Defined Subunit Arrangement and Rab Interactions Are Required for Functionality of the HOPS Tethering Complex

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Within the endomembrane system of eukaryotic cells, multisubunit tethering complexes together with their corresponding Rab-GTPases coordinate vesicle tethering and fusion. Here, we present evidence that two homologous hexameric tethering complexes, the endosomal CORVET (Class C core vacuole/endosome transport) and the vacuolar HOPS (homotypic vacuole fusion and protein sorting) complex, have similar subunit topologies. Both complexes contain two Rab-binding proteins at one end, and the Sec1/Munc18-like Vps33 at the opposite side, suggesting a model on membrane bridging via Rab-GTP and SNARE binding. In agreement, HOPS activity can be reconstituted using purified subcomplexes containing the Rab and Vps33 module, but requires all six subunits for activity. At the center of HOPS and CORVET, the class C proteins Vps11 and Vps18 connect the two parts, and Vps11 binds both HOPS Vps39 and CORVET Vps3 via the same binding site. As HOPS Vps39 is also found at endosomes, our data suggest that these tethering complexes follow defined but distinct assembly pathways, and may undergo transition by simple subunit interchange.

Key words: CORVET, HOPS, Rab-GTPase, tethering, vacuole, Vps39, Vps41

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The endomembrane system of eukaryotic cells relies on the constant flux of proteins and lipids between different organelles. Fusion of vesicles with their target membranes or homotypic fusion of organelles is governed by highly specific processes. The initial and reversible contact of fusion-destined membrane compartments is mediated by membrane tethering. Generally, this process is carried out by large tethering factors acting together with specific Rab-GTPases, and results in irreversible docking of the opposing membranes (1,2). Membrane-anchored SNAREs then interact in trans to drive bilayer fusion, which is promoted by the tethering factors (3–6).

The endolysosomal system contains two homologous tethering complexes, the endosomal CORVET (659 kDa) and the vacuolar HOPS (633 kDa) complex, which both consist of six subunits, each present in one copy within the complex (7–9). Four of these are present in both complexes, namely the class C Vps proteins Vps11, Vps16, Vps18 and Vps33. Vps11 and Vps18 carry C-terminal RING domains, indispensable for function (10), whereas Vps33 is homologous to Sec1/Munc18 (SM) proteins and might bind SNAREs (11–13). Not surprisingly, the deletion of either class C protein results in massive vacuole fragmentation (10,14). In addition to these four subunits, the CORVET complex contains Vps3 and Vps8, and the HOPS complex the paralogous Vps39/Vam6 and Vps41/Vam2 subunits. Both complexes are Rab effectors (i.e. they bind to Rab proteins in their GTP-containing form). CORVET interacts via Vps8 with the endosomal Vps21 (8,15) and HOPS binds via Vps41 to the vacuolar Rab Ypt7 (7,16, this study). In addition, Vps3 seems to interact specifically with GDP-Vps21 (8), and Vps39 was reported to act as a guanine nucleotide exchange factor for Ypt7 (17). However, recent findings show that Vps39 does not alter Ypt7 activation in vivo (18), suggesting that tether function may be separate from Rab activation.

HOPS and CORVET not only share the class C core but can also form a chimeric complex containing both CORVET and HOPS subunits [i.e. Vps3-Vps41-class C; (8)]. Although previous data suggested some interactions within the complex, the topology of HOPS and CORVET (or the intermediate complex) had not been analyzed in detail. Here, we dissect the composition of both complexes by analyzing deletion mutants. Using overexpression strains, we purified HOPS and the identified subcomplexes, and used them as biochemical tools. Based on Rab interaction and vacuole fusion studies, we show that HOPS depends on all six subunits for activity. We thus derive a model on how HOPS and CORVET can bridge membranes during tethering, and may interconvert by subunit exchange during endosome maturation.
Figure 1: Legend on next page.
Results

Subunit interactions within the HOPS complex reveal the central role of Vps11 and Vps18

HOPS and CORVET are multisubunit complexes, which promote Rab- and SNARE-dependent fusion. We reasoned that insights into the function of the hexamer would only be obtained if we could determine the topological arrangement of the subunits by focusing initially on the HOPS complex. We therefore explored the interactions by analyzing the composition of the complex under different conditions. To purify the complex, we had established a robust protocol to isolate HOPS from yeast (8). The complex with one C-terminally tandem affinity purification (TAP)-tagged subunit is isolated via immunoglobulin G (IgG) beads and further resolved via gel filtration after Tobacco etch virus (TEV) cleavage of the tag. As shown in Figure 1A, the HOPS complex was purified as an apparent heterohexamer of ~700 kD in fractions 10 and 11 using the Vps41 subunit as a bait. We then analyzed, using the same protocol, whether stable subcomplexes would appear upon deletion of single HOPS subunits. When we deleted vps33, we could isolate a pentameric HOPS (Figure 1B), whereas deletion of vps16 yielded a tetramer of Vps41, Vps39, Vps11 and Vps18 (Figure 1C). This indicates that Vps33 is attached to the HOPS complex via Vps16. Moreover, Vps16 and Vps33 could also be detected as a subcomplex, when isolated from a vps18Δ strain, consistent with their peripheral association with HOPS (Figure 1D), and in agreement with previous findings (13).

For the integrity of the HOPS complex, the class C subunits Vps11 and Vps18 seem to have a critical role. Deletion of vps11 caused a complete loss of HOPS assembly, resulting in just Vps41 (Figure 1E) and Vps18 (Figure 1F) in our purifications. Obviously, Vps11 is at the center of the HOPS complex and indispensable for its integrity. In addition to the Vps16-33 subcomplex, we also identified a second subcomplex from vps18Δ cells, consisting of Vps39 and Vps11 (Figure 1G). We noticed that subcomplexes containing Vps18 or Vps11 did not elute at their expected molecular weight. For now, we ascribe this to a potential multimerization via their exposed C-terminal RING domains, if the proteins are not part of the hexameric tethering complex (19).

