Supplemental Data

Supplemental Figure Legends

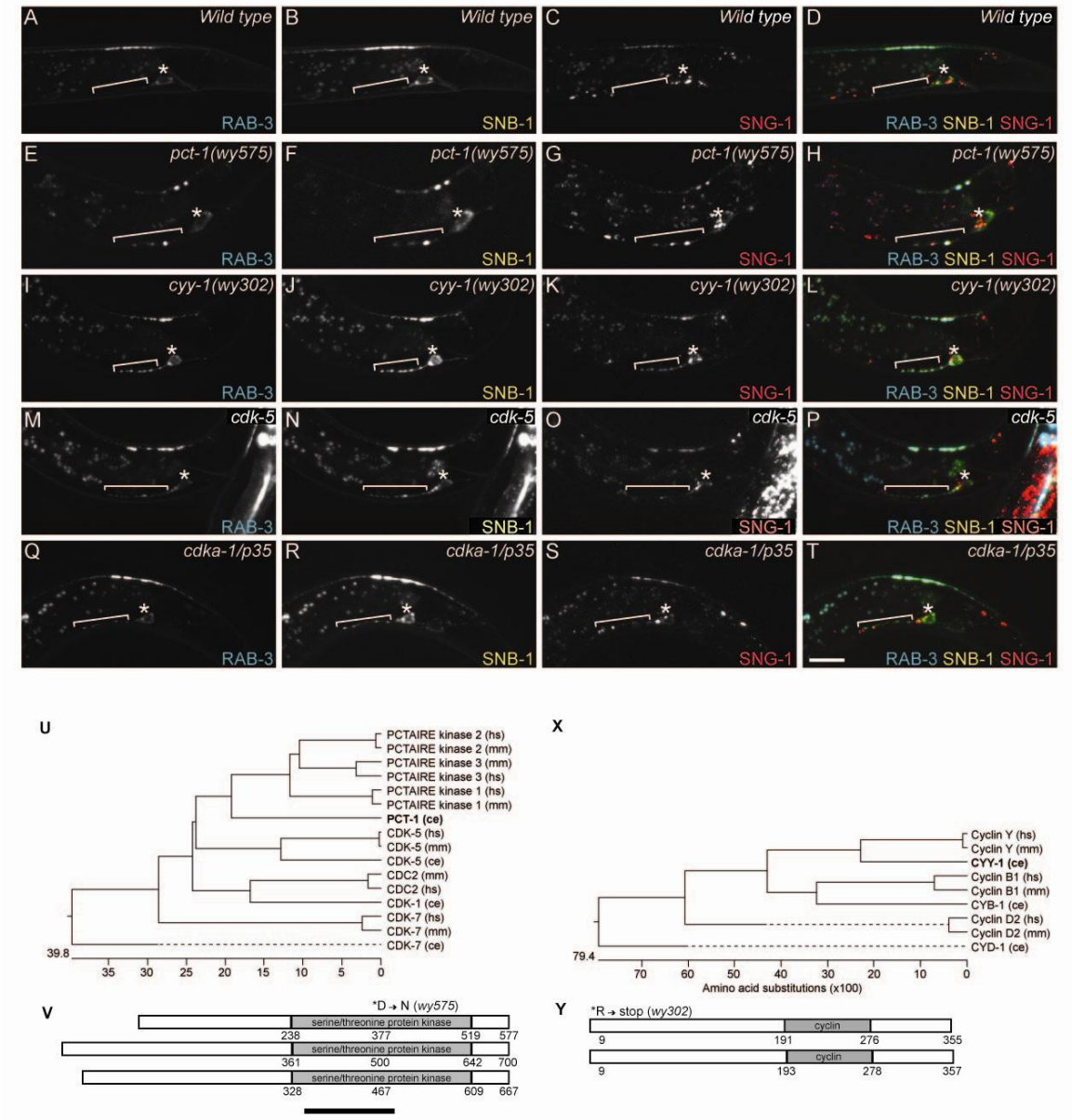

Figure S1. Mislocalization of Various Synaptic Vesicle Proteins in *wy575* and *wy302* Mutants and their Cloning, related to Figure 1.

(A-T) Synaptic vesicle proteins synaptobrevin/SNB-1, synaptogyrin/SNG-1 and RAB-3 mislocalize to the DA9 Dendrite in *pct-1(wy575), cyy-1(wy302), cdk-5(ok626),* and *cdka-1/p35(tm648)* Mutants. (A-D) Wild-type young larval L1 animal co-

expressing CFP::RAB-3, SNB-1::YFP, and SNG-1::mCherry in DA9(*wyIs109*). Note that all three markers are absent from the DA9 dendrite. (E-T) CFP::RAB-3, SNB-1::YFP, and SNG-1::mCherry in *pct-1(wy575)*, *cyy-1(wy302), cdk-5,* and *cdka-1/p35* mutant animals. The fluorescence in the middle of the worm is gut autofluorescence. Anterior, left and dorsal, top. Bracket, dendrite. Asterisk above cell body. Scale bar, 10μm.

