

Supplemental Materials and Methods

Antibody Production

Constructs for Mmp2 and Timp were optimized and ordered from GenArt. His-tagged Mmp2 and MBP-tagged Timp proteins were recombinantly expressed in *E. coli* and purified in the Vanderbilt Antibody and Protein Resource core (VAPR). The Mmp2 antigen was 162 amino acids immediately following the furin cleavage site (F186-Q298), which spans the catalytic domain included in all predicted isoforms:

MFSKYVLATLLALFAQSMCIQELSLPPEGSHSTAATRSKKAKTAISEDIMNYLMQFDY
LPKSDLETGALRTEDQLKEAIRSLQSFGNITVTGEIDSATARLIQKPRCGVGDRRSADSFS
PDNL YHEIGSNVRVRRFALQGPKWSRTDLTWSMVNR SMPDASKVERMVQTALDV
WANHSKLTREVYSDQADIQILFARRAHGDGYKFDGPGQVLAHAFYPGEGRGGDA
HFDADETWNFDGESDDSHGTNFLNVALHELGHSLGLAHSAIPDAVMFPWYQNNEV
AGNLPDDDRYGIQOLYGTKEKTWGPYKPQTTTTTTTMRAMIYRADKPA YWPWN
NPSNNPNNDRNARERQEEERRQEKEERRQEEERRHQEEERRQVEERQRQEEERWR
QEGERQEEENRRRKIEHKSQWERNPSKERNRPRERQEMERRQEGERQEGERQEGERQEDR
RRERERDRQLEWERRNRNGAREPVPTANTTPRPTNKPYPTVHRQHHHNKPRPKPD
SCMTYYDAISIIRGELFIFRGPYLWRIGTSGLYNGYPTEIRRHW SALPENLTKVDAVYEN
KQRQIVFFIGREYYVFNSVMLAPGFPKPLASLGLPPTLTHIDASFVWGHNNRTYMTSGTL
YWRIDDYTGQVELDYPRDMSIW SGVGYNIDA AFQYLDGKTYFFKNLGYWEFNDDRMK
VAHARA KLSARRWMQCARSANEV DDEQRWTASLVSEGEETGRSGSREL RINHFILSILL
LAIANWRS

The following codon optimized Mmp2 sequence was used:

TTTGCACTGCAGGGTCCGAAATGGTCACGTACCGATCTGACCTGGTCAATGGTTAAT
CGTAGCATGCCGGATGCAAGCAAAGTTGAACGTATGGTCAGACCGCACTGGATGT
TTGGGCAAATCATTCAAAACTGACCTTCGTGAAGTGTATAGCGATCAGGCAGATAT
TCAGATTCTGTTGCACGTCGTGCACATGGTGATGGTTACAAATTGATGGTCCGGG
TCAGGTTCTGGCACATGCATTTATCCGGGTGAAGGTGTTGGCGGAGATGCACATT
TGATGCAGATGAAACCTGGAATTTGATGGTAAAGTGATGATAGCCATGGCACCA
ATTTCTGAATGTTGCCCTGCATGAAC TGGGTCA TAGCCTGGGTCTGGCCC ATAGCG
CAATTCCGGATGCAGTTATGTTCCGTGGTATCAGAATAATGAAGTTGCAGGTAATC
TGCCGGATGATCGTTATGGTATT CAGCAG

The Timp antigen was 113 amino acids (P32-N144) as shown below:

MDLRKHLGLLTLVAVFAFYGRP ADACSCMPSHPQTHFAQADYVVQLRVLRKSDTI
EPGRTTYKVHIKRTYKATSEARRMLRDGRLSTPQDDAMCGINLDLGKVYIVAGRM

**PTLNICSYYKEYTRMTITERHGFSGGYAKATNCTVTPCFGERCFKGRNYADTCKWSP
FGKCETNYSACMPHKVQTVNGVISRCRWRRTQLYRKCMSNP**

The following codon optimized Timp sequence was used:

CCGAGTCATCCGCAGACCCATTTGCACAGGCAGATTATGTTGTTCAGCTGCGTGTT
CTGCGTAAAAGCGATAACCATTGAACCGGGTCGTACCACCTATAAAGTCATATTAAA
CGCACGTATAAACGACCGAGCGAACGACGTCGTATGCTGCGTGATGGTCGTCTGAG
CACACCCGAGGATGATGCAATGTGTGGTATTAATCTGGATCTGGCAAAGTGTATAT
TGTTGCAGGTCGTATGCCGACCCTGAATATTGTAGCTATTACAAAGAACACCCG
CATGACCATTACCGAACGTCATGGTTTAGCGGTGGTTATGCAAAAGCAACCAAAT

Rabbit polyclonal antibodies were raised against both sequences (Cocalico Biologicals, Inc.) and column affinity purified (VAPR).

Western blot analyses

SDS-PAGE Western blots were performed as previously described (Parkinson et al., 2013). The neuromusculature or brain/ventral nerve cord (CNS) was isolated from dissected larvae, and equal amounts of tissue were homogenized in buffer (67mM NaCl, 2M urea, 1mM EDTA, 1.3% SDS, Tris pH 8.0, Roche protease inhibitor cocktail) and centrifuged (20 min, 16kxg). Equal volumes of 2X NuPage sample buffer (Invitrogen) were added to supernatant and heated at 60°C for 15 mins with (Mmp1 and Mmp2) or without (Timp) 5% β-mercaptoethanol. Samples were loaded onto Bis-Tris SDS gels (Invitrogen), electrophoresed in 1X MES buffer (Mmp1 and Mmp2) or 1X MOPS (Timp) and transferred to nitrocellulose membranes (Biorad). Membranes were blocked for 1 hr at RT in either 2% BSA (Mmp1), 2% milk (Mmp2), or Odyssey blocking buffer (Timp) diluted TBS-T (10 mM Tris pH 8, 150 mM NaCl, 0.05% Tween 20). Primary antibodies were diluted in block, incubated overnight at 4°C and washed 6X in TBS-T for 5 mins the following day. All secondary antibodies were diluted 1:5000 in block, incubated for 1 hr at RT, washed 6X for 5 mins in TBS-T, and visualized using the Licor Odyssey Infrared Imaging System. Primary antibody concentrations used: mouse α-tubulin (1:1000; DSHB 12G10), mouse

α -Mmp1 catalytic cocktail (1:1:1 at 1:100; DSHB, 3B8D12/ 3A6B4/ 5H7B11), rabbit α -Mmp2 (1:1k, this study) and rabbit α -Timp (1:2k, this study). Secondary antibodies (all raised in goat) used: α -mouse680 (Invitrogen), α -mouse800 (Rockland), α -rabbit680 (Invitrogen) and α -rabbit800 (Rockland).

Immunocytochemistry imaging

Preparations fixed in ice-cold methanol for 5 mins (GluRIIA/B) or 4% PFA for 10 min at RT (all other labels) were processed with 0.1% Triton X-100 (permeabilized) or without detergent (extracellular labeling). Primary antibodies were incubated overnight at 4°C and then washed 3X for 10 mins. Secondary antibodies were incubated for 2 hr at RT, then washed 3X for 10 mins. Primary antibodies used: rabbit α -HRP (1:200; Sigma P7899), Cy3-conjugated goat α -HRP (1:250; Jackson Laboratories 123-165-021), Cy5-conjugated goat α -HRP (1:200; Jackson Laboratories 123-605-021), mouse α -Mmp1 (1:1:1 at 1:10; DSHB, 3B8D12/3A6B4/5H7B11), rabbit α -Mmp2 (1:1000; this study), rabbit α -Timp (1:500; this study), mouse α -GluRIIA (1:100; DSHB, 8B4D2(MH2B)), rabbit α -GluRIIB (1:1000; Chen and Featherstone, 2005; Marrus and DiAntonio, 2004), rabbit α -GluRIID (1:500; Chen and Featherstone, 2005), mouse α -BRP (1:100; DSHB, NC82), mouse α -Dlg (1:200; DSHB, DLG1), mouse α -Wg (1:2; DSHB, 4D4), rabbit α -DFz2-C (1:500; Mathew et al., 2005), and mouse α -Dlp (1:5; DSHB, 13G8). Secondary antibodies (all 1:500; Invitrogen) used: goat α -mouse (488, 568) and goat α -rabbit (488, 568). Samples were mounted in Fluoromount-G (Electron Microscopy Sciences). Entire NMJ Z-stack images were acquired with a Zeiss LSM 510 META laser-scanning confocal using 63X Plan Apo oil immersion objective. For intensity comparisons, images were obtained with the same settings and quantified using NIH ImageJ software.

