The tumor suppressor TMEM127 regulates insulin sensitivity in a tissue-specific manner

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Understanding the molecular components of insulin signaling is relevant to effectively manage insulin resistance. We investigated the phenotype of the TMEM127 tumor suppressor gene deficiency in vivo. Whole-body Tmem127 knockout mice have decreased adiposity and maintain insulin sensitivity, low hepatic fat deposition and peripheral glucose clearance after a high-fat diet. Liver-specific and adipose-specific Tmem127 deletion partially overlap global Tmem127 loss: liver Tmem127 promotes hepatic gluconeogenesis and inhibits peripheral glucose uptake, while adipose Tmem127 downregulates adipogenesis and hepatic glucose production. mTORC2 is activated in TMEM127-deficient hepatocytes suggesting that it interacts with TMEM127 to control insulin sensitivity. Murine hepatic Tmem127 expression is increased in insulin-resistant states and is reversed by diet or the insulin sensitizer pioglitazone. Importantly, human liver TMEM127 expression correlates with steatohepatitis and insulin resistance. Our results suggest that besides tumor suppression activities, TMEM127 is a nutrient-sensing component of glucose/lipid homeostasis and may be a target in insulin resistance.

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Although the mechanisms of insulin action have been extensively studied, how tissues communicate to control the actions of insulin in glucose homeostasis is not completely understood. Characterization of the molecular components of this inter-tissue interaction is relevant to understanding mechanisms of insulin resistance. This is a significant step toward addressing the growing epidemic of clinically relevant complications of insulin-resistant states in humans, including type 2 diabetes and nonalcoholic fatty liver disease.

The TMEM127 gene encodes a highly conserved transmembrane protein that has no ascribed function. We discovered inactivating TMEM127 mutations in the neuroendocrine tumors pheochromocytomas and, rarely, in renal carcinomas. TMEM127 colocalizes with intracellular endomembranes, including early and late endosomes and lysosomes, and loss of TMEM127 affects early to late endosomal vesicle progression, suggesting that TMEM127 may function in endocytosis and TMEM127 colocalizes with intracellular endomembranes, including early and late endosomes and lysosomes, and loss of TMEM127 affects early to late endosomal vesicle progression, suggesting that TMEM127 may function in endocytosis and TMEM127 forms complexes with lysosomal components of the mTORC1 pathway, a critical regulator of cell homeostasis.

To understand TMEM127’s contribution to whole-organism physiology we generated whole-body Tmem127 knockout mice (Tm-CMV-KO). These mice have reduced adiposity and are protected from age- and diet-dependent insulin resistance. Mice with liver-specific Tmem127 deletion (Tm-LKO) are also insulin sensitive and have reduced fat mass; unlike Tm-CMV-KO, Tm-LKO have increased hepatic lipogenic potential. In contrast, adipose-specific Tmem127 knockout mice (Tm-AKO) are insulin-resistant. Mechanistically, hepatic Tmem127 promotes hepatic gluconeogenesis and inhibits peripheral glucose uptake, whereas adipose Tmem127 inhibits both adipogenesis and hepatic gluconeogenesis. Both human and mouse liver TMEM127 expression correlate with states of insulin resistance and advanced hepatic steatosis, suggesting that TMEM127 may be a relevant target for treating insulin resistance and its hepatic complications.

### Results

**Whole-body Tmem127 null mice are small with low adiposity.** TMEM127 is ubiquitously expressed in both mice and humans (Supplementary Fig. 1A). We generated Tmem127 knockout (KO) mice by crossing mice carrying floxed Tmem127 alleles (Flx) with mice expressing Cre recombinase under a generic (CMV) promoter (Tm-CMV-KO, Fig. 1a). Loss of Tmem127 was confirmed by real-time PCR (Supplementary Fig. 1B). Mating of heterozygous Tmem127 mice produced offspring with a modest decrease in the expected number of KO mice (Supplementary Table 1, \( p = 0.03 \)) not detectable during embryogenesis.
Thermogenesis is impaired in TM-CMV-KO mice. In humans, germline loss-of-function TEMEM127 mutations confer susceptibility to neuroendocrine, sympathetic nervous system–derived tumors (pheochromocytomas) and renal cancers. However, TM-CMV-KO had no increased incidence of these, or other, tumors. Elevated catecholamines (epinephrine or norepinephrine), a hallmark of pheochromocytomas, increase lipolysis and contribute to weight loss in these patients. However, we did not find high epinephrine or norepinephrine levels in plasma (Supplementary Fig. 1F) or adrenals of the KO mice. In addition, unlike our previous observation in pheochromocytoma patients, we found no evidence of mTORC1 target upregulation in the KO mice adrenals (Supplementary Fig. S1G). Thus, the decreased body weight and adiposity of TM-CMV-KO were not the result of systemic hyperadrenergic levels or cancer-wasting effects.

Next, we evaluated caloric intake and energy expenditure, key contributors to reduced fat mass. We found that TM-CMV-KO food consumption was not significantly different from WT mice, but the KO had a small increase in resting metabolic rate (Table 1), consistent with higher energy expenditure. Two important components of energy expenditure are adaptive thermogenesis and physical activity. To determine whether TM-CMV-KO mice had enhanced thermogenesis, we examined their body temperature. Resting body temperature did not differ between WT and KO mice, but 24 h fasting resulted in a greater reduction in core body temperature in the KO mice (Fig. 1d). Moreover, serum levels of Fg21, a hormone known to increase in fasting states and enhance lipolysis and thermogenesis, were significantly lower in the KO mice in the fed state and remained lower than WT after 24 h of fasting and 4 h after refeeding (Supplementary Fig. 1H). We also found no upregulation of the thermogenic marker uncoupling protein-1 in brown and white adipose tissues of KO mice (Supplementary Fig. 11). Both the mass (Fig. 1e) and size (Supplementary Fig. 1K) of inguinal and epididymal white fat (iWAT and eWAT, respectively) were markedly smaller in TM-CMV-KO null than in WT mice. Histologically, we observed reduced size of brown and white adipocytes, but no morphological aberrations (Supplementary Fig. 1J). We concluded that increased thermogenesis does not account for the higher metabolic rate of TM-CMV-KO mice and, in fact these mice have an impaired thermogenic response to fasting. Excessive physical activity can also account for reduced fat mass. However, we found instead that TM-CMV-KO mice displayed significantly reduced locomotor activity (Table 1).

