LETTER

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Obesity-induced overexpression of miR-802 impairs glucose metabolism through silencing of *Hnf1b*

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Insulin resistance represents a hallmark during the development of type 2 diabetes mellitus and in the pathogenesis of obesity-associated disturbances of glucose and lipid metabolism[1–3]. MicroRNA (miRNA)-dependent post-transcriptional gene silencing has been recognized recently to control gene expression in disease development and progression, including that of insulin-resistant type 2 diabetes. The deregulation of miRNAs miR-143 (ref. 4), miR-181 (ref. 5), and miR-103 and miR-107 (ref. 6) alters hepatic insulin sensitivity. Here we report that the expression of miR-802 is increased in the liver of two obese mouse models and obese human subjects. Inducible transgenic overexpression of miR-802 in mice causes impaired glucose tolerance and attenuates insulin sensitivity, whereas reduction of miR-802 expression improves glucose tolerance and insulin action. We identify *Hnf1b* (also known as *Tcf2*) as a target of miR-802-dependent silencing, and show that short hairpin RNA (shRNA)-mediated reduction of Hnf1b in liver causes glucose intolerance, impairs insulin signalling and promotes hepatic gluconeogenesis. In turn, hepatic overexpression of Hnf1b improves insulin sensitivity in *Lepr*$^{db/db}$ mice. Thus, this study defines a critical role for deregulated expression of miR-802 in the development of obesity-associated impairment of glucose metabolism through targeting of *Hnf1b*, and assigns Hnf1b an unexpected role in the control of hepatic insulin sensitivity.

To identify novel miRNAs that are deregulated during obesity development and that may contribute to the development of obesity-associated insulin resistance and type 2 diabetes, we carried out 'miRNome' expression profiling using miRNA microarrays on RNA isolated from liver of two mouse models of obesity and insulin resistance: (1) high fat diet (HFD)-fed mice compared to normal chow diet fed mice, and (2) mice homozygous for the diabetes *db* mutation of the leptin receptor (*Lepr*$^{db/db}$) compared to wild-type controls. Out of 769 miRNA-specific probe sets, 334 (43.4%) and 311 (40.4%) miRNAs were detectable in liver of normal chow diet/HFD and control/*Lepr*$^{db/db}$ livers, respectively (Supplementary Fig. 1a, b). In the liver of HFD-fed mice, expression of 66 miRNAs was significantly changed compared to miRNAs in normal chow diet controls, of which 90.1% were increased and 9.9% decreased (Supplementary Table 1). In *Lepr*$^{db/db}$ livers, 156 miRNAs were significantly changed, the vast majority (99%) were upregulated in *Lepr*$^{db/db}$ mice compared to controls (Supplementary Table 2). This screen confirmed an increased expression of miRNAs previously associated with obesity-induced insulin resistance, such as miR-103 and miR-107(ref. 6), miR-143 (ref. 4) and miR-335 (ref. 7). In addition to these reported changes in obesity-associated miRNA expression, our screen revealed an even more pronounced overexpression of miR-802 in the liver of HFD and *Lepr*$^{db/db}$ mice compared to controls (Supplementary Tables 1 and 2). Increased miR-802 expression in obese mouse models was further confirmed by quantitative real-time PCR with reverse transcription (qRT–PCR) analyses, which revealed a 5.5-fold upregulation of miR-802 expression in the liver of HFD-fed mice and a 30-fold increase of miR-802 expression in the liver of *Lepr*$^{db/db}$ mice (Fig. 1a). Northern blot analyses confirmed miR-802 expression in the liver of control mice, and hepatic overexpression in HFD and *Lepr*$^{db/db}$ mice (Fig. 1a and Supplementary Fig. 1c). In addition, our analysis revealed that murine miR-802 expression is highly enriched in the liver of lean mice (Supplementary Fig. 1d). Moreover, we compared miR-802 expression in primary hepatocytes versus non-hepatocytes isolated from control mice, revealing that miR-802 expression was tenfold higher in hepatocytes versus non-hepatocytes, indicating that liver parenchymal cells represent the main source of miR-802 expression in this tissue (Supplementary Fig. 1e). miR-802 expression was also increased in other tissues of obese mice, such as in kidney, pancreatic islets, skeletal muscle, white adipose tissue (WAT) and the brain, particularly in *Lepr*$^{db/db}$ mice (Fig. 1a), although to a lesser extent than the overexpression observed in the liver. Next, we quantified miR-802 expression in liver of a cohort of human individuals. miR-802 levels were significantly increased in overweight (body mass index (BMI) $>$ 25) compared with lean individuals (Fig. 1b) and hepatic miR-802 expression significantly correlated with the BMI of these subjects (Fig. 1c). Taken together, hepatic expression of miR-802 is increased in dietary and genetic mouse models of obesity as well as in overweight human subjects.

