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Supplemental information

Ketone flux through BDH1 supports metabolic remodeling of skeletal and cardiac muscles in response to intermittent time-restricted feeding

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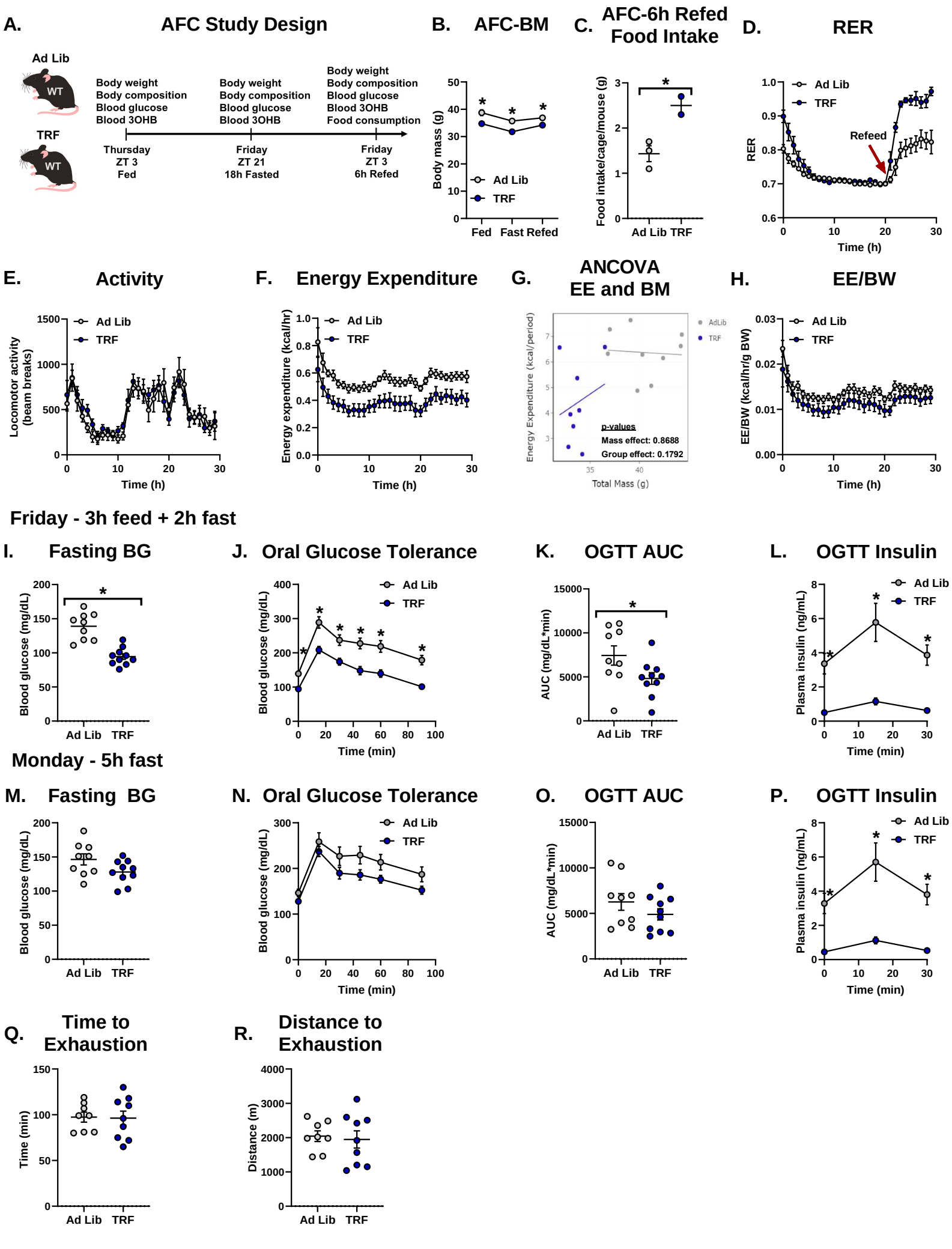
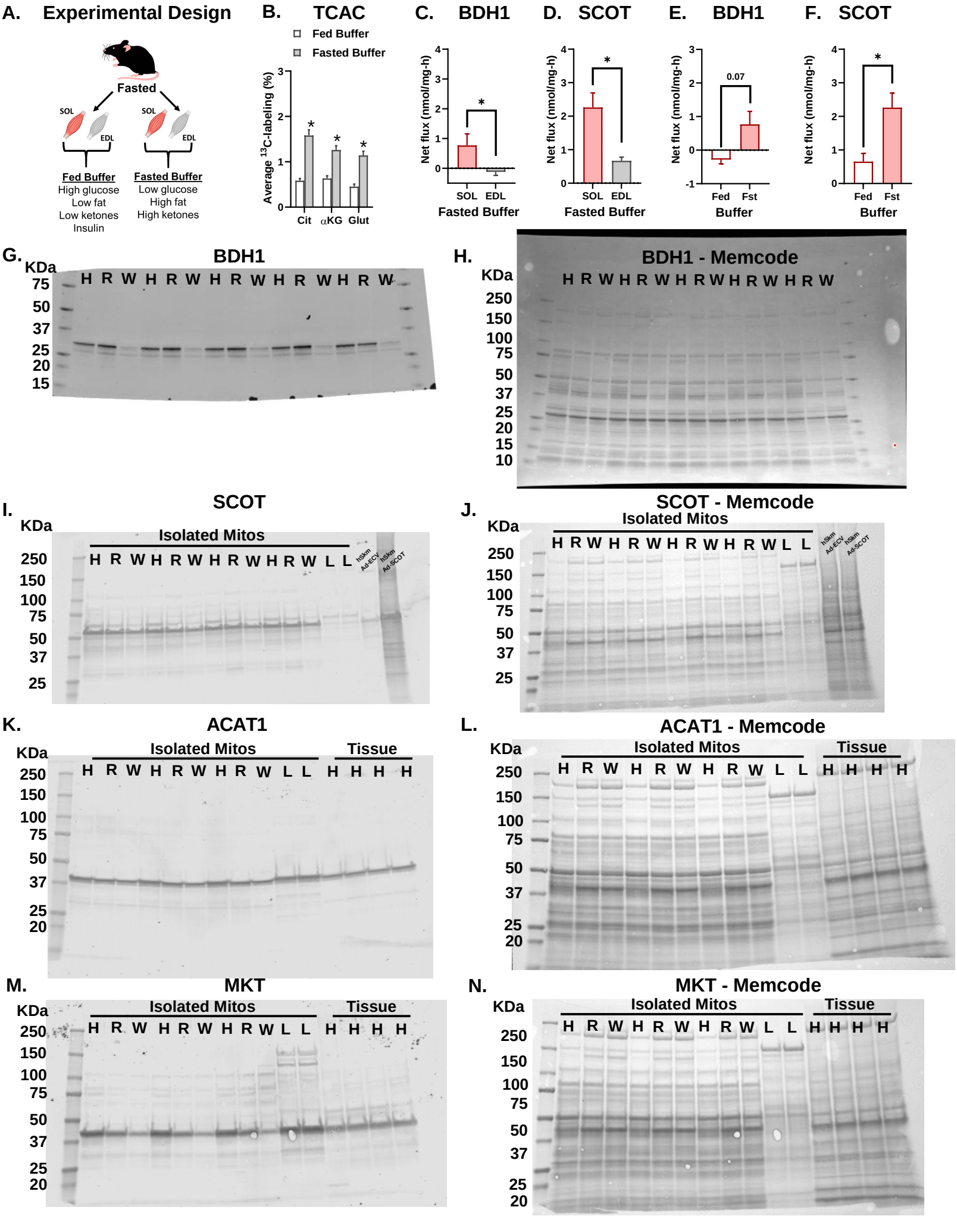
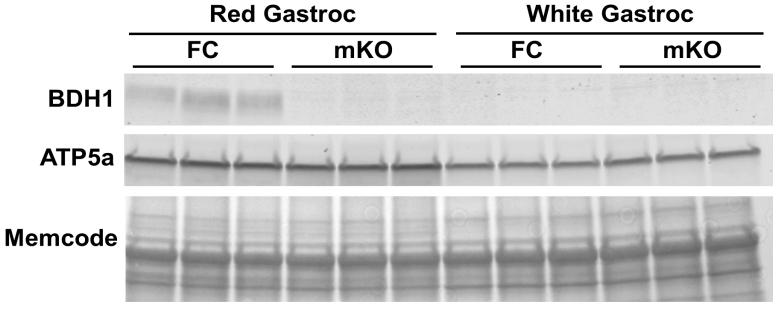

