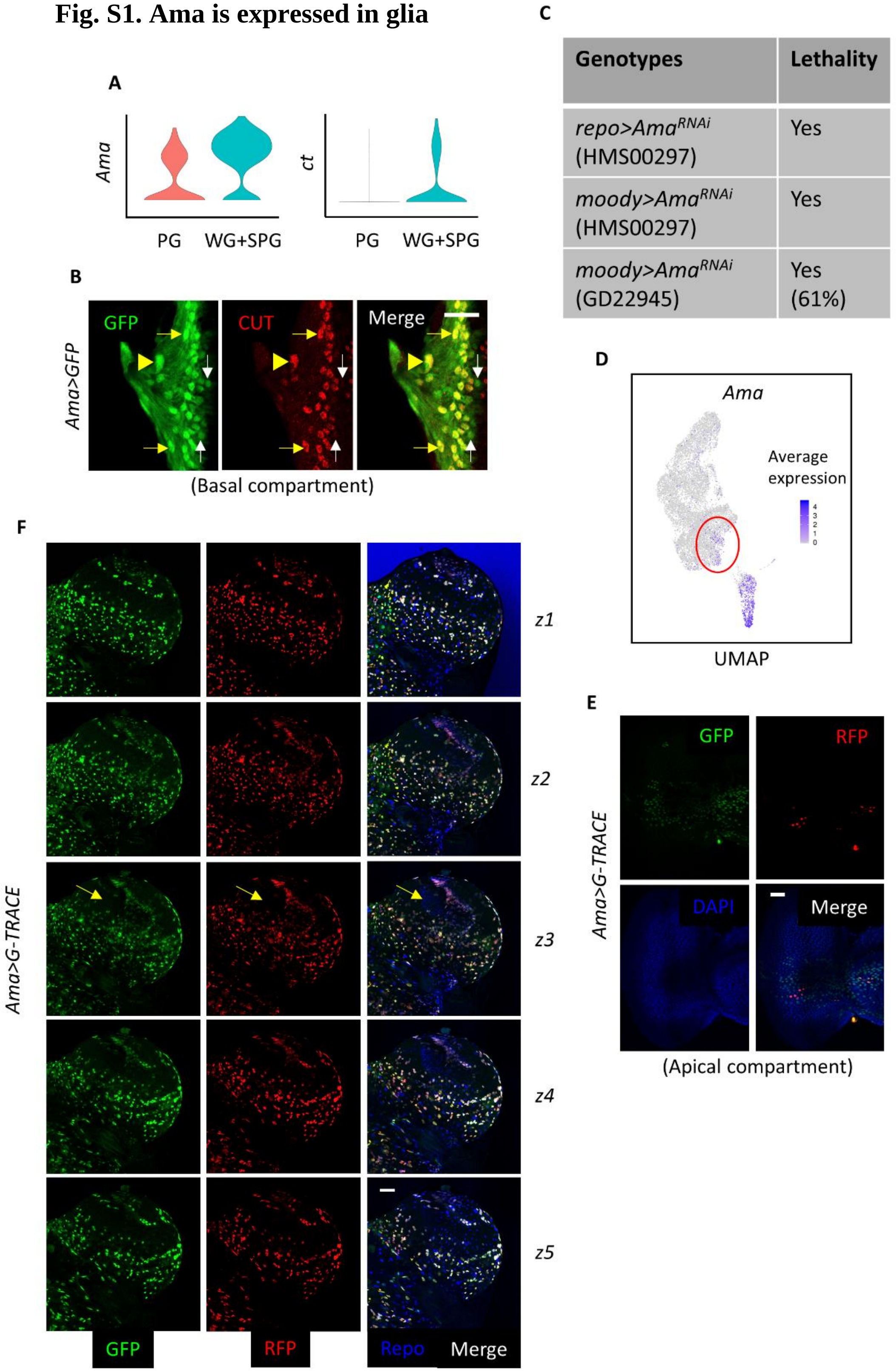
Supplementary Information

Fig. S1. Ama is expressed in glia



Genotypes	Lethality
repo>Ama^{RNAi} (HMS00297)	Yes
moody>Ama^{RNAi} (HMS00297)	Yes
moody>Ama^{RNAi} (GD22945)	Yes (61%)

Fig. S1. Ama is expressed in glia.

(A) Violin plot using scRNA-seq eye disc dataset shows *Ama* expression to be higher in WG+SPG than PG. The plot shows *ct* to be exclusively in WG+SPG.

(B) CUT Immunofluorescence using *Ama>GFP* eye discs reveals that *Ama* expression is higher in WG. CUT marks WG and SPG whereas GFP labels *Ama* expressing cells. White arrow labels PG, yellow arrow labels WG and yellow arrowhead labels SPG.

(C) Table showing lethality using two different *UAS-Ama*^{RNAi} lines (HMS00297 and GD22945).

(D) Feature plot on third instar eye disc UMAP for *Ama* shows some expression level in the ventral peripodial epithelium (VPE) outlined in red.

(E) *Ama>G-TRACE* showing lineage expression in VPE indicates that *Ama* expression is lost during VPE development. GFP lineage expressed is spread out in many cells in VPE whereas RFP, current expression, is in some cells in that area.

(F) *Ama>G-TRACE* z-stacks reveal that *Ama* is expressed in the same glial cells in the brain and shows lack of expression in glia in the medulla region of the optic lobe. Yellow arrow points at glia in the medulla of optic lobe. GFP displays lineage expression whereas RFP shows current expression. Scale bar is 20 µm.

Fig. S2. scRNA-seq in *Drosophila* control brains reveal a considerable amount of cellular heterogeneity.

