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Supplemental information

Mitochondrial dynamics regulate genome stability via control of caspase-dependent DNA damage

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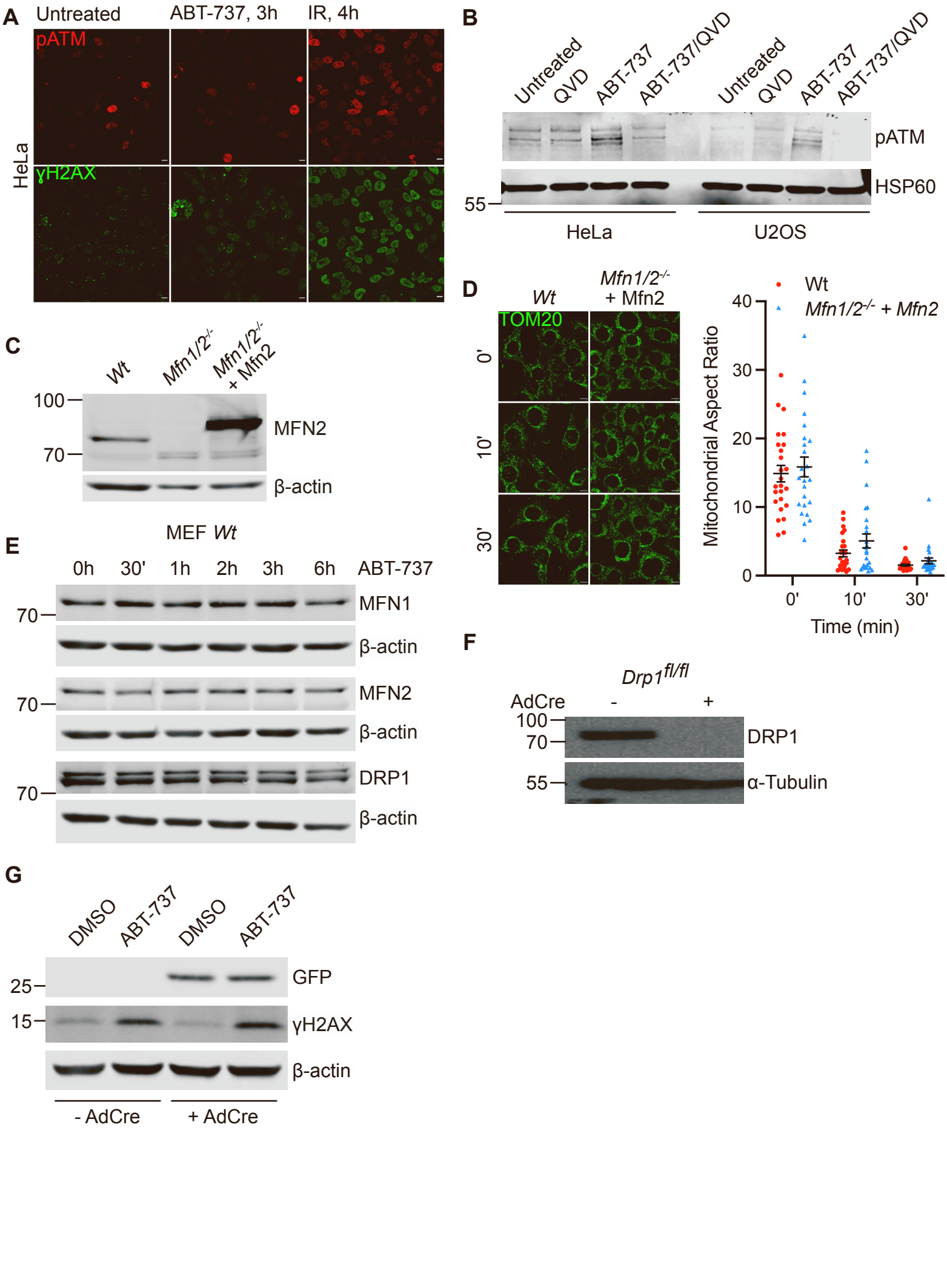

A
Untreated
ABT-737, 3h
IR, 4h
pATM
γH2AX
HeLa
B
Untreated
QVD
ABT-737
ABT-737/QVD
Untreated
QVD
ABT-737
ABT-737/QVD
pATM
HSP60
55
HeLa
U2OS
C
Wt
Mfn1/2-/-
Mfn1/2-/- + Mfn2
100
70
MFN2
β-actin
D
Wt
Mfn1/2-/- + Mfn2
TOM20
0'
10'
30'
Wt
Mfn1/2-/- + Mfn2
Mitochondrial Aspect Ratio
40
30
20
10
0
0'
10'
30'
Time (min)
E
MEF Wt
0h
30'
1h
2h
3h
6h
ABT-737
70
MFN1
β-actin
70
MFN2
β-actin
70
DRP1
β-actin
F
Drp1fl/fl
AdCre
-
+
100
70
DRP1
55
α-Tubulin
G
DMSO
ABT-737
DMSO
ABT-737
25
15
GFP
γH2AX
β-actin
- AdCre
+ AdCre

Figure S1. Mitochondrial dynamics regulate DNA damage. Related to Figure 1

A) Airyscan images of HeLa cells treated with 10 μM ABT-737 for 3 h, or 20 Gy irradiation for 4 h. Cells were immunostained with anti-pATM (red) or anti-γH2AX (green) antibody. Images representative of 3 independent experiments. Scale bar = 10 μm

B) Western blot of HeLa and U2OS cells treated with 10 μM QVD, 10 μM ABT-737 or a combination of both for 3 h. Blots were immunoblotted for pATM and HSP60 and a representative of 2 independent experiments.