(U-Y) Cloning of *wy575* and *wy302* mutants. (U) A balanced phylogenetic tree for PCT-1. (V) Schematic diagram of PCT-1 isoforms, *wy575* mutation, and *tm2175* deletion. (X) A balanced phylogenetic tree for CYY-1. CYB-1 and CYD-1 are *C. elegans* homologs of cyclin B and cyclin D respectively. (Y) Schematic diagram of CYY-1 isoforms and *wy302* mutation. Trees were generated using clustal analysis of full-length proteins in the software Megalign. The most closely related proteins from mouse (mm) and human (hs), and two known *C. elegans* cyclins and CDKs are included. Numbers in B and D indicate number of amino acids.

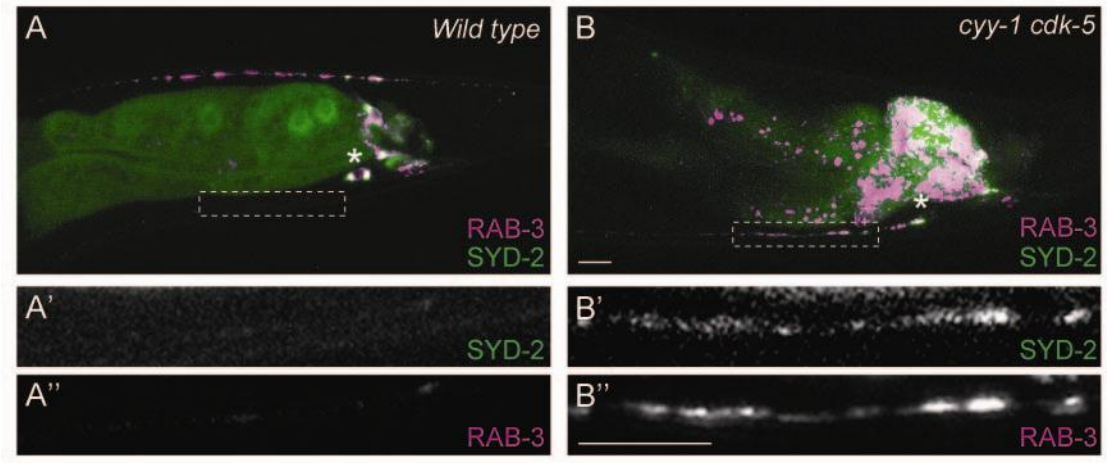

Figure S2. RAB-3 and SYD-2/Liprin-α Mislocalize to the DA9 Dendrite in *cyy-1 cdk-5* Mutant Animals, related to Figure 2.

(A, B) A wild-type (A) or *cyy-1 cdk-5* mutant animal (B) co-expressing GFP::RAB-3 and mCherry::SYD-2/liprin-α(*wyEx2055)* in DA9. (A'-B', A"-B") Higher magnification micrographs of the corresponding dotted boxes. Asterisk above cell body. Scale bar, 10μm.

