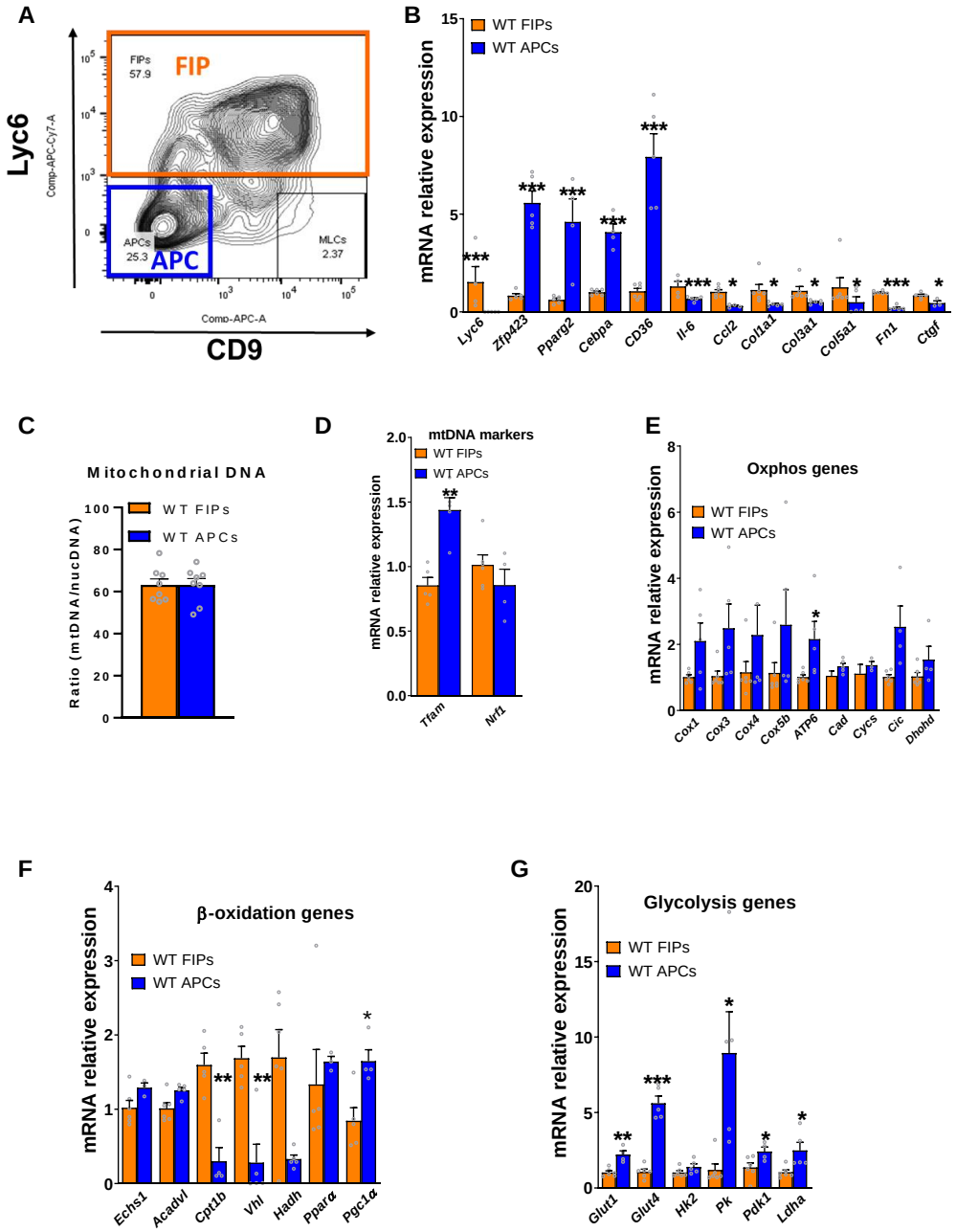
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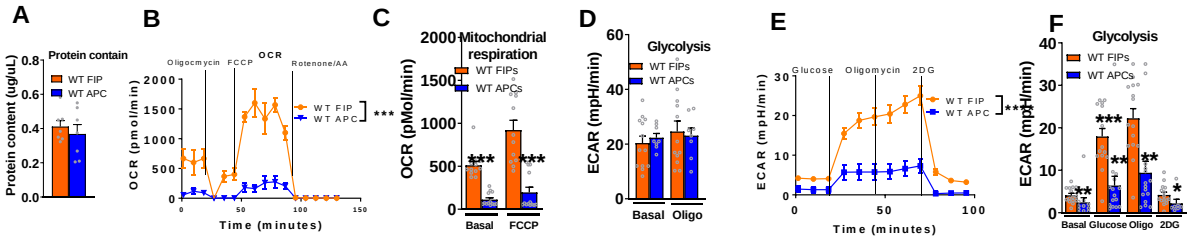
Lyc6
CD9
FIPs
57.9
FIP
APCs
25.3
APC
MLCs
2.37
B
WT FIPs
WT APCs
mRNA relative expression
Lyc6
Zfp423
Pparg2
Cebpa
CD36
Il-6
Ccl2
Col1a1
Col3a1
Col5a1
Fn1
Ctgf
C
Mitochondrial DNA
WT FIPs
WT APCs
Ratio (mtDNA/nucDNA)
D
mtDNA markers
WT FIPs
WT APCs
mRNA relative expression
Tfam
Nrf1
E
Oxphos genes
WT FIPs
WT APCs
mRNA relative expression
Cox1
Cox3
Cox4
Cox5b
ATP6
Cad
Cycs
Cic
Dhohd
F
β-oxidation genes
WT FIPs
WT APCs
mRNA relative expression
Echs1
Acadvl
Cpt1b
Vhl
Hadh
Pparα
Pgc1α
G
Glycolysis genes
WT FIPs
WT APCs
mRNA relative expression
Glut1
Glut4
Hk2
Pk
Pdk1
Ldha

