

Supplementary Materials:

THC Reduces Ki67-Immunoreactive Cells Derived from Human Primary Glioblastoma in a GPR55-Dependent Manner

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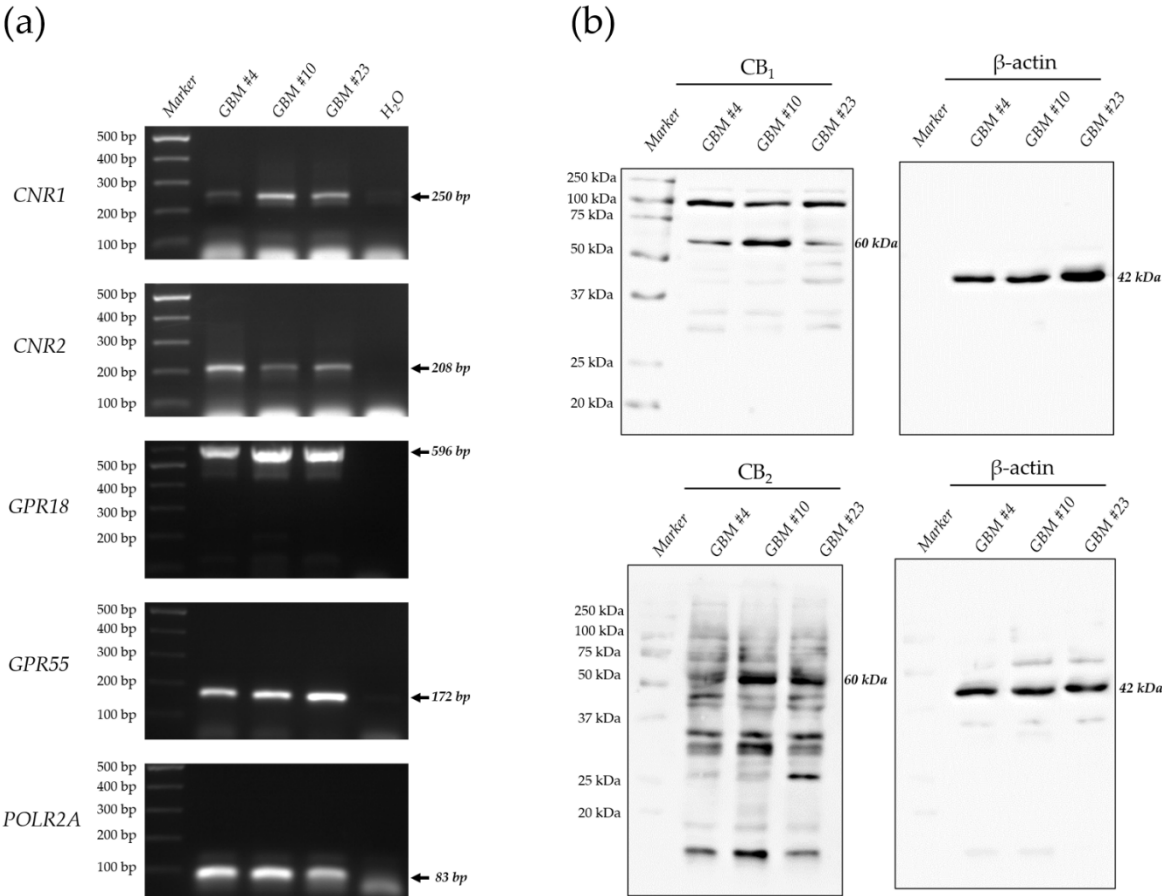


Figure 1. Original images of PCR products and Western Blots.

