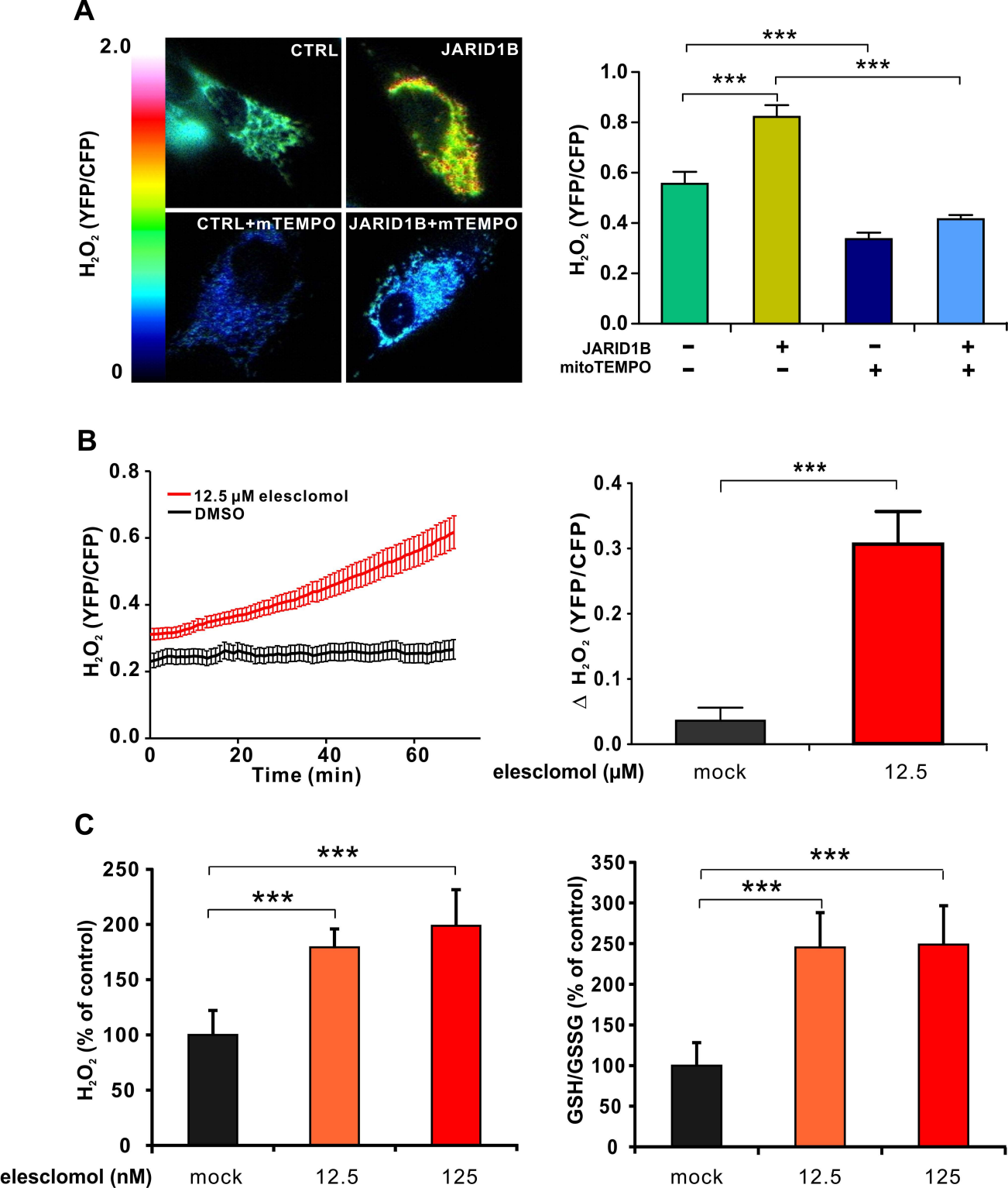
