

SUPPORTING INFORMATION

Therapeutic polymer-based Cannabidiol formulation: Tackling neuro-inflammation associated with ischemic events in the brain

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1 **Table S1: Variability of NPCBD physicochemical properties among batches.**

2 Hydrodynamic diameter (Dh, by intensity), polydispersity index (PDI); and zeta potential
3 values (δ).

BATCH #	CBD % w/w	Dh (nm)	PDI	δ (mV)
1	10	240.5 ± 5.9	0.22 ± 0.06	-21.0 ± 0.62
2		269 ± 5.9	0.16 ± 0.02	-26.3 ± 0.35
3		254.3 ± 0.6	0.13 ± 0.03	-31.0 ± 0.86
4		214.7 ± 2.1	0.07 ± 0.02	-18.1 ± 0.20

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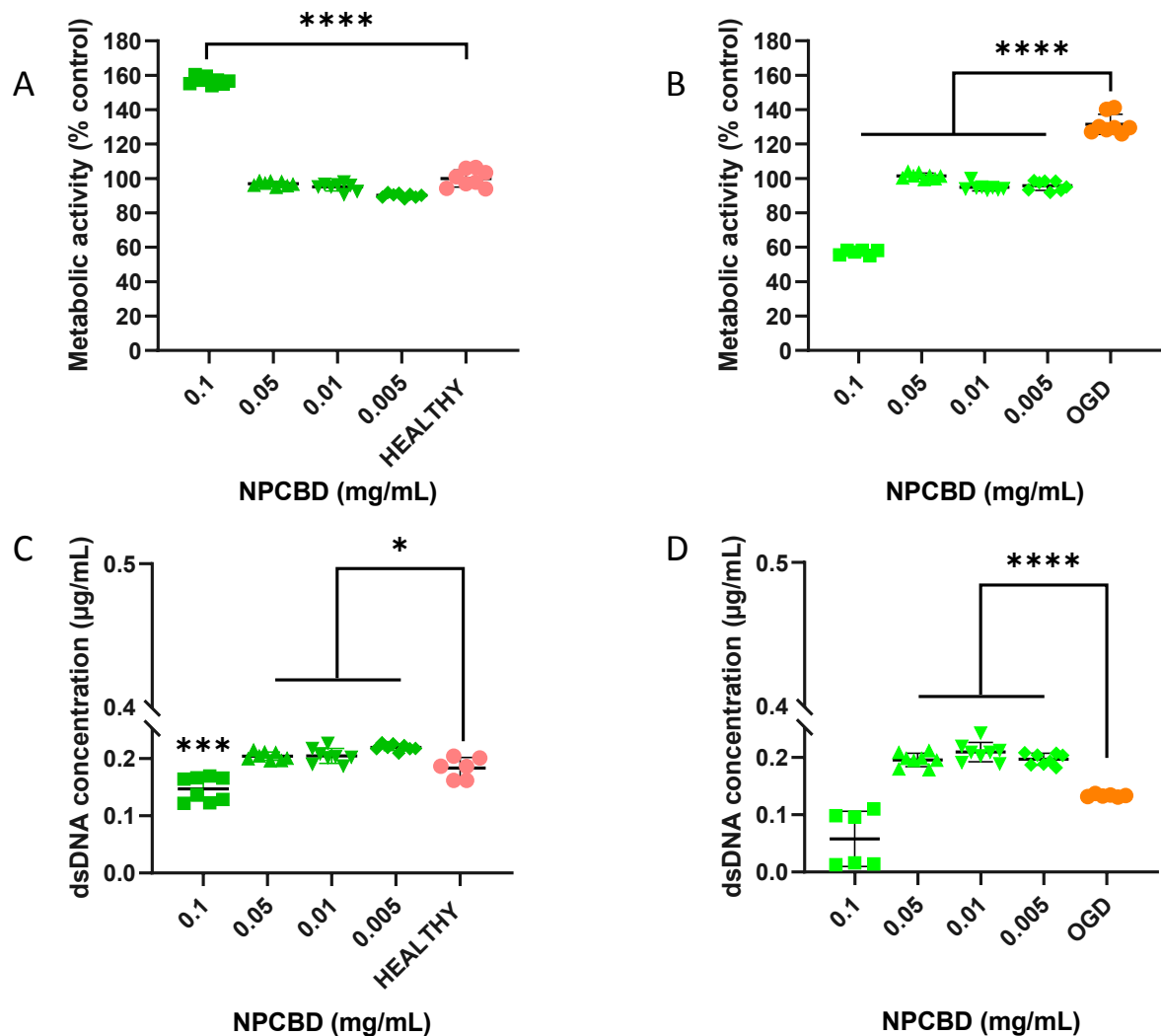
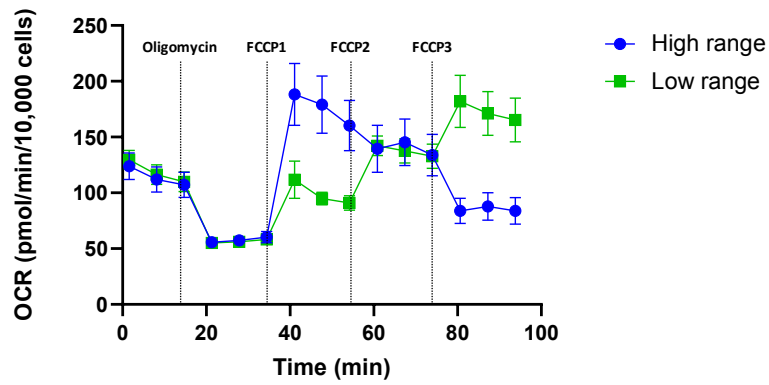
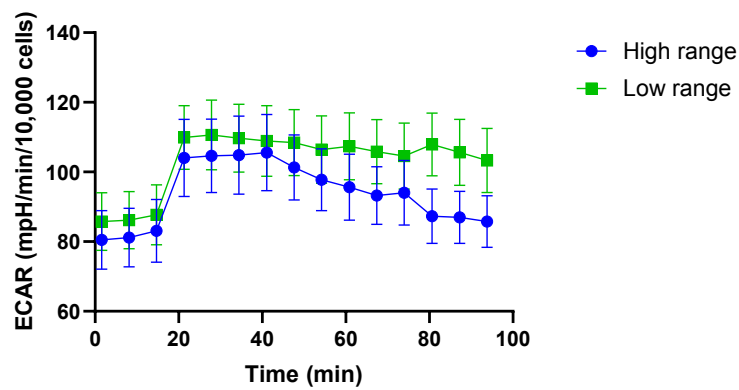


