# Supplementary Material For:

# A Ketogenic Diet Differentially Affects Neuron And Astrocyte Transcription

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**Table S1. From the neuron-enriched RNA samples, the thirty genes with the lowest uncorrected p-values.** A comparison between KD and Chow-fed mice neuron-enriched gene expression levels revealed the lowest uncorrected p-values for these genes. Black and red fonts indicate activated and suppressed genes respectively.

| Gene | P-Value |
|---|---|
| Ndufb5 | $6.0 \times 10^{-6}$ |
| Rnf115 | $1.39 \times 10^{-5}$ |
| 1700020I14Rik | 0.000147230 |
| Ctxn1 | 0.000168726 |
| Ubac2 | 0.000176338 |
| Gfra4 | 0.000210059 |
| B4galt3 | 0.000224228 |
| Rpusd1 | 0.000227457 |
| AK155692 | 0.000238010 |
| Rnf14 | 0.000246002 |
| Olfr1431 | 0.000275828 |
| Tst | 0.000282924 |
| Olfr385 | 0.000297638 |
| 2700029M09Rik | 0.000331136 |
| 4930413M19Rik | 0.000339662 |
| Slc16a4 | 0.000379751 |
| Ebag9 | 0.000383796 |
| Phpt1 | 0.000536413 |
| AK048085 | 0.000560823 |
| Mettl15 | 0.000581042 |
| Gm13769 | 0.000689679 |
| Pyroxd1 | 0.000693242 |
| Lars2 | 0.000723554 |
| Hist1h4m | 0.000763382 |
| Atg5 | 0.000779491 |
| Lipc | 0.000836519 |
| Dupd1 | 0.000904344 |
| Rps21 | 0.000965166 |
| Nmnat1 | 0.001008391 |
| Mt2 | 0.00105926 |

**Table S2. From the astrocyte-enriched RNA samples, the thirty genes with the lowest uncorrected p-values.** A comparison between KD and Chow-fed mice astrocyte-enriched gene expression levels revealed the lowest uncorrected p-values for these genes. Black and red fonts indicate activated and suppressed genes respectively.

| Gene | P-Value |
|---|---|
| Olfr1173 | 6.83E-05 |
| Gm9833 | 0.000609743 |
| Olfr1281 | 0.00064395 |
| Mup10 | 0.00078149 |
| Gm4340 | 0.001066191 |
| AK004434 | 0.00149721 |
| BC060616 | 0.001754201 |
| Olfr612 | 0.00189627 |
| Azgp1 | 0.001945021 |
| AK161373 | 0.001956496 |
| Serpina10 | 0.002214632 |
| Pcdha4 | 0.002240494 |
| Cabp4 | 0.002257912 |
| Cilp | 0.002389504 |
| Vmn1r68 | 0.00257838 |
| Olfr1447 | 0.00270419 |
| Mfsd6l | 0.003151691 |
| Oca2 | 0.003205539 |
| Srpx2 | 0.003285461 |
| Olfr1079 | 0.003368619 |
| Ntf3 | 0.003388646 |
| Hsf5 | 0.003490311 |
| Olfr1459 | 0.003701645 |
| Olfr685 | 0.003953845 |
| Vav1 | 0.004070868 |
| Fkbp14 | 0.0041769 |
| Gsdmc | 0.004207473 |
| AK157947 | 0.004295523 |
| Lao1 | 0.004322993 |
| Casr | 0.004376261 |

