SUPPLEMENTARY MATERIAL

RESULTS

abtm-1 depleted animals have a pleiotropic phenotype

abtm-1(RNAi) animals show a pleiotropic range of phenotypes. They have slow and reduced growth (Gro, for Growth rate abnormal); larvae reach adulthood 36-48 hours later than controls (data not shown) and adults are smaller in size (Figure S2). A small proportion of *abtm-1(RNAi)* animals also form aberrant gonads or lack gonads completely (Figure S2). Those worms which form functional gonads show an egg laying defect (Egl, for egg laying defective) (Figure S2), which may reflect the impairment of neurons and/or muscles involved in egg laying, or defective vulval and/or gonadal development.

XLSA/A patients show non-progressive cerebellar ataxia and uncoordinated limb movement. We therefore asked whether depletion of *abtm-1* disrupts locomotion in worms. Normal locomotion in *C. elegans* consists of rhythmic sinusoidal waves propagated along the body. In liquid environments this is manifest as a thrashing motion. We quantified basal locomotion levels using a thrashing assay. *abtm-1(RNAi)* worms performed 151.7±2.7 thrashes/min (N=24) compared to 167.4±2.6 of control worms (N=24), showing that these animals have a mild but significant (t-test $p<0.0005$) locomotion defect. This suggests that *abtm-1(RNAi)* worms have a mild impairment of either, or both, the body wall muscles and the locomotory nervous system.

C. elegans has a characteristic set of rhythmic behaviors, including two ultradian rhythms: pharyngeal pumping and defecation (1). Many Mit mutants disrupt one or both of these rhythms (2-4). Defecation is a tightly regulated process (5). The defecation motor program (DMP) occurs every 50 seconds (6) and consists of three coordinated muscle based steps. The cycle length and periodicity of this process are routinely measured by timing the first of these steps, pBoc, the posterior body contraction. We found that the defecation motor program of the *abtm-1(RNAi)* worms is disrupted. Although the mean period is only slightly increased ($p<0.001$) the rhythmicity of the cycle, as measured by the coefficient of variation (CV) (7), is substantially disrupted (Table 2). Moreover, as well as having disrupted defecation cycles, *abtm-1(RNAi)* worms are also constipated (data not shown), which suggests that the worms also have a defect in the final expulsion (Exp) step. Pharyngeal pumping was not significantly altered in *abtm-1(RNAi)* animals (data not shown).

EXPERIMENTAL PROCEDURES

Behaviour analysis

To quantify locomotion we used a thrashing assay. Individual worms were transferred into a microtiter well containing 60 µl of M9 buffer (8). Thrashes produced by each worm in a period of 30 seconds were counted after a 2 min equilibration period. A thrash was defined as a change in direction of bending at the midbody, as previously described (9). 24 worms were examined for each strain.

To measure defecation we determined the interval between successive pBocs for a minimum of 10 defecation cycles in 10 worms at 20°C as described before (7).

To measure pharyngeal pumping we counted contractions of the pharynx under the dissecting microscope at 20°C, as described previously (10). Ten L4 worms were followed during five periods of 30 s. Experiments were performed in the presence of food.

Table S1. Lifespan analysis of *abmt-1(RNAi)* animals in wildtype and *daf-16* mutant backgrounds.

Strain	Median lifespan (days)	N[a]	*p*-value
wt	19	219(16)	
wt; *cat(RNAi)*	19	234(19)	0.9628[b]
wt; *abtm-1(RNAi)*	23	252(42)	0.0001[C]
daf-16(mu86)	17	210(25)	
daf-16(mu86); *cat(RNAi)*	17	205(24)	0.5140 [b]
daf-16(mu86); *abtm-1(RNAi)*	18	231(29)	0.0001[C]

[a]Number of scored deaths (censored animals).
[b]Log-rank test *p*-value of pairwise comparison between *cat(RNAi)* control animals and the corresponding genetic background.
[c]Log-rank test *p*-value of pairwise comparison between *cat(RNAi)* control animals and *abtm-1(RNAi)* within the same genetic background.

