Supplemental Materials

Lrtm1 - A Novel Sensor of Insulin Signaling and Regulator of Metabolism and Activity

Yingying Yu[1]†, Guoxiao Wang[1]†, Wenqiang Chen[1], Xiangyu Liu[1], Vitor Rosetto Munoz[1], Weikang Cai[1,2], Antonio S. Gomes[1], C. Ronald Kahn[1]*

† Co-first authors

***Corresponding author:**

C. Ronald Kahn, M.D.

Joslin Diabetes Center

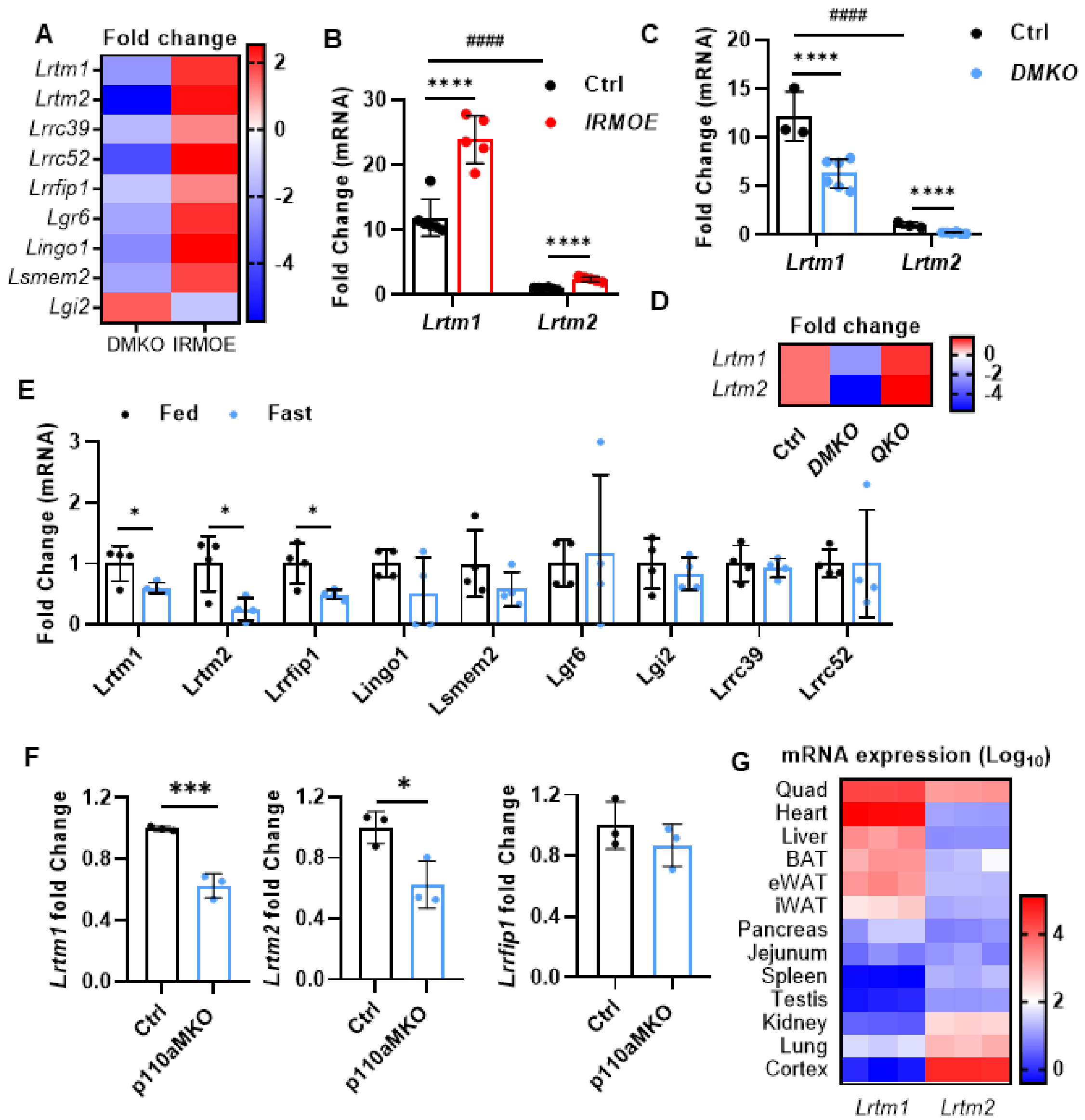
One Joslin Place, Boston, MA 02215

Phone: (617) 309-2635

Fax: (617) 309-2487

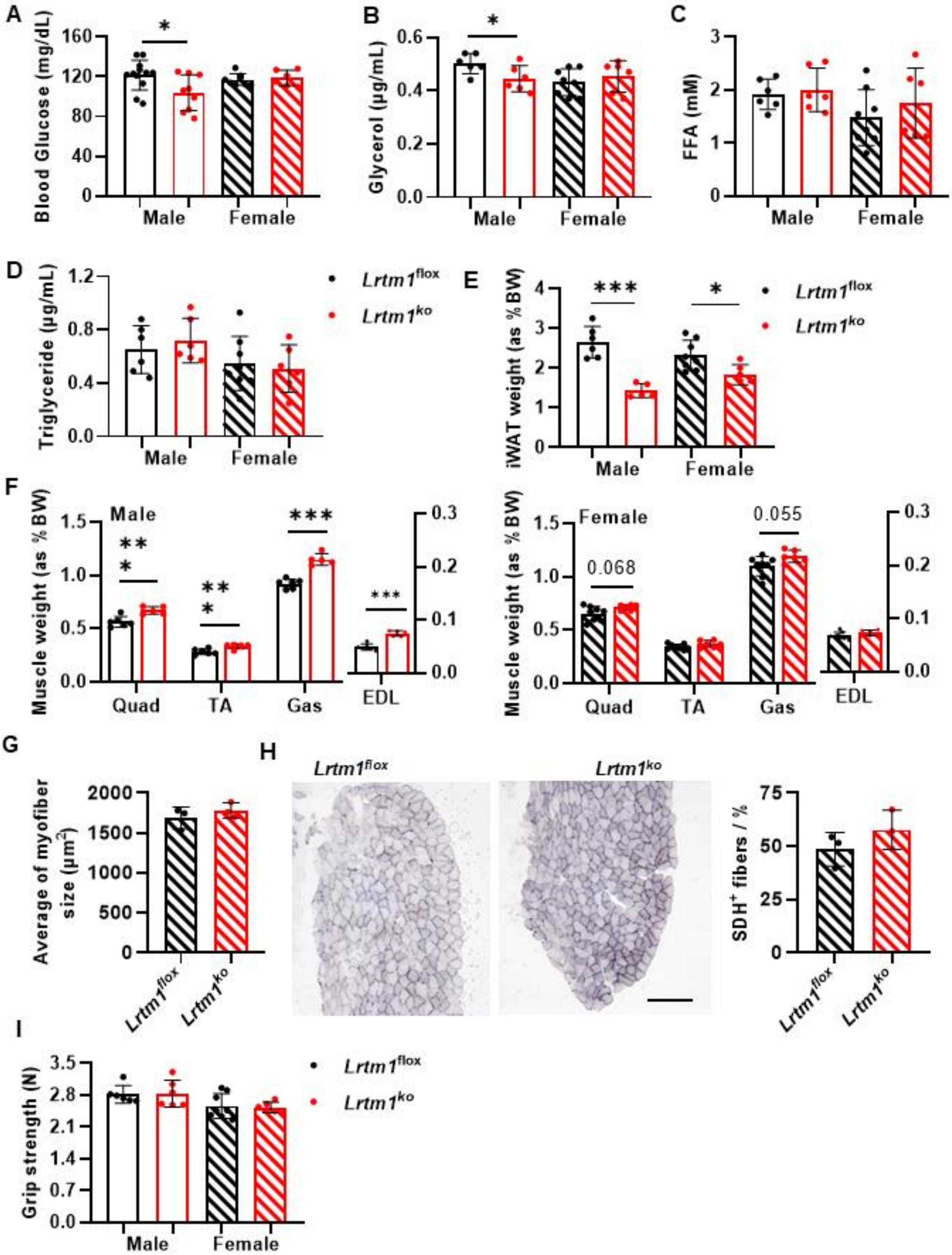
Email: C.Ronald.Kahn@joslin.harvard.edu

Fig. S1 *Lrtm1*, enriched in metabolic tissues, responses to the change of insulin signaling