C) Western blot of *Wt*, *Mfn1/2*$^{-/-}$ and *Mfn1/2*$^{-/-}$ + Mfn2 MEF immunoblotted for MFN2 and β-actin. Representative of 2 independent experiments.

D) Airyscan images of *Wt* or *Mfn1/2*$^{-/-}$ + Mfn2 MEF treated with 10 μM CCCP for the indicated times and immunostained with anti-TOM20 antibody. Mitochondrial aspect ratio data is from 3 independent experiments. Data are expressed as mean ± SEM.

E) Western blot of *Wt* MEF treated with 10 μM ABT-737 for 3 h, and immunoblotted for MFN1, MFN2, DRP1 and β-actin. Data are representative of 3 independent experiments.

F) Western blot of *Drp1*$^{fl/fl}$ MEF with and without adenoviral Cre infection. Lysates immunoblotted for DRP1 and α-tubulin.

G) Western blot of loxP-STOP-loxP MEF infected or not with adenoviral Cre to induce YFP expression. Cells were treated with 10 μM ABT-737 for 3 h and YFP expression analysed by GFP immunoblotting, DNA damage by γH2AX and β-actin for loading control. Data is representative of 3 independent experiments.

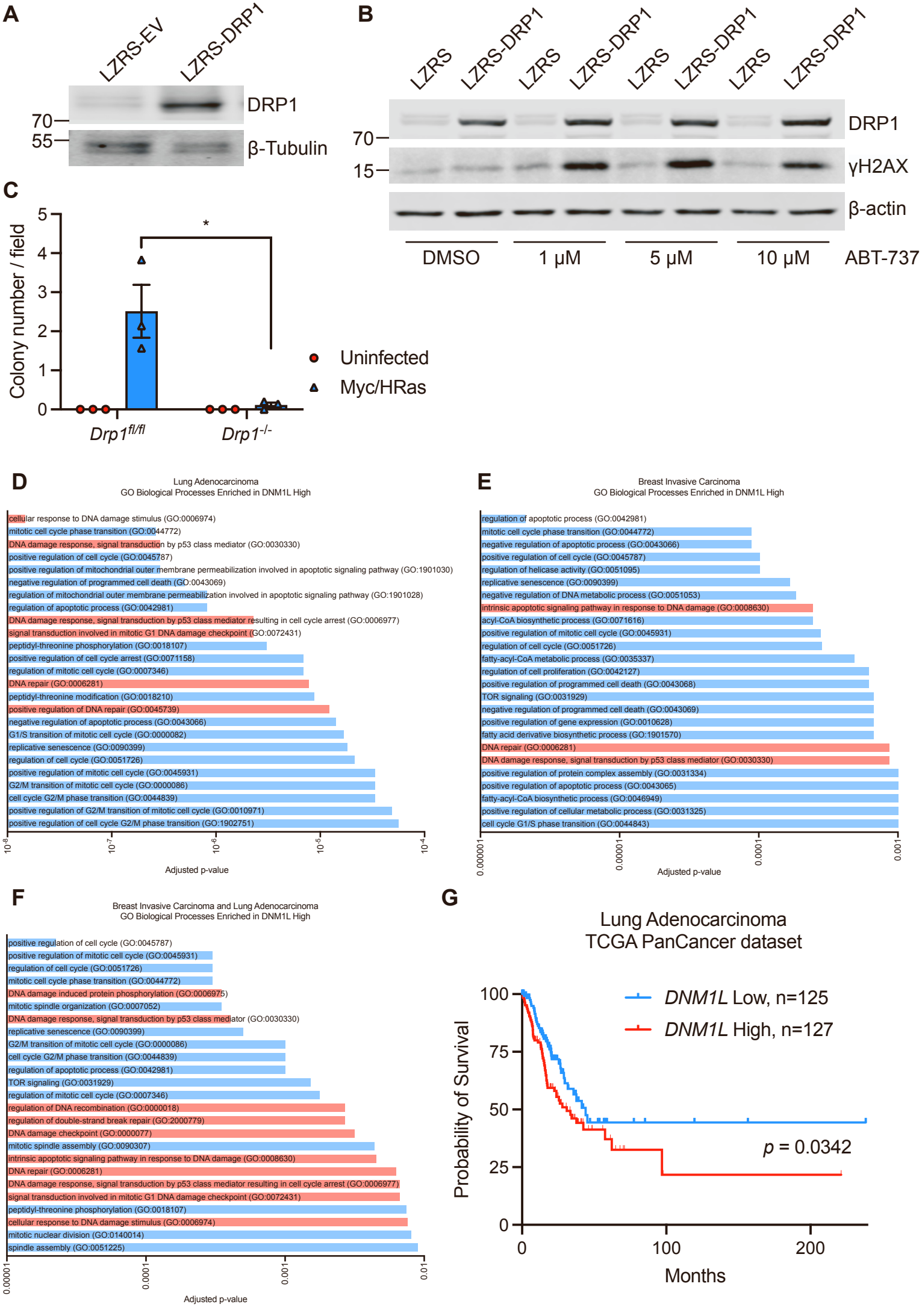
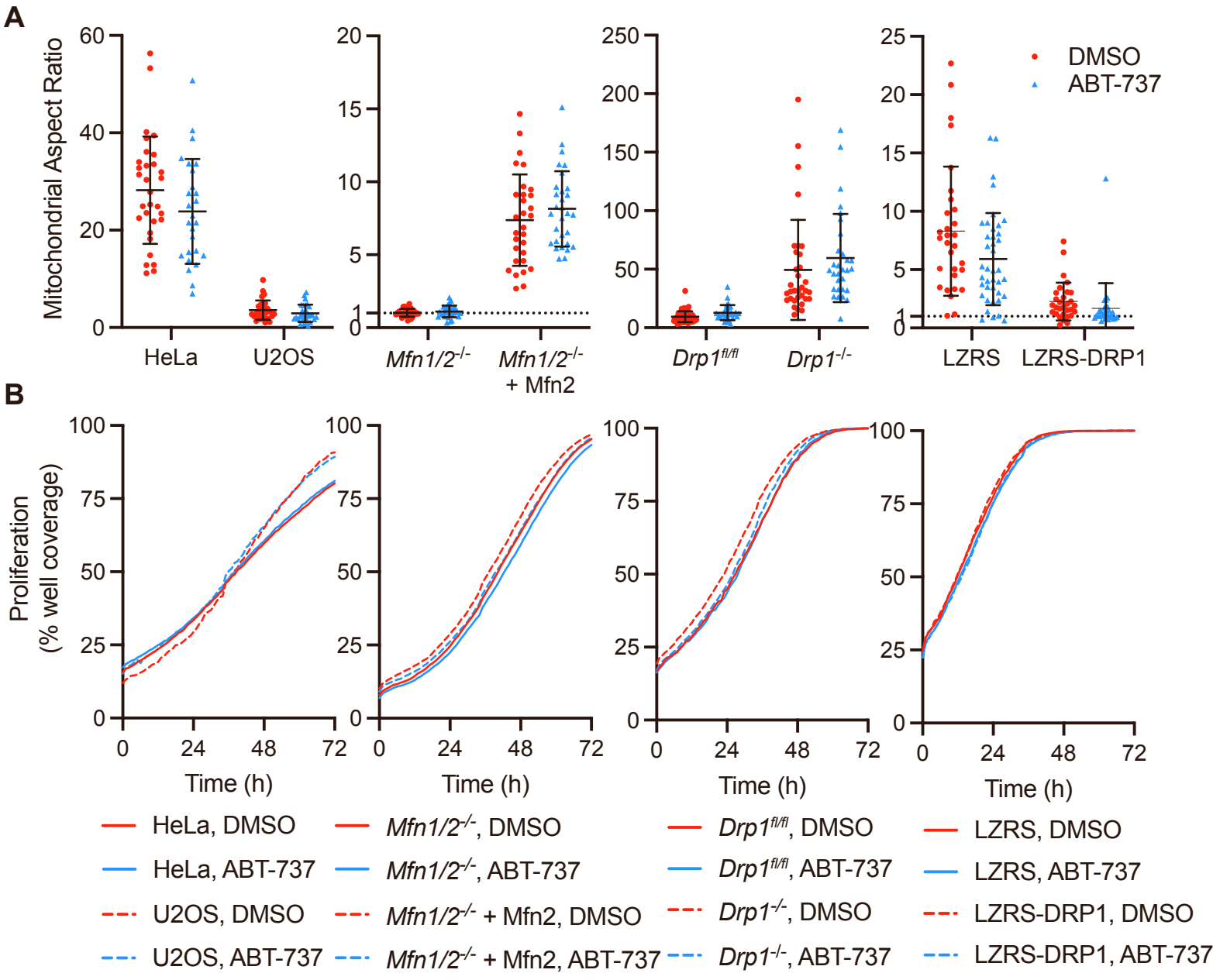
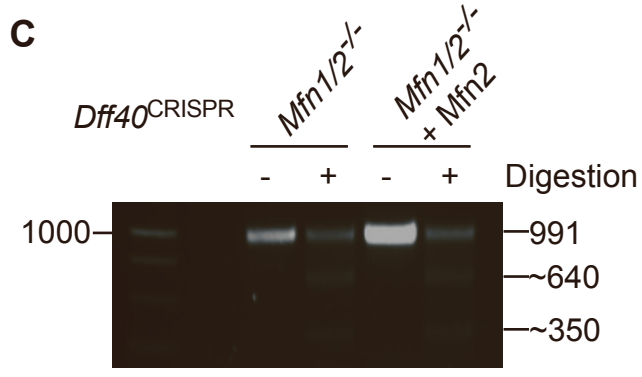

A
LZRS-EV
LZRS-DRP1
DRP1
β-Tubulin
70
55
B
LZRS
LZRS-DRP1
DRP1
γH2AX
β-actin
70
15
DMSO
1 μM
5 μM
10 μM
ABT-737
C
Colony number / field
Uninfected
Myc/HRas
Drp1fl/fl
Drp1-/-
D
Lung Adenocarcinoma
GO Biological Processes Enriched in DNM1L High
Adjusted p-value
E
Breast Invasive Carcinoma
GO Biological Processes Enriched in DNM1L High
Adjusted p-value
F
Breast Invasive Carcinoma and Lung Adenocarcinoma
GO Biological Processes Enriched in DNM1L High
Adjusted p-value
G
Lung Adenocarcinoma
TCGA PanCancer dataset
DNM1L Low, n=125
DNM1L High, n=127
p = 0.0342
Probability of Survival
Months

Figure S2. Mitochondrial fission regulates DNA damage, transformation and cancer survival. Related to Figure 2

