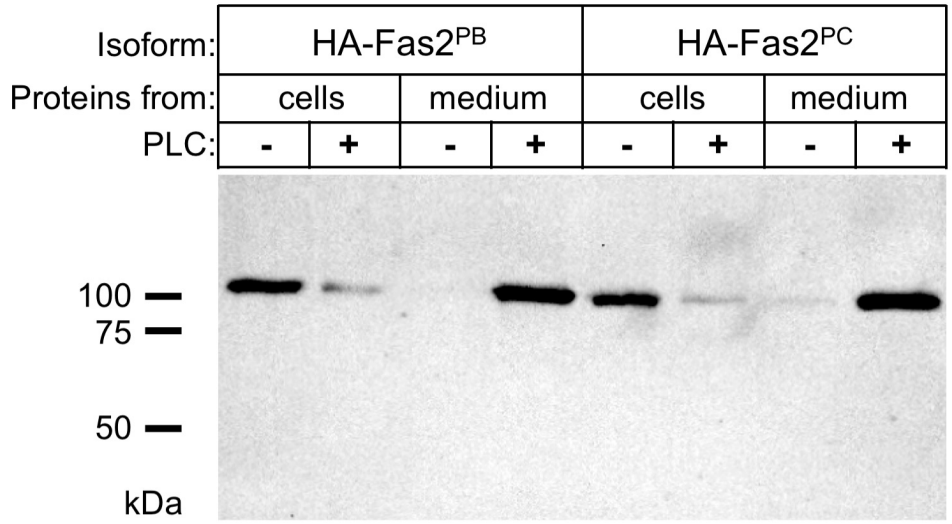


Figure S1 Fas2^{PB} and Fas2^{PC} are GPI linked proteins

Western blot analysis of S2 cells transfected with act5C-Gal4 together with either *UAS-HA-Fas2^{PB}* or *UAS-HA-Fas2^{PC}*. Cells were treated for 1 hour as indicated. Pellet and supernatant were analyzed separately. Both proteins are efficiently released to the supernatant (medium) by PiPLC treatment.

