Supplemental Figures and Legends

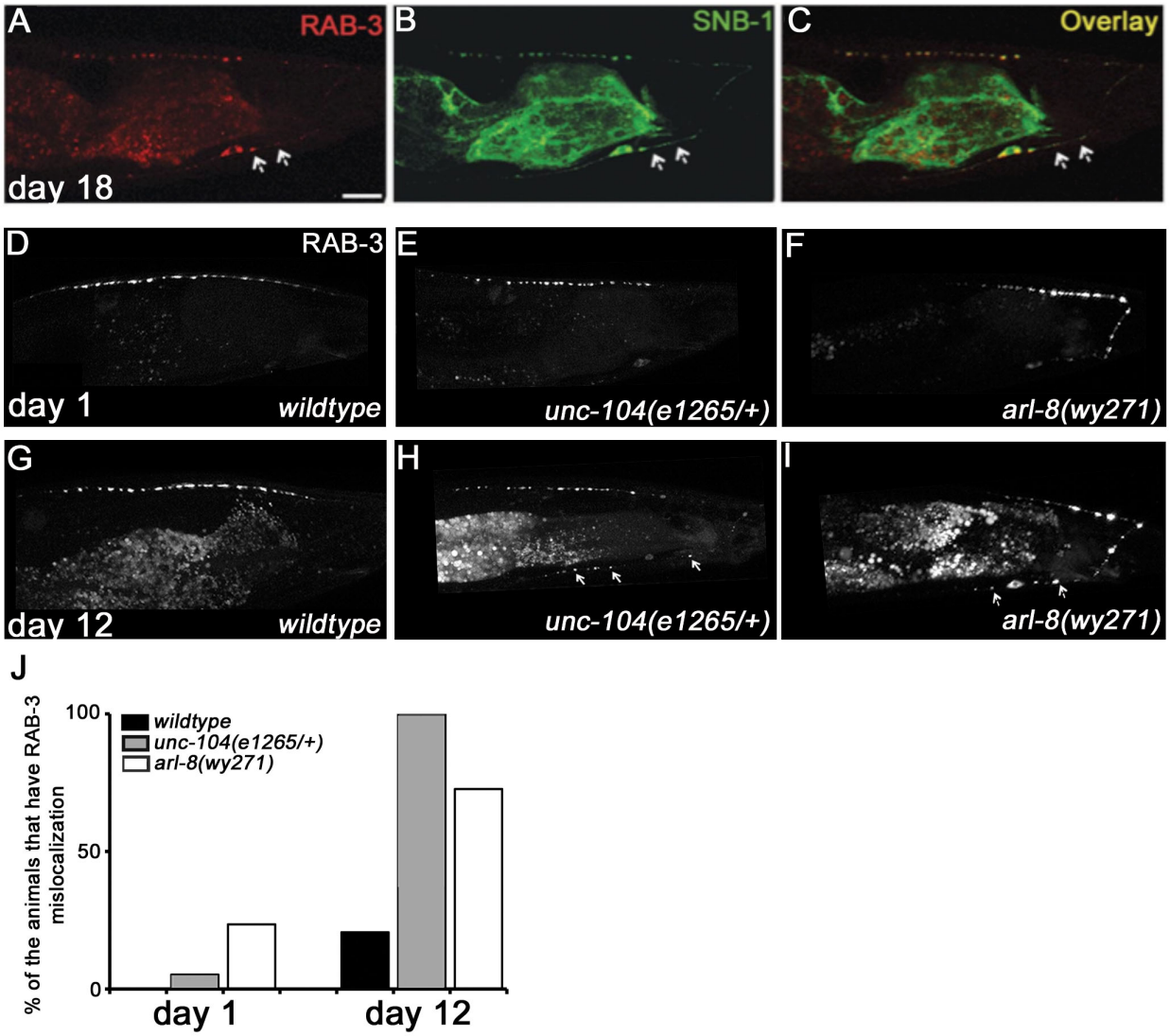

Figure S1, related to Figure 1 RAB-3 and SNB-1 are co-localized in aged animals, and is affected by unc-104 heterozygous mutants e1265/+ and mutations of UNC-104 regulator *arl-8*. (A-C) In DA9 neurons of day 18 animals, the SV-associated protein RAB-3 (A, red) and the SV transmembrane protein SNB-1 (B, green) are co-localized in both the presynaptic region and asynaptic region (arrows). (D-J) Confocal microscopy of distribution of the GFP:RAB-3 puncta in *wildtype* (D, J), *unc-104(e1265/+)*(E, H), and *arl-8(wy271)* (F, I) Middle row: day 1; bottom row: day 12. (J) Quantification of the percentage of worms that have RAB-3 mislocalization in either the dendritic or ventral axonal regions. Total animals analyzed: day 1: *wildtype*:15, *unc-104(e1265/+)*:19, *arl-8(wy271)*: 17; day 12: *wildtype*: 24, *unc-104(e1265/+)*: 18, *arl-8(wy271)*: 22. There is a significant increase in the percentage of worms that have RAB-3 mislocalization in *unc-104(e1265/+)* and *arl-8(wy271)* backgrounds.