**Table 1** Calorimetry features of adult Tmem127 knockout mice

| Measurement        | WT                  | KO                  | Student’s t test | ANCOVA |
|--------------------|---------------------|---------------------|------------------|--------|
| RMR (ml/hr/glbm)   | 1.636 ± 0.07 (n = 7)| 1.973 ± 0.128 (n = 6)| P = 0.05        | P = 0.09|
| Food consumption (kcal/mouse/day) | 13.97 ± 1.5 (n = 16) | 12.16 ± 0.65 (n = 32) | P = 0.31 | P = 0.3 |
| Locomotor activity (beam breaks, total count) | 29.206 ± 2399 (n = 5) | 20.359 ± 1315 (n = 5) | P = 0.01 | P = 0.03 |
| RQ                 | 0.948 ± 0.014 (n = 7) | 0.961 ± 0.006 (n = 6) | P = 0.43        | P = 0.6 |

Data are presented as mean ± standard error of the means (SEM). Number of mice used in each experiment (n) is indicated. Measurements obtained from males, ages 9–17 mo under a regular diet. RMR resting metabolic rate, calculated from the five lowest values of the adjusted oxygen consumption (VO2) measured in ml/hr/glbm (grams of lean body mass), RQ respiratory quotient, calculated by dividing carbon dioxide consumption (VCO2) by VO2 (RQ VO2/VCO2). ANCOVA analysis of covariance.
test was enhanced in the null animals (Fig. 3e). Together, the ability of Tm-CMV-KO mice to maintain normal glycemia in the presence of low insulin levels suggests that global Tmem127 loss leads to increased insulin sensitivity in vivo, and the hypoinsulinemia might contribute to the lower body mass of these mice.

We found that both fasting glucose (Supplementary Fig. 3B) and insulin (Supplementary Fig. 3C) levels tended to be lower in the younger Tm-CMV-KO animals compared with age-matched controls. However, unlike adult mice, glucose (Supplementary Fig. 3D) and insulin (Fig. 3f, Supplementary Fig. 3E) tolerance
was indistinguishable from WT in the younger cohort despite the lower body weight of KO mice. Interestingly, Tmem127 mRNA expression was higher in liver from older WT mice than younger animals (Fig. 3g). This profile suggests that Tmem127 expression might contribute to the age-related increase in insulin resistance.

The lower blood glucose of Tm-CMV-KO mice could be due to increased glucose uptake and metabolism by the liver and/or peripheral tissues, reduced hepatic gluconeogenesis or a combination of both. We measured the expression of genes in these pathways but found few changes in Glut4 expression or in the genes involved in glycolysis in WAT (Supplementary Fig. 4A), but a trend was noted toward increased Tm-CMV-KO Tmem127 but a trend was noted toward increased gene expression, suggestive of glycolysis, in muscle (Supplementary Fig. 4B) from Tmem127 KO mice. In the liver, Tm-CMV-KO had reduced expression of glucose transporter Glut2 and glycolytic (G6pase, G6pc) and pyruvate dehydrogenase (Pepck) genes (Fig. 4a), consistent with reduced hepatic glucose flow. The response of Tm-CMV-KO to a pyruvate challenge was significantly lower than that of controls (Fig. 4b), suggesting reduced gluconeogenic potential, even though expression of genes involved in gluconeogenesis was not changed under fed conditions (Fig. 4a). To further evaluate gluconeogenesis, we subjected the Tm-CMV-KO mice to overnight fasting, a condition expected to trigger a hepatic gluconeogenic response. Tmem127 KO mice displayed increased glucose clearance after overnight fasting (Fig. 4c). Moreover, the ability to initiate transcrion of G6pase (G6pc) and Pepck1 (Pck1) after 24-hour fasting was markedly attenuated in these mice (Fig. 4d). Based on these findings, we concluded that hepatic gluconeogenesis is diminished in mice lacking Tmem127.

We next analyzed liver lysates for phosphorylation of Akt, a key mediator of insulin signaling. Akt phosphorylation at serine 473 was higher in the liver lysates of KO mice (Fig. 4e). Serine 473 is phosphorylated by mTORC2, a critical mediator of insulin sensitivity in the cells. Akt phosphorylation was markedly higher in liver and muscle of null mice (Fig. 4g), in support of increased hepatic and peripheral insulin sensitivity.

**Tmem127 deletion protects from liver steatosis and insulin resistance.** We next asked whether Tmem127 deficiency would confer resistance to diet-induced obesity and its accompanying metabolic disruptions. We fed a cohort of WT and Tm-CMV-KO mice high-fat diet (HFD-60% kcal from fat) for 16 weeks. This regimen led to marked weight gain in both the WT and Tm-CMV-KO mice (Fig. 5a). In fact, the Tm-CMV-KO weight gain surpassed that of control animals, relative to their initial body weight (Supplementary Fig. 5B), indicating that the ability to expand fat depots was maintained in the null mice. The body weight-adjusted food intake was not significantly different between the two genotypes (Supplementary Fig. 5C), supporting higher feed efficiency (weight gain per calorie intake) of the null mice (Supplementary Fig. 5D). Although, similar to the WT controls, Tm-CMV-KO mice became glucose intolerant (Fig. 5b), their fasting insulin levels (Fig. 5c) and insulin glucose levels (Fig. 5d) were markedly lower than those of WT, suggesting that Tmem127 loss protected mice from HFD-induced insulin resistance, independent of the weight gain.

To gather insights into the mechanisms underlying the insulin sensitivity of Tm-CMV-KO mice we examined their liver, fat, and muscle. All mice increased fat depot weight at the end of the HFD challenge (Fig. 5e), but the visceral fat (eWAT) expansion was greater in KO compared with WT mice (Fig. 5e). In contrast, both the absolute and relative liver weight were lower in Tm-CMV-KO mice (Fig. 5e). Concordantly, null mice had reduced hepatic fat deposition (Fig. 5f) and hepatic triglyceride levels (Fig. 5g). Moreover, the expression of lipogenic and lipid storage enzymes trended lower in the KO liver (Fig. 5h), similar to the pattern seen in chow-fed KO. Likewise, glucose uptake remained low, without significant upregulation of gluconeogenic transcription (Fig. 5i). These data suggest that despite the augmented insulin sensitivity, the capacity to synthesize fatty acids was impaired in the KO liver.

