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## Supplemental information

# DEPDC5-dependent mTORC1 signaling mechanisms are critical for the anti-seizure effects of acute fasting

**Christopher J. Yuskaitis, Jinita B. Modasia, Sandra Schrötter, Leigh-Ana Rossitto, Karenna J. Groff, Christopher Morici, Divakar S. Mithal, Ram P. Chakrabarty, Navdeep S. Chandel, Brendan D. Manning, and Mustafa Sahin**

Figure S1. Effects of fasting on seizure susceptibility, systemic measurements, and brain weight in control and *Depdc5*cc+ mice, Related to Figure 1.

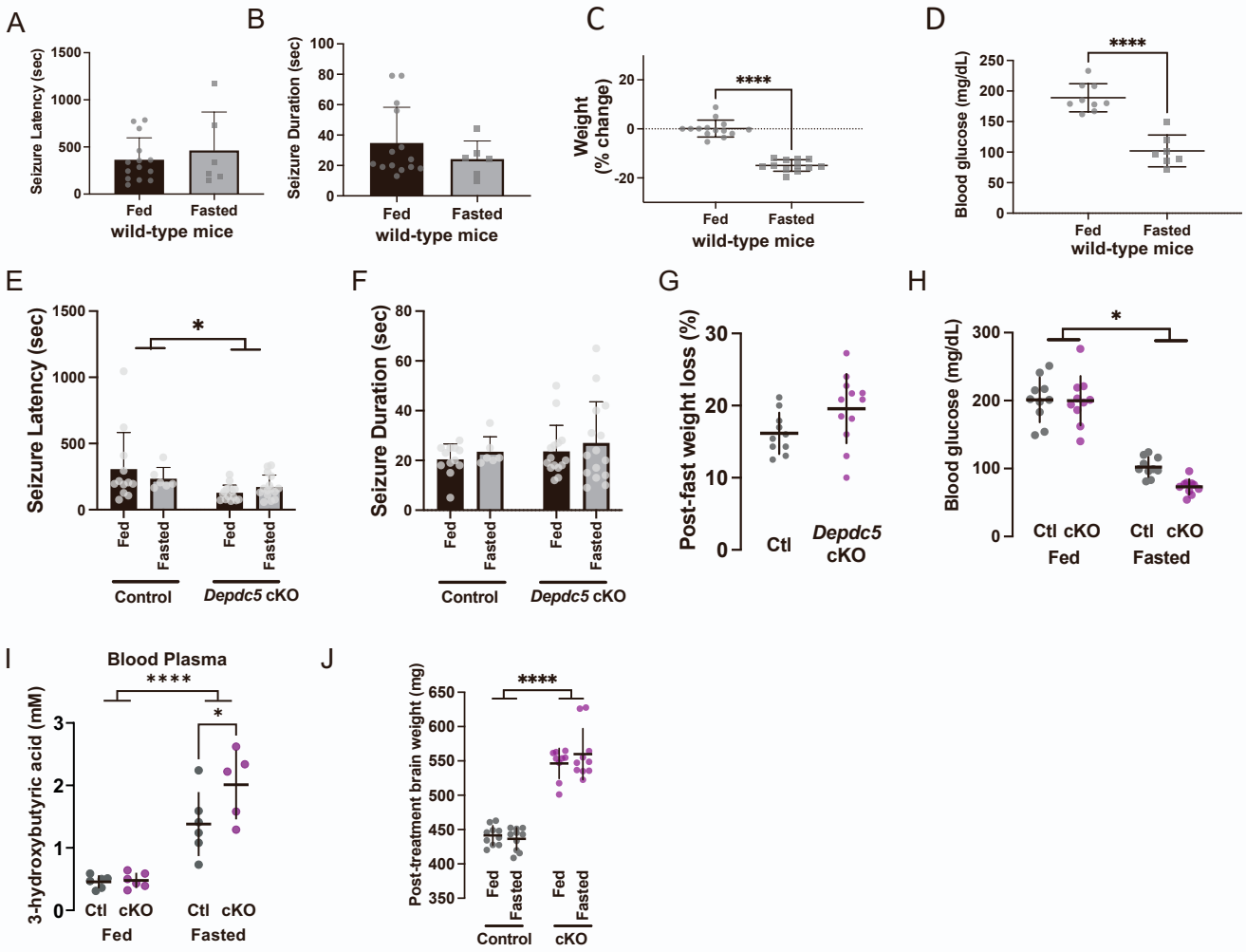


**Figure S1. Effects of fasting on seizure susceptibility, systemic measurements, and brain weight in control and *Depdc5*cc+ mice, Related to Figure 1.**

**(A)** Seizure latency in fed or fasted wild type mice treated with 65mg/kg PTZ (students t-test).

**(B)** Seizure duration in fed or fasted wild type mice treated with 65mg/kg PTZ (students t-test).

**(C)** Weight measurements after 24hr of fed or fasted conditions in wild-type mice (students t-test).

**(D)** Blood glucose measurements after 24hr of fed or fasted conditions in wild-type mice (students t-test).

**(E)** Seizure latency in fed or fasted control or *Depdc5*cc+ mice (cKO) treated with 65mg/kg PTZ (two-way ANOVA with Tukey's post hoc test, * $p = 0.015$ between fed/fasting state).

