Supplementary information:

Mesothelioma cells depend on the anti-apoptotic protein Bcl-xL for survival and are sensitized to ionizing radiation by BH3-mimetics

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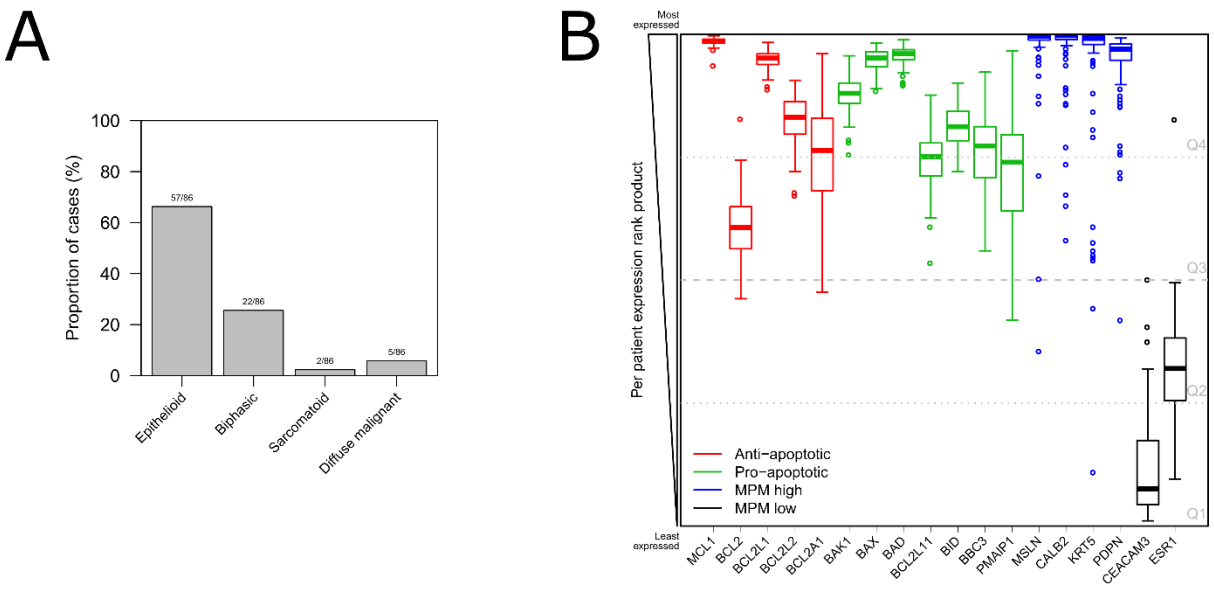
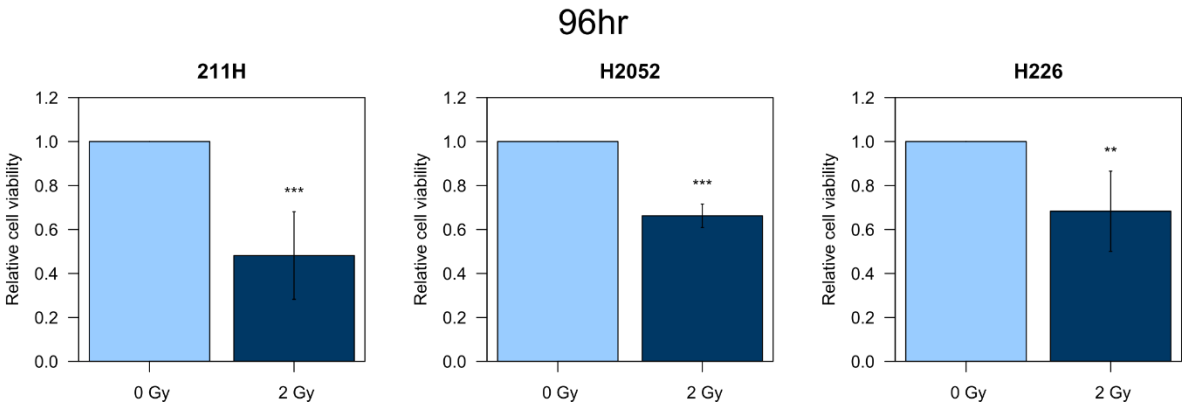


