SUPPLEMENTARY INFORMATION

Supplementary Fig. 1. **Expression levels of marker genes in interscapular BAT (IBAT) and epididymal WAT (eWAT).** Representative Northern blot. Total RNA (10 μg) isolated from IBAT and eWAT of NMRI mice housed at 22 ºC was used for each lane, and the blot was hybridized with aP2, PPARγ, PGC-1α, UCP1 and 18S rRNA probes.

Supplementary Fig. 2. **Expression levels of marker genes during spontaneous differentiation of white and brown adipocytes. (a)** White and brown adipocyte cultures were examined with phase-contrast microscopy after 7 days in culture. **(b)-(f)** Primary cultures of white and brown adipocytes were grown for the indicated number of days. Where indicated, 1 μM norepinephrine (NE) had been added 2 h before harvest. **(b)** Representative Northern blot. Total RNA (10 μg) was used per lane and the blot was hybridized with aP2, PPARγ, PGC-1α, UCP1 and 18S rRNA probes. **(c)** aP2, **(d)** PPARγ, **(e)** PGC-1α and **(f)** UCP1 mRNA levels during spontaneous differentiation of white and brown adipocytes. The aP2, PPARγ, PGC-1α and UCP1 mRNA levels were normalized to the 18S rRNA levels in each sample. The points represent means ± S.E. of three independent experiments, each performed in duplicate. The brown adipocyte control levels of aP2 and PPARγ and the NE-induced levels of PGC-1α and UCP1 at day 7 were set in each experiment to 100 %, and the white/brown adipocyte aP2, PPARγ, PGC-1α and UCP1 mRNA levels on different days were expressed relative to this value in each individual experiment. The aP2 ($p<0.0001$), the PGC-1α ($p<0.0001$), the PGC-1α ($p<0.0001$) and the UCP1 ($p<0.0001$) mRNA levels were significantly higher in brown adipocytes (two-way ANOVA with replicates, cell type vs. time in culture).

Supplementary Fig. 3. **Rosiglitazone does not favour growth of brown over white adipocytes.** Primary cultures of white adipocytes originating from UCP1-KO mice and brown adipocytes originating from UCP1-wild type mice were grown for 7 days separately or in co-culture in the indicated ratios in the absence (left panel) or presence of 1 μM rosiglitazone (right panel). Where indicated, 1 μM norepinephrine (NE) had been added 2 h before harvest. Representative Northern blot. Total RNA (10 μg) was used per lane and the blot was hybridized with the UCP1 and 18S rRNA probes. Arrows indicate wild type (WT) and knockout (KO) UCP1 transcripts. Since rosiglitazone in UCP1-KO cells induces a transcript that co-migrates with the predominant UCP1-WT transcript, the genetic identity of the cells was verified by RT-PCR (Suppl. Fig. 3).

Supplementary Fig. 4. **Verification of genetic identity of UCP1-KO and UCP1-WT adipocytes.** Rosiglitazone-treated samples from the same experiment as in Fig. 3 and Supplementary Fig. 2 were analysed by Reverse Transcriptase-PCR to validate the genotype of the UCP1-wild type and UCP1-KO cells. UCP1-KO mice had been generated by deleting exon 2 and part of exon 3 of UCP1 gene (Enerbäck et al. 1997). Thus, we used a forward primer annealing in exon 1 and a reverse primer annealing in exon 2 of UCP1 mRNA. PCR product was predicted to be 457 bp long in UCP1-WT mice and to be absent in UCP1-KO mice. The outcome of experiment confirmed genetic identity of UCP1-KO and UCP1-WT mice.

