Table S1. Transcription factors that regulate expression of genes that code for urea cycle enzymes..

Gene	Transcription Initiation	Regulatory Elements		References
		Promoter	Distal Enhancer	
NAGS	Sp1	CREB	HNF-1, NF-Y	1, 2
CPS1	TATA	GR	C/EBP, GR, HNF-3, CREB, P1, P2, P3	3, 4, 5
OTC	TATA	HNF-4, COUP-TF	C/EBP, HNF-4, COUP-TF	3, 6, 7, 8, 9
ASS	Sp1	AP2	CREB	3
ASL	Sp1	NF-Y		3
ARG1	Sp1	C/EBP, NF-Y, NF-1	C/EBP, P1[a], P2[a], NF-Y	3, 8, 9

[a]The P1 and P2 transcription factors that regulate expression of the ARG1 gene might not be the same as transcription factors designated P1 and P2 that regulate expression of the CPS1 gene.

References:

1. Heibel SK et al. (2012) *PLoS ONE*, 7(2): e29527
2. Heibel SK et al. (2011) *Hum Mutat*, 32(10): 1152-1160
3. Takiguchi M and Mori M (1995) *Biochem J*, 312(Pt 3): 649-659
4. Christoffels VM et al. (1998) *Mol Cell Biol*, 18(11): 6305-6315
5. Goping IS and Shore GC (1994) *J Biol Chem*, 269(5): 3891-3896
6. Nishiyori A et al. (1994) *J Biol Chem*, 269(2): 1323-1331
7. Inoue Y et al. (2002) *J Biol Chem*, 277(28): 25257-25265
8. Kimura T et al. (1998) *J Biol Chem*, 273(42):27505-27510.
9. Kimura T et al. (2001) *FEBS Lett*, 494(1-2):105-111.

