

## Supplemental data

### Supplemental figure legends:

**Supplemental Figure 1. Normal glucose tolerance in Glucagon-Cre mice and conserved glucose responses after insulin injection in  $\alpha$ Rheb<sup>Tg</sup> receiving Dox chow.** **A.** Intraperitoneal glucose tolerance test in 4-month-old Glucagon-Cre (n=6) and littermate wild-type (WT) controls (n=13) mice. **B.** Intraperitoneal glucose tolerance test in 1-month-old Rheb and littermate Glucagon-Cre;Rheb<sup>Tg Het</sup> male mice. **C.** Insulin tolerance test in 3-month-old Rheb and littermate Glucagon-Cre;Rheb<sup>Tg Het</sup> male mice. **D.** Insulin tolerance test in Rheb<sup>Tg</sup>+Dox (n=6) and  $\alpha$ Rheb<sup>Tg</sup>+Dox (n=10) male mice at 4 weeks of age. Data are shown as means  $\pm$  S.E.M.

**Supplemental Figure 2:  $\alpha$ Rheb<sup>Tg</sup> mice have decreased glucagon signaling with no changes in insulin secretion or insulin resistance.** **A.** Glucagon challenge test (20  $\mu$ g/kg) and **B.** AUC of glucagon challenge in 3-month-old Control mice (n= 6) and  $\alpha$ Rheb<sup>Tg</sup> (n= 6). **C.** Exogenous glucagon (100  $\mu$ g/kg) was directly injected into the portal vein of fasted control and  $\alpha$ Rheb<sup>Tg</sup> anesthetized mice. Western blot of pCREB (Ser133) in liver lysates collected at baseline (fasted, 0 min) and 5- and 10-min post-glucagon injection. **D.** Insulin secretion in response to glucose (GSIS) and Diazoxide (200  $\mu$ M) (Diaz) and in combination with KCl (30mM) (Diaz+KCl) in isolated islets from Control mice (n=10) and  $\alpha$ Rheb<sup>Tg</sup> (n=10). **E.** Western blot and quantification showing hepatic insulin sensitivity measured by pAKT (Ser473) after intraperitoneal insulin administration (1 U/kg). Data are shown as means  $\pm$  S.E.M. \*p<0.05. (Student's 2-tailed t-test). For A data are shown as means  $\pm$  S.E.M. \*p<0.05 (two-way ANOVA with Sidak's post-test).

**Supplemental Figure 3. Postnatal activation of Rheb 10 days after remove Dox.**

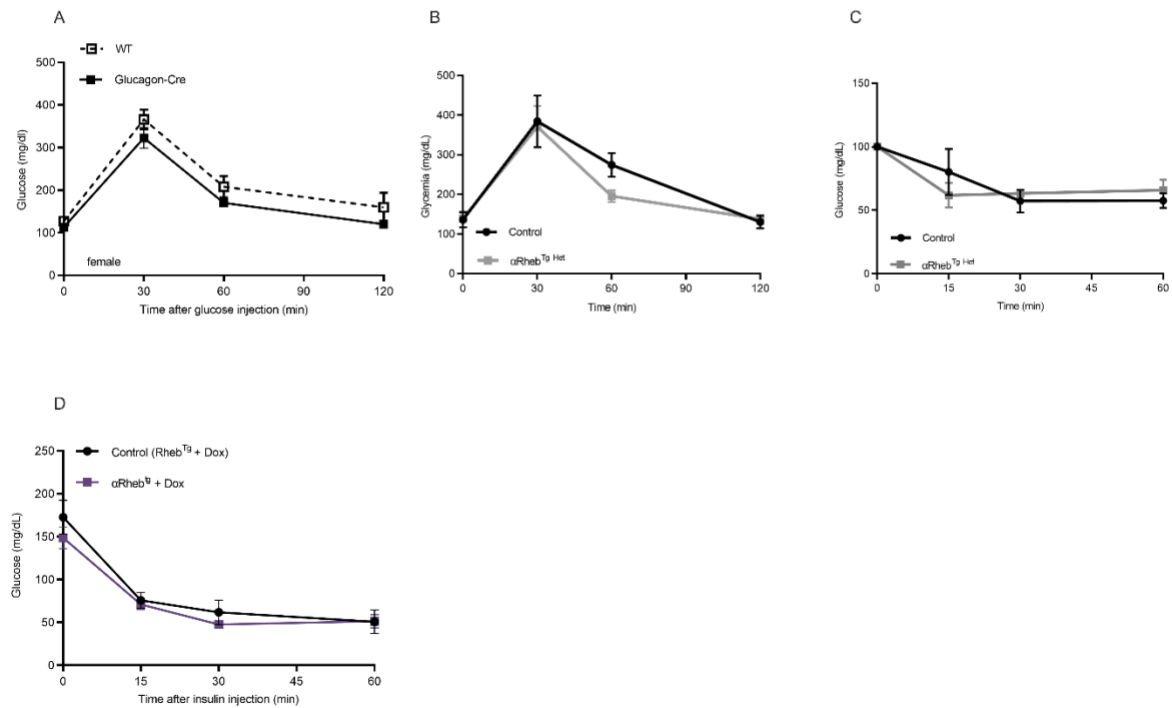
**A.** % of alpha cell number determined by glucagon+ cell measured by flow cytometry in dispersed islets from control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **B.** Glucagon expression determined by the mean fluoresce intensity (MFI) in dispersed islets in glucagon+ cells from control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=6 mice). **C.** Assessment of pS6<sup>Ser240</sup> by mean fluorescence intensity (MFI) measured by flow cytometry in dispersed alpha cells (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **D.**  $\alpha$ -cell size analyzed by flow cytometry using dispersed islets and quantified by forward scatter area (FSC-A) of control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **E.** RNA expression of hepatic key enzymes involved in gluconeogenesis in 6 hours fasted liver from control (n=6) and  $\alpha$ Rheb<sup>Tg</sup> (n=6) 10 days after removing Dox diet. Data are shown as means  $\pm$  S.E.M. \*p<0.05. (Student's 2-tailed *t* test).

