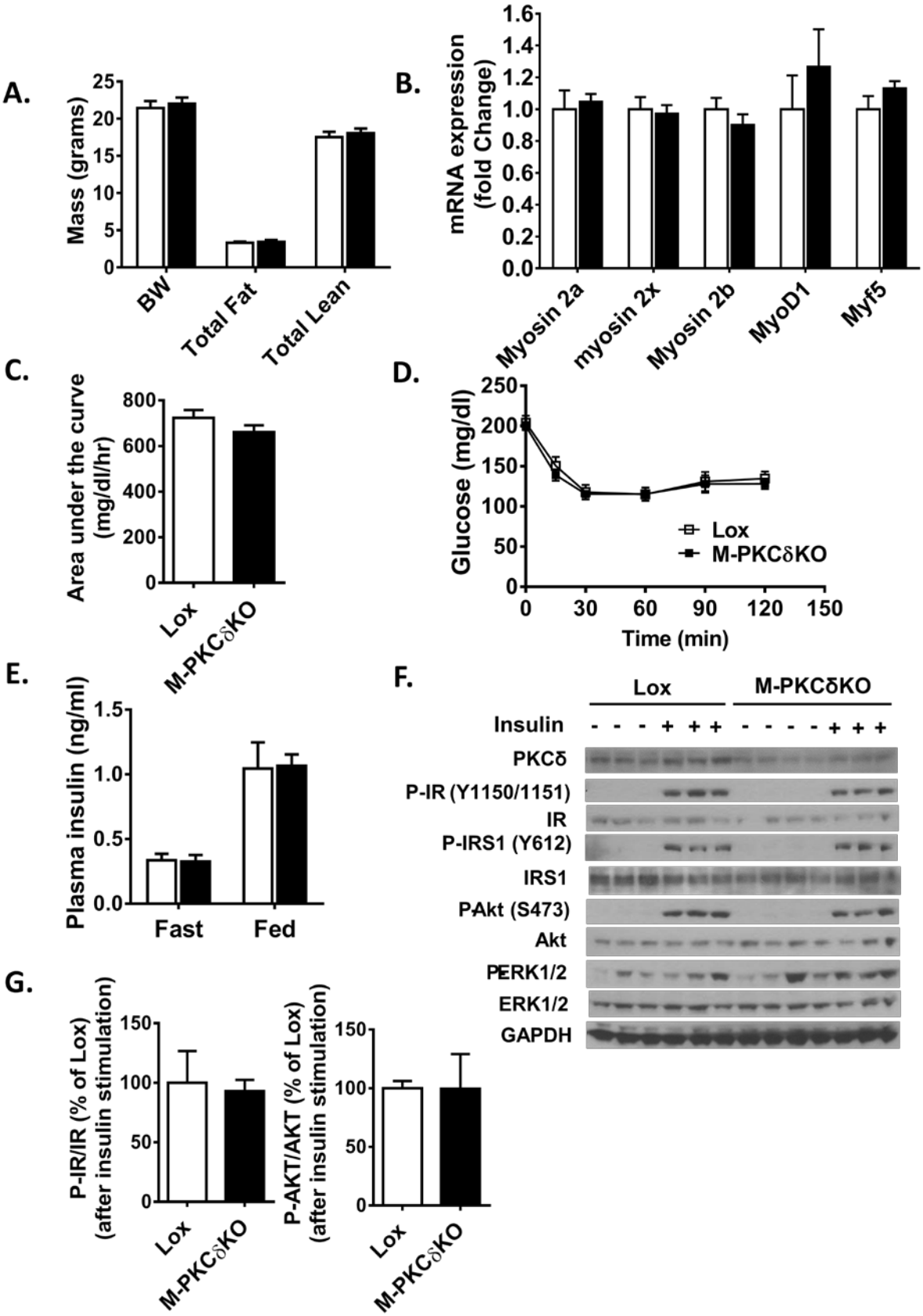
SUPPLEMENTARY DATA

Supplementary Figure S1. Insulin tolerance, gene expression of myogenesis markers and muscle fiber type markers and muscle insulin signaling in 10-week old mice. (A) Body weight, total fat and total lean mass of Lox and M-PKCδKO mice fed a chow diet were measured by DEXA analysis at 10-weeks of age. (B) Gene expression of muscle markers for muscle fiber type and myogenesis were assessed by qPCR in TA muscle of 10-week old Lox and M-PKCδKO mice. (C) Area under the curve of intraperitoneal glucose tolerance tests and (D) insulin tolerance tests were performed in Lox and M-PKCδKO mice. (E) Fasted and fed serum insulin levels in Lox and M-PKCδKO mice. (F) Western blot analysis of insulin signaling pathway in skeletal muscle of Lox and M-PKCδKO mice after intravenous insulin injection (200 U/kg of body weight). (G) Densitometric analysis of insulin receptor phosphorylation and AKT phosphorylation from panel F after insulin stimulation. White bars represent control mice; black bars represent M-PKCδKO mice. Data are mean ±SEM. n=6-8 per group in panels (A) – (E), and n = 3 per group in panels (F) and (G).

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure S2. Body composition and energy expenditure in M-PKCδKO mice on either a chow diet or high-fat diet. (A) Total lean and (B) total fat mass were assessed by DEXA in 4-month old Lox and M-PKCδKO mice fed with CD or HFD. (C) Food intake was measured by CLAMS in a 48 hr cycle after 10 weeks treatment with CD or HFD. (D) Spontaneous activity was measured in Lox and M-PKCδKO mice during 48 h cycle in metabolic cages. (E) Oxygen consumption (VO_2) and (F) respiratory exchange ratio (RER) were analyzed by metabolic cage assessment. (G) Gene expression of muscle markers for muscle fiber type and myogenesis were assessed by qPCR in TA muscle of Lox and M-PKCδKO mice fed either a CD or a HFD (n = 4 per group). n = 5 per group in panels (A) - (F). White bars represent control mice; black bars represent M-PKCδKO mice. Data are mean ±SEM.

