Expanded View Figures

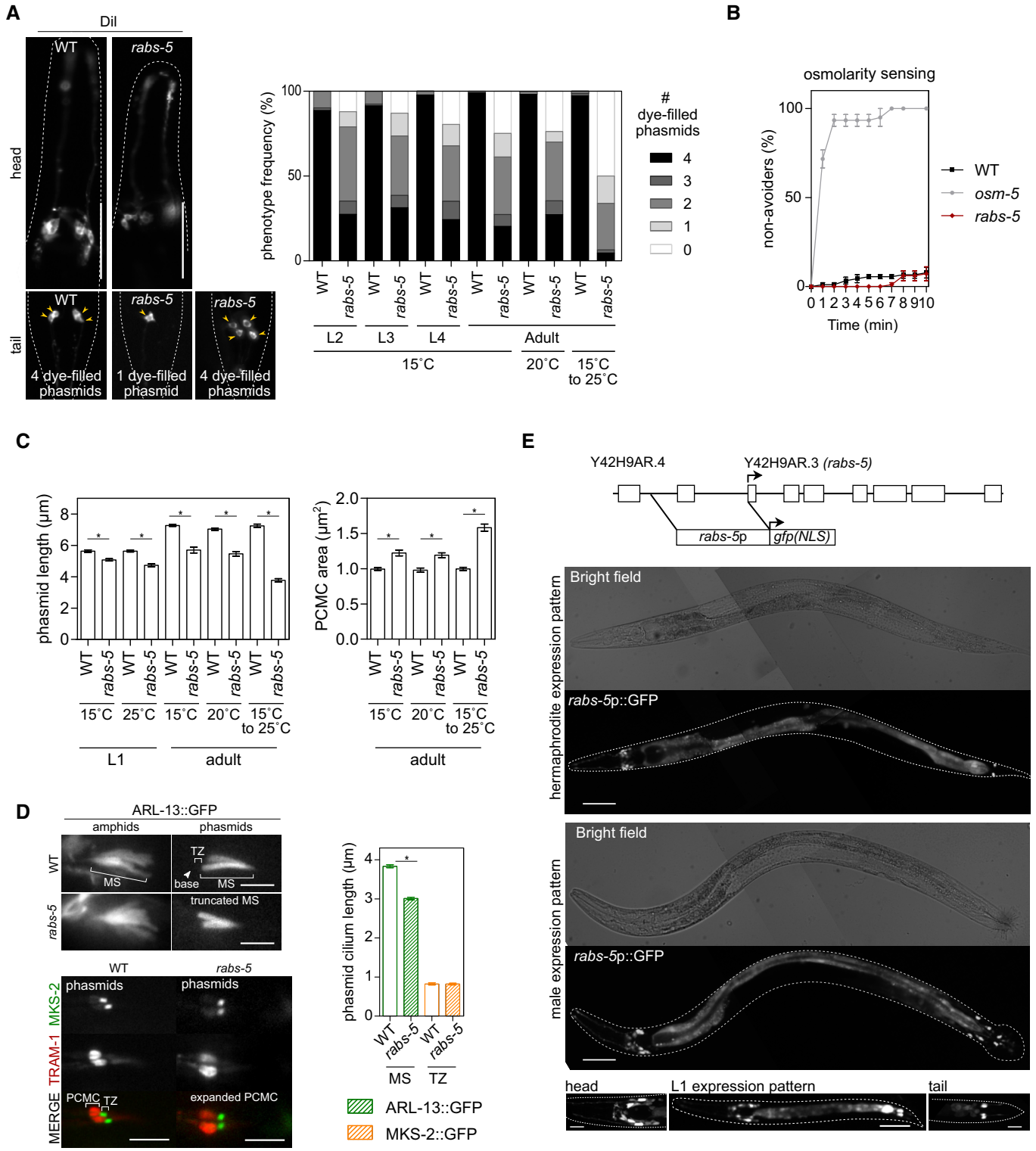

Figure EV1.

