

Supplemental Figure S1: (A) Immunoblot analysis of third instar larval homogenate using anti-Arfip antibody demonstrates that D42-Gal4 can drive the expression of Arfip from the *arfip*^{P1} insertion resulting in increases Arfip protein level by ~50-60% compared to wild type. Graph represents the quantification of Arfip protein normalized to actin of the indicated genotypes. (B) Immunoblots of co-immunoprecipitation analysis from S2 cells co-expressing FLAG-DNGLued and HA-Arfip fusion proteins incubated with anti-FLAG antibody coated beads. Inclusion of a competing FLAG peptide during incubation with beads completely abolished binding of Arfip-HA. Cell lysate of S2 cells co-expressing FLAG-DNGLued and HA-Arfip is shown as input. (C) Immunoblots of co-immunoprecipitation analysis from S2 cells co-expressing Arfip^{BAR}-FLAG and Arf6-HA fusion proteins incubated with anti-FLAG antibody coated beads. Inclusion of a competing FLAG peptide during incubation with beads completely abolished binding of Arf6-HA. Analysis of S2 cells expressing only Arf6-HA showed very low levels of non-specific binding to FLAG beads. Cell lysate of S2 cells co-expressing Arfip^{BAR}-FLAG and Arf6-HA or only Arf6-HA shows equivalent amount of protein materials added in each co-immunoprecipitation experiment.

Supplemental Movie S1: Live imaging of axonal transport of syb-GFP vesicles in wild type larvae. Representative movie of axonal tracts from a *OK6/UAS-syb-GFP; +/+* larvae. The larval VNC is located to the left of the movie.

Supplemental Movie S2: Live imaging of axonal transport of syb-GFP vesicles in *arfip*^{12/71} mutant larvae. Representative movie of axonal tracts from a *OK6/UAS-syb-GFP; arfip*^{12/71} larvae. The larval VNC is located to the left of the movie.

Supplemental Material

Table S1: Molecular and genetic information for inserts altering synapse growth

Insertion Genotype	Mutant Type ¹	Mutant Class ²	Insert ³	Predicted Gene ⁴	Predicted function ⁵
Enhancers¹					
<i>P{EP}CG17184^{EY11874}</i>	E2	L/G	5' UTR	<i>CG17184</i>	Membrane trafficking
<i>P{XP}CG17184^{d04252}</i>	E2	L	3' UTR	<i>CG17184</i>	Membrane trafficking
<i>P{EP}EY12448</i>	E2	n.d.	n.d.	none	n.a.
<i>PBac{PB}fan^{c04756}</i>	E2	L/G	5'	<i>fan</i>	vesicle-associated
<i>P{EP}EY20330</i>	E2	G	5'	<i>CG13813</i>	Kinase activity
<i>P{EP}klar^{EY01576}</i>	E1	L	CDS	<i>klar</i>	MT transport
<i>P{EP}bbg^{EY05191}</i>	E1	L	CDS	<i>bbg</i>	Disc development
<i>PBac{PB}Sirt2^{c03323}</i>	E1	L/G	5' UTR	<i>Sirt2</i>	Histone deacetylase
<i>P{EP}EY06888</i>	E1	n.d.	n.d.	none	n.a.
<i>P{EP}GRHR^{EY11371}</i>	E1	L/G	5' UTR	<i>GRHR</i>	Peptide receptor
Suppressors¹					
<i>P{EP}nesd^{EY11086}</i>	S3	L/G	5' UTR	<i>nesd</i>	CHO binding
<i>P{EP}Coop^{EY13293}</i>	S3	L/G	5' UTR	<i>Coop</i>	RNA transcription
<i>P{EP}CG42575^{EY04050}</i>	S2	L/G	5' UTR	<i>CG42575</i>	Transporter Activity
<i>P{EP}B4^{EY14645}</i>	S2	L	Intron	<i>B4</i>	Disc development
<i>P{EP}CG4562^{EY09703}</i>	S1	L/G	5' UTR	<i>CG4562</i>	Transporter Activity
<i>P{EP}TM9SF4^{EY00960}</i>	S1	L/G	5' UTR	<i>CG7364</i>	Transporter Activity

All data collected from P-element screen consisting of the indicated single copy insertion in trans to the *D42*, *DN^{Glued}* recombinant chromosome. Insertion genotypes obtained from Flybase.org.