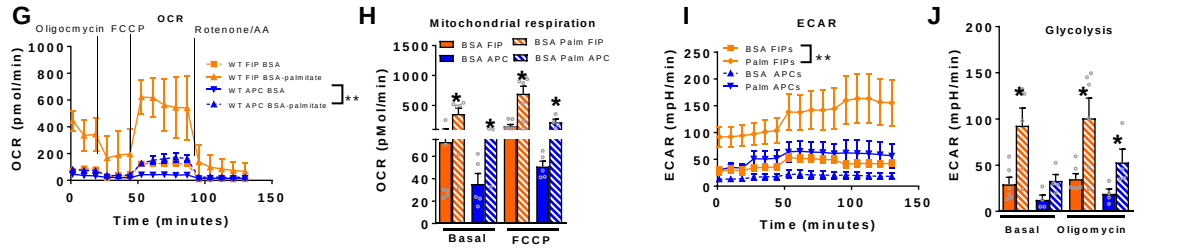
Supplemental Figure 1: FIPs and APCs gene profile from wild-type mice, Related to Figure 1

FIPs and APCs were sorted from epididymal WAT from 4-6 week-old male C57BL/6 mice using FACS by selecting $CD45^-$, $CD31^-$, $CD140b^+$, $Lyc6^+$ and $CD9^+$ (FIPs) and $CD45^-$, $CD31^-$, $CD140b^+$, $Lyc6^-$ and $CD9^-$ (APCs). **(A)** Flow cytometry graph representing the gating for FIPs and APCs for sorting **(B)** Gene expression of adipogenic, inflammatory and fibrosis markers compared between FIPs and APCs from WT mice. Data are presented in mRNA relative to FIPs **(C)** Mitochondrial DNA in FIPs and APCs from WT mice. Data are presented as the ratio between mtDNA and nuclear DNA. **(D-G)** Gene expression of FIPs and APCs from WT mice. Data are presented in mRNA relative to FIPs. **(D)** Gene expression of mitochondrial markers. **(E)** Gene expression of markers oxidative phosphorylation **(F)** Gene expression of fatty acid oxidative metabolism markers **(G)** Gene expression of glycolytic markers. Significance between FIPs and APCs from WT mice was calculated using a two-tailed student's t-test. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).

