Supplementary Information

Branched chain amino acid catabolism fuels adipocyte differentiation and lipogenesis

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Supplementary Results

Supplementary Table 1. Simplified network for Isotopomer Spectral Analysis (ISA)

Contribution to lipogenic AcCoA (%)	**Description**
AcM1.l (ab) → Ac (ab)	(AcCoA containing 1 ^{13}C tracer label)
AcM2.l (ab) → Ac (ab)	(AcCoA containing 2 ^{13}C tracer labels)
Ac.d (ab) → Ac (ab)	(unlabeled AcCoA)
7*Ac (ab) → Myr.s (abcdefghijklmn)	
8*Ac (ab) → Palm.s (abcdefghijklmnop)	
9*Ac (ab) → Oleate.s (abcdefghijklmnopqr)	
9*Ac (ab) → Stear.s (abcdefghijklmnopqr)	
***de novo* lipogenesis**	
Myr.s → Myr	Newly synthesized myristate
Myr.d → Myr	Pre–existing (unlabeled) myristate
0*Myr.s + 0*Myr.d → Myr.m	Mixing of pools for measurement
Palm.s → Palm	Newly synthesized palmitate
Palm.d → Palm	Pre–existing (unlabeled) palmitate
0*Palm.s + 0*Palm.d → Palm.m	Mixing of pools for measurement
Oleate.s → Oleate	Newly synthesized oleate
Oleate.d → Oleate	Pre–existing (unlabeled) oleate
0*Oleate.s + 0*Oleate.d → Oleate.m	Mixing of pools for measurement
Stearate.s → Stearate	Newly synthesized stearate
Stearate.d → Stearate	Pre–existing (unlabeled) stearate
0*Stearate.s + 0*Stearate.d → Stearate.m	Mixing of pools for measurement

Supplementary Results

Supplementary Table 2. Control and Low Gluc+AA media formulation

Amino Acid	Control (mM)	Low Gluc+AA (mM)
D–glucose	25	6
L–glutamine	4	1
L–arginine	0.4	0.1
L–cystine	0.2	0.05
Glycine	0.4	0.1
L–histidine	0.2	0.05
L–isoleucine	0.8	0.2
L–leucine	0.8	0.2
L–lysine HCl	0.8	0.2
L–methionine	0.2	0.05
L–phenylalanine	0.4	0.1
L–serine	0.4	0.1
L–threonine	0.8	0.2
L–tryptophan	0.078	0.02
L–tyrosine	0.4	0.1
L–valine	0.8	0.2

Supplementary Results

Supplementary Table 3. Metabolite fragment ions used for GC–MS analysis.

Metabolite	Carbons	Formula	Mass (m/z)
Leucine	23456	$C_{13}H_{32}NOSi_2$	274–280
Isoleucine	23456	$C_{11}H_{26}NSi$	200–206
Isoleucine	23456	$C_{13}H_{32}NOSi_2$	274–280
Valine	2345	$C_{12}H_{30}NOSi_2$	260–268
Valine	12345	$C_{13}H_{30}NO_2Si_2$	288–296
Methylmalonate	1234	$C_{12}H_{25}O_4Si_2$	289–295
Myristate (C14:0)	1–14	$C_{15}H_{30}O_2$	242–258
Pentadecanoate (C15:0)	1–15	$C_{16}H_{32}O_2$	256–272
Palmitate (C16:0)	1–16	$C_{17}H_{34}O_2$	270–284
Heptadecanoate (C17:0)	1–17	$C_{18}H_{37}O_2$	284–294
Oleate (C18:1)	1–18	$C_{19}H_{36}O_2$	296–310
Stearate (C18:0)	1–18	$C_{19}H_{38}O_2$	298–316

Supplementary Results

Supplementary Table 4. Primer sequences used in gene expression analysis.