**(F)** Seizure duration in fed or fasted control or *Depdc5*cc+ mice treated with 65mg/kg PTZ (two-way ANOVA with Tukey's post hoc test).

**(G)** Post-fasting weight loss was not different between *Depdc5*cc+ and littermate control mice (students t-test).

**(H)** Fasting mice had significantly reduced blood glucose compared to littermate fed mice, however no differences were noted between *Depdc5*cc+ and littermate controls (two-way ANOVA with Tukey's post hoc test, * $p < 0.0001$ between fed/fasting state).

**(I)** Plasma 3-hydroxybuterate levels in fed or fasted control or *Depdc5*cc+ mice (two-way ANOVA **** $p < 0.0001$ between fed/fasting state; post-hoc Tukey's * $p = 0.05$ fasted control versus fasted cKO).

**(J)** Brain weight was not changed in fasted mice but was significantly increased in *Depdc5*cc+ mice (two-way ANOVA with Tukey's post hoc test, **** $p < 0.0001$ between fed/fasting state).

For all experiments, individual symbols indicate individual animals for the above measurements, mean +/- S.D. with *p<0.05, **p<0.01, ****p<0.0001. Two-way ANOVA with Tukey's post hoc analysis unless otherwise indicated.

Figure S2. mTOR activity after nutrient manipulation in neuronal cultures and HEK293T cells, Related to Figure 2.

