Figure S1

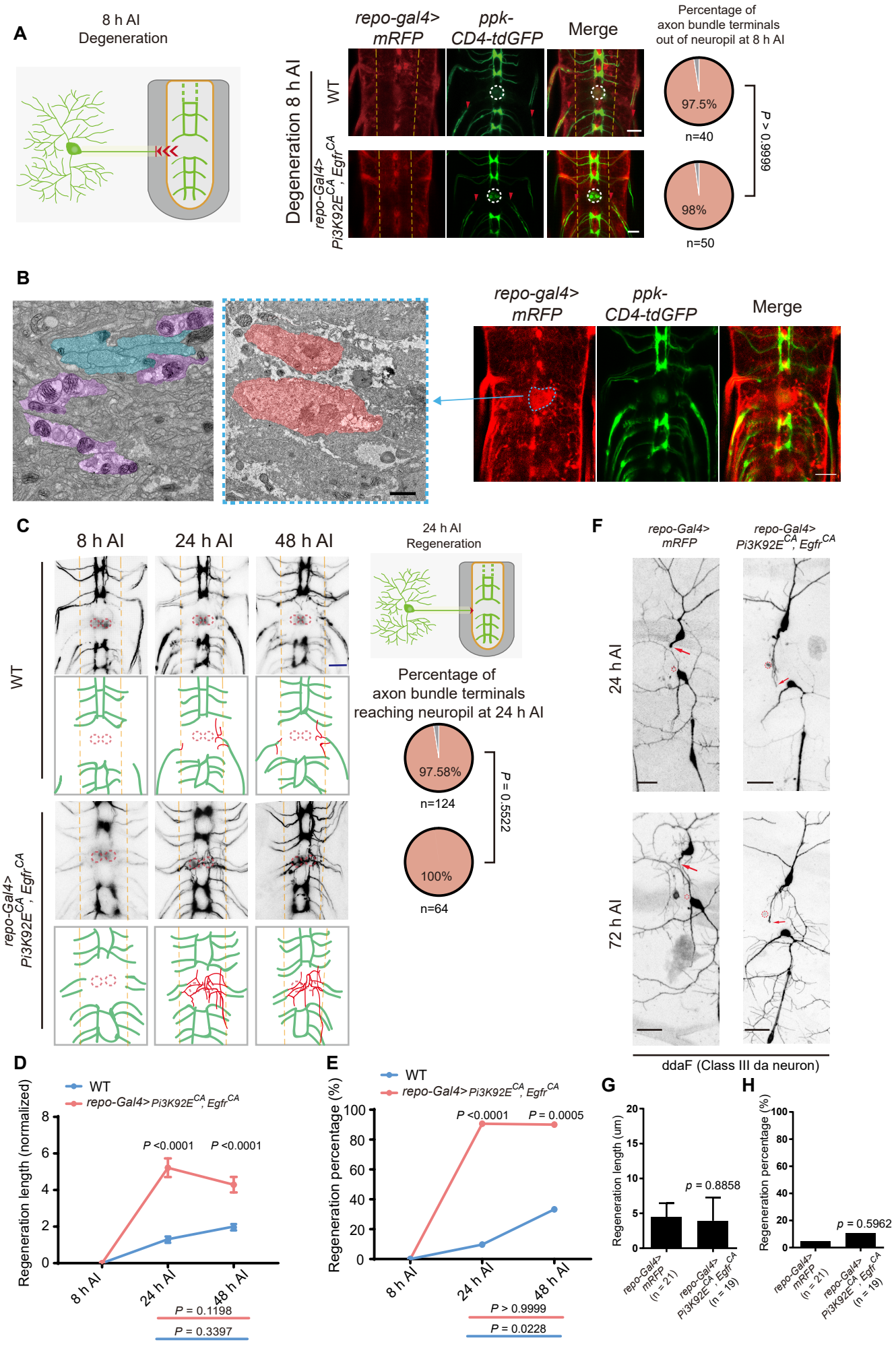

A
8 h AI
Degeneration
repo-gal4>
mRFP
ppk-
CD4-tdGFP
Merge
Degeneration 8 h AI
WT
repo-Gal4>
Pi3K92E^CA, Egfr^CA
Percentage of
axon bundle terminals
out of neuropil at 8 h AI
97.5%
n=40
98%
n=50
P > 0.9999
B
repo-gal4>
mRFP
ppk-
CD4-tdGFP
Merge
C
8 h AI
24 h AI
48 h AI
WT
repo-Gal4>
Pi3K92E^CA, Egfr^CA
24 h AI
Regeneration
Percentage of
axon bundle terminals
reaching neuropil at 24 h AI
97.58%
n=124
100%
n=64
P = 0.5522
F
repo-Gal4>
mRFP
repo-Gal4>
Pi3K92E^CA, Egfr^CA
24 h AI
72 h AI
ddaF (Class III da neuron)
D
WT
repo-Gal4>Pi3K92E^CA, Egfr^CA
Regeneration length (normalized)
P <0.0001
P <0.0001
P = 0.1198
P = 0.3397
8 h AI
24 h AI
48 h AI
E
WT
repo-Gal4>Pi3K92E^CA, Egfr^CA
Regeneration percentage (%)
P <0.0001
P = 0.0005
P > 0.9999
P = 0.0228
G
Regeneration length (um)
p = 0.8858
repo-Gal4>
mRFP
(n = 21)
repo-Gal4>
Pi3K92E^CA, Egfr^CA
(n = 19)
H
Regeneration percentage (%)
p = 0.5962

Figure S1. The CNS axon injury model in *Drosophila* larvae and regeneration induced by glial overexpression of Pi3K92E^{CA} and Egfr^{CA}, related to Figure 1.

(A) Schematic (left) and images (middle) show axons degenerating out of the neuropil at 8 h AI in both WT and larvae with glial overexpression of Pi3K92E^{CA} and Egfr^{CA}. Quantifications (right) show no difference in the percentage of axon bundle terminals out of the neuropil between the two groups (Fisher's exact test).

(B) Glial-scar-like tissue at 24 h AI in WT. Blue dotted circle demarcates the glial-scar-like region in the fluorescence images (right), and green autofluoresence indicates the lesion core which is located in the center of the glial-scar-like tissue. Electron microscopy (left) shows the hypertrophic glia in the glial-scar-like tissue. Axons (turquoise); Astrocytes (purple); Hypertrophic astrocytes (red). Scale bar: 1um.

