

Supplementary information

Glial AP1 is activated with aging and accelerated by traumatic brain injury

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Supplementary Table 1 | Genotypes for figures and extended data figures

Figure	Genotype (name in this paper) See footnote ^b	Additional experiment notes
Figure 1	<i>w¹¹¹⁸</i>	-
Figure 2	<i>TREdsRed</i>	-
Figure 3a	<i>TREdsRed</i> <i>repoGS>UAS-mCD8-GFP</i> <i>elavGS>UAS-mcd8-GFP</i>	geneSwitch (GS) flies maintained on RU486 for 3 d prior to collection
Figure 3b-e	<i>TREdsRed</i>	-
Extended Data Fig. 3a,b	<i>repoGS, TREdsRed>draper-RNAi</i>	see footnote ^a
Extended Data Fig. 3c	Experimental: <i>repoGS, TREdsRed>draper-RNAi</i> WT: <i>repoGS, TREdsRed>w¹¹¹⁸</i>	see footnote ^a
Figures 4a, 4c; Extended Data Fig. 3d,4d	<i>TREdsRed</i>	-
Extended Data Fig. 3e	<i>draper-/-; w¹¹¹⁸ (WT)</i>	-
Figure 4b; Extended Data Fig. 3g	<i>repoGS, TREdsRed>basket-RNAi</i>	see footnote ^a
Extended Data Fig. 3h	<i>daGS, TREdsRed>basket-RNAi</i>	see footnote ^a
Figure 4d	<i>repoGS, TREdsRed>rolled-RNAi</i>	see footnote ^a
Figure 4e; Extended Data Fig. 4e	<i>dFos-GFP</i>	-
Figure 4f; Extended Data Fig. 4e	<i>dJun-GFP</i>	-
Figure 4f; Extended Data Fig. 4c	<i>repoGS, TREdsRed>UAS-puckered</i>	see footnote ^a
Figure 4h	Experimental: <i>repoGS, TREdsRed>UAS-puckered</i> WT: <i>repoGS, TREdsRed>w¹¹¹⁸</i>	see footnote ^a
Figure 4g	Experimental: <i>repoGS>UAS-puckered</i> WT: <i>repoGS>w¹¹¹⁸</i>	Flies flipped to RU486 or vehicle at 12 dpi
Extended Data Fig. 4e	Experimental: <i>repoGS>UAS-puckered</i> Control: <i>repoGS>w¹¹¹⁸</i>	see footnote ^a
Figure 4j-n	<i>repoGS>UAS-puckered</i>	see footnote ^a
Extended Data Fig. 4h	Experimental: <i>repoGS>ERK-RNAi</i> Control: <i>repoGS>w¹¹¹⁸</i>	Flies flipped to RU486 or vehicle at 12 dpi
Extended Data Fig. 4i	<i>daGS>ERK-RNAi</i>	see footnote ^a
Extended Data Fig. 5a	<i>Ets21c-GFP</i>	-
Extended Data Fig. 5b	<i>repoGS>Ets21c-RNAi</i>	see footnote ^a
Extended Data Fig. 5c,d	<i>elavGS>UAS-puckered</i>	see footnote ^a
Extended Data Fig. 5e	<i>repoGS>basket-RNAi</i>	see footnote ^a
Figure 5	<i>TREdsRed</i>	-
Figure 6a,b; Extended Data Fig. 6b-d	<i>repoGS>UAS-0N4R</i>	see footnote ^a
Extended Data Fig. 6a	<i>elavGS>UAS-0N4R</i>	see footnote ^a
Figure 6c,d	Experimental: <i>repoGS, UAS-0N4R>UAS-puckered</i> Control: <i>repoGS, UAS-0N4R>w¹¹¹⁸</i>	Flies flipped to RU486 at 3 dpi
Extended Data Fig. e-g	Experimental: <i>repoGS, 0N4R>dFos-DN</i> Control: <i>repoGS, UAS-0N4R >w¹¹¹⁸</i>	Flies flipped to RU486 at 3 dpi

^a 0 d males maintained on RU486 or vehicle from 3 d prior to experimentation

^b Detailed genotypes of fly lines used in Supplementary Table 2. Carrot (>) indicates that lines from Supplementary Table 2 were crossed to achieve the indicated animals, thus the genotypes are heterozygous over a wildtype chromosome.

