Developmental Cell, Volume *56*

Supplemental information

Rheb mediates neuronal-activity-induced mitochondrial energetics through mTORC1-independent PDH activation

Wanchun Yang, Dejiang Pang, Mina Chen, Chongyangzi Du, Lanlan Jia, Luoling Wang, Yunling He, Wanxiang Jiang, Liping Luo, Zongyan Yu, Mengqian Mao, Qiuyun Yuan, Ping Tang, Xiaoqiang Xia, Yiyuan Cui, Bo Jing, Alexander Platero, Yanhui Liu, Yuquan Wei, Paul F. Worley, and Bo Xiao

Supplemental Information

Rheb Mediates Neuronal Activity-Induced Mitochondrial Energetics through mTORC1-independent PDH Activation

Authors: Wanchun Yang[1,3,5], Dejiang Pang[1,5], Mina Chen[1], Chongyangzi Du[1], Lanlan Jia[1], Luoling Wang[2], Yunling He[2], Wanxiang Jiang[1], Liping Luo[1], Zongyan Yu[2], Mengqian Mao[1], Qiuyun Yuan[1], Ping Tang[1], Xiaoqiang Xia[1], Yiyuan Cui[1], Bo Jing[2], Alexander Platero[4], Yanhui Liu[1,3], Yuquan Wei[1], Paul F. Worley[4,*], Bo Xiao[2,6,*]

Affiliations:

[1]Neuroscience & Metabolism Research, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, People's Republic of China.

[2]Department of Biology, School of Life Sciences, Brain Research Center, Southern University of Science and Technology, Shenzhen Key Laboratory of Gene Regulation and Systems Biology, Shenzhen 518055, People's Republic of China.

[3]Department of Neurosurgery, West China Hospital, Sichuan University, Chengdu 610041, People's Republic of China.

[4]The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

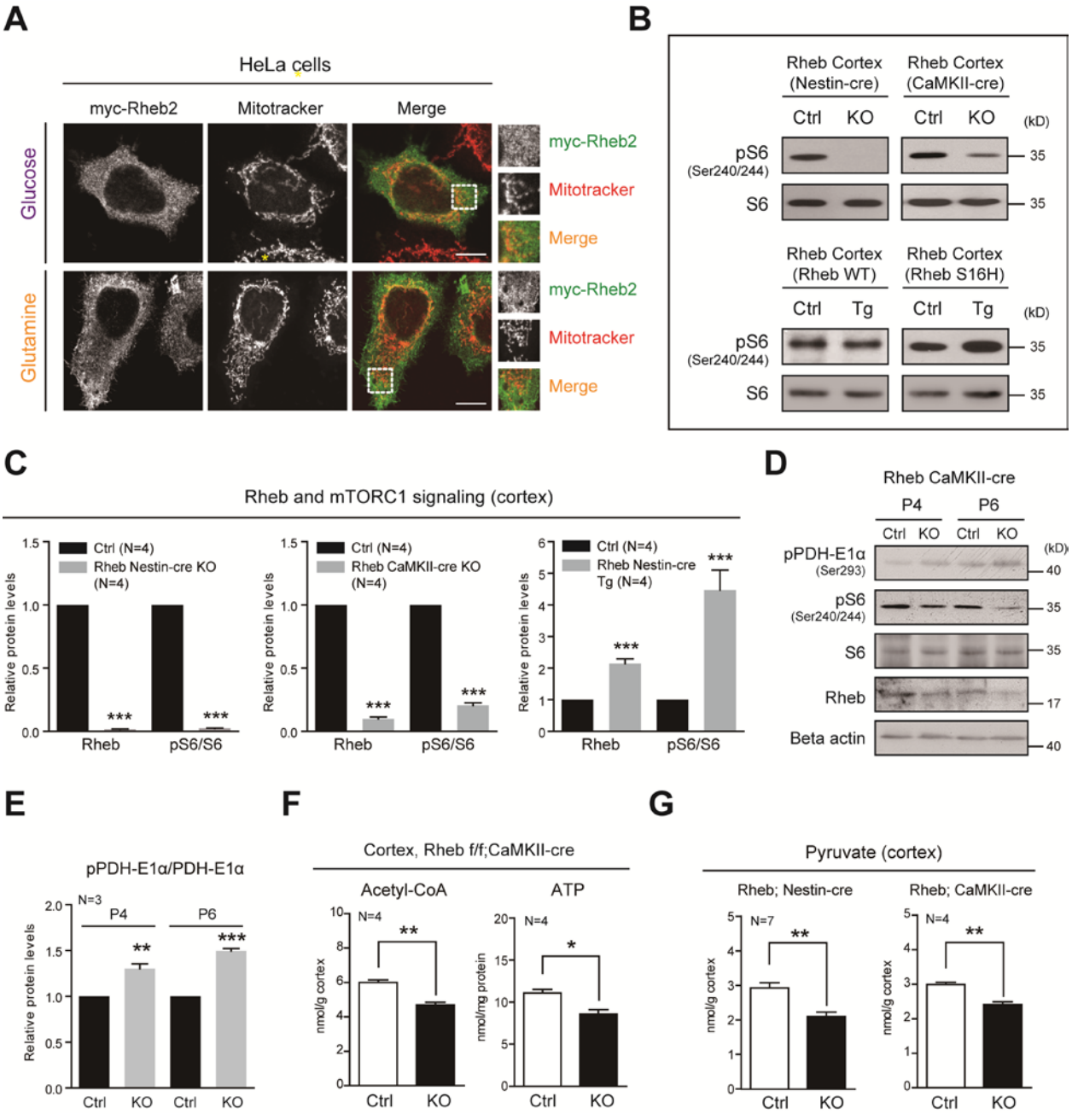
[5]These authors contributed equally to this work.

