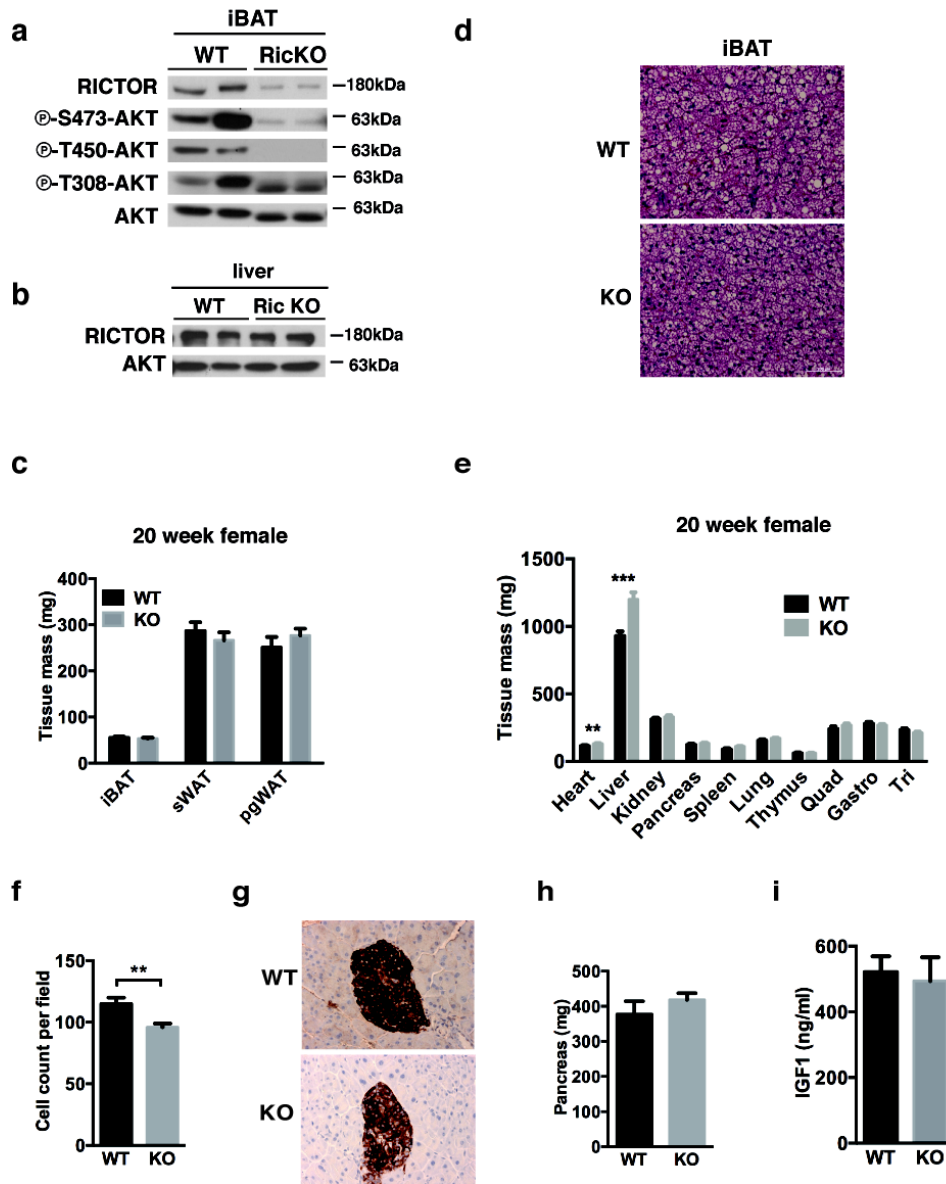


Supplementary Figure 1



Supplementary Figure 1. The general phenotypes of adipose tissue *Rictor* knockout mice.

(a) Western blot of RICTOR, AKT and phospho-AKT in iBAT of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice.

(b) Western blot of RICTOR and AKT in liver.

(c) Individual fat tissue mass of female mice at 20 weeks old. n=8-13.

(d) Representative images of H&E staining of iBAT.

(e) Individual lean tissue mass of female mice at 20 weeks old. n=8-13.

