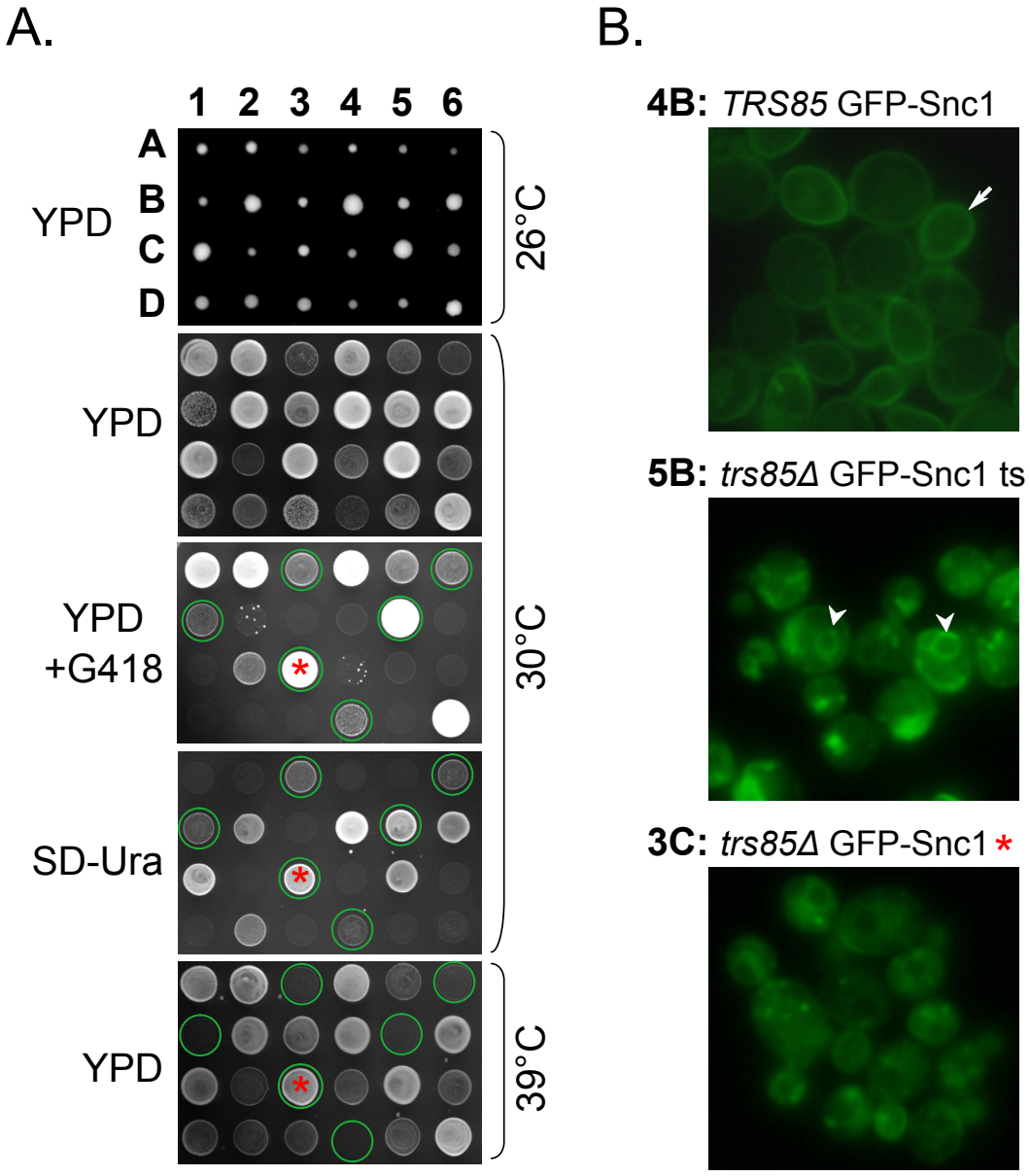
A.
1 2 3 4 5 6
A
B
C
D
YPD
26°C
YPD
YPD
+G418
SD-Ura
30°C
YPD
39°C
B.
4B: TRS85 GFP-Snc1
5B: trs85Δ GFP-Snc1 ts
3C: trs85Δ GFP-Snc1 *

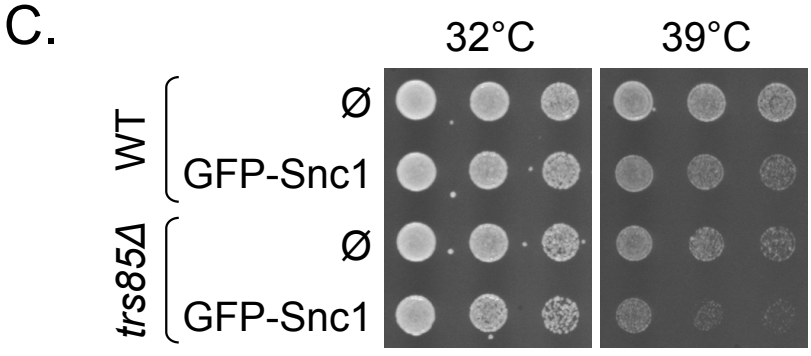
C.
32°C
39°C
WT
Ø
GFP-Snc1
trs85Δ
Ø
GFP-Snc1

Figure S2 A temperature-sensitive growth phenotype is linked to over-expression of GFP-Snc1 in *trs85Δ* mutant cells. A. Segregation of a temperature-sensitive growth phenotype with *trs85Δ* GFP-Snc1. The following two haploid strains were mated: *MATα* GFP-Snc1::*URA3* (YLY130) and *MATa trs85Δ*::*KAN*^{R} (YLY919) strain on YPD at 26°C. Diploids were selected, sporulated, dissected and grown on YPD at 26°C (top). Spores (A-D) from six consecutive tetrads (1-6) were inoculated in YPD medium and spotted on various plates, which were incubated at the indicated temperatures. Shown from top to bottom: Growth on YPD at 30°C, YPD+G418 (30°C) for *trs85Δ*::*KAN*^{R}, SD-Ura (30°C) for GFP-Snc1::*URA3*, and YPD at 39°C for temperature sensitivity. Green circles show *KAN*^{R} *URA3* spores. All *KAN*^{R} *URA3* spores are temperature sensitive for growth except for spore 3C (*). B. GFP-Snc1 localization in wild type and temperature-sensitive (ts) spores. Spores expressing GFP-Snc1 from panel A were examined using live-cell microscopy for their GFP-Snc1 phenotype at 37°C, as described for Figure 1B. The GFP-Snc1 phenotype is shown for three spores from top to bottom: 4B, *TRS85* GFP-Snc1 (arrows point to GFP-Snc1 on the PM in wild type cells); 5B, *trs85Δ* GFP-Snc1 ts (arrowheads point to the intracellular GFP-Snc1 rings); 3C, *trs85Δ* GFP-Snc1 not ts (*), GFP-Snc1 accumulates inside cells at a lower level than in ts cells. C. Temperature sensitive growth phenotype by expression of GFP-Snc1 from a plasmid in *trs85Δ* mutant cells. Wild type and *trs85Δ* mutant cells were transformed with a high-copy 2μ *URA3* empty plasmid, as a negative control, or the same plasmid expressing GFP-Snc1 from the *TPI* promoter. Shown is growth on SD-Ura plates at 32° and 39°C (10-fold serial dilutions from left to right). Over-expression of GFP-Snc1 in *trs85*Δ mutant cells results in a temperature-sensitive growth phenotype at 39°C. Results shown in this panel are representative of four independent transformants.