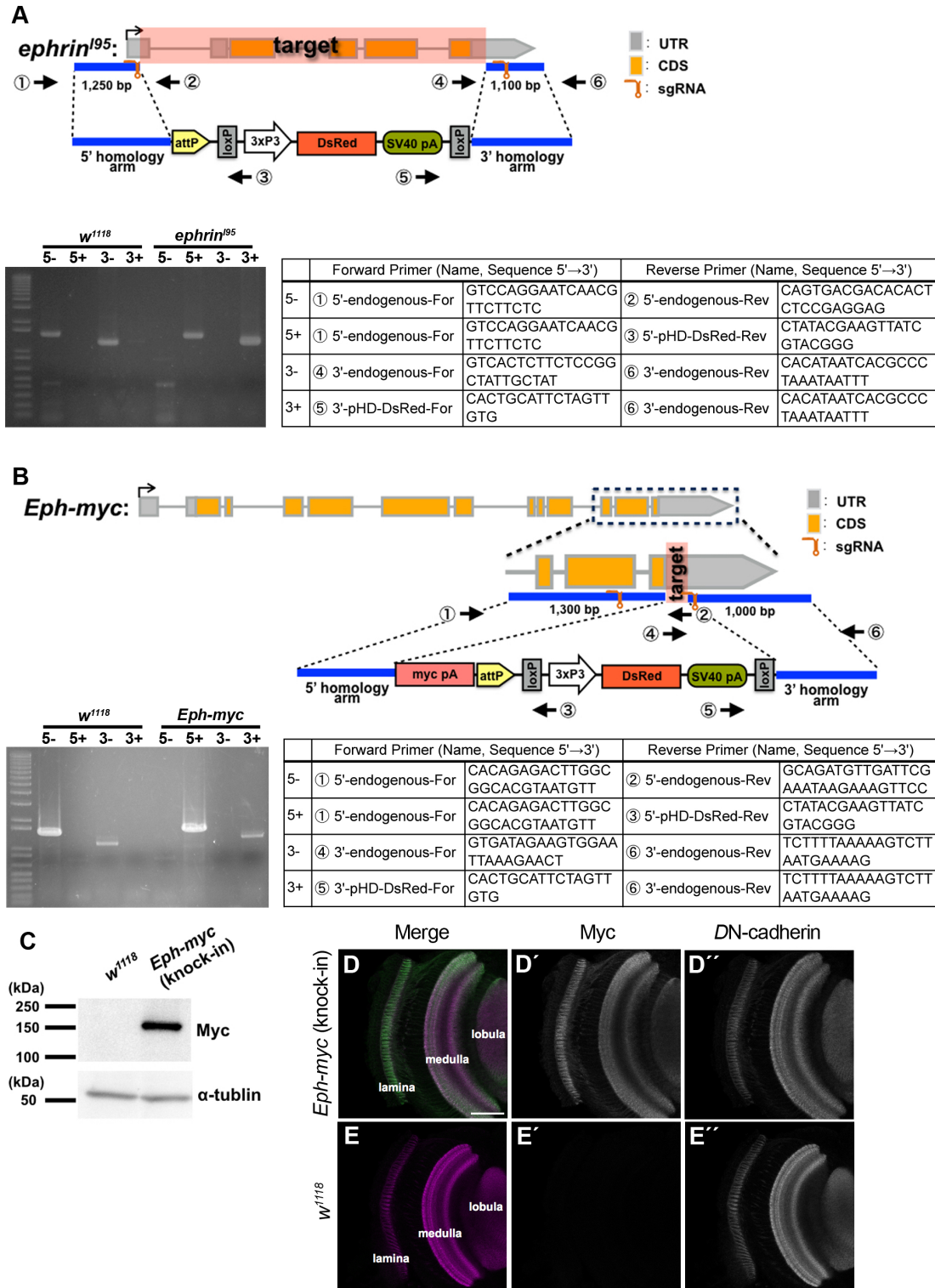


Supplemental information

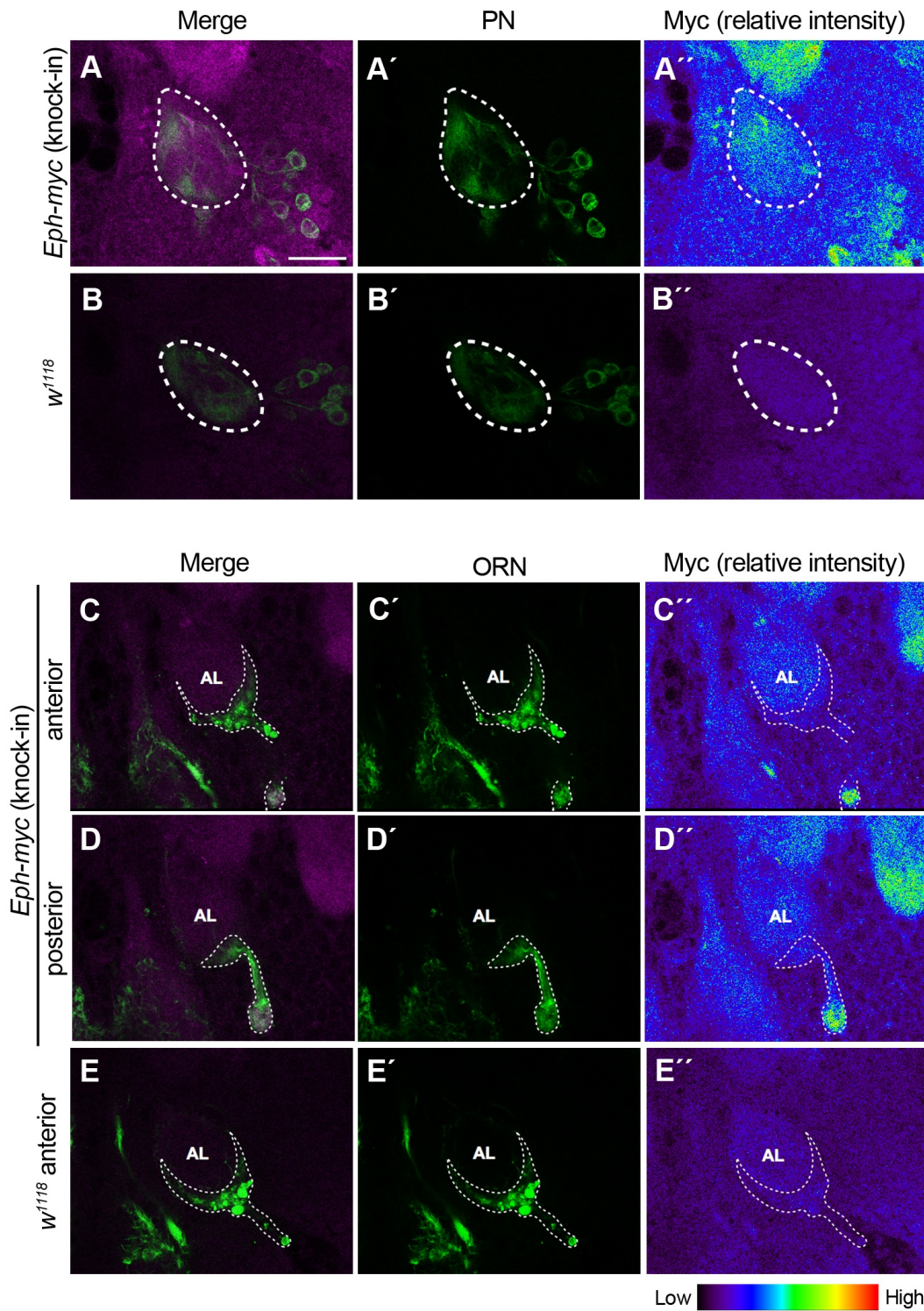
Anzo297424\_FigS1



**Figure S1. Generation of *ephrin*<sup>195</sup> and *Eph-myc* alleles.**

(A) *ephrin* knockout strategy for generating *ephrin*<sup>195</sup>. *ephrin* locus was replaced with the sequences from donor vector. Positions of the sgRNA and homology arms are shown in orange and blue, respectively. Primers used for genomic PCR are shown in arrows in the upper panel, and their sequences are listed in the lower table. The ‘-’ primer sets positively detect endogenous sequence, while ‘+’ primers positively detect sequence from the insert. (B) 7×Myc tag was knocked in to 3’ end of *Eph* coding sequence. The strategy is basically the same with A above. (C) Western blotting of *w*<sup>1118</sup> (control) or *Eph-myc* (knock-in) pupal brains (50 h APF) detected by anti-Myc antibody or anti- $\alpha$ -tubulin antibody. (D,E) *Eph-myc* expression in the optic lobe at 50 h APF. (D) *n*=5. (E) *n*=5. Scale bar, 50  $\mu$ m.