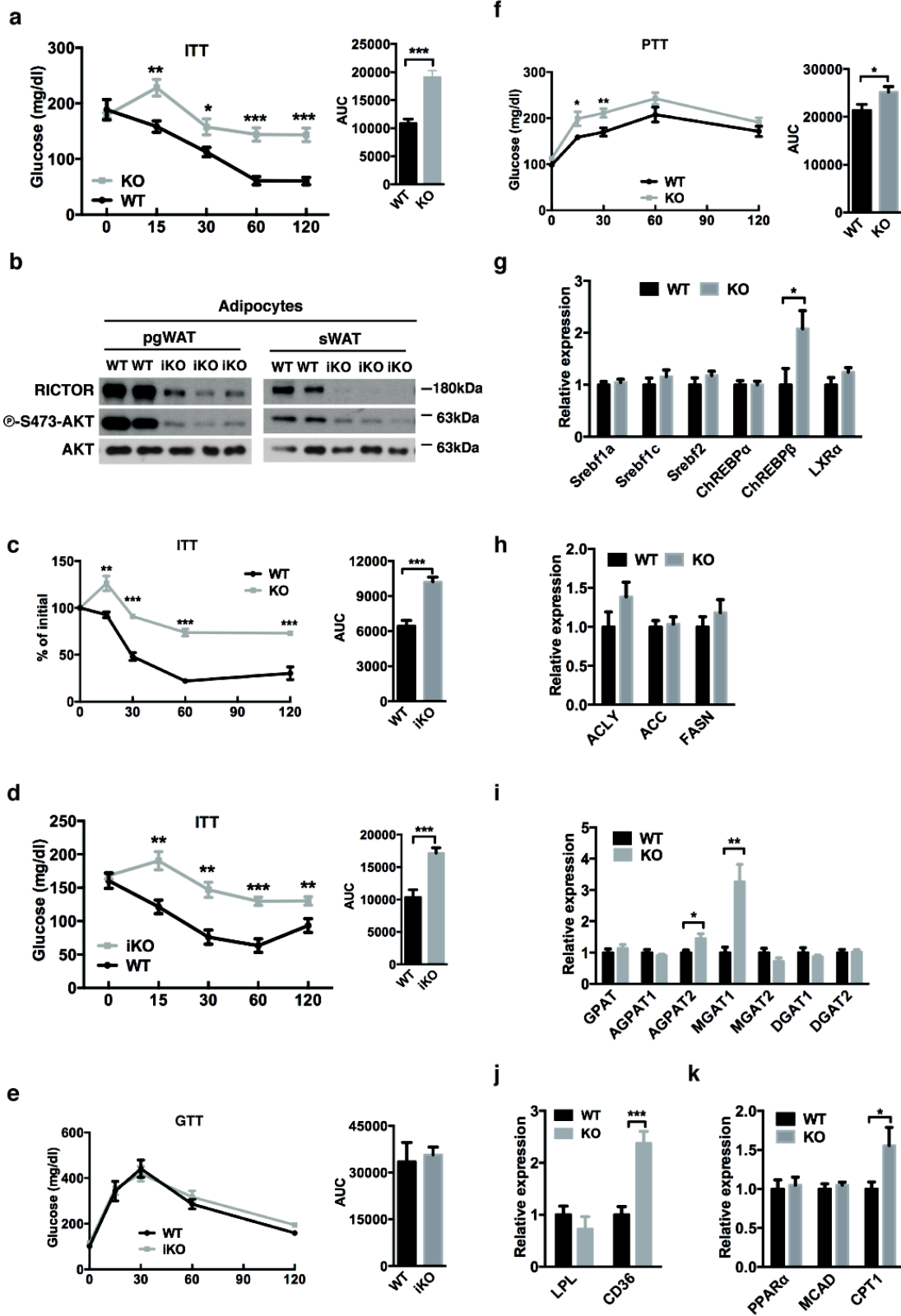
(f) Number of hepatocyte nuclei/field from liver H&E stained images. Four images from the liver of 4 mice were used in each group.

(g) Insulin staining of pancreas.

(h) Pancreas mass from samples used for in (g) and different from Figure 1(g). n=5.

(i) Plasma IGF1 level. n=6. Data were analyzed by Student's t test. Values are expressed as mean+sem. *p < 0.05; **p < 0.01; ***p < 0.001. Scale bar: 100μM.

Supplementary Figure 2

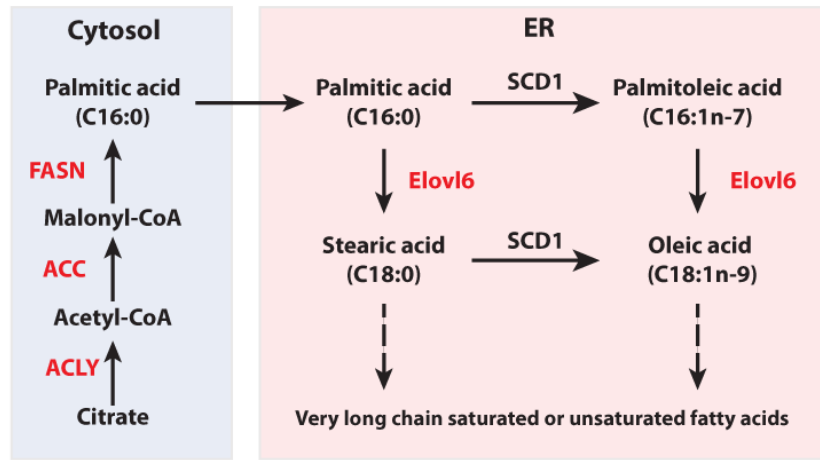


Supplementary Figure 2. Insulin resistance of inducible adipose tissue *Rictor* knockout mice.

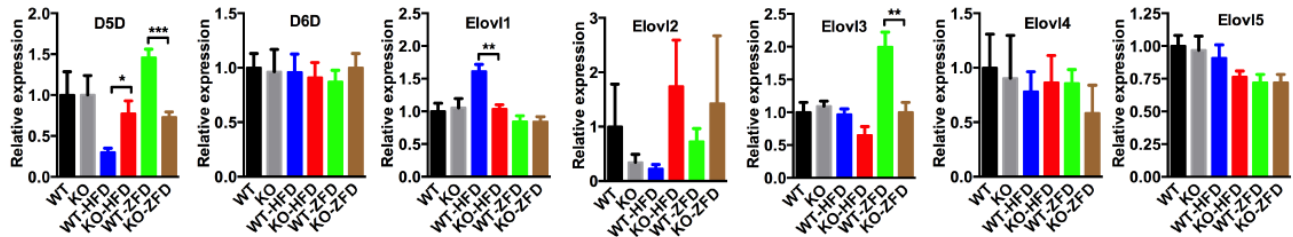
- (a) ITT of female *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice 8 weeks. n=6 or 8.
- (b) Western blot of RICTOR, AKT and phosphorylation of AKT at S473 of isolated adipocytes from *Rictor^{fl/fl}* (WT) and inducible *Rictor^{Adipoq-CreERT2}* (iKO) mice at 3 weeks post first tamoxifen injection.
- (c) ITT of mice at 5 weeks post first tamoxifen injection. n=6 or 8.
- (d) The raw data of (c).
- (e) GTT of mice at 6 weeks post first tamoxifen injection. n=6 or 8.
- (f) Pyruvate tolerance test (PTT) of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) male mice at 10 weeks old. n=7 or 8.
- (g-k) The relative mRNA expression of indicated genes in liver of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice. n=8. Data were analyzed by Student's t test. Values are expressed as mean±sem. *p < 0.05; **p < 0.01; ***p < 0.001.

