Supplementary Material
Recruitment of Scribble to the Synaptic Scaffolding Complex Requires GUK-holder a Novel DLG Binding Protein

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Supplementary Results

Expression of GUKH in the CNS and Epithelial Cells

We also observed anti-GUKH immunoreactivity in the CNS and in epithelial tissues. GUKH was first noticeable in clusters of cells in the CNS at embryonic stages 10 to 11 (Figure S1A). By stage 12, cells outlining the longitudinal connectives and commissures of the ventral ganglion became apparent (Figures S1B and S1C), and staining extended into the brain lobes. At stages 14/15, immunoreactivity increased in the nerve tracts, which were then distinctly more immunoreactive than neuronal cell bodies (Figure S1D). At this stage, GUKH staining in nerve tracts colocalized with DLG (Figures S1E–S1G). Labeling of sensory cells such as the chordotonal organs could be discerned by stage 15 (Figures S2A and S2B). Staining of lateral cell borders of most epithelial cells was also visible (Figure S2C).

GUKH was also observed in the larval CNS and imaginal discs (Figure S3). In the CNS, GUKH expression was observed in the ventral ganglion neuropil (Figure S3A), in two ventrally located cell clusters in the brain (Figure S3B), in the lamina (Figures S3C and S3D), and in dividing neuroblasts (Figure S3E, arrow). In neuroblasts, GUKH was asymmetrically distributed to the region of the neuroblast that gives rise to ganglion mother cells, in partial colocalization with DLG (Figures S3G–S3I). The ganglion mother cells in direct contact with the neuroblast also showed GUKH immunoreactivity in an asymmetric fashion (Figures S3G–S3I).

In imaginal discs, GUKH was observed at the periphery of epithelial cells (data not shown) but was particularly prominent in developing photoreceptors of the eye disc (Figures S3D and S3E). At this site, GUKH immunoreactivity was observed throughout photoreceptor projections into the lamina of the larval optic lobe (Figure S3D).

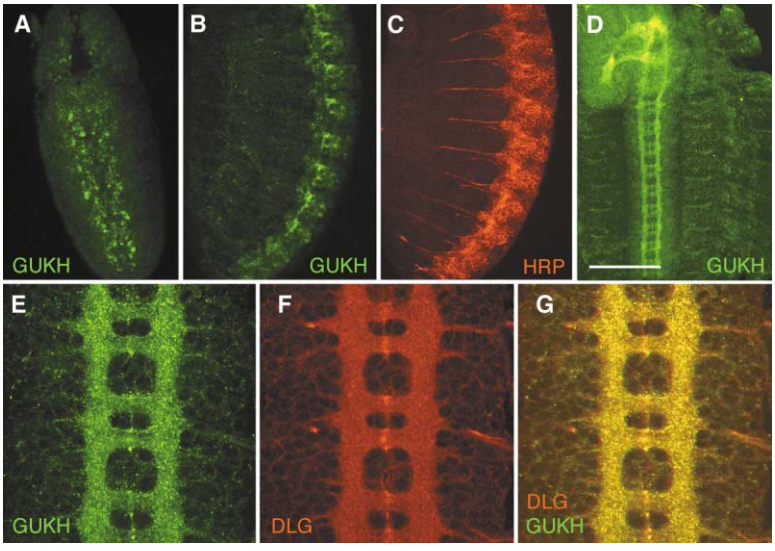

Figure S1. GUKH Immunoreactivity during Embryonic Development

(A) Dorsal view of a stage 10 to 11 whole-mount embryo showing clusters of GUKH immunoreactive cells in the CNS. (B) Lateral view of a stage 12 whole-mount embryo showing GUKH immunoreactivity in cells of the CNS. At this stage, peripheral nerves have begun to extend into the body wall, as demonstrated by anti-HRP double labeling (C), but no GUKH immunoreactivity is yet observed at neuronal projections. (D) Dorsal view of a stage 14/15 embryonic fillet showing strong GUKH immunoreactivity in commissural and longitudinal tracts. Note the relatively weaker staining of CNS cell bodies at this stage. (E–G) Higher magnification view of the CNS of a stage 14/15 embryonic fillet double stained with (E) anti-GUKH and (F) anti-DLG. (G) In this panel, the images in (E) and (F) have been merged to show areas of colocalization. Scale bar, 175 μm in (A)–(D) and 35 μm in (E)–(G).

