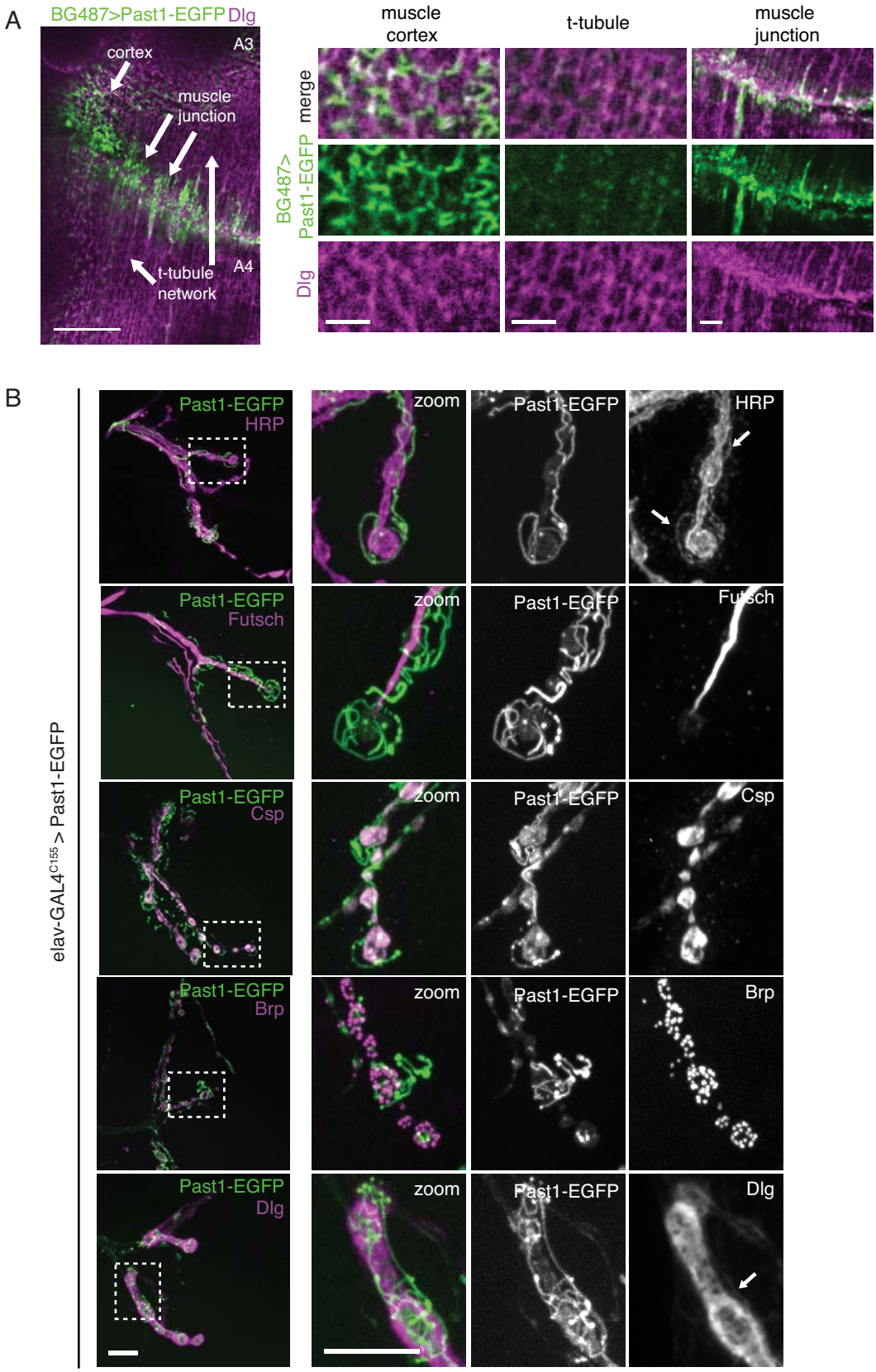
Supplemental Materials

Molecular Biology of the Cell

Koles et al.


