Gene profiling reveals a role for stress hormones in the molecular and behavioral response to food restriction

Supplemental Information

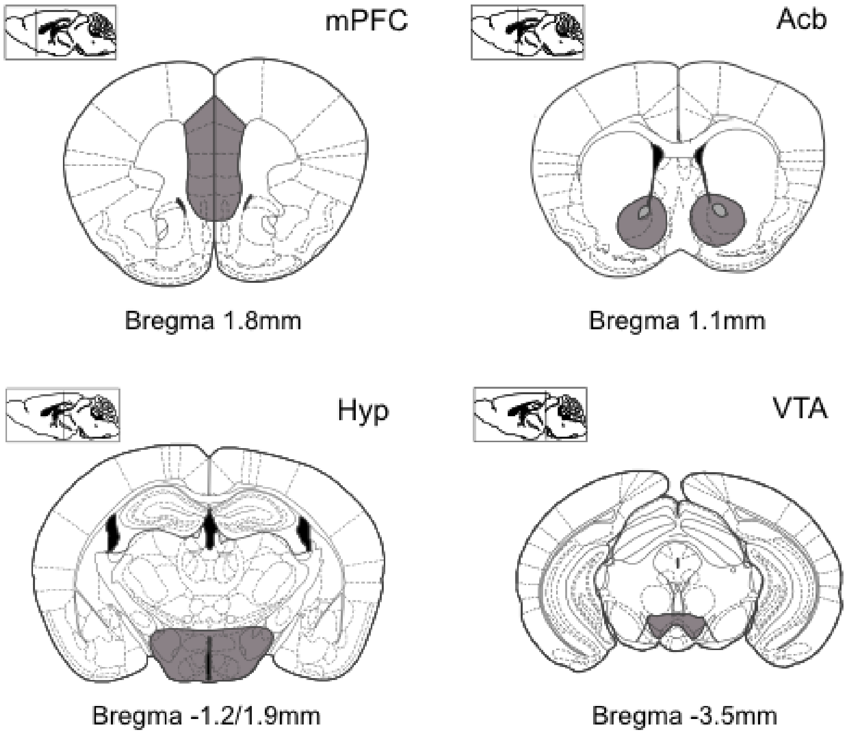

Figure S1. Brain regions dissected for microarray analysis. 1 mm brain slices used for microdissections. Templates adapted and reprinted with permission from Paxinos G, Franklin KBJ (2004): *The Mouse Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press. mPFC, medial prefrontal cortex; Acb, nucleus accumbens; Hyp, hypothalamus; VTA, ventral tegmental area.

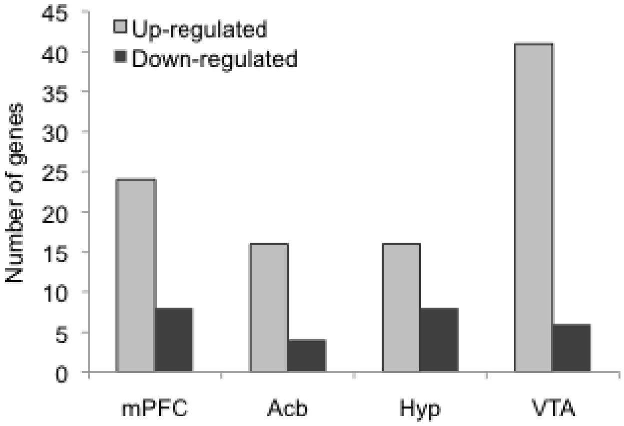

Figure S2. Gene expression changes in distinct regions of the mouse brain after a brief food restriction. Those changes +/- 1.5 fold change are shown. mPFC, medial prefrontal cortex; Acb, nucleus accumbens; Hyp, hypothalamus; VTA, ventral tegmental area.