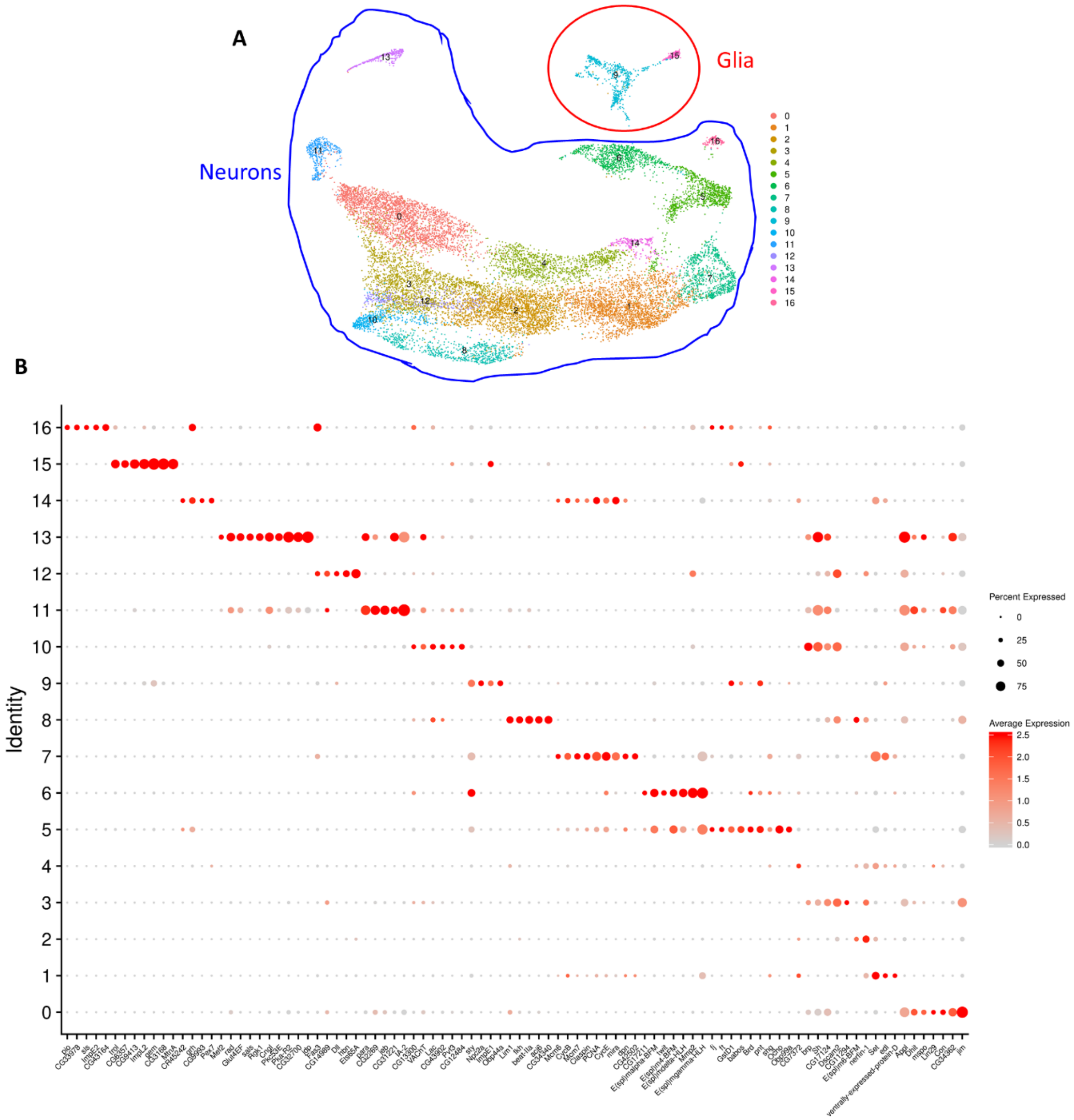


Fig. S2. scRNA-seq in *Drosophila* control brains reveal a considerable amount of cellular heterogeneity.

(A) UMAP of 16,553 cells in the control *repo*>+ brains identify 17 distinct cell clusters. The neuronal clusters are outlined in blue based on *elav* expression whereas glial clusters are outlines in red based on *repo* expression.

(B) Dot plot showing distinct biomarkers in each cluster in A.

Fig. S3. scRNA-seq supervised analysis in glial cells from *repo*>+ (control) brains reveal heterogeneity in glia.

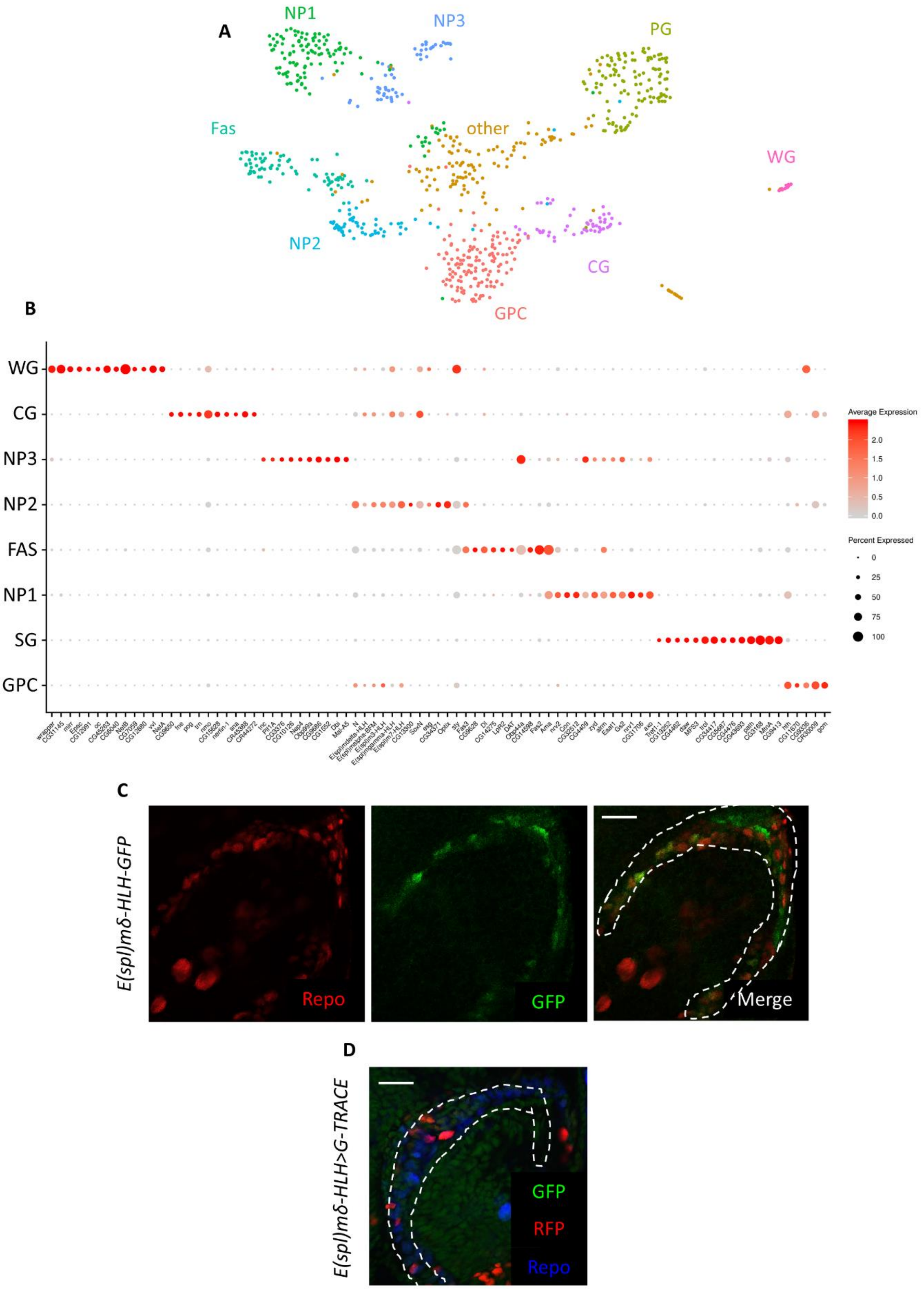


Fig. S3. scRNA-seq supervised analysis in glial cells from *repo*>+ (control) brains reveal heterogeneity in glia.

