

Supplementary Data

Isothiocyanates potentiate Tazemetostat-induced apoptosis by modulating the expression of apoptotic genes, members of Polycomb Repressive Complex 2 and levels of trimethylating lysine 27, at Histone 3, in human malignant melanoma cells

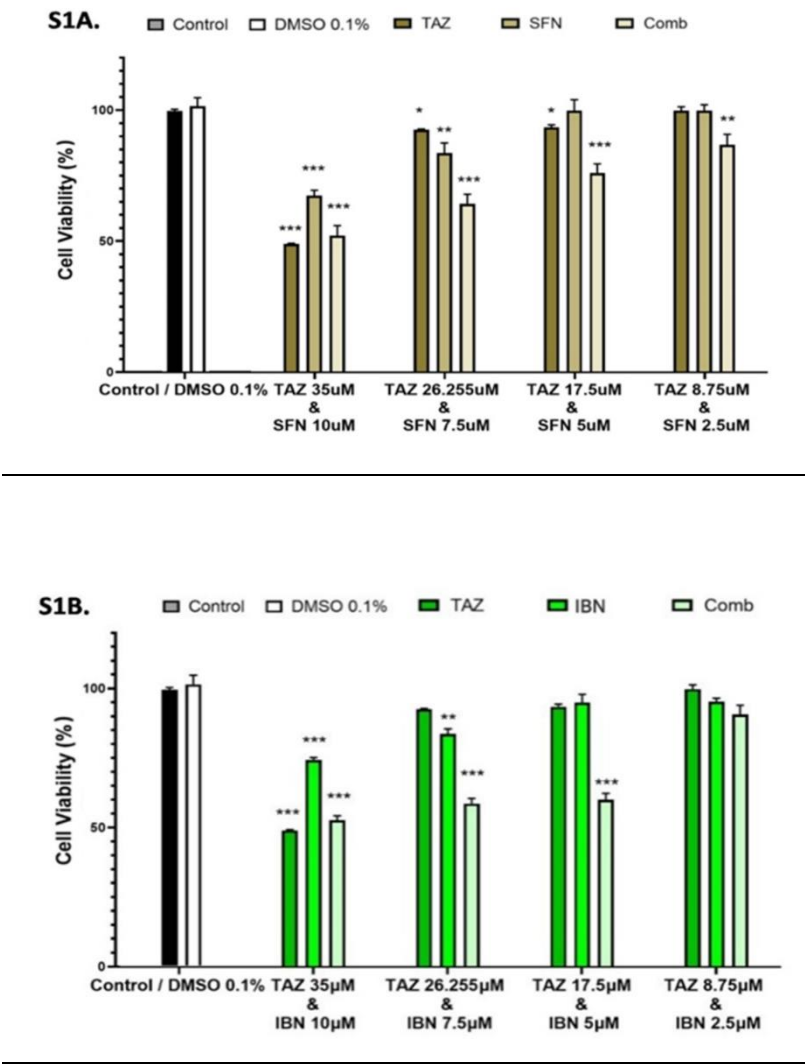
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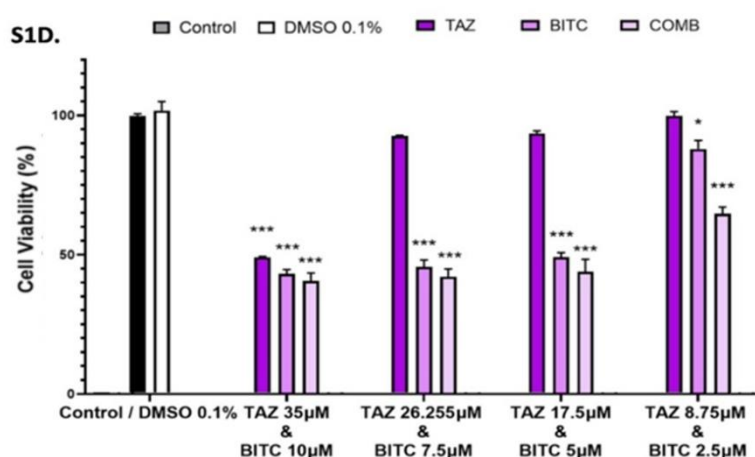
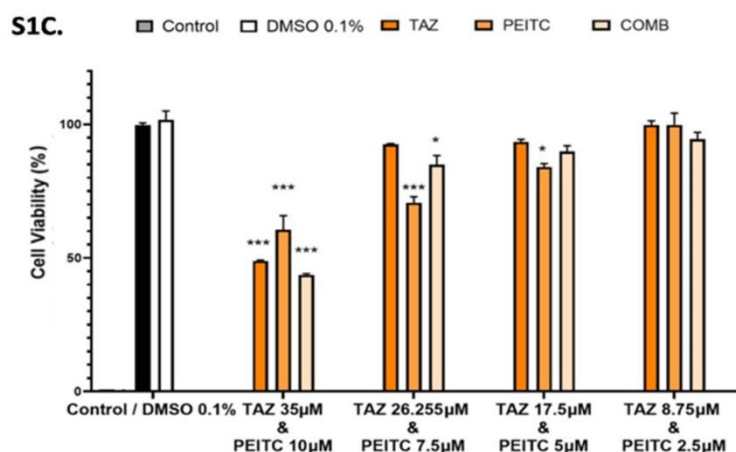
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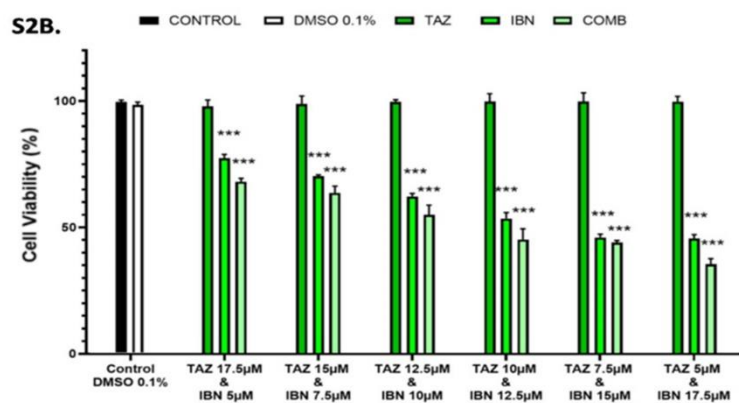
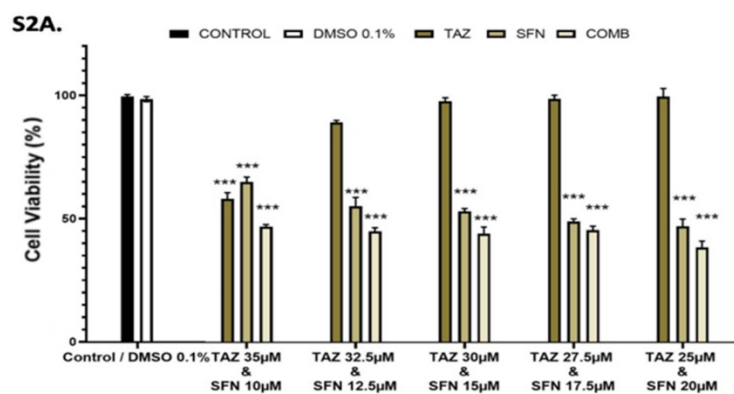
Figure S1