Figure EV1. *rabs-5* mutant ciliary phenotypes and *rabs-5* expression pattern (related to Fig 1).

A Representative images of the head and tail of WT and *rabs-5(ok1513)* worms subjected to a DiI uptake assay. Two extremes of the phasmid dye-filling phenotype are shown for *rabs-5* worms. Yellow arrowhead indicate dye-filled phasmid cell bodies. Anterior is to the left. Scale bars = 50 μm. Graph shows quantification of DiI uptake in the phasmid neurons of WT and *rabs-5(ok1513)* worms at different larval (L2, L3, and L4) and adult stages, grown at the indicated temperatures (the 15 to 25°C temperature upshift was performed on L4 larvae for 24 h). In each case, the number (0–4) of phasmid neurons taking up dye is scored. Bars show phenotype frequency (%) from three independent experiments ($n > 40$ per experiment).

B Osmotic avoidance phenotype for *rabs-5(ok1513)* worms, compared to WT (negative control) and *osm-5(p813)* (positive control) worms. Plot shows the fraction of worms that cross a hyperosmotic barrier over 10 min (non-avoiders). Data in plot represent mean ± SEM values from three independent experiments ($n = 30$ per condition).

C Quantification of phasmid cilium length (XBX-1::tdTomato) and PCMC (RPI-2::GFP) area of WT and *rabs-5(ok1513)* worms at the indicated developmental stage (L1 and adults) and grown at the indicated temperature (the 15 to 25°C temperature upshift was performed on L4 larvae for 24 h). Bars in graphs show mean ± SEM ($n = 45$). *$P < 0.001$ (unpaired Student's *t*-test; vs. WT).

D Representative images of the amphid and phasmid cilia of WT and *rabs-5(ok1513)* worms expressing *arl-13::gfp* (stains middle segment, MS) or *mks-2::gfp* (stains transition zone, TZ) + *tram-1::tdTomato* (stains periciliary membrane compartment, PCMC). Graph shows quantification of ARL-13 and MKS-2 signal lengths. Arrowead: ciliary base. Bars show mean ± SEM ($n = 45$). *$P < 0.001$ (unpaired Student's *t*-test; vs. WT). Scale bars = 2.5 μm (top panels) and 5.0 μm (bottom panels).

E Representative fluorescence and bright-field images of adult (hermaphrodite and male) and L1 larvae (hermaphrodites) expressing a transcriptional reporter for *rabs-5* (*rabs-5*p::*gfp*). Schematic shows the genomic organisation of the *rabs-5* gene and the upstream genomic sequence used for *rabs-5*p::*gfp*. Exons denoted by boxes. Arrow, start codon; NLS, nuclear localisation sequence. Anterior is to the left. Scale bars = 50 μm for adult images; 30 μm (centre image) and 10 μm (left and right images) for L1 images.

Figure EV2. Molecular basis of RABS-5 ciliary function and localisation in ciliated cells (related to Fig 2).

A Dominant-negative effect of *rabs-5(ΔFYVE)* overexpression on cilium integrity. Quantification of DiI uptake in the phasmid neurons of WT, and WT worms expressing *arl-13*p::*rfp::rabs-5(WT)* or *arl-13*p::*rabs-5(ΔFYVE)*. In each case, the number (0–4) of phasmid neurons taking up dye is scored. Bars show phenotype frequency (%) from three independent experiments ($n > 35$ per experiment).

B Representative images (Z-projects) of the phasmid neurons in worms co-expressing *arl-13*p::*rfp::eea-1* and *arl-13*p::*gfp::rab-5*. Arrowheads indicate colocalised RAB-5 and EEA-1 EEs. PCMC, periciliary membrane compartment. Anterior is to the left. Scale bars = 5 μm.

C Representative images (Z-projects) of the phasmid neurons in worms co-expressing *arl-13*p::*gfp::rab-5* and either *arl-13*p::*rfp::rabs-5(WT)*, *arl-13*p::*rfp::rabs-5(ΔZnF)*, *arl-13*p::*rfp::rabs-5(ΔFYVE)* or *arl-13*p::*rfp::rabs-5(ΔRBD)*. PCMC, periciliary membrane compartment. Anterior is to the left. Scale bars = 5 μm.