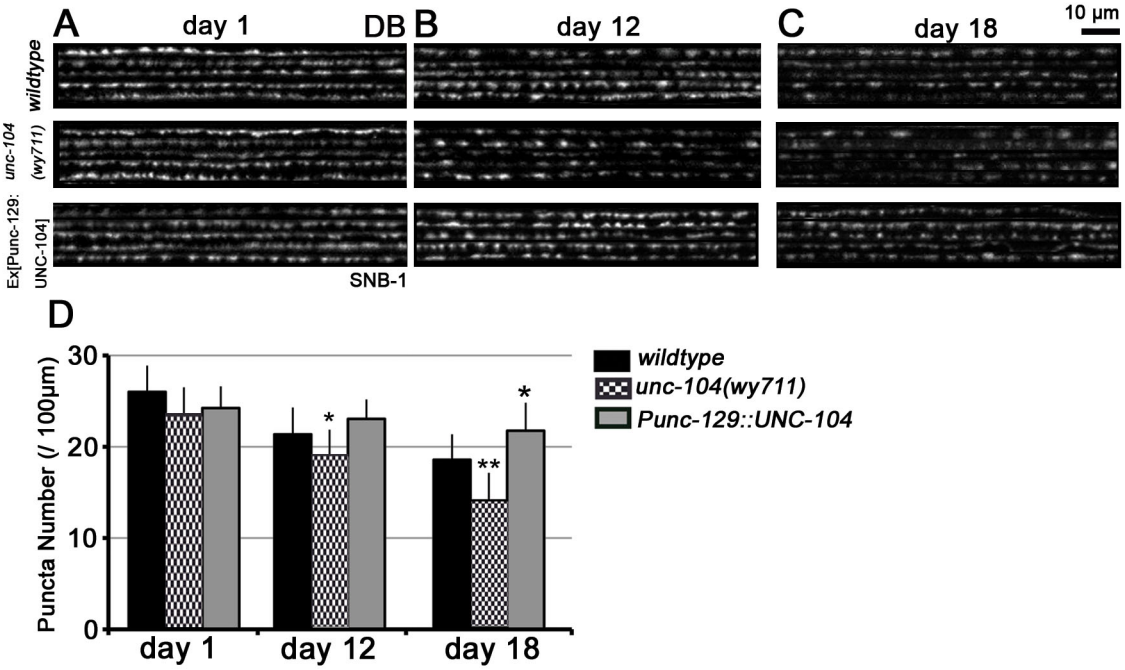

Figure S2, related to Figure 1 UNC-104 rescues aging-associated synapse loss in DB presynaptic regions cell autonomously. (A-C) Confocal images of SNB-1 puncta in the DB presynaptic region with age. upper row, *wildtype*; middle row, *unc-104(wy711);* bottom row, expression of UNC-104 specifically in DB neurons (P*unc-129*::UNC-104) can rescue the loss of SV density over age. (D) Quantification of the SNB-1 synapse density with age. day1: 16 (*wildtype*), 11 (*unc-104(wy711*)), 13 (*Punc-129::unc-104*); day 12: 14 (*wildtype*), 17 (*unc-104(wy711*)), 15 (*Punc-129::unc-104*); day 18: 11 (*wildtype*), 15 (*unc-104(wy711*)), 18 (*Punc-129::unc-104*).

