

Supplementary Figure 1. The antibodies AbDAF19N and AbDAF19C are specific for DAF-19

(A) Western blot on total worm protein from a mixed stage population. AbDAF19N detects a 120 kDa band in *wild type*, *daf-12* and *daf-19/mnC1* heterozygotes, but not in *daf-19* or *daf-19; daf-12* mutants.

(B-E) Immunocytochemistry stainings of the head regions of wild-type and *daf-19* adult worms. AbDAF19N and AbDAF19C detect DAF-19 in neurons of wild-type worms (B, D). The signal is lost in *daf-19* mutants (C, E). Arrowheads mark the location of the nerve ring and arrows the location of the cell bodies of amphid ciliated sensory neurons.

Supplementary Figure 2. Isoform-specific rescue of *daf-19*

(A-F) Single scan confocal micrographs showing the expression of DAF-19 and its direct targets, cilia proteins BBS-7 and OSM-5 in various transgenic *daf-19 (m86)* rescue lines.

(A-C) AbDAF19N detects DAF-19 in worms rescued for DAF-19A and for DAF-19A/B/C, but not in worms rescued for DAF-19C.

(D-F) AbDAF19C detects DAF-19 in all three rescue lines.

(C', C'', F', F'') Only DAF-19C is able to rescue the expression of the cilia proteins OSM-5 and BBS-7.

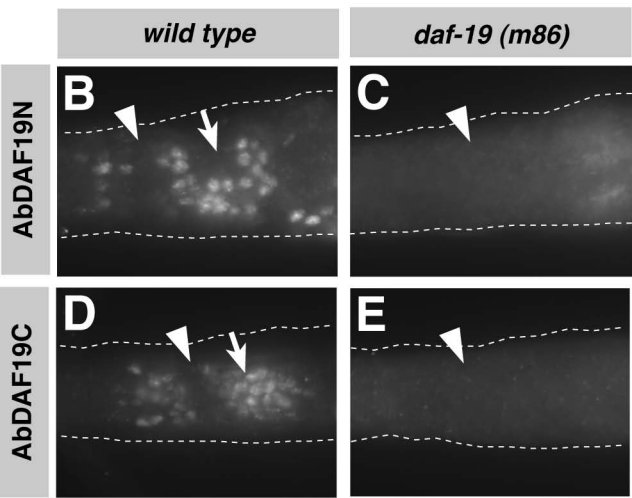
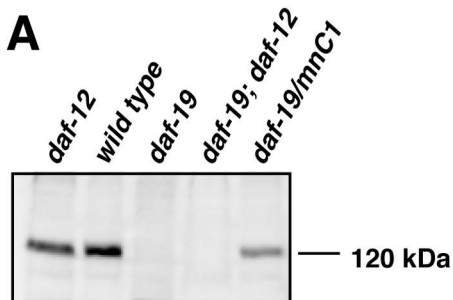
(B', B'', E', E'') DAF-19A, which is specific for non-ciliated neurons, does not rescue OSM-5 and BBS-7.

(A'''-F''') Schematics summarizing the expression of the three proteins DAF-19 (red), BBS-7 (green) and OSM-5 (white) in each transgenic line. Arrowheads depict ciliated sensory neurons, arrows depict non-ciliated neurons.