**Supplemental Figure 4. No changes in plasma urea and amino acid levels in control and  $\alpha$ Rheb<sup>Tg</sup> mice.** **A.** Body weight, **B.** Fasting (6 hours) urea levels, and **C.** Fasting (6 hours) amino acid levels from controls (n=6),  $\alpha$ Rheb<sup>Tg</sup> (n=6) and  $\alpha$ Rheb<sup>Tg</sup>+Dox (n=4) mice at 4 months of age. Data for A-D are shown as means  $\pm$  S.E.M.

**Supplemental Table 1. Antibodies**

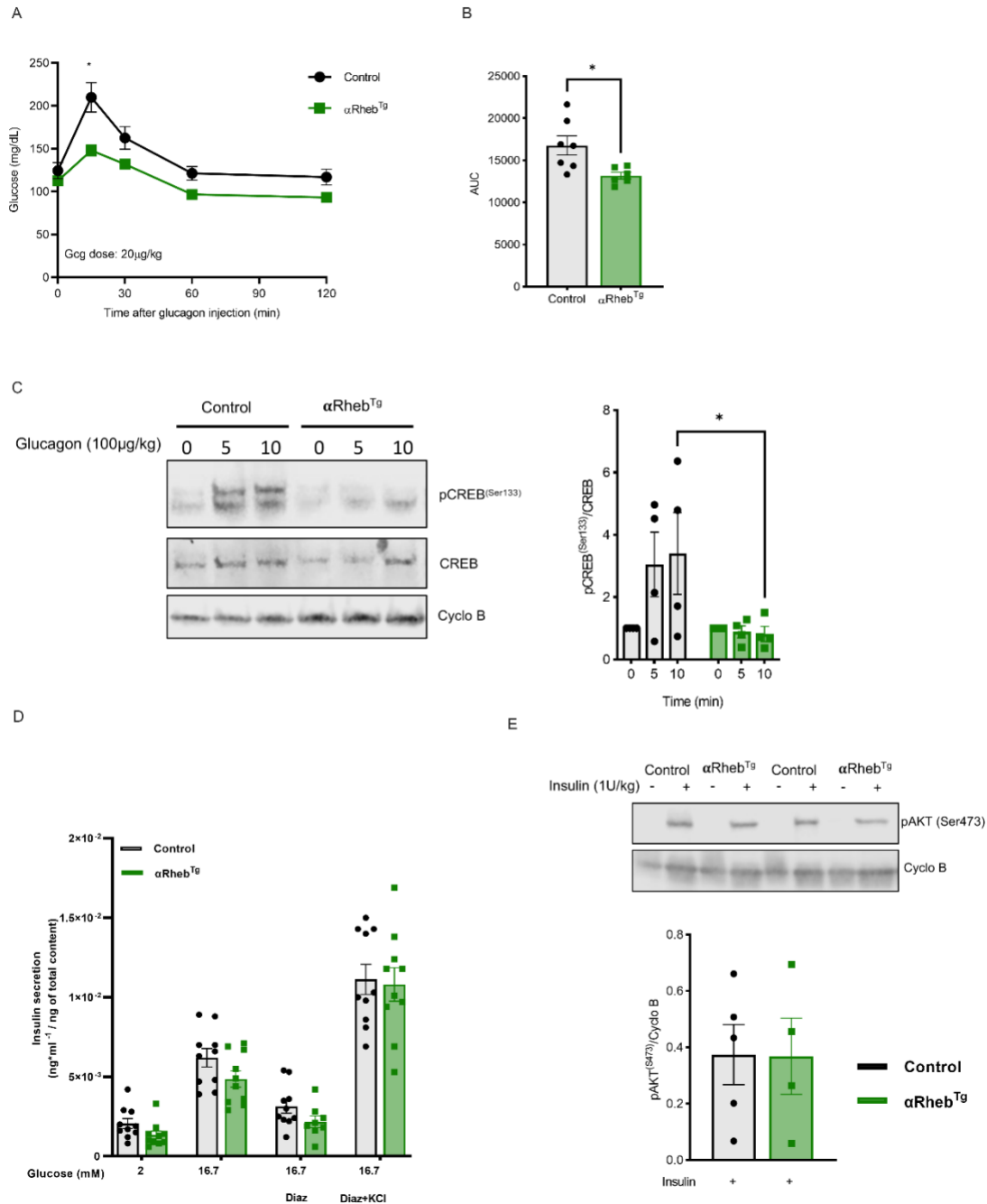
**Supplemental Table 2. Primer Sequences**

