Supplementary Information

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**Brown preadipocyte differentiation (WT-1 cell)**

- **Day 0**
- **Day 6**

**Microscopy**

- Oil Red O

**Relative expression**

- **Control**
- **α4-OE**

**Differentiated**

- **Control**
- **α4-OE**

**Relative expression**

- **Control**
- **α4-OE**

**UCP1 (fold change)**

- **Control**
- **α4-OE**

**Relative expression**

- **Control**
- **α4-OE**

**Average mitochondrial area (μm²)**

- **Control**
- **α4-OE**

**Relative expression**

- **Control**
- **α4-OE**

**EdU/DAPI (%)**

- **Control**
- **α4KD**

**h-UCP1**

- **Control**
- **α4-OE**

**Relative expression**

- **Control**
- **α4-OE**

**EdU/DAPI (%)**

- **Control**
- **α4KD**
**m**

- **Graph m**: Illustrates the cells per well (10^4) over time (hours) for Control and α4KD.
- **y-axis**: Cells per well (10^4).
- **x-axis**: Time (hours) from 0 to 120.
- **Legend**: - Control, α4KD.

**n**

- **Images n**: Show the Control and α4KD conditions with corresponding scales.

**p**

- **Graph p**: Depicts relative expression over insulin (Ins) conditions for Control and α4KD.
- **y-axis**: Relative expression (1.0 - 2.0).
- **x-axis**: Ins (−, +).

**o**

- **Comment o**: Highlights the importance of proper insulin (Ins) in the context of α4KD conditions.
- **Figure**: Depicts various proteins and their expressions over time.
  - **Proteins**: p-IRβ, p-Akt, p-S6, ERK1/2, GAPDH.
  - **Expression Levels**: Various bands indicating different expression levels.
  - **Time**: 5 min, 10 min, 30 min, 60 min.
Supplementary Figure 1. Knockdown of α4 in brown preadipocytes decreased insulin signaling.

(a) α4 expression (qPCR) from three different adipose tissues of 5-month-old C57BL/6J mice. Data are presented as mean ± SEM (One-way ANOVA post hoc Bonferroni test, ** p = 0.003, *** p < 0.0001, n = 8). (b) The bright-field microscopy image of the differentiated brown preadipocytes corresponds to Days 0 and 6; Scale bar = 100 μm. The results of Oil red O staining corresponding to Day 6 are also shown. (c) Densitometric analysis of α4 in lysates from brown preadipocyte cells during the differentiation process before or 2 and 6 days after induction of the differentiation. Box plots are defined in terms of minima and maxima by whiskers, and the center and bounds of box by quartiles (One-way ANOVA post hoc Bonferroni test, n = 4, *** p < 0.0001) (d) Bright field microscopy picture (left) and Oil red O staining (right) of the mouse differentiated brown adipocyte from Control and overexpressed 3XFlag-α4 (α4 OE) cells. The experiments were repeated independently three times. Scale bars, 100 μm. (e) Immunoblotting and densitometric analysis of UCP1 in lysates from differentiated adipocytes of α4-OE cells. Data are mean ± SEM (two-tailed Student t-test, * p = 0.02, n = 5 per group). (f) Mitochondrial markers in differentiated brown adipocytes from Control and 3XFlag-α4 transfected (α4-OE) mice cells. Data are mean ± SEM (two-tailed Student t-test, n = 5 per group, * p < 0.05). (g) Immunoblotting for α4 and Flag in lysates from Control human brown preadipocytes or cells overexpressed 3XFlag-α4 (α4-OE). (n = 5 biologically independent cell clones/group) (h) Densitometric analysis of α4 and Flag in human brown preadipocytes. Data are mean ± SEM of n = 5 (two-tailed Student t-test, *** p < 0.0001). (i) Representative Oil red O staining (left) and UCP1 mRNA expression (right) in human differentiated brown adipocytes from Control (n = 8) and 3XFlag-α4-OE cells (n = 8). Data are mean ± SEM (two-tailed Student t-test, *** p < 0.0001). (j) Representative electron microscopic images of mitochondria in differentiated brown adipocytes from Control and α4-OE cells. Scale bar, 1.0 μm. (bottom) Quantification of the average mitochondrial size from Control and α4OE brown adipocytes. Data are mean ± SEM (Two-tailed Student’s t-test, ** p = 0.001; n = 5). (k) Immunoblotting and densitometric analysis of α4 in lysates from preadipocytes depleted of α4 (α4 KD) by shRNA. Data are mean ± SEM (two-tailed Student t-test, ** p = 0.0001; five clones used in the experiments). (l) Representative images of EdU (Red) incorporation in Control and α4KD cells. Scale bars, 100 μm (left). Quantification of BrdU+ DAPI+ cells (right). Data are mean ± SEM (two-tailed Student t-test, five clones with the quadruplicate were used). (m) Cells were plated at a density of 20,000 cells per well and their number determined at 24h intervals for 5 consecutive days (left). Doubling time was calculated during the exponential growth phase between day 3 and day 4 after plating (right). Data are mean ± SEM (five clones were used in the experiments). (n) Oil red O staining of the mouse differentiated brown adipocyte from Control and α4 KD cells. The experiments were repeated independently three times. Scale bars, 80 μm. (o) Western blot analysis of insulin signaling in lysates from α4 KD preadipocytes 5 min after 100 nM insulin stimulation for indicated antibodies. Three clones used in three independent experiments. (p) Relative changes of α4 based on the densitometric immunoblotting analysis (Supplementary Figure 1i) of cell lysates of adipocytes from α4KD-treated and Control mice for 5 min using 100 nM insulin. Data are presented as mean ± SEM (One-way ANOVA post hoc Bonferroni test, * p = 0.02, *** p = 0.0005, Three clones used in three independent experiments.).
Supplementary Figure 2. Identification of α4 binding protein which regulates IR tyrosine phosphorylation.

