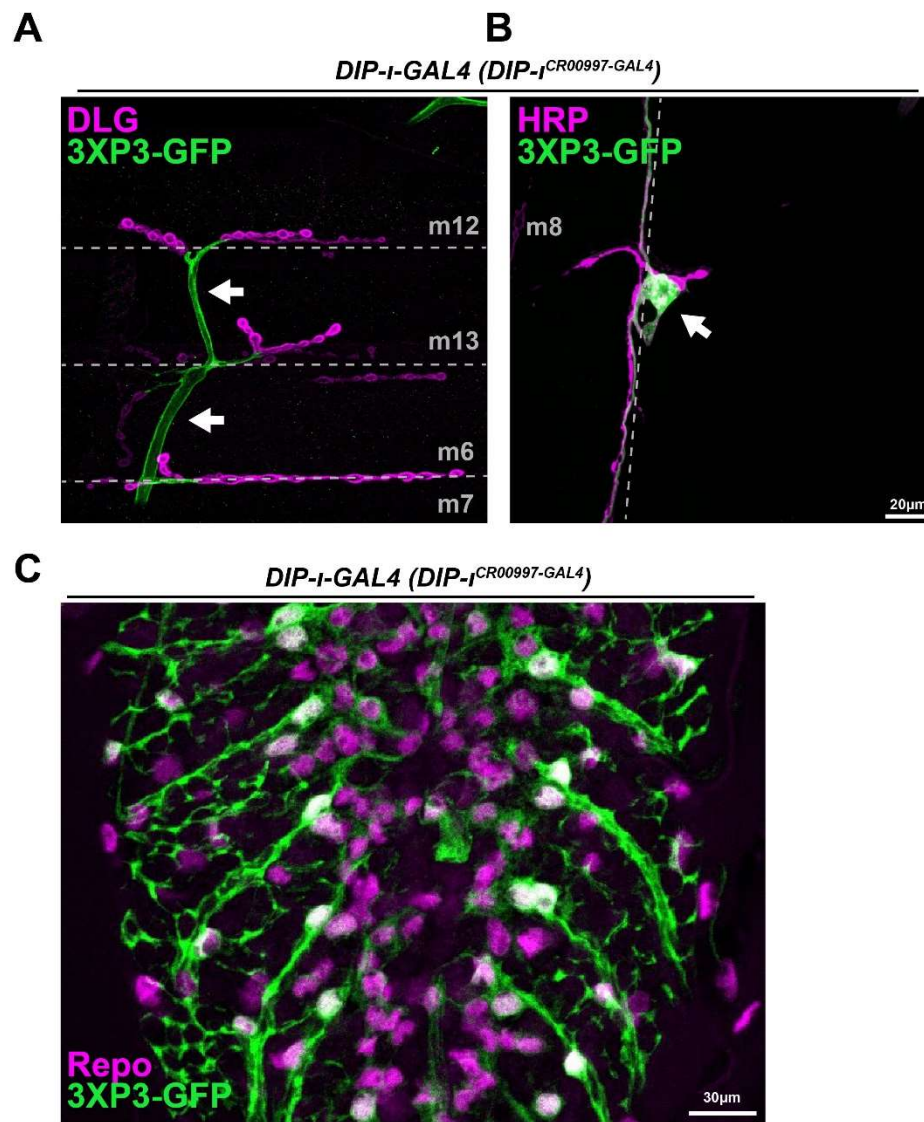**Fig. S1. Respective mRNA level in *dpr/DIP-GAL4* lines.**