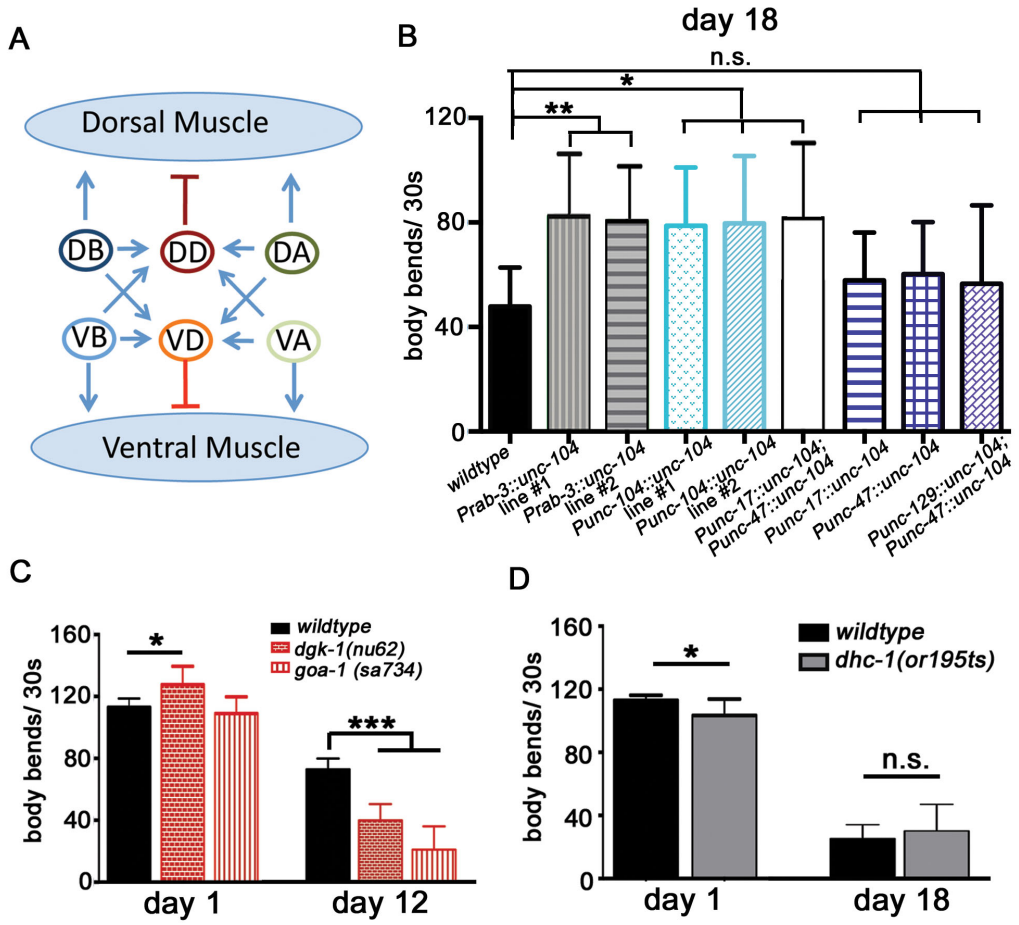

Figure S3, related to Figure 2 UNC-104 functions in motor neurons to maintain motor function during aging, and had distinctive effects from the hyperactive and SV transport mutants. (A) A schematic of the *C. elegans* motor circuit. DB, VB, DA, VA: cholinergic neurons; DD, VD: GABAergic neurons. (B) ExpressingUNC-104 from a pan-neuronal promoter (P*rab-3*), its endogenous promoter (P*unc-104*), or motorneurons alone (P*unc-17* and P*unc-47*), had similar effects in the improvement of body bend movements. In contrast, expressing UNC-104 in cholinergic neurons (P*unc-17*, DB, VB, DA, VA), or GABAergic neurons (P*unc-47*, DD, VD), or DB, DD, VD neurons (P*unc-129* and P*unc-47*), does not affect the motility defects in d18 animals. Total animals analyzed: *wildtype:* 25, *Prab-3::unc-104* line #1: 16, *Prab-3::unc-104* line #2: 16, *Punc-104::unc-104* line#1: 15, *Punc-104::unc-104* line#2: 14, *Punc-17::unc-104,Punc-47::unc-104:*11, *Punc-17::unc-104*: 