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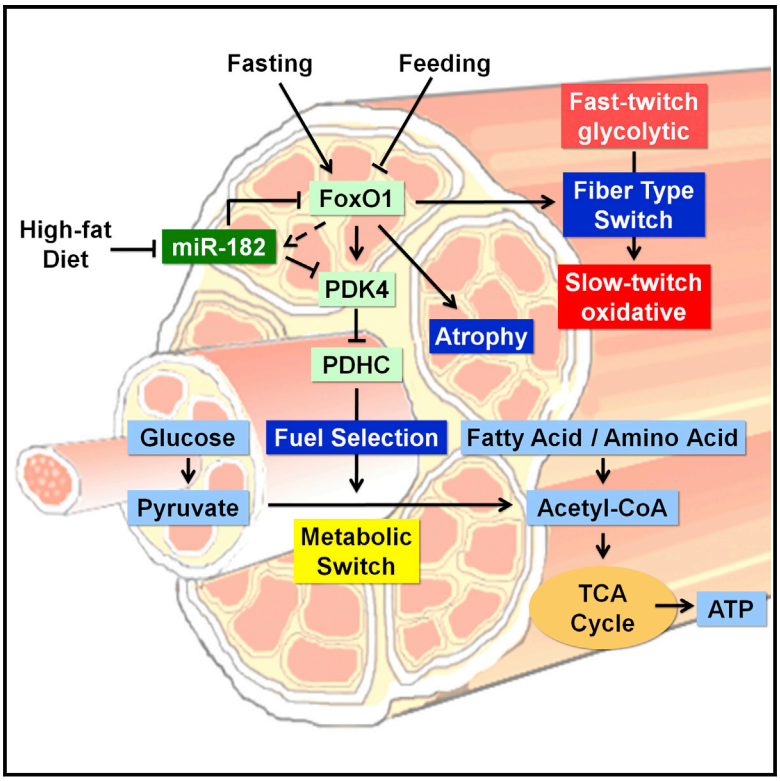
miR-182 Regulates Metabolic Homeostasis by Modulating Glucose Utilization in Muscle

Graphical Abstract



Highlights

- miR-182 is enriched in fast muscle, and its level correlates with blood glucose level
- Loss of miR-182 leads to muscle fiber-type switching and impaired glucose metabolism
- miR-182 regulates glucose utilization by modulating PDHC activity via FoxO1/PDK4
- Restoration of miR-182 expression improves glucose metabolism in mice fed a high-fat diet-fed

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In Brief

Zhang et al. observe that miR-182 is highly expressed in fast-twitch muscle and that mice lacking miR-182 exhibit muscle loss, fast-to-slow fiber-type conversion, and abnormal glucose homeostasis. Mechanistic studies reveal that miR-182 modulates glucose utilization in muscle by targeting FoxO1 and PDK4, which control fuel selection via PDHC.

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miR-182 Regulates Metabolic Homeostasis by Modulating Glucose Utilization in Muscle

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SUMMARY

Understanding the fiber-type specification and metabolic switch in skeletal muscle provides insights into energy metabolism in physiology and diseases. Here, we show that miR-182 is highly expressed in fast-twitch muscle and negatively correlates with blood glucose level. miR-182 knockout mice display muscle loss, fast-to-slow fiber-type switching, and impaired glucose metabolism. Mechanistic studies reveal that miR-182 modulates glucose utilization in muscle by targeting FoxO1 and PDK4, which control fuel selection via the pyruvate dehydrogenase complex (PDHC). Short-term high-fat diet (HFD) feeding reduces muscle miR-182 levels by tumor necrosis factor α (TNFα), which contributes to the upregulation of FoxO1/PDK4. Restoration of miR-182 expression in HFD-fed mice induces a faster muscle phenotype, decreases muscle FoxO1/PDK4 levels, and improves glucose metabolism. Together, our work establishes miR-182 as a critical regulator that confers robust and precise controls on fuel usage and glucose homeostasis. Our study suggests that a metabolic shift toward a faster and more glycolytic phenotype is beneficial for glucose control.

INTRODUCTION

Skeletal muscles are composed of different types of fibers exhibiting distinct contractile and metabolic properties. Myofiber types are characterized by different myosin heavy chain (MyHC) subtypes. For example, type I (slow oxidative) fibers express slow MyHC (MyHC I), whereas type II (fast glycolitic/oxidative) fibers express fast MyHC (MyHC IIa, IIx/d, and IIb) (Schiaffino and Reggiani, 1996). It has been widely accepted that type I fibers possess higher oxidative capacity compared to type II fibers and that enhancing oxidative metabolism has beneficial metabolic effects. However, a growing body of evidence suggests that an increase in glycolytic metabolism also has the capacity to improve glucose homeostasis (Gordon et al., 2009; Izumiya et al., 2008; LeBrasseur et al., 2011; Meng et al., 2013). It is still not clear whether obesity or diabetes is a cause or consequence of the transition from oxidative to glycolytic metabolism in muscle (Patti et al., 2003; Petersen et al., 2003). Therefore, understanding the molecular basis for fiber-type specification and metabolic switch in skeletal muscle may not only have implications for obesity and diabetes but also help us to develop new strategies to improve metabolism in disease.

Metabolic flexibility is the capacity for the organism to adapt fuel oxidation to fuel availability (Galgani et al., 2008). When the glucose level is low during fasting or after exercise, the major energy source for skeletal muscle is switched from carbohydrate to other energy sources, such as fatty acids or amino acids, to preserve glycogen storage and glucose levels for

glucose-dependent tissues (Cahill et al., 1966). Since metabolic flexibility is impaired in the muscle of insulin-resistant patients (Galgani et al., 2008; Kelley and Mandarino, 2000), the molecular mechanisms underlying the fuel usage have received increasing attention. The fuel selection occurs at the level of the pyruvate dehydrogenase complex (PDHC), which catalyzes pyruvate to form acetyl-CoA (coenzyme A) and links glycolysis to the tricarboxylic acid (TCA) cycle and ATP production (Zhang et al., 2014b). The PDHC activity largely depends on its phosphorylation status, which is under the control of pyruvate dehydrogenase kinase (PDK) isoenzymes. The isoform PDK4 is highly expressed in skeletal muscle and upregulated upon starvation and under pathological conditions associated with the switch from the utilization of glucose to fatty acids as an energy source, such as high-fat diet (HFD)-induced insulin resistance and type 2 diabetes (T2D) (Rinnankoski-Tuikka et al., 2012). In addition, the mRNA expression level of PDK4 is also regulated by the transcriptional factor, forkhead box O1 (FoxO1) (Furuyama et al., 2003). The regulatory role of the FoxO1/PDK4/PDHC axis in metabolic flexibility has been suggested.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate target gene expression post-transcriptionally. Recently, it has been shown that miRNAs modulate insulin action in multiple metabolic organs, including liver, adipose tissue, and muscle. Whether miRNAs control whole-body glucose homeostasis by targeting key genes involved in fuel selection in muscle is largely unknown. miR-182 has been previously reported as a potential signature miRNA that distinguished patients with impaired fasting glucose (IFG) and T2D. miR-182 is downregulated in the skeletal muscle of diabetic rats and obese mice (Karolina et al., 2011). However, whether the alteration of miR-182 expression is an early event during the development of metabolic disease is not clear. More importantly, the role of skeletal muscle miR-182 in glucose homeostasis and pathogenesis of T2D and the upstream stimuli that control the miR-182 expression remain unknown.

Here, we identified miR-182 as a miRNA enriched in fast-twitch muscles. Mice lacking miR-182 displayed a fast-to-slow muscle fiber conversion and abnormal glucose metabolism. The regulation of miR-182 on the FoxO1/PDK4/PDHC axis via direct targeting of both FoxO1 and PDK4 was established. Further studies revealed that miR-182 could affect the phosphorylation status and enzyme activity of PDHC in muscle, thereby modulating glucose utilization, while restoration of miR-182 expression in the muscle of diabetic mice improved glucose metabolism. Together, our data demonstrated that miR-182 plays an important role in muscle fiber-type switch and fuel selection and suggest miR-182 as a therapeutic target for metabolic diseases.

RESULTS

miR-182 Is Enriched in Fast-Twitch Muscles

To identify key miRNAs involved in metabolic regulation and myofiber specification of fast-twitch glycolytic and slow-twitch oxidative muscle, we compared the miRNA expression profiles between the glycolytic, fast (type II) fiber-enriched gastrocnemius (GAS) muscle and the oxidative, mixed fast/slow (type II/I) soleus (SOL) muscle, respectively (Figures S1A and S1B). The microarray analysis revealed a cluster of miRNAs differentially expressed in GAS and SOL muscles (Table S1), including miR-182, which is highly expressed in GAS muscles. Consistent with the early reports, we observed that miR-499 and miR-208b were enriched in SOL muscles (van Rooij et al., 2009; Zhang et al., 2014a). Some of the results from microarray analysis were further confirmed by qPCR analysis (Figure 1A). Absolute quantification results showed that miR-182 displayed a moderate level of expression in muscle, compared to others (Figures S1C and S1D). These results suggest that miR-182 is enriched in fast-twitch muscle and might be involved in fast-twitch myofiber development and/or glycolytic metabolism.

miR-182 Is Required for Maintaining Fast-Twitch Muscle Phenotype

A miR-182 knockout (KO) mouse model was used to test whether miR-182 plays a role in myofiber-type determination (Jin et al., 2009). Genotyping results and qPCR analysis in the muscle of miR-182 KO mice suggest that miR-182 has been deleted efficiently (Figures S1E and S1F). The body weights of KO mice and wild-type (WT) mice were compared over the development (from 2 to 18 weeks) (Figure S1G). A slight, but statistically significant, decrease of body weight was detected in the age-matched KO mice older than 10 weeks. The net weights of slow-twitch SOL muscles, fast-twitch tibialis anterior (TA), and GAS muscles were measured, respectively. The net weight, as well as the ratio of TA and GAS muscle weight to body weight, was significantly decreased in KO mice compared to WT mice (Figures 1B and 1C). In contrast, no significant difference of the weight of slow-twitch SOL muscles was detected between KO and WT mice (Figures S1H and S1I). Consistently, body composition analysis using nuclear magnetic resonance (NMR) exhibited a lower lean body mass in KO mice compared to WT mice (Figure 1D). These data imply that miR-182 may be involved in muscle mass maintenance of the fast-twitch glycolytic muscle but not of the slow-twitch oxidative muscle.

