

Supplementary Material

Title: Plant compounds inhibit the growth of cervical precancer cells containing episomal or integrant HPV DNA; tanshinone IIA synergizes with curcumin in cervical cancer cells

1. Combinations of turmeric (95% curcuminoids) plus ginger or carrageenan

1.1 Combination of turmeric (95% curcuminoids) plus ginger

. When increasing concentrations of both turmeric (95% curcuminoids) and ginger were combined, at a dose of ginger 0.8 $\mu\text{g/ml}$, the percent viable cells decreased from 92.64% with ginger alone to 82.29% with curcumin 0.2 $\mu\text{g/ml}$, to 73.22% with curcumin 0.8 $\mu\text{g/ml}$, to 55.37% with curcumin 2.0 $\mu\text{g/ml}$, to 11.40% with curcumin 10 $\mu\text{g/ml}$ ($p < 0.01$) ((Fig. 5 A,B). Thus, curcumin enhances the growth inhibitory effect of ginger on the human cervical cancer cell line Hela.

1.2 Combination of turmeric (95% curcuminoids) plus carrageenan

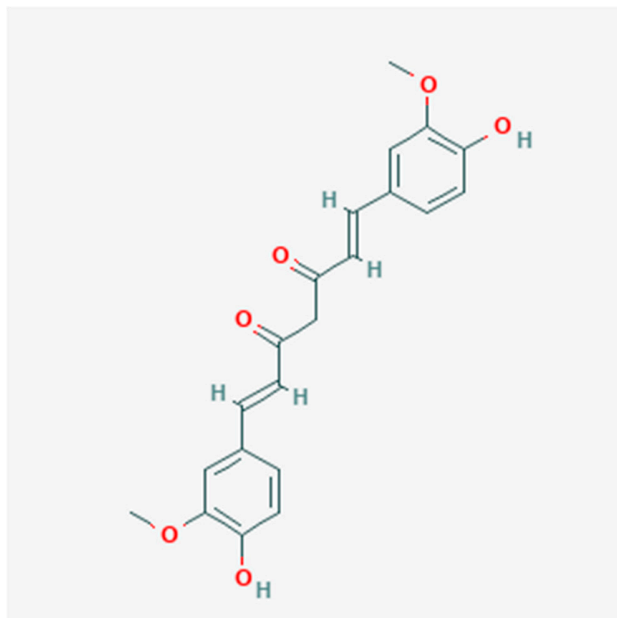
When increasing concentrations of both turmeric (95% curcuminoids) and carrageenan were combined, at a dose of carrageenan 2 μM , the percent viable cells decreased from 48.5% with carrageenan alone to 37.2% with curcumin 0.1 $\mu\text{g/ml}$, to 49.9% with curcumin 0.4 $\mu\text{g/ml}$, to 32.8% with curcumin 1.6 $\mu\text{g/ml}$, to 19.6% with curcumin 4 $\mu\text{g/ml}$ ($p < 0.01$) (Fig. 5 C,D). Thus, curcumin enhances the growth inhibitory effect of carrageenan on the human cervical cancer cell line Hela.

Supplementary Material Figures

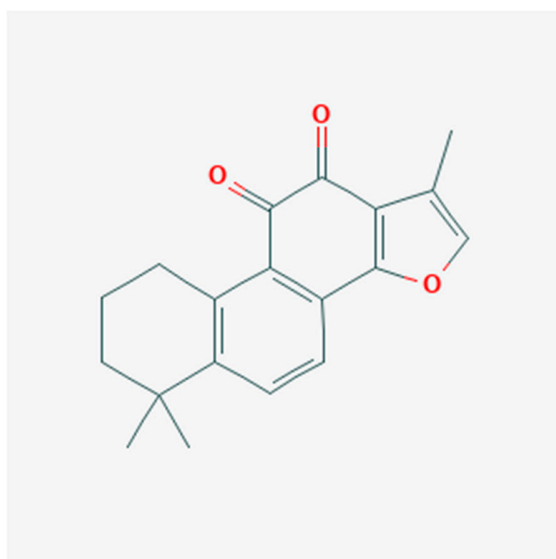
Figure S1. Structures of curcumin, tanshinone IIA and dihydromethysticin and HPLC of turmeric (95% curcuminoids)

- A. Curcumin
- B. Tanshinone IIA
- C. Dihydromethysticin
- D. HPLC of turmeric (95% curcuminoids)

