Supplementary material

A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*

Rachel Kraut, Kaushiki Menon and Kai Zinn

Current Biology 20 March 2001, **11**:417–430

Supplementary materials and methods

Live screening of third instar larvae

Homozygous lethal second chromosome EP insertions were balanced over *CyO-actin-GFP* (Bloomington Stock Center), and most homozygous lethal third chromosome insertions were balanced over *TM6B* (*Tubby*) so that EP-containing larvae could be recognized by the absence of the Tubby marker. Homozygous virgin females were crossed to the driver line for EPs on the X chromosome. For X chromosome lethals and some on the third that were balanced over TM3, we examined twice as many (eight) GFP-containing F1 larvae, given that those bearing EP insertions could not be separated from balancer larvae. Only about 2% of EPs are embryonic/early larval lethal when crossed to C155;GFP. The C155;GFP driver lines were homozygous for C155-GAL4 (on the X) [S104] and, on the second chromosome, for either UAS-GFP^{S65T} (a gift of Haig Keshishian and Barry Dickson) or UAS-2×-EGFP (a gift of Marc Halfon; this construct has two copies of E-GFP with an IRES sequence upstream of the second EGFP). The driver lines were of approximately equal brightness, but the 2×-EGFP driver appeared to be more resistant to bleaching. The crosses of the drivers with the EP lines were carried out at 25°C or 29°C on a blue *Drosophila* medium to reduce background autofluorescence of the gut (Carolina Biological Supply). Live larvae were temporarily paralyzed by keeping them on ice for >1 hr, and were then mounted in 70% glycerol in PBS under a 24 × 60 mm coverslip; alternatively, larvae were placed in 90% glycerol and partially squashed under the coverslip without placing them on ice (this procedure increases the brightness of the GFP signal). Live larvae were examined with a 40× water immersion lens, using an Endow GFP filter set (Chroma) on a Zeiss Axioplan microscope.

For many of the homozygous-viable second and third chromosome EP lines, we performed a behavioral prescreen to enrich for lines with GOF phenotypes. For crosses that were prescreened, five virgin females of the driver line were crossed to the EP lines, and vials were kept moist. We classified the EP lines according to the fecundity of the cross with the driver line and/or the crawling ability of the F1 larvae, as all F1 offspring from the driver cross were genetically identical. By conducting pilot screens, we observed that when cultured under equivalent conditions, crosses that produced less mobile or fewer than normal larvae (subnormal crosses) were greatly enriched for motor neuron defects compared to crosses that produced many larvae that crawled high up onto the vial walls. On the second chromosome, we eliminated about 400 lines based on this prescreen, and about 350 on the third chromosome. Thus, about 750 lines have not been anatomically screened, and a small number of these are likely to display phenotypes that would have been scored as abnormal had they been screened anatomically. The ID numbers of the ~750 EPs that were not screened anatomically are available on request. The behavioral prescreen only worked with genetically identical larvae. If larvae from a subnormal cross were mixed with wild-type larvae, their behavioral phenotypes were usually rescued. Thus, for homozygous lethal autosomal EP insertions and X-linked insertions, F1 larvae of the appropriate genotype from every cross were examined.

We examined four F1 larvae (30–40 hemisegments) of the appropriate genotype from the cross of the EP line to the driver; a line was considered abnormal if more than two hemisegments per larva showed errors in pathfinding and/or missing or abnormal synapses for the branches we scored. The occurrence of pathfinding or synaptic abnormalities in the driver line alone, or in wt x driver F1 larvae, was rare (<<1 abnormal synapse/larva). Lines displaying phenotypes were recrossed for confirmation. Images in Figures 1, 4, 5, and S1 of live GFP-expressing larvae were taken with a 40× water immersion objective on a Zeiss Axioplan microscope with an Optronics Magnafire camera (Olympus). Composites from several focal planes were constructed using Adobe Photoshop.

Antibody staining of wild-type and mutant larvae

Mutant lines were selected and rebalanced over green balancers (w; In(2LR)noc4LScorv9R, b1/CyO, P{w+mC=ActGFP}JMR1 and w; Sb1/TM3, P{w+mC=ActGFP}JMR2, Ser1). We examined the following mutant lines: P{ry+t7.2=PZ}poe^{1} cn1/CyO; ry^{506} (*pushover*); egl^{1286}/SM5 (*egalitarian*); cn^{1} P{ry+t7.2=PZ}ttv^{5282}/CyO; ry^{506}, and ttv^{681} (*tout-velu*); cn^{1} P{ry+t7.2=PZ}α-Adaptin^{06694}/CyO; ry^{506} (*α-adaptin*); w;k^{09507}/SMZ (*wunen*, from Ken Howard); pum^{01688}/TM3 (*pumilio*); pbl^{09645}/TM3 (*pebble*); *Ptp10D^{1}*; Abl^{1}/TM6B, Abl^{4}/TM6B (*Abl*); cdi^{R47}/TM3 (*cdi*, from Steve Crews); Kr-h1^{k0441}/CyO (*Kr-h1*); amn^{EP971} (*amnesiac*; other *amn* alleles were also examined, but these had weaker phenotypes). The stocks were obtained from the Bloomington Stock Center except where indicated.

For lines where non-Act-GFP third instar larvae survived, these were selected and filleted as follows (see [S1, S2]): larvae in Ringer's solution in an Eppendorf tube were placed in a 68°C water bath for 40 s, then on ice. They were pinned down using cut tungsten wire on Sylgard-coated dishes (Dow Corning) in Ringer's solution, then fixed for 20 min in phosphate buffer with 4% formaldehyde. This was washed out with PBS, 0.1% Triton X-100 several times. Larvae were stained with a mixture of mAbs 22C10 [S3] and 1D4 [S4] in PBS, 0.1% Tween 20. This was followed by thorough washing in PBS and incubation with Oregon Green–coupled secondary antibody (Molecular Probes; 1:500) and rhodamine-phalloidin (Sigma; 1:2000 dilution in PBS without detergent) to label the muscles. Overexpression larvae from the EP x driver crosses were dissected in the same manner and stained with anti-GFP antibody (Molecular Probes; 1:2000) followed by Oregon Green–coupled secondary antibody and rhodamine-phalloidin. Larvae were mounted in 70% glycerol in PBS with 1 mg/ml phenylenediamine to preserve fluorescence. Images were taken as a *z*-series on a Zeiss 510 confocal microscope and projected using Zeiss software. Volume-rendered images in Figure 2 were generated by projecting two separate 0.7 μ slice *z*-series of anti-GFP-stained nerves and rhodamine-phalloidin-stained muscles of larval fillets using the Imaris program (Bitplane AG, Zurich).

In situ hybridization

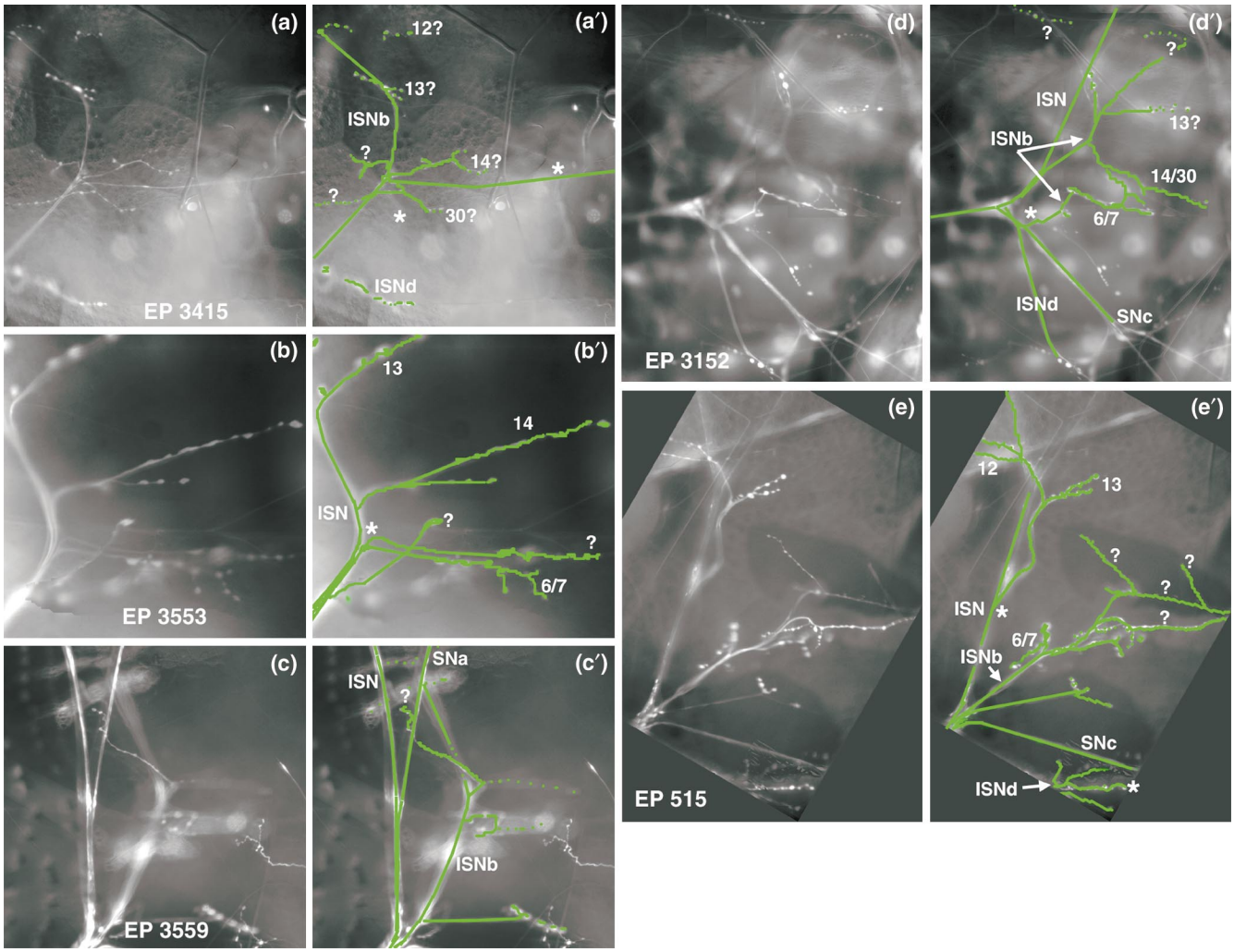
In situ hybridization of RNA probes to wild-type and EP x driver embryos was performed essentially as described [S5]. Probes were made with the Ambion Megascript kit using cDNA clones listed on the BDGP web site; clone numbers for the new genes are indicated in Table S5. cDNA clones were obtained from Research Genetics, and each cDNA was partially sequenced to confirm its identity before probe generation.

Database searching

Flanking sequences surrounding EP insertions were obtained from the Berkeley Drosophila Genome Project (BDGP) web site or from Guochun Liao of the BDGP. We searched the *Drosophila* databases with these sequences, and located known or predicted genes within the immediate vicinity of the EPs. We also identified EST and full-length cDNA sequences in these regions.

To determine the relationships between the predicted genes (CGs in GadFly) and other sequences in the database, we conducted blastp searches of the nr (nonredundant) and *Drosophila* genome databases with the CG protein sequences. We also searched >10 kb of DNA sequence adjacent to each EP for homology to protein sequences in the database using blastx. Over 3000 separate BLAST searches were conducted and analyzed in order to assemble a complete picture of the

Figure S1



Complex phenotypes in F1 EP x driver larvae. All panels show ventral muscle regions. The wild-type is shown in (a,a′) of Figure 4. **(a,a′)** EP3415, overexpressing *pebble*. This phenotype is characterized primarily by extra synaptic and axonal branches, some of which cross segment borders (*). **(b,b′)** EP3553, overexpressing CG1210 (encodes a PDK-like kinase). Abnormal branches are observed (?); the 6/7 synapse appears to be doubled (*). **(c,c′)** EP3559, overexpressing CG5643 (encodes a PP2A regulatory B subunit). ISNb has a very abnormal structure, with few recognizable branches. **(d,d′)** EP3152, overexpressing CG6811 (encodes p50-RhoGAP). ISNb is split into two tracts that leave the ISN at different points (arrows). One of these appears to wander dorsally and innervate 6/7 (*), while the other (out of focus) may innervate 12 and 13. **(e,e′)** EP0515, overexpressing CG7719 (encodes a kinase). ISNb is again split into two tracts that leave the ISN at different points. One of these (upper *) innervates 12 and 13, while the other (labeled ISNb) has an unrecognizable structure, but appears to innervate 6/7 and other muscles (possibly 14 and 30). ISNd, which is normally linear, is also split into a spray of branches (lower *).

homologies that are displayed by the genomic regions around these EPs.