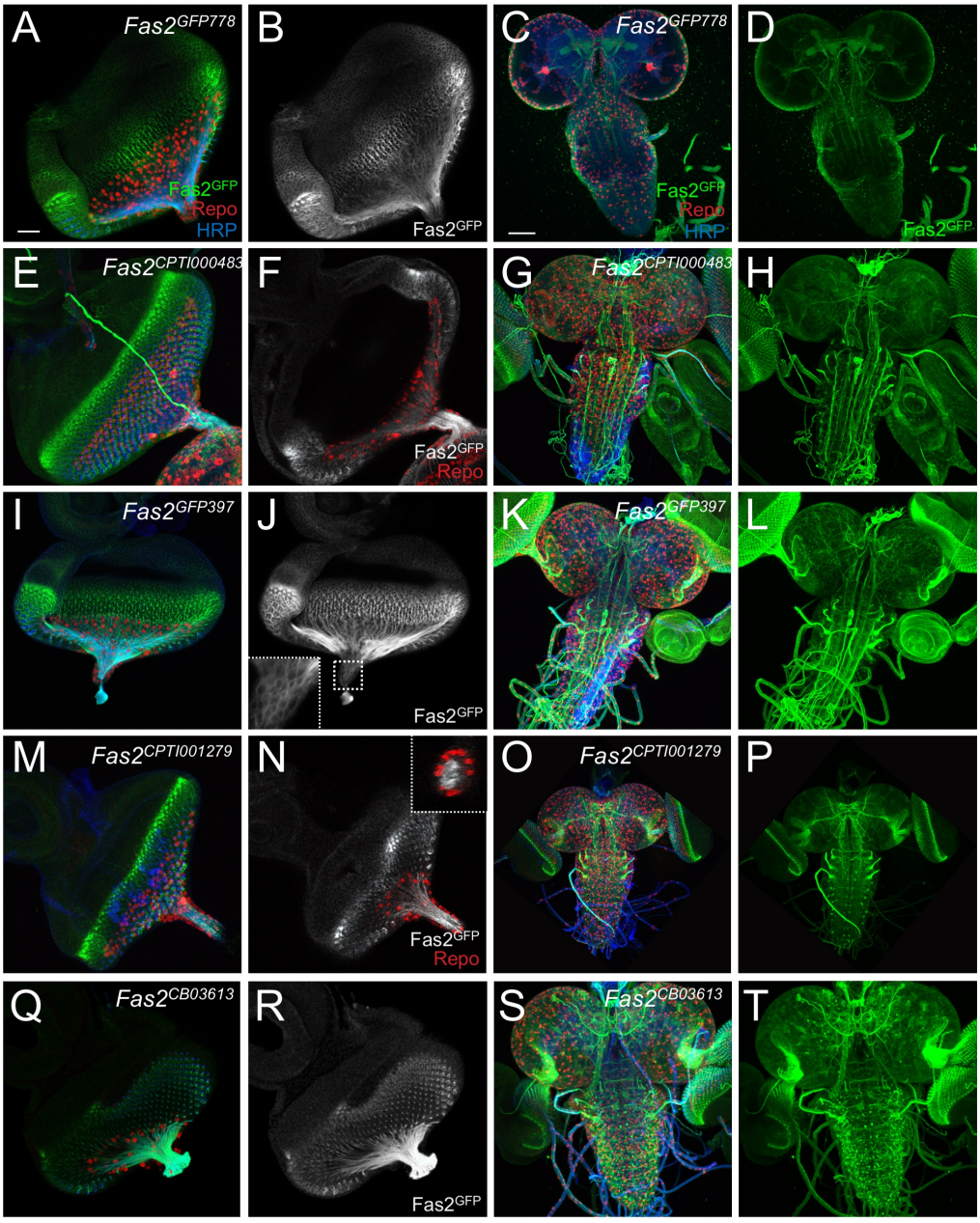


Figure S2 GFP expression associated with different *Fas2* gene traps

Larval expression pattern of the different gene trap insertion lines used in this study. Eye-imaginal discs are shown on the left, larval third instar brains are shown on the right. Specimens are stained for GFP expression (green), Repo expression (red) and

HRP expression (blue). A-D) *Fas2*^{GFP778}, E-H) *Fas2*^{CPTI000483}, I-L) *Fas2*^{GFP397}, M-P)^{CPTI001279}, Q-T) *Fas2*^{CB03613}. The inset in (J) shows the optic stalk to visualize the glial expression domain. The inset in (N) shows a cross section through the optic stalk to visualize the neuronal expression domain. Scale bar for eye imaginal discs 20 µm, scale bar for larval brain 50 µm. n>10 animals per genotype were analyzed.

