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Supplementary Materials for

Neuronal activity drives FMRP- and HSPG-dependent matrix metalloproteinase function required for rapid synaptogenesis

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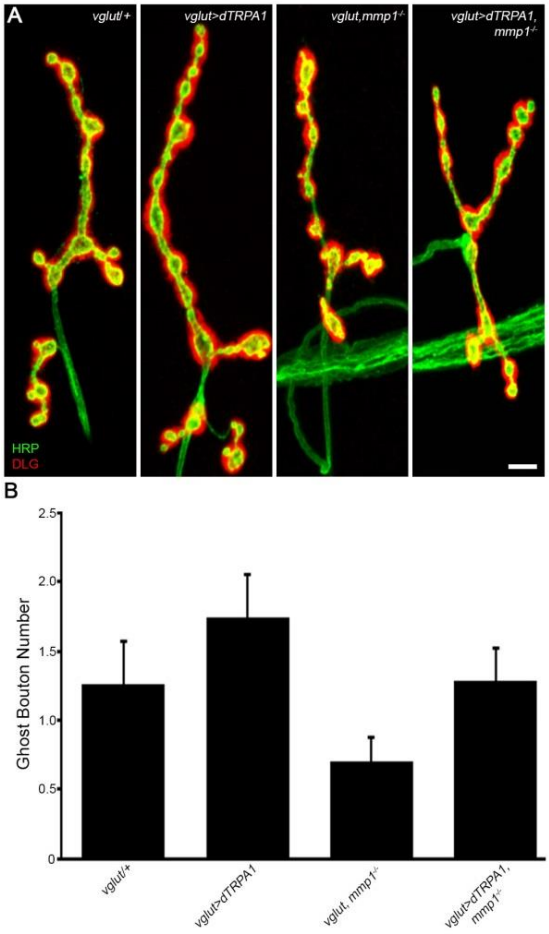

Fig. S1. Temperature controls for dTRPA1 activity-induced synaptic bouton formation. (A) Animals from the 4 genotypes shown were raised at 18°C to the wandering third instar stage, and then the NMJs were co-labeled for presynaptic HRP and postsynaptic DLG. Scale bar: 5 μm. **(B)** Quantification of ghost bouton number: *vglut-Gal4*/+ (*n*=23, 1.26±0.31), *vglut-Gal4>UAS-dTRPA1* (*n*=35, 1.74±0.31), *vglut-Gal4,mmp1*^{Q112*}/*mmp1*^{Q112*} (*n*=34, 0.71±0.17) and *vglut-Gal4>UAS-dTRPA1,mmp1*^{Q112*}/*mmp1*^{Q112*} (*n*=42, 1.29±0.24). The significance was determined by nonparametric ANOVA (Kruskal-Wallis) and Dunn's multiple comparisons post-test. All comparisons were non-significant (*P*>0.05). Data show mean ± SEM from at least 3 independent replicates, with *n* representing the NMJ number.

