Appendix

Transneuronal Dpr12/DIP-δ interactions facilitate compartmentalized dopaminergic innervation of *Drosophila* mushroom body axons

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List of genotypes used in this study:

hsFLP is y,w,hsFLP122; tdT is tdTomato; mtdT-HA is mtdTomato-3xHA; 40A and G13 are FRTs on 2L and 2R respectively; R71G10 is GMR71G10-Gal4; R18H09 is GMR18H09-Gal4; R58E02 is GMR58E02-Gal4; R10G03 is GMR10G03-Gal4; R48H011 is GMR48H011-Gal4; G80 is TubP-Gal80. Males and females were used interchangeably but only the female genotype is mentioned.

Figure 1
(C) y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/+
(D) y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/ TRiP.JF03210(dpr12-RNAi)
(E) y, w; R71G10, UAS-mCD8-GFP/+; UAS-Cas9.C
(F) y, w; R71G10, UAS-mCD8-GFP/+; UAS-Cas9.C/dpr12-gRNA
(G-I) y, w; UAS-mCD8-GFP/+; R71G10/+
(J) y, w; UAS-mCD8-GFP/+; R71G10/UAS-Dpr12
(K-M) y, w; 40A, *dpr12*$^{\Delta 50\text{-}81}$, G13/40A, *dpr12*$^{\Delta 50\text{-}81}$, G13; R71G10, UAS-mCD8-GFP/+
(N) y, w; 40A, *dpr12*$^{\Delta 50\text{-}81}$, G13/40A, *dpr12*$^{\Delta 50\text{-}81}$, G13; R71G10, UAS-mCD8-GFP/UAS-Dpr12

Figure 2
(A-C, G-I) hsFLP, UAS-mCD8-GFP; Gal80, FRT40A/FRT40A; R71G10, UAS-Dcr-2/+
(D-F, J-L) hsFLP, UAS-mCD8-GFP; Gal80, FRT40A/FRT40A; R71G10, UAS-Dcr-2/ TRiP.JF03210 (dpr12-RNAi)
(O) y, w; UAS-mCD8-GFP/+; R18H09/+

Figure 3
(A-C) y, w; Dpr12 ^{GFSTF}/+
(D) y, w; UAS-CD4-tdT/Dpr12 ^{GFSTF}; R18H09/+
(E-G) y, w;; DIP-δ ^{GFSTF}/+
(H) y, w; UAS-CD4-tdT/+; DIP-δ ^{GFSTF}/R18H09

Figure 4
(A-C) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ*$^{T2A\text{-}Gal4}$/+
(D) y, w; R71G10-QF2, QUAS-mtdT-HA/UAS-*DIP-δ*; *DIP-δ*$^{T2A\text{-}Gal4}$/+
(E-G) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ*$^{T2A\text{-}Gal4}$/*DIP-δ*$^{T2A\text{-}Gal4}$
(H) y, w; R71G10-QF2, QUAS-mtdT-HA/UAS-*DIP-δ*; *DIP-δ*$^{T2A\text{-}Gal4}$/*DIP-δ*$^{T2A\text{-}Gal4}$
(J) y, w; UAS-*DIP-δ-RNAi*/R71G10-QF2, QUAS-mtdT-HA; Repo-Gal4, UAS-mCD8-GFP/+
(K) y, w; UAS-*DIP-δ-RNAi*/R71G10-QF2, QUAS-mtdT-HA; OK107-Gal4
(L) C155-Gal4; UAS-*DIP-δ-RNAi*/R71G10-QF2, QUAS-mtdT-HA
(M) y, w; UAS-*DIP-δ-RNAi*/R71G10-QF2, QUAS-mtdT-HA; *DIP-δ*$^{T2A\text{-}Gal4}$/+

Figure 5
(A) UAS-mCD8-RFP; R53C03-p65.AD/+; DIP-δ ^{GFSTF}/R24E12-GAL4.DBD
(B) UAS-mCD8-RFP; R53C03-p65.AD/UAS-DTi; DIP-δ ^{GFSTF}/R24E12-GAL4.DBD

(C) y, w; UAS-CD4-tdT/+; DIP-δ ^{GFSTF}/R58E02
(D) y, w; UAS-CD4-tdT/UAS-DTi; DIP-δ ^{GFSTF}/R58E02
(E-G) hsFLP, UAS-mCD8-GFP; Gal80, FRT40A/FRT40A; *DIP-δ$^{T2A-Gal4}$*/+

