Excessive glucose production by the liver is a key factor in the hyperglycemia observed in type 2 diabetes mellitus (T2DM). Here, we highlight a novel role of liver kinase B1 (Lkb1) in this regulation. We show that mice with a hepatocyte-specific deletion of Lkb1 have higher levels of hepatic amino acid catabolism, driving gluconeogenesis. This effect is observed during both fasting and the postprandial period, identifying Lkb1 as a critical suppressor of postprandial hepatic gluconeogenesis. Hepatic Lkb1 deletion is associated with major changes in whole-body metabolism, leading to a lower lean body mass and, in the longer term, sarcopenia and cachexia, as a consequence of the diversion of amino acids to liver metabolism at the expense of muscle. Using genetic, proteomic and pharmacological approaches, we identify the aminotransferases and specifically Agxt as effectors of the suppressor function of Lkb1 in amino acid-driven gluconeogenesis.
The liver plays a major role in maintaining normal glycemia, by regulating the processes of glycogen breakdown (glycogenolysis), glycogen synthesis (glycogenogenesis) and glucose synthesis (gluconeogenesis) to produce glucose during fasting and to store glucose in the postprandial period. During fasting, glucose is initially generated by glycogenolysis, and then by gluconeogenesis from various gluconeogenic precursors, including amino acids. The liver is the principal organ capable of glucose synthesis. During fasting, glucose is initially generated by glycogenolysis, and then by gluconeogenesis from various gluconeogenic precursors, including amino acids. However, hepatic gluconeogenesis is not restricted to the fasted state; it is also a normal physiological event in the fed state, as part of the metabolism of excess dietary amino acids, the carbon moiety of which is stored as glycogen, via the indirect pathway of glycogen metabolism of excess dietary amino acids, the carbon moiety of which is stored as glycogen, via the indirect pathway of glycogen synthesis.

Lkb1 is a master upstream kinase that directly phosphorylates and activates AMP-activated protein kinase (AMPK) and 12 kinases related to AMPK, known as AMPK-related kinases (ARKs), including the salt-inducible kinases (SIKs). AMPK is a key intracellular energy sensor and regulator of multiple metabolic processes. Shaw et al. showed that the loss of Lkb1 in the liver results in hyperglycemia, due to an enhancement of gluconeogenesis, and identified Ampk as the enzyme responsible for induction of the gluconeogenic program by Lkb1 deficiency. However, subsequent studies called the role of Ampk into question, instead identifying Siks as the effectors of gluconeogenesis suppression by Lkb1 in the liver.

Here, using mice with a hepatocyte-specific Lkb1 deficiency, we characterize a new role of Lkb1 in the control of hepatic amino acid catabolism, identifying Lkb1 as a suppressor of gluconeogenesis from amino acids. We show that, in addition to its known effects on hepatic gluconeogenesis, the loss of hepatic Lkb1 increases the uptake and catabolism of amino acids in the liver and, ultimately, their utilization as substrates for gluconeogenesis. This is the case not only during fasting, but also during the postprandial period, identifying Lkb1 as a suppressor of postprandial gluconeogenesis. The increase in hepatic amino acid extraction in mutant mice is associated with a decrease in plasma amino acid concentration, ultimately affecting protein homeostasis in the skeletal muscle. Indeed, mutant mice have a lower lean body mass and muscle amino acid content than wild-type mice. In the longer term, the impairment of amino acid metabolism leads to the development of sarcopenia and cachexia, causing premature death in more than 60% of the mutant mice. Using genetic, proteomic, and pharmacological approaches, we identify aminotransferases and, specifically, Agrp as effectors, of the suppressor function of Lkb1 in amino acid-driven gluconeogenesis. We find that this effect of Lkb1 occurs independently of Ampk and identify a phosphorylated RNA-binding protein network controlled by Lkb1 as the key element in controlling the expression of enzyme of amino acid catabolism at the translational level.

Results

Hepatic loss of Lkb1 induced hyperglycemia and sarcopenia. Tamoxifen was injected into adult Lkb1fl/fl;TTR-CreTam and control Lkb1fl/fl mice to induce the deletion of Lkb1 specifically in the hepatocytes. The mutant mice are referred to hereafter as Lkb1KOlived mice. Their phenotypes were analyzed 15 days later, to characterize the immediate phenotype resulting from Lkb1 deletion in the liver. Western blots confirmed the absence of Lkb1 in the livers of Lkb1KOlived mice. As expected, Ampk phosphorylation levels were much lower in the livers of mutant mice than in those of controls.

Tamoxifen-treated Lkb1KOlived mutant mice presented hyperglycemia in the fasted state and were glucose-intolerant in intraperitoneal (IP) glucose tolerance tests, consistent with the findings of a previous study. (Fig. 1b). Plasma insulin concentrations did not differ significantly between mutant mice and controls, for any of the nutritional states considered. No significant differences in plasma and hepatic triglyceride levels were observed between mutant and wild-type mice (Supplementary Fig. 1b, c). Mutant mice had slightly, but significantly heavier livers (Fig. 1d) with preserved liver function (Supplementary Fig. 1d, e) and a normal architecture on liver histology examination (Supplementary Fig. 1g). However, the PAS staining of liver sections associated with the determination of glycogen content revealed a much higher level of glycogen accumulation in mutant than in control mice, in the fasted state (Fig. 1e). We investigated this increase in glycogen content in Lkb1KOlived mice, by studying two critical enzymes involved in the control of glycogen storage: glycogen synthase (Gys2), which is involved in glycogen synthesis, and glycogen phosphorylase (Pygl), which is involved in glycogen degradation. During the fasting period, the active form of Pygl (pS15Pygl) was much less abundant in Lkb1KOlived mice than in controls, suggesting a much lower levels of glycogen degradation; at the same time levels of the Gys2 protein were significantly higher. Given the role of glucagon as a counter-regulator of insulin signaling for glucose homeostasis, we checked its plasma levels in the fasting and refeed state and did not find any significant difference in the insulin/glucagon ratio, suggesting that a deregulation of glucagon secretion was not involved in the phenotype of the Lkb1KOlived mice (Supplementary Fig. 1f). Together, these findings can account for the higher glycogen content of the livers of fasted Lkb1KOlived mice.

We also monitored Lkb1KOlived mice for several months, to analyze their long-term phenotypes. About 6 months after tamoxifen injection, more than 60% of the mutant mice died from severe sarcopenia and cachexia (Fig. 1g). Mutant mice had a much lower body weight (Fig. 1i), with a striking lack of skeletal muscle and adipose tissue (Fig. 1h). Tibialis and gastrocnemius muscle weights were strongly decreased, and morphometric analysis showed that sarcopenia was linked to a smaller muscle fiber size (Supplementary Fig. 1h).