17, *Punc-47::unc-104*: 18, *Punc-129::unc-104*, *Punc-47::unc-104:*13. (C) The hyperactive mutants *dgk-1* and *goa-1* show increased body bend activities in bouts of 5-10 seconds and have slightly increased body bend activity over the period of 30s. Nevertheless, these mutants have more severe motility decline with age. Total animals analyzed: day1: *wildtype*: 15, *dgk-1(nu62)*: 7, *goa-1(sa734)*: 9; day12: *wildtype*: 25, *dgk-1(nu62)*: 16, *goa-1(sa734)*: 8. (D) Reduced function of DHC-1, a cytoplasmic dynein complex component, causes slight motility defects at d1, but does not affect the movements in d18 animals. Total animals analyzed: *dhc-1(or195ts)*: day 1:6, day18:6*, $P<0.05$; ***, $P<0.001$. n.s., not significant. One-way ANNOVA. The error bar stands for 95% confidence intervals.

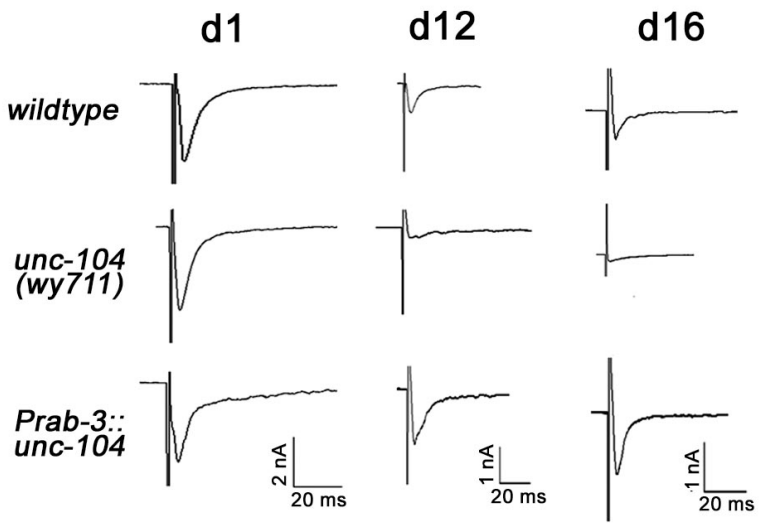

Figure S4, related to Figure 3 representative traces of evoked PSCs of *wildtype*, *unc-104(wy711)*, *wyEx6415 (Prab-3::unc-104mcherry)* over time.

