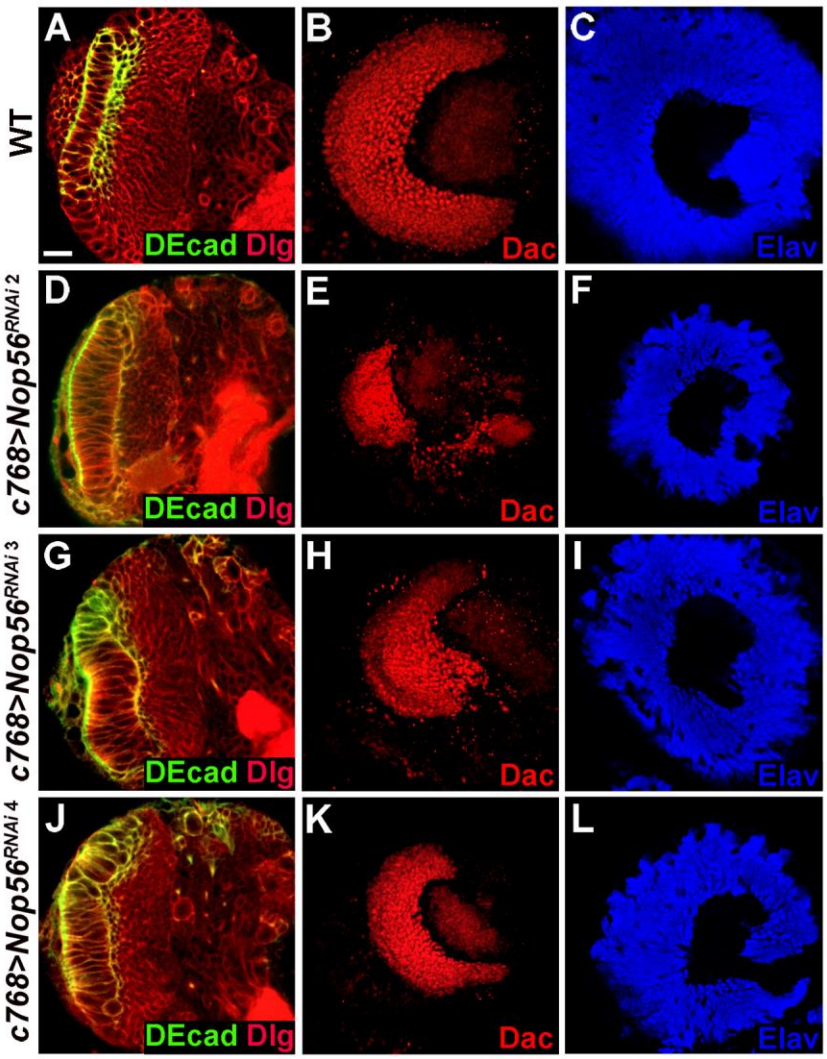


Figure S1 *Nop56* RNAi knockdown causes defects in medulla and lamina development. Brains dissected from late-third instar larvae cultured at 31° were stained with the markers indicated. Three different *UAS-Nop56^{RNAi}* constructs were expressed using *c768-Gal4*. Expression of these RNAi constructs inhibited neuroepithelial proliferation, resulting in elongated NEs (D, G, J), smaller lamina (E, H, K), and smaller medulla (F, I, L), compared to wild type (A-C). (A, D, G, J) Frontal view, lateral is to the left, medial to the right; (B, C, E, F, H, I, K, L) lateral view, anterior is to the left, dorsal is up. Scale bar: 20μm.