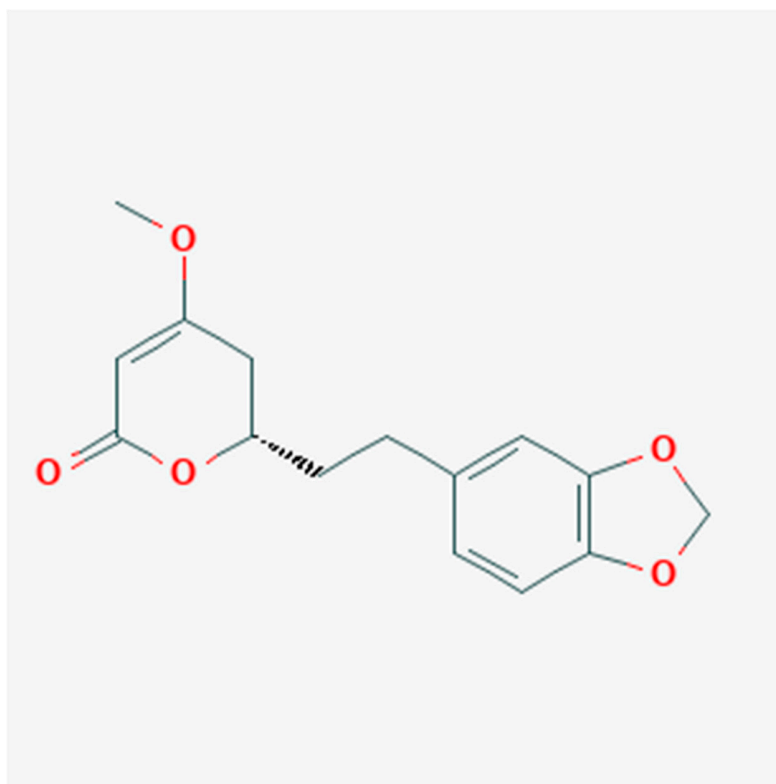
A)



B)



C)



D)

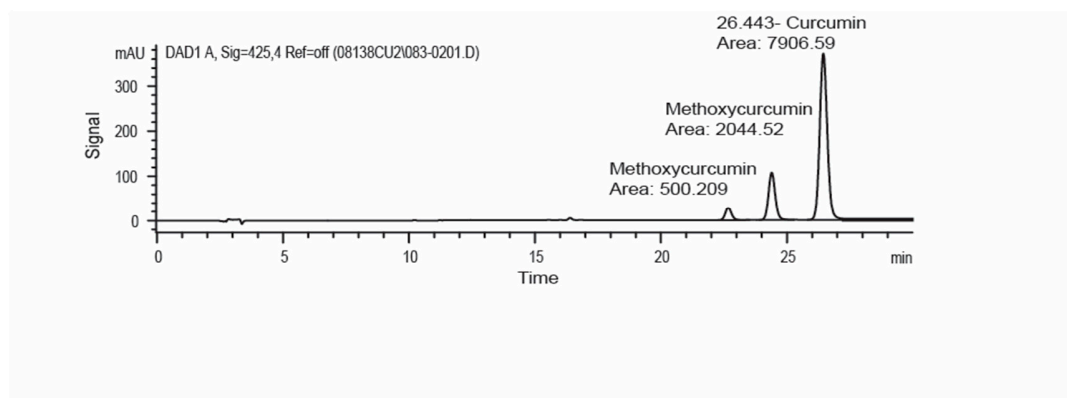


Figure S2. Growth inhibitory activity of combinations of herbal components: turmeric (95% curcuminoids) and A,B) ginger; C,D) carrageenan, on HeLa cells

A,B) Combination of turmeric (95% curcuminoids) plus ginger;

A) x-axis: (95% curcuminoids);;

B) x-axis; ginger (20% total pungent compounds calculated as gingerols and shogaol)

C,D) Combination of turmeric (95% curcuminoids) plus carrageenan;

C) x-axis: (95% curcuminoids);

D) x-axis; carrageenan;

We treated HeLa cells with all combinations of 4 concentrations of each of the agents tested and a solvent control [19]. Cells were exposed to increasing concentrations of agents for 96 h and the number of viable cells determined by: A,B) EZQUANT assay; C,D) MTT assay;

