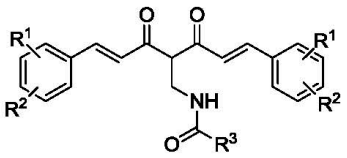
Supplementary materials

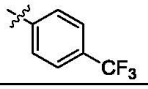
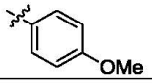
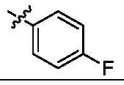
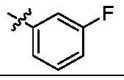
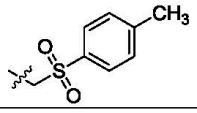
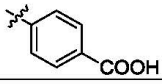
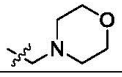
Supporting materials and methods

Cell confluency screening

A549 cells (6 x 10^3) were seeded into 96-well plates (Corning Life Sciences) in DMEM/F12 10 % FCS (Gibco). Cells were cultured overnight before treatment. Effects of curcumin and analogs were examined in concentrations presented in the figure after 72h incubation in 100 µl media. We monitored the cell confluency by JuLI™ FL Live Cell Analyser V.0 (NanoEntek) according to the instructions of the manufacturer. We present the confluency determined by the JuLI™ FL Live Cell Analyser after 72h treatment. Data were analyzed by GraphPad Prism® 5.

Supplementary figures:



Compound	R^1	R^2	R^3	Molecular weight
C501	3-OH			441
C502	3-OH			509
C503	3-OH			472
C504	3-OH			459
C505	3-OH			459
C509	3-OH			414
C510	3-OH			420
C513	3-OH			534
C514	3-OH			485
C515	3-OH			505
C516	3-OH			465
C517	3-F			418
C518	4-F			418
C519	2-F			418
C520	3-OMe	4-OH		474
C521	3-OH	4-OMe		474

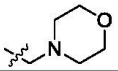
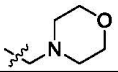
C524	3-OMe	4-OMe		502
C525	3-OH	3-OH		446
C526	4-COOH			470
C529	4-COOH			521
C530	3-OH	3-OH		497
C532	3-OH			418
C533	3-OH			415

Figure S1. Chemical structure of curcumin analogs.

