Figure S1

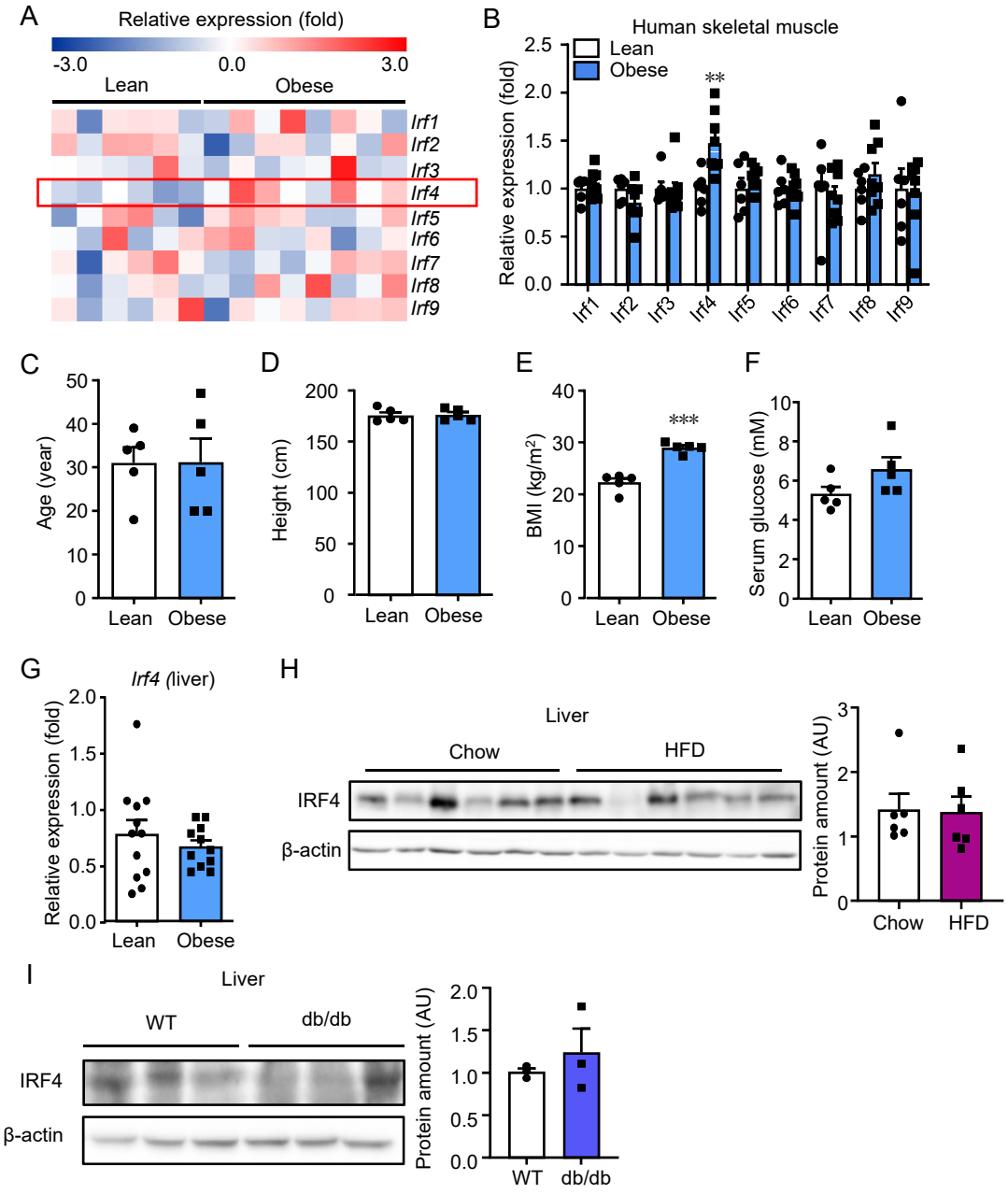
A
Relative expression (fold)
-3.0
0.0
3.0
Lean
Obese
Irf1
Irf2
Irf3
Irf4
Irf5
Irf6
Irf7
Irf8
Irf9
B
Human skeletal muscle
Lean
Obese
Relative expression (fold)
**
C
Age (year)
D
Height (cm)
E
BMI (kg/m²)
***
F
Serum glucose (mM)
G
Irf4 (liver)
Relative expression (fold)
H
Liver
Chow
HFD
IRF4
β-actin
Protein amount (AU)
I
Liver
WT
db/db
IRF4
β-actin
Protein amount (AU)