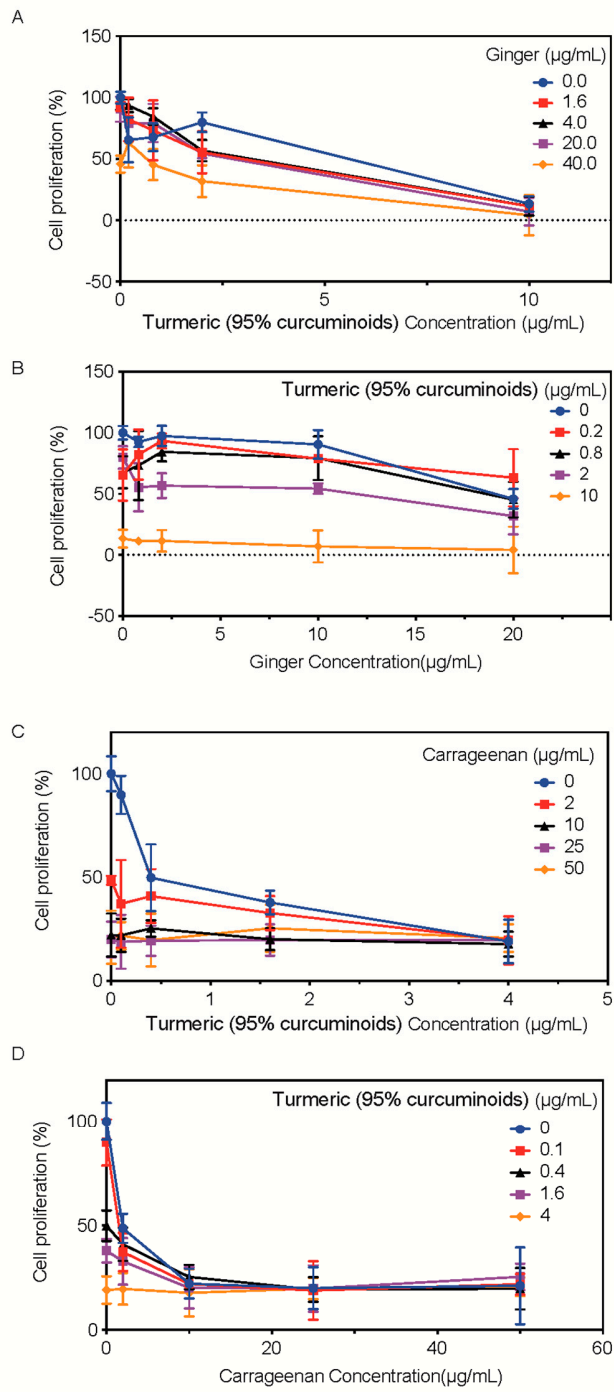
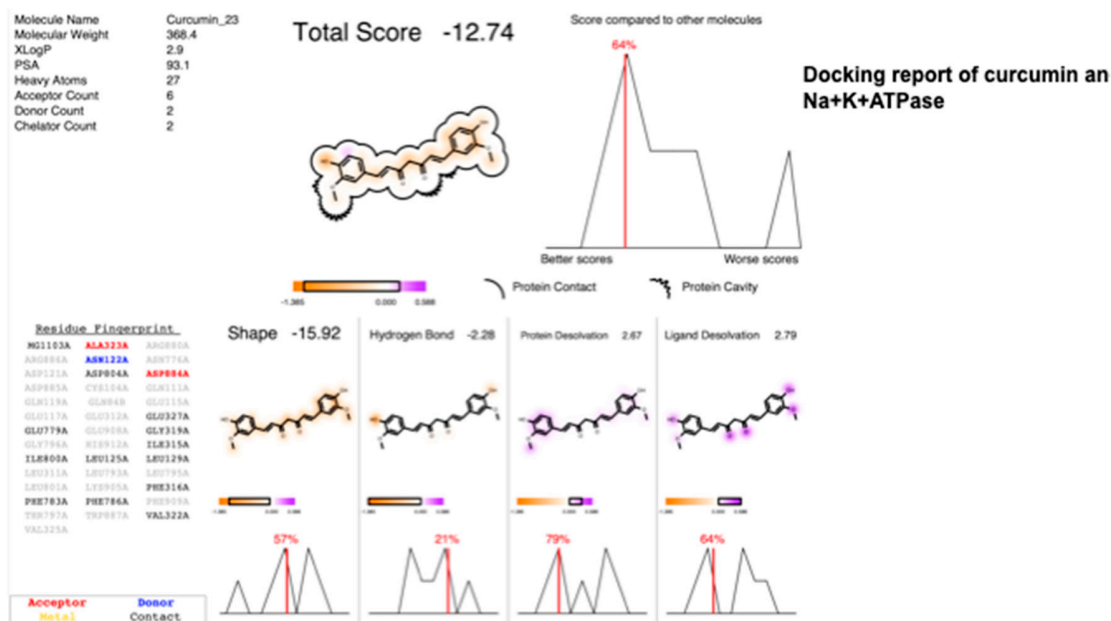