To address whether increased miR-802 expression may contribute to the development of insulin resistance, we overexpressed miR-802 in the murine hepatoma cell line Hepa1-6 (Supplementary Fig. 2a). This resulted in a diminished ability of insulin to phosphorylate Akt, a central signalling node of insulin action (Fig. 2a). To further address potential mechanisms leading to insulin resistance upon miR-802 overexpression, we investigated the messenger RNA expression of insulin signalling mediators and known inducers of insulin resistance in the presence of miR-802 overexpression. This analysis revealed unaltered expression of mRNAs for *Akt1*, *Igf1r*, *Insr* and *Irs1* (Supplementary Fig. 2c), but showed significantly increased mRNA expression of suppressor of cytokine signalling *Socs1* and *Socs3* upon miR-802 overexpression (Fig. 2b). Because deregulation of hepatic glucose production represents a key step in the development of type 2 diabetes, we assessed the effect of miR-802 expression on the transcriptional regulation of glucose 6 phosphatase (*G6pc*) and phosphoenolpyruvate carboxykinase 1 (*Pck1*). This analysis revealed that in addition to inducing insulin resistance, overexpression of miR-802 enhanced both basal and forskolin-induced expression of *G6pc* (Fig. 2c), but not *Pck1* (Supplementary Fig. 2b).

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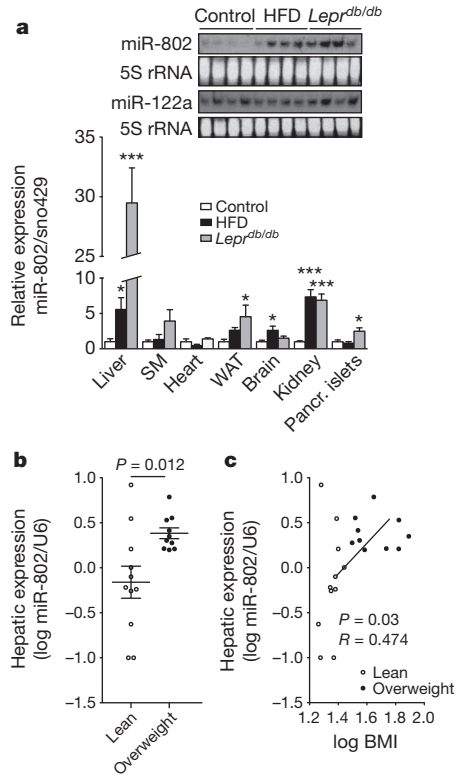

Figure 1 | miR-802 expression is increased in obese mice and humans. **a**, Comparative qRT–PCR analysis (bottom) of miR-802 expression in high fat diet (HFD)-fed mice (liver, $n = 6$; skeletal muscle (SM), $n = 8$; heart, $n = 6$; white adipose tissue (WAT), $n = 8$; brain, $n = 8$; kidney, $n = 7$; pancreatic islets, $n = 7$), $Lepr^{db/db}$ (liver, $n = 6$; SM, $n = 8$; heart, $n = 6$; WAT, $n = 7$; brain, $n = 8$; kidney, $n = 6$; pancreatic islets, $n = 5$) and chow-fed control mice (liver, $n = 6$; SM, $n = 8$; heart, $n = 7$; WAT, $n = 7$; brain, $n = 8$; kidney, $n = 14$; pancreatic islets, $n = 12$). Northern blot analysis (top) of miR-802 expression in liver of control, HFD-fed and $Lepr^{db/db}$ mice ($n = 4$ in each group). **b**, qRT–PCR quantification of human miR-802 expression in liver of lean ($n = 11$) and obese ($n = 10$) individuals. **c**, Correlation between hepatic miR-802 levels and BMI ($n = 21$) in lean ($n=11$) and obese ($n=10$) individuals. Expression was normalized to *sno429* in mice and to *U6* in humans; all error bars indicate mean ± s.e.m.; and $^*P < 0.05$, $^{***}P < 0.001$.