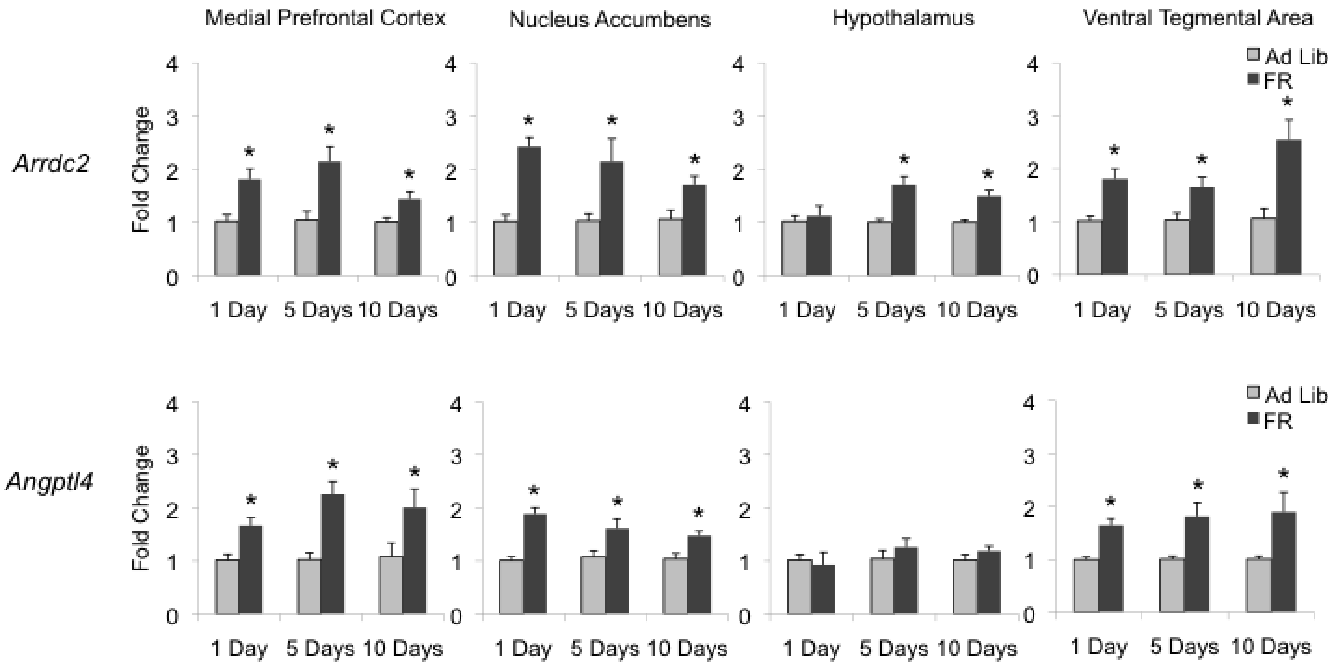

Figure S3. Time course of changes in gene expression are rapid and persistent. *Angptl4* and *Arrdc2* gene expression was analyzed in independent cohorts of 1, 5, and 10 day food restricted mice. *Angptl4* was significantly up-regulated at all three time points in the medial prefrontal cortex, nucleus accumbens and ventral tegmental area, but was not significantly up-regulated at any time point in the hypothalamus. *Arrdc2* was significantly up-regulated at all three time points in the medial prefrontal cortex, nucleus accumbens and ventral tegmental area, and was up-regulated at 5, and 10 day time points in the hypothalamus. The 5-day nucleus accumbens data is identical to that shown in Figure 2 and is shown here again for completeness. Ad Lib, *ad libitum* fed; FR, food restricted. * $p < 0.05$, $n = 5$ mice per group.