Supplementary Figure 3

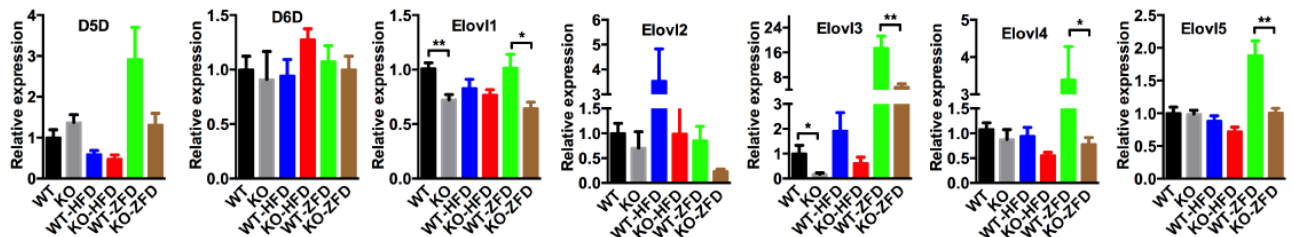
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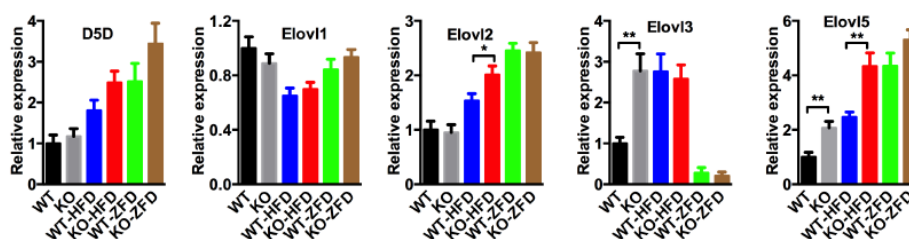
b pgWAT



c sWAT



d Liver

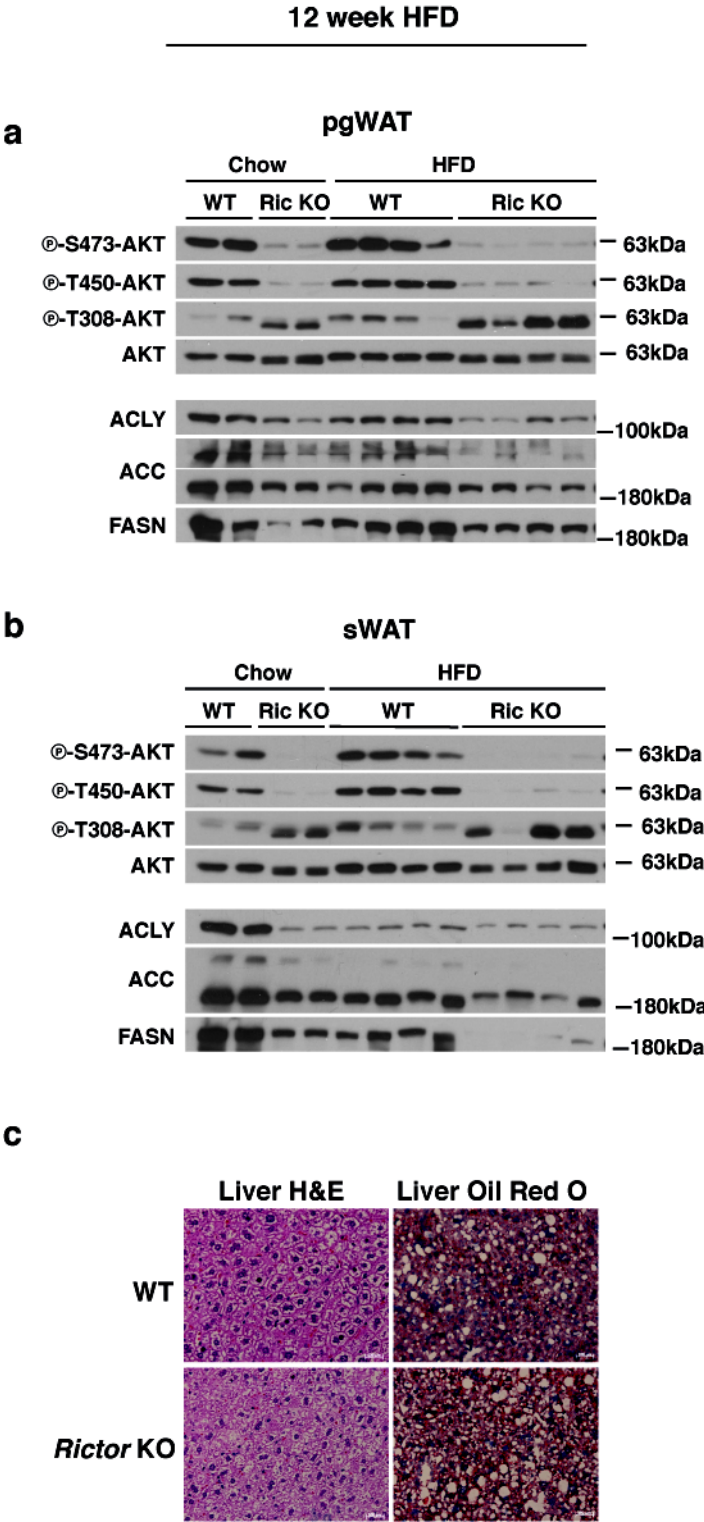


Supplementary Figure 3. The role of adipose mTORC2 in DNL and elongation.

(a) Model of DNL and elongation in adipose tissue. Genes with red color are regulated by adipose tissue mTORC2.