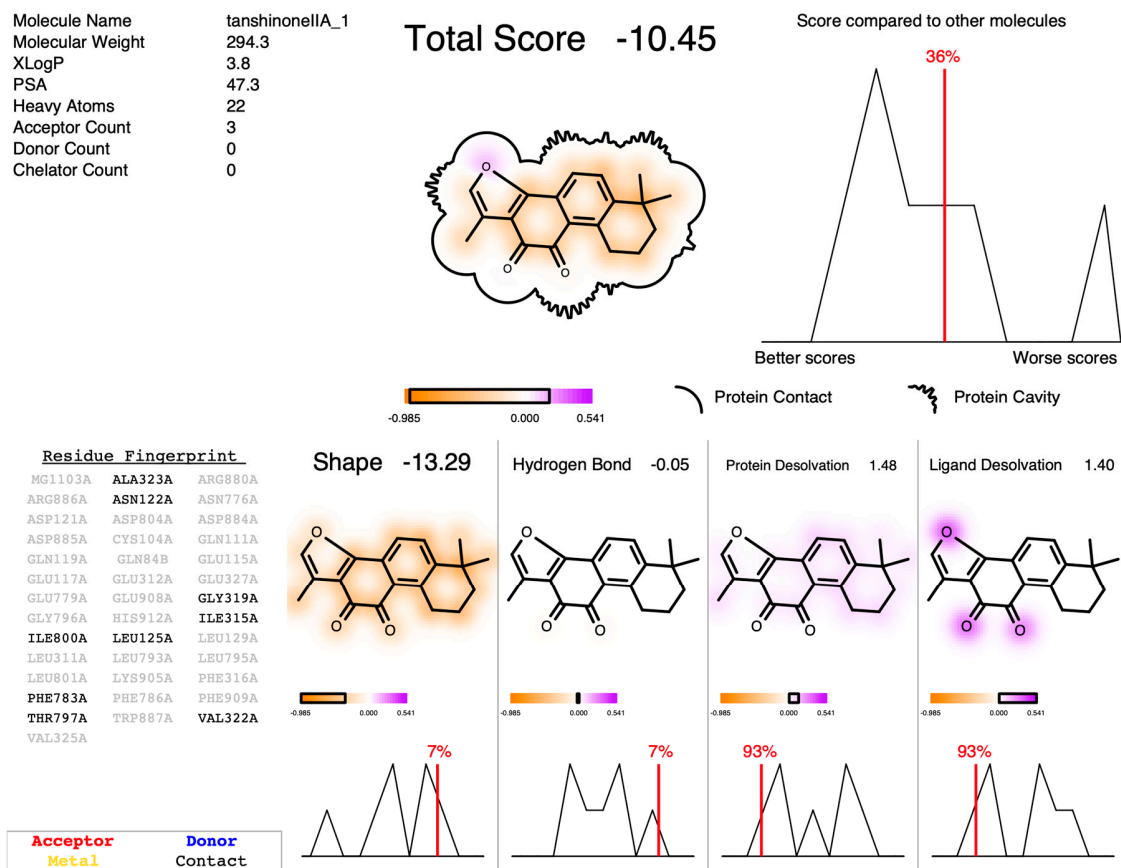