We then asked if we could obtain the same subcomplexes or the entire HOPS complex by sequentially overproducing the individual subunits in yeast. As shown in Figure 1H, overexpression of specific combinations of HOPS subunits under the strong, inducible GAL1 promoter revealed the same stable 1:1 subcomplexes of Vps39–Vps11 and Vps16–Vps33 (Figure 1H, lanes 2 and 3). We wondered if we could sequentially assemble the HOPS complex, starting from the Vps39-11 subcomplex. The additional co-overexpression with Vps18 or Vps18–Vps41 resulted in a stoichiometric, but detectable association (lanes 4 and 5) of these two subunits. As we never detected any direct interaction between Vps41 and Vps39 upon co-overexpression, we exclude at this point a contribution of Vps39 to Vps41 binding to the complex. However, Vps41 binds to Vps18 in a yeast two-hybrid assay (Figure 1J), but could not be coisolated with Vps18 upon co-overexpression (Figure 1J, lane 2). This suggests that Vps41 requires at least Vps11 and Vps18 for its association with the HOPS complex, and further binding partners seem to promote the stability of this interaction (see below).

As Vps16 is the binding partner for Vps33 in the HOPS complex (Figure 1B,C), we reasoned that the co-overexpression of additional Vps16 should result in a pentameric complex. Surprisingly, Vps16 only bound to other HOPS proteins in the presence of overproduced Vps33, leading to a fully assembled complex, which then contained similar amounts of all six subunits (Figure 1H, lane 7). Within this assembled complex, Vps16 and Vps33 appear to stabilize the interactions of Vps18 and Vps41. Our combined analysis of the HOPS complex is consistent with the assembled topological arrangement of the HOPS

Figure 1: Analysis of the HOPS topology. A–G) Deletion analysis reveals insight into HOPS topology. A) HOPS purification via TAP-tagged Vps41. Isolation of the complexes was carried out via TAP purification and subsequent gel filtration (for details, see Materials and Methods). Fractions from the gel filtration were TCA precipitated, resolved on gradient SDS–PAGE gels and stained with Coomassie. The identity of the bands was confirmed by mass spectrometry and western blotting (Figures 1 and 2). Asterisks indicate copurified proteins (exemplified in A for all purifications), which most likely interacted with the bead matrix and were all identified as chaperones. CbP, calmodulin-binding peptide, which is left on the TAP-tagged protein after TEV cleavage. The strain background is indicated to the right together with a model of the obtained subcomplexes. Models of the HOPS are shown to the right. Lost or deleted subunits are indicated in gray. B–D) Identification of the Vps16–Vps33 subcomplex on HOPS. Isolation of Vps41 from vps33A (B) and vps16Δ (C) cells. Isolation of Vps33 from vps18Δ cells (D). E and F) Vps11 and Vps18 are critical for the holocomplex assembly. Isolation of non-assembled Vps41 (E) and Vps18 (F) from vps11A cells. G) Identification of the Vps39–Vps11 and Vps3–Vps11 subcomplexes. Isolation of Vps11 from vps18Δ cells. Note that Vps39 and Vps3 comigrate in the upper band. H) Purification of co-overexpressed subcomplexes from yeast. Mini-scale TAPs were performed (see Materials and Methods), and the resulting EGTA eluates were analyzed by SDS–PAGE and Coomassie staining. Note that the yields of the subcomplexes differ for each isolation, and were adjusted to show the relative abundance of each component in the complex. HOPS subunits were overexpressed and tagged with a TAP tag (+T) as indicated. In some experiments, overproduced Vps18 carried a hemagglutinin (HA) tag (+HA). I) Co-overexpression of Vps41 and Vps18. The experiment was performed as in (H), using a strain with the indicated subunits under the control of the GAL1 promoter. The abbreviation ‘+’ indicates the TAP-tagged subunit used in the pull-down assay. J) Yeast two-hybrid analysis of HOPS subunit interactions. Growth on the QDO selection plates indicates interactions between the proteins. Note that Vps16-33 interactions were not detected here (DDO, double dropout selection plates). See Materials and Methods for details.
complex, where Vps11 and Vps18 at the center connect Vps39 and Vps41 at one end to Vps16 and Vps33 at the opposite side of the complex.

**HOPS and CORVET compositions differ upon deletion of the effector subunit**

Our observations suggested that Vps41 as one of the Ypt7-interacting proteins might have a central role in HOPS assembly. We therefore asked how the HOPS complex would form in the absence of Vps41. Surprisingly, a deletion in vps41 resulted in a similar Vps39–Vps11 complex as observed before, here also associated with some Vps18 (Figure 2A). This is not because of the use of Vps39 as the bait protein, as we could purify the entire HOPS complex efficiently via Vps39 from wild-type cells (Figure 2B). Thus, Vps41 is a crucial subunit for HOPS assembly.

