SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Genetic mosaics. The following *Drosophila* genotypes were used to generate loss of function clones:

hs-FLP; FRT42B mtv^{6}/ FRT42B Ubi.GFP

hs-FLP; FRT42B mtv^{6}/ FRT42B Ubi.GFP; hh-lacZ/+

hs-FLP; FRT42B mtv^{6}/ FRT42B Ubi.GFP; dpp-lacZ/+

hs-FLP; FRT42B Df(2R)en[E] mtv^{6}/ FRT42B Ubi.GFP

hs-FLP; FRT42B Df(2R)en[E] mtv^{6}/ FRT42B Ubi.GFP; hh-lacZ/+

hs-FLP; FRT42B Df(2R)en[E] mtv^{6}/ FRT42B Ubi.GFP; dpp-lacZ/+

hs-FLP; FRT42B Df(2R)en[E]/ FRT42B Ubi.GFP; hh-lacZ/+

hs-FLP; FRT42B mtv^{6}/ FRT42B Ubi.GFP; hh-lacZ/ c765-GAL4 UAS-Smo5A

hs-FLP tub-Gal4 UAS-GFP; FRT42B mtv^{6}/ FRT42B Gal80; UAS-Ptc/ hh-lacZ

hs-FLP; FRT42B ptc^{s2} mtv^{6}/ FRT42B Ubi.GFP

hs-FLP; FRT42B ptc^{s2} mtv^{6}/ FRT42B Ubi.GFP; hh-lacZ/+

hs-FLP; FRT42B ptc^{s2} / FRT42B Ubi.GFP; hh-lacZ/+

hs-FLP tub-Gal4 UAS-GFP; FRT42B ptc^{s2} mtv^{6}/ FRT42B Gal80; UAS-Nintra hh-lacZ

hs-FLP tub-Gal4 UAS-GFP; FRT42B ptc^{s2} mtv^{6}/ FRT42B Gal80; UAS-mam-DN hh-lacZ/+

hs-FLP; FRT82B Ubi-GFP hh-lacZ Df(3R)Espl22 / FRT82B πmyc

hs-FLP; hh-lacZ; FRT82B Ubi-GFP P(ci+)/ FRT82B πmyc; ci^{94}

hs-FLP; FRT82B Ubi-GFP hh-lacZ Df(3R)Espl22 / FRT82B P(ci+); ci^{94}

hs-FLP/MS1096-Gal4; UAS-Ci^{Cell}; FRT82B Ubi-GFP hh-lacZ Df(3R)Espl22/ FRT82B πmyc

The FLP/FRT technique (Xu and Rubin, 1993) was used to generate loss of function clones. The MARCM technique was combined to simultanesouly express different transgenes in these clones (Lee and Luo, 2001). Larvae of the appropriate genotypes were heat shocked for 1 h at 37°C, at several larval stages. The clones were visualized in discs by either loss of GFP expression or expression of GFP.

Yeast-two-hybrid: Yeast two-hybrid interaction assays were performed as described in (Goldstein et al., 1999). Yeast strain EGY048 (*MAT**a** trp1 ura3 his3 leu2::p3LexAop-LEU2*), containing an integrated *LEU2* reporter gene and upstream *LexA* operators, and transformed with *pSH18-34* (*URA3* plasmid *LexAop-lacZ*), and kindly provided by Z. Paroush, was

transformed with the *LexA(202+pl)* plasmid, driving expression of LexA-fusion baits, and with the *pJG4-5* (2µ *TRP1*) vector that allows galactose-inducible expression of proteins fused to an activation domain. Reporter gene activation was analysed on ura−his−trp−leu− growth plates, including galactose and raffinose, to select for activation of the *LexAop-LEU2* gene and on X-Gal indicator plates for *lacZ* expression. At least three colonies were assayed from each transformation.

GST pull-down: Sequence encoding Mtv was inserted into the *pGEX-4T-3* vector. GST-Hairy construct has been described previously (Paroush et al., 1994) and was kindly provided by Gerardo Jiménez. Expression of GST fusion proteins in the *E. coli* strain *BL21* and binding assays to ^{35}S-methionine-labeled Groucho protein (prepared using the TNT system, Promega) were performed as described previously (Paroush et al., 1994).

LEGENDS TO SUPPLEMENTARY FIGURE

Supplementary Fig 1. (A, B and E) Clones of cells mutant for *engrailed/invected* (*Df(2R)en[E]*, A and B) ot *ptc* (*ptc^{S2}*, E) labelled by the absence of the GFP marker (green). *hh-lacZ* (light blue or white) was not ectopically expressed in these clones. (C) The Hh signaling pathway was blocked by overexpression of a dominant negative version of Smoothened in all wing cells (Smo-5A). Note reduced levels of Patched (Ptc, red) expression. *hh-lacZ* (light blue) expression was not affected. (D, F and G) Expression of Patched (D), Mam-dn (F) or Nintra (G) under the control of the *apterous-Gal4* driver did not affect expression of *hh-lacZ* (blue). Ptc (red, in D), and GFP (green, in F and G) are shown to label the Gal4 expressing domain.

Supplementary Fig 2. (A) Full-length Groucho does not interact with Mtv in yeast, regardless of which of the two proteins is expressed as a DNA-binding domain fusion or as an activation-domain fusion. E(spl)-m7 and Huckebein are positive controls (Goldstein et al., 1999). Dmcdc2 serves as a negative control (Goldstein et al., 1999). β-galactosidase activity (blue colonies), visualized on X-Gal indicator plates, is indicative of protein-protein interaction. (B) Pull-down assay using the indicated GST fusions and *in vitro* translated Groucho labeled with ^{35}S-methionine. Groucho binds Hairy, as previously shown (Paroush et al., 1994), but not Mtv. Negative control shows no interaction between GST and Groucho. Lanes were loaded with 1/3 of the protein used in the binding reactions. Expression of GST fusion proteins was confirmed by Commassie (data not shown).

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