SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Neurogenesis in the main OPC and the tOPC. Related to Figure 1

A, B, Neurogenesis in the main OPC. **A,** Side view of a larval optic lobe (L3). Anterior (A) is at the top, Posterior (P) at the bottom. D, dorsal; V, ventral; M, medial; L, lateral; NBs, neuroblasts. The Outer proliferation Center (OPC) can be divided into two regions, based on their neurogenesis mode: the main OPC (purple) and the tOPC defined by *wg-gal4* expression (green). Main OPC neuroblasts sequentially express five transcription factors as they age: Hth, Ey, Slp, D and Tll. **B,** Neuronal diversity in the main OPC is generated by a combination of temporal patterning and Notch-mediated binary cell fate decisions. All Notch^{ON} neurons express Apterous (green). Picture: neurons produced during the Ey time window: Ey-expressing Notch^{OFF} neurons (blue) are intermingled with Apterous-expressing Notch^{ON} neurons (green). **C-I,** neurogenesis in the tOPC. All pictures are posterior views of L3 optic lobes **C,** Two hypotheses could explain the clustered organization of tOPC neurons. **D,E,** Class 1 neurons all co-express Dll (red, **D**), Salm (magenta, **D**) and Runt (white, **E**).**F,** Class 1 neurons in the ventral tOPC also express D (yellow). Dashed lines depict the tOPC. **G,** *ey-gal4* (green) is expressed in all Ey neuroblasts of the OPC (Cyan, arrows). Dll is in magenta. Dashed lines depict *ey-gal4* expression domain. Straight lines depict the tOPC. **H,** *slp-gal4* (green) is specifically expressed in the tOPC region (dashed lines): it is strongly expressed in all Slp-expressing neuroblasts (red) of the dorsal tOPC (arrow) and weakly expressed in most of the Slp-expressing neuroblasts of the ventral tOPC (arrowhead).**I,** Class 4 neurons co-express Toy (red) and D (yellow). Dashed lines depict the tOPC. NBs=Neuroblasts, GMCs=Ganglion Mother Cells, N=Neurons.

Figure S2: Notch status of tOPC neurons. Related to Figure 2

Posterior views of L3 optic lobes. **A-C,** Hey is a Notch sensor in the optic lobes. **A,** Hey (red) is lost in *su(H)* mutant clones (dashed lines, arrows). **B,** Control brain. **C,** *insc-gal4>Notch^{ICD}*. Hey (red) expression expands when Notch^{ICD} is ubiquitously expressed**. D, E,** *su(H)* mutant clones (dashed lines). Class 1 Salm/Runt (magenta, **D**) and Class 2 Svp (cyan, **E**) neurons are not affected when Notch signal is abolished (arrows). **F**, *insc-gal4>Notch^{ICD}*. Class 3 Toy and Class 4 Toy/D neurons (red) are not affected when Notch signaling is ectopically activated (arrows). At the opposite, Class 2 Svp neurons are lost (cyan, arrowheads).

Figure S3: Neuronal apoptosis in the tOPC. Related to Figure 3

Posterior views of L3 optic lobes. Dashed lines depict the tOPC. **A,** Cleaved Caspase-3 is expressed in Class 2, 3 and 4 clusters. A small number of Notch^{ON} Toy-expressing neurons (red) are found within the Notch^{OFF} Class 2 cluster. These neurons all express Cleaved-Caspase-3 (green, white arrows). A small number of Notch^{OFF} Svp neurons (Cyan) all expressing Cleaved-Caspase-3 are found in the Notch^{ON} Class 3 and Class 4 clusters (yellow arrows). **B, C,** In *wg>p35* brains, Notch^{OFF} Svp neurons (cyan) become completely intermingled with Notch^{ON} Hey positive (green in **B** and **C**) Toy-expressing (red, **C**) neurons.