A
BG487>Past1-EGFP Dlg
cortex
A3
muscle junction
t-tubule network
A4
muscle cortex
t-tubule
muscle junction
merge
BG487> Past1-EGFP
Dlg
B
elav-GAL4C155 > Past1-EGFP
Past1-EGFP HRP
Past1-EGFP Futsch
Past1-EGFP Csp
Past1-EGFP Brp
Past1-EGFP Dlg
zoom
Past1-EGFP
HRP
Futsch
Csp
Brp
Dlg

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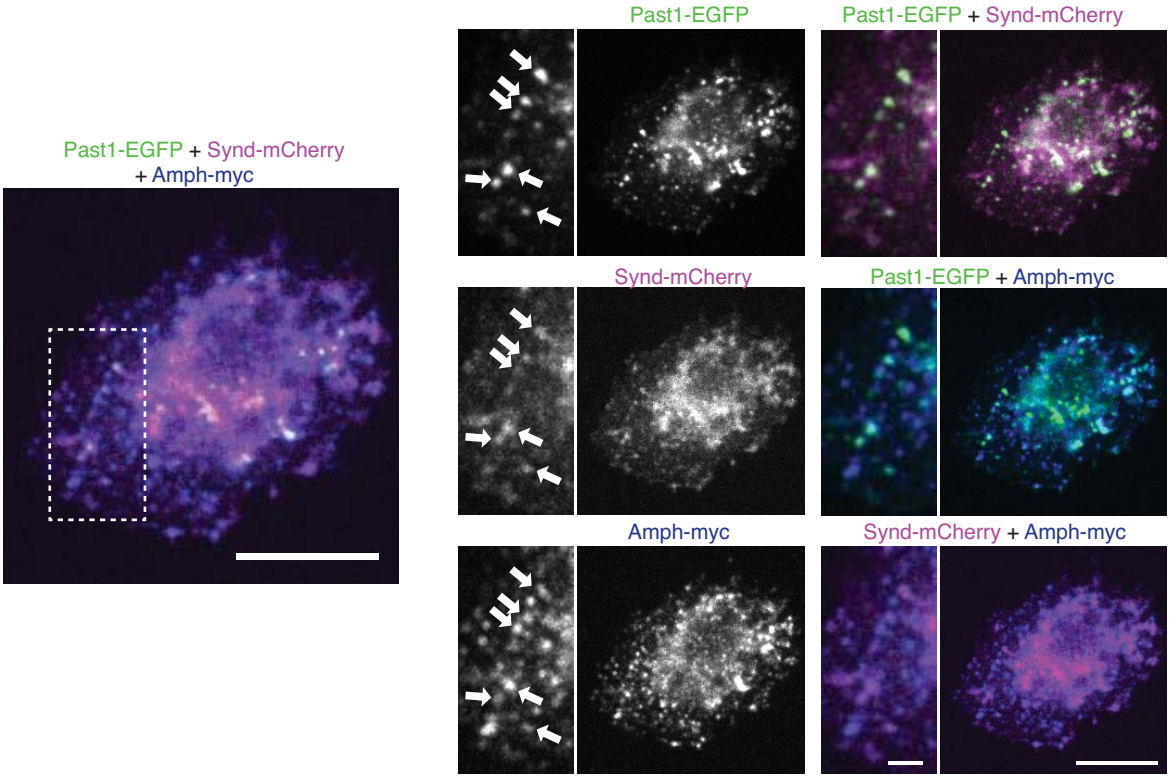