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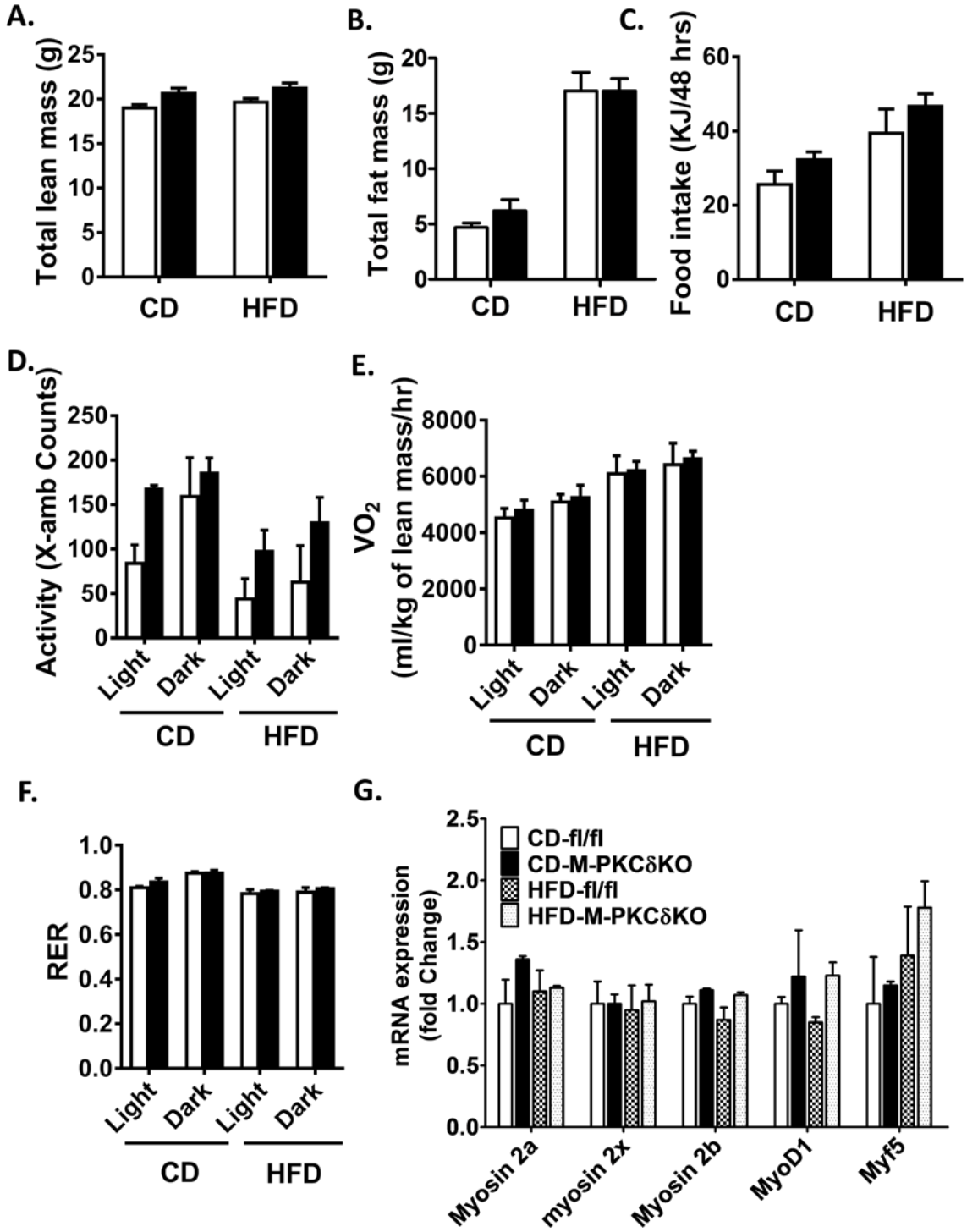
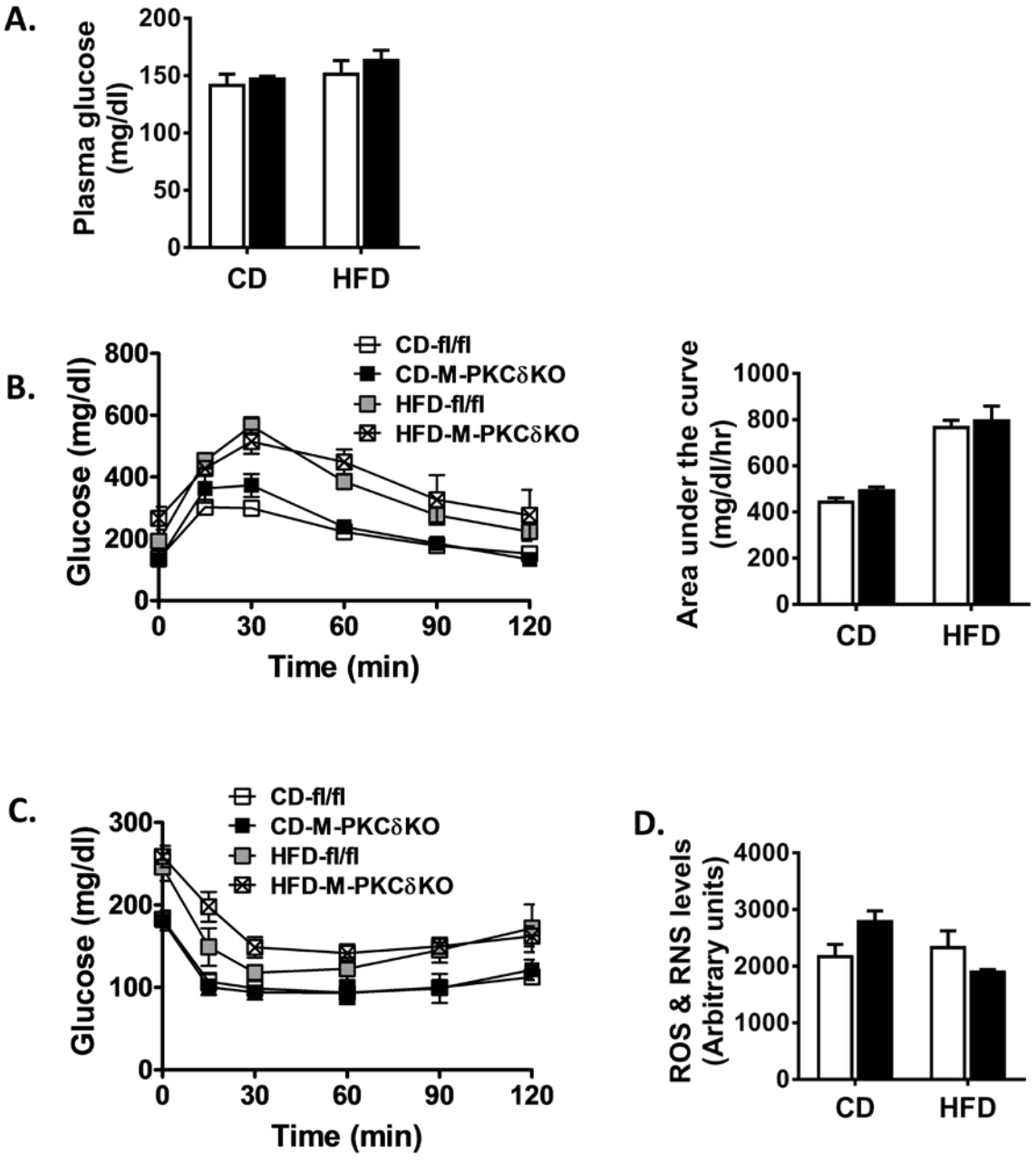