Over all, our results show that the specific deletion of Lkb1 in hepatocytes leads to the impairment of glucose homeostasis, with hyperglycemia and an abnormal accumulation of glycogen during the fasting period. In the longer term, the hepatocyte-specific inactivation of Lkb1 led to severe cachexia and sarcopenia, resulting in death in more than 60% of the animals.

Body composition of mutant mice reveals lower lean body mass. The cachectic and sarcopenic phenotype developing in Lkb1KOlived mice over time clearly suggests that hepatic Lkb1 loss is responsible for changes in the relationships between the liver and other organs, such as muscles. We monitored changes in body composition in Lkb1KOlived and control mice by MRI for nine weeks after tamoxifen injection, to confirm these peripheral consequences of liver-specific Lkb1 loss. No significant difference was found between mutant and control mice in terms of overall body weight gain, but the mutant mice displayed significant modifications of their body composition, with a lower lean mass...
and a higher fat mass (Fig. 2a–c). The monitoring of mice in indirect calorimetry cages revealed no significant differences in food intake or total energy expenditure between LKBKO\textsuperscript{livad} and control mice, and significantly lower levels of locomotor activity during the night in LKBKO\textsuperscript{livad} mice than in their control littermates, consistent with a positive energy balance (Fig. 2d–g). In addition, mutant mice displayed a whole-body shift in substrate preference toward higher levels of carbohydrate oxidation.

**Fig. 1 Short- and long-term phenotypes of Lkb1KO\textsuperscript{livad} mice.** a Immunoblot showing the efficient inactivation of Lkb1 in the livers of Lkb1KO\textsuperscript{livad} (KO, n = 3) mice and lower levels of Ampk phosphorylation compared to control litterate (WT, n = 3). β-actin is the loading control. Representative blot of four independent experiments. b-f Short-term phenotype, with impaired glucose homeostasis. Animals were analyzed 15 days after the injection of tamoxifen. b Blood glucose levels of fasted mice (WT: n = 14, KO: n = 16) and refeed (KO: n = 15, WT: n = 16). c Plasma insulin concentration in fasted (WT: n = 14, KO: n = 16) and refeed (WT: n = 10, KO: n = 13) mice. d Ratio of liver weight to total body weight (LW/TW) in fasted (WT: n = 14, KO: n = 16) and refeed (WT: n = 5, KO: n = 5) mice. e Periodic acid Schiff staining of sections of the liver from fasted mice (scale bar: 100 μm), and glycogen content in fasted (WT: n = 8, KO: n = 14) and refeed (WT: n = 3, KO: n = 3) mice. f Immunoblot of Pygl phosphorylation (pS15) and Gys2 levels in fasted and refeed mice. Quantification was performed with FUJI multigauge software and normalized to Gapdh, n = 3 mice per group. Representative blot of two independent experiments. g-j Long-term phenotype, with sarcopenia and cachexia. g Kaplan–Meier survival curves of mutant (KO, n = 29) and control (WT, n = 10) mice. h Gross morphology of a control littermate (WT) and a cachexic mutant (KO) showing a lack of fat mass (red arrow) and muscle sarcopenia (green arrow) in the mutant mice. i Total weight of female (KO: n = 4) and male (KO: n = 5) mutant mice at the time of euthanasia due to cachexia, relative to those of their control littermates (WT, female: n = 3, male: n = 4). j Weight of tibialis (TA) and gastrocnemius (GAS) muscles of male mutant mice (KO, n = 3) at the time of euthanasia due to cachexia, relative to those of their control littermates (WT, n = 4). All graphical data are means values ± SD. P values were determined by unpaired two-tailed t-test. *p ≤ 0.05; **p ≤ 0.01. Source data are provided as a Source data file.
Indeed, the respiratory exchange ratio (RER), a key indicator of substrate utilization, was consistent with an enhanced contribution of carbohydrates to energy expenditure specifically at the end of the day, at a time at which wild-type animals were preferentially making use of lipid oxidation (Fig. 2h and Supplementary Fig. 2). Analysis of the expression level of key ubiquitin ligases as well as Akt phosphorylation at Ser473 in the skeletal muscle of mutant mice strongly suggested that the decrease of the lean mass observed in mutant mice did not involve either catabolic or anabolic processes but rather reflects a switch in metabolic substrate utilization, consistent with an enhanced contribution of carbohydrates to energy expenditure (Fig. 2h).

**Lkb1 controls hepatic gluconeogenesis and amino acid catabolism.** Studies based on inhibition of the gluconeogenic program have characterized Lkb1 as a suppressor of hepatic gluconeogenesis7–10. Consistent with these findings, pyruvate tolerance tests revealed higher levels of gluconeogenesis in Lkb1KOlivad mice, associated with higher levels of expression for several of the target genes of Foxo-1, a master transcriptional activator of hepatic gluconeogenesis14,15 (Fig. 3a, b).

Given the complex metabolic phenotype of the mutant animals, which presented hyperglycemia, but with a defect in lean mass leading to sarcopenia in the long term, we performed proteomic analyses to decipher the role of Lkb1 in the control of liver metabolism. We used a label-free quantification (LFQ) method to characterize the liver proteome of Lkb1KOlivad mice and controls. This LFQ analysis was performed on the livers of fasted and refeed animals. We identified 177 (63 upregulated and 114 downregulated) and 322 (165 upregulated, 156 downregulated) proteins differentially expressed (DE) between the
liver of mutant and control mice in the fasted and re-fed states, respectively (−1<FC (Log2) >1, adjusted p value <0.05), (Supplementary Data 1 and Supplementary Data 2).

Ingenuity pathway analysis (IPA) identified gluconeogenesis and amino acid metabolism, and, more specifically, amino acid catabolism, as the top canonical pathways enriched in the DE proteins upregulated in the livers of fasted Lkb1 mutant mice, and in re-fed mutant mice (Fig. 3c, d). Most amino acids can be used as precursors of gluconeogenesis in the liver, as shown in Fig. 3e. In this scheme, we indicate the various enzymes involved in amino acid metabolism upregulated in the livers of the mutant animals. The heatmap of the DE proteins linked to amino acid catabolism (Tat, Pah, Hgd, Hpd, Kynu, Sds, Sdsl, Cth, Cbs, Pccb, Gnmnt, Mat1a, Ahcy, Aass), aminotransferases (Got1, Agxt, Gpt2), amino
acid uptake (Slc7a2, Slc25a15, Slc25a22, Slc38a3, Slc38a4) and the urea cycle (Oat, Glu2, Asl, Ass1) revealed a number of proteins deregulated in both the fasting and refeed states, indicating that Lkb1 downregulates the levels of proteins involved in amino acid catabolism regardless of feeding status (Fig. 3f and Supplementary Data 1, Supplementary Data 2). Western-blot analyses on primary hepatocytes from Lkb1KOlivad and control mice revealed an upregulation of proteins involved in amino acid catabolism similar to that observed with mouse liver tissues (Supplementary Fig. 3a). This finding indicates that Lkb1 controls amino acid catabolism in a cell-autonomous manner.