A) Western blot of MEF expressing LZRS empty control, or LZRS-DRP1. Lysates immunoblotted for DRP1 and β-tubulin.
B) MEF stably overexpressing LZRS-DRP1 or LZRS empty vector were treated with the indicated dose of ABT-737 for 3 h and lysates immunoblotted for DRP1, γH2AX and β-actin. Data are representative of 3 independent experiments.
C) *Drp1*$^{fl/fl}$ and *Drp1*$^{-/-}$ MEF infected with viruses encoding Myc and HRas were cultured in soft agar and colony number quantified. Data are from 3 independent experiments, expressed as mean ± SEM and analysed using student's t-test.
D) Gene ontology (GO) biological processes significantly up-regulated in DNM1L high expressing lung adenocarcinoma samples. Bars in red represent GO biological processes related to DNA damage and are expressed as adjusted p-values.
E) Gene ontology (GO) biological processes significantly up-regulated in DNM1L high expressing breast invasive carcinoma samples. Bars in red represent GO biological processes related to DNA damage and are expressed as adjusted p-values.
F) Gene ontology (GO) biological processes significantly up-regulated in DNM1L high expressing lung adenocarcinoma and breast invasive carcinoma samples. Bars in red represent GO biological processes related to DNA damage and are expressed as adjusted p-values.
G) Survival of lung adenocarcinoma patients. DNM1L Low refers to those patients in the lowest quartile of DNM1L mRNA expression, and DNM1L High refers to patients in the highest quartile. Significance is calculated by log-rank (Mantel-Cox) test.
Statistics: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$


A
Mitochondrial Aspect Ratio
HeLa
U2OS
Mfn1/2-/-
Mfn1/2-/- + Mfn2
Drp1fl/fl
Drp1-/-
LZRS
LZRS-DRP1
DMSO
ABT-737
B
Proliferation (% well coverage)
Time (h)
HeLa, DMSO
HeLa, ABT-737
U2OS, DMSO
U2OS, ABT-737
Mfn1/2-/-, DMSO
Mfn1/2-/-, ABT-737
Mfn1/2-/- + Mfn2, DMSO
Mfn1/2-/- + Mfn2, ABT-737
Drp1fl/fl, DMSO
Drp1fl/fl, ABT-737
Drp1-/-, DMSO
Drp1-/-, ABT-737
LZRS, DMSO
LZRS, ABT-737
LZRS-DRP1, DMSO
LZRS-DRP1, ABT-737


C
Dff40CRISPR
Mfn1/2-/-
Mfn1/2-/- + Mfn2
Digestion
1000
991
~640
~350

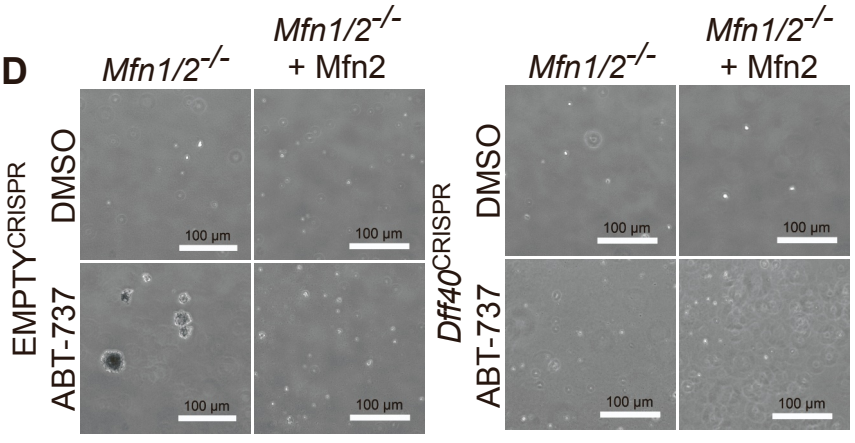

D
Mfn1/2-/-
Mfn1/2-/- + Mfn2
EMPTYCRISPR
Dff40CRISPR
DMSO
ABT-737
100 µm

Figure S3. Impact of mitochondrial fusion and fission machinery modulation on mitochondrial morphology, growth and CAD-dependent transformation. Related to Figure 4

A) Mitochondria aspect ratio of indicated cell lines treated with or without 10 μM ABT-737 for 3 h. Cells were immunostained for TOM20 and imaged using Airyscan microscopy. Mitochondrial aspect ratio was calculated from 3 independent experiments and expressed as mean (SD).
B) Proliferation of indicated cell lines treated with or without 10 μM ABT-737 for 3h, after which time the media was changed to media not containing ABT-737. Cells were imaged for 72 h.
C) T7 endonuclease I mismatch assay to assay for CAD/*Dff40* deletion.
D) Representative images of anchorage-independent growth of *Mfn1/2*$^{-/-}$ and *Mfn1*$^{-/-}$ MEF with and without CRISPR-Cas9-mediated *Dff40* deletion. Cells were passaged twenty times in 10 μM ABT-737. Scale bar = 100 μm. Data are quantified in **Figure 2K**.