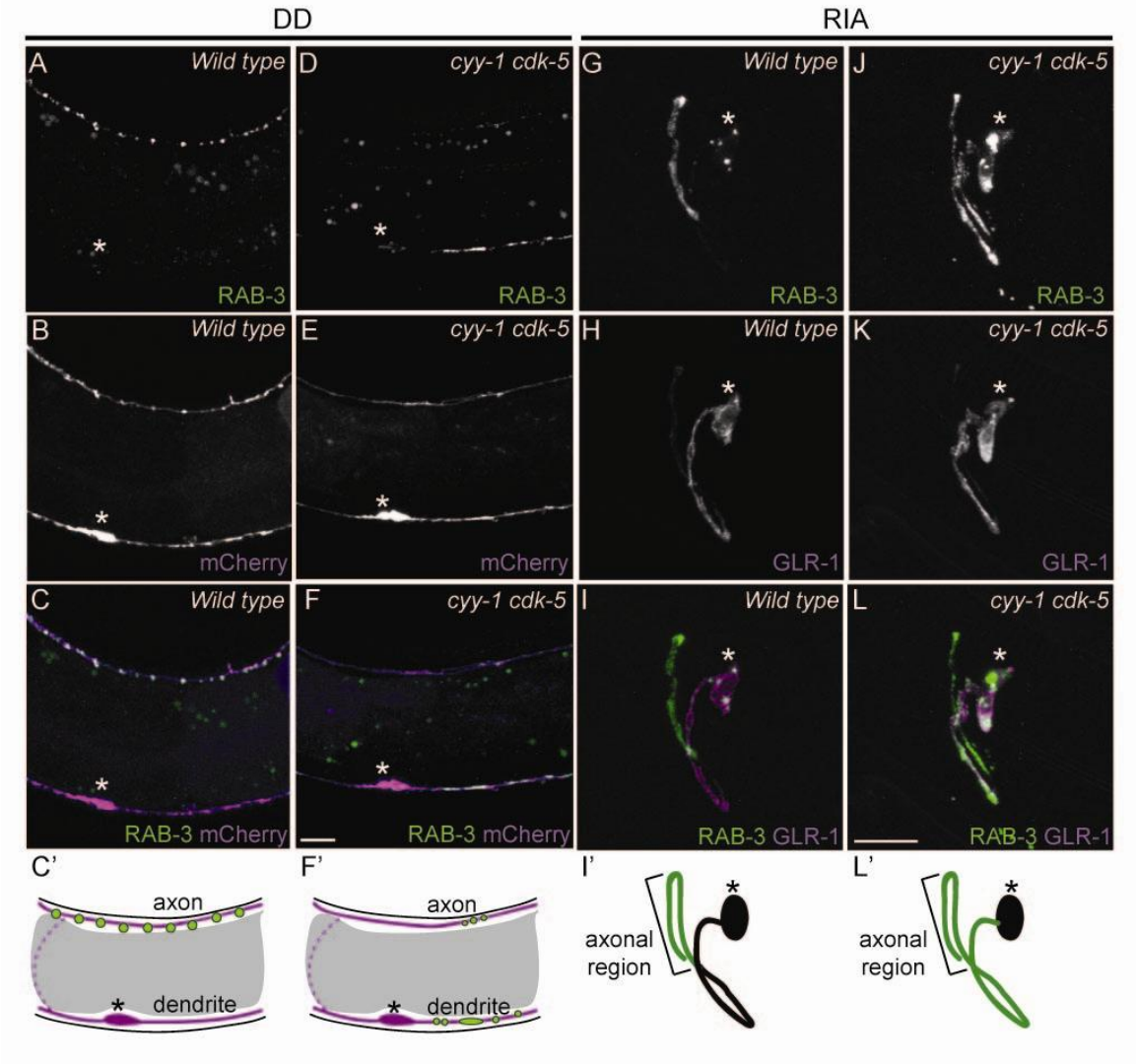

Figure S3. Synaptic Vesicle-Associated RAB-3 Mislocalizes to the Dendrite in DD and RIA Neurons in *cyy-1 cdk-5* Mutant Animals, related to Figure 3.

(A-F) Micrographs (A-C) and schematic diagram (C') of a wild-type adult animal co-expressing GFP::RAB-3 and mCherry in DD neurons(*wyIs202*). (D-F') GFP::RAB-3 and mCherry in a *cyy-1 cdk-5* mutant animal. (G-L') Micrographs and schematic diagram of wild-type (G-I') or *cyy-1 cdk-5* mutant animals (J-L') co-expressing mcherry::RAB-3 (green) and postsynaptic receptor GLR-1::GFP (purple) in

RIA(*wyIs93*). GLR-1 is unaffected while RAB-3 is present in the postsynaptic domain of RIA. Asterisk above cell body. Scale bar, 10μm.

