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Supplemental Information

The Lipid Handling Capacity of Subcutaneous Fat Is Programmed by mTORC2 during Development

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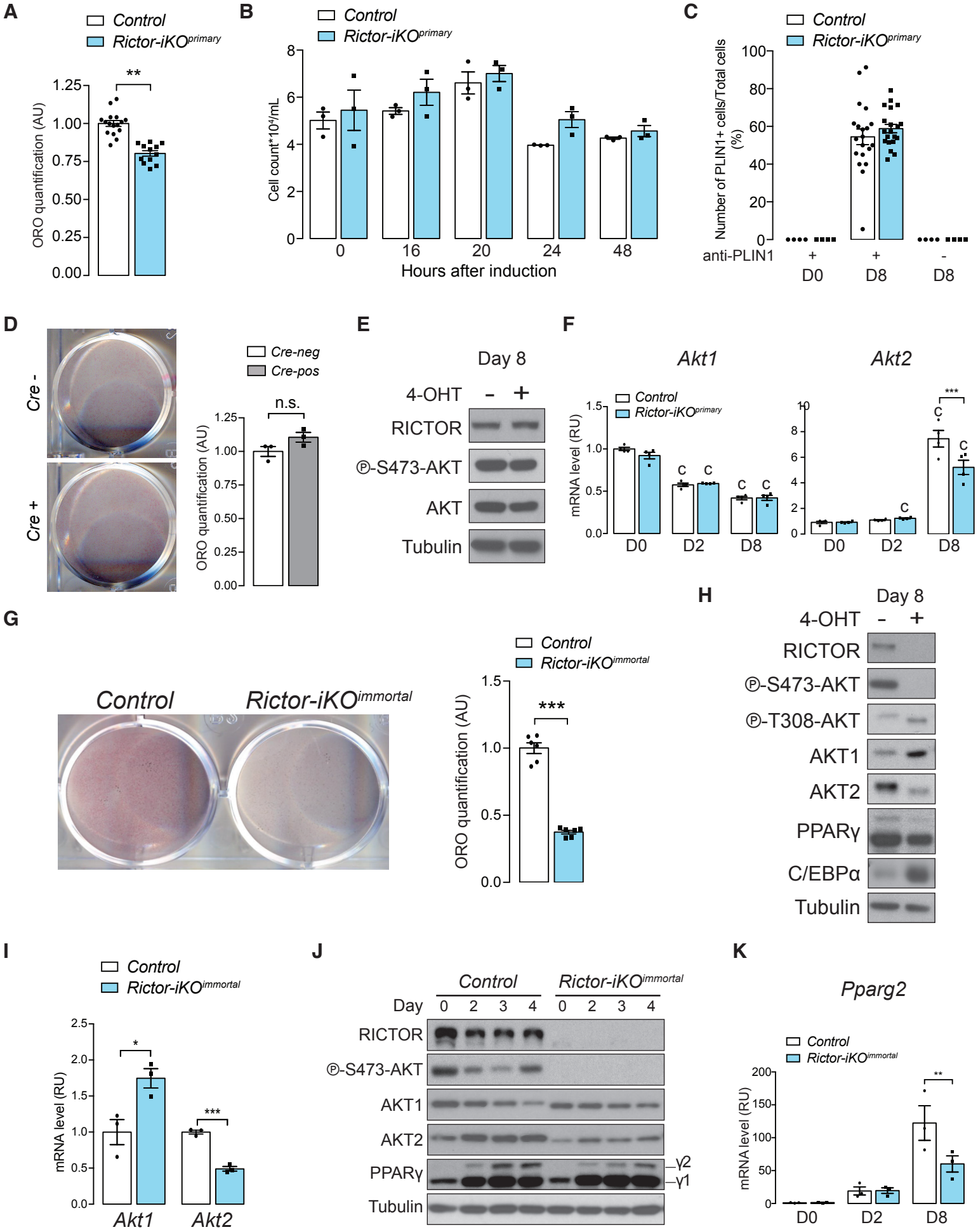
A
Control
Rictor-iKOprimary
ORO quantification (AU)
**
B
Control
Rictor-iKOprimary
Cell count*10⁴/mL
Hours after induction
C
Control
Rictor-iKOprimary
Number of PLIN1+ cells/Total cells (%)
anti-PLIN1
D0
D8
D8
D
Cre -
Cre +
Cre-neg
Cre-pos
n.s.
ORO quantification (AU)
E
Day 8
4-OHT
RICTOR
Ⓟ-S473-AKT
AKT
Tubulin
F
Akt1
Akt2
Control
Rictor-iKOprimary
mRNA level (RU)
D0
D2
D8
G
Control
Rictor-iKOimmortal
ORO quantification (AU)
H
Day 8
4-OHT
RICTOR
Ⓟ-S473-AKT
Ⓟ-T308-AKT
AKT1
AKT2
PPARγ
C/EBPα
Tubulin
I
Control
Rictor-iKOimmortal
mRNA level (RU)
Akt1
Akt2
J
Control
Rictor-iKOimmortal
Day
RICTOR
Ⓟ-S473-AKT
AKT1
AKT2
PPARγ
γ2
γ1
Tubulin
K
Pparg2
Control
Rictor-iKOimmortal
mRNA level (RU)
D0
D2
D8

Figure S1, related to Figure 1.

A Quantification (quant) of the Oil Red O (ORO) staining of differentiated control (EtOH) and *Rictor-iKO*$_{primary}$ (4-OHT) cells after isopropanol extraction and direct ORO content measurement (data represent mean ± SEM; $^{**}p < 0.01$). See also **Figure 1B**.

B Cell number quantification of control *and Rictor-iKO*$_{primary}$ cells at indicated time points during the first 48 hours of differentiation (data represent mean ± SEM).

C Percentage of Perilipin 1-positive (PLIN1+) cells per image taken from the immunofluorescence staining in **Figure 1C** (data represent mean ± SEM).

D ORO staining of differentiated primary *preadipocytes isolated from a UBC-Cre*^{ERT2} mouse. *Right panel*: quantification (quant) of the Oil Red O (ORO) staining. (data represent mean ± SEM; $^{**}p < 0.01$). Cre-: cells treated with EtOH (without Cre). Cre+: cells treated with 4-OHT (with Cre expression). n.s.: not significant.

E Western blot of lysates from differentiated (Day 8) *UBC-Cre*^{ERT2} cells with (4-OHT-treated) or without (EtOH-treated) Cre expression.

F Relative mRNA expression by RT-PCR of *Akt* isoforms in control and *Rictor-iKO*$_{primary}$ cells at the indicated differentiation days (data represent mean ± SEM; $^{***}p < 0.001$; c: $^{***}p < 0.001$ when compared to D0 cells).

G ORO staining of differentiated control and *Rictor-iKO*$_{immortal}$ cells. *right panel*: Quantification of the ORO staining of differentiated control and *Rictor-iKO*$_{immortal}$ cells after isopropanol extraction and direct ORO content measurement (data represent mean ± SEM; $^{***}p < 0.001$).

H Western blot of lysates from differentiated (Day 8) control and *Rictor-iKO*$_{immortal}$ cells.

I Relative mRNA expression by RT-PCR of *Akt* isoforms in differentiated control and *Rictor-iKO*$_{immortal}$ cells (data represent mean ± SEM; $^{*}p < 0.05$, $^{***}p < 0.001$).

