Baf60c drives glycolytic metabolism in the muscle and improves systemic glucose homeostasis through Deptor-mediated Akt activation

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A shift from oxidative to glycolytic metabolism has been associated with skeletal muscle insulin resistance in type 2 diabetes1–5. However, whether this metabolic switch is deleterious or adaptive remains under debate6–8, in part because of a limited understanding of the regulatory network that directs the metabolic and contractile specification of fast-twitch glycolytic muscle. Here we show that Baf60c (also called Smarcd3), a transcriptional cofactor enriched in fast-twitch muscle, promotes a switch from oxidative to glycolytic myofiber type through DEP domain–containing mTOR-interacting protein (Deptor)-mediated Akt activation. Muscle-specific transgenic expression of Baf60c activates a program of molecular, metabolic and contractile changes characteristic of glycolytic muscle. In addition, Baf60c is required for maintaining glycolytic capacity in adult skeletal muscle in vivo. Baf60c expression is significantly lower in skeletal muscle from obese mice compared to that from lean mice. Activation of the glycolytic muscle program by transgenic expression of Baf60c protects mice from diet-induced insulin resistance and glucose intolerance. Further mechanistic studies revealed that Deptor is induced by the Baf60c-Six4 transcriptional complex and mediates activation of Akt and glycolytic metabolism by Baf60c in a cell-autonomous manner. This work defines a fundamental mechanism underlying the specification of fast-twitch glycolytic muscle and illustrates that the oxidative-to-glycolytic metabolic shift in skeletal muscle is potentially adaptive and beneficial in the diabetic state.

Skeletal myofibers show remarkable diversity and plasticity in energy metabolism and contractile functions. Slow-twitch myofibers are rich in mitochondria and have high oxidative capacity, whereas fast-twitch fibers generate ATP primarily through glycolysis9,10. The regulatory network that drives the formation of slow-twitch muscle fibers centers on the transcriptional coactivator peroxisome proliferative activated receptor γ, coactivator-1α (PGC-1α) and its transcriptional partners10–12. However, the mechanisms that regulate the specification of fast-twitch glycolytic muscle remain undefined. To identify candidate regulators of glycolytic muscle formation, we analyzed gene expression profiles of soleus and tibialis anterior muscles (GSE10347)13, which contain oxidative and mixed myofibers, respectively. This analysis revealed a cluster of transcription factors and cofactors enriched in tibialis anterior muscle (Fig. 1a), including Baf60c (encoded by Smarcd3), a subunit of the SWI/SNF chromatin-remodeling complex14. Compared to its expression in the soleus muscle, Baf60c expression was significantly higher in the gastrocnemius, quadriceps, tibialis anterior, extensor digitorum longus (EDL) and plantaris, muscles that are enriched for glycolytic fibers (Fig. 1b). To test whether Baf60c regulates the glycolytic muscle program, we generated muscle-specific Baf60c transgenic mice using a muscle creatine kinase (MCK) promoter12. Two transgenic lines showed approximately twofold to threefold higher amounts of Baf60c protein in several muscles compared to wild-type (WT) mice (Fig. 1c), which is within its physiological range of expression in different muscles, but not in other tissues, including brown and white adipose tissues, pancreas, heart and liver (data not shown). Skeletal muscle from transgenic mice appeared paler in color than that of WT mice. Fiber-type analysis indicated that the percentage of type Ila myofiber in the plantaris muscle was slightly but significantly lower in transgenic mice compared to WT mice, whereas type Iib myofiber content was higher in transgenic muscles (Supplementary Fig. 1). Muscle fiber size was similar in both groups.

