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Supplemental Information

Loss of RNA-Binding Protein *Sfpq* Causes Long-Gene Transcriptopathy in Skeletal Muscle and Severe Muscle Mass Reduction with Metabolic Myopathy

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Supplemental Figures and Legends

Figure S1

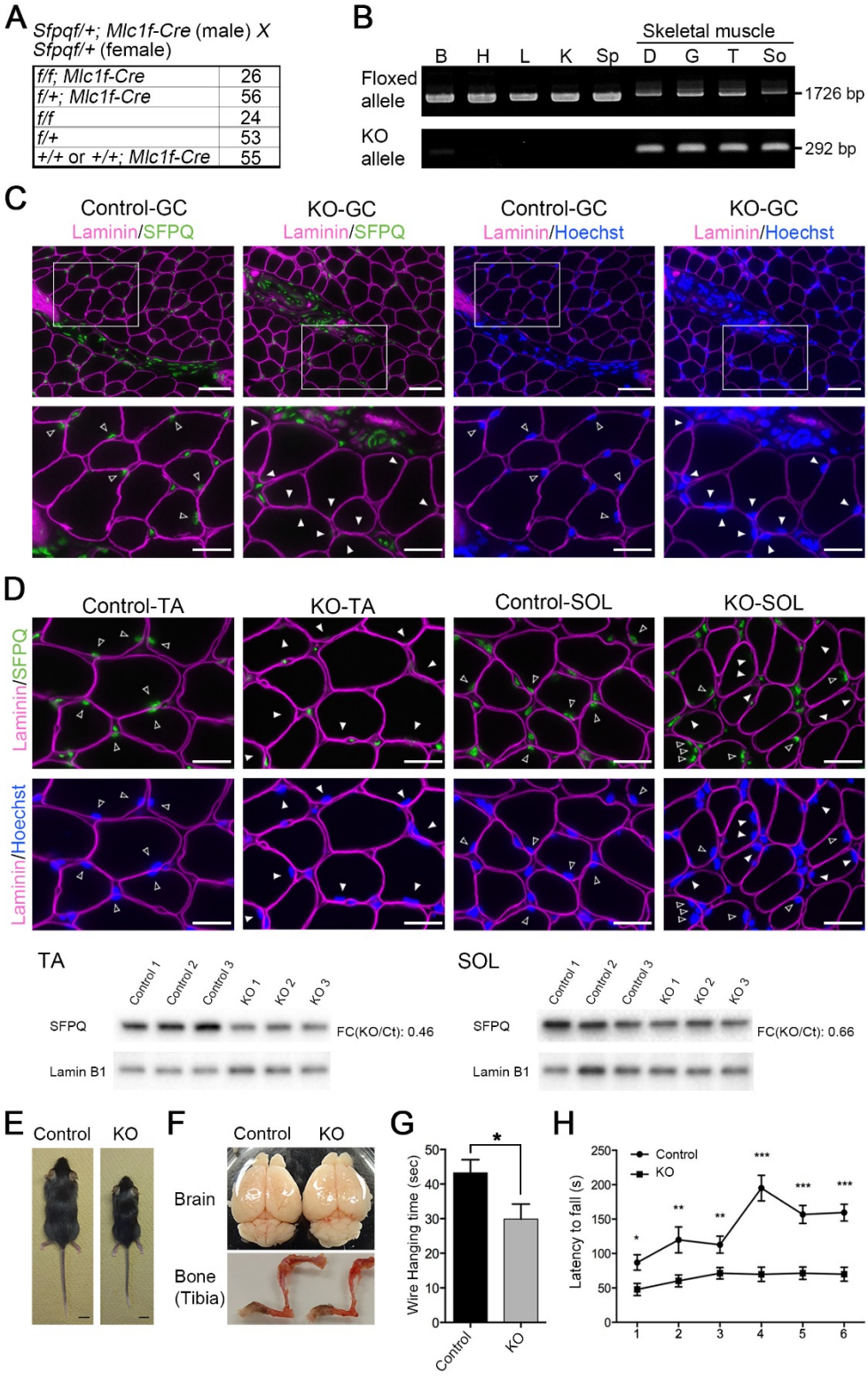
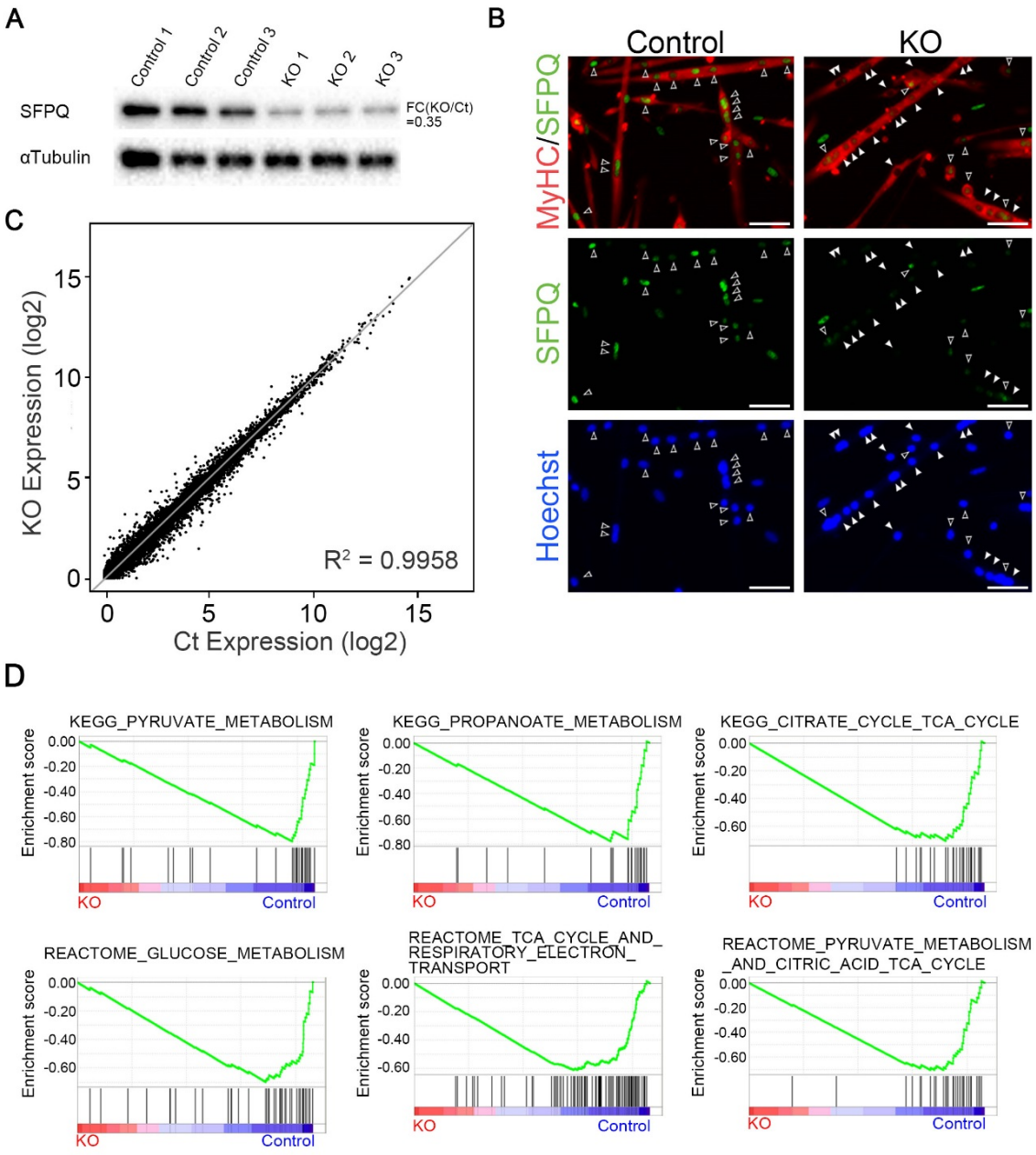