Figure S1. **Association between IRF4 and obesity. A** and **B**, The expression of IRFs in microarray experiments using human skeletal muscle tissues from obese and lean subjects. The data were extracted from GEO GSE474 (n=6-8, ***p*<0.01). **C-F**, The age, height, BMI and serum glucose of subjects with lean or obesity (n=5, ****p*< 0.001). **G**, The expression of Irf4 using human liver data from GEO GSE 126848 (n=11-12). **H**, Western blot analysis of the expression of IRF4 in Liver of mice on HFD or chow diet. Protein amount was quantified using Image J (n=6). **I**, Western blot analysis of the expression of IRF4 in Liver of db/db mice and WT mice. Protein amount was quantified using Image J (n=3). All results are expressed as means ± SEM. BMI, body mass index.

Figure S2

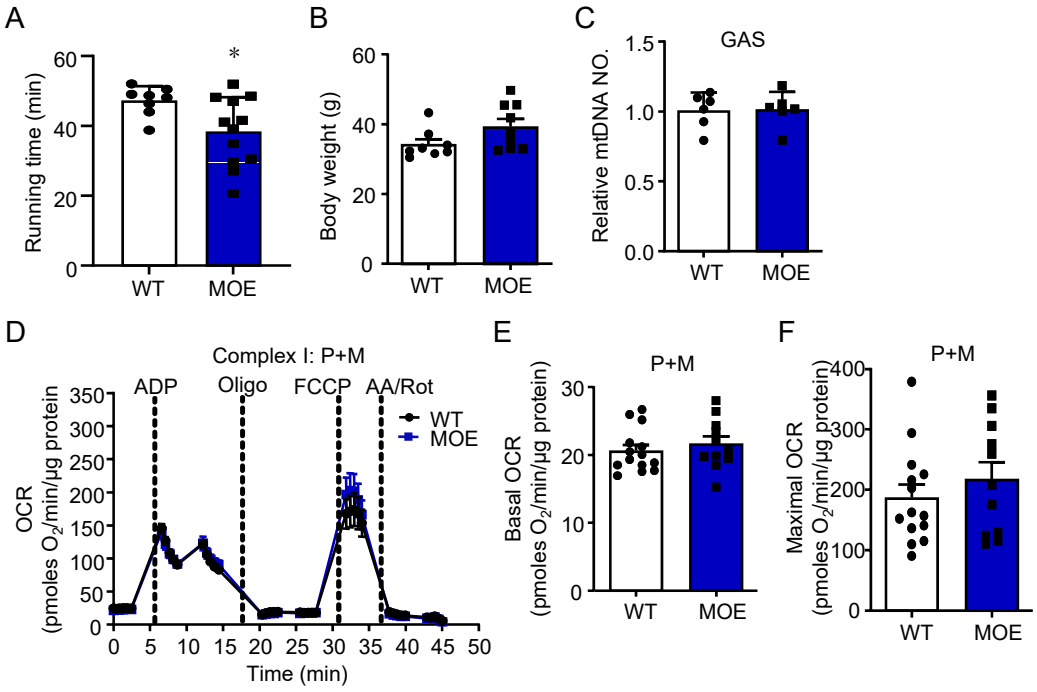
A
Running time (min)
60
40
20
0
*
WT
MOE
B
Body weight (g)
60
40
20
0
WT
MOE
C
GAS
Relative mtDNA NO.
1.5
1.0
0.5
0.0
WT
MOE
D
Complex I: P+M
ADP
Oligo
FCCP
AA/Rot
WT
MOE
OCR
(pmoles O2/min/µg protein
350
300
250
200
150
100
50
0
0 5 10 15 20 25 30 35 40 45 50
Time (min)
E
P+M
Basal OCR
(pmoles O2/min/µg protein)
30
20
10
0
WT
MOE
F
P+M
Maximal OCR
(pmoles O2/min/µg protein)
400
300
200
100
0
WT
MOE

Figure S2. **Overexpression of IRF4 in skeletal muscle promotes DIO. A**, The running time of male MOE and WT mice (n=7-12, *p<0.05). **B**, The body weight of male MOE and WT mice on 8-week HFD (n=8). **C**, The relative mitochondria DNA copy number of GAS in MOE and WT mice (n=6). **D**, Seahorse assays of OCR in the isolated mitochondria from GAS of MOE and WT mice (n=11-14). **E** and **F**, The basal and maximal OCR of mitochondria from GAS in MOE and WT mice (n=11-14). All results are expressed as means ± SEM. P, pyruvate; M, malate; GAS, gastrocnemius.