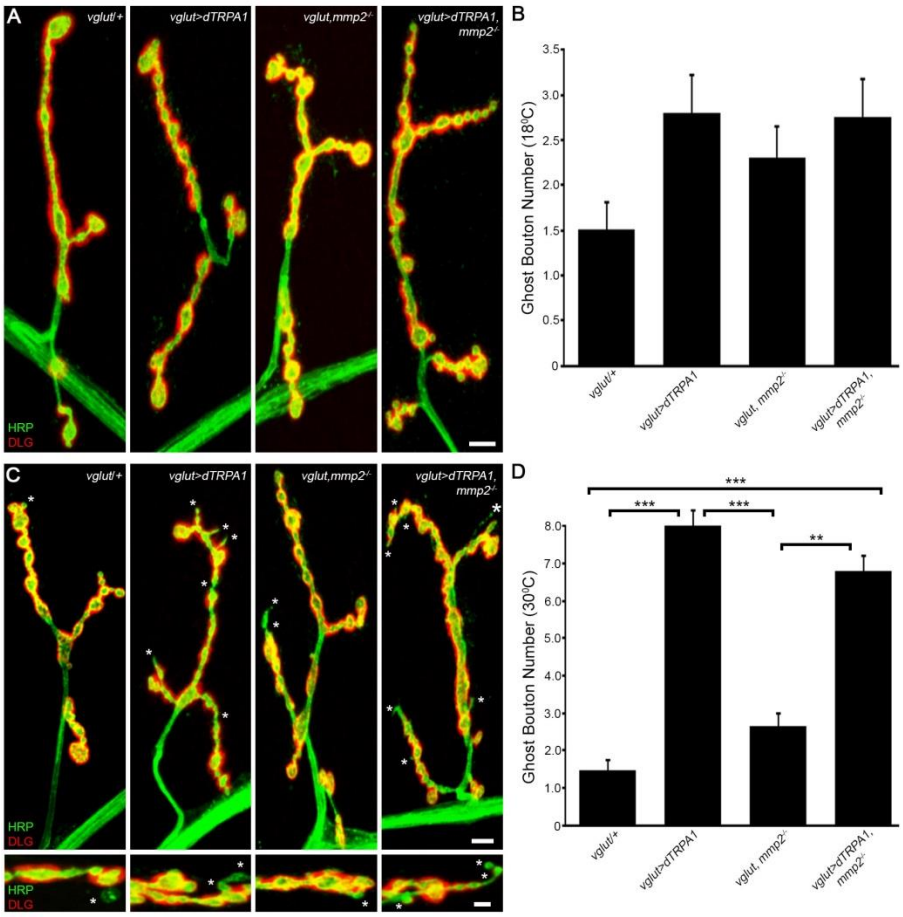

Fig. S2. Mmp2 is not required for activity-dependent synaptic bouton formation. (A and C) NMJs co-labeled for HRP and DLG at 18°C (A) or after dTRPA1 activity stimulation (C) in the 4 genotypes shown. Scale bars: 5 μm. Higher magnification images of synaptic boutons are shown in (C). Scale bar: 2 μm. White asterisks mark ghost boutons. **(B)** Quantification of the ghost bouton number at constant 18°C: *vglut-Gal4*/+ (*n*=29, 1.52±0.3), *vglut-Gal4>UAS-dTRPA1* (*n*=25, 2.8±0.42), *vglut-Gal4, mmp2^{W307*}/Df(2R)BSC132* (*n*=23, 2.3±0.34) and *vglut-Gal4>UAS-dTRPA1,mmp2^{W307*}/Df(2R)BSC132* (*n*=20, 2.75±0.42). Significance was determined by nonparametric ANOVA (Kruskal-Wallis) and Dunn's multiple comparisons post-test. All comparisons were non-significant (*P*>0.05). **(D)** Quantified ghost bouton number following dTRPA1 activation (1-hour at 30°C): *vglut-Gal4*/+ (*n*=26, 1.46±0.31), *vglut-Gal4>UAS-dTRPA1* (*n*=28, 8.0±0.83), *vglut-Gal4, mmp2^{W307*}/ Df(2R)BSC132* (*n*=26, 2.65±0.36) and *vglut-Gal4>UAS-dTRPA1,mmp2^{W307*}/Df(2R)BSC132* (*n*=24, 6.79±0.79). Significance was determined by nonparametric ANOVA (Kruskal-Wallis) and Dunn's multiple comparisons post-test, and indicated as ***P<0.001 and **P<0.01. Non-significant (P>0.05) comparisons 1) *vglut-Gal4*/+ vs. *vglut-Gal4, mmp2^{W307*}/Df(2R)BSC132* and 2) *vglut-Gal4>UAS-dTRPA1* vs. *vglut-Gal4> UAS-dTRPA1,mmp2^{W307*}/Df(2R)BSC132* are not shown. Data show mean ± SEM from at least 3 independent replicates, with *n* = NMJ number.

