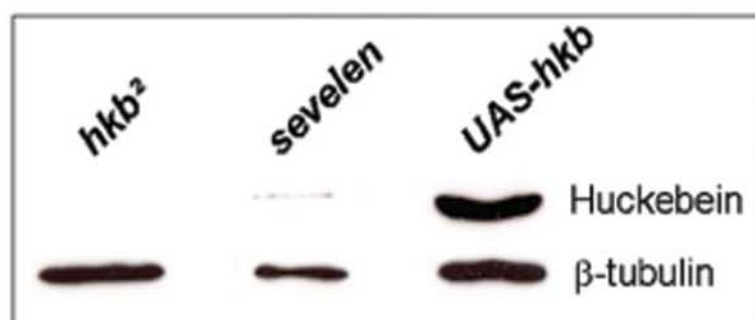


Sup. 1: Gcm-Hkb interaction by yeast two-hybrid system.

(A-I) Test of Gcm-Huckebein protein interaction using the yeast two-hybrid system based on LexA system. Yeast cells (L40) were co-transfected with pBTM116 plasmid containing the N-terminal part of Gcm (aminoacids 1-263) (pBTM116-Gcm) and pASV4 plasmid containing full length Hkb (pASV4-Hkb). (A-C) Positive controls show yeast growth on plates lacking leucine and tryptophan (SD-Trp-Leu): (A) pBTM116-Gcm + pASV4-Hkb, (B) pASV4-Hkb + pBTM116 (empty plasmid carrying Tryptophan encoding gene), (C) pBTM116-Gcm + pASV4 (empty plasmid carrying Leucine encoding gene). (D-F) X-gal assay. Only yeast co-transfected with pBTM116-Gcm and pASV4-Hkb give rise to a blue coloration (D) indicating of Gcm-Hkb interaction. (G-I) Auxotrophy assay on medium lacking Histidine. Only yeast co-transfected with pBTM116-Gcm and pASV4-Hkb grow on selective medium, confirming Gcm-Hkb interaction.



Sup. 2: *hkb*² is a null allele.

Quantitative analysis of Hkb protein levels by Western Blot. Extracts are from *hkb* (*hkb*²), wild type (Sevelen) or *sca-GAL4*; *UAS-hkb* (*UAS-hkb*) embryos. Note that Hkb-specific signal is not detected in *hkb* embryos. Anti-βtubulin was used to normalize loading.



Sup. 3: *hkb* overexpression does not affect the first NGB1-1A division.

Ventral views of WT (A) or *hkb* overexpressing embryos (UAS-*hkb* in B) at stage 16. Anti-Even skipped (*eve*) labeling to reveal aCC/pCC neurons (Broadus et al., 1995). A single abdominal segment is shown. Square brackets indicate aCC and pCC neurons. Scale bar: 10 μ m.