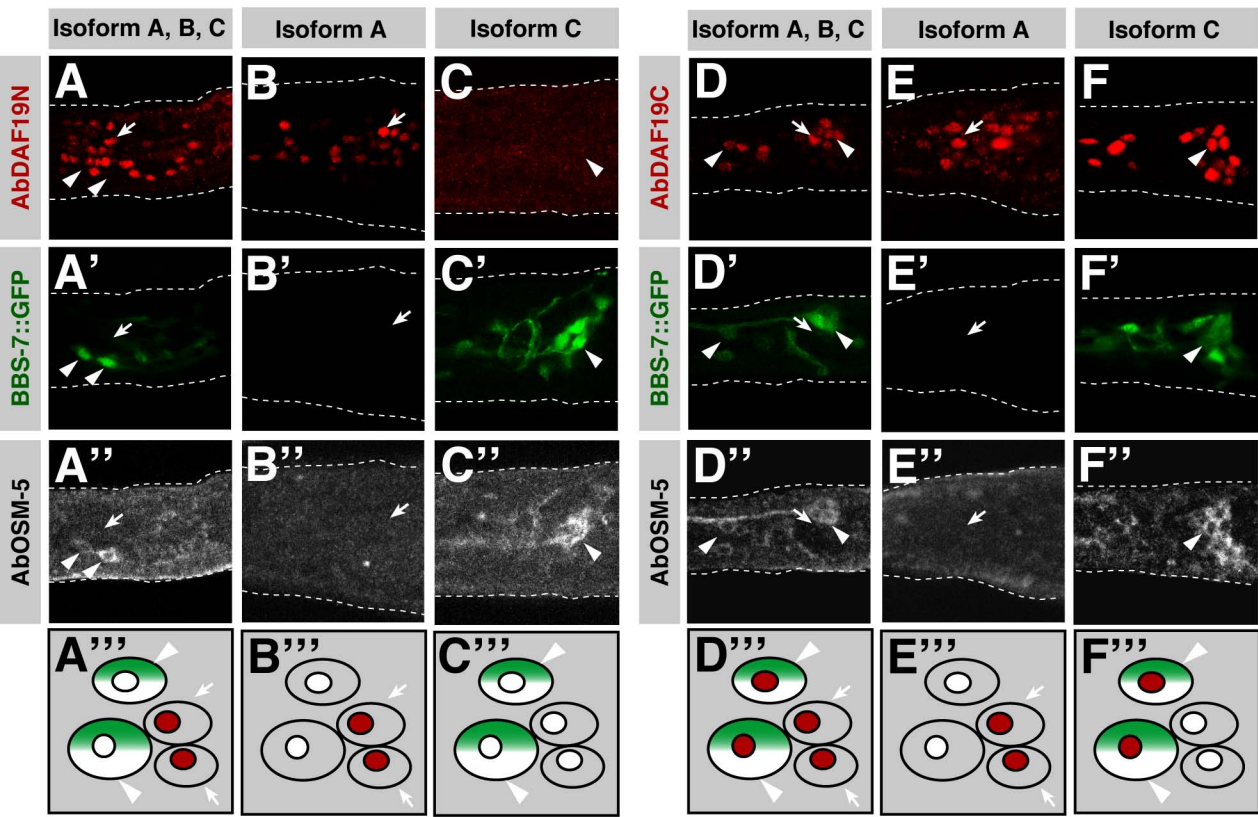
Supplementary Figure 3. Paralysis assays examining different cilia mutants and the effect of ectopic *daf-19a* expression in muscles

(A) The lack of sensory input (cilia mutants *che-13* and *osm-5*) does not cause resistance to aldicarb. (B) Ectopic expression of *daf-19a* in muscles (from the *unc-54* promoter) does not alter the paralysis phenotype of *daf-19*. Three independent transgenic lines (line 1-3) were tested.