(a) Flag-tagged α4 associated with the protein complex immunoprecipitated using Flag beads from the lysates of brown adipocytes as in (Fig. 2a) and subjected to SDS-PAGE with silver staining.

(b) Proteomics screening of α4 binding proteins. For the mass spectroscopic proteomic analysis, we compared the Control (n = 1) and α4 overexpressing (n = 2, biological replicates) samples.

(c) Flag-tagged α4 immunoprecipitation showed the interaction between Mid2 with α4 as well as that between PP6 and α4 in brown preadipocytes.
| Abbreviation | Name                  | Group       |
|--------------|-----------------------|-------------|
| CE           | Cholesteryl ester     | Neutral Lipids |
| MAG          | Monoacylglycerol      |             |
| DAG          | Diacylglycerol        |             |
| TAG          | Triacylglycerol       |             |
| PC           | Phosphatidylcholine   | Phospholipids |
| PE           | Phosphatidylethanolamine |         |
| PI           | Phosphatidylinositol  |             |
| LPC          | Lysophosphatidylcholine |       |
| LPE          | Lysophosphatidylethanolamine |     |
| SM           | Sphingomyelin         | Sphingolipids |
| CER          | Ceramide              |             |
| HCER         | Hexosylceramide       |             |
| L Cer        | Lactosylceramide      |             |
| DCER         | Dihydroceramide       |             |