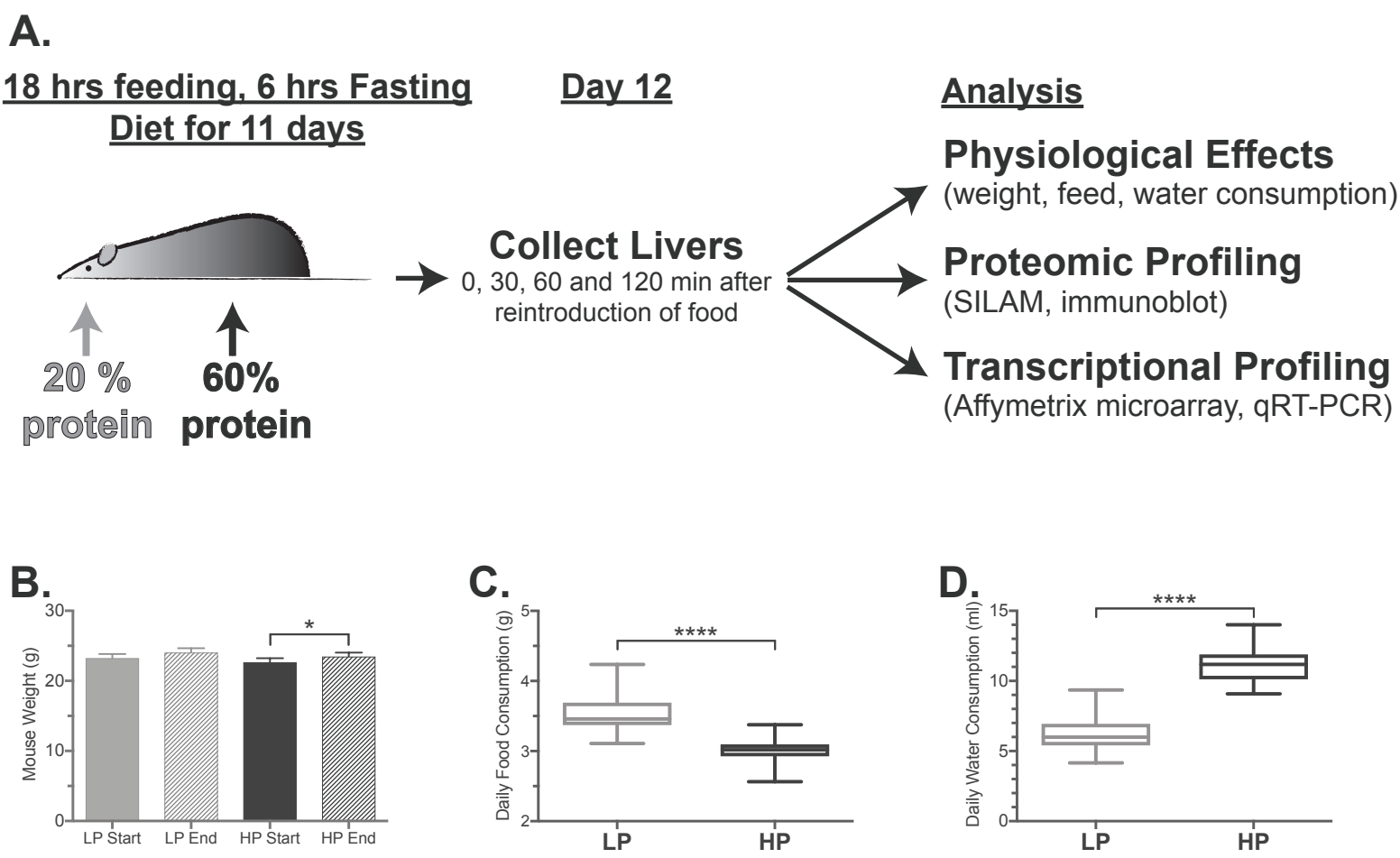

Figure S1. A. Experimental design for identifying signaling pathways that regulate expression of urea cycle genes. Mice were fed either LP (light gray) or HP (dark gray) diets on a time-restricted schedule.
B. The average weight of animals at the beginning (solid) and the end (hatched) of experiment.
C. Average daily food consumption of mice fed either LP or HP. **D.** Average daily water consumption of mice fed either LP or HP. **** – p<000.1.