J Western blot of lysates from control and *Rictor-iKO*$_{immortal}$ cells at early differentiation time points (day 0 through day 4). γ1: PPARγ1 isoform; γ2: PPARγ2.

K Relative mRNA expression by RT-PCR of *Akt* isoforms in control and *Rictor-iKO*$_{immortal}$ cells at indicated differentiation days (data represent mean ± SEM; $^{**}p < 0.01$).

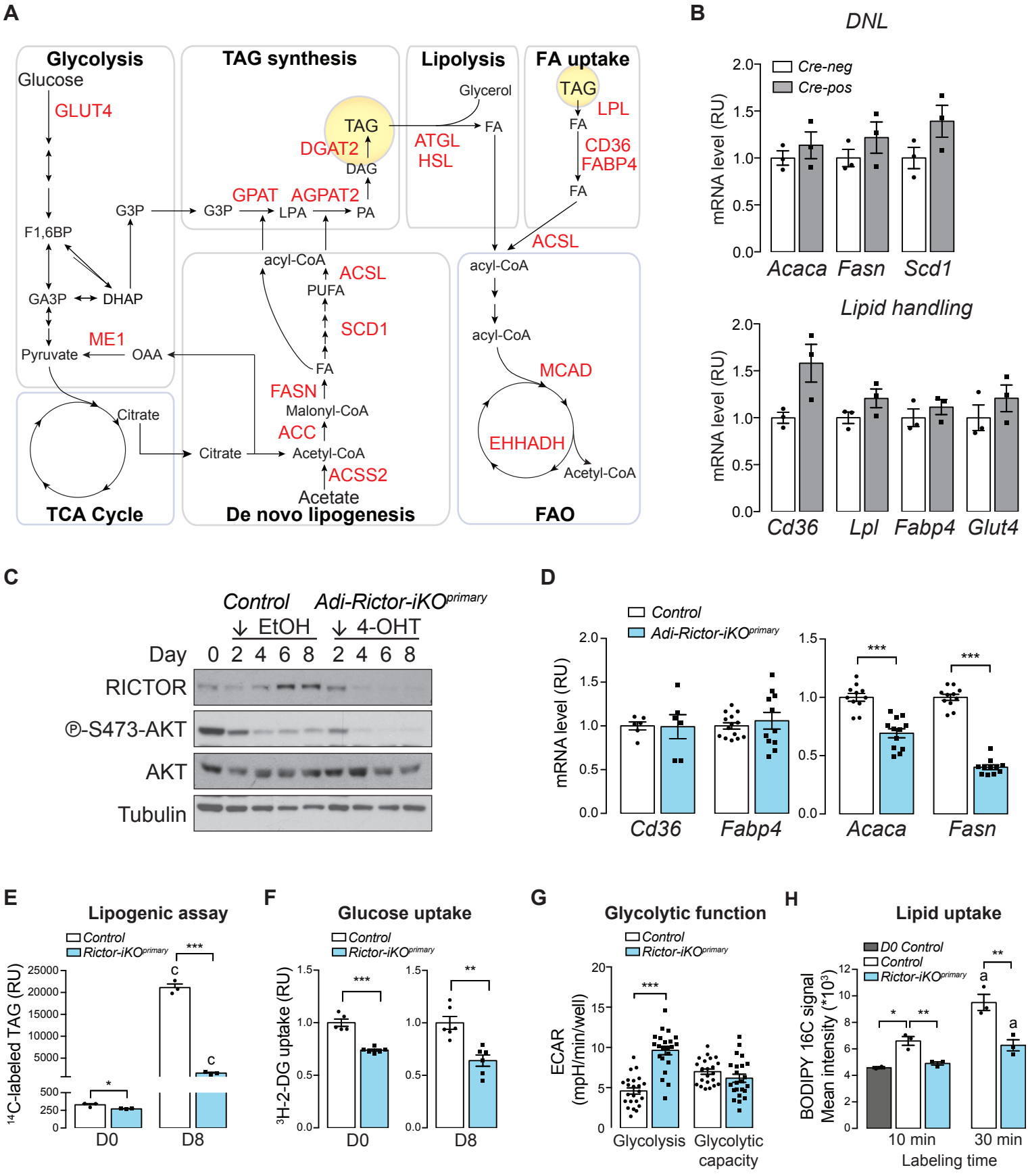
A
Glycolysis
Glucose
GLUT4
F1,6BP
G3P
GA3P
DHAP
ME1
Pyruvate
OAA
Citrate
TCA Cycle
TAG synthesis
TAG
DGAT2
DAG
GPAT
AGPAT2
G3P
LPA
PA
acyl-CoA
ACSL
PUFA
SCD1
FA
FASN
Malonyl-CoA
ACC
Citrate
Acetyl-CoA
ACSS2
Acetate
De novo lipogenesis
Lipolysis
Glycerol
TAG
ATGL
HSL
FA
FA uptake
TAG
LPL
FA
CD36
FABP4
FA
ACSL
acyl-CoA
acyl-CoA
MCAD
EHHADH
Acetyl-CoA
FAO
B
DNL
Cre-neg
Cre-pos
mRNA level (RU)
2.0
1.5
1.0
0.5
0.0
Acaca
Fasn
Scd1
Lipid handling
mRNA level (RU)
Cd36
Lpl
Fabp4
Glut4
C
Control
Adi-Rictor-iKOprimary
↓ EtOH
↓ 4-OHT
Day 0 2 4 6 8 2 4 6 8
RICTOR
Ⓟ-S473-AKT
AKT
Tubulin
D
Control
Adi-Rictor-iKOprimary
mRNA level (RU)
***
***
Cd36
Fabp4
Acaca
Fasn
E
Lipogenic assay
Control
Rictor-iKOprimary
14C-labeled TAG (RU)
25000
20000
15000
10000
5000
500
250
0
*
***
c
c
D0
D8
F
Glucose uptake
Control
Rictor-iKOprimary
3H-2-DG uptake (RU)
1.5
1.0
0.5
0.0
***
**
D0
D8
G
Glycolytic function
Control
Rictor-iKOprimary
ECAR (mpH/min/well)
20
15
10
5
0
***
Glycolysis
Glycolytic capacity
H
Lipid uptake
D0 Control
Control
Rictor-iKOprimary
BODIPY 16C signal Mean intensity (*10³)
12
10
8
6
4
2
0
*
**
**
a
a
10 min
30 min
Labeling time

Figure S2, related to Figure 2.

A Pathway clusters of the *Rictor*-required adipogenic genes modified by KEGG pathway analysis. Genes highlighted in red: down-regulatory genes identified as PPARγ/ChREBP/SREBP1 targets. Also see **Figure 2C**. FAO: fatty acid oxidation, TCA cycle: tricarboxylic acid cycle; Abbreviations of metabolites: F1,6BP: fructose-1,6-bisphosphate, GA3P: glyceraldehyde 3-phosphate, DHAP: dihydroxyacetone phosphate, LPA: lysophosphatidic, PA: phosphatidic acid, DAG: diacylglycerol, TAG: triacylglycerol, FA: fatty acid, PUFA: polyunsaturated fatty acid. Abbreviations of gene names: GLUT4: Glucose transporter type 4, ME1: Malic enzyme 1, GPAT: Glycerol-3-phosphate acyltransferase, AGPAT2: 1-acylglycerol-3-phosphate O-acyltransferase 2, DGAT2: Diacylglycerol O-acyltransferase 1, ACSS2: Acyl-CoA Synthetase Short Chain Family Member 2, ACC: Acetyl-CoA carboxylase, FASN: Fatty acid synthase, SCD1: Stearoyl-CoA desaturase 1, ACSL: Acyl-CoA synthetase long-chain, HSL: Hormone-sensitive lipase. ATGL: Adipose triglyceride lipase, LPL: Lipoprotein lipase, FABP4: Fatty acid binding protein 4, MCAD: Medium-chain acyl-CoA dehydrogenase, EHHADH: Enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase.