| **Table S3. KEGG molecular pathway changes.** | | | |
|---|---|---|---|
| **Neurons: Activated** ***Pathway (q-value)*** | **Neurons: Suppressed** ***Pathway (q-value)*** | **Astrocytes: Activated** ***Pathway (q-value)*** | **Astrocytes: Suppressed** ***Pathway (q-value)*** |
| Protein processing in endoplasmic reticulum (0.00066) | | Hematopoietic cell lineage (0.00783) | Axon guidance (0.00003) |
| Insulin signaling pathway (0.00122) | | Cytokine-cytokine receptor interaction (0.00960) | Glutamatergic synapse (0.000031) |
| Oxidative Phosphorylation (0.00122) | | Retinol metabolism (0.03533) | Circadian entrainment (0.000122) |
| Adrenergic signaling in cardiomyocytes (0.00122) | | Steroid hormone biosynthesis (0.04085) | Spliceosome (0.000151) |
| Dopaminergic synapse (0.00122) | | Complement and coagulation cascades (0.04085) | Long-term potentiation (0.000354) |
| cAMP signaling pathway (0.00122) | | | Phosphatidylinositol signaling system (0.000420) |
| Autophagy-animal (0.00122) | | | GABAergic synapse (0.000420) |
| MAPK signaling pathway (0.00122) | | | Oxytocin signaling pathway (0.000420) |
| ErbB signaling pathway (0.00122) | | | Retrograde endocannabinoid signaling (0.000497) |
| Oocyte meiosis (0.00125) | | | Insulin secretion (0.000591) |
| Ras signaling pathway (0.00156) | | | Dopaminergic synapse (0.000591) |
| Ribosome (0.00167) | | | GnRH signaling pathway (0.000844) |
| Long-term potentiation (0.00167) | | | Cholinergic synapse (0.000844) |
| Retrograde endocannabinoid signaling (0.00175) | | | mRNA surveillance pathway (0.000844) |
| Oxytocin signaling pathway (0.00184) | | | Protein processing in endoplasmic reticulum (.00138) |
| Glutamatergic synapse (0.00195) | | | ErbB signaling pathway (0.00314) |
| GABAergic synapse (0.00195) | | | Thyroid hormone signaling pathway (0.00319) |
| Endocytosis (0.00227) | | | Autophagy – animal (0.00319) |
| Neurotrophin signaling pathway (0.00227) | | | Adrenergic signaling in cardiomyocytes (0.00411) |
| Circadian entrainment (0.00235) | | | Gap junction (0.00533) |
| Axon guidance (0.00235) | | | MAPK signaling pathway (0.00533) |

| | | | |
|---|---|---|---|
| Estrogen signaling pathway (0.00272) | | | Endocytosis (0.00533) |
| Gap junction (0.00280) | | | cAMP signaling pathway (0.00576) |
| Rap1 signaling pathway (0.00286) | | | Inositol phosphate metabolism (0.00578) |
| Apelin signaling pathway (0.00405) | | | RNA transport (0.00578) |
| GnRH signaling pathway (0.00405) | | | Insulin signaling pathway (0.00863) |
| Regulation of actin cytoskeleton (0.00405) | | | Ubiquitin mediated proteolysis (0.00863) |
| Cholinergic synapse (0.00418) | | | Endocrine and other factor-regulated calcium reabsorption (0.00863) |
| Thyroid hormone signaling pathway (0.00529) | | | Calcium signaling pathway (0.00863) |
| Longevity regulating pathway – multiple species (0.00541) | | | Neurotrophin signaling pathway (0.00880) |
| Phospholipase D signaling pathway (0.00541) | | | Phospholipase D signaling pathway (0.0103) |
| Insulin secretion (0.00541) | | | Aldosterone synthesis and secretion (0.0103) |
| mTOR signaling pathway (0.00564) | | | Ribosome biogenesis in eukaryotes (0.0103) |
| cGMP-PKG signaling pathway (0.00620) | | | Progesterone-mediated oocyte maturation (0.0103) |
| Cardiac muscle contraction (0.0063) | | | Focal adhesion (0.0108) |
| Longevity regulating pathway (0.00781) | | | RNA degradation (0.0116) |
| Mitophagy – animal (0.00786) | | | Synaptic vesicle cycle (0.0126) |
| Gastric acid secretion (.00853) | | | Long-term depression (0.0141) |
| Progesterone-mediated oocyte maturation (0.00902) | | | cGMP-PKG signaling pathway (0.0188) |
| Fc gamma R-mediated phagocytosis (0.00980) | | | VEGF signaling pathway (0.0191) |
| Synaptic vesicle cycle (0.01014) | | | Aminoacyl-tRNA biosynthesis (0.0207) |
| Tight junction (0.01023) | | | Lysine degradation (0.0244) |
| EGFR tyrosine kinase inhibitor resistance (0.01107) | | | Endocrine resistance (0.0245) |
| Glucagon signaling pathway (0.01146) | | | Longevity regulating pathway (0.0253) |
| HIF-1 signaling pathway (0.01255) | | | Oocyte meiosis (0.0286) |

