



**Figure S1. Localization of vacuolar SNAREs in retromer mutants.** (a-c) Localization of GFP-tagged SNARE proteins was investigated in wild-type and *vps35Δ* cells. (a) Chromosomal *VT11* was N-terminally tagged with GFP under control of the intermediate *PHO5* promoter. (b,c) *GFP-PEP12* and *GFP-NYV1* under control of the *TPII* promoter were expressed from a centromeric plasmids pCU2701 (Pep12) and pCU2702 (Nyv1). Size bar, 5  $\mu$ m.

**Table S1. *Saccharomyces cerevisiae* strains used in this study.**

Strain	Genotype	Reference
BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	EUROSCARF library
BY4727	MATalpha <i>his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0</i>	EUROSCARF library
BJ3505	MATa <i>pep4Δ::HIS3 prb1-Δ1.6R lys2-208 trp1Δ101 ura3-52 gal2</i>	Haas <i>et al.</i> , 1995
DKY6281	MATalpha <i>leu2-3 leu2-112 ura3-52 his3-Δ200 trp1-Δ101 lys2-801 suc2-Δ9 PHO8::TRP1</i>	Haas <i>et al.</i> , 1995
SEY6210	MATalpha <i>leu2-3,122 ura3-52 his3-Δ200 trp-Δ901 lys2-801 suc2-Δ GAL1</i>	Robinson <i>et al.</i> , 1988
CUY4411	BY4727; <i>VPS17::GFP-kanMX4</i>	Balderhaar <i>et al.</i> , 2010
CUY4412	BY4727; <i>VPS26::GFP-kanMX4</i>	Balderhaar <i>et al.</i> , 2010
CUY4571	BY4727; <i>VPS10::GFP-kanMX6</i>	Balderhaar <i>et al.</i> , 2010
CUY4573	BY4741; <i>vps26Δ::kanMX6 VPS10::GFP-HIS3</i>	Markgraf <i>et al.</i> , 2009
CUY6133	BY4727; <i>VPS35::yeGFP-hphNT1</i>	This study
CUY6597	BJ3505; <i>vps35Δ::hphNT1</i>	This study
CUY6599	BJ3505; <i>vps17Δ::hphNT1</i>	This study
CUY7569	BJ3505; <i>vps26Δ::kanMX6</i>	This study
CUY7573	BJ3505; <i>vps17Δ::hphNT1 vps5Δ::kanMX6</i>	This study
CUY7575	BJ3505; <i>vps5Δ::kanMX6</i>	This study
CUY7837	BY4727; <i>VPS29::GFP-TRP1</i>	This study
CUY7848	SEY6210; <i>VPS26::3xmCherry-hphNT1</i>	This study
CUY9003	BY4741; <i>VPS10::natNT1-GAL1pr VPS10::GFP-HIS3 vps26Δ::kanMX6</i>	This study
CUY9004	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6</i>	This study
CUY9062	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vac8Δ::hphNT1</i>	This study
CUY9063	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vps5Δ::hphNT1</i>	This study
CUY9064	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 VPS35::mCherry-TRP1</i>	This study
CUY9065	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 ypt7Δ::hphNT1</i>	This study
CUY9204	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 YPT7::URA3-PHO5pr-mCherry</i>	This study
CUY9209	BY4741; <i>VMA2::yeGFP-hphNT1</i>	This study
CUY9212	BY4741; <i>VMA2::yeGFP-hphNT1 vps35Δ::kanMX6</i>	This study
CUY9213	BY4727; <i>VPS10::GFP-kanMX6 ypt7Δ::hphNT1</i>	This study
CUY9221	BY4741; <i>VAM3::URA3-PHO5pr-GFP</i>	This study
CUY9223	BY4727; <i>VPS10::natNT1-GALSpr VPS10::GFP-kanMX6</i>	This study
CUY9330	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 VPS1::mCherry-TRP1</i>	This study
CUY9331	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 YPT6::URA3-PHO5pr-mCherry</i>	This study
CUY9332	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 mon1Δ::LEU2</i>	This study
CUY9346	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vps1Δ::hphNT1</i>	This study
CUY9347	BJ3505; <i>vps1Δ::hphNT1</i>	This study
CUY9348	BY4727; <i>VPS10::GFP-kanMX6 ypt7Δ::hphNT1 vps26Δ::TRP1</i>	This study
CUY9399	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 VPS8-3xmCherry-hphNT1</i>	This study
CUY9400	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 apl5Δ::hphNT1</i>	This study
CUY9411	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 myp1Δ::TRP1</i>	This study
CUY9417	BY4727; <i>VPS26::GFP-kanMX4 vps1Δ::natNT1</i>	This study
CUY9418	BY4727; <i>VPS35::yeGFP-hphNT1 vps1Δ::natNT1</i>	This study

CUY9498	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vac7Δ::TRP1</i>	This study
CUY9499	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 erv14Δ::TRP1</i>	This study
CUY9500	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 btn2Δ::TRP1</i>	This study
CUY9501	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 btn1Δ::TRP1</i>	This study
CUY9502	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 btn3Δ::TRP1</i>	This study
CUY9511	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 SEC7::mCherry-hphNT1</i>	This study
CUY9563	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 VPS17::mCherry-TRP1</i>	This study
CUY9569	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vps29Δ::hphNT1</i>	This study
CUY9570	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 vps35Δ::TRP1</i>	This study
CUY9576	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 msb3Δ::TRP1</i>	This study
CUY9577	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 gyp7Δ::TRP1</i>	This study
CUY9581	BY4727; <i>VPS10::natNT1-GAL1pr VPS10::GFP-kanMX6 msb4Δ::TRP1</i>	This study
CUY9623	BY4727; <i>VPS17::GFP-kanMX4 vps1Δ::TRP1</i>	This study
CUY9624	BY4741; <i>VAM3::URA3-PHO5pr-GFP vps1Δ::hphNT1</i>	This study
CUY9705	BY4727; <i>VPS10::GFP-kanMX6 mvp1Δ::TRP1</i>	This study
CUY9708	BY4727; <i>VPS10ΔC(1-1490aa)::GFP-TRP1</i>	This study
CUY9709	BY4727; <i>VPS10::natNT1-GAL1pr VPS10ΔC(1-1490aa)::GFP-TRP1</i>	This study

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