(C) Left, axon degeneration at 8 h AI and regeneration at 24 and 48 h AI of C4da neurons in the VNC in both WT and larvae with glial overexpression of Pi3K92E^{CA} and Egfr^{CA}. Regenerated axons are in red in the neuropil (demarcated by the orange dashed lines), and the red dotted circles show the injury sites. Right, graphs show that, at 24 h AI, there is no difference in the percentage of axon bundle terminals reaching the neuropil between the two groups (Fisher's exact test).

(D) Quantification of normalized regenerated axon length for genotypes and time points presented in (C), n for WT = 44, 62, 18 and n for Pi3K92E^{CA} + Egfr^{CA} = 70, 32, 20 lesioned segments, two-way ANOVA with Tukey's test.

(E) Quantification of regeneration percentage for genotypes and time points presented in (C), Fisher's exact test.

(F) Images show ddaF (Class III da neuron) regeneration in the PNS of control (*repo-Gal4, UAS-mRFP*; *NompC-QF, QUAS-mCD8GFP*) and larvae with glial overexpression of Pi3K92E^{CA} and Egfr^{CA}. Class III da neuron are labelled by *NompC-QF>mCD8GFP*. The red dotted circles show the injury sites and the red arrows indicate axon tips. Injury is confirmed at 24 hours AI and regeneration is assessed at 72 hours AI.

(G) Quantification of normalized regenerated axon length for genotypes presented in panel (F), unpaired two-tailed Student's t-test.

(H) Quantification of regeneration percentage for genotypes presented in panel (F), Fisher's exact test.

Scale bars, 20 μm (unless indicated otherwise). Data are expressed as mean ± s.e.m.

Figure S2

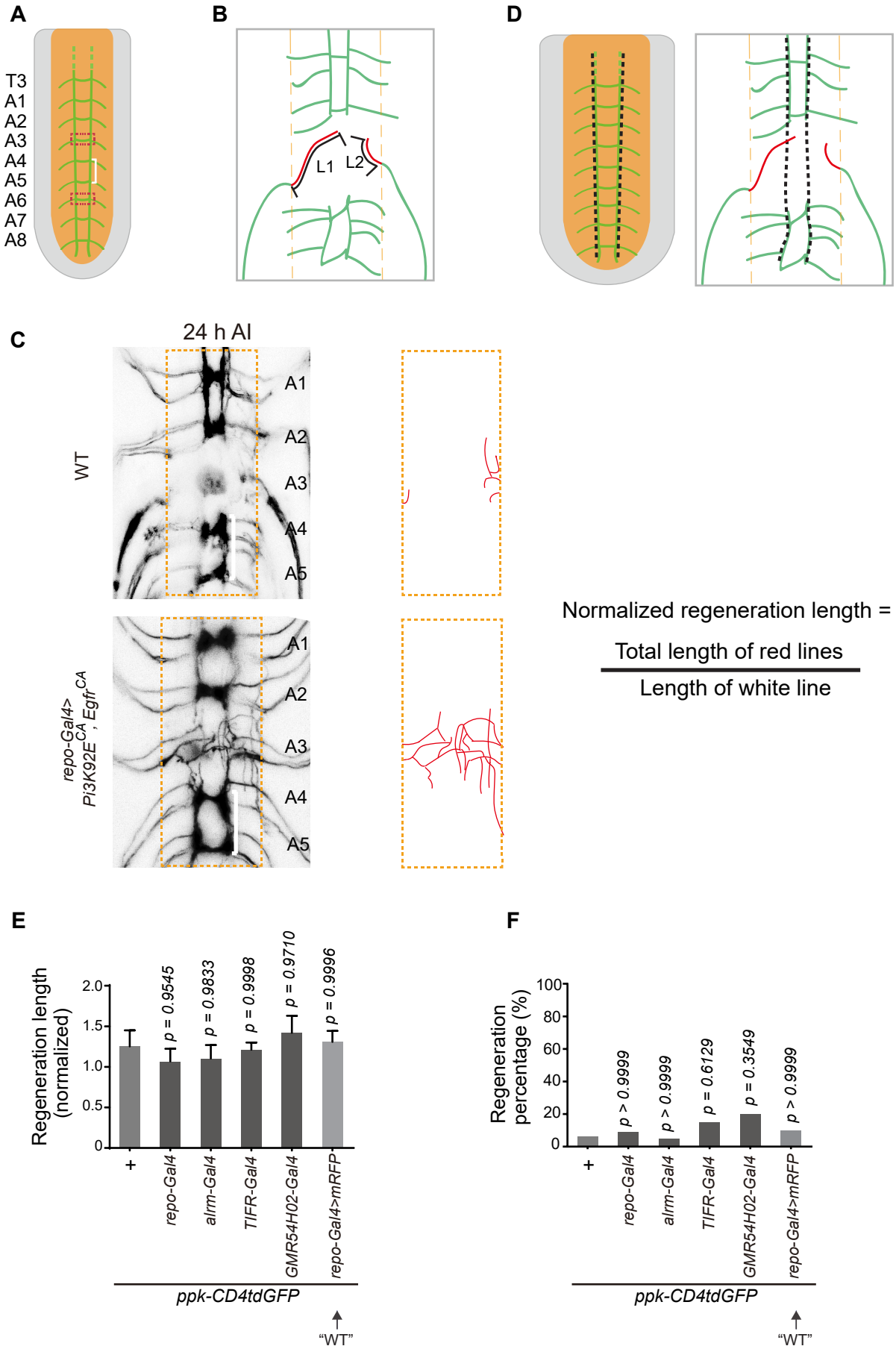

A
T3
A1
A2
A3
A4
A5
A6
A7
A8
B
L1
L2
D
C
24 h AI
WT
repo-Gal4>
PI3K92E^CA, Egfr^CA
A1
A2
A3
A4
A5
Normalized regeneration length =
Total length of red lines
Length of white line
E
Regeneration length (normalized)
2.0
1.5
1.0
0.5
0
p = 0.9545
p = 0.9833
p = 0.9998
p = 0.9710
p = 0.9996
+
repo-Gal4
alrm-Gal4
TlFR-Gal4
GMR54H02-Gal4
repo-Gal4>mRFP
ppk-CD4tdGFP
"WT"
F
Regeneration percentage (%)
100
80
60
40
20
0
p > 0.9999
p > 0.9999
p = 0.6129
p = 0.3549
p > 0.9999
+
repo-Gal4
alrm-Gal4
TlFR-Gal4
GMR54H02-Gal4
repo-Gal4>mRFP
ppk-CD4tdGFP
"WT"

Figure S2. Quantification of CNS axon regeneration, related to Figure 1, 3, 4, 5, 6 and STAR Methods.