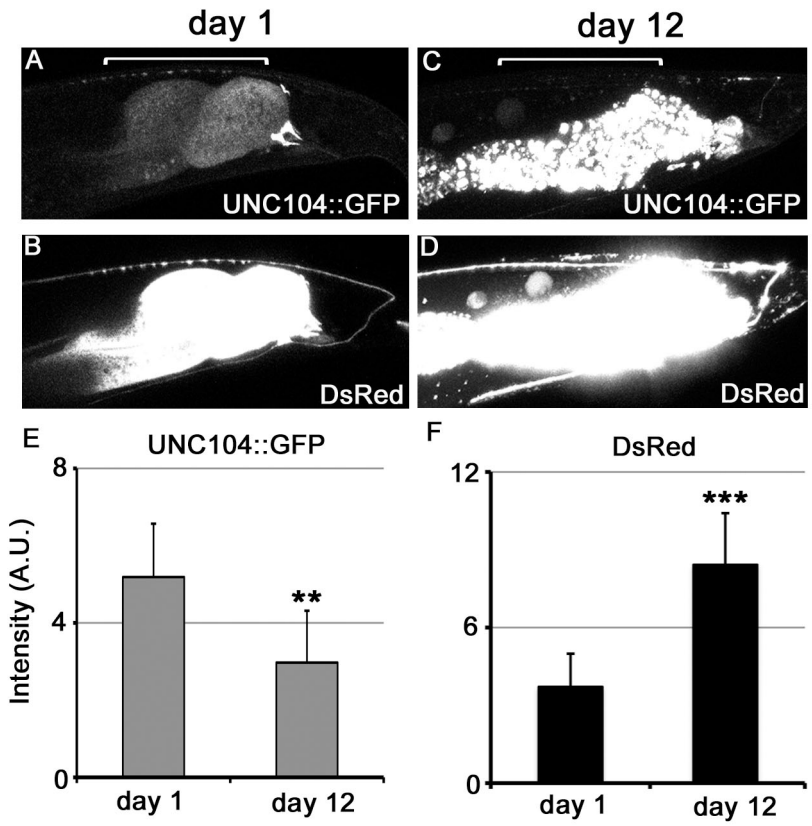

Figure S5, related to Figure 4 The steady state level of UNC-104 protein decreases in DA9 synaptic region with age. (A-D) Confocal images of expression of UNC-104::GFP in DA9 neuron, which are labeled with DsRED. (P*itr-1*::UNC-104GFP, P*itr-1*::DsRed). (E) Quantification of UNC-104GFP and DsRED fluorescence in day 1and day 12 animals. The steady state level of UNC-104 is specifically decreased in the synaptic region (marked by a bracket) in day 12 animals. Total animals analyzed: day1:9, day 12:10. **: P< 0.01. ***: P<0.001. Unpaired student t test.

