Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/45/eabb7272/DC1

Supplementary Materials for

Metabolic determinants of cellular fitness dependent on mitochondrial reactive oxygen species

Hyewon Kong, Colleen R. Reczek, Gregory S. McElroy, Elizabeth M. Steinert, Tim Wang, David M. Sabatini, Navdeep S. Chandel*

*Corresponding author. Email: nav@northwestern.edu

Published 4 November 2020, *Sci. Adv.* **6**, eabb7272 (2020)
DOI: 10.1126/sciadv.abb7272

This PDF file includes:

Supplementary Materials

Figure S1.

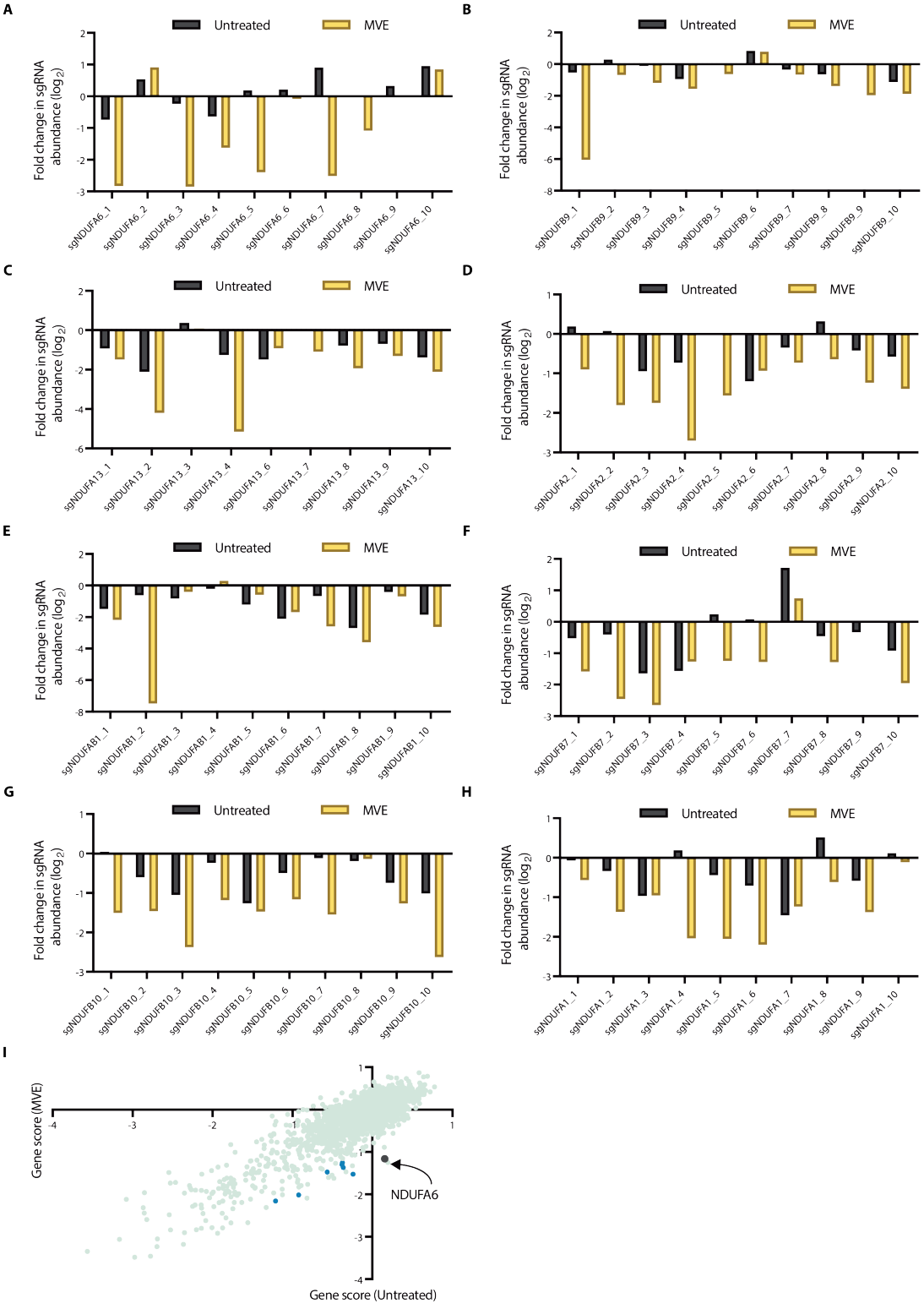

Fig. S1. A CRISPR-based negative selection screen identifies multiple subunits of mitochondrial complex I to be essential for cellular fitness in the presence of MVE.

(A-H) Changes in the abundance of individual sgRNAs targeting mitochondrial complex I subunit genes; *NDUFA6* (A), *NDUFB9* (B), *NDUFA13* (C), *NDUFA2* (D), *NDUFAB1* (E), *NDUFB7* (F), *NDUFB10* (G), and *NDUFA1* (H), in the presence (yellow) or absence (grey) of MVE. **(I)** Gene scores of all genes targeted by the metabolic sgRNA library in untreated (X-axis) versus MVE-treated (Y-axis) cell populations. Mitochondrial complex I subunit hits, *NDUFA6, NDUFB9, NDUFA13, NDUFA2, NDUFAB1, NDUFB7, NDUFB10*, and *NDUFA1*, are highlighted in blue. Among them, *NDUFA6*, indicated with an arrow, exhibited the largest differential gene score.

Figure S2.