(A) UMAP of 760 repo>+ glial identifies 8 distinct glial clusters:

GPC: Glia Progenitor Cells, display *gcm* as a top marker which was shown to be expressed in these progenitor cells (Chotard et al., 2005; Soustelle and Giangrande, 2007)

SG: Surface glia, have top markers such as *CG3168*, *trol* (Ariss et al., 2018), and *Tret1-1* (Volkenhoff et al., 2015) shown to be expressed at the surface of the brain

NP1: Neuropil associated glia 1, express neuropil glia specific markers such as *Eaat1* and *Gs2*, and *alrm* (Luna et al., 2017; Yildirim et al., 2019). This cluster displays a high expression level of genes involved in cell oxidation-reduction homeostasis such as NADH/NADPH production and recycling. For instance, transketolase (*CG8036*) is an oxireductase gene in the pentose phosphate pathway, which is important for NADPH production, is highly expressed in NP1. *Nmdmc* is also a top marker that functions to regenerate NADH. *Mtacp1* is another marker in NP1 which recycles NAD and the thioredoxin peroxidase, *Jafrac1*, is a gene highly expressed in this cluster which utilizes NADPH to neutralize reactive oxygen species. This is interesting since glial cells are involved in multiple neuronal homeostatic processes (Yildirim et al., 2019). NP1 are likely clustering by this biological function.

NP2: Neuropil associated glia 2, display *Notch* (*N*) as a top marker and *E(spl)* genes which are expressed when Notch signaling is activated. Two *E(spl)mδ-HLH* reporters (Suppl. Fig. 3c-d) show that NP2 represents neuropil associated glia in the optic lobe.

NP3: Neuropil associated glia 3, also expresses the neuropil glia Eaat1 and Gs2, and alrm like NP2. This cluster however expresses a multitude of genes involved in the enzymatic activity of macromolecule and peptide breakdown. For instance, Acetylcholine esterase (*Ace*), metallopeptidase *Nep4*, Sphingomyelin phosphodiesterase (*CG3376*), Alpha-glucosidase (*Mal-A5*), and serine protease rhomboid (*rho*) are top markers in this cluster. This is interesting since glial cells have been shown to clear neuronal debris (Doherty et al., 2009). Not only does NP3 have markers that promote marcomolecule breakdown, but the cluster also expresses *crq*, *Arc1* (Ashley et al., 2018*)*, *myo* (Awasaki et al., 2011), *cathD* (Eissenberg et al., 2011) which are genes involved in neuronal clearance, remodeling, and lysosomal peoteolysis. These findings suggest that NP3 groups glia involved in neural clearance.

We interestingly observe that *zyd*, a specific marker of CG (Melom and Littleton 2013), to be specifically expressed in NP1 and NP3 suggesting that some CG function in either in cellular homeostasis or neural clearance.

CG: Cortex glia, displays *nerfin-1* and *trn* as top markers which have been shown to be expressed near the medulla cortical region in the optic lobe between the brain surface and neuropil (Xu et al., 2017; Chang et al., 1993). Hence, this cluster was labeled as CG.

Fas: Fasciclin cluster, reveals to have top markers of different cell types such as *Fas2*, *alrm*, and *Ama*. For example, *Fas2* is highly expressed in surface glia (DeSalvo et al., 2014), *alrm* is a neuropil associated glia marker (Doherty et al., 2009), and *Ama* is expressed in multiple glial cell types. Since *Fas2*, *Fas3*, and *Ama* are all cell adhesion genes, the Fas cluster is likely pooling glia by that biological function.

WG: Wrapping Glia, originate from the eye disc (and optic stalk) or the peripheral nervous system (PNS). Since the dissection included some part of the optic stalk and PNS, Drop-seq captured these glial cells. WG displays top makers such as *wrapper* (Noordermeer et al., 1998), *NetA*, *NetB* (Harris et al., 1996), *vvl* (Wheeler et al., 2006) and *Epac* (Kearney et al., 2004) that have been shown to be expressed in midline glia. Midline glia are a category of wrapping glia that surround neuronal axons (Yildirim et al., 2019). This cluster also shares common top markers with recently published scRNA-seq data on WG in the third instar eye-disc (Ariss et al., 2018) such as *CG9336*, *Clic*, and *nuf*.

The “other” cluster represents low quality cells that predominantly express ribosomal genes.

(B) Dot plot showing top markers in each glial cluster in repo>+ brains

(C) *E(spl)mδ-HLH-GFP* reporter shows GFP expression in neuropil glia in the optic lobe indicating that NP2 are neuropil associated glia in that region of the brain. Repo labels glia.

(D) An *E(spl)mδ-HLH-Gal4* reporter was crossed to *UAS-G-TRACE* shows RFP (current expression of *E(spl)mδ-HLH* to be in neuropil glial cells in the optic lobe. Glia are labeled with Repo. GFP labels lineage expression of *E(spl)mδ-HLH*. Scale bar is 20 µm.

Fig. S4. Supervised scRNA-seq analysis using in *repo*>+ and *repo*>*Ama*^{RNAi} glia identifies distinct cellular perturbations in gene expression.