We also observed that the percentile of type I fiber was increased in both the GAS and SOL muscles of KO mice, compared to WT mice, by ATPase staining (Figures 1E–1G), suggesting the involvement of miR-182 in muscle fiber-type specification. Consistently, miR-182 deficiency led to a fast-to-slow shift in MyHC expression (Figure 1H). The shift from fast- to slow-twitch muscle phenotype in mice lacking miR-182 was further validated by immunostaining analysis (Figures 1I–1K). We did not observe any changes in the cross-sectional area of muscle fibers from the SOL and GAS muscles of KO mice compared to WT mice (Figure S1J). We did not find any alteration in the mRNA expression of nuclear respiratory factor 1 (NRF1), which is located close to miR-182 on the chromosome, and its target gene CPT1b in KO mice, suggesting that NRF1 is not involved (Figure S1K). These results suggest that miR-182 is required for maintaining the fast-twitch muscle phenotype.

FoxO1 Is a Target Gene of miR-182 in Skeletal Muscle

FoxO1, a key regulator involved in muscle fiber-type determination and muscle atrophy, was noticed as a direct target gene of miR-182 (Guttilla and White, 2009; Kamei et al., 2004; Kitamura

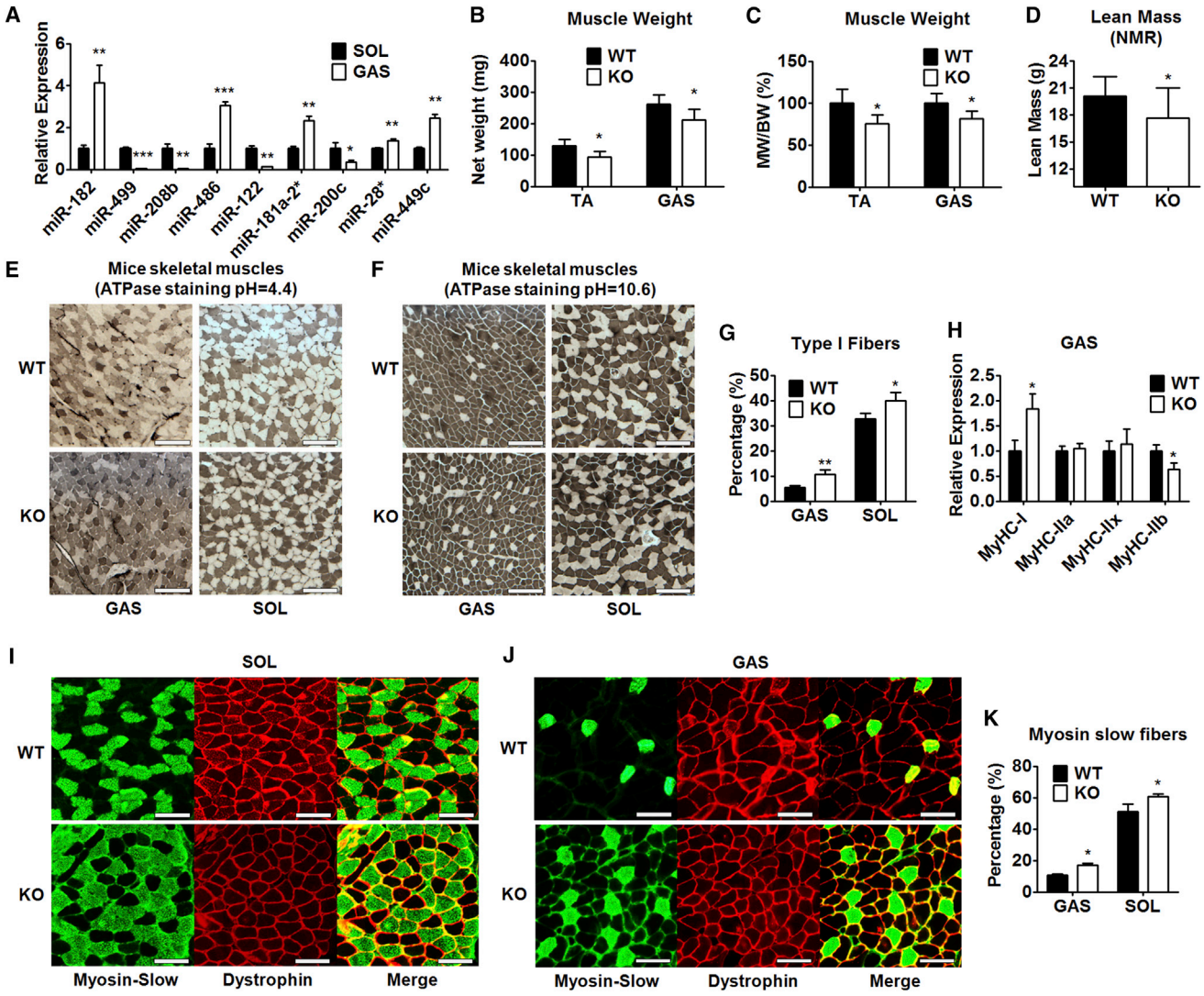

Figure 1. miR-182 Is Required for Maintaining Fast-Twitch Muscle Phenotype

(A) qPCR analysis of miRNA expression in the SOL and GAS muscles of mice (n = 3). Means ± SD are shown. *p < 0.05 versus SOL muscle; **p < 0.01 versus SOL muscle; ***p < 0.001 versus SOL muscle.

(B and C) The net weights of fast-twitch TA and GAS muscles (B) and the ratios of TA and GAS muscle weight (MW) to body weight (BW) (C) in WT and KO mice (n = 5).

(D) Analysis of lean mass of WT and KO mice by NMR technique (n = 13–16).

(E and F) Metachromatic ATPase staining of GAS and SOL muscles of WT and KO mice under different experimental condition as indicated. Scale bars, 150 μm.

(G) Percentage of type I fibers in SOL muscle of WT and KO mice according to the ATPase staining (n = 3).

(H) qPCR analysis of the mRNA expression of MHC isoforms in the GAS muscle of WT and KO mice (n = 3).

(I and J) Immunostaining of the SOL (I) and GAS (J) muscles of WT and KO mice using antibody against myosin-slow (green) and dystrophin (red). Scale bars, 100 μm.

(K) Percentage of myosin-slow fibers in the SOL muscle of WT and KO mice according to the immunostaining (n = 3).

Means ± SD are shown for (B)–(D), (G), (H), and (K). *p < 0.05 versus WT mice; **p < 0.01 versus WT mice.

See also Figure S1.

et al., 2007; Stittrich et al., 2010). Whether miR-182 affected muscle phenotype directly through FoxO1 was investigated. KO mice had a higher level of FoxO1 protein in both fast-twitch GAS and slow-twitch SOL muscles, compared to WT mice (Figure 2A). An elevated level of FoxO1 mRNA was also observed in the GAS muscles of KO mice (Figure 2B). Loss of miR-182 resulted in an increase of FoxO1 transcripts in the primary myotubes from KO and WT mice (Figure 2C). Consistent with the data in Figure 1E–1K, we observed higher protein levels of Myh7 (slow fiber marker) and mRNA levels of two slow fiber markers, myoglobin (MB) and Troponin I Type 1 (Tnni1), in the muscles or muscle cells from KO mice, compared to WT mice

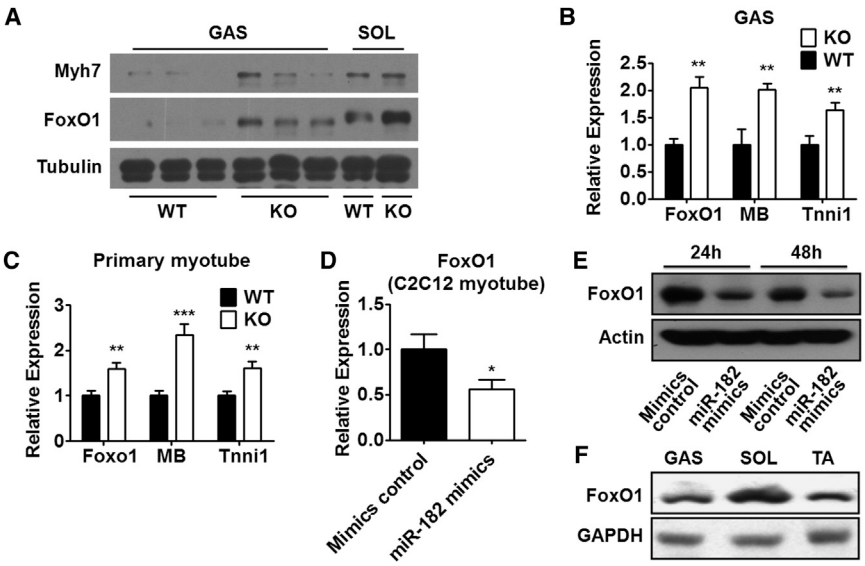

Figure 2. FoxO1 Is a Target Gene of miR-182 in Skeletal Muscle

(A) Western blot analysis of FoxO1, Myh7, and Tubulin protein expression in the GAS and SOL muscles of WT and KO mice.

