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Supplemental Information

Cell-Autonomous Regulation of Astrocyte Activation by the Circadian Clock Protein BMAL1

Brian V. Lananna, Collin J. Nadarajah, Mariko Izumo, Michelle R. Cedeño, David D. Xiong, Julie Dimitry, Chak Foon Tso, Celia A. McKee, Percy Griffin, Patrick W. Sheehan, Jeffery A. Haspel, Ben A. Barres, Shane A. Liddelow, Joseph S. Takahashi, Ilia N. Karatsoreos, and Erik S. Musiek

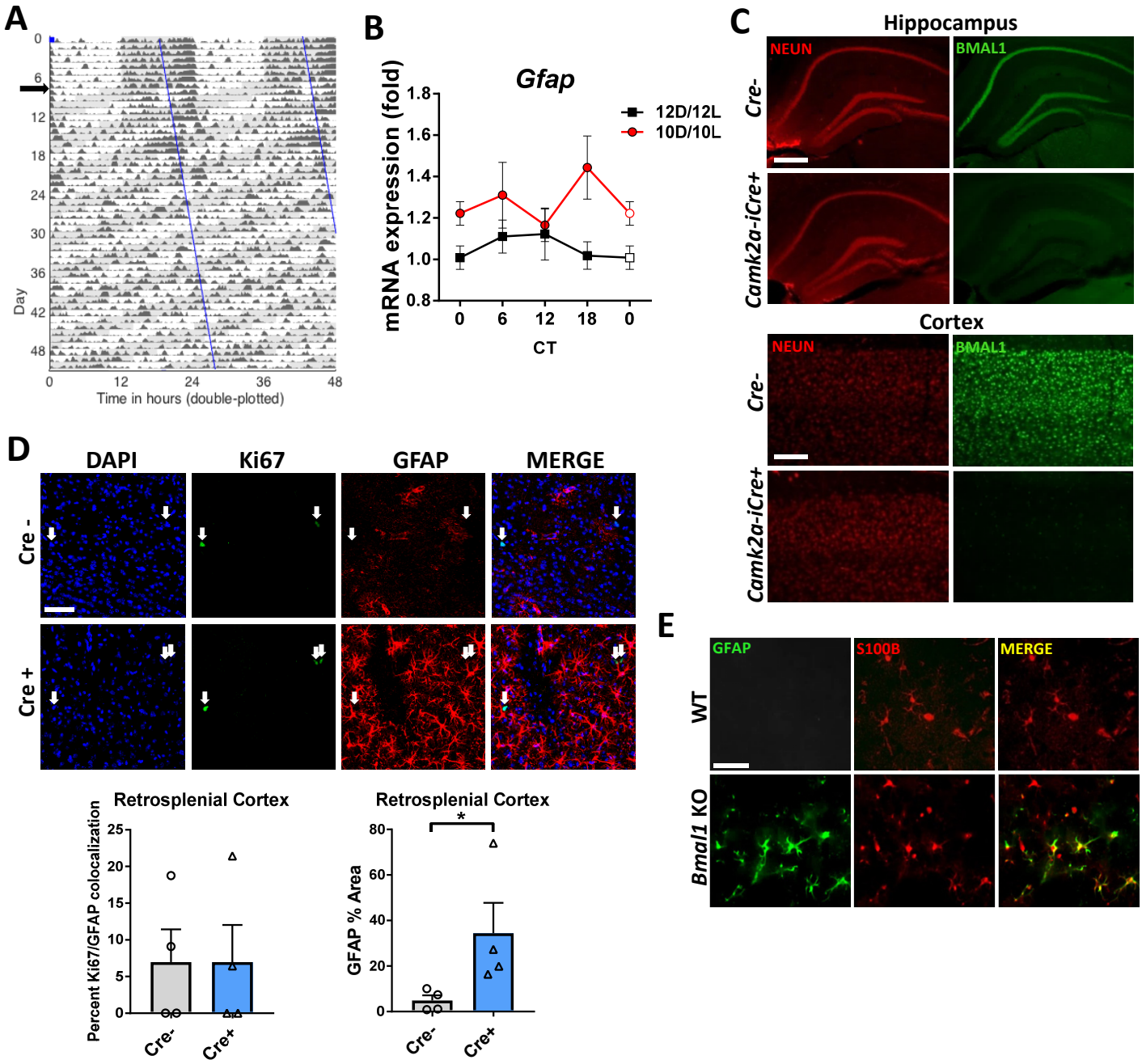


Figure S1. Related to Figure 1; Characterization of mouse models with astrogliosis induced by loss of *Bmal1*.

