SUPPLEMENTAL INFORMATION - MORI, ET AL

SUPPLEMENTAL FIGURES

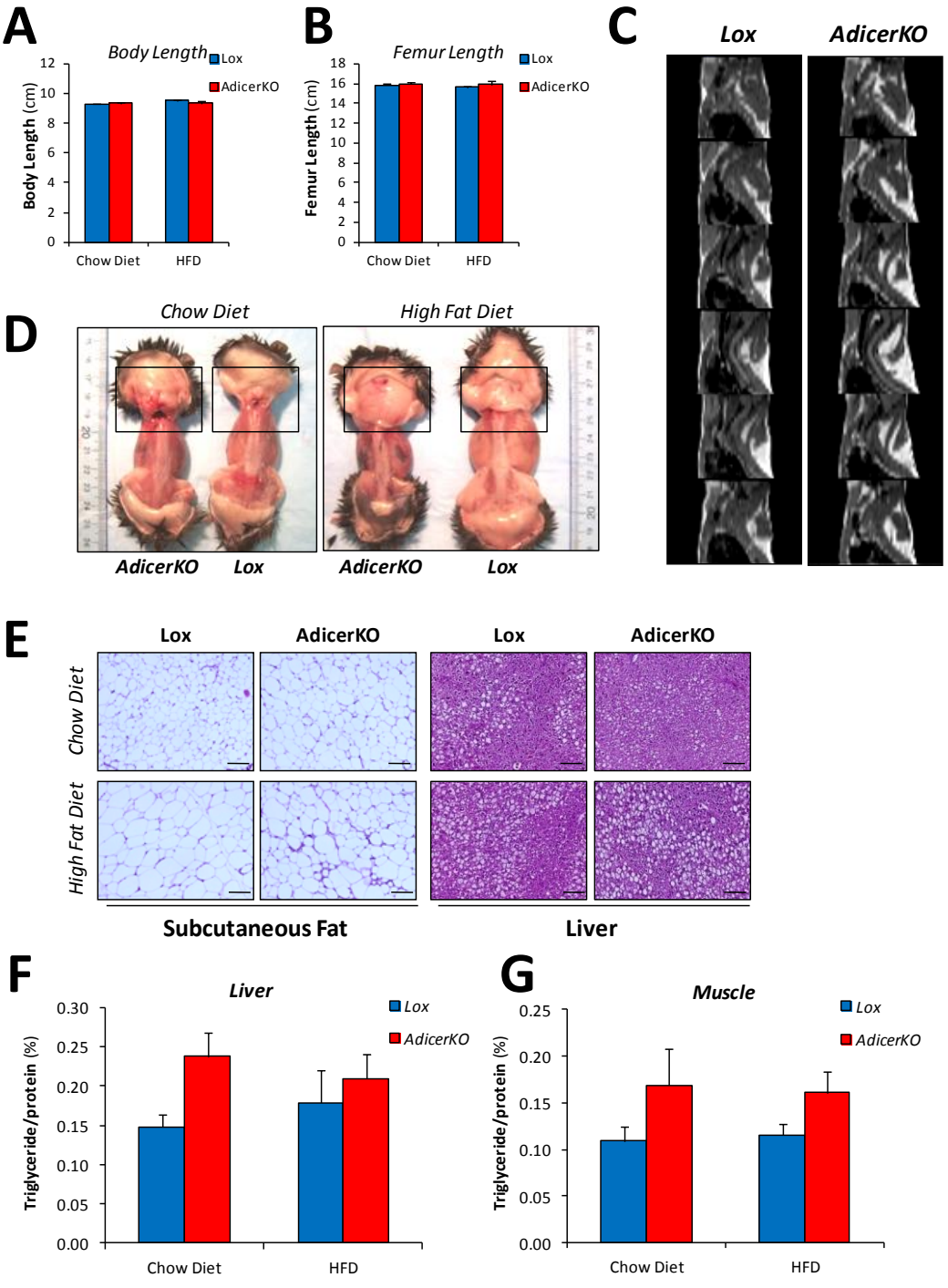

Figure S1. Fat-specific Dicer knockout mice exhibit partial lipodystrophy (AdicerKO).

(A,B,D-G) Ten-week old AdicerKO and Lox control mice were put on a high fat diet (HFD) or maintained on a chow diet for 5 months prior to the sacrifice (N = 5-12 animals per group).

(A) Body length and (B) femur length at sacrifice. * $P < 0.05$.

(C) Magnetic Resonance Imaging was performed in 4 month-old mice feeding chow diet. Figures show a sagittal plane representative of one mouse of each genotype. Fat is evidenced by the lighter tones.

(D) Images of the dorsocervical region and the enlarged interscapular fat pads of AdicerKO mice at sacrifice.

(E) Hematoxylin-eosin staining of subcutaneous fat of the flank and liver of male mice. Representative images (200x magnification) of at least 3 animals per group.

(F,G) Triglyceride content was measured in (F) liver and (G) quadriceps muscle and normalized by protein content.

Values are mean ± SEM.