| | | | |
|---|---|---|---|
| Sphingolipid signaling pathway (0.01255) | | | Vasopressin-regulated water reabsorption (0.0291) |
| Adherens junction (0.01322) | | | Sphingolipid signaling pathway (0.0295) |
| Aldosterone synthesis and secretion (0.01342) | | | Cardiac muscle contraction (0.0365) |
| Phosphatidylinositol signaling system (0.01608) | | | Tight junction (0.0365) |
| Endocrine resistance (0.01652) | | | Ras signaling pathway (0.0377) |
| Ubiquitin mediated proteolysis (0.01652) | | | Aldosterone-regulated sodium reabsorption (0.0437) |
| Salivary secretion (0.01705) | | | Apelin signaling pathway (0.0505) |
| Focal adhesion (0.01706) | | | mTOR signaling pathway (0.0519) |
| Endocrine and other factor-regulated calcium reabsorption (0.01706) | | | Mannose type O-glycan biosynthesis (0.0546) |
| Melanogenesis (0.01811) | | | Rap1 signaling pathway (0.0550) |
| Carbon metabolism (0.01901) | | | Estrogen signaling pathway (0.0585) |
| Vasopressin-regulated water reabsorption (0.01957) | | | Circadian rhythm (0.0621) |
| AMPK signaling pathway (0.02239) | | | Gastric acid secretion (0.0635) |
| Hippo signaling pathway (0.02239) | | | EGFR tyrosine kinase inhibitor resistance (0.0711) |
| Lysosome (0.02428) | | | Glucagon signaling pathway (0.0725) |
| FoxO signaling pathway (0.03066) | | | Autophagy – other (0.0801) |
| Spliceosome (0.03303) | | | Glycerophospholipid metabolism (0.0864) |
| Calcium signaling pathway (0.03987) | | | Other types of O-glycan biosynthesis (0.0864) |
| Wnt signaling pathway (0.04063) | | | Serotonergic synapse (0.0864) |
| Fatty acid metabolism (0.04197) | | | Fc gamma R-mediated phagocytosis (0.0966) |
| Chemokine signaling pathway (0.04309) | | | HIF-1 signaling pathway (0.0967) |
| Aldosterone-regulated sodium resorption (0.04319) | | | Adherens junction (0.0967) |
| Renin secretion (0.04605) | | | |
| Regulation of lipolysis in adipocytes (.04667) | | | |
| Apoptosis (0.04692) | | | |

| | | | |
|---|---|---|---|
| Various types of N-glycan biosynthesis (.05137) | | | |
| Prolactin signaling pathway (0.05196) | | | |
| Signaling pathways regulating pluripotency of stem cells (.05221) | | | |
| Thyroid hormone synthesis (.05381) | | | |
| Inflammatory mediator regulation of TRP channels (0.05805) | | | |
| Long-term depression (0.05805) | | | |
| Collecting duct acid secretion (0.06277) | | | |
| Pancreatic secretion (0.06277) | | | |
| Mineral absorption (0.06920) | | | |
| Platelet activation (0.07028) | | | |
| T cell receptor signaling pathway (0.07028) | | | |
| Cell cycle (0.07140) | | | |
| Circadian rhythm (0.07768) | | | |
| VEGF signaling pathway (0.07988) | | | |
| SNARE interactions in vesicular transport (.07988) | | | |
| Leukocyte transendothelial migration (0.08187) | | | |
| Fc epsilon RI signaling pathway (0.08296) | | | |
| N-glycan biosynthesis (0.08296) | | | |
| PI3K-Akt signaling pathway (0.084185) | | | |
| Adipocytokine signaling pathway (0.08419) | | | |
| B cell receptor signaling pathway (0.08741) | | | |
| Serotonergic synapse (0.08997) | | | |
| Inositol phosphate metabolism (0.09228) | | | |
| Glycosphingolipid biosynthesis – lacto and neolacto series (0.09572) | | | |
| RNA transport (0.0967) | | | |
| Cellular sensescence (0.0967) | | | |