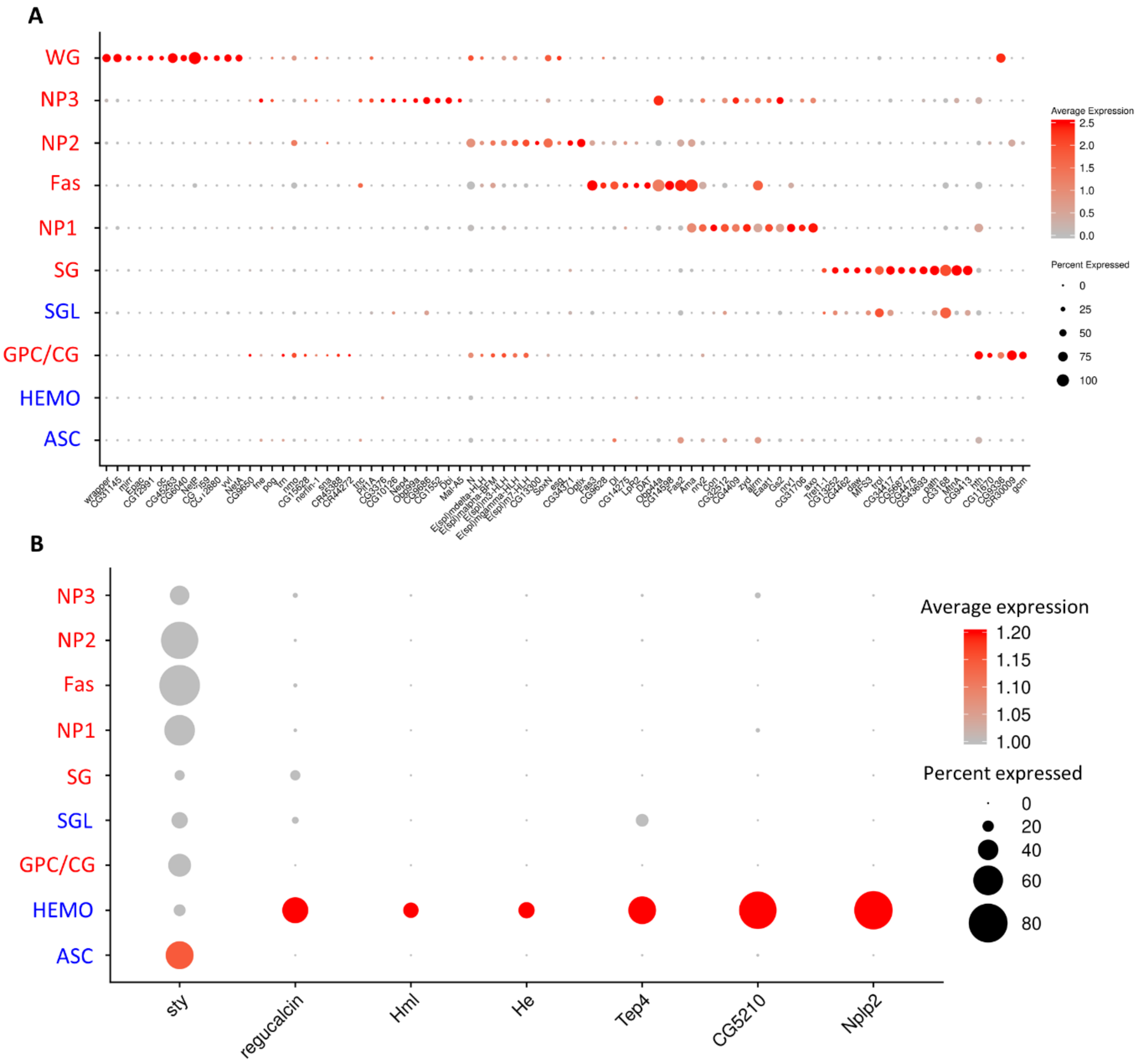


Fig. S4. Supervised scRNA-seq analysis using in *repo*>+ and *repo>Ama*^{RNAi} glia identifies distinct cellular perturbations in gene expression.

(A) Dot plot showing of top markers in the supervised analysis similar to Figure 2F however showing WG and HEMO in *repo*>+ and *repo>Ama*^{RNAi}. Red clusters are observed in the *repo*>+ supervised analysis. Blue clusters are additional clusters that appeared after pooling control cells with *repo>Ama*^{RNAi} brains.

(B) Dot plot showing expression of hemocyte markers in the HEMO cluster as well as sty highly expressed in ASC. Blue clusters are additional clusters that appeared after pooling control cells with *repo>Ama*^{RNAi} brains.

Fig. S5. Ama depletion affects neuronal cells.

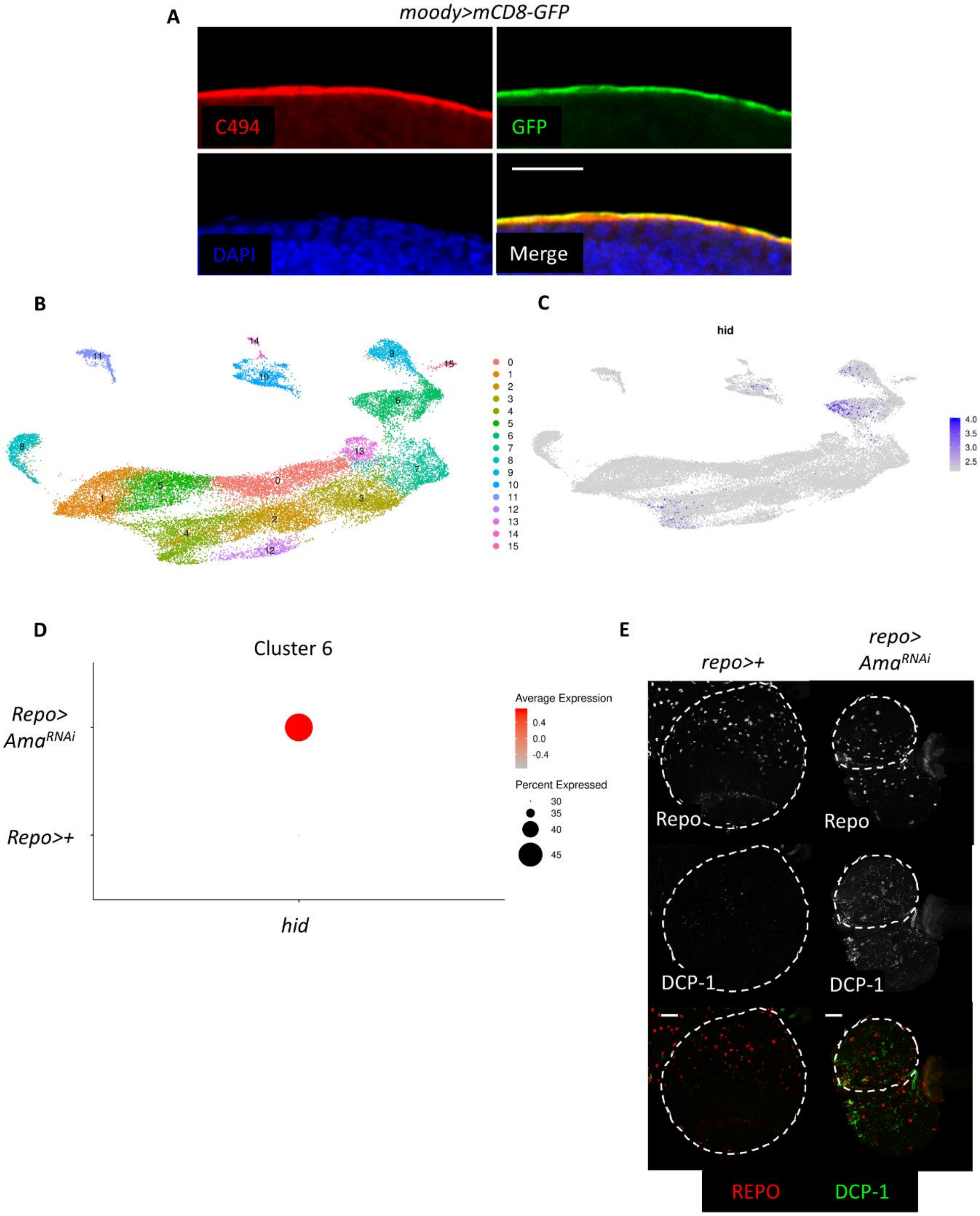


Fig. S5. Ama depletion affects neuronal cells.

(A) C494 immunofluorescence in moody>mCD8GFP brains shows that C494 colocalizes with SPG membranes. GFP labels SPG membranes

(B) UMAP of pooled *repo>+* and *repo>Ama*^{RNAi} 25,700 cells

(C) Feature plot of UMAP in B showing expression of the pro-apoptotic gene hid predominantly in cluster 6.

(D) Dot plot showing that expression originated from *repo>Ama*^{RNAi} cells.

(E) Cleaved DCP-1 immunofluorescence shows a prominent neuronal apoptotic signal in *repo>Ama*^{RNAi} showing the whole brains from Figure 3H. Scale bar is 20 µm.

Fig. S6. Ama affects Sty in the brain.

