



**Figure S4 She1 is required for sorbitol rescue of *cdc15-2* cells, is phosphorylated in response to sorbitol stress, and is phosphorylated in part by Hog1.** (A) Serial dilutions of cells of the indicated genotype were spotted onto YPD plates, or YPD plates containing 1.5M sorbitol (SORB). (B) She1 is rapidly phosphorylated in response to sorbitol treatment. She1-myc expressing cells were treated with 1M sorbitol, cells were harvested at indicated timepoints, TCA precipitated, and immunoblots of Phos-Tag gels were probed with anti-myc and Pgk1 antibodies. Arrow at right indicates the phospho-species of She1 that is enriched after sorbitol treatment, especially at the 2min timepoint. (C) Hog1 is phosphorylated in a cell-cycle dependent manner. Immunoblots of TCA-precipitated whole cell extracts from Hog1-GFP expressing cells were probed with antibodies against phosphorylated Hog1, GFP (total Hog1), and Clb2. Cells were collected every 15 minutes after hydroxyurea (HU) arrest and washout. ASY: asynchronously growing cultures that were not stressed, or stressed for 5 minutes with 1M Sorbitol to induce Hog1 phosphorylation. (D) Serial dilutions of cells of the indicated genotype were spotted onto YPD plates. (E) She1-myc is expressed at approximately equal levels in *SHE1* and *she1-2A* cells. TCA-precipitated extracts were run on an SDS-PAGE gel without Phos-Tag, and immunoblotted with anti-myc and Pgk1 antibodies. (F) She1-myc expressing cells of a WT or *hog1Δ* background were arrested in S-phase with hydroxyurea (HU), and harvested every 15min after HU washout. TCA-precipitated extracts were run on Phos-Tag gels, and immunoblotted with anti-myc antibodies. Shown are two different immunoblots, from gels that were run at the same time in the same gel-box, and processed alongside each other. The immunoblot images are aligned here according to molecular weight markers, so the band size is comparable between the blots. The double-headed arrow between the blots points to a phospho-She1 species that is enriched in WT cells, but not in the *hog1Δ* mutant.