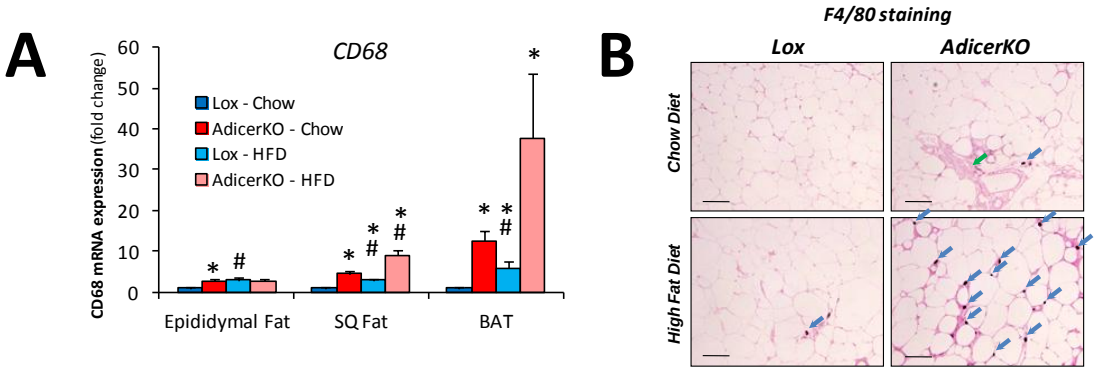

Figure S2. AdicerKO mice show signs of adipose tissue inflammation.

Ten-week old AdicerKO and Lox control mice were put on a high fat diet (HFD) or maintained on a chow diet for 5 months prior to the sacrifice (N = 6-12 animals per group).

(A) *CD68* mRNA was measured in epididymal fat, subcutaneous fat of the flank region (SQ fat) and interscapular brown adipose tissue (BAT) as a surrogate of macrophage infiltration.

(B) F4/80 immunohistochemistry with eosin counterstaining. Positive cells are highlighted by blue arrows. Fibrosis is indicated by the green arrow. Representative images (200x magnification) of 2 animals per group.

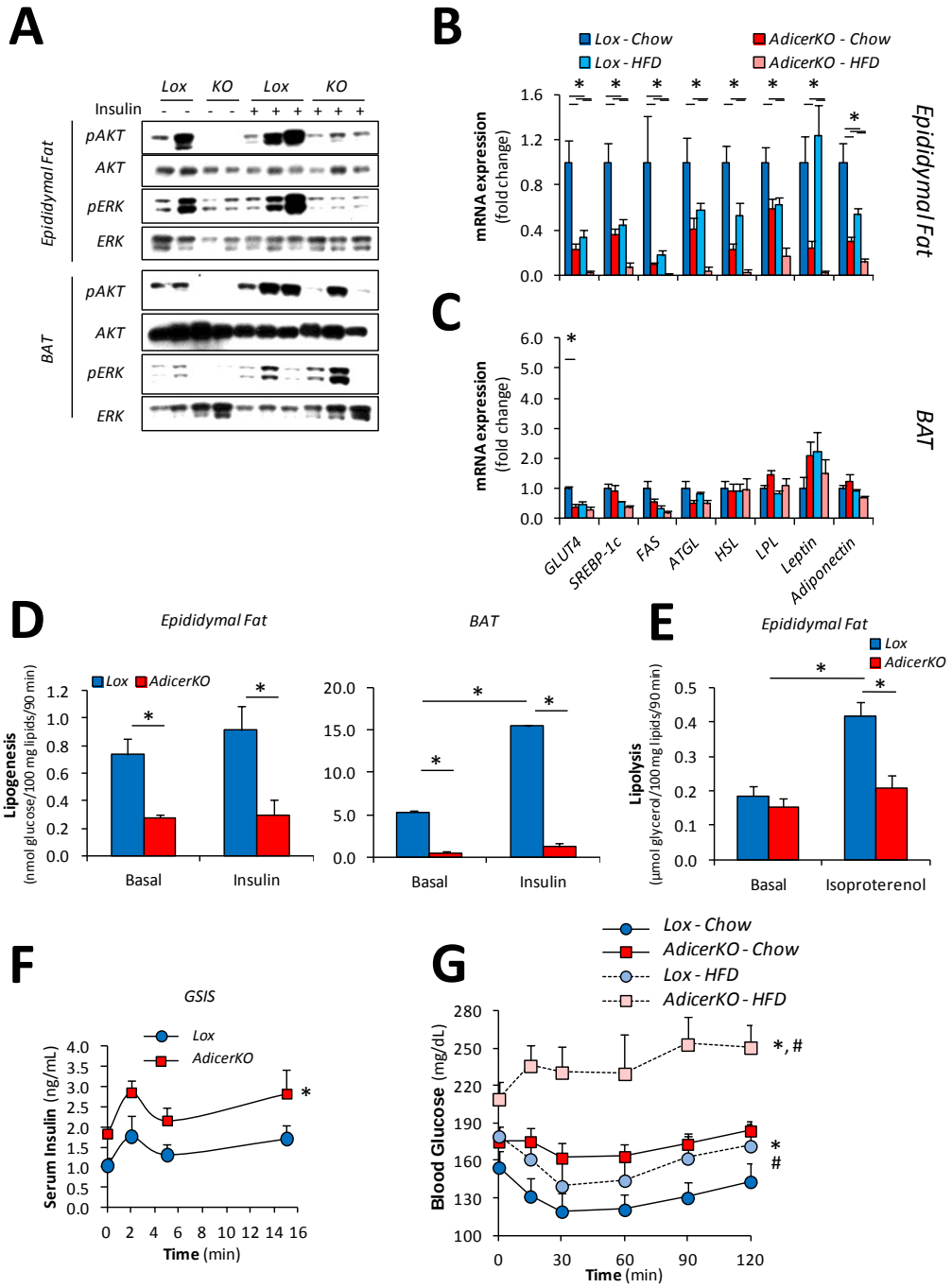

Figure S3. AdicerKO mice are insulin resistant.

