Supplementary data

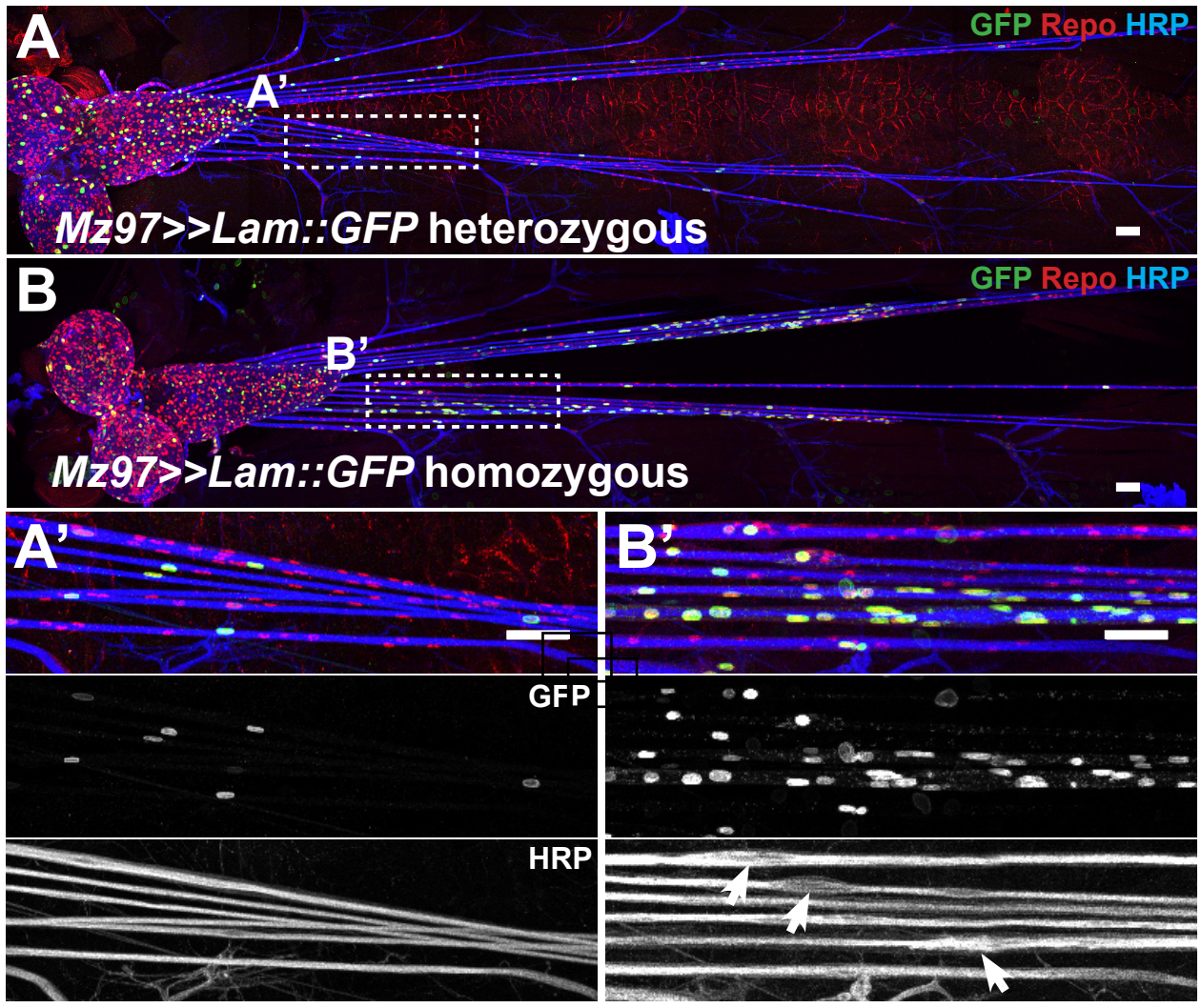


Figure S1 (related to Figure 1 and 2): **The *Mz97-Gal4* insertion affects the number of subperineurial glial nuclei**

(A,B) Confocal projections of third instar larval filet preparations stained as indicated. **(A)** The subperineurial glia-specific driver line *Mz97-Gal4* labels only few glial nuclei along the NER of abdominal nerves. The white dashed box is shown in higher magnification in **(A')**. **(B)** Homozygous *Mz97-Gal4* animal. The number of subperineurial glial nuclei increases. The white dashed box is shown in higher magnification in **(B')**. Note the axonal defasciculation in homozygous *Mz97-Gal4* animals (arrows). Scale bars are 50 μm.

