

Regulation of membrane scission in yeast endocytosis

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Abstract

Introduction

Clathrin-mediated endocytosis (CME) is an endocytic process by which cargo from the cell exterior is incorporated into a Clathrin-coated vesicle that is then transported into the cell (*Bitsikas et al., 2014*). Over 50 different proteins are involved in reshaping a flat plasma membrane into an invagination that eventually separates from the plasma membrane to form a vesicle (*Kaksonen and Roux, 2018*). In yeast, CME is the only pathway for uptake of cargo, and involves a similar membrane transformation as in other eukaryotes. Most mammalian CME proteins have homologues in yeast; these proteins establish endocytic sites and form the mechanical link between membrane and actin proteins (*Kaksonen and Roux, 2018*). Actin proteins nucleate and polymerize a branched actin network, producing the forces that form tubular invaginations in yeast (*Kübler et al., 1993; Kaksonen et al., 2003*). Forces that drive the final transition from tubular invagination to spherical vesicle in multicellular eukaryotes are provided by the GTPase Dynamin (*Grigliatti et al., 1973; Sweitzer and Hinshaw, 1998; Ferguson et al., 2007; Takei et al., 1995; Galli et al., 2017*). Dynamin is now known to interact via its proline-rich domain with the SH3 domains of crescent-shaped N-BAR proteins like Endophilin and Amphiphysin (*Grabs et al., 1997; Cestra et al., 1999; Farsad et al., 2001; Ferguson et al., 2009; Meinecke et al., 2013*). Conformation changes of Dynamin recruited to N-BAR molecules cause constriction of the underlying invaginated membrane, resulting in vesicle formation (*Shupliakov et al., 1997; Zhang and Hinshaw, 2001; Zhao et al., 2016*).

Yeast dynamin-like protein Vps1 has a major role in several membrane trafficking pathways (*Rothman et al., 1990; Peters et al., 2004; Hoepfner et al., 2001*), and been proposed to interact with endocytic proteins (*Nannapaneni et al., 2010; Yu and Cai, 2004; Smaczynska-de Rooij et al., 2012*). Others have failed to observe Vps1 at endocytic sites (*Goud Gadila et al., 2017; Kishimoto et al., 2011*), so the role of Vps1 in CME remains debated. In yeast cells, what then causes membrane scission is thus unclear, although some components of a scission mechanism have been identified, like the yeast N-BAR Rvs complex (*Munn et al., 1995; Kaksonen et al., 2005; D'Hondt et al., 2000; Kishimoto et al., 2011*). The Rvs complex (Rvs) is heterodimeric complex of the proteins Rvs161 and Rvs167. The two Rvs proteins are homologues of N-BAR proteins Amphiphysin and Endophilin (*Friesen et al., 2006; Youn et al., 2010*). Deletion of Rvs167 reduces scission efficiency by nearly 30% and reduces the invagination lengths at which scission occurs (*Kaksonen et al., 2005; Kukulski et al., 2012*). Apart from the canonical N-BAR domain which forms a crescent-shaped structure, Rvs167 has a Glycine-Proline-Alanine rich (GPA) region and a C-terminal SH3 domain (*Sivadon et al., 1997*). The GPA region is thought to act as a linker with no other known function, while loss of the SH3 domain affects budding pattern and actin morphology (*Sivadon et al., 1997*). Most Rvs

43 deletion phenotypes can be rescued by expression of the BAR domains alone (*Sivadon et al., 1997*),
44 suggesting that the BAR domains are the functional unit of the Rvs complex.

45 The Rvs complex can tubulate liposomes in vitro, indicating that their BAR domains can impose
46 curvature on membranes (*Youn et al., 2010*). However, Rvs arrives at endocytic sites when mem-
47 brane tubes are already formed: curvature sensing rather than generation is the likely interaction of
48 the complex with endocytic sites (*Kukulski et al., 2012; Picco et al., 2015*). Rvs molecules arrive at
49 endocytic sites a few seconds before scission, and disassemble rapidly at the time of scission (*Picco*
50 *et al., 2015*), consistent with a role in vesicle formation. While it is shown to be involved in the last
51 stages of endocytosis, a mechanistic understanding of the influence of Rvs on scission remains
52 incomplete.

53
54 In this work we test several previously proposed scission models that allow a major role for
55 Rvs. Although the yeast Dynamin Vps1 lacks a canonical BAR-protein binding site (*Bui et al.,*
56 *2012; Moustaq et al., 2016*), it may be recruited via a different mechanism and induce scission.
57 Synaptojanins may selectively hydrolyze lipids at endocytic sites, causing line tension between two
58 lipid types that results in scission (*Liu et al., 2009*). Protein friction generated along the membrane
59 invagination has been proposed as a mechanism by which scission may occur (*Simunovic et al.,*
60 *2017*). We used quantitative live-cell imaging and genetic manipulation in *Saccharomyces cerevisiae* to
61 test these theories and investigate the function of Rvs in endocytosis. We found that Rvs is recruited
62 to endocytic sites by both BAR and SH3 domains. Of several potential actin-interacting binding
63 partners of the SH3 domains such as Myo3, Myo5, Vrp1, Abp1 (*Lila and Drubin, 1997; Colwill et al.,*
64 *1999; Madania et al., 1999; Liu et al., 2009*). we found that type I myosin Myo3 interacts with Rvs
65 SH3 domains. Our data also suggests that the aforementioned theories of membrane scission are
66 unlikely to sever the membrane in yeast, and that the forces required for scission are generated by
67 the actin network.

68 Results

69 **Rvs167, rather than Vps1 influences coat movement**

70 Yeast Dynamin-like protein Vps1 does not contain a proline-rich domain, which in mammalian cells
71 is required for recruitment to endocytic sites (*Grabs et al., 1997; Cestra et al., 1999; Farsad et al.,*
72 *2001; Meinecke et al., 2013*). In spite of the lack of a stereotypical interaction domain, some works
73 have reported its recruitment to endocytic proteins, including to N-BAR protein Rvs167 (*Yu and*
74 *Cai, 2004; Nannapaneni et al., 2010; Rooij et al., 2010*). The question of whether or not Vps1 has a
75 function at endocytic sites has been obfuscated by potential tagging-induced dysfunction of Vps1
76 molecules. Vps1 tagged both N- and C-terminally with GFP constructs failed to co-localize with
77 endocytic protein Abp1 in our hands, consistent with other work that observed localization only
78 with other parts of the trafficking pathway (*Goud Gadila et al., 2017*). We argued that even if tagging
79 Vps1 induced defects in its localization and/or function, its contribution to endocytosis could be
80 examined by observing the dynamics of other endocytic proteins in cells lacking Vps1. The canonical
81 interaction partner of Vps1, Rvs167, localizes to endocytic sites and influences scission efficiency,
82 although its exact role is unclear (*Kukulski et al., 2012; Picco et al., 2015*). In order to determine
83 the roles of these proteins in endocytic scission, we studied cells lacking Vps1 and Rvs167, and
84 compared against wild-type (WT) cells (Fig.1A-C).

85
86 Vps1 deletion was confirmed by sequencing the gene locus, and these cells also showed a previously
87 reported (*Rothman and Stevens, 1986*) growth phenotype at high temperatures (Fig.S1). Scission
88 efficiency was quantified by tracking the endocytic coat protein Sla1 tagged at the C-terminus with
89 eGFP (Fig.1C). Upon actin polymerization, the endocytic coat moves into the cytoplasm along with
90 the membrane as it invaginates (*Skruzny et al., 2012*). Movement of coat protein Sla1 thus acts as a
91 proxy for the growth of the plasma membrane invagination. Membrane retraction, that is, inward

92 movement and subsequent retraction of the invaginated membrane back towards the cell wall is a
 93 scission-specific phenotype (*Kaksonen et al., 2005*). Retraction rates do not significantly increase in
 94 *vps1Δ* cells compared to the WT (Fig.1B).