Supplementary Table 2. *Drosophila* genotype information

Name in this paper	ID**	Full Genotype	Reference	Notes
<i>w¹¹¹⁸</i>	RRID:BDSC_5905	<i>w[1118]</i>	-	-
<i>basket-RNAi</i>	RRID:BDSC_53310	<i>y[1] sc[*] v[1] sev[21];;P{y[+t7.7] v[+t1.8]=TRiP.HMC03539}attP2</i>	-	-
<i>daGS, TREdsRed</i>	-	<i>w[1118]/w[*];daughterlessGeneSwitch[+mc], P{y[+t7.7] w[+mC]=TRE-DsRedT4}attP16/Sm6a,Cy;</i>	this paper	-
<i>draper-/-</i>	RRID:BDSC_67033	<i>draper^{Δ5}</i>	-	Courtesy of Marc Freeman
<i>draper-RNAi</i>	RRID:BDSC_67034	<i>y[1] w[*]; P{w[+mC]=UAS-drpr.dsRNA}2/CyO</i>	-	-
<i>dFos-DN</i>	RRID_BDSC_38657	<i>w[1118]; PBac{y[+mDint2] w[+mC]=kay-GFP.FLAG}VK00037</i>	-	-
<i>dJun-GFP</i>	RRID_BDSC_50755	<i>w[1118]; PBac{y[+mDint2] w[+mC]=Jra-GFP.FLAG}VK00033/TM3, Sb[1]</i>	-	-
<i>elavGS</i>	-	<i>y*w*;;elavGeneSwitch</i>	-	-
<i>ERK-RNAi</i>	RRID_BDSC_34855	<i>y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00173}attP2</i>	-	-
<i>Ets21c-GFP</i>	RRID_BDSC_38639	<i>w[1118]; PBac{y[+mDint2] w[+mC]=Ets21c-GFP.FLAG}VK00033/TM3, Sb[1]</i>	-	-
<i>Ets21c-RNAi</i>	RRID:BDSC_39069	<i>y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01989}attP2</i>	-	-
<i>MREdsRed</i>	RRID:BDSC_59013	<i>w[*]; P{y[+t7.7] w[+mC]=mRE-DsRedT4}attP40</i>	Chatterjee & Bohmann, 2012.	-
<i>repoGS*</i>	-	<i>w¹¹¹⁸;;P{repoGS}attP154</i>	-	Courtesy of Herve Tricoire; referred to as <i>repoGS2301</i>
<i>repoGS, TREdsRed</i>	-	<i>w[1118]/w[*]; P{repoGeneSwitch}attP40, P{y[+t7.7] w[+mC]=TRE-DsRedT4}attP16/Sm6a,Cy;</i>	This paper	Courtesy of Herve Tricoire; referred to as <i>repoGS16</i>
<i>repoGS,UAS-0N4R</i>	-	<i>w[1118];; P{repoGeneSwitch}attP154, UAS-tau.WT (0N4R)/Tm6c,Sb[1]</i>	This paper	-
<i>rolled-RNAi</i>	RRID:BDSC_34855	<i>y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00173}attP2]</i>	-	-
<i>TREdsRed</i>	RRID:BDSC_59012	<i>w[*]; P{y[+t7.7] w[+mC]=TRE-DsRedT4}attP16</i>	Chatterjee & Bohmann, 2012.	Courtesy of Marc Freeman
<i>UAS-mcd8-GFP</i>	-	<i>yw; UAS-mCD8-GFP/CyO;</i>	-	-
<i>UAS-puckered</i>	-	<i>y-w-;;UAS-puckered</i>	-	Courtesy of Marc Freeman
<i>UAS-0N4R</i>	-	<i>w[1118];;UAS-tau.WT (0N4R)</i>	-	Courtesy of Mel Feany

