

Supplementary Materials: The Cytotoxic Effects of Cannabidiol and Cannabigerol on Glioblastoma Stem Cells May Mostly Involve GPR55 and TRPV1 Signalling

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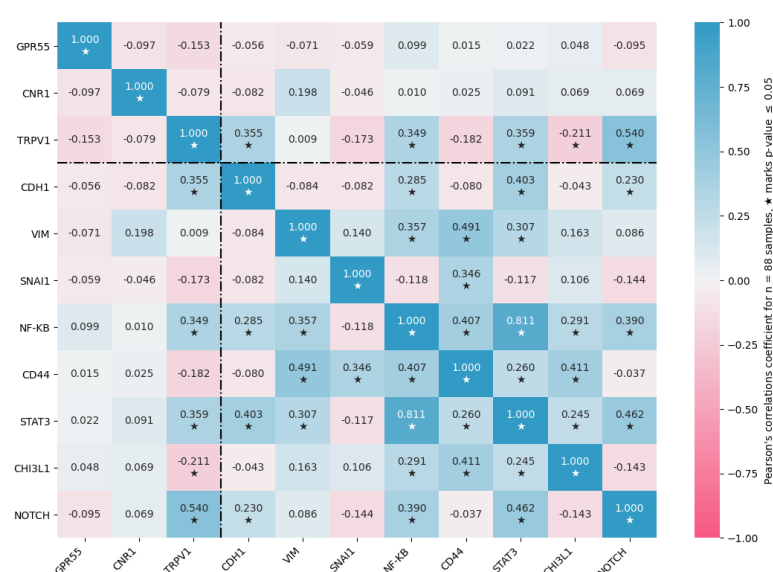


Figure S1. Correlation matrix of *GPR55*, *TRPV1*, and *CNR1* with epithelial-to-mesenchymal transition markers. These markers are *CDH1*, *vim*, *SNAI1*, *NF-κβ*, *CD44*, *STAT3*, *CHI3L1*, and *NOTCH*. *CNR1* and *GPR55* did not significantly correlate with any of these markers. *TRPV1* correlated with *CDH1* ($r=0.355$), *NF-κβ* ($r=0.349$), *STAT3* ($r=0.359$), *CHI3L1* ($r=-0.211$), and most strongly with *NOTCH* ($r=0.540$).

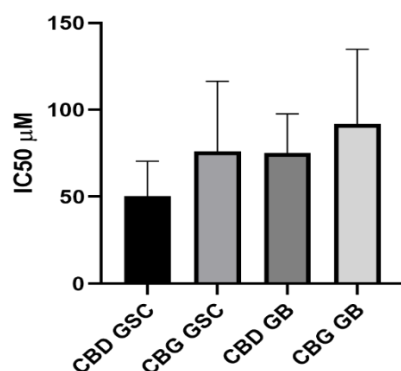


Figure S2. Mean IC₅₀ values for glioblastoma stem cell (GSC) and glioblastoma (GB) cell lines treated with cannabidiol (CBD) and cannabigerol (CBG), dissolved in base emulsion. Bars represent the mean±SEM of the cytotoxicity of CBD on GSCs (n=8), CBG on GSCs (n=8), CBD on GB cells (n=10), and CBG on GB cells (n=10). Statistical analyses were performed with GraphPad Prism software.