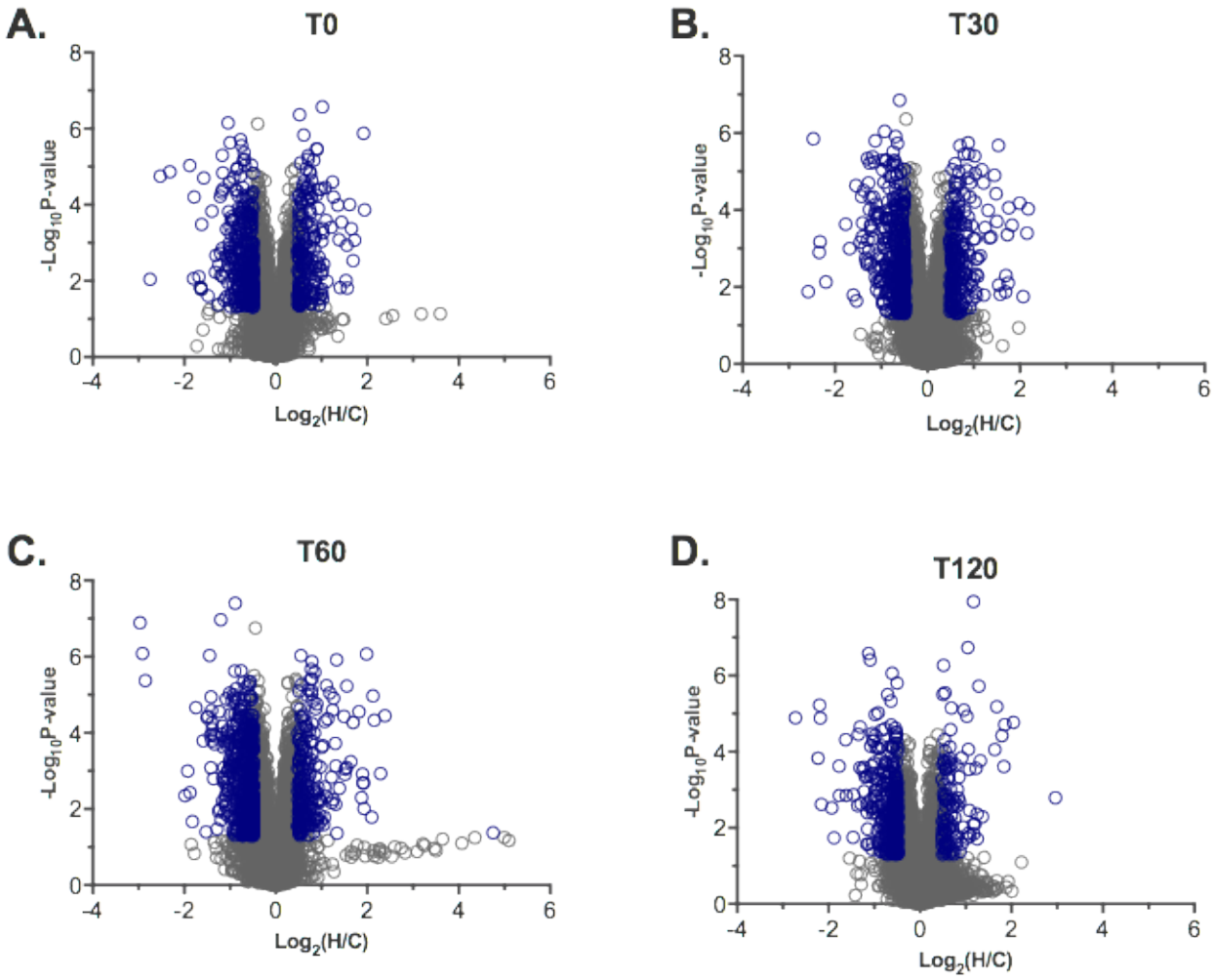

Figure S2. Scatter plot of statistical significance vs. fold change of mRNA expression in the livers of mice fed either HP or LP diets. Expression of mRNA was measured at fasting (A) and 30 (B), 60 (C) and 120 (D) min. after introduction of food. Blue – probe sets that had fold change ≥ ±1.4 and $p<0.05$. Gray – all other probesets.

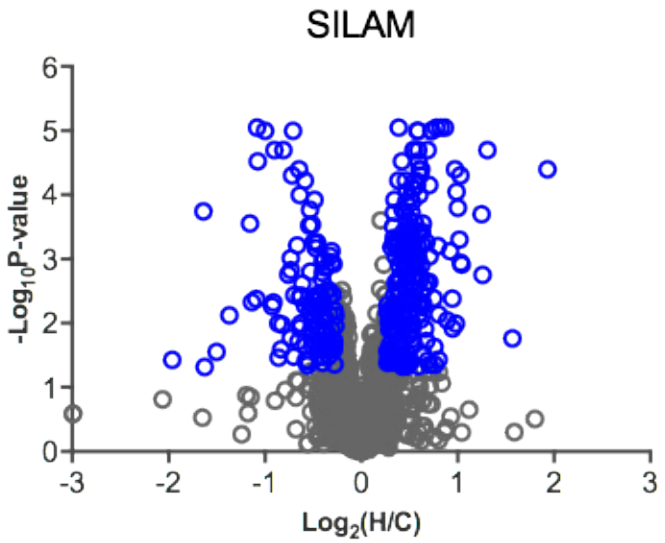

Figure S3. Scatter plot of statistical significance vs. fold change of protein expression in the livers of mice fed either HP or LP diets. Protein expression was measured at fasting. Blue – proteins that had fold change ≥ ±1.2 and $p<0.05$. Gray – all other proteins.

