

Table 1 supplementary. CI and synergy values for inhibition of SF126 and U251 cell proliferation. Incubations were carried out as described in the Material and Methods. The IC₂₀ and IC₈₀ used for cell treatments were predicted using the equations described under the pharmacological and statistical analyses section in the Methods. The combinations used in SF126 cells were: THC 3.9 μM (EC₈₀) + CBD 1.4 μM (EC₈₀) and THC 1.6 μM (EC₂₀) + CBD 1.1 μM (EC₂₀). The combinations used in U251 cells were: THC 5.4 μM (EC₈₀) + CBD 0.9 μM (EC₈₀) and THC 1.7 μM (EC₂₀) + CBD 0.4 μM (EC₂₀). CI values were calculated as described in the Material and Methods with a value of <1, 1, and >1 indicating synergism, additivity, and antagonism, respectively. Synergy, fold represents 1/CI.

Cell line	Drug combination	CI values	Synergy, fold
SF126	Δ ⁹ -THC EC ₈₀ + CBD EC ₈₀	0.22 ± 0.13	4.5
SF126	Δ ⁹ -THC EC ₂₀ + CBD EC ₂₀	1.04 ± 0.24	1
U251	Δ ⁹ -THC EC ₈₀ + CBD EC ₈₀	0.27 ± 0.13	3.7
U251	Δ ⁹ -THC EC ₂₀ + CBD EC ₂₀	0.29 ± 0.16	3.4

Figure 1 supplementary. Time course analysis of cannabinoid-dependent increases in apoptosis and ROS. The number of U251 cells positive for annexin (apoptosis) staining after one and two days treatment were measured using FACS analysis. For A) one or B) two days cells were treated with: A) CBD (0.4 μ M), Δ^9 -THC (1.7 μ M), or a combination of CBD (0.4 μ M) and Δ^9 -THC (1.7 μ M), denoted (THC/CBD). % control was calculated as positive annexin staining of the treated cells minus control cells. The production of ROS was also measured using 2'-7'Dichloro-dihydrofluorescein in U251 cells treated for C) one or D) two days with the treatments described above in addition to the presence or absence of 20 μ M TCP. Data are the mean of at least 3 independent experiments; bars, \pm SE. Data were compared using a one-way ANOVA with a Bonferroni's multiple comparison post-hoc analyses. (*) indicates statistically significant differences from control ($p < 0.05$).

Figure 2 supplementary. The combination of Δ^9 -THC and CBD selectively down-regulates pERK. The effects of cannabinoids on pERK were analyzed using Western analysis and α -tubulin was used as a loading control (LC).