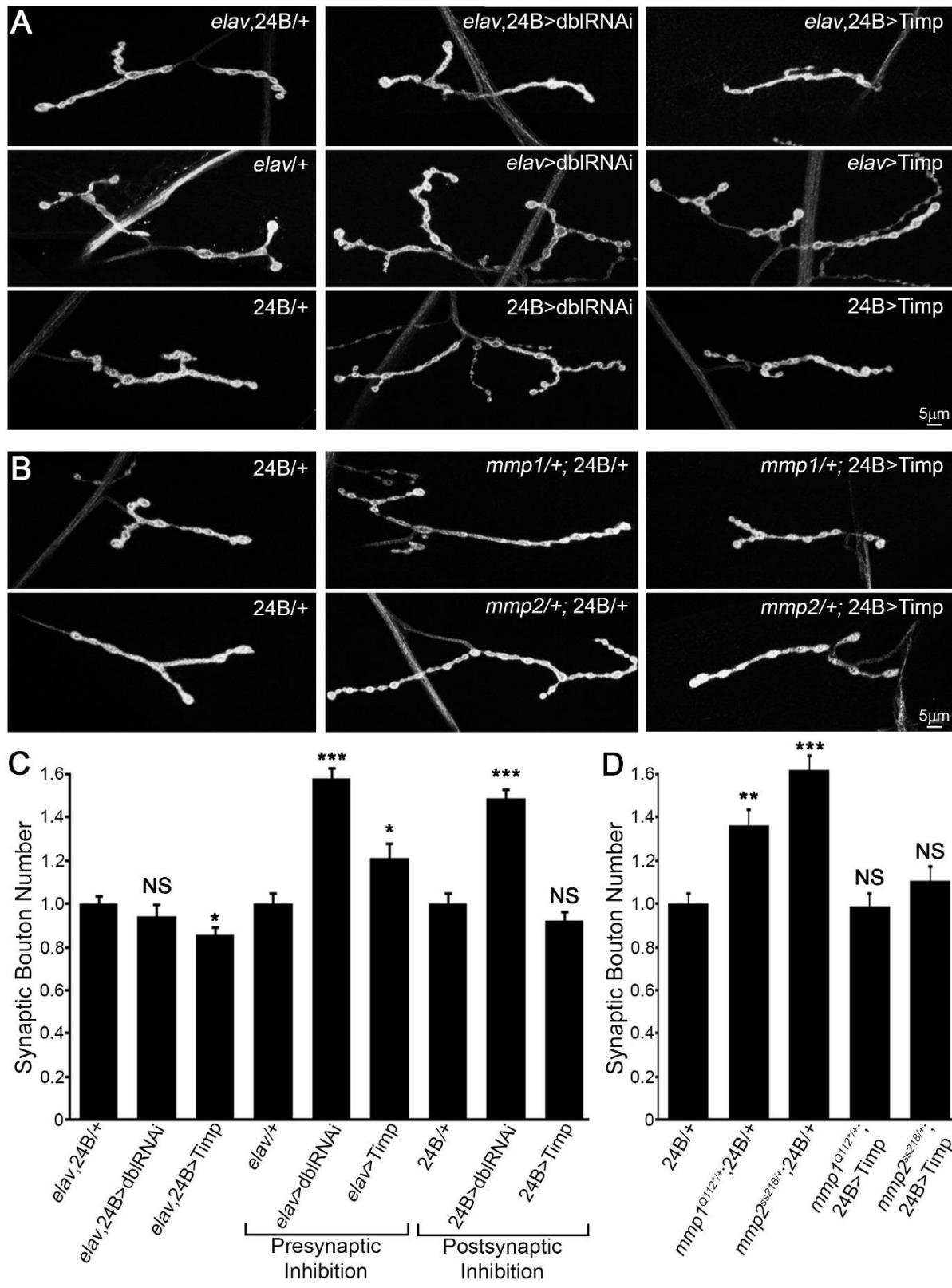


Fig. S1. Postsynaptic Timp expression suppresses NMJ morphology defects. A)

Representative wandering 3rd instar NMJs labeled for synaptic markers α -HRP and α -Dlg. Top row:

Recombined dual pre- and postsynaptic *elav*, 24B/+ transgenic control (left), *elav*, 24B>*mmp1*+2^{RNAi} knockdown (dblRNAi; center) and *elav*, 24B>UAS-Timp (Timp; right).

Middle row: *elav*/+ transgenic control (left), *elav*>dblRNAi (center), and *elav*>Timp (right).

Bottom row: 24B/+ transgenic control (left), 24B>dblRNAi (center), and 24B>Timp. **B)** NMJs

labeled for synaptic markers α -HRP and α -Dlg in specified genotypes. Top row: 24B/+ transgenic control (left), *mmp1*^{Q112*/+}; 24B/+ (center) and *mmp1*^{Q112*/+}; 24B>Timp (right).

Bottom row: 24B/+ transgenic control (left), *mmp2*^{ss218*/+}; 24B/+ (center) and *mmp2*^{ss218*/+};

24B>Timp (right). **C)** Quantification of synaptic bouton number normalized to controls from

genotypes shown in above (A). **D)** Quantification of synaptic bouton number normalized to controls from genotypes shown in above (B). Significance: *p<0.05, **p<0.01, ***p<0.001 and not significant (NS). See Table S1A for raw data values and sample sizes.