Figure S1. Seven days treatment NPCBD dose response after a six h OGD and reperfusion model of rat primary cortical cells. dsDNA concentration was determined by Picogreen™ analysis and metabolic activity of the culture was determined by alamarBlue™ after three days of treatment with NPCBD in healthy conditions (A and C) or after OGD exposure (B and D). Data are represented as mean ± SEM, N≥4 experimental replicates. *p<0.05, **p<0.01, ***p<0.001 vs Healthy or OGD cells. Ordinary One way ANOVA followed by Dunnet's post-hoc test.

A



B



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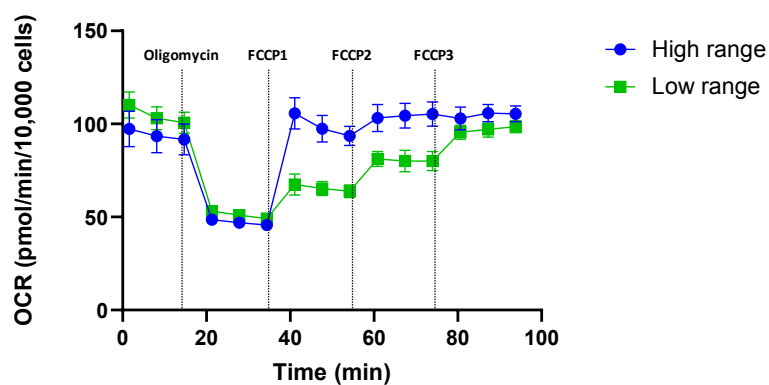
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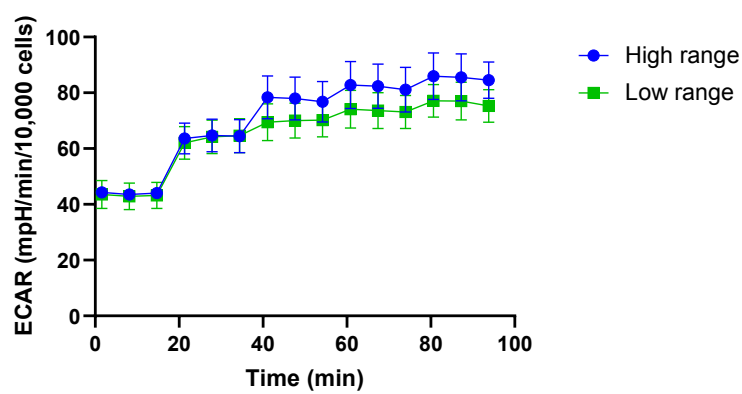
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Figure S2. FCCP Optimization with the XF Cell Mito Stress Test on HMC3. Continuous (A) OCR values (pmol/min/10,000 cells) (B) ECAR values (mpH/min/10,000 cells). Data are represented as mean $\pm$ SEM, N $\geq$ 3 experimental replicates.