Furthermore, a heatmap of the critical enzymes of gluconeogenesis (Pc, Pck1, Fbp1, G6pc) showed a strong upregulation of their protein levels in the refeed state in mutant animals, highlighting the key suppressor role of Lkb1 in controlling hepatic gluconeogenesis not only during fasting periods, but also during the normal postprandial process (Supplementary Fig. 3b). These results highlight a new role for Lkb1 as a suppressor of hepatic amino acid catabolism, in addition to its known role as a suppressor of gluconeogenesis, not only during the fasting period but also during the processing of nutrients after food intake.

Lkb1 controls amino acid availability in hepatic gluconeogenesis. The higher levels of hepatic amino acid catabolism in Lkb1KOlivad mice led us to explore the capacity of the liver to produce glucose from amino acids in Lkb1-deficient mice.

The main fate of alanine in the liver is to support gluconeogenesis. We, therefore, performed alanine tolerance tests (ATT) and determined glucose levels in the bloodstream after the IP injection of a bolus of alanine in fasted mice. In WT animals, alanine injection had a significant and modest effect on plasma glucose concentration, which rapidly returned to normal levels. However, in Lkb1KOlivad mice, we observed a large increase in glycemia, which remained high for more than 3 h after alanine injection (Fig. 4a).

The results obtained for primary cultures of hepatocytes from Lkb1KOlivad mice provided further evidence of the greater ability of Lkb1-deficient hepatocytes than of wild-type hepatocytes to produce glucose from amino acids. Hepatocytes from mutant mice produced larger amounts of glucose following the addition of pyruvate and lactate to the culture medium, consistent with the known role of Lkb1 as a suppressor of hepatic gluconeogenesis. However, glucose production levels were also higher in Lkb1-deficient hepatocytes following the addition of the three gluconeogenic amino acids tested: alanine, glutamine, and serine (Fig. 4b).

Consistent with these findings, measurements of metabolic fluxes in liver explants from fasted Lkb1KOlivad mice and controls incubated with [14C]alanine showed that the hepatocytes of mutant mice performed both de novo glucose synthesis and glycogen synthesis from alanine more efficiently than those of wild-type mice (Fig. 4c).

This greater utilization of amino acids for de novo glucose synthesis in Lkb1KOlivad mice was associated with a lower plasma concentration of most amino acids and, for some, a lower hepatic content compared to controls (Fig. 5a, b). Surprisingly, alanine content was maintained in both the serum and liver, possibly due to the massive release of alanine by the muscles during fasting, at much higher levels than for any of the other amino acids. Given the role of the muscle as the most important source of amino acids for gluconeogenesis during fasting and the lower lean mass of mutant mice observed on MRI, we also determined the amino acid content of the tibia: in mutant mice, this muscle had an amino acid profile with deficits very similar to those observed in the liver (Fig. 5c). This lower muscle amino acid content is probably a direct consequence of the lower plasma amino acid concentration in these mice, as this concentration is known to affect protein turnover in muscle. Hepatic and plasma urea and ammonia levels were similar in Lkb1KOlivad mice and controls, suggesting that the capacity of mutant animals to clear the nitrogen released by amino acid catabolism was preserved (Fig. 5d).

Collectively, our data demonstrate an enhancement of the ability of the liver of Lkb1-deficient mice to synthesize glucose from amino acids during fasting, but also during feeding, with the storage of this glucose in the form of glycogen. Our data also provide strong evidence that the control, by Lkb1, of the use of amino acids for gluconeogenesis in the liver controls the partitioning of amino acids between the liver and muscle. Hepatic Lkb1 thus appears to be a major player in the regulation of whole-body amino acid metabolism through its control of the use of amino acids in hepatic gluconeogenesis.

Aminotransferases including Agxt are effectors of gluconeogenesis mediated by Lkb1. Aminotransferases are frequently involved in the first step of amino acid metabolism for glucose production. The aspartate aminotransferase Gt1 was one of the top-ranking upregulated DE proteins for all the nutritional states tested, and Gpt2 was also upregulated in the liver of reed mutant mice (Fig. 3f and Supplementary Data 1, Supplementary Data 2), identifying aminotransferases as potential critical effectors of the function of Lkb1 as a suppressor of amino acid-driven gluconeogenesis. We thus assessed the effect of amino-oxyacetic acid (AOA), a pan-aminotransferase inhibitor, on glucose homeostasis, in mutant and control animals in vivo. AOA treatment rescued the fasting hyperglycemia phenotype of mutant mice (Fig. 6a). Consistent with these data, AOA treatment led to a significantly lower peak blood glucose concentration in ATTs, and a faster return to basal levels than in untreated mutant mice (Fig. 6b).

We also investigated the involvement of alanine glyoxylate aminotransferase (Agxt) in the phenotype of Lkb1KOlivad mice. Indeed, Agxt was one of the four genes we identified as commonly induced in the liver of mice bearing the specific Lkb1 deletion, not
Fig. 4 Liver gluconeogenesis from amino acids in Lkb1KOlivad (KO) mice. a Blood glucose levels in alanine tolerance test (KO, n = 6, WT: n = 8). The area under the curve (AUC) of glucose level is shown. Mice were fasted for 24 h before the ATT. Data are presented as mean values ± SD. P values were determined by unpaired two-tailed t-test. **p ≤ 0.001. b Glucose production from glutamine, alanine or serine in primary cultures of hepatocytes. Glucose production was normalized against protein content and is expressed as a percentage of the glucose produced by WT hepatocytes incubated in the absence of gluconeogenic substrates. Data are means ± SD of six biologically independent cell culture. P values were determined by unpaired two-tailed t-test. **p ≤ 0.01; ***p ≤ 0.001. c [14C]-glucose and [14C]-glycogen synthesis from [14C]-alanine in cultured liver explants from fasted mutant (KO, n = 3) and fasted control (WT, n = 3) mice. Data are means ± SD. P values were determined by unpaired two-tailed t-test. **p ≤ 0.01. For all the experiments described in this figure, animals were analyzed 15 days after the injection of tamoxifen. Source data are provided as a Source data file.