A. qRT-PCR results of homozygous viable *dpr/DIP-GAL4* lines. mRNA levels were double normalized to control animal and Rpl32 internal control.
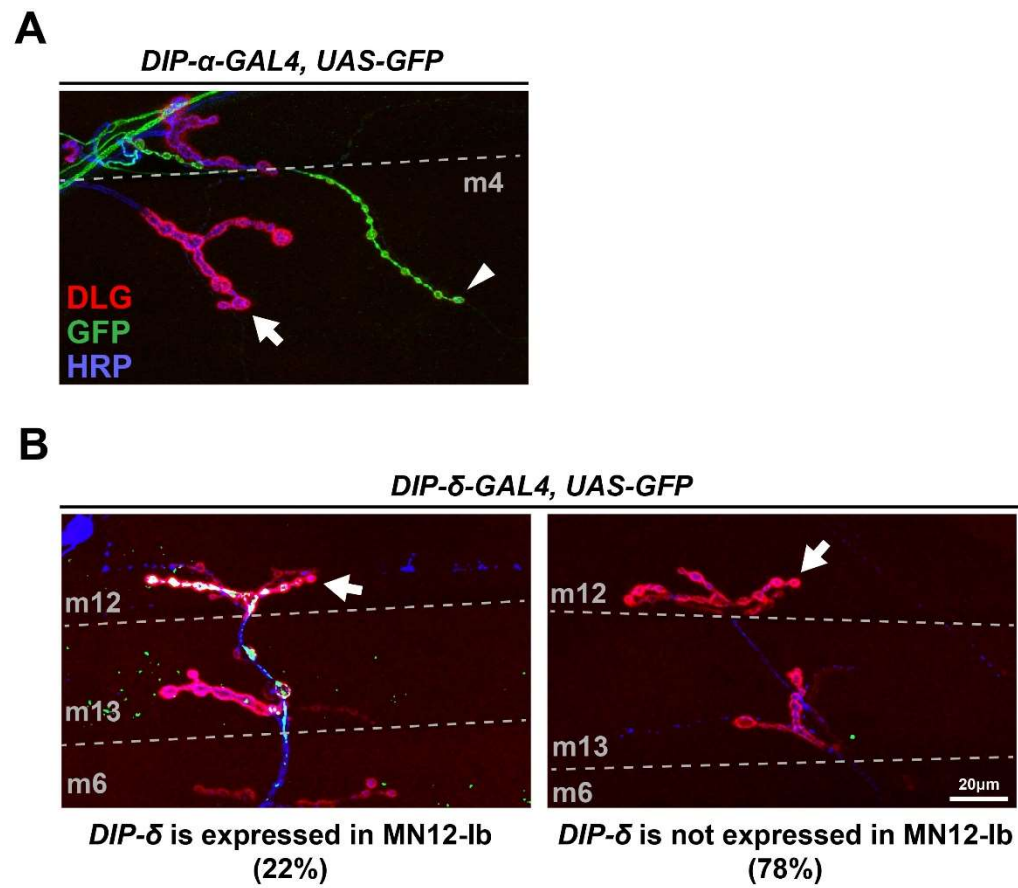
B. qRT-PCR results of homozygous lethal *dpr/DIP-GAL4* lines. mRNA levels were double normalized to control animal and Rpl32 internal control.

Fig. S2. Expression of CRIMIC 3XP3-GFP in nervous system.

A-B. Representative images showing the expression of 3XP3-GFP in peripheral glial cells (arrow) and the lbd SN (arrow).
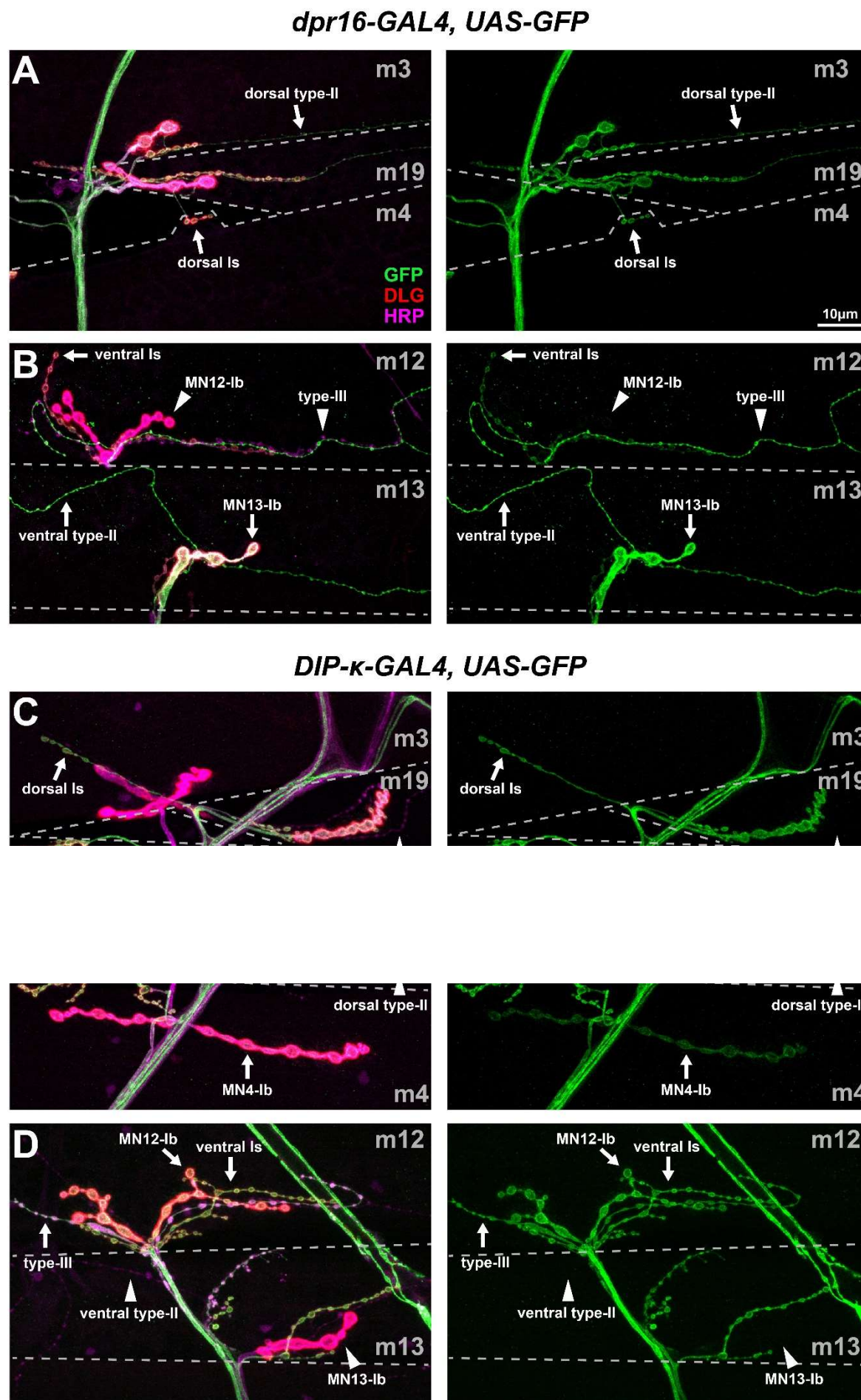
C. Representative image showing the expression of 3XP3-GFP in some glial cells in the VNC.

