

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/m^2 accordingly. BMI was calculated by $\text{Weight}(\text{kg})/\text{Height}(\text{m})^2$. 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: $\text{Fat Mass}(\text{kg})/\text{Height}(\text{m})^2$; FFMI: $\text{FFM}(\text{kg})/\text{Height}(\text{m})^2$, where $\text{FFM} = \text{fat-free mass}(\text{lean mass}(\text{kg}) + \text{bone mass}(\text{kg}))$; TMI: $\text{TTM}(\text{kg})/\text{Height}(\text{m})^2$, where $\text{TTM} = \text{total trunk mass}$; and TFMI: $\text{TFM}(\text{kg})/\text{Height}(\text{m})^2$, where $\text{TFM} = \text{trunk fat mass}$. 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 (41). 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 $Lmna^{\text{FL}}$ and $Lmna^{\text{ADKO}}$ mice. Individual adipocyte areas were quantified using MetaMorph Microscopy Automation & Image Analysis Software 2.0 (Molecular Devices, Sunnyvale, CA) as previously described (42). Frequency distribution curves were generated using a bin range of 0 to $4000 \mu\text{m}^2$ and bin width of $500 \mu\text{m}^2$.

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 μ M), isoproterenol (1 μ M) or norepinephrine (1 μ M) 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 4°C with gentle agitation for 2 weeks in 14% EDTA, changing the solution every 2 days. Tissue sections (5 μ m) were stained with hematoxylin and eosin as previously described (42).

Adipocyte and stromal vascular cell fractionation

Using a protocol modified from Rodbell (43), 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 μ m 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 μ Ci/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

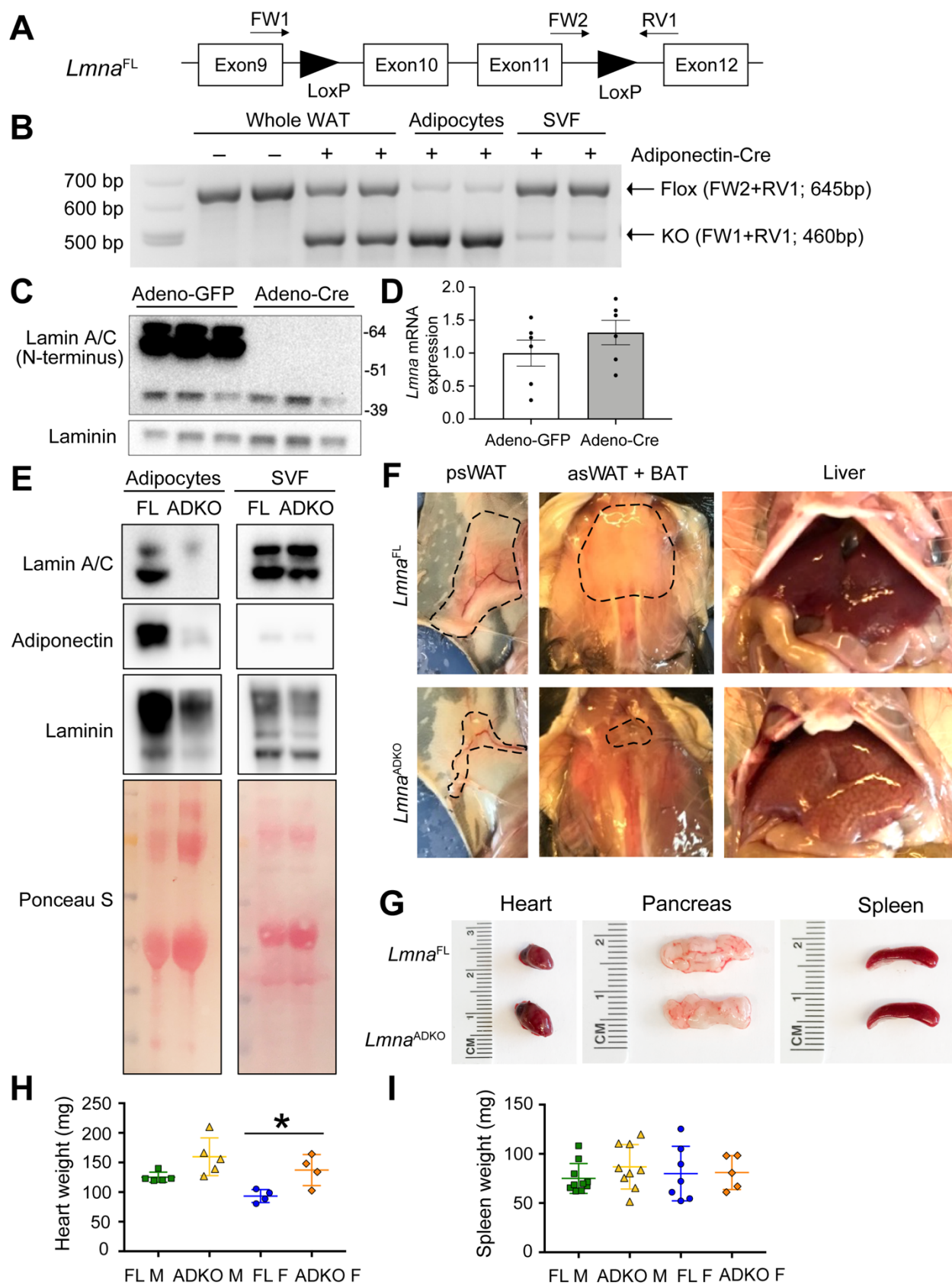
Quantitative RT-PCR measurements were performed as described previously (44; 45). Briefly, total RNA was isolated and purified from cultured cells using RNA-STAT60 (Tel-Test, Alvin, TX, USA) according to the manufacturer's instructions. M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) was 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