Figure S1. Phenotypic analysis of *Sfpq* KO mice, related to Figure 1. (A) Offspring ratio of male *Sfpq* conditional knockout mice obtained by crossing *Sfpq*$^{f/+}$*;Mlc1f-Cre* male mice with *Sfpq*$^{f/+}$ female mice (from P7 to P14). *f*, floxed; +, wild type. **(B)** Examination of Cre excision in several tissues from *KO* mice. Genotyping polymerase chain reaction (PCR) was performed to detect the floxed (Floxed allele; 1726 bp) and *Sfpq* knockout alleles after Cre excision [KO allele; 292 bp]. B, brain; D, diaphragm; G, gastrocnemius; H, heart; K, kidney; L, liver; Sp, spleen; T, tibialis anterior; So, soleus (female). **(C, D)** Immunostaining for SFPQ (green) and Laminin (purple) nuclear counterstained with Hoechst 33342 on gastrocnemius muscles (GC) (C), tibialis anterior (TA) and soleus muscles (SOL) (D, upper panel) from 1-month-old male KO and control mice. Scale bars: 50 μm. Boxed areas indicated in upper panels are shown with higher magnification in lower panels. Scale bar = 25 μm. Open arrow heads indicate nuclei in myofiber which are positively stained with SFPQ antibody and closed white arrow heads indicate nuclei which are negative for SFPQ. Western Blotting (WB) for SFPQ protein expression in TA and SOL muscle samples from 1-month-old male KO and control mice (n = 3 in each genotype). Lamin B1 was used as control for the equal sample loading. Averaged fold changes (FCs) between *Sfpq*-KO and control (KO/Ct) mice were indicated (D, lower panel). **(E)** Gross appearance of male KO and control mice at P35. Scale bars: 10 mm. **(F)** Representative photos of brain and bone (tibia) from 1-month-old male KO and control mice. **(G)** Wire hang test performed on male KO (n = 19) and control (n = 20) mice from P26 to P30. The test lasted 60 s. Data are presented as mean ± SEM. *$p < 0.05$ (Student's t test) **(H)** Rotarod test performed on male KO (n = 15) and control (n = 20) mice from P26 to P30. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control (Student's t test or Welch's t test).

Figure S2



E

Gene	Disease
Phkb (*phosphorylase kinase beta*)	Glycogen storage disease IXb
Gbel [*glucan (1,4-alpha-), branching enzyme 1*]	Glycogen storage disease IV
Pcx (*pyruvate carboxylase*)	Pyruvate carboxylase deficiency
Hlcs (*holocarboxylase synthetase*)	Multiple carboxylase deficiency
Pcca (*propionyl-Coenzyme A carboxylase, alpha polypeptide*)	Propionic acidaemia
Suclg2 (*succinate-Coenzyme A ligase, GDP-forming, beta subunit*)	Methylmalonic aciduria
Lpin1 (*lipin 1*)	Myoglobinuria recurrent

Figure S2. Transcriptome analysis and GSEA on *Sfpq* KO myotubes, related to Figure 3 and 4. (A) WB showing SFPQ protein expression in KO and control myotubes which were induced differentiation for 4 days. α-Tubulin was used as the control for equal sample loading. Averaged FC between *Sfpq*-KO and control (KO/Ct) myotubes was indicated. **(B)** Immunostaining for SFPQ (green) and MyHC (red) nuclear counterstained with Hoechst 33342 on KO and control myotubes which were induced differentiation for 4 days. Open arrow heads indicate nuclei in MyHC-positive differentiated myotubes which are positively stained with SFPQ antibody and closed white arrow heads indicate nuclei which are negative for SFPQ. Scale bars: 50 μm. **(C)** Scatterplot showing mRNA expression in KO (n = 3) and control myotubes (n = 3) determined by polyA-selected mRNA-seq. X- and y-axes show $\log_2$ of average mRNA expression in three control and three KO myotubes, respectively. Ct, control; KO, knockout; R^2, coefficient of determination. **(D)** Representative GSEA plot of the gene sets in Figure 3C and 3D. **(E)** Downregulated genes in KO myotubes and their association with inherited metabolic diseases. SFPQ target genes, which showed 3´-site downregulation, were shown with red characters.