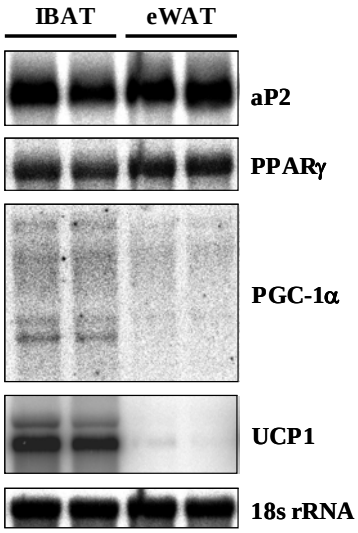
Forward primer: CTGGGCTTAACGGGTCCTC

Reversed primer: GGCAGACCGCTGTACAGTTT

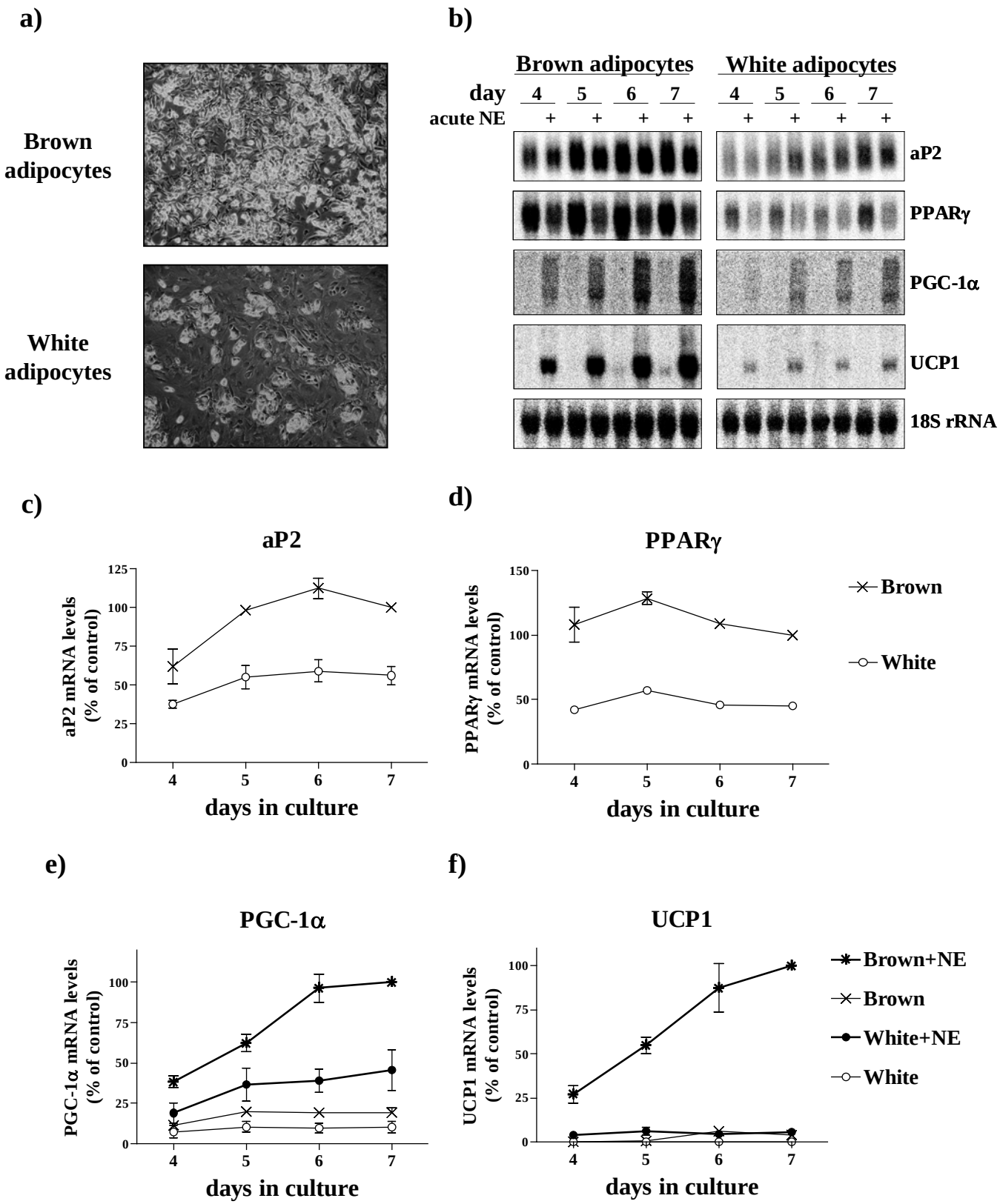
Supplementary Fig. 5. **Only a subset of cells in rosiglitazone-treated white adipocyte cultures is UCP1-positive and demonstrates NE-induced UCP1-dependent thermogenesis –** To improve

contrast, the black background in Fig. 6ab was altered to white (no areas with triple staining were observed in this study).

