

**Essential Amino Acid Ingestion Alters Expression of Genes Associated with
Amino Acid Sensing, Transport, and mTORC1 Regulation in Human Skeletal Muscle**

Online Supplementary Materials

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Differential Gene Expression Data			EAA		CQ		EAA vs. CQ	
Gene	Protein/Complex Name	Protein Function	%Change ¹	p-val. ²	%Change ¹	p-val. ²	%dif. ³	p-val. ⁴
Amino Acid Transporters and Subunits								
SLC38A10	SNAT10	sodium-dependent amino acid/proton antiporter	57 ± 15	0.019	-20 ± 9	0.001	77	0.001
SLC38A9	SNAT9	arginine sensor upstream of ragulator	31 ± 9	0.025	<i>24 ± 10</i>	<i>0.058</i>	6	0.645
SLC7A1	ATRC1 (CAT1)	cationic amino acid transporter 1	-8 ± 11	0.473	-37 ± 11	0.016	29	<i>0.081</i>
SLC7A8	LAT2	neutral amino acid antiporter light subunit	<i>41 ± 17</i>	<i>0.077</i>	-8 ± 6	0.241	49	0.018
Positive Regulators of mTORC1								
C7orf59	Ragulator	aka: LAMTOR4, GEF to RAG A/B	18 ± 6	0.042	5 ± 10	0.632	13	0.216
WDR24	GATOR2	Inhibits GATOR1	21 ± 4	0.007	8 ± 8	0.373	13	0.194
WDR59	GATOR2	Inhibits GATOR1	9 ± 5	0.15	-6 ± 3	0.152	15	0.034
MIOS5	GATOR2	Inhibits GATOR1	16 ± 4	0.012	14 ± 15	0.393	2	0.904
Negative Regulators of mTORC1 and mTORC1 Subunits								
DDIT4	REDD1 ⁵	cellular oxygen sensing, modulates Akt	209 ± 35	0.004	44 ± 28	0.170	164	0.005
DDIT4L	REDD2 ⁵	cellular oxygen sensing, modulates Akt	-13 ± 12	0.331	18 ± 5	0.022	-31	0.035
DEPDC5	GATOR1 ⁵	GAP to RAG A/B	20 ± 3	0.002	<i>24 ± 11</i>	<i>0.080</i>	-4	0.738
NPRL3	GATOR1 ⁵	GAP to RAG A/B	<i>14 ± 7</i>	<i>0.098</i>	15 ± 5	0.028	-1	0.993
NPRL2	GATOR1 ⁵	GAP to RAG A/B	12 ± 2	0.003	-2 ± 3	0.538	14	0.007
TBC1D7	TSC1-TSC2 ⁵	GAP to Rheb;	2 ± 5	0.364	-8 ± 3	0.039	<i>11</i>	<i>0.078</i>
TSC1	TSC1-TSC2 ⁵	GAP to Rheb;	15 ± 2	0.003	3 ± 5	0.548	<i>12</i>	<i>0.092</i>
TSC2	TSC1-TSC2 ⁵	GAP to Rheb;	<i>11 ± 4</i>	<i>0.062</i>	-19 ± 7	0.049	30	0.008
AKT1S1	PRAS40 ⁵	mTORC1 subunit--kinase inhibitor	29 ± 7	0.014	4 ± 8	0.647	26	0.037
MLST8	MLST8	mTORC1 subunit	16 ± 5	0.007	1 ± 7	0.884	15	0.129
mTOR	mTOR	serine/threonine kinase mTORC subunit	30 ± 10	0.044	'-6 ± 8	0.465	36	0.020
RPTOR	RAPTOR	scaffold for recruiting mTORC1 substrates	27 ± 9	0.041	'-4 ± 4	0.368	31	0.009
Important for Autophagy								
PIK3C3	PI3K	mediates formation of PIP3, autophagosome	14 ± 4	0.026	6 ± 6	0.401	8	0.345
TFEB	TFEB	Transcription factor positive for autophagy	96 ± 19	0.007	-8 ± 15	0.614	104	0.002

Table S1 Gene Expression Results. **Bold** indicates significant p-value ($p < 0.5$), *Italics* indicate a trend ($p < 0.1$), EAA indicates group fed essential amino acids, CQ indicates group fed essential amino acids while under the influence of chloroquine, EAA vs. CQ indicates a comparison between the changes seen in both groups. ¹ percent change from basal to fed condition, ² p-value from 2-tail paired student's t-test, ³ percent difference (EAA %change - CQ% change), ⁴ p-value taken from 2x2 Repeated Measures ANOVA between-subjects comparison by group, ⁵ a negative regulator of mTORC1.