[6]Lead Contact.

*Correspondence: Bo Xiao (xiaob@sustech.edu.cn);

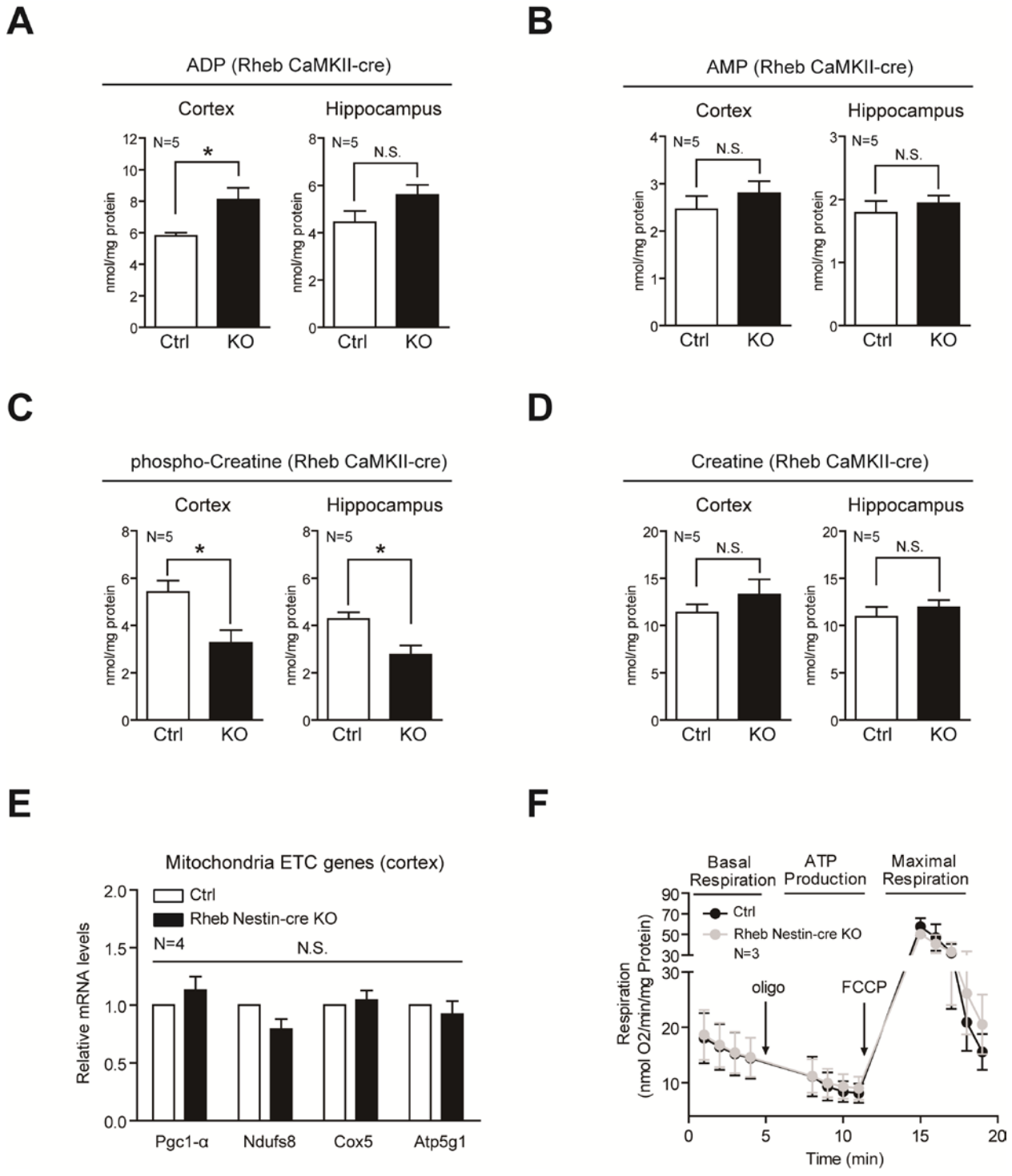
Paul. F. Worley (pworley@jhmi.edu).