To determine whether Baf60c regulates myofiber energy metabolism, we performed histochemical staining for α-glycerophosphate dehydrogenase (α-GPDH) and succinate dehydrogenase (SDH), which are enriched in glycolytic and oxidative myofibers, respectively9. Transgenic muscles had higher α-GPDH and lower SDH enzymatic activities compared to WT muscles (Fig. 1d). We observed this shift from oxidative to glycolytic metabolism in all muscles examined, including the tibialis anterior, EDL and soleus. Whereas intermyofibrillar mitochondria content was similar in transgenic and...
WT myofibers, subsarcolemmal mitochondrial content was markedly lower in transgenic myofibers (Fig. 1e). Accordingly, mitochondrial DNA content was lower in transgenic muscles (Fig. 1f). The activity of lactate dehydrogenase (LDH) and glycolytic flux were significantly elevated in transgenic muscle (Fig. 1g). LDH isoenzyme analysis indicated that the amounts of isoenzymes containing LDH-B, which is enriched in oxidative myofibers, were lower in transgenic plantaris compared to WT plantaris (Supplementary Fig. 4a). The NADH-to-NAD\(^+\) ratio was higher in transgenic muscle, whereas the ATP-to-AMP ratio and AMPK activity were similar in transgenic and WT muscles. (c) Immunobots of total muscle protein lysates (top) and the appearance of skeletal muscles from WT and transgenic (Tg) mice (bottom). (f) Transmission electron micrographs of tibialis anterior muscle. Arrows point to subsarcolemmal mitochondria. SS, subsarcolemmal; IM, intermyofibrillar. Scale bars, 500 nm. (g) LDH activity in plantaris muscle (n = 7–9 mice per group). (h) qPCR analysis of gene expression in plantaris muscle (n = 7–8 mice per group). (i) Tibialis anterior muscle glycogen content in running time–matched mice (n = 6 mice per group). All data are shown as the mean ± s.e.m. *P < 0.05 by two-tailed Student’s t-test.

WT myofibers, subsarcolemmal mitochondrial content was markedly lower in transgenic myofibers (Fig. 1e). Accordingly, mitochondrial DNA content was lower in transgenic muscles (Fig. 1f). The activity of lactate dehydrogenase (LDH) and glycolytic flux were significantly elevated in transgenic muscle (Fig. 1g). LDH isoenzyme analysis indicated that the amounts of isoenzymes containing LDH-B, which is enriched in oxidative myofibers, were lower in transgenic plantaris compared to WT plantaris (Supplementary Fig. 4a). The NADH-to-NAD\(^+\) ratio was higher in transgenic muscle, whereas the ATP-to-AMP ratio and AMPK activity were similar in WT and transgenic muscles. Blood lactate concentrations were similar between the two groups under ad libitum conditions but remained elevated in the transgenic group during starvation. Further, postprandial blood lactate concentrations after an intraperitoneal injection of 3-mercaptopropionic acid, an inhibitor of phosphoenolpyruvate carboxykinase 1 (PEPCK), were higher in transgenic mice. This shift from oxidative to glycolytic metabolism was accompanied by higher glycolytic and lower oxidative gene expression (Fig. 1h and Supplementary Figs. 2c and 3).

Fast-twitch muscle generates ATP primarily through glycolysis and is more susceptible to exercise-induced fatigue. To determine whether transgenic mice have a higher glycogen utilization rate during exercise compared to WT mice, we subjected mice to nonexhaustion treadmill running. Although basal glycogen content was similar in the two groups, transgenic mice showed more rapid glycogen depletion during running than WT control mice (Supplementary Fig. 4a and Fig. 1i). In a separate study, transgenic mice reached exhaustion significantly earlier than WT control mice and had shorter total running time and distance (Fig. 1j and data not shown). Blood lactate concentrations after exercise were elevated by approximately 50% in Baf60c transgenic mice. We conclude from these studies that Baf60c is sufficient to activate a program of molecular, metabolic and contractile changes characteristic of fast-twitch glycolytic myofibers.