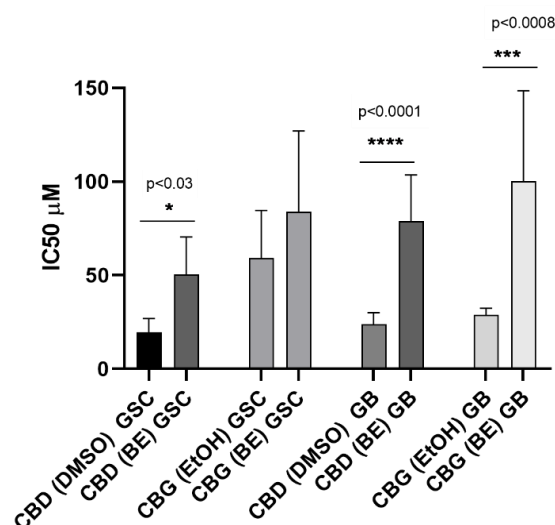


Figure S3. Mean IC₅₀ values for glioblastoma stem cell (GSC) and glioblastoma (GB) cell lines treated with cannabidiol (CBD) and cannabigerol (CBG), dissolved in ethanol/DMSO and base emulsion. Bars represent the mean \pm SEM of the cytotoxicity of CBD and CBG on nine GSCs and ten GB cells. CBD/CBG in base emulsion had significantly higher IC₅₀ values than in ethanol/DMSO. Statistical analyses were performed with GraphPad Prism software.

Table S1. List of assays used for RT-qPCR analysis (Thermo Fisher Scientific, USA).

Gene name	Protein name	Assay ID
<i>ACSBG1</i>	Acyl-CoA Synthetase Bubblegum Family Member 1	Hs00209500_m1
<i>ALYREF</i>	Aly/REF Export Factor	Hs00362329_m1
<i>CCL5</i>	Chemokine (C-C motif) ligand 5	Hs00982282_m1
<i>CCR3</i>	C-C Motif Chemokine Receptor 3	Hs00266213_s1
<i>CCR5</i>	C-C Motif Chemokine Receptor 5	Hs00152917_m1
<i>CD44</i>	CD44 molecule	Hs00174139_m1
<i>CD9</i>	CD9 antigen	Hs00233521_m1
<i>CDH1</i>	Cadherin 1	Hs01023895_m1
<i>CEBPA</i>	CCAAT Enhancer Binding Protein Alpha	Hs01072228_m1
<i>CHI3L1</i>	Chitinase-3-like protein 1; YKL-40	Hs01072228_m1
<i>CNR1</i>	Cannabinoid Receptor 1	Hs00275634_m1
<i>CNR2</i>	Cannabinoid Receptor 2	Hs00275635_m1
<i>COL1A1</i>	Collagen Type I Alpha 1 Chain	Hs00164004_m1
<i>COL1A2</i>	Collagen Type I Alpha 2 Chain	Hs01028956_m1
<i>CST7</i>	Cystatin F	Hs00175361_m1
<i>DAB2</i>	DAB Adaptor Protein 2	Hs01120074_m1
<i>DPYSL2</i>	Dihydropyrimidinase Like 2	Hs00265851_m1
<i>ERBB3</i>	Erb-B2 Receptor Tyrosine Kinase 3	Hs00176538_m1
<i>FREM2</i>	FRAS1 Related Extracellular Matrix 2	Hs0087621_m1
<i>FUT4</i>	Fucosyltransferase 4	Hs01106466_s1
<i>GFAP</i>	Glial Fibrillary Acidic Protein	Hs00909233_m1
<i>GPR55</i>	G Protein-Coupled Receptor 55	Hs00271662_s1
<i>ID1</i>	Inhibitor Of DNA Binding 1	Hs03676575_s1
<i>KCNF1</i>	Potassium Voltage-Gated Channel Modifier Subfamily F Member 1	Hs00266908_s1
<i>NFKB1</i>	Nuclear Factor Kappa B Subunit 1	Hs00765730_m1

<i>NOTCH1</i>	Neurogenic locus notch homolog protein 1	Hs01062014_m1
<i>OLIG2</i>	Oligodendrocyte Transcription Factor 2	Hs00377820_m1
<i>P2RX7</i>	Purinergic Receptor P2X 7	Hs00175721_m1
<i>POU5F1B</i>	POU Class 5 Homeobox 1B; OCT4-PG1 (OCT4 pseudo-gene)	Hs01596605_s1
<i>PROM1</i>	Prominin-1; CD133 antigen	Hs01009259_m1
<i>S100A4</i>	S100 Calcium Binding Protein A4	Hs00243202_m1
<i>SNAIL1</i>	Snail Family Transcriptional Repressor 1	Hs00195591_m1
<i>SOX2</i>	SRY-Box Transcription Factor 2	Hs01053049_m1
<i>SOX10</i>	SRY-Box Transcription Factor 10	Hs00366918_m1
<i>SPRY</i>	Sprouty RTK Signaling Antagonist 1	Hs00398096_m1
<i>STAT3</i>	Signal Transducer and Activator Of Transcription 3	Hs01047578_m1
<i>STMN4</i>	Stathmin 4	Hs00229288_m1
<i>TGFB1</i>	Transforming growth factor beta 1	Hs00998133_m1
<i>THBS1</i>	Thrombospondin 1	Hs00962908_m1
<i>TRIM28</i>	Tripartite Motif Containing 28	Hs00232212_m1
<i>TRPV1</i>	Transient Receptor Potential Cation Channel Subfamily V Member 1	Hs00218912_m1
<i>TUBB3</i>	Tubulin Beta 3 Class III	Hs00801390_s1
<i>TUFM</i>	Tu Translation Elongation Factor, Mitochondrial	Hs00607042_m1
<i>VIM</i>	Vimentin	Hs00958111_m1

