

1 **METHODS**

2 **Human tissue sampling**

3 The investigation conforms to the principles outlined in the Declaration of Helsinki.
4 The institutional ethics committee approved the study, and all patients provided written
5 informed consent for the use of cardiac tissue samples. Human ventricular muscle tissue
6 was obtained from freshly explanted hearts of six end-stage heart failure patients
7 undergoing cardiac transplantation as a result of ischemic or dilated cardiomyopathy
8 and from six donor hearts that could not be transplanted due to clinical reasons and
9 from aortic stenosis valve operation with Morrow resection. Detailed patient
10 characteristics are provided in the online supplement (Table S1). Healthy human hearts
11 (no pre-existing cardiac disease and noncardiac reason of death) that initially were planned for
12 transplantation but in the end were rejected by the surgeon served as controls. Reasons for
13 rejection were organ mismatch, elevated serum C-reactive protein levels, local hypokinesia.
14 Available information about heart donor individuals are given in the supplementary table S2.

15

16 **Transverse aortic constriction (TAC) in mice**

17 The investigation conforms to the Guide for the Care and Use of Laboratory Animals
18 (NIH publication No. 85–23, revised 1996) and was performed in accordance with the
19 ethical standards laid down in the Declaration of Helsinki 1964. Surgery was done using
20 a minimally invasive approach. Briefly, 8-week-old female FVBN mice were
21 anesthetized using intraperitoneal injections of a mixture of xylazine and ketamine. A
22 27 gauge needle was tied against the aorta using a 5-0 non-absorbable suture. Sham
23 animals underwent the same procedure except banding of the transverse aorta.

1 **Generation of *Fto* cKO mice**

2 In order to create *Fto*-flox mice, the B6Dnk;B6Brd;B6N-Tyr^{c-Brd}
3 *Fto*^{tm1a(EUCOMM)Wtsi/WtsiCnbc} strain (Wellcome Trust Sanger Institute) was crossed with
4 C57BL/6N-Tg(CAG-Flpo)1Afst/Mmucd strain [Kranz A, et al., Genesis. 2010
5 Aug;48(8):512-20] to first remove the lacZ-neomycin cassette. The obtained mice were
6 back-crossed with C57BL/6N wildtype mice to get rid of the *Flpo* allele finally
7 resulting in mice where Exon 3 of *Fto* is flanked by loxP sites (*Fto*^{fl/fl}).

8 The conditional *Fto* knock-out was created by mating male *Fto*^{fl/fl} mice with the
9 cardiomyocyte specific *αMHC-Cre* females (*αMHC-Cre*, Jackson no. 011038,
10 C57BL/6N and C57BL/6J mixed background; [Agah et al. 1997]). For experiments,
11 resulting *αMHC-Cre*⁺;*FTO*^{fl/fl} mice (Cre+ fl/fl) bearing the homozygous *Fto* knock-out
12 were used.

13 As wildtype control *αMHC-Cre*⁺ mice (Cre+ +/+) as well as *αMHC-Cre*⁻;*Fto*^{fl/fl} mice
14 (Cre- fl/fl) were used to rule out Cre- recombinase and loxP system derived effects.

15

16 **Echocardiography**

17 The mice were anaesthetized using 1.5% isoflurane, and echocardiography was
18 performed using a VS-VEVO 660/230 (Visualsonics, Toronto, Canada). 2D guided M-
19 mode images were recorded in the long-axis view at the left mid-ventricular level. The
20 examiner was blinded towards group assignment.

21

22

1 **Murine LV-Isolation**

2 Subsequent to the Echocardiography, mice were anesthetized with Isoflurane and killed
3 via cervical dislocation. The heart was isolated and flushed with 0.9% NaCl through
4 the aorta, the LV was separated and snap-frozen in liquid nitrogen.

6 **Western blot analysis**

7 Up to 30 mg of snap-frozen tissue from murine or human LV was homogenized with
8 the MICCRA D-9 (MICCRA; #090000) in ice-cold RIPA buffer (Merck; #20-188)
9 supplemented with protease- and phosphatase inhibitors (Roche, Basel, CH,
10 #04693159001; #04906845001). Protein concentration was determined with the Pierce
11 BCA Protein Assay Kit (Thermo Fisher Scientific; #23225).

