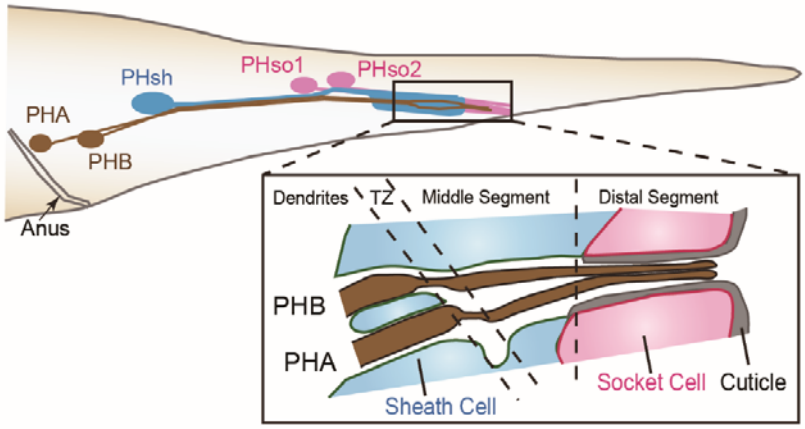
Supplementary information, Figures S1-S2, Table S1 and Data S1 This file includes: Supplementary information, Figures S1-S2, Supplementary information, Table S1, and Supplementary information, Data S1 Materials and Methods.