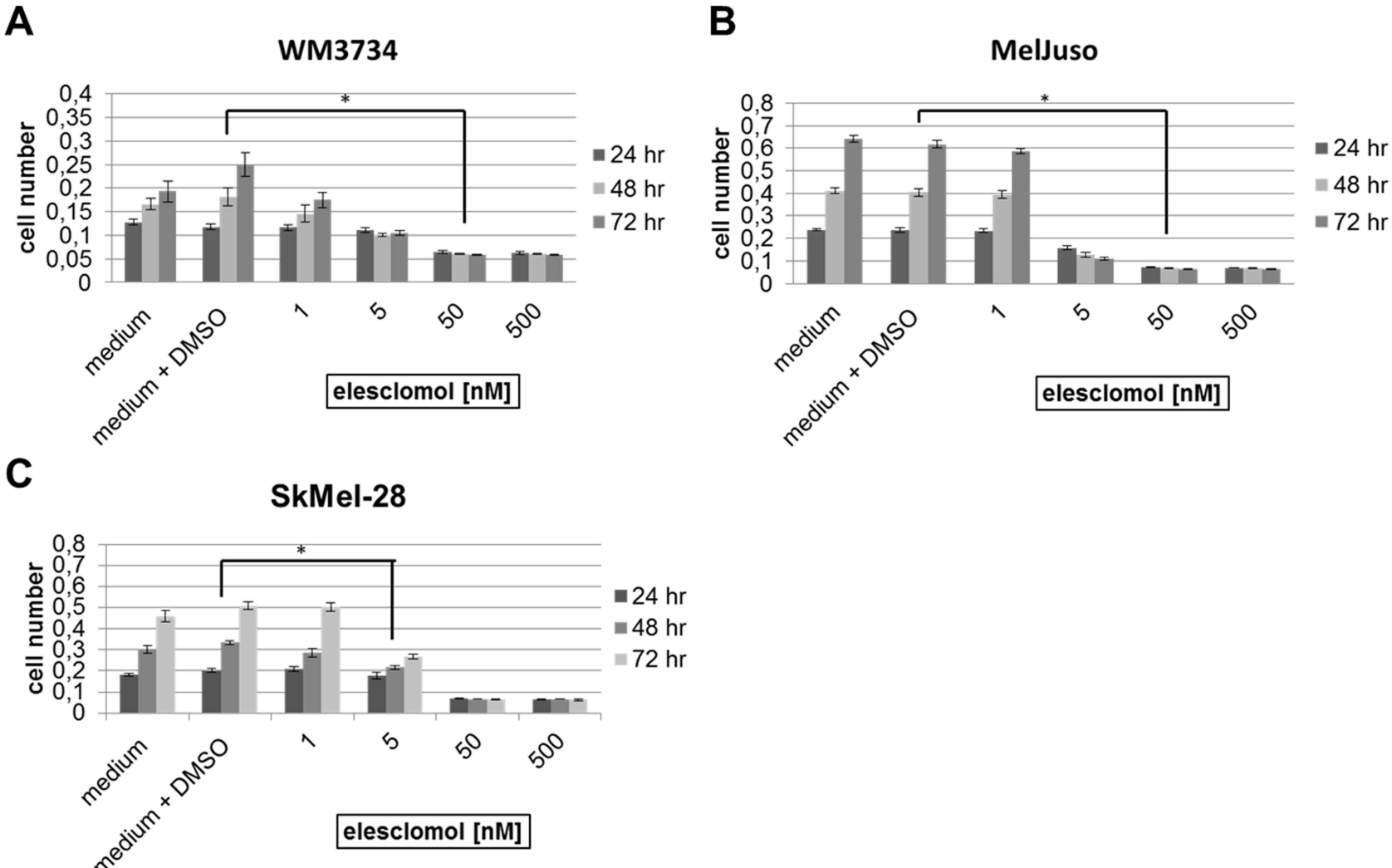
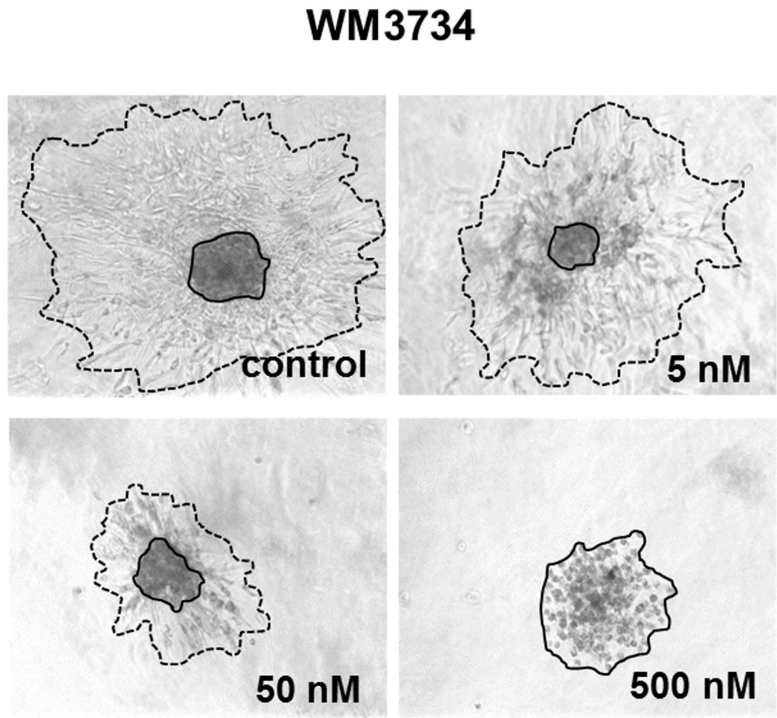