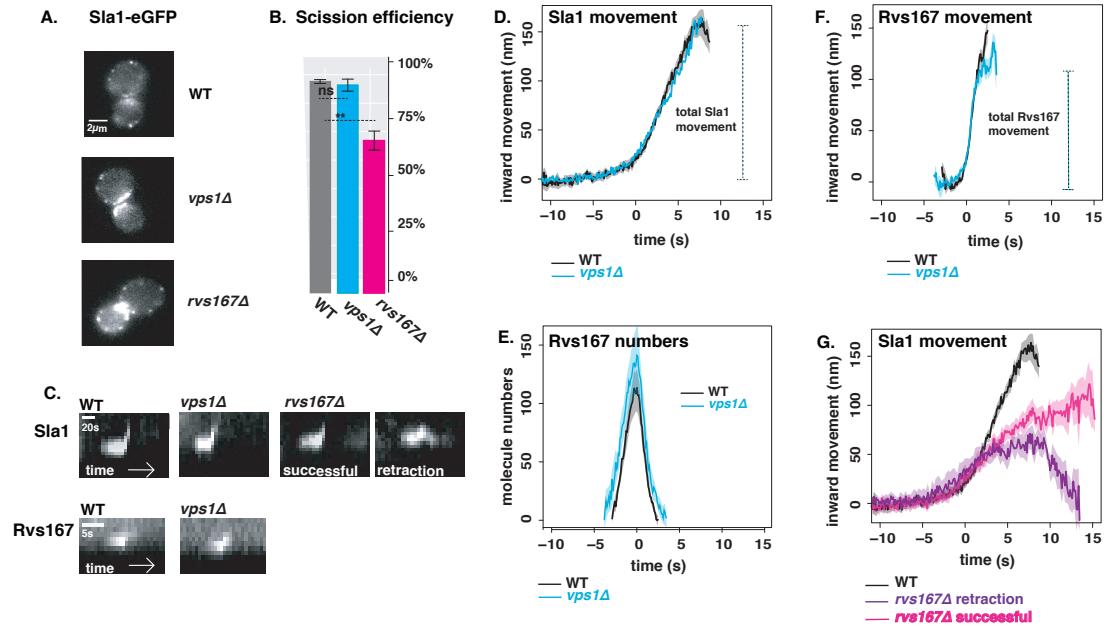


Figure 1. *vps1Δ* and *rvs167Δ* deletion **A:** Single slices from time-lapse movies of WT, *vps1Δ*, and *rvs167Δ* cells expressing Sla1-eGFP. **B:** Scission efficiency in WT, *vps1Δ*, and *rvs167Δ* cells. Error bars are standard deviation, p values from t-test, *= p < 0.05, **= p < 0.01, ***=p<0.001. **C:** Representative kymographs of Sla1-eGFP and Rvs167-eGFP patches in WT, *vps1Δ*, and *rvs167Δ* cells from time-lapse movies. **D:** Averaged centroid positions of Sla1-eGFP in WT and *vps1Δ* cells. **E:** Number of Rvs167 molecules in WT and *vps1Δ* cells. **F:** Averaged centroid positions of Rvs167-eGFP in WT and *vps1Δ* cells. **G:** Averaged centroid positions of Sla1-eGFP in WT, and successful and retracted Sla1-eGFP positions in *rvs167Δ* cells. All averaged positions were aligned in x axis to begin inward movement at time=0(s), and aligned in the y axis to a starting position = 0(nm). Shading on plots show 95% confidence intervals

95
 96 The total movement of the endocytic coat (Fig.1C,D) gives an indication of when invagination has
 97 undergone scission: greater movement would imply defects in the scission mechanism. In order to
 98 study this movement , the averaged centroid trajectory of about 50 Sla1-eGFP patches in *vps1Δ* and
 99 WT cells were tracked and compared (Fig.1D). In brief: yeast cells expressing fluorescently-tagged
 100 endocytic proteins were imaged at the equatorial plane. Since membrane invagination progresses
 101 perpendicularly to the plane of the plasma membrane, proteins that move into the cytoplasm
 102 during invagination do so in the imaging plane. Centroids of Sla1 patches- each patch being an
 103 endocytic site- were tracked in time and averaged. This provided an averaged centroid that could be
 104 followed with high spatial and temporal precision (*Picco et al., 2015*). Averaged centroid movement
 105 of Sla1-eGFP in WT cells was linear to about 140nm (Fig.1D). Sla1 movement in *vps1Δ* cells had the
 106 same magnitude of movement (Fig.1D). In spite of slight differences in the rates of movement, the
 107 total movement- and so the depth of endocytic invagination- did not change.

108
 109 Centroid tracking has shown that the number of molecules of Rvs167 peaks at the time of scission,
 110 and is followed by a rapid loss of fluorescent intensity, simultaneous with a sharp jump of the
 111 centroid into the cytoplasm (Fig.1E,F), (*Picco et al., 2015*). This jump, also seen in Rvs167-GFP
 112 kymographs (Fig.1C), is interpreted as loss of protein on the membrane tube, causing an apparent
 113 spatial jump of the centroid to protein localized at the base of the newly formed vesicle. Number of

114 molecules of Rvs167 recruited to endocytic sites in *vps1Δ* cells is not significantly different from
115 that recruited to WT cells (Fig.1E). Kymographs of Rvs167-GFP (Fig.1C), as well as Rvs167 centroid
116 tracking (Fig.1F) in *vps1Δ* cells showed the same jump as in WT.

117
118 The Rvs complex is composed of Rvs161 and Rvs167 dimers (?) so deletion of Rvs167 effectively
119 removes both proteins from endocytic sites. We quantified the effect of *rvs167Δ* on membrane
120 invagination (Fig.1A-C,G). Only 73% of Sla1 patches undergo successful scission in *rvs167Δ* cells
121 (Fig.1B). Similar scission rates have been measured in other experiments (*Kaksanen et al., 2005*)
122 and suggest failed scission in the remaining 27% of endocytic events. Coat movement both of
123 retractions and of successful endocytic events were quantified (Fig.1G) as described earlier. Sla1
124 centroid movement in both successful and retracting endocytic events in *rvs167Δ* cells look similar
125 to WT up to about 50nm (Fig.1G). In WT cells, Abp1 intensity begins to drop at scission time (Fig.S2).
126 Similarly, in successful endocytic events, Abp1 intensity drops after Sla1 centroid has moved about
127 100nm suggesting that scission occurs at invagination lengths between 60 -100 nm (Fig.S4). That
128 membrane scission occurs at shorter invagination lengths than in WT is corroborated by the smaller
129 vesicles formed in *rvs167Δ* cells by Correlative light and electron microscopy (CLEM) (*Kukulski et al.,
130 2012*). CLEM has moreover shown that Rvs167 localizes to endocytic sites after the invaginations
131 are about 60nm long (*Kukulski et al., 2012*). Sla1 movement in *rvs167Δ* indicates therefore that
132 membrane invagination is unaffected till Rvs is supposed to arrive.

133 **Synaptojanins likely influence vesicle uncoating, but not scission dynamics.**

134 As Vps1 did not appear to influence membrane scission, we proceeded to test another scission
135 model. The lipid hydrolysis model proposes that deletion of yeast synaptojanins would inhibit
136 scission, which would result in longer invaginations (*Liu et al., 2009*). Three Synaptojanin-like
137 proteins have been identified in *S. cerevisiae*: Inp51, Inp52, and Inp53. Inp51-eGFP exhibits a diffuse
138 cytoplasmic signal, Inp52-eGFP localizes to cortical patches that are endocytic sites and Inp53
139 localizes to patches within the cytoplasm (Fig.2A). Since Inp52 localizes to endocytic sites, we began
140 with determining the spatial and temporal recruitment of Inp52 within the endocytic machinery. We
141 aligned the averaged centroid of Inp52 in space and time to other endocytic proteins (*Picco et al.,
142 2015*). In order to do this, we imaged Inp52-eGFP simultaneously with Abp1-mCherry, and did the
143 same with Sla1-eGFP and Rvs167-eGFP. Using Abp1 as the common reference frame, we were able
144 to compare the arrival of the different proteins with respect to that of Abp1. We assigned as time
145 =0 (s) the fluorescent intensity maximum of Abp1, which in WT cells is concomitant with membrane
146 scission, and also coincides with the maximum of the Rvs167 fluorescent intensity (S2). On the
147 y axis, 0 (nm) indicates the position of the Sla1 centroid; positions of the other centroids are in
148 relation to the Sla1 centroid. Inp52 molecules arrived in the late stage of endocytosis after Rvs167
149 arrival, and localized to the invagination tip, suggesting a potential role in membrane scission
150 (Fig.2B).

151 Inp53 was not investigated further, as its localization conformed with other literature that found
152 that it is involved in the golgi trafficking pathway and not endocytosis (*Bensen et al., 2000*). Although
153 we were unable to observe localization of Inp51 at endocytic sites, deletion of Inp51 has been
154 shown to exacerbate the effect of *inp52Δ* on membrane retraction (*Liu et al., 2009*), so both Inp51
155 and Inp52 were tested as potential scission regulators.

156

157

158 Dynamics of Sla1-eGFP and Rvs167-eGFP in *inp51Δ* and *inp52Δ* cells were compared against
159 the WT (Fig.2C-E). Scission efficiency did not significantly decrease in *inp51Δ* compared to the WT,
160 but showed a slight decrease in *inp52Δ* cells (Fig.2C). Total movement of Sla1 and Rvs167 centroids
161 in *inp51Δ* were the same as in WT (Fig.2 D,E), while Rvs167 assembly and disassembly took longer
162 (Fig.S5). Rvs167 centroid in *inp51Δ* cells appeared to persist after movement compared to the WT,
163 likely because of a delay in Rvs167 disassembly from the newly formed vesicle. In *inp52Δ* cells,