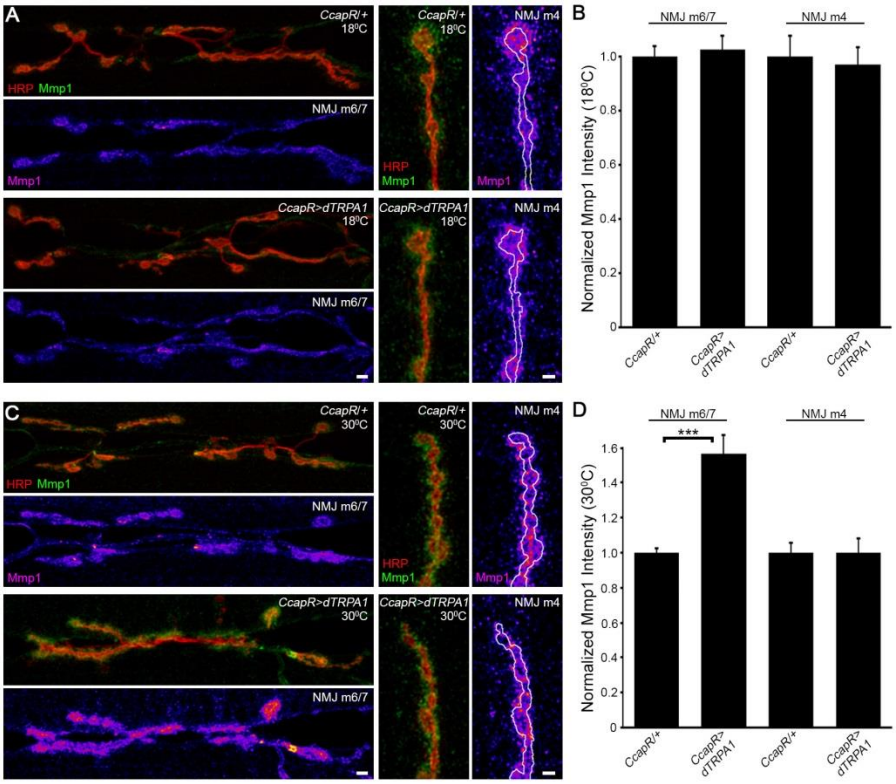

Fig. S3. Mmp1 is rapidly and specifically increased after dTRPA1 neuronal stimulation. (A and C) Images show NMJs co-labeled with HRP and Mmp1 at m6/7 NMJ (scale bar: 5 μm) and m4 NMJ (scale bar: 2 μm) in animals reared at dTRPA1-restrictive 18°C (A) or following dTRPA1 stimulation at 30°C for 1 hour (C) in the genotypes indicated. Mmp1 intensity is shown as a heat map. **(B)** Quantification of normalized Mmp1 intensity in the dTRPA1-restrictive condition at m6/7 and m4 NMJs: *CcapR-Gal4/+* m6/7 NMJ (*n*=12, 1.0±0.04) vs. *CcapR-Gal4>UAS-dTRPA1* m6/7 NMJ (*n*=12, 1.03±0.05) and *CcapR-Gal4/+* m4 NMJ (*n*=16, 1.0±0.08) vs. *CcapR-Gal4>UAS-dTRPA1* m4 NMJ (*n*=14, 0.97±0.07). Significance determined by Unpaired t-test with Welch correction was non-significant for both (*P*>0.05) and is not shown. **(D)** Quantification of normalized Mmp1 intensity in the dTRPA1-permissive condition at m6/7 and m4 NMJs: *CcapR-Gal4/+* m6/7 NMJ (*n*=16, 1.0±0.03) vs. *CcapR-Gal4>UAS-dTRPA1* m6/7 NMJ (*n*=13, 1.57±0.1) and *CcapR-Gal4/+* m4 NMJ (*n*=21, 1.0±0.05) vs. *CcapR-Gal4>UAS-dTRPA1* m4 NMJ (*n*=24, 0.99±0.08). Significance determined by Unpaired t-test with Welch correction (NMJ m6/7), indicated by ***P<0.001. Data show mean ± SEM from at least 3 independent replicates, with *n* = NMJ number.