(A) For each larvae, 2 photons laser induced lesion is on the A3 and A6 segments (red dotted rectangle).

(B) To quantify regeneration length, the length of regenerated axons inside the neuropil (red lines indicate regenerated axon and the neuropil is demarcated by the orange dashed lines) of injured bundles from both-sides are measured. Their sum (as L1 + L2 shown in the schematics) is normalized by the distance between the A4 and A5 segments (white line in (A)).

(C) Samples of axon regeneration length quantification for WT and glial overexpression of $Pi3K92E^{CA}$ and $Egfr^{CA}$ following the rules depicted in (B).

(D) Quantification of regeneration percentage. The two black dotted lines mark the location of C4da neuron axon terminals. Regenerated axons that extend into this region are more likely to contribute to functional recovery and are thus considered as regenerated (the regenerated axon on the left is regarded as regenerated, but not the one on the right). For each segment, as long as one axon has regenerated, it is regarded as a regenerated lesioned segment.

(E) Quantification of normalized regenerated axon length of C4da neurons in flies with "*ppk-CD4tdGFP*" *(*used for labelling C4da neurons), "*ppk-CD4tdGFP; repo-Gal4*", "*ppk-CD4tdGFP; alrm-Gal4*", "*ppk-CD4tdGFP; TIFR-Gal4*", "*ppk-CD4tdGFP; GMR54H02-Gal4*" and the WT group (*ppk-CD4tdGFP; repo-Gal4, UAS-mRFP*). n = 16, 22, 20, 20, 20, 62 lesioned segments from 8, 11, 10, 10, 10, 31 larvae respectively for each genotype, one-way ANOVA with Dunnett's test.

(F) Quantification of regeneration percentage for genotypes in (E), Fisher's exact test.

Figure S3

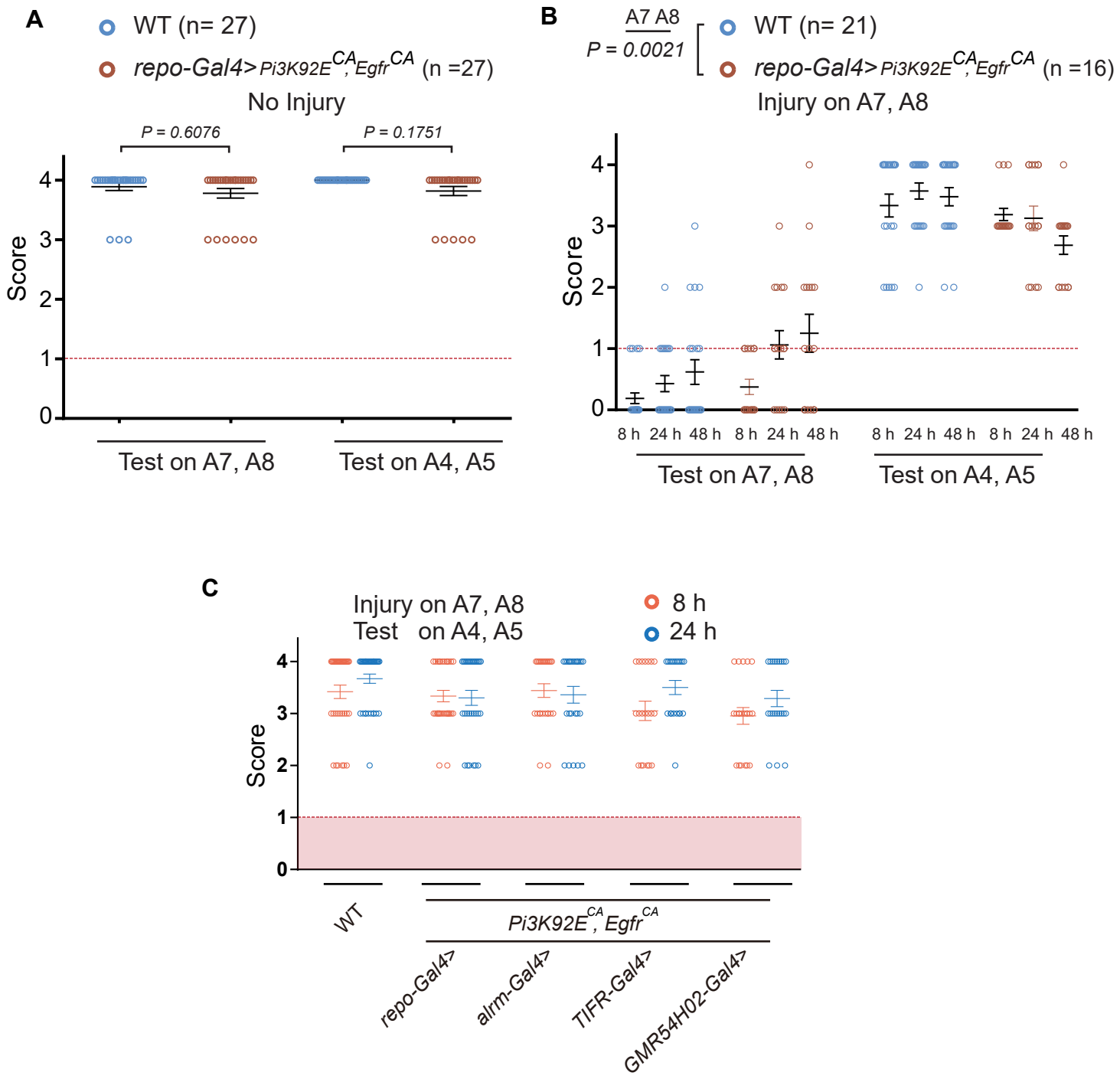

A
WT (n= 27)
repo-Gal4> Pi3K92E^CA, Egfr^CA (n =27)
No Injury
P = 0.6076
P = 0.1751
Score
Test on A7, A8
Test on A4, A5
B
A7 A8
P = 0.0021
WT (n= 21)
repo-Gal4> Pi3K92E^CA, Egfr^CA (n =16)
Injury on A7, A8
Score
8 h 24 h 48 h 8 h 24 h 48 h
8 h 24 h 48 h 8 h 24 h 48 h
Test on A7, A8
Test on A4, A5
C
Injury on A7, A8
Test on A4, A5
8 h
24 h
Score
WT
Pi3K92E^CA, Egfr^CA
repo-Gal4>
alrm-Gal4>
TIFR-Gal4>
GMR54H02-Gal4>

Figure S3. Reprogramming glial cells promotes functional recovery after injury in the *Drosophila* CNS, related to Figure 2.