### j: Serum

- **Control**
- **Ai-α4KO**

### k: Serum

- **Control**
- **Ai-α4KO**

### m: Serum

- **Control**
- **Ai-α4KO**

### n: WAT

- **Control**
- **Ai-α4KO**

### o: WAT

- **Control**
- **Ai-α4KO**

### p: WAT

- **Control**
- **Ai-α4KO**
Supplementary Figure 3. Acute loss of α4 in adipocytes altered serum lipid profiles. (a) Western blot analysis and (b) densitometric analysis of α4 in inguinal WAT (upper) and interscapular BAT (bottom) of Control and Ai-α4KO mice at day 3. Data are mean ± SEM (two-tailed Student t-test, **p = 0.003, ***p = 0.0004, n = 3). (c) YBX1 mRNA expression measured by real-time qPCR in iWAT (n = 9 per genotype) and BAT (n = 12 per genotype) from Control and Ai-α4KO. Data are mean ± SEM (two-tailed Student t-test, iWAT: ***p = 0.0006, BAT: ***p < 0.0001). Western blot analysis of insulin signaling (d) and densitometric analysis of phosphorylated IR, phosphorylated Akt and PTP1B in inguinal WAT (e) of 3-months-old fasted Control and Ai-α4KO mice 10 min after i.v. insulin stimulation (5 IU per mouse) or control saline (n = 4). Data are mean ± SEM (One-way ANOVA post hoc Bonferroni test: *p < 0.05; **p < 0.01; ***p < 0.001). (f) TUNEL staining results corresponding to iWAT and BAT sections from Control and Ai-α4KO mice on Day 3. Scale bars =50 μm. (g) Diameter distribution of isolated eWAT adipocytes in Control and Ai-α4KO at day 6. Data are mean ± SEM (two-tailed Student t-test, *, p < 0.05; **, p < 0.01; *** p < 0.001, n = 8/group). (h) Lipolysis assessed by FFA release from eWAT of Control and Ai-α4KO mice on Day 6. Samples were incubated ex vivo in the presence or absence of 10 mM isoprenaline, and FFA release into the medium was quantified. (Two-tailed Student's t-test, ***p = 0.0006; n = 6/group). (i) Quantified lipid classes and their abbreviations. (j) Heatmap of top 50 lipid species from serum differentially regulated between Control and Ai-α4KO in mice (n = 5 per group) at 1 week. Lipid class concentrations of Serum (k) and iWAT (n) from Ai-α4KO expressed as 1.0 of Control. Fatty acid concentrations of the indicated chain lengths were quantified in Serum (l) and iWAT (o) from Ai-α4KO expressed as 1.0 of Control. Ceramide species of the indicated chain lengths were quantified in Serum (m) and iWAT (p) from Ai-α4KO expressed as 1.0 of Control. Data are mean ± SEM (two-tailed Student t-test, *p < 0.05; **p < 0.01; ***p < 0.001, n = 5 per group).
Supplementary Figure 4. Acute loss of α4 reduces a Mitochondrial Biogenesis gene signature in adipose tissues.

(a) Volcano plot showing the distribution of differentially regulated genes in iWAT (left) and BAT (right), with log ratio of fold change in Control versus fold change in Ai-α4KO on x axis and -log10 q value on y axis. (b) Genes involved in the TCA cycle pathway in iWAT and BAT were listed in the heatmap, and the color intensities indicate Z-score of each sample by gene. (c) Top ten directionally down-regulated GO pathways between Control and Ai-α4KO in iWAT (left) and BAT (right). (d) Rectal temperature of Control (n = 6) and Ai-α4KO (n = 5) mice on Day 10 during a 3-h exposure to an environment at 4 °C. Data are mean ± SEM (Two-tailed Student’s t-test: * p < 0.05; ** p < 0.01; *** p < 0.001).
BAT GO pathway UP
- Immune system process
- Inflammatory response
- Positive regulation of interleukin-6 production
- Neutrophil chemotaxis
- Chemokinesis
- Negative regulation of NF-kappa B transcription factor activity
- Positive regulation of tumor necrosis factor production

iWAT GO pathway UP
- Immune system process
- Cell cycle
- Cell division
- Defense response to virus
- Innate immune response
- Positive regulation of interleukin-1 beta production
- Chromosome segregation
- Positive regulation of interleukin-6 production

iWAT TNF signaling pathway/NF-kappa B signaling pathway

BAT NF-kappa B signaling pathway

iWAT Cytokine-cytokine receptor interaction

f

BAT

Ccl3
Ccl6
Ccl8
Ccl9
Cxcl2
Cxcl12
Cxcl13
Cxcl14

Relative expression

p = 0.001
p = 0.002
p = 0.001
p = 0.001

CD11c F4/80

Control Ai-α4KO

Control Ai-α4KO
Supplementary Figure 5. Acute loss of α4 induces inflammatory chemokines and chemokine receptors gene signatures in adipose tissues.

