SUPPLEMENTARY INFORMATION

METHODS

Dominant suppressor screen for factors that interact with *hairy*

Full length *hairy* cDNA was introduced into pUAST (Brand and Perrimon, 1993) to create *UAS-hairy*. A transgene insertion on chromosome 2 was recovered using standard P-element transformation methods. *w; GMR-Gal4, UAS-h/ GMR-Gal4, UAS-h; FRT80B, FRT82B/ FRT80B, FRT82B* males were fed a 1% sucrose solution containing 25 mM EMS for 14-16 h. 24 h after mutagen treatment, the males were crossed to homozygous *w; GMR-Gal4,UAS-h; e* females and allowed to mate for 4 days. Adult progeny with suppression of the *GMR-Gal4,UAS-h* eye phenotype were retested by crossing to *w; GMR-Gal4,UAS-h; e*, and balanced by crossing to *w; GMR-Gal4, UAS-h/CyO; Sb/TM3*. We did not identify any mutations that enhanced the ectopic Hairy phenotype. A pilot screen of 3000 flies produced 3 mutations mapping to chromosome 2 that were strong suppressors of the phenotype. Analysis indicated that these mutations were likely to be mutations in either the *GMR-Gal4* or *UAS-hairy* transgenes, and so subsequent mutations mapping to chromosome 2 were not analysed further.

One mutation recovered from chromosome 3 (phenotype segregates with e^+) in the pilot screen failed to complement gro^{E48}, a strong hypomorphic allele of a known co-factor for Hairy. Thus all subsequent mutations recovered from chromosome 3 were tested for complementation to gro^{E48}. Mutations from chromosome 3 that complemented *gro* were tested with *glass*, since the product of *glass* drives expression of GMR-Gal4. We recovered 62 mutations from the screen of approximately 137,000 flies, which comprised of 50 alleles of *gro* and 8 alleles of *glass*. The remaining 4 mutations represented single alleles of different loci and were not characterised further.

Germline clones and embryo analysis

The *FRT/FLP/ovo*^{D} system (Chou et al., 1993) to was used to generate germline clones homozygous for alleles of *gro* as described previously (Jimenez et al., 1997). In situ hybridisation and immunohistochemistry were performed as described by (Jennings et al., 2006).

Imaginal disk clonal analysis

The FRT/FLP system was used to generate and visualise homozygous *gro* mutant clones in imaginal wing disks. Clones of homozygous mutant *gro* cells were marked by loss of GFP. The *vgQ-lacZ* reporter is described in Kim et al., 1996. Primary antibodies used were polyclonal rabbit anti-β-galactosidase (ICN/Cappel; 1:2,500) and mouse anti-GFP (JL8, Clontech; 1/500).

Molecular characterisation of *gro* alleles

RNA was isolated from pools of 20 adult flies heterozygous for each mutant allele and the parental *FRT82B* chromosome allele using the RNeasy kit (Qiagen). This RNA was transcribed into cDNA using the Titan OneTube RT-PCR system (Roche), with primers: 5'-CCGGCAACATACCAGCCTAAC and 5'-AAACTGCATGGTTTTGTGGAT and cloned into pCR4-TOPO (Invitrogen). Pools of cDNA clones (>100) for each allele were made and translated using the TNT T7 Coupled Reticulocyte Lysate System (Promega) with L-[^{35}S]methionine. The products of the translations were analysed by SDS-PAGE (see below) and autoradiography. Each pool was expected to contain a mix of wild type and mutant cDNAs since the RNA was isolated from heterozygous flies. Alleles producing pools of cDNA that gave an obviously truncated product were identified (20/50), and the screen repeated with individual cDNAs to identify mutant clones for sequencing.

To locate the mutation in flies not producing a truncated product, the coding region of genomic DNA from heterozygous for the mutant chromosome and the parental *FRT82B* chromosome was amplified in 150-200 bp fragments using PCR and analysed using single stranded conformational polymorphism (SSCP) as described previously (Jennings et al., 2004). Primer sequences are available on request. Genomic PCR fragments containing a polymorphism were cloned into pCR4-TOPO. Individual clones were analysed by SSCP to determine which contained the mutation and then sequenced. Where possible, individual cDNAs were isolated and sequenced to confirm the nature of the mutation, although some mutant cDNAs were not recovered, presumably due to nonsense mediated mRNA decay (Gatfield et al., 2003).

To estimate the relative stability of *MB36* transcripts *in vivo*, we used SSCP analysis to detect a polymorphism in *gro* between *MB36* and the wild-type *gro* allele of the *ss* e^4 *ro* chromosome. cDNAs was made from pooled adult *MB36/ss* e^4 *ro* flies by PCR with the following primers: Forward, GACATCTCGCAACCGGGCAACAAG; Reverse, GCCGATGTTAGTTCCGCCTTTATGCG, and individual cloned cDNAs examined by SSCP electrophoresis to estimate the relative abundance of *MB36* transcripts. From 126 cDNAs, only 13 derived from the *MB36* chromosome, all of which were longer, as determined by electrophoresis and/or DNA sequencing, due to the intronic sequences that create a translational frameshift. Thus, *MB36* transcripts are indeed unstable and non-coding.

***In vitro* protein analyses**

SDS PAGE was performed using 4-12% NuPage Bis-Tris Gels (Invitrogen) and MOPS SDS running buffer. Gels were transferred to Hybond-ECL (GE Healthcare) membranes for Western blot analysis using the manufacturer's protocol.

Blue-native (BN)-PAGE was carried out with minor modifications to the published method (Schagger and von Jagow, 1991). Addition of the negatively-charged dye Coomassie Blue G-250 causes a charge shift to native proteins, reduces non-specific aggregation, and maintains oligomeric state. Samples were mixed with an equal volume of 2x Tris-glycine native sample buffer (Invitrogen) containing 0.1% Coomassie Blue G-250 and run at 125 V on a 4-12% Tris-glycine gel (Invitrogen) with 1 x Tris-glycine Native Running Buffer (25 mM Tris-base, 192 mM Glycine) containing 0.005% Coomassie blue G-250 in the cathode buffer. The NativeMark ladder (Invitrogen) was used as the molecular weight standard. For western blot analyses from gels run under BN conditions, gels and membranes were washed separately for 10 minutes in 1 x Tris-glycine transfer buffer (Tris-base 12 mM, Glycine 96 mM, 20% methanol) containing 0.05% SDS, before blotting at 15 V for at least 2 h in transfer buffer lacking SDS.

GST binding assays were performed as described previously (Jennings et al., 2006).

Western blots of embryonic extracts

For SDS-PAGE blots, 15 μl of extract, made from 2 embryos per μl of sample buffer, was loaded per well. For BN-PAGE blots, 10 μl of extract, made from 4 embryos per μl of sample was loaded per well. Native extracts were made by grinding ~400 embryos in 100μl chilled native sample buffer for 1 min with a mortar and pestle in the presence of small glass beads. α-Groucho antibody (Delidakis et al., 1991); from the Developmental Studies Hybridoma Bank (DSHB), University of Iowa) was used at 1:5 dilution, and the E7 β-tubulin antibody (Chu and Klymkowsky, 1989 DSHB) at 1:2000. Bound antibody was detected using ECL Plus (GE Healthcare).

Supplementary Figure S1: Quantification of MB12 protein *in vivo*

Levels of Gro protein in MB12 germline clone and wild-type embryos were compared Western blotting of serial dilutions of wild-type extract run alongside MB12 extract. The E7 (β-tubulin) antibody was used as a loading control.

References

Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**, 401-415.

Chou, T.B., Noll, E. and Perrimon, N. (1993) Autosomal *P[ovoD1]* dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development*, **119**, 1359-1369.

Chu, D.T. and Klymkowsky, M.W. (1989) The appearance of acetylated alpha-tubulin during early development and cellular differentiation in *Xenopus*. *Dev Biol*, **136**, 104-117.

Delidakis, C., Preiss, A., Hartley, D.A. and Artavanis-Tsakonas, S. (1991) Two genetically and molecularly distinct functions involved in early neurogenesis reside within the Enhancer of split locus of *Drosophila melanogaster*. *Genetics*, **129**, 803-823.

Gatfield, D., Unterholzner, L., Ciccarelli, F.D., Bork, P. and Izaurralde, E. (2003) Nonsense-mediated mRNA decay in *Drosophila*: at the intersection of the yeast and mammalian pathways. *EMBO J*, **22**, 3960-3970.

Jennings, B.H., Pickles, L.M., Wainwright, S.M., Roe, S.M., Pearl, L.H. and Ish-Horowicz, D. (2006) Molecular recognition of transcriptional repressor motifs by the WD domain of the Groucho/TLE corepressor. *Mol Cell*, **22**, 645-655.

Jennings, B.H., Shah, S., Yamaguchi, Y., Seki, M., Phillips, R.G., Handa, H. and Ish-Horowicz, D. (2004) Locus-specific requirements for Spt5 in transcriptional activation and repression in *Drosophila*. *Curr Biol*, **14**, 1680-1684.

Kim J, Sebring A, Esch JJ, Kraus ME, Vorwerk K, Magee J, Carroll SB. (1996) Integration of positional signals and regulation of wing formation and identity by *Drosophila vestigial* gene. *Nature*. **382** 133-8.

Jimenez, G., Paroush, Z. and Ish-Horowicz, D. (1997) Groucho acts as a corepressor for a subset of negative regulators, including Hairy and Engrailed. *Genes Dev*, **11**, 3072-3082.

Schagger, H. and von Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal Biochem*, **199**, 223-231.

Supplementary Figure S1