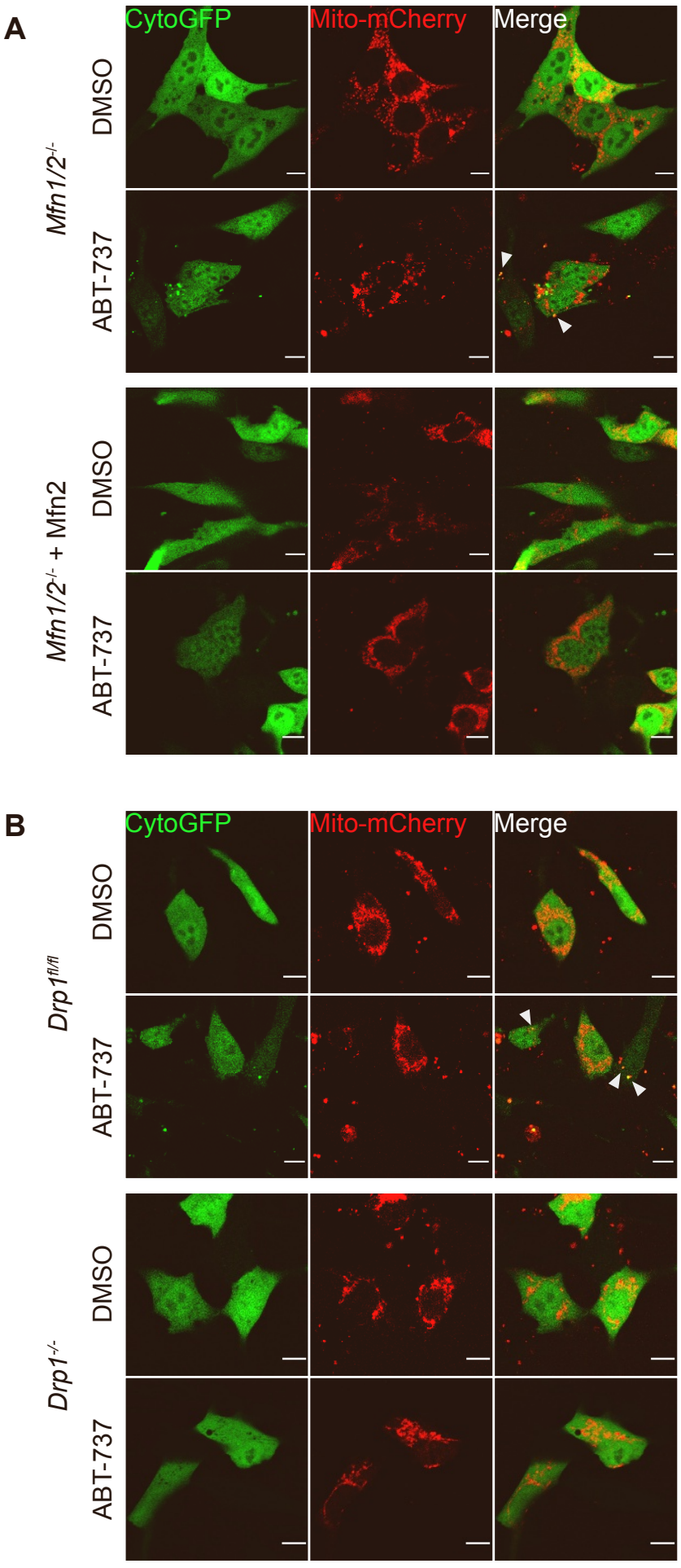

A
CytoGFP
Mito-mCherry
Merge
DMSO
ABT-737
Mfn1/2-/-
Mfn1/2-/- + Mfn2
B
Drp1fl/fl
Drp1-/-

Figure S4. Visualisation of minority MOMP in cells with modulated mitochondrial dynamics. Related to Figure 5.

A) Confocal images of *Mfn1/2*$^{-/-}$ and *Mfn1/2*$^{-/-}$ + Mfn2 cells transiently expressing cytoGFP and mito-mCherry. Cells were treated with dimeriser and treated or not with 10 μM ABT-737 for 3 h before fixation, imaging and quantification of minority MOMP. Images are quantified in **Figure 3E**.

B) Confocal images of *Drp1*$^{fl/fl}$ and *Drp1*$^{-/-}$ cells transiently expressing cytoGFP and mito-mCherry. Cells were treated with dimeriser and treated or not with 10 μM ABT-737 for 3 h before fixation, imaging and quantification of minority MOMP. Images are quantified in **Figure 3F**.

