Supplementary Material

Metabolic remodeling in cardiac hypertrophy and heart failure with reduced ejection fraction occurs independent of transcription factor EB in mice

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure 1. TFE3-induced *Ppargc1a* expression is regulated by class IIa HDACs and PKD. (**A**) Luciferase assays performed with cell extracts of COS-7 cells transfected with Hs_*Ppargc1a*-Luc and increasing amounts of FLAG-TFEB (TFEB; 50, 100, 200, and 400 ng) as indicated or control (-) plasmid. Luciferase activity was normalized to expression of CMV-LacZ and expressed as fold increase. Data are represented as mean ± SD. Two-tailed *t*-test; ###$p<0.001$ vs. control transfection with pcDNA3.1; *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. transfection with TFEB only. (**B-D**) Luciferase assays performed with cell extracts of COS-7 cells transfected with Hs_*Ppargc1a*-Luc, FLAG-TFEB (TFEB) and increasing amounts (50, 100, 200, and 400 ng) of MYC-HDAC4 (HDAC4), MYC-HDAC5 (HDAC5), or MYC-HDAC7 (HDAC7) as indicated or control (-) plasmid. Luciferase activity was normalized to expression of CMV-LacZ and expressed as fold increase. Data are represented as mean±SD. One-way ANOVA $q<0.0001$ for (B); ###$q<0.001$ vs. control transfection with pcDNA3.1; *$q<0.05$; **$q<0.01$; ***$q<0.001$ vs. transfection with TFEB only. (**E-G**), COS-7 cells were transfected with expression plasmids encoding FLAG-TFEB, (E) HDAC4-MYC, (F) HDAC5-MYC, or (G) HDAC7-MYC, or constitutively active (ca) PKD1 (left panel), caPKD2 (middle panel), and caPKD3 (right panel) proteins, as indicated, together with the Hs_*Ppargc1a*-Luc reporter construct. Values were normalized to expression of CMV-LacZ and calculated as the fold increase in luciferase/CMV-LacZ ratio compared with the reporter alone. Data are represented as mean ± SD. One-way ANOVA $q<0.0001$ for (E-G); *$q<0.05$; **$q<0.01$; ***$q<0.001$. n=5.

Supplementary Figure 2. Deletion of TFEB leads to an increased expression of cardiac stress and remodeling markers and a decreased cardiac function. (**A**) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of *Tfeb*, *Tfe3* and *Mitf* expression from WT and cKO mice. mRNA expression was normalized to *Gapdh*. (**B**) Western blot analysis with anti-TFEB and anti-GAPDH antibodies (left; n.s.=nonspecific signal). GAPDH was used as loading control. Bar graph showing the ratio of the relative densities of TFEB and GAPDH protein contents. (**C**) Heart, lung and liver weights of WT and cKO mice normalized to tibia length. (**D**) Echocardiographic data for left ventricular ejection fraction (LVEF) and fractional shortening (FS) from WT and cKO mice. (**E**) Representative images of Hematoxylin and Eosin (H&E) stained heart cross-sections of WT and cKO mice. (**F**) Wheat Germ Agglutinin (WGA) stained histological cross-sections of hearts from WT and cKO mice (Scale bar, 100 µm) and (**G**) myocyte cross-sectional area (MCSA) measured on WGA stained sections with Image J. (**H**) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of indicated genes from WT and cKO mice. mRNA expression was normalized to *Gapdh*. (**I**) Western blot analysis with anti-β-MyHC and anti-GAPDH antibodies. GAPDH was used as loading control. Bar graph showing

the ratio of the relative densities of β-MyHC and GAPDH protein contents. (**J**) Representative images of Picrosirius Red stained (PSR, left) heart cross-sections of WT and cKO mice; scale bar, 1 mm. Fibrotic area (right) was measured with Image J. (**K**) qRT-PCR analysis of indicated genes from WT and cKO mice. mRNA expression was normalized to *Gapdh*. Data are presented as Mean±SD (WT: n=10, cKO: n=8; *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001).