A. Representative actigraph of a WT mouse housed in 12h:12h light:dark, then switched to 10h:10h light:dark at black arrow, leading to behavioral arrhythmicity.

B. Cortical qPCR showing mRNA levels from 10h:10h light:dark WT mice from Fig. 1A, S1A reveals increased *Gfap* expression at most timepoints throughout the circadian day.

C. Hippocampal (top, scale bars = 150μm) and cortical (bottom, scale bars = 50μm) staining of NEUN (red) and BMAL1 (green) from 4mo *Camk2a-iCre+;Bmal*$^{f/f}$ mice and Cre- controls shows complete loss of neuronal BMAL1 in Cre+ animals.

D. Representative images (top) and quantification (bottom) illustrating minimal localization of the cell division marker Ki67 staining with GFAP+ astrocytes in cerebral cortex from 4 mo *Nestin-Cre+;Bmal1*$^{f/f}$ (NBmal1 KO) mice (left graph), despite large increases in GFAP+ cells as compared to Cre- controls (right graph). Scale bar= 50μm.

E. NBmal1 KO mice have increased GFAP+ activated astrocytes in the cerebral cortex without major changes in number of total (S100B+) astrocytes. Scale bar = 50μm.

All data represent mean+SEM. *p<0.05 by 2-tailed T-test.

