Liu et al., http://www.jcb.org/cgi/content/full/jcb.201506081/DC1

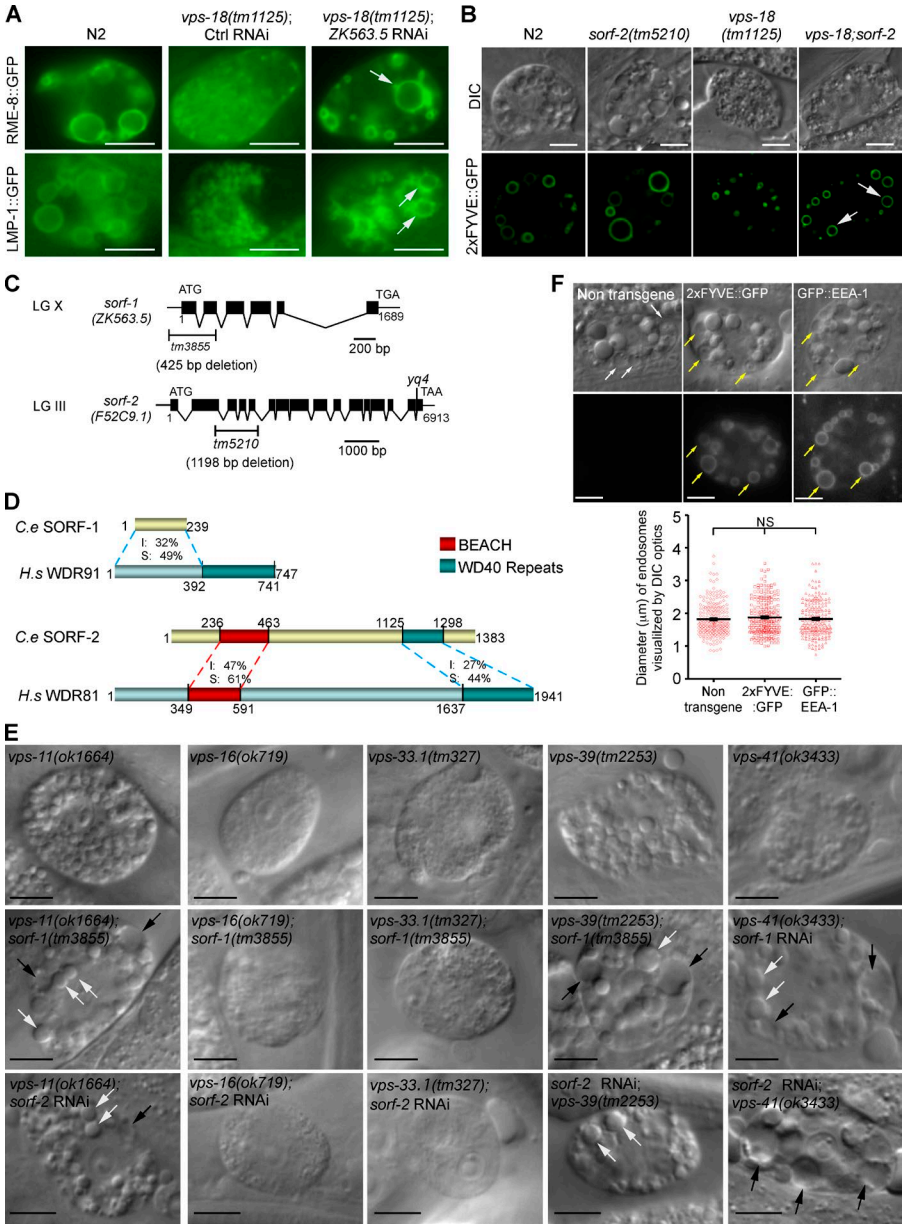


Figure S1. **Identification of *sorf-1* and *sorf-2*.** (A) RNAi of *ZK563.5* increases the sizes of endosomes (labeled with RME-8::GFP) and lysosomes (labeled with LMP-1::GFP) in *vps-18(tm1125)* coelomocytes. Arrows indicate endosomes and lysosomes in *ZK563.5* RNAi–treated *vps-18(tm1125)* coelomocytes. Ctrl, control. (B) *sorf-2(tm5210)* rescues the small endosome phenotype of *vps-18(tm1125)* coelomocytes. Endosomes are marked with 2xFYVE::GFP. DIC and fluorescence images are shown for each genotype. Arrows indicate endosomes in *vps-18(tm1125);sorf-2(tm5210)* double mutants. (C) Schematic representation of *sorf-1* and *sorf-2* genes. Solid bars represent exons, and thin lines represent introns. The *tm3855* deletion in the *sorf-1* gene and the *tm5210* deletion and *yq4* mutation in the *sorf-2* gene are indicated. (D) Schematic comparison of *C. elegans* SORF-1 and SORF-2 with human WDR91 and WDR81. The BEACH domain and WD40 repeats are indicated in different colors. The amino acid identity (I) and similarity (S) between compared protein domains are indicated. (E) Representative DIC images of coelomocytes in single CORVET/HOPS complex mutants (top row) and with simultaneous depletion of *sorf-1* [*sorf-1(tm3855)*, middle row] or *sorf-2* (*sorf-2* RNAi, bottom row). Black and white arrows indicate enlarged endosomes or lysosomes, respectively. (F) Representative DIC and fluorescence images of endosomes in coelomocytes from wild type (nontransgene) or animals carrying transgenes expressing 2xFYVE::GFP or GFP::EEA-1 (top). White and yellow arrows indicate some endosomes in nontransgenes and some GFP-positive endosomes in transgenic animals. Diameters (mean ± SEM) of endosomes were measured under DIC optics and are shown in the bottom panel. NS, no statistical significance. Each symbol represents an endosome. Bars, 5 μm.