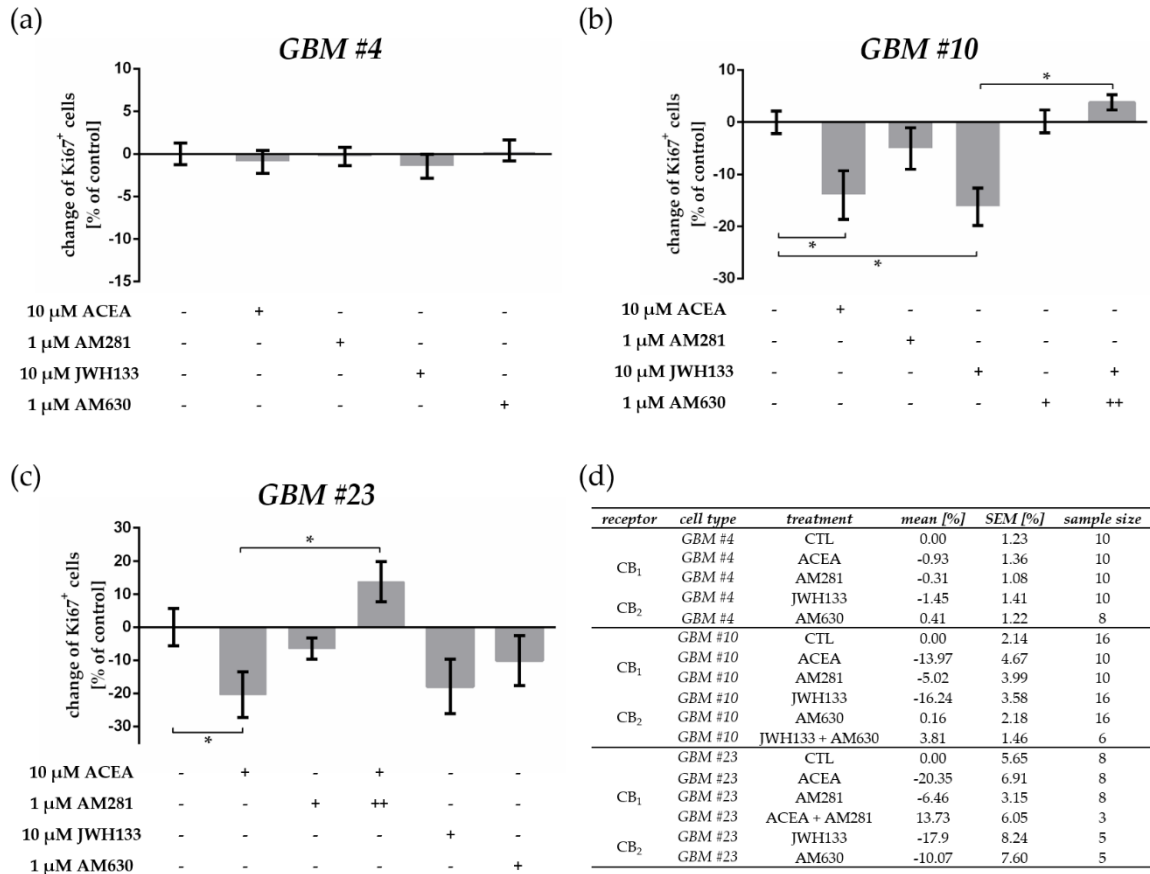


Figure S2: Influence and receptor dependence of CB₁ and CB₂ ligands on the percentage of Ki67⁺ cells. The cannabinoids ACEA (CB₁-agonist, 10 μ M), JWH133 (CB₂-Agonist, 10 μ M), AM281 (CB₁-antagonist, 1 μ M) and AM630 (CB₂-antagonist, 1 μ M) were used to target CB₁ and CB₂ receptor in GBM #4, GBM #10 and GBM #23. (a) Impact of CB₁- and CB₂-ligands on the number of Ki67⁺ cells of GBM #4. Cells remained unaffected after treatment with ACEA, JWH133, AM281 and AM630 for 24 h. (b) Impact of CB₁- and CB₂-ligands on number of Ki67⁺ cells of GBM #10. Application of ACEA and JWH133 led to a significant decreased number of Ki67⁺ cells compared to the control group. AM281 and AM630 alone caused no changes. By co-application with AM630, the effect of JWH133 was significantly abolished and CB₂-activation by JWH133 was identified as underlying mechanism. (c) Impact of CB₁- and CB₂-ligands on number of Ki67-immunoreactive cells of GBM #23. Treatment with different CB₁ and CB₂ ligands revealed a reduced number of Ki67-labelled cells upon ACEA exposure in comparison to the control group. This process was dependent on the activation of CB₁, since CB₁-antagonist AM281 prevented ACEA-mediated effect when both were co-applied. JWH133, AM281 and AM630 alone caused no significant changes. (d) Exact measurement values and sample sizes after treatment with CB₁- and CB₂-ligands. To completely block CB₁ and CB₂, antagonists were applied 15 min (++) before agonists were subsequently added. Data are means \pm SEM of N=3-8 independent experiments performed in duplicate. Significance was chosen for $p < 0.05$. The asterisk denotes significant results regarding the respective measurement indicated with the bar.

