﻿\subsection{**Rvs BAR domains recognize membrane curvature in-vivo**}

The curved tertiary structure and liposome binding assays of N-BAR domains have suggested that they may have a preference for curved membrane that match their own intrinsic curvature. Alternately, they may also impose their curvature on flat membrane and induce curvature formation. The curvature interaction of Rvs167 in vivo has not been tested. In order to do so, we deleted the SH3 domain of Rvs167 (henceforth BAR-GPA) and observed the localization of Rvs167 with and witout the SH3 domain. The GPA region is a disordered region that has no previously reported function and was retained to ensure proper folding and function of the BAR domain. Endogenously tagged Rvs167-eGFP and BAR-GPA-eGFP and Abp1-mCherry in WT and sla2deletion cells are compared. Sla2 acts as the molecular linker between forces exerted by the actin network and the plasma membrane (ref. Skruzny). Sla2deletion cells therefore contain polymerizing actin network at endocytic patches, but the membrane remains flat and endocytosis fails. In these cells, the full-length Rvs167 protein co-localizes with Abp1-mCherry, indicating that it is recruited to endocytic sites. BAR-GPA-eGFP localization is removed, except for rare transient patches that do not co-localize with Abp1-mCherry, indicating that in the absence of membrane curvature, the BAR domains cannot localize to endocytic sites.

﻿\subsection{**Rvs SH3 domains contribute to curvature independent localization**}

We have shown that BAR domains need membrane curvature to localize. Full-length Rvs167, however, is recruited to endocytic patches in sla2deletion cells. This indicates that a second interaction- that is not the BAR-curvature dependent- recruits the protein to endocytic sites. This interaction must come from the SH3 region, showing that Rvs localization is dependent on both BAR as well as SH3 domain interactions. Absence of the SH3 domain also reduces total recruitment of Rvs and Abp1 protein, giving the SH3 domain an important and surprising role in regulating the late stage of endocytosis.

﻿\subsection{**SH3 domains are recruited by Myosin 5**}

SH3 domains have been shown to interact with several proteins in the actin module of endocytosis: Las17, type I myosins, and Vrp1 all have genetic or physical interactions with Rvs167 SH3 domains (Lila and Drubin, 1997; Colwill et al., 1999, Madania et al., 1999; Liu et al., 2009).

We tested the interaction by studying the localization of full-length Rvs167 in cells with one of these proteins deleted, and treated with LatA to reproduce the situation in which BAR-curvature interaction is removed.

Deletion of neither Las17 nor Myo3 in combination with LatA treatment does not remove the localization of Rvs167. Deletion of Vrp1 and Myo5, with LatA treatment removes localization of Rvs167. Since Vrp1 is required for the recruitment of Myo5 (refMyo5), SH3 domains likely interact with Myo5 rather than Vrp1.