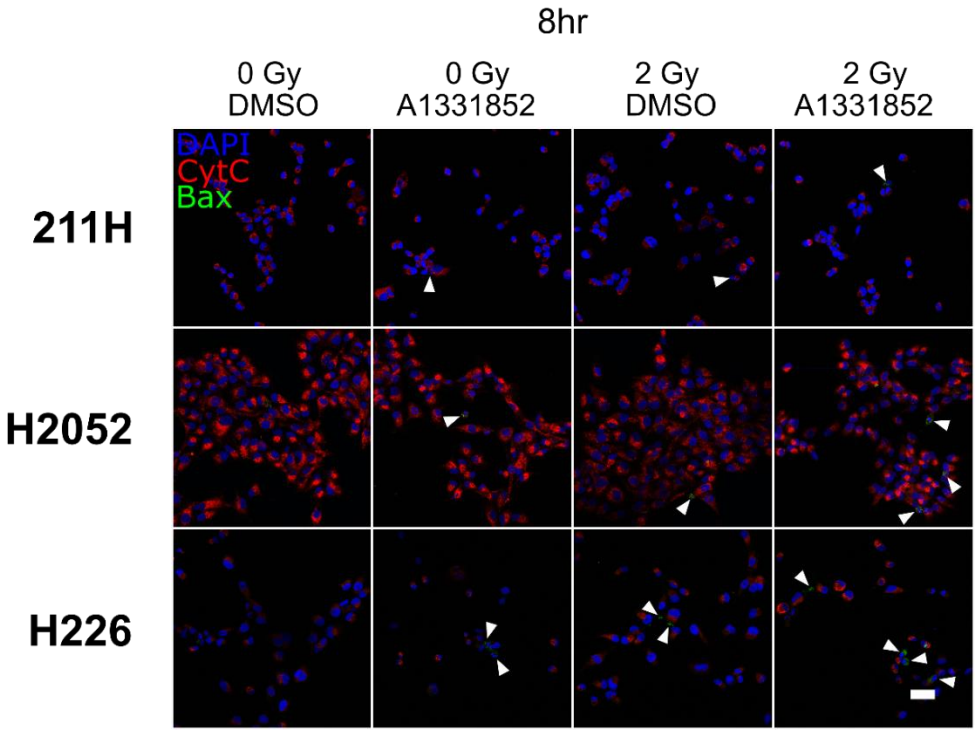
Supplementary Figure S1. Mesothelioma cases in TCGA.

(A) The proportion of TCGA cases (*n*=86) of each of the major histological subtype of MPM analysed for gene expression. (B) The expression rank product of the genes encoding major apoptotic proteins and diagnostically useful markers of MPM was determined. Box and whiskers plotted according to the Tukey method. Expression quartiles are indicated (Q1-4).