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**Figure 2: HOPS and CORVET composition upon deletion of the effector subunits.** A) Loss of HOPS Vps41 results in the accumulation of a Vps39–Vps11 subcomplex. Isolation of Vps39 from vps41Δ (A) and wild-type cells (B). Models of the HOPS and CORVET are shown to the right. Lost or deleted subunits are indicated in gray. C) CORVET assembly is independent of the HOPS complex integrity. Isolation of Vps11 from vps41Δ cells. Note that Vps11 will also copurify Vps39. In addition, Vps3 as part of the CORVET is lost from the complex during gel filtration as described before (8). Purification of the CORVET from wild-type cells has been shown before (8). D) The hybrid complex of Vps41-Vps3-class C can be isolated via Vps41 from vps39Δ cells. As in (C), Vps3 is lost during gel filtration and appears in fractions 13 and 14. E and F) Loss of CORVET subunits results in hexamer and pentamer complexes. E) Isolation of the Vps41-Vps3-class C hybrid via Vps3 from vps8Δ cells, similar to previous findings (8). F) Isolation of the Vps8-class C pentamer from vps3Δ cells. Vps39 was not detected in the eluate (not shown). G) Yeast two-hybrid analysis of Vps8 interactions within the CORVET. Growth on the QDO selection plates indicates interactions. H) Co-overexpression of all class C subunits results in two dimeric complexes. Co-overexpression and isolation were performed as in Figure 1H.
We wondered whether integrity of the CORVET complex would also be affected if the closely related HOPS fails to assemble. Here, we used Vps11 as the bait protein in vps41Δ cells, which under these conditions would purify both Vps39 (Figure 2A) and the entire CORVET complex, if assembled. Indeed, we obtained the CORVET complex, and Vps39, which comigrates with Vps3 (Figure 2C). Some of the missing Vps3 in lane 11 was recovered in fraction 13/14, as Vps3 easily dissociates from the CORVET complex during gel filtration (8) (Figure 2C). We have also previously shown that the deletion of vps39 results in a chimeric complex, in which Vps3 takes the position of Vps39, thereby generating a Vps3–Vps41 hybrid complex (Figure 2D) (8). A similar hybrid complex can also be obtained, if the CORVET is purified from vps8Δ cells (Figure 2E), consistent with our previous analysis (8). However, in the absence of Vps3, we did not observe the incorporation of the homologous Vps39 into the complex, but rather a pentameric CORVET complex, consistent with the stability of the remaining CORVET on gel-filtration columns after loss of Vps3. This indicates that CORVET follows a different assembly pathway than HOPS. Similar to Vps41, Vps8 also binds Vps18 in yeast two-hybrid assays, but also shows interactions with Vps11 and itself (Figure 2G), potentially because of interactions of the C-terminal RING domains (19). It is therefore likely that the HOPS and CORVET complex have a similar topological arrangement. As HOPS and CORVET both share the class C proteins, we finally asked if the class C proteins assemble into an independent unit if co-overexpressed, but did only obtain the just identified dimeric Vps16–Vps33 complex (Figure 2H, lane 1) and some substoichiometric Vps11–Vps18 complex (lane 2), dependent on the bait protein used for pull down.

In summary, both tethering complexes appear to have similar intermolecular interactions, but different fates if paralogous subunits are deleted. Our data show that Vps8 is not as critical for CORVET assembly as Vps41 is for HOPS assembly. At the center of each complex are the class C proteins, which depend on the CORVET- and HOPS-specific subunits to assemble into one complex. All these data strongly argue against the class C subunits forming an independent core complex. Of the class C proteins, Vps11 is at the center of the HOPS complex and indispensable for its integrity, even more than Vps41. Our data also indicate that CORVET can assemble independently of the HOPS complex. However, if assembly fails, the HOPS complex disassembled into at least two subcomplexes: Vps16–Vps33 and Vps39–Vps11 (with some Vps18).

**HOPS activity depends on Vps39 and can be reconstituted from subcomplexes**

Having determined the topological arrangement of the HOPS and CORVET complex, we asked if we could determine the activity of the complexes and its subcomplexes. For now, we focused on the activity of the HOPS complex, as no in vitro assay is yet available to analyze CORVET function. For this, we purified HOPS and the indicated subcomplexes via the TAP protocol as outlined in Materials and Methods, and used them as biochemical tools. The biological activity of the HOPS complex can be tested by a fusion assay with vacuoles from vps11-1 ts cells, which depend on supplemented HOPS for activity (20). When added to these vacuoles, our purified HOPS complex rescued fusion activity, but neither one of the purified proteins or subcomplexes did so (Figure 3A,B). This indicated that only the fully assembled HOPS complex can be active in fusion.

Besides the CORVET complex, we also identified an intermediate complex from cells lacking Vps39, consisting of Vps41–Vps3-class C. We therefore asked if this complex, which lacks Vps39, might still rescue fusion. The complex was co-overproduced and purified as a hexamer from yeast and showed the same stoichiometry as the HOPS complex (Figure 3D). We then compared its activity to the HOPS complex and could rescue fusion of vps11-1 vacuoles up to 25% (Figure 3C). This suggests that Vps39 is required for full activity of the complex, and cannot be compensated for by Vps3.

We finally tested if fusion activity could be obtained by reconstituting the HOPS complex from the identified subcomplexes. We used two subcomplexes, Vps16–Vps33 and Vps11–Vps18–Vps39–Vps41. Within the latter complex, Vps41 was substoichiometric, but we reasoned that reconstitution with two complexes would reduce the assembly options. As shown before, neither complex alone can rescue activity if titrated into the fusion assay (Figure 3A,B). However, we reproducibly obtained a partial rescue (about 30% compared to HOPS) in fusion activity if the two complexes were added together at the same concentration as the HOPS complex to the fusion reaction (Figure 3E). We therefore conclude that the assembled hexameric HOPS complex can be reconstituted from two subcomplexes and that the complete hexamer is required for optimal fusion activity.

**Crosstalk of Vps41 and Vps39 with the Rab-GTPase Ypt7**

The HOPS tethering complex has been implicated as a SNARE chaperone and Rab-interacting complex (7,12,21). The HOPS subunits Vps39 (17) and Vps41 can interact with Ypt7 (16). Our in vivo studies also pointed to Vps41 as the Ypt7 effector subunit of the HOPS (22). However, it was not clear, which nucleotide form of Ypt7 would bind Vps41 or Vps39, or if the nucleotide specificity of either protein would depend on its incorporation into the HOPS complex, or whether additional Rab-binding proteins would be present in the complex.

To determine which subunits of the HOPS complex would bind to Ypt7 in its respective GTP- or GDP-loaded state, we used a pull-down approach with glutathione S-transferase (GST)-tagged Rab-GTPases preloaded with the indicated nucleotide. With purified Vps39, we detected specific
binding to Ypt7, but not Vps21 (Figure 4A). This interaction was independent of the nucleotide status (Figure 4A), but specific for the Rab-GTPase Ypt7 as revealed by yeast two hybrid (Y2H) (Figure 4B) (17). As a control, Vps16 and Vps33 did not show any specific interaction (Figure 4C,D). In contrast, Vps41 bound specifically only to Ypt7-GTPyS (Figure 4E). Previous yeast two-hybrid studies had identified the N-terminal 500 amino acid residues of Vps41 as the Ypt7-binding site (16). Indeed, with both recombinantly produced full-length Vps41 or N-terminal portions of Vps41 (1-484 or 1-503), but not the C-terminal portion (504-992), we detected specific binding to Ypt7-GTPyS (Figure 4F). We therefore conclude that the HOPS contains two Rab-binding proteins: Vps41 is the effector subunit, whereas Vps39 binds Ypt7 independent of the nucleotide.