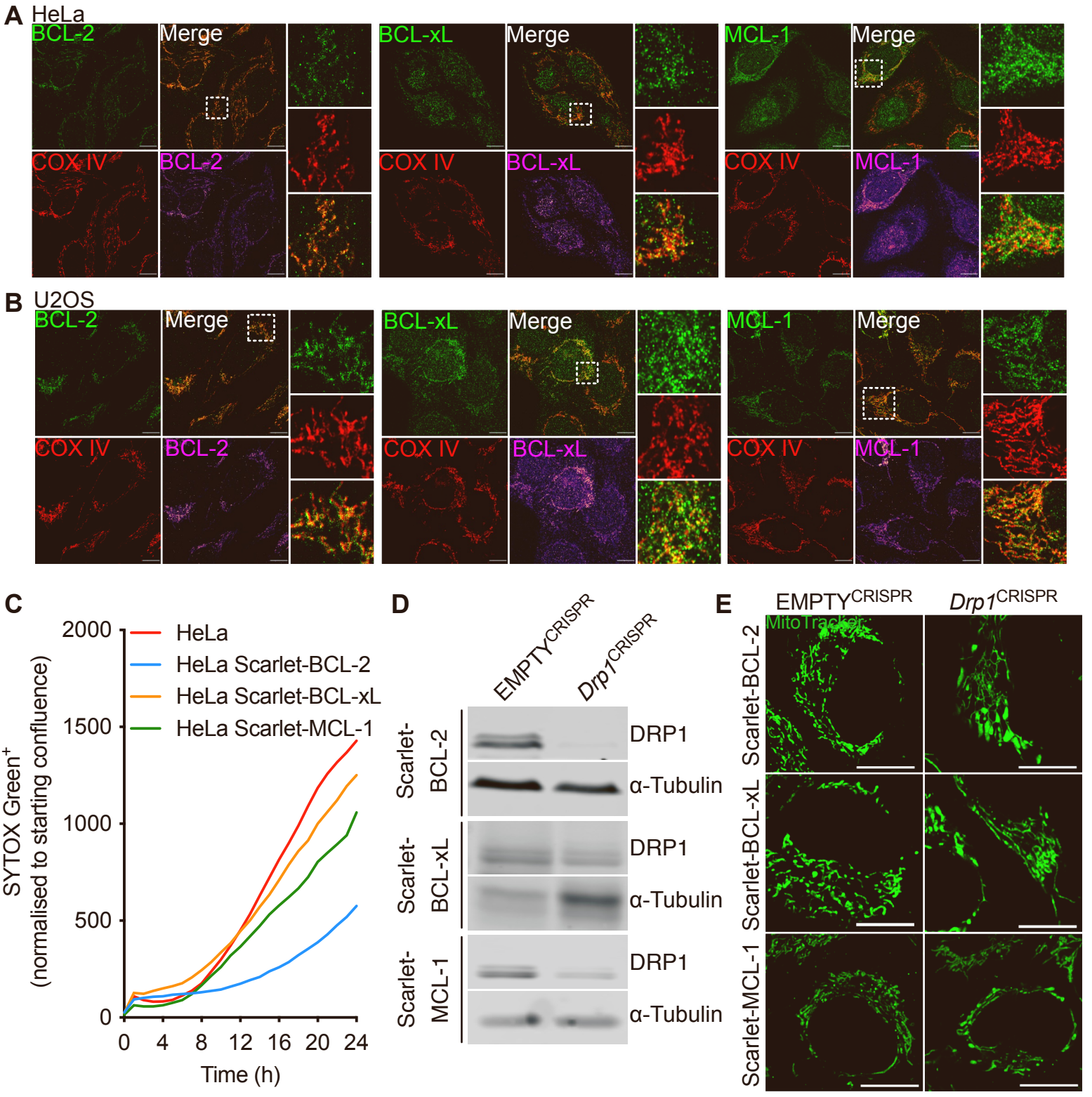

A
HeLa
BCL-2
Merge
COX IV
BCL-2
BCL-xL
Merge
COX IV
BCL-xL
MCL-1
Merge
COX IV
MCL-1
B
U2OS
BCL-2
Merge
COX IV
BCL-2
BCL-xL
Merge
COX IV
BCL-xL
MCL-1
Merge
COX IV
MCL-1
C
SYTOX Green+ (normalised to starting confluence)
2000
1500
1000
500
0
0
4
8
12
16
20
24
Time (h)
HeLa
HeLa Scarlet-BCL-2
HeLa Scarlet-BCL-xL
HeLa Scarlet-MCL-1
D
EMPTYCRISPR
Drp1CRISPR
Scarlet-BCL-2
DRP1
α-Tubulin
Scarlet-BCL-xL
DRP1
α-Tubulin
Scarlet-MCL-1
DRP1
α-Tubulin
E
EMPTYCRISPR
Drp1CRISPR
MitoTracker
Scarlet-BCL-2
Scarlet-BCL-xL
Scarlet-MCL-1
F
BCL-2
BCL-xL
MCL-1
Scarlet/MitoTracker StDev Ratio
0.6
0.4
0.2
0.0
0.3
0.2
0.1
0.0
2.0
1.5
1.0
0.5
0.0
EMPTYCRISPR
Drp1CRISPR

Figure S5. Characterisation of BCL-2 family knockin cells. Related to Figure 6.

A) Airyscan images of HeLa cells immunostained for BCL-2, BCL-xL or MCL-1 (green) in combination with COX IV (red). Also shown are BCL-2, BCL-xL or MCL-1 images with a magenta LUT applied to visualise heterogeneity. Scale bar = 10 μm. Data are representative of 3 independent experiments.

B) Airyscan images of U2OS cells immunostained for BCL-2, BCL-xL or MCL-1 (green) in combination with COX IV (red). Also shown are BCL-2, BCL-xL or MCL-1 images with a magenta LUT applied to visualise heterogeneity. Scale bar = 10 μm. Data are representative of 3 independent experiments.

C) Live-cell IncuCyte analysis of HeLa and HeLa Scarlet-MCL-1 knockin cells treated with 10 μM ABT-737 and 2 μM S63845. Cells were incubated with SYTOX Green and SYTOX Green positivity was measured over time and normalised to starting confluency.

D) Western blots of HeLa Scarlet-BCL-2, BCL-xL and MCL-1 knockin cells with CRISPR-Cas9-mediated DRP1 deletion. Lysates immunoblotted for DRP1 and α-tubulin as a loading control.

E) Representative Airyscan images of mitochondrial structure in HeLa Scarlet-BCL-2, BCL-xL and MCL-1 knockin cells with CRISPR-Cas9-mediated DRP1 deletion. Scale bar = 10 μm. Data are representative of at least 2 independent experiments.

F) Quantification of standard deviation of Scarlet to MitoTracker signal in HeLa Scarlet BCL-2, BCL-xL and MCL-1 cells, with or without CRISPR-Cas9-mediated DRP1 deletion. Data are expressed as borderless points for individual cells and points with borders represent summary data for n = 2 biological replicates for BCL-2 and BCL-xL, and n = 3 for MCL-1. The line represents the mean.