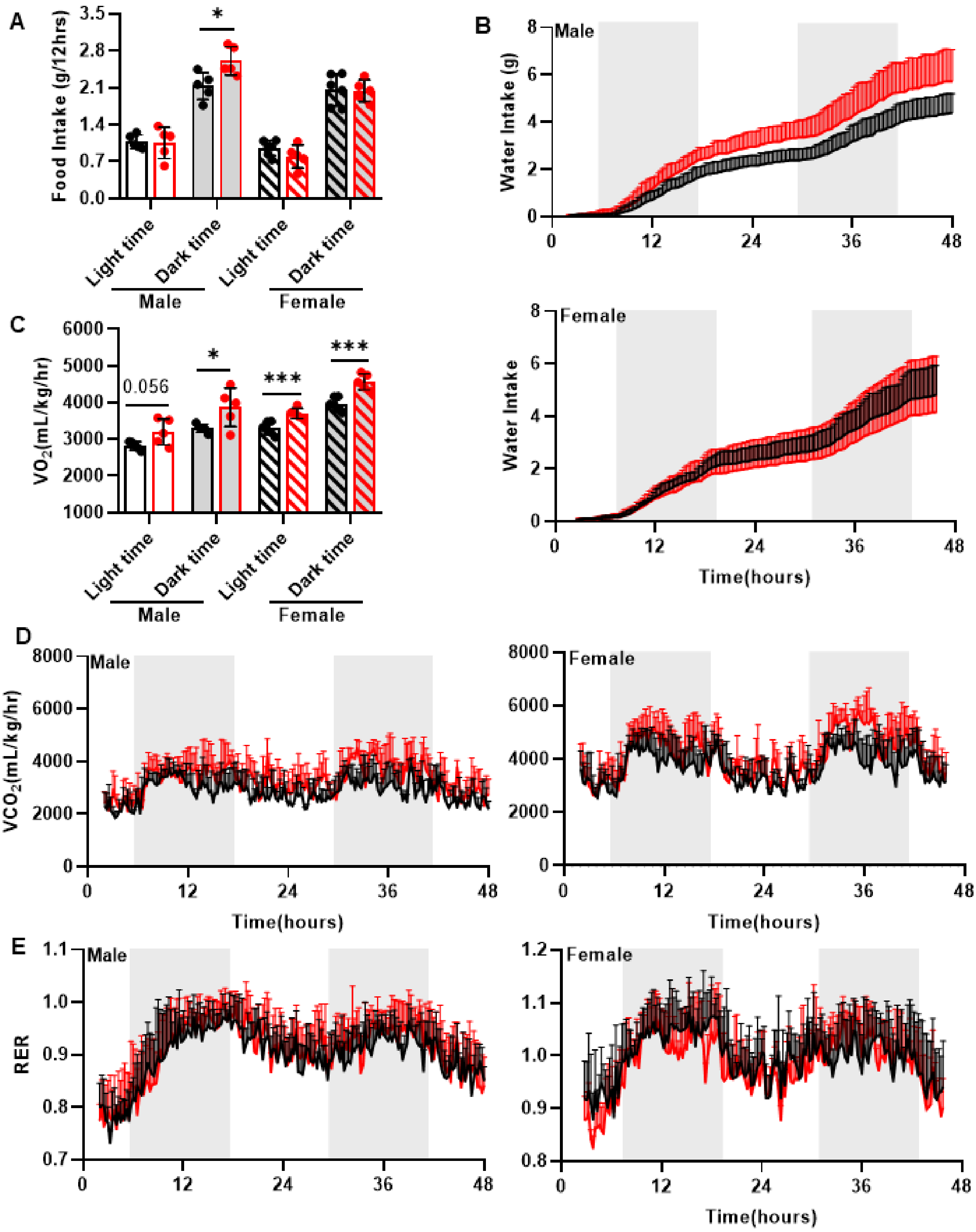
A: Heatmap illustrating the fold-change of differentially expressed genes of the leucine-rich repeat (LRR) family, derived from RNA sequencing results of skeletal muscle in InsR/IGF1R muscle-specific double knockout mice (*DMKO*) and InsR muscle-specific overexpression mice (*IRMOE*), compared to their respective control groups (Ctrl). *B:* Dot blots displaying the fold-change of *Lrtm1* and *Lrtm2* from skeletal muscle mRNA sequencing in Ctrl and IR muscle-specific overexpression (*IRMOE*) mice, normalized to the relative expression of *Lrtm2* in Ctrl mice. *C:* Dot blots showing fold-change of *Lrtm1* and *Lrtm2* from skeletal muscle sequencing in Ctrl and *DMKO* mice, normalized to the *Lrtm2*'s relative expression in Ctrl mice. *D:* Heatmap illustrating the fold-change of *Lrtm1* and *Lrtm2* based on RNA sequencing of skeletal muscle in Ctrl, InsR/IGF1R muscle-specific double knockout (DMKO), and InsR/IGF1R/Foxo1, 3, 4 quintet knockout (QKO) mice. *E:* Dot blots showing fold change of genes in LRR family from skeletal muscle mRNA sequencing in random fed and 24hr-fast mice. *F:* Dot blots showing fold change of *Lrtm1*, *Lrtm2*, and *Lrrfip1* from skeletal muscle mRNA in Ctrl and *p110*α muscle specific knockout mice (*p110αMKO*). *G:* Heatmap showing q-PCR analysis results of *Lrtm1* and *Lrtm2* mRNA expression (Log_{10}) in different tissues. All the data are from male mice and presented as mean ± SD, student *t* test, *$P < 0.05$, ***$P < 0.005$, ****$P<0.001$.