(a) Top directionally up-regulated GO pathways between Control and Ai-α4KO in BAT (upper) and iWAT (bottom). Heatmap of Genes involved in the cytokine-cytokine receptor interaction (b) in iWAT and TNF/NF-kappa B signaling pathways in iWAT (c) and BAT (d) were listed in the heatmap, and the color intensities indicate Z-score of each sample by gene. (e) Expression levels of inflammatory cytokines and inflammasome components measured by real-time qPCR in iWAT from Control and Ai-α4KO at day 10 (n = 11 per group). Expression levels of genes for chemokines were measured by real-time qPCR in BAT (f) and iWAT (g) from Control and Ai-α4KO (n = 12 per group). (h) Flow cytometry gating strategy of macrophage populations in the adipose tissues. Representative dot plots showing Single cells, Live cells and C+ cells were sorted for CD45+ and F4/80+ and were then sorted on the basis of expression of CD11c and CD206. (i) Percent of macrophages (F4/80+) infiltrated in iWAT and BAT in Control and Ai-α4KO mice by flowcytometric analysis (n = 8 per group). Staining of iWAT and BAT sections from Control and Ai-α4KO mice on Day 6 for (j) F4/80 and (k) cleaved-caspase3. (l) TUNEL staining results corresponding to iWAT and BAT sections from Control and Ai-α4KO mice on Day 6. Scale bars = 50 μm. The red arrow shows TUNEL positive cells. Experiments in j–l were repeated three times independently. All data are mean ± SEM (two-tailed Student t-test). Statistical significance is shown as p < 0.05 (the exact p value) or p < 0.001 (***)
Supplementary Figure 6. Adipose tissue biology in Control and Ai-α4KO mice.
(a) UCP1 expression in iWAT from Control and Ai-α4KO mice on Days 74. Scale bars = 100 μm.
(b) Strategy for identifying newly developed adipocytes in Ai-α4KO mice in combination with floxed mTmG mouse. (c) Rectal temperature in male Control and Ai-α4KO mice at 30-min intervals during the 3-h exposure of mice aged 12 wks to an environment at 4°C. Data are mean ± SEM (Two-tailed Student’s t-test: ** p = 0.002; n = 5)
Figure 1

**a**

Concentration

Body fat

| Control | A-α4KO |
|---------|--------|
| ![Image](image1.png) | ![Image](image2.png) |

**b**

iWAT (g) | eWAT (g) | BAT (g)

| Control | A-α4KO | Control | A-α4KO | Control | A-α4KO |
|---------|--------|---------|--------|---------|--------|
| ![Image](image3.png) | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |

**c**

iWAT | eWAT | BAT

| Control | A-α4KO | Control | A-α4KO | Control | A-α4KO |
|--------|--------|--------|--------|--------|--------|
| ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) | ![Image](image10.png) |

**d**

Liver

| Control | A-α4KO |
|---------|--------|
| ![Image](image11.png) | ![Image](image12.png) |

**e**

Rectal temperature | Interscapular temperature

| Control | A-α4KO | Control | A-α4KO |
|---------|--------|---------|--------|
| ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | ![Image](image16.png) |

**f**

Control | A-α4KO

| ![Image](image17.png) | ![Image](image18.png) |

**g**

Fed | Fasted

| Control | A-α4KO | Control | A-α4KO |
|--------|--------|--------|--------|
| ![Image](image19.png) | ![Image](image20.png) | ![Image](image21.png) | ![Image](image22.png) |

**h**

Blood glucose (mg/dl)

| Control | A-α4KO |
|---------|--------|
| ![Image](image23.png) | ![Image](image24.png) |

**i**

Decrease of initial serum glucose (%)

| Control | A-α4KO |
|---------|--------|
| ![Image](image25.png) | ![Image](image26.png) |
Supplementary Figure 7. Aα4KO mice showed cold intolerance, diabetes, pancreatic islet hyperplasia and fatty liver.

(a) Body fat percentage calculated by micro-CT imaging of Control (n = 4) and Aα4KO (n = 4). Box plots are defined in terms of minima and maxima by whiskers, and the center and bounds of box by quartiles (two-tailed Student t-test, *** p = 0.0009).

(b) Tissue weights of iWAT, eWAT and BAT in 3 months old Control (n = 8) and Aα4KO (n = 8). Data are mean ± SEM (two-tailed Student t-test, *** p < 0.0001).

(c) HE stained sections of iWAT, eWAT and BAT in Control and Aα4KO. Scale bars, 400 μm.

(d) Tissue weights of Liver in 3 months old Control (n = 8) and Aα4KO (n = 8). Data are mean ± SEM (two-tailed Student t-test, *** p < 0.0001) (left). HE stained sections of Liver in Control and Aα4KO. Scale bars, 100 μm (right).

(e) Rectal and interscapular temperature in Control and Aα4KO during a 3 h exposure to 4℃ environment. Data are mean ± SEM (two-tailed Student t-test, *, p < 0.05; **, p < 0.01; *** p < 0.001; **** p < 0.0001, n = 5 per group).

(f) Thermal images using a FLIR E8 Infrared Camera showing surface temperature over interscapular BAT after 2 h at 4℃ between Control and Aα4KO.