(B) qPCR analysis of FoxO1, MB, and Tnni1 mRNA expression in the GAS muscle of WT and KO mice (n = 3). Means ± SD are shown. **p < 0.01 versus WT mice.

(C) qPCR analysis of FoxO1, MB, and Tnni1 mRNA expression in primary myotubes derived from WT and KO mice (n = 3). Means ± SD are shown. **p < 0.01 versus WT myotubes; ***p < 0.001 versus WT myotubes.

(D) qPCR analysis of FoxO1 mRNA expression in C2C12 myotubes transfected with miR-182 mimics. Means ± SD are shown. *p < 0.05 versus cells transfected with mimics control.

(E) Western blot analysis of FoxO1 protein expression in C2C12 myotubes 24 or 48 hr after transfection with miR-182 mimics or mimics control.

(F) Western blot analysis of FoxO1 protein levels in the GAS, SOL, and TA muscles of mice.

See also Figure S2.

(Figures 2A–2C). In addition, overexpression of miR-182 using specific mimics reduced both mRNA and protein levels of FoxO1 in C2C12 myotubes (Figures 2D and 2E). Moreover, overexpression of miR-182 inhibited the luciferase activity of the reporter containing the FoxO1-3′ UTR with an miR-182-responsive element (MRE) in C2C12 myotubes (Figure S2A). As expected, we observed the highest mRNA and protein levels of FoxO1 in slow-twitch SOL muscles, in which the expression of miR-182 was the lowest (Figures 2F, S2B, and S2C). These results suggest that FoxO1 is a target of miR-182 in skeletal muscle and might be responsible for the atrophy phenotype and the shift from fast- to slow-twitch myofiber type in the muscle of KO mice.

miR-182 Plays a Regulatory Role in Glucose Homeostasis

FoxO1 regulates the muscle fuel usage by modulating PDHC activity via PDK4 (Furuyama et al., 2003). We speculated that the upregulation of FoxO1 due to miR-182 loss would impair metabolic flexibility and lead to abnormal glucose homeostasis. As expected, our results revealed that KO mice exhibited impaired glucose tolerance (Figures 3A and 3B), indicating that miR-182 is required for maintaining normal glucose homeostasis. We then determined the miR-182 expression pattern in the GAS muscles of mice with different blood glucose levels. We found that miR-182 expression was upregulated in the GAS muscles of mice fasted overnight, which was accompanied by a drop in blood glucose level (Figures 3C and S3A). In contrast, the expression of miR-182 was downregulated in the GAS muscles of mice after HFD feeding for 2 months, which led to an increase in blood glucose level (Figures 3D and S3B). Furthermore, in a cohort of human subjects with normal glucose levels, a moderate negative correlation ($r^2 = 0.44$, $p < 0.0001$) between the level of miR-182 in muscle specimens and the blood glucose level was observed (Figure 3E), further suggesting an association of miR-182 expression with the maintenance of glucose homeostasis.

The effect of glucose and insulin on the expression of miR-182 in C2C12 myotubes was investigated. Interestingly, we found that decreasing the glucose concentration in the culture medium induced the expression of miR-182, while insulin treatment decreased miR-182 levels (Figures 3F and 3G). We then tested whether FoxO1, as a downstream gene of insulin signaling, could affect the expression of miR-182. As expected, overexpression of FoxO1 stimulated the expression of miR-182, while downregulation of FoxO1 by specific small interfering RNA (siRNA) repressed the expression of miR-182 in C2C12 myotubes (Figures S3C and S3D).

PDK4 Is a miR-182 Target in Skeletal Muscle

To test whether the effect of miR-182 on glucose metabolism was solely dependent on FoxO1, we introduced a FoxO1 expression plasmid lacking 3′ UTR into the C2C12 myotubes transfected with an agomir RNA (agomiRNA) for miR-182 (agomiR-182) or a control agomiRNA. Interestingly, we observed that overexpression of FoxO1 was unable to totally attenuate the repressive effect of miR-182 on both mRNA and protein expression of PDK4, suggesting that FoxO1 was not solely responsible for the effect of miR-182 on PDK4 (Figures 4A and 4B). We identified a putative miR-182 target site in the PDK4-3′ UTR, which is highly conserved across different species (Figure 4C). Overexpression of miR-182 repressed the luciferase activity of a reporter containing PDK-3′ UTR with an miR-182-responsive element in HEK293T cells and C2C12 myoblasts, respectively (Figures 4D and S4A). In contrast, overexpression of miR-182 had no repressive effect on a reporter with a mutation in miR-182 responsive element (Figure 4D).

As expected, overexpression of miR-182 decreased both the mRNA and protein levels of PDK4 in C2C12 myotubes (Figures 4E and 4F), while loss of miR-182 resulted in an elevation in PDK4 mRNA levels (Figure 4G) and protein levels (Figure 4H) in the GAS muscles of KO mice compared to WT mice. Similar

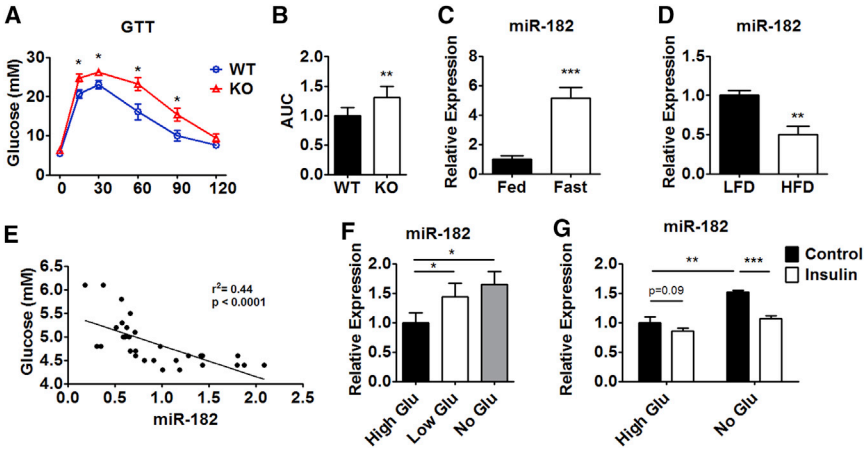

Figure 3. miR-182 Plays a Regulatory Role in Glucose Homeostasis

(A and B) Glucose tolerance test (GTT) (A) and area under the curve (AUC) data for GTT (B) in WT and KO mice (n = 5–8). Means ± SD are shown. *p < 0.05 versus WT mice; **p < 0.01 versus WT mice.

(C) qPCR analysis of miR-182 expression in the GAS muscle of fed and fasted mice (n = 3). Means ± SD are shown. ***p < 0.001 versus fed mice.

(D) qPCR analysis of miR-182 expression in the GAS muscle of low-fat diet (LFD)- and HFD-fed mice (n = 3). Means ± SD are shown. **p < 0.01 versus LFD-fed mice.

(E) Correlation between relative miR-182 levels and blood glucose levels in a group of humans with normal blood glucose levels (n = 16).

(F) qPCR analysis of miR-182 expression in the C2C12 myotubes cultured in medium with high-glucose (High Glu) concentration, low-glucose (Low Glu) concentration, or without glucose (No Glu). Means ± SD are shown. *p < 0.05 versus control group as indicated.

(G) qPCR analysis of miR-182 expression in the C2C12 myotubes treated with insulin in the presence of high-glucose medium (High Glu) or in the absence of glucose (No Glu). Means ± SD are shown. **p < 0.01; ***p < 0.001 versus control group as indicated.

See also Figure S3.

results were obtained from the GAS muscles of female KO mice (Figure S4B). Since it is known that the expression of FoxO1 and PDK4 are elevated upon fasting, it is not surprising to see a larger upregulation of FoxO1 and PDK4 in the GAS muscles of KO mice upon fasting (Figure S4C). In addition, both protein and mRNA levels of PDK4 were higher in slow-twitch SOL muscle than those in fast-twitch GAS muscle (Figures 4I and 4J). These results indicated that PDK4 is a direct target gene of miR-182 in skeletal muscle and might mediate the metabolic effect of miR-182.

miR-182 Modulates Glucose Utilization in Skeletal Muscle

Since the PDHC activity largely depends on its phosphorylation status by PDK isoenzymes, we speculated that miR-182 could affect the phosphorylation status of PDHC. To test this hypothesis, we investigated the effect of miR-182 on the phosphorylation status of PDHC. As expected, overexpression of miR-182 decreased the protein levels of FoxO1, PDK4, and the phosphorylated alpha subunit of pyruvate dehydrogenase (p-PDHA1), while inhibition of miR-182 increased the protein levels of FoxO1, PDK4, and p-PDHA1 in C2C12 myotubes (Figure 5A) and L6 myotubes (Figure S5A). In KO mice, an increase in p-PDHA1 protein levels was observed in GAS muscles (Figure 5B). Importantly, we found that overexpression of miR-182 by using agomiR-182 rescued the phenotype of increased FoxO1, PDK4, and p-PDHA1 levels in primary myotubes from KO mice, suggesting that the altered expression of FoxO1, PDK4, and p-PDHA1 is as a direct effect of de-repression via miR-182 (Figure 5C).