D Representative time-lapse images of the dendrite region (head ciliated neurons) in worms expressing *arl-13*p::*rfp::rabs-5(ΔFYVE)*. Red arrowhead indicates an enlarged RFP-positive vesicle moving towards the cilium. Asterisks, non-moving foci as a reference. Anterior is to the left. Scale bars = 5 μm.

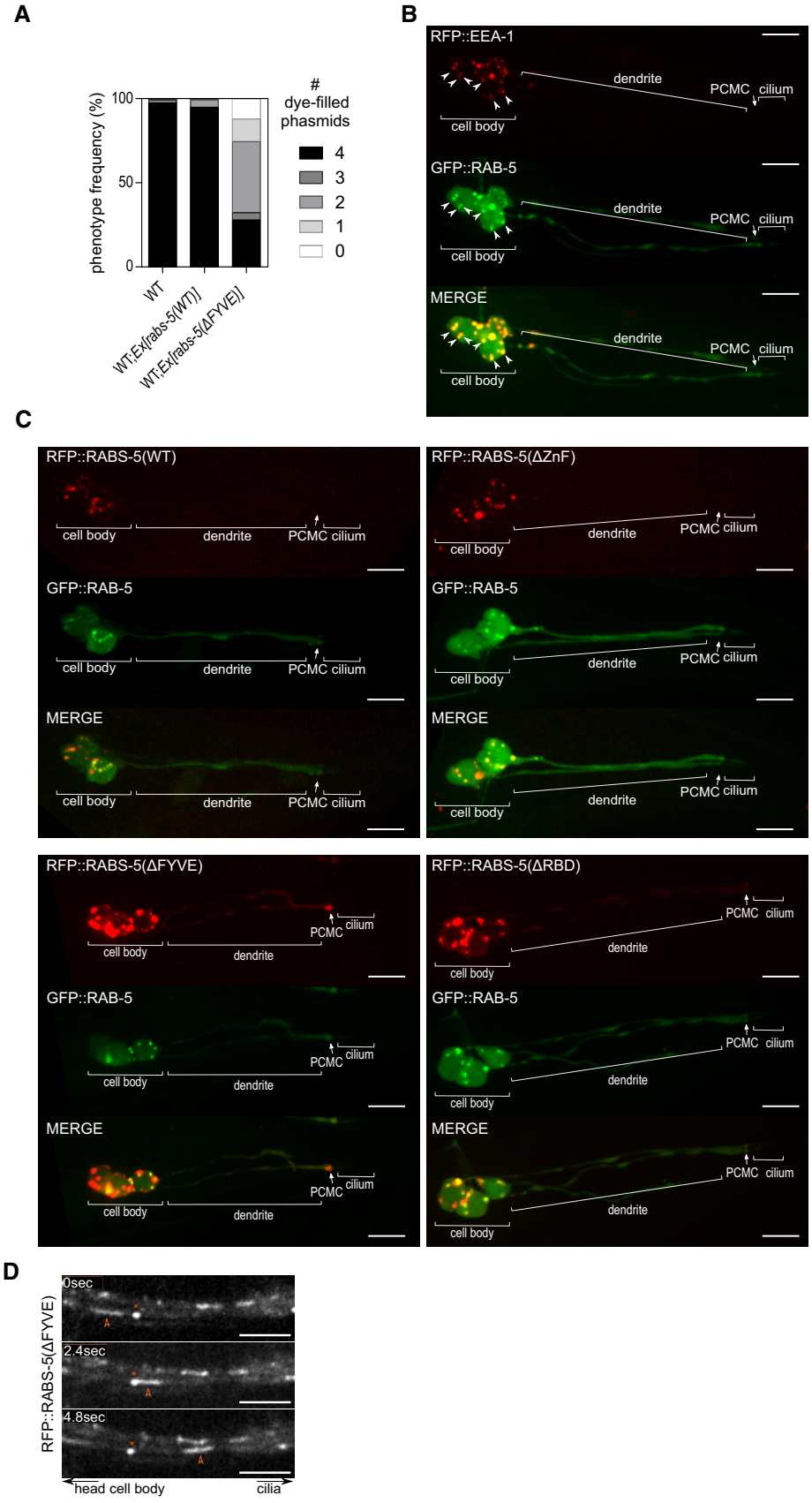

Figure EV2.

