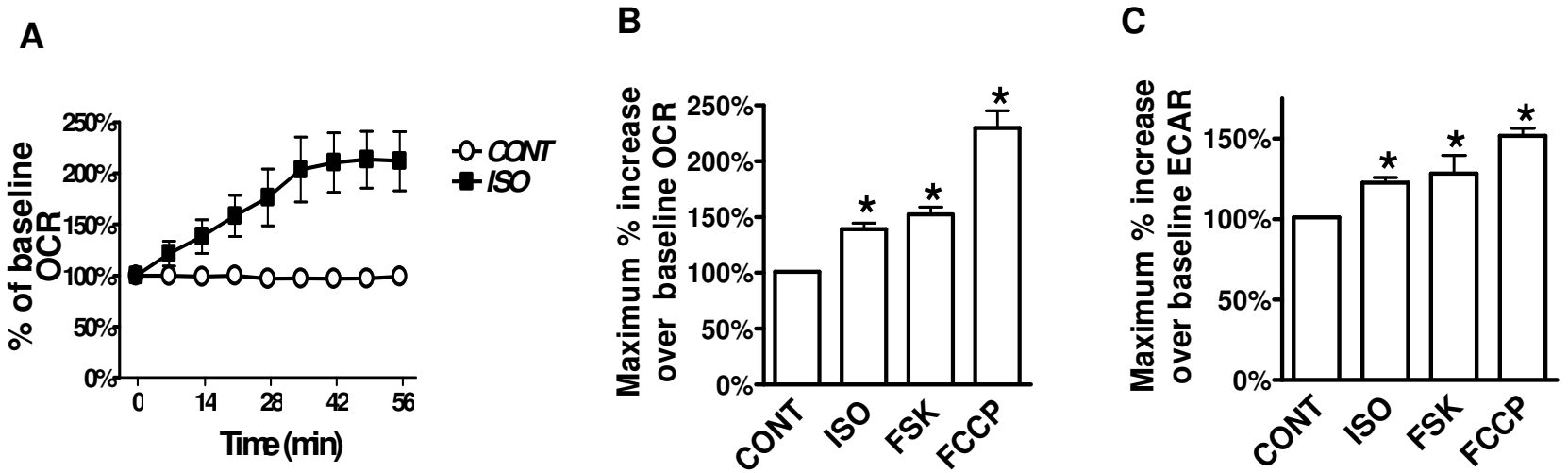
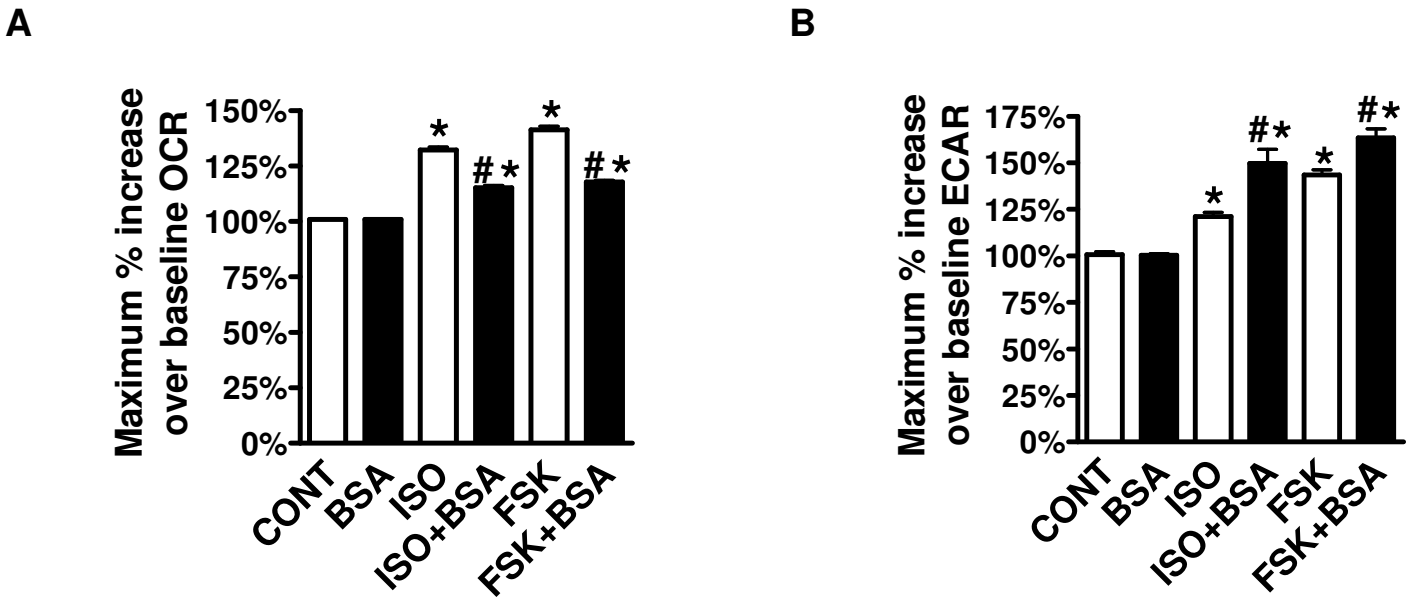
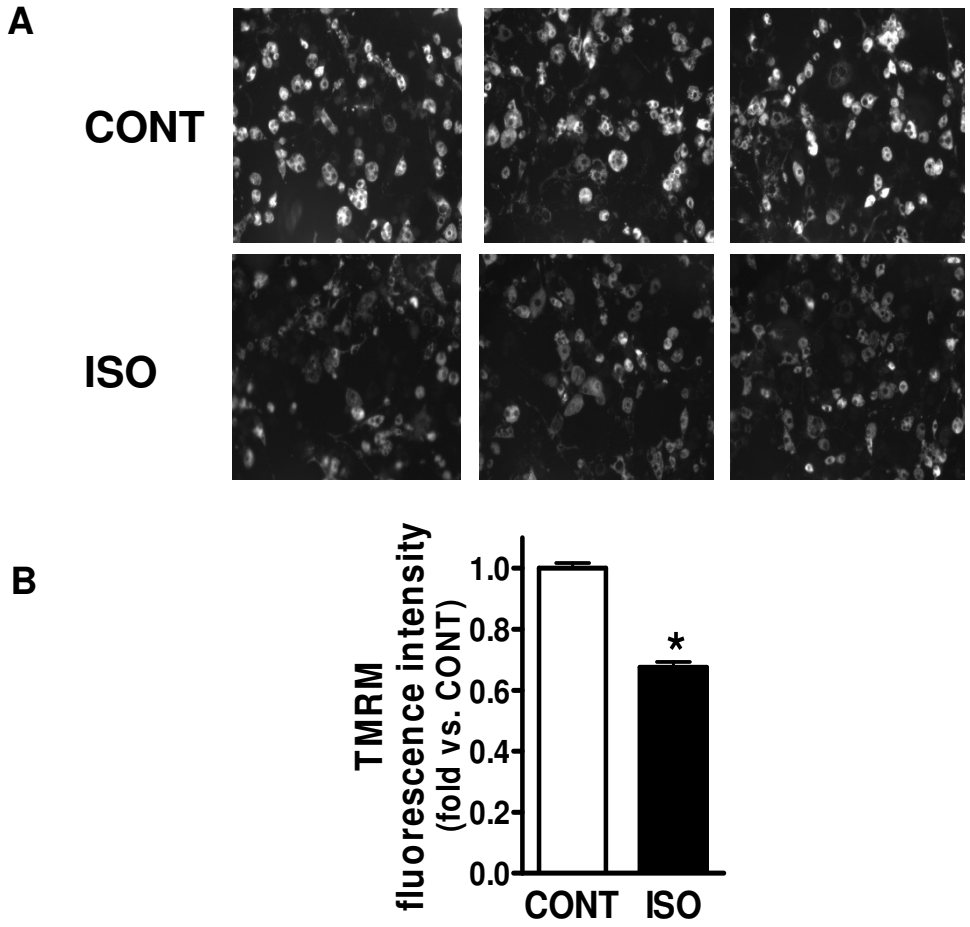


