Nakatsu et al., http://www.jcb.org/cgi/content/full/jcb.201409064/DC1

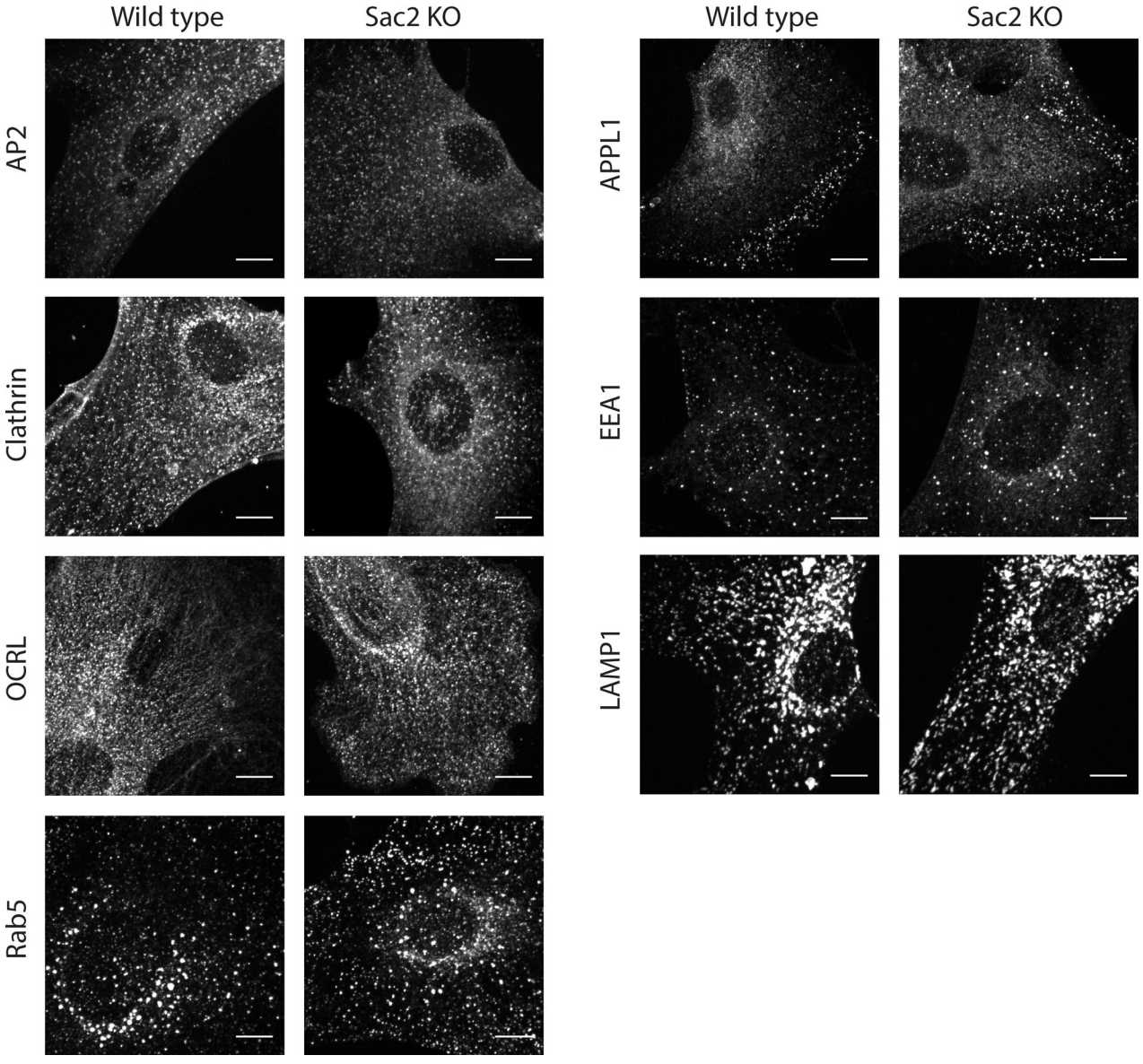


Figure S1. **Localization of endocytic proteins in WT and Sac2 KO MEFs.** Cells were immunostained for the proteins indicated. Clathrin, clathrin heavy chain; AP2, α-adaptin. The immunoreactive patterns are similar in WT and KO cells. Bars, 10 µm.