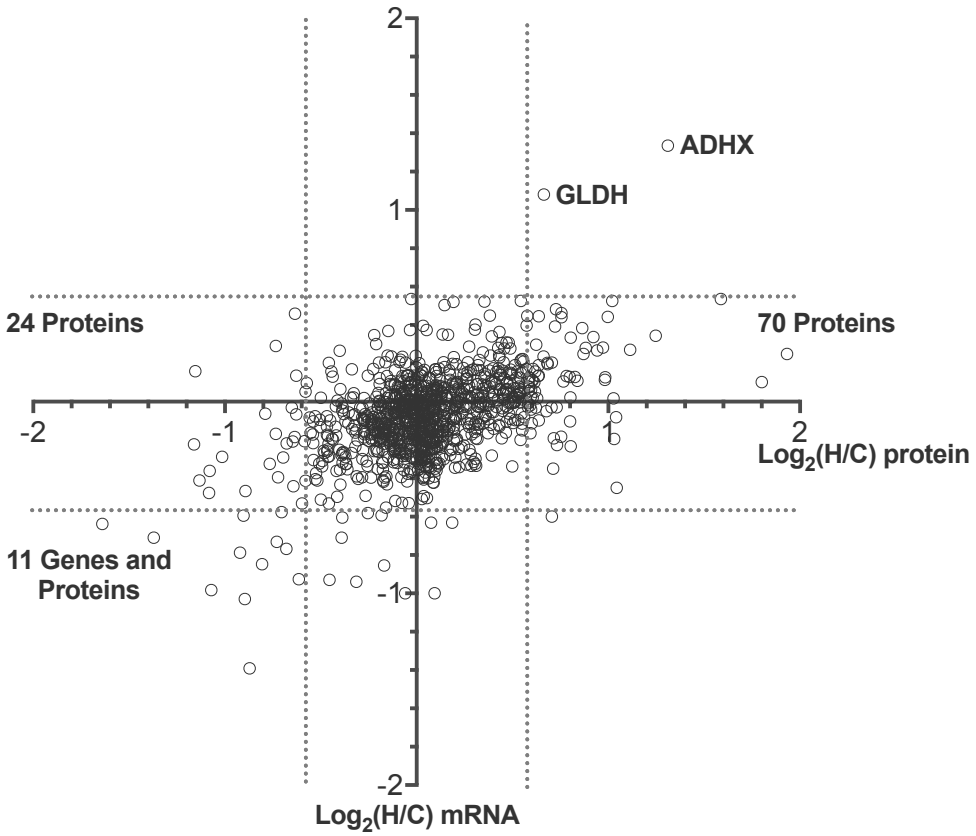

Figure S4. Correlation between changes in abundance of mRNA and proteins in livers of mice fed either HP or LP diets. ADH – aldehyde dehydrogenase X, GLDH – glycine dehydrogenase. Hatched lines indicate ±Log21.5.