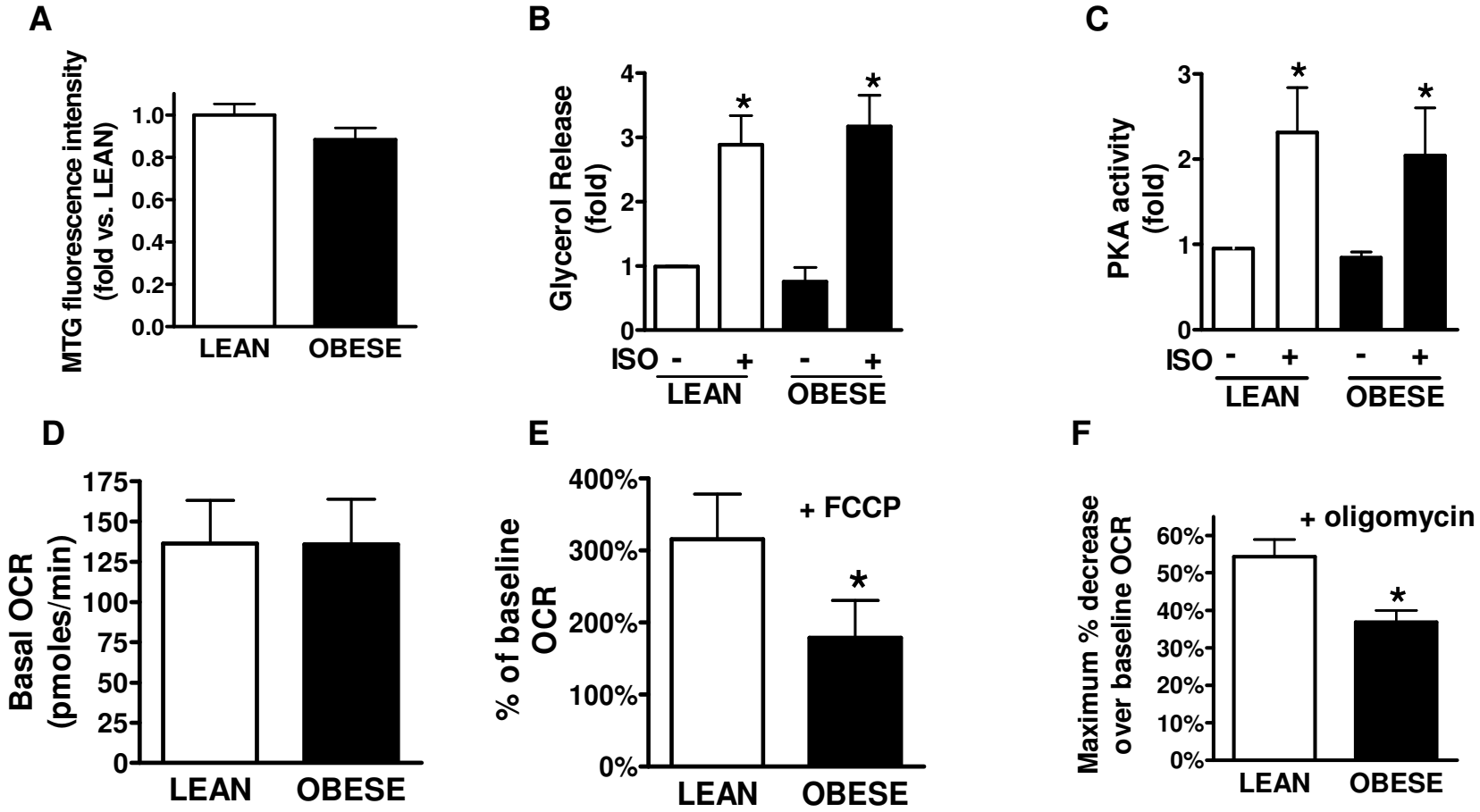
Supp. Figure 1. (**A**) Representative image of the percent increase in OCR in response to ISO, relative to baseline, in gonadal WAT from C57BL/6J mice. WAT was analyzed by the XF24 as described in Methods. At 0 min, ISO (1 μM, squares) or DMEM (CONT, circles) were injected. OCR measurements before ISO injection were set as 100 %. The data is the mean ± SD of 2 mice, each analyzed in 5 wells. Histograms summarizing the maximal percent increase in OCR (**B**) or ECAR (**C**) of mouse adipocyte cell line **3T3-L1** in response to DMEM (CONT), ISO (1 μM), FSK (10 μM),) or FCCP (0.6 μM) injections. Data is a mean of 3 experiments with 4-5 replicates. *, $p < 0.01$ compared with CONT.



