

Supplemental data for:

## **Resveratrol Treatment Enhances the Cellular Response to Leptin by Increasing OBRb Content in Palmitate-Induced Steatotic HepG2 Cells**

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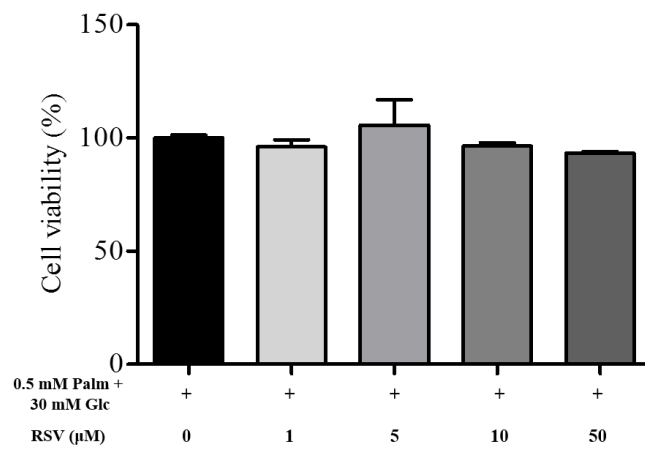
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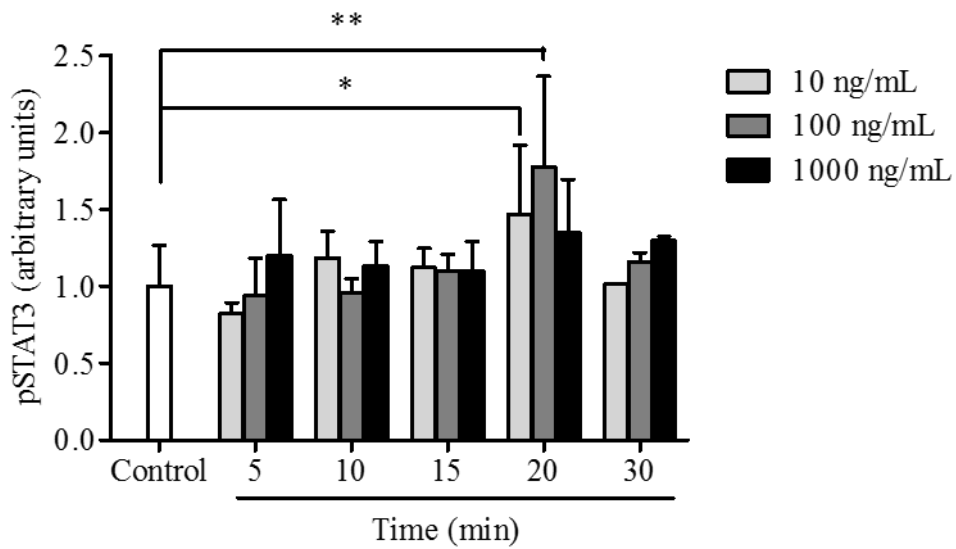
**Table S1.** Human-specific primer sequences used for qPCR analysis.

Name	Direction	Primer sequences (5'-3')	Primer length (nucleotides)	T <sub>m</sub> (°C)
<i>ACC</i>	FW	gctgaaccagcactctgat	19	47
	RV	ccttgacataaggctccagc	20	52
<i>ATF4</i>	FW	gggttctccagcgacaaggctaag	24	61
	RV	aacagggcatccaagtcaactc	23	58
<i>CHOP</i>	FW	agggagaaccaggaacggaaaca	24	61
	RV	tcctgcttgagccgttcattctct	24	60
<i>CPT1a</i>	FW	cgggggaatgtcaagagggtt	20	55
	RV	caagaaatgtcgacagagcc	20	56
<i>FAS</i>	FW	tctactacaagctgctgtgcc	20	51
	RV	aacctctcccagggtatgcga	20	54
<i>Il-6</i>	FW	gcttccctcaggatgcttgt	20	51
	RV	attaactggggtgcctgctc	20	54
<i>iNOS</i>	FW	caagcagcagaatgagctccc	20	52
	RV	ctgggtcctctggtaaac	20	50
<i>OBRe</i>	FW	aattgttctgggcacacagg	20	54
	RV	gagagaccacagttgtggc	20	49
<i>PPAR<math>\alpha</math></i>	FW	aggctgcaagggtcttctt	20	55
	RV	tcgggatgtcacacaacgc	19	55
<i>PPIA</i>	FW	ctcctttgagctgtttgcag	20	50
	RV	caccacatgcttgcaccc	19	54
<i>PTP1b</i>	FW	tgggaaatgcaggaggtct	20	53
	RV	ccacgacccgacttctaact	20	51
<i>SCD1</i>	FW	acttgagctgtgggtgagg	20	54
	RV	cgctggcacatcaactcac	20	54
<i>SIRT1</i>	FW	tggcacagatcctcgaacaa	20	54
	RV	catgaaacagacaccccagc	20	53
<i>SOCS3</i>	FW	caggaatgtagcagcgatggaa	22	57
	RV	cctgtccagcccaataactga	21	56
<i>sXBP1</i>	FW	gagttaagacagcgcttggg	20	52
	RV	gatgttctggagggtgaca	20	52
<i>TNF-<math>\alpha</math></i>	FW	gctgcactttggagtgatcg	20	52
	RV	gtgtgccagacacccctatct	20	53