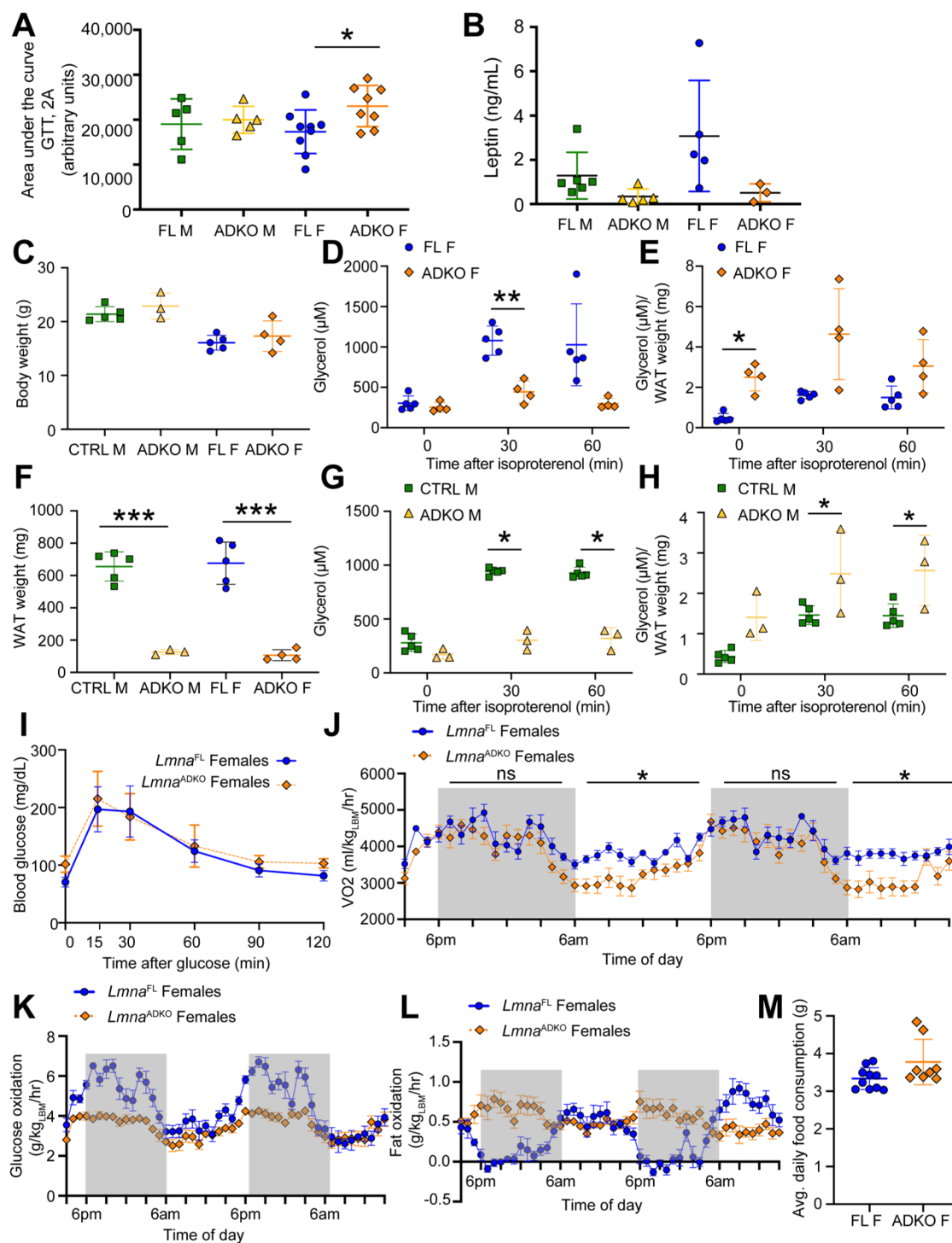
Energy balance measurements were performed by the University of Michigan Animal Phenotyping Core using a 16-cage Comprehensive Lab Animal Monitoring System unit (Columbus Instruments, OH, USA). Mice were placed in chambers individually and acclimated for 24 hours before measurement. O₂ consumption, CO₂ 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