A



B



1

2 **Figure S3. FCCP Optimization with the XF Cell Mito Stress Test on PCC.** Continuous (A)
 3 OCR values (pmol/min/10,000 cells) (B) ECAR values (mpH/min/10,000 cells). Data are
 4 represented as mean $\pm$ SEM, N $\geq$ 3 experimental replicates.

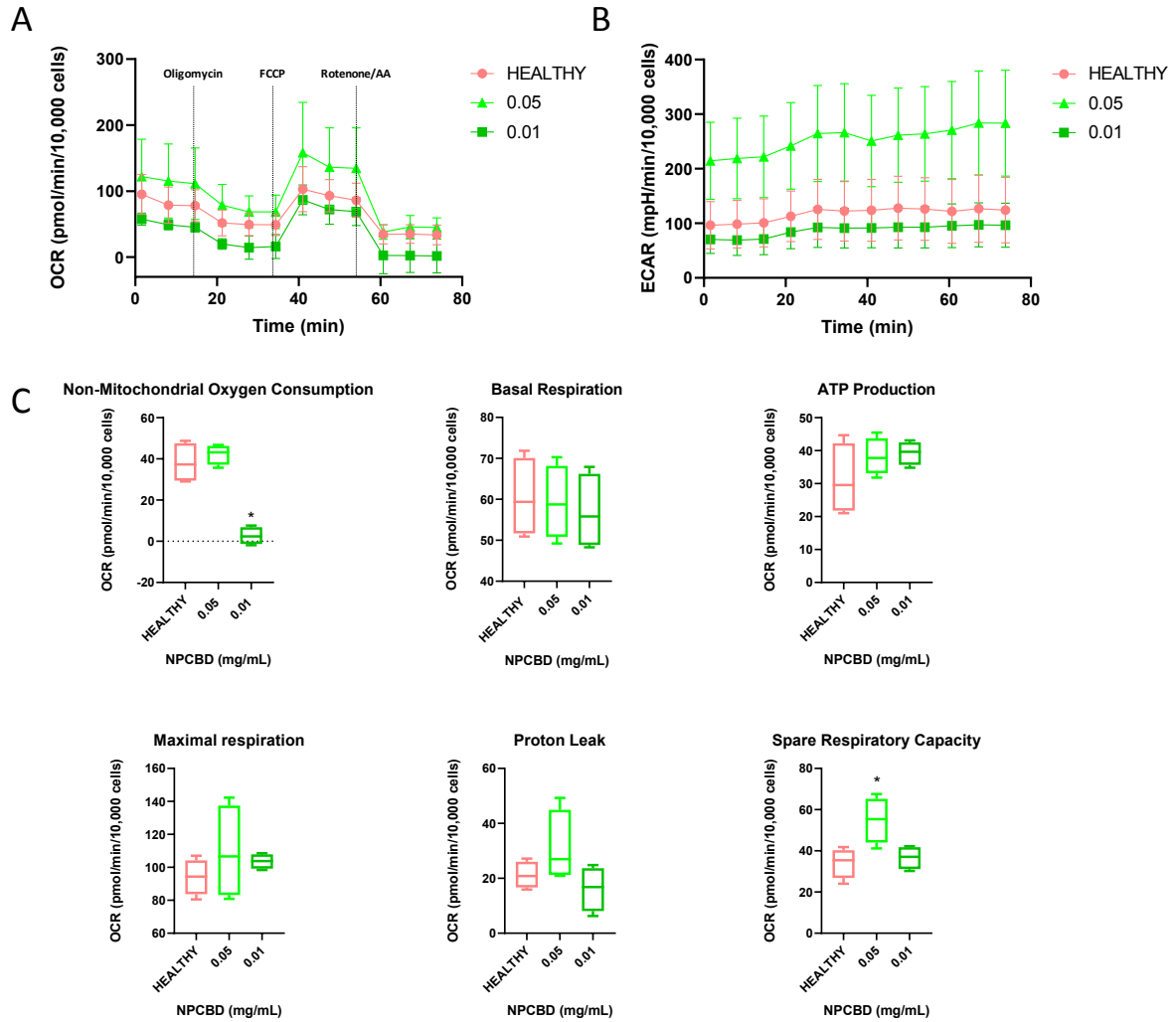


Figure S4. Cell Mito Stress analysis of mitochondrial respiratory capacity in HMC3 cells under normoxia. Cells were incubated 30 min before the experiment in an XF assay medium supplemented with 5 mM glucose and 2 mM glutamine and subsequently injected with oligomycin (1 μ M), FCCP (1.5 μ M), antimycin (1 μ M) and rotenone (1 μ M). Continuous (A) (A) OCR values (pmol/min/10,000 cells) (B) ECAR values (mpH/min/10,000 cells), (C) and OCR parameters three days after the treatment are reported. ($n \geq 3$, $*p < 0.05$).