Most of the 35 new genes that are adjacent to EPs appear to correspond to single CGs. The actual proteins that are encoded by some of these genes may differ from those described here, however, as CGs are preliminary annotations. Gene prediction programs can successfully predict exons, but they often group these exons into genes in an incorrect manner, and do not always select the correct exon borders (splice sites). They also cannot predict complex transcriptional maps with features such as overlapping genes [S6, S7]. Table S4 describes the evidence for the size of each of the predicted proteins, and notes potential errors in the CG predictions.

Supplementary references

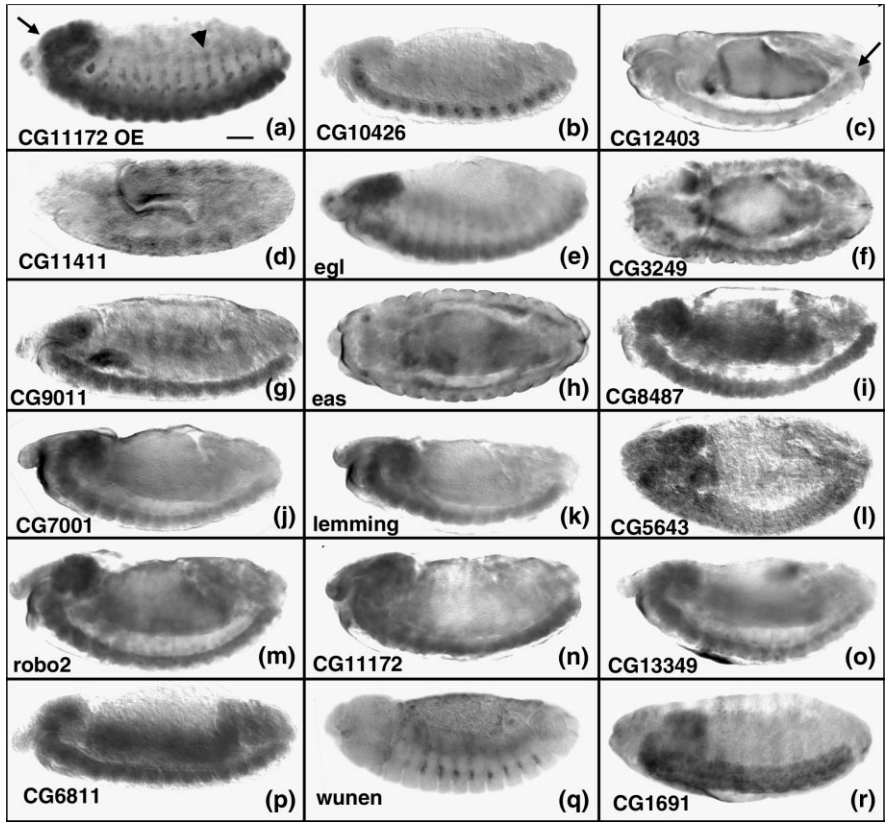
S1. Johansen J, Halpern ME, Johansen KM, Keshishian H: **Stereotypic morphology of glutamatergic synapses on identified muscle cells of *Drosophila* larvae.** *J Neurosci* 1989, **9:**710-725.

S2. Johansen J, Halpern ME, Keshishian H: **Axonal guidance and the development of muscle fiber-specific innervation in *Drosophila* embryos.** *J Neurosci* 1989, **9:**4318-4332.

S3. Zipursky S, Venkatesh T, Teplow DB, Benzer S: **Neuronal development in the *Drosophila* retina: monoclonal antibodies as molecular probes.** *Cell* 1984, **36:**15-26.

S4. Van Vactor D, Sink H, Fambrough D, Tsoo R, Goodman CS: **Genes that control neuromuscular specificity in *Drosophila*.** *Cell* 1993, **73:**1137-1153.

Figure S2



Embryonic expression patterns of new genes and known genes. Digoxigenin-labeled cRNA probes were hybridized to collections of EP x driver (a) and wild-type embryos (all other panels). Hybridization was detected using the alkaline phosphatase color reaction. Lateral views of whole-mount embryos are shown (anterior to the left, dorsal up), except where indicated. The CNS is the ventral band (arrow in [a]); PNS neurons indicated in (a) by an arrowhead. **(a)** A stage 14 EP1353 x driver embryo. The color reaction was allowed to proceed for 0.5 hr for (a), because overexpression is at much higher levels than endogenous expression, and for 1–3 hr for all other panels. Descriptions of new gene expression patterns are in Table S5. **(b)** CG10426/PPI 5-Ptase, stage 13. **(c)** CG12403/Vha68, stage 15 (negative control; this gene exhibits no expression in the CNS). **(d)** CG11411, stage 11. Segmentally repeated spots along the germ band are in the neuroblast layer. **(e)** *egalitarian*, stage 13; this gene is selectively expressed in the CNS during embryogenesis. **(f)** CG3249/PKA anchor protein, dorsal view, stage 14. **(g)** CG9011/DBEACH1, stage 14. **(h)** *easily shocked*, ventral view, stage 15; this gene is expressed at the highest levels in somatic and visceral mesoderm and the salivary placodes (dots at anterior end). **(i)** CG8487/sec7-GEF, stage 14. **(j)** CG7001/Bin4 kinase, stage 14. **(k)** *lemming*, stage 15; this gene is expressed in the CNS and elsewhere at lower levels. **(l)** CG5643/PP2A B subunit, stage 16. **(m)** *roundabout 2*, stage 15. **(n)** CG11172/Rel domain protein, stage 14. **(o)** CG13349/surface protein, stage 14. **(p)** CG6811/p50Rho-GAP, stage 13. **(q)** *wunen*, stage 14; this focal plane shows expression in segmentally repeated epidermal clusters. **(r)** CG1691/mRNA localization protein, stage 16. The scale bar represents 40 μm.

S5. Broadus J, Doe CQ: **Evolution of neuroblast identity: seven-up and prospero expression reveal homologous and divergent neuroblast fates in *Drosophila* and *Schistocerca*.** *Development* 1995, **121:**3989-3996.

S6. Ashburner M, Misra S, Roote J, Lewis SE, Blazej R, Davis T, *et al.*: **An exploration of the sequence of a 2.9-Mb region of the genome of *Drosophila melanogaster*: the Adh region.** *Genetics* 1999, **153:**179-219.

S7. Reese MG, Hartzell G, Harris NL, Ohler U, Abril JF, Lewis SE: **Genome annotation assessment in *Drosophila melanogaster*.** *Genome Res* 2000, **10:**483-501.

S8. GonzalezGaitan M, Jackle H: **Role of *Drosophila* alpha-adaptin in presynaptic vesicle recycling.** *Cell* 1997, **88:**767-776.

S9. Henkemeyer MJ, West SR, Gertler FB, Hoffman FM: **A novel tyrosine kinase-independent function of *Drosophila abl* correlates with proper subcellular localization.** *Cell* 1990, **63:**949-960.

S10. DeZazzo J, Sandstrom D, de Belle S, Velinzon K, Smith P, Grady L, *et al.*: **nalyot, a mutation of the *Drosophila* Myb-related Adf1 transcription factor, disrupts synapse formation and olfactory memory.** *Neuron* 2000, **27:**145-158.

S11. DeZazzo J, Xia SZ, Christensen J, Velinzon K, Tully T: **Developmental expression of an amn(+) transgene rescues the mutant memory defect of amnesiac adults.** *J Neurosci* 1999, **19:**8740-8746.

S12. Feany MB, Quinn WG: **A neuropeptide gene defined by the *Drosophila* memory mutant amnesiac.** *Science* 1995, **268:**869-873.

S13. Tatei K, Cai H, Ip YT, Levine M: **Race: a *Drosophila* homologue of the angiotensin converting enzyme.** *Mech Dev* 1995, **51:**157-168.

S14. Gellon G, Harding KW, McGinnis N, Martin MM, McGinnis W: **A genetic screen for modifiers of Deformed homeotic function identifies novel genes required for head development.** *Development* 1997, **124:**3321-3331.

S15. Adamson AL, Shearn A: **Molecular genetic analysis of *Drosophila* ash2, a member of the trithorax group required for imaginal disc pattern formation.** *Genetics* 1996, **144:**621-633.

S16. Mosrin-Huaman C, Canaple L, Locker D, Decoville M: **DSP1 gene of *Drosophila melanogaster* encodes an HMG-domain protein that plays multiple roles in development.** *Dev Genet* 1998, **23:**324-334.

S17. Ganetzky B, Wu CF: **Indirect suppression involving behavioral mutants with altered nerve excitability in *Drosophila melanogaster*.** *Genetics* 1982, **100:**597-614.

S18. Matthews BB, Crews ST: ***Drosophila* center divider gene is expressed in CNS midline cells and encodes a developmentally regulated protein kinase orthologous to human TESK1.** *DNA Cell Biol* 1999, **18:**435-448.

S19. D'Avino PP, Thummel CS: **crooked legs encodes a family of zinc finger proteins required for leg morphogenesis and ecdysone-regulated gene expression during *Drosophila* metamorphosis.** *Development* 1998, **125:**1733-1745.

S20. Olivier JP, Raabe T, Henkemeyer M, Dickson B, Mbamalu G, Margolis B, *et al.*: **A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of ras guanine nucleotide exchange, Sos.** *Cell* 1993, **73:**179-192.

S21. Simon MA, Dodson GS, Rubin GM: **An SH3-SH2-SH3 protein is required for p21-Ras1 activation and binds to sevenless and Sos proteins in vitro.** *Cell* 1993, **73:**169-178.

S22. Knust E, Tietze K, Campos-Ortega JA: **Molecular analysis of the neurogenic locus Enhancer of split of *Drosophila melanogaster*.** *EMBO J* 1987, **6:**4113-4123.

S23. Pavlidis P, Ramaswami M, Tanouye MA: **The *Drosophila* easily shocked gene: a mutation in a phospholipid synthetic pathway causes seizure, neuronal failure, and paralysis.** *Cell* 1994, **79:**23-33.

S24. Rottgen G, Wagner T, Hinz U: **A genetic screen for elements of the network that regulates neurogenesis in *Drosophila*.** *Mol Gen Genet* 1998, **257:**442-451.

S25. Dorn R, Morawietz H, Reuter G, Saumweber H: **Identification of an essential *Drosophila* gene that is homologous to the translation initiation factor-Eif-4a of yeast and mouse.** *Mol Gen Genet* 1993, **237:**233-240.

S26. Grenningloh G, Rehm EJ, Goodman CS: **Genetic analysis of growth cone guidance in *Drosophila*: fasciclin II functions as a neuronal recognition molecule.** *Cell* 1991, **67:**45-57.

S27. Lin DM, Fetter RD, Kopczyinski C, Grenningloh G, Goodman CS: **Genetic analysis of fasciclin II in *Drosophila*: defasciculation, refasciculation, and altered fasciculation.** *Neuron* 1994, **13:**1055-1069.

S28. Fischer-Vize JA, Rubin GM, Lehmann R: **The fat facets gene is required for *Drosophila* eye and embryo development.** *Development* 1992, **116:**985-1000.

S29. Auld VJ, Fetter RD, Broadie K, Goodman CS: **Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in *Drosophila*.** *Cell* 1995, **81:**757-767.

S30. Montell DJ, Goodman CS: ***Drosophila* laminin: sequence of B2 subunit and expression of all three subunits during embryogenesis.** *J Cell Biol* 1989, **109:**2441-2453.