**Lkb1 controls amino acid-driven gluconeogenesis via phosphorylation of RNA binding proteins.** We searched how Lkb1 may repress the hepatic amino acid-driven gluconeogenesis. We first looked at the possible involvement of Ampk. Using hepatocyte-specific Ampkα1/a2 null mice, we showed that Ampk did not control the expression of several enzymes of the amino acid metabolism such as Agxt, Got1 and Oat, strongly suggesting that Lkb1 controlled the hepatic amino acid-driven gluconeogenesis independently of Ampk (Fig.7a). To elucidate the mechanism by which Lkb1 suppress hepatic gluconeogenesis from amino acids, we used a quantitative phosphoproteomic approach to characterize the phosphoproteome of the liver of mutant Lkb1KOlivad in both the fasted and refeed state. Our comprehensive analysis identified 7851 phosphopeptides from 1952 phosphoproteins (4383 phosphopeptides for 1387 proteins in fasted liver and 4690 phosphopeptides for 1414 proteins in refeed liver). Differential expression analysis using Perseus software identified significant changes (t-test, p value <0.01) of differentially expressed (DE) phosphoproteins in the fasted and refeed state. We also retained all the phosphopeptides that were not quantified (designated NaN in Supplementary Data 4) in any of the KO samples whereas quantified in the WT samples, and vice versa (WT versus KO). They likely represent phosphopeptides that were dramatically controlled by Lkb1 (Supplementary

only in the adult liver (Lkb1KOlivad), but also in the embryonic liver21 (Supplementary Data 3, Supplementary Fig. 4a). Agxt is also known to be involved in the synthesis of glucose from serine, in addition to its role in glyoxylate detoxification22, as schematized on Fig.6c.

Western blotting and RT-qPCR analyses confirmed that Agxt was induced in the liver of mutant mice, in both the fasted and refeed states, at both the protein and mRNA levels (Fig. 6d, e).

We investigated Agxt function, by studying Lkb1- and Agxt-deficient DKO mice obtained by crossing AgxtKO mice bearing a complete inactivation of Agxt23 with Lkb1KOlivad mice (Supplementary Fig. 4b). DKO mice had a better fasting hyperglycemia phenotype than Lkb1KOlivad mice, but no improvement in postprandial hyperglycemia was observed highlighting that the function of Lkb1 may differ between the fasted and refeed state (Fig. 6f). Metabolic analyses of the DKO mice revealed no major changes compared to the Lkb1KOlivad mice, specifically for the glycogen content, indicating that the sole Agxt deletion is not sufficient to rescue the glycogen phenotype of Lkb1KOlivad mice (Supplementary Fig. 4c–e, g–i). Follow-up of the mice for a longer period revealed much higher survival rates for DKO mice than for Lkb1KOlivad mice, with the lethal cachexic phenotype developing significantly later in DKO mice (Fig. 6f).

Thus, both the pharmacological inhibition of aminotransferases and genetic Agxt inactivation partly rescued the fasting hyperglycemia phenotype of Lkb1KOlivad mice. The deletion of Agxt also delayed the premature death of Lkb1KOlivad mice. Overall, these results indicate that aminotransferases including Agxt are key effectors of the suppressor function of Lkb1 in amino acid-driven gluconeogenesis.
**Fig. 5 Plasma, liver, and muscle amino acid content of Lkb1KO^lived^ mice.**

a. Plasma amino acid concentration in fasted mutant (KO: n = 13, red bar) and fasted control (WT: n = 7, gray bar) mice.

b. Hepatic amino acid content of fasted mutant mice (KO: n = 10, red bar) and fasted control mice (WT: n = 9, gray bar).

c. Muscle (tibialis) amino acid content of fasted mutant mice (KO: n = 10, red bar) and fasted control mice (WT: n = 9, gray bar).

d. Ammonia and urea level in the plasma and ammonia level in the liver of Lkb1KO^lived^ (KO, n = 13, red bar) and controls (WT, n = 7, gray bar). All data are presented as mean values ± SD. P values were determined by unpaired two-tailed t-test. ns: not significant; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. For all the experiments described in this figure, animals were analyzed 15 days after the injection of tamoxifen.
 Altogether we found 739 DE phosphosites (639 downregulated, 100 upregulated) in the fasted state and 477 DE phosphosites (59 downregulated, 418 upregulated) in the refed state. Among the common molecules 78.5% have an opposite regulation. On a protein scale, this phosphosites matched with 415 DE proteins for the fasted state, 308 for the refed state with 121 common proteins. (Supplementary Data 4). As expected, in fasted mutants, we observed that most of the DE proteins had reduced phosphorylation, Lkb1 being a kinase. However, in the refed mutants, most of the DE proteins were hyperphosphorylated, highlighting that Lkb1 acts differently between the fasted and refed states (Supplementary Data 4). Importantly, less than 5% of the significantly regulated phosphopeptides were located on proteins whose expression levels were observed to

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**Fig. 6 Aminotransferases including Agxt are crucial effectors of the suppressor function of Lkb1 in amino acid-driven gluconeogenesis.**

- **a, b** Pharmacological inhibition of aminotransferase, in vivo, by AOA. Lkb1KOlovad (KO) and WT mice were left untreated (−) or were treated with AOA (10 mg/kg) 2 h before the determination of fasting blood glucose levels (a) or the alanine tolerance test (b). The area under the curve (AUC) of glucose level in the ATT is shown. AOA-treated animals, KO: n = 5, WT: n = 6. Animals not treated with AOA, KO: n = 5, WT: n = 4. Mice were fasted for 22 h before treatment with AOA or were left untreated for the same time period. **c** Scheme of the dual function of Agxt. **d, e** Agxt protein and mRNA levels in Lkb1KOlovad (KO) mice. **d** immunoblot of Agxt in fasted and refed mutant (KO, n = 3) and control (WT, n = 3) mice. Representative blot of two independent experiments. **e** Agxt gene expression levels, as assessed by RT-qPCR in fasted (KO: n = 10, WT: n = 6) and refed (KO: n = 7, WT: n = 5) mice. **f, g** Phenotypes of mice lacking both Lkb1 and Agxt in the hepatocytes (DKO), as compared with mice with a single deficiency of Lkb1 in hepatocytes (Lkb1KOlovad, KO) and controls (WT and AgxtKO). **e** Glycemia in fasting (WT: n = 11, KO: n = 19, DKO: n = 17, AgxtKO: n = 18) and refed (WT: n = 8, KO: n = 6, DKO: n = 10, AgxtKO: n = 12) mice. **f** Kaplan-Meier survival curves for Lkb1KOlovad mice (n = 18), mice lacking both Lkb1 and Agxt (DKO n = 23) and controls (WT, n = 10). All graphical data are means ± SD. P values were determined by unpaired two-tailed t-test. ns: not significant. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001. Source data are provided as a Source data file.