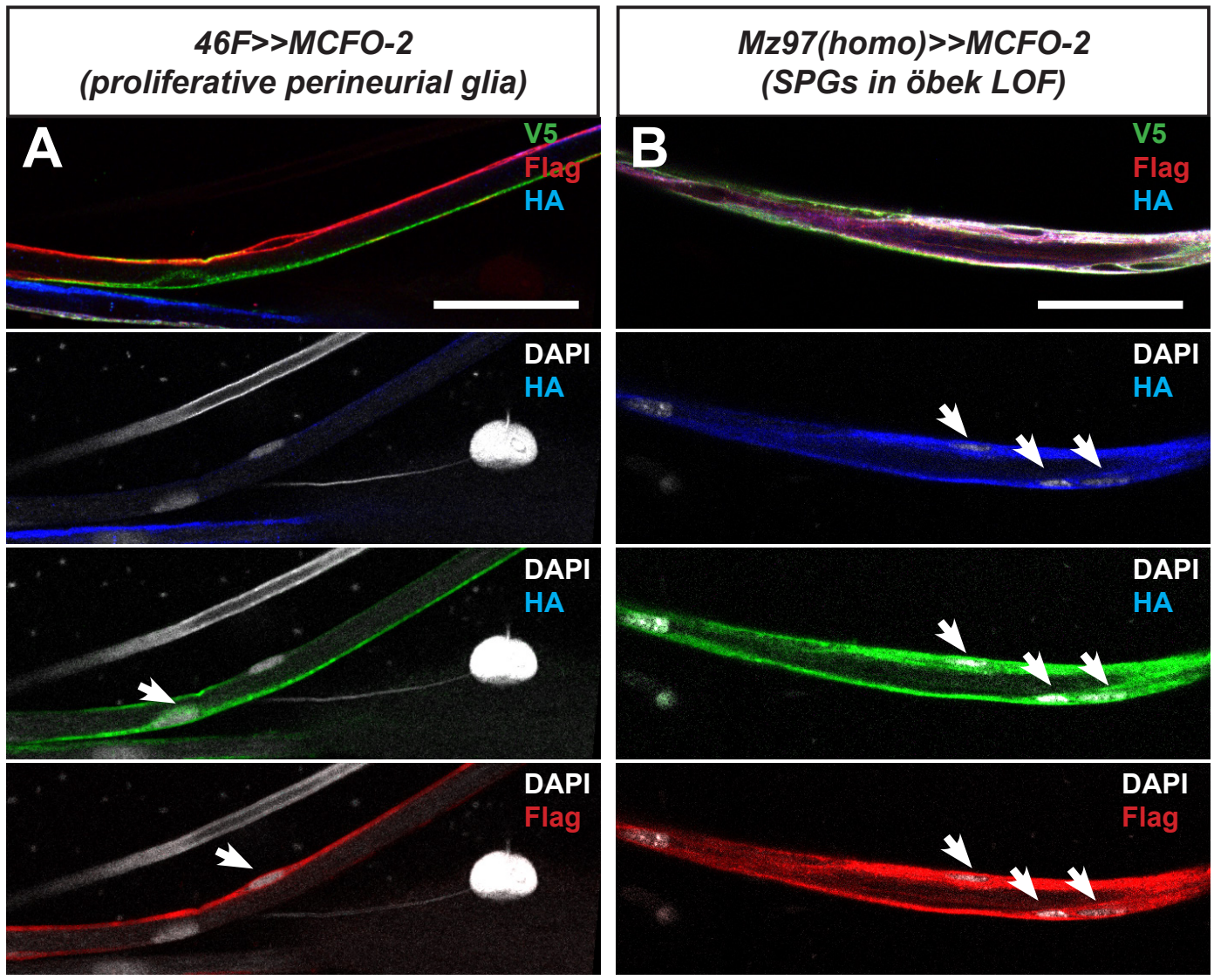


Figure S2 (related to Figure 2)**: Multicolor stochastic labeling of peripheral glial cells**

For multicolor stochastic label *MCFO-2* flies (Nern et al., 2015) **(B)** *46F-Gal4* or **(A)** *Mz97-Gal4*. A single confocal section is shown. **A)** Two distinctly colored perineurial glial cells are shown in a single confocal section through an abdominal nerve, containing each one nucleus (arrow). **(B)** A homozygous *Mz97-Gal4* animal was imaged representing an *öbek* mutant. An increased number of SPG nuclei (arrows) is found in a single SPG cell that is characterized by specific set of colors (n>20 animals). **(**Scale bar is 50 µm.