**Table S4. KEGG pathology analysis.** Black font=activated; red font=suppressed.

| **Implicated in Neurons**<br>***Pathology or Disease (q-value)*** | **Implicated in Astrocytes**<br>***Pathology or Disease (q-value)*** |
|---|---|
| Alzheimer’s disease (0.00066) | Morphine addiction (0.00309) |
| Parkinson’s disease (0.00167) | Choline metabolism in cancer (0.00309) |
| Huntington’s disease (0.00202) | Type II diabetes mellitus (0.00319) |
| Renal cell carcinoma (0.00216) | Nicotine addiction (0.0108) |
| Non-alcoholic fatty liver disease (0.00272) | Amphetamine addiction (0.0116) |
| Proteoglycans in cancer (0.00405) | Bacterial invasion of epithelial cells (0.0149) |
| Alcoholism (0.00472) | Central carbon metabolism in cancer (0.0195) |
| Morphine addiction (0.00602) | Renal cell carcinoma (0.0243) |
| Amphetamine addiction (0.00970) | Staphylococcus aureus infection (0.0340) |
| Bacterial invasion of epithelial cells (0.0108) | Glioma (0.0551) |
| Fluid shear stress and atherosclerosis (0.0115) | Dilated cardiomyopathy (0.0585) |
| Prostate cancer (0.0114) | Endometrial cancer (0.0635) |
| Endometrial cancer (0.0161) | MicroRNAs in cancer (0.0704) |
| Colorectal cancer (0.0170) | Proteoglycans in cancer (0.0760) |
| Type II diabetes mellitus (0.0171) | Insulin resistance (0.0888) |
| Choline metabolism in cancer (0.0204) | |
| MicroRNAs in cancer (0.0213) | |
| Glioma (0.0250) | |
| Insulin resistance (0.0250) | |
| Chagas disease (0.0350) | |
| Central carbon metabolism in cancer (0.0407) | |
| Pancreatic cancer (0.0588) | |
| Chronic myeloid leukemia (0.0613) | |
| Salmonella infection (0.0696) | |
| Prion disease (0.0714) | |
| Tuberculosis (0.0734) | |
| Dilated cardiomyopathy (0.0734) | |
| HTLV-I infection (0.0842) | |
| Amyotrophic lateral sclerosis (0.0983) | |
| Nicotine addiction (0.0983) | |

**Table S5. IPA disease states.** Gene name with associated p-value and z-score displayed in parenthesis and brackets respectively. Disease states predicted to be at decreased risk by IPA software are marked in red.

| Implicated in Neurons (p-value)[z-score] | Implicated in Astrocytes (p-value)[z-score] |
|---|---|
| Abnormality of cerebral cortex (0.0000035) [-1.172] | Abnormal morphology of epithalamus (0.00121) |
| Abnormal morphology of cerebral cortex (0.00000861) | Abnormal morphology of habenula (0.00747) |
| Abnormal morphology of hippocampus layer (0.0000205) | Wallerian degeneration (0.0158) |
| Abnormal morphology of hippocampus (0.0000511) | Abnormal morphology of inferior olivary nucleus (0.0232) |
| Abnormal morphology of corpus callosum (0.00015) | Loss of GABAergic neurons (0.0337) |
| Huntington Disease (0.000167) | Cell death of pyramidal neurons (0.0337) |
| Disorder of basal ganglia (0.000212) | Edema of brain (0.0376) |
| Dyskinesia (0.000265) | Lack of olfactory bulb (0.0456) |
| Abnormal morphology of brain (0.000608) | Brain lesion (0.0476) [1.745] |
| Abnormal morphology of stratum pyramidale (0.00074) | |
| Neurodegeneration of cerebral cortex (0.000813) [-1.765] | |
| Abnormal morphology of central nervous system (0.000871) | |
| Movement Disorders (0.00119) [-1] | |
| Abnormal morphology of pyramidal neurons (0.0013) | |
| Abnormal function of nervous system (0.00216) | |
| Agenesis of corpus callosum (0.00237) | |
| Hypoplasia of forebrain (0.00267) | |
| Neurodegeneration of central nervous system (0.00283) [-3.183] | |
| Neuroinflammation of brain (0.00339) [-1.192] | |
| Hypoplasia of brain (0.00381) [-2.919] | |
| Abnormal morphology of nervous system (0.00388) | |
| Degeneration of central nervous system (0.00421) [-3.316] | |
| Neurodegeneration of brain (0.00434) [-3.039] | |
| Neuropathy of brain (0.00439) | |
| Abnormal morphology of telencephalon (0.00529) | |
| Congenital malformation of brain (0.00542) [-3.08] | |
| Damage of hippocampus (0.00557) [1.406] | |
| Abnormal morphology of Purkinje cells (0.00632) | |
| Hypoplasia of telencephalon (0.00701) | |
| Degeneration of brain (0.00704) [-3.029] | |
| Abnormal morphology of cerebral ventricles (0.00903) | |
| Neurodegeneration of thalamus (0.0101) | |
| Abnormal morphology of cerebellar cortex (0.0102) | |
| Degeneration of postsynaptic terminals (0.0112) | |
| Edema of nervous tissue (0.0112) | |
| Abnormal morphology of dorsal striatum (0.0112) | |
| Damage of CA1 neuron (0.0112) | |
| Apoptosis of CA1 neuron (0.0112) | |
| Abnormal morphology of forebrain (0.0128) | |
| Abnormal morphology of oligodendrocytes (0.016) | |
| Abnormal morphology of hippocampal commissure (0.0161) | |