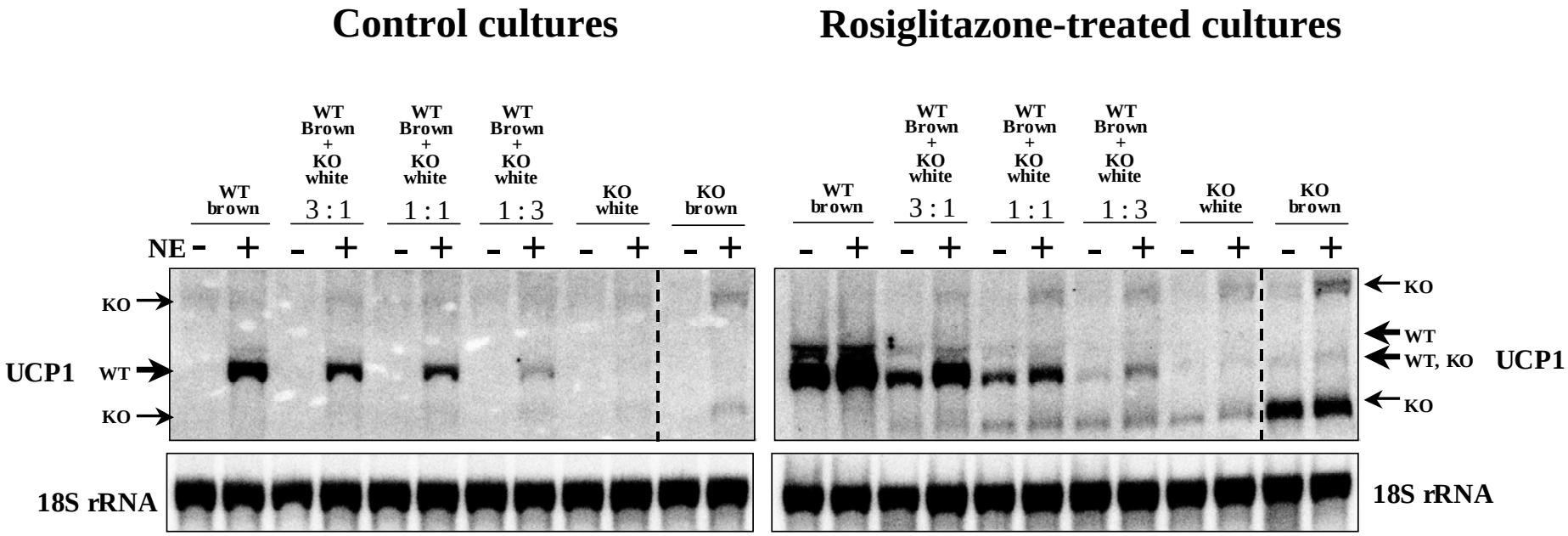
Petrovic_SupplementaryFig1.



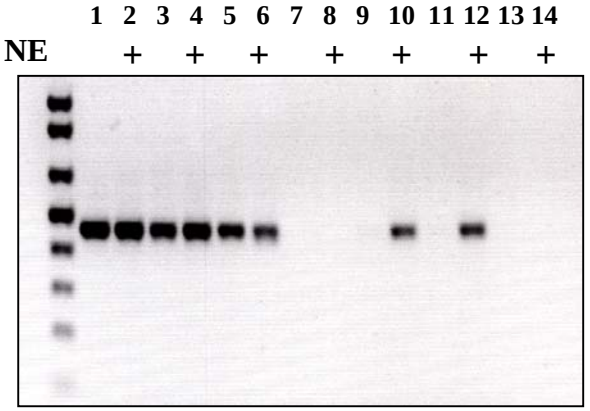
Petrovic_SupplementaryFig2.



Petrovic_SupplementaryFig3.



Petrovic_SupplementaryFig4.



1, 2 – brown adipocytes - WT

3, 4 – brown adipocytes - WT; white adipocytes - KO; 3:1

5, 6 – brown adipocytes - WT; white adipocytes - KO; 1:1

7, 8 – brown adipocytes - KO

9, 10 – brown adipocytes - KO; white adipocytes - WT; 1:1

11, 12 –white adipocytes - WT

13, 14 – white adipocytes - KO

Petrovic_SupplementaryFig5.

