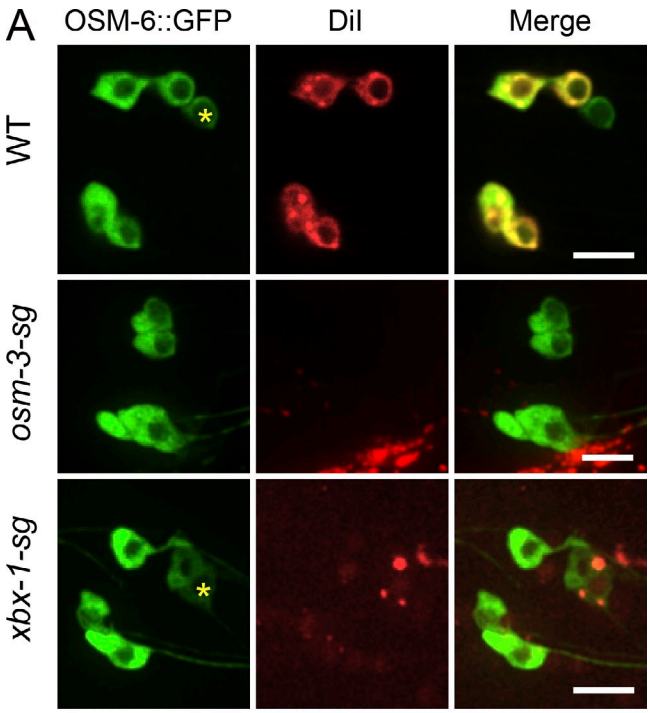
Li et al., http://www.jcb.org/cgi/content/full/jcb.201411041/DC1



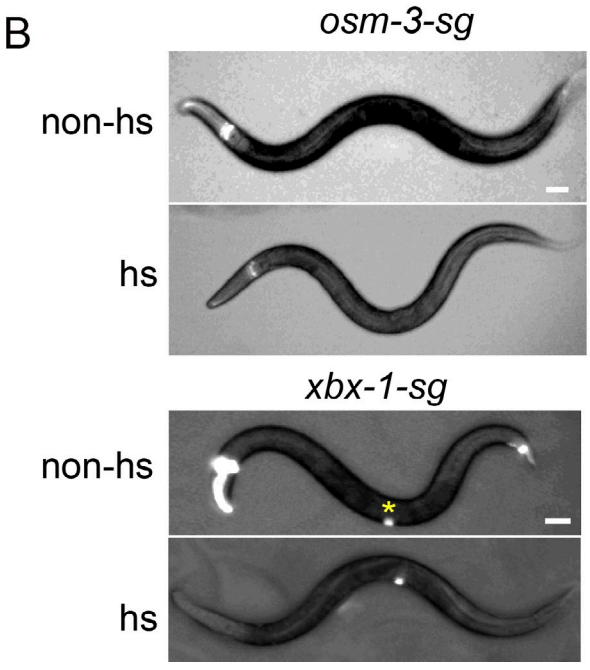


Figure S1. **The Dyf phenotype in *osm-3-sg* or *xbx-1-sg* animals.** (A) The dye-filling phenotype of four phasmid neurons (PHA/B/L/R) of WT, *osm-3-sg*, and *xbx-1-sg* conditional mutants under a 100× objective lens. The asterisk indicates a PQR neuron, which does not take up DiI in WT animals. Bar, 5 µm. (B) The Dyf defects in *osm-3-sg* and *xbx-1-sg* conditional mutants as observed under a fluorescence stereoscope. The head and the tail of the ciliated sensory neurons are stained by the red fluorescent DiI dye in WT animals, whereas a dye-filling defect is observed in conditional mutant animals. The asterisk indicates the transgenic marker. Non-hs, nonheat shock; hs, heat shock. Bar, 50 µm.

