

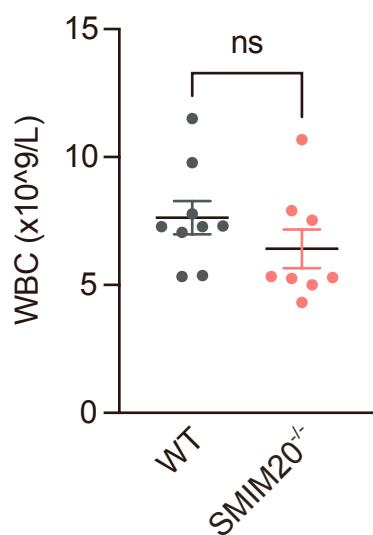
Supplemental information

**SMIM20 promotes complex IV biogenesis
and Ca²⁺ signaling in mice heart**

Angela Boshnakovska, Julius Ryan Pronto, Tanja Gall, Abhishek Aich, Jan Prochazka, Zuzana Nichtova, Radislav Sedlacek, Izzatullo Sobitov, Sofia Ainatzi, Christof Lenz, Dörthe M. Katschinski, Henning Urlaub, Niels Voigt, Peter Rehling, and Laura S. Kremer

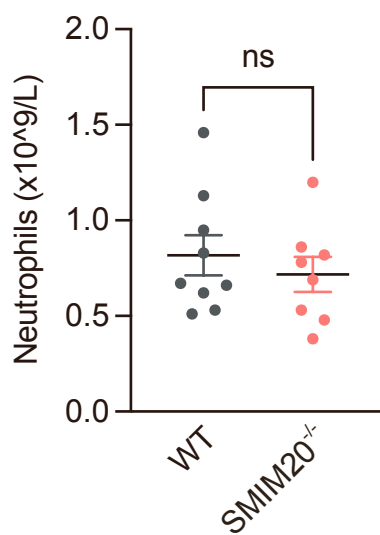
A

White blood cell count



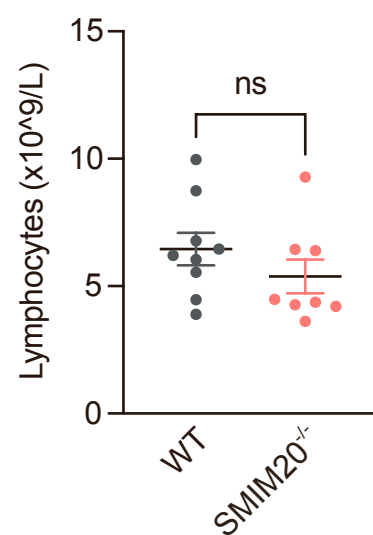
B

Neutrophil cell count



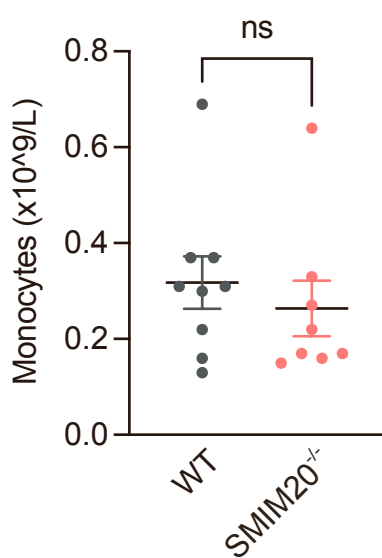
C

Lymphocyte cell count



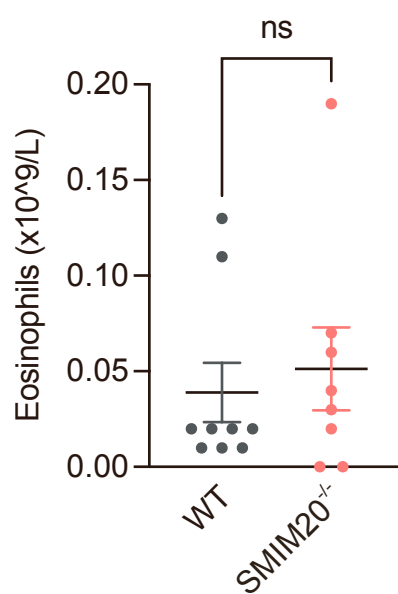
D

Monocyte cell count