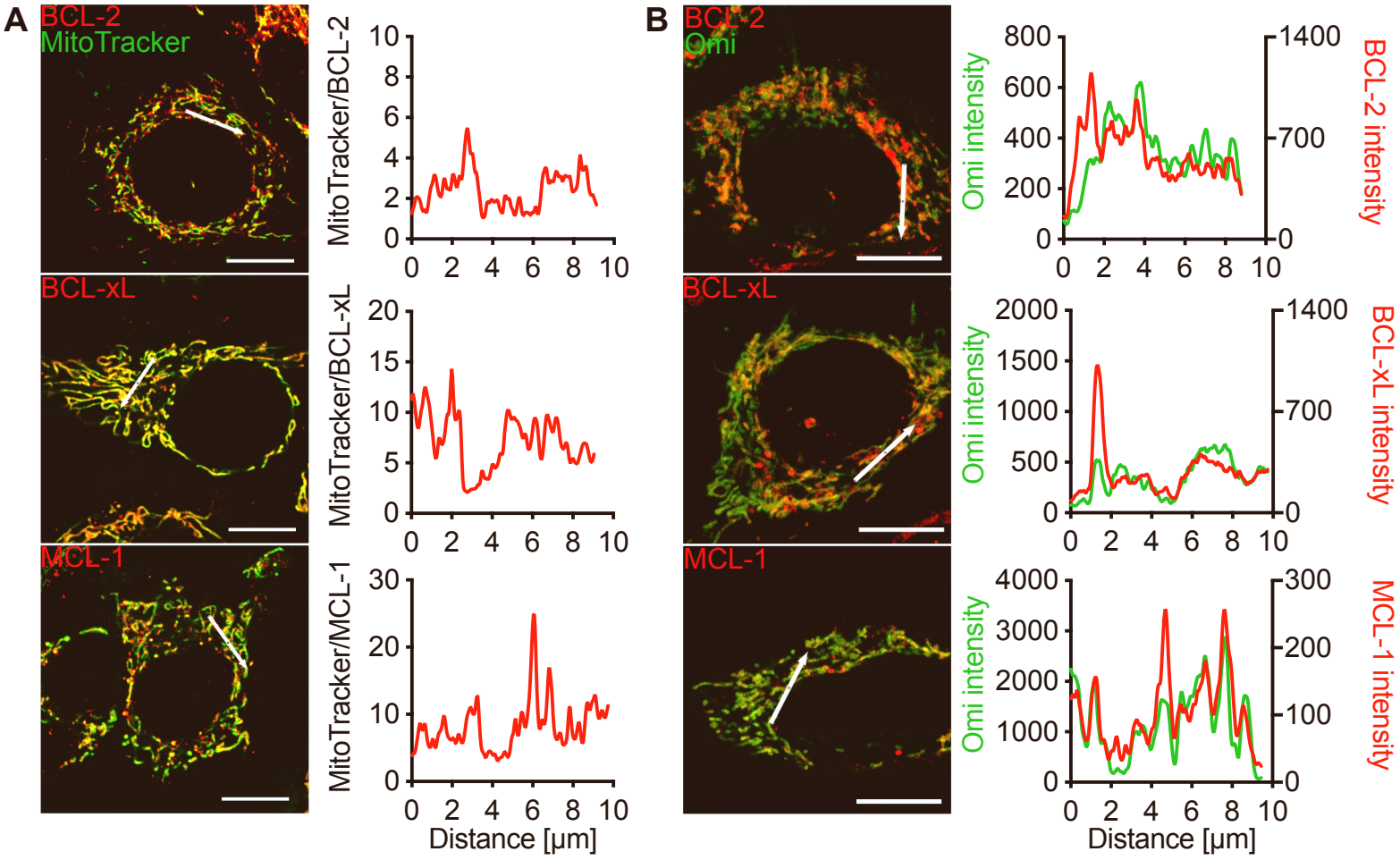
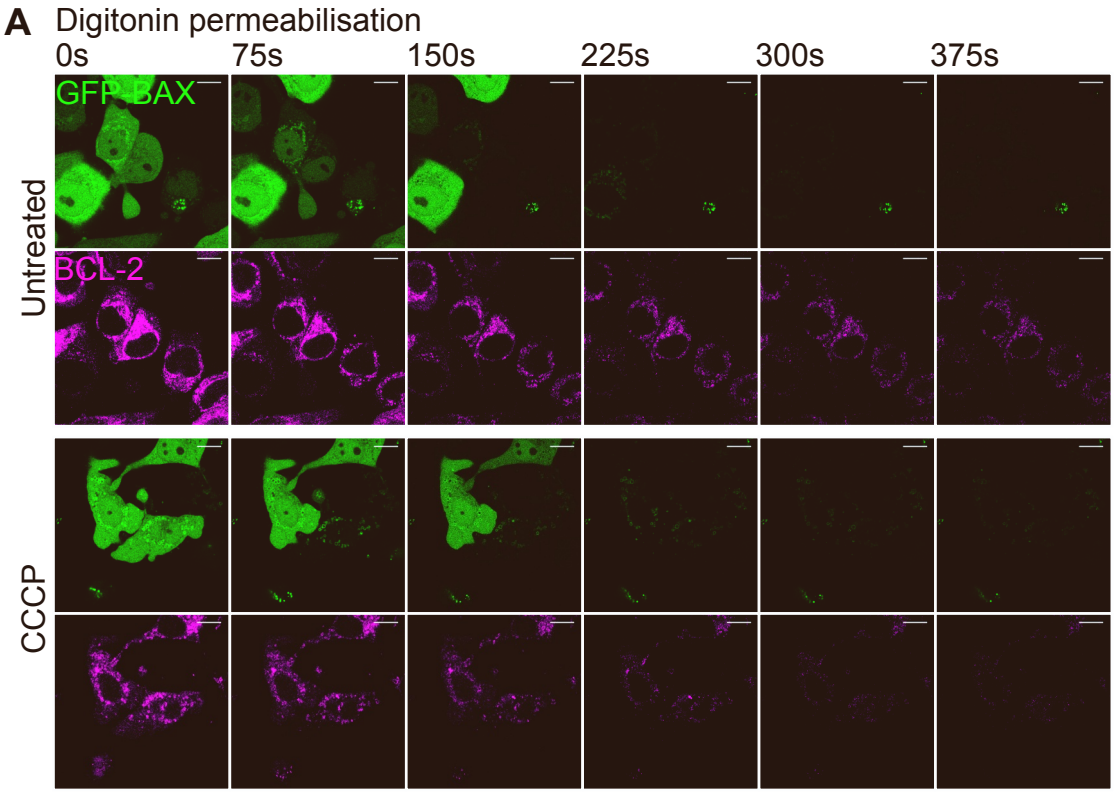