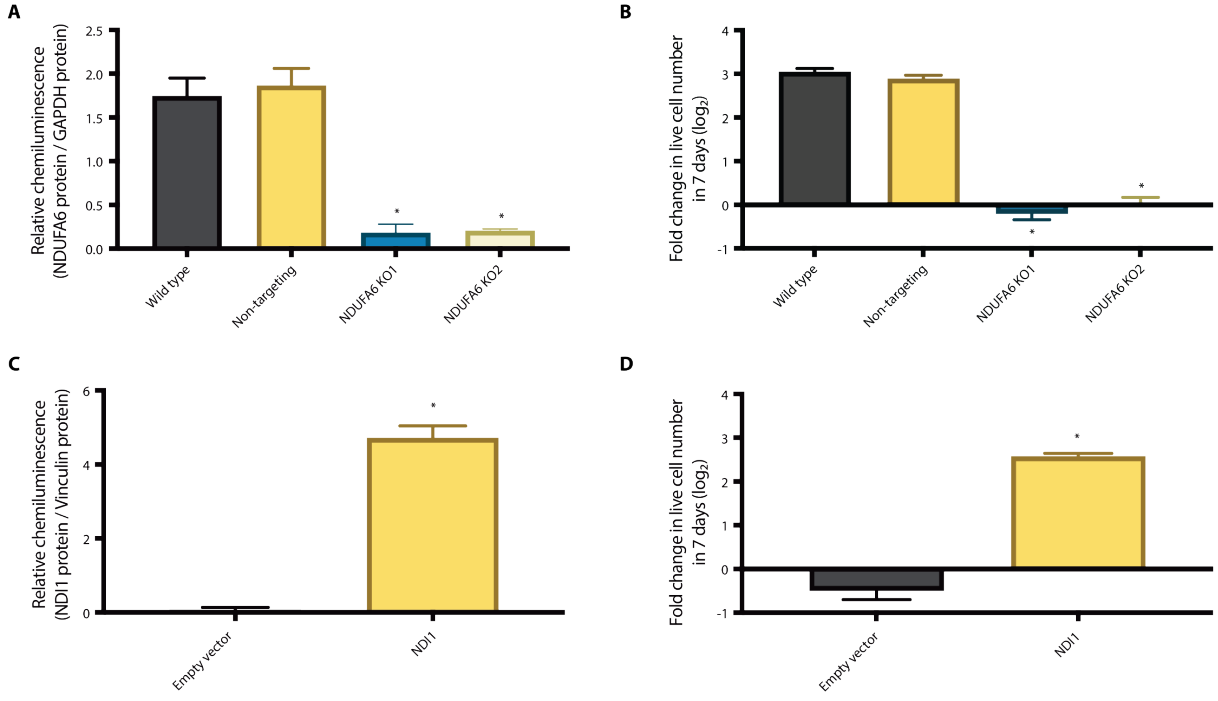

Fig. S2. CRISPR-mediated deletion of the *NDUFA6* gene impairs mitochondrial complex I function.

(A) Simple Western analysis of NDUFA6 protein in wild type, non-targeting control, and two NDUFA6-null (NDUFA6 KO1 and KO2) Jurkat cell lines. GAPDH was used as a loading control. (n=3, mean+s.e.m., *P=0.0003 compared to non-targeting) **(B)** Wild type, non-targeting control, and two NDUFA6-null (NDUFA6 KO1 and KO2) Jurkat cell lines were cultured in galactose media for 7 days, and the population doublings were assessed. (n=4, mean+s.e.m., *P<0.0001 compared to non-targeting) **(C)** Simple Western analysis of NDI1 protein expression in the NDUFA6 KO1 cell line with ectopic expression of either the empty vector or NDI1. Vinculin was used as a loading control. (n=3, mean+s.e.m., *P=0.0002 compared to empty vector) **(D)** NDUFA6 KO1 Jurkat cells expressing either empty vector or NDI1 were cultured in galactose media for 7 days, and the population doublings were assessed. (n=4, mean+s.e.m., *P<0.0001 compared to empty vector)

Figure S3.

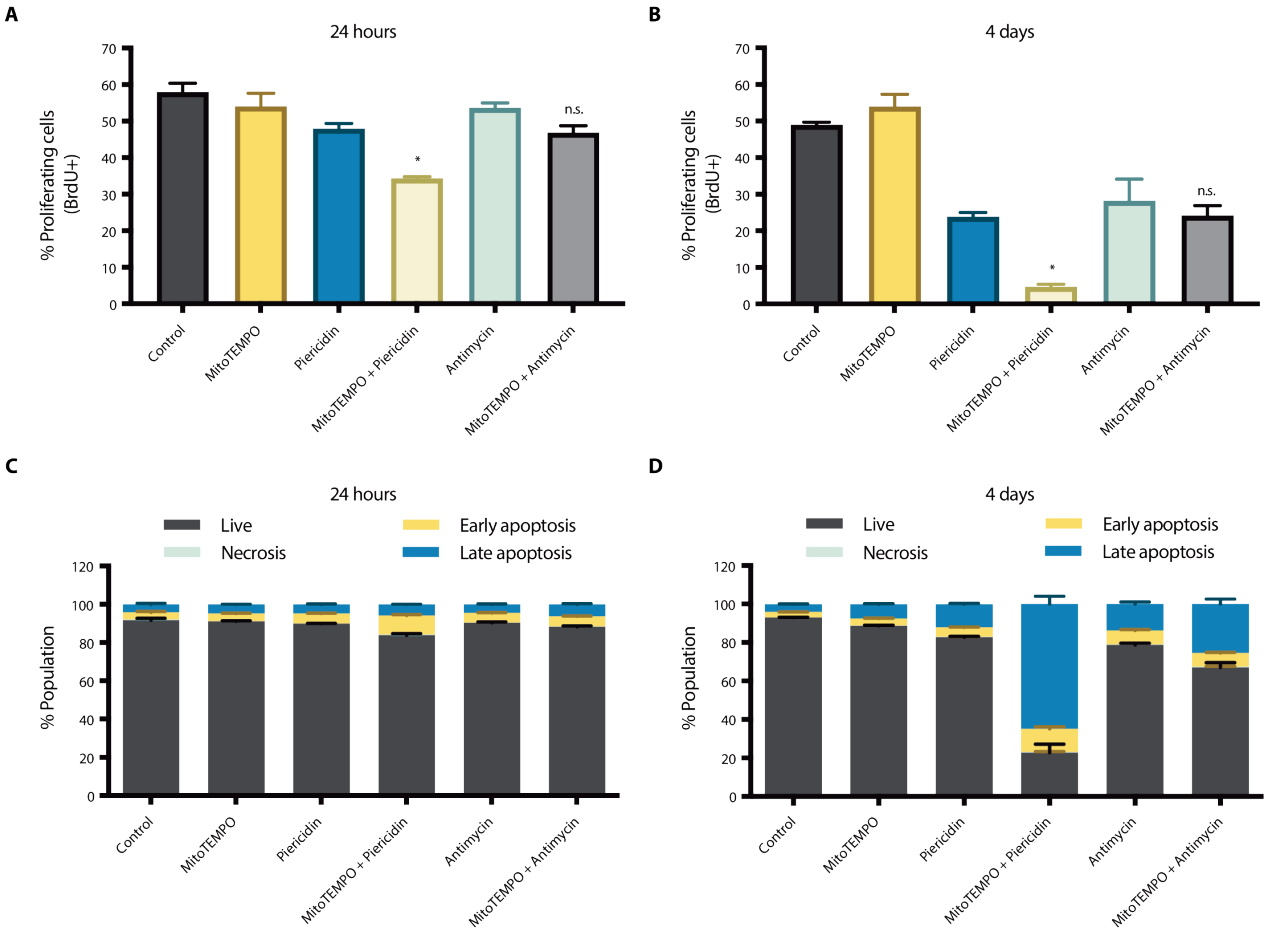
A
24 hours
% Proliferating cells (BrdU+)
70
60
50
40
30
20
10
0
*
n.s.
Control
MitoTEMPO
Piericidin
MitoTEMPO + Piericidin
Antimycin
MitoTEMPO + Antimycin
B
4 days
% Proliferating cells (BrdU+)
*
n.s.
Control
MitoTEMPO
Piericidin
MitoTEMPO + Piericidin
Antimycin
MitoTEMPO + Antimycin
C
24 hours
Live
Necrosis
Early apoptosis
Late apoptosis
% Population
120
100
80
60
40
20
0
Control
MitoTEMPO
Piericidin
MitoTEMPO + Piericidin
Antimycin
MitoTEMPO + Antimycin
D
4 days
Live
Necrosis
Early apoptosis
Late apoptosis
% Population
Control
MitoTEMPO
Piericidin
MitoTEMPO + Piericidin
Antimycin
MitoTEMPO + Antimycin

Fig. S3. Inhibition of mitochondrial complex I, but not mitochondrial complex III, synergizes with a mito-antioxidant to impair cellular fitness.