Figure S4: Cross regulations and role of tOPC temporal factors. Related to Figure 4

Posterior views of L3 optic lobes. **A,** *dll* mutant clones (dashed lines)**.** Removing *dll* (magenta) does not cause expression of Hth (blue) in the tOPC. **B**, In *ey* mutant clones (cyan, dashed lines), Dll expression does not expand (magenta, arrowhead). **C,** Control brain showing expression of Ey (Cyan), Slp (red), D (yellow) and E-cad (white). **c',** Removing *ey* leads to the loss of Slp and D in neuroblasts (arrows). **D,** *slp* mutant clones (dashed lines). Removing Slp leads to the loss of D (yellow) and the expansion of Ey (cyan, arrow) in neuroblasts. **E,** *D* mutant clones (dashed lines). Removing D (yellow) leads to the expansion of Slp in neuroblasts (arrows). **F,** Summary of the cross-regulations between tOPC temporal factors. **G**, *dll* mutant clones (dashed lines). Class 1 neurons (Salm, magenta) are not lost in *dll* clones. **H, I,** *slp* mutant clones (dashed lines). **H,** Svp expression (cyan, arrows) is neither lost nor expanded in *slp* mutants.**I,** $Notch^{ON}$ Hey-positive neurons (white) are lost in *slp* mutants. **J**, When p35 is expressed in *slp* clones (dashed lines), the death of Hey neurons (white) is rescued, indicating that Slp is required for survival of $Notch^{ON}$ neurons. **K,** *D* mutant clones (dashed lines). Class 4 Toy/D neurons (yellow) are lost in *D* mutants (arrows) and are replaced by Class 3 Toy neurons (red).

<u>Figure S5</u>: tOPC temporal factors and the cell death pathway. Related to Figure 5

Posterior views of L3 optic lobes. **A, B,** Like with P35 expression, removing *Dronc* rescues the death of tOPC neurons and generates an intermingling between $Notch^{OFF}$ Svp neurons (cyan in **A** and **B**) and $Notch^{ON}$ Hey-expressing neurons (green in **B**) in the Ey, Slp (not shown) and D temporal window (**A**). **C, C',** *wg>hid-RNAi.* Decreasing Hid levels has not effect on the neurons produced during the Ey time window (**C**). By contrast, decreasing Hid levels rescues the death of the $Notch^{OFF}$ Svp neurons (cyan) during the Slp and D time window (**C'**). This phenotype is more pronounced in the ventral tOPC (arrow in **C'**).

<u>Figure S6</u>: The tOPC produces neurons for three different optic ganglia

A, Schematics representing a larval optic lobe (left) and an adult optic lobe (right). The tOPC region is in green (larval lobe). Plug=lobula plug; IPC=Inner Proliferation Center. The main OPC gives rise to the medulla (blue), the inner part of the main OPC gives rise to the lamina (yellow), the IPC and the Plug give rise to the lobula and lobula plate (pink). **B,B',** *wg*>FLEXAMP clones. tOPC neuroblasts produce neurons for three different optic ganglia (arrows 1, 2=medulla, arrow 3=lobula and arrow 4=lobula plate). **C, C'** *slp*>FLEXAMP clones. These clones contain a subset of the neuronal subtypes observed in *wg*>FLEXAMP clones (arrows 2, 3 and 4). Neuropils are visualized with N-Cad (magenta). (M)=Medulla, (Lo)=Lobula, (Lop)=Lobula plate, (CB)=Central Brain. Dashed lines show the neuropils.

<u>Figure S7</u>: Neuronal subtypes produced in the tOPC. Related to Figure 6

A-E, *slp*>FLEXAMP clones in adult brains. N-Cad (magenta) stains the neuropils, arrows point the neuronal cell bodies and dashed lines show the neuropils. **A**, Toy-expressing Mt4 neurons (red) send a long projection in M7, some perpendicular V-shaped projections in M6 and connect the central brain (blue arrowhead, **A'**). **B,** Two Toy-expressing Pm7a neurons (red) identified with white and cyan arrows. These neurons send short projections into M7. **C,** A giant Toy-expressing Pm7c neuron (red) projecting into M7. **D,** One Pm7b neuron (white arrow) and several LCN6 neurons (yellow arrows). LCN6 neurons send characteristic axonal projections towards the central brain (blue arrowhead). **E,** The two types of

Class 4 Toy/D neurons. Some of the Toy/D-expressing neurons (yellow) are Lccn2 neurons projecting in lobula plate layer Lop2 whereas others seem to project in Lop3.