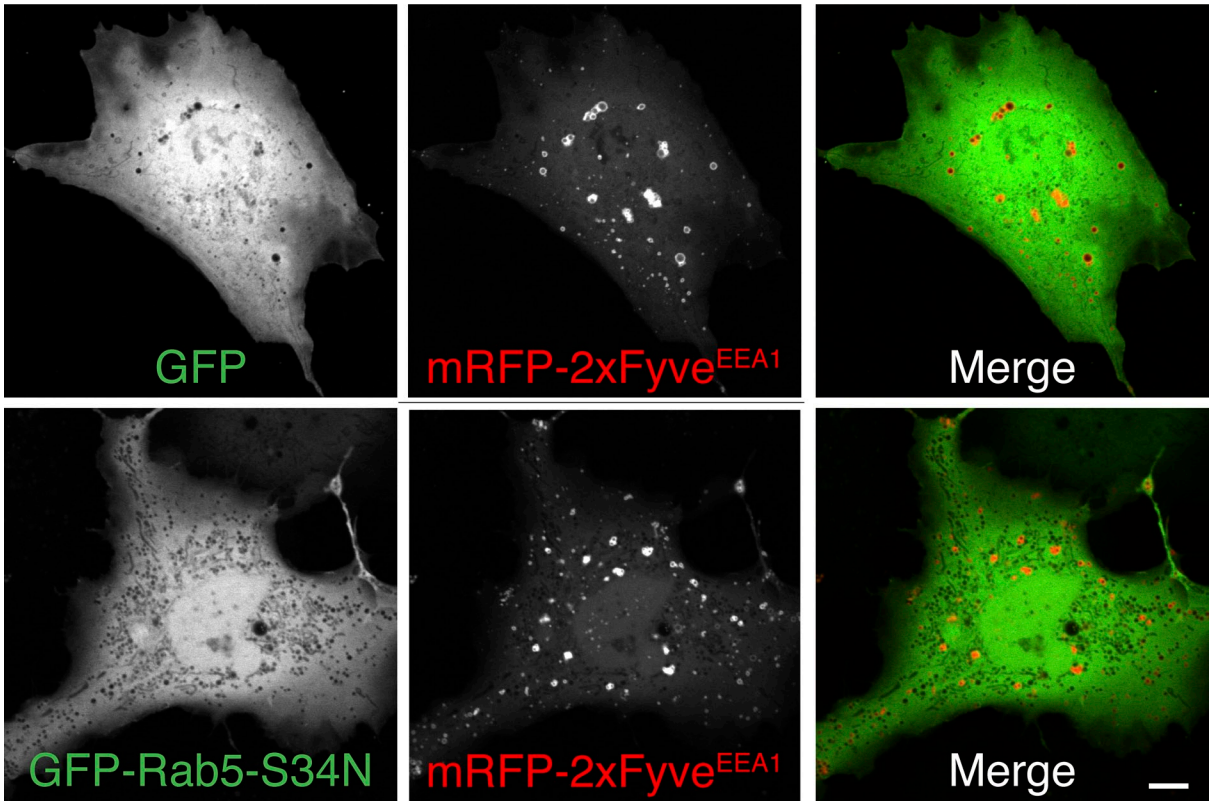
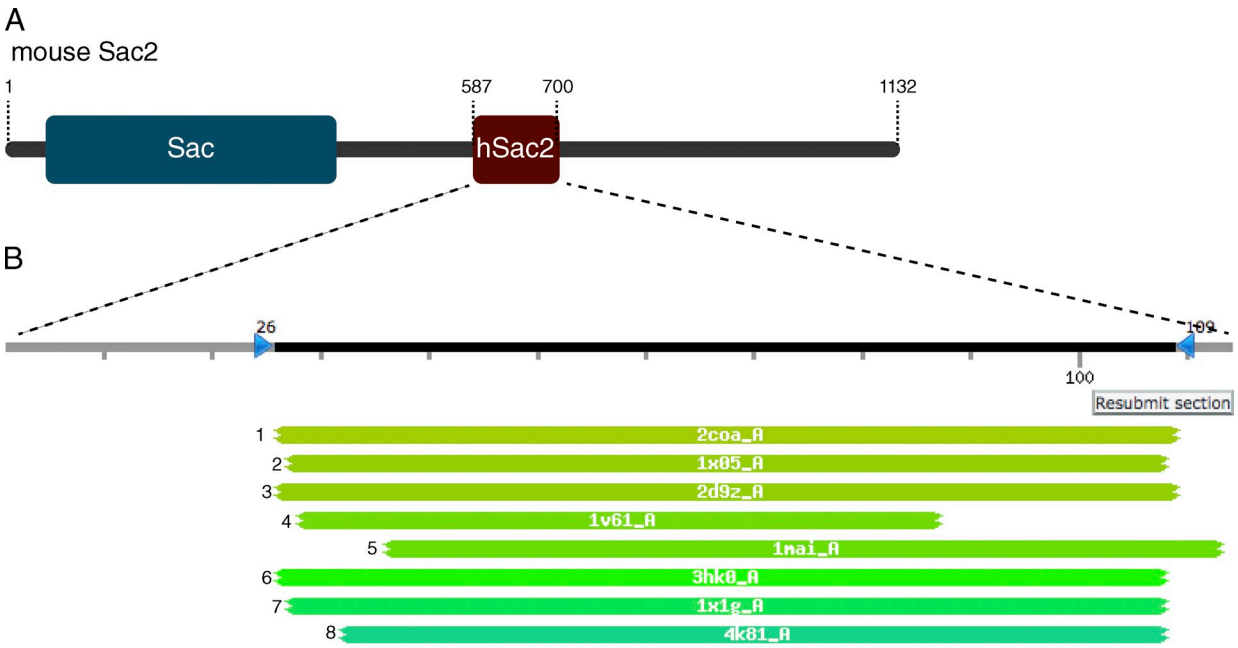