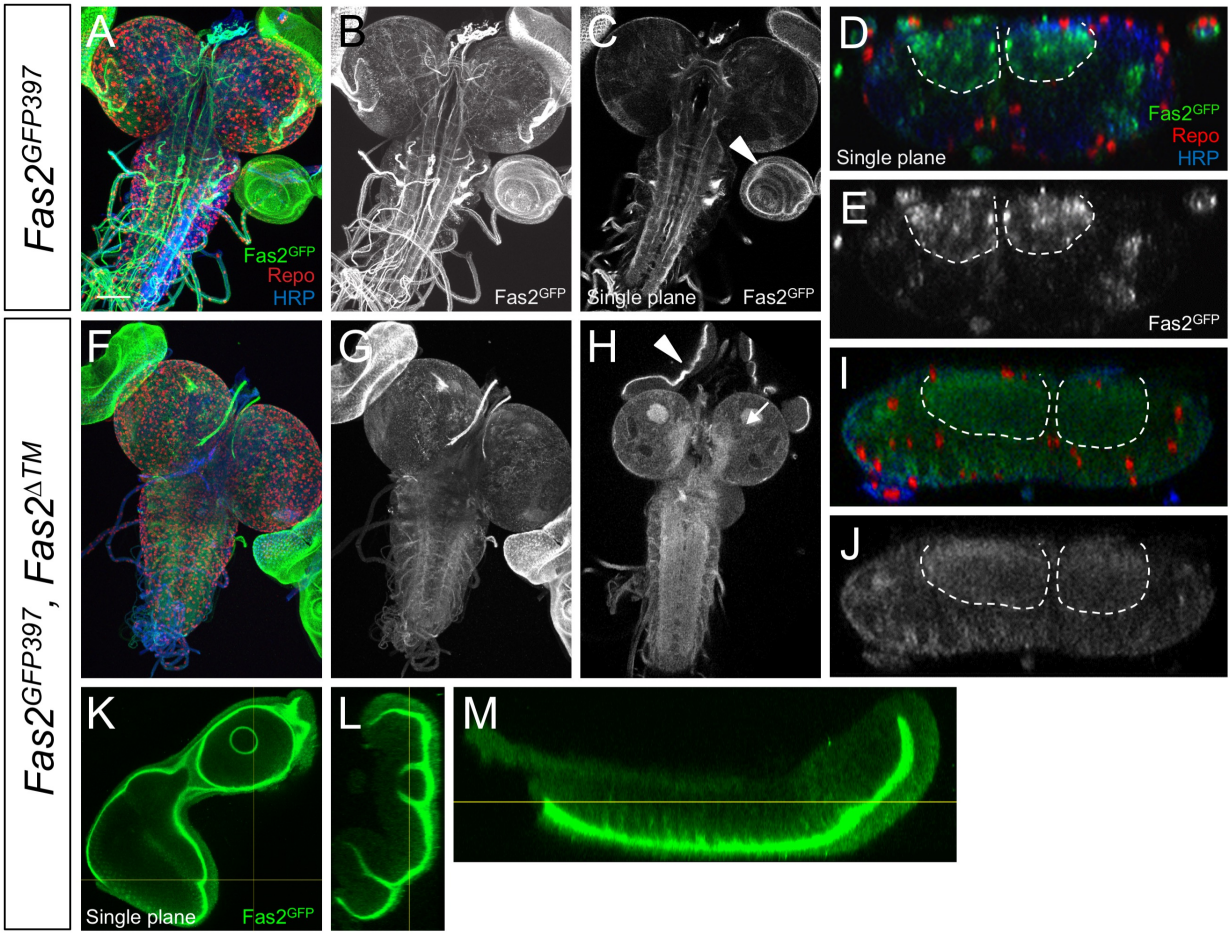


Figure S3 Expression of *Fas2^{PB}* changes in dependence of *Fas2^{TM}*

A-M) Expression of the *Fas2^{GFP397}* gene trap element. GFP expression is in green, Repo staining is shown in red, and HRP expression is shown in blue. Scale bar is 50 µm. A-C) Third instar larval brain. Note the strong neuronal expression in the eye-imaginal discs. C) In a single confocal plane enhanced GFP expression is detected at the apical domain of imaginal disc cells (arrowhead). D,E) Note the strong neuronal expression of the Fas2^{TM} isoforms. Some GFP expression is detected throughout the neuropil. The dashed line indicates the neuropil boundary. F-H) Third instar instar larval brains of mutant *Fas2^{GFP397}, Fas2$^{•TM}$* animals. The clear expression of Fas2 in CNS fascicles is lost. In addition, diffuse expression in the mushroom bodies is detected (arrow in H). I,J) The expression along axonal membranes in the neuropil is lost. Instead diffuse expression throughout the entire nervous system can be

detected. The dashed line indicates the neuropil boundary. K-M) Single confocal plane of an eye-imaginal disc of a mutant *Fas2*^{GFP397}, *Fas2*$^{\cdot TM}$ animal. Note the strong expression of Fas2 in the interior lumen of the imaginal disc. n>10 animals per genotype were analyzed.

