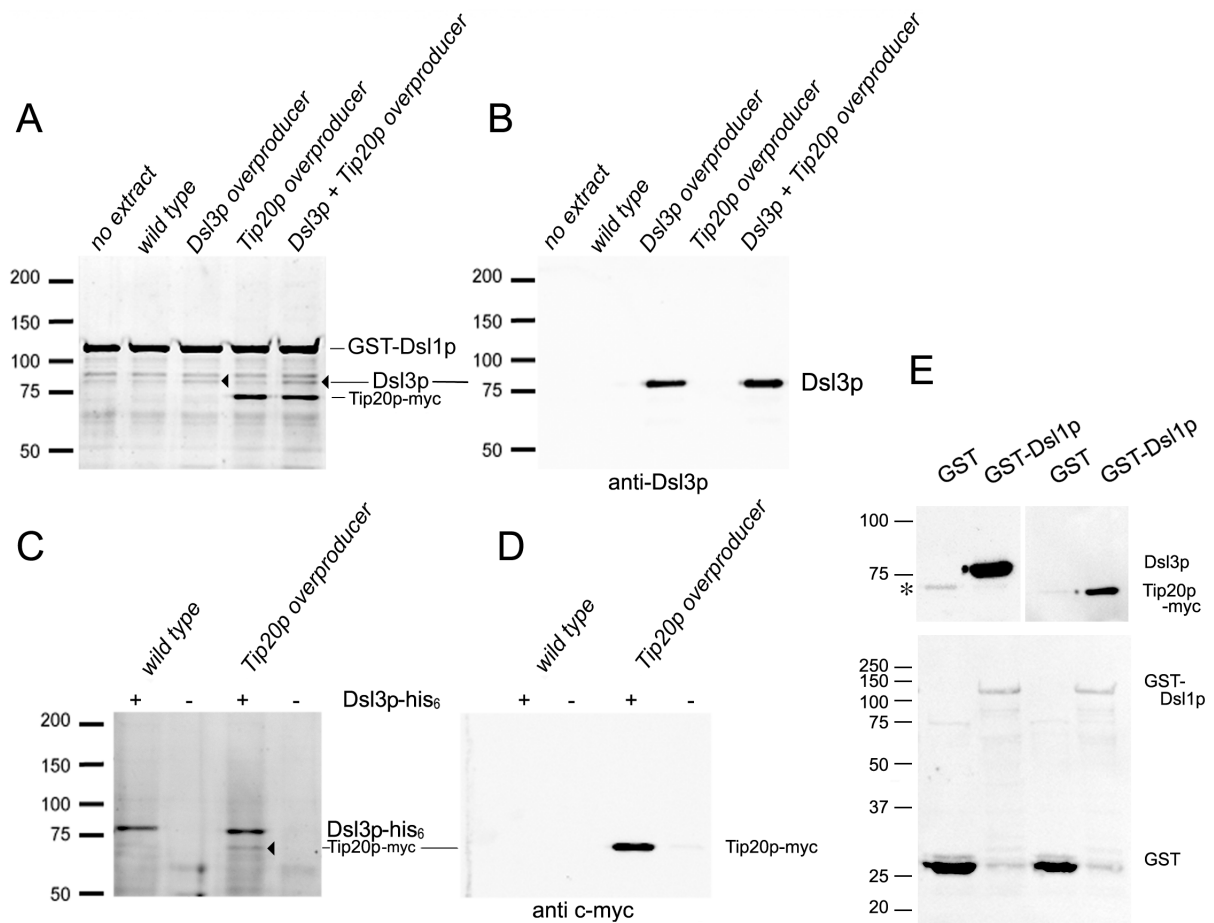
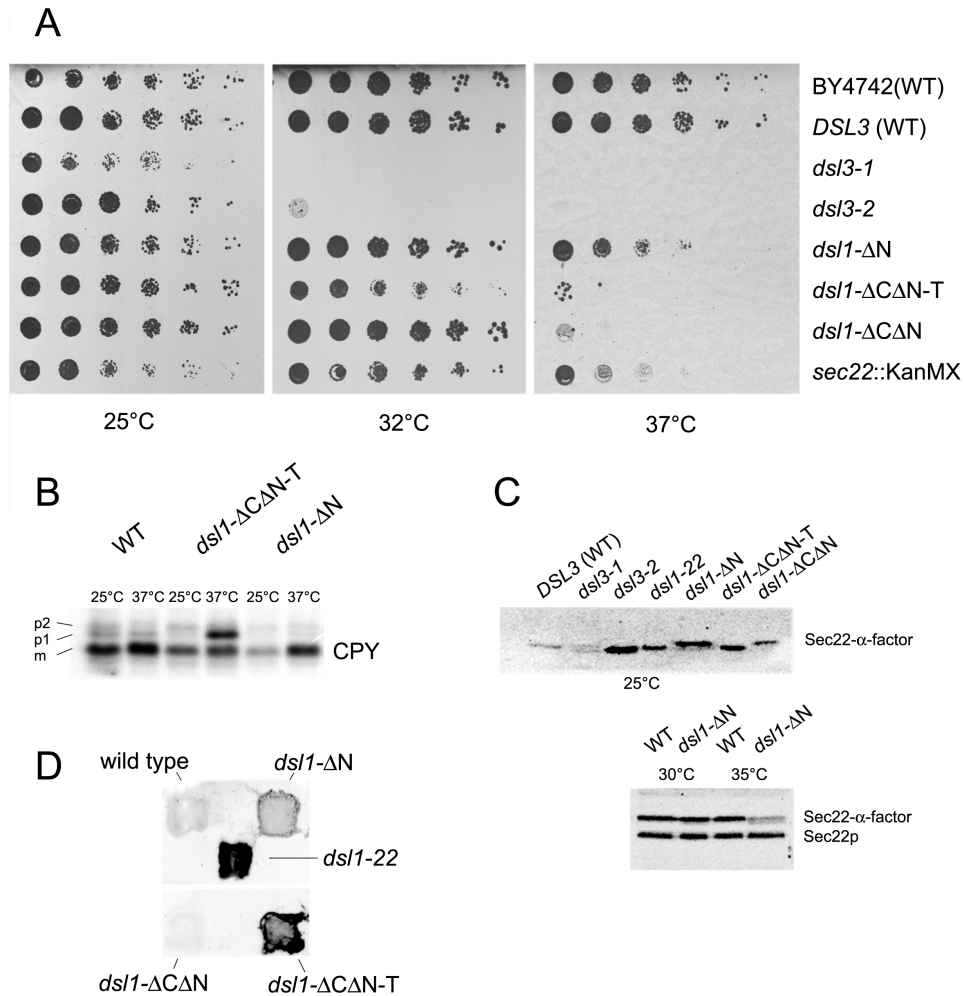