(A-B) Jurkat cells supplemented with pyruvate and uridine were treated with 1 μM Piericidin or 1 μM Antimycin +/- 400 μM MitoTEMPO for 24 hours (A) or 4 days (B), and the percentage of BrdU incorporation into the proliferating cell population was measured. (n=3, mean+s.e.m., *P= 0.0093 (A) or *P= 0.0114 (B) compared to Piericidin alone, $^{n.s.}$P=0.6588 (A) or $^{n.s.}$P>0.9999 (B) compared to Antimycin alone) **(C-D)** Jurkat cells supplemented with pyruvate and uridine were treated with 1 μM Piericidin or 1 μM Antimycin +/- 400 μM MitoTEMPO for 24 hours (C) or 4 days (D), and the percentage of Annexin V- and/or propidium iodine (PI)-incorporated apoptotic cell populations were measured. Live: Annexin V negative and PI negative; Necrosis: Annexin V negative and PI positive; Early apoptosis: Annexin V positive and PI negative; Late apoptosis: Annexin V positive and PI positive. (n=4, mean+s.e.m.)

Figure S4.

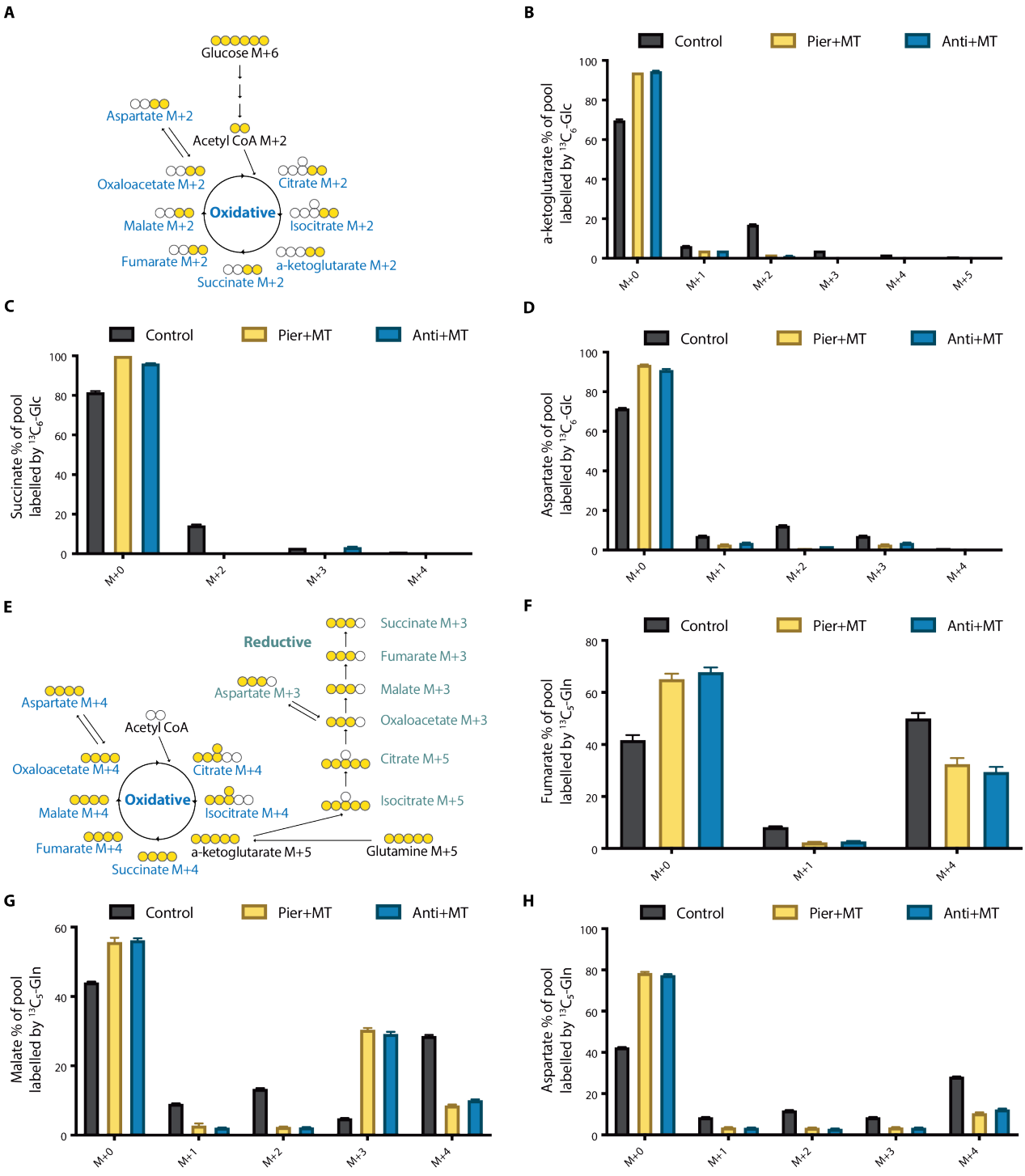
A
Glucose M+6
Aspartate M+2
Acetyl CoA M+2
Oxaloacetate M+2
Citrate M+2
Oxidative
Malate M+2
Isocitrate M+2
Fumarate M+2
a-ketoglutarate M+2
Succinate M+2
B
Control
Pier+MT
Anti+MT
a-ketoglutarate % of pool labelled by $^{13}C_6$-Glc
M+0
M+1
M+2
M+3
M+4
M+5
C
Succinate % of pool labelled by $^{13}C_6$-Glc
D
Aspartate % of pool labelled by $^{13}C_6$-Glc
E
Reductive
Succinate M+3
Fumarate M+3
Aspartate M+3
Malate M+3
Oxaloacetate M+3
Aspartate M+4
Acetyl CoA
Citrate M+5
Oxaloacetate M+4
Citrate M+4
Isocitrate M+5
Malate M+4
Oxidative
Isocitrate M+4
Fumarate M+4
a-ketoglutarate M+5
Glutamine M+5
Succinate M+4
F
Fumarate % of pool labelled by $^{13}C_5$-Gln
G
Malate % of pool labelled by $^{13}C_5$-Gln
H
Aspartate % of pool labelled by $^{13}C_5$-Gln

Fig. S4. Inhibition of mitochondrial complex I or mitochondrial complex III in the presence of a mito-antioxidant induces reductive TCA cycle.

