Supplemental Materials

Molecular Biology of the Cell

Russo et al.

RUSSO ET AL., SUPPLEMENTAL FIGURES AND LEGENDS

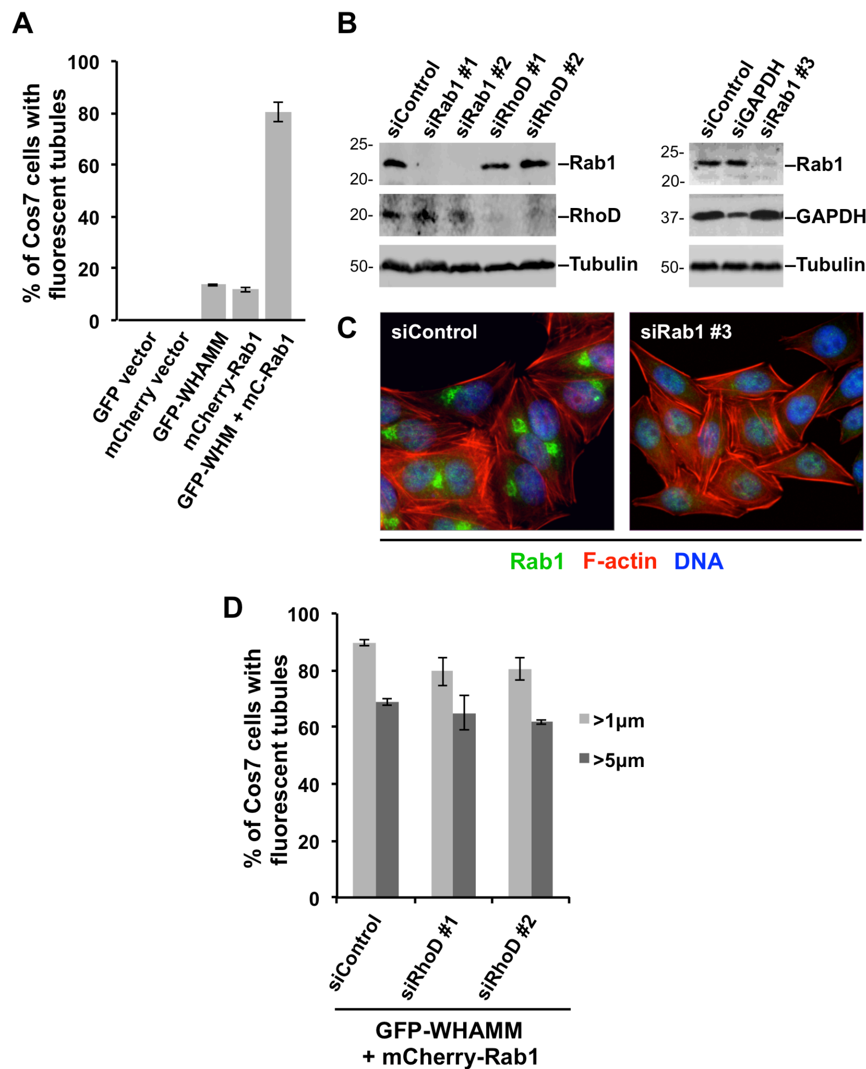

Fig.S1. Membrane tubulation and expression of Rab1 and RhoD.
A. Cos7 cells were transfected with plasmids encoding GFP- or mCherry-fusion proteins. The % of cells with fluorescent tubular structures >1 μm in length was quantified. Each bar represents the mean +/- SD from 3-4 experiments in which 50-75 cells were examined per experiment. Similar enhancement in tubulation of WHAMM-associated membranes upon co-expression with Rab1 was also observed in NIH3T3 cells and HFFs (not shown).
B. Cos7 cells treated with a control siRNA, GAPDH siRNA, independent pairs of siRNAs to Rab1a and Rab1b (#s 1-3), or independent siRNAs to RhoD (# 1-2) were subjected to SDS-PAGE and immunoblotted with antibodies to Rab1b, RhoD, GAPDH, and tubulin.
C. HeLa cells treated with a control siRNA or a pair of siRNAs to Rab1a and Rab1b were fixed and stained with anti-Rab1b antibodies, phalloidin to visualize F-actin, and DAPI to stain DNA.
D. Cos7 cells were co-transfected with plasmids encoding GFP-WHAMM plus mCherry-Rab1, and either a control siRNA or independent siRNAs to RhoD. The % of cells with fluorescent tubular structures >1 μm or >5 μm in length was quantified. Each bar represents the mean +/- SD from 3 experiments in which 55-80 cells were examined per experiment.