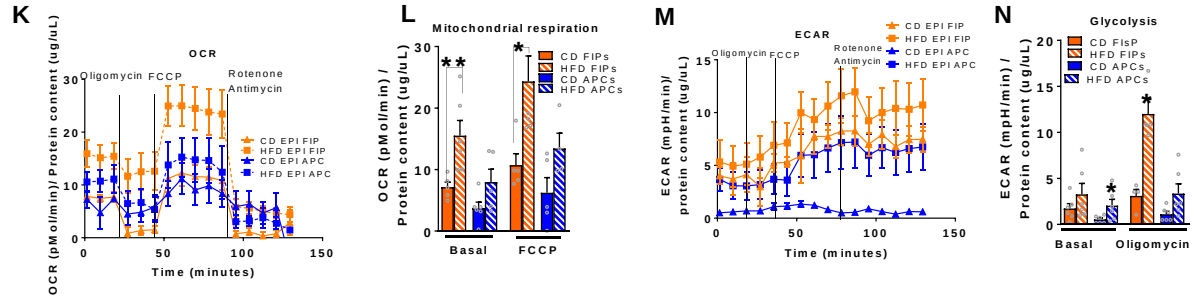
FIPs / APCs mitochondrial metabolism



4 h pretreatment with BSA Palmitate

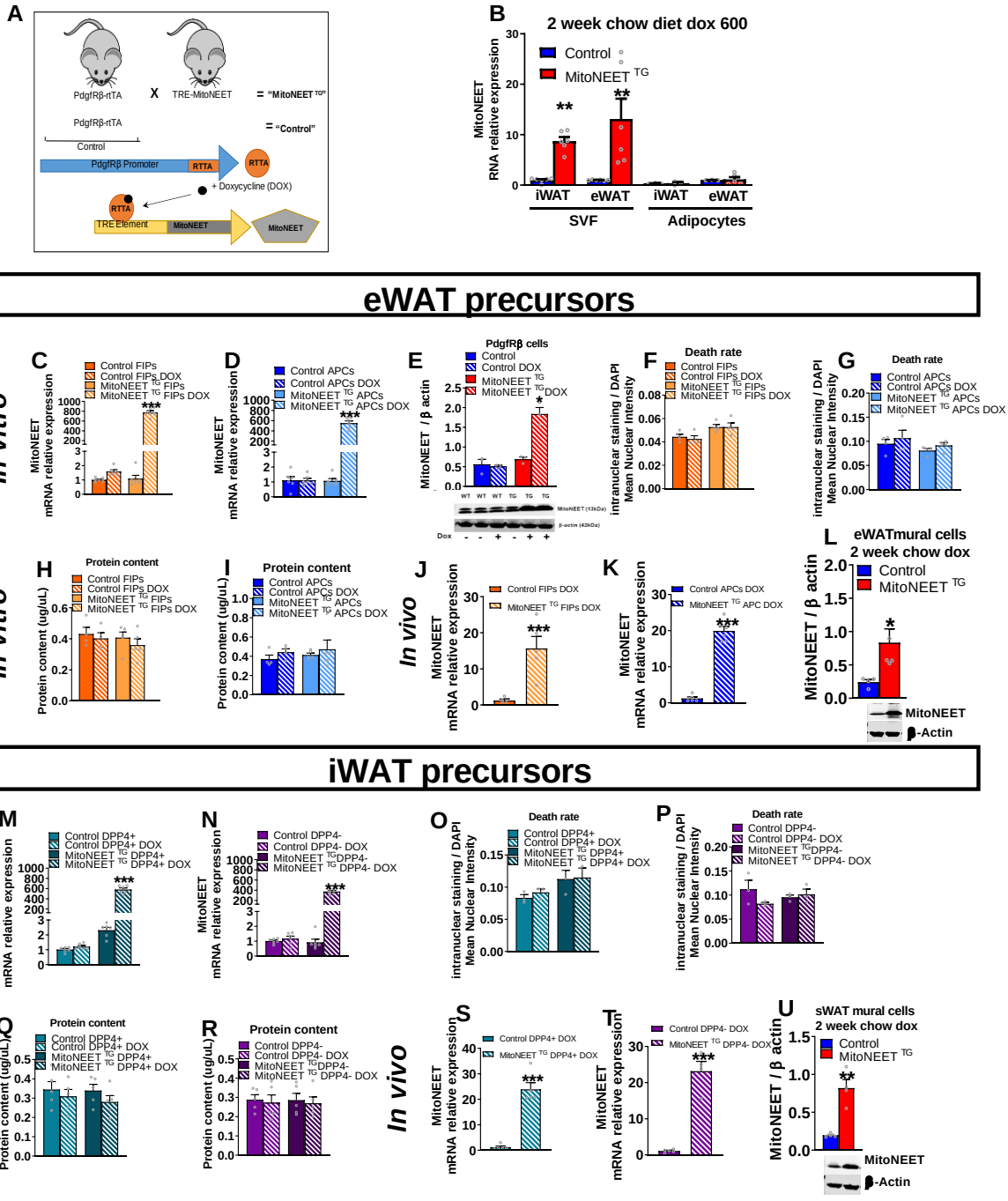


In vivo metabolic response to 3 day Control diet *vs* HFD



<u>Supplemental Figure 2:</u> FIPs and APCs have different metabolic capacities and are regulated differentially after 3 days of HFD feeding or by BSA-palmitate treatment, Related to Figure 1

FIPs and APCs were sorted from epididymal WAT (eWAT) from 4-6 week-old male C57BL/6 mice using FACS by selecting $CD45^-$, $CD31^-$, $CD140b^+$, $Lyc6^+$ and $CD9^+$ (FIPs) and $CD45^-$, $CD31^-$, $CD140b^+$, $Lyc6^-$ and $CD9^-$ (APCs). **(A)** Protein content of 80,000 FIPs or APCs plated in a seahorse plate for 24h (n=6). Data are presented in µg/µL. **(B-C)** Oxygen consumption rate of 80,000 FIPs and APCs measured. Data are presented in pmol/min **(D)** Glycolysis of 80,000 FIPs and APCs evaluated by extracellular acidification rate (ECAR). Data are presented in mpH/min. **(E-F)** Glycolysis stress analysis of 80,000 FIPs and APCs. Data are presented in mpH/min. **(G-J)** FIPs and APCs treated with 7 µM BSA or 0.2mM palmitate combined with 7µM BSA for 4 hours and then evaluated by Seahorse analysis. **(G-H)** Oxygen consumption rate of 80,000 FIPs and APCs pretreated with BSA or BSA-palmitate for 4 hours. Data is presented in pmol/min. **(I-J)** Glycolysis of 80,000 FIPs and APCs pretreated 4 hours with BSA or BSA-palmitate evaluated by ECAR. Data are presented in mpH/min. **(K-N)** FIPs and APCs sorting by FACS from the eWAT of wild type mice fed either control diet (CD) or high-fat diet (HFD) for 3 days. **(K-L)** Oxygen consumption rate of <u>freshly</u> isolated FIPs and APCs following 3 days of CD or HFD feeding. Data are presented in pmol/min per protein content (µg/µL). **(M-N)** Glycolysis of <u>freshly</u> isolated FIPs and APCs following 3 day chow or HFD feeding evaluated by ECAR. Data are presented in mpH/min. Significance in (B, E,G,I,K,M) was calculated usin a 2-way Anova with Tukey's post-test for multiple comparisons. Significance in (A,C,D,F,H,J, L,N) was calculated using a two-tailed student's t-test. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).