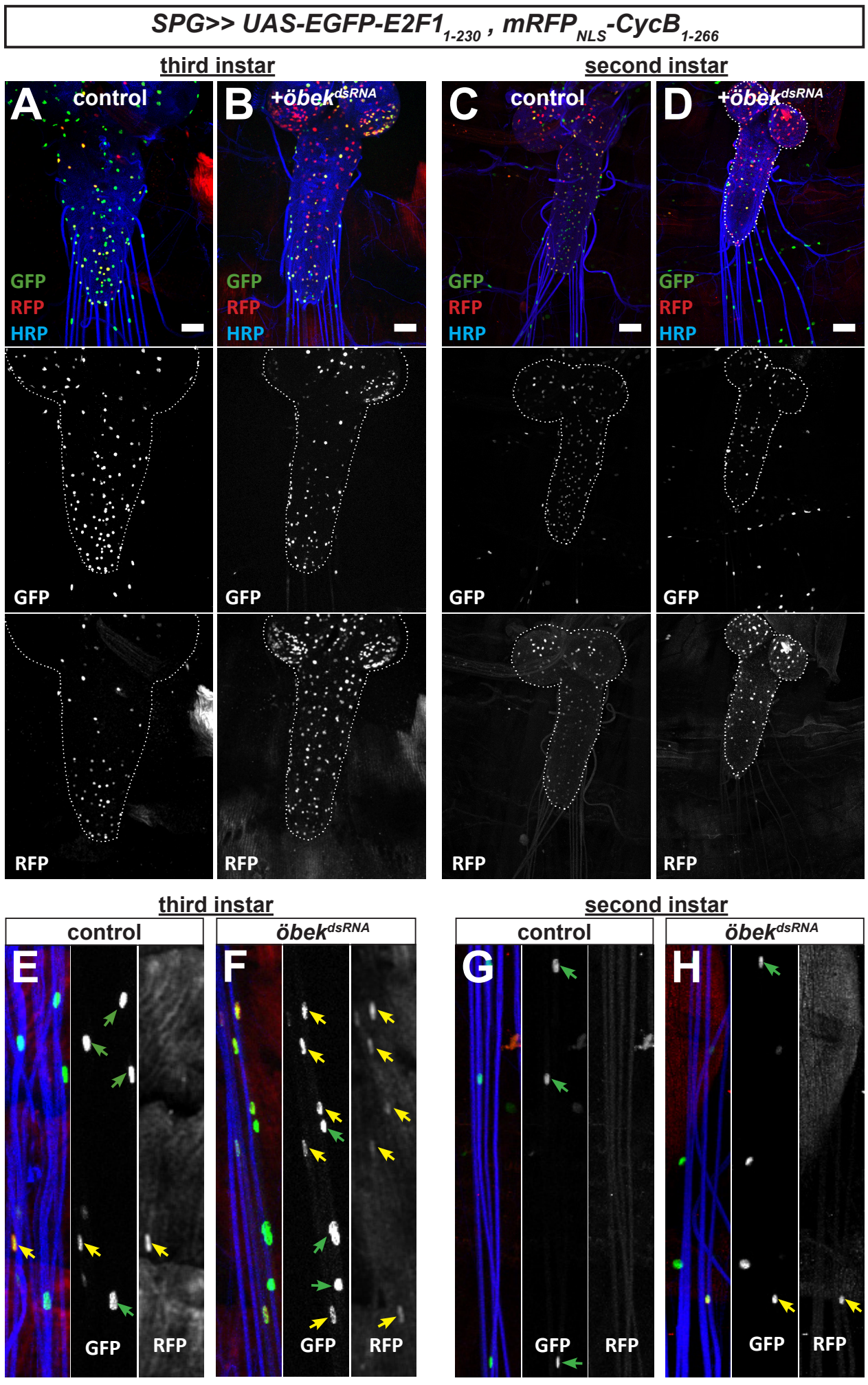


Figure S3 (related to Figure 2)**: *öbek* knockdown affects the cell cycle status of the subperineurial glia**

The Fly-FUCCI system (Zielke et al., 2014) was used to detect the cell cycle status of the subperineurial glial cells. Third instar and second instar larval brains / peripheral nerves are stained as indicated. Anterior is up. **(A-D)** Larval CNS preparations. **(E-H)** Images of abdominal nerves taken about 500 µm posterior to the ventral nerve cord. **(A,C)** Control animals. *UAS-EGFP-E2F1, mRFP-CycB* is expressed in subperineurial glial cells using the *SPG-Gal4* driver. Upon concomitant knockdown of *öbek* in the subperineurial glia, expression of RFP-CyclinB predominates, indicating that S-phase is initiated **(B,D)**. **(E-H)** In abdominal nerves the green arrows label the *mRFP-CycB* negative nuclei, whereas the yellow arrows label the *mRFP-CycB* positive nuclei. Note that upon suppression of Öbek the number of “yellow” nuclei increases (F,H). Scale bars are 50 µm.

