



Figure S3 Accumulation of intra-cellular GFP-Snc1 in *ypt1ts* and *trs85Δts* mutant cells is different from its accumulation in *ypt31Δ/32ts* and *trs130ts*. A. Accumulation of internal GFP-Snc1 in *ypt1ts* and *trs85Δts* mutant cells. Wild type and *ypt1ts*, *trs85Δts*, *ypt31Δ/32ts* and *trs130ts* mutant cells expressing chromosomally-tagged GFP-Snc1 were grown to mid-log phase in YPD medium at 26°C (left) or shifted to 37°C for 1.5 hours (right). GFP-Snc1 localization was visualized by live-cell fluorescence microscopy. In wild type cells, GFP-Snc1 localizes mainly to the PM. In all four mutant strains GFP-Snc1 accumulates inside cells. Whereas the intracellular GFP-Snc1 accumulation is evident as puncta in *ypt31Δ/32ts* and *trs130ts* mutant cells already at the permissive temperature, in *ypt1ts* and *trs85Δts* it is apparent as rings mostly at the restrictive temperature. Arrows point to GFP-Snc1 on PM, and arrowheads point to internalized GFP-Snc1. DIC images (on each side) show the contour of cells; Bar, 7 μm. B. Quantification of data presented in panel A. Shown is percent of cells containing an internal GFP-Snc1 ring. At least 100 cells were counted in at least three fields for each strain; error bars represent STDEV.