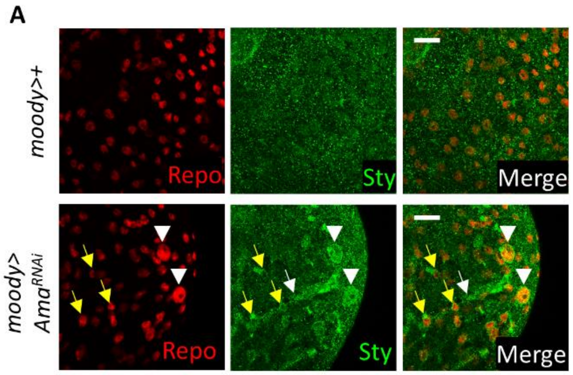


Fig. S6. Ama affects Sty in the brain.

(A) Sty immunofluorescence in SG shows an increase in Sty especially at the membranes in *moody>Ama*^{RNAi} (GD22945). White arrowheads point at SPG cells whereas white arrow points at SPG membranes. Yellow arrows point at PG cells. Repo labels all glia.

Fig. S7. Ama over expression in the eye affects RTK signaling.

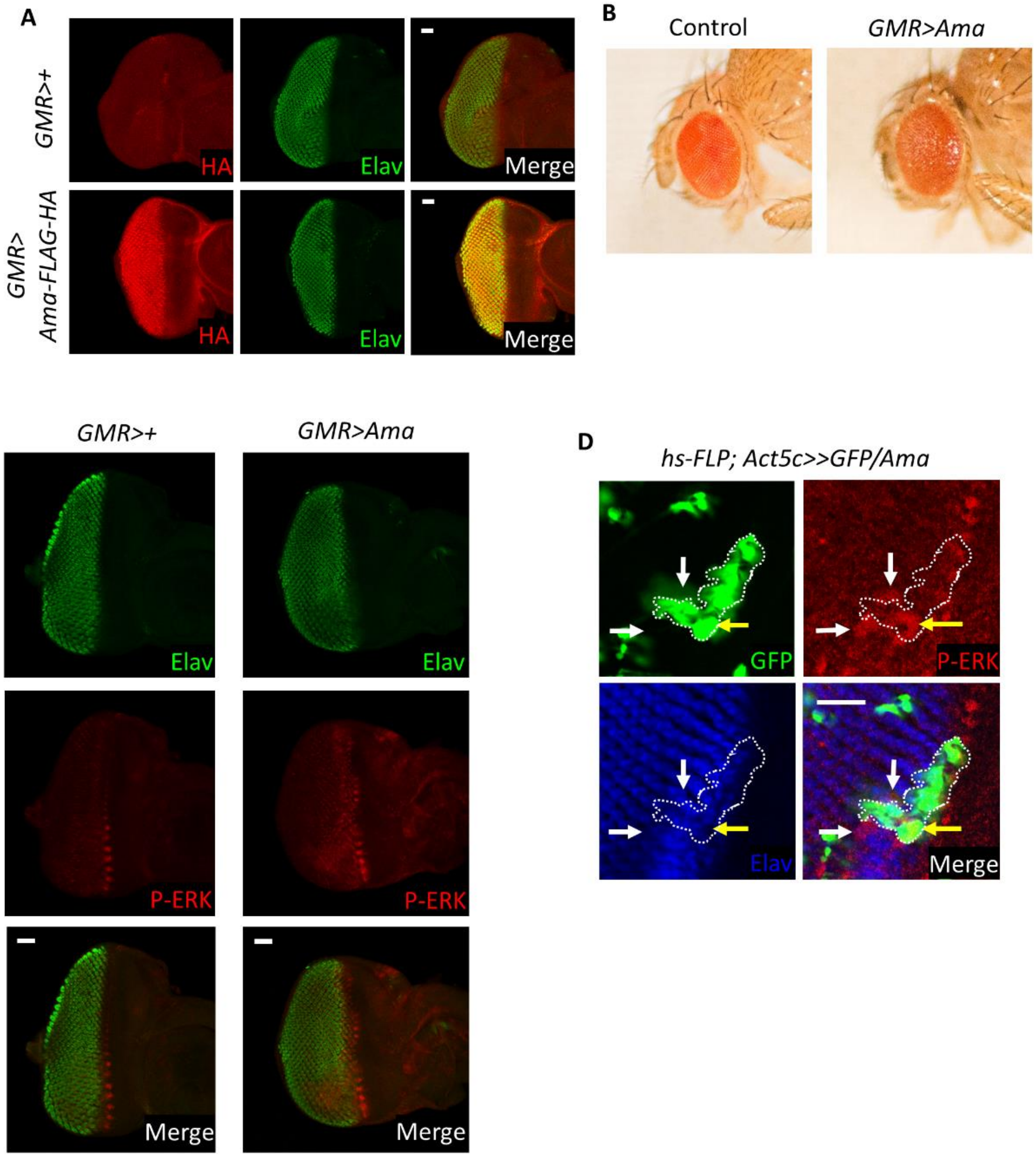


Fig. S7. Ama over expression in the eye affects RTK signaling.

(A) HA immunofluorescence shows HA signal after over expressing HA-tagged Ama in the eye disc. Elav labels photoreceptors.

(B) Ama over expression in the eye disc results in a mild rough eye phenotype

(C) Immunofluorescence showing that Ama over expression increases P-ERK levels in the eye disc. Elav labels photoreceptors.

(D) P-ERK immunofluorescence in a clone of cells using FLP-Out to over express Ama. GFP labels the clone of cells in the eye disc that over express Ama. Yellow arrow points at cell autonomous increase of P-ERK. White arrows point at cell non-autonomous increase of P-ERK. Elav labels photoreceptors. Scale bar is 20 µm.

Fig. S8. Sty knockdown in *Ama* depleted glia does not suppress apoptosis or recover SPG membranes

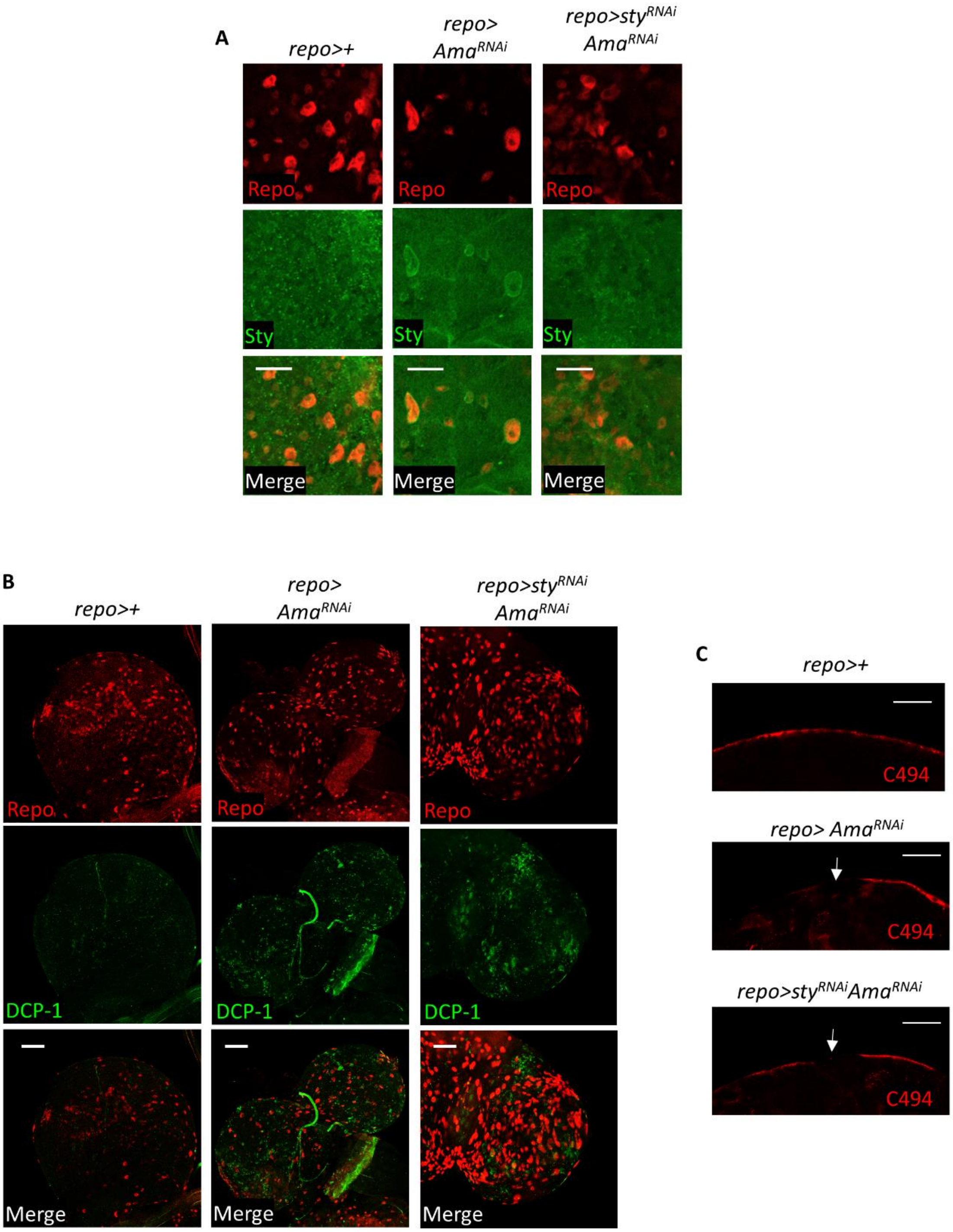


Fig. S8. Sty knockdown in *Ama* depleted glia does not suppress apoptosis or recover SPG membranes.

(A) Immunofluorescence shows a decrease in Sty levels in glia in *repo>sty*^{RNAi} *Ama*^{RNAi} relative to *repo>Ama*^{RNAi}. Repo labels glia.

(B) Cleaved DCP-1 immunofluorescence shows a neuronal apoptosis in both *repo>sty*^{RNAi} *Ama*^{RNAi} and *repo>Ama*^{RNAi} brains. Repo labels glia.

(C) C494 immunostaining shows that knockdown of *Ama* and *sty* in glial cells results in discontinuous SPG membranes and does not rescue the *Ama* single knockdown phenotype. Scale bar is 20 µm.

Supplementary tables

Table S1: Top markers in the scRNA-seq clusters in repo>+ BRAINS

Click here to Download Table S1

Table S2: Average expression of each gene in every clusters in repo>+ BRAINS

Click here to Download Table S2

Table S3: Top markers in the scRNA-seq clusters in repo>+ GLIA

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Table S4: Average expression of each gene in every clusters in repo>+ GLIA

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Table S5: Top markers in the scRNA-seq clusters in the pooled repo>+ and repo>AmaRNAi GLIA

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Table S6: Average expression of each gene in every clusters in the pooled repo>+ and repo>AmaRNAi GLIA

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Table S7: List of cell barcodes used in the supervised glial cell scRNA-seq analyses

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Table S8: Raw numbers of glial cells expressing Ama in Figure 1E

	Glial cells expressing *Ama* in wild type brains		
	Brain1	Brain2	Brain3
% *Ama* expressing glia	59.19811321	55.84269663	57.69230769
% *Ama* negative glia	40.80188679	44.15730337	42.30769231

	Ama positive	*Ama* negative	
Average %	57.57770584	42.42229416	values used in the manuscript are in yellow
Standard deviation	1.372237902	1.372237902	
Standar error	0.792261922	0.792261922	

Table S9: Raw numbers of PH3 positive glia in Figure 3D

Quantification the number of glia cells co-labeled with Repo and PH3 from Figure 3C following Ama depletion.

Relative number of PH3 glia	***repo>+***	***repo>AmaRNAi***	
brain 1	0.831776	0.327103	brain 1
brain 2	1.056075	0.317757	brain 2
brain 3	1.130841	0.252336	brain 3
brain 4	0.841121	0.28972	brain 4
brain 5	1.140187	0.299065	brain 5
		0.233645	brain 6
Average	1	0.286604	
Standard deviation	0.136717	0.033552	
Standar error	0.061142	0.013698	

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>AmaRNAi	*Variable 1*	*Variable 2*
Mean	1	0.286604
Variance	0.023364	0.001351
Observations	5	6
Pooled Variance	0.011135	
Hypothesized Mean Difference	0	
df	9	
t Stat	11.1649	
P(T<=t) one-tail	7.1E-07	
t Critical one-tail	1.833113	
P(T<=t) two-tail	1.42E-06	
t Critical two-tail	2.262157	

Table S10: Raw Sty fluorescence values in glia nuclei in Figure 4C

Sty relative fluorescent units in each surface glia nuclei		
repo>+	***repo>AmaRNAi***	***repo>Ama***
0.488413	0.852759902	0.594492186
0.698653	0.903633239	0.564371078
0.745242	1.008561493	0.608561493
0.702874	1.098109437	0.620712259
0.976074	1.004676446	0.684967425
1.577825	0.991966106	0.564099285
1.378632	1.411103561	0.602805868
1.50273	1.534945441	0.623733962
0.855686	1.136608178	0.628626244
0.774116	1.39364483	0.620680283
0.870538	1.003877053	0.614413046
1.014509	0.937943163	0.698716975
1.149207	0.882657181	0.617962349
0.917671	0.880690675	0.54488189
0.783836	1.094112475	0.564514969
0.842736	0.893225149	0.605188057
1.001047	0.834501779	0.579239778
1.030305	0.984819537	0.604900276
1.088549	0.848475159	0.615548183
1.190104	1.786706103	0.702522083
1.014765	1.578272513	0.534873496
1.105416	0.862016867	0.526463887
0.990303	0.887325633	0.452663975
1.031152	0.86764459	1.022870618
1.261681	0.926288021	1.056764859
1.405876	1.00873736	1.170982054
0.993709	1.105975459	1.179887286
1.166649	1.351740677	1.135313162
1.17095	1.024517367	1.070978057
1.202734	0.975786402	1.205467844
1.348527	0.90496023	1.23330269
1.33748	1.217234901	1.119900875
1.381206	0.821247852	1.23843479
1.495791	0.817506695	1.281857788
1.389296	0.890571166	1.325312762
1.421016	0.932059635	1.151045206
1.325505	1.036524242	1.257204525
1.381446	1.100651505	1.166761261