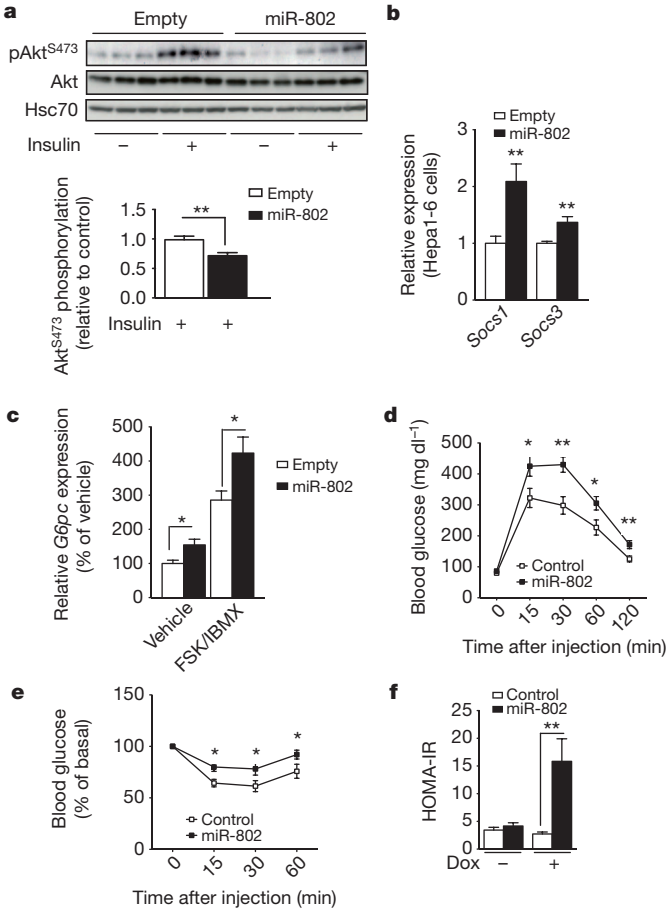

Figure 2 | Overexpression of miR-802 impairs insulin action and glucose metabolism. **a**, Immunoblot analysis (top) and quantification (bottom) of insulin-stimulated Akt Serine 473 phosphorylation versus total Akt protein levels in Hepa1-6 cells in the absence (empty vector) or presence of miR-802 overexpression. For quantification, the mean value for insulin-stimulated intensity of cells transfected with empty vector was set to 1. The results were obtained from three independent experiments, each performed in triplicate and a representative immunoblot is shown. Hsc70 is also known as Hspa8. **b**, qRT–PCR analysis of *Socs1* and *Socs3* expression in Hepa1-6 cells upon transfection with control or miR-802 expression vector. **c**, qRT–PCR analysis of *G6pc* expression in Hepa1-6 cells upon transfection with empty vector or miR-802 expression vector following stimulation with vehicle or forskolin (FSK) and 3-isobutyl-1-methylxanthine (IBMX). **d**, Glucose tolerance tests of miR-802 ($n = 11$) and control mice ($n = 11$) treated with doxycycline. **e**, Insulin tolerance tests of miR-802 ($n = 10$) and control mice ($n = 9$) after doxycycline administration. **f**, HOMA-IR of miR-802 ($n = 11$) and control littermates ($n = 11$). Dox, doxycycline. In all panels error bars indicate mean ± s.e.m.; $^*P < 0.05$, $^{**}P < 0.01$.

To verify whether overexpression of miR-802 also causes insulin resistance and impairs glucose metabolism *in vivo*, we aimed to mimic the obesity-associated increase of miR-802 expression via transgenic overexpression of miR-802 in lean mice. To do this we generated mice with the capacity for doxycycline (Dox)-induced overexpression of miR-802 (hereafter referred to as miR-802 mice, Supplementary Fig. 2d–f). Insertion of the transgenic miR-802 expression cassette in the absence of Dox stimulation did not alter body weight, glucose tolerance or insulin tolerance (Supplementary Fig. 2g–i). Upon Dox administration, we observed an increase of miR-802 expression in the liver, skeletal muscle, white adipose tissue, pancreatic islets and kidney of transgenic compared to control animals (Supplementary Fig. 2j). Profiling of global miRNA-expression of Dox-treated control and transgenic mice revealed no major differences in global miRNA-expression between both groups, indicating that transgenic overexpression of miR-802 does not interfere with transcription and processing of small RNAs in a non-specific manner (Supplementary Fig. 2k). Thus, transgenic overexpression of miR-802 mimicked the miR-802 expression observed in obese mice, with the exception of a higher degree of miR-802 expression in skeletal muscle and white adipose tissue compared to the obese state. Although we detected no differences in blood glucose concentrations (Supplementary Fig. 2l), transgenic mice developed glucose intolerance and insulin resistance upon miR-802 overexpression (Fig. 2d, e). Furthermore, homeostatic model assessment of insulin resistance (HOMA-IR) indices of mice overexpressing miR-802 were significantly increased (Fig. 2f). Collectively, overexpression of miR-802 in mice impairs glucose homeostasis *in vivo*.