A
BCL-2
MitoTracker
BCL-xL
MCL-1
MitoTracker/BCL-2
MitoTracker/BCL-xL
MitoTracker/MCL-1
Distance [μm]
B
BCL-2
Omi
BCL-xL
MCL-1
Omi intensity
BCL-2 intensity
BCL-xL intensity
MCL-1 intensity
Distance [μm]

Figure S6. Mitochondrial localisation of BCL2-family knockins. Related to Figure 5.

A) Airyscan images of HeLa Scarlet-BCL-2, BCL-xL and MCL-1 knockin cells and MitoTracker. Line scans show ratio of MitoTracker to Scarlet-BCL-2. Scale bar = 10 μm. Data representative of 3 independent experiments.

B) Airyscan images of HeLa Scarlet-BCL-2, BCL-xL and MCL-1 knockin cells and Omi-GFP. Line scans show ratio of Omi-GFP intensity (green) and Scarlet-BCL-2 intensity (red). Scale bar = 10 μm. Data representative of 3 independent experiments.


A
Digitonin permeabilisation
0s
75s
150s
225s
300s
375s
Untreated
GFP-BAX
BCL-2
CCCP

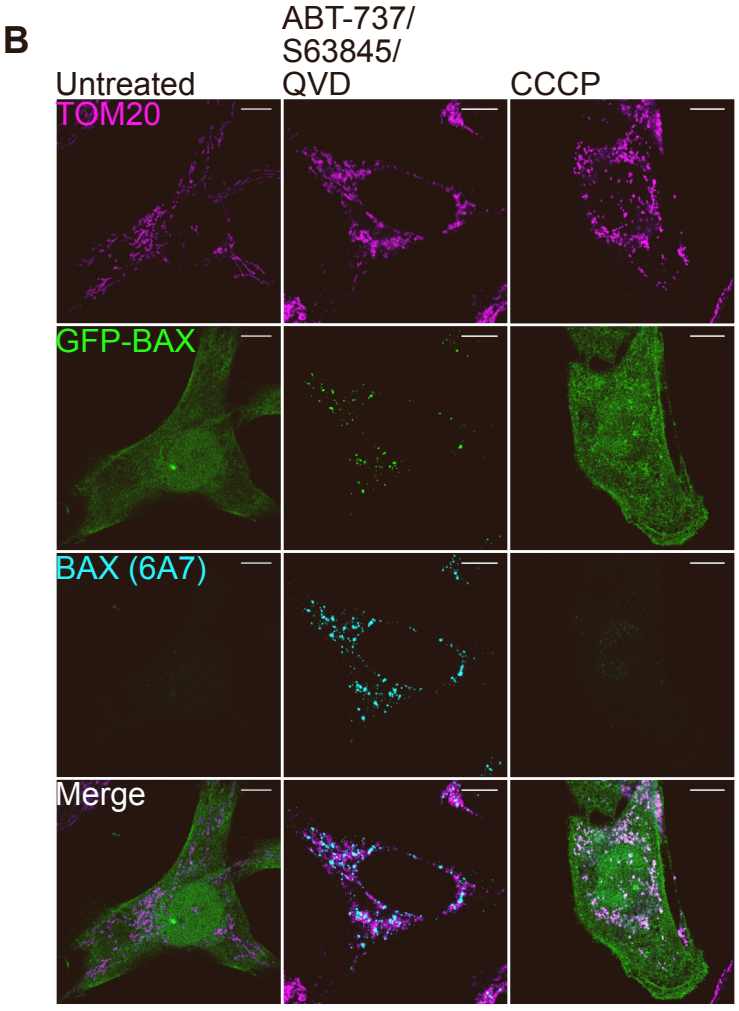

B
Untreated
ABT-737/
S63845/
QVD
CCCP
TOM20
GFP-BAX
BAX (6A7)
Merge

Figure S7. Mitochondrial dysfunction causes BAX accumulation on the mitochondrial outer membrane, but not BAX pore formation. Related to Figure 6.

A) Images of HeLa Scarlet-BCL-2 knockin (magenta) cells stably overexpressing GFP-BAX (green) treated 10 μM CCCP for 30 min before digitonin permeabilisation. Scale bar = 10 μm. See also **Videos S4, S5**.

B) Airyscan images of fixed HeLa cells stably overexpressing GFP-BAX (green) and treated with 10 μM CCCP or 10 μM ABT-737, 2 μM S63845 and 10 μM QVD for 3 h. Cells were immunostained with anti-TOM20 (magenta) and anti-BAX 6A7 (cyan). Scale bar = 10 μm. Images representative of 3 independent experiments.