0.999257	1.136224469	0.978072665
1.067812	1.058427595	1.059610696
0.912906	1.011615172	0.954698429
0.926656	1.213941405	0.932906991
0.883824	1.058203765	0.925200847
0.953307	1.556816819	0.723833886
1.017147	1.390783005	0.823326272
0.882098	0.992014069	1.050225828
0.916775	1.362036852	0.726120149
0.995563	1.649178624	0.683256725
0.932331	1.764482993	0.682633199
0.969327	1.552803869	0.921923338
0.885935	1.735800791	0.806938727
0.662552	1.860458052	0.765562173
0.898901	1.100747432	0.680474839
1.017227	0.815444262	0.712786282
1.125017	1.049858108	0.721435709
0.864191	0.964914665	0.796978296
1.155698	0.82014469	0.709844518
0.763692	1.618402014	0.878692194
0.948431	1.052496103	0.722826652
1.003206	1.602829849	0.629425637
0.809545	1.282465326	0.689412047
0.613342	1.329773372	0.587889204
0.716607	1.218849674	0.708885247
0.558823	0.873128422	0.570366521
0.506543	1.2903953	0.509340901
0.492202	1.66273632	0.489196211
0.880323	1.030896519	0.534377873
1.09982	1.041640353	0.493688797
1.079739	1.511651145	0.533306687
0.448955	1.351436908	0.580502818
	1.469667053	0.598856869
	0.854662457	0.507598225
	1.233286702	0.501267037
	0.959398857	0.518693793
	1.854382669	0.529437627
	1.084088093	1.062664375
	1.520508414	1.054622487
	1.478156601	0.955226028
	1.146041009	1.153507334
	1.219041528	1.066309605
	1.006499061	1.132371398

	1.132691155	1.212054838
	1.450369719	1.224285543
	1.393325073	1.155345937
		1.111011631
		1.115855949
		1.179903274
		0.980278988
		0.96264439
		0.988800512
		0.9018426
		0.85128902
		0.92005276
		0.983476558
		1.045285583
		1.192181942
		1.189783764
		0.970398497
		1.077037452
		1.173476158
		0.936712099
		1.054046924
		0.937399576
		1.065462249
		1.127974739
		0.793556897
		0.820800192
		0.840129502
		0.683064871
		0.823662017
		0.921523642
		0.532699149
		0.997034254
		1.412190735
		1.141708302
		1.271034014
		0.535241217
		0.610735841
		0.538406811
		0.574427435
		0.617882409
		0.622247092
		0.784395859
		0.745465446

		0.67057836
		0.608017906
		0.55060554
		0.53217155
		0.309284943
		0.294528159
		0.871929334
		0.86660538
		1.027922779
		0.809976418
		0.878020704
		0.745001799
		0.817554658
		0.767416763
		0.781390143
		0.71942124
		0.853974979
		0.750325752
		0.798529118
		0.899412447
		0.615756025
		0.73213158
		0.673088453
		0.780430873
		0.987905192
		1.035628922
		1.182205524
		1.237459531
		1.17160558
		1.164331108
		1.024293537
		0.933306687
		0.853143611
		0.80207842
		0.631839802
		1.003173588
		1.118797714
		0.816259643

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>AmaRNAi	*Variable 1*	*Variable 2*
Mean	1	1.173271361
Variance	0.067123103	0.081617651
Observations	70	84
Pooled Variance	0.075037889	
Hypothesized Mean Difference	0	
df	152	
t Stat	-3.908542022	
P(T<=t) one-tail	6.97296E-05	
t Critical one-tail	1.654940175	
P(T<=t) two-tail	0.000139459	
t Critical two-tail	1.975693928	

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>Ama	*Variable 1*	*Variable 2*
Mean	1	0.839470546
Variance	0.067123103	0.059037504
Observations	70	162
Pooled Variance	0.061463184	
Hypothesized Mean Difference	0	
df	230	
t Stat	4.526992124	
P(T<=t) one-tail	4.80062E-06	
t Critical one-tail	1.651505638	
P(T<=t) two-tail	9.60124E-06	
t Critical two-tail	1.970331773	

Table S11: Raw P-ERK fluorescence values in glia nuclei in Figure 4E

P-ERK relative fluorescent units in each surface glia nuclei		
repo>+	***repo>AmaRNAi***	***repo>Ama***
0.825735	0.338684925	1.111179318
0.631023	0.432912289	1.611521662
0.481242	0.407744068	0.728296541
0.489812	0.336914178	1.305914296
0.570983	0.358399244	0.731767206
1.272719	0.448542085	0.913398654
0.628781	0.399622241	1.079494747
0.623728	0.342344469	1.252225239
0.704592	0.37391099	0.965198914
0.770039	0.358564514	1.2383898
0.776603	0.390083815	1.352921733
0.770818	0.346830362	1.248447645
0.748979	0.415724236	1.277912879
0.626892	0.345838744	1.14180144
0.750277	0.334671231	1.05774997
1.35651	1.371809704	0.654798725
0.754079	1.699091016	1.23860229
0.939511	1.017093614	1.81933656
1.371456	1.541376461	1.233290048
1.929288	0.977263605	1.299492386
0.844174	0.854869555	0.94843584
0.857679	0.84495337	1.507378114
0.91125	0.805902491	1.423515524
1.159627	0.714461103	0.934907331
0.87992	1.03234565	1.497391099
1.139582	1.468209184	1.321095502
0.94624	0.852508559	1.654893165
0.823941	0.871325699	1.661763664
0.762531	1.082068233	1.490898359
0.670547	1.003258175	1.567252981
1.04212	0.97431236	0.656356983
0.901027	0.937268327	1.107023964
1.216409	0.989871326	1.123362059
1.049605	0.490662259	1.485609727
0.894747	0.352543974	2.567323811
0.911415	0.440349427	1.887097155
0.962318	0.402455436	0.928272931
1.093779	0.404367843	1.884358399

1.163027	0.478668398	1.645732499
1.513422	0.363357337	1.086199976
1.027057	0.369779247	1.181466179
0.952568	0.420233739	0.814402078
0.826065	0.488419313	1.201180498
1.178609	0.522039901	0.959343643
0.997568	0.532806044	1.015606186
1.558187	0.510400189	1.287947114
1.408665	0.513988903	1.131625546
1.180238	0.727234093	0.958517294
0.997356	0.543855507	0.843938142
0.902562	0.344563806	1.167111321
0.652768	0.343973557	0.950324637
1.404297	0.272695077	0.879990556
0.884571	0.371880534	0.562578208
0.71923	0.541211191	0.521803801
0.720977	0.498429937	0.588407508
0.685515	0.704001889	0.736796128
1.126124	0.489765081	0.500908984
0.648991	0.517837327	0.921119112
1.516562	0.473497816	0.463180262
0.711888	0.434989966	0.905276827
0.749427	0.446865777	0.825073781
0.774997	0.493094086	0.678621178
0.935946	0.478148979	0.779294062
0.836737	0.513327824	0.834659426
0.905017	0.446086649	1.21933656
1.368552	0.372423563	0.926785503
1.4879	0.357242356	1.058670759
0.954858	0.58715618	1.190910164
0.771762	0.548010861	1.301192303
0.955849	0.36146854	0.98087593
0.707	0.369873687	0.913280604
0.73741	0.602809586	0.893495455
0.777075	0.564089246	0.828945815
0.933467	0.414378468	0.743595797
0.959414	0.191051824	0.569330658
1.106977	0.208853736	0.731058907
0.871515	0.388738047	1.063983001
0.86344	0.228874985	1.204367843
2.00765	0.281100224	1.230149923
1.816716	0.220233739	1.146004014
1.082139	0.372636052	0.901333963