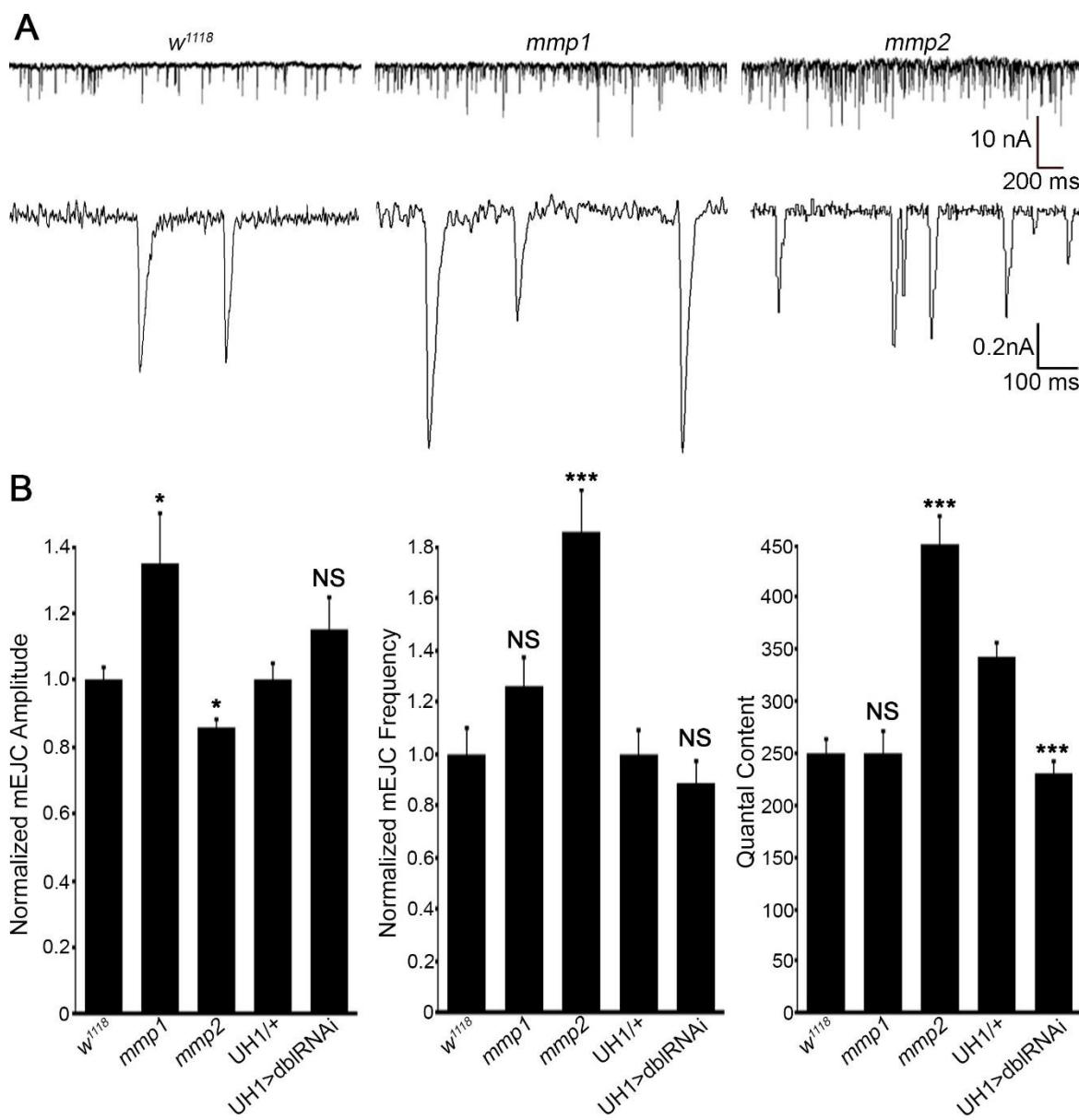


Fig. S2. Mmp1 and Mmp2 regulate spontaneous SV fusion rate and response amplitude. A) Representative spontaneous miniature EJC (mEJC) two-electrode voltage-clamp (TEVC) recording traces in genetic control (w^{1118}), $mmp1$ ($mmp1^{Q273*}$) and $mmp2$ ($mmp2^{W307*Df}$) mutants. Top: Sample long time scale recordings of mEJC events for all 3 genotypes. Bottom: Magnified mEJC event traces for all 3 genotypes. **B)** Quantification of mEJC amplitude (left), mEJC frequency (middle) and quantal content (right) normalized to respective control conditions. Significance indicated as * $p<0.05$, *** $p<0.001$ and not significant (NS). See Table S2B for raw data values and sample sizes.

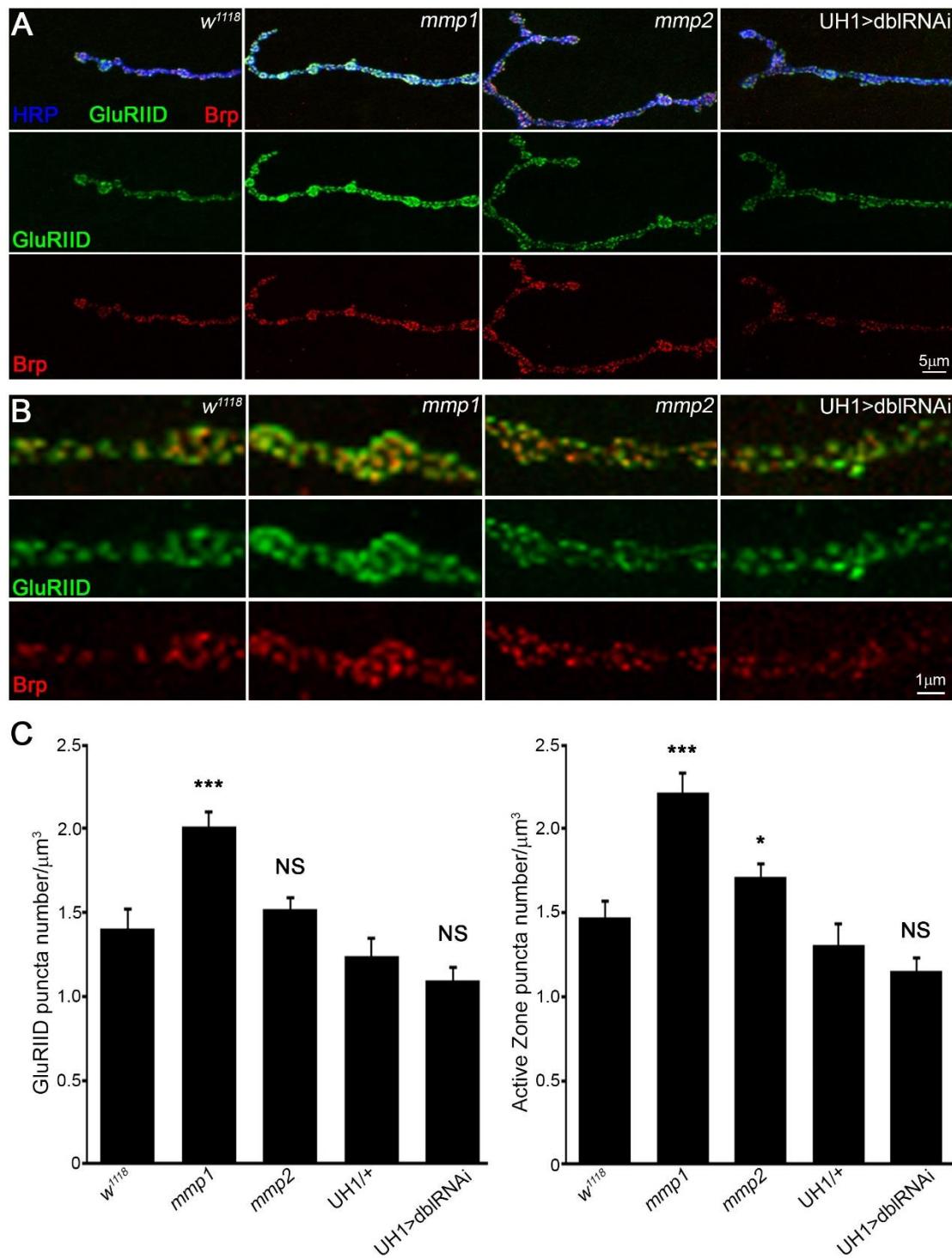


Fig. S3. Loss of Mmp1 and Mmp2 differentially regulate NMJ molecular assembly. A) Representative low magnification images wandering 3rd instar NMJs triple-labeled for synaptic membrane marker α -HRP (blue), core glutamate receptor subunit (GluRIID, green) and presynaptic active zone marker Bruchpilot (Brp, red) in genetic background control (w^{1118}), *mmp*

mutants ($mmp1^{Q112^*/Q273^*}$ and $mmp2^{ss218/Df}$) and the double *mmp* RNAi condition (UH1>*mmp1+2*^{RNAi} (dblRNAi)). **B)** Higher magnification images of synaptic boutons from the same genotypes. **C)** Quantification of GluRIID puncta number (left) and Brp puncta number (right) normalized to bouton volume. Significance indicated as * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and not significant (NS) based on Mann-Whitney tests. See Tables S1B and S2C for raw data values and sample sizes.