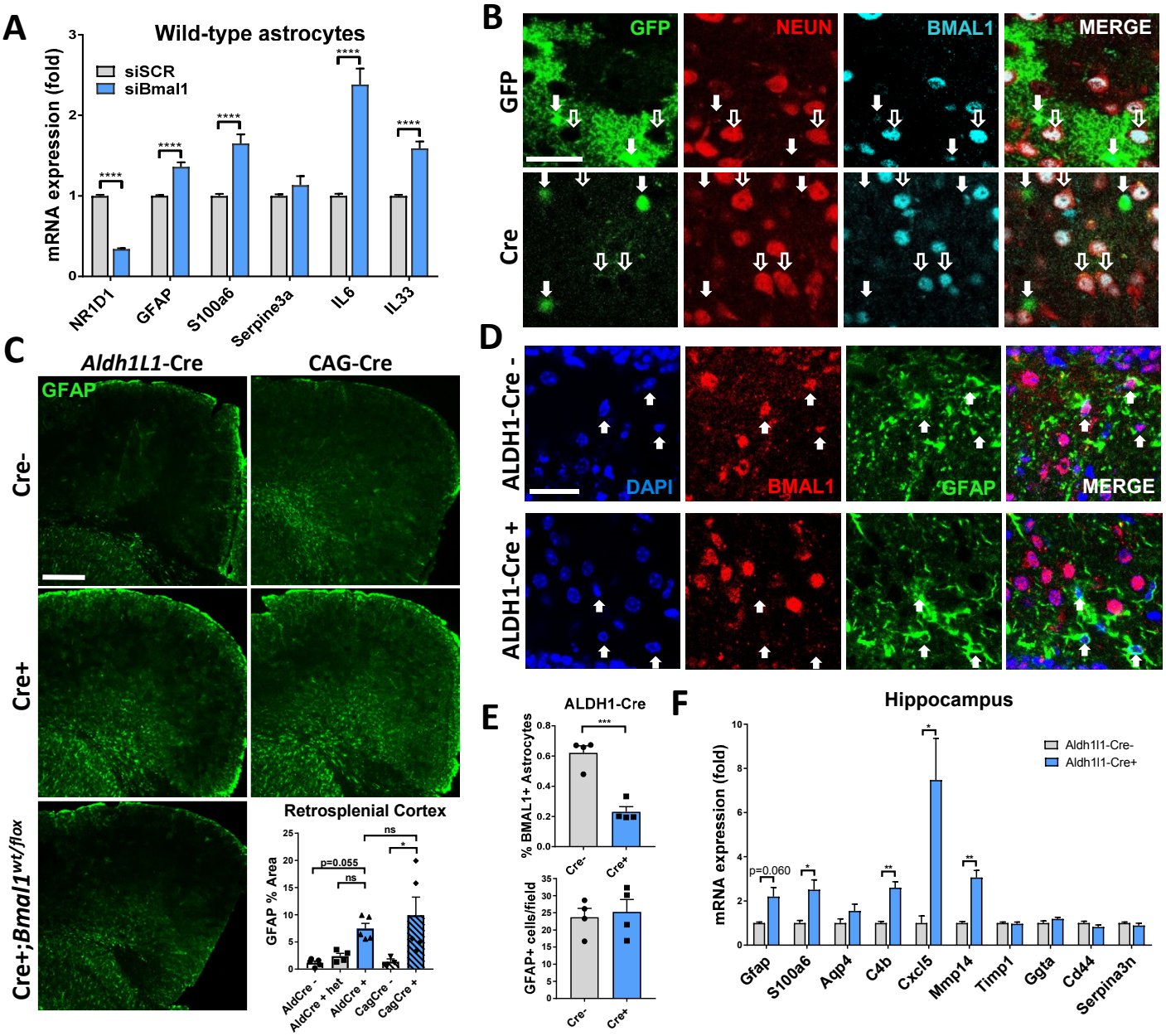


Figure S2. Related to Figure 2; Characterization of astrogliosis seen with loss of *Bmal1* in *vitro* and *in vivo*.

A. qPCR showing mRNA levels of several astrocyte activation and inflammatory genes 7 days after treatment of WT astrocytes with non-targeting siRNA (siSCR) or siRNA targeting *Bmal1* (siBmal1). N=12-15 experiments.

B. Representative cortical images from mice in Fig. 2F-H. *Top row*. AAV8-GFAP-GFP-infected cells (solid arrows) show