Figure 6
(A-D) y, w; Dpr12 ^{GFSTF}/+; *DIP-δ$^{T2A-Gal4}$*/*DIP-δ$^{T2A-Gal4}$*
(E-H) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; DIP-δ^{GFSTF}/+
(I) y, w; Dpr12 ^{GFSTF}/+; UAS-DIP-δ-T2A-tdT/+
(J) y, w; Dpr12 ^{GFSTF} /R30G08-p65.AD; R48B03-GAL4.DBD/ UAS-DIP-δ-T2A-tdT

Figure 7
(A) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R10G03/UAS-mCD8-GFP
(B) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R10G03/UAS-mCD8-GFP
(C) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R48H011/UAS-mCD8-GFP
(D) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R48H011/UAS-mCD8-GFP
(E) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R18H09/UAS-mCD8-GFP
(F) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R18H09/UAS-mCD8-GFP
(G) w^{1118}
(H) w^{1118}; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13

Figure 8
(A) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{T2A-Gal4}$*/*DIP-δ$^{1-119}$*
(B) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{T2A-Gal4}$*/*DIP-δ$^{1-119}$*, UAS-DIP-α
(C) y,w; Dpr12 ^{GFSTF}/+; *DIP-δ$^{1-119}$*, UAS-DIP-α/+
(D) y,w; Dpr12 ^{GFSTF}/+; *DIP-δ$^{1-119}$*, UAS-DIP-α/*DIP-δ$^{T2A-Gal4}$*
(E) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; *DIP-δ$^{T2A-Gal4}$*/ UAS-mtdT
(F) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; *DIP-δ$^{T2A-Gal4}$*/ UAS-DIP-α-T2A-tdT

Figure EV1
(A)
y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/ TRiP.JF03306(Dpr5-RNAi)
y, w; UAS-mCD8-GFP/+; R71G10/ TRiP.JF03172 (Dpr8-RNAi)
y, w; UAS-mCD8-GFP/+; R71G10/ TRiP.HMS00288 (Dpr9-RNAi)
y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/ TRiP.JF02920 (Dpr10-RNAi)
y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/ TRiP.JF03210(Dpr12-RNAi)
y, w; UAS-mCD8-GFP/+; R71G10/ TRiP.GL01238 (Dpr17-RNAi)
y, w; R71G10, UAS-mCD8-GFP/+; R71G10, UAS-Dcr-2/ TRiP.JF03283}attP2 (Dpr18-RNAi)
y, w; UAS-mCD8-GFP/+; R71G10/ TRiP.JF02923 (Dpr20-RNAi)
(D)
y, w; 40A, *Dpr12$^{Δ50-81}$*, G13/40A, *Dpr12$^{Δ50-81}$*, G13; R71G10, UAS-mCD8-GFP/+
y, w; 40A, *Dpr12$^{Δ50-81}$*, G13/40A, *Dpr12$^{Δ50-81}$*, G13; R71G10, UAS-mCD8-GFP/+
y, w; 40A, *Dpr12$^{Δ50-81}$*, G13/40A, *Dpr12$^{Δ50-81}$*, G13; R71G10, UAS-mCD8-GFP/+
y, w; UAS-mCD8-GFP/+; R71G10/+

Figure EV2

(A) hsFLP, UAS-mCD8-GFP; Gal80, FRT40A/FRT40A; R71G10, UAS-Dcr-2/+
(B) hsFLP, UAS-mCD8-GFP; Gal80, FRT40A/FRT40A; R71G10, UAS-Dcr-2/ TRiP.JF03210 (Dpr12-RNAi)
(D) y, w; UAS-mCD8-GFP/R30G08-p65.AD; R48B03-GAL4.DBD/ +
(E) y, w; UAS-mCD8-GFP/+; R18H09/ +
(F) y, w; UAS-mCD8-GFP/+; R48H011/+

Figure EV3
(C-D) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{T2A-Gal4}$/ DIP-δ$^{1-119}$*
(E) y, w; R71G10-QF2, QUAS-mtdT-HA/UAS-*DIP-δ*; *DIP-δ$^{T2A-Gal4}$/DIP-δ$^{T2A-Gal4}$*
(F) C155-Gal4; UAS-Cas9.P2/+
(G) C155-Gal4; UAS-Cas9.P2/TKO.GS02451(*gRNA DIP-δ)*
(H) y, w; R71G10, UAS-mCD8-GFP/ +; UAS-Cas9.C/+
(I) y, w; R71G10, UAS-mCD8-GFP/ TKO.GS02451(*gRNA DIP-δ)*; UAS-Cas9.C/+