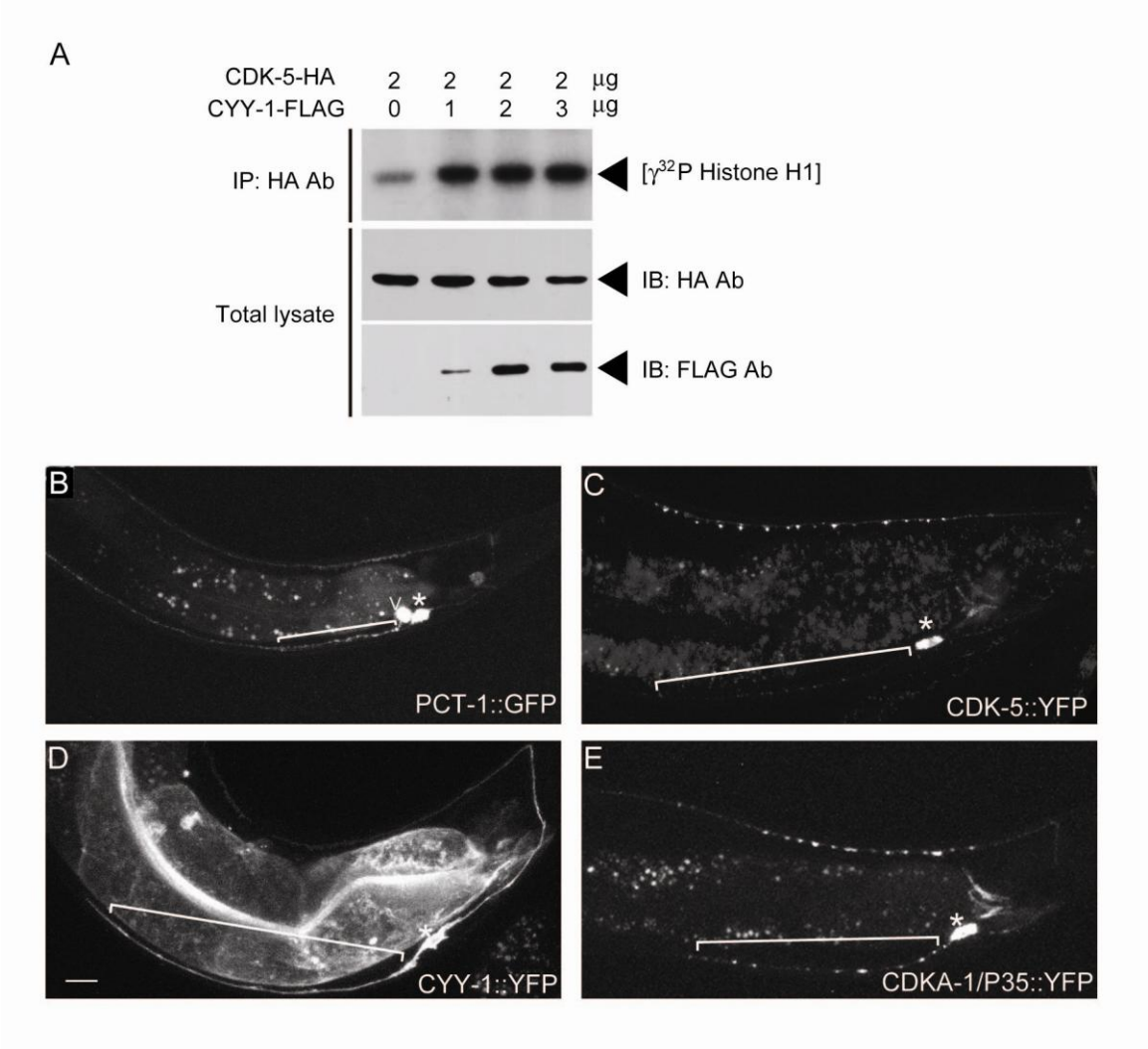

Figure S4. CYY-1 can activate CDK-5 and Subcellular localization of PCT-1, CYY-1, CDK-5, and CDKA-1/p35, related to Figure 4.

(A) *C. elegans* CDK-5-HA and CYY-1-FLAG were co-expressed in HEK 293T cells as indicated. Expression of CDK-5 alone exhibits no kinase activity, while co-expression with CYY-1 significantly increases the kinase activity of CDK-5. Lysate was immunoprecipitated with HA antibody and then subjected to kinase assay using Histone H1 protein as a substrate.

(B) PCT-1::GFP expressed in DA9 and VA12 neurons (*wyEx2774*). (C) CDK-5::YFP expressed in DA9 (*wyEx2160*). (D) CYY-1::YFP expressed in DA9 (*wyEx2789*). (E) CDKA-1/p35::YFP expressed in DA9 (wyEx2460).

