

Supplementary Materials

Table S1. Chemicals, enzymes and other reagents

Name	Company	Cat. Number
Phenothiazonium salt, methylene blue	Sigma-Aldrich	M9140
Cell lysis buffer (10x)	Cell Signaling Technology	9803
4x Laemmli sample buffer	Bio-RAD	161-0747
Cell staining buffer	BioLegend	420201
Fixation buffer	BioLegend	420801
Collagenase	Merck/Sigma Aldrich	C9407-100 mg
DNase	Merck/Sigma Aldrich	DN25-100 mg
Red Blood cell lysis buffer	Roche	4257400
0.05 % Trypsin-EDTA (1x)	Gibco	25300-054
Cultrex growth factor reduced BME type2 (Matrigel)	Biotechne UK	3533-010-02
Granzyme B inhibitor IV	Calbiochem	36805
WST1 assay kit	Sigma	5015944001
FITC Annexin V Apoptosis Detection	BioLegend	640914
Beads	Invitrogen	01-1111-42

Table S2. Cell media and components

Name	Company	Cat. Number
DMEM	Thermo Fisher Scientific	21969-035
DMEM-F12 (a phenol red-free medium)	Thermo Fisher Scientific	2104025
RPMI 1640	Thermo Fisher Scientific	21875034
Advanced DMEM/F12	Thermo Fisher Scientific	12634028
Foetal bovine serum	Thermo Fisher Scientific	10500064
Penicillin/Streptomycin	Thermo Fisher Scientific	15140122
L-Glutamine	Gibco	25030-024
Recombinant human IL-2 (rhIL-2)	Gibco	PHC0027
LEAF anti-human CD3 antibody	BioLegend	300314
Ultra-LEAF anti-human CD28 antibody	BioLegend	302934

Table S3. Antibodies for immunoblotting

Antibody	Company	Cat. Number
Bcl2	abcam	ab182858
Calreticulin (D3E6)	Cell Signaling Technology	12238
β Actin	Cell Signaling Technology	3700
Rabbit IgG HRP linked	Cell Signalling Technology	7074

Mouse IgG HRP linked	Cell Signaling Technology	7076
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Table S4. Antibodies r immunofluorescence

Antibody	Company	Cat. Number
Calreticulin (D3E6)	Cell Signaling Technology	12238
Granzyme B (496B)	Thermo Fisher Scientific	14-8889-82
Pan-Cytokeratin (AE1/AE3) (PanCK)	abcam	ab27988
Goat anti-Rabbit Ig (Alexa Fluor488)	Thermo Fisher Scientific	A-11012
Donkey anti-Rat IgG H&L (Alexa Fluor647)	abcam	ab1501555
Goat anti-Mouse Ig (Alexa Fluor594)	Thermo Fisher Scientific	A-11032

Table S5. Immunofluorescence probes and dye

Probe/Dye	Company	Cat. Number
GrzB probe H5	The University of Edinburgh	-
ActinGreen 488 ready probes	Thermo Fisher Scientific	R37110
Toto™ 3-Iodide probe	Invitrogen	T3604
Nuc Blue Fixed cells stain ready probe	Thermo Fisher Scientific	R37606
CellEvent caspase3/7 Green Ready Probe Reagent	Thermo Fisher Scientific	R37111
CellTracker™ Red CMTPX dye	Thermo Fisher Scientific	C34552
Zombie-UV kit	BioLegend	423101

Table S6. Antibodies for flow cytometry

Antibody	Company	Cat. Number
PE anti-Calreticulin (EPR3924)	abcam	205977
FITC anti-HLA-ABC	BioLegend	311404
APC anti-CD54 (ICAM1)	BioLegend	353112
APC/Cy7 anti-CD45	BioLegend	368516
FITC anti-CD326 (EpCAM)	BioLegend	324204
BV421 anti-CD8a	BioLegend	301035
FITC anti-CD8a	BioLegend	301006
FITC anti-Mouse IgG isotype control	BioLegend	4002008
PE anti-Rabbit IgG isotype control	abcam	209478
APC anti-Mouse IgG isotype control	BioLegend	400120
BV421 anti-Mouse IgG isotype control	BioLegend	400157

Table S7. Human lung cancer organoid non complete media formulations

ADF+++ media	Working Concentration	Company
Advanced DMEM/F12	1x	Life Technologies

1000x Glutamax	1x	Life Technologies
1M Hepes	1mM	Life Technologies
Penicillin/Streptomycin	100U/130µg per ml	Life Technologies

