Gamma-interferon-inducible lysosomal thiol reductase maintains cardiac immuno-metabolic homeostasis in heart failure

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ABSTRACT:

Heart failure (HF) remains the leading cause of death globally. The progression of HF is characterized by cardiac mitochondrial dysfunction and aberrant inflammatory responses. Although the lysosome has been recognized as the central player for maintaining immuno-metabolic homeostasis in diverse organs in health and disease, the mechanistic insights into the regulation of lysosome-dependent immuno-metabolism in the heart are lacking. Lysosomal reductase Gamma Interferon-Inducible Thiol Reductase (GILT) is the only identified lysosomal reductase that controls more than 11 lysosomal enzymes, and a single nucleotide polymorphism in the coding sequence of GILT has been implicated in promoting cardiovascular risk. Here, we show that GILT expression and activity are reduced in hearts from human patients and mice with HF, respectively. Moreover, cardiomyocyte-specific deletion of GILT (GILT-cKO) results in left ventricular remodeling and dysfunction in the setting of pressure overload. At the cellular level, cardiac GILT deficiency leads to impaired mitochondrial respiration, elevated mitochondrial oxidative stress, and increased NLR Family Pyrin Domain Containing 3(NLRP3)-dependent inflammation in the heart. Mechanistically, inhibition of NLRP3 in primary cardiomyocytes ameliorated the mitochondrial dysfunction in the GILT deficient cells, implicating a causative role of the lysosome-inflammasome axis on regulating cardiac mitochondrial function. Together, these findings elucidate a functional link between cardiac lysosomes, inflammatory responses and mitochondrial respiration. Knowledge gained from this study might speed the development of therapeutic agents to treat patients with HF.

INTRODUCTION:

The pathophysiology of Heart Failure (HF) is characterized by altered cardiac metabolism and activation of inflammatory pathways in the heart. Our current understanding of how these processes interact in the setting of HF remains incompletely understood. Intracellularly, macromolecule catabolism, nutrient sensing and inflammatory regulation are fine-tuned by the lysosome. Although defective lysosomal function has been implicated in a wide range of

cardiovascular diseases (CVDs)[1], delineating the physiological role of lysosomal function *in vivo* has been a challenge. This is in part attributed to the complexity of the lysosome, which contains more than 60 enzymes that act upon a multitude of substrates. As such, murine studies of individual lysosomal enzymes using gain- or loss-of-function have contradicting observations with regard to cardiac pathophysiology. Gamma-Interferon-Inducible Lysosomal Thiol Reductase (GILT) holds a central place in maintaining lysosomal organelle homeostasis as it is the only identified reductase in this compartment[2]. To date, GILT has been primarily recognized as a regulator of immune cell antigen presentation[2], but its role in the heart in the context of HF is essentially unknown. Notably, a single nucleotide polymorphism (SNP) in the coding sequence of *IFI30,* which encodes GILT, has been predicted to alter its enzymatic activity and is associated with CVD risk factors[3]. Here we report a novel function of GILT in maintaining immuno-metabolic homeostasis in response to pressure overload conditions.

RESULTS

To evaluate the pathophysiological relevance of GILT in the setting of HF, we measured *IFI30* transcript expression in cardiac samples from healthy human donors or patients with HF with reduced ejection fraction (HFrEF; EF<30%). The *IFI30 gene* transcript was significantly suppressed in human failing hearts (Figure [A]). Similarly, analysis of a publicly available RNA-Seq dataset (GSE54681) revealed reduced *Ifi*30 transcript levels in mouse hearts with HFrEF (Figure [B]). Accordingly, Gilt enzymatic activity was diminished in hearts from HFrEF mice (Figure [C&D]). To determine the necessity of Gilt in cardiac pathophysiology, we generated a mouse strain with loxP sites flanking exons 4 through 7 of *Ifi30* (Gilt fl/fl; C57BL/6J background) and recombined the alleles in cardiomyocytes by crossing these mice with α-Myosin heavy chain (α-MHC) Cre mice (Gilt-cKO) (Figure [E]). Gilt-KO mice display normal cardiac function at baseline (Figure [F]). After transverse aortic constriction (TAC), Gilt-cKO mice developed worsened cardiac dysfunction and LV dilation compared to its littermate controls (Gilt fl/fl, Figure [G-J]). Histologically, cardiac deficiency of Gilt led to increased myocardial fibrosis (Figure [K-M]).

Together, these data demonstrate that loss of GILT in the heart worsens TAC-mediated cardiac dysfunction and exacerbates pathological remodeling.

