

### **Supplementary Table 1**

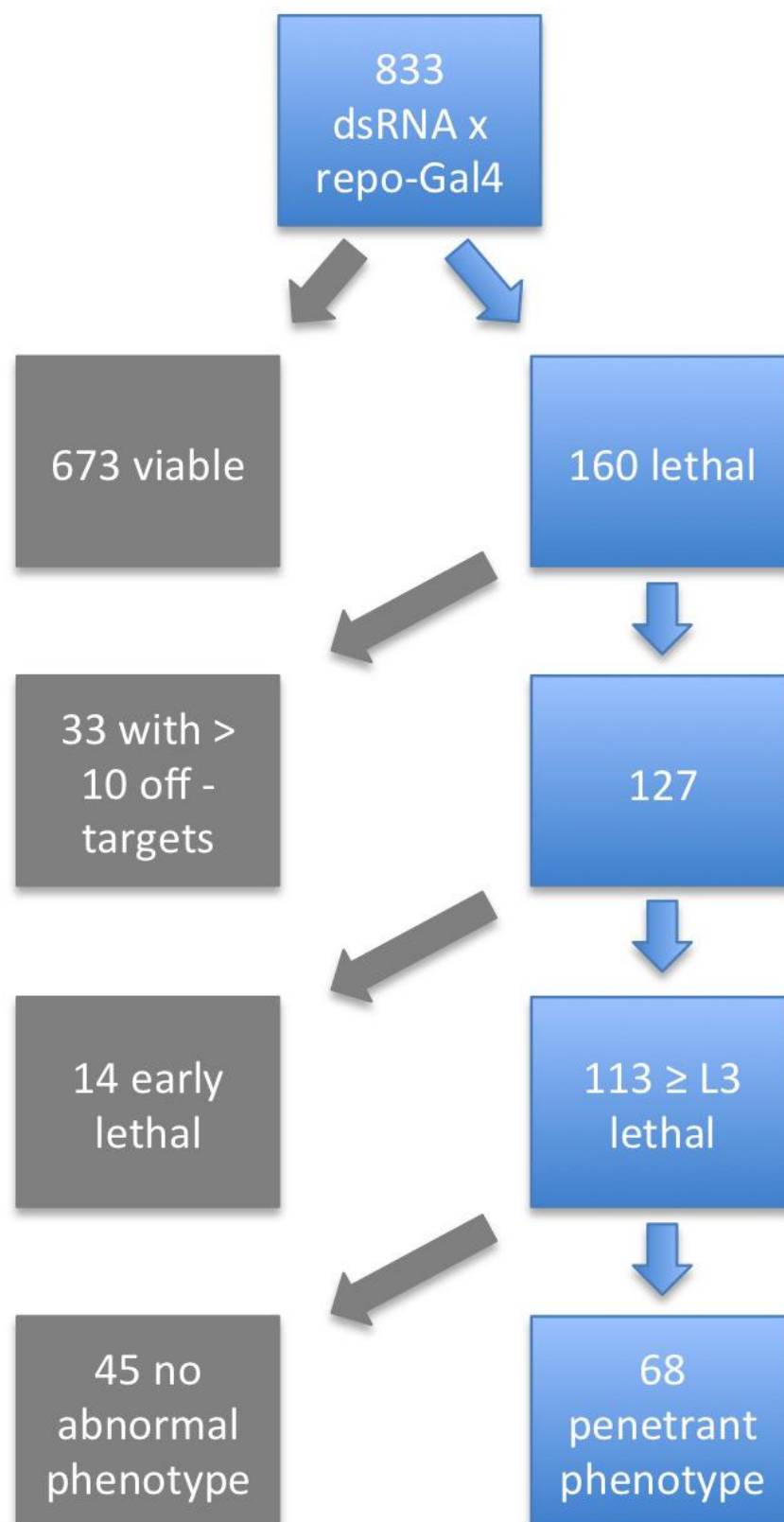
A list of all transgenes screened in this study. ID: Transformant identification number of the respective line; Phenotype upon pan-glial expression, let: lethal; sl, semi lethal; v: viable.