whole-cell GFP expression, persistent colocalized nuclear BMAL1, and fail to colocalize with neuronal nuclei (hollow arrows). *Bottom row*. AAV8-GFAP-Cre infected cells show only nuclear GFP expression (solid arrows, Cre^{eGFP} fusion protein), loss of nuclear BMAL1 and fail to colocalize with neuronal nuclei (hollow arrows). Scale bar= 30µm.

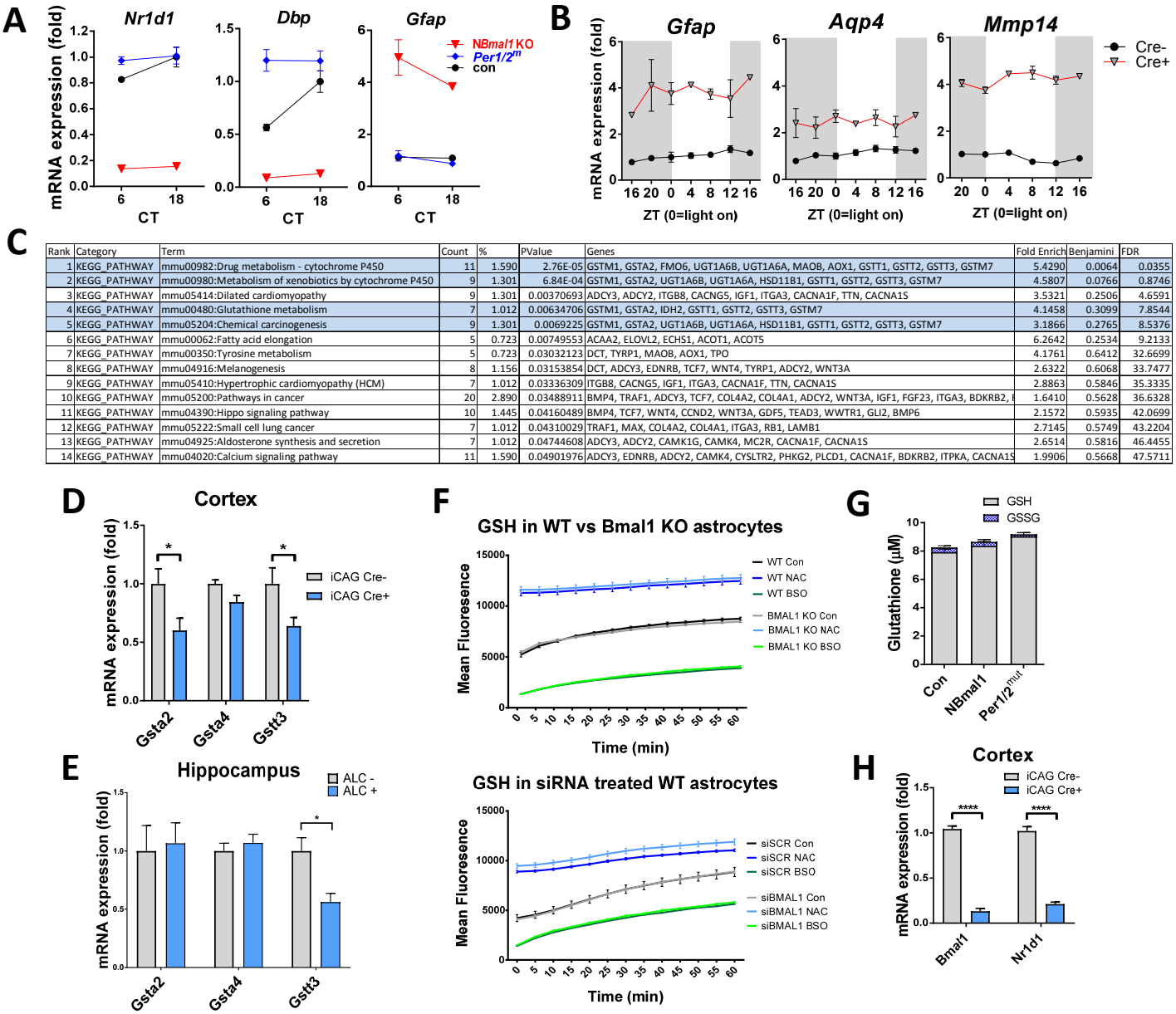
C. Representative images, quantification of %GFAP area in retrosplenial cortex of mice in Fig. 2H. Scale bar = 100µm.

