EPRS is a critical mTORC1–S6K1 effector that influences adiposity in mice

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Metabolic pathways that contribute to adiposity and ageing are activated by the mammalian target of rapamycin complex 1 (mTORC1) and p70 ribosomal protein S6 kinase 1 (S6K1) axis2,3. However, known mTORC1–S6K1 targets do not account for observed loss-of-function phenotypes, suggesting that there are additional downstream effectors of this pathway4–6. Here we identify glutamyl-poly-tRNA synthetase (EPRS) as an mTORC1–S6K1 target that contributes to adiposity and ageing. Phosphorylation of EPRS at Ser999 by mTORC1–S6K1 induces its release from the aminoacyl tRNA multisynthetase complex, which is required for execution of noncanonical functions of EPRS beyond protein synthesis7,8. To investigate the physiological function of EPRS phosphorylation, we generated Eprs−/− mice bearing phospho-deficient Ser999-to-Ala (S999A) and phospho-mimetic S999D mutations. Homozygous S999A mice exhibited low body weight, reduced adipose tissue mass, and increased lifespan, similar to S6K1-deficient mice9–11 and mice with adipocyte-specific deficiency of raptor, an mTORC1 constituent12. Substitution of the EprsS999A allele in S6K1-deficient mice normalized body mass and adiposity, indicating that EPRS phosphorylation mediates S6K1-dependent metabolic responses. In adipocytes, insulin stimulated S6K1-dependent EPRS phosphorylation and release from the multisynthetase complex. Interaction screening revealed that phospho-EPRS binds SLC27A1 (that is, fatty acid transport protein 1, FATP1)13–15, inducing its translocation to the plasma membrane and long-chain fatty acid uptake. Thus, EPRS and FATP1 are terminal mTORC1–S6K1 axis effectors that are critical for metabolic phenotypes.

Phosphorylation of aminoacyl-tRNA synthetases is emerging as a prevalent regulator of noncanonical functions beyond aminoacylation7,16. Vertebrate EPRS contains two distinct aminoacylation domains, connected by a non-catalytic linker, and first enters the tRNA multisynthetase complex, which is required for execution of noncanonical functions of EPRS (Fig. 1f, Extended Data Fig. 1d). Rapamycin, an mTORC1 inhibitor18, inhibited S6K1 phosphorylation at the classical Thr389 activation site, and suppressed EPRS Ser999 phosphorylation (Fig. 1g), as did short interfering RNA (siRNA) targeting the mTORC1 constituent, raptor, but not the mTORC2 constituent, rictor18 (Extended Data Fig. 1e). To verify the mTORC1 requirement, S6K1 was suppressed with siRNA targeting the 3′ UTR, and Myc-tagged S6K1 bearing a phospho-defective mutation at Thr389 was introduced (Extended Data Fig. 1f, g). The phospho-defective S6K1(T389A) mutant, but not the phospho-mimetic T389E mutant, was unable to bind and phosphorylate EPRS (Extended Data Fig. 1g, h). These results establish the requirement for mTORC1-activated S6K1 to phosphorylate EPRS at Ser999 (Fig. 1a).

To investigate the physiological role of EPRS phosphorylation, homozgyous phospho-defective (Ser999A-to-Ala; denoted as EprsA/A) and phospho-mimetic (Ser999D-to-Asp; EprsD/D) knock-in mice in C57BL/6 background were generated by homologous recombination and verified (Extended Data Fig. 2a, b). Progeny of heterozygous crosses of both models exhibited near-Mendelian genotype distribution, indicating no apparent developmental defects (Extended Data Fig. 2c). Importantly, adult male and female EprsA/A, but not EprsD/D, mice exhibited about 15–20% lower weight than wild-type EprsA/A mice (Fig. 2a, Extended Data Fig. 2d). The weight difference was not observed in embryonic or early developmental stages, but began at 8–10 weeks (Fig. 2a, Extended Data Fig. 2e, f). Marked lifespan increase was observed for both genders of EprsA/A mice; the combined median increase was about 118 days or 15% (Fig. 2b and Extended Data Fig. 3a). Cox regression analysis revealed genotype, not gender, date of birth, or parental ID, as the most dominant predictor of longevity with a hazard ratio substantially greater than one (Extended Data Fig. 3b). No change in lifespan was observed in EprsD/D mice (Extended Data Fig. 3c–e).

The body length of EprsA/A mice was same as wild-type, however, the opened abdominal cavity revealed reduced adipose tissue in EprsA/A mice (Extended Data Fig. 4a, b). The mass of major white adipose tissue (WAT) depots and interscapular brown adipose tissue (IBAT) in EprsA/A was about half that of in EprsA/A mice, but other major organs were unchanged (Fig. 2c); no mass differences were observed in EprsD/D mice (Extended Data Fig. 4c). Reduced adipocyte size and unaltered...
number was observed in Eprs<sup>A/A</sup> mice (Fig. 2d, Extended Data Fig. 4d, e). Lipolysis and fatty acid β-oxidation were greater in Eprs<sup>A/A</sup> (Extended Data Fig. 4f, g). Serum glucose, triglycerides, and free fatty acid levels were unaltered in Eprs<sup>A/A</sup> mice, but insulin level was reduced (Extended Data Fig. 4h). High-fat diet (~60% total calories from fat) feeding induced EPRS phosphorylation in wild-type adipose tissue, and

interscapular brown adipose tissue (IBAT), from 20-week-old male mice; excised calf muscle comprised gastrocnemius, soleus and plantaris from 20-week-old male mice; and double-knockout mice. c, IFNγ-induced S6K-L-EPRS interaction determined by co-immunoprecipitation. d, Recombinant full-length S6K1 directly phosphorylates Ser999 determined by [γ-<sup>32</sup>P]ATP labelling and by phospho-specific antibodies. g, Rapamycin (10 nM) blocks S6K1 activation and Ser999 phosphorylation in U937 cells.

