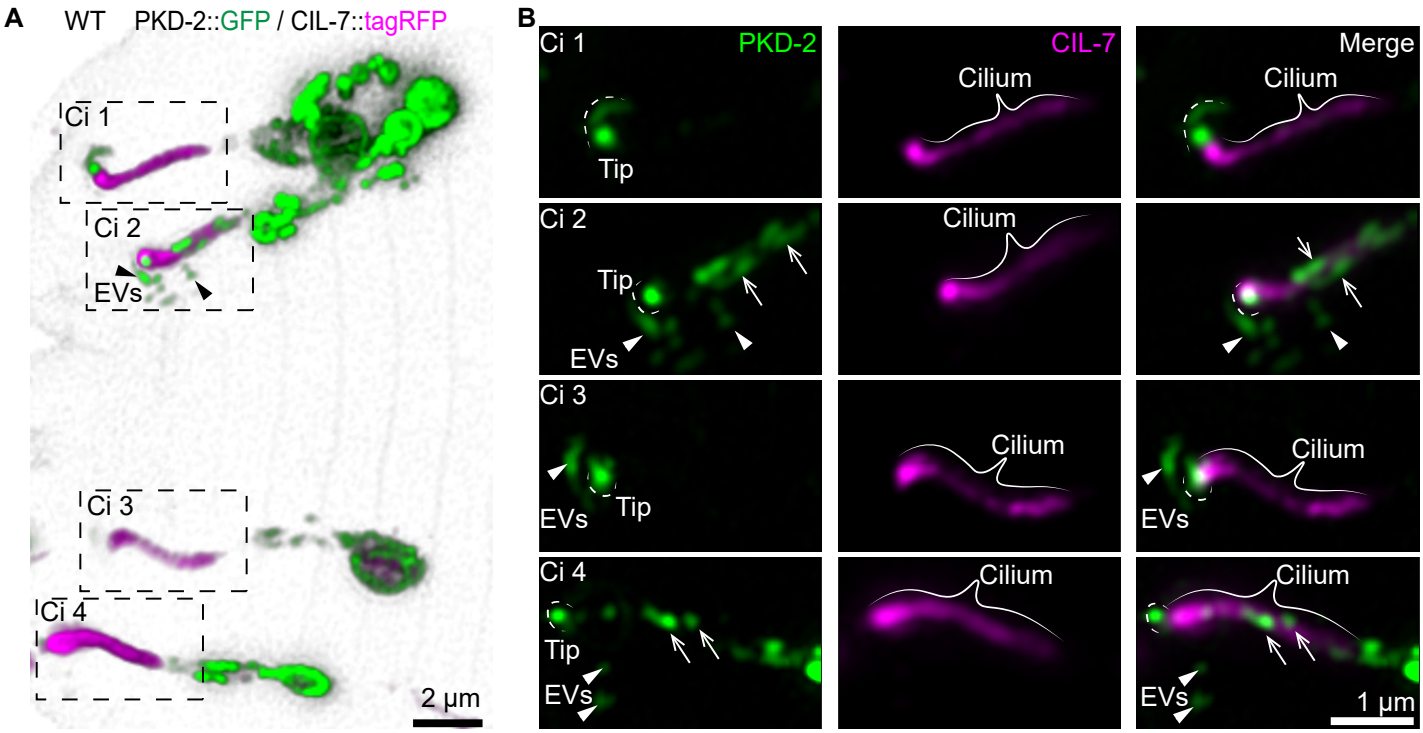

Figure S1. CIL-7-mediated enrichment of PKD-2 at the ciliary tip is required for biogenesis of environmental EVs, related to Figure 2.

A, Three-dimensional rendering model of a male head with all four CEM cilia showing PKD-2::GFP and CIL-7::tagRFP distributions in tip EVs, cilia, and base EVs. Each cilium is numbered and shown on close-up images on panel B. B, PKD-2::GFP enrichment at the ciliary tip is outlined by white dashed lines. White arrowheads indicate environmental EVs. White arrows indicate PKD-2::GFP captured along the ciliary shaft of Ci 2 and Ci 4. Scale bar 2 µm for A, 1 µm for B.