Figure S3

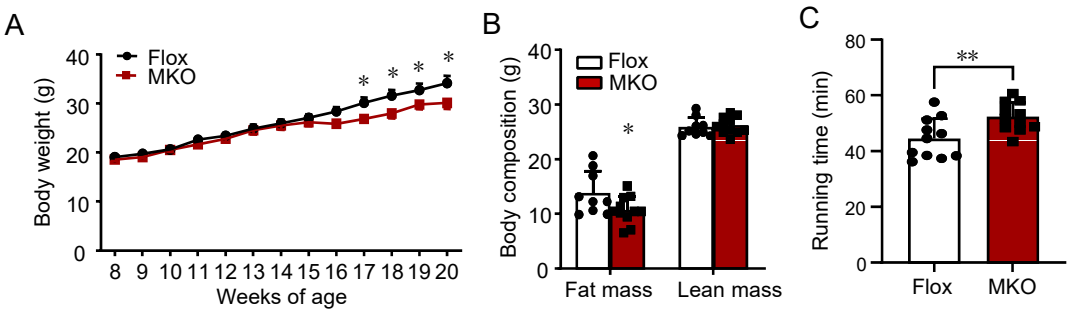
A
Flox
MKO
Body weight (g)
40
30
20
10
0
8 9 10 11 12 13 14 15 16 17 18 19 20
Weeks of age
* * * *
B
Flox
MKO
Body composition (g)
40
30
20
10
0
*
Fat mass
Lean mass
C
Running time (min)
80
60
40
20
0
**
Flox
MKO

Figure S3. **Ablation of IRF4 in skeletal muscle attenuates DIO. A**, The body weight of female MKO and Flox mice on HFD (n=8-10, *p<0.05). **B**, The body composition of female MKO and Flox mice on 12-week HFD (n=8-10, *p<0.05). **C**, The running time of male MKO and Flox mice (n=10-11, *p<0.05). All results are expressed as means ± SEM.

