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Supplementary Materials for

Nutrient Signaling in Protein Homeostasis: An Increase in Quantity at the Expense of Quality

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The PDF file includes:

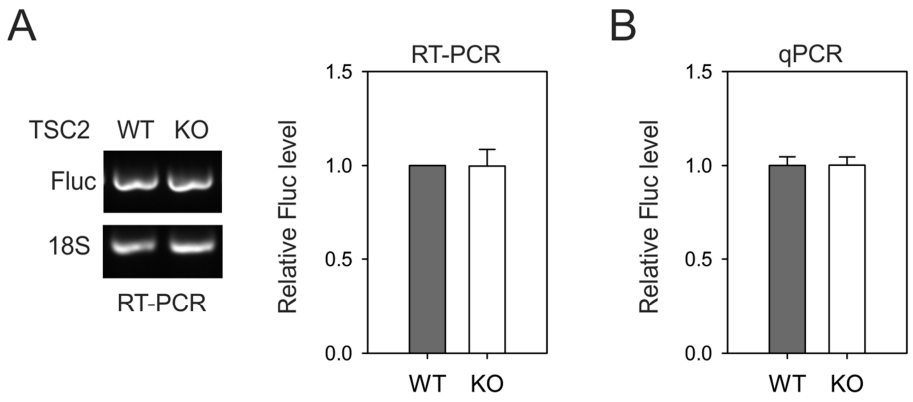

Figure S1. Quantification of Fluc mRNA abundance in wild-type and TSC2 knockout cells.

(A) Total RNA was extracted from TSC2 wild-type and knockout MEFs transfected with Fluc plasmids followed by RT-PCR with Fluc and 18*S* primers (left panel). Quantification of Fluc mRNA abundance is normalized to 18*S* rRNA (right panel) (mean ± SEM; n=3 independent experiments for PCR).

(B) RNA was extracted as in (A) and quantitative RT-PCR used to measure abundance of Fluc mRNA normalized to β-actin (mean ± SEM; n=3 independent experiments for qPCR).

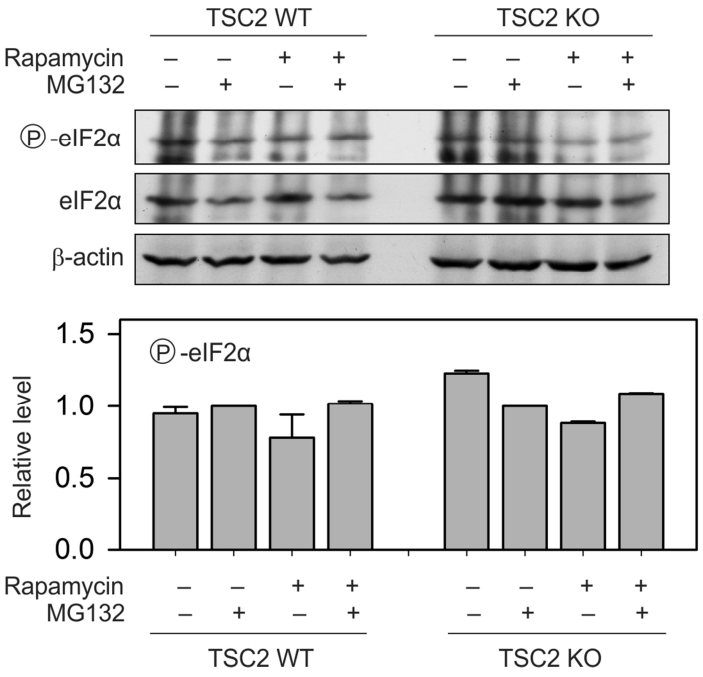

Figure S2. Phosphorylation status of eIF2α in wild-type and TSC2 knockout cells. TSC2 wild-type and knockout cells were treated with 10 nM Rapamycin and/or 5 μM MG132 for 15 hours. Whole cell lysates were immunoblotted using antibodies as indicated (top panel). Phosphorylation of eIF2α was normalized to total eIF2α (mean ± SEM; n=3 independent experiments).

