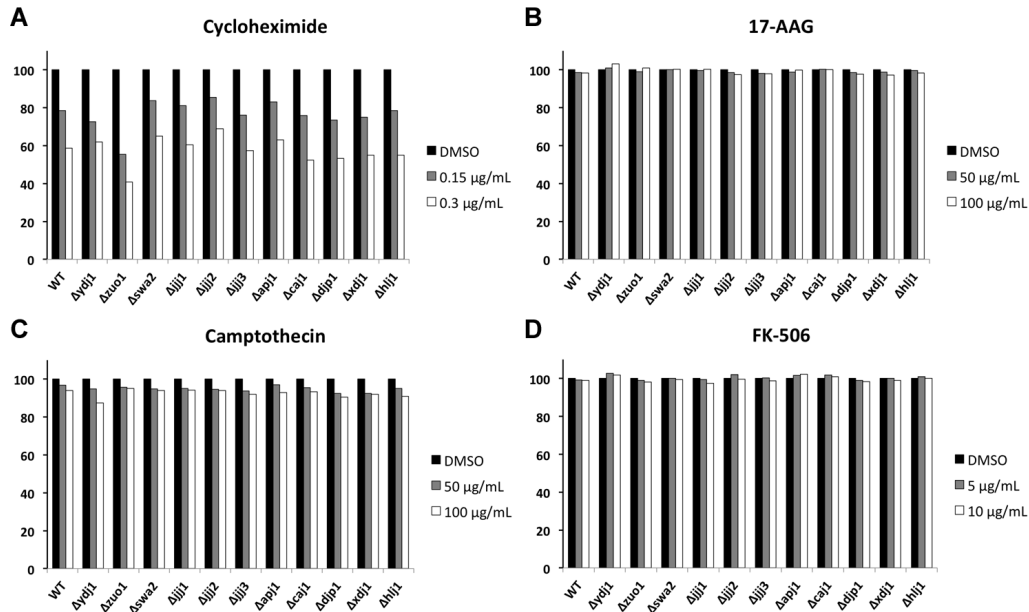
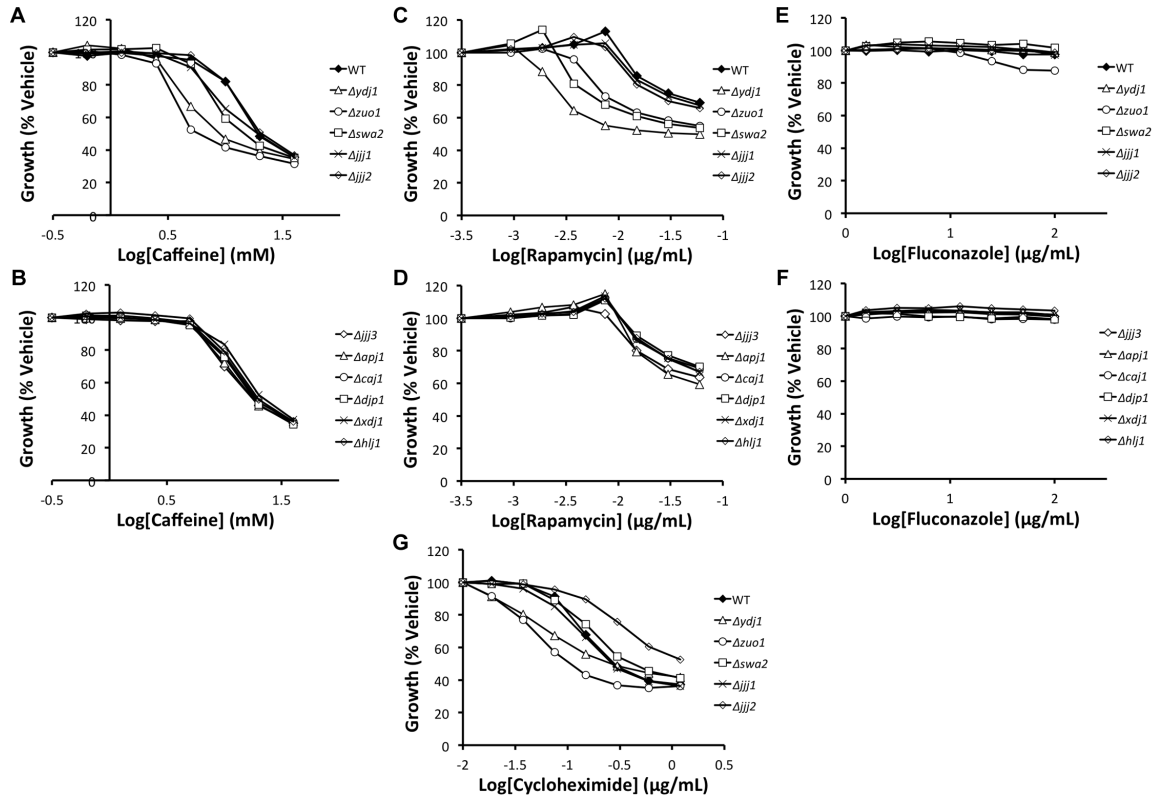