Figure S4

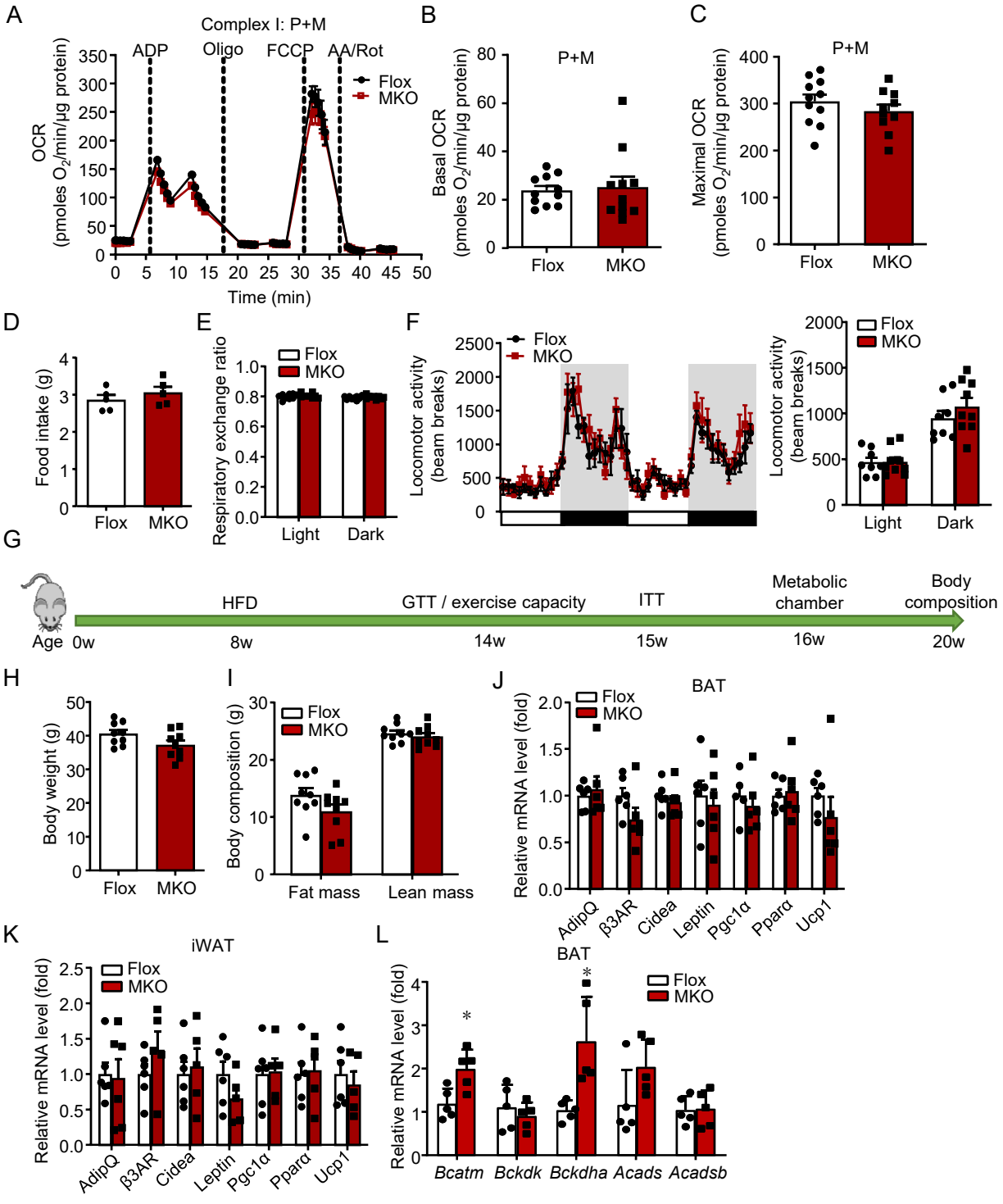
A
Complex I: P+M
ADP
Oligo
FCCP
AA/Rot
OCR (pmoles O2/min/μg protein)
Flox
MKO
Time (min)
B
P+M
Basal OCR (pmoles O2/min/μg protein)
Flox
MKO
C
P+M
Maximal OCR (pmoles O2/min/μg protein)
Flox
MKO
D
Food intake (g)
Flox
MKO
E
Respiratory exchange ratio
Flox
MKO
Light
Dark
F
Locomotor activity (beam breaks)
Flox
MKO
Locomotor activity (beam breaks)
Flox
MKO
Light
Dark
G
Age
0w
HFD
8w
GTT / exercise capacity
14w
ITT
15w
Metabolic chamber
16w
Body composition
20w
H
Body weight (g)
Flox
MKO
I
Body composition (g)
Flox
MKO
Fat mass
Lean mass
J
BAT
Relative mRNA level (fold)
Flox
MKO
AdipQ
β3AR
Cidea
Leptin
Pgc1α
Pparα
Ucp1
K
iWAT
Relative mRNA level (fold)
Flox
MKO
AdipQ
β3AR
Cidea
Leptin
Pgc1α
Pparα
Ucp1
L
BAT
Relative mRNA level (fold)
Flox
MKO
Bcatm
Bckdk
Bckdha
Acads
Acadsb

Figure S4. **IRF4 in skeletal muscle has no effects on food intake, RER or locomotor activity. A**, Seahorse assays of complex I OCR in the isolated mitochondria from GAS of MKO and Flox mice (n=11). **B** and **C**, The basal and maximal OCR of mitochondrial complex I from GAS in MKO and Flox mice (n=11). **D**, Food intake of male MKO and Flox mice on 12-week HFD (n=5). **E**, Respiratory exchange ratio of male MKO and Flox mice on 8-week HFD (n=9). **F**, The locomotor activity of male MKO mice and Flox mice on 8-week HFD (n=9). **G**, The timeline of mice experiment. **H**, The body weight of male MKO and Flox mice on 8-week HFD (n=9). **I**, The body composition of male MKO and Flox mice on 8-week HFD (n=9-10). **J** and **K**, The mRNA expression of thermogenic genes in BAT and iWAT of male MKO and Flox mice (n=6). **L**, The mRNA expression of BCAAs catabolic genes in BAT of MKO and Flox mice (n=5, $*p<0.05$). All results are expressed as means ± SEM. P, pyruvate; M, malate; GAS, gastrocnemius; HFD, high fat diet; BAT, brown adipose tissue; iWAT, inguinal white adipose tissue.