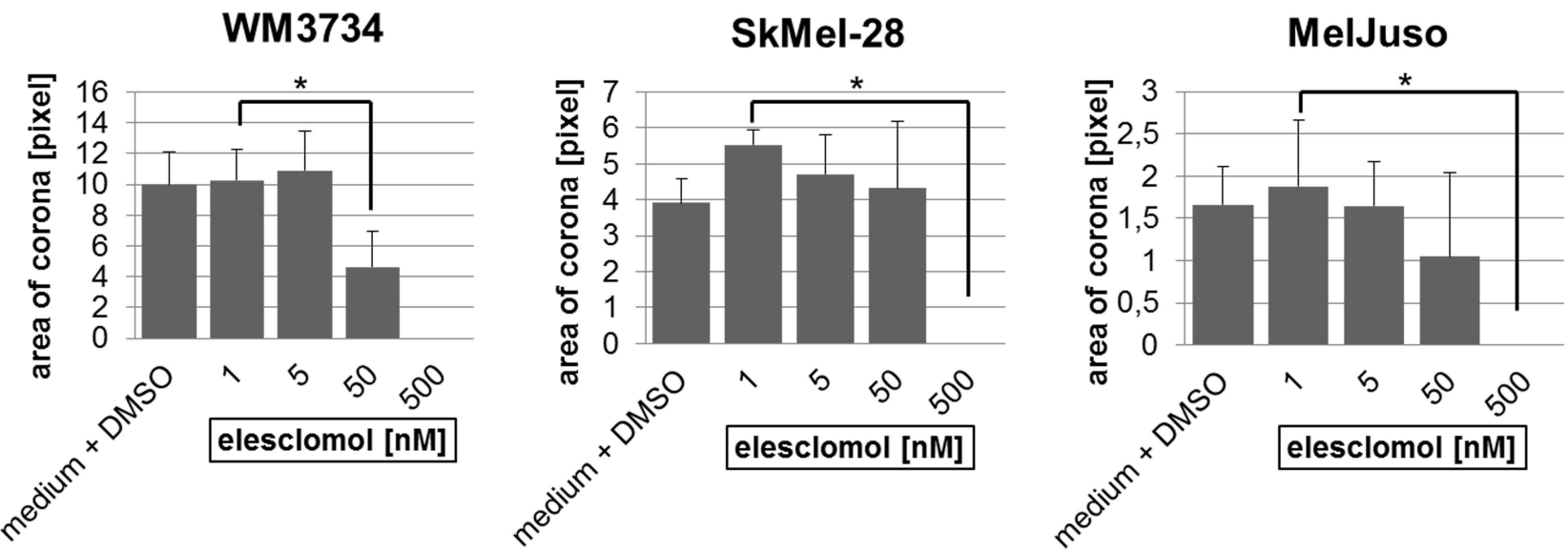