12 20µg of protein samples were first denatured and then separated using 4-20% TGX
13 Gels (Bio-Rad; #4568096, followed by transfer onto a Nitrocellulose Membrane (Bio-
14 Rad, Hercules, CA; #1620214). Membranes were incubated with anti-FTO (1:1000;
15 NBP2-29512, Novus Biologicals, Bio-Techne, Minneapolis, MN), anti-METTTL3
16 (1:1000; 15073-1-AP, Proteintech, Rosemont, IL), anti-METTTL14 (1:1000; SC-
17 247960, Santa Cruz Biology, Dallas, TX), anti-METTTL16 (1:1000; PA5-54185,
18 Thermo Fisher Scientific, Waltham, MA), anti-CALM1 (1:1000, LS-C331329-50,
19 LifeSpan BioSciences, Seattle, WA), anti-Calm1 (1:1000, ab45689, Abcam,
20 Cambridge, United Kingdom), anti-Smyd1 (1:10000, GTX119484, Genetex, Irvine,
21 CA) and anti-GAPDH (1:30000; MAB374; Merck, Burlington, MA) [all in 1%
22 Milk/TBST]. ECL anti-Rabbit igG (1:10000; Amersham/GE-Healthcare Life Sciences,
23 Little Chalfont, UK), ECL anti-Mouse igG (1:10000; Amersham/GE-Healthcare Life

1 Sciences, Little Chalfont, UK) and ECL anti-Goat igG (1:10000; Agilent, CA) were
2 used as secondary antibodies. Antibody was detected using the SuperSignal™ West
3 Femto Maximum Sensitivity Substrate (34095; Thermo Fisher Scientific, Waltham,
4 MA). Bands of protein of interest were normalized to GAPDH.

5

6 **Me-RIP-seq**

7 Total RNA from the left ventricles was isolated with Trizol Reagent (Thermo Fisher
8 Scientific, #15596018) following manufacture's instruction. After DNase treatment
9 rRNA was removed using NEBNext rRNA Depletion Kit (NEB, #E6310, Ipswich,
10 MA) and purified large RNA fraction (>200 bp) was fragmented to about 80 nt by RNA
11 Fragmentation Reagent at 70°C for 12 min (#AM8740, Thermo Fisher Scientific,
12 Waltham, MA). 8% of RNA samples was kept as inputs and the rest was subjected to
13 immunoprecipitation in 10mM Tris-HCl pH 7.4, 150mM NaCl and 0.1% (vol/vol)
14 Igepal CA-630 buffer supplemented with 200 U RNasin Plus RNase Inhibitor (#2611,
15 Promega) and 2mM RVC (#R3380, Sigma-Aldrich). 5 µg of anti-m6A polyclonal
16 antibody (Synaptic Systems, #202003) conjugated to Dynabeads Protein G (Thermo
17 Fisher Scientific, #10003D) was added to the RNA and was incubated at 4°C ON. The
18 beads were washed with 1x IP buffer three times, and m6A RNA was eluted twice with
19 6.7mM N6-methyladenosine (Sigma-Aldrich, M2780) in 1x IP buffer. The eluted RNA
20 fragments were purified with ZymoResearch RNA Clean and Concentrator-5 (Zymo
21 Research, #R1015), cDNA libraries were prepared from input and immunoprecipitated
22 samples using TruSeq Stranded Total RNA Library Prep Kit (#20020596, Illumina)
23 and sequenced on Illumina HiSeq 2000.

1 **Analysis of NGS data**

2 For RNA methylation analysis first adapters were trimmed from the original reads and
3 low quality reads were removed. The remaining reads were mapped to the mouse
4 genome (mm10) using STAR tools. Mapped reads were sorted and indexed with
5 SAMtools and generated bam files of input and immunoprecipitated samples were
6 generated. Peaks showing significant enrichment in the immunoprecipitated samples
7 vs. corresponding input samples for all submitted replicates were detected using
8 MeTPeak package [12]. Only those with false discovery rate (FDR) values equal to or
9 less than 0.05 were considered as real m6A peaks. All peaks were assigned based on
10 their position in mRNA, in particular 5' untranslated region (5'UTR), coding sequence
11 (CDS) and 3' untranslated region (3'UTR). Few peaks mapped to non-coding RNAs
12 were identified as well.