Impaired mitochondrial function has been linked to insulin resistance in skeletal muscle. However, whether the shift from oxidative to glycolytic metabolism is deleterious for metabolic homeostasis remains unclear. In fact, transgenic activation of the mitochondrial oxidative program by PGC-1\(\alpha\) is not sufficient to improve skeletal muscle insulin sensitivity in mice. Analyses of Baf60c expression revealed that its protein amounts were significantly lower in skeletal muscle from diet-induced and genetic obese mice compared to lean control mice (Fig. 2a). To determine whether cytokines regulate Baf60c expression, we treated C2C12 myotubes with myostatin (Mstn), interleukin-6 (IL-6) or tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)). TNF-\(\alpha\)-induced IL-6 expression in myotubes. Although Mstn and IL-6 had modest effects, treatment with TNF-\(\alpha\) significantly decreased
Baf60c expression in C2C12 and primary human myotubes (Fig. 2b,c). Chromatin immunoprecipitation (ChIP) assays indicated that TNF-α treatment markedly reduced the amounts of acetyl-histone H3 and trimethylation of H3 lysine 4 (H3K4me3), epigenetic markers that are associated with transcriptionally active chromatin, in the proximal Smarcd3 (Baf60c) promoter (Fig. 2d). In contrast, dimethylation of H3 lysine 9 (H3K9me2), a repressive chromatin mark, was augmented in response to treatment with TNF-α. These results suggest that Smarcd3 (Baf60c) expression may be repressed in insulin-resistant states as a result of epigenetic signaling downstream of pro-inflammatory cytokines.

To assess the role of glycolytic muscle metabolism in insulin resistance, we subjected WT and transgenic mice to high-fat diet (HFD) feeding. Both groups developed obesity, with the transgenic mice gaining slightly less body weight (Fig. 2e). Compared to WT mice, Baf60c transgenic mice had significantly lower fasting blood glucose and insulin concentrations (Fig. 2e). The concentrations of plasma lipids, including triglycerides, ketone body and nonesterified fatty acids, were higher in transgenic mice (Supplementary Fig. 4b). Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) indicated that transgenic mice were more insulin sensitive and had better glucose tolerance compared to WT mice (Fig. 2f). Using hyperinsulinemic-euglycemic clamp studies, we found that the glucose infusion rate and whole-body glucose turnover were higher in the transgenic group. In addition, 2-deoxyglucose uptake was higher in transgenic gastrocnemius muscle (303 ± 17 nmol g⁻¹ min⁻¹ (mean ± s.e.m.) for transgenic compared to 237 ± 17 nmol g⁻¹ min⁻¹ for WT; P < 0.05). Hepatic glucose production rates were indistinguishable between the two groups (Fig. 2g). Skeletal muscle triglyceride content, myokine gene expression and adipose tissue histology were similar in the two groups, whereas transgenic mice had less hepatic steatosis (Fig. 2h,i and Supplementary Fig. 4c,d). The mRNA levels of fatty β-oxidation genes were elevated, whereas those of lipogenic genes were lower, in the livers from transgenic mice (Supplementary Fig. 5). As such, genetic activation of the glycolytic muscle program protects mice from diet-induced metabolic dysregulation, suggesting that a shift from an oxidative to a glycolytic phenotype in skeletal myofiber per se does not lead to insulin resistance. Given that resistance training increases glycolytic muscle mass and function and improves metabolic parameters in patients with diabetes17,18, our findings raise the possibility that the oxidative-to-glycolytic shift may have a previously unappreciated role in skeletal muscle adaptation in type 2 diabetes.

Recent studies demonstrated that conditional Akt activation in muscle is sufficient to enhance glycolytic muscle growth and improve metabolic parameters19. Notably, the phosphorylation of Thr308 and Ser473 residues in Akt was markedly enhanced in transgenic quadriceps (Fig. 3a). To identify Baf60c target genes that mediate Akt activation, we compared gene expression profiles of WT and transgenic quadriceps and found that expression of Deptor (also called Depdc6), which was recently identified as an Akt activator and mTOR inhibitor20, was markedly elevated in transgenic muscles (Fig. 3a and Supplementary Fig. 6a,b). Deptor mRNA and protein expression were also highly enriched in glycolytic muscles (Fig. 3b).
To establish the cell-autonomous nature of Akt activation by Deptor, we performed gain- and loss-of-function studies in cultured C2C12 myotubes differentiated from transduced myoblasts. Retroviral-mediated overexpression of Baf60c robustly stimulated Deptor mRNA and protein expression and enhanced Akt phosphorylation in cultured myotubes (Fig. 3c and Supplementary Fig. 6c). Conversely, RNAi-mediated knockdown of Baf60c mRNA resulted in lower Deptor expression and attenuated Akt phosphorylation. These activities of Baf60c are probably not the result of alterations in muscle differentiation. In fact, adenoviral-mediated expression of Baf60c in differentiated C2C12 myotubes also stimulated Deptor expression and Akt phosphorylation (Supplementary Fig. 7). Further, Baf60c dose-dependently stimulated DEPTOR expression and Akt phosphorylation in cultured primary human myotubes, suggesting that this pathway is conserved between rodents and humans (Fig. 3d).