Data 4). Altogether we found 739 DE phosphosites (639 downregulated, 100 upregulated) in the fasted state and 477 DE phosphosites (59 downregulated, 418 upregulated) in the refed state. Among the common molecules 78.5% have an opposite regulation. On a protein scale, this phosphosites matched with 415 DE proteins for the fasted state, 308 for the refed state with 121 common proteins. (Supplementary Data 4). As expected, in fasted mutants, we observed that most of the DE proteins had reduced phosphorylation, Lkb1 being a kinase. However, in the refed mutants, most of the DE proteins were hyperphosphorylated, highlighting that Lkb1 acts differently between the fasted and refed states (Supplementary Data 4). Importantly, less than 5% of the significantly regulated phosphopeptides were located on proteins whose expression levels were observed to
change confirming that the majority of the observed changes in the phosphoproteome are not driven by changes in protein expression (Supplementary Fig. 5a). IPA analysis revealed an enrichment for proteins involved in the regulation of RNA post-transcriptional modification and protein synthesis (Fig. 7b), indicating that Lkb1 could control amino acid catabolism at a post-transcriptional level. Consistent with this result, we did not find any increase in the expression of genes encoding proteins involved in hepatic amino acid metabolism in a microarray analysis on the livers of fasted LKB1\textsuperscript{KO/livad} mice, except for Agxt (Supplementary Data 3), indeed suggesting a control of protein expression at the translational level. Because the control of mRNA translation relies around messenger ribonucleoprotein (mRNP) complexes, we focused on the RNA binding proteins

![Diagram](image-url)

**IPA for phosphoproteome of fasted mutant mice**

| Name                                           | p-value range     |
|------------------------------------------------|-------------------|
| RNA post-transcriptional modification          | 2.44E-06 – 3.43E-16 |
| Cellular death and survival                    | 1.36E-03 – 2.60E-14 |
| Cellular assembly and organization             | 1.34E-03 – 2.12E-12 |
| Gene expression                                | 5.06E-04 – 3.81E-11 |
| Cellular function and maintenance              | 1.34E-03 – 1.82E-07 |

**IPA for phosphoproteome of refed mutant mice**

| Name                                           | p-value range     |
|------------------------------------------------|-------------------|
| Cellular assembly and organization             | 2.36E-03 – 3.13E-09 |
| Cell death and survival                        | 1.36E-03 – 2.13E-09 |
| RNA post-transcriptional modification          | 1.36E-04 – 2.16E-09 |
| Cell death and survival                        | 2.32E-03 – 1.03E-08 |
| Cellular development                           | 2.16E-03 – 1.10E-07 |

**IPA for common phosphoproteome**

| Name                                           | p-value range     |
|------------------------------------------------|-------------------|
| Protein synthesis                              | 7.69E-03 – 5.53E-06 |
| Cellular movement                              | 1.57E-02 – 9.67E-06 |
| Cellular assembly and organization             | 1.64E-02 – 1.22E-05 |
| Cellular function and maintenance              | 1.56E-02 – 1.22E-05 |
| RNA post-transcriptional modification          | 5.88E-04 – 2.35E-05 |

**Refed**

![Graph](image-url)
RBP (RNA binding protein) that are key actors of the mRNP complexes. Since mRNP are highly dynamic and phosphorylation is a key regulatory mechanism for mRNP remodeling, the identification of the phosphosites of the RBP that are controlled by Lkb1 appears to be an interesting clue to understand how Lkb1 control the hepatic amino acid catabolism at the translational level. We compared the DE phosphoproteins identified in the fasted and refed state to the canonical RBP repertoire, published by Beckmann et al. and found a strong enrichment in RBP in both the fasted and refed state (Fig. 7c). 22 RBP were common to the fasted and refed state although with different phosphosites and opposite regulation for 82% of them (Supplementary Data 4). Heatmap of the significantly differentially phosphorylated RBP confirmed that most of the phospho-RBP controlled by Lkb1 were downregulated in the fasted liver while they were upregulated in the refed liver and involved different phosphopeptides for each common phosphoproteins (Fig. 7d, Supplementary Fig. 5b). Interestingly, we found in the phosphoprotein network of Lkb1 many regulators of translation including eukaryotic initiation and elongation factors (Eif3b, Eif3g1, Eif4b, Eif4g1, Eif4g2, Eif5b, Eef1d) as well as factors such as Larpl, Larp4, Lsm14, Npm1, Nolci, Pum1, Ybx1 (Fig. 7d and Supplementary Data 4). As a further step towards identifying a regulatory link between Lkb1-dependent amino acid catabolism and RBP biology, we constructed a network map integrating the interactions between: (1) Lkb1, (2) the differentially phosphorylated RBP and (3) the differentially phosphorylated kinases and phosphatases, (4) the proteins of amino acid catabolism and involved in gluconeogenesis that are controlled at the translational level. This approach identified three main clusters of interactions, i.e Lkb1 with the phosphorylated kinases/ phosphatases, the phosphorylated RBP and the Lkb1 metabolic targets (Supplementary Fig. 5c). This analysis revealed distinct mechanisms by which Lkb1 controls expression of its metabolic targets in the fasted and refed state. While direct interactions between Lkb1 and phosphorylated RBP, such as Hsp90aa1 as well as indirect paths involving kinases could be observed in the fasted state, only indirect paths could be observed in the refed state between Lkb1 and the phosphorylated RBP (Supplementary Fig. 5c).

Agxt performs different functions of glyoxylate detoxification and neoglucogenesis depending on its location in peroxisomes and mitochondria. In the mitochondria, it is mainly involved in neoglucogenesis. The variable localisation of Agxt depends on its location in peroxisomes and mitochondria. In the mitochondria, it is mainly involved in neoglucogenesis. The variable localisation of Agxt depends on its location in peroxisomes and mitochondria. In the mitochondria, it is mainly involved in neoglucogenesis.
amino acids is a normal postprandial process not limited to fasting periods.\textsuperscript{3,31}

