Supplementary Materials: Biophysical Studies and In Vitro Effects of Tumor Cell Lines of Cannabidiol and Its Cyclodextrin Inclusion Complexes

Kyriaki Hatziagapiou, Kostas Bethanis, Eleni Koniari, Elias Christoforides, Olti Nikola, Athena Andreou, Aimilia Mantzou, George P. Chrousos, Christina Kanaka-Gantennbein and George I. Lambrou

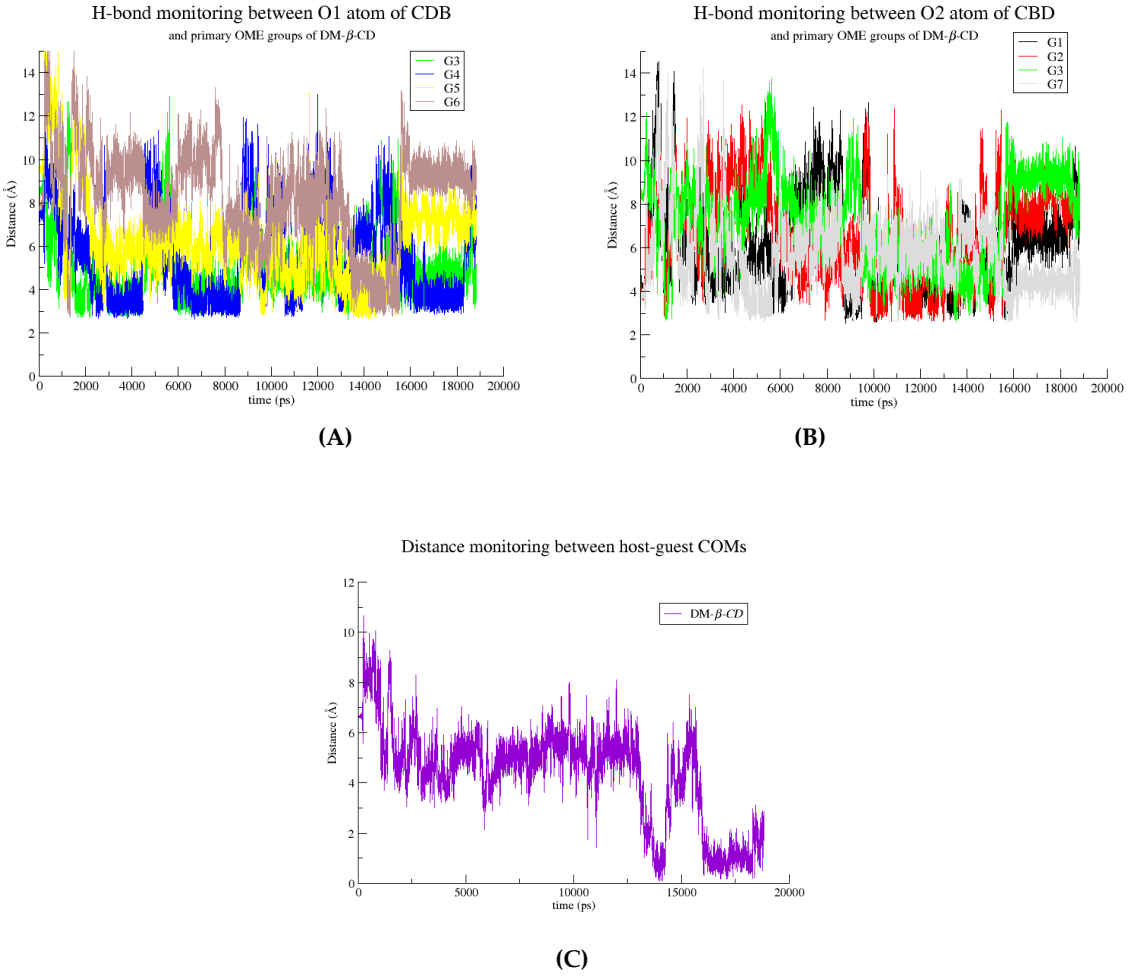
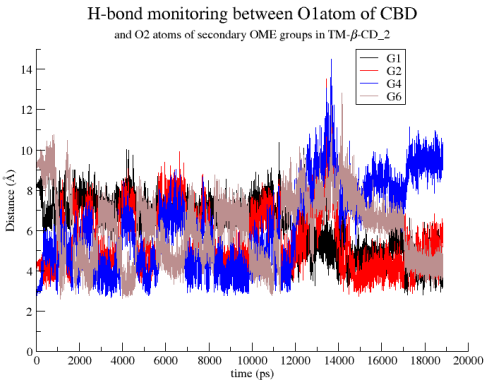
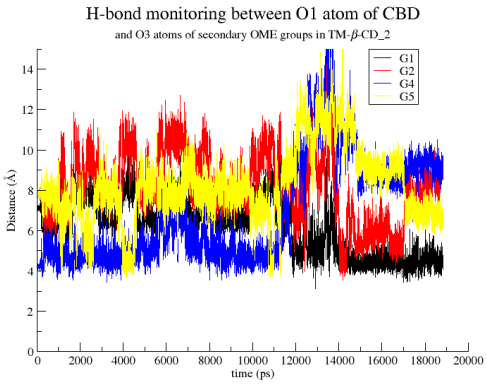


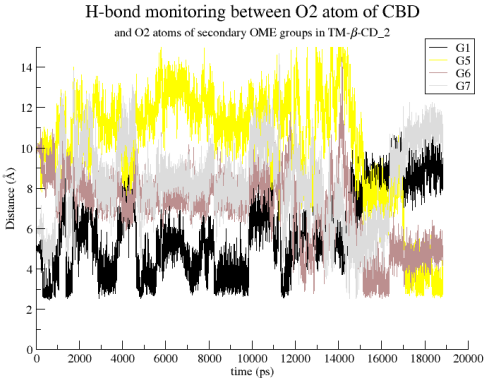
Figure S1. (**A**,**B**) H-bond monitoring between O1 and O2 atoms of CBD and primary methoxy groups (OME) of the host (glucose units G3, G4, G5 and G6) and (**C**) plot of host-guest Centre of Mass (COM) distance. Rapid decrease at 14 ns and 16 ns indicates the deeper immersion of the guest inside the DM-β-CD cavity.