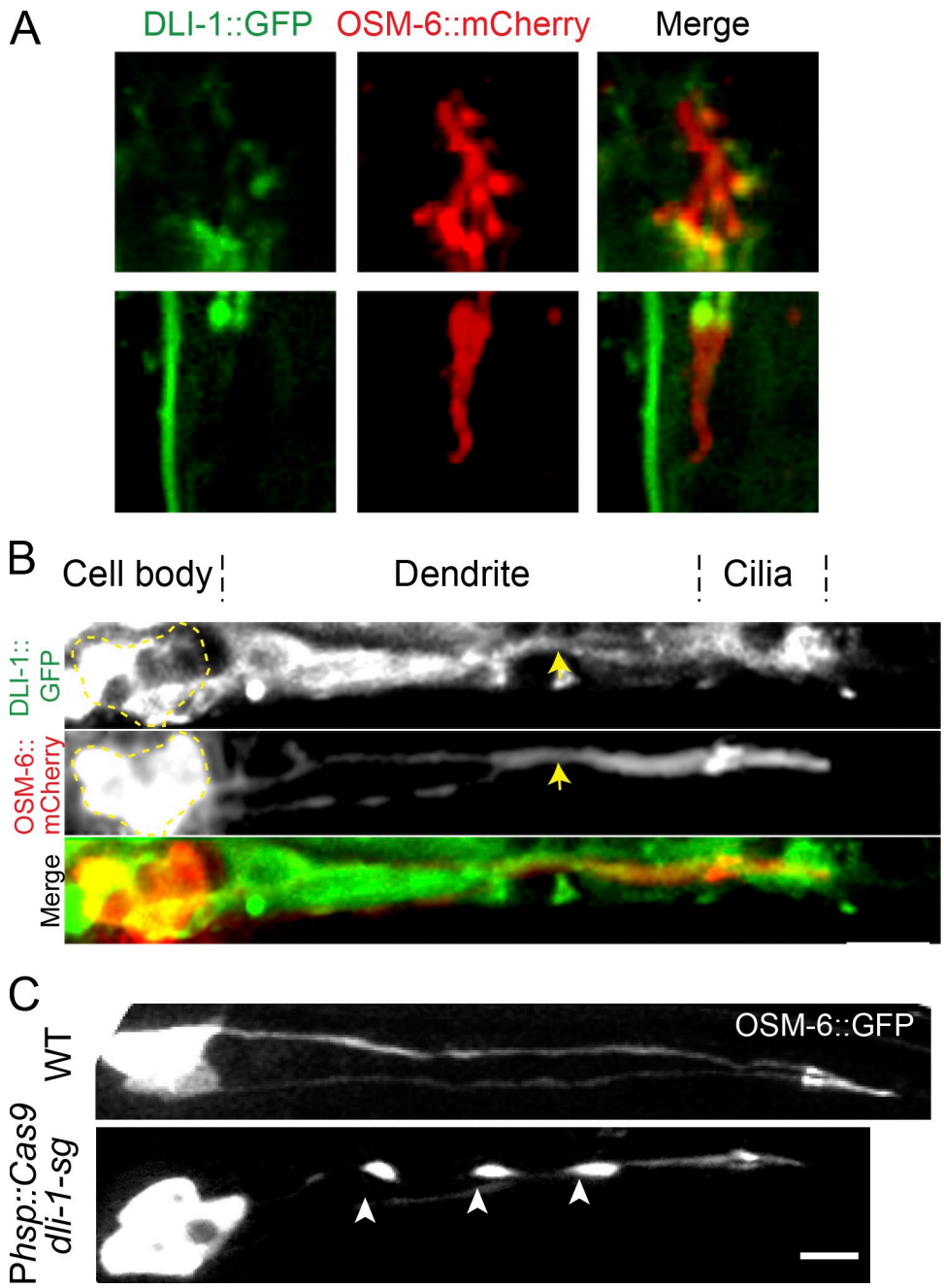


Figure S2. **DLI-1 functions in *C. elegans* ciliated neurons.** (A and B) Animals expressing P*dli-1::dli-1::GFP* illustrate that DLI-1 does not enter the cilia (A) but localizes in the cell body and dendrites of ciliated neurons (B, arrows). The broken outlines indicate the junctions between segments within ciliated neurons. (C) Aggregates of OSM-6::GFP (arrows) along the dendrite are observed in *dli-1* conditional mutant animals. Bar, 5 µm.