One unforeseen consequence of unrestrained hepatic gluconeogenesis from amino acids was the impact on muscle physiology observed in mice with a hepatocyte-specific Lkb1 deficiency. Our data indicated that the decrease in lean body mass was neither associated with activation of catabolic pathways nor changes in insulin signaling in skeletal muscle. This led us to hypothesize that the decrease in lean body mass observed in the mutant animals is an indirect consequence of the higher levels of amino acid extraction for glucose synthesis in these animals, leading to a decrease in plasma amino acid concentration. Indeed, plasma amino acid availability has been shown to make a major contribution to muscle protein turnover. High amino acid availability, as observed in the postprandial state, favors muscle anabolism by activating protein synthesis through control over the translation initiation step of protein synthesis,\textsuperscript{32} whereas low amino acid availability leads to an inhibition of muscle protein synthesis, resulting in a net release of amino acids from the muscle.\textsuperscript{33} Indeed, mice with a knockout of the Klf15 transcription factor, an important regulator of branched-chain amino acid catabolism,\textsuperscript{34} have low levels of hepatic amino acid catabolism associated with high hepatic and plasma amino acid concentrations, resulting in a significantly higher lean mass than observed in control animals.\textsuperscript{35} This mutant is, thus, the mirror image of Lkb1KO\textsuperscript{livada} mice. Similarly, glucagon is known to stimulate hepatic amino acid catabolism for gluconeogenesis;\textsuperscript{36} in all models of glucagon deficiency, the phenotype of the mutant mice mirrors that of Lkb1KO\textsuperscript{livada}, with the operation of a similar amino acid catabolism program and increases in the levels of most amino acids in the plasma, and many amino acids in the liver.\textsuperscript{37–39} However, the impact on muscle is less striking in these mutants, which display only a slight increase in lean mass.\textsuperscript{40} However, we cannot exclude the possibility that mechanisms other than amino acid availability per se are involved in these modifications to relationships between the liver and muscle observed in Lkb1KO\textsuperscript{livada} mice; indeed, hepatic Lkb1 may control the synthesis of hepatokines acting on peripheral tissues.

The accumulation of glycogen in fasted Lkb1KO\textsuperscript{livada} mice was surprising and revealed a defect in glycogen use during fasting in these mice. Fasted mutant mice displayed much lower levels of phosphorylation of the glycogen phosphorylase (Pygl), indicative of lower levels of glycogen degradation. The reason for this inactivity of Pygl remains unknown, but hyperglycemia has been shown to be a strong suppressor of glycogenolysis.\textsuperscript{41} Of note, we observed an increase in the glycogen synthesize (Gys2) content both in the fasted and fed state in the liver of mutant animals (Fig. 1f and Supplementary Data 1, Supplementary Data 2). Our data indicate that the activity of Gys2 may not be controlled by a phosphorylation event, but only by an increase in Gys2 total protein level. In the fasted state, Gys2 was not identified as differentially phosphorylated between mutant and control in our quantitative phosphoproteomic analysis. In the refed state, we found an increase in Gys2 phosphorylation at the Ser11 site in mutant liver, but this phosphosite is described as inhibitory for Gys2 activity (Supplementary Table 4). Flux analyses with 14C-labeled alanine demonstrated higher levels of glycogen synthesis in fasted Lkb1-deficient hepatocytes, revealing the ability of these cells to synthesize glycogen from alanine via the indirect pathway.\textsuperscript{2} Thus, both a decrease in glycogenolysis and an increase in glycogen synthesis explain the persistence of glycogen during fasting in these mutants. The enhanced rerouting of amino acids to liver glycogen observed in mutant animals probably explains the moderate increase in hyperglycemia observed in these animals despite unrestricted amino acid-driven gluconeogenesis.

Our data showed that Lkb1 controls hepatic amino acid-driven gluconeogenesis both in the fasted and refed state. These results highlighted a critical role of Lkb1 in the control of postprandial gluconeogenesis. Although postprandial gluconeogenesis is critical for the body to cope with excess of dietary amino acids, this aspect and the contribution of Lkb1 in its control have been poorly studied. We have shown that it is independent of the Ampk signaling. The in silico analysis of our phosphoproteomics data sets identified a Lkb1-dependent network of phosphorylated RBP that supports the control of the amino acid catabolism at the translation level. Interestingly, the mechanism by which Lkb1 controls liver gluconeogenesis from amino acids during the fasting state differ from that used during postprandial gluconeogenesis. Even if similar phosphoproteins are used in the two nutritional states, the phosphorylation sites and the regulation were clearly distinct.

We identified aminotransferases as effectors of the function of Lkb1 in the control of amino acid-driven gluconeogenesis. The fasting hyperglycemia phenotype of the mutant mice was partially rescued by treatment with a pan inhibitor of aminotransferases, AOAA, consistent with the key role of aminotransferases which are frequently involved in the first step of amino acid catabolism. Consistent with our data, a recent study showed that inhibition of the hepatic alanine transaminase reduced amino acid gluconeogenesis and was associated with a reduced postprandial blood glucose and the rescue of the hyperglycemia phenotype of different models of diabetes.\textsuperscript{42} In addition our data highlight a role for Agxt a serine-pyruvate transaminase involved in both gluconeogenesis from serine and glyoxylate detoxification. AGXT mutations cause primary hyperoxaluria,\textsuperscript{43} however, no changes of glucose metabolism have been reported in patients with such mutations. In our experiments, Agxt deletion partially rescued the fasting hyperglycemia phenotype of Lkb1KO\textsuperscript{livada} mice. We were able to monitor the phenotype of the DKO mice, in which the Agxt gene was deleted, over longer time periods. Agxt deletion greatly delayed the development of cachexia in Lkb1KO\textsuperscript{livada} mice. This result highlights the crucial role of gluconeogenesis deregulation in the development of the lethal phenotype in mutant mice. Interestingly, recent studies have identified transaminases as effectors of other metabolic functions of Lkb1 involved in the epigenetic reprogramming occurring during malignant transformation,\textsuperscript{44} or during the glial fate specification of neural crest cells.\textsuperscript{45} Together with our data, these results highlight a key unsuspected role of aminotransferases in Lkb1 function.

Hepatic gluconeogenesis is frequently deregulated in DT2M, so the identification of Lkb1 as a suppressor of hepatic amino acid-driven gluconeogenesis should open up new avenues for the treatment of DT2M.

Methods

Ethical compliance statement. All animal procedures were carried out according to legal regulations (Ministère de la Recherche, de l’Enseignement Supérieur et de l’Innovation) and approved by ethics committee at the University Paris Descartes (Project APAFIS 8722 and 8612).

