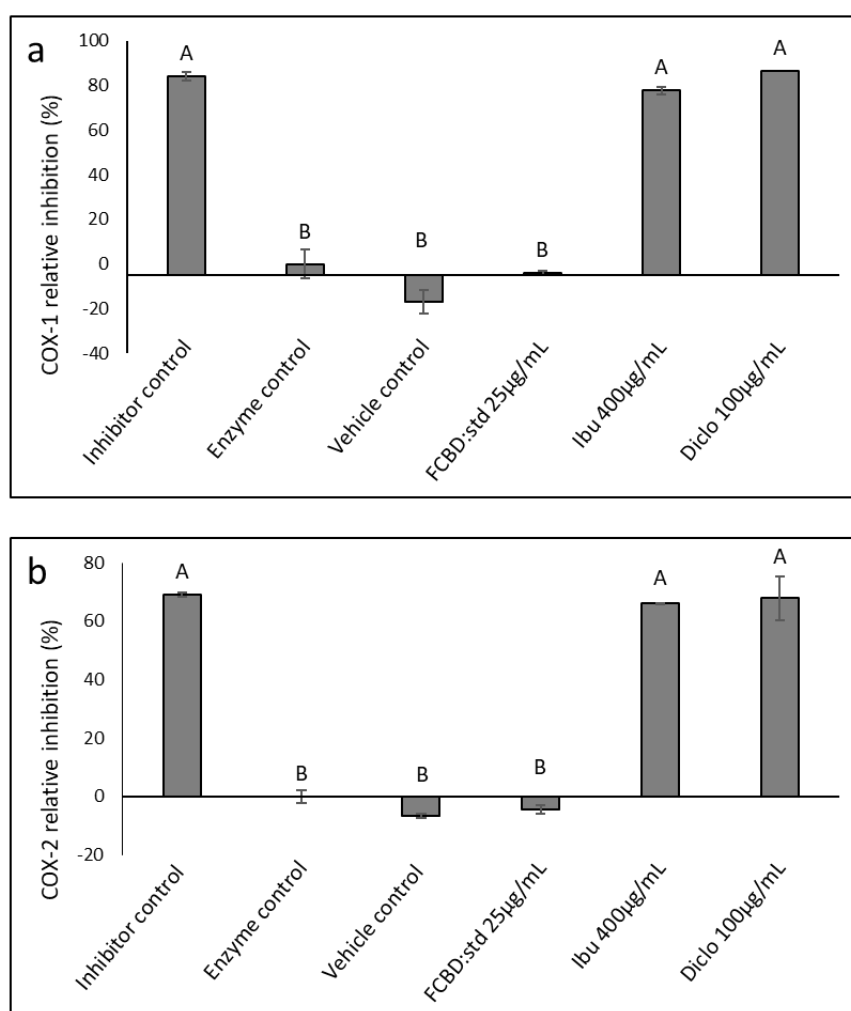


# Phytocannabinoids act synergistically with non-steroidal anti-inflammatory drugs reducing inflammation in 2D and 3D in vitro models

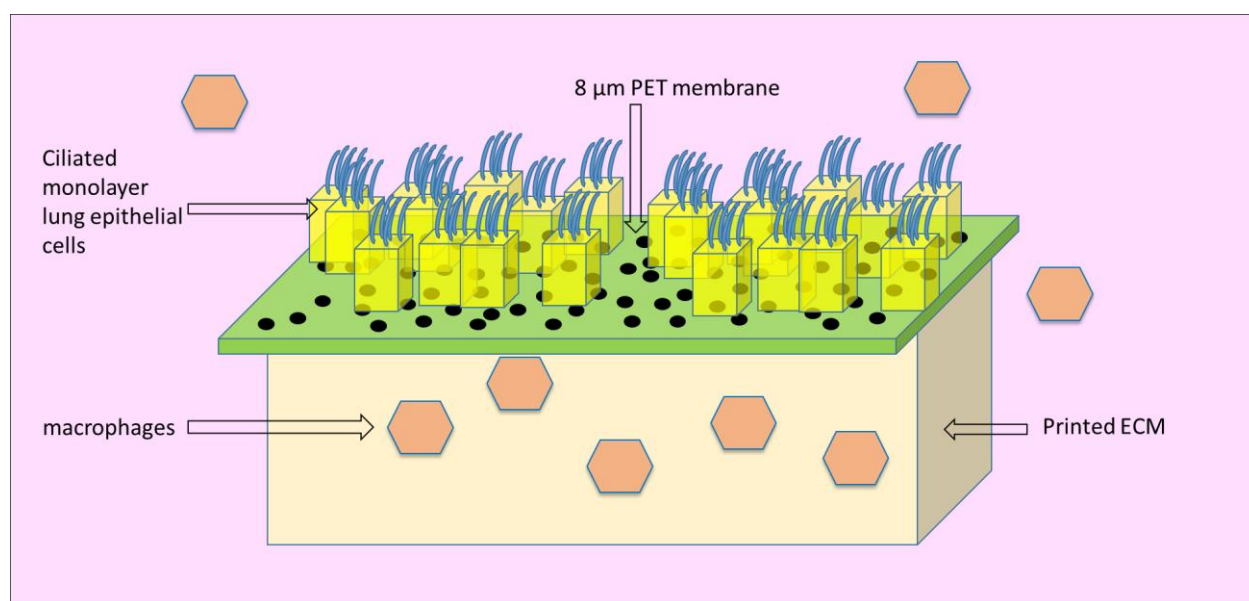
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## Supplementary Data