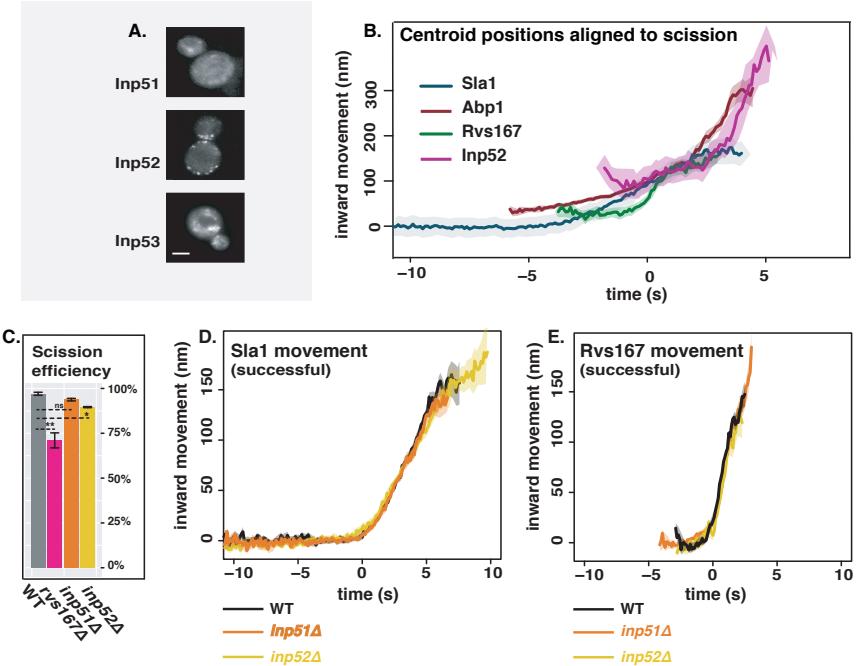


Figure 2. Involvement of yeast Synaptojanin-like proteins in endocytosis **A:** Cells endogenously tagged with Inp51-, Inp52-, and Inp53-eGFP. **B:** Inp52 centroid trajectory was aligned in space and time to other endocytic proteins. **C:** Sla1 retraction rates in *inp51Δ* and *inp52Δ* cells compared to WT and *rvs167Δ*. Error bars are standard deviation, with p values from t-test, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. **D:** Averaged centroid positions of Sla1-eGFP in WT, *inp51Δ*, and *inp52Δ* cells. **E:** Averaged centroid positions of Rvs167-eGFP in WT, *inp51Δ*, and *inp52Δ* cells.

164 Sla1 movement had the same magnitude and rate as in WT, but Sla1-eGFP signal is persistent after
 165 inward movement scission (Fig.2D). Rvs167 and Sla1 disassembly were delayed in *inp52Δ* cells
 166 compared to WT (Fig.S5). This data are consistent with Synaptojanin involvement in assembly and
 167 disassembly of coat and scission proteins at endocytic sites rather than in membrane scission.

168 Rvs BAR domains recognize membrane curvature in-vivo

169 So far Rvs167 remains the protein that has a major influence on scission efficiency and movement
 170 of Sla1. Rvs can tubulate liposomes in vitro (*Youn et al., 2010*), but its interaction with membrane
 171 curvature in vivo has not so far been tested. Recruitment of the Rvs complex to endocytic sites, and
 172 BAR-membrane interaction was thus investigated further. The SH3 domain has known interactions
 173 with proteins within actin network (*Lila and Drubin, 1997; Colwill et al., 1999; Madania et al., 1999;*
 174 *Liu et al., 2009*). We removed the contribution of the SH3 by deleting the domain (Fig.3A) and
 175 observed the localization of Rvs167 $\Delta sh3$ compared to full-length Rvs167. Endogenously tagged
 176 Rvs167-eGFP and Rvs167 $\Delta sh3$ -eGFP colocalization with Abp1-mCherry in WT and *sla2Δ* cells were
 177 compared (Fig.3B). Sla2 acts as the molecular linker between forces exerted by the actin network
 178 and the plasma membrane (*Skrzuny et al., 2012*). *sla2Δ* cells therefore contain a polymerizing
 179 actin network at endocytic patches, but the membrane has no curvature, and endocytosis fails.
 180 In these cells, the full-length Rvs167 co-localizes with Abp1-mCherry, indicating that it is recruited
 181 to endocytic sites independently of membrane curvature (Fig.3B, “*sla2Δ*”). Rvs167 $\Delta sh3$ does not
 182 localize to the plasma membrane except for rare transient patches that do not co-localize with
 183 Abp1-mCherry: Rvs167 $\Delta sh3$ is not recruited to endocytic sites in the absence of curvature in *sla2Δ*
 184 cells.

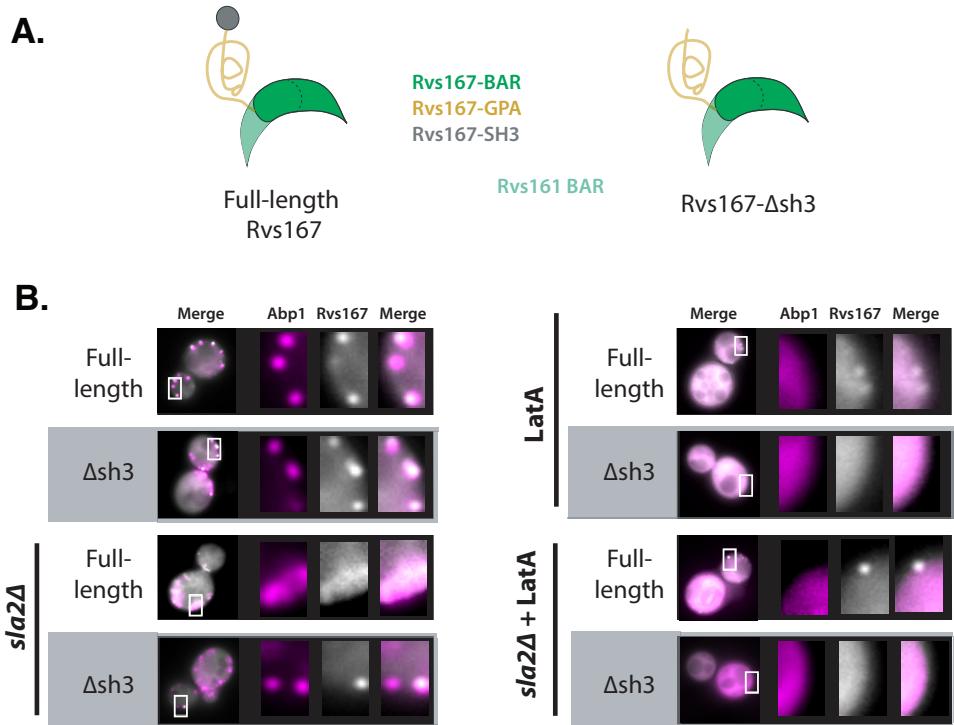


Figure 3. Localization of Rvs167 BAR domain **A:** Schematic of Rvs protein complex with and without the SH3 domain. **B:** Localization of full-length Rvs167 and Rvs167 Δ sh3 in WT, *sla2Δ*, LatA treated, and LatA treated *sla2Δ* cells. Scale bar=2 μ m.

185 Rvs SH3 domains have an actin and curvature independent localisation

186 In order to test if genetic interactions of SH3 domains are prevalent in *in vivo* endocytosis, we
 187 tested the localization of Rvs167 and Rvs167 Δ sh3 in LatA treated cells (Fig.3B, “LatA”). Plasma
 188 membrane localization of Rvs167 remains upon LatA treatment, and transient patches continue to
 189 exist in *sla2Δ* cells treated with LatA (Fig3B, “*sla2Δ+ LatA*”). Rvs167 Δ sh3 does not localize to the
 190 plasma membrane in either case. Thus, localization of full-length Rvs167 in the presence of LatA is
 191 due to the SH3 domain. This indicates that the SH3 domain is able to recruit Rvs molecules to the
 192 plasma membrane in an actin- and curvature-independent manner.

193 SH3 domains are likely recruited by Myosin 3

194 Type I myosins Myo3 and Myo5, and Vrp1 have known genetic and/or physical interactions with
 195 Rvs167 SH3 domains (*Lila and Drubin, 1997; Colwill et al., 1999; Madania et al., 1999; Liu et al., 2009*). We tested the interaction between these proteins and the Rvs167 SH3 region by studying
 196 the localization of full-length Rvs167 in cells with one of the genes for these proteins deleted, and
 197 treated with LatA. By using LatA we expected to reproduce the situation in which BAR-curvature
 198 interaction is removed (Fig.4B). Then, if we lost SH3 interaction because we removed the protein
 199 with which it interacts, we would lose localization of Rvs167 completely. Deletion of neither Vrp1
 200 nor Myo5 in combination with LatA treatment removes the localization of Rvs167. Deletion of
 201 Myo3 with LatA treatment removes localization of Rvs167, indicating that SH3 domains interact at
 202 endocytic sites with Myo3.

A. Rvs167-eGFP

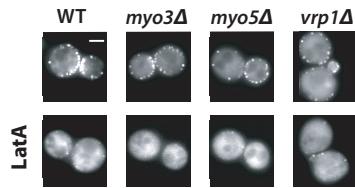


Figure 4. Localization of the SH3 domain Localization of full-length Rvs167-eGFP in WT, *myo3Δ*, *myo5Δ*, and *vrp1Δ* cells. Scale bars=2μm.

204 what about the differences in *myo5* and *myo3* number...

205 **Loss of Rvs167 SH3 domain affects coat and actin dynamics**

206 Since the Rvs167 SH3 domain has an influence on the recruitment of the Rvs complex to endocytic
207 sites, we wondered if the domain also affects later stages of invagination formation endocytic
208 dynamics. We compared dynamics of coat and scission markers in WT and *rvs167Δsh3* cells (Fig.5).
209 Movement of Sla1 centroid is slower and reduced in *rvs167Δsh3* cells compared to WT (Fig4A,B).
210 The movement of Rvs167textitΔsh3 centroid is smaller than that of full-length Rvs167 (Fig.5A,B),
211 consistent with the formation of shorter invaginations suggested by the reduced Sla1 movement in
rvs167Δsh3 cells.