**Figure 2 | Reduced adiposity and extended lifespan of Eprs<sup>A/A</sup> mice.** a, Growth curves of Eprs<sup>S/S</sup> (males, mean ± s.e.m., n = 14 per group; *P < 0.0001, two-way ANOVA) and Eprs<sup>D/D</sup> (n = 10 per group) mice. b, Kaplan–Meier survival curves for male (n = 52 per group) and female (n = 54 per group) mice; MC χ<sup>2</sup> = 7.92, P = 0.005; GBW χ<sup>2</sup> = 8.91, P = 0.003), and gender-combined (n = 106 per group; MC χ<sup>2</sup> = 15.28, P < 0.0001; GBW χ<sup>2</sup> = 14.96, P = 0.0001) Eprs<sup>S/S</sup> and Eprs<sup>A/A</sup> mice. c, Weights of white adipose tissues (WAT) (epididymal, EWAT; inguinal, IWAT; perirenal, PRAT; inguinal, RWAT) and interscapular brown adipose tissue (IBAT), from 20-week-old male mice; excised calf muscle comprised gastrocnemius, soleus and plantaris from 20-week-old male mice; and double-knockout mice. c, IFNγ-induced S6K-L-EPRS interaction determined by co-immunoprecipitation. d, Recombinant full-length S6K1 directly phosphorylates Ser999 determined by [γ-<sup>32</sup>P]ATP labelling and by phospho-specific antibodies. g, Rapamycin (10 nM) blocks S6K1 activation and Ser999 phosphorylation in U937 cells.
body weight gain was substantially reduced in Eprs<sup>A/A</sup> mice (Extended Data Fig. 4i, j). These characteristics of Eprs<sup>A/A</sup> mice approximately phenocopy S6K1-null and adipocyte-specific, raptor-deficient mice, suggesting EPRS as mTORC1–S6K1 effector contributing to obesity-related phenotypes<sup>9,10,12</sup>. To test this relationship, we bred phosphomimetic Eprs<sup>D/D</sup> alleles into S6K1<sup>−/−</sup> mice generated on a C57BL/6 background by targeted disruption<sup>9</sup>. Like the previously characterized S6K1<sup>−/−</sup> line<sup>20,21</sup>, the mice were small; however, obesity-related phenotypes were not investigated. Gene deletion was verified by genotyping and protein absence in adipocytes (Extended Data Fig. 5a, b). As before, this S6K1<sup>−/−</sup> line exhibited reduced body weight in embryos and early developmental stages<sup>9</sup> (Extended Data Fig. 5c). Lifespan extension of about 170 days or 22% was observed in combined genders of S6K1<sup>−/−</sup> mice (<sup>16,22</sup>). Older (about 600 days old) S6K1<sup>A/A</sup> mice exhibited slower glucose clearance following bolus injection of glucose, but faster clearance following insulin injection (Extended Data Fig. 6a, b). This paradoxical result is similar to observations in S6K1<sup>−/−</sup> mice<sup>10,22</sup>. Incomplete restoration might indicate contributions of other established or as-yet unidentified mTORC1–S6K1 targets<sup>9,10,21</sup>.

Carbohydrate metabolism was investigated in Eprs mutant mice by glucose and insulin tolerance tests (GTT and ITT, respectively). Young adult (112 days old) Eprs<sup>A/A</sup> mice exhibited slower glucose clearance following bolus injection of glucose, but faster clearance following insulin injection (Extended Data Fig. 6a, b). This paradoxical result is similar to observations in S6K1<sup>−/−</sup> mice<sup>10,22</sup>. Older (about 600 days old) Eprs<sup>A/A</sup> mice metabolized glucose more quickly, as analysed by both GTT and ITT (Extended Data Fig. 6c, d). Glucose metabolism was identical in Eprs<sup>D/D</sup> and wild-type mice, both young and old (Extended Data Fig. 6a–d). Food intake, faecal lipid excretion, and ketone body formation were essentially identical in Eprs<sup>A/A</sup>, Eprs<sup>D/D</sup>, and wild-type mice (Extended Data Fig. 6e–i). O<sub>2</sub> consumption (VO<sub>2</sub>) and CO<sub>2</sub> release (VCO<sub>2</sub>) were substantially higher in Eprs<sup>A/A</sup> mice compared to wild-type, both in the dark (as expected for nocturnal animals) and light phases, as observed in S6K1<sup>−/−</sup> mice<sup>9</sup> (Extended Data Fig. 7a, b). The respiratory exchange ratio trended towards lower indicative of greater fat utilization (Extended Data Fig. 7c). Energy expenditure determined as heat output was significantly higher in Eprs<sup>A/A</sup> mice (Extended Data Fig. 7d). Energy balance in Eprs<sup>D/D</sup> mice was not significantly different from the wild-type (Extended Data Fig. 7e–h). Female Eprs mutant mice showed O<sub>2</sub> consumption similar to males (Extended Data Fig. 7i).

In view of reduced Eprs<sup>A/A</sup> adipocyte size, and critical role of EPRS in translation, we established that total protein synthesis in adipocytes was not disrupted (Extended Data Fig. 8a). Importantly, insulin, a principal agonist of mTORC1–S6K1 activation and adipocyte anabolic activity<sup>23</sup> induced robust Ser999 phosphorylation in adipocytes from Eprs<sup>A/A</sup> mice; phosphorylation was abrogated in Eprs<sup>A/A</sup> mice despite S6K1 activation as detected by phosphorylation of S6K1 and its RPS6 target (Fig. 3a). Like myeloid cells, EPRS phosphorylation in adipocytes required mTORC1 and S6K1 (Extended Data Fig. 8b). EPRS phosphorylation in mouse adipocytes or differentiated 3T3-L1 cells was not stimulated by IFN<sub>γ</sub>, the activator of EPRS and GAIT pathway in myeloid cells (Extended Data Fig. 8c). Likewise, insulin did not induce GAIT complex assembly or repress <em>in vitro</em> translation of GAIT element-bearing transcript in differentiated 3T3-L1 cells (Extended Data Fig. 8d, e). To determine the potential role of inflammation in the lean phenotype of Eprs<sup>A/A</sup> mice, we determined white blood cell levels and found no statistically significant differences between Eprs<sup>A/A</sup> and wild-type mice in any leukocyte type (Extended Data Fig. 8f).

Analysis
of an anti-cytokine array showed differential expression of CC27 only, but the difference was not validated by immunoblot (Extended Data Fig. 8g, h).

Insulin-stimulated long-chain fatty acid (LCFA) uptake was significantly reduced in Eprsa/A mice but not Eprsa/D mouse adipocytes; glucose uptake was essentially identical (Fig. 3b). Insulin-stimulated LCFA uptake did not require EPRS phosphorylation in liver, heart, muscle, or BMDM (Extended Data Fig. 9a). Notably, a requirement for S6K1 in insulin-stimulated EPRS phosphorylation and LCFA uptake in adipocytes was observed (Extended Data Fig. 9b). Fatty acid binding proteins and transporters, together with lipolytic enzymes and a glycolic transporter, were screened to identify potential downstream targets of phospho–EPRS. Insulin-dependent interaction of EPRS was observed exclusively with the fatty acid transporter, FATP1, a principal mediator of insulin-stimulated LCFA uptake in adipocytes [13,14] (Fig. 3c). Insulin reduced EPRS association with two multisynthase complex constituents, AIMP3 and lysyl tRNA synthetase, establishing stimulus-dependent release from the parental complex (Fig. 3c). Interaction between phospho–EPRS and FATP1 was confirmed by co-immunoprecipitation, and LCFA uptake measured (mean ± s.e.m., n = 4 experiments in duplicate) (right). Binding of EPRS domains to FATP1 was detected by co-immunoprecipitation, and LCFA phosphorylation determined by 32P-labelling (left). Binding of EPRS domains (ERS, PRS, and linker), and insulin-stimulated LCFA uptake measured (mean ± s.e.m., n = 3 experiments in duplicate). c. Insulin induces translocation of Ser999-phosphorylated EPRS and FATP1 to membranes. Anti-GAPDH and anti-Na+/K+ ATPase antibodies verified cytosolic and membrane isolation. d. Effect of knockdown of EPRS (left) and FATP1 (targeting the coding sequence (CDS), right) on translocation to membrane. e. Schematic of mTORC1–S6K1 activation of EPRS- and FATP1-mediated LCFA uptake in adipocytes. MSC, multisynthase complex; TG, triglycerides.