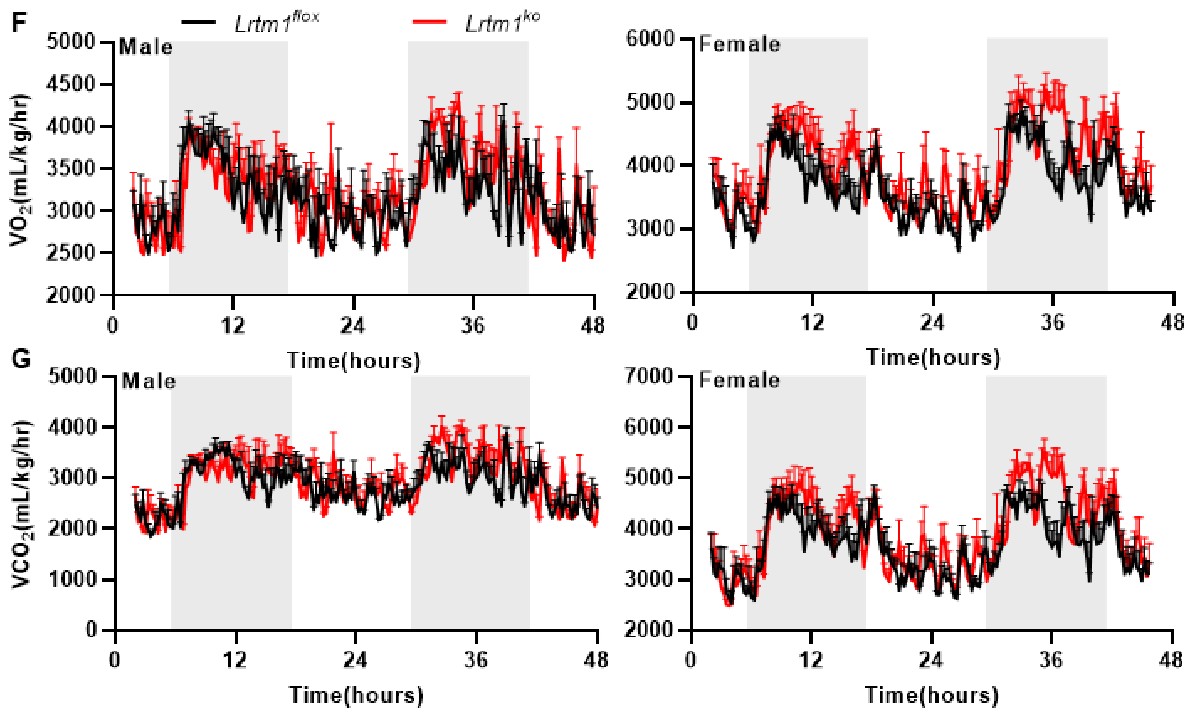
Fig. S2 Effects of *Lrmt1* whole body knockout on tissue weight and muscle fiber type



A: Overnight fasting blood glucose level of 4-month-old control and *Lrtm1*^{ko} mice, (*n* = 6-12). *B, C, D:* Plasma glycerol, free fatty acid, and triglyceride levels in overnight fasted control and *Lrtm1*^{ko} mice at around 4-month-old age, (*n* = 6-9). *E:* Inguinal adipose tissue (iWAT) weight from 6-month-old control and *Lrtm1*^{ko} mice normalized by body weight, (*n* = 5-8). *F:* Weight of different skeletal muscles from 6-month-old control and *Lrtm1*^{ko} mice normalized by body weight, (*n* = 5-8). *G:* Quantification of muscle fiber size as shown in Fig.1I, *n* > 50 fibers per mice, three to four mice per group were quantitated, (*n* = 3-4). *H:* SDH staining and quantification of the percentage of SDH highly positive myofibers in soleus muscle from 6-month-old female control and *Lrtm1*^{ko} mice, scale bar=200 μm, (*n* = 3). *I:* Grip strength in 4-month-old control and *Lrtm1*^{ko} mice, (*n* = 5-8). Data from male mice shown in open bar and data from female mice shown in striped bar. Data represent mean ± SD, student *t* test, **P* < 0.05, ***P* < 0.01, ****P* < 0.005.