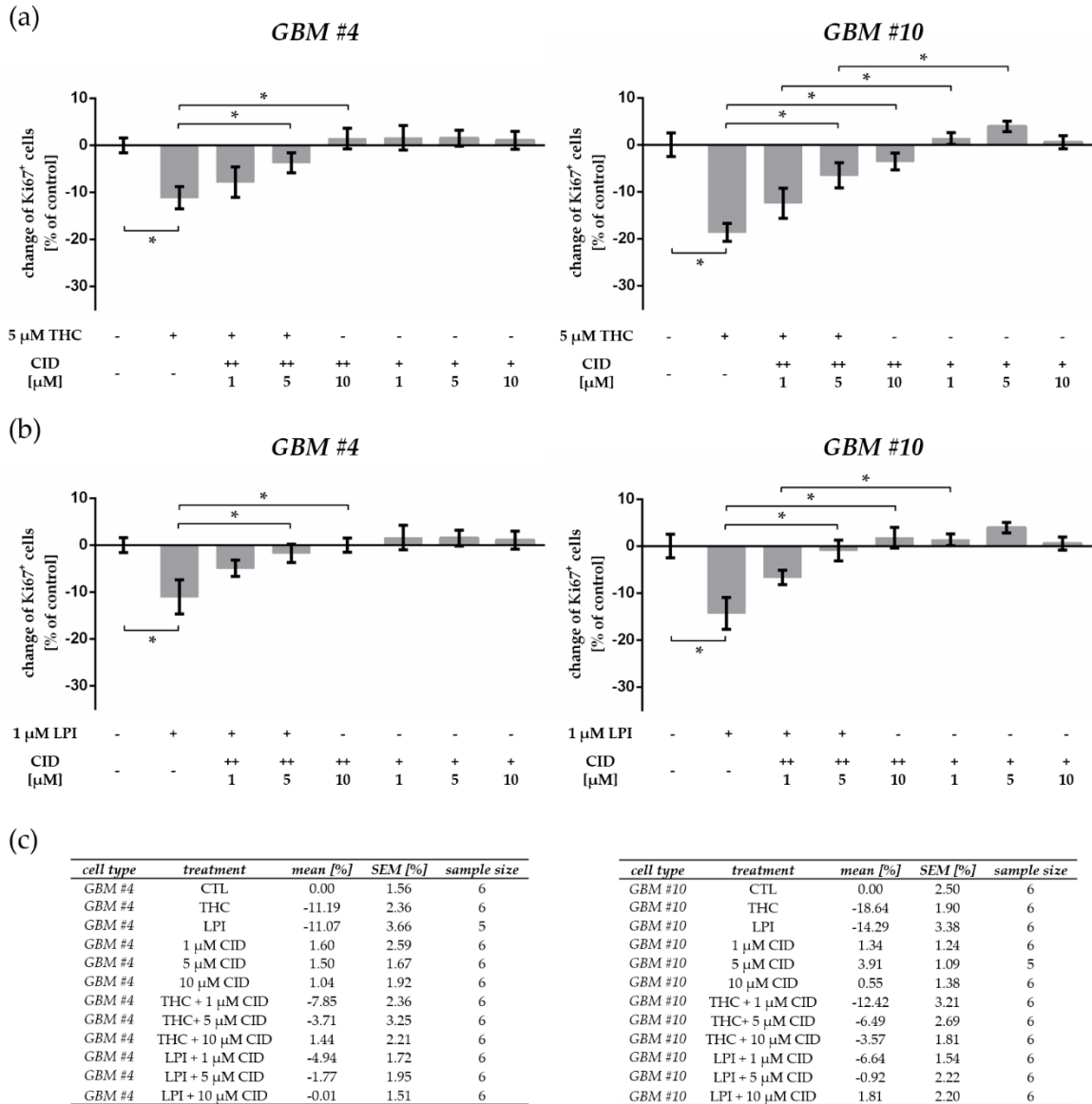


Figure S3: Establishment of GPR55 antagonist CID16020046 (CID). **(a)** Influence of increasing concentration of CID in presence of THC. The effects of THC were significantly abolished after pre-treatment with 5 μM or 10 μM CID in *GBM #4* and *GBM #10*. CID alone had no significant effects. **(b)** Influence of increasing concentration of CID in presence of LPI. 5 μM or 10 μM CID were sufficient to block LPI effects completely. CID alone had no significant effects. **(c)** Exact measurement values and sample sizes after treatment with increasing concentration of CID. Data are means ± SEM of N=3 independent experiments performed in duplicate. To completely block GPR55 CID were applied 15 min (++) before THC or LPI were subsequently added. Significance was chosen for $p < 0.05$. The asterisk denotes significant results regarding the respective measurement indicated with the bar.