Figure S3

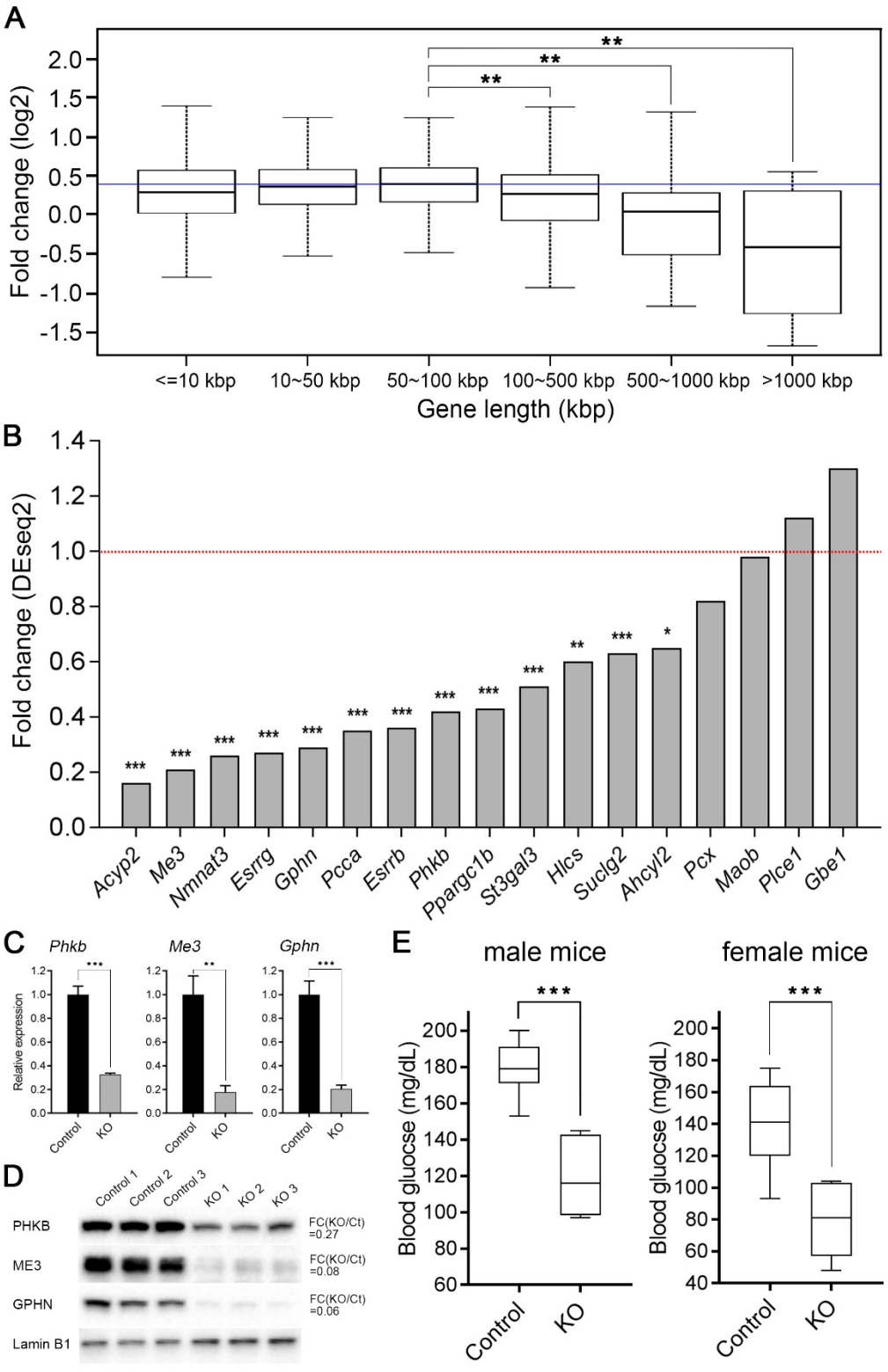
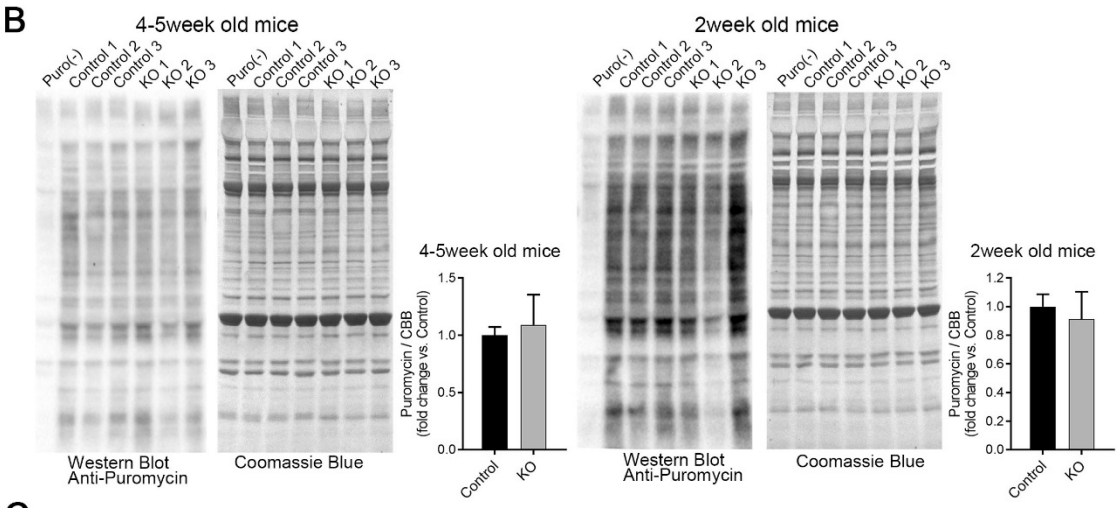

A
Fold change (log2)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
**
**
**
<=10 kbp
10~50 kbp
50~100 kbp
100~500 kbp
500~1000 kbp
>1000 kbp
Gene length (kbp)
B
Fold change (DEseq2)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Acyp2
Me3
Nmnat3
Esrrg
Gphn
Pcca
Esrrb
Phkb
Ppargc1b
St3gal3
Hlcs
Suclg2
Ahcyl2
Pcx
Maob
Plce1
Gbe1
C
Phkb
Me3
Gphn
Relative expression
Control
KO
D
Control 1
Control 2
Control 3
KO 1
KO 2
KO 3
PHKB
ME3
GPHN
Lamin B1
FC(KO/Ct) =0.27
FC(KO/Ct) =0.08
FC(KO/Ct) =0.06
E
male mice
female mice
Blood glucose (mg/dL)
Control
KO

Figure S3. *In vivo* transcriptome analysis in *Sfpq* KO mice and blood glucose measurement related to Figure 3 and 4. (A) Box plots showing length-dependent gene expression changes in KO-GC muscle from P35 male mice (n = 3). Bins were defined according to gene length. $^{**}p < 0.001$ for changes between two bins (Mann-Whitney U test). The baseline was drawn at the mean value in 50-100 kbp genes (fold change = 0.40). **(B)** The fold changes of mRNA levels in KO muscles for all downregulated genes > 100 kbp in GSEA identified metabolic pathway genes and transcriptional regulators in KO myotubes. Fold changes and q values were calculated using DESeq2 for analyzing RNA-seq data of GC muscle samples from P35 male KO and control mice (n = 3). $^{*}q < 0.05$, $^{**}q < 0.001$ and $^{***}q < 0.001$. **(C)** RT-qPCR quantification of indicated genes in GC muscle samples from P35 male KO and control mice (n = 3). Data are presented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$ (Student's t test). **(D)** WB for PHKB, ME3 and GPHN proteins on TA muscles from 1-month-old male KO and control mice (n = 3). Lamin B1 was used as control for the equal sample loading. Averaged FCs between *Sfpq* KO and control (Ct) mice (KO/Ct) were indicated. **(E)** Blood glucose concentrations were measured in 4-5-week-old male (*left*) and female (*right*) KO and control mice after 6-hour fast. (male: n = 6, female: n = 7). $^{***}p < 0.001$ (Student's t test)