# Kraynack et al., Supplemental Figure S1



**GST-Dsl1p and Dsl3p-his<sub>6</sub> produced in *E. coli* bind Tip20p overproduced in yeast cells. GST-Dsl1p also recruits Dsl3p from extracts of overexpressing cells.** GST-Dsl1p and Dsl3p-his<sub>6</sub> were expressed and purified from *E. coli*. Purified proteins were loaded to glutathione Sepharose or Ni-NTA agarose beads. The equivalent of 2 mg protein from wild type yeast cells (cI3-ABYS-86) overexpressing the indicated proteins were added. Extracts were prepared using HKET buffer (*Materials and Methods*). After 1 h incubation at 4°C, beads were washed four times with HKET buffer. SDS-gels were stained with SYPRO® Red (Cambrex) (A and C) and blotted for immunodetection of Dsl3p (B) or Tip20p-myc (D). Overproduced Tip20p-myc does not bind to another his<sub>6</sub>-tagged protein, his<sub>6</sub>-ARF1 (unpublished data). (E) No Dsl3p and no Tip20p-myc bind to GST alone. The upper panel shows immunoblots of an 8% polyacrylamide gel. The asterisk marks a contaminating *E. coli* protein at 75 kDa that cross-reacted with the anti-Dsl3p antibody in this experiment. The lower panel shows a Ponceau Red stained 12% polyacrylamide gel showing the amount of GST-Dsl1p and GST used in this control experiment.