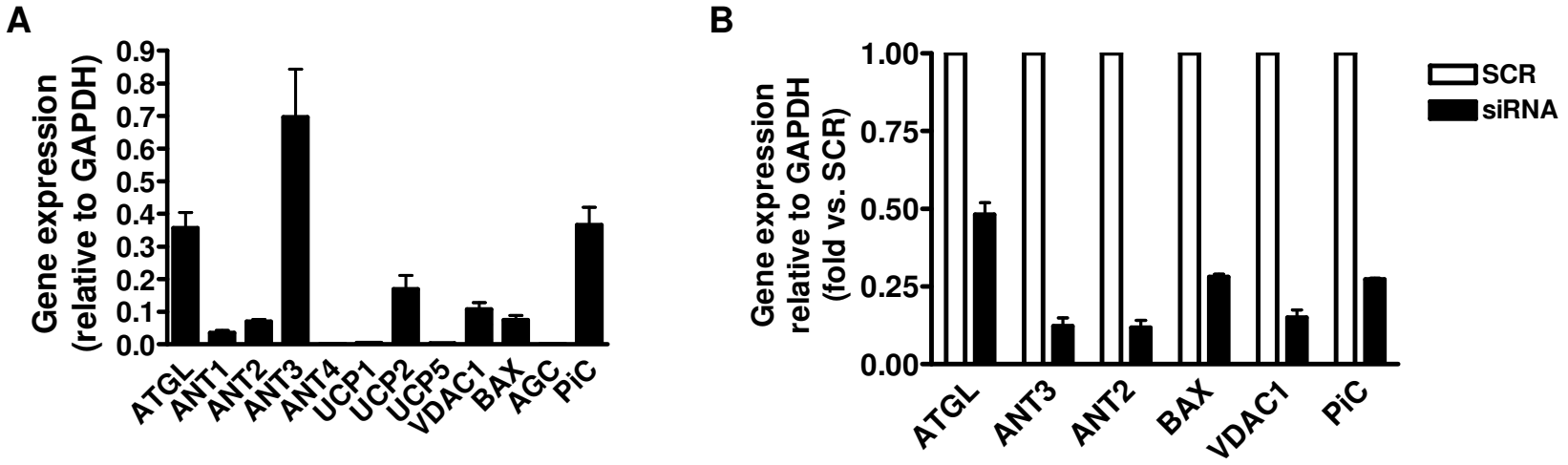
Supp. Figure 2. Histograms summarizing the maximal percent increase in OCR (**A**) or ECAR (**B**) of mouse adipocyte cell line **3T3-F442A** in response to DMEM (CONT), ISO (1 μM) or FSK (10 μM) injections. Where indicated, cells were pretreated with FA-free BSA (5 %, 1 hour). Data is the mean of 3 experiments. *, $p < 0.01$ compared with CONT; #, $p < 0.01$ compared with respective samples without BSA.



