Supplemental Material

Fig. S1 Validation of microarray results by in situ hybridization

(A-B) Example of MB neurons labeled with OK107-GAL4 driven UAS-mCD8::GFP expression before (A; arrowheads) and after (B; open arrowheads) laser-capture microdissection.

(C-J) In situ hybridization of genes regulated by ecdysone signaling. Both *CG12880* (C-D) and *CG31324* (E-F) are upregulated in 0 hour APF pupae compared to early third instar larvae (L). Conversely, *Rab27* (G-H) and *Gapdh1* (I-J) are downregualted in 0 hour APF pupae.

(K-P) In situ hybridization for *boule* mRNA expression in MB γ neurons in wt third instar larval brains (K-L), wt 0 hour APF pupal brains (M-N) and EcRDN-expressing MB γ neurons in 0 hour APF pupal brains (O-P). Panels (K, M and O) show staining with antisense *boule* probe and panels (L, N and P) show staining with sense control probe.

(Q) Table of genes from microarray that were validated by fluorescent in situ hybridization showing fold expression changes from microarray experiments (see Fig. 1A for comparisons) and results of in situ hybridization.

Panels for in situ hybridizations show confocal z-projections from 15 μm cryosections through MB neuron cell bodies. All images for the same gene (antisense and sense probes) are from in situ hybridizations carried out under the same conditions and imaged at the same gain. Yellow dashed outlines indicate MB γ neurons as defined by 201Y-GAL4 driven mCD8::GFP expression and DAPI staining (not shown). Arrow in (P) denotes the calyx region, which is composed of MB dendrites and is devoid of cell bodies.

Fig. S2 Validation of *bol*^{40} null mutation

(A-B) Boule antibody staining in testes (left) and brain (right) of bol^{1}/+ (A_1), bol^{1} (A_2), bol^{40}/+ (B_2) and bol^{40} (B_2) flies. Arrows point to region of positive staining around the antennal lobes.

(C) Quantification of bol^{40} mutant fertility. A single male or 3 virgins of the genotypes listed were independently crossed to 6 virgin female or 3 male w^{1118} flies, allowed to mate for 3 days and removed. Vials with progeny were scored as fertile.

Fig. S3 Misexpression of *Drosophila* polyA-binding protein in MB neurons does not inhibit axon pruning

(A) Axon lobes of wt MB neurons in the adult brain labeled with OK107-Gal4 driven mCD8::GFP (green) and immunostained for endogenously expressed FasII protein (red), which marks γ and α/β axons.

(B) MB neurons expressing transgenic *Drosophila* polyA-binding protein tagged with three FLAG epitopes (*UAS-PABP*; Yang et al., *Nucleic Acids Res.* 33:17, 2005) show defects in dorsal lobe axon morphology, but not in axon pruning (compare to Fig. 6H). Similarly, our gain-of-function screen uncovered several other RNA binding proteins that gave diverse phenotypes when misexpressed in MB neurons but did not inhibit axon pruning. These RNA binding proteins, which include *Fmr1*, *pumilio*, *split ends* and *hephaetus,* have different RNA binding motifs than Boule.

Panels (A_2 and B_2) show immunostaining against FLAG, and panels (A_3 and B_3) are show a merged image of mCD8::GFP (green), FasII (red) and FLAG (blue) staining. Scale bar is 50 μm.

List of Tables

Table S1 – Genes upregulated by EcR in MB neurons at the onset of pruning

Ecdysone-induced genes. Genes were considered as ecdysone-induced if they showed a statistically significant increase in expression in wt MB neurons at 0 or 5 hours APF compared to larval neurons, and in wt neurons at 0 or 5 hours APF compared to EcRDN neurons. The majority (67%) of ecdysone-induced genes are present only at 0 hours APF, while 9.6% are present only at 5 hours APF and 23.5% are present at both time points. Genes highlighted in blue were verified by in situ hybridization (see Fig. S1).

Table S2 – Genes downregulated by EcR in MB neurons at the onset of pruning

Ecdysone-repressed genes. Genes that showed a statistically significant decrease in expression between wt neurons at 0 or 5 hours APF compared to larval neurons and in wt neurons at 0 or 5 hours APF compared to EcRDN neurons. Similar to ecdysone-induced genes, 53% of the ecdysone-repressed genes are present at 0 hours APF only, 18% at 5 hours APF only and 29% at both time points. Genes highlighted in blue were verified by in situ hybridization (see Fig. S1).

Table S3 – GO analysis of Ecdysone upregulated genes.

(A) Table with EASE scores for GO terms from the GO slim essential list (Tomancak et al., 2007) that are enriched in the upregulated population of genes. Terms are sorted by fold change, which represents the fold increase in the frequency of finding genes from the list (List Total) that belong to a particular GO terms (Pop Hits) compared to the expected frequency derived from the total population of genes on the array (Pop Total). The P value is the modified Fisher's exact p value given by DAVID (Dennis et al., 2003).

(B-D) Tables of the genes that were identified for the GO terms in (A) separated by Biological Process, Cellular Component or Molecular Function. Affymetrix probe sets and corresponding gene names are listed.

Table S4 – GO analysis of Ecdysone downregulated genes.

(A) Table with EASE scores for GO terms from the GO slim essential list (Tomancak et al., 2007) that are enriched in the downregulated population of genes. Same conventions as Table S3.

(B-D) Tables of the genes that were identified for the GO terms in (A) separated by Biological Process, Cellular Component or Molecular Function. Affymetrix probe sets and corresponding gene names are listed.

Table S5 – Primer sequences