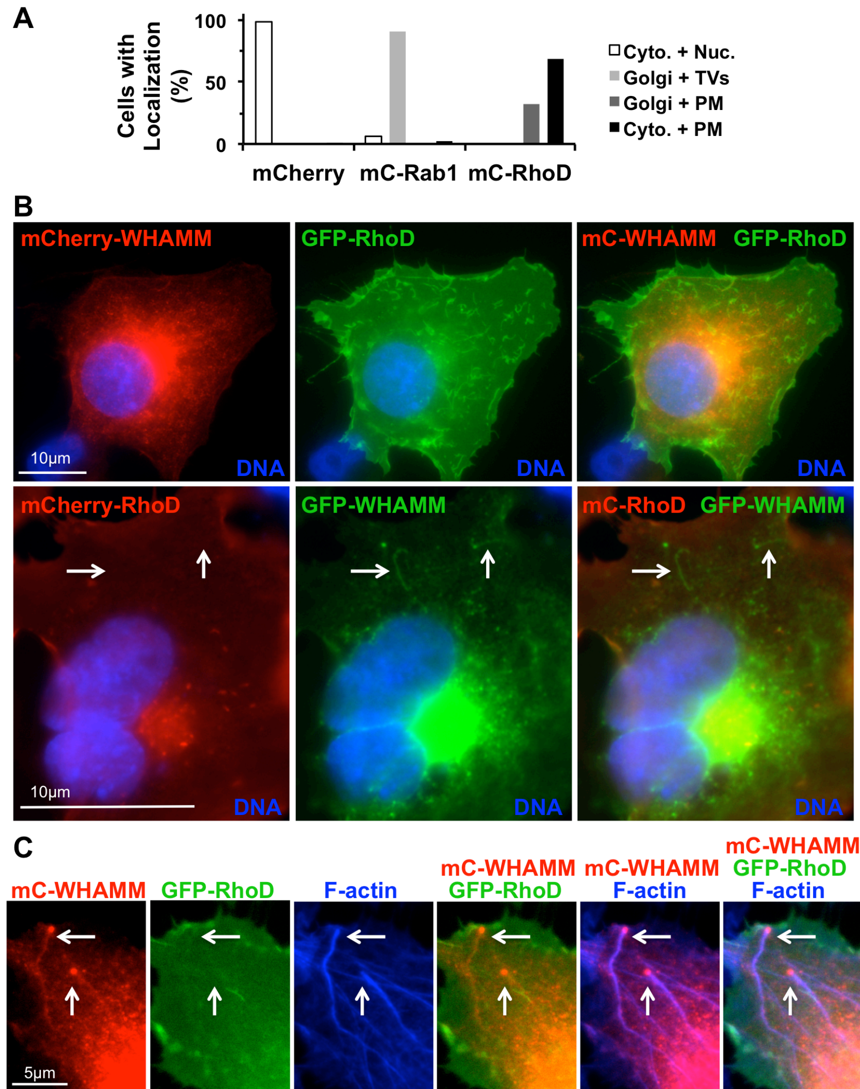

Fig.S2. WHAMM and RhoD do not co-localize along tubulo-vesicular structures.
A. Cos7 cells were transfected with plasmids encoding mCherry, mCherry-Rab1a, or mCherry-RhoD, fixed, stained with phalloidin and DAPI, and examined by fluorescence microscopy. The % of cells with cytosolic + nuclear (Cyto. + Nuc.), Golgi-like + tubulo-vesicular (Golgi + TVs), Golgi-like + plasma membrane (Golgi + PM), or cytosolic + plasma membrane (Cyto. + PM) mCherry fluorescence was then scored. 250-350 cells were examined for each construct.
B. Cos7 cells were co-transfected with plasmids encoding mCherry- and GFP-tagged versions of WHAMM or RhoD, fixed, and treated with DAPI to label DNA (blue). The top panels show a primarily cytosolic + plasma membrane localization for RhoD, while the bottom panels show a Golgi-like + plasma membrane localization for RhoD. Arrows highlight sites of WHAMM-associated tubulo-vesicular membranes that did not exhibit mCherry-RhoD localization.
C. Cos7 cells co-transfected as in part B were stained with phalloidin to detect actin filaments along WHAMM-associated tubulo-vesicular membranes. GFP-RhoD did not localize to these structures.

