**APPENDIX A. SUPPORTING INFORMATION**

**Supplementary Figure 1**: **Effects of miR-125b-5p over expression and inhibition.**

hMADS adipocytes transfected with miR-125b-5p mimics (A) or anti-miR-125b-5p (B) or their respective control at day 14, and mRNA levels of marker genes were analysed at day 18. Histograms display mean ± SEM of 4 independent experiments.

**Supplementary Figure 2**: **Effects of miR-125b-5p over expression and inhibition on OXPHOS protein expression.**

Brite hMADS adipocytes transfected with miR-125b-5p mimic or control mimic (A) and white hMADS adipocytes transfected with miR-125b-5p LNA inhibitor or control LNA inhibitor (B) at day 14. Protein levels of representative subunits of each of the 5 oxidative phosphorylation complexes were quantified by immunoblot analysis (C) of isolated mitochondria at day 18. Plots and histograms display mean ± SEM, \*: p<0.05, data are from 3 independent experiments (4 replicates per experiment).

**Supplementary Figure 3**: **Effects of miR-125b-5p over expression and inhibition on mitochondrial fission.**

hMADS adipocytes were transfected with miR-125b-5p mimics, anti-miR-125b-5p or their respective control at day 14, and proteins were extracted at day 18. Left panel displays representative blot for total and phosphorylated DRP1, as well as β-tubulin as loading control. Ratio between phospho(Ser616)-DRP1 and total DRP1 was evaluated using band optic density (od) intensity. Histograms display mean ± SEM of 3 independent experiments.

**Supplementary Figure 4**: **Analysis of non-injected anterior scWAT.**

10 week-old C57BL/6 male mice received miR-125b-5p or control mimics (A), or anti-miR-125b-5p or anti-miR control (B) in the subcutaneous WAT. Levels of Ucp1 and representative brite and white adipocyte markers mRNA were determined by RT-qPCR in anterior scWAT (non-injected) from mice treated with CL316,243.

**Supplementary Figure 5**: **Effects of miR-125b-5p injection in posterior scWAT.**

Subcutaneous WAT of 10 week-old C57BL/6 male mice injected with miR-125b-5p or control mimics were used for protein analysis. (A) PlnA/B, Cs and Ucp1 protein levels as well as (B) protein levels of representative subunits of oxidative phosphorylation complex I, II, III and V were quantified by immunoblot analysis. Complex IV signal was too low to be quantified. Histograms display mean ± SEM of 4 fat pads, \*: p<0.05.

**Supplementary Table 1. Sequence of primers used for gene expression analysis, and for mitochondrial and genomic DNA quantification.**

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| --- | --- | --- |
| **Human oligonucleotide sequences** | | |
| **name** | **Reverse primer** | **Forward primer** |
| CIDEA | CGTTAAGGCAGCCGATGAA | GCGAGAGTCACCTTCGACTTG |
| UCP1 | CCAGGATCCAAGTCGCAAGA | GTGTGCCCAACTGTGCAATG |
| UCP1’ | GGAAAGAAACAGCACCTAGTTTAGGAAGCA | CGTCAAGCCTTCGGTTGTTGCTATTATTCTG |
| CPT1-M | GAGCAGCACCCCAATCAC | AACTCCATAGCCATCATCTGCT |
| PAT2 | CCTGCCACTGTATGCACATC | TAGTCCATGCATCACCGTGT |
| CITED1 | GAGGCCTGCACTTGATGTC | CACGGAGCTATCTCTTTGGT |
| PLN5 | CTACGAGCACTCTGTGGGGA | GGTCTATCAGCTCCAGCGTCT |
| TBX1 | ACGACAACGGCCACATTATTC | CCTCGGCATATTTCTCGCTATCT |
| CD36 | GGGAAAGTCACTGCGACATGAT | ACGTCGGATTCAAATACAGCATAGA |
| ELOVL3 | TTGGACCTTGACTTCTGCAA | GGGCTATGGGGAATGAGG |
| FABP3 | AGTTGGGGGTGGAGTTCGATGAGAC | GCAGTGCCGTGGGTGAGTGTC |
| ADPQ | GCAGTCTGTGGTTCTGATTCCATAC | GCCCTTGAGTCGTGGTTTCC |
| COX2 | TGCCCTTTTCCTAACACTCACAA | CGCCGTAGTCGGTGTACTCG |
| COX4 | CGTTATCATGTGGCAGAAGCA | CCACCCACTCTTTGTCAAAGC |
| COX7A1 | TGGACAGAGGAGGACTACGC | CACTCGGTTCTGAAAGCGGT |
| COX10 | CACACTCTCTCCTCACGCCTC | TTCTTTCAAGATACCAGACAGAGC |
| ND1 | CCCTAAAACCCGCCACATCT | GAGCGATGGTGAGAGCTAAGGT |
| ND4 | CCATTCTCCTCCTATCCCTCAAC | CACAATCTGATGTTTTGGTTAAACTATATTT |
| MFN2 | TCCCTGCTAGGAGTTGCTGTAC | CACCTCAGCCCATGTGTCTCTT |
| TFAM | AATATGGTGCTGAGGAGTGTTAAAAG | TAACTGGTTTCCTGTGCCTATCC |
| PGC1α | CTGTGTCACCACCCAAATCCTTAT | TGTGTCGAGAAAAGGACCTTGA |
| CytC | TGGCCCCTCCCATCTACAC | ATCCTTGGCTATCTGGGACATG |
| TBP | CAAACCGCTTGGGATTATATTCG | ACGCCAGCTTCGGAGAGTTC |
| 36B4 | TGCATCAGTACCCCATTCTATCAT | AGGCAGATGGATCAGCCAAGA |

