SUPPLEMENTARY FIGURE LEGENDS

Fig. S1 Chelerythrine remains effective in inducing loss of $\Delta\Psi_m$ in the absence of Bax and Bak. (A) Data from Fig. 2A (chelerythrine) and 2B (etoposide) were plotted as the ratio of red (FL2) versus green (FL1) fluorescence. The mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. (B) WT (*left panel*) and DKO (*right panel*) MEFs were treated with indicated concentrations of etoposide. After 16 h, cells were harvested, stained with TMRE, and analyzed by flow cytometry. Fluorescence emissions (FL2-Height) of control (thick line), 6.25 μM (dotted line) and 12.5 μM (thin line) etoposide are shown. Data are representative of at least 3 experiments.

Fig. S2 Staurosporine induced Cyt. C release and Bax translocation and integration in WT MEFs. (A) Bax translocation and integration induced by staurosporine coincided with Cyt. C release in WT MEFs. MEFs were treated with 1 μM staurosporine for the indicated durations and harvested for cytosolic and pellet fractions as described in Fig. 3A. For Bax and VDAC integration analysis, mitochondria isolated from staurosporine-treated (1 μM) WT and DKO MEFs for the indicated durations were resuspended in 0.1 M $NaCO_3$ (pH 10.5) and incubated on ice for 20 min followed by sonication for 5 min. Mitochondria were repelleted by centrifugation (100,000 rpm, 20 min) and immunobloted for the indicated proteins. (B) DKO MEFs were resistant to staurosporine-induced Cyt. C release. DKO MEFs were treated with 1 μM staurosporine for indicated durations and analyzed as described in (A).

Fig. S3. Chelerythrine does not trigger necrosis in MEFs. (A) Caspase inhibitor Q-VD-OPH inhibited ROS elevation triggered by chelerythrine, but not H_2O_2 in DKO MEFs. DKO MEFs were treated with 500 μM H_2O_2 (*left panel*) or 10 μM chelerythrine (*right panel*) in the presence (red line) or absence (green line) of 20 μM Q-VD-OPH for 16 h. Cells were then harvested, washed with PBS and stained with 1 μM CM-H_2DCFDA in PBS at 37°C for 30 min in the dark. Excess, unreacted dye was removed by washing cells with PBS before resuspending them in 1 ml of ice-cold PBS. Fluorescence of stained cells (FL1-Height) was determined on channel FL1 of a flow cytometer. Fluorescence emission of control non-treated cells (black line) is also shown. (B) PARP inhibition resulted in resistance to nitrogen mustard but not chelerythrine-induced cell death in WT MEFs. WT MEFs were treated with indicated concentrations of nitrogen mustard (*left panel*) or chelerythrine (*right panel*) in the presence (*triangles*: 10 μM; *circles*: 50 μM) or absence (*squares*) of PARP inhibitor (DPQ) for 4 h. Cell viability was then determined using WST assay.

Fig. S4. The effect of 3MA on loss of $\Delta\Psi_m$ in chelerythrine-treated DKO MEFs. DKO MEFs were treated with 10 μM chelerythrine or 20 μM of etoposide in the presence (+3MA) or absence (-3MA) of 10 mM 3MA for 48 h, stained with JC-1 (10 μM) and the red and green fluorescence were examined by fluorescence microscopy (*right panel*). Composite images of red fluorescence that indicate J-aggregates in live cells or green fluorescence due to low $\Delta\Psi_m$ are shown. Bright-field microscopy of the cells is shown in the left panel. Data are representative of at least 3 experiments.

Fig. S5. Gossypol fails to exert direct effect on mPTP opening in MEFs. (A) Gossypol was less effective in inducing the caspase-3 activation and the appearance of sub-G1 DNA in the absence of Bax and Bak. WT (*square symbols*) and DKO (*triangle symbols*) MEFs were treated with indicated concentrations of gossypol before they were harvested at 16 h or 48 h for caspase-3 activation (*open symbols broken lines*) or DNA content (*closed symbols solid lines*) assays, respectively. Fold caspase-3 activation *versus* control and percentages of cells with sub-G1 DNA (mean ± s.d., n=3) are shown. (B) Gossypol failed to induce direct Cyt. C release from isolated mitochondria. Mitochondria were isolated from WT and DKO MEFs, and incubated at room temperature with the indicated concentrations of gossypol. Supernatant (S/N) and mitochondrial fractions were then subjected to SDS-PAGE, and immunoblotted with Cyt. C and HSP60 antibodies. (C) Gossypol did not induce swelling of mitochondria isolated from WT and DKO MEFs. Mitochondria from WT (*left panel*) and DKO MEFs (*right panel*) were treated with gossypol (25 μM and 50 μM), and monitored for

mitochondrial swelling (by light scatter). Control non-treated mitochondria are also shown. Lines represent values of one experiment performed at least 3 times.