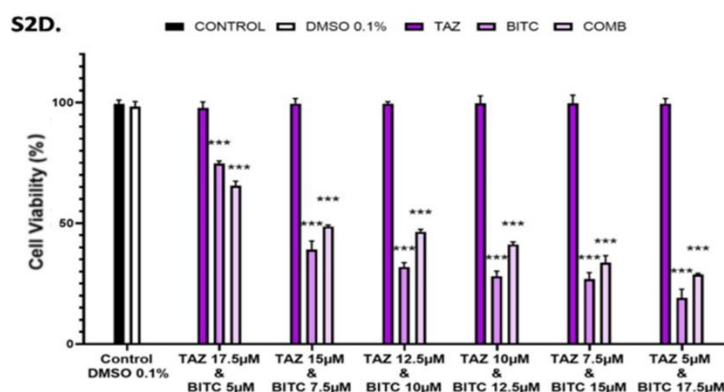
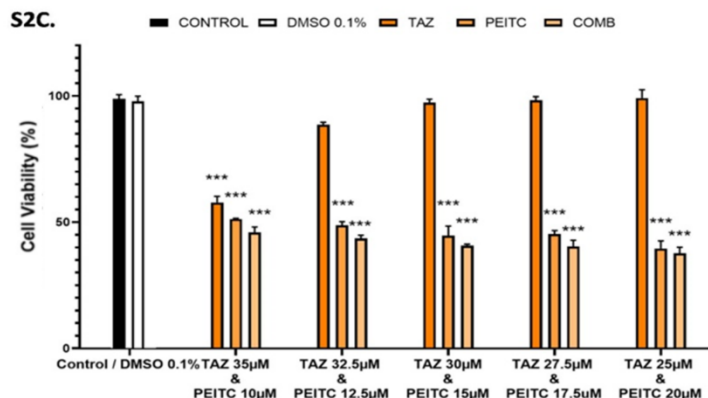




Cell viability levels of A375 cells under Exposure Protocol 1. Cell viability levels of A375 cells were recorded following different exposure conditions between TAZ alone, each ITC alone or between TAZ combined with SFN (S1A), IBN (S1B), PEITC (S1C) and BEITC (S1D). Specifically, combinational exposure protocols between TAZ and each ITC were applied as follows: i) 100% the EC_{50} concentration of TAZ (35µM) : 100% the EC_{50} value of each ITC (10µM); ii) 75% of the EC_{50} concentration of TAZ (26.255µM) : 75% of EC_{50} value of each ITC (7.55µM); iii) 50% of EC_{50} concentration of TAZ (17.5µM) : 50% of EC_{50} value of each ITC (5µM); iv) 25% of EC_{50} concentration of TAZ (6.75µM) : 25% of EC_{50} value of each ITC (2.5µM). Cell viability was determined by using the Alamar Blue assay. All data are expressed as means of five replicates \pm SEM and are representative of three independent experiments. Statistical significance is indicated by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ relative to the corresponding 0.1% DMSO controls.