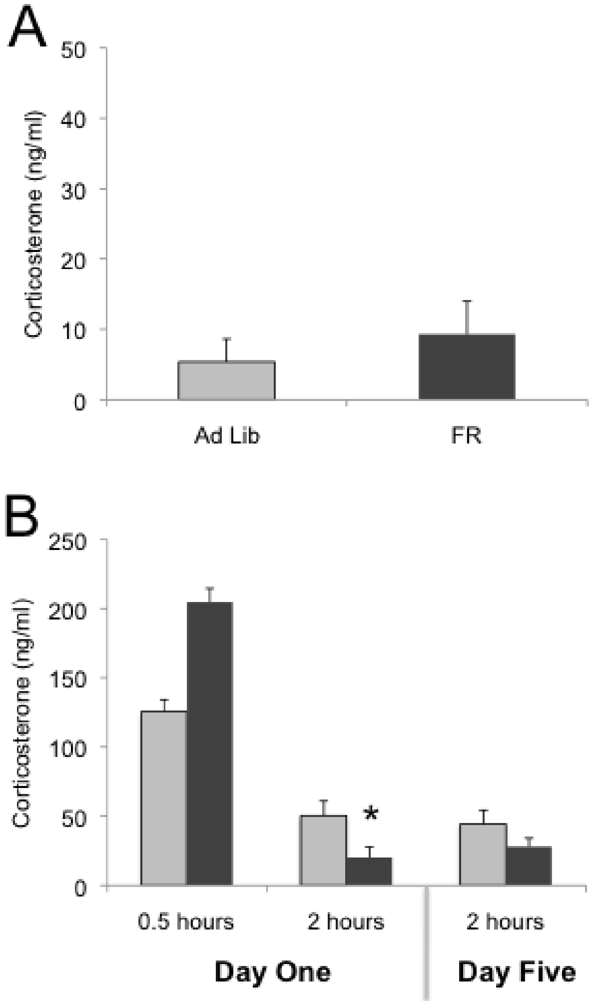

Figure S4. Corticosterone (CORT) levels support role in mediating observed gene expression. **(A)** Blood plasma corticosterone levels were measured in adrenalectomized animals provided with *ad libitum* diet (Ad Lib) or food restricted for 5 days (FR). There is no significant difference between Ad Lib and FR indicating corticosterone is virtually absent in adrenalectomized animals. $n = 5$ mice per group. **(B)** Blood plasma corticosterone levels were measured in Ad Lib fed animals receiving intraperitoneal injections of vehicle (Veh, gray bars) or corticosterone (black bars) at different time points. As expected, levels are initially quite high at 0.5 hours and after another 1.5 hours suggest negative feedback. After the fifth day of injection, there is no significant difference between the Veh and CORT groups. * $p < 0.05$, $n = 2$ for 0.5 hours and $n = 5$ for the other time points.

