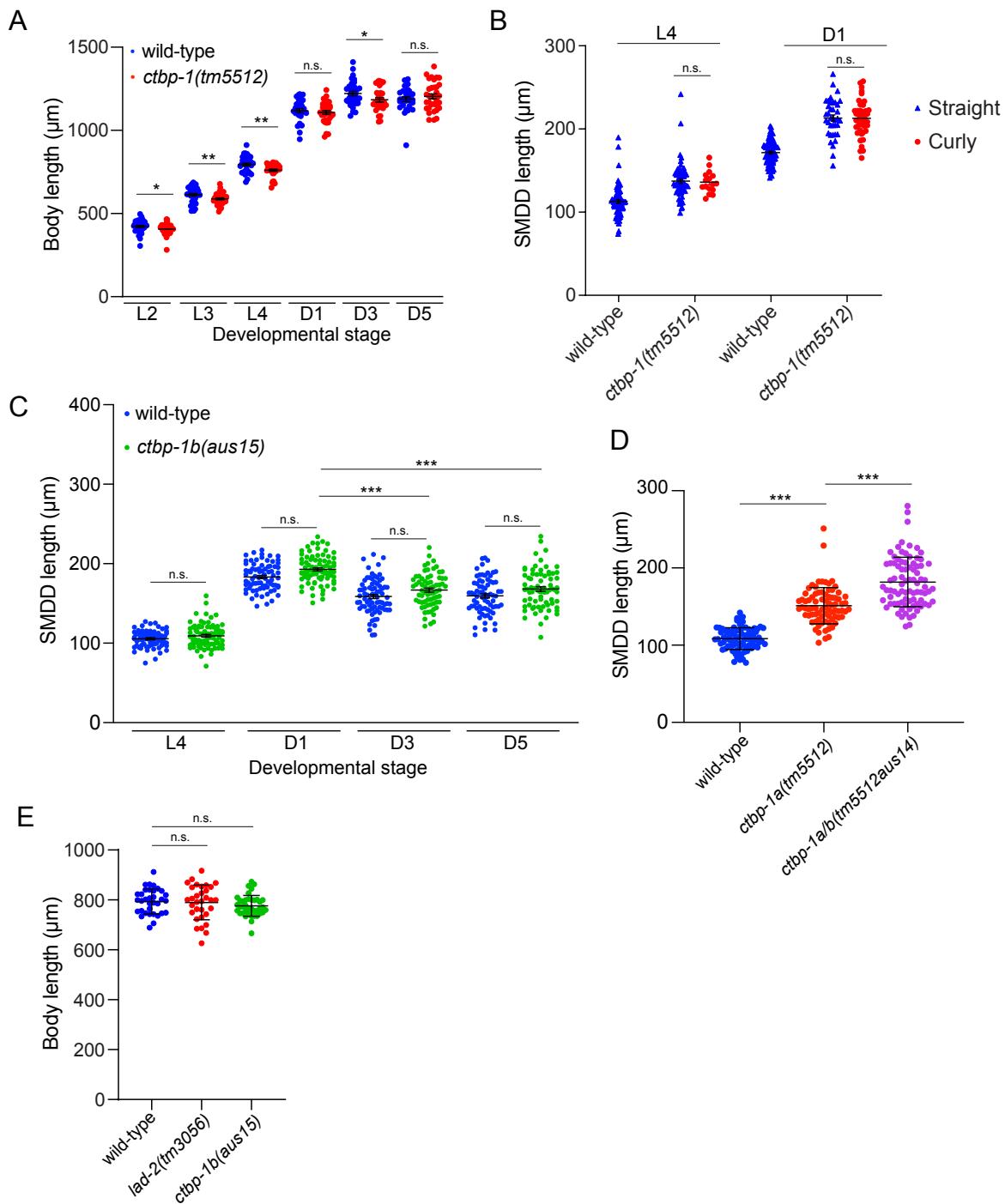


## SUPPLEMENTARY INFORMATION

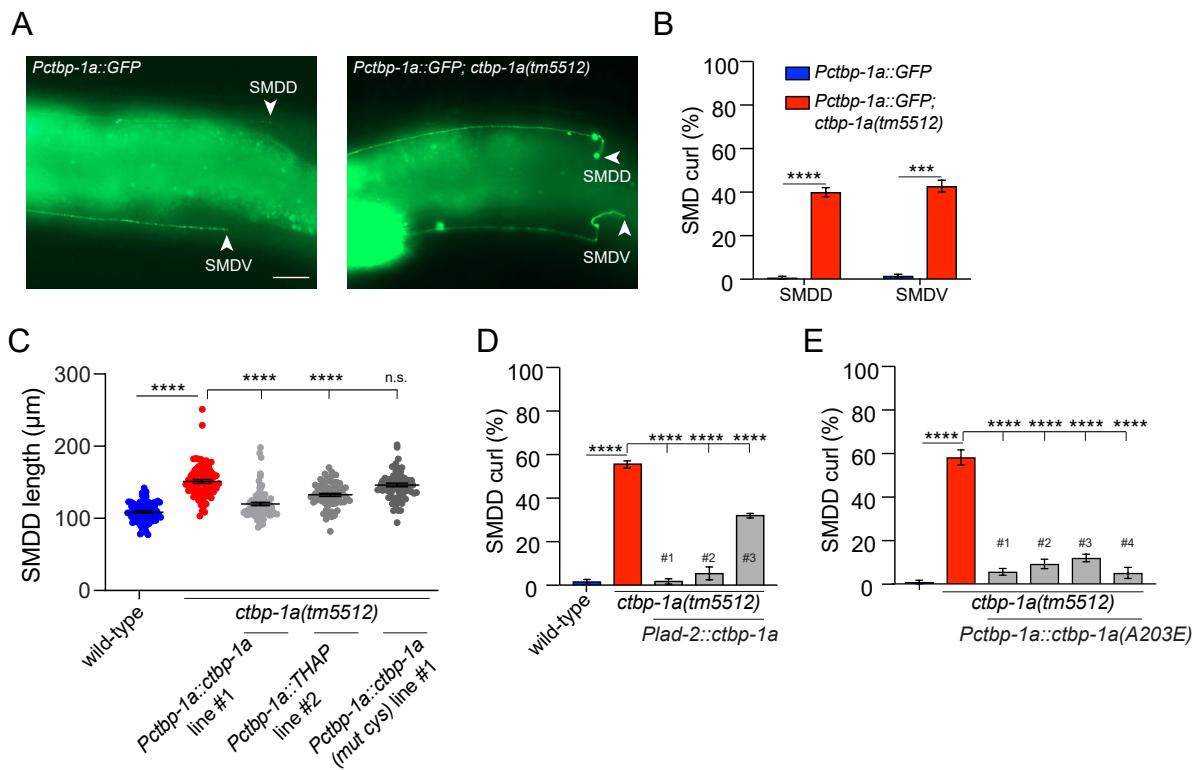
### SUPPLEMENTARY FIGURES



**Figure S1. Worm body and SMDD length measurements**

(A) Quantification of body length in wild-type and *ctbp-1(tm5512)* animals. Data presented as mean  $\pm$  S.E.M (bar) of 2 biological replicates, n = 29-42 animals for each developmental stage. \*p < 0.05, \*\*p < 0.01, n.s. - not significant (student's t-test).

- (B) Quantification of SMDD axon length in wild-type and *ctbp-1a(tm5512)* animals. Straight axons = blue triangles, curly axons = red dots. *ctbp-1a(tm5512)* mutant axons are the same length whether curly or straight. Data presented as individual straight or curly axons with mean  $\pm$  S.E.M (bar). n=78-80 axons for each developmental stage. n.s. - not significant (student's t-test).
- (C) Quantification of SMDD axon length in wild-type and *ctbp-1b(aus15)* animals. Data presented as individual axon lengths (points) presented as mean  $\pm$  S.E.M (bar) of 2 biological replicates, n = 70-80 axons for each developmental stage. \*\*\*p<0.001, n.s. - not significant (student's t-test).
- (D) Quantification of SMDD axon length in wild-type, *ctbp-1a(tm5512)* and *ctbp-1a/b(tm5512aus14)* animals. Data presented as individual axon lengths (points) presented as mean  $\pm$  S.E.M (bar) of 2 biological replicates, n = 70-84 axons. \*\*\*p<0.001.
- (E) Quantification of body length in wild-type, *lad-2(tm3056)* and *ctbp-1(aus15)* animals at the L4 stage. Data presented as mean  $\pm$  S.E.M (bar) of 2 biological replicates, n = 29-44. n.s. - not significant (student's t-test).



