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Supplemental Information

GSK3β Regulates Brain Energy Metabolism

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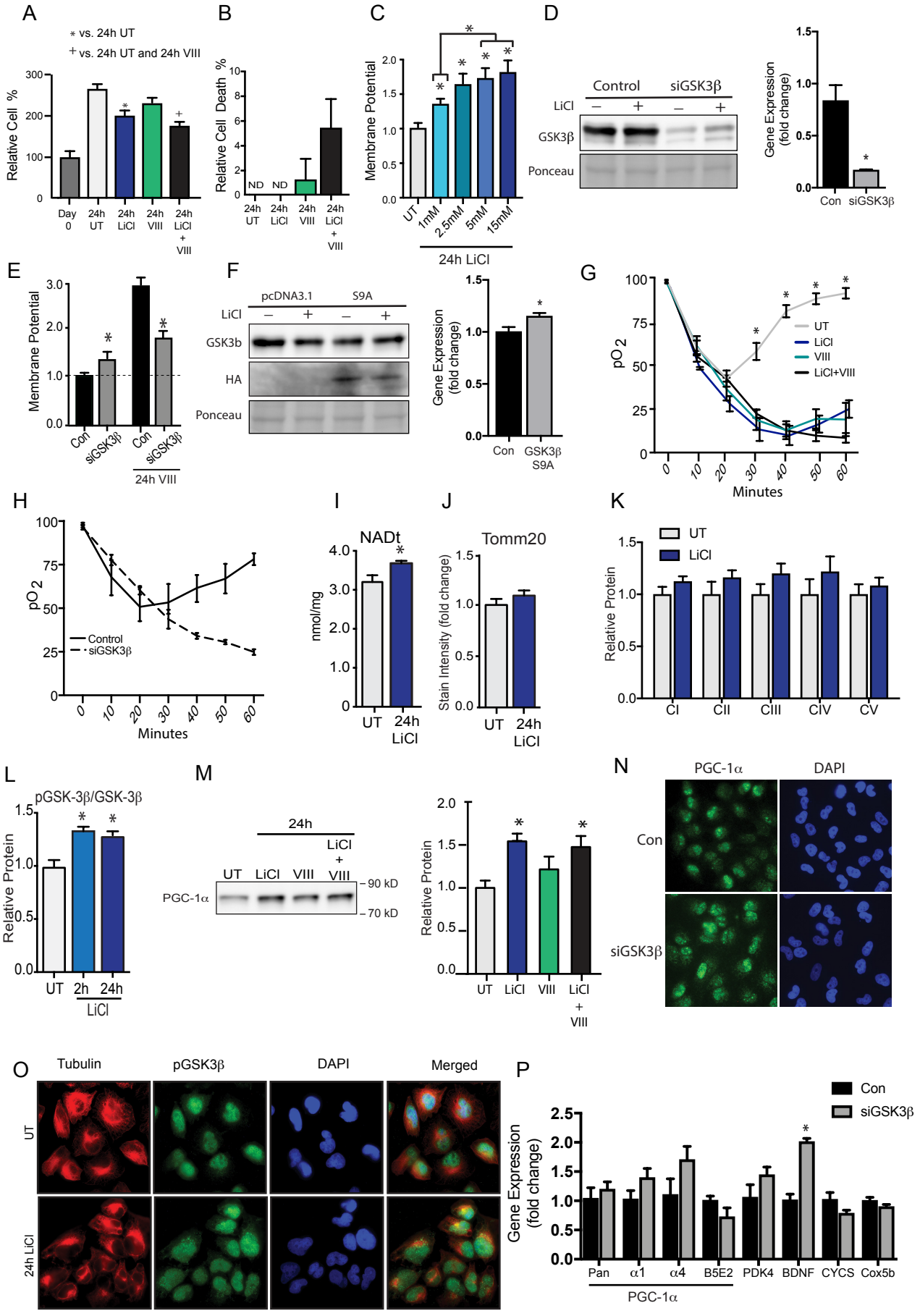

