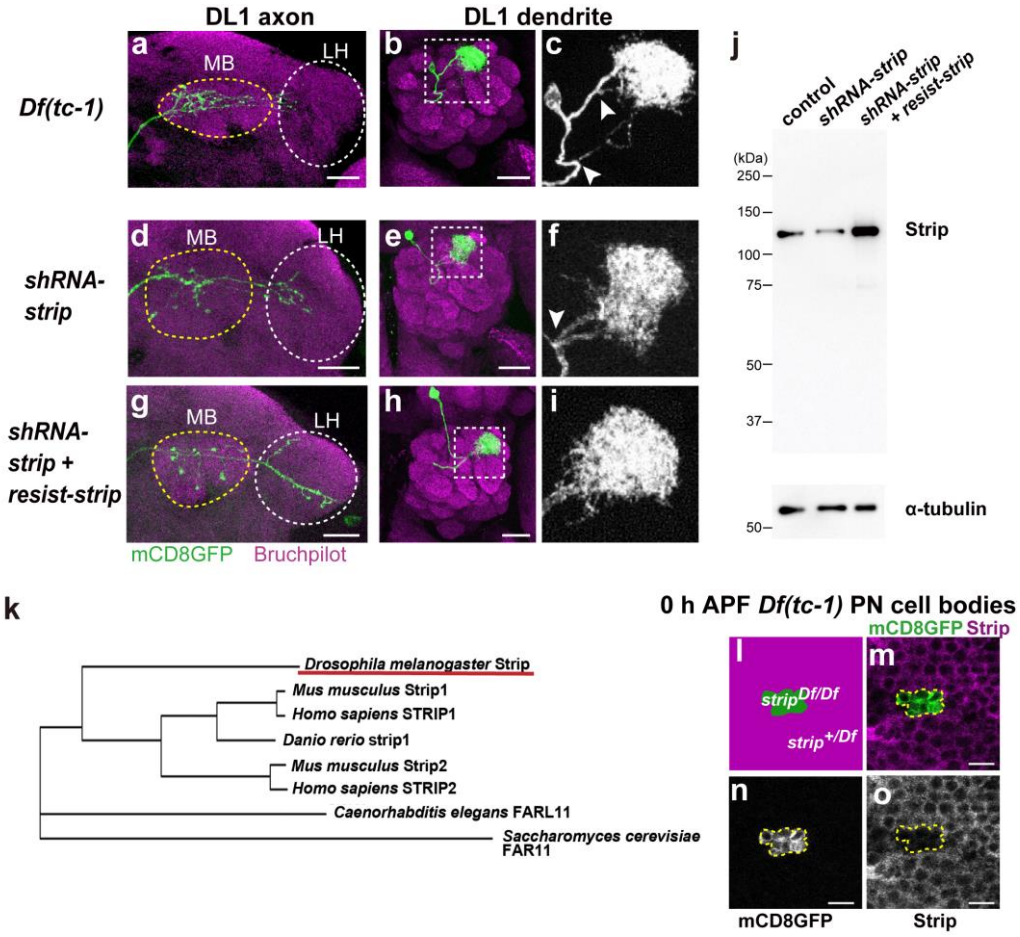
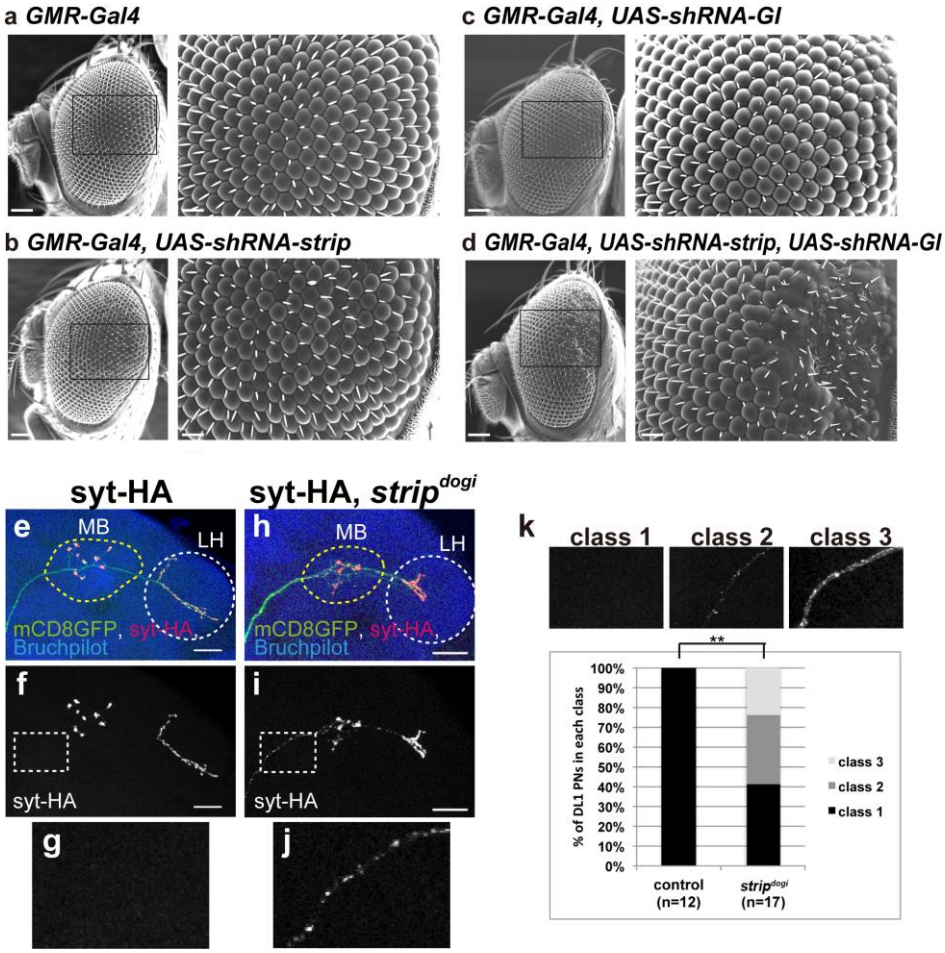