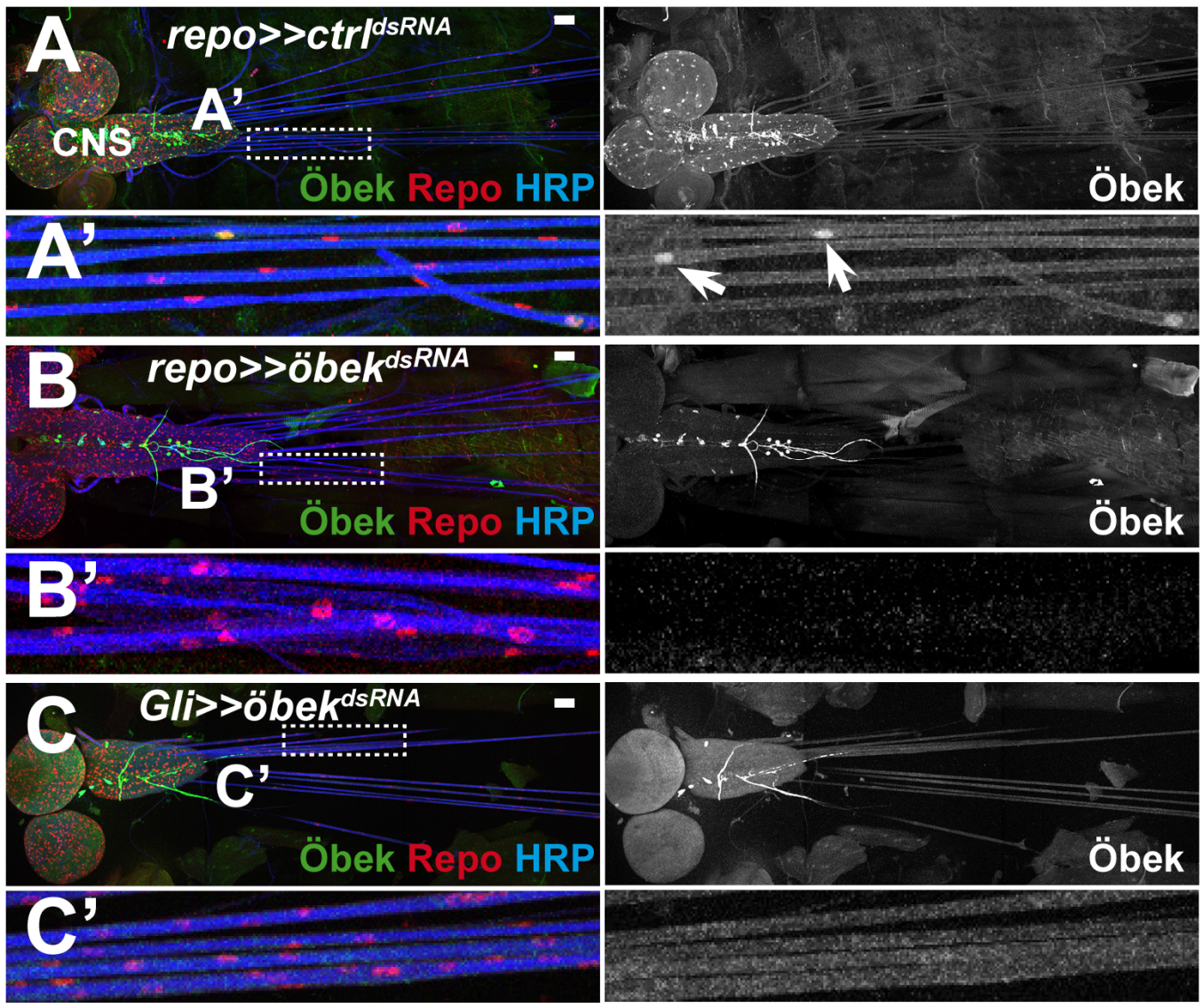


Figure S4 (related to Figure 4)**: Expression of Öbek in larval nerves**

(A-C) Confocal projections of third instar larval filet preparations, stained as indicated. The white dashed box indicates the enlarged area shown in A'-C'. **(A,A')** Control animal. Weak cytoplasmic Öbek expression is found along the nerves. In addition, some glial nuclei show strong nuclear Öbek expression (arrows). **(B,B')** Upon *repo-Gal4* mediated *öbek* knockdown, no specific Öbek expression can be detected along the peripheral nerves. **(C,C')** Upon *Gli-Gal4* mediated *öbek* knockdown only in the subperineurial glia, no nuclear expression is detected but the weak cytoplasmic Öbek expression remains. Scale bars are 50 µm.

