Supplementary Table 1: Immunoblot antibodies

Protein:	**Company:**	**Catalog #:**
Lamin A/C	Cell Signaling Technology	4777
Lamin A/C (N-terminal)	Proteintech	10298-1-AP
Adiponectin	Sigma	A6354
FABP4	R&D Systems	1443
Laminin	Novus	NB300-144
Tubulin	Thermo Fisher	MA180017
HSL (total)	Cell Signaling Technology	4107
p-HSL (Ser563)	Cell Signaling Technology	4129
AKT (total)	Cell Signaling Technology	9272
p-AKT (Ser476)	Cell Signaling Technology	9271
Perilipin	Abcam	ab3526
ATGL	Cell Signaling Technology	2138
p-PKA substrates	Cell Signaling Technology	9624
Mac2	Thermo Fisher	50-112-2869
Caveolin1	Cell Signaling Technology	3238

Supplementary Methods

Human subjects and study design

Body composition was evaluated by body weight measured with an electronic Filizola platform scale with a precision of 0.1 kg. Height was measured using a fixed stadiometer to the nearest 0.1 cm. BMI was calculated as kg/m² accordingly. BMI was calculated by Weight(kg)/Height(m)^2. We also calculated the FMI (fat mass index), FFMI (free fat mass index), trunk mass index (TMI) and trunk fat mass index (TFMI) using the following equations: FMI: Fat Mass (kg)/ Height (m)^2; FFMI: FFM (kg)/ Height (m)^2, where FFM = fat-free mass (lean mass (kg) + bone mass (kg)); TMI: TTM (kg)/ Height (m)^2, where TTM = total trunk mass; and TFMI: TFM (kg)/ Height (m)^2, where TFM = trunk fat mass. Using the MatchControls function of R's e1071 package, we performed a 1:1 nearest neighbor case-control match. The matching parameters were age, sex, and BMI, with the goal of avoiding impacts of different body weights on bone mass between groups. Matching outcomes were evaluated by visualizing histograms and comparing parameters between the two cohorts.

Immunoblot analysis

Immunoblots were performed as previously described (1). In brief, 5-20 μg of cell or tissue protein extract was separated by SDS-PAGE, transferred onto PVDF membranes, and immunoblotted with primary antibodies listed in Supplementary Table 1.

Quantification of adipocyte size

Histomorphometrical analyses of adipocyte size and number was performed on hematoxylin and eosin-stained tissue sections from psWAT and gWAT depots of *Lmna*^{FL} and *Lmna*^{ADKO} mice.

Individual adipocyte areas were quantified using MetaMorph Microscopy Automation & Image Analysis Software 2.0 (Molecular Devices, Sunnyvale, CA) as previously described (2). Frequency distribution curves were generated using a bin range of 0 to 4000 μm^2 and bin width of 500 μm^2.

Lipolysis assays

Fully differentiated primary adipocytes were equilibrated in HBSS buffer + 2% fatty acid free BSA for 1 hour, then treated with either vehicle (DMSO), forskolin (5 μM), isoproterenol (1 μM) or norepinephrine (1 μM) in HBSS buffer + 2% fatty acid free BSA. After a 2 hour treatment, glycerol concentrations in the conditioned media were measured using the free glycerol kit from Sigma Aldrich (St Louis, MO). For immunoblot analysis, cell lysates were collected following 15 minutes of treatment. For *in vivo* lipolysis assays, mice were injected with 10 mg/kg isoproterenol, blood was collected at the indicated timepoints, and circulating glycerol concentrations in serum were determined using the free glycerol kit from Sigma Aldrich (St Louis, MO).

Histology

Tissues were fixed in 10% neutral-buffered formalin for 24 hours and processed for paraffin embedding. Bones from *Lmna*^{FL} and *Lmna*^{ADKO} mice were washed 3x with water following overnight fixation. Decalcification prior to processing was at 4ºC with gentle agitation for 2 weeks in 14% EDTA, changing the solution every 2 days. Tissue sections (5 μm) were stained with hematoxylin and eosin as previously described (2).

Adipocyte and stromal vascular cell fractionation

Using a protocol modified from Rodbell (3), psWAT and gWAT were isolated and combined from *Lmna*^{FL} and *Lmna*^{ADKO} mice, minced with scissors, and digested with 1 mg/ml collagenase (type I; Worthington Biochemical, Lakewood, NJ) in Krebs-Ringer-HEPES buffer +3% fatty acid-free BSA (Gold Biotechnology, St. Louis, NJ). After 1 h digestion at 37°C, the cell suspension was filtered through 100 μm cell strainers. Adipocytes and stromal vascular fraction were separated by differential centrifugation (100 x g for 8 min) and washed with Krebs-Ringer-HEPES buffer containing 3% BSA. For primary culture, stromal vascular cells were isolated from the psWAT, and cultured following the adipogenesis protocol described above.

Insulin-mediated glucose uptake

Cultured adipocytes were incubated in D-PBS + 0.7% fatty acid free BSA for 4 hours, then treated with insulin for 30 minutes (0, 2, 10, 20, and 100 nM in D-PBS + 0.7% fatty acid free BSA). The cells were then stimulated with 0.1 μCi/ml [^{14}C]-2-deoxy-glucose. After 5 minutes, the reaction was stopped by addition of 200 mM unlabeled 2-deoxyglucose. The cells were then washed twice with PBS and lysed with 0.1% SDS. Cell lysates were cleared by centrifugation and radioactivity in supernatant was measured using a scintillation counter. Labeled glucose uptake was normalized to total protein content per well.

Glucose and insulin tolerance tests and serum analysis

For glucose tolerance tests, animals were fasted for 16 hours, then administered 1 mg/kg glucose intraperitoneally. For insulin tolerance tests, animals were fasted for 4 hours, then administered 0.75 U/kg insulin intraperitoneally. Following administration of glucose or insulin, blood glucose concentrations were monitored at the indicated timepoints using Contour® next blood glucose

strips (Bayer AG, Leverkusen, Germany). Serum insulin and leptin concentrations were measured by ELISA (Crystal Chem, Elk Grove Village, IL for insulin; PreproTech, Rocky Hill, NJ for leptin). Circulating triacylglycerol concentrations were measured using a colorimetric assay from Cayman Chemical (Ann Arbor, MI), and circulating glycerol levels were determined using the free glycerol detection kit from Sigma Aldrich (St. Louis, MO).

