

## **SI Appendix for**

### **Hippocampal neurons' cytosolic and membrane-bound ribosomal mRNA profiles are differentially regulated by learning and subsequent sleep**

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Datasets S1 – S11

## **SI Materials and Methods.**

### **Mouse husbandry, handling, and behavioral procedures**

All animal husbandry and experimental procedures were approved by the University of Michigan Institutional Animal Care and Use Committee (PHS Animal Welfare Assurance number D16-00072 [A3114-01]). For all studies, mice were maintained on a 12:12h light/dark cycle (lights on at 8 AM) with food and water provided *ad lib*. *B6.Cg-Tg(Camk2a-cre)T29-1Stl/J* mice (Jackson) were crossed to *B6N.129-Rpl22<sup>tm1.1P<sup>sam</sup></sup>/J* mice (Jackson) to express HA-tagged Rpl22 protein in Camk2a+ neurons. The resultant offspring thus expressed transgenes on a mixed C57Bl/6N and C57Bl6/J genetic background, due backcrossing of the two parent lines onto these two strains. Available data suggest that these parent mouse lines could show differences in phenotypes such as circadian rhythms and exploratory behaviors, which could in turn affect CFM measures. Offspring of crosses between these parent stains typically present with intermediate phenotypes for these features (1-5). Our mixed-background mice show SD-induced deficits in CFM consolidation, consistent with prior findings (6-10), as shown in **Figure S3**.

Double-transgenic mice were individually housed with beneficial enrichment for one week prior to experiments and were habituated to daily handling (5 min/day) for five days prior to experiments. For RNA seq experiments, mice were randomly assigned to one of four groups: HC + Sleep ( $n = 8$ ), HC + SD ( $n = 7$ ), CFC + Sleep ( $n = 8$ ), CFC + SD ( $n = 7$ ). Each mouse's bilateral hippocampi comprised one biological replicate for sequencing, and no samples were pooled between mice. Beginning at lights-on (8 AM), half of the mice underwent single-trial CFC as described previously (8, 11, 12). Briefly, at lights on (ZT 0) mice were placed in a novel conditioning chamber (Med Associates) and were allowed 2.5 min of free exploration time prior to delivery of a 2-s, 0.75 mA foot shock through the chamber's grid floor. After 3 min total in the chamber, mice were returned to their original home cage (HC). As a control for the effects of learning, HC controls remained in their home cage during this time. HC + SD or CFC + SD mice were then kept awake continuously by gentle handling (SD; consisting of cage tapping, nest disturbance, and if necessary, stroking with a cotton-tipped applicator) over the next 3 h for all RNA seq studies, or 5 h for all qPCR experiments. HC + Sleep and CFC + Sleep mice were permitted *ad lib* sleep in their home cage for the same time interval. For behavioral experiments to validate effects of SD on CFM consolidation in double-transgenic mice, CFC was carried out at ZT 0 as described above. Following CFC, mice were returned to their home cages and randomized into Sleep ( $n = 15$ ) and SD ( $n = 13$ ) groups. SD mice underwent a 6-h period of gentle handling SD, and were allowed subsequent recovery sleep in their home cage until ZT0 the

following day, at which point all mice were returned to the CFC chamber for CFM assessment, using previously-described methods (8, 9, 11, 12).

### **Translating Ribosome Affinity Purification (TRAP)**

RiboTag TRAP was performed as previously described (13) by indirect conjugation (14), separating membrane-bound and free-floating ribosomes (15). Briefly, following 3 h *ad lib* sleep or SD, mice were sacrificed with an overdose of pentobarbital (Euthasol). Brains were extracted and bilateral hippocampi were dissected in ice cold dissection buffer (1x HBSS, 2.5 mM HEPES [pH 7.4], 4 mM NaHCO<sub>3</sub>, 35 mM glucose, 100µg/ml cycloheximide). Each mouse's hippocampal tissue was then transferred to glass dounce column containing 1 ml homogenization buffer (10 mM HEPES [pH 7.4], 150 mM KCl, 10 mM MgCl<sub>2</sub>, 2 mM DTT, 0.1 cOmplete™ Protease Inhibitor Cocktail [Sigma-Aldrich, 11836170001], 100 U/mL RNasin® Ribonuclease Inhibitors [Promega, N2111], and 100 µg/mL cycloheximide) and manually homogenized on ice. Homogenate was transferred to 1.5 ml LoBind tubes (Eppendorf) and centrifuged at 4°C at 1000 g for 10 min. The resulting supernatant (cytosolic fraction) was transferred to a new LoBind tube while the pellet (MB fraction) was resuspended in homogenization buffer. 10% NP40 was then added to the samples and incubated 5 min on ice, after which both MB and cytosolic fractions were centrifuged at 4°C at maximum speed for 10 min. The resulting supernatant from both MB and cytosolic fractions was then separated into Input (~50µL), Camk2a+ (~400µL), and pS6+ fractions (~500µL). For isolating ribosomes from Camk2a+ populations, fractions were incubated with 1:40 anti-HA antibody (Abcam, ab9110) (16). To isolate ribosomes from highly active (pS6+) neurons fractions were incubated with 1:25 anti-pS6 244-247 (ThermoFisher 44-923G) (17). Antibody binding of the homogenate-antibody solution occurred over 1.5 h at 4°C with constant rotation.

For affinity purification, 200 µl/sample of Protein G Dynabeads (ThermoFisher, 10009D) were washed 3 times in 0.15M KCl IP buffer (10 mM HEPES [pH 7.4], 150 mM KCl, 10 mM MgCl<sub>2</sub>, 1% NP-40) and incubated in supplemented homogenization buffer (+10% NP-40). Following this step, supplemented buffer was removed, homogenate-antibody solution was added directly to the Dynabeads, and the solution was incubated for 1 h at 4°C with constant rotation. After incubation, the RNA-bound beads were washed four times in 900µL of 0.35M KCl (10mM HEPES [pH 7.4], 350 mM KCl, 10 mM MgCl<sub>2</sub>, 1% NP40, 2 mM DTT, 100 U/mL RNasin® Ribonuclease Inhibitors [Promega, N2111], and 100 µg/mL cycloheximide). During the final wash, beads were placed onto the magnet and moved to room temperature. After removing the supernatant, RNA was eluted by vortexing the beads vigorously in 350 µl RLT (Qiagen, 79216). Eluted RNA was purified using RNeasy Micro kit (Qiagen).

### Quantitative real-time PCR (qPCR)

For qPCR, RNA from each sample (i.e., bilateral hippocampi from one mouse) was converted into cDNA using the SuperScript IV Vilo Master Mix (Invitrogen 11756050). qPCR was performed on diluted cDNA that employed either Power SYBR Green PCR Mix (Invitrogen 4367659) or TaqMan Fast Advanced Master Mix (Invitrogen 4444557). For TRAP enrichment values, each sample was normalized to the geometric mean of *Pgk1* and *Gapdh* housekeeping transcripts and then normalized to the corresponding Input sample (TRAP enrichment =  $2^{(\Delta Ct_{\text{target}} - \Delta Ct_{\text{housekeeping}})}$ ). Effects of SD were assessed by normalizing all groups' expression to the HC + Sleep group. Effects of CFC were quantified by normalizing CFC + Sleep to HC + Sleep and normalizing CFC + SD to HC + SD. Primers for mRNAs quantified are listed below.