Figure S1. (related to Fig. 1). Data show the impact of lithium on cell proliferation (A) and (B) cell death. (C) JC-1 measurement of mitochondrial membrane potential following LiCl dose-response in H4 glioma. (D) GSK3β protein and gene expression 48 hours following GSK3β siRNA transfection. (E) JC-1 measurement of mitochondrial membrane potential following GSK3b inhibitor VIII (15μM) in H4 glioma with GSK3β interference. (F) GSK3β protein and gene expression 48 hours following GSK3β–S9A transfection. (G) Basal oxygen consumption over time in H4 glioma treated with DMSO, LiCl (15mM), inhibitor VIII (15μM), and both LiCl (15mM) and inhibitor VIII (15μM), and (H) in H4 glioma with GSK3β interference. (I) Total NAD (NADt) levels, and immunodetection of Tomm 20 (J), complexes I, II, III, IV, and V proteins of the ETS (K), and pGSK3β/GSK3β ratio (L) following the indicated LiCl treatment (15mM) in H4 glioma. (M) Detection of PGC-1α protein in H4 glioma following the indicated treatment. (N) Immunodetection of PGC-1μM in H4 glioma with GSK3β interference. (O) Immunodetection of tubulin and pGSK3β following 24h LiCl treatment in H4 glioma. (P) Gene expression of PGC-1μM and indicated transcripts in H4 glioma with GSK3β interference. (n= 3-6 biological replicates per assay; data shown as average +/- SEM; *p<0.05 ANOVA, independent sample t-test).

