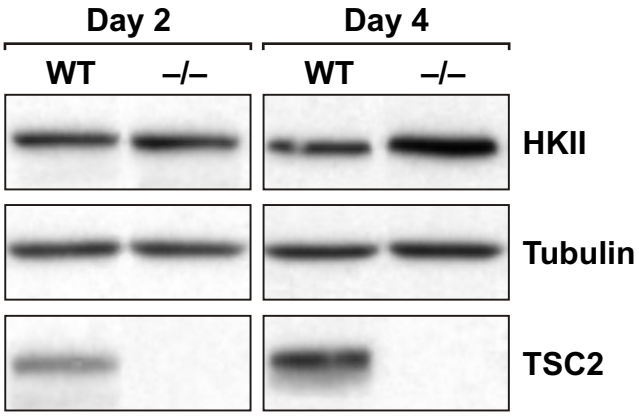
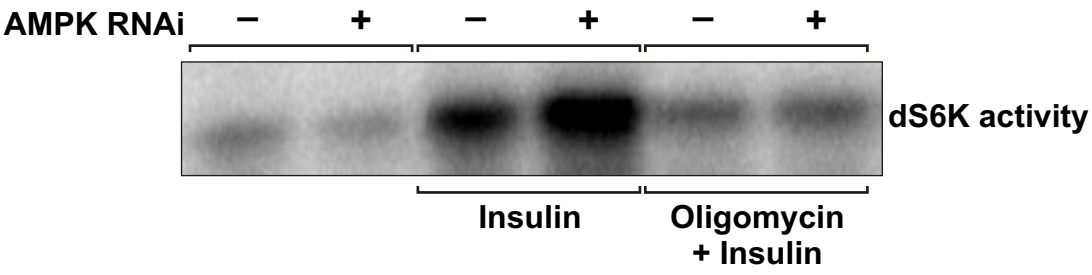


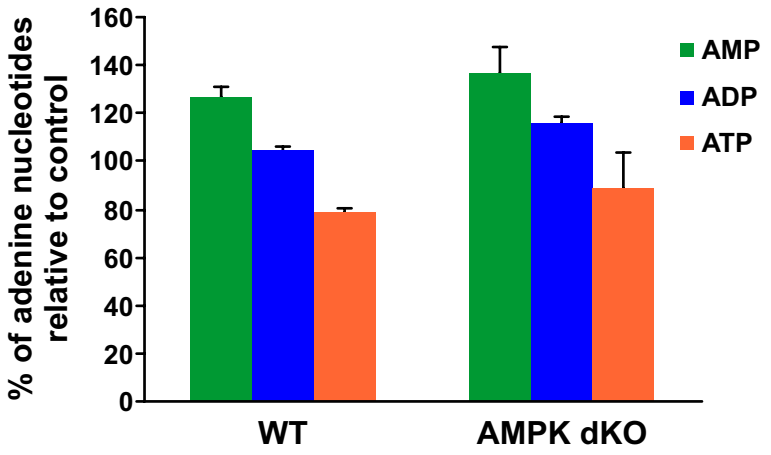
Supplemental Figure S1: Hexokinase-II levels increases as a function of TSC2$^{-/-}$ cell confluency

TSC2$^{+/+}$ and *TSC2*$^{-/-}$ MEFs at day 2 and day 4 post seeding. Immunoblots were performed as described in Experimental Procedures and probed with the indicated antibodies



Supplemental Figure S2: Oligomycin affects dS6K activity independent of dAMPK.