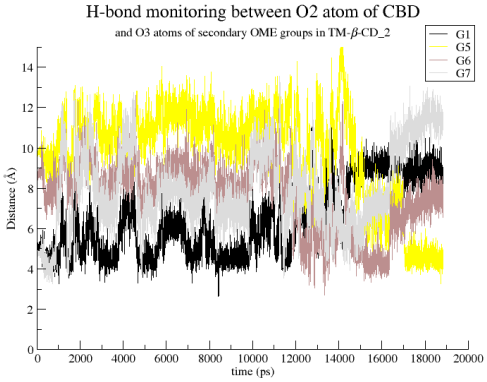
(A)



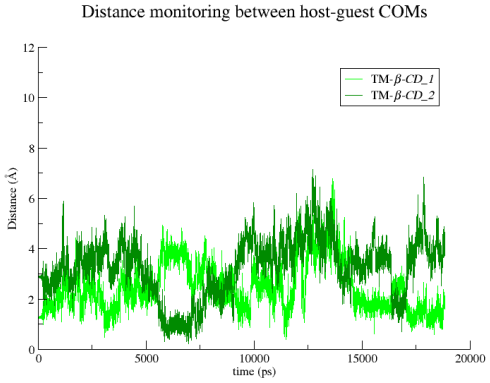
(B)



(C)



(D)



(E)

Figure S2. (**A**–**E**). H-bond monitoring between the hydroxyls of the benzenediol group of the guest and the secondary methoxy groups (OME) of the hosts and (**E**) plots of hosts-guest Centre of Mass (COM) distances.

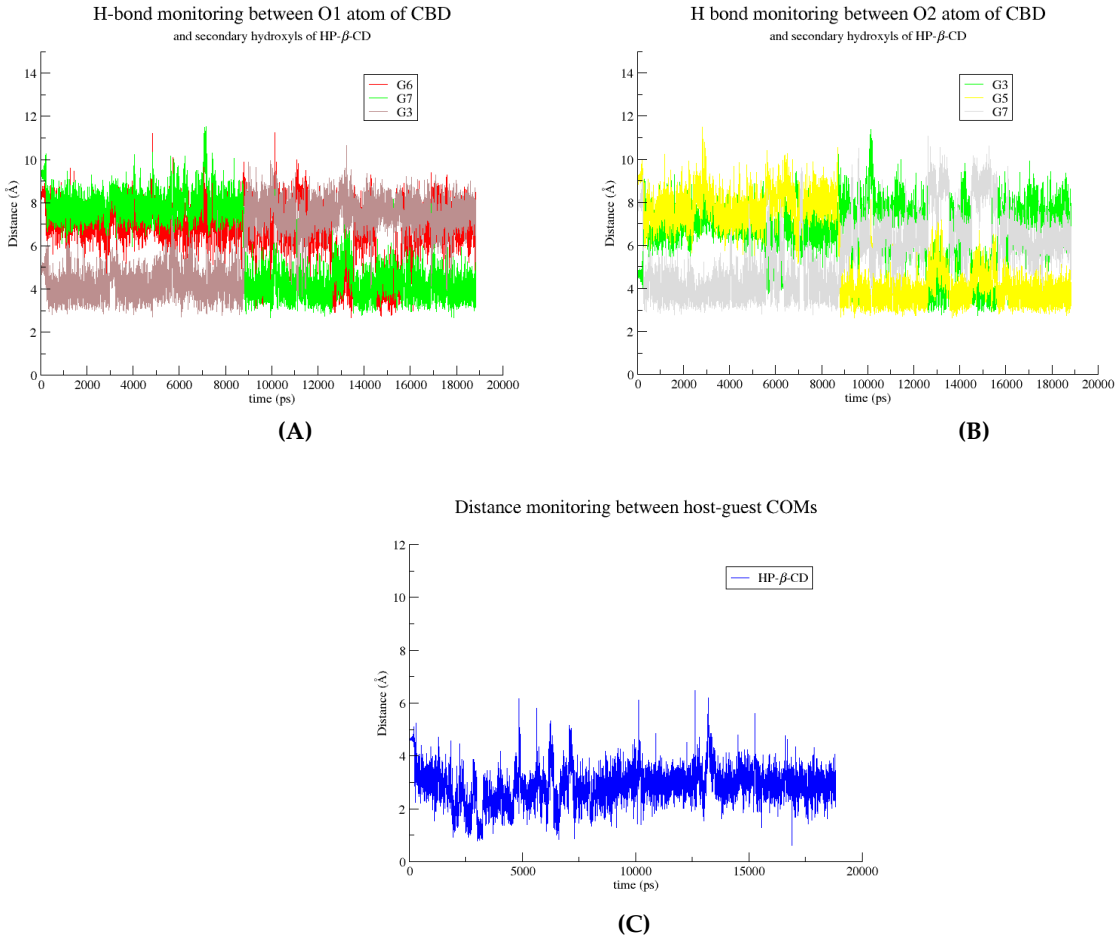


Figure S3. (**A**,**B**) H-bond monitoring between the hydroxyls of the benzenediol group of the guest and the secondary hydroxyl groups of the host and (**C**) plot of host-guest Centre of Mass (COM) distance.