Table S8. Human lung cancer organoid complete media formulations

Component	Signalling pathway		Supplier	Catalogue number	Final concentration
	Activation	Blocking			
R-Spondin 1	Wnt/β-Catenin signalling		Peprotech	120-38	150 ng·ml ⁻¹
FGF 7	FGFR2b signalling		Peprotech	100-19	25 ng·ml ⁻¹
FGF 10	FGFR2b signalling		Peprotech	100-26	100 ng·ml ⁻¹
Noggin	<i>TGF-β signalling</i>		Peprotech	120-10C	100 ng·ml ⁻¹
hEGF	Growth, proliferation		R&D Systems	PHG0311	50 ng ml ⁻¹
CIR-99021	WNT signalling		Merck	SML1046-25mg	3µM
A83-01	<i>TGF-β signalling</i>		Tocris	2939	100 nM
Y-27632	<i>ROCK signalling</i>		Abmole	Y-27632	10 µM
SB202190	<i>p38 MAPK signalling</i>		Sigma	S7067	10 µM
B27 supplement	Insulin signalling		Gibco	17504-44	1x
N-Acetylcysteine	Antioxidant		Sigma	A9165-5g	1.25 mM
Nicotinamide	Co-enzyme precursor		Sigma	N0636	10 mM
GlutaMax 100x	Nutrient		Invitrogen	12634-034	1x
Hepes	Buffer		Invitrogen	15630-056	10 mM
Penicillin/Streptomycin	Antibiotics		Invitrogen	15140-122	100 U·ml ⁻¹ / 100 µg·ml ⁻¹
Primocin	Antibiotic		Invitrogen	Ant-pm-1	50 µg·ml ⁻¹
Advanced DMEM/F12	Base medium		Invitrogen	12634-034	1x

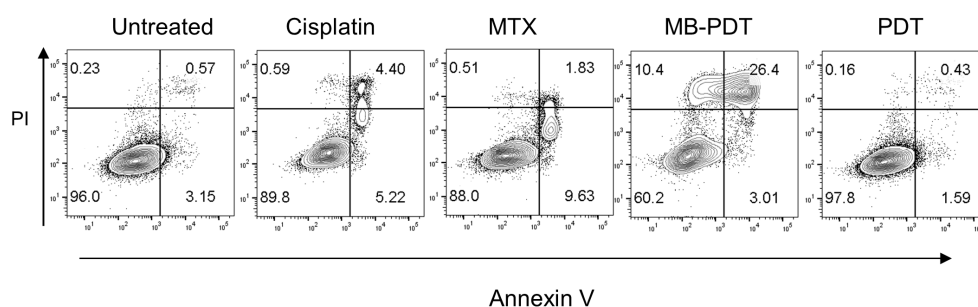


Figure S1. MB-PDT reduced A549 cell viability. Apoptosis demonstrated in A549 cells 24 hours post treatment by Annexin V assay. Representative histograms of flow cytometry showing PI (Y-axis) vs Annexin AF488 (X-axis) acquired on 5L LSR flow cytometry.

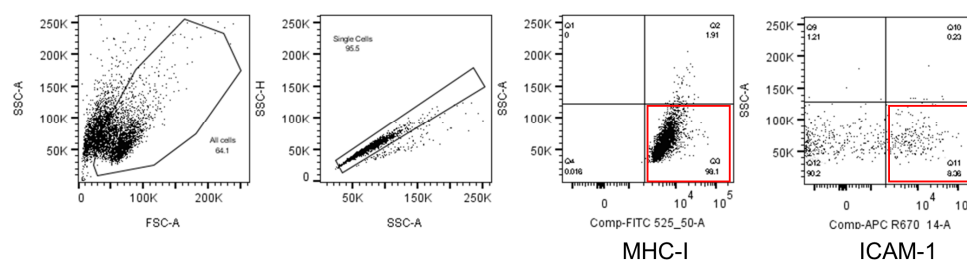


Figure S2. MB-PDT upregulated expression of MHC-I and ICAM-1, as markers of ICD. Gating strategy for flow cytometry of the expression of MHC-I and ICAM-1 in A549 cells 24h post-treatment. The expression of MHC-I and ICAM-1 was investigated in A549 cells 24 and 48 hours post-treatment by flow cytometry. Data were acquired on 5L LSR.

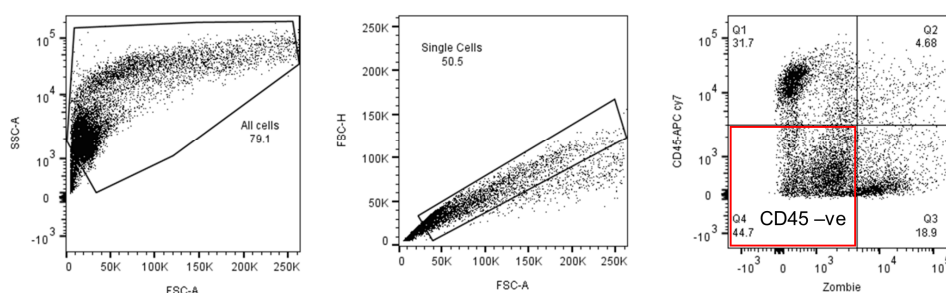


Figure S3. MB-PDT activated cytotoxic effect of CD8⁺ T cells. Gating strategy for flow cytometry of H1299 cells in co-culture with activated CD8⁺ T cells for 48 hours. Pre-gating was done on CD45-APC-cy7 cells and Zombie according to their light scatter characteristics using the 5L LSR flow cytometry.