A.
AFC Study Design
Ad Lib
WT
TRF
WT
Body weight
Body composition
Blood glucose
Blood 3OHB
Thursday
ZT 3
Fed
Body weight
Body composition
Blood glucose
Blood 3OHB
Friday
ZT 21
18h Fasted
Body weight
Body composition
Blood glucose
Blood 3OHB
Food consumption
Friday
ZT 3
6h Refed
B.
AFC-BM
Body mass (g)
Ad Lib
TRF
Fed
Fast
Refed
C.
AFC-6h Refed
Food Intake
Food intake/cage/mouse (g)
Ad Lib
TRF
D.
RER
RER
Ad Lib
TRF
Refeed
Time (h)
E.
Activity
Locomotor activity (beam breaks)
Ad Lib
TRF
Time (h)
F.
Energy Expenditure
Energy expenditure (kcal/hr)
Ad Lib
TRF
Time (h)
G.
ANCOVA
EE and BM
Energy Expenditure (kcal/period)
Total Mass (g)
AdLib
TRF
p-values
Mass effect: 0.8688
Group effect: 0.1792
H.
EE/BW
EE/BW (kcal/hr/g BW)
Ad Lib
TRF
Time (h)
Friday - 3h feed + 2h fast
I.
Fasting BG
Blood glucose (mg/dL)
Ad Lib
TRF
J.
Oral Glucose Tolerance
Blood glucose (mg/dL)
Ad Lib
TRF
Time (min)
K.
OGTT AUC
AUC (mg/dL*min)
Ad Lib
TRF
L.
OGTT Insulin
Plasma insulin (ng/mL)
Ad Lib
TRF
Time (min)
Monday - 5h fast
M.
Fasting BG
Blood glucose (mg/dL)
Ad Lib
TRF
N.
Oral Glucose Tolerance
Blood glucose (mg/dL)
Ad Lib
TRF
Time (min)
O.
OGTT AUC
AUC (mg/dL*min)
Ad Lib
TRF
P.
OGTT Insulin
Plasma insulin (ng/mL)
Ad Lib
TRF
Time (min)
Q.
Time to Exhaustion
Time (min)
Ad Lib
TRF
R.
Distance to Exhaustion
Distance (m)
Ad Lib
TRF