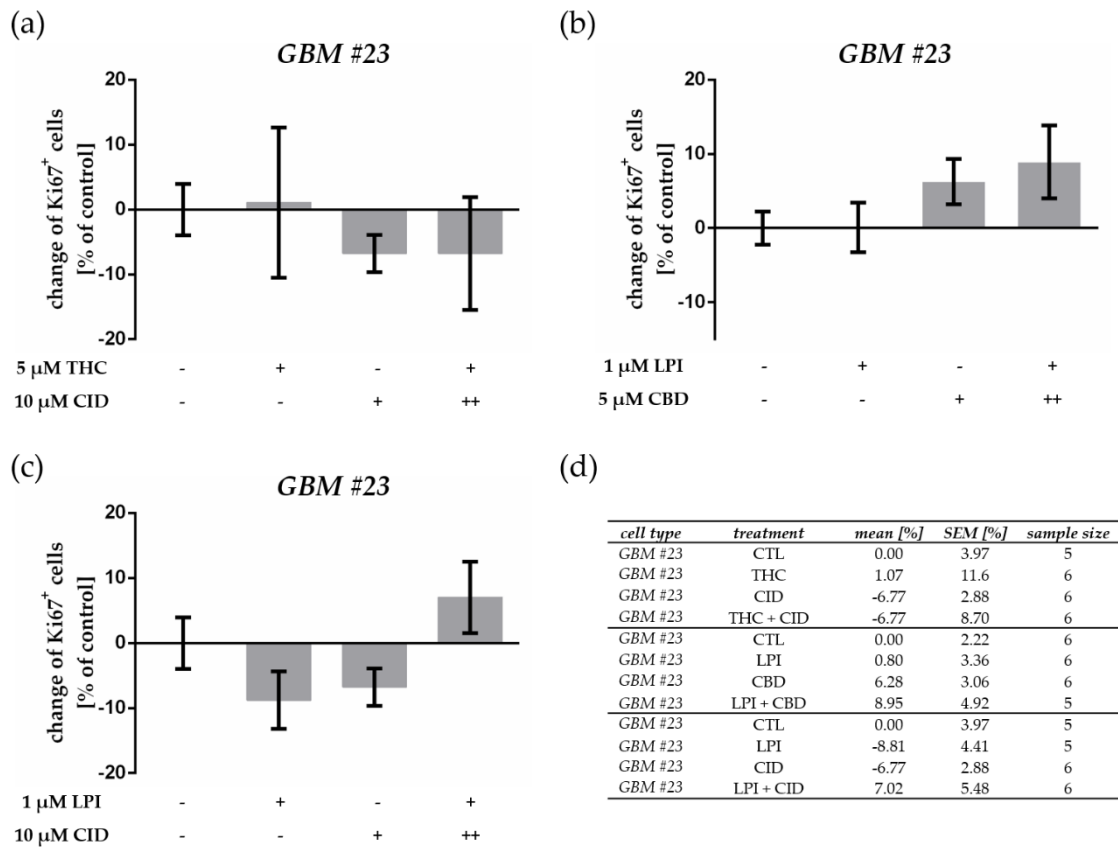


Figure S4: Impact of GPR55 ligands on the percentage of Ki67⁺ cells in GBM #23. **(a)** Impact of THC and CID16020046 (CID) on the number of Ki67-labelled GBM cells. Application of THC (5 μM) did not alter the percentage of Ki67⁺ cells alone and in combination with CID (10 μM). CID alone displayed also no significant effects. **(b)** Impact of LPI and CBD on the number of Ki67-labelled GBM cells. Cells treated with LPI (1 μM) for 24 h were unaffected through the number of Ki67⁺ cells. The combination with GPR55-antagonist CBD had also no significant effects on GBM #23. **(c)** Impact of LPI and CID on the number of Ki67-labelled GBM cells. Graph demonstrates the effects of CID (10 μM) alone and LPI (1 μM) in presence of CID. CID alone had no influence on the growth fraction of GBM #23 and did not altered LPI-acting when both were co-applied. **(d)** Exact measurement values and sample sizes after treatment with CB₁- and CB₂-ligands. Data are means \pm SEM of N=3 independent experiments performed in duplicate. To completely block GPR55 CBD and CID were applied 15 min (++) before THC or LPI were subsequently added.

Table S1. Data in patients and primary tumor samples of investigated GBM.

		<i>GBM #4</i>	<i>GBM #10</i>	<i>GBM #23</i>	
primary tumor sample	sex	female	male	female	
	age	50	56	83	
	tumor location	temporooccipital	temporal	central	
	tumor origin	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	
	MGMT ¹ promotor	methylated	methylated	unmethylated	
	IDH1 ² status (R132H)	wildtype	wildtype	wildtype	
patient-derived cells	MGMT promotor	methylated	methylated	methylated	
	IDH1 status (R132H)	wildtype	wildtype	wildtype	
	stem cell marker	CD133 ⁺ cells	<1%	<1%	<1%
		SOX2 mRNA	detectable	detectable	detectable