(g) Oxygen consumption (VO2) measured in metabolic cages with Control (n = 6) and Aα4KO (n = 6) mice under fed (upper) and fasted (bottom) condition. Dark phase is the 12h period of day during which the lights were off. (two-tailed Student t-test). All data are represented as mean ± SEM.

(h) GTT and GTT AUC for Control (n = 13) and Aα4KO (n = 14). Data are mean ± SEM (two-tailed Student t-test, ** p = 0.004).

(i) ITT and the decrease of AUC in Control (n = 12) and Aα4KO (n = 14). Data are mean ± SEM (two-tailed Student t-test, * p = 0.02).

(j) Tissue weights of iWAT, eWAT and BAT in Control and Aα4KO mice fed a chow diet or HFD. Box plots are defined in terms of minima and maxima by whiskers, and the center and bounds of box by quartiles (One-way ANOVA post hoc Bonferroni test, *** p < 0.001, Control CD (n = 10), Aα4KO CD (n = 13), Control HFD (n = 11) and Aα4KO HFD (n = 9)).

(k) Serum insulin levels of Control and Aα4KO mice fed a chow diet or HFD. Data are mean ± SEM (One-way ANOVA post hoc Bonferroni test, CD: * p = 0.02, HFD: * p = 0.01, Control CD (n = 7), Aα4KO CD (n = 8), Control HFD (n = 9) and Aα4KO HFD (n = 8))

(l) Pancreas tissue sections in Control and Aα4KO mice fed a chow diet or HFD. Scale bars, 400 mm.

(m) Tissue weights of Liver in Control and Aα4KO. Box plots are defined in terms of minima and maxima by whiskers, and the center and bounds of box by quartiles (One-way ANOVA post hoc Bonferroni test, ** p = 0.002, *** p < 0.0001, Control CD (n = 10), Aα4KO CD (n = 13), Control HFD (n = 11) and Aα4KO HFD (n = 9)).

(n) Liver sections from Control and Aα4KO mice fed a chow diet or HFD stained with H&E (upper panel; Scale bars, 400 mm.) and Oil red O (bottom; Scale bars, 200 mm.).