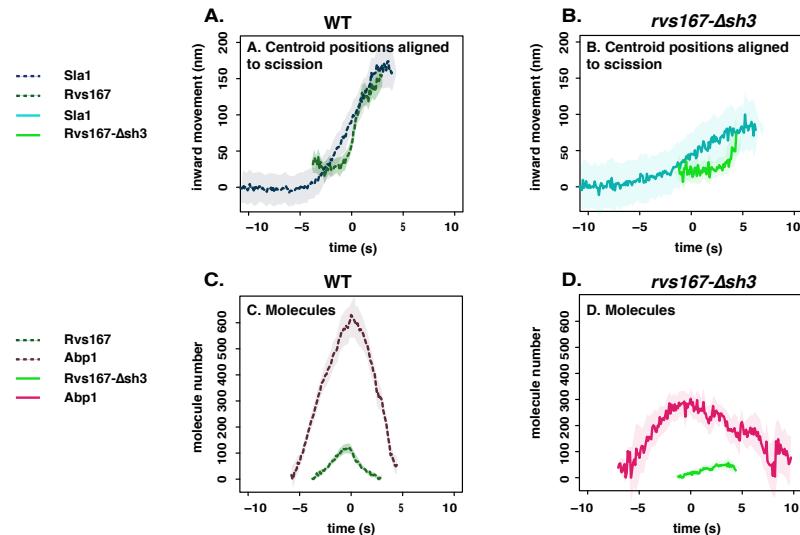


Figure 5. Endocytic dynamics in *rvs167Δsh3* cells **A,B:** Averaged centroid positions aligned in x axis so that time=0(s) is the peak of fluorescent intensity of Abp1 in respective strains. Centroids are aligned in y axis so that Sla1 begins at y=0 (nm), and Rvs167 and Rvs167Δsh3 positions are determined with respect to Sla1 centroids. **C,D:** Numbers of molecules in WT and *rvs167Δsh3* cells, aligned so that time=0(s) is the maximum of fluorescent intensity of Abp1 in the corresponding strains.

212
 213 There is delay in Rvs167Δsh3 recruitment compared to the onset of Abp1 assembly in *rvs167Δsh3*
 214 cells compared to WT (Fig.5 C,D). In WT cells, Rvs167 and Abp1 molecule number peaks are also co-
 215 incident: the actin network begins disassembling as soon as scission occurs (Fig.5C). Asynchronous
 216 peaks in *rvs167Δsh3* cells indicates a disruption in the feedback between actin network dynamics
 217 and membrane scission. Rvs167textitΔsh3 accumulation begins however, when Abp1 molecule
 218 numbers in the mutant are about the same as in WT (about 300 copies, Fig.5C,D). . Both Rvs167
 219 and Rvs167Δsh3 molecules arrive at endocytic sites when the Sla1 centroid is 20-30 nm away from

220 its starting position. This would mean the endocytic coat has moved about 30 nm when both WT
221 and mutant forms of Rvs are recruited. That *Rvs167Δsh3* recruitment anticipates a certain growth
222 of the invagination and amount of Abp1 suggests that the Rvs complex is recruited to a specific
223 geometry of membrane invagination, and that *Rvs167Δsh3* recruitment is delayed because invagi-
224 nations in these cells take longer to acquire this specific geometry. Recruitment of *Rvs167Δsh3* is
225 reduced to half of *Rvs167* (Fig.5C,D), although cytoplasmic concentration of both are similar (Fig.S6).
226 Recruitment therefore is unlikely to be limited by cytoplasmic expression of the mutant protein.
227 Abp1 disassembly is slowed down in *rvs167Δsh3* cells compared to WT, and recruitment is reduced
228 to 50% of WT recruitment (Fig.5C,D), indicating disruption of actin network dynamics.

229 **Increased BAR domain recruitment corresponds to increased membrane move-
230 ment**

231 Since removal of *Rvs167* in *rvs167Δsh3* cells, and the reduced amount of *Rvs167Δsh3* recruited in
232 *rvs167Δsh3* cells results in decreased Sla1 movement, we wondered if Sla1 movement would scale
233 with amount of Rvs recruited to endocytic sites. We titrated the amount of Rvs expressed in cells by
234 endogenously duplicating the *Rvs167* and *Rvs161* genes (Huber et al. 2014) in diploid and haploid
235 yeast cells (Fig.5) . We thus made diploid strains with 4x copies of both the Rvs genes (4xRVS), 2x
236 copies (WT diploid cells, 2xRVS), and 1x copy (1xRVS). Number of molecules of *Rvs167* recruited
237 to endocytic sites increases with gene copy number (Fig5A). “Excess” Rvs recruited to endocytic
238 sites in the 4xRVS case does not change the rate or total movement of Sla1, or of *Rvs167* (Fig.6B,C)
239 compared to the WT (2xRVS). In the case of 1xRVS, Sla1 movement is slightly reduced after 100nm
240 (Fig.6B). Magnitude of *Rvs167* inward movement was similar in all three, but the *Rvs167-eGFP* signal
241 was lost immediately after the inward movement in the 1xRVS case, unlike in the 4xRVS and 2xRVS
242 cases, likely because fewer molecules are recruited (Fig.6A). Unlike in the *rvs167Δsh3* case, Abp1
243 and *Rvs167* peaks were concomitant in all three strains, with similar amounts of Abp1 recruited
244 irrespective of Rvs gene copies (Fig.6D). Thus was there no apparent disruption of the actin network,
245 or of the coupling between scission and actin network disassembly. Adding more Rvs than in the
246 WT diploid case did not lead to differences in Sla1 movement, although reducing the amount of Rvs-
247 as in the 1xRVS case- marginally decreased movement.

248 In haploid cells, we duplicated the full-length *Rvs167* gene, as well as *rvs167Δsh3* gene (Fig5E-H).
249 We thus produced strains with 2x copies of the Rvs genes (2xRVS), 1x copy of each (WT haploid,
250 1xRVS), 2x copies of the *rvs167Δsh3* gene (2xBAR), or 1 copy of *rvs167Δsh3* gene (1xBAR). Amount of
251 WT and mutant *Rvs167* molecules recruited at endocytic sites varied in these strains between 50
252 and 180 copies (Fig5E). Sla1 dynamics remained the same in Rvs duplicated strain (2xRVS) as in the
253 WT (Fig.6F). In the 2xBAR case, the amount of *Rvs167Δsh3* molecules recruited to endocytic sites
254 increased (Fig.6E), as did Sla1 movement, as well the inward jump of *Rvs167* (Fig.6F,G), compared
255 to 1xBAR. Total Abp1 numbers recruited were reduced in 1xBAR (that is *rvs167Δsh3*), compared to
256 the 2xBAR, 1xRVS and 2xRVS (Fig5H). Higher Abp1 numbers corresponds to larger Sla1 centroid
257 movement in both diploid and haploid cells (Fig.6C, D, G, H), suggesting a correlation between the
258 maximum number of Abp1 recruited and total invagination length.

259 **Discussion**

260 Recruitment and function of the Rvs complex has been studied in this work, and several existing
261 models for membrane scission have been tested. We propose that Rvs is recruited to endocytic
262 sites via interactions between the Rvs BAR domains and invaginated membrane, and that SH3
263 mediated protein-protein interactions are required for efficient recruitment of Rvs. We found that
264 arrival of Rvs at the membrane invagination scaffolds the membrane and prevents membrane
265 scission. WT invagination lengths depend on recruitment of a critical number of Rvs molecules.
266 Both timing and recruitment efficiency appear crucial to Rvs function.

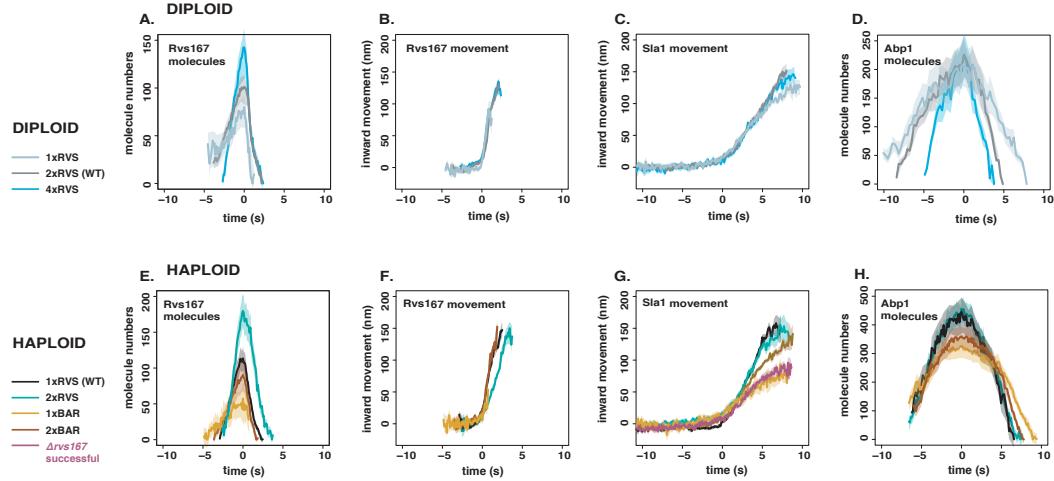


Figure 6. RVS duplication in haploid and diploid cells **A:** Recruitment of Rvs167 in diploid strains with different copy number of Rvs167 and Rvs161 genes. **B:** Rvs167 centroid positions in these strains **C:** Sla1 centroid positions in these strains. **D:** Abp1 molecule numbers in same strains, with only one Abp1 allele tagged. **E,F:** Recruitment and centroid positions of Rvs167 and Rvs167 $\Delta sh3$ in haploid strains. **G:** Sla1 centroid positions in these strains. **H:** Abp1 recruitment in the same strains. All centroid positions were aligned in the time axis so that time=0(s) corresponds to beginning of inward movement of each average centroid. Centroids were aligned in the y axis so that y=0(nm) corresponds to the beginning of the average centroid position