Figure S1. Rheb regulates neuronal PDH activity; related to Figure 1 and Figure 2.



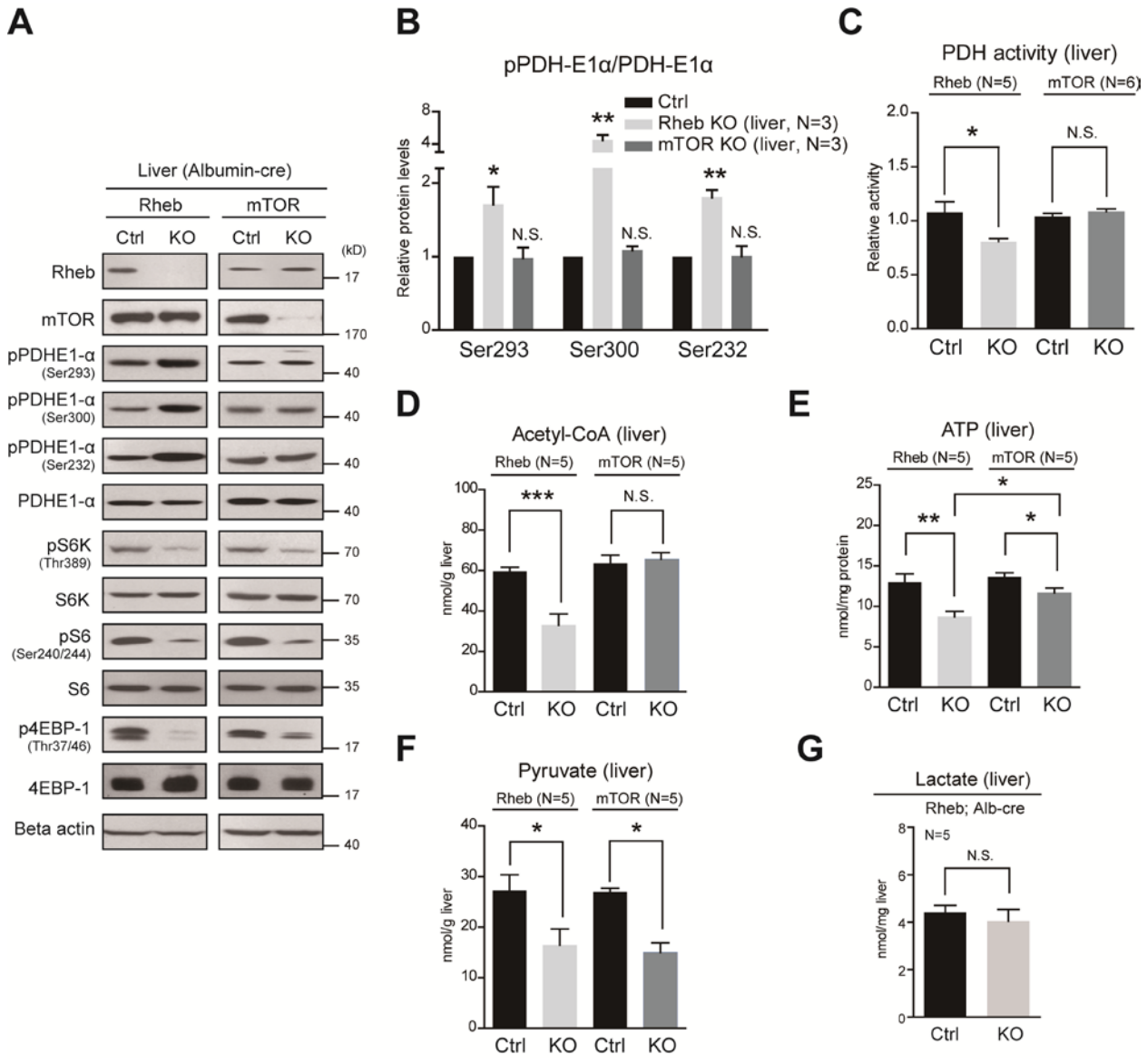
(A) Immunofluorescence showing that myc-Rheb2 (green) was not colocalized with Mitotracker (red) in mitochondria of HeLa cells. **(B-C)** Western blots (**B**) and quantification (**C**) showing the reduction and elevation of pS6 in the cortex of *Rheb* KO and Tg mice, respectively. **(D-E)** Western blots (**D**) and quantifications (**E**) indicating increase of PDH phosphorylation in the cortex of *Rheb* CaMKII-cre KO mice at P4 and P6. N=3 pairs of mice. pPDH-E1α (Ser293), P4, p=0.0046; P6, p<0.001. **(F)** Acetyl-CoA and ATP reductions in the cerebral cortex of *Rheb* CaMKII-cre KO mice. N=4 pairs of mice. Acetyl-CoA, p=0.0011; ATP, p=0.0106. **(G)** Decreased pyruvate levels in the cerebral cortex of *Rheb* Nestin-cre (N=7 pairs of mice, p=0.0015) and CaMKII-cre (N=4 pairs of mice, p=0.0011) KO mice. All data represent mean ± SEM. Statistical analysis was performed by using two-tailed Student's *t* test (C,E,F,G), and *p<0.05, **p<0.01, and ***p<0.001.