Fig. S6. Sodium azide- and FCCP-induced cytotoxicity is dependent on Bax and Bak. (A - B) WT (*filled symbols*) and DKO (*empty symbols*) MEFs were treated with indicated concentrations of sodium azide (A) and FCCP (B) before they were harvested at 16 h for WST assays. Percentages of cell death (mean ± s.d., n=3) are shown.

Fig. S1

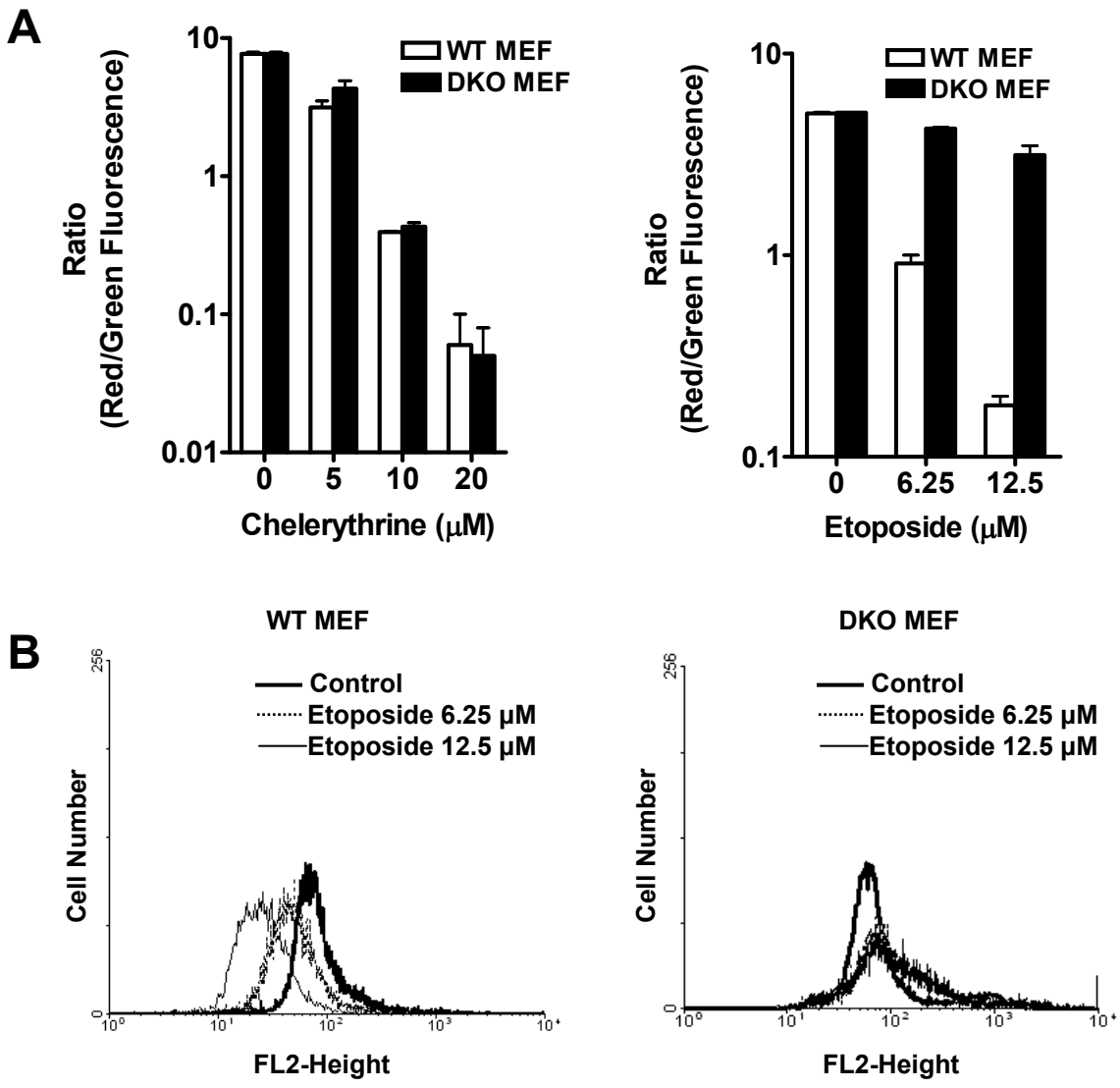
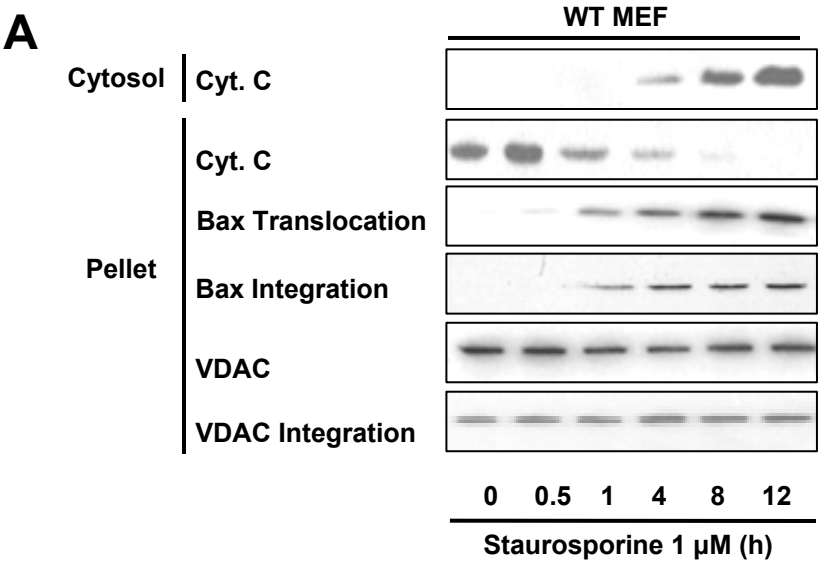

