TFE3 REGULATES WHOLE BODY ENERGY METABOLISM IN COOPERATION WITH TFEB

Nunzia Pastore\textsuperscript{1,2}, Anna Vainshtein\textsuperscript{1,2}, Tiemo J. Klisch\textsuperscript{1,2}, Andrea Armani\textsuperscript{3}, Tuong Huynh\textsuperscript{1,2}, Niculin J. Herz\textsuperscript{1,2}, Elena V. Polishchuk\textsuperscript{4}, Marco Sandri\textsuperscript{3,5} and Andrea Ballabio\textsuperscript{1,2,4,6}

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Appendix Material and Methods

AAV vectors production and injection
The human TFE3 coding sequence was cloned into the pAAV-CMV-EGFP plasmid (Tessitore et al, 2008) by replacing the enhanced green fluorescence protein (EGFP) sequence. The AAV vectors were produced and characterized by the Telethon Institute of Genetics and Medicine (TIGEM, Naples, Italy) AAV Vector Core. pAAV2.1-CMV-hTFE3 and pAAV2.1-CMV-EGFP were triple-transfected in subconfluent 293 cells along with pAd-Helper and pack2/1 packaging plasmids as described previously (Xiao et al, 1999). Recombinant vectors were purified by two rounds of CsCl gradient centrifugation, as described previously (Xiao et al, 1999). Vector titers, expressed as genome copies per milliliter (GC/ml), were assessed by both PCR quantification and dot-blot analysis. Mice were intramuscularly (i.m.) injected with a total dose of $10^{11}$ GC of AAV2.1 vector preparation. Muscles were collected 8 weeks after injection and frozen in liquid nitrogen for subsequent analysis.

Protein Carbonyls detection
Carbonylation of liver proteins was detected by using the OxyBlot Protein Oxidation Detection Kit from Millipore following the manufacturer’s instructions.
Appendix Figure S1. TFE3 depletion alters energy expenditure

A  Energy expenditure (EE) in WT (black line) (n=5) and Tfe3 KO mice (red line) (n=4). Grey areas indicate dark periods (6 PM to 6 AM). Data are presented as mean ± SEM.

B  Bar graph represents average EE values during day and night (n=5 per group). Data are presented as mean ± SEM. Student’s two-tailed t-test: Day *p=0.05; Night *p=0.017.
Appendix Figure S2

Appendix Figure S2. Energy expenditure

A  Energy expenditure (EE) in WT (black line) and Tfe3 KO mice (red line) after 1 month of HFD (*n*=5 per group). Grey areas indicate dark periods (6 PM to 6 AM). Data are presented as mean ± SEM.

B  Bar graph represents average EE values during day and night (*n*=5 per group). Data are presented as mean ± SEM.
Appendix Figure S3. TFE3 regulates thermogenesis genes in BAT

A Uncoupling protein (Ucp) and β-oxidation gene expression in brown adipose tissues (BAT) from WT and Tfe3 KO mice fed a HFD for 18 weeks (n=10 per group). Dashed line represents WT mice fed a chow diet. Data are presented as mean ± SEM. Student’s two-tailed t-test: Tfe3 ***P<0.0001; Ucp1 *P=0.0213; Ucp3 ***P=0.0006; Ppara *P=0.0114.

B UCP1 immunoblot and relative quantification from WT and Tfe3 KO mice fed a HFD for 18 weeks. Data are presented as mean ± SEM. Student’s two-tailed t-test: **P=0.0011.
Appendix Figure S4. TFE3 regulates mitochondrial function

A  Overall protein carbonylation of WT and Tfe3 KO livers revealed by Oxyblot. A representative immunoblot for carbonylated proteins is depicted on the left, and densitometric quantification of the carbonylated proteins is represented by the graph on the right. Data are presented as mean ± SEM. Student’s two-tailed t-test: Tfe3 **P=0.0462.

B  Quantification of the RoGFP fluorescence intensity 405/488 emission ratio. ROS production was monitored by transfection of the ROS sensor Matrix-roGFP plasmid in WT (n=7) and Tfe3 KO (n=9) MEFs. Data are presented as mean ± SEM. Student’s two-tailed t-test: *P=0.0110.
C-D  Mitochondrial membrane potential of WT \((n=4)\) and \(Tfe3\) KO \((n=7)\) HeLa (C), and WT \((n=5)\) and \(Tfe3\) KO \((n=8)\) MEFs (D) and representative images of TMRM signal. Where indicated, oligomycin (olm) or protonophore carbonylcyanide-p-trifluoromethoxyphenyl hydrazone (FCCP) were added. Data are presented as mean ± SEM.
Appendix Table S1. Metabolic parameters of 2-months-old WT and *Tfe3* KO mice 24h after fasting. ALT, AST, CPK and LDH values are reported in U/L along with the relative *p* value calculated by Student’s two-tailed *t*-test.