Supp. Figure 3. (**A**) Confocal microscopy images of 3T3-L1 adipocytes treated with ISO (1 μM, 30 min) or not (CONT) and stained with TMRM, as described in Methods. (**B**) Histogram summarizing TMRM fluorescence intensity. Data is a mean of 50 images collected from 3 independent experiments. *, $p < 0.001$, compared with CONT.



Supp. Figure 4. (**A**) Histogram summarizing MitoTracker Green (MTG) fluorescence intensity in human adipocytes. Cells were pooled from 5 lean subjects (BMI 21.7-24.6, LEAN) or 5 obese subjects (BMI 30-35.5, OBESE). Data is a mean of at least 100 cells collected from 3 independent experiments and calculated by ImagePro Plus software, as described in Methods. (**B**) Histogram summarizing glycerol release of human adipocytes in the presence or absence of ISO (1 μM, 2 hours), as described in Methods. Results are presented as fold vs. untreated LEAN. Data is a mean of 4 human subjects with 3 replicates each. *, $p < 0.01$, compared with the respective control. (**C**) Histogram summarizing PKA activity of human adipocytes, in the presence or absence of ISO (1 μM, 15 min), as described in Methods. Results are presented as fold vs. untreated LEAN. Data is a mean of 4 human subjects with 3 replicates each. *, $p < 0.05$, compared with the respective control. (**D**) Basal OCR levels of human adipocytes. (**E**) Percent OCR increase in human adipocytes in the presence of FCCP (0.6 μM). OCR measurements before FCCP injection were set as 100 %. (**F**) Percent OCR decrease from basal in human adipocytes in the presence of oligomycin (Oligo, 1 μg/ml). In (**D, E, F**) data is a mean of 12 lean subjects (BMI 21.7-24.6, LEAN) or 9 obese subjects (BMI 30-35.5, OBESE). *, $p < 0.01$, compared with LEAN.