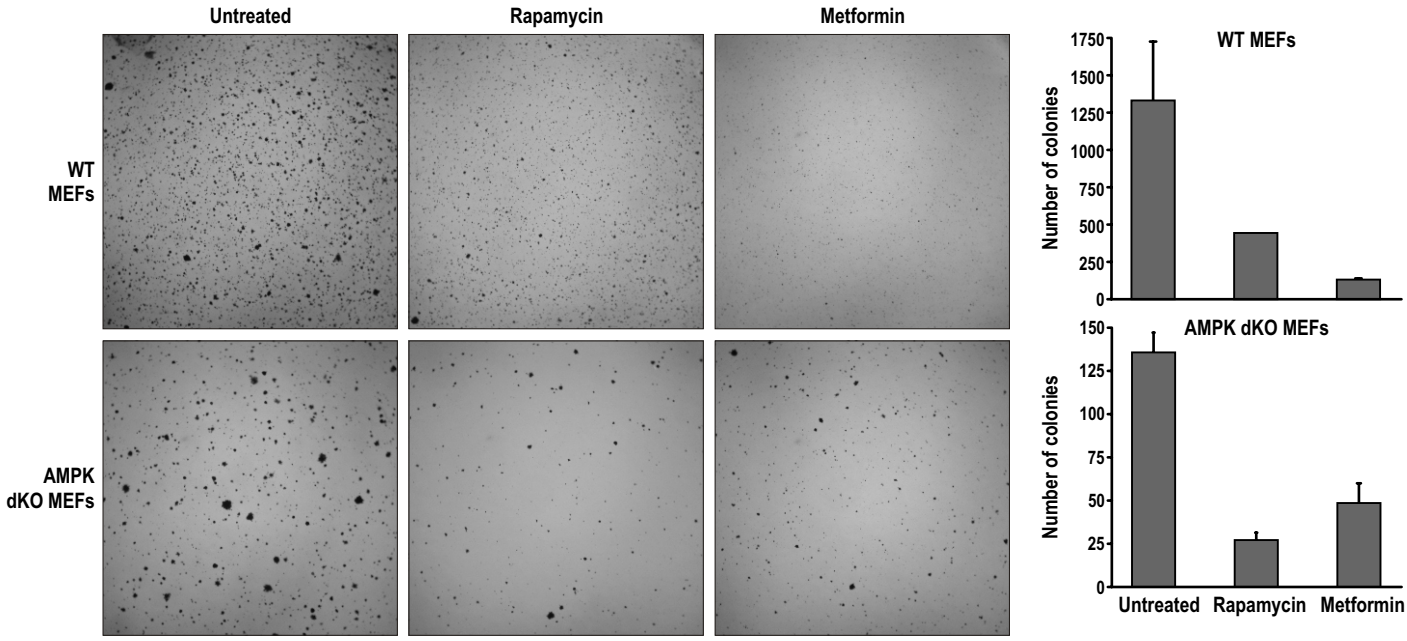
Analysis of dS6K activity from protein extracts of Kc167 cells employing H2B as a substrate (Radimerski et al., 2002). Treatment with dsRNA was performed essentially as described (Radimerski et al., 2002) , with an incubation time of 7 days. All primers were designed starting with the T7 RNA polymerase binding site as follows: 5'-TTAATACGACTCACTATAGGGAGA-3'. dAMPK sense-primer 5'- TTCGGCAAGGTGAAGATC-3', and anti-sense-primer 5'- CACTTGCAGCATCTGACA- 3'. Cells were treated with Insulin 100nM for 30min or 10 M Oligomycin for 15min followed by 100nM Insulin for 30min.



Supplemental Figure S3A: Relative AMP, ADP and ATP levels in WT and AMPK dKO MEFs