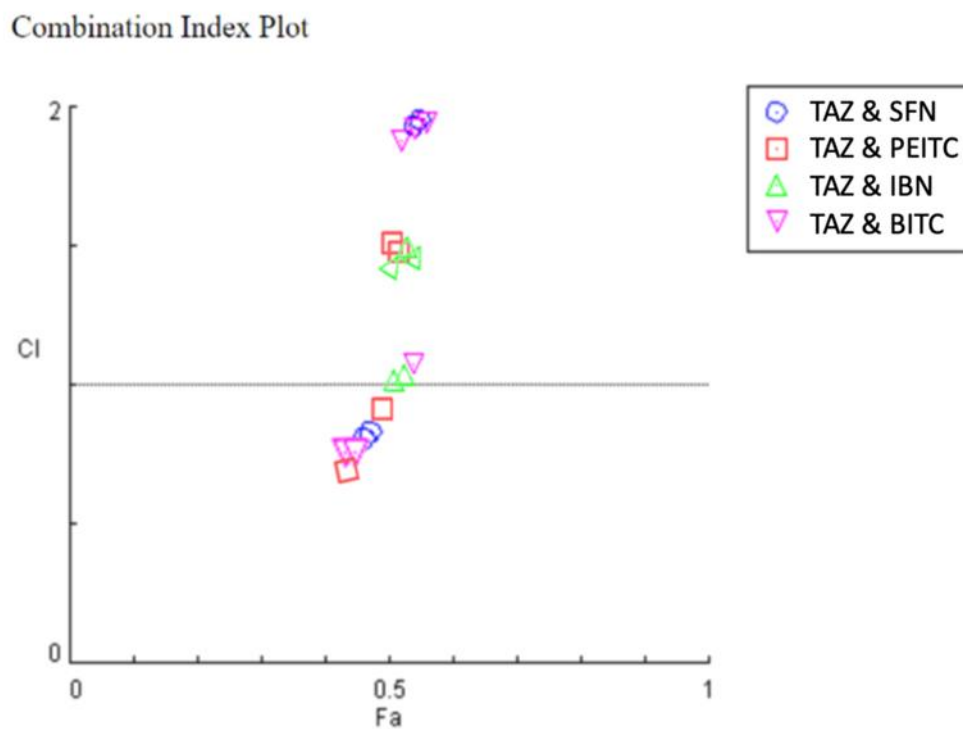
Figure S2





Cell viability levels of A375 cells under Exposure Protocol 2. Cell viability levels of A375 cells were recorded following different exposure conditions between TAZ alone, each ITC alone or between TAZ combined with SFN (S2A), IBN (S2B), PEITC (S2C) and BEITC (S2D). Specifically, A375 cells were exposed to gradually decreasing concentrations of the EC₅₀ concentration of TAZ (by 2.5 μM) together with a parallel gradual increase of the EC₅₀ concentrations of each ITC (by 2.5 μM) until reached a final concentration of 20 μM, at the most. All data are expressed as means of five replicates ± SEM and are representative of three independent experiments. Statistical significance is indicated by *p<0.05, **p<0.01, ***p<0.001 relative to the corresponding 0.1% DMSO controls.