(A) Schematic representation of the glucose-driven oxidative TCA cycle. Labelled carbons (^{13}C) are indicated in yellow. Oxidative TCA cycle of [U-$^{13}C_6$]glucose results in M+2 pools of TCA-cycle metabolites, as well as aspartate. **(B-D)** Jurkat cells treated with vehicle (Control), Piericidin and MitoTEMPO (Pier+MT), or Antimycin and MitoTEMPO (Anti+MT) for 24 hours were labelled for 8 hours with [U-$^{13}C_6$]glucose, and the percentages of labelled α-ketoglutarate (B), succinate (C), and aspartate (D) pools were assessed. (n=5, mean+s.e.m.) **(C)** Schematic representation of the glutamine-driven oxidative (blue) and reductive (green) TCA cycles. Labelled carbons (^{13}C) are indicated in yellow. Metabolism of [U-$^{13}C_5$]glutamine results in fully labeled M+5 α-ketoglutarate. Oxidative TCA cycle of M+5 α-ketoglutarate generates M+4 pools of TCA-cycle metabolites, as well as aspartate. Reductive TCA cycle of M+5 α-ketoglutarate produces M+5 pool of (iso)citrate, followed by M+3 pool of malate. **(F-H)** Jurkat cells treated with vehicle (Control), Pier+MT, or Anti+MT for 24 hours were labelled for 8 hours with [U-$^{13}C_5$]glutamine, and the percentages of labelled fumarate (F), malate (G), and aspartate (H) pools were assessed. (n=5, mean+s.e.m.)

Figure S5.

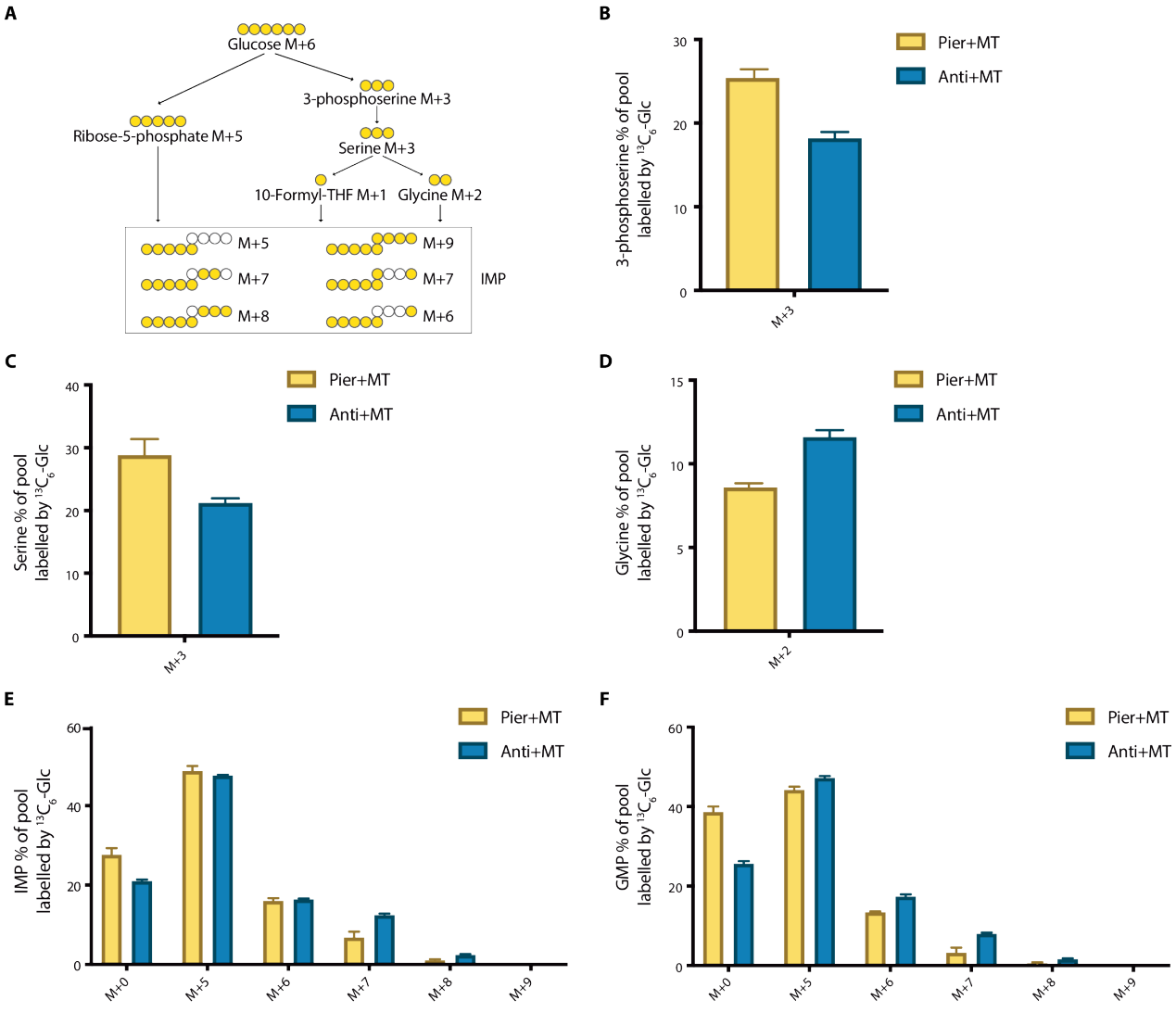
A
Glucose M+6
3-phosphoserine M+3
Ribose-5-phosphate M+5
Serine M+3
10-Formyl-THF M+1
Glycine M+2
M+5
M+9
M+7
M+7
M+8
M+6
IMP
B
Pier+MT
Anti+MT
3-phosphoserine % of pool labelled by $^{13}C_6$-Glc
M+3
C
Pier+MT
Anti+MT
Serine % of pool labelled by $^{13}C_6$-Glc
M+3
D
Pier+MT
Anti+MT
Glycine % of pool labelled by $^{13}C_6$-Glc
M+2
E
Pier+MT
Anti+MT
IMP % of pool labelled by $^{13}C_6$-Glc
M+0
M+5
M+6
M+7
M+8
M+9
F
Pier+MT
Anti+MT
GMP % of pool labelled by $^{13}C_6$-Glc
M+0
M+5
M+6
M+7
M+8
M+9

Fig. S5. Inhibition of mitochondrial complex I in the presence of a mito-antioxidant dysregulates one carbon metabolism and purine biosynthesis.

(A) Schematic representation of the glucose-driven one carbon metabolism and purine biosynthesis. Labelled carbons (^{13}C) are indicated in yellow. Metabolism of [U-$^{13}C_6$]glucose generates M+3 pools of 3-phosphoserine and serine, followed by M+2 pool of glycine. M+2 glycine, when metabolized with fully labelled M+5 ribose-5-phosphate, can produce M+7 pool of purine precursor, IMP. **(B-F)** Jurkat cells treated with Piericidin and MitoTEMPO (Pier+MT), or Antimycin and MitoTEMPO (Anti+MT) for 24 hours were labelled for 8 hours with [U-$^{13}C_6$]glucose. The percentages of M+3 3-phosphoerine (B), M+3 serine (C), and M+2 glycine (D), labelled by glucose-driven one carbon metabolism were examined. Additionally, the percentages of labelled IMP purine precursor (E) and GMP purine (F) pools were assessed. (n=5, mean+s.e.m.).