Conversely, and in keeping with the diet-induced fat mass increase (Fig. 5e), the expression of lipogenic and fat storage enzymes in fat tissue did not differ between null and WT animals (Supplementary Fig. 5E). However, a notable change was that both glucose transport and glycolytic enzyme expression were significantly increased in WAT from the HFD-fed null mice, indicative of increased glucose disposal (Fig. 5i). These data suggest that adipose expansion and increased glucose clearance in fat tissue contributes to the improved insulin sensitivity of the HFD-fed Tm-CMV-KO. Overall, these data are consistent with an effect of Tmem127 in promoting hepatic lipogenesis and inhibiting adipose glucose uptake and glycolysis.

**Tmem127 is required for hepatic lipogenesis transcription.** We hypothesized that reduced hepatic fat content contributes to the improved insulin tolerance of Tm-CMV-KO mice. Hepatic lipid synthesis is controlled by transcriptional activities and/or levels of lipogenesis-promoting transcription factors and adiogenic factors. We found that Srebfl mRNA expression in liver was similar between the control and null mice (Fig. 5k), but the expression of Pparg, Cebpa and, to a greater degree, Chrebpa, were significantly downregulated in the Tm-CMV-KO liver, both under a regular diet and after HFD (Fig. 5k). HFD-fed KO mice also had significant decrease in both Lxra (Nrlh3) and Lxrb (Nrlh2), suggesting a coordinated downregulation of the transcriptional lipogenic program in the KO mouse liver (Fig. 5k). In contrast, the expression of these transcription factors was not significantly changed in iWAT or muscle (Supplementary Fig. 5G), suggesting a liver-specific defect. Taken together, our results point to reduced transcriptional input as a possible driver of diminished hepatic lipogenic capacity of the Tm-CMV-KO mice.
Tm-LKO and Tm-AKO mice have differential insulin sensitivity. Based on the findings above, we sought to evaluate the tissue-specific impact of Tmem127 deficiency. We generated mice lacking Tmem127 exclusively in the liver (Tm-LKO) or in fat tissue (Tm-AKO), by crossing Tmem127 Flx mice with mice expressing Cre under the albumin or adiponectin promoter, respectively (Fig. 6a). Both mice were viable and tissue-specific deletion of Tmem127 was confirmed by protein expression (Fig. 6b).

Body weight of these two mouse strains was not different from Flx controls at young ages (Fig. 6c), but adult Tm-LKO had lower fat/lean mass ratio, whereas adult Tm-AKO had increased fat/lean mass ratio (Fig. 6d). In addition, the Tm-LKO mice had lower fasting glucose (Fig. 6e) and insulin (Fig. 6f) levels relative to controls. In contrast, although Tm-AKO mice showed no difference in glucose levels (Fig. 6e), their fasting circulating insulin was elevated (Fig. 6f). Tm-LKO were more insulin
sensitive, even at a young age (Fig. 6g), whereas adult Tm-AKO developed insulin (Fig. 6g) and glucose (Fig. 6h) intolerance. Accordingly, the homeostatic model assessment of insulin resistance index (HOMA-IR) was higher in Tm-AKO, whereas that of Tm-LKO mice was lower, than controls (Supplementary Fig 6A). In keeping with this profile, Tm-AKO mice released more insulin in response to glucose, compared with controls (Supplementary Fig. 6B). These observations suggest that Tm-LKO is insulin sensitive and Tm-AKO is insulin-resistant.

**Tm-LKO has increased insulin signaling.** Under chow-fed conditions, glucose transporter genes were upregulated in liver, muscle, and adipose tissue of the Tm-LKO (Fig. 7a), as was glycolytic gene Pfkp (Fig. 7a). In addition, both liver and fat tissue...
had a trend toward higher AKT phosphorylation levels (Fig. 7b). These results support increased glucose flux and insulin signaling in these mice, phenocopying the Tm-CMV-KO. Since a feature of the latter was decreased fatty-acid gene/protein expression and concomitant coordinated downregulation lipogenic transcription factors, we next examined these pathways. However, unlike the Tm-CMV-KO, expression of several fatty acid synthesis genes (Fig. 7c) and lipogenic transcription factors (Fig. 7d) was instead upregulated in the liver of Tm-LKO and agreed with increased hepatic triglyceride levels in these mice (Supplementary Fig. 6C).

Nutritional and insulin sensing are impaired in Tm-AKO.

During prolonged fasting, glucose is synthesized almost exclusively from gluconeogenesis13,21. We evaluated the gluconeogenic potential of Tm-AKO by measuring their hepatic gene expression after fed or prolonged fasting states. We found that hepatic gluconeogenic gene transcription in the fed state reached levels similar to those observed after fasting in the Tm-AKO (Fig. 7e), suggesting that baseline hepatic gluconeogenesis was not suppressed in conditions of nutrient supply, as in controls. In addition, expression of fatty-acid synthesis (Fig. 7f) and lipogenic transcription factors (Fig. 7g) were attenuated relative to control mice. These findings are consistent with higher hepatic gluconeogenesis and reduced hepatic lipogenesis under regular diet, supportive of hepatic insulin resistance, although we did not find appreciable differences in the levels of AKT phosphorylation (Supplementary Fig. 7A) or Rictor expression (Supplementary Fig. 7B) in Tm-AKO mice relative to controls under those conditions. In contrast, we observed high expression of Glut4 mRNA (Fig. 7h) and high levels of ACC protein (Fig. 7i) in adipose tissue, indicative of high glucose uptake in fat tissue and increased adipogenesis, respectively, both of which are associated with peripheral insulin sensitivity22,23. This agrees with the higher fat mass observed in the Tm-AKO (Fig. 6d). In indirect calorimetry, these mice displayed no change in resting metabolic rate (Supplementary Fig. 7C) but had reduced respiratory quotient (RQ, Fig. 7e), a measure of preferential use of fatty acids, as opposed to carbohydrates, as energy substrate. Collectively, these results suggest that adipose Tmem127 is relevant for the proper regulation of energy sensing, utilization and production. Although these data need to be evaluated under conditions of dietary stress and the contribution of other tissues still remain to be fully assessed, they support a model whereby Tm-AKO mice have adipose insulin sensitivity and hepatic insulin resistance.

Cell autonomous and non-cell autonomous roles of TMEM127.