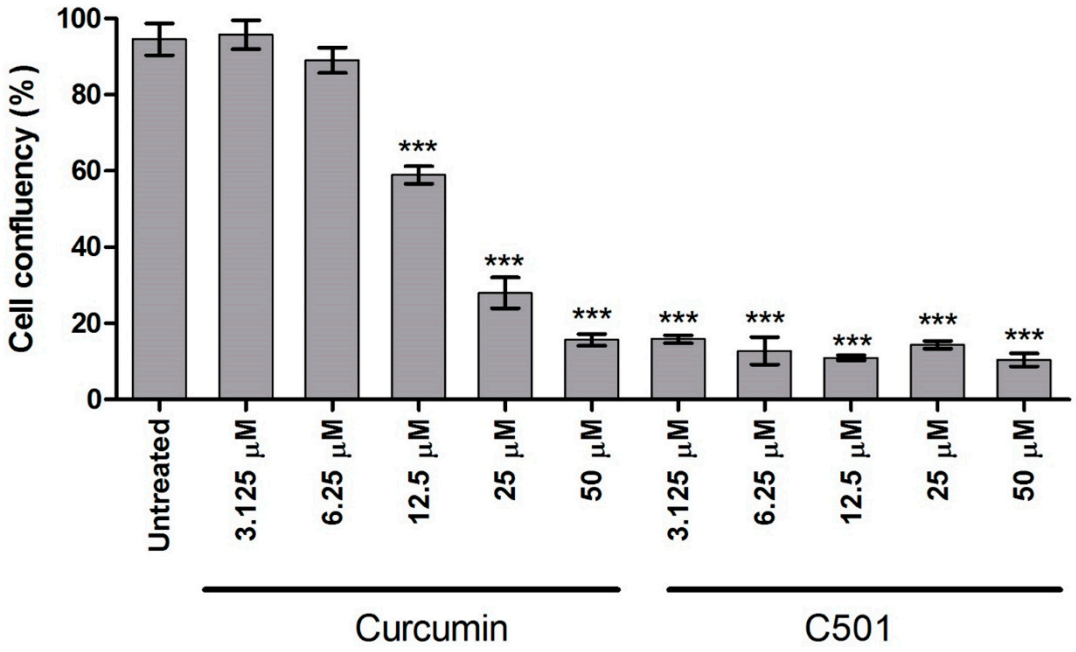
