Supplemental Materials

Molecular Biology of the Cell

Gerganova et al.

Figure S1

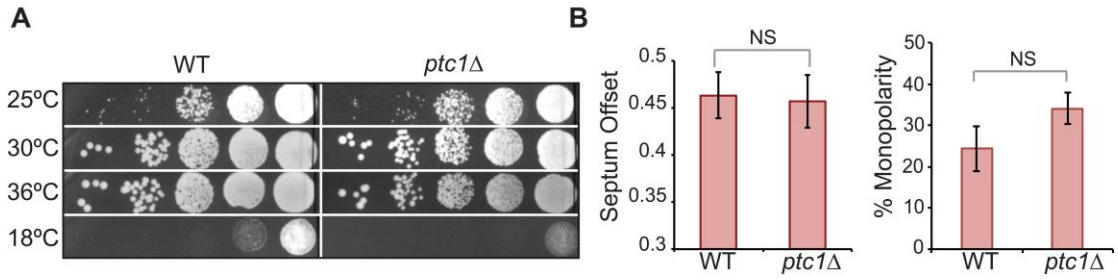

Figure S1: *ptc1*Δ cells are not temperature sensitive. A. Serial dilution assay of wild type and *ptc1*Δ cells at different temperatures. **B.** Septum offset quantification in wild type and *ptc1*Δ cells. More than 120 cells per strain were counted from 2 independent experiments. Right: percentage of monopolar cells from wild type and *ptc1*Δ strains. More than 100 septated cells were counted from 3 independent experiments. *p-values* determined using student t-test.

Figure S2

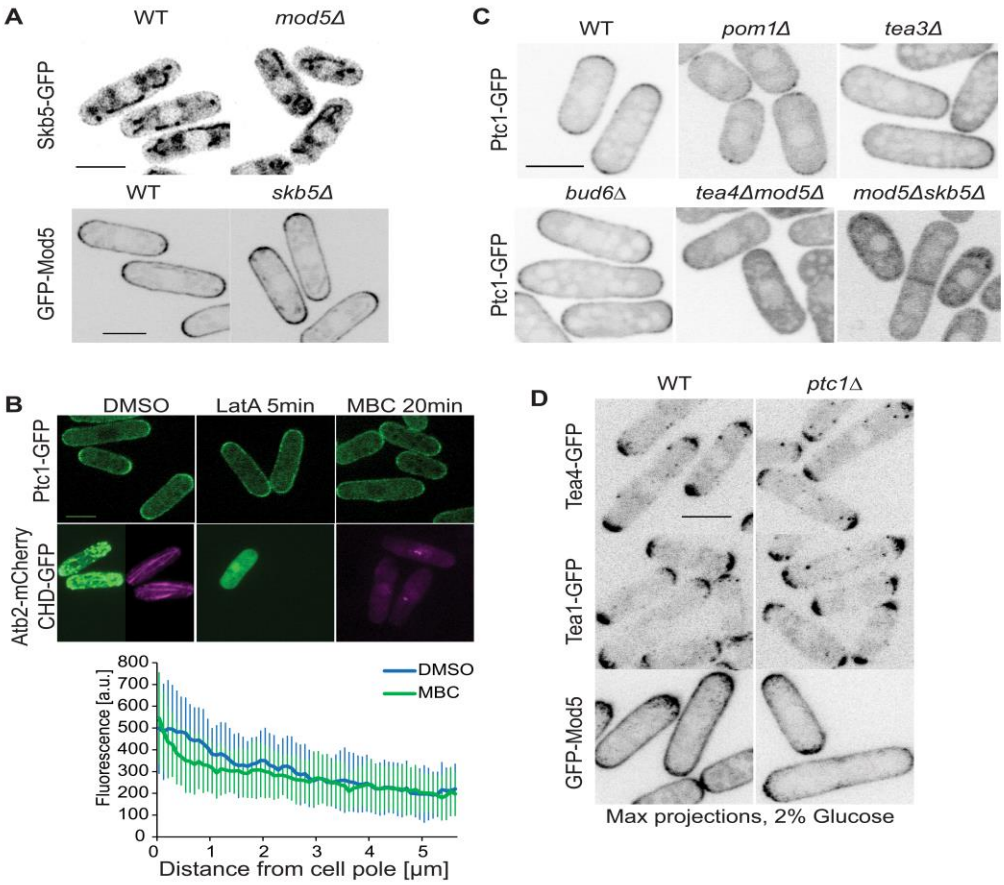

Figure S2: Investigation of Ptc1 localization. A. Localization of Skb5-GFP in WT and *mod5Δ* cells and GFP-Mod5 in WT and *skb5Δ* cells (widefield microscopy). Insets show magnifications of selected cell poles. **B.** Localization of Ptc1-GFP in MBC and LatA treated cells. Top row: Cells were treated with DMSO, 200µM LatA for 5', and 25µg/ml MBC for 20' and imaged using confocal microscope. Max projections of 14 z-slices are shown. Middle row: Cells with labeled mCherry-Atb2 and CHD-

GFP were treated and imaged similarly as above to serve as controls for effective depolymerization of MTs and F-actin upon drug treatment. LatA treatment effectively removed actin cables and patches. MBC treatment effectively destroyed interphase MTs. Bottom: average profile from quantification of Ptc1-GFP fluorescence intensity along the cortex of 16 cells treated with MBC or DMSO control for 20min. Quantifications done on sum projections of 14 z-slices. Error bars are standard deviations. **C.** Localization of Ptc1-GFP in WT, *pom1*Δ*, bud6*Δ, *tea3Δ*, *tea4Δmod5Δ* and *mod5*Δ*skb5*Δ cells. Note the residual localization of Ptc1 at the division site of *mod5*Δ*skb5*Δ cells. **D.** Localization of Tea4-GFP, Tea1-GFP and GFP-Mod5 in WT and *ptc1*Δ. Scale bars: 5µm.