Sustained activation of proinflammatory signaling cascades and release of IL-1β results in recruitment and activation of inflammatory immune cells, a critical factor for the progression of HF[4]. Gilt deficiency significantly increased macrophage infiltration in the heart after TAC (Figure [N&O]). Notably, level of Il-1β was significantly increased in Gilt-cKO heart homogenates after TAC and in Gilt-deficient primary cardiomyocytes exposed to a proinflammatory stimulus (LPS), respectively (Figure [P&Q]). NLR family pyrin domain containing 3 (Nlrp3) inflammasome activation is the primary source of Ik-1β. Indeed, we found that loss of Gilt in cardiomyocytes increased TAC-induced Nlrp3 and Caspase-1 expression (Figure [R]). Together, these results indicate an anti-inflammatory role of Gilt, which is mediated in part by regulating inflammatory signaling via I1-1β. These results further strengthen the notion that cardiomyocyte-intrinsic inflammation may be a crucial contributor to HF progression[4].

It has been shown that cardiac Nlrp3 activation is associated with mitochondrial defects in HF. To determine whether deletion of Gilt alters cardiac mitochondrial respiratory capacity, we performed high resolution respirometry analysis (Oxygraph-2k, Oroboros) in freshly dissected permeabilized cardiac fibers from unstressed Gilt KO and its littermate control mice. Gilt deletion diminished respiration of cardiac fibers in ADP-stimulated palmitoyl-carnitine and malate-driven state-3 respiration (Figure [S]). This finding is in line with a metabolomic analysis, which revealed an impaired cardiac TCA cycle flux in Gilt-cKO TAC mice (Figure [T]). However, this metabolic defect was not due to reduced expression of proteins within the mitochondrial oxidative phosphorylation (OxPhos) complex (Figure [U]). Excessive mitochondrial reactive oxygen species (ROS) can impair mitochondrial structure and function, and contribute to heart pathologies[5]. Intracellularly, Gilt deficiency results in reduced mitochondrial respiration in cardiomyocytes, which was ameliorated with a mitochondrial-specific ROS scavenger, Mito-TEMPO (Figure [S]). Lastly, to address whether Gilt deficiency-associated Nlrp3 activation could induce mitochondrial

respiratory defects, we treated primary cardiomyocytes with a potent Nlrp3 inhibitor, MCC950, and measured mitochondrial ROS in the presence of isoproterenol. Notably, inhibiting Nlrp3 reduced basal and isoproterenol-induced mitochondrial ROS in Gilt-deficient cardiomyocytes (Figure [V&W]).

Our study demonstrates a previously unrecognized role of Gilt in the regulation of lysosomal function by linking cardiomyocyte-intrinsic immune action with metabolic homeostasis in the setting of pressure overload stress (Figure [X]). These findings offer a platform for dissecting the contribution of lysosomal dysfunction in HF and identify potential targets for therapeutic intervention for patients with HF.

FIGURE LEGEND

Gilt is required for maintaining immune and metabolic homeostasis in the failing heart.

A. The expression of the *IFI30* transcript in the heart from donors with non-heart failure (non-HF) and HF with reduced ejection fraction (HFrEF; EF<30%) determined by qRT-PCR. N=6-8 samples/group. **B.** *Ifi30* transcript levels determined by RNA-Seq analysis (GSE54681; RStudio) from sham (non-HF) and different stages of transient HFrEF; N = 3 samples/group. **C.** *In vitro* GILT enzyme activity in cardiac lysate from 15-week-old sham and HFrEF mice determined by the reduction of the $F(ab')_2$ fragment of IgG into heavy (H) and light (L) chains. IgG (red): non reducing control. N = 3 male mice/group. **D.** Quantification of relative intensity of the $F(ab')_2$ and H/L fractions in (C). **E.** *Ifi30* expression in the heart determined by qRT-PCR. N = 3 male mice (C57BL/6J background)/group at 8 weeks of age. **F.** Echocardiography in male Gilt fl/fl and Gilt-cKO mice (C57BL/6J background). N = 3 mice/group at 15 weeks of age. **G-J.** Echocardiography in Gilt fl/fl TAC and Gilt-cKO TAC mice. N = 9-15 male mice/group at 15 weeks of age 5 weeks post-TAC. **K.** Representative images of intact hearts from Gilt fl/fl TAC and Gilt-cKO TAC mice; **L.** Masson's trichrome staining from Gilt fl/fl TAC and Gilt-cKO mice and **M.** quantification of fibrotic tissue. N = 4-5 male mice/group 15 weeks of age. **N.** Representative confocal microscopy