A
PdgfRβ-rtTA
X
TRE-MitoNEET
= "MitoNEET TG"
PdgfRβ-rtTA
Control
= "Control"
PdgfRβ Promoter
RTTA
RTTA
+ Doxycycline (DOX)
RTTA
TRE Element
MitoNEET
MitoNEET
B
2 week chow diet dox 600
Control
MitoNEET TG
MitoNEET RNA relative expression
iWAT
eWAT
iWAT
eWAT
SVF
Adipocytes
eWAT precursors
In vitro
C
Control FIPs
Control FIPs DOX
MitoNEET TG FIPs
MitoNEET TG FIPs DOX
MitoNEET mRNA relative expression
D
Control APCs
Control APCs DOX
MitoNEET TG APCs
MitoNEET TG APCs DOX
MitoNEET mRNA relative expression
E
PdgfRβ cells
Control
Control DOX
MitoNEET TG
MitoNEET TG DOX
MitoNEET / β actin
WT WT WT TG TG TG
MitoNEET (13kDa)
β-actin (42kDa)
Dox - - + - + +
F
Death rate
Control FIPs
Control FIPs DOX
MitoNEET TG FIPs
MitoNEET TG FIPs DOX
intranuclear staining / DAPI
Mean Nuclear Intensity
G
Death rate
Control APCs
Control APCs DOX
MitoNEET TG APCs
MitoNEET TG APCs DOX
intranuclear staining / DAPI
Mean Nuclear Intensity
In vitro
H
Protein content
Control FIPs
Control FIPs DOX
MitoNEET TG FIPs
MitoNEET TG FIPs DOX
Protein content (ug/uL)
I
Protein content
Control APCs
Control APCs DOX
MitoNEET TG APCs
MitoNEET TG APCs DOX
Protein content (ug/uL)
In vivo
J
Control FIPs DOX
MitoNEET TG FIPs DOX
MitoNEET mRNA relative expression
K
Control APCs DOX
MitoNEET TG APC DOX
MitoNEET mRNA relative expression
L
eWATmural cells
2 week chow dox
Control
MitoNEET TG
MitoNEET / β actin
MitoNEET
β-Actin
iWAT precursors
In vitro
M
Control DPP4+
Control DPP4+ DOX
MitoNEET TG DPP4+
MitoNEET TG DPP4+ DOX
MitoNEET mRNA relative expression
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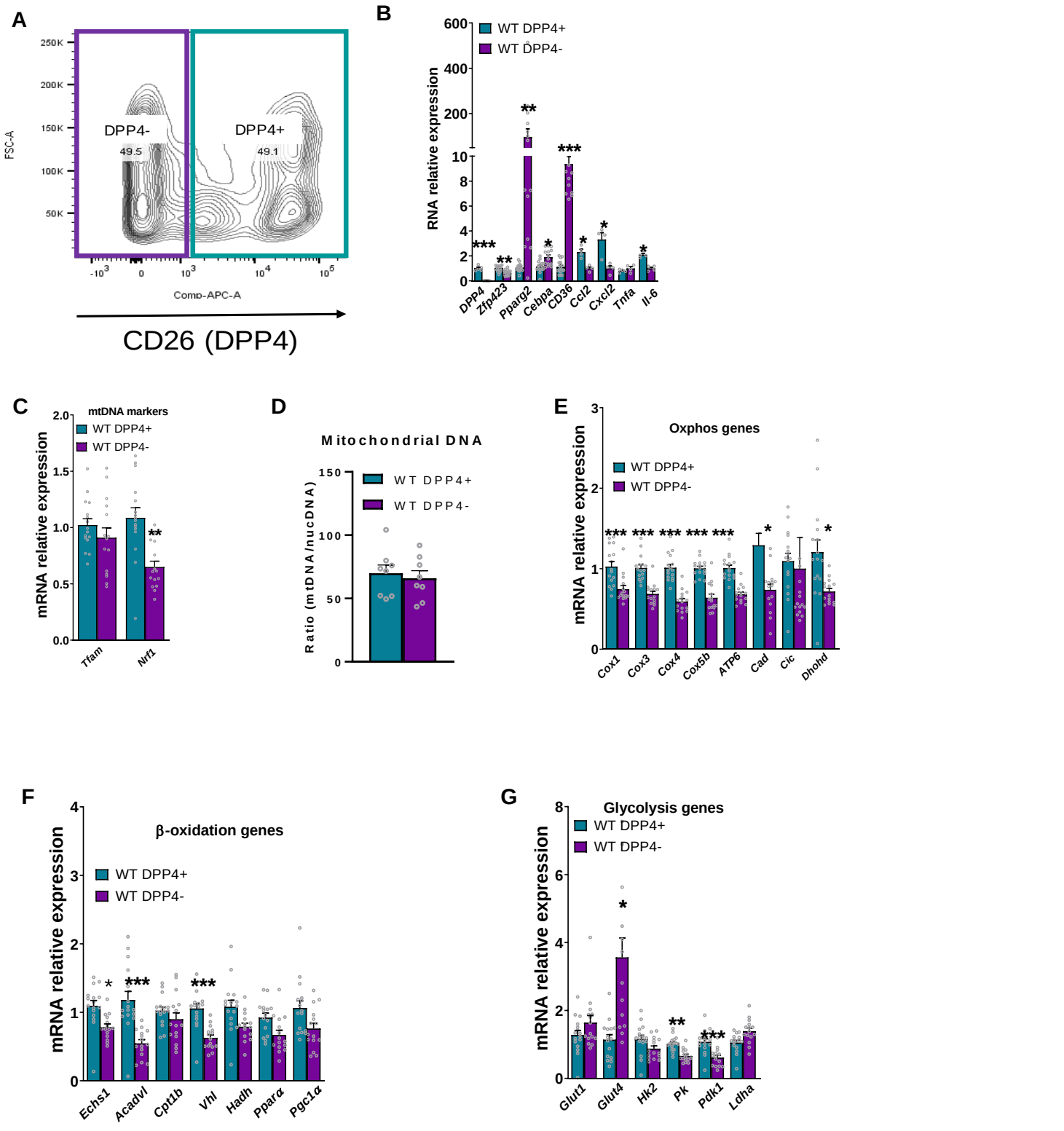
Control DPP4-
Control DPP4- DOX
MitoNEET TGDPP4-
MitoNEET TG DPP4- DOX
MitoNEET mRNA relative expression
O
Death rate
Control DPP4+
Control DPP4+ DOX
MitoNEET TG DPP4+
MitoNEET TG DPP4+ DOX
intranuclear staining / DAPI
Mean Nuclear Intensity
P
Death rate
Control DPP4-
Control DPP4- DOX
MitoNEET TGDPP4-
MitoNEET TG DPP4- DOX
intranuclear staining / DAPI
Mean Nuclear Intensity
In vitro
Q
Protein content
Control DPP4+
Control DPP4+ DOX
MitoNEET TG DPP4+
MitoNEET TG DPP4+ DOX
Protein content (ug/uL)
R
Protein content
Control DPP4-
Control DPP4- DOX
MitoNEET TGDPP4-
MitoNEET TG DPP4- DOX
Protein content (ug/uL)
In vivo
S
Control DPP4+ DOX
MitoNEET TG DPP4+ DOX
MitoNEET mRNA relative expression
T
Control DPP4- DOX
MitoNEET TG DPP4- DOX
MitoNEET mRNA relative expression
U
sWAT mural cells
2 week chow dox
Control
MitoNEET TG
MitoNEET / β actin
MitoNEET
β-Actin

