Zhang et al., http://www.jcb.org/cgi/content/full/jcb.201104141/DC1

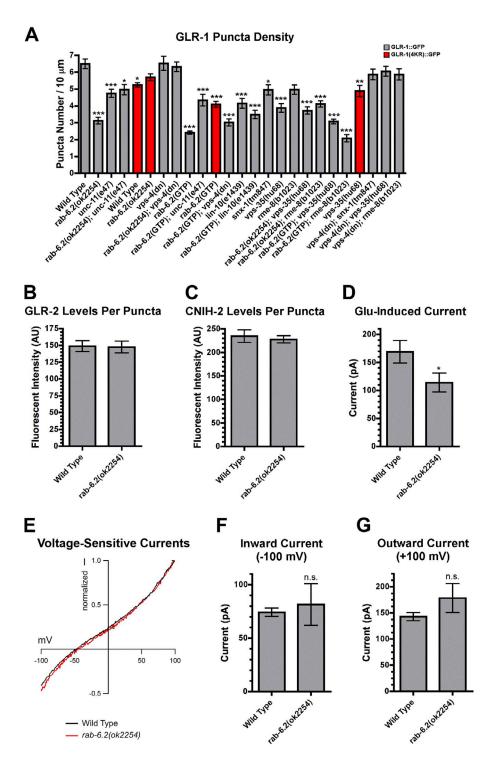


Figure S1. **Supplemental quantification data**. (A) The mean GLR-1 puncta density along the ventral cord dendrites (puncta per 10 µm dendrite length) is shown for the indicated genotypes and the indicated reporter. (B and C) The mean fluorescent intensity of puncta for GFP::GLR-2 (B) and CNIH-2::GFP (C) for the given genotypes is indicated. ANOVA followed by Dunnett's multiple comparison to wild type (\*\*\*, P < 0.001; \*\*, P < 0.01; \*, P < 0.05). n = 15–35 animals. (D) Mean peak current amplitudes for wild type and rab-6.2(ok2254) mutants. Student's t test (\*, P = 0.027). n = 8–12 animals. (E) The current-voltage relationship for representative recordings from AVA neurons from either a wild-type animal or a rab-6.2(ok2254) mutant. (F and G) Mean voltage ramp currents for –100 mV (F) and 100 mV (G) are shown. n.s. indicates no significant difference by Student's t test. AU, arbitrary unit.

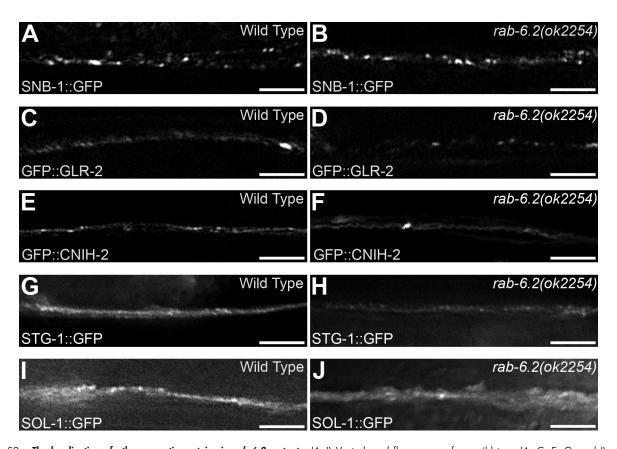


Figure S2. The localization of other synaptic proteins in rab-6.2 mutants. (A–J) Ventral cord fluorescence from wild type (A, C, E, G, and I) or rab-6.2(ok2254) mutants (B, D, F, H, and J) expressing the synaptic vesicle protein SNB-1 (synaptobrevin)::GFP (A and B), GFP::GLR-2 (C and D), GFP::CNIH-2 (cornichon; E and F), STG-1::GFP (stargazin; G and H), and SOL-1::GFP (I and J). Bars,  $5 \mu m$ .

The LIN-10 PTB Domain Interacts With Rabs		
Prey	Gene ID	Interaction
RAB-1(WT)	C39F7.4	+
RAB-1(GTP)		+
RAB-1(GDP)		+
RAB-6.1(WT)	F59B2.7	+
RAB-6.1(GTP)		+
RAB-6.1(GDP)		-
RAB-6.2(WT)	T25G12.4	+
RAB-6.2(GTP)		+
RAB-6.2(GDP)		-
RAB-8(WT)	D1037.4	-
RAB-8(GTP)		+
RAB-8(GDP)		+
RAB-10(WT)	T23H2.5	-
RAB-10(GTP)		-
RAB-10(GDP)		-
RAB-11.1(WT)	F53G12.1	-
RAB-14(WT)	K09A9.2	+
AEX-6(WT)	Y87G2A.4	-
RAB-21(WT)	T01B7.3	-
RAB-28(WT)	Y11D7A.4	-
RAB-30(WT)	Y45F3A.2	-
RAB-33(WT)	F43D9.2	-
RAB-35(WT)	Y47D3A.25	-
RAB-37(WT)	W01H2.3	-
RAB-39(WT)	D2013.1	-
K02E10.1	K02E10.1	-
F11A5.4	F11A5.4	-
F11A5.3	F11A5.3	-
Y71H2AM.12	Y71H2AM.12	-

Figure S3. The LIN-10 PTB domain interacts with RAB-6.2 in a yeast two-hybrid assay. The table lists yeast two-hybrid interaction data using the LIN-10 PTB domain as bait and the indicated Rab small GTPase as prey. WT indicates the wild-type version of the protein. GTP indicates the GTP-locked mutant version of the protein. GDP indicates the GDP-locked mutant version of the protein. The plus sign indicates interaction as scored by both growth on selection media and the presence of  $\beta$ -galactosidase activity. The minus signs indicate no interaction as scored by failed growth on selection media and the absence of  $\beta$ -galactosidase activity.