Table S2. Strains used in this work

Strain	Genotype	Reference
Bristol N2[a]	Reference wild type	(11)
SU93[a]	*jcIs1*IV	(12)
NL2098[a]	*rrf-1(pk1417)*I	(13)
KR344[a]	*let-363(h98) dpy-5(e61) unc-13(e450)I; sDp2(I;f)*	(14)
KN259[a]	*huIs33[sod-3::GFP; pRF4(rol-6(su1006)]*	(15)
FP11	*frh-1(ok610)*II/*mln[mls14dpy-10(e128)]II*	(3)
TJ356	*zIs356[daf-16p::daf-16-gfp; rol-6(su1006)]*	(16)
TM2721[b]	*abtm-1(tm2721)*I/+	This work
FP8	*abtm-1(tm2721)*I; *sDp2*(I;f)	This work
FP1	*ipEx1[abtm-1::GFP1; pRF4(rol-6(su1006); gDNA]*	This work
FP4	*ipEx4[abtm-1::GFP2; pRF4(rol-6(su1006); gDNA]*	This work

[a] Strains supplied by the *Caenorhabditis* Genetics Centre (University of Minnesota, MN).

[b] TM2721 was supplied by the NBRP (Tokyo Women's Medical University School of Medicine).

Table S3. Primers used to amplify DNA sequences to produce *dsRNA in vitro*

Gene	Forward primer/sequence (5'-3')	Reverse primer/sequence (5'-3')	DNA fragment (bp)
abtm-1	FP12/AGTCTTCGCAAAAGTCGCGC	FP13/GAGTGAAATTGAGCAGAGCC	506
Y62E10A.6	FP501/TTAGCAATTGTGGGCTCCGG	FP498/CGCGGAGCTCCTTGATTGT	610
lpd-8	FP503/TGCTCACAAGACTGAACCGG	RACE3'[a]	666
B0205.6	FP505/AATTGAGCCAGGATCTCCGC	FP497/CGGCATAGTTCACCAATTTG	522
Y73F8A.27	FP499/TGGCGATGAGAGCTAAAGG	RACE3' [a]	491
Y39B6A.3	FP507/ATGTCAAAATTCGGTGGAGC	RACE3'[a]	390
Y45F10D.4	FP509/CTTCAAATCAGTTCAGCCGC	FP496/GGCGAGCATTGAACAGTGAA	400

[a]In these cases we used a sequence-specific forward primer, designed based on the WormBase prediction of the genes, combined with RACE3' to obtain a fragment of the ORF.

