Increased mitochondrial respiration of adipocytes from metabolically unhealthy obese compared to healthy obese individuals

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Supplemental legends

Figure S1: Potential underlying pathomechanisms, n=4/group; mean ±SEM; mRNA expression normalized to RPS13 of genes pivotal for: lipogenesis (panel A), lipolysis (B), β- oxidation (C), respiratory chain enzymes of complexes I-IV (D), and mitochondrial biogenesis (E), analyzed in adipocytes. Panel F – citrate synthase activity in preadipocytes and differentiated adipocytes.

Figure S2: Western Blots for protein levels of selected genes, n=4/group, if not stated otherwise; relative band intensity values (mean ±SD) are normalized to tubulin; proteins include important proteins for A) Complex II, B) Complex III (n=3 vs. 4), and C+D) mitochondrial biogenesis. On the right side, representative original western blots are shown.

Figure S3: mitochondrial DNA encoded genes; mean ±SD; mRNA expression normalized to RPS13; n=2 vs.3; ND1 representing complex I, COI = complex IV, ATP6 = complex V.

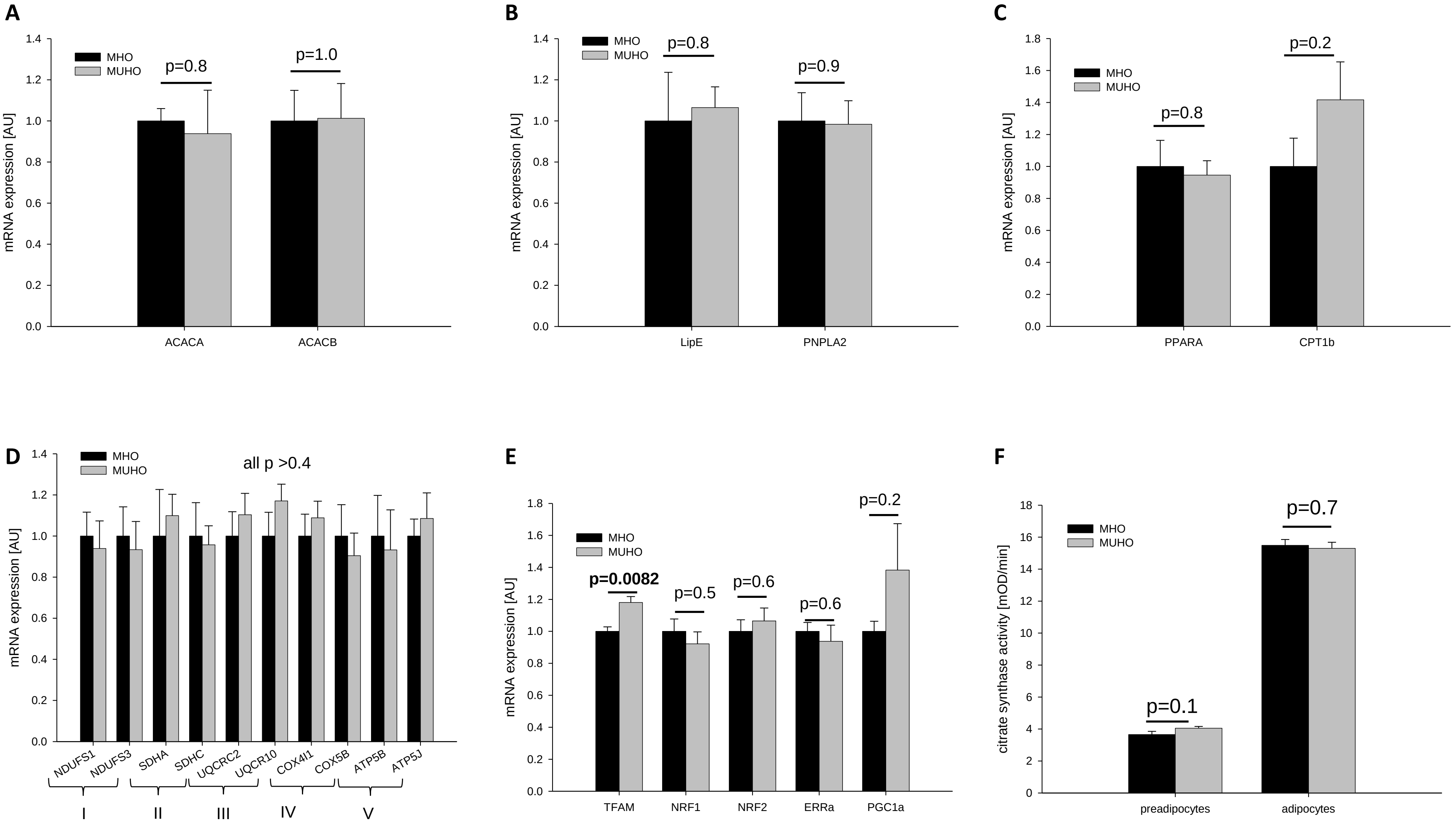
Figure S4: additional pathomechanistic hypotheses; panel A) lipolysis, B) cAMP, and C) ROS; mean ±SD; each n=2 vs. 3; A) and c) normalized to protein.