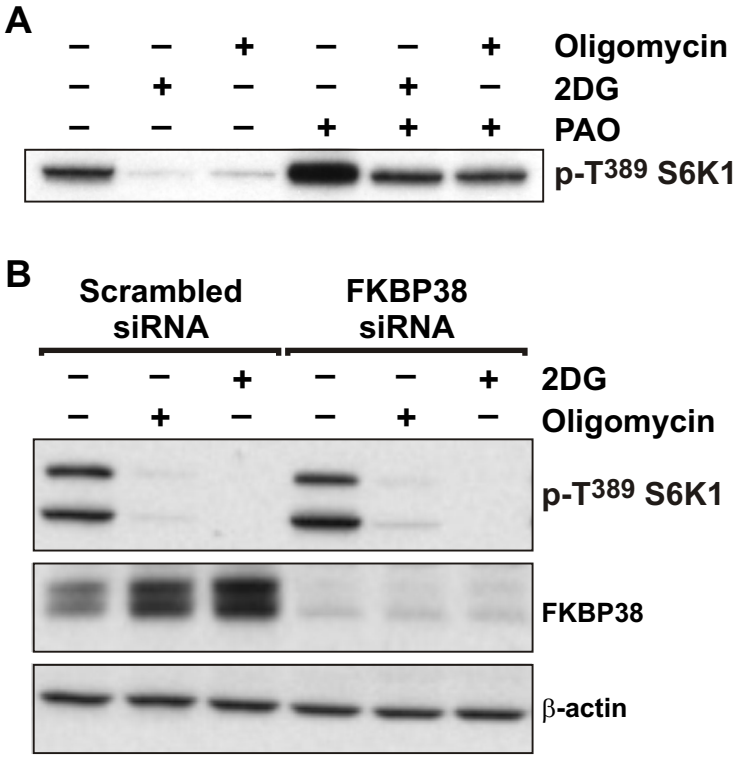
WT and AMPK dKO MEFs were treated with 5mM Phenformin for 1h. AMP, ADP and ATP levels are reported as a percentage to non-treated WT or AMPK dKO MEFs, respectively. AMP, ADP and ATP values of both cell types were similar prior treatment. Error bars represent SEM of three independent experiments. Comparison of WT and AMPK dKO cells treated with Phenformin gave a p value of 0.444 for AMP, 0.019 for ADP and 0.024 for ATP in a Two tails T-test .

ATP measurements were carried out from 5 x10^5 cells grown and treated as described in Experimental Procedures. Following PBS washes, cells were extracted with 60 µl 1N perchloric acid and immediately scrapped off the plates into microfuge tubes. The extracts were then incubated on ice for an additional 5min, followed by a centrifugation at 4°C for 5min. The supernatants were isolated carefully and neutralized with a 1:4 (v:v) mix of bromophenol blue and 3M K_2CO_3 , by adding a tenth of the supernatant volume, or until the liquid became green to blueish in color, but not yellow. Samples were centrifuged at 14000 rpm at room temperature and further filtered through a 0.2µM spin filter to remove any residual precipitate. The filtrate was either analyzed immediately or kept at -80°C until use. The chromatographic system used was from Shimadzu instruments Class V-P. 20 µl of samples were injected into the anion exchange column Partisphere 5 SAX from Whatman and separated using buffer A: 10mM $(NH_4)H_2PO_4$ pH3.7 and buffer B: 480m $(NH_4)H_2PO_4$ pH3.7. The separation sequence was 8 min 0% buffer B, 12 min 5% B, 15min 35% B, 20min 45 % B, 25min 50% B, 27min 100% B, 43min 100% B and 44min 0% B. The flow rate was 1.25 ml/min and the detection was performed with UV Vis



Supplemental Figure S3B: Metformin blocks colony formation of AMPK deficient MEFs.