A
2.0
0
H2O2 (YFP/CFP)
CTRL
JARID1B
CTRL+mTEMPO
JARID1B+mTEMPO
***
***
***
1.0
0.8
0.6
0.4
0.2
0.0
H2O2 (YFP/CFP)
JARID1B - + - +
mitoTEMPO - - + +
B
0.8
0.6
0.4
0.2
0.0
H2O2 (YFP/CFP)
12.5 μM elesclomol
DMSO
0
20
40
60
Time (min)
***
0.4
0.3
0.2
0.1
0.0
Δ H2O2 (YFP/CFP)
elesclomol (μM) mock 12.5
C
***
***
250
200
150
100
50
0
H2O2 (% of control)
elesclomol (nM) mock 12.5 125
***
***
350
300
250
200
150
100
50
0
GSH/GSSG (% of control)
mock 12.5 125

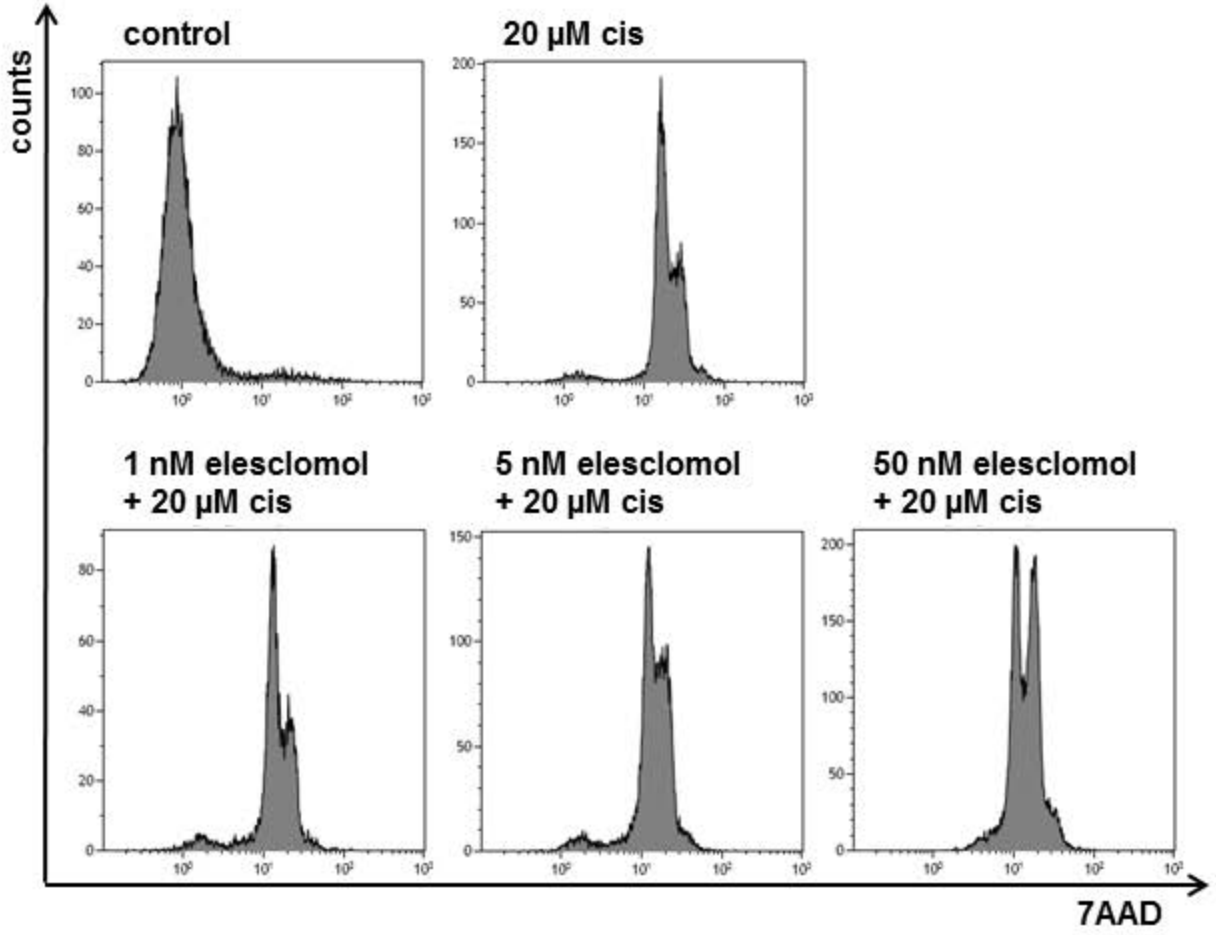

A
WM3734
cell number
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
*
24 hr
48 hr
72 hr
medium
medium + DMSO
1
5
50
500
elesclomol [nM]
B
MelJuso
cell number
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
24 hr
48 hr
72 hr
medium
medium + DMSO
1
5
50
500
elesclomol [nM]
C
SkMel-28
cell number
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
24 hr
48 hr
72 hr
medium
medium + DMSO
1
5
50
500
elesclomol [nM]