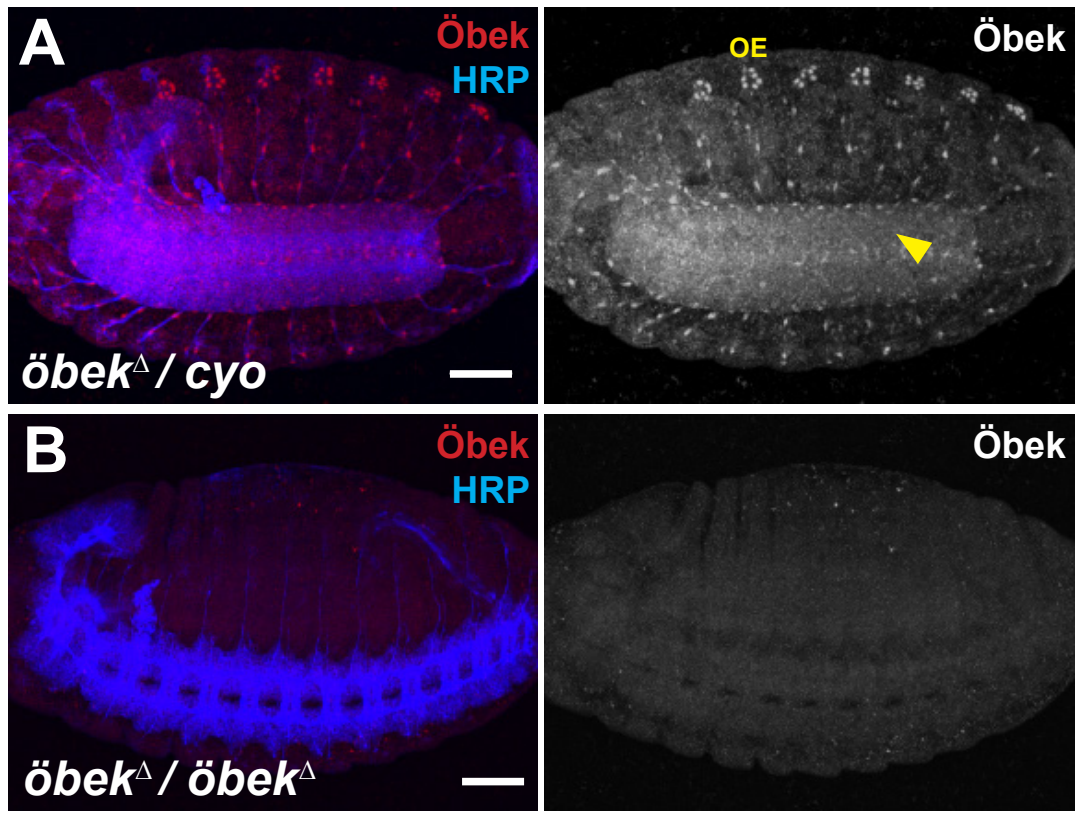
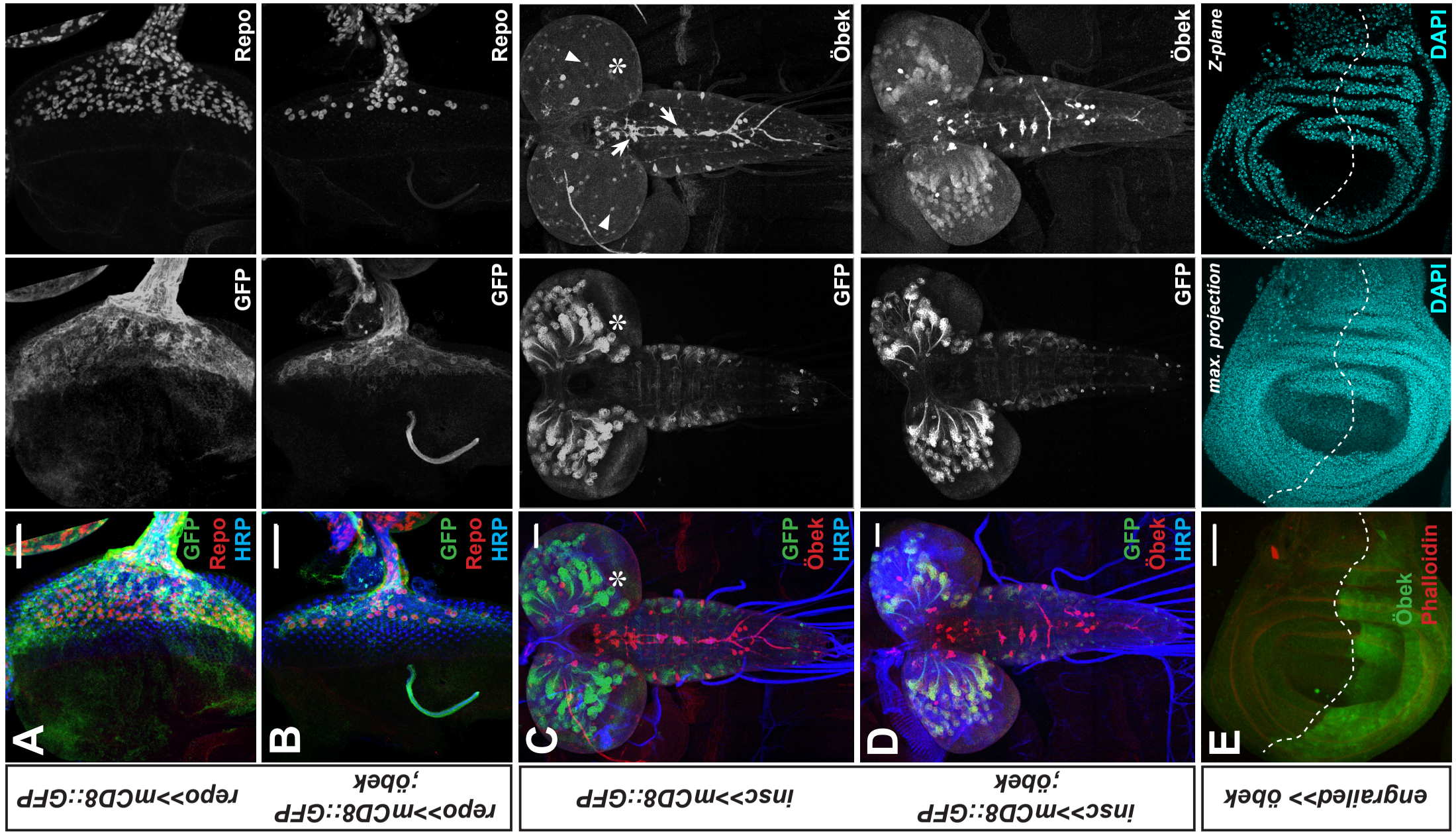


Figure S5 (related to Figure 4)**: Öbek null mutants are embryonic lethal**

Confocal projections of late stage embryos. Embryos are stained for Öbek protein expression as indicated. **(A)** Embryos heterozygous for the deficiency allele *öbek*$^{\Delta}$ show strong Öbek expression in the nuclei of some glial cells and the oenocytes (OE). Weaker cytoplasmic staining is detected throughout the embryo, including the ventral nerve cord (arrowhead). **(B)** Embryos homozygous for the deficiency allele *öbek*$^{\Delta}$. No specific Öbek expression can be detected.



Development: doi:10.1242/dev.164111: Supplementary information

Figure S6 (related to Figure 4): **High Öbek levels block proliferation only in glial cells**

(A-B) Confocal projections of third instar eye imaginal discs, stained as indicated. **(A)** In control eye imaginal discs many glial nuclei are labeled by Repo expression. **(B)** Upon panglial overexpression of *öbek*, the number of Repo expressing nuclei is significantly reduced. **(C-D)** Confocal projections of third instar larval brains. **(C)** The progeny of neuroblasts expressing *insc-Gal4* are labeled with CD8::GFP (*insc>>mCD8::GFP*). Öbek protein is detected by specific antisera. Öbek expression is detected in few neurons and their axons, including the neurohemal organ (arrows), and some glial cells (arrowheads). In contrast, no Öbek expression is found in neuroblasts (asterisk). **(D)** Upon expression of Öbek using *insc-Gal4*, Öbek protein expression can be detected in neuroblasts. However, no difference in the size of the brain is observed suggesting that neuroblast proliferation is not impaired. **(E)** Confocal projection of third instar wing imaginal disc where ectopic Öbek expression is induced only in the posterior compartment using *en-Gal4*. Although strong cytoplasmic Öbek expression can be detected in the posterior compartment, no differences in cell number between anterior and posterior compartments are found using DAPI labeling.