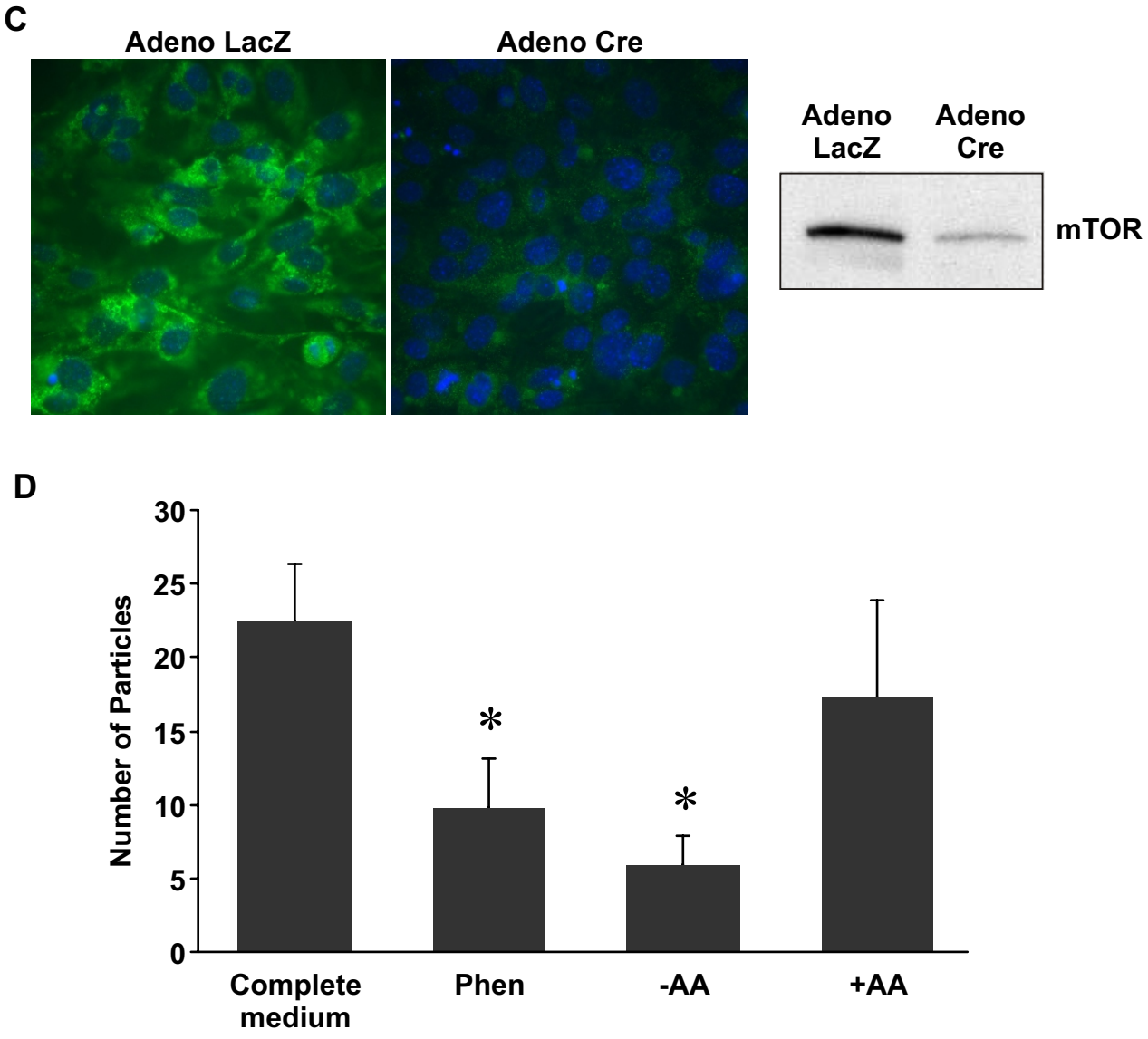
AMPK WT and AMPK dKO MEFs were grown in soft agar, treated with 20 nM Rapa or 10 mM Metformin for 4 weeks and stained with MTT. Number of colonies were quantified.



Supplemental Figure S4A, B: Role of oxidizing agents and FKBP38 in mTORC1 signaling upon energy depletion.

(**A**) HEK293 cells were pretreated with 5 μM Phenylarsine Oxide (PAO) for 5 min followed by treatment for 15 min with 100 mM 2DG or 10 μM Oligomycin.