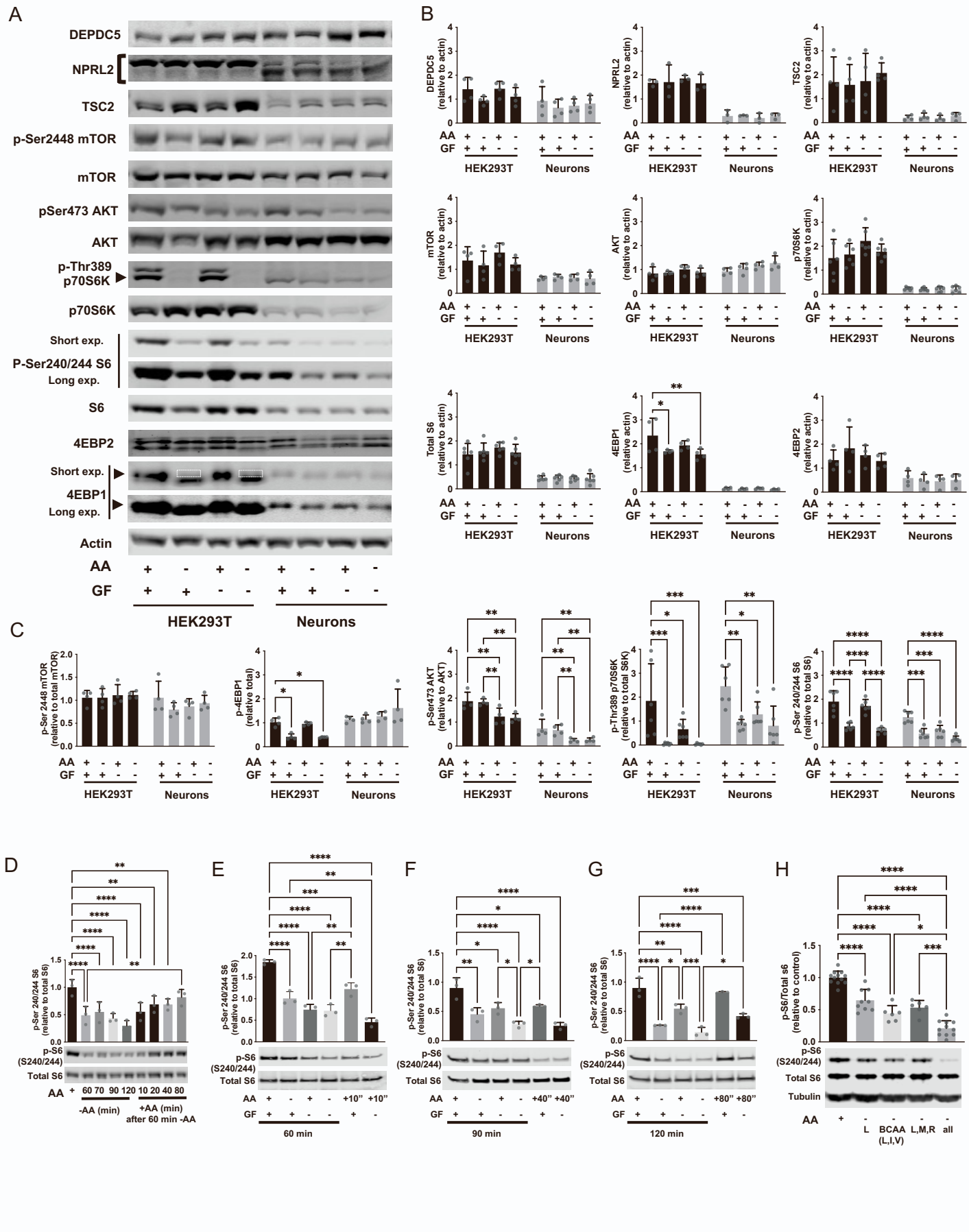


**Figure S2. mTOR activity after nutrient manipulation in neuronal cultures and HEK293T cells, Related to Figure 2.**

**(A)** Representative immunoblots from 20ug protein from either HEK293T or DIV12 primary cortical neurons treated for 60 min in the indicated conditions.

**(B)** Quantification of total protein amounts normalized to actin was unchanged except for 4EBP1 in HEK293T cells by amino acid withdrawal.

**(C)** Quantification of phosphorylated proteins from the immunoblots, normalized to their respective total protein amounts. Note that p-4EBP1 runs at a higher molecular weight (upper band indicated by the arrowhead and outlined absent bands) and divided by the total of both bands.

**(D)** Kinetics of mTORC1 reduction in primary neuronal cultures after withdrawal of AA in GF containing media, and restoration of mTORC1 activity 1x AA added back at the indicated times after 60 min AA withdrawal.

**(E-G)** Primary cortical neuron mTORC1 signaling after AA and/or GF withdrawal for 60, 90, or 120 minutes. **(F)** 90 minutes of AA and GF withdrawal by 90 min demonstrate an additive effect on the reduction of mTORC1 activity. **(G)** 80 min of reintroducing AA only partially rescues mTOR activity.