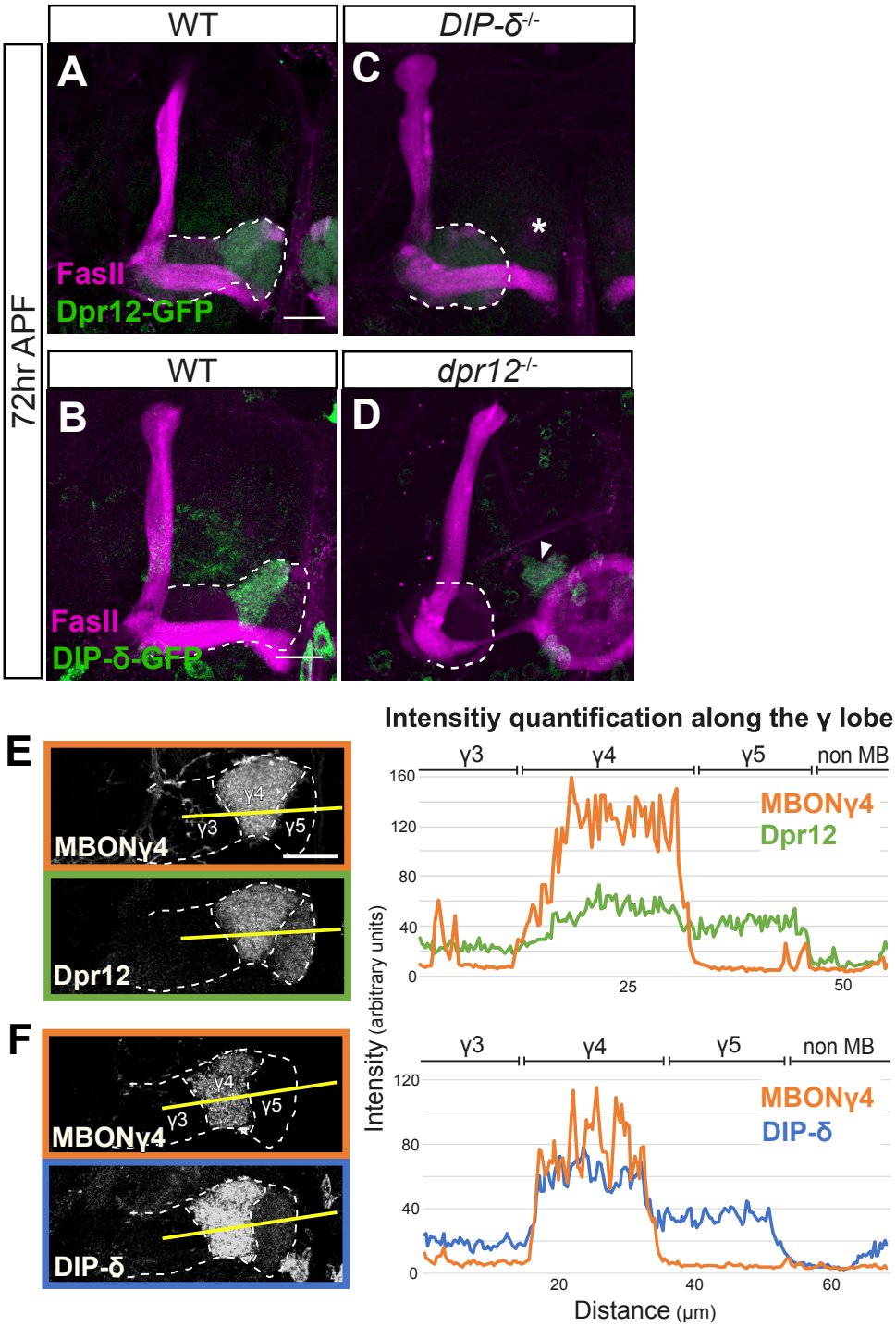
Figure EV4
(A-C) y, w; QUAS-GFP, UAS-mtdT-HA/UAS-tdT; R58E02/R58E02-QF2
(D-F) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{T2A-Gal4}$*/+

