Supplemental Tables

Table S1: Copper, iron, and zinc content of diets.

Diet	Cu (μg/g)	Fe (μg/g)	Zn (μg/g)
Control TD.08584	8.41 ± 0.99	97.8 ± 3.44	42.3 ± 2.04
CuD TD.08585	0.43 ± 0.05	88.0 ± 0.73	41.0 ± 0.94
FeD TD.09337	7.39 ± 2.19	7.10 ± 0.70	43.3 ± 2.39

Metal content of diets was determined by wet ashing and flame AAS and represents the mean ± SEM of 3 separate diet samples.

Table S2: qPCR primer sequences

Gene	NCBI GenBank ID	qPCR Primer Sequences	Position	Product length (bp)
Pvalb	NM_022499.2	F: 5’-AGCCTTTACTGCTGCAGACTCCTT-3’ R: 5’-AGCTCATCCTCCTCAATGAAGCCA-3’	63-86 203-180	141
Enpp6	NM_001107311.1	F: 5’-ATGGGTCAGAACCCTTGTGGATCA-3’ R: 5’-AGTCGAGAGCATCGCTAACTGCAT-3’	419-442 592-569	174
Hr	NM_024364.2	F: 5’-TGGCCCTTGTAGGAAATGTCAGGA-3’ R: 5’-TTTCAGCTTGGTGTGATGGCTTGG-3’	1469-1492 1592-1569	124
Mbp	NM_001025291.1	F: 5’-GACTCACACACGAGAACT-3' R: 5’-CCAGCTAAATCTGCTGAG-3'	333-350 497-480	165
Mobp	NM_012720.1	F: 5’-TTCACCTTCCTCAACTCCAAGCGT-3’ R: 5’-TGCTCCTCCTTCTCAATCTGGTCT-3’	201-224 336-313	136
Agt	NM_134432.2	F: 5’-ATCAACAGGTTTGTGCAGGCTGTG-3’ R: 5’-TGTTGTCCACCCAGAACTCATGGA-3’	924-947 1086-1063	163
Dio3	NM_017210.3	F: 5’-TGTCAGCGCAGCAAGAGTACTTCA-3’ R: 5’-ATAGGCACCATATGCGGAACTGCT-3’	677-700 773-750	97
Mct8	NM_147216.1	F: 5’-CATTGCGTTTGAACTGGTGGG-3’ R: 5’-GCATCAGAGGGACGAAGAAAAGG-3’	1317-1327 1519-1497	203
Dio2	NM_031720.3	F: 5’-GAAGGACTACGCTGTGTCTGGAAC-3’ R: 5’-TCGCTGAACCAAAGTTGACCAC-3’	735-758 948-927	214
Oatp1c1	NM_053441.1	F: 5’-TCACTCCCAACATCTCTCCGTG-3’ R: 5’-GTCTTCACTGGCGAAATCATCC-3’	823-845 1040-1019	218
Rc3	NM_024140.2	F: 5’-TGCTCCAAGCCAGACGACGATATT-3' R: 5’-GGCCACACTCTCCACTCTTTATCT-3'	274-297 406-283	133
Egr1	NM_012551.2	F: 5’-TCTGAATAACGAGAAGGCGCTGGT-3’ R: 5’-ACAAGGCCACTGACTAGGCTGAAA-3’	243-266 401-378	159
Dpysl3	NM_012934.1	F: 5’-TTGGGAAGGACAACTTCACGGCTA-3’ R: 5’-TCGCTGTCAGAACCCACAGCTATT-3’	1107-1130 1302-1279	196
Dbm	NM_013158.2	F: 5’-TGGAATCTTGGAGGAGATGTGCGT-3’ R: 5’-TGCCGAACCGGTTTACTATGTGGA-3’	1458-1481 1585-1562	128
Crym	NM_053955.1	F: 5’-TGCAAGGAGATGTTCGGGTCTGTT-3’ R: 5’-TGCTTCATGAGCTCGTCATCCAGT-3’	570-593 756-733	187
Dmt1	NM_013173.1	F: 5’-TGTGGCCTGGCGTTACG-3’ R: 5’-CGCAGAAGAACGAGGACCAA-3’	4102-4118 4165-4146	64
TfR1	NM_022712.1	F: 5’-GAATACGTTCCCCGTTGTTGA-3’ R: 5’-ATCCCCAGTTCCTAGATGAGCAT-3’	991-1001 1057-1035	67