SUPPLEMENTAL METHODS

Immunohistochemistry

Larval or adult brains were dissected in 1XPBS and fixed in 4% Formaldehyde for 30 minutes (larval) or 50 minutes (adult) on ice. Brains were then washed three times in PBX (1% PBS, 0.3% triton, 2% horse serum), incubated in primary antibody solution overnight at 4°C, washed three times and incubated in secondary antibody solution 3 hours at room temperature. They were finally washed overnight and mounted in Slowfade. Images were acquired using a Leica SP5 confocal microscope.

Genetics and fly strains

Experiments	Crosses	Source
wild-type **hsFLP clones**	*y,w,hs-FLP; wg-gal4/CyO; MKRS/TM6B* to *y,w,hs-FLP; UAS>CD2,y+>CD8::GFP/CyO; TM2/TM6B*	
Su(H) **MARCM clones**	*y,w,hs-FLP,UAS-CD8::GFP; FRT40A,tub-Gal80/CyO; tub-gal4/TM6B* to *w; FRT40A,su(H)$^{\Delta 47}$/CyO*	F. Schweisguth
Notch^{ICD} over-expression	*y,w; insc-gal4/CyO; tub-gal80^{ts}/TM6B* to *w;;UAS-Notch^{ICD}/TM6B*	S. Bray
P35 **over-expression**	*y,w; wg-gal4/CyO; tub-gal80^{ts}/TM6B* to *w;UAS-P35/CyO*	Bloomington # 5072
dll **MARCM clones**	*y,w,hs-FLP, UAS-nu::GFP,tub-gal4; FRT42D,tub-gal80/CyO* to *y,w,hs-FLP; dll^{SA-1}/CyO*	R. Mann
dll **over-expression**	*w; tub-gal80^{ts}/CyO; slp-gal4/TM6B* to *w; Sp/CyO; UAS-dll/TM6B*	R. Mann
ey **clones**	*hs-FLP;; FRT80B/TM6B; ey$^{J5.71}$/Ln(4)ci^{D}* to *w; FRT80B,ey-rescue,ubiGFP/TM6B*	Li et al. 2013
ey **RNAi**	*y,w; wg-gal4/CyO; tub-gal80^{ts}/TM6B* to *w;; UAS-ey-RNAi^{JF02501}*	Bloomington # 29339
ey **RNAi with P35**	*y,w; wg-gal4/CyO; tub-gal80^{ts}/TM6B* to *w; UAS-P35/CyO; UAS-ey-RNAi^{JF02501}*	
ey **over-expression**	*y,w; insc-gal4/CyO; tub-gal80^{ts}/TM6B* to *w;; UAS-ey/CyO*	Bloomington # 6294
slp **MARCM clones**	*y,w,hs-FLP,UAS-CD8::GFP; FRT40A,tub-Gal80/CyO; tub-gal4/TM6B* to *w; FRT40A,slp^{S37A}/SM6^TM6B*	A. Tomlinson
slp **MARCM clones with P35**	*y,w,hs-FLP,UAS-CD8::GFP; FRT40A,tub-Gal80/CyO; tub-gal4/TM6B* to *w; FRT40A,slp^{S37A}; UAS-P35/SM6^TM6B*	Bloomington # 5073
D **mutant clones**	*y,w,hs-FL ;; FRT2A,ubiGFP/TM6B* to *FRT2A,D^{87}/TM6B*	J. Nambu
def(3L)H99 **MARCM clones**	*y,w,hs-FLP,UAS-GFP; tub-gal4/CyO ; FRT80B,tub-Gal80/TM6B* to *w;; def(3L)H99,FRT80/TM6B*	D. Ryoo
hid **RNAi**	*y,w; wg-gal4/CyO; tub-gal80^{ts}/TM6B* to *w; UAS-hid-RNAi*	S.Cohen/VDRC