## Supplemental data

### Figure S1. Comparison of YRab1 knockdown efficiency using four different knockdown constructs.

We used the *engrailed*-gal4 driver to express each knockdown construct in a striped pattern in the epidermis of YRab1 larvae and imaged the endogenous YFP signal in heat-fixed third-instar wandering larvae. UAS-mCherry was used to label *engrailed*-gal4 expressing cells.

(A-D') Micrographs of epidermis in third-instar larvae showing a portion of epidermal cells not expressing *engrailed*-gal4 next to cells that express it (labelled by mCherry). (A-D) YFP signal from YRab1 (green), (A'-D') Overlays of YRab1 with mCherry (red).

(E) Quantification of knockdown efficiency. Bars show the difference in mean YRab1 pixel intensity in an area showing mCherry signal compared to YRab1 signal in an area showing no mCherry signal.

### Figure S2. Alignment of EYFP and GFP-IR1 sequences.

The alignment was generated using NCBI BLASTn from the GFP-IR1 sequence as reported on NIG-Fly and EYFP as reported on Addgene (http://addgene.org).