To determine cell autonomous and non-cell autonomous roles of Tmem127 in the liver, we examined hepatic cells (human HepG2) lacking TMEM127 via CRISPR-CAS9, engineered with previously reported guide RNAs24 (Supplementary Fig. 8, Fig. 8a). HepG2 cells are derived from a well-differentiated human hepatoblastoma and retain characteristics of quiescent hepatocytes25. We observed that insulin induced AKT phosphorylation was increased in TMEM127 KO HepG2 cells (Fig. 8a). Furthermore, in response to a pyruvate challenge, the KO cells failed to induce gluconeogenic gene expression to the level of control cells (Fig. 8b), in favor of lower hepatic glucose production and similar to the in vivo profile of Tm-CMV-KO. In these cells, we also investigated the expression program of lipid synthesis and found that transcription of both fatty-acid synthesis genes and transcription factors followed the upward trend (Fig. 8c), though to a lesser extent, than that seen in the in vivo Tm-LKO model. To independently verify the cell autonomous effects of hepatic Tmem127 loss, we examined primary hepatocytes derived from Tm-CMV-KO mice. These cells showed partial overlap with the fatty-acid synthesis gene expression seen in vivo, without reaching statistical significance (Fig. 8d). Taken together, our in vivo and in vitro models suggest that hepatic Tmem127 has a cell autonomous role promoting hepatic gluconeogenesis but has less effect toward hepatic lipogenesis and therefore the reduced lipogenic potential of the whole-body KO liver is likely due to extra-hepatic (non-cell autonomous) effects.

Tmem127 deficiency activates mTORC2. As shown above, Tmem127 loss results in increased mTORC2 activation (Fig. 4e–h and Fig. 8a). Several features of Tmem127 deficiency are anticorrelated with those observed in the liver-specific Rictor deletion model, the unique component of mTORC2 and a key mediator of metabolic homeostasis13. To further investigate the role of mTORC2 in the increased insulin signaling observed on the Tmem127 models, we knocked down RICTOR by shRNA in TMEM127-deleted HepG2 cells (Fig. 8e). In agreement with increased insulin signaling, cells null for TMEM127 had higher AKT activation after insulin stimulation (Fig. 8e). However, RICTOR knockdown was able to downregulate mTORC2 targets in both control and TMEM127 KO cells. Interestingly, we observed higher levels of endogenous TMEM127 protein after RICTOR knockdown, suggesting that RICTOR and/or mTORC2 might regulate TMEM127 expression (Fig. 8e). Overall, our results suggest that RICTOR functions downstream of TMEM127 and support a reciprocal regulation of mTORC2 toward TMEM127 expression.

Tmem127 expression correlates with insulin resistance states. We noticed that TMEM127 expression increases after insulin stimulation in liver cells in vitro (Fig. 8a, e) and considered that this may be related to its role in vivo. Also, as shown above, hepatic Tmem127 was higher in adult mice when compared with young, more insulin-sensitive mice (Fig. 3g). Hence, we examined Tmem127 expression in several mouse models of insulin...
resistance. We found that liver Tmem127 transcription increased after HFD in WT mice (Fig. 9a), but this change was reversed when these mice were placed on a regular diet for an additional 10 weeks (Fig. 9a). Interestingly, after HFD Tmem127 expression in adipose tissue varied depending on the type of fat depot (Supplementary Fig. 9A, B), suggesting a complex regulation in adipose tissue. We next examined hepatic Tmem127 mRNA in two well-known genetic models of diabetes, obesity, and insulin...
Fig. 5 Tmem127 loss protects against high-fat diet (HFD)-induced hepatic steatosis and insulin resistance. a Weekly body weight of WT and Tmem127 KO male mice on a 60% kcal fat diet for 16 weeks (n = 7–8 per genotype, starting age 5–7 months); b GTT and AUC (inset graph) of HFD-fed adult male WT (n = 8) and KO (n = 10) mice; c fasting (6 h) serum insulin of HFD-fed WT and KO male mice (n = 10 per genotype, 9–11 mo-old); d ITT and AUC (inset) of HFD-fed male WT and KO mice (n = 6 per genotype, 9–11 months); e absolute weight of liver, inguinal white adipose tissue (iWAT), epididymal WAT (eWAT), and relative weight of these organs to total body weight in HFD-fed WT (n = 4) and KO (n = 6) mice (9–11 months old); f representative hematoxylin and eosin (HE) sections of liver from HFD-fed WT and KO mice, scale bar is 500 µm; g hepatic triglyceride content in HFD-fed adult WT (n = 6) and KO (n = 7) mice (9–11 mo-old); h relative liver mRNA expression of the indicated fatty-acid synthesis, storage, oxidation, and transport gene expression from HFD-fed male WT and KO mice (n = 6 per genotype, 9–11 mo-old) measured by RT-PCR; i Relative liver mRNA expression of the indicated glucose transporter, glycolysis and gluconeogenesis genes from HFD-fed male WT (n = 5) and KO (n = 6) mice (9–11 mo-old); j relative iWAT mRNA expression of the indicated glucose transporter and glycolysis genes from HFD-fed male WT (n = 4) and KO (n = 7) mice (9–11 mo-old); k relative liver mRNA expression of the indicated lipogenic transcription factor genes from Chow or HFD-fed male WT (n = 6 per diet) and KO (n = 9 per diet) mice (9–11 mo-old); Data were analyzed by Student’s t test or analysis of variance (ANOVA). Values are expressed as mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; a = p = 0.06. For 5 k, ‘ comparisons between diet; * comparisons between genotype # or *P < 0.05, ## or **P < 0.01, ### or ***P < 0.001. See also Supplementary Fig. 5A–G. Source data are provided as a Source Data file showing that expression of lipogenic transcription factors or fatty-acid synthesis genes are not decreased in primary hepatocytes or in human hepatic cells null for TMEM127.

Our results point to a complex tissue-specific effect of Tmem127 on insulin sensitivity, with partially antagonistic effects in liver and fat (Fig. 9g). Although liver Tmem127 is likely involved in hepatic gluconeogenesis in a cell autonomous manner, adipose Tmem127 coordinates hepatic insulin regulation and adipogenesis. We also propose that the ability to respond to changes in nutritional status is impaired in mice lacking adipose tissue Tmem127 and this may account for the hepatic insulin resistance of this model.