**(H)** Immunoblots and quantification of mTORC1 activity in primary neuronal cultures demonstrating no additive response by combinations of amino acids branch chain amino acids (BCAA) leucine, isoleucine (I), valine (V), or L, M, R.

For all experiments, graphs of mean +/- S.D. with symbols representing individual replicates from at least three independent experiments, one-way ANOVA with Tukey's post hoc analysis. *$p<0.05$, **$p<0.01$, ****$p<0.001$, ****$p<0.0001$.

Figure S3. Effects of mTOR activity and localization in neuronal cultures after DEPDC5 loss, Related to Figure 3.

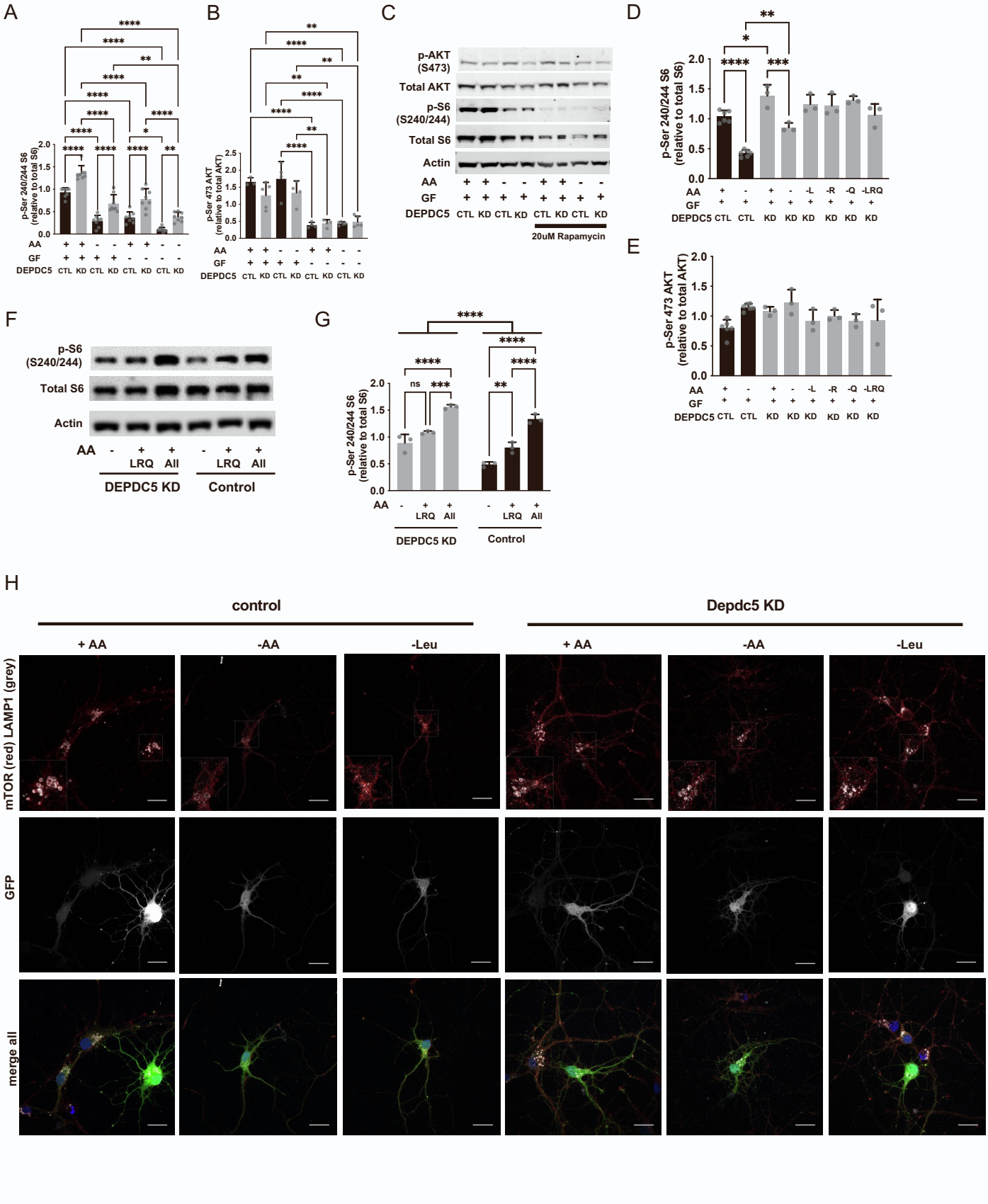


**Figure S3. Effects of mTOR activity and localization in neuronal cultures after DEPDC5 loss, Related to Figure 3.