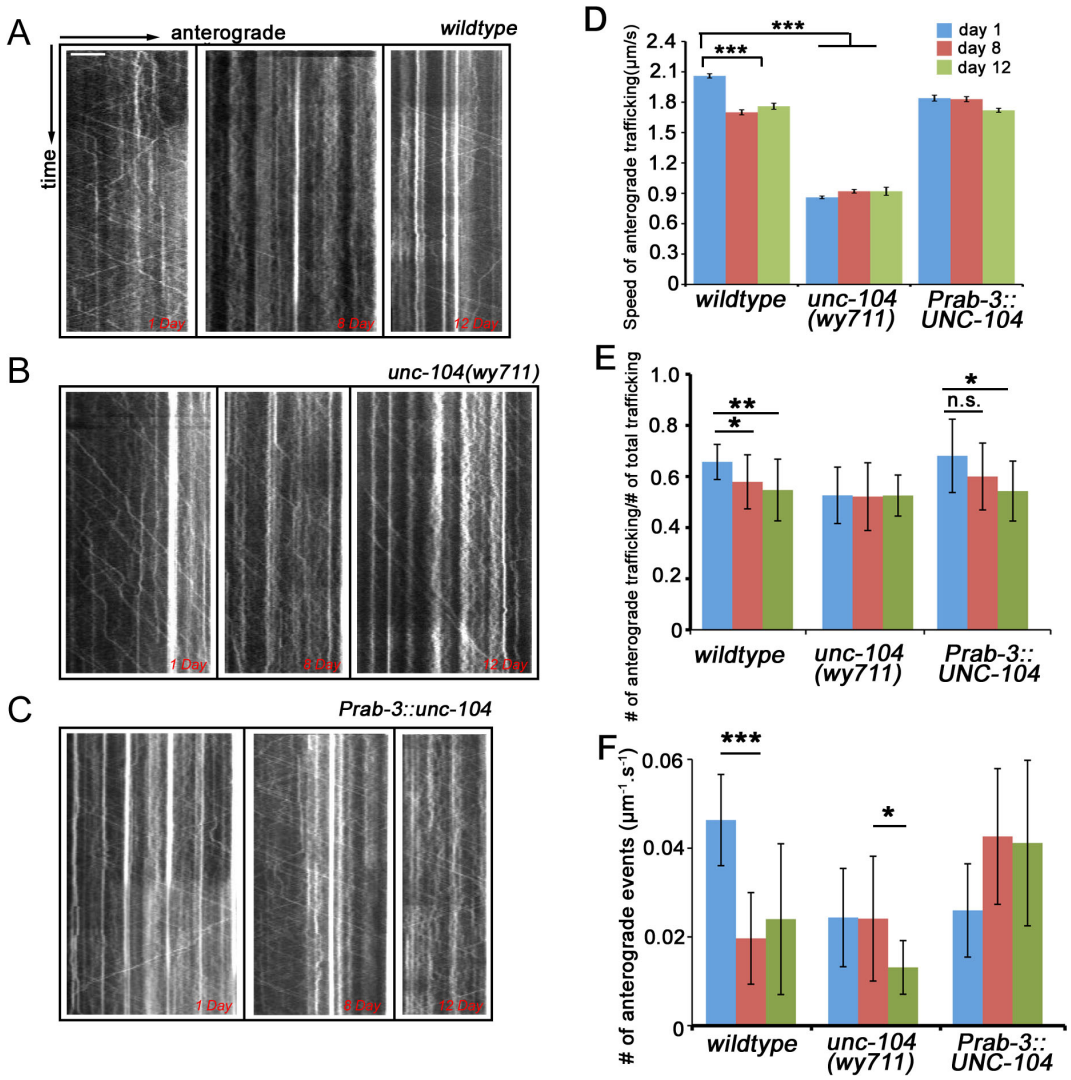

Figure S6, related to Figure 4 kymograph analyses of synaptic vesicle trafficking in young and aged worms. (A-C) Representative kymograph of *wildtype* (A), *unc-104(wy711)* (B), and *Prab-3::UNC-104*(C). (D-F) Quantification of the speed of anterograde trafficking (D), the percentage of anterograde events during aging (E), and the number of anterograde movements (F). Total animals analyzed: day1: *wildtype*: 13, *unc-104(wy711)*: 21, *Prab-3::unc-104*:12; day8: *wildtype*: 24; *unc-104(wy711)*: 22; *Prab-3::unc-104*: 16; day12: *wildtype*: 17, *unc-104(wy711)*: 9, *Prab-3::unc-104*:11.