267 **BAR domains sense *in vivo* membrane curvature and time recruitment of Rvs**
 268 The curved structure of Endophilin and Amphiphysin BAR dimers ([Peters et al., 2004; Mim et al., 2012](#))
 269 In the absence of membrane curvature - in *sla2* Δ cells - Rvs167 $\Delta sh3$ domains do not localize
 270 to endocytic sites (Fig.5B). This demonstrates for the first time that the BAR domain senses and
 271 requires membrane curvature to localize to endocytic sites. Rvs167 $\Delta sh3$ has a similar average
 272 lifetime at endocytic sites as full length Rvs167 (Fig.5C,D). However, time alignment with Abp1 shows
 273 that there is a delay in the recruitment of Rvs167 $\Delta sh3$, (Fig.5B). Sla1 moves inwards at a slower rate
 274 in *rvs167* $\Delta sh3$ cells, so it takes longer for the membrane in these cells to reach the same invagination
 275 length as in WT. We propose that Rvs recruitment is timed to specific membrane invagination length-
 276 therefore to a specific membrane curvature- accounting for the delay in recruitment. The timing of
 277 recruitment is therefore provided by the BAR domain.

278 **SH3 domains allow efficient and actin independent recruitment**
 279 Rvs167 $\Delta sh3$ accumulates to about half the WT number (Fig.5C,D), even though the same cyto-
 280 plasmic concentration is measured (Fig.5 supplement), indicating that loss of the SH3 domain
 281 decreases the efficiency of recruitment of Rvs. In *sla2* Δ cells, full-length Rvs167 forms patches
 282 on the membrane (Fig.3B). Since Rvs167 $\Delta sh3$ does not localize to the plasma membrane in *sla2* Δ
 283 cells, localization of the full-length protein must be mediated by the SH3 domain. That full-length
 284 Rvs167 is able to assemble and disassemble at cortical patches in *sla2* Δ cells without the curvature-
 285 dependent interaction of the BAR domain (Fig.3B) indicates that the SH3 domain can mediate
 286 both the recruitment and disassembly of Rvs at endocytic sites. In *sla2* Δ cells treated with LatA
 287 (Fig.3B), both membrane curvature and actin-interacting proteins are removed from endocytic sites.
 288 Full-length Rvs167 in these cells still shows transient localizations at the plasma membrane: the
 289 SH3 domain is able to localise the Rvs complex in an actin and curvature independent manner.

290 **Loss of SH3 domain disrupts endocytic actin network dynamics**
 291 In WT cells, the Abp1 and Rvs167 fluorescent intensities reach maxima concomitantly (Fig.5C,D),
 292 and the consequent decay of both coincide. Coincident disassembly indicates that upon vesicle

293 scission, the actin network is immediately disassembled. Membrane scission occurs around the
294 intensity peak of the two proteins (*Kukulski et al., 2012; Picco et al., 2015*). This coincident peak is
295 lost in bar-gpa cells: Rvs167 Δ sh3 average fluorescent intensity peaks several seconds after Abp1
296 intensity starts to drop, and the decay of Abp1 is prolonged, taking nearly double the time as in WT.
297 Although it is not clear what the decoupling of Abp1 and Rvs167 Δ sh3 peaks means, the changes in
298 Abp1 dynamics suggests a strong disruption of the actin network dynamics.

299 **Rvs acts as a membrane scaffold preventing membrane scission**

300 Invaginations in *rvs167* Δ cells undergo scission when the Sla1 centroid has moved about 80nm
301 (Fig.1F), compared to the WT lengths of 140nm. This shows that enough forces are generated at
302 80nm to cause scission. Since invagination lengths of *rvs167* Δ cells are increased by overexpression
303 of the Rvs167 Δ sh3 domains (Fig.6E-G), we think that localization of Rvs BAR domains to the
304 membrane tube stabilizes the membrane (*Boucrot et al., 2012; Dmitrieff and Nédélec, 2015*) This
305 allows the invagination to grow until actin polymerization produces enough forces to sever the
306 membrane. The requirement for Rvs scaffolding cannot be removed by reducing turgor pressure
307 (Fig.6supplement? or 7?), so the function of the scaffold is not to counter turgor pressure. There is
308 a limit to the stabilization by BAR domains: in diploid strains with 4 copies of each RVS gene, the
309 same amount of actin is recruited before scission. The invagination lengths are the same as in the
310 other strains even though more Rvs is recruited. It is possible that the nature of the Rvs complex
311 interaction with the membrane changes after a certain amount of Rvs is recruited.

312 If enough forces are generated at 80nm, why is scission efficiency decreased in *rvs167* Δ compared to WT?
313 Forces from actin may be at a threshold when the invagination is at 80nm. There
314 could be enough force to sever the membrane, but not enough to sever reliably. The Rvs scaffold
315 then keeps the network growing to accumulate enough actin to reliably cause scission. Controlling
316 membrane tube length could also be a way for the cell to control the size of the vesicles formed,
317 and therefore the amount of cargo packed into the vesicle.

318 **What causes membrane scission?**

319 We looked for changes in the dynamics of Sla1 and Rvs167 that would indicate a scission defect
320 in various mutant strains: longer invaginations than in WT, so Sla1 centroid movements of over
321 140nm, and a bigger inwards jump of Rvs167 centroid, indicating that a longer invagination has
322 been cut. In *vps1* Δ cells, no major changes are seen in Sla1 or Rvs167 dynamics. We conclude that
323 even if Vps1 is recruited to endocytic sites, it is not necessary for Rvs localization or function, and is
324 not necessary for scission.

325 In the lipid hydrolysis model, synaptojanins hydrolyze PIP₂ molecules that are not covered by
326 BAR domains, resulting in a boundary between hydrolyzed and non-hydrolyzed PIP₂. Interfacial
327 forces generated at this lipid boundary causes scission (*Liu et al., 2006*). Deleting synaptojanins
328 Inp51 and Inp52 should increase invagination lengths if scission was driven by lipid hydrolysis.
329 Sla1 and Rvs centroid dynamics shows that deletion of neither Inp51 nor Inp52 result in scission
330 delay. In *inp51* Δ cells, Rvs assembly is slightly slower than that in WT: Inp51 could play a role in
331 Rvs recruitment. In the *inp52* Δ strain, about 12% of Sla1-GFP tracks retract, this could suggest
332 a moderate influence of Inp52 on scission. Rvs centroid persists after scission in *inp52* Δ cells:
333 disassembly of Rvs after scission is delayed. Sla1 signal also persists for longer after scission in the
334 *inp52* Δ than in WT cells, suggesting that post-scission disassembly of proteins from the vesicle is
335 inhibited in *inp52* Δ cells. Inp52 likely plays a role in recycling endocytic proteins from the vesicle to
336 the plasma membrane.

337 A protein-friction model has proposed that BAR domains induce a frictional force on the mem-
338 brane, causing scission (ref!). If more BAR domains were added to the membrane tube at a faster
339 rate, the frictional force generated as the membrane is pulled under it should increase, and the
340 membrane should rupture faster. That is, membrane scission should occur as soon as WT forces
341 are generated on the tube. In Rvs duplicated cells, adding up to 1.6x the WT amount of Rvs at faster

342 rates to membrane tubes does not affect the length at which the membrane undergoes scission
343 (Fig.6E). We think that protein friction does not contribute significantly to membrane scission in
344 yeast endocytosis.

345
346 We observed that the maximum amount of Abp1 measured in all the diploid strains is about 220
347 molecules (Fig.6D). Since only one allele of Abp1 is fluorescently tagged in these strains, the total
348 amount of Abp1 recruited is about 440 ± 20 molecules. In WT haploid cells, the maximum number
349 of Abp1 measured is 460 ± 20 molecules. We propose that recruitment of a similar amount of Abp1
350 before scission in all these strains indicates that scission is dependent on the amount of Abp1,
351 and correspondingly, on the amount of actin recruited. We propose that actin supplies the forces
352 necessary for membrane scission. The membrane invagination continues until the “right” amount
353 of actin is recruited. The amount of force necessary is determined by the physical properties of the
354 membrane like membrane rigidity, tension, and proteins accumulated on the membrane (**Dmitrieff**
355 **and Nédélec, 2015**). Vesicle scission releases membrane-bound Rvs, resulting in release of the SH3
356 domains along with BAR domains. Release of the SH3 domains could indicate to the actin network that
357 vesicle scission has occurred, beginning disassembly of actin components.

358 **Model for membrane scission**

359 We propose that Rvs is recruited to sites by two distinct mechanisms. SH3 domains cluster Rvs at
360 endocytic sites, increasing the efficiency with which the BAR domains sense curvature on tubular
361 membranes. BAR domains bind to endocytic sites by sensing tubular membrane. Membrane
362 shape is stabilized by BAR-membrane interaction against fluctuations that could cause scission.
363 This prevent actin forces from rupturing the membrane, and the invaginations continue to grow in
364 length as actin continues to polymerize.