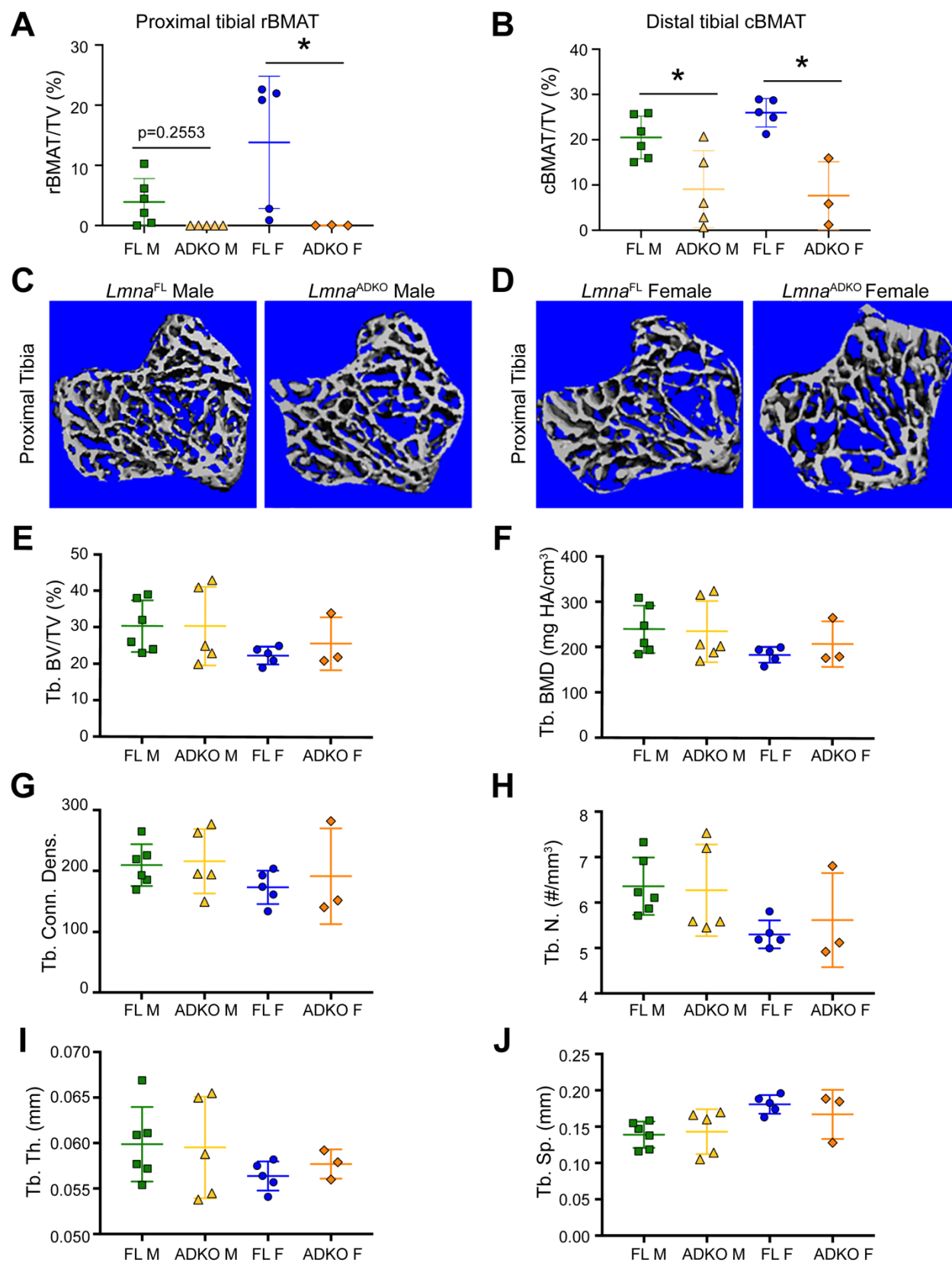
Fatty acid uptake was assessed with a previously-described, modified β -oxidation protocol (46). Cultured adipocytes were washed 2x with DMEM, serum-starved for four hours, then incubated with an excess of [9,10- 3 H] palmitic acid (Perkin Elmer) conjugated to BSA in serum-free DMEM containing 25 μ M 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.