Figure S4

A Protein degradation markers

	Gene symbol	FC(KO/Ct)	*q* value
Ubiqitin-Proteasome	*Fbxo32* (*F-box protein 32*) (*Atrogin1*)	1.17	0.76
	Trim63 (*tripartite motif-containing 63*)(*Murf1*)	1.08	0.72
	Foxo1 (*forkhead box O1*)	1.00	1.00
	Foxo3 (*forkhead box O3*)	1.13	0.60
	Fbxo40 (*F-box protein 40*)	0.91	0.52
Calpain-Calpastatin	*Capn1* (*calpain 1*)	1.21	0.07
	Capn2 (*calpain 2*)	1.22	0.08
	Capn3 (*calpain 3*)	0.89	0.46
Lysosome-Autophagy	*Map1lc3b* (*microtubule-associated protein 1 light chain 3 beta*) (*LC3b*)	0.98	0.93
	Lamp2 (*lysosomal-associated membrane protein 2*)	0.82	0.32
	Sqstm1 (*sequestosome 1*) (*p62*)	1.18	0.14
	Ctsl (*cathepsin L*)	1.44	0.0004***
	Gabarapl1 (*gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1*)	1.04	0.88
	Atg4b (*autophagy related 4B, cysteine peptidase*)	1.05	0.84
	Atg12 (*autophagy related 12*)	0.83	0.34
	Bnip3 (*BCL2/adenovirus E1B interacting protein 3*)	0.99	0.94
	Bnip3l (*BCL2/adenovirus E1B interacting protein 3-like*)	1.20	0.20
	Becn1 (*beclin 1, autophagy related*)	0.93	0.71



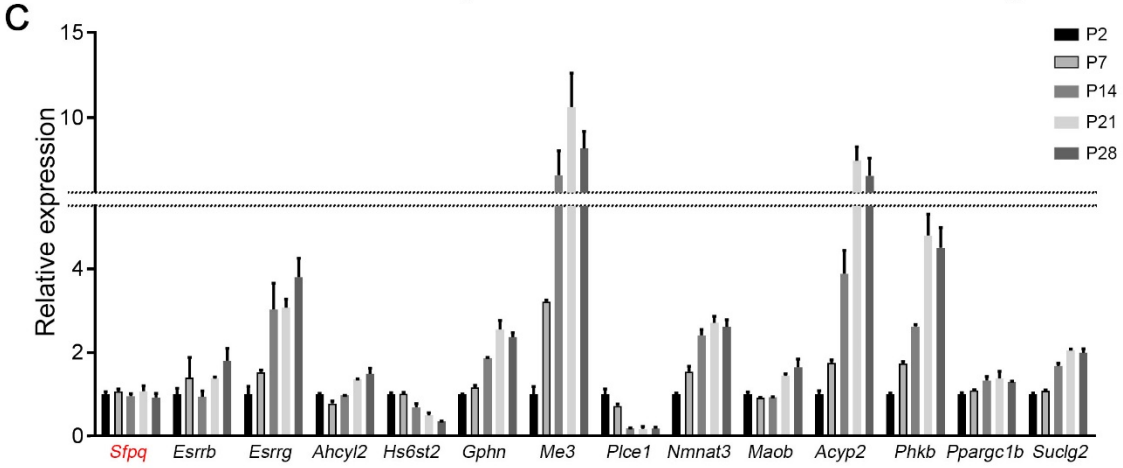


Figure S4. Catabolic and anabolic activities in KO muscle tissues, expression change of SFPQ target genes in during postnatal muscle development, related to Figure 3 and 4. **(A)** FCs and *q* values of genes relating to protein degradation. FCs and *q* values were calculated using DESeq2 for RNA-seq data of GC muscle samples from P35 male KO and control mice ($n = 3$). ***$q < 0.001$. **(B)** WB for Puromycin-labeled newly synthesized protein from 4-5 week (*left*) and 2 week (*right*) old male KO and control mice ($n = 3$). Coomassie Blue staining (CBB) of membranes was conducted to verify equal sample loading. Intensity of total lanes in WB of Puromycin antibody was measured and adjusted by CBB stained signal to measure the total synthesized proteins per loaded proteins, and relative fold changes of synthesized proteins were plotted. Data are presented as mean ± SD. There were no significant differences between KO and Control (Student's t test). **(C)** The mRNA expression levels of *Sfpq* and SFPQ target genes, which showed 3′-site downregulation, in control (wild-type) GC muscle samples collected at indicated developmental stages ($n = 2$). Data was shown as relative mRNA levels of those in postnatal day 2 (P2) for comparison. Data are presented as mean ± SD.

Supplemental Table

Table S1. Genes downregulated in KO myotubes in the GSEA, related to Figure 4.

Gene symbol	Fold change	*p* value	Gene length	Regulatory pathway	*
Genes extracted from downregulated KEGG gene sets					
Gmds	0.52	2E-06	**518959**	Fructose and mannose metabolism	
Gphn	0.54	4E-09	**458118**	Folate biosynthesis	
Pcca	0.69	0.0006	**355617**	Carbon metabolism, etc.	1)
Plce1	0.68	0.0010	**260909**	Inositol phosphate metabolism	
Gbe1	0.59	0.0012	**255772**	Starch and sucrose metabolism	2)
Suclg2	0.72	0.0005	**245838**	Carbon metabolism, etc.	3)
Me3	0.49	5E-09	**221542**	Pyruvate metabolism, etc.	
St3gal3	0.72	0.0014	**202794**	Mannose type O-glycan biosynthesis	
Hlcs	0.69	0.0002	**158551**	Biotin metabolism	4)
Ahcyl2	0.61	6E-07	**143868**	Cysteine and methionine metabolism	
Acyp2	0.55	2E-05	**143405**	Pyruvate metabolism, etc.	
Nmnat3	0.53	1E-05	**114846**	Nicotinate and nicotinamide metabolism	
Pcx	0.71	2E-05	**111281**	Carbon metabolism, etc.	5)
Maob	0.70	0.0008	**108086**	Arginine and proline metabolism, etc.	
Plpp3	0.66	0.0007	75421	Glycerolipid metabolism, etc.	
Lpin1	0.58	0.0004	54102	Glycerolipid metabolism, etc.	6)
Acsl1	0.68	0.0010	45086	Fatty acid metabolism	
Kyat3	0.59	0.0009	43864	Tryptophan catabolism, etc.	
Galnt5	0.59	2E-06	41017	Mucin type O-glycan biosynthesis	
Pon3	0.62	6E-06	35382	Metabolic pathways	
Idh1	0.69	1E-07	27864	2-Oxocarboxylic acid metabolism	
Got1	0.53	2E-06	24854	2-Oxocarboxylic acid metabolism	
Bdh2	0.54	0.0001	23206	Butanoate metabolism	
Oat	0.71	0.0009	18924	Arginine and proline metabolism, etc.	
Extl1	0.55	8E-06	16175	Glycosaminoglycan biosynthesis	
Hmox1	0.67	0.0009	6976	Porphyrin and chlorophyll metabolism	
Cycs	0.75	0.0020	3912	Sulfur metabolism	
Cox7a1	0.59	0.0003	1860	Oxidative phosphorylation	
Genes extracted from downregulated Reactome gene sets (except for above genes)					
Hs6st2	0.62	9E-07	**294966**	Metabolism of carbohydrates	
Phkb	0.69	3E-06	**219641**	Metabolism of carbohydrates, etc.	7)
Slc25a12	0.65	0.0018	93260	Gluconeogenesis, etc.	
D2hgdh	0.76	0.0029	27122	TCA cycle, etc.	