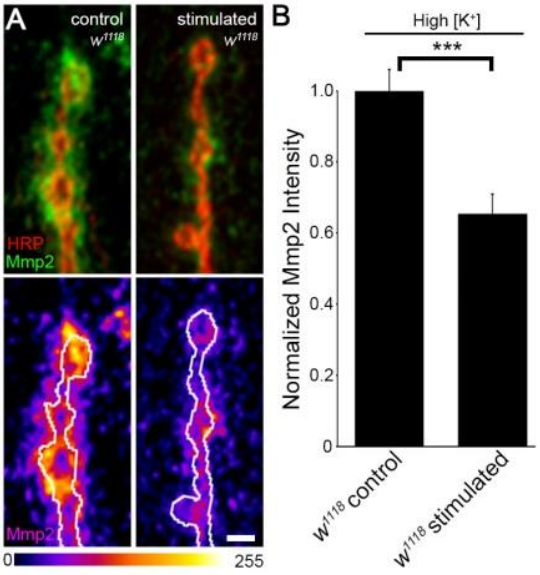

Fig. S4. Synaptic Mmp2 is rapidly reduced after acute neuronal stimulation. (A) Images show NMJs co-labeled for HRP and Mmp2 in either the w^{1118} mock-treated (control) or following 10 minute high [K^+] stimulation. Heat map shows Mmp2 alone (with intensity scale below) and HRP synaptic outlines in white. Scale bar: 2 μm. (B) Quantification of Mmp2 fluorescent intensity normalized to the unstimulated controls: w^{1118} control (unstimulated, *n*=25, 1.0±0.06) vs. w^{1118} stimulated (high [K^+], *n*=28, 0.66±0.06). Significance was determined by Unpaired t-test, indicated as ***P<0.001. Data show mean ± SEM from at least 3 independent experimental replicates, with *n* = NMJ number.

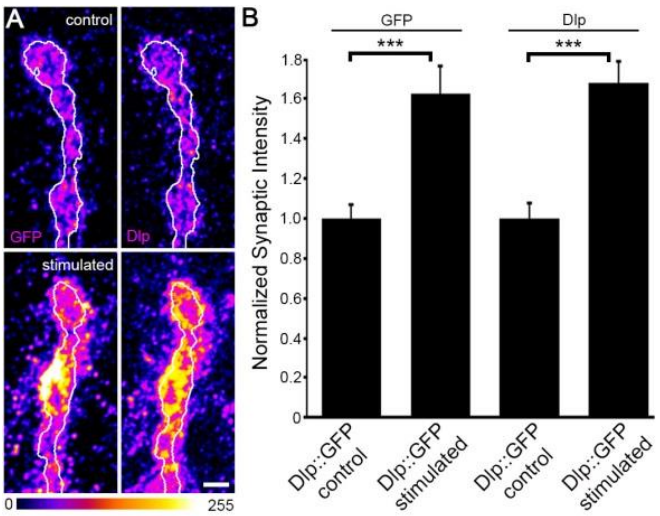

Fig. S5 Synaptic Dlp is rapidly increased after acute neuronal stimulation. (A) Images show NMJs from Dlp::GFP animals co-labeled for GFP and Dlp under standard conditions (control) or following 10 minutes high [K^+] stimulation. Dlp::GFP and Dlp are shown as a heat map (with intensity scale below) and HRP synaptic outlines in white. Scale bar: 2 μm. **(B)** Quantification of GFP and Dlp fluorescence intensities normalized to unstimulated controls. GFP: control (*n*=24, 1.0±0.07) vs. stimulated (*n*=23, 1.62±0.14) and Dlp: control (*n*=14, 1.0±0.07) vs. stimulated (*n*=15, 1.68±0.11). Significance was determined by Unpaired t-test with Welch correction, indicated as ***P<0.001. Data show mean ± SEM from at least 3 independent replicates, with *n* = NMJ number.

