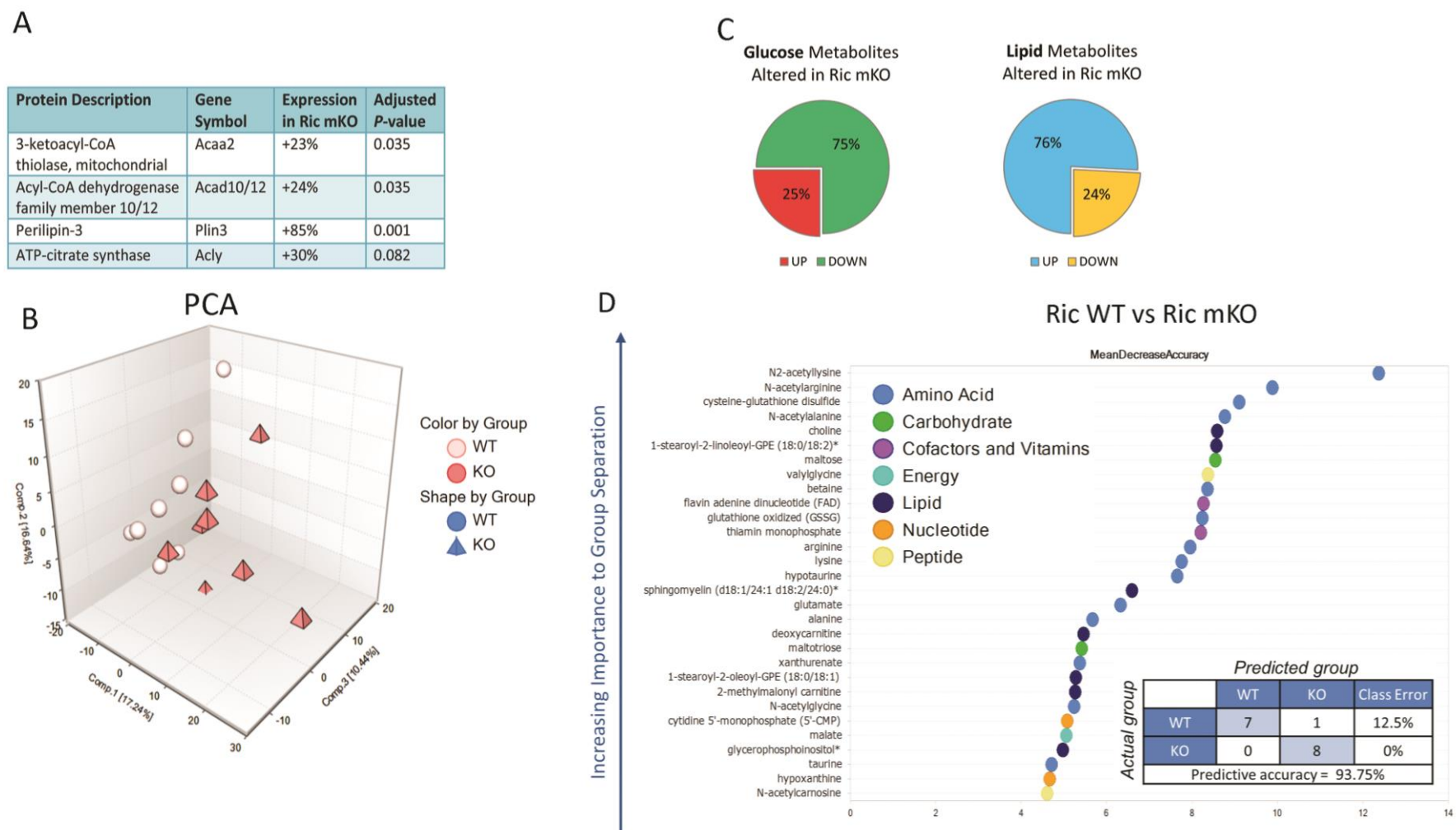
