Supplementary figure legend

Figure S1. Identification of genes required for development of the larval optic lobe.

(A-B) PCNA-3xEmGFP allows rapid identification of optic lobe neuroepithelial cells. (A) The

PCNA::3XEmGFP expression pattern in central brain neuroblasts and optic lobe neuroepithelial cells was indistinguishable from the pattern of BrdU incorporation following a 3-hour pulse labeling. Thus, PCNA::3XEmGFP reliably and reproducibly labeled all proliferating cells (yellow arrow). The yellow dotted line separated the central brain from the optic lobe. The scale bar = 50 μm. (B) PCNA-3XEmGFP co-localizes with BrdU incorporated during a 3-hour pulse labeling in brain neuroblasts (yellow arrow). Yellow arrowhead = ganglion mother cell.

(C-E) The optic lobe absent mutant. (C) The lateral projection view of the third instar *csw* mutant larval optic lobe reveals absence of optic lobe neuroepithelia and neuroblasts. The scale bar = 20 μm. (D) A dorsoventral single confocal optical section of a third instar *csw* mutant larval optic lobe shows a small cluster of primitive optic lobe neuroepithelial cells. (E) A three-dimensionally reconstructed model of the *csw* mutant stained for Phall reveals the absence of the neuropil in the optic lobe. The neuropil seen is a part of the central brain neuropil.

(F-H) The optic lobe expanded mutant. (F) The lateral projection view of the third instar $ft^{3477/G\text{-}rv}$ mutant larval optic lobe reveals dramatically expanded optic lobe neuroepithelia. (G) The dorsoventral single confocal optical section of the third instar $ft^{3477/G\text{-}rv}$ mutant larval optic lobe shows partially overlapped optic lobe neuroepithelia. (H) A three-dimensionally reconstructed model of the $ft^{5072/G\text{-}rv}$ mutant optic lobe stained for Phall reveals the presence of the neuropil.

(I-K) The optic lobe premature lost mutant. (I) The lateral projection view of the third instar $o\text{-}fut1^{2834/Df}$ mutant larval optic lobe reveals prematurely formed neuroblasts replacing the

swath of neuroepithelia. (J) The dorsoventral single confocal optical section of a third instar *o-fut1*$^{2834/Df}$ mutant larval optic lobe shows prematurely formed neuroblasts located on the surface of the larval optic lobe. (K) A three-dimensionally reconstructed model of the *o-fut1*$^{2834/Df}$ mutant optic lobe stained for Phall reveals the fragmented neuropil.

(L) The *o-fut1*^{2834} mutant allele contains a nucleotide substitution (G->A; highlighted in red) at the splicing acceptor site in the first intron of the *o-fut1* gene.

(M-N') Failure to properly process the Notch protein in the *o-fut1* mutant neuroblast. (M-M') The Notch protein is abundantly detected in the cortex of a wild type brain neuroblast. (N-N') The Notch protein mainly localizes in the cytoplasm of *o-fut1* mutant brain neuroblasts.

(O) The Aph-1^{5072} mutant protein contains an amino acid substitution (G->R; highlighted in red) at the amino acid residue 15.

(P-S') The onset of the prematurely lost optic lobe phenotype coincides with loss of E(spl)mγ-GFP expression in the *o-fut1*$^{2834/Df}$ mutant. (P) In the wild type second instar (L2) larvae, E(spl)mγ-GFP is detected predominantly in neuroblasts in the central brain. (Q) In the wild type third instar (L3) larvae, E(spl)mγ-GFP is detected in neuroblasts in the central brain and in transitioning neuroepithelial cells and in neuroblasts in the optic lobe. The dotted yellow line indicates the boundary between the larval optic and the larval brain. (R) In the *o-fut1*$^{2834/Df}$ mutant second instar (L2) larvae, E(spl)mγ-GFP is detectable only in some neuroblasts in the central brain. Please note the presence of the larval optic lobe as indicated by the PatJ staining. (S) In the *o-fut1*$^{2834/Df}$ mutant third instar (L3) larvae, E(spl)mγ-GFP is undetectable in any neuroblasts in the central brain and in any neuroblasts in the optic lobe. Dpn serves as the neuroblast marker. Please note the absence of the larval optic lobe as

indicated by the absence of PatJ staining. The scale bar = 20 μm.

Figure S2. *Notch* is a critical regulator of neuroepithelial cell maintenance.

(A-B') *Notch* functions cell autonomously to maintain the neuroepithelial cell identity. (A-A') Wild type neuroepithelial cells in a GFP-marked mosaic clone become converted into neuroblasts located medially from neuroepithelia. The higher magnification image of the boxed area is shown in A'. (B-B') Neuroepithelial cells in a *Notch* mutant mosaic clone become prematurely converted into neuroblasts, which delaminate inward away from the rest of neuroepithelia and locate in the cortex of future medulla. The higher magnification image of the boxed area is shown in B'. The scale bar = 10 μm.

(C-D') Down-regulation of *Notch* is necessary for conversion of neuroepithelial cells into neuroblasts. (C-C') Wild type neuroepithelial cells in the GFP-marked mosaic clone undergo conversion into neuroblasts synchronously with those located outside of the clone. The higher magnification image of the boxed area is shown in C'. (D-D') Neuroepithelial cells over-expressing $Notch_{intra}$ within the clone show unrestrained expansion beyond the medial edge of neuroepithelia and become surrounded by neuroblasts located outside of the clone. Please note that a few neuroblasts outside of the clone appear to overlap with the clone due to the lateral projection view. The higher magnification image of the boxed area is shown in D'. The scale bar = 20 μm.

Figure S3. E(spl)mγ-GFP marks transitioning neuroepithelial cells.

(A-A') E(spl)mγ-GFP is detected in transitioning neuroepithelial cells but is undetectable in

immature neuroblasts (dotted yellow line).

Supplementary movie 1. Three-dimensional model of the wild type third instar larval optic lobe stained for PatJ and GFP driven by the *GH146-Gal4* driver to allow visualization of neuroepithelia and neuroblasts.

Supplementary material and methods

pcna::3XEmgfp: The *pcna::3XEmgfp* transgene is generated by replacing *egfp* in the *pcna::egfp* described in the Thacker et al., 2003 with three copies of the emerald green fluorescent protein. The *pcna::3XEmgfp* transgenic fly lines were generated in the laboratory of Dr. R. Duronio, UNC, Chaple Hill.

Genetic screen: We generated and screened six thousand EMS-induced mutant lines carrying homozygous lethal mutations on the second chromosome of the fly genome. We identified one new allele for each one of the following genes *cnk*, *ft*, *o-fut1* and *aph-1*. In addition, we screened the entire collection of the P-element induced lethal mutations available from the Bloomington stock center, and identified one allele of *cnk* and two alleles of *csw* that show the optic lobe absent phenotype.