Table S2. Details of the GBM patients operated at the Department Neurosurgery of the University Medical Centre Ljubljana, Slovenia, used for cell viability assays.

Patient	Gender	Age (Years)	KPV	MGMT promoter methylation	Mutations				Chromosomal abnormalities
					IDH1	TP53	EGFR mutation/ amplification	Other	
K26	F	75	60	N.A.	wt	mut	N.A.	N.A.	N.A.
NIB138	F	51	90	N.A.	wt	wt	-	-	-
NIB140	M	54	80	no	wt	wt	del eks 2-7 (EGFRvIII)	-	-
NIB142	F	56	80	N.A.	wt	mut	-	-	-
NIB167	M	84	30	no	wt	mut	-	-	-
NIB180	M	56	90	no	N.A.	N.A	ampl	mut pTERT C228T, del CDKN2A and CDKN2B, ampl PDGFRA, mut PDGFRA I317S, mut. AVCR1 S440G, mut. SETD2 K1706E	ampl. 7, del 10q
NIB182	M	74	80	yes	N.A.	N.A	-	mut pTERT C228T, PIK3R1 R631*	ampl 7, del 10q
NIB185	M	40	90	no	N.A.	mut R213*	-	mut pTERT C228T; C242F	ampl 7, del 10q
NIB220	M	56	N.A	no	wt	wt	-	mut pTERT (C228T) mut PIK3CA p.(Gly118Asp) (G118D) ampl EGFR, MET and PIK3CA; del CDKN2A in CDKN2B	ampl 7, del 10q, part del 1p and 19q.
NIB237	M	65	N.A	no	wt	mut p.(Arg267Trp) (R267W) and p.(Arg342Ter) (R342*)	-	mut pTERT (C228T). mut PTEN p.(Gly36Val)(G36V). FGFR1 p.(Arg455Cys) (R455C)	ampl 7, part del 1p and del 10q in 19q.
NIB249	M	47	N.A	no	wt	mut p.(Pro190Leu) (P190L)	mut p.(Arg108Lys)(R108K); ampl EGFR	mut pTERT (C228T) ampl MET and PIK3CA; del CDKN2A and CDKN2B mut PTEN p.(Ile122Phe)(I122F).	del 10q
NIB253	F	58	N.A	yes	wt	del	mut p.(Arg108Lys) (R108K) and p.(Gly598Val) (G598V); ampl EGFR	ampl MET, MDM2, MDM4, PIK3CA, PIK3R1 and SETD2. del CDKN2A, CDKN2B, FGFR1, and CIC; mut PTEN p.(Glu242Gly) (E242G).	ampl 7 and del 10q in 19q.
NIB255	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
NIB258	M	66	N.A	no	wt	ampl	-	mut pTERT c.-124C>T (C228T). mut PTEN p.(Pro248ThrfsTer5) (P248Tfs*5). ampl PDGFRA, MDM2, PIK3CA, PIK3R1, ACVR1 and CIC, del CDKN2A and CDKN2B.	ampl 7 and LOH 10q.

¹ Abbreviations: mut, mutated; del, deletion; part del, partial deletion; ampl, amplification; (-), No mutations identified, negative; wt, wild-type; LOH, loss of heterozygosity; N.A., Not performed.