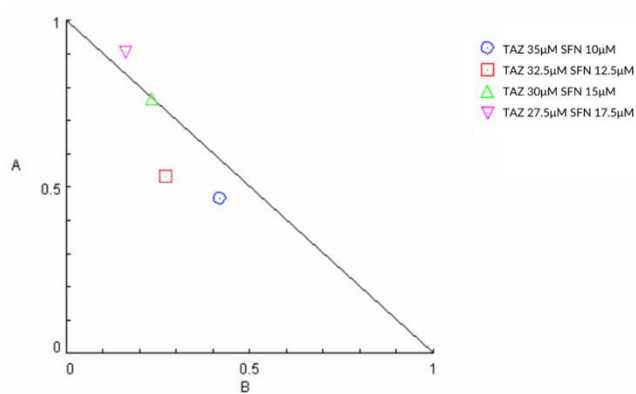
Figure S3



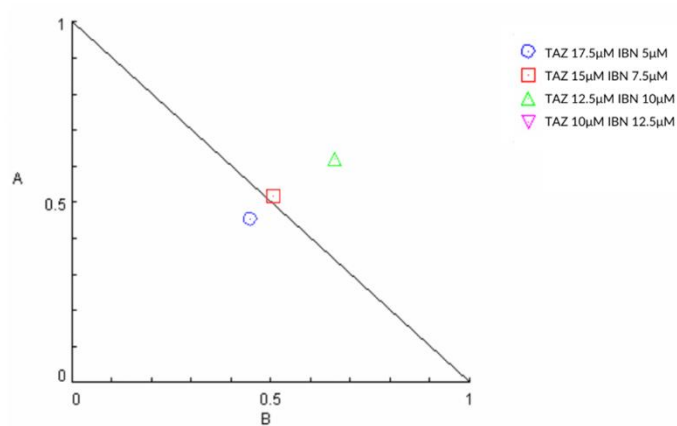
Combination Index plot of combinatorial treatments of TAZ with different ITCs in A375 cells. Combination Index (CI) plot descriptive of the combinatorial exposure of TAZ with each ITC against A375 melanoma cells. The interaction of each individual combinatorial exposure was measured using the CompuSyn software, V.20 (Biosoft, Cambridge, UK) where $CI < 1$ is descriptive of a synergistic, $CI = 1$ an additive and $CI > 1$ an antagonistic interaction.

Figure S4.

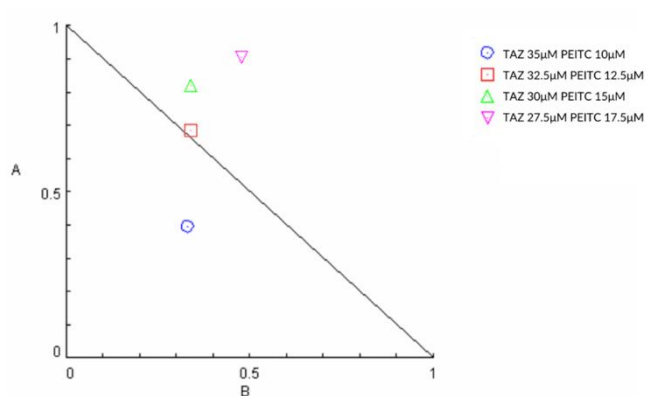
S4A. Isobologram TAZ+SFN



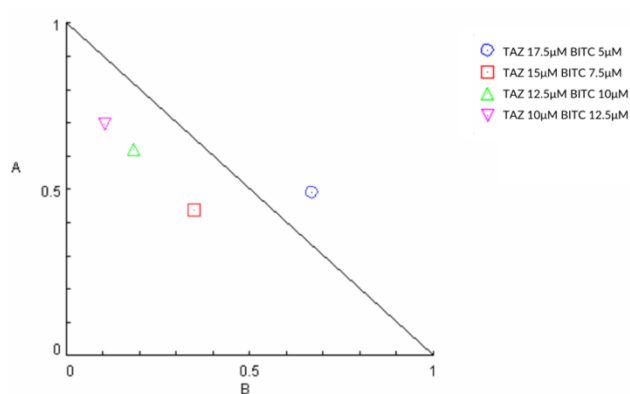
S4B. Isobologram TAZ+IBN



S4C. Isobologram TAZ+PEITC



S4D. Isobologram TAZ+BITC



Normalized isobolograms showing the interaction between TAZ and different ITCs in inhibiting cell growth of A375 melanoma cells. Isobologram analysis was based on the results of cell viability, following treatments of A375 cells with combined exposure conditions of TAZ and SFN (A), IBN (B), PEITC (C) and BITC (D), according to experimental protocol 2 (Fig. S2). Combination data points that are below the line are synergistic, above the line are antagonistic and on the line are additive. The interaction of each individual combinatorial exposure was measured using the CompuSyn software, V.20 (Biosoft, Cambridge, UK).

Table S1. Cell viability levels following various combinatorial exposure protocols with TAZ and each of the ITCs in HaCaT cells. The rationale for the indicated combinatorial exposure conditions was based on their efficacy to reduce the viability levels of A375 cells around their EC₅₀ value (45-65%) while inducing a safe cytotoxic profile against HaCaT cells (over 80%). To this end, bold highlighted conditions were selected, as optimum, and utilized in the next series of experiments. All data are expressed as means of five replicates \pm SEM and are representative of three independent experiments.

EXPOSURE PROTOCOL	CELL VIABILITY LEVELS
Control	100.00 \pm 2.89
0.1% DMSO	98.10 \pm 2.32
TAZ (35.0μM)+ SFN (10.0μM)	86.51 \pm 2.00
TAZ (32.5 μ M)+ SFN (12.5 μ M)	79.37 \pm 0.90
TAZ (30.0 μ M)+ SFN (15.0 μ M)	63.01 \pm 3.19
TAZ (15.0μM)+ IBN (7.5μM)	90.12 \pm 1.85
TAZ (12.5 μ M)+ IBN (10.0 μ M)	79.39 \pm 1.63
TAZ (10.0 μ M)+ IBN (12.5 μ M)	72.34 \pm 0.97
TAZ (35.0μM)+ PEITC (10.0μM)	91.11 \pm 1.80
TAZ (32.5 μ M)+ PEITC (12.5 μ M)	75.01 \pm 3.23
TAZ (30.0 μ M)+ PEITC (15.0 μ M)	69.06 \pm 4.28
TAZ (15.0μM)+ BITC (7.5μM)	82.45 \pm 2.97