*Inherited metabolic disease identified in Human Gene Mutation Database: 1) propionic acidemia, 2) glycogen storage disease IV, 3) methylmalonic aciduria, 4) multiple carboxylase deficiency, 5) pyruvate carboxylase deficiency, 6) myoglobinuria recurrent, and 7) glycogen storage disease IXb.

Transparent Methods

Mice.

Sfpq-floxed mice (accession no. CDB0981K; http://www2.clst.riken.jp/arg/mutant%20mice%20list.html) were generated as previously described (http://www2.clst.riken.jp/arg/Methods.html) (Takeuchi et al., 2018) and were crossed with *Mlc1f-Cre* mice to generate skeletal muscle-specific knockout mice (Bothe et al., 2000). Genotyping PCR was performed using genomic DNA from a finger as previously described (Takeuchi et al., 2018). Cre-mediated excision of the floxed *Sfpq* DNA segments was determined by PCR amplification of genomic DNA isolated from mouse tissues using primers P763-2 and P766. *Cre* transgenes were detected by PCR using primers P1005 and P720 along with those for the internal control (*interleukin-2*). Genotyping primer positions are shown below. Animal care and experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all experimental protocols were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine and RIKEN, Kobe Branch.

Sfpq-recombinant alleles
P763-2: 5'-TTGGTGCTGTCTTCCTAGTCTGCATTTAG-3'
P766: 5'-GTATTCATAACACTGGCATGATGGCTCACACCTATA-3'

Mlc1f-Cre with internal control
P1005:5'-CAAGTGTCACTCTGACAGGTTCTTCTGGAGGAG-3'
P720: 5'-TCCATGAGTGAACGAACCTGGTCGAAATCAG-3'
P715: 5'-CTAGGCCACAGAATTGAAAGATCT-3'
P716: 5'-GTAGGTGGAAATTCTAGCATCATCC-3'

Behavioral tests.

Behavioral tests were performed as previously described (Matsuo et al., 2009; Miyakawa et al., 2001). A grip-strength meter (O'Hara & Co., Tokyo, Japan) was used to measure forelimb grip strength. Mice were lifted by their tails to allow their forepaws to grip a wire grid, and with the bodies of the mice parallel to the surface of the table, the mice were gently and continuously pulled back by their tails until they released the grid. The maximum force applied by the forelimbs to grasp the grid was recorded in Newtons (N). The grip-strength test was performed three times for each mouse, and the highest measured value was used for analytical purposes. During the wire hang test, each mouse was placed on a wire mesh that was then inverted and waved gently in order to force the mouse to grip the wire. The time required for mice to fall was recorded, with a 60-s

cut-off time. For the rotarod test, an accelerating rotarod (UGO Basile Accelerating Rotarod) that accelerated from 4 to 40 rpm over a 5-min period was used. The mice were placed on the rotating drums (3 cm diameter), and the time each mouse was able to maintain its balance on the rod was record. The speed of the rotarod accelerated from 4 to 40 rpm over a 5-min period.

Measurement of blood glucose concentration.

Following 6 hour fast, blood glucose concentration was measured from tail vain using Accu-Chek ST Meter (Roche Diagnostics K.K., Tokyo, Japan).

Measurement of glycogen content.

The glycogen content of muscles was measured by modifications of a previously described procedure (Chan and Exton, 1976; Iwasaki et al., 2012; Quinlan et al., 2010). GC Muscles were removed from the KO and control mice. Then, they were incubated in 200µl 1M KOH per 40mg muscle at 70°C for 1hour. Following digestion 75 µL of saturated Na_2SO_4 and 1.725 mL of ethanol was added on ice (final volume 2 mL). Samples were precipitated by centrifuging at 13000 rpm for 10 mins at 4°C. The supernatant was removed and the pellet dissolved in 200 µL dH_2O. Samples were reprecipitated with 1.8 mL ethanol. The supernatant was removed and pellet was dried. The pellets were resuspended in 400 µL Glycogen Hydrolysis Buffer (Abcam, Cambridge, UK). The amount of glycogen was determined with Glycogen Assay Kit (Abcam).

Surface sensing of translation (SUnSET).

For all *in vivo* measurements of protein synthesis with SUnSET (Goodman et al., 2011), puromycin (Merck Millipore, Billerica, MA, USA) stock solution (75mM) was prepared and diluted to the appropriate volume, which was needed to inject mice with 0.04 µmol/g body weight in 200 µl of phosphate-buffered saline (PBS), and subsequently administered into the animals via intraperitoneal injection. At exactly 30 min after injection, muscle tissues were extracted and either frozen in liquid nitrogen for WB analysis as detailed below. After capturing the appropriate image, the membranes were stained with Coomassie Blue (Nacalai Tesque, Kyoto, Japan) to verify equal loading in all lanes. Densitometric measurements were performed by the density of each total lane volume.

H&E staining.

After mice were sacrificed, skeletal muscles were isolated, embedded in tragacanth gum (Wako Pure Chemical Industries, Osaka, Japan), and flash frozen in isopentane cooled in liquid nitrogen. Cryosections (7 µm) of skeletal muscle tissue were stained with H&E as previously described (Tanihata et al., 2008).

Modified Gomori trichrome staining.

Cryosections (10 μm) from skeletal muscle tissue were stained in Gomori solution followed by Harris Hematoxylin (Muto Pure Chemicals, Tokyo, Japan). Gomori solution consists of fast green FCF (Kanto Chemical, Tokyo, Japan), chromotrope 2R (Sigma-Aldrich, St. Louis, MO, USA), phosphotungstic acid (Kanto Chemical) and acetic anhydride (Malicdan et al., 2009).

NADH-TR staining.

Cryosections (10 μm) from skeletal muscle tissue were incubated with NADH-TR staining solution [0.8 mg/ml β-NADH (Sigma-Aldrich) and 1 mg/ml nitro blue tetrazolium (Sigma-Aldrich) in 50 mM Tris–HCl (pH 7.4)] at 37°C. After staining, sections were washed with acetone solution followed by deionized water, and mounted with Glycerol Gelatin (Sigma-Aldrich).

Periodic acid Schiff staining.