images (panels) and relative quantification (below) of F4/80 macrophage marker staining in frozen heart sections (N = 3, age-matched male mice at 15 weeks of age). **O.** Flow cytometry analysis of single cells isolated from 15-week-old Gilt fl/fl and Gilt-cKO hearts; quantification represents a gated population of Cd45+/F4/80+ cells (macrophages). **P-Q.** Concentration of IL-1β determined by ELISA in hearts from Gilt fl/fl TAC and Gilt-cKO TAC mice (P, N = 5 male mice/group at 15 weeks of age) and primary cardiomyocytes from Gilt fl/fl and Gilt KO mice stimulated with LPS (500 ng/mL) for 50 min and ATP (5 mM) for 10 min (Q, N = 3 male mice/group at 16 weeks of age). **R.** Representative western blot (left) and quantified densitometry analysis (right) of Nlrp3 and Caspase-1 in hearts from Gilt fl/fl TAC and Gilt-cKO TAC mice normalized to Actb. **S.** High resolution respirometry (Oroboros) in freshly resected heart digested fibers from Gilt fl/fl and Gilt KO mice at 16 weeks of age. N = 8 cardiac samples from individual mice/group. Pal/Mal – palmitoyl-carnitine/malate at 0.075 mM; ADP – adenosine diphosphate at 5 mM. **T.** Metabolomic analysis in hearts of Gilt fl/fl TAC and Gilt-cKO TAC mice as in [G], N = 3 mice/group. **U.** Normalized densitometry analysis (U) of mitochondrial oxidative phosphorylation complex (OxPhos) proteins in hearts from Gilt fl/fl TAC and Gilt-cKO TAC mice at 15 weeks of age. N = 5 male mice/group. **V-W**. Representative confocal microscopy images (V) and quantification (W) of mitochondrial superoxide levels (MitoSOX; at 500 nM) in primary cardiomyocytes under different treatments. MCC950: 1 μM for 2 hrs.; Isoproterenol: 100 nM for 15 min. Images of MitoSOX in each cell were normalized to its surface area in ImageJ. **X.** Proposed model of Gilt-dependent regulation of cardiac immuno-metabolic homeostasis. All data are presented as means ± SEM. * indicates statistical significance compared to the non-HF groups in (A-D), to the Gilt fl/fl groups with same treatments in (E-I, M-R, and S&W); # indicates MitoTEMPO effects in equivalent groups of cells in (S) and MCC950 effects in equivalent cells in (W). Red and green boxes in (T) represent statistically significant increases or decreases of metabolites in Gilt-cKO TAC versus Gilt fl/fl TAC, respectively. Statistical analysis was performed by using Student's t-test in panels

in (A-J, M-P, and R), and Two-way ANOVA with Tukey's multiple comparisons test in (Q, S and W), p-value <0.05 was set as the threshold for statistical significance.

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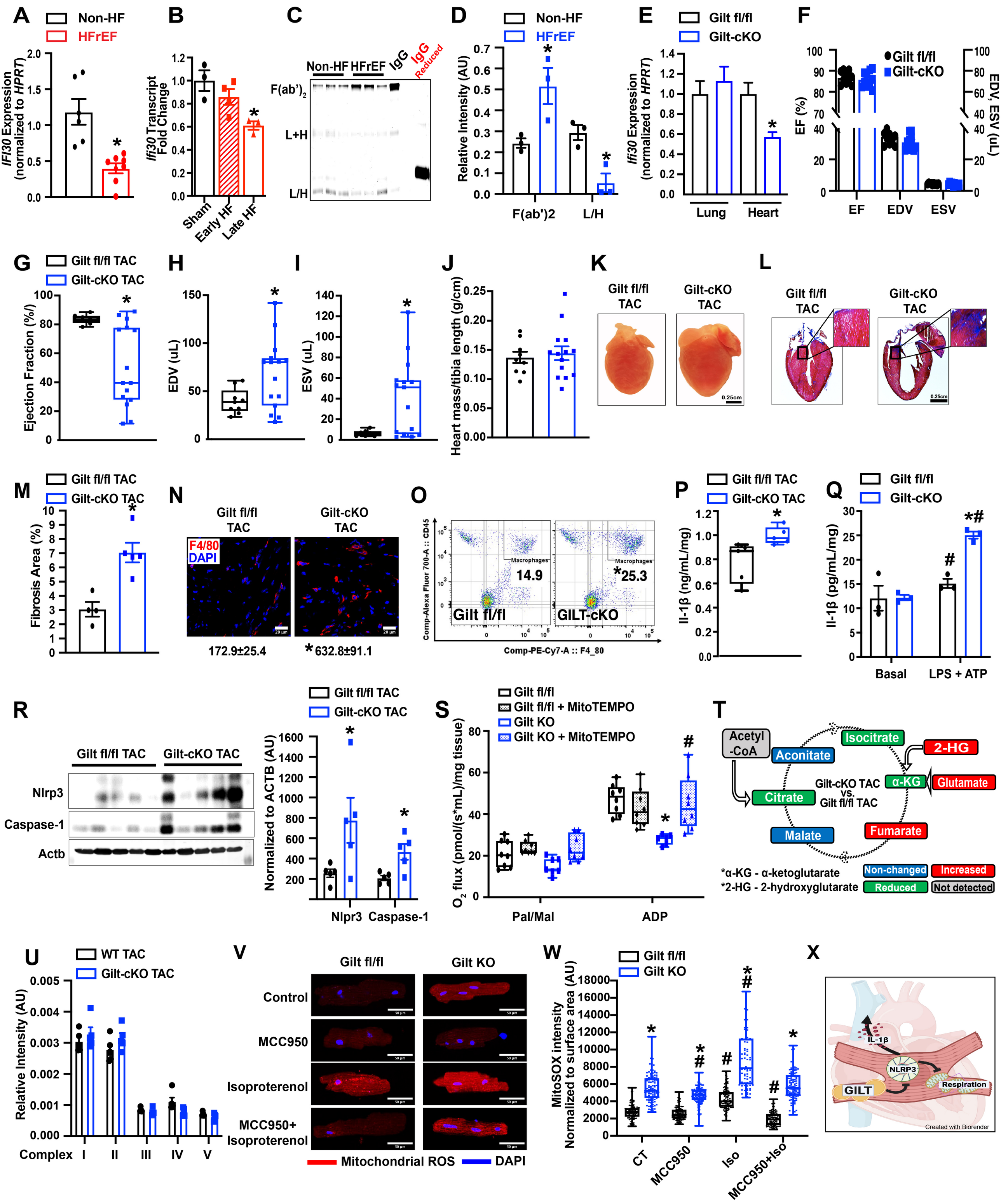
Figure