B Relative mRNA expression by RT-PCR of genes in the DNL and lipid handling pathways in differentiated *primary UBC-Cre$_{ERT2}$* cells with (Cre-pos, 4-OHT-treated) or without (Cre-neg, EtOH-treated) Cre expression (data represent mean ± SEM). See also **Figure 1A**.

C Western blot of lysates from control and *Rictor-iKO$_{primary}$* cells with *Rictor* deleted from D2 (*Adi-Rictor-iKO$_{primary}$*) at indicated differentiation time points. Arrow: Starting point of 4-OHT exposure.

D Relative mRNA expression by RT-PCR of genes in the dnl and lipid handling pathways in control and *Adi-Rictor-iKO$_{primary}$* cells. Data represent mean ± SEM; $^{***}p < 0.001$.

E Lipogenic assay measured by 14C-glucose incorporation into 14C-TAG of undifferentiated (D0) and differentiated (D8) control *and Rictor-iKO$_{primary}$* cells. Data represent mean ± SEM; $^{*}p < 0.05$, $^{***}p < 0.001$. c: $p < 0.001$ relative to its D0 counterpart. RU: relative unit (calculated from CPM per μg of protein).

F ^{3}H-2-deoxyglucose (^{3}H-2-DG) uptake of undifferentiated (D0) and differentiated (D8) control *and Rictor-iKO$_{primary}$* cells. Data represent mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$. RU: relative unit (calculated from CPM per minute per μg of protein).

G Glycolytic function determined by extracellular acidification rate (ECAR) of differentiated control *and Rictor-iKO$_{primary}$* cells. Data represent mean ± SEM; $^{***}p < 0.001$.

H BODIPY FL C16 lipid uptake of undifferentiated (D0) and differentiated (D8) control *and Rictor-iKO$_{primary}$* cells incubated in either 10 or 30 minutes. Mean intensity was determined by flow cytometry. Data represent mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$. a: $p < 0.05$ when compared to its counterpart in 10-minute group.

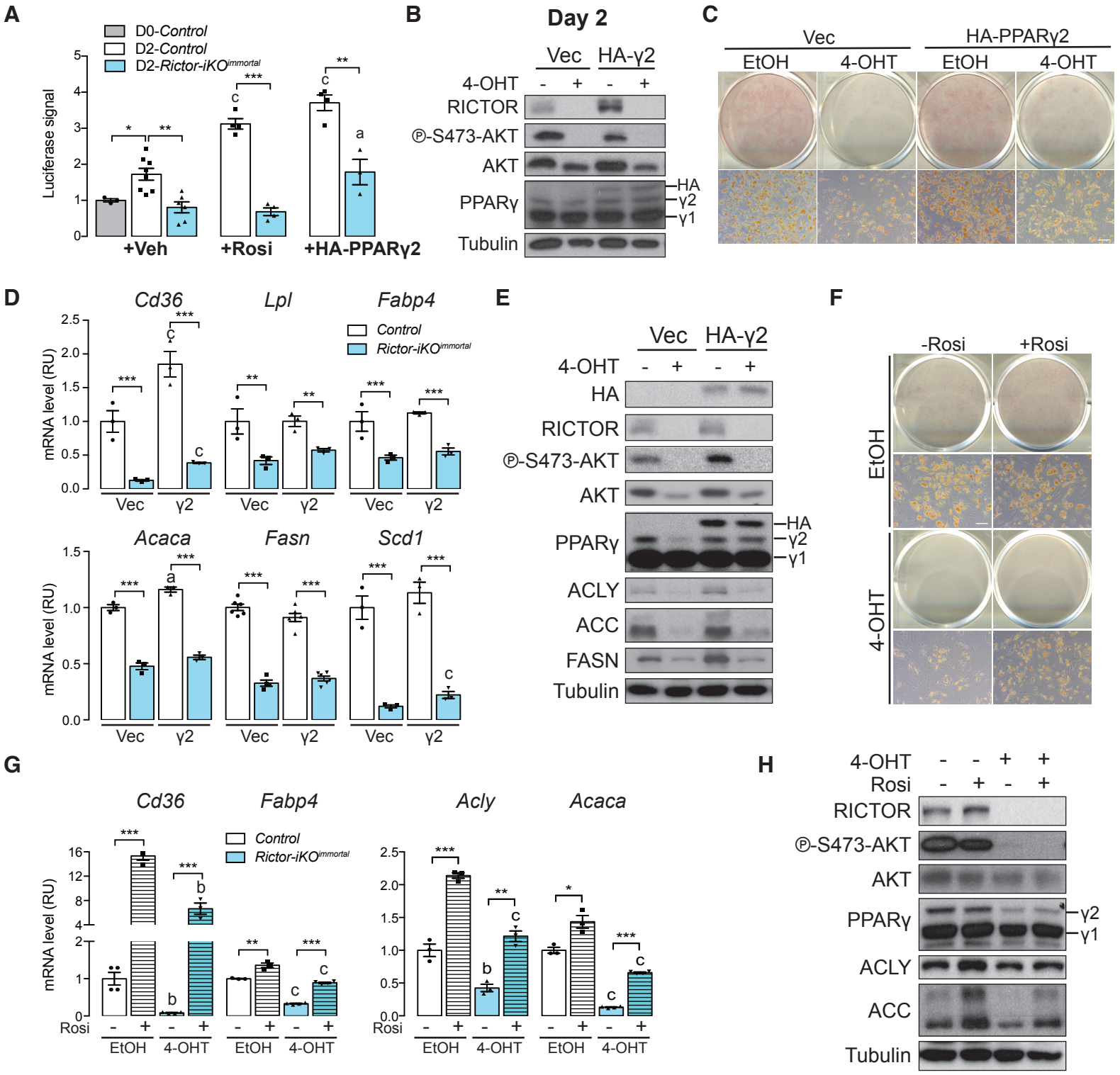
A
D0-Control
D2-Control
D2-Rictor-iKOimmortal
Luciferase signal
+Veh
+Rosi
+HA-PPARγ2
B
Day 2
Vec
HA-γ2
4-OHT
RICTOR
Ⓟ-S473-AKT
AKT
PPARγ
Tubulin
HA
γ2
γ1
C
Vec
HA-PPARγ2
EtOH
4-OHT
D
Cd36
Lpl
Fabp4
Control
Rictor-iKOimmortal
mRNA level (RU)
Vec
γ2
Acaca
Fasn
Scd1
E
Vec
HA-γ2
4-OHT
HA
RICTOR
Ⓟ-S473-AKT
AKT
PPARγ
ACLY
ACC
FASN
Tubulin
F
-Rosi
+Rosi
EtOH
4-OHT
G
Cd36
Fabp4
Acly
Acaca
Control
Rictor-iKOimmortal
mRNA level (RU)
Rosi
EtOH
4-OHT
H
4-OHT
Rosi
RICTOR
Ⓟ-S473-AKT
AKT
PPARγ
ACLY
ACC
Tubulin
γ2
γ1

Figure S3, related to Figure 3.

