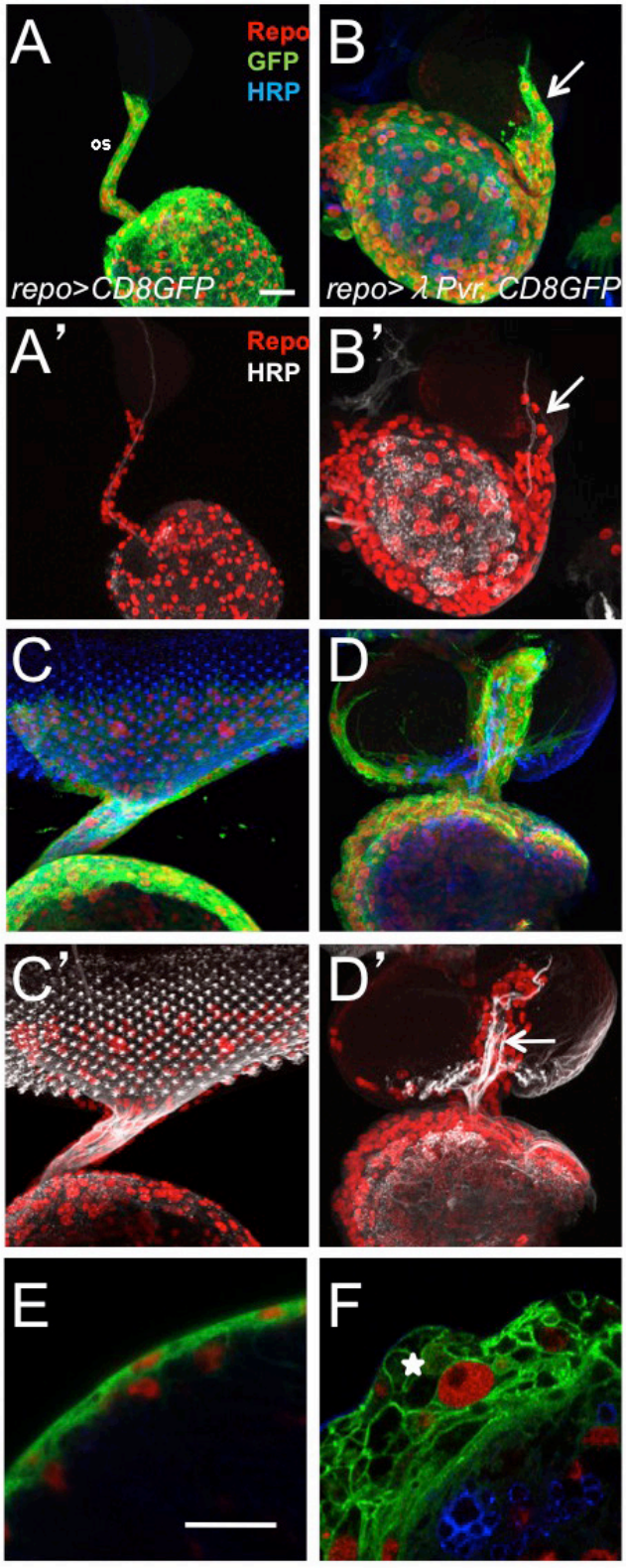
Supplementary Methods

Quantitative RT-PCR (qRT-PCR)

