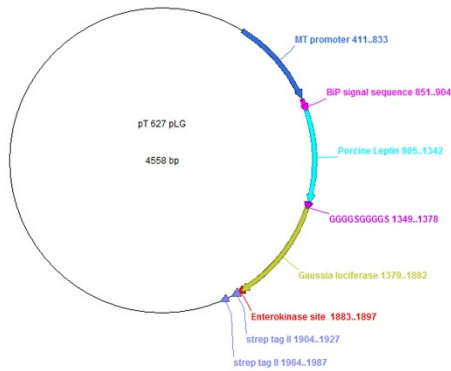
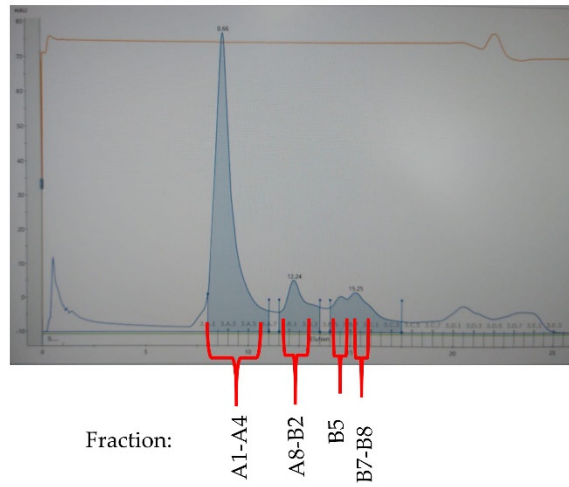


