**SUPPLEMENTARY INFORMATION**

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**Suppl. Fig. 1**

**a**

<table>
<thead>
<tr>
<th>CST#3360</th>
<th>liver</th>
<th>heart</th>
<th>sk mu</th>
<th>brain</th>
<th>MEFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST#4357</td>
<td>RagA</td>
<td>RagB</td>
<td>RagC</td>
<td>β-actin</td>
<td></td>
</tr>
</tbody>
</table>

**Alignment of mouse RagA to RagB**

<table>
<thead>
<tr>
<th>RagA</th>
<th>RagB</th>
</tr>
</thead>
<tbody>
<tr>
<td>47aa</td>
<td>80aa</td>
</tr>
<tr>
<td>127aa</td>
<td>160aa</td>
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<tr>
<td>207aa</td>
<td>240aa</td>
</tr>
<tr>
<td>254aa</td>
<td>287aa</td>
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<tr>
<td>313aa</td>
<td>346aa</td>
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<table>
<thead>
<tr>
<th>Mouse RagA to RagB</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

**b**

Knock-in construct

- Knock-in construct
- **GTP**
- **GTP**
- **GTP**
- **GTP**
- **GTP**
- **GTP**

**c**

Effect of serum withdrawal in MEFs

- RagA+/+
- RagA<sup>GTP/GTP</sup>
- re-addition of insulin (min)
- - FBS 6 h
- 10 60 120 180
- 10 60 120 180

**d**

3T3 of primary MEFs

- **/+/**
- **GTP/+**
- **GTP/GTP**

**e**

e13.5 embryos

- /+/**
- GTP/+**
- GTP/GTP**

**f**

<table>
<thead>
<tr>
<th>liver</th>
<th>skin</th>
<th>heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>RagA&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RagA&lt;sup&gt;GTP/GTP&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

**IDENTITY:** 98%

**alignment of mouse RagA to RagB**

**RagA**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>RagA</td>
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<tr>
<td>β-actin</td>
<td>β-actin</td>
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<tr>
<td>RagC</td>
<td>RagC</td>
</tr>
<tr>
<td>RagB</td>
<td>RagB</td>
</tr>
<tr>
<td>T389-S6K1</td>
<td>T389-S6K1</td>
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<tr>
<td>S6K1</td>
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<td>S473-Akt</td>
<td>S473-Akt</td>
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<tr>
<td>Act</td>
<td>Act</td>
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</tbody>
</table>

**Identification:** 98% excluding the first 30

![Image](image-url)
Supplementary Figure 1. (A) (Top) Protein levels of RagA, RagB and RagC in tissues and MEFs. Note that significant RagB levels are found only in brain. (Bottom) Alignment of mouse RagA to RagB proteins. Except for the n-terminus extension in RagB, identity is ~98%. (B) Strategy for RagA knock-in construct and recombination. Southern blot of ES cells was performed using EcoRV as restriction enzyme, which cuts in the transcriptional STOP cassette, and a 5’ probe. (C) MEFs of all genotypes show inhibition of mTORC1 activity upon serum withdrawal. Cells were deprived of FBS for 6 h and FBS was re-added for indicated times points and whole-cell protein extracts were obtained. (D) 3T3 protocol performed in RagA+/+, RagAGTP/+ and RagAGTP/GTP MEFs. (E) Representative pictures of RagA+/+, RagAGTP/+ and RagAGTP/GTP E13.5 embryos. (F) Hematoxylin & eosin staining of liver, skin, heart and brain of RagA+/+, RagAGTP/+ and RagAGTP/GTP neonates. (G) (Top) Lack of inhibition of mTORC1 activity by 1 h fasting in RagAGTP/GTP neonatal skeletal muscle from leg and diaphragm. (Bottom) Akt signaling in 1 h fasted neonatal liver and heart. (H) High mTORC1 activity (p-S6) in tissues from RagAGTP/GTP neonates (versus RagA+/+ and RagAGTP/+ neonates) after 10 h fasting. (I) mRNA expression by qRT-PCR in livers from RagA+/+ (n=2), RagAGTP/+ (n=5) and RagAGTP/GTP (n=4) neonates; data are mean ± SEM. (J) Suppression of mTORC1 activity by rapamycin in liver and heart from RagA+/+, RagAGTP/+ and RagAGTP/GTP neonates.
Supplementary Figure 2. (A) Western blot of genes involved in glycogen metabolism. (B) Reduced levels of plasma glutamine and alanine in and RagA<sup>GTP/GTP</sup> neonates fasted for 10 h (n=4, n=4 and n=3, respectively; data are mean ± SEM). (C) Similar levels of plasma lactate in RagA<sup>GTP/GTP</sup> (n=3) versus RagA<sup>+/+</sup> and RagA<sup>GTP/+</sup> (n=4) neonates fasted 10 h, data are mean ± SD. (D) Proficient gluconeogenesis in neonates by amino acid substrates. Fifteen-percent alanine in PBS was injected after 5.5 h of fasting and 45 min later; glycaemia was measured immediately before the first injection and 75 min later. All neonates, regardless of the genotype, undergo a significant increase in glycaemia, reflecting the ability to execute gluconeogenesis from amino acid substrates (+/+: n=9, G/+: n=26; G/G: n=11; some values overlap).
Supplementary Information

