Supplemental Material

Myotube Protein Content Associates with Intracellular L-Glutamine Levels

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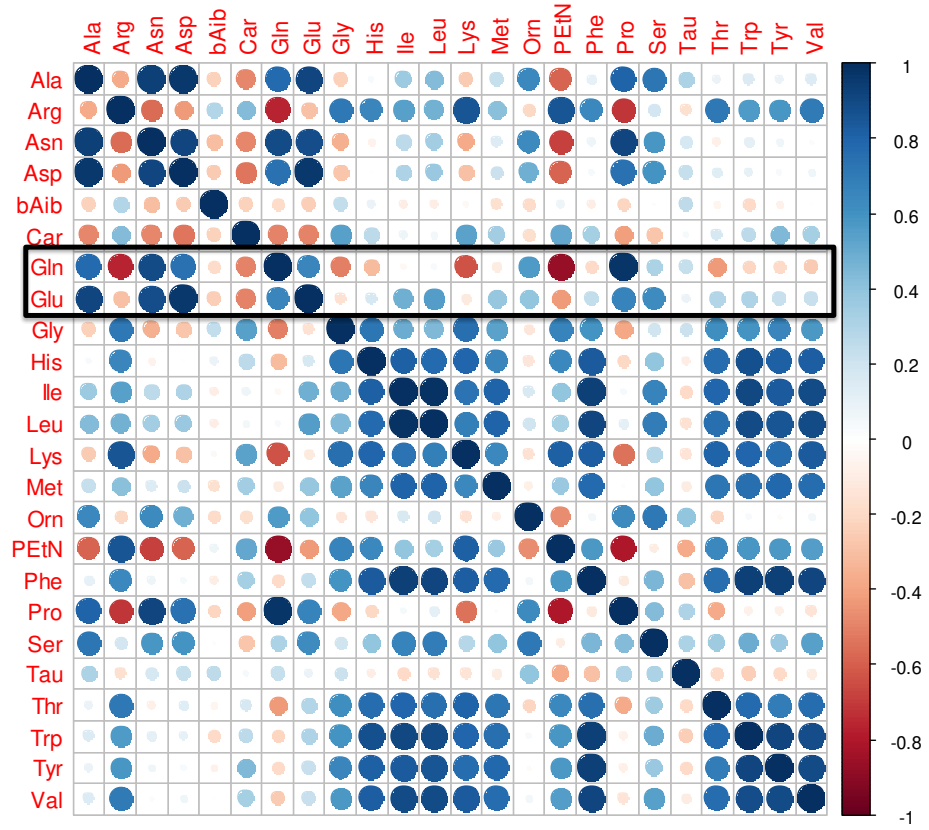
Figure S1. High Correlation (blue) between L-glutamine or L-glutamate levels and intracellular contents (A) or uptake (B) of amino acids and products of amino acid metablism in C_2C_{12} myotubes cultured in absence of glutamine (no addition and no addition plus glutamine synthase inhibitor) for 48 hours. C2C12 cells were cultivated in proliferation (DMEM low glucose -5.5 mM and 2 mM L-glutamine with 10% FBS for 2 days) and differentiation (DMEM low glucose – 5.5 mM and 2 mM L-glutamine with 2% horse serum for 4 days) conditions. Cells differentiated to myotubes were treated with different L-glutamine concentrations in DMEM with low glucose (5.5 mM) and 2% horse serum for 2 days. Figures refer to the following amino acids: alanine (Ala), arginine (Arg), asparagine (Asn), aspartate (Asp), β-aminoisobutyric acid (bAib), carnosine (Car), glutamine (Gln), glutamate (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), o-phosphoethanolamine (PEtN), phenylalanine (Phe), proline (Pro), serine (Ser), taurine (Tau), threonine (Thr), triptophan (Trp), tyrosine (Tyr) and valine (Val). Results were analyzed using Pearson correlation.

