﻿\subsection{**Vps1 does not influence coat or scission dynamics**}

﻿Endocytic membrane scission in mammalian cells is understood to be driven by constriction of the tubule neck by the Gtpase Dynamin (Grigliatti et al., 1973; Poodry and Edgar, 1979; van der Bliek and Meyerowrtz, 1991). Mammalian Dynamin is recruited to endocytic sites via their proline-rich domains (PRD) to SH3 domains of N-BAR proteins amphiphysin and endophilin (Grabs et al., 1997; Cestra et al., 1999; Farsad et al., 2001; Meinecke et al., 2013, Ferguson, 2009). In yeast, the Dynamin-like protein Vps1 is essential for vacuolar protein sorting, and does not contain a PRD. It is however, reportedly recruited to endocytic sites and interacts with endocytic proteins (refAyscough, Yu, 2004; Nannapaneni et al., 2010; Goud Gadila et al., 2017). Vps1 tagged both N- and C-terminally with GFP constructs failed to co-localize with endocytic proteins in our hands (Fig.1 supplement), indicating that Vps1 may not play a role in yeast endocytosis. To test whether absence of Vps1 influences scission, endocytic dynamics are observed in cells lacking Vps1 and compared against WT cells. In Fig.1d, retraction of Sla1 in \textit{vps1$\Delta$} and wild-type cells is quantified, suggesting that retraction rates do not increase in the absence of Vps1.

In Fig.1e, the averaged centroid trajectory of Sla1-eGFP is tracked in ~50 endocytic sites in \textit{vps1$\Delta$} and WT cells. Centroid movement of Sla1-eGFP in WT cells shows a linear movement to about 140nm. Sla1 movement in \textit{vps1$\Delta$} cells has the same magnitude of inward movement to about 140nm. In spite of slight differences in the rates of inward movment, that the total inward movment does not change indicates that plasma membrane dynamics remain uninfluenced by the absence of Vps1.

Centroid tracking has shown that the number of molecules of Rvs167 peaks at the time of scission, and is followed by a rapid loss of fluorescent intensity, simultaneous with a sharp jump of the centroid into the cytoplasm (ref.Andrea). This jump, also seen in Rvs167-GFP kymographs (Fig.1c), is interpreted as loss of protein on the membrane tube, causing an apparent spatial jump to the protein localized at the base of the newly formed vesicle. Kymographs of Rvs167-GFP (Fig.1c), as well as Rvs167 centroid tracking (Fig.1e) in Vps1 deleted cells show the same jump, indicating that vesicles are formed in the same position in Vps1 deletion cells as in WT cells. From the absence of ch