A
Ratio
(Red/Green Fluorescence)
10
1
0.1
0.01
WT MEF
DKO MEF
0
5
10
20
Chelerythrine (μM)
Ratio
(Red/Green Fluorescence)
10
1
0.1
WT MEF
DKO MEF
0
6.25
12.5
Etoposide (μM)
B
WT MEF
Control
Etoposide 6.25 μM
Etoposide 12.5 μM
Cell Number
256
0
FL2-Height
DKO MEF
Control
Etoposide 6.25 μM
Etoposide 12.5 μM
Cell Number
256
0
FL2-Height

Fig. S2


A
WT MEF
Cytosol
Cyt. C
Pellet
Cyt. C
Bax Translocation
Bax Integration
VDAC
VDAC Integration
0 0.5 1 4 8 12
Staurosporine 1 μM (h)

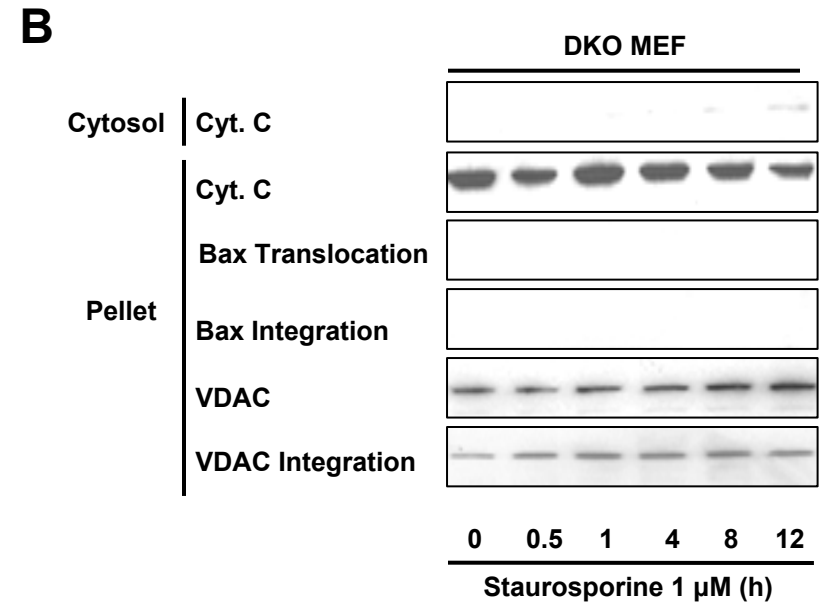

B
DKO MEF
Cytosol
Cyt. C
Pellet
Cyt. C
Bax Translocation
Bax Integration
VDAC
VDAC Integration
0 0.5 1 4 8 12
Staurosporine 1 μM (h)