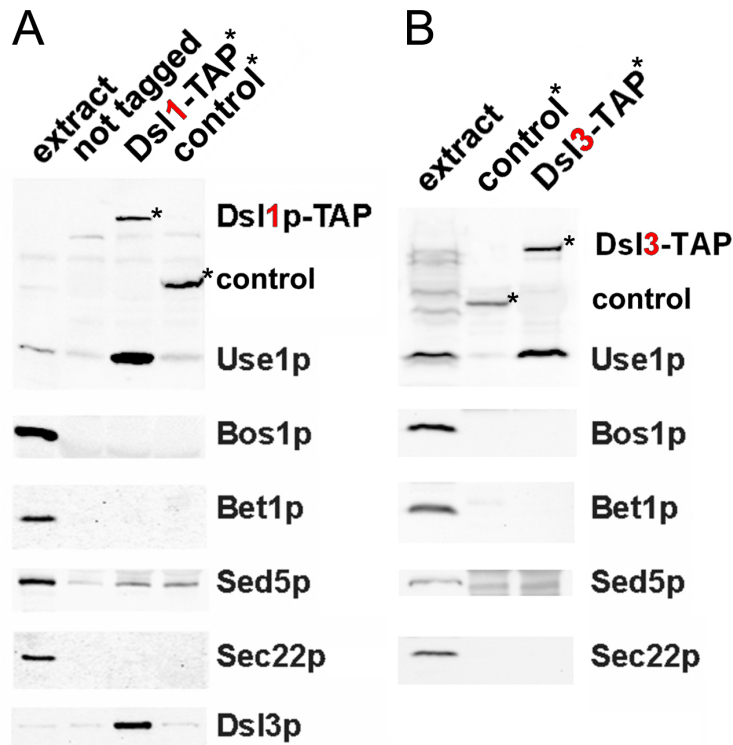
## Kraynack et al., Supplemental Figure S2



**Phenotypes of N-terminally truncated *dsl1* mutants.** (A) Growth of wild type and mutants. Colonies shown in lane 3-5 represent  $\Delta dsl3$  strains carrying a centromeric vector expressing wild type and mutant versions of *DSL3*. *dsl1-ΔN* (lane 5) cells produce a plasmid encoded N-terminally truncated version of Dsl1p ( $\Delta 8-317$ ) in a *dsl1Δ* background. *dsl1-ΔCΔN* and *dsl1-ΔCΔN-T* mutants express a split *DSL1* gene created by integration of a plasmid at the *DSL1* locus (pRS305- $\Delta Ndsl1$ ; *LEU2*, integrating vector; see main text). In *dsl1-ΔCΔN-T* mutants the TAP-tag is fused to the C-terminal fragment, while in *dsl1-ΔCΔN* mutants the C-terminus is not modified. (B) CPY pulse chase experiment to analyze ER-Golgi forward transport in *dsl1-ΔN* mutants. The pulse chase experiment was performed in parallel to the experiment shown in Figure 3C. (C) Sec22- $\alpha$ -factor mislocalization assay as in Figure 3B. (D) BiP secretion assay as in Figure 3A. Cells were incubated at 35°C for 24 hours.