Figure S2. Rheb regulates neuronal energy production; related to Figure 2.



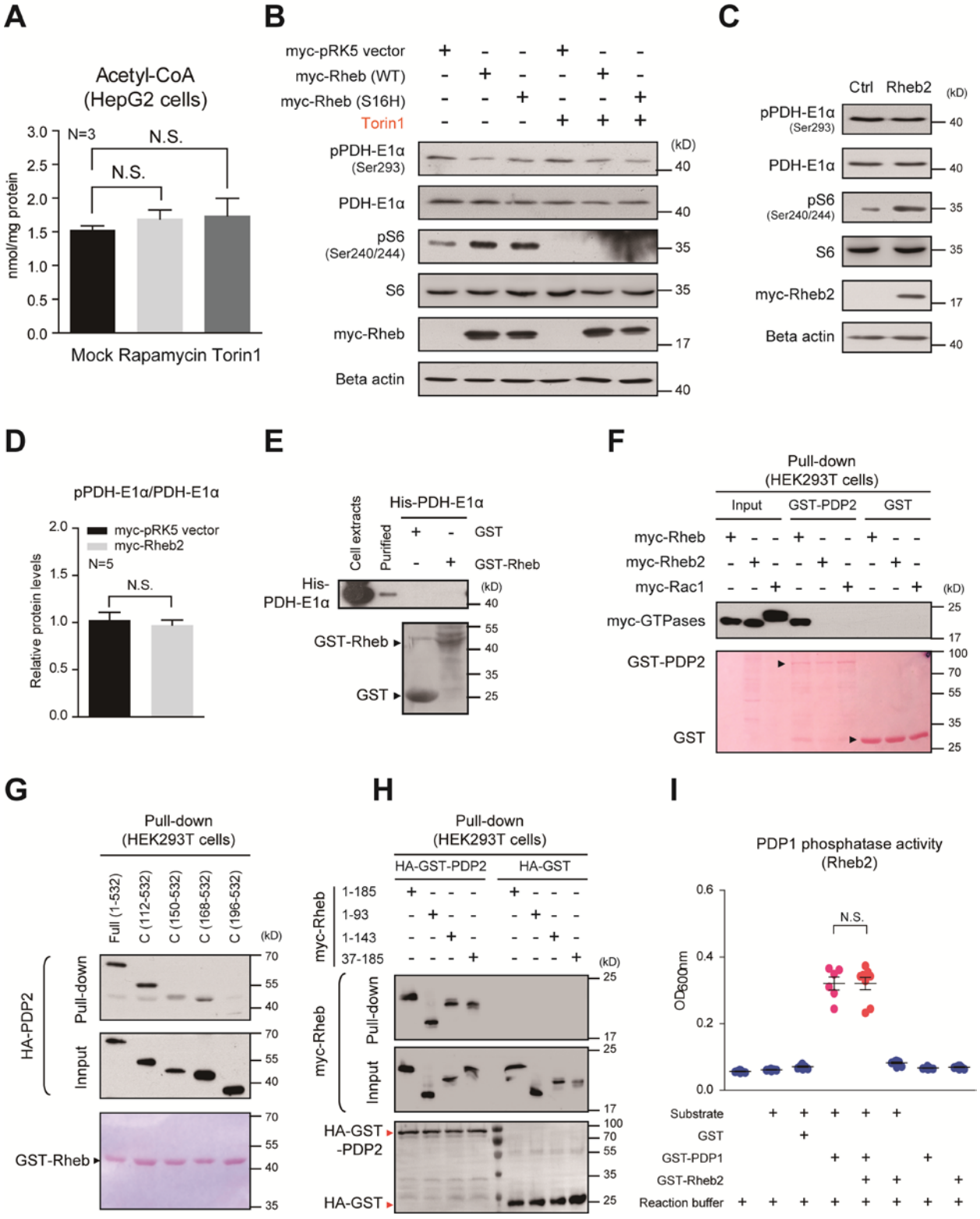
(A-D) Increased ADP levels in the cerebral cortex of *Rheb* CaMKII-cre KO mice, and decreased phospho-creatine levels in the cerebral cortex and hippocampus of *Rheb* CaMKII-cre KO mice. N=4 pairs of mice. **(E)** qRT-PCR results demonstrating that mRNA levels of mitochondria electron transport chain (ETC) genes are not altered in the cortex of *Rheb* Nestin-cre KO mice. N=4 pairs of mice. Pgc1-α, p=0.3473; Ndufs8, p=0.1001; Cox5, p=0.6384; Atp5g1, p=0.5464. **(F)** Seahorse assay demonstrating that basal and maximal respiration are not altered in synaptosomes prepared from the cortex of *Rheb* Nestin-cre KO mice. N=4 pairs of mice. All data represent mean ± SEM. Statistical analysis was performed by using two-tailed Student's *t* test (A,B,C,D,E), and *$p<0.05$.

