Supplemental information

Activation and expansion of presynaptic signaling foci drives presynaptic homeostatic plasticity

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Supplemental Figure 1, related to Figures 1 and 2. Non-normalized values for electrophysiology data.

A) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 1F. No significant changes (absence of curarine) were observed between conditions with the exception of RGD application that slightly potentiates baseline quantal content compared to control (p < 0.05). Regarding this difference, we note that RGD peptides are not as specific to ITGB1 as compared to the antibodies used in this experiment. B) Cumulative EPSC values for data presented in main Figure 1G and H. C) Fluorescence intensity of ab 9EG7 (recognizes activated ITGβ1) normalized to NMJ synaptic area of indicated genotypes and treatments related to main Figure 2G. D) Representative images for wild type NMJ in the presence and absence of curarine as indicated, stained with antibodies as indicated. Different NMJ are shown plus minus curarine. Right: Fluorescence intensity of ab AIIIB2 (anti-ITGβ1) normalized to synaptic area of wild type NMJs, -/+ Curarine, related to main figure 2G. Synaptic area in C and D defined by AChR receptor fields labeled with Bungarotoxin conjugated to a fluorophore. Significance determined by Mann-Whitney. E) Representative voltage clamp traces recorded from NMJs of the indicated genotypes, with and without EGTA-AM (50 µM) treatments, as indicated. F) Quantification of paired pulse ratios (EPC4/EPC1) of indicated genotypes and treatments. G) Paired pulse ratio of each EPC within a train of the indicated genotypes and treatments. H) Representative traces recorded from the NMJs of indicated genotypes and treatments. I-K) Quantification mEJC and EJC parameters: mEPC decay Tau (I), EPC width half max (J) and EPC time to peak (K). Note: panels E-K are related to characterizing synaptic transmission of ITGβ1 cKO mutants in main Figure 2C-E. NMJs were stimulated at 100 Hz for paired pulse ratio assay. Averaged data represent mean ± SEM. Significance (unless otherwise indicated) determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings performed at 2 mM [Ca2+]c.
Supplemental Figure 2, related to Figure 2. Non-normalized values and imaging.

**A)** Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 3C.  
**B)** Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 3I.  
**C)** Structured Illumination Microscopy (SIM) single confocal plane of endogenous MysGFP protein trap (green) and the presynaptic membrane marker anti-HRP (magenta), regions of co-localization are highlighted (arrow heads). Image is a single plane taken from projection image shown in main Figure 3A. Scale bar 5 µm.  
**D)** Data for mEPSP and quantal content underlying the normalized values presented in main Figure 3G.  
**E)** Data for quantal content underlying the normalized values presented in main Figure 3K, L.  
**F)** Representative images and quantification of co-localization of Mys-GFP (endogenously tagged) and anti-Mys antibody staining, shown in main Figure 3A (top right panels). Twelve synapses from three animals were analyzed for co-localization quantification. Scale bars represent 5 µm. Averaged data represent mean ± SEM.  
Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings at 0.3 mM [Ca2+]e.

**Note:** See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

**Panel A)** mEPSP: control vs. mys<sup>b8</sup> (p=0.002). mEPP control vs. mys<sup>142</sup> (p<0.001)  
**Panel A)** EPP: control vs. mys<sup>142</sup> (p=0.04).  
**Panel A)** Quantal content: no differences between control and experimental genotypes.
Supplemental Figure 3, related to Figure 3. Non-normalized values.

A) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 3D. B) Representative traces for indicated genotypes in the absence and presence of PhTx (see main Figure 3D). C) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 3E. Muscle RNAi causes a significant decrease in EPSP and quantal content (p<0.01), but a significant homeostatic modulation of quantal content persists. Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings at 0.3 mM [Ca2+].

Note: See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes in the absence of PhTx. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

Panel A) mEPSP: control vs mys1/42 (p<0.01) vs mys RNAi 1 (p=0.003) vs mys RNA2i (p<0.01) vs muscle rescue (p<0.01)
Panel A) EPSP: control vs mys1/42 (p=0.003) vs mys RNAi 1 (p=0.002) vs mys RNA2i (p=0.005)
Panel A) Quantal Content: no significant differences
Panel C) mEPSP: no significant differences
Panel C) EPSP: control vs MN RNAi 1 (p<0.01) control vs MN RNAi 2 (p<0.01)
Panel C) Quantal Content: control versus MN RNAi 1 (p<0.01) control versus MN RNAi 1 (p=0.01)
Supplemental Figure 4, related to Figure 4. Anatomical analysis of NMJ growth.

A) Images of control and mys mutant Drosophila NMJs stained with postsynaptic DLG (magenta) and presynaptic BRP (green). Images acquired using super-resolution structured illumination microscopy (SIM) to optimally visualize and quantify BRP puncta. Inset shows that mys mutant BRP appear qualitatively similar to wild type. Scale bar is 5 µm; inset 1 µm.

B) Average data for bouton number per NMJ for each indicated genotype, at muscles 6/7, abdominal segment A2. There is a small reduction in bouton number in the mys LOF mutant and no change in the GOF allele.

C) Average data for active zone number, determined by semi-automated quantification of all individual BRP puncta per NMJ at muscles 6/7, abdominal segment A2. There are no significant differences.

D) Representative images of synaptic boutons at muscle 6/7, abdominal segment A2, intended to show the integrity of individual synaptic boutons and BRP puncta (active zone) organization within boutons. Only the mysS196A heterozygous mutant (CRISPR KI) shows evidence of anatomic dis-organization.