Does the reported interaction of the vacuolar Rab Ypt7 with the HOPS complex reflect identical interactions as observed for Vps39 and Vps41? Intriguingly, we detected strong HOPS interaction with the GTP form of Ypt7 and a weaker interaction with the nucleotide-free form, regardless of whether we purified HOPS via Vps39 (Figure 4G) or Vps41 (Figure 4H), comparable to isolated Vps41 (Figure 4E). We wondered why no interaction with the GDP-bound form was detectable, as was observed for...
Vps39 (Figure 4A). We reasoned that Vps39 might have lost its capability to bind Ypt7 in the assembled HOPS complex. Indeed, binding of the Vps39–Vps11 (Figure 4I) or the Vps39-11-18 intermediate (Figure 4J) to any form of Ypt7 was reduced to background levels.

If Vps41 is the main Ypt7-binding site from within the HOPS complex, it should be maintained if Vps39 is missing. We therefore tested the purified Vps41-Vps3-class C complex (Figure 3D; 8) in the Rab pull-down assay, and observed specific binding only to Ypt7-GTP (Figure 4K), in agreement with this proposal. Our data therefore indicate that Vps39 may interact with Ypt7 only outside the HOPS complex, presumably before the complete assembly.

**Colocalization of HOPS subunits as an indicator of interactions**

Our data thus far indicate that Vps39 is required for HOPS activity, but binds Ypt7 only prior to its integration into the HOPS complex. We therefore wondered if we may trace Vps39 and its interaction partner Vps11 to obtain evidence for its connection to Ypt7 in vivo. Although the Vps39–Vps11 subcomplex does not bind Ypt7 in vitro, we reasoned that this analysis may nevertheless provide insight into their subcellular localization, and potentially, the site of HOPS assembly. As deletion of Vps39, Ypt7 or Vps11 leads to a massive fragmentation of the vacuole, we decided to monitor the co-overexpressed subunits, using one green fluorescent protein (GFP) or red fluorescence protein (RFP)-tagged subunits as a marker.

In wild-type cells, Vps11 localizes to endosomal membranes and to the vacuole (23). Upon overexpression, the surplus Vps11 (visualized by an RFP tag) was found primarily in the cytoplasm (Figure 5A). This situation remained unchanged upon co-overexpression of Vps41 (Figure 5D). In contrast, dot-like structures became predominant upon co-overexpression of Vps39 (Figure 5B). Both Vps11 and Vps39 colocalize in these structures (Figure 5C), and as they could be transiently stained with FM4-64, they most likely represent endocytic structures (Figure 5J). Similarly, the localization of overexpressed Vps18-RFP was cytosolic (Figure 5E–G), and only concentrated into dot-like structures upon co-overexpression of Vps11 and Vps39 (Figure 5H) or Vps11, Vps39 and Vps41 (Figure 5I). Thus, Vps39 and Vps11 accumulate with Vps18 in endocytic structures, in agreement with their observed interaction by co-overexpression or in vps41Δ cells (Figures 1H and 2A). As the excess Vps39 and Vps11 likely interfered with HOPS assembly, we asked if cargo sorting to the vacuole would be affected. Indeed, these cells accumulated some endocytic cargo, like Cps1 in these structures (Figure 5K), and secreted some carboxypeptidase Y (CPY), a soluble hydrolase normally sorted to the vacuole lumen (Figure 5L). However, cells had, under those conditions, a functional AP-3 (adapter protein complex 3) pathway, which targets proteins directly from the Golgi to the vacuole (not shown).

As Vps39 binds to Ypt7 (Figure 4A) (17), we followed GFP-tagged Vps21 and Ypt7 upon Vps39 (and Vps11) overexpression. While GFP-Vps21 localization was unaffected by Vps39 (Figure 5M), GFP-Ypt7 strongly accumulated on membranes (Figure 5N), indicating a cellular crosstalk between Ypt7 and Vps39 (and Vps11).

Taken together, these data indicate that Vps39 is able to strongly recruit Vps11 to intracellular membranes, and the Vps11–Vps39 subcomplex recruits Vps18, substantiating the results from the deletion and overexpression experiments. Moreover, our data indicate that Vps39 relocalizes Ypt7 to endosomal membranes, suggesting that the endosome serves as an assembly site for the HOPS complex in vivo.

**A common binding motif for (HOPS-)Vps39 and (CORVET-)Vps3 at the Vps11 C-terminal region**

Next, we analyzed if we could identify a critical interacting region for Vps39 and Vps11 assembly that may provide insight into the composition or the transition of CORVET into HOPS. Previous yeast two-hybrid studies had suggested that Vps39 and Vps11 interact via their C-terminal regions (17). To address this more directly, we co-overexpressed and purified Vps39, Vps11 or truncated variants of either one. For Vps11, the interaction site required residues 890 to 927 (Figure 6A), and for Vps39 residues 901 to 974 (Figure 6B).

The CORVET subunit Vps3 can displace Vps39 from the HOPS complex (8), suggesting that it may bind to Vps11 as well. We indeed observed an interaction of Vps11 and Vps3 by yeast two-hybrid analysis, and also identified the Vps3 C-terminal region (residues 758-980) as the Vps11-binding site (Figure 6C). More specifically, the Vps11-binding site relies upon Vps3 residues 967-986 (Figure 6E), whereas Vps3 binding is supported by the same region in Vps39 (residues 890-927, Figure 6D), which is also needed for Vps39 binding. Indeed, Vps39 residues 870 to 977 and Vps3 residues 901 to 1011 show the highest extent of identity in sequence alignment (Figure 6F), and these sequences correspond to the regions identified for the interaction with Vps11 (Figure 6B,E). The binding of Vps11 via the same binding site (residues 890-927) to two subunits of the homologous tethering complexes can explain why Vps3 can compete with Vps39 in vivo.