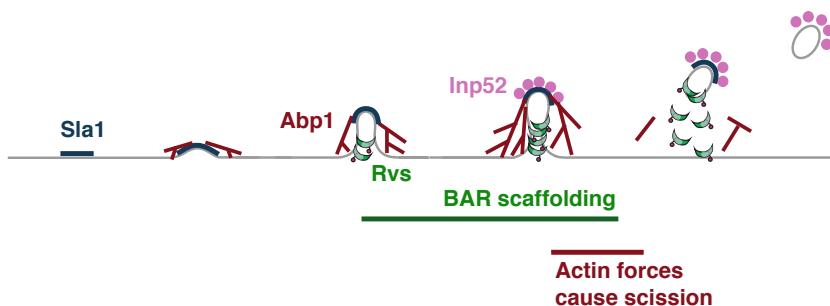


Figure 7. Model for yeast endocytic scission

365 As actin continues to polymerize, enough forces are generated to overcome the resistance to
366 membrane scission provided by the BAR scaffold. The membrane ruptures, and vesicles are formed.
367 Synaptojanins may help recruit Rvs at endocytic sites: Inp51 and Inp52 have proline rich regions
368 that could act as binding sites for Rvs167 SH3 domains. They are involved in vesicle uncoating
369 post-scission, likely by dephosphorylating PIP₂ and inducing disassembly of PIP₂-binding endocytic
370 proteins. Eventually phosphorylation regulation allows endocytic proteins to be reused at endocytic
371 sites, while the vesicle is transported elsewhere into the cell.

372 **Methods and Materials**

373 **Homologous recombination with PCR cassette insertion**

374 Tagging or deletion of endogenous genes was done by homologous integration of the product
375 of a Polymerase Chain Reaction using appropriate primers and a plasmid containing a selection
376 cassette and fluorescent tag, or only selection cassette for gene deletions. Primers were designed

377 according to Janke et al, 2004. PCRs used the Velocity Polymerase for fluorescent tagging, and Q5
378 for gene deletions using the NAT cassette. All fluorescently tagged genes have a C-terminus tag and
379 are expressed endogenously. Gene deletions and fluorescent tags are checked by PCR. Vps1del and
380 gene duplications were confirmed by sequencing.

381 **Live-cell imaging and electron microscopy**

382 Sample preparation for live imaging

383 40µL Concanavalin A (ConA) was incubated on a coverslip for 10 minutes. 40µL Yeast cells incubated
384 overnight at 25C in imaging medium SC-TRP was added to the coverslip after removing the ConA,
385 and incubated for another 10 minutes. Cells were then removed, adhered cells were washed 3x in
386 SC-TRP, and 40µL SC-TRP was finally added to the coverslip to prevent cells from drying.

387 Sample preparation for live imaging in LatA and sorbitol treated cells

388 Cells went through the same procedure as above till the last washing step. Instead of SC-TRP, 100x
389 diluted LatA, or Sorbitol at a final concentration of 0.2M in SC-TRP was added to the adhered cells.
390 For LatA experiments, cells were incubated in LatA for 10 minutes before imaging. For sorbitol
391 treatments, cells were imaged within 5 minutes of adding sorbitol.

392 Epifluorescent imaging for centroid tracking

393 Live-cell imaging was performed as in (*Picco et al., 2015*) Picco et al., 2015. All images were obtained
394 at room temperature using an Olympus IX81 micro- scope equipped with a 100x/NA 1.45 PlanApo
395 objective , with an additional 1.6x magnification lens and an EMCCD camera. The GFP channel was
396 imaged using a 470/22 nm band-pass excitation filter and a 520/35 nm band-pass emission filter.
397 mCherry epifluorescence imaging was carried out using a 556/20 nm band-pass excitation filter and
398 a 624/40 band-pass emission filter. GFP was excited using a 488 nm solid state laser and mCherry
399 was excited using a 561 nm solid state laser. Hardware was controlled using Metamorph software.
400 For single-channel images, 80-120ms was used as exposure time. All dual-channel images were
401 acquired using 250ms exposure time. Si- multaneouous dual-color images were obtained using a
402 dichroic mirror, with TetraSpeck beads used to correct for chromatic abberation.

403 Epifluorescent imaging for molecule number quantification

404 Images were acquired as in Picco et al., 2015. Z-stacks of cells containing the GFP-tagged protein
405 of interest, incubated along with cells containing Nuf2-GFP, were acquired using 400ms exposure
406 using a mercury vapour lamp, on a CCD camera. Z stacks were spaced at 200nm.

407 **Live-cell image analysis**

408 Images were processed for background noise using a rolling ball radius of 90 pixels. Particle
409 detection, and tracking was performed for a particle size of 6 pixels, using scripts that com-
410 bine background subtraction with Particle Tracker and Detector, that can be found on ImageJ
411 (<http://imagej.nih.gov>). Further analysis for centroid averaging, alignments between dual-color
412 images and single channel images, for alignment to the reference Abp1 were done using scripts
413 written in Matlab (Mathworks) and R (www.r-project.org), written originally by Andrea Picco, and
414 modified by me. Details of analysis can be found at (*Picco et al., 2015*). All movement and intensity
415 plots from centroid tracking show the average centroid with 95% confidence interval. All molecule
416 number quantifications report either the median or maximum number of molecules with standard
417 error of mean. Maximum number is preferred over median in cases when the rate of change of
418 fluorescent intensity of two populations being compared are not similar, and the lifetime of the
419 protein populations being compared are not similar. The median in this case underreports the
420 differences in protein accumulation.

421 **Cytoplasmic background quantification**

422 On a maximum intensity projection of time-lapse images, the average pixel intensity within a circle
423 of set radius in the cytoplasm was measured. This circle is manually arranged so that cortical
424 patches were excluded, and mean intensity was acquired for about 10 cells of each cell type. A
425 fixed area outside the cells was drawn, and mean intensity was calculated to establish "background
426 intensity". This background intensity was then subtracted from the mean intensity to obtain a
427 rough measure of cytoplasmic intensity. There are some caveats with this quantification: the cells
428 were not incubated in the same field of view, cellular autofluorescence is assumed to be equal for
429 the different strains.

430 **Citations**

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439 **Acknowledgments**

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441 the acknowledgments section.

442 **References**

- 443 Bensen, E. S., Costaguta, G., and Payne, G. S. (2000). Synthetic genetic interactions with temperature-sensitive
444 clathrin in *Saccharomyces cerevisiae*. Roles for synaptojanin-like Inp53p and dynamin-related Vps1p in
445 clathrin-dependent protein sorting at the trans-Golgi network. *Genetics*, 154(1):83–97.
- 446 Bitsikas, V., Corrêa, I. R., and Nichols, B. J. (2014). Clathrin-independent pathways do not contribute significantly
447 to endocytic flux. *eLife*, 3:e03970.
- 448 Boucrot, E., Pick, A., Camdere, G., Liska, N., Evergren, E., McMahon, H. T., and Kozlov, M. M. (2012). Membrane
449 Fission Is Promoted by Insertion of Amphipathic Helices and Is Restricted by Crescent BAR Domains. *Cell*,
450 149(1):124–136.
- 451 Bui, H. T., Karren, M. A., Bhar, D., and Shaw, J. M. (2012). A novel motif in the yeast mitochondrial dynamin Dnm1
452 is essential for adaptor binding and membrane recruitment. *The Journal of cell biology*, 199(4):613–22.
- 453 Cestra, G., Castagnoli, L., Dente, L., Minenkova, O., Petrelli, A., Migone, N., Hoffmüller, U., Schneider-Mergener, J.,
454 and Cesareni, G. (1999). The SH3 domains of endophilin and amphiphysin bind to the proline-rich region of
455 synaptojanin 1 at distinct sites that display an unconventional binding specificity. *The Journal of biological
456 chemistry*, 274(45):32001–7.
- 457 Colwill, K., Field, D., Moore, L., Friesen, J., and Andrews, B. (1999). In Vivo Analysis of the Domains of Yeast Rvs167p
458 Suggests Rvs167p Function Is Mediated Through Multiple Protein Interactions. *Genetics*, 152(3):881–893.
- 459 D'Hondt, K., Heese-Peck, A., and Riezman, H. (2000). Protein and Lipid Requirements for Endocytosis. *Annual
460 Review of Genetics*, 34(1):255–295.
- 461 Dmitrieff, S. and Nédélec, F. (2015). Membrane Mechanics of Endocytosis in Cells with Turgor. *PLoS Comput Biol*,
462 11(10):e1004538.
- 463 Farsad, K., Ringstad, N., Takei, K., Floyd, S. R., Rose, K., and De Camilli, P. (2001). Generation of high curvature
464 membranes mediated by direct endophilin bilayer interactions. *The Journal of Cell Biology*, 155(2):193–200.
- 465 Ferguson, S. M., Brasnjo, G., Hayashi, M., Wölfel, M., Collesi, C., Giovedi, S., Raimondi, A., Gong, L. W., Ariel, P.,
466 Paradise, S., O'Toole, E., Flavell, R., Cremona, O., Miesenböck, G., Ryan, T. A., and De Camilli, P. (2007). A selective
467 activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. *Science*, 316(5824):570–574.