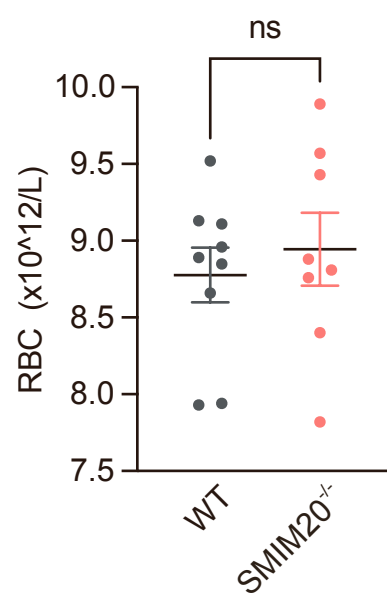
E

Eosinophil cell count



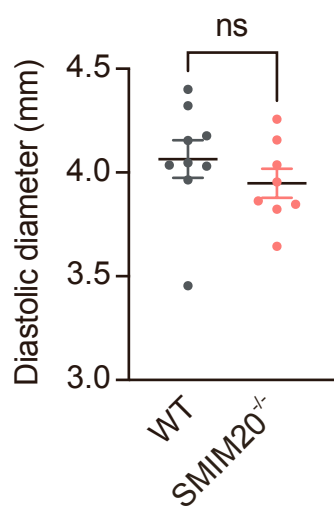
F

Red blood cell count



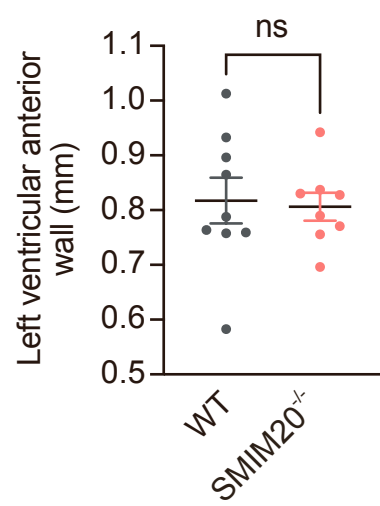
G

Diastolic diameter



H

Thickness in systole



Thickness in systole

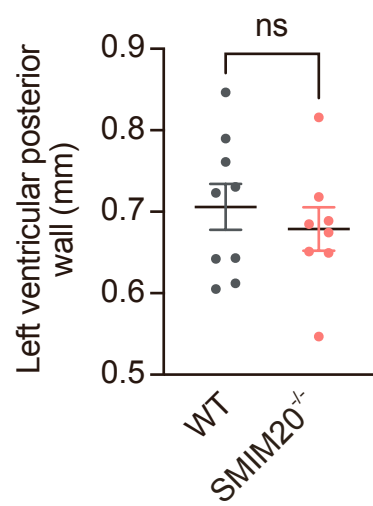


Figure S1: Characterization of the SMIM20^{-/-} mouse model, related to Figures 1 and 2

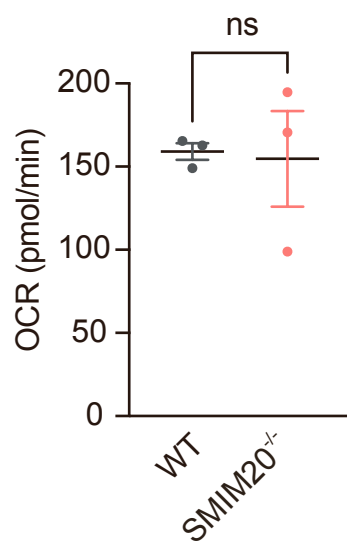
(A-F) Quantification of (A) white blood cells, (B) neutrophils, (C) lymphocytes, (D) monocytes, (E) eosinophils and (F) red blood cells of 15-week-old male WT and SMIM20^{-/-} mice. n = WT (9) and SMIM20^{-/-} (8), mean ± SEM, Unpaired t-test, ns - non significant.