Figure EV3. *ups-45* mutant ciliary phenotypes and RABS-5-dependent VPS-45 localisations in ciliated cells (related to Fig 3).

A Representative images of amphid and phasmid cilia from WT, *rabs-5(ok1513)*, *rabx-5(tm1215)*, *rme-6(b1014)*, *eea-1(ok1040)*, and *rme-1(b1045)* worms expressing OSM-6::GFP (marks ciliary axoneme) or RPI-2::GFP (marks the PCMC). Phasmid cilium lengths and PCMC areas quantified in graphs; bars show mean ± SEM (n = 45). *P < 0.001 (unpaired Student's t-test; vs. WT). For images, anterior is to the top. All images identically scaled. PCMC, periciliary membrane compartment. Scale bars = 5 μm.

B Quantification of phasmid PCMC area in WT and *rabs-5(ok1513)* worms expressing *arl- 13*p::*gfp*::*rab-5(Q78L)* or *arl-13*p::*gfp*::*rab-5(S33N)*. Both GFP::RAB-5(Q78L)/(S33N) markers stain the PCMC, allowing its area to be measured. Bars show mean ± SEM (n = 40). *P < 0.001 (unpaired Student's t-test; vs. WT).

C Quantification of DiI uptake in the phasmid neurons of the WT and *ups-45(tm0246)* worms at different larval (L2, L3 and L4) and adult stages, grown at the indicated temperatures (the 15 to 25°C temperature upshift was performed on L4 larvae for 24 h). In each case, the number (0–4) of phasmid neurons taking up dye is scored.

D Quantification of roaming and osmotic avoidance behaviours in *ups-45(tm0246)* worms. *bbs-8(nx77)* and *osm-5(p813)* worms are positive controls. Bars show roaming score as mean ± SEM (n = 60). *P < 0.001 (unpaired Student's t-test; vs. WT). Osmotic avoidance plots show the fraction of worms that cross a hyperosmotic barrier over 10 min. Plots show mean ± SEM from three independent experiments (n = 30).

E Quantification of phasmid cilium length (using XBX-1::tdTomato marker) and PCMC area (using RPI-2::GFP marker) in WT and *ups-45(tm0246)* worms at L1 and adult stages, grown at the indicated temperatures (the 15 to 25°C temperature upshift was performed on L4 larvae for 24 h). Bars in graphs show mean ± SEM (n = 45). *P < 0.001 (unpaired Student's t-test; vs. WT).

F Representative fluorescence and bright-field images of hermaphrodite worms expressing a transcriptional reporter for *ups-45* (*ups-45*p::*gfp*). High-magnification images show GFP expression in head (around the 2nd pharyngeal bulb where amphid neuronal cell bodies lie) and tail neurons. Anterior is to the left. Scale bars = 20 μm. Schematic shows the genomic organisation of *ups-45*, the position of the *tm0246* deletion (grey shading) and the upstream genomic sequence used for *rabs-5*p::*gfp*. Exons denoted by boxes. Arrow, start codon; NLS, nuclear localisation sequence.

G Representative images (Z-projects) of the phasmid neurons of worms co-expressing *arl-13*p::*ups-45*::*gfp* and either *arl-13*p::*rfp*::*rabs-5(WT)* or *arl-13*p::*rfp*::*rabs-5(ΔFYVE)*. PCMC, periciliary membrane compartment. Anterior is to the left. Scale bars = 5 μm.