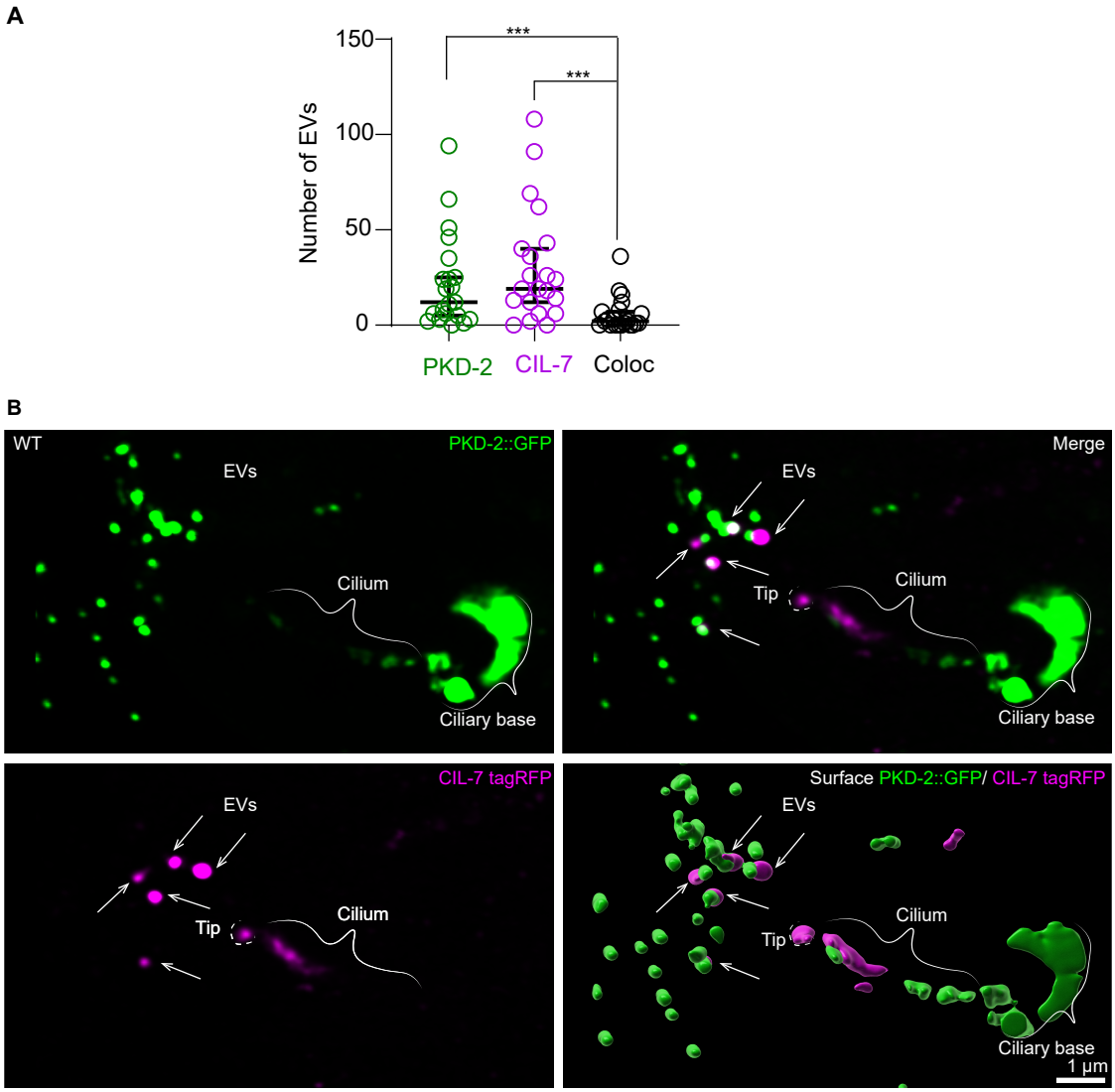

Figure S2. PKD-2 and CIL-7 are sorted to distinct environmental EVs, related to Figure 2 and Figure 4.