Cryosections (10 μm) from skeletal muscle tissue were fixed with Carnoy's fixative (60% methanol, 30% chloroform, and 10% acetic acid) and incubated for 10 min in periodic acid solution (Sigma-Aldrich). Sections were incubated in Schiff's reagent (Sigma-Aldrich) for 10 min and dehydrated.

Immunohistochemistry.

Cryosections (7 μm) obtained from the middle portion of frozen muscles were dried and fixed with 4% paraformaldehyde for SFPQ at room temperature (RT) and acetone for 10 min at −20°C for other 1st antibodies, and pre-incubated in PBS containing 10% goat serum and 0.5% Triton X-100 for 15 min at RT. Primary antibodies were applied overnight at 4°C with 15% goat serum followed by 30 min at RT with secondary antibodies. The antibodies used were raised polyclonal for SFPQ (1:800) (Takeuchi et al., 2018), mouse monoclonal anti-MyHC type I [1:100; BA-D5-c, the Developmental Studies Hybridoma Bank maintained by the University of Iowa (DSHB), Iowa City, IA, USA], mouse monoclonal anti-MyHC type IIA (1:200; SC-71-c, DSHB), mouse monoclonal anti-MyHC type IIB (1:200; BF-F3-c, DSHB) and rat monoclonal laminin 2 (1:200; 4H8-2: Cat#ALX-804-190-C100, Enzo Life Sciences, Farmingdale, NY, USA), followed by Chicken anti-Rat Alexa Fluor 488 (1:400, Cat#A21470, Thermo Fisher Scientific), Goat anti-Mouse Alexa Fluor 568 (1:400 Cat#A11031, Thermo Fisher Scientific), Goat anti-Mouse Alexa Fluor 488 (1:400, Cat#A21042, Chicken anti-Rat, Thermo Fisher Scientific), Chicken anti-Rat Alexa Fluor 594 (1:400, Cat#A21471, Thermo Fisher Scientific), Goat anti-Rabbit Alexa Fluor 488 (1:400, Cat#A11070, Thermo Fisher Scientific) and Goat anti-Rat Alexa Fluor 647 (1:400, Cat#A21247, Thermo Fisher Scientific). Sections were stained with Hoechst 33342 (Nacalai

Tesque) for nuclear staining and mounted using ProLong Gold Antifade Reagent (Thermo Fisher Scientific). Mounted sections were observed using a fluorescence microscope (model BZ-9000 or BZ-X710; Keyence, Osaka, Japan). The fiber cross-sectional area and number of MyHC isoform-positive fibers were determined from three animals, with a minimum of 1800 myofibers assayed per section using Hybrid Cell Count software (BZ-II Analyzer; Keyence).
All histological analysis was carried out using three independent samples.

Cell preparation and culture.

Mouse primary myoblasts were cultured according to a previously reported method (Rando and Blau, 1994; Tanihata et al., 2008). Briefly, limb muscles of 1-month-old KO ($n = 3$) male mice and littermate controls ($n = 3$) were removed and minced into a slurry in PBS using dissecting scissors. Cells were enzymatically dissociated by adding 0.2% collagenase type 2 (Worthington Biochemical, Freehold, NJ, USA) followed by passage through an 18-gauge needle and filtration through 100- and 40-μm nylon meshes (Falcon; BD Biosciences, Franklin Lakes, NJ, USA). After pre-plating for 1.5 h on non-coated dishes, primary myoblasts were cultured alone with growth medium (GM) composed of F-10 containing 20% fetal bovine serum, 1% penicillin-streptomycin (Nacalai Tesque), and 2.5 ng/ml basic fibroblast growth factor (Thermo Fisher Scientific, Waltham, MA, USA) in collagen-coated dishes (Iwaki, Tokyo, Japan). To induce differentiation, the medium was changed to differentiation medium (DM) composed of 2% horse serum in Dulbecco's Modified Eagle Medium and cultured for 4 days. GM was changed every 24 h, and DM was changed on days 1 and 3 after induction of differentiation.

Immunocytochemistry.

Differentiated primary myoblasts on day 4 after inducing differentiation were fixed with 4% paraformaldehyde in PBS and pre-incubated in PBS 3% goat serum / 1% bovine serum albumin (BSA) / 0.1% Triton X-100 for 30 min at RT. Primary antibodies in 3% goat serum and 1% BSA were applied overnight at 4°C followed by 1 hr at RT with secondary antibodies. The antibodies used were raised polyclonal for SFPQ (1:2000) (Takeuchi et al., 2018), mouse monoclonal anti-sarcomeric MyHC (1:250; MF-20, DSHB), followed by Goat anti-Rabbit Alexa Fluor 488 (1:400, Cat#A11070, Thermo Fisher Scientific) and Goat anti-Mouse Alexa Fluor 555 (1:500, Cat#A21425, Thermo Fisher Scientific). Sections were stained with Hoechst 33342 (Nacalai Tesque) for nuclear staining. Cells were observed using a fluorescence microscope (model BZ-X710; Keyence). Analysis was carried out using three independent samples.

WB (Western blotting)

Proteins were extracted from primary cells and mouse skeletal muscle tissue using sample

buffer (Nacalai Tesque), and the lysates were denatured at 95°C for 3 min, except in the case of detection of mitochondrial OXPHOS complexes. The lysates were then resolved by SDS-PAGE (SuperSep Ace gel; Wako Pure Chemical Industries) and transferred to a polyvinylidene difluoride membrane (Pall Corporation, Port Washington, NY, USA). Antibody reactions were performed with Can Get Signal immunoreaction enhancer solution (Toyobo, Osaka, Japan). Immunoreactivity was visualized with Chemi-Lumi One Super (Nacalai Tesque) or ImmunoStar LD (Wako Pure Chemical Industries) and a ChemiDoc MP imaging system (Bio-Rad, Hercules, CA, USA). The following primary antibodies were used in this study: raised polyclonal for SFPQ (1:2000 or 20000) (Takeuchi et al., 2018), mouse monoclonal antibodies cocktail for OXPHOS complexes (1:1000, Cat#ab110413, Abcam), mouse monoclonal anti-α-tubulin (1:10000, DM1A, Cat#MS-581-P1, Thermo Fisher Scientific), rabbit monoclonal anti-LaminB1 (1:1000, D4Q4Z:Cat#12586, Cell Signaling Technology, Danvers, MA, USA), mouse monoclonal anti-Puromycin (1:1000, 3RH11:Cat#PEN-MA001, Cosmo Bio Co., Ltd., Tokyo, Japan), rabbit monoclonal anti-ME3 (1:500, EPR10378:Cat#ab172972, Abcam), rabbit polyclonal anti-PHKB (1:2000, Cat#13400-1-AP, Proteintech Group, Inc., Chicago, IL, USA), rabbit polyclonal anti-GPHN (1:1000, Cat#14304, Cell Signaling Technology). Horseradish peroxidase-conjugated anti-rabbit (Cat#NA934) and anti-mouse IgG (Cat#ab5887) secondary antibodies were purchased from GE Healthcare Life Sciences (Pittsburgh, PA, USA) and Abcam, respectively.