H Representative time-lapse images of the dendritic region (ciliated phasmid neurons) in worms expressing *arl-13*p::*ups-45*::*gfp* and *arl-13*p::*rfp*::*rabs-5(ΔFYVE)*. Arrowhead denotes a double labelled vesicle trafficking towards the cilium. Anterior is to the right. Scale bars = 2 μm.

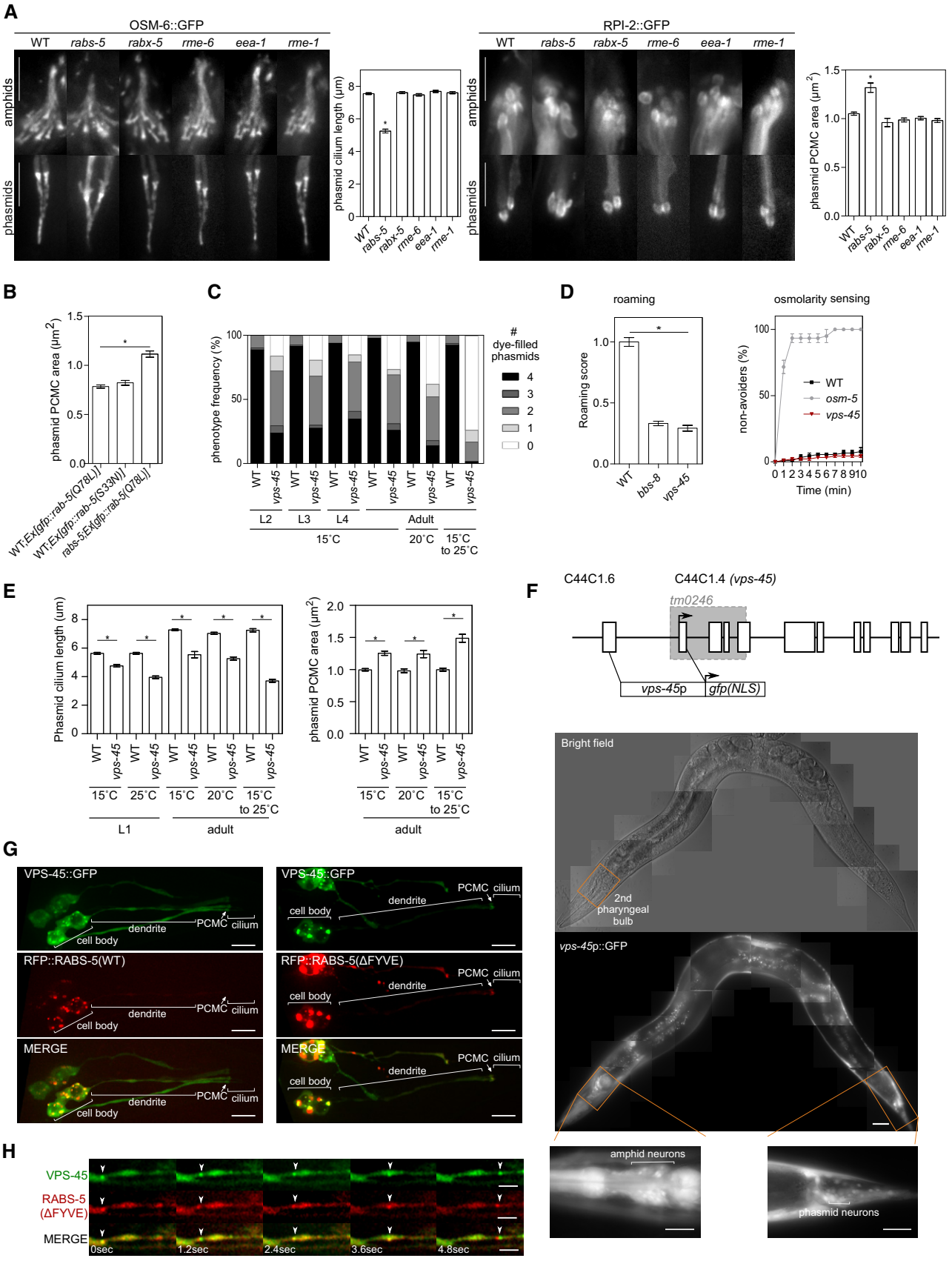

Figure EV3.

