



**Figure S3: Ptc1 acts on both Mkk1 and Mkk2. A)** Western blotting analysis of cell extracts from the wild type strain YPH499 and the isogenic *mkk1* $\Delta$  and *mkk2* $\Delta$  strains transformed with pYES3-GFP or pYES3-GFP-PTC1 and pRS316 or pRS316-BCK1-20. Cells were grown to mid-log phase in raffinose-based medium, then galactose was added to a final 2% for 2 h. Phospho-Slt2, GFP-fused proteins, and G6PDH (loading control) were detected with anti-phospho-p42/44, anti-GFP and anti-G6PDH antibodies, respectively. **B)** Western blotting analysis in the same strains as in A, transformed with plasmids transformed with pEG-(KG) or pEG(KG)-PTC1 and YCplac112 (-) or YCplac112-PKC1<sup>AAA</sup>. **C)** Western blotting in the wild type strain BY4741 and the isogenic *mkk1* $\Delta$  and *mkk2* $\Delta$  strains, transformed with pEG-(KG) or pEG(KG)-PTC1. Cells were cultured at 24°C (-) or at 39 °C (+) for one hour.