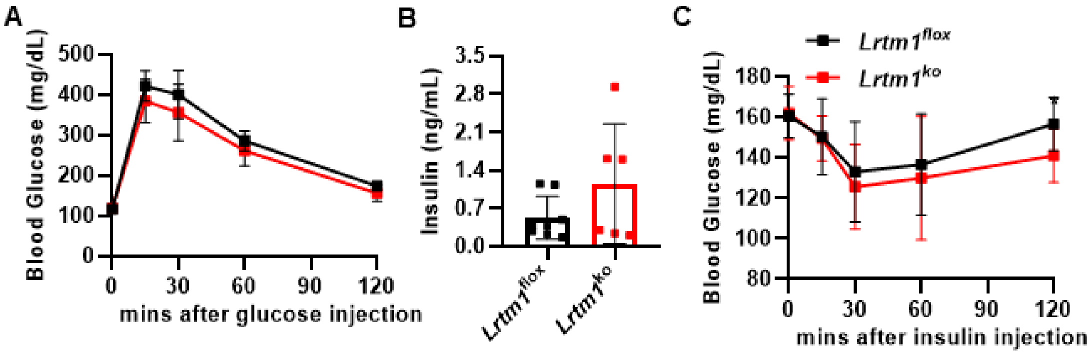
Fig. S3 *Lrtm1* whole body deficiency increases food intake and energy expenditure





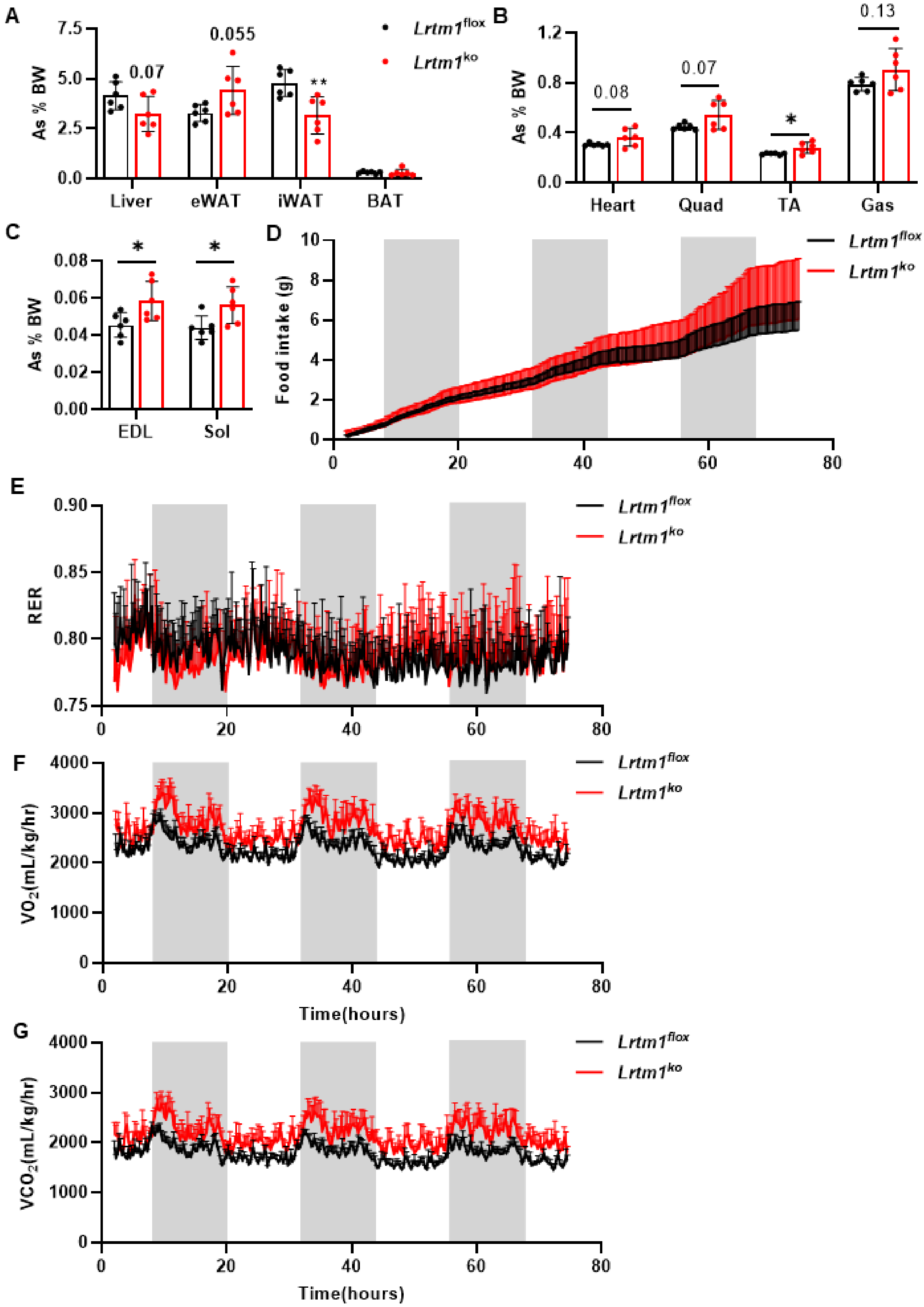
A: Average food intake, *B:* Cumulative water intake, *C:* Average O_2 consumption rate (normalized by body weight), *D:* CO_2 production rate (normalized by body weight), *E:* Respiratory exchange ratio, *F:* O_2 consumption raw data, and *G:* CO_2 production raw data in 5-month-old control and *Lrtm1*^{ko} mice fed chow diet with 12h light and dark cycle (gray background) for two days, (n = 5-6). Data from male mice shown in open bar and data from female mice shown in striped bar. Data represent mean ± SD, student *t* test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