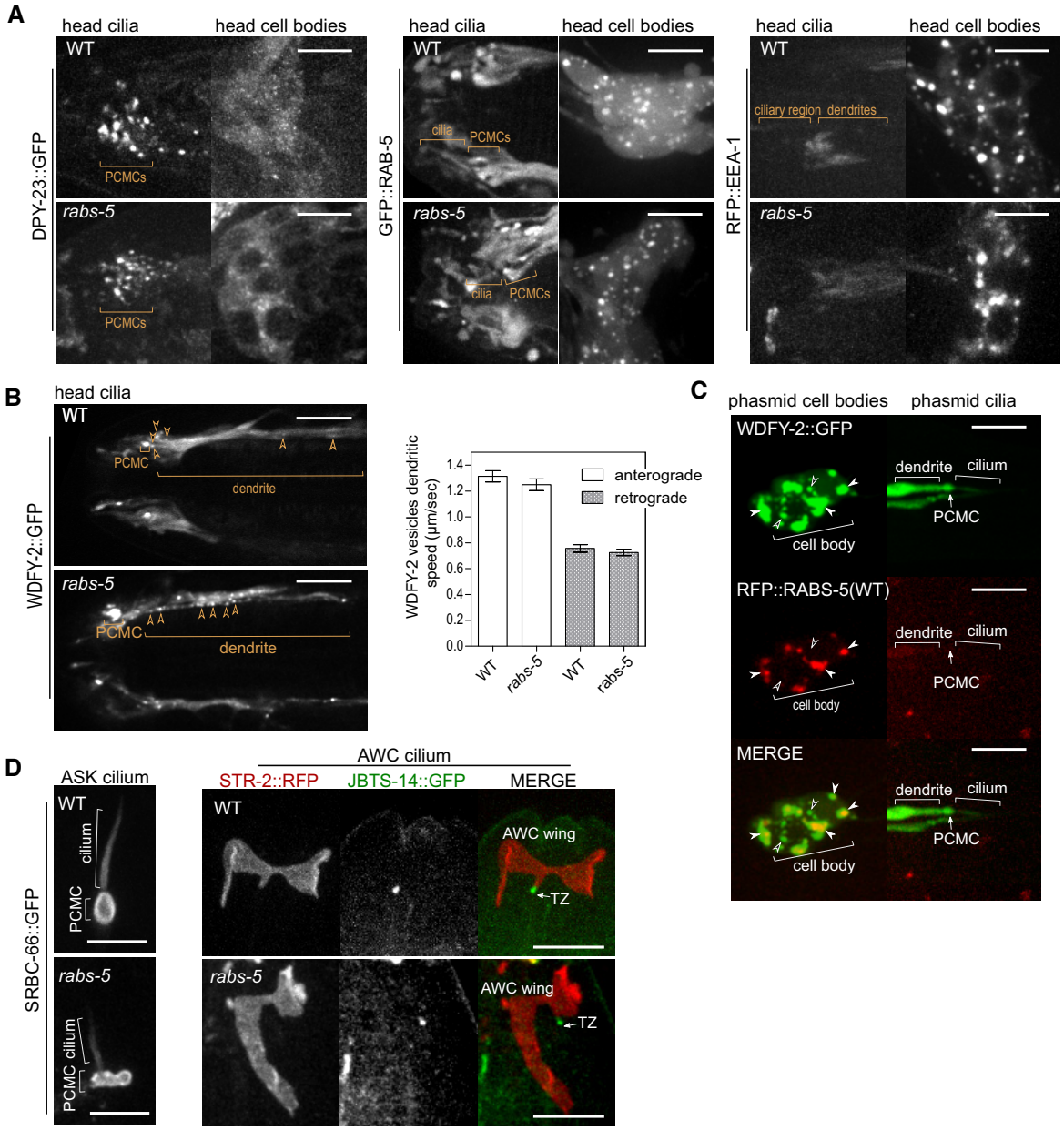

Figure EV4. Localisation of reporters of endocytic proteins and ciliary transmembrane receptors in *rabs-5* mutant (related to Fig 4).