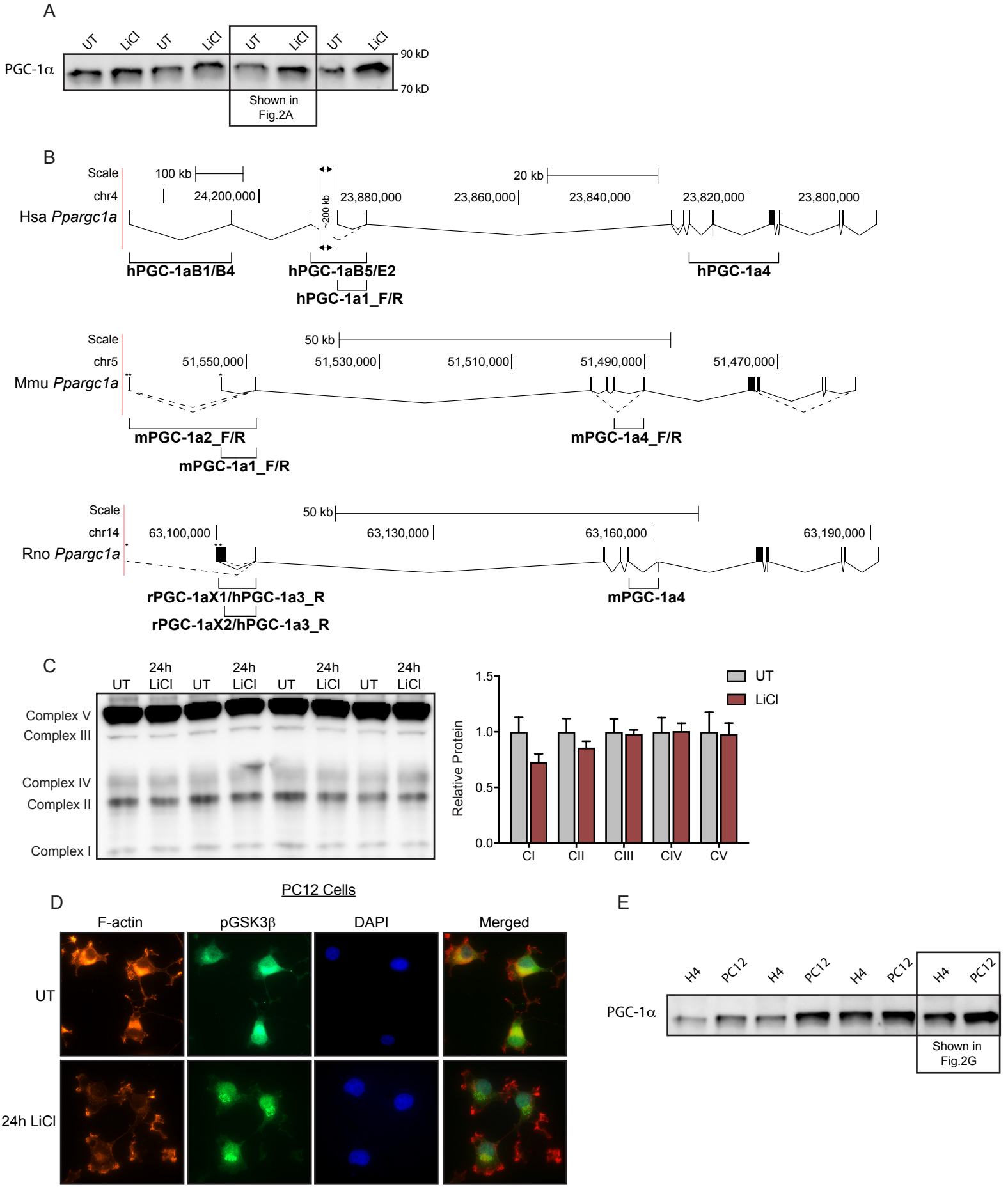

Figure S2. (related to Fig. 2). (A) Immunodetection of PGC-1α following 24h LiCl treatment in PC12-derived neurons (B) Gene structures of PGC-1α exons and alternative splicing for human, mouse and rat genomes. Brackets below each genome represent primer pairs used (Primer Table) to detect different PGC-1α isoforms throughout study. (*) indicates possible and validated transcriptional start sites. (C) Complexes I, II, III, IV, and V proteins of the ETS following the 24h LiCl treatment. (D) Immunodetection of tubulin and pGSK3β following 24h LiCl treatment in PC12-derived neurons. (n= 3-6 biological replicates per assay; data shown as average +/- SEM).