(A) Four-month old mice were injected with either saline or 10U insulin and AKT and ERK phosphorylation were assessed by western blotting in epididymal adipose tissue and interscapular brown adipose tissue (BAT).

(B,C,G) Ten-week old AdicerKO and Lox control mice were put on a high fat diet (HFD) or maintained on a chow diet for 5 months prior to the sacrifice (N = 6-12 animals per group).

(B,C) Gene expression analyses in (C) epididymal and (D) interscapular brown adipose tissues.

(D) Lipogenesis and (E) lipolysis were assessed in isolated adipocytes of the indicated tissues of 4-month old mice (N = 8 per group).

(F) Glucose-stimulated insulin secretion (GSIS). Mice were 4-month old and were feeding a chow diet when the experiment was performed (N = 7-9 mice per group).

(G) Intraperitoneal insulin tolerance test was performed using 1U insulin/Kg body weight injection.

* $P < 0.05$. Values are mean ± SEM.

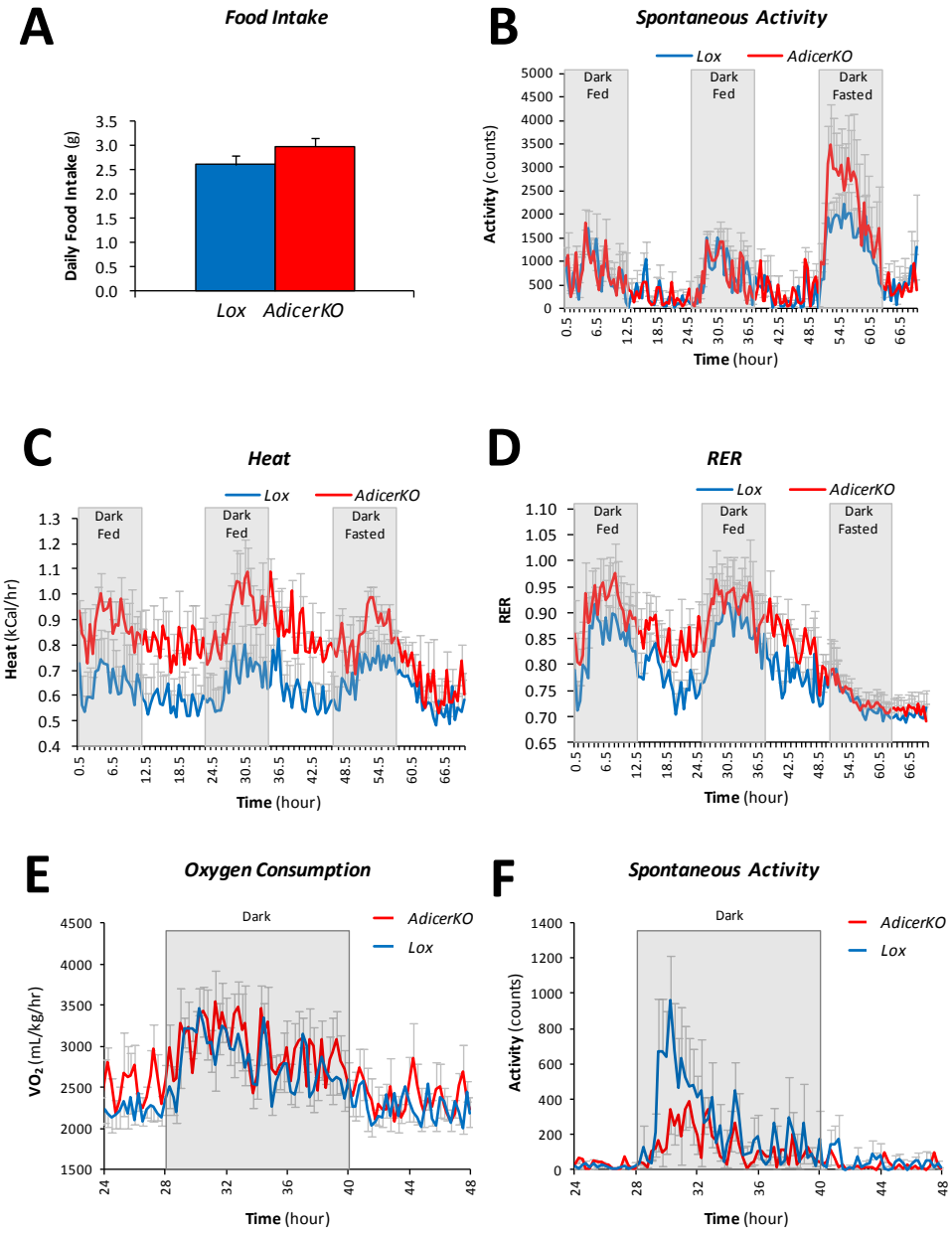

Figure S4. Bioenergetic profile of AdicerKO mice.