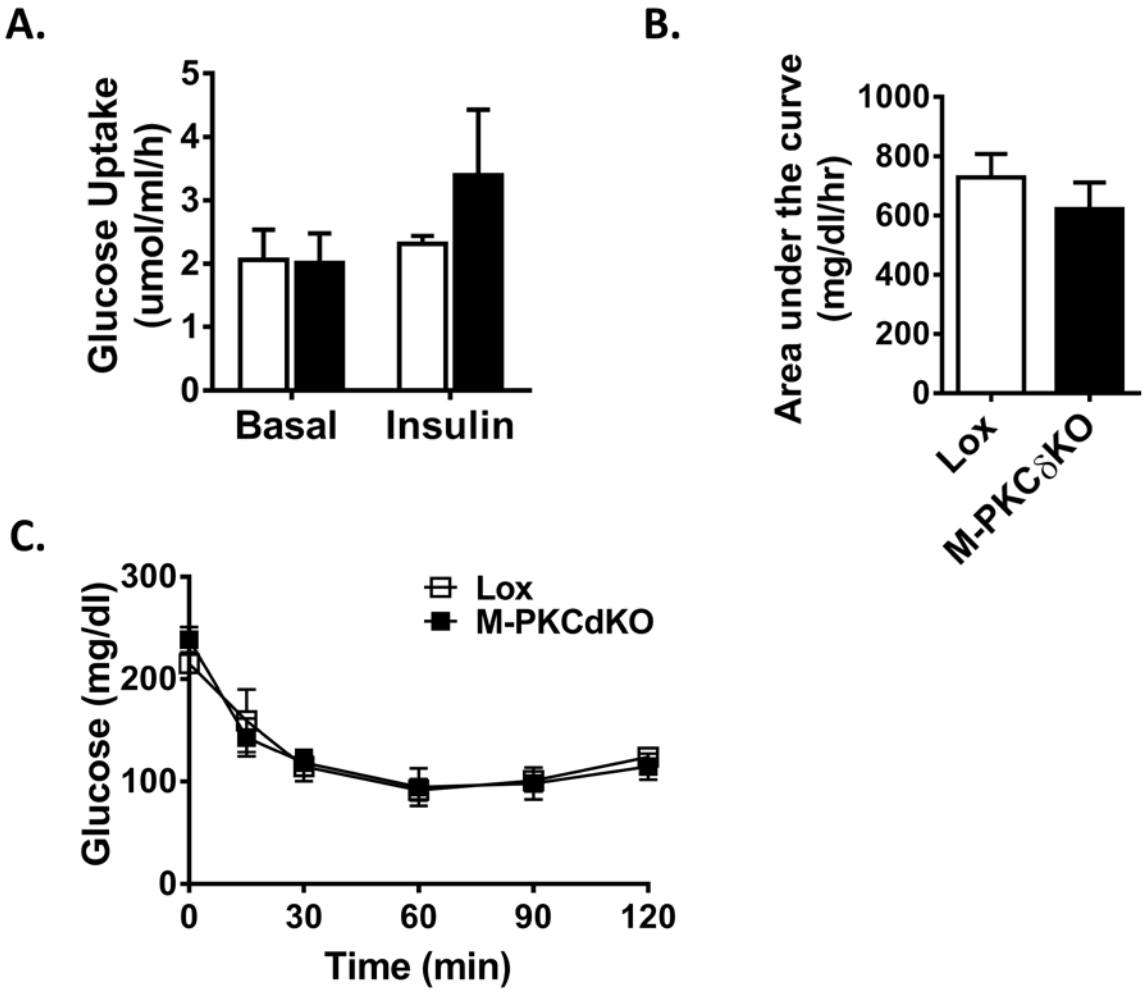
SUPPLEMENTARY DATA

Supplementary Figure S3. Muscle-specific knockout of PKCδ does not alleviate HFD-induced muscle insulin resistance. (A) Glucose levels were measured in 4-month old Lox and M-PKCδKO mice fed either a CD or HFD. (B) Intraperitoneal glucose tolerance tests and its area under the curve, and (C) insulin tolerance tests were performed in 4-month old Lox and M-PKCδKO mice fed either a CD or HFD from 6-weeks of age. (D) Reactive oxygen and reactive nitrogen species were measured in TA muscles of Lox and M-PKCδKO mice fed either a CD or HFD. White bars represent control mice; black bars represent M-PKCδKO mice. Data are mean ±SEM. n= 5 per group in panels (A) to (D).