We found that Tmem127 actions on insulin are independent of obesity. Indeed, the capacity of whole-body Tmem127 null mice to remain insulin-sensitive despite diet-induced weight gain suggest that its deficiency may facilitate healthy adipose expansion after a dietary challenge, which in turn may lead to reduced hepatic fat deposition and improved hepatic and peripheral insulin sensitivity. An examination of the effect of dietary stress in the liver-specific or fat-specific Tmem127 loss, and precise quantification of glucose uptake will be relevant to further shed light on insulin- and non-insulin-dependent tissue homeostasis. In addition, as neither liver nor adipose deficiency of Tmem127 fully recapitulates the phenotype of Tm-CMV KO, it is likely that other tissues involved in insulin homeostasis, including islet beta cells, muscle, hypothalamus, and macrophages, for example, might contribute to the metabolic outcome of global loss of Tmem127. Future studies should address their role in these metabolic pathways. Likewise, as our results support non-cell autonomous actions of Tmem127 loss, such as the effect of adipose Tmem127 on hepatic gluconeogenesis, it remains to be determined what factors, e.g., hormones, cytokines, metabolites, lipid composition, etc, mediate the tissue response.

The Tmem127 liver and fat loss profile is reminiscent of the complex actions of mTORC2 in these two tissues: loss of mTORC2-mediated by Rictor deletion in the liver results in age-dependent impairment of glucose metabolism owing to reduced Akt signaling. Moreover, adipose mTORC2 regulates liver lipogenesis via modulation of Chrebp and promotes glucose uptake into adipocytes. This mTORC2-mediated cross-talk between liver and adipose tissue leads to increased carbohydrate flux away from the liver under diet stress, strikingly similar to the Tm-CMV-KO profile after HFD (Fig. 5i). The mechanisms through which Tmem127 interfaces with mTORC2 remain to be elucidated. However, the observation that Rictor deficiency leads to accumulation of Tmem127 suggest possible feedback regulation, in support of an interaction between these two molecules.

We were able to exclude increased locomotor activity as a major contributor to the reduced fat mass and higher metabolic resistance, in which the metabolic disruption exists even under a regular diet, the Db/Db and Ob/Ob mice. Tmem127 expression was higher in the liver of homozygous Db/Db (Fig. 9b) and Ob/Ob (Fig. 9c) mice relative to their controls. We also evaluated liver of Ob/Ob mice treated with pioglitazone, a thiazolidinedione with insulin sensitizing properties, and found that this treatment normalized Tmem127 mRNA levels (Fig. 9c). Taken together, these findings support an association of Tmem127 liver expression with states of insulin resistance. Importantly, these data also suggest that the increase in Tmem127 expression is reversible with the improvement of insulin sensitivity, either by diet (from HFD to chow) or by pharmacological intervention.

At last, to assess the translatability of our findings to human pathology, we examined liver biopsies from patients with histologically proven nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH) and found that this treatment normalized Tmem127 mRNA levels (Fig. 9c). Taken together, these findings support an association of Tmem127 liver expression with states of insulin resistance. Importantly, these data also suggest that the increase in Tmem127 expression is reversible with the improvement of insulin sensitivity, either by diet (from HFD to chow) or by pharmacological intervention.

Discussion
In this study, we uncover the Tmem127 tumor suppressor as an unsuspected regulator of insulin signaling and lipogenesis. Whole-body loss of Tmem127 in mice results in low adiposity, improved glucose metabolic profile, and low insulin levels, which may contribute to the reduced body mass. Loss of Tmem127 enhances insulin sensitivity and protects from diet-induced insulin resistance likely due to reduced hepatic gluconeogenesis and increased glucose clearance in fat tissue, although other mechanisms may also be involved. Moreover, these mice have low hepatic lipogenic gene expression, and we were able to show that this feature is not the primary determinant of their favorable insulin profile, as liver-specific Tmem127 deletion also promotes insulin sensitivity but without reduced hepatic fatty-acid synthesis expression. This conclusion is corroborated by ex vivo data showing that expression of lipogenic transcription factors or fatty-acid synthesis genes are not decreased in primary hepatocytes or in human hepatic cells null for TMEM127.
rate of these mice. In fact, Tm-CMV-KO mice had decreased physical activity, similar to the phenotype of liver Tsc1 KO mice, which have elevated mTORC1 activity and augmented energy expenditure. However, we found that Tmem127 KOs had an impaired capacity to induce Fgf21. The mechanisms underlying this abnormal response and the increased energy expenditure of Tmem127 KO will need to be explored, but collectively they support an mTORC1-independent mechanism.

Other genes involved in oncogenesis, including Pten, Akt, Bad, also have key contributions to metabolism, and metabolic...
reprogramming is now recognized as a hallmark of cancer\textsuperscript{34–36}. Future studies of Tmem127 should integrate its tumor suppressor functions with its role in cellular glucose and insulin homeostasis. It is plausible that Tmem127 activities in endosomal trafficking and lysosome positioning may be linked to its ability to sense nutrients and modulate glucose metabolism\textsuperscript{3,5}. Interestingly, mTORC2 activity was recently shown to rely on lysosomal distribution\textsuperscript{37}, much like the mTORC1 dependency on a multiprotein lysosomal-anchored complex and its distribution in response to nutrients\textsuperscript{38}. We previously identified TMEM127 associations with components of this mTORC1 lysosomal complex and effects on lysosomal positioning\textsuperscript{5}. It remains to be...
Fig. 7 Liver-specific Tmem127 deletion increases hepatic and peripheral insulin sensitivity. a Relative liver, muscle, and iWAT mRNA expression of the indicated glucose transporter and glycolysis genes from Fix (n = 5) and LKO (n = 11) adult mice (9–12 mo old); b western blot of liver and inguinal white adipose tissue lysates from chow-fed LKO and Fix adult male mice, probed with phosphorylated Ser473 and Thr308 Akt and total Akt; c relative liver mRNA expression of the indicated fatty-acid synthesis genes from LKO and AKO adult male mice, represented as ratio over Flx (n = 12 Fix, 11 LKO and 7 AKO, 9–12 mo); d relative liver mRNA expression of the indicated lipogenic transcription factor genes from LKO and AKO adult male mice (n = 11 Fix, 12 LKO and 7 AKO, 9–12 mo). e Real-time (RT) PCR of liver G6Pase gene expression in control or AKO 9–12 mo old male mice starved of food for 6 h and re-fed for 3 h (fed) or starved for 24 h (fasted), mice or feed condition (WT (n = 4, KO n = 7); f RT-PCR of liver of the indicated fatty acid synthesis genes from chow-fed adult male Fix (n = 11) and AKO (n = 5) mice; g RT-PCR of inguinal fat (iWAT) Glut4 gene expression from chow-fed, adult male Fix (n = 11) and AKO (n = 5) mice; h western blot of inguinal white adipose tissue lysates from chow-fed LKO and Fix adult male mice (n = 3 per genotype), probed with total ACC and a loading control; i respiratory quotient (RQ) of Fix (n = 5) or AKO (n = 7) chow-fed, adult male mice, data collected for 48 h and sorted by day or night cycle. Data were analyzed by Student’s t test. Values are expressed as mean ± s.e.m. *P < 0.05; **P < 0.01. See also Supplementary Fig. 7A–C. Source data are provided as a Source Data file.

determined if TMEM127 interacts with the mTORC2 complex at the lysosome and whether this putative interaction mediates any of the metabolic changes we observed in vivo.