### Figures



**Supplementary Figure S1.** Relative inhibition level (%) of (a) COX-1 (b) COX-2 activity in the presence of FCBD:std, ibuprofen (Ibu) or diclofenac (Diclo). Vehicle control is 1% methanol (1% DMSO vehicle control is not significantly different from 1% methanol, not shown).



**Supplementary Figure S2.** Structure of the 3D printed model. In green: 8  $\mu$ m PEM membrane attached to the printed ECM. Designed and illustrated by Yoav Koltai.

## Tables

Treatments (all treated by TNF $\alpha$ )	IL-8 level per cell in treatment vs. vehicle + TNF $\alpha$ control (% mean $\pm$ SE; n=3)	Fold change in activity vs. ibuprofen
Ibu 100 $\mu$ g/mL	131.54 $\pm$ 5.89	
Ibu 200 $\mu$ g/mL	108.52 $\pm$ 5.79	
Ibu 300 $\mu$ g/mL	107.85 $\pm$ 5.51	
Ibu 400 $\mu$ g/mL	89.74 $\pm$ 3.39	
Ibu 500 $\mu$ g/mL	78.45 $\pm$ 0.84	
FCBD:std 20 $\mu$ g/mL	570.69 $\pm$ 2.68	
FCBD:std 30 $\mu$ g/mL	245.34 $\pm$ 1.58	
FCBD:std 20 $\mu$ g/mL + Ibu 100 $\mu$ g/mL	490.01 $\pm$ 75.75	0.3

FCBD:std 20 µg/mL + Ibu 200 µg/mL	147.21±9.51	0.7
FCBD:std 20 µg/mL + Ibu 300 µg/mL	29.75±6.46	3.6
FCBD:std 20 µg/mL + Ibu 400 µg/mL	25.28±3.60	3.6
FCBD:std 20 µg/mL + Ibu 500 µg/mL	13.71±1.82	5.7
FCBD:std 30 µg/mL + Ibu 100 µg/mL	65.44±3.98	2.0
FCBD:std 30 µg/mL + Ibu 200 µg/mL	78.98±6.46	1.4
FCBD:std 30 µg/mL + Ibu 300 µg/mL	46.87±11.67	2.3
FCBD:std 30 µg/mL + Ibu 400 µg/mL	25.91±3.93	3.5

**Supplementary Table S1a.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.8%)+TNF $\alpha$  controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with ibuprofen (Ibu) in relation to ibuprofen only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means  $\pm$  standard error (SE, n=3) are shown.

Treatments (all treated by TNF $\alpha$ )	IL-8 level per cell in treatment vs. vehicle + TNF $\alpha$ control (% mean $\pm$ SE; n=3)	Fold change in activity vs. budesonide
Bud 50 ng/mL	66.82±1.80	
Bud 100 ng/mL	66.83±8.22	
Bud 250 ng/mL	72.72±5.33	
Bud 500 ng/mL	55.54±4.09	
Bud 1000 ng/mL	54.11±1.60	

FCBD:std 30 µg/mL	140.32±10.26	
FCBD:std 30 µg/mL + Bud 50 ng/mL	33.73±5.23	2.0
FCBD:std 30 µg/mL + Bud 100 ng/mL	65.32±6.70	1.0
FCBD:std 30 µg/mL + Bud 250 ng/mL	62.98±2.58	1.2
FCBD:std 30 µg/mL + Bud 500 ng/mL	75.52±5.64	0.7
FCBD:std 30 µg/mL + Bud 1000 ng/mL	57.18±18.36	0.9

**Supplementary Table S1b.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF $\alpha$  controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with budesonide (Bud) in relation to budesonide only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means  $\pm$  standard error (SE, n=3) are shown.