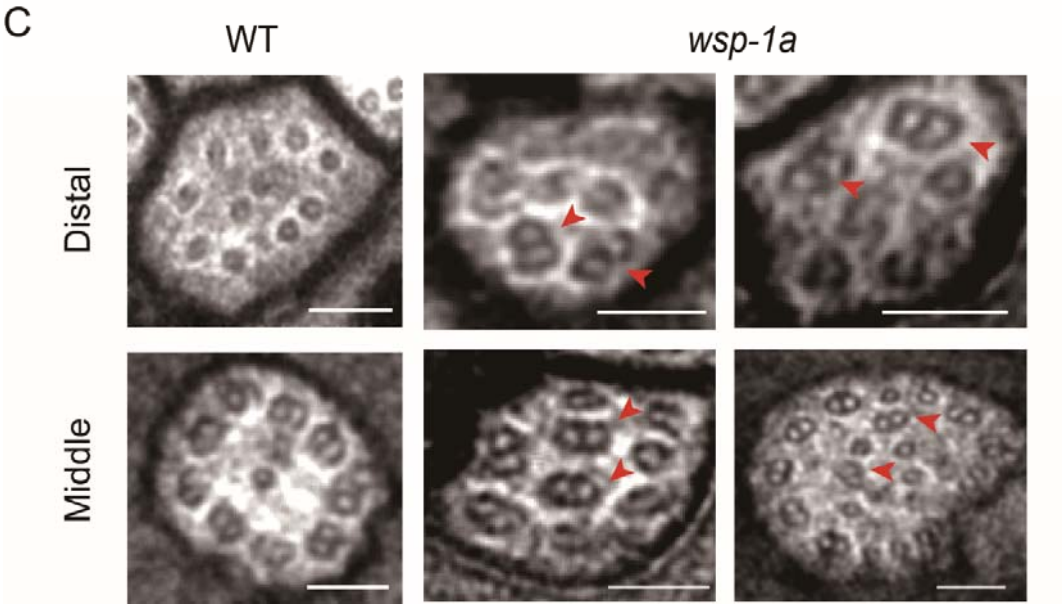
A



B

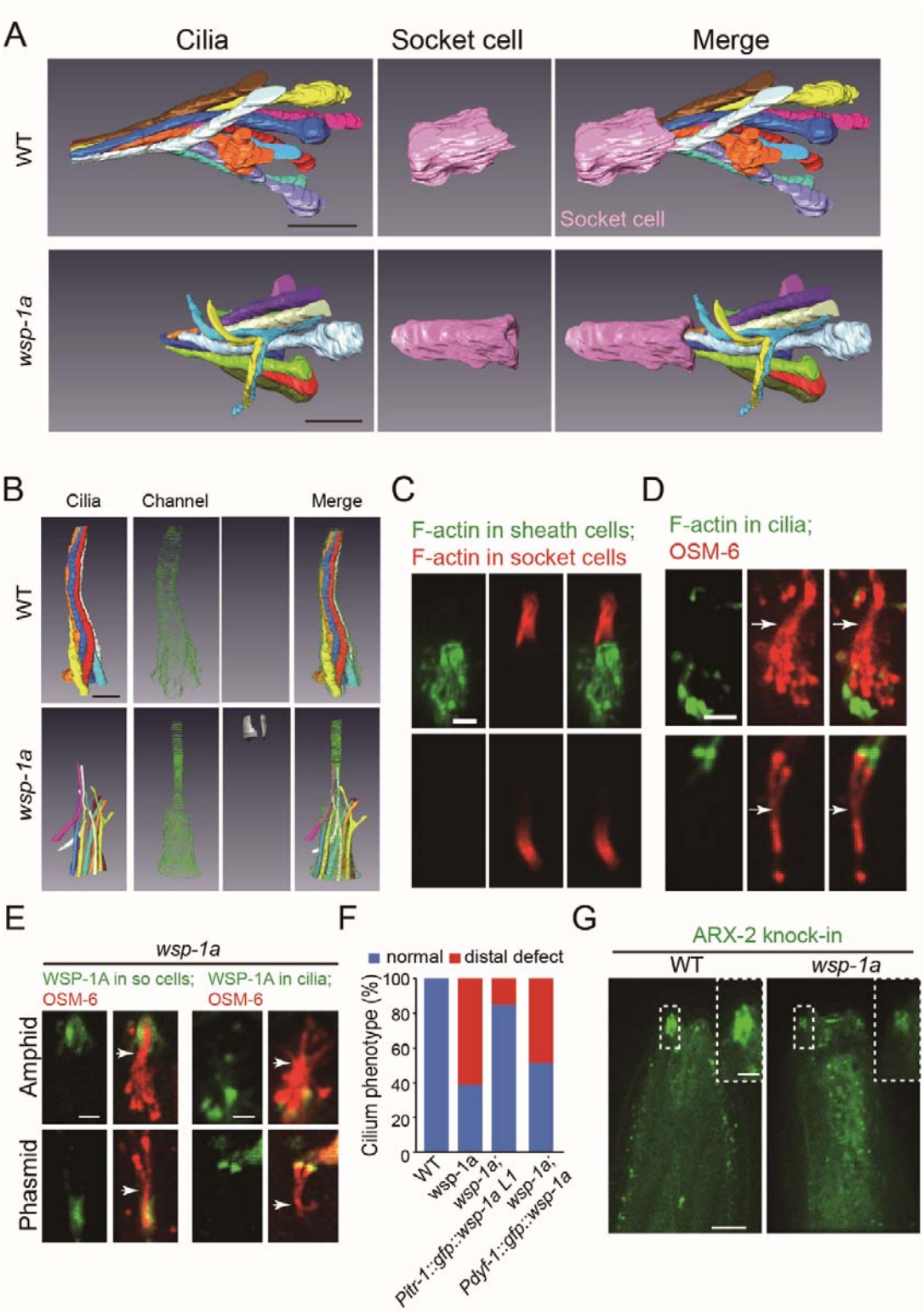
Gene	Allele	Homology (*H. sapiens*)	Dye filling defect (%)		N
			amphid	phasmid	
wsp-1a	*gm324*	N-WASP	0	55	390
wsp-1b	*gk206830*	N-WASP	0	7	520
unc-34	*e315*	VASP	0	0	224
unc-60	*e723*	cofilin	0	0	342
dbn-1	*ok925*	mABP1	0	0	289

C



Supplementary information, Figure S1. The *C. elegans* phasmid and actin regulators that are involved in ciliogenesis.

(**A**) Schematic of the *C. elegans* phasmid. Top: two glial cells including: the socket cell (PHso, pink) and the sheath cell (PHsh, blue). Each phasmid has 2 neurons (PHA and PHB, brown). The area in the rectangle is enlarged at the bottom. Bottom: dendrite endings develop cilia that enter the sensory channel formed by the glial cells at the tip. TZ: transition zone. Based on Perkins et al., 1986 [6]. (**B**) The dye-filling screen of mutants with defective actin regulators. (**C**) The axonemal microtubule structure of the ciliary middle or distal segments. Red Arrowhead: doublet microtubules. Scale bar, 100 nm.