Figure S3. Molecular docking of tanshinone IIA and curcumin to **Na+K+ - ATPase**

A) Tanshinone IIA

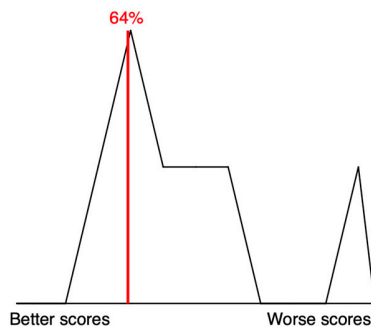
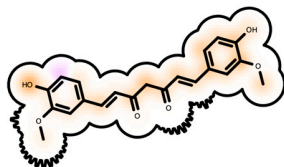


B) Curcumin

Molecule Name Curcumin_23
Molecular Weight 368.4
XLogP 2.9
PSA 93.1
Heavy Atoms 27
Acceptor Count 6
Donor Count 2
Chelator Count 2

Total Score -12.74

Score compared to other molecules



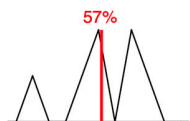
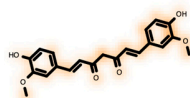
Protein Contact

Protein Cavity

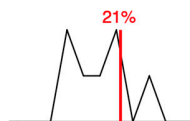
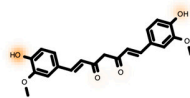
Residue Fingerprint

MG1103A	ALA323A	ARG880A
ARG886A	ASN122A	ASN776A
ASP121A	ASP804A	ASP884A
ASP885A	CYS104A	GLN111A
GLN119A	GLN84B	GLU115A
GLU117A	GLU312A	GLU327A
GLU779A	GLU908A	GLY319A
GLY796A	HIS912A	ILE315A
ILE800A	LEU125A	LEU129A
LEU311A	LEU793A	LEU795A
LEU801A	LYS905A	PHE316A
PHE783A	PHE786A	PHE909A
THR797A	TRP887A	VAL322A
VAL325A		

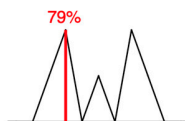
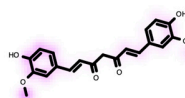
Shape -15.92



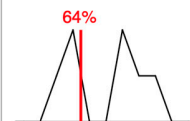
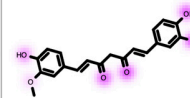
Hydrogen Bond -2.28



Protein Desolvation 2.67



Ligand Desolvation 2.79



Acceptor
Metal

Donor
Contact

Supplementary Material Tables

Table S1. Primer sequences used in RT-PCR.

Gene name	Primer direction	Sequence	Reference
TP53	Forward	CACATGACGGAGGTTGTGAG	*
	Reverse	ACACGCAAATTCCTTCCAC	
E1	Forward	AACGTGTTGCGATTGGTGTA	Straub et al., 2014
	Reverse	TACGCAATTTTGGAGGCTCT	
E2	Forward	TGGAAGTGCAGTTTGATGGA	Straub et al., 2014
	Reverse	CCGCATGAACCTCCCATACT	
E4	Forward	GACTATCCAGCGACCAAGATCAG	Egawa et al., 2017
	Reverse	CTGAGTCTCTGTGCAACAACTTAGTG	
E6	Forward	CTGCAATGTTTCAGGACCCA	[13]
	Reverse	TCATGTATAGTTGTTTGCAGCTCTGT	
E7	Forward	AAGTGTGACTCTACGCTTCGGTT	Kalantari et al., 2008
	Reverse	GCCCATTAACAGGTCTTCCAAA	
P21	Forward	TGGAGACTCTCAGGGTCGAAA	Al-Haj et al., 2012
	Reverse	AGTTGAGAATGAAGGTGGATGA	
MDM2	Forward	CAGGAGACGTGAAGATGCTG	IDT**
	Reverse	AGTTGAGAATGAAGGTGGATGA	
GAPDH	Forward	CAGCCTCAAGATCATCAGCA	*
	Reverse	GTCTTCTGGGTGGCAGTGAT	
b-actin	Forward	TGCCGACAGGATGCAGAAG	Ma et al., 2014
	Reverse	CTCAGGAGGAGCAATGATCTTGA	
HPV16E5	Forward	GTCTGTGTCTACATACACATCATTAAATAC TATTG	Scott et al., 2020
	Reverse	GTAATTAAAAAGCGTGCATGTGTATGT	

*The mRNA sequences were obtained from the public GeneBank database (www.ncbi.nlm.nih.gov), and the primers were designed using Primer 3 software obtained from The Massachusetts Institute of Technology (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

** The primer sequences were designed by Integrated DNA Technologies, Inc. (IDT).

Table S2. Effect of Tanshinone IIA on the relative level of Hpv16 and p53 mRNAs in W12 cells.