Supplemental Figure 1



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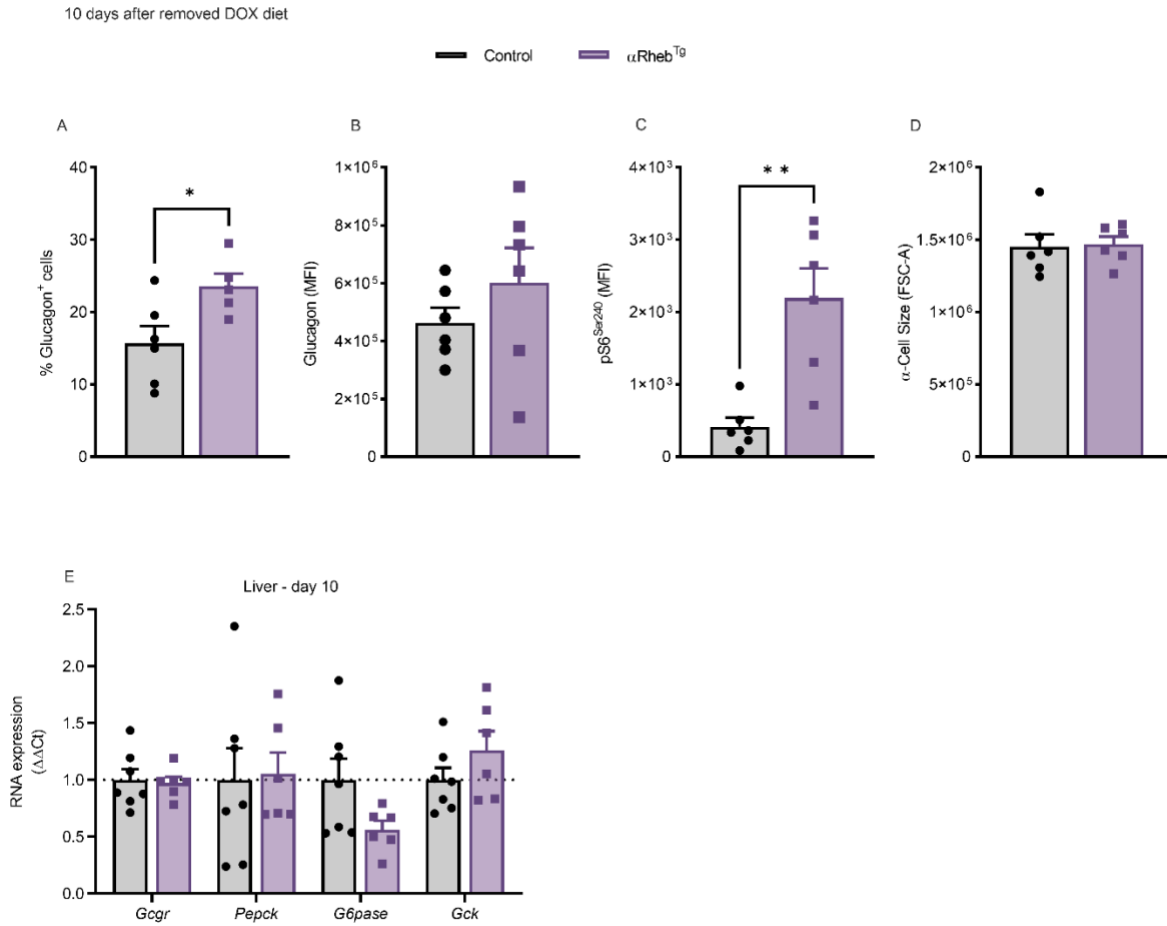
Supplemental Figure 2