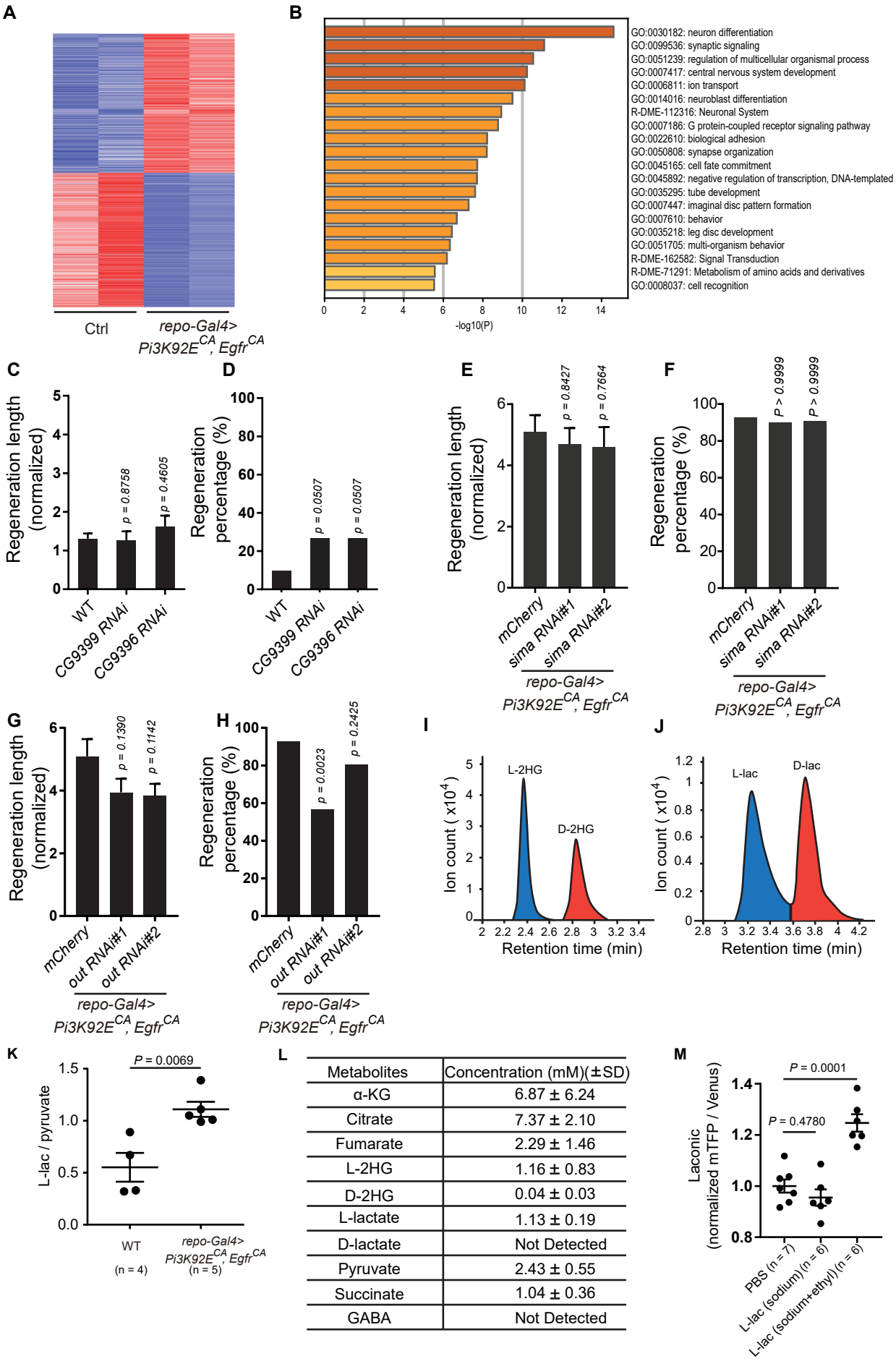
(A) Behavioral tests on uninjured larvae from WT or glial overexpression of $Pi3K92E^{CA}$, $Egfr^{CA}$ (one-way ANOVA with Tukey's test).

(B) Behavioral tests on injured larvae from WT or glial overexpression of $Pi3K92E^{CA}$, $Egfr^{CA}$ at 8 h, 24 h and 48 h AI. Tests on A4 and A5 are the positive control (two-way ANOVA).

(C) Behavioral scores of tests on A4 and A5 of larvae shown in **Figure 2D**.

Data are expressed as mean ± s.e.m.

Figure S4



Metabolites	Concentration (mM)(±SD)
α-KG	6.87 ± 6.24
Citrate	7.37 ± 2.10
Fumarate	2.29 ± 1.46
L-2HG	1.16 ± 0.83
D-2HG	0.04 ± 0.03
L-lactate	1.13 ± 0.19
D-lactate	Not Detected
Pyruvate	2.43 ± 0.55
Succinate	1.04 ± 0.36
GABA	Not Detected

Figure S4. Glycolysis is critical for axon regeneration in the CNS induced by reprogramming glial cells, related to Figure 3, 4.

(A, B) RNA sequencing analysis of CNS tissues collected from control (*repo-Gal4, UAS-mRFP*) or glial overexpression of $Pi3K92E^{CA}$ and $Egfr^{CA}$. Heat map (A) shows significantly differentially expressed genes (DEGs = 2403, fold-change > 2, probability of equally expressed < 0.05) from the two groups (two individual experiments for each group, 70-100 larvae CNS tissues for each experiment). GO analysis of DEGs (B).

(C, D) Normalized regenerated axon length (C) and regeneration percentage (D) of flies with glial *CG9399* or *CG9396* RNAis, and WT as control, n = 62, 26, 26 lesioned segments for each genotype; regeneration length, one-way ANOVA with Dunnett's test, adjusted *P* value showed in graphs; regeneration percentage, Fisher's exact test.