Figure S5: Original western blot; blots in figure S2 were taken from this blot. Black framed parts correspond to figure S2.

Figure S6: FCCP titration experiments showing the mitochondrial stress test under various FCCP concentrations (in µM), showing that optimal conditions were used in this study; n=5.

Table S1: Primer sequences used for quantitative real-time PCR.

fig. S1


A
mRNA expression [AU]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
MHO
MUHO
p=0.8
p=1.0
ACACA
ACACB
B
mRNA expression [AU]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
MHO
MUHO
p=0.8
p=0.9
LipE
PNPLA2
C
mRNA expression [AU]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
MHO
MUHO
p=0.8
p=0.2
PPARA
CPT1b
D
mRNA expression [AU]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
MHO
MUHO
all p >0.4
NDUFS1
NDUFS3
SDHA
SDHC
UQCRC2
UQCR10
COX4I1
COX5B
ATP5B
ATP5J
I
II
III
IV
V
E
mRNA expression [AU]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
MHO
MUHO
p=0.0082
p=0.5
p=0.6
p=0.6
p=0.2
TFAM
NRF1
NRF2
ERRa
PGC1a
F
citrate synthase activity [mOD/min]
0
2
4
6
8
10
12
14
16
18
MHO
MUHO
p=0.1
p=0.7
preadipocytes
adipocytes