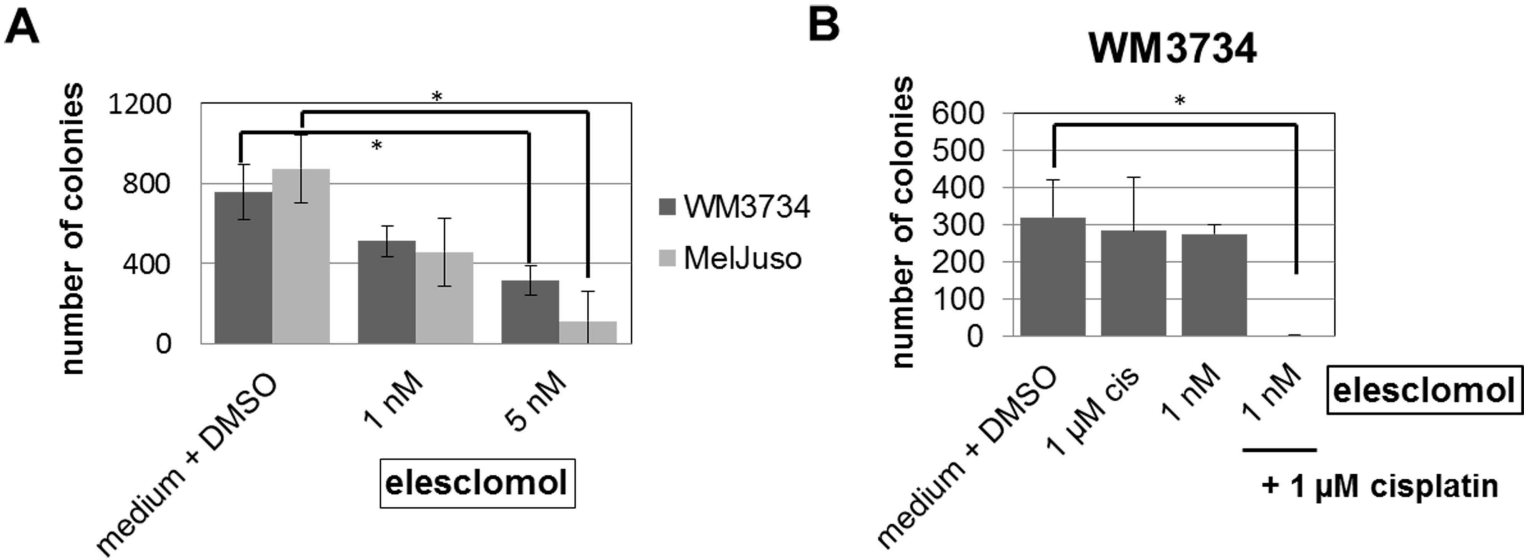
A

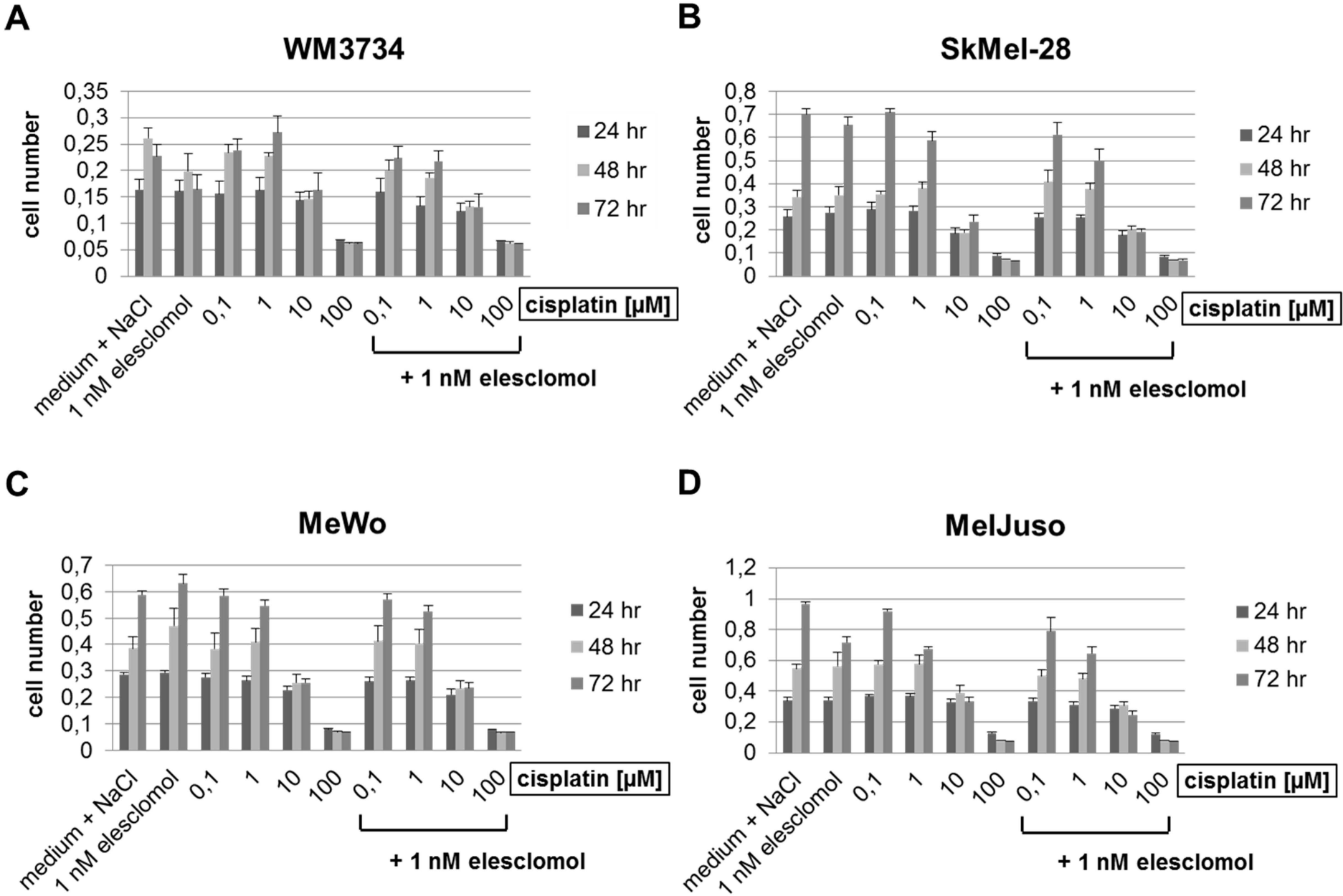

WM3734
control
5 nM
50 nM
500 nM

B


WM3734
SkMel-28
MelJuso
area of corona [pixel]
*
medium + DMSO
1
5
50
500
elesclomol [nM]


counts
control
20 µM cis
1 nM elesclomol
+ 20 µM cis
5 nM elesclomol
+ 20 µM cis
50 nM elesclomol
+ 20 µM cis
7AAD