We next examined the effects of Baf60c on glycolytic metabolism in C2C12 myotubes. Retroviral-mediated Baf60c expression markedly increased, whereas RNAi-mediated knockdown decreased, the rate of glucose consumption and lactate production (Fig. 3e,f). Glycolytic flux measurements using [5-3H]glucose indicated that Baf60c augmented glycolysis in an Akt-dependent manner (Fig. 3g). Consistently, glycolytic flux was significantly lower in C2C12 myotubes expressing shRNA targeting Smarcd3 (Baf60c) compared to myotubes expressing scramble shRNA (Fig. 3h).

Further, Smarcd3 (Baf60c) knockdown attenuated insulin-stimulated Akt activation and glycolytic flux in transfected myotubes (Supplementary Fig. 8a,b). Similarly to Baf60c, retroviral-mediated expression of Deptor resulted in a marked increase in Akt phosphorylation, whereas Deptor RNAi knockdown had the opposite effect (Supplementary Fig. 8c). To evaluate the role of Deptor in mediating Akt activation by Baf60c, we sequentially transduced C2C12 myoblasts with retroviruses expressing scramble or Deptor shRNA and then performed a second round of transduction with vector or Baf60c retroviruses. In scramble-transduced myotubes, Baf60c transduction led to higher Deptor expression and Akt phosphorylation compared to myotubes transduced with vector (Fig. 3i). In contrast, RNAi-mediated knockdown of Deptor nearly abolished the enhancement of Akt phosphorylation by Baf60c. Measurements of lactate production indicated that Deptor knockdown severely impaired the ability of Baf60c to increase lactate secretion from transfected myotubes. Taken together, these results demonstrate that the Baf60c-Deptor pathway regulates Akt activation and glycolytic metabolism in myotubes in a cell-autonomous manner.

Recent work has implicated homeobox transcriptional factors Six1 and Six4 in the regulation of fast-twitch muscle genes21. Using a Genomatix motif analysis, we identified one putative Six4 binding site located 1.6 kb upstream of the transcription start site of Deptor. Six4 and Baf60c cooperatively stimulated the expression of endogenous Deptor mRNA in cultured myotubes and augmented promoter activity through the predicted Six4 binding site (Fig. 4a,b). ChIP assays revealed that Six4 and Baf60c were recruited to the Six4 binding site through direct physical interaction (Fig. 4c,d). Notably, H3K4me3 was markedly higher and H3K9me2 was lower in the proximal Deptor promoter in myotubes transfected with Baf60c compared to those transduced with vehicle.