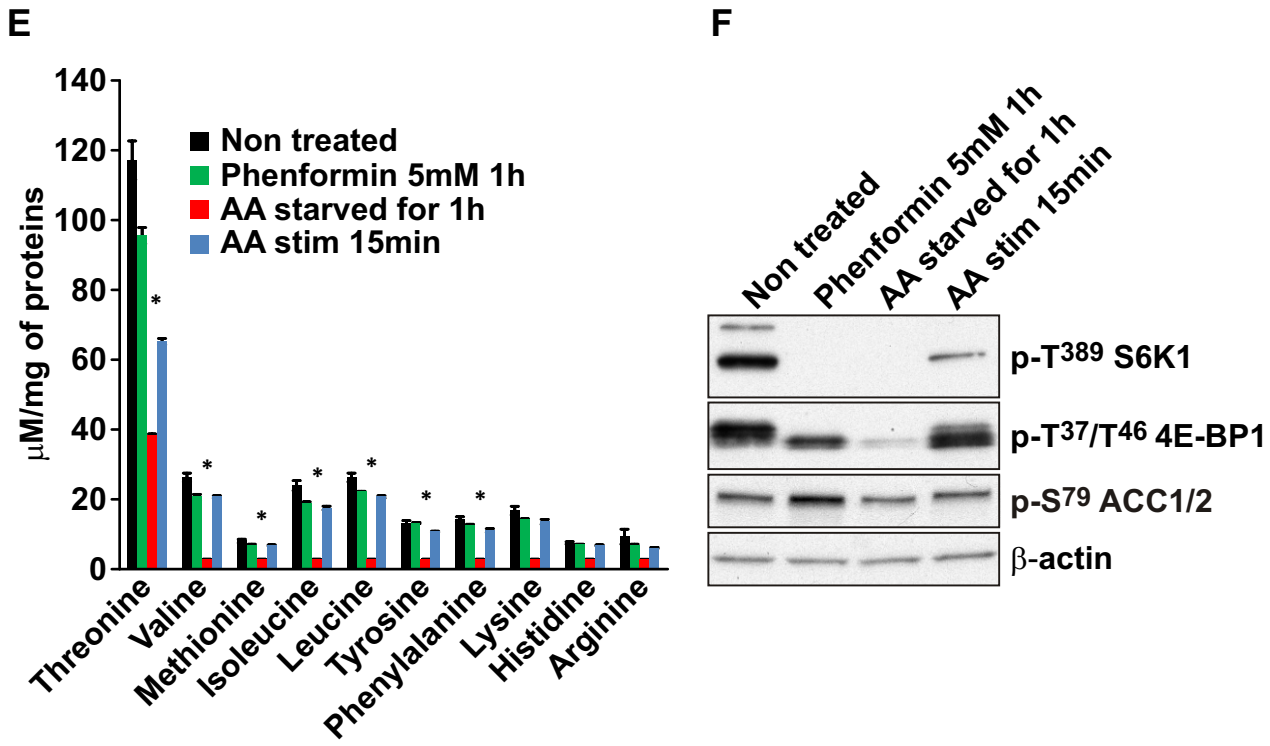
(**B**) HEK293 cells transfected with siRNA oligos for 48h followed by treatment of 30 min Oligomycin 10 μM or 100 mM 2DG.



Supplemental Figure S4C, D: Validation of the mTOR antibody and quantification of confocal images

(**C**) mTORfl/fl MEFS were infected with either Adenovirus LacZ or Adenovirus Cre and processed in an immunofluorescence assay with an mTOR antibody (green) and co-stained with DAPI for DNA content (blue). Western blot analysis of the cell extracts probed with mTOR antibody