¹– Refers to the effects of inserts on viability when in trans to *D42*, *DN^{Glued}* at 25C. Enhancers (E) *enhance* the effects of the *D42*, *DN^{Glued}* allele on viability, and suppressors (S) have the opposite effects on viability. 1= weakest, 3= strongest. The lethality due to one copy of the *D42*, *DN^{Glued}* is ~30%. Note that E2 = 100% lethality and E1 = < 60% lethality. S3 = 100% viability.

²–Predicted mutant class of insertion based on P-element type and location of insertion. L equals loss-of-function and G equals gain-of-function. Because many of the P-elements landed in 5' UTR, inserts could be either L or G (L/G).

³– Refers to the position of the insert in the reported gene based on genetic information provided by Flybase.org. UTR=untranslated mRNA, CDS=coding sequence of mRNA. 5' refers to insertion site relative to gene.

⁴– Candidate genes predicted by the proximal location of insert and genes determined from the physical map data present at Flybase.org for each insert. # indicates insert location verified by iPCR.

⁵– Gene function data obtained from Flybase.org for gene listed in predicted gene column.

Table S2: Syb-GFP vesicle axonal transport analyses

Genotype¹	n²	% particles moving³	Axonal Density⁴
wt	43 (8)	55.8 (76/136)	0.0673 (0.0037)
<i>arfip</i> ^{12/71}	56 (11)	57.6 (90/156)	0.0586 (0.0028)

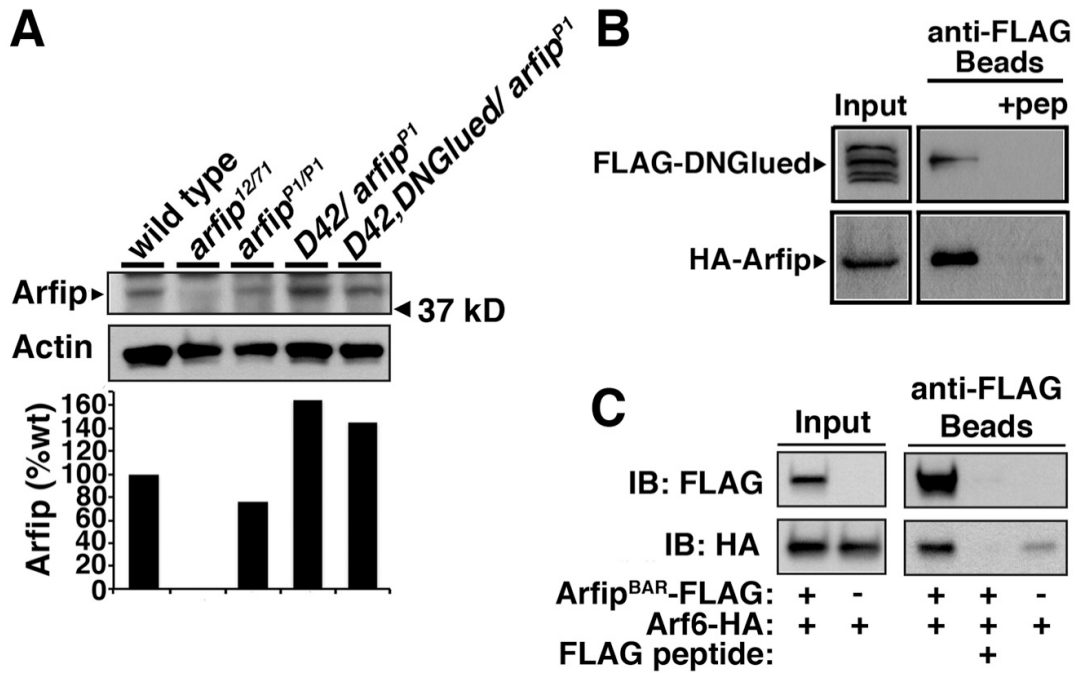
¹-Refers only to the genotype of 3rd chromosome, both genotypes have the following 2nd chromosome genotype: *OK6-Gal4/UAS-syb-GFP*.

²-Indicates the total number of axons analyzed for movement and density. The number of animals analyzed is presented within the parenthesis.

³-Represents the percentage of particles within a given region of interest that move in either direction during acquisition.

⁴-Represents the average number of syb-GFP transport vesicles per length of axon (particle/um). Values are not significantly different between wt and *arfip* mutants.

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