**

**(A, B)** Quantification of immunoblots from Figure 3A. **(A)** DEPDC5 knockdown (KD) have increased mTORC1 signaling compared scramble control (CTL) in primary cortical neurons after 90min amino acid (AA) and/or growth factor (GF) withdrawal**. (B)** Reduction in mTORC2 by growth factor (GF) withdrawal occurs in both CTL and DEPDC5 KD neurons.

**(C)** 24hr 20 uM rapamycin treatment further reduces pS6 after AA withdrawal in both DEPDC5 KD and control neurons.

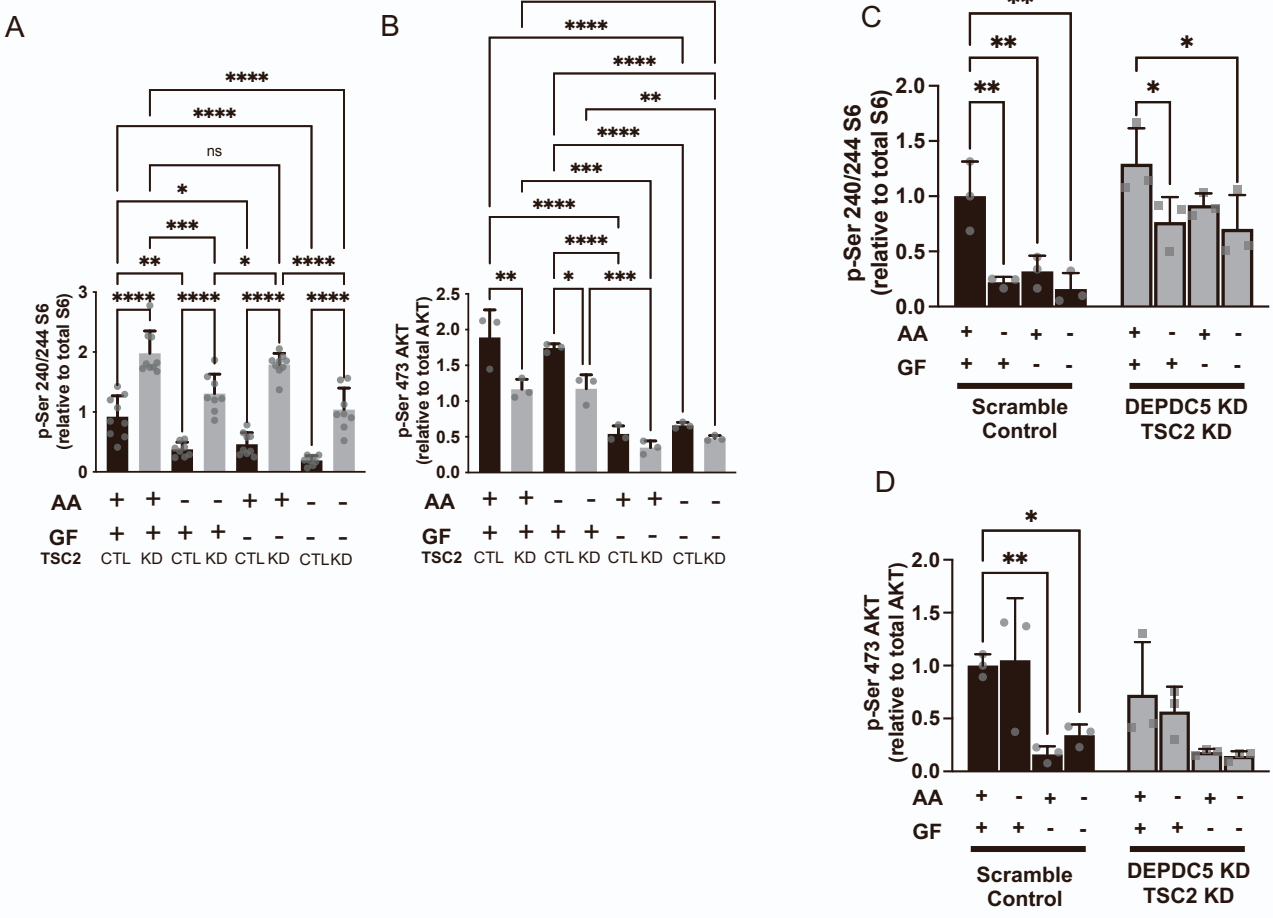
**(D, E)** Quantification of immunoblots from Figure 3C. **(D)** mTORC1 (pS240/244-S6) is insensitive to withdrawal of leucine (L), arginine (R), and/or glutamine (Q) in DEPDC5 KD but sensitive to withdrawal in CTL primary cortical neurons. **(E)** mTORC2 (pS473-AKT) is unchanged by withdrawal of leucine (L), arginine (R), and/or glutamine (Q) in CTL and DEPDC5 KD primary cortical neurons.

