FIGURE S1: Cyclin C is exported from the nucleus in slt2Δ cells under high stress. (A) Representative images of the progression of cyclin C-YFP export in DAPI stained wild type cells. In the absence of stress cyclin is located in the nucleus (upper panels). The “intermediate” phenotype (middle panels) is characterized by cyclin C being predominantly located just alongside the nucleus (orange arrow) cyclin C and well being discreet foci in the cytoplasm (pink arrow). The “export” phenotype (bottom panels) is characterized by cyclin C being predominantly exported from the nucleus and located in the cytoplasm. (B) Field view (collapsed deconvolved 0.2 µM slices) of fluorescence microscopy conducted on mid-log phase on slt2Δ kdx1Δ (RSY1737) cells expressing YFP-cyclin C (pBK37) following (2 h) 1.2 mM H2O2 treatment. The cells were fixed, stained with DAPI and then examined by fluorescence microscopy. (C) The percent of wild type (RSY10) and slt2Δ (RSY1006) cells (mean ± s.e.m.) within the population displaying at least 3 cytoplasmic cyclin C foci is given before and following H2O2 (0.4 and 1.2 mM) treatment for 2 h. At least 200 cells were counted per timepoint from 3 individual isolates. (D) Representative images (collapsed de-convolved 2µ slices) of slt2Δ cells harboring cyclin C-YFP after 2 h 1.2mM H2O2 stress.
FIGURE S2: (A) Fixed cell images of the whole cell wild type cells (RSY10) expressing cyclin C-YFP and Nop1-RFP. Cells were harvested and stained with DAPI following 1.2mM H₂O₂ at the time points indicated. (B) Field view (collapsed deconvolved 0.2 μM slices) of fluorescence microscopy conducted on mid-log phase on bck1Δ ste11Δ (BLY478) cells expressing YFP-cyclin C (pBK37) following (2 h) 1.2 mM H₂O₂ treatment. The cells were fixed, stained with DAPI and then examined by fluorescence microscopy.
FIGURE S3: the cell wall sensor Sho1 may be able to transmit the stress signal to Ste11 following H$_2$O$_2$ stress. (A) sho1$\Delta$ (RSY1988) and bck1$\Delta$ sho1$\Delta$ (RSY1989) cultures expressing myc-cyclin C (pLR337) were grown to mid-log phase (0 hr) then treated with 1.2 mM H$_2$O$_2$ for the indicated times. Cyclin C levels were determined by Western blot analysis of immunoprecipitates. Tub1 levels were used as a loading control. (B) Cell viability assays on wild type sho1$\Delta$ and bck1$\Delta$ sho1$\Delta$ strains following treatment with 2mM H$_2$O$_2$ for 2 hrs. Decreasing dilutions of the cells (represented by the arrow) were plated on YPDA media and the surviving colonies photographed after 2 days at 30°C. (C) Cyclin C exhibits the “intermediate” localization phenotype in the bck1$\Delta$ sho1$\Delta$ strain following 1.2 mM H$_2$O$_2$ stress. Fluorescence microscopy was conducted on mid-log phase on bck1$\Delta$ sho1$\Delta$ cells expressing YFP-cyclin C (pBK37) following (2 h) 1.2 mM H$_2$O$_2$ treatment. The cells were fixed, stained with DAPI and then examined by fluorescence microscopy. Representative images (collapsed deconvolved 0.2 µM slices) of the results obtained are shown.