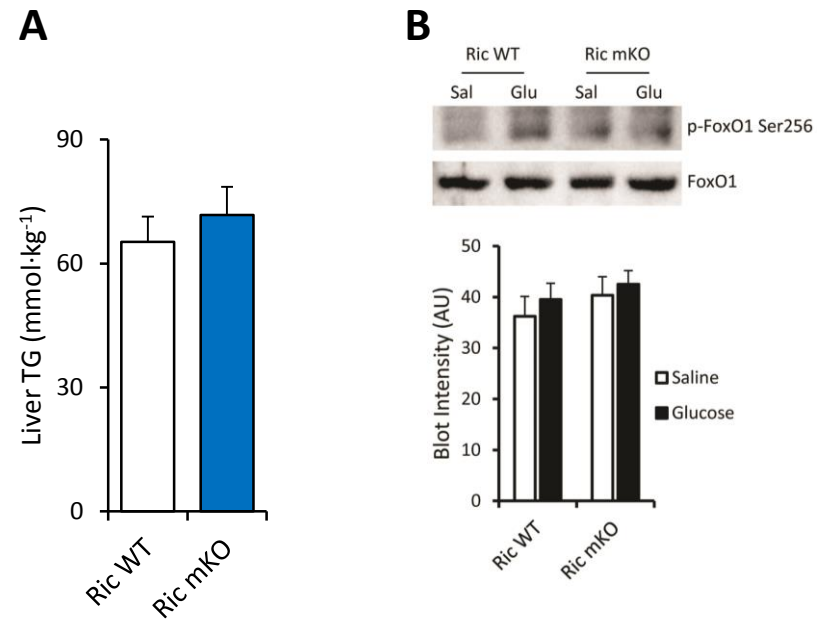


