

Cannabidiol Potentiates p53-Driven Autophagic Cell Death in Non-Small Cell Lung Cancer Following DNA Damage: A Novel Synergistic Approach Beyond Canonical Pathways

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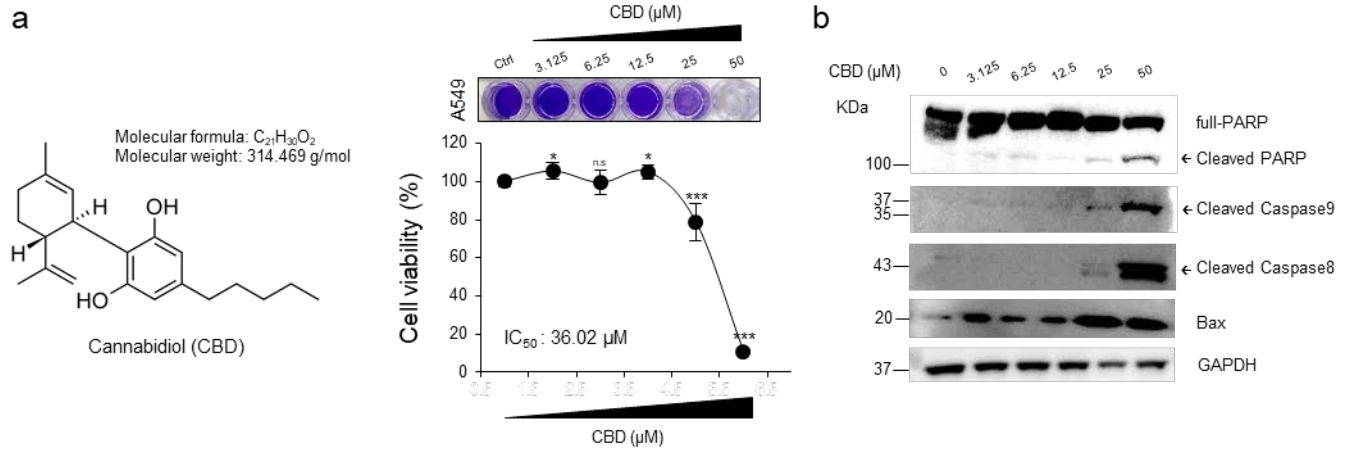
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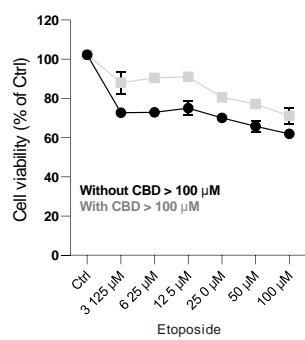
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Supplementary Figures

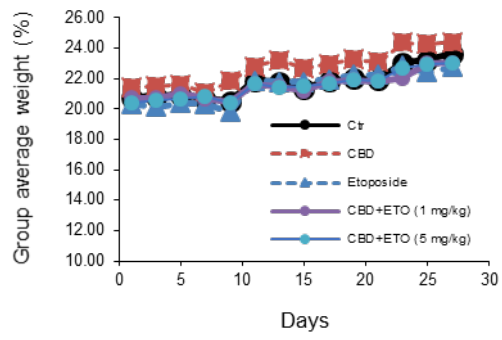


Supplementary Fig. 1 Low concentrations of CBD do not induce cell death in A539 cells. (a) Chemical structure of CBD (*left*). Cell viability is shown with a point plot at the indicated dose in A549 cells for 48 h (*right*). **(b)** Expression levels of cleaved PARP, caspases 8 and 9, and Bax are evaluated by Western blot. Protein levels are normalized to those of GAPDH.