**(F, G)** Immunoblot and quantification from DEPDC5 knockdown (KD) or scramble control (CTL) primary cortical neurons starved of amino acids for 90 minutes followed by addition of all amino acids or a mixture of leucine (L), arginine (R), and/or glutamine (Q) demonstrates mTORC1 signaling in DEPC5 KD neurons is insensitive to addition of LQR but retain signaling from all amino acids.

**(H)** Representative images of mTOR lysosomal localization in primary neurons stained with mTOR (red) and the lysosomal marker, LAMP1 (grey) after incubation with or without AA and/or GF for 90 min transduced with GFP labeled scramble or DEPDC5 KD lentivirus (green). Scale bars = 20 μm, 2x inset.

For all experiments, graphs of mean +/- S.D. with symbols representing individual replicates from at least three independent experiments, two-way ANOVA with Tukey's post hoc analysis. *$p<0.05$, **$p<0.01$, ****$p<0.001$, ****$p<0.0001$.

Figure S4. Effects of mTOR activity and localization in neuronal cultures after TSC2 loss, Related to Figure 3.



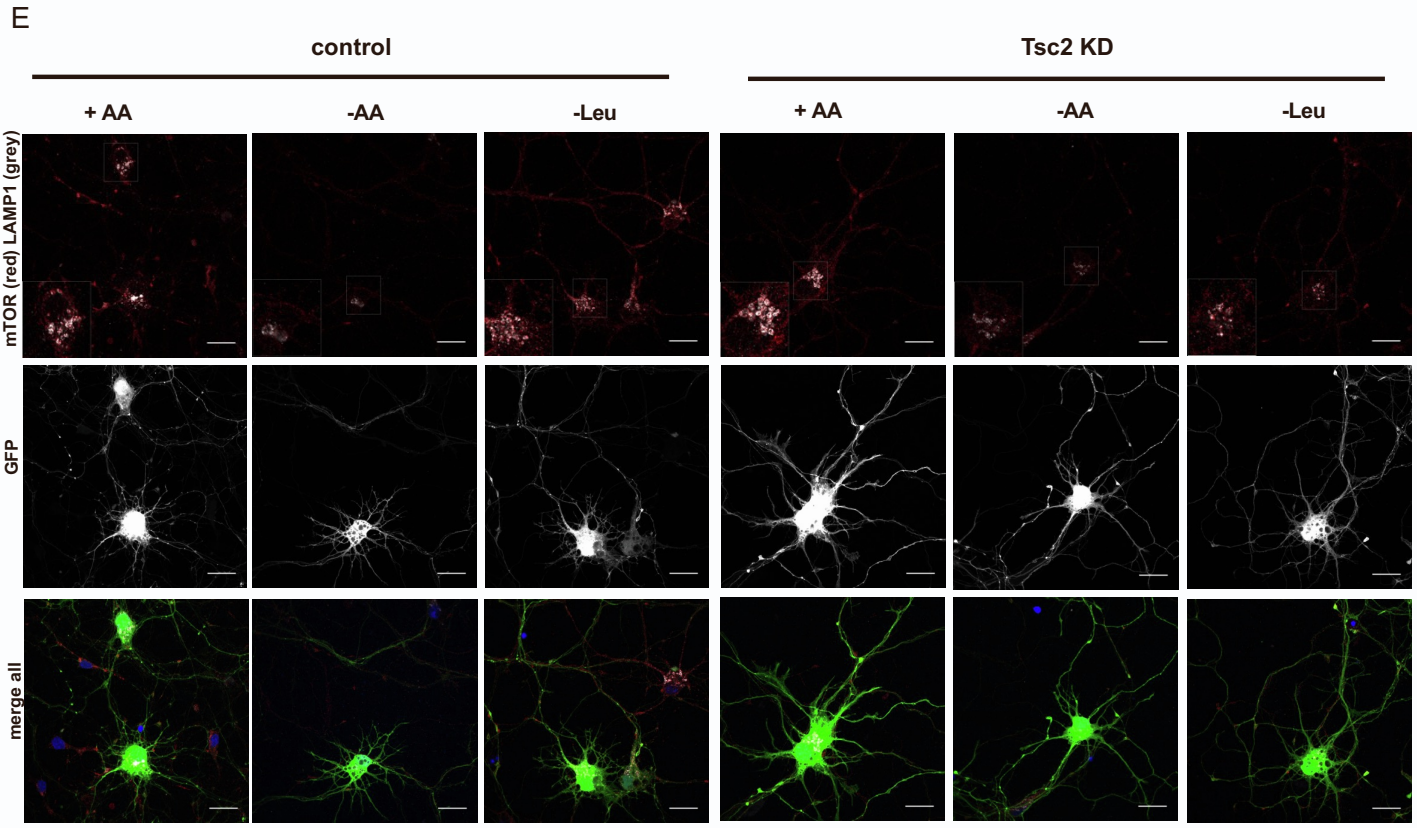