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<th>LDH</th>
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<td>Fed</td>
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<td></td>
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</tr>
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<td></td>
<td>WT</td>
<td>45±13.4</td>
<td>67.75±14.6</td>
<td>273±110.7</td>
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<tr>
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<td>KO</td>
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<td>60.5±9.6</td>
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<td>0.69</td>
<td>0.44</td>
<td>0.81</td>
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<tr>
<td></td>
<td>Fasted</td>
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<tr>
<td></td>
<td>WT</td>
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<td>KO</td>
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<td>0.067</td>
<td>0.024</td>
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# Appendix Table S2. Gene ontology analysis of the 136 mitochondria-related genes identified as TFE3 targets (Betschinger et al, 2013) ($p \ 2.38*E-23$).

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<th>Biological Processes in which the TFE3 Mitochondrial Targets are enriched:</th>
<th>N GENES</th>
<th>SYMBOLS</th>
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<tr>
<td>GO:0000266--mitochondrial fission</td>
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<td>FIS1, BAX, MUL1</td>
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<tr>
<td>GO:0000302--response to reactive oxygen species</td>
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<td>GPX4, BCL2, TXNRD2, PRDX5, SOD2, GLRX2</td>
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<td>GO:0001666--response to hypoxia</td>
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<td>LONP1, BCL2, ABAT, SOD2</td>
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<td>GO:0001836--release of cytochrome c from mitochondria</td>
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<td>BID, BAK1, BBC3, BCL2, BAX, SOD2</td>
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<tr>
<td>GO:0006006--glucose metabolic process</td>
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<td>PDK1, PDK4, PCK2, OGDH, CPT1A</td>
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<tr>
<td>GO:0006066--alcohol metabolic process</td>
<td>6</td>
<td>PDK1, CPT1B, PDK4, PCK2, OGDH, CPT1A</td>
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<td>GO:0006084--acetyl-CoA metabolic process</td>
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<td>NNT, ACO2, SUCLG1, CS, IDH2, SUCLA2</td>
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<td>GO:0006119--oxidative phosphorylation</td>
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<td>GO:0006120--mitochondrial electron transport, NADH to ubiquinone</td>
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<td>GO:0006139--nucleobase, nucleoside, nucleotide and nucleic acid metabolic process</td>
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<td>GO:0006886~intracellular protein transport</td>
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<td>GO:0006979~response to oxidative stress</td>
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<td>GO:0015985--energy coupled proton transport, down electrochemical gradient</td>
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<td>GO:0070585--protein localization in mitochondrion</td>
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Appendix Table S3. Metabolic parameters in WT and Tfe3 KO mice fed a HFD for 18 weeks. ALT, AST, ALP, CPK, LDH and glucose values are reported in U/L along with the relative p value calculated by Student’s two-tailed t-test.

<table>
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<tr>
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<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>CPK</th>
<th>LDH</th>
<th>Glucose</th>
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<tr>
<td>WT</td>
<td>318±89.8</td>
<td>259.5±76.6</td>
<td>126±19.5</td>
<td>375.75±76.4</td>
<td>611.25±119.8</td>
<td>284.33±25.5</td>
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<td>KO</td>
<td>563.3±124.4</td>
<td>686.33±187.6</td>
<td>299.5±64.8</td>
<td>215.5±62.2</td>
<td>3289±1334.7</td>
<td>171±21.2</td>
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<td>p value</td>
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<td>0.042</td>
<td>0.155</td>
<td>0.094</td>
<td>0.0417</td>
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<tr>
<td>WT HDAd-TFE3 early</td>
<td>99.75±26.7</td>
<td>127.75±18.9</td>
<td>70.75±18.9</td>
<td>556.5±174.6</td>
<td>373.75±76.8</td>
<td>262.5±23.1</td>
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<td>WT HDAd-TFE3 late</td>
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<td>139.7±27</td>
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<td>259.25±98.7</td>
<td>365.3±54.8</td>
<td>212.25±21.7</td>
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<td>p value</td>
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<td>0.260</td>
<td>0.789</td>
<td>0.387</td>
<td>0.165</td>
<td>0.313</td>
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### Appendix Table S4. Primers used for qPCR analysis