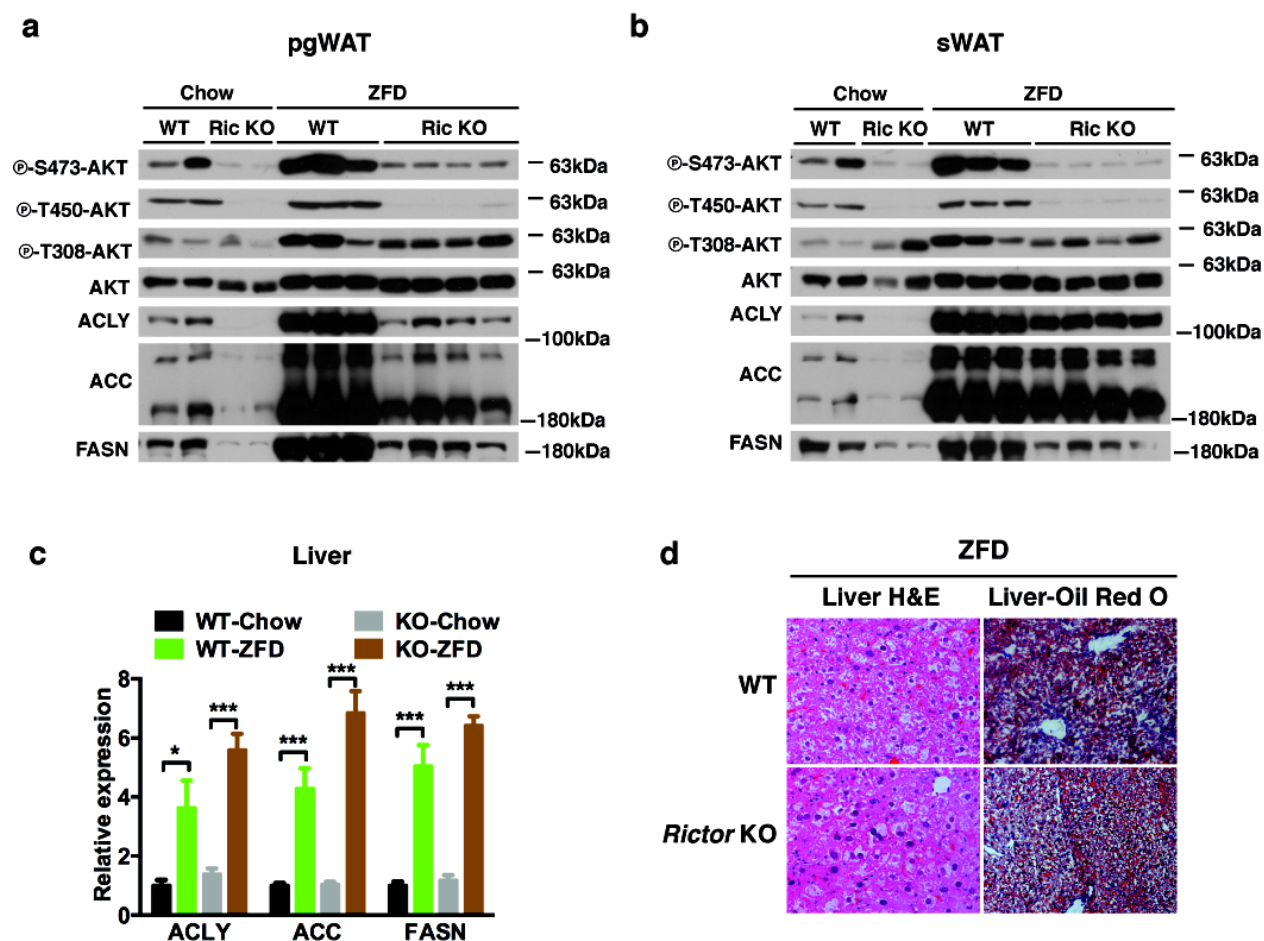
(b-d) The relative mRNA expression of indicated genes in pgWAT (b), sWAT (c) and liver (d) of *riCTOR^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) under chow, HFD or ZFD. n=8. Data were analyzed by Student's t test. Values are expressed as mean±sem. *p < 0.05; **p < 0.01; ***p < 0.001.

Supplementary Figure 4



Supplementary Figure 4. HFD represses DNL in WAT and induces fatty liver.
 (a-b) Western blot of indicated proteins of pgWAT (a) and sWAT (b) of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice under chow and HFD.
 (c) The representative images of liver H&E staining and liver Oil Red O staining of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice under HFD for 12 weeks. Scale bar: 100µM.