<u>Supplemental Figure 3:</u> Validation of PdgfRβ-rtTA TRE-MitoNEET (MitoNEET ^{TG}) mouse model. Related to Figure 1 and Figure 3

(A) Breeding strategy of the mouse model PdgfRβ-rtTA (Control) or PdgfRβ-rtTA x TRE-MitoNEET (MitoNEET ^{TG}) mice. **(B)** *In vivo* validation: Control and MitoNEET^{TG} were fed 2 weeks with chow diet containing 600 mg/kg of doxycycline and tissues were harvest to evaluate mRNA expression of *Cisd1* (MitoNEET) gene between control and MitoNEET^{TG} mice (n=6). **(C-I)** Control or MitoNEET^{TG} cells were isolated by FACS from eWAT, plated at confluency and treated with or without 4.4 µM doxycycline for 24 hours. **(C-D)** *In vitro* evaluation of *Cisd1* (MitoNEET) gene expression of FIPs **(C)** or APCs **(D)** from control or MitoNEET ^{TG} mice (n=6). Data are presented in mRNA expression relative to untreated control cells. **(E)** Protein expression of MitoNEET *in vitro* from PdgfRβ+ cells from control or MitoNEET^{TG} mice (n=3). Data are presented as a ratio between MitoNEET and β-actin protein expression. **(F-G)** *In vitro* death rate of FIPs (F) or APCs (G) from control or MitoNEET^{TG} mice (n=4). Data are presented as the ratio between intranuclear staining and DAPI. **(H-I)** *In vitro* protein content of FIPs (H) or APCs (I) from control or MitoNEET^{TG} mice plated in seahorse plate (n=4-5). **(J-L)** Control or MitoNEET^{TG} cells were isolated by FACS from eWAT of mice fed 1 week with chow diet containing 600 mg/kg of doxycycline. **(J-K)** Gene expression of Cisd1 (MitoNEET) from FIPs (n=4) (J) or APCs (n=5) (K). Data are presented in mRNA expression relative to control cells. **(L)** Protein expression of MitoNEET *in vivo* from PdgfRβ+ cells from control or MitoNEET^{TG} mice (n=4). Data are presented as a ratio between MitoNEET and β-actin protein expression. **(M-R)** Control or MitoNEET^{TG} cells were isolated by FACS from sWAT, plated at confluency and treated with or without 4.4 µM doxycycline for 24 hours. **(M-N)** *In vitro* mRNA expression of *Cisd1*(MitoNEET) of DPP4+ (n=6) **(M)** or DPP4- cells (n=6) **(N)** from control or MitoNEET^{TG} mice. Data are presented in mRNA expression relative to untreated control cells. **(O-P)** *In vitro* death rate of DPP4+ (n=3) (F) or DPP4- cells (n=3) (G) from control or MitoNEET^{TG} mice. Data are presented as the ratio between intranuclear staining and