Next, we assessed the functional contribution of increased miR-802 expression to the development of insulin resistance by reducing miR-802 expression in obese mice. We synthesized locked nucleic acids (LNA) specifically targeting the seed sequence of miR-802, as well as appropriate control LNA in which four nucleotides of the seed sequence were mutated. C57BL/6 mice, which had been receiving a HFD for 12 weeks, were injected with either anti-miR-802 or control LNA. We observed an 80% reduction of hepatic miR-802 expression in mice that had received the anti-miR-802 LNA compared to those receiving control LNA (Fig. 3a). Although anti-miR-802 treatment also efficiently reduced miR-802 expression in the kidney of obese mice, it only slightly reduced miR-802 expression in skeletal

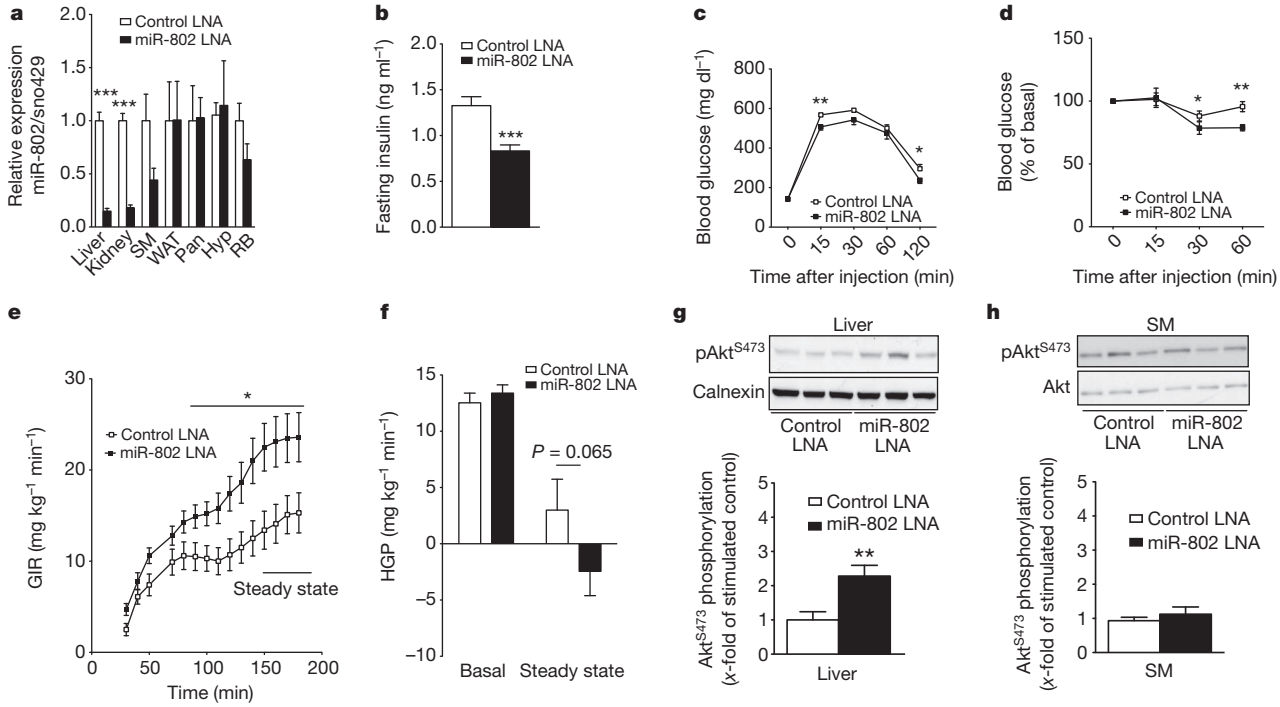

Figure 3 | Suppression of miR-802 expression improves obesity-associated insulin resistance and glucose intolerance. **a**, miR-802 expression after intravenous injection of HFD-fed mice with miR-802 locked nucleic acids (LNA) (liver, $n = 9$; kidney, $n = 10$; SM, $n = 6$; WAT, $n = 6$; pancreatic islets, $n = 3$; hypothalamus (Hyp), $n = 10$; rest of brain (minus the hypothalamus) (RB), $n = 9$) or control LNA (liver, $n = 7$; kidney, $n = 8$; skeletal muscle, $n = 6$; WAT, $n = 6$; Pan, $n = 4$; Hyp, $n = 7$; RB, $n = 6$). **b**, Serum insulin levels of HFD-fed mice treated with miR-802 LNA ($n = 8$) or control LNA ($n = 8$). **c**, Glucose tolerance tests of HFD-fed mice administered with miR-802 LNA ($n = 19$) or control LNA ($n = 17$). **d**, Insulin tolerance tests of HFD-fed mice treated with miR-802 LNA ($n = 14$) or control LNA ($n = 16$). **e**, Glucose infusion rates (GIR) during euglycaemic–hyperinsulinaemic clamps in HFD-fed mice treated with control LNA ($n = 9$) or miR-802 LNA ($n = 12$). **f**, Hepatic glucose production (HGP) of HFD-fed mice treated with control LNA ($n = 9$) or miR-802 LNA ($n = 12$) before (basal) and during (steady state) euglycaemic–hyperinsulinaemic clamp analysis. $P = 0.065$ in unpaired one-tailed (based on the improved insulin sensitivity of miR-802 LNA treated mice) Student's *t*-test. **g**, **h**, Immunoblot analysis of insulin-stimulated Akt^{S473} phosphorylation in liver (**g**) and skeletal muscle (**h**) of HFD-fed mice treated with control and miR-802 LNA (top). Densitometric quantification of Akt phosphorylation in liver (**g**) and skeletal muscle (**h**) of control ($n = 9$) or miR-802-LNA-treated mice ($n = 12$) (bottom). In all panels error bars indicate mean ± s.e.m.; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

muscle and did not affect miR-802 expression in the pancreas, WAT, hypothalamus or other brain areas (Fig. 3a). Anti-miR-802 treatment had no effect on body weight, body fat content or circulating serum leptin concentrations (Supplementary Fig. 3a–c) as well as no effect on adipocyte size or gene expression of pro-inflammatory mediators in WAT (Supplementary Fig. 3d–f). On the other hand, the obesity-associated rise in serum insulin concentrations was diminished in HFD animals treated with miR-802 LNA (Fig. 3b). In accordance with this, glucose