TAZ (12.5μM)+ BITC (10.0μM)	79.55 ± 4.00
TAZ (10.0μM)+ BITC (12.5μM)	73.62 ± 4.06

Table S2. Fold change in expression levels of genes involved in intrinsic and extrinsic apoptosis in A375 cells exposed, for 48h, to TAZ as a single agent and/or in combined exposures with each ITC. Fold changes in gene expression levels were determined by Real-Time PCR (RT-PCR). All data are expressed as means of three replicates ± SEM and are representative of three independent experiments. Gene expression data were normalized to β-actin, using the 2-ΔΔCt method and were expressed as fold-change compared to untreated (control) samples.

INTRINSIC PATHWAY					
	TAZ	TAZ+SFN	TAZ+IBN	TAZ+PEITC	TAZ+BITC
<i>CASP3</i>	1.515±0.019	1.888±0.052	1.78±0.001	1.852±0.002	1.81±0.068
<i>BID</i>	0.99±0.020	1.114±0.033	1.337±0.038	1.08±0.032	1.576±0.002
<i>BAX</i>	2.44±0.006	2.062±0.080	1.752±0.067	1.83±0.012	2.16±0.002
<i>XIAP</i>	0.89±0.001	0.59±0.029	0.742±0.137	0.84±0.210	0.705±0.190
<i>PMAIP1</i>	1.57±0.900	1.0877±0.012	1.147±0.058	0.942±0.002	1.212±0.015
<i>BAD</i>	1.24±1.160	2.539±0.305	4.635±0.002	2.1±0.342	1.786±0.642
<i>CYCS</i>	1.22±0.009	1.314±0.122	1.348±0.002	1.42±0.124	1.568±0.095
<i>BAK1</i>	5.064±0.021	2.26±0.860	5.301±0.127	1.19±0.089	3.17±0.011
<i>APAF1</i>	4.992±0.032	5.902±0.100	5.121±0.100	6.21±0.901	6.121±0.567
<i>CASP9</i>	1.229±0.403	1.166±0.089	1.786±0.040	1.09±0.003	1.642±0.012
<i>CASP2</i>	2.745±0.395	2.37±0.036	2.194±0.025	2.42±0.213	2.173±0.075
<i>DIABLO (SMAC)</i>	1.896±0.058	1.075±0.019	1.608±0.007	1.103±0.013	1.426±0.008
<i>BCL2L1 (BIM)</i>	0.082±0.009	1.371±0.076	1.355±0.124	1.124±0.035	1.310±0.039
<i>CASP6</i>	1.494±0.038	2.063±0.256	2.495±0.155	2.032±0.421	5.211±0.081
<i>CASP7</i>	1.05±0.102	1.103±0.562	1.058±0.139	1.432±0.242	1.037±0.024
<i>MCL1</i>	0.104±0.021	0.592±0.066	0.077±0.039	0.720±0.042	0.966±0.064

<i>BCL2</i>	1.029±0.030	1.016±0.480	0.980±0.020	1.092±0.132	1.033±0.052
<i>BCL2L1</i>	0.439±0.039	0.972±0.003	1.099±0.031	0.801±0.002	0.745±0.0355