Individual EPRS domains, catalytic ERS and PRS, and the connecting linker, were expressed in adipocytes in which endogenous mouse EPRS was repressed by 3′-UTR-specific siRNA (Fig. 4a). Insulin induced linker phosphorylation and interaction with FATP1, which in turn enhanced LCFA uptake (Fig. 4a). FATP1 failed to bind the S999A mutant linker, but bound the S999D mutant even without insulin stimulation; LCFA uptake likewise required Ser999 phosphorylation (Fig. 4b). As shown by others, insulin induced FATP1 translocation from cytoplasm to membranes[13] (Fig. 4c). Insulin-stimulated translocation of phospho–EPRS phosphorylation as EPRS and FATP1 localization in membranes was reduced in Eprsa/A adipocytes; in contrast, both translocated to membranes in Eprsa/D adipocytes even in the absence of insulin (Extended Data Fig. 10c). EPRS knockdown markedly reduced FATP1 membrane localization, but not vice versa, indicating EPRS is critical for FATP1 translocation (Fig. 4d). Membrane fractionation showed insulin induced FATP1 and EPRS translocation specifically to plasma membranes (Extended Data Fig. 10d).

The metabolic phenotype of Eprsa/A mice captures several salient features of S6K1−/− mice. They exhibit unaltered food intake, small adipocytes, reduced adiposity and body weight, improved glucose homeostasis in adults, and increased energy expenditure. Adipocytes from both mouse types have reduced insulin-stimulated EPRS binding to FATP1 and LCFA uptake, and enhanced basal lipolysis. We observed extended lifespan in Eprsa/A and S6K1−/− mice of both genders; a previous report showed significant longevity increase in female S6K1−/− mice, with similar trend for males[10]. However, S6K1−/− mice have reduced body weight at an earlier stage in development, and reduced muscle mass[20,22]. These differences might be explained by the breadth of S6K1 targets with multiple functions in diverse organ systems[6,11,26]. The phenotypic relationship between Eprsa/A and Fatp1−/− mice is less clear. Both mice exhibit markedly reduced adipocyte size and epididymal fat mass[13]. However, reduced body...
weight was not observed in Fatp1−/− mice. Possibly, the difference is explained by genetic background, compensatory upregulation of an alternative LCAF uptake mechanism in a knockout,27 or by calculation of body weight as fraction of initial weight that can reduce apparent differences at later times.13

Adipocyte triglyceride accumulation is largely determined by relative fluxes through catalytic reactions driving LCAF uptake, its esterification to glycerol backbones, glyceride lipolysis, and β-oxidation. Phospho-EPRS enhances the first step by activating FATP1, and EPRS knockdown inhibits adipocyte LCAF uptake by ~25% and 60–70%, respectively, suggesting EPRS stimulates FATP1-independent uptake mechanisms.18,29 Although stable interaction of EPRS was detected only with FATP1, we have not eliminated the possibility that EPRS activates transport by indirect mechanisms or by transient interactions not captured by immunoprecipitation. We also provide evidence that EPRS phosphorylation contributes to lipid accumulation by inhibiting catalytic reactions, that is, lipolysis and fatty acid oxidation.

The mTORC1–S6K1 axis is central to metabolic pathways,1–2, but established axis targets do not account for all observed loss-of-function metabolic phenotypes.4–6. Our studies reveal an unexpected adipogenic pathway resulting from mTORC1–S6K1 activation of EPRS (Fig. 4e). EPRS belongs to the S6K1 substrate class, site-specifically phosphorylated despite the lack of the established kinase recognition sequence, R/KXXRXS/T.7 Phospho-EPRS contributes to adiposity through membrane translocation and activation of FATP1, which stimulates LCAF uptake by an unresolved mechanism that might feature facilitated transport, or intracellular metabolic trapping by long-chain fatty acyl coenzyme A synthetase activity of FATP1 or by ligation of FATP1 to a distinct acyl coenzyme A synthetase.30 The binding of FATP1 to the non-catalytic linker domain in EPRS is consistent with the known binding promiscuity of linker WHEP domains, and the recent recognition that late-evolving, appended domains are largely responsible for non-canonical functions of multiple RNA synthetases.16. The noncatalytic domain represents a potential therapeutic target for obesity and ageing-related disorders.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Methods

Data reporting. No statistical methods were used to determine sample size. Reagents. Affinity-purified antibodies against total and Ser886 and Ser999 phospho-sites of EPRS were generated as described. Antibody against phospho-Ser was from Meridian Life Science. Antibodies specific for the C terminus of S6K1 and N terminus of S6K2 were purchased from Abcam and LifeSpan, respectively. Antibodies against PDK1, DMPK, PKN, GAPDH, caveolin1, CD36, FAT, GLUT4, His-tag, β-actin and FATP1, FATP3, and FATP4 were from Santa Cruz. Antibodies specific for FATP4 and FABPS were from RockD and for FABPpm and GOT2 was from GenEx. All other antibodies and rapamycin were from Cell Signaling. SignalSilence siRNAs targeting RSK1, AKT and S6K1 were from Cell Signaling, and those targeting raptor and rictor were from Santa Cruz. The 3′-UTR-specific duplex siRNAs, 5′-UUAGAUCAGGAUCCUCUCAG-3′ and 5′-GCCUAAUAAUCAGGAAUA-3′, targeting mouse EPRS were from Origene. Smart pool siRNA targeting the coding sequence of mouse FATP1 (SL27a1) was from Dharmacon and 3′-UTR-specific triliner siRNA targeting human S6K1 was from Origene.

Plasmids and proteins. Recombinant wild-type and Ser-to-Ala (S886A and S999A) mutant His-tagged linker proteins spanning Pro683 to A1023 of human EPRS were expressed and purified as described. Recombinant active S6K1 (ref. 32) and RSK1–3 were from Cell Signaling; Akt1 and Akt2 were from EMD Millipore. Mouse EPRS domains ER (Met1 to Gln682), linker (Pro683 to A1023), and RSK (Leu1024 to Tyr1512) were cloned into pCDNA3 vector with an N terminus Flag tag using full-length mouse EPRS cDNA (Origene) as template. Flag-tagged mouse wild-type linker and linker with Ser999-to-Ala (S999A) and Ser999-to-Asp (S999D) mutations were generated as described. Full-length human S6K1 cDNA in pCMV6-ENTRY vector was purchased from Origene and recloned, deleting the 23-amino acid N terminus nuclear localization signal, and adding an in-frame upstream His tag and a downstream Myc tag in pcDNA3. Specific Thr389-to-Ala (T389A) and Thr389-to-Glu (T389E) mutations were introduced using primers with the desired mutation and GENEART Site-Directed Mutagenesis System (Invitrogen).

Cell culture. Human U937 mononuclear cells (CRL 1593.2; ATCC authenticated by STR DNA profiling) were cultured in RPMI 1640 medium and 10% fetal bovine serum (FBS) with penicillin and streptomycin at 37 °C in 5% CO2. Bone-marrow-derived macrophages (BMDM) were flushed from femur and tibia marrows of 8–12-week-old C57BL/6 mice (from G. Thomas) and then cultured for one week in RPMI 1640 medium containing 10% FBS and 20% L929 cell-conditioned medium at 37 °C and 5% CO2. Confluent cells were treated with 500 U ml−1 of penicillin and streptomycin at 37 °C in 5% CO2. 106 cells per well) in Williams’ medium for another 24 h. Before experiments, cells were incubated for 4 h in serum-free DMEM and then with 100 nM insulin. Adult mouse cardiac cells were isolated by sequential plating using non-perfusion adult cardiomyocyte isolation kit (Cellutron) after isolation. Isolated cardiomyocytes were incubated for 24 h in serum-containing A5 medium, and then with serum-free A5 medium for another 24 h. Before experiments, cells were incubated for 4 h in serum-free DMEM and then with 100 nM insulin.