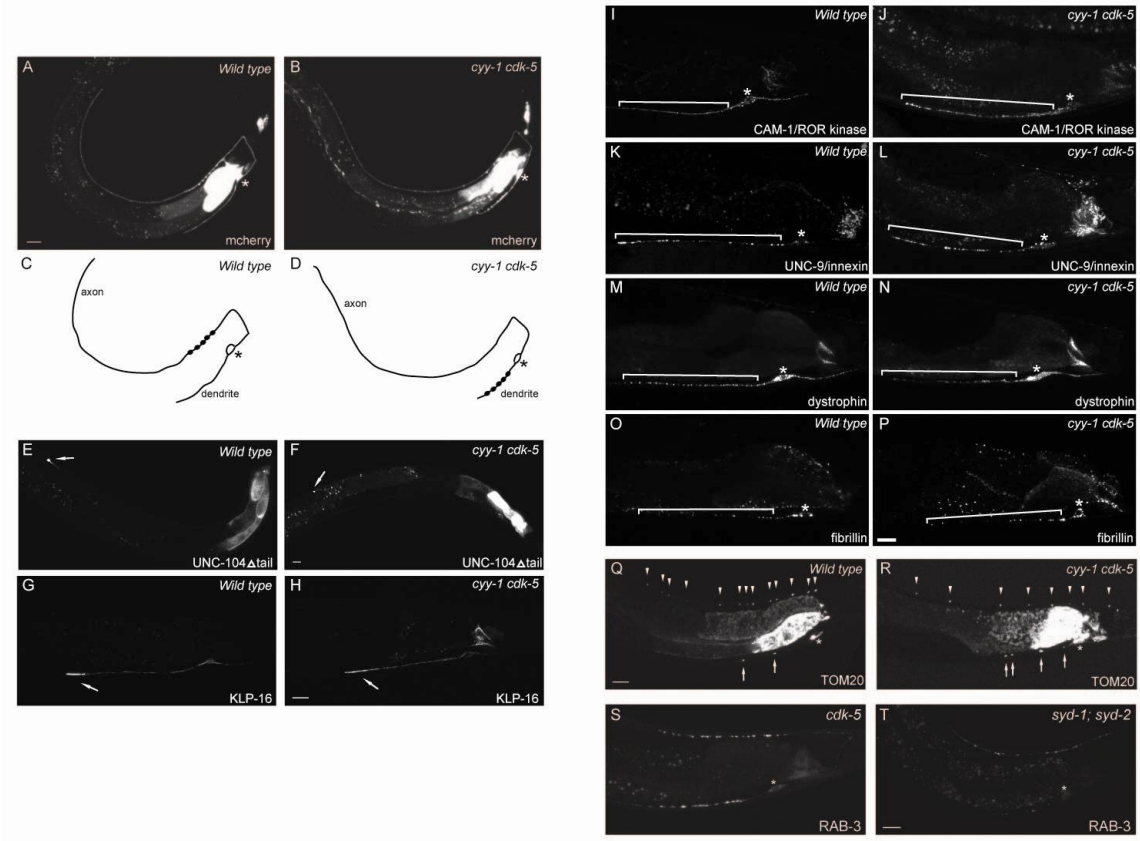

Figure S5. Many Aspects of Subcellular Specifications are Normal in *cyy-1 cdk-5* Mutants, related to Figure 5.

(A-D) DA9 axon and dendrite projection is normal in *cyy-1 cdk-5* mutant animals. Wild-type (A) or *cyy-1 cdk-5* (B) mutant animals expressing mCherry(*wyEx1902)* in DA9. (C, D) Schematic diagrams of DA9 guidance and morphology.(E-H) The anterograde motor UNC-104/KIF1A localizes to the tip of the dorsal axon while the minus-end Motor KLP-16/KIFC3 is enriched at the tip of the DA9 dendrite in wild-type and *cyy-1 cdk-5* Mutant Animals. This pattern is consistent with a mixed MT polarity in dendrite, where the preferred orientation is with their minus-ends oriented toward the tip of the dendrite. (E, F) Wild-type or *cyy-1 cdk-5* mutant

animals expressing UNC-104 Δtail (1-389)::GFP(*wyEx2992)* in DA9. (G, H) Wild-type or *cyy-1 cdk-5* mutant animals expressing KLP-16::YFP(*wyEx3128)* in DA9. Arrow, accumulation of motor protein. (I-P) Four dendritic proteins localize appropriately in *cyy-1 cdk-5* Mutant Animals. Animals expressing CAM-1::YFP (I, J, *wyEx403*), UNC-9::YFP (K, L, *wyEx1054*), DYS-1::YFP (M, N, *wyEx2430*), or fibrillin::YFP (O, P, *wyEx2396*) in DA9. (Q-R) TOM20, a Subunit of the Mitochondrial Translocase Complex is Still Present in the Axon in *cyy-1 cdk-5* Mutant Animals. A wild-type (Q) or *cyy-1 cdk-5* mutant animal (R) expressing TOM20::YFP(*wyEx2406)* in DA9. Arrowheads, axonal TOM20 puncta. Arrows, dendritic TOM20 puncta. (S-T) Presynaptic assembly mutants have a different phenotype when compared to *cdk-5* Mutant Animals. A *cdk-5* (S) or *syd-1(ju82); syd-2/liprin-α(wy5)* mutant animal (T) expressing GFP:RAB-3(*wyIs85)* in DA9. Asterisk indicates cell body. Scale bars, 10μm.

