Figure S5

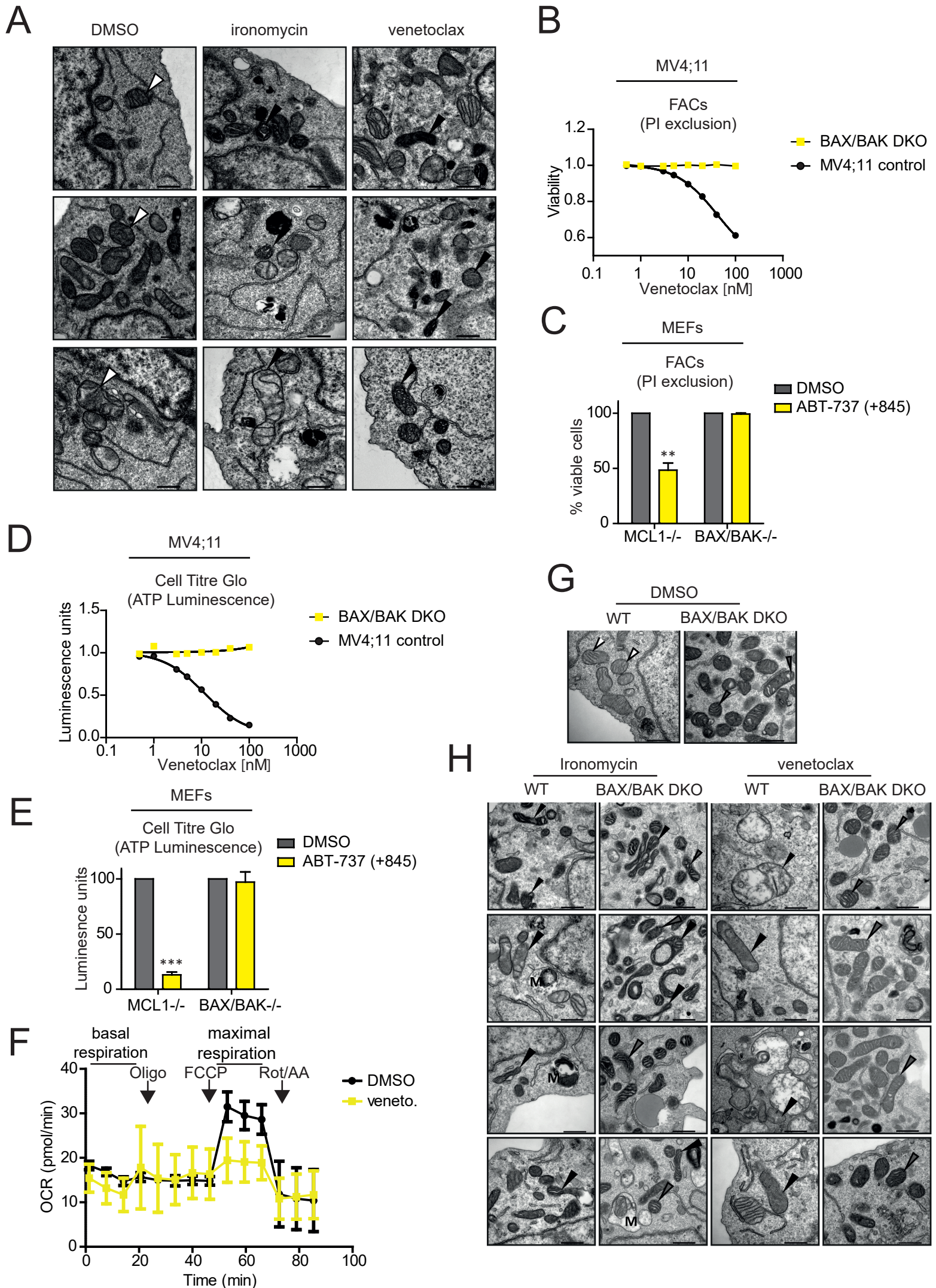
A
DMSO
ironomycin
venetoclax
B
MV4;11
FACs
(PI exclusion)
Viability
1.2
1.0
0.8
0.6
0.1
1
10
100
1000
Venetoclax [nM]
BAX/BAK DKO
MV4;11 control
C
MEFs
FACs
(PI exclusion)
% viable cells
100
50
0
**
MCL1-/-
BAX/BAK-/-
DMSO
ABT-737 (+845)
D
MV4;11
Cell Titre Glo
(ATP Luminescence)
Luminescence units
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Venetoclax [nM]
BAX/BAK DKO
MV4;11 control
E
MEFs
Cell Titre Glo
(ATP Luminescence)
Luminesnce units
100
50
0
***
MCL1-/-
BAX/BAK-/-
DMSO
ABT-737 (+845)
F
basal
respiration
maximal
respiration
Oligo
FCCP
Rot/AA
OCR (pmol/min)
40
30
20
10
0
0
20
40
60
80
100
Time (min)
DMSO
veneto.