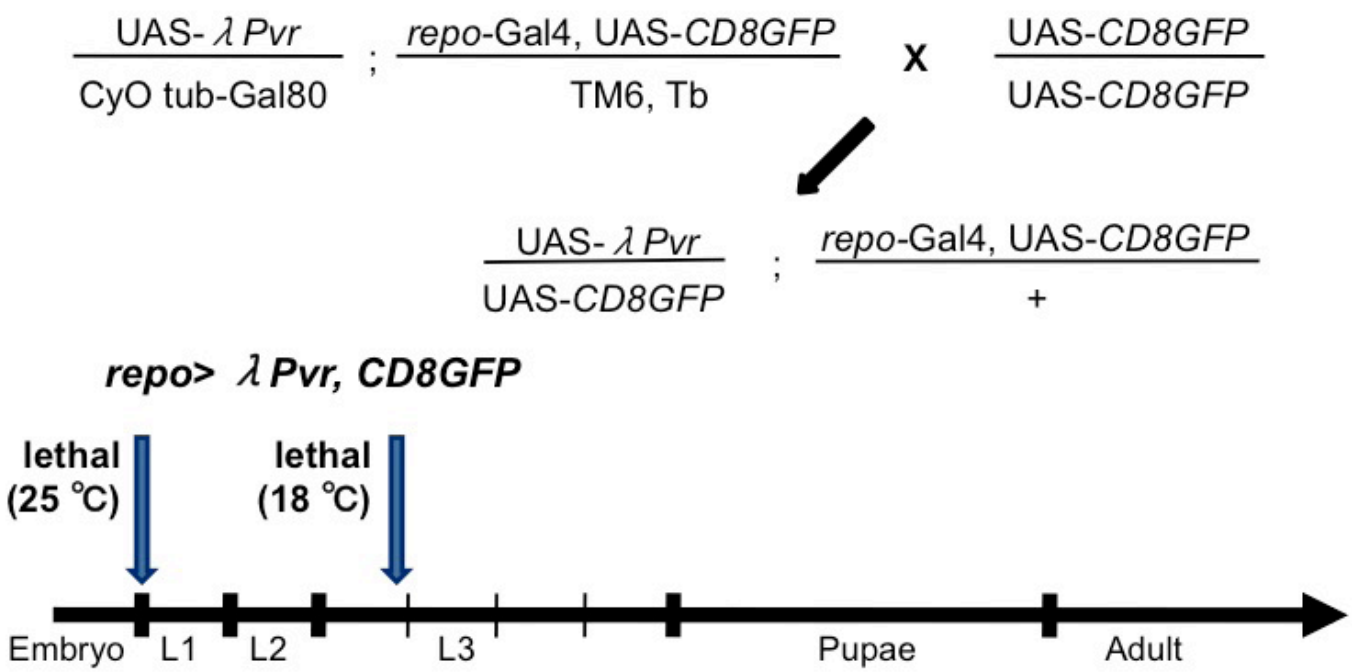
For the quantitative RT-PCR we used the following primers:
reaction *lox* (5' GTCTGCGCAGCCCACGGAAA; 3' TACCGGAAGGTGGCCCAGGG), *lox2* (5' CATTCACATGGCAGATGCGG; 3' GAATCCCACTCGTCATCGCA), *myospheroid* (5' TGTCGAGCGGATAGCACATC; 3' ACTCGCATGTACCGTGATCG), *Actin5c* (5' GAGCGCGGTTACTCTTTCAC; 3' ACTTCTCCAACGAGGAGCTG), *RpL32* (5' GTTCGATCCGTAACCGATGT; 3' CCAGTCGGATCGATATGCTAA), *Gapdh1* (5' CGGACGGTAAGATCCACAAC; 3' CCGCCCAGAACATCATCC), *elav* (5' GCGCACAAACCTTATTGTCA; 3' CGGATTGAGAGGATCGATGT), *EF1*, *Gapdh2*, *alphaTub84B*, *RpL13A*, *Ef1a48D* and *Hsp70* for SYBR green-monoplex reaction, and *lox* (Dm02151941_g1; FAM-labeled), *Act5c* (Dm02361909_s1; VIC-labeled) and *RpL32* (Dm02151827_g1; VIC-labeled) for TaqMan Gene Expression Assays for duplex reaction (Applied Biosystems).
Human materials were examined with TaqMan Gene Expression Assays (Applied Biosystems) LOX (Hs00942480_m1), LOXL1 (Hs00173746_m1) and LOXL4 (Hs00260059_m1) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression using primers GAPDH-fwd 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-rev 5'-CATACCAGGAAATGAGCTTGACAA-3', and GAPDH-probe 5'-CTGGCATTGCCCTCAACGACCA-3'. Rat materials were analyzed using LOX (Rn00566984_m1), LOXL1 (Rn01418038_m1) and LOXL4 (Rn01410863_m1) and normalized to GAPDH expression using primers GAPDH-fwd 5'-CAAGAAGGTGGTGAAGCAG-3', GAPDH-rev 5'-CAAAGGTGGAAGAATGGGAG -3' and GAPDH-probe 5'-ACTAAAGGGCATCCTGGGCTACACTGAGGAC-3'. All experiments were performed in triplicate.