A, Analysis of colocalization of PKD-2 and CIL-7 on environmental EVs released from ciliary tips of adult wild-type isolated males. PKD-2 and CIL-7 are mostly sorted into separate EVs. The graph contains paired data where each data point represents number of EVs released from a single male head. Median values with 95% confidence intervals are indicated. *** $p<0.001$ by Friedman test, n=21. B, Environmental EVs are observed in the vicinity of the releasing wild-type cilium. The image is captured at the moment of CIL-7 enrichment at the ciliary tip (outlined by dashed white line) that is about to separate from the cilium. White arrows point to CIL-7 EVs. Note each of the CIL-7 EVs stays connected with PKD-2 EVs after their release. Scale bar, 1 µm.

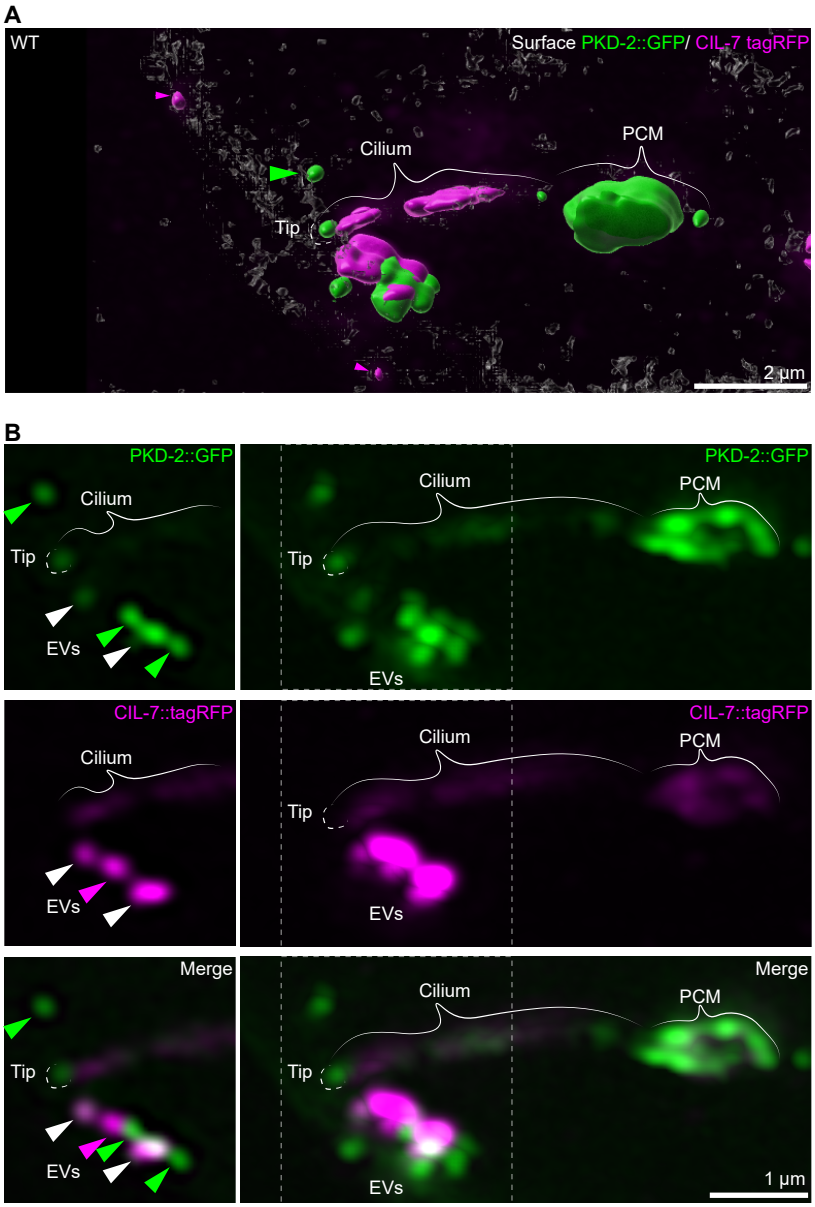

Figure S3. Simultaneous release of PKD-2 and CIL-7 environmental EVs, related to Figure 2.