Supplementary Figure 5



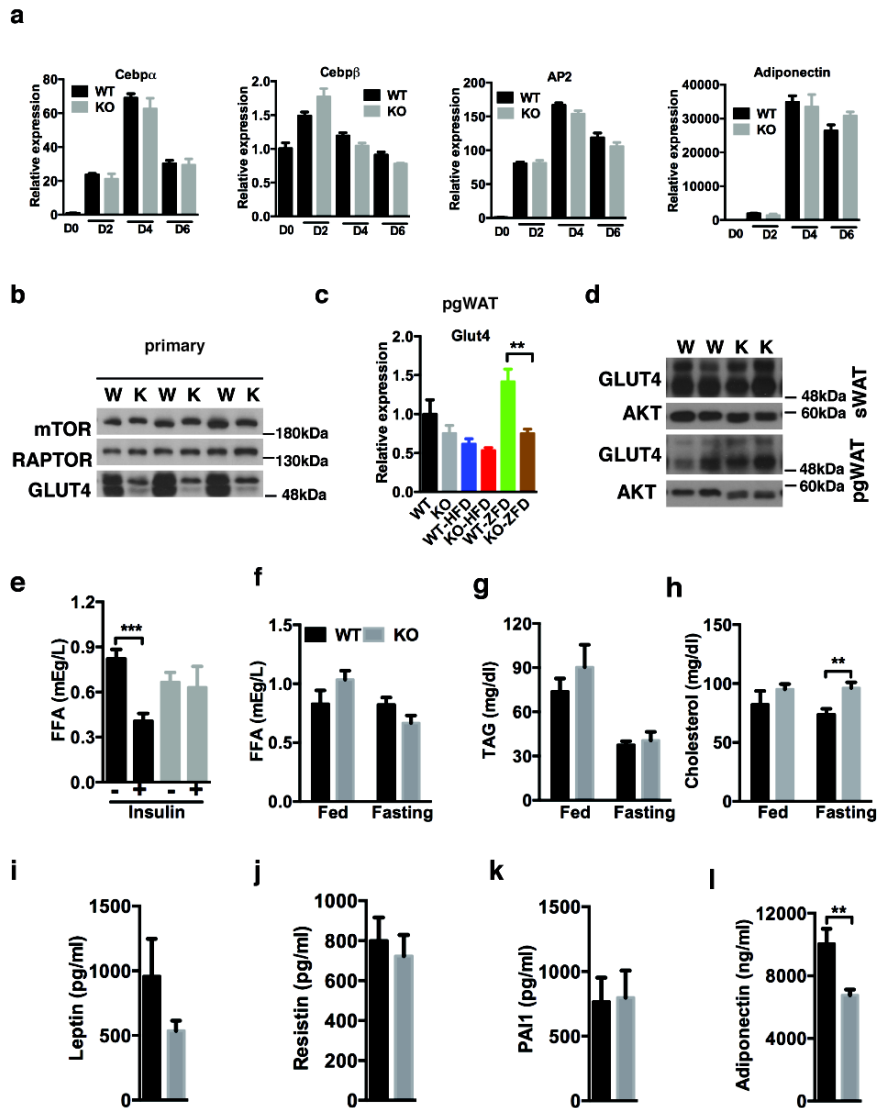
Supplementary Figure 5. ZFD increases hepatic DNL and causes fatty liver.

(a-b) Western blot of indicated proteins in pgWAT (a) and sWAT (b) of *Rictor^{fl/fl}* (WT) and *Rictor^{Adipoq-Cre}* (KO) mice under chow and ZFD for 12 weeks started at 8 weeks old.

(c) The relative mRNA expression of *acly*, *acc* and *fasn* in liver of mice under ZFD for 12 weeks. n=8. Data were analyzed by Student's t test between groups. Values are expressed as mean±sem.

(d) The representative images of liver H&E staining and liver Oil Red O staining of mice under ZFD for 12 weeks. *p < 0.05; **p < 0.01; ***p < 0.001. Scale bar: 100µM.

Supplementary Figure 6



Supplementary Figure 6. Plasma metabolites of adipose tissue *Rictor* knockout mice

(a) The relative mRNA expression of indicated genes in differentiated cells at various time points. n=3.

(b) The expression of indicated proteins in differentiated adipocytes. n=3.

(c) The relative expression of *Glut4* gene in pgWAT of *Rictor*^{f/f} (WT) and *Rictor*^{Adipoq-Cre} (KO) mice. n=8.

(d) The GLUT4 protein level measured by western blotting in sWAT and pgWAT.

(e) The plasma FFA level after 6 hour fasting with or without insulin injection for 15 minutes.

(f) The plasma FFA level under random feeding and 6 hour fasting.

(g) The plasma TAG level under random feeding and 6 hour fasting.

(h) The plasma cholesterol level under random feeding and 6 hour fasting.

(i) The plasma leptin level under random feeding.

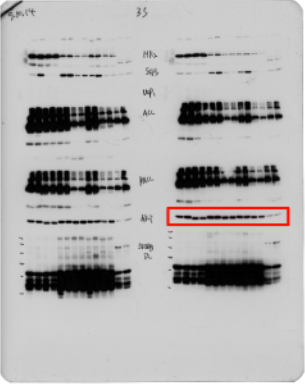
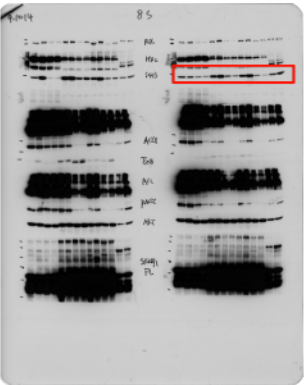
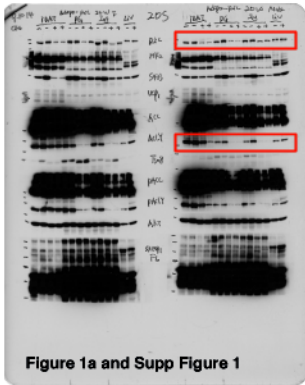
(j) The plasma resistin level under random feeding.

(k) The plasma PAI1 level under random feeding.

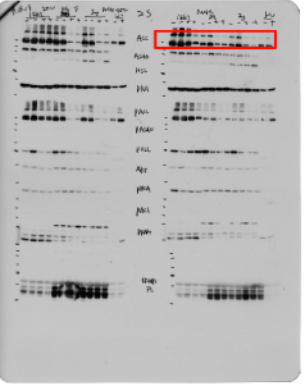
(l) The plasma adiponectin level under random feeding. n=6. Data were analyzed by Student's t test. Values are expressed as mean+sem. *p < 0.05; **p < 0.01; ***p < 0.001.