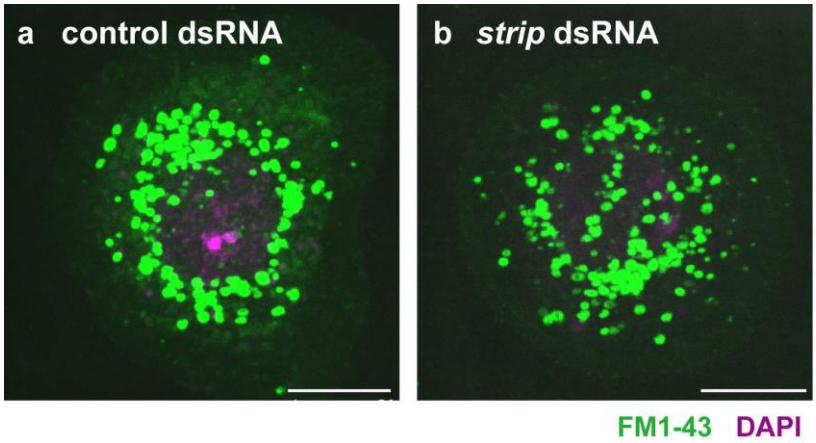
Supplementary Figure 1 Evolutionarily conserved Strip is expressed at projection neurons (PNs) and regulates dendrite branching and axon elongation

(a–i) Representative images of *Df(tc-1)* DL1 PNs (a–c), DL1 PNs expressing *shRNA-strip* (d–f), and DL1 PNs expressing *shRNA-strip* and cDNA of strip that is resistant to *shRNA-strip* (*resist-strip*) simultaneously (g–i). DL1 PNs are labelled in green. Magenta: Bruchpilot staining. Yellow and white dotted circles indicate the mushroom body (MB) and the lateral horn (LH), respectively. Scale bar: 25 µm. (c), (f), and (i) are magnified images of dotted rectangles of (b), (e), and (h), respectively. Only green channels are shown in grey scale. (j) Evaluation of *shRNA-strip* using *Drosophila* S2 cells. (k) Phylogenetic tree of *strip* and its homologs. (l–o) Schematic image (l) and cell bodies of a MARCM neuroblast clone for *Df(tc-1)* induced at 0–24 h after larval hatching and dissected at 0 h after puparium formation (m–o, yellow dotted circles). Green: *Df(tc-1)*-homozygous PN cell bodies. Magenta: anti-Strip antibody staining. Scale bar: 10 µm.