<b><u>Target</u></b>	<b><u>Forward Primer</u></b>	<b><u>Reverse Primer</u></b>
Gapdh	GTGTTTCCTCGTCCCGTAGA	AATCCGTTACACCCGACCTT
Pgk1	TCGTGATGAGGGTGGACTTC	ACAGCAGCCTTGATCCTTTG
Arc	CCAGATCCAGAACCACATGAA	GAGAGTGTACCCTCACTGTATTG
cFos	GAAGAGGAAGAGAAACGGAGAAT	CTTGGAGTGTATCTGTCAGCTC
Homer1a	GCATTGCCATTTCCACATAGG	ATGAACTTCCATATTTATCCACCT TACTT
Glua1	AGTGACGCTCGGGACCACAC	CTCTGGAAGGCCTCCGCCAT
Bdnf	GGTCACAGCGGCAGATAAA	TCAGTTGGCCTTTGGATACC
Hspa5	CCGAGAACACGGTCTTCGAT	ATTCCAAGTGCCTCCGATGA
Grin2a	CGTAGAGGATGCCTTGGTCA	CCATAGCCTGTGGTGGCAAA
Grin2b	CGGCCTGAGTGACAAGAAGT	TCCTCTCTGTGCTGCCATTG

Vglut1	CCAGCATCTCTGAGGAGGAG	GGCTGAGAGATGAGGAGCAG
Parv	GTCGATGACAGACGTGCTCA	TTGTGGTCTGAAGGAGTCTGC
Sst	CTCGGACCCCAGACTCCGTC	CTCGGGCTCCAGGGCATCAT
Mbp	CCTTGACTCCATCGGGCGCT	CTTCTGGGGCAGGGAGCCAT
Gfap	TCCTGGAACAGCAAACAAG	CAGCCTCAGGTTGGTTTCAT

**Target**                      **Catalog # for ThermoFisher Taqman**  
**Probes**

Gapdh	Mm99999915_g1
Pgk1	Mm00435617_m1
FosB	Mm00500401_m1
ΔFosB	Custom-AP47Y2V
Homer1	Mm01282664_m1
Homer1a	Custom-APT2DGG
Atf3	Mm00476033_m1
Egr3	Mm00516979_m1
1700016P03Rik	Mm01253067_m1

**RNA-seq and expression analysis**

RNA-Seq was carried out at the University of Michigan's DNA Sequencing Core. Amplified cDNA libraries were prepared using Takara's SMART-seq v4 Ultra Low Input RNA Kit (Takara

634888) and sequenced on Illumina's NovaSeq 6000 platform. Libraries were run with onboard cluster generation, and together comprised 40% of a NovaSeq S4 200 cycle kit (v1.5; ~16–20 billion paired-end reads per flow cell, quality score  $\geq$  90% of bases higher than Q30). Sequencing reads (50 bp, paired-end) were mapped to *Mus musculus* using Star v2.6.1a and quality checked with Multiqc (v1.6a0). Reads mapped to unique transcripts were counted with featureCounts (18).

Differential expression analyses were run with Deseq2 on all 30 hippocampal samples, with bilateral hippocampi from each mouse constituting a biological replicate (19). Analyses were run with an initial filtering step (removing rows with  $< 10$  counts) and with betaPrior = False. To test differences between subcellular fractions within their respective cell population, the design of the GLM was set to compare differences between supernatant and pellet-associated expression of transcripts. Camk2a+, pS6+, and Input samples were analyzed separately (e.g., Camk2a+[supernatant/pellet]). To quantify effects of SD and CFC on expression, the design was switched, and each cell population and subcellular fraction was analyzed separately. The same two-factor design was used to analyze the effects of an animal's state (Sleep or SD) and learning (CFC or HC) on RNA expression. The design compared the effects of SD alone by combining HC and CFC animals. This strategy accounted for differential effects of learning experiences on SD-driven changes to transcript profiles (20). SD-driven transcript changes identified with this strategy were compared with SD-driven changes in mRNA and proteins found in recent prior studies(21-23), as show in **Figure S5**. In contrast, the effect of learning (CFC) was assessed separately in CFC + Sleep and CFC + SD mice.

To characterize the differences between the effects of SD and CFC, significantly altered transcripts were analyzed using Ingenuity's Pathway Analysis (IPA). GO analyses were performed in IPA and DAVID's Functional Annotation tool. For subcellular fraction comparisons, 2000 of the top cytosolic ( $\text{Log}_2\text{FC} > 0$ ) and MB ( $\text{Log}_2\text{FC} < 0$ ) differentially-expressed transcripts (ranked by adjusted  $p$  values) were run through IPA's Canonical Pathways analysis. To characterize differences in common metabolic pathways between cytosolic and MB fractions, hierarchical clustering was used to visualize the most differentially-expressed transcripts. Since signaling pathways were less overlapping between the MB and cytosolic fraction, they were ranked by enrichment  $p$  values. Those transcripts were then run through DAVID's Functional Annotation tool, selecting for cellular composition to describe the cellular compartment the corresponding protein relates to. Data were plotted in Fragments Per Million (FPM) and their correlation value (R) calculated in the ViDger R package (24).

## **Immunohistochemistry**

To characterize HA and pS6 expression in the hippocampus, experimentally naive animals were sacrificed and perfused with 1xPBS followed by 4% paraformaldehyde. 50 $\mu$ M coronal sections containing dorsal hippocampus were blocked in normal goat serum for 2 h and incubated overnight using a biotin conjugated anti-HA (Biolegend 901505, 1:500), anti-pS6 244-247 (ThermoFisher 44-923G, 1:500), and anti-parvalbumin (Synaptic Systems 195 004, 1:500) antibodies. Sections were then incubated with secondary antibodies - Streptavidin-Alexa Fluor® 647 (Biolegend 405237), Fluorescein (FITC) Goat Anti-Rabbit IgG (H+L) (Jackson 111-095-003), and Alexa Fluor® 555 Goat Anti-Guinea pig IgG H&L (Abcam ab150186). Immunostained sections were coverslipped in ProLong Gold Antifade Reagent (ThermoFisher, P36930) for imaging with a Leica SP5 laser scanning confocal microscope.