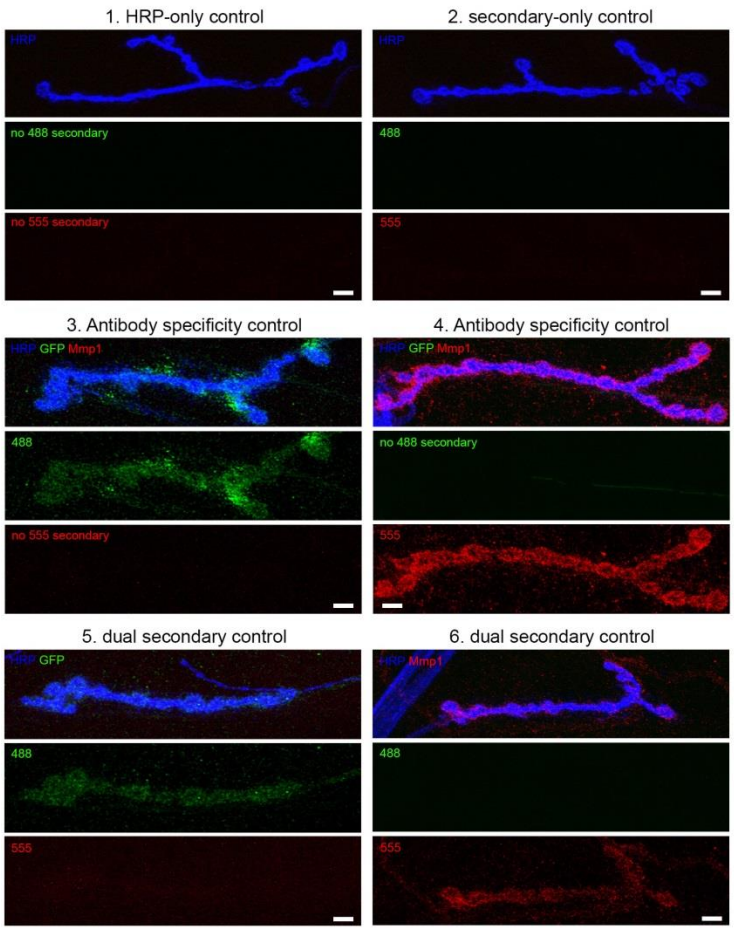

Fig. S6. Synaptic Mmp1 and Dlp imaging controls at the NMJ terminal. Confocal images of Dlp::GFP NMJs labeled with denoted primary (specified in top panels) and secondary antibodies (specified in the middle and bottom panels), processed side-by-side with studies shown in Figure 3. Scale bars: 2 μm. All conditions included HRP::Cy5 to mark NMJs. Controls included: control 1 terminals labeled with HRP alone; control 2 terminals labeled with HRP and secondary antibodies only (rabbit::488 and mouse::555); control 3 terminals labeled with three primary antibodies (HRP, GFP and Mmp1) but incubated with rabbit::488 secondary only; control 4 terminals labeled with three primary antibodies (HRP, GFP, and Mmp1) and then incubated with mouse::555 secondary only; control 5 terminals labeled with the two primary antibodies (HRP and GFP) and then incubated with the two secondary antibodies (rabbit::488 and mouse::555); and control 6 terminals labeled with two primary antibodies (HRP and Mmp1) and then incubated with the two secondary antibodies (rabbit::488 and mouse::555).