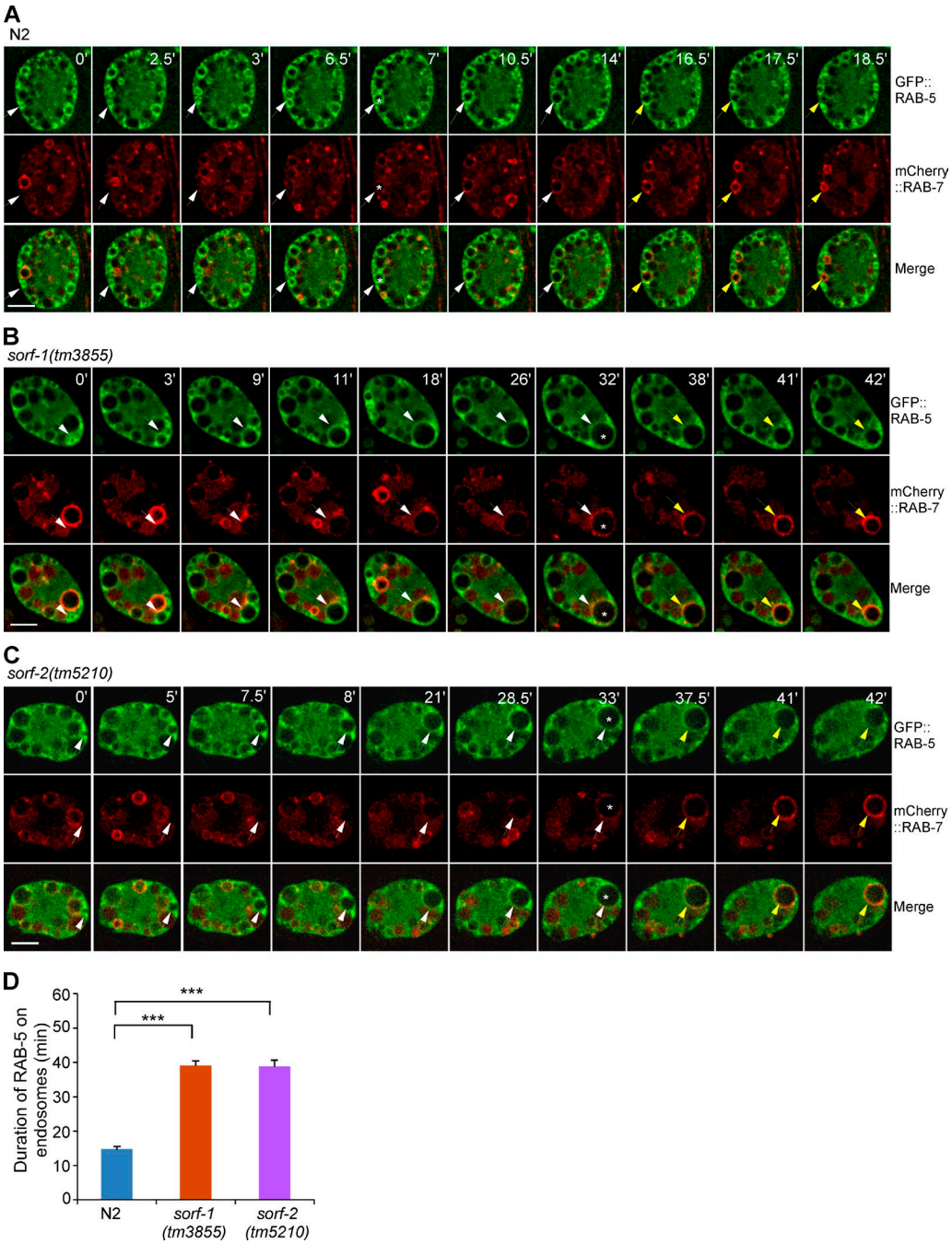
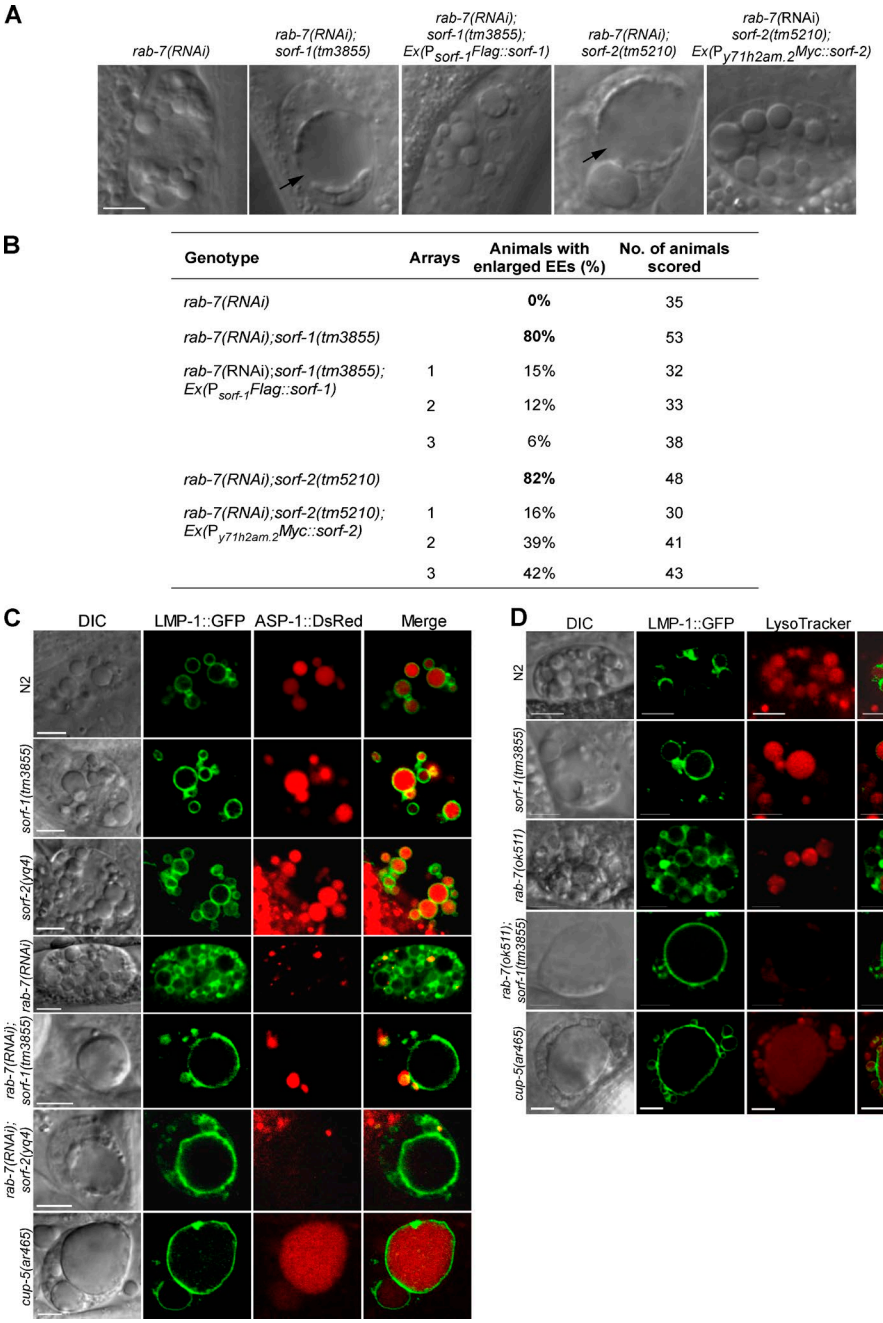


Figure S2. **Time-lapse analysis of dynamic changes of GFP::RAB-5 and mCherry::RAB-7 on endosomes in coelomocytes.** (A) N2. (B) *sorf-1(tm3855)*. (C) *sorf-2(tm5210)*. Asterisks indicate time points at which endosomes reached peak sizes. White and yellow arrows indicate the chased endosomes before and after enrichment of RAB-7, respectively. Bars, 5 µm. (D) Quantification of duration of GFP::RAB-5 on endosomes. 25 or more endosomes were examined for each genotype. ***, $P < 0.001$.