Bdnf	NM_012513.3	F: 5’-GATGCTCAGCAGTCAAGTGCCTTT-3’ R: 5’-GACATGTTTGCGGCATCCAGGTAA-3’	948-971 1034-1011	87
Bdnf IV	EF125679.1	F: 5’-AACAGGAGTACATATCGGCCACCA-3’ R: 5’-GCAGCCTTCATGCAACCGAAGTAT-3’	186-209 417-394	232
Bdnf VI	EF125680.1	F: 5’-ACCAGGAGCGTGACAACAATGTGA-3’ R: 5’-GCAGCCTTCATGCAACCGAAGTAT-3’	284-307 425-402	142
Gapdh	NM_017008.3	F: 5’-TGCACCACCAACTGCTTAGC-3' R: 5’-GGCATGGACTGTGGTCATGAG-3'	524-543 610-590	87

Genes: Parvalbumin, *Pvalb*; Ectonucleotide pyrophosphatase/phosphodiesterase 6, *Enpp6*; Hairless, *Hr*; Myelin basic protein, *Mbp*; Myelin-associated oligodendrocyte basic protein**,** *Mobp*; Angiotensinogen, *Agt*; Type 3 deiodinase, *Dio3*; Monocarboxylate transporter 8, *Mct8*; Type II deiodinase, *Dio2*; Organic anion transporting polypeptide 1c1, *Oatp1c1*; Neurogranin, *Rc3*; Early growth response factor 1, *Egr1*; Dihydropyrimidinase-like 3, *Dpysl3*; Dopamine beta-monooxygenase, *Dbm*; Mu crystallin, *Crym*; Divalent metal transporter 1, *Dmt1*; Transferrin receptor 1, *TfR1*; Brain-derived neurotrophic factor (total), *Bdnf*; Brain-derived neurotrophic factor isoform IV, *Bdnf IV*; Brain-derived neurotrophic factor isoform VI, *Bdnf VI*; Glyceraldehyde 3-phosphate dehydrogenase, *Gapdh.* F = Forward, R = Reverse