Treatments (all treated by TNF $\alpha$ )	IL-8 level per cell in treatment vs. vehicle + TNF $\alpha$ control (% mean $\pm$ SE; n=3)	Fold change in activity vs. dexamethasone
Dexa 250 ng/mL	82.04±6.30	
Dexa 500 ng/mL	101.36±28.93	
Dexa 1000 ng/mL	71.0±0.80	
Dexa 2000 ng/mL	77.316±11.95	
Dexa 4000 ng/mL	65.876±2.53	
FCBD:std 30 µg/mL	130.936±10.40	
FCBD:std 30 µg/mL + Dexa 250 ng/mL	25.486±1.39	3.2
FCBD:std 30 µg/mL + Dexa 500 ng/mL	36.90±11.64	2.7

FCBD:std 30 µg/mL + Dexa 1000 ng/mL	24.31±9.64	2.9
FCBD:std 30 µg/mL + Dexa 2000 ng/mL	20.85±14.65	3.7

**Supplementary Table S1c.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNFα controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with dexamethasone (Dexa) in relation to dexamethasone only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means ± standard error (SE, n=3) are shown.

Treatments (all treated by TNFα)	IL-8 level per cell in treatment vs. vehicle + TNFα control (% mean±SE; n=3)	Fold change in activity vs. Diclofenac
Diclo 5 µg/mL	117.80±6.35	
Diclo 25 µg/mL	95.26±2.16	
Diclo 50 µg/mL	89.29±2.53	
Diclo 75 µg/mL	76.02±1.99	
Diclo 100 µg/mL	74.37±3.19	
FCBD:std 20 µg/mL	318.04±14.08	
FCBD:std 30 µg/mL	347.11±2.66	
FCBD:std 20 µg/mL + Diclo 50 µg/mL	163.03±3.78	0.5
FCBD:std 20 µg/mL + Diclo 75 µg/mL	92.29±2.45	0.8
FCBD:std 20 µg/mL + Diclo 100 µg/mL	76.48±1.45	1.0
FCBD:std 30 µg/mL + Diclo 5µg/mL	296.57±12.83	0.4
FCBD:std 30 µg/mL + Diclo 25 µg/mL	228.66±6.32	0.4

FCBD:std 30 µg/mL + Diclo 50 µg/mL	72.64±3.45	1.2
FCBD:std 30 µg/mL + Diclo 75 µg/mL	37.24±1.89	2.0
FCBD:std 30 µg/mL + Diclo 100 µg/mL	39.27±1.51	1.9

**Supplementary Table S1d.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 1%)+TNF $\alpha$  controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with diclofenac (Diclo) in relation to diclofenac only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means  $\pm$  standard error (SE, n=3) are shown.

		FCBD:std (µg/mL)						
		0	5	10	20	30	40	
a	Ibuprofen (µg/mL)	0	E	E	E	E	E	E
		100	E	E	E	C	C	E
		200	E	E	E	AB	C	DE
		300	E	E	E	A	C	E
		400	E	E	E	AB	C	E
		500	E	E	DE	B	CD	E
		FCBD:std (µg/mL)						
		0	5	10	20	25	30	
b	Budesonide (ng/mL)	0	ABCDE	ABCDE	ABCDE	ABCDE	ABCDE	ABCDE
		50	ABCDE	ABCDE	ABCDEF	ABCDEF	DEF	A
		100	ABCDE	ABCDEF	ABCDEF	ABCDEF	ABCDEF	ABC
		250	ABCDE	ABCDEF	EF	ABCDEF	BCDEF	AB
		500	ABCDE	ABCDEF	CDEF	EF	F	ABCDE
		1000	ABCDE	ABCDEF	BCDEF	DEF	ABCDEF	ABCD
		FCBD:std (µg/mL)						
		0	5	10	20	25	30	
c		0	ABC	ABC	ABC	ABC	ABC	ABC
		250	ABC	ABC	DE	F	AB	A