Figure S2. **PI3P-positive endosomes in control cells and in cells expressing Rab5-S34N.** The overall abundance and distribution of endosomes, as revealed by PI3P labeling with 2xFyve^{EEA1}, is similar in COS7 cells expressing GFP and in cells expressing a dominant-negative mutant of Rab5 (GFP-Rab5-S34N). Bar, 5 µm.



		Probability	E-value	P-value	Score
1: 2coa_A	PH domain from Protein Kinase C, D2 type	79.5	14	0.00041	25.3
2: 1x05_A	PH domain from Pleckstrin	78.9	8	0.00024	25.2
3: 2d9z_A	PH domain from Protein kinase C	78.9	12	0.00036	25.9
4: 1v61_A	PH domain from Rac/Cdc42 GEF6	76.9	9.5	0.00029	25.9
5: 1mai_A	PH domain from Phospholipase C delta-1	76.3	2.6	0.000078	28.4
6: 3kh0_A	PH domain from Grb10	71.2	29	0.00088	25.7
7: 1x1g_A	PH domain from Pleckstrin2	65.0	13	0.00039	23.8
8: 4k81_A	PH domain from Grb14	61.8	50	0.0015	24.4

Figure S3. **Predicted structural similarity of the hSac2 domain of Sac2 to PH domains.** (A) Domain structure of Sac2. (B) Protein domains with the greatest structural similarity to the hSac2 domain of Sac2 as predicted using the HHpred program (http://toolkit.tuebingen.mpg.de/hhpred).

