Figure S1

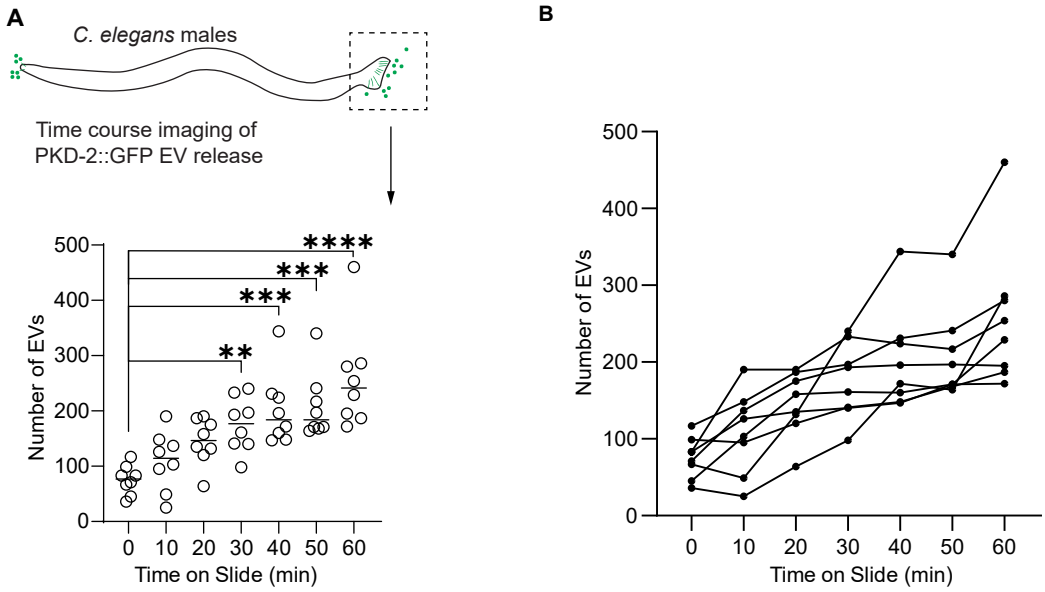


Figure S1 PKD-2::GFP ciliary EV release dynamics, related to Figure 1.

(A) Quantification of EV numbers during time-lapse imaging of an hour. Each circle represents total number of EVs released by one animal from the tail at the time point. n= 8 animals. Statistical analysis was performed using one-way ANOVA with Bonferroni correction. ** denotes $p < 0.01$ and **** denotes $p < 0.0001$.

(B) Individual trajectories depicting the EV release pattern from the male tail at various time points. Each trajectory represents EV counts from a single animal. The EV count at new time points includes newly released EVs in addition to previously released EVs that remain visible post-photobleaching. Photobleaching may explain the decline in EV counts among certain animals where newly released EVs are fewer than previously released EVs, which would have been photobleached multiple times.