We then analyzed the enzyme activity of PDHC in the GAS muscles of KO mice and C2C12 myotubes transfected with an antagomir specific for miR-182 (Ant-182) or agomiR-182. As expected, both miR-182 deficiency in KO mice and downregulation of miR-182 in C2C12 myotubes resulted in a lower enzyme activity of PDHC compared to control groups, while overexpression of miR-182 in C2C12 myotubes led to a higher activity (Figure 5D). Since PDHC catalyzes the key step in glucose oxidation, we measured the glucose oxidation by using [U-^{14}C] D-glucose. As expected, either lacking miR-182 or inhibition of miR-182 by Ant-182 suppressed the glucose oxidation, while overexpression of miR-182 by agomiR-182 transfection enhanced the glucose oxidation in myocytes (Figure 5E). These results suggest that miR-182 modulates glucose oxidation through regulating PDHC via PDK4.

We also performed in vivo indirect calorimetry to measure the fuel utilization in KO mice. Consistent with the inhibition of PDHC and decreased glucose oxidation in muscle cells, KO mice displayed a lower respiratory exchange ratio (RER), suggesting that carbohydrate utilization was reduced (Figure 5F). In agreement of these findings, we found that pyruvate was accumulated when miR-182 was lacking or inhibited, while pyruvate content was reduced after miR-182 overexpression in myotubes (Figure 5G). The observation that acetyl-CoA levels were elevated in C2C12 myotubes overexpressing miR-182 was consistent with the increased enzyme activity of PDHC (Figure S5B). In contrast, acetyl-CoA levels were also elevated in myotubes lacking miR-182 or treated with Ant-182, indicating that the usage of other energy sources might be increased to compensate the defect in glucose utilization due to the inhibition of PDHC (Figure S5B). This result was consistent with the indirect calorimetry data from KO mice, further suggesting that miR-182 modulates fuel selection.

Interestingly, qPCR analysis revealed that agomiR-182 treatment restored the MyHC expression, suggesting that the effect of miR-182 on muscle fiber-type conversion is direct (Figure 5H). The role of miR-182 in regulating muscle glycolytic phenotype and metabolism was further investigated by measuring glycolysis and basal respiration. Both the glycolysis capacity and the rate of glycolysis were inhibited by Ant-182 transfection in C2C12 myotubes (Figures 5I and S5C). The opposite results were obtained in C2C12 myotubes transfected with agomiR-182, although the change in the rate of glycolysis did not reach

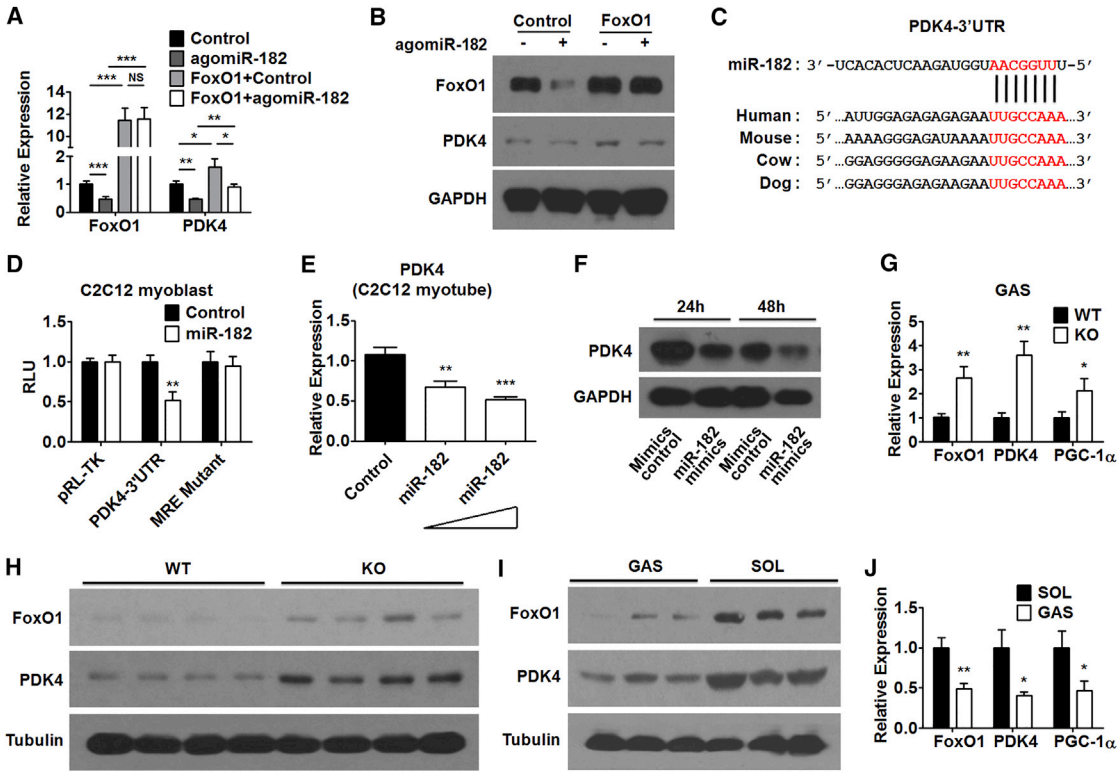

Figure 4. PDK4 Is a miR-182 Target in Muscle

(A) qPCR analysis of FoxO1 and PDK4 mRNA expression in C2C12 myotubes transfected with agomiR-182 mimics and/or FoxO1 expression vector lacking 3′ UTR (n = 3). Means ± SD are shown. *p < 0.05; **p < 0.01; ***p < 0.001; NS, not significant.

(B) Western blot analysis of FoxO1, PDK4, and GAPDH protein expression in C2C12 myotubes transfected with agomiR-182 and/or FoxO1 expression vector lacking 3′ UTR.

(C) Sequence alignment of miR-182 and the PDK4 3′ UTR from various species.

(D) Analysis of the effect of miR-182 mimics on the activity of the reporter containing PDK4 3′UTR or PDK4 3′ UTR mutated at the miR-182 regulatory element (MRE mutant) by luciferase assay in C2C12 myoblasts (n = 3). Means ± SD are shown. **p < 0.01 versus cells transfected with mimics control. RLU, relative light units.

(E) qPCR analysis of PDK4 mRNA expression in C2C12 myotubes transfected with increasing amounts of miR-182 mimics (4 nM and 10 nM; n = 3). Means ± SD are shown. **p < 0.01 versus cells transfected with mimics control; ***p < 0.001 versus cells transfected with mimics control.

(F) Western blot analysis of PDK4 protein expression in C2C12 myotubes 24 or 48 hr after transfection with miR-182 mimics or mimics control (n = 3).

(G) qPCR analysis of FoxO1, PDK4, and PGC-1α mRNA expression in the GAS muscle of WT and KO mice (n = 3). Means ± SD are shown. *p < 0.05 versus WT mice; **p < 0.01 versus WT mice.

(H) Western blot analysis of FoxO1, PDK4, and Tubulin protein expression in the GAS muscle of WT and KO mice (n = 4).

(I) Western blot analysis of FoxO1, PDK4, and Tubulin protein expression in the GAS and SOL muscles of mice (n = 3).

(J) qPCR analysis of FoxO1, PDK4, and PGC-1α mRNA expression in the GAS and SOL muscles of mice (n = 3). Means ± SD are shown. *p < 0.05 versus SOL muscle; **p < 0.01 versus SOL muscle.

See also Figure S4.

statistical significance (Figures 5I and S5C). Consistent with the role of miR-182 in promoting a glycolytic metabolic phenotype, the basal respiration was increased after Ant-182 treatment, while it was decreased after agomiR-182 transfection (Figure 5J).

Although the aforementioned in vitro results demonstrated that miR-182 modulates glucose oxidation in skeletal muscle in a cell-autonomous manner, we could not totally rule out the possibility that the effect of miR-182 in other metabolic tissues also contributed to the phenotype observed in miR-182 whole-body KO mice used in this study. We then tested whether FoxO1 and PDK4 were affected in the liver and adipose tissue of KO mice. Interestingly, only PDK4 protein expression was altered in the adipose tissue of KO mice (Figures S5D–S5G), suggesting that the elevation of PDK4 in adipose tissues might also contribute to the altered fuel usage in KO mice. The elevation of PDK4 in adipose tissues might also contribute to the increased fat mass observed in KO mice (Figure S5H), since PDK4 could redirect glucose from oxidation toward triglyceride synthesis (Barquissau et al., 2016). In addition, we did not observe any changes in liver triglyceride level in KO mice (Figure S5I).