**Figure 3** Baf60c activates the Akt pathway through Deptor in a cell-autonomous manner. (a) Immunoblots of total protein lysates from WT and transgenic quadriceps. pAkt, phosphorylated Akt; pS6K, phosphorylated S6K; pS6, phosphorylated S6. (b) Deptor mRNA and protein expression in the indicated muscles. Data are shown as the mean ± s.e.m. *P < 0.05 by two-tailed Student’s t test for soleus compared to other muscles. (c) Immunoblots of total lysates from transduced C2C12 myotubes. Veh, vehicle; scrb, scramble siRNA; siBaf60c, Baf60c-specific siRNA. (d) qPCR analysis of DEPTOR mRNA and immunoblots of total lysates from transduced primary human myotubes. (e,f) Glucose and lactate concentrations in culture medium from transduced C2C12 myotubes. (g) Glycolytic flux in transduced C2C12 myotubes treated with vehicle or 10 μM of the Akt1 and Akt2 inhibitor (Akti) for 1 h. BAF, Baf60c. (h) Glycolytic flux in transduced C2C12 myotubes under basal conditions. (i) Immunoblots of protein lysates from transduced C2C12 myotubes (top) and lactate concentrations in culture medium from transduced C2C12 myotubes (bottom). siDeport, Deptor-specific siRNA. Data are shown as the mean ± s.d. and are representative of three independent experiments (d-i). *P < 0.01 by two-tailed Student’s t test.
with vector (Fig. 4e). These results suggest that Baf60c functions as a coactivator for Six4 and stimulates Deptor transcription through altering local chromatin structure and the epigenetic landscape.

To evaluate the importance of Baf60c in glycolytic muscle metabolism, we performed in vivo RNAi-mediated knockdown in skeletal muscle using a mouse strain that transgenically expresses the coxsackie B adenovirus receptor (MCK-CAR mice). The efficiency of adenoviral expression model. Baf60c was recently shown to regulate cardiac metabolism in adult mice22,23. We injected purified adenoviral vectors expressing Smarcd3 (Baf60c) shRNA intramuscularly into the tibialis anterior in one leg and injected control shRNA in the contralateral leg. Compared to vector (Fig. 4f), Baf60c mRNA and protein expression were significantly lower endogenous Baf60c mRNA and protein expression (Fig. 4f). Accordingly, Deptor mRNA and protein expression were also significantly lower (Fig. 4f), suggesting that Baf60c is required for maintaining normal Deptor expression in vivo. Akt phosphorylation at both sites was diminished in response to RNAi-mediated knockdown of Smarcd3 (Baf60c). To explore whether Baf60c is required for muscle glycolytic metabolism, we performed histochemical staining of COX (middle) and immunoblots of total protein lysates from transduced tibialis anterior muscle (right). Shown are representative blots and densitometric analysis of Baf60c in transiently transfected HEK293T cells. IP, immunoprecipitation; Myc-Baf60c, Myc-tagged Baf60c; Baf60c and Six4 at the putative Six4 binding site on the proximal Deptor promoter (Fig. 4a). Both Baf60c and Deptor mRNA and protein levels in tibialis anterior muscle transduced with indicated adenoviral vectors (n = 6 mice per group) were significantly lower (Fig. 4f). (g) LDH enzymatic activity in transduced tibialis anterior muscle (n = 8 mice per group). (h) Representative histochemical staining of COX (left), SDH (middle) and α-GPDH (right) activities on frozen sections of transduced tibialis anterior muscle (n = 8 mice per group). Scale bar, 100 µm. (i) Model depicting the Baf60c-Deptor-Akt regulatory cascade in metabolic and contractile specification of fast-twitch glycolytic muscle. Data are shown as the mean ± s.e.m. (f,g). *P < 0.01 by two-tailed Student’s t test.