A, Surface rendered image of PKD-2::GFP and CIL-7::tagRFP distribution along the cilium of a young adult male. PKD-2::GFP is enriched at the ciliary base and the ciliary tip. CIL-7::tagRFP is captured at a moment of forming a larger protrusion from the ciliary tip that still houses multiple PKD-2::GFP EVs not completely separated yet from the CIL-7-enriched launchpad. B, Split view of channels used to produce the surface rendered image on panel A. Left column shows a select focal plane from the Z-stack depicted at the right column; the featured area is marked by vertical dashed. On the single focal plane PKD-2 and CIL-7 EVs are alternating with each other within one string budding from the ciliary tip. Green arrowhead indicates the sole PKD-2 environmental EV; magenta arrowheads indicate the sole CIL-7 environmental EVs; white arrowheads show points of PKD-2 and CIL-7 colocalization. Scale bar, 2 µm in A, 1 µm in B.

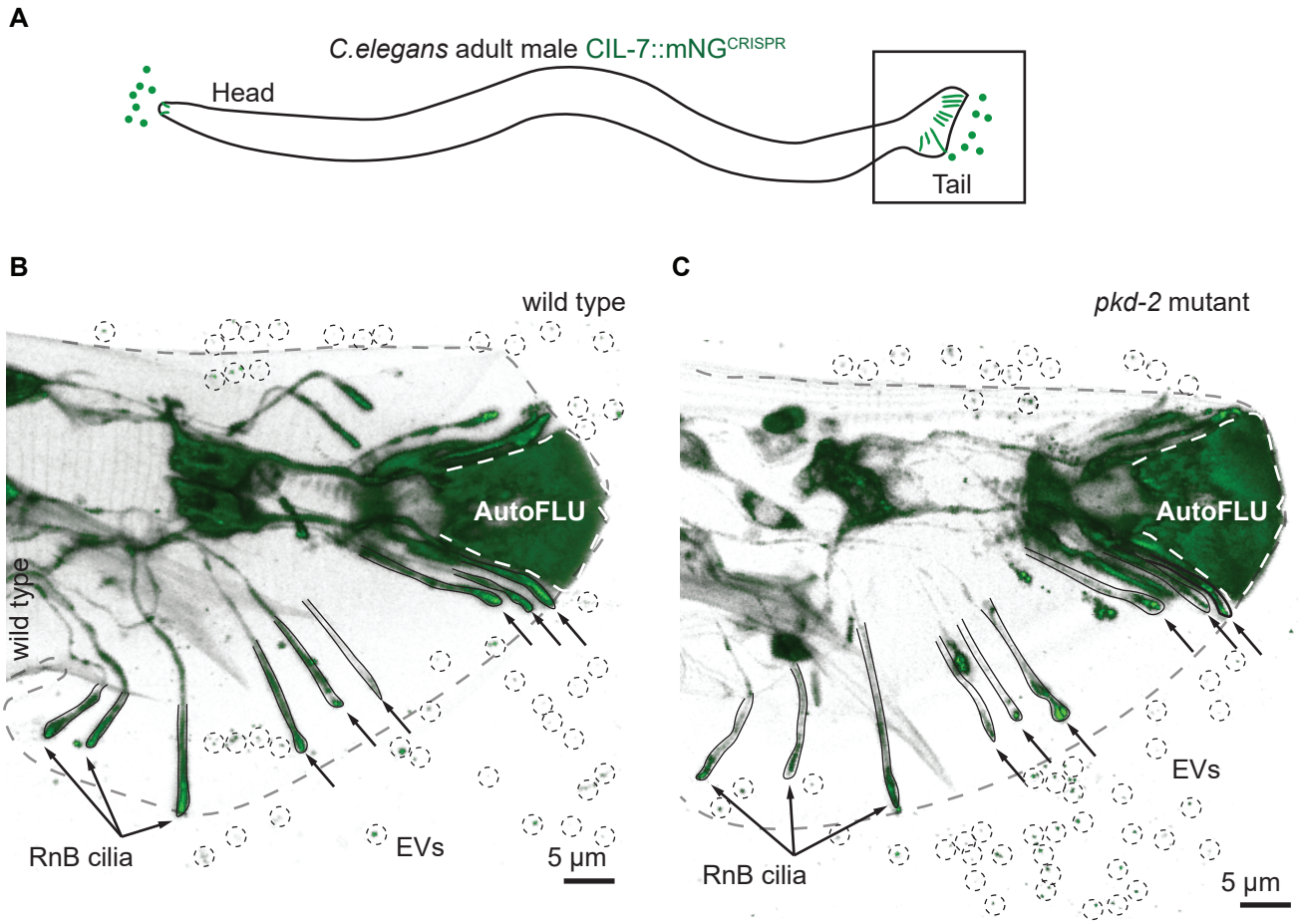

Figure S4. PKD-2 is not essential for CIL-7 EV biogenesis, related to Figure 2.
A, Cartoon of C. elegans adult male releasing EVs from the sensory neurons, and the imaged areas in the tail. B-C, Representative images of C. elegans adult male tail releasing CIL-7::mNeonGreen EVs from the EV releasing neurons (EVNs). CIL-7::mNeonGreen transgenic animals were generated by CRISPR in wild type (strain name PT3602), the transgenic line was crossed into the pkd-2 mutant to generate the strain PT3621. In the wild type (B) and the pkd-2 mutant (C), CIL-7 EVs are released by male-specific RnB (n=1-9) cilia (arrows point to the ciliary tips). Both ciliary localization and EV biogenesis in the RnB cilia look similar in wild