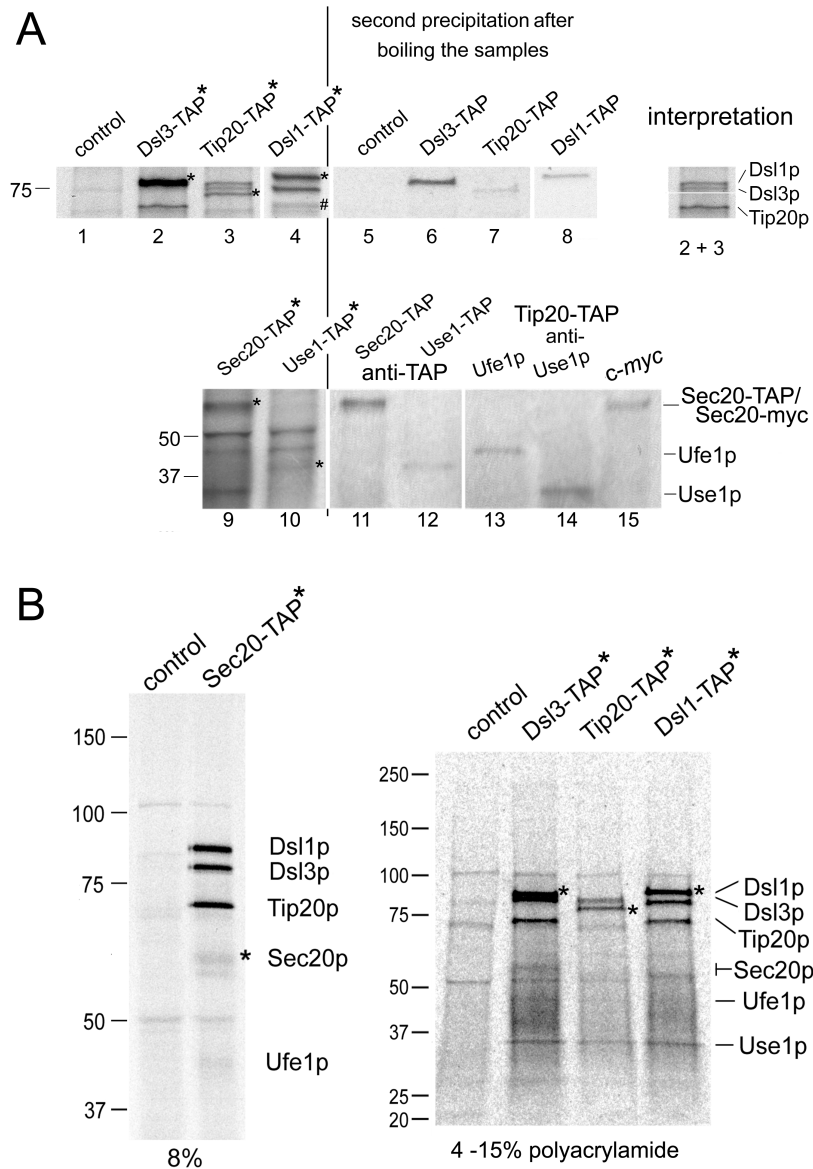
In summary, *dsl1-ΔN* mutants show only slight growth and ER retention defects. However, the *dsl1-ΔN* mutation aggravates the growth defect of *sec22Δ* mutants (our unpublished data). In *dsl1-ΔN* cells expressing in addition the N-terminal 58 kDa fragment of Dsl1p the growth defect became stronger (*dsl1-ΔCΔN*). In *dsl1-ΔCΔN-T* mutants the presence of the C-terminal TAP tag causes an even stronger growth defect and specific retention defects..

Kraynack et al., Supplemental Figure S3



**SNAREs involved in ER-Golgi anterograde transport do not bind to Dsl1p or Dsl3p.** The pull-down experiment was performed as described in legend to Figure 5. Cells producing no tagged protein as well as cells expressing TAP-tagged YOR164c were used as a negative controls. 1/20 the of the input was loaded in the first lane (extract). (A) Dsl1-TAP, (B) Dsl3-TAP. The TAP-tagged proteins are marked by asterisks.

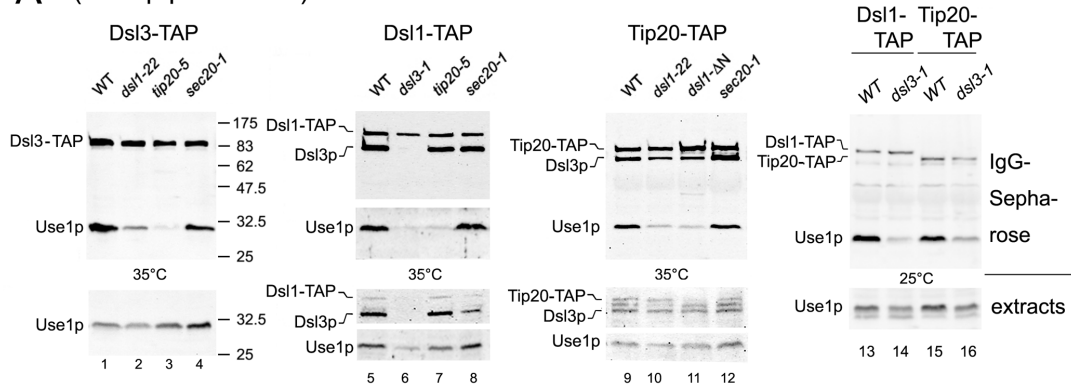
# Kraynack et al., Supplemental Figure S4