A recent study reported that in vivo ablation of mesodermal transcription factor T-box 15 (Tbx15), which is highly and specifically expressed in glycolytic myofibers, led to a decrease in muscle size and glycolytic fibers and an increase in oxidative fibers, adiposity, and glucose intolerance (Lee et al., 2015). Since

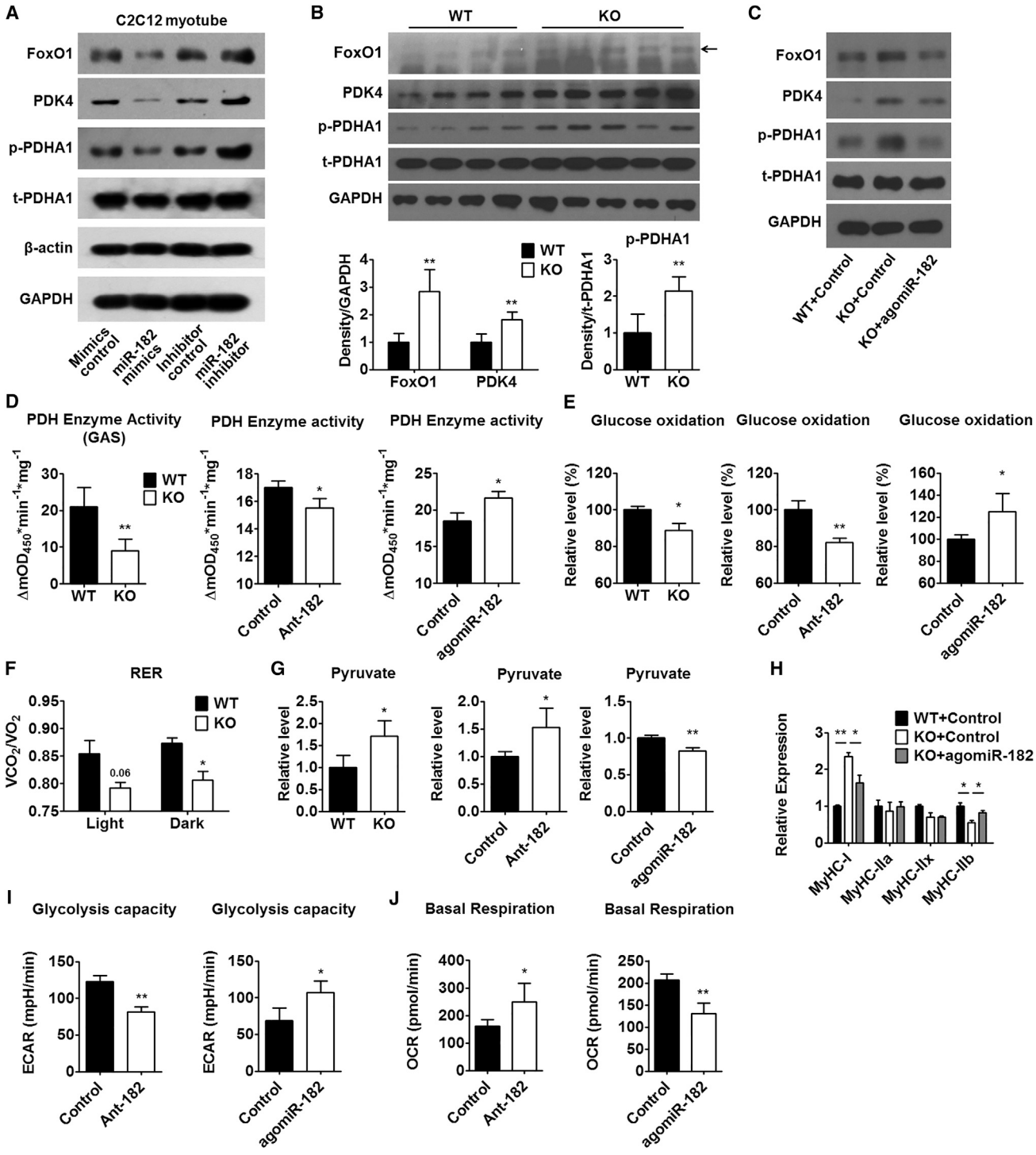

Figure 5. miR-182 Modulates Glucose Utilization in Muscle

(A) Western blot analysis of FoxO1, PDK4, p-PDHA1, t-PDHA1, β-actin, and GAPDH protein levels in C2C12 myotubes transfected with miR-182 mimics or miR-182 inhibitor as indicated.

(B) Western blot analysis of FoxO1, PDK4, p-PDHA1, t-PDHA1, and GAPDH protein levels in the GAS muscle of WT and KO mice (n = 4–5). Relative protein levels of FoxO1 and PDK4, and p-PDHA1 were normalized to those of GAPDH and t-PDHA1, respectively. Means ± SD are shown. **p < 0.01. The band corresponding to FoxO1 is indicated by the arrow.

(C) Western blot analysis of FoxO1, PDK4, p-PDHA1, t-PDHA1, and GAPDH protein expression in primary myotubes derived from WT and KO mice, which were transfected with agomiR-182 as indicated.

(legend continued on next page)

some of the phenotypes we found in the muscle, liver, and adipose tissue of KO mice were similar to those in Tbx15 knockout mice, we tested whether Tbx15 could mediate the effect of miR-182. However, we did not detect any changes in the mRNA levels of Tbx15 (Figure S5J), suggesting that Tbx15 might not be involved in the phenotypic alteration in KO mice.

TNFα Reduces miR-182 Levels Shortly after HFD Feeding

Since tumor necrosis factor α (TNFα) level is increased in diabetic mice (Borst and Conover, 2005), we tested whether it was one of the signals regulating miR-182 expression in the HFD-induced diabetic mouse model. We found that TNFα reduced miR-182 levels in a dose-dependent manner in C2C12 myotubes, which was accompanied by an increase in both FoxO1 and PDK4 mRNA transcripts (Figure 6A; Figure S6A). Our in vivo study also showed that, in the GAS muscles of mice after HFD feeding for 2 months, an upregulation of TNFα (Figure 6B), FoxO1, and PDK4 (Figure 6C) and a downregulation of miR-182 (Figure 3D) were observed. These results indicate that the increased levels of TNFα in the muscle of HFD-fed mice might contribute to the downregulation of miR-182 and the upregulation of FoxO1 and PDK4. To exclude the possibility that the change of muscle fiber-type composition—but not the elevated levels of TNFα, due to the long-term HFD feeding (2 months)—altered the expression of miR-182/FoxO1/PDK4, short-term HFD feeding for 1 and 2 weeks was performed, which did not cause a myofiber-type switch (Figure S6B). We found that 1-week HFD feeding was able to significantly change the levels of TNFα and miR-182 (Figures 6D and 6E), the mRNA levels of FoxO1 and PDK4, and the protein levels of FoxO1 (Figures 6F, 6G, and S6C) in GAS muscles. The increase in the protein levels of PDK4 and p-PDHA1 reached statistical significance in the GAS muscles of mice after 2 weeks of HFD feeding (Figures 6G and S6C). These data indicate that the dysregulation of the miR-182/FoxO1/PDK4 axis due to the elevated levels of TNFα contributes to metabolic inflexibility at a very early stage of HFD feeding and might be involved in disease initiation.

Overexpression of miR-182 Improves Glucose Metabolism

To test whether miR-182 could serve as a therapeutic target for metabolic diseases, HFD-fed mice were treated with agomiR-182. The elevation of miR-182 expression induced by agomiR-182 treatment was confirmed in the muscle of HFD-fed mice (Figure S6D). Interestingly, agomiR-182 treatment improved glucose tolerance in these diabetic mice without changing the body weight gain (Figures 6H and S6E). These agomiR-182-treated mice also displayed a smaller increase in fasting glucose level upon the HFD feeding compared to control mice (Figure 6I). As expected, the downregulation of FoxO1 and PDK4 and reduction of PDHA1 phosphorylation were observed in the muscle of mice treated with agomiR-182 (Figures 6J and 6K). A slow-to-fast shift in MHC expression upon agomiR-182 treatment was also detected (Figure 6L), suggesting that restoration of miR-182 in the HFD-fed mice triggers an oxidative-to-glycolytic metabolic shift in muscle, which is able to improve glucose metabolism. To be noted, we did not detect any changes in the mRNA expression of miR-182 and its target genes in the liver of HFD-fed mice treated with agomiR-182 (Figure S6F). The beneficial effect of agomiR-182 treatment on glucose metabolism was also seen in *ob/ob* mice (Figures S6G–S6I).

FoxO1 and miR-182 Form a Feedback Loop under Different Nutritional Status

The finding that both of the levels of miR-182 and its target genes (FoxO1/PDK4) increased (Figures 3C and S4C) in the muscle of mice upon fasting prompted us to determine the levels of miR-182 and FoxO1/PDK4 under different feeding conditions. Interestingly, we found that both mRNA and protein levels of either FoxO1 or PDK4 were increased in the GAS muscles of mice after an overnight fast and then decreased upon refeeding (Figures 7A–7C), which shared a similar expression pattern with miR-182 (Figure 7D). Given that FoxO1 is a positive regulator of miR-182 (Figures S3C and S3D), we propose that FoxO1 and miR-182 form a feedback loop and maintain glucose homeostasis under different nutritional status.

Taken together, our data indicate that skeletal muscle miR-182 regulates glucose homeostasis through controlling the activity of PDHC via targeting FoxO1/PDK4 in a cell-autonomous manner (Figure 7E). Under different feeding conditions, FoxO1 and miR-182 form a feedback loop and confer robust and precise controls on fuel usage and glucose metabolism (Figure 7F). Upon HFD feeding, downregulation of miR-182 by TNFα might contribute to aberrant fuel selection and metabolic inflexibility (Figure 7G).