Genotype	Arrays	Animals with enlarged EEs (%)	No. of animals scored
rab-7(RNAi)		**0%**	35
rab-7(RNAi);sorf-1(tm3855)		**80%**	53
rab-7(RNAi);*sorf-1(tm3855); Ex*(P_{sorf-1}*Flag::sorf-1)*	1	15%	32
	2	12%	33
	3	6%	38
rab-7(RNAi);sorf-2(tm5210)		**82%**	48
rab-7(RNAi);sorf-2(tm5210); Ex($P_{y71h2am.2}$*Myc::sorf-2)*	1	16%	30
	2	39%	41
	3	42%	43

Figure S3. **Characterization of giant endosomes induced by loss of *rab-7* and *sorf-1* or *sorf-2*.** (A) Representative DIC images of coelomocytes in *rab-7* RNAi–treated animals as indicated. Arrows indicate enlarged endosomes. Bar, 5 µm. (B) Quantification of the rescuing effects of *rab-7 RNAi*-treated *sorf-1(tm3855)* and *sorf-2(tm5210)* mutants by Flag::SORF-1 and Myc::SORF-2 transgenes. Animals with at least one coelomocyte showing abnormally enlarged endosomes were scored. Three independent transgenic arrays were analyzed for each transgene. EEs, early endosomes. (C) Representative images of the labeling of endocytic organelles by LMP-1::GFP and ASP-1::DsRed in N2, *sorf-1(tm3855)*, *sorf-2(yq4)*, *rab-7(RNAi)*, *rab-7(RNAi);sorf-1(tm3855)*, *rab-7(RNAi);sorf-2(yq4)*, and *cup5(ar465)* mutants. Both DIC and fluorescence images are shown for each genotype. In N2, *sorf-1(tm3855)*, and *sorf-2(yq4)* coelomocytes, LMP-1::GFP-positive organelles are also labeled with ASP-1::DsRed, indicating that these organelles are lysosomes. LMP-1::GFP-positive organelles in *rab-7(RNAi)* coelomocytes and the abnormally enlarged organelles in *rab-7(RNAi);sorf-1(tm3855)* and *rab-7(RNAi);sorf-2(yq4)* mutants are, however, not positive for ASP-1::dsRed, suggesting that they are not lysosomes. As a positive control, the abnormally enlarged LMP-1::GFP-positive lysosomes are also positive for ASP-1::DsRed in *cup-5(ar465)* mutants. (D) Representative images of LysoTracker red staining of LMP-1::GFP-positive organelles in N2, *sorf-1(tm3855)*, *rab-7(ok511)*, *rab-7(ok511);sorf-1(tm3855)*, and *cup-5(ar465)* coelomocytes. Note that LMP-1::GFP-positive organelles in *rab-7(ok511)* mutants are only partially positive for LysoTracker red staining, and the giant LMP-1::GFP-positive organelles are negative for LysoTracker red in *rab-7(ok511);sorf-1(tm3855)* coelomocytes. This contrasts with the LMP-1::GFP-positive vesicles in N2, *sorf-1(tm3855)* and *cup-5(ar465)* cells, which show strong LysoTracker staining. Bars, 5 µm.