A Representative images (Z-projects) of the head region of WT and *rabs-5(ok1513)* worms expressing *arl-13p::dpy-23::gfp*, *arl-13p::gfp::rab-5*, or *arl-13p::rfp::eea-1*. DPY-23::GFP, GFP::RAB-5 and RFP::EEA-1 distribution is unaffected by *rabs-5* loss. Left panels show the head ciliary regions; right panels show the head cell body regions. PCMC, periciliary membrane compartment. Anterior is to the left. Scale bars = 5 µm.

B Single frame from a time-lapse movie of the head region in WT and *rabs-5(ok1513)* worms expressing *arl-13p::wdfy-2::gfp*. Arrowhead indicates WDFY-2-positive early endosomes (EE) in the dendrites of ciliated neurons. Graph shows quantification of the speed WDFY-2-positive EEs traffic in the dendrites, both in the anterograde (towards the cilium) and retrograde (away from the cilium) directions. Bars show mean ± SEM values (n > 100 positive WDFY-2 EE speed measured for each condition). Anterior is to the left. Scale bars = 5 µm.

C RABS-5 and WDFY-2 colocalise in the ciliated neuronal cell bodies. Representative images (Z-projects) of the phasmid neurons in worms co-expressing *arl-13p::wdfy-2::gfp* and *arl-13p::rfp::rabs-5*. Left panels show phasmid cell body region; right panels show phasmid ciliary region. White arrowhead: colocalised RABS-5 and WDFY-2. Unfilled arrowheads: WDFY-2 and RABS-5 signals that do not colocalise. PCMC, periciliary membrane compartment. Anterior is to the left. Scale bars = 5 µm.

D Representative images (Z-projects) of SRBC-66::GFP in ASK cilia and STR-2::RFP in AWC (wing shaped) cilia in WT and *rabs-5(ok1513)* using worms expressing *srbc-66::gfp* or *str-2::rfp* + *str-2p::jbts-14::gfp* (marks the transition zone, TZ), respectively. PCMC, periciliary membrane compartment. Anterior is to the top. Scale bars = 5 µm.