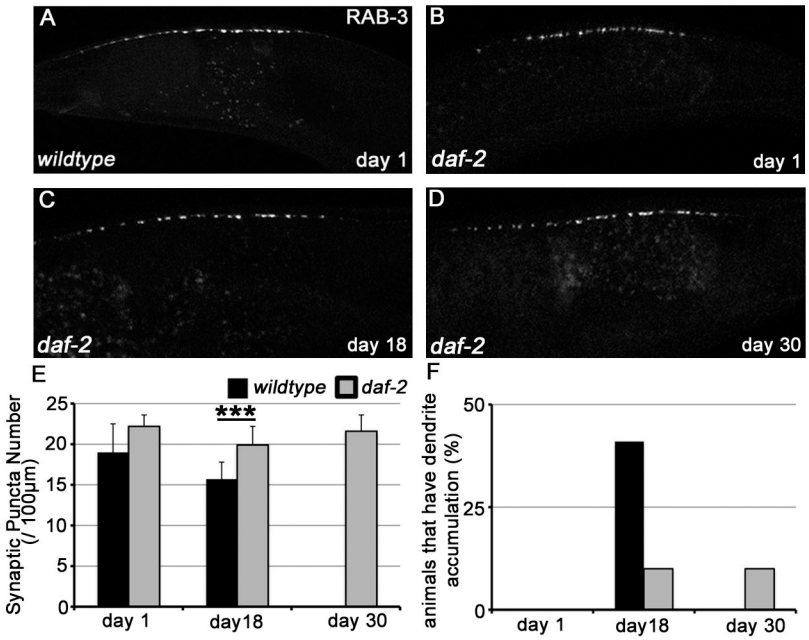

Figure S7, related to Figure 7. DA9 synapses are preserved in aged *daf-2* mutants. (A-D) Confocal images of DA9 RAB-3 puncta of *wildtype* (A) and young (B) and aged (C, D) *daf-2* mutants. (E-F) Quantification of RAB-3 puncta in the synaptic region (E) and mislocalization (F) in *daf-2* animals. Student's t test. ***: P<0.001. Total animals analyzed: day1: 8, day 18: 11, day 30:12.

Strain	Mean Llifesapn ± S.D. (days)	Median Lifespan	75% Lifespan	n (assayed)	P value against control
wildtype	17.8±1.0	18	20	187	
unc-104(wy711)	15.4±1.4	17	18	153	0.0011
unc-104(e1265/+)	16.5±1.0	17	18	71	0.103
wyEx6415 (Prab-3::unc-104 line #1)	19.3±0.6	21	23	93	<0.0001
wyEx6228 (Prab-3::unc-104 line #2)	19.5±0.7	21	23	43	<0.0001
daf-16(mu86)	11±1.4	12	13	254	
daf-16(mu86);unc-104(wy711)	11±0.4	12	13	200	0.9402
daf-16(mu86);wyEx6415	12.3±1.3	12	15	109	0.4053
hsf-1(sy441)	9.5±2.2	11	13	244	
hsf-1(sy441);unc-104(wy711)	4.9±0.4	6	9	194	<0.0001
hsf-1(sy441);wyEx6415	12.8±1.3	14	16	182	<0.0001
daf-2(e1370)	35.3±0.6	36	42	113	
daf-2(e1370);unc-104(wy711)	39.5±1.7	40	45	176	0.004
daf-2(e1370);wyEx6415	38.7±0.7	39	43	123	0.0289

Table S1, related to Figure 6 lifespan analyses

Supplemental Experimental Procedures

Culture and strains

Worms were raised on NGM plates at 20°C, using OP50 *E.coli* as a food source. N2 Bristol was used as the wild-type reference strain. The mutant strains CB1265 *unc-104(e1265)*, *CB1370 daf-2(e1370), syd-2(ju37), unc-10(e102), nab-1(ok943), unc-57(ok310),unc-11(e47),dsh-1(ok1445),lin-44(n1792), daf-16(mu86), hsf-1(sy441), skn-1(zu67)/nT1[unc-(n754)let-](IV;V), daf-2(e1370),*were obtained through the *Caenorhabditics* Genetics Center. The *wy711* mutant was described as previously[1].

For aging experiments, worms were transferred every 2-3 days to fresh NGM plates to separate the adults from the larvae. Transgenic constructs were cloned into the pSM vector, a derivative of pPD49.26 (A.Fire) with extra cloning sites. The transgenic strains were generated using standard techniques.