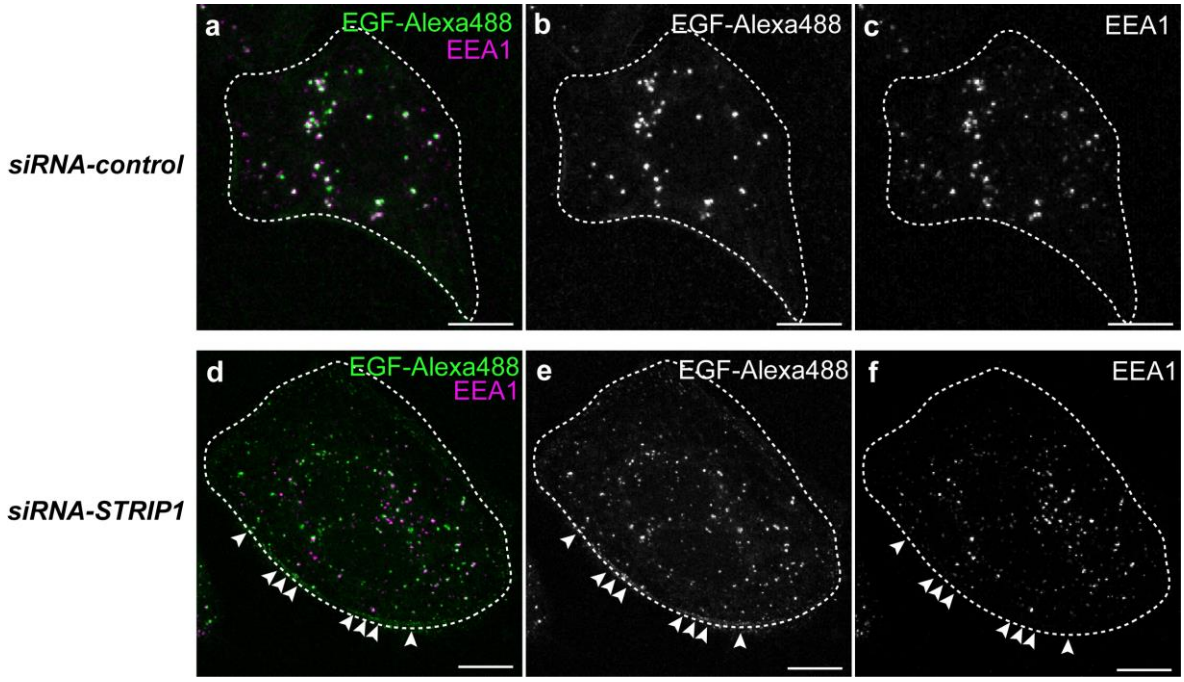


Supplementary Figure 2 Strip genetically interacts with *Gl* in eye development and is involved in intracellular transport

(a–d) Representative images of compound fly eyes (a), expressing *shRNA-strip* (b), *shRNA-Gl* (c), or *shRNA-strip* and *shRNA-Gl* (d). Right: a magnified view of the rectangles in the left images. Scale bars: left, 66.6 µm; right, 20 µm. (e–j) HA-tagged synaptotagmin (syt-HA), a representative cargo transported by the dynein/dynactin complex, was expressed in wild-type (WT) (e–g) and *strip*^{dogi} PNs (h–j) and detected by the anti-HA antibody. green: DL1 MARCM clone, red: syt-HA, blue: Bruchpilot. Yellow and white dotted circles indicate the mushroom body (MB) and the lateral horn (LH), respectively. (g) and (j) are magnified views of the dotted rectangles in (f) and (i), respectively. All single-cell clones were induced at 0–24 h ALH. Scale bar: 25 µm. (k) All the single-cell clones were categorized by their syt-HA levels in axon stalks (class 1–3) by a blind test. **p = 0.0046, chi-square test.

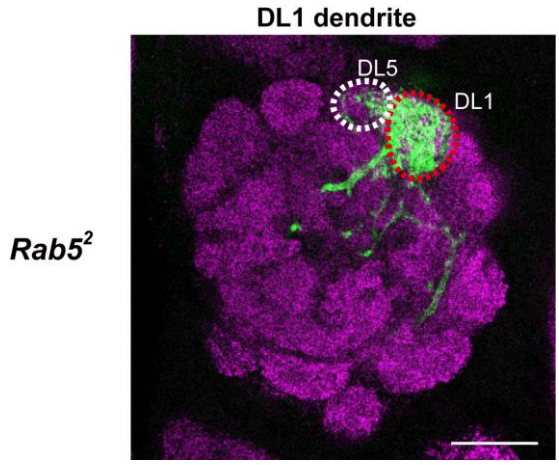


Supplementary Figure 3 Endocytosis was not inhibited in *strip* dsRNA-treaded cells *Drosophila* S2 cells were treated with control (a) or *strip* dsRNA (b) for 8 days and assayed for endocytosis with a lipophilic styryl compound, FM1-43 dye (green). Magenta: DAPI staining.