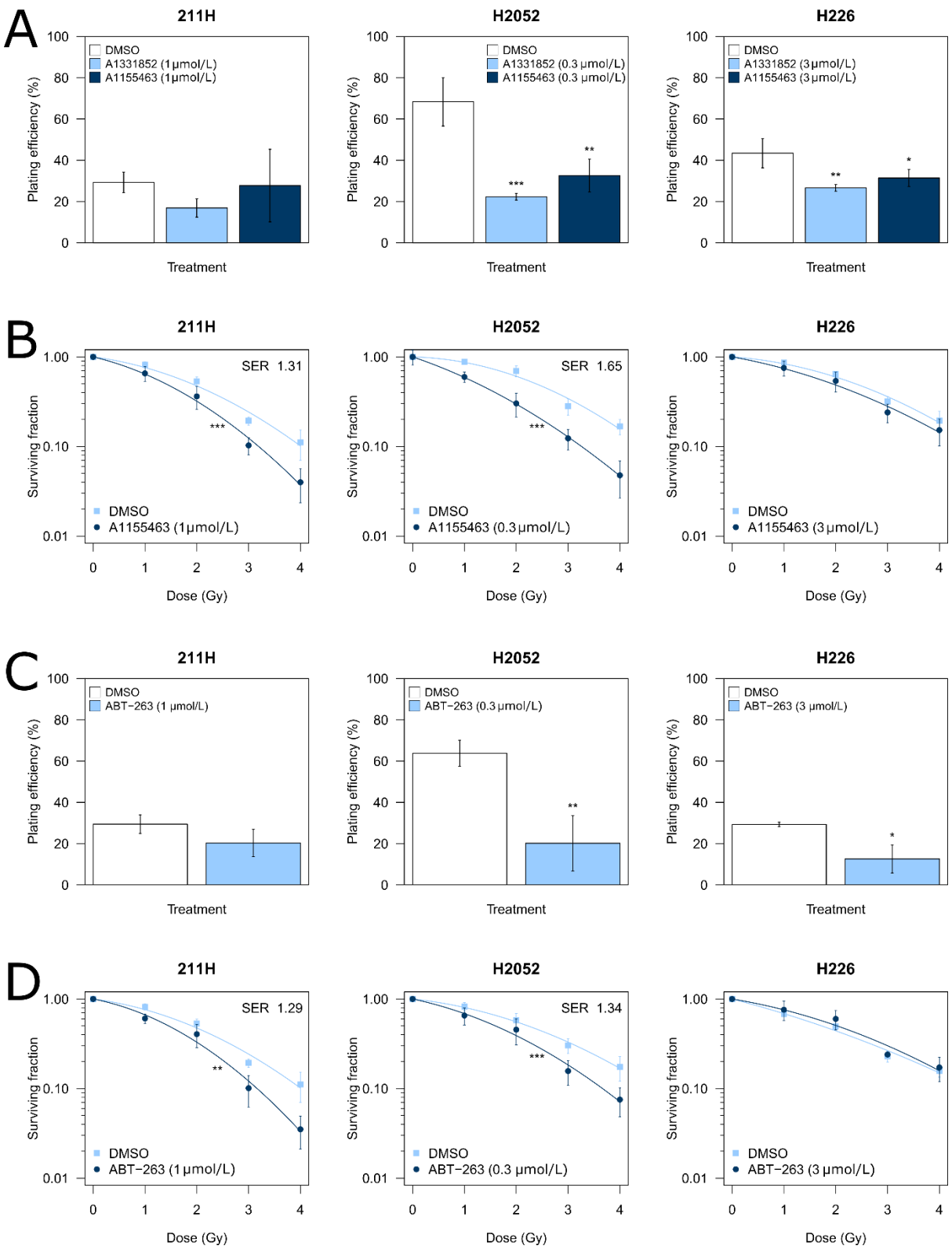
Supplementary Figure S2. IR reduces mesothelioma cell viability.

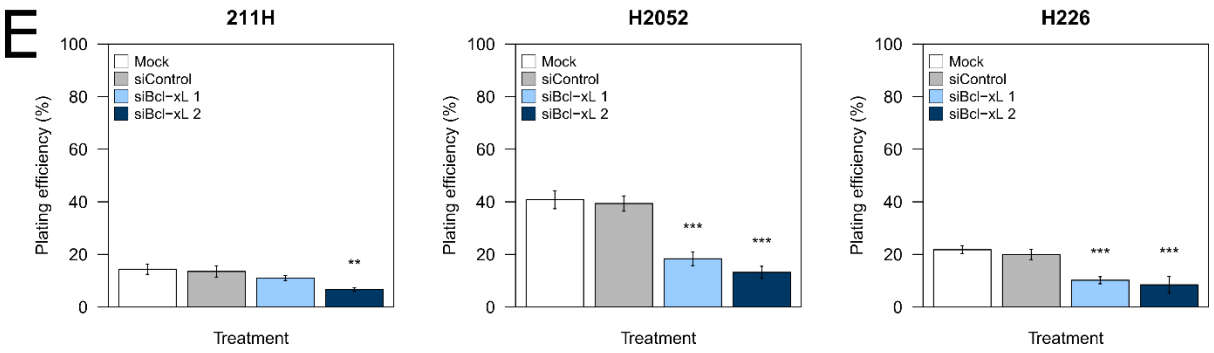
The effect of IR-alone on the viability of MPM cells was determined. Cells were treated with 2 Gy of IR and assayed for relative viability after 96 hours. Bars represent the mean±SD of 3 independent experiments and were compared by t-test. *** P<0.001, ** P<0.01.



Supplementary Figure S3. Induction of MOMP in mesothelioma cells treated with A1331852 and IR.

