Supporting Information

Swan et al. 10.1073/pnas.0914658107

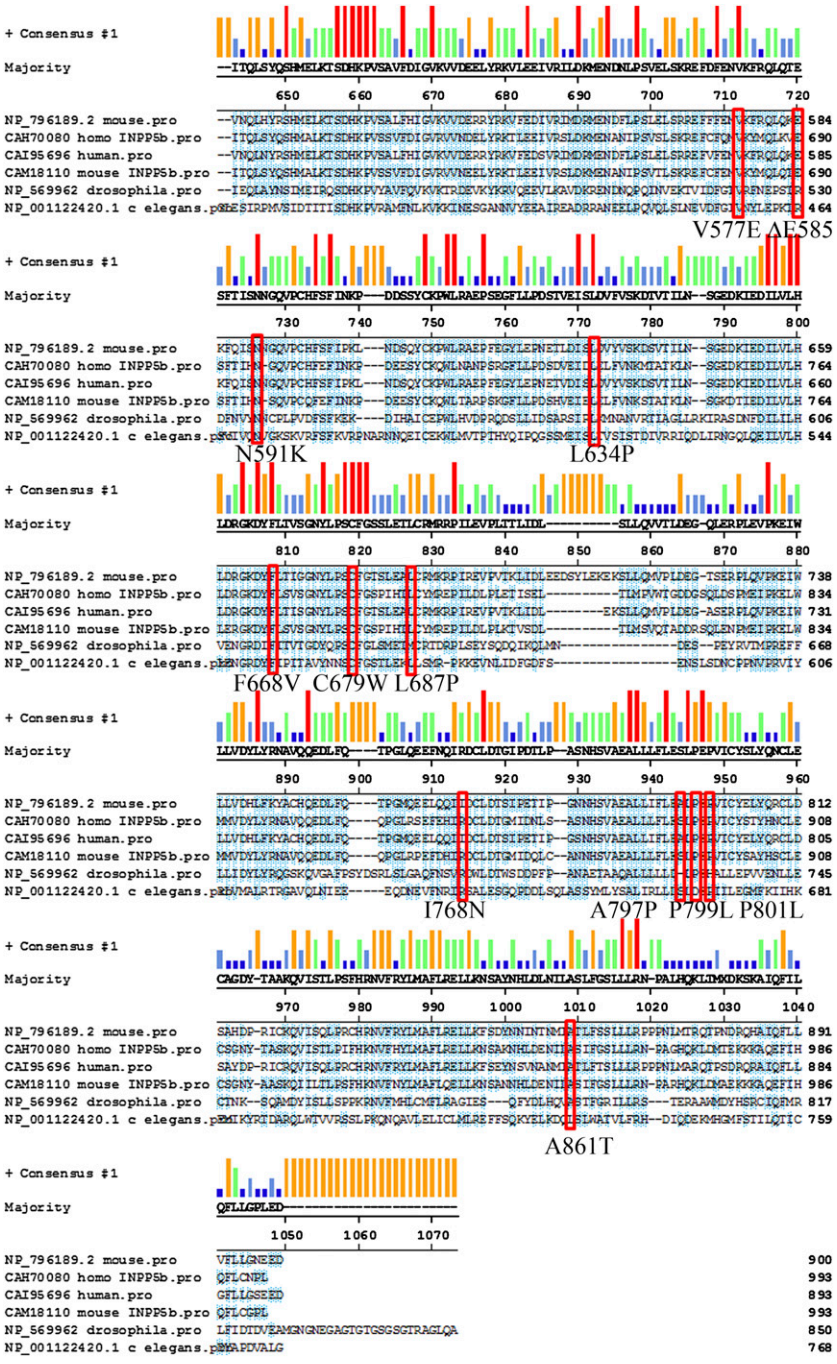


Fig. S1. Conservation of the ASH-RhoGAP–like module of OCRL and INPP5b. Boxed residues are mutations examined in this study (Fig. 4 and Fig. S2).

