Figure S1

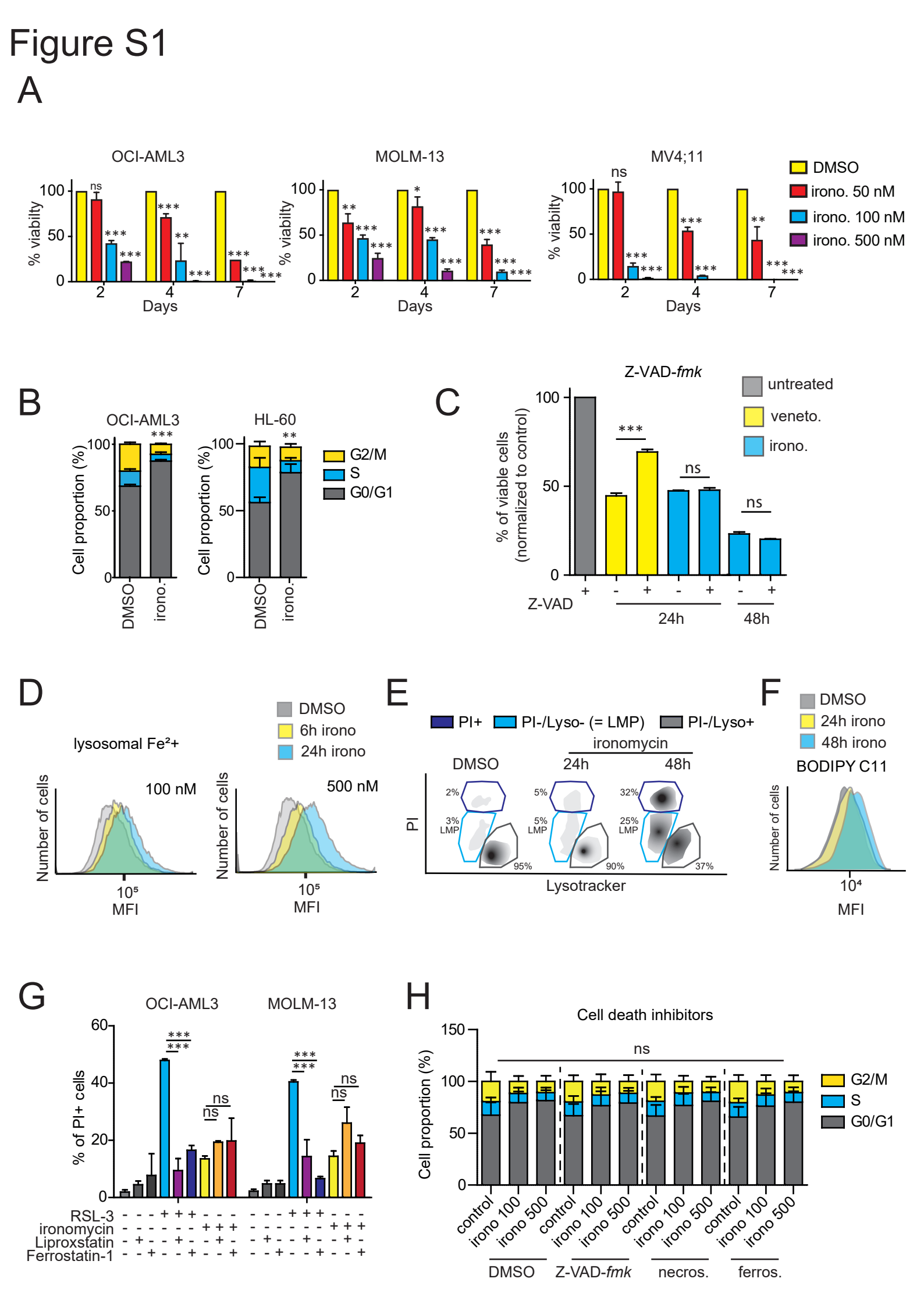
A
OCI-AML3
MOLM-13
MV4;11
% viabilty
Days
DMSO
irono. 50 nM
irono. 100 nM
irono. 500 nM
B
OCI-AML3
HL-60
Cell proportion (%)
DMSO
irono.
G2/M
S
G0/G1
C
Z-VAD-fmk
% of viable cells (normalized to control)
untreated
veneto.
irono.
Z-VAD
24h
48h
D
lysosomal Fe²+
DMSO
6h irono
24h irono
100 nM
500 nM
Number of cells
MFI
E
PI+
PI-/Lyso- (= LMP)
PI-/Lyso+
ironomycin
DMSO
24h
48h
PI
Lysotracker
2%
3% LMP
95%
5%
5% LMP
90%
32%
25% LMP
37%
F
DMSO
24h irono
48h irono
BODIPY C11
Number of cells
MFI
G
OCI-AML3
MOLM-13
% of PI+ cells
RSL-3
ironomycin
Liproxstatin
Ferrostatin-1
H
Cell death inhibitors
Cell proportion (%)
G2/M
S
G0/G1
control
irono 100
irono 500
DMSO
Z-VAD-fmk
necros.
ferros.

Figure S1| (related to Figure 1). Ironomycin induces potent cell death in acute myeloid leukaemia through a non-classical cell death pathway. A, Cell viability assessed by FACS (PI) in OCI-AML3, MOLM-13 and MV4;11 cell lines treated with ironomycin (n=3 biological replicates, means ± SD; *p < 0.05, **p < 0.01, ***p < 0.001). **B**, Cell cycle analysis using DAPI on OCI-AML3 and HL60 cell lines after 24 hours of DMSO or 500 nM (n=3 biological replicates, means ± SD; **p < 0.01, ***p < 0.001). **C**, Effect on cell death of the pan-caspase inhibitor Z-VAD-*fmk*. MV4;11 cells were pretreated with 50 μM Z-VAD-*fmk* for 30 minutes and treated with 500 nM ironomycin or 50 nM venetoclax. Cell death was assessed by FACS (PI, n=3 biological replicates, means ± SD, *** indicates p<.0005). **D**, FACS plots showing lysosomal Fe^{2+} using a lysosomal FACS turn-on probe (60) upon ironomycin treatment in MOLM-13 cells. Fe2+ specifically reduces Rhonox-M to a Rhodamine B derivative, which fluoresces. One representative experiment is shown. **E**, FACS plot showing lysosomal membrane permeabilization (LMP) using lysotracker (Lyso) in MOLM-13 cell line. We treated cells with DMSO or 500 nM ironomycin. LMP is associated with a loss of lysotracker staining. We show one single representative experiment **F**, FACS plots showing lipid peroxidation using BODIPY C11 staining at 24 and 48 hours of 500 nM ironomycin treatment and DMSO control (48 h) in MOLM-13 cells. One representative experiment is shown. **G**, Effect on cell viability of the ferroptosis inhibitors ferrostatin-1 or liproxstatin. MOLM-13 cell line was pretreated with 20 μM ferrostatin-1 or 10 μM liproxstatin for 30 minutes and treated with 500 nM ironomycin or 30 nM RSL-3 for the indicated times. Cell death (PI positive cells) was assessed by FACS staining (n=3 biological replicates, means ± SD, ***p < 0.001). **H**, Cell cycle analysis using DAPI staining by FACS in AML cell lines without treatment or after 24 hours of 500 nM ironomycin. Cells were pretreated with 50 μM Z-VAD-*fmk*, 10 μM necrostatin-1 or 20 μM ferrostatin-1 for 30 minutes before ironomycin treatment (n=3 biological replicates in OCI-AML3, HL-60 and MOLM-13 cell lines).