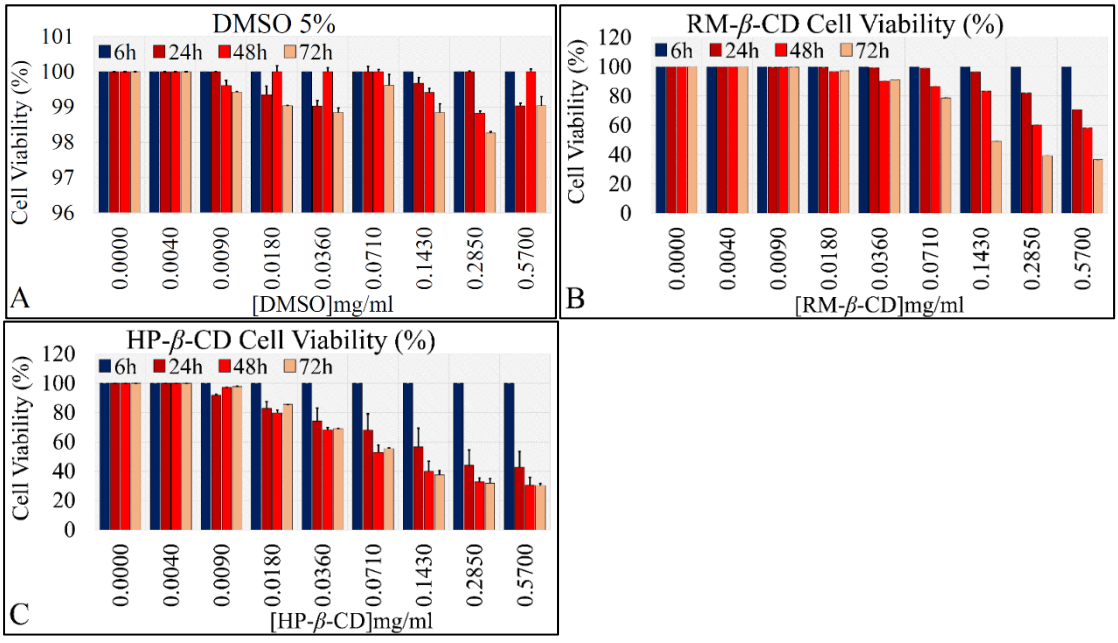


Figure S4. Dose-dependent effect of vehicles on A172 cells. Analyzing drug effects with respect to concentration for each time point studied (6, 24, 48, and 72 hours), revealed information in the role of the tested CD-vehicles. (**A**) DMSO treatment exhibited no significant cytotoxic effect as cell

viability did not fall below 97% for all concentrations and at all time points, (**B**) In the case of RM-*β*-CD and (**C**) HP-*β*-CD, cell viability was reduced in a dose- and time-dependent manner for concentrations ≥ 0.036, and ≥ 0.018, respectively. The two vehicles, manifested a cytotoxic effect by themselves, as they reduced cell viability down to approximately 30% for the higher concentrations. Note that no *p*-values are presented since the main focus was on the vehicle's effects.