### **Supplementary Figure Legends.**

**Figure S1. Cytosolic and membrane protein-encoding transcripts are more abundant in supernatant and pellet ribosomal fractions, respectively.** (A) Volcano plot of transcripts differentially expressed between pellet (red) and supernatant (blue) cell fractions of Camk2a+ neurons. Data are shown for  $n = 30$  biological replicates from 30 total mice across the 4 treatment groups. Of the 28,071 transcripts detected, 7,651 (27%) were significantly ( $p_{adj} < 0.1$ ) more abundant in the supernatant (cytosolic) fraction, and 10,911 (39%) were significantly more abundant in the pellet (MB) fraction. Complete transcript list in **Dataset S1**. (B) Volcano plot of transcripts differentially expressed between pellet (red) and supernatant (blue) cell fractions from pS6+ neurons. Of the 34,657 transcripts detected, 8,030 (23%) were significantly ( $p_{adj} < 0.1$ ) enriched in the supernatant (cytosolic) fraction, and 14,244 (41%) were significantly enriched in the pellet (MB) fraction. Complete transcript list in **Dataset S2**. (C-D) Top 10 cellular component localizations (from DAVID) of the 2000 transcripts most differentially expressed (based on adjusted  $p$  value) between MB and cytosolic fractions from Camk2a+ (C) or pS6+ (D) neurons. (E-F) Top 20 most-enriched signaling and metabolic pathways represented by the 2000 most-differentially-expressed transcripts in Camk2a+ (E) or pS6+ (F) cytosolic or MB fractions. (G) Illustration of the synaptogenesis signaling pathway (IPA) protein components, with proteins' color coded to indicate corresponding transcripts' that are localized preferentially in cytosolic (red) and MB (blue) fractions of Camk2a+ neurons. Functional category analysis available in **Dataset S1**. (H) Log2FC values indicating differences in the abundance of transcripts for proteins in Creb1 signaling pathway, including upstream activators or inhibitors of Creb signaling, between the cytosolic and membran fractions. Transcripts for pathway components selectively localized in the cytosolic or MB fractions of pS6+ neurons are shown in red and blue, respectively, with corresponding proteins subcategorized by encoded protein type. Functional category analysis available in **Dataset S2**.

**Figure S2. Transcripts enriched in cytosolic and MB fractions from Input (whole hippocampus).** (A) Volcano plot of transcripts significantly enriched in pellet (red) and supernatant (blue) cell fractions. Of the 27,773 transcripts detected, and 8,310 (30%) showed significant enrichment in the supernatant (cytosolic) fraction, 9,285 (33%) showed enrichment in the pellet (MB) fraction. Complete transcript list in **Dataset S3**. (B) Top 10 cellular component localizations (from DAVID) of the 2000 transcripts which were most significantly enriched (based on  $p_{adj}$  value) in either pellet (MB) or supernatant (cytosolic) fractions. (C) Top 20 most-enriched



signaling and metabolic pathways represented by the 2000 most-enriched transcripts in Input cytosolic or MB fractions. **(D)** Log<sub>2</sub>FC values indicating enrichment of transcripts from the ubiquitin signaling pathway in the cytosolic (red) or MB (blue) fractions. Functional category analysis available in **Dataset S3**

**Figure S3. Post-CFC SD disrupts CFM consolidation in *B6N.129-Rpl22<sup>tm1.1P<sub>sam</sub></sup>/J* × *B6.Cg-Tg(Camk2a-cre)T29-1Stl/J* mice.** **Top:** Experimental design. All mice underwent single-trial CFC at lights on (ZT 0) and were subsequently returned to their home cages; SD mice were kept awake via gentle handling over the first 6 h post-CFC. CFM was assessed 24 h later, as described previously(8, 9, 11, 12). **Bottom:** CFM consolidation was significantly disrupted in SD mice ( $n = 13$ ), compared with mice allowed *ad lib* sleep ( $n = 15$ ). \* indicates  $p = 0.036$ , Student's t-test.

**Figure S4. mRNAs altered by SD and prior learning on cytosolic ribosomes.** **Left:** Proportional and overlapping Venn diagrams of transcripts significantly altered by SD, CFC + Sleep, and CFC + SD in cytosolic fractions from Camk2a+ neurons, pS6+ neurons, and Input. **Right:** Volcano plots of Deseq2 results for transcripts measured in each condition.

**Figure S5. Overlap of SD-altered transcripts with previously-characterized SD-altered mRNAs and proteins.** Venn diagrams indicate degree of overlap for transcripts altered by SD in the present study and those previously reported for RNA seq of whole hippocampus following SD (Gaine et al., 2021) (21), for Camk2a+ TRAP-seq following SD (Lyons et al., 2020) (22), and for RNA seq and proteomics of whole forebrain synaptoneurosomes (characterizing transcripts which lose circadian rhythmicity in the context of SD; Noya et al., 2019) (23). Transcripts in each section are listed in **Dataset S11**.

**Figure S6. Creb1 target transcripts are upregulated on cytosolic ribosomes after SD.** **(A)** Z-scores for the 5 upstream transcriptional regulators whose target transcripts were most significantly affected by SD, ranked by  $p_{adj}$  values. **(B)** Networks of Creb1 transcriptional targets altered by SD in Camk2a+ (**top**) and pS6+ (**bottom**) neurons. Color of arrows from Creb1 to transcripts indicates the predicted direction of transcriptional regulation - orange (with red transcript symbols) denotes transcripts predicted to be upregulated by Creb1 which are upregulated following SD; blue (with green transcript symbols) indicates transcripts predicted to be repressed by Creb1 which are repressed by SD; yellow indicates SD-related changes that do not match predicted regulation by Creb1; grey indicates undermined effects of Creb1 on transcript

levels. **(C)** Relative Creb1 network regulation  $p_{\text{adj}}$  values and z-scores are plotted for cytosolic ribosomes' transcripts altered by SD, CFC + Sleep, and CFC + SD in Camk2a+ and pS6+ cell populations. Upstream regulator analysis results are included in **Dataset S9**. Cytosolic input CREB network analyses are available in **Figure S6**.

**Figure S7. Input CREB upstream regulator analysis.** Networks of Creb1 transcriptional targets altered by SD in the cytosolic fraction taken from whole hippocampal homogenate (Input). Color of arrows indicates predicted regulation by Creb1 following SD whereas the color of the gene symbol indicates  $\text{Log}_2$  FC in expression following SD. Orange arrows pointing to red symbols indicates genes increased by Creb1 that are also increased by SD while blue arrows connote transcripts predicted to be repressed by Creb1 which are also repressed by SD. Yellow arrows encompass all SD-related changes that do not match predicted regulation by Creb1; grey indicates undetermined effects of Creb1 on transcript levels.

**Figure S8. CFC-induced alterations in *Fosb* and *Homer1* splice variants are occluded by post-CFC SD in Camk2a+ neurons.** **(A)** *Fosb* and  $\Delta F_{\text{osb}}$  expression for CFC and HC mice after 5 h of subsequent sleep or SD is shown for cytosolic fractions of the 3 cell populations. **Top, left:** Expression in the 4 conditions relative to values from HC + Sleep mice (Two-way ANOVA, CFC/SD, df = 18, \*\*\* p-value < 0.001). **Top, right:** Relative enrichment/de-enrichment for *Fosb* and  $\Delta F_{\text{osb}}$  in Camk2a+ and pS6+ neurons, relative to Input, for the 4 conditions. **Bottom:** Expression of *Fosb* and  $\Delta F_{\text{osb}}$  following CFC conditions relative to same-state (SD or Sleep) home-cage (HC) conditions (t-test,  $n = 5/\text{group}[\text{HC}]$ ,  $n = 6/\text{group}[\text{CFC}]$ ) #, \*, \*\*, and \*\*\* indicate  $p < 0.1$ ,  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. **(B)** *Homer1* and *Homer1a* in cytosolic fractions in the 4 conditions, normalized as described in **(A)**.