(E, F) Normalized regenerated axon length (E) and regeneration percentage (F) of flies with glial *sima* RNAis (two RNAis) in the *repo-Gal4>Pi3K92E*^{CA}, *Egfr*^{CA} background at 24 h AI, with glial mCherry expression as control (*UAS-mCherry*; *repo-Gal4, UAS-Pi3K92E*^{CA}, *UAS-Egfr*^{CA}; *ppk-CD4tdGFP*), n = 28, 20, 22 lesioned segments for each genotype; regeneration length, one-way ANOVA with Dunnett's test, adjusted *P* value showed in graphs; regeneration percentage, Fisher's exact test.

(G, H) Normalized regenerated axon length (G) and regeneration percentage (H) of flies with glial *out* RNAis (two RNAis) in the *repo-Gal4>Pi3K92E*^{CA}, *Egfr*^{CA} background at 24 h AI, with glial mCherry expression as control (*UAS-mCherry*; *repo-Gal4, UAS-Pi3K92E*^{CA}, *UAS-Egfr*^{CA}; *ppk-CD4tdGFP*), n = 28, 30, 26 lesioned segments for each genotype; regeneration length, one-way ANOVA with Dunnett's test, adjusted *P* value showed in graphs; regeneration percentage, Fisher's exact test.

(I, J) Chiral derivatization was performed to allow separation of enantiomers for 2HG (I) and lactate (J) by liquid chromatography-mass spectrometry (LC-MS). The graphs show separated spikes of mixed reference standards of L-2HG and D-2HG, or L-lactate and D-lactate.

(K) Ratio of L-lactate/pyruvate in the WT and *repo-Gal4>Pi3K92E*^{CA}, *Egfr*^{CA} groups. Metabolites are measured from the CNS tissues of fly larvae and each data point represents one experiment using 50-100 larval CNS tissues (unpaired two-tailed Student's t-test).

(L) Concentrations of metabolites in the hemolymph samples of WT larvae measured by gas chromatography-mass spectrometry (GC-MS) or LC-MS.

(M) Quantification of fluorescence intensity ratio of mTFP/Venus from glial cells in the VNC of flies expressing glial Laconic (*repo-Gal4, UAS-Laconic*). VNCs were treated with PBS, sodium lactate (160 mM) or lactate mixture (140 mM sodium L-lactate + 20 mM ethyl L-lactate) for 40 minutes *in vitro* (one-way ANOVA with Dunnett's test, adjusted *P* value showed in graphs).

Data are expressed as mean ± s.e.m.

Figure S5

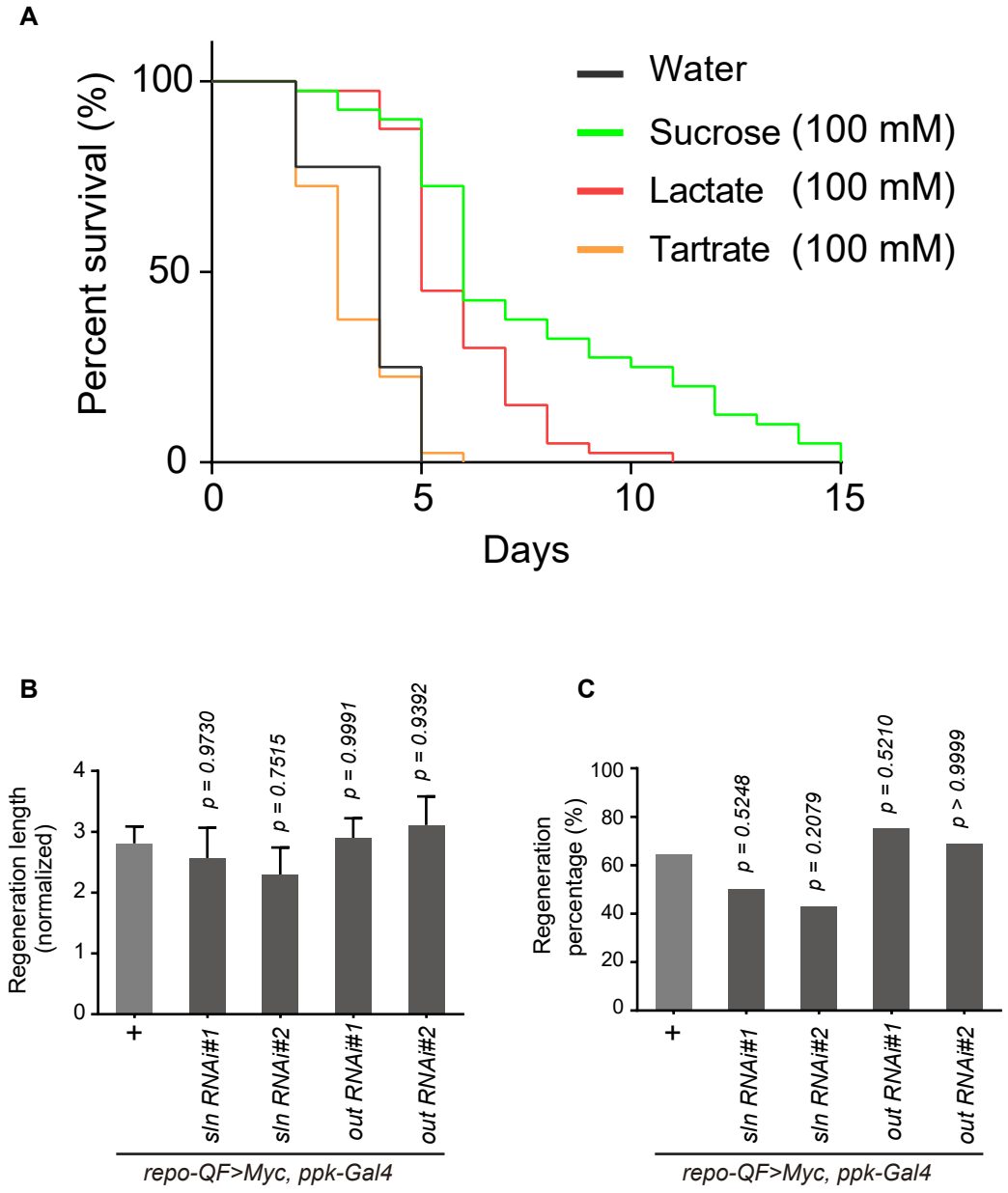

A
Percent survival (%)
100
50
0
0
5
10
15
Days
Water
Sucrose (100 mM)
Lactate (100 mM)
Tartrate (100 mM)
B
Regeneration length (normalized)
4
3
2
1
0
p = 0.9730
p = 0.7515
p = 0.9991
p = 0.9392
+
sln RNAi#1
sln RNAi#2
out RNAi#1
out RNAi#2
repo-QF>Myc, ppk-Gal4
C
Regeneration percentage (%)
100
80
60
40
20
0
p = 0.5248
p = 0.2079
p = 0.5210
p > 0.9999
+
sln RNAi#1
sln RNAi#2
out RNAi#1
out RNAi#2
repo-QF>Myc, ppk-Gal4

Figure S5. L-tartrate is not a viable energy source for *Drosophila* larvae, and axon regeneration of C4da neurons with neuronal *Sln* or *out* RNAis when glia is reprogrammed by Myc, related to Figure 5 and Discussion.