**Figure S2. SMDD/V analysis and *ctbp-1a(tm5512)* phenotypic rescue**

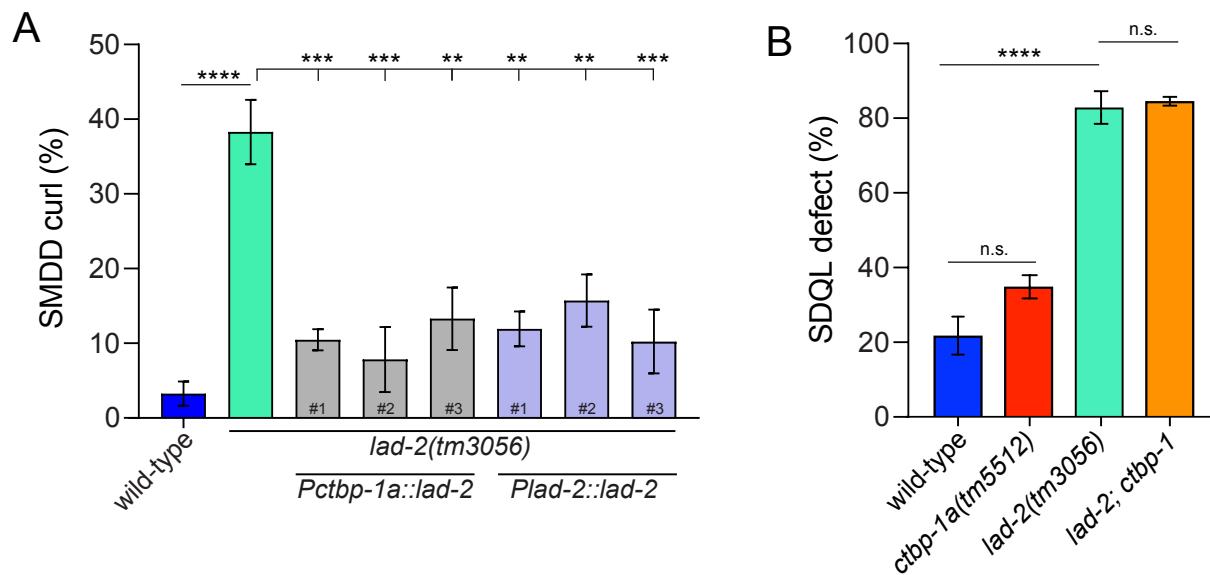
(A) Expression of the *Pctbp-1a::GFP* transcriptional reporter, showing the SMDD and SMDV axons (arrowheads) in wild-type and *ctbp-1a(tm5512)* mutant animals. Anterior is to the left, ventral is down. Scale bar, 20  $\mu\text{m}$ .

(B) Quantification of SMDD or SMDV curls in wild-type and *ctbp-1a(tm5512)* mutant day 1 adults using the *Pctbp-1a::GFP* reporter. Data presented as mean  $\pm$  S.E.M (bar) of 3 biological replicates,  $n > 100$  axons. \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  (unpaired t-test).

(C) Quantification of SMDD axon length of wild-type and *ctbp-1a(tm5512)* rescue animals at the L4 stage: *ctbp-1a* promoter driving *ctbp-1a* cDNA (line #1 from Figure 2E), THAP domain-only (line #2 from Figure 2H) or *ctbp-1a(mut cys)* (line #1 from Figure 2G). Data presented as individual axon lengths (points) presented as mean  $\pm$  S.E.M (bar) of 2 biological replicates,  $n = 70-80$ . \*\*\*\* $p < 0.0001$ , n.s. - not significant (student's t-test).

(D) Quantification of the SMDD axon curl phenotype of *ctbp-1a(tm5512)* rescue: expression of *ctbp-1a* cDNA under the *lad-2* promoter rescues the *ctbp-1a(tm5512)* SMDD curl phenotype of day 2 adults (3 independent transgenic rescue lines in grey). Data presented as mean  $\pm$  S.E.M (bar) of 3 biological replicates,  $n > 50$  animals. \*\*\*\* $p < 0.0001$  (one-way ANOVA with Tukey's correction).

