPD agar plates and grown at room temperature for 4 days. Water-cell suspensions were prepared for each strain and imaged on an Evos (details) microscope at 400x.