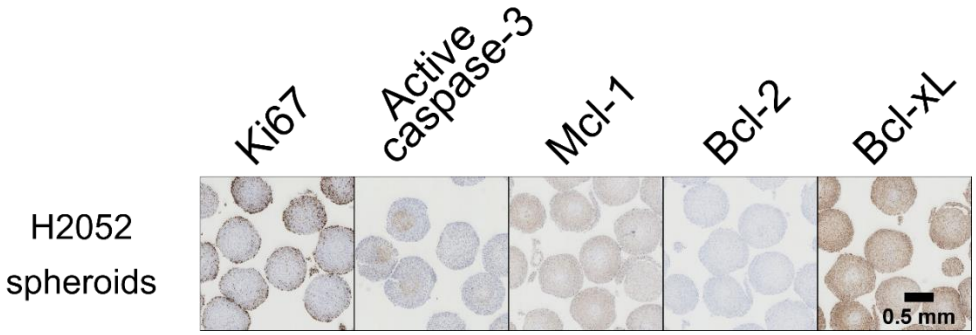
Induction of MOMP was assessed by immunofluorescent staining of Bax (green) and Cytochrome C (red), 8 hours after exposure to A1331852 and IR (2 Gy). Cells undergoing MOMP are characterized by presence of punctate Bax and release of Cytochrome C (arrowheads). Nuclei were counterstained with DAPI (blue), scale bar 50 µm. Images represent 3 independent experiments.


A
211H
H2052
H226
Plating efficiency (%)
Treatment
DMSO
A1331852 (1 µmol/L)
A1155463 (1µmol/L)
A1331852 (0.3 µmol/L)
A1155463 (0.3 µmol/L)
A1331852 (3µmol/L)
A1155463 (3µmol/L)
B
Surviving fraction
Dose (Gy)
SER 1.31
SER 1.65
DMSO
A1155463 (1µmol/L)
A1155463 (0.3µmol/L)
A1155463 (3µmol/L)
C
ABT−263 (1 µmol/L)
ABT−263 (0.3 µmol/L)
ABT−263 (3 µmol/L)
D
SER 1.29
SER 1.34
ABT−263 (1 µmol/L)
ABT−263 (0.3 µmol/L)
ABT−263 (3 µmol/L)



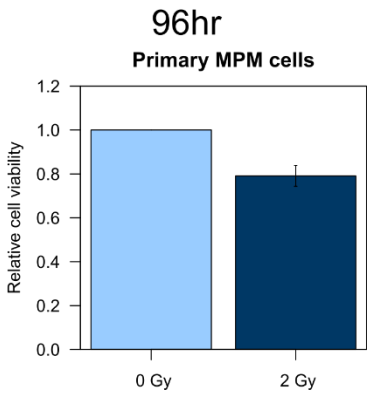
Supplementary Figure S4. Inhibition of Bcl-xL radiosensitizes mesothelioma cells.

(A) Clonogenic plating efficiency was determined for A1331852 and A1155463 treated MPM cells. (B) The clonogenic survival of cells treated with A1155463 and IR was measured. Surviving fraction was calculated using the plating efficiency of vehicle-only or drug-only treated cells. (C) The clonogenic plating efficiency was determined for ABT-263 treated MPM cells. (D) The clonogenic survival of cells treated with ABT-263 and IR was measured. (E) The clonogenic plating efficiency was determined for siRNA treated MPM cells. Bars represent the mean±SD of 3 independent experiments and were compared by one-way ANOVA. Points represent the mean±SD of 3 independent experiments. For clonogenic assays, data were fitted by linear quadratic model and the curves compared by F-test. *** P<0.001, ** P<0.01, ** P<0.05 compared to vehicle (DMSO) or mock.



Supplementary Figure S5. Mesothelioma spheroids express Bcl-xL.

H2052 mesothelioma spheroids were grown for 17 days before fixation, embedding and sectioning. Sections were stained for Ki67, active caspase-3 and Bcl-2 proteins, and were counterstained with haematoxylin.



Supplementary Figure S6. Primary mesothelioma cells treated with IR.

The effect of IR-alone on the viability of primary MPM cells was determined 96 hours after irradiation. Cells were treated with 2 Gy of IR and assayed for relative viability after 4 days. Bars represent the mean±SD of 2 technical replicates from a single experiment.