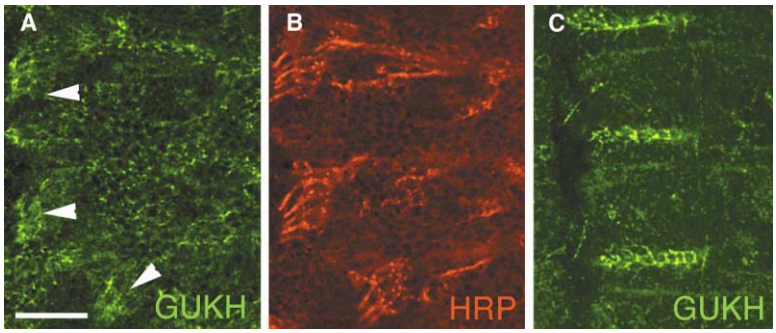

Figure S2. GUKH Immunoreactivity in Chordotonal Organs and Epithelial Cells

(A–C) View of the embryonic epidermis showing (A) GUKH expression at the border of epithelial cells and at chordotonal organs (arrowheads), (B) chordotonal organs stained with anti-HRP, and (C) prominent staining of lateral cell borders at the ventro-lateral segmental furrows. Scale bar, 25 μm.

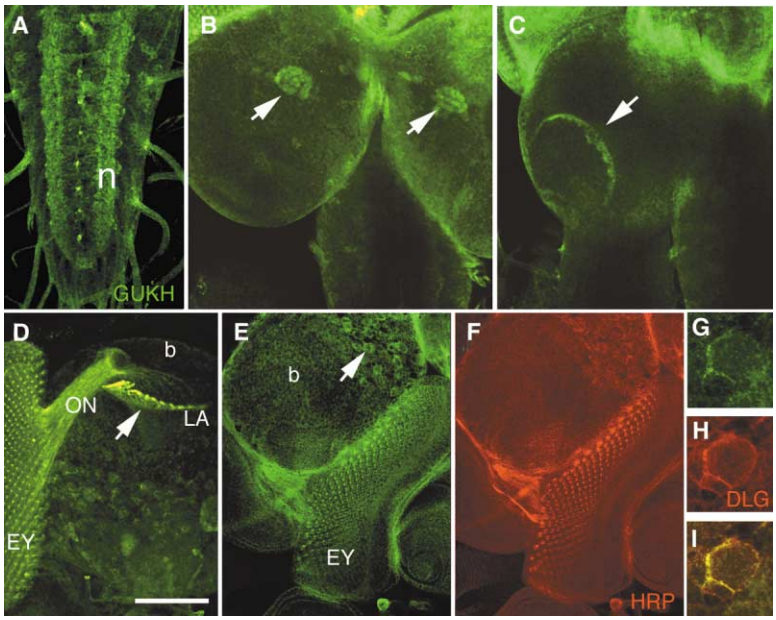

Figure S3. GUKH Expression in the Larval CNS and Eye Disc

GUKH expression in (A) the ventral ganglion neuropil (n), (B) brain lobe cell clusters (arrows), (C and D) the lamina region of the optic lobe (arrow), and (E) in neuroblasts (arrow) and photoreceptors in the eye disc (EY). (F) shows the same preparation as in (E) but labeled with anti-HRP. (G–I) Larval neuroblasts double stained with (G) anti-GUKH and (H) anti-DLG. (I) shows a merged image from (G) and (H). ON, optic nerve; LA, lamina; b, brain. Calibration bar is 45 μm in (A)–(F) and 8 μm in (G)–(I).