Fig. S3

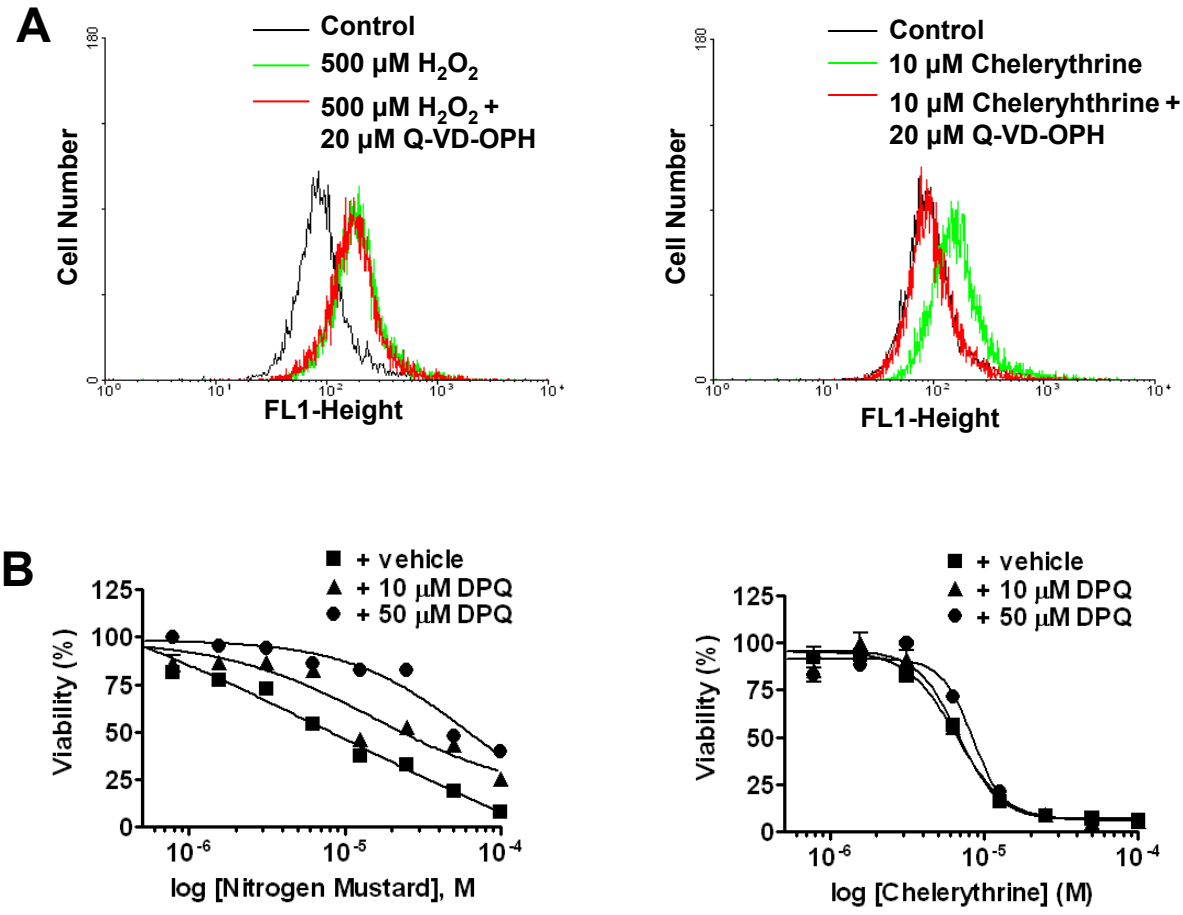

A
Control
500 μM H_2O_2
500 μM H_2O_2 +
20 μM Q-VD-OPH
Cell Number
FL1-Height
Control
10 μM Chelerythrine
10 μM Chelerythrine +
20 μM Q-VD-OPH
Cell Number
FL1-Height
B
+ vehicle
+ 10 μM DPQ
+ 50 μM DPQ
Viability (%)
log [Nitrogen Mustard], M
+ vehicle
+ 10 μM DPQ
+ 50 μM DPQ
Viability (%)
log [Chelerythrine] (M)