Anzo297424\_FigS2

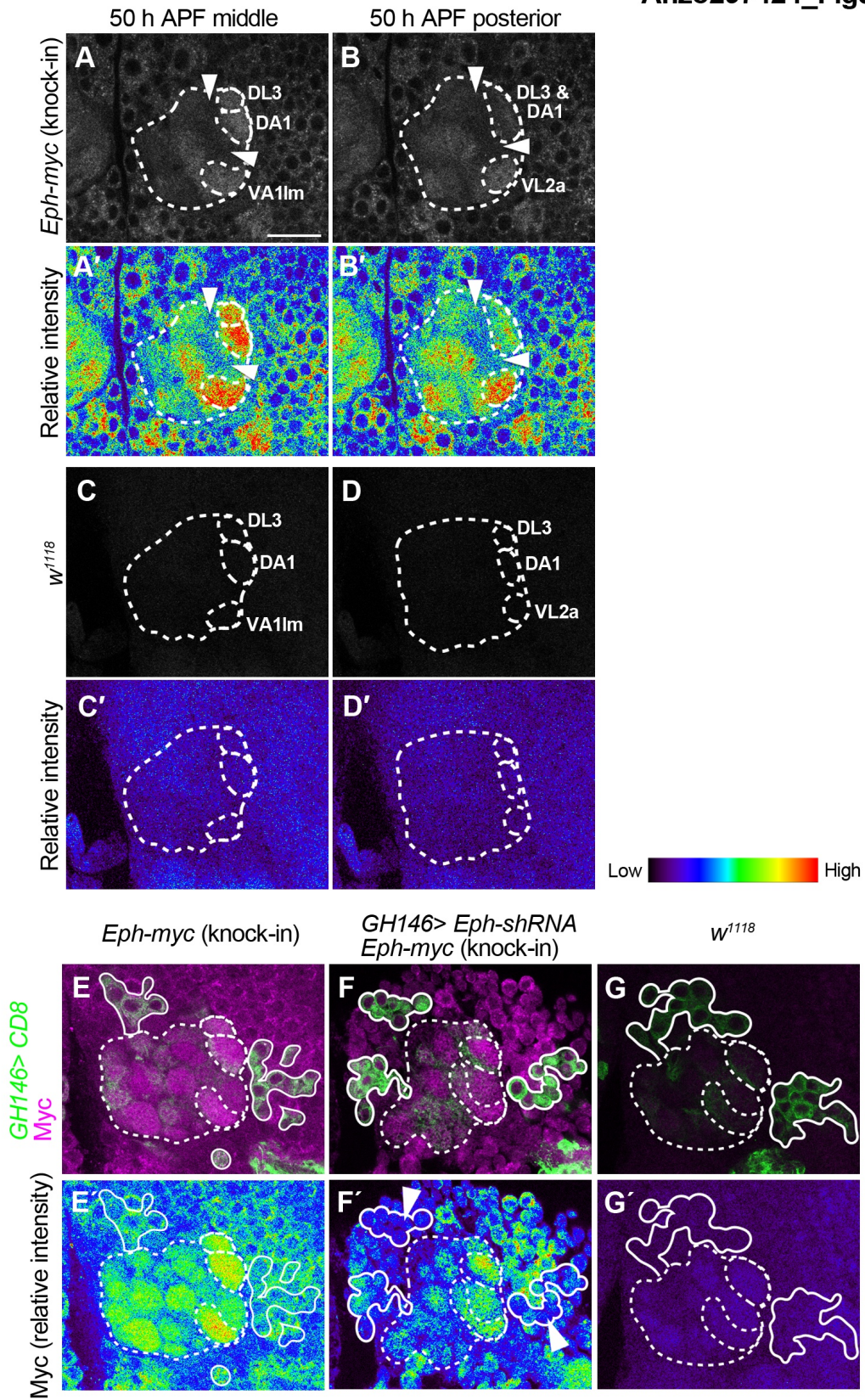


**Figure S2. Eph-myc signals inside the AL are co-localized with PN- or ORN-specific marker at 18 h APF.**

(A,B) *Eph-myc* (knock-in) with *GHI46-Gal4*, *UAS-mCD8-GFP* (PNs are labeled) was stained with

anti-Myc (magenta in merged image, and heatmap in the right image) and anti-mCD8 (green) antibodies. Dotted circle indicates the developing AL. (A)  $n=5$ . (B)  $n=5$ . (C-E) *Eph-myc* (knock-in) with *peb-Gal4*, *UAS-mCD8-GFP* (ORNs are labeled) was stained with anti-Myc (magenta in merged image, and heatmap in the right image) and anti-mCD8 (green) antibodies. The outline of innervating ORNs is shown in dotted line. AL: antennal lobe. (C,D)  $n=7$ . (E)  $n=8$ . Scale bar, 25  $\mu\text{m}$ .

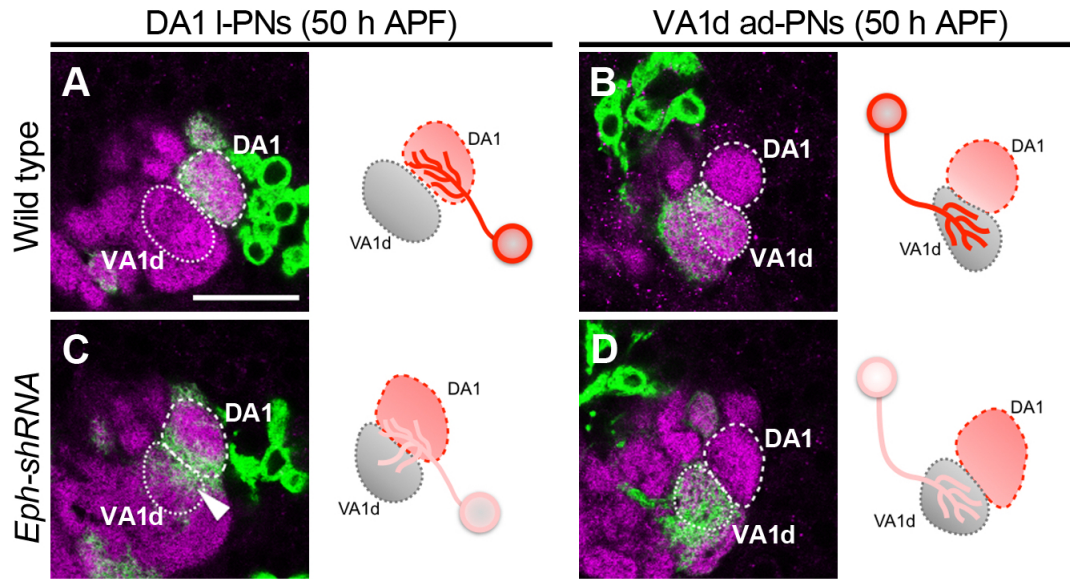
Anzo297424\_FigS3



**Figure S3. Eph is highly expressed in the developing PNs that send dendrites to the glomeruli related to reproductive behaviors.**