Supplementary Figure 3. Deletion of TFEB leads to an impaired regulation of genes involved in energy homeostasis. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of indicated metabolic genes (**A-B**) and genes involved in OXPHOS (**C**) and mitochondrial fission and fusion (**D**) from WT and cKO mice. mRNA expression was normalized to *Gapdh*. (**E**) Representative images of PAS stained heart cross-sections of WT and cKO mice; left: overview, scale bare 1mm; middle: higher magnification, scale bar 100 µm (corresponding to inset from left), right: quantification of glycogen content with Image J. (**A-E**) Data are presented as mean ± SD (WT: n=10, cKO: n=8; *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001). (**F**) Volcano plots of differentially expressed genes in WT and cKO hearts. Genes that are significantly upregulated (red, q-value < 0.05), downregulated (blue, q-value < 0.05) or unchanged (gray, not significant (n.s.)) are indicated. The grid line on the y axis indicates the significant threshold of $-\log_{10}(q\text{-value}) = 1.3$.

Supplementary Figure 4. PO-induced cardiomyocyte hypertrophy is comparable between WT and cKO mice in the LVH model. (**A**) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of MiT/TFE genes in hearts of WT and cKO mice after 21 days of Sham or TAC surgery as indicated. mRNA expression was normalized to *Gapdh*. (**B**) Kinetics of left ventricular mass as determined by echocardiography at indicated time points after Sham and TAC surgery, respectively, of WT and cKO mice. (**C**) Representative images of Hematoxylin and Eosin (H&E) stained cardiac cross-sections of WT and cKO mice; scale bar, 100 µm. (**D-G**) Frequency-distribution histograms of myocyte cross-sectional areas (MCSA) measured with Image J on Wheat Germ Agglutinin (WGA) stained sections comparing WT_Sham and WT_TAC (D), cKO_Sham and cKO_TAC (E), WT_Sham and cKO_Sham (F), and WT_TAC and cKO_TAC (G). Data are presented as mean ± SD (21 days, WT Sham: n=10, WT TAC: n=13, cKO Sham: n=10, cKO TAC: n=15; *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001).

Supplementary Figure 5. PO-induced cardiomyocyte hypertrophy is attenuated in cKO mice in the HFrEF model. (**A**) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of MiT/TFE genes in hearts of WT and cKO mice after 56 days of Sham or TAC surgery as indicated. mRNA expression was normalized to *Gapdh*. (**B**) Kinetics of left ventricular mass as determined by echocardiography at indicated time points after Sham and TAC surgery, respectively, of WT and cKO mice. (**C**) Representative images of Hematoxylin and Eosin (H&E) stained cardiac cross-sections of WT and cKO mice; scale bar, 100 µm. (**D-G**) Frequency-distribution histograms of myocyte cross-sectional areas (MCSA) measured with Image J on Wheat Germ Agglutinin (WGA) stained sections comparing WT_Sham and WT_TAC (D), cKO_Sham and cKO_TAC (E), WT_Sham and cKO_Sham (F), and WT_TAC and cKO_TAC (G). Data are presented as mean ± SD (56 days, WT Sham: n=10, WT TAC: n=6, cKO Sham: n=8, cKO TAC, n=6; *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001).

Supplementary Figure 6. Proteomics data of WT and cKO mice after 56 days of Sham or TAC surgery. (**A**) Correlation between the cKO_TAC/cKO_Sham, (**B**) cKO_TAC/WT_TAC and (**C**) cKO_Sham/WT_Sham ratios and the WT_TAC/WT_Sham ratios. Ratio data is presented as single points. Correlation was calculated with Pearson r and p value. Simple linear regression is shown with 95% CI. Single protein analysis of (**D**) cardiac stress and remodeling markers and (**E**) proteins of the

energy metabolism, mitochondrial fission and fusion, and ETC. (56 days, WT Sham: n=10, WT TAC: n=6, cKO Sham: n=8, cKO TAC: n=6; *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001).