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| **Mice oligonucleotide sequences** | | |
| **name** | **Reverse primer** | **Forward primer** |
| 36b4 | TCC AGG CTT TGG GCA TCA | CTTTATCAGCTGCACATCACTCAGA |
| Cpt1m | GGCTCCAGGGTTCAGAAAGT | TGCCTTTACATCGTCTCCAA |
| Fabp4 | CTTGTGGAAGTCACGCCTTT | AAGAGAAAACGAGATGGTGACAA |
| Ucp1 | CACCTTCCCGCTGGACACT | CCTGGCCTTCACCTTGGAT |
| Cidea | CTGTCTCAATGTCAAAGCCACG | GAACTGTCCCGTCATCTGTGC |
| Pln1 | AGCGTGGAGAGTAAGGATGTC | CTTCTGGAAGCACTCACAGG |
| Citrate synthase | AGCCCTCAACAGTGAAAGCAA | CTTGGCAATGAGGTCCAT |
| Pgc1α | GTAAATCACACGGCGCTCTT | GAAAGGGCCAAACAGAGAGA |
| Pdk4 | ATCGCCAGAATTAAACCTCACAC | TGGATTGGTTGGCCTGGA |
| Prdm16 | CGTGGAGAGGAGTGTCTTCAG | ACAGGCAGGCTAAGAACCAG |

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| --- | --- | --- |
| **Human oligonucleotide sequences for mitochondrial and genomic DNA quantification** | | |
| **name** | **Reverse primer** | **Forward primer** |
| hLPL (gDNA) | TTCTGGATTCCAATGCTTCGA | CGAGTCGTCTTTCTCCTGATGAT |
| hNADH (mDNA) | GAGCGATGGTGAGAGCTAAGGT | CCCTAAAACCCGCCACATCT |

**Supplementary Table 2. Mass Spectrometry protein analysis of mitochondrial enriched fraction from miR-ctr and miR-125b-5p transfected hMADS brite adipocytes.**

**Supplementary Table 3. List of mRNA potentially targeted by miR-125b-5p and decreased at mRNA level after miR-125b-5p transfection in hMADS brite adipocytes.** Global mRNA expression profiling was performed using microarrays and downregulated mRNAs were analyzed for the number of distinct miR-125 seed match types. Further, the miRNA-target prediction tools TargetScan, Diana microT, ElMMo, rna22, miRanda ([www.microrna.org](http://www.microrna.org)), PITA and PicTar were queried for predicted direct interactions of miR-125b-5p and the respective mRNA to get a cumulative number of algorithms which are predicting a distinct miRNA-mRNA interaction. The whole microarray and whole data set is available on GEO site under the number GSE80816.