Dexamethasone (ng/mL)	500	ABC	ABC	DE	F	BC	AB
	1000	ABC	ABC	DE	DEF	A	AB
	2000	ABC	ABC	DEF	EF	CD	AB
	4000	ABC	ABC	DE	EF	AB	A
d	FCBD:std (µg/mL)						
		0	5	10	20	30	40
	0	G	G	G	G	G	G
	5	G	G	G	EF	CD	G
	25	G	G	G	G	DE	G
	50	G	G	G	CD	A	FG
	75	G	G	G	BC	A	G
	100	G	G	G	B	A	G

**Supplementary Table S2.** Different letters signify delta values that are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference test (HSD;  $P \leq 0.05$ ). Delta values calculated according to the Bliss model between the experimental (observed) and the calculated (expected) values of the synergistic interactions between FCBD:std with ibuprofen (a), budesonide (b), dexamethasone (c) or diclofenac (d) on anti-inflammatory activity of KG1 cells following combined treatments. Delta between observed and experimental values is graphically presented in Figure 1.

Treatments (all treated by TNFα)	IL-8 level per cell in treatment vs. vehicle + TNFα control (% mean±SE; n=3)	Fold change in activity vs. ibuprofen
FCBD:std 20 µg/mL	95.79±2.32	
Ibu 300 µg/mL	110.46±2.80	
Ibu 400 µg/mL	104.41±5.89	
Ibu 500 ug/mL	98.30±1.12	
FCBD:std 20 µg/mL + Ibu 300 µg/mL	72.69±1.84	1.5

FCBD:std 20 µg/mL + Ibu 400 µg/mL	50.97±1.70	2.0
FCBD:std 20 µg/mL + Ibu 500 µg/mL	44.65±4.10	2.2

**Supplementary Table S3a.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.8%)+TNFα controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with ibuprofen (Ibu) in relation to ibuprofen only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means ± standard error (SE, n=3) are shown.

Treatments (all treated by TNFα)	IL-8 level per cell in treatment vs. vehicle + TNFα control (% mean±SE; n=3)	Fold change vs. to budesonide
Bud 50 ng/mL	98.71±0.99	
Bud 100 ng/mL	105.00±1.34	
FCBD:std 5 µg/mL	90.50±0.87	
FCBD:std 10 µg/mL	96.81±1.40	
FCBD:std 20 µg/mL	82.71±0.28	
FCBD:std 5 µg/mL + Bud 100 ng/mL	57.32±2.83	1.8
FCBD:std 10 µg/mL + Bud 50 ng/mL	65.77±1.06	1.5
FCBD:std 10 µg/mL + Bud 100 ng/mL	66.25±2.19	1.6
FCBD:std 20 µg/mL + Bud 100 ng/mL	53.79±0.85	2.0

**Supplementary Table S3b.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNFα controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with budesonide (Bud) in relation to budesonide only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means ± standard error (SE, n=3) are shown.



Treatments (all treated by TNF $\alpha$ )	IL-8 level per cell in treatment vs. vehicle + TNF $\alpha$ control (% mean $\pm$ SE; n=3)	Fold change in activity vs. dexamethasone
Dexa 250 ng/ mL	64.66 $\pm$ 1.14	
Dexa 500 ng/mL	60.51 $\pm$ 1.69	
Dexa 1000 ng/mL	59.77 $\pm$ 0.97	
Dexa 2000 ng/mL	63.46 $\pm$ 6.59	
Dexa 4000 ng/mL	64.84 $\pm$ 4.31	
FCBD:std 10 $\mu$ g/mL	116.98 $\pm$ 4.28	
FCBD:std 10 $\mu$ g/mL + Dexa 250 ng/mL	60.97 $\pm$ 0.31	1.1
FCBD:std 10 $\mu$ g/mL + Dexa 500 ng/mL	58.07 $\pm$ 2.42	1.0
FCBD:std 10 $\mu$ g/mL + Dexa 1000 ng/mL	48.46 $\pm$ 0.96	1.2
FCBD:std 10 $\mu$ g/mL + Dexa 2000 ng/mL	59.13 $\pm$ 4.59	1.1
FCBD:std 10 $\mu$ g/mL + Dexa 4000 ng/mL	56.39 $\pm$ 2.11	1.1

**Supplementary Table S3c.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF $\alpha$  controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with dexamethasone (Dexa) in relation to dexamethasone only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means  $\pm$  standard error (SE, n=3) are shown.