Scale bars are 50 μm.

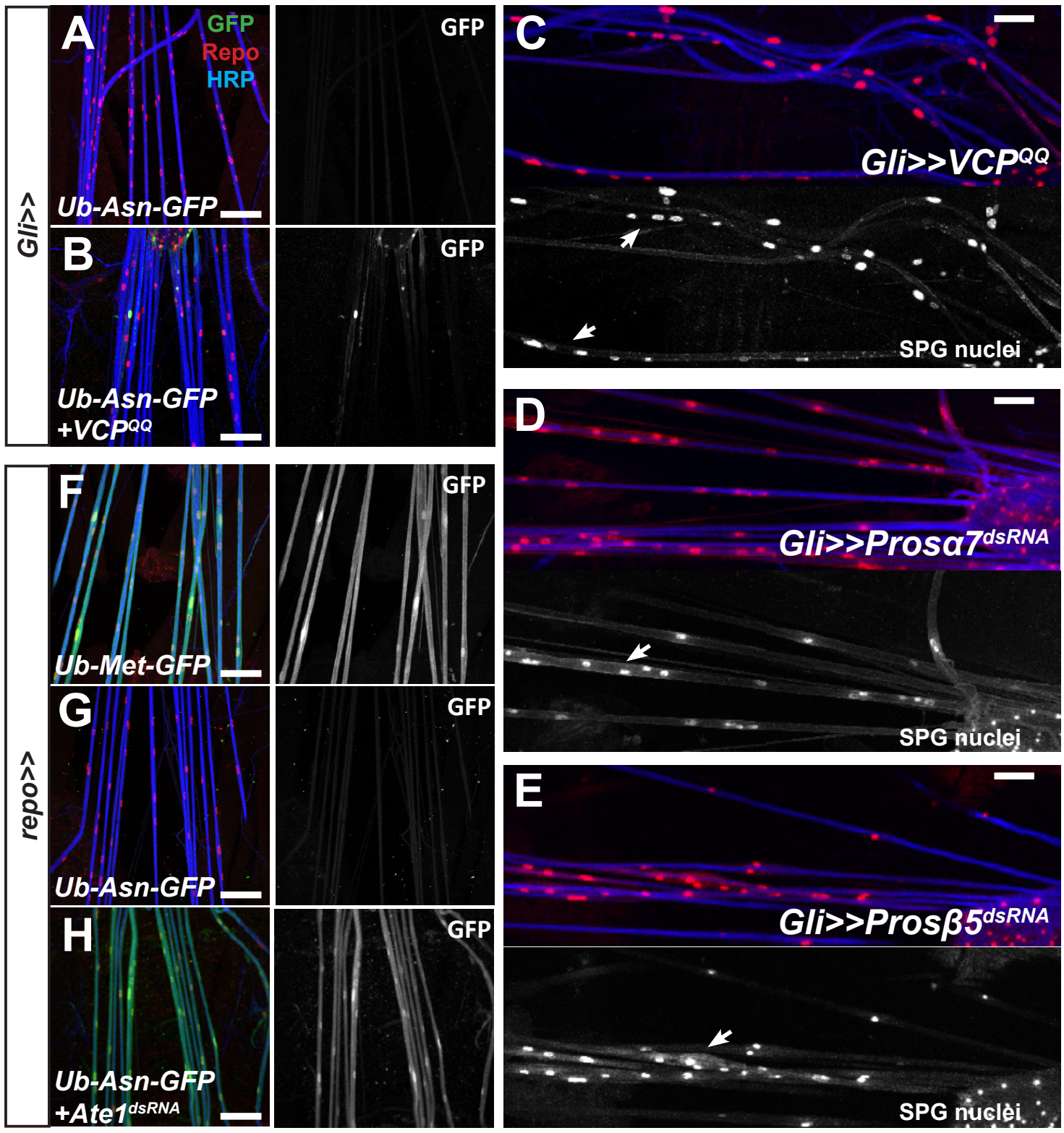
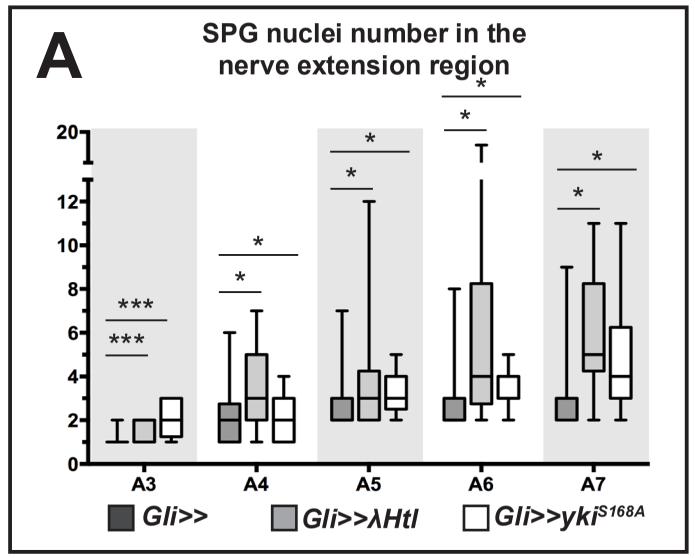
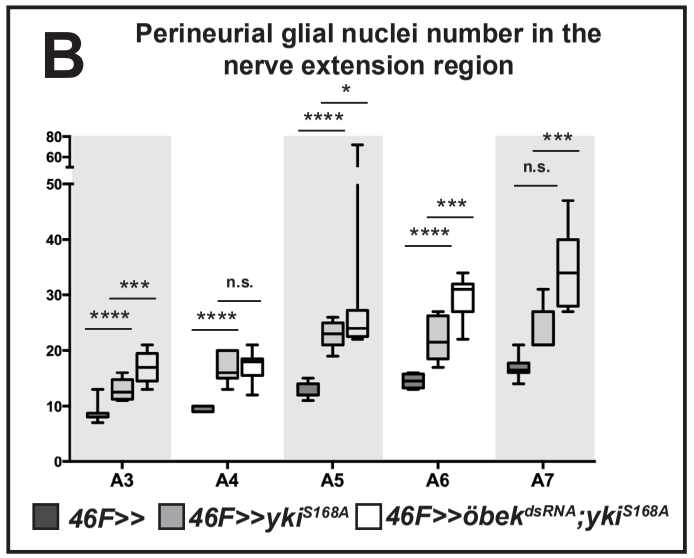
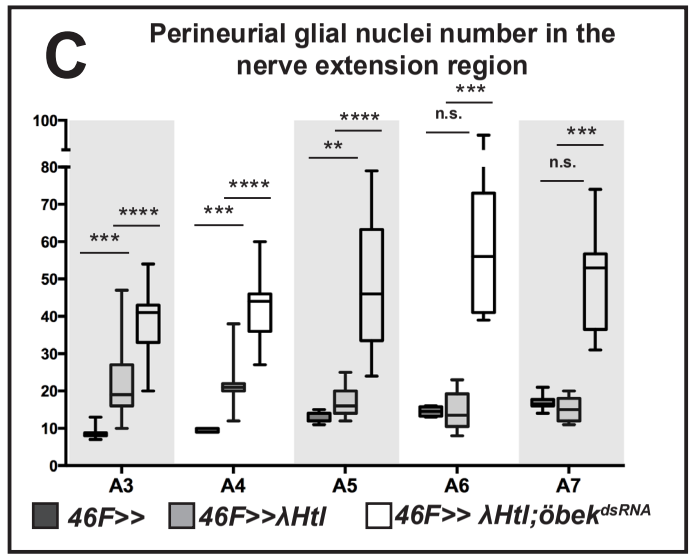


