Supplemental Fig. 1

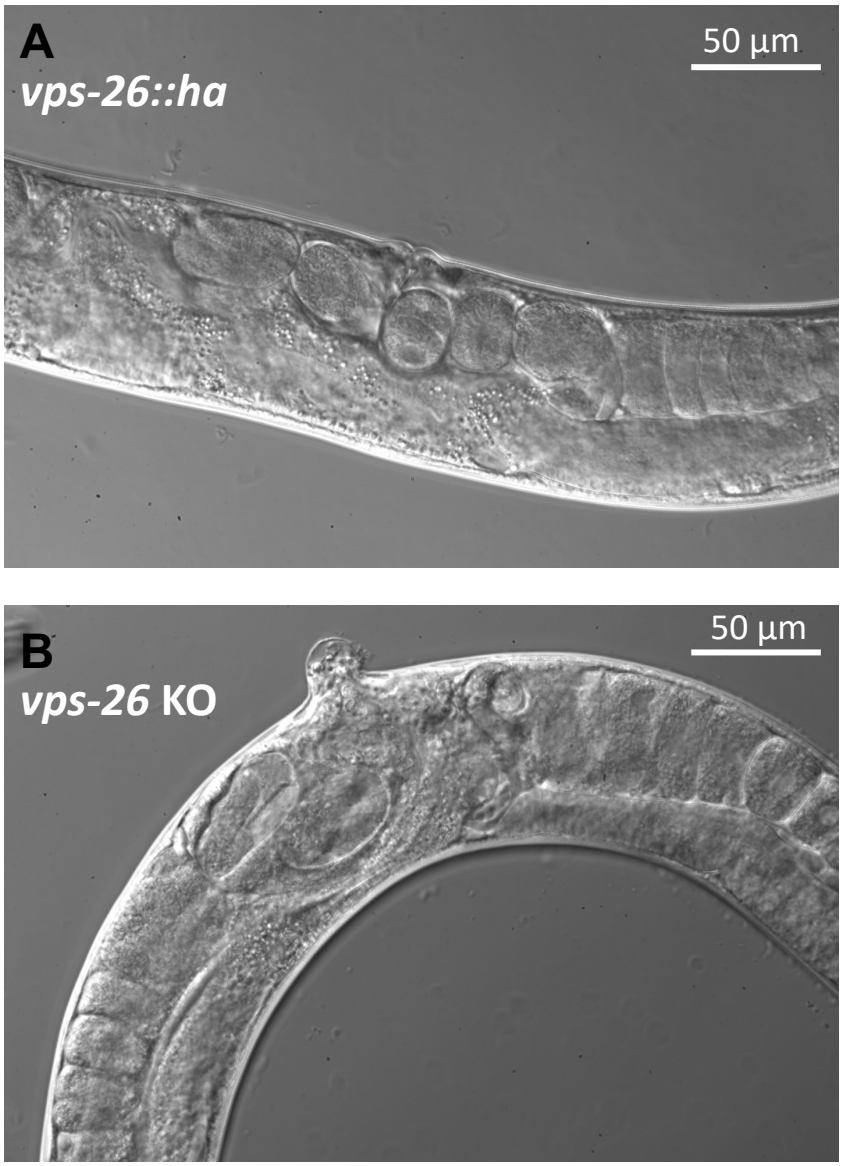

Fig. S1. Effect of *vps-26* knockout on vulva development in adult worms. (A) A representative DIC image of a normal adult *vps-26::ha* vulva. (B) A representative DIC image of an abnormal protruding vulva that is often seen in *vps-26* knockout worms.