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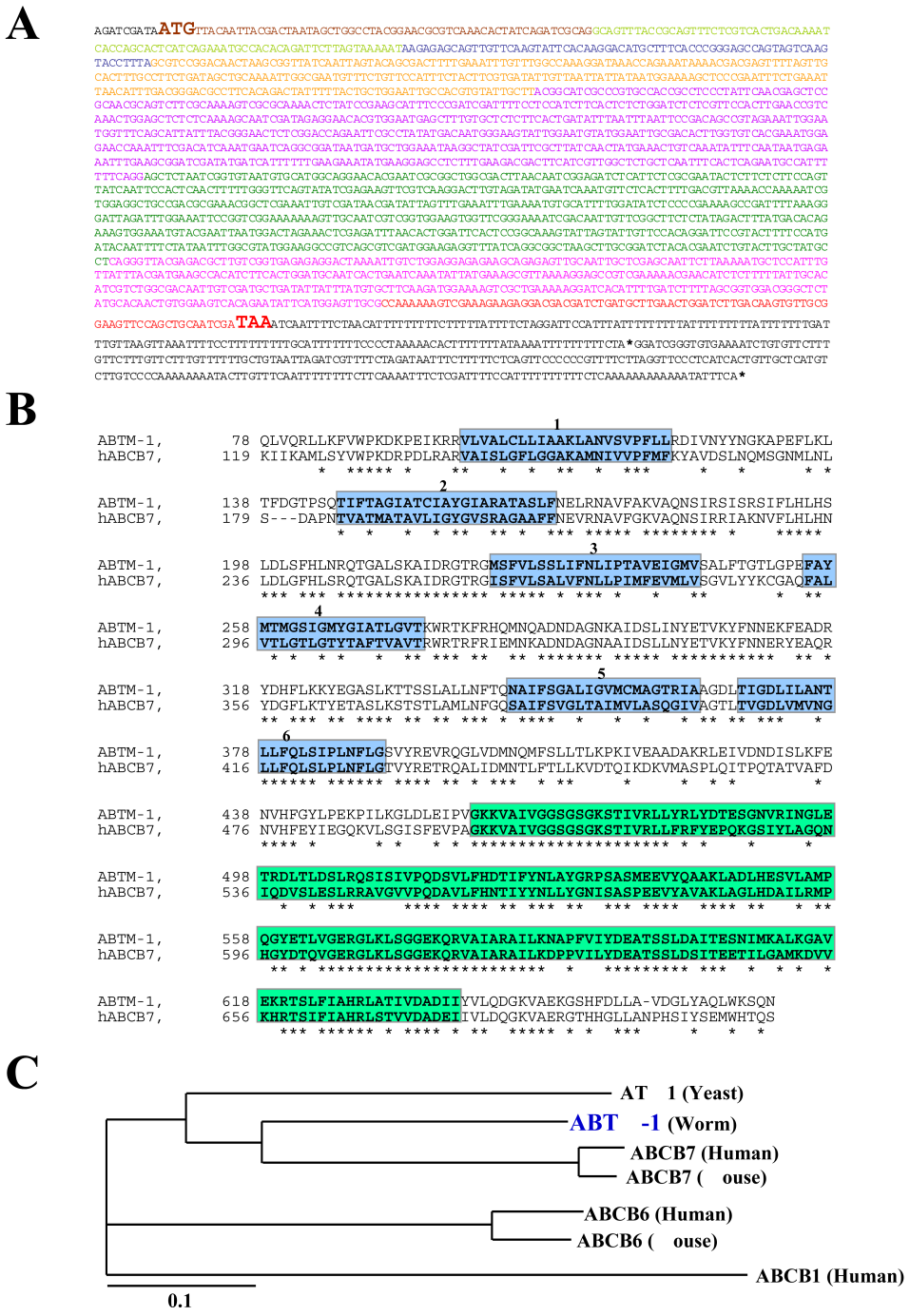

Figure S1. ABTM-1 is the *C. elegans* homologue of ABCB7. *A*, sequence of the cDNA of the *abtm-1* gene. UTRs are represented in black. The start and stop codons are highlighted. Exons are shown in different colors within the coding sequence. Two different 3' cDNA ends were identified by RT-PCR. The positions of the poly(A) tract, in each cDNA, are marked by an asterisk. *B*, alignment between the C-terminal regions of ABTM-1 and ABCB7 shows that the sequences are very similar (51% identity using Blast to align two sequences (17)). The six transmembrane domains and the ATP binding domain are indicated, by the blue and green backgrounds respectively. Asterisks indicate identical amino acids in the two proteins. *C*, phylogenetic tree, from an alignment of several proteins of the ABCB family, shows that ABTM-1 is clustered with Atm1p from yeast and ABCB7 from mice and humans, further suggesting that this is homologue of ABCB7 in *C. elegans*. The bar shows relative phylogenetic distance between peptides.