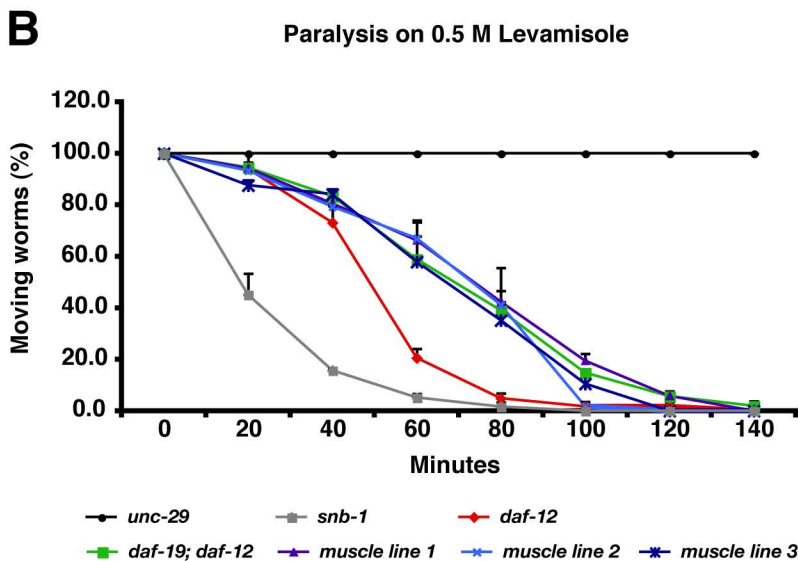
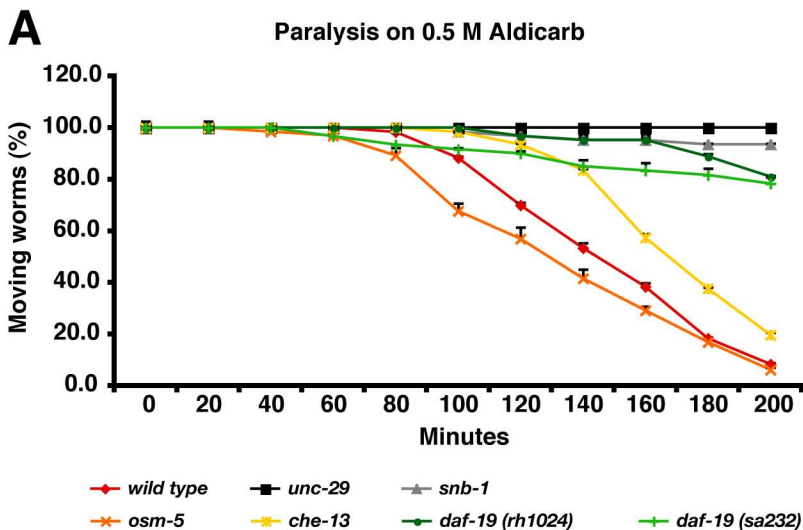
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table 1. Co-expression analysis of DAF-19 A/B in *gfp* reporter lines

Worm strains that express *gfp* in specific subsets of neurons were stained with AbDAF19N, the antibody specific for DAF-19A/B, which specifically labels non-ciliated neurons. The only exceptions are FLP neurons, which can be considered as hybrid neurons since they bear characteristics of both ciliated sensory neurons as well as non-ciliated touch sensory neurons (Wu et al. (2001), Genes Dev 15, 789-802). In the neurons HSN, AVJ or AIN and AIM or RIH none of the DAF-19 isoforms was detected. We cannot rule out, that DAF-19 expression in those neurons is below detection level. Note that AVJ or AIN (marked with *adIs1240*), AIM or RIH (marked with *mgIs42*) and RIR (marked with *akEx51*) have not been clearly identified in the original publications.

	Promoter	Cilia	AbDAF19N
Dopaminergic neurons (<i>egIs1</i>)	<i>dat-1</i>		
ADEL/R, CEPD (2), CEPV (2), PDE (2)		√	—
Serotonergic neurons (<i>mgIs42</i>)	<i>tph-1</i>		
NSM		—	√
ADFL/R		√	—
GABAergic neurons (<i>oxIs12</i>)	<i>unc-47</i>		
AVL, D motoneurons (19), DVB, RMEV (2), RMEL/R, RMED (2), RIS		—	√
Glutamatergic neurons (<i>adIs1240</i>)	<i>eat-4</i>		
ADAL/R, ALML/R, AUAL/R, AVM, LUAL/R, PLML/R, PVDL/R, PVR		—	√
FLPL/R		√	√
ASHL/R, ASKL/R, IL1 (6), OLQD (2), OLQV (2)		√	—
Ciliated neurons (<i>kyIs4</i>)	<i>ceh-23</i>		
ADFL/R, ADLL/R, AFDL/R, ASEL/R, ASGL/R, ASHL/R, AWCL/R,		√	—
BAGL/R, PHAL/R, PHBL/R		√	—
AIYL/R, CANL/R		—	√
Interneurons (<i>akIs3</i>)	<i>nmr-1</i>		
AVAL/R, AVDL/R, AVEL/R, AVG, PVCL/R, RIML/R		—	√
Interneurons (<i>akEx130</i>)	<i>glr-1</i>		
AIBL/R, AVAL/R, AVBL/R, AVDL/R, AVEL/R, AVG, PVCL/R,		—	√
RIML/R, RMDL/R, RMDD (2), RMDV (2), SMDD (2), SMDV (2),		—	√
URYD (2), URYV (2)		—	√
Interneurons (<i>akEx51</i>)	<i>glr-2</i>		
AIAL/R, AIBL/R, AVAL/R, AVDL/R, AVEL/R, AVG, M1, PVCL/R,		—	√
RIAL/R, RIGL/R, RIR, RMDD (2), RMDV (2)		—	√
XXX cell (<i>dhIs64</i>)	<i>daf-9</i>		
XXXL/R		—	—