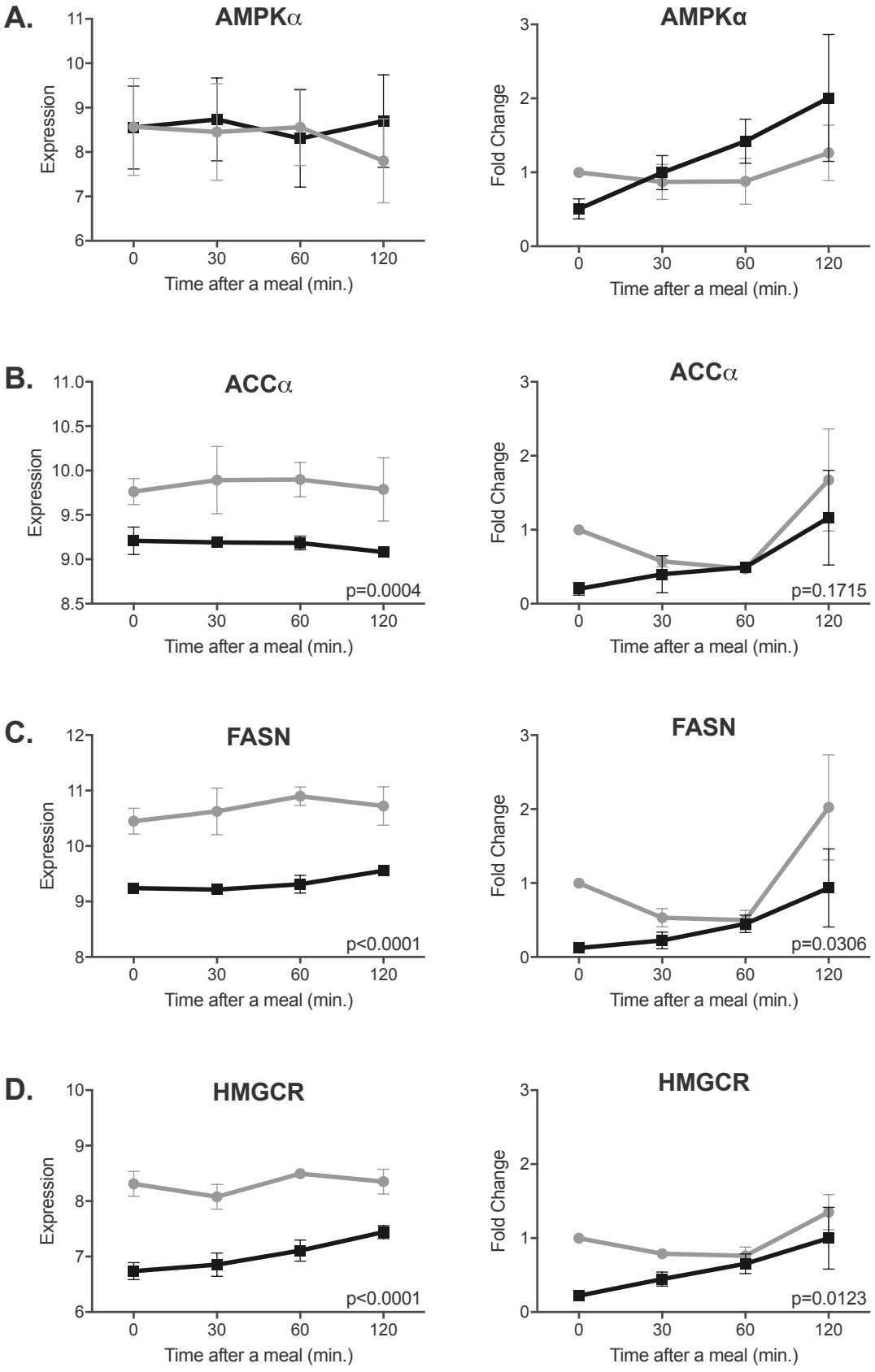

Figure S5. Expression changes of AMPKα (**A**), ACCα (**B**), FASN (**C**) and HMGCR (**D**) mRNA in the livers of mice fed either HP (dark grey) or LP (light gray) diets. Abundance of mRNA was measured at fasting and 30, 60 and 120 min. after introduction of food. Graphs on the left show expression measurements using oligonucleotide microarrays. Graphs on the right show expression measurements using quantitative RT-PCR in a different cohort of mice. Each data point is a mean and associated SEM of 4 measurements.

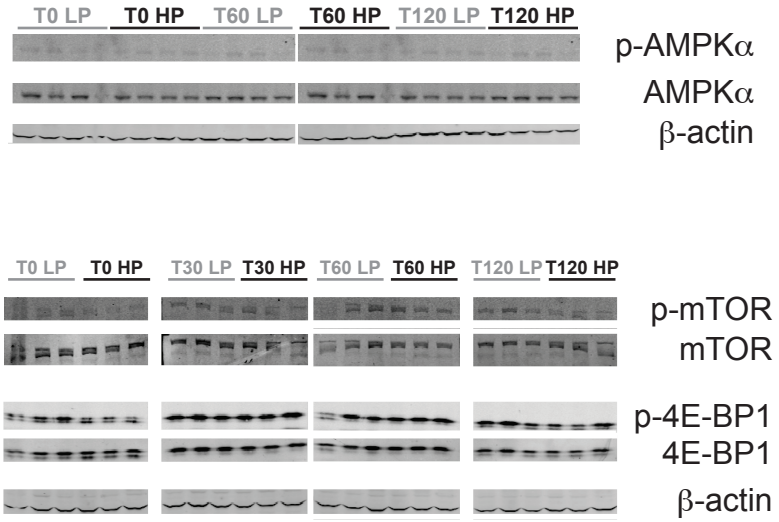

Figure S6. Quantification of hosphorylated AMPKα subunit, mTOR and 4E-BP1 using immunoblotting. Total liver proteins were probed with antibodies spoecific for phospho-Thr^{172}, phpsho-^{Thr2446} and phospho Thr$^{37/46}$ in the AMPKα, mTOR and 4E-BP1, respectively, follwed by probing for the total amounts of the three proteins. Data were normalized to β-actin.