S31. Garcia-Alonso L, Fetter RD, Goodman CS: **Genetic analysis of laminin A in *Drosophila*: extracellular matrix containing laminin A is required for ocellar axon pathfinding.** *Development* 1996, **122:**2611-2621.

S32. Schmid AT, Tinley TL, Yedvobnick B: **Transcription of the neurogenic gene mastermind during *Drosophila* development.** *J Exp Zool* 1996, **274:**207-220.

S33. Schuldt AJ, Brand AH: **Mastermind acts downstream of Notch to specify neuronal cell fates in the *Drosophila* central nervous system.** *Dev Biol* 1999, **205:**287-295.

S34. Baumgartner S, Littleton JT, Broadie K, Bhat MA, Harbecke R, Lengyel JA, *et al.*: **A *Drosophila* neurexin is required for septate junction and blood-nerve barrier formation and function**. *Cell* 1996, **87:**1059-1068.

S35. Rothwell WF, Fogarty P, Field CM, Sullivan W: **Nuclear-fallout, a *Drosophila* protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization.** *Development* 1998, **125:**1295-1303.

S36. Inoue YH, Avides MD, Shiraki M, Deak P, Yamaguchi M, Nishimoto Y, *et al.*: **Orbit, a novel microtubule-associated protein essential for mitosis in *Drosophila melanogaster***. *J Cell Biol* 2000, **149:**153-165.

S37. Prokopenko SN, Saint R, Bellen HJ: **Tissue distribution of PEBBLE RNA and Pebble protein during *Drosophila* embryonic development.** *Mech Dev* 2000, **90:**269-273.

S38. Prokopenko SN, Brumby A, O'Keefe L, Prior L, He YC, Saint R, *et al.*: **A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila***. *Genes Dev* 1999, **13:**2301-2314.

S39. Yu F, Morin X, Cai Y, Yang X, Chia W: **Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization.** *Cell* 2000, **100:**399-409.

S40. Richards S, Hillman T, Stern M: **Mutations in the *Drosophila* pushover gene confer increased neuronal excitability and spontaneous synaptic vesicle fusion.** *Genetics* 1996, **142:**1215-1223.

S41. Tian S-S, Tsoulfas P, Zinn K: **Three receptor-linked protein-tyrosine phosphatases are selectively expressed on central nervous system axons in the *Drosophila* embryo.** *Cell* 1991, **67:**675-685.

S42. Yang X, Seow KT, Bahri SM, Oon SH, Chia W: **Two *Drosophila* receptor-like tyrosine phosphatase genes are expressed in a subset of developing axons and pioneer neurons in the embryonic CNS.** *Cell* 1991, **67:**661-673.

S43. Sun Q, Bahri S, Schmid A, Chia W, Zinn K: **Receptor tyrosine phosphatases regulate axon guidance across the midline of the *Drosophila* embryo.** *Development* 2000, **127:**801-812.

S44. Schmucker D, Jackle H, Gaul U: **Genetic analysis of the larval optic nerve projection in *Drosophila*.** *Development* 1997, **124:**937-948.

S45. Simpson JH, Bland KS, Fetter RD, Goodman CS: **Short-range and long-range guidance by slit and its robo receptors: a combinatorial code of robo receptors controls lateral position.** *Cell* 2000, **103:**1019-1032.

S46. Rajagopalan S, Vivancos V, Nicolas E, Dickson BJ: **Selecting a longitudinal pathway: robo receptors specify the lateral position of axons in the *Drosophila* CNS.** *Cell* 2000, **103:**1033-1045.

S47. Simpson JH, Kidd T, Bland KS, Goodman CS: **Short-range and long-range guidance by slit and its robo receptors: robo and robo2 play distinct roles in midline guidance.** *Neuron* 2000, **28:**753-766.

S48. Rajagopalan S, Nicolas E, Vivancos V, Berger J, Dickson BJ: **Crossing the midline: roles and regulation of robo receptors**. *Neuron* 2000, **28:**767-777.

S49. Scaerou F, Aguilera I, Saunders RDC, Kane N, Blottiere L, Karess R: **The rough deal protein is a new kinetochore component required for accurate chromosome segregation in *Drosophila*.** *J Cell Sci* 1999, **112:**3757-3768.

S50. Beumer KJ, Rohrbough J, Prokop A, Broadie K: **A role for PS integrins in morphological growth and synaptic function at the postembryonic neuromuscular junction of *Drosophila*.** *Development* 1999, **126:**5833-5846.

S51. Grotewiel MS, Beck CDO, Wu KH, Zhu XR, Davis RL: **Integrin-mediated short-term memory in *Drosophila*.** *Nature* 1998, **391:**455-460.

S52. Arora K, Dai H, Kazuko SG, Jamal J, O'Connor MB, Letsou A, *et al.*: **The *Drosophila* schnurri gene acts in the Dpp/TGF beta signaling pathway and encodes a transcription factor homologous to the human MBP family.** *Cell* 1995, **81:**781-790.

S53. Kania A, Salzberg A, Bhat M, Develyn D, He YC, Kiss I, *et al.*: **P-element mutations affecting embryonic peripheral nervous system development in *Drosophila melanogaster*.** *Genetics* 1995, **139:**1663-1678.

S54. Senti KA, Keleman K, Eisenhaber F, Dickson BJ: **Brakeless is required for lamina targeting of R1-R6 axons in the *Drosophila* visual system.** *Development* 2000, **127:**2291-2301.

S55. Rutledge BJ, Zhang K, Bier E, Jan YN, Perrimon N: **The *Drosophila* spitz gene encodes a putative EGF-like growth factor involved in dorsal-ventral axis formation and neurogenesis.** *Genes Dev* 1992, **6:**1503-1517.

S56. Huang Z, Shilo BZ, Kunes S: **A retinal axon fascicle uses spitz, an EGF receptor ligand, to construct a synaptic cartridge in the brain of *Drosophila*.** *Cell* 1998, **95:**693-703.

S57. Bellaiche Y, The I, Perrimon N: **Tout-velu is a *Drosophila* homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion.** *Nature* 1998, **394:**85-88.

S58. Zhang N, Zhang J, Purcell KJ, Cheng Y, Howard K: **The *Drosophila* protein Wunen repels migrating germ cells.** *Nature* 1997, **385:**64-67.

S59. Wills Z, Marr L, Zinn K, Goodman CS, Van Vactor D: **Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the *Drosophila* embryo.** *Neuron* 1999, **22:**291-299.

S60. Schuster CM, Davis GW, Fetter RD, Goodman CS: **Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin-II controls synaptic stabilization and growth.** *Neuron* 1996, **17:**641-654.

S61. Salzberg A, Prokopenko SN, He Y, Tsai P, Pal M, Maroy P, *et al.*: **P-element insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: mutations affecting embryonic PNS development.** *Genetics* 1997, **147:**1723-1741.

S62. Sun Q, Schindelholz B, Knirr M, Schmid A, Zinn K: **Complex genetic interactions among four receptor tyrosine phosphatases regulate axon guidance in *Drosophila*.** *Mol Cell Neurosci* 2001, in press.

S63. Huang AM, Rubin GM: **A misexpression screen identifies genes that can modulate RAS1 pathway signaling in *Drosophila melanogaster*.** *Genetics* 2000, **156:**1219-1230.

S64. Schaefer M, Shevchenko A, Knoblich JA: **A protein complex containing inscuteable and the G alpha-binding protein Pins orients asymmetric cell divisions in *Drosophila*.** *Curr Biol* 2000, **10:**353-362.

S65. Moore MS, DeZazzo J, Luk AY, Tully T, Singh CM, Heberlein U:

Ethanol intoxication in *Drosophila*: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* 1998, **93:**997-1007.

S66. Su YC, Maurel-Zaffran C, Treisman JE, Skolnik EY: **The Ste20 kinase misshapen regulates both photoreceptor axon targeting and dorsal closure, acting downstream of distinct signals.** *Mol Cell Biol* 2000, **20:**4736-4744.

S67. Ruan WJ, Pang P, Rao Y: **The SH2/SH3 adaptor protein dock interacts with the Ste20-like kinase misshapen in controlling growth cone motility.** *Neuron* 1999, **24:**595-605.

S68. Abdelilah-Seyfried S, Chan YM, Zeng CY, Justice NJ, Younger-Shepherd S, Sharp LE, *et al.*: **A gain-of-function screen for genes that affect the development of the *Drosophila* adult external sensory organ**. *Genetics* 2000, **155:**733-752.

S69. Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, *et al.*: **Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF**. *Science* 1999, **286:**316-318.

S70. Wan HI, DiAntonio A, Fetter RD, Bergstrom K, Strauss R, Goodman CS: **Highwire regulates synaptic growth in *Drosophila***. *Neuron* 2000, **26:**313-329.

S71. Kim YH, Choi CY, Lee SJ, Conti MA, Kim Y: **Homeodomain-interacting protein kinases, a novel family of co-repressors for homeodomain transcription factors.** *J Biol Chem* 1998, **273:**25875-25879.

S72. Zhu WC, Hanes SD: **Identification of *Drosophila* bicoid-interacting proteins using a custom two-hybrid selection.** *Gene* 2000, **245:**329-339.

S73. Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM: **Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A**. *Science* 1999, **283:**2089-2091.

S74. Kai M, Wada I, Imai S, Sakane F, Kanoh H: **Cloning and characterization of two human isozymes of Mg2+-independent phosphatidic acid phosphatase.** *J Biol Chem* 1997, **272:**24572-24578.

S75. Kanoh H, Kai M, Wada I: **Molecular characterization of the type 2 phosphatidic acid phosphatase**. *Chem Phys Lipids* 1999, **98:**119-126.

S76. Roberts R, Sciorra VA, Morris AJ: **Human type 2 phosphatidic acid phosphohydrolases. Substrate specificity of the type 2a, 2b, and 2c enzymes and cell surface activity of the 2a isoform.** *J Biol Chem* 1998, **273:**22059-22067.

S77. Asano T, Mochizuki Y, Matsumoto K, Takenawa T, Endo T: **Pharbin, a novel inositol polyphosphate 5-phosphatase, induces dendritic appearances in fibroblasts**. *Biochem Biophys Res Commun* 1999, **261:**188-195.

S78. Cadavid AL, Ginzel A, Fischer JA: **The function of the *Drosophila* fat facets deubiquitinating enzyme in limiting photoreceptor cell number is intimately associated with endocytosis.** *Development* 2000, **127:**1727-1736.

S79. Leverson JD, Joazeiro CA, Page AM, Huang HK, Hieter P, Hunter T: **The APC11 RING-H2 finger mediates E2-dependent ubiquitination**. *Mol Biol Cell* 2000, **11:**2315-2325.

S80. Makino Y, Yoshida T, Yogosawa S, Tanaka K, Muramatsu M, Tamura T: **Multiple mammalian proteasomal ATPases, but not proteasome itself, are associated with TATA-binding protein and a novel transcriptional activator, TIP120.** *Genes Cells* 1999, **4:**529-539.

S81. Wigge P, McMahon HT: **The amphiphysin family of proteins and their role in endocytosis at the synapse.** *Trends Neurosci* 1998, **21:**339-344.

S82. Razzaq A, Su Y, Mehren JE, Mizuguchi K, Jackson AP, Gay NJ, *et al.*: **Characterisation of the gene for *Drosophila* amphiphysin**. *Gene* 2000, **241:**167-174.

S83. Fujita Y, Shirataki H, Sakisaka T, Asakura T, Ohya T, Kotani H, *et al.*: **Tomosyn: a syntaxin-1-binding protein that forms a novel complex in the neurotransmitter release process.** *Neuron* 1998, **20:**905-915.

S84. Masuda ES, Huang BCB, Fisher JM, Luo Y, Scheller RH: **Tomosyn binds t-SNARE proteins via a VAMP-like coiled coil**. *Neuron* 1998, **21:**479-480.

S85. Kwak E, Gerald N, Larochelle DA, Vithalani KK, Niswonger ML, Maready MD, *et al.*: **LvsA, a protein related to the mouse beige protein, is required for cytokinesis in *Dictyostelium***. *Mol Biol Cell* 1999, **10:**4429-4439.