Finally, and of translational relevance, we found that hepatic TMEM127 expression correlates with fatty liver disease and insulin-resistant states both in mice and in humans. Importantly, we saw that hepatic Tmem127 expression in mice is responsive to dietary or pharmacological interventions that ameliorate insulin resistance. These findings support TMEM127 as a potential target for insulin resistance which might be amenable to therapeutic modulation.

Methods

Generation of Tmem127 knockout mice models. All animal studies were performed after receiving ethical approval of the University of Texas Health San Antonio (UTHSA) IACUC (Institutional Animal Care and Use Committee), and in compliance with all relevant ethical regulations for animal testing and research. Mice with recombinant Tmem127 allele (Tmem127fl/fl) were generated by crossing Tmem127+/− mice with CMV-Cre transgenic mice of C57BL6 J background (Tg (CMV-cre)1Ggn; Jackson Laboratory)19, Tmem127+/− heterozygotes were mated to obtain WT and KO mice. For generating liver- and adipose-specific Tmem127 deletion, the Tmem127fl/fl mice were crossed with Albumin-Cre (Tg(Alb-cre) IDir; Jackson Laboratory)20 and Adiponectin-Cre (Tg(Adipoq-cre)1Evdr; Jackson Laboratory)21 transgenic mice, respectively. Tmem127fl/fl/cre−/− (referred to hereafter as TM-LKO) and Tmem127fl/fl/cre−/− (TM-AKO) mice were compared with littermate ‘ Fix’ controls (Tmem127fl/fl/cre+/−). These strains are available under an MTA agreement. Leptin receptor knockout (Db/db) and control (Db/+ ) mice were obtained from Jackson Laboratories.

Diet and treatment. Mice were housed according to UTHSCSA guidelines, and procedures were approved by the Institutional Animal Care and Use Committee (IACUC), in compliance with the standards for the use of laboratory animals. Mice were fed standard rodent chow (Harlan) ad libitum unless mentioned otherwise. Mice on an HFD regimen were fed a diet containing 60% calories fat (D12492, Research Diets) for 16 weeks. A subset of this group was switched back to chow diet for 10 additional weeks. For the pioglitazone studies, 12-week old Ob/Ob mice were given ad libitum access to food containing pioglitazone for 4 weeks (diet was custom-made by Research Diets to contain 300 mg pioglitazone/kg diet, 10% calories fat). On average mice consumed 25 mg/kg/day of pioglitazone, which previously has been shown to significantly improve hepatic and adipose tissue insulin sensitivity29.

Body temperature, body composition, and energy expenditure. Core body temperature was measured using a rectal probe connected to Animal Temperature Controller (#ATC200; World Precision Instruments). Fat mass, lean mass, and percentage of fat were determined using Dual-Energy X-ray Absorptiometry (GE Medical Systems). For energy expenditure measurement, mice were placed in metabolic cages, and energy expenditure was measured by indirect calorimetry using the MARS system from Sables Systems (Las Vegas, NV) for the TM-CMV-, KO, and the Columbus CLAMS system (Columbus, OH) for the TM-AKO mice. Oxygen consumption (VO2), carbon dioxide production (VCO2), and RQ are measured for each mouse. Animals are habituated during the first 16h and measurements and data recorded during the subsequent 24h. Total activity and sleep behavior were measured by Open-Field activity monitoring. The number of beam breaks across the X–Y axis is measured to determine total activity. Sleep and sleep fragmentation assessments are based on a model developed by Pack et al for high-throughput phenotyping of sleep in mice42. The technique uses activity/inactivity assessments as a measure of sleep, with sleep defined as any bout of inactivity of ≥ 40 s. Based on their results, we measure activity across a 24-hour period, distinguishing between light and dark phases to assess sleep and sleep fragmentation.

Human samples. Samples and clinical data were obtained after signed informed consent in accord with ethical standards through Institutional Review Board-approved research repositories at the Brooke Army Medical Center and the UTHSA. A total of 52 individuals (20 males; 53.4%); ages 20–64 and average BMI of 31.3 (range, 25.7–37.6) were included in the study. Based on their liver histology, seven individuals with no liver disease were used as controls, 22 patients were classified as having steatosis with stage 0 fibrosis (NAFLD), and 24 patients had NASH stage 1–3 fibrosis, according to well-established histopathological criteria38. Twelve individuals (23%) had diabetes. Information available from these patients included gender, BMI, insulin, and glucose; HOMA-IR and NAFLD score were derived from these parameters (Supplementary Table 4).

RNASEq. Whole-genome transcriptomic analyses were performed on liver tissue obtained by biopsies from seven healthy controls, 21 patients with nonalcoholic fatty liver disease (NAFLD/NN) and 24 patients with NASH (nonalcoholic steatohepatitis), of which 16 were stages 0/1, five were stage 2, and three were stage 3. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with DNase I digestion according to the manufacturer’s instructions. RNA quantity and purity were determined by spectrophotometry (260/280 = 1.8–2.0) (NanoDrop) and integrity (RIN) was determined using an Agilent 2100 Bioanalyzer with an RNA 6000 Nano assay (Agilent Technologies, Palo Alto, CA). Samples with RIN ≥ 7.0 were selected for RNA sequencing. Double-stranded cDNA library was prepared starting from 1 μg of total RNA input according to the TrueSeq RNA v2 sample preparation kit protocol (Illumina, San Diego, CA). In brief, mRNA was selected using poly-T oligo-attached magnetic beads and then fragmented. First and second cDNA strands were synthesized and end-repaired. Multiplexed adaptors were ligated after 3′-adenylation. Double-stranded cDNA templates were enriched by PCR. Libraries were validated using a DNA High Sensitivity assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and quantified by a Kapa Library quantification kit (Kapa Biosystems, Woburn, MA). Libraries were clustered using the Illumina cBot (Illumina, San Diego, CA) and then paired-end sequenced (2 × 101 bp) on an Illumina HiSeqTM 2000 (Illumina, San Diego, CA). Base calling and quality filtering were performed using the CASAVA v1.8.2 (Illumina, San Diego, CA) pipeline. Sequences were aligned and mapped to the UCSC hg19 build of the Homo sapiens genome (from Illumina genomes) using tophat v2.0.1. 7 Gene counts for 23,239 unique, well-curated genes were obtained using HTSeq framework v0.5.3P3 (http://www.huber.embl.de/users/anders/ HTSeq/doc/history.html). Gene counts were normalized to FPKM. NCBI EST BLAST was used to identify the R package, DESeq v1.10.18.XX. A generalized linear model based on the negative binomial distribution with the likelihood ratio test was used to test for associations between Tmem127 expression and: disease states (controls, NAFLD, and NASH), insulin levels, or HOMA-IR scores39. P value < 0.05 was considered significant. Both normalized and raw counts are provided as Supplementary Table 4 and Source files.