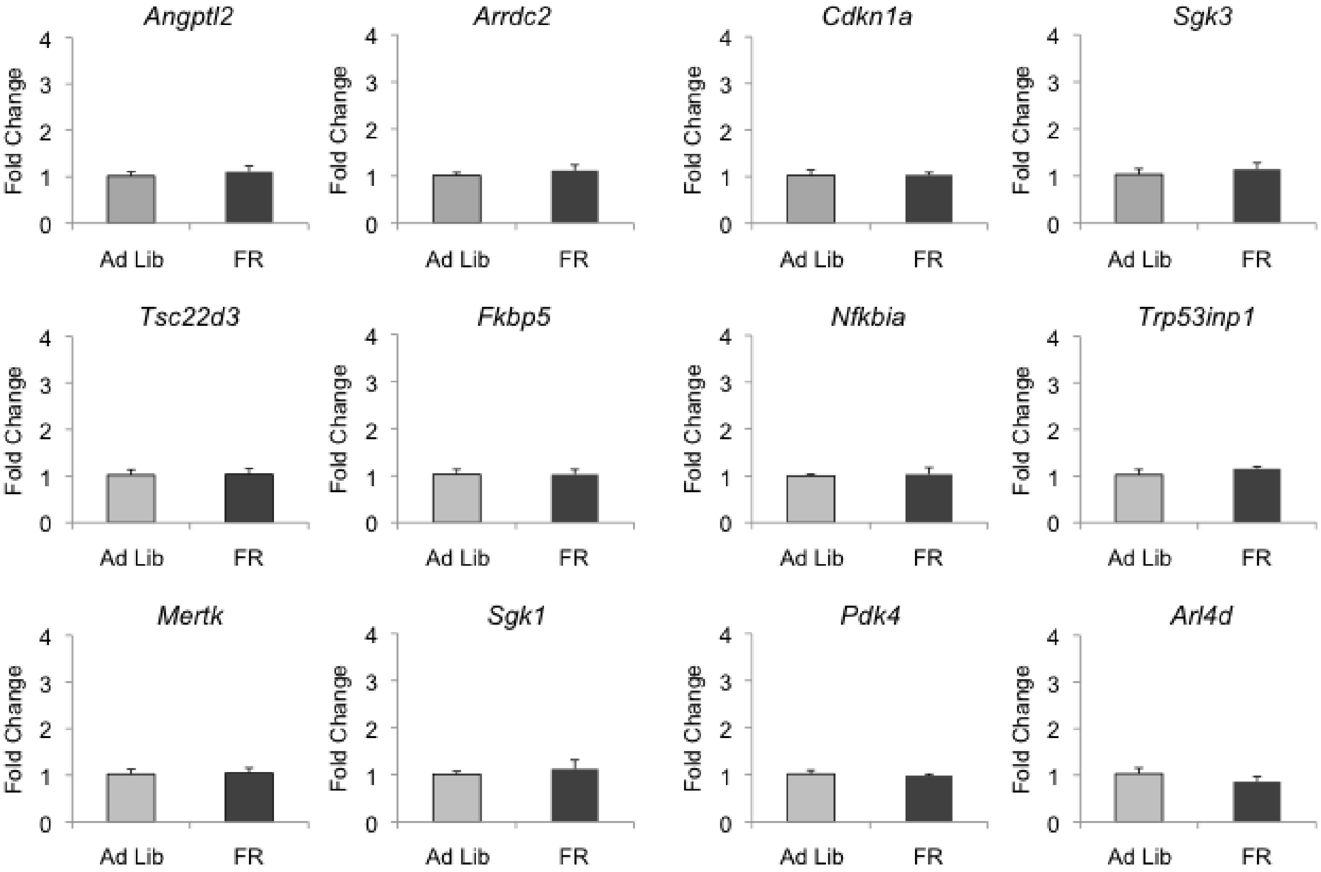

Figure S5. Adrenal glands are necessary for gene up-regulation during food restriction. Within the nucleus accumbens, gene expression of adrenalectomized mice food restricted (FR) for 5 days was not significantly different than adrenalectomized mice fed *ad libitum* (Ad Lib). The *Cdkn1a*, *Angptl4* and *Arrdc2* data are the same as in Figure 2 and are shown here again for completeness. n = 5 mice per group.