(A-D) Six-month old females were subjected to CLAMS analysis in which (A) food intake, (B) spontaneous activity, (C) heat production and (D) respiratory exchange ratio (RER, VCO_2/VO_2) were measured (N = 4 mice per group). Values are mean ± SEM.

(E,F) Six-month old males were subjected to CLAMS analysis in which (E) oxygen consumption (normalized by lean mass) and (F) spontaneous activity were measured (N = 5 mice per group). Values are mean ± SEM.

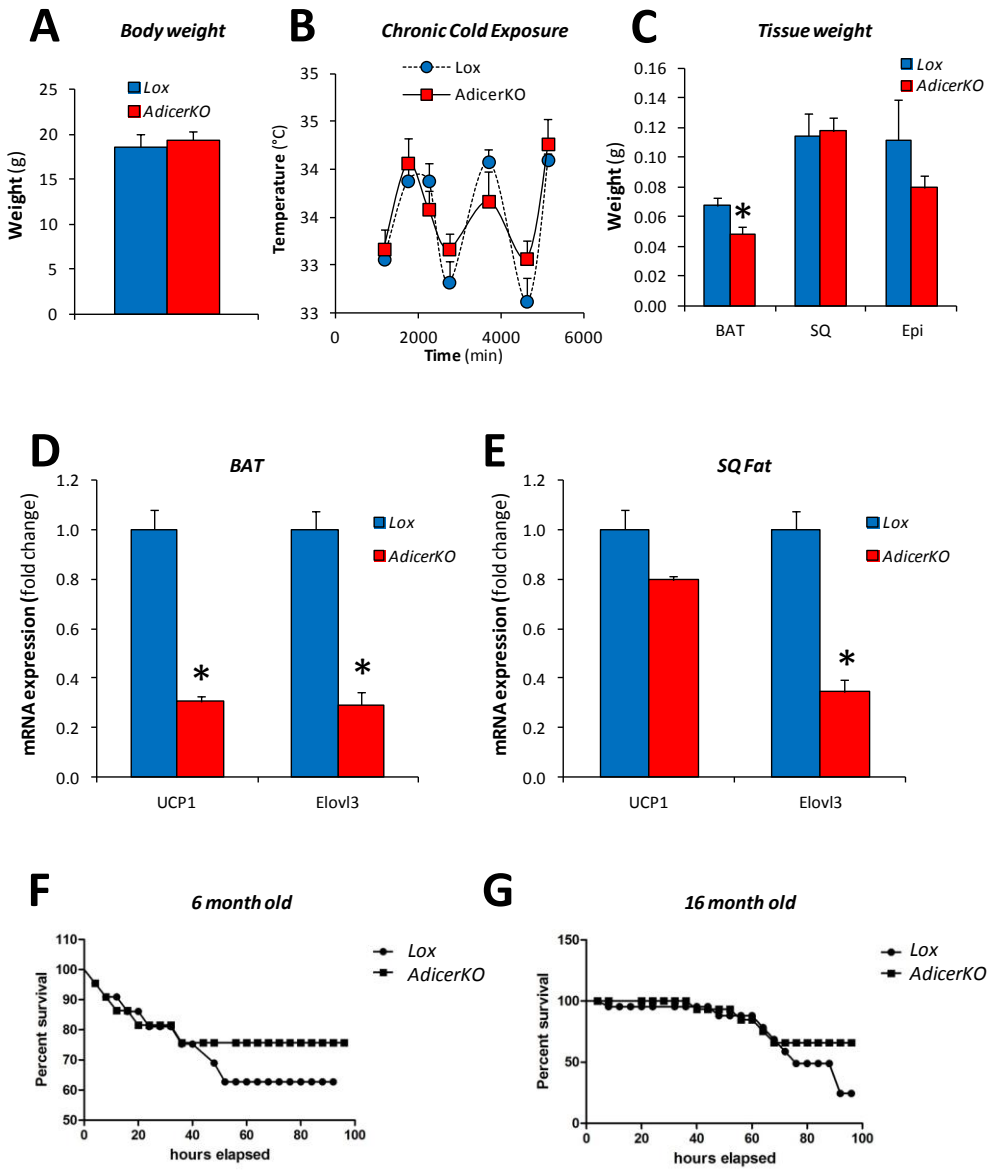

Figure S5. Age-related metabolic responses in AdicerKO mice.

(A-E) Two-month old mice were subjected to 4-day cold exposure and (A) body weight, (B) rectal temperature, and (C) tissue weight were measured. *UCP1* and *Elovl3* mRNA expression was measured in (D) interscapular brown adipose tissue (BAT) and in (E) flank subcutaneous white adipose tissue (SQ fat). N = 5-6 animals per group. Values are mean ± SEM.

(F,G) Survival of mice in response to intraperitoneal injection of paraquat. (F) 6 months of age. (G) 16 months of age. N = 8-12 animals per group.