Supplemental Figure 1. TRF physiology, Related to Figure 1. (**A**) Acute fasting challenge (AFC) study design. (**B**) Body mass during the AFC in the fed, 18h fasted (fast) and 6h refed states (refed). (**C**) Food intake during the AFC 6h refeeding period. (**D**) Respiratory exchange ratio (RER) during a 19h fasted and 6h refed challenge. The x-axis represents time (h) in the metabolic cages. (**E**) Locomotor activity. (**F**) Energy expenditure (EE) during a 19h fasted and 6h refed challenge. (**G**) Analysis of co-variance (ANCOVA) of CLAMS energy expenditure and body mass during the dark cycle. (**H**) EE/BW. (**I**) Fasting blood glucose (BG) at the start of the oral glucose tolerance test (OGTT) on Friday after a 3h pair feed + 2h fast at TRF week 10. (**J**) Friday oral glucose tolerance. (**K**) Friday OGTT area under the curve (AUC) with baseline subtracted. (**L**) Friday plasma insulin during the OGTT. (**M**) Fasting blood glucose at the start of the oral glucose tolerance test (OGTT) on Monday after a 5h fast. (**N**) Monday oral glucose tolerance. (**O**) Monday OGTT AUC with baseline subtracted. (**P**) Monday plasma insulin during the OGTT. Treadmill exercise (**Q**) time and (**R**) distance to exhaustion. (**B**-**F** and **H**-**P**) Data are represented as mean ± SEM. (**B** and **C**) N=10 per group. (**D**-**H**) N=8-9 per group. (**I**-**P**) N=9-10 per group. (**Q** and **R**) N=9 per group. (**B** and **C**, **I**-**R**) Data were analyzed by two-tailed Student's t-test. (*) represents a significant difference between Ad Lib and TRF mice. *P ≤ 0.05. (**G**) Data were analyzed by ANCOVA. N represents biological replicates.

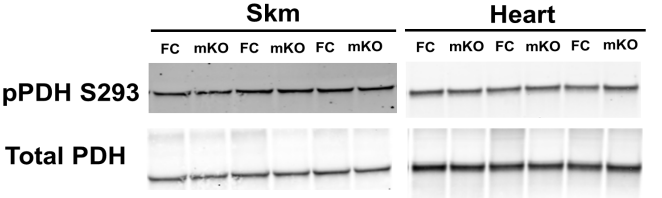

A. Experimental Design
Fasted
SOL
EDL
Fed Buffer
High glucose
Low fat
Low ketones
Insulin
Fasted Buffer
Low glucose
High fat
High ketones
B. TCAC
Fed Buffer
Fasted Buffer
Average 13C-labeling (%)
Cit
αKG
Glut
C. BDH1
D. SCOT
E. BDH1
F. SCOT
Net flux (nmol/mg-h)
SOL
EDL
Fasted Buffer
Fed
Fst
Buffer
0.07
G. BDH1
KDa
H R W H R W H R W H R W H R W
H. BDH1 - Memcode
I. SCOT
Isolated Mitos
hSkm Ad-ECV
hSkm Ad-SCOT
J. SCOT - Memcode
K. ACAT1
Tissue
L. ACAT1 - Memcode
M. MKT
N. MKT - Memcode

Supplemental Figure 2. Comparison of 3OHB oxidation in fasted muscles incubated in fed or fasted buffer, Related to Figure 2. (**A**) Experimental design. (**B**) Average ^{13}C-labeling (%) of TCAC intermediates from fasted EDL muscles incubated in fed or fasted buffer. (**C**) BDH1 forward flux (3OHB → AcAc) estimated as the uptake of unlabeled 3OHB from the buffer and (**D**) SCOT (reverse BDH1) flux (AcAc → 3OHB) estimated as the appearance of M4 3OHB in fasted SOL and EDL muscles incubated in fasted buffer. (**E**) BDH1 and (**F**) SCOT net flux in fasted SOL muscles incubated in fed or fasted buffer. Uncropped western blot images of (**G**) BDH1, (**I**) SCOT **K**) ACAT1 and (**M**) MKT and (**H**, **J, L** and **N**) corresponding memcode images (protein loading control). (**B**-**G**) N=5-6 per group. (**G**-**N**) N=3-5/group. (**B**-**F**) Data were analyzed by two-tailed Student's t-test. (*) represents significant differences between fed and fasted mice. *P≤0.05. N represents biological replicates.