| | |
|---|---|
| Abnormal morphology of Purkinje's layer of cerebellum (0.0178) | |
| Enlargement of axons (0.0226) | |
| Neuroinflammation (0.0237) [-0.162] | |
| Abnormal morphology of white matter (0.0237) | |
| Neurodegeneration of hippocampus (0.0254) [-0.19] | |
| Astrocytosis (0.0269) [-0.437] | |
| Abnormal morphology of neuroglia (0.0307) | |
| Apoptosis of cerebral cortex cells (0.0313) [-0.749] | |
| Abnormal morphology of disorganized dorsal root ganglion (0.0314) | |
| Damage of dopaminergic neurons (0.0314) | |
| Infection of neurons (0.0314) | |
| Neurodegeneration (0.0365) [-3.442] | |
| Damage of cerebral cortex (0.0402) [1.085] | |
| Abnormal morphology of hippocampal CA1 region (0.0412) | |
| Apoptosis of hippocampal cells (0.0438) [-0.124] | |
| Damage of pyramidal neurons (0.0443) | |
| Lack of hippocampal commissure (0.0443) | |
| Brain atrophy (0.047) [-1.746] | |

## Supplementary Figure Legends

**Figure S1. Overall cell enrichment procedure.**

**Figure S2. Low expression of microglia, oligodendrocyte, and endothelium-specific gene transcripts.** A) Low levels of *Itgam*, *Aif1*, *Ptprc*, and *Cxcr3* indicate limited microglia contamination. B) Low levels of *Olig1*, *Olig2*, *Olig3*, and *Sox10* indicate limited oligodendrocyte contamination. C) Low levels of *Tek*, *Vcam1*, *Pecam1*, and *Vwf* indicate limited endothelial cell contamination. N = 12, 11 for neuron and astrocyte samples respectively.

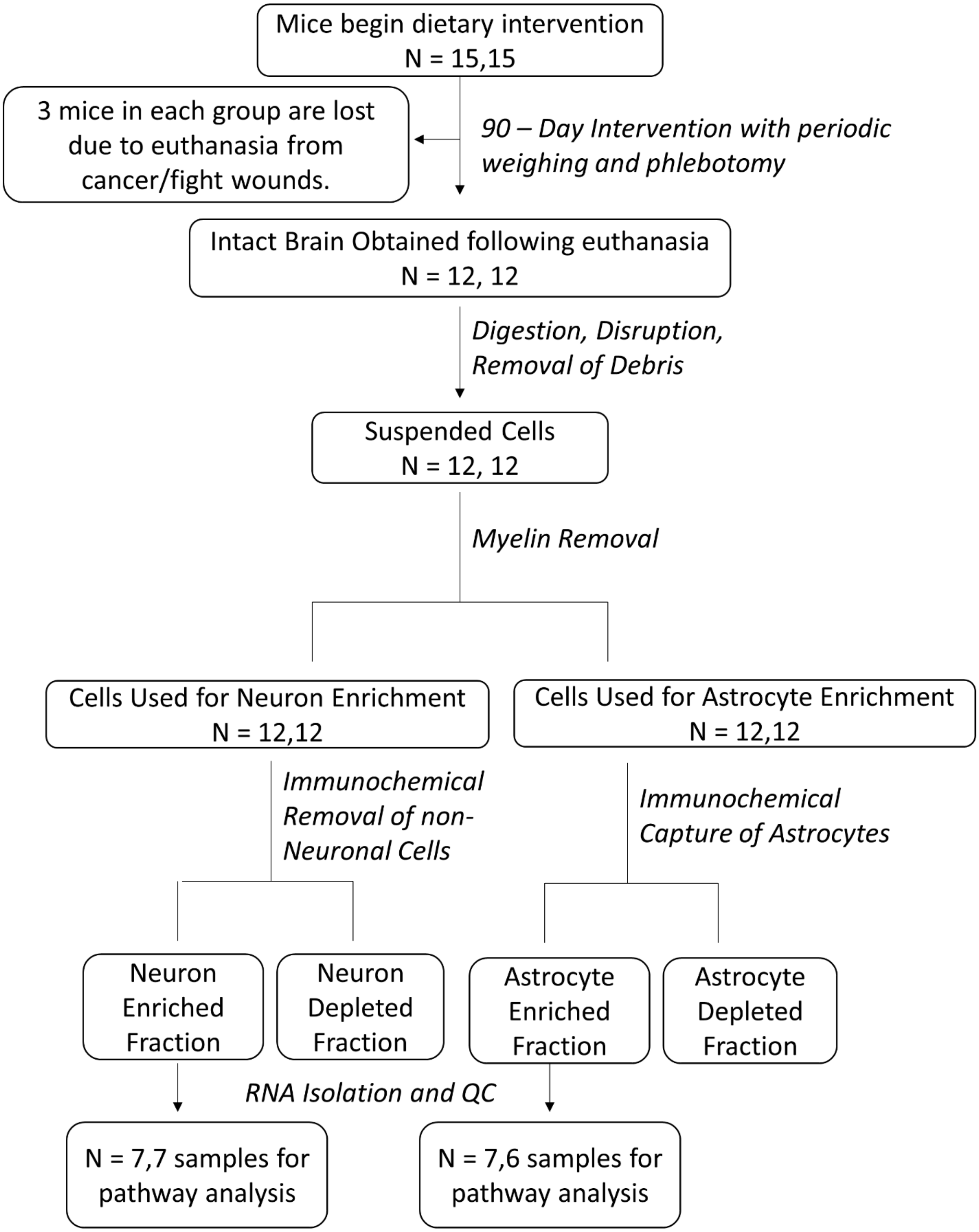
Mice begin dietary intervention
N = 15,15
3 mice in each group are lost due to euthanasia from cancer/fight wounds.
90 – Day Intervention with periodic weighing and phlebotomy
Intact Brain Obtained following euthanasia
N = 12, 12
Digestion, Disruption, Removal of Debris
Suspended Cells
N = 12, 12
Myelin Removal
Cells Used for Neuron Enrichment
N = 12,12
Cells Used for Astrocyte Enrichment
N = 12,12
Immunochemical Removal of non-Neuronal Cells
Immunochemical Capture of Astrocytes
Neuron Enriched Fraction
Neuron Depleted Fraction
Astrocyte Enriched Fraction
Astrocyte Depleted Fraction
RNA Isolation and QC
N = 7,7 samples for pathway analysis
N = 7,6 samples for pathway analysis