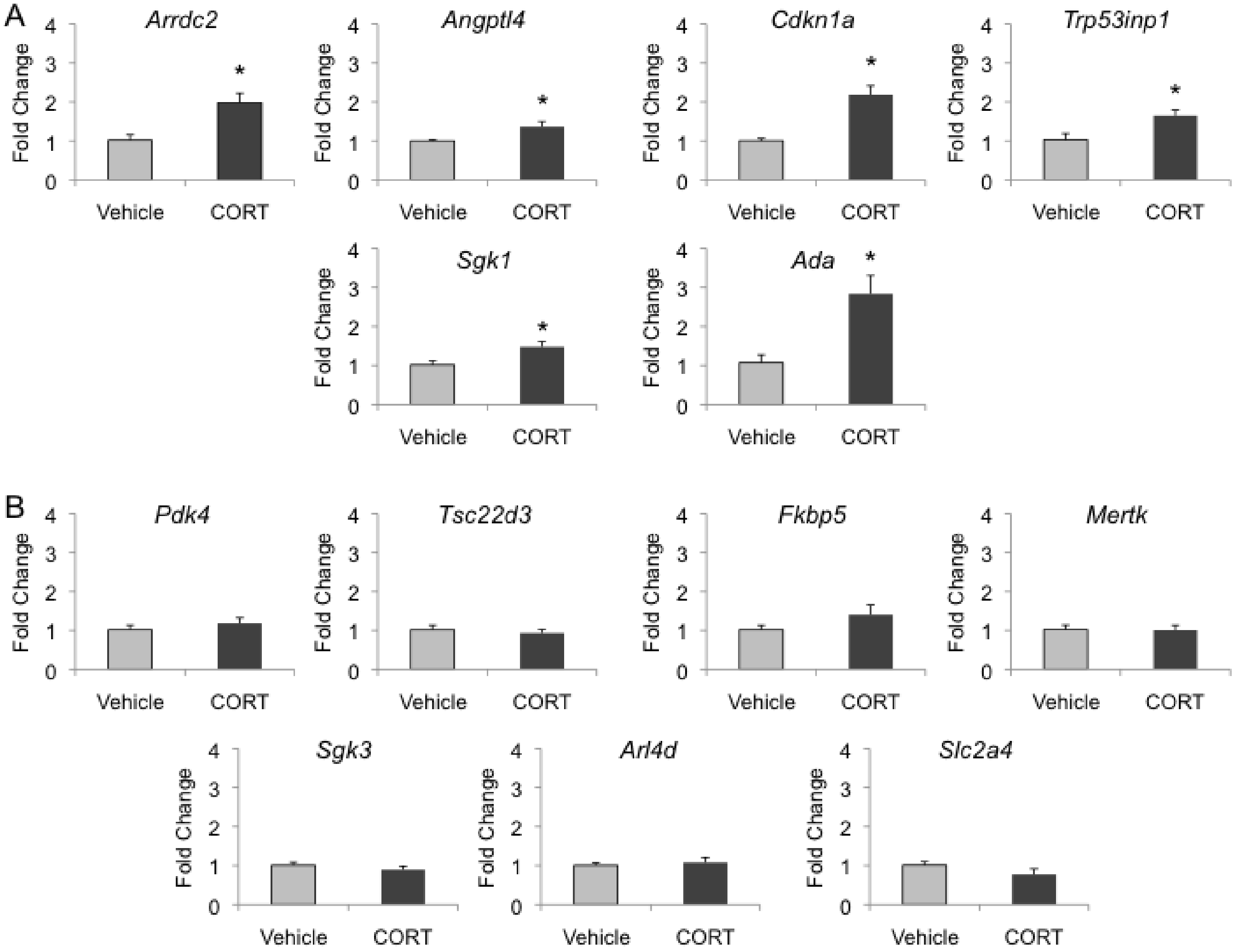

Figure S6. Repeated corticosterone exposure is sufficient to up-regulate a subset of qPCR validated genes. Animals were i.p. injected with vehicle or corticosterone for 5 days and gene expression changes were measured in the nucleus accumbens. **(A)** A subset of validated genes shows an up-regulation upon corticosterone treatment in the fully fed state. **(B)** However, another subset of genes was not up-regulated in the same animals. The *Arrdc2* and *Pdk4* data are the same as in Figure 3 and are shown here again for completeness. CORT, corticosterone; i.p., intraperitoneal; qPCR, quantitative polymerase chain reaction. * $p < 0.05$, $n = 5$ mice per group.

Table S1. Gene lists by brain region showing those regulated +/- 1.5 fold.