Materials and Methods

RNA sample preparation and Microarray Processing

Hierarchical Clustering Explorer (HCE) 3.5 power analysis tool (Seo, et al. 2006) was used to determine that three replicates would be sufficient to allow for detection of significant differences in mRNA levels for 60-80% of probe sets present on each microarray.

RNA was isolated from frozen livers using TRIzol reagent (Invitrogen). The quality of isolated RNA was tested by determining the ratio of absorbance at 260 and 280nm, and visualization of the 28S and 18S ribosomal RNA. Purified RNA was converted to double-stranded cDNA using One-Cycle cDNA Synthesis Kit (Affymetrix) according to manufacturer's instructions, and cDNA was amplified to ensure greater than 4-fold amplification. The cDNA was transcribed and biotin tagged with the IVT labeling kit (Affymetrix), according to manufacturer's instructions. The cRNA was purified with the RNAeasy Kit (QIAGEN), and fragmented to approximately 200 bp. To ensure proper fragmentation, the IVT fragmented and un-fragmented samples were visualized on an agarose gel. Following completion of all quality control checks, cRNA was hybridized to GeneChip Mouse Genome 430 2.0 (Affymetrix) and the microarray chips were washed and stained on an Affymetrix Fluidics Station 400 according to manufacturer's instructions. Fluorescent images were scanned using a Hewlett-Packard G2500A Gene Array Scanner. Hybridization, washing and scanning of chips was carried out within the Center for Genetic Medicine's Expression Profiling core facility.

Analysis of Microarray Data

Affymetrix image data was analyzed using Probe Microarray Suite (MAS) version 5.0 and dCHIP (Li and Wong 2001) probe set algorithms to highlight genes both algorithms identified as having significant expression level change from the control and to eliminate false positives. Genes identified by each algorithm were imported into Partek software package (Partek Incorporated), normalized by Log2, and analyzed by 2-way ANOVA with p-value of $P<0.05$. Comparisons for the time-restricted feeding study included low protein T0 vs. high protein T0, low protein T30 vs. high protein T30, low protein T60 vs. high protein T60, low protein T120 vs. high protein 120.

ANOVA results were imported into Ingenuity Pathway Analysis (IPA) software (Ingenuity) using Affymetrix probe set IDs as identifiers and absolute fold-change for

observation values. The genes with fold-change cutoffs of less than -1.4 and more than 1.4 were imported into IPA for pathway analysis.

Validation of Gene Expression Data

RNA was extracted from mouse liver tissues as described previously. Reverse transcription and cDNA synthesis was performed using Oligo-dT 15, random hexamer primers (Invitrogen Life Technologies, Carlsbad, CA) and Superscript III reverse transcriptase (200 U/μg of RNA, Invitrogen Life Technologies) at 42 °C for 1 h.

The expression of transcripts with fold changes outside of expression value cutoffs (±1.4-fold) were validated using Applied Biosystems 384 custom gene card array. 64 genes (Table S1) were assayed by quantitative PCR using 7900HT Fast Real-Time PCR System (Applied Biosystems, Inc.) and included myosin heavy chain 9 as the normalization control that does not change due to diet and 18S RNA as the manufacturer's control. The $\Delta\Delta C_t$ method was used to calculate the difference in expression levels for each gene (Livak and Schmittgen 2001).