E) Average data for bouton number per NMJ for each indicated genotype, at muscles 6/7, abdominal segment A2. Although all of the represented mys mutations block expression of PHP, only two reduce bouton numbers, thereby separating integrin-related function during synaptic development from integrin related activity during the acute induction of PHP.

F) Average data for active zone number, determined by semi-automated quantification of all individual BRP puncta per NMJ at muscles 6/7, abdominal segment A2. Again, every mutant blocks PHP, but only a single mutant affects active zone number. Finally, we note that the semi-automated quantification of active zone number is statistically similar whether we utilize SIM (A-C) or standard deconvolution confocal microscopy (D-F). Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.
Supplemental Figure 5, related to Figures 3 and 5. Non-normalized values and protein localization.

A) Data for mEPSP and quantal content underlying the normalized values presented in main Figure 5D. There are no significant differences in the absence of PhTx comparing experimental genotypes to control. B) Data comparing synaptic mys transgene expression to endogenous mys expression (mys^{GFP} CRISPR KI). Data related to main Figure 3A and 5A-C. C and D) Data for mEPSP, EPSP and quantal content related to the genetic interaction data presented main Figure 5D. There are no significant differences in the absence of PhTx comparing experimental genotypes to control. E) Representative images and (G-I) quantification of wild type and mutant mys expression (CRISPR KIs and Ubi transgenes) by western blot (G), quantified in (H), and immuno-staining at the NMJ quantified (Mys intensity normalized to HRP area) related to main figure 3J-L. Scale bar represents 5 µm. Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings at 0.3 mM [Ca^{2+}]e.

Note: See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes in the absence of PhTx. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

Panel A) No significant differences among baseline mEPSP or Quantal Content.
Panel C) No significant differences among mEPSP, EPSP or Quantal Content averages.
Supplemental Figure 6, related to Figure 5A and B. Non-normalized values and image quantification.

A) Representative image of PlexB Myc tagged protein (green) localization when expressed in the presynaptic terminal (magenta). This UAS-Myc PlexB transgene was driven pre-synaptically by OK371-Gal4. B) Representative images (single plane) of PlexB-Flag tagged protein (green) localization in the PlexB FLAG protein trap. PlexB-FLAG localizes within the NMJ (arrows), defined by DLG staining (magenta). C) PlexB-FLAG synaptic puncta are not observed in control NMJs (single plane images). The control genotype is a wild type animal treated with the same histological protocol. D) Western blot of cell lysate from Drosophila larval brains for indicated genotypes. Actin serves as a loading control. E) Quantification of PlexB puncta per synaptic bouton in the PlexB-FLAG protein trap line (black bar graph) compared to Myc-PlexB tagged protein (green) when over expressed in the presynaptic neuron (OK371-Gal4). F-I) Raw and normalized data for mEPSP, EPSP and quantal content for indicated genotypes. Note that transgene expression (UAS-PlexB V5 and UAS-PlexBΔsema V5) is driven by the OK371-Gal4 driver. Scale bars represent 5 μm. Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings at 0.3 mM [Ca2+]e.

Note: See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes in the absence of PhTx. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

Panel F) No significant differences among baseline.
Panel G) No significant differences among baseline.
Panel H) No significant differences among baseline.
Supplemental Figure 7, related to Figures 6 and 7. Non-normalized values and protein localization data.

A) Representative traces related to main Figure 6F. B) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 6F. C) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 7D. EPSP amplitudes in the absence of PhTx are not significant (p=0.077). D) Data underlying the normalized values presented in main Figure 7C,D. E) Data for mEPSP, EPSP and quantal content underlying the normalized values presented in main Figure 6G. No significant differences are observed for mEPSP, EPSPs or quantal contents comparing (in the absence of PhTx) controls with experimental genotypes. F) Representative images of synaptic Talin protein localization for indicated KI and Ubi transgenes, related to main figure 7. G) Schematic of Talin GFP KI and Ubi transgenes, indicating where the protein is tagged for images in F. H) Quantification of synaptic Talin protein localization for indicated KI and Ubi transgenes in F, related to main figure 7. Scale bars represent 5 µm. Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant. Recordings at 0.3 mM [Ca2+]e.

Note: See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes in the absence of PhTx. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

Panel B) No significant differences among baseline.
Panel C) No significant differences among baseline.
Panel E) No significant differences among baseline.
A trend is apparent for EPSP: MN Gal4 vs Talin RNAi (p=0.07)
Supplemental Figure 8, related to Figure 8. Non-normalized values for genetics and PHP induction.

A) Data for EPSP amplitudes underlying the normalized values presented in main Figure 8B.

B) Western blot of Multiplexin (Anti-GFP) and ES (Anti-GFP) protein from immunoprecipitation of Mys (Anti-Mys, Ab CF.6G11).

C) Average mEPSP, EPSP and quantal content for recordings of indicated genotypes related to data presented in main Figure 8H, I. Data are averages of 10 NMJ recordings from 10 animals per genotype. Averaged data represent mean ± SEM. Significance determined by One-way Anova with Tukey correction for multiple comparisons: *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

Recordings at 0.3 mM [Ca2+]e.

Note: See below for additional statistically significant difference, not indicated on the figure, comparing control to experimental genotypes in the absence of PhTx. Tests were performed according to One-way Anova with Tukey correction for multiple comparisons.

Panel A) No significant differences among baseline.