Fig.S4 *Lrtm1* affects insulin action in female mice



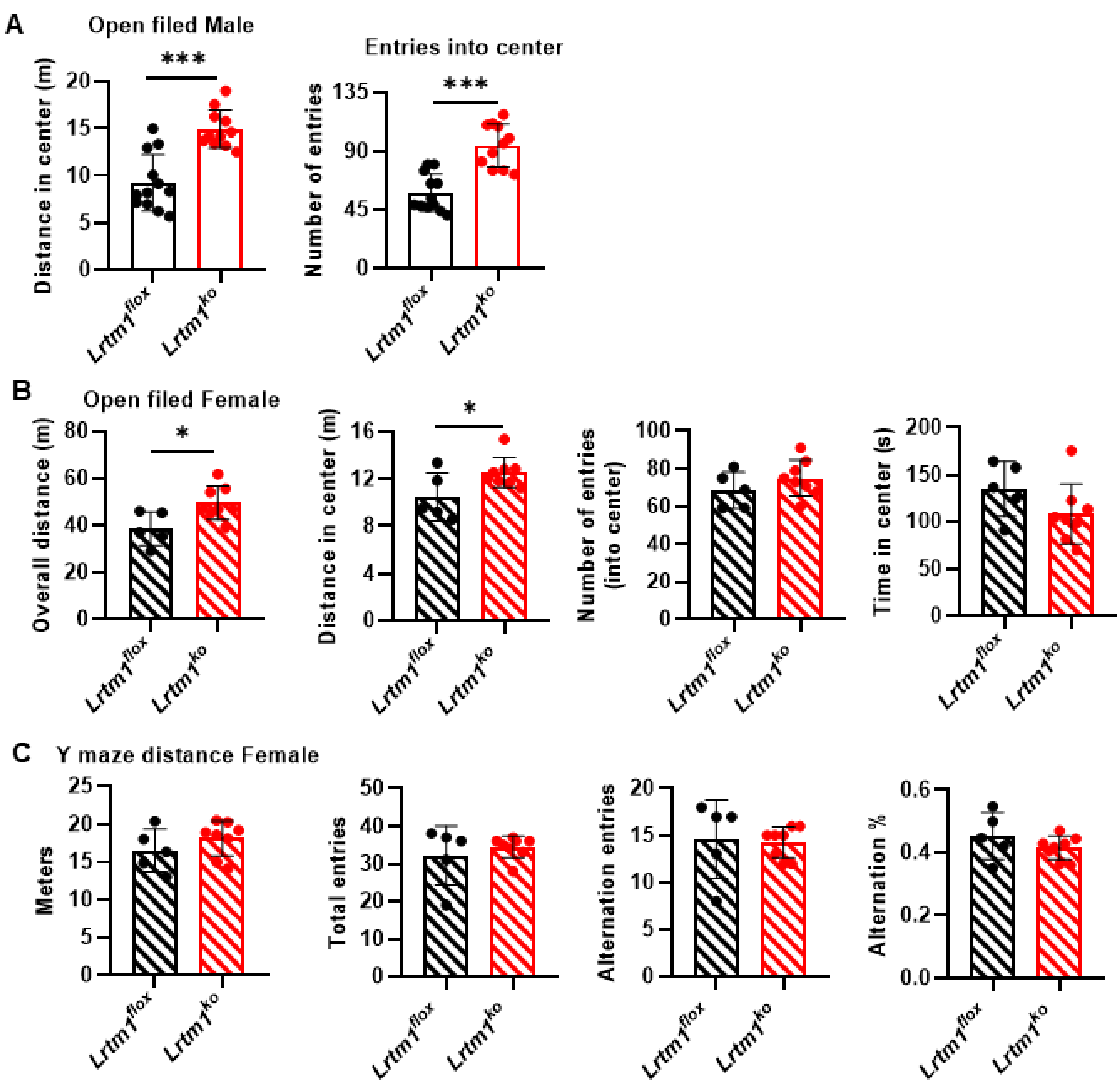
A: IP glucose tolerance tests in 4-month-old control and *Lrtm1*^{ko} female mice on chow diet, (n = 6-7). *B:* Plasma insulin levels after overnight fast in 4-month-old control and *Lrtm1*^{ko} female mice on chow diet, (n = 6-8). *C:* I.P. Insulin tolerance tests in 5-month-old control and *Lrtm1*^{ko} female mice on chow diet, (n = 6-7). Data represent mean ± SD, student t test, *$P < 0.05$.

Fig. S5 Tissue weight and metabolic phenotype in *Lrtm1* deficient mice on HFD



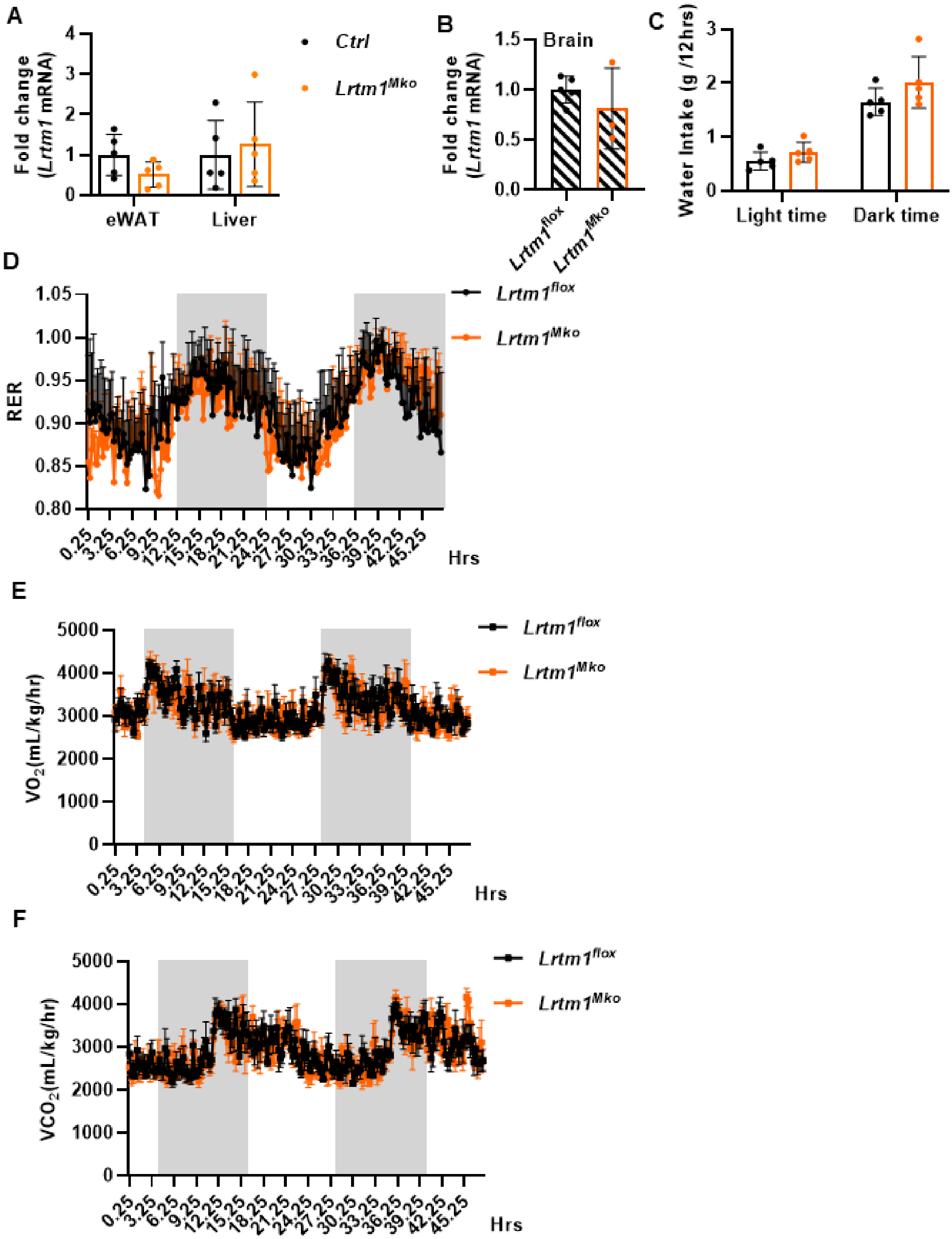
A, B, C: Weight of different tissues normalized to body weight in control and *Lrtm1*^{ko} male mice on HFD for 15 weeks, (*n* = 5-6). *D, E:* Cumulative food intake and respiratory exchange ratio, and *F*: O_2 consumption raw data, and *G:* CO_2 production raw data in control and *Lrtm1*^{ko} male mice on HFD for 13 weeks, (n = 5-6). Data represent mean ± SD, student *t* test, *$P < 0.05$, **$P < 0.01$.