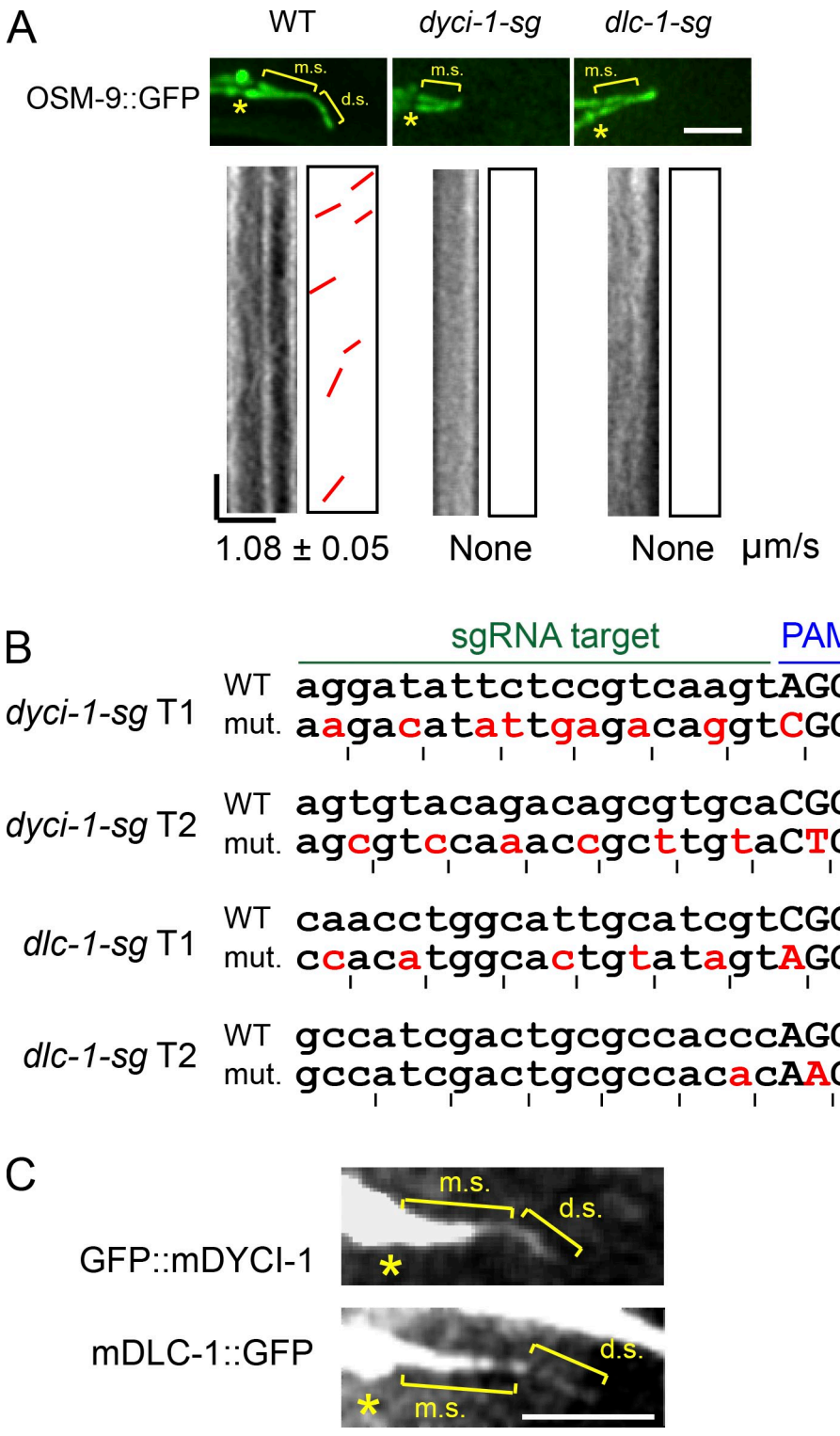


Figure S3. **GFP-tagged DYCI-1 and DLC-1 with synonymous mutations rescued ciliary phenotypes in the corresponding conditional mutants.** (A) The retrograde movement of OSM-9::GFP in cilia of WT, *dyci-1-sg*, and *dlc-1-sg* conditional mutant animals. (top) OSM-9::GFP localization. (bottom) Kymographs and corresponding lines of the movement. Bars: (micrograph bar on top) 5 μm; (kymograph horizontal bar) 2 μm; (vertical bar) 5 s. (B) Synonymous mutations (red) of the target sites in *dyci-1* and *dlc-1*. (C) The localization of GFP-tagged DYCI-1 and DLC-1 with synonymous mutations in cilia of *dyci-1-sg* and *dlc-1-sg* conditional mutant animals. Bar, 5 μm. m.s., middle segment; d.s., distal segment. The asterisks indicate the transition zone.