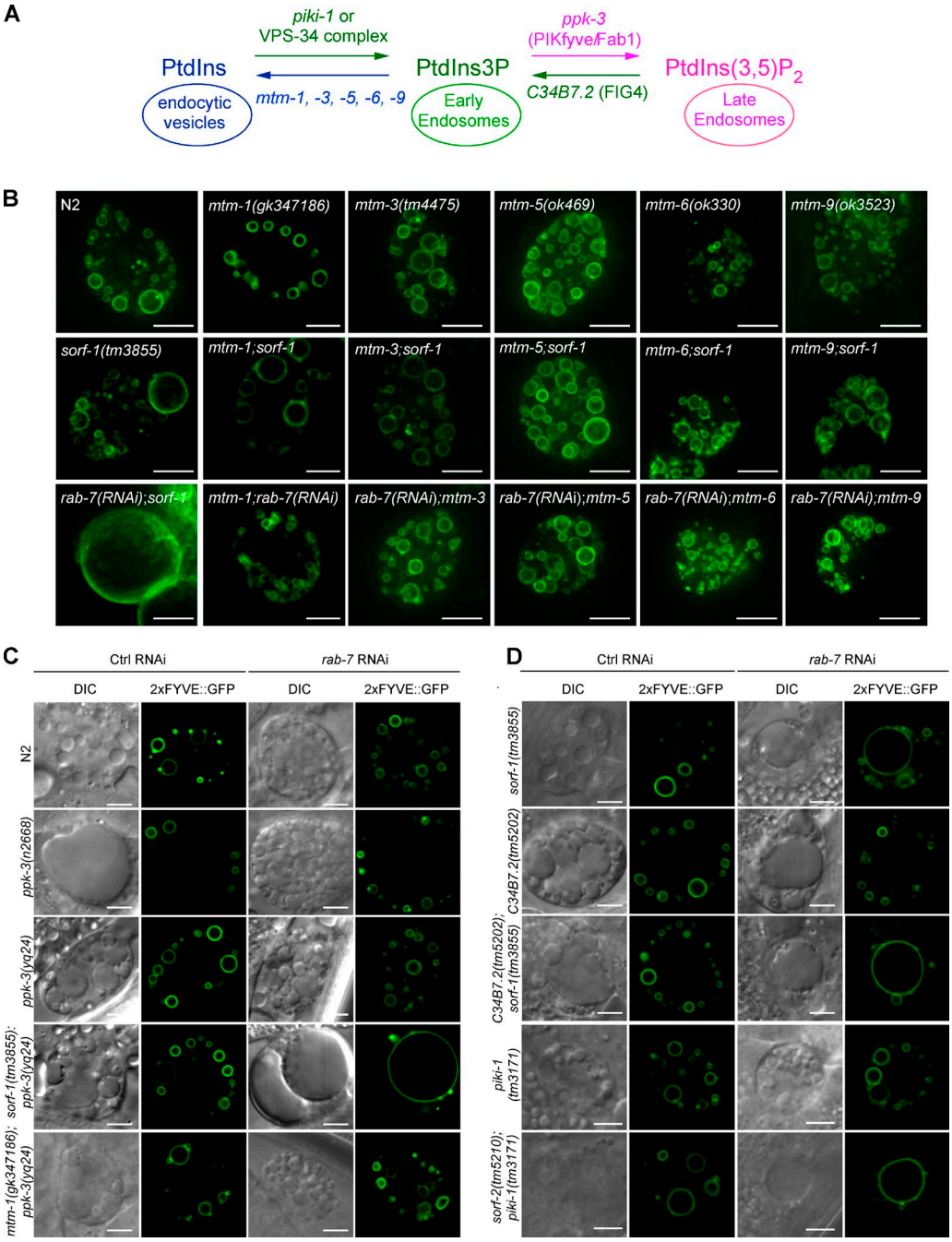


Figure S4. **Genetic interaction analysis of *sorf-1* or *sorf-2* with genes involved in endosomal PtdIns3P metabolism.** (A) Summary of endosomal PtdIns3P metabolism mediated by phosphoinositide kinases and phosphatases. (B) Representative confocal images of 2xFYVE::GFP-labeled endosomes in coelomocytes of strong loss-of-function mutants of *mtm-1*, *-3*, *-5*, *-6*, and *-9* (top row) and with simultaneous depletion of *sorf-1* (*sorf-1(tm3855)*; middle row) or *rab-7* (*rab-7* RNAi; bottom row). Images of 2xFYVE::GFP-labeled endosomes in coelomocytes are also shown for N2, *sorf-1(tm3855)*, and *rab-7(RNAi);sorf-1(tm3855)* animals. (C) Representative DIC and fluorescence (2xFYVE::GFP) images of endosomes in coelomocytes of N2, *ppk-3(n2668)*, *ppk-3(yq24)*, *ppk-3(yq24);sorf-1(tm3855)*, and *mtm-1(gk347186);ppk-3(yq24)* animals. Animals were treated with control (Ctrl) RNAi and *rab-7* RNAi. (D) Representative DIC and fluorescence (2xFYVE::GFP) images of endosomes in coelomocytes of N2, *sorf-1(tm3855)*, *C34B7.2(tm5202)*, *C34B7.2(tm5202);sorf-1(tm3855)*, *piki-1(tm3171)*, and *sorf-2(tm5210);piki-1(tm3171)* animals. Animals were treated with control RNAi and *rab-7* RNAi. Bars: (B–D) 5 µm.