S86. Han JD, Baker NE, Rubin CS: **Molecular characterization of a novel A kinase anchor protein from *Drosophila melanogaster*.** *J Biol Chem* 1997, **272:**26611-26619.

S87. Parmentier ML, Woods D, Greig S, Phan PG, Radovic A, Bryant P, *et al.*: **Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in *Drosophila***. *J Neurosci* 2000, **20:**U1-U5.

S88. The I, Bellaiche Y, Perrimon N: **Hedgehog movement is regulated through tout velu-dependent synthesis of a heparan sulfate proteoglycan.** *Mol Cell* 1999, **4:**633-639.

S89. Toyoda H, Kinoshita-Toyoda A, Selleck SB: **Structural analysis of glycosaminoglycans in *Drosophila* and *Caenorhabditis elegans* and demonstration that tout-velu, a *Drosophila* gene related to EXT tumor suppressors, affects heparan sulfate in vivo.** *J Biol Chem* 2000, **275:**2269-2275.

S90. Hofmann K: **A superfamily of membrane-bound O-acyltransferases with implications for Wnt signaling.** *Trends Biochem Sci* 2000, **25:**111-112.

S91. Mach JM, Lehmann R: **An Egalitarian-BicaudalD complex is essential for oocyte specification and axis determination in *Drosophila*.** *Genes Dev* 1997, **11:**423-435.

S92. Gao XS, Neufeld TP, Pan DJ: ***Drosophila* PTEN regulates cell growth and proliferation through PI3K-dependent and -independent pathways.** *Dev Biol* 2000, **221:**404-418.

S93. Sonoda J, Wharton RP: **Recruitment of Nanos to hunchback mRNA by Pumilio**. *Genes Dev* 1999, **13:**2704-2712.

S94. Wharton RP, Sonoda J, Lee T, Patterson M, Murata Y: **The Pumilio RNA-binding domain is also a translational regulator**. *Mol Cell* 1998, **1:**863-872.

S95. Lie YS, Macdonald PM: **Apontic binds the translational repressor bruno and is implicated in regulation of oskar mRNA translation.** *Development* 1999, **126:**1129-1138.

S96. Fyrberg C, Becker J, Barthmaier P, Mahaffey J, Fyrberg E: **A family of *Drosophila* genes encoding quaking-related maxi-KH domains.** *Biochem Genet* 1998, **36:**51-64.

S97. Ward RE, Lamb RS, Fehon RG: **A conserved functional domain of *Drosophila* coracle is required for localization at the septate junction and has membrane-organizing activity.** *J Cell Biol* 1998, **140:**1463-1473.

S98. Yee GH, Hynes R: **A novel tissue-specific integrin subunit b-v expressed in the midgut of *Drosophila melanogaster*.** *Development* 1993, **118:**845-858.

S99. Paululat A, Goubeaud A, Damm C, Knirr S, Burchard S, Renkawitz-Pohl R: **The mesodermal expression of rolling stone (rost) is essential for myoblast fusion in *Drosophila* and encodes a potential transmembrane protein.** *J Cell Biol* 1997, **138:**337-348.

S100. Fernandez-Funez P, Nino-Rosales ML, de Gouyon B, She WC, Luchak JM, Martinez P, *et al.*: **Identification of genes that modify ataxin-1-induced neurodegeneration**. *Nature* 2000, **408:**101-106.

S101. Sigrist SJ, Thiel PR, Reiff DF, Lachance PED, Lasko P, Schuster CM: **Postsynaptic translation affects the efficacy and morphology of neuromuscular junctions**. *Nature* 2000, **405:**1062-1065.

S102. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, *et al.*: **3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase.** *Curr Biol* 1997, **7:**776-789.

S103. Hernandez G, Diez del Corral R, Santoyo J, Campuzano S, Sierra JM: **Localization, structure and expression of the gene for translation initiation factor eIF-4E from *Drosophila melanogaster*.** *Mol Gen Genet* 1997, **253:**624-633.

S104. Lin DM, Goodman CS: **Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance.** *Neuron* 1994, **13:**507-523.

Table S1

Phenotypes displayed by EP lines crossed to C155;GFP.

EP line	Pathfinding	Excess/ectopic synapses	Reduced/abnormal synapses
EP(x) 0346	X		X
EP(x) 0355	X		X
EP(3) 0381	X	X	X
EP(3) 0430			X
EP(x) 0438			X
EP(3) 0515	X	X	X
EP(3) 0549	X		
EP(3) 0568	X		
EP(3) 0643	X		
EP(2) 0644			X
EP(2) 0682	X		X
EP(2) 0737	X		X
EP(x) 0764	X		X
EP(2) 0765		X	
EP(x) 0770	X	X	
EP(x) 0779			X
EP(x) 0784			X
EP(3) 0809	X	X	
EP(2) 0815	X		X
EP(3) 0851	X		X
EP(3) 0888	X	X	
EP(2) 0896	X		X
EP(x) 0912			X
EP(2) 0938			X
EP(2) 1011	X		X
EP(x) 1016	X		X
EP(3) 1082	X	X	X
EP(x) 1172	X		X
EP(x) 1201	X		
EP(2) 1242			X
EP(x) 1321		X	X
EP(x) 1335	X		X
EP(x) 1336	X	X	X
EP(x) 1353	X		X
EP(x) 1359	X		X
EP(x) 1400	X		X
EP(x) 1433			X
EP(x) 1462	X	X	X
EP(x) 1463	X		X
EP(x) 1493	X		X
EP(x) 1508	X		X
EP(x) 1534	X		X
EP(x) 1562			X
EP(x) 1571		X	X
EP(x) 1592		X	X
EP(x) 1603	X		X
EP(x) 1624	X		X
EP(2) 2028		X	X
EP(2) 2077	X		X
EP(2) 2153	X		X
EP(2) 2171	X		X
EP(2) 2208		X	X
EP(2) 2226			X
EP(2) 2235	X		X
EP(2) 2237			X
EP(2) 2247	X		

(*continued*)

Table S1

Continued

EP line	Pathfinding	Excess/ectopic synapses	Reduced/abnormal synapses
EP(2) 2253		X	X
EP(2) 2270		X	
EP(2) 2289	X		
EP(2) 2299			X
EP(2) 2306	X		X
EP(2) 2315		X	
EP(2) 2324	X	X	
EP(2) 2339	X	X	
EP(2) 2353	X		X
EP(2) 2372			X
EP(2) 2431	X		X
EP(2) 2443			X
EP(2) 2461	X	X	
EP(2) 2477	X	X	
EP(2) 2575	X		X
EP(2) 2582	X		
EP(2) 2587	X	X	
EP(2) 2591	X		X
EP(2) 2607			X
EP(2) 2609	X	X	X
EP(2) 2632	X	X	X
EP(3) 3038	X		X
EP(3) 3077	X	X	X
EP(3) 3101	X		
EP(3) 3109	X	X	
EP(3) 3113	X	X	X
EP(3) 3152	X	X	
EP(3) 3208	X		X
EP(3) 3221	X	X	
EP(3) 3289	X		X
EP(3) 3292	X	X	
EP(3) 3303	X		X
EP(3) 3319	X		
EP(3) 3340	X		
EP(3) 3373	X	X	
EP(3) 3403	X		X
EP(3) 3408	X		
EP(3) 3415		X	
EP(3) 3472	X		X
EP(3) 3474	X		X
EP(3) 3503	X		X
EP(3) 3524	X		
EP(3) 3527	X		
EP(3) 3539	X		X
EP(3) 3548	X	X	X
EP(3) 3553	X	X	
EP(3) 3559	X		X
EP(3) 3561	X	X	
EP(3) 3571	X		
EP(3) 3587	X		
EP(3) 3592	X		X
EP(3) 3630	X		X
EP(3) 3636	X		
EP(3) 3657	X		
EP(3) 3662	X		
EP(3) 3678	X		X
EP(3) 3697	X		X

Table S2

Insertion sites of EP elements identified by the screen.

	X	2nd	3rd
Known gene: +/+	7	16	16
Known gene: −/+	0	3	0
CG: +/+	14	16	25
CG: −/+	4	3	2
No nearby gene	2	2	4

Table S3

Known genes identified in the screen.

GENE	EP	Expressed in CNS?	LOF affects nervous system?	Best BLASTP E value versus another species	EST?
α-adaptin, CG4260	EP(2) 0896	YES [8]	YES [8]	E=0.0 (H.s.)	YES
Abl, CG4032	EP(3) 3101	YES [9]	YES [9, 59]; this work, Figure 6	0.0 (H.s)	YES
Adf1 (nalyot), CG15845	EP(X) 0815	YES [10]	YES [10]	3e-7 (C.e.)	YES
amnesiac, CG11937	EP(X) 0346, 1571	YES [11]	YES (behavioral, [12, 65]; anatomical, this work, Figure 6)	no significant homology (nsh; >e-3)	NO
Ance, CG8827	EP(2) 2171	NO [13]	unknown	e-152 (H.s.)	YES
apontic, CG5393	EP(2) 2339	YES [14]	unknown	nsh	YES
ash2, CG6677	EP(3) 3472	ubiquitous?; [15]	unknown	e-138 (H.s.)	YES
bang senseless, CG12223	EP(X) 0355	YES [16]	YES; behavioral,[17]	6e-46 (H.s.)	YES
center divider, CG6027	EP(3) 3319	YES [18]	YES (our unpublished results)	8e-83 (H.s.)	YES
crooked legs, CG14938	EP(2) 2226	YES [19]	unknown	e-132 (H.s.)	YES
drk, CG6033	EP(2) 2477	YES; ubiquitous, [20, 21]	YES; alters cell fates, [20, 21]	2e-75 (H.s.)	YES
HLHm7, CG8361	EP(3) 3587	YES [22]	unknown; only GOF mutants exist	4e-15 (Xenopus)	YES
easily shocked, CG3525	EP(X) 0770	YES; ubiquitous; Fig. S2	YES; behavioral, [17, 23]	1e-77 (H.s.)	YES
egalitarian, CG4051	EP(2) 0938	YES; this work, Fig. S2	YES (this work; Table S4)	2e-31 (C.e.)	YES
eif-4A, CG9075	EP(2) 1011	YES; ubiquitous, [24, 25]	YES; LOFs are dominant modifiers of Notch function, [24]	e-162 (H.s.)	YES
eif-4E, CG4035	EP(3) 0568	YES; ubiquitous,[103]	unknown	2e-47 (Aplysia)	YES
Fasciclin 2, CG3665	EP(X) 1462	YES [26]	YES [27]	2e-63 (H.s.)	YES
fat facets, CG1945	EP(3) 0381	YES ([28] and CK EST in situ [BDGP])	YES; affects neuronal cell fates in the eye [28]	0.0 (H.s.)	YES
Gliotactin, CG3903	EP(2) 2306	CNS glia [29]	YES [29]	2e-69 (H.s.)	YES
Kr-h1, CG9167	EP(2) 2289	YES (note 1)	unknown	1e-46 (H.s.)	YES
Laminin A, CG10236	EP(3) 3678	CNS glia/ECM; [30]	YES [31]	0.0 (C.e.)	YES
lemming, CG18042	EP(2) 2077	YES; this work, Fig. S2	unknown	1e-37 (H.s.)	YES
mastermind, CG8118	EP(2) 2575	YES [32]	YES [33]	nsh	YES
misshapen, no CG	EP(3) 0549, 0609	YES [66, 67]	YES [66, 67]	e-163 (C.e.)	YES
Neurexin, CG6827	EP(3) 0809	YES [34]	YES [34]	0.0 (H.s.)	YES
nuclear fallout, CG7867	EP(3) 3077	ubiquitous?; [35]	unknown; but note that nuf is a 502aa cytoskeletal protein [35] and EP is inserted in an intron within coding region. EP could drive expression of a C-terminal coiled-coil region of up to 192aa, and this might act as a dominant negative; so elav-GAL4 driven EP phenotype may represent a nervous system LOF for nuf.	1e-20 (H.s.)	YES
orbit, no CG	EP(3) 3403	ubiquitous?	YES [36]	e-101 (H.s.)	YES
pebble, CG8114	EP(3) 3415	YES [37]	YES [38]	e-136 (H.s.)	YES
pins (Rapsynoid), CG5692	EP(3) 3113	YES [39, 87]	YES [39, 87]	e-163 (H.s.)	YES
purity-of-essence (pushover/Calossin), CG14472	EP(2) 0737	YES (note 1)	YES [40]	0.0 (H.s.)	YES
Ptp10D, CG1817	EP(X) 1172	YES [41–43]	YES [43], as a double mutant; as a single mutant, this work, Fig. 6	e-144 (H.s.)	YES
pumilio, CG9755	EP(3) 3038	YES (this work)	YES; [44], this work, Table S4	e-160 (H.s.)	YES
rough deal, CG1569	EP(3) 3048	ubiquitous?; [49]	unknown	2e-23 (H.s.)	NO
roundabout 2, no CG	EP(2) 2582	YES [45–48]; in situ, Figure S2	YES [45–48]	e-130 (H.s.)	YES
scab (Volado), CG8095 (−/+)	EP(2) 2591	YES [50, 51]	YES [51]	4e-60 (H.s.)	YES
schnurri, CG7734 (−/+)	EP(2) 0644	YES; widespread late embryonic and larval expression; [52]	YES [53]	3e-35 (H.s.)	YES
scribbler (brakeless), CG5580	EP(2) 2461	YES; ubiquitous in eye disc [54]	YES [54]	nsh	YES
spitz, CG10334	EP(2) 2632	YES; mRNA ubiquitous but enriched in PNS and ventral midline; [55]	YES [56]	6e-6 (H.s.)	YES
tout-velu, CG10117	EP(2) 0765	ubiquitous?; [57]	YES; this work, Figure 6	0.0 (H.s.)	YES
Uba1, CG1782 (−/+)	EP(2) 2609	YES (note 1)	unknown	0.0 (H.s.)	YES
wunen, CG8804	EP(2) 2208	YES [58]	YES; this work, Table S4	5e-54 (H.s.)	YES