Quantitative RT-PCR

Quantitative RT-PCR measurements were performed as described previously (4,5). Briefly, total RNA was isolated and purified from cultured cells using RNA-STAT60 (Tel-Test, Alvin, TX, USA) according to the manufacturer's instructions. M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) was used to reverse-transcribe 1 ug RNA to cDNA. qRT-PCR was performed using aPCRBIO SyGreen Mix (Innovative Solutions, Beverly Hills, MI, USA) on a StepOnePlus System (Applied Biosystems, Foster City, CA, USA). All primers were validated with cDNA titration curves prior to use; qPCR product specificities were confirmed by melting curve analysis and gel electrophoresis. Gene expression was normalized to peptidylprolyl isomerase A (PPIA) mRNA expression. The qPCR primer sequences, 5' to 3', for *Lmna* were Forward: GAGACGCGGCTTGTGGAG; Reverse: AGCTTGGCGGAGTATGTCTTTT.

CLAMS analyses

Energy balance measurements were performed by the University of Michigan Animal Phenotyping Core using a 16-cage Comprehensive Lab Animal Monitoring System unit (Columbus Instruments, OH, USA). Mice were placed in chambers individually and acclimated for 24 hours before measurement. O_2 consumption, CO_2 production, food intake, and locomotor activity were

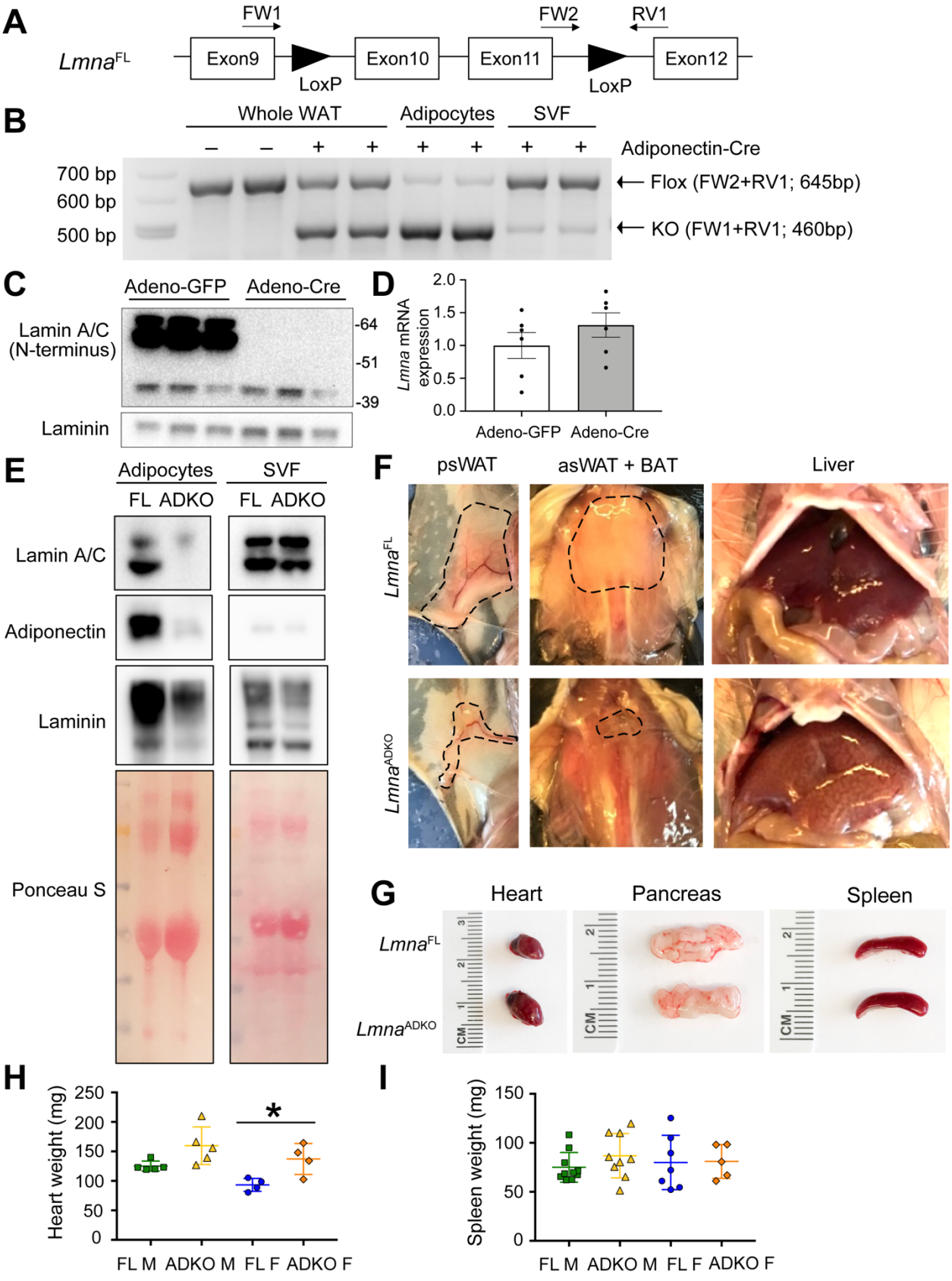
recorded during a period of 48 hours. Mice were provided with access to normal chow diet and water *ad libitum* throughout the study with an ambient temperature of 23˚C.