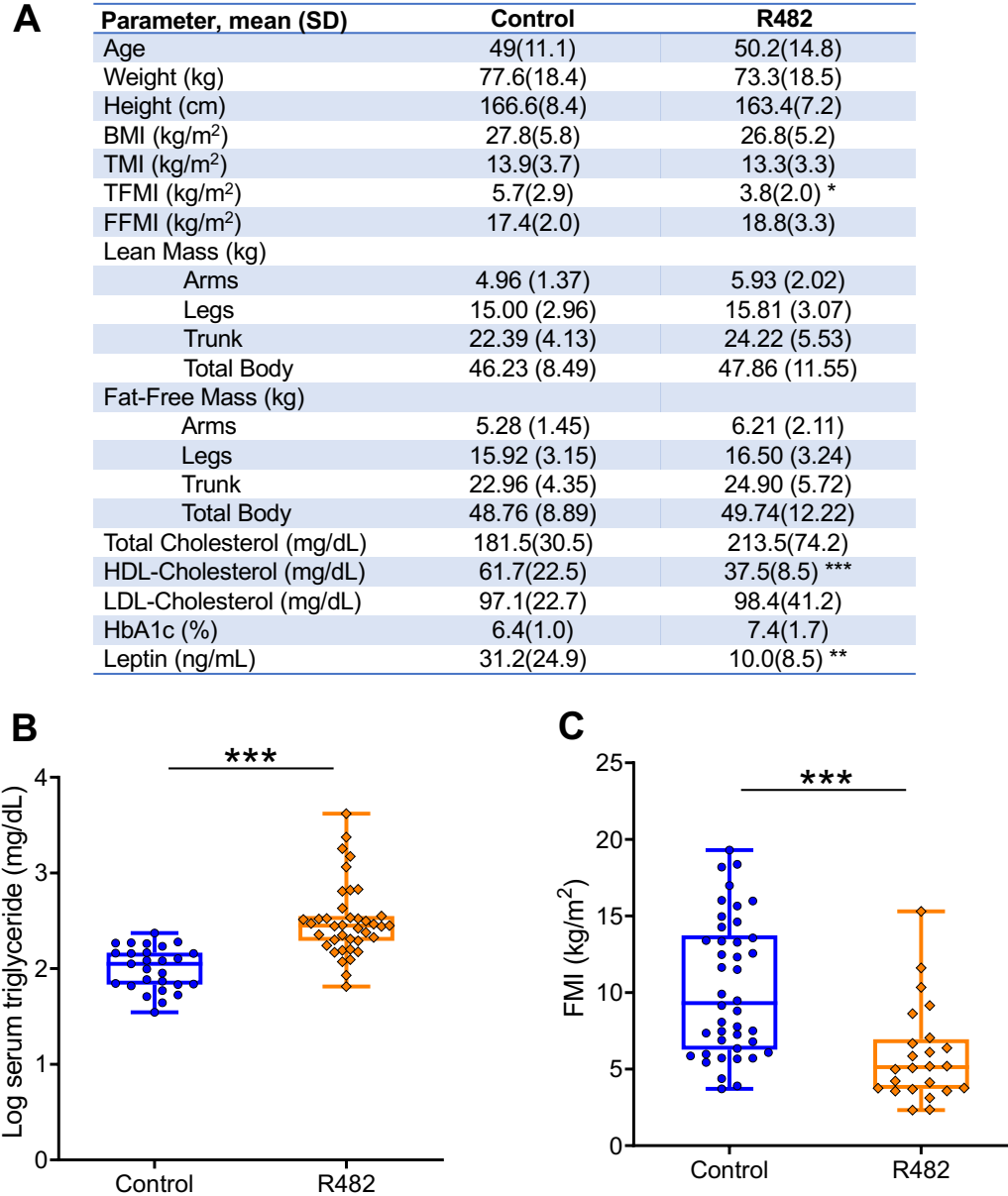
Supplementary Fig. 1 – Adipocyte-specific deletion of lamin A/C leads to loss of adipose tissue in mice. A, schematic of the *Lmna* floxed allele indicating the position of LoxP sites and genotyping primers. B, genotyping PCR from *Lmna*^{FL} and *Lmna*^{ADKO} mice demonstrating Cre-mediated recombination 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.



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 \pm SEM. * $p < 0.05$, *** $p < 0.001$.

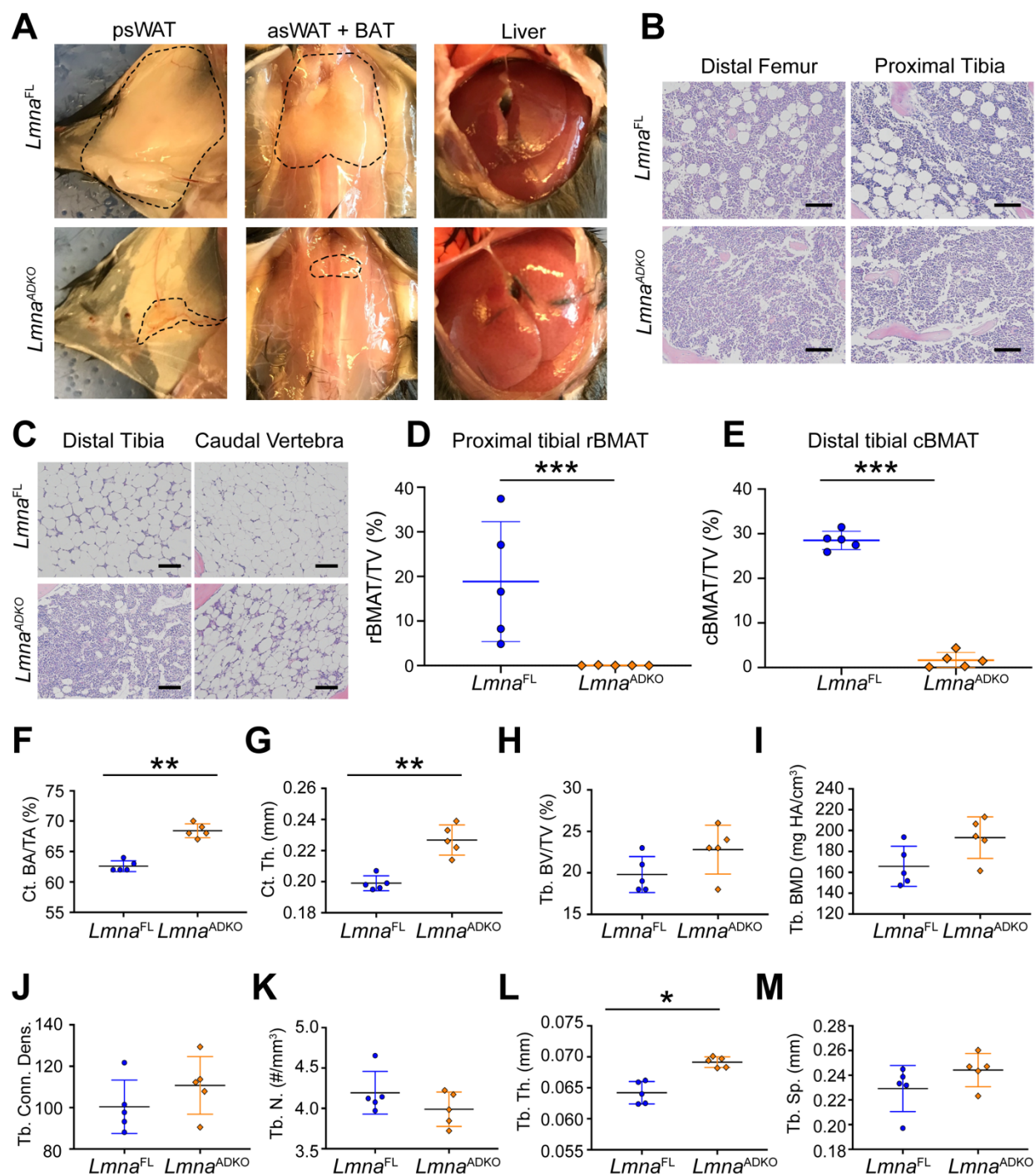


Supplementary Fig. 3 – Adipocyte-specific deletion of *Lmna* reduces tibial BMAT, but does not influence trabecular bone in 12-14 week-old mice on a normal chow diet. A-B, osmium μ CT quantification of BMAT in A, proximal tibia, and B, distal tibia. Statistics were performed on \log_{10} normalized data. C-D, representative μ CT scans of the proximal tibial trabecular bone of C, male and D, female *Lmna*^{FL} and *Lmna*^{ADKO} mice. E-J, μ CT 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. * $p < 0.05$, ** $p < 0.1$.

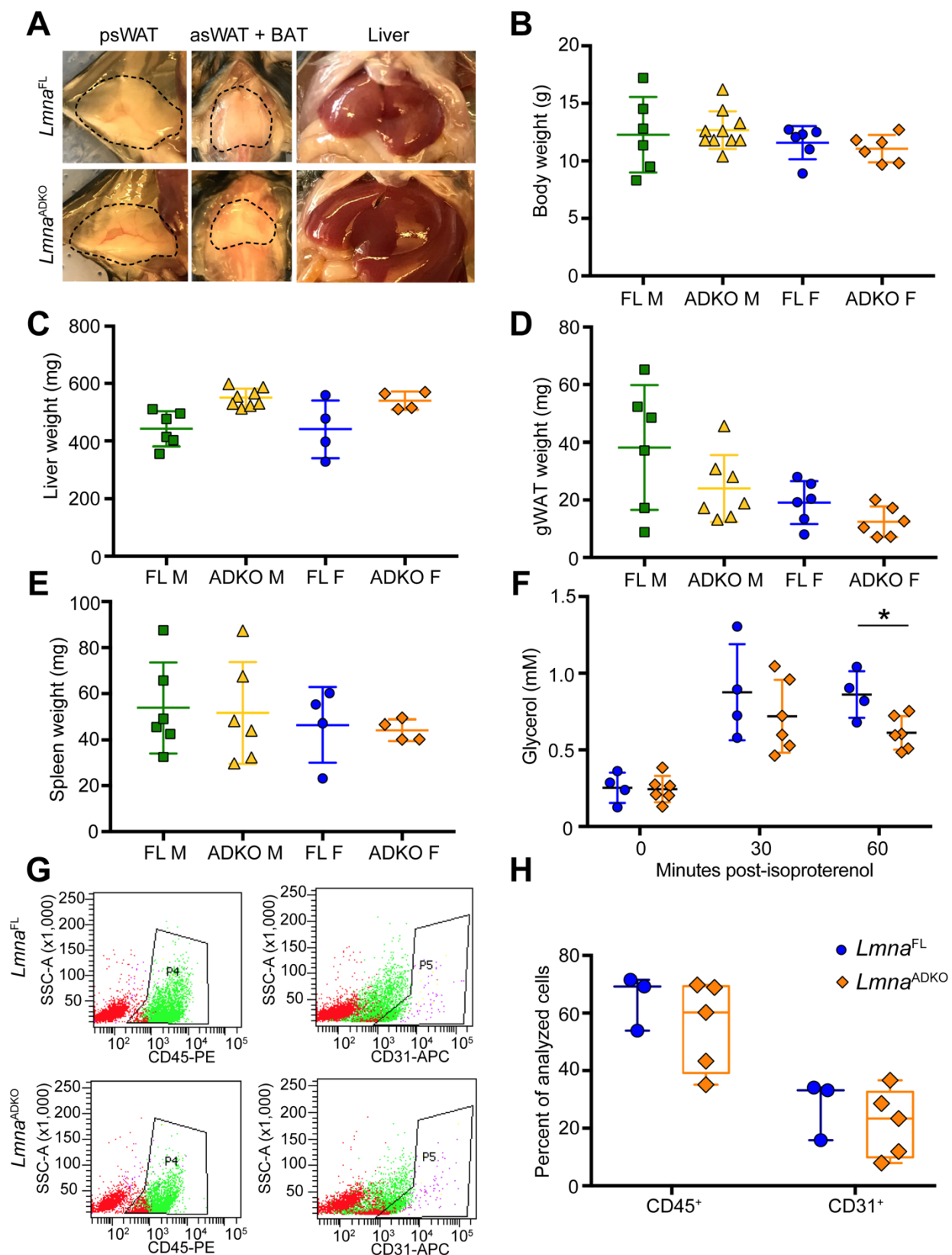


Supplementary Fig. 4 – Patients with R482 mutation have impaired metabolic homeostasis and less fat mass compared to controls. A, metabolic and body composition comparison between control and R482 groups. B, serum triglycerides were higher in R482 compared to control, and C, Fat Mass Index (FMI) was 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.

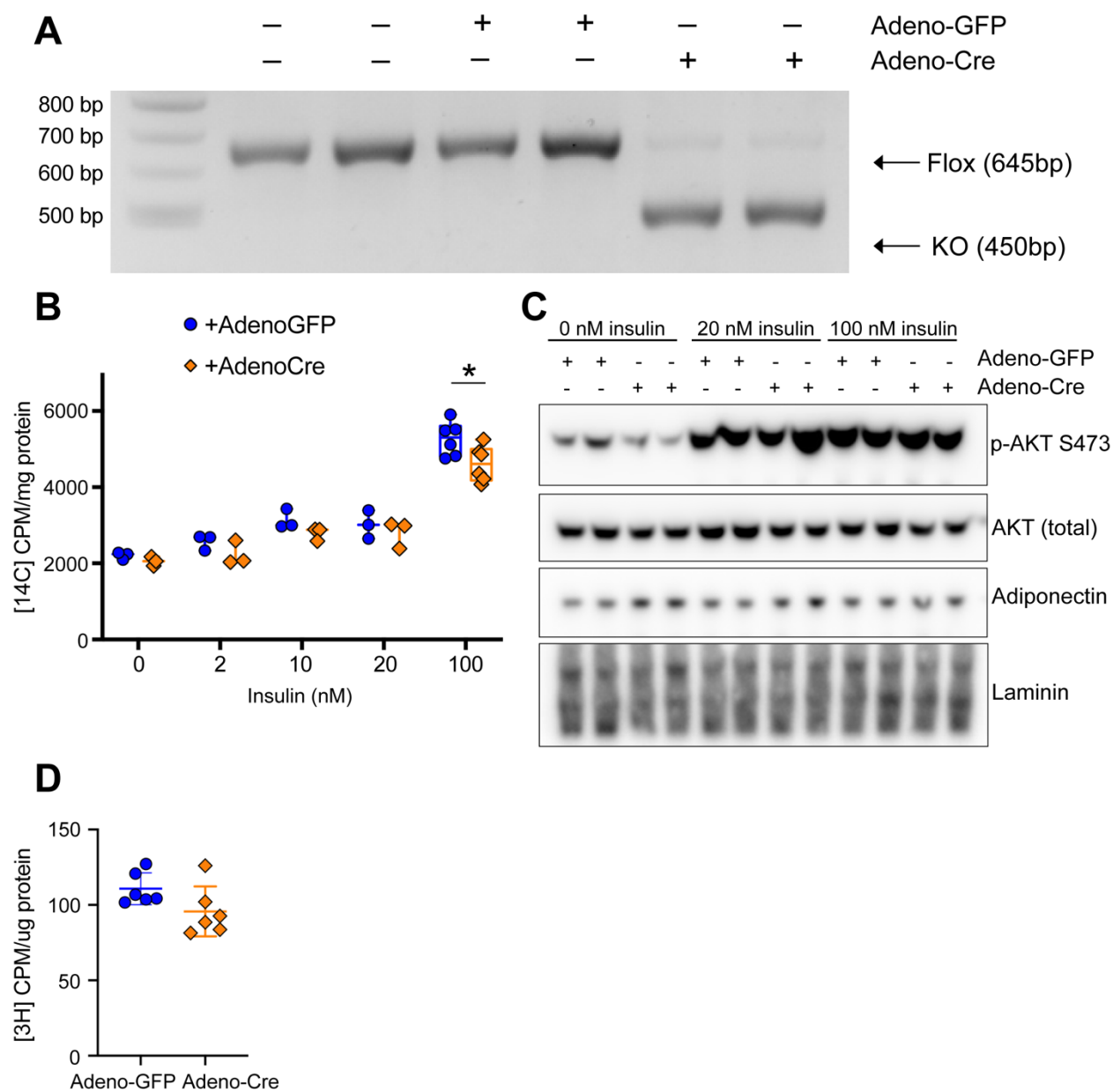
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



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 μ m. D-E, osmium μ CT quantification of BMAT in D, proximal tibia, and E, distal tibia of HFD-fed *Lmna*^{FL} and *Lmna*^{ADKO} mice. Statistics were performed on log₁₀ normalized data. F-M, μ CT 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.



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.



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 [¹⁴C]-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 [³H]-palmitic acid in mature adipocytes following 20 minute incubation with radiolabeled fatty acid (n = 6). *p<0.05.