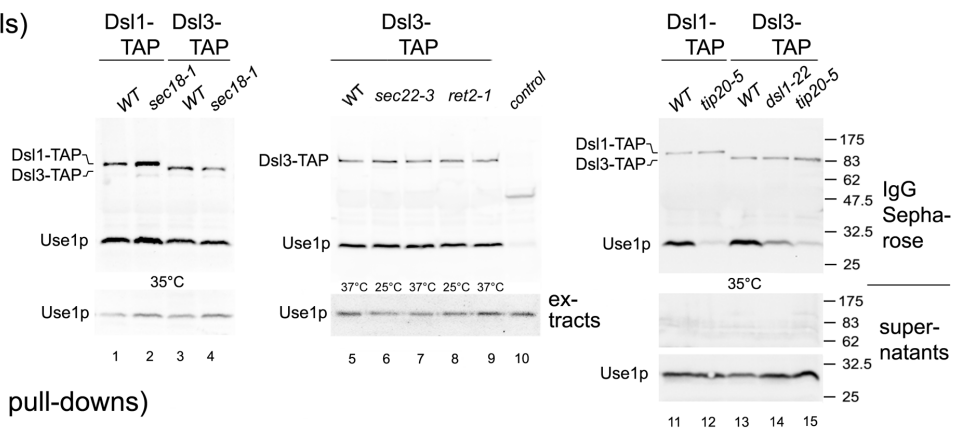
**Identification of proteins purified by tandem affinity purification (Figure 6).** (A) Aliquots of the tandem affinity purifications were heated to destroy protein complexes. The antibodies indicated above lane 5-8 and 11-15 were used for immunoprecipitations to identify particular proteins. Proteins carrying the TAP-tag are marked by an asterisk. Note that the anti-TAP antibody (Open Biosystems) is directed against that part of the tag that is exposed after TEV protease cleavage. Tip20p in lane 4 represents wild type Tip20p and plasmid-encoded Tip20-myc (marked #). The protein shown in lane 15 is from a Tip20-TAP producing strain that expresses a myc-tagged *SEC20* allele. (Control: YOR164c-TAP as in Supplemental Figures S3, S5 and S6). (B) One-percentage gel showing a preparation using an extract from a Sec20-TAP expressing strain as well as a 4-15% gradient gel with samples from Dsl3-TAP, Tip20-TAP and Dsl1-TAP strains. The corresponding gradient and one-percentage gels of these or equivalent preparations are shown in Figure 6.