Fatty acid uptake

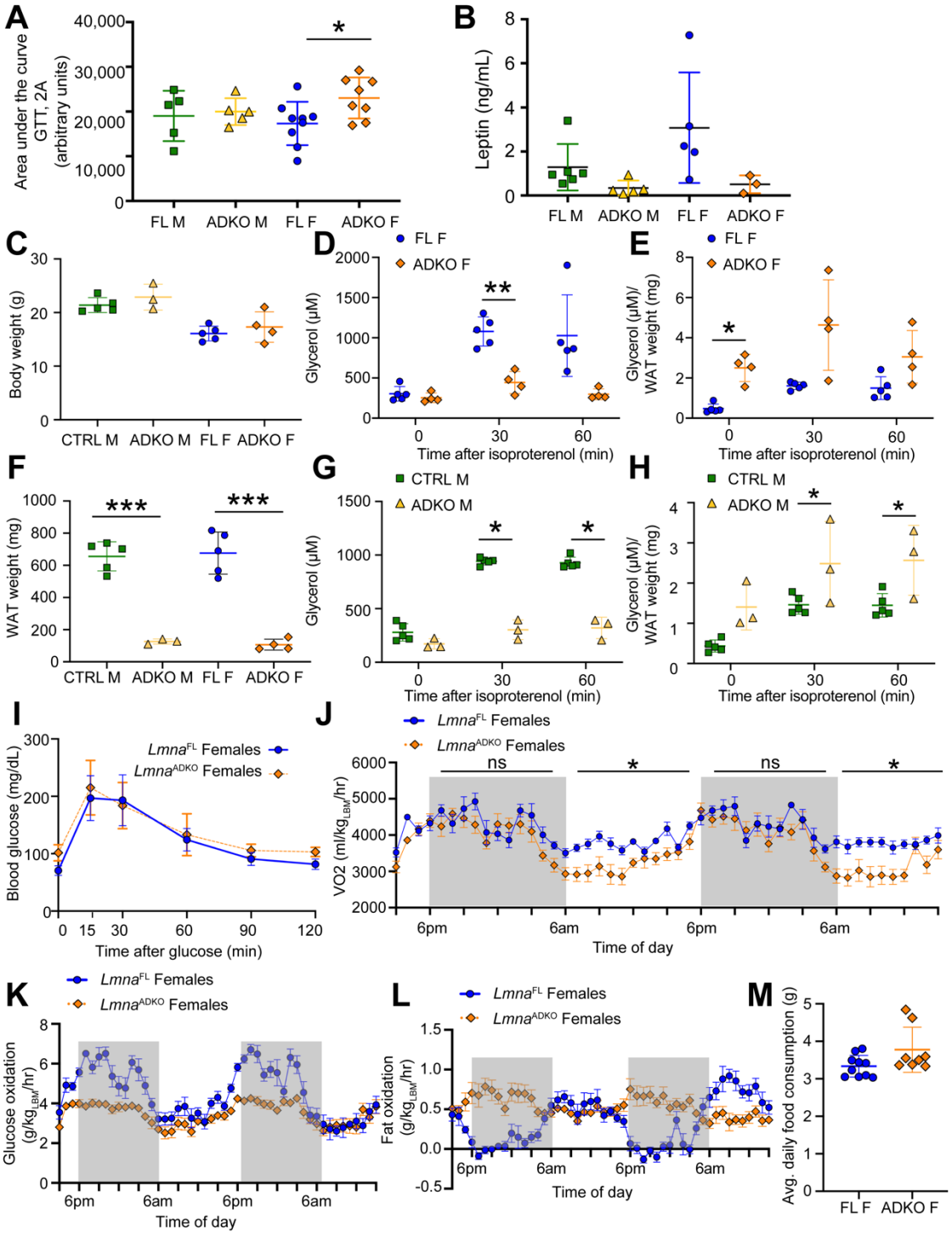
Fatty acid uptake was assessed with a previously-described, modified ß-oxidation protocol (6). Cultured adipocytes were washed 2x with DMEM, serum-starved for four hours, then incubated with an excess of [9,10-3H] palmitic acid conjugated to BSA in serum-free DMEM containing 25 uM etomoxir (Cayman Chemical) for 20 minutes. Cells were lysed in buffer with 1% SDS, lysate was collected in scintillation vials and mixed with scintillation cocktail Bio-Safe II (RPI Research Product International), and radioactivity was measured with a scintillation counter. Protein was then quantified using a BCA kit (Thermo Scientific, Rockford, IL, USA) according to manufacturer's instructions.

Supplementary references cited:

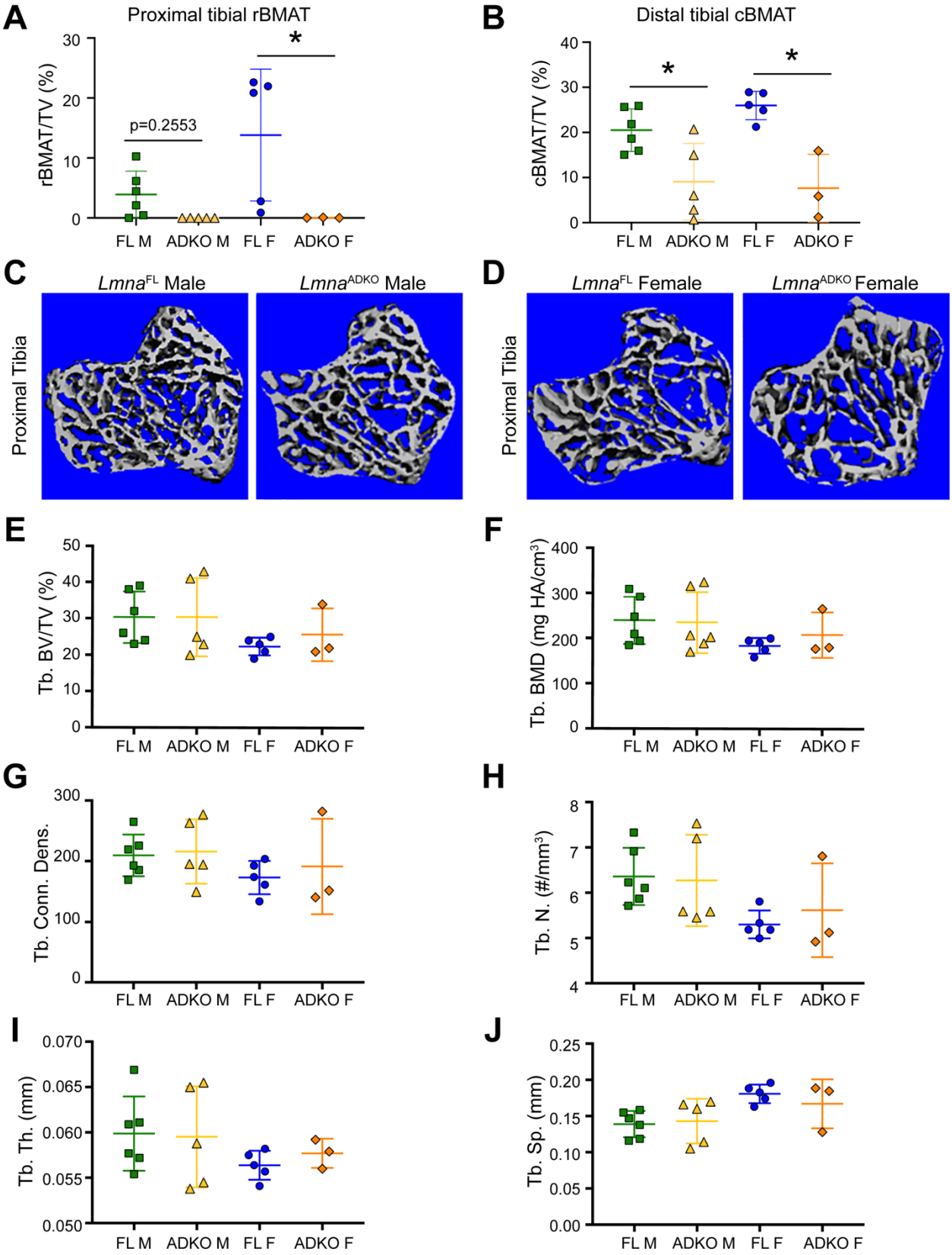
1. Cawthorn, W. P., Bree, A. J., Yao, Y., Du, B., Hemati, N., Martinez-Santibañez, G., and Macdougald, O. A. (2012) Wnt6, Wnt10a and Wnt10b inhibit adipogenesis and stimulate osteoblastogenesis through a β-catenin-dependent mechanism. *Bone* **50**, 477-489
2. Parlee, S. D., Lentz, S. I., Mori, H., and Macdougald, O. A. (2014) Quantifying size and number of adipocytes in adipose tissue. *Methods in enzymology* **537**, 93-122
3. RODBELL, M. (1964) METABOLISM OF ISOLATED FAT CELLS. I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS. *The Journal of biological chemistry* **239**, 375-380
4. Bagchi, D. P., Nishii, A., Li, Z., DelProposto, J. B., Corsa, C. A., Mori, H., Hardij, J., Learman, B. S., Lumeng, C. N., and MacDougald, O. A. (2020) Wnt/beta-catenin signaling regulates adipose tissue lipogenesis and adipocyte-specific loss is rigorously defended by neighboring stromal-vascular cells. *Mol Metab* **42**, 101078
5. Bagchi, D. P., Li, Z., Corsa, C. A., Hardij, J., Mori, H., Learman, B. S., Lewis, K. T., Schill, R. L., Romanelli, S. M., and MacDougald, O. A. (2020) Wntless regulates lipogenic gene expression in adipocytes and protects against diet-induced metabolic dysfunction. *Mol Metab* **39**, 100992
6. Mori, H., Dugan, C. E., Nishii, A., Benchamana, A., Li, Z., Cadenhead, T. S. t., Das, A. K., Evans, C. R., Overmyer, K. A., Romanelli, S. M., Peterson, S. K., Bagchi, D. P., Corsa, C. A., Hardij, J., Learman, B. S., El Azzouny, M., Coon, J. J., Inoki, K., and MacDougald, O. A. (2021) The molecular and metabolic program by which white adipocytes adapt to cool physiologic temperatures. *PLoS Biol* **19**, e3000988


A
FW1
FW2
RV1
Lmna^FL
Exon9
LoxP
Exon10
Exon11
LoxP
Exon12
B
Whole WAT
Adipocytes
SVF
– – + + + + + + Adiponectin-Cre
700 bp
600 bp
500 bp
← Flox (FW2+RV1; 645bp)
← KO (FW1+RV1; 460bp)
C
Adeno-GFP
Adeno-Cre
Lamin A/C (N-terminus)
-64
-51
-39
Laminin
D
Lmna mRNA expression
2.0
1.5
1.0
0.5
0.0
Adeno-GFP
Adeno-Cre
E
Adipocytes
SVF
FL ADKO FL ADKO
Lamin A/C
Adiponectin
Laminin
Ponceau S
F
psWAT
asWAT + BAT
Liver
Lmna^FL
Lmna^ADKO
G
Heart
Pancreas
Spleen
Lmna^FL
Lmna^ADKO
H
Heart weight (mg)
250
200
150
100
50
0
*
FL M ADKO M FL F ADKO F
I
Spleen weight (mg)
150
100
50
0
FL M ADKO M FL F ADKO F

Supplementary Fig. 1 – Adipocyte-specific deletion of lamin A/C leads to loss of adipose tissue in mice. A, schematic of the *Lmna* floxed allele indicating the position of LoxP sites and genotyping primers. B, genotyping PCR from $Lmna^{FL}$ and $Lmna^{ADKO}$ mice demonstrating Cre-mediated recombination of the floxed allele. C, immunoblot and D, qPCR analyses of *Lmna* expression in $Lmna^{ADKO}$ adipocytes infected with adeno-GFP or adeno-Cre. E, immunoblot analyses of fractionated adipocytes and SVF cells isolated from the psWAT of 4 week-old $Lmna^{FL}$ and $Lmna^{ADKO}$ mice. F, representative images of male $Lmna^{FL}$ and $Lmna^{ADKO}$ mice during necropsy. Dashed lines indicate perimeter of the labeled tissue. G, representative images of heart, pancreas, and spleen from 12-14 week-old $Lmna^{FL}$ and $Lmna^{ADKO}$ male mice. Organ weights of 12-14 week-old $Lmna^{FL}$ and $Lmna^{ADKO}$ mice, for H, heart, and I, spleen. *$p<0.05$.