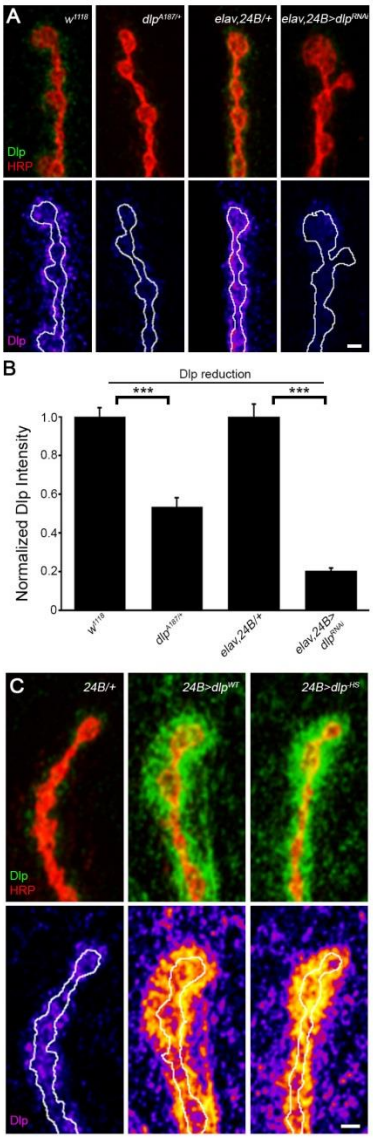

Fig. S7. Synaptic Dlp changes with bidirectional *dlp* genetic manipulations. (A) Images show NMJs co-labeled for HRP and Dlp in the 2 indicated *dlp* reduction conditions compared to matched genetic controls. Dlp is shown in a heat map and HRP synaptic outlines in white. Scale bar: 2 μm. **(B)** Quantified Dlp fluorescence intensity normalized to controls: w^{1118} (*n*=21, 1.0±0.04), $dlp^{A187/+}$ (*n*=23, 0.54±0.04), *elav-Gal4, 24B-Gal4*/+ (*n*=9, 1.0±0.06) and *elav-Gal4, 24B-Gal4>UAS-*dlp^{RNAi} (*n*=11, 0.21±0.01). Significance was determined by Unpaired t-test (w^{1118} vs. $dlp^{A187/+}$) or Unpaired t-test with Welch correction, indicated as ****P*<0.001. Data show mean ± SEM from at least 3 independent replicates, with *n* representing NMJ number. **(C)** NMJ images co-labeled as in (A) for the 2 indicated *dlp* overexpression conditions compared to transgenic control. Scale bar: 2 μm. *n*=5 NMJs.

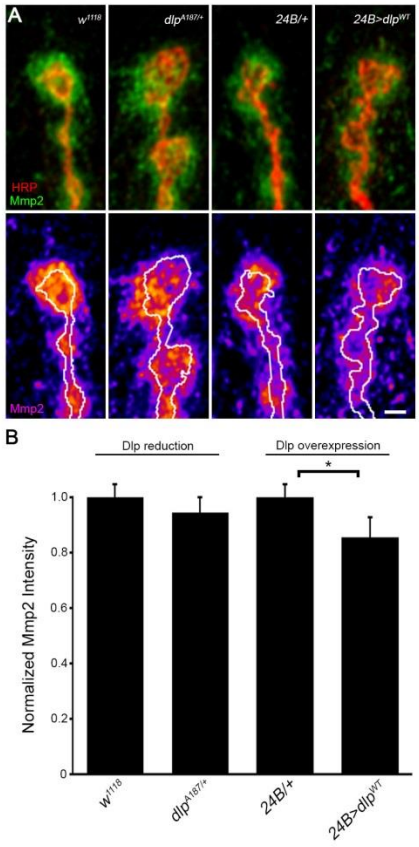

Fig. S8. Synaptic Mmp2 changes with bidirectional *dlp* genetic manipulations. (A) Images show NMJs co-labeled for HRP and Mmp2 in the indicated *dlp* genotypes compared to matched controls. Mmp2 is shown in a heat map and HRP synaptic outlines in white. Scale bar: 2 μm. **(B)** Quantification of Mmp2 fluorescence intensity normalized to matched genetic controls: w^{1118} (n=39, 1.0±0.05) vs. $dlp^{A187/+}$ (n=44, 0.94±0.06), and *24B-Gal4/+* driver control (n=29, 1.0±0.05) vs. *24B-Gal4>UAS-*dlp^{WT} (n=31, 0.86±0.07). Significance determined by Unpaired t-test (w^{1118} vs. $dlp^{A187/+}$) was non-significant (P>0.05; not shown). Significance determined by the Mann-Whitney U-Test (*24B-Gal4/+* vs. *24B-Gal4>UAS-*dlp^{WT}) indicated as *P<0.05. Data show mean ± SEM from at least 3 independent replicates, with n = NMJ number. See Figure S7 for Dlp abundance in the genetic manipulations reported here.