Supplementary Figure 7 Original Films

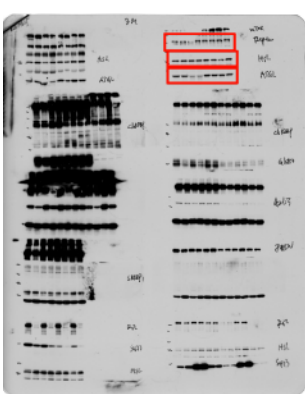
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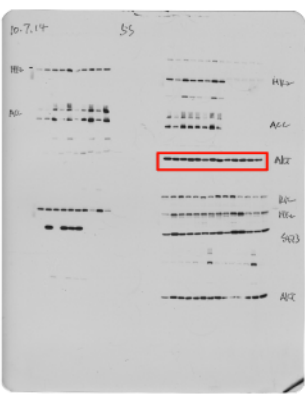
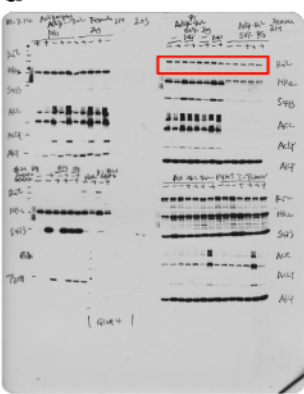
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c

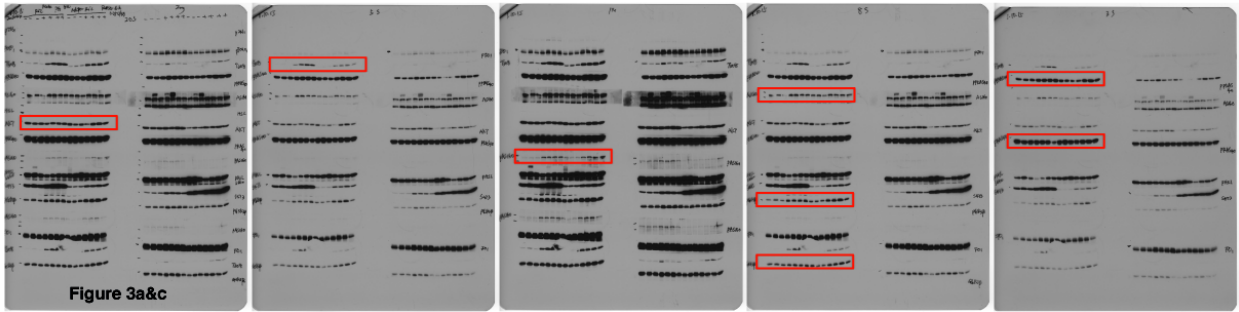


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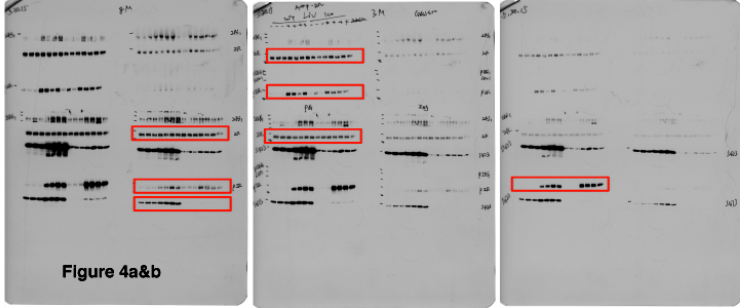


Supplementary Figure 8 Original Films

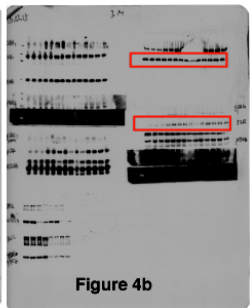
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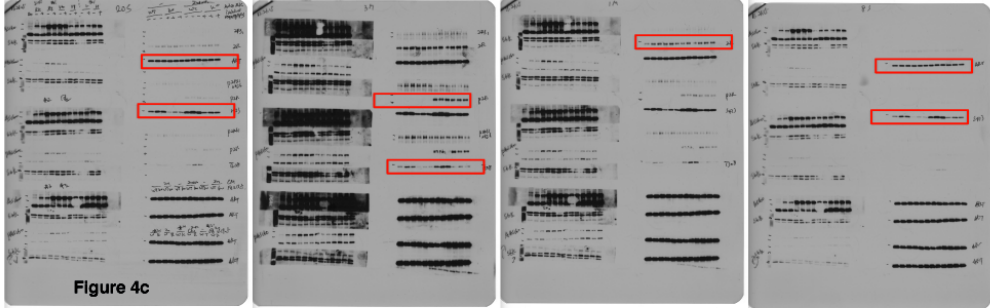
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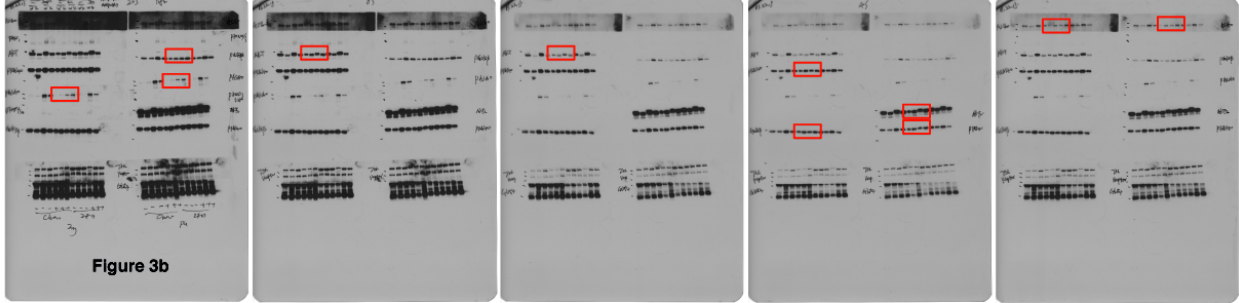
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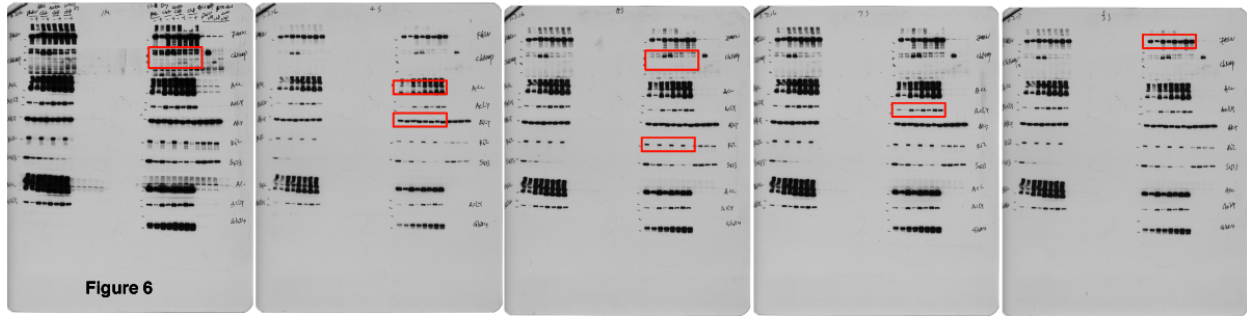