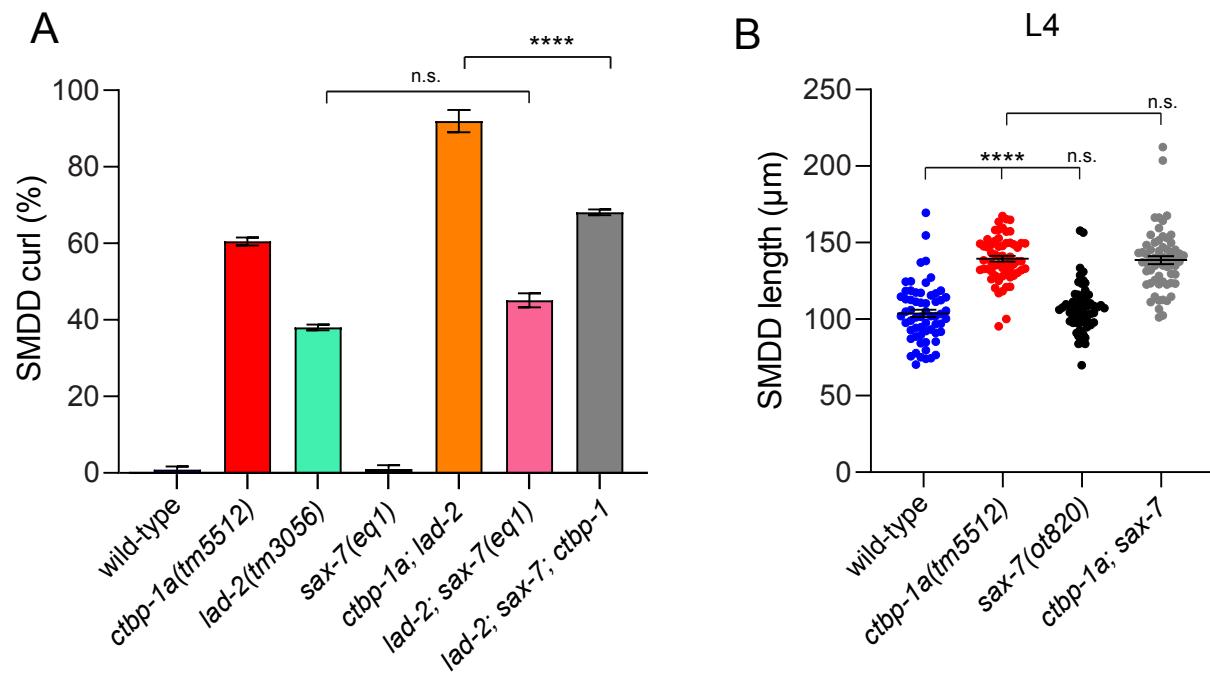
(E) Quantification of the SMDD axon curl phenotype of *ctbp-1a(tm5512)* rescue: expression of *ctbp-1a(A203A)* cDNA under the *ctbp-1a* promoter rescues the *ctbp-1a(tm5512)* SMDD curl phenotype of day 2 adults (4 independent transgenic rescue lines in grey). Data presented as mean ± S.E.M (bar) of 3 biological replicates, n> 50 animals. \*\*\*\*p<0.0001 (one-way ANOVA with Tukey's correction).



**Figure S3. *lad-2* mutant rescue double mutant analysis with *ctbp-1a***

(A) Quantification of SMDD axon curl phenotype of *lad-2(tm3056)* rescue: expression of *lad-2* cDNA under the *lad-2* or *ctbp-1* promoter rescues the *lad-2(tm3056)* SMDD curl phenotype of day 2 adults. (3 independent transgenic rescue lines in grey *Pctbp-1a::lad-2* and blue *Plad-2::lad-2*). Data presented as mean ± S.E.M (bar) of 3 biological replicates, n> 50 animals. \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 (one-way ANOVA with Tukey's correction).