Total RNA extraction from skeletal muscle.

GC muscles were isolated and frozen in liquid nitrogen. Frozen muscle tissues were powdered in liquid nitrogen, lysed by Buffer RLT (QIAGEN, Valencia, CA, USA), and digested by proteinase K. Total RNA was extracted using an RNeasy mini kit (QIAGEN) according to manufacturer protocol.

Reverse Transcription-quantative Polymerase Chain Reaction (RT)-qPCR.

First-strand cDNAs were synthesized with random primers (Takara, Shiga, Japan), and qPCR was performed using FastStart Universal SYBR-Green Master (Hoffmann-La Roche, Inc., Nutley, NJ, USA) according to the manufacturer's protocol. Cell culture data were normalized to *TATA box binding protein* (*Tbp*) expression. Mouse data were normalized to *hypoxanthine guanine phosphoribosyl transferase* (*Hprt*) expression. PCR primer pairs were as follows.

Dmd 3' F (HO255)	CTCTCCATTTCACAGCATACCAC
Dmd 3' R (HO256)	AAACCGTGGATAAGTGCTCTATG
Dmd 5' F (HO261)	CCCTCCATTCTAACCGATGTG
Dmd 5' R (HO262)	ATTTGATCACACAGGTATGCGC

Acyp2 3' F (H0583)	AGCCAACCCAAGATTAGCAGGC
Acyp2 3' R (H0584)	GTTCCGAGGTCAGCGTCTGTAAC
Acyp2 5' F (H0585)	ACTTTGTCAGAGGTTGTAGCCCC
Acyp2 5' R (H0586)	TGGCCTGGGAGTGTACTTCAGC
Ahcyl2 3' F (H0607)	TTGTCCTAAGTCTGTCTGCACGG
Ahcyl2 3' R (H0608)	AGTAAAGCGCCCAATGGCTCAC
Ahcyl2 5' F (H0609)	CTCGGGAAACCCTCTGAGTTGC
Ahcyl2 5' R (H0610)	TGCAATGGCTAGAAAGGAAGGGC
Esrrb 3' F (H0655)	TTCCCAGCTTAACGAACCCAGC
Esrrb 3' R (H0656)	CTGCCTTTCCAGGTACAGCTCC
Esrrb 5' F (H0657)	AAAGCATCCATCCCTGTGTGGC
Esrrb 5' R (H0658)	GGGACCCACTGATTCCAAACGAG
Esrrg 3' F (H0699)	GTTGCATCCGCACTTGGCTTG
Esrrg 3' R (H0700)	AATCACTCCCCTGCCAACAAGG
Esrrg 5' F (H0663)	TGTCTGGTGCCTTTTGTGTGGAG
Esrrg 5' R (H0664)	CCAGCGCCTATTCTTAGCACCTG
Gmds 3' F (H0625)	GATTTGGGGTGGGAGCACATTTAC
Gmds 3' R (H0626)	AAGGGAGACCCACATAGCTGGAC
Gmds 5' F (H0627)	GGCATGAACTCCTTGGGTGAGG
Gmds 5' R (H0628)	GCAGTGGTTAGCCTTATCTGTTGC
Gphn mRNA F (H0635)	ACTCCGGTGCTTGGTACAGAAATC
Gphn mRNA R (H0636)	ATCAGCAGCTCGAACAGCATAGCC
Gphn 3' F (H0631)	TGACTGAATAGGAGAAAGAAGACAC
Gphn 3' R (H0632)	ACCCATTTTCACTGAGGTTTGAGC
Gphn 5' F (H0633)	CTGCATGTCTGATTCCGTTGCG
Gphn 5' R (H0634)	GAACCTCAGCCGAAACCCCTAC
Hs6st2 3' F (H0691)	TTCATGCCTGGTGTGGATACGG
Hs6st2 3' R (H0692)	AGGTGGTGGCATCTGTTCTGTG
Hs6st2 5' F (H0693)	CCGCAAGGACTGGACACTACAC
Hs6st2 5' R (H0694)	AACAAGCCTGGTCTTTGGGACC
Maob 3' F (H0601)	CACCTTCTGGAATGACTCCCACC
Maob 3' R (H0602)	CCAGCTCTTCAAGGTTCTTTACTCC
Maob 5' F (H0603)	CAGGTCCAAAGACTGCCCATCC
Maob 5' R (H0604)	CATGGAGTCTGCCTGACCTTCC
Me3 mRNA F (H0581)	CTGTCCTGCTAGATGTTGGCACC
Me3 mRNA R (H0582)	TCATCATACTCCTCCCCACGGAC
Me3 3' F (H0673)	TCACAATGTCGGTACAAGGAGCC
Me3 3' R (H0674)	CAGCCTGGAGTATGATGGAGGAC
Me3 5' F (H0579)	GGGCTTGGGACGAAACCTATGG
Me3 5' R (H0580)	TCTCCATGTCAAGAGGGGGACG
Nmnat3 3' F (H0619)	GACTAAACCCCACAGGACTGCC