**Figure S9. CFC-induced alterations in activity-dependent transcripts in Sleep and SD mice.** Expression of activity-regulated transcripts (*Cfos*, *Atf3*, *Arc*, and *Erg1*) and lncRNA *1700016P03Rik* for CFC and HC mice after 5 h of subsequent sleep or SD is shown for cytosolic fractions of Camk2a+ and pS6+ neurons. **Top:** Expression in the 4 conditions relative to values from HC + Sleep mice (Two-way ANOVA, CFC/SD, df = 18) \*, \*\*, and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. **Bottom:** Expression for the CFC conditions relative to same-state (SD or Sleep) HC conditions.

**Figure S10. mRNAs altered by SD and prior learning on MB ribosomes.** *Left:* Proportional and overlapping Venn diagrams of transcripts significantly altered by SD, CFC + Sleep, and CFC + SD in MB fractions from Camk2a+ neurons, pS6+ neurons, and Input. *Right:* Volcano plots of Deseq2 results for transcripts measured in each condition.

**Figure S11. Overview of data in Supplementary Datasets.**

**Supplementary Datasets.**

**Dataset S1 - Transcripts enriched in cytosolic or MB ribosomal fractions of Camk2a+ neurons.**

**Dataset S2 - Transcripts enriched in cytosolic or MB ribosomal fractions of pS6+ neurons.**

**Dataset S3 - Transcripts enriched in cytosolic or MB ribosomal fractions of Input.**

**Dataset S4 - Transcripts altered by SD and CFC in Camk2a+ neurons.**

**Dataset S5 - Transcripts altered by SD and CFC in pS6+ neurons.**

**Dataset S6 - Transcripts altered by SD and CFC in Input.**

**Dataset S7 - Venn diagram values.**

**Dataset S8 - Canonical/molecular functions altered by SD (cytosolic fractions).**

**Dataset S9 - CREB upstream regulator analysis (cytosolic fractions).**

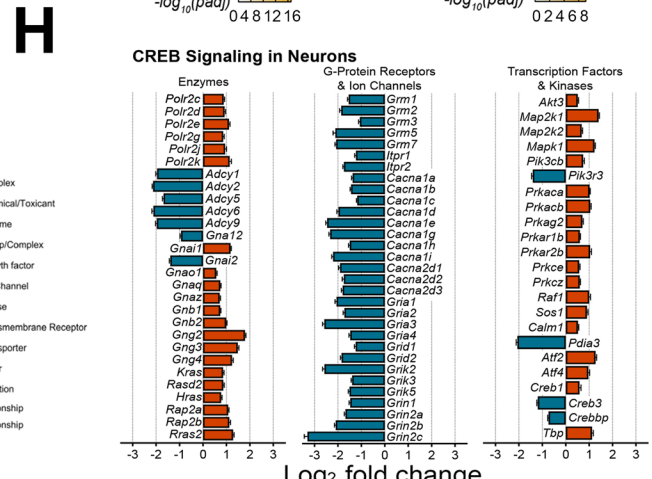
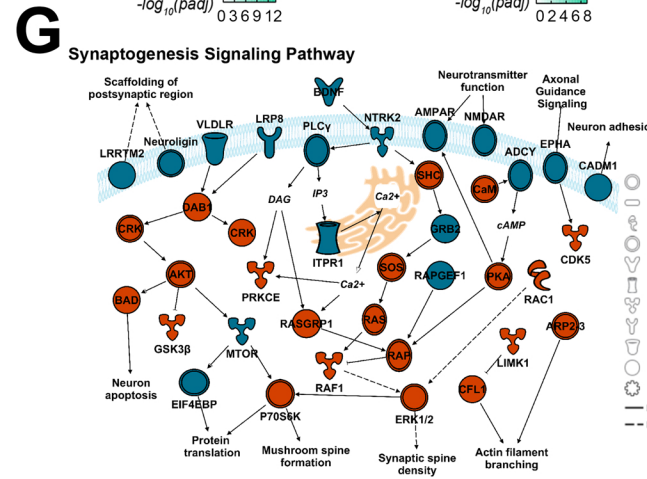
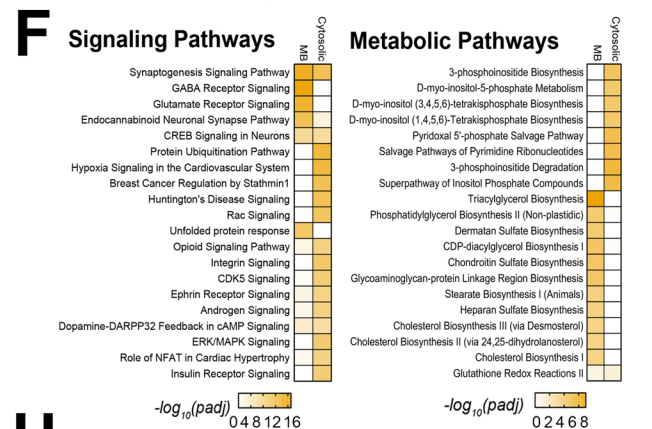
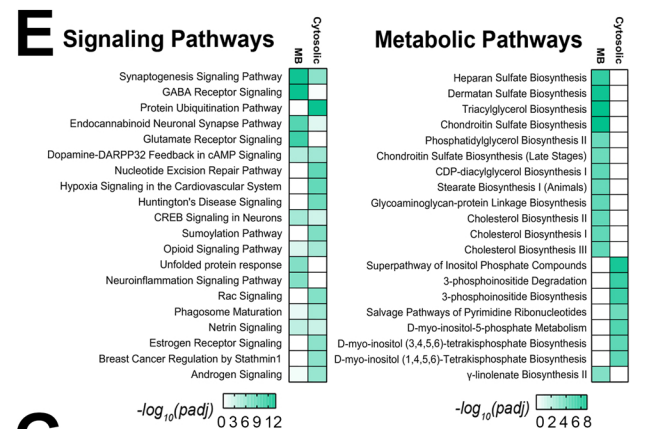
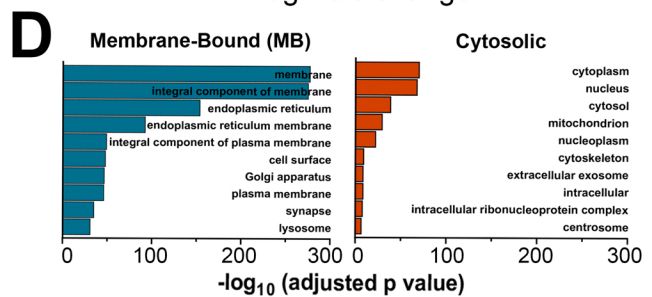
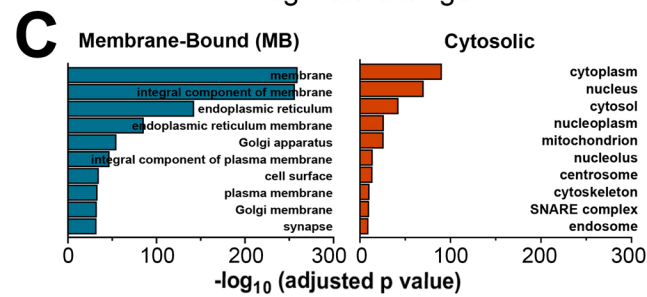
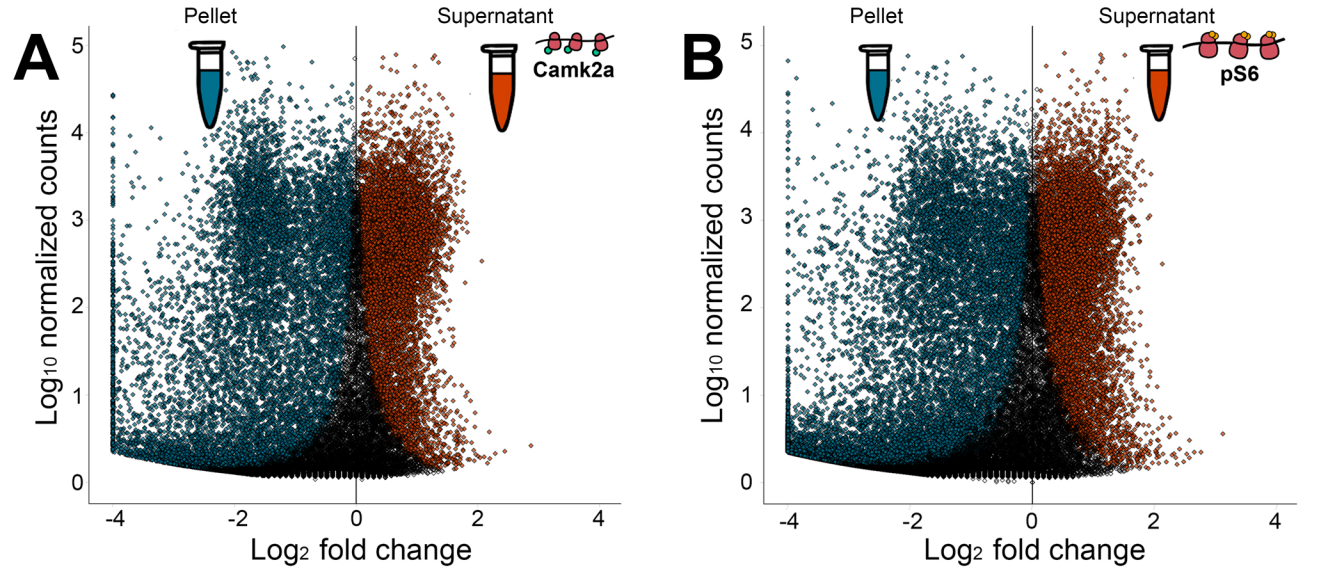
**Dataset S10 - Canonical/molecular functions altered by SD and CFC (MB fractions).**