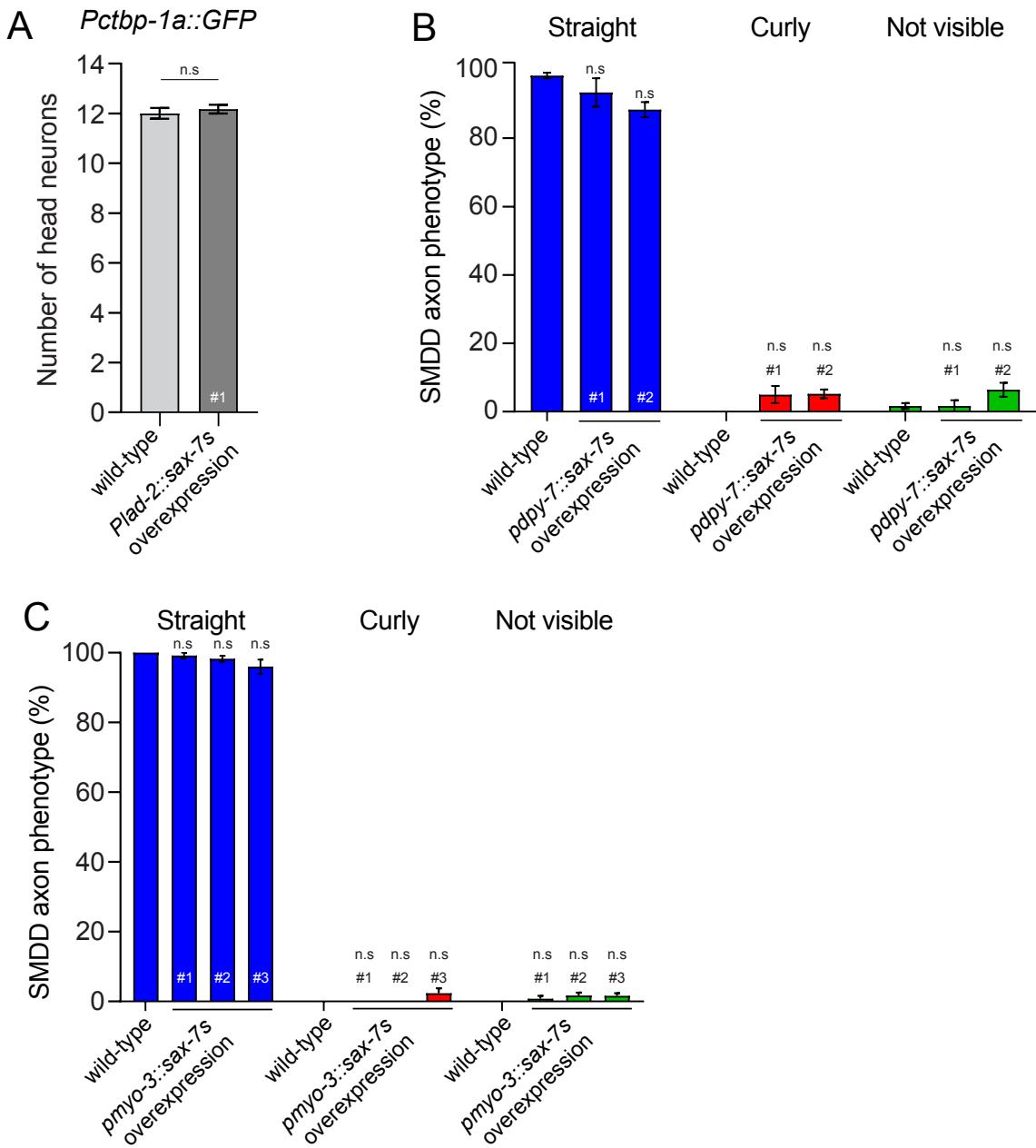
(B) Quantification of the SDQL axon defects in genotypes indicated in day 1 adults. Data presented as mean ± S.E.M (bar) of 3 biological replicates, n> 50 animals. Data presented as mean \*\*\*\*p<0.0001, n.s. - not significant (one-way ANOVA with Tukey's correction).



**Figure S4. *sax-7* mutant SMDD curl and length analysis**

(A) Quantification of SMDD axon curl defect of the triple *sax-7*; *lad-2*; *ctbp-1* mutant compared to single and double mutant combinations. \*\*\*p<0.0001, n.s. - not significant (one-way ANOVA with Tukey's correction).

(B) Quantification of SMDD axon length in wild-type and *sax-7(ot820)* mutant L4 larvae. Data presented as individual axon lengths (points) with mean ± S.E.M (bar) of 2 biological replicates, n= 59-64 axons for each developmental stage. \*\*\*p<0.0001, n.s. - not significant (student's t-test).

**Figure S5. *sax-7s* overexpression analysis**

(A) Quantification of *Pctbp-1a::GFP*-expressing head neurons in wild-type L4 larvae +/- the *Plad-2::sax-7s* overexpression transgene (line #1 from Figure 4E-F). Data presented as mean  $\pm$  S.E.M (bar) of 2 pooled biological replicates, n= 29-34 animals. n.s. - not significant (student's t-test).

(B-C) Quantification of SMDD axon curl phenotype (%) of *sax-7s* overexpression in hypodermis (*Pdpy-7::sax-7s* lines 1-2, B) and muscles (*Pmyo-3::sax-7s* lines 1-3, C). Data presented as mean  $\pm$  S.E.M (bar) of 3 biological replicates, n>50 animals. n.s. - not significant (one-way ANOVA with Dunnett's correction for each phenotype).

**SUPPLEMENTARY TABLES****Table S1. Quantification of body length and SMDD axon length in wild-type animals**

Mean body length and SMDD axon length ( $\mu\text{m}$ ) at larval stages L2-L4 and adult days D1-D5 in wild-type animals. n of animals or axons shown for each developmental stage. Percentage (%) change denotes the change in length from the previous developmental stage (right-hand column).