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Genes of Interest (p<0.10)			EAA		CQ		EAA vs. CQ	
Gene	Protein Name	Protein Function	%Change ¹	p-val. ²	%Change ¹	p-val. ²	%dif. ³	p-val. ⁴
Amino Acid Transporters and Subunits								
SLC38A4	SNAT4	sodium-dependent neutral amino acid transporter	114 ± 59	0.126	-2 ± 11	0.847	<u>116</u>	<u>0.063</u>
SLC36A1	PAT1	amino acid symporter; positive regulator of mTOR	<u>27 ± 11</u>	<u>0.07</u>	20 ± 21	0.381	7	0.792
Positive Regulators of mTORC1								
LAMTOR1	Ragulator	aka: p18; anchors RAGULATOR to lysosome	15 ± 12	0.147	-4 ± 4	0.414	<u>19</u>	<u>0.06</u>
LAMTOR2	Ragulator	aka: p14	<u>21 ± 9</u>	<u>0.071</u>	-0.4 ± 6	0.942	<u>22</u>	<u>0.059</u>
LAMTOR3	Ragulator5	aka: MP1	14 ± 20	0.533	<u>-11 ± 5</u>	<u>0.061</u>	25	0.226
FLCN	Folliculin	Folliculin subunit, GAP towards RAG C/D	149 ± 76	0.144	11 ± 14	0.459	<u>138</u>	<u>0.058</u>
Important for Lysosome								
LAMP2	LAMP2	lysosome maintenance of acidic environment	26 ± 18	0.228	<u>18 ± 9</u>	<u>0.098</u>	8	0.681
MAP1LC3	LC3A	lysosome membrane formation	<u>54 ± 21</u>	<u>0.064</u>	35 ± 24	0.207	18	0.59

Table S2 Genes of Interest (p<0.1)

Italics indicate a trend ($p < 0.1$), EAA indicates group fed essential amino acids, CQ indicates group fed essential amino acids while under the influence of chloroquine, EAA vs. CQ indicates a comparison between the changes seen in both groups. ¹ percent change from basal to fed condition, ² p-value from 2-tail paired student's t-test, ³ percent difference (EAA %change - CQ% change), ⁴ p-value taken from 2x2 Repeated Measures ANOVA between-subjects comparison by group.