Supplementary information, Figure S2. WSP-1A regulates the morphology of the socket cells.

(**A**) Side views of a 3D reconstruction from the aligned longitudinal section FIB-SEM images show trajectories of cilia and the socket cells in WT and *wsp-1a* mutants.

Colors: cilia. Pink: socket cells. Scale bar, 2 µm. (**B**) Side views of a 3D reconstruction from the aligned cross section FIB-SEM images show trajectories of cilia and the channel from the distal tip to the transition zone in WT and *wsp-1a* mutants. Colors: cilia. Grey: highly electron dense material. Green: channel. Scale bar, 1 µm. (**C**) Expression of F-actin marker *gfp::Utrophin* in the socket cells (P*itr-1* promoter, red) and the amphid sheath cells (P*vap-1* promoter, green). (**D**) Expression of F-actin marker *gfp::Utrophin (CH)* (green) in cilia (P*dyf-1* promoter). Cilia were labeled with OSM-6::mCherry (red). Arrows indicate the boundaries of the ciliary distal and middle segments. (**E**) Fluorescence images of GFP::WSP-1A in socket (so) cells (left) or cilia (right) in *wsp-1a* mutants. Arrows indicate the middle and distal segment boundaries. (**F**) Quantifications of (**E**) cilium phenotype. n = 40-50. (**G**) Localization of ARX-2 (green) in the amphid in WT or *wsp-1a* mutant animals. Scale bar, 5 µm. Insert box, ARX-2 in the socket cell. Scale bar, 2 µm.

Supplementary information, Table S1A. Primers for Molecular analysis

Gene	CRISPR-Cas9 targets (PAM)	Primers (For: forward; Rev: reverse)
arx-2 knock-in	GCAAGATGTATGGCCAAACTTGG	For :ATAGAAGCCCCACCAAGCCG Rev: AACGTTTGAATGAGAGTGGG
wsp-1 knock-in	CGGATCATCAAAACATATGTCGG	For: CCCTGAATTTAATACTCTCTCCTCTGCC Rev: CATCTCATTTTCTTGTGAATTGAGCTC

Supplementary information, Table S1B. Plasmids and Primers in This Study

Plasmid Name	**Primer 5'**	**Primer 3'**	**Notes**
pDD162-P*eft-3*::Cas9+PU6::*arx-2*-knock-in-sg	ATGTATGGCCAAACTGTTTTAGAGCTAGAAATAGCAAG	AGTTTGGCCATACATCTTGCAAGACATCTCGCAATAGG	PCR from pDD162-P*eft-3::Cas9+ PU6::Empty sgRNA*
pDD162-P*eft-3*::Cas9+P*U6::wsp-1*-knock-in-sg	CATCAAAACATATGTGTTTTAGAGCTAGAAATAGCAAG	ACATATGTTTTGATGATCCGCAAGACATCTCGCAATAGG	PCR from pDD162-P*eft-3::Cas9+ PU6::Empty sgRNA*
pPD95.77-*arx-2::gfp* knock-in template	GAACTATACAAATAGATATTGTGACTTTATTTTCGTG	GAAGAGTAATTGGACAATTATCTAGAGGTAGGCGG	The 3'arm sequences were amplified from N2 and cloned into pPD95.77-*arx-2*-5' arm::GFP via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*arx-2*-5' arm::GFP	GTACCGGTAGAAAAAGAAATACTGACGCGTTCCAG	ACCAAGCTTGGGTCTAGCTTTGATTCCAAGCTTAGCCATGCAGC	The 5'arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*wsp-1*-5'arm::GFP	GTACCGGTAGAAAAAAATTCAATGCTGCAAGCGCG	TTCTCCTTTACTCATATGTTTTGATGATCCGAGG	The 5' arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*gfp::wsp-1* knock-in template	CCCAAGCTTGGTACCATGTCGGTATATCCTCCCAC	GAAGAGTAATTGGACCTCTCAATTCATCGGTGCTC	The 3' arm sequences were amplified from N2 and cloned into pPD95.77-*wsp-1*-5' arm::GFP via In-Fusion Advantage PCR Cloning Kit.