Supplemental Fig. 2

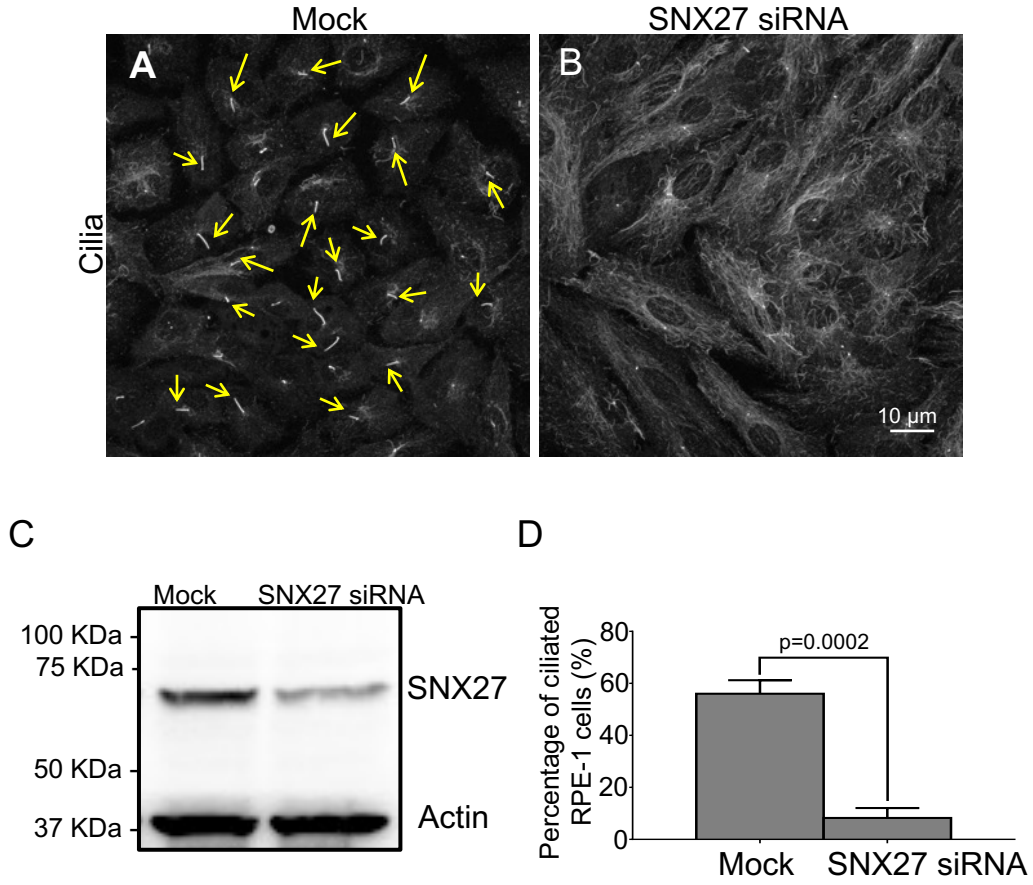

Fig. S2. SNX27 is required for normal ciliogenesis. (A-D) RPE-1 cells were Mock-treated (A) or SNX27-siRNA-treated (B) for 48 h, and serum-starved for 24 h to induce ciliogenesis. Cells were then fixed and immunostained with acetylated-tubulin to identify cilia/centrioles. Compared to Mock-treated cells (A), fewer cilia were generated upon depletion of SNX27 (B). (C) SNX27 siRNA-depletion efficacy was determined by immunoblotting. (D) The percentages of ciliated RPE-1 cells from either Mock- or siRNA-treated cover-slips were quantified and presented as a bar graph. The p-value was calculated for comparison between Mock- and siRNA-treated cells; n=3 experiments (>100 cells quantified for each experiment). Error bars denote standard deviation.