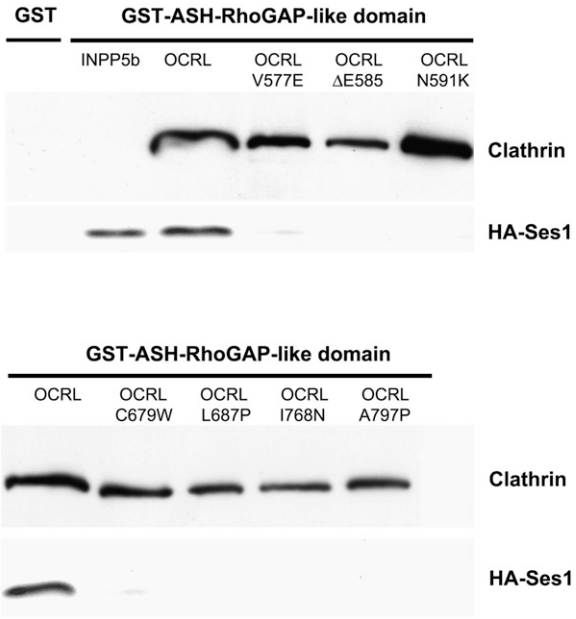


Fig. S2. Western blots of GST pulldowns from extracts of HA-Ses1–transfected Cos7 cells showing that HA-Ses1 binds to the ASH-RhoGAP–like domains of WT OCRL and INPP5b but not to GST or to the mutant ASH-RhoGAP–like domains of OCRL harboring the patient mutations V577E, ΔE585, N591K, C679W, L687P, I768N, and A797P. All these mutations were shown previously to abolish APPL1 binding (1, 2). Clathrin heavy chain specifically binds the OCRL ASH-RhoGAP–like domain and not INPP5B or GST alone, as expected (1).

1. Erdmann KS, et al. (2007) A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway. *Dev Cell* 13:377–390.
2. McCrea HJ, et al. (2008) All known patient mutations in the ASH-RhoGAP domains of OCRL affect targeting and APPL1 binding. *Biochem Biophys Res Commun* 369:493–499.

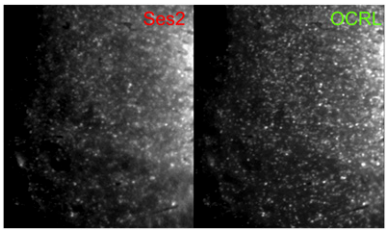


Fig. S3. tagRFP-t-Ses2 and GFP-OCRL colocalize on endosomal structures. Widefield microscopy. Magnification is 63×.

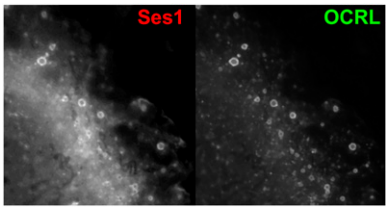


Fig. S4. GFP-OCRL and tagRFP-t-Ses1 colocalize on macropinosomes generated by overexpression of $Hras^{V12G}$. Widefield image from live cells. Magnification is 63×