Figure S3

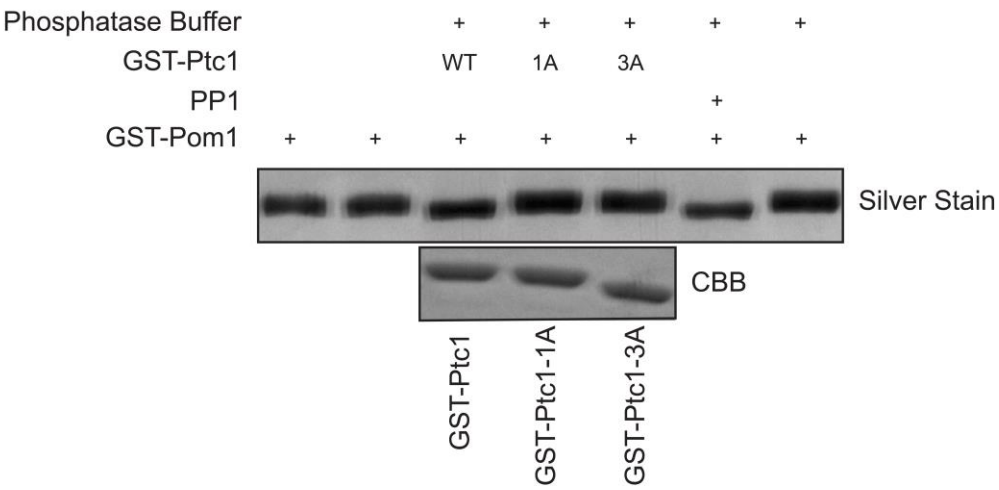

Figure S3: Ptc1 phosphatase activity in vitro. In-vitro phosphatase assay. Purified proteins were incubated in phosphatase buffer at 37°C for 1.5h. Top: GST-Pom1 shift revealed using silver staining. Bottom: Loading control for GST-Ptc1 proteins revealed using Coomassie blue staining (CBB).

Figure S4

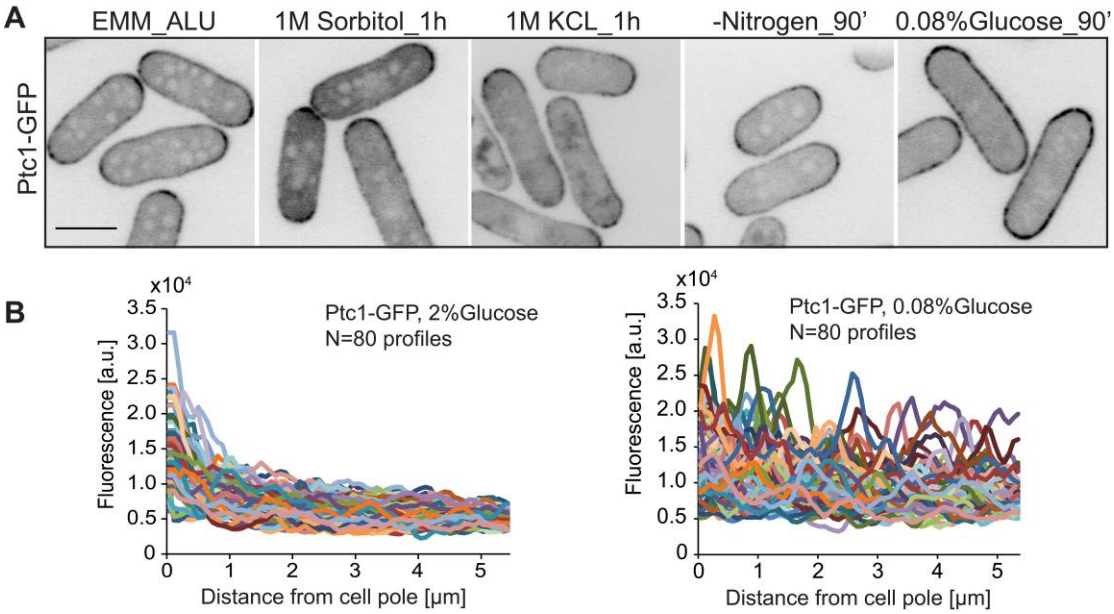

Figure S4: Controls for Pom1 and Ptc1 localization. A. Localization of Ptc1-GFP in different stresses. Cells were grown in EMM-ALU for 24h and then treated with 1M sorbitol, or 1M KCL for 1h before imaging. For nutrient starvation, cells grown in EMM-ALU were washed 3 times and shifted to EMM-ALU lacking nitrogen, or in EMM-ALU with 0.08% glucose for 90' before imaging. Sum projections of 5 confocal images acquired over time are shown. Scale bar: 5µm. **B.** Traces of individual Ptc1-GFP fluorescence profiles for n=20 cells grown in EMM-ALU 2% glucose for 24h (left panel) and shifted to 0.08% glucose for 1h before imaging (right panel). Quantified on mid-plane sum projections of 5 consecutive snapshots. Error bars are standard deviations.

Table S1: Cell length at division of indicated genotypes

Table S2: *S. pombe* strains used in this study.