Nmnat3 3' R (H0620)	TCTCTGGACAACTAACAGGCCAC
Nmnat3 5' F (H0621)	AAGCCACTGTTCTAAGATGGGACTG
Nmnat3 5' R (H0622)	GAGATACCTTCACCCCTGCTGTG
Phkb mRNA F (H0563)	ACCAACTTTGTCGGATATGACC
Phkb mRNA R (H0564)	CCAGTTCAGGGTTTCTTTCCAGC
Phkb 3' F (H0677)	AATCTGTGGCCTTTGACCTGGC
Phkb 3' R (H0678)	TCATGCTCGCTCTTGGGAAGAC
Phkb 5' F (H0561)	GAAATTGGCCCCTTCCCTAGCC
Phkb 5' R (H0562)	GTTCAGGACACTCGGACTGCTG
Plce1 3' F (H0717)	TTACGGTGCCACAAAGTAGCGAG
Plce1 3' R (H0718)	TGGTCCCACTAGAAGCTCTTTTGG
Plce1 5' F (H0639)	TGTCTATTAGCTTTGGCGAGGC
Plce1 5' R (H0640)	AGGGAGAAGAGAGGAAAGACGGAC
Ppargc1b 3' F (H0649)	CTCAGAGTTTCCTGGGGAGCAG
Ppargc1b 3' R (H0650)	ACATGGGATGAACCTATCGCCC
Ppargc1b 5' F (H0651)	TCCTCCCGCAGGTAGAATCCAG
Ppargc1b 5' R (H0652)	AAAGTACGGCAGCAAGTGCCAG
Suclg2 3' F (H0679)	TTGGAGTGCCAGGGACAATACAG
Suclg2 3' R (H0680)	CTTTTCAGATATGCCCGGCTGAC
Suclg2 5' F (H0591)	GAGGTTTCTCAGCCGCTTACTGG
Suclg2 5' R (H0592)	ACAGACAAGCACAAGGGGGTC
Idh1 3' F (H0667)	TTTTGAAACCTACTCTTCGGGC
Idh1 3' R (H0668)	TGCACACATTCACAGGAGACCC
Idh1 5' F (H0669)	GATGAACTCAGTGGCAAGGCCC
Idh1 5' R (H0670)	CTCCCAAAGCGATCCCAACCTC
Tbp-F (H0681)	ATCAGATGTGCGTCAGGCGTTC
Tbp-R (H0682)	CGTCTTCAATGTTCTGGGTTATCTTC
Gbe1 3' F (H0565)	ACGTGCTAGTCTGAGGGGACAC
Gbe1 3' R (H0566)	AGCACAAGCCGGTGAACTCAAG
Gbe1 5' F (H0567)	TCTTGCACCCTCCTGTTTGCTC
Gbe1 5' R (H0568)	GCAAAGGTCGCTCTTCGATGTTG
Hlcs 3' F (H0595)	GTGTGCTCGTTTTCCTTTGCCC
Hlcs 3' R (H0596)	TGGGCTTCGGGTCTGCTTTTAG
Hlcs 5' F (H0597)	ACTCACCCTTTAGGAACACCGC
Hlcs 5' R (H0598)	TACTGTTGTGCTGGCTCTGACG
Pcca 3' F (H0701)	TCAGGCCGACTGCGTTTATCAG
Pcca 3' R (H0702)	CATGGCTGCACACAGACTTCTC
Pcca 5' F (H0703)	GTCCGAGCTTTACCTGCACTCC
Pcca 5' R (H0704)	GATGCCCCTTCAAAGTCGTTGC
Pcx 3' F (H0571)	GGGAGCTGTTAGCAAGAAGATGGG
Pcx 3' R (H0572)	CCACCCTTTAACCCCTGCCAAC

Pcx 5' F (HO573)	CCCACATTGGCCTTAATCCTGC
Pcx 5' R (HO574)	TGACAGACTGGAGTACGAGCCC
St3ga/3 3' F (HO643)	GAGTGCTGCTCACGACTTGATG
St3ga/3 3' R (HO644)	TTGAGGCTTTACTGGAGGCGAG
St3ga/3 5' F (HO645)	TTTGGTTAGGCGGATGTCCCAC
St3ga/3 5' R (HO646)	ACTCAGTTTTACCACCTCCCCG
Hprt mRNA F (HO199)	GGACCTCTCGAAGTGTTGGATAC
Hprt mRNA R (HO200)	GCTCATCTTAGGCTTTGTATTTGGC

RNA-seq.

Total RNA was extracted from differentiated primary myoblasts or skeletal muscles using the RNeasy Mini kit (Qiagen). The quality of isolated total RNA was examined using Agilent RNA 6000 Nano kit and BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA), with RNA integrity numbers ranging from ~9.50 to 9.80 (primary myotubes) or ~7.60 to 8.70 (skeletal muscle). Total RNA (5 μg) was purified using a Dynabeads mRNA DIRECT Micro kit or a RiboMinus Eukaryote System v.2 (Life Technologies, Carlsbad, CA, USA) to prepare polyA-selected mRNA or RNA-Seq Ribo(−) RNA. Purified RNA was used for library preparation using the Ion Total RNA-Seq kit v.2 (Life Technologies). High-throughput sequencing was performed on the Ion Proton system (Thermo Fisher Scientific). Experiments were performed with biological triplicate.

Bioinformatics analysis of RNA-seq data.

We used mRNA-seq data (using polyA-selected mRNA) to evaluate gene expression. Quality controls were performed as previously described (Takeuchi et al., 2018), and reads that passed the quality control were mapped to the mouse genome sequence mm10 using STAR aligner (Dobin et al., 2013). We used the GRCm38.p4 Refseq gene model in this study. For quantification of gene expression levels, we initially calculated number of reads per 1-kb exonic genomic region per 10M reads for each gene, followed by scaling to transcripts per million (TPM) values. A gene was defined as expressed when the TPM value exceeded 2 for at least one condition. We used DEseq2 to analyze differentially expressed genes (http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html.) (Love et al., 2014). Scatterplots showing average gene expression levels in *Sfpq* KO and control samples and relationships between FCs in gene expression and pre-mRNA lengths were tested and plotted using the R statistical package (http://www.r-project.org/).

Bioinformatics analysis of RNA-seq Ribo(−).

Bioinformatics analysis was performed as previously described (Takeuchi et al., 2018), with some

modifications. For visualization using the Integrated Genome Browser (http://bioviz.org/igb/), we prepared 10-kbp windows and calculated the RPK10M and relative expression values for KO vs. control myotubes for only intron reads from RNA-seq data. We determined pre-mRNA ratios between KO and control myotubes by calculating the average depths of RNA-seq reads using a window size of 10 kbp. We split mapping results (BAM files) into exon-mapped reads and others (i.e., mainly intron-mapped reads) with samtools (http://www.htslib.org/), and reads mapped to exons were excluded to detect changes in pre-mRNA levels. Relative expression values were smoothed around +4 regions.

GSEA.

Expressed genes (10,810 genes; TPM ≥ 2.0 for at least one condition) were used as input for GSEA (http://software.broadinstitute.org/gsea/index.jsp) (Subramanian et al., 2005) using GSEA 2.0 with default parameters (except for the permutation type, which was changed to "gene set") and the KEGG and Reactome gene sets from the Molecular Signatures Database curated gene sets (v.6.0; http://software.broadinstitute.org/gsea/msigdb/). The "METABOLIC_PATHWAYS" gene set from KEGG pathway mmu01100 was added to the KEGG gene sets. Gene symbols in the KEGG and Reactome gene sets were changed to mouse-specific gene symbols in mm10.

Statistics.

Values are presented as mean ± SD or SEM. Statistical significance was evaluated with a two-tailed Student's or Welch's t test to analyze differences between two experimental groups ($p < 0.05$ was considered significant). In Figure 1F, 3A and S3A, statistical significance was evaluated with Mann-Whitney U test ($p < 0.01$ was considered significant). Statistical analysis of RNA-seq was evaluated with DEseq2 to analyze differentially expressed genes (http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html.) (Love et al., 2014). Statistical analysis of GSEA data was evaluated with GSEA 2.0 (http://software.broadinstitute.org/gsea/index.jsp) (Subramanian et al., 2005) with default parameters (except for the permutation type, which was changed to "gene set").

Data and Software Availability.

RNA-seq data are registered in the NCBI SRA with accession number GSE106827. For calculating reads per kilo base per million mapped reads (RPKM) and TPM in-house scripts were used. All data and scripts not included here are available from the corresponding author upon reasonable request.

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