[Click here to Download Table S1](#)

### **Supplementary Table 2**

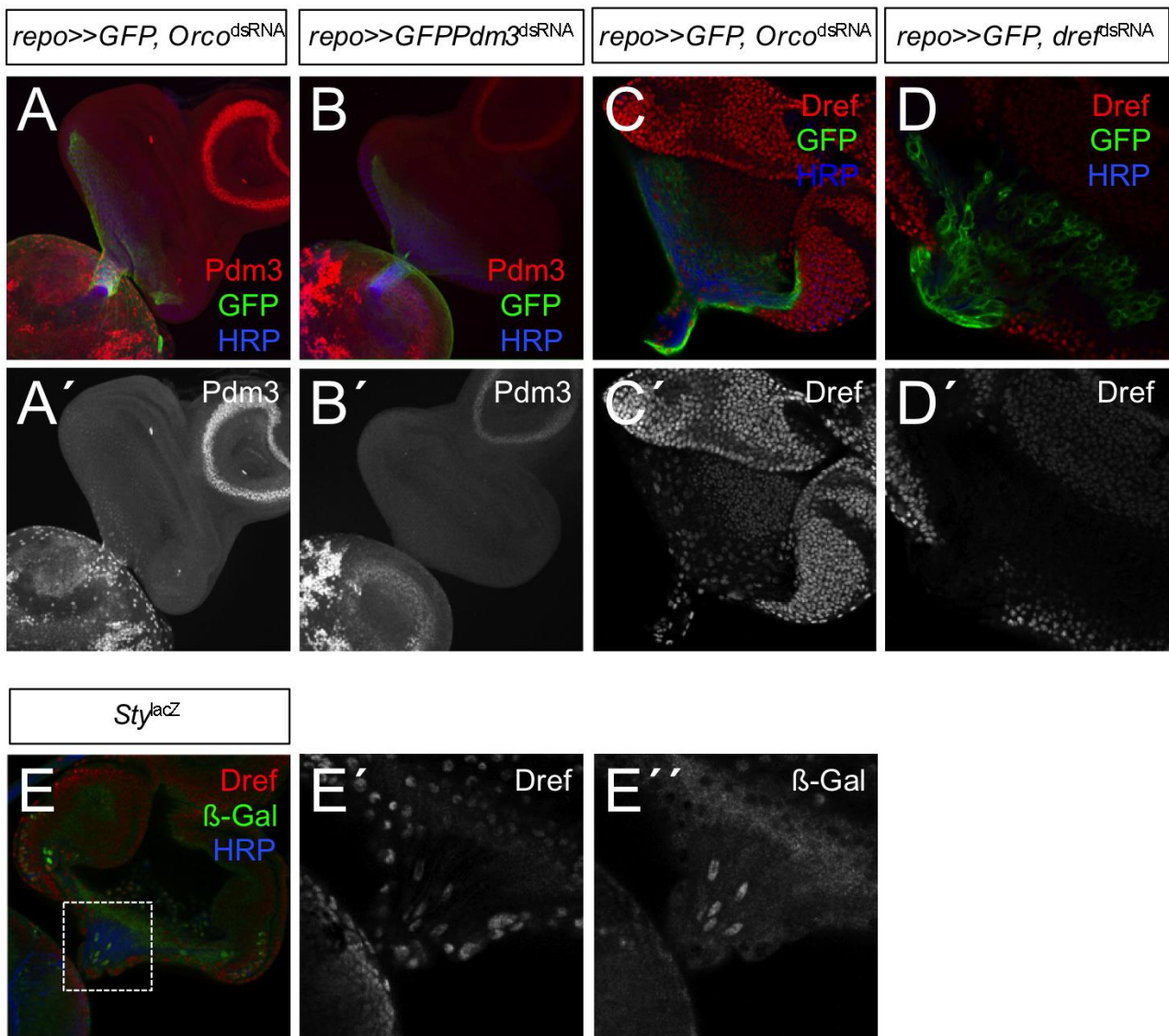
List of all transgenes with low numbers of predicted off targets that resulted in a late lethal phenotype when expressed pan-glially. The transformant identification number (ID), the CG number, the common name and the number of predicted off targets (OT) is given. For all genotypes, confocal analysis of third instar larval eye imaginal disc was performed. The phenotypes were classified according to the following criteria. Cell number (Cell No.): The number of eye disc glial cells in wild type is about 300 (wt). Reduction of glial cell number down to 30 cells was considered as fewer cells (fewer). Below 30 glial cells per eye imaginal disc is indicated in the adjacent column (< 30). Cell morphology defects were noted in perineurial glial cells (PG) or wrapping glial cells (WG). For some lines only very subtle morphological defects were found (\*, asterisk). Most glial cells on the eye disc maintain cell contact to their neighbors (wt). In some cases we noted the formation of single glial cells lacking cell contacts to other glial cells (single).

