Supporting Information

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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 63x.



Fig. 54. GFP-OCRL and tagRFP-t-Ses1 colocalize on macropinosomes generated by overexpression of Hras^{V12G}. Widefield image from live cells. Magnification is $63 \times$

+ Consensus #1	والدرج والد		الاستاب الاللا	
Consensus #1				FH
Majority	PEEGGPRP	AGRPL PPRRLOGPAST.L.L	AGOSPVSPE	TSCESBLHD
najorrey				
	250 2	60 270 280	290 300	310 320
maccaca 109a	TFRPGPEP	PPPPPRRRAS	APHGPLD	VAPFARLHE 215
pan 109a	TFRPGPEP	PPPPPRRRAS	APHGPLD	MAPFARLHE 229
fam109a homo	TFRPGPEP	PPPPPRRRAS	APHGPLD	MAPFARLHE 229
tetraodon 109a	RAISQKCPTWLR	RNALITPLNGPATR	VGEWPLVDLD	PLQDFQRLHD 219
monodelphis 109a	PSLANGTLPRTGHEGAP	RPP <mark>PIPPRR</mark> KP <mark>SSG</mark> PSPAPLLVPAPL-	LVPETPFTPG	ATPFSRLHD 229
sus 109a	PATPAAAAPASL	PAPPLPPRRRAS	VP <mark>NGP</mark> LD	SVPFTQLHE 199
gallus 109a	GVDRLHDDGDGEANP	RPP <mark>PLPPRR</mark> RA <mark>S</mark> S	GDTGTAE	ISAFCRLHE 216
gallus 109b	DRD <mark>G</mark> GHT	KPP <mark>PLPPRR</mark> RS <mark>AAGS</mark> SAP	ALQESPVSPE	TTCFAKLHH 229
bos 109b	PMGLVEEGGSRP	AGRGLAEWELQGPASLLLG	RGQSPVSPE	TSCFSTLHE 229
mouse 109b	SIGTVEERGIRP	IGRDLTEWELQGPASLLLS	MGQSPVSPE	SSCFSTLHD 229
rat 109b	SMGTVEERGIRP	IGRDLTEWELQGPASLLLS	MGQSPVSPE	TSCFSTLHD 229
fam109b hsap	PVGLVEEAGSRS	AGWGLAEWELQGPASLLLG	KGQSPVSPE	TSCFSTLHD 229
tetraodon 2	RAISQKCPTWLR	RNALITPLNGPATR	V <mark>GEWP</mark> LVDLD	PLQDFQRLHD 219
macaca 109b	PVGLVEEGGSRS	SGRGLAEWELQGPASLLLG	RGQSPVSPE	TSCFSTLHD 229
monodelphis dom 109b	PRLPAPSTR	RRLADSYPWGPAGPPLN	GGDSPVSPE	TSCFSKLHG 221
ornithorhychus 109b	PNSGGPMG	EPP <mark>PLPP</mark> PRHPTGRGSLPA	LGESPVSPE	TACFARLHD 223
sus 109b	PLGLVEEGGSRP	TGRGMAEWELQGPANFLLG	RGQSPVSPE	TSCFSTLHD 229
canis 109b	PEDLVEERGSRP	AGRGLAEWQGPAGLFLG	RRQSPSLPE	TSYFSMLHN 227
ses PA	LMSKQDANGNGSPSGTPRA	QRRPTPAPAIANSVFYPDVRDPGAA	NNNHSTVNGAERQRF	RQVKALEEFARNHE 272
aedes cg12393	PVPTGHQPPSESITTSTNS	SNRPIQATTNSTSGGSALNMVDLLA	IVGDGTDGTAT	TAPSEEYTFEEMHR 262
anopheles cg12393	AAPTVHSPTVPDIGKLSPS	ADPTADGPVSRKATVTATKSSDAIA	<mark>SMAN</mark> GDQQ <mark>Q</mark> RPLEF	RGSVASFSFEAMHS 263
dpseu cg12393	LVIKQDGNG <mark>NGSP</mark> HGTPKA	HRRPAPQPVTSAFYIDHQSSETAGE	RPTVLPRN <mark>ANNNH</mark> IDSAEQKRF	RQAKAKEDFNRNHE 278
+ Consensus #1 Consensus #1 Majority	WYGQEIMELRREWLQ-RAK	QS0 P		
	330 3	40 350 360		
maccaca 100a	CYCOETRAL PCOMT S- CPU			235
nan 109a	CYGOETRALRGOWLS-SRV	OP		249
fam109a homo	CYGOETRALRGOWLS-SRV	OP		249
tetraodon 109a	YYGOOVKEAREEWRKSOGK	EEORGEGEVTDLG		251
monodelphis 109a	WYGOETLALRSEWOO-SRG	OP		249
sus 109a	WYGOEVRALRSOWLR-SOA	OT.		219
gallus 109a	RYGREVAOLRODWOH-ROC	OHLP		238
gallus 109b	WYGOEIAALROEWOE-ROK	RGLP		251
bos 109b	WYGREIMELRRAWLO-RAO	ENOPECKDODRP		259
mouse 109b	WYGKEIMELRRGWQQ-RAK	GSOTENKSONRP		259
rat 109b	WYGKEIMELRRRWQQ-RAK	GSOPESKPHNRP		259
fam109b hsap	WYGQEIVELRQCWQK-RAQ	GSI SKCEEQDRP		259
tetraodon 2	YYGQQVKEAREEWRKSKGK	EEORGEGEVTDLGVKPRTRK.		259
macaca 109b	WYGQEIMELRQCWQQ-RAQ	RSQPKCEEQDKP		259
monodelphis dom 109b	WYGQEITGLRREWLQARAL	AGI RAVKGS		249
ornithorhychus 109b	WFGQEIAELRREWRAQRRG	QS		244
sus 109b	WYGQEIMELRRVWLQ-RAQ	GSOPEHEQQDRP		259
canis 109b	WYGQEIVELRQRWLQ-RAW	ASI LERENRDGH		257
ses PA	RFRRELMPDVSAYRERQ	-GOPLIQL		296
aedes cg12393	QLGVPVLADLKARKMAMEK	SEOPLIMF		289
anopheles cg12393	ALGVPVLADLCKWNASRQE	LE <mark>G</mark> QQQQQ		290
dpseu cg12393	HFRKELMPGIVAYRATQ	-KOPLI		300

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).

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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

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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