Table S1. ***C. elegans* strains used in this study**

Strain name	Genotype	Method
SP2101	ncl-1(e1865) unc-36(e251); osm-6(p811); mnIs17[Posm-6::osm-6::GFP; unc-36(+)]	CGC
JT11069	xbx-1(ok279)	CGC, a 1,600-bp deletion
CX3716	lin-15B(n765); kyIs141[Posm-9::osm-9::gfp; lin-15(+)]	CGC
GOU1348	casEx1605[Phsp-16.2::Cas9+PU6::che-3 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Micoinjection and cross with *mnIs17*
GOU1411	casEx5520[Phsp-16.2::Cas9+PU6::lis-1-T1/2 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1417	casEx5512[Phsp-16.2::Cas9+PU6::dlc-1-T1/2 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1418	casEx5514[Phsp-16.2::Cas9+PU6::dyci-1-T1/2 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1419	casEx5513[Phsp-16.2::Cas9+PU6::dli-1-T1/2 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1421	casEx5516[Phsp-16.2::Cas9+PU6::dylt-3 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1422	casEx5517[Phsp-16.2::Cas9+PU6::dyrb-1 sgRNA; Podr-1::dsRed; unc-76(+)]; mnIs17	Microinjection and cross with *mnIs17*
GOU1423	casEx5534[Phsp-16.2::Cas9+PU6::osm-1-T1/2 sgRNA; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; mnIs17	Microinjection and cross with *mnIs17*
GOU1442	casIs482[Phsp-16.2::Cas9+PU6::dyci-1 sgRNA T1/2; Podr-1::dsRed (+)]; mnIs17	Cross with *mnIs17*
GOU1444	casEx1629[Phsp-16.2::Cas9+PU6::che-11 sgRNA; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; mnIs17	Microinjection and cross with *mnIs17*
GOU1448	casEx1633[Pxbx-1::xbx-1::YFP; rol-6(su1006) (+)];casIs482	Microinjection and cross with *casIs482*
GOU1453	xbx-1(ok279); casEx1630[Pdyf-1::gfp::dyci-1; Pdyf-1::osm-6::mCherry; rol-6(su1006) (+)]	Microinjection
GOU1461	casEx5524[Phsp-16.2::Cas9+PU6::osm-12-T1/2 sgRNA; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; mnIs17	Microinjection and cross with *mnIs17*
GOU1460	casEx1629[Phsp-16.2::Cas9+PU6::xbx-1 sgRNA; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; mnIs17	Microinjection
GOU1463	casEx1629[Phsp-16.2::Cas9+PU6::xbx-1 sgRNA; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; casIs487[Pdyf-1::Cas9; Podr-1::dsRed;unc-76(+)]; mnIs17	Cross with *casIs487*
GOU1464	casEx5544[Phsp-16.2::Cas9+PU6::osm-3 sgRNA; Pdyf-1::osm-6::mCherry; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]	Microinjection
GOU1465	casEx5535[Pdlc-1::dlc-1::GFP;Pdyf-1::osm-6::mCherry; unc-76(+)]	Microinjection
GOU1466	casEx5537[Pdylt-3::dylt-3::GFP;Pdyf-1::osm-6::mCherry; unc-76(+)]	Microinjection
GOU1467	casEx5527[Pdyf-1::GFP::dyci-1;Pdyf-1::osm-6::mCherry; rol-6(su1006) (+)]	Microinjection
GOU1468	casEx5523[Pdyci-1::dyci-1::GFP; Pdyf-1::osm-6::mCherry; rol-6(su1006) (+)]	Microinjection
GOU1469	casEx5538[Pdli-1::dli-1::GFP; Pdyf-1::osm-6::mCherry; unc-76(+)]	Microinjection
GOU1470	casEx5547[Pdyf-1::GFP::dli-1; Pdyf-1::osm-6::mCherry; unc-76(+)]	Microinjection
GOU1471	casEx5548[Plis-1::lis-1::GFP; Pdyf-1::osm-6::mCherry]	Microinjection
GOU1472	casEx5549[Pdyf-1::GFP::lis-1; Pdyf-1::osm-6::mCherry]	Microinjection
GOU1473	casEx5540[Phsp-16.2::Cas9+PU6::osm-3 sgRNA; Pdyf-1::Cas9; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24]; mnIs17	Microinjection
GOU1474	casIs482[Phsp-16.2::Cas9+PU6::dyci-1 sgRNA T1/2; Podr-1::dsRed (+)]	Microinjection and integration
GOU1475	casIs487[Pdyf-1::Cas9; Podr-1::dsRed; unc-76(+)]	Microinjection and integration
GOU1568	casIs509; kyIs141	Cross with *kyIs141*
GOU1570	casIs482; casEx1642[Pdyf-1::gfp::mdyci-1; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24; rol-6(su1006) (+)]	Microinjection
GOU1571	casIs509[Phsp-16.2::Cas9+PU6::dlc-1 sgRNA T1/2; Podr-1::dsRed; unc-76(+)]; casEx1643[Pdlc-1::mdlc-1::GFP; Pegl-17::Myri-mCherry; Pegl-17::mCherry::his-24; rol-6(su1006) (+)]	Microinjection and integration
GOU1584	casIs482; kyIs141	Cross with *kyIs141*

CGC, *Caenorhabditis* Genetics Center.

Table S2. **PCR products for construction of transgenic *C. elegans***

PCR product	5′ primer (5′ to 3′)	3′ primer (5′ to 3′)	Template
P*dylt-3::dylt-3::GFP*	CAGCGTGAGTGAGAAGTTGGTCTAG	AAGGGCCCGTACGGCCGACTAGTAGG	N_2 Genomic DNA