A Luciferase reporter gene assay with PPRE-responsive element (PPRE-Luc) in control and *Rictor-iKO*$_{immortal}$ cells with indicated treatment or transduction at day 2. Rosi: Rosiglitazone (data represent mean ± SEM; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; a: $*p < 0.05$ when compared to vehicle-treated cells; c: $***p < 0.001$ when compared to vehicle-treated cells).

B Western blot of lysates from control and *Rictor-iKO*$_{immortal}$ at day 2. HA-γ2: cells transduced with HA-PPARγ2.

C ORO staining of differentiated control and *Rictor-iKO*$_{immortal}$ cells overexpressing empty vector (Vec) or HA-PPARγ2 plasmid (scale bar = 50 μm).

D Relative mRNA expression of indicated genes from differentiated control and *Rictor-iKO*$_{immortal}$ cells overexpressing empty vector (vec) or HA-PPARγ2 (γ2) plasmid (data represent mean ± SEM; $**p < 0.01$, $***p < 0.001$; a: $*p < 0.05$ when compared to vector-expressing cells; c: $***p < 0.001$ when compared to vector-expressing cells).

E Western blot of lysates from differentiated control and *Rictor-iKO*$_{immortal}$ cells overexpressing empty vector (Vec) or HA-PPARγ2 (HA-γ2) plasmid.

F ORO staining of differentiated control and *Rictor-iKO*$_{immortal}$ cells (4-OHT-treated) with or without rosiglitazone (Rosi) supplement during differentiation (scale bar = 50 μm).

G Relative mRNA expression of indicated genes from differentiated control and *Rictor-iKO*$_{immortal}$ cells (4-OHT-treated) with or without Rosiglitazone (Rosi) supplement (data represent mean ± SEM; $**p < 0.01$, $***p < 0.001$; a: $*p < 0.05$ when compared to its counterpart in non-Rosi group; b: $**p < 0.01$; c: $***p < 0.001$).

H Western blot of lysates from differentiated control and *Rictor-iKO*$_{immortal}$ cells with or without Rosiglitazone (Rosi) supplement.

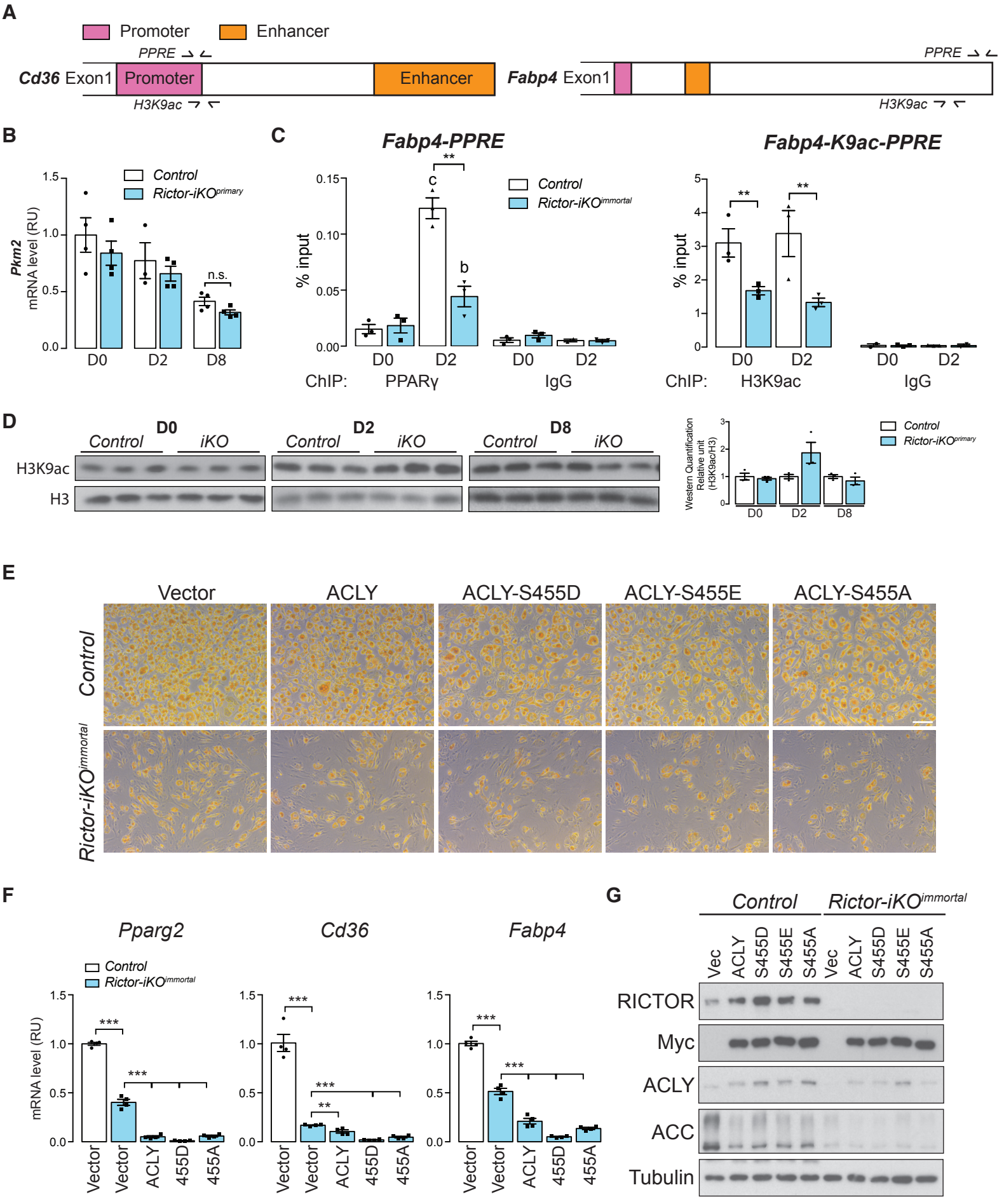
A
Promoter
Enhancer
PPRE
Cd36 Exon1
Promoter
Enhancer
H3K9ac
Fabp4 Exon1
PPRE
H3K9ac
B
Pkm2
mRNA level (RU)
Control
Rictor-iKOprimary
n.s.
D0
D2
D8
C
Fabp4-PPRE
Fabp4-K9ac-PPRE
% input
Control
Rictor-iKOimmortal
ChIP: PPARγ
IgG
ChIP: H3K9ac
IgG
D
D0
D2
D8
Control
iKO
H3K9ac
H3
Western Quantification
Relative unit
(H3K9ac/H3)
Control
Rictor-iKOprimary
E
Vector
ACLY
ACLY-S455D
ACLY-S455E
ACLY-S455A
Control
Rictor-iKOimmortal
F
Pparg2
Cd36
Fabp4
Control
Rictor-iKOimmortal
mRNA level (RU)
Vector
Vector
ACLY
455D
455A
G
Control
Rictor-iKOimmortal
Vec
ACLY
S455D
S455E
S455A
RICTOR
Myc
ACLY
ACC
Tubulin

Figure S4, related to Figure 3 and Figure 4.