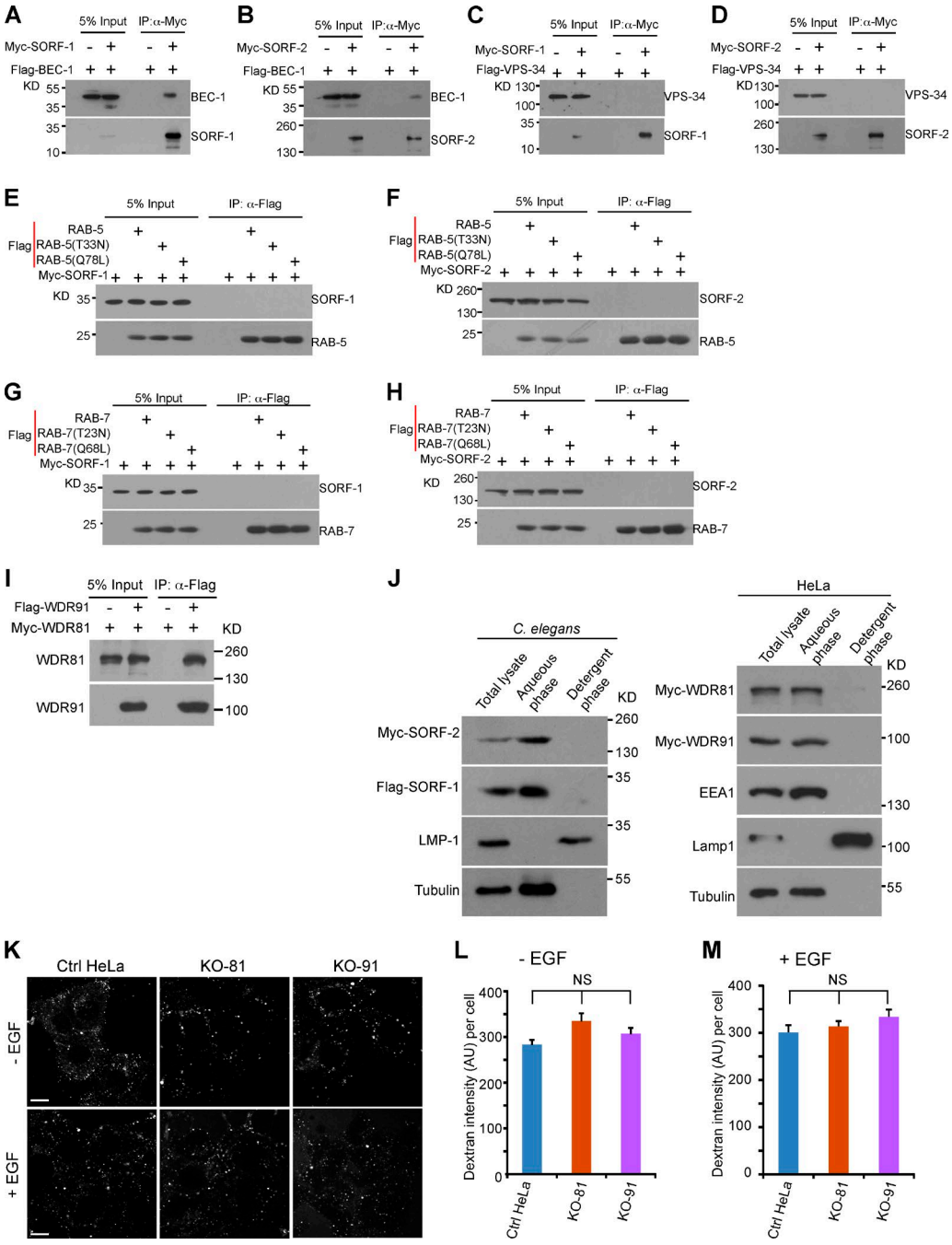
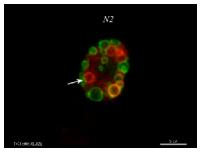
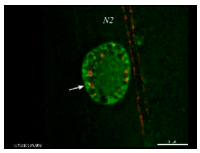


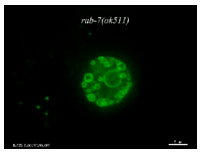
Figure S5. **Interaction analysis of SORF-1 or SORF-2 with BEC-1, VPS-34, RAB-5, or RAB-7 and characterization of human WDR91 and WDR81.** (A and B) Flag-BEC-1 was coexpressed with Myc-SORF-1 (A) or Myc-SORF-2 (B) in HEK293 cells and immunoprecipitated with Myc antibody. (C and D) Flag-VPS-34 was coexpressed with Myc-SORF-1 (C) or Myc-SORF-2 (D) in HEK293 cells and immunoprecipitated with Myc antibody. (E and F) Co-IP of Flag-tagged RAB-5 or its GDP-bound form (T33N) or GTP-bound form (Q78L) with Myc-SORF-1 (E) or Myc-SORF-2 (F). (G and H) Co-IP of Flag-tagged RAB-7 or its GDP-bound form (T23N) or GTP-bound form (Q68L) with Myc-SORF-1 (G) or Myc-SORF-2 (H). In E–H, proteins were coexpressed in HEK293 cells and IPs were performed with Flag antibody. Precipitated proteins were detected with Flag and Myc antibodies in all panels. (I) Myc-WDR81 coimmunoprecipitated with Flag-WDR91. Protein expression and IPs were performed as in A–H. (J) Phase separation analysis of *C. elegans* SORF-1 and SORF-2 (left) and mammalian WDR91 and WDR81 (right). LMP-1 and Lamp1 were used as the membrane protein controls in *C. elegans* and HeLa cells, respectively. (K–M) Knockout of WDR91 or WDR81 does not affect macropinocytosis. Control (Ctrl), KO-91, and KO-81 HeLa cells were incubated with dextran 405 (1 mg/ml) without or with EGF (50 ng/ml) for 1 h or 10 min, respectively. After extensive washing, cells were fixed and imaged for internalized cargos (M). Bars, 5 µm. Quantifications of internalized dextran 405 are shown in L and M. 80 or more cells were analyzed for each treatment. Data were generated from three independent experiments. AU, arbitrary units; NS, no statistical significance.



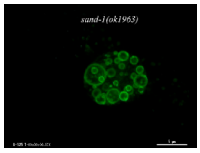
Video 1. **Time-lapse monitoring of early-to-late endosome conversion in coelomocytes of N2, *sorf-1(tm3855)*, *sorf-2(tm5210)*, and *sorf-1;sorf-2* animals.** Early and late endosomes were labeled with 2xFYVE::GFP and mCherry::RAB-7, respectively. Images were taken every 30 s with a *Z*-series of 30 sections (0.5 µm/section) for 1 h using a DeltaVision microscopy system (DV Elite). Video was generated by projection of 20 sections. Image frames are shown at 8 fps. Images shown in Fig. 3 (A–D) were derived from this image sequence.



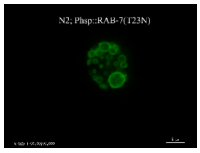
Video 2. **Time-lapse recording of endosomal RAB-5-to-RAB-7 switch in N2, *sorf-1(tm3855)*, and *sorf-2(tm5210)* coelomocytes.** GFP::RAB-5 and mCherry::RAB-7 were used to label early and late endosomes, respectively. Images were taken as described previously, and video was generated by projection of five sections. Image frames are shown at 8 fps. Images shown in Fig. S2 (A–C) were derived from this image sequence.



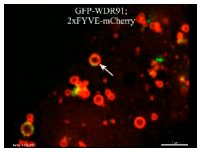
Video 3. **Time-lapse recording of endosomal PtdIns3P indicated by 2xFYVE::GFP in *rab-7(ok511)*, *rab-7(ok511);sorf-1(tm3855)*, and *rab-7(ok511);sorf-2(tm5210)* coelomocytes.** Images were taken every 30 s with a *Z*-series of 30 sections (0.5 µm/section) for 2 h. Video was generated by projection of 30 sections. Image frames are shown at 10 fps. Images shown in Fig. 5 D were derived from this image sequence.



Video 4. **Time-lapse recording of endosomal PtdIns3P indicated by 2xFYVE::GFP *sand-1(ok1963)*, *sand-1;sorf-1*, and *sand-1;sorf-2* coelomocytes.** Images and video were generated as in Video 3. Images shown in Fig. 5 E were derived from this image sequence.



Video 5. **Time-lapse recording of fusion of early endosomes indicated by 2xFYVE::GFP in coelomocytes of N2, *sorf-1(tm3855)*, and *sorf-2(tm5210)* animals expressing P_{hsp}::RAB-7(T23N).** Images were taken every 30 s with a *Z*-series of 30 sections (0.5 µm/section) for 4 h. Video was generated by projection of 30 sections. Image frames are shown at 20 fps. Images shown in Fig. 6 (A–C) were derived from this image sequence.



Video 6. **Live-cell imaging of dynamic changes of PtdIns3P (2xFYVE-mCherry) and GFP-WDR91 or GFP-WDR81 on endosomes.** HeLa cells were transiently transfected with vectors expressing 2xFYVE-mCherry and GFP-WDR91 or GFP-WDR81 and maintained at 37°C in glass-bottom dishes placed in a humidified chamber (Chamlide) supplemented with 5% CO_2. 12 h later, images were taken every 30 s with a *Z*-series of 20 sections (0.4 µm/section) for 90 min using DV Elite. Video was generated by projection of five sections. Image frames are shown at 10 fps. Images shown in Fig. 8 D were derived from this image sequence.