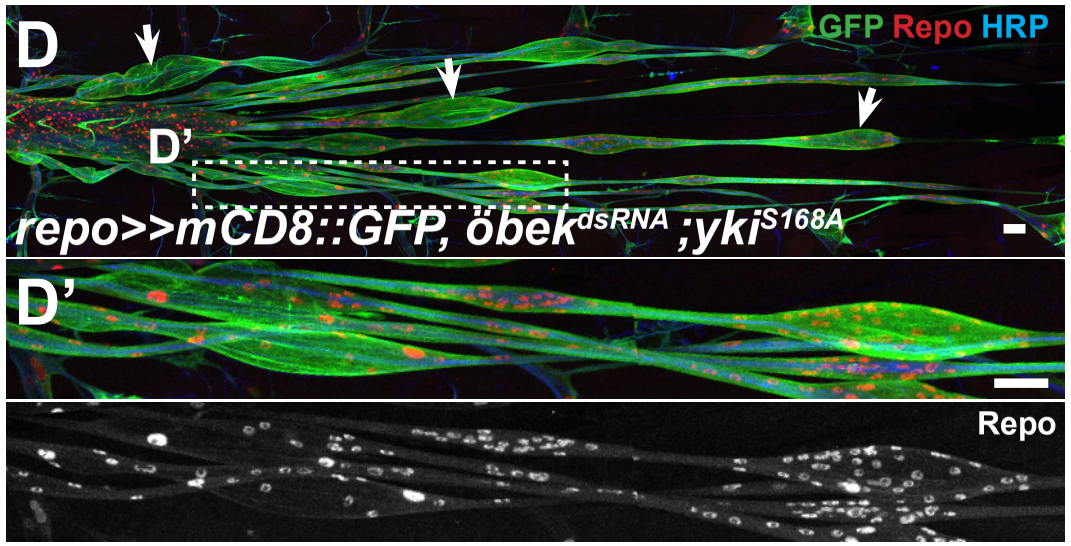
Figure S7 (related to Figure 5)**: *Ate1* knockdown stabilizes the Asn-GFP reporter**

