Supplementary materials

Modulation of STAT3 signalling, cell redox defences and cell cycle checkpoints by β-caryophyllene in cholangiocarcinoma cells: possible mechanisms accounting for doxorubicin chemosensitization and chemoprevention

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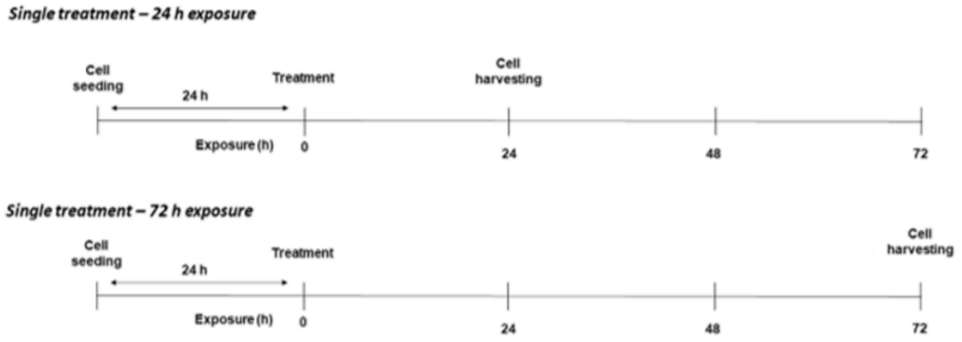

Figure S1. Schedule of the long-term exposures of 24 and 72 h.

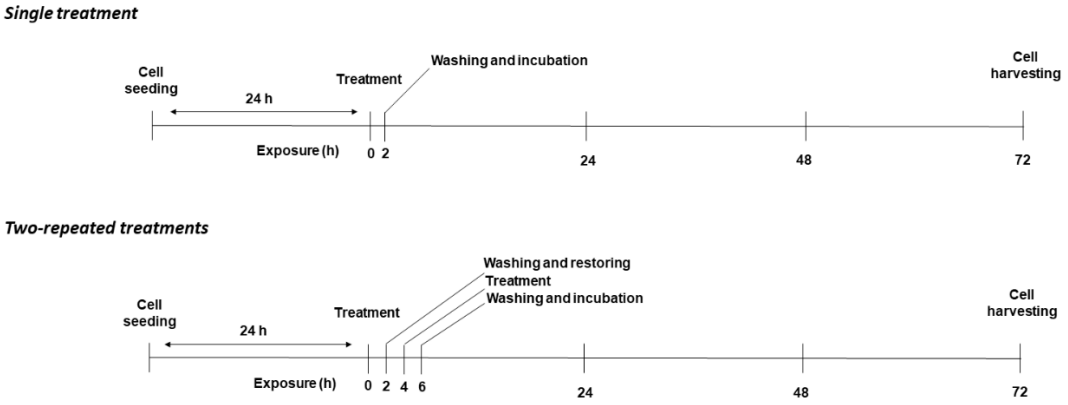

Figure S2. Schedule of the metronomic treatment. The cells were subjected to a short and/or two repeated exposure of 2 h followed by a recovery time of 72 h.