Table S1. **WormBase ID of mutants and transgenic arrays**

Linkage group	Mutants or transgenes	WormBase ID
LGI	*mtm-1(gk347186)*	WBVar00636319
LGI	*C34B7.2(tm5202)*	WBVar00317442
LGII	*rab-7(ok511)*	WBVar00091798
LGII	*vps-11(ok1664)*	WBVar00092872
LGII	*vps-18(tm1125)*	WBVar00250142
LGII	*tbc-2(tm2241)*	WBVar00251159
LGIII	*mtm-3(tm4475)*	WBVar02143901
LGIII	*vps-33.1(tm327)*	WBVar00249375
LGIII	*vps-16(ok719)*	WBVar00092001
LGIII	*sorf-2(tm5210)*	WBVar00317463
LGIV	*sand-1(ok1963)*	WBVar00093157
LGIV	*bec-1(ok700)*	WBVar00091984
LGV	*unc-76(e911)*	WBVar00143591
LGV	*mtm-9(ok3523)*	WBVar00094510
LGV	*vps-39(tm2253)*	WBVar00251168
LGX	*vps-41(ok3433)*	WBVar00296633
LGX	*mtm-5(ok469)*	WBVar00091759
LGX	*mtm-6(ok330)*	WBVar00091628
LGX	*ppk-3(n2668)*	WBVar00090540
LGX	*piki-1(tm3171)*	WBVar00251938
LGX	*sorf-1(tm3855)*	WBVar00252449
LGX	*Y34B4A.2(gk280145)*	WBVar00503388
—	*cdIs85*($P_{unc-122}$*2xfyve::gfp*)	WBTransgene00000251
—	*cdIs97*($P_{unc-122}$*mCherry::cup-5*)	WBTransgene00000252
—	*cdIs131*($P_{unc-122}$*gfp::rab-5*)	WBTransgene00000254
—	*bIs34*(P_{rme-8}*gfp::rme-8*)	WBTransgene00000153
—	*pwIs50*(P_{lmp-1}*lmp-1::gfp*)	WBTransgene00001789
—	*pwIs126*(P_{eea-1}*gfp::eea-1*)	WBTransgene00001804
—	*opIs334*(P_{ced-1}*yfp::2xfyve*)	WBTransgene00001421
—	*tmIs225* (P_{asp-1}*asp-1::dsRed*)	—
—	*qxIs605* (P_{vps-34}*Flag::vps34*)	—

—, not applicable.

Table S2. ***C. elegans* expression constructs**

Vector	Promoter (insertion sites)	Gene (insertion sites)	Backbone
P_{sorf-1}Flag-*sorf-1*	*sorf-1* promoter (2 kb; SphI–BamHI)	*sorf-1* ORF with Flag (BamHI–NheI)	pPD49.26
$P_{y71h2am.2}$Myc-*sorf-2*	*y71h2am.2* promoter (5 kb; SphI–XbaI)	*sorf-2* ORF with Myc (XmaI)	pPD49.26
P_{ctns-1}*mCherry::rab-7*	*ctns-1* promoter (2.8 kb; BamHI)	*rab-7* ORF (SacI)	pPD49.26-mcherry
P_{hsp}*::rab-7(T23N)*	*hsp-16* promoter	*rab-7* ORF (T23N) (KpnI–EcoRV)	pPD49.78, pPD49.83
P_{cc1}*cfp::2xfyve*	*unc-122* promoter (800 bp; BamHI)	2xFYVE (Hrs) (SacI)	pPD49.26-cfp
P_{ctns-1}*rme-8::mCherry*	*ctns-1* promoter (2.8 kb; BamHI)	*rme-8* cDNA (XmaI)	pPD49.26-mcherry
P_{ctns-1}*yfp::bec-1*	*ctns-1* promoter (2.8 kb; BamHI)	*bec-1* ORF (KpnI)	pPD49.26-yfp

Table S3. **Bacterial and mammalian expression constructs**

Vector	Gene (cDNA)	Insertion site	Backbone
pET28a-SORF-2	SORF-2	SalI–NotI	pET28a
pET28a-SORF-1	SORF-1	BamHI–XhoI	pET28a
pMALc2X-SORF-1	SORF-1	EcoRI–SalI	pMALc2X
pCMV-MycSORF-1	SORF-1	EcoRI–SalI	pCMV-Myc
pCMV-MycSORF-2	SORF-2	SalI–NotI	pCMV-Myc
pCMV-mycSORF-2 (1–498)	SORF-2 (1–498)	XbaI–PstI	pCMV-Myc
pCMV-mycSORF-2 (491–1273)	SORF-2 (491–1273)	XbaI–PstI	pCMV-Myc
pCMV-mycSORF-2 (1120–1383)	SORF-2 (1120–1383)	XbaI–PstI	pCMV-Myc
pCMV-Tag2B-SORF-1	SORF-1	BamHI–XhoI	pCMV-Tag2B
pCMV-Tag2B-RAB-5	RAB-5	EcoRI–XhoI	pCMV-Tag2B
pCMV-Tag2B- RAB-7	RAB-7	EcoRI–XhoI	pCMV-Tag2B
pEGFP-c1-SORF-1	SORF-1	XhoI–BamHI	pEGFP-c1
pEGFP-c1-WDR91	WDR91	XhoI–EcoRI	pEGFP-c1
pEGFP-c1-WDR81	WDR81	XhoI–XbaI	pEGFP-c1
pCMV-Tag2B-WDR91	WDR91	XhoI–EcoRI	pCMV-Tag2B
pCMV-Tag3B-WDR91	WDR91	XhoI–BamHI	pCMV-Tag3B
pCMV-Tag3B-WDR91	WDR81	XhoI–BamHI	pCMV-Tag3B