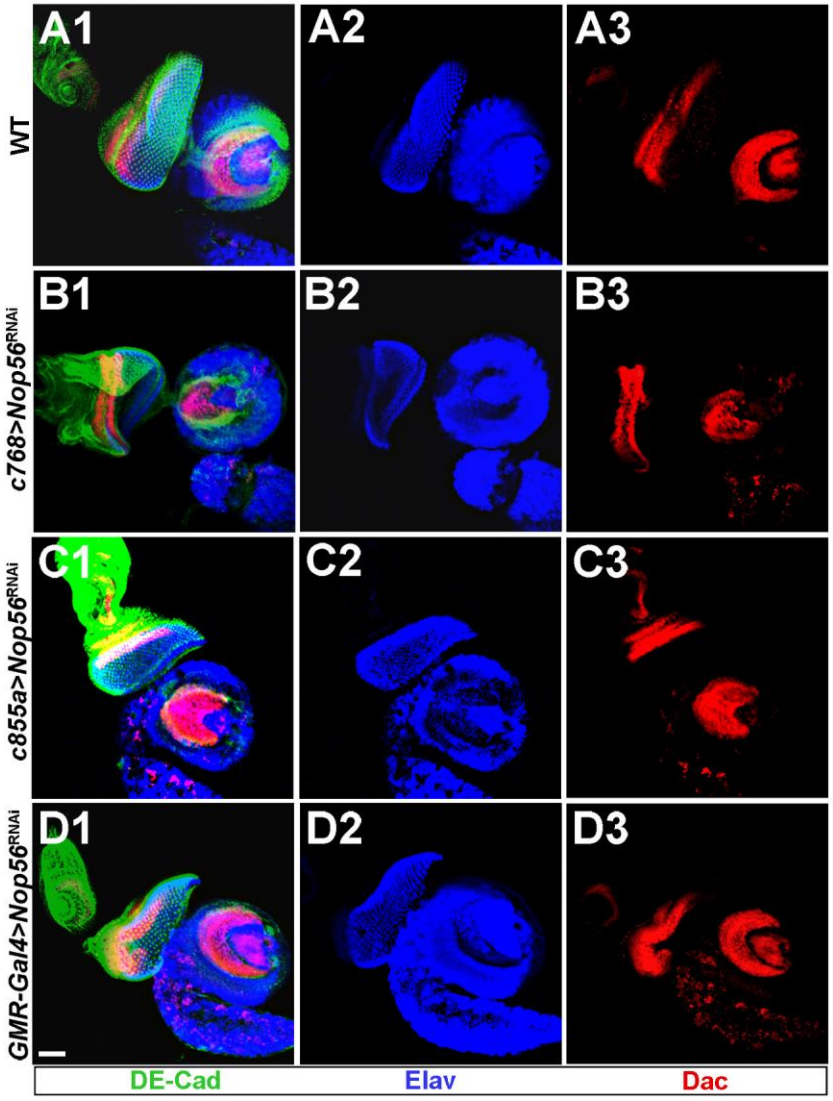


Figure S2 The defects in *Nop56* RNAi optic lobes do not result from the eye. Brain lobes with attached eye imaginal discs were dissected from late-third instar larvae cultured at 31°, and stained with the markers indicated. (A) Wild type eye disc and optic lobe. (B) *c768-Gal4/UAS-Nop56^{RNAi}* eye disc having a normal number of differentiated photoreceptors (B1, B2) projected to a smaller lamina (B3). (C) *c855a-Gal4/UAS-Nop56^{RNAi}* eye disc having a normal number of differentiated photoreceptors (C1, C2) projected to an enlarged lamina (C3). (D) *GMR-Gal4/UAS-Nop56^{RNAi}* eye disc and optic lobe developed normally. Lateral view, anterior is to the left, dorsal is up. Scale bar: 40μm.