Immunohistochemistry

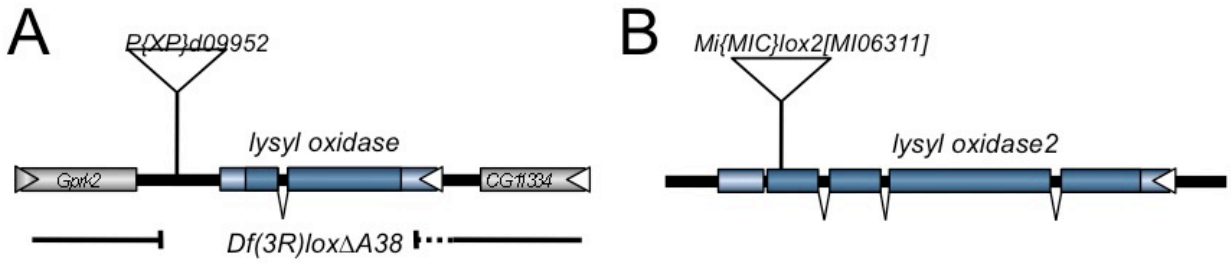
Generally, fly specimens were mounted in Vectashield (Vector Laboratories). The following antibodies were used: Mouse anti-Repo antibodies (1:5) were obtained from the Developmental Studies Hybridoma bank (Iowa City, IA), anti-Nidogen (1:2,000, gift of S. Baumgartner). Rabbit anti-GFP (1:1,000, Invitrogen) and goat anti-HRP (DyLight^{TM649} conjugated AffiniPure HRP) (1:500, Dianova GmbH) and Alexa 488, 568 or 647 (1:1,000, Molecular Probes)) and biotinylated goat anti-rabbit secondary antibodies (1:500, Dako) were used as secondary antibodies. Anti-hLOX (1:1,500, Abnova), anti-hLOXL1 (1:400, Deciphergen Biotechnology), anti-hLOXL4 (1:400, Enzo Life Sciences), anti-Ki67 (1:100, Acris Antibodies). For mammalian tissue and cells, staining was done using the ABC method (Vector Laboratories) and sections were counterstained with hematoxylin.



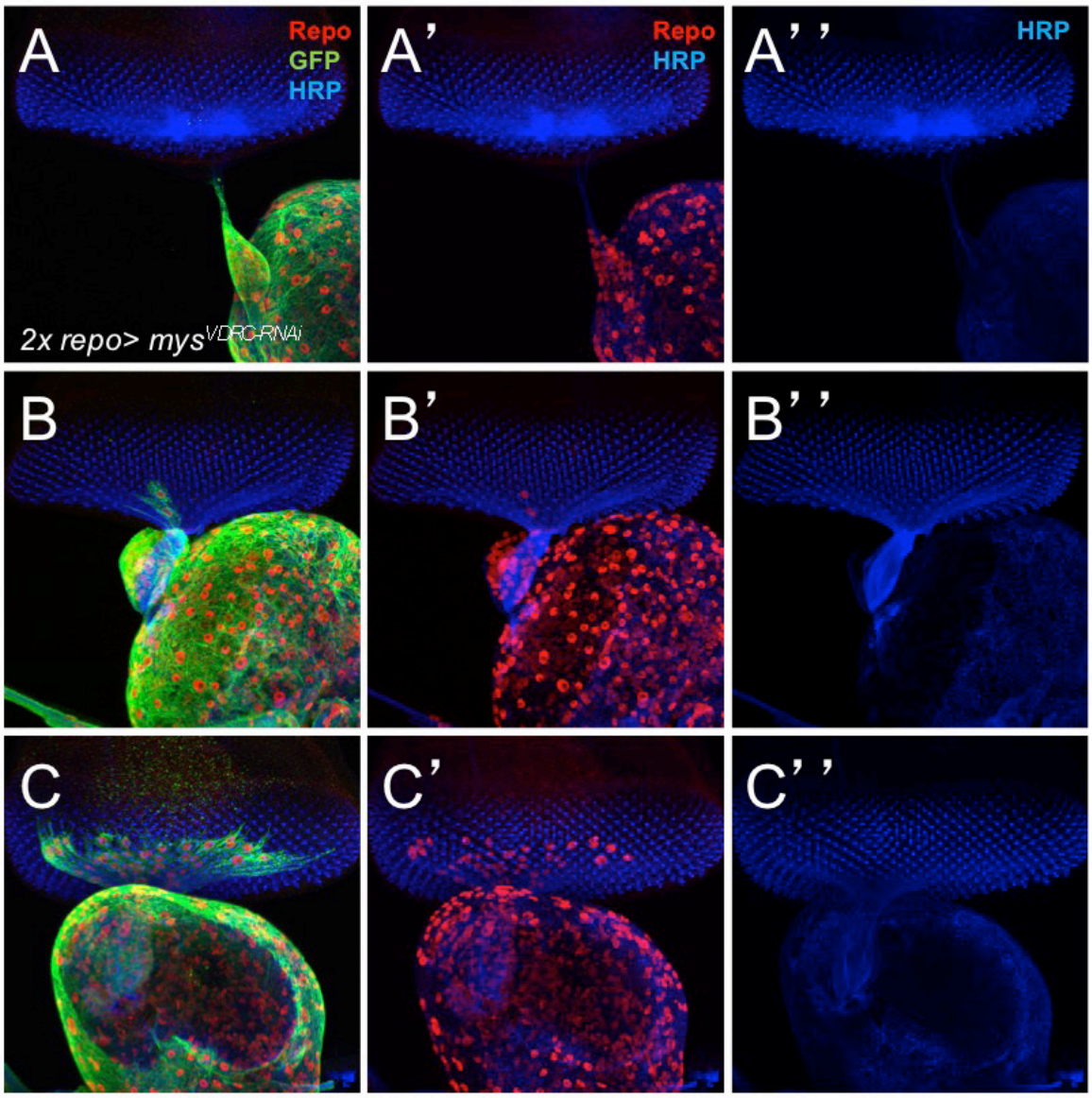
Supplementary Figure 1. PVR causes early onset of glial migration. Staining of eye imaginal discs for expression of CD8G-FP specifically expressed by all glial cells (green), Repo protein to highlight all glial nuclei (red), and the HRP antigen which is found on all neuronal membranes (blue or grey). Scale bars are 20 μm. **A**) Second instar larva. Glial migration onto the eye disc has not yet started. os, optic stalk. **B**) Second instar larva overexpressing activated PVR in all glial cells. The arrow denotes glial cells prematurely migrating onto the eye disc. **A',B'**) Only glial nuclei and neuronal membranes are shown. **C,C'**) Third instar larva. Note that all photoreceptor cells born in the eye imaginal disc project through the optic stalk towards the brain. **D,D'**) Third instar larva overexpressing activated PVR in all glial cells. Ectopic glial migration can be observed. In addition aberrant axonal projections can be seen (arrow). **E,F**) PVR affects surface glia in the brain. **E**) In larvae of the genotype *repo*-Gal4, UAS-*CD8GFP* glial cells show a normal appearance on the brain surface. **F**) Following pan-glial expression of activated PVR a multilayered organization of glial cells is detected and larger glial nuclei are seen (asterisk). Same magnification as in (**E**).



