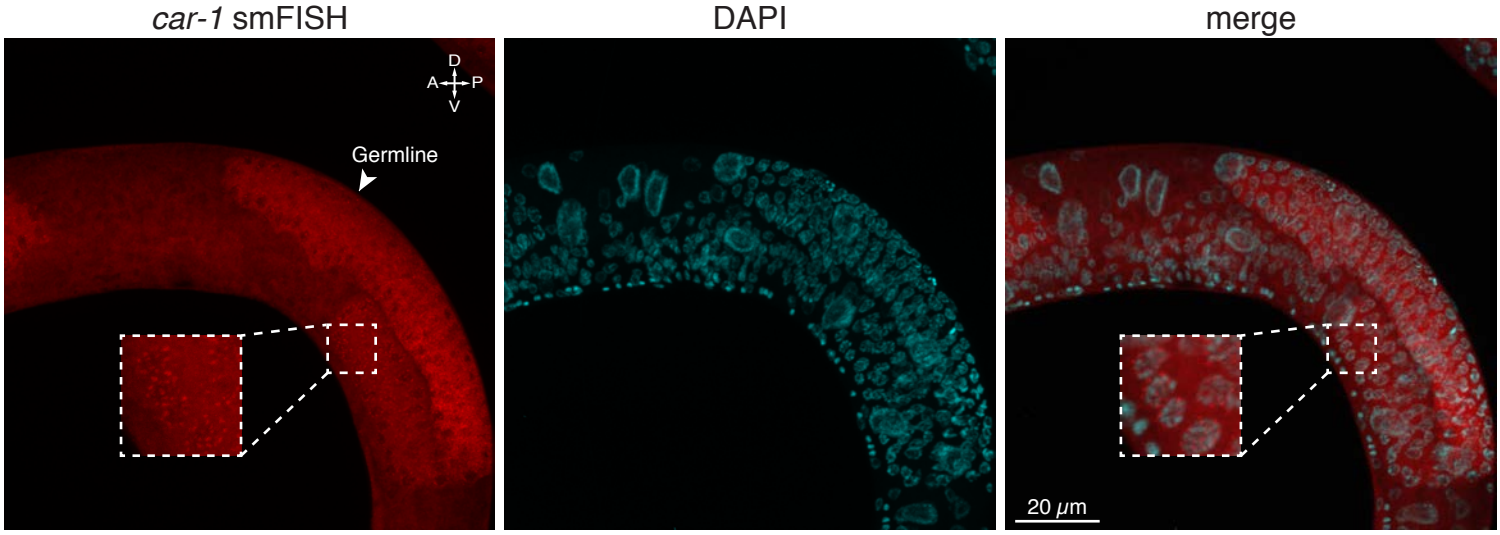
car-1 smFISH
DAPI
merge
D
A
P
V
Germline
20 μm

Figure S1. *car-1* mRNAs are present in the *C. elegans* germline. Related to Figure 1.

smFISH of *car-1* mRNAs showing cytoplasmic puncta in the germline. DAPI counterstain for DNA. Scale bar, 20 μm.