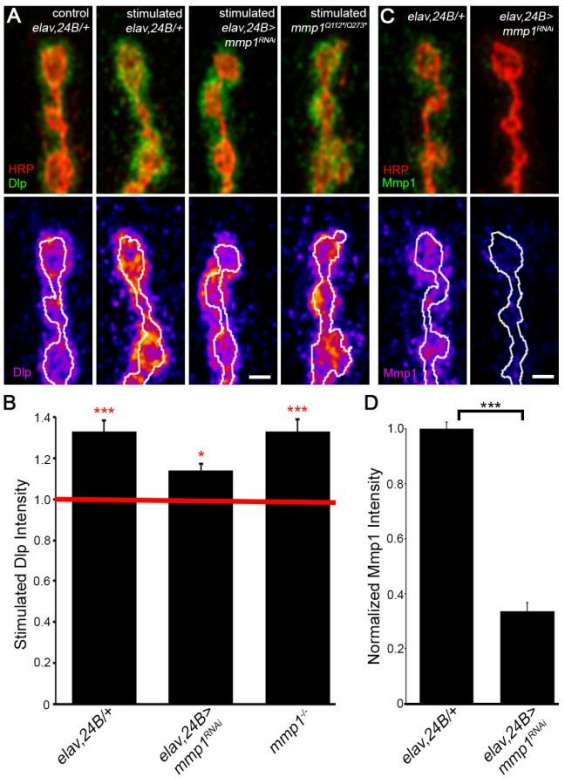

Fig. S9. Activity-dependent synaptic Dlp increase occurs in the absence of Mmp1. (A) Images show NMJs co-labeled for HRP and Dlp in the indicated genotypes, mock treated (unstimulated) or stimulated with high [K^+]. Dlp is shown in a heat map and HRP synaptic outlines in white. Scale bar: 2 μm. **(B)** Quantification of stimulated Dlp intensity (bar graphs) normalized to unstimulated controls (red line): *elav-Gal4, 24B-Gal4/+* control (unstimulated, *n*=14, 1.0±0.05) vs. stimulated *elav-Gal4, 24B-Gal4/+* (*n*=14, 1.33±0.05), and *elav-Gal4, 24B-Gal4>UAS-mmp1^{RNAi}* (unstimulated, *n*=21, 1.0±0.04) vs. stimulated *elav-Gal4, 24B-Gal4>UAS-mmp1^{RNAi}* (*n*=20, 1.14±0.04) and unstimulated *mmp1^{Q112*}/mmp1^{Q273*}* (*n*=22, 1.0±0.04) vs. stimulated *mmp1^{Q112*}/mmp1^{Q273*}* (*n*=25, 1.33±0.06). **(C)** Images show NMJs co-labeled for HRP and Mmp1 in the indicated genotypes. Heat map shows Mmp1 alone and HRP synaptic outlines in white. Scale bar: 2 μm. **(D)** Quantification of Mmp1 intensity: *elav-Gal4, 24B-Gal4/+* control (*n*=18, 1.0±0.02) and *elav-Gal4, 24B-Gal4>UAS-mmp1^{RNAi}* (*n*=21, 0.34±0.03). (B,D) Significance determined by Unpaired t-test, indicated as **P*<0.05 and ****P*<0.001. Data show mean ± SEM from at least 3 independent replicates, with *n* = NMJ number.

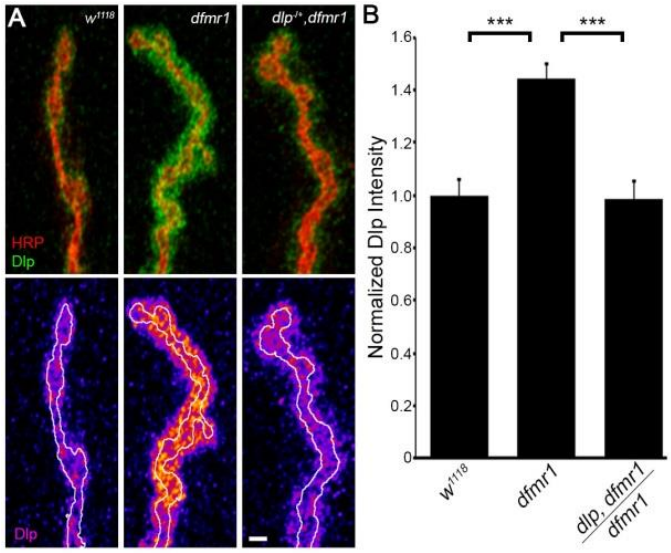

Fig. S10. Synaptic Dlp in FXS disease model is restored by single-copy *dlp* coremoval. (A) Images show NMJs co-labeled for HRP and Dlp in the 3 indicated genotypes. Dlp is shown in a heat map and HRP synaptic outlines in white. Scale bar: 2 μm. **(B)** Quantification of normalized Mmp1 fluorescence intensity: w^{1118} control (n=13, 1.0±0.06), $dfmr1^{50M/50M}$ (n=16, 1.44±0.06) and $dlp^{A187/+}$, $dfmr1^{50M/50M}$ (n=20, 0.98±0.07). Significance was determined by nonparametric ANOVA (Kruskal-Wallis) and Dunn's multiple comparisons post-test, indicated as ***P<0.001. The non-significant (P>0.05) comparison for w^{1118} vs. $dlp^{A187/+}$, $dfmr1^{50M/50M}$ is not shown. Data show mean ± SEM from at least 3 independent replicates, with n = NMJ number.