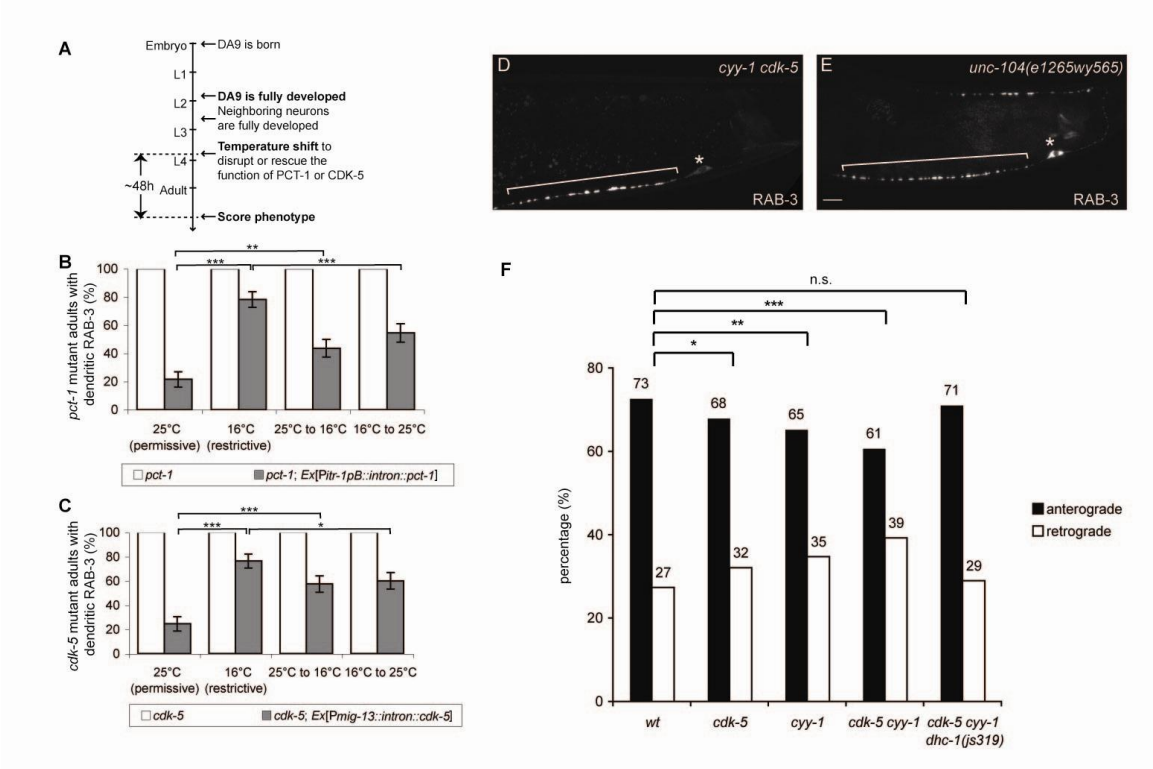

Figure S6. PCT-1 and CDK-5 Maintain the Balance between Anterograde and Retrograde SVP Transport, related to Figure 6.

(A-C) PCT-1 and CDK-5 are required to maintain the polarized localization of GFP::RAB-3. (A) Experimental time line. (B, C) *pct-1* (white columns in B) or *cdk-5* (white columns in C) mutant animals have mislocalized RAB3 in dendrites at 16-25°C. The mislocalization defect of *pct-1* or *cdk-5* mutant animals respectively is rescued at 25 °C, not 16 °C in early or later stage by $P_{itr\text{-}1pB}$*::intron::pct-1* (black columns in B, *wyEx2904*) or $P_{mig\text{-}13}$*::intron::cdk-5* (black columns in C, *wyEx2923*) transgenes . Error bars represent standard error of proportion. n > 100. ***, $p < 0.0005$. **, $p < 0.005$. *, $p < 0.05$. χ^2 test.

(D-E) *unc-104/kif1a(e1265wy565)* mutants have a weaker mislocalization defect than *cyy-1 cdk-5* double mutants. A *cyy-1 cdk-5* double mutant animal (D) or *unc-104/kif1a(e1265wy565)* mutant animal (E) expressing GFP::RAB-3(*wyIs85*). Asterisk below cell body. Scale bar, 10μm.

(F) Percentage of total SVPs moving in the anterograde (black) or retrograde (white) direction. While the balance between anterograde and retrograde transport is most dramatically affected in the *cdk-5 cyy-1* double mutant, either single mutant displays an intermediate phenotype significantly different from *wild type* (*wt*). The *dhc-1(js319)* allele rescues the imbalance in SVP transport in the *cdk-5 cyy-1* mutant background. ***, $p < 0.0005$; **, $p < 0.005$; *, $p < 0.05$, Fisher's exact test.

