



Figure S2 Mad2 kinetochore SPIs.

(A) A set of 88 kinetochore and kinetochore-associated GFP-tagged proteins were retested (Table S1). Mad2-GBP is compared with *mad2ΔC*-GBP. The dashed line indicates a mean log growth ratio (LGR) of 0.4 and the control strains, indicated in red, are untagged BY4741, File S2.

(B) The *MAD3* gene was deleted from 22 GFP-tagged kinetochore strains and the WT and *mad3Δ* strains were retested for their sensitivity to Mad2-GBP versus the *mad2ΔC*-GBP control. The growth defects of Ipl1-GFP, Sli15-GFP and Bir1-GFP with Mad2-GBP are suppressed by *mad3Δ* (File S2).

(C-F) Imaging of cells containing Mad2-GBP, *mad2-RQ/AA* or GBP plasmids and GFP/YFP-tagged proteins shows that Mad2-GBP and *mad2-RQ/AA*-GBP are recruited to Ipl1-GFP (C), Bir1-YFP (D) (note that GBP does not bind CFP), Nkp2-GFP (E), and Ame1-GFP (F). Fluorescence contrast differs between images and the scale bar is 5 μm.

(G) The growth effects of the Cse4-Mad2 SPI (and controls) are shown.

(H) The growth effects of the Ipl1-Mad2 SPI (and controls) are shown both in wild-type cells and *mad3Δ* cells.

(I) The growth effects (LGR compared to GBP control) of all the Mad2-GBP constructs (WT and mutants) with GFP strains that showed the strongest growth defects (see File S2 for all the data). C-Mad2 is a constitutively closed version of Mad2 (mutation of leucine 7 to alanine) and *mad2-TA/VN* is constitutively open version of Mad2 that cannot bind C-Mad2 (mutations of threonine 133 and valine 188 to alanines).