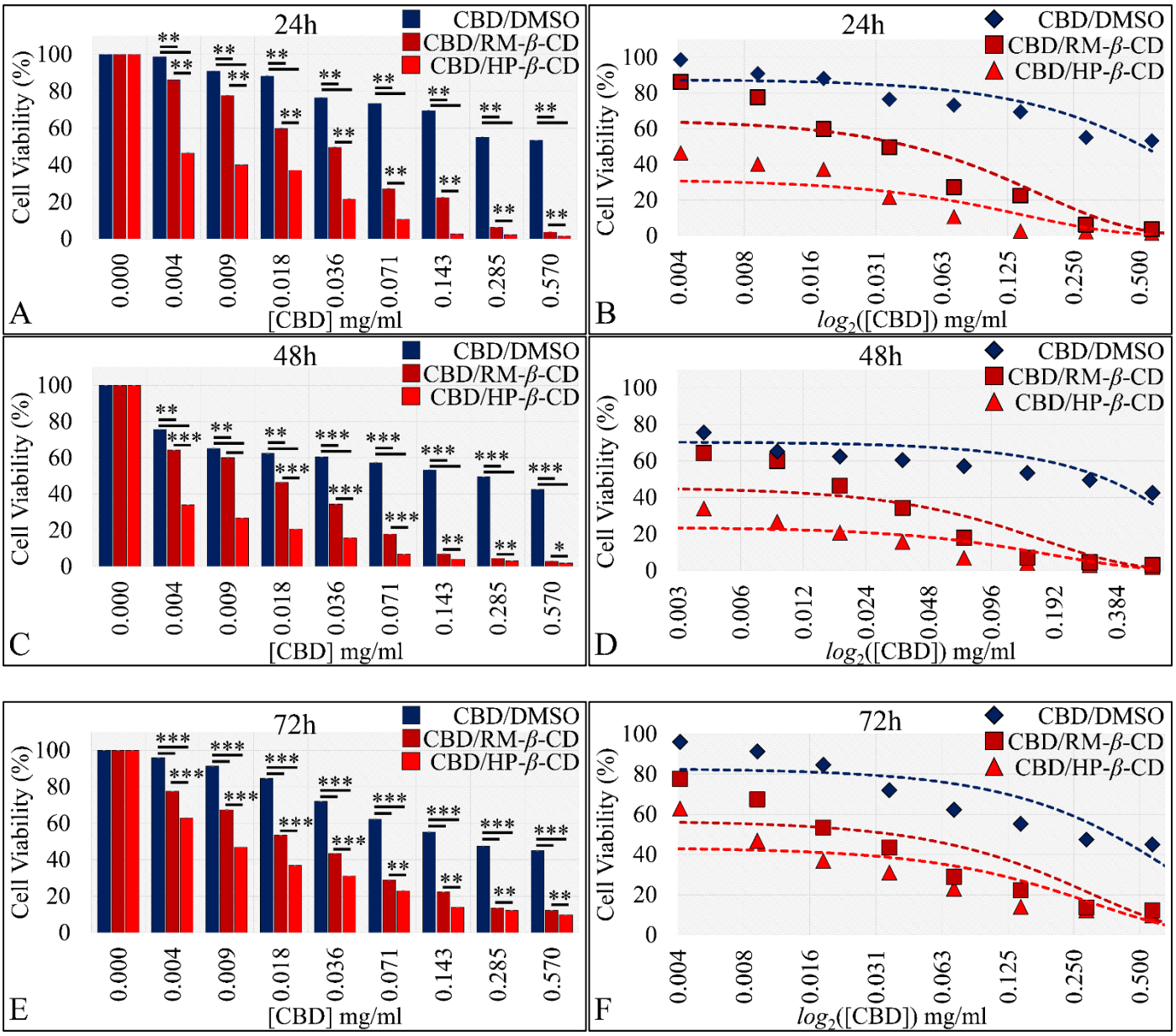


Figure S5. Comparative dose-dependent effect of CBD and CBD inclusion complexes on A172 cells. The comparative effectiveness of the inclusion complexes was investigated and presented together. The CBD/vehicle effectiveness appeared to follow the same pattern at all time points and in particular CBD/HP-*β*-CD was the most effective treatment, followed by CBD/RM-*β*-CD and CDB/DMSO. This effect was evident at all time points that is 24 hours (**A**,**B**), 48 hours (**C**,**D**) as well as 72 hours (**E**,**F**) * depicts a significance at the $p < 0.05$ level; ** depict a significance at the $p < 0.01$ level; *** depict a significance at the $p < 0.001$ level.

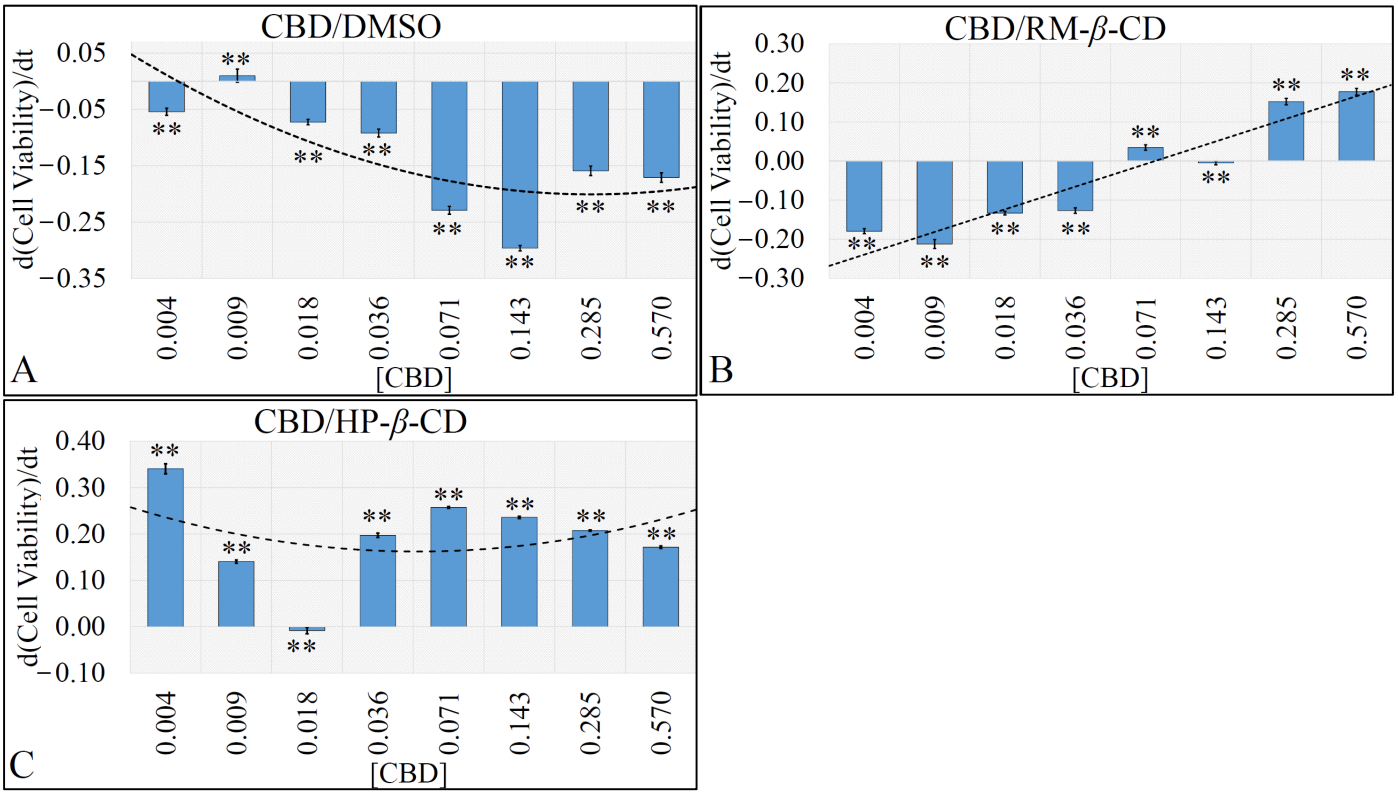


Figure S6. The comparative recovery velocity of CBD complexes. We calculated the "speed" by which cells recover after treatment, using the Equation (S1).