Gene Name	Forward sequence	Reverse Sequence
Pparγ (*Pparg*)	TTCGCTGATGCACTGCCTAT	ACAGACTCGGCACTCAATGG
Adiponectin (*Adipoq*)	GACACCAAAAGGGCTCAGGA	GCCCTTCAGCTCCTGTCATT
Leptin (*Lep*)	GGGAGGAAAATGTGCTGGAGAC	AAGCCCAGGAATGAAGTCCAA
Glucose transporter type 4 (*Glut4*)	AGCCTCTGATCATCGCAGTG	ACTAAGAGCACCGAGACCAAC
Perilipin 4 (*Plin4*)	GTGTCCACCAACTCACAGATG	GGACCATTCCTTTTGCAGCAT
Branched–chain ketoacid dehydrogenase (*Bckdha*)	TGGCTAGATCTCACCCCAGCA	AGAGAATGCGGTCCATGGTG
18S rRNA	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA

Supplementary Results

Supplementary Table Legends

Table 1. Summary of Isotopomer Spectral Analysis (ISA) network for determination of substrate contribution to lipogenic AcCoA and rate of *de novo* lipogenesis in differentiated adipocytes.

Table 2. Control and Low Gluc+AA media formulation denoting those substrates with differing concentrations. All other trace components, lipids, vitamins, salts, etc. present in DMEM media were unchanged.

Table 3. Fragment ions formula and m/z range used to quantify metabolite abundances.

Table 4. Primer sequences used in gene expression analysis.

Supplementary Figure Legends

Supplementary Figure 1. Adipocyte differentiation reprograms intracellular metabolism

(a) Phase contrast image of differentiated 3T3–L1 adipocytes with Oil Red O staining of lipids.
(b) Palmitate labeling from [U–$^{13}C_6$]glucose tracer in 3T3–L1 pre–adipocytes and adipocytes.
(c) Palmitate labeling from [U–$^{13}C_5$]glutamine tracer in 3T3–L1 pre–adipocytes and adipocytes.
(d) Absolute lipogenic flux to palmitate synthesis from [U–$^{13}C_6$]glucose and [U–$^{13}C_5$]glutamine in 3T3–L1 pre–adipocytes and adipocytes.
(e) Efflux of glutamine synthesized from [U–$^{13}C_6$]glucose.
Data shown in **(b)**–**(e)** are 3 technical replicates representative of 3 biological replicates. Data presented in **(b)**–**(e)** represent mean ± s.d.

Supplementary Figure 2. Mitochondrial metabolism is supported by BCAA catabolism

(a) Summary of BCAA catabolism and carbon atom transitions from each BCAA tracer.
Enzyme key: 1: Branched chain amino transferase, 2: Branched–chain ketoacid DH/lipoamide, 3: Isovaleryl–CoA DH, 4: Acyl–CoA DH (ACAD) short branched–chain (ACADSB) or ACAD short–chain (ACADS), 5: ACAD8 or ACADSB or ACADS, 6: Methylcrotonyl–CoA carboxylase, 7: Methylglutaconyl–CoA hydratase, 8: Enoyl–CoA hydratase, 9: 3–hydroxy–2–methylbutyryl–CoA DH, 10: 3–hydroxyisobutyryl–CoA hydrolase, 11: Hydroxymethylglutaryl–CoA lyase, 12: Acetyl–CoA acyltransferase, 13: 3–hydroxyisobutyrate DH, 14: Methylmalonate semialdehyde DH, 15: Propionyl–CoA carboxylase, 16: Methylmalonyl–CoA mutase; Abbreviations: αKG: α–ketoglutarate; glut: glutamate; Oac: oxaloacetate; FA: fatty acid; AdoCbl: 5'–adenosylcobalamin, DH: dehydrogenase.
(b) Mole percent enrichment (MPE) of citrate from [U–$^{13}C_6$]glucose and [U–$^{13}C_6$]leucine over 3–hour time points.
(c) MPE of TCA cycle intermediates from [U–$^{13}C_6$]leucine and [U–$^{13}C_6$]isoleucine.
(d) Citrate labeling from [U–$^{13}C_6$]leucine in 3T3–L1 adipocytes in the presence of tracer for 24 hours beginning on the indicated day of differentiation.
Data shown in **(b)**–**(d)** are 3 technical replicates representative of 3 biological replicates. Data presented in **(b)**–**(d)** represent mean ± s.d.

Supplementary Figure 3. BCAA catabolites are oxidized and used for DNL

(a) Ratio of the substrate–specific oxygen consumption rate (OCR) of adipocytes to pre–adipocytes. Substrate key: M: malate; KIC–M: keto–isocaproate–malate; KMV–M: keto–methylvalerate–malate; KIV: keto–isovalerate; P–M: pyruvate–malate; S–R: succinate–rotenone;

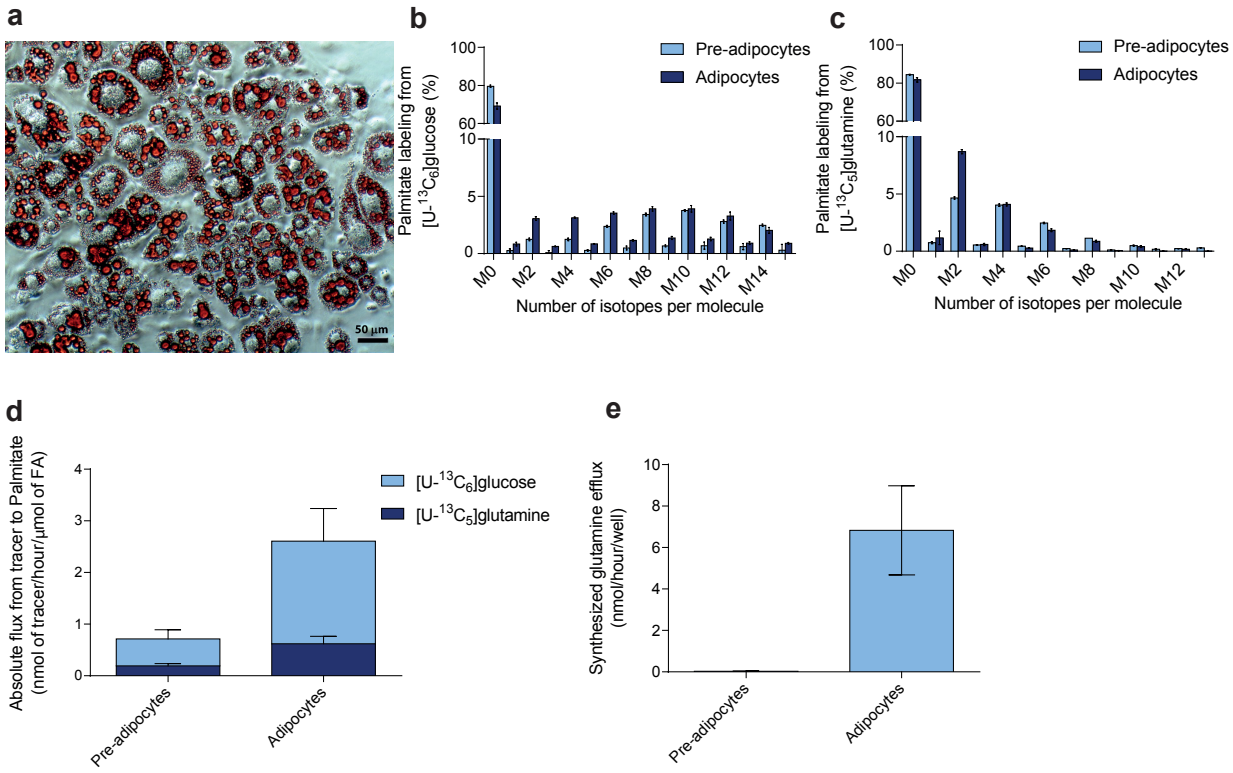
G–M: glutamate–malate. Data shown are from at least 5 biological replicates, each with 4 technical replicates; *represents $p<0.05$, **represents $p<0.01$, and ***represents $p<0.001$ analyzed via one–way ANOVA with Holm–Sidak's multiple comparisons test.
(b) Contribution to lipogenic AcCoA from [U–$^{13}C_6$]leucine in 3T3–L1 adipocytes in the presence of tracer for 24 hours beginning on the indicated day of differentiation.
(c) Even–chain fatty acid accumulation in 3T3–L1 adipocytes in the presence of tracer for 24 hours beginning on the indicated day of differentiation.
Data shown in **(a)** represent at least 5 biological replicates, each with 4 technical replicates. Data in **(b)–(c)** are 3 technical replicates representative of 3 biological replicates. Data plotted in **(a)–(c)** represent mean ± s.d.

Supplementary Figure 4. Protein catabolism supports BCAA metabolism
(a) Total intracellular FA in 3T3–L1 cells between control and Low Gluc+AA conditions.
(b) FA growth rates obtained via ISA analysis in control and Low Gluc+AA conditions.
(c) Sum of M1–M5 labeled glutamine in [U–$^{13}C_6$]glucose tracer medium in control or Low Gluc+AA conditions. ***represents $p<0.001$ determined via Student's t–test.
Data in **(a)–(c)** are 3 technical replicates representing 3 biological replicates and are presented as mean ± s.d.

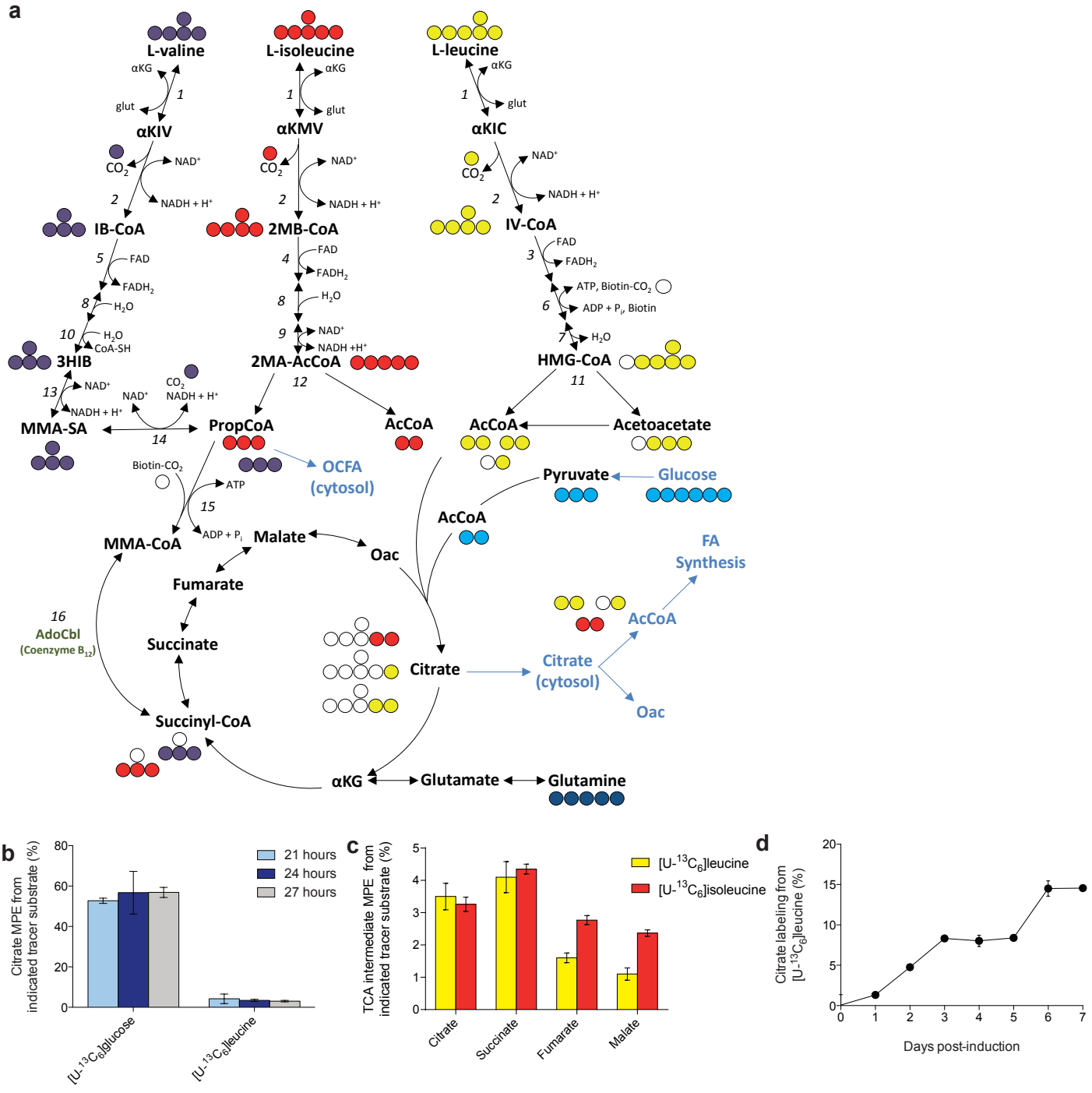
Supplementary Figure 5. Cobalamin supplementation alters 3T3–L1 metabolism
(a) Representative GC/MS chromatogram of BCAAs, norvaline internal standard, and methylmalonate (MMA) under normal culture conditions.
(b) Methylmalonate (MMA) and **(c)** C15:0 labeling from [U–$^{13}C_6$]isoleucine and [U–$^{13}C_5$]valine in 3T3–L1 adipocytes.
(d) C17:0 abundance in cells cultured in DMEM +10% FBS and above cobalamin concentrations beginning on Day 0 of differentiation.
(e) MMA level in 3T3–L1 adipocytes after culture with 500 nM cobalamin or 100 nM AdoCbl. Asterisks denote significance compared to control after statistical analysis via two–way ANOVA.
(f) Fumarate labeling from [U–$^{13}C_5$]valine in control and +cobalamin conditions.
(g) Malate labeling from [U–$^{13}C_5$]valine in control and +cobalamin conditions.
Mole percent enrichment (MPE) in TCA intermediates from **(h)** [U–$^{13}C_5$]valine and **(i)** [U–$^{13}C_6$]glucose in 3T3–L1 adipocytes in normal culture conditions with and without 500 nM cobalamin supplementation.
Data in **(b)–(i)** are 3 technical replicates representing 3 biological replicates and are presented as mean ± s.d. *represents $p<0.05$, **represents $p<0.01$, and ***represents $p<0.001$ determined via Student's t–test unless otherwise noted.

Supplementary Figure 6.
(a) Full, uncropped Western blot images.

Supplementary Figure 1



Supplementary Figure 2



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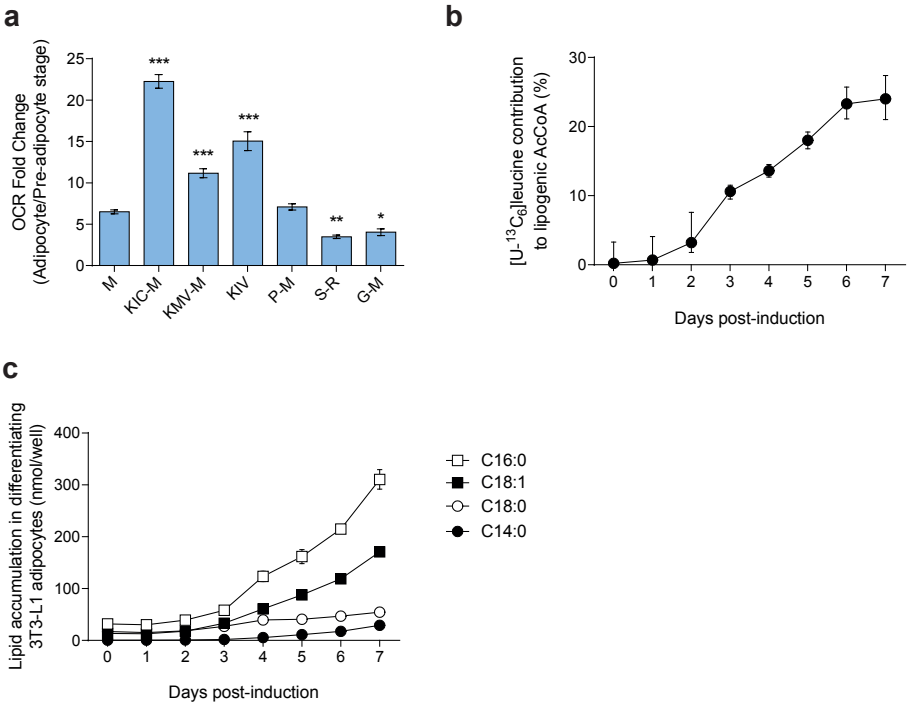

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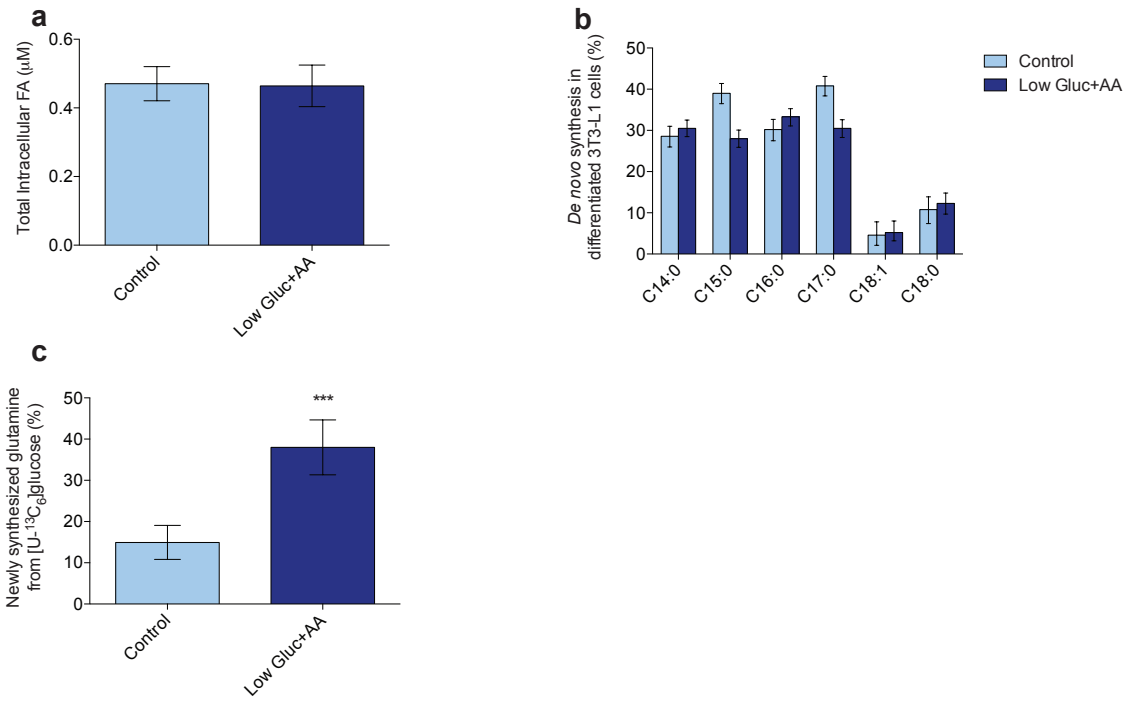

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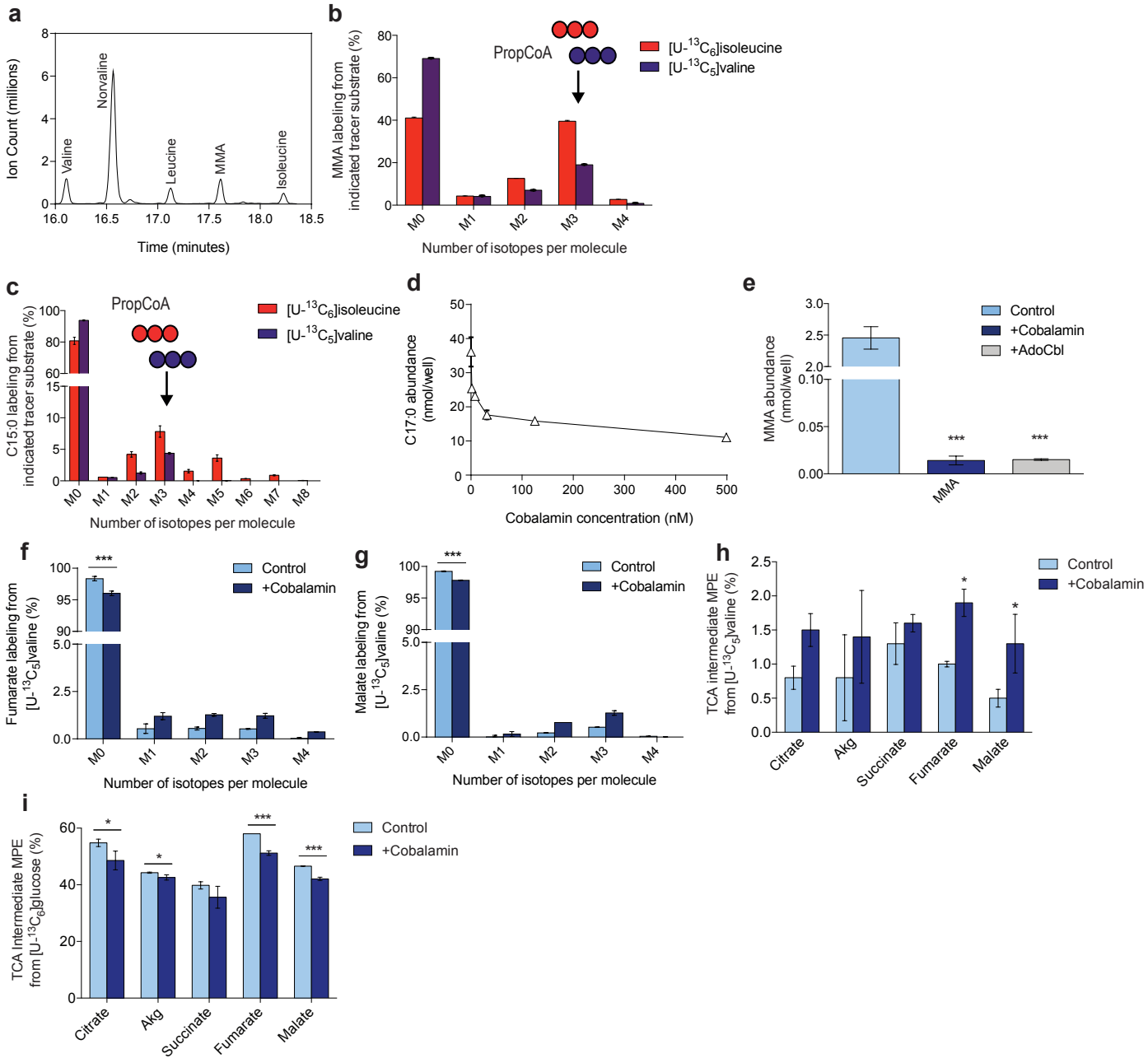
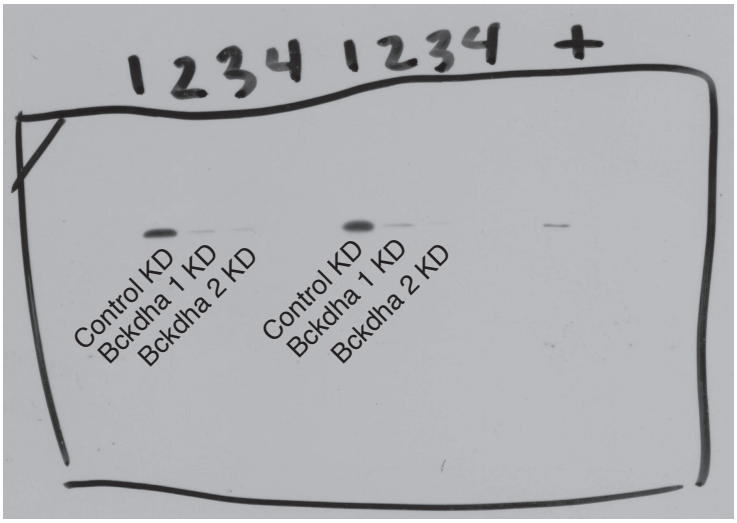


Supplementary Figure 6

a

Bckdha



β-Actin