Gene Symbol	Accession	Fold Change	*P* value
Medial Prefrontal Cortex			
Creg2	NM_170597	3.538	0.049
Frmd3	NM_172869	2.067	0.047
Hif3a	Q0VBL6	2.042	0.049
Insm1	NM_016889.3	2.031	0.045
Ccdc148	XM_906580.3	1.930	0.047
Folh1	NM_016770.3	1.876	0.049
Angptl4	NM_020581	1.865	0.046
Grhl1	NM_145890	1.815	0.045
Kctd8	NM_175519	1.769	0.045
Trp53inp1	NM_021897	1.762	0.045
Trank1	XM_001481067.1	1.760	0.045
Tsc22d3	NM_001077364.1	1.742	0.045
Rin1	Q921Q7	1.660	0.043
Dner	NM_152915	1.641	0.044
Arrdc2	NM_027560	1.636	0.044
Arrdc2	NM_134133	1.618	0.043
2010002N04Rik	NM_134133	1.551	0.004
Padi6	Q8K3V4	1.550	0.045
Amy1	P00687	1.546	0.044
Scn3a	NM_018732.3	1.540	0.043
Zfp189	NM_145547	1.528	0.042
Nucleus Accumbens			
Insm1	NM_016889.2	1.928	0.044
Nuak2	NM_028778	1.882	0.001
Angptl4	NM_020581.1	1.861	0.033
Antxr2	NM_133738.1	1.832	0.008
Ada	P03958	1.822	0.003
Mro	NM_027741.2	1.821	0.033
Tsc22d3	NM_001077364.1	1.767	0.002
Arrdc2	NM_027560.1	1.719	0.000
Flnb	Q80X90	1.704	0.021
Ly6d	P35459	1.699	0.017
Zic3	NM_009575.2	1.696	0.040
Gsta3	P30115	1.600	0.000
Pnma3	NM_153169	1.585	0.032
Khdrbs2	NM_133235	1.551	0.021
Pdk4	O70571	1.525	0.006
Rragb	NM_001004154.1	1.501	0.007
Wasf3	NM_145155	-1.704	0.023
Hltf	NM_009210	-1.643	0.030
Nkd1	NM_027280	-1.641	0.048
Gpx3	Q91WR8	-1.590	0.021

Gene Symbol	Accession	Fold Change	*P* value
Ttc5	NM_177625	1.526	0.042
4833442J19Rik	NM_177101.4	1.517	0.034
Slc16a9	NM_025807	1.508	0.041
Cenpf	NM_001081363.2	-2.542	0.001
Gabre	NM_017369	-1.590	0.005
Bptf	NM_176850.2	-1.533	0.005
Fam189b	NM_001014995.1	-1.530	0.006
Cdkl5	Q8BWI8	-1.511	0.005
Lasp1	NM_010688.4	-1.503	0.015
Hypothalamus			
Hr	Q61645	2.198	0.001
Ptprd	Q64487	2.011	0.028
Sult1a1	P52840	2.009	0.032
1810029B16Rik	1810029B16Rik	1.908	0.018
Arrdc2	NM_027560.1	1.794	0.001
Dcun1d4	NM_178896.4	1.786	0.019
Mdga2	NM_207010.1	1.767	0.039
Tsc22d3	NM_001077364.1	1.693	0.021
Sgk1	Q9WVC6	1.676	0.021
Cntnap2	Q9CPW0	1.671	0.015
Cdkn1a	P39689	1.667	0.024
Tsc22d3	NM_001077364.1	1.587	0.021

Gene Symbol	Accession	Fold Change	*P* value
Ventral Tegmental Area			
Tmc7	NM_172476	2.516	0.013
Oprk1	P33534	2.455	0.000
Arrdc2	NM_027560	2.222	0.000
Ada	P03958	2.176	0.000
Sgk3	Q9ERE3	2.122	0.007
Mxra7	NM_026280.2	2.120	0.034
Sgk1	Q9WVC6	2.116	0.041
Cdkn1a	P39689	2.081	0.031
Hif3a	Q0VBL6	2.027	0.027
Pdk4	O70571	2.000	0.025
Pdk4	O70571	1.990	0.017
Il12rb1	Q60837	1.958	0.001
Mgp	P19788	1.925	0.033
Angptl4	NM_020581	1.862	0.015
Cdh9	P70407	1.802	0.034
Usp31	XM_357781.4	1.754	0.039
Hr	Q61645	1.740	0.039
Cpeb3	NM_198300	1.739	0.003
Mertk	Q60805	1.721	0.004
Kcns3	NM_173417	1.716	0.008
Fam83d	NM_027975	1.676	0.040
Neurog2	P70447	1.670	0.040