EXTRINSIC PATHWAY					
	TAZ	TAZ+SFN	TAZ+IBN	TAZ+PEITC	TAZ+BITC
<i>FASL</i>	1.102±0.096	1.002±0.515	1.003±0.095	1.142±0.002	1.022±0.351
<i>FAS</i>	1.375±0.124	1.039±0.742	1.393±0.145	1.462±0.075	0.879±0.052
<i>FAIM</i>	1.045±0.040	0.838±0.481	0.916±0.145	0.920±0.003	0.913±0.012
<i>TNFRSF10</i> (<i>TRAIL</i>)	2.107±0.415	1.612±0.762	6.268±0.081	1.529±0.052	1.990±0.137
<i>TNFRSF10D</i>	0.745±0.395	0.837±0.036	0.794±0.025	0.842±0.213	0.873±0.075
<i>TNFRSF10C</i>	0.839±0.162	0.836±0.014	0.907±0.163	0.739±0.034	0.943±0.005
<i>TNFRSF10CB</i>	1.048±0.325	1.037±0.021	1.065±0.133	1.092±0.031	0.913±0.061
<i>TNFRSF10A</i>	1.622±0.001	1.348±0.047	1.160±0.070	1.423±0.004	1.450±0.091
<i>FADD</i>	1.102±0.001	1.068±0.133	1.047±0.120	1.342±0.103	1.052±0.188
<i>CASPASE8</i>	1.57±0.900	1.877±0.012	1.547±0.058	1.942±0.002	1.612±0.015
<i>CASPASE 10</i>	1.22±0.009	1.314±0.122	1.348±0.002	1.420±0.124	1.568±0.095
<i>C-FLAR</i>	0.351±0.075	0.303±0.009	0.406±0.049	0.323±0.122	0.461±0.026
<i>TNF</i>	1.657±0.463	2.147±0.038	2.090±0.020	2.682±0.001	1.583±0.354
<i>TNFRSF1A</i> (<i>TNFR1</i>)	1.55±0.928	9.143±0.051	5.214±0.008	5.130±0.053	4.655±0.020
<i>TRADD</i>	4.992±0.032	5.902±0.100	5.121±0.100	6.210±0.901	6.121±0.567
<i>TRAF2</i>	3.252±0.347	1.331±0.054	2.514±0.028	1.582±0.042	1.092±0.055
<i>BIRC2</i> (<i>CIAP1</i>)	1.412±0.026	0.795±0.074	0.825±0.024	0.069±0.120	0.610±0.077
<i>BIRC3</i> (<i>CIAP2</i>)	0.676±0.054	0.495±0.174	0.401±0.174	0.492±0.005	0.592±0.303
<i>TRAF1</i>	0.821±0.141	0.992±0.050	1.010±0.625	1.021±0.013	1.272±0.426
<i>TRAF5</i>	1.44±0.699	5.469±0.403	3.855±0.658	5.992±0.341	1.405±0.017
<i>TNFAIP3/A20</i>	0.902±0.001	0.899±0.029	0.842±0.137	0.732±0.210	0.705±0.190

Table S3. Fold change in expression levels of genes involved in intrinsic and extrinsic apoptosis in Colo-679 cells exposed, for 48h, to TAZ as a single agent and/or in combined exposures with each ITC. Fold changes in gene expression levels were determined by Real-Time PCR (RT-PCR). All data are expressed as means of three replicates \pm SEM and are representative of three independent experiments. Gene expression data were normalized to β -actin, using the $2^{-\Delta\Delta C_t}$ method and were expressed as fold-change compared to untreated (control) samples.

INTRINSIC PATHWAY					
	TAZ	TAZ+SFN	TAZ+IBN	TAZ+PEITC	TAZ+BITC
<i>CASP3</i>	1.219\pm0.031	1.530\pm0.014	1.359\pm0.032	1.629\pm0.328	1.721\pm0.032
<i>BID</i>	1.241\pm0.039	1.429\pm0.039	1.429\pm0.012	1.420\pm0.932	1.672\pm0.084
<i>BAX</i>	1.924\pm0.042	1.948\pm0.029	1.602\pm0.892	2.019\pm0.382	1.982\pm0.042
<i>XIAP</i>	1.121\pm0.029	0.913\pm0.079	0.929\pm0.249	0.719\pm0.029	0.902\pm0.249
<i>PMAIP1</i>	1.103\pm0.204	1.231\pm0.029	1.001\pm0.039	1.429\pm0.329	1.182\pm0.092
<i>BAD</i>	0.924\pm0.021	2.012\pm0.039	2.952\pm0.042	2.443\pm0.042	1.292\pm0.034
<i>CYCS</i>	1.029\pm0.029	1.210\pm0.082	1.492\pm0.095	1.329\pm0.320	1.029\pm0.291
<i>BAK1</i>	2.293\pm0.129	2.420\pm0.193	1.234\pm0.391	1.829\pm0.093	2.219\pm0.032
<i>APAF1</i>	3.211\pm0.013	3.992\pm0.031	4.829\pm0.009	4.90\pm0.003	5.230\pm0.002
<i>CASP9</i>	1.682\pm0.311	1.428\pm0.021	1.929\pm0.032	1.429\pm0.203	1.824\pm0.249
<i>CASP2</i>	1.839\pm0.049	3.572\pm0.103	2.453\pm0.042	2.824\pm0.192	3.529\pm0.127
<i>DIABLO</i> (SMAC)	1.244\pm0.049	1.294\pm0.294	1.432\pm0.029	1.320\pm0.032	1.120\pm0.039
<i>BCL2L1</i> (BIM)	0.903\pm0.024	1.423\pm0.049	1.329\pm0.082	1.439\pm0.049	1.728\pm0.039
<i>CASP6</i>	1.210\pm0.042	1.932\pm0.194	1.329\pm0.032	1.235\pm0.241	2.103\pm0.391
<i>CASP7</i>	1.330\pm0.432	1.492\pm0.239	1.029\pm0.029	1.782\pm0.092	1.324\pm0.042

<i>MCL1</i>	0.429 ±0.249	0.892 ±0.023	0.530 ±0.040	0.920 ±0.093	1.103 ±0.078
<i>BCL2</i>	1.392 ±0.204	1.284 ±0.029	1.392 ±0.920	1.923 ±0.033	1.299 ±0.029
<i>BCL2L1</i>	0.892 ±0.042	0.823 ±0.320	0.929 ±0.194	0.702 ±0.320	1.203 ±0.013