(D) Total PDHA activities were determined in the skeletal muscle (GAS) from WT or KO mice (n = 5) or in the C2C12 myotubes (n = 3) transfected with Ant-182 or agomiR-182 by using a PDH activity microplate assay kit and were normalized to total protein. ΔmOD_{450}, milli-optical density units at 450 nm. Means ± SD are shown. *p < 0.05; **p < 0.01.

(E) Relative levels of glucose oxidation were measured in primary myotubes derived from WT or KO mice (n = 3–4) or in the C2C12 myotubes (n = 3) transfected with Ant-182 or agomiR-182 for 48 hr. Means ± SD are shown. *p < 0.05; **p < 0.01.

(F) Respiratory exchange ratios (RERs) were analyzed by performing indirect calorimetry using WT and KO mice (n = 4). VCO_2/VO_2, ratio of carbon dioxide production to oxygen consumption. Means ± SD are shown. *p < 0.05.

(G) Relative levels of pyruvate were examined in primary myotubes derived from the GAS muscles of WT or KO mice (n = 3) or in the C2C12 myotubes (n = 3) transfected with Ant-182 or agomiR-182 for 48 hr. Means ± SD are shown. *p < 0.05; **p < 0.01.

(H) qPCR analysis of the mRNA expression of MHC isoforms in primary myotubes derived from the GAS muscles of WT and KO mice (n = 3), which were transfected with agomiR-182 as indicated. Means ± SD are shown. *p < 0.05; **p < 0.01.

(I and J) Glycolysis capacity (I) and basal respiration (J) were analyzed in the C2C12 myotubes (n = 3–5) transfected with Ant-182 or agomiR-182. The extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR) measurements were performed using a Seahorse XF instrument. Means ± SD are shown. *p < 0.05; **p < 0.01.

See also Figure S5.

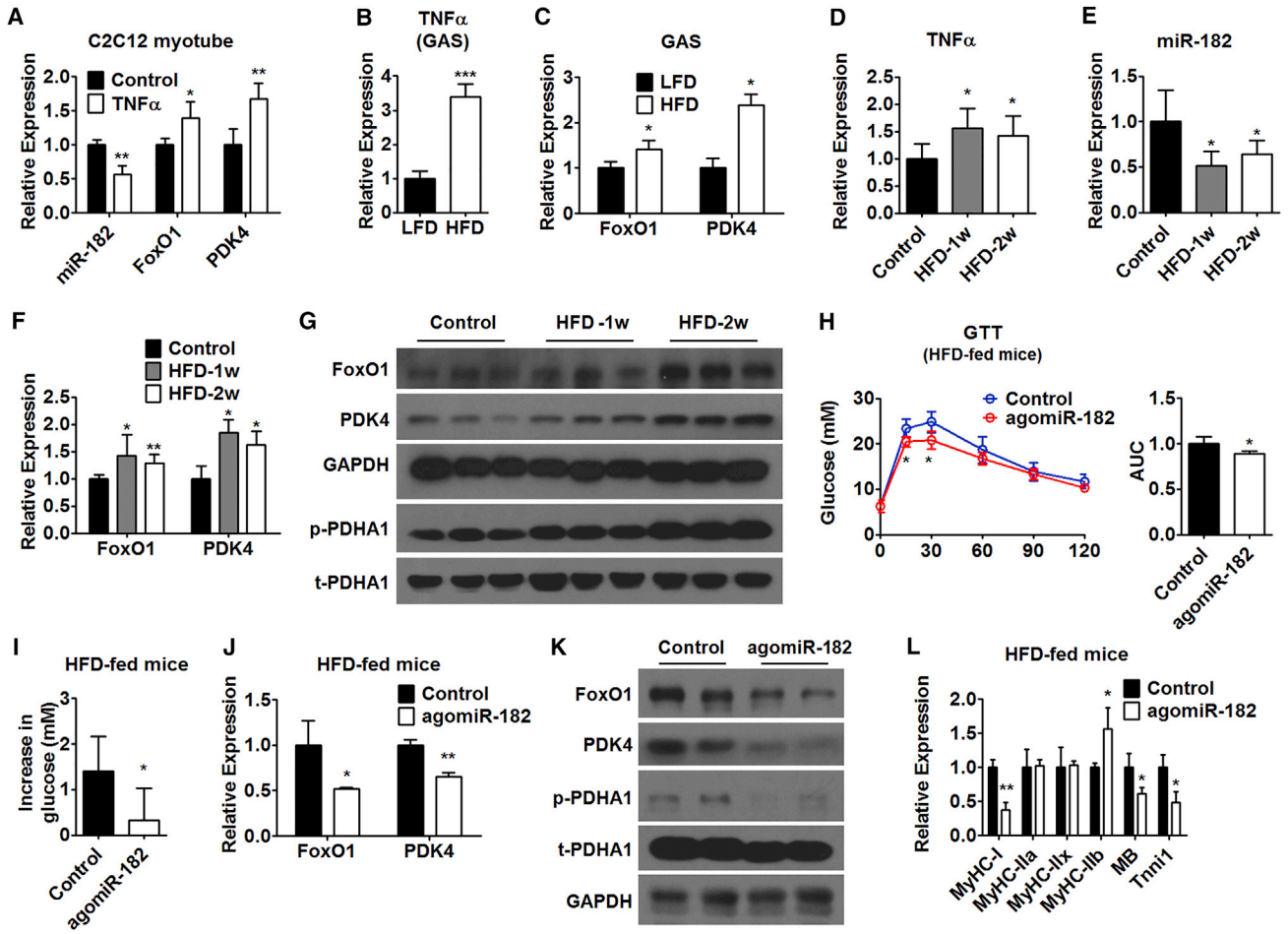

Figure 6. Restoration of miR-182 Expression Improves Glucose Metabolism in HFD-Fed Mice

(A) qPCR analysis of miR-182, FoxO1, and PDK4 mRNA levels in C2C12 myotubes treated with TNFα (n = 4). Means ± SD are shown. *p < 0.05 versus cells treated with vehicle (control); **p < 0.01 versus control.

(B and C) qPCR analysis of TNFα (B), FoxO1, and PDK4 (C) mRNA expression in the GAS muscle of mice after HFD feeding for 2 months (n = 3). Means ± SD are shown. *p < 0.05 versus LFD-fed mice; ***p < 0.001 versus LFD-fed mice.

(D–F) qPCR analysis of TNFα (D), miR-182 (E), FoxO1, and PDK4 (F) expression levels in the GAS muscle of mice after 1 or 2 weeks of HFD feeding (HFD-1w or HFD-2w) (n = 6). Means ± SD are shown. *p < 0.05 versus mice fed with regular chow diet (Control); **p < 0.01 versus control.

(G) Western blot analysis of FoxO1, PDK4, p-PDHA1, t-PDHA1, and GAPDH protein levels in GAS muscle of mice fed with HFD for 1 or 2 weeks (HFD-1w or HFD-2w) (n = 3).

(H) Glucose tolerance test (GTT) (left) and area under the curve (AUC) data for GTT (right) in HFD-fed mice treated with agomiR-182 (n = 4). Means ± SD are shown. *p < 0.05.

(I) Increase in blood glucose levels in HFD-fed mice after agomiR-182 treatment (n = 8). Means ± SD are shown. *p < 0.05.

(J) qPCR analysis of miR-182, FoxO1, and PDK4 in GAS muscle of HFD-fed mice treated with agomiR-182 (n = 3). Means ± SD are shown. *p < 0.05; **p < 0.01.

(K) Western blot analysis of FoxO1, PDK4, p-PDHA1, t-PDHA1, and GAPDH protein levels in GAS muscle of HFD-fed mice treated with agomiR-182.

(L) qPCR analysis of the mRNA expression of MHC isoforms in GAS muscle of HFD-fed mice treated with agomiR-182 (n = 3). Means ± SD are shown. *p < 0.05; **p < 0.01 versus HFD-fed mice treated with agomiRNA control.

See also Figure S6.

DISCUSSION

During feeding and fasting cycles, carbohydrates and fatty acids are the two primary substrates in oxidative metabolism. Metabolic flexibility, which coordinates the glucose/fat oxidation switch to homeostatic signals, is essential for maintaining metabolic homeostasis (Galgani et al., 2008). The loss of metabolic flexibility responding to energy demands and nutrient availability leads to metabolic disease. Fuel partitioning is mainly controlled by the PDHC activity, which is suppressed by PDK4 through phosphorylation (Patel and Korotchkina, 2006). Therefore, inappropriate suppression of PDHC activity in skeletal muscle promotes the development of hyperglycemia by fueling excessive gluconeogenesis. However, whether and how miRNAs are involved in the substrate selection for oxidation is largely unknown. Here, we report an important role for miR-182 in