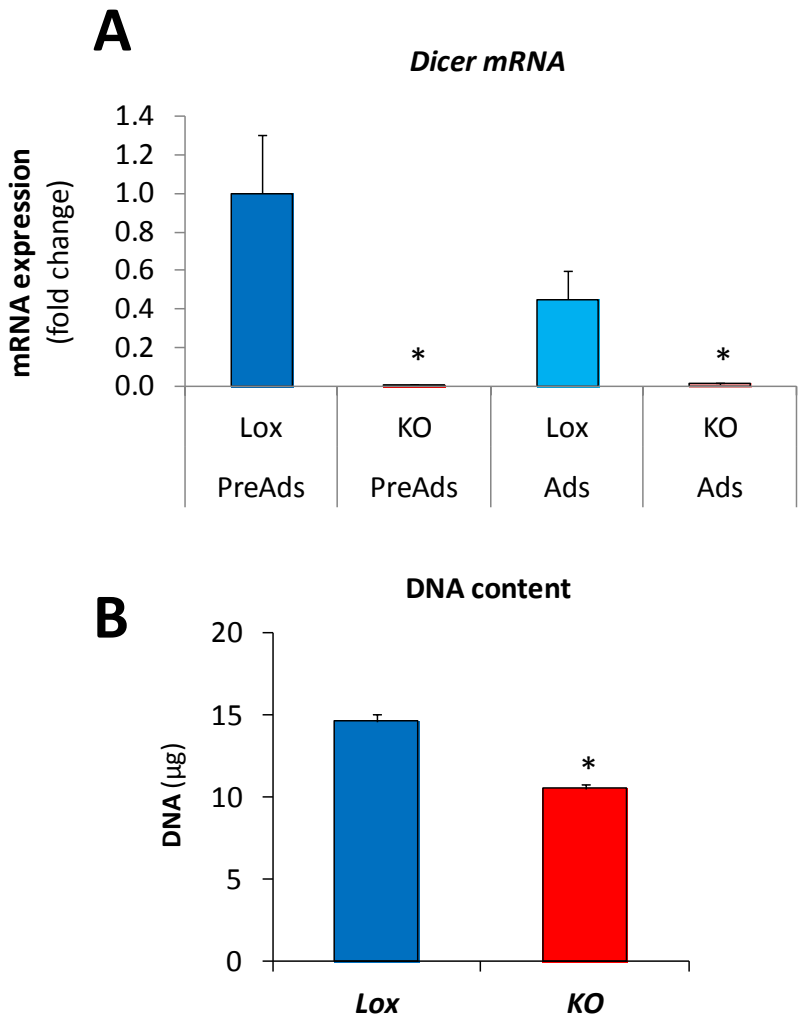

Figure S6. Characterization of Dicer knockout adipocytes.

Immortalized interscapular brown preadipocytes of *Dicer*$^{lox/lox}$ mice were transduced with adenoviruses harboring GFP (Lox) or Cre recombinase (Dicer knockout, KO) and, 4 days later (PreAds, day 0), cells were differentiated in vitro into adipocytes (Ads, day 8).

(A) *Dicer* mRNA expression as assessed by qPCR (N = 3 per group).

(B) DNA content per well of a 6-well plate of day 8 differentiated adipocytes (N = 3 per group).

* $P < 0.05$. Values are mean ± SEM.

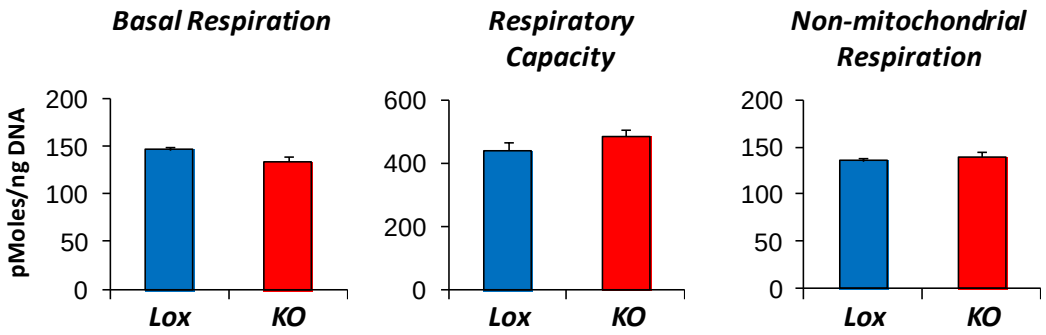

Figure S7. Bioenergetic profile of Dicer knockout in adipocytes.

Immortalized interscapular brown preadipocytes of *Dicer*$^{lox/lox}$ mice were transduced with adenoviruses harboring GFP (Lox) or Cre recombinase (Dicer knockout, KO) and, 4 days later, cells were differentiated in vitro into adipocytes. Basal respiration, respiratory capacity and non-mitochondrial respiration were measured using the Seahorse Bioscience XF24-3 Extracellular Flux Analyzer (N = 5 per group). Values are mean ± SEM.