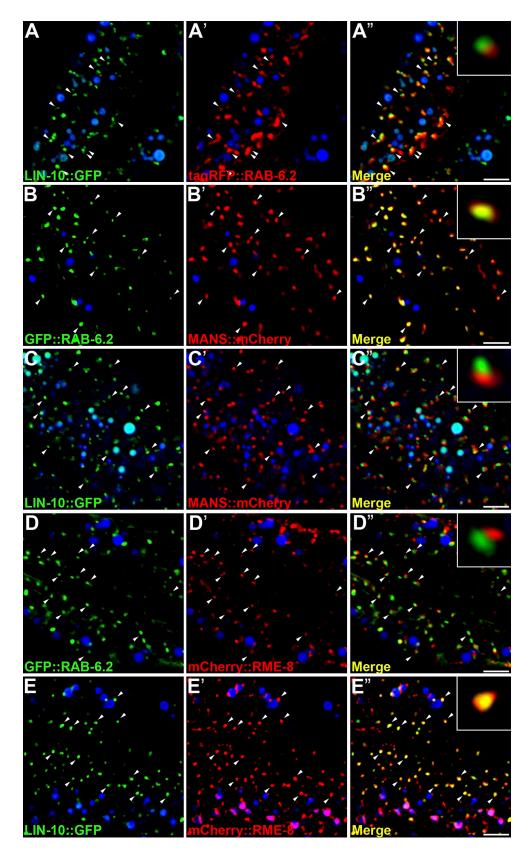


Figure S4. RAB-6.2 is localized to Golgi in intestinal epithelial cells. GFP-, tagRFP-, or mCherry-tagged proteins in intestinal epithelial cells. (A–E′) LIN-10:: GFP (A, C, and E), GFP::RAB-6.2 (B and D), tagRFP::RAB-6.2 (A′), MANS::mCherry (B′ and C′), and mCherry::RME-8 (D′ and E′). Intestinal autofluorescent lysosome-like organelles are shown in the DAPI channel (blue). Bona fide fluorescence from GFP- or mCherry-tagged marker proteins was identified as fluorescence acquired in the green or red channels that also did not overlap with fluorescence acquired in the DAPI channel. Arrowheads indicate colocalization. Bars, 5 µm. Typical puncta for the indicated genotype are magnified in each inset.

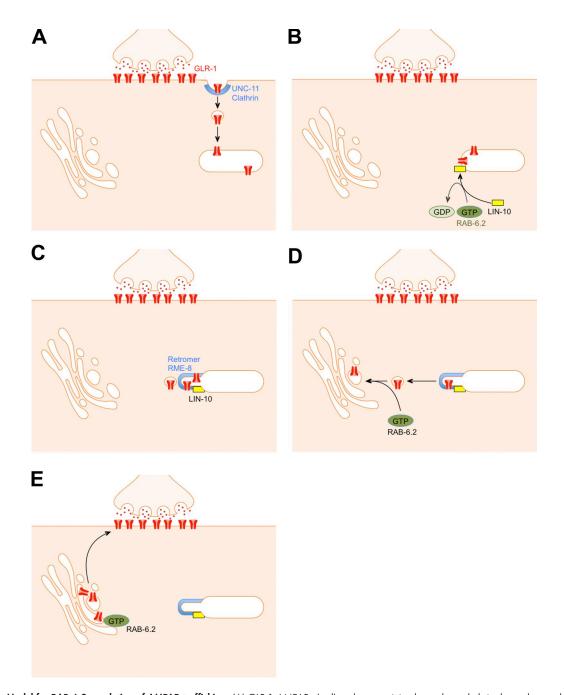


Figure S5. Model for RAB-6.2 regulation of AMPAR trafficking. (A) GLR-1 AMPARs (red) undergo activity-dependent, clathrin-dependent endocytosis in a process that requires UNC-11/AP180 (blue). (B) RAB-6.2 (green), in its GTP-bound form, interacts with LIN-10 (yellow), delivering LIN-10 to early endosomes. (C) LIN-10, along with the retromer complex and RME-8 (blue), sequester AMPARs into endosomal tubules that in turn give rise to retrograde cargo vesicles for the receptors. (D) RAB-6.2, in its GTP-bound form, regulates the trafficking of these cargo vesicles to Golgi, including dendritic outpost Golgi as well as cell body Golgi. (E) Once at Golgi, the receptors can affiliate with new coreceptors and be resorted back to synaptic membranes.