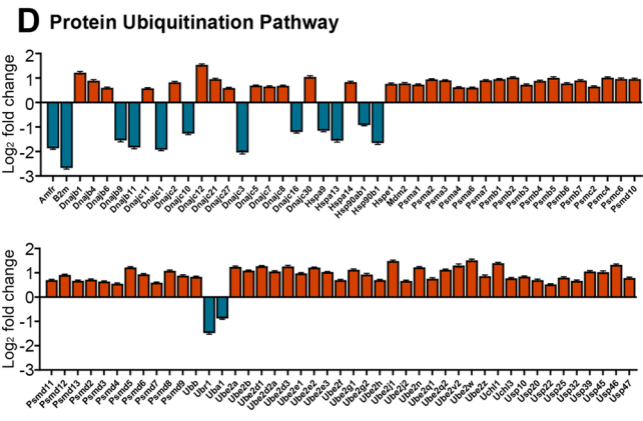
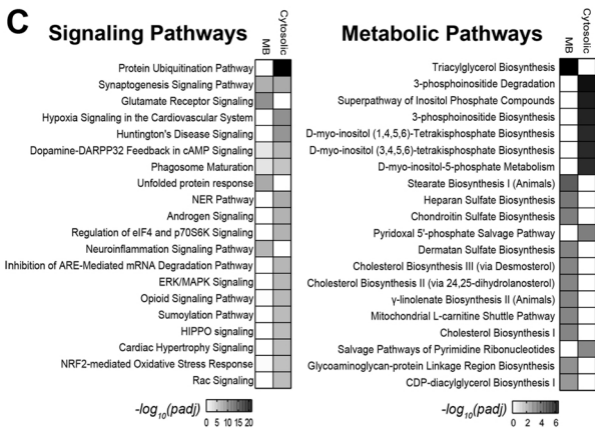
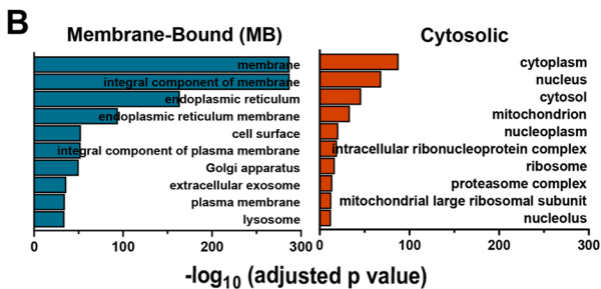
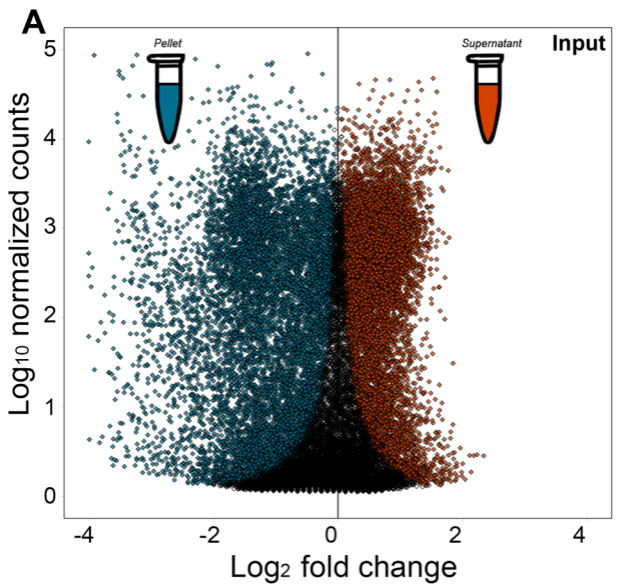
**Dataset S11 – Venn diagram values corresponding to Figure S4.**