Figure S2

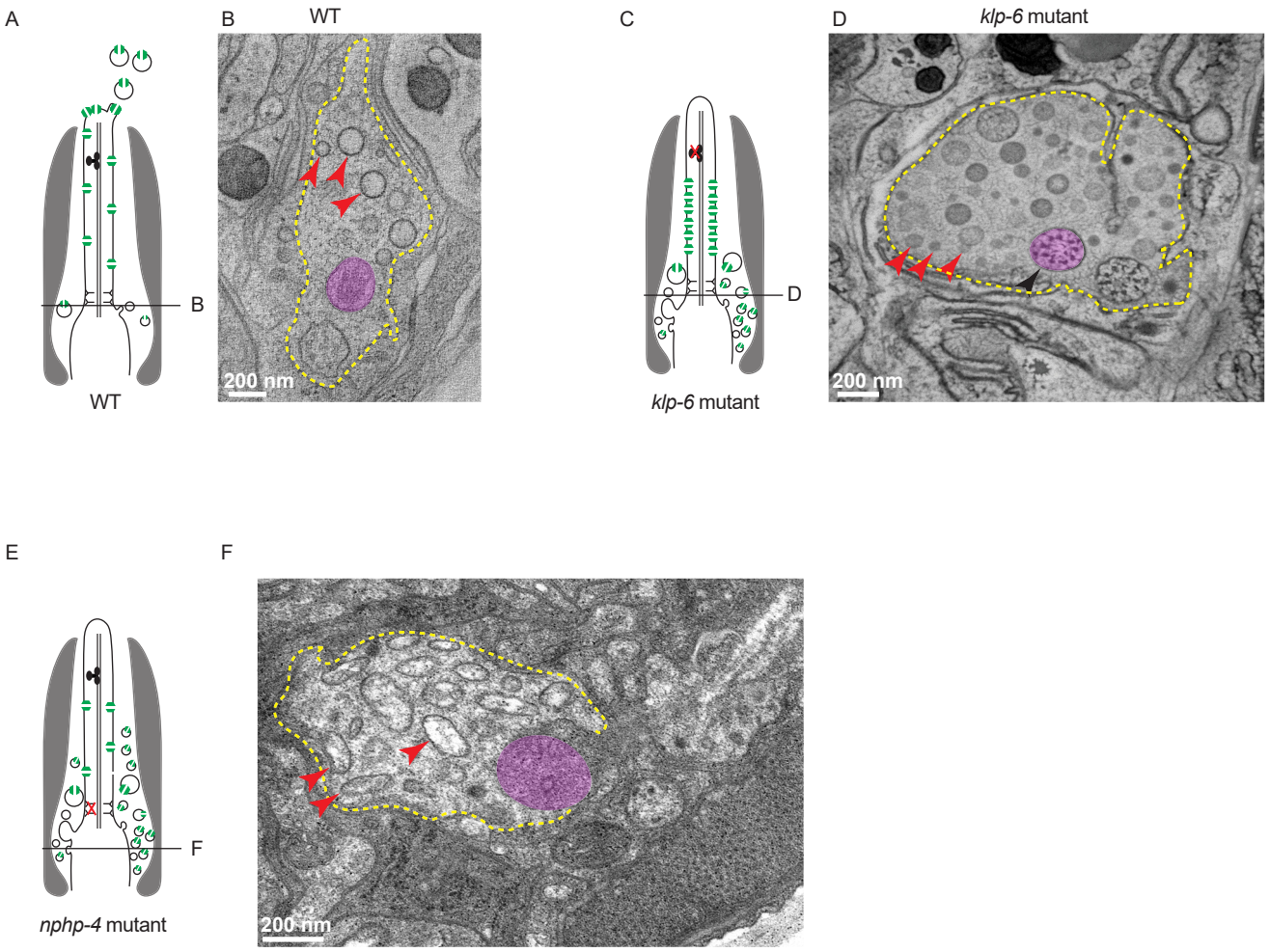


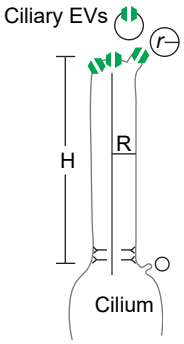
Figure S2 *nphp-4* mutant has excessive internal EVs, related to Figure 3.

(A, C, E) Schematic representation of a cephalic male (CEM) cilium, illustrating its relationship to the ciliary tip and base EVs in wild type, *klp-6* and *nphp-4* mutants respectively. The environmentally-released EVs originate from the ciliary tip, while the ciliary base EVs are located in the lumen formed by the surrounding glia (depicted in gray). Green circle with a channel denotes PKD-2. Black lines indicate the approximate position of the cross-section transmission electron microscopy (TEM) image shown in corresponding panels. Modified from [S1-2].
(B, D) TEM image of a cross section through a ciliary region in wild-type and *klp-6* mutant respectively . The *klp-6* mutant accumulates excess EVs in the lumen (reproduced from [S1]).
(F) TEM image of *nhph-4* mutant ciliary region. In the *nphp-4* mutant, more EVs accumulates in the lumen surrounding the ciliary base. TEM image is from the same TEM series shown in Figure 4i [S3].

In all TEM images, the red arrowheads indicate EVs. The CEM cilium is highlighted in magenta. The glial lumen encircling the CEM cilium is outlined by a yellow dashed line. Scale bars represent 200 nm.

Figure S3

A



Area of ciliary EV membrane = $4\pi r^2$
r = radius of ciliary EV ≈ 50 nm

Area of ciliary membrane = $2\pi RH+\pi R^2$
R = radius of cilium ≈ 100 nm
H = height of cilium ≈ 3 µm

Area Ratio of ciliary to EV membrane

$$= \frac{2\pi RH+\pi R^2}{4\pi r^2} \approx 61$$

B

TTM-5 expression in hermaphrodite

Neuron	Expression
PHA	281.671
IL2_LR	262.148
PVT	137.746
ASG	122.193
IL2_DV	86.219
PHB	68.52
ASEL	63.28