A

DIP-α-GAL4, UAS-GFP

m4

DLG
GFP
HRP

B

DIP-δ-GAL4, UAS-GFP

m12

m13

m6

DIP-δ is expressed in MN12-Ib
(22%)

m12

m13

m6

20µm

DIP-δ is not expressed in MN12-Ib
(78%)

Fig. S3. Expression of *DIP-α* and *DIP-δ* in MNs.

A. Representative image showing the expression of *DIP-α* in Is MNJ (arrowhead) but not in adjacent Ib NMJ (arrow).

B. Representative images showing the varied expression of *DIP-δ* in MN12-Ib (arrow). Note that 22% MN12-Ib express *DIP-δ* (left) whereas 78% do not express (right).

Fig. S4. Expression of *dpr16* and *DIP-κ* in type-II and type-III MNs.

A-B. Representative images showing the expression of *dpr16* in dorsal and ventral type-II MNs, dorsal and ventral Is MNs and MN13-Ib (arrows), but not in MN12-Ib and type-III MN (arrowheads).

C-D. Representative images showing the expression of *DIP-κ* in dorsal and ventral Is MNs, MN4-Ib, MN12-Ib and type-III MN (arrows), but not in dorsal and ventral type-II MNs (arrowheads).
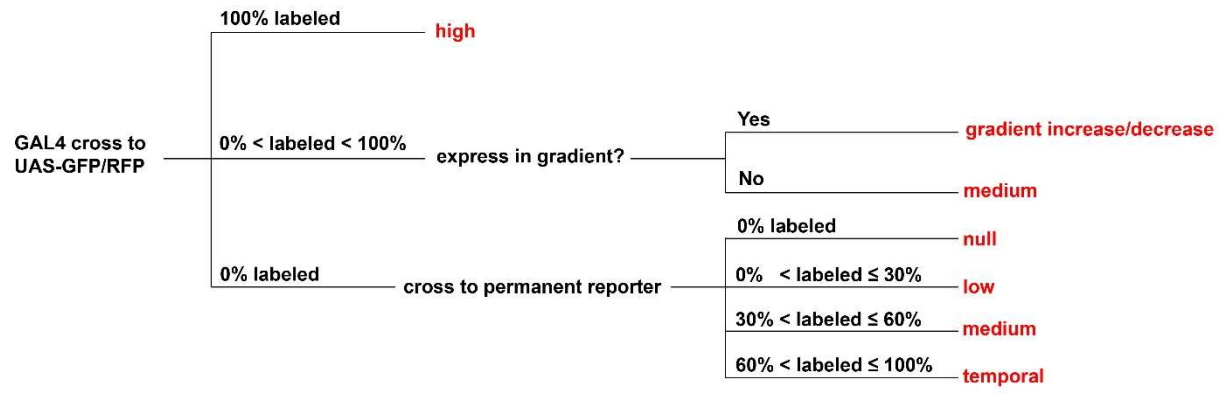
Fig. S5. Criteria to determine the expression of GAL4 in a certain MN/SN.

A graphical flow chart depicting how we scored the *dpr*/*DIP* expression data into categories.

A *dpr19-GAL4, UAS-mCherry*
(first instar)