<b>BODY LENGTH</b>		<b>n</b>	<b>Mean</b>	<b>SEM</b>	<b>% change</b>
L2	wild-type	40	422.8	5.7	
L3	wild-type	38	613.1	7.6	145.0
L4	wild-type	34	794.1	8.6	129.5
D1	wild-type	29	1118	13.0	140.8
D3	wild-type	31	1222	13.7	109.3
D5	wild-type	29	1188	14.3	97.2

<b>SMDD AXON LENGTH</b>		<b>n</b>	<b>Mean</b>	<b>SEM</b>	<b>% change</b>
L2	wild-type	76	44.54	1.143	
L3	wild-type	91	68.16	1.4	153.0
L4	wild-type	80	112.8	2.1	165.5
D1	wild-type	77	171.7	1.7	152.2
D3	wild-type	75	157.6	2.4	91.8
D5	wild-type	77	157.7	2.2	100.1

**Table S2. Quantification of body length**

Mean body length and SMDD axon length ( $\mu\text{m}$ ) at larval stages L2-L4 and adult days D1-D5 in wild-type and *ctbp-1* (*tm5512*) mutants. n of animals or axons shown for each developmental stage. Difference between mean of wild-type and *ctbp-1a*(*tm5512*) at each developmental stage (*ctbp-1*(*tm5512*)/wild-type) shown (right-hand column).

BODY LENGTH		n	Mean	S.E.M	<i>ctbp-1(tm5512)</i> /wild-type
L2	wild-type	40	422.8	5.7	96.2
	<i>ctbp-1(tm5512)</i>	38	406.6	5.3	
L3	wild-type	38	613.1	7.6	96.0
	<i>ctbp-1(tm5512)</i>	42	588.6	5.2	
L4	wild-type	34	794.1	8.6	95.7
	<i>ctbp-1(tm5512)</i>	33	760.1	7.3	
D1	wild-type	29	1118	13.0	99.1
	<i>ctbp-1(tm5512)</i>	34	1108	11.1	
D3	wild-type	31	1222	13.7	96.9
	<i>ctbp-1(tm5512)</i>	31	1184	12.8	
D5	wild-type	29	1188	14.3	101.3
	<i>ctbp-1(tm5512)</i>	34	1204	14.5	
SMDD AXON LENGTH		n	Mean	S.E.M	<i>ctbp-1(tm5512)</i> /wild-type
L2	wild-type	76	44.54	1.143	101.5
	<i>ctbp-1(tm5512)</i>	73	45.22	1.186	
L3	wild-type	91	68.2	1.4	129.9
	<i>ctbp-1(tm5512)</i>	86	88.5	1.9	
L4	wild-type	80	112.8	2.1	121.5
	<i>ctbp-1(tm5512)</i>	79	137.1	2.3	
D1	wild-type	77	171.7	1.7	124.1
	<i>ctbp-1(tm5512)</i>	80	213.0	2.5	
D3	wild-type	75	157.6	2.4	158.7
	<i>ctbp-1(tm5512)</i>	75	250.1	4.4	
D5	wild-type	77	157.7	2.2	158.1
	<i>ctbp-1(tm5512)</i>	75	249.4	4.9	