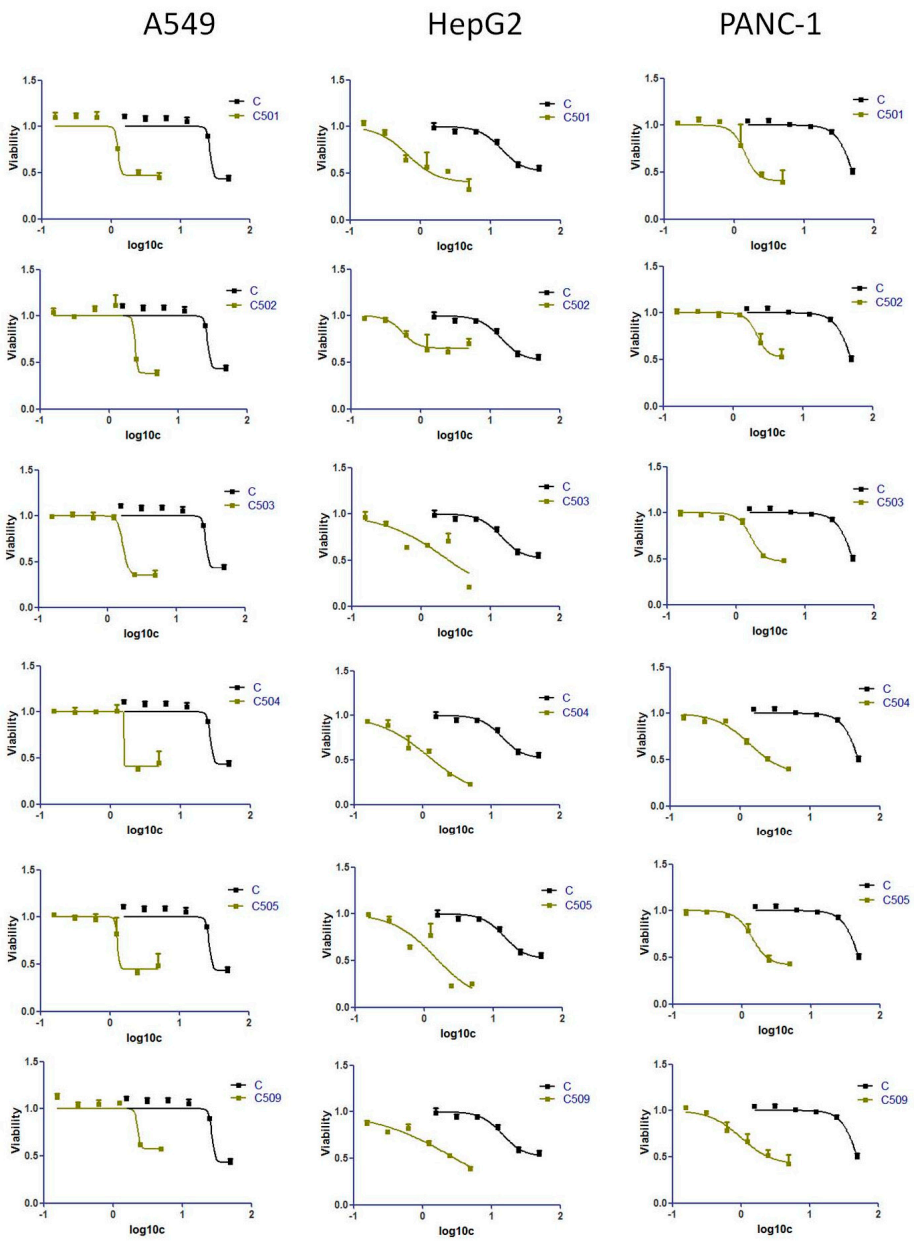
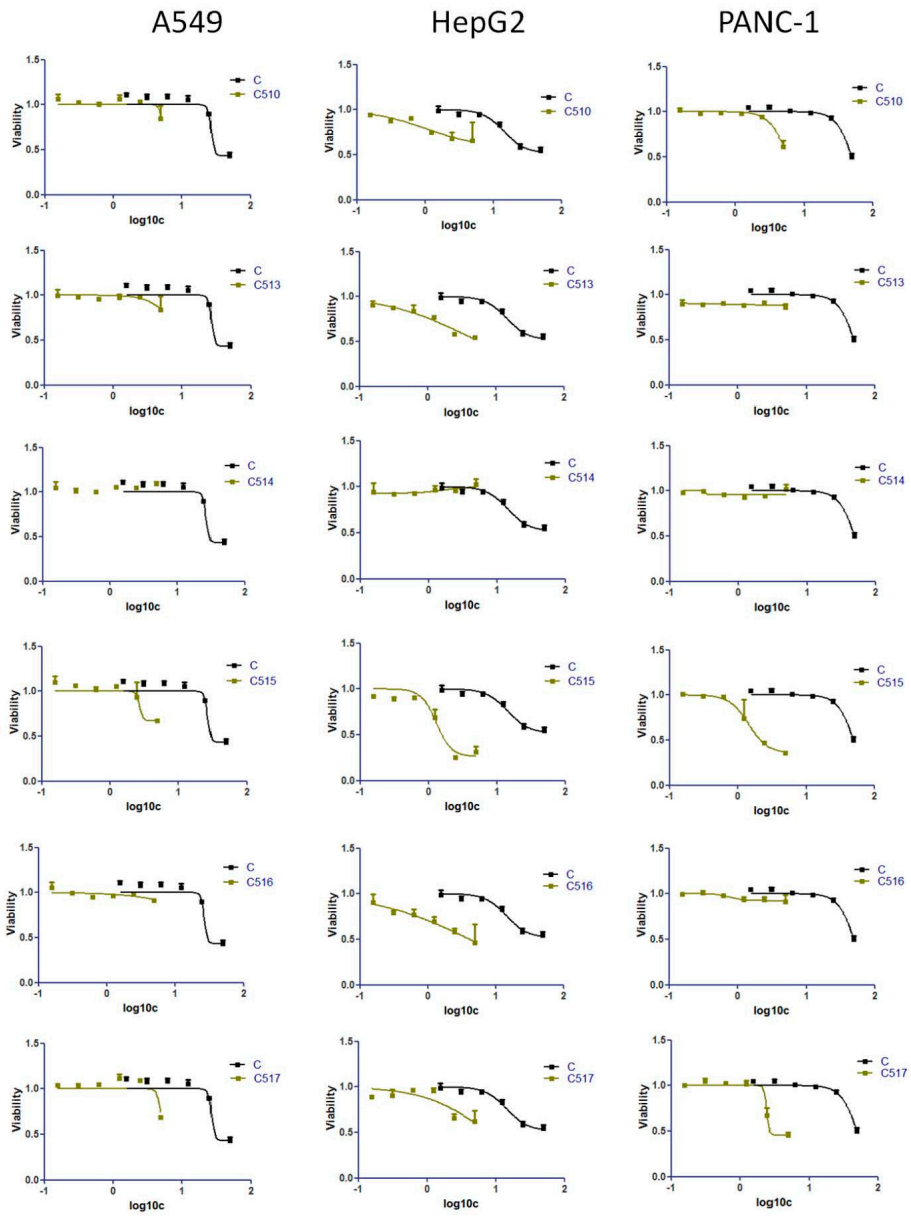