DAPI. **(Q-R)** *In vitro* protein content of DPP4+ (n=4-5) (Q) or DPP4- cells (n=5) (R) from control or MitoNEET^{TG} mice plated in seahorse plate. **(S-U)** Control or MitoNEET^{TG} cells were isolated by FACS from sWAT of mice fed 1 week with chow diet containing 600 mg/kg of doxycycline. **(S-T)** Gene expression of Cisd1 (MitoNEET) from DPP4+ (n=5) (S) or DPP4- cells (n=4-5) (T). Data are presented in mRNA expression relative to control cells. **(U)** Protein expression of MitoNEET *in vivo* from PdgfRβ+ cells from control or MitoNEET^{TG} mice (n=4). Data are presented as a ratio between MitoNEET and β-actin protein expression. Significance in (C-I; M-R) was calculated using a Kruskal-Wallis (One-Way Anova) test with Dunn's post-test for multiple comparisons. Significance in (B; J-L; S-U) was calculated using a two-tailed student's t-test. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).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150K
100K
50K
DPP4-
49.5
DPP4+
49.1
-10^3
0
10^3
10^4
10^5
Comp-APC-A
CD26 (DPP4)
B
WT DPP4+
WT DPP4-
RNA relative expression
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400
200
10
8
6
4
2
0
DPP4
Zfp423
Pparg2
Cebpa
CD36
Ccl2
Cxcl2
Tnfa
Il-6
C
mtDNA markers
WT DPP4+
WT DPP4-
mRNA relative expression
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1.5
1.0
0.5
0.0
Tfam
Nrf1
D
Mitochondrial DNA
WT DPP4+
WT DPP4-
Ratio (mtDNA/nucDNA)
150
100
50
0
E
Oxphos genes
WT DPP4+
WT DPP4-
mRNA relative expression
3
2
1
0
Cox1
Cox3
Cox4
Cox5b
ATP6
Cad
Cic
Dhohd
F
β-oxidation genes
WT DPP4+
WT DPP4-
mRNA relative expression
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3
2
1
0
Echs1
Acadvl
Cpt1b
Vhl
Hadh
Pparα
Pgc1α
G
Glycolysis genes
WT DPP4+
WT DPP4-
mRNA relative expression
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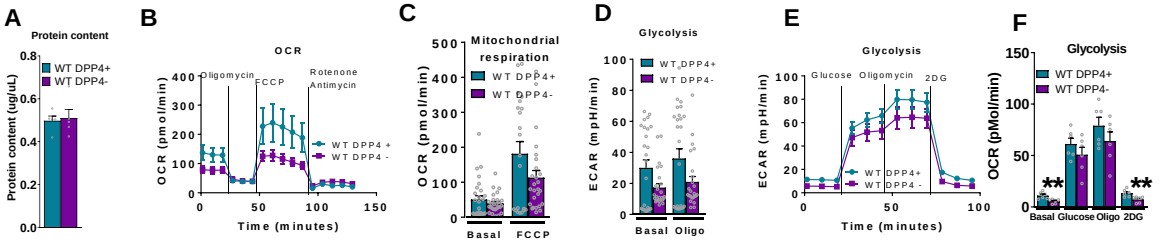
6
4
2
0
Glut1
Glut4
Hk2
Pk
Pdk1
Ldha

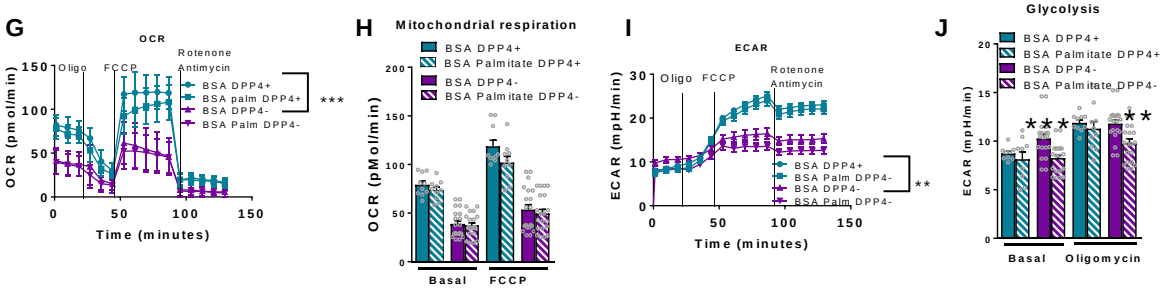
<u>Supplemental Figure 4:</u> DPP4^{+} and DPP4^{-} gene profile from wild-type mice, Related to Figure 3