(G) Diastolic diameter of heart from 15-week-old WT and SMIM20^{-/-} male mice n = WT (9) and SMIM20^{-/-} (8), mean ± SEM, Unpaired t-test, ns - non significant.

(H) Left ventricular anterior (left) and posterior (right) heart wall thickness during diastole from 15-week-old male WT and SMIM20^{-/-} mice. n = WT (9) and SMIM20^{-/-} (8), mean ± SEM, Unpaired t-test, ns - non significant.

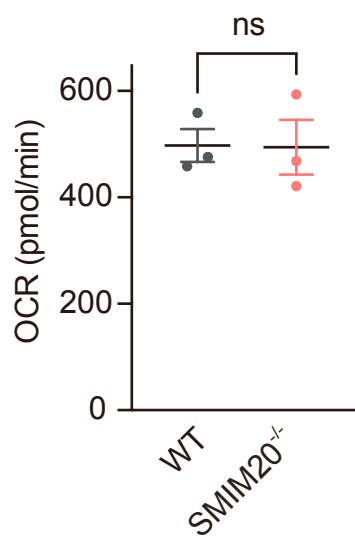
A

Pyruvate based respiration



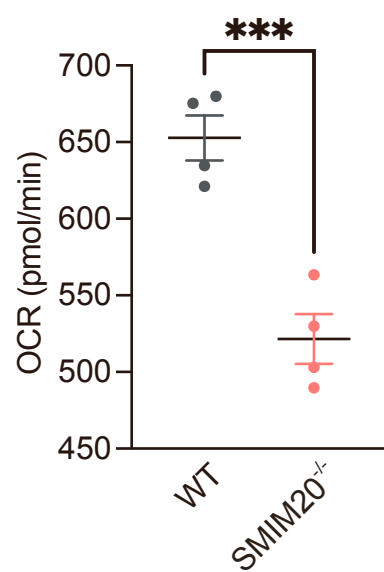
B

Succinate based respiration



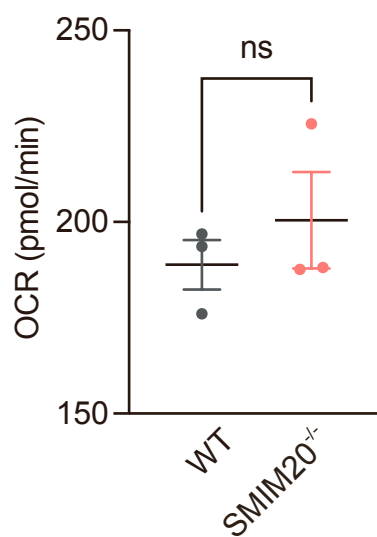
C

FAO based respiration



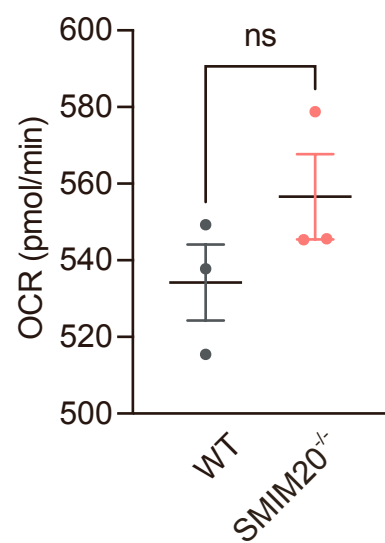
D

Pyruvate based respiration



E

Succinate based respiration

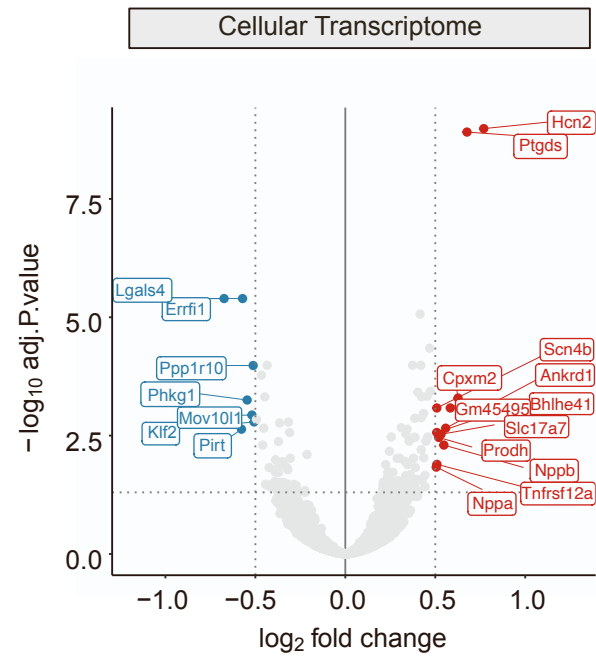


Supplementary Figure. 2

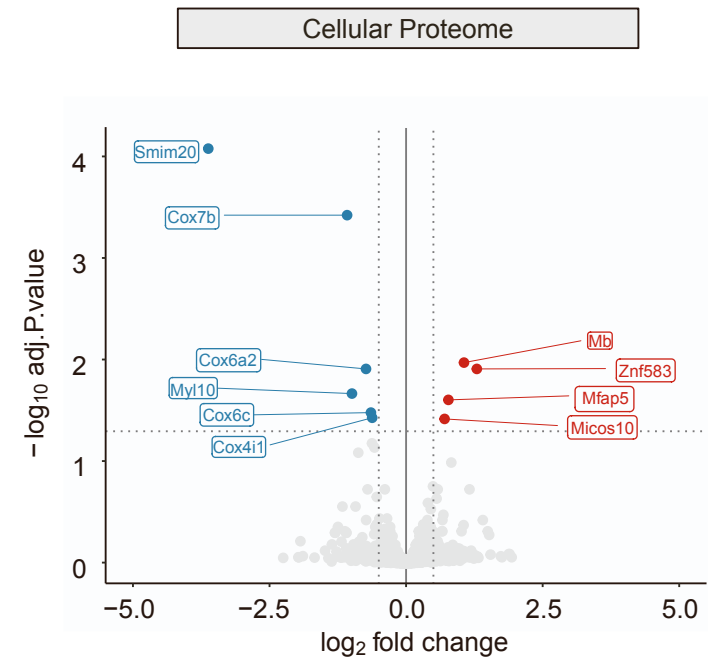
Figure S2: Molecular analysis of the SMIM20^{-/-} mouse mitochondria, related to Figure 4

- (A) Oxygen consumption rate recorded for isolated heart mitochondria from 15-week-old male WT and SMIM20^{-/-} mice where pyruvate was used as a substrate. n = 3, mean ± SEM, Unpaired t-test, ns - non-significant.
- (B) Oxygen consumption rate recorded for isolated heart mitochondria from 15-week-old male WT and SMIM20^{-/-} mice where succinate was used as a substrate. n = 3, mean ± SEM, Unpaired t-test, ns - non-significant.
- (C) Oxygen consumption rate recorded for isolated liver mitochondria from 15-week old WT and SMIM20^{-/-} male mice where palmitoyl CoA/carnitine/malate/ADP was used as substrate. ETO was used as a negative control. n = 4, mean ± SEM, Unpaired t test, ns - non significant; p*** = 0.0010.
- (D) Oxygen consumption rate recorded for isolated liver mitochondria from 15-week old male WT and SMIM20^{-/-} mice where pyruvate was used as a substrate. n = 3, mean ± SEM, Unpaired t-test, ns - non-significant.
- (E) Oxygen consumption rate recorded for isolated liver mitochondria from 15-week old male WT and SMIM20^{-/-} mice where succinate was used as a substrate. n = 3, mean ± SEM, Unpaired t-test, ns - non-significant.