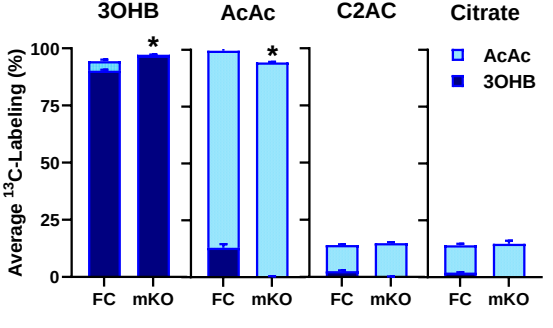
A. Tissue BDH1 Expression



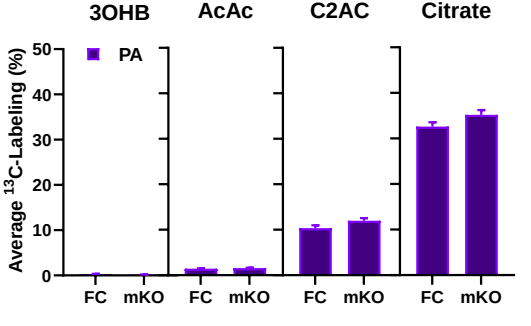
B. Tissue pPDH and PDH



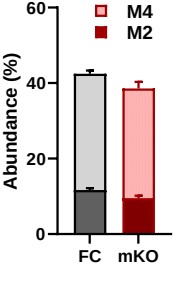
C. Labeling from [^{13}C]Ketones



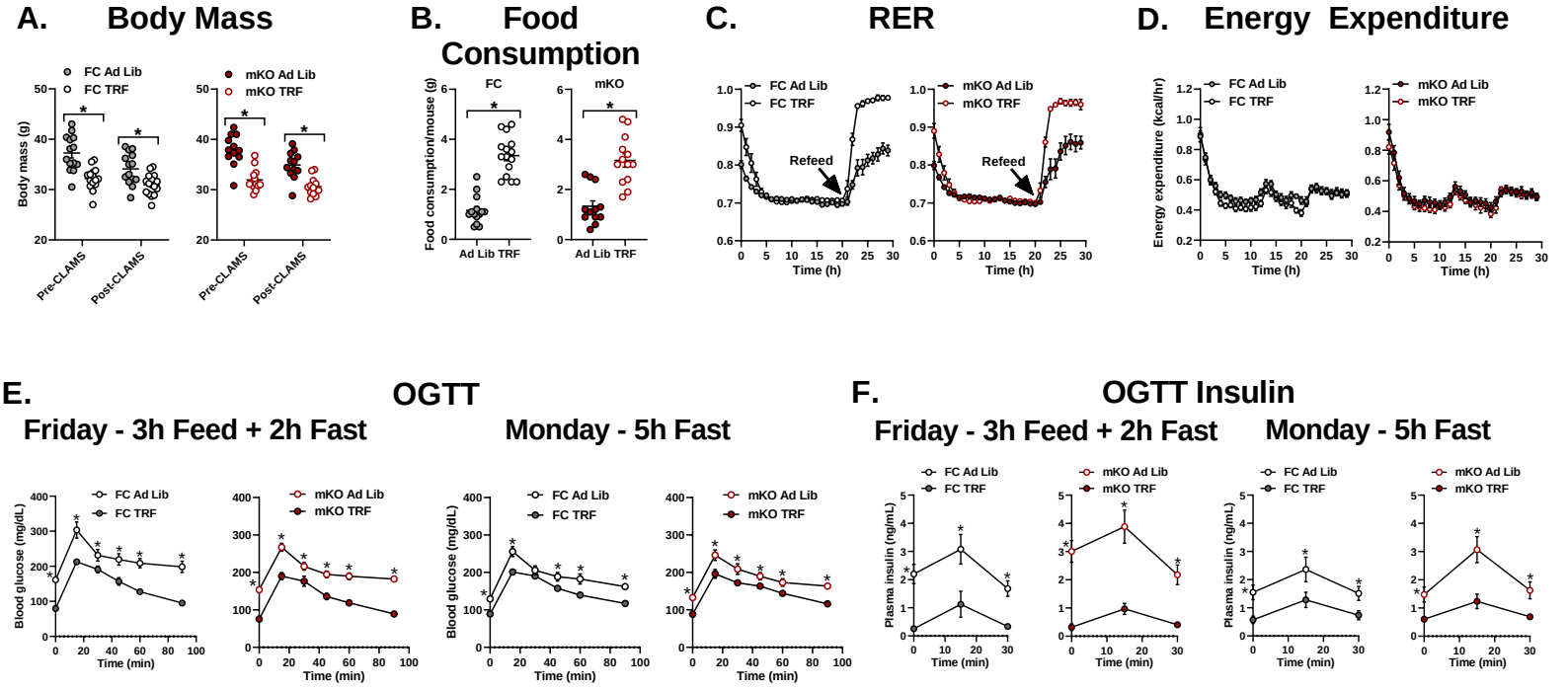
D. Labeling from [^{13}C]Palmitate



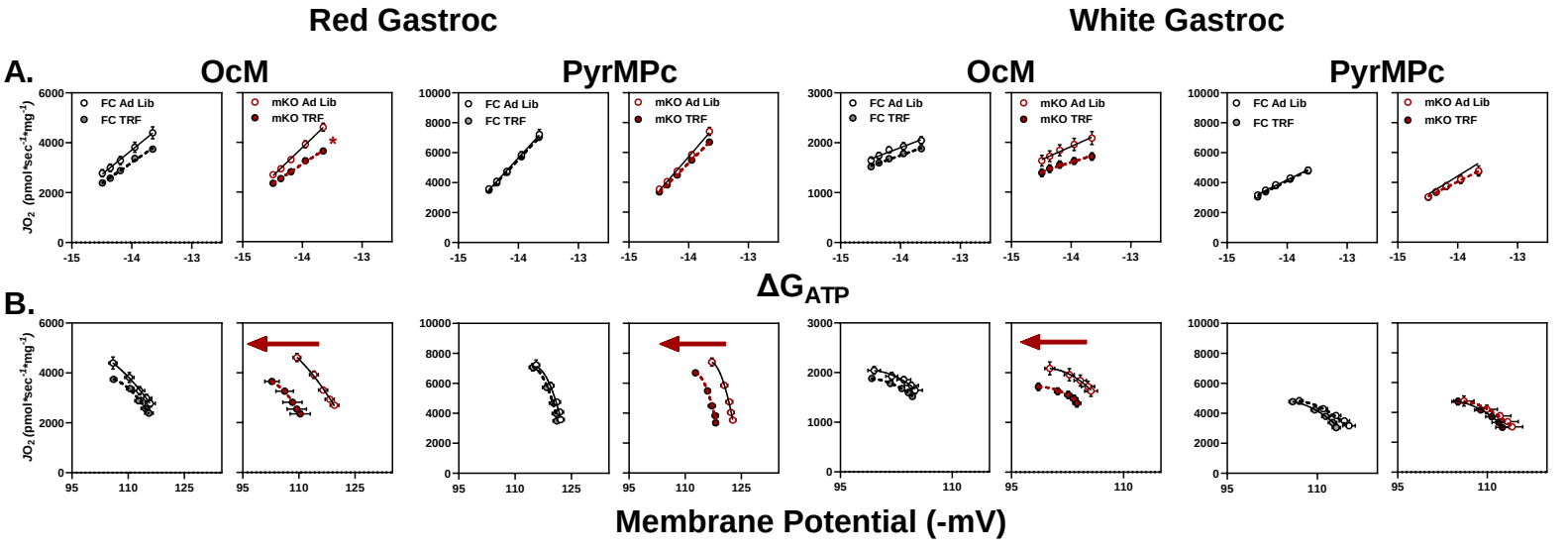
E. C4OHAC MID



Supplemental Figure 3. Ketone and palmitate ^{13}C labeling in EDL muscles from fasted FC and mKO mice, Related to Figure 3. (**A**) BDH1 expression in red and white gastrocnemius (Gastroc) from FC and mKO mice. **B**) Levels of phosphorylated (pPDH) and total PDH measured in skm and heart from 18h fasted FC and mKO mice. (**C**) Total ketone and (**D**) palmitate average ^{13}C-labeling (%) of 3OHB, AcAc, C2AC and citrate (Cit) in FC and mKO fasted EDL muscles. (**E**) EDL total ketone labeling of C4OHAC represented as MID (**A**) N=3 per group. (**B**) Representative image with N=3 per group. (**C**-**E**) Data are represented as mean ± SEM. N=6-8 per group and data were analyzed by two-tailed Student's t-test. (**C**-**E**) (*) represents significant differences between FC and mKO mice. *P≤0.05. N represents biological replicates.