Supplemental Methods

Constructs and Transgenic Worms.

wyEx2288,9, 2776,7: a AscI-KpnI PCR fragment containing the *cyy-1* genomic sequence was subcloned into P*mig-13::yfp::unc-5 3'utr* pSM to make P*mig-13::cyy-1 stop::yfp::unc-5 3'utr*. A SphI-AscI PCR fragment containing P*itr-1 pB* was subcloned into *cyy-1stop::yfp::unc-5 3'utr* pSM. P*itr-1 pB::cyy-1stop::yfp::unc-5 3'utr* was injected at 10ng µl-1(*wyEx2288,9*) or 20ng µl-1(*wyEx2276,7)* with P*odr-1::gfp* at 20ng μl^{-1} into *cyy-1;wyIs85* or *pct-1;wyIs85* animals. The four arrays are separate lines obtained from two injections.

P*itr-1 pB* primers 5' GAAAGGGGCCGCCATCTATTCCAGAGTTCGTTCCCGAGC and 3' CTTTCCGGCGCGCCCAATTCGTGTGCTTCCACCACCAC

cyy-1 primers 5' GAAAGGGGCGCGCCATGGGAAATTCATCGTGTTGTCTG and 3' GAAAGGGGTACCCTACGAGAGAACAGCCGGATG

wyEx2506,7: an AscI-KpnI PCR fragment containing the *pct-1c* genomic sequence was subcloned into Pmig-13 delta pSM vector to make P*mig-13::pct-1c::unc-54 3'utr*. This construct was injected with P*odr-1::gfp* (both at 20ng μl^{-1}) into *wyIs85* animals. The two arrays are separate lines obtained from one injection. *pct-1c* primers 5' GAAAGGGGCGCGCCGTATACTTTCTCATTTCCATCG and 3' GAAAGGGGTACCGCATATTATCATTTTTCTGGG

wyEx2706: an AscI-KpnI PCR fragment containing the *pct-1a* cDNA obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/) was subcloned into P*itr-1 pB::yfp::unc-5 3'utr* pSM to make P*itr-1 pB::pct-1a stop::yfp::unc-5 3'utr*. This

construct was injected at 40ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *wyIs85* animals. *pct-1a* primers 5' GAAAGGGGCGCGCCATGAAGAAGCTTAAACG and 3' GAAAGGGGTACCTAAGTGTGATGACTCGAAT

wyEx2904, 2923: An AscI PCR fragment obtained from pAC13, a generous gift from M. Chalfie, containing *mec-2 intron9* was subcloned into P*itr-1 pB::pct-1a::unc-5 3'utr(wyEx2904)* or P*mig-13::cdk-5::unc-54 3'utr(wyEx2923)*. Both plasmids were injected at 10ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *pct-1; wyIs85* or *cdk-5; wyIs85* mutants. The two arrays are separate lines obtained from one injection. *mec-2 intron9* primers 5' GAAAGGGGCGCGCCCACCGCCTAAAGTGTAAGTTTTC and 3' GAAAGGGGCGCGCCGACGGTGGCTCCTCACTGAAAAC

wyEx2624,6: The *cdk-5* entry clone was obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector P*mig-13::gateway::yfp*(Klassen and Shen, 2007) and P*mig-13::gfp::gateway* using the gateway strategy with LR clonase (Invitrogen) to make P*mig-13::cdk-5::yfp* and P*mig-13::gfp::cdk-5*. The AscI-Kpn-1 fragment containing *cdk-5* was subcloned into *mig-13::unc-54 3'utr* pSM and the resulting plasmid was injected at 5ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *cdk-5; wyIs85*. The two arrays are separate lines obtained from one injection.

wyEx2286,7: P*itr-1::cdk-5::unc-54 3'utr* was injected at 10ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *cdk-5; wyIs85* animals. The two arrays are separate lines obtained from one injection.

wyEx2860,1: a AscI-KpnI PCR fragment containing the *cdka-1* genomic sequence was subcloned into P*itr-1 pB::yfp::unc-5 3'utr* pSM and P*itr-1 pB::cdka-1 stop::yfp::unc-5*

3'utr was injected at 10ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *cdka-1; wyIs85* animals. The two arrays are separate lines obtained from one injection. *cdka-1* primers 5' GAAAGGGGCGCGCCATGGGCGCAAATTTGACGTC and 3' GAAAGGGGTACCTCATTCGGAACTTGAACAATGCTTG

wyEx2774, 2160, 2789, 2460: P*mig-13::pct-1c::gfp* (cloned as *wyEx2506*, 20ng µl^{-1}) (*wyEx2774*), P*itr-1pB::cdk-5::yfp* (cloned as *wyEx2624*, 50ng µl^{-1}) (*wyEx2160*), P*itr-1pB::cyy-1::yfp* (cloned as *wyEx2288*, 50ng µl^{-1}) (co-inject with P*itr-1pB::mCherry*, 20ng µl^{-1}), P*itr-1pB::cdka-1/p35::yfp* (cloned as *wyEx2860*, 15ng µl^{-1}) were injected with P*odr-1::gfp* at 20ng µl^{-1} into into wild-type animals.