Figure S1



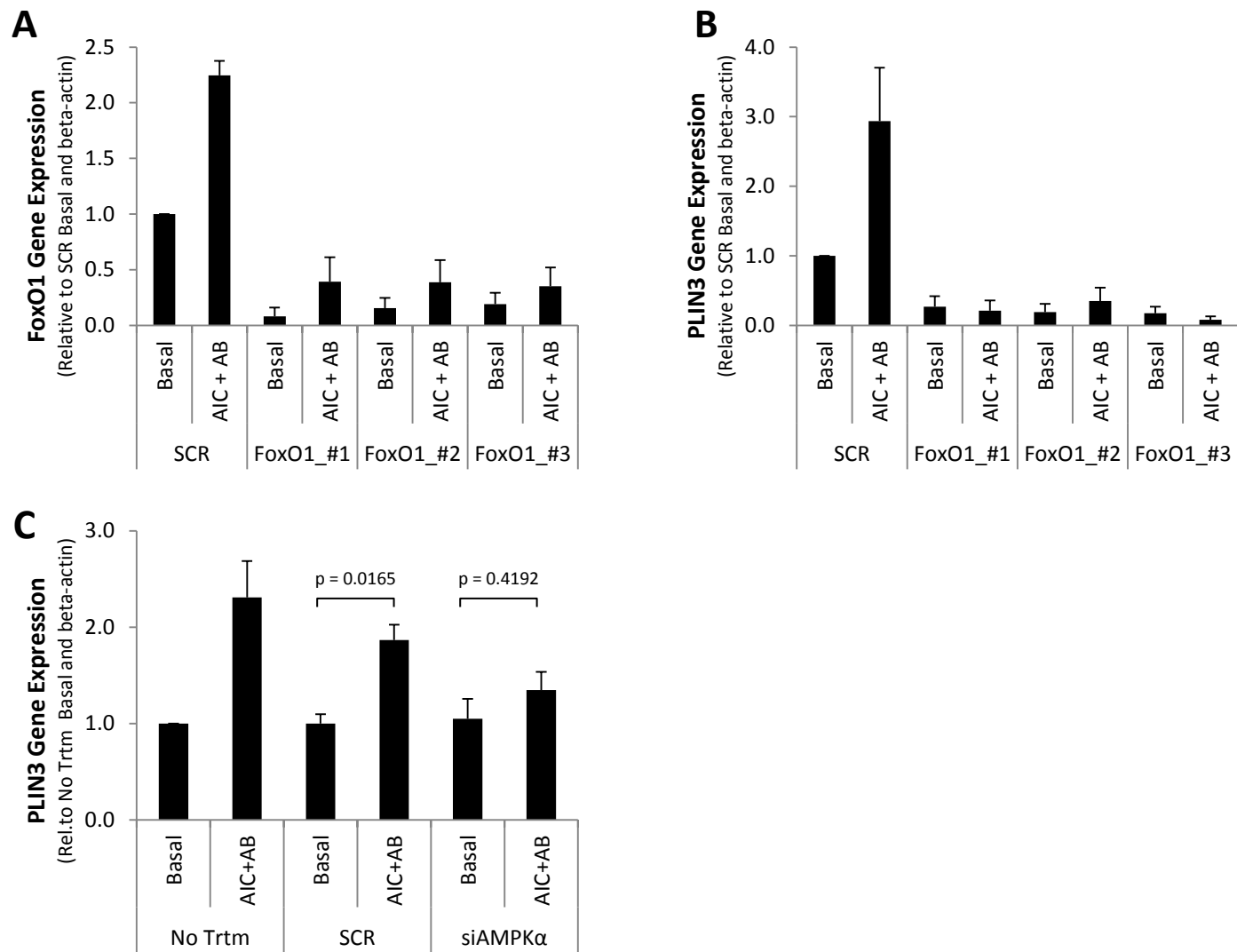
**Figure S1 related to Figure 1:** (A) Lipid metabolism proteins that were differentially expressed in Ric WT vs Ric mKO muscle as identified in the proteome analysis by MS. (B) Principal component analysis of the metabolome revealed a distinct separation between Ric WT and Ric mKO muscles suggesting that mTORC2-deficient muscle possess a unique biochemical signature. (C) Directional changes of glucose and lipid metabolites that were altered based on  $p < 0.1$  as identified by the metabolomic screen. (D) Random forest analysis (a supervised classification technique based on an ensemble of decision trees) resulted in a predictive accuracy of 93.75% (shown in the inset table). A value of 93.75% is greater than one would expect by random chance alone (50%), suggesting that these metabolites may be of interest as candidate biomarkers associated with mTORC2 expression. Listed are the top 30 metabolites based on their importance in separating the two groups.

Figure S2



**Figure S2 related to Figure 1 and 2:** **(A)** Liver triglyceride levels (n = 6-8). **(B)** Representative blot and quantification of p-FoxO1 Ser256 in quadriceps muscle lysate from Ric WT and Ric mKO mice that had been injected with saline or glucose (n = 8-9). FoxO1 total serves as loading control.

Figure S3



**Figure S3 related to Figure 5: (A,B)** The effect of FoxO1 knockdown using individual FoxO1 (FoxO1 (1), FoxO1 (2), FoxO1 (3)) siRNA sequences (methods online) on gene expression of FoxO1 and PLIN3 under control (Ctr) conditions and in response to AMPK activation by AICAR (AIC) plus A769662 (AB) (n = 3) as described in methods online. **(C)** The effect of AMPK $\alpha$  knockdown on PLIN3 expression following AMPK activation by AICAR (AIC) plus A769662 (AB).

