



Article scientifique

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Supplemental data

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PTEN down-regulation by unsaturated fatty acids triggers hepatic steatosis
via an NF-kappaBp65/mTOR-dependent mechanism

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SUPPLEMENTAL TABLE I:**Primary antibodies:**

Protein targeted	Host	Clone	Provider	Catalogue number
PTEN	Mouse	A2B1	SantaCruz (CA, USA)	SC-7974
PTEN	Mouse	6H2.1	(DakoCytomation, Switzerland)	M3627
SHIP2	Goat	-	SantaCruz (CA, USA)	SC-14504
NF- κ Bp65	Rabbit	-	SantaCruz (CA, USA)	SC-372
Insulin receptor (IR)	Rabbit	-	SantaCruz (CA, USA)	SC-711
IRS1	Rabbit	-	SantaCruz (CA, USA)	SC-559
IRS2	Rabbit	-	Calbiochem BadSoden, Germany)	420293
phospho(Tyr632)-IRS1	Goat	-	SantaCruz (CA, USA)	SC-17196
phospho(Tyr1146)-insulin receptors	Rabbit	-	Cell Signaling (MA, USA)	3021
phospho(Ser473)-Akt	Rabbit	-	Cell Signaling (MA, USA)	9271
ERK1/2	Rabbit	-	Cell Signaling (MA, USA)	9102
phospho(Thr202/Tyr204)-ERK1/2	Mouse	E10	Cell Signaling (MA, USA)	9106
mTOR	Rabbit	-	Cell Signaling (MA, USA)	2972
phospho(Ser2448)-mTOR	Rabbit	-	Cell Signaling (MA, USA)	2971
phospho(Ser276 or Ser468)-NF- κ Bp65	Rabbit	-	Cell Signaling (MA, USA)	3037
phospho(Thr183/Tyr185)-JNK	Mouse	G7	SantaCruz (CA, USA)	SC-6254
phospho(Thr172)-AMPK	Rabbit	-	Cell Signaling (MA, USA)	2531
AMPK	Rabbit	-	Cell Signaling (MA, USA)	2532
I κ B α	Rabbit	-	Cell Signaling (MA, USA)	9242
p70-S6K	Rabbit	-	Cell Signaling (MA, USA)	9202
phospho(Thr389)-p70-S6K	Rabbit	-	Cell Signaling (MA, USA)	9205
actin	Mouse	C4	Chemicon (CA, USA)	MAB1501 R

Secondary antibodies:

Protein targeted	Host	Provider	Catalogue number
HRP-conjugated anti-mouse antibodies	Goat	Biorad (Basel, Switzerland)	170-6516
HRP-conjugated anti-rabbit antibodies	Goat	Biorad (Basel, Switzerland)	170-6515
Alexa Fluor 488 conjugated anti-rabbit IgG	Goat	Molecular Probes (Invitrogen, Basel, Switzerland)	A-11008
HRP conjugated anti-Goat	Rabbit	Sigma (St Louis, USA)	A-5420

Other reagents:

Name	Provider	Catalogue number
Rapamycin	Calbiochem (BadSoden, Germany)	553211
LY294002	Cell Signaling (MA, USA)	9901
BAY11-70859	Calbiochem (BadSoden, Germany)	196870
SP-600125	Calbiochem (BadSoden, Germany)	420119
PD98059	Cell Signaling (Allschwil, Switzerland)	9900
PTD-p65-P1	Imgenex (San Diego, USA)	IMG-2001
Fatty acids	Sigma (St Louis, USA)	O1208, P9417, P0500, L1376
[¹⁴ C]-Oleic acid (51 mCi/mmol)	Sigma (St Louis, USA)	O5011
FuGene HD reagent	Roche (Basel, Switzerland)	04 709 705 001
GPO-PAP kit	Roche (Basel, Switzerland)	12016648-122
ECL reagent	Amersham (Dübendorf, Switzerland)	RPN2135
A-sepharose beads	GE Healthcare (Uppsala, Sweden)	17-0780-01
G-sepharose beads	GE Healthcare (Uppsala, Sweden)	17-0618-01
Oil-Red O	Sigma (St Louis, USA)	75087
Trizol Reagent	Invitrogen (Carlsbad, CA)	15596-018
Qiagen RNeasy FFPE kit	Qiagen (Basel, CH)	74404
Qproteome FFPE Tissue kit	Qiagen (Basel, CH)	37623
random hexamer primers	Invitrogen (Carlsbad, CA)	48190-011
SuperScript II Reverse Transcriptase	Invitrogen (Carlsbad, CA)	18064-022
Quantitect SYBR®Green PCR kit	Qiagen (Basel, CH)	204143

Plasmids:

Insert	Plasmid	Source	References
PTEN	EGFP-C2	R. Cowburn (Karolinska Institute, Sweden)	Kerr F et al., FEBS Lett 2006, 580:3121-8.
PTEN _{G129E} mutant	EGFP-C2	R. Cowburn (Karolinska Institute, Sweden)	Kerr F <i>et al.</i> , FEBS Lett 2006, 580:3121-8.
NF-κB _{mut} 1-450 mutant	pCMV4	S.C. Sun (PennState Univ., USA)	Harhaj EW <i>et al.</i> , Mol Cell Biol 1999, 19:7088-95.
Flag-NF-κBp65	pRcRSV	G. Haegeman (Gent Univ., Belgium)	Vermeulen L et al., Embo J 2003, 22:1313-24.
NF-κB _{mut} S276A mutants	pRcRSV	G. Haegeman (Gent Univ., Belgium)	Vermeulen L et al., Embo J 2003, 22:1313-24.

Diets for rats:

Diet	Metabolized energy	Provider
Standard	2.61 kcal/g	RMI, Hersteller, UK
High-fat	4.31 kcal/g	2154 KLIBA NAFAG diet, Provimi Kliba, Kaiseraugst, Switzerland