Supplementary Table 2. Quantification of signals in antibody stainings against SNB-1 and UNC-64 in *wild type*, *daf-19* mutant and rescue worms, and two different cilia mutants, *che-11* and *che-13* (see also Figure 6). Numbers are given in percentage as strong / medium / weak staining when compared to UNC-10, which was used as a co-marker since it is unchanged in *wild type* and *daf-19*. At least 40 animals were scored for each genotype and developmental stage.

SNB-1 expression	L1/L2			L3/L4			Adults		
	<u>strong</u>	<u>medium</u>	<u>weak</u>	<u>strong</u>	<u>medium</u>	<u>weak</u>	<u>strong</u>	<u>medium</u>	<u>weak</u>
<i>wild type</i>	68	32	0	30	67	3	65	35	0
<i>che-13 (e1805)</i>	63	37	0	23	75	7	63	37	0
<i>che-11 (e1810)</i>	43	52	5	13	85	2	60	40	0
<i>daf-19 (m86)</i>	20	77	3	10	88	2	17	63	20
<i>daf-19 (rh1024)</i>	25	68	7	12	80	8	12	75	13
<i>daf-19 (sa232)</i>	20	73	7	8	85	7	10	60	30
<i>daf-19 (m86) + daf-19a</i>	52	41	7	22	73	5	67	24	9

UNC-64 expression	L1/L2			L3/L4			Adults		
	<u>strong</u>	<u>medium</u>	<u>weak</u>	<u>strong</u>	<u>medium</u>	<u>weak</u>	<u>strong</u>	<u>medium</u>	<u>weak</u>
<i>wild type</i>	40	57	3	33	67	0	69	27	5
<i>che-13 (e1805)</i>	30	70	0	28	72	0	76	22	2
<i>che-11 (e1810)</i>	23	75	2	28	70	2	66	32	2
<i>daf-19 (m86)</i>	28	65	7	20	75	5	22	40	38
<i>daf-19 (rh1024)</i>	30	63	7	28	57	15	10	47	43
<i>daf-19 (sa232)</i>	30	65	5	23	67	10	15	34	51
<i>daf-19 (m86) + daf-19a</i>	24	67	9	22	69	9	57	38	5

Supplementary Table 3. Published lists of predicted x-box genes (Blacque et al., 2005 [OB], Chen et al., 2006 [NC], Efimenko et al., 2005 [EE]) were searched for potential functions at synapses or in vesicles as summarized in this Table. Genes marked in grey were investigated in detail in this study.