Figure S5

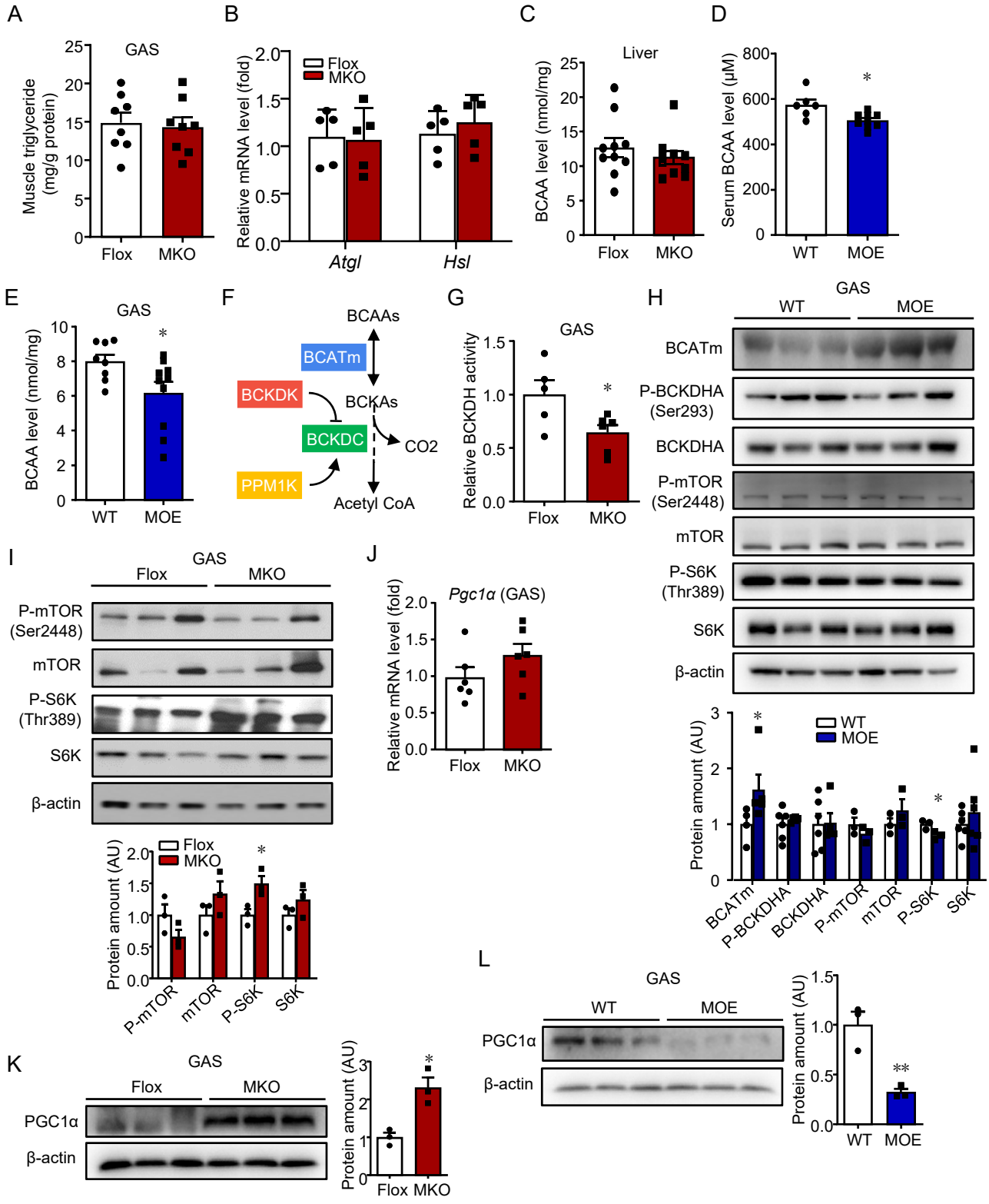
A
GAS
Muscle triglyceride (mg/g protein)
Flox
MKO
B
Flox
MKO
Relative mRNA level (fold)
Atgl
Hsl
C
Liver
BCAA level (nmol/mg)
Flox
MKO
D
Serum BCAA level (μM)
WT
MOE
E
GAS
BCAA level (nmol/mg)
WT
MOE
F
BCAAs
BCATm
BCKDK
BCKAs
BCKDC
CO2
PPM1K
Acetyl CoA
G
GAS
Relative BCKDH activity
Flox
MKO
H
GAS
WT
MOE
BCATm
P-BCKDHA (Ser293)
BCKDHA
P-mTOR (Ser2448)
mTOR
P-S6K (Thr389)
S6K
β-actin
Protein amount (AU)
BCATm
P-BCKDHA
BCKDHA
P-mTOR
mTOR
P-S6K
S6K
I
GAS
Flox
MKO
P-mTOR (Ser2448)
mTOR
P-S6K (Thr389)
S6K
β-actin
Protein amount (AU)
P-mTOR
mTOR
P-S6K
S6K
J
Pgc1α (GAS)
Relative mRNA level (fold)
Flox
MKO
K
GAS
Flox
MKO
PGC1α
β-actin
Protein amount (AU)
Flox
MKO
L
GAS
WT
MOE
PGC1α
β-actin
Protein amount (AU)
WT
MOE