- 468 Ferguson, S. M., Raimondi, A., Paradise, S., Shen, H., Mesaki, K., Ferguson, A., Destaing, O., Ko, G., Takasaki, J.,
469 Cremona, O., O' Toole, E., and De Camilli, P. (2009). Coordinated actions of actin and BAR proteins upstream
470 of dynamin at endocytic clathrin-coated pits. *Developmental cell*, 17(6):811–822.
- 471 Friesen, H., Humphries, C., Ho, Y., Schub, O., Colwill, K., and Andrews, B. (2006). Characterization of the Yeast
472 Amphiphysins Rvs161p and Rvs167p Reveals Roles for the Rvs Heterodimer In Vivo. *Molecular Biology of the
473 Cell*, 17(3):1306–1321.
- 474 Galli, V., Sebastian, R., Moutel, S., Ecard, J., Perez, F., and Roux, A. (2017). Uncoupling of dynamin polymerization
475 and GTPase activity revealed by the conformation-specific nanobody dynab. *eLife*, 6:e25197.
- 476 Goud Gadila, S. K., Williams, M., Saimani, U., Delgado Cruz, M., Makaraci, P., Woodman, S., Short, J. C., McDermott,
477 H., and Kim, K. (2017). Yeast dynamin Vps1 associates with clathrin to facilitate vesicular trafficking and
478 controls Golgi homeostasis. *European Journal of Cell Biology*, 96(2):182–197.
- 479 Grabs, D., Slepnev, V. I., Songyang, Z., David, C., Lynch, M., Cantley, L. C., and De Camilli, P. (1997). The SH3
480 domain of amphiphysin binds the proline-rich domain of dynamin at a single site that defines a new SH3
481 binding consensus sequence. *The Journal of biological chemistry*, 272(20):13419–25.
- 482 Grigliatti, T. A., Hall, L., Rosenbluth, R., and Suzuki, D. T. (1973). Temperature-Sensitive Mutations in Drosophila
483 melanogaster XIV. A Selection of Immobile Adults *. *Molec. gen. Genet.*, 120:107–114.
- 484 Hoepfner, D., van den Berg, M., Philippse, P., Tabak, H. F., and Hettema, E. H. (2001). A role for Vps1p, actin, and
485 the Myo2p motor in peroxisome abundance and inheritance in *<|>Saccharomyces cerevisiae</i>*. *The Journal
486 of Cell Biology*, 155(6):979–990.
- 487 Kaksonen, M. and Roux, A. (2018). Mechanisms of clathrin-mediated endocytosis.
- 488 Kaksonen, M., Sun, Y., and Drubin, D. G. (2003). A pathway for association of receptors, adaptors, and actin
489 during endocytic internalization. *Cell*, 115(4):475–487.
- 490 Kaksonen, M., Toret, C. P., and Drubin, D. G. (2005). A modular design for the clathrin- and actin-mediated
491 endocytosis machinery. *Cell*, 123(2):305–320.
- 492 Kishimoto, T., Sun, Y., Buser, C., Liu, J., Michelot, A., and Drubin, D. G. (2011). Determinants of endocytic
493 membrane geometry, stability, and scission. *Proceedings of the National Academy of Sciences of the United
494 States of America*, 108(44):E979–E988.
- 495 Kübler, E., Riezman, H., Riezman, H., and Riezman, H. (1993). Actin and fimbrin are required for the internalization
496 step of endocytosis in yeast. *The EMBO journal*, 12(7):2855–62.
- 497 Kukulski, W., Schorb, M., Kaksonen, M., and Briggs, J. G. (2012). Plasma Membrane Reshaping during Endocytosis
498 Is Revealed by Time-Resolved Electron Tomography. *Cell*, 150(3):508–520.
- 499 Lila, T. and Drubin, D. G. (1997). Evidence for physical and functional interactions among two *Saccharomyces
500 cerevisiae* SH3 domain proteins, an adenylyl cyclase-associated protein and the actin cytoskeleton. *Molecular
501 Biology of the Cell*, 8(2):367–385.
- 502 Liu, J., Kaksonen, M., Drubin, D. G., and Oster, G. (2006). Endocytic vesicle scission by lipid phase boundary
503 forces. *Proceedings of the National Academy of Sciences of the United States of America*, 103(27):10277–82.
- 504 Liu, J., Sun, Y., Drubin, D. G., and Oster, G. F. (2009). The Mechanochemistry of Endocytosis. *PLoS Biol*,
505 7(9):e1000204.
- 506 Madania, A., Dumoulin, P., Grava, S., Kitamoto, H., Scharer-Brodbeck, C., Soulard, A., Moreau, V., and Winsor,
507 B. (1999). The *Saccharomyces cerevisiae* Homologue of Human Wiskott-Aldrich Syndrome Protein Las17p
508 Interacts with the Arp2/3 Complex. *Molecular Biology of the Cell*, 10(10):3521–3538.
- 509 Meinecke, M., Boucrot, E., Camdere, G., Hon, W.-C., Mittal, R., and McMahon, H. T. (2013). Cooperative recruitment
510 of dynamin and BIN/amphiphysin/Rvs (BAR) domain-containing proteins leads to GTP-dependent membrane
511 scission. *The Journal of biological chemistry*, 288(9):6651–61.
- 512 Mim, C., Cui, H., Gawronski-Salerno, J. A., Frost, A., Lyman, E., Voth, G. A., and Unger, V. M. (2012). Structural
513 basis of membrane bending by the N-BAR protein endophilin. *Cell*, 149(1):137–145.
- 514 Moustaq, L., Smaczynska-de Rooij, I. I., Palmer, S. E., Marklew, C. J., and Ayscough, K. R. (2016). Insights into
515 dynamin-associated disorders through analysis of equivalent mutations in the yeast dynamin Vps1. *Microbial
516 cell (Graz, Austria)*, 3(4):147–158.

- 517 Munn, A. L., Stevenson, B. J., Geli, M. I., and Riezman, H. (1995). end5, end6, and end7: Mutations that cause
518 actin delocalization and block the internalization step of endocytosis in *Saccharomyces cerevisiae*. *Molecular*
519 *Biology of the Cell*, 6(12):1721–1742.
- 520 Nannapaneni, S., Wang, D., Jain, S., Schroeder, B., Highfill, C., Reustle, L., Pittsley, D., Maysent, A., Moulder, S.,
521 McDowell, R., and Kim, K. (2010). The yeast dynamin-like protein Vps1: vps1 mutations perturb the internal-
522 ization and the motility of endocytic vesicles and endosomes via disorganization of the actin cytoskeleton.
523 *European Journal of Cell Biology*, 89(7):499–508.
- 524 Peters, C., Baars, T. L., Bühler, S., and Mayer, A. (2004). Mutual control of membrane fission and fusion proteins.
525 *Cell*, 119(5):667–78.
- 526 Picco, A., Mund, M., Ries, J., Nédélec, F., and Kaksonen, M. (2015). Visualizing the functional architecture of the
527 endocytic machinery. *eLife*, page e04535.
- 528 Rooij, I. I. S.-d., Allwood, E. G., Aghamohammadzadeh, S., Hettema, E. H., Goldberg, M. W., and Ayscough,
529 K. R. (2010). A role for the dynamin-like protein Vps1 during endocytosis in yeast. *Journal of Cell Science*,
530 123(20):3496–3506.
- 531 Rothman, J. H., Raymond, C. K., Gilbert, T., O'Hara, P. J., and Stevens, T. H. (1990). A putative GTP binding protein
532 homologous to interferon-inducible Mx proteins performs an essential function in yeast protein sorting. *Cell*,
533 61(6):1063–1074.
- 534 Rothman, J. I. and Stevens, T. H. (1986). Protein Sorting in Yeast: Mutants Defective in Vacuole Biogenesis
535 Mislocalize Vacuolar Proteins into the Late Secretory Pathway. Technical report.
- 536 Shupliakov, O., Löw, P., Grabs, D., Gad, H., Chen, H., David, C., Takei, K., De Camilli, P., and Brodin, L. (1997).
537 Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. *Science (New York,*
538 *N.Y.*, 276(5310):259–63.
- 539 Simunovic, M., Manneville, J.-B., Renard, H.-F. O., Johannes, L., Bassereau, P., Callan, A., Correspondence, J.,
540 Evergren, E., Raghunathan, K., Bhatia, D., Kenworthy, A. K., Voth, G. A., Prost, J., Mcmahon, H. T., and Callan-
541 Jones, A. (2017). Friction Mediates Scission of Tubular Membranes Scaffolded by BAR Proteins. *Cell*, 170:1–13.
- 542 Sivadon, P., Crouzet, M., and Aigle, M. (1997). Functional assessment of the yeast Rvs161 and Rvs167 protein
543 domains. *FEBS letters*, 417(1):21–27.
- 544 Skruzny, M., Brach, T., Ciuffa, R., Rybina, S., Wachsmuth, M., and Kaksonen, M. (2012). Molecular basis for
545 coupling the plasma membrane to the actin cytoskeleton during clathrin-mediated endocytosis. *Proceedings*
546 *of the National Academy of Sciences of the United States of America*, 109(38):E2533–42.
- 547 Smaczynska-de Rooij, I. I., Allwood, E. G., Mishra, R., Booth, W. I., Aghamohammadzadeh, S., Goldberg, M. W., and
548 Ayscough, K. R. (2012). Yeast Dynamin Vps1 and Amphiphysin Rvs167 Function Together During Endocytosis.
549 *Traffic*, 13(2):317–328.
- 550 Sweitzer, S. M. and Hinshaw, J. E. (1998). Dynamin Undergoes a GTP-Dependent Conformational Change Causing
551 Vesiculation. *Cell*, 93(6):1021–1029.
- 552 Takei, K., McPherson, P. S., Schmid, S. L., and Camilli, P. D. (1995). Tubular membrane invaginations coated by
553 dynamin rings are induced by GTP-γS in nerve terminals. *Nature*, 374(6518):186–190.
- 554 Youn, J.-Y., Friesen, H., Kishimoto, T., Henne, W. M., Kurat, C. F., Ye, W., Ceccarelli, D. F., Sicheri, F., Kohlwein, S. D.,
555 McMahon, H. T., and Andrews, B. J. (2010). Dissecting BAR Domain Function in the Yeast Amphiphysins Rvs161
556 and Rvs167 during Endocytosis. *Molecular Biology of the Cell*, 21(17):3054–3069.
- 557 Yu, X. and Cai, M. (2004). The yeast dynamin-related GTPase Vps1p functions in the organization of the actin
558 cytoskeleton via interaction with Sla1p. *Journal of Cell Science*, 117(17):3839–3853.
- 559 Zhang, P. and Hinshaw, J. E. (2001). Three-dimensional reconstruction of dynamin in the constricted state. *Nature*
560 *Cell Biology*, 3(10):922–926.
- 561 Zhao, W.-D., Hamid, E., Shin, W., Wen, P. J., Krystofiak, E. S., Villarreal, S. A., Chiang, H.-C., Kachar, B., and Wu, L.-G.
562 (2016). Hemi-fused structure mediates and controls fusion and fission in live cells. *Nature*, 534(7608):548–52.
- 563
- 564