Fig. S6 *Lrtm1* whole body deficiency alters behavior activity



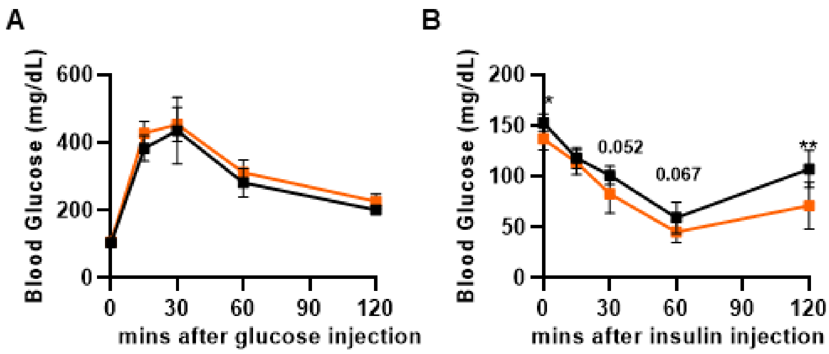
A: The overall distance and entries in center of 11-13-week-old control and $Lrtm1^{ko}$ male mice measured by open field test for 10 mins, (n = 11-12). *B:* The overall distance travelled, distance travelled while in the center, number of entries into center, and time in center of 11-13-week-old female mice monitored by open field test for 10 mins. *C:* Total distance, total entries, alternation of entries, and alternation ratio to different arms of 14-16-week-old control and $Lrtm1^{ko}$ female mice in Y maze for 5 mins. Data represent mean ± SD, student *t* test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

Fig. S7 *Lrtm1* skeletal muscle deficiency has no effect on water intake and RER in male mice



A: q-PCR analysis of *Lrtm1* in eWAT and liver from 4-month-old control and *Lrtm1*^{Mko} male mice. *B:* q-PCR analysis of Lrtm1 in Brain from 2-6 month old control and *Lrtm1*^{Mko} female mice. *C:* The water intake, *D:* respiratory exchange ratio, *E:* O_2 consumption raw data, and *F:* CO_2 production raw data in 5-month-old control and *Lrtm1*^{Mko} male mice on chow diet monitored by metabolic cages, (n = 5). Data represent mean ± SD.

Fig. S8 *Lrtm1* muscle specific knockout mildly improves insulin sensitivity in female mice



A: Glucose tolerance tests in 4-month-old control and *Lrtm1*^{Mko} female mice on chow diet, (*n* = 6-8). *B:* Insulin tolerance tests in 4.5- month-old control and *Lrtm1*^{Mko} female mice on chow diet, (*n* = 6-8). Data represent mean ± SD, student *t* test, *P < 0.05, **P < 0.01.