**Discussion**

Since its identification, the physiological function of the HOPS complex has been extended from a Rab effector to a GTP exchange factor (GEF) and SNARE chaperone (3,7,13,17,24), though detailed knowledge of each of these activities is still scarce. Here, we provide novel insights into the protein–protein interactions within the HOPS and CORVET complexes and link them to their respective Rab-GTPases, focusing primarily on the stable...
HOPS complex. Within this study, we provide several important new insights.

(i) Both tethering complexes have similar subunit arrangements (Figure 7A). Our data show that Vps41 and Vps39 are likely situated at one end of the HOPS complex, Vps16 and Vps33 at the opposite end. Vps11 as a central subunit is required with Vps18 as a linker (Figure 7A). In the CORVET complex, Vps8 and Vps3 replace Vps41 and Vps39 (8), and likely have a similar arrangement like HOPS. This arrangement can explain how a tethering complex can bridge two membranes by binding the Rab-GTPase and SNAREs via opposite ends. This model is consistent with recent in vitro observations of HOPS-dependent
liposome fusion (25). Presumably, CORVET and HOPS both use Vps33 as the SNARE interaction site, but may require additional binding sites to decode the right SNARE combination. Potentially, post-translational modifications in HOPS or CORVET are necessary for this distinction.

(ii) As shown for the CORVET complex, HOPS contains two Rab-binding proteins. Vps41 is the effector subunit of the HOPS complex, which binds Ypt7-GTP (Figure 4), whereas the Ypt7-interaction site of Vps39 seems to be quenched once it is incorporated into the complex (Figure 4). Likewise, CORVET has two Rab-binding proteins: one provided by the Vps41 paralog Vps8, the effector subunit of the CORVET complex (15), and one by Vps3, which seems to bind to Vps21-GDP or -GTP, depending on the conditions (8) (F. A., C. U., unpublished data). Presently, it is not clear how the Vps39 or Vps3 binding specificity for each Rab contributes to Rab recruitment, tether assembly or function. However, the different fate of each tethering complexes upon vps3 or vps39 deletion also suggests functional differences between the two proteins. Future experiments will need to clarify these issues.

(iii) Both the CORVET and HOPS complex rely on the class C proteins as an assembly platform. Our data show a central role of Vps11, which binds both CORVET Vps3 and HOPS Vps39 via the same binding site (Figure 6). In agreement with this observation, Vps3 overexpression displaces Vps39 from the HOPS complex and results in a class C–Vps41–Vps3 complex (8). Moreover, if Vps11 is missing, the HOPS complex (and likely also the CORVET complex) disassembles (Figure 1), resulting in a massive fragmentation of vacuoles (10). However, the class C proteins only assemble into one complex in the presence of the additional CORVET and/or HOPS subunits (Figures 1 and 2). This suggests that CORVET and HOPS assembly follows a defined order, and occurs potentially only at membranes. At least in vitro, two HOPS subcomplexes can combine to promote fusion (Figure 3). Importantly, our deletion analyses suggest that this order differs for CORVET and HOPS as deletions in the effector subunit have different outcomes: CORVET converts into the Vps41–Vps3-class hybrid, HOPS into subcomplexes, one of which is the identified Vps39–Vps11–Vps18 (Figure 2A), another presumably Vps16–Vps33 (Figure 1D). Importantly, deletion of the second Rab-binding protein (discussed below) has also different outcomes for HOPS and CORVET. If HOPS Vps39 is missing, Vps3 fills the gap, resulting in the Vps41–Vps3-class hybrid, whereas a pentameric Vps8–class C complex has been isolated from vpsΔ cells. Although suggested previously (8,9), our current data are not in agreement with a symmetric replacement of HOPS and CORVET subunits. We consider it likely that the observations of our deletion analyses relate to the assembly and turnover at membranes, a task for future studies.

Previous data suggested that HOPS may combine Rab activation (via Vps39; (17)) and Rab-binding activities (as shown now via Vps41) into one protein complex (7). Similar ideas were developed for CORVET (8,9). Indeed, the HOPS subunit Vps39 is a Ypt7-binding protein (17) (Figure 4), as long as it is not part of any complex (Figure 4), and seems to localize to endosomes (Figure 5) or endosome–vacuole contact sites (22). Vps39 also promotes Ypt7 accumulation at endosomal sites (Figure 5), suggesting that its function on Ypt7 may precede its role as a member of the HOPS complex. However, Vps39 is not the long-assumed GEF subunit, but seems to cooperate with the Ypt7 GEF, which we recently identified (Nordmann et al., submitted). Thus, Vps39 may cooperate with the GEF to couple Ypt7 activation to HOPS assembly/binding at endosomes. This consideration agrees with recent findings on Vps39 in metazoan cells (18). Similar to Vps39, CORVET Vps3 is also a Rab-binding protein (15), and purified Vps3 binds Vps21-GTP (F. A., C. U., unpublished data). Following the initial GEF idea, we tested for GEF activity of Vps39 for Vps21, but did not detect any activity (F. A., D. M., C. U., unpublished observations). Indeed, the well-characterized Vps9/Rabex5 protein may be the only Vps21-GEF (26,27). Thus, we postulate that Rab activation, and CORVET- and HOPS-mediated tethering function, resides in different protein (complexes).