A
number of colonies
1200
800
400
0
*
*
WM3734
MelJuso
medium + DMSO
1 nM
5 nM
elesclomol
B
WM3734
number of colonies
600
500
400
300
200
100
0
*
medium + DMSO
1 μM cis
1 nM
1 nM
elesclomol
+ 1 μM cisplatin


A
WM3734
cell number
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
24 hr
48 hr
72 hr
medium + NaCl
1 nM elesclomol
0,1
1
10
100
0,1
1
10
100
cisplatin [µM]
+ 1 nM elesclomol
B
SkMel-28
cell number
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
24 hr
48 hr
72 hr
medium + NaCl
1 nM elesclomol
0,1
1
10
100
0,1
1
10
100
cisplatin [µM]
+ 1 nM elesclomol
C
MeWo
cell number
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
24 hr
48 hr
72 hr
medium + NaCl
1 nM elesclomol
0,1
1
10
100
0,1
1
10
100
cisplatin [µM]
+ 1 nM elesclomol
D
MelJuso
cell number
1,2
1
0,8
0,6
0,4
0,2
0
24 hr
48 hr
72 hr
medium + NaCl
1 nM elesclomol
0,1
1
10
100
0,1
1
10
100
cisplatin [µM]
+ 1 nM elesclomol

Supplemental Figure Legends

Supplemental Figure 1. Mitochondrial ROS measurement in melanoma cells. (A) H_2O_2 levels in control (CTRL) or JARID1B-transfected WM3734 cells (48 hr after transfection) were visualized and measured using the H_2O_2 sensitive mitochondria-targeted sensor mitoHyPer. Left panel shows representative images of mitoHyPer signals. Treatment with the mitochondria-targeted antioxidant and free radical scavenger mito-Tempo counteracts the JARID1B-induced increase in H_2O_2. Cells transfected with empty pBIND vector were used as control (CTRL). Right panel shows quantitated results of at least 13 cells per condition. Asterisks indicate statistical significance with $p<0.01$. (B) Measurement of mitochondrial H_2O_2 levels in WM3734 cells by mitoHyPer under continuous elesclomol exposure. Elesclomol was preincubated for 20 min. (left panel). Net increase of mitochondrial H_2O_2 production 70 min after elesclomol addition (right panel). Control group was treated with DMSO. (C) Relative H_2O_2 levels (left panel) and GSH/GSSG ratios (right panel) in WM3734 cells treated with elesclomol for 48 hr at the indicated concentrations. Control cells were incubated with 100 nM mito-TEMPO for 20 min to fully reduce the protein sensors. Mitochondrial H_2O_2 production and GSH/GSSG ratio were calculated as % of control. The intensities of fully reduced mitoHyPer and mitoGrx1-roGFP were taken as 100%. Error bars represent standard error of mean (SEM).

Supplemental Figure 2. Elesclomol treatment inhibits melanoma cell growth *in vitro.* Short-term, two-dimensional cytotoxicity assays of different melanoma cell lines (A,B,C) treated with increasing elesclomol concentrations for 24, 48 and 72 hr. The graphs show representative examples of three independently performed experiments. Error bars represent the standard deviation, asterisks represent $p< 0.05$.

Supplemental Figure 3. Elesclomol inhibits growth of melanoma spheroids and invasion into collagen. (A) Three-dimensional collagen-embedded melanoma cell spheroids were treated with increasing concentrations of elesclomol over 4 days. (B) Digital quantitation of the coronal area of invading melanoma cells (subtraction of solid from dashed delineated areas as shown in panel A) revealed a significant reduction starting from 50 nM of elesclomol in WM3734 cells and 500 nM in SkMel-28 and MelJuso cells. Error bars represent the standard deviation, asterisks represent $p< 0.05$.