Supplemental Fig. 3

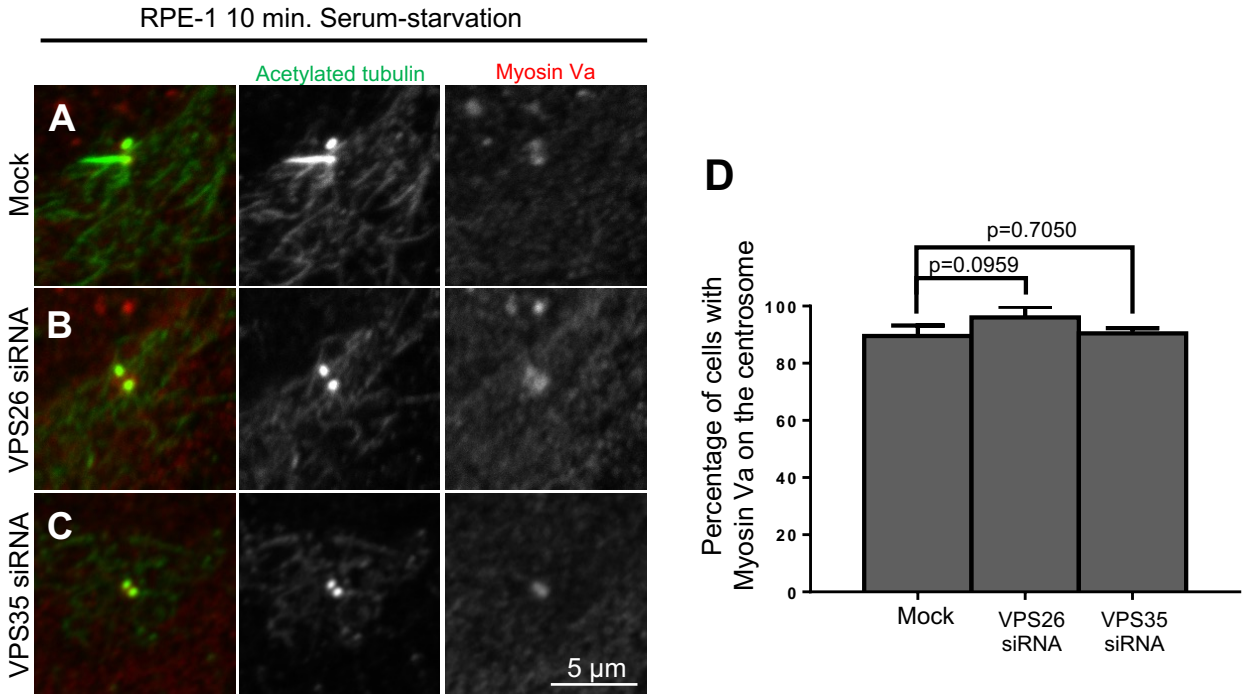

Fig. S3. Recruitment of preciliary vesicles to the centrosome is independent of the retromer. (A-D) RPE-1 cells were Mock-treated (A), VPS26-siRNA-treated (B), or VPS35-siRNA-treated (C), and serum-starved for 10 min to induce ciliogenesis. Immunoblots demonstrating siRNA depletion of these proteins is shown in Fig. 6. Cells were then fixed and immunostained with antibodies to acetylated-tubulin (green) to identify cilia/centrioles and Myosin Va (red) to mark preciliary vesicles. Myosin Va was detected on the centrosome in (A) Mock-treated, (B) VPS26-depleted and (C) VPS35-depleted cells. (D) The bar graph indicates the percentage of cells showing recruitment of Myosin Va onto cilia/centrioles. n=3 experiments (>100 cells quantified for each experiment). Error bars denote standard deviation.