Figure 4: HOPS contains two Rab-binding proteins. A and B) Vps39 interacts with Ypt7 in a nucleotide-independent manner. A) Rab pull down with Vps39. GST-Rab proteins were loaded with the respective nucleotide, bound to GSH beads and used for the pull-down experiment. Vps39 was purified from a VPS39-TAP overexpression strain using the same TAP purification method as for Figure 1H. Bound proteins were eluted, analyzed by SDS–PAGE and western blotting using anti-CbP antibodies or Coomassie staining (to visualize Rab-GTPases). All subsequent Rab pull-down experiments (C–J) were performed in the same way, using TEV-cleaved eluates or lysates after a 20 000 × g spin (K) from the respective overexpression or wild-type (lysate) strains, and were performed at least 3 × with similar results. B) Yeast two-hybrid analysis of the interaction of Vps39 with Rab wild-type proteins and different Rab mutants, which are locked in either nucleotide state (T22N and S21N GDP-locked; G68L and G66L GTP-locked). DDO, double dropout medium corresponding to SDC-ura-leu; QDO, quadruple dropout medium corresponding to SDC ura-leu-his-ade. C and D) Interaction of purified Vps16 or Vps33 via TAP purification reveals no Rab interaction. Rab pull downs were performed as in (A), using the indicated purified proteins isolated from a strain that overexpressed protein(s) listed in the header. Eluates were blotted and decorated against the TAP tag. The TAP-tagged protein was Vps16 (C) and Vps33 (D). See Materials and Methods for details. E and F) Vps41 binds Ypt7-GTP. Rab pull downs were performed as before using purified Vps41 from yeast (E) or Vps41 full-length or fragments recombinantly expressed in E. coli (F). G and H) HOPS preferentially binds Ypt7-GTP. Rab pull downs were performed with the purified complexes as described in Figure 1H and in the TAP protocol. K) Binding of the Vps41-3–class C complex to Ypt7. Lysate from the vps39 Δ strain, in which Vps41-TAP is part of the intermediate complex (8), shown in blot, was incubated with the indicated Rabs. Interactions of the intermediate complex with the Rabs were quantified (right, n = 3).
Figure 5: Codependence of Vps39, Vps11 and Vps18 localization in cells.

A–D) Vps11 localization is affected by Vps39. Strains with an RFP-tagged version of Vps11 under the control of the GAL1 promoter were grown in YPG medium and analyzed for Vps11 localization using fluorescence microscopy. Overexpression of RFP-Vps11 alone (A) or with Vps39 (B), GFP-Vps39 (C) or Vps41 (D). E–I) Vps18 clusters upon co-overexpression with Vps11 and Vps39. Localization of overproduced Vps18-RFP (E), or upon co-overexpression with Vps11 (F), Vps39 (G), Vps11 and Vps39 (H), or Vps11, Vps39 and Vps41 (I). Note that vacuole rings are only observed in (I). J) Colocalization of Vps39-GFP with the endocytic dye FM4-64. Cells co-overexpressing Vps11 and Vps39-GFP were stained with FM4-64, and visualized by fluorescence microscopy. K) Cargo sorting is affected by the co-overexpression of Vps11 and Vps39. Vps11-RFP was co-overexpressed with Vps39, and GFP-tagged Cps1 was followed. In several cells, GFP-Cps1 was observed in dots, which colocalized with Vps11-RFP, presumably resembling endosomes. Despite apparent delay, correct luminal sorting was unaffected as GFP-Cps1 appeared in the vacuole lumen (not shown). L) CPY secretion from cells co-overexpressing the indicated subunits. Cells were grown on YPD to repress protein expression or YPG to induce overexpression, and patched onto nitrocellulose. Secreted CPY was then analyzed by western blotting (19). M and N) Localization of GFP-tagged Rabs Vps21 and Ypt7 upon (co-)overexpression of Vps11 and Vps39. Cells were stained with FM4-64 as described and then analyzed by fluorescence microscopy. Expression levels of GFP-Ypt7 are shown below part (N). Note that Vps39 overexpression leads to partial vacuole fragmentation in cells with GFP-tagged Ypt7. Similar observations have been obtained before when the interaction of Vps41 with membranes was impaired (22).

In combination, our data thus show important new insights into the tether organization and function at the endosome and vacuole. The elucidation of how Rab activation is coordinated with tether assembly/function during endolysosomal transport and endosomal maturation will be a major focus of future studies.

Materials and Methods

Yeast strains
Details on all strains (Table S1) are given in Supporting Information.

Tandem affinity purification
TAP was performed as described (28) using the following buffer: 50 mM HEPES/KOH, pH 7.4, 300 mM NaCl, 0.15% NP-40 (Igepal CA-630; Sigma-Aldrich) and 2 mM MgCl₂. Gel-filtration experiments of TEV protease eluates were performed as before (8) using a 10 × 300 mm Superose 6 analytical grade column at 0.3 mL/min in the same buffer.

Tandem affinity purification (mini-scale)
This method is based on the TAP protocol (29). In brief, yeast cell lysates were prepared from 500 optical density (OD)₆₀₀ equivalents of cells by thoroughly vortexing cells in lysis buffer [50 mM HEPES/KOH, pH 7.4, 300 mM NaCl, 0.15% NP-40 (Igepal CA-630; Sigma-Aldrich), 2 mM MgCl₂, 2 mM DTT] and 2 mg/ml lysozyme. Cell debris was removed by centrifugation at 10,000 g for 10 min.
Figure 6: Identification of the interaction site between Vps11 and its binding partners Vps3 and Vps39. A and B) Mapping of the Vps39–Vps11 interaction site. Mini-TAP purifications of co-overexpression strains were performed as before using either TAP-tagged Vps11 or Vps39 as the bait. Samples were analyzed by SDS–PAGE and coomassie staining. Expression levels were assessed by western blot using the same strain background with an additional TAP tag on the bait protein, to obtain comparable staining of bands by decoration with anti-TAP antibody (Open Biosystems). C) Yeast two-hybrid analysis of the Vps3–Vps11 interaction. Left: Interaction of Vps3 with Vps11 and Vps18. Right: Fragments of the Vps3 sequence were analyzed for interactions with full-length Vps11. D and E) Mapping of the Vps3–Vps11 interaction site was performed as in (A and B). F) Sequence alignment of C-terminal fragments of Vps39 and Vps3. An alignment of the full-length sequences of the two proteins using the LALIGN algorithm (http://www.ch.embnet.org/software/LALIGN_form.html) yielded the depicted fragment alignment with the indicated sequence identity. Other parts of the protein sequences were omitted by LALIGN due to the standard threshold settings of the algorithm. G) Model of the interaction sites in Vps11, Vps3 and Vps39.
Membrane binding via Rab-GTP

CORVET

HOPS

Rab binding and effector complex integrator

SNARE interaction

Membrane binding via SNAREs

Figure 7: Model of CORVET and HOPS topology and function. CORVET and HOPS have three distinct modules: (i) the Rab module; Vps41 and Vps8 are Rab effectors, Vps39 and Vps3 are Rab-binding proteins; (ii) the integration module of Vps11 and Vps18 and (iii) the SNARE interaction module of the Sec1-like protein Vps33. Both complexes may bridge membranes by binding Rab-GTP and SNAREs on opposite ends of the complex.