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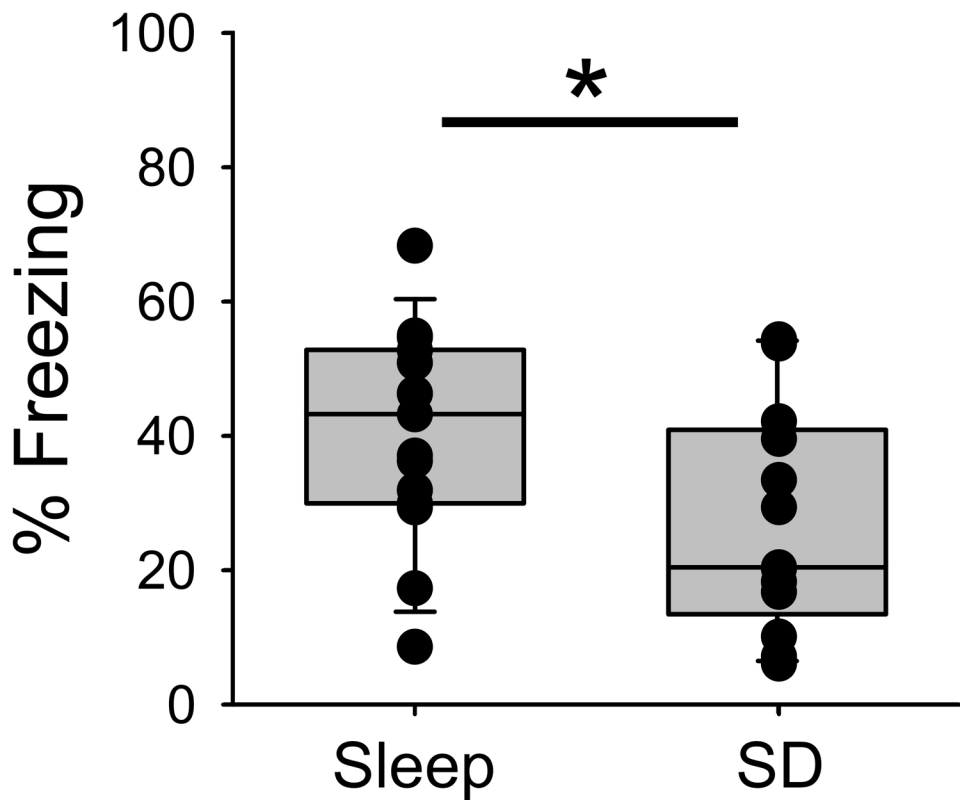
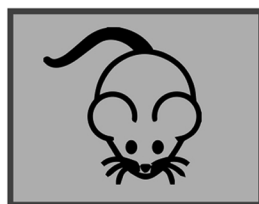
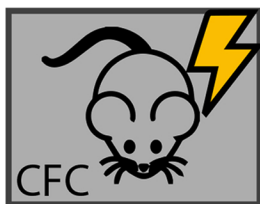
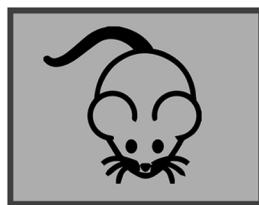
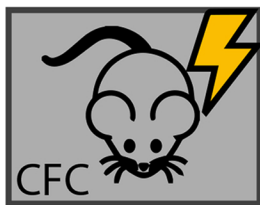
21. M. E. Gaine *et al.*, Altered hippocampal transcriptome dynamics following sleep deprivation *Mol Brain* **14** (2021).
22. L. C. Lyons, S. Chatterjee, Y. Vanrobaeys, M. E. Gaine, T. Abel, Translational changes induced by acute sleep deprivation uncovered by TRAP-Seq *Mol Brain* **13** (2020).
23. S. B. Noya *et al.*, The Forebrain Synaptic Transcriptome Is Organized by Clocks but Its Proteome Is Driven by Sleep *Science* **366** (2019).
24. A. McDermaid, B. Monier, J. Zhao, B. Liu, Q. Ma, Interpretation of differential gene expression results of RNA-seq data: review and integration. *Briefings in Bioinformatics* **20**, 2044-2054 (2019).







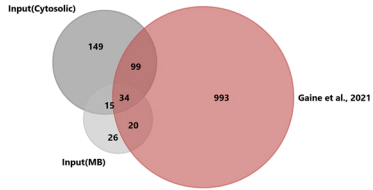
ZT0 . . . . . ZT0



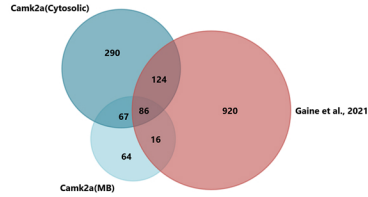


## Input

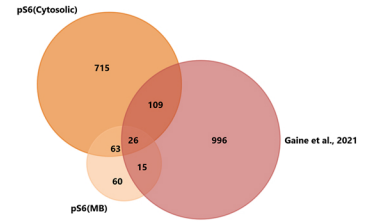
Gaine et al., 2021(hippocampal transcripts)



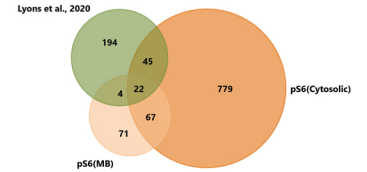
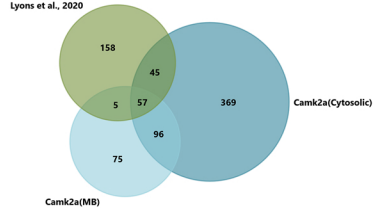
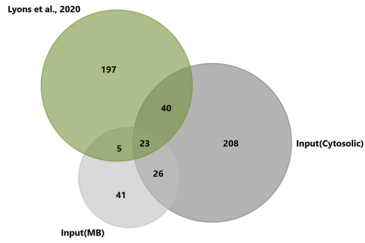
## Camk2a+



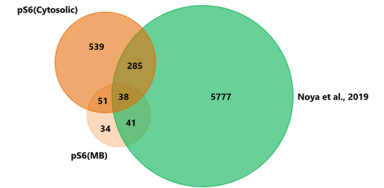
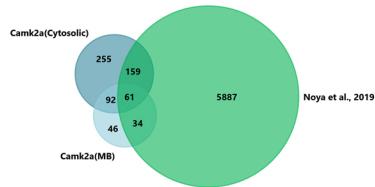
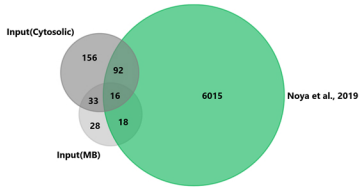
## pS6+



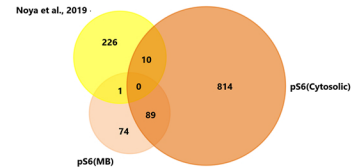
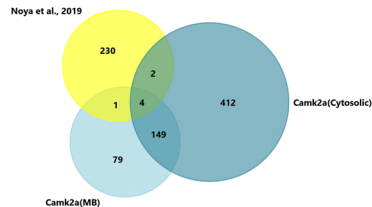
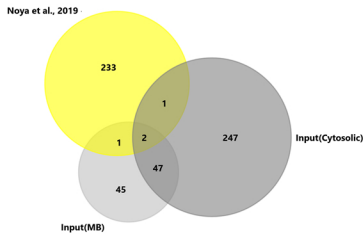
Lyons et al., 2020 (hippocampal Camk2a+ TRAP transcripts)