Mice. Mice with an inducible specific deletion of Lkb1 in hepatocytes (Lkb1KO\textsuperscript{livada} mice) were obtained by crossing Lkb1fl/fl mice (FVB/N background)\textsuperscript{46} with TTR-CreTam (FVB/N background) mice expressing an inducible Cre-recombinase under the control of the hepatocyte-specific transthyretin promoter.\textsuperscript{21} The control mice for this study were their Cre-negative littermates (Lkb1fl/fl, Cre- ). AgktKO mice (C57Bl6/N background) were a gift from Anna Verbast (University of Antwerp, Belgium) and Eduardo Salido (Tenerife, Spain).\textsuperscript{33} The DKO mice were obtained by crossing LKBBKO\textsuperscript{livada} mice with AgktKO mice. Liver double knockout of Ampk1α and Ampk2α catalytic subunits was achieved by crossing Ampk1αlox/lox (C57Bl6/N background) mice with Ampk2αlox/lox mice (C57Bl6/N background) then Alfp-Cre transgenic mice (C57Bl6/N background) to generate Ampk1αlox/lox,α2lox/lox (control) and Ampk1αlox/lox,α2lox/lox-Alfp-Cre (liver Ampk1α/2 KO) mice.\textsuperscript{44}
Mice were housed in colony cages in SPF conditions, under a 12-h light/12-h dark cycle, in a controlled-temperature environment (21 °C) with 30% humidity. They were fed ad libitum with a standard laboratory diet (SD, 65% carbohydrate, 11% lipids, and 24% proteins; SAFE 03, FRANCE). In most cases, animals were studied either directly after 18 h of fasting (fasted state) or 4 h after the initiation of refeeding with a Chow diet (reefed state). For long-term experiments, animals were fed with a standard diet ad libitum.

We injected two doses of tamoxifen (1.5 mg/mouse of tamoxifen Sigma-Aldrich, T5648) in corn oil IP into 8- to 12-week-old mice. Most studies were performed on male mice 15 days after injection. For the survival analysis, both males and females were monitored for up to 300 days. For all experiments, age-matched control CD1 mice were used as controls. For short-term experiments, livers were removed after each experiment and frozen in liquid nitrogen. For information on the number of animals in each experiment, please refer to the corresponding figure legend.

For AOA (O-(carboxymethyl)hydroxylamine hemichloride, Sigma-Aldrich C13408-1G) was dissolved in PBS, and injected into the animals via the IP route (10 mg/kg) 2 h before blood glucose determinations or alanine tolerance tests.

Serum and liver biochemistry. Serum samples were obtained by the immediate centrifugation of blood collected from the orbital sinus into heparin or EDTA. Plasma for biochemical or hormonal analyses was stored at −70 °C until use. Insulin and glucagon were determined with the MO DIABETES INSULIN SET and MO DIABETES GLUCAGON SET (BIO-RAD, respectively), by immunonassay on a Luminex apparatus (Biorad). The kits for determining alanine aminotransferase (ALT) and triglyceride (TG) levels were purchased from DiaSys. Capillary blood glucose level was determined with an Accu-Check II Glucometer (Roche Diagnostics).

Glycogen quantification. The liver was homogenized and deproteinized by treatment with 4% (w/v) perchloric acid treatment. Hepatic glycogen content was then assessed as described by Roehrig and Allred. Glycogen was first hydrolyzed with u-amylglucosidase, and the glucose generated was then converted into 6-phospho-D-gluconolactone by hexokinase treatment followed by glucose-6-phosphate dehydrogenase treatment in the presence of NADP. NDPPI production was then assayed by spectrophotometry at 340 nm.

Glucose, pyruvate, and alanine tolerance tests. The IP glucose tolerance test (GTT) was performed on fasted male mice. Animals received an IP injection of 2 g of glucose/kg body weight (BW). Blood was drawn from the tail vein. Blood glucose levels were determined at various time points after glucose injection with the Accu-Check II Glucometer (Roche Diagnostics). Similar protocols were used to perform pyruvate tolerance test (PTT) (injection of 2 g pyruvate/kg BW) and the alanine tolerance test (ATT) (injection of 2 g alanine/kg BW). The fasting period was 18 h for GTT and PTT, but 24 h for ATT.

Analyses of energy expenditure and body composition. Mice were analyzed for total energy expenditure (EE), oxygen consumption (VO2) and carbon dioxide production (VCO2), respiratory exchange rate (RER, VCO2/VO2), food intake (g), and energy expenditure (kcal/h). Subsequently, each value was expressed by total body weight or total lean body mass measured by EchoMRI. Body mass composition (lean tissue mass, LBM), fat mass, free water and total body water content were analyzed by MRI with the EchoMRI 100 system (Whole Body Composition Analyzers, EchoMRI, Houston, USA) according to the manufacturer’s instructions.

Analysis was performed in Excel XP, on the extracted raw value of VO2 (ml/h), VCO2 (ml/h), and energy expenditure (kcal/h). Subsequently, each value was expressed by total body weight or total lean body mass measured by EchoMRI.

For the respirometry analysis of the DKO mice compared to KO mice, metabolic rates were measured using an 8-cage Promethion metabolic phenotyping system (SSI) as described in Lark et al. Animals had free access to drink and food hoppers in an ambient temperature of 22 ± 0.5 °C with light from 7 am to 7 pm. Mouse cage behavior, including roaming (XZY beam breaks), food and water intake (to 1 mg) were monitored continuously at a sample rate of 1 sample/sec for all sensors and cages simultaneously via an error-correcting control area network (CAN). Air was pulled from the cages at a controlled mass flow rate of 2 L/min. O2 and CO2 were continuously monitored for assessment of EE. Air calibration was made accordingly to the manufacturer using a 100% nitrogen as zero reference and with a span gas containing of known concentration of CO2 (Air Liquide, S.A. France). Data were stored in raw, unprocessed form for later analysis using analysis scripts run on ExpeData analytical software (SSI). This allowed complete and traceable control of the analytical process, the equations used, the baseline algorithms employed, and all other aspects of data transformation and final data extraction.

Immuno blot analysis. Total protein extracts were obtained from 100 mg of frozen mouse liver or from mouse primary hepatocytes homogenized in lysis buffer (50 mM Hepes, 150 mM NaCl, 5 mM EDTA, 30 mM NaF, 50 mM NAM (1% Triton, 1 mM DTT, protease inhibitor cocktail (Pierce #32933, Thermo Fisher Scientific)) supplemented with phosphate inhibitor cocktail (Pierce #8867, Thermo Fisher Scientific) in a bead mill, with the Tissue Lyser disruption system (Qiagen, Hilden, Germany). Proteins were resolved by SDS–PAGE, transferred to nitrocellulose and blocked by incubation with 5% BSA or 5% milk. Blots were incubated with specific primary antibodies overnight at 4 °C, washed, incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (Cell Signaling) and developed by enhanced chemiluminescence techniques (Amer sham). Immunoblot bands were captured using the ImageQuant Technologies (LRK1: clone D60CS, 1:1000; p-Ampk (T172), 50081, 1:1000; Ampk, 5831, 1:1000; Akt, p-Akt (S473), 4060, 1:1000; Gys2, 3886, 1:1000, Abcam (Oat, ab113769, 1:200; Aggt, ab178708, 1:2000), and Santa Cruz (Gadph, FL-335, 1:1000; Got1, sc-515641, 1:10000; p-Pygl (S15), 1:100 was obtained from MRC PPU reagents and services. The antibody against Agt for the immune gold analysis was from Abcam, ab178708, 1:200).