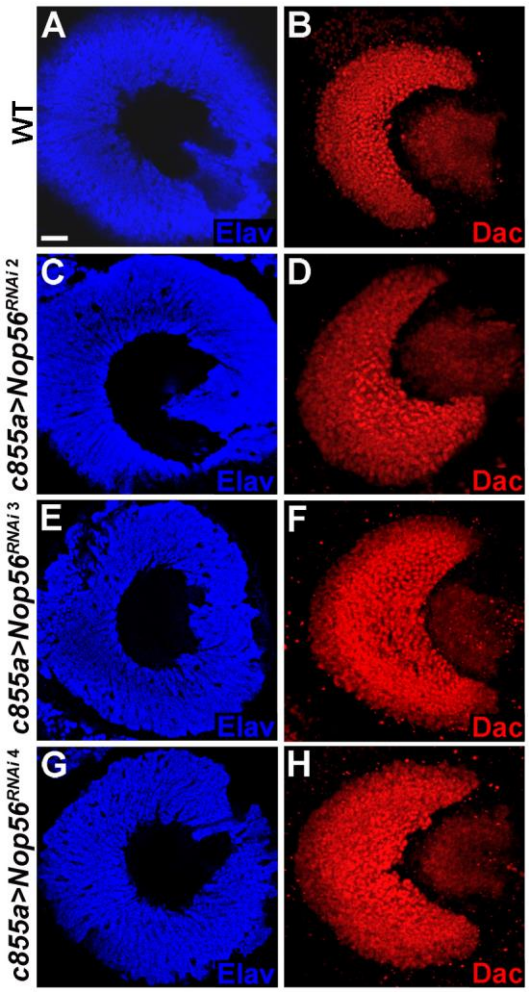
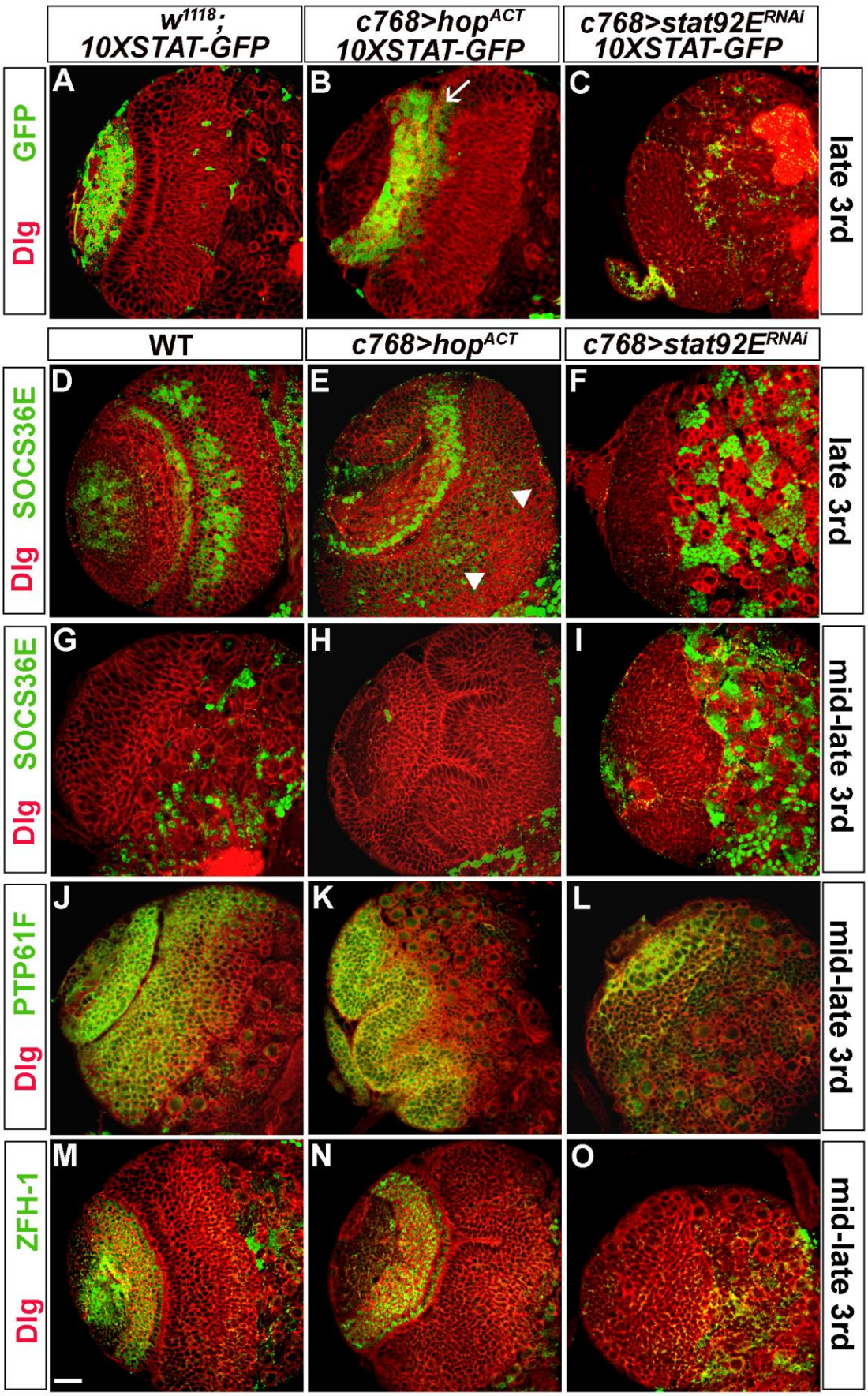


Figure S3 *Nop56* RNAi enhances lamina neurogenesis. Brains dissected from late-third instar larvae cultured at 31° were stained with Elav and Dac. (A, B) Wild type medulla (A) and lamina (B); (C-H) The expression of three different *UAS-Nop56^{RNAi}* constructs using *c855a-Gal4* led to enlarged lamina (D, F, H) as compared with wild type (B). Lateral view, anterior is to the left, dorsal is up. Scale bar: 20μm.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Dlg GFP
A
B
C
late 3rd
WT
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c768>stat92ERNAi
Dlg SOCS36E
D
E
F
late 3rd
Dlg SOCS36E
G
H
I
mid-late 3rd
Dlg PTP61F
J
K
L
mid-late 3rd
Dlg ZFH-1
M
N
O
mid-late 3rd