A Graphic showing the primer binding site (arrows) used in the ChIP assays relative to the promoter and enhancer regions of indicated genes.

B Relative mRNA expression of *Pkm2* in differentiated control and *Rictor-iKO*$_{primary}$ cells (data represent mean ± SEM; n.s: not significant).

C PPARγ/PPAR-responsive element (PPRE) interaction and H3K9 acetylation (H3K9ac) identified by chromatin IP (ChIP) at *Fabp4* promoter in control and *Rictor-iKO*$_{immortal}$ cells (data represent mean ± SEM; $^{**}p < 0.01$; b: $^{**}p < 0.01$ when compared to D0 cells; c: $^{***}p < 0.001$ when compared to D0 cells). ChIP with IgG were used as negative controls.

D Western blot of extracted histones from control and *Rictor-iKO*$_{primary}$ cells at indicated days during differentiation. Right panel: quantification of H3K9 acetylation signals compared to total H3 (data represent mean ± SEM).

E Oil Red O (ORO) staining of differentiated control (EtOH) and *Rictor-iKO*$_{immortal}$ (4-OHT) cells overexpressing empty vector (Vec), Myc-ACLY, Myc-ACLY-S455D, Myc-ACLY-S455E, or Myc-ACLY-S455A (scale bar = 100 μm).

F Relative mRNA expression by RT-PCR of indicated genes in differentiated control and *Rictor-iKO*$_{immortal}$ cells overexpressing empty vector (Vec), ACLY, ACLY-S455D, ACLY-S455E, or ACLY-S455A (data represent mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$).

G Western blot of lysates from differentiated control and *Rictor-iKO*$_{immortal}$ cells overexpressing empty vector (Vec), ACLY or indicated ACLY mutants.

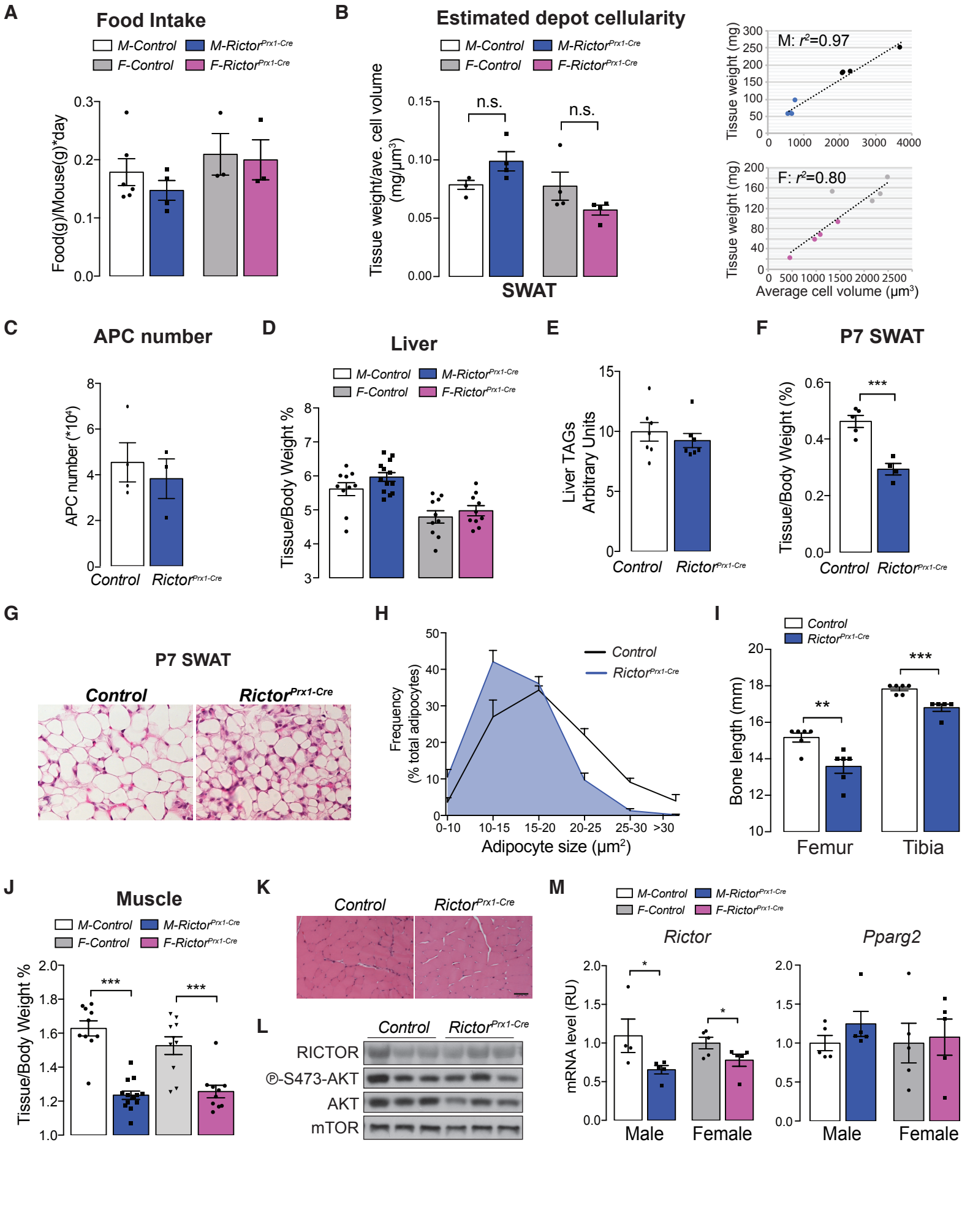
A
Food Intake
M-Control
M-Rictor Prx1-Cre
F-Control
F-Rictor Prx1-Cre
Food(g)/Mouse(g)*day
B
Estimated depot cellularity
Tissue weight/ave. cell volume (mg/μm3)
n.s.
n.s.
SWAT
Tissue weight (mg)
M: r2=0.97
F: r2=0.80
Average cell volume (μm3)
C
APC number
APC number (*10^4)
Control
Rictor Prx1-Cre
D
Liver
Tissue/Body Weight %
E
Liver TAGs Arbitrary Units
F
P7 SWAT
Tissue/Body Weight (%)
***
G
P7 SWAT
Control
Rictor Prx1-Cre
H
Frequency (% total adipocytes)
Adipocyte size (μm2)
0-10
10-15
15-20
20-25
25-30
>30
I
Bone length (mm)
Femur
Tibia
**
***
J
Muscle
Tissue/Body Weight %
K
L
RICTOR
Ⓟ-S473-AKT
AKT
mTOR
M
Rictor
Pparg2
mRNA level (RU)
Male
Female

Figure S5, related to Figure 6.

A Food intake of control and *Rictor*$^{Prx1\text{-}Cre}$ mice at 8 weeks of age (N = 3-6; data represent mean ± SEM).

B Estimated depot cellularity in SWAT from control and *Rictor*$^{Prx1\text{-}Cre}$ mice at 8 weeks of age (N = 4; data represent mean ± SEM). *right panel*: linear correlation of tissue weight versus average cell volume. r_2 = square of correlation. n.s.: not significant, M: male, F: female.