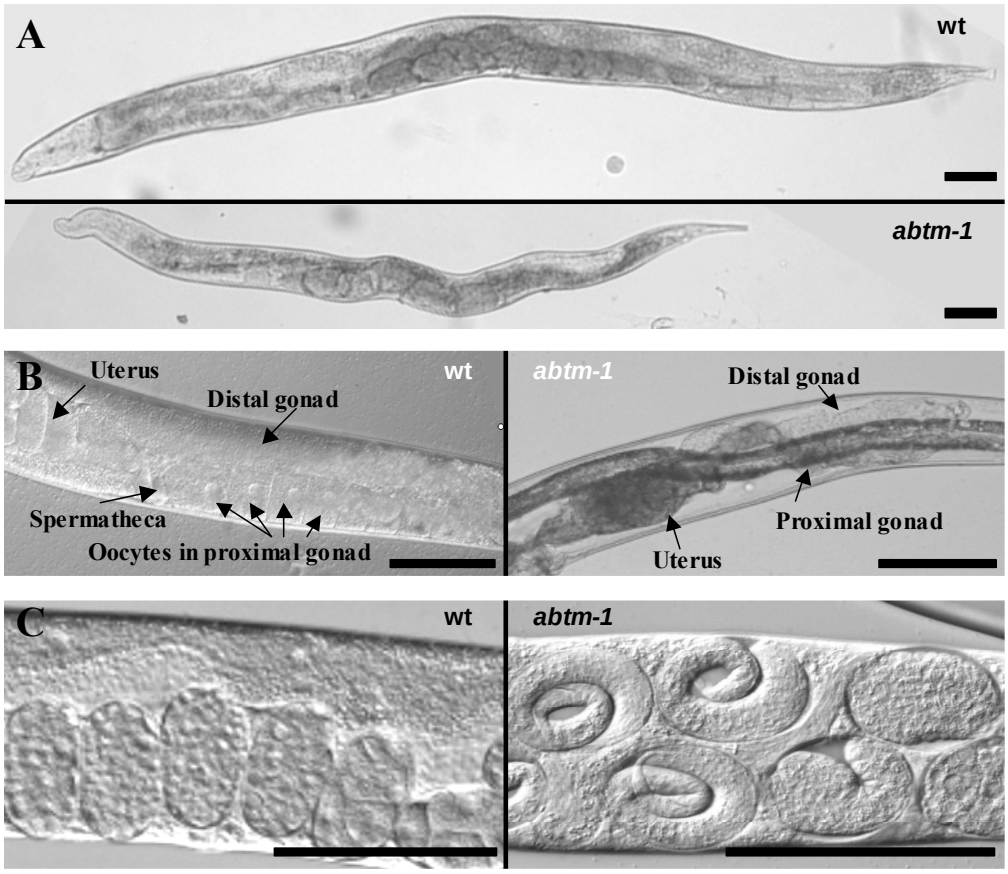

Figure S2. *abtm-1(RNAi)* adults have pleiotropic phenotypic defects. *A*, a wild type adult worm (top) compared with a typical *abtm-1(RNAi)* animal (bottom). *abtm-1(RNAi)* adults are paler and smaller in size than wild type animals. *B*, some *abtm-1(RNAi)* adults (right) have abnormal gonads. The gonad of a wild type worm is shown for comparison (left). *C*, *abtm-1(RNAi)* adults are Egl, as judging by the presence of embryos in late developmental stages in the adult (right) which is not usually observed in wild type animals (left). All scale bars represent 50 μm.

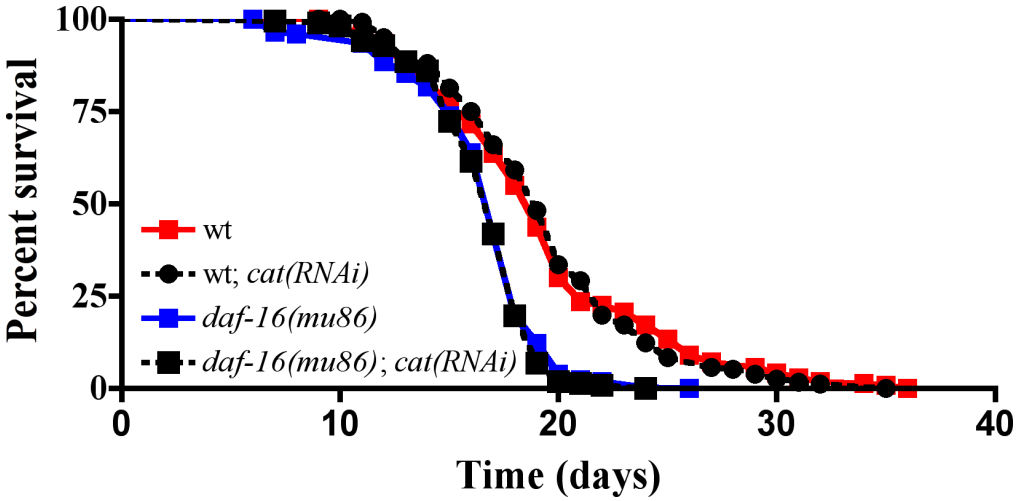

Figure S3. *cat(RNAi)* does not alter lifespan in wild type or *daf-16(mu86)* backgrounds. *cat(RNAi)* was performed on wild type and *daf-16(mu86)* and their lifespan compared to untreated animals.