B *dpr19-GAL4, UAS-mCherry*
(third instar)
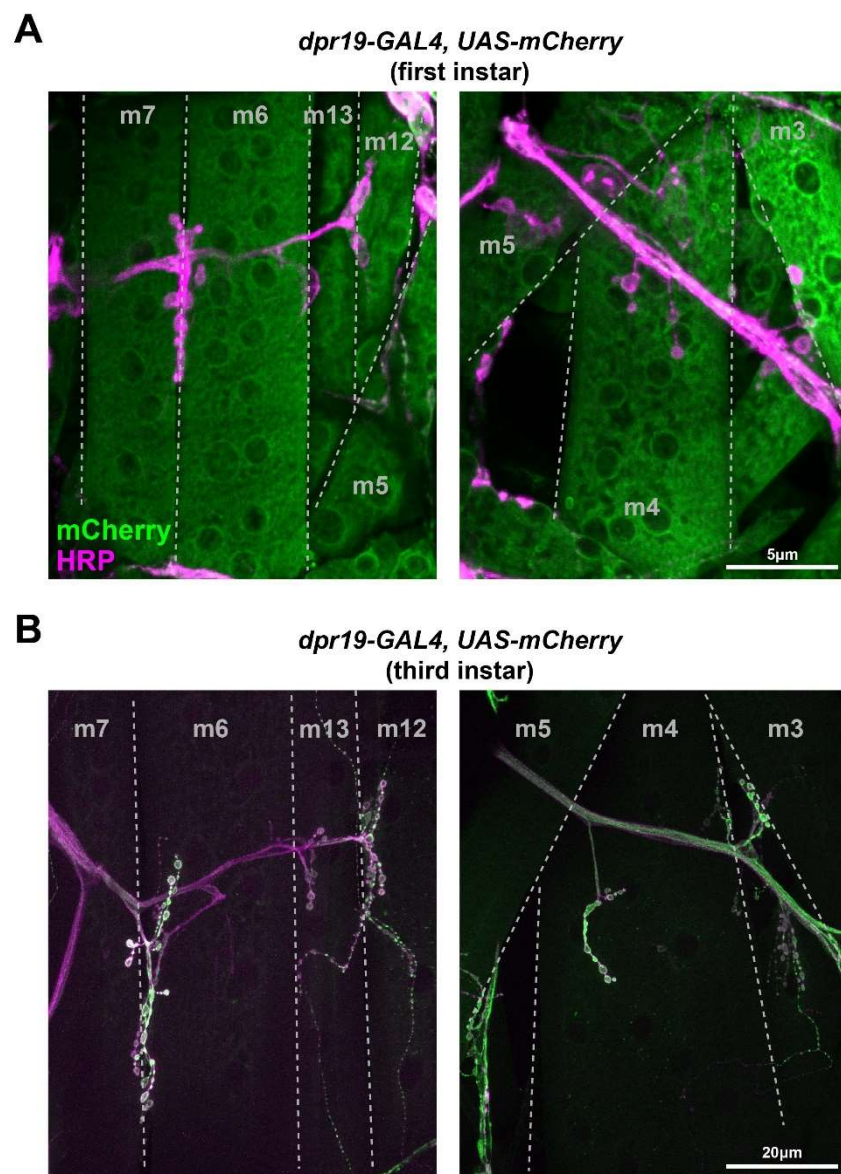
Fig. S6. *dpr19* **is expressed in muscles in early larval development.**

A. Representative images of *dpr19-GAL4>mCherry* in first instar larvae. *dpr19* is expressed in ventral (left) and dorsal (right) muscles.

B. Representative images of *dpr19-GAL4>mCherry* in third instar larvae. *dpr19* is not expressed in ventral (left) or dorsal (right) muscles.
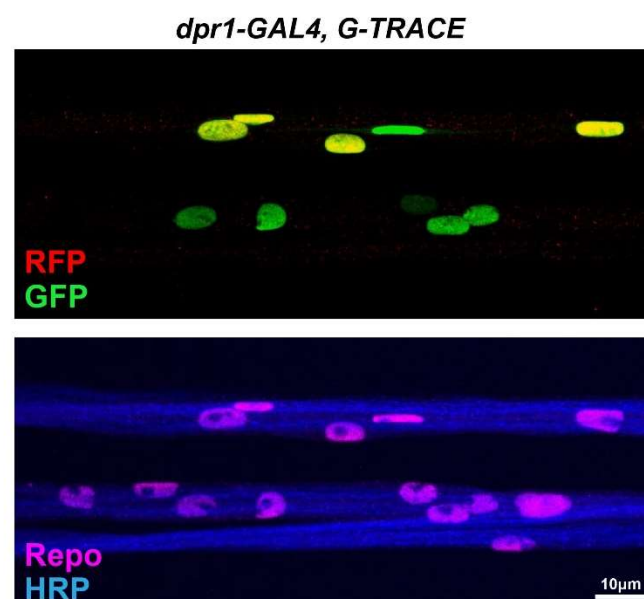

dpr1-GAL4, G-TRACE

Fig. S7. The G-TRACE system revealed *dpr1* **expression in peripheral glial cells.** *dpr1* is the only *dpr/DIP* expressed in peripheral glial cells. *dpr1-GAL4* is expressed in subsets of peripheral glial cells as indicated by some glial nuclei labeled by both GFP and RFP, some only by GFP, and some lacking both GFP and RFP.
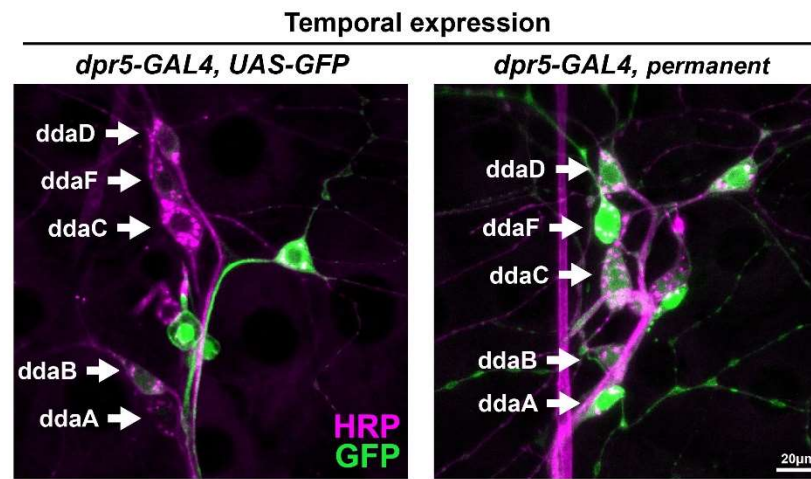
Fig. S8. Temporal expression of *dpr5* in the dorsal da neuron cluster.