1 mM DTT, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 1xFY protease inhibitor mix (Serva) together with glass beads in a Disrupter Genie for 2 × 10 min, followed by a centrifugation at 20 000 × g at 4 °C. The supernatant was centrifuged for 60 min at 100 000 × g and the cleared lysate loaded onto 25 μL of prewashed IgG beads. After 1 h of incubation at 4 °C, the beads were washed 3 × with 1 mL lysis buffer containing 0.5 mM DTT, but lacking protease inhibitors. Bound proteins were eluted by TEV protease treatment for 1 h at 16 °C. TEV eluates were either analyzed by SDS–PAGE and Coomassie staining or loaded onto 25 μL of prewashed calmodulin–Sepharose beads, and incubated for 1 h at 4 °C. The beads were subsequently washed 3 × with 1 mL lysis buffer, bound proteins were eluted by incubation with 20 mM EGTA in lysis buffer for 20 min at 30 °C, and analyzed by SDS–PAGE and Coomassie staining.

**Vacuole fusion assay**

Vacuoles were purified from the tester strains BJ3505 (pep4Δ1) and DKY8281 (pho8Δ1) or vps17-A1 temperature-sensitive (ts) tester strains (CSY9 and CSY10; kindly provided by W. Wickner) as described (20). Fusion reactions containing 3 μg of each vacuole type were performed in fusion reaction buffer (10 mM PIPES/KOH, pH 6.8, 5 mM MgCl2, 125 mM KCl, 0.2 mM sorbitol), containing 80 mM purified Vam7 and an ATP-regenerating system. Reactions were incubated for 90 min at 26 °C, and then developed as described (30). TEV eluates from standard TAPs (see Materials and Methods) were used to supplement the indicated (sub)complexes.

**Microscopy**

For GFP/RFP microscopy, yeast cells were grown to mid-log phase in YPD (yeast peptone dextrose) medium, YPG (yeast peptone galactose) medium for overexpression of the indicated gene or selective medium, collected by centrifugation, washed once with PBS and immediately analyzed by fluorescence microscopy. Staining of the cells with FM4-64 was performed as described (31). In brief, cells were incubated with 30 μM FM4-64 for 30 min, washed with the corresponding medium and chased for 1.5 h in fresh medium. Images were acquired with a Leica DM5500 B microscope equipped with a SPOT Pursuit camera using GFP, RFP, FM4-64 and DIC (differential interference contrast) filters. Pictures were processed using Adobe Photoshop CS3.

**Yeast two-hybrid analysis**

Y2H assays were carried out as described (31). Combinations of pACT2 and pB T9-Y2H vectors carrying the DNA sequence of the indicated proteins were transformed into the yeast strain PJ69-4A and plated onto synthetic media lacking leucine and tryptophane (double dropout, DDO). Transformants were successively transferred first onto medium lacking leucine, tryptophane, histidine and adenine (quadruple dropout, QDO) and afterwards onto DDO medium containing 2% glucose. For each Y2H vector combination, four clones were analyzed. An interaction between tested proteins results in the capability to grow on QDO medium.

**Acknowledgments**

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Table S1:** Strains used in this paper.

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CUY2725  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::natNT2-GAL1pr VPS39::TAP-TRP1
this study

CUY2836  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS16pr::natNT2-GAL1pr VPS18pr::kanMX-GAL1pr-3HA VPS11::TAP-Ura3
this study

CUY2837  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS16pr::natNT2-GAL1pr VPS18pr::kanMX-GAL1-3HA VPS18::TAP-Ura3
this study

CUY2838  MATalpha his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 VPS33pr::TRP1-GAL1pr VPS33::TAP-Ura3
this study

CUY2916  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS3::KanMX-GAL
this study

CUY2989  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS16pr::natNT2-GAL1pr VPS18pr::kanMX-GAL1pr-3HA VPS33::TAP-Ura3
this study

CUY3021  MATalpha his3Δ200/met15Δ0 trp1Δ63 ura3Δ0/ura3Δ0 lys2Δ0 leu2Δ0 VPS33pr::TRP1-GAL1pr VPS11pr::HIS3-GAL1pr VPS16pr::natNT2-GAL1pr VPS18pr::kanMX-GAL1pr-3HA VPS11::TAP-Ura3
this study
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this study

CUY3364  MATa/α his3Δ200/his3Δ200 leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63 ura3Δ0/ura3Δ0 VPS41pr::TRP1-GAL1pr VPS39pr::kanMX-GAL1pr VPS11pr::HIS3-GAL1Pr VPS18pr::kanMX-GAL1Pr-3HA VPS11::TAP-URA3

this study

CUY3396  MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS11::HIS3-GAL VPS39::natNT2-GAL1pr VPS18::TRP1-GAL1

this study

CUY3407  MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS41pr::TRP1-GAL1pr

this study

CUY3408  MATa his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS39pr::kanMX-GAL1Pr VPS41::URA3-PHO5-GFP

this study

CUY3491  MATα his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS11pr::kanMX-GAL1Pr VPS11::TAP-URA3