Supplemental Fig. 4

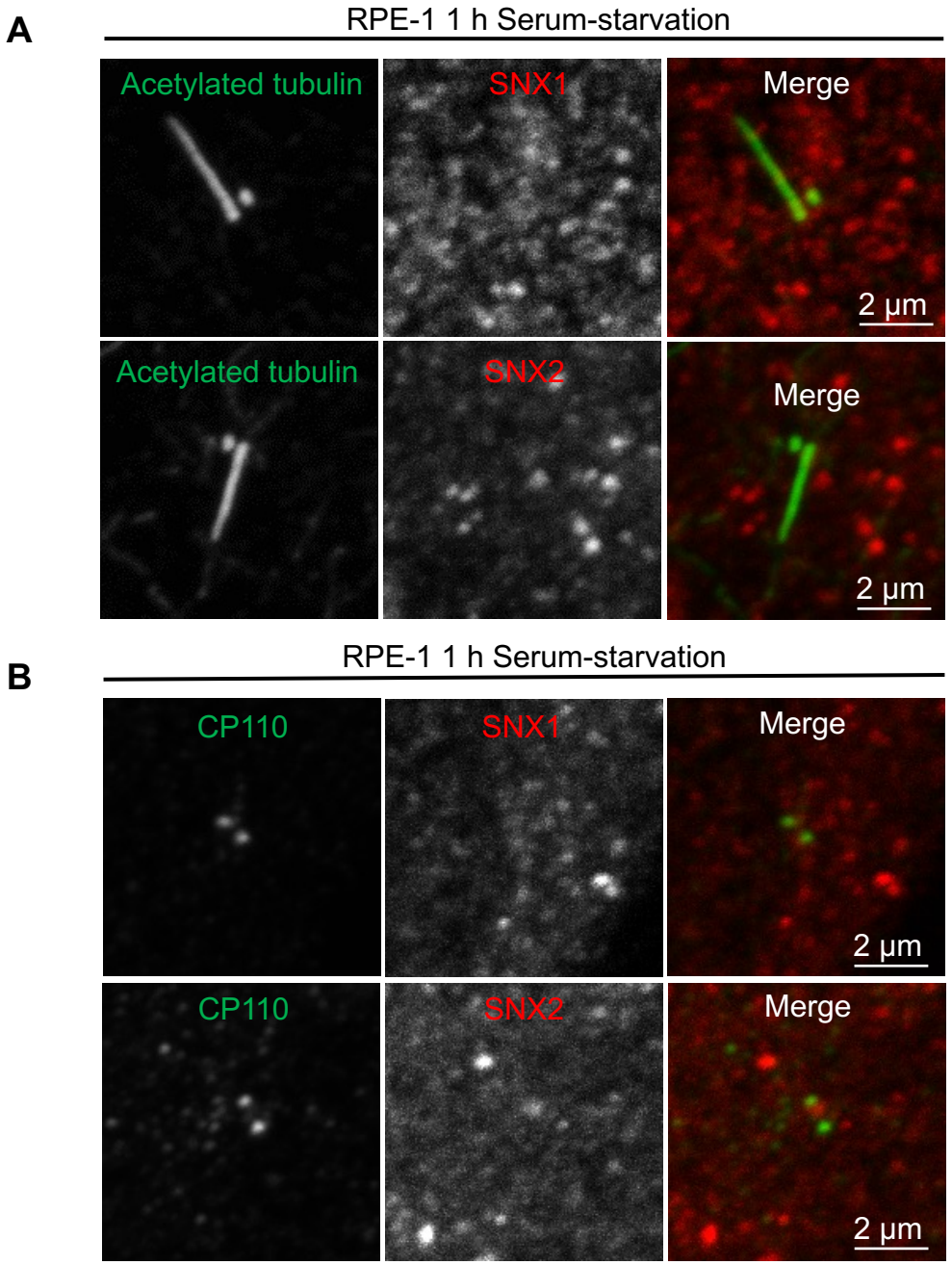

Fig. S4. SNX1 and SNX2 display minimal co-localization with cilia/basal bodies or CP110. (A) RPE-1 cells were fixed and immunostained using anti-acetylated-tubulin antibodies (green) and anti-SNX1 or anti-SNX2 antibodies (red). (B) RPE-1 cells were fixed and immunostained using anti-CP110 antibodies (green) and anti-SNX1 or anti-SNX2 antibodies (red). Immunofluorescence images were obtained by confocal microscopy. (C-D) Lack of CP110 co-immunoprecipitation with SNX1 and SNX2. RPE-1 cell lysates were subjected to immunoblotting (Input) or immunoprecipitation using anti-CP110 antibodies or rabbit anti-GFP antibodies (negative control). Immunoprecipitated proteins were immunoblotted with anti-CP110 (C and D), anti-SNX1 (C), and anti-SNX2 antibodies (D).

Table S1.

STRAIN	GENOTYPE	METHOD	RESOURCE
N2	WT	N/A	CGC
IYR010	*vps-26(luv10[vps-26::ha]) IV*	CRISPR	This study
IYR021	*vps-26(luv21) IV*	CRISPR	This study
PY6100	*oyIs59 Is[osm-6p::gfp] III*	N/A	Piali Sengupta Lab
IYR025	*oyIs59 Is[osm-6p::gfp] III; vps-26(luv21) IV*	Crossing	This study

Table S2.