(o) mRNA levels of genes involved in gluconeogenic enzymes and inflammation after 12 weeks of the HFD (n = 10/group) or CD (n = 10/group). Box plots are defined in terms of minima and maxima by whiskers, and the center and bounds of box by quartiles (One-way ANOVA post hoc Bonferroni test, * p = 0.04, *** p < 0.0001).
**Supplementary Figure 8. Uncut blots.** The red sections indicate blot results shown in Supplementary Figure 1.
**Supplementary Figure 9. Uncut blots.** The red sections indicate blot results shown in Supplementary Figure 2 and Supplementary Figure 3.
| Gene       | Direction | Sequence                      | Gene       | Direction | Sequence                      |
|------------|-----------|-------------------------------|------------|-----------|-------------------------------|
| TBP        | Forward   | ACCCTTCACCAATGACTCCTATG       | h-UCP1     | Forward   | ACCCGCAAGGAAAGAAGACGC        |
| TBP        | Reverse   | TGACTCGACGAAATCGCTTG          | h-UCP1     | Reverse   | TCAGATTGGAGAGTCTGCGCTCTC    |
| FAS        | Forward   | GAGGACACTCAAGTGGCTGA          | NFKB1      | Forward   | ATGCCAGAGCTGATCCCTACG       |
| FAS        | Reverse   | GTGAGGTTGCTGGCTGCTGT          | NFKB1      | Reverse   | TTGTGACAGTGGATTTCTGCTG      |
| AP2        | Forward   | GATGCGCTTTGTGGGAAACT          | NFKB2      | Forward   | GCCCGGAAACAGATCTACTACTCT    |
| AP2        | Reverse   | CTGTCGTCGCTGGATATT            | NfkB2      | Reverse   | TCAGACACACAGCCACACTG        |
| ATGL       | Forward   | ACTGGGCTCTATCCCTCTCT          | Nrf3       | Forward   | ATTACCCGACCAGAAGG           |
| ATGL       | Reverse   | AACTTGATGCTGGTGGTTGT          | Nrf3       | Reverse   | TCAGCAGAAAGATCCACCAGA       |
| Adiponectin| Forward   | GATGGCTCCCGCTCCTTTT           | PTP1B      | Reverse   | GGAACCTGGGAGCCTTTACTCAG     |
| Adiponectin| Reverse   | GCCCTCCAGGGCTCCTTT            |           |           |                               |
| Leptin     | Forward   | GGCTTCAACCCCATCTCTGA          | Cxcl2      | Forward   | CCAACACACAGGCTACAG          |
| Leptin     | Reverse   | TGCCATCTCGGACATTGTGT          | Cxcl2      | Reverse   | GGCCTACACTCAAGCTCGT         |
| Glu4       | Forward   | ATCTCGTGACCTGTGGCTCT          | Cxcl10     | Forward   | CCAAGTTGGTGACCTTATTTTC      |
| Glu4       | Reverse   | CTCTAAAGGAGGCACAGAAGC         | Cxcl10     | Reverse   | GGCTCGACAGGATATTCCAA        |
| PPARγ      | Forward   | TGTTATGGGTGAAACTCTGGG         | Cxcl12     | Forward   | TGGCTACTGAGCTGAAACCA        |
| PPARγ      | Reverse   | AGACCTGATCGGAGATTGTG          | Cxcl12     | Reverse   | TTTCTCAGCCCTGCAACACTC       |
| Elovi3     | Forward   | GACTTAAAGGCGCCCTTTT           | Cxcl13     | Forward   | GGCAGCAGATCTTGCAGAAGA       |
| Elovi3     | Reverse   | ATCCGCCTGCTGCTCTGTA           | Cxcl13     | Reverse   | GCGGCAGATCTTGCAGACTG        |
| Cidea      | Forward   | ATCAACACTGCTGGCTTAGC          | Cxcl14     | Forward   | GAGATGGTATGCTCTAGACACC      |
| Cidea      | Reverse   | ACTACCCGCTGTCCTCCTCT          | Cxcl14     | Reverse   | CGTTCACAGGATCTCAGACCTC      |
| Glu2       | Forward   | AGCTCCTCTGGTGAAGG             | Col3       | Forward   | TTCTCTGCACTGACAGCTTTG       |
| Glu2       | Reverse   | ATCAAGAGGGCTGCTAGTA           | Col3       | Reverse   | GTGGAATCTTCCGGCTTAG         |
| SREBP1c    | Forward   | ATCTCCTAGAGCGAGGCTTG          | Col5       | Forward   | GCTGCTTTGCTACCTCTCCTCC      |
| SREBP1c    | Reverse   | TATTTAGCAACTGAGATCCATCAA      | Col5       | Reverse   | TCAGTGAACACACAGACTG         |
| FBP1       | Forward   | CCATGATAATCGGAGCTTGA          | Col6       | Forward   | GCTGCGCTTACACAAGAATTG       |
| FBP1       | Reverse   | CCTTCAGGAGGTCAGTACAG          | Col6       | Reverse   | GCTGCGCTTACACAAGAATTG       |
| m-UCP1     | Forward   | ACTGCCACACTCTCAGCTATT         | Col8       | Forward   | TACTGCCAGTCTGCTTGCCTGCC     |
| m-UCP1     | Reverse   | CTGGTCCTCACTCAGGATTGG         | Col8       | Reverse   | AAGGGGAGCTTCCAGTACAGATGTA   |
| Adrb3      | Forward   | GTCGAATGAGGGTACGGAGCT         | Col9       | Forward   | CACCTCTCTCCTCTCATTACA       |
| Adrb3      | Reverse   | TAGAAGAGGAGAGCCACTGAG         | Col9       | Reverse   | AGTCTGAGAAGCCCATGGAA        |
| PRDM16     | Forward   | CAGCAGCTGAGGACCCATTG          | Tnfα       | Forward   | AGCGGATGATCCAGAAACG         |
| PRDM16     | Reverse   | GCGTGCACTCGGCTTGTG            | Tnfα       | Reverse   | AGATAAGAATCCGGCTAGG         |
| PGC1α      | Forward   | CCCTGCCAATCTGGGTAAAGCC        | IL-6       | Forward   | TAGCTGCTTACACCCAATTTCC      |
| PGC1α      | Reverse   | TGCTGCTGTCTTGTTTTTCT          | IL-6       | Reverse   | TTGCTCTAGCACTCTATCCCT       |
| CEBPα      | Forward   | CAAGAACAGAAAGGCTACTG          | IL-1b      | Forward   | GCAACTCTGCTGCTCAGACTCAA     |
| CEBPα      | Reverse   | GTGACCTGACTGTTGACAGCAC        | IL-1b      | Reverse   | ATCTTGGGTGAGTCTCTGCTG       |
| Tfarm      | Forward   | AGTCCACAGGCTGTAATGT          | F4/80      | Forward   | CTGGAATGACGAGCTGTAAGG       |
| Tfarm      | Reverse   | GGCGACATCTCGACCC              | F4/80      | Reverse   | AGAGGCTGATGACGATGTTG        |
| Myd88      | Forward   | TCTAGTTCCATCACCTCGTGT         | CD11c      | Forward   | CTTGATAGCTCCTCTGCTG        |
| Myd88      | Reverse   | AAACTCGAGTGGGGCTAGC           | CD11c      | Reverse   | GCACACTGTTGCGGACACTA       |
| TLR4       | Forward   | ATGGCTGAGGGTCTACACC          | CCL2       | Forward   | TTAAGGATCAGGACAGACCAA       |
| TLR4       | Reverse   | GAGGCAATTTTTGTCTCCACA         | CCL2       | Reverse   | GCATGATCGAGATCTACGCCG      |
| Caspase1   | Forward   | ACAAGGCGACGGAGCTATG          | TGFβ1      | Forward   | AAGGCTGAGGCTGAGCTGCTG       |
| Caspase1   | Reverse   | TCCCGATCGATCTCCTGGAATG        | TGFβ1      | Reverse   | GCCCTGGATACAAACTATGG        |
| IL-18      | Forward   | GACTTTGGCTGAACCTTCAAGG        | Col1a1     | Forward   | CTCGAGGATGTCAGAGAACAC       |
| IL-18      | Reverse   | CAGGCTGCTTTGGCTCACAGA         | Col1a1     | Reverse   | TTGATCCAGGAAAGCCTTTGG       |
## Supplementary Table 2. Quantitative Real-Time PCR primer sequences