(A-D) Anti-Myc staining of pupal brain at 50 h APF. (A'-D') Relative intensity of anti-Myc staining shown in pseudocolors. (A-B') *w<sup>118</sup>; Eph-myc* (*n*=7). High Myc signal was observed in DL3, DA1, VA11m, and VL2a glomeruli. Myc signal was markedly low at the neighboring region of Eph-positive glomeruli (arrowheads). (A) Middle section (posterior than Fig. 2G). (B) Posterior section (posterior than Supplemental Fig. S2A). (C-D') Anti-Myc staining against *w<sup>118</sup>* (negative control; *n*=8). (E-G) Anti-Myc staining was performed at 50 h APF. (E'-G') Relative intensity of anti-Myc staining shown in pseudocolors. (E) *Eph-myc* (knock-in) (*n*=8). (F) *UAS-Eph-shRNA* was driven by *GHI46-Gal4* together with *UAS-mCD8-GFP* in *Eph-myc* (knock-in) background (*n*=9). Decreased Myc signal was observed in *GHI46*-positive PN cell bodies (arrowheads). (G) *w<sup>118</sup>* (*n*=8). Scale bar, 25  $\mu$ m.

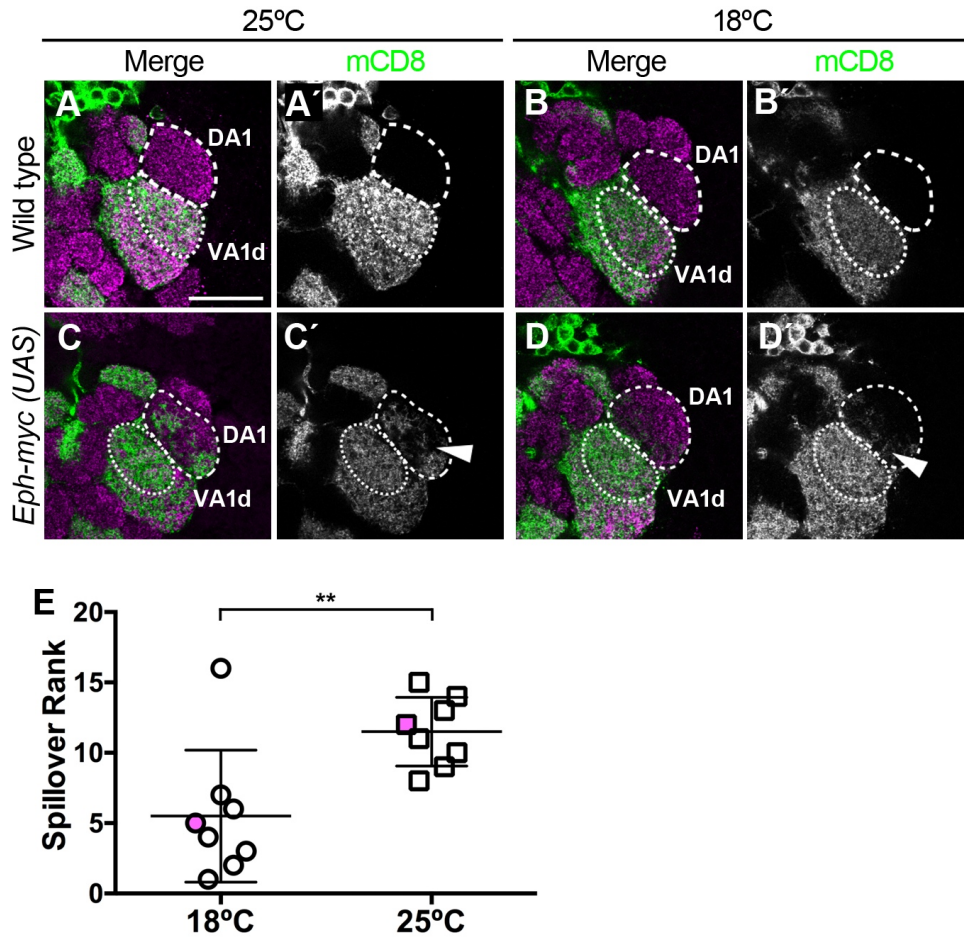
## Anzo297424\_FigS4



**Figure S4. Eph is cell-autonomously functions from the developmental stage.**

(A,B) Wild-type PN neuroblast clones at 50 h APF, labeled by *mCD8-GFP* driven by *GHI46-Gal4*-driven MARCM. Magenta represents *DN-cadherin* staining. I-PNs (A,  $n=3$ ) and ad-PNs (B,  $n=10$ ) are shown. (C,D) *Eph* knockdown at 50h APF. (C) Dendritic spillover phenotype of DA1 PN expressing *Eph-shRNA* was observed at 50 h APF ( $n=8$ ). (D) VA1 PNs expressing *Eph-shRNA* did not show any spillover ( $n=10$ ). Scale bar, 25  $\mu\text{m}$ .