ddaA, ddaC, ddaF and ddaD are not labeled in *dpr5-GAL4>GFP* larvae but are robustly labeled in the cross to the permanent reporter. Therefore, *dpr5* is temporally expressed in these SNs. ddaB is labeled in the cross to the real-time reporter with a low frequency, thus *dpr5* is considered as low expression in ddaB.
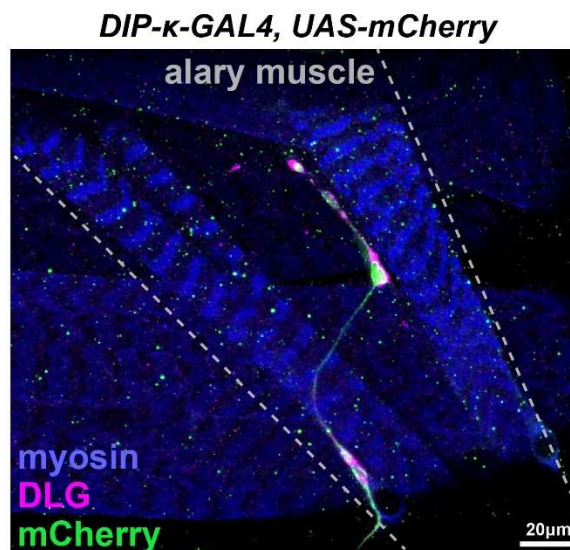
Fig. S9. The alary MN also expresses *dprs* and *DIPs*.

Representative image showing *DIP-κ* expression in the alary MN. Alary MNs display features of excitatory MNs as they have DLG accumulation around boutons.
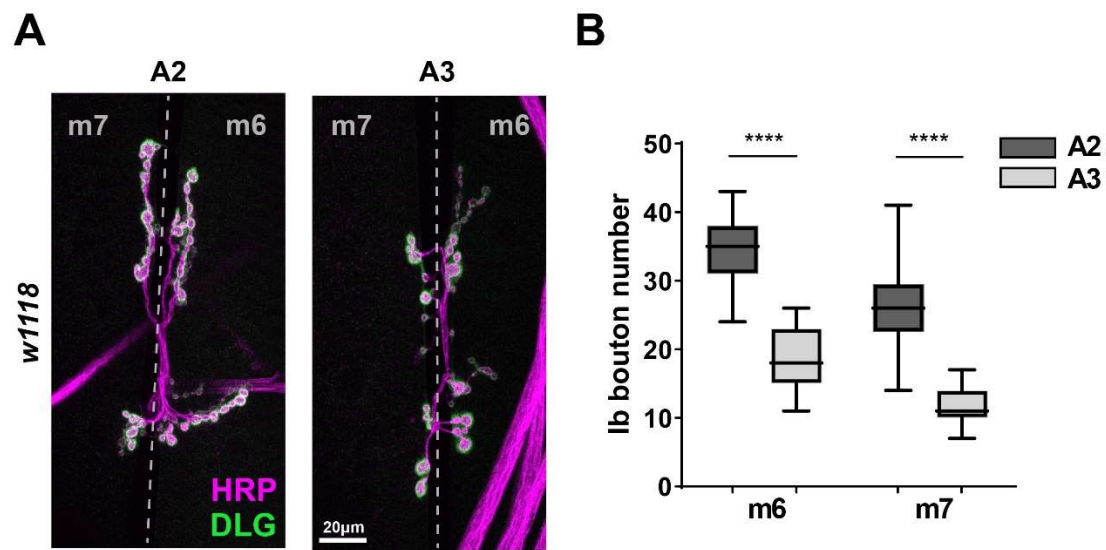
Fig. S10. Larger NMJs on m6 and m7 in A2.