e

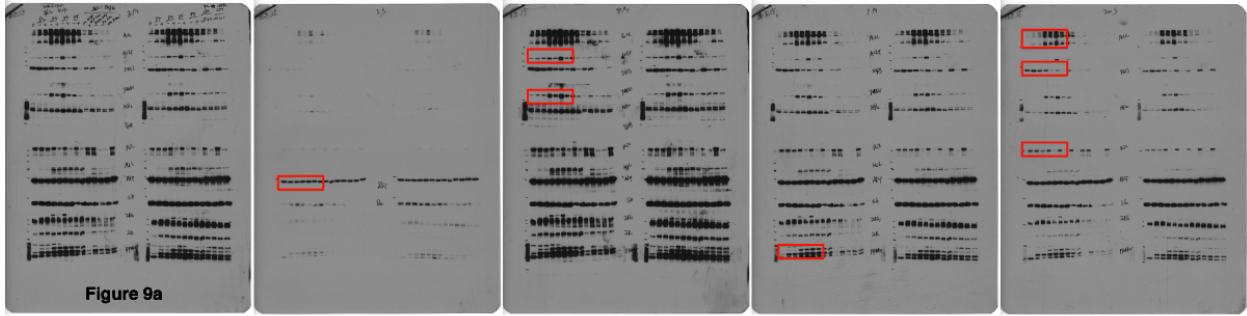


Supplementary Figure 9 Original Films

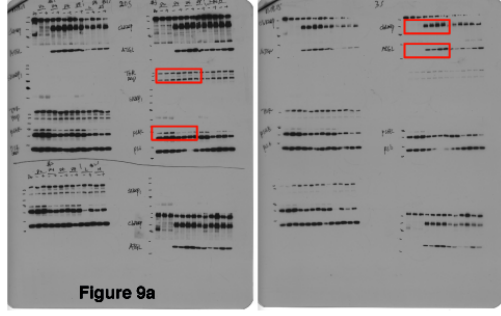
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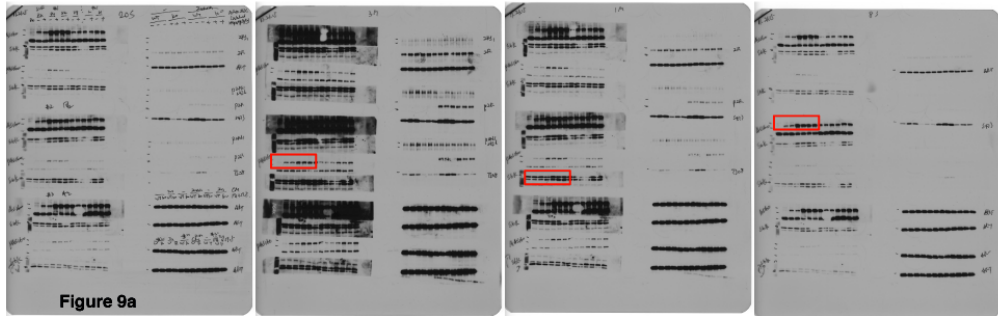
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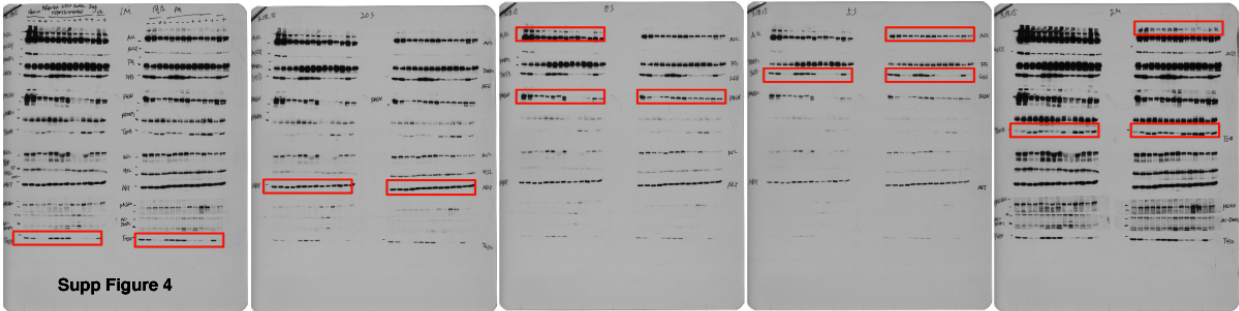


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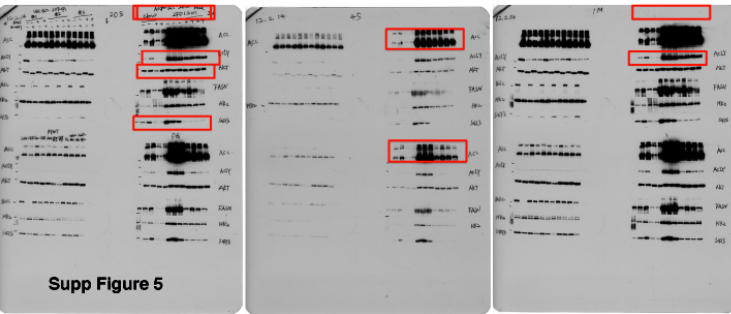