Supplemental Figure Legends

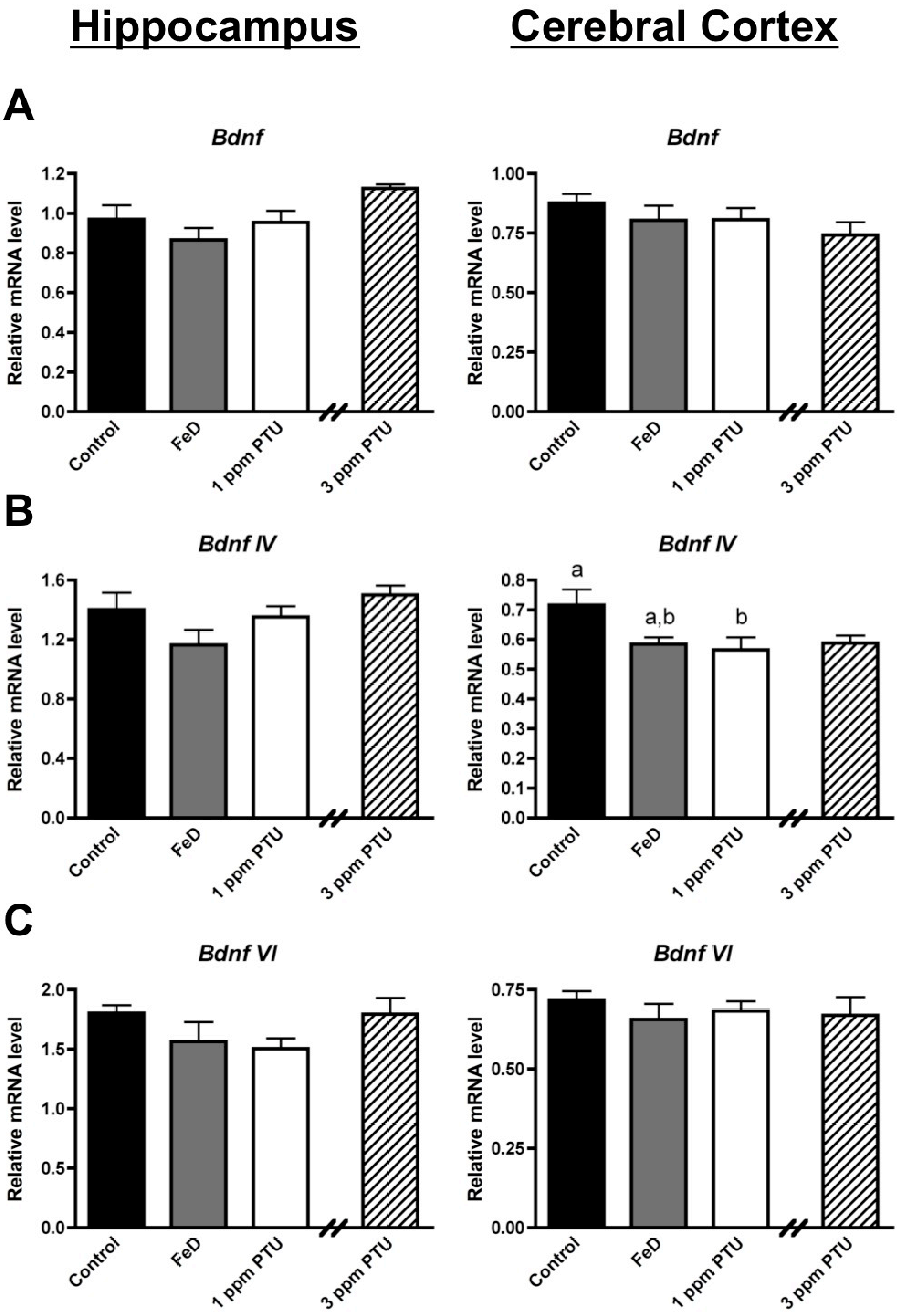
Supplemental Figure 1: *Bdnf* mRNA expression is not altered in the Fe- or TH-deficient neonatal cerebral cortex or hippocampus. Hippocampi or cerebral cortices were harvested from P10 male pups (n = 8 – 10), total RNA was extracted, and cDNA was synthesized. Quantitative real-time PCR was performed for (A) Brain-derived neurotrophic factor (total), *Bdnf* (B) Brain-derived neurotrophic factor isoform IV, *Bdnf IV* (C) Brain-derived neurotrophic factor isoform VI, *Bdnf VI*. Relative mRNA levels are calculated relative to an internal control cDNA sample. Data are presented as the mean ± SEM. Groups not sharing a common superscript are significantly different by one-way ANOVA and Tukey's or Scheffe's multiple comparison test ($P < 0.05$). Cu deficiency did not impact serum and brain TH concentrations or TH-responsive gene expression and thus was excluded from mRNA expression analysis. The 3 ppm PTU group served as a positive hypothyroid control. Asterisks indicate statistical difference between 3 ppm PTU and control.