C

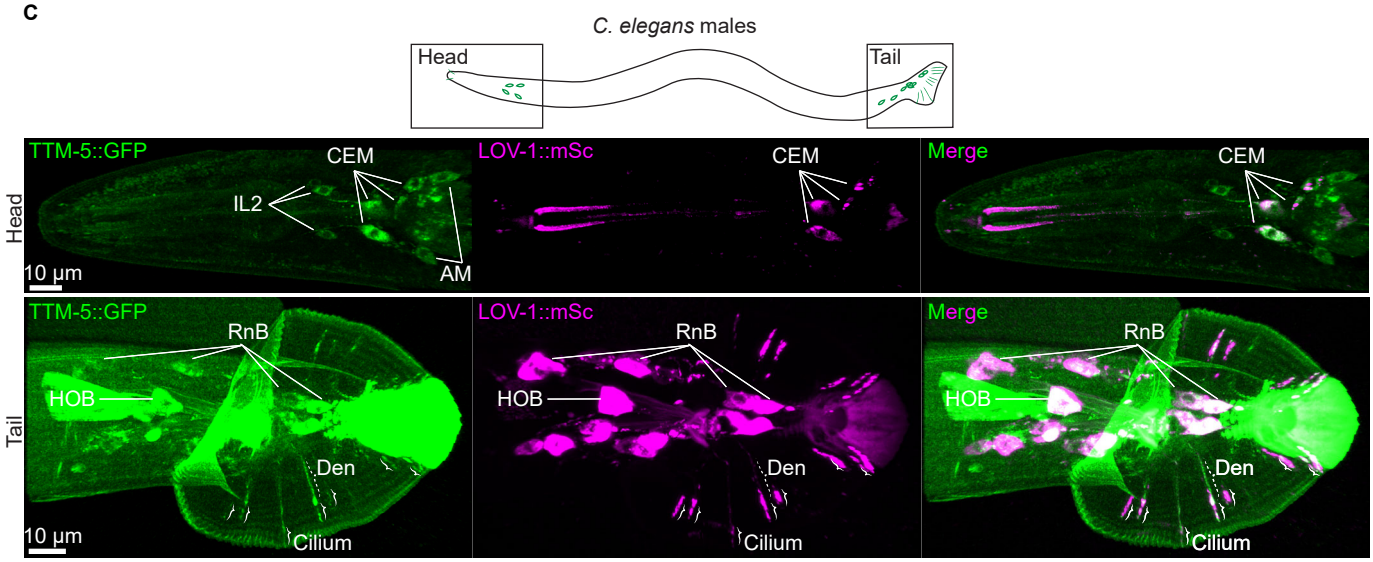


Figure S3 Estimation of ciliary EV membrane requirements and analysis of *ttm-5* expression, related to Figure 4.

(A) A calculation of the ratio between ciliary and EV membrane surface areas.
(B) Single cell RNAseq data from CENGEN APP (CeNGENApp) listed IL2 neurons, the hermaphrodite EV releasing neurons as top expressing neurons of *ttm-5* in the hermaphrodite *C. elegans* [S4].
(C) Images of *C. elegans* males showing the co-expression of TTM-5::GFP (green) and LOV-1::mSc (magenta) in male specific EV releasing neurons. Top panel, schematic of *C. elegans* male-specific EV releasing neurons. The black rectangles indicate the imaged areas in the head and the tail. Lower panel, In the head, TTM-5::GFP is expressed in the inner labial type 2 (IL2) neurons, the cephalic male (CEM) neurons, and some unidentified amphid (AM) neurons. In the tail, TTM-5 is expressed in the male-specific EV releasing neurons, the HOB, and ray type B (RnBs) neurons. TTM-5::GFP localizes to the cilia in all EVNs but is not visibly detected in EVs. Dashed bracket indicate the dendrite (Den), and solid brackets indicate cilia.