Figure S6.

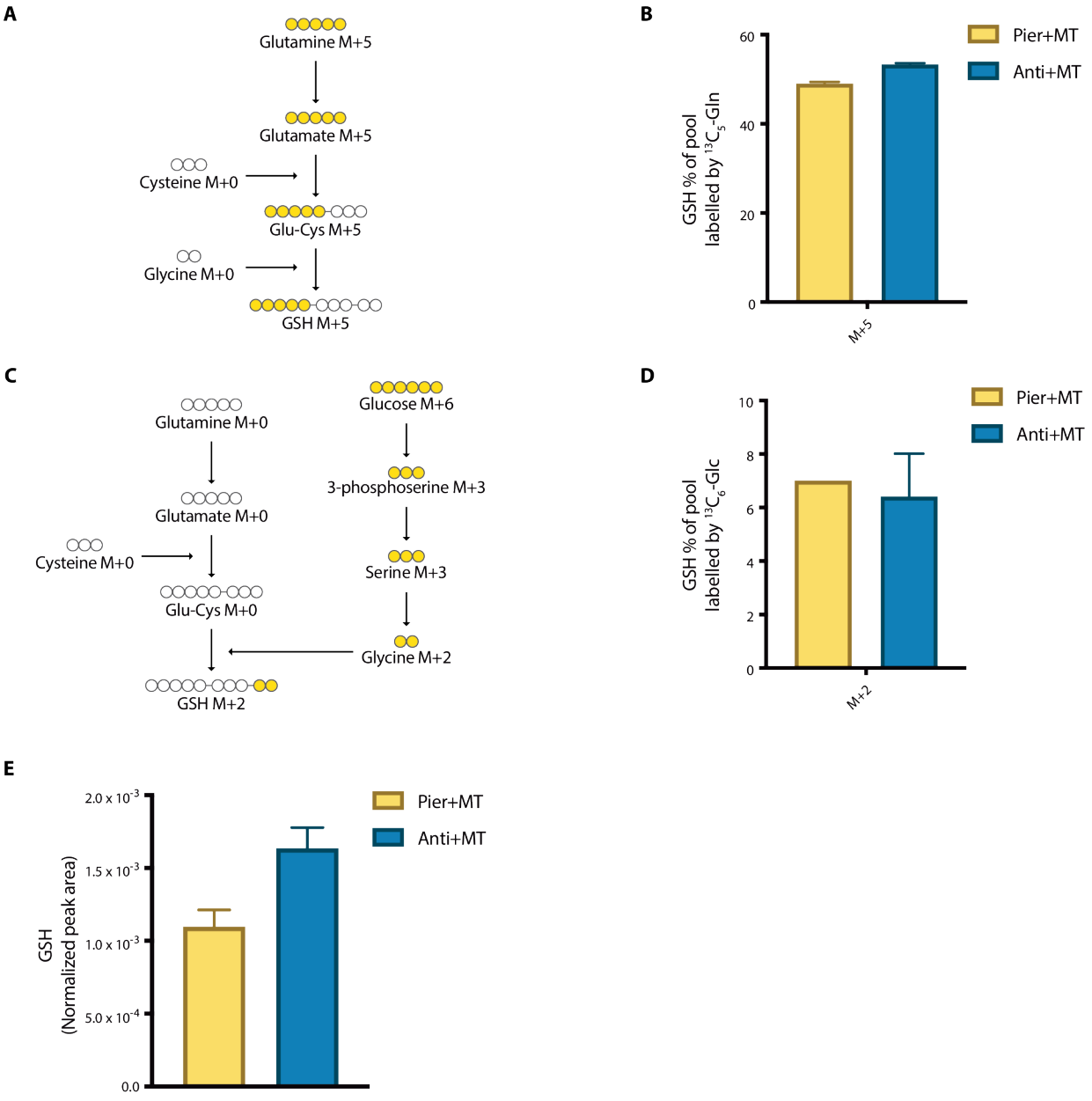
A
Glutamine M+5
Glutamate M+5
Cysteine M+0
Glu-Cys M+5
Glycine M+0
GSH M+5
B
Pier+MT
Anti+MT
GSH % of pool labelled by $^{13}C_5$-Gln
60
40
20
0
M+5
C
Glutamine M+0
Glucose M+6
3-phosphoserine M+3
Glutamate M+0
Cysteine M+0
Serine M+3
Glu-Cys M+0
Glycine M+2
GSH M+2
D
Pier+MT
Anti+MT
GSH % of pool labelled by $^{13}C_6$-Glc
10
8
6
4
2
0
M+2
E
Pier+MT
Anti+MT
GSH (Normalized peak area)
2.0 x 10^{-3}
1.5 x 10^{-3}
1.0 x 10^{-3}
5.0 x 10^{-4}
0.0

Fig. S6. Glutathione (GSH) content and synthesis in cells treated with Piericidin or Antimycin in combination with MitoTEMPO.

(A) Schematic representation of the glutamine-derived GSH synthesis. Labelled carbons (^{13}C) are indicated in yellow. Metabolism of [U-$^{13}C_5$]glutamine results in M+5 pool of GSH. **(B)** Jurkat cells treated with Piericidin and MitoTEMPO (Pier+MT), or Antimycin and MitoTEMPO (Anti+MT) for 24 hours were labelled for 8 hours with [U-$^{13}C_5$]glutamine, and the percentage of M+5 labelled GSH was assessed. (n=5, mean+s.e.m.) **(C)** Schematic representation of the glucose-derived GSH synthesis. Labelled carbons (^{13}C) are indicated in yellow. Metabolism of [U-$^{13}C_6$]glucose results in M+2 pool of GSH. **(D)** Jurkat cells treated with Pier+MT or Anti+MT for 24 hours were labelled for 8 hours with [U-$^{13}C_6$]glucose, and the percentage of M+2 labelled GSH was assessed. (n=5, mean+s.e.m.) **(E)** GSH levels in Jurkat cells treated with Pier+MT or Anti+MT for 24 hours. (n=6, mean+s.e.m.)

Figure S7.