All studies using cultured cells were repeated at least three times. The number of replicates was estimated from comparable published studies that gave statistically significant results.

Transfection. U937 cells (1 × 10⁷), PBMs and differentiated 3T3-L1 adipocytes (5 × 10⁶ cells for both) were transfected with endotoxin-free plasmid DNAs or siRNAs (target-specific and scrambled control) using nucleofector (100 μl solution V for U937 cells and PBMs and 100 μl solution L for 3T3-L1 adipocytes) from Amaza nuclease kit (Lonza) following the manufacturer’s protocol. Transfected cells were immediately transferred to pre-warmed Opti-MEM media for 6 h and then to RPMI 1640 (for U937 cells and PBMs) and DMEM (for 3T3-L1 adipocytes) containing 10% FBS supplemented with penicillin, streptomycin, and gentamicin (G418; 20 μg ml−1) for 18 to 24 h before treatment with insulin and inhibitors.

In vitro kinase and phosphorylation assays. Cell lysates or purified active kinases were pre-incubated with recombinant EPRS linker (wild-type and mutant) for 5 min in kinase assay buffer (50 mM Tris-HCl (pH 7.6), 1 mM dithiothreitol, 10 mM MgCl2, 1 mM CaCl2, and phosphatase inhibitor cocktail) and phosphorylation was initiated by addition of 5 μCi [γ-32P]ATP (Perkin-Elmer) for 15 min, and terminated using SDS gel-loading buffer and heat denaturation. Phosphorylated proteins were detected after resolution on Tris-glycine SDS–PAGE, fixation in 40% methanol and 10% acetic acid, and autoradiography. Immunoblot with anti-His tag antibody to detect EPRS linker served as control. To assay kinase activity using peptide substrates, 50 μM of synthetic peptides were phosphorylated with 1 μCi [γ-32P]ATP in kinase assay buffer. Equal volumes were spotted onto P81-phosphocellulose squares, washed in 0.5% H2O2 and 32P incorporation determined by scintillation counting.

Immunocomplex kinase assay. U937 cell lysates were pre-cleared using protein A-sepharose beads for 4 h, and washed three times with kinase assay buffer supplemented with 0.1% Triton X-100. The immunocomplex was captured by incubating with protein A-sepharose beads for 4 h, and washed three times with kinase assay buffer supplemented with 0.1% Triton X-100. The immunocomplex was resuspended in kinase assay buffer and used to phosphorylate EPRS linker as above, and 32P incorporation into peptide substrates was determined by scintillation counting. Target peptides for S6K1, RSK1, MSK1, SGK494, NDR1, MRCKα, RIK, RSK1, ROCK1 and 2 (RRLSSLRA), GRK2 (CKCLGDEQAEEDSLDLESDE), LAT51 (CCKNRNRLSVA), MAST1 (KKGRDYMTRMQG), PRKX (RRRLSFAEPG), DMPK (KKGRDYMTRMQG), and PDK1 (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC) were generated from SignalChem; for MK2 (KKLNRNLTLVA) from Enzo Life Sciences; for PKA (RRKASGP), SGK1/1 (RPRAATF), PKK/PCN (HPLSRTLSVAAK), PKG, (RIKISEFDRPLR), and Cdk5 (PKTPKAKKL) were from Santa Cruz.

Mouse husbandry. All mice were housed in microisolator cages (maximum 5 cage of same-sex littermates) and maintained in climate/temperature- and photoperiod-controlled barrier rooms (22 ± 0.5 °C, 12–12–dark–light cycle) with unrestricted access to water and standard rodent diet (Harlan Teklad 2918) deriving 24, 18 and 58 kcal% from protein, fat and carbohydrate, respectively. Mice were fed standard rodent diet unless otherwise indicated. The number of animals used in each experiment is indicated in the figure legends. Animals were euthanized by cervical dislocation. All studies were performed in compliance with procedures approved by the Cleveland Clinic Lerner Research Institute Institutional Animal Care and Use Committee.

Generation of mice with Eprs999A and Eprs999D knock-in mutations. Genetically-modified EPRS phospho-deficient S999A and phospho-mimetic S999D knock-in mice were generated (Xenogen Biosciences, Taconic). The RP23-999A and 999D knock-in mice were generated (Xenogen Biosciences, Taconic). The RP23-
expression cassette for negative selection. The targeting vector was electroporated into C57BL/6 embryonic stem cells and screened with G418. Positive expanded clones with confirmed mutation were selected. Neo was deleted by FLP electroporation, and blastocysts injected. Male chimaeras were bred with C57BL/6 wild-type females, and resulting F1 heterozygotes interbred to generate homozygotes in C57BL/6 background. Genotyping was done using forward primer 5’-CAGCATAGAGGTTGGCAATAAAGG-3’ and reverse primer 5’-TTTCTGAACACACACATCGACAGACTC-3’. For all experiments the wild-type genotypes, Eprs+/− and Eprs−/− were generated exclusively by breeding heterozygotes (Eprs+/− and Eprs−/−), and most experiments shown use male mice unless otherwise indicated. Mice were not randomized and studies were performed unblinded with respect to mouse genotype.

**Generation of mice with knock-in of Eprs**

In microisolator cages (maximum 5 per cage of same-sex littermates) with routine between February 2011 and December 2013 from 23 S6K1−/− mice (112, the data were coded as follows: genotype, Eprs−/− and Eprs+/−) were generated from crosses of S6K1−/− heterozygotes.

**Recruitment and determination of Eprs−/− mouse longevity.** Male and female mice of Eprs−/− and Eprs+/− genotypes were recruited (n = 212 total mice) exclusively from crosses of heterozygotes (Eprs+/−). All mice were housed in microisolator cages (maximum 5 per cage of same-sex littermates) with routine cage maintenance as above. Weaned mice (>21 days), born between June 2010 and December 2012 from 40 heterozygous parents, were monitored daily and weighed biweekly for the entire duration of their life. Mice that spontaneously developed conditions common in the C57BL/6 strain, such as malocclusion and hydrocephalus, were sacrificed and excluded from the study. Assessments of deterioration in general health and quality of individual life were made in consultation with veterinary services of the Biological Resources Unit (BRU) of the Cleveland Clinic Lerner Research Institute. Severely sick and moribund mice that were judged to not survive another 48 h were euthanized with this date considered date of death, and included in the longevity analysis. Mice euthanized owing to imminent death include 11.5% (6 of 52) male and 11.1% (6 of 54) female of Eprs−/− genotype, and 7.7% (4 of 52) male and 9.3% (5 of 54) female of Eprs+/− genotype. Longevity was analysed by Kaplan–Meier survival curves from 212 mice (52 male and 54 female of each genotype, Eprs−/− and Eprs+/−) using known birth and death dates. Statistical differences were evaluated by log-rank Mantel–Cox and Gehan–Breslow–Wilcoxon tests using GraphPad Prism 5.