wyEx2694,5: The *unc-104* entry clone was kindly provided by Dieter Klopfenstein and inserted into the destination vector P*mig-13::gateway* to make P*mig-13::unc-104.* The resulting plasmid was injected at 50ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into *cyy-1 cdk-5; wyIs85* animals. The two arrays are separate lines obtained from one injection.

wyEx2992: a Nhe1-Kpn1 PCR fragment containing *unc-104(1-389)* sequence was subclone into *Pitr-1pB::GFP::unc-54 3'UTR pSM* to make P*itr-1 pB::unc-104(1-389)::gfp)*. This construct was injected at 20 ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into into wild-type animals. *unc-104(1-389)* primers 5' GAAAGGGCTAGCATGTCATCGGTTAAAGTAGCTG and 3' GAAAGGGGTACCGTCTCCTGTACGTCTGTGACA

wyEx3128: The *klp-16* entry clone was inserted into the destination vector P*itr-1 pB::gateway::yfp* to make P*itr-1 pB::klp-16::yfp.* The resulting plasmid was injected at 10ng µl^{-1} with P*odr-1::gfp* at 20ng µl^{-1} into wild-type animals.

wyEx2793: A SphI-AscI fragment containing P*mig-13* was subcloned into *gfp::rab-3::unc-54 3'utr* pSM and the resulting P*mig-13::gfp::rab-3* construct was injected at 4ng µl^{-1} with P*odr-1::gfp* at 40ng µl^{-1} into wild-type animals.

Electron Microscopy.

Ten animals were placed in a type-A 100µm-deep specimen carrier (BAL-TEC) filled with bacteria and then capped with the flat side of a type-B specimen carrier. The specimens were frozen instantaneously in a BAL-TEC HPM 010 high-pressure freezer (BAL-TEC, Liechtenstein) and transferred into a cryovial containing anhydrous acetone with 1% osmium tetroxide, 0.1% uranyl acetate, and 1% water. Freeze-substitutions were carried out in a Leica EM AFS2 with the following program: 48 hours at -90ºC, 5ºC/hour to -20ºC (14 hours), 16 hours at -20ºC, and 10ºC/hour to 20ºC (4 hours). The animals were then infiltrated and embedded in epon-araldite. 200-300 contiguous ultra-thin (33nm) sections were collected from regions around the anterior reflex of the gonad of multiple animals using an Leica Ultra 6 microtome. The sections were then stained with 2.5% uranyl acetate for 4 minutes and imaged on a Hitachi H-7100 electron microscope equipped with a GATAN orius CCD camera.

Temperature Shift Experiments.

pct-1(tm2175);wyIs85; wyEx2904(Pitr-1pB::intron::pct-1) and *pct-1(tm2175);wyIs85* (Figure S6B) or *cdk-5(ok626); wyIs85; wyEx2904(Pitr-1pB::intron::cdk-5)*and *cdk-5(ok626); wyIs85* (Figure S6C) animals were cultured at 16°C or 25°C for multiple generations before being shifted to a different temperature. These animals in the L3 and

early L4 larval stages were placed at 16°C for three days or at 25°C for two days before the phenotype was analyzed. Scoring was done in gravid adults.

Co-immunoprecipitation assay

The cells were lysed in lysis buffer (in mM) (HEPES, pH 7.4, 25; $MgCl_2$, 1; NaCl, 300; EDTA, 1; EGTA, 1) supplemented with 0.5% Nonidet P-40 and various protease inhibitors. The cell lysates were then incubated with anti-FLAG (Sigma) or anti-HA (Roche) antibodies (2 μg) at 4 °C for 2 hours, followed by incubation with 40 μl of protein G sepharose (GE Healthcare) at 4 °C for 1 hour. The samples were washed with lysis buffer and resuspended in SDS sample buffer. Proteins were co-immunoprecipitated and detected using Western blot analysis

In vitro kinase assay.

The cells were lysed using lysis buffer (HEPES, pH 7.4, 25mM; EGTA, 1mM; DTT, 1mM) supplemented with 0.2% Nonidet P-40 and various protease inhibitors.
The kinase assay was performed at 30 °C for 30 min in kinase buffer (20 mM MOPS, pH 7.4, 15 mM $MgCl_2$, 100 μM ATP) containing 1 μCi of [γ-^{32}P] ATP (PerkinElmer). Phosphorylated proteins were separated by 15% SDS-PAGE and visualized by autoradiography.