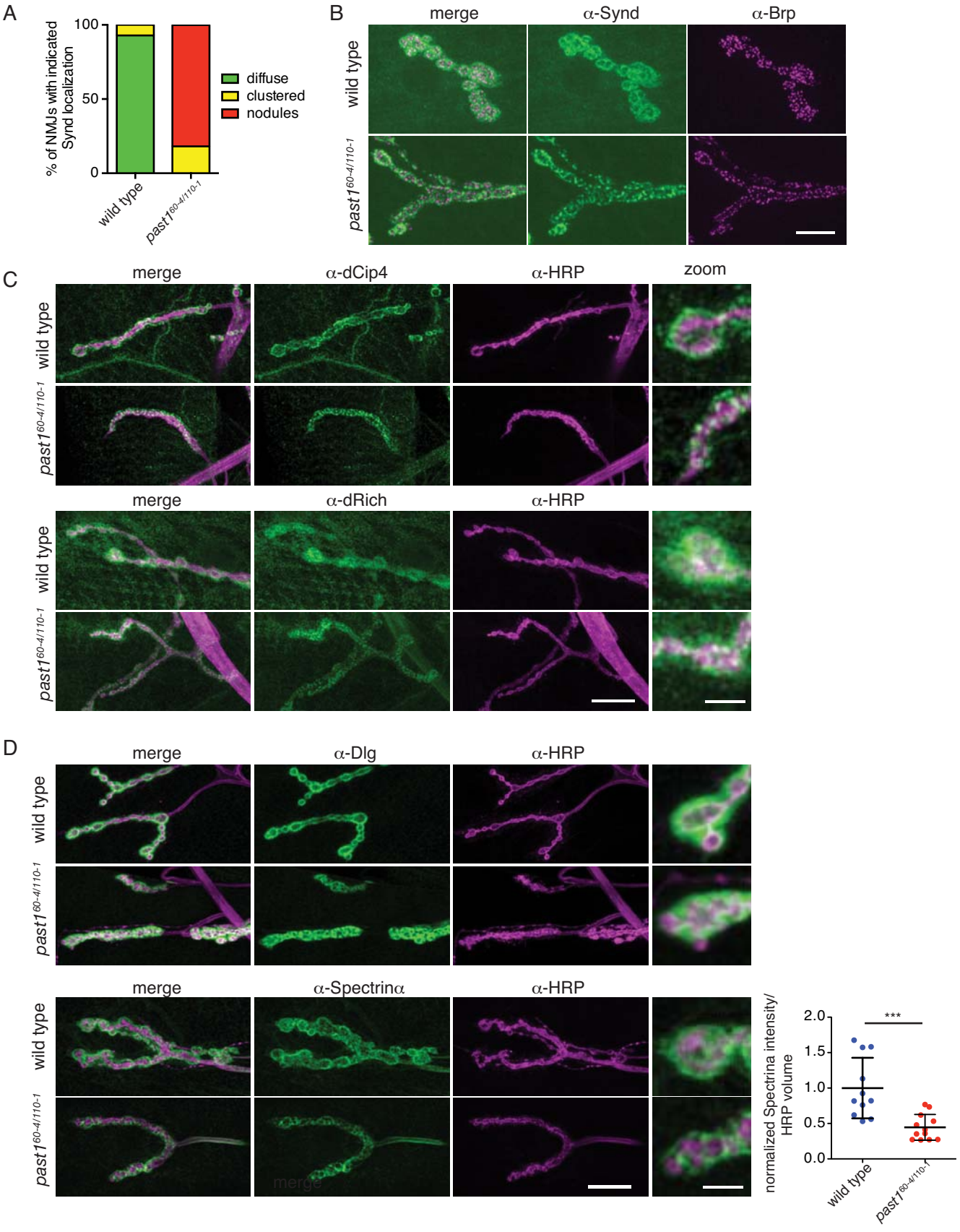
A
% of NMJs with indicated Synd localization
100
50
0
diffuse
clustered
nodules
wild type
past1 60-4/110-1
B
merge
α-Synd
α-Brp
wild type
past1 60-4/110-1
C
merge
α-dCip4
α-HRP
zoom
wild type
past1 60-4/110-1
merge
α-dRich
α-HRP
wild type
past1 60-4/110-1
D
merge
α-Dlg
α-HRP
wild type
past1 60-4/110-1
merge
α-Spectrinα
α-HRP
wild type
past1 60-4/110-1
normalized Spectrina intensity/ HRP volume
2.0
1.5
1.0
0.5
0.0
***
wild type
past1 60-4/110-1

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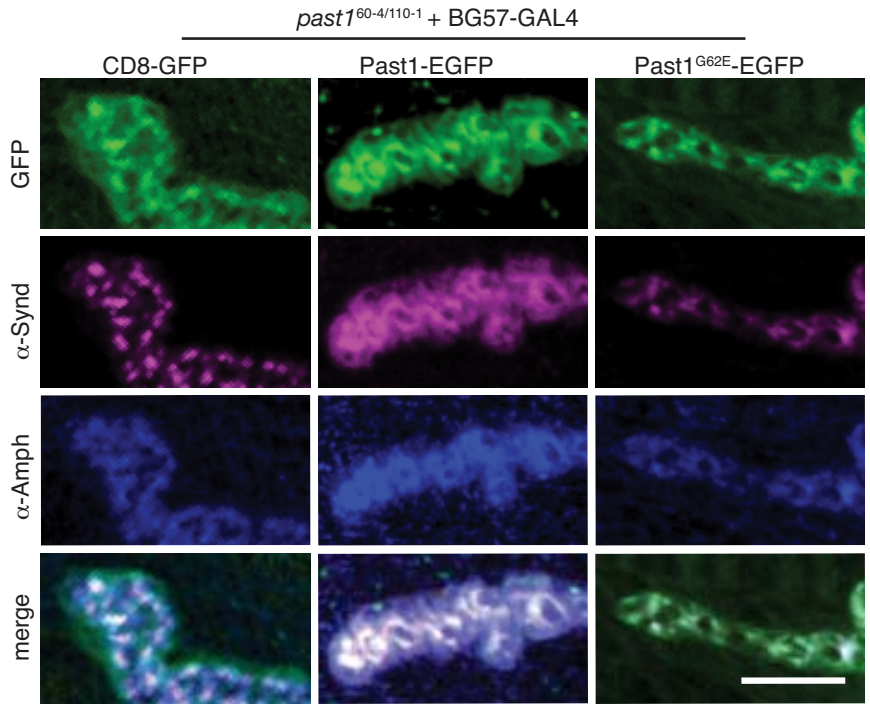