Supplementary Table 3 | Primer sequences

Target gene	Assay	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Reference ^a
geneSwitch	PCR	gggctacgaagtcaaacc	tctgccagaaggaaacacc	Goodman et al., 2019
pUAS	PCR	agcaaccaagtaaatcaactgc	ttcatcagttccataggttg	Goodman et al., 2019
basket	qPCR	tacggcccataggatcagggtg	tgctgggtgatagatcgtgaag	PA60065
dsRed	qPCR	ccgaggacgtcatcaaggagtt	accttcagcttgccggtctg	This paper
draper	qPCR	gcagatgcctgaataactcctc	tccttcattccatgccgtag	PP31489
dorsal	qPCR	atccgtgtggatccgtttaa	aatcgaccgaattcagatc	PD70364
dFos	qPCR	agaccggccagagtgttct	gccagtgctcctcgatg	PP6106
Ets21c	qPCR	caacgacgacgaaccaa	ggtcgcgttgacgaatc	doi:10.1038/srep34725
MMP1	qPCR	gaaggctcggacaacgag	gtcgttgactggatgacg	doi:10.1038/srep34725
puckered	qPCR	gccacatcagaacatcaagc	ccgtttccgtgcatctt	doi:10.1038/srep34725
RpL32 (house keeping)	qPCR	tgtcctccagcttaagatgaccatc	ctgggctgcgccattgtg	Gabler et al., 2004
upd2	qPCR	tttccggcaaatcagagatcc	gcgcttgataactcgtccttg	PP29178

^aPxxxxxx refers to IDs from FlyPrimerBank (<https://www.flyrnai.org/flyprimerbank>)

Yanhui Hu, Richelle Sopko, Marianna Foos, Colleen Kelley, Ian Flockhart, Noemie Ammeux, Xiaowei Wang, Lizbeth Perkins, Norbert Perrimon, and Stephanie E Mohr. 2013. "FlyPrimerBank: an online database for *Drosophila melanogaster* gene expression analysis and knockdown evaluation of RNAi reagents." *G3* (Bethesda), 3, 9, Pp. 1607-16.

Supplementary Table 4 | Antibody information

Antigen	Source and catalog #	Lot #	Western immunoblot dilution	Whole mount IF dilution	Paraffin IF dilution
Primary antibodies					
aTubulin	DHSB, AA4.3	11/5/15-51ug/ml	1:10,000	-	-
AT100	Invitrogen, MN1060	UH2803903	-	-	1:100
AT8	Invitrogen, MN1020	UH2805521	-	-	1:100
dsRed	ThermoFisher, MA515257	TE264883	1:1000	-	-
elav	DSHB, Rat-Elav-7E8A10	12/27/18-23ug/ml	-	1:20	-
ERK	Sigma, 5670	21190726	1:5000	-	-
GFP	Invitrogen, A11122		-	1:500	
Tau	DAKO, A0024	20024929	1:20,000	-	-
JNK	Santa Cruz Biotechnology, sc571 (discontinued now)	J209	1:500	-	-
pERK	Cell Signaling, 4370	24	1:2000	-	-
pJNK	Promega, V7931	L019	1:5000	-	1:200
repo	DSHB, 8D12	12/6/19-54ug/ml	-	1:5	-
Secondary antibodies					
Goat anti-rabbit AlexaFluor 488	Invitrogen, A11008	1911238	-	1:200	1:200
Goat anti-mouse AlexaFluor 488	Invitrogen, A32723	2120125	-	1:200	1:200
Goat anti-rat AlexaFluor 546	Invitrogen, A-111081	1921310	-	1:200	1:200
Goat anti-mouse AlexaFluor 594	Invitrogen, A11032	1887003	-	1:200	1:200
Goat anti-mouse AlexaFluor 647	Invitrogen, A21236	2011576	-	1:200	1:200
Goat anti-rabbit HRP	Jackson ImmunoResearch, 111-035-144	127629	1:5000	-	-
Goat anti-mouse IgG H&L HRP	Abcam, ab6789	NA	1:5000	-	-