[Click here to Download Table S2](#)



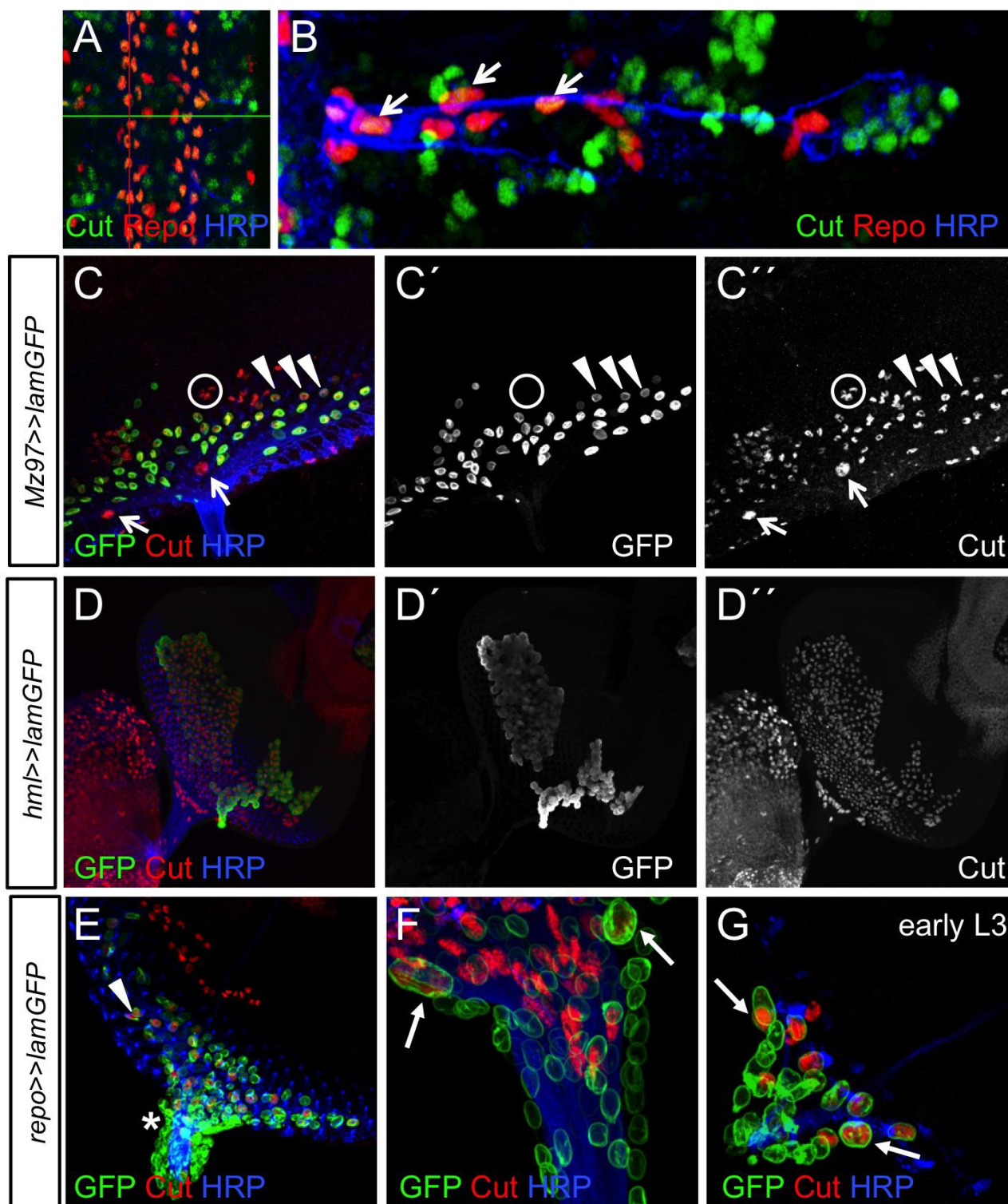
**Supplementary Figure 1: Flow diagram of the RNAi screen**