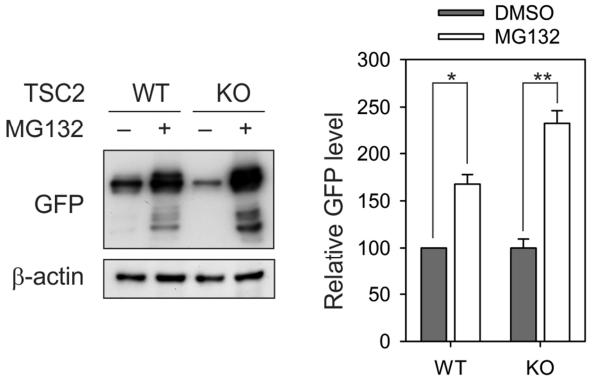

Figure S3. Features of GFP in wild-type and TSC2 knockout cells.

TSC2 wild-type and knockout cells transfected with myc-GFP plasmids were treated with 5 μM MG132 for 15 hours. Whole cell lysates were immunoblotted as indicated (left panel). The abundance of GFP was normalized to that of β-actin (mean ± SEM; n=3 independent experiments for blot; *P = 0.022, **P = 0.0077, Paired t-test).

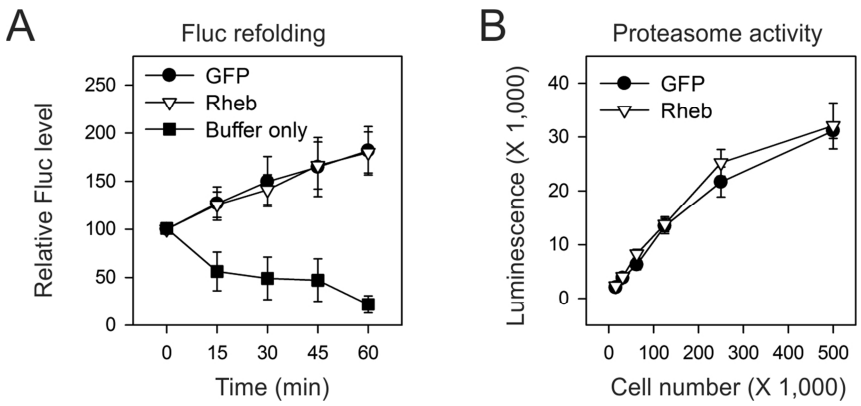

Figure S4. Chaperone and proteasome activity in cells expressing Rheb.

(A) Heat-denatured Fluc proteins were incubated with whole cell lysates derived from HEK293 cells expressing myc-GFP or myc-Rheb at room temperature. Fluc refolding was monitored by measuring Fluc activity at the time points indicated. Relative Fluc activities are presented (mean ± SEM; n=3 independent experiments).

(B) HEK293 cells expressing myc-GFP or myc-Rheb were plated at the indicated concentrations and the intracellular chymotrypsin activity was measured by the luminescent reagent Proteasome-Glo (mean ± SEM; n=3 independent experiments).

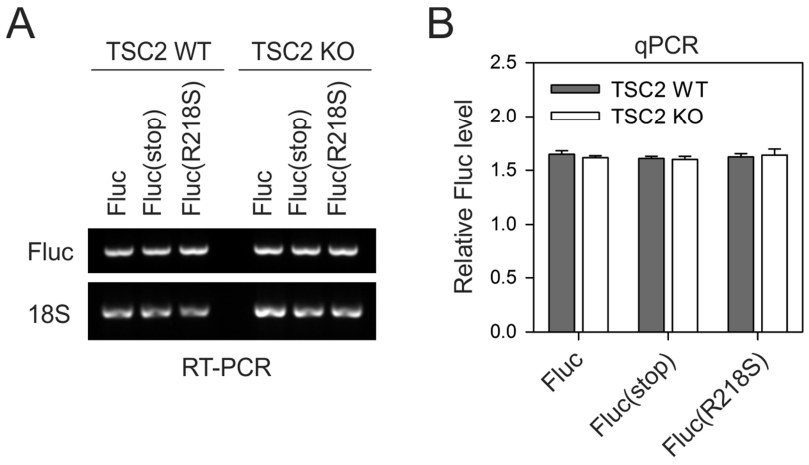

Figure S5. Quantification of mRNA abundance of Fluc mutants in wild-type and TSC2 knockout cells.