DPP4^{+} and DPP4^{-} cells were sorted from subcutaneous WAT (sWAT) from 4-6 week-old male C57BL/6 mice (WT mice) using FACS by selecting for CD45^{-}, CD31^{-}, CD140b^{+}, DPP4^{+} and CD45^{-}, CD31^{-}, CD140b+, DPP4^{-}. **(A)** Gating strategy to sort DPP4+ and DPP4- cells based on the Cd26 (DPP4) marker **(B)** Gene expression of adipogenic and inflammatory markers in DPP4^{+} and DPP4^{-} cells isolated from the sWAT of WT mice. **(C)** Gene expression of mitochondrial markers in DPP4+ and DPP4- cells from the sWAT of WT mice **(D)** Mitochondrial DNA in DPP4^{+} and DPP4^{-} cells from the sWAT of WT mice. Data are expressed as the ratio of mtDNA to nuclear DNA. **(E)** Gene expression of oxidative phosphorylation markers in DPP4+ and DPP4- cells from the sWAT of WT mice. **(F)** Gene expression of fatty acid oxidative metabolism markers in DPP4+ and DPP4- cells from the sWAT of WT mice **(G)** Gene expression of glycolytic markers. Data are presented as relative expression of markers in DPP4^{+} cell to DPP4^{-} cells. Significance between FIPs and APCs from WT mice was calculated using a two-tailed student's t-test. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).