**Recruitment and determination of Eprs+/− mouse longevity.** Male and female mice of Eprs−/− and Eprs+/− genotypes were recruited (n = 89 total mice) exclusively from crosses of heterozygotes (Eprs+/−). All weaned mice (>21 days) born between February 2011 and September, 2014 from 23 Eprs+/− parents) were housed in microisolator cages (maximum 5 per cage of same-sex littermates) with routine cage maintenance and health monitoring as above. Mice killed owing to imminent death (as described above) include 8.7% (2 out of 23) male and 9.5% (2 out of 21) female of Eprs−/− genotype, and 8.3% (2 out of 24) male and 4.8% (1 out of 21) female of Eprs+/− genotype. Longevity was analysed by Kaplan–Meier survival curves from 89 mice (23, 21 male and 24, 21 male of genotype, Eprs−/− and Eprs+/−, respectively) using known birth and death dates and statistical analysis, as above.

**Recruitment and determination of S6K1−/− mouse longevity.** Male and female mice of S6K1+/− and S6K1−/− genotypes were recruited (n = 112 total mice) exclusively from crosses of heterozygotes (S6K1+/−). All weaned mice (>21 days born between February 2011 and December 2013 from 23 S6K1+/− parents) were housed in microisolator cages (maximum 5 per cage of same-sex littermates) with routine cage maintenance and health monitoring as above. Mice killed owing to imminent death (as described above) include 13.8% (4 out of 29) male and 10.3% (3 out of 29) female of S6K1+/− genotype, and 14.3% (4 out of 28) male and 14.3% (3 out of 21) female of S6K1−/− genotype. Longevity estimation was analysed by Kaplan–Meier survival curves from 112 mice (29, 29 male and 28, 26 female of genotype, S6K1+/− and S6K1−/−, respectively) using known birth and death dates and statistical analysis as above.

**Longevity analysis by Cox proportional hazard (CPH) regression.** Univariate and multivariate CPH regression models were performed to analyse the effects of 4 variables; genotype, date of birth (DOB), gender, and parental identity (PID), on longevity of mice recruited for the study. The independent variables were fitted as categorical variables in the model. Genotype and gender were coded as binary variables. DOB and PID were coded as multiple categories. For CPH regression analysis of Eprs−/− and Eprs+/− mice (n = 212), the data were coded as follows: genotype, Eprs−/− (1) and Eprs+/− (0); gender, male (0) and female (1). On the basis of unique occurrences, DOB and PID were categorized into 79 (0–78, 0 being the DOB for oldest mice in the study) and 40 (1–40) categories, respectively. Oldest DOB category represents the reference for DOB. PID-1 was considered reference for PID variable. Models were fit using Cox proportional hazards regression in R package ‘survival’ using coxph function. Univariate model was built fitting each of the four variables individually and multivariate model was built fitting all four variables simultaneously. For CPH regression analysis of Eprs−/− and Eprs+/− mice (n = 89), the data were coded as follows: genotype, Eprs−/− (1) and Eprs+/− (0); gender, male (0) and female (1). On the basis of unique occurrences, DOB and PID were categorized into 38 (0–37, 0 being the DOB for oldest mice in the study) and 23 (1–23) categories, respectively. For CPH regression analysis of S6K1−/− and S6K1−/− mice (n = 112), the data were coded as follows: genotype, S6K1−/− (1) and S6K1−/− (0); gender, male (0) and female (1). On the basis of unique occurrences, DOB and PID were categorized into 36 (0–35, 0 being the DOB for oldest mice in the study) and 23 (1–23) categories, respectively.

**Scanning electron microscopy.** Scanning electron microscopy was performed by the Cleveland Clinic Imaging Core. WAT from 20-week-old male mice was fixed using 2.5% glutaraldehyde and 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4 °C. Tissues were washed three times in PBS followed by post-fixation with 1% osmium tetroxide in PBS for 1 h at 4 °C. Finally, the tissues were dehydrated through graded alcohol (50, 70, 90, and 100%), twice in ethanol:hexamethyldisilzane (HMDS; 1:1), and three times in 100% HMDS for 10 min each, and dried at room temperature. Samples were mounted on aluminium stubs and coated with palladium-gold using a sputter-coater, and viewed at X500 magnification with a Jeol JSM 5310 Electron Microscope (EOL).

**Histochemistry.** Adipose tissue from 20-week-old male mice were fixed in formalin, dehydrated in ethanol, embedded in paraffin, and cut at 5-μm thickness. Sections were deparaffinized, rehydrated, and stained with haematoxylin and eosin by the Cleveland Clinic Histology Core. Stained tissues were visualized with Leica DM2500 microscope, captured with Micropublisher 5.0 RTV digital camera (QImaging) using a 5X objective lens for magnification, and QCapture Pro 6.0 (QImaging) software for image acquisition.

**Determination of adipose tissue cell number.** Adipocytes from 100 mg EWAT of 20-week-old male mice were isolated as described above and suspended in DMEM. Cells were counted in a haemocytometer.

**Lipolysis in primary adipocytes.** Basal lipolysis in primary adipocytes from Eprs−/−, Eprs+/−, and Eprs+/− EWAT was measured by glycerol release using adi- polysis assay kit (Cayman).

**Fatty acid oxidation.** Fatty acid oxidation in EWAT of 20-week-old male mice was performed as described. Explants were placed in an Erlenmeyer flask (Kimble-chase Kontes) containing the reaction mixture (DMEM with 0.1 μCi of 14C]oleic acid, 100 mM t-carnitine, and 0.2% fat-free BSA), and conditioned for 5 min in a 37 °C CO2 incubator. The flask was sealed with a rubber stopper containing a centre-well (Kimble-chase Kontes) fitted with a loosely folded filter paper moistened with 0.2 ml of 1 M NaOH, and incubated for 5h at 37 °C. CO2 in the filter paper was trapped by addition of 200 μl of perchloric acid to the reaction mixture followed by incubation at 55 °C for 1 h. Radioactivity in the filter paper was determined by scintillation counting.

**Food intake studies.** At 16 weeks, mice were individually housed and given standard rodent diet and water ad libitum. Cumulative food intake was measured by weighing the mouse and food every second day for 30 consecutive days.

**Glucose and insulin tolerance tests.** Intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT) in Eprs−/−, Eprs+/−, and Eprs+/− mice were determined as described. Briefly, GTT was done after an overnight (12 h) fast followed by intraperitoneally injecting 2 mg/kg body weight, Sigma). ITT was performed in 6-h fasted mice by injection of 0.75 U/kg body weight of insulin (Sigma). Blood glucose was determined using a commercial glucometer (Contour, Bayer).

**Blood and serum measurements.** Serum triglycerides, free fatty acids, glucose, and insulin in 12-h fasted and in 1-h post-prandial (fed) mice were determined using commercially available kits. Serum triglycerides, free fatty acids, and glucose kits were from Wako. Insulin was determined using enzyme-linked immunosassay-based, ultra-sensitive mouse insulin kit (Crystal). Determination of serum α-hydroxybutyrate (for ketone body analysis) from 6-h fasted mice was done using colorimetric assay kit from Cayman. White blood cell counts in blood freshly collected by cardiac puncture in the presence of 10 mM EDTA were determined using Advia hematometry system.