$$v_{Cell\_Recovery} = \frac{Cytotoxicity_{[CBD],t_2} - Cytotoxicity_{[CBD],t_1}}{t_2 - t_1} \quad \text{(S1)}$$

where, $\mathcal{V}$, is the calculated recovery speed at mg/ml/h. Calculations between the endpoint of 72 hours and 24 hours for all concentrations and all CBD inclusion complexes were performed. (**A**) Cells treated with CBD/DMSO did not recover alltogether and the observed differences were significant ($p < 0.001$). (**B**) Cells exposed to CBD/RM-β-CD for concentrations 0.071–0.57 mg/ml exchibited a cellular rescue, whereas for concentrations 0.004–0.036 mg/ml they did not demonstrate such behaviour. (**C**) Cells exposed to CBD/HP-β-CD manifested the "fastest" recovery rate. Yet, although the recovery rate under CBD/HP-β-CD treatment was high, its effectiveness still surpassed all other CBD inclusion complexes. The asterisks ** depict a significance at the $p < 0.01$ level. The asterisks above all bars indicate that the differences are significant among all concentrations and in all combinations.

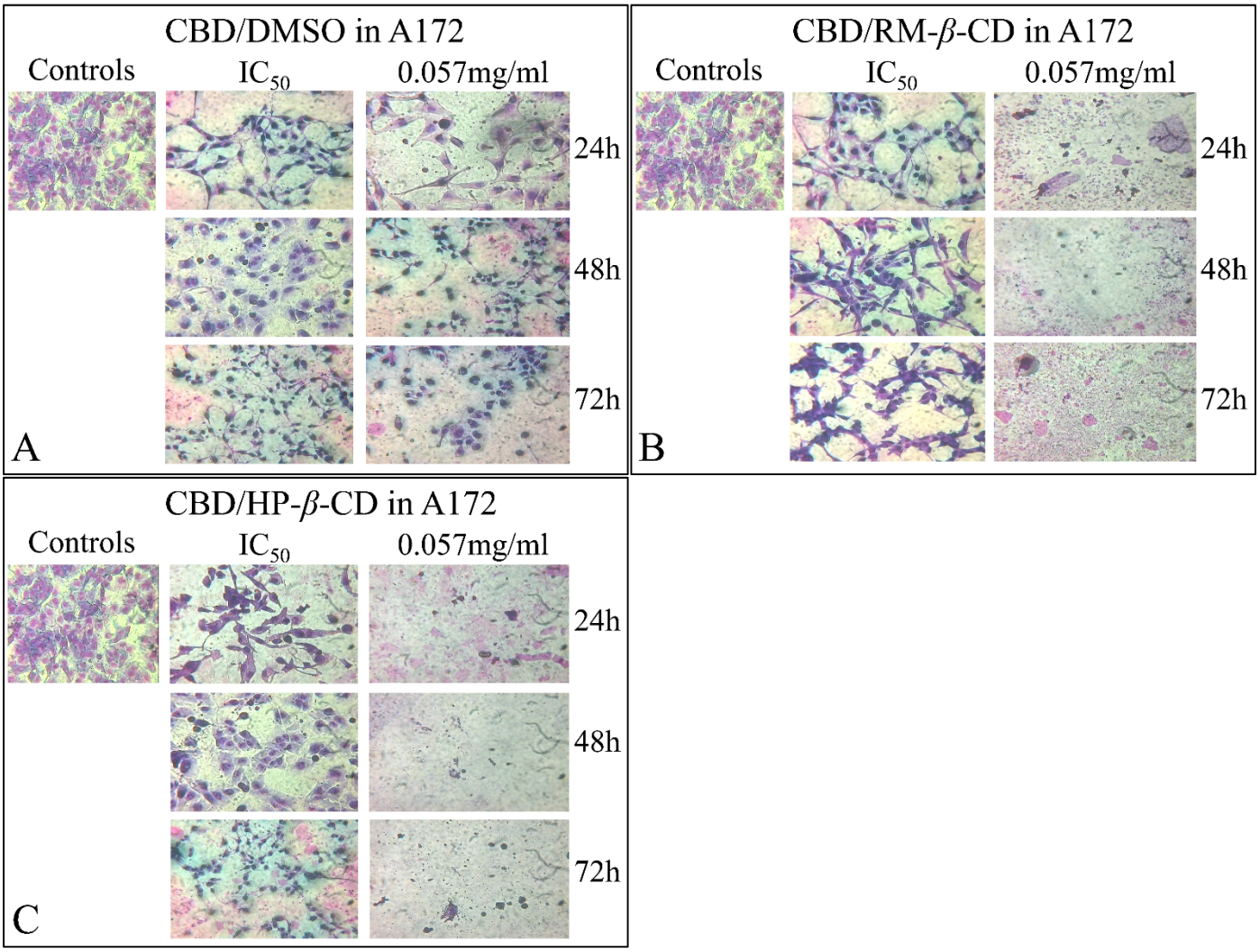


Figure S7. Microscopic examination of A172 cells exposed to CBD, and its inclusion complexes. Microscopy confirmed the cell viability when A172 were exposed to **(A)** CBD/DMSO, **(B)** CBD/RM-*β*-CD complex and **(C)** CBD/HP-*β*-CD complex.