Figure S5. **IRF4 regulates macronutrients metabolism and mTOR pathway in skeletal muscle. A**, GAS TG level (n=8). **B**, qPCR analysis of lipolytic genes expression (n=5). **C**, The BCAA level in liver of MKO and Flox mice (n=10). **D** and **E**, Serum and GAS BCAA level of MOE and WT mice (n=6-10, **p*<0.05). **F**, BCAAs metabolism pathway in skeletal muscle. **G**, The relative BCKDH activity in GAS of male MKO and Flox mice on HFD (n=5-6, **p*<0.05). **H**, Western blot analysis of BCAA catabolism and mTOR signaling pathway in GAS from MOE and WT mice on HFD (n=3, **p*< 0.05). **I**, Western blot analysis of mTOR signaling pathway in GAS from MKO and Flox mice on HFD. Protein amount was quantified using Image J (n=3, **p*< 0.05). **J**, The mRNA expression of Pgc1α in GAS of MKO and Flox mice (n=6). **K**, Western blot analysis of the expression of PGC1α in GAS of MKO and Flox mice. Protein amount was quantified using Image J (n = 3, **p*< 0.05). **L**, Western blot analysis of the expression of PGC1α in GAS of MOE and WT mice (n=3, ***p*< 0.01). All results are expressed as means ± SEM. GAS, gastrocnemius.