Table S1

ACC#	Protein Description	Gene Symbol	Ric mKO Ric WT
Q62087	Serum paraoxonase/lactonase 3	Pon3	-101%
Q9JME5	AP-3 complex subunit beta-2	Ap3b2	-63%
Q9Z0J4	Nitric oxide synthase	Nos1	-50%
Q9Z0J4-2	N-NOS-2 Nitric oxide synthase		
Q9Z0J4-3	NNOS beta Nitric oxide synthase		
Q9Z0J4-4	NNOS gamma Nitric oxide synthase		
Q9Z0J4-5	NNOS mu Nitric oxide synthase		
O08689	Growth/differentiation factor 8	Mstn	-47%
P55012	Solute carrier family 12 member 2	Slc12a2	-31%
Q8R1G2	Carboxymethylenebutenolide homology	Cmb1	-29%
P82350	Alpha-sarcoglycan	Sgca	-29%
Q9QUH0	Glutaredoxin-1	Glrx	-28%
D3Z7P3	Glutaminase kidney Iso	Gls	-25%
D3Z7P3-2	Iso 2 Glutaminase kidney Iso		
P29758	Ornithine aminotransferase	Oat	-22%
Q9DAV9	Trimeric intracellular cation channel type B	Tmem38b	-22%
Q91YW3	DnaJ homolog subfamily C member 3	Dnajc3	-22%
Q05BC3	Echinoderm microtubule-associated protein-like 1	Eml1	-19%
Q05BC3-2	Iso 2 Echinoderm microtubule-associated protein-like 1		
Q05BC3-3	Iso 3 Echinoderm microtubule-associated protein-like 1		
Q9DB05	Alpha-soluble NSF attachment protein	Napa	18%
Q6VGS5	Protein Daple	Ccdc88c	19%
Q6VGS5-2	Iso 2 Protein Daple		
Q9IJZ3	SH3 and multiple ankyrin repeat domains protein 3	Shank3	22%
Q8R164	Valacyclovir hydrolase	Bphl	
Q61362	Chitinase-3-like protein 1	Chi3l1	23%
Q8BWT1	3-ketoacyl-CoA thiolase mitochondrial	Acaa2	
Q8K370	Acyl-CoA dehydrogenase family member 10	Acad10	24%
D3Z2B3	Acyl-CoA dehydrogenase family member 12	Acad12	
P15105	Glutamine synthetase	Glul	26%
Q5NBX1	Protein cordon-bleu	Cobl	37%
Q5NBX1-2	Iso 2 Protein cordon-bleu		
Q5NBX1-3	Iso 3 Protein cordon-bleu		
Q5NBX1-4	Iso 4 Protein cordon-bleu		
Q3KNY0	Ig-like and fibronectin type III domain-containing protein 1	Igfn1	37%
Q3KNY0-2	Iso 2 Ig-like and fibronectin type III domain-containing protein 1		
Q3KNY0-3	Iso 3 Ig-like and fibronectin type III domain-containing protein 1		
Q3KNY0-4	Iso 4 Ig-like and fibronectin type III domain-containing protein 1		
Q9QXY6	EH domain-containing protein 3	Ehd3	45%
Q9EQP2	EH domain-containing protein 4	Ehd4	
Q3KNY0	Ig-like and fibronectin type III domain-containing protein 1	Igfn1	49%
P17563	Selenium-binding protein 1	Selenbp1	53%
P16015	Carbonic anhydrase 3	Ca3	62%
Q9ERT9	Protein phosphatase 1 regulatory subunit 1A	Ppp1r1a	65%
P09541	Myosin light chain 4	Myl4	72%
Q9DBG5	Perilipin-3	Plin3	85%

**Table S1:** Protein groups differentially expressed in Ric mKO muscle compared to Ric WT muscle (adjusted P value < 0.05) (n = 8). Gray bars on the left encompass the protein groups that shared peptide sequences identified and quantified by unbiased MS-based proteomics.