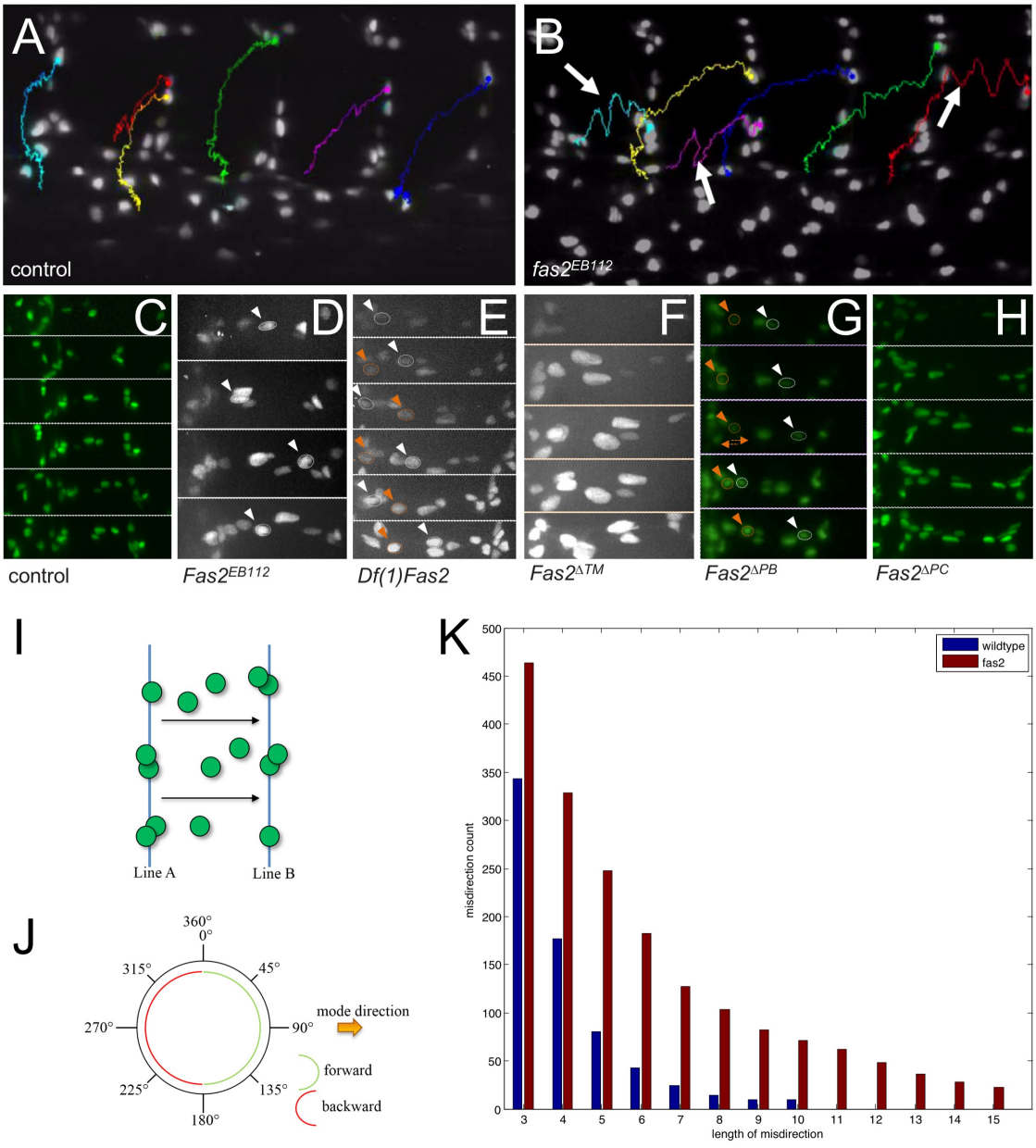


Figure S4 Fas2^{PB} is required for correct positioning of glial nuclei during embryonic development

The Figure shows stills of movies of embryonic development of the genotypes as indicated. Glial nuclei were imaged using a *repo-stinger::GFP* fusion. A) In wild type animals, peripheral glial cells born in the CNS/PNS transition zone move outwards to peripheral positions. The colored lines follow the movement of an individual glial cell. Note the straight migration towards the periphery. B) *Fas2*^{EB112} mutant animal. Note the backwards movement of glial nuclei in several segments (arrows). C-H) Consecutive frames of a movie showing the migration of the peripheral glial cells. C) Control embryo. D) *Fas2*^{EB112} mutant animal. E) *Df(1)Fas2* mutant animal. F) *Df(1)Fas2*$^{\Delta f}$ mutant

animal. G) *Fas2*$^{\Delta a}$ and (H) *Fas2*$^{\Delta a}$ mutant animal. In *Fas2* mutants affecting the expression of Fas2^{PB} (*Fas2*^{EB112}, *Df(1)Fas2*, *Fas2*$^{\Delta a}$) glial nuclei often move backwards as indicated (white and orange arrowheads). For detailed imaging see supplementary movies 1-6. I) Schematic view of glial movement from line A to line B. All movements from A to B in angles as indicated in (J) where rated as forward, movements from line B to line A where rated as backward. K) Quantification of length of backwards movement. The x-axis gives the length of misdirection in frames of movies imaged with 1 frame per minute. The y-axis indicates number of sequences observed in the different movies. 30-40 individual glial cell nuclei were tracked per movie. *control*: n= movies from 4 embryos, *"go*^{EB112}: n=movies from 7 embryos.