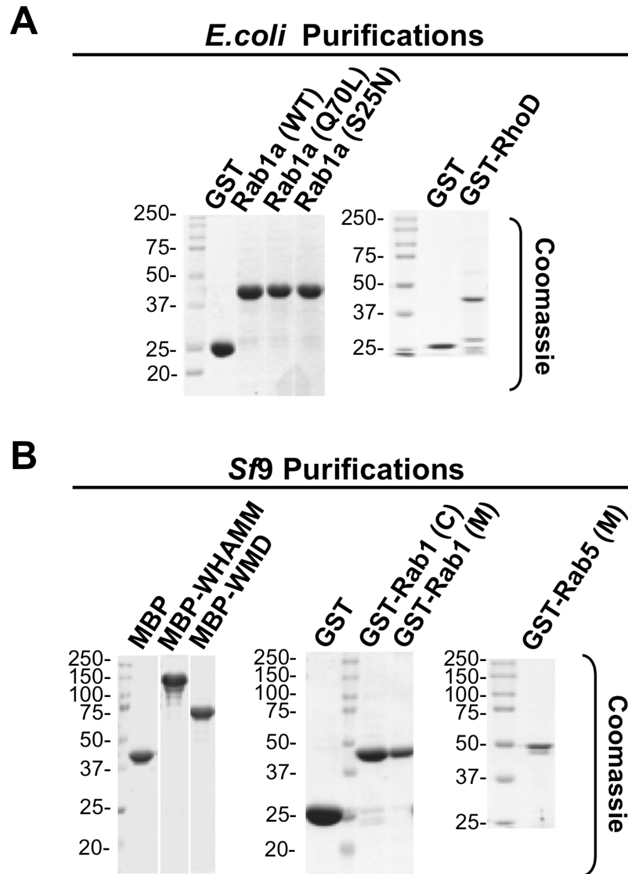

Fig.S3. Purification of GST- and MBP-fusion proteins from *E.coli* or *Sf*9 insect cells.
A. Recombinant forms of GST, GST-RhoD, or GST-tagged versions of wild type (WT), constitutively active (Q70L), or inactive (S25N) Rab1a were expressed in *E.coli*, purified using glutathione affinity beads, subjected to SDS-PAGE, and stained with Coomassie blue.
B. Recombinant forms of MBP or MBP-tagged full-length WHAMM or the WMD were expressed in *Sf*9 insect cells and purified using amylose affinity beads. Recombinant forms of GST, GST-Rab1a, or GST-Rab5 were also expressed in *Sf*9 insect cells, and GST was purified from whole cell extracts using glutathione affinity beads. For GST-Rab1a and GST-Rab5, the cells were first fractionated into their membrane and cytosolic components. Membrane-extracted (M) and cytosolic (C) GST-fusions were then purified separately using glutathione affinity beads. All purified proteins were analyzed by SDS-PAGE and Coomassie blue staining.

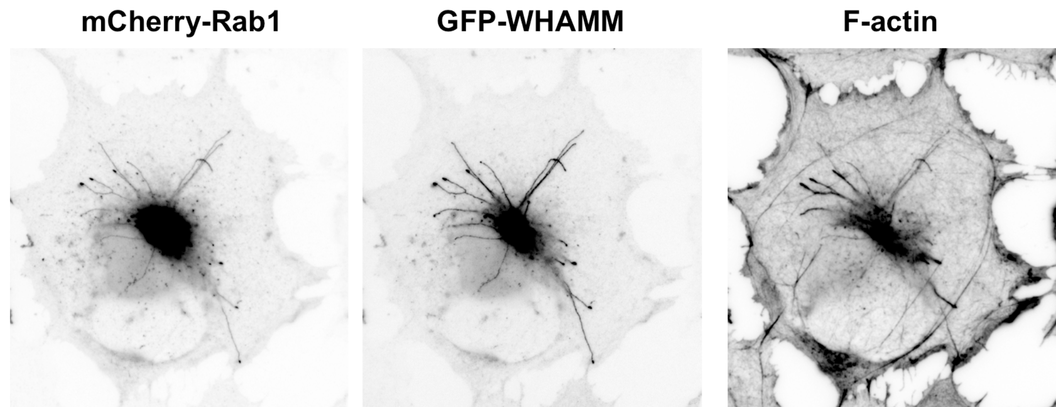

Fig.S4. Actin filaments are present along tubulo-vesicular structures.
Cos7 cells co-expressing mCherry-Rab1a and GFP-WHAMM were stained with phalloidin to label actin filaments. These inverted panels correspond to the colorized images in Fig.7A.