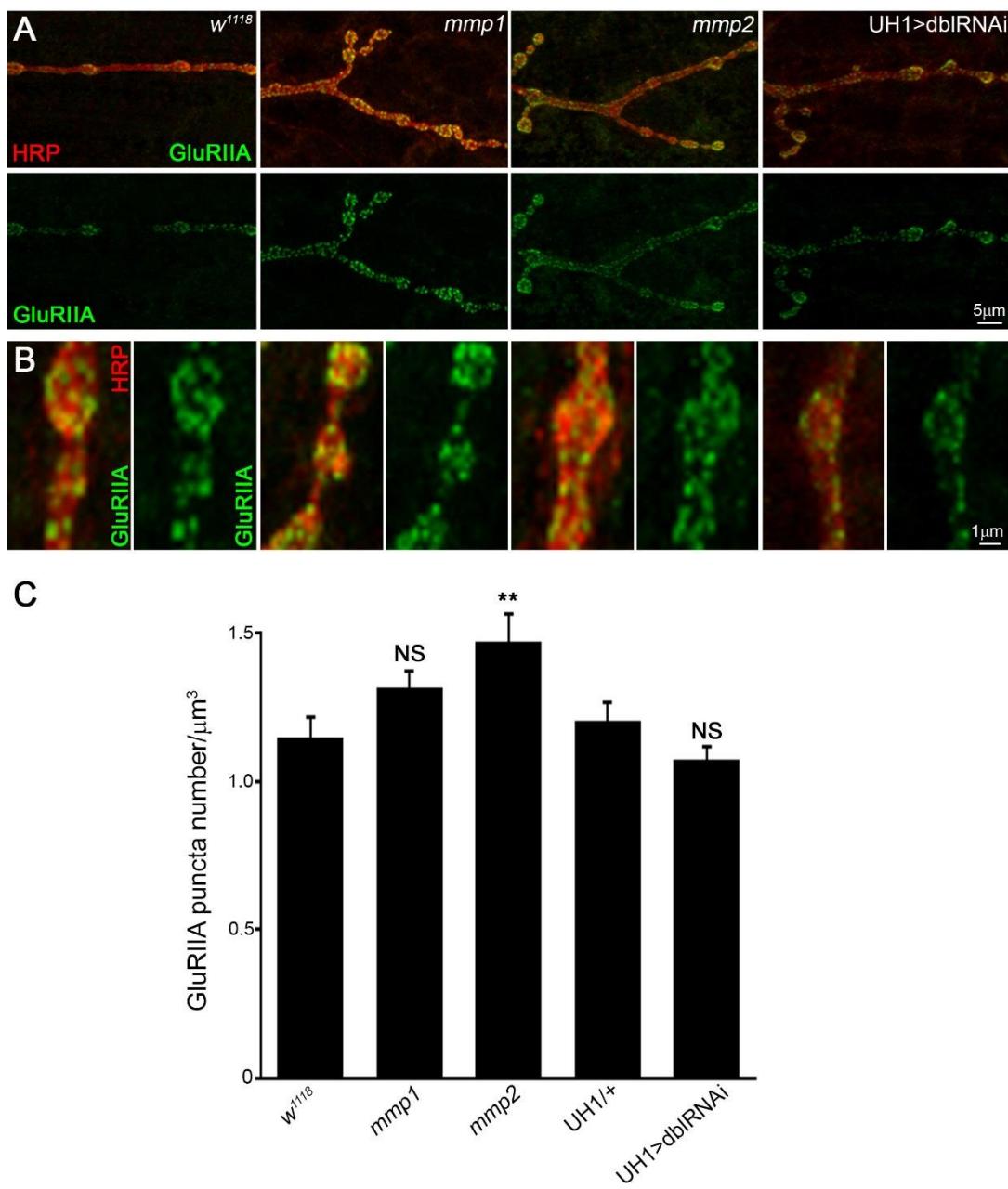


Fig. S4. Mmp2 negatively regulates GluRIIA-containing receptors. **A)** Representative low magnification images of wandering 3rd instar NMJs co-labeled for the GluRIIA subunit (green) and the synaptic membrane marker α -HRP (red) in genetic background control (*w¹¹¹⁸*), *mmp* mutants (*mmp1*^{Q112*/Q273*} and *mmp2*^{ss218/Df}) and *UH1>mmp1+2^{RNAi}* condition (dblRNAi). Bottom row shows GluRIIA subunit (green) labeling alone. **B)** Higher magnification images of synaptic boutons from the same genotypes. Right panels show GluRIIA subunit (green) labeling alone. **C)** Quantification of GluRIIA puncta number normalized to bouton volume. Significance indicated as ** $p<0.01$ and not significant (NS). See Tables S1B and S2C for raw data values and sample sizes.

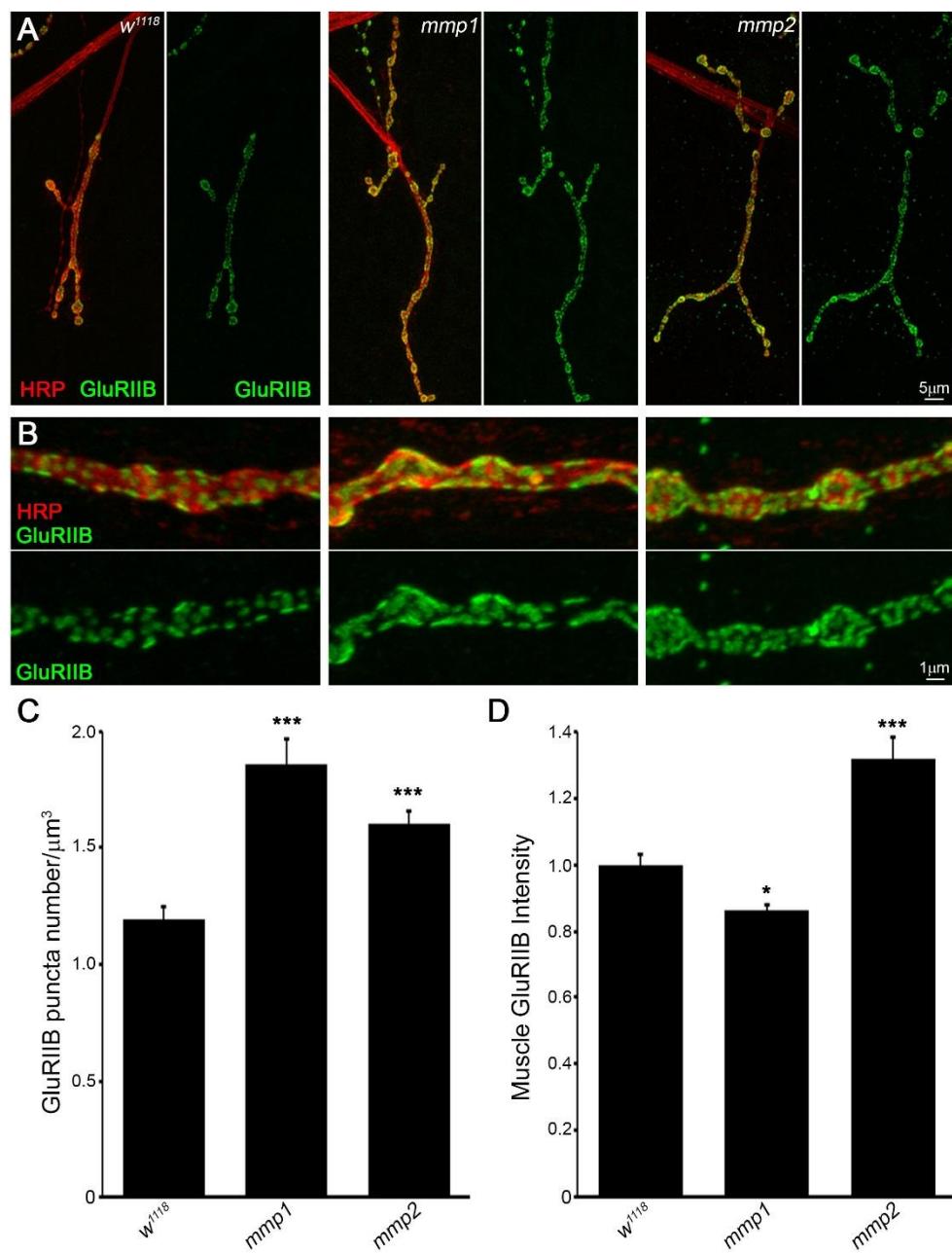


Fig. S5. Mmp1 and Mmp2 negatively regulate GluRIIB-containing receptors. **A)** Representative low magnification images of wandering 3rd instar NMJs co-labeled for the GluRIIB subunit (green) and synaptic membrane marker α-HRP (red) in genetic background control (*w¹¹¹⁸*, left), *mmp1* (*mmp1^{Q112*/Q273*}*, middle) and *mmp2* (*mmp2^{ss218/Df}*, right). **B)** Higher magnification images of synaptic boutons from the same genotypes. **C)** Quantification of GluRIIB puncta number normalized to bouton volume. **D)** Quantification of GluRIIB intensity in the muscle of each genotype normalized to genetic control. Significance indicated as *p<0.05, ***p<0.001 and not significant (NS). See Tables S1B and S2C for raw values and sample sizes.