1.127659	0.279376697	0.78075788
1.304167	0.326384134	1.128131271
1.200897	0.286861055	1.050903081
1.035273	0.254232086	1.242875694
1.634848	0.340384842	0.768645969
1.369803	0.330704757	1.730563098
1.179105	0.361633809	1.876944871
1.275009	0.287238815	1.255436194
1.313989	0.325935545	0.750442687
1.42871	0.301074253	1.160004722
1.183591	0.310069649	1.455672294
1.637776	0.319773344	1.753771692
1.343737	0.295030103	1.419690709
1.001204	0.255034825	0.675811592
1.355236	0.261952544	0.913469484
1.161752	0.468728603	0.866202337
1.378397	0.335922559	1.247337977
1.497604	0.287734624	1.727375753
1.288726	0.249155944	1.307803093
0.945508	0.291606658	1.239948058
0.83669	0.360099162	1.315594381
0.826113	0.305819856	1.127635462
1.283957	0.29035533	1.0002361
0.843206	0.260772046	1.207201039
1.582293	0.415558966	0.98038012
0.795325	0.449510093	0.798748672
0.937079	0.45132806	0.739841813
1.038862	0.527942392	1.330185338
1.296518	0.538212726	1.652437729
1.22302	0.439310589	1.200779129
1.079802	0.459213788	1.529547869
1.16867	0.41938378	1.089458151
1.155991	0.43860229	1.383236926
1.133845	0.546853972	1.062117814
0.986802	0.636406564	0.713493094
0.937174	0.384606304	1.159532523
0.667123	1.029040255	1.033927517
0.802219	0.372187463	
0.580711	0.359060323	
0.680085	0.274442215	
0.746901	0.342840279	
0.638366	0.326714674	
0.986566	0.261173415	

0.676945	0.262259473	
0.619573	0.430220753	
1.293	0.388997757	
1.22819	0.417872742	
1.437634	0.561987959	
1.224247	0.75483414	
1.274041	0.553110613	
1.293094	0.478951718	
1.49418	0.766473852	
1.459237	0.389682446	
1.283107	0.478432298	
1.20772	0.511604297	
1.254468	0.403659544	
0.718003	0.243890922	
0.753347	0.267996695	
0.599386	0.165836383	
0.680439	0.229063865	
0.905206	0.208027388	
0.614497	0.216314485	
1.095101	0.242049345	
0.650407	0.295620352	
0.826372	0.318687286	
0.494463	0.315570771	
1.477393	0.443465943	
1.038673	0.266745367	
0.987085	0.264101051	
0.644741	0.396127966	
1.337245	0.287215205	
1.195939	0.30317554	
1.044103	0.205666391	
1.177877	0.241789635	
0.932428	0.226655649	
1.431496	0.246275528	
1.29876	0.1744068	
1.220116	0.157525676	
0.532098	0.181112029	
0.603046	0.177641365	
0.614945	0.186329831	
0.658576	0.210482824	
0.630717	0.204816433	
0.513186	0.200684689	
0.550254	0.203990084	
0.632747	0.171880534	

0.623256	0.220942038	
0.724448	0.301310353	
0.805855	0.352921733	
0.880061	0.465234329	
	0.451233621	
	0.2319915	

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>AmaRNAi	*Variable 1*	*Variable 2*
Mean	0.999988816	0.447412628
Variance	0.096133469	0.067234827
Observations	171	173
Pooled Variance	0.081599649	
Hypothesized Mean Difference	0	
df	342	
t Stat	17.93865624	
P(T<=t) one-tail	1.73814E-51	
t Critical one-tail	1.649321259	
P(T<=t) two-tail	3.47629E-51	
t Critical two-tail	1.966924645	

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>Ama	*Variable 1*	*Variable 2*
Mean	0.999988816	1.126518991
Variance	0.096133469	0.123494811
Observations	171	118
Pooled Variance	0.107287744	
Hypothesized Mean Difference	0	
df	287	
t Stat	-3.227819109	
P(T<=t) one-tail	0.000695988	
t Critical one-tail	1.650180211	
P(T<=t) two-tail	0.001391976	
t Critical two-tail	1.968264113	

Table S12: Raw glial cell count in Figure 5B

Relative number of glia in each brain	*repo>+*	*repo>AmaRNAi*	*repo>Ama*	*repo>styRNAi AmaRNAi*	*repo> rlSEM AmaRNAi*
	0.927316	0.281235	1.022328	0.31829	0.408551
	1.1962	0.195724	0.682185	0.39905	0.378147
	0.938717	0.124466	1.167696	0.360095	0.349644
	0.937767	0.166271		0.495962	0.394299
Average	1	0.191924	0.957403	0.393349	0.38266
Standard deviation	0.113364	0.057445	0.203456	0.065768	0.021888
Standar error	0.056682	0.028722	0.117465	0.032884	0.010944

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>AmaRNAi	*Variable 1*	*Variable 2*
Mean	1	0.191924
Variance	0.017135	0.0044
Observations	4	4
Pooled Variance	0.010768	
Hypothesized Mean Difference	0	
df	6	
t Stat	11.01307	
P(T<=t) one-tail	1.67E-05	
t Critical one-tail	1.94318	
P(T<=t) two-tail	3.33E-05	
t Critical two-tail	2.446912	

t-Test: Two-Sample Assuming Equal Variances

repo>+ vs repo>Ama	*Variable 1*	*Variable 2*
Mean	1	0.957403
Variance	0.017135	0.062092
Observations	4	3
Pooled Variance	0.035118	
Hypothesized Mean Difference	0	
df	5	
t Stat	0.297617	
P(T<=t) one-tail	0.388983	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.777965	

t Critical two-tail	2.570582

t-Test: Two-Sample Assuming Equal Variances

repo>AmaRNAi vs repo>styRNAi AmaRNAi	*Variable 1*	*Variable 2*
Mean	0.191924	0.393349
Variance	0.0044	0.005767
Observations	4	4
Pooled Variance	0.005084	
Hypothesized Mean Difference	0	
df	6	
t Stat	-3.99526	
P(T<=t) one-tail	0.003579	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.007158	
t Critical two-tail	2.446912	

t-Test: Two-Sample Assuming Equal Variances

repo>AmaRNAi vs repo> rlSEM AmaRNAi	*Variable 1*	*Variable 2*
Mean	0.191924	0.38266
Variance	0.0044	0.000639
Observations	4	4
Pooled Variance	0.002519	
Hypothesized Mean Difference	0	
df	6	
t Stat	-5.37411	
P(T<=t) one-tail	0.000853	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.001705	
t Critical two-tail	2.446912	