pDONR-*Pdyf-1::gfp::Utrophin::unc-54 3'utr*	GGAAGTGGTAGCGGTGCCAAGTATGGAGAACATGAAGC	GAAGAGTAATTGGACTTAGTCTATGGTGACTTGCTGAG	Utrophin was amplified and cloned into pDONR-*unc-54 3'utr* via In-Fusion Advantage PCR cloning kit
pDONR-*Pvap-1::gfp::Utrophin::unc-54 3'utr*	cctactgtagaggagatgttgagc	ctgtgaaaatgaacgcacgcctgc	*Pvap-1* was amplified and cloned into pDONR-*gfp::Utrophin::unc-54 3'utr* via In-Fusion Advantage PCR cloning kit
pPD95.77-*Pvap-1::gfp::wsp-1a::unc-54 3'utr*	cctactgtagaggagatgttgagc	ctgtgaaaatgaacgcacgcctgc	*Pvap-1* was amplified and cloned into pPD95.77-*gfp::wsp-1a::unc-54 3'utr* via In-Fusion Advantage PCR cloning kit
pPD95.77-*Pitr-1::gfp::wsp-1a::unc-54 3'utr*	CGTTCCCGAGCATTATGAATgtaag	GGCCGTCGTTTCGTTTTCGTctg	*Pitr-1* was amplified and cloned into pPD95.77-*gfp::wsp-1a::unc-54 3'utr* via In-Fusion Advantage PCR cloning kit

Supplementary information, Table S1C. *C. elegans* Strains in this Study

Strain name	Genotype	Method	Purpose
N2	wild-type		
GOU2047	*cas607 [arx-2::gfp knock-in]*	Microinjection	To visualize endogenous ARX-2
GOU2049	*cas723 [gfp::wsp-1 knock-in]*	Microinjection	To visualize endogenous WSP-1
NG324	*wsp-1a(gm324)*	CGC	*wsp-1a* mutant
VC20008	*wsp-1b(gk206830)*	CGC	*wsp-1b* mutant
CB315	*unc-34 (e315)*	CGC	*unc-34* mutant
CB723	*unc-60(e723)*	CGC	*unc-60* mutant
RB1004	*dbn-1(ok925)*	CGC	*dbn-1* mutant
SP2101	*mnIs17 [osm-6::gfp + unc-36(+)]*	CGC	To visualize cilia
GOU1757	*casIs550[Pdyf-1::osm-6::mCherry]*	Microinjection	To visualize cilia
GOU1929	*wsp-1a(gm324); SP2101[Pdyf-1::osm-6::gfp]*	Cross	To visualize cilia in *wsp-1a* mutant
GOU1926	*cas723 [gfp::wsp-1a knock-in]; casIs550[Pdyf-*	Cross	To visualize cilia and

	1::osm-6::mCherry]		WSP-1A
GOU1813	*cas607 [arx-2::gfp knock-in]; casIs550[Pdyf-1::osm-6::mCherry]*	Cross	To visualize cilia and ARX-2
GOU2278	*casEX3401[Pitr-1::gfp::wsp-1a + Pdyf-1::osm-6::mCherry + Pegl-17::Myri::mCherry + Pegl-17::mCherry::his-24]; wsp-1a (gm324)*	Microinjection	To rescue *wsp-1a* mutant
GOU2279	*casEX3402[Pdfy-1::gfp::wsp-1a + Pdyf-1::osm-6::mCherry + Pegl-17::Myri::mCherry + Pegl-17::mCherry::his-24]; wsp-1a (gm324)*	Microinjection	To rescue *wsp-1a* mutant
GOU2280	*casEX6077 [Pdyf-1::gfp::utrophin + Pdyf-1::osm-6::mCherry + rol-6(su1006)]*	Microinjection	To visualize F-actin in cilia
GOU1967	*casEX6085[Pvap-1::gfp::utrophin + Pitr-1::mCherry::utrophin + Pdyf-1::osm-6::mCherry]*	Microinjection	To visualize F-actin in glial cells
GOU2285	*casEX6085[Pvap-1::gfp::utrophin + Pitr-1::mCherry::utrophin + Pdyf-1::osm-6::mCherry]; wsp-1a(gm324)*	Cross	To visualize F-actin in glial cells in *wsp-1a* mutant