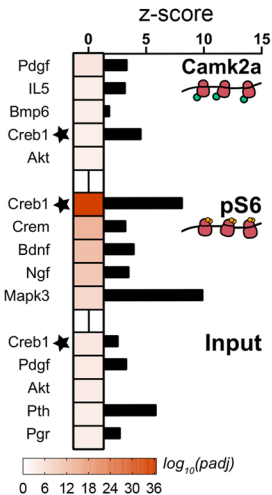
Noya et al., 2019 (forebrain synaptoneurosome transcripts)



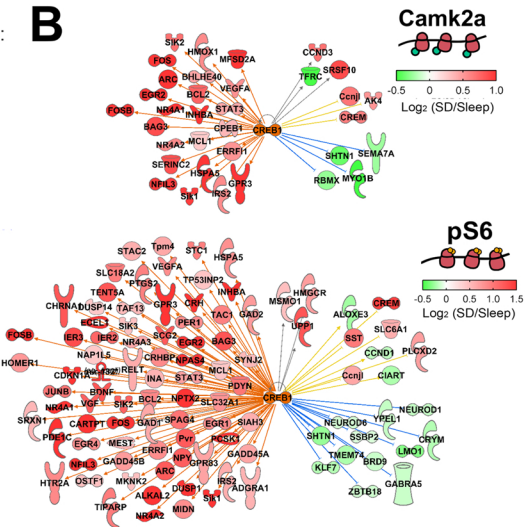
Noya et al., 2019 (forebrain synaptoneurosome proteins)



# A Predicted upstream regulators:

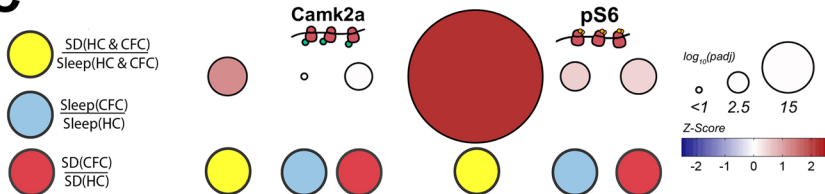


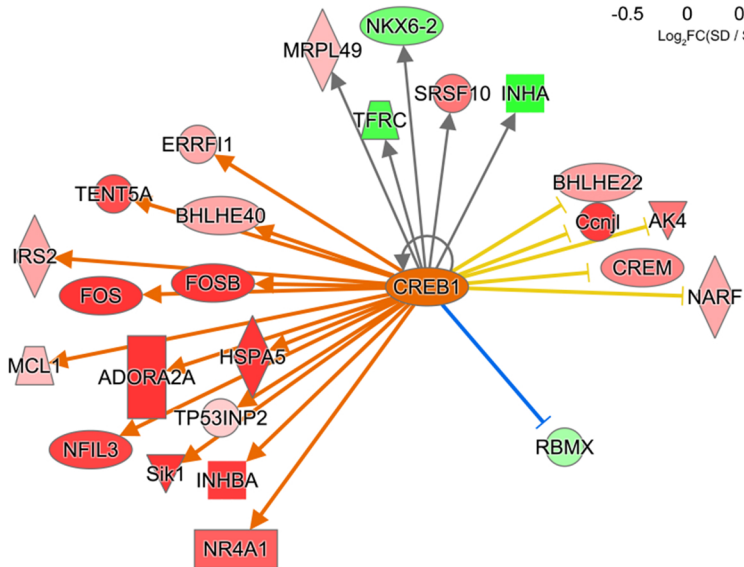
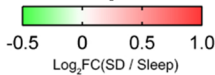
# B

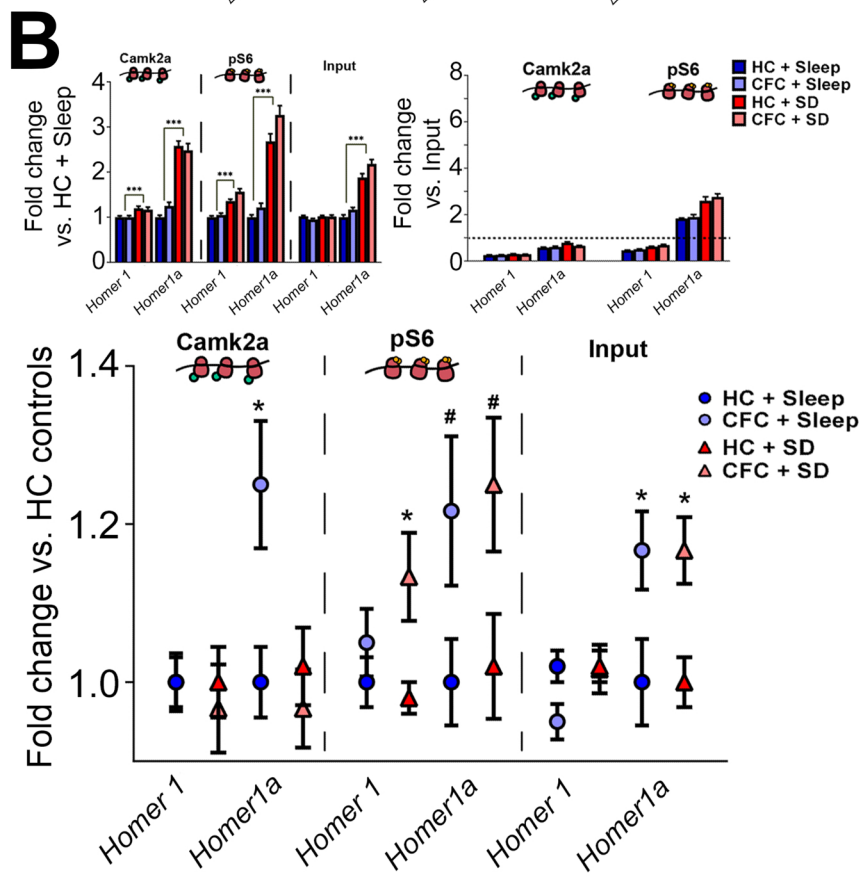
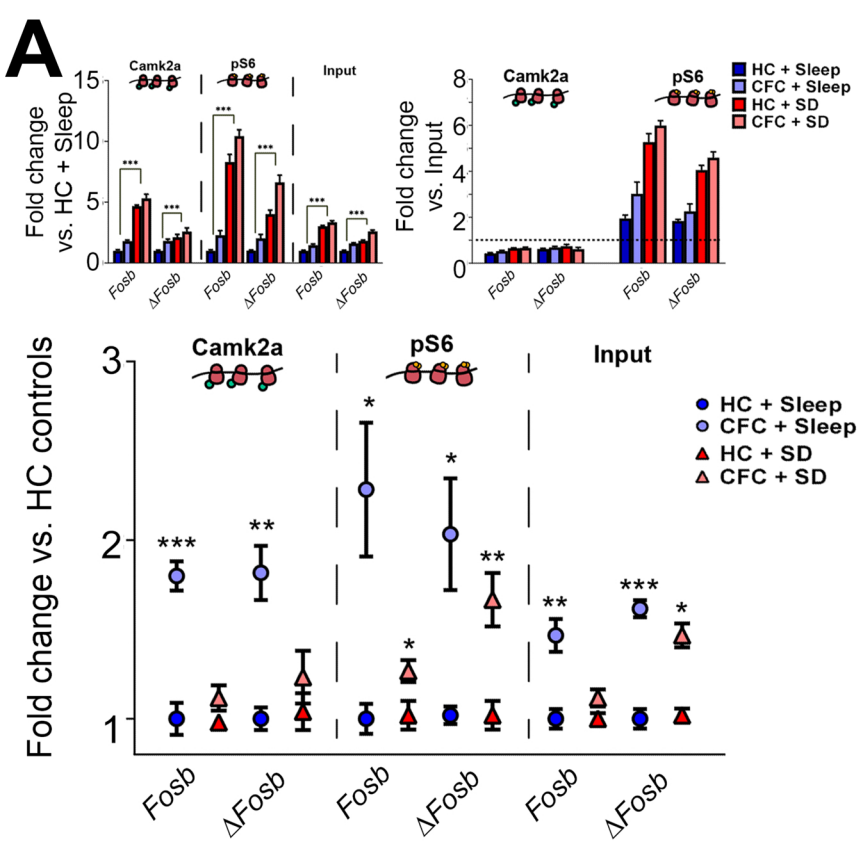