EST refers only to *Drosophila* ESTs that are perfect matches to the genes. Note 1: Expression analysis from Meister and Braun, 1995, personal communication to FlyBase available at http://flybase.bio.indiana.edu/.bin/fbpcq.html?FBrf0083714. H.s., *Homo sapiens*; M.m., *Mus musculus*; X.l., *Xenopus laevis*; C.e., *Caenorhabditis elegans*; A.t., *Arabidopsis thaliana*. See Flybase for further information and additional references concerning these genes.

Table S4

Annotations for genes identified in the screen.

GENE	EP	Molecular information	Comments
GTPases/GEFs/GAPs/GTP binding proteins			
CG9366, RhoL	EP(3) 0888	190 aa protein, a member of Rac/Rho/Cdc42 family of small GTPases that regulate cytoskeletal structure. Protein defined by ESTs and homology. Closest C.e. relatives are RAC-1 and CED-10 (both E=1e-52). Closest fly relative is Cdc42, 3e-52. EP inserted at -39 relative to mRNA start.	
pebble (Rho-GEF), CG8114	EP(3) 3415	Rho-GEF involved in actin reorganization. GEFs facilitate GTP-GDP exchange onto small GTPases. Closest C.e. relative is T19E10.1b, E=1e-47. EP inserted in 5' UTR.	GOF phenotype: ISNb has ectopic synaptic branches, including some that cross segment borders (Figure S1). Also recovered in a GOF screen for EPs affecting adult PNS (ES organs) when driven in SOPs by sca-GAL4 [68]. Could not examine LOF in larvae due to embryonic lethality.
CG 8487, sec7 family GEF	EP(2) 2028	2040 aa predicted protein; ortholog of a human brefeldin A target protein (GBF); E=0.0. Closest C.e. relative is C24H11.7, E=0.0. CG8487 is a member of the Gea(S.c)/GBF/GNOM(A.t.) subfamily of Sec7-related GEFs, These GEFs act on ARFs, which are small GTPases that regulate clathrin coat assembly and are required for protein transport through the ER-Golgi system. ARFs may also be involved in endocytosis. The closest fly relative of CG8487 is CG7578 (E=6e-42); this appears to be the fly ortholog of the Sec7/BIG subfamily of Sec7-like GEFs, which is distinct from the GBF subfamily; thus CG8487 is likely to be the only member of the GBF family in the fly. N terminus of CG8487 defined by homology to GBFs; C terminus by ESTs. A null mutation in A.t. GNOM causes developmental cell polarity defects [69]. There are 5 sec7 domain proteins in the fly. EP inserted 295 nt 5' to predicted ATG.	GOF phenotype includes ISNb pathfinding defects and ectopic ISNb synaptic branches (Figure 4). Expressed in CNS: Figure S2, Table S5.
CG3862, RCC GEF-related protein	EP(2) 1242	454 aa predicted protein related to RCC (regulator of chromosome condensation) protein family; these contain 7 repeats of the RCC domain, and some are GEFs for Ran family nuclear GTPases. Most closely related human protein is retinitis pigmentosa GTPase regulator (E=7e-19), which is mutated in X-linked retinitis pigmentosa. There are 10 RCC domain proteins in the fly. Most closely related fly protein is CG11734 (E=5e-23), but this is a much bigger protein (4669 aa predicted). The recently described Highwire protein (a fusion of CG9049 and CG9041), which regulates neuromuscular synaptogenesis, is also related (E=4e-5) and contains RCC domains; it is very large (5233 aa; [70]). Size of CG3862 protein not clearly defined by homology, but defined by ESTs. EP inserted 843 nt 5' to predicted ATG.	Expression pattern: Table S5.
CG18640 (Bj1), RCC GEF-related protein (−/+)	EP(3) 3630	RCC protein, the apparent ortholog of vertebrate RCC (E=1e-83). Also related to CG3862 (E=2e-13) and Highwire (E=5e-7). Protein size defined by cDNAs and homology. EP is inserted in the −/+ orientation in the 5' UTR. No nearby genes in +/+ orientation.	Strong GOF phenotype in which ISNb is sometimes absent and the ISN innervates the VLMs. May be a neural LOF for CG18640 due to generation of an antisense transcript.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG 6811, p50Rho-GAP	EP(3) 3152	Apparent ortholog (E=4e-66) of mammalian p50Rho-GAP, a GTPase-activating protein for Rho family GTPases Rho, Rac, and Cdc42, which control cytoskeletal reorganization. Protein size defined by ESTs and homology. Has a single C.e. ortholog, W02B12.8, E=8e-63. Most closely related fly protein is CG3208, E=2e-19. EP inserted in 5' UTR, 1188 nt 5' to predicted ATG.	GOF phenotype (Figure S1) includes axonal pathfinding errors such as ISNb bypass, and abnormal ISNb synaptic branches. Expressed in CNS: Figure S2, Table S5.
CG5521, related to tuberous sclerosis Rap-GAP	EP(3) 0527	Large predicted protein (1958 aa; CG translation is 672aa, but this is incorrect). C-terminal half is closely related (E=e-171) to human KIAA1272 protein, 1059 aa (aa849-1845 of fly protein=aa109-1056 of H.s. protein). C terminus of fly protein is related to tuberous sclerosis (TSC2 or tuberin) human tumor suppressor gene (E=2e-15) and to other Rap-GAPs; also to Drosophila Gigas (E=1e-14). Rap1 is a small GTPase that is thought to be an antagonist of Ras signaling. No ESTs, so protein is only a prediction; but the N and C termini are likely to go together because human KIAA1219 protein is homologous to both ends; this protein is the apparent ortholog of D.m. protein BcDNA: GH09358. EP is inserted 513 nt upstream of predicted ATG.	
CG 2017, GP-1 related	EP(3) 3503	Apparent ortholog (E=e-139) of GTPBP2, a mammalian member of the GP-1 family of GTP binding proteins. Also closely related to GP-1, and to a fly protein, Dgp-1 (E=2e-97). Protein size defined by ESTs and homology. 3 alternatively spliced mRNAs, all encoding the same predicted protein; EP is inserted into 5' UTR of smallest mRNA, which corresponds to the 1st intron of the 2 larger mRNAs. EP should thus drive entire coding region.	ISN synaptic branches (but not the main nerves) cross over segmental borders in GOF (like EP3636 phenotype in Figure 5). Expression pattern: Table S5.
CG11411, 8D8.1	EP(X) 1336	Large predicted protein (2118aa) with an ATP/GTP binding motif; no significant homologies to vertebrate or C.e. proteins. C terminus of protein defined by EST; N terminus is only a prediction. Closest fly relative is CG4790 (E=2e-7). EP inserted 198 nt upstream of predicted ATG.	Strong ISNb pathfinding GOF phenotype. Expressed in neuroblasts: Figure S2, Table S5.
Kinases and phosphatases			
Abl (tyrosine kinase), CG4032	EP(3) 3101	Tyrosine kinase; EP inserted 153 nt upstream of ATG.	GOF phenotype (ISNb fusion bypass): Figure 3. LOF phenotype (missing synapse on muscle 12): Figure 6.
Ptp10D (Receptor tyrosine phosphatase), CG1817	EP(X) 1172	Receptor tyrosine phosphatase expressed in CNS neurons, including motor neurons [62]; EP inserted in 5' UTR.	GOF phenotype (axonal pathfinding errors): Figure 3. LOF phenotype (ISN truncations and crossovers): Figure 6. Protein is overexpressed in motor axons when EP crossed to driver (Q. Sun and K.Z., unpublished).
center divider (S/T kinase), CG6027	EP(3) 3319	S/T kinase orthologous to human TESK ; EP inserted in 5' UTR.	Strong ISNb and ISN pathfinding GOF phenotype (Figure 4). Could not examine LOF in larvae due to embryonic lethality. LOF has embryonic motor axon phenotype, as revealed by mAb 1D4 staining (K.M., unpublished).