The numbers of lines screened and the numbers obtained for the indicated phenotypes are given. For details see text.



### Supplementary Figure 2: Glial expression of Pdm3 and Dref

Eye imaginal discs were stained as indicated. **A** Control eye imaginal disc expressing dsRNA directed against the *Orco* gene in all glial cells. **A'** Weak Pdm3 expression is noted in the perineurial glia. **B,B'** Upon suppression of *Pdm3* expression no glial Pdm3 can be noted. **C** Control eye imaginal disc expressing dsRNA directed against the *Orco* gene in all glial cells. **C'** Dref expression is noted in most glia. **D,D'** Upon suppression of *Dref* expression no glial Dref protein can be noted. **E** Dref is expressed in wrapping glia which are marked by  $\beta$ -Galactosidase expression driven by the *Sty<sup>lacZ</sup>* enhancer trap insertion. **E'** Dref expression. **E''**  $\beta$ -Galactosidase expression in the *Sty* expression domain.

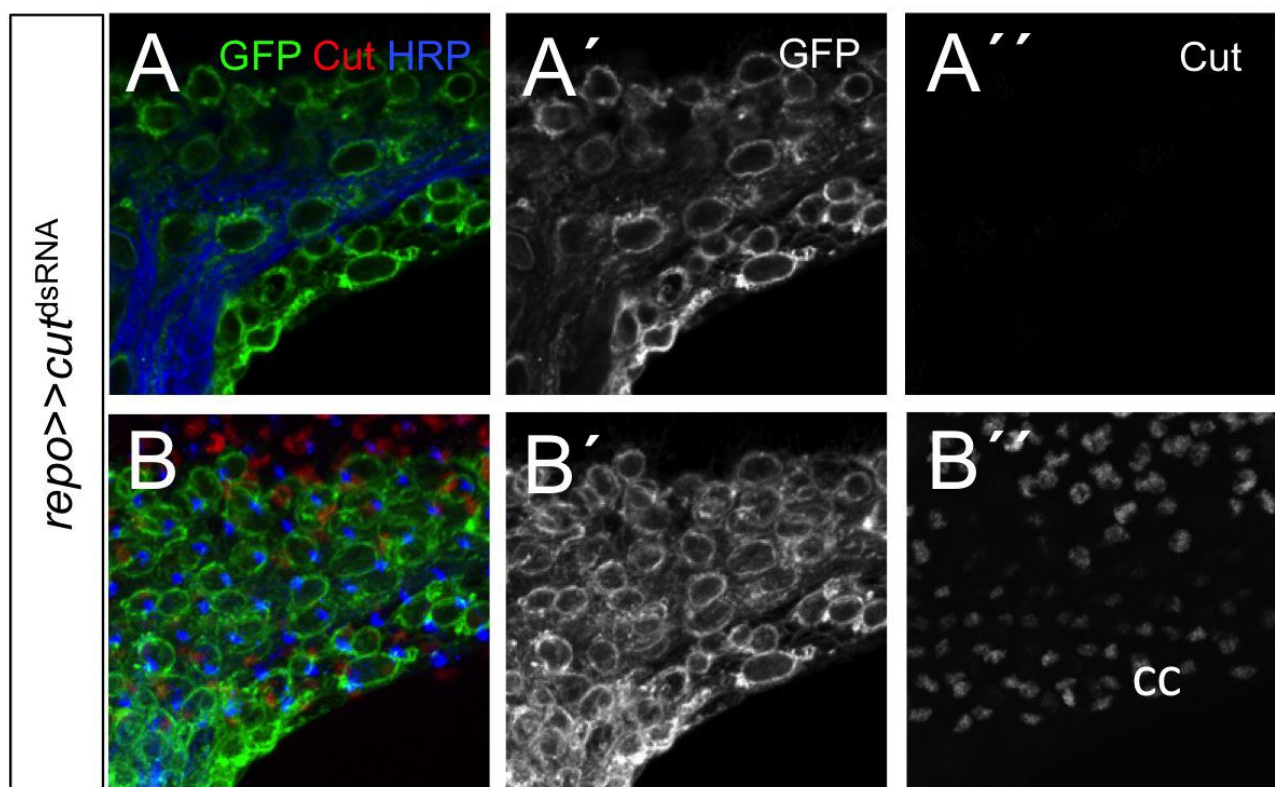


### Supplementary Figure 3: *cut* is expressed in glial cells throughout development

**A,B** Wild type embryos were stained for Cut (green), glial cell nuclei are labeled with anti-Repo (red) and neuronal membranes are labeled with HRP (blue). Anterior is up. **A** During

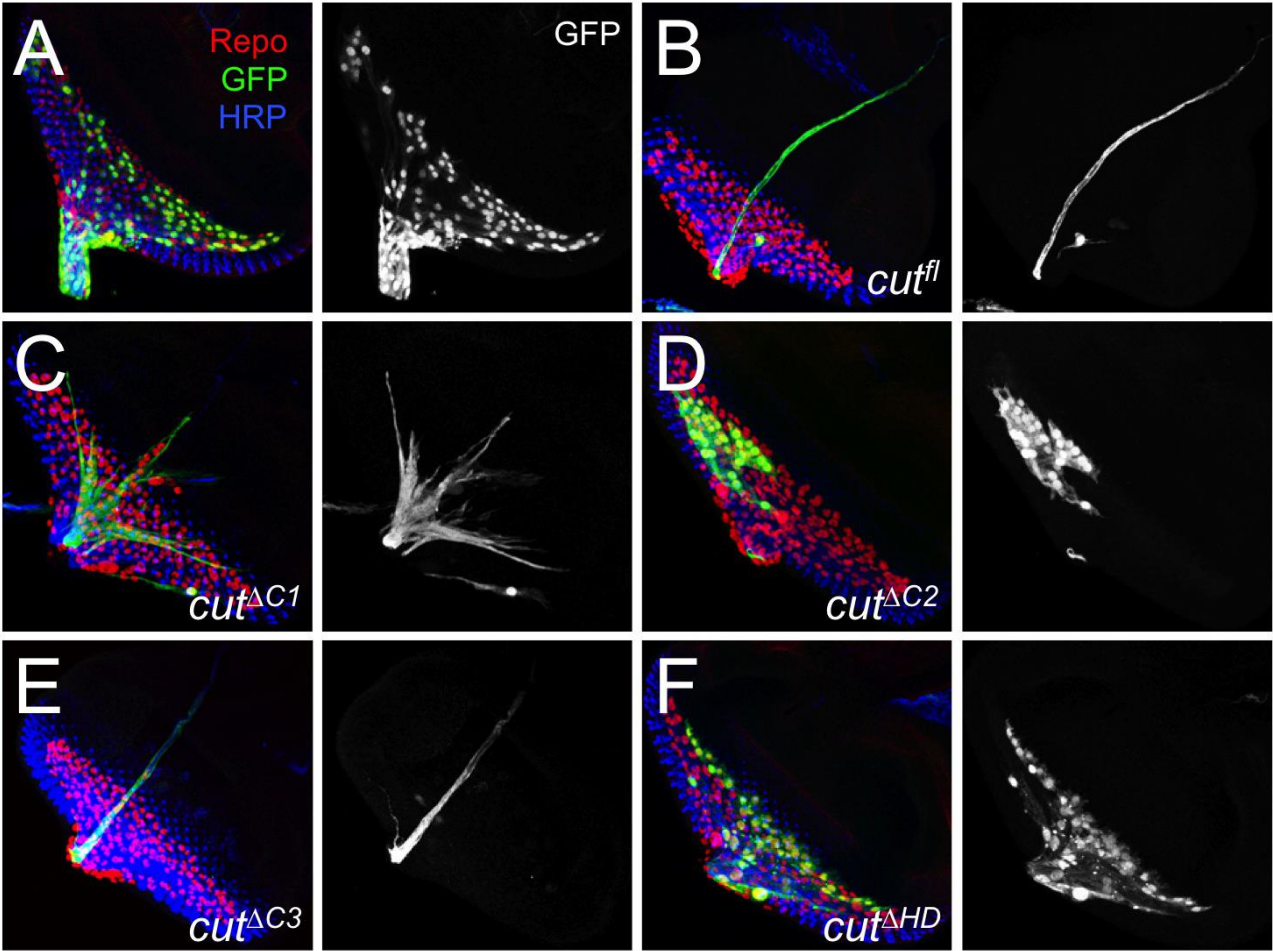
stage 14 *cut* is expressed by the longitudinal glia within the CNS. These cells correspond to the astrocyte and the ensheathing glia of the third instar larval brain. **B** In the PNS, *Cut* is expressed in the peripheral glial cells ePG1, ePG5 and ePG9 (arrows). **C** *Cut* (red) is expressed in the wrapping glia of the eye imaginal disc labeled by *Mz97>>lamGFP* expression (green) (arrowheads). In addition, *Cut* is found in the carpet glial cells (arrows) and in hemocytes attached to the eye disc (white circle). **D** GFP expression directed by hemocyte specific Gal4 driver *hml-Gal4* overlaps with *Cut* expression. **E-G** Glial cell nuclei express GFP (green, *repo>>lamGFP*). **E** *Cut* expression is absent in perineurial glia around the stalk (asterisk), the arrowhead denotes *Cut* expression in the wrapping glia. **F** *Cut* is expressed in both carpet glial cell nuclei (arrows). **G** Already in early third instar eye discs, *Cut* is expressed in the carpet glia (arrows).