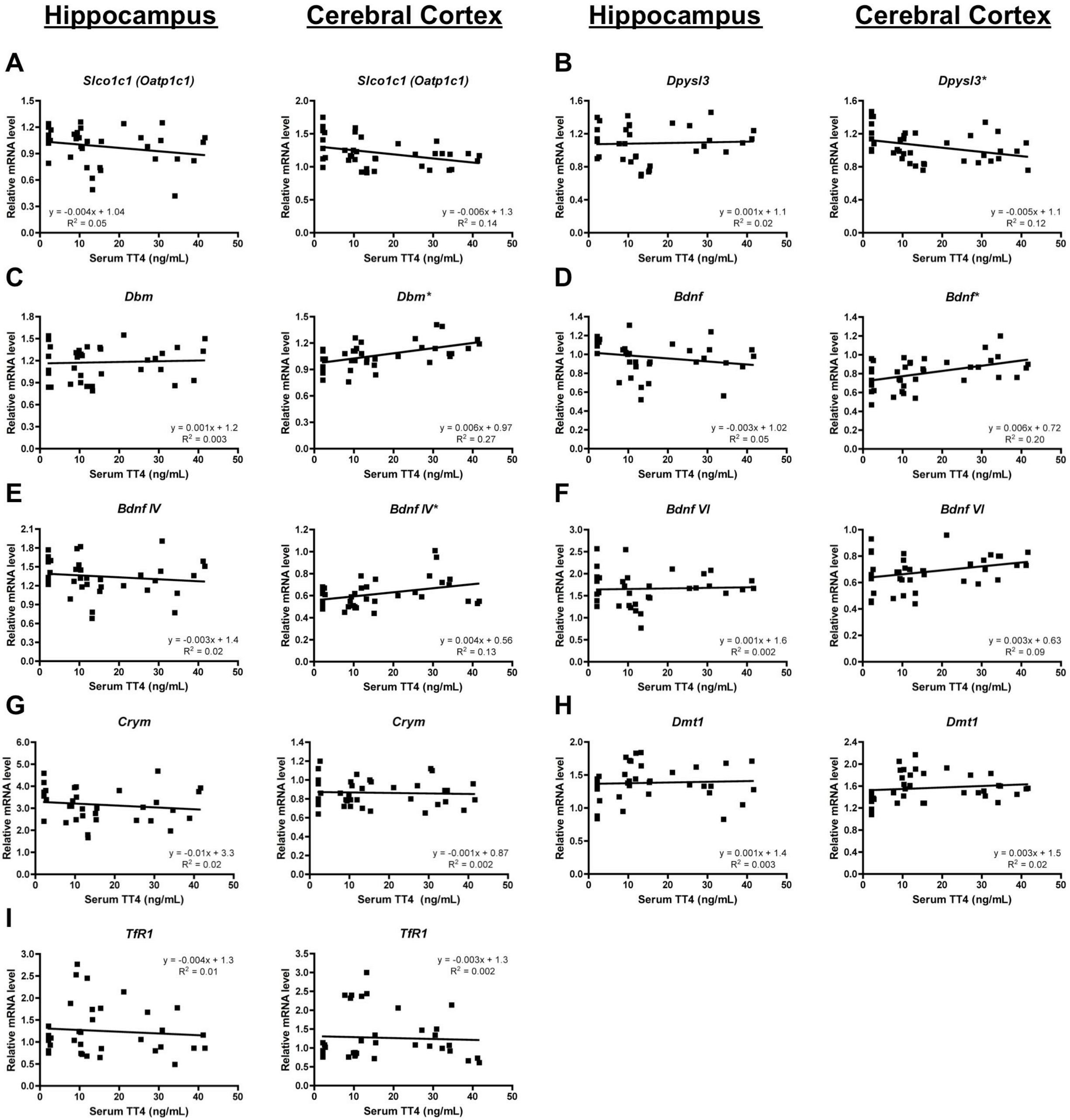
Supplemental Figure 2: Relationship between serum total T4 and brain regional mRNA levels. Linear regression scatter plots demonstrating the relationship between serum total T4 concentrations and either hippocampus or cerebral cortex mRNA levels in individual rat pups are shown for several genes. (A) Organic anion transporting polypeptide 1c1, *Oatp1c1* (B) Dihydropyrimidinase-like 3, *Dpysl3* (C) Dopamine beta-monooxygenase, *Dbm* (D) Brain-derived neurotrophic factor (total), *Bdnf* (E) Brain-derived neurotrophic factor isoform IV, *Bdnf IV* (F) Brain-derived neurotrophic factor isoform VI, *Bdnf VI* (G) Mu crystallin, *Crym* (H) Divalent metal transporter 1, *Dmt1* (I) Transferrin receptor 1, *TfR1*. The slope, y-intercept, and Pearson correlation R^2 values are shown for each linear regression analysis. Asterisks indicate relationships with slopes that significantly deviate from zero ($P < 0.05$).

