

Supplementary material

Article: Researching new drug combinations with senolytic activity using senescent human lung fibroblasts MRC-5 cell line.

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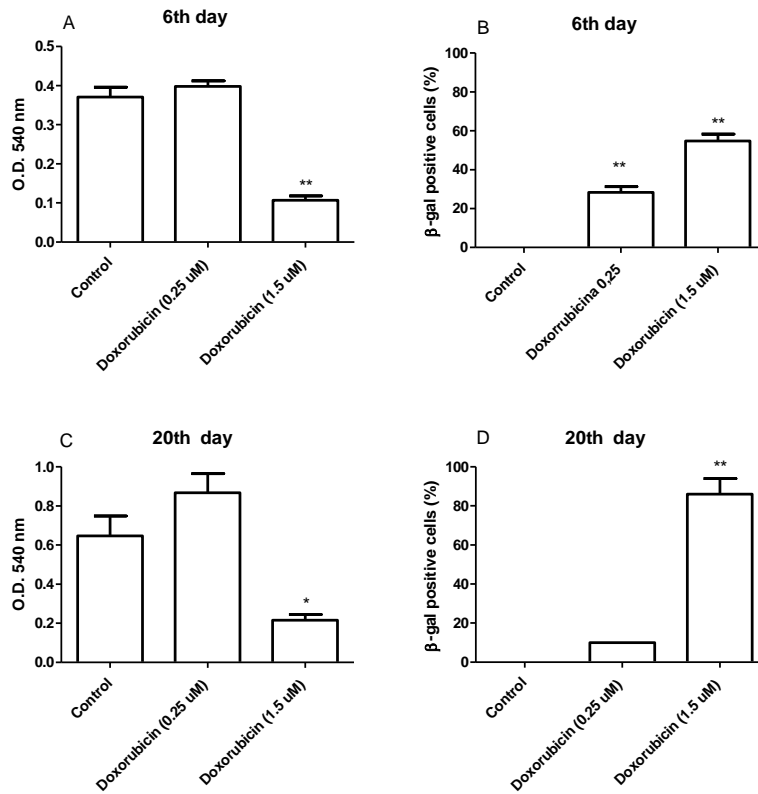


Figure S1: Proliferation and β -gal positive MRC-5 cells after 6 and 20 days of 24 h treatment with doxorubicin 0.25 and 1.5 μ M). The experiment was carried out in triplicate with one repetition. * p<0.05 when compared to non-treated MRC-5 cells and ** p<0.01 when compared to non-treated MRC-5 cells

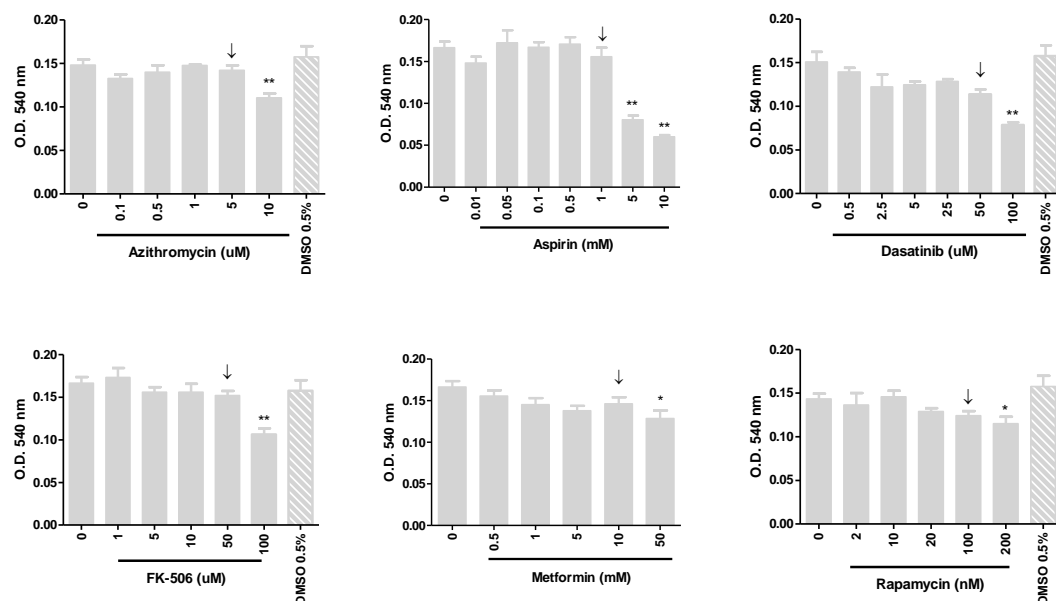


Figure S2: Cytotoxicity evaluated in control MRC-5 cells incubated during 24 h with a concentration range of the drugs Azithromycin (0.1-10 μM), Aspirin (0.01-10 mM), Dasatinib (0.5-100 μM), FK-506 (1-100 μM), Metformin (0.5-50 mM) and Rapamycin (2-200 nM). The experiment was carried out in triplicate with one repetition. * $p < 0.05$ and ** $p < 0.01$ when compared to non-treated MRC-5 cells. The arrows show the concentration chosen for each drug for subsequent assays.

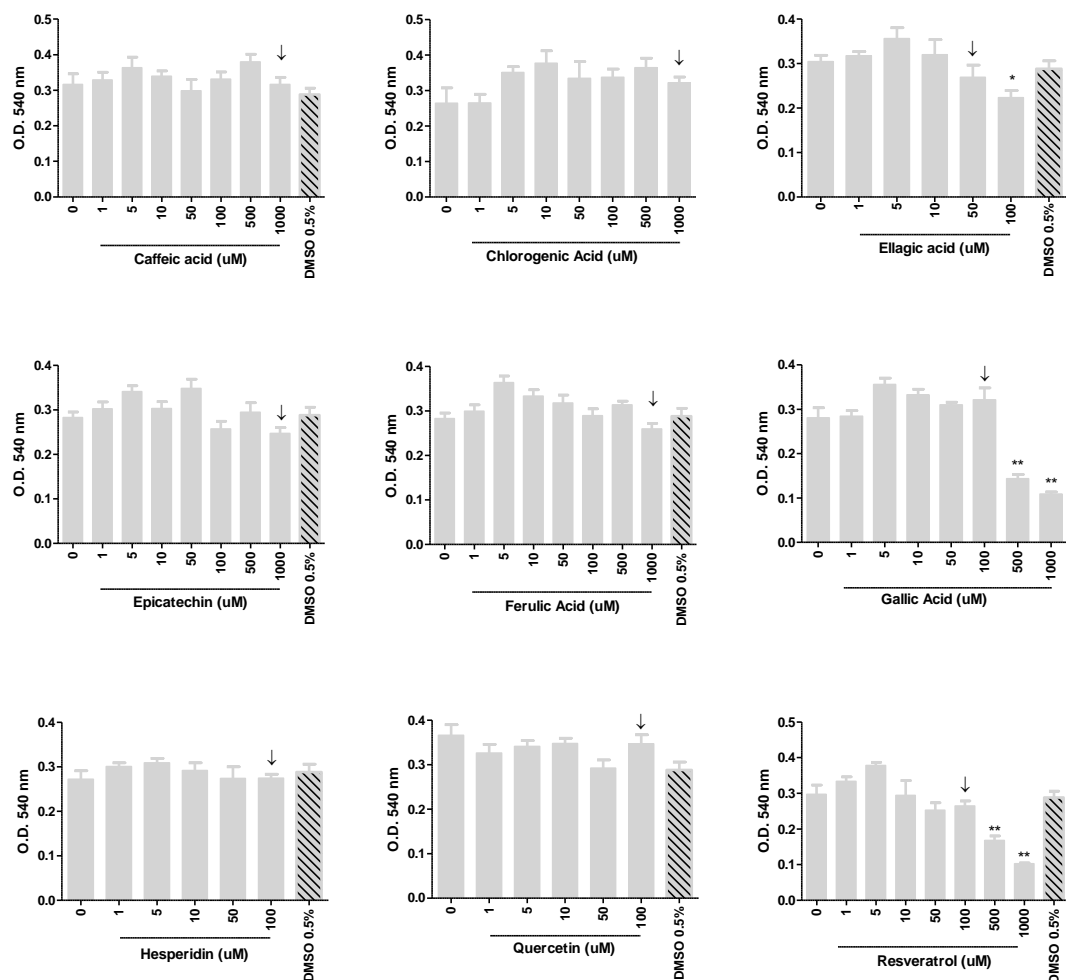


Figure S3: Cytotoxicity evaluated in control MRC-5 cells incubated during 24 h with a concentration range of the polyphenols caffeic acid (1-1000 μ M), chlorogenic acid (1-1000 μ M), ellagic acid (1-100 μ M), epicatechin (1-1000 μ M), ferulic acid (1-1000 μ M), gallic acid (1-1000 μ M), hesperidin (1-100 μ M), quercetin (1-100 μ M) and resveratrol (1-1000 μ M). The experiment was carried out in triplicate with one repetition. ** p<0.01 and * p<0.05 when compared to non-treated MRC-5 cells. The arrows show the concentration chosen for each polyphenol for subsequent assays.