Western Blots

fig. S2

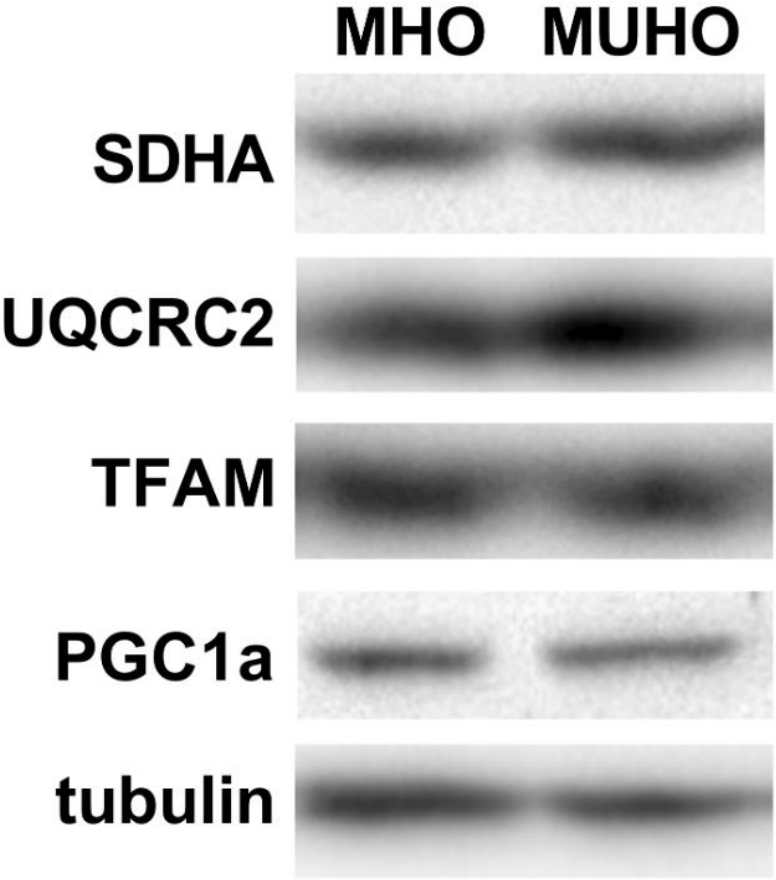


fig. S3

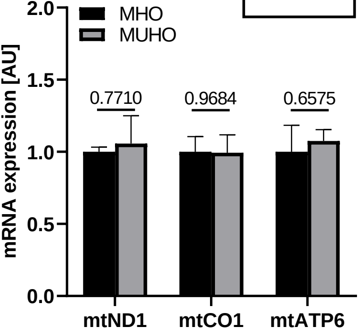

■ MHO
▨ MUHO
mRNA expression [AU]
2.0
1.5
1.0
0.5
0.0
0.7710
0.9684
0.6575
mtND1
mtCO1
mtATP6

fig. S4

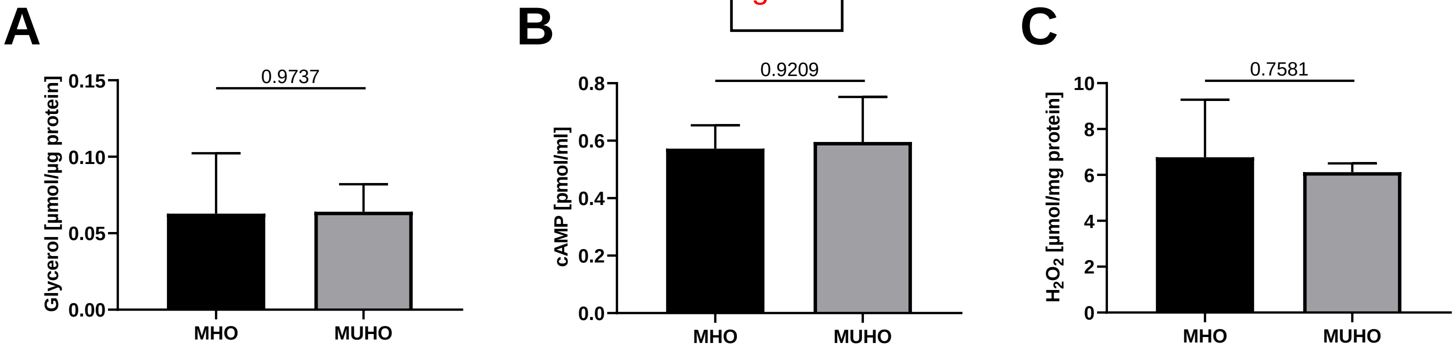

A
Glycerol [µmol/µg protein]
0.15
0.10
0.05
0.00
0.9737
MHO
MUHO
B
cAMP [pmol/ml]
0.8
0.6
0.4
0.2
0.0
0.9209
MHO
MUHO
C
H_2O_2 [µmol/mg protein]
10
8
6
4
2
0
0.7581
MHO
MUHO