Gene Symbol	Accession	Fold Change	*P* value
Rnf144b	NM_146042.3	1.548	0.012
Rbm3	O89086	1.532	0.017
Fkbp5	Q64378	1.515	0.023
Arl4d	Q99PE9	1.510	0.001
Pdgfd	NM_027924	1.502	0.031
Clstn2	Q9ER65	-1.753	0.020
Ramp2	NM_019444.2	-1.712	0.009
C530008M17Rik	Q80TH6	-1.711	0.007
Micall1	Q8BGT6	-1.687	0.011
Luzp1	NM_024452	-1.652	0.006
Ankrd11	NM_001081379.1	-1.560	0.014
Ankrd11	NM_001081379.1	-1.555	0.018
Rnf165	XM_129042.8	-1.510	0.048
Olfr985	NM_146855	-1.508	0.001

Gene Symbol	Accession	Fold Change	*P* value
Ccdc3	NM_028804	1.635	0.013
Nfkbia	Q9Z1E3	1.635	0.007
Cadps2	NM_153163	1.625	0.014
Fkbp5	Q64378	1.613	0.017
2610042L04Rik	Q8BNC0	1.610	0.018
Lrrc8	Q80WG5	1.603	0.000
Mt1	P02802	1.596	0.002
Dcn	P28654	1.595	0.035
Arl4d	Q99PE9	1.582	0.006
Dedd2	Q8QZV0	1.578	0.012
Slc2a4	P14142	1.556	0.002
Txnip	Q91X82	1.549	0.024
Cyp1b1	Q64429	1.544	0.048
Slc39a4	NM_028064	1.539	0.041
Slitrk1	Q810C1	1.529	0.025
Itpk1	NM_172584	1.523	0.015
Tns	Q7TSV1	1.510	0.024
Vcam1	P29533	1.509	0.008
Edn1	P22387	1.506	0.019
Lrrc33	NM_146069.4	1.505	0.011
Cadps2	NM_153163	1.434	0.014
Calml4	NM_138304	-2.358	0.038
Ddo	NM_027442.5	-2.128	0.018

Gene Symbol	**Accession**	**Fold Change**	***P* value**	**Gene Symbol**	**Accession**	**Fold Change**	***P* value**
				Spc25	NM_025565.2	-2.041	0.009
				Lrrc23	NM_013588	-1.554	0.046
				Onecut2	NM_194268	-1.517	0.038
				Arl15	NM_172595.4	-1.516	0.023

Table S2. qPCR validation of array genes by brain region

Gene Symbol	Accession	Array Fold Change	qPCR Fold Change
Medial Prefrontal Cortex			
Creg2	NM_170597	3.538	n.s.
Frmd3	NM_172869	2.067	n.s.
Angptl4	NM_020581	1.865	3.893
Grhl1	NM_145890	1.815	n.s.
Trp53inp1	NM_021897	1.762	1.602
Tsc22d3	NM_001077364.1	1.742	1.682
Rin1	Q921Q7	1.660	n.s.
Dner	NM_152915	1.641	n.s.
Arrdc2	NM_027560	1.636	1.634
Padi6	Q8K3V4	1.550	n.s.
Amy1	P00687	1.546	n.s.
Cenpf	NM_001081363.2	-2.542	n.s.
Gabre	NM_017369	-1.590	n.s.
Bptf	NM_176850.2	-1.533	n.s.
Cdkl5	Q8BWI8	-1.511	n.s.
Lasp1	NM_010688.4	-1.503	n.s.
Nucleus Accumbens			
Angptl4	NM_020581.1	1.861	2.298
Ada	P03958	1.822	2.459
Mro	NM_027741.2	1.821	n.s.
Tsc22d3	NM_001077364.1	1.767	1.864
Arrdc2	NM_027560.1	1.719	2.043
Flnb	Q80X90	1.704	n.s.
Zic3	NM_009575.2	1.696	n.s.
Khdrbs2	NM_133235	1.551	n.s.
Pdk4	O70571	1.525	1.956
Wasf3	NM_145155	-1.704	n.s.
Hypothalamus			
Ptprd	Q64487	2.011	n.s.
Arrdc2	NM_027560.1	1.794	2.462