Figure S3. Deletion of Rheb, not mTOR, decreases hepatic PDH activity and acetyl-CoA production; related to Figure 2.



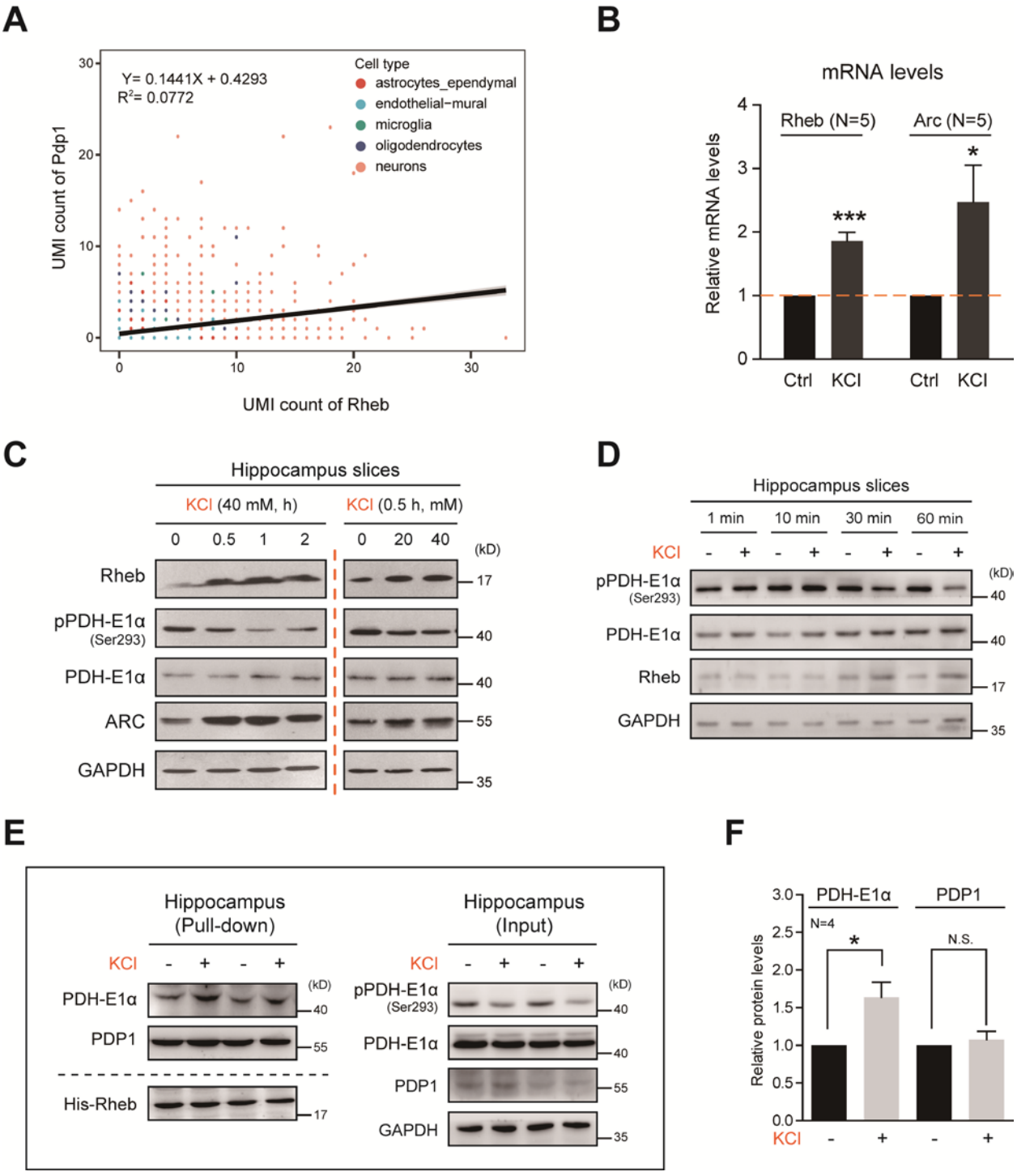
(A-B) Western blots (**A**) and quantification (**B**) demonstrating the effect of deletion of Rheb or mTOR on PDH phosphorylation and mTORC1 signaling in liver of Alb-cre deleter mice (*Rheb*$^{f/f}$;*Alb-cre* or *mTor*$^{f/f}$;*Alb-cre*). Ctrl (*Rheb*$^{f/+}$ *or Rheb*$^{f/f}$, *mTor*$^{f/+}$ or *mTor*$^{f/f}$). **(C)** Decrease of PDH activity in the liver of *Rheb* Alb-cre KO, but not in *mTOR* Alb-cre KO mice. Rheb, N=5, $p<0.05$; mTOR, N=6, N.S. (no statistical significance). **(D)** Decrease of acetyl-CoA level in the liver of *Rheb* Alb-cre KO, but not in *mTOR* Alb-cre KO mice. Rheb, N=5, $p<0.001$; mTOR, N=5, N.S.. **(E)** Decrease of ATP levels in the liver of *Rheb* Alb-cre KO and *mTOR* Alb-cre KO mice. N=5, Rheb, Ctrl vs KO, $p<0.01$; mTOR, Ctrl vs KO, $p<0.05$; Rheb KO vs mTOR KO, $p<0.05$. **(F)** Decrease of pyruvate levels in the liver of *Rheb* Alb-cre KO and *mTOR* Alb-cre KO mice. Rheb, N=5, $p<0.05$; mTOR, N=5, $p<0.05$. **(G)** Lactate level in the liver of *Rheb* Alb-cre KO is not different than control. N=5 pairs of mice. $p=0.5323$. All data represent mean ± SEM. Statistical analysis was performed by using two-tailed Student's *t* test (B,G) or one-way ANOVA with Tukey post hoc test (C,D,E,F), and *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Figure S4. mTORC1 signaling does not alter PDH activity; related to Figures 3-5.