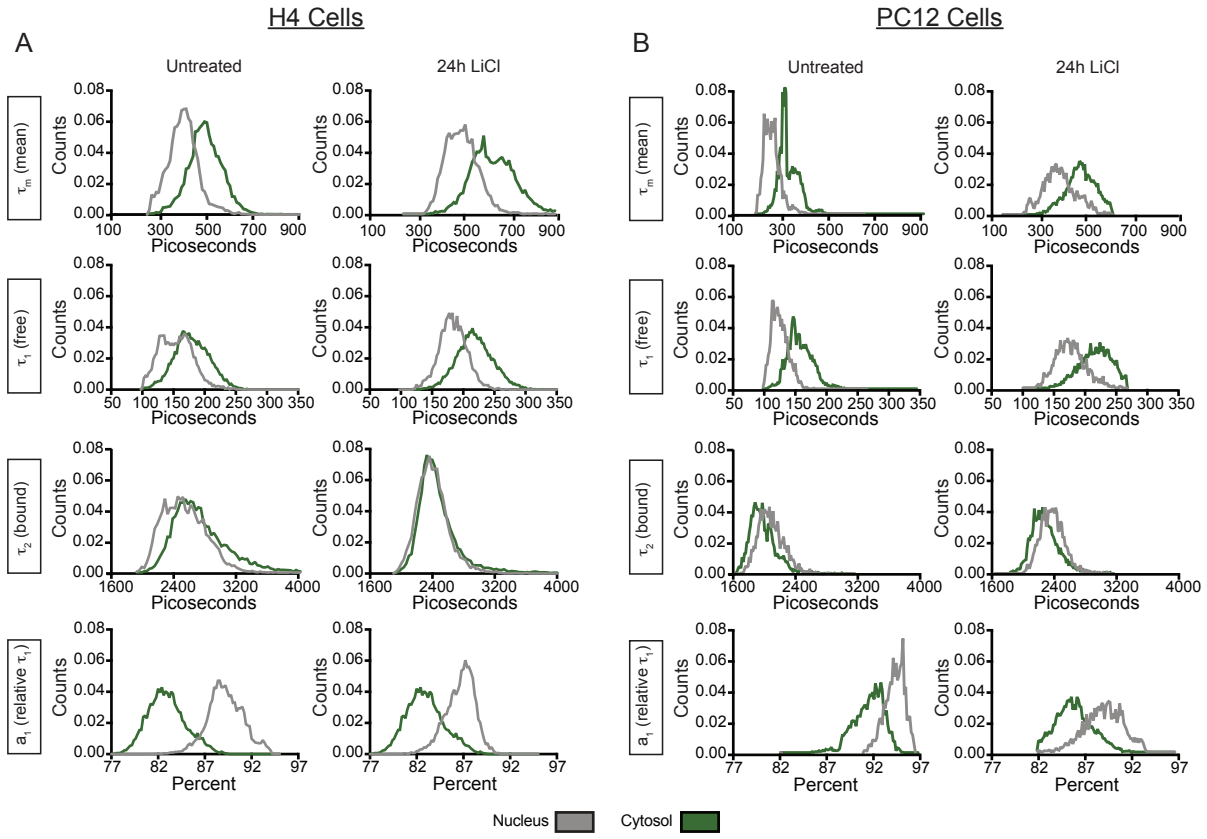

Figure S3. (related to Fig. 3). Impact of GSK3β inhibition on fluorescent lifetime components parameters in H4 glioma and PC12-derived neurons. (A) Distributions of mean fluorescence lifetime τm (top rows), short component τ_1 (upper middle rows), long component τ_2 (lower middle rows), and a_1, the relative contribution of τ_1 to τ_m (bottom row) following LiCl treatment (15mM) within the nucleus and cytoplasm of (A) H4 glioma and (B) PC12-derived neurons. (n= 6-8 biological replicates per measure; data shown as distribution or as average +/- SEM; *p<0.05, linear mixed model).