Table S3. **Plasmids for construction of transgenic *C. elegans***

Plasmid name	5′ primer (5′ to 3′)	3′ primer (5′ to 3′)	Notes
pDD162-***Phsp-16.2:***::*Cas9+ PU6::dyci-1-T1 sgRNA*	GATATTCTCCGTCAAGTGTTTTAGAGCTAGAAATAGC	CTTGACGGAGAATATCCTCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dyci-1-T2 sgRNA*	TGTACAGACAGCGTGCAGTTTTAGAGCTAGAAATAGC	GCACGCTGTCTGTACACTCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dlc-1-T1 sgRNA*	ACCTGGCATTGCATCGTGTTTTAGAGCTAGAAATAGC	CGATGCAATGCCAGGTTGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dlc-1-T2 sgRNA*	CATCGACTGCGCCACCCGTTTTAGAGCTAGAAATAGC	GGTGGCGCAGTCGATGGCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dli-1-T1 sgRNA*	AGCAAATGCTGTGCTTCGTTTTAGAGCTAGAAATAGC	AAGCACAGCATTTGCTGTCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dli-1-T2 sgRNA*	TCGACAGACGAAGAAGTGTTTTAGAGCTAGAAATAGC	CTTCTTCGTCTGTCGAAGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dylt-3 sgRNA*	CACAATTTCTGTGTGTCGTTTTAGAGCTAGAAATAGC	ACACACAGAAATTGTGTACAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::dyrb-1 sgRNA*	CATGATTGCTCCAGACAGTTTTAGAGCTAGAAATAGC	GTCTGGAGCAATCATGATCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::lis-1-T1 sgRNA*	ACATGAGTTTGTCGGAGGTTTTAGAGCTAGAAATAGC	TCCGACAAACTCATGTTGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::lis-1-T2 sgRNA*	GATATCAAGCCACTAGGGTTTTAGAGCTAGAAATAGC	CTAGTGGCTTGATATCATCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::osm-3 sgRNA*	GGCACTGTGTTTGCCTAGTTTTAGAGCTAGAAATAGC	AGGCAAACACAGTGCCATCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::xbx-1 sgRNA*	CGAGTCATTTCGCATTGTTTTAGAGCTAGAAATAGC	ATGCGAAATGACTCGTCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::che-3 sgRNA*	AGTCGGGATTTCCAATTGTTTTAGAGCTAGAAATAGC	TTGGAAATCCCGACTTCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::che-11 sgRNA*	GTAGATTGGAGCCACGAGTTTTAGAGCTAGAAATAGC	GTGGCTCCAATCTACTCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::osm-1-T1 sgRNA*	AGTTCCTCCGTACAATTGTTTTAGAGCTAGAAATAGC	TTGTACGGAGGAACTTGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::osm-1-T2 sgRNA*	TGCCAGAAGAGGTGCCGTTTTAGAGCTAGAAATAGC	GCACCTCTTCTGGCAGACAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::osm-12-T1 