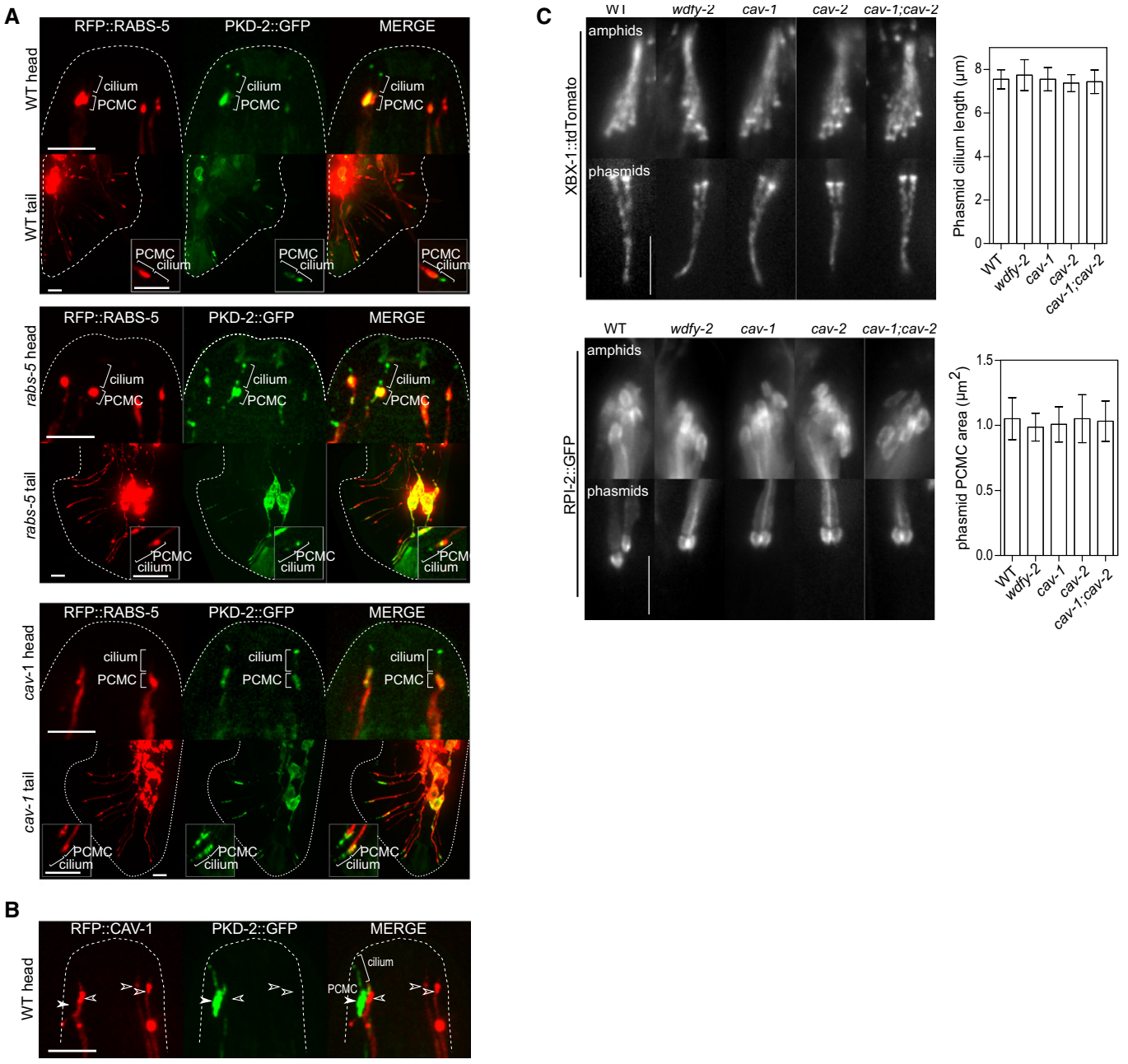

Figure EV5. PKD-2::GFP localisation and cilium structure phenotypes in worms with *rabs-5*, *wdfy-2*, *cav-1* or *cav-2* mutations (related to Fig 5).

A Representative images (Z-projects) of the head and tail regions of WT, *rabs-5(ok1513)* and *cav-1(ok2089)* male worms expressing *pkd-2*p::*rfp*::*rabs-5* and *pkd-2*::*gfp*. Insets show one pair of ray neuronal cilia at higher magnifications. PCMC, periciliary membrane compartment. Anterior is to the top. Scale bars = 5 and 2.5 µm (insets).

B Images of the CEM (head) cilia of a male worm expressing *pkd-2*p::*rfp*::*cav-1* and *pkd-2*::*gfp*. In this example, RFP::CAV-1 is expressed in three CEMs (unfilled arrowheads); the 4th CEM neuron lacks RFP::CAV-1 expression (filled arrowhead). PKD-2::GFP ciliary signals are only retained in CEMs where CAV-1 is not overexpressed. Anterior is to the top. Scale bars = 5 µm.

C Representative images of the amphid (head) and phasmid (tail) cilia in WT, *wdfy- 2(ok3592)*, *cav-1(ok2089)*, *cav-2(hc191)* and *cav-1(ok2089);cav-2(hc191)* worms expressing XBX-1::tdTomato (marks ciliary axonemes and basal body) or RPI-2::GFP (marks the PCMC). Graphs shows quantification of phasmid ciliary length and PCMC area. Bars show mean ± SD (n = 30). PCMC, periciliary membrane compartment. Anterior is to the top. All images identically scaled. Scale bars = 5 µm.