Figure S4 Expression of previously reported targets in the optic lobe. Brains from late-third instar (A-F) or mid-late third instar larvae (G-O) cultured at 25^{o} were stained with the markers indicated. (A-C) The *10XSTAT92E-GFP* reporter is strongly expressed in the lamina, but virtually undetectable in the NEs (A); the reporter expression weakly increased in the NEs of JAK activated brains (B, indicated by arrow), but was not detectable in JAK inactivated brains which had no NEs (C). (D-F) At late-third instar, SOCS36E protein is strongly expressed in the NEs, medulla neuroblasts, LPCs, and some developing lamina neurons, as well as strongly expressed in neurons of the central brain (D). SOCS36E expression did not increase cell autonomously in the NEs of JAK activated brains (E, arrowheads indicate SOCS36E expression in the overgrown neuroepithelium), but was undetectable in JAK inactivated brains which essentially lost all NEs (F). (G-I) At mid-late third instar, SOCS36E protein is not expressed in the optic lobe, but is strongly expressed in neurons and neuroblasts of the central brain. (J-L) PTP61F is strongly expressed in the NEs, medulla neuroblasts, LPCs, and the lamina, and is also expressed in central brain neuroblasts (J); PTP61F expression did not appear to increase cell autonomously in the NEs and lamina cells of JAK activated brains (K), and was not reduced in the residual NEs of JAK inactivated brains (L). (M-O) Zfh-1 protein is expressed in the lamina (M); Zfh-1 expression did not increase in JAK activated brains (N), but was undetectable in JAK inactivated brains which had no lamina (O). Frontal view, lateral is to the left, medial to the right. Scale bar: 20μm.