tolerance tests revealed an improvement of glucose tolerance upon miR-802 inhibition (Fig. 3c). Moreover, insulin sensitivity was also improved upon inhibition of miR-802 (Fig. 3d). To further examine the exact mechanisms and cell type through which reducing miR-802 expression improves glucose metabolism, we performed euglycaemic–hyperinsulinaemic clamps in HFD-fed mice, which had received either control or anti-miR-802 LNA. Reduction of miR-802 expression significantly increased glucose infusion rate by 1.5-fold during the clamp (Fig. 3e and Supplementary Fig. 4a), whereas analysis of insulin-stimulated glucose uptake in skeletal muscle and WAT did not reveal any differences between mice receiving control or anti-miR-802 LNA (Supplementary Fig. 4b). By contrast, the ability of insulin to suppress glucose production was enhanced in mice receiving anti-miR-802 compared to those receiving control LNA, although this effect did not reach statistical significance (Fig. 3f). Moreover, the ability of insulin to inhibit *G6pc* expression in the liver as well as *G6pc* and *Pck1* expression in the kidney was enhanced upon inhibition of miR-802 expression (Supplementary Fig. 4c). Consistent with improved hepatic insulin sensitivity, insulin-stimulated Akt phosphorylation was enhanced in the liver (Fig. 3g), but not in the skeletal muscle of anti-miR-802 treated animals during the clamp (Fig. 3h). Taken together, the findings show that reduction of miR-802 expression in diet-induced obese mice results in improvement of insulin sensitivity predominantly by increasing insulin action in the liver.

We aimed to identify those mRNA(s) that are targeted by miR-802 and serve as its molecular effector(s) in disease progression. Using a stringent bioinformatics approach, we identified 26 putative murine miR-802 target genes (Supplementary Fig. 5a), among which the gene encoding hepatocyte nuclear factor 1 beta (*Hnf1b;* also known as transcription factor 2, *Tcf2*) harboured a miR-802 binding site, which is also conserved in the human *HNF1B* gene (Supplementary Fig. 5b). HNF1B has been causally linked to the development of maturity onset diabetes of the young (MODY) type 5 (ref. 8) and variants in *HNF1B* have also been linked to predisposition for type 2 diabetes[9]. Inclusion of the *Hnf1b* 3′ untranslated region (UTR) into a luciferase reporter construct reduced luciferase activity compared to a reporter lacking the *Hnf1b* 3′ UTR upon transfection into Hepa1-6 cells (Fig. 4a and Supplementary Fig. 5c). Overexpression of miR-802 led to a further reduction of luciferase activity when the reporter construct contained the *Hnf1b* 3′ UTR (Fig. 4a). In contrast, mutation of the conserved miR-802 binding motif abrogated reduced luciferase expression (Fig. 4a). Moreover, overexpression of miR-802 in Hepa1-6 cells led to reduced Hnf1b protein expression (Supplementary Fig. 5d). *In vivo*, hepatic Hnf1b protein expression was reduced by 50% in $Lepr^{db/db}$ mice, which exhibit increased miR-802 expression compared to controls (Fig. 4b), whereas hepatic Hnf1b protein expression was significantly increased in mice that had been treated with anti-miR-802 LNA