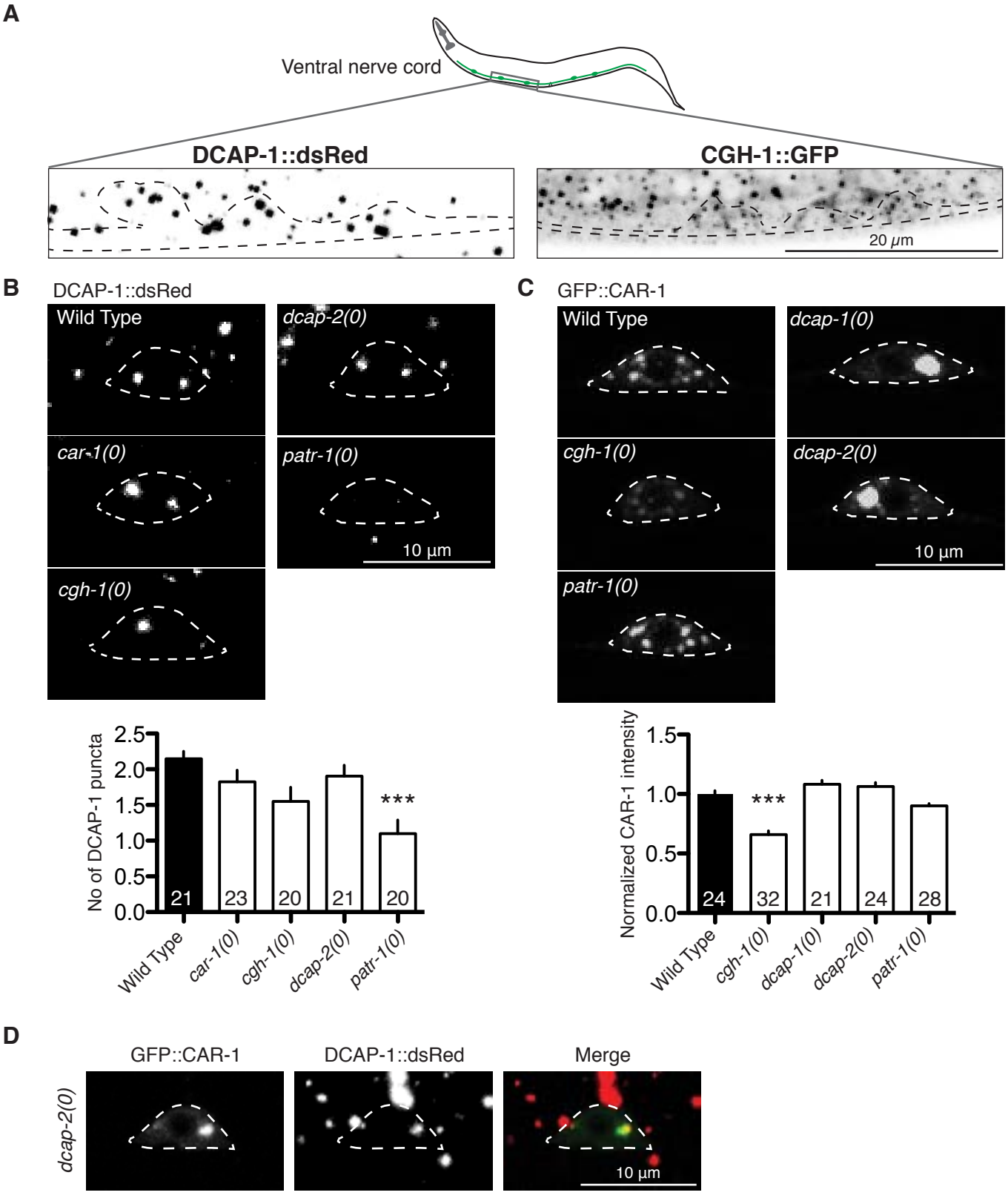
A
Ventral nerve cord
DCAP-1::dsRed
CGH-1::GFP
20 μm
B
DCAP-1::dsRed
Wild Type
dcap-2(0)
car-1(0)
patr-1(0)
10 μm
cgh-1(0)
C
GFP::CAR-1
Wild Type
dcap-1(0)
cgh-1(0)
dcap-2(0)
10 μm
patr-1(0)
No of DCAP-1 puncta
2.5
2.0
1.5
1.0
0.5
0.0
21
23
20
21
20
***
Wild Type
car-1(0)
cgh-1(0)
dcap-2(0)
patr-1(0)
Normalized CAR-1 intensity
1.5
1.0
0.5
0.0
24
32
21
24
28
***
Wild Type
cgh-1(0)
dcap-1(0)
dcap-2(0)
patr-1(0)
D
GFP::CAR-1
DCAP-1::dsRed
Merge
dcap-2(0)
10 μm

Figure S2. Localization of DCAP-1 and CAR-1 in mRNA decay mutants. Related to Figure 1.

A) Localization of DCAP-1 [P*dcap-1*-DCAP-1*::dsRed(bpIs37)*] or CGH-1 [*Pcgh-1*-CGH-1::GFP*(dhIs1000)*] within the ventral nerve cord of cholinergic motor neurons (outlined). The cholinergic motor neurons are labeled with P*unc-17*-GFP(*vsIs48*) or P*unc-17*-mCherry(*nuIs321*). DCAP-1 and CGH-1 showed similar localization in GABAergic motor neurons (not shown). The ventral nerve cord is outlined. Scale bar, 20 μm.

B) Top shows confocal images of DCAP-1::dsRed(*bpIs37*) localization in indicated mRNA decay mutants. Bottom shows quantitation of DCAP-1 puncta in the PLM cell body. Sample size is indicated in the bar.

C) Top shows confocal images of GFP::CAR-1 (*juSi338*) localization in indicated mRNA decay mutants. Bottom shows quantitation of DCAP-1 puncta in the PLM cell body. Statistics: one-way ANOVA with Bonferroni's post test; ***p<0.001.

D) The loss of *dcap-2* results in accumulation of CAR-1 into enlarged puncta, which colocalizes with the DCAP-1 granules. Scale bar, 10 μm.