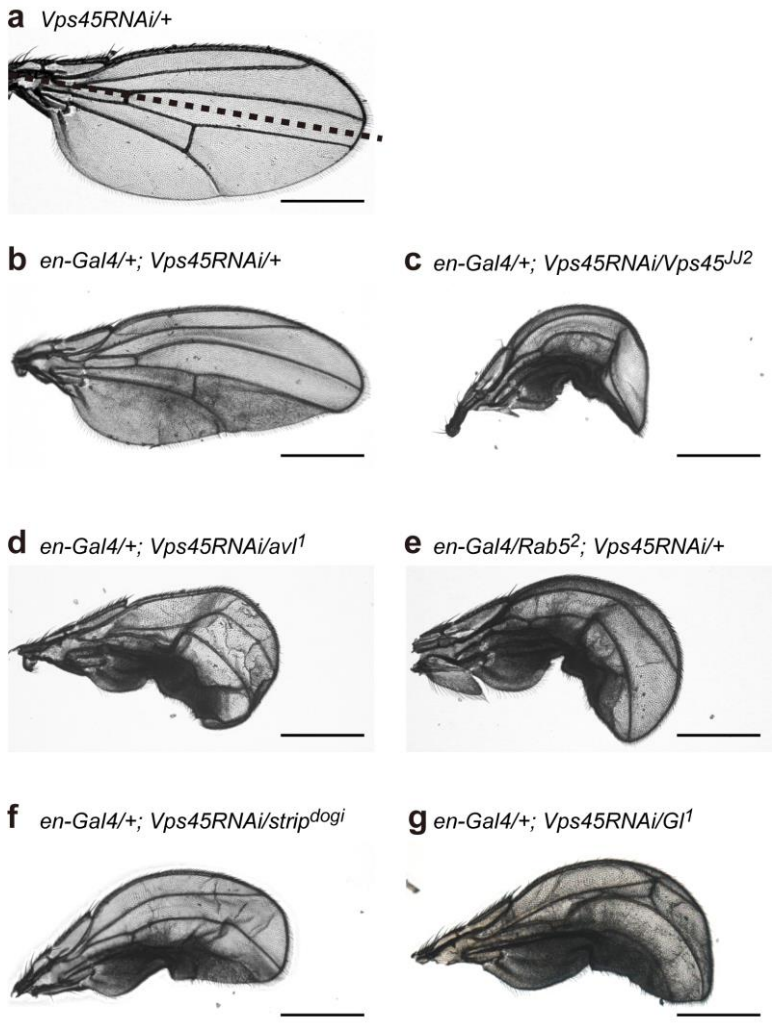
Supplementary Figure 4 EGF-Alexa488-positive vesicles localized at cell periphery in *STRIP1* siRNA-treated HeLa cells were EEA1 negative

Control (a–c) or *STRIP1* (d–f) siRNA-treated HeLa cells were stimulated with EGF-Alexa488 for 3 min, rinsed and incubated with DMEM for 27 min and fixed. Magenta: EEA1, green: EGF-Alex488. Scale bar: 10 μm. The outlines of cells are indicated by dotted circles. EGF-Alexa488-positive vesicles localized at cell periphery are indicated by arrowheads.



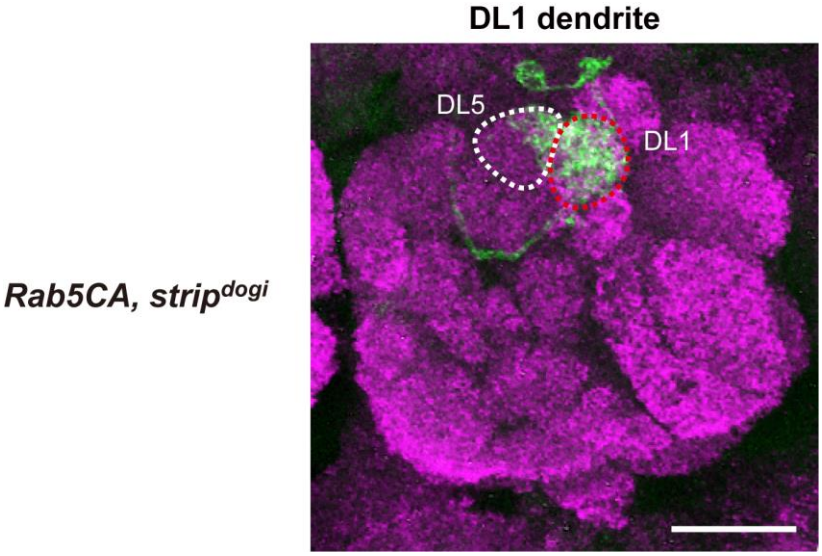
Supplementary Figure 5 Some dendrites of *Rab5*^{2} DL1 PNs targeted both DL1 and DL5 glomeruli

A *Rab5*^{2} DL1 PN (green) whose dendrite targeted not only DL1 (red dotted circle), but also DL5 (white dotted circle) glomeruli. Magenta: Bruchpilot staining. Scale bar: 25 μm.