A.
Body Mass
FC Ad Lib
FC TRF
mKO Ad Lib
mKO TRF
Body mass (g)
Pre-CLAMS
Post-CLAMS
B.
Food Consumption
FC
mKO
Food consumption/mouse (g)
Ad Lib TRF
C.
RER
Refeed
Time (h)
D.
Energy Expenditure
Energy expenditure (kcal/hr)
E.
OGTT
Friday - 3h Feed + 2h Fast
Monday - 5h Fast
Blood glucose (mg/dL)
Time (min)
F.
OGTT Insulin
Plasma insulin (ng/mL)

Supplemental Figure 4. BDH1 flux in skeletal and cardiac muscles does not alter iTRF-induced metabolic flexibility during an acute fasting challenge (AFC), Related to Figure 5. (**A**) Body mass before (pre-CLAMS) and after (post-CLAMS) the CLAMS run. (**B**) CLAMS food consumption per mouse in the 6h refed period. (**C**) Respiratory exchange ratio (RER) and (**D**) energy expenditure in the CLAMS during a 19h fasted and 6h refed challenge. (**E**) Oral glucose tolerance tests (OGTT) Friday after a 3h pair feed + 2h fast (left) and Monday after a 5h fast (right). (**F**) Plasma insulin during the OGTT on Friday after a 3h pair feed + 2h fast (left) or Monday after a 5h fast (right). (**A**-**D**) N=12-16 per group. (**E**-**F**) N=12-15 per