Supplementary Figure 7. Analysis of proteomics data from hearts of WT and cKO mice after 56 days of Sham or TAC surgery. (**A**) Heatmaps of canonical metabolic pathways and (**B**) canonical signaling pathways with significant enrichment of regulated proteins are shown. Data are presented as heat maps (q-value ≤ 0.05, FC=1.3). Hatched marked boxes indicate that changes are not significant. (**C**) KEGG pathway of FAO related proteins (highlighted in red arrows) as shown in A.

1.2 Supplementary Tables

Supplementary Table 1: PCR primers for genotyping.

Name	Primer sequence
Tcfeb_forward	GTAGAACTGAGTCAAGGCATACTGG
Tcfeb_reverse	GGGTCCTACCTACCACAGAGCC
αMHC-Cre_forward	CGGCACTCTTAGCAAACCTC
αMHC-Cre_reverse	AGGCAAATTTTGGTGTACGG

Supplementary Table 2: qRT-PCR primers.

Name	Primer sequence
Acta1_forward	AGCCGGTGCTCTCCTACTG
Acta1_reverse	CGTCGCACATGGTGTCTAGT
Atp5a1_forward	CGGGCTGAGGAATGTTCA
Atp5a1_reverse	CCAAGTTCAGGGACATACCC
Col1a1_forward	TGTAAACACCCCAGCGAAGAA
Col1a1_reverse	CTGAGTTGCCATTTCCTTGGA
Col3a1_forward	CTCACCCTTCTTCATCCCACTCTTA
Col3a1_reverse	ACATGGTTCTGGCTTCCAGACAT
Cox4_forward	TCACTGCGCTCGTTCTGAT
Cox4_reverse	CGATCGAAAGTATGAGGGATG
Cpt1b_forward	GAGTGACTGGTGGGAAGAATATG
Cpt1b_reverse	GCTGCTTGCACATTTGTGTT
Drp1_forward	TCCCAATTCCATTATCCTCGC
Drp1_reverse	CATCAGTACCCGCATCCATG
Esrra_forward	GCCTCCAATGAGTGTGAGATC
Esrra_reverse	TTTGTACTTCTGCCGTCCG
Esrrb_forward	TGTGTTCCTCATCAACTGGG
Esrrb_reverse	CAGCTTGTCATCGTATGGGAG
Esrrg_forward	GGATGGGCAAAACATATTCCAG
Esrrg_reverse	GTTCATCCTCAAACGAAAGCG
Fis1_forward	AAGGGAGCAAAGAGGAACAG
Fis1_reverse	GCCCTCGCACATACTTTAGAG
Gys1_forward	CTGTCCTGTTCGGCTTCCT
Gys1_reverse	CCACATACGGCTTCTCTTCG
Hk2_forward	GCCAGCCTCTCCTGATTTTAGTGT
Hk2_reverse	GGGAACACAAAAGACCTCTTCTGG
Mfn1_forward	CATTGCGTTTCGGTTTTCCC
Mfn1_reverse	GAAGGAGCAGTAGGAGTTGAAG
Mfn2_forward	CGAGGCTCTGGATTCACTTC
Mfn2_reverse	CAACCAGCCAGCTTTATTCC
Mitf_forward	AAAAGTCAACCTCTGAAGAGCAG
Mitf_reverse	GCGTAGCAAGATGCGTGAT
MT-Co1_forward	TCCACTATTTGTCTGATCCGTACT
MT-Co1_reverse	AGTAGTATAGTAATGCCTGCGGCTA
MT-Cytb_forward	CATTTATTATCGCGGCCCTA
MT-Cytb_reverse	TGGGTTGTTTGATCCTGTTTC
MT-Nd1_forward	CTAGCAGAAACAAACCGGGC
MT-Nd1_reverse	CCGGCTGCGTATTCTACGTT
MT-Nd4_forward	CCAAACTCCATGAAGCTTCATAGG