**Supplemental Figure 2:  $\alpha$ Rheb<sup>Tg</sup> mice have decreased glucagon signaling with no changes in insulin secretion or insulin resistance. A.** Glucagon challenge test (20  $\mu$ g/kg) and **B.** AUC of glucagon challenge in 3-month-old Control mice (n= 6) and  $\alpha$ Rheb<sup>Tg</sup> (n= 6). **C.** Exogenous

glucagon (100  $\mu\text{g/kg}$ ) was directly injected into the portal vein of fasted control and  $\alpha\text{Rheb}^{\text{Tg}}$  anesthetized mice. Western blot of pCREB (Ser133) in liver lysates collected at baseline (fasted, 0 min) and 5- and 10-min post-glucagon injection. **D.** Insulin secretion in response to glucose (GSIS) and Diazoxide (200  $\mu\text{M}$ ) (Diaz) and in combination with KCl (30mM) (Diaz+KCl) in isolated islets from Control mice (n=10) and  $\alpha\text{Rheb}^{\text{Tg}}$  (n=10). **E.** Western blot and quantification showing hepatic insulin sensitivity measured by pAKT (Ser473) after intraperitoneal insulin administration (1 U/kg). Data are shown as means  $\pm$  S.E.M. \* $p < 0.05$ . (Student's 2-tailed  $t$  test). For A data are shown as means  $\pm$  S.E.M. \* $p < 0.05$  (two-way ANOVA with Sidak's post test).

Supplemental Figure 3

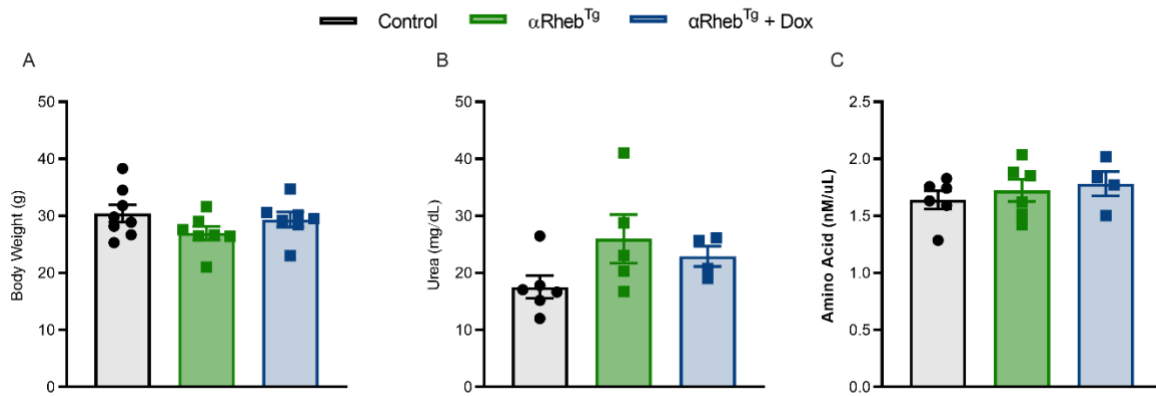


### Supplemental Figure 3. Postnatal activation of Rheb 10 days after remove Dox.

**A.** % of alpha cell number determined by glucagon<sup>+</sup> cell measured by flow cytometry in dispersed islets from control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **B.** Glucagon expression determined by the mean fluoresce intensity (MFI) in dispersed islets in glucagon<sup>+</sup> cells from control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=6 mice). **C.** Assessment of pS6<sup>Ser240</sup> by mean fluorescence intensity (MFI) measured by flow cytometry in dispersed alpha cells (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **D.**  $\alpha$ -cell size analyzed by flow cytometry using dispersed islets and quantified by forward scatter area (FSC-A) of control (n=6 mice) and  $\alpha$ Rheb<sup>Tg</sup> (n=5 mice). **E.** RNA expression of hepatic key

enzymes involved in gluconeogenesis in 6 hours fasted liver from control (n=6) and  $\alpha$ Rheb<sup>Tg</sup> (n=6) 10 days after removing Dox diet. Data are shown as means  $\pm$  S.E.M. \*p<0.05. (Student's 2-tailed *t* test).