fig. S5

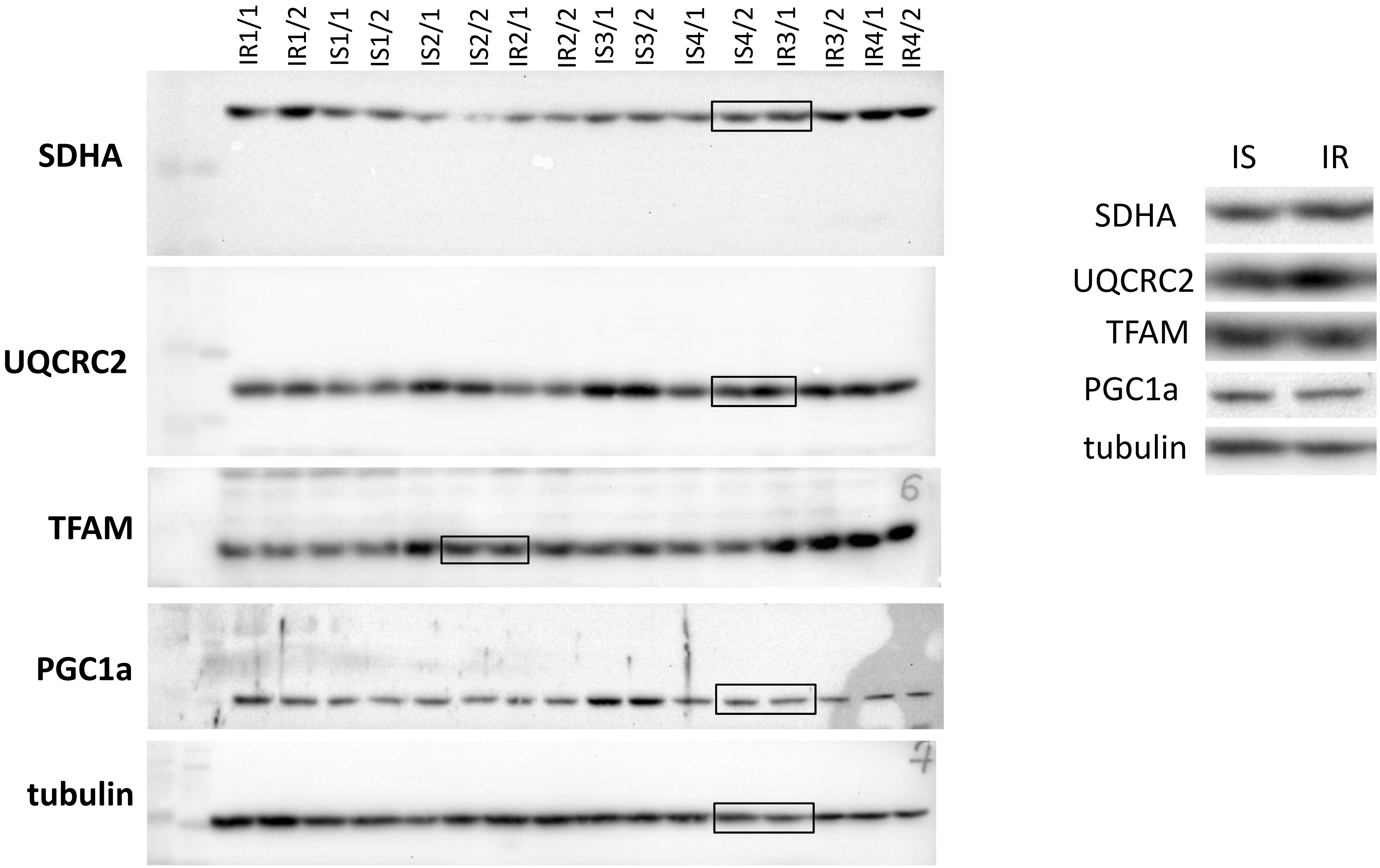