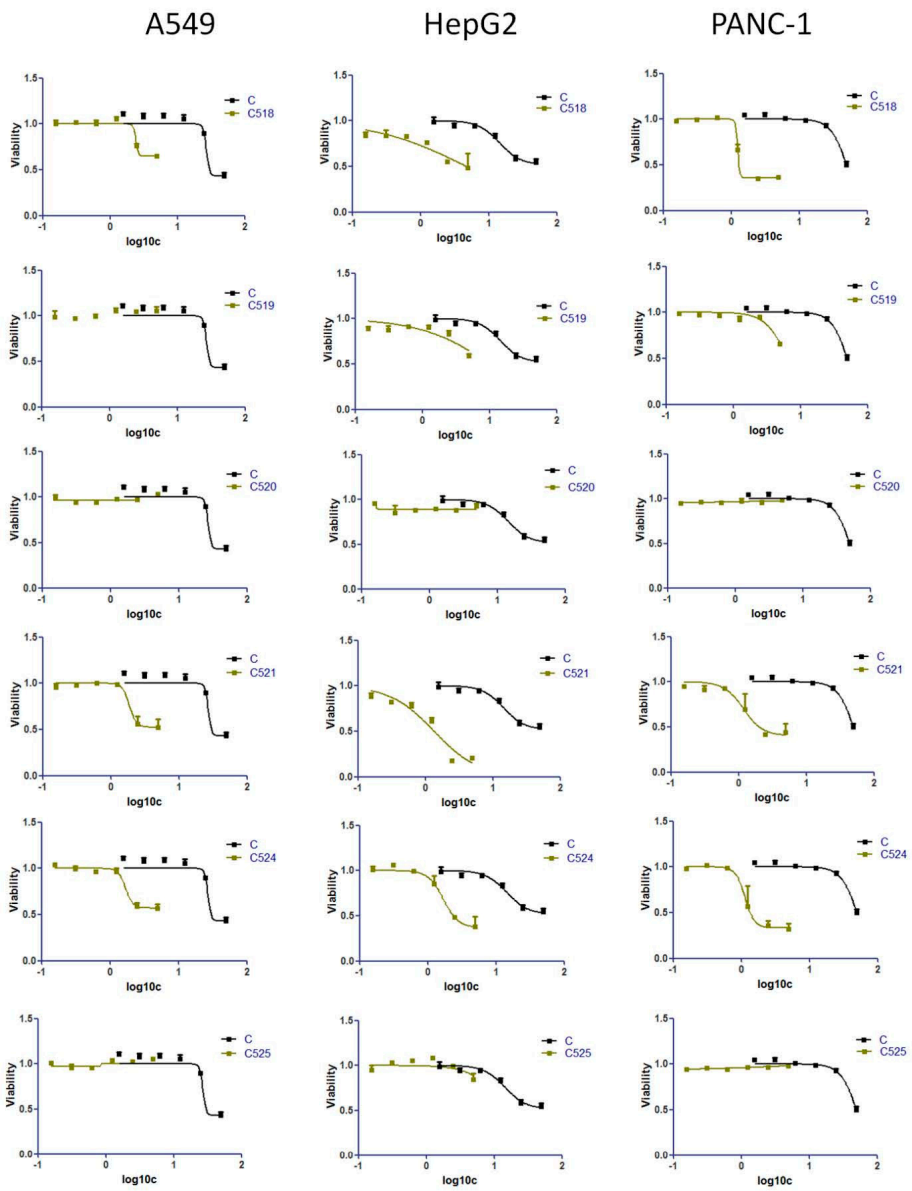


Figure S2. Curcumin and C501 hampered the viability of A549 cells. Cell confluency data of cells treated (from left to right: untreated, 3.125, 6.25, 12.5, 25 and 50 µM) in duplicates for 72h, recorded by Juli^{TM}. The arithmetic mean values of two samples ± SD are presented. For experimental details see Supporting materials and methods.


A549
HepG2
PANC-1
C
C501
C502
C503
C504
C505
C509
Viability
log10c


A549
HepG2
PANC-1
C
C510
C513
C514
C515
C516
C517
Viability
log10c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HepG2
PANC-1
C
C518
C519
C520
C521
C524
C525
Viability
log10c

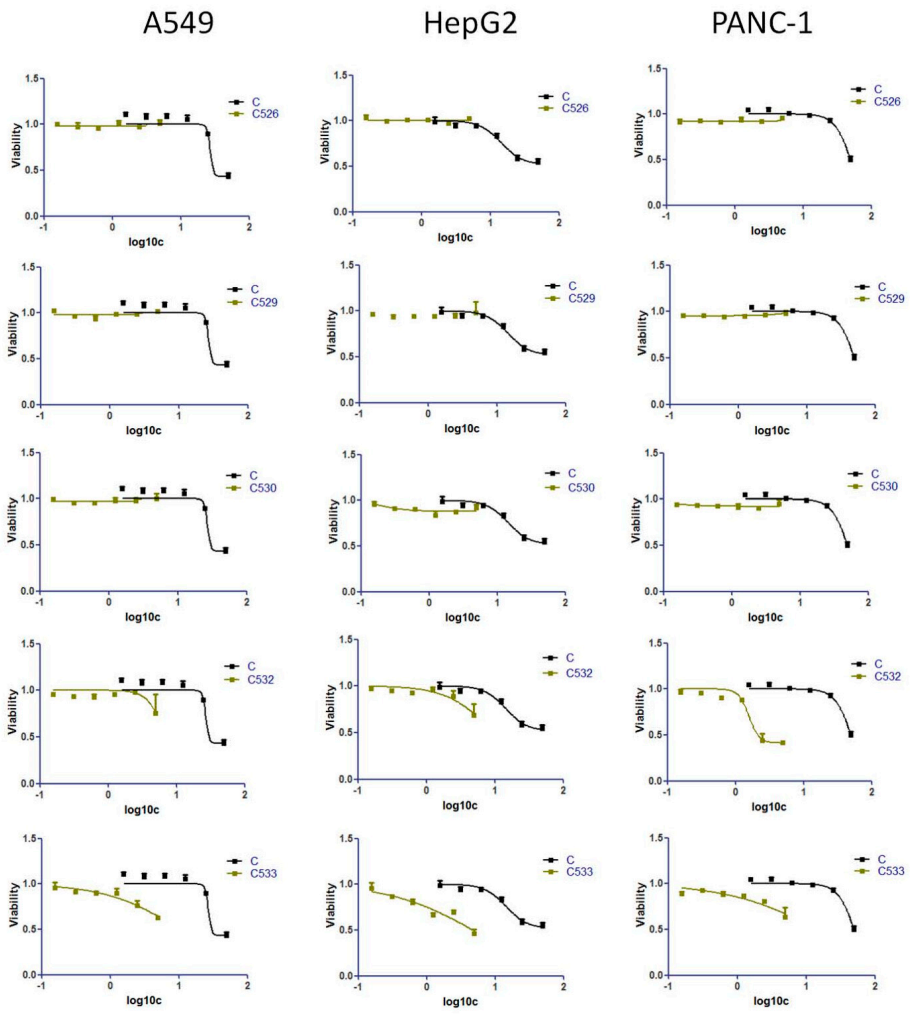
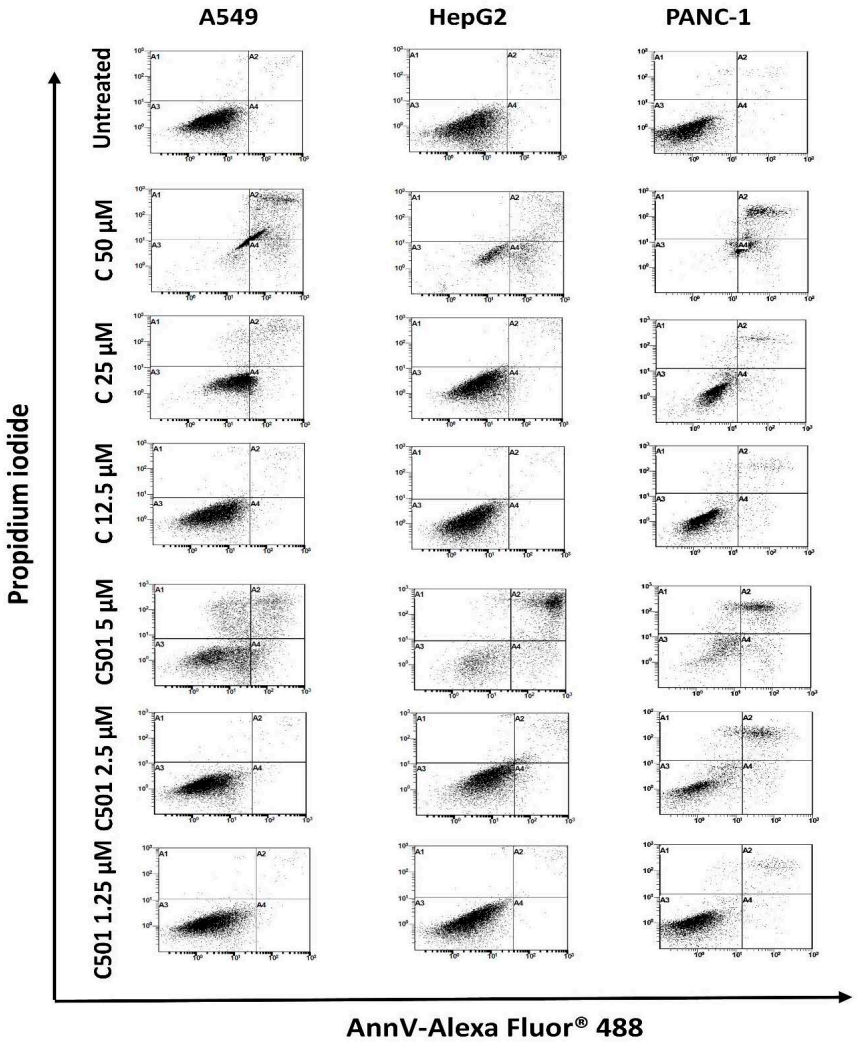
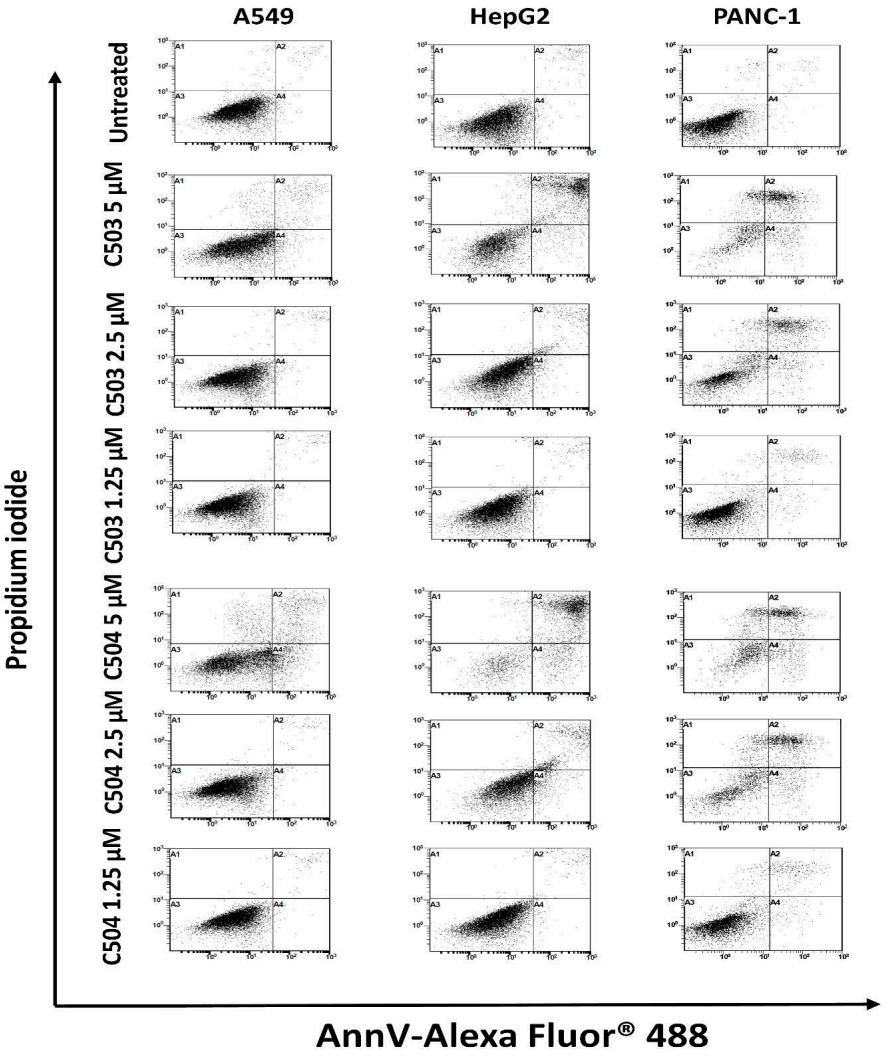
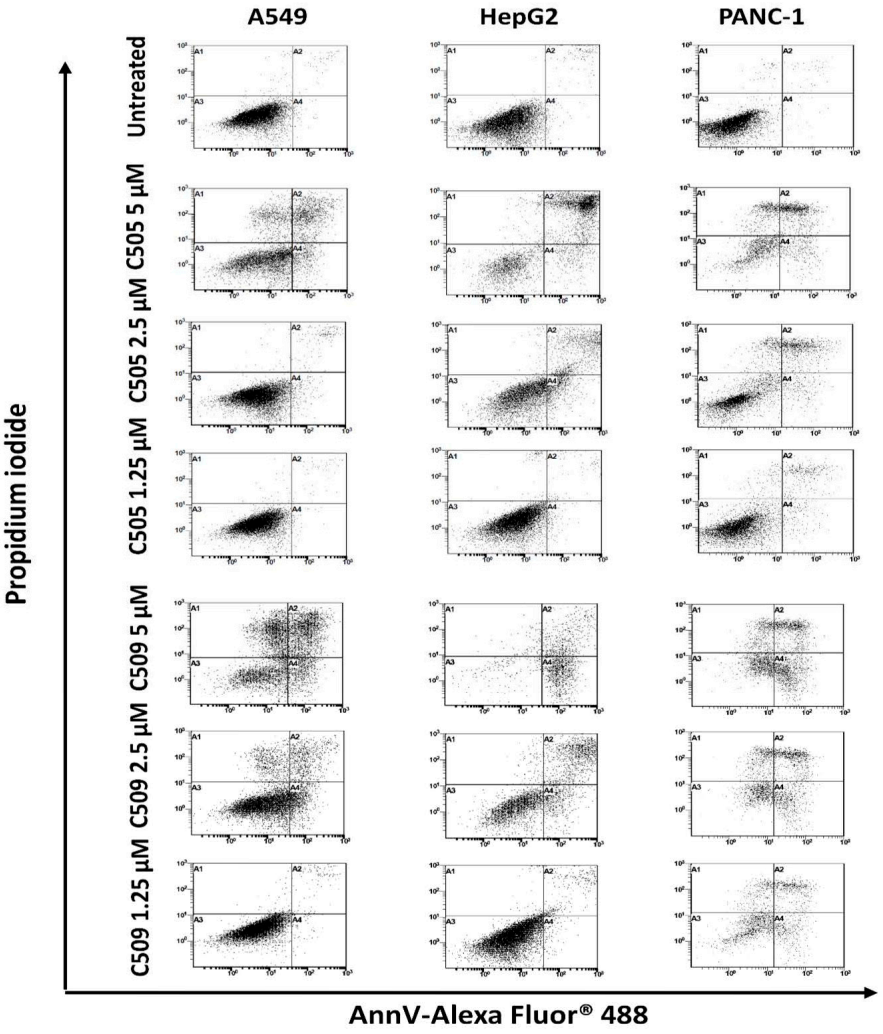