565 **Supplementary Material**

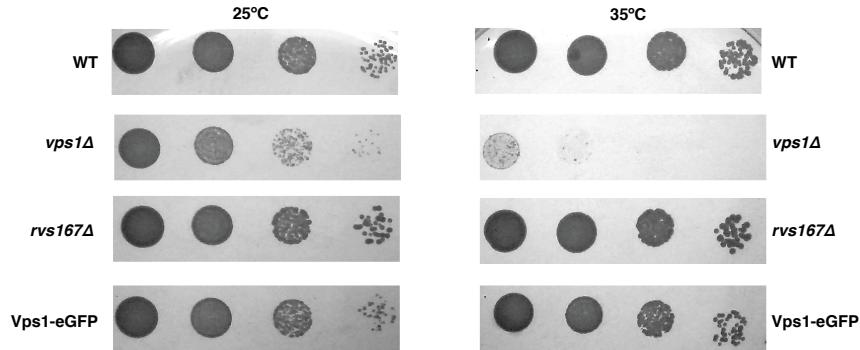


Figure S1. Growth assay of WT, *vps1* Δ , *rvs167* Δ , and cells expressing Vps1-eGFP at 25°C and 35°C

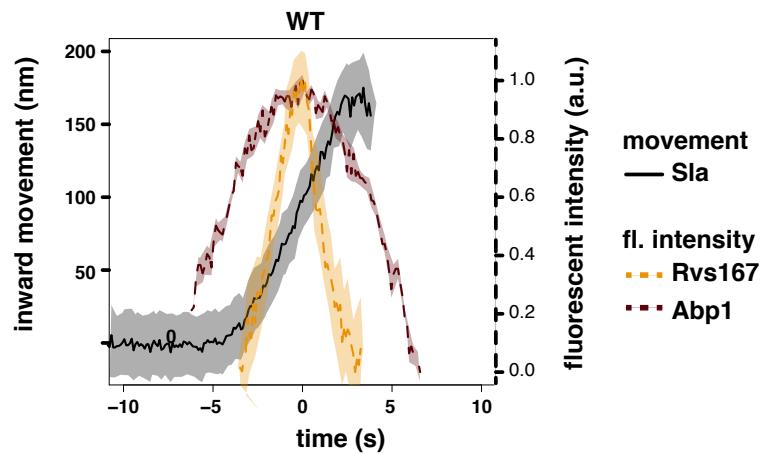


Figure S2. Sla1 centroid aligned so that time=0(s) is the maximum of Abp1 fluorescent intensity in WT cells (Picco et al., 2015), normalized Abp1 and Rvs167 fluorescent intensities in WT cells

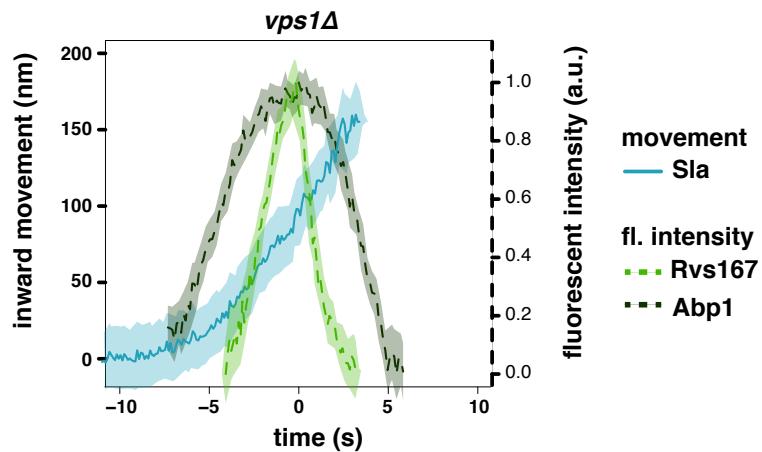


Figure S3. Sla1 centroid aligned so that time=0(s) is the maximum of Abp1 fluorescent intensity in *vps1* Δ cells (Picco et al., 2015), normalized Abp1 and Rvs167 fluorescent intensities in *vps1* Δ cells

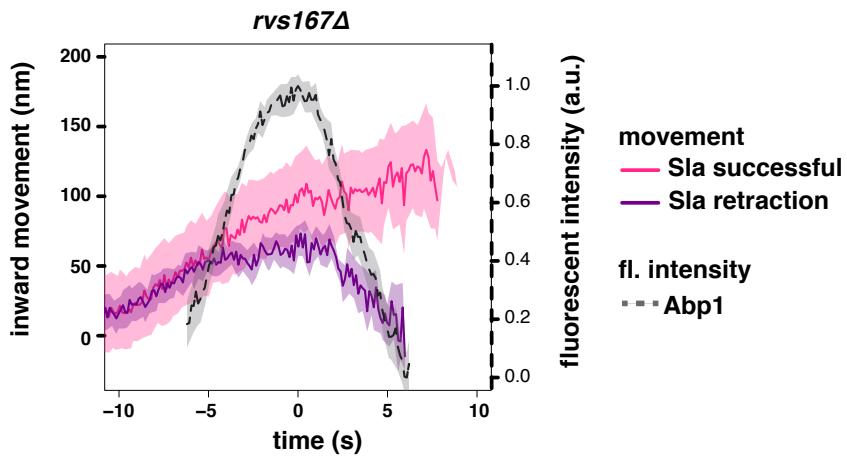


Figure S4. Sla1 centroid aligned so that time=0(s) is the maximum of Abp1 fluorescent intensity in *rvs167 Δ* cells (Picco et al., 2015), normalized Abp1 fluorescent intensity in *rvs167 Δ* cells

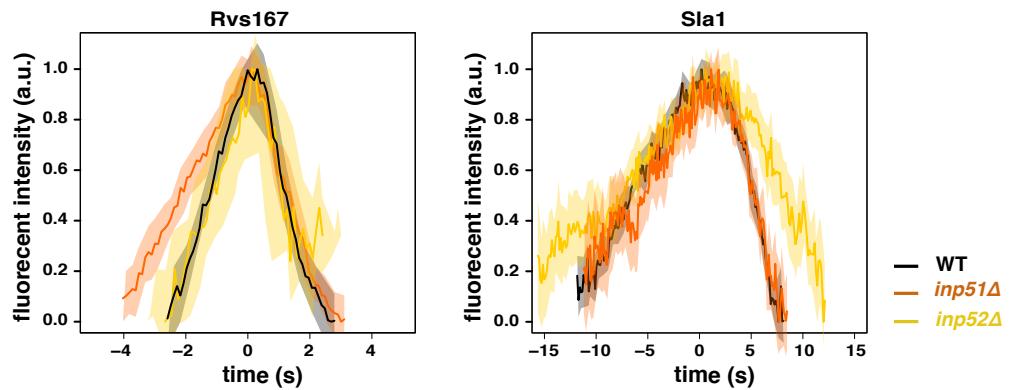


Figure S5. Normalized Rvs167 and Sla1 fluorescent intensities aligned in time so that time=0(s) is the maximum of each corresponding fluorescent intensity profile

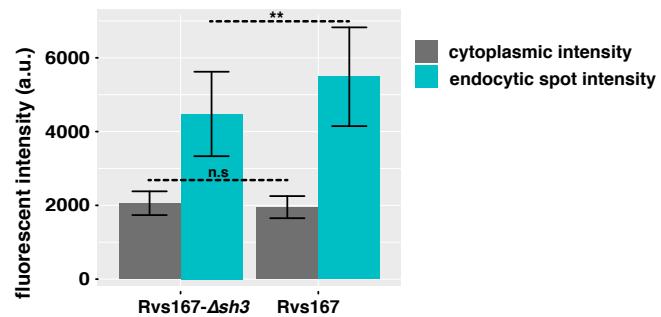


Figure S6. Cytoplasmic intensity and intensity of endocytic patches of Rvs167 and Rvs167 Δ sh3 in WT and *rvs167 Δ sh3* cells. Error bars are standard deviation, p values from t-test, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.