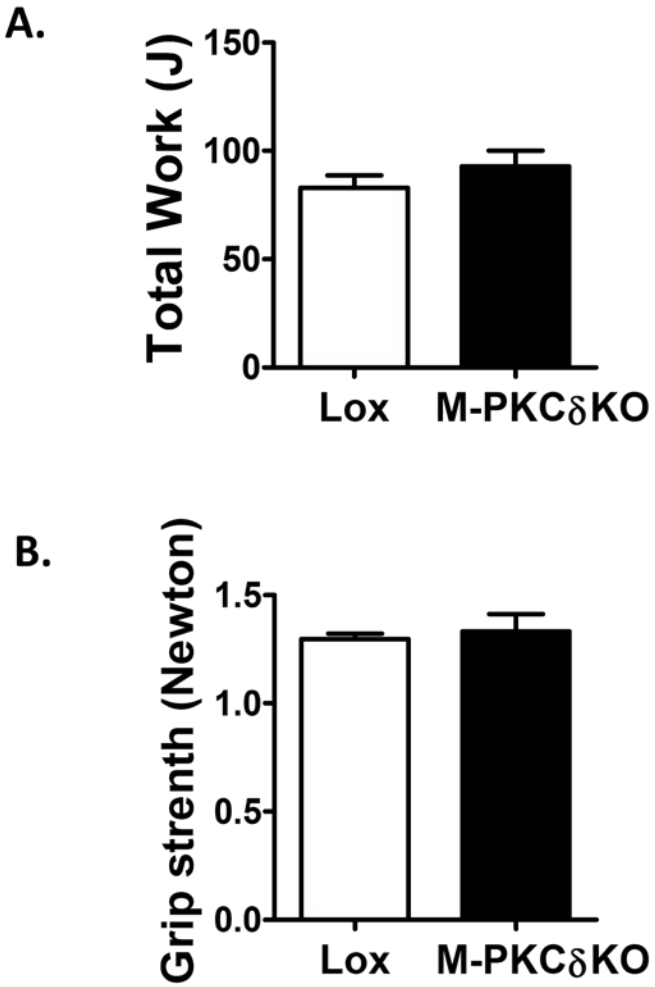
SUPPLEMENTARY DATA

Supplementary Figure S4. *Ex vivo* glucose uptake in EDL and insulin tolerance of M-PKCδKO mice at 6- to 7-months of age. (A) Rates of ^{3}H-2-deoxy-glucose uptake into muscles in the presence or absence of 2.5 mU/ml of insulin (n = 4-5). (B) Area under the curve of intraperitoneal glucose tolerance tests (GTT), and (C) insulin tolerance tests performed in Lox and M-PKCδKO mice at 7-months of age (n = 5). White bars represent Lox mice; black bars represent M-PKCδKO mice. Data are mean ±SEM.



SUPPLEMENTARY DATA

Supplementary Figure S5. Exercise capacity and muscle grip strength of mice at 7-months of age. (A) Maximal exercise capacity was measured in 7-month old Lox and M- PKCδKO mice that were exercised on a treadmill until exhaustion. (B) Forelimb grip strength was determined in Lox and M-PKCδKO mice. Data are mean ±SEM.



SUPPLEMENTARY DATA

Supplementary Figure S6. PKCδ protein are increased in muscle of 15-month old mice. (A) PKCδ protein content in TA muscle of 10-week, 4- and 15-month old Lox and M-PKCδKO mice by western blot analysis. PKCδ (B) and CD31 (C) mRNA expression in gastrocnemius (Gastro), tibialis anterior (TA) and liver endothelial cells (Endothelial) from control mice. Data are mean ±SEM. * $P < 0.05$ by two-way ANOVA.