Gene	Type of oligo	Sequence (5' to 3')
dpy-10 (Paix et al, 2015)	co-CRISPR crRNA	GCUACCAUAGGCACCACGAG
vps-26	KO crRNA#1	ACAAUAAAUUUCACAUUUAC
vps-26	KO crRNA#2	AUGGCGAUGCUUUUCGGCUU
vps-26	C-terminal HA-tag crRNA	GAAGAAUCAGAAUUAUCGUC
dpy-10 (Arribere et al, 2014)	*dyp-10(cn64)* repair oligo	CACTTGAACTTCAATACGGC
		AAGATGAGAATGACTGGAAA
		CCGTACCGCATGCGGTGCCTA
		TGGTAGCGGAGCTTCACATGG
		CTTCAGACCAACAGCCTAT
vps-26	KO repair oligo	GTTTATTTTCTGGAAAATAA
		ACAATAAATTTCACATTTAC
		TAAGTAGCCAATCAGCAGAA
		ATTCAAATTCGGCTCTCAAAT
		GAGGAT
vps-26	HA repair oligo	AATCGCCAAGATCGGATCCA
		AAAAGTGGATCAACAAGTCC
		TGATGACAACAGTGACAGTA
		GTTACCCATATGATGTTCCAG
		ATTACGCTTAGAGATAGAGAT

		AGTATTTCGATGCAATTAAAT
		CATTTT
vps-26	KO screening primer 1	CCTTGGGATGAAGCAGTTCC
vps-26	KO screening primer 2	TCCAGTAACTGATTCTCCATC
vps-26	HA screening primer 1	GGAAGTAACTCTCTGGCGAA
vps-26	HA screening primer 2	GAGAAACAAAACAAACGGGG

Table S3.

Antibodies	Host	Manufacturer	Catalogue#	Application	Dilution
Acetylated-tubulin	Mouse	Sigma	T7451	IF	1:100
Acetylated-tubulin	Rabbit	Cell signaling	5335	IF	1:100
Alpha tubulin	Mouse	Santa Cruz Biotechnology	sc-32293	IB, IF	1:200
CP110	Rabbit	Protein Tech	12794-1-AP	IF, IB,IP	1:200 (IF) 1:2000(IB)
Myosin Va	Rabbit	Novus	NBP1-92156	IF	1:500
GFP	Mouse	Roche	1184460001	IF	1:200
GFP	Rabbit	Pierce	PA1-980-A	IP	
HA	Rabbit	Cell Signaling	3724S	IB, IF	1:1000
Actin	Mouse	Novus	NB600-535	IB	1:5000
MICAL-L1	Mouse	Novus	H00085377-B01P	IF	1:200
MICAL-L1	Rabbit	LifeTein	RB1794	IB	1:1000
GAPDH-HRP	Mouse	Protein Tech	HRP60004	IB	1:2000
VPS26	Rabbit	Abcam	Ab23892	IB	1:1000
VPS35	Rabbit	Abcam	Ab157220	IF,IB	1:200 (IF) 1:1000 (IB)
SNX1	Rabbit	Novus	NBP2-13359	IF, IB	1:200 (IF) 1:500
SNX2	mouse	BD Transduction Laboratories	611308	IF, IB	1:200 (IF) 1:500
SNX5	Rabbit	Abcam	Ab180520	IB	1:500
SNX27	Mouse	Abcam	Ab77799	IB	1:800
Mouse HRP light chain only	Goat	Jackson	115-035-174	IB	1:7000
Rabbit HRP	Donkey			IB	1:5000
Mouse IRDye 680 RD	Goat	LI-COR	926-68070	IB	1:14000
Rabbit IRDye 800CW	Donkey	LI-COR	926-32213	IB	1:14000

Mouse Alexa 488	**Goat**	**Molecular Probe**	**A11029**	**IF**	**1:500**
Rabbit Alexa 568	**Goat**	**Molecular Probe**	**A11036**	**IF**	**1:500**
Mouse Alexa 568	**Rabbit**	**Molecular Probe**	**A11061**	**IF**	**1:500**
RabbitAlexa488	**Goat**	**Molecular Probe**	**A11034**	**IF**	**1:500**
Mouse Alexa 568	**Goat**	**Thermo Fisher**	**A-11004**	**IF**	**1:1000**
Rabbit Alexa 488	**Goat**	**Thermo Fisher**	**A-11034**	**IF**	**1:1000**