		<i>MSI1</i> mRNA	detectable	detectable	detectable
		<i>NES</i> mRNA	detectable	detectable	detectable
		<i>CD44</i> mRNA	detectable	detectable	detectable

¹O-6-methylguanine-DNA methyltransferase; ² isocitrate dehydrogenase 1

Table S2. Exact measurement values and sample sizes after treatment with THC and CBD.

cell type	treatment	mean [%]	SEM [%]	sample size
<i>GBM #4</i>	CTL	0.00	2.08	10
<i>GBM #4</i>	THC	-16.68	2.39	9
<i>GBM #4</i>	CBD	-4.09	1.54	8
<i>GBM #4</i>	THC + CBD	-4,75	3.33	7
<i>GBM #10</i>	CTL	0.00	2.27	7
<i>GBM #10</i>	THC	-9.11	2.62	8
<i>GBM #10</i>	CBD	-1.69	2.17	7
<i>GBM #10</i>	THC + CBD	-0.55	1.78	6
<i>GBM #23</i>	CTL	0.00	2.07	10
<i>GBM #23</i>	THC	-1.39	4.05	9
<i>GBM #23</i>	CBD	1.27	2.96	9
<i>GBM #23</i>	THC + CBD	-4.55	2.91	10

Table S3. Exact measurement values and sample sizes after treatment with THC in presence of different antagonists.

receptor	cell type	treatment	mean [%]	SEM [%]	sample size
CB ₁ /CB ₂	<i>GBM #4</i>	CTL	0.00	3.17	12
	<i>GBM #4</i>	THC	-27.66	1.36	6
	<i>GBM #4</i>	AM281 + AM630	10.28	0.78	6
	<i>GBM #4</i>	THC + AM281 + AM630	-25.41	1.99	6
	<i>GBM #10</i>	CTL	0.00	3.06	12
	<i>GBM #10</i>	THC	-21.06	3.08	6
	<i>GBM #10</i>	AM281 + AM630	7.42	2.57	6
	<i>GBM #10</i>	THC + AM281 + AM630	-25.58	2.47	6
GPR18	<i>GBM #4</i>	CTL	0.00	3.36	6
	<i>GBM #4</i>	THC	-19.02	3.70	6
	<i>GBM #4</i>	O-1918	-7.07	3.39	6
	<i>GBM #4</i>	THC + O-1918	-19.58	3.13	6
	<i>GBM #10</i>	CTL	0.00	1.96	6
	<i>GBM #10</i>	THC	-20.49	4.64	6
	<i>GBM #10</i>	O-1918	-4.118	3.08	6
	<i>GBM #10</i>	THC + O-1918	-27.95	3.69	6
GPR55	<i>GBM #4</i>	CTL	0.00	1.56	6
	<i>GBM #4</i>	THC	-11.19	2.36	6
	<i>GBM #4</i>	CID	1.04	1.92	6
	<i>GBM #4</i>	THC + CID	1.44	2.21	6
	<i>GBM #10</i>	CTL	0.00	2.50	6
	<i>GBM #10</i>	THC	-18.64	1.90	6
	<i>GBM #10</i>	CID	0.55	1.38	6
	<i>GBM #10</i>	THC + CID	-3.57	1.81	6