**Table S3. Strains used in this study**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Wild-type, Bristol strain	CGC	N2
<i>lad-2(tm3056) IV; otEx331[Plad-2::GFP]</i>	Wang et al., 2008	LH247
<i>rhl54[Pglr-1::GFP] III</i>	This study	HRN169
<i>rhl54 III; cbp-1(tm5512) X</i>	This study	HRN226
<i>rhl54 III; cbp-1(tm5512aus14) X</i>	This study	HRN551
<i>rhl54 III; cbp-1(aus15) X</i>	This study	HRN552
<i>otEx331[Plad-2::GFP]</i>	This study	HRN575
<i>rhl54 III; lad-2(tm3056) IV; cbp-1(tm5512) X</i>	This study	HRN585
<i>rhl54 III; lad-2(tm3056) IV</i>	This study	HRN586
<i>otEx331; lad-2(tm3056) IV; cbp-1(tm5512) X</i>	This study	HRN587
<i>otEx331; cbp-1(tm5512) X</i>	This study	HRN588
<i>rhl54 III; sax-7(nj48) IV</i>	This study	RJP4005
<i>rhl54 III; sax-7(nj48) IV; cbp-1(tm5512) X</i>	This study	RJP4006
<i>cbp-1(tm5512) X</i>	This study	RJP4072
<i>rpEx1739 (Pctbp-1a::GFP + Ptx-3::dsRed2) line 1</i>	This study	RJP4076
<i>rpEx1740 (Pctbp-1a::GFP + Ptx-3::dsRed2) line 2</i>	This study	RJP4077
<i>rpEx1741 (Pctbp-1a::GFP + Ptx-3::dsRed2) line 3</i>	This study	RJP4078
<i>rpEx1742 (Pctbp-1a::GFP + Ptx-3::dsRed2) line 4</i>	This study	RJP4079
<i>rhl54 III; sax-7(eq1) IV</i>	This study	RJP4080
<i>rhl54 III; sax-7(eq1) IV; cbp-1(tm5512) X</i>	This study	RJP4081
<i>rhl54 III; sax-7(nj53) IV</i>	This study	RJP4082
<i>rhl54 III; sax-7(nj53) IV; cbp-1(tm5512) X</i>	This study	RJP4083
<i>cbp-1(tm5512) X; rpEx1739 (Pctbp-1a::GFP)</i>	This study	RJP4164
<i>rhl54 III; sax-7(eq1) IV; lad-2(tm3056) IV</i>	This study	RJP4250
<i>rhl54 III; sax-7(eq1) IV; lad-2(tm3056) IV; cbp-1(tm5512) X</i>	This study	RJP4251
<i>rhl54 III; cbp-1(tm5512) X; rpEx1812 (Pctbp-1a::cbp-1a::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4270
<i>rhl54 III; cbp-1(tm5512) X; rpEx1813 (Pctbp-1a::cbp-1a::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4271
<i>rhl54 III; cbp-1(tm5512) X; rpEx1814 (Pctbp-1a::cbp-1a::mCherry + Punc-122::GFP) line #3</i>	This study	RJP4272
<i>rhl54 III; cbp-1(tm5512) X; rpEx1815 (Pctbp-1a::cbp-1b::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4278
<i>rhl54 III; cbp-1(tm5512) X; rpEx1816 (Pctbp-1a::cbp-1b::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4279
<i>rhl54 III; cbp-1(tm5512) X; rpEx1817 (Pctbp-1a::cbp-1b::mCherry + Punc-122::GFP) line #3</i>	This study	RJP4280
<i>rhl54 III; cbp-1(tm5512) X; rpEx1842 (Pctbp-1a::cbp-1a THAP only::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4322
<i>rhl54 III; cbp-1(tm5512) X; rpEx1843 (Pctbp-1a::cbp-1a THAP only::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4323
<i>rhl54 III; rpEx1847 (Pdp-7::sax-7s + Punc-122::GFP) line #1</i>	This study	RJP4327
<i>rhl54 III; rpEx1848 (Pdp-7::sax-7s + Punc-122::GFP) line #2</i>	This study	RJP4328
<i>rhl54 III; cbp-1(tm5512) X; rpEx1855 (Pctbp-1a::cbp-1a (C5A,C10A)::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4339
<i>rhl54 III; cbp-1(tm5512) X; rpEx1856 (Pctbp-1a::cbp-1a (C5A,C10A)::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4340
<i>rhl54 III; cbp-1(tm5512) X; rpEx1857 (Pctbp-1a::cbp-1a (C5A,C10A)::mCherry + Punc-122::GFP) line #3</i>	This study	RJP4341
<i>rhl54 III; sax-7(ot820) IV; cbp-1(tm5512) X</i>	This study	RJP4349
<i>rhl54 III; sax-7(ot820) IV</i>	This study	RJP4360
<i>rhl54 III; cbp-1(tm5512) X; rpEx1881 (Pctbp-1a::cbp-1a (A203E)::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4401
<i>rhl54 III; cbp-1(tm5512) X; rpEx1882 (Pctbp-1a::cbp-1a (A203E)::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4402
<i>rhl54 III; cbp-1(tm5512) X; rpEx1883 (Pctbp-1a::cbp-1a (A203E)::mCherry + Punc-122::GFP) line #3</i>	This study	RJP4403
<i>rhl54 III; rpEx1891 (Plad-2::sax-7s + Punc-122::GFP) line #1</i>	This study	RJP4418
<i>rhl54 III; rpEx1892 (Plad-2::sax-7s + Punc-122::GFP) line #2</i>	This study	RJP4419
<i>rhl54 III; rpEx1893 (Plad-2::sax-7s + Punc-122::GFP) line #3</i>	This study	RJP4420
<i>rhl54 III; rpEx1894 (Plad-2::sax-7s + Punc-122::GFP) line #4</i>	This study	RJP4421
<i>rhl54 III; lad-2(tm3056); rpEx1891 (Plad-2::sax-7s + Punc-122::GFP) line #1</i>	This study	RJP4483
<i>rhl54 III; cbp-1(tm5512) X; rpEx1874 (Plad-2::cbp-1a::mCherry + Punc-122::GFP) line #1</i>	This study	RJP4384
<i>rhl54 III; cbp-1(tm5512) X; rpEx1875 (Plad-2::cbp-1a::mCherry + Punc-122::GFP) line #2</i>	This study	RJP4385
<i>rhl54 III; cbp-1(tm5512) X; rpEx1876 (Plad-2::cbp-1a::mCherry + Punc-122::GFP) line #3</i>	This study	RJP4386
<i>rhl54 III; rpEx2037 (Pctbp-1a::sax-7s) line #1</i>	This study	RJP4573
<i>rhl54 III; lad-2(tm3056) IV; rpEx2040 (Pctbp-1a::lad-2 + Punc-122::GFP) line #1</i>	This study	RJP4576
<i>rhl54 III; lad-2(tm3056) IV; rpEx2041 (Pctbp-1a::lad-2 + Punc-122::GFP) line #2</i>	This study	RJP4577
<i>rhl54 III; lad-2(tm3056) IV; rpEx2042 (Pctbp-1a::lad-2 + Punc-122::GFP) line #3</i>	This study	RJP4578
<i>rhl54 III; lad-2(tm3056) IV; rpEx2043 (Plad-2::lad-2 + Punc-122::GFP) line #1</i>	This study	RJP4579
<i>rhl54 III; lad-2(tm3056) IV; rpEx2044 (Plad-2::lad-2 + Punc-122::GFP) line #2</i>	This study	RJP4580
<i>rhl54 III; lad-2(tm3056) IV; rpEx2045 (Plad-2::lad-2 + Punc-122::GFP) line #3</i>	This study	RJP4581
<i>rpEx1739 (pctbp-1a::GFP); rpEx1891 (plad-2::sax-7s cDNA)</i>	This study	RJP4610
<i>rhl54 III; sax-7(ot820) IV; cbp-1(tm5512) X; rpEx2037 (Pctbp-1a::sax-7s) line #1</i>	This study	RJP4611
<i>rhl54 III; rpEx2073 (Pmyo-3::sax-7s + Punc-122::GFP) line #1</i>	This study	RJP4633
<i>rhl54 III; rpEx2074 (Pmyo-3::sax-7s + Punc-122::GFP) line #2</i>	This study	RJP4634
<i>rhl54 III; rpEx2075 (Pmyo-3::sax-7s + Punc-122::GFP) line #3</i>	This study	RJP4635