C Adipocyte precursor (APC) number isolated from SWAT using cell surface markers staining followed by FACS analysis (N= 4; data represent mean ± SEM).

D Liver weight relative to body weight of control and *Rictor*$^{prx1\text{-}Cre}$ mice at 8 weeks of age (N= 10-11; data represent mean ± SEM).

E TAG content of livers from 8-week-old female mice. Data represent mean ± SEM.

F SWAT weight relative to body weight of control and *Rictor*$^{Prx1\text{-}Cre}$ mice at postpartum day 7 (P7) (N =4; data represent mean ± SEM; ***p < 0.001).

G H&E stains of adipose tissues from postpartum day 7 (P7) mice.

H Adipocyte size distribution in indicated depot (N = 4; more than 100 adipocytes were calculated from each mouse; data represent mean ± SEM).

I Femur and Tibia length from 8-week-old male mice (N =6; data represent mean ± SEM; **p < 0.01, ***p < 0.001).

J Muscle (quadriceps) weight relative to body weight of control and *Rictor*$^{prx1\text{-}Cre}$ mice at 8 weeks of age (N= 10-11; data represent mean ± SEM; ***p < 0.001).

K H&E stains of muscles from 8-week-old mice (scale bar = 100 μm).

L Western blot of lysates from muscles of control and *Rictor*$^{Prx1\text{-}Cre}$ mice.

M Relative mRNA expression by RT-PCR of *Rictor* and *Pparg2* in bone marrow adipose tissues isolated from control and *Rictor*$^{Prx1\text{-}Cre}$ mice (N= 4-5; data represent mean ± SEM; *p < 0.05).

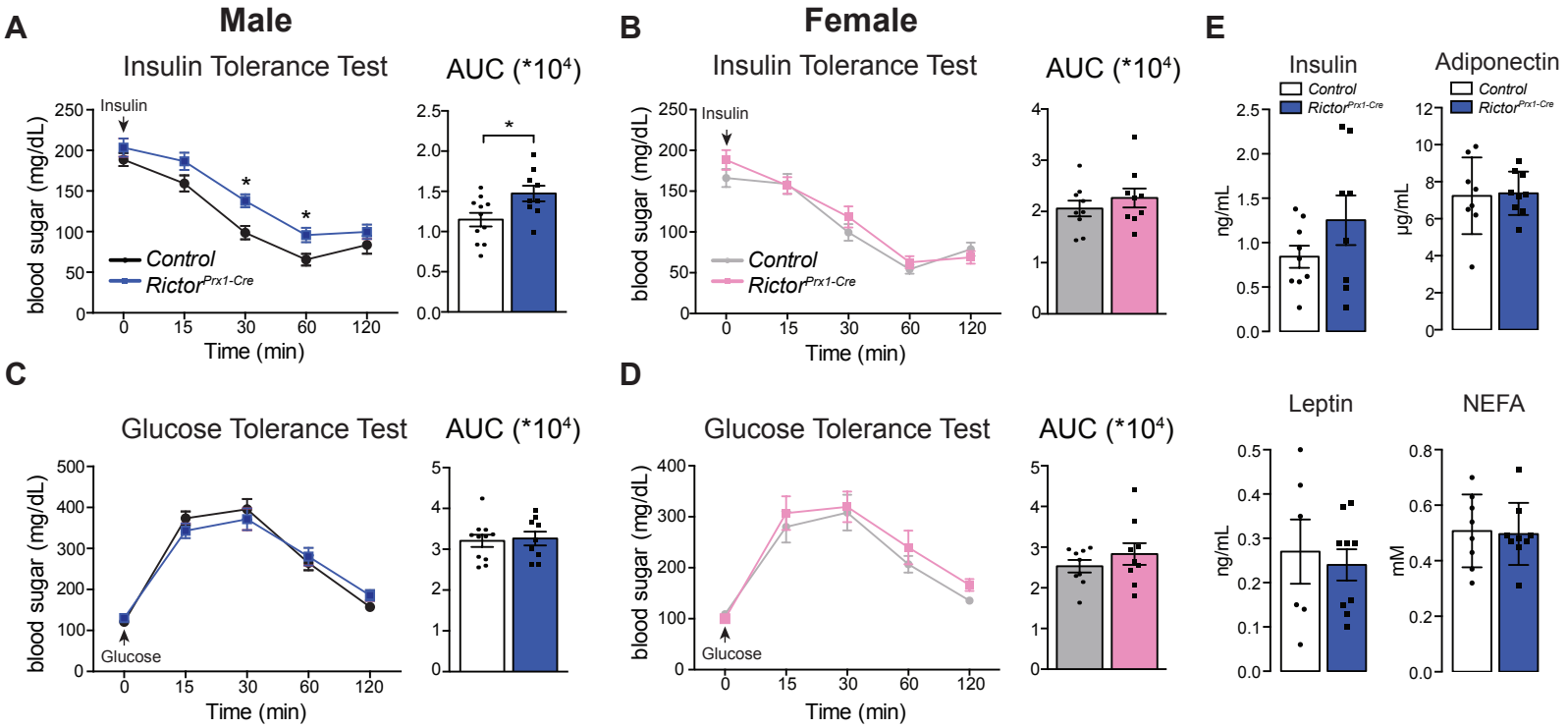
A
Male
Insulin Tolerance Test
AUC (*10⁴)
Insulin
blood sugar (mg/dL)
Control
Rictor Prx1-Cre
Time (min)
B
Female
Insulin Tolerance Test
AUC (*10⁴)
C
Glucose Tolerance Test
AUC (*10⁴)
Glucose
D
Glucose Tolerance Test
AUC (*10⁴)
E
Insulin
Adiponectin
Leptin
NEFA
ng/mL
µg/mL
mM

Figure S6, related to Figure 6.
A-B Insulin tolerance test (left) with area under curve (AUC, right) from 8-week-old male (**A**) and female (**B**) mice (n = 9-11; data represent mean ± SEM; $*p < 0.05$)
C-D Glucose tolerance test (left) with area under curve (AUC, right) from 8-week-old male (**C**) and female (**D**) mice (n = 9-11; data represent mean ± SEM; $*p < 0.05$)
E Plasma insulin, adiponectin, leptin, and non-esterified fatty acids (NEFA) levels ad libitum fed 8-week-old male mice (n = 7-9; bars represent mean ± SEM).