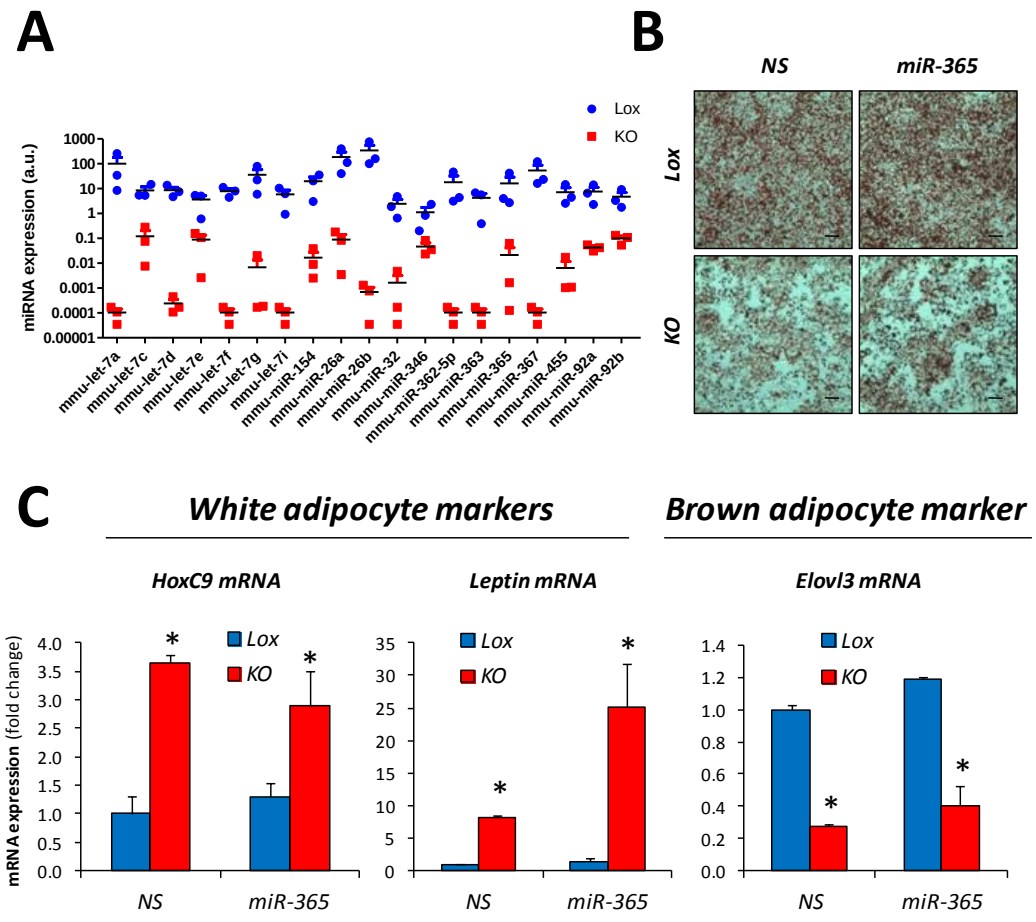

Figure S8. Role of miRNAs in the identity switch phenotype of Dicer knockout adipocytes.

Immortalized interscapular brown preadipocytes of *Dicer*$^{lox/lox}$ mice were transduced with adenoviruses harboring GFP (Lox) or Cre recombinase (Dicer knockout, KO). (A) miRNA expression was measured at the end of the adipocyte differentiation protocol. (B,C) Three days after the adenoviral transduction, Lox and Dicer knockout preadipocytes were transfected with *miR-365* mimic or a non-silencing control (NS). Adipocyte differentiation started in the following day (day 0) and (B) oil-red O staining was performed (200x using optical microscope) and (C) adipocyte gene expression markers were measured when cells were fully differentiated (day 8) (N = 3 per group). * $P < 0.05$. Values are mean ± SEM.

SUPPLEMENTAL TABLES

Table S1. Hyperinsulinemic-euglycemic clamps in 5-month old AdicerKO mice.

	Lox	**AdicerKO**	
	Mean ± SEM	Mean ± SEM	*P value*
Body Weight (g)	23 ± 1	22 ± 1	0.502
Fat Mass (g)	2.6 ± 0.3	2.1 ± 0.2	0.153
Lean Mass (g)	22.7 ± 0.8	23.0 ± 1.3	0.836
Basal Glucose (mg/dl)	96 ± 9	116 ± 10	0.170
Clamp Glucose (mg/dl)	122 ± 5	125 ± 6	0.705
Glucose Turnover (mg/kg/min)	80.8 ± 3.0	67.0 ± 3.2*	*0.010*
Glycolysis (mg/kg/min)	55.6 ± 2.0	43.0 ± 5.9	0.071
Glycogen Synthesis (mg/kg/min)	25.2 ± 3.5	24.0 ± 4.9	0.850
Skeletal Muscle Glucose Uptake (mg/kg/min)	666 ± 85	442 ± 58	0.055
Heart Glucose Uptake (mg/kg/min)	2146 ± 76	1849 ± 93*	*0.033*

N = 6 animals per group. * $P < 0.05$

Table S2. Body Composition of 6-week old AdicerKO mice.