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
misshapen (S/T kinase), no CG.	EP(3) 0549, 0609	Ste20-like S/T kinase related to C.e. MIG-15 and mouse MINK-1. LOF phenotype: R1-6 growth cones fail to stop at lamina targets [66, 67]. EP is 20 nt upstream of mRNA start.	Also identified in a GOF screen for modifiers of a dominant-negative KSR kinase rough eye phenotype [63].
CG7719-related, S/T kinase	EP(3) 0515	866aa predicted protein, most closely related to a family of kinases that includes the fly Wts/Lats tumor suppressor kinase (E=7e-35); but all other kinases are homologous only to sequences at the N and C termini of CG7719. aa 234-703 of CG7719 are not homologous to any other sequences. aa234-703 are also not represented by ESTs; ESTs encode the N terminus only to aa 168. Removing the nonhomologous aa to create a new predicted protein improves the scores versus mammalian kinases from 9e-36 to 2e-72 and also greatly changes the relative scores of the homologous kinases. We conclude that the CG7719 prediction may be incorrect, and that the new prediction is most closely related to a family of kinases that includes IRE in A.t. (closest relative, E=e-80) and syntrophin-associated kinases in the mouse. Closest fly relative of the new prediction is CG6498, E=8e-67; this is a large predicted protein (2139aa) that may be the true ortholog (E=e-140) of the syntrophin-associated kinase family. EP inserted 343 nt 5′ to predicted ATG.	GOF phenotype: ISNb pathfinding defects, including fusion bypass, and ectopic synaptic branches (Figure S1). Expression pattern: Table S5.
CG1210 (Pk61C), S/T kinase related to PDKs	EP(3) 3553	Apparent ortholog (E=3e-72) of mammalian 3-phosphoinositide-dependent protein kinase-1 (PDK1) [102]. Protein defined by cDNA sequences. Several alternate 5' exons, and mRNAs encode at least two different proteins; EP inserted 35 nt 5' to start of 2nd alternate 5' exon (transcript CT42509).	GOF phenotype includes ectopic ISNb synaptic branches and ISNb axonal pathfinding errors (Figure S1); also intersegmental crossovers of the entire ISN. Expression pattern: Table S5.
CG17090, homeodomain-interacting S/T kinase	EP(3) 3571	Apparent ortholog (E=0.0) of mammalian homeodomain-interacting kinases, which are transcriptional corepressors [71]. Protein size defined by ESTs. Closest fly relative is minibrain kinase (E=9e-54). EP inserted in 2nd intron; would drive expression of a fragment lacking the first 103aa (1320 total aa).	Probably a GOF phenotype since the missing 103 aa sequence at the N terminus is not homologous to other proteins.
CG6297, JIL-1 S/T kinase	EP(3) 3657	Tandem S/T kinase associated with chromosomes, apparent ortholog of nuclear mitogen/stress activated human kinase RKLP, E=e-153, and closely related to other members of the S6 kinase family; closest fly relative is S6KII, e-100. EP inserted in 5' UTR, 269 nt 5' to ATG. Protein defined by cDNA sequences.	A mutation in this gene is listed in Flybase but there is no published information on phenotype.
CG7001, Bin4 S/T kinase	EP(X) 0438	Also most closely related to S6 kinase family, but much less similar to human S6Ks (E=5e-39) than is JIL-1. Protein size defined by cDNA sequences. EP inserted 350 nt 5' to cDNA start. Originally identified as a protein that interacts with Bicoid in a 2-hybrid screen [72]; so might be a nuclear protein.	GOF phenotype includes abnormal bouton shapes. Expression pattern: Figure S2, Table S5.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG5643, PP2A (S/T phosphatase) regulatory B subunit	EP(3) 3559	An ortholog of the B' or B56 family of mammalian PP2A regulatory B subunits (E=e-168 vs the B56 ϵ isoform). Protein size defined by BDGP cDNA sequence. There are several families of mammalian PP2A B subunits, but these families are not homologous to each other. The B'/B56 subunits are implicated in regulation of β-catenin signaling [73]. There are 2 fly genes, CGs 7913 (E=5e-59) and 4924 (E=1e-42), which also encode B'/B56 subunits related to CG5643. The B'/B56 subunit family is not homologous to the B (PR55) family of PP2A B subunits; the only member of this family in the fly is the twins gene, CG6235. EP inserted in 5' UTR.	GOF phenotype: ectopic and missing ISNb synaptic branches; bouton numbers are also reduced (Figure S1). Also recovered in PNS GOF screen [68]. Expression pattern: Figure S2, Table S5.
wunen (lipid phosphatase), CG8804	EP(2) 2208	Transmembrane phosphatidate phosphatase (PAP). PAPs dephosphorylate phosphatidic acid, producing the second messenger DAG. PAPs can also dephosphorylate sphingosine and ceramide-containing lipids, regulating the amounts of other second messengers. Wun is required in gut epithelium for generation of a nonpermissive signal that forces primordial germ cells to coalesce and migrate away from Wun-expressing regions; it might regulate a DAG or other lipid messenger produced by these gut cells [58, 74]. PAPs are thought to be ectoenzymes acting on exogenous substrates and/or outer leaflet lipids (their enzymatic domains map to extracellular loops; [75, 76]), so this lipid signal might be a direct repellent for the germ cells. Protein defined by published sequences. Most closely related human protein is PAP2a (E=5e-54). EP inserted 4772 nt upstream of wun ATG, but there are no intervening cDNAs or CGs.	wunen has an LOF phenotype in which synaptic structure is simpler than normal and boutons have distorted shapes (R.K., unpublished). Wun mRNA is expressed at low levels in the CNS [58]; also expressed in ectodermal patches (Figure S2). Wun mRNA is overexpressed in CNS in F1 EP2208 x driver embryos (R.K., unpublished).
CG8805, Wunen2/Tunen, lipid phosphatase	EP(2) 2607	See notes above for wunen. This is the next 5' gene. E=2e-35 versus closest mammalian relative. Wun and Wun2 are part of a fly gene family with 8 members: CG8805 adjacent to wun (most closely related, E=1e-76), CGs 11425, 11426, 11437, 11438, and 11440 at 79E4, and CG12746 at 83B3. Wun apparently has a function that cannot be substituted for by Wun2, since the wun LOF mutant has a synaptic phenotype. 7 of the 8 fly genes are related to human PAPs 2a, 2b, and 2c with the same relative scores. CG12746 is the most divergent member of the family, being much more closely related to a set of A.t. (E=2e-32) and fission yeast PAPs than it is to mammalian PAP2s (E=1e-6), and it might thus have a distinct substrate specificity and/or function. EP inserted 7.2 kb 5' to ATG of CG8805, but there are no intervening cDNAs, CGs, or homologies. Protein size defined by EST contig sequence.	CK clone in situ (clot 177) shows that Wun2 mRNA is expressed in CNS (BDGP). Distance between EP and Wun2 makes it unclear if Wun2 overexpression causes GOF phenotype.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG10426, pharbin-like inositol polyphosphate 5-phosphatase	EP(3) 3636	An inositol polyphosphate 5-phosphatase; apparent ortholog of the protein called pharbin in the rat (E=5e-85); less closely related (E=2e-30) to synaptojanin, a phosphatase in the same family required for synaptic vesicle recycling in the mouse. Protein size defined by ESTs. CG10426 and pharbin both have C-terminal membrane-targeting CaaX motifs. Pharbin overexpression in fibroblasts induces dendritic extensions [77]. Synaptojanin is the ortholog (E=0.0) of CG6562. Synaptojanin has an N-terminal Sac1-like inositol phosphatase domain that acts on the 3, 4, and 5 positions, and a C-terminal 5-phosphatase; this is the segment that is homologous to pharbin. There are 5 5-phosphatase domains in the fly, and 4 Sac1-like domains; CG6562 is the only protein with both. EP inserted in 1st intron, upstream of entire coding region.	GOF phenotype includes intersegmental crossovers of the ISN terminal arbors (Figure 5). Expressed in CNS: Figure S2, Table S5.
Protein degradation/trafficking/modification			
fat facets (ubiquitin C-terminal hydrolase), CG1945	EP(3) 0381	May be involved in regulation of endocytosis through deubiquitination of Liquid Facets, the fly epsin ortholog [78]; Epsin interacts with the AP2 clathrin adaptor complex. EP inserted in 1st intron, upstream of ATG.	GOF phenotype includes abnormal ISN synaptic branches (Figure 5); the number of boutons is also reduced. Also recovered in PNS GOF screen [68].
Uba 1 (E1 ubiquitin-activating enzyme), CG1782 (−/+)	EP(2) 2609	Activation of ubiquitin by E1s is required for subsequent ligation of ubiquitin to other proteins. EP inserted in −/+ orientation at nt 601 of mRNA, 5' to ATG. There are at least 2 closely related fly genes: CG7528 (Uba2) and CG13343. No nearby genes in +/+ orientation to EP.	Might be a neural LOF for Uba1; Uba1 mutations confer lethality but no other phenotype has been published.
lemming, (RING finger protein), CG18042	EP(2) 2077	Related (E=4e-19) to yeast APC11p, a known E3 ubiquitin-protein ligase [79]. RING finger proteins often function as E3s, which are adaptor subunits that are required for binding of protein substrates to the E2 ubiquitin ligase. EP inserted 456 nt 5′ to cDNA start.	Expressed in CNS: Figure S2.
CG1341 (Rpt1), MSS1-related AAA ATPase	EP(2) 2153	Apparent ortholog (E=0.0) of vertebrate AAA ATPase MSS1, a 26S proteasome regulatory subunit that also functions as a transactivator for HIV Tat protein. N terminus defined by ESTs; C terminus by homology. When bound in a complex to Tat and TATA binding protein MSS1 is apparently associated with other proteasomal AAA ATPases but not with the proteasome itself [80]. Related to many other AAA ATPase genes in the fly. EP inserted in 5' UTR.	
α-***adaptin*** (clathrin adaptor subunit), CG4260	EP(2) 0896	Large subunit of AP2 clathrin adaptor; EP inserted 118 bp upstream of cDNA start.	
CG 8487, sec7-GEF family protein	EP(2) 2028	see notes above	
CG10426, pharbin-like inositol polyphosphate phosphatase	EP(3) 3636	see notes above	