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<tr>
<th>Gene</th>
<th>Sequences (5'-&gt;3')</th>
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| Tfe3      | forward AGGATCAAGAGCTGGGCCAC  
           | reverse CCGGCTCTCCAGGTCTTTG  |
| Pepck     | forward GGCAGATGCATGGCCTGGATGA  
           | reverse TGCTTCACTGAGGTGCGGCA  |
| G6Pc      | forward TGGTAGCCCTGTCTTTTCTTG  
           | reverse TTCCAGCATTCACACTTTCCT  |
| Gsk3      | forward TGCCAGCAAGGTAACCACAG  
           | reverse CGGTTCTTTAAATCGGTCTCTG  |
| Opa1      | forward ATACTGGGATCTGCTGG  
           | reverse AAGTCAGGCAACATCCTCTT  |
| Drp1      | forward TCAGATCGTCTGTAGTGGA  
           | reverse TCTTCTGTGGAAACGTGGAC  |
| Fis1      | forward AAGTATGTCGAGGGCTGT  
           | reverse TGCCATCCAGTCATCCTTC  |
| Mfn1      | forward CACTGCTCTCTCTAAACCA  
           | reverse AGGGAGCAACATCCTGGA  |
| Mfn2      | forward A1GTATTACCAGGAGCTGGAC  
           | reverse AACTGCTTCTCCGTCTGAC  |
| Cd36      | forward GGCATAAGGCAGGACACTGCC  
           | reverse GCACATGCGCTGGACAGAC  |
| Cpt1a     | forward GGCATAAAACGCAGACCTTCTC  
           | reverse CAGTGCCATCTCTGAGTAC  |
| Cyp7a1    | forward CACCATCTGTGGTGCCAC  
           | reverse ATGCGATTCCCTCAGAGCTGA  |
| Fgl21     | forward ATCAGGGAGGATGGAAAGTG  
           | reverse AGCTCCATCTCGTGTCGGCA  |
| Pgc1a     | forward GAATCAAGCCACTAGACACCG  
           | reverse CATTCCTCTGAGCGCTTCTG  |
| ApoA4     | forward CAGGATGCGATGCTAAGTCAG  
           | reverse AGCTGTACGGCAAGGGCAAC  |
| Cyp17a1   | forward GACTGTCCAGTGACTGAACCT  
           | reverse AGACGCTTCCGGTGGCGCA  |
| Cyp4a10   | forward GCTACTCAAGAGCTTCCAGC  
           | reverse CCAGAAACCTTAGGAAAAGGCAC  |
| Cyp4a14   | forward CAGCTACCAAGGGGCTTCCAG  
           | reverse GGCAAAACGTCATCAGGAGGAC  |
| Fasn      | forward AGAAGCCATGTTGGGAAGATT  
           | reverse AGCAGGGAGGCAAGGACAA  |
| Srebp1c   | forward AGCTGTCCGGTGTACGTCTG  
           | reverse GAGACTTGGGACACTGGCTTG  |
| Ucp1      | forward CAAAACAGAAGGGATGCGCAGAA  
<pre><code>       | reverse TCTGGACTGACGTAGGAG  |
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<tr>
<th>Gene</th>
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<th>Reverse Sequence</th>
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<td>Ucp2</td>
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<td>TTGGCGGTATCCAGAGGGAA</td>
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<td>Ucp3</td>
<td>GTGCTGAGATGGTGACCTACG</td>
<td>GCGTTCATGTATCGGGGTCTTTA</td>
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<td>Ppara</td>
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<td>ATCTCCGCAAACACAGCTCTCCCT</td>
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<td>CATAGCAAGGCAAAGTTCCAC</td>
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<td>Gapdh</td>
<td>AACACTGAGCATCTCCCTCA</td>
<td>GTGGGTGCAGCGAACTTTAT</td>
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<td>S16</td>
<td>AGGAGCGATTTGCTGGTGTTGG</td>
<td>GCTACCAGGGCCTTTGAGATG</td>
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Appendix Table S5. Primary antibodies used for immunoblots and staining.

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<th>Antigen</th>
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<th>Dilution</th>
<th>Source</th>
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<td>Sigma Aldrich</td>
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<td>TFEB</td>
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<td>1:1000</td>
<td>Bethyl Laboratories</td>
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<td>1:3000</td>
<td>Cell Signaling</td>
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<td>1:3000</td>
<td>Invitrogen</td>
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<td>Santa Cruz</td>
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<tr>
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<td>DYSTROPHIN</td>
<td>Mouse</td>
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<td>Novocastra</td>
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<tr>
<td>UCP1</td>
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<td>1:5000</td>
<td>Abcam</td>
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