Sample Preparation and Mass Spectrometry

Protein lysates were prepared from frozen and ground mouse tissue by homogenization of tissue in 50mM Tris-acetate buffer, pH 7.5 containing 250mM sucrose, 1mM EDTA, 1% Nonident P40 (NP40), and Complete Mini Protease Inhibitor Cocktail (Roche). Aliquots of 50 μg of total liver proteins from mice labeled with $^{13}C_6$-lysine (Rayavarapu, et al. 2013) were added to 50 μg of total liver proteins from experimental animals, resolved by SDS-PAGE, stained with Bio-Safe Coomassie (Bio-Rad, Hercules, CA), and 1mm wide sections were excised from the gel. Proteins in gel slices were digested with trypsin as previously described (Jensen, et al. 1999). Concentrated peptides from each band were injected via an autosampler (6 μl) and loaded onto a Symmetry C18 trap column (5μm, 300 μm i.d. x 23 mm, Waters) for 10 min. at a flow rate of 10 μL/min, 0.1% formic acid. The samples were subsequently separated by a C18 reverse-phase column (3 μm, 200A, 100 μm x 15 cm, Magic C18, Michrom Bioresources) at a flow rate of 300 nl/min. using an Eksigent nano-hplc system (Dublin, CA). The mobile phases consisted of water with 0.1% formic acid (A) and 90% acetonitrile (B). A 65 min. linear gradient from 5 to 60% B was employed. Eluted peptides were introduced into the mass spectrometer via Michrom Bioresources CaptiveSpray. The spray voltage was set at 1.4 kV and the heated capillary at 200°C. The LTQ-Orbitrap-XL (ThermoFisherScientific) was operated in data-dependent mode with dynamic exclusion in which one cycle of experiments consisted of a full-MS in the Orbitrap

(300-2000 m/z) survey scan in profile mode, resolution 30,000, and five subsequent MS/MS scans in the LTQ of the most intense peaks in centroid mode using collision-induced dissociation with the collision gas (helium) and normalized collision energy value set at 35%.

Database Search and SILAM Ratio Measurement.

For protein identification and quantification we used Integrated Proteomics Pipeline (IP2) version 1.01 software developed by Integrated Proteomics Applications, Inc. (http://www.integratedproteomics.com/). Mass spectral data were uploaded into IP2 software. Files from each lane were searched against the forward and reverse Uniprot mouse database (UniProt release 15.4, June 2009) for tryptic peptides allowing one missed cleavage, and possible modification of oxidized methionine (15.99492 Da) and heavy lysine (6.0204 Da). IP2 uses the Sequest 2010 (06_10_13_1836) search engine. Mass tolerance was set at +/- 30 ppm for MS and +/- 1.5 Da for MS/MS. Data were filtered based on a 3% false discovery rate. All the bands from each lane were summed in the analysis. Census software version 1.77, built into the IP2 platform, was used to determine the ratios of unlabeled and labeled peptide pairs using an extracted chromatogram approach. The distribution of ratios was plotted and correction factors applied to adjust for error in sample mixing. Data were checked for validity by using regression correlation better than 0.98 for each peptide pair.

Validation of Protein Profiling Data

Mouse liver tissue was homogenized in an extraction buffer containing 50mM Tris-acetate pH 7.5, 250 mM sucrose, 1mM EDTA, 1% Nonidet P40 and anti-protease mixture (Roche), and diluted to a protein concentration of 20 mg/ml. NAGS was probed in 20 µg of total liver proteins, which were resolved by SDS-PAGE and transferred to a nitrocellulose filter. Filters were blocked with blocking buffer (Pierce) containing 0.5% Surfact-Amps 20 (Pierce). The filter was probed with polyclonal antibody raised against recombinant mouse NAGS at 1:5,000 dilution for 1 hr. at room temperature and washed with Tris buffered saline containing 0.005% Tween-20 (TBST). The filter was then incubated for 1 hr. at room temperature with donkey horseradish peroxidase (HPRT). Nags bands were visualized using SuperSignal West Pico kit (Pierce) according to the manufacturer's instructions. Cps1 was probed in 150 ng of total liver protein using rabbit anti-CPS1 primary antibody (AbCam) at 1:5000 dilution and HPRT-conjugated donkey anti-rabbit secondary antibody (Pierce). Otc was probed in 250 ng of total liver protein using rabbit primary antibody raised against recombinant Ots at 1:5000 dilution and HPRT-

conjugated donkey anti-rabbit secondary antibody (Pierce). ASS was probed in 250 ng of total liver protein using goat anti-ASS primary antibody (Santa Cruz Inc.) at 1:500 dilution and HPRT-conjugated donkey anti-goat secondary antibody (Promega). ASL was probed in 40 μg of total liver protein using goat anti-ASL primary antibody (Santa Cruz Inc.) at 1:500 dilution and HPRT-conjugated donkey anti-goat secondary antibody (Promega). Vinculin was probed with mouse anti-vinculin primary antibody (Sigma) at 1:1000 dilution and HPRT-conjugated goat anti-mouse secondary antibody (Bio-Rad) at 1:2000 dilution. Cps1, Otc, Ass, Asl and vinculin were visualized with the ECL Western Blotting Substrate (Pierce) according to the manufacturer's instructions.