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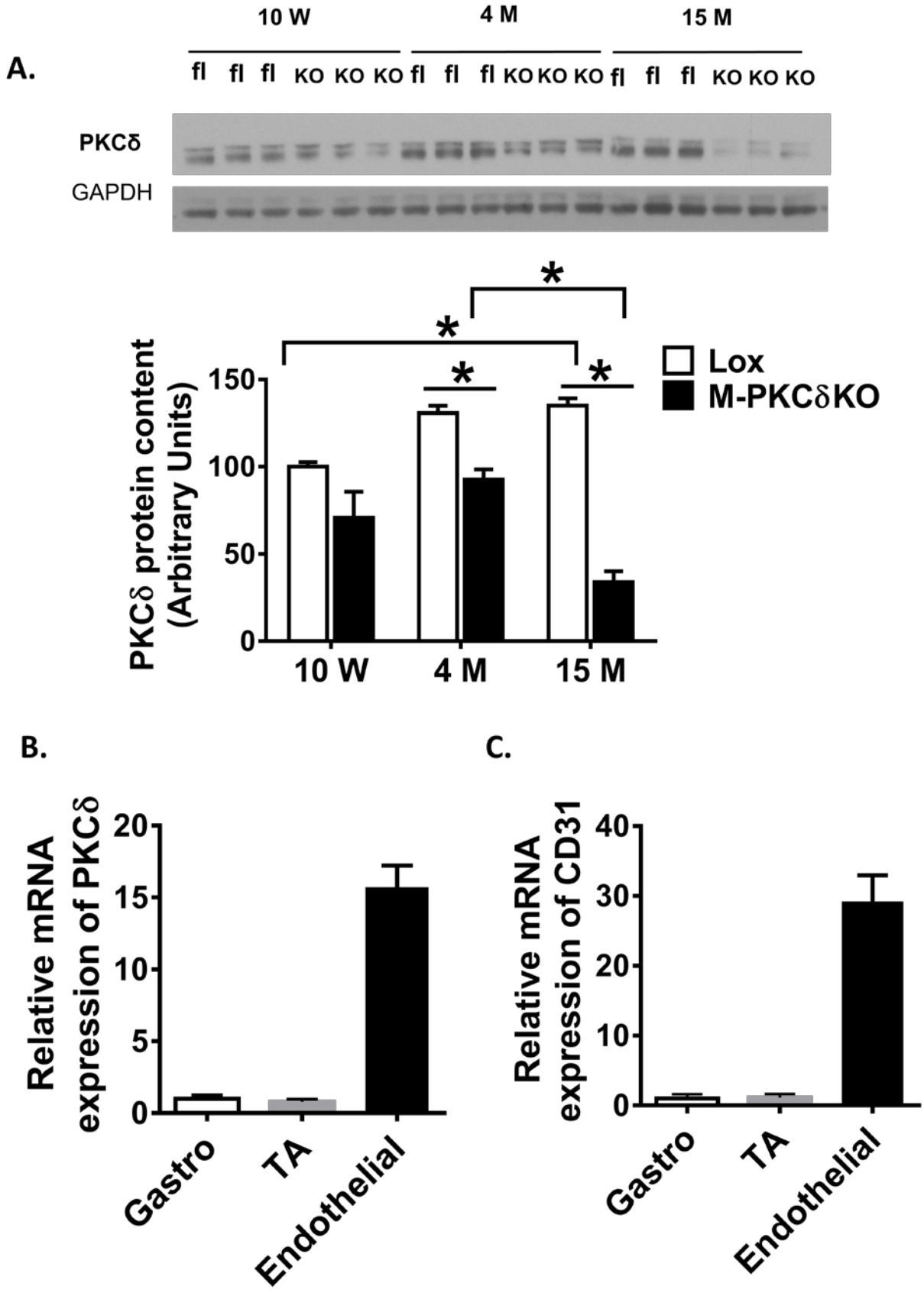

SUPPLEMENTARY DATA

Supplementary Figure S7. Plasma insulin and energy expenditure of mice at 15-months of age. (A) Fast and fed insulin levels were measured in 15-month old control and M-PKCδKO mice (n=6 per group). (B) mRNA levels of muscle fiber type markers were measured in TA muscle of 15-month old Lox and M-PKCδKO mice by qPCR (n = 5 per group). (C) Food intake in 15-month old Lox and M-PKCδKO mice (n=12 per group). (D) Spontaneous activity was measured in Lox and M-PKCδKO mice over 48 h in CLAMS metabolic cages (n=12 per group). (E) respiratory exchange ratio (RER) from the same analysis. (F) Basal and ADP-dependent (State 3) respiratory rates of isolated mitochondria from hindlimb muscle were measured using a Seahorse X24 Flux Analyzer in the presence of pyruvate/malate (n=4 per group) as described in *Materials and Methods*. (G) Basal and State 3 respiratory rates of isolated mitochondria from hindlimb muscle were measured with palmitoyl-carnitine/malate (fatty acid) as substrate (n=4 per group). (H) Respiratory control ratio (RCR) of isolated mitochondria was measured with pyruvate/malate (PM) or palmitoyl-carnitine/malate (PC) as substrate (n=4 per group). OCR, oxygen consumption rate. White bars represent control mice; black bars represent M-PKCδKO mice. Data are mean ±SEM.