Fig. S4

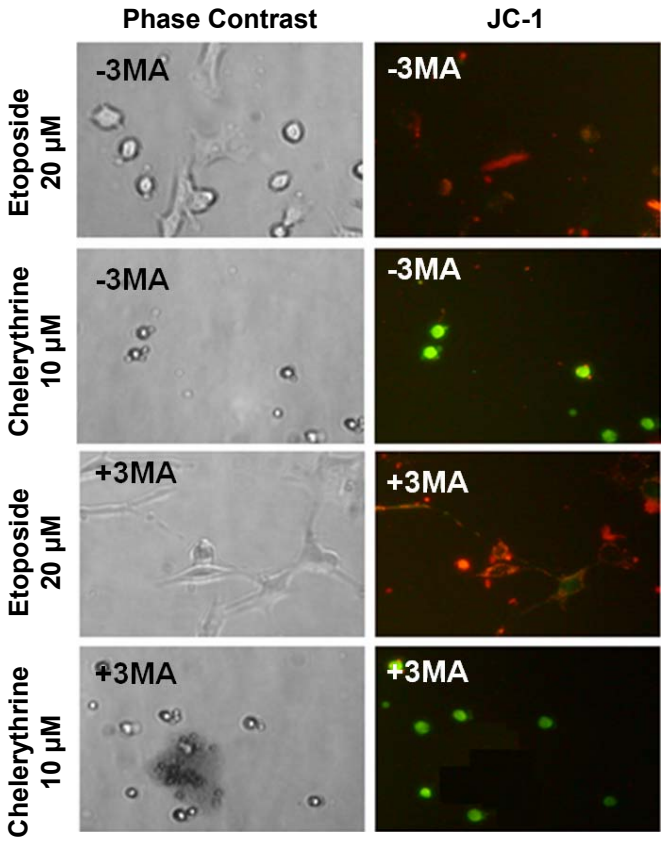

Phase Contrast
JC-1
Etoposide 20 μM
Chelerythrine 10 μM
Etoposide 20 μM
Chelerythrine 10 μM
-3MA
-3MA
-3MA
-3MA
+3MA
+3MA
+3MA
+3MA