Table S1

	Segment A3, muscle 4	
	w (wild type)	*past1*$^{60\text{-}4/110\text{-}1}$
Number of NMJs	24	23
Mean bouton number (s.e.m.)	57.2 (3.8)	39.6 (3.9)
p value vs wild-type (t-test)		0.0022
Mean muscle area (1000 μm^2) (s.e.m.)	52.8 (2.8)	46.3 (2.1)
p value vs wild-type (t-test)		0.07
	Segment A3, muscle 6/7	
	w (wild type)	*past1*$^{60\text{-}4/110\text{-}1}$
Number of NMJs	24	20
Mean bouton number (s.e.m.)	87.2 (6.0)	66.8 (5.1)
p value vs wild-type (t-test)		0.0147
Mean muscle area (1000 μm^2) (s.e.m.)	78.1 (2.7)	66.8 (3.1)
p value vs wild-type (t-test)		0.0082

SUPPLEMENTARY LEGENDS

Figure S1. (A) Localization of endogenous Past1 and Past1-EGFP to the muscle cortex and muscle-muscle attachment sites, but not to t-tubules. Larvae expressing Past1-EGFP (green) under the control of the BG487-GAL4 muscle driver (which has highest expression in the most anterior segments) were stained with α-Dlg antibodies (magenta). Left image shows a single 60x spinning disk confocal slices of segment A3 and A4, muscles 6 and 7, optically sectioned at an angle through the muscle to demonstrate cortex, t-tubules, and muscle junctions. Right images show enlarged views of each structure. Scale bars are 20 µm (left) or 5 µm (right). (B) Presynaptic expression ($elav^{C155}$-GAL4) of Past1-EGFP induces extrasynaptic membrane tubules. Images show 2D projections of 100X spinning disk confocal stacks of NMJs on muscles 12 and 13 from $elav^{C155}$; +; UAS-Past1-EGFP/+ larvae, labeled with Past1-EGFP (green) and the indicated antibodies (magenta). Extrasynaptic tubules do not contain Futsch, Csp, or Brp, but do contain α-HRP-positive presynaptic membrane and some Dlg (arrows). Scale bars are 10 µm.

Figure S2. Past1, Amph and Synd co-localization in heterologous cells. Expression of Past1-EGFP, Synd-mCherry, and Amph-A in S2 cells spread on Concanavalin A. Images show single 100X confocal slices ~1 µm from the cell-coverslip interface from representative cells. Scale bars in large images are 10 µm; scale bar in magnified views to the left of large images (representing area in dashed rectangle) is 2 µm.

Figure S3. Postsynaptic organization in *past1* mutant NMJS. (A) Blind quantification of Synd localization from maximum intensity projections of NMJs, in wild type (n=14) and *past1* mutants (n=11). (B) Synd nodules in *past1* mutants do not co-localize with active zones. Images show maximum intensity projections of 100X confocal stacks from representative muscle 4 NMJs labeled with the indicated antibodies. Scale bar is 10 µm. (B) dCip4 and dRich localize to nodules in *past1* mutants. Images show maximum intensity projections of 60X confocal stacks from representative muscle 4 NMJs labeled with the indicated antibodies. Scale bar is 20 µm in large images and 5 µm in magnified images. (C) Dlg and spectrin are misorganized in *past1* mutants, and spectrin levels are reduced. Scale bar is 20 µm.

Figure S4. Past1 ATP-binding is required to rescue Synd nodule formation. (A) Rescue of Synd nodules by muscle (BG57-GAL4) re-expression of Past1-EGFP but not mCD8-GFP or $Past1^{G62E}$-EGFP. Images show maximum intensity projections of 60X confocal stacks from a representative muscle 4 NMJs labeled with the indicated antibodies. mCD8-GFP is expressed more highly than Past1-EGFP and is therefore shown at a lower intensity to visualize localization. Scale bar is 10 µm.

Table S1. Bouton number and muscle area measurements for wild type and *past1* mutants.