(A) Survival rate of w^{1118} larvae fed with water, sucrose, L-lactate or L-tartrate (n = 40 for each group).

(B) Quantification of normalized regenerated axon length in flies with *ppk-Gal4* as control (*ppk-Gal4*; *repo-QF, QUAS-Myc*; *ppk-CD4tdGFP*), C4da neuronal *Sln* RNAis and *out* RNAis, in the background of *repo-QF>Myc*, two RNAis for each gene knockdown, n = 28, 16, 14, 16, 16 lesioned segments from 14, 8, 7, 8, 8 larvae respectively for each genotype, one-way ANOVA with Dunnett' s test.

(C) Quantification of regeneration percentage for genotypes in (B), Fisher's exact test.

Figure S6

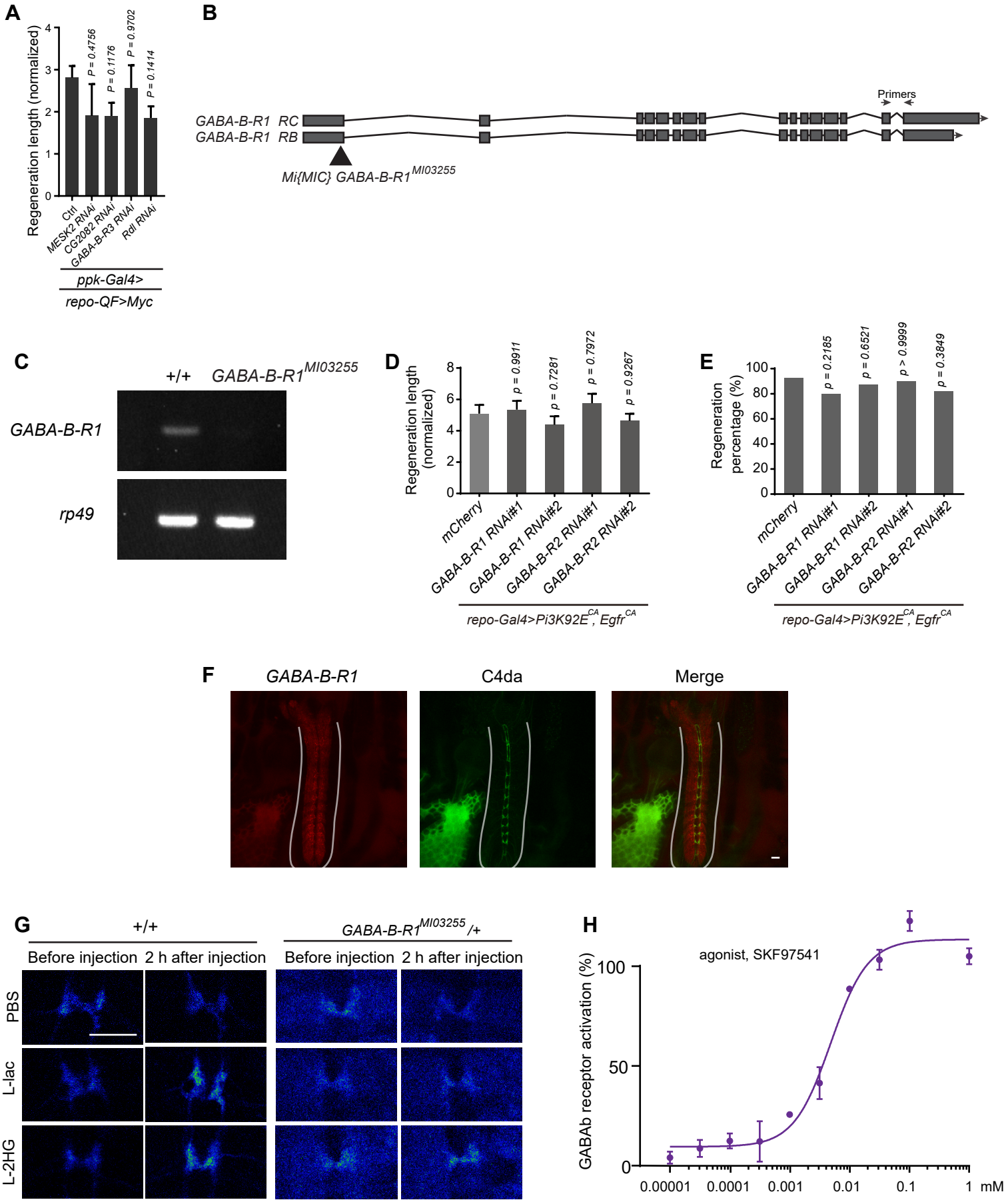

A
Regeneration length (normalized)
P = 0.4756
P = 0.1176
P = 0.9702
P = 0.1414
Ctrl
MESK2 RNAi
CG2082 RNAi
GABA-B-R3 RNAi
Rdl RNAi
ppk-Gal4>
repo-QF>Myc
B
Primers
GABA-B-R1 RC
GABA-B-R1 RB
Mi{MIC} GABA-B-R1MI03255
C
+/+
GABA-B-R1MI03255
GABA-B-R1
rp49
D
Regeneration length (normalized)
p = 0.9911
p = 0.7281
p = 0.7972
p = 0.9267
mCherry
GABA-B-R1 RNAi#1
GABA-B-R1 RNAi#2
GABA-B-R2 RNAi#1
GABA-B-R2 RNAi#2
repo-Gal4>Pi3K92ECA, EgfrCA
E
Regeneration percentage (%)
p = 0.2185
p = 0.6521
p > 0.9999
p = 0.3849
mCherry
GABA-B-R1 RNAi#1
GABA-B-R1 RNAi#2
GABA-B-R2 RNAi#1
GABA-B-R2 RNAi#2
repo-Gal4>Pi3K92ECA, EgfrCA
F
GABA-B-R1
C4da
Merge
G
+/+
GABA-B-R1MI03255/+
Before injection
2 h after injection
Before injection
2 h after injection
PBS
L-lac
L-2HG
H
agonist, SKF97541
GABAb receptor activation (%)
100
50
0
0.00001
0.0001
0.001
0.01
0.1
1 mM

Figure S6. Axon regeneration induced by reprogramming glial cells depends on $GABA_B$ receptor in C4da neurons, and L-lactate and L-2HG regulate cAMP level in neurons through $GABA_B$ receptor, related to Figure 6.