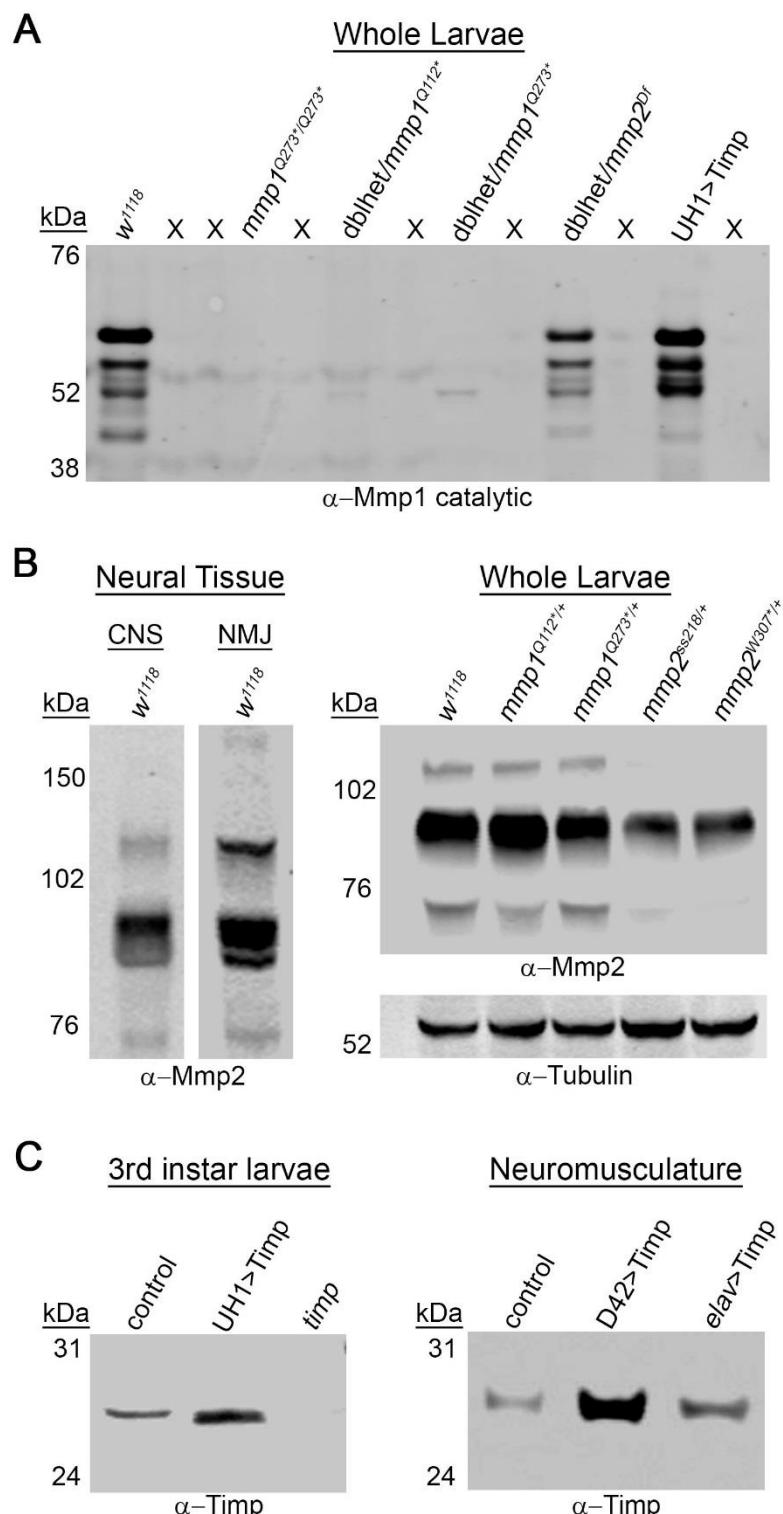


Fig. S6. Western blot characterization and specificity of Mmp2 and Timp antibodies. **A)** Western blot shows Mmp1 immunoreactivity in wandering 3rd instar whole larval tissue from genetic background control (*w¹¹⁸*), strong Mmp1 hypomorph (*mmp1^{Q273*}*), three different *mmp1^{Q112*},mmp2^{W307*}* double, heterozygous (dblhet) dosage combinations (dblhet/*mmp1^{Q112*}*; dblhet/*mmp1^{Q273*}*; dblhet/*mmp2^{Df}*) and the ubiquitous double inhibition condition (UH1>Timp).

The 74kDa band corresponds to a predicted GPI-anchored Mmp1 isoform while the 64, 52 and 46 kDa bands correspond to secreted and activated Mmp1 isoforms. **B)** Left: α -Mmp2 Western blots from 3rd instar central nervous system (CNS, left) and neuromusculature (NMJ, right) lysates from control (w^{1118}). Right: Whole larvae α -Mmp2 Western blots from w^{1118} , two *mmp1* heterozygotes (*mmp1*^{Q112*/+}, *mmp2*^{Q273*/+}) and two *mmp2* heterozygotes (*mmp2*^{ss218/+}, *mmp2*^{W307*/+}). Predominant ~90kDa band corresponds to predicted Mmp2 activated/processed form, and fainter ~120kDa band corresponds to predicted full-length/pre-processed Mmp2 form. Source of the ~76 kDa Mmp2 immunoreactive band is unknown. **C)** Left: α -Timp Western blot of wandering 3rd instar larvae shows a single ~28kDa band at the predicted Timp molecular weight in transgenic control animals (UH1/+), which is elevated by transgenic Timp overexpression (UH1>Timp) and absent in *timp* null mutants. Right: α -Timp Western blot of isolated neuromusculature from transgenic control (D42/+), motor neuron D42>Timp and pan-neuronal *elav*>Timp shows endogenous and overexpressed Timp.