this study

CUY3645  MATa/α his3Δ200/his3Δ200 leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1Pr VPS16pr::natNT2-GAL1Pr VPS18pr::kanMX-GAL1Pr-3HA VPS41pr::TRP1-GAL1pr VPS39pr::kanMX-GAL1pr VPS33pr::HIS3-GAL1pr VPS39::TAP-URA3

this study

CUY3742  MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPS39::GFP-HIS3 VPS39pr::kanMX-GAL1pr

this study

CUY3789  MATa/α his3Δ200/his3Δ200 leu2Δ0/leu20 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS18::kanMX-GAL1pr VPS39::GFP-TRP1

this study

CUY3834  MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS18::kanMX-GAL1-3HA VPS18::TAP-URA3

this study

CUY3916  MATa/α his3Δ200/his3Δ200 leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS33pr::TRP1-GAL1pr VPS16pr::kanMX-GAL1Pr-3HA

this study

CUY4256  MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL VPS11::MARS-KanMX

this study
CUY4264  MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met1Δ0/met1Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS39::KanMX- Gal1Pr VPS41::URA3-PHO5-GFP VPS11pr::HIS3-GAL VPS11::MARS-KanMX this study

CUY4265  MATa/alpha his3Δ200/his3Δ200 leu2Δ0 leu2Δ0 lys2Δ0 met1Δ0/met1Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS39::KanMX- Gal1Pr VPS39::GFP-TRP1 VPS11pr::HIS3-GAL VPS11::MARS-KanMX this study

CUY4291  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11::TAP-Ura3 this study

CUY4292  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11(Δ928-1029)::TAP-URA3 this study

CUY4293  MATalpha his3Δ200 leu2Δ0 lys2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11(Δ891-1029)::TAP-URA3 this study

CUY4294  MATa his3Δ1 leu2Δ0 met1Δ0 ura3Δ0 VPS3::kanMX-GAL1pr VPS3::TAP-URA3 this study

CUY4295  MATa his3Δ1 leu2Δ0 met1Δ0 ura3Δ0 VPS3::kanMX-GAL1pr VPS3(Δ987-1011)::TAP-URA3 this study

CUY4296  MATa his3Δ1 leu2Δ0 met1Δ0 ura3Δ0 VPS3pr::kanMX-GAL1pr VPS3(Δ968-1011)::TAP-URA3 this study

CUY4297  MATa his3Δ200 leu2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS39pr::KanMX- Gal1Pr VPS39::TAP-URA3 this study

CUY4298  MATa his3Δ200 leu2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS39pr::KanMX- Gal1Pr VPS39(Δ1019-1049)::TAP-URA3 this study

CUY4299  MATa his3Δ200 leu2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS39pr::KanMX- Gal1Pr VPS39(Δ975-1049)::TAP-URA3 this study

CUY4300  MATa his3Δ200 leu2Δ0 met1Δ0 trp1Δ63 ura3Δ0 VPS39pr::KanMX- Gal1Pr VPS39(Δ902-1049)::TAP-URA3 this study

CUY4301  MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met1Δ0/met1Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11::TAP-Ura3 VPS39pr::KanMX- Gal1Pr VPS39::TAP-URA3 this study
CUY4302 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-Gal1Pr this study

CUY4303 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11(Δ928-1029)::TAP-URA3 VPS39pr::KanMX-Gal1Pr VPS39::TAP-URA3 this study

CUY4304 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11(Δ891-1029)::TAP-URA3 VPS39pr::KanMX-Gal1Pr this study

CUY4305 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-Gal1Pr VPS39::TAP-URA3 this study

CUY4306 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-Gal1Pr this study

CUY4307 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-Gal1Pr VPS39::TAP-URA3 this study

CUY4308 MATa/alpha his3Δ1/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-GAL1pr VPS3::TAP-URA3 this study

CUY4309 MATa/alpha his3Δ1/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX-GAL1pr this study

CUY4311 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX- Gal1Pr VPS39(Δ1019-1049)::TAP-URA3 this study

CUY4312 MATa/alpha his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 VPS11pr::HIS3-GAL1pr VPS39pr::KanMX- Gal1Pr VPS39(Δ975-1049)::TAP-URA3 this study
CUY4324 MATa/alpha his3Δ1/His3Δ200 leu2Δ0/Leu2Δ0 lys2Δ0/met15Δ0/Met15Δ0 trp1Δ63/Trp1Δ63 ura3Δ0/Ura3Δ0 VPS11pr::HIS3-GAL1pr VPS3pr::kanMX-GAL1pr VPS3(Δ987-1011)::TAP-URA3 this study

CUY4325 MATa/alpha his3Δ1/His3Δ200 leu2Δ0/Leu2Δ0 lys2Δ0/met15Δ0/Met15Δ0 trp1Δ63/Trp1Δ63 ura3Δ0/Ura3Δ0 VPS11pr::HIS3-GAL1pr VPS11::TAP-Ura3 VPS3pr::kanMX-GAL1pr VPS3(Δ968-1011)::TAP-URA3 this study

CUY4326 MATa/alpha his3Δ1/His3Δ200 leu2Δ0/Leu2Δ0 lys2Δ0/met15Δ0/Met15Δ0 trp1Δ63/Trp1Δ63 ura3Δ0/Ura3Δ0 VPS11pr::HIS3-GAL1pr VPS3pr::kanMX-GAL1pr VPS3(Δ968-1011)::TAP-URA3 this study

CUY4327 MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VPS39::TAP-URA3 VPS16::ProtA-KanMX this study

CUY4328 MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VPS39::TAP-URA3 VPS16::TEV-ProtA-KanMX this study

CUY4329 MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VPS39::TAP-URA3 VPS33::ProtA-KanMX this study

CUY4330 MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VPS39::TAP-URA3 VPS33::TEV-ProtA-KanMX this study

CUY4806 MATa his3Δ200 leu2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VAM6::KanMX-Gal1Pr YPT7::TRP1-PHO5-GFP this study

CUY4807 MATa his3Δ200 leu2Δ0 met15Δ0 trp1Δ63/ura3Δ0 VPS11::KanMX-GAL1Pr YPT7::TRP1-PHO5pr-GFP this study