(A) Normalized regenerated axon length for flies with RNAis targeting screened candidates possibly interacting with L-lactate in neurons in the *repo-QF>Myc* background (control: *ppk-Gal4*; *repo-QF, QUAS-Myc*; *ppk-CD4tdGFP*), n = 28, 6, 22, 12, 16, one-way ANOVA with Dunnett's test, adjusted *P* value shown in graphs.

(B) Schematics showing the MiMIC insertion in $GABA\text{-}B\text{-}R1^{MI03255}$. Primers spanning exons 15 and 16 are used in (C) to detect the transcript level of *GABA-B-R1*.

(C) *GABA-B-R1* transcription is reduced in $GABA\text{-}B\text{-}R1^{MI03255}$ mutants. PCR templates are cDNA derived from homogenized whole larvae. *Ribosomal protein 49 (rp49)* is used as loading control (n = 3 replicates).

(D) Quantification of normalized regenerated axon length of C4da neurons in flies with glial mCherry expression as control (*UAS-mCherry*; *repo-Gal4, UAS-*$Pi3K92E^{CA}$, *UAS-*$Egfr^{CA}$; *ppk-CD4tdGFP*), glial *GABA-B-R1* RNAis and glial *GABA-B-R2* RNAis in the *repo-Gal4>*$Pi3K92E^{CA}$, $Egfr^{CA}$ background at 24 h AI in the VNC. Two RNAis for each gene knockdown, n = 28, 20, 24, 20, 22 lesioned segments from 14, 10, 12, 10, 11 larvae for each genotype respectively, one-way ANOVA with Dunnett's test.

(E) Quantification of regeneration percentage for genotypes in (D), Fisher's exact test.

(F) GABA-B-R1 is labeled by $GABA\text{-}B\text{-}R1^{MI01930\text{-}GFSTF.0}$, a GFP-tagged *GABA-B-R1* allele, and C4da neuron axons are labeled by *ppk-CD4tdTomato.* VNC is demarcated by the white line.

(G) Representative images for data in **Figure 6I.**

(H) GABA-B receptor activation by SKF97541 (agonist for GABA-B receptor) at a concentration range from 0.00001-1 mM, which is revealed by ^{35}S-GTPγS assay for G protein coupled receptors with Human recombinant GABBR1a and GABBR2. Activation by SKF97541 is defined as positive. X-axis shows as Log10.

Scale bars, 20μm. Data are expressed as mean ± s.e.m.