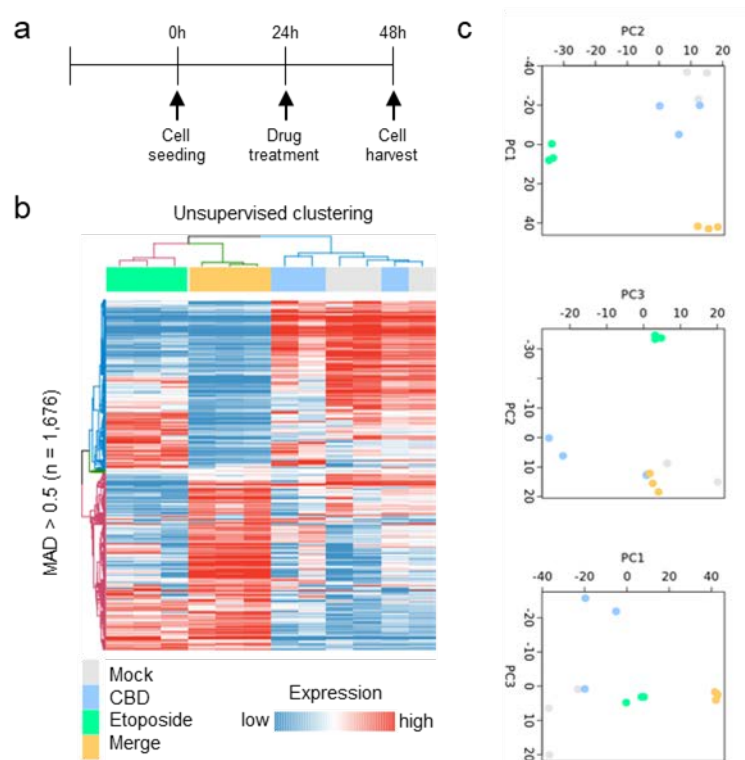


Supplementary Fig. 2 Assessment of combination treatment efficacy using WST-8 assay on normal lung fibroblast cells.

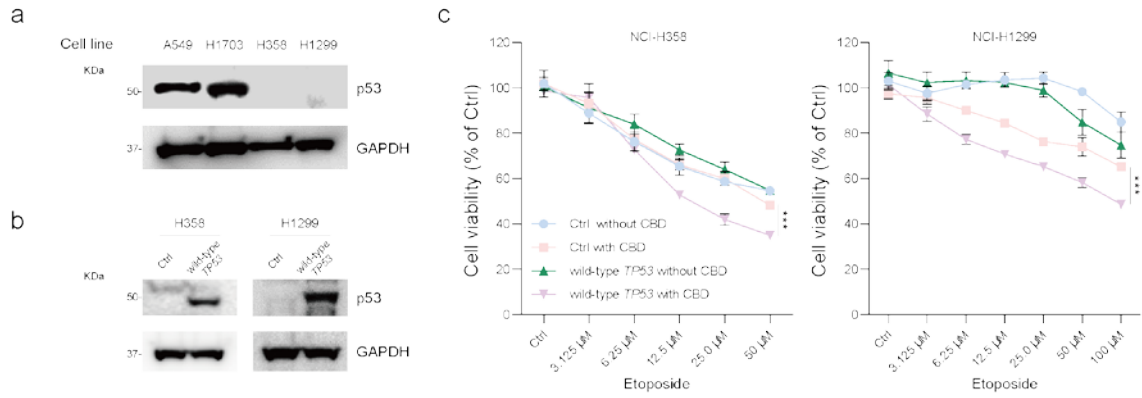
A plot shows the cell viability of HEL299 cells treated with etoposide, with or without CBD, for 48 h. IC₅₀ values are indicated.



Supplementary Fig. 3 Body weight change during *in vivo* experiment. A plot shows the body weight change during *in vivo* experiment.



Supplementary Fig. 4 Unsupervised clustering heatmap and PCA. (a) The schematic illustrates the workflow and details of sample preparation process of A549 cells for RNA-seq. A549 cells treated with CBD (15 μ M), etoposide (20 μ M), and their combination (merge) at 24 h. (b) A heatmap shows the unsupervised clustering of transcriptome data, demonstrating that the transcriptome of variably expressed genes (median absolute deviation > 0.5, n = 1,676) shows distinct patterns based on treatment status. (c) Principal component analysis using these variable genes reveals that groups are notably distributed in distinct clusters.



Supplementary Fig. 5 Establishment of p53-overexpressing cell lines and assessment of combination treatment efficacy using WST-8 assay. (a) Western blot analysis of p53 and GAPDH in NSCLC cell lines (A549, NCI-H1703, NCI-H358, and NCI-H1299). (b) Western blot analysis of p53 and GAPDH in control cells (Ctrl) and established wild-type *TP53*-overexpressing cells. (c) Plots show the cell viability of Ctrl and wild-type *TP53*-overexpressing cells treated with etoposide, with or without CBD, for 48 h in NCI-H358 and NCI-H1299, respectively. Statistical significance is indicated (***) $P < 0.001$; Student's t-test).