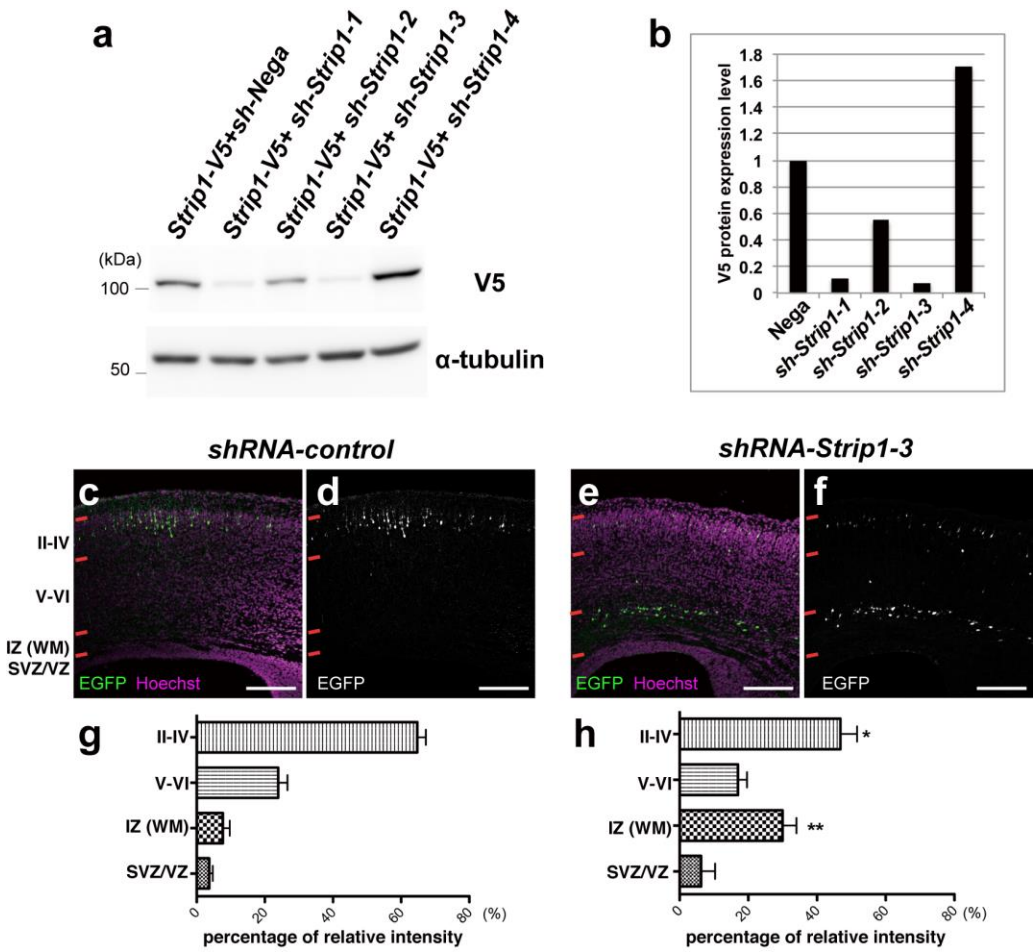
Supplementary Figure 6 Genetic interactions among *strip*, *Gl*, and regulators of early endosome fusion

Genetic interaction tests in a sensitized background generated by driving expression of a *Vps45* RNAi at posterior compartment of wing. (a) Control wing. The dashed line indicates the boundary of anterior-posterior compartment. (b) *Vps45* RNAi was expressed at posterior compartment of wing. (c–g) One copy of the wild-type *Vps45* (c), *avl* (d), *Rab5* (e), *strip* (f), or *Gl* (g) was removed from the flies expressing *Vps45* RNAi in the posterior compartment of their wing. Scale bar: 500 μm



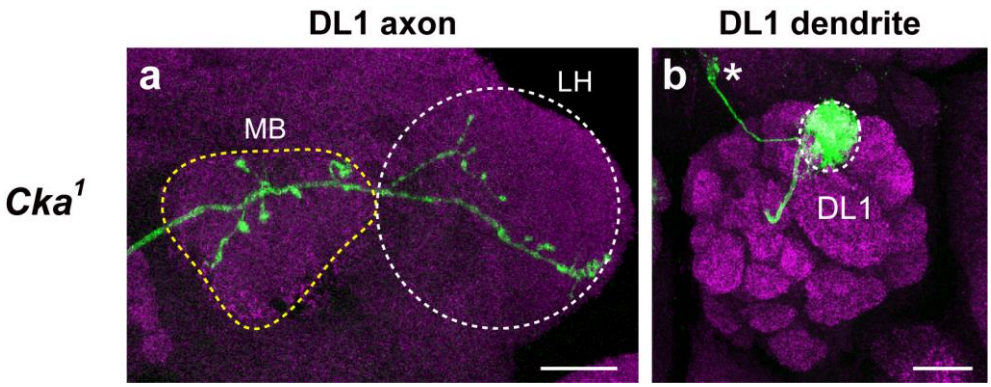
Supplementary Figure 7 Expression of a constitutively active form of Rab5 did not suppress dendrite branching defects of *strip*^{dogi} PNs

A *strip*^{dogi} DL1 PN (green) expressing constitutively active form of Rab5 (Rab5CA, Rab5Q88L) whose dendrite targeted not only DL1 (red dotted circle), but also DL5 (white dotted circle) glomeruli. Magenta: Bruchpilot staining. Scale bar: 25 µm.



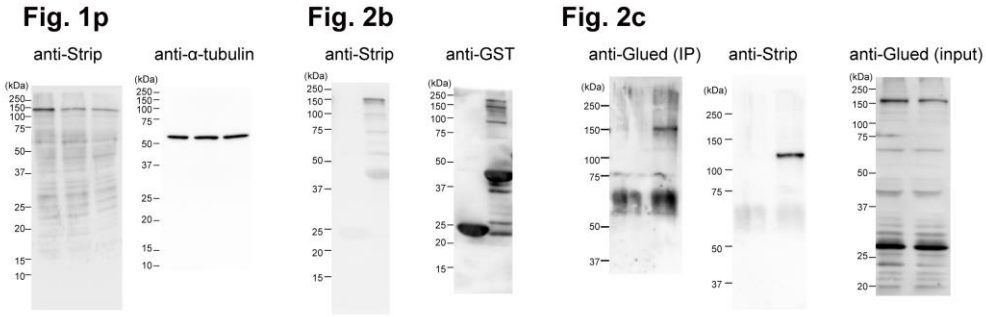
Supplementary Figure 8 The mouse orthologue of *strip*, *Strip1,* is involved in neuronal positioning at the mice cerebral cortices

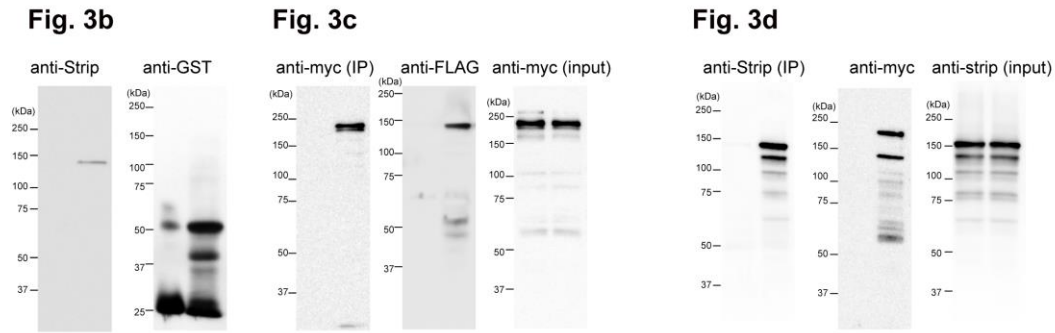
(a) Efficiency of shRNA targeting *Strip1*. HEK293T cells were transfected with *pcDNA3-Strip1-V5* and *shRNA-Strip1-1/2/3/4* for 24 h. (b) The densitometric quantification of Strip1-V5 of (a). (c–f) Cerebral cortices at P0, electroporated with the indicated plasmids plus pEGFP at E14. Short red bars indicate the borders between II–IV, V–VI, IZ, and SVZ/VZ. (c,e) Green: EGFP, Magenta: Hoechst staining. (d,f) Only green channels are shown in grey scale. (g,h) cell migration, estimated by recording the EGFP fluorescence intensity in distinct regions of the cerebral cortex using Leica SP5 software. Each bar represents the mean percentage of relative intensity ±SEM. Control, n=4; *Strip1; n=6*. II–IV, layers II–IV of the cortical plate; V–VI, layers V–VI of the cortical plate; IZ, intermediate zone; WM, white matter; SVZ/VZ, subventricular zone/ventricular zone. *p = 0.0135, **p = 0.0013, *t*-test (compared with control). Scale bar: 200 μm.

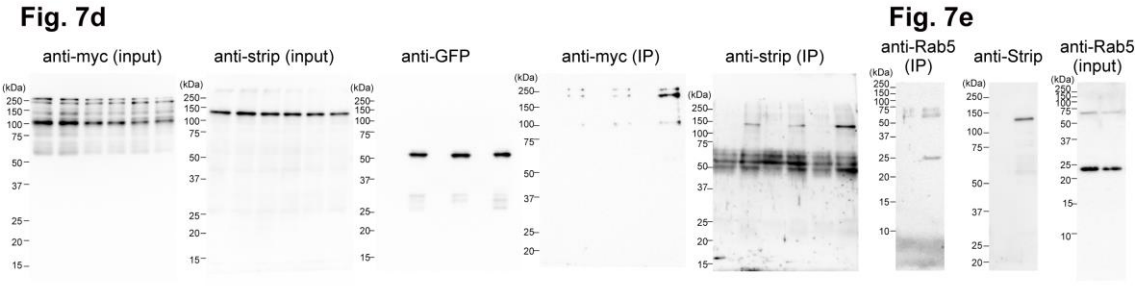


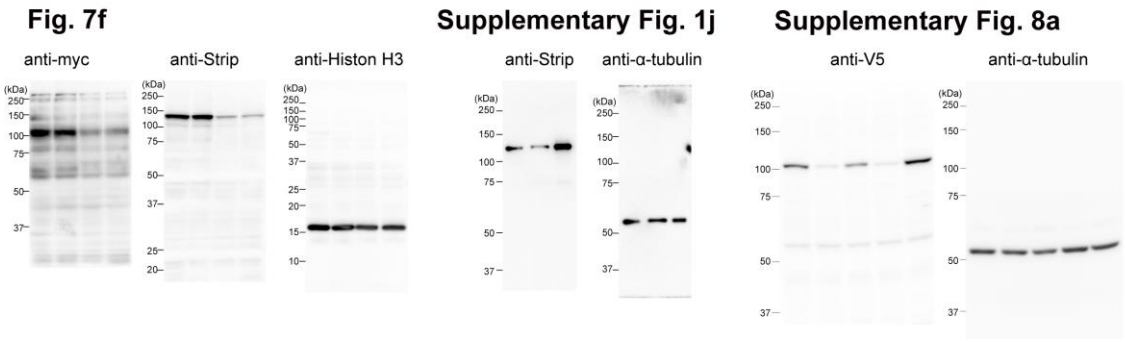
Supplementary Figure 9 *Cka*^{1} PNs did not exhibit defects in axon elongation or dendrite branching.

Representative image of *Cka*^{1} DL1 PN (green). (a) Axon. Yellow and white dotted circles indicate the mushroom body (MB) and the lateral horn (LH), respectively. (b) Dendrite. Asterisk: cell body. Magenta: Bruchpilot staining. Scale bar: 25 µm.









Supplementary Figure 10 Full blots for all western blots.

Supplementary Table 1. Genotypes list.

Brief Summary	Figure	Genotype
PNs expressing mCD8-GFP (WT)	Fig. 1b, 1c, 1g, 1k	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/ FRT^{2A}*
strip^{dogi} PNs	Fig.1d, 1h, 1l, 2d–f	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/ strip^{dogi}, FRT^{2A}*
Df(tc-1) PNs	Supplementary Fig. 1a–c, m–o	*y w, hs-flp122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/ Df(tc-1), FRT^{2A}*
MARCM rescue with *strip*	Fig. 1e, 1i, 1m	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-strip; tubP-Gal80, FRT^{2A}/ strip^{dogi}, FRT^{2A}*
MARCM rescue with *Strip1*	Fig. 1f, 1j, 1n	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-Strip1; tubP-Gal80, FRT^{2A}/ strip^{dogi}, FRT^{2A}*
PNs expressing *shRNA-strip*	Supplementary Fig. 1d–f	*y w, hs-FLP122, UAS-mCD8-GFP/ y w (or Y); GH146-Gal4, UAS-mCD8GFP/ UAS-shRNA-strip; tubP-Gal80, FRT^{2A}/ FRT^{2A}*
MARCM rescue with *resistant-strip*	Supplementary Fig. 1g–i	*y w, hs-FLP122, UAS-mCD8-GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-shRNA-strip, UAS-resist-strip ; tubP-Gal80, FRT^{2A}/ FRT^{2A}*
Gl^{1} PNs	Fig. 2g–i	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/Gl^{1}, FRT^{2A}*
Gl^{1} and *strip^{dogi}* PNs	Fig. 2j–m	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/ strip^{dogi}, Gl^{1}, FRT^{2A}*
PNs expressing *shRNA-spri*	Fig. 3e–g	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-shRNA-spri; tubP-Gal80, FRT^{2A}/ FRT^{2A}*
strip^{dogi} PNs expressing *shRNA-spri*	Fig. 3h–k	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-shRNA-spri; tubP-Gal80, FRT^{2A}/ strip^{dogi}, FRT^{2A}*
Gl^{1} PNs expressing *shRNA-spri*	Fig. 3l–o	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); GH146-Gal4, UAS-mCD8-GFP/ UAS-shRNA-spri; tubP-Gal80, FRT^{2A}/ Gl^{1}, FRT^{2A}*

Control eye	Supplementary Fig. 2a	*y w;; GMR-Gal4/+*
shRNA-strip expressing eye	Supplementary Fig. 2b	*y w; UAS-shRNA-strip/+; GMR-Gal4/+*
shRNA-Gl expressing eye	Supplementary Fig. 2c	*y w; UAS-shRNA-Gl/+; GMR-Gal4/+*
shRNA-strip and *shRNA-Gl* expressing eye	Supplementary Fig. 2d	*y w; UAS-shRNA-strip/ UAS-shRNA-Gl; GMR-Gal4/+*
PNs expressing syt-HA	Supplementary Fig. 2e–g	*y w, hs-FLP122, UAS-mCD8-GFP/ UAS-syt-HA; GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/FRT^{2A}*
$strip^{dogi}$ PNs expressing syt-HA	Supplementary Fig. 2h–j	*y w, hs-FLP122, UAS-mCD8-GFP/ UAS-syt-HA; GH146-Gal4, UAS-mCD8-GFP/ +; tubP-Gal80, FRT^{2A}/ $strip^{dogi}$, FRT^{2A}*
PNs expressing GFP-FYVE	Fig. 4a–c	*y w, hs-FLP122/ y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ UAS-GFP-myc-2xFYVE; tubP-Gal80, FRT^{2A}/ FRT^{2A}, UAS-GFP-myc-2xFYVE*
$strip^{dogi}$ PNs expressing GFP-FYVE	Fig. 4d–f	*y w, hs-FLP122/ y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ UAS-GFP-myc-2xFYVE; tubP-Gal80, FRT^{2A}/ $strip^{dogi}$, FRT^{2A}, UAS-GFP-myc-2xFYVE*
$Rab5^{2}$ PNs	Fig. 7a, Supplementary Fig. 5	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); tubP-Gal80, FRT^{40A}, GH146-Gal4, UAS-mCD8-GFP/ $Rab5^{2}$, FRT40A*
MARCM rescue with YFP-Rab5	Fig. 7b, c	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); tubP-Gal80, FRT^{40A} , GH146-Gal4, UAS-mCD8-GFP/ $Rab5^{2}$, FRT^{40A}; UAS-YFP-Rab5-WT /+*
PNs expressing mCD8-RFP (WT)	Fig. 7h	*y w, hs-FLP122 / y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ +; tubP-Gal80, FRT^{2A}/ FRT^{2A}*
$strip^{dogi}$ PNs (RFP-labelled)	Fig. 7i	*y w, hs-FLP122/ y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ +; tubP-Gal80, FRT^{2A}/ $strip^{dogi}$, FRT^{2A}*
$strip^{dogi}$ PNs (RFP-labelled) expressing *Rab5CA*	Fig. 7j, Supplementary Fig. 7	*y w, hs-FLP122/ y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ UAS-YFP-Rab5CA (Rab5Q88L); tubP-Gal80, FRT^{2A}/ $strip^{dogi}$, FRT^{2A}*

strip^{dogi} PNs (RFP-labelled) expressing *Rab5DN*	Fig. 7k	*y w, hs-FLP122/ y w (or Y); GH146-Gal4, UAS-mCD8-RFP/ UAS-YFP-Rab5DN (Rab5S43N); tubP-Gal80, FRT*^{2A}*/ strip*^{dogi}*, FRT*^{2A}
Control wing	Supplementary Fig. 6a	*Vps45RNAi/ + (Sb)*
Vsp45 RNAi expressing wing	Supplementary Fig. 6b	*engrailed (en)-Gal4 / +; Vps45RNAi/ +*
Vps45 RNAi expressing wing at *Vps45* heterozygous background	Supplementary Fig. 6c	*en-Gal4 / +; Vps45RNAi/ Vps45*^{JJ2}
Vps45 RNAi expressing wing at *avl* heterozygous background	Supplementary Fig. 6d	*en-Gal4/ +; Vps45RNAi/ avl*^{1}
Vps45 RNAi expressing wing at *Rab5* heterozygous background	Supplementary Fig. 6e	*en-Gal4/ Rab5*^{2}*; Vps45RNAi/+*
Vps45 RNAi expressing wing at *strip* heterozygous background	Supplementary Fig. 6f	*en-Gal4/ + ; Vps45RNAi/ strip*^{dogi}
Vps45 RNAi expressing wing at *Gl* heterozygous background	Supplementary Fig. 6g	*en-Gal4/ + ; Vps45RNAi/ Gl*^{1}
Cka^{1} PNs	Supplementary Fig. 9	*y w, hs-FLP122, UAS-mCD8GFP/ y w (or Y); tubP-Gal80, FRT*^{40A} *, GH146-Gal4, UAS-mCD8-GFP/ Cka*^{1}*, FRT40A*