Tsc22d3	NM_001077364.1	1.693	1.870
Sgk1	Q9WVC6	1.676	2.080
Cntnap2	Q9CPW0	1.671	n.s.
Cdkn1a	P39689	1.667	2.823
Tsc22d3	NM_001077364.1	1.587	1.870
Fkbp5	Q64378	1.515	2.754
Arl4d	Q99PE9	1.510	2.237
Luzp1	NM_024452	-1.652	n.s.
Ventral Tegmental Area			
Arrdc2	NM_027560	2.222	2.616
Ada	P03958	2.176	3.402
Sgk3	Q9ERE3	2.122	2.674
Sgk1	Q9WVC6	2.116	2.967
Cdkn1a	P39689	2.081	3.388
Pdk4	O70571	2.000	2.171
Pdk4	O70571	1.990	2.171
Angptl4	NM_020581	1.862	3.081
Mertk	Q60805	1.721	2.014
Nfkbia	Q9Z1E3	1.635	1.784
Fkbp5	Q64378	1.613	2.102
Arl4d	Q99PE9	1.582	2.294
Slc2a4	P14142	1.556	1.309
Slc39a4	NM_028064	1.539	2.274
Onecut2	NM_194268	-1.517	-1.631
Arl15	NM_172595.4	-1.516	n.s.

n.s., no significant change in expression; qPCR, quantitative polymerase chain reaction.

Table S3. qPCR primer sequences

Gene Symbol	Gene Name	Primer Sequence
Ada	adenosine deaminase	F - ACGCAGACCCAGAGAGCTT R - TCCAGGTGGACGTGTAACTC
Angptl4	angiopoietin-like 4	F - AAGATGACCCAGCTCATTGG R - ACTGTGCCTCTCCCCTTCTT
Arl4d	ADP-ribosylation factor-like 4D	F - GACGAGTCGGTGTCTGGTT R - GGCCAAGAAGAGGAGACCTT
Arrdc2	arrestin domain containing 2	F - CGGAAGGTGTTCACTGTCATT R - CACGTGTGCAGTACCAGGAT
Cdkn1a	cyclin-dependent kinase inhibitor 1a	F - TCCACAGCGATATCCAGACA R - AGAGACAACGGCACACTTTG
Fkbp5	FK506 binding protein 5	F - GGGAGATGGACACCAAAGAA R - TCTCTGACAGGCCGTATTCC
Mertk	c-mer proto-oncogene tyrosine kinase	F - GATGGCTACTCCCCACTTCA R - ATGTGGCGAAGCAGAGGTAT
Nfkbia	nuclear factor kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	F - CAGCTGACCCTGGAAAATCT R - GCCTCCAAACACACAGTCAT
Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	F - AGGGAGGTCGAGCTGTTCT R - GACGTTCTTTCACAGGCATTT
Sgk1	serum/glucocorticoid regulated kinase 1	F - CTGGGATGATCTCATCAATAAGAAG R - GGTAAACTCGGGATCGAAGT
Sgk3	serum/glucocorticoid regulated kinase 3	F - TTCCACCTCCATTTAACCCTAA R - ACTGGCATTCACGATGGAAT
Slc2a4	solute carrier family 2 (faciliated glucose transporter), member 4	F - GACGGACACTCCATCTGTTG R - CTCAAAGAAGGCCACAAAGC
Slc39a4	solute carrier family 39 (zinc transporter), member 4	F - GAACTGGACGGCCTGTTAAA R - TGGACTCCAGGACTGATTCTG
Tbp	TATA box binding protein	F - AAAGGGAGAATCATGGACCAGAACAA R - TGGACTAAAGATGGGAATTCCAGGAG
Trp53inp1	transformation related protein 53 inducible nuclear protein 1	F - ACAACTCCTGTCCTGGTCTCA R - AATGTGCTTCCCCATTTCAC
Tsc22d3	TSC22 domain family, member 3	F - GGTGGCCCTAGACAACAAGA R - TCAAGCAGCTCACGAATCTG

F, forward; R, reverse; qPCR, quantitative polymerase chain reaction. All primers were designed to cross introns and were tested to be efficient.