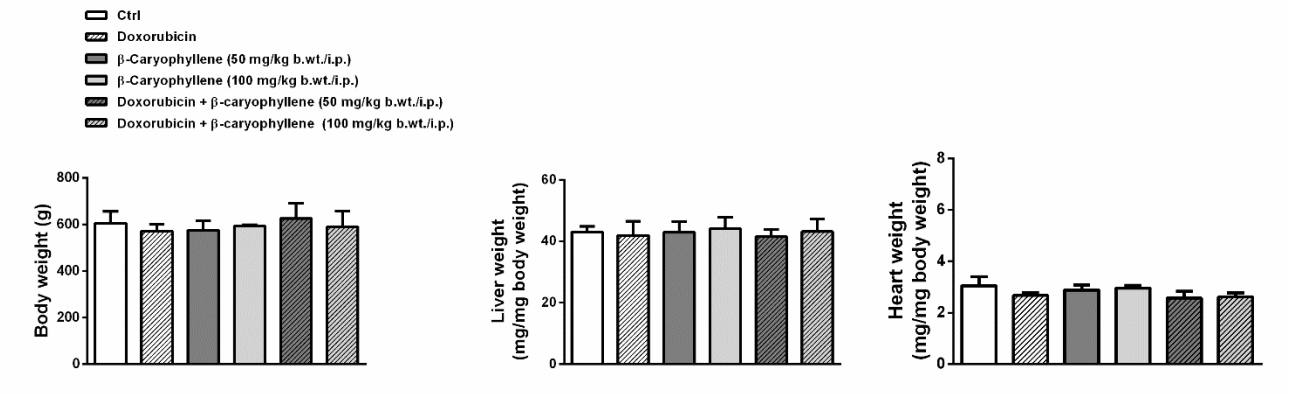

Figure S3. Effect of a single administration of β-caryophyllene (50 and 100 mg/kg b.wt./i.p. in olive oil; single dose), doxorubicin (3 mg/kg b.wt./i.p. in physiological solution; single dose), and their combinations on body, heart and liver weight in Sprague Dawley rats (Charles River, Lecco, Italy). Data are expressed as mean ± SD (n = 6 in each group). To perform the study, 36 rats were randomly assigned to the experimental groups and subjected to the treatment with doxorubicin (dose chosen according to literature data [1]) and two doses of β-caryophyllene (50 and 100 mg/kg b.wt./i.p.),

selected to be about 1000- and 2000-folds higher than that used in the combination experiments in liver cancer cells. The dose of 50 mg/kg b.wt./i.p. was previously used for the analogue β-caryophyllene oxide [2]. After dosing, animals were daily observed for one week, then sacrificed in accordance with the International Animal Welfare Legislation (Directive 2010/63/EU, 2010). Thereafter, liver and heart were taken and weighted, being the main organs affected by doxorubicin toxicity. Under our experimental conditions, the treatments did not induce animal death, and no toxicity signs were highlighted at the necroscopic analysis compared the control group.