Figure S6

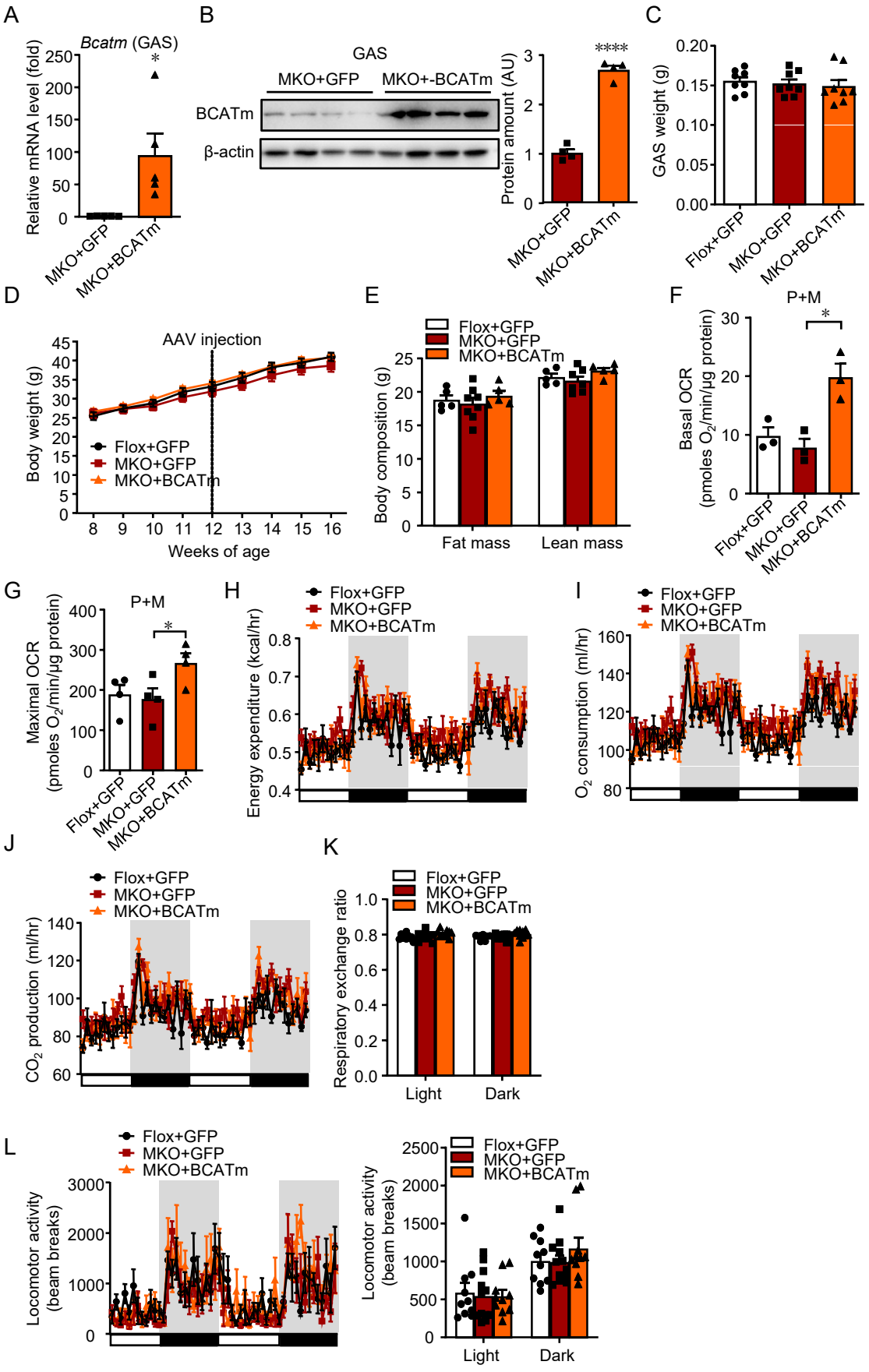
A
Bcatm (GAS)
Relative mRNA level (fold)
MKO+GFP
MKO+BCATm
B
GAS
MKO+GFP
MKO+-BCATm
BCATm
β-actin
Protein amount (AU)
****
C
GAS weight (g)
Flox+GFP
MKO+GFP
MKO+BCATm
D
AAV injection
Body weight (g)
Weeks of age
E
Body composition (g)
Fat mass
Lean mass
F
P+M
Basal OCR
(pmoles O2/min/μg protein)
G
Maximal OCR
H
Energy expenditure (kcal/hr)
I
O2 consumption (ml/hr)
J
CO2 production (ml/hr)
K
Respiratory exchange ratio
Light
Dark
L
Locomotor activity
(beam breaks)

Figure S6. **BCATm contributes to IRF4-induced metabolic phenotype. A**, qPCR analysis of *Bcatm* in GAS of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice (n=5, **p*<0.05). **B**, Western blot analysis of the expression of BCATm in GAS of MKO+AAV-BCATm mice and MKO+AAV-GFP mice. Protein amount was quantified using Image J (n=4, *****p*<0.0001). **C**, The weight of GAS in MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on HFD on 8-week HFD (n=8). **D**, The body weight of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on HFD (n=8). **E**, The body composition of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on HFD (n=5-8). **F** and **G**, The basal and maximal OCR of mitochondrial complex I from GAS in AAV injected mice (n=3). **H-J**, The energy expenditure, oxygen consumption, and carbon dioxide production of male MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on 8-week HFD (n=10-12, body weight matched). **K**, Respiratory exchange ratio of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on 8-week HFD (n=10-12). **L**, The locomotor activity of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice on 8-week HFD (n=10-12). All results are expressed as means ± SEM. GAS, gastrocnemius.