Supplemental Figure 4



**Supplemental Figure 4. No changes in plasma urea and amino acid levels in control and  $\alpha$ Rheb<sup>Tg</sup> mice.** **A.** Body weight, **B.** Fasting (6 hours) urea levels and **C.** Fasting (6 hours) amino acid levels from controls (n=6),  $\alpha$ Rheb<sup>Tg</sup> (n=6) and  $\alpha$ Rheb<sup>Tg</sup>+Dox (n=4) mice at 4 month of age. Data for A-D are shown as means  $\pm$  S.E.M.

**Supplemental Table 1. Antibodies**

| <b>Antibody</b>    | <b>Specie</b>      | <b>Catalog number</b> | <b>Source</b>  | <b>Application</b> |
|--------------------|--------------------|-----------------------|----------------|--------------------|
| Creb               | Mouse              | 9104S                 | Cell signaling | WB                 |
| Cyclo B            | Rabbit             | PA1027A               | Thermo Fisher  | WB                 |
| Glucagon           | Mouse              | Ab10988               | Abcam          | IFC                |
| Glucagon           | Rabbit             | Ab932                 | EMD Millipore  | IFC                |
| Glucagon-BV421     | Human/Mouse        | 565891                | BD Biosciences | FC                 |
| Insulin            | Guinea Pig         | ir002                 | Dako           | IFC                |
| Insulin-APC        | Human/Mouse/Bovine | IC1417A               | R&D Systems    | FC                 |
| pAkt (Ser473)      | Rabbit             | 4060S                 | Cell signaling | WB                 |
| pCreb (Ser133)     | Rabbit             | 9198S                 | Cell signaling | WB                 |
| pS6 (S240) – PE600 | Mouse              | 560430                | BD Biosciences | FC                 |
| Ghost Dye Red 780  |                    | 13-0856-T500          | Tonbo          | FC                 |

IFC = Immunofluorescent Chemistry

FC=Flow Cytometry

**Supplemental Table 2. Primer Sequences**

| <b>Gene</b>   | <b>Forward</b>          | <b>Reverse</b>           |
|---------------|-------------------------|--------------------------|
| <i>Cps1</i>   | CATGGAACATCCAGCCGAATTGG | CATGGAACATCCAGCCGAATTGG  |
| <i>Gcgr</i>   | ACGGTACAGCCAGAAGAT      | CTACCAGCAACCAGCAATAG     |
| <i>Pepck</i>  | ATCATCTTTGGTGGCCGTAG    | ATCTTGCCCTTGTGTTCT       |
| <i>G6pase</i> | CCGGTGTTTGAACGTCATCT    | CAATGCCTGACAAGACTCCA     |
| <i>Gck</i>    | CTGTTAGCAGGATGGCAGCTT   | TTTCCTGGAGAGATGCTGTGG    |
| <i>Fas</i>    | AGAAGCCATGTGGGGAAGATT   | AGCAGGGACAGGACAAGACAA    |
| <i>Sds</i>    | CACTGGCCTCGCTGGTTGTCATT | GTGGCCAGGGCAGCAGCAGAT    |
| <i>Gpt1</i>   | CTTTGAAAGCAGTGCAGCGT    | AAATCAGGCCTACACCCAGC     |
| <i>Pc</i>     | ATCCAGCGGCGGCACCAGA     | GCGGGAATTGACCTCGATGAAGTA |
| <i>Got1</i>   | TCTGACCGTGGTCGGAAAAG    | TTTGGTGGCGTGAACTACGA     |
| <i>Crtc2</i>  | GGTGGTTCTCTGCCCAATGT    | AGTCAGAGCTTGTCTTCGC      |
| <i>Arg1</i>   | TATGACGTGAGAGACCACGG    | CTTCCAAGTCCAGACTGTG      |
| <i>Oat</i>    | TGGCGGTTTATACCCTGTGT    | AAACCTCAAGAGCCGCAATG     |
| <i>Ass1</i>   | GAGTGGGTTCGCATTGACATC   | GGGTCTCGTAGATACCTCGG     |
| <i>Nnmt</i>   | ATATTCTGCCTGGGTGCTGT    | AGGCTCCTGGTTCCTTCTTC     |
| <i>Fgf21</i>  | AGATCAGGGAGGATGGAACA    | TCAAAGTGAGGCGATCCATA     |
| <i>18S</i>    | GCAATTATCCCCATGAACG     | GGGACTTAATCAACGCAAGC     |