A
Non-HF
HFrEF
IFI30 Expression (normalized to HPRT)
B
Ifi30 Transcript Fold Change
Sham
Early HF
Late HF
C
Non-HF HFrEF IgG IgG Reduced
F(ab')2
L+H
L/H
D
Non-HF
HFrEF
Relative Intensity (AU)
F(ab')2
L/H
E
Gilt fl/fl
Gilt-cKO
Ifi30 Expression (normalized to HPRT)
Lung
Heart
F
Gilt fl/fl
Gilt-cKO
EF (%)
EDV, ESV (uL)
EF
EDV
ESV
G
Gilt fl/fl TAC
Gilt-cKO TAC
Ejection Fraction (%)
H
EDV (uL)
I
ESV (uL)
J
Heart mass/tibia length (g/cm)
K
Gilt fl/fl TAC
Gilt-cKO TAC
0.25cm
L
Gilt fl/fl TAC
Gilt-cKO TAC
0.25cm
M
Gilt fl/fl TAC
Gilt-cKO TAC
Fibrosis Area (%)
N
Gilt fl/fl TAC
Gilt-cKO TAC
F4/80
DAPI
20 µm
172.9±25.4
632.8±91.1
O
Comp-Alexa Fluor 700-A :: CD45
Macrophages
14.9
25.3
Gilt fl/fl
GILT-cKO
Comp-PE-Cy7-A :: F4_80
P
Gilt fl/fl TAC
Gilt-cKO TAC
Il-1β (ng/mL/mg)
Q
Gilt fl/fl
Gilt-cKO
Il-1β (pg/mL/mg)
Basal
LPS + ATP
R
Gilt fl/fl TAC
Gilt-cKO TAC
Nlrp3
Caspase-1
Actb
Normalized to ACTB (AU)
Nlpr3
Caspase-1
S
Gilt fl/fl
Gilt fl/fl + MitoTEMPO
Gilt KO
Gilt KO + MitoTEMPO
O2 flux (pmol/(s*mL)/mg tissue)
Pal/Mal
ADP
T
Acetyl -CoA
Aconitate
Isocitrate
2-HG
Citrate
Gilt-cKO TAC vs. Gilt fl/fl TAC
α-KG
Glutamate
Malate
Fumarate
*α-KG - α-ketoglutarate
*2-HG - 2-hydroxyglutarate
Non-changed
Increased
Reduced
Not detected
U
WT TAC
Gilt-cKO TAC
Relative Intensity (AU)
Complex I
II
III
IV
V
V
Gilt fl/fl
Gilt KO
Control
MCC950
Isoproterenol
MCC950+ Isoproterenol
50 µm
Mitochondrial ROS
DAPI
W
Gilt fl/fl
Gilt KO
MitoSOX intensity Normalized to surface area (AU)
CT
MCC950
Iso
MCC950+Iso
X
IL-1β
NLRP3
GILT
Respiration
Created with Biorender