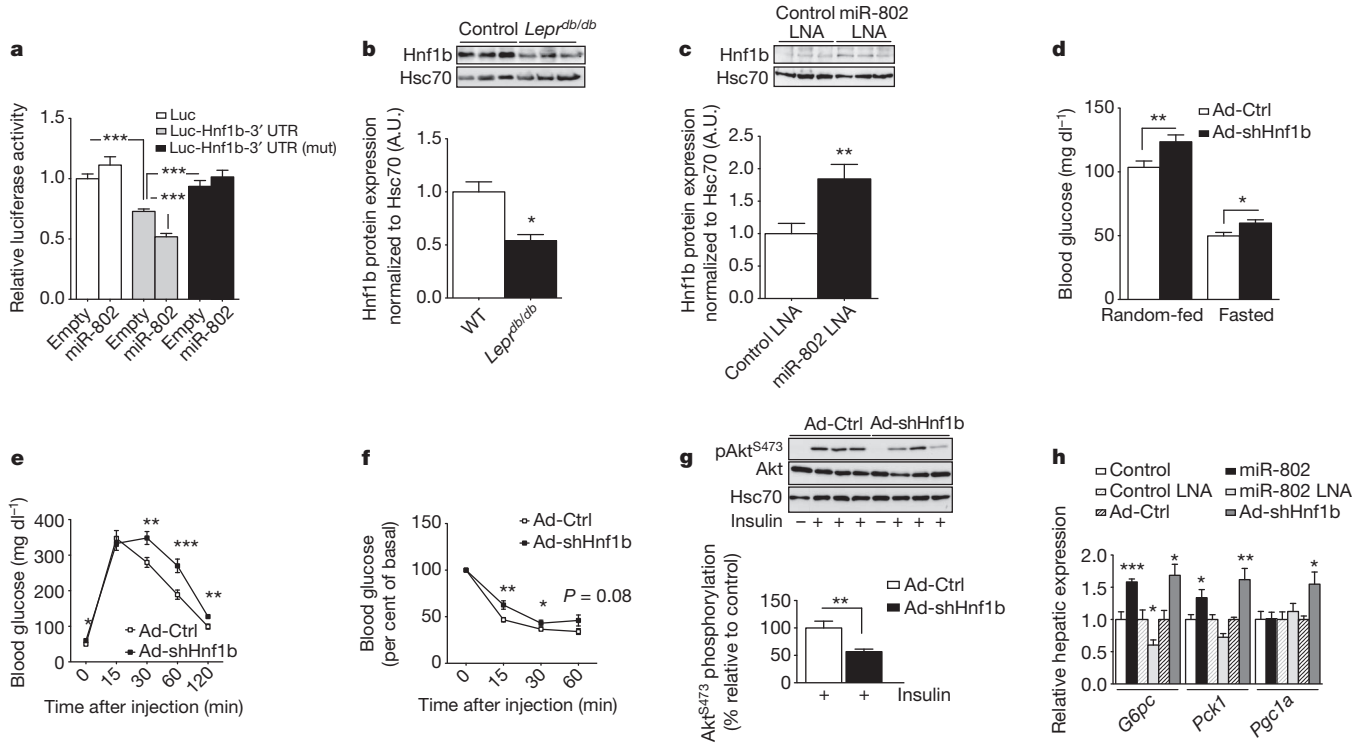

Figure 4 | Silencing of the miR-802 target *Hnf1b* impairs insulin action and glucose metabolism. **a**, Relative luciferase activity of the firefly reporter constructs containing either the wild-type or mutated 3′ UTR of the murine *Hnf1b* gene. Firefly luciferase activity was normalized to the activity of *Renilla* luciferase. Luciferase activity of the luciferase-only containing construct was set to 1. One-way ANOVA was carried out followed by Bonferroni post-hoc analysis, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. **b**, Immunoblot analysis (top) and densitometry quantification (bottom) of hepatic Hnf1b expression in the liver of $Lepr^{db/db}$ ($n = 3$) and control mice ($n = 3$). **c**, Representative immunoblot analysis (top) and densitometric quantification (bottom) of hepatic Hnf1b expression in HFD-fed control LNA treated mice ($n = 10$) and miR-802 LNA treated mice ($n = 9$). **d**, Blood glucose concentrations of random-fed and overnight-fasted mice treated with Ad-shHnf1b ($n = 20$) or Ad-Ctrl ($n = 20$). **e**, Glucose-tolerance tests of mice treated with Ad-shHnf1b ($n = 20$) or Ad-Ctrl ($n = 20$). **f**, Insulin tolerance tests of mice administered Ad-shHnf1b ($n = 20$) or Ad-Ctrl ($n = 20$). **g**, Representative immunoblot analysis (top) and quantification (bottom) of insulin-stimulated Akt Serine 473 phosphorylation versus total Akt protein levels in liver of mice delivered Ad-shHnf1b ($n = 7$) or Ad-Ctrl ($n = 7$). For quantification, the mean value for insulin-stimulated intensity of control animals was set to 100%. **h**, qRT–PCR analysis of *G6pc*, *Pck1* and *Pgc1a* mRNA expression in liver of Dox-treated miR-802 transgenic ($n = 6$) versus control animals ($n = 6$); HFD-fed mice treated with miR-802 LNA ($n = 9$) and control LNA ($n = 7$); and wild-type mice treated with Ad-shHnf1b ($n = 9$) and Ad-Ctrl ($n = 9$). In all panels error bars indicate mean ± s.e.m.; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(Fig. 4c). Functionally, shRNA-mediated silencing of *Hnf1b* in Hepa1-6 cells led to enhancement of basal and forskolin-induced *G6pc* and *Pck1* expression (Supplementary Fig. 6a) and to increased expression of *Socs1* and *Socs3* (Supplementary Fig. 6b), similar to what was observed upon miR-802 overexpression. Taken together, these experiments define *Hnf1b* as a target of miR-802-dependent post-transcriptional silencing in liver *in vitro* and *in vivo*.

