



### Supplementary Figure 5 GAL4-inducible RNAi directed against *dab* and *appl*

To specifically lower the amount of Dab and APPL, we generated transgenic flies with RNAi constructs which can be expressed under the control of GAL4. For this purpose, fragments of the respective cDNAs were amplified by PCR with the following primers:

Dab- $\gamma$ 17up (GACTCTAGAGGAGCAGTACCCACTGAGA)

Dab- $\gamma$ 17lo (GACTCTAGATCGAAAACCTACCTGGAAGAG)

Dab- $\gamma$ 3up (GACTCTAGAGCCATGTCACTAATCCACCT)

Dab- $\gamma$ 3lo (GACTCTAGAGGAAAGCAGCCATCAGA)

APPL-Hup (GACTCTAGATTGGCACTGCTCAGGTT)

APPL-Hlo (GACTCTAGATCCTCGTAGTTGTCACCCTC)

Products were subcloned into the GAL4-inducible pWIZ vector as suggested by the authors (Lee and Carthew, 2003). Several transgenic lines were generated and tested for their ability to suppress phenotypes induced by the ectopic expression of the *dab* *wt* cDNA in the *Drosophila* eye (data not shown) and to reduce the amount of overexpressed Dab and APPL protein (GMR-GAL4; western blot analysis). The RNAi-effect of the different chromosomal insertions showed a broad variation in the overall reduction. Therefore independent lines were used for further analysis, e.g. recombined with *sca*-GAL4. Overexpression of the *dab* cDNA results in three major immuno-reactive bands as indicated. The neuronal protein Elav served as loading control.

Lee YS & Carthew RW (2003) Making a better RNAi vector for *Drosophila*: use of intron spacers. *Methods* **3**: 322-329.