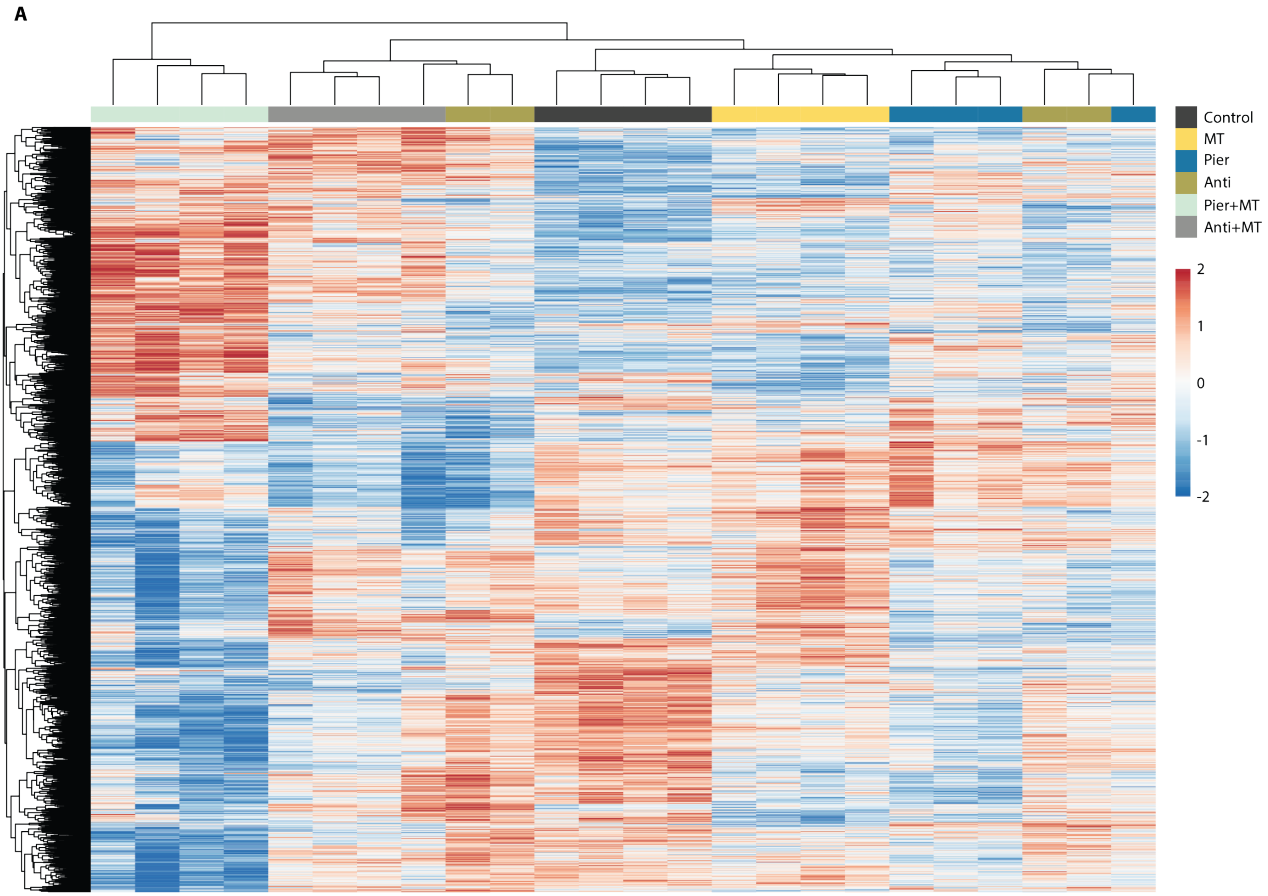

Fig. S7. Combination of mitochondrial complex I inhibition and a mito-antioxidant induces a unique transcriptomic signature.

(A) Heatmap of unsupervised clustering, representing RNA transcripts whose abundances were significantly different among Jurkat cells treated with vehicle (Control), MitoTEMPO (MT), Piericidin (Pier), Antimycin (Anti), Piericidin and MitoTEMPO (Pier+MT), or Antimycin and MitoTEMPO (Anti+MT) for 24 hours. The relative abundance of each transcript is depicted as z-score across rows. (Red: high; Blue: low) (n=4, FDR≤0.05)

Figure S8.

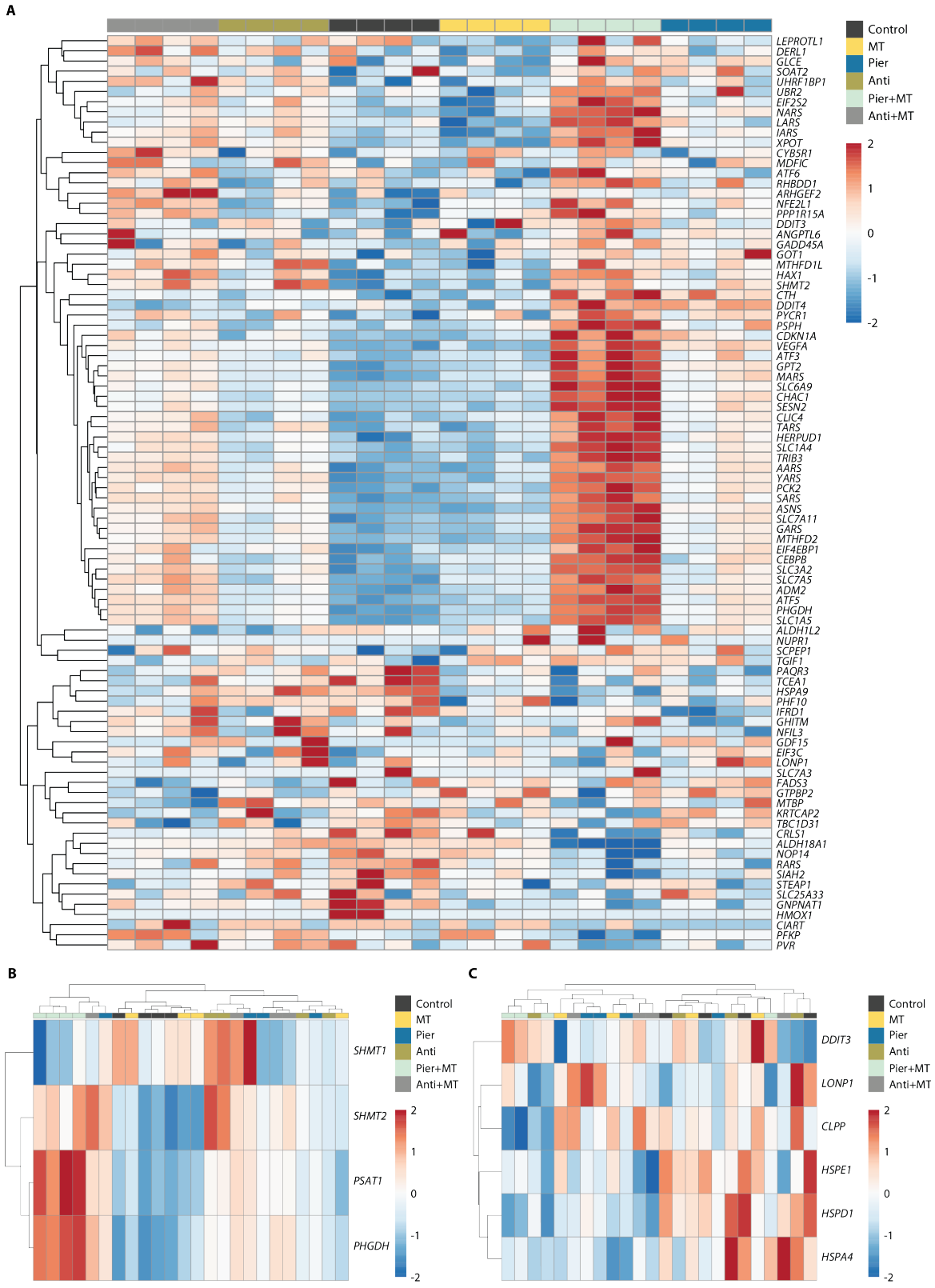
A
LEPROTL1
DERL1
GLCE
SOAT2
UHRF1BP1
UBR2
EIF2S2
NARS
LARS
IARS
XPOT
CYB5R1
MDFIC
ATF6
RHBDD1
ARHGEF2
NFE2L1
PPP1R15A
DDIT3
ANGPTL6
GADD45A
GOT1
MTHFD1L
HAX1
SHMT2
CTH
DDIT4
PYCR1
PSPH
CDKN1A
VEGFA
ATF3
GPT2
MARS
SLC6A9
CHAC1
SESN2
CLIC4
TARS
HERPUD1
SLC1A4
TRIB3
AARS
YARS
PCK2
SARS
ASNS
SLC7A11
GARS
MTHFD2
EIF4EBP1
CEBPB
SLC3A2
SLC7A5
ADM2
ATF5
PHGDH
SLC1A5
ALDH1L2
NUPR1
SCPEP1
TGIF1
PAQR3
TCEA1
HSPA9
PHF10
IFRD1
GHITM
NFIL3
GDF15
EIF3C
LONP1
SLC7A3
FADS3
GTPBP2
MTBP
KRTCAP2
TBC1D31
CRLS1
ALDH18A1
NOP14
RARS
SIAH2
STEAP1
SLC25A33
GNPNAT1
HMOX1
CIART
PFKP
PVR
Control
MT
Pier
Anti
Pier+MT
Anti+MT
2
1
0
-1
-2
B
SHMT1
SHMT2
PSAT1
PHGDH
C
DDIT3
LONP1
CLPP
HSPE1
HSPD1
HSPA4