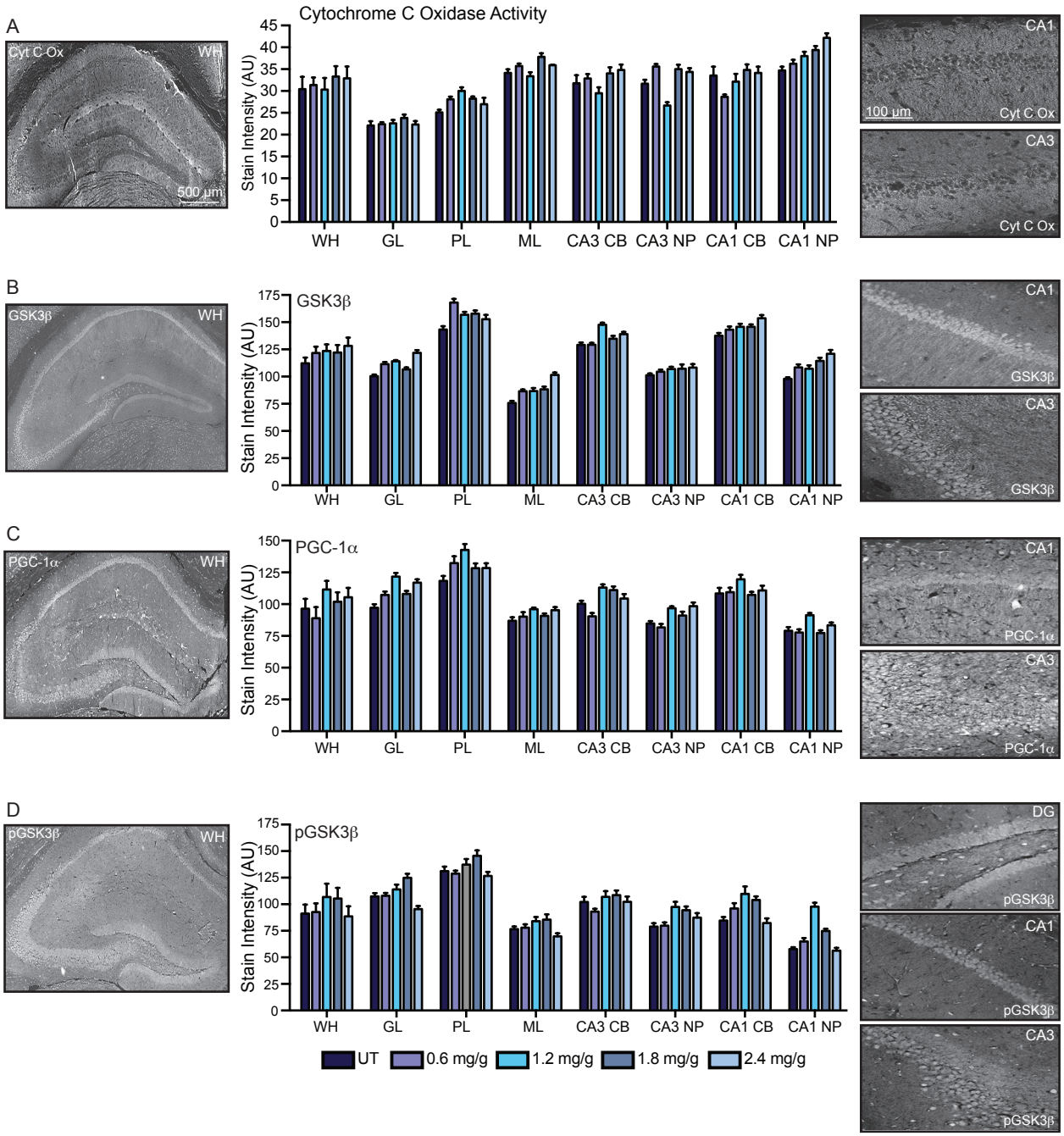

Figure S4. (related to Fig. 4). Representative images and quantification of (A) Cytochrome C oxidase activity (B) GSK3β protein immunodetection (C) PGC-1α immunodetection, and (D) pGSK3β immunodetection in the indicated hippocampal regions of mice fed the Li_2CO_3.(n = 4-6 mice per Li_2CO_3 dosage; data shown as average +/- SEM or distributions; *p<0.05, linear mixed models. WH, whole hippocampus; DG, dentate gyrus; GL, granular layer; PL, polymorphic layer; ML, molecular layer; CB, cell bodies; NP, neuropil.).

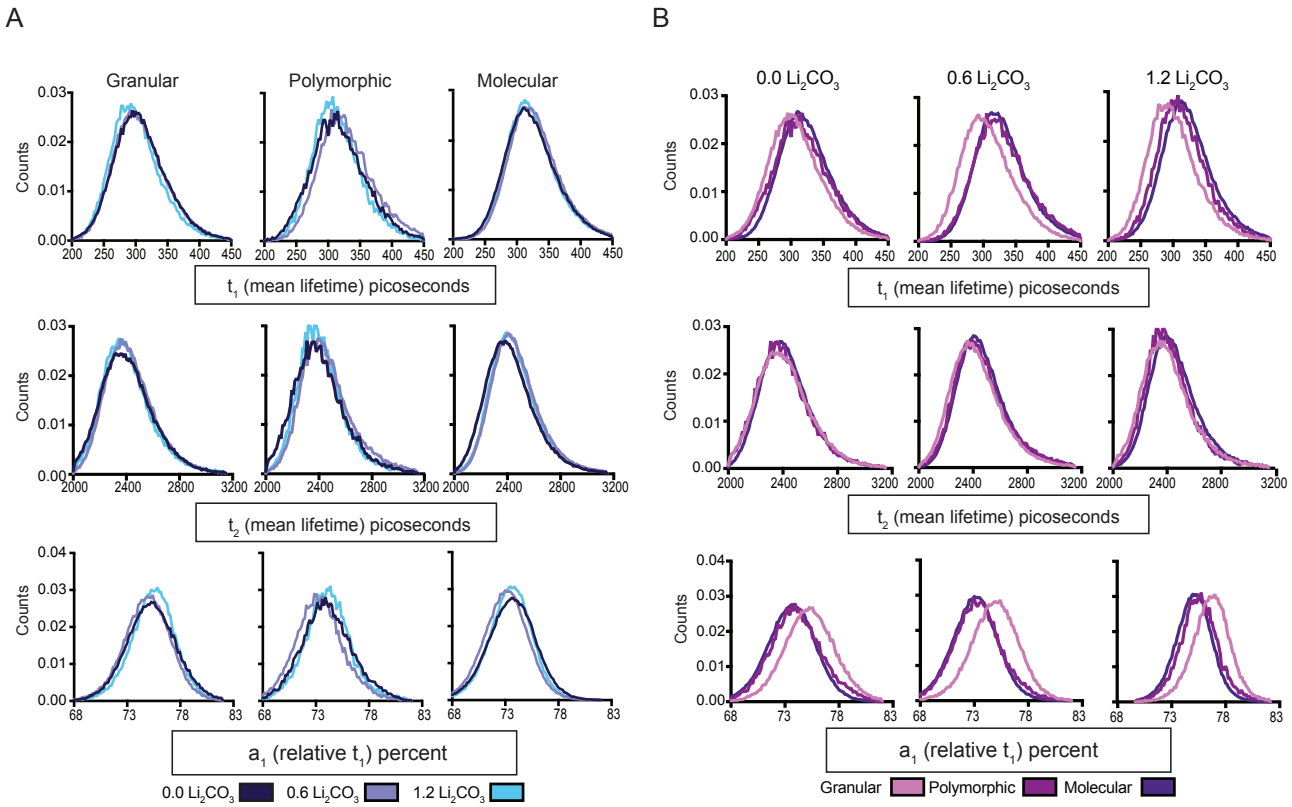

Figure S5. (related to Fig. 4). (A, B) Distributions of fluorescence lifetime short component τ_1 (upper middle rows), long component τ_2 (lower middle rows), and a_1, the relative contribution of τ_1 to τ_m (bottom row) separated by (A) region and (B) dosage in the dentate gyrus of mice fed Li_2CO_3. (n = 4-6 mice per Li_2CO_3 dosage; data shown as average +/- SEM or distributions; *p<0.05, linear mixed models.)