Supplementary information, Data S1 Materials and Methods

***C. elegans* strains, genetics and DNA manipulations**

C. elegans strains were maintained on the nematode growth medium (NGM) plates seeded with *E. coli* OP50 at 20°C. CRISPR-Cas9-assisted conditional knock-in animals were produced as described previously [1]. We amplified the 1-1.5kb upstream and downstream homologous repair templates from the N2 genomic DNA and inserted them into pPD95.77 by In-Fusion Advantage PCR Cloning Kit (Clontech). The knock-in worms were examined by PCR and Sanger sequencing. Transgenic worms were generated by the germline microinjection of DNA plasmids or PCR products at 10 - 50 ng/µl. Our knock-in animals of ARX-2 and WSP-1A do not show any obvious abnormality in the overall development of *C. elegans*. The primers, plasmids, strains and PCR products were list in Tables S1-S3.

Dye-filling assay

Young adult worms were washed and collected into M9 buffer, and incubated with 20 µg/ml of DiI or DiO solution for 30-60 min in dark at room temperature. The worms were transferred to NGM plates and scored for dye filling defective (dye) uptake 1 hr later.

Specimen preparation

The young adult WT and the distal defective *wsp-1a* mutant animals were fixed in M9 solution containing 2.5% glutaraldehyde, 1% paraformaldehyde, and 0.1 M sucrose for 1 days at 4℃. The samples were post-fixed for 1 hr on ice with 1.5% potassium ferrocyanide and 1% aqueous osmium tetroxide for 20 min followed by another 1hr of 1 % OsO4 alone. They were stained en block in 1% uranyl acetate at 4℃ overnight, dehydrated with up-graded ethanol serials, and embedded in resin SPIPON 812.

TEM, FIB-SEM and image processing

TEM experiments were performed according to standard procedures, as described in Schouteden, 2015 [2] . Briefly, ultrathin (~70 nm) sections were cut on Leica UC6

ultramicrotome and placed on formvar-coated copper grids and counterstained with uranyl acetate and lead citrate. Images were captured by FEI Tecnai Sprit 120 kV transmission electron microscope operated at 100 kV.

FIB-SEM experiments were performed as described previously with a few modifications [3]. For FIB-SEM, blocks were trimmed to expose the longitudinal head area. On Helios NanoLab 600i FIB-SEM (FEI), a layer of platinum about 1 μm thick was deposited on a surface perpendicular to the block face to be imaged. The block face was imaged using an electron beam with 3 keV acceleration voltage, 3 nA current, and 3 μs/pixel dwell time. After the block face was imaged, gallium ion beam with an acceleration voltage of 30 keV and a current of 3 nA was used to mill the 50 nm thick superficial layer from the block face for the next round of imaging and milling. After the entire volume was acquired, the images were imported into Amira software and aligned [4]. The sensory compartments were manually traced and annotated using Amira v5.2 software (https:// www.amira.com/).

Live cell imaging

Young adult worms were anesthetized with 0.2 mmol/L levamisole in M9 buffer, and were mounted on 3% agarose pads at 20 °C [5]. Our live cell imaging system includes an Axio Observer Z1 microscope (Carl Zeiss MicroImaging, Inc.) equipped with a 100×, 1.45 N.A. objective or an Olympus IX83 microscope equipped with a 150×, 1.45 N.A. oil objective, an EM CCD camera (Andor iXon+ DU-897D-C00-#BV-500), and a Sapphire CW CDRH USB Laser System with a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). Time-lapse images were acquired with an exposure time of 200 msec by μManager software (https://www.micro-manager.org/) and processed and quantified by ImageJ software (http://rsbweb.nih.gov/ij/).

Quantifications and statistical analysis

The Student's *t*-test was used to examine significant differences in the sensory compartment between WT and mutants as indicated in figure legends.

Reference

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