MT-Nd4_reverse	GATGATGTGAGGCCATGTGCG
Myh6_forward	GCCAAGACTGTCCGGAATGA
Myh6_reverse	TGGAAGATCACCCGGGACTT
Myh7_forward	CGCATCAAGGAGCTCACC
Myh7_reverse	CTGCAGCCGCAGTAGGTT
Ndufb8_forward	TCCCTTCCTACCAGCCTGT
Ndufb8_reverse	GAGCAGGAAAACAGGAATGC
Nppa_forward	GGGGGTAGGATTGACAGGAT
Nppa_reverse	ACACACCACAAGGGCTTAGG
Nppb_forward	GCACAAGATAGACCGGATCG
Nppb_reverse	CTTCAAAGGTGGTCCCAGAG
Nrf1_forward	TGGAGTCCAAGATGCTAATGG
Nrf1_reverse	GCGAGGCTGGTTACCACA
Ppara_forward	CTGAGACCCTCGGGGAAC
Ppara_reverse	AAACGTCAGTTCACAGGGAAG
Ppargc1a_forward	TGAAAGGGCCAAACAGAGAG
Ppargc1a_reverse	GTAAATCACACGGCGCTCTT
Pppargc1b_forward	CCACAGCCCACTTCCAGA
Pppargc1b_reverse	CCAAGAGAGTCGCTTTGTGA
Sdha_forward	CCCTGAGCATTGCAGAATC
Sdha_reverse	TCTTCTCCAGCATTTGCCTTA
Sdhb_forward	ACTGGTGGAACGGAGACAAG
Sdhb_reverse	CCTCTGTGAAGTCGTCTCTGG
Slc2a1_forward	TTGTTGTAGAGCGAGCTGGA
Slc2a1_reverse	TTCAAAGAAGGCCACAAAGC
Slc2a4_forward	GTCGGGTTTCCAGCAGAT
Slc2a4_reverse	GGCATTGATAACCCCAATGT
Tfam_forward	CAAAGGATGATTCGGCTCAG
Tfam_reverse	AAGCTGAATATATGCCTGCTTTTC
Tfeb_forward	GAGCTGGGAATGCTGATCC
Tfeb_reverse	CTTGAGGATGGTGCCTTTGT
Tfe3_forward	GCTCAAAAGCCAACCCCTAT
Tfe3_reverse	GGTGTGGCCTGCAGTGATA
Uqcrc2_forward	TGGGCTCTTTGGAATTTACAC
Uqcrc2_reverse	TTGGTTGTAGGCAGCATTGA

Supplementary Table 3: LC-MS/MS parameters (A) and Spectronaut parameters for peptide/Protein identification and intensity extraction (B).

Supplementary Table 3A: LC-MS/MS parameters (data independent mode; quantitative data).

Data independent acquisition (DIA)	
Reversed phase liquid chromatography	**Ultimate 3000 RSLC (Thermo Scientific)**
Trap column	75 µm inner diameter, packed with 3 µm C18 particles (Acclaim PepMap100, Thermo Scientific)
Analytical column	75 µm inner diameter, packed with 2.6 µm C18 particles (Accucore, 25 cm, Thermo Scientific)
Flow rate	300 nl/min
Column oven temperature	40°C
Buffer system	binary buffer system consisting of 0.1% acetic acid in HPLC-grade water (buffer A) and 100% ACN in 0.1% acetic acid (buffer B)
Gradient	gradient of buffer B: 2min 2% to 5 %, 8min 7%, 60min 7% to 25%, 5min 25 to 40%, 2 min 40% to 90%, 6 min 90%, 2 min 90% to 2%, 10 min 2%
Mass spectrometer	Exploris 480
Operation mode	Data-independent
Electrospray	Nanospray Flex Ion Source
Full MS	
MS scan resolution	120,000
Normalized AGC target	300%
Maximum ion injection time for the MS scan	60 ms
Scan range	350 to 1,200 m/z
Spectra data type	profile
dd-MS2	
Resolution	30,000
Normalized MS/MS AGC target	3,000
Maximum ion injection time for the MS/MS scans	auto
Spectra data type	profile
Selection for MS/MS	1
Isolation window	65 windows m/z 13 (overlap m/z 2)
Fixed first mass	200
Dissociation mode	higher energy collisional dissociation (HCD)
Normalized collision energy	fixed, 30
Dissociation mode	HCD

Supplementary Table 3B: Spectronaut parameters for peptide/Protein identification and intensity extraction.