13 For differential methylation analysis we used in-house developed pipeline. In brief,
14 peaks overlapping or unique for treated and control samples were detected using
15 BEDTools and fold changes with joined p values were calculated for the hypo- and
16 hypermethylated peaks. mRNAs showing significant (p-value < 0.05) and at least 2 fold
17 change in the methylation levels for all peaks detected for the given transcript are
18 considered in this study.

19 For the comparison of transcriptome of control and affected heart tissue, differential
20 expression analysis was conducted of input samples using DESeq2. Genes with
21 transcripts levels showing 2 fold and/more changes in their transcripts levels with padj
22 values equal to or less than 0.05 were considered as differentially expressed.

1

2 **qRT-PCR verification of differentially methylated transcripts**

3 RNA was reverse transcribed into cDNA by using Transcriptor First Strand cDNA
4 Synthesis Kit (Roche, Mannheim, Germany) followed by quantitative Real-Time PCR
5 analysis on LightCycler 480 System (Roche, Mannheim, Germany). LightCycler 480
6 parobes master mix was used in reaction with FAM-labeled probes from the Universal
7 Probe Library: #48 for *CALM1* (forward primer 5'- taccacgaaccctcagc -3' and reverse
8 primer 5'- gaccaaatttacattcggtgttca-3'), #89 for *Calm1* (forward primer 5'-
9 gctgcaggatatgatcaacg-3' and reverse primer 5'- agaactctgggaagtcaatggt-3'), #40 for
10 *Gata6* (forward primer 5'- ggtctctacagcaagatgaatgg-3' and reverse primer 5'-
11 tggcacaggacagtccaa-3'), #96 *Rnd3* (forward primer 5'- cggacagatgtcagcacatt-3' and
12 reverse primer 5'- tctgcttgccatatttgc-3'), #17 for *Smyd1* (forward primer 5'-
13 catgtttcacacgcagatga-3' and reverse primer 5'- aggtgcaggaagtctatgtagga-3'). Ratios
14 between immunoprecipitated and input RNAs for normal mouse and human heart
15 tissues were set to one.

16

17 **Analysis of polysome-associated RNAs**

18 Polysomes were prepared from the heart tissue of five TAC and five control animals as
19 described [13]. Briefly, tissue samples were lysed in a buffer containing 50 mM Tris-
20 HCl pH 7.4, 10 mM MgCl₂, 100 mM NaCl, 1% Triton-X-100, 1 mM DTT, RNase
21 inhibitors and 100 µg/ml cycloheximide using a MICCRA D-1 homogenizer. Cell
22 debris were removed by centrifugation at 20,000 x g for 10 min at 4 °C. The soluble
23 whole cell extracts were separated on 10-50% sucrose density gradients (prepared in

1 50 mM Tris-HCl pH 7.4, 10 mM MgCl₂, 100 mM NaCl, 1 mM DTT and 100 µg/ml
2 cycloheximide) in an SW-40Ti rotor by centrifugation for 3 h at 35,000 rpm. Fractions
3 containing polysomes (determined by monitoring the absorbance of each fraction at
4 260 nm) were pooled and RNAs were extracted using
5 phenol:chloroform:isoamylalcohol (25:24:1), ethanol precipitated and resuspended in
6 water. The cDNA libraries were constructed using TruSeq Stranded Total RNA Library
7 Prep Kit (#20020596, Illumina) and sequenced on Illumina HiSeq 2000. DESeq2
8 package was used to detect enriched and depleted transcripts in polysomal bound RNA
9 vs. input samples and those with at least 2 fold change and *p*_{adj} values equal to or less
10 than 0.05 were reported as transcripts translated differentially.

11

12 **Pathway analysis**

13 We used ClueGO plug-in (version 2.3.2) in the open source software platform
14 Cytoscape for creation and visualization of functionally grouped networks of GO terms
15 and pathways. Parameters were following: Network Specificity – medium, Bonferroni
16 corrected p-value < 0.01. GO categories form “Biological Process” were extracted and
17 plotted with their p-values.

18

19 **Data availability**

20 The raw sequencing and processed data reported in this study have been deposited in
21 NCBI’s Gene Expression Omnibus and are accessible through GEO series accession

- 1 number GSE131296
- 2 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131296>).