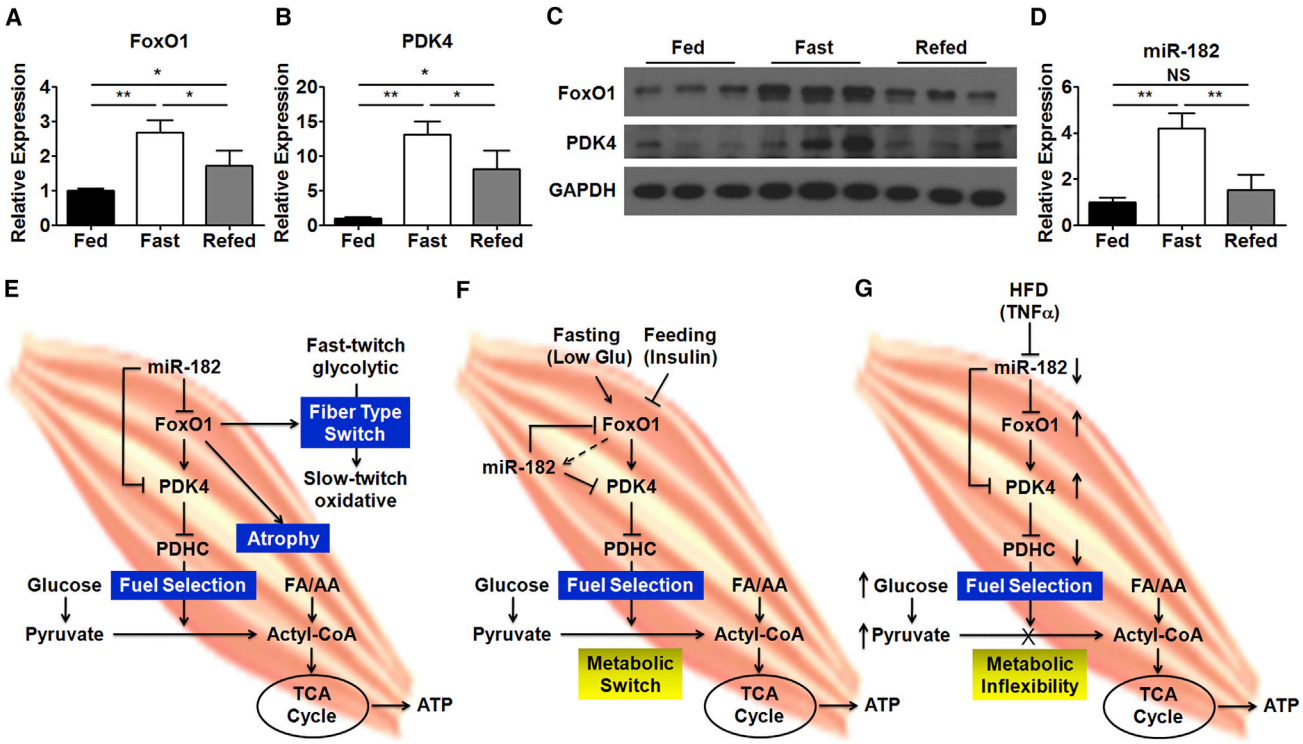

Figure 7. Working Hypothesis for the Regulation of Glucose Utilization by miR-182

(A and B) qPCR analysis of FoxO1 (A) and PDK4 (B) mRNA expression in the GAS muscle of fed, fasted, and refed mice (n = 3). Means ± SD are shown. *p < 0.05; **p < 0.01.

(C) Western blot analysis of FoxO1, PDK4, and GAPDH protein levels in GAS muscle of fed, fasted, and refed mice (n = 3).

(D) qPCR analysis of miR-182 expression in the GAS muscle of fed, fasted, and refed mice (n = 3). Means ± SD are shown. **p < 0.01; NS, not significant.

(E) Schematic diagram of the working model of skeletal muscle miR-182.

(F) Schematic diagram of the FoxO1-miR-182 feedback loop in maintaining glucose homeostasis under different nutritional status.

(G) Schematic diagram of the miR-182-mediated metabolic inflexibility upon HFD feeding.

regulating fuel usage through the FoxO1/PDK4/PDHC axis in skeletal muscle. Our data suggest that miR-182 could be a therapeutic target for improving glycemic control for patients with diabetes.

The PDK4 gene, as a major direct target of FoxO1, is frequently altered in a variety of diseases. For example, PDK remains in the "starvation" mode and keeps a high level in skeletal muscles during obesity development, even though plentiful nutrients are available (Frier et al., 2011). mRNA transcripts of PDK4 were upregulated in the muscle of T2D patients compared to the healthy subjects after overnight fasting (Kulkarni et al., 2012). Besides that, the methylation status of PDK4 promoter was reduced in T2D patients, suggesting that epigenetic modification is also involved in the T2D pathogenesis (Kulkarni et al., 2012). In this study, our finding that downregulation of miR-182 in HFD-fed mice was accompanied with an increased PDK4 expression suggests a miRNA-based mechanism for HFD-associated hyperglycemia.

miRNAs, together with transcription factors, form a complicated and highly interconnected network to mediate biological processes. Since miRNA processing is faster than protein translation, miRNAs are ideally suited to serve in loops that confer robustness and precision (Ebert and Sharp, 2012). In this study, we demonstrated that FoxO1 and miR-182 formed a feedback loop to regulate the PDHC activity under different feeding conditions. Upon fasting, the starvation signal directly stimulates the expression of FoxO1 and PDK4 but, at the same time, promotes inhibition at a post-transcriptional level by inducing miR-182 expression (Figure 7F). Thus, miR-182 acts as a sensor of energy stress and can confer cell-exquisite temporal and quantitative precision over cell signaling. In this way, miR-182 serves as a guard to avoid overactivation of PDK4 and excessive inhibition of PDHC activity, thereby allowing accurate tuning of glucose homeostasis.

Since the dietary-induced impairment of PDHC activation in skeletal muscles is one of the causative factors for insulin resistance and other metabolic syndromes (Gorter et al., 2004), inhibitors targeting FoxO1/PDK4 or agents to restore miR-182 will improve the glucose metabolism in patients. We have shown that administration of agomiR-182 could suppress the expression of FoxO1/PDK4, reduce the phosphorylation of PDHC in the muscle of mice under HFD feeding, and eventually derepress the glycolytic program and alleviate the metabolic inflexibility in these mice, as shown by improved glucose metabolism. Our data not only reinforce the concept that modulation of PDK4 expression could have significant beneficial effects on nutrient

handling in obesity but also support the notion that the oxidative-to-glycolytic metabolic shift in skeletal muscle is beneficial and could alleviate metabolic inflexibility.

Our in vitro results demonstrated that miR-182 modulates glucose oxidation in skeletal muscle in a cell-autonomous manner. However, we could not rule out the possibility that the role of miR-182 in other metabolic tissues might also contribute to the dysregulation of glucose metabolism in KO mice. Given that skeletal muscle accounts for 90% of insulin-stimulated glucose uptake, adipose tissue accounts for only 10%, glucose is stored as glycogen in skeletal muscle, and fatty acids are stored as triglyceride in adipose tissue, it is likely that the impaired glucose homeostasis observed in miR-182 KO mice was mainly due to the defects in muscle. In addition, we only observed the changes in the protein levels of PDK4 in the adipose tissue of KO mice. Moreover, the protein levels of p-PDHA1 did not change significantly in the adipose tissue of KO mice (Figures S5D–S5G). These findings further suggest that the metabolic phenotype observed in KO mice was due to the abnormal glucose ultilization caused by miR-182 deficiency.

FoxO3, another member of the forkhead box O family, is also known to play an important role in muscle atrophy and autophagy (Mammucari et al., 2007; Zhao et al., 2007). Recently, miR-182 has been shown to control FoxO3 levels and correlated with glucocorticoid-induced autophagy genes (Hudson et al., 2014). Therefore, it is possible that the dysregulation of FoxO3 due to miR-182 deficiency might also contribute to the muscle loss in KO mice. Further study is required to determine whether autophagy was involved in the muscle atrophy in KO mice.

Disease is often the result of an aberrant or inadequate response to physiologic and pathophysiologic stress (Mendell and Olson, 2012). miRNAs often profoundly influence the responses of tissues to physiologic and pathophysiologic stress (Leung and Sharp, 2010). In this study, we demonstrate that FoxO1 and miR-182 form a feedback loop to control fuel usage and maintain glucose homeostasis, establishing miR-182 as a critical regulator that confers robustness and precision on the regulation of fuel usage and glucose homeostasis. Our data suggest that HFD-induced down-regulation of miR-182 contributes to the upregulation of FoxO1/PDK4, which leads to metabolic inflexibility and abnormal glucose homeostasis, while restoration of miR-182 expression is able to alleviate metabolic inflexibility and improves glucose metabolism in HFD-fed mice. Our studies reinforce the notion that promoting a metabolic shift toward a faster and more glycolytic phenotype is beneficial in diabetes and, thus, constitutes a promising approach to combat metabolic disease.

EXPERIMENTAL PROCEDURES

See the Supplemental Experimental Procedures for additional details.

In Vivo Study

8- to 12-week-old male mice were used in this study. All experimental procedures and protocols were reviewed and approved by the Institutional Review Board of the Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (permit number: 2011-AN-14). miR-182 KO, *ob/ob*, and wild-type mice were maintained as described previously (Liu et al., 2014). Muscle weight was measured using a NMR Analyzer (Bruker). The human muscle specimens of quadriceps were obtained during surgery from patients receiving total hip replacement at the Department of Orthopedics, Zhongshan Hospital. All the procedures were reviewed and approved by the Ethics Committee of Zhongshan Hospital, Fudan University, and informed consent was obtained from all patients. A detailed description for the glucose-tolerance test, HFD feeding, chemically modified miRNA oligonucleotide treatment, in vivo indirect calorimetry measurement, and tissue collection can be found in the Supplemental Experimental Procedures.

Cell Culture and Transfection

A detailed description of the primary myoblast isolation, the culture and differentiation of C2C12 and L6 myoblasts, transfection using Lipofectamine 2000 (Invitrogen), insulin and TNFα treatment, luciferase assays, and the plasmids and RNA oligonucleotide used in this study can be found in the Supplemental Experimental Procedures.