A. Representative images showing larger type-Ib NMJs on m6 and m7 in A2 compared to A3.

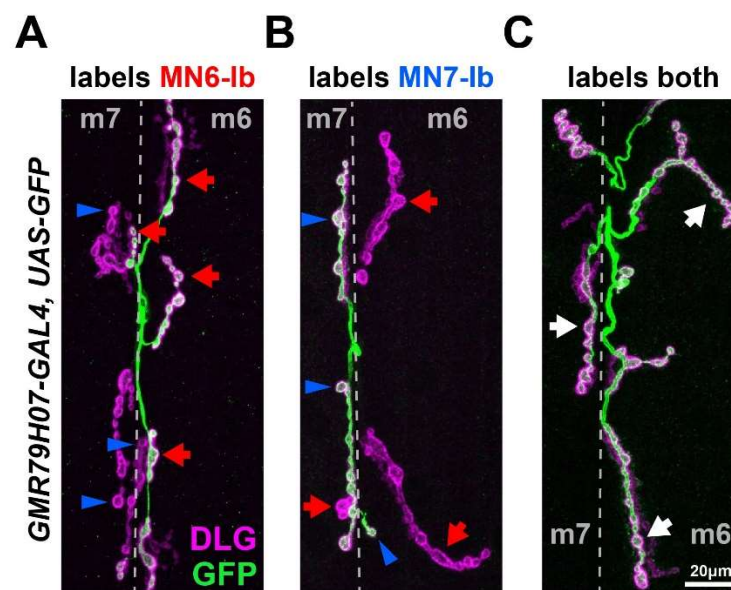B. Bouton number counts from m6 and m7 in A2 and A3 confirmed that A2 NMJs are double the size of A3 (n = 17 and 15 for A2 and A3, respectively, ****p<0.0001).

Fig. S11. *GMR79H07-GAL4* randomly labels MN6-Ib and MN7-Ib.

A previous study reported that *GMR79H07-GAL4* labels type-Ib NMJs on m6 in A2 (Aponte-Santiago et al., 2020). We crossed this driver to *UAS-GFP* and found inconsistent expression patterns since it (A) sometimes only labels MN6-Ib (red arrows), (B) sometimes only labels MN7-Ib (blue arrowheads), or (C) sometimes labels both MNs (white arrows). These expression patterns support the existence of MN6-Ib and MN7-Ib and their dual innervation properties.

Table S1. *dpr/DIP-GAL4* lines information

GAL4 line	GAL4 derived from	Insertion site	Genotype	Sources
DIP-α-GAL4	MI10680 (T2A-GAL4)	6th coding intron	$y^1\ w^*\ Mi\{Trojan\text{-}GAL4.1\}DIP\text{-}\alpha^{MI10680\text{-}TG4.1}$	GDP$^{\#}$
DIP-β-GAL4	MI01971 (GT-GAL4)	5' UTR intron	$y^1\ w^*\ Mi\{GT\text{-}GAL4\}DIP\text{-}\beta^{MI01971\text{-}GAL4}$	(BL#90316)
DIP-γ-GAL4	MI03222 (GT-GAL4)	5' UTR intron	$Mi\{GT\text{-}GAL4\}DIP\text{-}\gamma^{MI03222\text{-}GAL4}$	(BL#90315)
DIP-δ-GAL4	MI08287 (T2A-GAL4)	4th coding intron	$Mi\{Trojan\text{-}GAL4.1\}DIP\text{-}\delta^{MI08287\text{-}TG4.1}$	(BL#90320)
DIP-ε-GAL4	MI11827 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}DIP\text{-}\varepsilon^{MI11827\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	(BL#67502)
DIP-ζ-GAL4	MI03838 (T2A-GAL4)	2nd coding intron	$Mi\{Trojan\text{-}GAL4.0\}DIP\text{-}\zeta^{MI03838\text{-}TG4.0}$	(BL#90317)
DIP-η-GAL4	MI07948 (T2A-GAL4)	4th coding intron	$Mi\{Trojan\text{-}GAL4.1\}DIP\text{-}\eta^{MI07948\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	(BL#90318)
DIP-θ-GAL4	MI03191 (T2A-GAL4)	2nd coding intron	$Mi\{Trojan\text{-}GAL4.1\}DIP\text{-}\theta^{MI03191\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	GDP$^{\#}$
DIP-ι-GAL4	CR00997 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}DIP\text{-}\iota^{CR00997\text{-}TG4.1}$	(BL#83243)
DIP-κ-GAL4	CR01146 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}DIP\text{-}\kappa^{CR011146\text{-}TG4.1},Gpo3^{CR01114\text{-}TG4.1X}/CyO,Dfd\text{-}YFP$	(BL#83252)
DIP-λ-GAL4	CR70096 (T2A-GAL4)	2nd coding intron	$Tl\{CRIMIC\text{-}TG4.0\}DIP\text{-}\lambda^{CR70096\text{-}TG4.0}$	This study
dpr1-GAL4	MI12729 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr1^{MI12729\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	GDP$^{\#}$
dpr2-GAL4	MI05656 (T2A-GAL4)	4th coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr2^{MI05656\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	GDP$^{\#}$
dpr3-GAL4	MI05963 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr3^{MI05963\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	GDP$^{\#}$
dpr4-GAL4	CR00485 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}dpr4^{CR00485\text{-}TG4.1}/TM6,Sb,Hu,Dfd\text{-}YFP$	(BL#79271)
dpr5-GAL4	MI11085 (T2A-GAL4)	2nd coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr5^{MI11085\text{-}TG4.1}$	GDP$^{\#}$
dpr6-GAL4	MI01358 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr6^{MI01358\text{-}TG4.1}$	GDP$^{\#}$
dpr7-GAL4	MI05719 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr7^{MI05719\text{-}TG4.1}$	(BL#78385)
dpr8-GAL4	MI11830 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr8^{MI11830\text{-}TG4.1}$	This study
dpr9-GAL4	MI03594 (T2A-GAL4)	3rd coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr9^{MI03594\text{-}TG4.1}$	GDP$^{\#}$
dpr10-GAL4	MI03557 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr10^{MI03557\text{-}TG4.1}$	GDP$^{\#}$
dpr11-GAL4	MI01743 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr11^{MI01743\text{-}TG4.1}$	GDP$^{\#}$
dpr12-GAL4	MI01695 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr12^{MI01695\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	GDP$^{\#}$
dpr13-GAL4	MI05577 (T2A-GAL4)	2nd coding intron	$Mi\{Trojan\text{-}GAL4.0\}dpr13^{MI05577\text{-}TG4.0}/CyO,actin\text{-}GFP$	This study
dpr14-GAL4	CR00516 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}dpr14^{CR00516\text{-}TG4.1}$	(BL#80586)
dpr15-GAL4	MI01408 (T2A-GAL4)	3rd coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr15^{MI01408\text{-}TG4.1}/TM6,Sb,Hu,Dfd\text{-}YFP$	(BL#66827)
dpr16-GAL4	MI05173 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr16^{MI05173\text{-}TG4.1}$	GDP$^{\#}$
dpr17-GAL4	MI08707 (T2A-GAL4)	1st coding intron	$Mi\{Trojan\text{-}GAL4.1\}dpr17^{MI08707\text{-}TG4.1}/TM6,Sb,Hu,Dfd\text{-}YFP$	(BL#76200)
dpr18-GAL4	CR01009 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}dpr18^{CR01009\text{-}TG4.1}$	(BL#83245)
dpr19-GAL4	CR00996 (T2A-GAL4)	1st coding intron	$Tl\{CRIMIC\text{-}TG4.1\}dpr19^{CR00996\text{-}TG4.1}/CyO,Dfd\text{-}YFP$	(BL#83242)