group. (**A**-**F**) Data were analyzed by two-tailed Student's t-test. (*) represents significant differences between FC Ad Lib and FC TRF or mKO Ad Lib and mKO TRF mice. *P≤0.05. N represents biological replicates.


Red Gastroc
White Gastroc
A.
OcM
PyrMPc
OcM
PyrMPc
FC Ad Lib
FC TRF
mKO Ad Lib
mKO TRF
JO2 (pmol*sec-1*mg-1)
B.
ΔGATP
Membrane Potential (-mV)

Supplemental Figure 5. mBDH1 deficiency compromises respiratory efficiency in mitochondria from red gastrocnemius muscle, Related to Figure 6. (**A**-**B**) Freshly isolated mitochondria from red or white gastrocnemius (gastroc) muscles were used to assay mitochondrial energetics. (**A**) JO_2 vs. Gibb's Free Energy of ATP hydrolysis (ΔG_{ATP}), (**B**) mitochondrial respiratory efficiency represented as JO_2 plotted against $\Delta\Psi$ measured in red or white gastroc mitochondria fueled by octanoyl-carnitine + malate (OcM) or pyruvate + palmitoyl-carnitine + malate (PyrMPc). (**A**-**B**) Data are means ± SEM. (**A**-**B**) N=4-6 per group. (**B**) The red (mKO) arrows indicate the direction of the respiratory efficiency shift. *P≤0.05. N represents biological replicates.