Table S2

Biochemical Name	KEGG	HMDB	PubChem	Ric mKO / Ric WT	Super Pathway	Sub Pathway
ergothioneine	<a href="#">C05570</a>	<a href="#">HMDB03045</a>	3032311	10.45	Xenobiotics	Food Component/Plant
galactose 1-phosphate	<a href="#">C00446</a>	<a href="#">HMDB00645</a>	123912	2.66	Carbohydrate	Fructose, Mannose and Galactose Metabolism
S-lactoylglycithione	<a href="#">C03451</a>	<a href="#">HMDB01066</a>	440018	2.47	Amino Acid	Glutathione Metabolism
riboflavin (Vitamin B2)	<a href="#">C00255</a>	<a href="#">HMDB00244</a>	493570	2.02	Cofactors and Vitamins	Riboflavin Metabolism
phosphopantetheine	<a href="#">C01134</a>	<a href="#">HMDB01416</a>	987	1.98	Cofactors and Vitamins	Pantothenate and CoA Metabolism
xanthosine	<a href="#">C01762</a>	<a href="#">HMDB00299</a>	64959	1.88	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
cysteine-glutathione disulfide	--	<a href="#">HMDB00656</a>	4247235	1.84	Amino Acid	Glutathione Metabolism
thiamin diphosphate	<a href="#">C00068</a>	<a href="#">HMDB01372</a>	1132	1.75	Cofactors and Vitamins	Thiamine Metabolism
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	--	--	--	1.69	Lipid	Phospholipid Metabolism
gamma-glutamylglutamate	<a href="#">C05282</a>	<a href="#">HMDB11737</a>	92865	1.66	Peptide	Gamma-glutamyl Amino Acid
leucylglycine	--	--	79070	1.62	Peptide	Dipeptide
acetyl CoA	<a href="#">C00024</a>	<a href="#">HMDB01206</a>	444493	1.55	Lipid	Fatty Acid Metabolism
hypotaurine	<a href="#">C00519</a>	<a href="#">HMDB00965</a>	107812	1.54	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
choline	<a href="#">C00114</a>	<a href="#">HMDB00097</a>	305	1.53	Lipid	Phospholipid Metabolism
valylglycine	--	--	136487	1.52	Peptide	Dipeptide
1-palmitoyl-GPI (16:0)*	--	<a href="#">HMDB61695</a>	--	1.49	Lipid	Lysolipid
AICA ribonucleotide	<a href="#">C04677</a>	<a href="#">HMDB01517</a>	65110	1.48	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
1-docosahexaenoylglycerol (22:6)	--	<a href="#">HMDB11587</a>	--	1.44	Lipid	Monoacylglycerol
N-acetyl glycine	--	<a href="#">HMDB00532</a>	10972	1.42	Amino Acid	Glycine, Serine and Threonine Metabolism
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	--	<a href="#">HMDB05320</a>	5283496	1.42	Lipid	Phospholipid Metabolism
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	--	<a href="#">HMDB05322</a>	9546747	1.42	Lipid	Phospholipid Metabolism
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	--	--	9546749	1.41	Lipid	Phospholipid Metabolism
spermidine	<a href="#">C00315</a>	<a href="#">HMDB01257</a>	1102	1.40	Amino Acid	Polyamine Metabolism
gamma-glutamylphenylalanine	--	<a href="#">HMDB00594</a>	111299	1.38	Peptide	Gamma-glutamyl Amino Acid
2-methylmalonyl carnitine	--	<a href="#">HMDB13133</a>	53481628	1.38	Lipid	Fatty Acid Synthesis
hydroxybutyrylcarnitine*	--	<a href="#">HMDB13127</a>	53481617	1.38	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
glycerophosphoinositol*	--	--	--	1.38	Lipid	Phospholipid Metabolism
squalene	<a href="#">C00751</a>	<a href="#">HMDB00256</a>	638072	1.35	Lipid	Sterol