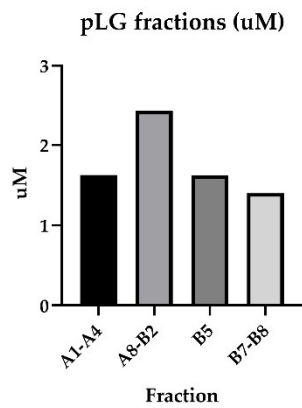
A



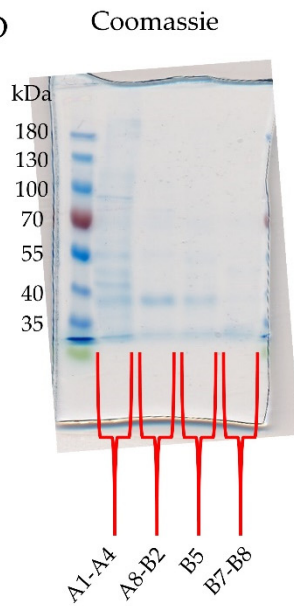
B



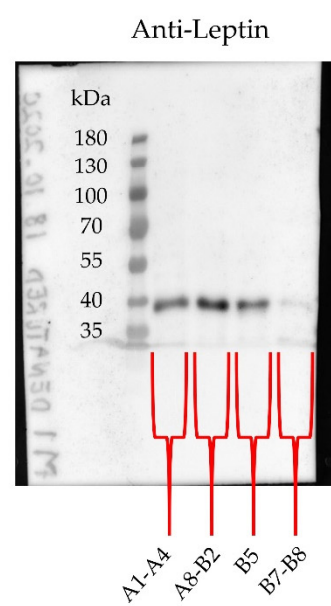
C



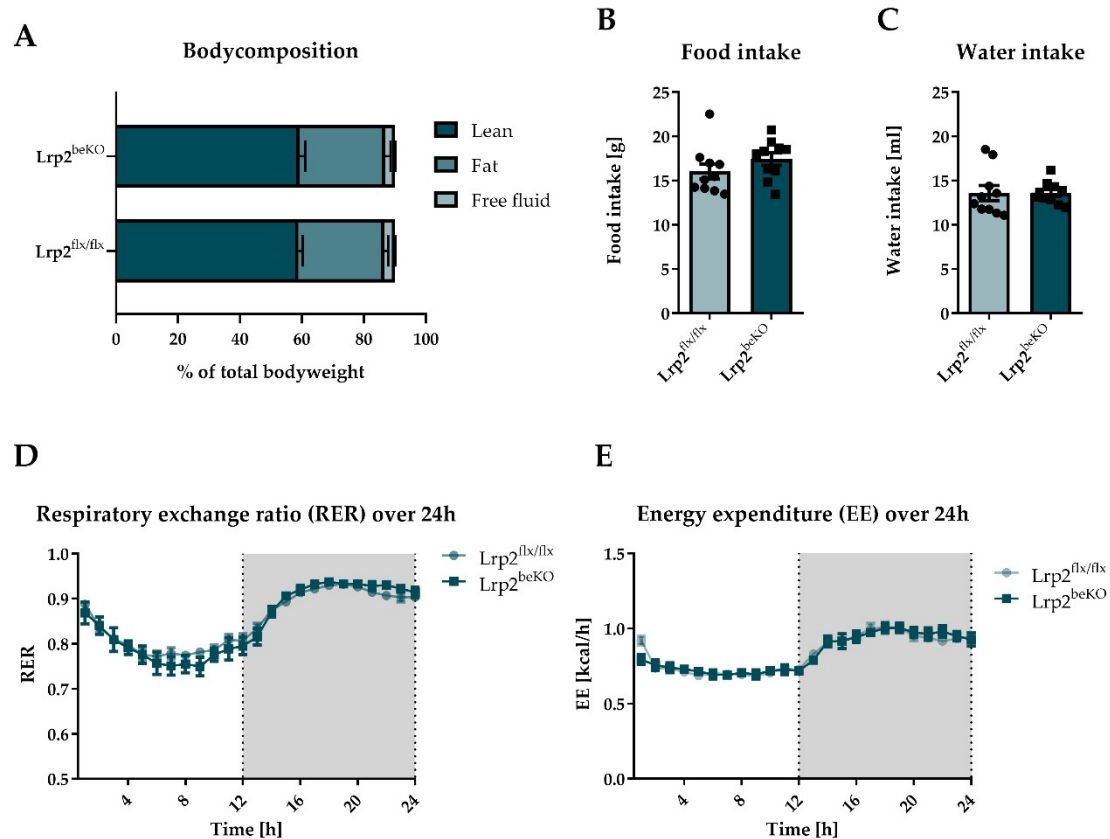
D



E



**Figure S1. Purification of the pLG fusion protein from pT627 pLG.** pLG was purified from the pT627 insect expression plasmid (A). The fractions that resulted (B) all illustrated luciferase activity between 1-2.5uM (C) when plotted against a standard curve of recombinant gaussia luciferase. However, the purity of fraction A1-A4 was compromised as illustrated in the Coomassie stain (D), and the quantity was low in fraction B7-B8 as was observed both in the coomassie (D) and anti-leptin stain (E). For these reasons, fraction A8-B2 and B5 were the fractions that were tested on LepRb overexpressing CHO cells.



**Figure S2. Indirect calorimetry and body composition of Lrp2<sup>beKO</sup> compared to Lrp2<sup>flx/flx</sup> mice.** No difference was observed in any body composition parameters (A) n=10. Correspondingly, food (B) and water (C) intake were similar in Lrp2<sup>beKO</sup> and Lrp2<sup>flx/flx</sup> mice (both n=10). Respiratory exchange ratio (RER, D) and energy expenditure (EE, E) also did not differ between groups, both n=10. Food intake and water intake represent the sum of the last 3 days in calorimetry. RER and EE are the average of each corresponding hour for each corresponding mouse over the last 3 days in calorimetry. Results are presented as mean  $\pm$  SEM \*p<.05 \*\*p<.01 \*\*\*p<.001.