sgRNA*	ACTGATTTCGCTCAAGTGTTTTAGAGCTAGAAATAGC	CTTGAGCGAAATCAGTTCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDD162-***Phsp-16.2***::*Cas9+ PU6::osm-12-T2 sgRNA*	ATACCCTGTTCAATCGTGTTTTAGAGCTAGAAATAGC	CGATTGAACAGGGTATCCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pDONR-***Pdyf-1***::*Cas9*	TGTAAGCTTGTCAAAATGGACAAAAAATACAGCATCGG	GAAGAGTAATTGGACTTAGGCGTAGTCTGGGACGT	PCR from pDD162-***Phsp-16.2***::*Cas9+ PU6::Empty sgRNA*
pPD95.77-***Pdyci-1***::*dyci-1::GFP*	GTACCGGTAGAAAAACGGTAGATTCTGCAAGTTTGCGTG	TTCTTCTCCTTTACTCATGTTATAATTCTCCGCTTCCTCATTCG	PCR from N_2
pPD95.77-***Pdlc-1***::*dlc-1::GFP*	AGTGACCTGTTCGTTCTCCCTTTGGAGCCTCAATCGGTA	CCCTCCACCTCCGCCTCCACCTCCAGACTTGAATAGCAGGATGGCGA	PCR from N_2
pPD95.77-***Pdli-1***::*dli-1::GFP*	AGTGACCTGTTCGTTGTCAAATGGAACAGTTTGTAGATCGGATTGAC	CCCTCCACCTCCGCCTCCACCTGCATCACTGTCCCGGGGTTGAGG	PCR from N_2
pPD95.77-***Plis-1***::*lis-1::GFP*	AGTGACCTGTTCGTTTGTTTGCACACAATATTTCTCACGACC	CCCTCCACCTCCGCCTCCACCACGGCATTCCCAAACTTTGCAC	PCR from N_2
pDONR-***Pdyf-1***::*GFP::dyci-1*	GATGAACTATACAAAATGTCAGAACTGAGGAAACTCGAA	GAAGAGTAATTGGACCATGTTATAATTCTCCGCTTCCTCA	PCR from N_2
pDONR-***Pdyf-1***::*dli-1::GFP*	TGTAAGCTTGTCAAAATGCCACCAACTGCGCAACCACTGG	CCCTCCACCTCCGCCTCCACCTGCATCACTGTCCCGGGGTTGAGG	PCR from N_2
pDONR-***Pdyf-1***::*GFP::lis-1*	GATGAACTATACAAAATGAGTTTGTCGGAGAGGCAAAAAG	GAAGAGTAATTGGACTCAACGGCATTCCCAAACTTTGC	PCR from N_2
pDONR-***Pdlc-1***::*dlc-1::GFP*	AGTGACCTGTTCGTTCTCCCTTTGGAGCCTCAATCGGTA	CCCTCCACCTCCGCCTCCACCTCCAGACTTGAATAGCAGGATGGCGA	PCR from N_2
pDONR-***Pdyf-1***::*GFP::dyci-1*^{mT1}	ACCTGTCTCAATATGTCTTCGACCTCATTCGATGAAAG	CATATTGAGACAGGTCGGTATTTCAACTGAGCCAACAG	PCR from pDONR-P*dyf-1::GFP::mdyci-1*
pDONR-***Pdyf-1***::*GFP::dyci-1*$^{mT1+mT2}$	GTACAAGCGGTTTGGACGCTGGAAAATTCCAAGTCGAC	CCAAACCGCTTGTACTGATAACAAAGATTCGGTAAGC	PCR from pDONR-P*dyf-1::GFP::dyci-1*^{mT1}
pDONR-***Pdlc-1***::*dlc-1*^{mT1}*::GFP*	GTGTTGCACAATCTATTGCGTCCTGTTGCATGTCATC	TAGATTGTGCAACACAAGCCCTCGAGAAATACAACAT	PCR from pDONR-P*dlc-1::dlc-1::GFP*
pDONR-***Pdlc-1***::*dlc-1*$^{mT1+mT2}$*::GFP*	TATACAGTGCCATGTGGGGTTGTATTTCTTGTCGAACT	ACATGGCACTGTATAGTAGGAAGAAACTTTGGAAGCTAC	PCR from pDONR-P*dlc-1::dlc-1*^{mT1}*::GFP*