GDP$^{\#}$: *Drosophila Gene Disruption Project*

Table S2. mRNA level and primer information

Gene	Tested Genotype	(norm.) mRNA level	Primer	Sequence
DIP-α	*DIP-α^MI10680-GAL4*	0.02±0.02	*DIP-α-qPCR-F*	GGAGTACCGCCATCGGTCTC
			DIP-α-qPCR-R	GGCTTCGACGTGACACTCG
DIP-β	*DIP-β^MI01971-GAL4*	0.55±0.11	*DIP-β-qPCR-F*	GAGCACGTGGTCATCCGAAG
			DIP-β-qPCR-R	GGAGGCAATGCACATGTAGGC
DIP-γ	*DIP-γ^MI03222-GAL4*	0.16±0.04	*DIP-γ-qPCR-F*	GAGAGCCTCCGATCAGACCG
			DIP-γ-qPCR-R	CCTGCACATCGATACAACCC
DIP-δ	*DIP-δ^MI08287-GAL4*	0.35±0.16	*DIP-δ-qPCR-F*	GGCAACTATCGATGCATCTCG
			DIP-δ-qPCR-R	GTCGTTGCGTGATGAGGGTATG
DIP-ε	*DIP-ε^MI11827-GAL4/CyO,Dfd-YFP*	0.20±0.06	*DIP-ε-qPCR-F*	CGGCCAAGACCCAGTATGG
			DIP-ε-qPCR-R	GATTTTGTTGCCGTCATCGCG
DIP-ζ	*DIP-ζ^MI03838-GAL4*	0.04±0.004	*DIP-ζ-qPCR-F*	GTGGAAGCCACAGTCGGATTG
			DIP-ζ-qPCR-R	GAGGCAGTGGTTGGAGGATATG
DIP-η	*DIP-η^MI07948-GAL4/CyO,Dfd-YFP*	0.41±0.23	*DIP-η-qPCR-F*	GGCAGTAACGTGACGCTCAAATG
			DIP-η-qPCR-R	CGAGGGAGGGACTCCATTGG
DIP-θ	*FRT40A, DIP-θ^MI03191-GAL4*	0.08±0.10	*DIP-θ-qPCR-F*	CTCCTGCAGAACGTAACGGTG
			DIP-θ-qPCR-R	GCGTGAGTTATGCTCATGCG
DIP-ι	*DIP-ι^CR00997-GAL4*	0.89±0.20	*DIP-ι-qPCR-F*	GGGATGCCCTACTCACGTGTG
			DIP-ι-qPCR-R	CATCCGCGATCCGATTCCTG
DIP-κ	*DIP-κ^CR011146-GAL4/CyO,Dfd-YFP*	0.51±0.30	*DIP-κ-qPCR-F*	GACACGCAGACGATCCTGTC
			DIP-κ-qPCR-R	CGTGTTCACTTGGCACATGTAC
DIP-λ	*DIP-λ^CR70096-GAL4*	1.50±0.05	*DIP-λ-qPCR-F*	CACCCACATGGTCTCACTAAATCC
			DIP-λ-qPCR-R	CTCTGGATTATGTTCTGGGTGG
dpr1	*dpr1^MI12729-GAL4/CyO,Dfd-YFP*	0.92±0.10	*dpr1-qPCR-F*	GAGCCCAAGATGTCCCTGTC
			dpr1-qPCR-R	GAATATGTTGCCCAGCTCGTGG
dpr2	*dpr2^MI05656-GAL4/CyO,Dfd-YFP*	0.93±0.27	*dpr2-qPCR-F*	CGTCGGCGCAGGATATTGG
			dpr2-qPCR-R	GATGACATTGACCACCACGCTG
dpr3	*dpr3^MI05963-GAL4/CyO,Dfd-YFP*	0.55±0.24	*dpr3-qPCR-F*	GGACGCTGCATGTGAAGGC
			dpr3-qPCR-R	GCCTTGAAGTGCAGATCGGG
dpr4	*dpr4^CR00485-GAL4/TM6,Sb,Hu,Dfd-YFP*	0.56±0.20	*dpr4-qPCR-F*	CGGATTCGGGCAACTACACG
			dpr4-qPCR-R	GCACGGACGTGGATGAAAGG
dpr5	*dpr5^MI11085-GAL4*	0.13±0.02	*dpr5-qPCR-F*	CACGGAGCCCAAGATCAGTC
			dpr5-qPCR-R	CGTGTCCTTGTGCCACAGC
dpr6	*dpr6^MI01358-GAL4*	0.23±0.03	*dpr6-qPCR-F*	CCATCACCAGGACACGGAGG
			dpr6-qPCR-R	GACACGCCGCCTCTTGATG
dpr7	*dpr7^MI05719-GAL4*	0.18±0.10	*dpr7-qPCR-F*	GCACAGCCAAGAGACAGTGG
			dpr7-qPCR-R	GCCAGGGCAATAGTGCTATCCC
dpr8	*dpr8^MI11830-GAL4/FM7,Dfd-YFP*	0.49±0.19	*dpr8-qPCR-F*	GAATTTGGGCAATCGCACG