## Supplementary methods.

### 1. Expression and purification of pLGH and pLG (Accompanying Figure S1)

CHO cells were cultured in DMEM F12 (PAN-Biotech GmbH, Aidenbach, Germany) with 5 % fetal calf serum (FCS, Sigma-Aldrich, St. Louis, USA) and 1 % penicillin-streptomycin (pen/strep, Sigma-Aldrich). The pLGH mammalian expression plasmid was transfected into CHO grown in 10-cm diameter cell culture plates using calcium phosphate precipitation. Briefly, 400 ng/cm<sup>2</sup> pLGH plasmid in 50 µl sterile water was added to 62.5 µl 2 M CaCl<sub>2</sub> and 387.5 µl sterile water. 2xBBS (0.5 ml, 50 mM BES, 280 mM NaCl, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.95) was added to the tube. After 3 min the mixture was added to the media and cells were incubated for 24 h at 37°C with 3 % CO<sub>2</sub>. The media was then changed, and CO<sub>2</sub> was turned up to 5 %. The cells were incubated another 24 h and then detached with trypsin 0.05 %/EDTA 0.02 % in PBS (PAN-Biotech GmbH) for 2-3 min and seeded into a T75 flask. At this point, the cells were incubated with puromycin (8 µg/ml, Sigma-Aldrich) to stabilize the transfection. The cells were incubated with puromycin, changing the media every 2-3 days. After two weeks the cells were trypsinized as above and seeded into 15x T175 flasks with DMEMF12 containing 2.5 % FCS. Since the pLGH was secreted into the media, the cells were incubated for 48 h and the media collected for purification. The media was centrifuged at 1000 x g to pellet any debris. The Hispur Ni-NTA 3 ml spin column kit (Thermo Fisher Scientific, Waltham, USA) was then used with some modification for media extraction. An equal volume of equilibration buffer was added to the media together with 3 ml Ni-NTA beads from the kit, prepared as per kit instructions. The media/buffer was incubated at 10 °C shaking at 150 rpm over-night. The beads were pelleted at 700 x g 4 °C and the kit protocol was resumed.

For production in *Drosophila* Schneider 2 (S2) cells stable transfectants were generated as previously described [55]. Briefly, the pLG expression plasmid was co-transfected with a dominant selectable marker encoding puromycin acetyltransferase using Effectene (Qiagen) according to the manufacturer's recommendation. For protein production an approximate culture volume of 2,4 l was induced with a final concentration of 4 µM CdCl<sub>2</sub> at a density of 8 × 10<sup>6</sup> cells per ml. Six days after induction the supernatant was concentrated to a volume of 300 ml and applied to a 8 ml Strep-Tactin XT column according to the manufacturer's recommendation. Subsequently, the protein was further purified by size exclusion chromatography using a Superdex 200 Increase 10/300 column pre-equilibrated with PBS.

### 2. Western blot

The western blots were performed using 10 % polyacrylamide gels. All samples were blotted on two membranes, one membrane was then used for staining phosphorylated protein and the other for non-phosphorylated protein. The membranes were stained with Ponceau S (Sigma-Aldrich), imaged, and then washed 3x 10min in TBS-T. The membranes were then blocked in blocking buffer (5 % bovine serum albumin (Sigma-Aldrich) in TBS-T) for 90 min. The primary antibodies (Table S1) were incubated cold in blocking buffer over-night on a roller. The membranes were then washed 3x 10 min in TBS-T and the secondary antibody (Table S1) was incubated for 90min diluted in blocking buffer. The membranes were washed 3x 10 min and developed using Enhanced chemiluminescence SuperSignal™ (Thermo Fisher Scientific) according to the kit instructions. The imaging was done on a Fusion Solo S (Vilber Lourmat Sté, Collégien, France).

**Table S1.** Primary and secondary antibodies used for western blot. Dilutions are given for antibodies that are delivered in constituted form, and pg/ml for lyophilized antibodies

Target protein	Vendor	Catalog Nr	Raised in	Dilution
pERK 1/2	CellSignalling <sup>1</sup>	9101	Rabbit	1:7500
ERK 1/2	CellSignalling <sup>1</sup>	4695	Rabbit	1:10000
pSTAT 3	CellSignalling <sup>1</sup>	9145	Rabbit	1:1000
STAT 3	CellSignalling <sup>1</sup>	9139	Mouse	1:1000
Leptin	Peprotech <sup>2</sup>	500-P68	Rabbit	50pg/ml
Rabbit Ig	Dako <sup>3</sup>	P0448	Goat	1:2000
Mouse Ig	Dako <sup>3</sup>	P0161	Rabbit	1:2000

<sup>1</sup> Cell Signaling Technology, Inc., Danvers, USA <sup>2</sup>PeprTech, New Jersey, USA <sup>3</sup> DakoCytomation, Denmark