Fig. S5

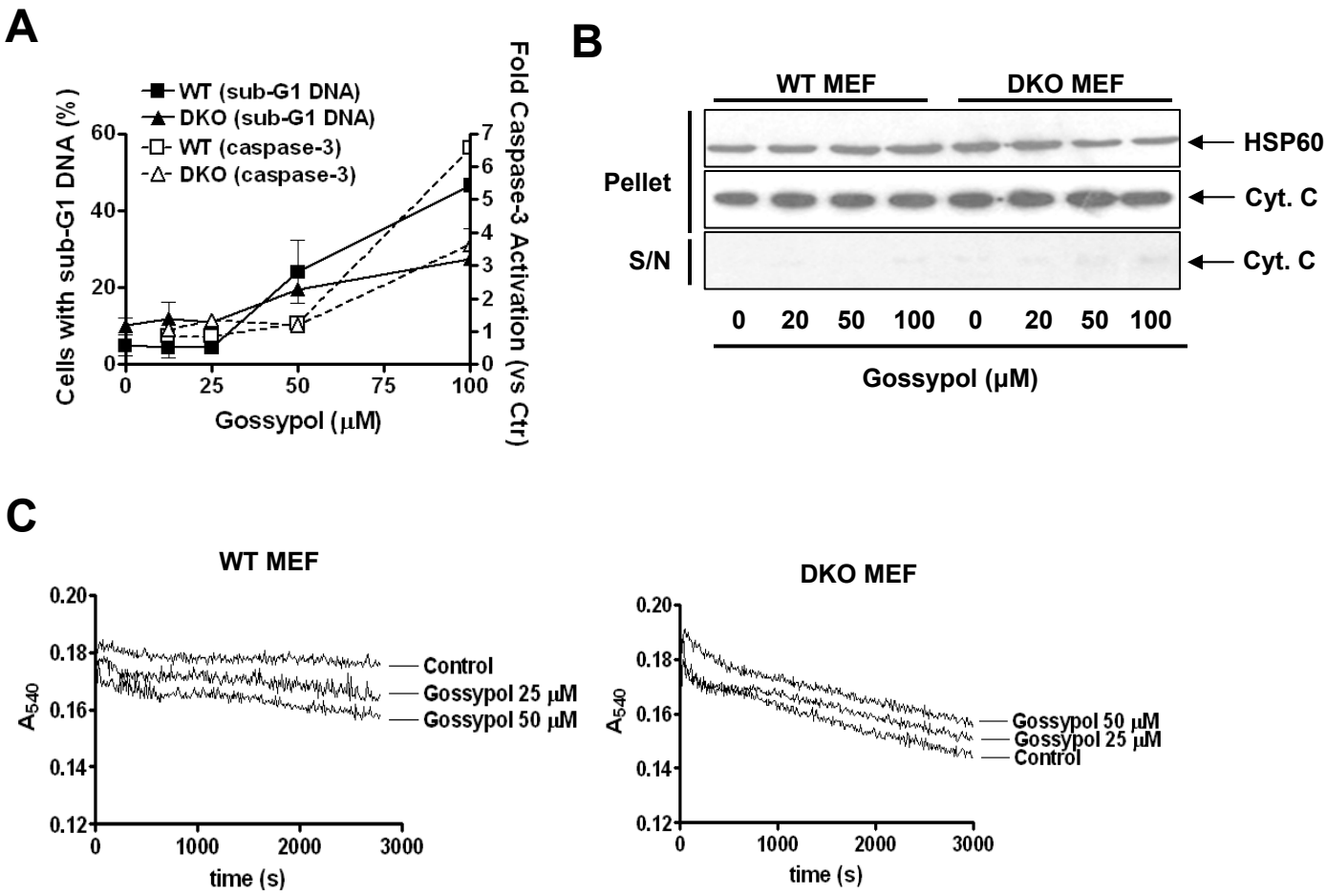
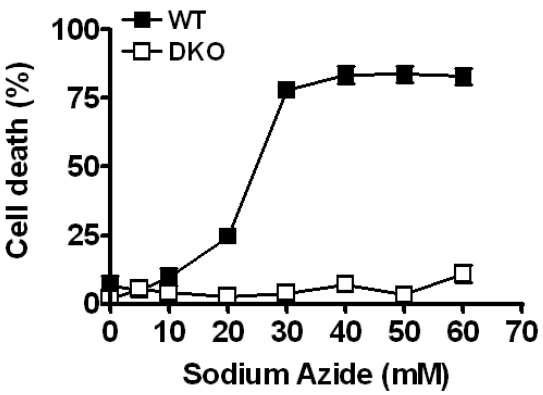
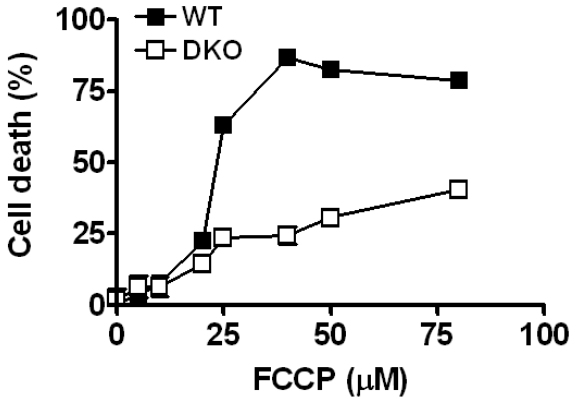

A
WT (sub-G1 DNA)
DKO (sub-G1 DNA)
WT (caspase-3)
DKO (caspase-3)
Cells with sub-G1 DNA (%)
Fold Caspase-3 Activation (vs Ctr)
Gossypol (μM)
B
WT MEF
DKO MEF
Pellet
S/N
HSP60
Cyt. C
Cyt. C
0 20 50 100 0 20 50 100
Gossypol (μM)
C
WT MEF
Control
Gossypol 25 μM
Gossypol 50 μM
A540
time (s)
DKO MEF
Gossypol 50 μM
Gossypol 25 μM
Control
A540
time (s)

Fig. S6

A


WT
DKO
Cell death (%)
100
75
50
25
0
0 10 20 30 40 50 60 70
Sodium Azide (mM)

B


WT
DKO
Cell death (%)
100
75
50
25
0
0 25 50 75 100
FCCP (μM)