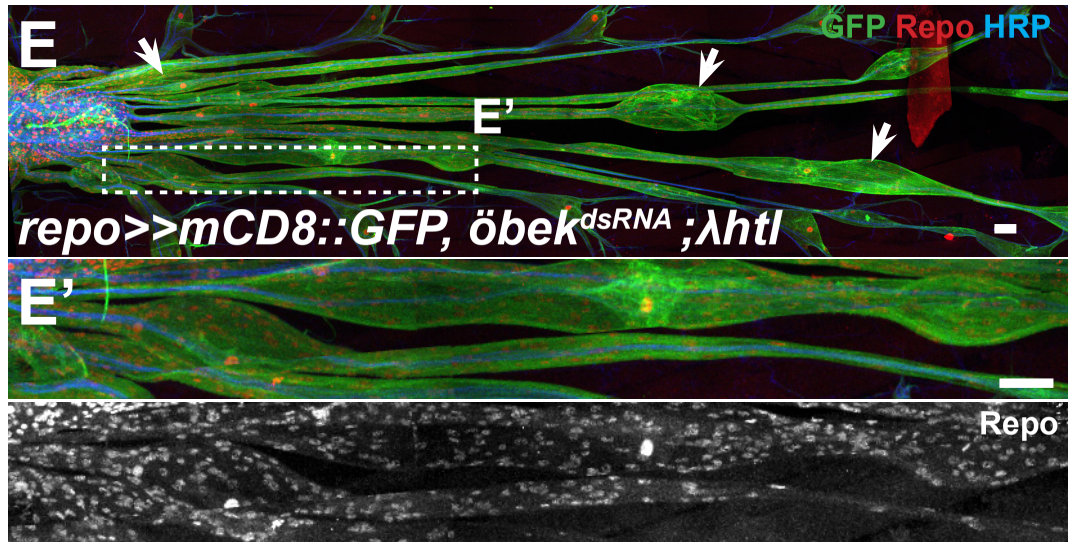
Confocal projections of third instar larval filet preparations stained as indicated. *UAS-cherry*^{dsRNA} was used as control to obtain equal Gal4/UAS ratios. The images are taken about 100 µm posterior to the ventral nerve cord, anterior is up. **(A-C)** Expression of a dominant-negative form of valosin-containing protein (VCP^{QQ}) only in the subperineurial glia leads to stabilization of Ub-Asn-GFP **(B)** and also results in additional SPG nuclei **(C)** (arrows indicate additional subperineurial glial nuclei) **(D,E)** RNAi-mediated silencing of proteasomal subunits *Prosα7* or *Prosß5* only in the subperineurial glia using *Gli-Gal4* led to occasional nerve bulges with an increased number of subperineurial glial nuclei (arrows). **(F)** Panglial expression of Ub-Met-GFP generates stable GFP expression (control). **(G)** Ub-Asn-GFP expression generates an unstable GFP and almost no fluorescence is detected. **(H)** Suppression of arginyl-transferase (*Ate1*) expression stabilizes the Ub-Asn-GFP reporter. Scale bars are 50 µm.











Development: doi:10.1242/dev.164111: Supplementary information

Figure S8: (related to Figure 6): **Öbek counteracts Yorkie and FGF-signaling to regulate glial growth**

Quantification of glial nuclei number along the abdominal nerves A3 - A7 in control animals and larvae expressing activated Htl or Yorkie in **(A)** the SPGs using Gli-Gal4 or the perineurial glial cells **(B,C)**. The effects of concomitant öbek knockdown are indicated **(B,C)**. **(D,E)** Confocal projections of third instar larval filet preparations stained as indicated. Anterior is to the left. The area in white dashed box **(D,E)** is shown in higher magnification **(D',E')**. **(D,D')** Panglial expression of *yki*^{S168A} concomitant to a RNAi-based panglial knockdown of *öbek*. Many glial nuclei and a prominent bulging phenotype can be detected on all nerves (arrows). (**E,E'**) Panglial expression of *λhtl* concomitant to a RNAi-based panglial knockdown of *öbek*. A very large number of glial nuclei and a prominent bulging phenotype can be detected on all nerves (arrows). , *** p≤ 0.001, ** p≤ 0.01, * p≤ 0.05; n≥7 Scale bar is 50 µm.

Table S1 (related to Figure 5)**: C-value quantifications in SPGs per NER**

Total C-values in SPGs in the nerve extension region of peripheral nerves calculated using DAPI labeling as a read-out for DNA-amount. One row does not correspond to nerves from one animal.

Total C-value of SPG nuclei per nerve extension region									
Gli>> (control)					***Gli>>öbek^{dsRNA}***				
A3	**A4**	**A5**	**A6**	**A7**	**A3**	**A4**	**A5**	**A6**	**A7**
21C	16C	16C	10C	16C	38C	40C	44C	62C	91C
10C	7C	14C	10C	16C	20C	32C	40C	28C	20C
4C	14C	37C	30C	21C	41C	106C	57C	119C	52C
14C	6C	19C	42C	65C	48C	78C	99C	62C	65C
7C	7C	14C	52C	17C	40C	29C	79C	42C	58C
5C	11C	64C	9C	26C	8C	41C	60C	33C	46C
9C	7C	16C	39C	12C	15C	14C	34C	13C	28C
12C	10C	12C	15C	4C	72C	218C	424C	111C	100C
10C	-	15C	13C	26C	18C	132C	130C	173C	164C
6C	-	65C	49C	12C	123C	200C	53C	41C	56C
9C	-	27C	36C	21C	41C	181C	32C	38C	75C
-	-	-	15C	-	-	-	-	-	-
-	-	-	27C	-	-	-	-	-	-