Supplemental Information

Release and targeting of polycystin-2-carrying ciliary extracellular vesicles

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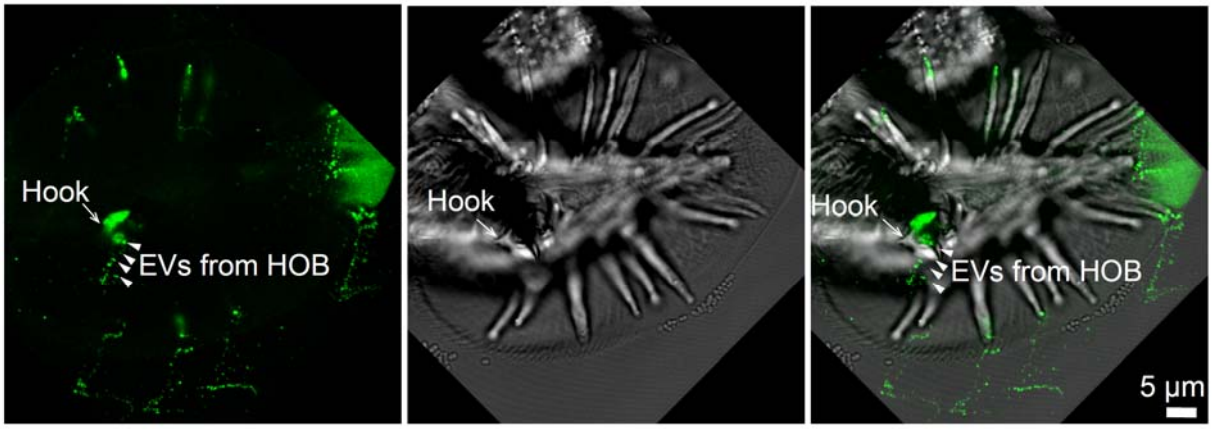

Figure S1. Release of the PKD-2::GFP-labeled EVs from the HOB sensory cilium of the male tail.
Optical sections through *C. elegans* male tail positioned ventrally to the bare coverslip. The hook structure with the HOB sensory cilium is labeled with an arrow, and PKD-2::GFP EVs released from the HOB cilium are labeled with arrowheads.

	Side of male tail adjacent to coverslip			Side of male tail opposite to coverslip			Two proportion Z-test	
Ray identity	Total number of rays examined	Rays that release EVs	%	Total number of rays examined	Rays that release EVs	%	Z-value	*p*-value
Ventral and dorsal rays	52	28	54%	46	2	4%	5.3	$p < 0.0001$
Ventral rays	12	11	92%	30	1	3%	5.7	$p < 0.0001$
Dorsal rays	40	17	43%	16	1	6%	2.6	$p < 0.01$

Table S1. Statistical analysis of the frequencies of PKD-2::GFP EV releasing events from ray B type ciliated neurons of the male tail when positioned either adjacent or opposite to the coverslip.

Supplemental Experimental Procedures

Strains

All *C. elegans* strains were maintained under standard conditions as described in [S1]. The mating assay used uncoordinated hermaphrodites of the CB369 (*unc-51* (e369)) strain to serve as easy mating targets for males. The males were of the PT443 strain (*myIs1*[P*pkd-2*::PKD2::GFP+P*unc-122*::GFP] I; *pkd-2(sy606)* IV; *him-5(e1490)* V) that carries a loss-of-function *pkd-2* allele together with an integrated rescuing array encoding GFP-labeled PKD-2 protein [S2].

Scoring of the PKD-2 EV release was performed using the PT3112 strain (*pha-1(e2123)* III; *him-5(e1490)* V, *myIs4*[P*pkd-2*::PKD2::GFP+P*unc-122*::GFP] V; *myEx888*[CIL-7::tagRFP + pBX1]) [This work].

Mating assay tracking sperm and EV transfer

Male worms were pre-stained by soaking in 10 μM MitoTracker Ted CMXRos dye (ThermoFisher Scientific #M7512) buffered with the isosmotic M9 buffer (22 mM KH_2PO_4, 43 mM Na_2HPO_4, 85 mM NaCl, 1 mM $MgSO_4$) for 7 hours in the dark to label their spermatozoa [S3]. Prior to introduction to the hermaphrodites, males were allowed to crawl on a clean plate to remove any excess of the dye. Mating was conducted on an agar plate with a bacterial lawn of 1 cm in diameter generated with 15 μl of overnight OP50 *E. coli* culture. Ten unstained L4 *unc-51 (e369)* hermaphrodites were placed together with fifty pre-stained males and allowed to mate for 24 hours in the dark at 22^{o}C. Following mating, hermaphrodites were mounted in a 0.5 μl drop of 10 mM levamisole (prepared in the M9 buffer) placed on a 5% agarose pad (prepared with ultra-pure water and agarose, Sigma #A9539) for further imaging.

Mounting of males for quantitative scoring of the EV release

For regular imaging of the EV release, males were mounted on agarose-layered glass slides in the same way as described above for the hermaphrodites [S4]. In order to diminish mechanical stimulation and test the hypothesis about mechanoresponsive EV release, we developed the double agarose sandwich protocol, where not only the microscope glass slide is layered with 5% agarose, but the coverslip also has a thin padding made from agarose gel. To prepare the padded coverslips with the thinnest possible agarose pad, 20 μl of 5% agarose gel were dropped on a pre-heated to 90^{o}C coverslip and pressed with another coverslip. Then, the coverslip-agarose sandwich was cooled to ambient temperature and was carefully separated so that only one piece of the coverslip was left covered with the agarose gel padding. Imaging of male worms mounted with the agarose-padded coverslips was performed in 1 mM levamisole. After each imaging session, the agarose-padded coverslip was replaced with a bare coverslip to conduct a second imaging session on the same male to score its response to a bare coverslip. Both imaging sessions were performed within a 30-minute timeframe.

Fluorescent imaging and quantification

Images were acquired using Zeiss LSM880 confocal microscope with Airyscan high-resolution detector. Image processing included Airyscan processing performed with the accompanying Zeiss software ZEN 2 (Blue version). Surface rendering of the hermaphrodite images was obtained with Imaris software. PKD-2::GFP EVs were quantified using ZEN Blue imaging analysis software.

Statistical analysis

Non-parametric Kruskal-Wallis test was used to test the hypothesis that the double agarose sandwich mounting protocol results in significantly different number of EVs released from cilia of a male tail.

Two-proportion Z-test was used to test the hypothesis that frequencies of EV release events from male ray cilia facing or not facing the bare coverslip are significantly different (Table S1). Lateral ray 3 was also producing EVs but was excluded from the analysis as its position relative to the coverslip could not be established unequivocally.

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Author Contributions

Study conception and design: J.W., P.W.S., M.M.B. Acquisition of data: J.W. Analysis and interpretation of data: J.W., A.G., I.A.N., M.M.B. Writing of manuscript: J.W., I.A.N., M.M.B.

Declaration of Interests

The authors declare no conflicts of interests.

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