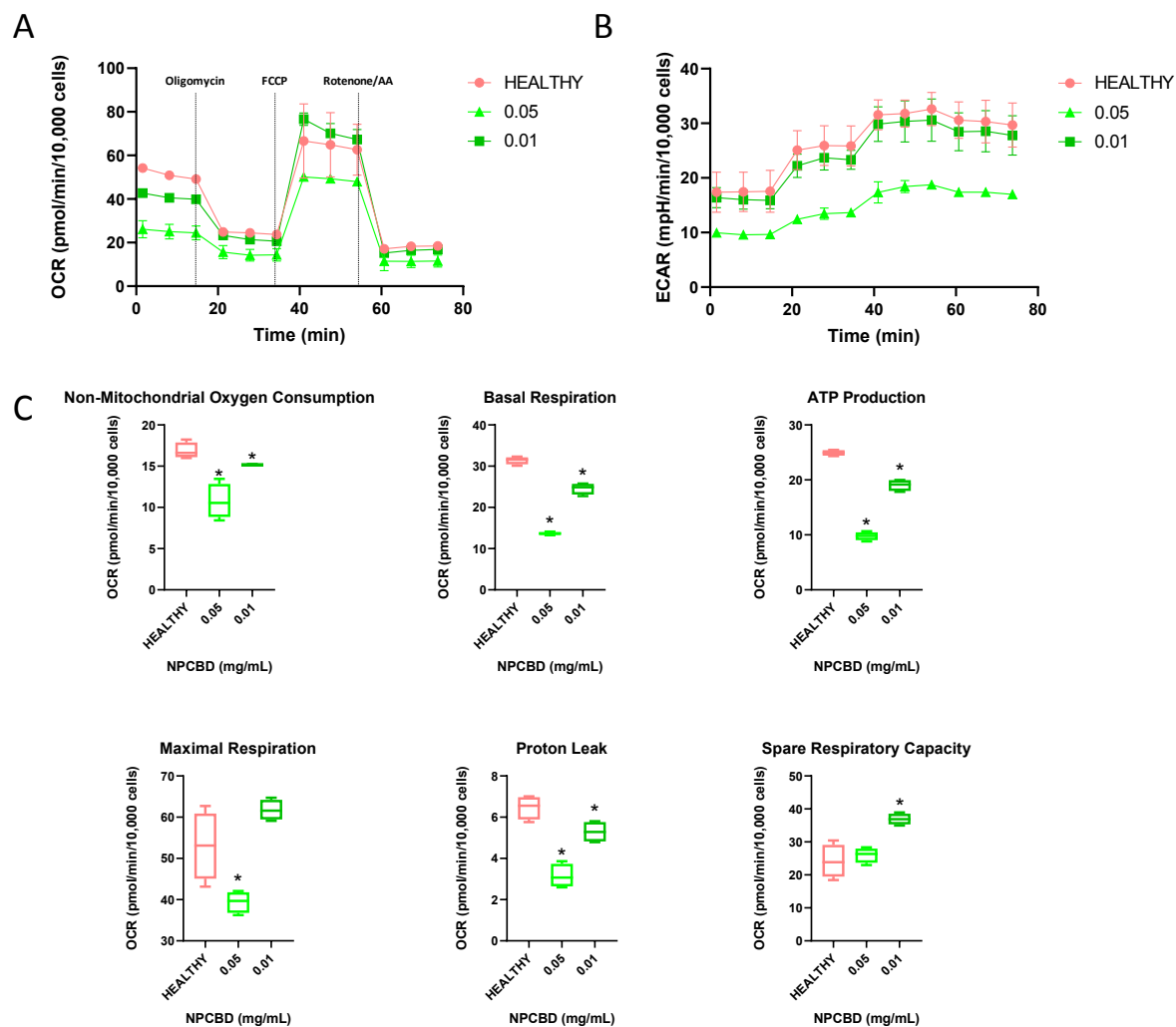


Figure S5. Cell Mito Stress analysis of mitochondrial respiratory capacity in PCC cells under normoxia. Cells were incubated 30 min before the experiment in an XF assay medium supplemented with 5 mM glucose and 2 mM glutamine and subsequently injected with oligomycin (1 μ M), FCCP (1.5 μ M), antimycin (1 μ M) and rotenone (1 μ M). Continuous (A) OCR values (pmol/min/10,000 cells) (B) ECAR values (mpH/min/10,000 cells), (C) and OCR parameters three days after the treatment are reported. ($n \geq 3$, $*p < 0.05$).