D. Representative confocal images of *Aldh1l1-Cre+;Bmal$^{f/f}$* mice and Cre- controls. DAPI+, GFAP+ (solid arrows) astrocytes colocalize with BMAL1 in Cre- mice, but not in Cre+ mice. Scale bar= 30µm.

E. Quantification of DAPI/GFAP/BMAL1+ cells (top) or GFAP+ cells per field of view (bottom) in *Aldh1l1-Cre+;Bmal$^{f/f}$* mice vs Cre- controls in hippocampus. Each data point = 1 mouse. 6 fields/mouse with an average of 24.5 GFAP+ cells/field, totaling 1,176 cells counted. Note that there is not an increase in GFAP+ cells in hippocampus, just the size and shape of these cells.

F. mRNA levels of astrocyte activation markers in hippocampus from *Aldh1l1-Cre+;Bmal$^{f/f}$* mice vs Cre- controls.

All data represent mean+SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by 2-tailed T-tests (A), (E), and (F) or 1-way ANOVA (D) with Hom-Sidak correction for multiple comparisons when applicable.



Rank	Category	Term	Count	%	PValue	Genes	Fold Enrich	Benjamini	FDR
1	KEGG_PATHWAY	mmu00982:Drug metabolism - cytochrome P450	11	1.590	2.76E-05	GSTM1, GSTA2, FMO6, UGT1A6B, UGT1A6A, MAOB, AOX1, GSTT1, GSTT2, GSTT3, GSTM7	5.4290	0.0064	0.0355
2	KEGG_PATHWAY	mmu00980:Metabolism of xenobiotics by cytochrome P450	9	1.301	6.84E-04	GSTM1, GSTA2, UGT1A6B, UGT1A6A, HSD11B1, GSTT1, GSTT2, GSTT3, GSTM7	4.5807	0.0766	0.8746
3	KEGG_PATHWAY	mmu05414:Dilated cardiomyopathy	9	1.301	0.00370693	ADCY3, ADCY2, ITGB8, CACNG5, IGF1, ITGA3, CACNA1F, TTN, CACNA1S	3.5321	0.2506	4.6591
4	KEGG_PATHWAY	mmu00480:Glutathione metabolism	7	1.012	0.00634706	GSTM1, GSTA2, IDH2, GSTT1, GSTT2, GSTT3, GSTM7	4.1458	0.3099	7.8544
5	KEGG_PATHWAY	mmu05204:Chemical carcinogenesis	9	1.301	0.0069225	GSTM1, GSTA2, UGT1A6B, UGT1A6A, HSD11B1, GSTT1, GSTT2, GSTT3, GSTM7	3.1866	0.2765	8.5376
6	KEGG_PATHWAY	mmu00062:Fatty acid elongation	5	0.723	0.00749553	ACAA2, ELOVL2, ECHS1, ACOT1, ACOT5	6.2642	0.2534	9.2133
7	KEGG_PATHWAY	mmu00350:Tyrosine metabolism	5	0.723	0.03032123	DCT, TYRP1, MAOB, AOX1, TPO	4.1761	0.6412	32.6699
8	KEGG_PATHWAY	mmu04916:Melanogenesis	8	1.156	0.03153854	DCT, ADCY3, EDNRB, TCF7, WNT4, TYRP1, ADCY2, WNT3A	2.6322	0.6068	33.7477
9	KEGG_PATHWAY	mmu05410:Hypertrophic cardiomyopathy (HCM)	7	1.012	0.03336309	ITGB8, CACNG5, IGF1, ITGA3, CACNA1F, TTN, CACNA1S	2.8863	0.5846	35.3335
10	KEGG_PATHWAY	mmu05200:Pathways in cancer	20	2.890	0.03488911	BMP4, TRAF1, ADCY3, TCF7, COL4A2, COL4A1, ADCY2, WNT3A, IGF1, FGF23, ITGA3, BDKRB2,	1.6410	0.5628	36.6328
11	KEGG_PATHWAY	mmu04390:Hippo signaling pathway	10	1.445	0.04160489	BMP4, TCF7, WNT4, CCND2, WNT3A, GDF5, TEAD3, WWTR1, GLI2, BMP6	2.1572	0.5935	42.0699
12	KEGG_PATHWAY	mmu05222:Small cell lung cancer	7	1.012	0.04310029	TRAF1, MAX, COL4A2, COL4A1, ITGA3, RB1, LAMB1	2.7145	0.5749	43.2204
13	KEGG_PATHWAY	mmu04925:Aldosterone synthesis and secretion	7	1.012	0.04744608	ADCY3, ADCY2, CAMK1G, CAMK4, MC2R, CACNA1F, CACNA1S	2.6514	0.5816	46.4455
14	KEGG_PATHWAY	mmu04020:Calcium signaling pathway	11	1.590	0.04901976	ADCY3, EDNRB, ADCY2, CAMK4, CYSLTR2, PHKG2, PLCD1, CACNA1F, BDKRB2, ITPKA, CACNA1S	1.9906	0.5668	47.5711

Figure S3. Related to Figure 3; Pathway analysis, GSTx, and GSH with *Bmal1* deficiency.

A. Expression of BMAL1 transcriptional targets and *Gfap* from microarray in Fig. 3A at CT 6 and 18.

B. Expression of astrocyte activation transcripts *Gfap*, *Aqp4,* and *Mmp14* in cortex of NBmal1 KO (Cre+, red) and Cre- control mice (black) at 4 hour timepoints across the circadian cycle. No clear circadian oscillations were observed in Cre- or Cre+ mice. N=3-4 mice/genotype/timepoint.

C. KEGG pathway analysis of NBmal1 KO microarray data. Blue = pathways related to glutathione homeostasis.

D. qPCR of mRNA expression of glutathione transferases in the cortex of *iCAG-Cre+;Bmal1$^{f/f}$* mice (Fig. 3F), 9 days after tamoxifen treatment, normalized to Cre-, *Bmal$^{f/f}$* controls.

E. qPCR of mRNA expression of glutathione transferases in hippocampus of *Aldh1l1-Cre+;Bmal1$^{f/f}$* mice (Fig. 2H).

F. GSH levels in Bmal1 KO (top) and siBmal1 treated WT (bottom) astrocyte enriched cultures along with appropriate controls as measured by fluorometric intracellular glutathione detection assay.

G. Quantification of GSH and GSSG levels in cortical tissue (CT 10) from control, NBmal1 KO, and *Per1/2^{mut}* mice. None of the averages were significantly different.

H. mRNA expression of *Bmal1* and *Nr1d1* in the cortex of *iCAG-Cre+;Bmal1$^{f/f}$* mice used in Fig. 3G, 9 days after tamoxifen treatment, normalized to Cre-, *Bmal$^{f/f}$* controls.

All data represent mean±SEM. *p<0.05, ****p<0.001 by 2-tailed T-test with Holm-Sidak correction for multiple comparisons when applicable.