#### Supplementary Figure 4: Efficiency of the *cut* knockdown

**A, B** Single sections of confocal stacks of third instar eye discs of the genotype *repo-Gal4, UAS-CD8GFP UAS-cut<sup>dsRNA</sup>*. Glial cell membranes are labeled in green, neuronal membranes in blue and Cut expression is shown in red. Glial expression of *cut<sup>dsRNA</sup>* removes Cut expression from glial cells (A-A''). In the same eye disc, Cut expression is detectable in the cone cells (cc, B-B'').



**G**

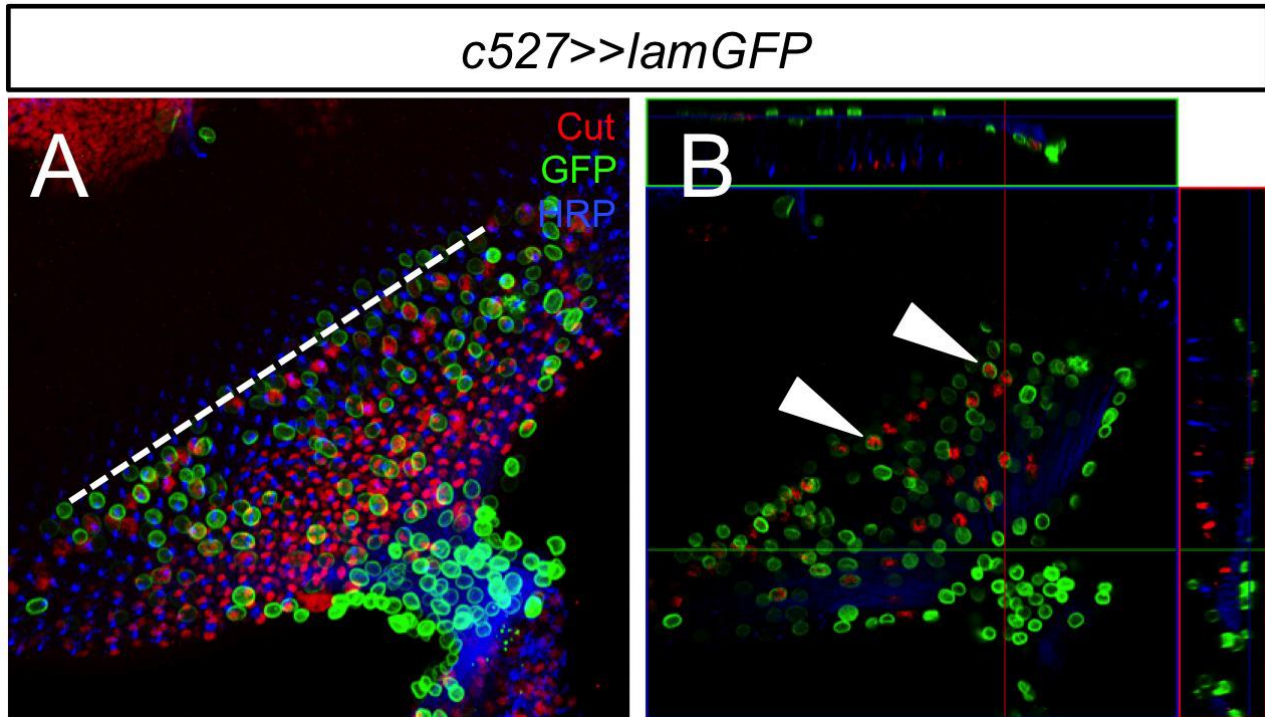
	Expression in	
	all glial cells viability	single glial cells morphology
<i>Cut<sup>fl</sup></i>	lethal	spikes
<i>Cut<sup>ΔHD</sup></i>	viable	wild type
<i>Cut<sup>ΔC1</sup></i>	escaper	spikes
<i>Cut<sup>ΔC2</sup></i>	lethal	wild type
<i>Cut<sup>ΔC3</sup></i>	lethal	spikes
<i>Cut<sup>ΔCterm</sup></i>	viable	wild type

**Supplementary Figure 5: Structure function analysis for Cut**

The flip out system was used to express Cut deletion constructs (see Figure 5A). *repoflp6-*