## Anzo297424\_FigS5

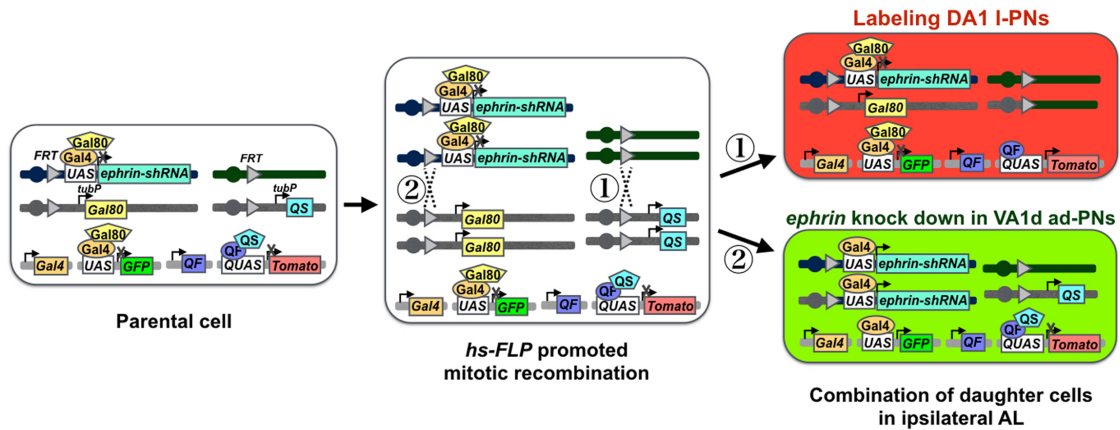


**Figure S5. Severity of VA1d dendritic spillover phenotype depends on the expression level of ectopic Eph.**

(A,B) Wild-type VA1d ad-PNs labeled in flies raised at 25°C (A,  $n=7$ ) or 18°C (B,  $n=13$ ). Magenta represents presynaptic marker Bruchpilot. (C,D) VA1d dendritic spillover phenotype by *Eph-myc* overexpression flies raised at 25°C (C,  $n=8$ ) or 18°C (D,  $n=8$ ). (E) Rank order of VA1d dendritic spillover at different temperature as indicated. The VA1d dendritic spillover degree to DA1 was blindly ranked with the lowest score denoting the mildest and highest score denoting the severest. Student's t-tests were performed to determine significance (\*\* $p<0.05$ ). Magenta symbols indicate the examples shown in C-D'.



Anzo297424\_FigS6



**Figure S6. Scheme representing the principle of independent-double-MARCM.**

*Gal4*-driven MARCM and *QF*-driven MARCM were simultaneously performed in the same individual. In the parental cell, both *Gal80* and *QS* were expressed under *tub-P*, thus *Gal4* and *QF* activities are both repressed. *hs-FLP* promoted mitotic recombination at either ① or ② followed by chromosome segregation produce differently labeled daughter cells. The ALs incidentally bearing I-PNs labeled in red and ad-PNs labeled in green were analyzed; DA1 I-PNs devoid of *QS* but carries *Gal80* resulted in the expression of *mtdTomato* (①), and VA1d ad-PNs lacking *Gal80* but having *QS* resulted in the expression of *ephrin-shRNA* and *mCD8-GFP* (②).