Spectronaut 14.10	
Computer Name: AGVOE-SPECTRONA	
User Domain Name: AGVOE-SPECTRONA	
User Name: spectronaut	
Analysis Mode: UI	
Analysis Type: directDIA	
Analysis Date: 20-April-2021	
Settings Used:	
Pulsar Search\Peptides	
Toggle N-terminal M:	True
Min Peptide Length:	7
Max Peptide Length:	52
Missed Cleavages:	2
Digest Type:	Specific
Enzymes / Cleavage Rules:	Trypsin/P
Pulsar Search\Labeling	
Channels:	
Channel 1:	False
Channel 2:	False
Channel 3:	False
DIA Analysis\Data Extraction	
MS1 Mass Tolerance Strategy:	Dynamic
Correction Factor:	1
MS2 Mass Tolerance Strategy:	Dynamic
Correction Factor:	1
Intensity Extraction MS1:	Maximum Intensity
Intensity Extraction MS2:	Maximum Intensity
DIA Analysis\XIC Extraction	
XIC IM Extraction Window:	Dynamic
Correction Factor:	1
XIC RT Extraction Window:	Dynamic
Correction Factor:	1
Pulsar Search\Modifications	
Max Variable Modifications:	5
Database	
Original File:	Uniprot_2_2021.fasta (Mus musculus)
Select Modifications:	
Fixed Modifications:	Carbamidomethyl (C)
Variable Modifications:	Acetyl (Protein N-term), Oxidation (M)

DIA Analysis\Calibration

MS1 Mass Tolerance Strategy:	System Default
MS2 Mass Tolerance Strategy:	System Default
Precision iRT:	True
iRT <-> RT Regression Type:	Local (Non-Linear) Regression
Exclude Deamidated Peptides:	True
MZ Extraction Strategy:	Maximum Intensity
Allow source specific iRT Calibration:	True

DIA Analysis\Identification

Generate Decoys:	True
Decoy Limit Strategy:	Dynamic
Library Size Fraction:	0.1
Decoy Method:	Mutated
Preferred Fragment Source:	NN Predicted Fragments
Machine Learning:	Per Run
Exclude Duplicate Assays:	True
Precursor PEP Cutoff:	1
Protein Qvalue Cutoff (Experiment):	0.01
Protein Qvalue Cutoff (Run):	0.05
Exclude Single Hit Proteins:	False
Pvalue Estimator:	Kernel Density Estimator
Precursor Qvalue Cutoff:	0.001
Single Hit Definition:	By Stripped Sequence

DIA Analysis\Quantification

Interference Correction:	True
MS1 Min:	2
MS2 Min:	3
Exclude All Multi-Channel Interferences:	True
Only Identified Peptides:	True
Protein LFQ Method:	Automatic
Major (Protein) Grouping:	by Protein Group Id
Minor (Peptide) Grouping:	by Stripped Sequence
Minor Group Top N:	False
Minor Group Quantity:	Sum precursor quantity
Major Group Top N:	True
Min:	2
Max:	3
Major Group Quantity:	Mean peptide quantity
Quantity MS-Level:	MS2
Quantity Type:	Area
Proteotypicity Filter:	None
Data Filtering:	Qvalue percentile
Fraction:	0.25
Imputing Strategy:	No Imputing