**Fecal lipid excretion.** Lipid content in mouse faeces was determined after extraction with chloroform:methanol (2:1)45,46.
GAIT system activity assay by in vitro translation. GAIT system activity in insulin-treated adipocytes was determined by in vitro translation of capped poly(A)-tailed Luc-Gp GAIT and T7 gene 10 reporter RNAs as described15,17. Gel-purified RNAs were incubated with lysates from U937 monocytes and differentiated 3T3-L1 adipocytes in the presence of rabbit reticulocyte lysate and [35S]methionine. Translation of the two transcripts was determined following resolution on 10% SDS–PAGE and autoradiography.

Serum cytokine determination. Cytokine levels in mouse serum (100 μg protein) were determined using mouse cytokine antibody array C3 kit (RayBiotech). Assay of liver lipid content. Mouse liver triglyceride content was determined by measurement of glycerol following saponification in ethanol KOH (2:1, ethanol: 30% KOH)46. For assessment of total neutral lipid, freshly isolated liver slices were frozen in OCT, 5-μm sections stained with Oil Red O, and analysed by densitometry using NIH image J as described46.

Energy metabolism by indirect calorimetry. Mouse energy metabolism was determined by indirect calorimetry using the Oxymax CLAMS system (Columbus Instruments) in the Rodent Behavioural Core of the Cleveland Clinic Lerner Research Institute. Mice were housed individually in CLAMS cages and allowed to acclimate for 48 h with unrestricted access to food and water. Thereafter, O2 consumption (VO2), CO2 release, RER and heat generation were recorded for 24 h spanning a single light–dark cycle.

32P metabolic labelling. Adipocytes from 500 mg WAT from wild-type and Eptf/Aad mice were labelled with 150 μCi of 32P-orthophosphate (MP Biomedicals) in phosphate-free DMEM medium in absence of insulin (100 nM) for 4 h. EPRS was immunoprecipitated with antibodies cross-linked to protein A-sepharose beads (Sigma) in 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Triton X-100, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and protease/phosphatase inhibitor cocktail. Immunoprecipitated beads were washed with 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.1% Triton X-100, and then in 50 mM Tris-HCl (pH 7.6) and 150 mM NaCl. 32P incorporation in immunoprecipitated proteins was determined by Tris-glycine SDS–PAGE, fixation and autoradiography.

Determination of protein synthesis by labelling with [14C]Glut and [14C]Pro. Adipocytes (0.25 × 10^6 cells) were pre-incubated in serum-free DMEM for 4 h. Subsequently, the medium was supplemented with 2.5 μCi [14C]Glu or [14C]Pro (Perkin-Elmer), and cells incubated for additional 6 h. Adipocytes were lysed and 14C incorporation determined by trichloroacetic acid precipitation and scintillation counting.

Determination of protein synthesis by [35S]Met/Cys metabolic labelling. Mouse adipocytes (0.25 × 10^6 cells) were pre-incubated in methionine-free RPMI medium (Invitrogen) with 10% FBS for 30 min. [35S]Met/Cys (250 μCi/μL, Perkin-Elmer) was added and incubated at 37°C with 5% CO2 for 15 min. Labelled cells were lysed in RIPA buffer (Thermo Fisher) and analysed by Tris-glycine SDS–PAGE, fixation and autoradiography.

Immunoblot analysis. Cell lysates or immunoprecipitates were denatured in Laemmli sample buffer (Bio-Rad) and resolved on Tris-glycine SDS–PAGE, and autoradiography. Immunoblots shown are typical of experiments independently carried out at least three times.

Co-immunoprecipitation. Pre-cleared cell lysates (1 mg) were incubated with antibody cross-linked to protein A-sepharose beads in detergent-free buffer containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, and EDTA-free protease/ phosphatase inhibitor cocktail. Immunoprecipitates were analysed by Tris-glycine SDS–PAGE and immunoblotting either after washing the beads three times in the same buffer or after elution, followed by neutralization with 0.2 M glycine-HCl (pH 2.6) or 50 mM Tris-HCl (pH 8.5), respectively.

Fatty acid uptake assays. Fatty acid uptake assay kit (QBT, Molecular Devices) that utilizes fluorescent bodipy-C12, a LCFA analogue, was used to determine fatty acid uptake46. Differentiated 3T3-L1 adipocytes were plated at 5 × 10^4 cells per well in a 96-well plate. Adipocytes were first incubated in serum-free Hank's balanced salt (HBS) solution for 4 h, and then with 100 mM insulin and bodipy-C12 for an additional 4 h. After 30 min, relative fluorescence was read at 485 nm excitation and 515 nm emission wavelength in bottom-read mode (SpectraMax GeminiEM, Molecular Devices).

LCFA uptake was also determined in differentiated 3T3-L1 adipocytes as cellular accumulation of [14C]oleate (Perkin-Elmer). Adipocytes (10,000 cells) were seeded in a 24-well plate in DMEM with 10% calf serum overnight. Cells were serum-deprived for 4 h, treated with 100 mM insulin for 3.5 h, and then with 50 μM of [14C]oleate in HBS containing 0.1% fatty acid-free BSA for 30 min51,52. Cells were washed extensively in cold HBS with 0.1% fatty acid-free BSA to remove unincorporated [14C]oleate, lysed in RIPA buffer (Thermo Fisher), and centrifuged at 2000 rpm for 5 min. Supernatant radioactivity was determined by scintillation counting and normalized to protein. LCFA uptake by mouse WAT, hepatocytes, cardiac cells, BMDM, and soleus muscle strips were measured using essentially the same method51.

Glucose uptake assay. Adipocytes from wild-type and mutant mice were pre-incubated for 4 h in serum- and glucose-free DMEM and then rinsed with Krebs–Ringer buffer containing 20 mM HEPES (pH 7.4), 5 mM sodium phosphate, 1 mM MgSO4, 1 mM CaCl2, 136 mM NaCl, and 4.7 mM KCl53,54. Adipocytes were incubated for 4 h in the presence of 1 μg of [14C]2-deoxy-d-glucose (DG; Perkin-Elmer) and 100 nM insulin in the same buffer supplemented with 100 mM unlabeled 2-DG (Sigma). Uptake was stopped using ice-cold PBS containing 50 mM cytochalasin, followed by four washes with PBS. Lysate radioactivity was determined by scintillation counting.

Separation of cytosolic and membrane fractions. Membrane fraction from differentiated 3T3-L1 adipocytes was isolated by phase partitioning using Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Thermo-Scientific).

Isolation of plasma membranes. Plasma membrane fractions from 3T3-L1 adipocytes were prepared as described44. Differentiated 3T3-L1 adipocytes were washed in buffer containing 250 mM sucrose, 10 mM Tris (pH 7.4), and 0.5 mM EDTA. Lysates were prepared by homogenization in the same buffer supplemented with protease and phosphatase inhibitor cocktail, and centrifuged at 16,000g for 20 min at 4°C. The re-suspended pellet was layered onto a solution containing 1.12 M sucrose, 10 mM Tris (pH 7.4), and 0.5 mM EDTA, and centrifuged at 150,000g for 20 min at 4°C. The resulting pellet was suspended in RIPA buffer (Sigma) and plasma membrane was obtained by centrifugation at 74,000g for 20 min at 4°C.