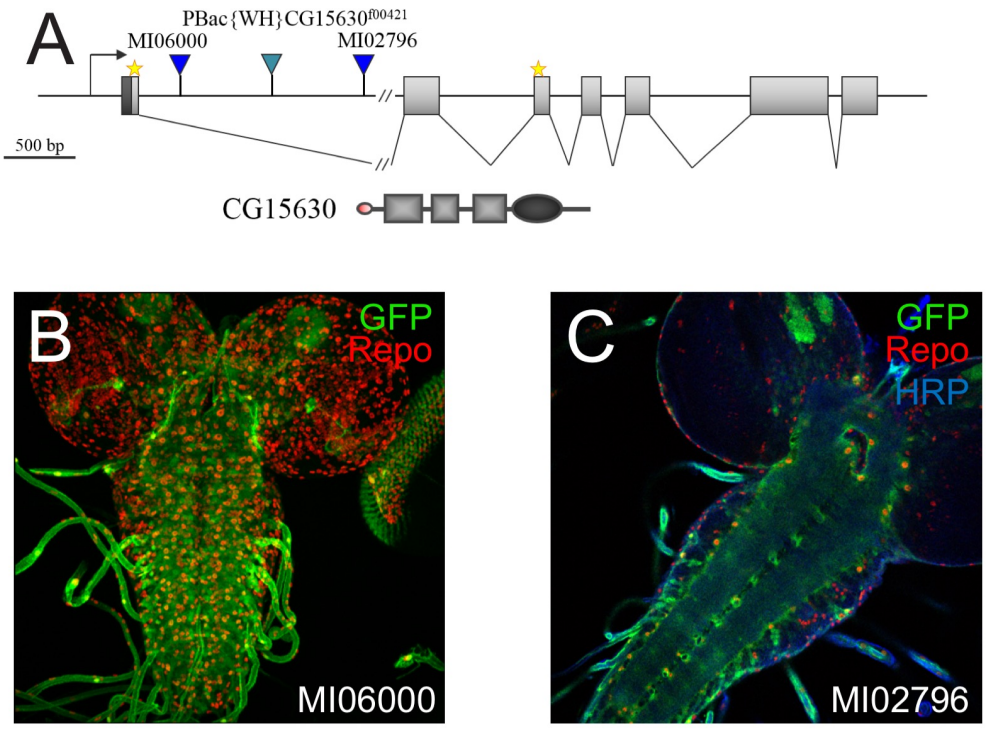


Figure S5 Expression of *CG15630* in the larval nervous system

A) Schematic view of the CG1530 gene locus. The insertion of two MiMIC transposon insertions is indicated. Transcription is from left to right. B) GFP expression directed by the MiMIC insertion MI06000. Green: GFP expression, red show expression of the Repo protein which labels glial cell nuclei. C) GFP expression directed by the MiMIC insertion MI02796. Staining is as in (B). n>6 brains were analyzed per genotype.

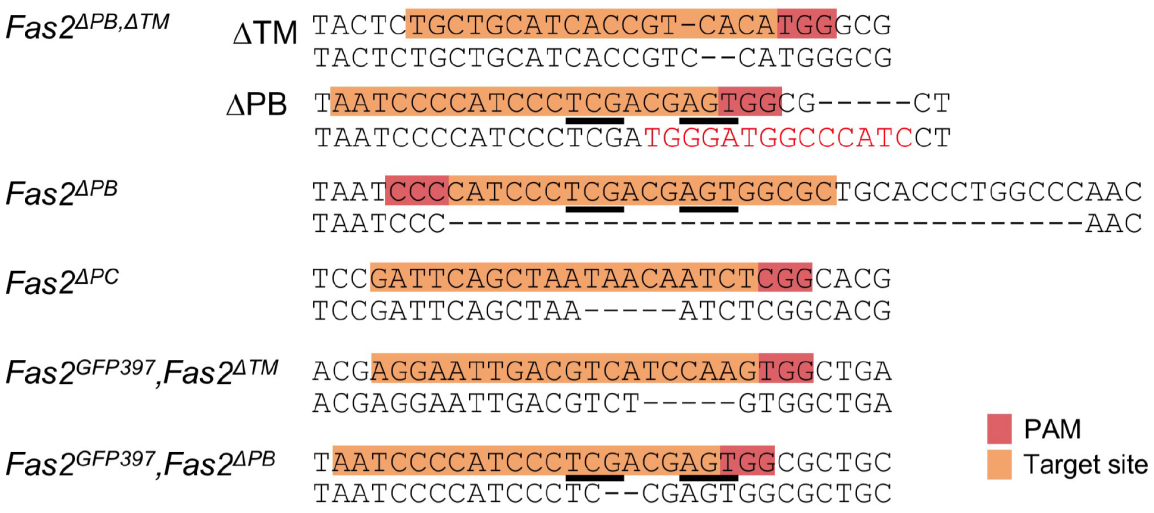
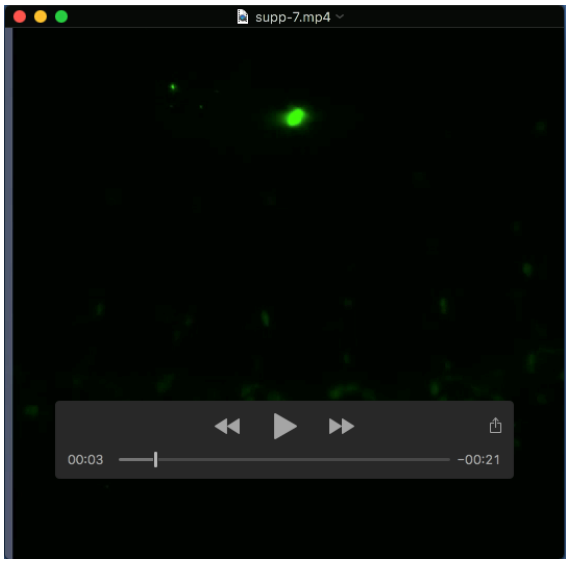