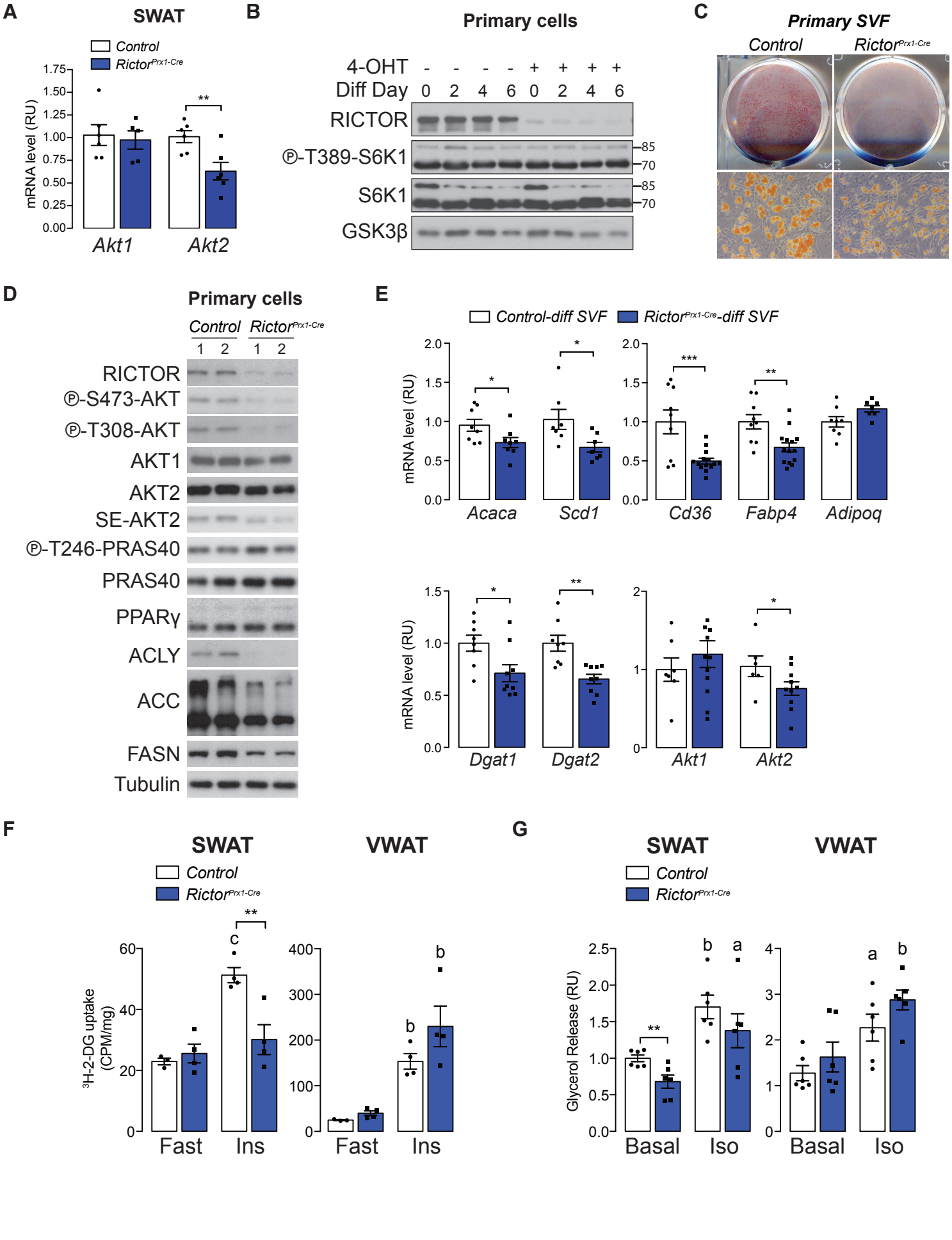
A
SWAT
Control
Rictor Prx1-Cre
mRNA level (RU)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
**
Akt1
Akt2
B
Primary cells
4-OHT - - - - + + + +
Diff Day 0 2 4 6 0 2 4 6
RICTOR
Ⓟ-T389-S6K1
S6K1
GSK3β
85
70
85
70
C
Primary SVF
Control
Rictor Prx1-Cre
D
Primary cells
Control
Rictor Prx1-Cre
1 2 1 2
RICTOR
Ⓟ-S473-AKT
Ⓟ-T308-AKT
AKT1
AKT2
SE-AKT2
Ⓟ-T246-PRAS40
PRAS40
PPARγ
ACLY
ACC
FASN
Tubulin
E
Control-diff SVF
Rictor Prx1-Cre -diff SVF
mRNA level (RU)
Acaca
Scd1
Cd36
Fabp4
Adipoq
Dgat1
Dgat2
Akt1
Akt2
F
SWAT
VWAT
Control
Rictor Prx1-Cre
3H-2-DG uptake (CPM/mg)
Fast
Ins
G
SWAT
VWAT
Control
Rictor Prx1-Cre
Glycerol Release (RU)
Basal
Iso

Figure S7, related to Figure 7.
A Relative mRNA expression by RT-PCR of *Akt* isoforms in SWAT from 8-week-old control and *Rictor*$^{Prx1\text{-}Cre}$ mice (N= 6-7; data represent mean ± SEM; **p < 0.01).
B Western blot of total and phospho-S6K1 in lysates from controls and *Rictor-iKO*$_{primary}$ cells at indicated time points during adipogenesis.
C Oil Red O (ORO) staining of differentiated primary preadipocytes isolated from control and *Rictor*$^{Prx1\text{-}Cre}$ mice.
D Western blot of lysates from differentiated primary preadipocytes isolated from control and *Rictor*$^{Prx1\text{-}Cre}$ mice. Each sample is from an individual mouse with the indicated genotype.
E Relative mRNA expression by RT-PCR of indicated genes from differentiated primary preadipocytes isolated from control and *Rictor*$^{Prx1\text{-}Cre}$ mice (N= 8; data represent mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001).
F ^{3}H-2-Deoxyglucose uptake (^{3}H-2-DG) of SWAT and VWAT with or without insulin (ins) stimulation from control and *Rictor*$^{Prx1\text{-}Cre}$ mice. Data represent mean ± SEM; **p < 0.01. b: p < 0.01 while its fasted counterpart is compared; c: p < 0.001.
G Lipolytic function measured by glycerol release from SWAT and VWAT with or without isoproterenol (iso) stimulation from control and *Rictor*$^{Prx1\text{-}Cre}$ mice. Data represent mean ± SEM; **p < 0.01. a: p < 0.05 while its counterpart under basal condition is compared; b: p < 0.01.

Table S1: Gene ontology biological processes and KEGG pathway enrichment analysis of *Rictor*-dependent genes, related to Figure 2.

GO term Biology Processes			
Rictor-required (Down-regulatory) genes			
GO term	GO #	Gene count	FDR
D2			
Cell adhesion	0007155	8	3.20E-02
D8			
Lipid metabolic process	0006629	22	9.80E-09
Metabolic process	0008152	19	4.90E-06
Oxidation-reduction process	0055114	21	6.10E-05
Fatty acid metabolic process	0006631	10	2.10E-03
Triglyceride metabolic process	0006641	6	1.10E-02
Inflammatory response	0006954	12	4.20E-02
Rictor-suppressed (Up-regulatory) genes			
GO term	GO #	Gene count	FDR
D2			
Complement activation	0006956	6	7.70E-06
Immune system process	0002376	11	1.50E-02
KEGG pathways			
Rictor-required (Down-regulatory) genes			
Pathway		Gene count	FDR
D0			
ECM*-receptor interaction		6	2.90E-03
D2			
ECM-receptor interaction		6	9.40E-04
D8			
PPAR signaling pathway		12	1.10E-07
Fatty acid metabolism		8	8.40E-05
Metabolic pathways		30	2.00E-03
Propanoate metabolism		5	7.10E-03
Glycerolipid metabolism		6	1.20E-02
Fat digestion and absorption		5	9.40E-04
Rictor-suppressed (Up-regulatory) genes			
Pathway		Gene count	FDR
D2			
Staphylococcus aureus infection		7	1.70-E04
Complement and coagulation cascades		6	4.00E-02

*ECM: extracellular matrix

Table S2: Bone structure analysis, related to Figure 6.