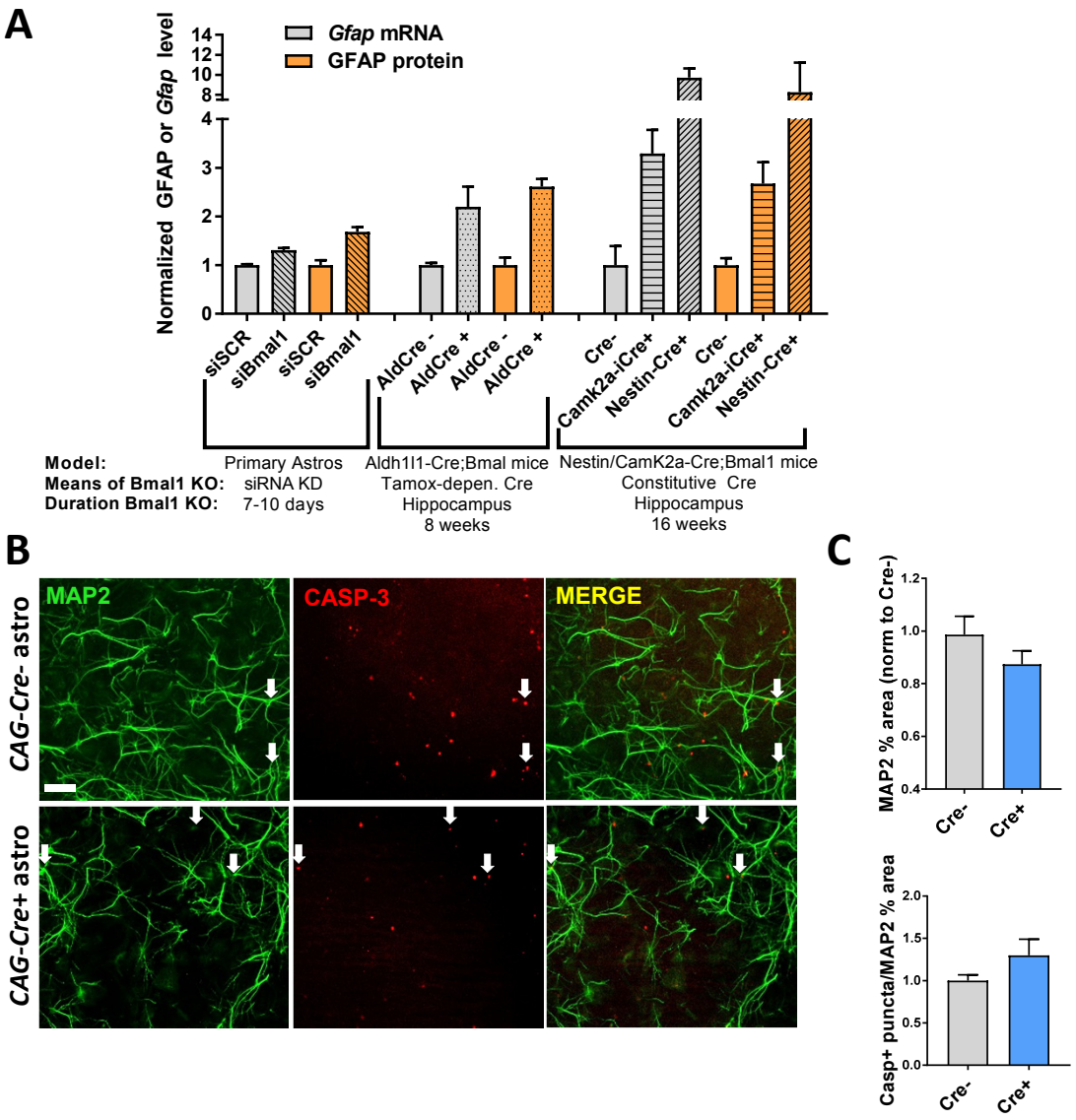


Figure S4. Related to Figures 3 and 4; Comparison of *Gfap* mRNA and GFAP protein across experiments; Further characterization of neuronal support deficits observed with loss of *Bmal1*.

A. Comparison of *Gfap* mRNA (quantified by qPCR) and GFAP protein (quantified by immunofluorescence) across experiments in this manuscript. Note that in both cultures cells and two distinct Cre-Lox mouse models, *Gfap* mRNA and GFAP protein are very closely related. Data is shown separately in Figs. 1 and 2.

B. Representative images showing MAP2+ (green) and cleaved-Caspase-3+ (CASP-3, red, arrows) WT neurons (grown as in Fig. 4A) at DIV 7. Scale bar = 100μm.

C. Quantification of MAP2 percent area (top) and cleaved-Caspase-3+ (bottom) neurons from (C) shows non-significant trends toward decreased MAP2 and increased cleaved-Caspase-3+ neurons when neurons are plated on BMAL1-deficient astrocytes (Cre+).

All data represent mean+SEM.