A
Area under the curve GTT, 2A (arbitrary units)
40,000
30,000
20,000
10,000
0
*
FL M
ADKO M
FL F
ADKO F
B
Leptin (ng/mL)
8
6
4
2
0
FL M
ADKO M
FL F
ADKO F
C
Body weight (g)
30
20
10
0
CTRL M
ADKO M
FL F
ADKO F
D
FL F
ADKO F
Glycerol (µM)
2000
1500
1000
500
0
**
0
30
60
Time after isoproterenol (min)
E
FL F
ADKO F
Glycerol (µM)/ WAT weight (mg)
8
6
4
2
0
*
0
30
60
Time after isoproterenol (min)
F
WAT weight (mg)
1000
800
600
400
200
0
***
***
CTRL M
ADKO M
FL F
ADKO F
G
CTRL M
ADKO M
Glycerol (µM)
1500
1000
500
0
*
*
0
30
60
Time after isoproterenol (min)
H
CTRL M
ADKO M
Glycerol (µM)/ WAT weight (mg)
4
3
2
1
0
*
*
0
30
60
Time after isoproterenol (min)
I
Lmna^FL Females
Lmna^ADKO Females
Blood glucose (mg/dL)
300
200
100
0
0
15
30
60
90
120
Time after glucose (min)
J
Lmna^FL Females
Lmna^ADKO Females
VO2 (ml/kg_LBM/hr)
6000
5000
4000
3000
2000
ns
*
ns
*
6pm
6am
6pm
6am
Time of day
K
Lmna^FL Females
Lmna^ADKO Females
Glucose oxidation (g/kg_LBM/hr)
8
6
4
2
0
6pm
6am
6pm
6am
Time of day
L
Lmna^FL Females
Lmna^ADKO Females
Fat oxidation (g/kg_LBM/hr)
1.5
1.0
0.5
0.0
-0.5
6pm
6am
6pm
6am
Time of day
M
Avg. daily food consumption (g)
5
4
3
2
1
0
FL F
ADKO F

Supplementary Fig. 2 – *Lmna*^{ADKO} mice display mild metabolic dysregulation on a normal chow diet. A, area under the curve for glucose tolerance tests from 14 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice. B, circulating leptin concentrations in 12-14 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice. C, body weights. D, G, circulating glycerol concentrations in 10-12 week-old female (D) and male *Lmna*^{CTRL} and *Lmna*^{ADKO} mice (G). E, *Lmna*^{CTRL} and *Lmna*^{ADKO} mice at the indicated timepoints following isoproterenol administration (1mg/kg, i.p.). F, total WAT tissue weight in 10-12 week-old *Lmna*^{CTRL} and *Lmna*^{ADKO} mice. Total WAT weight was calculated by combining gWAT, iWAT, asWAT, perirenal WAT, retroperitoneal WAT, and interscapular WAT. I, glucose tolerance test for ~6 month-old *Lmna*^{FL} and *Lmna*^{ADKO} female mice. H, circulating glycerol concentrations per total WAT tissue weight in 10-12 week-old female (E) and male (H) *Lmna*^{CTRL} and *Lmna*^{ADKO} mice at the indicated timepoints following isoproterenol administration (1mg/kg, i.p.). I, glucose tolerance test for ~6 month-old female *Lmna*^{FL} and *Lmna*^{ADKO} mice. J-L, metabolic data assessed by CLAMS for ~6 month-old female *Lmna*^{FL} and *Lmna*^{ADKO} mice. J, oxygen consumption rate normalized to lean body mass. Statistical analysis was by ANCOVA with lean body mass as a covariate. K, glucose oxidation and L, fat oxidation. M, average food consumption for 7 days in ~6 month-old female *Lmna*^{FL} and *Lmna*^{ADKO} mice. Data in panels J-L are presented as mean ± SEM. *$p<0.05$, ***$p<0.001$.

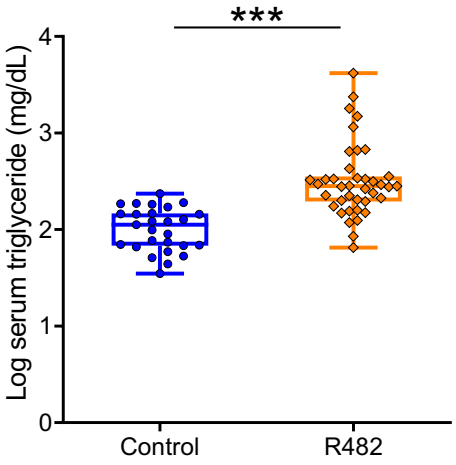

A
Proximal tibial rBMAT
rBMAT/TV (%)
p=0.2553
*
FL M
ADKO M
FL F
ADKO F
B
Distal tibial cBMAT
cBMAT/TV (%)
*
*
C
Lmna^FL Male
Lmna^ADKO Male
Proximal Tibia
D
Lmna^FL Female
Lmna^ADKO Female
Proximal Tibia
E
Tb. BV/TV (%)
F
Tb. BMD (mg HA/cm³)
G
Tb. Conn. Dens.
H
Tb. N. (#/mm³)
I
Tb. Th. (mm)
J
Tb. Sp. (mm)

Supplementary Fig. 3 – Adipocyte-specific deletion of *Lmna* reduces tibial BMAT, but does not influence trabecular bone in 12-14 week-old mice on a normal chow diet. A-B, osmium μCT quantification of BMAT in A, proximal tibia, and B, distal tibia. Statistics were performed on $\log_{10}$ normalized data. C-D, representative μCT scans of the proximal tibial trabecular bone of C, male and D, female *Lmna*^{FL} and *Lmna*^{ADKO} mice. E-J, μCT analyses of bones from *Lmna*^{FL} and *Lmna*^{ADKO} mice, with E, quantification of trabecular bone volume fraction, F, bone mineral density, G, connective density, H, number, I, thickness, and J, separation. *$p<0.05$, **$p<0.1$.