Gene Model	Gene	X-box	Distance to the ATG	Reference	Predicted function/Homolog
F42G8.11	<i>sph-1</i>	GTTTCT AC AGTAAC	- 13	OB	Synaptophysin
F41B4.4b	<i>glr-6</i>	GTTTTT TT AAAAAC	- 28	EE	Glutamate receptor
T12A2.15b		GTTTCC AT AACTAC	- 51	EE	C2 domain vesicle protein
C18E9.10		GTTTCT AT GATAAC	- 53	EE	SFT-2 domain protein, membrane protein involved in Golgi to ER transport
F17E9.8		GTTACT GT AGAAAC	- 68	OB	Neuronal acetylcholine receptor protein, alpha-6 chain precursor
B0395.3		ATCACT AT AGTAAC	- 88	EE/OB	Splice isoform 1 of P43155 Carnitine O-acetyltransferase
F23H12.1	<i>snb-2</i>	GTCTCC AT ATCAAA	- 154	OB/NC	Vesicle-associated membrane protein 2
T12A2.15a		GTTTCC AT AACTAC	- 187	OB	C2 domain vesicle protein
M03E7.5		ATTATT TT AAAAAC	- 195	EE	Golgi SNAP complex vSNARE
C40C9.2	<i>acr-9</i>	TTTTCA AT AGCAAC	- 211	OB/NC	Neuronal acetylcholine receptor protein, alpha-7 chain precursor
C31H5.3	<i>acr-19</i>	GTTTCA AT AGAAAT	- 280	OB/NC	Neuronal acetylcholine receptor protein, alpha-7 chain precursor
Y57A10A.16		GTTTTA AT GACACA	- 312	NC	Trafficking protein particle complex 5
Y69A2AR.2a	<i>ric-8</i>	GTTTCG AT GCAAAT	- 377	NC	Synembryn
C09E8.1		GTTGCC AT GATAAC	- 411	EE/NC	Sodium neurotransmitter symporter
F59E12.8		GTATTT AT AAAAAC	- 420	EE	NMDA receptor subunit
ZK455.3		GTTTCG TT CGCAAC	- 448	EE/OB	Galanin receptor type 2, catione/carnitine transporter
F37A4.7d	<i>rbf-1</i>	GTCTCC AA GGAAAC	- 478	EE/NC	Rabphilin-3A
H35N03.1	<i>exp-1</i>	GTTTTT AT GGCCAC	- 481	EE/OB	Splice isoform 1 of GABA receptor beta-3 subunit precursor
T05C12.2	<i>acr-14</i>	GTAAC T AC GGTAAC	- 496	EE	Neuronal acetylcholine receptor protein, alpha-7 chain precursor
F35D2.5a	<i>syd-1</i>	GTCAC T AT AACAA C	- 530	EE/OB/NC	7h3 protein
F48E3.7	<i>acr-22</i>	GTCTAC AT GCCAAC	- 534	OB	Neuronal acetylcholine receptor protein, alpha-9 chain precursor
F08F8.8		ATTCT T TT GAAAC	- 540	EE	Golgi vSNARE
ZK1098.5		TTCTCC AT GGCAAG	- 598	OB	Trafficking protein particle complex subunit 3
T01B11.3	<i>syn-4</i>	GTGTCC AT GACAA C	- 676	OB/NC	Syntaxin 1B2
F37A4.7a	<i>rbf-1</i>	GTAACC AC GATAAC	- 804	OB	Rabphilin-3A
Y58G8A.1		GTTTCC GT AGTAAT	- 822	OB	Acetylcholine receptor protein, beta chain precursor
F55A4.1		GTTTTT TT AAAAAC	- 862	EE	Vesicle protein sec-22
F12F6.6	<i>sec-24.1</i>	ATATTT AT AGGAAC	- 868	EE	COPII protein
T22F7.3		GTTACT GT AGCAAT	- 934	OB	Splice isoform Alpha of P10646 Tissue factor pathway inhibitor precursor
T22F7.1		GTTATC TT GGTAAC	- 966	EE/NC	Carrier protein (synaptic vesicle protein)
C08G5.4	<i>snt-6</i>	GTTTCT AT GCCAAT	- 967	OB	Synaptotagmin VII
C18E9.2		GTTATC AT AGAAAC	- 991	OB	Translocation protein-1
F55A4.4		GTAACA AT AGTAAC	- 1026	OB	39k3 protein
T23H2.2	<i>snt-4</i>	ATTACC TT GCCAAC	- 1226	OB	Synaptotagmin IV
F09E8.7	<i>lev-1</i>	GCTTCC AT AGAAAT	- 1399	OB/NC	Neuronal acetylcholine receptor protein, alpha-4 chain precursor

Supplementary Table 4. Strains and transgenes (extra-chromosomal and integrated arrays) used for this study