ethanolamine	<a href="#">C00189</a>	<a href="#">HMDB00149</a>	700	1.32	Lipid	Phospholipid Metabolism
adenosine 3',5'-diphosphate	<a href="#">C00054</a>	<a href="#">HMDB00061</a>	159296	1.32	Nucleotide	Purine Metabolism, Adenine containing
N-acetylalanine	<a href="#">C02847</a>	<a href="#">HMDB00766</a>	88064	1.30	Amino Acid	Alanine and Aspartate Metabolism
thiamin monophosphate	<a href="#">C01081</a>	<a href="#">HMDB02666</a>	3382778	1.30	Cofactors and Vitamins	Thiamine Metabolism
glutathione, oxidized (GSSG)	<a href="#">C00127</a>	<a href="#">HMDB03337</a>	65359	1.29	Amino Acid	Glutathione Metabolism
suberate (octanedioate)	<a href="#">C08278</a>	<a href="#">HMDB00893</a>	10457	1.29	Lipid	Fatty Acid, Dicarboxylate
flavin adenine dinucleotide (FAD)	<a href="#">C00016</a>	<a href="#">HMDB01248</a>	643975	1.28	Cofactors and Vitamins	Riboflavin Metabolism
xanthine	<a href="#">C00385</a>	<a href="#">HMDB00292</a>	1188	1.25	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
flavin mononucleotide (FMN)	<a href="#">C00051</a>	<a href="#">HMDB01520</a>	710	1.25	Cofactors and Vitamins	Riboflavin Metabolism
hypoxanthine	<a href="#">C00262</a>	<a href="#">HMDB00157</a>	790	1.24	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
pyridoxal phosphate	<a href="#">C00018</a>	<a href="#">HMDB01491</a>	1051	1.24	Cofactors and Vitamins	Vitamin B6 Metabolism
glutamate	<a href="#">C00025</a>	<a href="#">HMDB00148</a>	611	1.23	Amino Acid	Glutamate Metabolism
arachidonate (20:4n6)	<a href="#">C00219</a>	<a href="#">HMDB01043</a>	444899	1.23	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
betaine	<a href="#">C00719</a>	<a href="#">HMDB00043</a>	247	1.22	Amino Acid	Glycine, Serine and Threonine Metabolism
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)*	--	--	5287971	1.21	Lipid	Phospholipid Metabolism
malate	<a href="#">C00149</a>	<a href="#">HMDB00156</a>	525	1.20	Energy	TCA Cycle
cytidine 5'-monophosphate (5'-CMP)	<a href="#">C00055</a>	<a href="#">HMDB00095</a>	6131	1.17	Nucleotide	Pyrimidine Metabolism, Cytidine containing
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	--	--	9547087	1.15	Lipid	Phosphatidylserine (PS)
sphingomyelin (d18:1/24:1, d18:2/24:0)*	--	--	--	1.15	Lipid	Sphingolipid Metabolism
arabonate/xyfonate	--	--	--	1.14	Carbohydrate	Pentose Phosphate Pathway
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	--	--	--	1.14	Lipid	Phospholipid Metabolism
guanosine 5'- monophosphate (5'-GMP)	<a href="#">C00144</a>	<a href="#">HMDB01397</a>	6804	1.14	Nucleotide	Purine Metabolism, Guanine containing
kynurenine	<a href="#">C00328</a>	<a href="#">HMDB00684</a>	161166	1.11	Amino Acid	Tryptophan Metabolism
creatine	<a href="#">C00791</a>	<a href="#">HMDB00562</a>	568	1.11	Amino Acid	Creatine Metabolism
phosphate	<a href="#">C00009</a>	<a href="#">HMDB01429</a>	1061	1.07	Energy	Oxidative Phosphorylation
creatine	<a href="#">C00300</a>	<a href="#">HMDB00064</a>	586	1.05	Amino Acid	Creatine Metabolism
taurine	<a href="#">C00245</a>	<a href="#">HMDB00251</a>	1123	1.03	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism

Table S2: Metabolites that were increased (p &lt; 0.05) in Ric mKO compared to Ric WT muscle (n = 8).

Table S3

Biochemical Name	KEGG	HMDB	PubChem	Ric mKO / Ric WT	Super Pathway	Sub Pathway
1,2-dipalmitoyl-GPC (16:0/16:0)	--	<a href="#">HMDB000564</a>	452110	0.93	Lipid	Phospholipid Metabolism
pipicolate	<a href="#">C00408</a>	<a href="#">HMDB000070</a>	849	0.92	Amino Acid	Lysine Metabolism
alanine	<a href="#">C00041</a>	<a href="#">HMDB001161</a>	5950	0.90	Amino Acid	Alanine and Aspartate Metabolism
anserine	<a href="#">C01262</a>	<a href="#">HMDB001194</a>	112072	0.89	Peptide	Dipeptide Derivative
N-acetylneuraminate	<a href="#">C00270</a>	<a href="#">HMDB00230</a>	439197	0.87	Carbohydrate	Aminosugar Metabolism
methionine sulfoxide	<a href="#">C02989</a>	<a href="#">HMDB002005</a>	156980	0.86	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
carnosine	<a href="#">C00386</a>	<a href="#">HMDB000033</a>	439224	0.85	Peptide	Dipeptide Derivative
aspartate	<a href="#">C00049</a>	<a href="#">HMDB001191</a>	5960	0.84	Amino Acid	Alanine and Aspartate Metabolism
N-methylproline	--	--	557	0.83	Amino Acid	Urea cycle, Arginine and Proline Metabolism
4-guandimobutanoate	<a href="#">C01035</a>	<a href="#">HMDB003464</a>	500	0.80	Amino Acid	Guandino and Acetamidol Metabolism
deoxycarnithine	<a href="#">C01181</a>	<a href="#">HMDB011161</a>	134	0.80	Lipid	Carnitine Metabolism
N6, N6-trimethyllysine	<a href="#">C03753</a>	<a href="#">HMDB01325</a>	440120	0.77	Amino Acid	Lysine Metabolism
arginine	<a href="#">C00062</a>	<a href="#">HMDB000517</a>	232	0.76	Amino Acid	Urea cycle, Arginine and Proline Metabolism
galactitol (dulcitol)	<a href="#">C01697</a>	<a href="#">HMDB00107</a>	11850	0.76	Carbohydrate	Fructose, Mannose and Galactose Metabolism
betaine aldehyde	<a href="#">C00576</a>	<a href="#">HMDB01252</a>	249	0.75	Amino Acid	Glycine, Serine and Threonine Metabolism
malotetraose	<a href="#">C02052</a>	<a href="#">HMDB01296</a>	446495	0.74	Carbohydrate	Glycogen Metabolism
lysine	<a href="#">C00047</a>	<a href="#">HMDB001182</a>	5962	0.73	Amino Acid	Lysine Metabolism
xanthurenate	<a href="#">C02470</a>	<a href="#">HMDB000881</a>	5699	0.70	Amino Acid	Tryptophan Metabolism
N-acetylcarnosine	--	<a href="#">HMDB12881</a>	9903482	0.70	Peptide	Dipeptide Derivative
UDP-glucose	<a href="#">C00029</a>	<a href="#">HMDB00286</a>	8629	0.70	Carbohydrate	Nucleotide Sugar
sorbitol	<a href="#">C00794</a>	<a href="#">HMDB00247</a>	5780	0.68	Carbohydrate	Fructose, Mannose and Galactose Metabolism
N-acetylarginine	<a href="#">C02582</a>	<a href="#">HMDB04620</a>	67427	0.63	Amino Acid	Urea cycle, Arginine and Proline Metabolism
malotriose	<a href="#">C01835</a>	<a href="#">HMDB01262</a>	439586	0.62	Carbohydrate	Glycogen Metabolism
oleoylcarnithine	--	<a href="#">HMDB005065</a>	6441392;53477789	0.60	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
maltose	<a href="#">C00208</a>	<a href="#">HMDB001163</a>	10991489	0.54	Carbohydrate	Glycogen Metabolism
N6-carboxymethyllysine	--	--	--	0.52	Carbohydrate	Advanced Glycation End-product
linoleoylcarnitine*	--	<a href="#">HMDB006469</a>	6450015	0.52	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
N2-acetyllysine	<a href="#">C12989</a>	<a href="#">HMDB004446</a>	92907	0.38	Amino Acid	Lysine Metabolism

Table S3: Metabolites that were decreased (p < 0.05) in Ric mKO compared to Ric WT muscle (n = 8).