			dpr8-qPCR-R	CGCAATGTCCAATCCTCGG
dpr9	*dpr9^{MI03594-GAL4}*	0.08±0.09	*dpr9-qPCR-F*	GGGCGATACGACCACATCG
			dpr9-qPCR-R	CTCCCCTGGAAACGGAATGG
dpr10	*dpr10^{MI03557-GAL4}*	0.06±0.05	*dpr10-qPCR-F*	GGGCTGTCGTGTCAAGCATC
			dpr10-qPCR-R	GCCCACTTGATCTGCAGGG
dpr11	*dpr11^{MI01743-GAL4}*	0.30±0.06	*dpr11-qPCR-F*	GTGTCCAGCTGCAAGTTGTGG
			dpr11-qPCR-R	GGTTGGGATCCAGTTGCGTG
dpr12	*dpr12^{MI01695-GAL4}/CyO,Dfd-YFP*	0.82±0.05	*dpr12-qPCR-F*	CGGCATGTACGAGTGCCAG
			dpr12-qPCR-R	CGTACTGCGGTGGTGTAGGAC
dpr13	*dpr13^{MI05577-GAL4}/CyO,actin-GFP*	0.92±0.06	*dpr13-qPCR-F*	GTTCAGTGCCACGCACTTGAAG
			dpr13-qPCR-R	CAGCGTTGAACCTGGGGTTA
dpr14	*dpr14^{CR00516-GAL4}*	0.04±0.02	*dpr14-qPCR-F*	GGTGGTGCATGTGCTGAACG
			dpr14-qPCR-R	CACTTGTCCCAAGCCCAATCC
dpr15	*FRT82B, dpr15^{MI01408-GAL4}*	0.57±0.09	*dpr15-qPCR-F*	CCATTGTCCTCCACGTGCTG
			dpr15-qPCR-R	CTTGTGGTGGCTTTGCTGGTG
dpr16	*dpr16^{MI05173-GAL4}*	0.99±0.38	*dpr16-qPCR-F*	CACTCCGGCAACTACACGTG
			dpr16-qPCR-R	CCCGTGACCCAGTCTGTGG
dpr17	*FRT82B, dpr17^{MI08707-GAL4}*	0.01±0.01	*dpr17-qPCR-F*	CGGCGCAACCTGACAATGC
			dpr17-qPCR-R	GCATGCGCACCCATGAAACG
dpr18	*dpr18^{CR01009-GAL4}*	0.33±0.11	*dpr18-qPCR-F*	GCCGTGTCGGTATGCTCAAG
			dpr18-qPCR-R	CGCCAATTGTTCGGGTACTGG
dpr19	*dpr19^{CR00996-GAL41}/Cyo,Dfd-YFP*	0.55±0.13	*dpr19-qPCR-F*	CCTGAATCCCTCGGTCAGTG
			dpr19-qPCR-R	CATGGCCGCCGTTTTCTCAC
Rpl32	ALL		*Rpl32-qPCR-F*	ATGCTAAGCTGTCGCACAAATG
			Rpl32-qPCR-R	GTTCGATCCGTAACCGATGT