	Lox	**AdicerKO**	
	Mean ± SEM	Mean ± SEM	*P value*
Body Weight (g)	21.5 ± 1.8	21.3 ± 1.2	0.954
Blood Glucose (mg/dL)	230 ± 5.3	218.7 ± 6.8	0.257
Epididymal Fat (g)	0.30 ± 0.03	0.24 ± 0.03	0.210
Inguinal Fat (g)	0.23 ± 0.01	0.26 ± 0.08	0.694
Interscapular Brown Fat (g)	0.08 ± 0.03	0.08 ± 0.00	0.902
Liver (g)	1.00 ± 0.09	0.91 ± 0.04	0.423
Brain (g)	0.45 ± 0.02	0.44 ± 0.01	0.789
Femur (cm)	1.4 ± 0.0	1.4 ± 0.1	0.643

N = 3 animals per group.

Table S3. Body Composition of iaP2DicerKO mice.

		Lox			iaP2DicerKO		
Total		*Mean*	*±*	*SEM*	*Mean*	*±*	*SEM*
	Body Weight (g)	26.50	±	0.76	29.30	±	0.51*
	Total (g)	27.01	±	0.71	28.71	±	0.50*
	Fat Mass (g)	5.84	±	0.40	5.94	±	0.48
	Lean Mass (g)	21.19	±	0.46	22.77	±	0.30*
	% Fat	21.29	±	1.16	20.67	±	1.35
Abdomen							
	Total (g)	7.11	±	0.11	7.56	±	0.23*
	Fat Mass (g)	1.54	±	0.14	1.41	±	0.18
	Lean Mass (g)	5.59	±	0.09	6.13	±	0.20*
	% Fat	20.84	±	1.77	18.54	±	2.02

Eight-month old male mice were subjected to DEXA scan. Total, calculated total mass. *, $P < 0.05$. N = 6-7 animals per group.

Table S4. Serum profile of 8-month old iaP2DicerKO mice.

	Lox			iaP2DicerKO		
	Mean	±	SEM	Mean	±	SEM
Insulin (ng/mL)	0.91	±	0.18	1.20	±	0.19
IGF-1 (ng/mL)	84.70	±	27.07	119.16	±	17.21
Leptin (ng/mL)	7.11	±	1.19	10.90	±	1.88
T3 (ng/mL)	1.23	±	0.07	1.18	±	0.10
TG (mg/dL)	73.68	±	4.91	93.86	±	8.75
Cholesterol (mg/dL)	111.44	±	5.21	110.98	±	2.97
FFA (mEq/L)	1.50	±	0.09	1.59	±	0.16

TG, triglycerides. FFA, free fatty acids. *, $P < 0.05$. N = 6-7 animals per group.

Table S5. Gene Set Enrichment Analysis of Dicer KO vs. Lox preadipocytes .

NAME	SIZE	NOM p-val	FDR q-val
CTACCTC,LET-7A,LET-7B,LET-7C,LET-7D,LET-7E,LET-7F,MIR-98,LET-7G,LET-7I	270	0.000	0.045
GGCAGAC,MIR-346	27	0.008	0.108
GGGCATT,MIR-365	83	0.000	0.085
ATAACCT,MIR-154	46	0.007	0.144
GGCACAT,MIR-455	43	0.004	0.116
GTGCAAT,MIR-25,MIR-32,MIR-92,MIR-363,MIR-367	233	0.000	0.149
TACTTGA,MIR-26A,MIR-26B	232	0.000	0.149
CAAGGAT,MIR-362	52	0.030	0.246

miRNAs which targets are enriched among the genes up-regulated in Dicer KO preadipocytes. Size: size of the gene set, representing the number of genes predicted to be targeted by the miRNA or miRNA family. NOM p-val, nominal p-value. FDR q-val, false discovery rate q-value. A q-value < 0.25 was considered significant.

Table S6. Demographic and Clinical Characteristics of HIV-Infected Participants and controls of the Boston cohort.

	HIV lipodystrophy	*Control*
N	6	14
Age (years)	51 ± 3	43 ± 4
Lipohypertrophic [n (%)]	3 (50)	0
Lipoatrophic [n (%)]	3 (50)	0
Duration HIV (yrs)	16 ± 2	0
Duration ART use (yrs)	11 ± 2	0
PI use [n (%)]	4 (67)	0
NRTI use [n (%)]	5 (83)	0
NNRTI [n (%)]	1 (17)	0
CD4 cell count (#/mm3)	450 ± 95	ND
HIV RNA viral load (copies/mL, $\log_{10}$)	2.2 ± 0.5	ND
Detectable HIV RNA viral load [n (%)]	1 (17)	ND
BMI (kg/m^2)	23.7 ± 2.1	30.9 ± 2.4
Weight (kg)	67 ± 7	89 ± 7.7

All data are reported as mean ± SEM. ART, antiretroviral therapy; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor. ND, not determined.

Table S7. Demographic and Clinical Characteristics of HIV-Infected Participants and controls of the Finnish cohort.

