

A

MTVQPAEMAENGSRVDPVTA SPGRAPPGLPANQMPAMGNQQHHGNQQHH
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 NKFNSLP RPRPGTRLQGIRSRMGDRLVDENDGNTSAPEFDVRRGRDLRQR
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 NSYD FYTSTSTVSSSRPFANGGNNYHTLGTYRAQYAAATNPFLDAFDEKP
 GSNNGNAHGEEKLGADKGGHRAAVMAFQ**SGDSKNGSDEYDDIK**

B

Protein	Mass (D)	Score
gil6094287; CG5406	230344	113/787
Still life		
gil7302107; CG1976-PA	195330	296/265
RhoGAP100F (DSYD-1)		
gil54641571; GG11661	112442	162/341
Neural conserved at 73F		
gil6066229; CG1975	51979	326/226
Drep2		

Figure S1. **MS/MS identification of DSYD-1.** (A) Amino acid sequence of DSYD-1, with the peptides identified in BRP coimmunoprecipitations via MS/MS highlighted in red. LD28013 of *dsyd-1* is indicated in gray. The peptide comprising the DSYD-1 antibody epitope is highlighted in blue. (B) Proteins found to coprecipitate with BRP in two independent approaches.

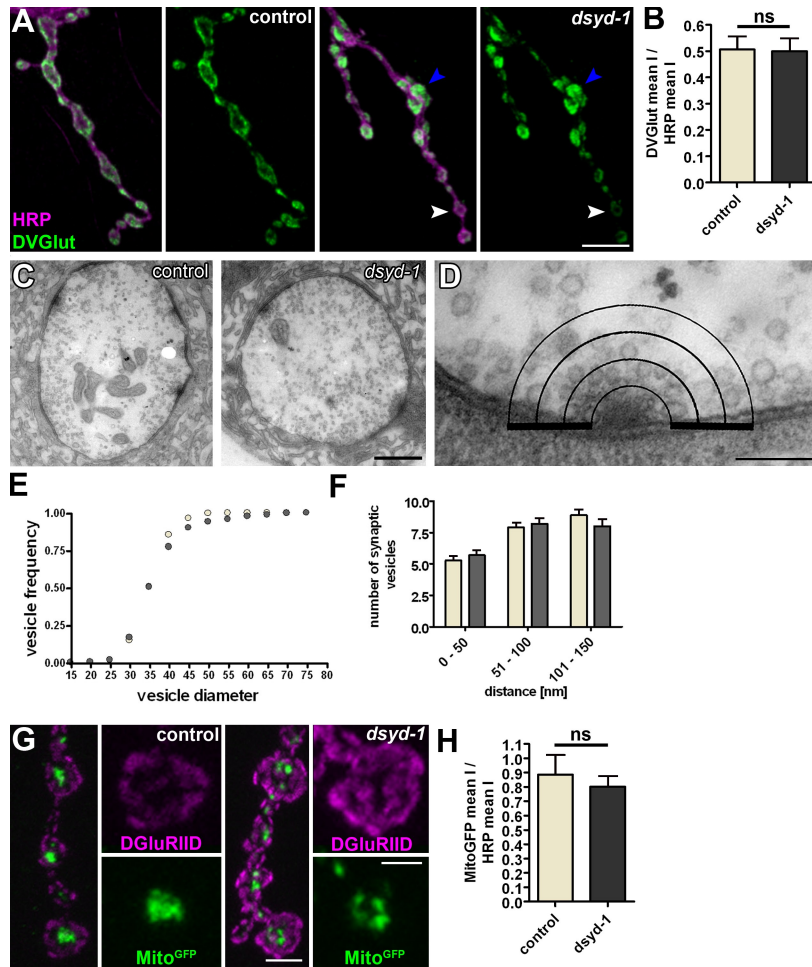


Figure S2. **SV distribution and size is mildly affected in *dsyd-1*.** (A) HRP and DVGlut costaining of control and *dsyd-1* NMJs. Mean DVGlut intensity is comparable between mutant and control. Bar, 5 μ m. (B) Control: 0.51 ± 0.05 , $n = 7$; *dsyd-1*: 0.50 ± 0.05 , $n = 10$, $P = 0.88$ according to a Mann-Whitney test. DVGlut distribution seems to be inhomogeneous in *dsyd-1* mutants (A; blue arrowhead indicates bouton with strong reactivity, white arrowhead indicates bouton with weak reactivity). (C–F) An ultrastructural analysis (bouton overview shown in C). The relative cumulative frequency of vesicle diameter in *dsyd-1* mutants and controls is comparable (E), as is the SV distribution surrounding the T bar at AZs (D and F). (G) Co-labeling for MitoGFP and DGluRIID. (H) The mean intensity of MitoGFP is not altered significantly (control: 0.89 ± 0.14 , $n = 10$; *dsyd-1*: 0.80 ± 0.08 , $n = 10$, $P = 0.97$ according to a Mann-Whitney test). Error bars indicate the SEM. ns, $P > 0.05$. Bars: (A) 5 μ m; (C) 700 nm; (D) 150 nm; (G) 1 μ m; (G, insets) 500 nm.

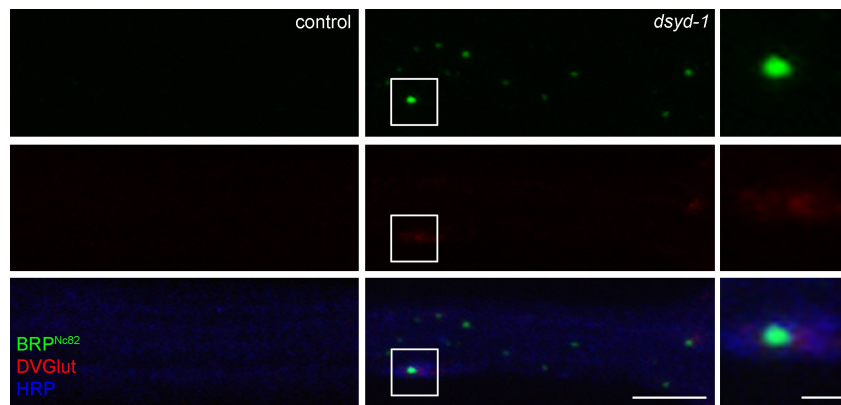


Figure S3. **Ectopic BRP and DVGlut colocalizing in *dsyd-1* axon.** Some axonal BRP-positive spots are also positive for the SV marker DVGlut in *dsyd-1*. Panels on the right show enlarged views of the boxed regions. Bars: (left) 3 μ m; (right) 500 nm.