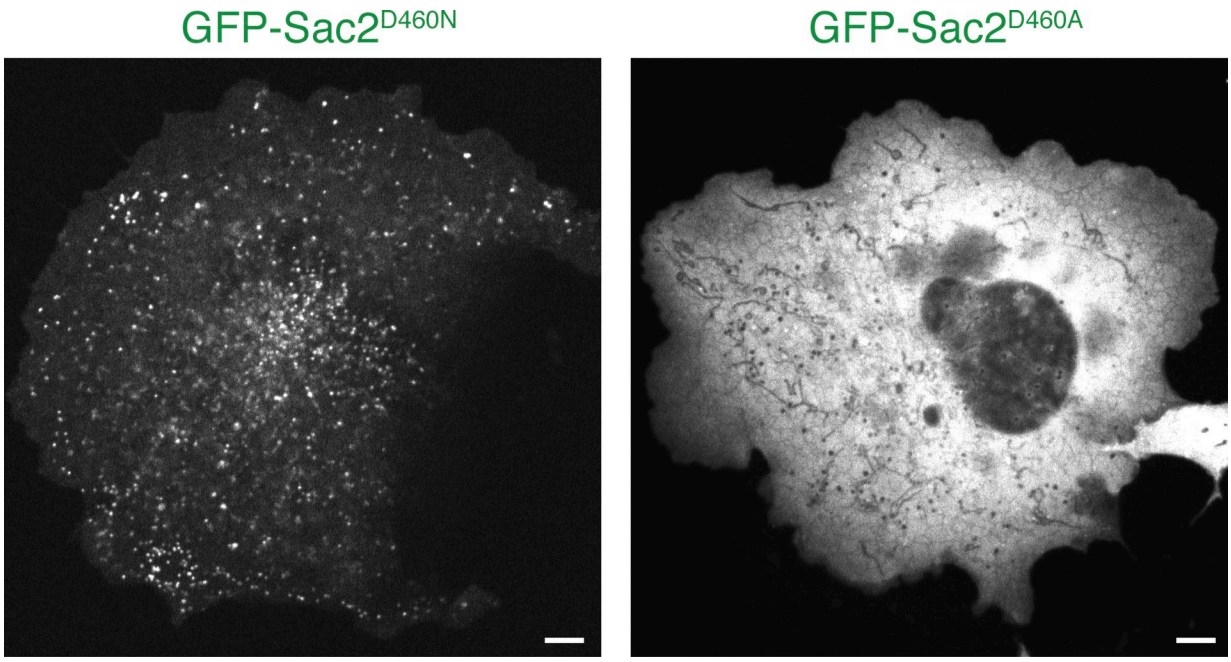


Figure S4. **The disrupting effect of the D460A mutation on the subcellular localization of Sac2.** While GFP-Sac2^{D460N} has the same punctate intracellular distribution as GFP-Sac2^{WT} (see Fig. 1), GFP-Sac2^{D460A} has a diffuse cytosolic localization. In the crystal structure of yeast Sac1, the carboxyl group of conserved aspartate D394 in the catalytic site (corresponding to D460 in mouse and human Sac2) forms a hydrogen bond with the main chain amide proton of L304, a residue conserved in mouse and human Sac2 (Manford et al., 2010). This suggests that although the replacement of the aspartate with asparagine may be compatible with folding (although not with catalytic activity), its replacement with a hydrophobic residue may result in misfolding. Bars, 5 µm.