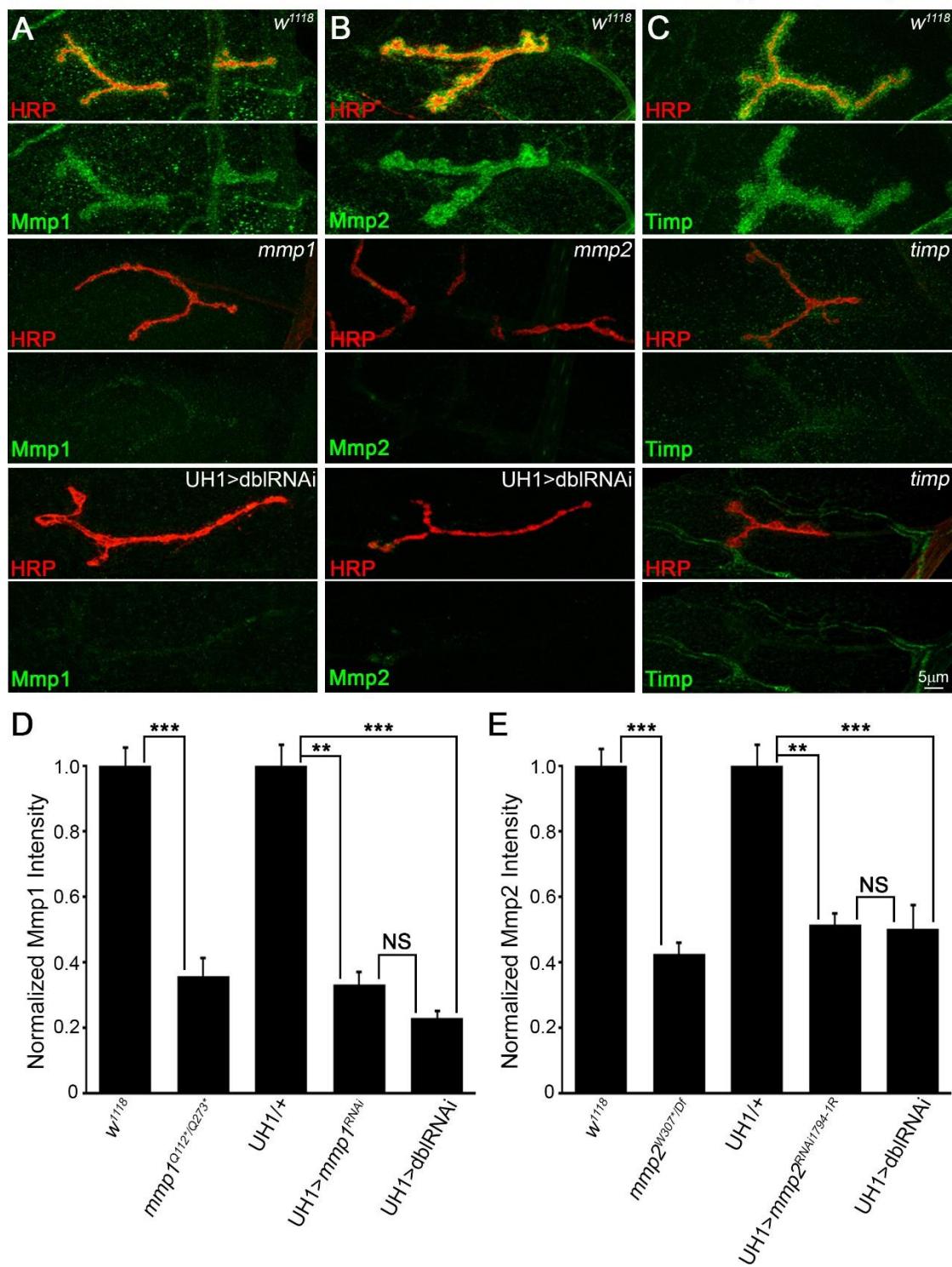


Fig. S7. Imaging characterization and specificity of Mmp2 and Timp antibodies. **A)** Representative images of α -Mmp1 (green) co-labeled with the NMJ marker α - HRP (red) in w^{1118} genetic control (top), $mmp1^{Q112^*/Q273^*}$ mutant (middle) and with $UH1 > mmp1 + 2^{RNAi}$ (dblRNAi, bottom panel). **B)** α -Mmp2 (green) co-labeled with α -HRP (red) in w^{1118} (top),

mmp2^{W307*/Df} mutant (middle) and UH1>dblRNAi, (bottom). **C)** α -Timp (green) co-labeled with α -HRP (red) in *w*¹¹¹⁸ (top) and *tmp* mutants (middle and bottom panels). **D)** Quantification of Mmp1 fluorescent intensity normalized to genetic controls (*w*¹¹¹⁸, UH1) for *mmp1*^{Q112*/Q273*}, UH1>*mmp1*^{RNAi} and UH1>dblRNAi. **E)** Quantification of Mmp2 fluorescent intensity normalized to genetic controls (*w*¹¹¹⁸, UH1) for *mmp2*^{W307*/Df}, UH1>*mmp2*^{RNAi1794-1R} and UH1>dblRNAi. Significance indicated as **p<0.01, ***p<0.001 and not significant (NS). See Tables S4A for raw data values and sample sizes.

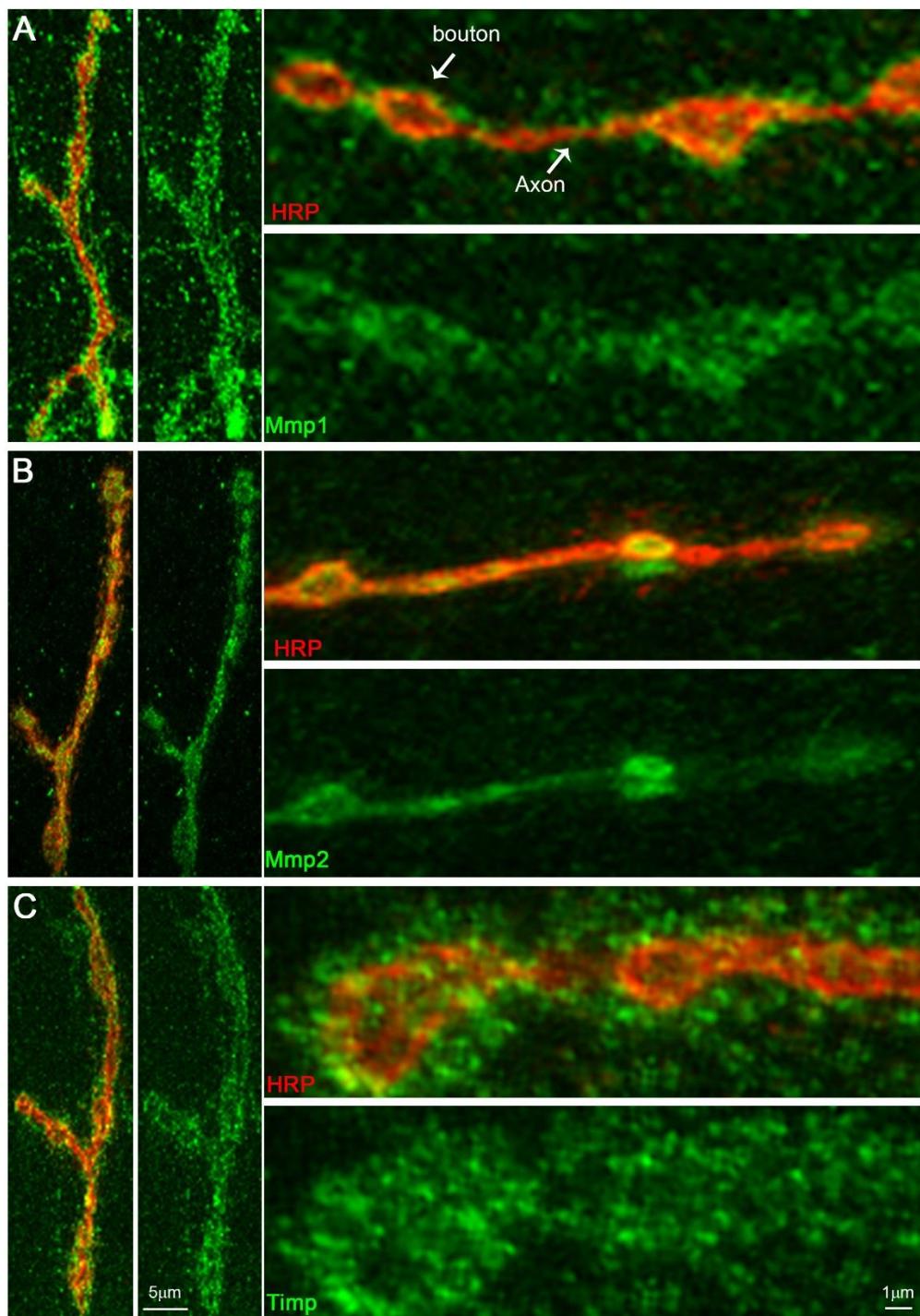


Fig. S8. Characterization of Mmp1, Mmp2 and Timp localization at the NMJ.

Representative wandering 3rd instar NMJ images from *w¹¹¹⁸* control animals showing (A) α -Mmp1 (green), (B) α -Mmp2 (green) and (C) α -Timp (green) relative to α -HRP presynaptic membrane marker (red) at low (left) and high (right) magnifications. Arrows label a representative bouton and axon/inter-bouton space.