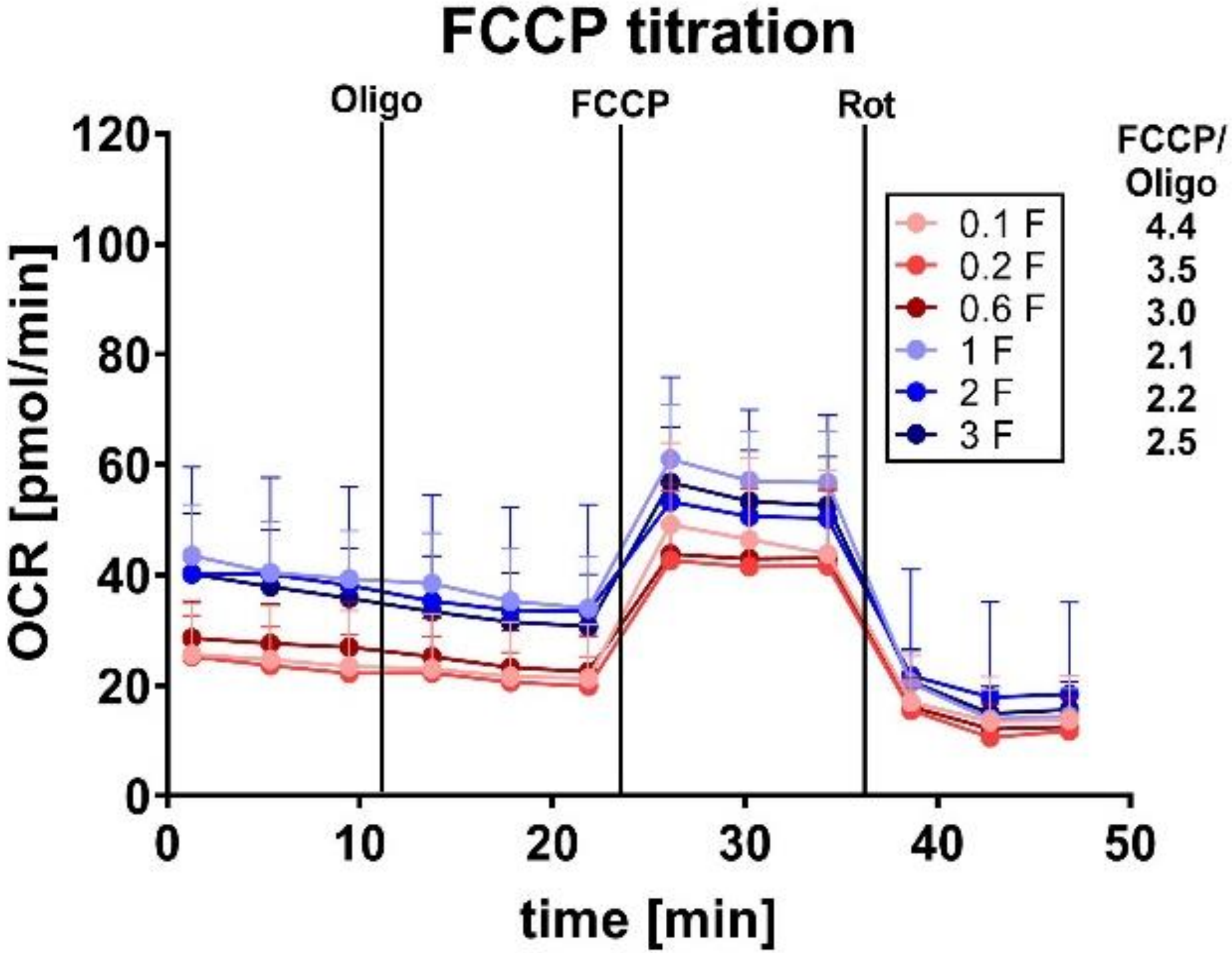