Table S1. (related to Fig. 1). Lithium treatment alters cellular respiration in H4 glioma cells.

Time-point (minute)	ANOVA	UT vs. 2h LiCl (p-value)	UT vs. 24h LiCl (p-value)	2h vs. 24h LiCl (p-value)
(Basal) 6	$F_{(2, 11)} = 14.99$; p = 0.001*	.029*	.001*	.106
12	$F_{(2, 11)} = 14.878$; p = 0.001*	.037*	.001*	.083
18	$F_{(2, 11)} = 21.073$; p = 0.000*	.008*	.000*	.085
(oligomycin) 24	$F_{(2, 11)} = 17.798$; p = 0.001*	.002*	.001*	.864
30	$F_{(2, 11)} = 8.266$; p = 0.009*	.043*	.009*	.575
36	$F_{(2, 11)} = 12.025$; p = 0.003*	.220	.002*	.034*
(FCCP) 42	$F_{(2, 11)} = 31.328$; p = 0.000*	.001*	.000*	.264
48	$F_{(2, 11)} = 24.093$; p = 0.000*	.003*	.000*	.124
54	$F_{(2, 11)} = 20.090$; p = 0.000*	.007*	.000*	.140
(Rotenone/Antimycin) 60	$F_{(2, 11)} = 10.513$; p = 0.004*	.979	.010*	.007*
66	$F_{(2, 11)} = 11.285$; p = 0.004*	.979	.006*	.008*
72	$F_{(2, 11)} = 11.825$; p = 0.004*	.912	.006*	.010*

*p<0.05

Table S2. (related to Fig. 3). Lithium regulates NAD(P)H metabolism in H4 glioma and PC12-derived neurons.

Lifetime Component	Lithium Main Effect	Cellular Compartment Main Effect	Lithium x Cellular Compartment Interaction
H4 glioma Cells			
τ_m	$F_{(1,24)}$=397.64; p<0.0001*	$F_{(1,24)}$=1442.81; p<0.0001*	$F_{(1,24)}$=39.10; p<0.0001*
τ_1	$F_{(1,24)}$=22.687; p<0.0001*	$F_{(1,24)}$=1065.98; p<0.0001*	$F_{(1,24)}$=20.40; p<0.0001*
τ_2	$F_{(1,24)}$=52.63; p<0.0001*	$F_{(1,24)}$=99.67; p<0.0001*	$F_{(1,24)}$=38.26; p<0.0001*
a_1	$F_{(1,24)}$=351.75; p<0.0001*	$F_{(1,24)}$=2088.94; p<0.0001*	$F_{(1,24)}$=13.10; p<0.0001*
PC12-derived neurons			
τ_m	$F_{(1,20)}$=1347.86; p<0.0001*	$F_{(1,20)}$=1421.16; p<0.0001*	$F_{(1,20)}$=36.90; p<0.0001*
τ_1	$F_{(1,20)}$=753.53; p<0.0001*	$F_{(1,20)}$=1795.27; p<0.0001*	$F_{(1,20)}$=33.72; p<0.0001*
τ_2	$F_{(1,20)}$=367.49; p<0.0001*	$F_{(1,20)}$=230.66; p<0.0001*	$F_{(1,20)}$=0.11; P=0.7461
a_1	$F_{(1,20)}$=1049.76; p<0.0001*	$F_{(1,20)}$=1720.55; p<0.0001*	$F_{(1,20)}$=19.28; p<0.0001*

*p<0.05

Table S3. (related to Fig. 4). Dietary Li_2CO_3-induced body mass and composition changes.