**Figure S1**

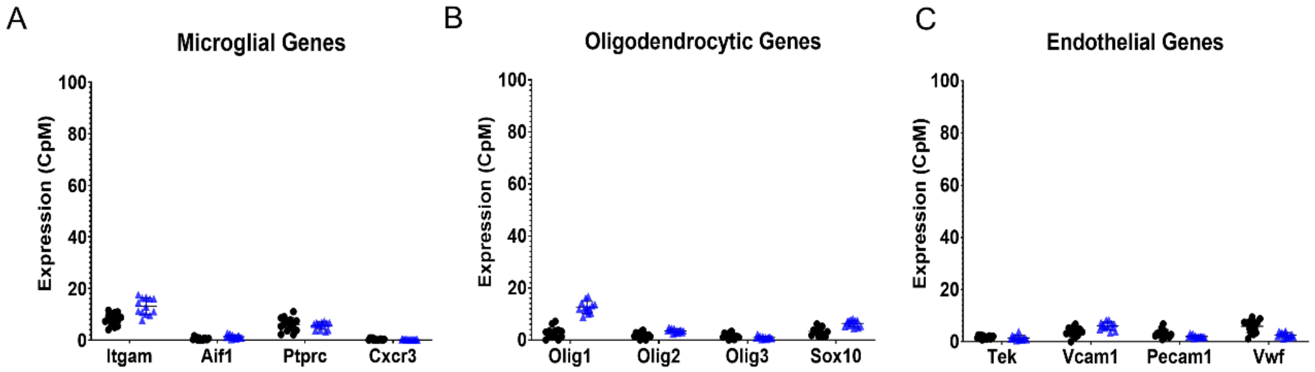
A
Microglial Genes
Expression (CpM)
100
80
60
40
20
0
Itgam
Aif1
Ptprc
Cxcr3
B
Oligodendrocytic Genes
Expression (CpM)
100
80
60
40
20
0
Olig1
Olig2
Olig3
Sox10
C
Endothelial Genes
Expression (CpM)
100
80
60
40
20
0
Tek
Vcam1
Pecam1
Vwf

**Figure S2**