Supplementary Figure 2. Crossing scheme. A fly strain with genotype {*UAS-λPvr / CyO tub-Gal80; repo-Gal4 UAS-CD8GFP/TM6 Tb*} was generated. Here no Gal4-mediated expression is observed due to the activity of the Gal80 protein. Upon crossing to flies carrying no Gal80 element all non-TM6 progeny express activated PVR and CD8GFP in all glial cells. Depending on the culture temperature, these flies die as late embryos/early L1 (25°C) or as young L3 larvae (18°C).



Supplementary Figure 3. Genomic organization of *lox* and *lox2*. A) Schematic organization of the Drosophila *lox* gene. The P-element used to generate the small deficiency is indicated. The deletion *Df(3R)lox*$^{\Delta A38}$ removes the *lox* gene but leaves the neighboring transcription units intact. **B**) Schematic organization of the Drosophila *lox2* gene. The insertion site of the Minos element close to the LTQ domain is indicated. This insertion likely disrupts the the catalytic function of the Lox2 protein.



Supplementary Figure 4. *myospheroid* affects glial migration onto the eye imaginal disc. Eye imaginal discs where we suppressed *myosheroid* expression through RNAi were stained for expression of CD8GFP specifically expressed by all glial cells (green), Repo protein to highlight all glial nuclei (red), and the HRP antigen which is found on all neuronal membranes (blue). Upon pan-glial silencing of *myospheroid* three different phenotypic classes were observed. **A**) In the most extreme case, no glial migration onto the eye disc was noted. **B**) In an intermediate class dramatically reduced migration of 10 or less glial cells on the eye disc was noted. **C**) In a third class the number of glial cells moving onto the eye disc was reduced but migration distances appeared relatively normal.

Table S1. Summary of all AFM measurements

Genotype	Number of eye discs	Number of measurements	Median (Pa)	Mean (Pa)	sd	sem
w^{1118}	10	488	383.71	425.55	226.27	10.24
$lox^{\Delta A38}$	4	165	221.79	221.28	89.38	6.96
repo-Gal4>	2	82	335.78	356.26	163.3	18.03
repo>mycLox	4	170	431.49	522.12	315.89	24.82
repo>mys^{dsRNA}	2	42	183.96	210.61	100.73	15.54
Fak^{CG1}	6	205	177.59	327.3	332.22	23.2
repo>Integrin	7	225	410.96	479.16	279.42	18.46

sd, standard deviation; sem, standard error of the mean. Pa, Pascal.