Experiments		
W12 cells type	type 1	type 2
W12 cells clone number	20822	20862
Relative level of mRNA Gapdh	1+/-0.14	1+/-0.13
E1	0.46+/-0.05	0.46+/-0.19
E2	0.44+/-0.02	0.54+/-0.11
E4	0.78+/-0.03	0.51+/-0.15
E6	0.84+/-0.17	0.72+/-0.09
E7	0.78+/-0.07	0.66+/-0.12
p53*	1.96+/.04	2.53+/-0.18

The experiments were performed on different clones of W12 cells. W12 cells were treated with Tanshinone IIA at 5 uM for 24 hours; extracts were prepared and analyzed by Real-time RT-PCR, as described in Materials and Methods. Fold change indicates relative expression in Tanshinone IIA treated versus control cells.

*separate experiment

Statistical Analysis of the Figures

In the interest of space, we have included portions of the statistical analyses of some figures, as examples, and can provide complete analyses upon request.

Non-significant: yellow

Table S3. Statistical analysis for Figure 4 C,D.

Growth inhibitory activity of combinations of phytochemicals: Turmeric (95% curcuminoids) plus tanshinone IIA on HeLa cells;

	Mean1	Mean2	Mean3	Mean4	Mean5
Tanshinone IIA	0	0.2	0.8	2	8

ANOVA based T-test									
T(Mean1-Mean2)	T(Mean1-Mean3)	T(Mean1-Mean4)	T(Mean1-Mean5)	T(Mean2-Mean3)	T(Mean2-Mean4)	T(Mean2-Mean5)	T(Mean3-Mean4)	T(Mean3-Mean5)	T(Mean4-Mean5)
9.244	17.759	17.862	17.974	8.515	8.617	8.730	0.103	0.215	0.113
11.379	18.900	18.718	19.100	7.521	7.339	7.721	0.181	0.201	0.382
13.381	21.872	21.754	22.195	8.490	8.373	8.813	0.118	0.323	0.441
11.849	20.550	21.759	20.099	8.701	9.910	8.250	1.209	0.450	1.660
1.283	6.341	6.752	6.757	5.058	5.469	5.474	0.411	0.416	0.005

P value of ANOVA based T-test (2 tails)									
T(Mean1-Mean2)	T(Mean1-Mean3)	T(Mean1-Mean4)	T(Mean1-Mean5)	T(Mean2-Mean3)	T(Mean2-Mean4)	T(Mean2-Mean5)	T(Mean3-Mean4)	T(Mean3-Mean5)	T(Mean4-Mean5)
5.11789E-14	1.38764E-28	9.76622E-29	6.65697E-29	1.24972E-12	7.96087E-13	4.85857E-13	0.918379099	0.830012622	0.910637472
5.30154E-18	3.02019E-30	5.49025E-30	1.56431E-30	9.69837E-11	2.13387E-10	4.03802E-11	0.856729397	0.841440061	0.703609347
1.46564E-21	2.78945E-34	3.95737E-34	1.07372E-34	1.39139E-12	2.32972E-12	3.37331E-13	0.906769854	0.747478511	0.660724239
7.43366E-19	1.54711E-32	3.90001E-34	6.33781E-32	5.52644E-13	2.82095E-15	3.98529E-12	0.230316815	0.653681207	0.101124138
0.203505383	1.55371E-08	2.69861E-09	2.64257E-09	2.91986E-06	5.70493E-07	5.59344E-07	0.682035832	0.678465295	0.996106365

**Table S4. Statistical analysis for Figure 5A (Table S2):
RT-PCR analysis of RNA obtained after treating W12 cells with tanshinone IIA;**

mRNA	p value
GAPDH	1.00
HPV-E1	1.00
HPV-E2	0.1963
HPV-E4	0.0378
HPV-E6	0.3407
HPV-E7	0.2090
p53	0.0059

Table S5. Statistical analysis for Figure 5C:

Genes significantly altered by curcumin (DMSO-8 vs Cur-8)	
Pro-Apoptotic genes;	P value
PRUNE2	0.0072
PPP2CA	0.0001
MAD1L1	0.0021
HMGB1	0.1154
Anti-Apoptotic genes;	P value
PEG10	0.0002
NOTCH1	0.0518
USP47	0.0066
NR4A2	0.0042

References

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