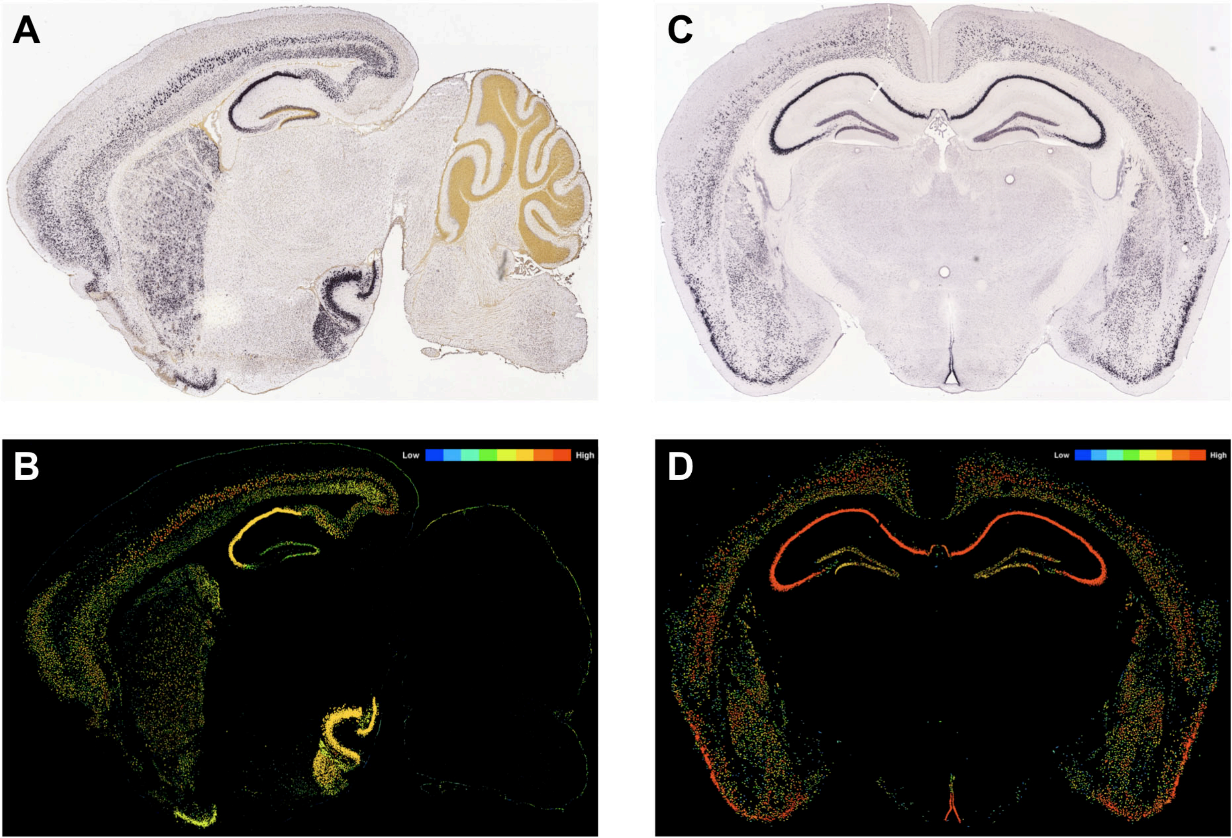
Supplemental Figure 3: Crym mRNA is highly expressed in the adult and neonatal hippocampus and the tanycytes lining the 3rd ventricle. *In situ* hybridization images demonstrating Crym mRNA expression patterns in the mouse brain were downloaded with permission from the Allen Brain Atlas (ABA) website (Reference a). A description of the site and the methods used by ABA can be found in Reference b. A) The image shows Crym mRNA expression by *in situ* hybridization in a postnatal day 14 mouse brain sagital section. B) The same image as in panel A is shown using ABA's expression analysis tool. C) The image shows Crym mRNA expression by *in situ* hybridization in an adult mouse brain coronal section. D) The same image as in panel A is shown using ABA's expression analysis tool. In panels A and C, cells with high Crym mRNA expression have significant silver grain deposition and are black. In panels B and D, red coloring indicates cells with high Crym mRNA expression, whereas blue indicates cells with low Crym expression as depicted in the legend in the upper right hand corner of each image. References:

A. Allen Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. © 2009. Available from: http://www.brain-map.org.

B. Lein, E.S. et al., Genome-wide atlas of gene expression in the adult mouse brain, Nature 445: 168-176 (2007), doi: 10.1038/nature05453


Hippocampus
Cerebral Cortex
A
Bdnf
Relative mRNA level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
FeD
1 ppm PTU
3 ppm PTU
Bdnf
Relative mRNA level
1.00
0.75
0.50
0.25
0.00
Control
FeD
1 ppm PTU
3 ppm PTU
B
Bdnf IV
Relative mRNA level
1.6
1.2
0.8
0.4
0.0
Control
FeD
1 ppm PTU
3 ppm PTU
Bdnf IV
Relative mRNA level
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
a,b
b
Control
FeD
1 ppm PTU
3 ppm PTU
C
Bdnf VI
Relative mRNA level
2.0
1.5
1.0
0.5
0.0
Control
FeD
1 ppm PTU
3 ppm PTU
Bdnf VI
Relative mRNA level
0.75
0.50
0.25
0.00
Control
FeD
1 ppm PTU
3 ppm PTU


Hippocampus
Cerebral Cortex
Hippocampus
Cerebral Cortex
A
Slco1c1 (Oatp1c1)
y = -0.004x + 1.04
R^2 = 0.05
Slco1c1 (Oatp1c1)
y = -0.006x + 1.3
R^2 = 0.14
B
Dpysl3
y = 0.001x + 1.1
R^2 = 0.02
Dpysl3*
y = -0.005x + 1.1
R^2 = 0.12
C
Dbm
y = 0.001x + 1.2
R^2 = 0.003
Dbm*
y = 0.006x + 0.97
R^2 = 0.27
D
Bdnf
y = -0.003x + 1.02
R^2 = 0.05
Bdnf*
y = 0.006x + 0.72
R^2 = 0.20
E
Bdnf IV
y = -0.003x + 1.4
R^2 = 0.02
Bdnf IV*
y = 0.004x + 0.56
R^2 = 0.13
F
Bdnf VI
y = 0.001x + 1.6
R^2 = 0.002
Bdnf VI
y = 0.003x + 0.63
R^2 = 0.09
G
Crym
y = -0.01x + 3.3
R^2 = 0.02
Crym
y = -0.001x + 0.87
R^2 = 0.002
H
Dmt1
y = 0.001x + 1.4
R^2 = 0.003
Dmt1
y = 0.003x + 1.5
R^2 = 0.02
I
TfR1
y = -0.004x + 1.3
R^2 = 0.01
TfR1
y = -0.003x + 1.3
R^2 = 0.002
Relative mRNA level
Serum TT4 (ng/mL)
0
10
20
30
40
50


A
C
B
Low
High
D
Low
High