IR1/1
IR1/2
IS1/1
IS1/2
IS2/1
IS2/2
IR2/1
IR2/2
IS3/1
IS3/2
IS4/1
IS4/2
IR3/1
IR3/2
IR4/1
IR4/2
SDHA
UQCRC2
TFAM
6
PGC1a
tubulin
7
IS
IR
SDHA
UQCRC2
TFAM
PGC1a
tubulin

fig. S6


FCCP titration
Oligo
FCCP
Rot
OCR [pmol/min]
120
100
80
60
40
20
0
time [min]
0
10
20
30
40
50
0.1 F
0.2 F
0.6 F
1 F
2 F
3 F
FCCP/
Oligo
4.4
3.5
3.0
2.1
2.2
2.5

gene	**sequence 5' - 3'**
ACACA up	CATTCCGAGCAAGGGATAAG
ACACA do	CAGAGCAGGCTCCAGATGA
ACACB do	TGCGGAACATCTCATAGGC
ACACB up	TCGGGTCATCGAGAAGGT
ATP5B do	ACCAGAATTTCCTGCTCAACA
ATP5B up	TGATGAAAGAGGTCCCATCAA
ATP5J do	AAGCTCCCTCTCCAGCTCTT
ATP5J up	CAGAAACTCTTTGTGGACAAGATTAG
COX4I1 do	CAGACAGGTGCTTGACATGG
COX4I1 up	TCCACCTCTGTGTGTGTACGA
COX5B do	TGTATGGGTCCAGTCCCTTCT
COX5B up	TCCATGGCATCTGGAGGT
CPT1b do	GAAGGAGGAACCCACTGTTG
CPT1b up	CCTGGAAGAAACGCCTGAT
ESRRA do (ERRa)	TCAACCACCAGCAGATGAGA
ESRRA up (ERRa)	GTGGGCGGCAGAAGTACA
LipE do	TTCGTTCCCCTGTTGAGC
LipE up	CGCTGGAGGAGTGCTTCTT
NDUFS1 do	AAGAAGATAATTGGAACGCAAATC
NDUFS1 up	TCTGACACCTTATGCACTGAAGA
NDUFS3 do	AACTCATTGAAGCAGGACACCT
NDUFS3 up	AGCAGCTCTCAGCTTTTGGA
NFE2L2 up (NRF2)	GAGACAGGTGAATTTCTCCCAAT
NFE2L2 do (NRF2)	TTTGGGAATGTGGGCAAC
NRF1 do	TGGAGGGTGAGATACAGAGGA
NRF1 up	TATCCGGAAGAGGCAACAAA
PGC1a do	ATAAATCACACGGCGCTCTT
PGC1a up	TGAGAGGGCCAAGCAAAG
PNPLA2 do	CCCTGCTTGCACATCTCTC
PNPLA2 up	CTCCACCAACATCCACGAG
PPARa do	GTCCCCGCAGATTCTACATT
PPARa up	GAAGCTGTCCTGGCTCAGAT
SDHA do	CCAGCGTTTGGTTTAATTGG
SDHA up	GGACCTGGTTGTCTTTGGTC
SDHC do	CTGCACTCAAAGCAATACCAGT
SDHC up	TCCTCTGTCTCCCCACATTAC
TFAM do	GAATCAGGAAGTTCCCTCCA
TFAM up	GAACAACTACCCATATTTAAAGCTCA
UQCR10 do	CTCCAGCTCTGGGCAAAC
UQCR10 up	GGGGTGACAGTGGAGTAGAGAA
UQCRC2 do	TGCACTTGTGAAATGGTTCTG
UQCRC2 up	TGTCCTGACTATAGGATTGGAAAA
mtND1 up	AACCTCTCCACCCTTATCACAA
mtND1 do	TCATATTATGGCCAAGGGTCA
mtCO1 up	CCCTCCCTTAGCAGGGAAC

mtCO1 do	TGAAATTGATGGCCCCTAAG
mtATP6 up	GCCGCAGTACTGATCATTCTATT
mtATP6 do	GGGTGGTGATTAGTCGGTTG

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