	Male			Female		
	Control	*Rictor*$^{Prx1\text{-}Cre}$	*P*-Value	Control	*Rictor*$^{Prx1\text{-}Cre}$	*P*-Value
Tb thickness (mm)	0.0513±0.00584	0.04334±0.00496	0.0396*	0.0407±0.00819	0.0397±0.00328	0.782
Tb spacing (mm)	0.246±0.0218	0.289±0.147	0.500	0.362±0.0919	0.376±0.0603	0.785
Tb number (1/mm)	4.132±0.321	4.006±1.459	0.840	2.975±0.829	2.721±0.364	0.529
BV/TV	0.101±0.225	0.0986±0.0702	0.938	0.037±0.0259	0.0275±0.0091	0.421
Cort thickness (mm)	0.185±0.00850	0.1426±0.0178	0.00127**	0.171±0.00985	0.1315±0.00706	0.00073***
Cort/Total Area	0.529±0.00371	0.500±0.0335	0.223	0.540±0.0517	0.501±0.0233	0.134
Marrow Area (mm^2)	0.610±0.00985	0.505±0.0461	0.0578	0.4133±0.0271	0.387±0.0399	0.272

*P <0.05; **P<0.01; P<0.001

Tb: trabecular; BV: bone volume; TV: total volume; Cort: cortical.

Table S3: Bone marrow analysis and osmium stain quantification, related to Figure 5.

		Male			Female		
		Control	*Rictor*$_{Prx1\text{-}Cre}$	*P*-Value	Control	*Rictor*$_{Prx1\text{-}Cre}$	*P*-Value
Prox.	MV (mm_3)	6.659±0.723	4.564±0.678	0.00287**	3.895±0.437	3.59±0.409	0.316
	MAT (mm_3)	0.03465±0.04	0.0788±0.088	0.405	0.0693±0.0673	0.0077±0.00816	0.039*
Dist.	MV (mm_3)	1.429±0.0856	1.218±0.281	0.196	1.184±0.222	1.104±0.110	0.462
	MAT (mm_3)	0.82±0.289	0.771±0.687	0.876	0.789±0.377	0.579±0.239	0.306

*P <0.05; **P<0.01.

Prox.: proximal; Dis.: distal; MV: marrow volume; MAT: marrow adipose tissue.

Table S4: Primer sequences, related to the STAR Methods section.

RT-PCR (mouse)		
Site		Sequence (5'-3')
TBP	Forward	ACGGACAACTGCGTTGATTTT
	Reverse	ACTTAGCTGGGAAGCCCAAC
Pparg2	Forward	TGGCATCTCTGTGTCAACCATG
	Reverse	GCATGGTGCCTTCGCTGA
Cebpa	Forward	CAAGAACAGCAACGAGTACCG
	Reverse	GTCACTGGTCAACTCCAGCAC
Cebpb	Forward	TCGGGACTTGATGCAATCC
	Reverse	AAACATCAACAACCCCGC
Cebpd	Forward	GCTTTGTGGTTGCTGTTGAA
	Reverse	ATCGACTTCAGCGCCTACA
Akt1	Forward	CACGCTACTTCCTCCTCAAG
	Reverse	CTCTGTCTTCATCAGCTGGC
Akt2	Forward	CCTTCCATGTAGACTCTCCAG
	Reverse	CCTCCATCATCTCAGATGTGG
Acly	Forward	CTCACACGGAAGCTCATCAA
	Reverse	ACGCCCTCATAGACACCATC
Acaca	Forward	GGAGATGTACGCTGACCGAGAA
	Reverse	ACCCGACGCATGGTTTTCA
Fasn	Forward	GCTGCGGAAACTTCAGGAAAT
	Reverse	AGAGACGTGTCACTCCTGGACTT
Cd36	Forward	TGGCCTTACTTGGGATTGG
	Reverse	CCAGTGTATATGGCTCATCCA
Fabp4	Forward	GATGCCTTTGTGGGAACCT
	Reverse	CTGTCGTCTGCGGTGATTT
Lpl	Forward	GGCCAGATTCATCAACTGGAT
	Reverse	GCTCCAAGGCTGTACCCTAAG
Glut4	Forward	GTGACTGGAACACTGGTCCTA
	Reverse	CCAGCCAGTTGCATTGTAG
Scd1	Forward	CCCTGCGGATCTTCCTTATC
	Reverse	TGTGTTTCTGAGAACTTGTGGTG
Dgat1	Forward	GAGGCCTCTCTGCCCCTATG
	Reverse	GCCCCTGGACAACACAGACT
Dgat2	Forward	CCGCAAAGGCTTTGTGAAG
	Reverse	GGAATAAGTGGGAACCAGATCA
Hsl	Forward	CAGTGTGACCGCCAGTTC
	Reverse	ACCTCAATCTCAGTGATGTTCC
Mcad	Forward	GCCAAGATCTATCAGATTTATGAAGG
	Reverse	AGCTATGATCAGCCTCTGAATTTGT
Perilipin1	Forward	CTGTGTGCAATGCCTATGAGA
	Reverse	CTGGAGGGTATTGAAGAGCCG
Adipoq	Forward	TGTTCCTCTTAATCCTGCCCA
	Reverse	CCAACCTGCACAAGTTCCCTT

Chrebpa	Forward	CGACACTCACCCACCTCTTC	
	Reverse	TTGTTCAGCCGGATCTTGTC	
Chrebpb	Forward	TCTGCAGATCGCGTGGAG	
	Reverse	CTTGTCCCGGCATAGCAAC	
Pkm2	Forward	TCGCATGCAGCACCTGATT	
	Reverse	CCTCGAATAGCTGCAAGTGGTA	
Rictor	Forward	TCGATCTGACCCGAGAACCTT	
	Reverse	GTTATTCAGATGGCCCAGCTTTT	
ChIP-qPCR primers			
Site		Sequence (5'-> 3')	Reference
Cd36-PPRE	Forward	CCAACGGAACTGATTTGAGC	(Lefterova et al., 2010)
	Reverse	TTGCTGCTACACTCCAGCAT	
Fabp4-PPRE	Forward	AATGTCAGGCATCTGGGAAC	(Lefterova et al., 2010)
	Reverse	GACAAAGGCAGAAATGCACA	
H3K9ac-Cd36-PPRE	Forward	GAGCCGCCCCTTCTATACTT	(Lefterova et al., 2010)
	Reverse	TGTTGGGACAGACCAATCAG	
H3K9ac-Fabp4-PPRE	Forward	TTCTGACTCCTGGCCTGAAC	(Lefterova et al., 2010)
	Reverse	TGCCCTCTCAGGTTTCATTT	
Pkm2-PPRE	Forward	GCAGCCAGCCTGTAAGGGCA	(Panasyuk et al., 2012a)
	Reverse	GCGAAGACAGGAAAACAGTGGGT	
Insulin	Forward	CTTCAGCCCAGTTGACCAAT	N/A
	Reverse	AGGGAGGAGGAAAGCAGAAC	
Chr. 15	Forward	AGCGTGGCCTTGGCAGCAAA	(Zhang et al., 2012)
	Reverse	TGCGATTGGCTTCCTCTCCCC	