# Kraynack et al., Supplemental Figure S5

## A (Use1p pull-downs)



## B (controls)



## C (Ufe1p pull-downs)

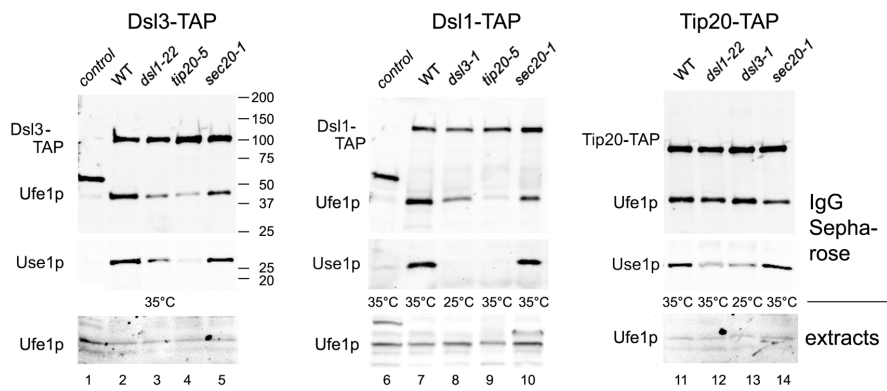
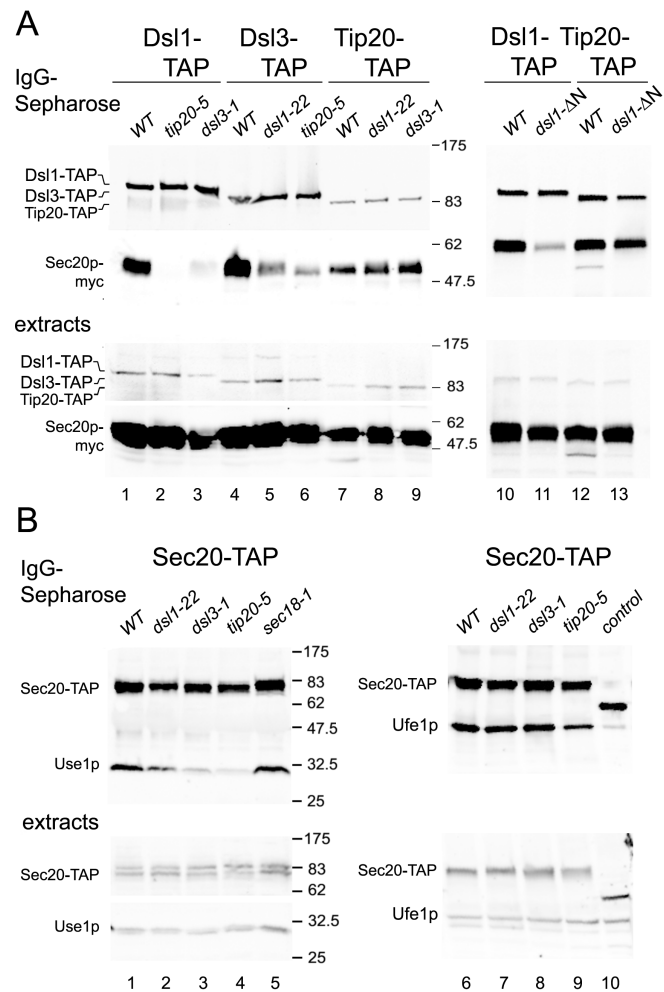


Figure legend see next page

**Binding of SNARE proteins Use1p and Ufe1p to the Dsl1 complex is affected by mutations in subunits of the 'Dsl1 complex in a very specific manner.** Mutant strains were obtained by tetrad analysis after crossing the original TAP-tagged strains (Ghaemmaghami *et al.*, 2003) with mutants having the genetic background of BY4742. Extracts were prepared and processed as described in *Materials and Methods*. Samples marked 'extracts' represent 1/10 the of the total input of the pull-down experiment and were applied to the same gel as the pull-down fractions displayed above. Lanes marked 'supernatants' contain amounts of the acetone-precipitated supernatant equivalent to the pull-down fractions were loaded. **(A)** (lane 1-12) Mutant and wild type cells were shifted to the restrictive temperature of 35°C one hour before harvesting of cells. The strains used were the wild type strains (Ghaemmaghami *et al.*, 2003) and mutants strains derived from these strains by tetrad analysis ('D3T-d1', 'D3T-t20/5', 'D3T-s20', 'D1T-d3', D1T-t20/5', D1T-s20', T20T-d1', T20T-d1N', and 'T20Ts20'; Table 1). (lane 5-12) These immunoblots were probed with both anti-Use1p and anti-Dsl3p antibodies. (lane 13-14) *DSL3* and *dsl3-1* mutant cells carrying a chromosomal deletion of *DSL3* and expressing wild type or mutant alleles from centromeric vectors (pBR12 or pRS315-*dsl3-1*) were analyzed (strain 'D1T-d3' or 'T20-d3'). Cells were grown at 25° and not shifted to the restrictive temperature to avoid degradation of Use1p during growth. In independent experiments we proved that Use1p is not degraded during the incubation of extracts with IgG Sepharose (Supplemental Figure S7). **(B)** Control experiments to prove the validity of the approach. (lane 1-10) The *sec18-1*, *sec22-3* and *ret2-1* ( $\delta$ -COP) mutation do not reduce the interaction of Use1p with members of the 'Dsl1p complex'. Cells were shifted to the restrictive temperature of 35°C or 37°C for one hour as indicated below the panels. (lane 11-15) After the incubation with IgG Sepharose the supernatants of these samples were analyzed to rule out the possibility the Use1p becomes unstable during the incubation step. **(C)** The interaction of Ufe1p with TAP-tagged Dsl3p, Dsl1p and Tip20p expressed in wild type and mutant cells was analyzed as described above. To prevent unspecific binding the ratio of extract to Sepharose beads was raised as described in *Materials and Methods*. As mentioned above, *dsl3-1* mutant cells were kept at room temperature and not shifted to the restrictive temperature before harvesting. A control strain expressing an unrelated TAP-tagged protein (YOR164cp, strain YSC1178-7502847) was included to prove that Ufe1p did not bind unspecifically to IgG Sepharose (see *Materials and Methods*). Lane 1-5 and 11-14 were analyzed in parallel. The different migration rates of TAP-tagged proteins may be due to the use of gradient gels for the experiments used in Figure 6C. The used were 'D3T-d1', 'D3T-t20/5', 'D3T-s20', 'D1T-d3', D1T-t20/5', D1T-s20', T20T-d1', T20T-d3', and 'T20Ts20' (Table 1).