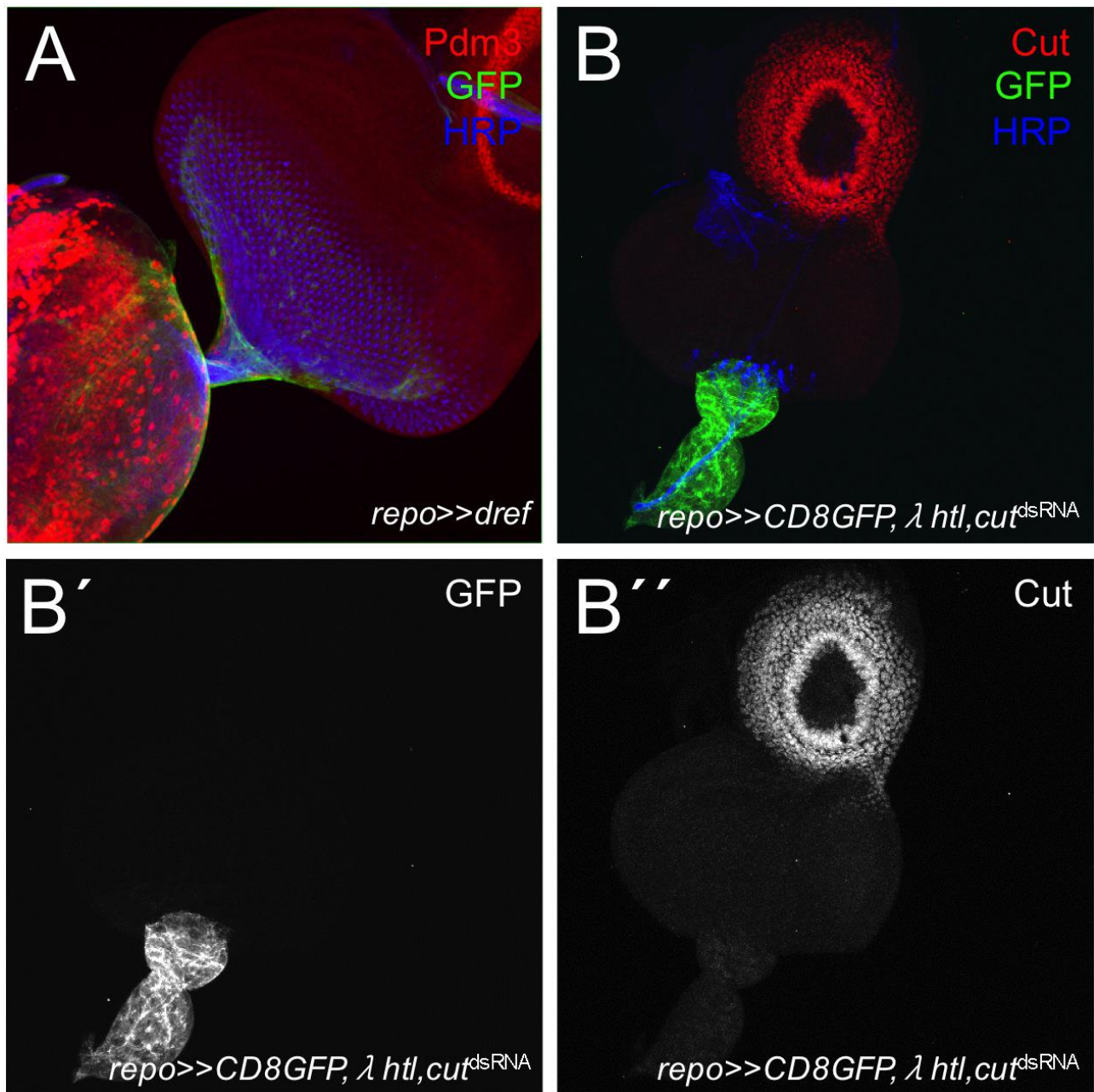
2, *tub>64>Gal4*, *UAS-Cut*, *UAS-GFP*. Flip out clones were identified by expression of GFP (green). Glial nuclei are shown in red and neuronal membranes are shown in blue. **A** Wild type clone. **B** Glial cells expressing full length *Cut<sup>fl</sup>* form long protrusions at the apical side of the eye disc. **C** Glial cells expressing *cut<sup>ΔC1</sup>* show intense ramifications at the basal side of the eye disc. **D** Glial cell clones expressing *cut<sup>ΔC2</sup>* are comparable to the wild type control. **E** Glial cells expressing *cut<sup>ΔC3</sup>* form long protrusions. **F** Glial cell clones expressing *cut<sup>ΔHD</sup>* are comparable to wild type. **G** Summary of the mis-expression data.





**Supplementary Figure 6: Cut expression in glial cells starts at the morphogenetic furrow**

**A** Projection of a confocal stack of a third instar eye disc. Perineurial glial cell nuclei are labeled in green (*c527>>lamGFP*) and neuronal membranes in blue (HRP staining). Cut expression is detected using anti-Cut antibody (red). Cut expression in glial cells starts close to the morphogenetic furrow (indicated by the dashed line). **B** Orthogonal section. Single glial cells close to the morphogenetic furrow express Cut (arrowheads).



**Supplementary Figure 7: Dref expression does not induce Pdm3 expression and knockdown of Cut is not able to suppress the activated FGF-receptor**

**A** Pan-glial overexpression of Dref does not induce the expression of Pdm3. **B** Eye imaginal discs expressing activated FGF-receptor develop tumor-like proliferation phenotypes also when *cut* expression is suppressed by RNAi. **B'** GFP expression showing the enlarged optic stalk. **B''** No Cut expression is seen. Note that the eye disc is rather young and almost no neuronal differentiation has yet occurred (blue staining in B).