Promoters are highlighted in bold.

Table S4. **Targets of CRISPR in *C. elegans***

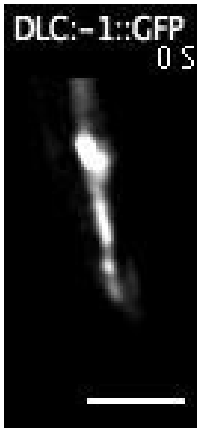
Gene	CRISPR–Cas9 targets (POM)	
	Target	Sequence
dyci-1	T1	AGGATATTCTCCGTCAAGT**AGG**
	T2	AGTGTACAGACAGCGTGCA**CGG**
dlc-1	T1	CAACCTGGCATTGCATCGT**CGG**
	T2	GCCATCGACTGCGCCACCC**AGG**
dli-1	T1	ACAGCAAATGCTGTGCTTC**CGG**
	T2	CTTCGACAGACGAAGAAGT**CGG**
dylt-3	T1	TACACAATTTCTGTGTGTC**CGG**
dyrb-1	T1	ATCATGATTGCTCCAGACA**AGG**
lis-1	T1	CAACATGAGTTTGTCGGAG**AGG**
	T2	ATGATATCAAGCCACTAGG**AGG**
osm-3	T1	ATGGCACTGTGTTTGCCTA**TGG**
xbx-1	T1	ACGAGTCATTTCGCATT**TGG**
che-3	T1	GAAGTCGGGATTTCCAATT**TGG**
osm-1	T1	CAAGTTCCTCCGTACAATT**TGG**
	T2	TCTGCCAGAAGAGGTGCC**TGG**
che-11	T1	GAGTAGATTGGAGCCACGA**CGG**
dyci-1	mT1	AAGACATATTGAGACAGGTC**GG**
	mT2	AGCGTCCAAACCGCTTGTA**CTG**
dlc-1	mT1	CCACATGGCACTGTATAGTA**GG**
	mT2	GCCATCGACTGCGCCACACA**AG**