| Gene   | Direction | Sequence            | Gene   | Direction | Sequence            |
|--------|-----------|---------------------|--------|-----------|---------------------|
| Ndufs1 | Forward   | AGGATATGTTCCGCAACACTGG | Ndufs1 | Reverse   | TCGATGACGCACACCTG   |
| Ndufs2 | Forward   | CAGCCAGATTTGAAATGGGCA | Ndufs2 | Reverse   | TGTTGGCACCAGGCTTCTCT |
| Ndufs4 | Forward   | CTGCCGTTCCGCTGTTAGAG | Ndufs4 | Reverse   | TGTTATTGCGAGCAGAACAA |
| Ndufs5 | Forward   | GACATAACGAAAAGCTGGGCA | Ndufs5 | Reverse   | TCGGCTCATCGTTTGTACCC |
| Ndufs7 | Forward   | GTTCAATGCTGATGTTAGGCTG | Ndufs7 | Reverse   | CAGGGCAAAGTTGATAGGC |
| Ndufs8 | Forward   | GTTCAATGGGTGACAGGCAAG | Ndufs8 | Reverse   | TCCATTGAAGATGTCCTGTCG |
| Cox5a  | Forward   | GCCGCTGTCTGTTCCATTC  | Cox5a  | Reverse   | GCATCAATGCTGTTGCTGAA |
| Cox5b  | Forward   | ACCCTAATCATGTCCTGGTC  | Cox5b  | Reverse   | CAGCCTAACAGATGACAG  |
| Cox6c  | Forward   | GCCTCTGCGGTTCCATTTG  | Cox6c  | Reverse   | TCTGCAATGCTGTTTCTT |
| Cox6a1 | Forward   | TCAACGTGTTTCCTCAGTCGC | Cox6a1 | Reverse   | AGGGTATGTTACGCTTCC |
| Cox7a1 | Forward   | GCTCTGGTCCGCTTTAGAC  | Cox7a1 | Reverse   | GTACTGGGGAGGTCATGTCG |
| Cox7a2 | Forward   | GCTGCCCCTTCTGCTGATT  | Cox7a2 | Reverse   | GGCATCCATTATCTGCTGGA |
| Cox7b  | Forward   | TTGCCCTTAGGCAAAACGC   | Cox7b  | Reverse   | TCATGGAACATTGGTCCTC |
| Cox8b  | Forward   | TGGGGGATCTGACCCATAGT  | Cox8b  | Reverse   | AGTGGGCTAAAGCCATCA |
| NdufAB1| Forward   | GGACCAGTTCTGATGCTTG   | NdufAB1| Reverse   | AAACCCAAATTCGTTCTCATG |