Data availability. All data generated are included in the published article and its supplementary information files. Additional statistical data sets generated are available from the corresponding author upon request.
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Extended Data Figure 1 | Identification of S6K1 as EPRS Ser999 kinase.

a, Screening of AGC kinase group members for phosphorylation of EPRS Ser999 by immunocomplex kinase assay and [γ-32P]ATP-labelling with S886A linker target in U937 cells. Kinase activity using kinase-specific substrate is shown (bottom; mean ± s.e.m., n = 3).

b, Specificity of S6K1 for Ser999 phosphorylation, determined by 32P incorporation in wild-type (WT), S999A and S886 linker.

c, siRNA targeting S6K1 inhibits IFNγ-stimulated EPRS phosphorylation in U937 cells determined by 32P-labelling (mean ± s.e.m., n = 3). d, Active recombinant kinases used for in vitro phosphorylation of linker bearing S886A (Ser999 P-acceptor) mutation shows site-specific phosphorylation by S6K1. e, Raptor not rictor is required for Ser999 phosphorylation. f, siRNA targeting the S6K1 3′-UTR inhibits S6K1 expression and phosphorylation of EPRS Ser999, but not Ser886 (mean ± s.e.m., n = 3). g, Phosphorylation of S6K1 Thr389 is required for phosphorylation of EPRS. Cells were co-transfected with siRNA targeting the 3′-UTR to knock down endogenous S6K1, and with myc-tagged wild-type or mutant S6K1 cDNA; IFNγ-stimulated EPRS phosphorylation determined by 32P-labelling (mean ± s.e.m., n = 3).

h, Cells treated as in e but followed by reciprocal co-immunoprecipitation.
Extended Data Figure 2 | Gene targeting and generation of Eprs<sup>A/A</sup> and Eprs<sup>D/D</sup> knock-in mice, and their body weight phenotypes. a, Knock-in mice bearing Ser999-to-Ala and -Asp mutations were generated by homologous recombination. b, Validation of wild-type (Eprs<sup>S/S</sup>) and EPRS knock-in mice by PCR genotyping (top) and sequence analysis (bottom). c, Genotype analysis of littermates. Total of 1,410 and 644 progeny from interbreeding Eprs<sup>S/A</sup> and Eprs<sup>S/D</sup> mice, respectively, were used at postnatal days P0, P4, and P21. d, Growth curves of Eprs<sup>A/A</sup> (mean ± s.e.m., n = 10 per group; P < 0.0001, two-way ANOVA) and Eprs<sup>D/D</sup> (n = 10 per group) female mice. e, Representative images (left) and weights (right) of wild-type (Eprs<sup>S/S</sup>; S/S) and Eprs<sup>A/A</sup> (A/A) mice on embryonic day E16.5 and post-embryonic development stages. Data shown are mean ± s.e.m.; n = 11 for E16.5 embryos, n = 10 for P0 and P10 mice, and n = 14 for 3, 12, 20, 30, and 50-week mice. f, Representative images (left) and weights (right) of 50-week Eprs<sup>S/S</sup> and Eprs<sup>D/D</sup> (D/D) mice (mean ± s.e.m.; n = 10 per group).
Extended Data Figure 3 | Lifespan analysis of Epr{superscript}S/S, Epr{superscript}A/A and Epr{superscript}D/D mice monitored from weaning (>21 days). a, Youngest and oldest 10% are the mean lifespan of the shortest- and longest-living 10% mice. Numbers of days are represented to nearest full day. Median and mean ± s.e.m.; are shown.

b, Cox proportional hazard (CPH) regression analyses of Epr{superscript}S/S and Epr{superscript}A/A mice shows genotype as the most significant predictor of increased longevity. Longevity relative to survival days (that is, age at death) was analysed in pooled mice by CPH regression model. The four independent variables genotype, date of birth (DOB), parental ID (PID), and gender were replaced with a set of category variables. In case of genotype and gender, category represents their presence or absence. DOB and PID data were divided into multiple categories as described in the supplementary methods. Independent variables were fitted into the CPH model individually (univariate) or simultaneously (multivariate). Shown are: β (the unstandardized regression coefficient) with standard error (s.e.), the degrees of freedom (df), and the significance for each model fit. \( \exp(\beta) \) for the covariate of interest is the predicted change in hazard ratio for a unit increase in the predictor, and its 95% confidence interval (CI).

c, Kaplan–Meier survival curves show no change in lifespan of male, female, or combined Epr{superscript}D/D mice. Male (MC \( \chi^2 = 0.003, P = 0.956 \); GBW \( \chi^2 = 0.001, P = 0.972 \)), female (MC \( \chi^2 = 0.158, P = 0.691 \); GBW \( \chi^2 = 0.206, P = 0.650 \)), and gender-combined (MC \( \chi^2 = 0.079, P = 0.779 \); GBW \( \chi^2 = 0.076, P = 0.783 \)). d, e, Survival and CPH regression analyses of Epr{superscript}S/S and Epr{superscript}D/D mice as described above in a and b.
Extended Data Figure 4 | Adipose tissue deposition and lipolysis in EPRS<sup>A/A</sup> and EPRS<sup>D/D</sup> mice. a, Length of mice was measured from head to beginning of tail using a digital caliper (Fisherbrand Traceable). Data shown are mean ± s.e.m., n = 15 for 20-week-old male mice. b, Ventral view of wild-type and Eprs<sup>A/A</sup> mouse abdominal cavity. c, Weights of adipose and non-adipose tissues from 20-week-old male Eprs<sup>A/A</sup> and control mice (mean ± s.e.m., n = 14 per group, P value from unpaired t-test). d, Scanning electron micrographs of EWAT in 20-week-old male Eprs<sup>SS</sup>, Eprs<sup>A/A</sup>, and Eprs<sup>D/D</sup> mice. e, Total adipocyte cell number in EWAT of Eprs<sup>A/A</sup> knock-in and wild-type mice. Data represent mean ± s.e.m., n = 5 per group. f, Elevated lipolysis in adipocytes from Eprs<sup>A/A</sup>, but not Eprs<sup>D/D</sup>, mice (mean ± s.e.m.; n = 6 per group). g, Elevated 3-oxidation in WAT explants from Eprs<sup>A/A</sup> mice as determined by release of 14CO2 from 14C-oleic acid (mean ± s.e.m.; n = 5 per group). h, Serum levels of insulin, glucose, triglycerides (TG) and free fatty acids (FFA) in 12-h fasted and 1-h post-prandial (fed) 16-week-old male mice (mean ± s.e.m., n = 10 per group, *P < 0.05, unpaired t-test). i, Growth curves of Eprs<sup>A/A</sup> and Eprs<sup>D/D</sup> mice (males, n = 12 per group, mean ± s.e.m., P < 0.001, two-way ANOVA) started at 6 weeks on an unrestricted high-fat diet (Harlan Teklad TD.06414) deriving 18, 60, and 21 kcal% from protein, fat, and carbohydrate, respectively. j, Phosphorylation of EPRS Ser999 in WAT from Eprs<sup>A/A</sup> and Eprs<sup>D/D</sup> mice after high-fat diet feeding for 24 weeks.
Extended Data Figure 5 | Adiposity and lifespan of S6K1−/− mice.
a, PCR genotyping of wild-type (S6K1+/+), heterozygous (S6K1+/−) and homozygous (S6K1−/−) mice. b, Immunoblot analysis of S6K1, EPRS, and FATP1 in S6K1−/− mice. e, Weight of S6K1−/− mice at embryonic day E16.5, and at postnatal days P0 and P10. d, Kaplan–Meier survival curves shows increased longevity in male, female or combined S6K1−/− mice.