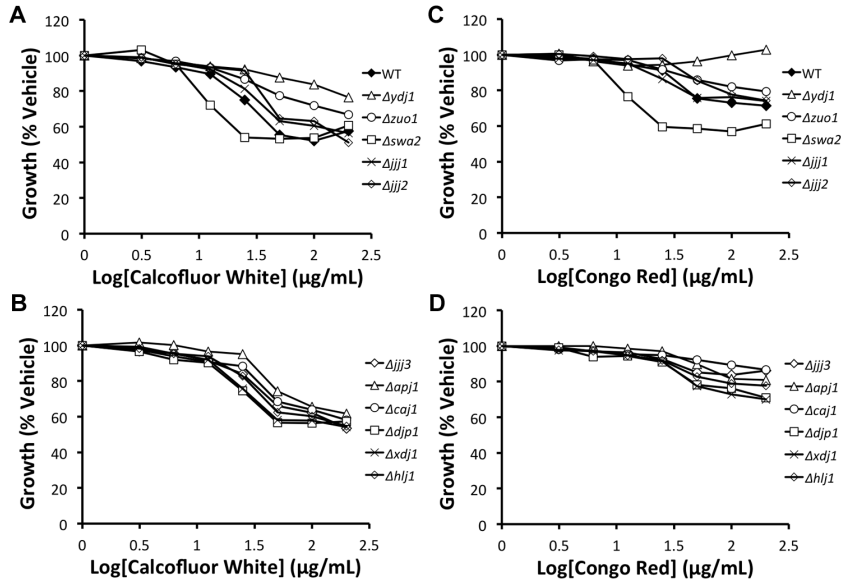
## Supplemental Information



**Figure S1.** Cycloheximide, 17-AAG, camptothecin, and FK-506 were tested in liquid media at concentrations 2-fold greater than those tested in the solid media assay, to identify any phenotypes that might have been missed at the lower concentration. Only cycloheximide had an effect on some strains, and this result was further explored with dose-response curves (see Fig. S2G and Fig 4).



**Figure S2.** Dose-response curves for all 11 J protein deletion strains in the presence of four representative compounds. Deletion strains were grown in the presence of (A,B) caffeine (40-0 mM), (C,D) rapamycin (0.06-0  $\mu\text{g/mL}$ ), (E,F) fluconazole (100-0  $\mu\text{g/mL}$ ), and (G) cycloheximide (1.2-0  $\mu\text{g/mL}$ ). In general, the liquid media assay only reproduced the more dramatic findings from the solid media study, suggesting that it is a less sensitive assay for this specific purpose.



**Figure S3.** Testing of cell wall inhibitors in the liquid media assay is prone to artifacts. Deletion strains were grown in the presence of calcofluor white (CW) or congo red (CR) (200-0  $\mu\text{g/mL}$ ) and OD used to estimate cell growth. In this platform, optical density is a misleading measurement because, as confirmed by light microscopy, cell wall inhibitors cause turbidity and cell clumping.<sup>1</sup> Thus, although  $\Delta ydj1$  is clearly sensitive to CW and CR in solid media (see Fig. 1), it appears resistant in the liquid assay. Interestingly,  $\Delta swa2$  was sensitive to CR and C, suggesting that its mechanism is distinct from that of  $\Delta ydj1$  and  $\Delta zuo1$ .

1. R. J. Karreman and G. G. Lindsey, *Can J Microbiol*, 2007, **53**, 1203-1210.