**Figure S4. Effects of mTOR activity and localization in neuronal cultures after TSC2 loss, Related to Figure 3.**

**(A,B)** Quantification of immunoblots from Figure 3F. TSC2 knockdown (KD) have **(A)** increased mTORC1 signaling compared scramble control (CTL) in primary cortical neurons after 90min amino acid (AA) and/or growth factor (GF) withdrawal, and **(B)** reduction in mTORC2 in TSC2 KD primary cortical neurons (KD) compared to scramble control (CTL) at baseline but are still responsive to growth factor (GF) withdrawal.

**(C-D)** Quantification of immunoblots from Figure 3J. Double knockdown of DEPDC5 and TSC2 (DTKD) in primary neurons demonstrating **(C)** mTORC1 is minimally sensitive to AA but not GF withdrawal, and **(D)** mTORC2 retains sensitivity to GF withdrawal.

**(E)** Representative images of mTOR lysosomal localization in primary neurons stained with mTOR (red) and the lysosomal marker, LAMP1 (grey) after incubation with or without AA and/or GF for 90 min transduced with GFP labeled scramble or TSC2 KD lentivirus (green). Scale bars = 20 μm, 2x inset.

For all experiments, graphs of mean +/- S.D. with symbols representing individual replicates from at least three independent experiments, two-way ANOVA with Tukey's post hoc analysis. *$p<0.05$, **$p<0.01$, ****$p<0.001$, ****$p<0.0001$.