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG8604, Amphiphysin	EP(2) 2443	Apparent ortholog (E=5e-52) of vertebrate amphiphysin, an SH3 domain protein that regulates endocytosis by clathrin-coated vesicles. Protein size defined by cDNA sequences. Amphiphysin binds to dynamin, AP2, clathrin, and synaptojanin [81]. Closest fly relative is CG15694, E=7e-6, so this is likely to be the only fly amphiphysin. EP inserted 402 nt upstream of predicted ATG, 46 nt upstream of predicted mRNA start.	Amphiphysin cDNA in situ shows little or no CNS expression [82]; surprising since this appears to be the only Amph gene in flies, and Amph in vertebrates is a central element in recycling of synaptic vesicles in neurons. Perhaps there is low-level CNS expression; otherwise GOF must be a misexpression phenotype. No published LOF mutants.
CG17762, Tomosyn	EP(X) 1359, 1562	CG17762 is the apparent ortholog (E=e-179) of rat tomosyn, and its N-terminal region is closely related (E=6e-53) to Lethal Giant Larvae (LGL); no other closely related proteins exist in fly. Protein size defined by ESTs. Rat tomosyn displaces Munc18 from syntaxin; it may then be replaced by VAMP/synaptobrevin, allowing formation of the SNARE complex required for membrane fusion [83]. EP is inserted in 2nd to last intron, and would overexpress last two exons of Tomosyn (aa 657-960). This is the region that interacts with syntaxin, but it is downstream of the LGL repeats.	Since EP would drive a C-terminal fragment that complexes to syntaxin but lacks the LGL repeats [84], this might act as a dominant negative; perhaps it cannot be replaced by VAMP after binding. Phenotype may thus be a tomosyn LOF.
CG9011/14001?, related to Chediak-Higashi syndrome/Beige, Dictyostelium LvsA, and a PKA anchor protein, Akap550 (provisionally denoted DM-BEACH1 in text)	EP(2) 2299	EP inserted 829 nt 5′ to predicted ATG of CG14001, a very short gene. 2 kb 3′ to 14001 ATG is 5′ end of CG9011 (1085 aa, but homology to related proteins extends much farther 3′; indeed GadFly shows BEACH, WD40, and FYVE domains that are not in the CG translation). Homology also extends 5′ to the predicted CG9011 ATG. Our analysis suggests that CG14001 and CG9011 may be parts of a larger gene encoding a protein of at least 3000aa. The only CG9011 EST does not define either the N or C terminus of this protein; CG14001 does not have an EST. Predicted 3000aa protein is most closely related to a C.e. ortholog (VT23B5.1/VT23B5.2; E=0.0) and to LvsA (E=e-146) from Dictyostelium, which is involved in cytokinesis [85]. The fly and C.e. proteins contain BEACH (Beige/Chediak-Higashi) homology domains and WD40 repeats. There are a total of 5 BEACH/WD40 proteins in the fly. CG11814 is the apparent ortholog of the human C-H syndrome (lethal human disease in which cytoplasmic granules accumulate) protein, which is hypothesized to be a 'lysosomal trafficking regulator (LysT)'.	Strong GOF synaptogenesis phenotype (Figure 3). Also recovered in PNS GOF screen [68]. CG9011 mRNA is overexpressed when EP is crossed to driver (Table S5), showing that the large predicted protein is likely to be responsible for the overexpression phenotype. Expressed in CNS: Figure S2, Table S5.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
		CG11814 has no clear C.e. ortholog. Akap550/CG6775 (PKA anchor protein) is predicted to be 3614aa and also contains BEACH and WD40 domains. It is not homologous to any other Akaps, however, and the biochemically characterized Akap550 [86] is an N-terminal fragment that lacks the BEACH/WD40 region; Akap550 has clear C.e. (F10F12.1) and human (LysT-like) orthologs (E=0.0). CG1332 is shorter (511 aa) and appears to be composed primarily of BEACH and WD40 domains; its closest C.e. relative is also F10F12.1. The final BEACH/WD40 protein is CG6734; its C.e. ortholog is F52C9.3/F52C9.1, and it is most closely related to mammalian neutral sphingomyelinase activators such as FAN, which are inducers of apoptosis.	
pins (Rapsynoid), CG5692	EP(3) 3113	TPR repeat protein that complexes with Inscuteable and a G-alpha subunit to regulate asymmetric cell divisions in the nervous system and elsewhere [39, 64, 87]. EP inserted 5′ to mRNA start; also −/+ within the first intron of an mRNA for CG5643 (PP2A B subunit, see above), which is the adjacent gene.	GOF phenotype includes ISNb axonal pathfinding errors and abormal synaptic geometries (Figure 4). In situ hybridization shows that Pins mRNA is overexpressed when EP3113 is crossed to driver (K.M., unpublished). Also possible that CG5643 expression is reduced by predicted antisense mRNA.
CG3249, PKA anchor protein with KH domain	EP(X) 1400	CG3249, apparent ortholog of vertebrate PKA anchor proteins (E=1e-32 with human Akap1). These proteins contain a KH/Tudor domain (RNA binding). N terminus defined by EST; C terminus not well defined, extends beyond homology region. EP inserted in 1st intron of CG3249, 5' to entire coding region. EP also −/+ to an overlapping gene, CG4165, which is a ubiquitin C-terminal hydrolase with a human ortholog (E=5e-27); CG4165 is more weakly related to Faf (E=2e-6).	Phenotype could be a GOF for A-kinase anchor, a partial LOF for CG4165 ubiquitin hydrolase, or a combination of the two. Expression pattern: Figure S2, Table S5.
tout-velu (EXT-like heparan sulfate copolymerase), CG10117	EP(2) 0765	A heparan sulfate copolymerase [88, 89], 760aa; homologous to mamalian multiple exostoses (EXT) tumor suppressors; type II membrane protein with only the extreme N terminus in the membrane; localized to intracellular membranes; required for Hh movement, probably because such movement requires a heparan sulfate proteoglycan. EP inserted in 5th intron, and would drive expression of the C-terminal 336 aa segment.	EPs might drive synthesis of a C-terminal fragment of up to 336 aa that would presumably be located within intracellular secretory vesicles, as it lacks the N-terminal membrane anchor. Since homology to EXTs extends across whole protein this fragment is probably inactive; perhaps it acts as a dominant negative. In situ hybridization shows that ttv mRNA is overexpressed in CNS when EP is crossed to driver (R.K., unpublished). Has a synaptic LOF phenotype: Figure 6.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG18445, Porcupine-related O-acyltransferase	EP(2) 2324	CG18445, encoding a predicted 722 aa multispan transmembrane protein related to fly Porcupine (Por) and Nessy and to C.e. Mom-1. N terminus defined by EST; C terminus not well defined, extends beyond homology region. Por and Mom-1 are required for Wg signaling. The Por family of proteins (membrane-bound O-acyltransferases or MBOATs) is related to O-acyltransferases, which transfer fatty acids onto lipids or lipopolysaccharides [90]. The substrate of Por/Mom-1 is unknown but could be carbohydrates on the Dally proteoglycan required for Wg targeting; or possibly Wg itself, although MBOATs are not known to have protein substrates. CG18445 has 2 C.e. orthologs that that are much more closely related to it (C54G7.2, E=9e-77; C08F8.4, E=3e-60) than are any of its fly or vertebrate relatives (E=2e-33 for most closely related mouse protein, C3F). We find at least 7 members of the MBOAT family in the fly: Por, CG9655 (nessy; most closely related to CG18445, E=9e-27), CG9526, CG18445, CG17937, CG8112, CG6023. EP inserted in 5′ UTR.	GOF phenotype includes abnormal ISNb synaptic geometries (Figure 5). Expression pattern: Table S5.
Putative signaling proteins with protein–protein interaction and/or Ca^{2+} binding domains			
drk (SH2-SH3 adapter), CG6033	EP(2) 2477	EP inserted 526 nt 5' of ATG.	
purity-of-essence (pushover/Calossin) (calmodulin binding), CG14472	EP(2) 0737	Huge Ca^{2+}/CaM binding protein; EP inserted 42 nt 5′ to ATG.	Weak synaptic bouton LOF phenotype (R.K., unpublished).
CG1435, CBP Ca^{2+} binding protein	EP(X) 1201	Member of a family of EF hand CBPs found only in invertebrates; related to PERVT. Protein size defined by ESTs. Closest relative is in polychaete marine worms, E=2e-23; no closely related fly sequences. EP inserted in 5' UTR.	Expression pattern: Table S5.
CG 6017, ankyrin domain	EP(3) 3292	Ortholog of human protein KIAA0946 (E=e-160); member of a family of ankryin repeat proteins which includes the closely related Hyph protein that interacts with huntingtin. Protein size defined by ESTs. CG6618 is also part of this family (E=e-50). EP inserted 444 nt 5' to predicted mRNA start.	Expression pattern: Table S5.
CG10977, MSP protein	EP(3) 3539	Weakly related (E=3e-8) to mamalian VAMP/synaptobrevin-associated (VAP) protein. Contains MSP (major sperm protein) domain; MSP domains associate to form filaments. N termimus defined by EST; C terminus not well defined, extends beyond homology region. Closest fly relative is Farinelli (E=4e-7), a testis-specific VAP-like protein required for male fertility. EP inserted 1404 nt 5′ to predicted ATG.	

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
Putative transcriptional regulators			
bang senseless (DSP1/ SSPR2) (HMG box transcriptional repressor), CG12223	EP(X) 0355	EP inserted in 5′ UTR	
crooked legs (Zn finger proteins), CG14938	EP(2) 2226	EP inserted in 5′ UTR	
Kr-h1 (Zn finger protein), CG9167	EP(2) 2289	EP inserted in 5' UTR	Also recovered in PNS GOF screen [68]. Could not examine LOF in larvae due to embryonic lethality.
Adf 1 (nalyot) (transcription factor), CG15845	EP(X) 0815	EP inserted in 5' UTR	Strong GOF phenotype involving ISNb/ISNd pathfinding errors and synaptic abnormalities. Also recovered in PNS GOF screen [68].
mastermind, CG8118	EP(2) 2575	Q-rich nuclear protein of unknown function. Mam LOF affects vMP2 fate and thus changes its axonal morphology [33]. EP inserted in 1st intron of largest mRNA isoform, upstream of ATG	Entire large Mam protein isoform should be overexpressed in EP2575 x driver embryos.
ash2 (trithorax-like transcriptional activator), CG6677	EP(3) 3472	572 aa trithorax-group protein. EP inserted in 4th intron, would drive expression of aa 232–572. This fragment would lack a PHD finger DNA binding domain located N-terminal to aa 200; would contain an intact SPRY domain, a region of unknown function found in trithorax-group proteins and in the ryanodine receptor.	ISNb is selectively affected in F1 EP3472 x driver embryos. EP should drive expression of the C-terminal half of the protein; since this lacks the putative DNA binding domain it might function as a dominant negative, so phenotype might represent a neural ash2 LOF phenotype.
HLHm7 (HLH protein), CG8361	EP(3) 3587	Part of the E(spl) complex; EP inserted 713 nt 5' to longest cDNA start.	
schnurri (Zn finger transcription factor), CG7734 (−/+)	EP(2) 0644	Very large transcription factor involved in DPP signaling; EP inserted in −/+ orientation in 1st intron, 11 bp 3′ to splice donor site. No nearby genes in +/+ orientation to EP.	EP should drive expression of an antisense transcript complementary to 1st exon of shn; this might reduce or block expression of Shn protein, so phenotype might be a neural LOF; shn LOFs affect PNS development [53].
scribbler (brakeless), nuclear protein, CG5580	EP(2) 2461	Nuclear protein required for R cell axon targeting in the optic lobe [96]. EP inserted 538 nt 5' to ATG.	
CG11172, Rel domain protein	EP(X) 1335, 1353, 1508	1419 aa predicted protein, highly related (E=1e-74) to mammalian nuclear factor of activated T cells (NFAT); but homology limited to Rel domain region (aa 465–748). Protein size defined by ESTs. Only distant fly relatives exist; the closest is the Rel domain protein Relish, CG11992 (E=0.36). EPs 1335 and 1508 are in 1st intron, EP 1353 is in 5' UTR of predicted mRNA. 1335 and 1508 would be predicted to drive expression of a protein lacking 44 aa at the N terminus.	Also recovered in PNS GOF screen [68]. Expressed in CNS: Figure S2, Table S5.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
CG4427, Sp1-like Zn finger protein	EP(2) 2237	Related to many Zn finger proteins; the closest fly relative is Sp1, CG1343. Closest human relative is an Sp1-like protein, E=8e-35. EP inserted 228 nt 5' to predicted ATG.	
CG17090, homeodomain-interacting S/T kinase	EP(3) 3571	see notes above	
RNA binding proteins and helicases			
egalitarian, CG4051	EP(2) 0938	Weak homology (E=3e-4) to Werner syndrome helicase; Egl/BicD complexes are involved in RNA localization during oogenesis [91]. EP inserted 18 nt 5' to cDNA start	GOF phenotype: type II synaptic arborization is greatly increased (Figure 5). Weak synaptic bouton LOF phenotype (R.K., unpublished). Expressed in CNS: Figure S2.
eIF-4A (helicase, translation factor), CG9075	EP(2) 1011	DEAD box helicase, translation initiation factor. As heterozygotes, LOFs enhance extra bristle phenotype conferred by ectopic expression of lethal-of-scute [24]. LOFs also suppress cell overgrowth phenotypes of dPten mutants [92]. It is thought that a decrease in eIF-4A expression selectively affects translation of certain mRNAs involved in growth control. Not clear what effects should be produced by overexpression. EP inserted 1.7 kb 5' to mRNA start.	EP is inserted in 1st (noncoding) intron of the next gene, chickadee (Profilin), in −/+ orientation; but phenotypes in EP1011 × driver embryos probably not due to inhibition of Profilin expression, even though lack of Profilin causes axon guidance phenotypes [59], because the only exon that could be affected by an antisense RNA from EP is ovary specific. Could not examine LOF in larvae due to embryonic lethality.
eIF-4E (mRNA cap binding protein), CG4035	EP(3) 0568	eIF-4E regulates translation of a subset of postsynaptic mRNAs. It is controlled by eIF-4EBPs, which sequester and inactivate eIF-4E; removal of 4EBPs activates translation of these mRNAs. 4EBP dissociation is caused by their phosphorylation by the rapamycin-sensitive mTOR kinase. eIF-4E is localized to aggregates on postsynaptic side of fly NMJs. Overexpression of eIF-4E increases bouton number and evoked postsynaptic current [101]. EP inserted 239 nt upstream of mRNA start.	