C

Maximum % increase over baseline OCR

	CONT	Knock-Down
UCP2 *	212 ± 17	212 ± 13
ANT2	156.5 ± 5.5	154 ± 4.1
ANT3	137 ± 4	136 ± 6
VDAC1	139 ± 4	137.8 ± 7
PiC	135 ± 4	136 ± 5

Supp. Figure 5. (**A**) Gene expression levels of the indicated genes were measured in subcutaneous human adipocytes as described in Methods. Data is a mean of 3-6 samples. Real-time PCR primers used for the indicated genes are summarized in Supp. Figure 6. (**B**) Gene expression levels of the indicated genes following transfection of human adipocytes with scrambled (SCR) or with the specific siRNA. Data is presented as fold vs. SCR of each gene. The siRNA probes (Applied Biosystems, Carlsbad, CA) and conditions for each gene are summarized in Supp. Figure 6. (**C**) Table summarizing the maximum percent increase of OCR in response to ISO and relative to baseline rates. Data is a mean of 2-3 experiments. AGC; aspartate/glutamate carrier, ANT; adenine nucleotide transferase, BAX; Bcl2-associated X protein, PiC; phosphate carrier, UCP; uncoupling protein, VDAC; voltage dependant anion channel. * Measured in gonadal WAT from *Ucp2*+/+ vs. *Ucp2*-/- mice as described in Methods.

A

Gene	Accession #	Forward primer	Reverse primer
ATGL	NM_020376	GCCGGGAGAAGATCACATC	ATGTTGGAGAGGGTGGTCAG
ANT1 (SLC25A4)	NM_001151	TCGTAGAATGATGATGCAGTCC	CTTGGCTCCTTCGTCTTTTG
ANT2 (SLC25A5)	NM_001152	AAGGACTTCCTGGCAGGTG	TCTGCAGTGATCTGCTTGCT
ANT3 (SLC25A6)	NM_001636	CGTGTACGATACGGCCAAG	CGAAGGGGTAGGACACCAC
ANT4 (SLC25A3)	NM_031291	GCAGCTATTCATGTCTGGAGTTAAT	CTCCACCAGAAGCCAGGTT
BAX	NM_138761	CAAGACCAGGGTGGTTGG	CACTCCCGCCACAAAGAT
VDAC1	NM_003374	TTCTCACCTAACACTGGGAAAAA	CCAGCAATGTCGAAATCCAT
GAPDH	NM_005276	CTATACAGCATCCTCCAGCACAA	GGCCCTCGTAGCACACCTT

B

Gene	ABI #
GAPDH	Hs99999905
UCP1	Hs00222453
UCP2	Hs01075224
UCP5	Hs01073976
PiC	Hs00358082
AGC	Hs00192604

C

Gene	siRNA ABI #	Concentration/ incubation time
ATGL	5' –GCGAGAAUGUCAUUAUAtt *	30 nM / 24h
ANT2	s1375	30 nM / 48h
ANT3	s1377	60 nM / 48h
BAX	s1888	30 nM / 48h
VDAC1	s14768	30 nM / 48h
PiC	s10428	30 nM / 48h

Supp. Figure 6. Tables summarizing the real-time PCR primers used for the indicated genes by SYBR Green (**A**) or TaqMan (**B**) gene Expression assays (Applied Biosystems). (**C**) Table summarizing the siRNA probes (Applied Biosystems, Carlsbad, CA) and conditions used for each gene. *, ATGL siRNA sequence was described previously (Ryden M, 2007, Am J Physiol Endocrinol Metab, 292:E1847-55) and was synthesized by Qiagen, Valencia, CA.