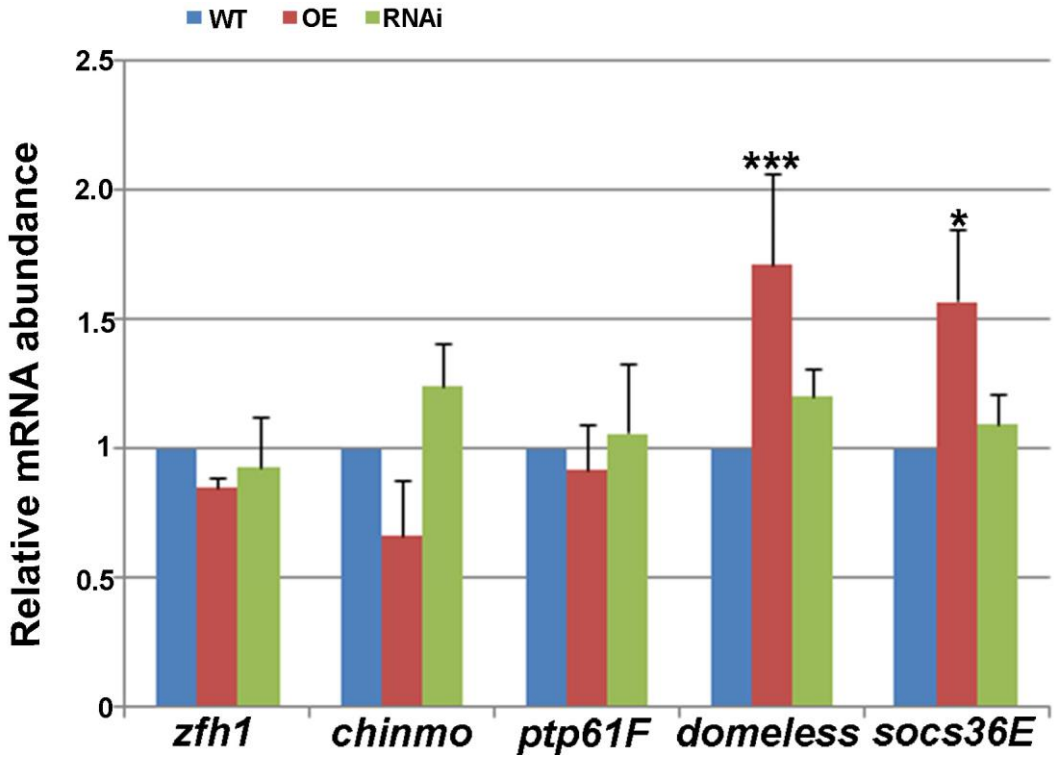

Figure S5 Relative expression levels of previously reported targets determined by quantitative PCR. Total RNAs were isolated from the CNS of late-third instar larvae cultured at 25°; reversed transcribed cDNAs were used as template in PCR using the SYBR Green PCR Master mix (Applied Biosystems). The signals were normalized to the internal reference, ribosome protein 49-encoding gene (*Rp49*). The PCR was run in triplicates per primer set, and repeated 4 independent times. *Zfh-1* and *PTP61F* RNA levels did not change in JAK activated or inactivated brains; *domeless* and *SOCS36E* RNA levels significantly increased in JAK activated brains, but did not decrease in JAK inactivated brains; *chinmo* expression was repressed by JAK signaling, although not statistically significant. WT: wild type; OE: *c768-Gal4/UAS-hop*$^{Tum\text{-}l}$; RNAi: *c768-Gal4/UAS-stat92E*^{RNAi}. ***: p <0.01; *: p<0.05.

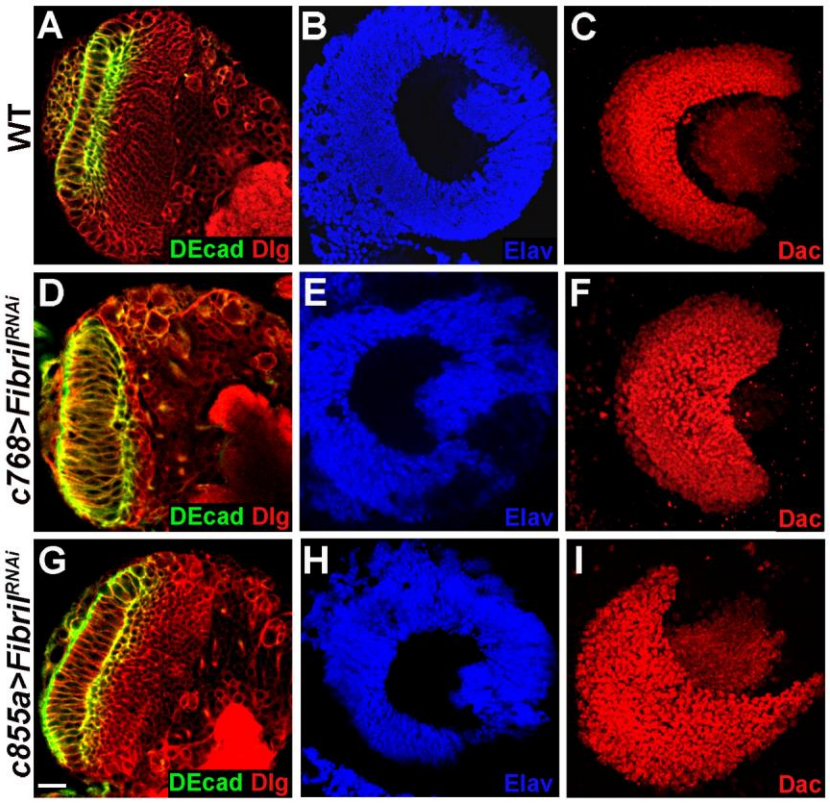

Figure S6 *Fibrillarin* RNAi causes defects in lamina and medulla development. Brains dissected from late-third instar larvae cultured at 31^{o} were stained with the markers indicated. (A-C) Wild type brains. (D-F) Expression of *Fibrillarin^{RNAi}* using *c768-Gal4* inhibited neuroepithelial proliferation, resulting in elongated NEs (D, 87%, n=32), a smaller medulla (E, 72%, n=29), and an enlarged lamina (F, 86%, n=29). (G-I) Expression of *Fibrillarin^{RNAi}* using *c855a-Gal4* weakly inhibited neuroepithelial proliferation, resulting in somewhat elongated NEs (G, 78%, n=27), slightly smaller medulla (H, 79%, n=21), and enlarged lamina (I, 95%, n=21). (A, D, G) Frontal view, lateral is to the left, medial to the right; (B, C, E, F, H, I) lateral view, anterior is to the left, dorsal is up. Scale bar: 20μm.

Tables S1-S2 are available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.155945/-/DC1

Table S1 Genes up or down regulated in *upd*-overexpressing brains

Table S2 Genes down or up regulated in hop^{M4} brains