Table S1. Raw values for NMJ morphology studies			
A. Synaptic Bouton Number			
	<i>Genotype</i>	<i>n</i>	<i>mean ± sem</i>
Genetic LOF	<i>w¹¹¹⁸</i> †	31	18.2 ± 0.99
	<i>w¹¹¹⁸</i>	23	21.5 ± 0.87
	<i>mmp1^{Q112*/Q112*}</i> †	13	23.4 ± 1.3
	<i>mmp1^{Q273*/Q273*}</i>	21	28 ± 1.1
	<i>mmp2^{W307*/Df}</i>	19	29.7 ± 1.3
	<i>mmp2^{ss218/Df}</i>	21	29.1 ± 1.3
Heterozygotes	<i>w¹¹¹⁸</i>	12	21.9 ± 1.2
	<i>mmp1^{Q112*/+}</i>	15	31.4 ± 1.6
	<i>mmp1^{Q273*/+}</i>	22	32.5 ± 1.6
	<i>mmp2^{W307*/+}</i>	20	34.1 ± 1.5
	<i>mmp2^{ss218/+}</i>	22	37.6 ± 1.2
	<i>mmp2^{W307*/+}, mmp1^{Q112*/+}</i> (dhet)	22	21.7 ± 1.1
	<i>mmp2^{W307*/+}, mmp1^{Q112*/Q112*}</i> †	12	25.0 ± 1.7
	<i>mmp2^{W307*/+}, mmp1^{Q112*/Q273*}</i>	20	31.2 ± 1.2
	<i>mmp2^{W307*/Df}, mmp1^{Q112*/+}</i>	22	17.8 ± 0.90
Ubiquitous Inhibition	UH1/+ †	21	18.9 ± 0.50
	UH1>dblRNAi	23	19.8 ± 1.5
	UH1>Timp †	18	16.8 ± 0.63
	UH1/+	23	21.4 ± 0.74
	UH1> <i>mmp1^{RNAi}</i>	22	25.3 ± 1.1
	UH1> <i>mmp2^{RNAi}</i>	35	28.3 ± 0.74
	UH1> <i>mmp2^{RNAi1794-1R}</i>	20	35.6 ± 1.3
Pre & Post Inhibition	<i>elav,24B/+</i>	24	25.1 ± 0.78
	<i>elav,24B>mmp1^{RNAi}</i>	20	35.8 ± 0.99
	<i>elav,24B>mmp2^{RNAi}</i>	21	31.3 ± 0.85
	<i>elav,24B>mmp2^{RNAi1794-1R}</i>	21	35 ± 1.0
	<i>elav,24B>dRNAi</i>	23	23.7 ± 1.3
	<i>elav,24B>Timp^{OE}</i>	22	21.5 ± 0.92
	<i>elav/+</i>	24	21.3 ± 0.94
Presynaptic Inhibition	<i>elav>mmp1^{RNAi}</i>	27	28.6 ± 0.96
	<i>elav>mmp2^{RNAi}</i>	26	25.5 ± 1.38
	<i>elav>mmp2^{RNAi1794-1R}</i>	14	36.9 ± 0.91
	<i>elav>dRNAi</i>	26	33.7 ± 0.99
	<i>elav>Timp^{OE}</i>	18	25.8 ± 1.5
	24B/+	29	21.1 ± 1.1
	24B> <i>mmp1^{RNAi}</i>	23	26 ± 1.1
Postsynaptic Inhibition	24B> <i>mmp2^{RNAi}</i>	21	25.4 ± 1.4
	24B> <i>mmp2^{RNAi1794-1R}</i>	17	30.8 ± 0.88
	24B/+	27	23.1 ± 1.06
	24B>dblRNAi	23	34.3 ± 0.91
	24B>Timp	23	21.3 ± 0.84

	24B/+	29	20.4 ± 0.97
	<i>mmp1</i> ^{Q112*/+} ; 24B/+	19	27.8 ± 1.52
	<i>mmp2</i> ^{ss218/+} ; 24B/+	18	33.1 ± 1.34
	<i>mmp1</i> ^{Q112*/+} ; 24B>Timp	21	20.1 ± 1.28
	<i>mmp2</i> ^{ss218/+} ; 24B>Timp	19	22.6 ± 1.33

	<i>Genotype</i>	<i>n</i>	<i>mean ± sem</i>
Dip Modulation	24B/+ †	20	19.4 ± 1.1
	<i>mmp1</i> ^{Q112*/Q273*} †	20	25.4 ± 1.4
	<i>mmp1</i> ^{Q112*/Q273*} ; 24B>dlp †	18	17.8 ± 0.83
	<i>w</i> ¹¹¹⁸	33	22.7 ± 0.67
	<i>mmp2</i> ^{W307*/Df} ; <i>dlp</i> ^{A187/+}	19	21.8 ± 0.2

B. Synaptic Ultrastructure

Morphology		Bouton Area (μm^2)		Bouton Volume (μm^3)		Muscle Surface Area ($\mu\text{m}^2 \times 10^4$)	
		Genotype	<i>n</i>	<i>mean ± sem</i>	<i>n</i>	<i>mean ± sem</i>	<i>n</i>
	<i>w</i> ¹¹¹⁸	31	8.9 ± 1.3	61	8.4 ± 0.6	35	4.8 ± 0.10
	<i>mmp1</i> ^{Q112*/Q273*}	47	3.7 ± 0.4	62	4.9 ± 0.3	31	3.0 ± 0.11
	<i>mmp2</i> ^{ss218/Df}	36	5.8 ± 0.5	70	7.5 ± 0.4	37	5.1 ± 0.12
	UH1/+	-	-	78	8.3 ± 0.5	41	4.6 ± 0.12
	UH1>dblRNAi	44	8.0 ± 0.7	83	8.8 ± 0.5	41	4.5 ± 0.12
Synaptic Vesicles		SV Density		SV's 0-250 nm		SV's 250-500 nm	
		Genotype	<i>n</i>	<i>mean ± sem</i>	<i>mean ± sem</i>	<i>mean ± sem</i>	<i>mean ± sem</i>
	<i>w</i> ¹¹¹⁸	31	34.5 ± 3.1	14.7 ± 0.5	18.6 ± 0.79		
	<i>mmp1</i> ^{Q112*/Q273*}	47	42.2 ± 3.2	15.7 ± 0.2	23.8 ± 1.4		
	<i>mmp2</i> ^{ss218/Df}	36	48 ± 3.1	18.5 ± 0.5	29.0 ± 1.3		
	UH1>dblRNAi	44	33.4 ± 2.4	15.6 ± 0.4	19.6 ± 1.2		

† Denotes size matched comparisons.

Table S2. Raw values for NMJ functional studies

A. Evoked Neurotransmission (TEVC)			
	<i>Genotype</i>	<i>n</i>	<i>Amplitude (nA)</i>
Genetic LOF	<i>w¹¹¹⁸</i>	10	191 ± 10
	<i>mmp1^{Q273*}</i>	10	244 ± 10
	<i>mmp1^{Q273*/Q273*}</i>	11	251 ± 21
	<i>mmp2^{W307*/Df}</i>	11	296 ± 18
	<i>mmp2^{ss218/Df}</i>	12	319 ± 13
Ubiquitous Inhibition	UH1/+	18	263 ± 9
	UH1>dblRNAi	11	202 ± 10
	UH1>Timp	10	192 ± 9
	UH1> <i>mmp1^{RNAi}</i>	16	292 ± 10
	UH1> <i>mmp2^{RNAi}</i>	12	310 ± 14
Cell-Targeted Inhibition	<i>elav</i> /+	10	264 ± 16
	<i>elav</i> > <i>mmp1^{RNAi}</i>	10	257 ± 14
	<i>elav</i> > <i>mmp2^{RNAi}</i>	11	281 ± 15
	24B/+	11	267 ± 8
	24B> <i>mmp1^{RNAi}</i>	12	301 ± 13
	24B> <i>mmp2^{RNAi}</i>	11	326 ± 21
	24B>dblRNAi	13	213 ± 17
	24B>Timp	11	210 ± 14
	24B/+	11	224 ± 13
	<i>mmp1^{Q112*/+; 24B/+}</i>	12	184 ± 13
	<i>mmp2^{ss218/+; 24B/+}</i>	11	224 ± 14
	<i>mmp1^{Q112*/+; 24B>Timp}</i>	13	199 ± 16
	<i>mmp2^{ss218/+; 24B>Timp}</i>	11	246 ± 15
Dlp Modulation	24B/+	11	224 ± 13
	<i>mmp1^{Q112*/Q273*}; 24B>dlp</i>	10	139 ± 17
	<i>w¹¹¹⁸</i>	11	194 ± 14
	<i>mmp2^{W307*/Df}</i>	10	313 ± 13
	<i>mmp2^{W307*/Df; dlp^{A187/+}}</i>	10	234 ± 12

B. Spontaneous mEJC Analysis							
Ubiquitous Inhibition	LOF	<i>Genotype</i>	<i>n</i>	<i>Amplitude (nA)</i>	<i>Frequency (Hz)</i>	<i>Quantal Content</i>	
		<i>w¹¹¹⁸</i>	15	0.77 ± 0.1	2.07 ± 1.1	250 ± 13	
		<i>mmp1^{Q273*/Q273*}</i>	19	1.0 ± 0.6	2.4 ± 1.2	251 ± 21	
		<i>mmp2^{W307*/Df}</i>	15	0.66 ± 0.1	3.9 ± 1.5	451 ± 27	
		UH1/+	15	0.67 ± 0.02	1.3 ± 0.1	393 ± 14	
		UH1> <i>mmp1^{RNAi}</i>	8	0.4 ± 0.02	0.7 ± 0.1	729 ± 24	
		UH1> <i>mmp2^{RNAi}</i>	11	1.5 ± 0.4	2.2 ± 0.4	207 ± 9.4	
		UH1>dblRNAi	10	0.71 ± 0.03	3.9 ± 0.6	285 ± 14	
		UH1/+	15	0.77 ± 0.05	9.3 ± 0.9	344 ± 12	
		UH1>dblRNAi	14	0.88 ± 0.08	8.5 ± 0.8	230 ± 12	