A


Ala
Arg
Asn
Asp
bAib
Car
Gln
Glu
Gly
His
Ile
Leu
Lys
Met
Orn
PEtN
Phe
Pro
Ser
Tau
Thr
Trp
Tyr
Val
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0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

B

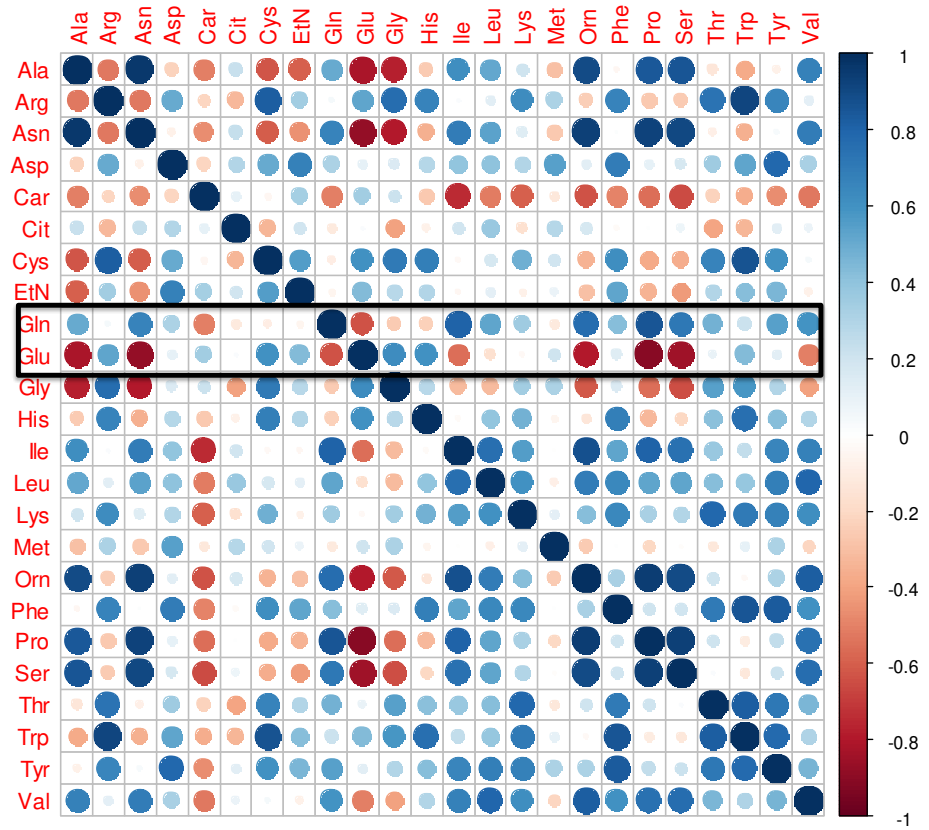
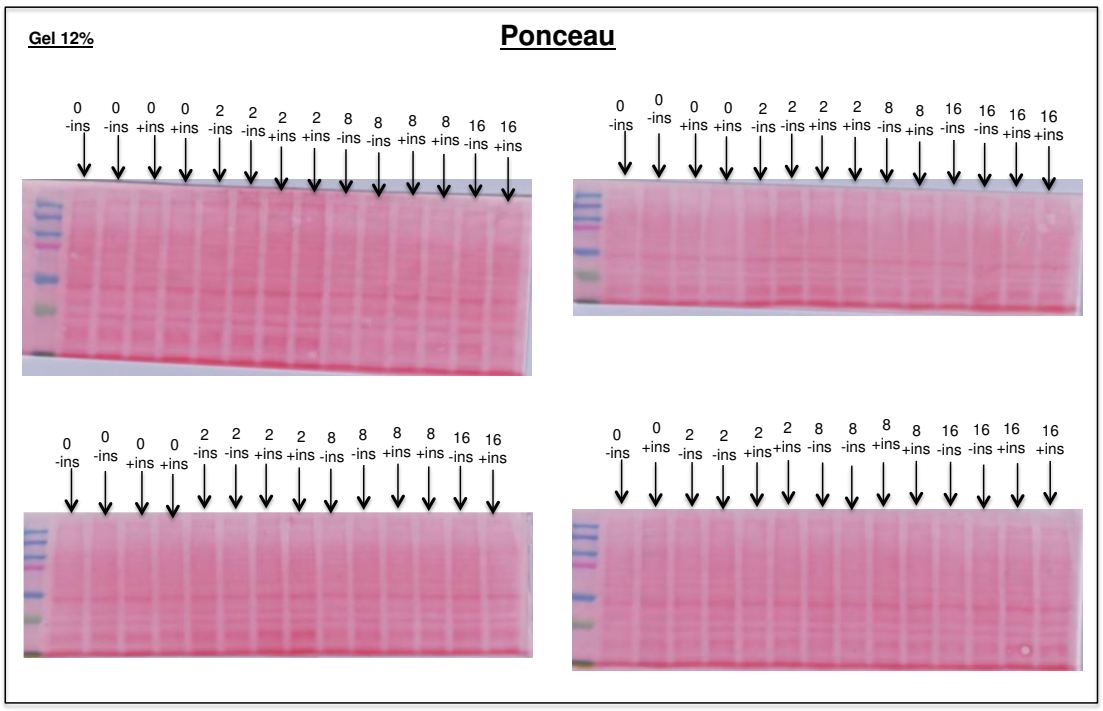
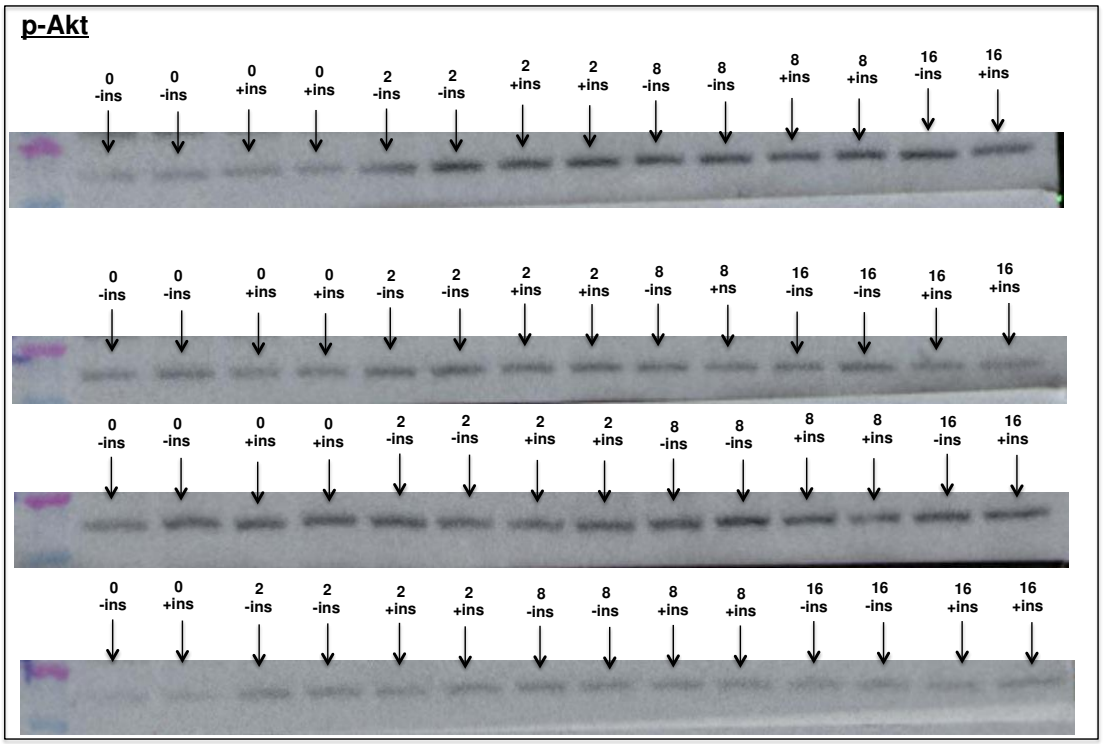