Li_2CO_3 Dosage (mg/g diet)	**Pre-intervention body mass (g)**	**Post-intervention body mass (g)**	**Body mass Δ (%)**	**Post-intervention body fat (%)**
0.0	25.74 ± 0.52	36.47 ± 0.7	42.17 ± 3.80	35.08 ± 1.48
0.6	26.31 ± 0.62	37.80 ± 0.7	46.40 ± 3.43	35.05 ± 0.87
1.2	26.02 ± 0.52	37.40 ± 0.89	45.84 ± 4.59	34.42 ± 1.02
1.8	25.92 ± 0.53	32.51 ± 0.81[a]	25.59 ± 2.79[a]	29.02 ± 1.39[a]
2.4	25.82 ± 0.52	26.84 ± 0.52[ab]	4.24 ± 2.50[ab]	21.40 ± 0.42[ab]

Mean ± SEM
[a] $p<0.001$ vs. 0.0, 0.6, 1.2 mg/g dosages; [ab] $p<0.001$ vs. 1.8 mg/g dosage

Table S4. (related to Fig. 4). Dietary Li_2CO_3 induced changes in hippocampal immunohistochemistry.

Stain	Lithium Dosage Main Effect	Region Main Effect	Dosage x Region Interaction
Cytochrome C Oxidase	$F_{(4,21)}$=14.22; p<0.0001*	$F_{(6,130)}$=188.6; p<0.0001*	$F_{(23,130)}$=102.98; P<0.0001*
PGC-1α	$F_{(4,21)}$=0.63; p=0.6486	$F_{(6,119)}$=58.98; p<0.0001*	$F_{(21,119)}$=27.80; p<0.0001*
GSK-3β	$F_{(4,21)}$=2.78; p=0.0536	$F_{(6,124)}$=352.45; p<0.0001*	$F_{(21,124)}$=2.29; p<0.0001*
Phospho-GSK3β	$F_{(4,22)}$=0.70; p=0.602	$F_{(6,124)}$=65.42; p<0.0001*	$F_{(22,124)}$=687.25; p<0.0001*

*p<0.05

Table S5. (related to Fig. 4). Dietary Li_2CO_3 induced changes in hippocampal NAD(P)H metabolism

Lifetime Component	Lithium Dosage Main Effect	Region Main Effect	Dosage x Region Interaction
τ_m	$F_{(2,13)}$=28.47; p<0.0001*	$F_{(2,13)}$=342.27; p<0.0001*	$F_{(4,13)}$=8.01; P=0.0018*
τ_1	$F_{(2,13)}$=4.05; p=0.0429*	$F_{(2,13)}$=96.65; p<0.0001*	$F_{(4,13)}$=3.81; P=0.0292*
τ_2	$F_{(2,13)}$=9.27; p=0.0032*	$F_{(2,13)}$=14.26; P=0.0005*	$F_{(4,13)}$=2.29; P=0.115
a_1	$F_{(2,13)}$=57.69; p<0.0001*	$F_{(2,13)}$=391.38; p<0.0001*	$F_{(4,13)}$=19.02; p<0.0001*

*p<0.05

Table S6. (Related to STAR Methods and Key Resources Table) List of primer sequences.