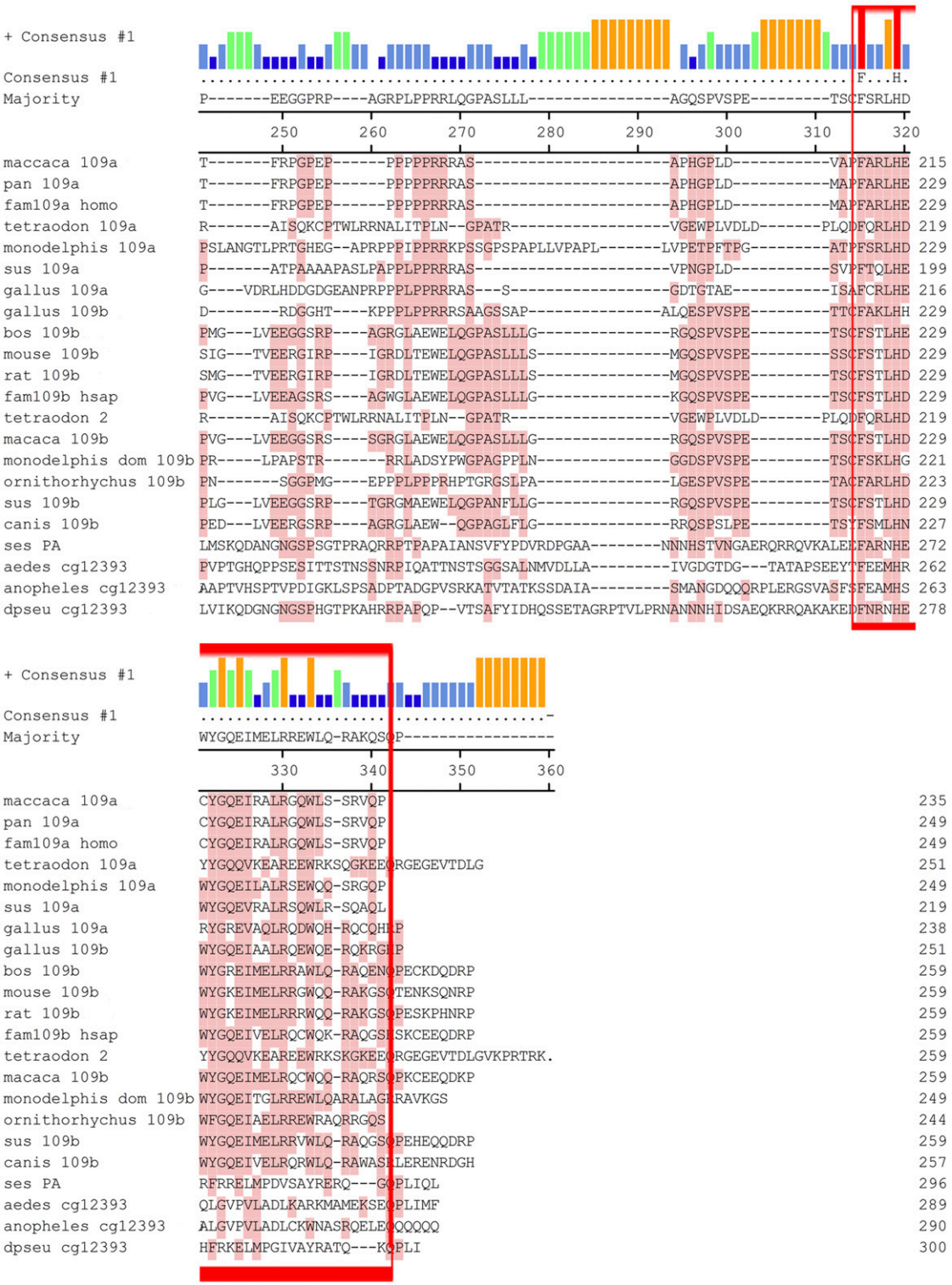
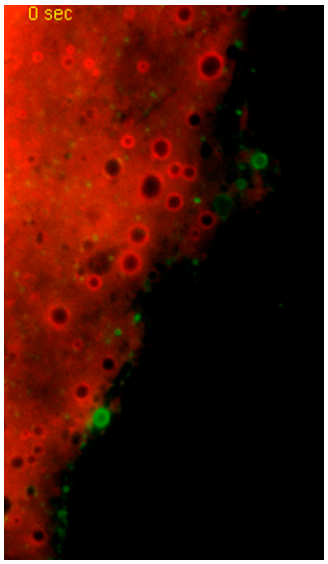
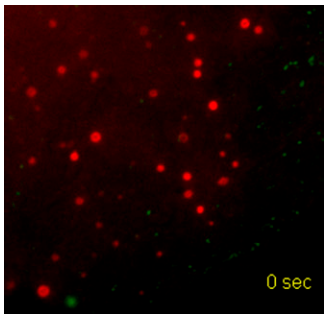


Fig. S5. Conservation of a region of homology in Ses1/Fam109a, Ses2/Fam109b, and invertebrate DSes/CG12393. The C-terminal region of Ses-like proteins is poorly conserved apart from the heavily conserved F-x-x-x-H motif (F&H) at or near the C terminus (red box).



Movie S1. A 0.2-Hz widefield microscopy movie of a Cos7 cell transfected with OCRL, TagRFP-T-Ses2, and GFP-APPL1. Spontaneously generated macropinosomes are initially APPL1 positive and then shed this protein. Some of them acquire Ses2 upon shedding APPL1.

Movie S1



Movie S2. A 0.2-Hz widefield microscopy movie of a Cos7 cell transfected with OCRL, TagRFP-T-Ses2, and GFP-APPL1. Spontaneously generated Ses2-positive macropinosomes shed Ses2 and acquire APPL1 on treatment (wortmannin**)** with the PI3′-kinase inhibitor wortmannin.

Movie S2