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
pumilio (mRNA binding), CG9755	EP(3) 3038	Contains 8 PUF RNA binding domains; involved in RNA localization in the embryo. Complexes with Nanos, and complex binds to hunchback mRNA and other targets. Also regulates translation [93, 94]. EP inserted in large 8th intron, upstream of all PUF domains.	EP should overexpress C-terminal regions of the Pum proteins (aa 662-1533 for large isoform); these contain all the RNA binding domains and might have wild-type function. The pum LOF (pumuckel in this paper) has a Bolwig nerve guidance phenotype [44]. Expressed in a subset of CNS cells, and LOF has a motor axon pathfinding phenotype (K.M., unpublished). A different EP in pum (3461) was recovered in a screen for enhancers of a rough eye phenotype induced by ataxin overexpression [100].
apontic (mRNA binding), CG5393	EP(2) 2339	Binds to Oskar mRNA together with Bruno [95]; inolved in RNA localization in the oocyte. EP inserted 315 nt 5' to start of LD45581 apontic cDNA clone.	GOF phenotype includes abnormal ISNb synaptic geometries and ectopic synaptic branches (Figure 5).
CG16788, RRM domain protein	EP(3) 1082	RRM (also known as RNP-1) domain-containing protein. Protein size defined by ESTs. Many close vertebrate and fly relatives, some of which are splicing factors, but no clear vertebrate ortholog. Closest H.s. relative has a score of E=4e-24. EP inserted 794 nt 5' to predicted ATG.	
CG1691, KH domain protein	EP(X) 1433	Apparent ortholog of vertebrate beta-actin mRNA zipcode binding protein (E=3e-88); this is also known as IGF-II mRNA binding protein and Vg1 mRNA binding protein. Protein size defned by ESTs. Closest fly relative is mushroom body expressed (mub), E=2e-13. EP inserted 1548 nt 5' to predicted ATG.	Strong GOF phenotype. Includes ISNb axonal pathfinding errors and missing synapses (Figure 4). Selectively expressed in CNS: Figure S2, Table S5.
CG3613, qkr58E-1, KH domain protein	EP(2) 2103	A member of an 8-gene family of fly KH domain RNA-binding proteins. Several are located in a cluster at 58E. Protein size defined by cDNA sequence. The family is related to the mouse quaking gene. Closest mammalian relative is mouse SLM-1, E=e-43. The fly family includes held out wings (how), which may regulate integrin function. EP inserted 287 nt 5' to predicted mRNA start.	qkr58E-1 is expressed in the CNS [96].
CG3249, PKA anchor protein with KH domain	EP(X) 1400	see notes above	
CG11486, polyA-nuclease related protein	EP(3) 3109	Closest relative is a C. elegans protein, E=2e-92; CG11486 and the C.e. protein are apparent orthologs (E=6e-49) of yeast Pan3p, a subunit of the polyA-nuclease that regulates the lengths of mRNA polyA tails. Protein size defined by ESTs. No close fly relatives. EP inserted 200 nt 5' to cDNA start, 980 nt 5' to predicted ATG.	Strong pathfinding GOF phenotype.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
Putative plasma membrane/secreted proteins			
amnesiac, CG11937	EP(X) 0346, 1571	Gene is predicted to encode a neuropeptide; but the protein has not been characterized and cDNAs have never been sequenced. EP(X) 0346 inserted 207 bp 3′ to presumptive ATG [65]. EP(X) 1571 141 bp 3′ to ATG. These EPs should overexpress fragments of the protein lacking the signal sequence, so we do not understand the origin of the GOF phenotype. These fragments are probably not dominant negatives since GOF and LOF phenotypes differ. Perhaps additional unpredicted proteins can be made from the amn gene. Other EPs: 1649, 0971,1639 are inserted >330 bp 3′ to ATG; 0367, 1442, 1609 are −/+ relative to coding.	1571 has a weaker GOF phenotype than 0346. The EPs inserted at more 3′ positions and the −/+ EPs do not produce GOF phenotypes. GOF phenotype (weak ISN synapses): Figure 3. LOF phenotype (missing boutons): Figure 6.
Fasciclin 2 (Ig-CAM), CG3665	EP(X) 1462	EP inserted 96 nt 5' to cDNA start.	The protein is overexpressed in axons when EP is crossed to driver (R.K., unpublished).
roundabout 2 (Ig/FN domain surface protein), no CG	EP(2) 2582	EP inserted 101 nt 5′ to mRNA start.	Protein is expressed on a subset of CNS axons [45–48]. In situ: Figure S2.
Gliotactin (surface protein), CG3903	EP(2) 2306	Esterase-related glial cell surface protein; EP inserted 42 nt 5′ to cDNA start.	Also recovered in PNS GOF screen [68].
Laminin A, CG10236	EP(3) 3678	The only fly laminin A chain; EP inserted 68 nt 5' to cDNA start.	
Neurexin (surface protein), CG6827	EP(3) 0809	Transmembrane protein at septate junctions; EP inserted into 1st intron, downstream of exon encoding ATG and signal sequence.	ISN terminal arbors cross between segments in F1 EP809 x driver larvae. Should overexpress a protein lacking N-terminal 35 aa, including signal sequence. Could be a dominant negative since C-terminus of Neurexin binds to Coracle [97]; Neurexin trapped within the cytoplasm might sequester Coracle.
Ptp10D, CG1817	EP(X) 1172	see notes above	see notes above
scab (Volado) (α-integrin PS3 subunit), CG8095 (−/+)	EP(2) 2591	Two mRNAs encode proteins with different N termini; EP inserted in −/+ orienation in 1st intron of large mRNA isoform; could make an antisense transcript complementary to 1st exon of large but not to small mRNA isoform. No nearby genes in +/+ orientation to EP.	EP is inserted in −/+ orientation; might reduce or block expression of large mRNA isoform by making an antisense transcript. The Vol-1 mutation, which affects expression of large but not of small mRNA isoform, produces a memory deficit; and the Vol-1 enhancer trap is expressed in mushroom body neurons [51].

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
spitz (DER tyrosine kinase ligand), CG10334	EP(2) 2632	EGF-related transmembrane protein that requires Rhomboid for cleavage and activation; EP inserted in 2nd intron, upstream of entire coding region	
CG1762, Integrin βν	EP(2) 2235	An integrin β subunit exclusively expressed in the gut [98]. Protein defined by cDNA sequence. Closest relative is C.e. pat-1 (E=e-109). EP inserted 1008 nt 5' to ATG.	GOF phenotype is quite strong; reduced and morphologically defective synapses. Since βν is not expressed in neurons must be a misexpression phenotype; it may be caused by association of βν with α integrin subunits expressed in growth cones and synapses [50], leading to abnormal integrin signaling. No published LOF mutations exist for this gene.
CG7607/CG14141?, an Ig domain protein	EP(3) 3548	EP inserted 2330 nt 5′ to predicted ATG. EP is also −/+ to a gene on the other strand, CG6199, whose mRNA ends <800 nt from EP site. CG6199 encodes a lysyl hydroxylase enzyme. CG7607 has a short coding region (167 aa), but it may be linked to the adjacent (3′) gene, CG14141 (147 aa predicted), which also encodes an Ig-domain protein. N terminus of CG7607 defined by EST. CG14141 is the most closely related sequence to CG7607 in the database (E=1e-39). CG14141 has no ESTs. We do not know whether the CG7607 product is secreted, transmembrane, or intracellular. CG7607 has a plausible N-terminal signal sequence, but neither CG7607 or CG14141 has an obvious transmembrane domain. The closest vertebrate relative is the receptor tyrosine phosphatase RPTPσ (E=1e-5).	Synapses are longer than normal in GOF larvae; could be due to GOF for CG7607, an LOF for CG6199, or a combination of the two.

(*continued*)

Table S4

Continued

GENE	EP	Molecular information	Comments
wunen (lipid phosphatase), CG8804	EP(2) 2208	see notes above	
CG8805, Wunen2/Tunen, lipid phosphatase	EP(2) 2607	see notes above	
CG13101, related to Rolling Stone	EP(2) 2247	CG13101 is a member of a 6-gene family related to Rolling Stone (Rost), a multispan transmembrane protein required for myoblast fusion [99]. Protein size defined by ESTs. CG13101 is immediately 3' to rost (CG9552). The other genes in the family are CG9555, CG17906, CG4480, and CG15639. CG9555 and CG17906 are particularly closely related (E=e-112) and are also adjacent genes. CG13101 is equally related to Rost, CG9555, and CG17906 (E=4e-37 with CG9555). The Rost gene family is not significantly related to any vertebrate or C. e. sequences. EP inserted 99 nt 5' to cDNA start.	
CG13349, Xoom-related membrane glycoprotein	EP(2) 2270	EP inserted 4555 nt 5' to predicted ATG. EP is also within the 3rd intron of the Cp1 gene (CG6692) encoding a cysteine proteinase, and is in the −/+ orientation to this gene; so would presumably make an antisense Cp1 transcript. CG13349 is the apparent ortholog (E=1e-47) of a group of approx. 400 aa vertebrate membrane glycoproteins that include Xenopus Xoom and mouse adhesion regulating molecule. N terminus defined by ESTs; C terminus by homology. The only closely related fly sequence (8e-29) is CG6789. CG13349 contains a signal sequence and a single predicted transmembrane domain.	GOF phenotype includes ISNb axonal pathfinding errors and ectopic synaptic branches (Figure 4). The origin of the phenotype is unclear, because the EP is within the Cp1 gene; it could be a GOF for CG13349, a neural LOF for Cp1 due to generation of an antisense RNA, or a combination of the two. In situ hybridization shows that CG13349 mRNA is in fact overexpressed when EP is crossed to driver. Expressed in CNS: Figure S2, Table S5.

Known genes are listed by name in italics, followed by a descriptor and a CG number. New genes are listed by CG number, followed by a descriptor. When a gene falls into two categories, the notes of columns 3 and 4 are only included for the first category, but the gene and EP are listed for both categories. The E values for the alignments of each of the new genes to their closest noninsect relatives are underlined. Some E values may be incorrect because the proteins actually encoded by the new genes are different from the CG annotations and/or from our revised annotations (see CG7719 for an example).

Table S5

Expression patterns of new genes identified in the screen.

Gene (EP)	Probe	Overexpression	Wild-type expression
CG8487 (2028)	SD10766	medium*	Expressed in CNS (generalized) at stage 13 and later, and elsewhere at somewhat lower levels (Figure S2i).
CG3862 (1242)	SD03439	medium	Expressed in midgut from stages 10–13; also a head patch at stage 9. CNS is at background levels.
CG6811 (3152)	CK00088	strong	Expressed in CNS (generalized) at stage 13 and later, and also in ectoderm (Figure S2p).
CG2017 (3503)	LD02491	medium	Expressed in visceral mesoderm at stages 12–15. Low-level overall staining, including the CNS, as well.
CG7719 (0515)	LD35132	strong	High levels of RNA in syncitial blastoderm (maternal loading?); also in germ cells up through stage 10–11. Also expressed at low levels in neuroblast layer at stages 10–11. Weak CNS and epidermal expression after stage 13.
CG1210/Pk61C (3553)	GH15751	strong	Expressed at stage 8 in a segmentally striped pattern (faint), plus expression in neuroectoderm. High-level expression in cell clusters that are probably the Malpighian tubule primordia from stages late 11 to 13; these cells are adjacent to hindgut. Hindgut also expresses RNA at stage 13 only. After stage 14, low-level ubiquitous expression including CNS.
CG5643 (3559)	LD34343	strong	Expressed in brain and ventral nerve cord (generalized) during stages 14 and later; also lower-level expression elsewhere (Figure S2l).
CG10426 (3636)	GH16681	strong	Segmentally repeated, large subset of CNS cells at stages 13–16 (Figure S2b); also stripes in epidermis that line up with CNS cell groups.
CG9011 (2299)	LP02084	strong	Expressed in CNS (generalized) at stage 13 and later, and elsewhere at slightly lower levels (Figure S2g).
CG18445 (2324)	SD01379	strong	Expressed in amnioserosa at stages 11–14; also a subset of CNS cells.
CG1435 (1201)	LD11255	strong	ubiquitous
CG6017 (3292)	LD10758	strong	Expressed in muscle precursors.
CG11172 (1335, 1353, 1508)	LD10950	strong (Figure S2a; note that this panel was developed for about 1/3 as long as the wild-type panels)	Expressed in CNS (generalized) at stage 12 and later, and elsewhere at lower levels (Figure S2n)
CG13349 (2270)	LD03370	medium	Ubiquitous expression, with higher levels in CNS (Figure S2o)
CG1691 (1433)	SD10340	strong	CNS-specific at stages 11–16; expressed in a subset of CNS cells (Figure S2r). Also expressed in a pair rule pattern in early embryos.
CG7001 (0438)	LD21956	strong	Ubiquitous expression, with slightly higher levels in CNS (Figure S2j)
CG3249 (1400)	LD26452	strong	Expressed in somatic and visceral mesoderm (Figure S2f). No detectable CNS expression.
CG11411 (1336)	GM13066	strong	Expressed in subsets of neuroblasts at stage 11, and in posterior midgut (Figure S2d). Little expression later in embryogenesis.
CG12403 (2353)	GH10636	strong	Expressed in proventriculus and midgut. Serves as an example of an embryo with no CNS signal (Figure S2c). CG12403 mRNA is overexpressed in EP2353 x driver embryos.
CG8805 (2607)	n/a	n/a	Expressed in CNS; see Table S4 annotation.
CG3613 (2103)	n/a	n/a	Expressed in CNS; see Table S4 annotation.

*Medium overexpression: endogenous and CNS/PNS overexpression patterns simultaneously visualizable in EP x driver embryos, although CNS/PNS overexpression is stronger. Strong overexpression: when color reaction has proceeded until dark CNS/PNS staining is observed in EP x driver embryos, only weak endogenous expression can be seen in wild-type embryos.