**Suppl. Fig. 3**

**a**

Selected autophagosomes and autophagolysosomes in hepatocytes from 1 h fasted RagA++ neonates

- AP

**b**

Liver 10 h fast, untreated rapamycin

- +/G/+G/+G
- ++/G/+G/++

**C**

Endogenous LC3B

- RagA++
- RagA^GTP/GTP

- +AA

- -AA 1 h
Supplementary Figure 3. (A) Representative electron micrographs of RagA$$^{+/+}$$ livers showing autophagosomes (AP) and autophagolysosomes (AL). Bar indicates 0.5 μm. (B) Effect of rapamycin on mTORC1 activity and autophagy markers in neonatal livers fasted for 10 h. (C) Immunofluorescence (IF) of endogenous LC3B in RagA$$^{+/+}$$ and RagA$$^{GTP/GTP}$$ MEFs starved of amino acids, as done in Fig. 3e for recombinant LC3B. Bar indicates 10 μm. (D) (Top) IF of recombinant TFEB and Lamp2 (as lysosomal marker) in MEFs deprived of amino acids. Bar indicates 10 μm. (Bottom) TFEB transcriptional program is partially impaired in RagA$$^{GTP/GTP}$$ MEFs upon amino acid withdrawal (n=3; data are mean ± SEM; p<0.05). (E) Triggering of autophagy by fetal bovine serum (FBS) withdrawal in MEFs. Cells were deprived of FBS for the indicated time points, and whole-cell protein extracts were obtained and mTORC1 activity (S6K, 4E-BP1 and ULK-1 phosphorylation) and LC3B determined by immunoblotting.
**Supplementary Information**

**a**
- Plasma amino acids
- Relative amino acid concentration
- 0 h, 1 h fast
- BCAA
- T37/46-4E-BP1
- P-T389-S6K1
- S6K1
- P-S79-ACC
- β-actin

**b**
- Genotype: RagA+/+ RagGTP/+ GTP/GTP
- Amino acids: essential, amino acids
- P-T389-S6K1
- S6K1
- P-S79-ACC
- ACC
- β-actin

**c**
- Genotype: AMPK+/+ AMPK<sup>ΔKD</sup>
- HEK-293T-RagBGTP
- HEK-293T
- P-T389-S6K1
- S6K1
- P-S757-ULK1
- 4E-BP1
- P-S79-ACC
- ACC
- P62
- β-actin

**d**
- Cells: HEK-293T
- HEK-293T-RagBGTP
- Addition: amino acids, glucose
- P-T389-S6K1
- S6K1
- P-S79-ACC
- ACC
- β-actin

**e**
- Quantification of S6K phosphorylation from Figure 4b

**f**
- Effect of amino acids esters in MEFs
- Starvation for: amino acids and glucose
- Native amino acids: +/+ G/G
- Amino acids esters: -

**g**
- Intracellular amino acids in MEFs after 1 h glucose starvation versus control
- Relative amino acid concentration (to +glucose)
- +/+ G/G
Supplementary Figure 4. (A) Decrease in plasma amino acids in all 1 h fasted neonates, (+/+: n=4 and 3, G/+: n=5 and 6, G/G: n=4 and 4, at 0 h and 1h, respectively, data are mean ± SEM). (B) Suppression of mTORC1 activity by amino acid deprivation in AMPK-DKO cells. (C) Time course of mTORC1 and AMPK activity in AMPK-wt, AMPK-DKO, RagA+/+ and RagAGTP/GTP MEFs deprived of glucose for 0.5, 2 and 4 h. (D) Control HEK-293T cells or those expressing RagBGTP were deprived of growth factors, glucose, amino acids, or glucose and amino acids for 1 h and re-stimulated with glucose and/or amino acids for 10 min. Whole cell lysates were analyzed by immunoblotting for the indicated proteins. (E) Quantification of the western blots on Figure 4b and Supplementary Fig. 4d. (F) Effect of amino acid esters on mTORC1 activity in wt MEFs. (G) Quantification of amino acids in RagA+/+ and RagAGTP/GTP MEFs deprived of glucose (n=1, relative to un-starved controls). (H) Effect of aminoimidazole carboxamide ribonucleotide (AICAR) on mTORC1 activity in RagA+/+ and RagAGTP/GTP MEFs. (I) (Left) Effect of amino acids or glucose on mTORC1 activity in GCN2-deficient MEFs. (Right) Effect of amino acid deprivation on GCN2 activity in RagA+/+ and RagAGTP/GTP MEFs. (J) mTOR localization by IF in HEK-293T-RagBGTP and control cells upon amino acid deprivation. (K) mTOR localization by IF in RagA+/+ and RagAGTP/GTP MEFs deprived of amino acids or glucose. Bar indicates 10 μm.