Figure 4 Baf60c is required for maintaining glycolytic metabolism in adult skeletal muscle. (a) Deptor mRNA expression in C2C12 myotubes transduced with the indicated adenoviral vectors. (b) Reporter gene assay with constructs containing either WT Deptor promoter or a Deptor promoter mutated at the Six4 binding site. (c) Relative enrichment of Baf60c and Six4 at the putative Six4 binding site on the proximal Deptor promoter. (d) Physical interaction of Six4 and Baf60c in transiently transfected HEK293T cells. IP, immunoprecipitation; Myc-Baf60c, Myc-tagged Baf60c; FH-Six4, Flag- and HA-tagged Six4. (e) ChIP assay in transduced myotubes using antibodies to H3K4me3 and H3K9me2. The locations of the qPCR primers are relative to the transcriptional start site of Deptor. (f) Baf60c and Deptor mRNA levels in tibialis anterior muscle transduced with indicated adenoviral vectors (n = 6 mice per group) (left) and immunoblots of total protein lysates from transduced tibialis anterior muscle (right). Shown are representative blots of two mice. (g) LDH enzymatic activity in transduced tibialis anterior muscle (n = 8 mice per group). (h) Representative histochemical staining of COX (left), SDH (middle) and α-GPDH (right) activities on frozen sections of transduced tibialis anterior muscle (n = 8 mice per group). Scale bar, 100 μm. (i) Model depicting the Baf60c-Deptor-Akt regulatory cascade in metabolic and contractile specification of fast-twitch muscle fibers (Fig. 4i). Both Baf60c and Deptor are enriched in fast glycolytic muscles and cell-autonomously enhance Akt activation. Previous work demonstrated that conditional activation of Akt in skeletal muscle leads to hypertrophic muscle growth19. The lack of muscle mass increase in Baf60c transgenic mice may be the result of relatively modest Akt activation compared to that in the Akt overexpression model. Baf60c was recently shown to regulate cardiac development and muscle gene expression through transactivating transcription factors, including Gata4, Tbx5, Nkx2-5 and MyoD24,25. Baf60a, a close homolog of Baf60c, was recently identified as a regulator of hepatic fatty acid β-oxidation26, raising the possibility that the Baf60 subunit may restrict SWI/SNF-mediated chromatin remodeling to selective transcriptional targets and elicit specific biological responses.

Genetic and pharmacological manipulations that increase mitochondrial oxidative metabolism have been demonstrated to improve metabolic homeostasis in both humans and rodent models of insulin resistance11,27-29. Given the strong association between oxidative myofiber metabolism and insulin sensitivity, why does genetic activation of the glycolytic muscle type ameliorate diet-induced metabolic dysregulation? One possibility is that the shift from oxidative to glycolytic myofiber metabolism may serve as an adaptive response to elevated glucose and insulin concentrations in the diabetic state. In fact, resistance training improves metabolic profiles in patients with diabetes through promotion of the growth and function of fast glycolytic muscle17,18. In rodents, inhibition of myostatin signaling leads to glycolytic muscle growth and beneficial effects on glucose homeostasis30. Thus, an inadequate adaptive increase of skeletal muscle
glycolytic capacity may exacerbate insulin resistance, whereas activation of this adaptive mechanism alleviates diet-induced disruption of metabolic homeostasis. Interestingly, Baf60c expression is lower in skeletal muscle from obese mice as a result of proinflammatory activity. Our work raises the possibility that expansion of skeletal muscle glycolytic capacity by increasing muscle mass and glycolytic activity, for example, through resistance training, may provide metabolic benefits in type 2 diabetes.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.D.L. and Z.-X.M. conceived the project and designed research. Z.-X.M., S.L. and I.W. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. H.J.K., Y.L., D.Y.J. and J.K.K. performed the studies. 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ONLINE METHODS

Animal studies. A Flag- and HA-tagged full-length mouse Baf60c coding sequence (FH-Baf60c) was placed downstream of the 4.8-kb mouse MCK promoter31. Transgenic mice were generated by pronuclear microinjection. Six independent founders were identified and crossed with C57BL/6 mice to generate stable transgenic lines. For in vivo knockdown study, heterozygous MCK-CAR mice were used. Mice were maintained in a 12-h dark, 12-h light cycle and fed with normal rodent chow or HFD (D12492, Research Diets). All animal studies were performed according to procedures approved by the University Committee on Use and Care of Animals. All experiments were performed on male mice unless otherwise indicated.