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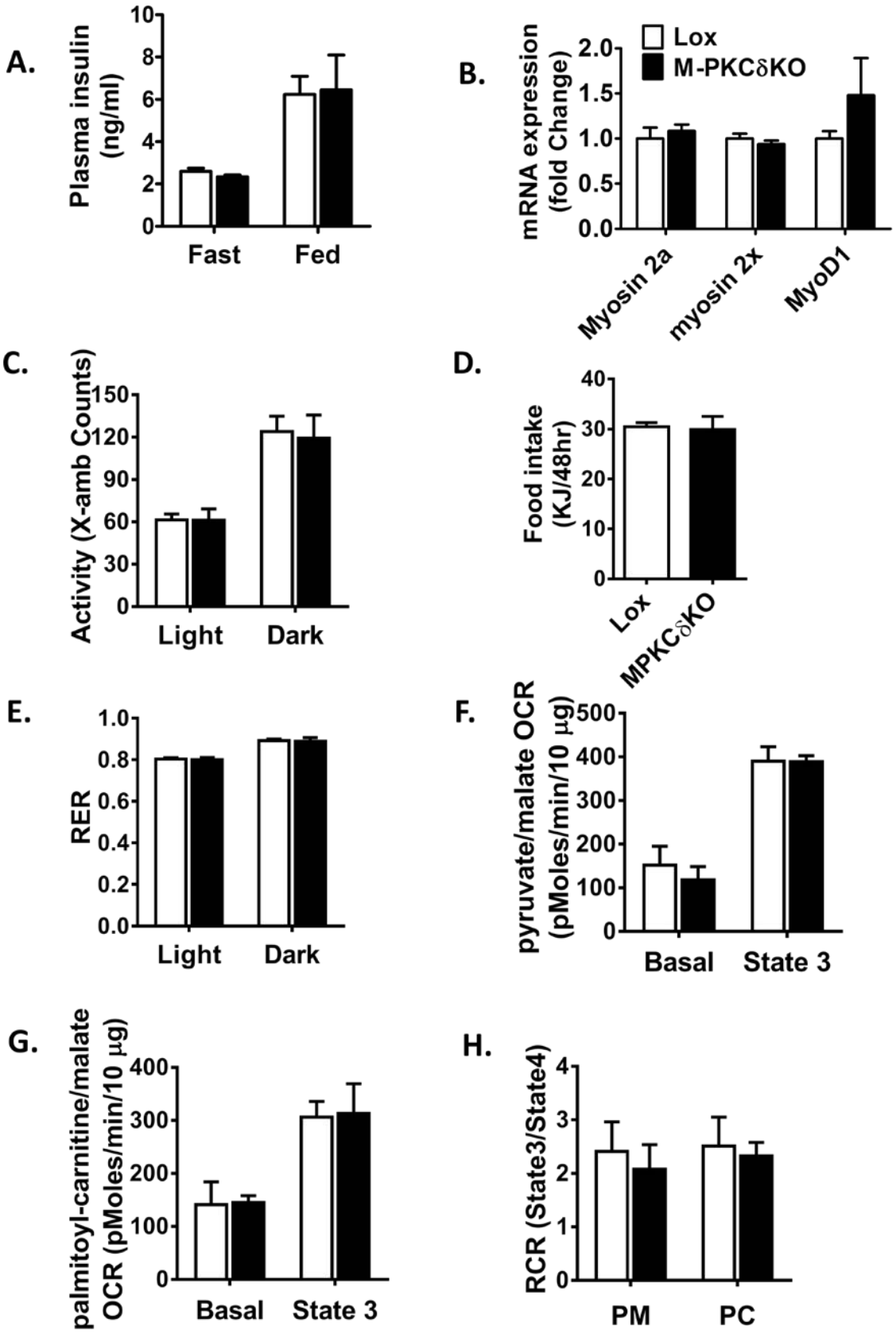

SUPPLEMENTARY DATA

Supplementary Figure S8. Impact of muscle-specific PKCδ deletion on mitochondrial protein content at 10-week, 4-months and 15-months of age. (A) Protein levels of mitochondrial marker (VDAC) and muscle oxidative phosphorylation complex subunits (mitochondrial complex I, II and IV) were assessed by western blot in TA muscles of Lox and M- PKCδKO mice at 10-weeks, 4-months and 15-months of age (n = 3 per group). (B) Densitometric quantification of western blots. White bars represent Lox mice; black bars represent M-PKCδKO mice. Data are mean ±SEM. * $P < 0.05$ by two-way ANOVA;

SUPPLEMENTARY DATA