C. Glutamate Receptors							
Essential GluR Subunit	<i>Genotype</i>	<i>n</i>	<i>Total AZ #</i>	<i>AZ Density</i>	<i>Total GluRIID #</i>	<i>GluRIID Density</i>	<i>Apposition (GluRIID:nc82)</i>
	<i>w¹¹¹⁸</i>	13	216 ± 14	1.47 ± 0.10	203 ± 14	1.4 ± 0.12	0.94 ± 0.03
	<i>mmp1^{Q112*/Q273*}</i>	13	216 ± 11	2.22 ± 0.11	201 ± 11	2.0 ± 0.09	0.90 ± 0.02
	<i>mmp2^{ss218/Df}</i>	13	295 ± 10	1.74 ± 0.08	263 ± 8	1.52 ± 0.07	0.90 ± 0.03
	UH1/+	16	173 ± 11	1.31 ± 0.12	178 ± 11	1.24 ± 0.11	0.95 ± 0.02
	UH1>dblRNAi	17	184 ± 7	1.15 ± 0.08	200 ± 11	1.12 ± 0.07	0.98 ± 0.02
Alternative GluR Subunits	<i>Genotype</i>	<i>n</i>	<i>GluRIIA Density</i>	<i>GluRIIA Muscle Intensity (A.U.)</i>	<i>n</i>	<i>GluRIIB Density</i>	<i>GluRIIB Muscle Intensity (A.U.)</i>
	<i>w¹¹¹⁸</i>	13	1.15 ± 0.07	1.0 ± 0.09	27	1.18 ± 0.05	1.0 ± 0.03
	<i>mmp1^{Q112*/Q273*}</i>	14	1.32 ± 0.05	0.90 ± 0.12	13	1.86 ± 0.11	0.86 ± 0.02
	<i>mmp2^{ss218/Df}</i>	14	1.47 ± 0.09	0.98 ± 0.11	24	1.60 ± 0.06	1.32 ± 0.06
	UH1/+	16	1.20 ± 0.06	1.0 ± 0.1	-	-	-
	UH1>dblRNAi	16	1.07 ± 0.05	0.59 ± 0.09	-	-	-

Table S3. Raw values for NMJ immunocytochemical studies						
A. IHC: NMJ Mmp Levels			Mmp1 Levels (A.U.)		Mmp2 Levels (A.U.)	
Genetic LOF	<i>w¹¹¹⁸</i>	n	mean ± sem	n	mean ± sem	
		19	1.0 ± 0.06	15	1.0 ± 0.05	
	<i>mmp1^{Q112*/Q273*}</i>	12	0.36 ± 0.06	10	0.45 ± 0.06	
	<i>mmp2^{W307*}</i>	-	-	15	0.43 ± 0.03	
	<i>mmp2^{ss218/Df}</i>	24	1.64 ± 0.09	11	0.4 ± 0.07	
Ubiquitous Knockdown	<i>UH1/+</i>	13	1.0 ± 0.06	25	1.0 ± 0.04	
	<i>UH1>mmp1^{RNAi}</i>	9	0.33 ± 0.04	-	-	
	<i>UH1>mmp2^{RNAi}</i>	-	-	15	0.56 ± 0.07	
	<i>UH1>mmp2^{RNAi1794-1R}</i>	-	-	11	0.51 ± 0.04	
	<i>UH1/+</i>	23	1.0 ± 0.07	22	1.0 ± 0.06	
	<i>UH1>dblRNAi</i>	24	0.23 ± 0.02	15	0.50 ± 0.07	
	<i>UH1/+</i>	8	1.0 ± 0.06	10	1.0 ± 0.12	
	<i>UH1>Timp</i>	7	0.50 ± 0.04	14	0.70 ± 0.04	
	<i>elav/+</i>	16	1.0 ± 0.08	-	-	
	<i>elav>mmp1^{RNAi}</i>	19	0.67 ± 0.05	-	-	
Muscle KD	<i>elav>mmp2^{RNAi}</i>	-	-	-	-	
	<i>elav>mmp2^{RNAi1794-1R}</i>	-	-	-	-	
	<i>24B/+</i>	15	1.0 ± 0.07	11	1.0 ± 0.09	
	<i>24B>mmp1^{RNAi}</i>	17	0.54 ± 0.06	11	0.44 ± 0.10	
	<i>24B>mmp2^{RNAi}</i>	11	1.4 ± 0.12	9	0.57 ± 0.07	
	<i>24B>mmp2^{RNAi1794-1R}</i>	12	1.5 ± 0.20	13	0.48 ± 0.06	

B. IHC: NMJ Timp Levels and Area						
Genotype			Timp Levels (A.U.)		Perisynaptic Timp Domain (μm^2)	
Genetic LOF	<i>w¹¹¹⁸</i>	n	mean ± sem	n	mean ± sem	
		15	1.0 ± 0.09	15	0.65 ± 0.07	
	<i>mmp1^{Q112*/Q273*}</i>	21	1.1 ± 0.10	21	0.69 ± 0.06	
	<i>mmp2^{ss218/Df}</i>	15	0.84 ± 0.06	15	1.4 ± 0.09	
	<i>tim^p</i>	6	0.43 ± 0.02	-	-	

C. IHC: NMJ Wg Signaling Pathway						
Extracellular Wg Ligand	<i>Genotype</i>	Intensity (A.U.)		% Wg-Expressing Boutons		
		<i>n</i>	<i>mean</i> \pm <i>sem</i>	<i>n</i>	<i>mean</i> \pm <i>sem</i> (%)	
	<i>w¹¹¹⁸</i>	31	1.0 \pm 0.03	31	68 \pm 5	
	<i>mmp1^{Q112*/Q273*}</i>	17	0.63 \pm 0.05	20	31 \pm 5	
	<i>mmp2^{W307*/Df}</i>	35	0.92 \pm 0.02	36	75 \pm 3	
	<i>mmp2^{ss218/Df}</i>	29	0.93 \pm 0.02	27	77 \pm 3	
	UH1/+	22	1.0 \pm 0.08	24	72 \pm 6	
	UH1> <i>mmp1^{RNAi}</i>	20	0.88 \pm 0.06	10	39 \pm 5	
	UH1>dblRNAi	22	0.97 \pm 0.08	23	74 \pm 8	

FrzC2 Receptor	<i>Genotype</i>	NMJ Intensity (A.U.)		Nuclear Intensity (A.U.)	
		<i>n</i>	<i>mean</i> \pm <i>sem</i>	<i>n</i>	<i>mean</i> \pm <i>sem</i>
	<i>w¹¹¹⁸</i>	20	1.0 \pm 0.06	20	1.0 \pm 0.05
	<i>mmp1^{Q112*/Q273*}</i>	19	1.16 \pm 0.07	19	1.25 \pm 0.08
	<i>mmp2^{ss218/Df}</i>	22	1.21 \pm 0.07	22	1.35 \pm 0.07
	UH1/+	26	1.0 \pm 0.03	26	1.0 \pm 0.03
	UH1>dblRNAi	22	0.88 \pm 0.08	22	1.08 \pm 0.08

			0.04		
Dlp Co-receptor	<i>Genotype</i>	Intensity (A.U.)	Perisynaptic Dlp Area:HRP Area		
		<i>n</i>	<i>mean</i> \pm <i>sem</i>	<i>n</i>	<i>mean</i> \pm <i>sem</i>
		22	1.0 \pm 0.06	23	0.60 \pm 0.04
	<i>mmp1</i> ^{Q112*/Q273*}	12	0.73 \pm 0.06	13	0.38 \pm 0.03
	<i>mmp2</i> ^{ss218/Df}	22	0.93 \pm 0.05	24	1.11 \pm 0.06
	UH1/+	26	1.0 \pm 0.02	35	0.58 \pm 0.04
	UH1>dblRNAi	26	0.93 \pm 0.07	35	0.59 \pm 0.02