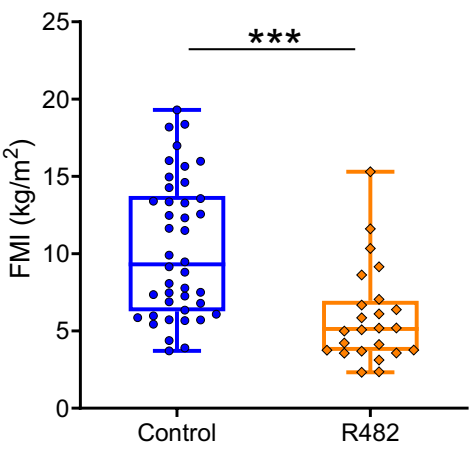
A

Parameter, mean (SD)	Control	R482
Age	49(11.1)	50.2(14.8)
Weight (kg)	77.6(18.4)	73.3(18.5)
Height (cm)	166.6(8.4)	163.4(7.2)
BMI (kg/m^2)	27.8(5.8)	26.8(5.2)
TMI (kg/m^2)	13.9(3.7)	13.3(3.3)
TFMI (kg/m^2)	5.7(2.9)	3.8(2.0) *
FFMI (kg/m^2)	17.4(2.0)	18.8(3.3)
Lean Mass (kg)		
Arms	4.96 (1.37)	5.93 (2.02)
Legs	15.00 (2.96)	15.81 (3.07)
Trunk	22.39 (4.13)	24.22 (5.53)
Total Body	46.23 (8.49)	47.86 (11.55)
Fat-Free Mass (kg)		
Arms	5.28 (1.45)	6.21 (2.11)
Legs	15.92 (3.15)	16.50 (3.24)
Trunk	22.96 (4.35)	24.90 (5.72)
Total Body	48.76 (8.89)	49.74(12.22)
Total Cholesterol (mg/dL)	181.5(30.5)	213.5(74.2)
HDL-Cholesterol (mg/dL)	61.7(22.5)	37.5(8.5) ***
LDL-Cholesterol (mg/dL)	97.1(22.7)	98.4(41.2)
HbA1c (%)	6.4(1.0)	7.4(1.7)
Leptin (ng/mL)	31.2(24.9)	10.0(8.5) **

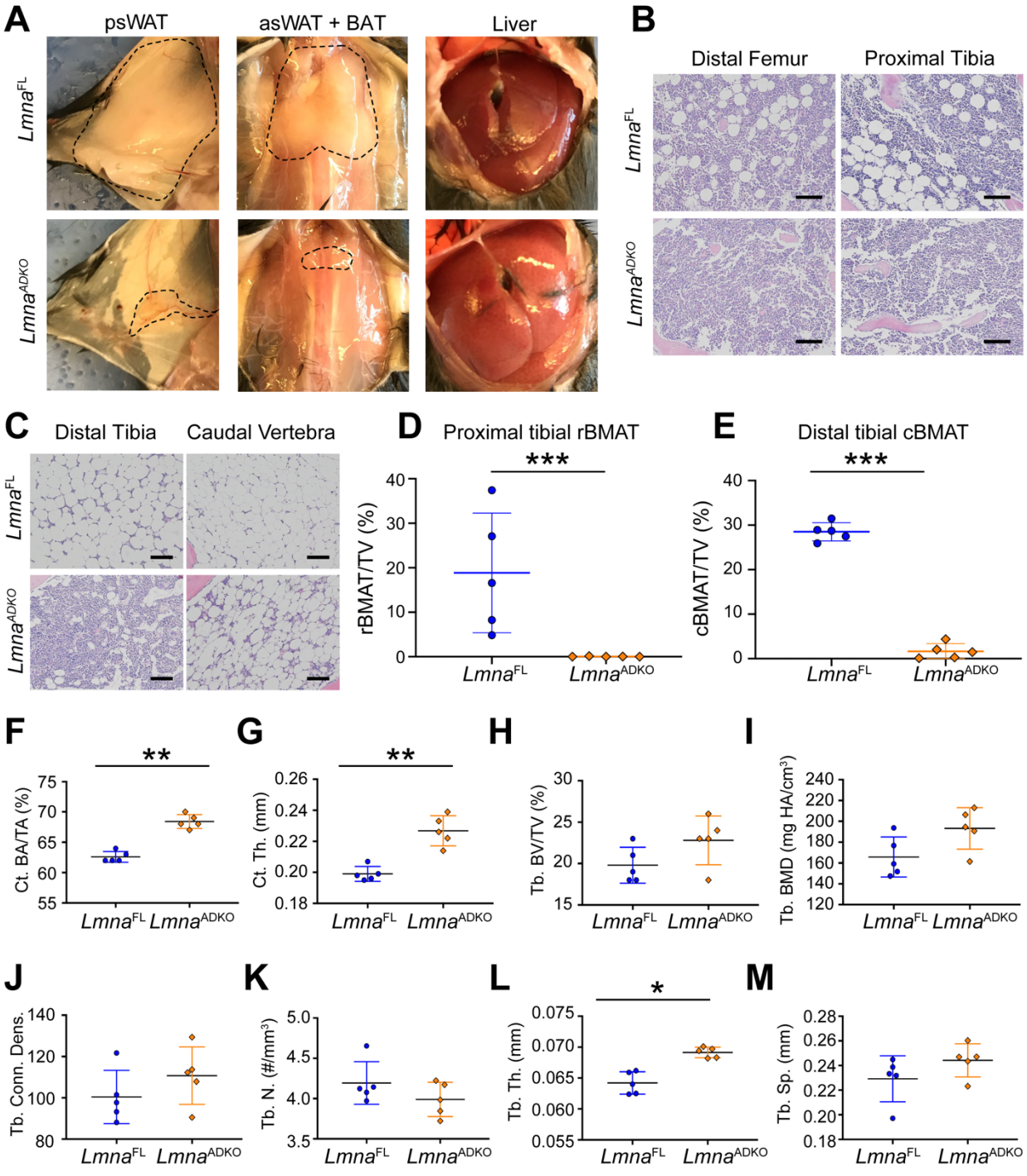
B



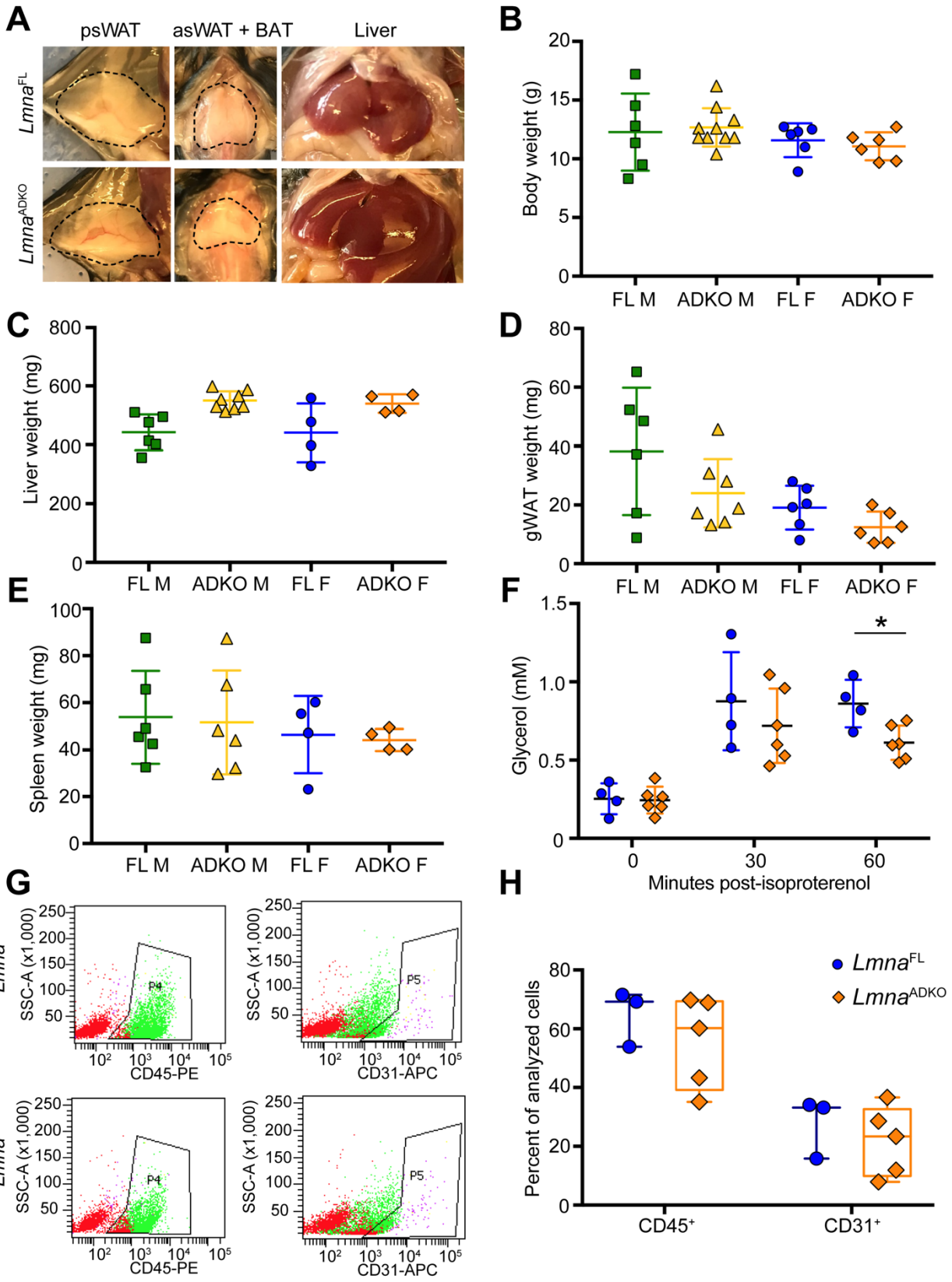
C



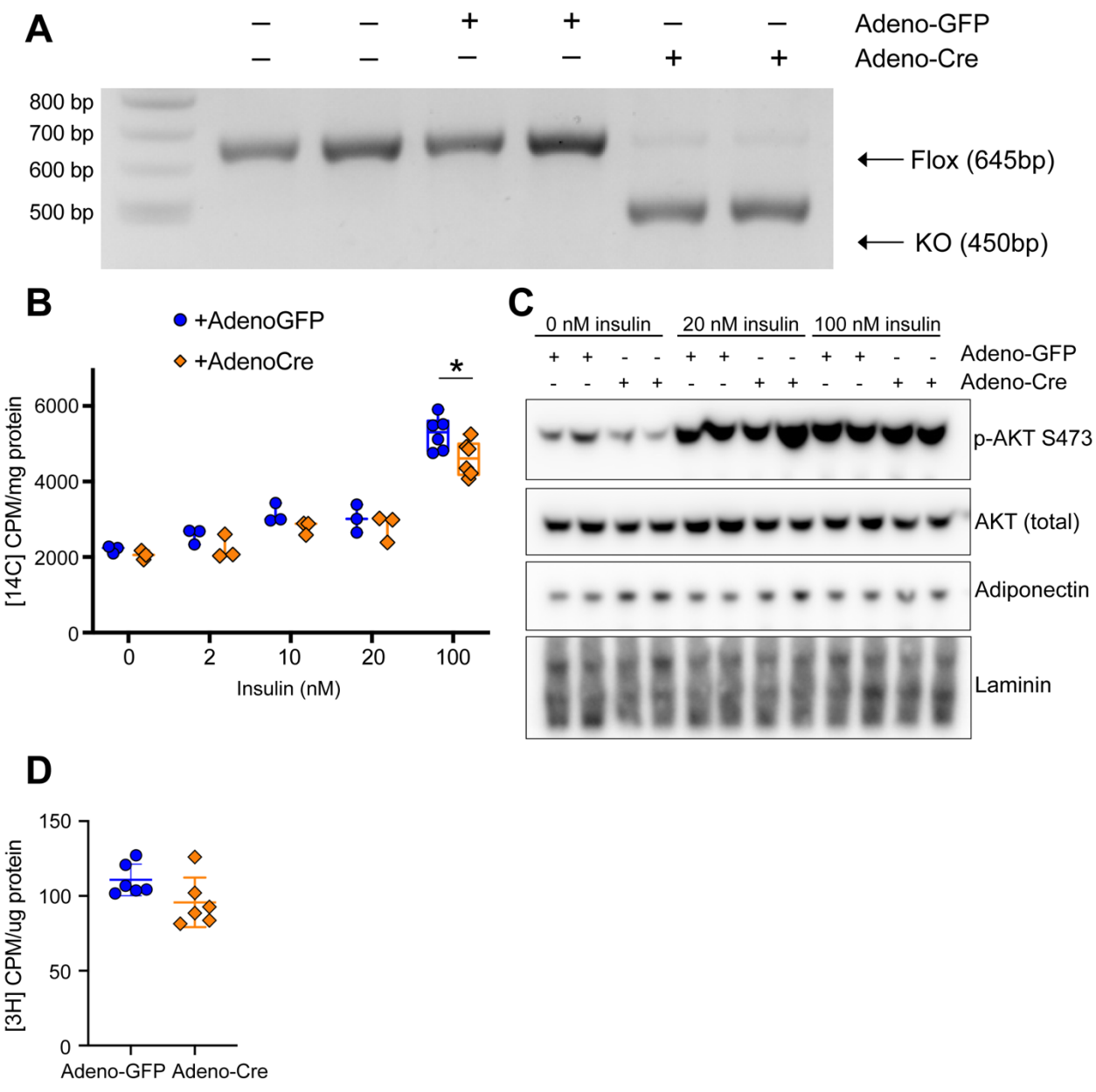
Supplementary Fig. 4 – Patients with R482 mutation have impaired metabolic homeostasis and less fat mass compared to controls. A, metabolic and body composition comparison between control and R482 groups. B, serum triglycerides were higher in R482 compared to control, and C, Fat Mass Index (FMI) was lower in R482 compared to control. BMI: body mass index; TMI: trunk mass index; TFMI: trunk fat mass index; FFMI: fat-free mass index; HbA1c: hemoglobin A1c. *p<0.05; **p<0.01; ***p<0.001.


A
psWAT
asWAT + BAT
Liver
LmnaFL
LmnaADKO
B
Distal Femur
Proximal Tibia
C
Distal Tibia
Caudal Vertebra
D
Proximal tibial rBMAT
rBMAT/TV (%)
***
E
Distal tibial cBMAT
cBMAT/TV (%)
***
F
Ct. BA/TA (%)
**
G
Ct. Th. (mm)
**
H
Tb. BV/TV (%)
I
Tb. BMD (mg HA/cm3)
J
Tb. Conn. Dens.
K
Tb. N. (#/mm3)
L
Tb. Th. (mm)
*
M
Tb. Sp. (mm)

Supplementary Fig. 5 – High fat diet challenge exacerbates the lipodystrophic phenotype in mice with adipocyte-specific deletion of lamin A/C. A, representative images of HFD-fed female *Lmna*^{FL} and *Lmna*^{ADKO} mice during necropsy. Dashed lines indicate perimeter of the labeled tissue. B-C, representative histological images of bone marrow sections stained with H&E, for B, distal femurs, proximal tibia, and C, distal tibia, caudal vertebrae from HFD-fed *Lmna*^{FL} and *Lmna*^{ADKO} mice. Scale bars, 200 μm. D-E, osmium μCT quantification of BMAT in D, proximal tibia, and E, distal tibia of HFD-fed *Lmna*^{FL} and *Lmna*^{ADKO} mice. Statistics were performed on $\log_{10}$ normalized data. F-M, μCT analysis of tibia from HFD-fed *Lmna*^{FL} and *Lmna*^{ADKO} mice. Quantification of F, cortical bone area per total tissue area for distal tibiae. G, cortical thickness of distal tibiae. H, trabecular bone volume fraction, I, bone mineral density, J, connective density, K, number, L, thickness, and M, separation. *$p<0.05$, **$p<0.1$, ***$p<0.001$.


A
psWAT
asWAT + BAT
Liver
Lmna^FL
Lmna^ADKO
B
Body weight (g)
FL M
ADKO M
FL F
ADKO F
C
Liver weight (mg)
D
gWAT weight (mg)
E
Spleen weight (mg)
F
Glycerol (mM)
Minutes post-isoproterenol
*
G
SSC-A (x1,000)
CD45-PE
CD31-APC
P4
P5
H
Percent of analyzed cells
CD45+
CD31+

Supplementary Fig. 6 – Adipose tissue develops postnatally in *Lmna*^{ADKO} mice and is progressively lost with aging. A, representative images of 4 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice during necropsy. Dashed lines indicate perimeter of the labeled tissue. B, body weights and tissue weights for 4 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice, including C, liver, D, gWAT, and E, spleen, F, circulating glycerol concentrations in 4 week-old *Lmna*^{FL} and *Lmna*^{ADKO} female mice at the indicated timepoints following isoproterenol administration (10mg/kg, i.p.). G, representative FACS plots for SVF cells from 4 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice stained with CD45 and CD31. H, quantification of CD45-positive and CD31-positive SVF cells from 4 week-old *Lmna*^{FL} and *Lmna*^{ADKO} mice. *$p<0.05$.


A
Adeno-GFP
Adeno-Cre
800 bp
700 bp
600 bp
500 bp
Flox (645bp)
KO (450bp)
B
+AdenoGFP
+AdenoCre
*
[14C] CPM/mg protein
6000
4000
2000
0
0
2
10
20
100
Insulin (nM)
C
0 nM insulin
20 nM insulin
100 nM insulin
Adeno-GFP
Adeno-Cre
p-AKT S473
AKT (total)
Adiponectin
Laminin
D
[3H] CPM/ug protein
150
100
50
0
Adeno-GFP
Adeno-Cre

Supplementary Fig. 7 – *Lmna* deletion does not affect adipogenesis of primary precursors. Primary precursors from *Lmna*^{FL} mice were treated with Adeno-GFP or Adeno-Cre prior to differentiation. A, PCR demonstrating efficient Adeno-Cre recombination at the *Lmna* locus. B, insulin-mediated uptake of [^{14}C]-2-deoxy-glucose in mature adipocytes following 30 minute treatment with the indicated concentrations of insulin. C, immunoblot for mature adipocytes following 15 minute treatment with the indicated concentrations of insulin (n = 3-6 wells per condition). D, uptake of [^{3}H]-palmitic acid in mature adipocytes following 20 minute incubation with radiolabeled fatty acid (n = 6). *$p<0.05$.