Figure S7

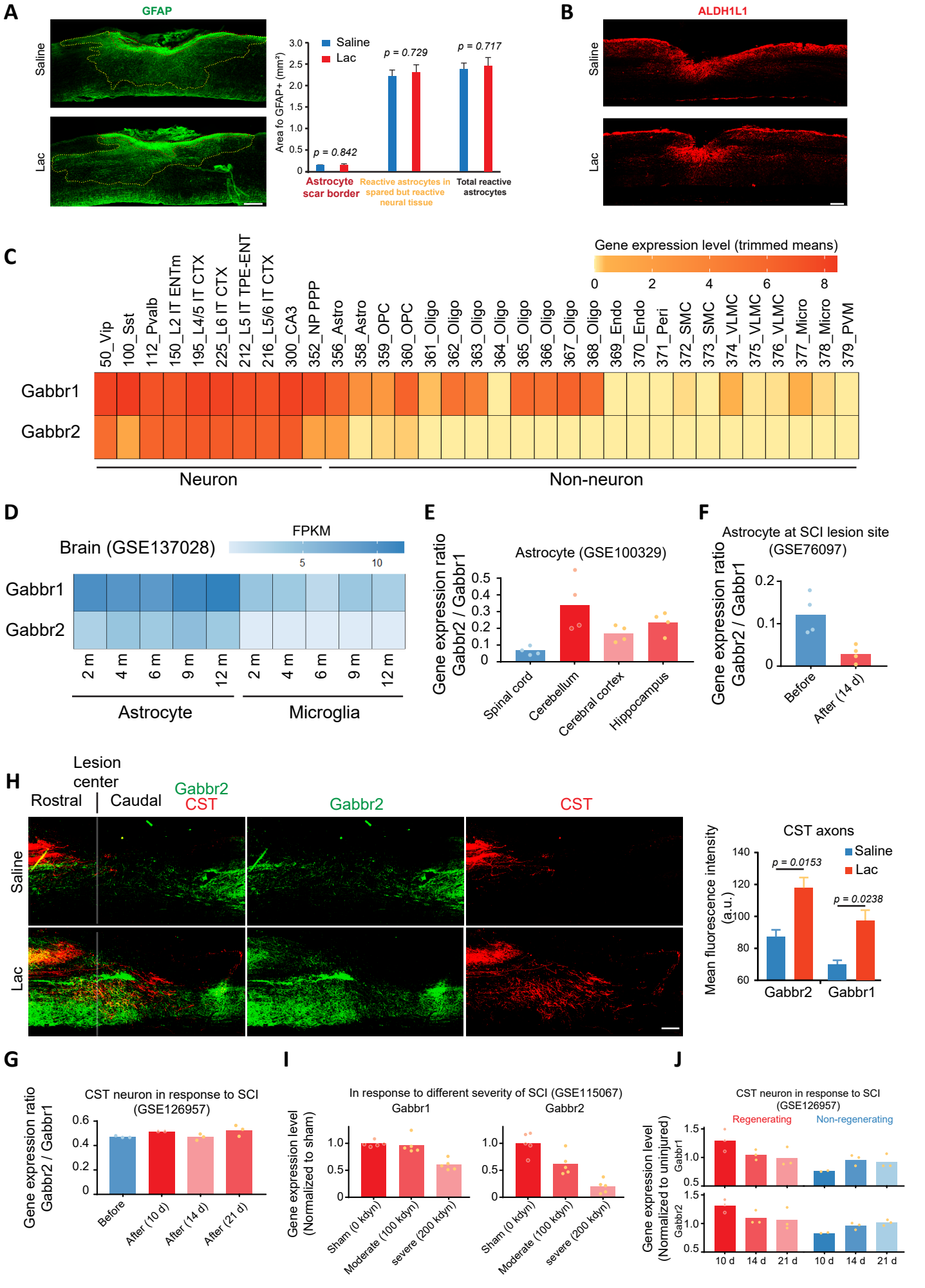

A
GFAP
Saline
Lac
Area fo GFAP+ (mm²)
Saline
Lac
p = 0.842
p = 0.729
p = 0.717
Astrocyte scar border
Reactive astrocytes in spared but reactive neural tissue
Total reactive astrocytes
B
ALDH1L1
Saline
Lac
C
Gene expression level (trimmed means)
50_Vip
100_Sst
112_Pvalb
150_L2 IT ENTm
195_L4/5 IT CTX
225_L6 IT CTX
212_L5 IT TPE-ENT
216_L5/6 IT CTX
300_CA3
352_NP PPP
356_Astro
358_Astro
359_OPC
360_OPC
361_Oligo
362_Oligo
363_Oligo
364_Oligo
365_Oligo
366_Oligo
367_Oligo
368_Oligo
369_Endo
370_Endo
371_Peri
372_SMC
373_SMC
374_VLMC
375_VLMC
376_VLMC
377_Micro
378_Micro
379_PVM
Gabbr1
Gabbr2
Neuron
Non-neuron
D
FPKM
Brain (GSE137028)
Gabbr1
Gabbr2
2 m
4 m
6 m
9 m
12 m
Astrocyte
Microglia
E
Astrocyte (GSE100329)
Gene expression ratio Gabbr2 / Gabbr1
Spinal cord
Cerebellum
Cerebral cortex
Hippocampus
F
Astrocyte at SCI lesion site (GSE76097)
Gene expression ratio Gabbr2 / Gabbr1
Before
After (14 d)
H
Lesion center
Rostral
Caudal
Gabbr2 CST
Gabbr2
CST
Saline
Lac
CST axons
Mean fluorescence intensity (a.u.)
Saline
Lac
p = 0.0153
p = 0.0238
Gabbr2
Gabbr1
G
CST neuron in response to SCI (GSE126957)
Gene expression ratio Gabbr2 / Gabbr1
Before
After (10 d)
After (14 d)
After (21 d)
I
In response to different severity of SCI (GSE115067)
Gabbr1
Gabbr2
Gene expression level (Normalized to sham)
Sham (0 kdyn)
Moderate (100 kdyn)
severe (200 kdyn)
J
CST neuron in response to SCI (GSE126957)
Regenerating
Non-regenerating
Gene expression level (Normalized to uninjured)
Gabbr1
Gabbr2
10 d
14 d
21 d

Figure S7. GFAP and ALDH1L1 staining at the lesion site, and expression of Gabbr1 and Gabbr2 in different cell types of murine CNS and its correlation with the axon regeneration potential, related to Figure 7.

(A) Quantification of GFAP staining area at the lesion site from the lactate treatment and saline control groups. Red dash line demarcates the astrocyte scar boarder and yellow dash line demarcates reactive astrocytes in spared but reactive neural tissue. Scale bar, 200 μm.

(B) ALDH1L1 staining at the lesion site of the lactate treatment and saline control groups. Scale bar, 200 μm.

(C) Gene expression of Gabbr1 and Gabbr2 from Mouse Whole Cortex and Hippocampus SMART-seq (the Allen mouse brain atlas, calculated as trimmed means), including all non-neuronal clusters and representative 10 neuronal clusters.

(D) Gene expression of Gabbr1 and Gabbr2 in astrocytes and microglia in mice of different ages from a previous study (Gene Expression Omnibus (GEO) accession code GSE137028, calculated as FPKM (fragments per kilobase of exon model per million reads mapped)). M, month.

(E) Gene expression ratio of Gabbr2 to Gabbr1 in astrocytes from spinal cord, cerebellum, cerebral cortex and hippocampus from a previous study (GSE100329).

(F) Gene expression ratio of Gabbr2 to Gabbr1 in astrocytes from spinal cord before and after SCI from a previous study (GSE76097).

(G) Gene expression ratio of Gabbr2 to Gabbr1 in CST neurons before and after SCI from a previous study (GSE126957).

(H) Quantification of mean fluorescence intensity of Gabbr2 and Gabbr1 in CST axons within an area around the lesion site (0.8 mm rostral and caudal to the lesion center), from the lactate treatment group (n = 4 mice) and saline control group (n = 3 mice). The representative images show Gabbr2 staining with BDA-labelled CST axons in the two groups. Two-tailed unpaired student's t-test. Scale bar, 200 μm.

(I) Gene expression of Gabbr1 and Gabbr2 in response to different severity of SCI from a previous study (GSE115067).

(J) Gene expression of Gabbr1 and Gabbr2 in CST neurons after SCI. Regenerating group refers to regenerated axons induced by neural progenitor cell grafts; non regenerating group refers to lesion only (GSE126957).

Table S1

ppk-CD4tdGFP; repo-Gal4>	Human homologs	Regeneration percentage (%)	*p* value, n (vs. WT, n =62 9.7%)
Pi3K92E^{CA}	*PI3K*	*66.7*	*<0.0001, 24*
Egfr^{CA}	*EGFR*	*54.5*	*<0.0001, 22*
stg	*CDC25A*	*52.6*	*<0.0001, 19*
btl^{CA}	*FGFR1*	*44.4*	*0.0006, 18*
htl^{CA}	*FGFR3*	*42.3*	*0.0004, 26*
sima	*HIF1A*	*43.8*	*0.0011, 16*
ci	*GLI3*	*42.9*	*0.0021, 14*
foxo	*FOXO1*	*42.9*	*0.0021, 14*
Smox	*SMAD3*	*41.7*	*0.0044, 12*
Stat92E	*STAT5*	*35.7*	*0.0124, 14*
p53^{DN}	*TP53*	*30*	*0.0714, 10*
Grn	*GATA2*	*16.7*	*0.4754, 12*
Su(H)	*RBPJ*	*0*	*0.17, 18*

Table S1. List of genes screened for axon regeneration in the CNS when they are expressed in glial cells (Fisher's exact test). CA, constitutively active; DN, dominant negative. Related to Figure 1.