Fig. S8. ISR-associated genes are largely upregulated in Jurkat cells treated with Piericidin and MitoTEMPO.

(A) Heatmap of all ISR signature genes in Jurkat cells treated with vehicle (Control), MitoTempo (MT), Piericidin (Pier), Antimycin (Anti), Piericidin and MitoTEMPO (Pier+MT), or Antimycin and MitoTEMPO (Anti+MT) for 24 hours. The relative abundance of each transcript is depicted as z-score across rows. (Red: high; Blue: low) (n=4) **(B-C)** Heatmap of unsupervised clustering, representing one carbon metabolism (B) or canonical mitochondrial UPR (UPR^{mt}) (C) associated genes in Jurkat cells treated with vehicle (Control), MT, Pier, Anti, Pier+MT, or Anti+MT for 24 hours. The relative abundance of each transcript is depicted as z-score across rows. (Red: high; Blue: low) (n=4)

Figure S9.

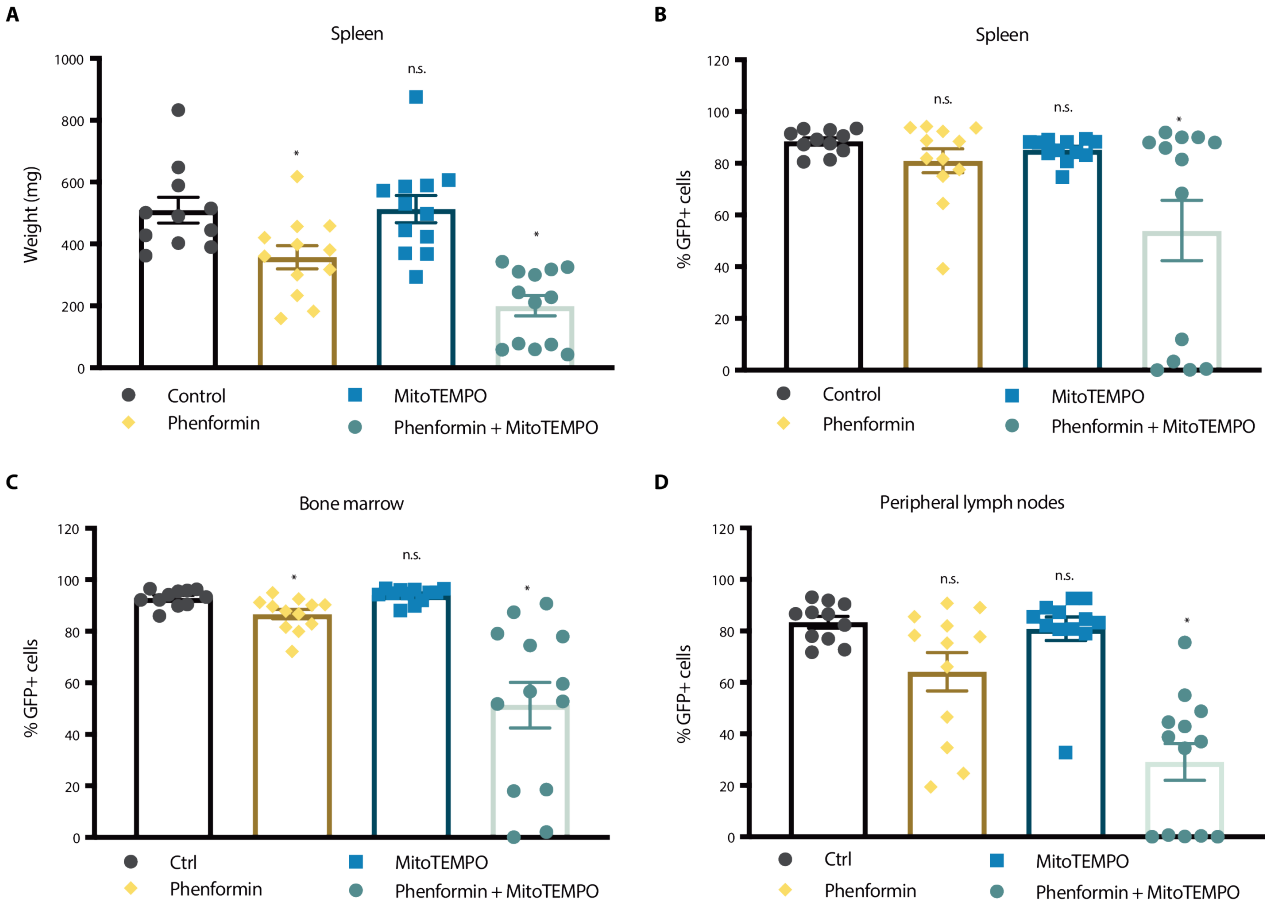
A
Spleen
Weight (mg)
1000
800
600
400
200
0
n.s.
*
*
Control
Phenformin
MitoTEMPO
Phenformin + MitoTEMPO
B
Spleen
% GFP+ cells
120
100
80
60
40
20
0
n.s.
n.s.
*
Control
Phenformin
MitoTEMPO
Phenformin + MitoTEMPO
C
Bone marrow
% GFP+ cells
120
100
80
60
40
20
0
*
n.s.
*
Ctrl
Phenformin
MitoTEMPO
Phenformin + MitoTEMPO
D
Peripheral lymph nodes
% GFP+ cells
120
100
80
60
40
20
0
n.s.
n.s.
*
Ctrl
Phenformin
MitoTEMPO
Phenformin + MitoTEMPO

Fig. S9. Combination of mitochondrial complex I inhibition and a mito-antioxidant diminishes percentage of leukemic cells *in vivo*.