Male (n = 29 per group; MC $\chi^2 = 4.919$, P = 0.027; GBW $\chi^2 = 4.660$, P = 0.031), female (n = 28 for S6K1+/+ and n = 26 for S6K1−/−; MC $\chi^2 = 7.927$, P = 0.005; GBW $\chi^2 = 7.277$, P = 0.007), and gender-combined (n = 26 for S6K1+/+ and n = 55 for S6K1−/−; MC $\chi^2 = 11.78$, P = 0.0006; GBW $\chi^2 = 11.01$, P = 0.0009). e, f, Lifespan and CPH regression analyses of S6K1+/+ and S6K1−/− mice as described above in Extended Data Fig. 3a, b.
Extended Data Figure 6 | Glucose metabolism, food intake and faecal lipid excretion in Eprs\(^{A/A}\) and Eprs\(^{D/D}\) mice. a, Glucose tolerance test (GTT) in 112-day-old Eprs\(^{S/S}\), Eprs\(^{A/A}\) and Eprs\(^{D/D}\) mice (mean ± s.e.m., \(n = 10\) per group). b, Insulin tolerance test (ITT) on mice as in a (mean ± s.e.m., \(n = 10\) per group). c, d, Same as a and b but using ~600-day-old mice (mean ± s.e.m., \(n = 9\) per group). e–g, Determination of food intake as g per mouse per day (left) or g per body weight (g) per day (right in male (e) and female (f) Eprs\(^{A/A}\) mice, and in male Eprs\(^{D/D}\) mice (g). All values represent mean ± s.e.m., \(n = 14\) per group. h, Faecal lipid excretion in Eprs\(^{S/S}\), Eprs\(^{A/A}\), and Eprs\(^{D/D}\) mice (mean ± s.e.m., \(n = 6\) per group). i, Serum ketone body (β-hydroxybutyrate) level in 6-h fasted mice.
Extended Data Figure 7 | Energy metabolism in Eprs<sup>S/S</sup> and Eprs<sup>A/A</sup> mice. 

**a**, **b**, Determination of VO<sub>2</sub> (left) and VCO<sub>2</sub> (right) in Eprs<sup>S/S</sup> and Eprs<sup>A/A</sup> male mice over a 24-h period. 

**c**, **d**, Respiratory exchange ratio (RER) (left) and heat generation (right) in 12-h light and dark cycles were determined (mean ± s.e.m., n = 6 per group). 

**e**–**h**, Same as **a**–**d** but comparing Eprs<sup>S/S</sup> and Eprs<sup>D/D</sup> male mice (mean ± s.e.m., n = 6 per group). 

**i**, Determination of VO<sub>2</sub> in female Eprs<sup>S/S</sup>, Eprs<sup>A/A</sup>, and Eprs<sup>D/D</sup> mice (mean ± s.e.m., n = 3 per group).
Extended Data Figure 8 | Absence of GAIT pathway in adipocytes and inflammatory response in EPRS<sup>A/A</sup> mice. a, Total protein synthesis determined by [35S]Met/Cys labelling (left), and by incorporation of [14C]Glu and [14C]Pro into TCA-precipitated proteins in adipocytes from EPRS<sup>S/S</sup>, EPRS<sup>A/A</sup>, and EPRS<sup>D/D</sup> mice. b, Effect of siRNA-mediated knockdown of S6K1, raptor, and rictor on phosphorylation of EPRS Ser999 in differentiated mouse 3T3-L1 adipocytes in presence of 100 nM insulin. c, Effect of IFNγ and insulin on phosphorylation of EPRS Ser999 in differentiated 3T3-L1 adipocytes or mouse primary adipocytes determined using phospho-specific EPRS antibody. d, GAIT complex formation in IFNγ-stimulated U937 cells and insulin-stimulated 3T3-L1 adipocytes by immunoprecipitation with anti-EPRS antibody and immunoblot with antibodies against GAIT complex constituents. Cytosolic lysates from IFNγ-treated U937 cells served as positive control. e, Determination of GAIT pathway activity in IFNγ-stimulated U937 cells and insulin-stimulated 3T3-L1 adipocytes by in vitro translation of a control (T7 gene 10) and GAIT element bearing (Luc-ceruloplasmin (Cp) GAIT element) reporter RNAs. f, White blood cells counts in blood from EPRS<sup>S/S</sup> and EPRS<sup>A/A</sup> mice by Advia hematology system (LUC, large unstained cells). g, Determination of cytokine levels in serum from EPRS<sup>S/S</sup> and EPRS<sup>A/A</sup> mice. Mouse cytokine antibody arrays were incubated with serum (100 μg, protein, right). Pixel intensity was determined by densitometry (right; mean ± s.e.m., 2 mice per group). h, Immunoblot analysis of selected cytokines in serum from EPRS<sup>S/S</sup> and EPRS<sup>A/A</sup> mice.
Extended Data Figure 9 | Tissue-specificity of insulin-stimulated LCFA uptake and EPRS-FATP1 interaction. a, $[^{14}C]$oleate uptake determined in insulin-stimulated hepatocytes, cardiac cells, soleus muscle strips, and BMDM from Eprs$^{S/S}$, Eprs$^{A/A}$, and Eprs$^{D/D}$ mice (mean ± s.e.m., n = 6 mice per group). b, Insulin-stimulated EPRS Ser999 phosphorylation (top), $[^{14}C]$oleate uptake (middle), and $[^{14}C]2$-deoxy-d-glucose (DG) uptake (bottom) in adipocytes from white adipose tissue of S6K1$^{+/+}$ and S6K1$^{-/-}$ mice (mean ± s.e.m., n = 5 mice per group). c, Efficiency of EPRS and FATP1 knockdown in 3T3-L1 adipocytes by siRNA targeting each mRNA alone and in combination, as determined by densitometry (NIH image J) of immunoblots shown in Fig. 3g (mean ± s.e.m., n = 4 experiments). d, Insulin-induced EPRS Ser999 phosphorylation, interaction with FATP1, and $[^{14}C]$oleate uptake in human adipocytes (mean ± s.e.m., n = 3 experiments in duplicate). e, Co-immunoprecipitation experiment to determine FATP1 binding to EPRS in lysates from multiple tissues as indicated. f, EPRS and FATP1 expression in male and female S6K1$^{-/-}$ mice (mean ± s.e.m., n = 3 mice per group). g, Lack of interaction of EPRS and FATP1 in insulin-stimulated adipocytes of S6K1$^{-/-}$ mice.
Extended Data Figure 10 | Hepatic lipids and FATP1/EPRS membrane localization in Eprs\textsuperscript{A/A} and Eprs\textsuperscript{D/D} mice. a, Optimum cutting temperature (OCT) compound-fixed liver slices from Eprs\textsuperscript{S/S}, Eprs\textsuperscript{A/A}, and Eprs\textsuperscript{D/D} mice were stained with H&E (top) or oil red O (bottom), and the latter quantitated by densitometry (right; mean ± s.e.m., n = 3 mice per group). b, Determination of liver triglycerides in wild-type and mutant mice (mean ± s.e.m., n = 5 mice per group). c, Insulin-inducible membrane localization of EPRS and FATP1 in adipocytes from wild-type and mutant mice. d, Membrane fractionation shows EPRS and FATP1 co-localizing in plasma membrane (mean ± s.e.m., n = 3 experiments).