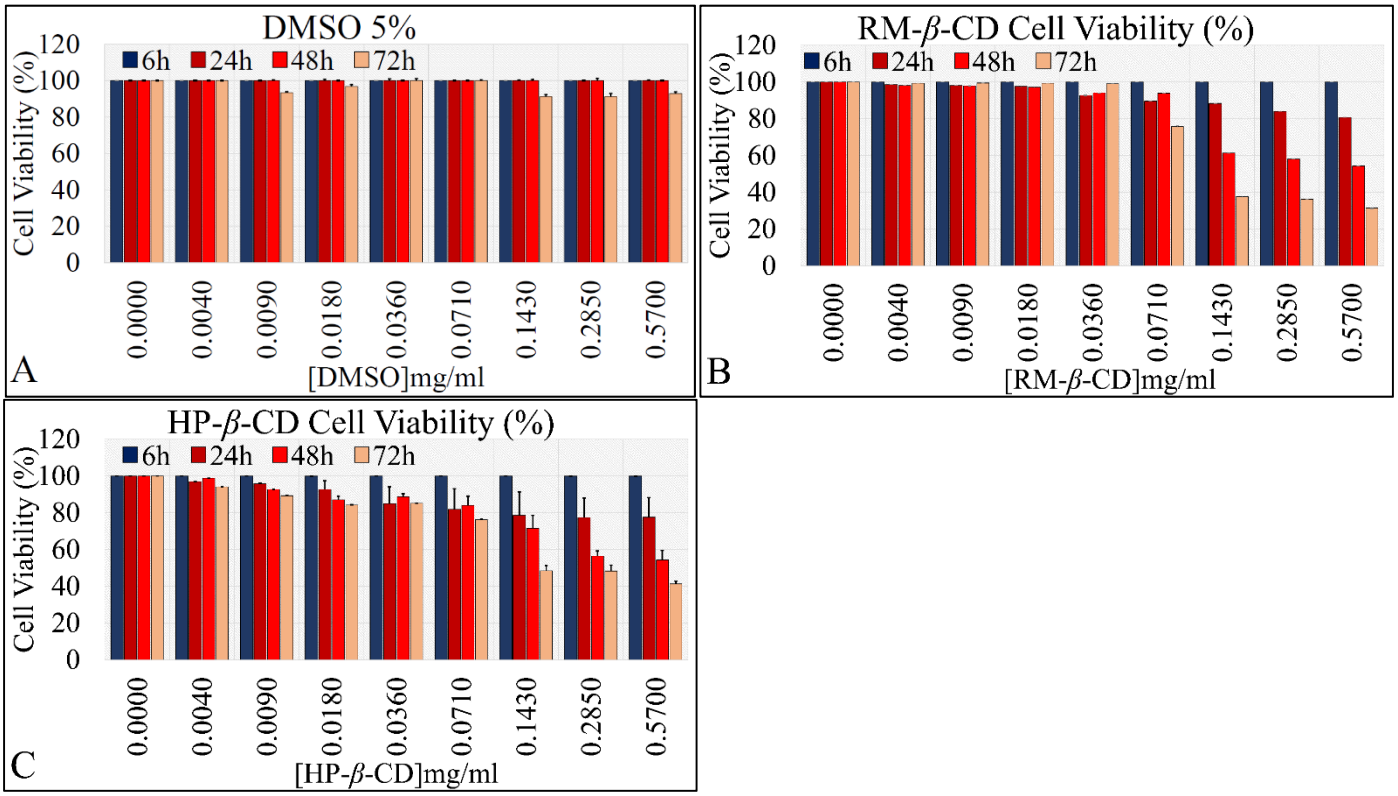


Figure S8. Dose-dependent effect of vehicles on TE671 cells. Analyzing drug effects with respect to concentration for each time point studied (6, 24, 48, and 72 hours), revealed information in the role of the tested CD-vehicles. **(A)** DMSO treatment exhibited no significant cytotoxic effect as cell viability did not fall below 90% for all concentrations and at all time points, **(B)** In the case of RM-*β*-CD and **(C)** HP-*β*-CD, cell viability was reduced in a dose- and time-dependent manner for concentrations ≥ 0.036, and ≥ 0.009, respectively. The two vehicles, manifested a cytotoxic effect by themselves, as they reduced cell viability down to approx. 30% for the higher concentrations. Note that no *p*-values are presented since the main focus was on the vehicle's effects.