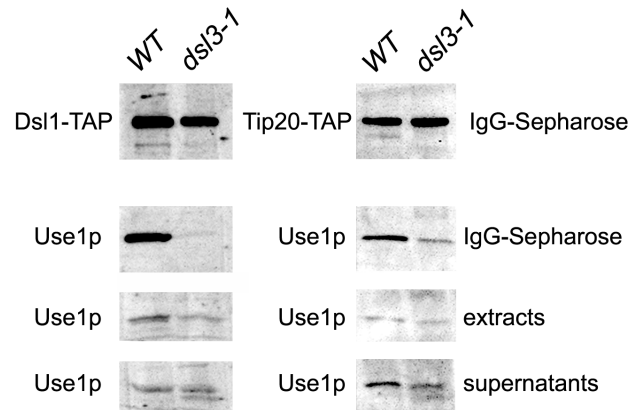
## Kraynack et al., Supplemental Figure S6



**Binding of myc-tagged Sec20p to the ‘Dsl1p complex’ resembles that of Ufe1p.**

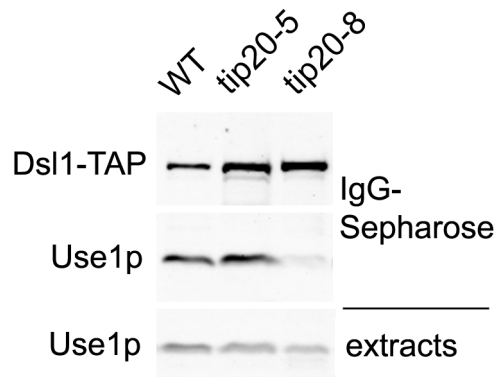
(A) Interaction of Sec20p-myc with TAP-tagged Dsl1p, Dsl3p and Tip20p expressed in mutant cells and analyzed as described in Figure 6. Sec20p-myc expressing mutant and wild type strains were created by crosses followed by tetrad analysis using a strain carrying a myc-tagged version of *SEC20* integrated at the *URA3* locus (strain ‘D1T-WT-Sm’, ‘D1T-t20/5-Sm’, ‘D1T-d3-Sm’, ‘D3T-WT-Sm’, ‘D3T-d1-Sm’, ‘D3T-t20/5-Sm’, ‘T20T-WT-Sm’, ‘T20T-d1-Sm’, ‘T20T-d3-Sm’, ‘T20T-d1N-Sm’, and ‘T20T-d1N-Sm’). All mutants except *dsl3-1* were shifted to the restrictive temperature of 35°C one hour before harvesting. Note that O-glycosylation of Sec20p is responsible for high apparent molecular weight of Sec20p fusion proteins and the diffuse appearance of the Sec20p bands. (B) Co-purification of Ufe1p and Ufe1p to TAP-tagged Sec20p from extracts of wild type and mutant cells as described in Figure S5.

## Kraynack et al., Supplemental Figure S7



**Use1p in extracts from *ds13-1* mutants is stable during the incubation with IgG-Sepharose.** The binding experiments were performed in small volumes (200  $\mu$ l). Aliquots of extracts and supernatants were loaded without prior acetone precipitation. Amounts loaded were: extracts 12.5%, supernatant 7%.