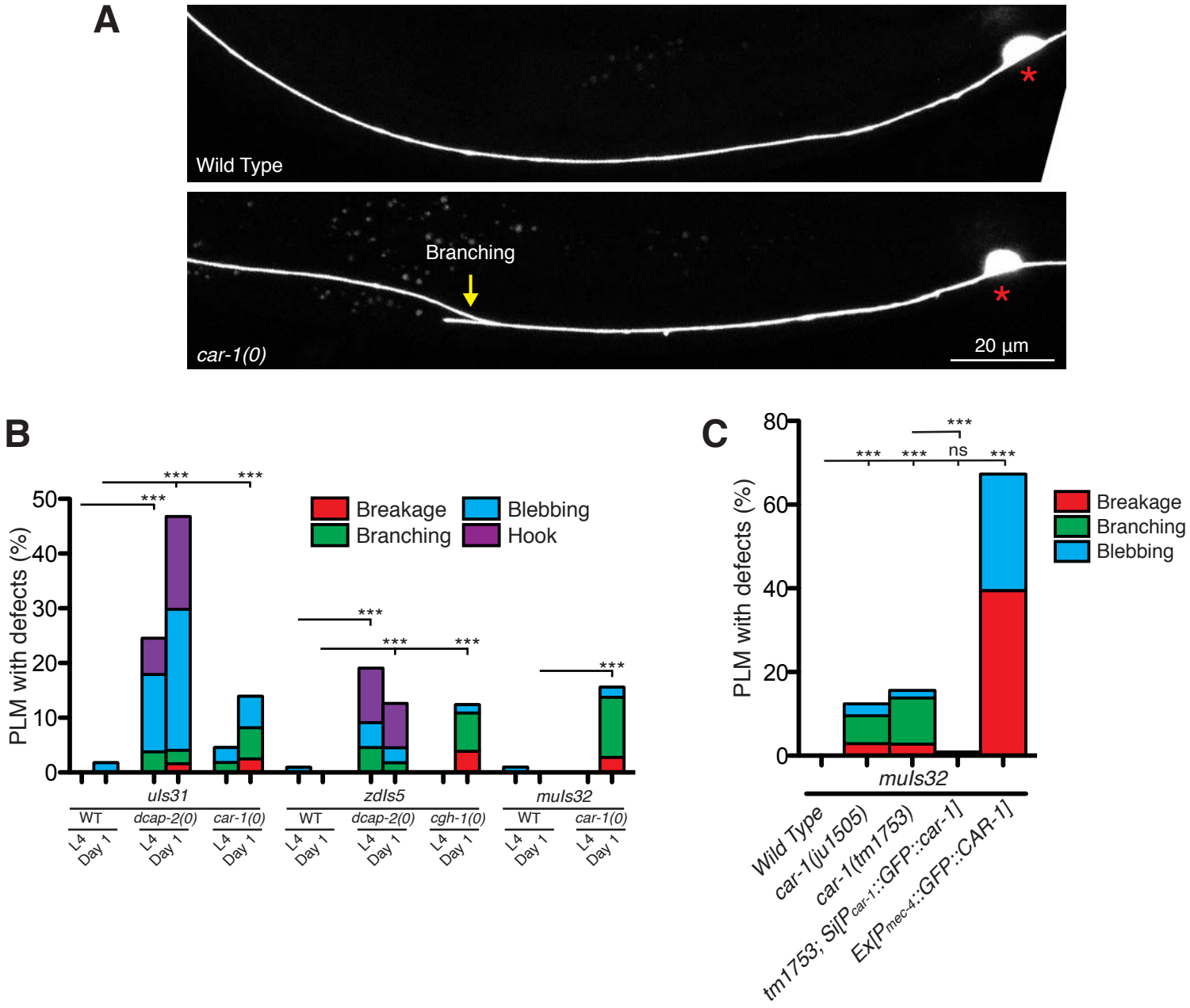
A
Wild Type
Branching
car-1(0)
20 µm
B
PLM with defects (%)
Breakage
Branching
Blebbing
Hook
uIs31
zdIs5
muIs32
WT
dcap-2(0)
car-1(0)
cgh-1(0)
L4
Day 1
C
PLM with defects (%)
ns
Breakage
Branching
Blebbing
muIs32
Wild Type
car-1(ju1505)
car-1(tm1753)
tm1753; Si[Pcar-1::GFP::car-1]
Ex[Pmec-4::GFP::CAR-1]

Figure S3. Re-expression of *car-1* rescues axonal morphology in *car-1(0)* mutants. Related to Figure 2.

A) Posterior end of the PLM axons in wild type or *car-1(0)* mutants showing branching phenotype. Red * marks position of the cell body, yellow arrowhead marks the position of branched axons. Anterior to the left.

B) Quantification of abnormal PLM phenotypes in wild type and mRNA decay mutants. TRNs were labeled with *Pmec-17-GFP(uIs31)*, *Pmec-4-GFP(zdIs5)* or *Pmec-7-GFP(muIs32).* N≥100. Statistics: Fisher's exact test; ** $p<0.01$, *** $p<0.001$; ns, not significant ($P>0.05$).

C) Abnormal PLM morphology of *car-1(0)* mutant was observed using two independent alleles, in two independent transgenic backgrounds (*uIs31* and *muIs32*). P*car-1*-GFP::CAR-1(*juSi343*) rescued the PLM defects in *car-1(0)* whereas over-expression of CAR-1 (Ex[P*mec-4*::GFP::CAR-1]) resulted in serious PLM defects. Day 1 adults were observed and quantified. N≥100. Statistics: Fisher's exact test; ***$p<0.001$; ns, not significant ($P>0.05$).

A

```
Ce_MICU-1       MLHCSFLRVIPIKNASKRLIIVRSLTSAPAKTSEAEKQQDSLKNIDAGEKYTALGNIRQKKDVFHYTDRASGVGYSHYSSSVYQHRPYIWPPLRKLY----------HWNYALVIAGMV-      109
Human_MICU1     ------------------------------------------------------------MFRLNSLSALAELAVGSRWYHGGSQPIQIR-------RRLM------------MVAFLGASAV-      44
Ce_C56A3.6      ------------------------------------------------------------------------------------------------MKRVLSRFWGFSTKHRQHIL      20
Human_MICU2     ------------------------------------------------------------------------------------MAAAAGSC---ARVAAWGGKLRRGLAVS      25
Human_MICU3     ------------------------------------------------------------------------MAALRRLLWPPPRVSPPLCAHQPLLGPWGRPAVTTLGLP      39
                                                                                                                          :

Ce_MICU-1       -----------ILMSDFEWLKDQIK-----------------SASLP--FRPE----ASQKE--EV-T---ESNGEVEEVKEKPKKKKLGFRERRIIEYE-DRLRLYSTPDKIFRYFATL      188
Human_MICU1     -----------TASTGLLWKRAHAE-----------------SPPCVDNLKSDIGDKGKNKDEGDV-CNHEKKTADLAPHPEEKKKKRSGFRDRKVMEYE-NRIRAYSTPDKIFRYFATL      134
Ce_C56A3.6      NQRIVERTS---------------ARNVAVLT-AAGIGTSIGAYQLIDTARAGILDGS----------------------------------DDASRHNVTTDHKLTKRELRFLQFASV      89
Human_MICU2     RQAVR---------------------------SPGPL----AAAVAGAALAGAGAAWHHS----------------RVSVAARDGSFTVSAQKNVEHGIIYIGKPSLRKQRFMQFSSL      96
Human_MICU3     GRPFSSREDEERAVAEAAWRRRRRWGELSVAAAAGGGLVGLVCYQLYGDPRAGSPATGRPSKSAATEPEDPPRGRGMLPIPVAAAKETVAI-GRTDIEDLDLY---ATSRERRFRLFASI      155
                                                  .                                                                 .        :  .  *  *:::

Ce_MICU-1       KIIDPNEDSGRFEVFMTPEDFLRSFTPGVMQPRRWGLDSFKNYN----PEKHKRHKFSDPDSIFYKLGENGLINFSDYLFLMTLLSTSHADFALAFKIFDVDGNGALDKEEFTKVQQLIM      304
Human_MICU1     KVIS---EPGEAEVFMTPEDFVRSITPNEKQPEHLGLDQYIIKRFDGKKISQEREKFADEGSIFYTLGECGLISFSDYIFLTTVLSTPQRNFEIAFKMFDLNGDGEVDMEEFEQVQSIIR      251
Ce_C56A3.6      E--------YDDVIYMSPMDFIDSLTLDAPRERVYRR-VLKEKDI-QRILKKTPPFRSGGKHFFRTMDQSGIISYSEYIFLLTLLTKSKAAFRIAFLMFDEDDNGNIDRDEFMLIRSLTS      199
Human_MICU2     E--------HEGEYYMTPRDFLFSVMFEQMERKTSVK-KLTKKDI-EDTLSGIQTA-GCGSTFFRDLGDKGLISYTEYLFLLTILTKPHSGFHVAFKMLDTDGNEMIEKREFFKLQKIIS      205
Human_MICU3     E--------CEGQLFMTPYDFILAVTTDEPKVAKTWK-SLSKQEL-NQMLAETPPVWKGSSKLFRNLKEKGVISYTEYLFLLCILTKPHAGFRIAFNMFDTDGNEMVDKKEFLVLQEIFR      265
                :           :*:* **: :.     .                                  :*  : : *:*.:::*:**  :*:. :  * :** ::* :.:  ::  **  ::.:

Ce_MICU-1       SQTTVGQRHRDHITP-------------------N-----------------------------------------QSFRVETNSALETYFFGKDGKGSLSSEKFIEFQERLQHDILK      362
Human_MICU1     SQTSMGMRHRDRPTT-------------------G-----------------------------------------NTLKSGLCSALTTYFFGADLKGKLTIKNFLEFQRKLQHDVLK      309
Ce_C56A3.6      SLRSTTRVQPSTASDEEDRRESCQLDAADYHFAVSRI---GADRLFTGADSYAVMFTIARMFASKAATLSGTRLVHKSEEEVRKQDTTLLLHLFGLRGNATLSFDEFQQFYENLQEELME      316
Human_MICU2     KQDDLMTVK-----TNET----------GYQE--------------------------------------------AIVKEPEINTTLQMRFFGKRGQRKLHYKEFRRFMENLQTEIQE      265
Human_MICU3     KKNEKREIK-----GDEEKRAMLRLQLYGYHSPTNSVLKTDAEELVS--RSYW---DTLRR--NTSQALFSDLAERADDITSLVTDTTLLVHFFGKKGKAELNFEDFYRFMDNLQTEVLE      373
                .      :                                                                    ::*   :**   :  *  ..* .*  .** :: :

Ce_MICU-1       MEFERRDALDNPDGLINEDSFAQLLLLHAQINEKKQKHMLKRVKRRFKGENLKGISFGETKAFFEFLYHIDDVDIALHFHKMAGMSIDAKLLQRVAVKVTGIPLSDHVVDVVITLFDDNL      482
Human_MICU1     LEFERHDPV---DGRITERQFGGMLLAYSGVQSKKLTAMQRQLKKHFKE--GKGLTFQEVENFFTFLKNINDVDTALSFYHMAGASLDKVTMQQVARTVAKVELSDHVCDVVFALFDCDG      424
Ce_C56A3.6      IEFYEFA---RGKTAISPVDFARLILRYSIVNFDDYHKYLQRVQE-KSDDDEPGISLSQWATFSRFLNNLAEFQSAVRLYVNSNVPVSEPEFARAVGCTIGKELDPVVVSMIFRIFDENN      432
Human_MICU2     MEFLQFS---KGLSFMRKEDFAEWLLFFTNTEN--KDIYWKNVRE--KLSAGESISLDEFKSFCHFTTHLEDFAIAMQMFSLAHRPVRLAEFKRAVKVATGQELSNNILDTVFKIFDLDG      378
Human_MICU3     IEFLSYS---NGMNTISEEDFAHILLRYTNVEN--TSVFLENVRY--SIPEEKGITFDEFRSFFQFLNNLEDFAIALNMYNFASRSIGQDEFKRAVYVATGLKFSPHLVNTVFKIFDVDK      486
                :**           :   .*.  :* .:  :        ..::   .     .::: :   * *  :: :.  *: :.  :   :    : :..  .    :.  : . :: :** :

Ce_MICU-1       DGKLSHEEMVAVMRRRMRRGLERPRDTGLFRLFDAVLECGKRAYHASPLPFY--------          534
Human_MICU1     NGELSNKEFVSIMKQRLMRGLEKPKDMGFTRLMQAMWKCAQETAWDFALPKQ--------          476
Ce_C56A3.6      DGTLSYPEFLAVMSDRLHRGLRGRLEKP--WGWKPFKNCVISEVSRA-------------          477
Human_MICU2     DECLSHEEFLGVLKNRMHRGLWVPQH----QSIQEYWKCVKKESIKGVKEVWKQAGKGLF          434
Human_MICU3     DDQLSYKEFIGIMKDRLHRGFRGYKT-V--QKYPTFKSCLKKELHSR-------------          530
                :  **  *::.::  *: **:               .*
```

B

MICU-1::GFP(*ju1783*)

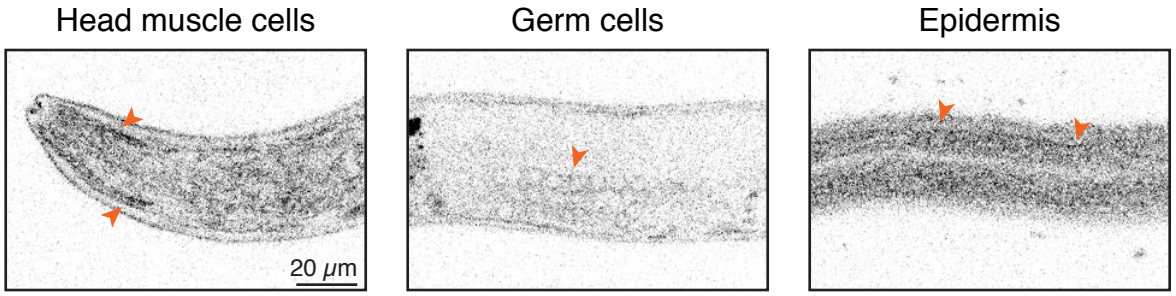

Figure S4. *C. elegans* MICU proteins are related to mammalian MICU. Related to Figure 6.

A) Multiple protein sequence alignment and phylogenetic tree for MICU1 family proteins. Accession numbers: *C. elegans* MICU-1 isoform D (Q95PZ2), Human MICU1 (Q9BPX6), *C. elegans* C56A3.6 (Q18874), human MICU2 (Q8IYU8), human MICU3 (Q86XE3). Sequence alignment and phylogenetic tree were generated using ClustalOmega (http://www.ebi.ac.uk/Tools/msa/clustalo/) with default settings. Among human MICU family members, MICU1 is closest to *C. elegans* MICU-1; MICU2 and MICU3 are equally related to C56A3.6.

B) Representative confocal images of endogenous MICU-1::GFP(*ju1783*) in head muscles, germ cells and the epidermis.

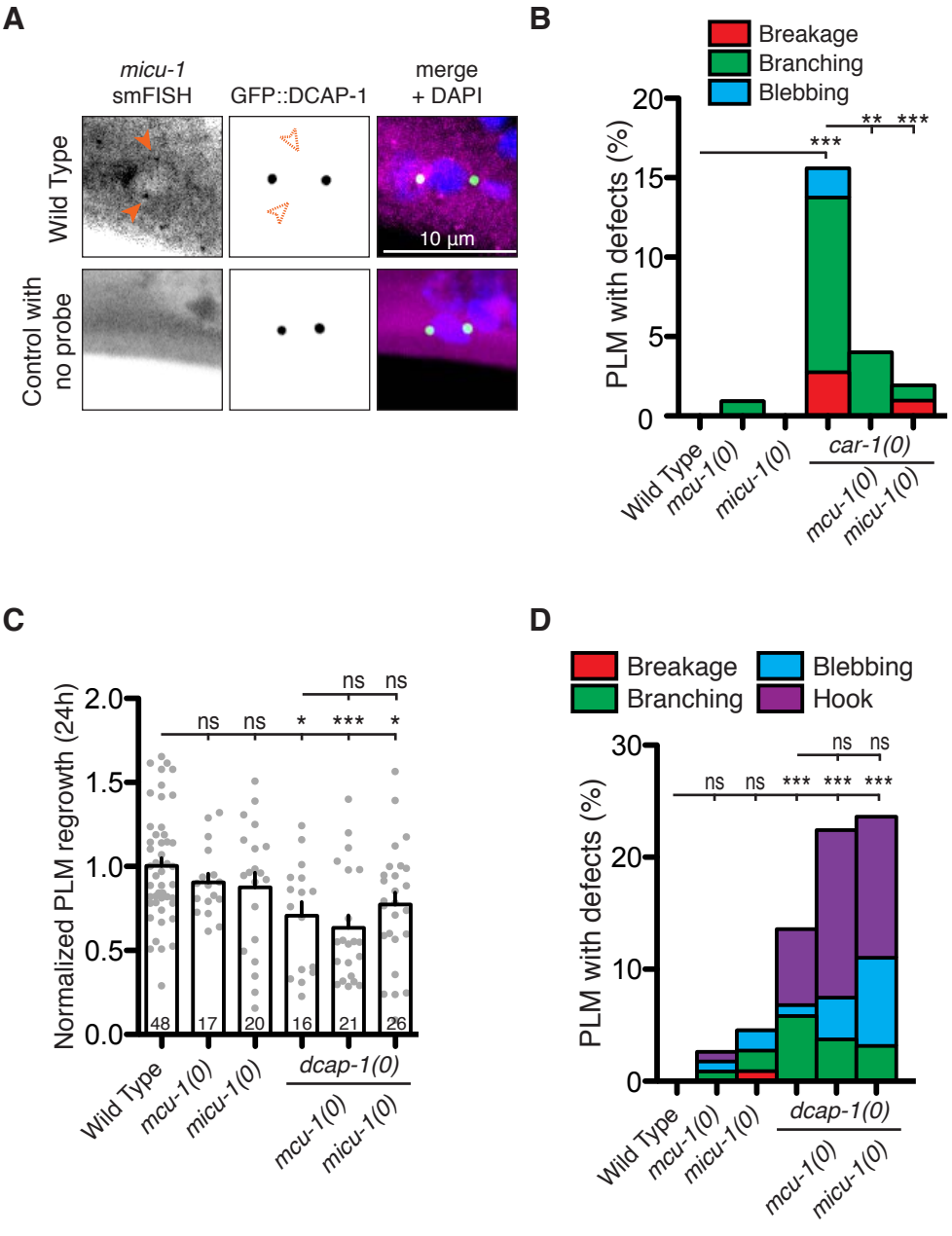
A
micu-1
smFISH
GFP::DCAP-1
merge
+ DAPI
Wild Type
Control with
no probe
10 µm
B
Breakage
Branching
Blebbing
PLM with defects (%)
20
15
10
5
0
***
** ***
Wild Type
mcu-1(0)
micu-1(0)
car-1(0)
mcu-1(0)
micu-1(0)
C
Normalized PLM regrowth (24h)
2.0
1.5
1.0
0.5
0.0
ns ns
ns ns * *** *
48
17
20
16
21
26
Wild Type
mcu-1(0)
micu-1(0)
dcap-1(0)
mcu-1(0)
micu-1(0)
D
Breakage
Branching
Blebbing
Hook
PLM with defects (%)
30
20
10
0
ns ns
ns ns *** *** ***
Wild Type
mcu-1(0)
micu-1(0)
dcap-1(0)
mcu-1(0)
micu-1(0)

Figure S5. Loss of *mcu-1* or *micu-1* does not suppress *dcap-1(0)* phenotypes. Related to Figure 6 and 7.

A) smFISH of *micu-1* mRNA. *micu-1* mRNAs do not colocalize with GFP::DCAP-1 expressed in TRNs [P*mec-4*-GFP::DCAP-1(*juEx8024*)].

B) Abnormal PLM morphology of *car-1(0)* mutant is rescued by deletion of *mcu-1* or *micu-1*. N≥100. Statistics: Fisher's exact test; **p<0.01; ***p<0.001.

C) Reduced axon regrowth in *dcap-1(0)* mutant is not rescued by deletion of *mcu-1* or *micu-1*. Statistics: one-way ANOVA with Bonferroni's posttest; *p<0.05; ***p<0.001; ns, not significant. Sample size is indicated in the bar.

D) Abnormal PLM morphology of *dcap-1(0)* mutant is not rescued by deletion of *mcu-1* or *micu-1*. N≥100. Statistics: Fisher's exact test; ***p<0.001; ns, not significant.

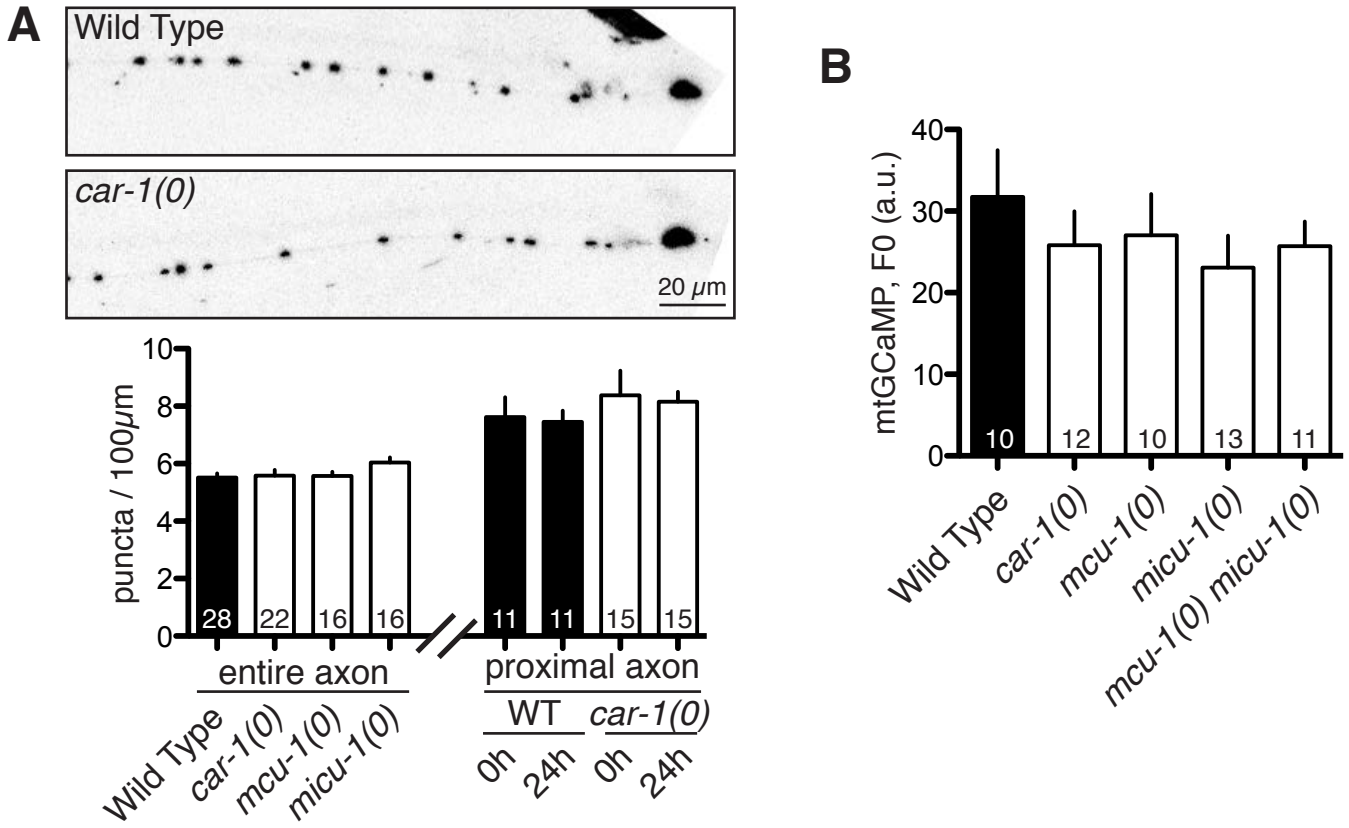
A
Wild Type
car-1(0)
20 μm
puncta / 100μm
10
8
6
4
2
0
28
22
16
16
11
11
15
15
entire axon
proximal axon
WT
car-1(0)
Wild Type
car-1(0)
mcu-1(0)
micu-1(0)
0h
24h
0h
24h
B
mtGCaMP, F0 (a.u.)
40
30
20
10
0
10
12
10
13
11
Wild Type
car-1(0)
mcu-1(0)
micu-1(0)
mcu-1(0) micu-1(0)

Figure S6. *car-1*, *mcu-1*, and *micu-1* mutants display normal axonal mitochondrial distribution and baseline $[Ca^{2+}]_{mt}$. Related to Figure 7.

A) Mitochondria density in PLM neurons was not affected in *car-1(0)*, *mcu-1(0)* and *micu-1(0)* mutants. Top shows representative images of mitochondria localization [P*mec-7*-tagRFP::mito*(jsIs1073)*] in PLM neurons in wild type and *car-1(0).* Bottom shows quantification of mitochondria puncta in PLM neurons in the entire uninjured axon (left), or proximal axon (right) at 0 h or 24 h post-axotomy. Sample size is indicated in the bar.

B) Baseline levels of mito-GCaMP (F0) in different mutants before axotomy. Sample size is indicated in the bar.

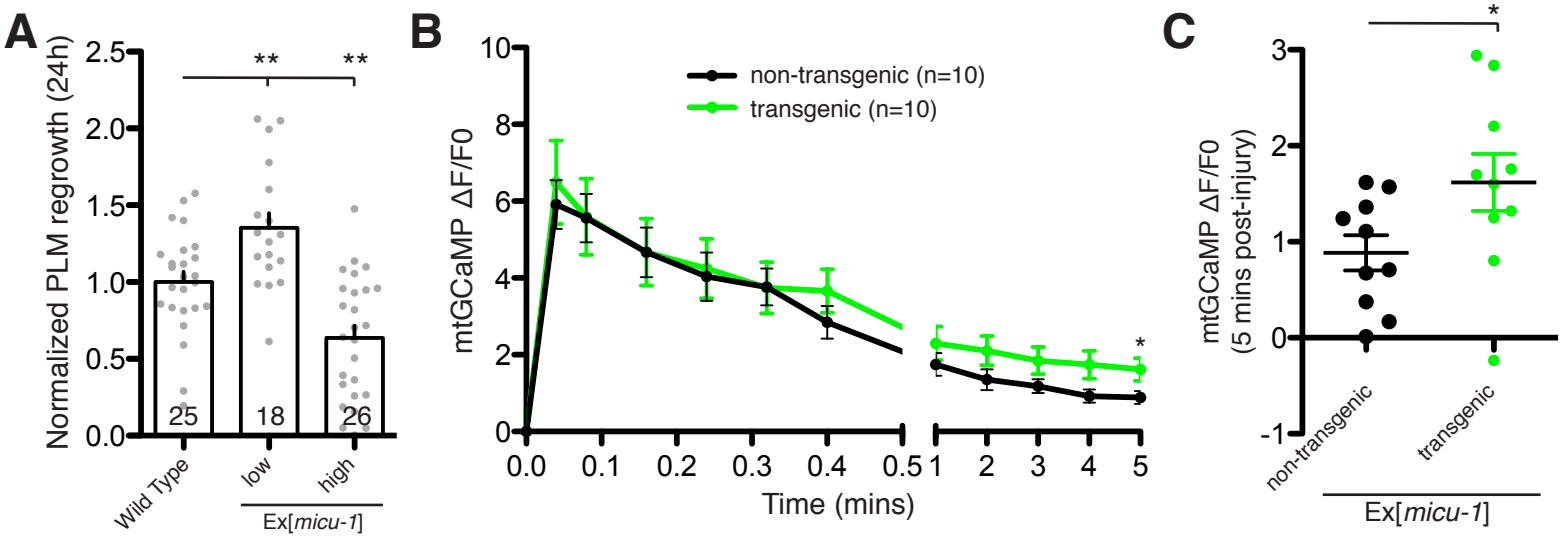
A
Normalized PLM regrowth (24h)
2.5
2.0
1.5
1.0
0.5
0.0
**
**
25
18
26
Wild Type
low
high
Ex[micu-1]
B
mtGCaMP ΔF/F0
10
8
6
4
2
0
non-transgenic (n=10)
transgenic (n=10)
*
0.0
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
Time (mins)
C
mtGCaMP ΔF/F0
(5 mins post-injury)
3
2
1
0
-1
*
non-transgenic
transgenic
Ex[micu-1]

Figure S7. Overexpression of *micu-1* phenocopies *car-1(0)* mutants in axon regrowth. Related to Figure 7.

A) Slight overexpression of *micu-1* (2ng/µl, *juEx8048*) results in increased PLM axon regrowth, whereas high overexpression of *micu-1* (10ng/µl, *juEx8050*) results in decreased PLM axon regrowth. Bars indicate mean ± SEM. Statistics: one-way ANOVA with Bonferroni's post test; **p<0.01.

B) mtGCaMP fluorescence intensity (ΔF/F0) over 5 minutes post-axotomy. Transgenic animals (*juEx8048*) show sustained $[Ca^{2+}]_{mt}$ uptake upon axon injury. Non-transgenic animals are siblings from the same strain. Statistics: Unpaired t-test against non-transgenic animals; *p<0.05.

C) mtGCaMP fluorescence intensity (ΔF/F0) at 5 minutes post-axotomy in genotypes indicated. Each dot represents a mtGCaMP in an animal. Statistics: Unpaired t-test; *p<0.05.

Gene name	Allele number	Breakpoints/Mutations
car-1	*ju1505*	AGTTTGAGCTTACCT…(698bp deletion+9bp insertion)...GCTCGTGATGATGTC
car-1	*ju1506*	TTTTCGTTATTTTTT... (1110bp deletion) ...CCGTTAGGCCAAATC
micu-1	*ju1155*	CGTTGATAAATCGACCGCGT...(1001bp deletion+218bp insertion)...ACAGTGGACGCTTTGAAGTT
micu-1	*ju1156*	CGCTCCGTTGATAAATCGAC…(1014bp deletion)...CGCTTTGAAGTTTTCATGAC

Table S2. Description of new alleles created in this study. Related to Figure 3 and 7.