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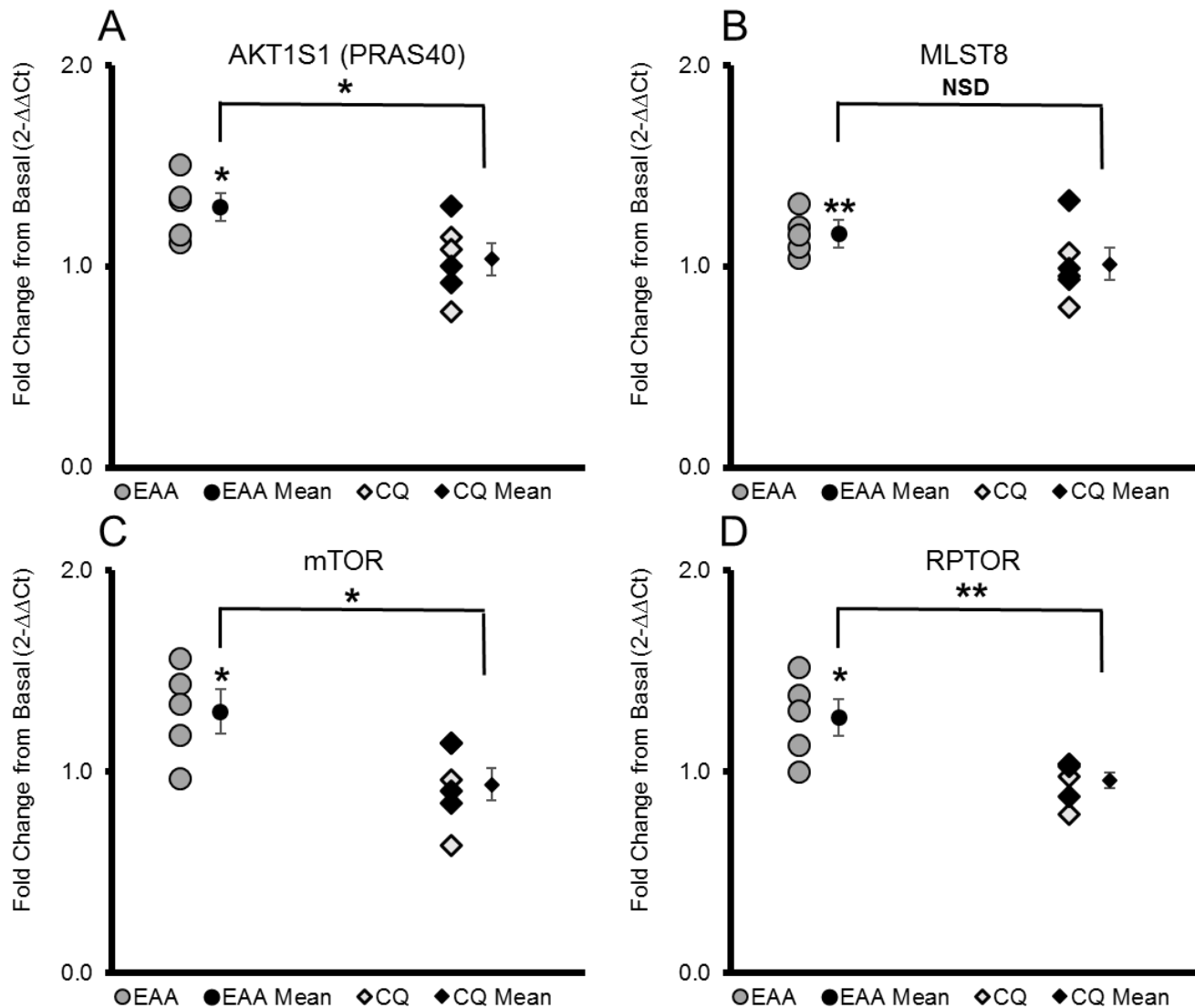


Figure S1 mTORC1 Subunits. Genes for four subunits of mTORC1 were upregulated in EAA, but not CQ, including: **A**) AKT1S1 (regulatory subunit PRAS40); **B**) MLST8 gene; **C**) serine/threonine kinase subunit gene mTOR; **D**) RPTOR (regulatory RAPTOR). x-axis: EAA = essential amino acid only group, CQ = essential amino acid plus chloroquine group, mean = mean of group ± standard error; y-axis units is fold change in gene expression from the basal to fed state using the 2-ΔΔCt method; bar indicates independent t-test comparison between EAA and CQ, symbols over means indicate paired t-test comparison of fold change with normalized baseline; * = $p \leq 0.05$, ** = $p \leq 0.01$, # = $p < 0.10$, NSD or no label = $p > 0.10$; the female subjects ($n=3$ in CQ) are delineated by darker data points.