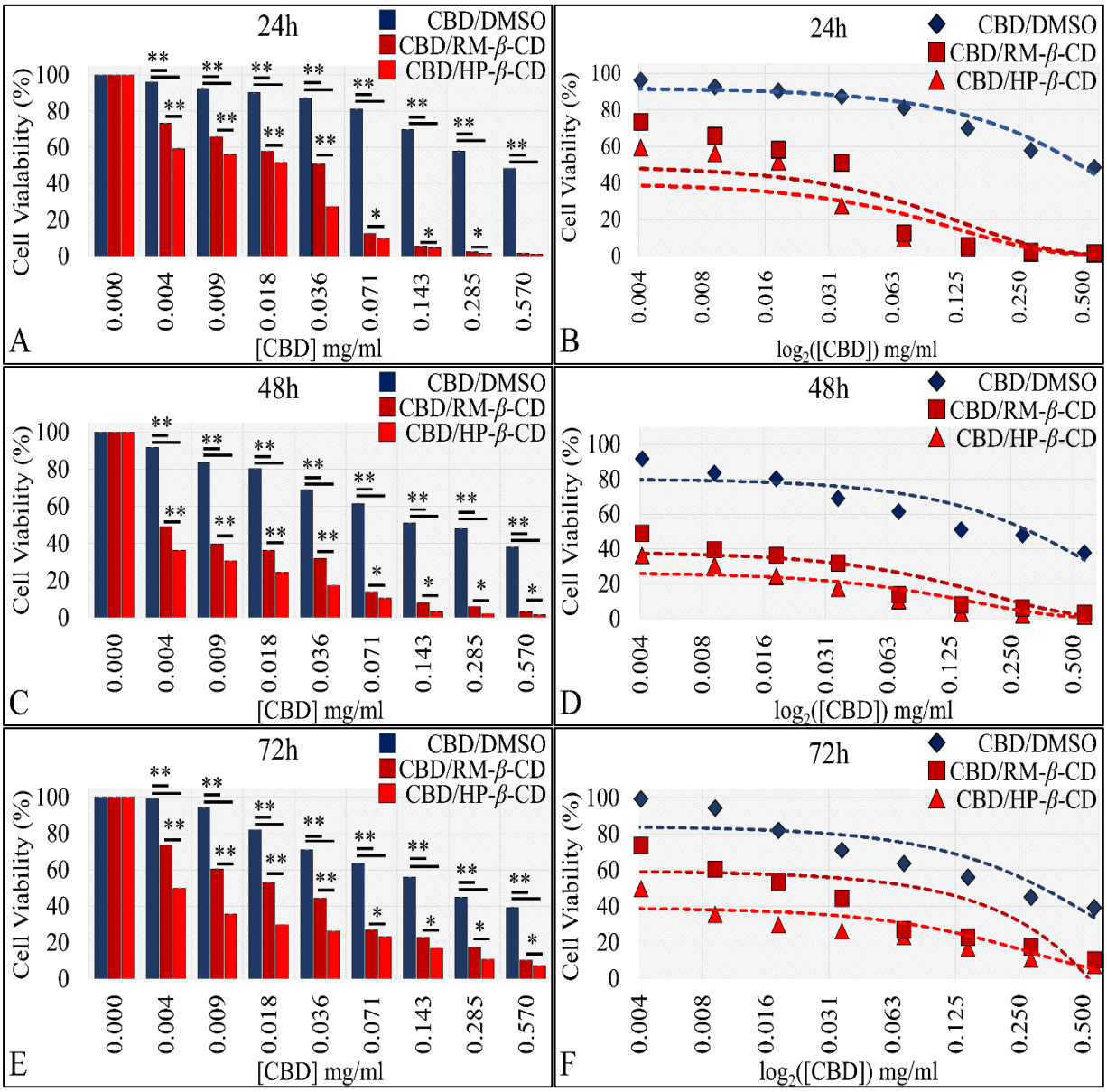


Figure S9. Comparative dose-dependent effect of CBD and CBD inclusion complexes on TE671 cells. The CBD/vehicle effectiveness appeared to follow the same pattern at all time points and in particular CBD/HP-*β*-CD was the most effective treatment, followed by CBD/RM-*β*-CD and CDB/DMSO. This effect was evident at all time points, i.e., 24 (**A**,**B**), 48 (**C**,**D**), and 72 hours (**E**,**F**); * depicts a significance at the $p < 0.05$ level; ** depict a significance at the $p < 0.01$ level.