Histological analysis. Skeletal muscle samples were immediately frozen with liquid nitrogen–cooled isopentane after dissection and sectioned on a cryostat microtome. Muscle fiber–typing immunofluorescence staining was performed with antibodies to MHC I (1:200, DSHB, BA-F8), MHC IIa (1:200, DSHB, SC-71) and MHC IIb (1:200, DSHB, BF-F3), as previously described32. SDH and cytochrome oxidase staining were performed as described33. COX staining was conducted as previously described34. Transmission electron microscopy was performed by the Microscopy and Image Analysis Laboratory at the University of Michigan. The images were taken using a Philips CM-100 transmission electron microscope.

qPCR and western blot analyses. Total RNA was extracted using TRizol (Invitrogen). Gene expression was assessed by qPCR analysis using the primers shown in Supplementary Table 1. Protein lysates were quantified, separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore) followed by immunoblotting with the primary antibodies listed below. Rabbit polyclonal antibody to Baf60c was generated with the recombinant GST fusion mouse Baf60c protein and affinity purified. Antibodies to myoglobin (1:1,000, sc-74525), palbocチン (1:500, sc-74484), HA (1:1,000, sc-66181) and ribosomal protein S6 (1:2,000, sc-74459) were from Santa Cruz. Antibodies to AKT (1:1,000, 4691), pAKT (Ser473) (1:1,000, 4060), pAKT (Thr382) (1:1,000, 2956), p70 S6 kinase (1:1,000, 2708), phosphorylated p70 S6 kinase (Thr389) (1:1,000, 9234) and p56 (Ser240 and Ser244) (1:1,000, 4838) were from Cell Signaling. Antibodies to Flag (1:1,000, M2), c-Myc (1:1,000, C-9956) and α-tubulin (1:1,000, T6199) were from Sigma. Antibodies to Deptor (1:1,000, 09-463) and BAF53a (1:1,000, 10341-1-AP) were from Millipore and Proteintech, respectively.

Treadmill running. We performed treadmill running studies using a motorized, speed-controlled treadmill system (Columbus Instruments). Running speed was set to 10 mm min−1 for 30 min and increased by 2 mm min−1 increments every 20 min. The inclination angle was level. Male mice were trained at a speed of 10 m min−1 in three sessions to acquire running skills before running tests. WT and MCK-Baf60c transgenic mice were allowed to run until exhaustion for the measurements of total running time and distance. Blood lactate concentrations were measured before the onset of the running study and immediately after exhaustion using a Lactate Pro blood lactate test meter (ARKRAY Inc.). In a separate study, transgenic mice were subjected to run until exhaustion with running time–matched WT mice used as controls. Mice were anesthetized, and skeletal muscles were dissected for the measurement of muscle glycogen content.

In vivo adenoviral transduction in skeletal muscle. Adenoviruses were purified and transduced into tibialis anterior muscle as previously described35. Briefly, MCK-CAR mice were injected intramuscularly into the tibialis anterior with adenoviruses (1 × 109 PFU in 25 μl) expressing scramble shRNA on one leg and Smarcd3 (Baf60c) shRNA on the contralateral leg. The injection was performed twice on 2 consecutive days. Tibialis anterior muscles were dissected 5 d after transduction.

ITT and GTT. For GTT, mice were fasted overnight (16 h) and injected intraperitoneally with a glucose solution (20% prepared in saline) at 2.0 g per kg body weight. Blood glucose concentrations were measured before and 15, 30, 60 and 120 min after glucose injection. For ITT, mice prefasted for 4 h were injected intraperitoneally with insulin at 1.0 U per kg body weight. Blood glucose concentrations were measured before and 20, 45, 90 and 120 min after insulin injection.

Hyperinsulinemic-euglycemic clamp. We performed the hyperinsulinemic-euglycemic clamp study as described35. Briefly, a 120-min clamp was conducted with a prime-continuous infusion of human insulin (Humulin, Eli Lilly and Co.) at a rate of 2.5 mIU kg−1 min−1 to raise plasma insulin concentration. Blood samples (20 μl) were collected at 20-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain plasma glucose at basal concentrations. Insulin-stimulated whole-body glucose turnover and metabolism were estimated using [3-3H]glucose (10 μCi bolus, 0.1 μCi min−1) infusion throughout the clamps. To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-d-[1-14C]glucose (2-[14C]DG; PerkinElmer Inc.) was administered as a bolus (10 μCi) at 75 min after the start of the clamps. Blood samples (20 μl) were taken at 80, 85, 90, 100, 110 and 120 min after the start of the clamps for the determination of plasma [3H]glucose, 3H2O and 2-[14C]DG concentrations. At the end of the clamp experiments, mice were anesthetized, and tissues were harvested for biochemical analysis.