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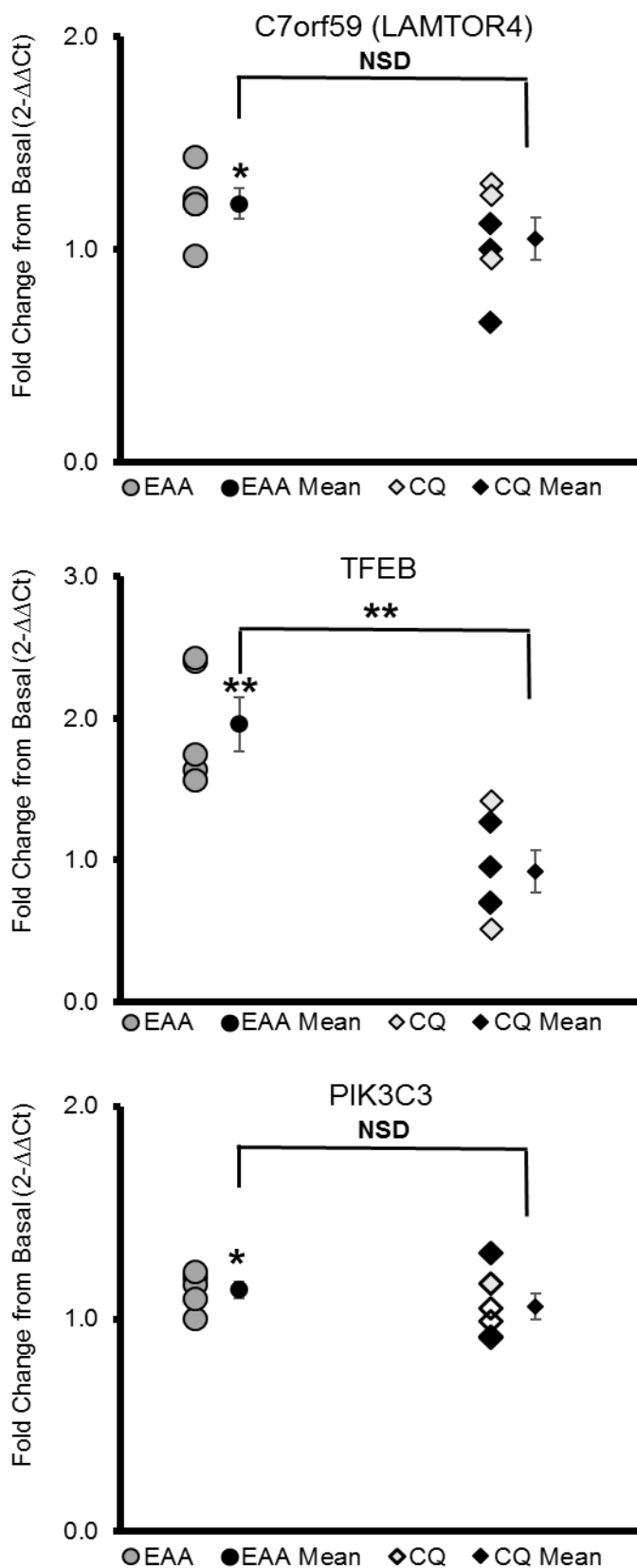


Figure S2 Other Genes. Autophagy related genes TFEB and PIK3C3 increased in EAA but not in CQ, as did the RAGULATOR subunit mRNA for LAMTOR4, C7orf59. x-axis: EAA=essential amino acid only group, CQ=essential amino acid plus chloroquine group, mean = mean of group ± standard error; y-axis units is fold change in gene expression from the basal to fed state using the $2^{-\Delta\Delta Ct}$ method; bar indicates independent t-test comparison between EAA and CQ, symbols over means indicate paired t-test comparison of fold change with normalized baseline; * = $p \leq 0.05$, ** = $p \leq 0.01$, # = $p < 0.10$, NSD or no label = $p > 0.10$; the female subjects (n=3 in CQ) are delineated by darker data points.

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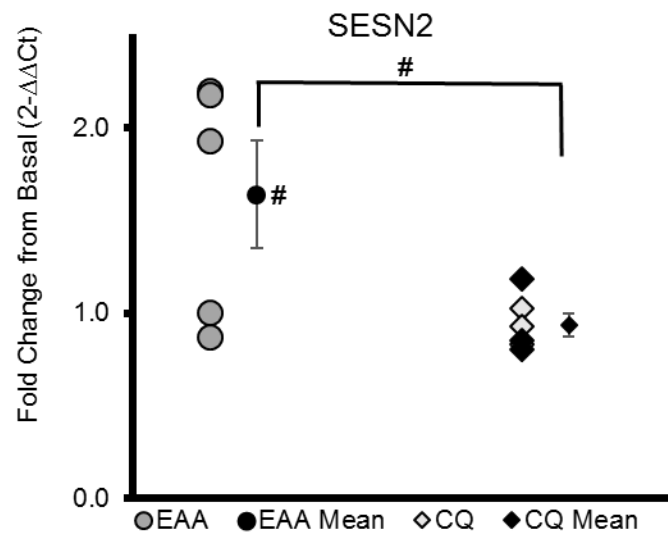


Figure S3 SESN2.

SESN2 gene codes for the Sestrin2 protein, one function of which is to act to inhibit GATOR2 (thus inhibiting mTORC1). Sestrin2 is deactivated with sufficient leucine. x-axis: EAA=essential amino acid only group, CQ=essential amino acid plus chloroquine group, mean = mean of group \pm standard error; y-axis units is fold change in gene expression from the basal to fed state using the 2- $\Delta\Delta$ Ct method; bar indicates independent t-test comparison between EAA and CQ, symbols over means indicate paired t-test comparison of fold change with normalized baseline; * = $p \leq 0.05$, ** = $p \leq 0.01$, # = $p < 0.10$, NSD or no label = $p > 0.10$; the female subjects (n=3 in CQ) are delineated by darker data points.