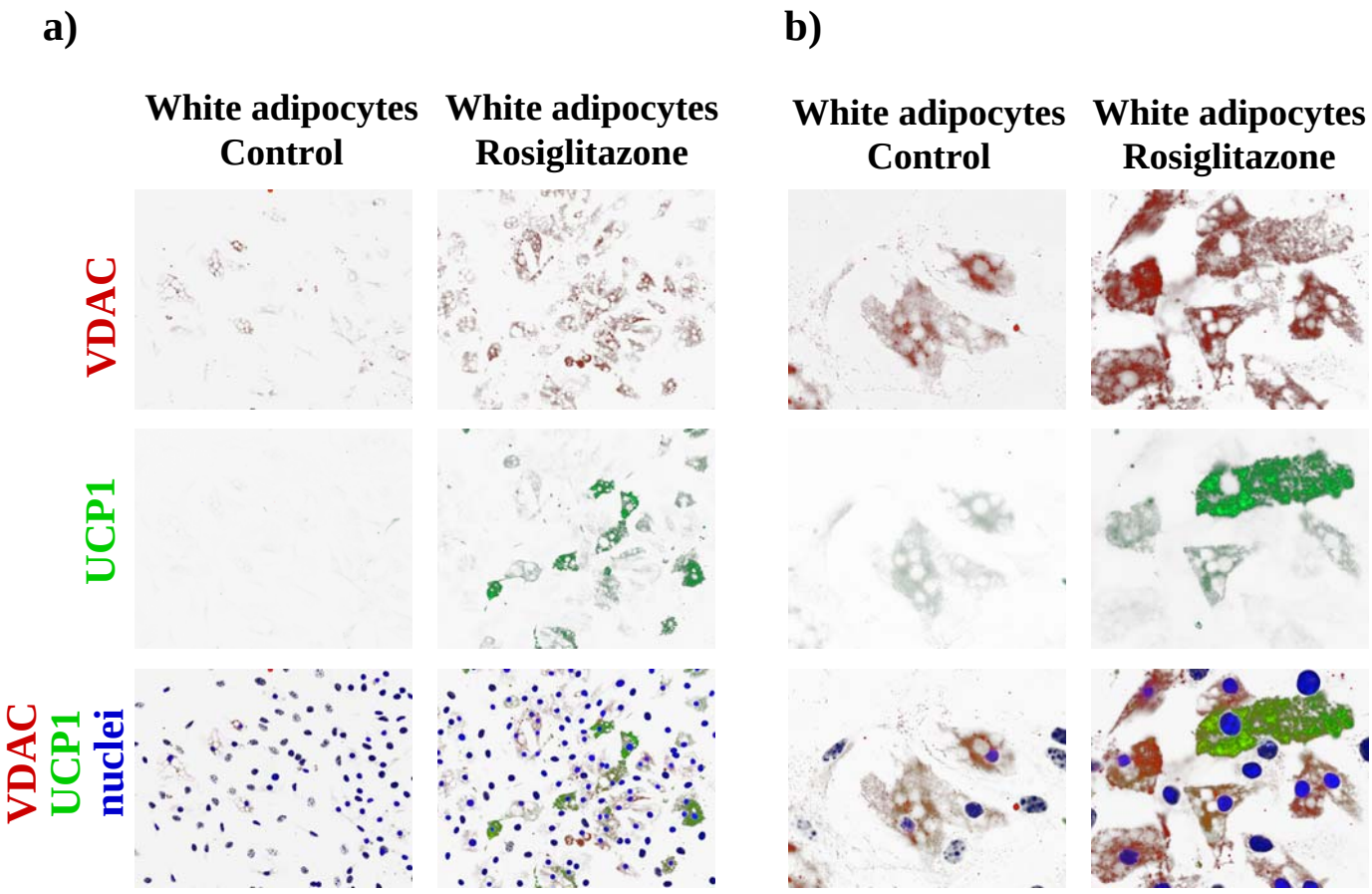


Table S1. Primers used for qRT-PCR analysis

Gene	Forward primer (5’ - 3’)	Reverse primer (5’ - 3’)
PPARα	CCGAGGGCTCTGTCATCA	GGGCAGCTGACTGAGGAA
CPT-1M	TGCCTTTACATCGTCTCCAA	GGCTCCAGGGTTCAGAAAGT
Elovl3	GCCTCTCATCCTCTGGTCCT	TGCCATAAACTTCCACATCCT
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
PGC-1α	GAAAGGGCCAAACAGAGAGA	GTAAATCACACGGCGCTCTT
UCP1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT
Myogenin	CTACAGGCCTTGCTCAGCTC	TGGGAGTTGCATTCACTGG
Zic1	AACCTCAAGATCCACAAAAGGA	CCTCGAACTCGCACTTGAA
Lhx8	GAGCTCGGACCAGCTTCA	TTGTTGTCCTGAGCGAACTG
Meox2	CTTTGACCCGCTTCCACTT	AATCTAGACCTCACTGAAAGACAGG
PRDM16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Igfbp3	GCAGCCTAAGCACCTACCTC	TCCTCCTCGGACTCACTGAT
DPT	CTGCCGCTATAGCAAGAGGT	TGGCTTGGGTACTCTGTTGTC
Tcf21	CATTCACCCAGTCAACCTGA	TTCCTTCAGGTCATTCTCTGG
Hoxc9	GCAGCAAGCACAAAGAGGAGAAG	GCGTCTGGTACTTGGTGTAGGG
TBP	ACGGACAACTGCGTTGATTT	TTCTTGCTGCTAGTCTGGATTG