Supplemental Materials

Molecular Biology of the Cell

Shanmugam *et al*.

PTP-3 phosphatase promotes intramolecular folding of SYD-2 to inactivate kinesin-3 UNC-104 in neurons

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Running title: PTP-3 controls SYD-2 binding to UNC-104

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SUPPLEMENTAL FIGURE LEGENDS

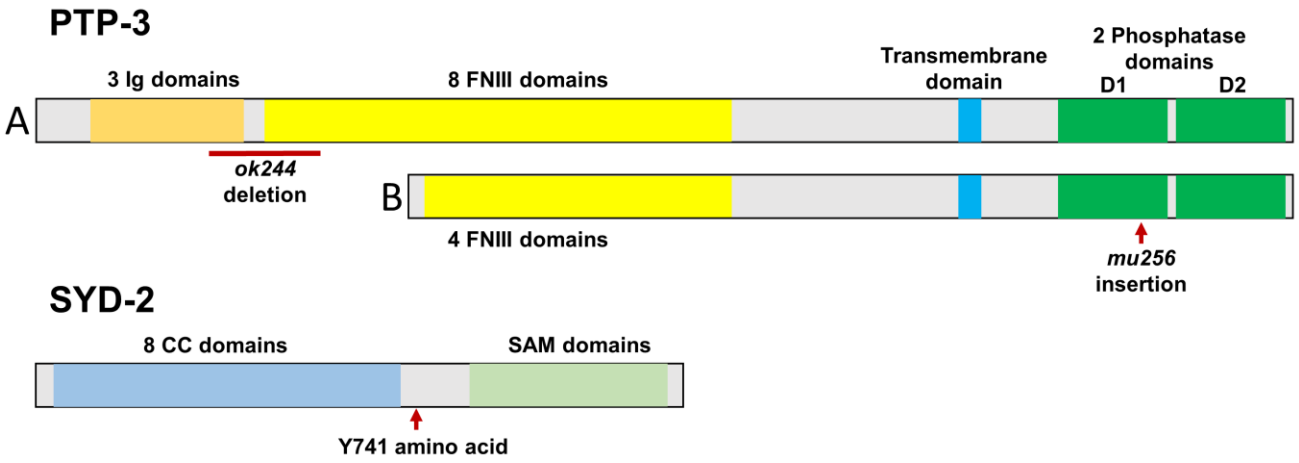


Figure S1. Domain structures of PTP-3A/B and SYD-2 with indicated mutant alleles.

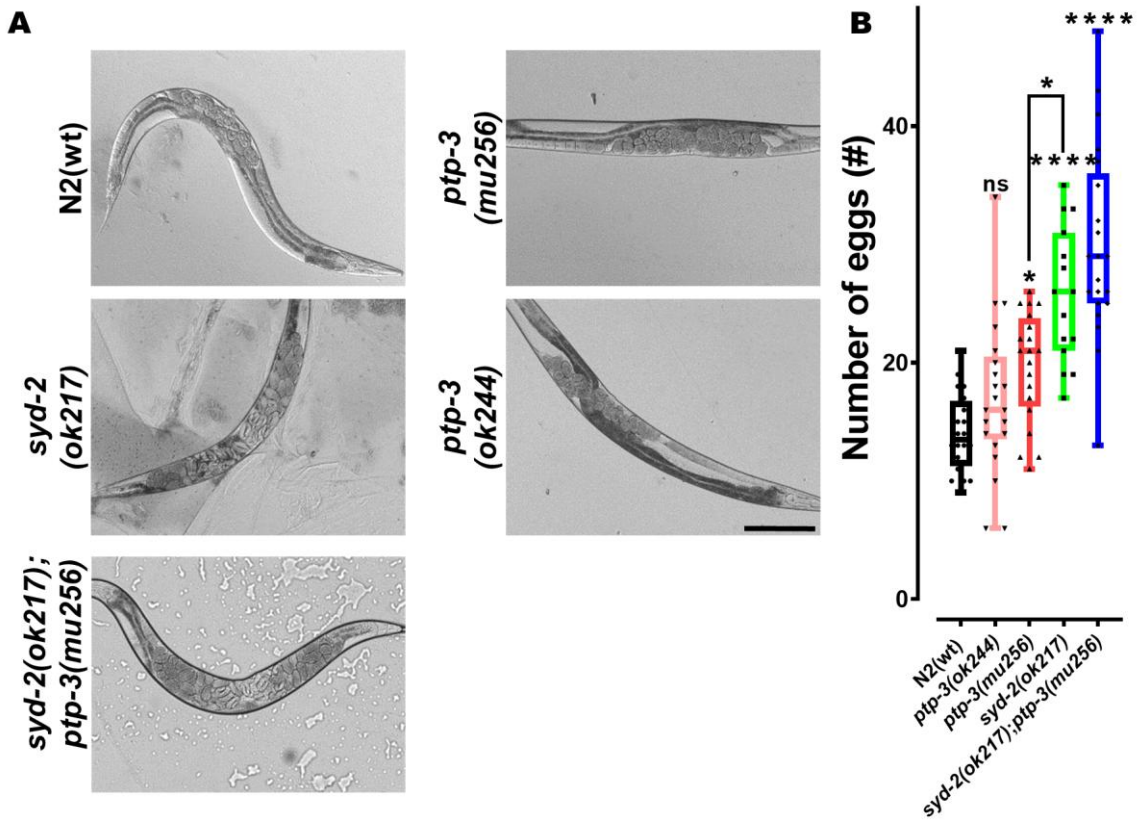


Figure S2. Mild egg retention phenotype in *ptp-3* mutants. (A) Images of worms with accumulated unlaid eggs inside the bodies of mutants (mild hatch-bag phenotypes). (B) Quantification of eggs re-

tained inside worms. Scale bar: 100 µm. Error bars: ± max. and min. range. One-way ANOVA with Dunnett's multiple comparison test. * p<0.05, ** p<0.005, *** p<0.001 and **** p<0.0001.