Muscle cell culture, retroviral infection and differentiation. C2C12 myoblasts were obtained from ATCC and cultured in DMEM containing 10% FBS. Pooled primary human skeletal myoblasts isolated from three healthy adult donors were purchased from Zen-Bio and maintained in skeletal muscle growth medium (SKM-M, Zen-Bio). Myoblasts were transduced with control retroviruses (vector or scramble shRNA) or retroviruses expressing Baf60c, Deptor or SiX4 or shRNAs targeting Smarcd3 (Baf60c) or Deptor and subjected to puromycin selection. For sequential retroviral transduction, myoblasts stably transduced with scramble or Deptor shRNA retroviruses were transduced again with retroviruses expressing vector control or Baf60c followed by neomycin selection. Myotube differentiation was initiated by switching confluent C2C12 or primary human myoblasts to DMEM containing 2% bovine growth serum or skeletal muscle differentiation medium (SKM-D, Zen-Bio), respectively. All studies were performed in differentiated myotubes.

Coimmunoprecipitation and ChIP. HEK293T cells were transiently transfected with FH-Six4 and Myc-tagged Baf60c for 24 h. Total lysates or immunoprecipitated proteins were analyzed by western blot using monoclonal antibodies to c-Myc (1:1,000, C-29356) or Flag (1:1,000, M2) from Sigma. ChIP assay was performed according to the protocol developed by Upstate Biotechnology as described36. Briefly, chromatin lysates were prepared from C2C12 myotubes after crosslinking with 1% formaldehyde. The samples were precleared with Protein G agarose beads and immunoprecipitated using antibodies to Baf60c (as described above), Six4 (Santa Cruz, sc-55766), acetyl-H3 (Upstate, 06-599), H3K4me3 (Upstate, 07-473) or H3K9me2 (Abcam, ab1220) or control IgG in the presence of BSA and salmon sperm DNA. Beads were washed extensively before reverse crosslinking. DNA was purified using a PCR Purification Kit (Invitrogen) and subsequently analyzed by qPCR using primers located in the proximal Smarcd3 (Baf60c) or Deptor promoter (the primers used are listed in Supplementary Table 2).

Luciferase reporter assay. The proximal Deptor promoter (~2258 to +23 relative to the transcriptional start site) was cloned into the pGL3 luciferase reporter vector. The Six3 binding site mutant was constructed by site-directed mutagenesis. C2C12 myoblasts were transiently transfected with the indicated plasmids using Lipofectamine 2000 and differentiated into myotubes before the luciferase assay. All the reporter assays were repeated at least three times in triplicates.

LDH activity assay and isoenzyme analysis. Skeletal muscle LDH activity was measured using an assay kit (BioVision). Briefly, frozen muscle tissue was homogenized in 0.25 ml cold assay buffer. After centrifugation at 10,000g for 15 min at 4 °C, the supernatant was collected for the enzymatic assay. The activities of LDH isoenzymes were determined as previously described36. Briefly, whole-cell lysates of plantarius muscles were loaded on a 6% native polyacrylamide gel. After electrophoresis, the gel was stained at room temperature for 30 min.
Measurement of glycolytic flux. The rate of glycolysis in isolated skeletal muscles and cultured myotubes were measured using d-[5-3H]glucose (American Radiolabeled Chemicals) as previously described with some modifications. Briefly, EDL muscles dissected from WT and transgenic male mice or cultured myotubes were incubated for 1 h at 37 °C in Krebs buffer with 10 µCi ml⁻¹ d-[5-3H]glucose plus 5 mM glucose for isolated muscles or 10 mM glucose for myotubes. 3H₂O produced by glycolysis was separated from D-[5-3H]glucose in the incubation buffer using the equilibration method and determined by liquid scintillation counting.

Statistics. Data were analyzed using two-tailed Student’s t test for independent groups. P < 0.05 was considered statistically significant.

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