The bands on the immuno blots were quantified with FUJi multi gauge software after incubation with a peroxidase-coupled secondary antibody and densitometric analysis.

Histology and immunohistochemistry. Mouse livers were cut into 3-mm-thick sections and fixed by incubating for 5% BSA and 5% milk. Blocks were incubated with specific primary antibodies overnight at 4 °C, washed, incubated with the corresponding peroxidase-conjugated secondary antibody and densitometric analysis.

RNA extraction and quantitative real-time PCR analysis. Total RNA was extracted from mouse tissues and cell lines with Trizol Reagent (Life Technologies) according to the manufacturer’s protocol. Reverse transcription was performed with 1 μg of total RNA and the Transcripter First Strand cDNA Synthesis Kit (Roche Diagnostics), with random hexamer primers. Quantitative PCR was performed on a Light Cycler 480 thermocycler (Roche). RNA levels were calculated with the 2-ΔCt method, with 18S as the internal control, relative to RNA levels in control littersmates. The PCR primers used are: Ppa1gatc5, 5′- TGAAGAGGGCCAAACAGAGAG-3′ (forward) and 5′-GTAATACTCA- GCCGGCTCTT-3′ (reverse); G6pc, 5′-TGTCGAGGGACGG-3′ (forward) and 5′-ACACAGCCCTTTCGT-3′ (reverse); Aggt2l, 5′-CACCTGGGCGGATGAATA-3′ (forward) and 5′-AGCACACGAGGAAACACT-3′ (reverse); Mnd2, 5′-CTGGGCAAGCATGGTGTTG-3′ (forward) and 5′-ATGGTGAGGATGTA-3′ (reverse); Pkh1, 5′-GGACCGCTTCTGTTTGGT-3′ (forward) and 5′-GCAAATTCTCGAG-3′ (reverse).

Microarray analysis. The cDNA was purified and fragmented. We checked for fragmentation with a 2100 Bioanalyzer, and the cDNA was then end-labeled with 153 biotin, using terminal transferase (WT terminal labeling kit, Affymetrix). The cDNA was then hybridized to GeneChip Mouse Gene 2.0 ST Arrays (Affymetrix) at 45 °C for 17 h. The chips were washed on a FS450 fluidic station FS450, according to specific protocols, and scanned with a GCS3000 7G. The image was analyzed with Expression Console software to obtain raw data (CEL files) and metrics for quality control. The data obtained have been deposited in the Gene Expression Omnibus (GEO) database. Microarray data were analyzed with R-based BRR-Array Tools, as described in Just et al.

Mass spectrometry (MS) for label free protein quantification (LFQ) and phosphoproteomics. We ground and homogenized 50 mg of liver tissue in 500 μl Tres/SDS buffer (Tres/SDS buffer: 50 mM Tris/HCl pH 8.5, 2% SDS) in an Ultra Turrax apparatus. The homogenate was incubated for 5 min at 95 °C, and centrifuged at 12,800 g for 15 min. The supernatant concentration of the supernatant was determined in a bicinchoninic acid assay (BCA, Pierce). We reduced and alkylated 50 μg of protein with 20 mM TCEP (tris(2-carboxyethyl)phosphine) and 50 mM chloroacetamide.
variable modifications of STY, acetylation of the protein N-terminus and methionine oxidation were set as conditions for razor peptides, and required at least two such peptides. Statistical analysis and quantification were performed using Maxquant version 1.6.6.0. The database used was a concatenation of murine sequences from the UniProt-SwissProt database (UniprotKB, release 2015-02). All proteomic data that support the finding of this study have been deposited in the repository PRIDE with the accession numbers PXD013478, PXD019755. The sequencing data that support the functional analyses of both proteomic and phosphoproteomic data were generated through the use of IBA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenyway-pathway-analysis, v49932394, Release Date: 2019-11-14). Enrichments were performed using overrepresentation analysis validated by a right-tailed Fisher’s exact test. The RPB list was added to the IPA database as a new term.

Interaction combined score > 0.4 was regarded as significant. Heatmap were done using the genesis software (https://genome.tugraz.at). Multigauge V3.0 (Fujifilm) for quantification of western blot.

Phosphoproteome analysis. Phosphopeptides were purified according to Humphrey et al. Briefly, livers were minced and homogenized in homogenization buffer (100 mM Tris/HCl, pH 8.00 containing 6 M guanidinium chloride). Proteins were quantified with the bicinchoninic acid assay and reduced by heating to 95 °C for 2 min with 10 mM TCEP and 40 mM chloroacetamide. Proteins were precipitated with cold acetone, resolubilized in 100 mM bicarbonate ammonium solution containing 10% trifluoroethanol and digested overnight with 60 µg trypsin. KCl, KH2PO4, AC Na and TFA were added to final concentrations of 300 mM, 5%, 1%, and 1% respectively. Samples were centrifuged at 20,000 × g for 10 min at room temperature and transferred to 30 µl TiO2 bead pellets. Samples with TiO2 beads were incubated for 5 min at 40 °C with continuous agitation, beads were recovered by centrifugation, washed with 50% ACN containing 1% TFA and transferred to C8 StageTips in 80% ACN containing 0.5% acetic acid. Peptides were eluted with 50% ACN containing 15% NH4OH and immediately acidified by TFA to pH 2. Peptides were dried and quantified by ion exchange chromatography with post-column ninhydrin derivatization, with a JLC-500/5 AminoTac amino acid analyzer (Jeol, Croissy sur Seine, France).

Data availability. The proteomic data were obtained using the Uniprot-Swissprot database (Uniprot, release 2015-02). All proteomic data that support the finding of this study have been deposited in the repository PRIDE with the accession numbers PXD013478, PXD019757, and PXD019755. The sequencing data that support the finding of this study have been deposited in the Gene Expression Omnibus with the accession number GSE75564 already described in21 and with the accession number GSE132536. Uncropped scans of Western blots are included in the Source Data file. Source data are provided with this paper.

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Author contributions
C.P., P.A.J., S.C., and P.B. conceived and performed experiments. C.P. and B.R. secured funding. C.P., P.B., and J.P.D.B., wrote the manuscript. R.G.P.D. and S.L. performed experiments for MRI investigation. M.I., F.G., and P.M. performed experiments for proteomic analysis. M.I.G. and C.P. performed ontology and data analyses, J.P.D.B., and S.M. performed the amino acid determinations. M.S., M.T., S.B., N.S., P.S., M.W., M.F., and A.S. performed experiments. M.V.C. provided the TTR-CreTam mice. All the authors approved the final version for submission.

Competing interests
The authors declare no competing interests.

Additional information
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