**Table S4. Plasmids used in this study**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cas9 expression vector ( <i>Peft-3::cas9::tbb-2</i> )	(Chen et al., 2013)	Addgene plasmid #48960
<i>ctbp-1</i> sgRNA 1 expression vector 5'-GGTGTAAATGAAGCTGTGG-3'	This study, modified from (Norris et al., 2015)	N/A
<i>ctbp-1</i> sgRNA 2 expression vector 5'-GCCAATGGTACTAACCGACG-3'	This study, modified from (Norris et al., 2015)	N/A
pCFJ90 ( <i>Pmyo-2::mCherry::unc-54</i> )	(Frokjaer-Jensen et al., 2008)	Addgene plasmid #19327
pCFJ104 ( <i>Pmyo-3::mCherry::unc-54</i> )	(Frokjaer-Jensen et al., 2008)	Addgene plasmid #19328
<i>Punc-122::GFP</i> ( <i>coel::GFP</i> )	(Miyabayashi et al., 1999)	Addgene plasmid #8937
RJP383 <i>Pctbp-1a::GFP</i>	This study	RJP383
RJP422 <i>Pctbp-1a::ctbp-1a::mCherry</i>	This study	RJP422
RJP423 <i>Pctbp-1a::ctbp-1b::mCherry</i>	This study	RJP423
RJP424 <i>Plad-2::ctbp-1a::mCherry</i>	This study	RJP424
RJP425 <i>Pdpy-7::ctbp-1a::mCherry</i>	This study	RJP425
RJP426 <i>Pctbp-1a::ctbp-1a(THAP only)::mCherry</i>	This study	RJP426
RJP514 <i>Pctbp-1a::ctbp-1a(A203E)::mCherry</i>	This study	RJP514
RJP427 <i>Pctbp-1a::ctbp-1a(C5A,C10A)::mCherry</i>	This study	RJP427
RJP414 <i>Pctbp-1a::sax-7s cDNA</i>	This study	RJP414
RJP515 <i>Plad-2::sax-7s cDNA</i>	This study	RJP517
RJP540 <i>Pmyo-3::sax-7s cDNA</i>	This study	RJP540
pRP13 <i>Pdpy-7::sax-7s cDNA</i>	(Pocock et al., 2008)	pRP13
RJP517 <i>Pctbp-1a::lad-2 cDNA</i>	This study	RJP517
RJP520 <i>Plad-2::lad-2 cDNA</i>	This study	RJP520