To gain further insights into how partial reduction of Hnf1b expression affects glucose metabolism, we performed glucose and insulin tolerance tests in heterozygous *Hnf1b*-deficient mice ($Hnf1b^{+/-}$) and control littermates[10]. However, these analyses revealed no differences in glucose or insulin tolerance between these groups of mice (Supplementary Fig. 7a, b). Surprisingly, analysis of *Hnf1b* mRNA and protein expression revealed only a negligible reduction of *Hnf1b* mRNA and unaltered protein expression in liver (Supplementary Fig. 7c, d) and kidney of $Hnf1b^{+/-}$ mice (Supplementary Fig. 7e, f). Interestingly, $Hnf1b^{+/-}$ mice exhibited reduced hepatic miR-802 expression (Supplementary Fig. 7g), potentially contributing to unaltered Hnf1b protein expression in these animals. Thus, we next aimed to acutely and efficiently reduce hepatic Hnf1b expression through generation of adenoviruses expressing shRNA targeting *Hnf1b* (Ad-shHnf1b) and control viruses (Ad-Ctrl). When administered intravenously to the control mice, injection of Ad-shHnf1b led to a significant, 40% reduction of hepatic *Hnf1b* mRNA expression (Supplementary Fig. 8a). Importantly, analysis of global miRNA expression in these animals revealed that pol-III-dependent expression of the Hnf1b-shRNA did not generally interfere with expression and processing of small RNA molecules (Supplementary Fig. 9). Ad-shHnf1b-mediated reduction of hepatic Hnf1b protein expression had no effect on body weight, fat mass, adipocyte size or WAT inflammation (Supplementary Fig. 8b–e), whereas it resulted in significantly increased blood glucose concentrations (Fig. 4d). Moreover, glucose and insulin tolerance tests (GTT and ITT, respectively) revealed a profound reduction in glucose tolerance and insulin sensitivity in Ad-shHnf1b-treated animals (Fig. 4e, f), and insulin-evoked phosphorylation of Akt was significantly impaired in these mice (Fig. 4g). In addition, microarray gene expression profiling of mRNAs isolated from liver of Ad-shHnf1b- and Ad-Ctrl-treated mice revealed the coordinate induction of key catabolic, fasting-associated gene ontology (GO) pathways such as gluconeogenesis, beta-oxidation of fatty acids, oxidative phosphorylation and the tricarbon acid cycle upon reduction of Hnf1b expression (Supplementary Fig. 10 a–d). qRT–PCR analyses confirmed the significant induction of *Pgc1a* and its gluconeogenic target genes *Pck1* and *G6pc* upon reduction of Hnf1b protein expression (Fig. 4h). Conversely, expression of *G6pc* and *Pck1* mRNA was reduced in anti-miR-802 LNA treated HFD mice and increased in the liver of mice with conditional overexpression of miR-802 (Fig. 4h).