Cross Run Normalization:	False
DIA Analysis\PTM Workflow	
PTM Localization:	True
Probability Cutoff:	0.75
PTM Analysis:	True
Multiplicity:	True
Run Clustering:	False
PTM Consolidation:	Sum
Flanking Region:	7
DIA Analysis\Workflow	
MS2 DeMultiplexing:	Automatic
Run Limit for directDIA Library:	-1
Method Evaluation:	False
Profiling Strategy:	iRT Profiling
Profiling Row Selection:	Minimum Qvalue Row Selection
Qvalue Threshold:	0.001
Profiling Target Selection:	Profile only non-identfied Precursor
Identification Criterion:	Qvalue
Threshold:	0.001
Carry-over exact Peak Boundaries:	False
Unify Peptide Peaks Strategy:	Select corresponding Peak
DIA Analysis\Protein Inference	
Protein Inference Workflow:	Automatic
Inference Algorithm:	IDPicker
DIA Analysis\Post Analysis	
Calculate Sample Correlation Matrix:	True
Calculate Explained TIC:	Quick
Differential Abundance Grouping:	Major Group (Quantification Settings)
Smallest Quantitative Unit:	Precursor Ion (Quantification Settings)
Use All MS-Level Quantities:	False
Differential Abundance Testing:	Paired *t*-test
Group-Wise Testing Correction:	False
Run Clustering:	True
Distance Metric:	Manhattan Distance
Linkage Strategy:	Ward's Method
Z-score transformation:	False
Order Runs by Clustering:	True
DIA Analysis\Pipeline Mode	
Post Analysis Reports:	
Scoring Histograms:	True
Data Completeness Bar Chart:	True
Run Identifications Bar Chart:	True
CV Density Line Chart:	True

CVs Below X Bar Chart:	True
Generate SNE File:	True
Store Iontraces in SNE:	False
Report Schema:	C_FunGene_complex (Normal)
Reporting Unit:	Across Experiment

Pulsar Search\Identification

Peptide FDR:	0.01
Protein Group FDR:	0.01
PSM FDR:	0.01

Pulsar Search\Tolerances

Tolerance Parameters:

Thermo Orbitrap:

Calibration Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1
Main Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1

TOF:

Calibration Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1
Main Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1

Thermo IonTrap:

Calibration Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1
Main Search:	Dynamic
MS1 Correction Factor:	1
MS2 Correction Factor:	1

Pulsar Search\Workflow

Use DNN Predicted Ion Mobility:	Auto
Fragment Ion Selection Strategy:	Intensity Based
In-Silico Generate Missing Channels:	False

Pulsar Search\Result Filters

Precursors:

Best N Fragments per Peptide:	True
Min:	6
Max:	10
Channel Count:	False
Modifications:	None
Amino Acids:	False

Best N Peptides per Protein Group:	False
FASTA Matched:	False
Missed Cleavage:	False
Peptide Charge:	False
Proteotypicity:	False
Fragment Ions:	
m/z:	True
Min:	300
Max:	1,800
Ion Charge:	False
Ion Loss Type:	False
Ion Type:	False
Ion AA Length:	True
N:	3
Relative Intensity:	True
Min:	5

Supplementary Table 4: Subunits of the electron transport chain.

Complex	Gene	Gen name
Complex I	*MT-Nd1*	NADH-ubiquinone oxidoreductase chain 1
	MT-Nd4	NADH-ubiquinone oxidoreductase chain 4
	Ndufb8	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial
Complex II	*Sdha*	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	Sdhb	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial
Complex III	*MT-Cytb*	Cytochrome b
	Uqcrc2	Cytochrome b-c1 complex subunit 2, mitochondrial
Complex IV	*Cox4*	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial
	MT-Co1	Cytochrome c oxidase subunit 1
Complex V	*Atp5a1*	ATP synthase subunit alpha, mitochondrial

Supplementary Table 5. Summarized echocardiography data from WT and cKO mice after 21 days of TAC.

Attached as “Table S5 - Echo - 21 days of TAC – eng.xlsx”

Supplementary Table 6. Summarized echocardiography data from WT and cKO mice after 56 days of TAC.

Supplementary Table 6A. WT_TAC vs. WT_Sham & cKO_TAC vs. cKO_Sham.

Supplementary Table 6B. cKO_Sham vs. WT_Sham & cKO_TAC vs. WT_TAC.

Attached as “Table S6 A,B - Echo - 56 days of TAC - eng.xlsx”