Figure EV5
(A) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R10G03/UAS-mCD8-GFP
(B) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, dpr12$^{Δ50-81}$, G13; R10G03/UAS-mCD8-GFP
(D) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R48H011/UAS-mCD8-GFP
(E) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R48H011/UAS-mCD8-GFP
(G) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R18H09/UAS-mCD8-GFP
(H) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R18H09/UAS-mCD8-GFP

Figure EV6
(A) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{1-119}$/DIP-δ$^{1-119}$*
(B) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{1-119}$/DIP-δ$^{1-119}$*, UAS-DIP-α
(C) y, w; R71G10-QF2, QUAS-mtdT-HA/+; *DIP-δ$^{T2A-Gal4}$/DIP-δ$^{T2A-Gal4}$*
(D) y, w; R71G10-QF2, QUAS-mtdT-HA/UAS-FasII; *DIP-δ$^{T2A-Gal4}$/DIP-δ$^{T2A-Gal4}$*

Appendix Figure S1
(A) y, w; Dpr12 ^{GFSTF}/+
(B) y, w;; DIP-δ ^{GFSTF}/+
(C) y, w; Dpr12 ^{GFSTF}/+; *DIP-δ$^{T2A-Gal4}$/DIP-δ$^{T2A-Gal4}$*
(D) y, w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; DIP-δ^{GFSTF}/+
(E) y, w; UAS-CD4-tdT/Dpr12 ^{GFSTF}; R18H09/+
(F) y, w; UAS-CD4-tdT/+; DIP-δ ^{GFSTF}/R18H09

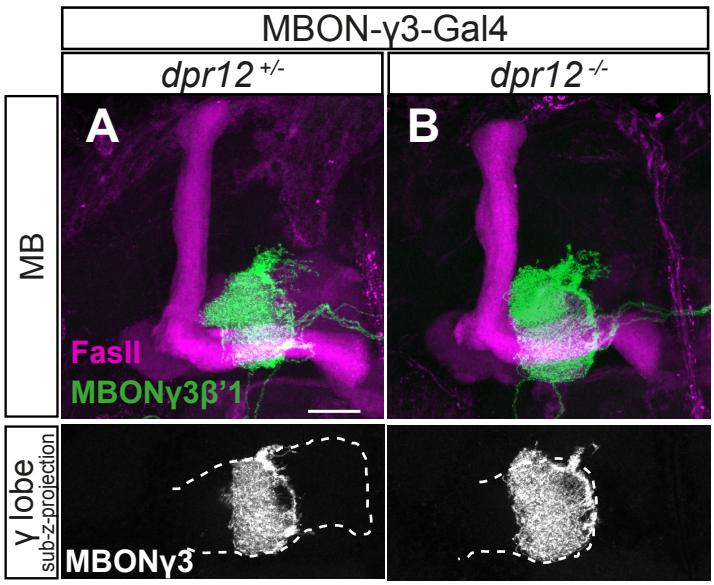
Appendix Figure S2
(A) y,w; 40A, *dpr12$^{Δ50-81}$*, G13/+; R94B10-GAL4.DBD, R52G04-p65.AD/ UAS-mCD8-GFP
(B) y,w; 40A, *dpr12$^{Δ50-81}$*, G13/40A, *dpr12$^{Δ50-81}$*, G13; R94B10-GAL4.DBD, R52G04-p65.AD/ UAS-mCD8-GFP



Appendix figure S1. Additional data regarding Dpr12 and DIP-δ protein localization, related to Figures 3 and 6.

(A-D) Confocal z-projections of brains expressing either MiMIC mediated Dpr12^{GFSTF} (Dpr12-GFP; A,C) or DIP-δ^{GFSTF} (DIP-δ-GFP; B,D) fusion proteins, in either WT (A-B), *DIP-δ$^{T2A-Gal4}$* homozygous mutant (C) or *dpr12$^{Δ50-81}$* homozygous mutant (D) animals at 72hr APF. Green is GFP, magenta is FasII. Dashed line depicts the medial γ-lobe, as determined by FasII staining. Asterisk demarcates the distal part of the lobe; arrowhead demarcates DIP-δ expression at the distal part of the lobe. Scale bar is 20μm.

(E-F) Intensity quantification of the indicated channels in the confocal z-projections that appear in Figure 3D (for E) and Figure 3H (for F). Intensity (arbitrary units) was quantified along a defined line that crosses the γ lobe from γ3 until it exits the MB (marked in yellow; distance is shown in μm). The MBON-γ4 channel (GMR18H09-Gal4>CD4-tdT) is shown in orange (E, F), Dpr12-GFP in green (E) and DIP-δ-GFP in blue (F). The rough division to γ-lobe zones is indicated above the graphs. Scale bar is 20μm.