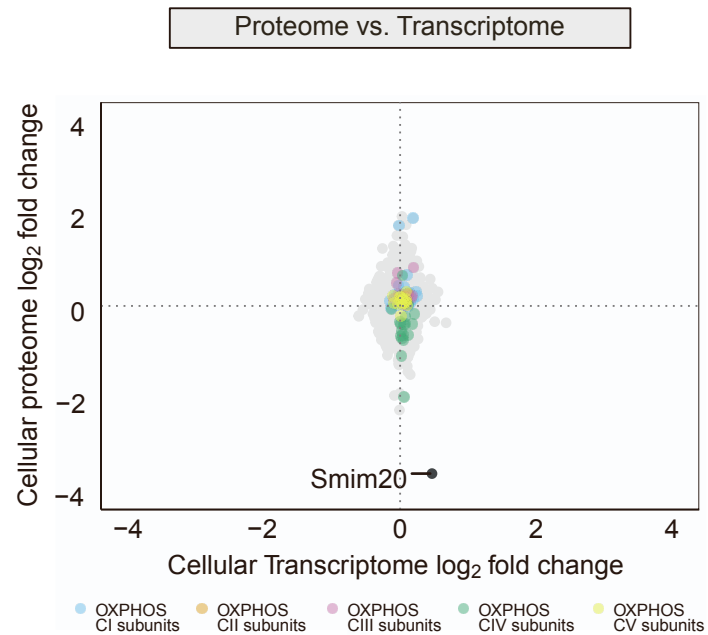
A



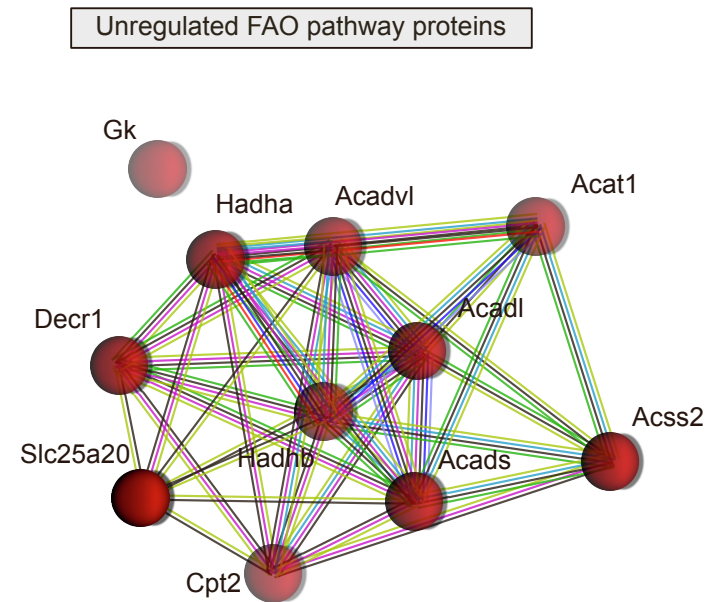
B



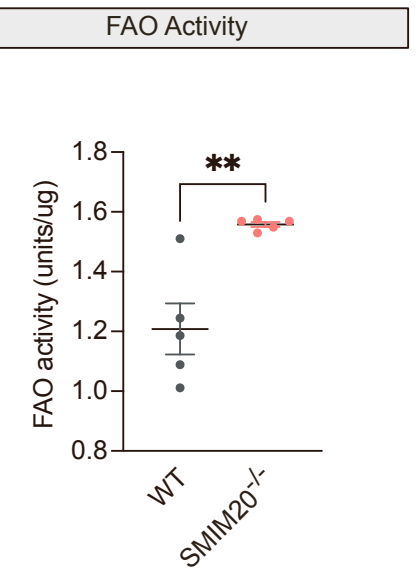
C



D



E



Supplementary Figure. 3

Figure S3: Proteomic analysis identified dysregulated metabolic pathways, related to Figure 5

(A) Volcano plot of RNA-seq analysis of the cellular transcriptomes of SMIM20^{-/-} mice compared against WT mice. n = WT (3) and SMIM20^{-/-} (5).

(B) Volcano plot of mass spectrometry analysis of the cellular proteomes of SMIM20^{-/-} mice compared against WT mice. n = WT (6) and SMIM20^{-/-} (4).