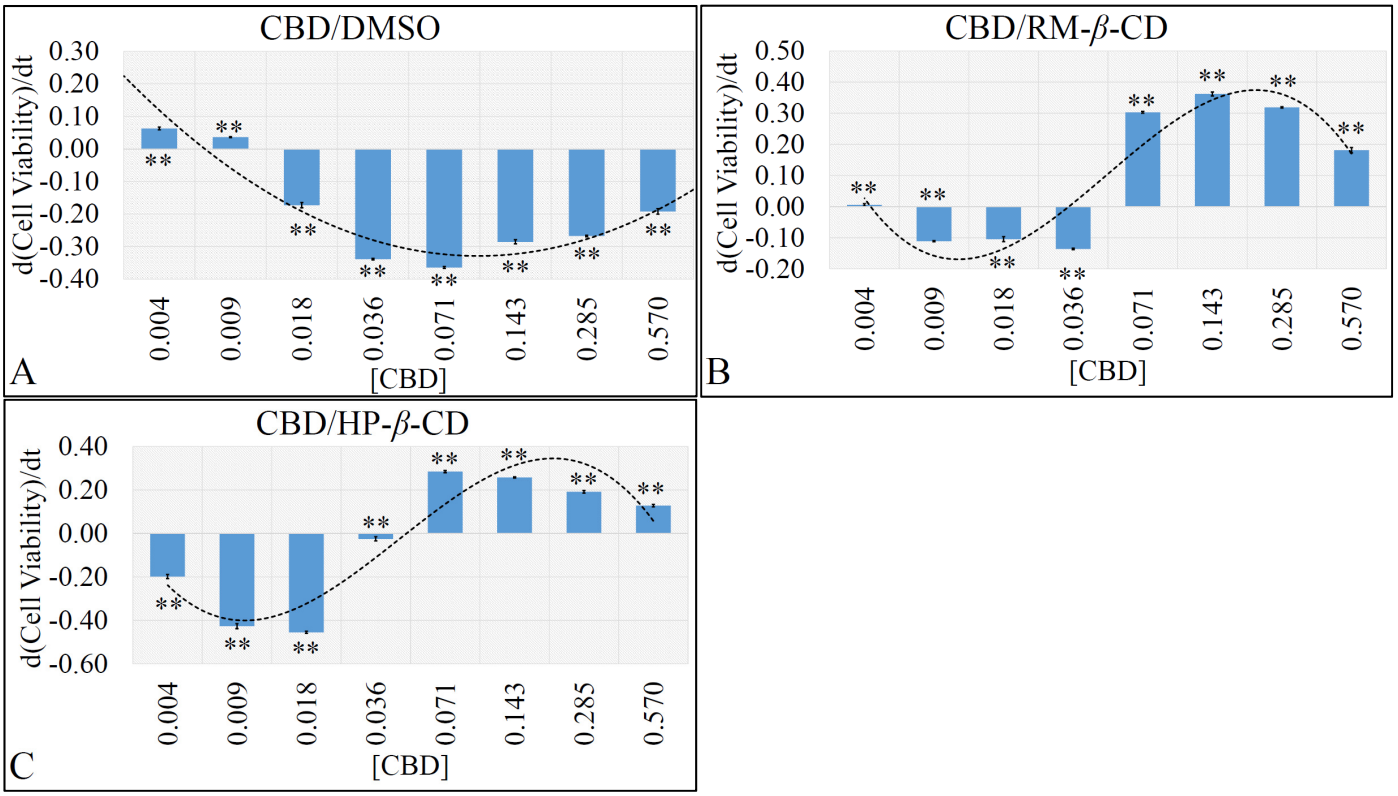


Figure S10. The comparative recovery velocity of CBD complexes in the TE671 cells. (**A**) Cells treated with CBD/DMSO did not recover all together and the observed differences were significant ($p < 0.001$). (**B**) Cells exposed to CBD/RM-*β*-CD manifested a cellular rescue at concentrations 0.071–0.57 mg/ml, whereas for the rest of the concentrations 0.004–0.036 mg/mL such rescue was not observed. (**C**) Cells treated with CBD/HP-*β*-CD manifested a similar behavior as the CBD/RM-*β*-CD complex, with a threshold at 0.036 mg/mL, in which they begun to recover. The asterisks ** depict a significance at the $p < 0.01$ level. The asterisks above all bars indicate that the differences are significant among all concentrations and in all combinations.

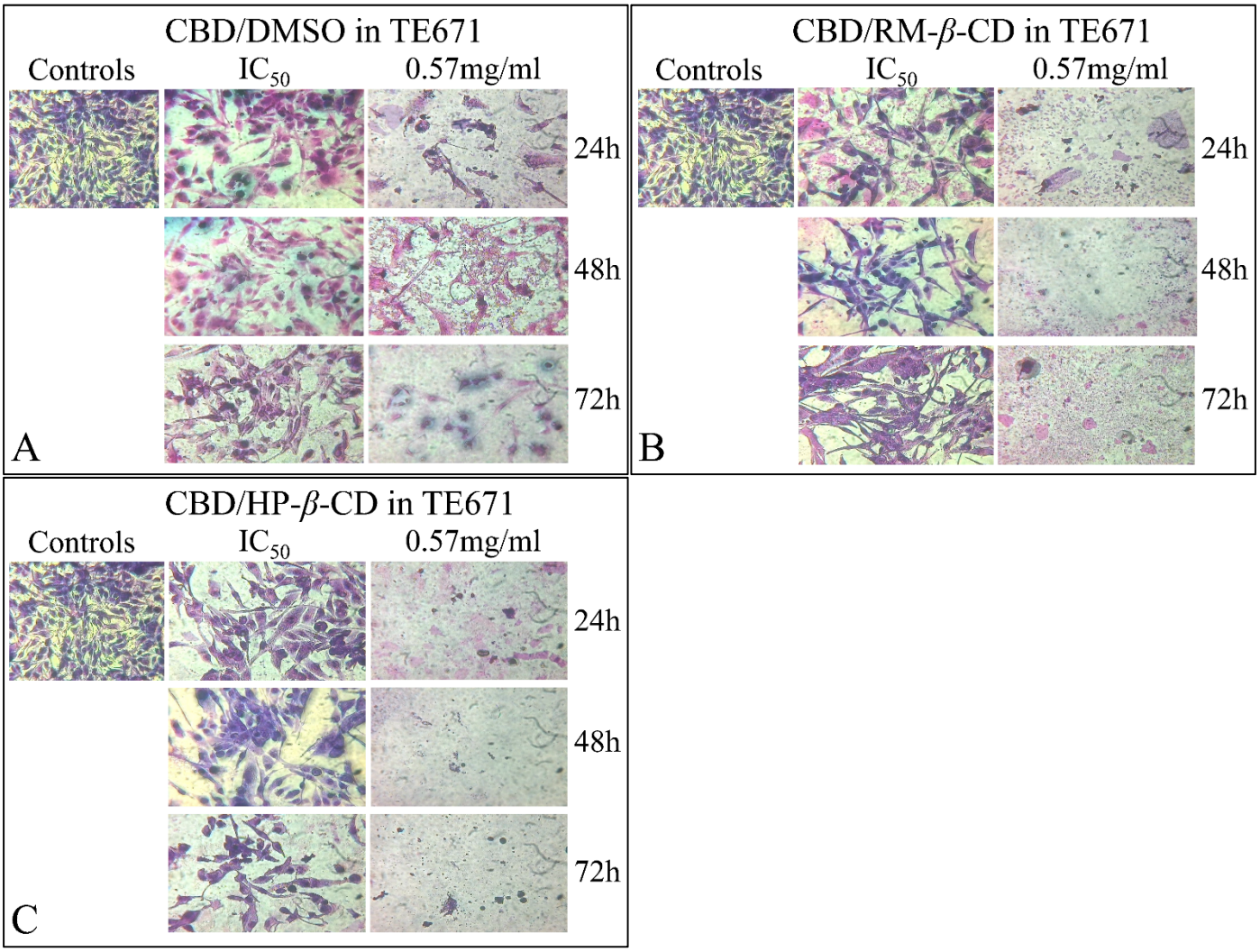


Figure S11. Microscopic examination of TE671 cells exposed to CBD, and its inclusion complexes. Microscopy confirmed the cell viability results for cells exposed to **(A)** CBD/DMSO, (**B**) CBD/RM-*β*-CD and **(C)** CBD/HP-*β*-CD inclusion complexes.