Figure S4

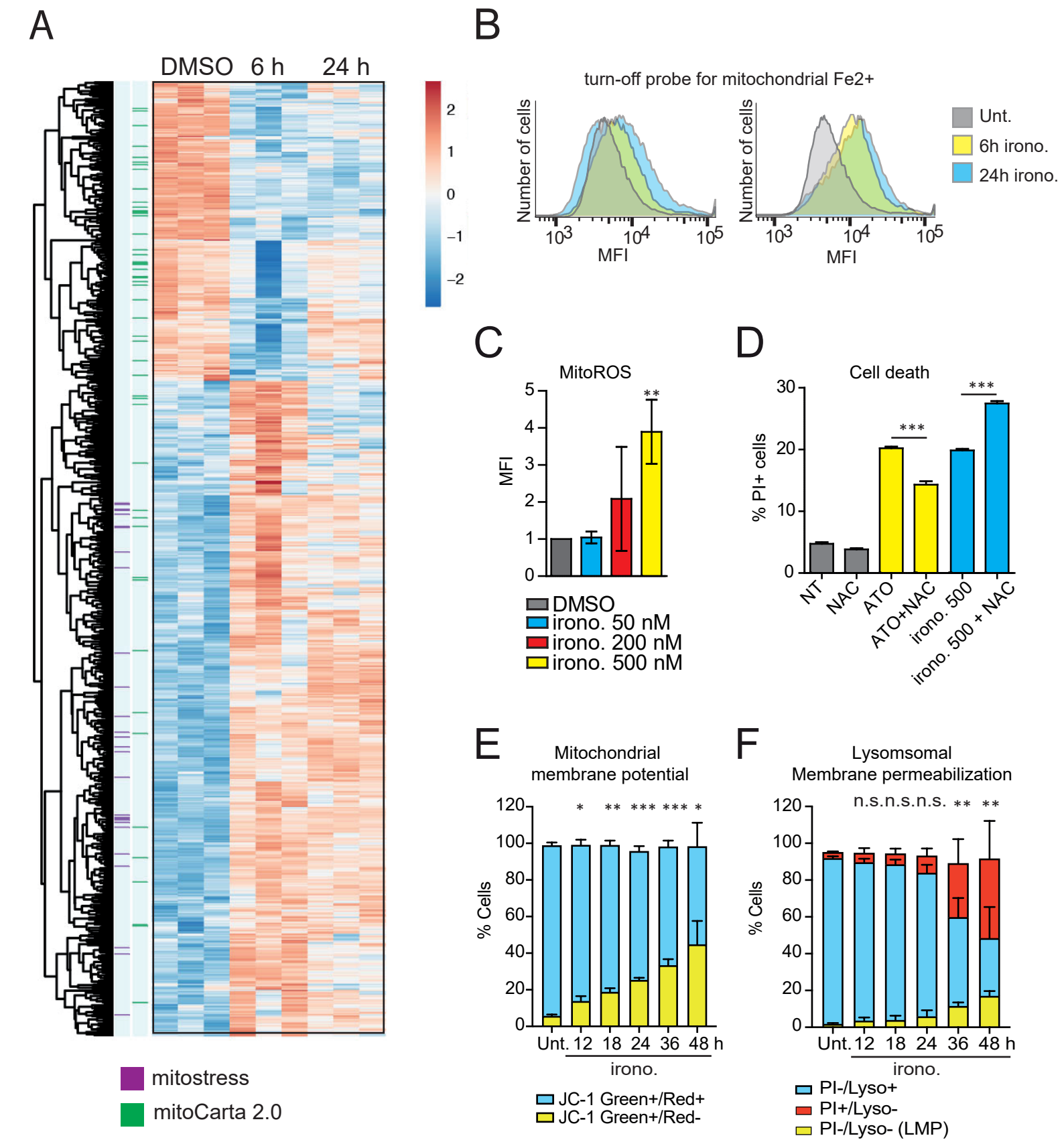
A
DMSO
6 h
24 h
2
1
0
−1
−2
mitostress
mitoCarta 2.0
B
turn-off probe for mitochondrial Fe2+
Number of cells
MFI
Unt.
6h irono.
24h irono.
C
MitoROS
MFI
DMSO
irono. 50 nM
irono. 200 nM
irono. 500 nM
D
Cell death
% PI+ cells
NT
NAC
ATO
ATO+NAC
irono. 500
irono. 500 + NAC
E
Mitochondrial
membrane potential
% Cells
Unt. 12 18 24 36 48 h
irono.
JC-1 Green+/Red+
JC-1 Green+/Red-
F
Lysomsomal
Membrane permeabilization
n.s.n.s.n.s.
% Cells
Unt. 12 18 24 36 48 h
irono.
PI-/Lyso+
PI+/Lyso-
PI-/Lyso- (LMP)

Figure S4| (related to Figure 4). Ironomycin induces mitochondrial stress through iron deprivation. A, Heatmap showing the differentially expressed genes in MV4;11 cells after 6 and 24 hours of treatment. MV4;11 cells were treated with either 500 nM ironomycin or vehicle (DMSO) followed by mRNA extraction (n=3 biological replicates). Genes encoding mitochondrial proteins are highlighted in green ("mitoCarta2"). Most of these genes are downregulated. Genes related to mitochondrial stress response are highlighted in purple ("mitostress"). All these genes are upregulated **B**, FACS plot showing an example of mitochondrial iron quantification using a mitochondrial specific turn-off probe in MOLM-13 (58). An increase in median fluorescence intensity (MFI) correlates with a decrease in mitochondrial iron. **C**, Measurement of mitochondrial ROS by FACS using Mitosox Red fluorescence dye in MV4;11 cell line. Cells were treated with ironomycin (500 nM) or DMSO for 24 h (n=3 biological replicates, means ± SD $^{**}p < 0.01$). **D**, Effect on cell viability of the ROS scavenger N-acetyl cysteine (NAC). MV4;11 cell line was pretreated with 10 mM NAC for 12 hours and treated with 500 nM ironomycin or 3 µM ATO for 24 hours. Cell death was assessed by FACS analysis (PI). Data are median fluorescence intensity (MFI) normalized on DMSO conditions (n=3 biological replicates, means ± SD, $^{***}p < 0.001$). **E**, Analysis of mitochondrial membrane potential (Δ Ψm) using JC-1 staining assessed by FACS after 24 hours of 500 nM ironomycin. Loss of JC-1 staining is associated with a loss of Δ Ψm. Data show mean ± SEM of 4 independent experiments (n=4 biological replicates, means ± SEM $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). **F**, FACS analysis of lysosomal membrane permeabilization (LMP) using lysotracker (Lyso) in MOLM-13 cell line. We treated cells with DMSO or 500 nM ironomycin at indicated times. LMP is associated with a loss of lysotracker staining. We show the mean ± SEM of four independent experiments (n=4 biological replicates, means ± SEM, $^{**}p < 0.01$).