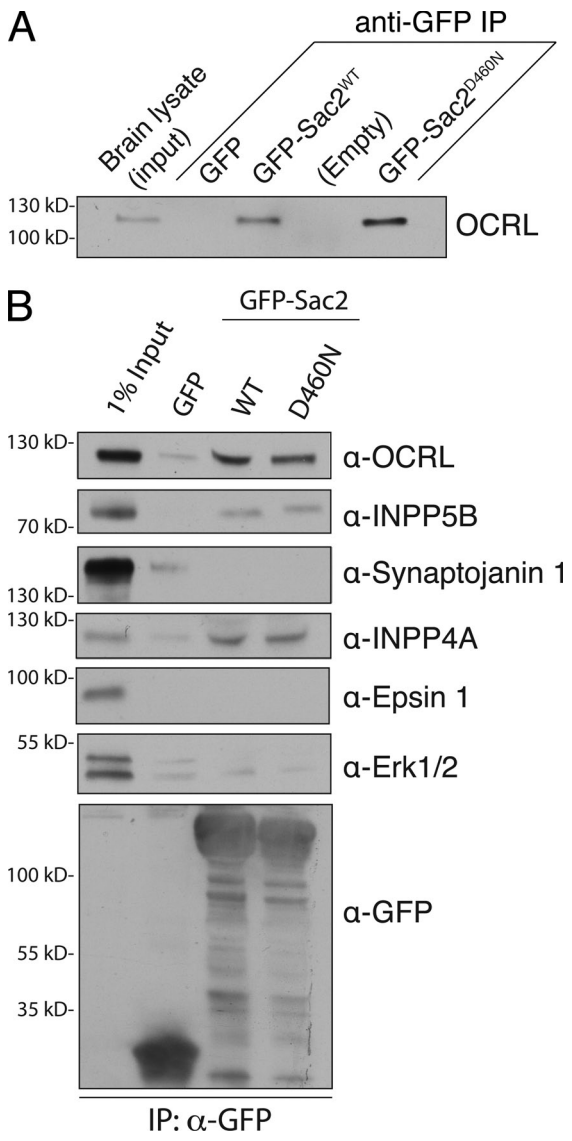
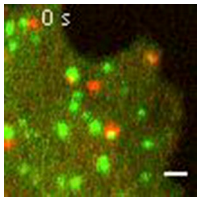


Figure S5. **Association of inositol phosphatases with Sac2 as revealed by immunoprecipitation and affinity purification.** (A) Western blotting of material used for malachite-based assay shown in Fig. 6 A, demonstrating the cosedimentation of OCRL in the anti-GFP immunoprecipitates generated from Expi293 HEK cells. (B) Lysates of COS-7 cells expressing GFP, GFP-Sac2^{WT}, or GFP-Sac2^{D460N} were subjected to anti-GFP immunoprecipitation, and the immunoprecipitates were incubated with COS7 cell lysates to explore for a further enrichment by affinity purification of inositol phosphatases. After elution, the affinity-purified material was assessed by immunoblotting using antibodies against the inositol phosphatases OCRL, INPP5B, synaptojanin 1, and INNP4A and against the control proteins epsin 1 and Erk1/2. Note that immunoprecipitates for GFP-Sac2^{WT} or GFP-Sac2^{D460N} specifically enriched OCRL, INPP5B, and INPP4A.



Video 1. **GFP-Sac2 dynamics at the late-stage clathrin-coated pits.** COS7 cells were transfected with GFP-Sac2 and CLC-mRFP. Live imaging by spinning-disk confocal microscopy (UltraView VoX system; PerkinElmer) demonstrates endocytic clathrin-coated pit dynamics showing recruitment of GFP-Sac2 (green) at clathrin-coated pits just when the clathrin signal (CLC-mRFP, red) is about to disappear. Bar, 2 µm.

Reference

Manford, A., T. Xia, A.K. Saxena, C. Stefan, F. Hu, S.D. Emr, and Y. Mao. 2010. Crystal structure of the yeast Sac1: implications for its phosphoinositide phosphatase function. *EMBO J.* 29:1489–1498. http://dx.doi.org/10.1038/emboj.2010.57