PCR and Western Blot Analysis

See the Supplemental Experimental Procedures for detail information.

Phenotype Characterization and Metabolic Profiling

A detailed description of the immunostaining and metabolic ATPase staining; the measurement of the muscle cross-sectional area; glucose oxidation, oxygen consumption, and glycolysis; PDH (pyruvate dehydrogenase) enzyme activity; and pyruvate, acetyl-CoA, and triglyceride can be found in the Supplemental Experimental Procedures.

Statistical Analysis

GraphPad Prism 5.0 software was applied to all statistical analyses. Data were presented as means ± SD. Student's t test was performed to assess the means of two groups are statistically significant from each other ($p < 0.05$).

ACCESSION NUMBERS

The accession number for the miRNA data reported in this study is GEO: GSE81976.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.06.040.

AUTHOR CONTRIBUTIONS

D.Z. and Yan Li contributed equally to this work; D.Z., Yan Li, Z.J., and H.Y. designed the research; D.Z., Yan Li, and Xuan Yao carried out most of the cell culture and animal studies and analyzed the data; Lei Zhao, Xiaohan Yao, and X.L. performed ATPase staining and immunostaining of muscles from mice; H.J., H.W., S.Z., C.Y., W.L., H.C., S.Y., Ling Zhang, and Yun Liu provided technical assistance; Q.L. and Yi Liu contributed to the collection of muscle specimens; Y.W., J.J., H.W., Jingya Li, Jia Li, N.I., Z.J., and H.Y. contributed to discussion and supervised the project; H.Y. wrote the manuscript. All authors participated in the interpretation of the data and production of the final manuscript.

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REFERENCES

Barquissau, V., Beuzelin, D., Pisani, D.F., Beranger, G.E., Mairal, A., Montagner, A., Roussel, B., Tavernier, G., Marques, M.A., Moro, C., et al. (2016). White-to-brite conversion in human adipocytes promotes metabolic reprogramming towards fatty acid anabolic and catabolic pathways. Mol. Metab. *5*, 352–365.

Borst, S.E., and Conover, C.F. (2005). High-fat diet induces increased tissue expression of TNF-alpha. Life Sci. *77*, 2156–2165.

Cahill, G.F., Jr., Herrera, M.G., Morgan, A.P., Soeldner, J.S., Steinke, J., Levy, P.L., Reichard, G.A., Jr., and Kipnis, D.M. (1966). Hormone-fuel interrelationships during fasting. J. Clin. Invest. *45*, 1751–1769.

Ebert, M.S., and Sharp, P.A. (2012). Roles for microRNAs in conferring robustness to biological processes. Cell *149*, 515–524.

Frier, B.C., Jacobs, R.L., and Wright, D.C. (2011). Interactions between the consumption of a high-fat diet and fasting in the regulation of fatty acid oxidation enzyme gene expression: an evaluation of potential mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. *300*, R212–R221.

Furuyama, T., Kitayama, K., Yamashita, H., and Mori, N. (2003). Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. Biochem. J. *375*, 365–371.

Galgani, J.E., Moro, C., and Ravussin, E. (2008). Metabolic flexibility and insulin resistance. Am. J. Physiol. Endocrinol. Metab. *295*, E1009–E1017.

Gordon, B.A., Benson, A.C., Bird, S.R., and Fraser, S.F. (2009). Resistance training improves metabolic health in type 2 diabetes: a systematic review. Diabetes Res. Clin. Pract. *83*, 157–175.

Gorter, P.M., Olijhoek, J.K., van der Graaf, Y., Algra, A., Rabelink, T.J., and Visseren, F.L.; SMART Study Group (2004). Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. Atherosclerosis *173*, 363–369.

Guttilla, I.K., and White, B.A. (2009). Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. J. Biol. Chem. *284*, 23204–23216.

Hudson, M.B., Rahnert, J.A., Zheng, B., Woodworth-Hobbs, M.E., Franch, H.A., and Price, S.R. (2014). miR-182 attenuates atrophy-related gene expression by targeting FoxO3 in skeletal muscle. Am. J. Physiol. Cell Physiol. *307*, C314–C319.

Izumiya, Y., Hopkins, T., Morris, C., Sato, K., Zeng, L., Viereck, J., Hamilton, J.A., Ouchi, N., LeBrasseur, N.K., and Walsh, K. (2008). Fast/glycolytic muscle fiber growth reduces fat mass and improves metabolic parameters in obese mice. Cell Metab. *7*, 159–172.

Jin, Z.B., Hirokawa, G., Gui, L., Takahashi, R., Osakada, F., Hiura, Y., Takahashi, M., Yasuhara, O., and Iwai, N. (2009). Targeted deletion of miR-182, an abundant retinal microRNA. Mol. Vis. *15*, 523–533.

Kamei, Y., Miura, S., Suzuki, M., Kai, Y., Mizukami, J., Taniguchi, T., Mochida, K., Hata, T., Matsuda, J., Aburatani, H., et al. (2004). Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. J. Biol. Chem. *279*, 41114–41123.

Karolina, D.S., Armugam, A., Tavintharan, S., Wong, M.T., Lim, S.C., Sum, C.F., and Jeyaseelan, K. (2011). MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. PLoS ONE *6*, e22839.

Kelley, D.E., and Mandarino, L.J. (2000). Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes *49*, 677–683.

Kitamura, T., Kitamura, Y.I., Funahashi, Y., Shawber, C.J., Castrillon, D.H., Kollipara, R., DePinho, R.A., Kitajewski, J., and Accili, D. (2007). A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. J. Clin. Invest. *117*, 2477–2485.

Kulkarni, S.S., Salehzadeh, F., Fritz, T., Zierath, J.R., Krook, A., and Osler, M.E. (2012). Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus. Metabolism *61*, 175–185.

LeBrasseur, N.K., Walsh, K., and Arany, Z. (2011). Metabolic benefits of resistance training and fast glycolytic skeletal muscle. Am. J. Physiol. Endocrinol. Metab. *300*, E3–E10.

Lee, K.Y., Singh, M.K., Ussar, S., Wetzel, P., Hirshman, M.F., Goodyear, L.J., Kispert, A., and Kahn, C.R. (2015). Tbx15 controls skeletal muscle fibre-type determination and muscle metabolism. Nat. Commun. *6*, 8054.

Leung, A.K., and Sharp, P.A. (2010). MicroRNA functions in stress responses. Mol. Cell *40*, 205–215.

Liu, W., Cao, H., Ye, C., Chang, C., Lu, M., Jing, Y., Zhang, D., Yao, X., Duan, Z., Xia, H., et al. (2014). Hepatic miR-378 targets p110α and controls glucose and lipid homeostasis by modulating hepatic insulin signalling. Nat. Commun. *5*, 5684.

Mammucari, C., Milan, G., Romanello, V., Masiero, E., Rudolf, R., Del Piccolo, P., Burden, S.J., Di Lisi, R., Sandri, C., Zhao, J., et al. (2007). FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab. *6*, 458–471.

Mendell, J.T., and Olson, E.N. (2012). MicroRNAs in stress signaling and human disease. Cell *148*, 1172–1187.

Meng, Z.X., Li, S., Wang, L., Ko, H.J., Lee, Y., Jung, D.Y., Okutsu, M., Yan, Z., Kim, J.K., and Lin, J.D. (2013). Baf60c drives glycolytic metabolism in the muscle and improves systemic glucose homeostasis through Deptor-mediated Akt activation. Nat. Med. *19*, 640–645.

Patel, M.S., and Korotchkina, L.G. (2006). Regulation of the pyruvate dehydrogenase complex. Biochem. Soc. Trans. *34*, 217–222.

Patti, M.E., Butte, A.J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R., et al. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc. Natl. Acad. Sci. USA *100*, 8466–8471.

Petersen, K.F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D.L., DiPietro, L., Cline, G.W., and Shulman, G.I. (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science *300*, 1140–1142.

Rinnankoski-Tuikka, R., Silvennoinen, M., Torvinen, S., Hulmi, J.J., Lehti, M., Kivela, R., Reunanen, H., and Kainulainen, H. (2012). Effects of high-fat diet and physical activity on pyruvate dehydrogenase kinase-4 in mouse skeletal muscle. Nutr. Metab. *9*, 53.

Schiaffino, S., and Reggiani, C. (1996). Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. *76*, 371–423.

Stittrich, A.B., Haftmann, C., Sgouroudis, E., Kühl, A.A., Hegazy, A.N., Panse, I., Riedel, R., Flossdorf, M., Dong, J., Fuhrmann, F., et al. (2010). The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. Nat. Immunol. *11*, 1057–1062.

van Rooij, E., Quiat, D., Johnson, B.A., Sutherland, L.B., Qi, X., Richardson, J.A., Kelm, R.J., Jr., and Olson, E.N. (2009). A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev. Cell *17*, 662–673.

Zhang, D., Wang, X., Li, Y., Zhao, L., Lu, M., Yao, X., Xia, H., Wang, Y.C., Liu, M.F., Jiang, J., et al. (2014a). Thyroid hormone regulates muscle fiber type conversion via miR-133a1. J. Cell Biol. *207*, 753–766.

Zhang, S., Hulver, M.W., McMillan, R.P., Cline, M.A., and Gilbert, E.R. (2014b). The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr. Metab. (Lond.) *11*, 10.

Zhao, J., Brault, J.J., Schild, A., Cao, P., Sandri, M., Schiaffino, S., Lecker, S.H., and Goldberg, A.L. (2007). FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab. *6*, 472–483.