Table S4. Exact measurement values and sample sizes after treatment with increasing concentrations of THC in presence of CID.

cell type	treatment	mean [%]	SEM [%]	sample size
<i>GBM #4</i>	CTL	0.00	1.36	6
<i>GBM #4</i>	0.1 μ M THC	-8.52	1.62	6
<i>GBM #4</i>	1 μ M THC	-6.45	1.69	6
<i>GBM #4</i>	5 μ M THC	-13.72	3.24	6
<i>GBM #4</i>	10 μ M THC	-12.53	1.56	5
<i>GBM #4</i>	0.1 μ M THC + CID	1.11	0.76	6
<i>GBM #4</i>	1 μ M THC + CID	-0.99	1.28	6
<i>GBM #4</i>	5 μ M THC + CID	2.21	1.22	6
<i>GBM #4</i>	10 μ M THC + CID	0.98	0.79	6
<i>GBM #4</i>	CID	0.44	0.61	5
<i>GBM #10</i>	CTL	0.00	3.14	6
<i>GBM #10</i>	0.1 μ M THC	-7.04	1.46	6
<i>GBM #10</i>	1 μ M THC	-8.97	1.95	6
<i>GBM #10</i>	5 μ M THC	-15.48	3.49	6
<i>GBM #10</i>	10 μ M THC	-16.92	2.19	5
<i>GBM #10</i>	0.1 μ M THC + CID	-2.65	3.10	6
<i>GBM #10</i>	1 μ M THC + CID	-2.80	2.66	6
<i>GBM #10</i>	5 μ M THC + CID	-1.26	1.35	6
<i>GBM #10</i>	10 μ M THC + CID	-5.42	2.4	6
<i>GBM #10</i>	CID	4.63	1.45	6

Table S5. Exact measurement values and sample sizes after treatment with LPI and LPI in presence of CBD and CID.

cell type	treatment	mean [%]	SEM [%]	sample size
<i>GBM #4</i>	CTL	0.00	2.26	6
<i>GBM #4</i>	LPI	-13.11	0.82	6
<i>GBM #4</i>	CBD	-1.70	1.29	6
<i>GBM #4</i>	LPI + CBD	-1.35	1.75	6
<i>GBM #10</i>	CTL	0.00	3.05	6
<i>GBM #10</i>	LPI	-13.28	1.72	6
<i>GBM #10</i>	CBD	2.81	0.54	6
<i>GBM #10</i>	LPI + CBD	3.66	1.76	6
<i>GBM #4</i>	CTL	0.00	1.56	6
<i>GBM #4</i>	LPI	-11.07	3.66	6
<i>GBM #4</i>	CID	1.04	1.92	6
<i>GBM #4</i>	LPI + CID	-0.01	1.51	6
<i>GBM #10</i>	CTL	0.00	2.50	6
<i>GBM #10</i>	LPI	-14.29	3.38	6
<i>GBM #10</i>	CID	0.55	1.38	6
<i>GBM #10</i>	LPI + CID	1.81	2.20	6

Table S6. Exact measurement values and sample sizes after treatment with LPI and THC co-application.

cell type	treatment	mean [%]	SEM [%]	sample size
<i>GBM #4</i>	CTL	0.00	3.36	6
<i>GBM #4</i>	LPI	-19.02	3.70	6
<i>GBM #4</i>	THC	-15.55	2.78	5
<i>GBM #4</i>	LPI + THC ¹	-18.69	2.60	6
<i>GBM #4</i>	THC + LPI ¹	-19.47	3.15	6
<i>GBM #10</i>	CTL	0.00	1.96	6
<i>GBM #10</i>	LPI	-20.49	4.64	6
<i>GBM #10</i>	THC	-14.97	4.32	6
<i>GBM #10</i>	LPI + THC ¹	-20.78	2.12	6
<i>GBM #10</i>	THC + LPI ¹	-19.69	4.21	6

¹15 min pre-treatment