We next investigated whether adenoviral overexpression of Hnf1b can affect the increased *G6pc* expression observed upon miR-802 overexpression in Hepa1-6 cells. Indeed, a 1.5-fold increase in *Hnf1b* expression reversed the increased *G6pc* mRNA expression upon concomitant miR-802 overexpression (Supplementary Fig. 11 a, b). Next,

we aimed to restore hepatic Hnf1b expression via adenovirus-mediated overexpression of Hnf1b in liver of $Lepr^{db/db}$ mice. Injection of a *Hnf1b*-expressing adenovirus resulted in a ninefold increase in hepatic *Hnf1b* expression compared to $Lepr^{db/db}$ mice, which had been injected with a GFP-expressing control vector (Supplementary Fig. 11c). Increasing hepatic Hnf1b expression lead to an improvement of insulin sensitivity in $Lepr^{db/db}$ mice and to a reduction of HOMA-IR indices (Supplementary Fig. 11d, e), supporting the model that miR-802-mediated reduction of hepatic Hnf1b expression contributes to the metabolic impairment observed upon development of obesity.

Our study reveals an important role for obesity-induced overexpression of miR-802 in the development of obesity-associated insulin resistance. Interestingly, the degree of increased hepatic expression of miR-802 in $Lepr^{db/db}$ mice exceeded that of miRNAs, whose upregulation has been described so far in the context of obesity-associated insulin resistance. The observation that overexpression of miR-802 causes insulin resistance and impairs glucose tolerance, whereas reducing miR-802 expression in obese mice improves these metabolic parameters, clearly indicates a functional role for increased miR-802 expression in the development of obesity-associated insulin resistance. Importantly, increased miR-802 expression is not restricted to murine obesity models, but is also detected in obese humans, thus characterizing miR-802 and its target gene(s) as potential new targets for the treatment of obesity-associated insulin resistance and type 2 diabetes. Among these potential target genes, we have functionally validated *Hnf1b* as a bona fide miR-802 target both *in vitro* and *in vivo*. Hnf1b constitutes a member of the homeodomain-containing superfamily of liver-enriched transcription factors, and truncated or loss-of-function *HNF1B* alleles cause MODY type 5 in humans[8,11]. In addition, some genome-wide association studies revealed an association between *HNF1B* variants with the susceptibility to develop type 2 diabetes, whereas others failed to observe this effect in different populations[12]. Although the functional consequences of dysfunctional *HNF1B* alleles are well understood for the development of MODY type 5 in humans, as they affect not only pancreatic β-cell function but also cause insulin resistance[13,14], surprisingly few studies have addressed the role of Hnf1b in liver. Our experiments clearly reveal an important role for Hnf1b in control of hepatic insulin sensitivity and glucose metabolism *in vivo*. We demonstrate that both overexpression of miR-802 or knockdown of *Hnf1b* leads to upregulation of hepatic *Socs1* and *Socs3* expression. These findings are consistent with earlier reports that Hnf1b represses *Socs3* expression and in turn enhances hepatocyte growth factor signalling in kidney[15]. Because both *Socs1* and *Socs3* are characterized mediators of insulin resistance *in vitro* and *in vivo*[16,17], miR-802-evoked, Hnf1b-dependent derepression of *Socs* transcription represents a candidate pathway to cause insulin resistance. Although the mechanism(s) of how Hnf1b controls hepatic glucose metabolism clearly requires further investigation, our study reveals an important role for miR-802- and Hnf1b-dependent regulation of insulin sensitivity and glucose metabolism *in vivo*.

METHODS SUMMARY

LNA synthesis and administration. Custom-made miRCURY locked nucleic acids (LNA) for *in vivo* application were designed and synthesized as unconjugated and fully phosphorothiolated oligonucleotides by Exiqon. The sequence of the LNA targeting miR-802 was fully complementary to the mature miRNA sequence: 5′-AATCTTTGTTACTG-3′ (miR-802 LNA); the appropriate mismatch LNA control was mutated in four nucleotide positions: 5′-TATGTTACTTACTG-3′ (control LNA). LNA were intravenously delivered to HFD-fed mice at a concentration of 25 mg kg^{-1} body weight in 1×PBS. Mice were injected on two consecutive days and killed two weeks after LNA administration.

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Supplementary Information is available in the online version of the paper.

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Author Information Gene expression data was deposited with Gene Expression Omnibus (GEO) under accession number GSE42188. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.C.B. (bruening@nf.mpg.de).