Figure S6 Details of the different isoform specific *Fas2* mutations

The figure shows the mutations generated using CRISPR/Cas9. The PAM and the target sequence are indicated by shading. The triangle indicates the predicted cleavage sites. The underlined triplets encode amino acids that can be used for GPI-anchor addition.



Movie 1

Stage 14 control embryo carrying a *repo-stGFP* element to label glial nuclei.

Movie 2

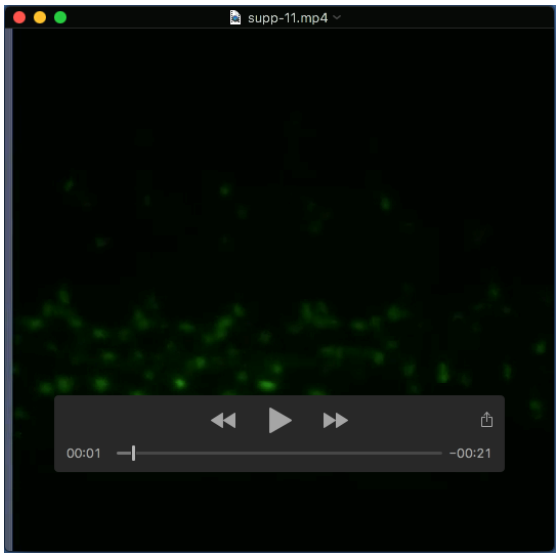
Stage 14 *Fas2*^{EB112} mutant embryo carrying a *repo-stGFP* element to label glial nuclei.

Movie 3

Stage 14 *Df(1)Fas2* mutant embryo carrying a *repo-stGFP* element to label glial nuclei.

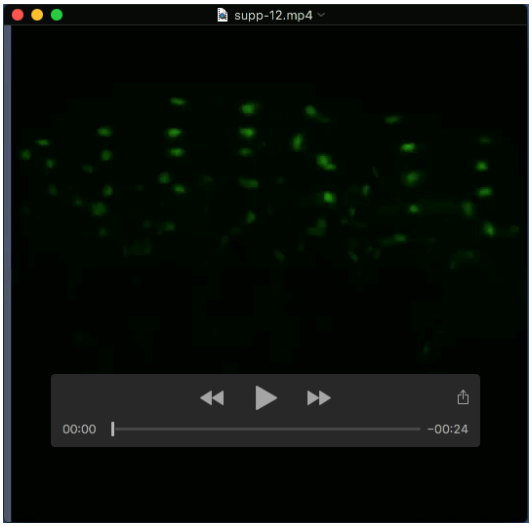
Movie 4

Stage 14 *Fas2*$^{\Delta a}$ mutant embryo carrying a *repo-stGFP* element to label glial nuclei.



Movie 5

Stage 14 *Fas2*$^{\Delta a}$ mutant embryo carrying a *repo-stGFP* element to label glial nuclei.



Movie 6

Stage 14 *Fas2*$^{\Delta a}$ mutant embryo carrying a *repo-stGFP* element to label glial nuclei.