Transgenic Lines and Molecular Cloning

wyEx3296 (Pitr-1::snb-1::yfp, Pitr-1:: cfp::rab-3): The plasmid was injected at 1 ng μL^{-1} with 40ng *Podr-1::rfp* into N2 worms.

wyEx5554 (Punc-129::snb-1::yfp, Punc-129::cfp::rab-3): The P*itr-1* promoter sequences in the plasmids *Pitr-1::snb-1::yfp* and *Pitr-1::cfp::rab-3* was replaced by the Punc-129 promoter between the restriction enzyme sites SphI and AscI. The plasmid was injected at 1.0 ng μL^{-1} with 40ng *Podr-1::rfp* into N2 worms.

wyEx6415 (Prab-3::unc-104::mcherry): The plasmid was injected at 20 ng μL^{-1} with 40ng Podr-1::gfp into N2 worms.

wyEx6187 (Punc-104::unc-104::mcherry): The P*rab-3* promoter in P*rab-3::mcherry::unc-104* was replaced by the P*unc-104* promoter (4.5 kb upstream of the unc-104 transcript) between the SphI and NheI sites. The plasmid was injected at 20 ng μL^{-1} with 40ng P*odr-1::gfp* into N2 worms.

wyEx6164 (Punc-17::unc-104::mcherry): The P*rab-3* promoter in P*rab-3::mcherry::unc-104* was replaced by the P*unc-17* promoter between the SphI and NheI sites. The plasmid was injected at 20 ng μL^{-1} with 40ng Podr-1::gfp into N2 worms.

wyEx6461 (Punc-104::myrGFP, Punc-104::unc-104::mcherry) The *Prab-3* promoter in P*rab-3::mcherry::unc-104*, and the *Punc-17* promoter in *Punc-17::myrGFP* was replaced by the P*unc-104* promoter (4.5 kb upstream of the unc-104 transcript) between the SphI and NheI sites. The plasmid was injected at 12 and 16 ng μL^{-1} into N2 worms.

Fluorescence Confocal Imaging and Quantification

Images of fluorescently tagged fusion proteins were captured in live *C. elegans* using a plan-Apochromat 40X 1.3 objective on a Zeiss LSM710 confocal microscope, using identical image and laser setting for each genotype. The distribution of GFP::RAB-3 puncta along the DA9 dorsal nerve cords were extracted from the confocal images with the "straighten to line" program in ImagJ64. The puncta number was calculated using the "analyze particles" function, with the threshold of 1 pixels.

Body bend assays

Individual worms were placed in a droplet (10 μl) of M9 buffer and allowed to recover for 20-30s. The number of body bends was calculated as the average of body bend movements counted

in two continuous 30s intervals. A body bend was defined as a change in the direction of the segment from the head to the midbody of an animal.

Learning and memory assays
The learning and memory behaviors were performed as previously described[S2].

Real-time RT-PCR
Total RNA was isolated from 50-100 adult worms by Trizol Reagent (Ambion #15596). cDNA was synthesized in a 20µl reaction volume from 1-2 µg of total RNA using the SuperScript III first-strand synthesis Super Mix (Invitrogen #11752). 1 µl of 1:20 dilution of cDNA was used as the template in a 10µl reaction volume from the SsoFast EvaGreen Supermix with Low ROX (bio-rad, #172-5211). The real-time PCR primers were designed using the probe-design software form Roche. Real-time PCR was performed in duplicates using the Bio-Rad CFX96 Real-Time System. Data were analyzed using the standard curve method. The entire experiments were repeated 6-10 times on independent RNA preparations.

Supplemental References

S1. Wu, Y.E., Huo, L., Maeder, C.I., Feng, W., and Shen, K. (2013). The balance between capture and dissociation of presynaptic proteins controls the spatial distribution of synapses. Neuron *78*, 994-1011.

S2. Kauffman, A.L., Ashraf, J.M., Corces-Zimmerman, M.R., Landis, J.N., and Murphy, C.T. (2010). Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. PLoS Biol *8*, e1000372.