Ala
Arg
Asn
Asp
Car
Cit
Cys
EtN
Gln
Glu
Gly
His
Ile
Leu
Lys
Met
Orn
Phe
Pro
Ser
Thr
Trp
Tyr
Val
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure S2. Membranes and blots of Western Blotting results. Ponceau S staining of nitrocellulose membranes after protein transfer from 12% polyacrylamide gels (A); Blots of p-Akt, (B) p-RPS6 (C) and p-4E-BP1 (D) contents in in C_2C_{12} myotubes cultivated in various glutamine concentration conditions (no addition or 2, 8 or 16 mM glutamine) for 48 hours stimulated with insulin (100nM) in the last hour.

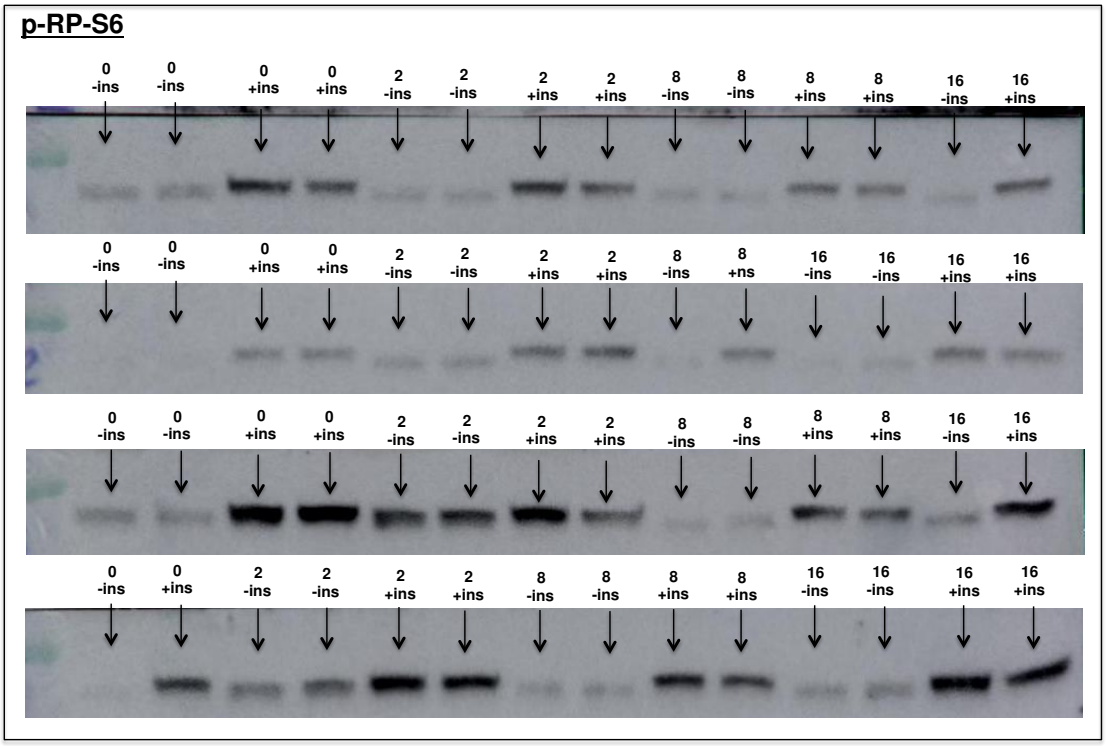
A



B p-Akt



C p-RP-S6



D p-4EBP1