Generation of Tmem127 knockout HepG2 cells with CRISPR/Cas9. Tmem127-null HepG2 cells were generated by CRISPR-Cas9 technology. Guide RNAs targeting the first and last coding exon of the Tmem127 gene (AGTATC GCCCCGCGGAGGC and CTTCGCCGTTGACTCTTCA, respectively) were cloned into pLentiCRISPRv2, BsmBI digestion, one vector system35. Cells were transduced with LentiCRISPRv2 expressing each guide or empty LentiCRISPRv2 as control, and single clones were selected with puromycin44. The KO status of these clones was verified by Sanger sequencing (Supplementary Fig. 8) and Western blot (Fig. 8a, e). Clone Tmem127 T2-2 was used for further experiments.
containing ice-cold HBSS/HEPES buffer (370 mM NaCl, 5 mM KCl, 0.33 mM MgCl₂, 5.5 mM CaCl₂), mice were euthanized during perfusion. Livers were transferred into Petri dishes and washed with HBSS/HEPES by low-speed centrifugation (500 rpm at 4 °C for 2 min). Freshly isolated hepatocytes were suspended in Williams’ medium supplemented with 10% fetal bovine serum and 100 U/ml penicillin and streptomycin. All cells were negative for Mycoplasma infection when tested using PCR-based protocol. HepG2 cells were transfected with lentivirus PTM-LKO.1 empty vector or PTM-LKO.1-Rictor-shRNA. Transduction efficiency was verified by Rictor western blot. For insulin treatment, 80% confluent cells were serum starved for 16 h and challenged with 100 nM insulin (#I9278; Sigma) for indicated time points and harvested for protein or RNA analysis.

**Cell culture, transfections, and transductions.** HepG2 cell line was obtained from ATCC (HB-8065), and mouse embryonic fibroblasts were isolated from Tmem127 mice using standard methods. HepG2 cells were cultured in Eagle’s Minimum Essential medium (MEM; #10-009-CV, Corning) supplemented with 10% fetal bovine serum and 100 U/ml penicillin and streptomycin. All cells were negative for Mycoplasma infection when tested using PCR-based protocol. HepG2 cells were transfected with lentivirus PTM-LKO.1 empty vector or PTM-LKO.1-Rictor-shRNA. Transduction efficiency was verified by Rictor western blot.

**RNA isolation and qPCR analysis.** RNeasy kits (Qiagen) were used for RNA isolation from liver and cell lines (RNeasy Mini Kit), muscle (RNeasy Fibrous Tissue Mini Kit) and fat tissues (RNeasy Lipid Tissue Mini Kit) using the manufacturer’s suggested protocol. One μg of total RNA was used for cDNA synthesis (High capacity cDNA synthesis Kit; Applied Biosystems). Quantitative PCR reactions containing the SYBR green mix (Applied Biosystems) were performed with StepOnePlus Real-time PCR System (Applied Biosystems). Triplicate runs of each sample were normalized to Tβh (mouse tissues) or TBP (human, cell lines) to determine relative expression levels. Primer sequences are available in the Supplementary Information.

**Immunoblotting.** Liver and muscle were lysed in cold RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% Sodium dodecyl sulfate, 10% Glycerol) supplemented with phosphatase and protease inhibitor cocktails with mechanical agitation using a hand-held homogenizer. Lysates were incubated on ice for 30 min and sonicated for 30 s on ice. Fat tissue samples were lysed in cold HNTG buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 1% TritonX100) with mechanical agitation and lysates incubated on ice for 30 min. Cells were lysed in cold RIPA buffer supplemented with phosphatase and protease inhibitor cocktails and mechanical agitation, followed by incubation on ice for 15 min. All lysates were centrifuged at 16,000 g for 15 min, supernatant was collected, and protein concentration measured by Bradford assay (#500-0006; Bio-Rad). Twenty μg of protein was separated on 8%, 10%, 12%, or 14% gradient Tris-Glycine gel by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Alternatively, some cell lysates were prepared by direct lysis with 1 x Laemmli buffer and separated on SDS-PAGE. Blots were probed with antibodies at the indicated dilutions: Acaca (3676, 1:10,000), Akt (9272, 1:1000), phospho-Akt,S473 (9271; 1:1000), phospho-Akt,T308 (10383; 1:1000), Rictor (9476; 1:1000), mTORC2 (2983; 1:1000), phospho-S6K,T389 (9206; 1:1000), S6K (9202; 1:1000), phospho-4EBP1,T377/46 (4549; 1:1000), from Cell Signaling Technologies; β-actin (A2228; 1:20,000) from Sigma-Aldrich, β-tubulin (ab6046; 1:10,000) from Abcam, and Tmem127 (A303-450A, 1:1000) from Bethyl laboratories. X-rays were scanned and quantified using Image software (NIH). Uncropped and unprocessed scans of the most important blots are available in the Source Data file.

**Tissue lysis and immunoprecipitation.** Liver tissue was lysed in ice-cold CHAPS buffer (0.3% CHAPS, 10 mM beta-glycerol phosphate, 10 mM sodium pyrophosphate, 40 mM HEPES (pH 7.4) and 2.5 mM MgCl₂) with EDTA-free protease inhibitor (Roche #1183617001). Lysates were cleared by centrifugation at 16,000 g.
at 4 °C for 10 min. Cleared lysates were normalized for protein content by Bradford assay, and 2–3 mg of protein lysate were incubated with mTOR antibody at manufacturer’s recommended dilutions at 4 °C overnight on a rotator, followed by addition of protein A agarose beads (Pierce #22811; 40 µl of 50% slurry per 1 mg of lysate) and continued incubation for 2 h at 4 °C. Beads were washed three times with CHAPs lysis buffer supplemented with 150 mM NaCl, eluted with 2 × Laemmli buffer by boiling at 100 °C for 10 min and separated on SDS-PAGE. Probed blots were quantified using ImageJ. Uncropped and unprocessed scans of the most important blots are available in the Source Data file.