# C

Creb1 upstream regulation:

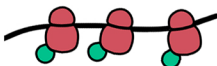
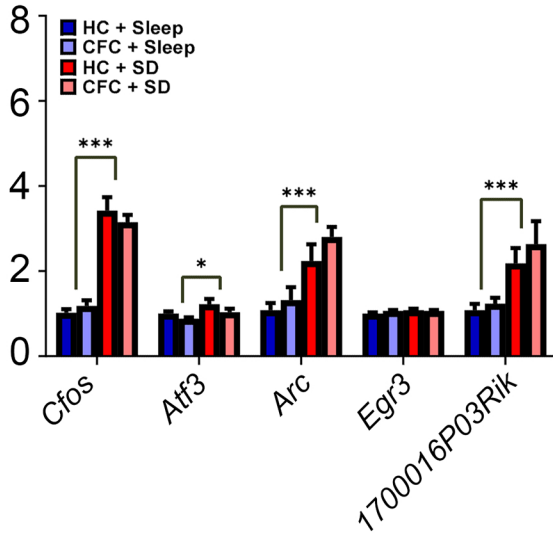


**Input**

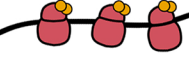
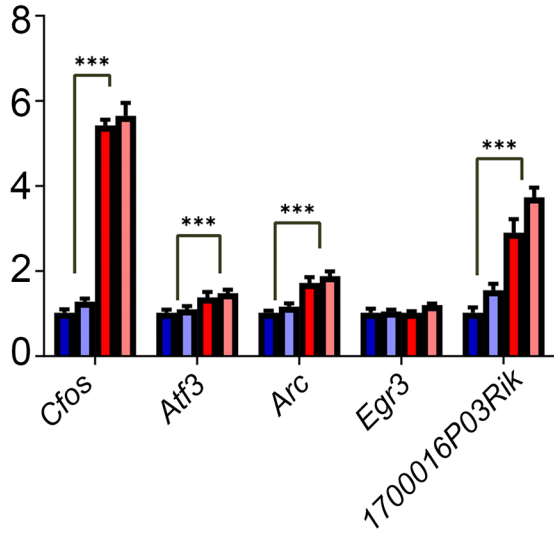


Fold change vs. HC + Sleep

## Camk2a

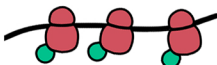
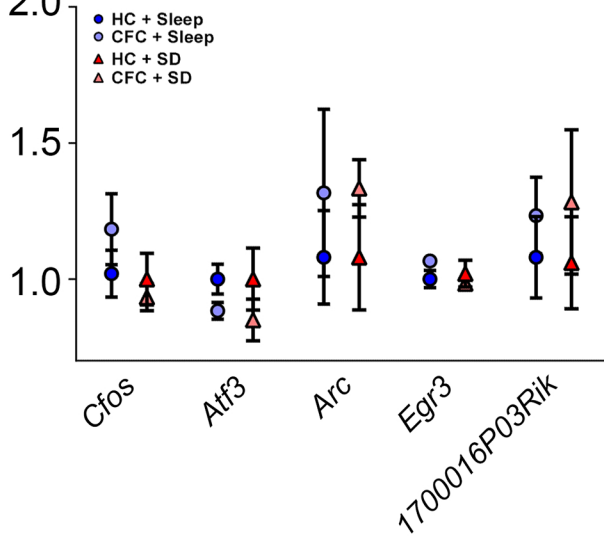



## pS6

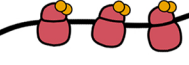
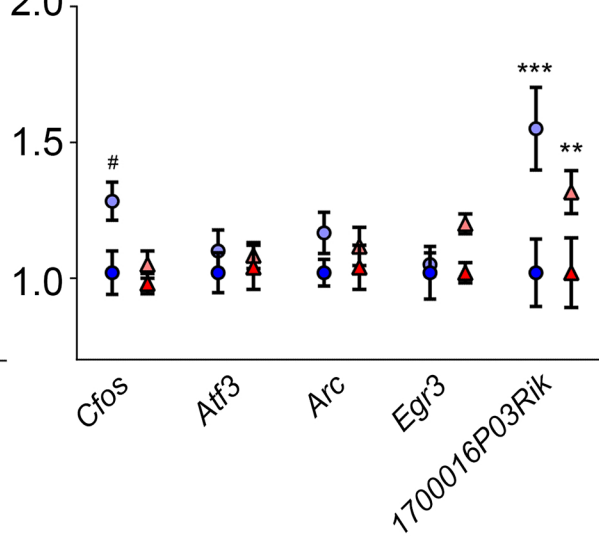



Fold change vs. HC controls

## Camk2a

## pS6

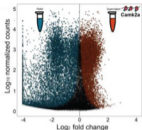







# Overview of Supplemental Tables

Supernatant vs. pellet:

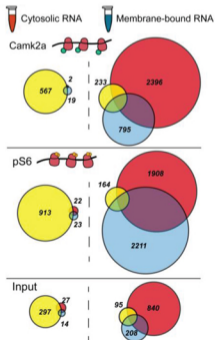


**Camk2a+** Dataset S1

**pS6+** Dataset S2

**Input+** Dataset S3

SD vs. CFC:



**Camk2a+** Dataset S4

**pS6+** Dataset S5

**Input+** Dataset S6

Venn Overlap Transcripts (ALL) Dataset S7

Canonical/Molecular Functions (Cytosolic) Dataset S8

CREB UR Analysis (Cytosolic) Dataset S9

Canonical/Molecular Functions (MB) Dataset S10

Venn Overlap Transcripts with Prior Studies Dataset S11