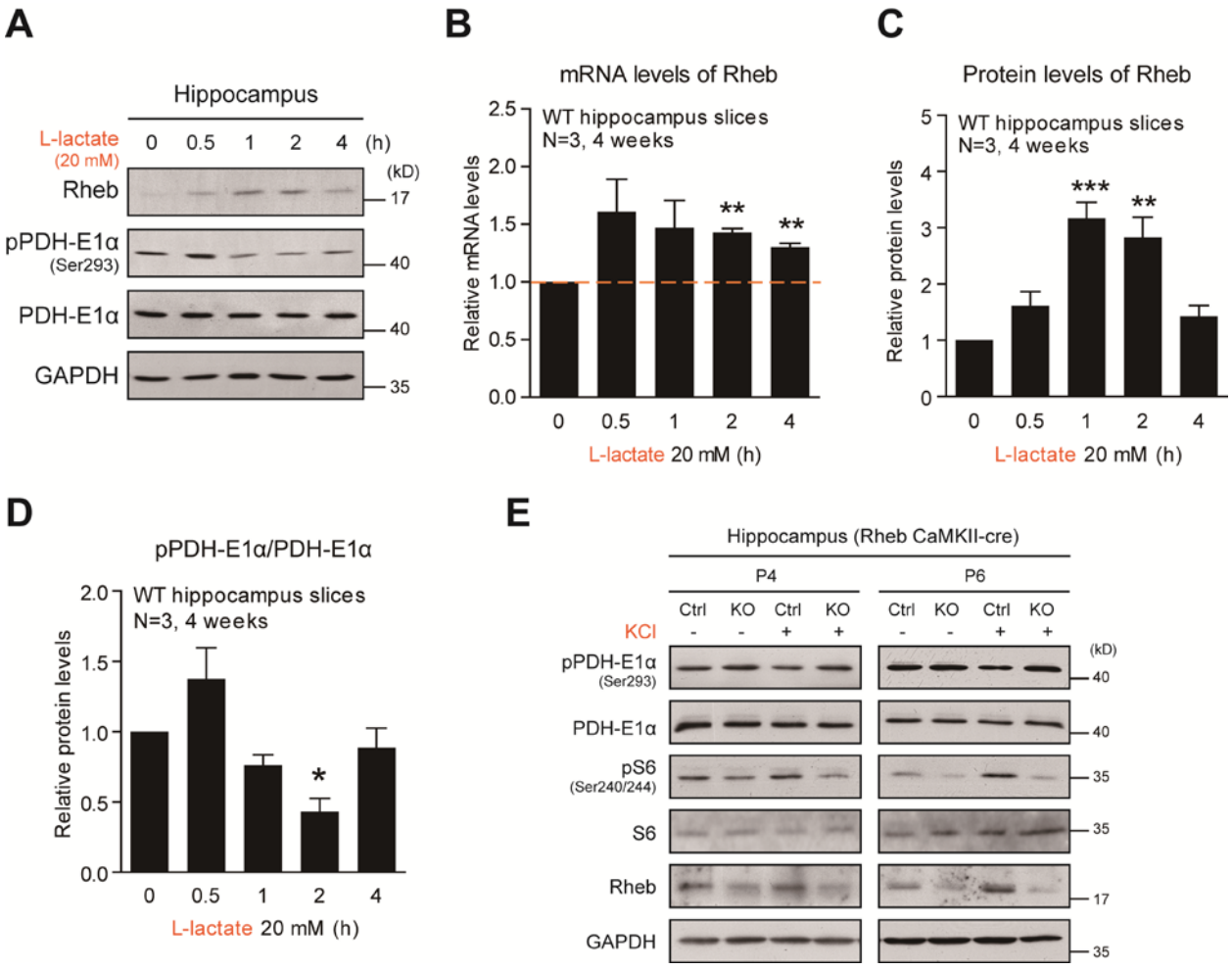
(A) Acetyl-CoA level is not altered by rapamycin (100 nM, 6 hours) or Torin1 (200 nM, 3 hours) treatment in HepG2 cells. N=3 independent experiments. **(B)** Western blots showing that transient overexpression of *Rheb* (WT or S16H) decreases PDH phosphorylation in HepG2 cells, and Torin1 (200 nM, 6 hours) treatment does not prevent the decrease. **(C-D)** Western blots (**C**) and quantification (**D**) showing that overexpression of myc-tagged Rheb2 does not alter PDH-E1α phosphorylation in HepG2 cells. pPDH-E1α (Ser293), N=5 independent experiments, p=0.5888. **(E)** Bacterial GST-Rheb does not pull-down recombinant His-PDH-E1α purified from HEK293T cells. **(F)** GST pull-down demonstrating that purified GST-PDP2 binds myc tagged-Rheb, but not -Rheb2 or -Rac1 transiently expressed in HEK293T cells. **(G)** GST pull-down mapping sequence of PDP2 required for interaction with Rheb. **(H)** GST pull-down to map sequence of Rheb required for interaction with PDP2. **(I)** Incubation of Rheb2 protein with PDP1 does not alter the phosphatase activity of PDP1. N=6 independent experiments. All data represent mean ± SEM. Statistical analysis was performed by using *t* test (D) or one-way ANOVA with Tukey post hoc test (A,I).