	Control (n =60)	**HIV/LA+** (n=19)	**HIV/LA-** (n=8)	***P* value** (LA+ vs LA-)
Women/men	39/21	1/18***	0/8**	1.0000
Age (*years*)	45 (24-65)	45 (41-52)	41 (34-51)	0.3004
BMI (*kg/m²)*	28.7 ± 0.7	24.1 ± 0.7**	24.3 ± 0.7*	0.8536
Waist-to-hip ratio	0.91 ± 0.01	1.00 ± 0.01***	0.94 ± 0.02	0.0069 *
Liver fat (*%*)	3.5 (1.6-11.1)	3.4 (1.3-19.5)	1.0 (0.8-1.3)*	0.0063 *
fP-glucose (*mmol/L*)	5.5 (5.1-5.9)	5.2 (4.9-5.8)	5.2 (5.0-5.7)	0.8944
HbA_{1C} (*%*)	5.6 (5.3-5.8)	5.1 (4.7-5.7)*	4.6 (4.2-4.8)***	0.0194 *
fS-insulin (*mU/L*)	6.7 (4.0-11.2)	10.0 (5.9-17.8)	4.8 (4.0-6.1)	0.0224 *
fS-C-peptide	0.68 (0.53-0.96)	0.73 (0.58-1.01)	0.44 (0.37-0.75)*	0.0526
HOMA-IR index	1.7 (0.9-2.7)	2.3 (1.0-4.3)	1.1 (0.9-1.5)	0.0631
fP-HDL cholesterol (*mmol/L*)	1.4 (1.2-1.7)	1.1 (1.0-1.3)***	1.9 (1.5-2.1)	0.0004 ***
fP-LDL cholesterol (*mmol/L*)	2.8 ± 0.1	2.9 ± 0.2	2.4 ± 0.3	0.2313
fP-triglycerides (*mmol/L*)	1.0 (0.8-1.7)	2.7 (1.9-4.0)***	1.3 (0.9-1.7)	0.0021 **
S-ALT	24.0 (17.0-42.5)	30.0 (20.0-54.0)	29.5 (22.0-30.5)	0.5590

Data are shown as mean ± SEM or median followed by the 25^{th} and 75^{th} percentile. ALT, alanine aminotransferase; BMI, body mass index; fP, fasting plasma; fS, fasting serum; HbA_{1C}, glycosylated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance (calculated from the formula: fasting glucose (mmol/L) × fasting insulin (mU/L) / 22.5; LDL, low-density lipoprotein. *P < 0.05, **P < 0.005, ***P < 0.0005. HIV/LA+, HIV-infected patients undergoing combination antiretroviral therapy and exhibiting loss of subcutaneous adipose tissue with or without accumulation of adipose tissue intra-abdominally or in the dorsocervical upper trunk. HIV/LA-, HIV-infected patients undergoing combination antiretroviral therapy without developing the aforementioned changes in body fat composition.

Table S8. Primer sequences.

Gene	Species	Forward	Reverse
Adiponectin	*Mouse*	GATGGCACTCCTGGAGAGAA	TCTCCAGGCTCTCCTTTCCT
aP2	*Mouse*	GATGCCTTTGTGGGAACCT	CTGTCGTCTGCGGTGATTT
ATGL	*Mouse*	ACTGTGGCCTCATTCCTCCT	AACTGGATGCTGGTGTTGGT
C/EBP-α	*Mouse*	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
C/EBP-β	*Mouse*	CCAAGAAGACGGTGGACAA	CAAGTTCCGCAGGGTGCT
CD68	*Mouse*	GCAGCACAGTGGACATTCAT	TTGCATTTCCACAGCAGAAG
CIDEA	*Mouse*	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Dicer	*Mouse*	AGATGCTTGGCGACTCCTT	TTGCTGACCTTTTTGCTTCTC
Dio2	*Mouse*	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Elovl3	*Mouse*	GGACTTAAGGCCCTTTTTGG	TTCCGCGTTCTCATGTAGGT
FAS	*Mouse*	GAGGACACTCAAGTGGCTGA	GTGAGGTTGCTGTCGTCTGT
GLUT4	*Mouse*	TGATTCTGCTGCCCTTCTGT	GGACATTGGACGCTCTCTCT
HSL	*Mouse*	ACGGATACCGTAGTTTGGTGC	TCCAGAAGTGCACATCCAGGT
Leptin	*Mouse*	GGGCTTCACCCCATTCTGA	TGGCTATCTGCAGCACATTTTG
LPL	*Mouse*	AGTAGACTGGTTGTATCGGG	AGCGTCATCAGGAGAAAGG
PGC-1α	*Mouse*	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
PPAR-γ	*Mouse*	TCAGCTCTGTGGACCTCTCC	ACCCTTGCATCCTTCACAAG
PRDM16	*Mouse*	ACATCCGTGTAGCGTGTTCC	GCACCAACAGTTCCTCTCCA
SREBP-1c	*Mouse*	ATCTCCTAGAGCGAGCGTTG	TATTTAGCAACTGCAGATATCCAA
UCP1	*Mouse*	CTGCCAGGACAGTACCCAAG	TCAGCTGTTCAAAGCACACA
Dicer	*Human*	TCAACAGCCAACAAAAGAACC	AATACAGAAGAGCGTGAACTGG
TBP	*Human*	TTCGGAGAGTTCTGGGATTG	CGAAGTGCAATGGTCTTTAGGT
36B4	*Human*	CATGCTCAACATCTCCCCCTTCTCC	GGGAAGGTGTAATCCGTCTCCACAG