EXTRINSIC PATHWAY					
	TAZ	TAZ+SFN	TAZ+IBN	TAZ+PEITC	TAZ+BITC
<i>FASL</i>	1.209 ±0.024	1.382 ±0.329	1.214 ±0.029	1.492 ±0.492	1.102 ±0.293
<i>FAS</i>	1.637 ±0.294	1.392 ±0.424	1.628 ±0.049	1.104 ±0.029	1.210 ±0.091
<i>FAIM</i>	1.103 ±0.043	1.121 ±0.032	0.820 ±0.140	0.792 ±0.129	0.924 ±0.029
<i>TNFRSF10</i> (<i>TRAIL</i>)	1.723 ±0.028	1.820 ±0.293	1.942 ±0.099	1.992 ±0.320	2.492 ±0.320
<i>TNFRSF10D</i>	1.102 ±0.293	1.021 ±0.029	0.920 ±0.092	0.992 ±0.104	1.201 ±0.039
<i>TNFRSF10C</i>	0.920 ±0.439	0.942 ±0.294	0.603 ±0.240	0.829 ±0.312	1.243 ±0.021
<i>TNFRSF10CB</i>	1.240 ±0.421	1.102 ±0.219	1.293 ±0.320	1.112 ±0.043	1.210 ±0.091
<i>TNFRSF10A</i>	1.213 ±0.029	1.429 ±0.093	1.482 ±0.329	1.392 ±0.328	1.943 ±0.201
<i>FADD</i>	1.204 ±0.391	1.039 ±0.019	1.102 ±0.102	1.428 ±0.210	1.129 ±0.210
<i>CASPASE8</i>	1.319 ±0.219	1.219 ±0.013	1.928 ±0.328	1.221 ±0.039	1.567 ±0.032
<i>CASPASE 10</i>	1.201 ±0.032	1.426 ±0.043	1.232 ±0.094	1.219 ±0.001	1.928 ±0.210
<i>C-FLAR</i>	0.582 ±0.192	0.792 ±0.021	0.672 ±0.024	0.910 ±0.211	0.674 ±0.031
<i>TNF</i>	1.205 ±0.059	2.882 ±0.002	2.320 ±0.149	2.253 ±0.112	1.924 ±0.032
<i>TNFRSF1A</i> (<i>TNFR1</i>)	1.832 ±0.053	3.249 ±0.104	3.243 ±0.003	4.243 ±0.043	5.252 ±0.039
<i>TRADD</i>	3.214 ±0.104	6.254 ±0.130	5.043 ±0.129	4.942 ±0.001	4.543 ±0.018
<i>TRAF2</i>	2.193 ±0.021	1.392 ±0.039	1.928 ±0.049	1.928 ±0.073	1.674 ±0.023
<i>BIRC2</i> (<i>CIAP1</i>)	1.829 ±0.037	1.203 ±0.206	0.922 ±0.042	0.822 ±0.102	0.782 ±0.001
<i>BIRC3</i> (<i>CIAP2</i>)	0.892 ±0.002	0.864 ±0.027	0.804 ±0.094	0.792 ±0.067	0.881 ±0.012
<i>TRAF1</i>	0.955 ±0.032	1.103 ±0.003	1.142 ±0.032	0.921 ±0.094	0.892 ±0.074
<i>TRAF5</i>	1.730 ±0.004	4.357 ±0.027	2.934 ±0.003	3.452 ±0.039	1.839 ±0.092
<i>TNFAIP3/A20</i>	0.993 ±0.024	0.928 ±0.018	0.911 ±0.049	0.892 ±0.032	0.983 ±0.072

Table S4. List of primer sequences used in RT-PCR experiments.