Supplemental Figure 4. Confirmation of cell death in culture supernatant. Flow cytometric 7AAD staining of the supernatant of WM3734 cell cultures that have been treated with

elesclomol and cisplatin (cis) over 72 hr at the indicated concentrations. Shown is one representative example from three independent experiments.

Supplemental Figure 5. Elesclomol treatment reduces the colony formation capacity and long-term growth of melanoma cells. (A) Significantly reduced colony formation of WM3734 and MelJuso cells, which have been pulse treated with elesclomol for 72 hr prior to embedding as single cells into soft agar. (B) Co-treatment of WM3734 cells with elesclomol and cisplatin at the indicated concentrations for 72 hr prior to soft agar embedding induced a dramatic loss of long-term growth. Colonies were counted manually after 4-5 weeks in triplicates. Error bars represent the standard deviation, asterisks represent $p < 0.05$.

Supplemental Figure 6. Addition of elesclomol to cisplatin does not significantly enhance the inhibition of two-dimensional melanoma cell growth *in vitro.* Short-term, two-dimensional cytotoxicity assays of different melanoma cell lines (A, B, C, D) co-treated with increasing cisplatin concentrations plus 1 nM of elesclomol at the indicated time points. The graphs show representative examples of three independently performed experiments. Error bars represent the standard deviation, asterisks represent $p < 0.05$.

Experimental Procedures

Melanoma cell lines and lentiviral construct

WM3734 (*BRAFV600E, NRAS wt*), SkMel-28 (*BRAFV600E*), MeWo (*BRAF wt, NRAS wt*) and MelJuso (*NRASQ61L*) were selected as melanoma cell lines representing common genetic aberrations in melanoma patients. Cells were kept under normal culture conditions (5% CO_2, 37°C) and maintained in either 2% FBS-substituted melanoma medium (WM3734, see also Roesch et al., 2010) or 10% FBS-substituted-RPMI medium (SkMel-28, MeWo, MelJuso). WM3734$^{JARID1Bprom\text{-}EGFP}$ cells have been stably transduced with the lentiviral vector pLU-JARID1Bprom-EGFP-Blast as previously described by us (Roesch et al., 2010). Continuous selection for positively transduced cells was ensured by blasticidin treatment once per month.

Drugs and chemical compounds

Cisplatin (Pharmacy of Saarland University Hospital) and elesclomol (Selleckchem, S1052; STA-4783) were used as test compounds. Mito-Tempo was purchased from Enzo Life Sciences. Elesclomol stocks were substituted with copper chloride (Sigma Aldrich, 751944-25G) to a final concentration of 10 mM in the assays.

Flow cytometry of J/EGFP signals

Flow cytometric detection of JARID1B promoter-driven EGFP signals of WM3734$^{JARID1Bprom\text{-}EGFP}$ cells was performed on a FACSCanto instrument (BD Biosciences) as reported earlier (Roesch et al., 2010, Roesch et al., 2013). After drug incubation for 72 hr, cells were harvested including the supernatant. Dead cells were detected by 7AAD staining. For reliable assessment of endogenous JARID1B expression, an analytical threshold was applied and set to the maximum 5% of J/EGFP fluorescence signals as described previously (Roesch et al., 2010, Roesch et al., 2013). Cells above the threshold were considered as JARID1B^{high} subpopulation.

Immunoblotting

Whole cell lysates of WM3734 cells were prepared according to standard procedures. Samples were loaded on 10% SDS-PAGE gels and electrophoretically transferred onto nitrocellulose or PVDF membranes (Roth). Primary rabbit polyclonal anti-JARID1B antibody (1:2000, Novus, NB 100-97821) and rabbit polyclonal GAPDH (1:8000 Santa-Cruz, sc-25778) were incubated at 4°C overnight in 5% milk plus 0.1% Tween-20 and counterstained with anti-rabbit horseradish peroxidase-conjugated secondary antibody (Dianova, 111-035-046) diluted 1:10,000 in 5% TBST-milk. Protein amounts were assessed using an enhanced