Figure S5. Rheb expression and PDH activity are concomitantly induced by neuronal activity; related to Figure 6.



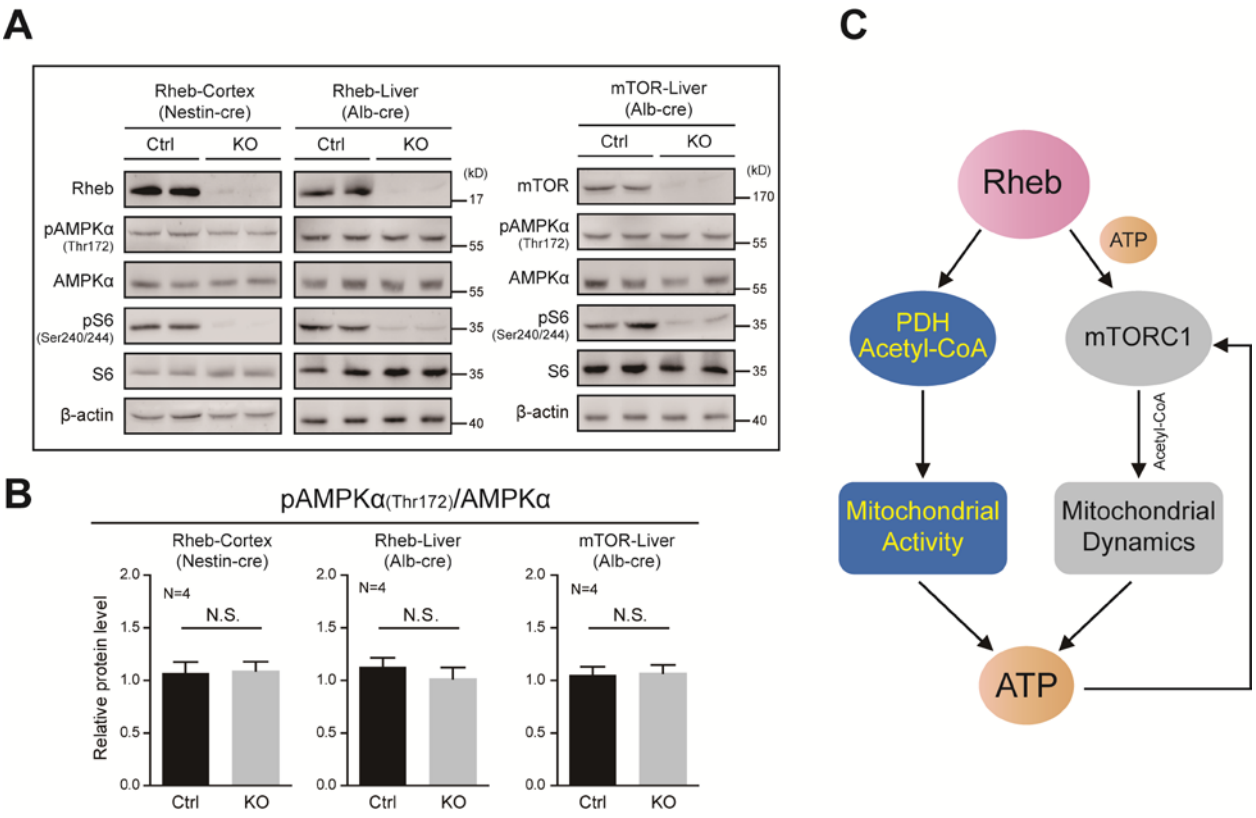
(A) Diagram showing that the expressive corrleation of Rheb and PDP1 was less strong than Rheb-PDH in mouse brain. **(B)** qRT-PCR demonstrating increase of mRNA levels of *Rheb* and *Arc* in acute hippocampal slices (WT mice) following KCl treatment (40 mM, 1 hour). N=5 independent experiments. Rheb, p=0.0002; Arc, p=0.0236. **(C)** Western blots demonstrating time-dependent decrease of PDH-E1α phosphorylation in acute hippocampal slices (WT mice) following KCl treatment. **(D)** Western blotting showing the induction of Rheb protein and PDH dephosphorylation by KCl in hippocampal slices of wildtype mice. **(E-F)** His-Rheb Pull-down (**E**) and quantification (**F**) showing increased PDH amount and comparable amount of PDP1 in the precipitates from hippocampal slices treated with KCl (40 mM, 1 hour) versus controls. Blots were results from a representative experiment in duplicate samples. N=4 independent experiments. All data represent mean ± SEM. Statistical analysis was performed by using Student's *t*-test (B,F), and *p<0.05 and ***p<0.001.

Figure S6. Lactate induces Rheb expression and PDH activation; related to Figure 6 and Figure 7.



(A-D) Western blots (**A**) and quantification (**B,C,D**) demonstrating induction of Rheb expression and pPDH-E1α decrease in acute hippocampal slices (WT mice) by L-lactate treatment (20 mM). N=3 independent experiments. **(E)** Western blots showing absence of change of PDH-E1α phosphorylation following KCl treatment (40 mM, 1 h) in acute hippocampal slices of *Rheb* CaMKII-cre KO mice (P4 and P6). All data represent mean ± SEM. Statistical analysis was performed by using one-way ANOVA with Dunnett (B,C,D) post hoc test, and *p<0.05, **p<0.01 and ***p<0.001.

Figure S7. Rheb/mTORC1 signaling on AMPK *in vivo*, and a model; related to Figure 2.



(A-B) Western blots (**A**) and quantifications (**B**) showing that AMPK phosphorylation was not altered in either *Rheb* or *mTOR* KO tissues. N=4 pairs of mice. Data represent mean ± SEM. Statistical analysis was performed by using Student's *t*-test. **(C)** Illustration showing Rheb coordinating mitochondrial activity and mTORC1-regulated mitochondrial dynamics.