GENE	FORWARD PRIMER	REVERSE PRIMER
INTRINSIC APOPTOTIC PATHWAY		
<i>CASP2</i>	5'- ATTGGATCCCTTGGGCACCTC-3'	5' ATCATGTCTGAGCGCGTGGG-3'
<i>CASP3</i>	5' TTATTCAGGCCTGCCGTGGT-3'	5'- AGCATGGCACAAAGCGACTG-3'
<i>CASP6</i>	5- TTTGGCTGCAATGAGCTCGG-3'	5'- GGCATCTGCGTGGCTAACAG-3'
<i>CASP7</i>	5'- AAGCTGAGGGAGCGTCCTAC-3'	5- ACCGGTCTGGCTTAGCATCC-3'
<i>CASP9</i>	5'-CGGTGACGCAAGAGCGAATC-3'	5- GATCAGCTGCCTGGCCTGAT-3'
<i>APAF1</i>	5'- GTTGGGTTCATGGTGTGATG-3'	5'- TTTGTCTCCCAGAGCCTGA-3'
<i>BAD</i>	5'- TCAGGGGCCTCGAGATCGG-3'	5'- TCCTGCTCACTCGGCTCAAA-3'
<i>BAK1</i>	5'- AGACCTGAAAAATGGCTTCG	5'- CGGAAAACCTCCTCTGTGTC-3'
<i>BCL2</i>	5'- AGTACCTGAACCGGCACCT-3'	5'- GCCGTACAGTTCCACAAAGG-3'
<i>BID</i>	5'- TGCAGCTCAGGAACACCA-3'	5'-TCTCCATGTCTCTAGGGTAGGC-3'
<i>BAX</i>	5'- ACCTTGACTTGATTAGTGCCTTCT-3'	5'- GGGTCATCAATGAACTTGAGC-3'
<i>XIAP</i>	5'- GCAAGAGCTCAAGGAGACCA-3'	5- AAGGGTATTAGGATGGGAGTTCA-3'
<i>MCL1</i>	5'- TTACGACGGGTGTTGGGGATGG-3'	5'- CTGCCCCAGTTTGTTACGCC-3'
<i>CYCS</i>	5'- TTCGGAGCGGGAGTGTTTCGT-3'	5'- TGTGGCACTGGGAACACTTCAT-3'

<i>DIABLO</i>	5'- TGACTGCAGTTGGTCTTTCAG-3'	5'- GCGGTTATAGAGGCCTGATCT-3'
<i>BCL2L11</i>	5'- ACTGGAGAGCTCATTGCAGAC-3'	5'- AAATACCAGGACCCGAAGGT-3'
<i>BCL2L1</i>	5'- CGTGGAAAGCGTAGACAAG-3'	5'- AAGAGTGAGCCCAGCAGAA-3'
<i>PMAIP1</i>	5'- CAAGAACGCTCAACCGAGCC-3'	5'- AGGAGTCCCCTCATGCAAGT-3'
EXTRINSIC APOPTOTIC PATHWAY		
<i>CASP8</i>	5'- GGCGGAGGGTCGATCATCTAT-3'	5'- TTCCTTCTCCCAGGATGACCC-3'
<i>CASP10</i>	5'- GGTAATAATGAGGGCAGCTGTGT-3'	5'- TGAACCCAAGCCACTGGAACA-3'
<i>FAS</i>	5'- TTTTCCTCATGGCTTCACCT-3'	5'- ATGTGGCTGTGCTCATTGAC-3'
<i>BIRC2</i>	5'- ATCGTGCGTCAGAGTGAGC-3'	5'- CGCCGACAAGGAGATACG-3'
<i>BIRC3</i>	5'- GCTTGTCTTGCTGGTGCAT-3'	5'- TCCCGAGATTAGACTAAGTCCCTT-3'
<i>C-FLAR</i>	5'- TCCATCTTGGGTGCGCCTTC-3'	5'- TCCGGGCCAGTCAACAGAAA-3'
<i>FADD</i>	5'- CCGAGCTCAAGTTCCTATGC-3'	5'- AGGTCTAGGCCGCTCTGC-3'
<i>FAIM2</i>	5'- ATCGGAGCGAAGCAGAGAGG-3'	5'- CACAGTGGTTGAGCATGGGC-3'
<i>FASLG</i>	5'- GAGTCTACCAGCCAGATGCACA-3'	5'- AGGCATGGACCTTGAGTTGG-3'
<i>TNFRSF10A</i>	5'- TACGCCCTGGAGTGACATCG-3'	5'- GACCCAAGCGCCAGAAACAC-3'
<i>TNFRSF10C</i>	5'- CCCTAAAGTTCGTCGTCGTC-3'	5'- TGGTGGCAGAGTAAGCTAGGA-3'
<i>TNF</i>	5'- TGCACTTTGGAGTGATCGGC-3'	5'- TTGTCACTCGGGGTTCGAGA-3'
<i>TNFAIP3</i>	5'- TGCACACTGTGTTTCATCGAG-3'	5'- ACGCTGTGGGACTGACTTTC-3'
<i>TNFRSF10D</i>	5'- AAGTTCGTCGTCTTCATCGTC-3'	5'- GATGGTGGCAGAGTCAACC-3'
<i>TNFRSF1B</i>	5'- CCAGTGCGTTGGACAGAAGG-3'	5'- ATGGCCACCAGGGGAAGAAT-3'
<i>TNFRSF10</i>	5'- TCACAGTGCTCCTGCAGTCT-3'	5'- GCCACTTTTGGAGTACTTGTCC-3'
<i>TRADD</i>	5'- TAGTGCAGCAGGAGGTGAGATG-3'	5'- CCAGCGAGGACTCCACAAAC-3'
<i>TRAF2</i>	5'- GCATACCCGCCATCTTCTC-3'	5'- CGCCGTTCAAGGTAGATACG-3'
<i>TRAF5</i>	5'- ATGCCACTTCCCTACTGCTC-3'	5'- CATGGCCCATACTCATCCTT-3'