(A) Weight of spleens from T-ALL recipients treated with vehicle (Control), Phenformin, MitoTEMPO, or Phenformin+MitoTempo for 15 days. (Control: n=11; Phenformin: n=12; MitoTEMPO: n=12; Phenformin+MitoTEMPO: n=13., mean±s.e.m., *P=0.0307 (Phenformin) or *P<0.0001 (Phenformin+MitoTEMPO) compared to control, $^{n.s.}$P value is reported in the material and methods) **(B, C, D)** Percentage of GFP+ T-ALL cells in the spleen (B), bone marrow (C), or peripheral lymph nodes (D) from T-ALL recipients treated with vehicle (Control), Phenformin, MitoTEMPO, or Phenformin+MitoTempo for 15 days. (Control: n=11; Phenformin: n=12; MitoTEMPO: n=12; Phenformin+MitoTEMPO: n=13., mean±s.e.m., *P=0.0195 (B); *P=0.0382 (C; Phenformin); *P<0.0001 (C; Phenformin+MitoTEMPO); *P<0.0001 (D) compared to control, $^{n.s.}$P values are reported in the material and methods)

Table S1. Human integrated stress response (ISR) associated genes

group	gene_id	symbol
gene.clic	ENSG00000090861	AARS
gene.clic	ENSG00000128165	ADM2
gene.clic	ENSG00000059573	ALDH18A1
gene.clic	ENSG00000136010	ALDH1L2
gene.clic	ENSG00000130812	ANGPTL6
gene.clic	ENSG00000116584	ARHGEF2
gene.clic	ENSG00000070669	ASNS
gene.clic	ENSG00000162772	ATF3
gene.clic	ENSG00000169136	ATF5
gene.clic	ENSG00000118217	ATF6
gene.clic	ENSG00000124762	CDKN1A
gene.clic	ENSG00000172216	CEBPB
gene.clic	ENSG00000128965	CHAC1
gene.clic	ENSG00000159208	CIART
gene.clic	ENSG00000169504	CLIC4
gene.clic	ENSG00000182372	CLN8
gene.clic	ENSG00000088766	CRLS1

gene.clic	**ENSG00000116761**	**CTH**
gene.clic	**ENSG00000159348**	**CYB5R1**
gene.clic	**ENSG00000175197**	**DDIT3**
gene.clic	**ENSG00000168209**	**DDIT4**
gene.clic	**ENSG00000136986**	**DERL1**
gene.clic	**ENSG00000125977**	**EIF2S2**
gene.clic	**ENSG00000184110**	**EIF3C**
gene.clic	**ENSG00000187840**	**EIF4EBP1**
gene.clic	**ENSG00000221968**	**FADS3**
gene.clic	**ENSG00000135842**	**FAM129A**
gene.clic	**ENSG00000116717**	**GADD45A**
gene.clic	**ENSG00000106105**	**GARS**
gene.clic	**ENSG00000130513**	**GDF15**
gene.clic	**ENSG00000165678**	**GHITM**
gene.clic	**ENSG00000138604**	**GLCE**
gene.clic	**ENSG00000100522**	**GNPNAT1**
gene.clic	**ENSG00000120053**	**GOT1**
gene.clic	**ENSG00000166123**	**GPT2**

gene.clic	**ENSG00000172432**	**GTPBP2**
gene.clic	**ENSG00000143575**	**HAX1**
gene.clic	**ENSG00000051108**	**HERPUD1**
gene.clic	**ENSG00000100292**	**HMOX1**
gene.clic	**ENSG00000113013**	**HSPA9**
gene.clic	**ENSG00000196305**	**IARS**
gene.clic	**ENSG00000006652**	**IFRD1**
gene.clic	**ENSG00000163463**	**KRTCAP2**
gene.clic	**ENSG00000133706**	**LARS**
gene.clic	**ENSG00000104660**	**LEPROTL1**
gene.clic	**ENSG00000196365**	**LONP1**
gene.clic	**ENSG00000166986**	**MARS**
gene.clic	**ENSG00000135272**	**MDFIC**
gene.clic	**ENSG00000172167**	**MTBP**
gene.clic	**ENSG00000120254**	**MTHFD1L**
gene.clic	**ENSG00000065911**	**MTHFD2**
gene.clic	**ENSG00000134440**	**NARS**
gene.clic	**ENSG00000082641**	**NFE2L1**

gene.clic	**ENSG00000165030**	**NFIL3**
gene.clic	**ENSG00000087269**	**NOP14**
gene.clic	**ENSG00000176046**	**NUPR1**
gene.clic	**ENSG00000089723**	**OTUB2**
gene.clic	**ENSG00000163291**	**PAQR3**
gene.clic	**ENSG00000100889**	**PCK2**
gene.clic	**ENSG00000067057**	**PFKP**
gene.clic	**ENSG00000130024**	**PHF10**
gene.clic	**ENSG00000092621**	**PHGDH**
gene.clic	**ENSG00000087074**	**PPP1R15A**
gene.clic	**ENSG00000146733**	**PSPH**
gene.clic	**ENSG00000073008**	**PVR**
gene.clic	**ENSG00000183010**	**PYCR1**
gene.clic	**ENSG00000113643**	**RARS**
gene.clic	**ENSG00000144468**	**RHBDD1**
gene.clic	**ENSG00000031698**	**SARS**
gene.clic	**ENSG00000121064**	**SCPEP1**
gene.clic	**ENSG00000130766**	**SESN2**

gene.clic	**ENSG00000182199**	**SHMT2**
gene.clic	**ENSG00000181788**	**SIAH2**
gene.clic	**ENSG00000115902**	**SLC1A4**
gene.clic	**ENSG00000105281**	**SLC1A5**
gene.clic	**ENSG00000171612**	**SLC25A33**
gene.clic	**ENSG00000168003**	**SLC3A2**
gene.clic	**ENSG00000196517**	**SLC6A9**
gene.clic	**ENSG00000151012**	**SLC7A11**
gene.clic	**ENSG00000165349**	**SLC7A3**
gene.clic	**ENSG00000103257**	**SLC7A5**
gene.clic	**ENSG00000167780**	**SOAT2**
gene.clic	**ENSG00000164647**	**STEAP1**
gene.clic	**ENSG00000113407**	**TARS**
gene.clic	**ENSG00000156787**	**TBC1D31**
gene.clic	**ENSG00000187735**	**TCEA1**
gene.clic	**ENSG00000177426**	**TGIF1**
gene.clic	**ENSG00000101255**	**TRIB3**
gene.clic	**ENSG00000024048**	**UBR2**

gene.clic	**ENSG00000065060**	**UHRF1BP1**
gene.clic	**ENSG00000112715**	**VEGFA**
gene.clic	**ENSG00000184575**	**XPOT**
gene.clic	**ENSG00000134684**	**YARS**