Appendix figure S2. MBON-γ3 innervation of the MB γ-lobe in Dpr12 mutant brains, related to Figure 7.

(A-B) Confocal z-projections of *dpr12*$^{\Delta 50\text{-}81}$ heterozygous (A) or homozygous (B) mutant brains expressing membrane-bound GFP (CD8-GFP) driven by MB083C (MBONγ3-Gal4) that is expressed in MBONγ3β'1 (MBON-09). The greyscale channels are sub-z-projections comprised of slices restricted to the γ-lobe (which is outlined in white).

Green and grey are CD8-GFP, magenta is FasII. Scale bar is 20μm.

Color	Type	Name	Projection Pattern	*neu*Print Body ID
●	γ-KC	KCγm	Medial γ-lobe	415511327
●		KCγm	Medial γ-lobe	415835070
●		KCγm	Medial γ-lobe	415835178
●		KCγm	Medial γ-lobe	415848228
●		KCγm	Medial γ-lobe	415852518
●		KCγm	Medial γ-lobe	415908516
●		KCγm	Medial γ-lobe	416189410
●		KCγm	Medial γ-lobe	416189500
●		KCγm	Medial γ-lobe	416598862
●		KCγm	Medial γ-lobe	415494049
●	γ5 PAM-DAN	PAM01	γ5 compartment	925799233
●		PAM01	γ5 compartment	1202397143
●		PAM01	γ5 compartment	5812982779
●		PAM15	γ5β'2a compartment	5813096699
●	γ4 PAM-DAN	PAM08	γ4 compartment	580317662
●		PAM08	γ4 compartment	988852391
●	γ4>γ1γ2 MBON	MBON05	γ4>γ1γ2 compartment	799586652
●	γ5β'2a MBON	MBON01	γ5β'2a compartment	612371421

Appendix Table S1. EM-skeletons used for model generation.

A list of the cell types, names, projection patterns, color coding and *neu*Print IDs for all the EM-based skeletons used to construct the models in Figure 1P and Movie EV1 (see methods for additional details).

Driver		Expressing cells	Expression during development			Source
Name in manuscript	Formal name		Larva	Pupa	Adult	
γ-Gal4	R71G10-Gal4	γ-KCs consistently α/β-KCs stochastically	✓	✓	✓	Chr. III: Bl. #39604 Chr. II: Alyagor et al., 2018
γ-QF2	R71G10-QF2	γ-KCs consistently α/β-KCs stochastically	✓	✓	✓	This study
DIP-δ-Gal4	DIP-δ-T2A-Gal4	DIP-δ-expressing cells including PAM-DANs targeting γ4/5 zones	✓	✓	✓	This study
PAM-DAN-Gal4	R58E02-Gal4	All PAM-DANs	pPAMs*	After 48hr APF	✓	BDSC #41347
PAM-DAN-γ3-Gal4	MB441B	PAM-DANs targeting γ3	X	ND	✓	BDSC #68251
PAM-DAN-γ4-Gal4	R10G03	PAM-DANs targeting γ4	ND	After 72hr APF	✓	BDSC #48271
PAM-DAN-γ5-Gal4	R48H11	PAM-DANs targeting γ5	ND	After 72hr APF	✓	BDSC #50396
MBON-γ4-Gal4	R18H09	MBON-γ4>γ1γ2 (MBON-05)	X	Not at 24hr APF (ND for other time points)	✓	BDSC #48830
MBON-γ4-Gal4	MB298B	MBON-γ4>γ1γ2 (MBON-05)	ND	After 24hr APF	✓	BDSC #68309
MBON-γ3-Gal4	MB083C	MBON-γ3β‘1 (MBON-09)	ND	ND	✓	BDSC #68287
Pan-Glia-Gal4	Repo-Gal4	All glia	✓	✓	✓	BDSC #7415
Pan-KC-Gal4	OK107-Gal4	All KCs (γ, α/β and α'/β')	✓	✓	✓	BDSC #854
Pan-Neuron-Gal4	C155-Gal4	All neurons	✓	✓	✓	BDSC #458

Appendix Table S2. List of drivers used in the current study.

The driver name (in the manuscript and formally), the cells in which it is expressed, and expression throughout development are depicted. ND; not determined. *pPAMs are primary-PAMs which are different from the adult PAM-DANs (data not shown).