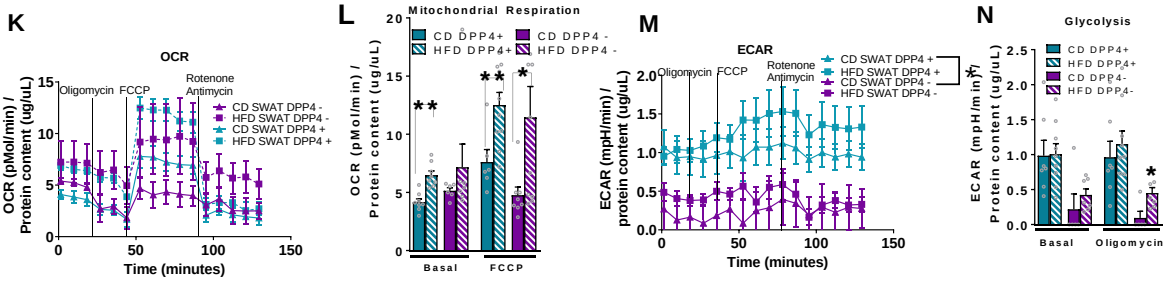
DPP4+ / DPP4- mitochondrial metabolism



4h pretreatment with BSA Palmitate

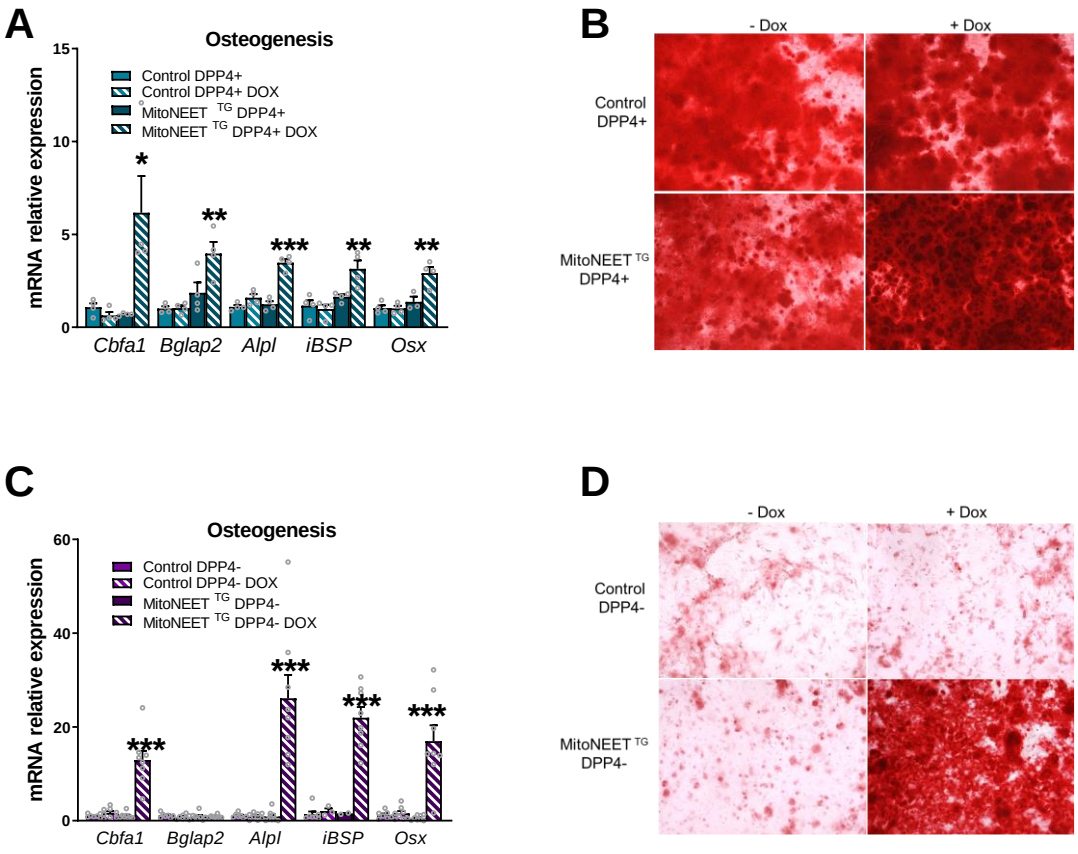


In vivo metabolic response to 3 day Control diet *vs* HFD



Supplemental Figure 5: DPP4$^+$ and DPP4$^-$ metabolic status in response to palmitate or three days of HFD, Related to Figure 3

DPP4$^+$ and DPP4$^-$ cells were sorted from sWAT from 4-6 week-old male C57BL/6 mice FACS by selecting CD45$^-$, CD31$^-$, CD140b$^+$, CD26$^+$ (DPP4$^+$) cells and CD45$^-$, CD31$^-$, CD140b$^+$, CD26$^-$ (DPP4$^-$) cells. (A) Protein content of DPP4+ and DPP4- cells plated in Seahorse plate. **(B-C)** Oxygen consumption rate of 80,000 DPP4$^+$ and DPP4$^-$ cells. Data are presented in pmol/min **(D)** Glycolysis of 80,000 DPP4$^+$ and DPP4$^-$ cells evaluated by extracellular acidification rate (ECAR). Data are presented in mpH/min. **(E-F)** Glycolysis stress analysis of 80,000 DPP4$^+$ and DPP4$^-$ cells. Data are presented in mpH/min. **(G-J)** DPP4$^+$ and DPP4$^-$ cells pretreated with 7µM BSA or 0.2mM palmitate combined with 7µM BSA for 4 hours: **(G-H)** Oxygen consumption rate of 80,000 DPP4$^+$ and DPP4$^-$ cells. Data are presented in pmol/min. **(I-J)** Glycolysis of 80,000 DPP4$^+$ and DPP4$^-$ evaluated by ECAR. Data are presented in mpH/min. **(K-N)** DPP4$^+$ and DPP4$^-$ cells sorted by FACS from the sWAT of WT mice fed either control diet (CD) or high-fat diet (HFD) for 3 days: **(K-L)** Oxygen consumption rate of freshly isolated DPP4$^+$ and DPP4- cells following 3 days of chow or HFD feeding. Data are presented in pmol/min per protein content **(H)** Glycolysis of freshly isolated DPP4$^+$ and DPP4$^-$ cells following 3 days of chow or HFD feeding evaluated by ECAR. Data are presented in mpH/min. Significance in (B, E,G,I,K,M) was calculated using 2-way Anova with Tukey's post-test for multiple comparisons. Significance in (A,C,D,F,H,J, L,N) was calculated using a two-tailed student's t-test. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).


A
Osteogenesis
mRNA relative expression
Control DPP4+
Control DPP4+ DOX
MitoNEET TG DPP4+
MitoNEET TG DPP4+ DOX
Cbfa1
Bglap2
Alpl
iBSP
Osx
B
- Dox
+ Dox
Control DPP4+
MitoNEET TG DPP4+
C
Osteogenesis
mRNA relative expression
Control DPP4-
Control DPP4- DOX
MitoNEET TG DPP4-
MitoNEET TG DPP4- DOX
Cbfa1
Bglap2
Alpl
iBSP
Osx
D
- Dox
+ Dox
Control DPP4-
MitoNEET TG DPP4-

Supplemental Figure 6: Mitochondrial activity greatly influences mesenchymal lineage determination in DPP4+ and DPP4-, Related to Figure 3

DPP4+ or DPP4- cells were isolated by FACS from sWAT of control or MitoNEET^{TG} mice**.** DPP4+ or DPP4- cells were plated at confluency and treated with or without 4.4 µM doxycycline for 24 hours. Cells were then differentiated into osteoblast for 14 days as described in Methods. **(A)** Gene expression of osteogenic markers in DPP4^{+} cells from control or MitoNEET^{TG} mice. Data are presented in mRNA relative expression to untreated control cells. **(B)** Alizarin Red S staining for evaluation of calcium deposits in DPP4+ cells. **(C)** Gene expression of osteogenic markers in DPP4^{-} cells from control or MitoNEET^{TG} mice. Data are presented in mRNA relative expression to untreated control cells. **(D)** Alizarin Red S staining for evaluation of calcium deposit in DPP4- cells. Significance in (A;C) was calculated using a Kruskal-Wallis (one-way Anova) test with Dunn's post-test for multiple comparisons. Error bars represent mean ± S.E.M. * ($P<0.05$), ** ($p<0.01$), *** ($p<0.0001$), **** ($p<0.00001$).