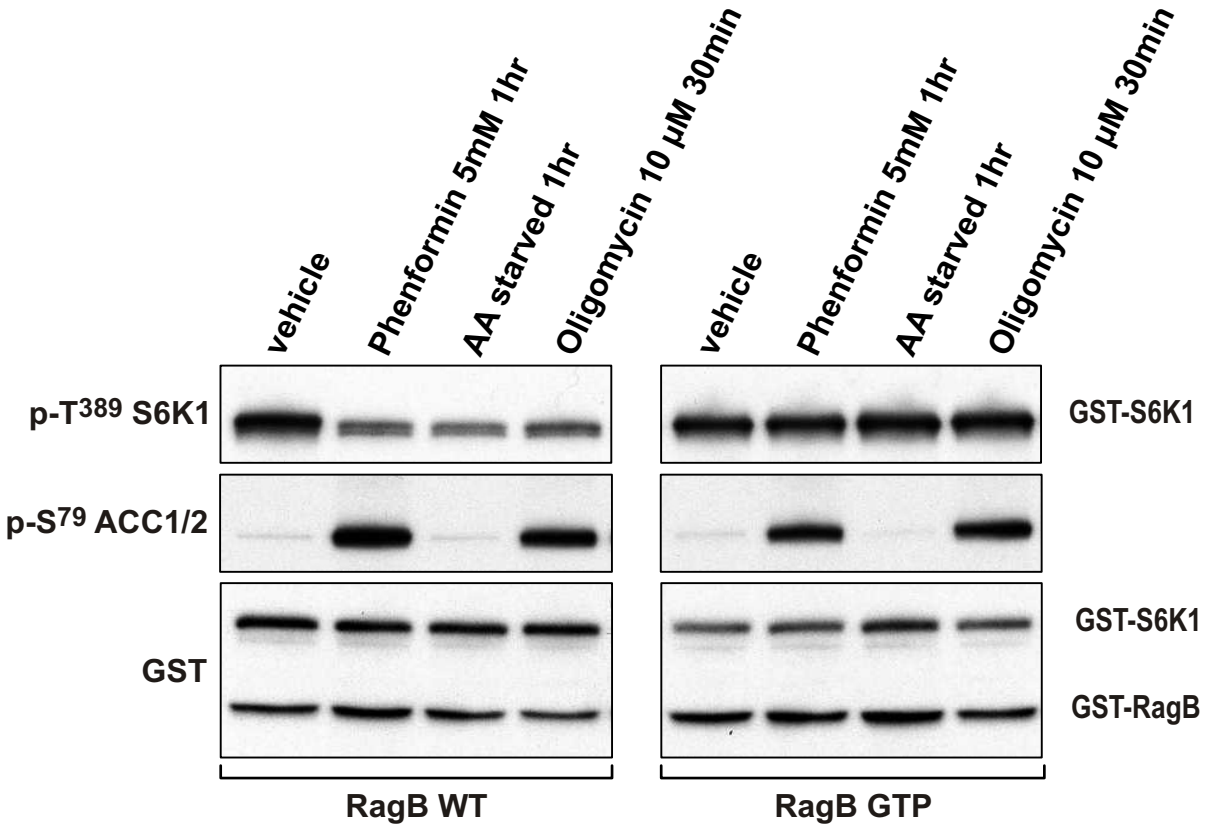
(**D**) Quantification of confocal images were performed using ImageJ software. Particles between the size of 10 and 10000 were counted from three different fields of each treatment using particle analyzer and nucleus counter. The (*) indicates $p<0.05$ using a one-tailed t-test.



Supplemental Figure S4E, F: Essential amino acids levels in HEK293T cells treated with Phenformin

(**E**) Amino acids (AA) quantification was normalized to protein content as described (Nobukini et al., 2005). Error bars represent SEM of two independent experiments. Two tails T-test *p<0.05

(**F**) Western blot analysis of extracts from cells treated as in A and probed with the indicated antibodies



Supplemental Figure S4G: HEK293T cells expressing epitope-tagged WT Rag GTPase or constitutively active Rag GTPase were treated with DMSO (**v**ehicle) for 1hr or 5mM Phenformin in DMSO for 1hr or 10 μM oligomycin in DMSO for 30min. AA deprivation for 1hr was used as a control. Western analyses were performed as described in Experimental Procedures and proteins were detected with indicated antibodies.