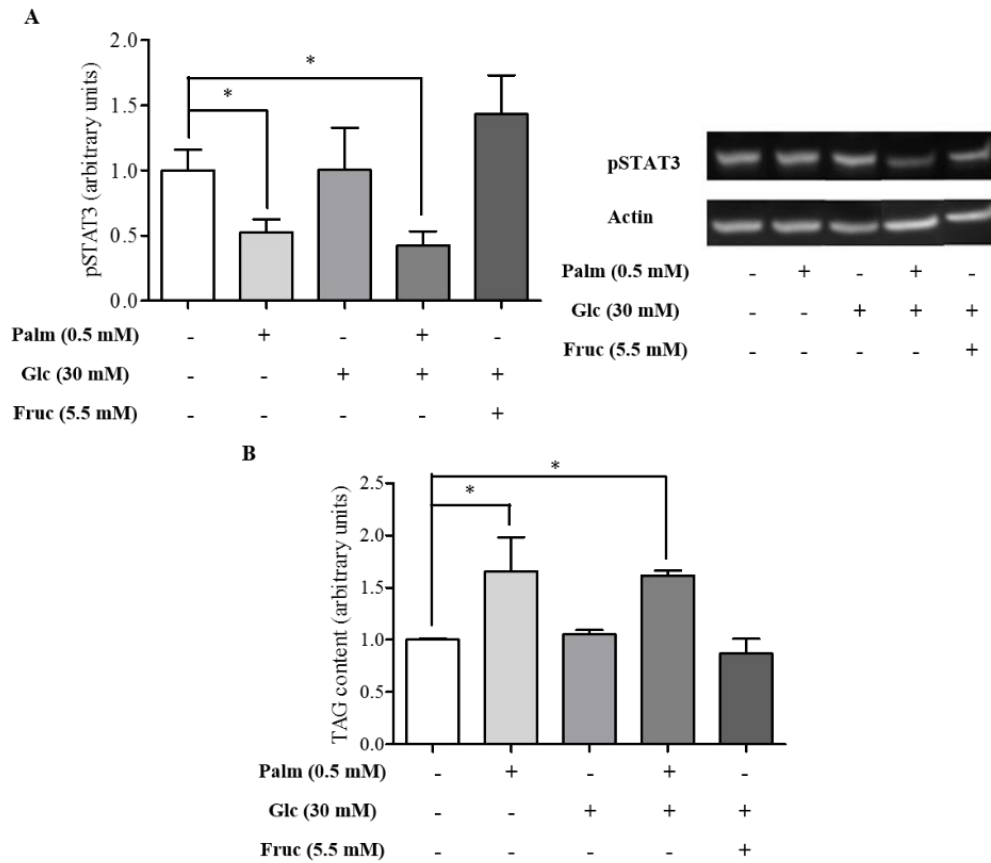
*Acc*: acetyl-CoA carboxylase, *Atf4*: activating transcription factor 4, *Chop*: DNA damage inducible transcript 3, *Cpt1a*: carnitine palmitoyltransferase 1a, *Fas*: fatty acid synthase, *Il-6*: interleukine 6, *iNos*: inducible nitric oxide synthase, *ObRb*: leptin receptor isoform b, *Ppara*: peroxisome proliferator activated receptor  $\alpha$ , *Ppia*: peptidylprolyl isomerase a, *Ptp1b*: protein-tyrosine phosphatase 1b, *Scd1*: stearoyl-CoA desaturase 1, *Sirt1*: NAD<sup>+</sup>-dependent deacetylase sirtuin-1, *Socs3*: suppressor of cytokine signaling 3 and *sXbp1*: x-box binding protein 1, *Tnf- $\alpha$* : tumor necrosis factor alpha.