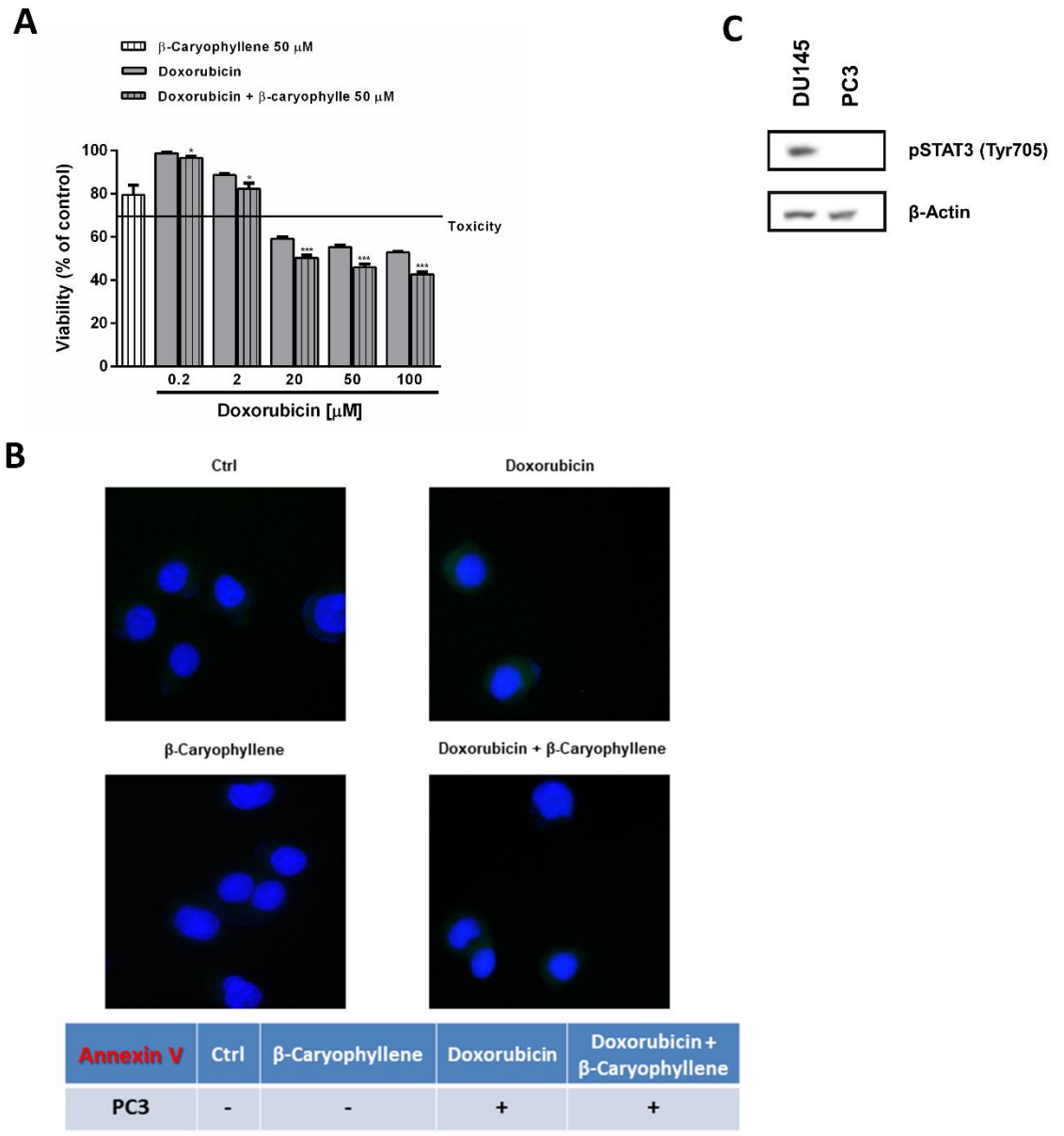

Figure S4. Cytotoxic effects of doxorubicin (0.2 - 100 μM) and its combination with β-caryophyllene (50 μM) in human prostatic carcinoma PC3 cells, lacking in the expression of STAT3 [3-4], after a single long-term exposure of 24 h. Our western blotting analysis confirmed that PC3 cells do not express STAT3. In these cells, the cytotoxic effect of doxorubicin was slightly increased in combination with β-caryophyllene, although no statistical difference was found in the IC50 values (48.4, C.L. 29.2-65.8 μM and 44.1, C.L. 19.8-72.1 μM for doxorubicin and doxorubicin plus β-caryophyllene, respectively). Furthermore, β-caryophyllene did not affect the doxorubicin-induced apoptosis as shown by the immunofluorescence images. (A) Cytotoxicity bar graph as measured by the MTT assay. Data are expressed as mean ± SE (standard error) of at least two experiments in which each treatment was tested at least in triplicate (n = 6). * $p < 0.05$ and *** $p < 0.001$ (t-Student test), significantly lower than doxorubicin. (B) Representative immunofluorescence images and semiquantitative analysis

of the apoptosis induced by β-caryophyllene (50 μM), doxorubicin (20 μM) and their combination compared the control in PC3 cells after a 24 h treatment, as measured by Annexin-V-FITC stain. The semiquantitative analysis has been carried out (four fields for each treatment) applying a previous published grading system [5]: 0%–5% = negative; 6%–10% = +/−; 11%–30% = +; 31%–60% = ++; >61% = +++. (C) A representative western blotting image, showing the lacking expression of the phospho(Tyr705) STAT3 in PC3 cells compared DU145 prostatic cancer cells. β-Actin was used as protein loading control.

References

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