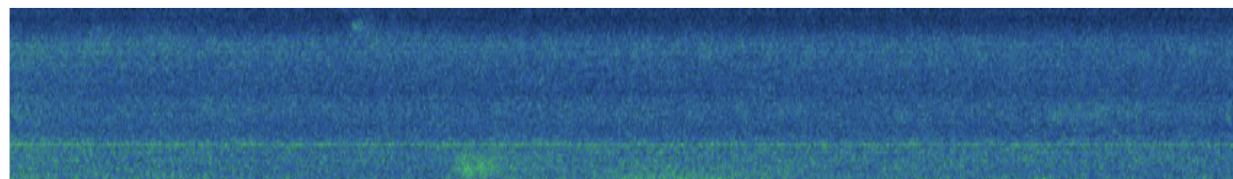
(C) Comparison of cellular transcriptomes and cellular proteomes log₂ fold changes from A and B.

(D) STRING analysis for the enriched proteins in the “Fatty Acid Beta Oxidation” process from the GSEA.

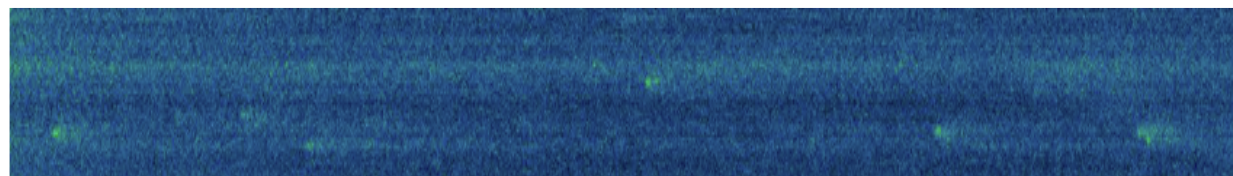
(E) Quantification of the activity of FAO enzymes in heart tissue lysate of 15-week old male WT and SMIM20^{-/-} mice. n = 5, mean ± SEM, Unpaired t-test, p** = 0.0036.

A

1000 ms
10 μ m



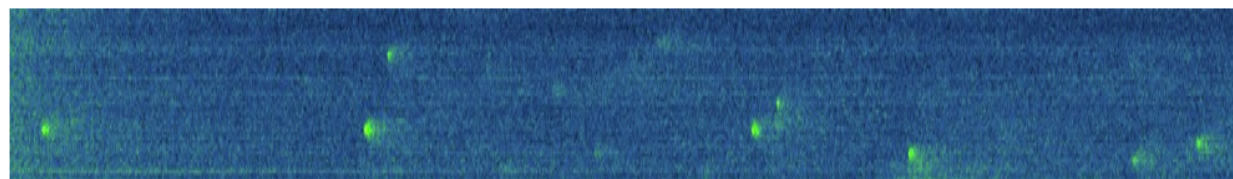
WT



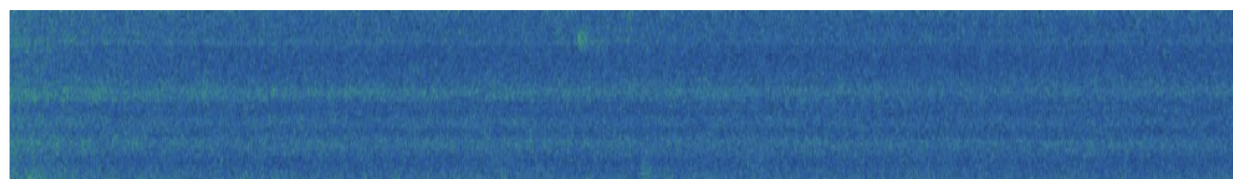
SMIM20^{-/-}

B

1000 ms
10 μ m



SMIM20^{-/-} + DMSO



SMIM20^{-/-} + MitoTEMPO

Figure S4: Dysregulated calcium handling in SMIM20^{-/-} mice, related to Figure 6

(A) Incidence of Ca²⁺ sparks in 15-week-old male WT and SMIM20^{-/-} mouse ventricular myocytes. Representative confocal line scans showing SR Ca²⁺ release

events in the form of Ca²⁺ sparks in WT and SMIM20^{-/-} mouse ventricular myocytes.

(B) Incidence of Ca²⁺ sparks in DMSO- and MitoTEMPO-treated 15-week-old male WT and SMIM20^{-/-} mouse ventricular myocytes. Representative confocal line scans showing SR Ca²⁺ release events in the form of Ca²⁺ sparks in WT and SMIM20^{-/-} mouse ventricular myocytes.