Bold text indicates PAM sequences. Underlined text indicates synonymous mutations of the CRISPR–Cas9 targets for the rescue experiments. Sequences are 5′ to 3′.

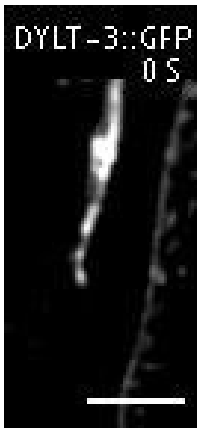
Table S5. **Primers for molecular analysis**

Target gene	5′ sequence (5′ to 3′)	3′ sequence (5′ to 3′)
osm-3	CAAGGACTTCACGTTCGATGGAGC	GTCAAAGAGTCAGGTCAAGGGC
xbx-1	CAGTATCAGAAGTTCGTCGT	CACCAATACAAGTCTAAGCTAG
dyci-1	GGCAGAGCTGAAATCTCAGCGG	CGCTCATCGTCAGTCTGGGTTCC
dlc-1	GGTTGACCGCAAGGCTGTGATCAA	GCACACTGAAGATCCTACGCCACC
dli-1	GCACGTGGAGCACACGATATCC	GCAGACGAACTTCTCCGAGGG
dylt-3	TGCCATGACGGACCGGAAATACTTT	ACTCATTGCCTTTACCGATTCTCGCC
dyrb-1	TCGTTCCTCGATTCACTGGCTGC	ACGTGGAGGGACACTGTTCTAAGCA
lis-1	CTGCAAAAAATCTGAATTTCCTTTTTCAATCTC	GATATTTCATCTCTTGAACCTTAATGAGCAGG

Video 1. **IFT of GFP::DYCI-1.** Transgenic *C. elegans* strain (GOU1467) expressing GFP-tagged DYCI-1 in cilia. Images were taken using a time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (CSU-X1 Spinning Disk Unit; Yokogawa Electric Corporation). Frames were taken continuously for 1.5 min. The display rate was 7 frames per second.



Video 2. **IFT of DLC-1::GFP.** Transgenic *C. elegans* strain (GOU1465) expressing GFP-tagged DLC-1 in cilia. Images were taken using a time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (CSU-X1 Spinning Disk Unit; Yokogawa Electric Corporation). Frames were taken continuously for 1.5 min. The display rate was 7 frames per second.



Video 3. **IFT of DYLT-3::GFP.** Transgenic *C. elegans* strain (GOU1466) expressing GFP-tagged DYLT-3 in cilia. Images were taken using a time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (CSU-X1 Spinning Disk Unit; Yokogawa Electric Corporation). Frames were taken continuously for 1.5 min. The display rate was 7 frames per second.

Video 4. **IFT of GFP::LIS-1.** Transgenic *C. elegans* strain (GOU1472) expressing GFP-tagged LIS-1 in cilia. Images were taken using a time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (CSU-X1 Spinning Disk Unit; Yokogawa Electric Corporation). Frames were taken continuously for 1.5 min. The display rate was 7 frames per second.