Figure S3. Dose-response curves of curcumin and curcumin analogs on A549, HepG2 and PANC-1 cells. Cells were treated with curcumin (C, 1.56, 3.125, 6.25, 12.5, 25 and 50 μM), and curcumin analogs (0.16, 0.3125, 0.625, 1.25, 2.5 and 5 μM) in duplicates for 72 h. Viability was examined by resazurin assay as described in the 3.2. Materials and methods. The arithmetic means of two samples ± SD are presented.


A549
HepG2
PANC-1
Untreated
C 50 µM
C 25 µM
C 12.5 µM
C501 5 µM
C501 2.5 µM
C501 1.25 µM
Propidium iodide
AnnV-Alexa Fluor® 488


A549
HepG2
PANC-1
Untreated
C503 5 µM
C503 2.5 µM
C503 1.25 µM
C504 5 µM
C504 2.5 µM
C504 1.25 µM
Propidium iodide
AnnV-Alexa Fluor® 488


A549
HepG2
PANC-1
Untreated
C505 5 µM
C505 2.5 µM
C505 1.25 µM
C509 5 µM
C509 2.5 µM
C509 1.25 µM
Propidium iodide
AnnV-Alexa Fluor® 488

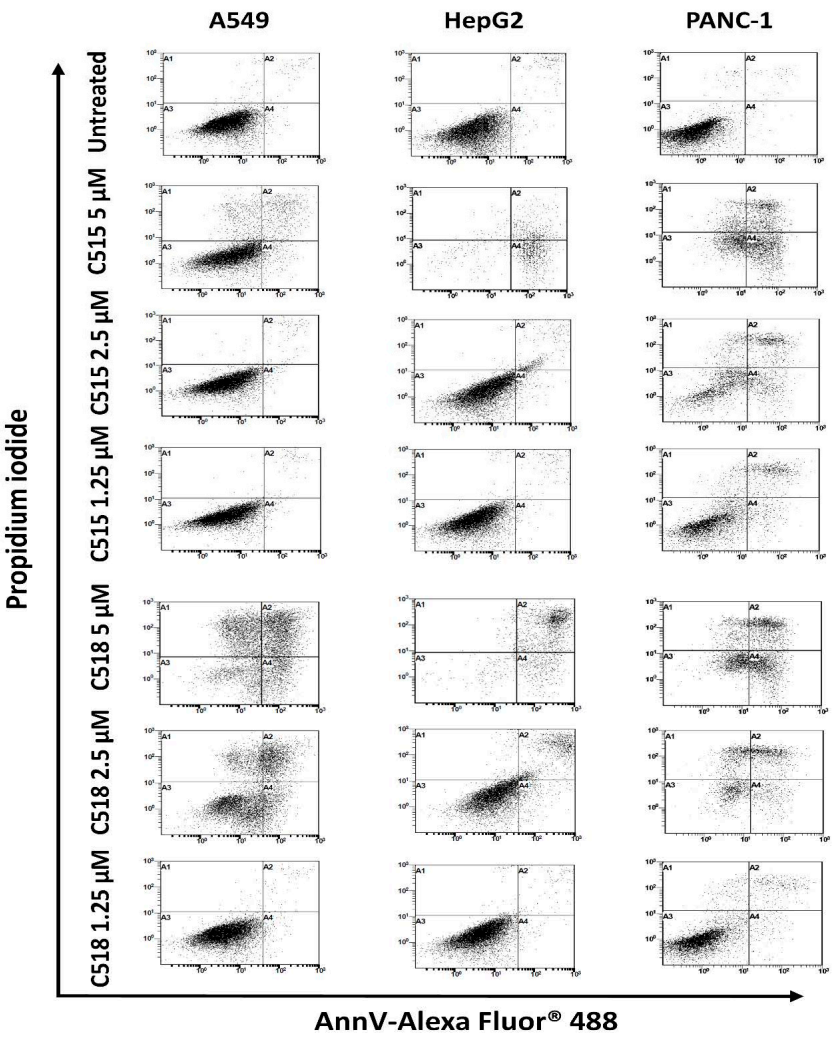


Figure S4. Detection of phosphatidylserine exposure on A549, HepG2 and PANC-1 cells. Representative dot plot images of cells treated with curcumin (C, 12.5, 25 and 50 µM), and curcumin analogs (1.25, 2.5, 5 µM) in duplicates for 72 h. Phosphatidylserine exposure was detected as described in 3.3.1. Materials and methods.

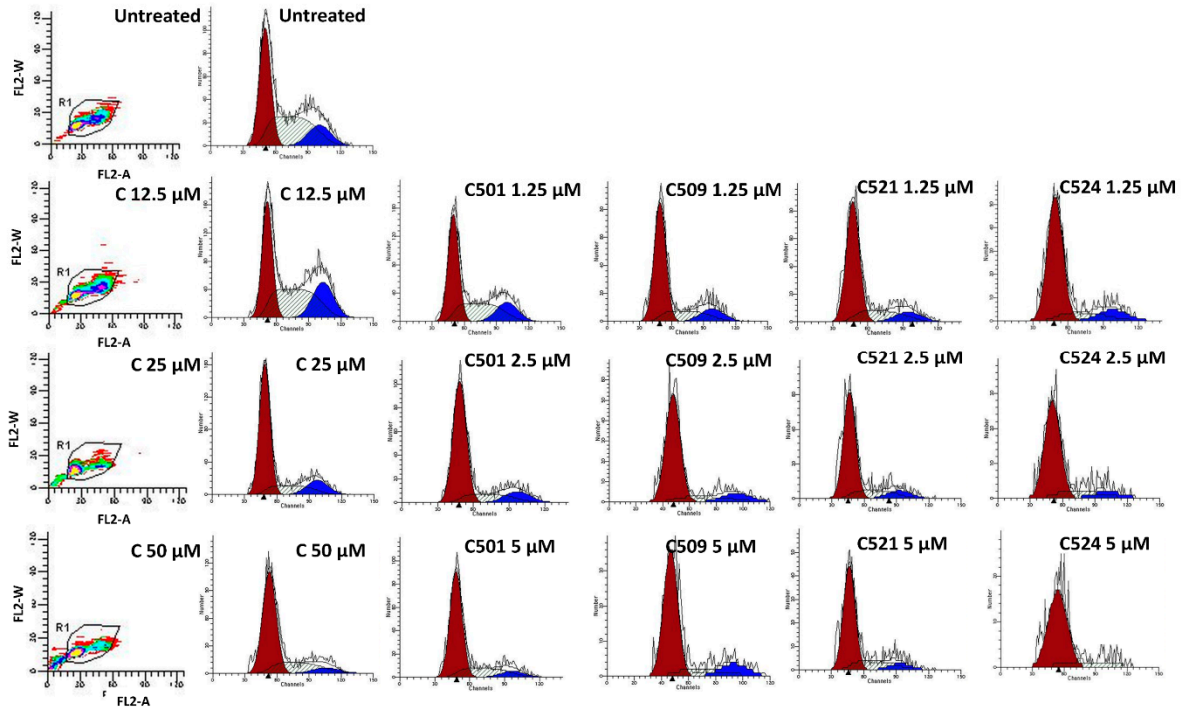


Figure S5. Cell cycle analysis of PANC-1 cells upon curcumin and curcumin analog treatment. Cells were treated with curcumin (C, 12.5, 25 and 50 µM) and curcumin analogs (1.25, 2.5 and 5 µM) in duplicate for 72 h. Representative images show cell cycle distribution analysis as described in the 3.3.2. Materials and methods.