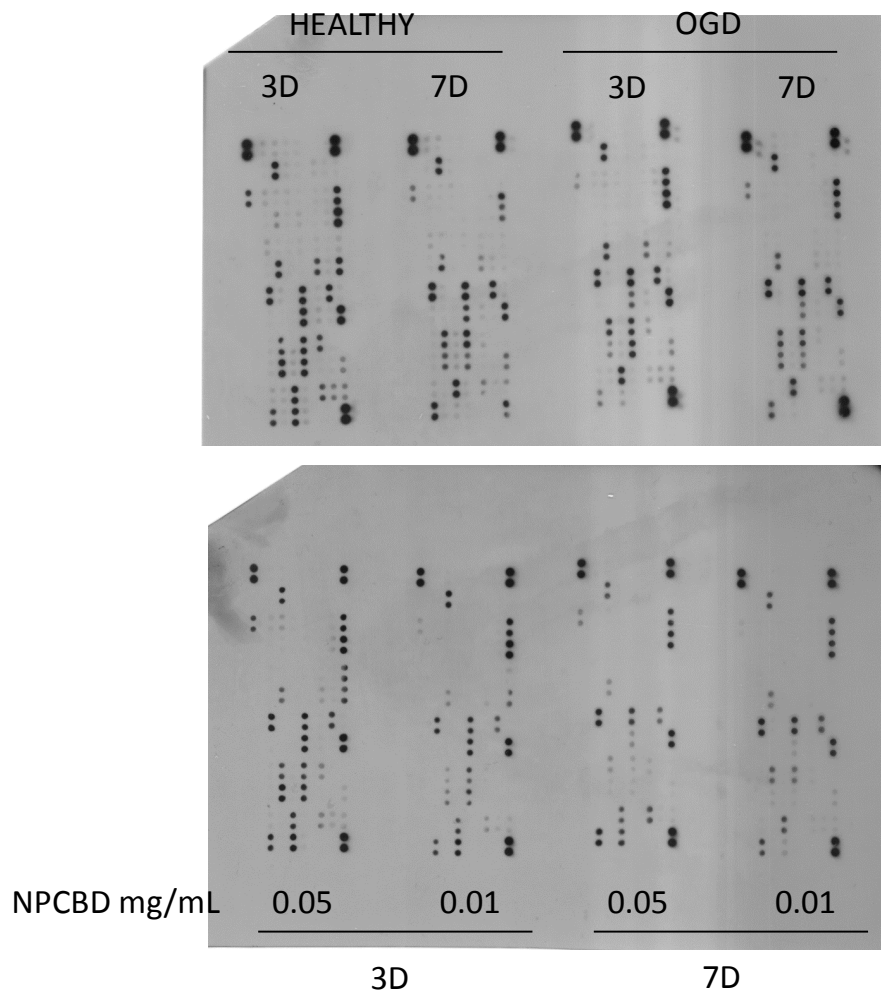
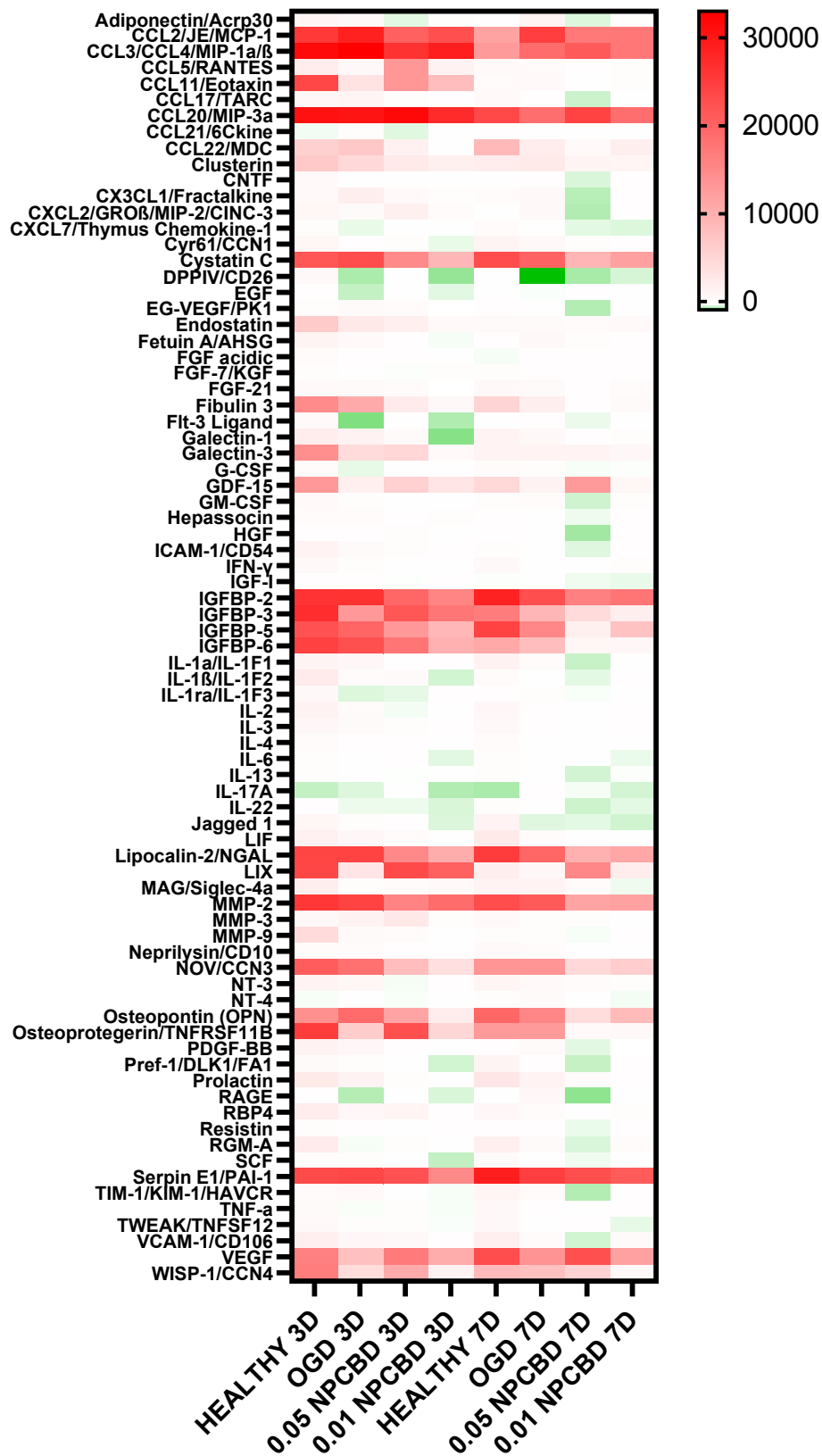


Figure S6. The Cytokine Array detects changes in cytokines and chemokines in the media describing the inflammatory phenotype of PCC after the treatments. Proteome profiler membranes of the assessed groups (Healthy, OGD, and OGD+NPCBD at 0.05 and 0.01 mg/mL) and at two timepoints (day-3, day-7). At least three experimental replicates were pulled supernatant and run for two technical replicates.



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Figure S7. Analysis of 79 analytes related to inflammation. The mean pixel density was analyzed by the proteome profile array of proteins secreted by PCC in the supernatant. Colors define the semi-quantitative values related to protein activation as highly expressed (red) and no expression (green). Treatment was given at two doses after OGD exposure, and the supernatant was analyzed at two time points, day three and day seven. The experiment was carried out in three biological replicates, and supernatants were pooled together to perform the proteome profiler array. Each analyte on the array was printed in duplicate. The values shown per time point are an average of both.