Table S1. Genotype list, Related to all Figures

Figure			Genotype
Figure 1	D-E	Wild type	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	F-G	<i>Eph<sup>X652</sup></i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4;; Eph<sup>X652</sup></i>
	H-I	<i>ephrin<sup>195</sup></i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4;; ephrin<sup>195</sup></i>
Figure 2	E-H	<i>Eph-myc</i> (knock-in)	<i>w;;; Eph-myc</i>
	I-L	<i>w<sup>1118</sup></i>	<i>w<sup>1118</sup></i>
Figure 3	A, C	<i>Eph-shRNA</i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	E-G	Wild type	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	H-J	<i>Eph-shRNA</i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	K	<i>Eph<sup>resi</sup></i> rescue	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; UAS-Flag-Eph<sup>resistant</sup>-HA/+</i>
	M-N	<i>Eph-myc</i> (UAS)	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-myc FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
Figure 4	B-D	Wild type	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP QUAS-mtdTomato-HA/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; FRT<sup>2A</sup> FRT<sup>82B</sup>/GH146-QF FRT<sup>82B</sup> tubP-OS</i>
	F-H	<i>ephrin-shRNA</i> in ad-PNs	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP QUAS-mtdTomato-HA/+; UAS-ephrin-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; FRT<sup>2A</sup> FRT<sup>82B</sup>/GH146-QF FRT<sup>82B</sup> tubP-OS</i>
Figure 5	A-B	<i>ephrin-shRNA</i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-ephrin-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	C	<i>ephrin<sup>resi</sup></i> rescue	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-ephrin-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; UAS-ephrin<sup>resistant</sup>-myc/+</i>
Figure 6	D	<i>Eph<sup>ALBD</sup></i> rescue	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; UAS-Eph<sup>resistant, ALBD</sup>-HA/+</i>
	E	<i>ephrin<sup>E320K</sup></i> rescue	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-ephrin-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4; UAS-ephrin<sup>resistant, E320K</sup>-myc/+</i>
Figure S1	D	<i>Eph-myc</i> (knock-in)	<i>w;;; Eph-myc</i>
	E	<i>w<sup>1118</sup></i>	<i>w<sup>1118</sup></i>
Figure S2	A	<i>Eph-myc</i> (knock-in)	<i>GH146-Gal4 UAS-mCD8-GFP/+;; Eph-myc/+</i>
	B	<i>w<sup>1118</sup></i>	<i>GH146-Gal4 UAS-mCD8-GFP/+</i>
	C-D	<i>Eph-myc</i> (knock-in)	<i>peb-Gal4 UAS-mCD8-GFP/+; tft or CyO/+;; Eph-myc/+</i>
	E	<i>w<sup>1118</sup></i>	<i>peb-Gal4 UAS-mCD8-GFP/+; tft or CyO/+</i>
Figure S3	A-B	<i>Eph-myc</i> (knock-in)	<i>w;;; Eph-myc</i>
	C-D	<i>w<sup>1118</sup></i>	<i>w<sup>1118</sup></i>
	E	<i>Eph-myc</i> (knock-in)	<i>GH146-Gal4 UAS-mCD8-GFP/+;; Eph-myc/+</i>
	F	<i>GH146&gt; Eph-shRNA</i> <i>Eph-myc</i> (knock-in)	<i>GH146-Gal4 UAS-mCD8-GFP/UAS-Eph-shRNA;; Eph-myc/+</i>
	G	<i>w<sup>1118</sup></i>	<i>GH146-Gal4 UAS-mCD8-GFP/+</i>
Figure S4	A-B	Wild type	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	C-D	<i>Eph-shRNA</i>	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-shRNA FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
Figure S5	A-B	Wild type	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>
	C-D	<i>Eph-myc</i> (UAS)	<i>hs-FLP<sup>122</sup> UAS-mCD8-GFP/+; UAS-Eph-myc FRT<sup>40A</sup>/tubP-Gal80 FRT<sup>40A</sup> GH146-Gal4</i>