Treatments (all treated by TNF $\alpha$ )	IL-8 level per cell in treatment vs. vehicle + TNF $\alpha$ control (% mean $\pm$ SE; n=3)	Fold change in activity vs. diclofenac
FCBD:std 5 $\mu$ g/mL	104.81 $\pm$ 8.13	
FCBD:std-10 $\mu$ g/mL	104.88 $\pm$ 8.45	
Diclo 50 $\mu$ g/mL	101.42 $\pm$ 1.08	

Diclo 100 µg/mL	94.20±1.37	
Diclo 200 µg/mL	87.45±1.74	
Diclo 300 µg/mL	67.56±0.81	
FCBD:std 5 µg/mL + Diclo 50 µg/mL	74.45±2.69	1.4
FCBD:std 10 µg/mL + Diclo 50 µg/mL	77.82±4.21	1.3
FCBD:std 5 µg/mL + Diclo 100 µg/mL	62.29±3.34	1.5
FCBD:std 5 µg/mL+Diclo 200 µg/mL	47.34±0.20	1.8
FCBD:std 5 µg/mL+Diclo 300 µg/mL	45.49±2.76	1.5
FCBD:std 10 µg/mL+Diclo 200 µg/mL	63.67±3.53	1.4

**Supplementary Table S3d.** IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 1%)+TNF $\alpha$  controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with diclofenac (Diclo) in relation to diclofenac only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means  $\pm$  standard error (SE, n=3) are shown.

		FCBD:std (µg/mL)					
		0	5	10	20	30	40
<b>a</b>	0	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH
	100	DEFGH	HI	BCDEFGH	CDEFGH	BCDE	BCDEFG
	200	DEFGH	HI	BCDEFG	BCDE	ABCD	BCDEF
	Ibuprofen (µg/mL)						BCDEFG
	300	DEFGH	GHI	BCDE	AB	ABC	H
	400	DEFGH	FGHI	EFGHI	A	ABCD	DEFGH
	500	DEFGH	CDEFGH	BCDE	A	BCDEF	I
		FCBD:std (µg/mL)					
		0	5	10	20	30	40
<b>b</b>	0	CDEFGH I	CDEFGH I	CDEFGHI	CDEFGHI	CDEFGH I	CDEFGHI
	Budonsonide (ng/mL)						
	50	CDEFGH I	ABCD	ABC	ABCDE	DEFGHI	EFGHI

Dexamethason e (ng/mL)	c	100	CDEFGH I	A	A	AB	CDEFGH	EFGHI
		250	CDEFGH I	ABCDEF	ABCDEFG	BCDEFG H	GHI	FGHI
		500	CDEFGH I	ABCDEF	ABCDEFG H	BCDEFG H	FGHI	HI
		1000	CDEFGH I	DEFGHI	BCDEFGH	EFGHI	HI	I
		FCBD:std (µg/mL)						
			0	5	10	20	30	40
		0	CD	CD	CD	CD	CD	CD
		250	CD	CD	ABC	D	ABCD	ABCD
		500	CD	ABCD	ABCD	D	BCD	ABCD
		1000	CD	ABCD	A	D	ABCD	ABCD
Diclofenac (µg/mL)	d	2000	CD	ABCD	ABC	BCD	ABCD	BCD
		4000	CD	ABCD	AB	BCD	ABCD	ABCD
		FCBD:std (µg/mL)						
			0	5	10	20	25	30
		0	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH
		50	DEFGH	ABC	ABC	ABCD	BCDE	HIJKL
		100	DEFGH	AB	CDEFG	BCDE	BCDE	HIJKL
		200	DEFGH	A	ABC	BCDE	CDEFG	HIJKL
		300	DEFGH	ABC	CDEFG	EFGHI	HIJKL	JKLMN
		400	DEFGH	BCDEF	EFGHI	GHIJK	KLMN	LMN

**Supplementary Table S4.** Different letters signify delta values that are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference test (HSD;  $P \leq 0.05$ ). Delta values calculated according to the Bliss model between the experimental (observed) and the calculated (expected) values of the synergistic interactions between FCBD:std with ibuprofen (a), budesonide (b), dexamethasone (c) or diclofenac (d) on anti-inflammatory activity of A549 cells following combined treatments. The delta between observed and experimental values is graphically presented in Figure 2.