H4 Primers (Human)	**Source**	**Catalog #**
NRF1	ThermoFisher	Hs00602161_m1
TFAM	ThermoFisher	Hs00273372_s1
PDK4	ThermoFisher	Hs01037712_m1
IDH3a	ThermoFisher	Hs00194253_m1
COX5b	ThermoFisher	Hs00426950_g1
CYCS	ThermoFisher	Hs01588974_g1
SCD1	ThermoFisher	Hs01682761_m1
FASN	ThermoFisher	Hs01005622_m1
ACACA	ThermoFisher	Hs01046047_m1
ACADL	ThermoFisher	Hs00155630_m1
ACADM	ThermoFisher	Hs00936584_m1
GSK3b	ThermoFisher	Hs01047719_m1
BDNF	ThermoFisher	Hs02718934_s1
18S F- GTAACCCGTTGAACCCCATT	UW Biotech Center	N/A
18S R- CCATCCAATCGGTAGTAGCG	UW Biotech Center	N/A
hPGC-1a Pan F- CAG CCT CTT TGC CCA GAT CTT	UW Biotech Center	N/A
hPGC-1a Pan R- TCA CTG CAC CAC TTG AGT CCA C	UW Biotech Center	N/A
hPGC-1a1 F- ATG GAG TGA CAT CGA GTG TGC T	UW Biotech Center	N/A
hPGC-1a1 R- GAG TCC ACC CAG AAA GCT GT	UW Biotech Center	N/A
hPGC-1a2 F- AGT CCA CCC AGA AAG CTG TCT	UW Biotech Center	N/A
hPGC-1a2 R- ATG AAT GAC ACA CAT GTT GGG	UW Biotech Center	N/A
hPGC-1a3 F- CTG CAC CTA GGA GGC TTT ATG C	UW Biotech Center	N/A
hPGC-1a3 R- CAA TCC ACC CAG AAA GCT GTC T	UW Biotech Center	N/A
hPGC-1a4 F- TCA CAC CAA ACC CAC AGA GA	UW Biotech Center	N/A
hPGC-1a4 R- CTG GAA GAT ATG GCA CAT	UW Biotech Center	N/A
PGC-1a B5E2 F- CCTGGCTGCTGCTTTGGTA	UW Biotech Center	N/A
PGC-1a B5E2 R- GCTGTCTGTATCCAAGTCGT	UW Biotech Center	N/A
PGC-1a B1B4 F- TACAACTACGGCTCCTCCTGG	UW Biotech Center	N/A
PGC-1a B1B4 R- TACCCTTCATCCATGGGGCTC	UW Biotech Center	N/A
PGC-1a4 R- CTGGAAGATATGGCACAT	UW Biotech Center	N/A
PC12 Primers (Rat)	**Source**	**Catalog #**
rPGC-1a Pan F- TCTGGGTGGATTGAAGTGGTG	UW Biotech Center	N/A
rPGC-1a Pan R- CGAATATGTTCGCGGGCTCA	UW Biotech Center	N/A
rPGC-1aX1 F- AGTGACAGCCCAGCCTAC	UW Biotech Center	N/A
rPGC-1aX1 R- CAATCCACCCAGAAAGCTGTCT	UW Biotech Center	N/A
rPGC-1aX2 F- TTGTGGACTCTGGTGAGATGG	UW Biotech Center	N/A
rPGC-1aX2 R- CAATCCACCCAGAAAGCTGTCT	UW Biotech Center	N/A
rPGC-1a4 F- TCACACCAAACCCACAGAGA	UW Biotech Center	N/A
rPGC-1a4 R- CTGGAAGATATGGCACAT	UW Biotech Center	N/A
rBDNF F- ATTAGCGAGTGGGTCACAGC	UW Biotech Center	N/A
rBDNF R- TGGCCTTTTGATACCGGGAC	UW Biotech Center	N/A
rCox4i1 F- GCCTAATTGGCAAGAGAGC	UW Biotech Center	N/A
rCox4i1 R- TGGGCCACATCAGGCAAG	UW Biotech Center	N/A
rCox8a F- GTCATGTCTTCCCTGACGC	UW Biotech Center	N/A
rCox8a R- AACACACGAAGCAGGAAGTG	UW Biotech Center	N/A
rCox5b F- ACCCGAATCTAGTCCCTTCC	UW Biotech Center	N/A
rCox5b R- CAGCCACAACCAGATGACAG	UW Biotech Center	N/A
rPDK4 F- AGCTGGTACATCCAGAGCCT	UW Biotech Center	N/A
rPDK4 R- TCGAACTTTGACCAGCGTGT	UW Biotech Center	N/A
rGSK3b F- AGAAGAGCCATCATGTCGGG	UW Biotech Center	N/A

rGSK3b R- CCAAAAGCTGAAGGCTGCTG	UW Biotech Center	N/A
Mouse Primers (hippocampus)	**Source**	**Catalog #**
mPGC-1a Pan F- TGATGTGAATGACTTGGATACAGACA	UW Biotech Center	N/A
mPGC-1a Pan R- GCTCATTGTTGTACTGGTTGGATATG	UW Biotech Center	N/A
mPGC-1a1 F- GGACATGTGCAGCCAAGACTCT	UW Biotech Center	N/A
mPGC-1a1 R- CACTTCAATCCACCCAGAAAGCT	UW Biotech Center	N/A
mPGC-1a2 F- CCACCAGAATGAGTGACATGGA	UW Biotech Center	N/A
mPGC-1a2 R- GTTCAGCAAGATCTGGGCAAA	UW Biotech Center	N/A
mPGC-1a4 F- TCACACCAAACCCACAGAAA	UW Biotech Center	N/A
mPGC-1a4 R- CTG GAA GAT ATG GCA CAT	UW Biotech Center	N/A