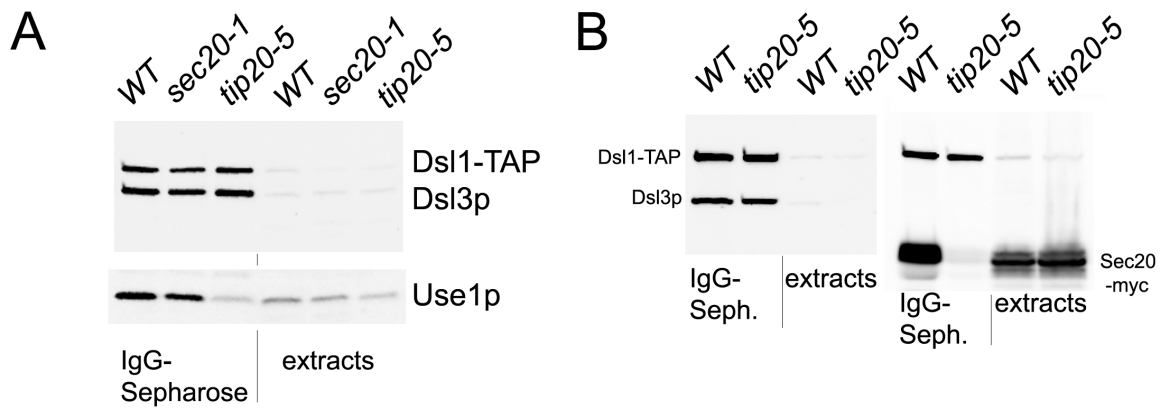
## Kraynack et al., Supplemental Figure S8



**Comparison of the effect of the *tip20-5* and *tip20-8* mutations at permissive temperature.** Binding of Use1p to Dsl1-TAP was analyzed by a pull-down experiment performed as described in legend of Figure S5. The cells were not shifted to the restrictive temperature.

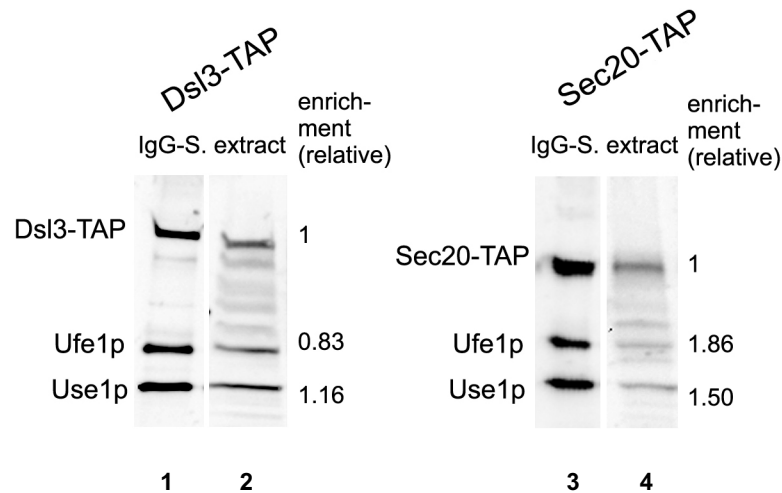


# Kraynack et al., Supplemental Figure S9



**The *tip20-5* mutation does not affect the interaction between Dsl1p and Dsl3p.** Experiments were performed as described in legend of Figure S5 and S6. Use1p and Sec20-myc binding was analyzed as a control.

## Kraynack et al., Supplemental Figure S10



**Comparable fractions of Ufe1p and Use1p bind to TAP-tagged Dsl3p or Sec20p as determined by a single step affinity purification.** Immunoblots from pull-down experiments with Dsl3-TAP and Sec20-TAP producing wild type cells were first incubated with anti-Ufe1p antibody followed by an incubation with anti-Use1p antibody. (The experiments are comparable to those shown in Supplemental Figure S5C, lane 2, and S6B, lane 1 and 6). The 'extract' lanes represent 10% of the input. We determined the relative enrichment of the proteins to avoid problems with the strongly varying immunoreactivity of proteins in the extract containing lanes (compare lane 2 and 4). The ratio of TAP-tagged protein in the IgG Sepharose fraction/extract fraction was set to 1. The results suggest that Use1p and Ufe1p bind equally efficient to TAP-tagged Dsl3p as well as TAP-tagged Sec20p. This would also suggest that the relatively low amount of Ufe1p compared to Use1p in tandem affinity preparations (Figure 6) may be due to loss of Ufe1p during the lengthy preparation (7 hours) or a lower labeling efficiency of Ufe1p.