Figure S4

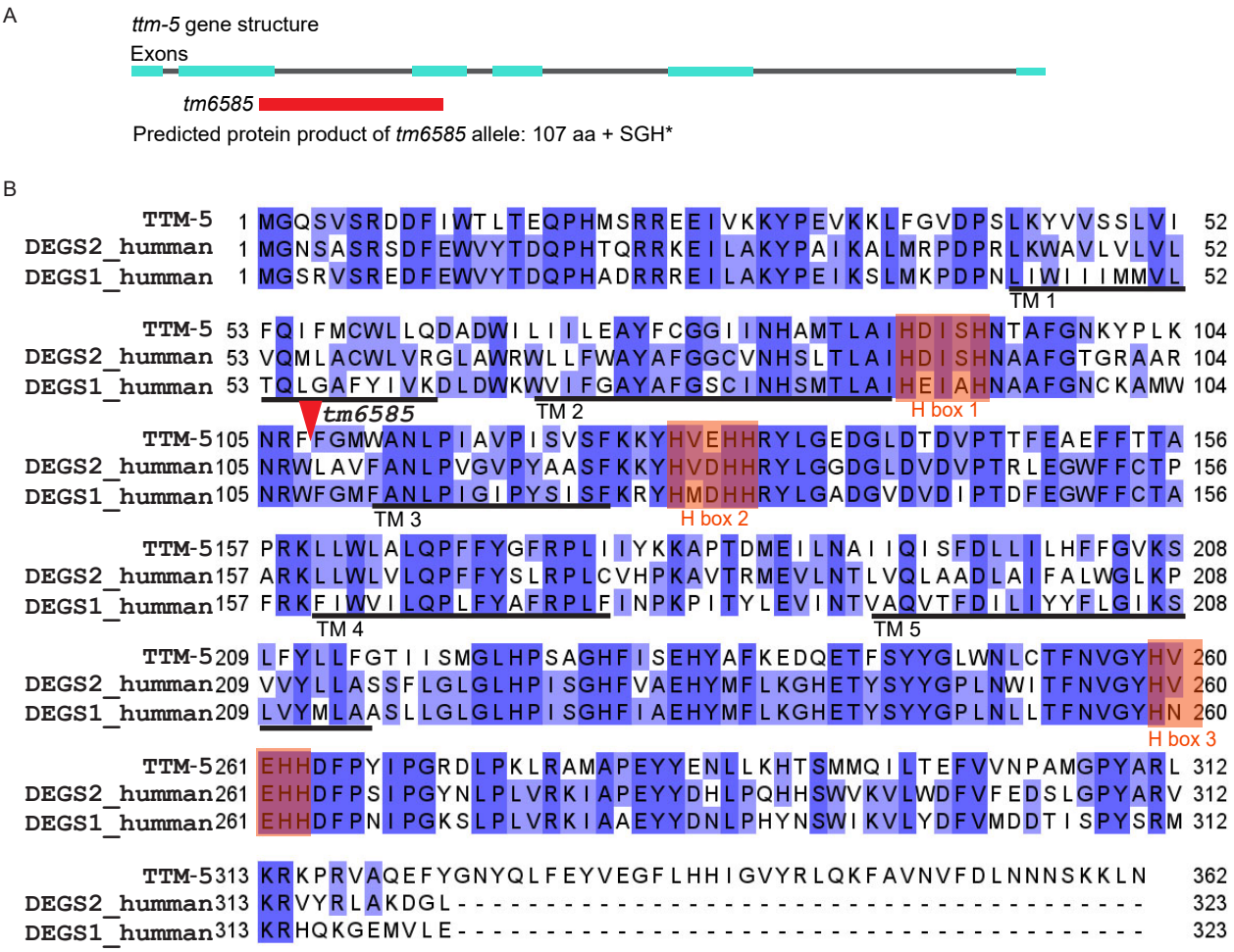


Figure S4 *ttm-5 (tm6585)* **lesion analysis, related to Figure 4.**

(A) The deletion allele *tm6585* removes sequences between exon 2 and exon 3, resulting in a truncated protein that retains 107 amino acids of the TTM-5 protein (indicated by red arrow in panel B), followed by three frame shift amino acids.

(B) Alignment of TTM-5 with its human orthologs DEGS1/2. TTM-5 and DEGSs are predicted to have five transmembrane domains. The allele *tm6585* is a null allele that eliminates two H boxes, which are histidine-rich motifs crucial for desaturase activity [S5].

Supplemental references:

[S1] Wang, J., Silva, M., Haas, L.A., Morsci, N.S., Nguyen, K.C., Hall, D.H., and Barr, M.M. (2014). *C. elegans* Ciliated Sensory Neurons Release Extracellular Vesicles that Function in Animal Communication. Curr Biol 24, 519-525. S0960-9822(14)00003-7 [pii]10.1016/j.cub.2014.01.002.

[S2] Wang, J., Nikonorova, I.A., Silva, M., Walsh, J.D., Tilton, P.E., Gu, A., Akella, J.S., and Barr, M.M. (2021). Sensory cilia act as a specialized venue for regulated extracellular vesicle biogenesis and signaling. Curr Biol 31, 3943-3951 e3943. 10.1016/j.cub.2021.06.040.

[S3] Jauregui, A.R., Nguyen, K.C., Hall, D.H., and Barr, M.M. (2008). The *Caenorhabditis elegans* nephrocystins act as global modifiers of cilium structure. J Cell Biol 180, 973-988.

[S4] Hammarlund, M., Hobert, O., Miller, D.M., 3rd, and Sestan, N. (2018). The CeNGEN Project: The Complete Gene Expression Map of an Entire Nervous System. Neuron 99, 430-433. 10.1016/j.neuron.2018.07.042.

[S5] Halim, N.F.A.A., Ali, M.S.M., Leow, A.T.C. Rahman R.N.Z.R.A. (2022). Membrane fatty acid desaturase: biosynthesis, mechanism, and architecture. Appl Microbiol Biotechnol 106, 5957–5972. https://doi.org/10.1007/s00253-022-12142-3