Figure S7

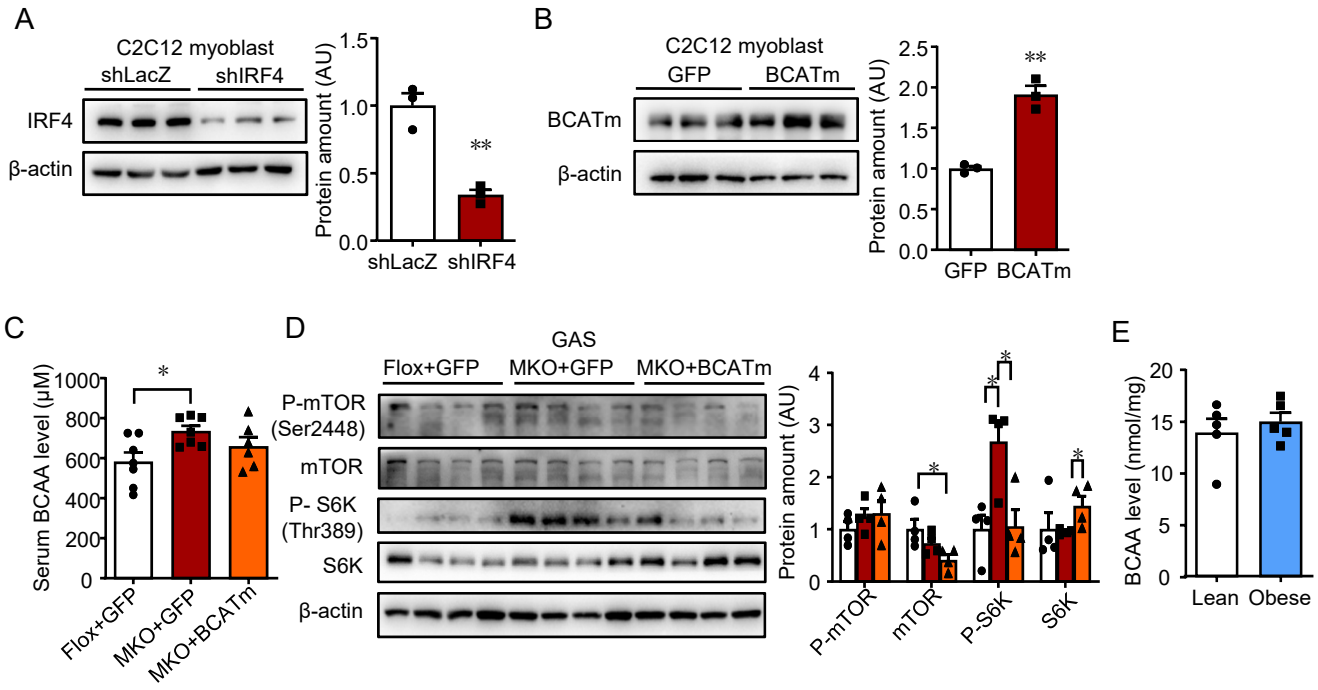
A
C2C12 myoblast
shLacZ
shIRF4
IRF4
β-actin
Protein amount (AU)
1.5
1.0
0.5
0.0
**
shLacZ
shIRF4
B
C2C12 myoblast
GFP
BCATm
BCATm
β-actin
Protein amount (AU)
2.5
2.0
1.5
1.0
0.5
0.0
**
GFP
BCATm
C
Serum BCAA level (μM)
1000
800
600
400
200
0
*
Flox+GFP
MKO+GFP
MKO+BCATm
D
GAS
Flox+GFP
MKO+GFP
MKO+BCATm
P-mTOR (Ser2448)
mTOR
P- S6K (Thr389)
S6K
β-actin
Protein amount (AU)
4
3
2
1
0
*
*
*
*
P-mTOR
mTOR
P-S6K
S6K
E
BCAA level (nmol/mg)
20
15
10
5
0
Lean
Obese

Figure S7. **BCATm accounts for changed BCAAs metabolism and mTOR pathway in IRF4-deleted skeletal muscle. A** and **B**, Western blot analysis of the expression of IRF4 and BCATm in C_2C_{12} myoblast. Protein amount was quantified using Image J (n=3, $^{**}p<0.01$). **C,** Serum BCAA level of male MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice (n=6-7, $^{*}p<0.05$). **D**, Western blot analysis of mTOR signaling pathway in GAS of MKO+AAV-BCATm mice, MKO+AAV-GFP mice, and Flox+AAV-GFP mice. Protein amount was quantified using Image J (n = 4, $^{*}p< 0.05$). **E**, The BCAA level in skeletal muscle of lean and obese subjects (n=5). All results are expressed as means ± SEM.