(A) RNA was extracted from TSC2 wild-type and knockout cells expressing wild-type or mutant Fluc plasmids followed by RT-PCR with Fluc and 18*S* primers.

(B) RNA was extracted as in (A) and quantitative RT-PCR used to measure abundance of Fluc mRNA normalized to β-actin (mean ± SEM; n=3 independent experiments for qPCR).

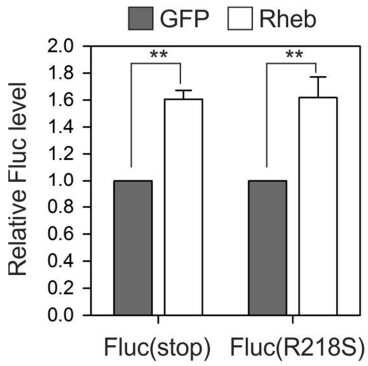

Figure S6. Translation fidelity in cells expressing Rheb.

HEK293 cells expressing myc-GFP or myc-Rheb were transfected with plasmids encoding Fluc mutants for 24 hours. Relative Fluc activity was normalized using wild-type Fluc (mean ± SEM; n=5 independent experiments; **$p < 0.01$, Ratio paired t-test).

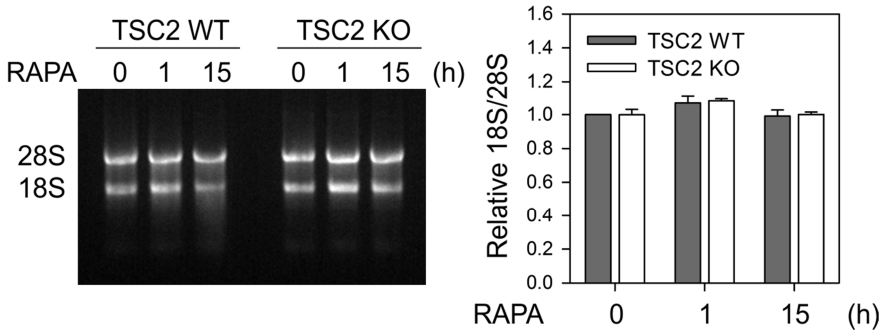

Figure S7. Ratio of 28*S* to 18*S* rRNAs in wild-type and TSC2 knockout cells.
RNA was extracted from wild-type and TSC2 knockout cells with or without 20 nM rapamycin treatment for the indicated times. RNA was run on a 1% agarose gel by electrophoresis (left panel). Relative ratios of 18*S* rRNA was compared to 28*S* rRNA (right panel) (mean ± SEM; n=3 independent experiments).

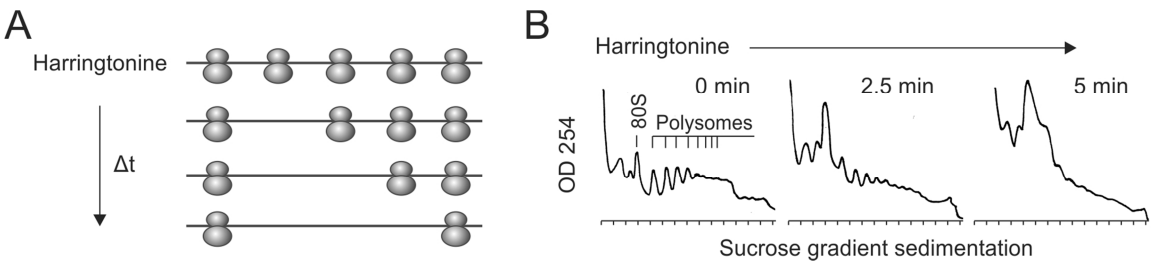

Figure S8. Measurement of ribosome dynamics during translation elongation

(A) Schematic of ribosome run-off in the presence of harringtonine.

(B) An example of polysome profiles of MEF cells in the presence of harringtonine (1 μg/ml) at the indicated times. Monosome (80*S*) and polysomes are highlighted and the P/M ratio change is quantified in Fig. 6B.