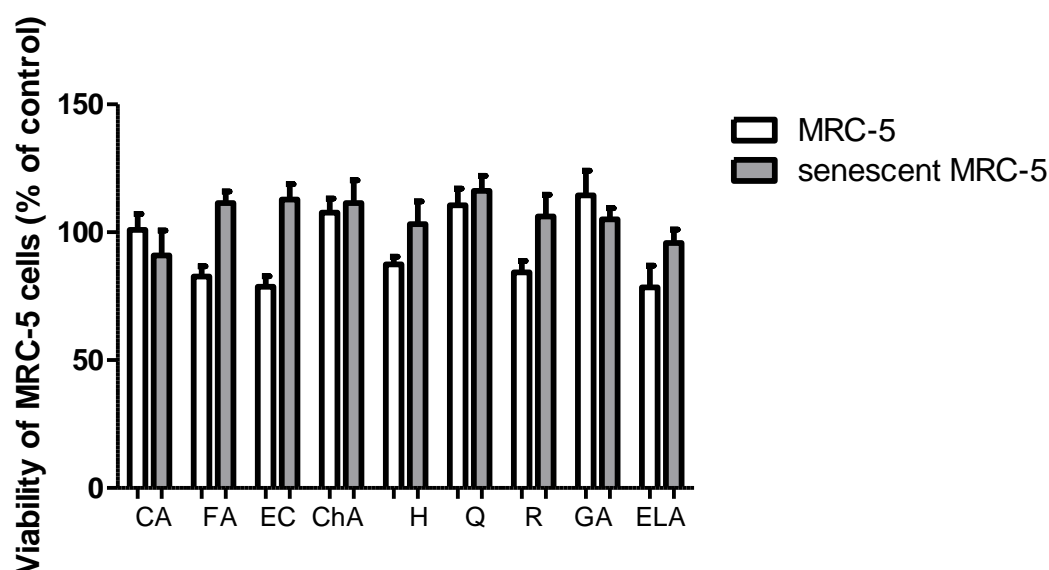


Figure S4: Cytotoxic activity in MRC-5 cells or senescent MRC-5 cells by isolated caffeic acid (CA, 1000 μ M), chlorogenic acid (ChA, 1000 μ M), ellagic acid (50 μ M), epicatechin (EC, 1000 μ M), ferulic acid (FA, 1000 μ M), gallic acid (100 μ M), hesperidin (100 μ M), quercetin (100 μ M) or resveratrol (100 μ M). The experiments were carried out in triplicate with one repetition.

Table S1: Primers sequence

Gene	Forward	Reverse
CDKN2A	GAGCAGCATGGAGCCTTC	CGTAACTATTCGGTGCGTTG
CDKN1A	TCACTGTCTTGTTACCTTGTC	GGCGTTTGGAGTGGTAGAAA
b-actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG

Table S2: Viability of control and senescent MRC-5 after 24 h dasatinib, ellagic acid, quercetin and resveratrol isolated or in combination and percentage of viability reduction of senolytic combinations.

Drug	Control MRC-5 viability	Senescent MRC-5 viability	% reduction
Dasatinib	81.9 \pm 1.1	80.4 \pm 5.8	9.8
Ellagic acid	78.5 \pm 8.4	95.8 \pm 5.3	-
Quercetin	110.6 \pm 6.4	116.1 \pm 5.9	-
Resveratrol	84.3 \pm 4.5	106.2 \pm 8.5	-
Dasatinib + Ellagic Acid	107.8 \pm 8.9	46.7 \pm 6.7	43.3
Dasatinib + Quercetin	105.3 \pm 7.7	51.7 \pm 6.5	49.2
Dasatinib + Resveratrol	84.43 \pm 0.4	37.1 \pm 7.4	43.9