type and in the pkd-2 mutant (n=4 animals for wild type, n=5 animals for the pkd-2 mutant). Circles indicate environmentally released EVs. “AutoFLU” and white dashed line indicate autofluorescence from the cuticulular fan of the male tail. Scale bar, 5 µm.

Tip EV PKD-2 relative fluorescence	Ciliary shaft PKD-2 relative fluorescence	PCM + ciliary base EV PKD-2 relative fluorescence	Tip EVs observed	Base EV observed
0.000	0.171	0.829	NO	Yes
0.000	0.208	0.792	NO	Yes
0.000	0.065	0.935	NO	Yes
0.000	0.118	0.882	NO	Yes
0.000	0.251	0.749	NO	Yes
0.000	0.011	0.989	NO	Yes
0.000	0.001	0.999	NO	Yes
0.000	0.000	1.000	NO	Yes
0.000	0.228	0.772	NO	Yes
0.000	0.034	0.966	NO	Yes
0.003	0.048	0.950	Yes	Yes
0.003	0.114	0.884	Yes	Yes
0.003	0.140	0.857	Yes	Yes
0.005	0.121	0.874	Yes	Yes
0.015	0.130	0.855	Yes	Yes
0.017	0.187	0.796	Yes	Yes
0.020	0.265	0.715	Yes	Yes
0.029	0.200	0.772	Yes	Yes
0.040	0.392	0.568	Yes	Yes
0.057	0.373	0.569	Yes	Yes
0.065	0.065	0.870	Yes	Yes
0.068	0.281	0.652	Yes	Yes
0.076	0.037	0.887	Yes	Yes
0.080	0.541	0.379	Yes	Yes
0.084	0.337	0.580	Yes	Yes
0.106	0.185	0.709	Yes	Yes
0.115	0.313	0.572	Yes	Yes
0.120	0.064	0.816	Yes	Yes
0.136	0.035	0.829	Yes	Yes
0.161	0.309	0.530	Yes	Yes
0.162	0.630	0.208	Yes	Yes
0.203	0.131	0.667	Yes	Yes
0.320	0.440	0.239	Yes	Yes
0.428	0.280	0.291	Yes	Yes
0.527	0.205	0.268	Yes	Yes

Table S1. Relative PKD-2 fluorescence intensity on the tip EVs, ciliary shaft, PCM and ciliary base EVs, related to Figure 1E-G. Data is sorted by value of PKD-2 relative fluorescence intensity on tip EVs in the order of smallest to largest. Each data row represents the fraction of PKD-2 in each compartment of one cilium as determined by relative fluorescence intensity.