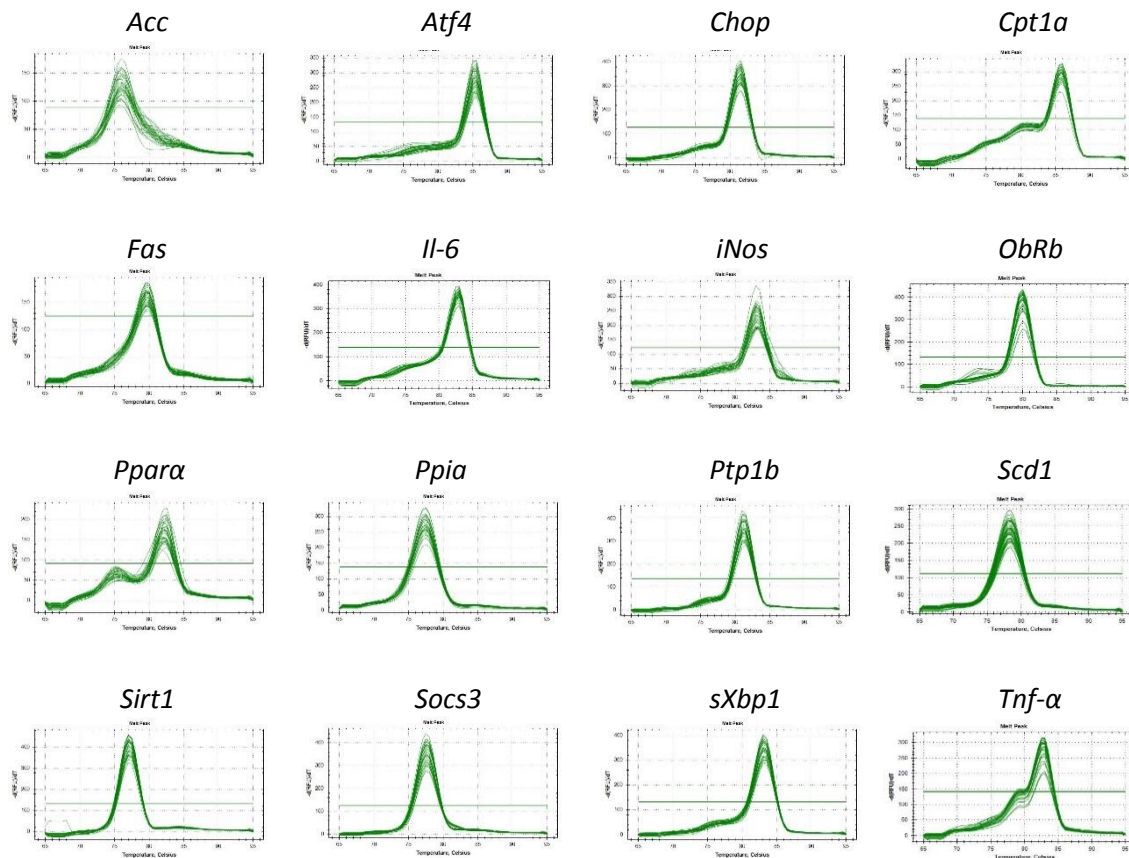
**Figure S1.** Cell viability by neutral red assay. Cells were incubated 48 h with Palm + Glc and the last 24 h were supplemented with 0, 1, 5, 10 and 50  $\mu\text{M}$  RSV. Data are expressed as the mean  $\pm$  SD of 3 replicates.



**Figure S2.** Activation of the leptin signalling pathway in HepG2 cells. pSTAT3 protein levels after the incubation of HepG2 cells with different leptin concentrations (10, 100 and 1,000 ng/mL) for different incubation times (5, 10, 15, 20 and 30 min). \*  $p < 0.05$  comparing leptin conditions to the control group as assessed by Student's t-test. Data are expressed as the mean  $\pm$  SD of 3 replicates.



**Figure S3.** Development of a leptin resistance state in HepG2 cells. (A) pSTAT3 protein levels and (B) TAG content in HepG2 cells incubated with different treatments to produce leptin resistance. Cells were treated with 0.5 mM Palm, 30 mM Glc, 0.5 mM Palm + 30 mM Glc and 30 mM Glc + 5.5 mM Fruc for 48 h. Leptin signalling was activated by the addition of 10 ng/mL leptin during the last 20 min of incubation. \*  $p < 0.05$  comparing treatment conditions to the control group as assessed by Student's t-test. Data are expressed as the mean  $\pm$  SD of 3 replicates. A representative WB image for each RSV incubation time was included.



**Figure S4.** Melt curves from one RT-qPCR plate. The figure shows melt curves of primers in one plate for quantitative RT-PCR. Note that each primer has only one melt curve, indicating the specificity of the primers. *ACC*: acetyl-CoA carboxylase, *ATF4*: activating transcription factor 4, *CHOP*: DNA damage inducible transcript 3, *CPT1A*: carnitine palmitoyltransferase 1a, *FAS*: fatty acid synthase, *Il-6*: interleukine 6, *INOS*: inducible nitric oxide synthase, *OBRb*: leptin receptor isoform b, *PPARα*: peroxisome proliferator activated receptor α, *PPIA*: peptidylprolyl isomerase a, *PTB1B*: protein-tyrosine phosphatase 1b, *SCD1*: stearyl-CoA desaturase 1, *SIRT1*: NAD<sup>+</sup>-dependent deacetylase sirtuin-1, *SOCS3*: suppressor of cytokine signaling 3 and *SXBP1*: x-box binding protein 1, *TNF-α*: tumor necrosis factor alpha.