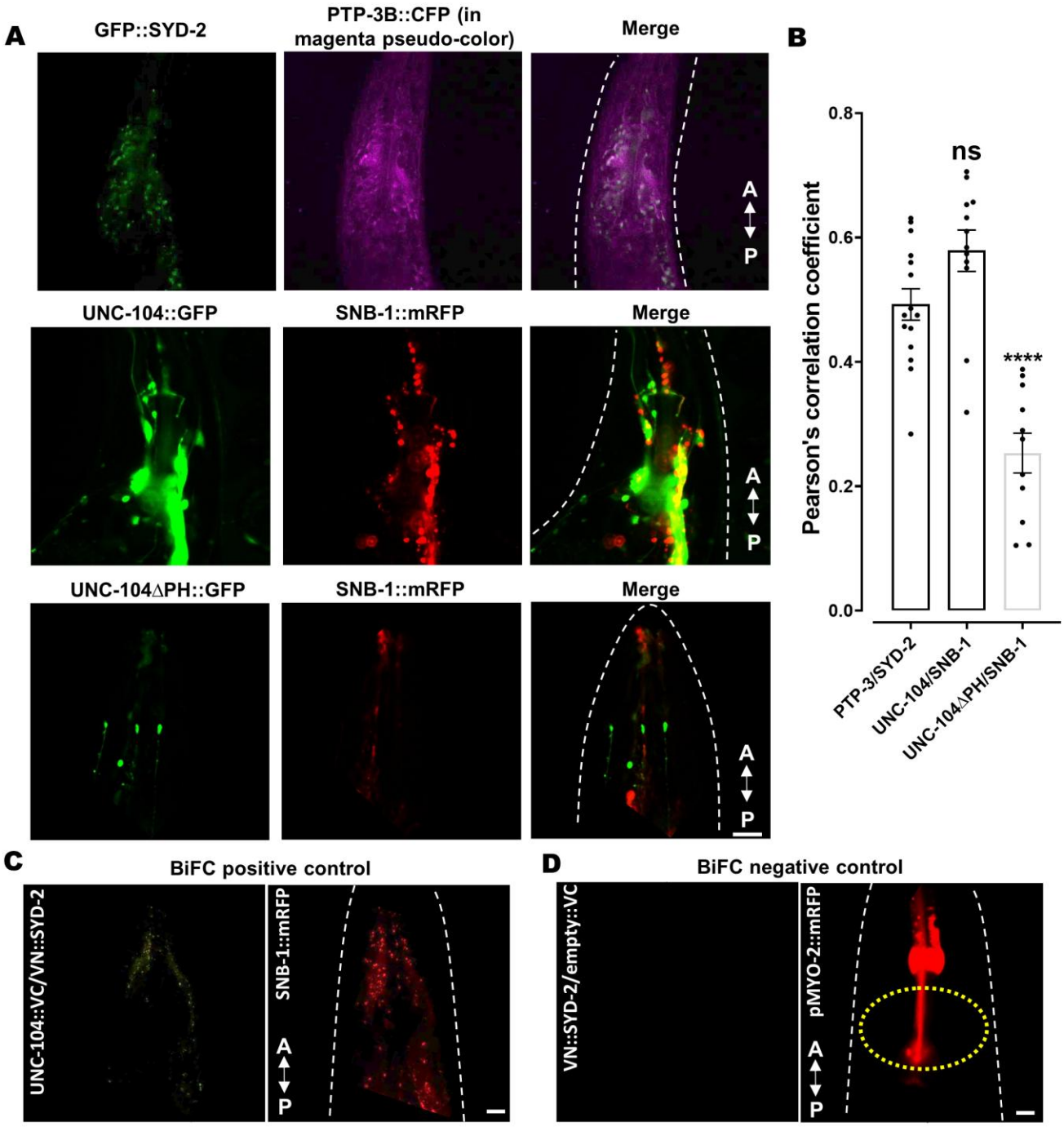
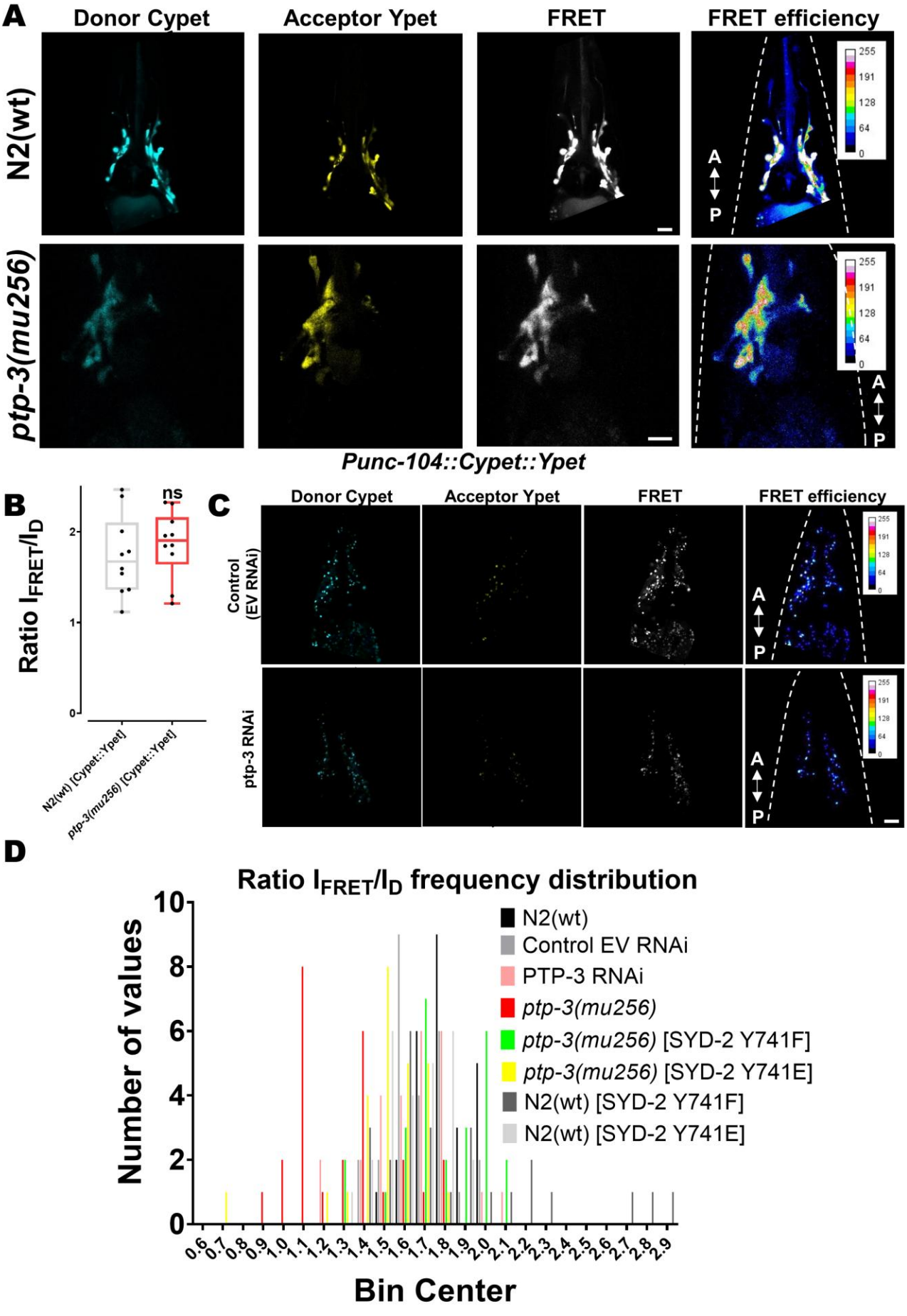


Figure S3. Control experiments for colocalization and BiFC studies. (A) Images of worms expressing SYD-2/PTP-3B, UNC-104/SNB-1 (positive control) and UNC-104ΔPH/SNB-1 (negative control). (B) Quantification of Pearson's correlation coefficient values from images shown in (A). (C)

Representative image of a nerve ring from a BiFC positive control worm (UNC-104::VN/VC::SYD2 with SNB-1::mRFP as neuronal marker). (D) Image of nerve ring region from a BiFC negative control worm (VN::SYD-2/empty::VC). Note that to avoid bleed through artifacts in negative control, we refrained from overexpressing any (red) fluorescent neuronal marker. Instead, we used a pharyngeal marker (*Pmyo-2::mrfp*) to locate the position of the nerve ring (yellow, dotted line) and screened through all confocal planes for BiFC signal at that location. Scale bar: 10 µm. Error bars: ± SEM. One-way ANOVA with Dunnett's multiple comparison test. **** $p<0.0001$.


A
Donor Cypet
Acceptor Ypet
FRET
FRET efficiency
N2(wt)
ptp-3(mu256)
255
191
128
64
0
A
P
Punc-104::Cypet::Ypet
B
Ratio I_FRET/I_D
ns
N2(wt) [Cypet::Ypet]
ptp-3(mu256) [Cypet::Ypet]
C
Donor Cypet
Acceptor Ypet
FRET
FRET efficiency
Control (EV RNAi)
ptp-3 RNAi
D
Ratio I_FRET/I_D frequency distribution
Number of values
Bin Center
N2(wt)
Control EV RNAi
PTP-3 RNAi
ptp-3(mu256)
ptp-3(mu256) [SYD-2 Y741F]
ptp-3(mu256) [SYD-2 Y741E]
N2(wt) [SYD-2 Y741F]
N2(wt) [SYD-2 Y741E]

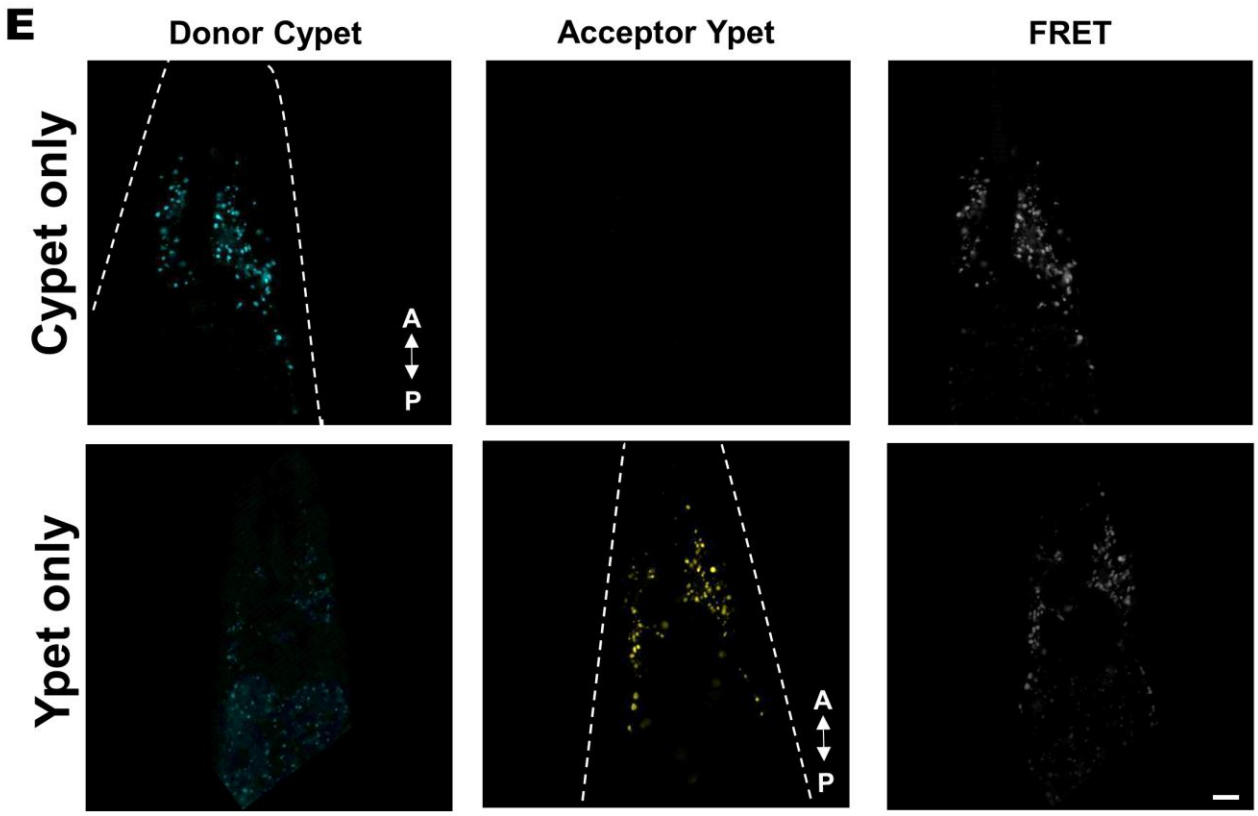


Figure S4. Control experiments for FRET studies. (A) FRET images of worms expressing Cypet::Ypet without the syd-2 gene in either N2(wt) or *ptp-3(mu256)* mutants. (B) Quantification of FRET ratios of images from (A) revealing no significant differences between N2(wt) and *ptp-3(mu256)* mutants. (C) FRET images of worms treated with either control (empty RNAi vector) or ptp-3 RNAi. (D) Frequency distribution of I_{FRET}/I_D ratio data from Figure 3C. (E) FRET images of bleed-through controls. Note that subtraction of bleed-through signal is not necessary in *intra*molecular FRET because the ratio of donor/acceptor is supposed to be always 1 (different from *inter*molecular FRET with varying ratios). Dotted, white line marks the contour of the worm head. Scale bar: 10 µm. Unpaired Student's t-test with Welch's correction.

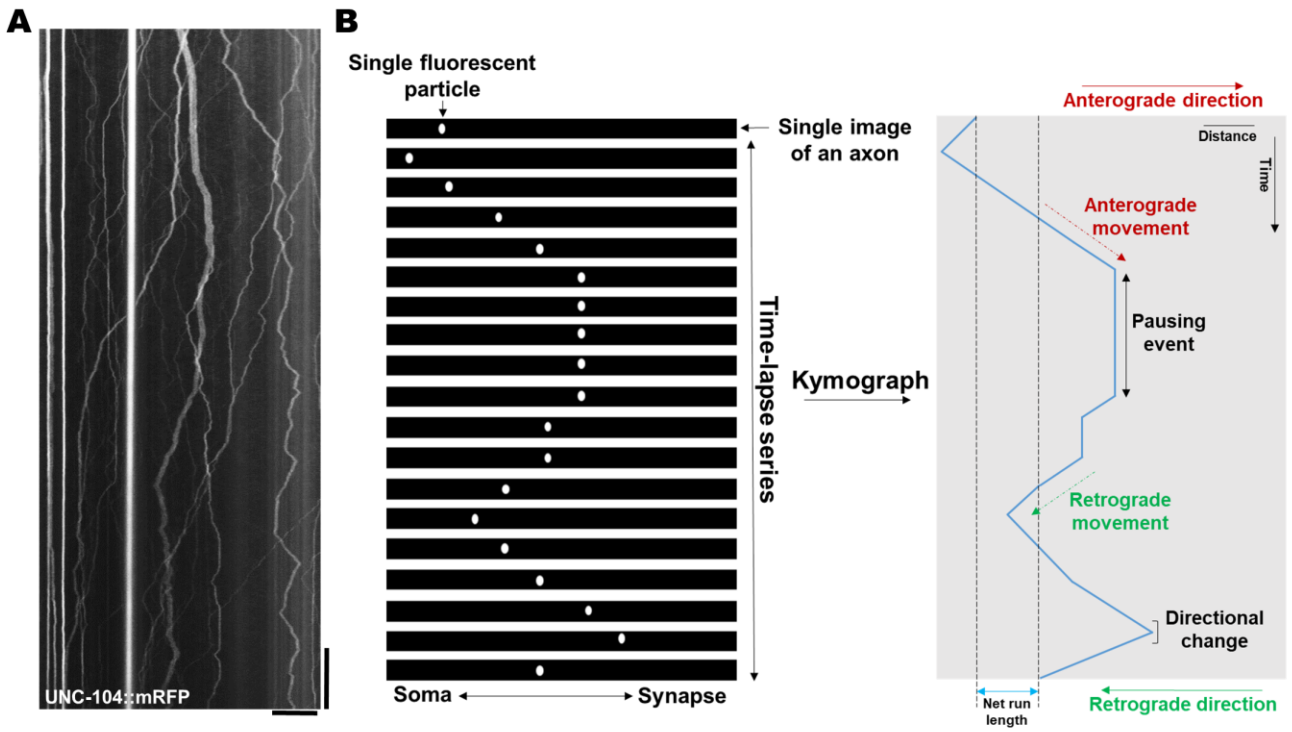


Figure S5. Kymograph description and definition of motility occurrences. (A) Example of a kymograph (UNC-104::mRFP motilities). (B) To generate a kymograph a video of a neuron is "resliced": All images that have been captured in this time-lapse series are stacked upon each other resulting in traces left behind by moving particles. In the right-hand cartoon, a blue line indicates the position of the fluorescent particle along the axon at increments of time. Red and green dotted arrows indicate anterograde and retrograde directions, respectively. Black double-headed arrow indicates a pausing event of the fluorescent particle. Square bracket reveals a directional change event. From the slope of the blue line in the kymograph, velocity can be calculated. Run length is the distance between two spatial positions of a particle. Total run length is the sum of all run lengths events of this particle. Net run length is marked by a blue double-headed arrow. Vertical scale bar: 25 sec and horizontal scale bar: 10 µm.

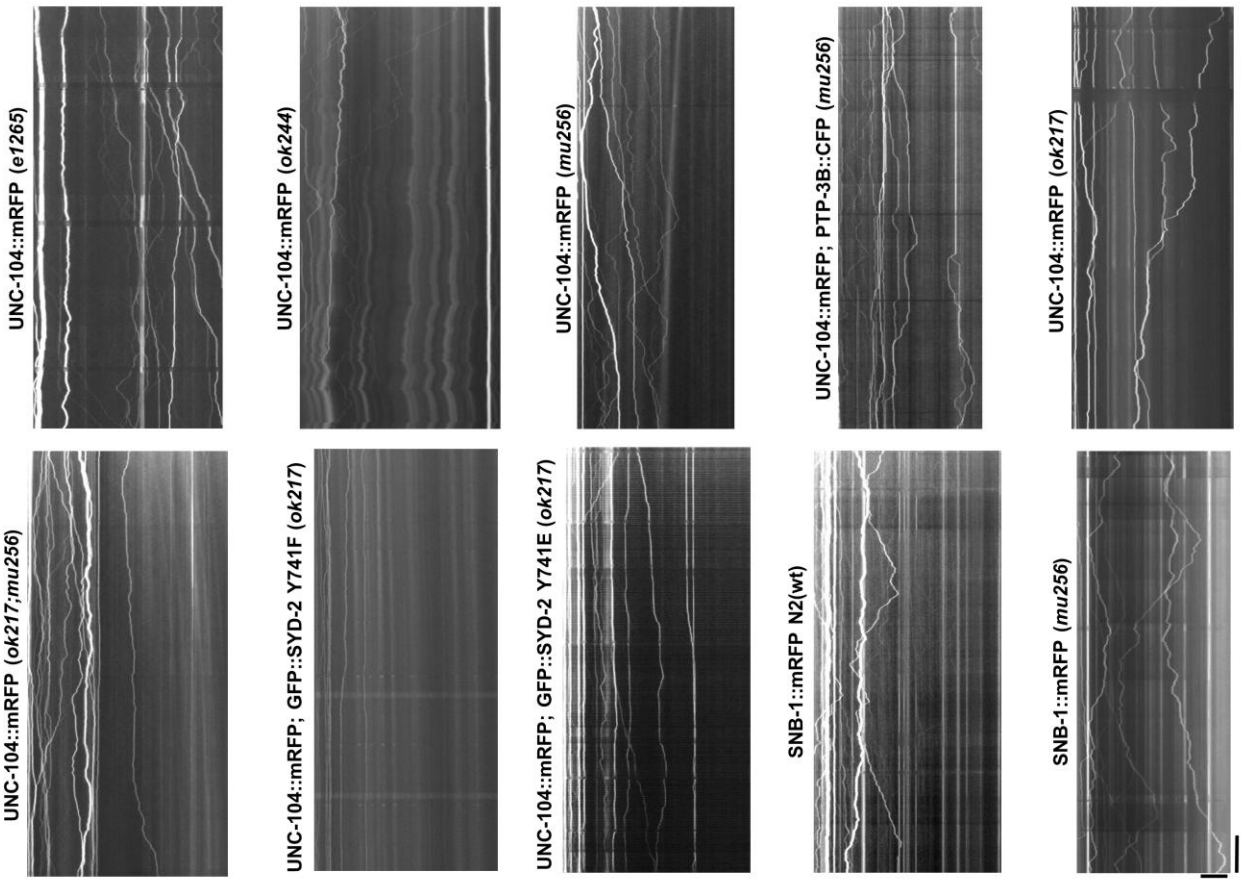


Figure S6. Examples of generated kymographs for this study. In this Figure, representative kymographs from worms expressing UNC-104::mRFP or SNB-1::mRFP in different genetic backgrounds are shown which have been used for motility analysis in Figure 6. Vertical scale bar: 25 sec and horizontal scale bar: 10 µm.

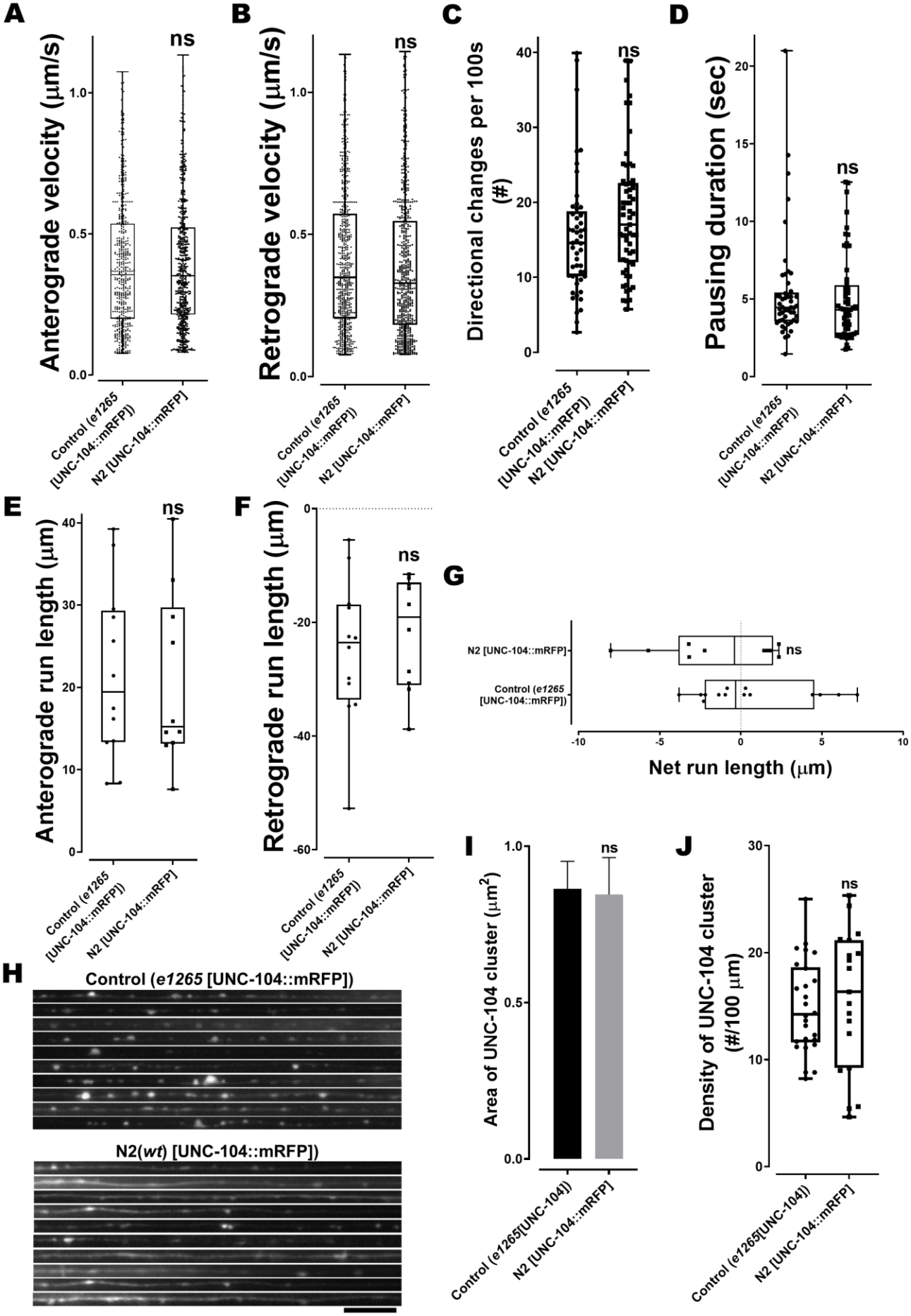
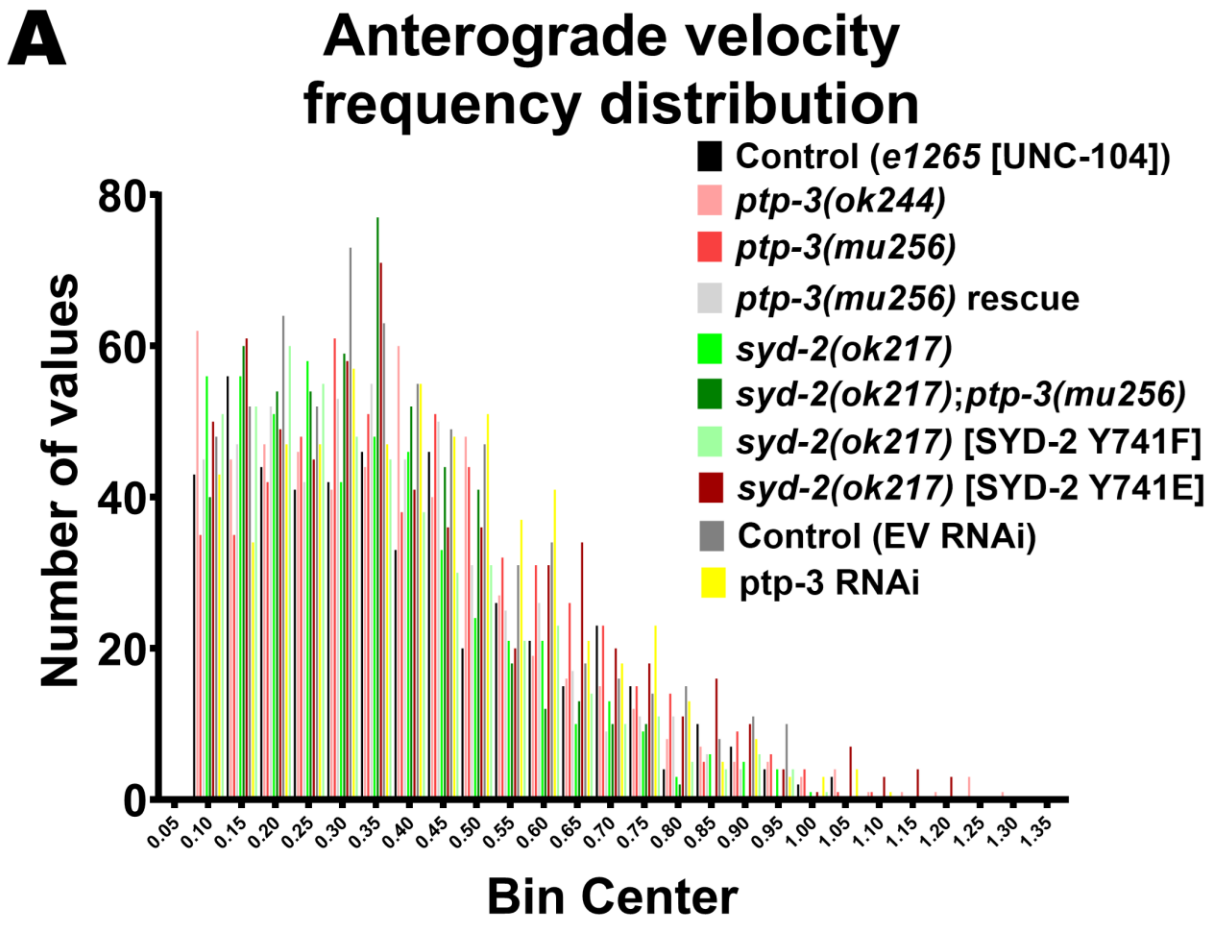
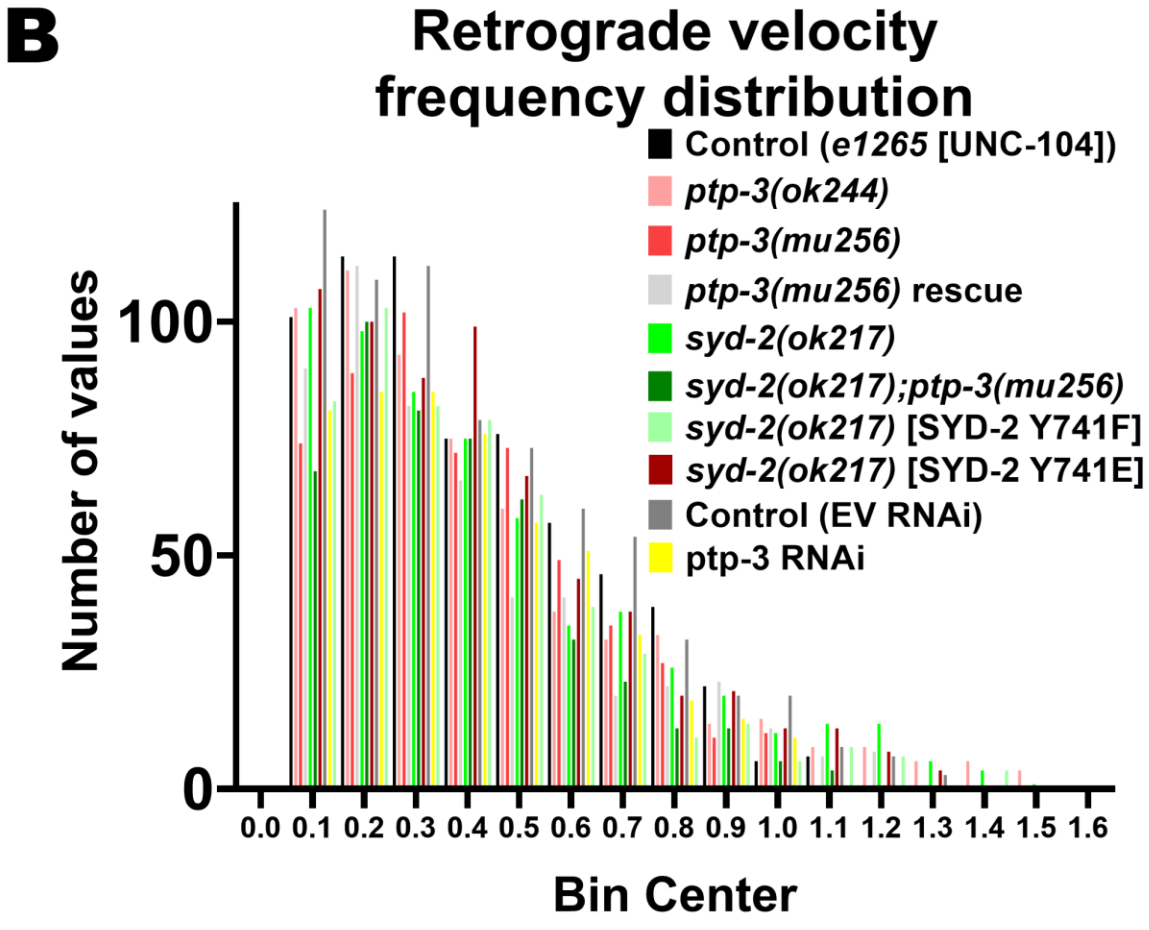
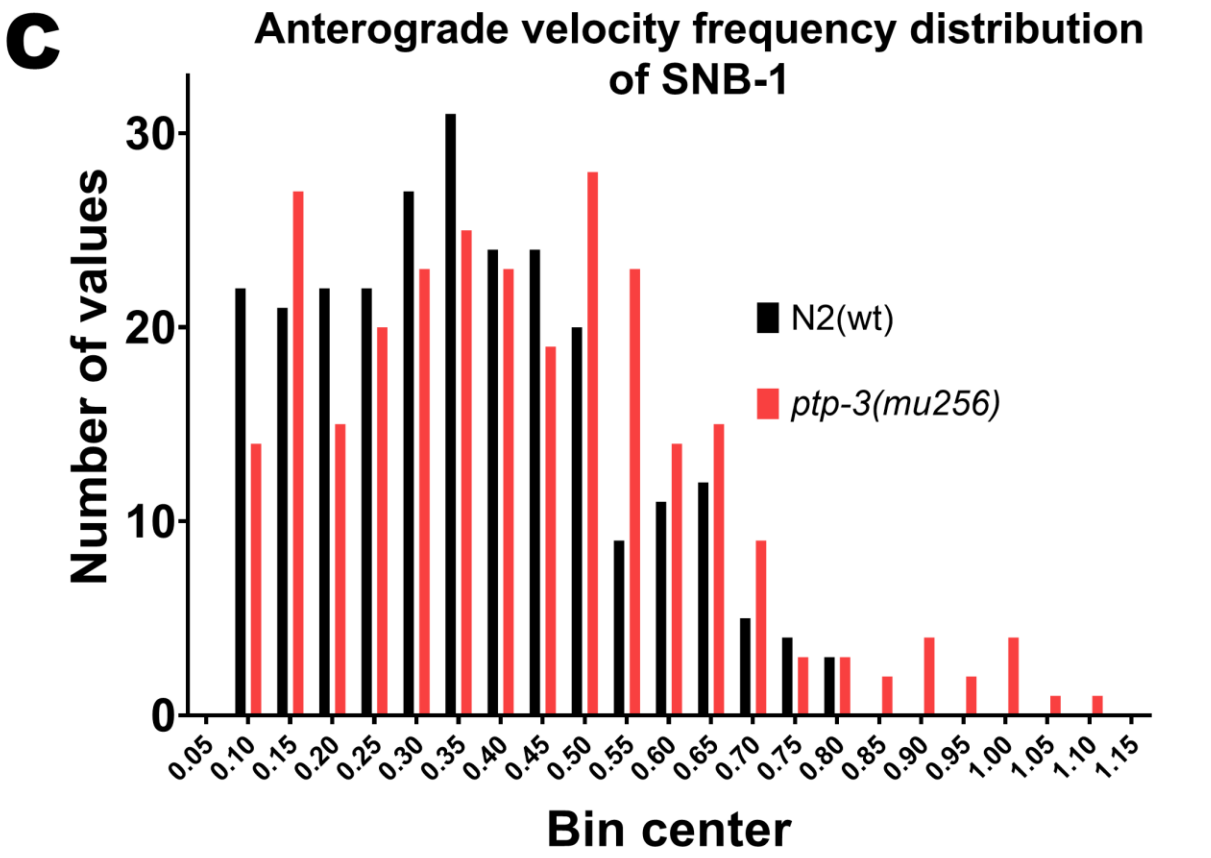

A
Anterograde velocity (μm/s)
ns
1.0
0.5
0.0
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
B
Retrograde velocity (μm/s)
ns
1.0
0.5
0.0
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
C
Directional changes per 100s (#)
ns
40
30
20
10
0
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
D
Pausing duration (sec)
ns
20
15
10
5
0
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
E
Anterograde run length (μm)
ns
40
30
20
10
0
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
F
Retrograde run length (μm)
ns
0
-20
-40
-60
Control (e1265 [UNC-104::mRFP])
N2 [UNC-104::mRFP]
G
N2 [UNC-104::mRFP]
ns
Control (e1265 [UNC-104::mRFP])
-10
-5
0
5
10
Net run length (μm)
H
Control (e1265 [UNC-104::mRFP])
N2(wt) [UNC-104::mRFP])
I
Area of UNC-104 cluster (μm2)
ns
1.0
0.5
0.0
Control (e1265[UNC-104])
N2 [UNC-104::mRFP]
J
Density of UNC-104 cluster (#/100 μm)
ns
30
20
10
0
Control (e1265[UNC-104])
N2 [UNC-104::mRFP]

Figure S7. Motility parameters and UNC-104 clustering in CB1265 (*e1265*)[UNC-104::mRFP] compared to *N2(wt)*[UNC-104::mRFP]. (A) Anterograde velocity, (B) retrograde velocity, (C) directional changes (motor reversals), (D) pausing duration, (E) anterograde run length, (F) retrograde run length, (G) net run length, (H) straightened sublateral neurites for cluster analysis, (I) area of UNC-104 particle in sublateral neurites, and (J) cluster density. Scale bar: 10 µm. Number of events in motility data > 1500 events. Unpaired Student's t-test.


A
Anterograde velocity frequency distribution
Control (e1265 [UNC-104])
ptp-3(ok244)
ptp-3(mu256)
ptp-3(mu256) rescue
syd-2(ok217)
syd-2(ok217);ptp-3(mu256)
syd-2(ok217) [SYD-2 Y741F]
syd-2(ok217) [SYD-2 Y741E]
Control (EV RNAi)
ptp-3 RNAi
Number of values
0
20
40
60
80
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00 1.05 1.10 1.15 1.20 1.25 1.30 1.35
Bin Center


B
Retrograde velocity frequency distribution
Control (e1265 [UNC-104])
ptp-3(ok244)
ptp-3(mu256)
ptp-3(mu256) rescue
syd-2(ok217)
syd-2(ok217);ptp-3(mu256)
syd-2(ok217) [SYD-2 Y741F]
syd-2(ok217) [SYD-2 Y741E]
Control (EV RNAi)
ptp-3 RNAi
Number of values
0
50
100
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
Bin Center


C
Anterograde velocity frequency distribution of SNB-1
Number of values
30
20
10
0
N2(wt)
ptp-3(mu256)
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
1.05
1.10
1.15
Bin center

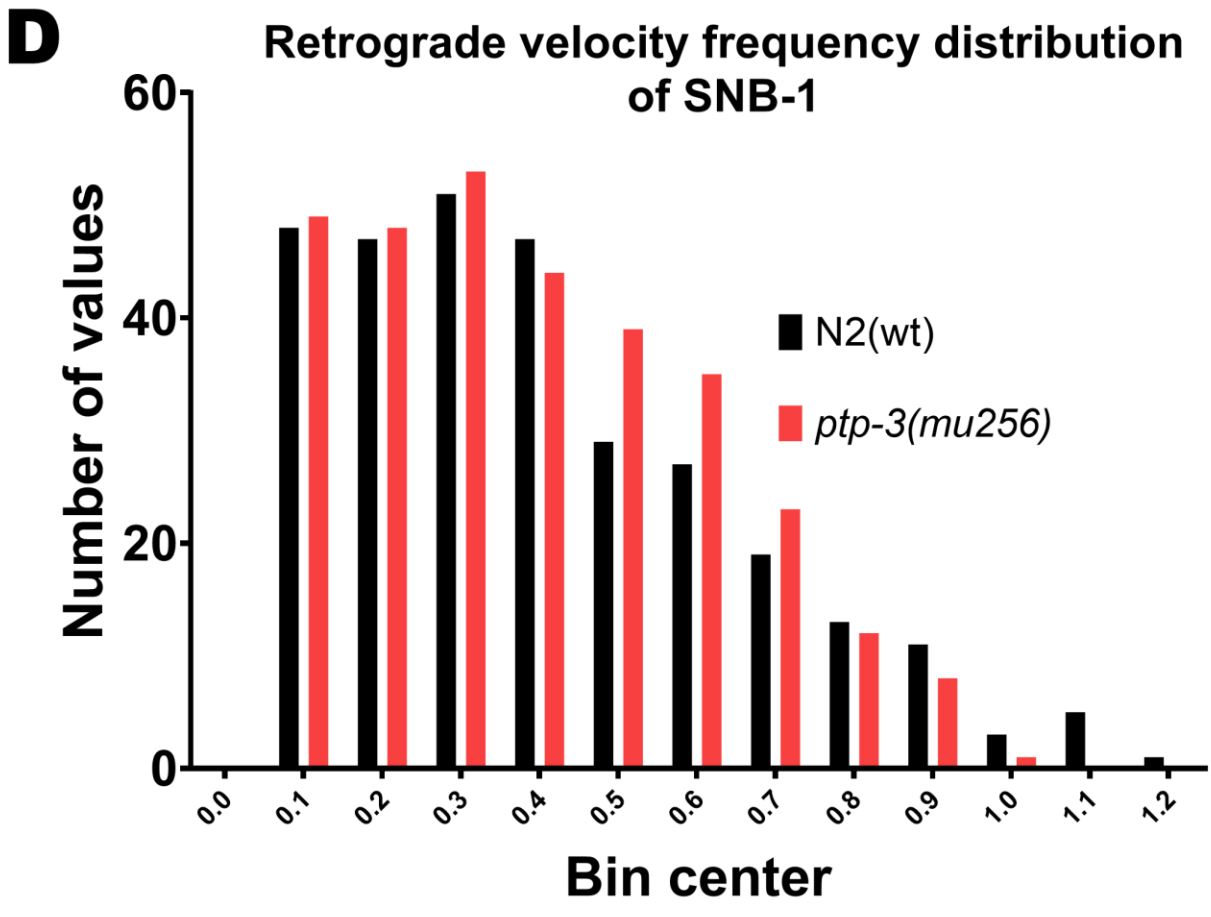

D
Retrograde velocity frequency distribution of SNB-1
Number of values
60
40
20
0
N2(wt)
ptp-3(mu256)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
Bin center

Figure S8. Frequency distribution of UNC-104 and SNB-1 velocity data. (A) Frequency distribution of UNC-104 anterograde velocity (data from Figure 6A). (B) Frequency distribution of UNC-104 retrograde velocity data (data from Figure 6B). (C) Frequency distribution of SNB-1 anterograde velocity data (data from Figure 6A). (D) Frequency distribution of SNB-1 retrograde velocity (data from Figure 6B).