Measurement of Protein Phosphorylation

Proteins were extracted from liver tissue using cell lysis buffer (Cell Signaling Technology) according to manufacturer's instructions with the modification for lysis of tissue samples. Briefly, 100mg of liver tissue was incubated with 2x lysis buffer and sonicated using 3 pulses of 15 s at 30% output. Tissue suspension was centrifuged and supernatent was collected for analysis. 30ug of whole liver lysate was separated under denaturing conditions by 4-20% SDS-Page gel electrophoresis and transferred to PVDF membranes. Membranes were blocked in 50% Blocking Buffer (Odyssey) and labeled with primary rabbit antibodies to phosphorylated and non-phosphorylated Ampkα, mTor, 4E-BP1 and β-actin proteins (Cell Signaling Technologies) at a 1:1000 dilution. The membranes were washed three times for 10 minutes each with TBS containing 0.1% Tween 20, followed by incubation with secondary antibody at a 1:10,000 dilution. Image analyses were performed using an Odyssey Imager (LiCor, Lincoln, NE).

Primary Hepatocyte Cultures

The Liver Tissue Cell Distribution System (LTCDS) is a National Institutes of Health (NIH)-funded collaborative network to provide human liver tissue from regional centers for distribution to scientific investigators. The regional centers have active liver transplant programs with human subjects' approval to provide portions of the resected pathologic liver for which the transplant is performed. All human tissues were collected with informed consent following ethical and institutional guidelines. Tissue dissociation and subsequent hepatocyte isolation procedures were developed by Seglen with modifications (Gramignoli, et al. 2012). Hepatocytes were allowed to adhere for 2 hours with Hepatocyte Maintenance Media (Lonza, Walkersville, MD) containing 10% FBS and penicillin and streptomycin. This was followed by maintenance culture for 1 days

in serum-free Hepatocyte Maintenance Media (Lonza, Walkersville, MD). Hepatocytes were treated with 5 μM AICAR with or with out 40 μM Compound C (Sigma-Alrich, St. Louis, MO) for 24 hours. Cell viability was determined using PrestoBlue Cell Viability Reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Cell pellets and supernatants were stored at -80°C until analysis.

Quantitative RT-PCR

RNA was extracted from cell pellets using RNeasy Minikit (Qiagen) according to the manufacturer's instructions. One microgram of RNA was reverse transcribed to cDNA using a modified MMLV-reverse transcriptase with RNase H+ activity (iScript, Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Real-time quantitative PCR reactions were carried out in 50μL using TaqMan systems (Applied Biosciences, Carlsbad, CA) using ABI 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA).

References

Gramignoli, R, Green, ML, Tahan, V, et al. 2012 Development and application of purified tissue dissociation enzyme mixtures for human hepatocyte isolation. *Cell Transplant* 21: 1245-1260

Jensen, ON, Wilm, M, Shevchenko, A and Mann, M 1999 Sample preparation methods for mass spectrometric peptide mapping directly from 2-D gels. *Methods Mol Biol* 112: 513-530

Li, J and Wong, L 2001 Emerging patterns and gene expression data. *Genome Inform Ser Workshop Genome Inform* 12: 3-13

Livak, KJ and Schmittgen, TD 2001 Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods* 25: 402-408

Rayavarapu, S, Coley, W, Cakir, E, et al. 2013 Identification of disease specific pathways using in vivo silac proteomics in dystrophin deficient mdx mouse. *Mol Cell Proteomics* 12: 1061-1073

Seo, J, Gordish-Dressman, H and Hoffman, EP 2006 An interactive power analysis tool for microarray hypothesis testing and generation. *Bioinformatics* 22: 808-814