**H&E staining and fat cell size measurement.** For H&E staining, adipose and liver tissues were fixed with a buffer containing 10% formalin for 24–48 hr and embedded in paraffin. Tissue sections (6 µm thick) were stained with H&E. Fat cell isometry was quantified by ImageJ-based measurements.
size measurement was performed according to the procedure described by Mul et al.48 The average diameter of adipocyte cell from 4–5 fields of imaged H&E sections was analyzed using Image J.

Fgf21 assays. Plasma Fgf21 was measured from fed and 24 hr fasted mouse plasma using an ELISA kit (#RD291108200R; BioVendor) according to the manufacturer’s instructions.

Insulin assays. Plasma insulin was measured from 6 h fasted mice or after stimulation with glucose using Multiplex MAP Mouse adipokine magnetic bead panel kit (#MADKMAG-71K, Millipore) according to the manufacturer’s instructions.

Triglyceride and free fatty-acid assays. Triglyceride Assay Kit (#ab65336, Abcam) was used to measure tissue (liver) and plasma triglycerides and Free Fatty Acid Quantitation Kit (#MAK044, Sigma) was used to measure plasma free fatty acids, according to each manufacturer’s instructions.

Hepatic glucose production assay. HepG2 TMEM127 KO or control cells were transferred to a 12-well plate at a density of 1 × 10⁶ cells/well and cultured for 28 h incubated in glucose-free Dulbecco’s modified Eagle’s medium (Thermo Fisher Gibco, catalog no. D1050) supplemented with 2 mM-pyruvate (Sigma, catalog no. P2256) or phosphate-buffered saline (PBS) overnight, then insulin 100 μU or PBS were added for 30 min and cells were harvested for RNA.

Catecholamine assays. Norepinephrine and epinephrine from plasma and adrenal tissue were measured using gas chromatography–mass spectrometry methods49.

Antibodies. The following antibodies were obtained from Cell Signaling Technology: phospho-S6 ribosomal protein (5235S/2636 (2211)), total S6 ribosomal protein (5G10) (2217), phospho-AKT/S473 (9271), phospho-AKT/T308 (#13038), total AKT (#2272), phospho-4EBP1 (#2855), Rictor (#2114), total AKT (#9272), phospho-AKT/S473 (#9271), phospho-AKT/T308 (#2983) and immunoprecipitation (#2972); alpha-tubulin was from Sigma (#A303-450A). The following antibodies were obtained from Cell Signaling Technology: ACACA/ACC (#3676), FASN (#3180), mTOR antibody for western (#2983) and (#13038), total AKT (#9272), phospho-4EBP1 (##2855), Rictor (#2114), total AKT (#9272), phospho-AKT/S473 (#9271), phospho-AKT/T308 (#2983) and immunoprecipitation (#2972); alpha-tubulin was from Sigma (#A303-450A).

Constructions. The following plasmids were obtained from Addgene: SV40 Large-T antigen (Addgene #14088), pCMV-VSV-G (#8454), pSPAX2 (#12260), pMD2G (#12263) LentCRISPRV2 (#52961), Ritorc, λ shRNA (#1853) and pTM-LKO1 (#8453).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Correspondence and requests for materials related to this study should be sent to dahia@uthscsa.edu. All data supporting the findings of this study are available within the paper and its Supplementary Information and as source data files, or from the corresponding author on reasonable request.

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Fig. 9 Hepatic TMEM127 expression correlates with states of insulin resistance in humans and mice. a Relative liver Tmem127 mRNA expression from adult WT male mice under chow (n = 6), high-fat diet (HFD, n = 6) for 16 weeks or after HFD followed by 10 weeks of chow (HFD > chow, n = 3 per genotype) measured by RT-PCR, b relative liver Tmem127 mRNA expression from adult male Db/db or age-matched heterozygous db mice (n = 6 per genotype), c relative liver Tmem127 mRNA expression of control (n = 8), db/db (n = 3) or ob/ob (n = 3) adult mice treated for 4 weeks with the insulin sensitizer pioglitazone. d Log2-transformed, normalized TMEM127 gene RNAseq read counts in liver biopsies of patients with the indicated status: CTLs = control individuals; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; the number of samples per group is indicated below the graph. The statistical test was the negative binomial generalized linear model implemented in DESeq with a likelihood ratio test to compare TMEM127 expression between the groups. The thick horizontal line represents median values, box represents 25th and 75th percentiles, whiskers represent minimum and maximum values, outlier samples are shown as dots; e correlation between TMEM127 mRNA levels shown in d and insulin levels (n = 52). Symbols represent individual patients control samples are shown in green, NAFLD are orange and NASH are red; f correlation between TMEM127 mRNA shown in d and the homeostatic model assessment insulin resistance (HOMA-IR) index. Symbols represent individual patients control samples are shown in green, NAFLD are orange and NASH are red; Spearman’s correlation test was used to test associations between TMEM127 and insulin levels (shown in e) or HOMA-IR (shown in f). *P < 0.05; **P < 0.01; ***P < 0.001; g working model of TMem127 function. The upper panel displays a summary of the effects of global loss of TMEM127 on insulin sensitivity and fat deposition under regular diet in young vs. adult mice or after high-fat diet (HFD). Adult knockout (KO) mice show higher hepatic and peripheral insulin sensitivity as well as diminished hepatic fat deposition even after a HFD challenge. The lower panel summarizes our findings in liver- or adipose-specific TMEM127 deficiency. Our data suggest that liver TMEM127 promotes hepatic glucose production and peripheral glucose uptake both by cell autonomous and non-cell autonomous pathways; fat TMEM127 inhibits adipogenesis potential and modulates hepatic gluconeogenesis. Furthermore, it is likely that TMem127 in other tissues contribute to the metabolic phenotype. See also Supplementary Fig. 9A, B and Supplementary Tables 3 and 4. Source data are provided as a Source Data file.
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Competing interests

The authors declare no competing interests.

Additional information

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