G
DMSO
WT
BAX/BAK DKO
H
Ironomycin
venetoclax
WT
BAX/BAK DKO
WT
BAX/BAK DKO
M
M
M

Figure S5| (related to Figure 5). Ironomycin induced mitochondrial dysfunction is BAX/BAK independent but cell death is BAX/BAK dependent. A, Transmission electron microscopy images of MV4;11 cells treated with or without 500 nM ironomycin or 50 nM venetoclax for 36 hours. White arrowheads – standard mitochondrial morphology; black arrowheads – changed mitochondrial morphology (cristae reduction and dilation, fragmentation, or dark condensed matrix). Scale bars, 20 μm top panel, 500 nm bottom panel. **B,** Cell death assessed by PI exclusion using FACS in MV4,11 WT and BAX/BAK DKO cell lines after 48 hours of venetoclax (n=3 biological replicates). **C,** Cell death assessed by PI exclusion using FACS in MEF cells treated for 48 hours with 1 μM ABT-737 + 20 μM S63845 (MCL-1 inhibitor, only for *Bax*$^{-/-}$ *Bak*$^{-/-}$ MEF cell line). We compared a *Bax*$^{-/-}$ *Bak*$^{-/-}$ with a control *Mcl-1*$^{-/-}$ MEF cell line (n=3 biological replicates, means ± SEM, *$p < 0.05$). **D**, ATP luminescence measured by Cell Titer-Glo® in MV4,11 WT and BAX/BAK DKO cell lines after 48 hours of venetoclax (n=3 biological replicates). **E,** ATP luminescence measured by Cell Titer-Glo® in MEF cells treated for 48 hours with with 1 μM ABT-737 + 20 μM S63845 (MCL-1 inhibitor, only for *Bax*$^{-/-}$ *Bak*$^{-/-}$ MEF cell line). We compared a *Bax*$^{-/-}$ *Bak*$^{-/-}$ with a control *Mcl-1*$^{-/-}$ MEF cell line, (n=3 biological replicates, means ± SEM, ***$p < 0.001$). **F**, Seahorse assay measuring mitochondrial basal and maximal respiration in WT MV4;11 cells. We treated the cells for 6 hours with 50 nM venetoclax (n=3 biological replicates). **G-H**, Transmission electron microscopy images of mitochondria in MV4;11 WT and BAX/BAK DKO cells treated with DMSO (**G**), 500 nM ironomycin or 50nM venetoclax for 16 hours (**H**). M – Mitophagosome; White arrowheads – standard mitochondrial morphology; grey arrowheads –changed morphology of Bax/Bak DKO cells (dark matrix with dilated cristae); black arrowheads – changed mitochondrial morphology (cristae reduction and dilation, condensed matrix, unusual shapes, circular mitochondria, or electron-lucent areas). Scale bars, 500 nm.