﻿\subsection{**Rvs167 deletion results in reduced coat movement**}

Deletion of the Rvs genes have resulted in the scission-specific phenotype of membrane retraction: inward movement and consequent retraction of the invaginated membrane back to the cell wall (ref.Marko). Since Rvs161 and Rvs167 form dimers (ref.Dominik), deletion of Rvs167 effectively removes both proteins from endocytic sites. We therefore quantified the effect of deletion of Rvs167 on membrane invagination. To follow the membrane invagination we used Sla1, an endocytic coat protein. Upon actin polymerization, the endocytic coat is pulled along with the membrane as it invaginates (ref.Skruzny?), and thus acts as a proxy for the behaviour of the plasma membrane. We endogenously tagged Sla1 at the N-terminus with eGFP in WT and \textit{rvs167$\Delta$} cells (Fig.1a), and tracked the dynamics of Sla1.

27\% of Sla1 patches that begin to form invaginations move inward and then retract in \textit{rvs167$\Delta$} cells (Fig.1d), consistent with retraction rates measured in other experiments (Kaksonen, Toret and Drubin, 2005), and suggesting failed scission in 27\% of endocytic events. Movement of the retractions and of successful endocytic events were quantified (Fig.1e) as described in Picco et. al, 2015. In brief, yeast cells expressing fluorescently-tagged endocytic proteins are imaged at the equatorial plane. Since membrane invagination progresses perpendicularly to the plane of the plasma membrane, proteins that move into the cytoplasm during invagination do so in the imaging plane. Centroids of Sla1 patches- each patch being an endocytic site- are tracked in time. Between 40-50 Sla1 centroids are averaged. This provides an averaged centroid that can be followed with high spatial and temporal resolution. When different endocytic proteins are simultaneously imaged with Actin Binding Protein Abp1, Abp1 provides a frame of reference to which all the other proteins can be aligned. Abp1 is used because it is abundant at endocytic sites and therefore easily imaged. Time=0 is established as the peak of the Abp1 fluorescence intensity in respective co-tagged strains strains (Fig.1, supplement). Abp1 fluorescent intensity maxima in wild-type cells is concomitant with the peak of Rvs167 fluorescent intensity and is time window in which scission occurs (ref2andrea, refwanda).

In WT cells, the averaged Sla1 centroid moves inward at a linear rate to a distance of 140nm from its starting position. After this, the centroid position shows increased noise, likely from random motion of the vesicle post-scission. Following scission, Sla1 molecules are disassembled from the endocytic vesicle (Fig1 supplement). Sla1 centroid movement in both successful and retracting endocytic events in \textit{rvs167$\Delta$} cells and WT look similar up to about 60nm (Fig.1e). Consequent movement in successful scission events slows dramatically, and invaginations appear to undergo scission between 60 -80 nm. CLEM has shown that Rvs167 localizes to endocytic sites after the invaginations are about 60nm long (Kukulski et al., 2012). Sla1 movement in \textit{rvs167$\Delta$} shows therefore that membrane invagination is unaffected till Rvs is supposed to arrive. Sla1 in \textit{rvs167$\Delta$} then continues to move at a much slower rate to about 80nm. That membrane scission occurs at shorter invagination lengths than in WT is corroborated by the smaller vesicles formed in \textit{rvs167$\Delta$} cells (Kukulski et al., 2012). This indicates that first, membrane scission can occur at invagination lengths of 80nm. Then, that the arrival of Rvs prevents membrane scission at 80nm and allows further membrane invagination. In retraction events, after inward movement, the Sla1 centroid moves back towards the starting position, that is, to the plasma membrane.