Supplementary Figure 10 Original Films

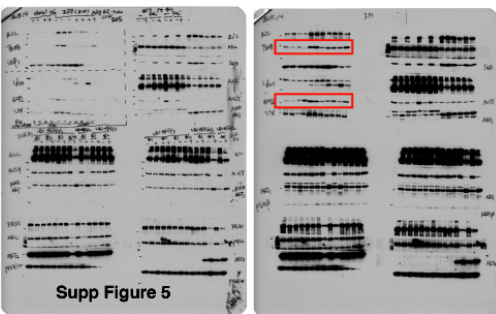
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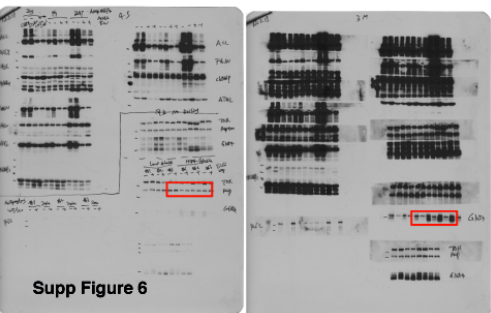
b



c



d



Supplementary Table 1

A comparison between the *Rictor*^{Adipoq-Cre} mice described in this study and previously described *Rictor*^{aP2-Cre} mice

Parameter Examined	Observed difference relative to control	
	<i>Rictor</i> ^{Adipoq-Cre}	<i>Rictor</i> ^{aP2-Cre*}
Total body mass	No difference	Increased
Adipose tissue mass	No difference	No difference
Liver mass	Increased	No difference
Hepatic TAGs	Slightly Increased	Normal/Slightly increased
Lean tissue mass	No difference	Increased
Circulating IGF-1	No difference	Increased/normal
Circulating Insulin	Elevated	Elevated
Leptin	Normal	Normal
Adiponectin	Decreased	Normal/Decreased
Islet mass	No difference	Increased
ITT	Severe Insulin Resistance	Normal/Slight Insulin resistance
GTT	Normal	Normal/Resistance (aged mice)
Muscle glucose uptake (clamp)	Normal	Decreased
Fat glucose uptake (clamp)	Decreased	Decreased
HGP (clamp)	Increased	Increased
GIR (clamp)	Decreased	Decreased
AS160 phosphorylation	Normal	Decreased
FOXO1 phosphorylation	Normal	Decreased
Basal fat lipolysis (ex vivo)	Elevated	Elevated
Basal fat lipolysis (primary cell)	Normal	n.d.
Response to HFD	Reduced adipose tissue growth	Increased adipose tissue growth
Response to ZFD	Reduced adipose tissue growth	n.d.
<i>Chrebpβ</i> expression in WAT	Decreased	n.d.
<i>DNL</i> genes in WAT	Decreased	n.d.
<i>Elovl6</i> expression in WAT	Decreased	n.d.
<i>Scd1</i> expression in WAT	normal	n.d.
Lipid composition (fat)	Altered	n.d.
Lipid composition (liver)	Altered	n.d.

* aP2-Cre;*Rictor* mice are described in Reference 17 & 18 of the Main text

BOLD Indicates key differences between models

n.d. Not determined

ITT Insulin tolerance test

GTT Glucose tolerance test

HGP Hepatic glucose production

GIR Glucose infusion rate

HFD High fat diet

ZFD Zero fat diet

DNL De novo lipogenesis

WAT White adipose tissue

Supplementary Table 2. Primer sequences for quantitative RT-PCR analysis

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Tbp</i>	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT
<i>Ppary</i>	TCAGCTCTGTGGACCTCTCC	ACCCTTGCATCCTTCACAAG
<i>Srebf1a</i>	TAGTCCGAAGCCGGGTGGGCGCCGG	GATGTCGTTCAAACCGCTGTGTGT C
<i>Srebf1c</i>	AAGCAAATCACTGAAGGACCTGG	AAAGACAAGCTACTCTGGGAG
<i>Srebf2</i>	GGATCCTCCCAAAGAAGGAG	TTCCTCAGAACGCCAGACTT
<i>Chrebpa</i>	CGACACTCACCCACCTCTTC	TTGTTCCAGCCGGATCTTGTC
<i>Chrebpβ</i>	TCTGCAGATCGCGTGGAG	CTTGTCGCCGCATAGCAAC
<i>Acly</i>	CTCACACGGAAGCTCCATAA	ACGCCCTCATAGACACCATC
<i>Acc</i>	GGAGATGTACGCTGACCGAGAA	ACCCGACGCATGGTTTTCA
<i>Fasn</i>	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
<i>Elovl6</i>	TCAGCAAAGCACCCGAAC	AGCGACCATGTCTTTGTAGGAG
<i>Scd1</i>	CCCTGCGGATCTTCCTTATC	TGTGTTTCTGAGAACTTGTGGTG
<i>Elovl1</i>	GTGGCCCAGCCCTACCTT	TGTGCAGTGAGACCAGGACAA
<i>Elovl2</i>	TCAATGCTTTCTTGACAACATG	GGTAAGAGTCCAGCAGGAACCA
<i>Elovl4</i>	GTGGGTGGCTGGAGGCCAAGCGTTTT TCGG	ATTCGTGGCCGTCTTTCCGGTTTTT GACTG
<i>D6D</i>	TATGGCAAGAAGAAGCTGAAATACCTG CCC	TGCTTACCTCCATAAATGAAGCTGC CGTCC
<i>D5D</i>	GGGTGGACTTGGCCTGGATGCTCAGC TTCT	GCTGCTATTGGTGAAGGTAAGCGT CCAACC
<i>Elovl5</i>	CCCCCGAGATACAAGAGTCA	GCAGGGTGAGTCCAAGGTTA
<i>Elovl3</i>	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA