Supplemental Figure Legends

Figure S1. Subcellular localization of Fmi\textsuperscript{E86} mutant protein

Wild-type embryos (A-A”) or fmi\textsuperscript{E86} homozygous embryos (B-B”) at stage 16 were double-stained for a pan-neuronal marker, anti-HRP (A and B; magenta in A” and B”; Jan and Jan 1982) and Fmi (A’ and B’; green in A” and B”). We found punctate signals in da neurons in the wild type as previously reported (bracket in A’; Sweeney et al., 2002) and also in the mutant (bracket in B’). Note that epidermal signals and neural signals are often difficult to separate in the wild type; one the other hand, epidermal signals were weak in the mutant embryo. In the CNS, Fmi\textsuperscript{E86} protein was distributed in the axon scaffold as in the wild type (data not shown). (C-D”) 31 hr APF pupal wing of the wild type (C-C”) or fmi\textsuperscript{E86}/fmi\textsuperscript{E59} (D-D”) was stained for DE-cadherin (C and D; Oda et al. 1993) and Fmi (C’ and D’). (C” and D”) Merged images. Distal is to the right and anterior is at the top. In wild-type cells, Fmi was localized along the proximal-distal cell interface (C’; green in C”; Usui et al., 1999). In contrast, Fmi\textsuperscript{E86} mutant protein resided almost uniformly at cell borders, and punctiform signals were prominent in the cytoplasm (D’; green in D”).

Figure S2. Member of the \textit{Drosophila} PET-LIM domain Family

(A) Schematic illustrations of domain structures of Prickle (Pk), Espinas (Esn) and dTestin. Each of these three proteins contains a PET domain and triple LIM domains. Pk and Esn have carboxyl-terminal CAAX motifs, but dTestin does not. Numbers indicate amino-acid identities between domains of Esn and those of Pk or dTestin. Esn-N and Esn-C indicate the polypeptide region used as antigens. (B) Flag-tagged JM of Fmi and Myc-tagged LIM of Pk were co-expressed in S2 cells. The cell lysates were blotted with
anti-Flag (Fmi input) or anti-Myc (Pk input). Flag-tagged JM was immunoprecipitated with anti-Flag and blotted with either anti-Myc (Pk co-IP) or anti-Flag (Fmi IP).

(C and D) Live images of dendritic arbors of class IV ddaC. The wild type (C) and

\( pk^{pK\text{sp}le-6}/pk^{pK\text{sp}le-13} \) (D). Arrows indicate crossings of dendritic branches. Genotype: (C) +; ppk-eGFP/ppk-eGFP, (D) \( pk^{pK\text{sp}le-6}/pk^{pK\text{sp}le-13} \), ppk-eGFP/ppk-eGFP. Scale bar represents 50 \( \mu \)m. (E and F) Quantitative analyses of the sum of branch lengths in each imaged zone (E) and the crossing index (F) of ddaC neurons of indicated genotypes. The \( pk \) mutant showed no dendritic crossing defect. Error bars indicate the mean ± s.d. The \( P \)-values are indicated in each graph (Student’s t-test). The number of cells counted for each genotype is indicated in E.

Figure S3. Esn protein is co-localized with Fmi in S2 cells

The Fmi-Esn interaction was investigated by expressing full-length proteins in S2 cells. We tagged Fmi and Esn with different fluorescent proteins (Fmi:3eGFP and mCherry:Esn, respectively). Expression of mCherry:Esn alone gave punctate signals that were positive for a Golgi marker ManII:GFP (A-A”). When both Fmi:3eGFP and mCherry:Esn were expressed in isolated cells, they were well co-localized in the intracellular puncta (B-B”). We then attempted to examine whether or not the localization of mCherry:Esn was altered when cells adhered to each other by way of Fmi-Fmi homophilic binding (Usui et al. 1999; Kimura et al. 2006). To answer this question, it was critical to mount a small number of cells that made contact with each other on a ConA-coated glass slide. However, we found that this mounting protocol made Fmi-mediated cell aggregates fall apart due to weak cell-cell binding. Nevertheless, in our best sample, Fmi was enriched at the cell-cell contact site where Esn was co-localized (C-C”). This result suggests that Esn could be
recruited to the cell interface by associating with Fmi C-tail.

**Figure S4. fz and RhoA are required for dendritic self-avoidance, and fmi genetically interacts with hpo.**

We studied whether or not self-avoidance requires fz, a member of core group genes, or the downstream components RhoA and Basket (Bsk)/JNK (A-D), and a genetic interaction between fmi and hpo, encoding an activator kinase of Trc (E and F). The sum of branch length (A, C, E), and the crossing index (B, D, F) were quantitated. fz null mutant larvae showed a significant increase in dendritic crossing (A, B), and expression of dominant-negative RhoA (RhoA^{T19N}) gave rise to remarkable elevation of dendritic crossing (C, D). In contrast, expression of dominant-negative Bsk (Bsk^{DN}) didn’t affect self-avoidance. fmi/hpo trans-heterozygote (fmi^{ES9}/hpo^{MGH4}) had a significant defect in suppression of dendritic crossing (E, F). Error bars indicate the mean ± s.d. The P-values are indicated in each graph (Student’s t-test [A, B, E, F], or one-way ANOVA and HSD post-hoc test [C, D]). (G) Quantitative analyses of the sum of branch length of each genotype (a paired graph of Fig. 6J). Error bars indicate the mean ± s.d., * P < 0.05, ** P < 0.01, *** P < 0.001 (one-way ANOVA and Tukey’s HSD post-hoc test). Magenta asterisks indicate statistically significant differences of the cohort from the wild type; and blue asterisks from the corresponding heterozygous controls. NS: Statistically not significant (P > 0.05). Genotype: (control in A and B) w; Gal4^{4-77} UAS-mCD8::GFP/++; +/+, (fz^{R52}/fz^{D21} in A and B) w; Gal4^{4-77} UAS-mCD8::GFP/++; fz^{R52}/fz^{D21}, (control in C and D) w; Gal4^{4-77} UAS-mCD8::GFP/+, (UAS-Bsk^{DN} in C and D) w/UAS-Bsk^{DN}; Gal4^{4-77} UAS-mCD8::GFP/+, (UAS-RhoA^{T19N} in C and D) w; Gal4^{4-77} UAS-mCD8::GFP/UAS-RhoA^{T19N}, (fmi^{ES9}/+ in E, F) w; fmi^{ES9}/+; ppk-eGFP/ppk-eGFP,
(fmi_E59/hpo^MGH4 in E and F) w; fmi_E59/hpo^MGH4; ppk-eGFP/ppk-eGFP. (+/trc^1 and +/trc^2 in G) w; +/+; trc^1 ppk-eGFP/ppk-eGFP and w; +/+; trc^2 ppk-eGFP/ppk-eGFP, (fmi_E59/trc^1 and fmi_E59/trc^2 in G) w; fmi_E59+/+; trc^1 ppk-eGFP/ppk-eGFP and w; fmi_E59+/+; trc^2 ppk-eGFP/ppk-eGFP.

Figure S5. *fmi* SOP-MARCM analysis and “*fmi* RNAi in *fmi* MARCM”

(A-C) Live images of dendritic arbors of class IV ddaC MARCM clone. The control clone (A), *fmi*E59 MARCM clone (B), and *fmi*E59 MARCM clone expressing *fmi* ds RNA (C). Arrows indicate crossings of dendritic branches. Genotype: (A) Gal4^{40}

\[UAS-Venus:pm SOP-flp^{42}/+; FRT^{G13}+/FRT^{G13} tubP-Gal80; +/+,(B) Gal4^{40}\]

\[UAS-Venus:pm SOP-flp^{42}/+; FRT^{G13} fmi^{E59}/FRT^{G13} tubP-Gal80; +/+,(C) Gal4^{40}\]

\[UAS-Venus:pm SOP-flp^{42}/+; FRT^{G13} fmi^{E59}/FRT^{G13} tubP-Gal80; UAS-fmi^RNAi/+\]. Scale bar represents 50 μm. (D and E) Quantitative analyses of the sum of branch lengths in each imaged zone (D) and the crossing index (E) of ddaC neurons of indicated genotypes. Error bars indicate the mean ± s.d. The *P*-values are indicated in each graph (one-way ANOVA and Tukey’s HSD post-hoc tests). The number of cells counted for each genotype is indicated in E.

References