A. STRAINS

Strain	Relevant transgene	Genotype
AA277	<i>daf-9::gfp</i>	[<i>lin-15</i> (n765); <i>dhIs64</i>]
BZ555	<i>dat-1::gfp</i>	[<i>egIs1</i>]
CB1072		[<i>unc-29</i> (<i>e1072</i>)]
CX2565	<i>ceh-23::gfp</i>	[<i>lin-15</i> (n765); <i>kyIs4</i>]
DA1240	<i>eat-4::gfp</i>	[<i>lin-15</i> (n765); <i>adIs1240</i>]
EG1285	<i>unc-47::gfp</i>	[<i>lin-15</i> (n765); <i>oxIs12</i>]
GR1366	<i>tph-1::gfp</i>	[<i>mgIs42</i>]
JT204		[<i>daf-12</i> (<i>sa204</i>)]
JT5010		(wild-type N2 Bristol)
JT6924		[<i>daf-19</i> (<i>m86</i>); <i>daf-12</i> (<i>sa204</i>)]
JT8651		[<i>daf-19</i> (<i>m86</i>)/ <i>mnC1</i> ; <i>lin-15</i> (n765)]
MT1642		[<i>lin-15</i> (n765)]
NM467		[<i>snb-1</i> (<i>md247</i>)]
OE3000		[<i>che-13</i> (<i>n1520</i>); <i>him-8</i> (<i>e1489</i>)]
OE3035		[<i>daf-19</i> (<i>sa232</i>); 6x outcrossed]
OE3059		[<i>daf-19</i> (<i>rh1024</i>); 6x outcrossed]
OE3063		[<i>daf-19</i> (<i>m86</i>); 6x outcrossed]
VM182	<i>glr-2::gfp</i>	[<i>lin-15</i> (n765); <i>akEx51</i>]
VM484	<i>nmr-1::gfp</i>	[<i>akIs3</i>]
VM763	<i>glr-1::gfp</i>	[<i>lin-15</i> (n765); <i>akEx130</i>]

B. EXTRA-CHROMOSOMAL AND INTEGRATED ARRAYS

Relevant transgene	Type of construct	Genotype
<i>B0395.3::gfp</i>	promoter <i>gfp</i> fusion	<i>ofEx301</i> , <i>ofEx302</i>
<i>C18E9.10::gfp</i>	promoter <i>gfp</i> fusion	<i>ofEx333</i> , <i>ofEx338</i>
<i>F17E9.8::gfp</i>	promoter <i>gfp</i> fusion	<i>ofEx324</i> , <i>ofEx325</i>
<i>glr-1::gfp</i>	translational <i>gfp</i> fusion	<i>akEx130</i>
<i>ida-1::gfp</i>	translational <i>gfp</i> fusion	<i>ofEx260</i> , <i>ofEx261</i>
<i>jnk-1::gfp</i>	translational <i>gfp</i> fusion	<i>ofEx61</i> , <i>ofEx62</i>
pGG20	<i>daf-19</i> intron 3- <i>gfp</i> fusion	<i>ofEx308</i> , <i>ofEx309</i>
pGG21	<i>daf-19</i> intron 4- <i>gfp</i> fusion	<i>ofEx310</i> , <i>ofEx311</i>
pGG14	genomic <i>daf-19</i> construct	<i>ofEx27</i> , <i>ofEx42</i>
pGG18	genomic <i>daf-19</i> construct	<i>ofEx375</i> , <i>ofEx376</i>
pGG67	<i>daf-19a</i> rescue construct	<i>ofEx316</i> , <i>ofEx165</i>
pTJ786	genomic <i>daf-19</i> construct	<i>ofEx168</i> , <i>ofEx169</i>
pTJ803	<i>daf-19c</i> rescue construct	<i>ofEx163</i> , <i>ofEx164</i>
<i>snb-1::gfp</i>	translational <i>gfp</i> fusion	<i>ofEx304</i> , <i>ofEx305</i>
<i>sng-1::gfp</i>	translational <i>gfp</i> fusion	<i>jsIs219</i>
<i>syd-1::gfp</i>	translational <i>gfp</i> fusion	<i>juIs40</i>
<i>unc-43::gfp</i>	translational <i>gfp</i> fusion	<i>ofEx263</i> , <i>ofEx297</i>
<i>unc-104::gfp</i>	translational <i>gfp</i> fusion	<i>ofEx305</i> , <i>ofEx306</i>
<i>unc-54::daf-19a</i>	muscle-specific <i>daf-19</i> expression	<i>ofEx585</i> to <i>587</i>