Supporting Information for

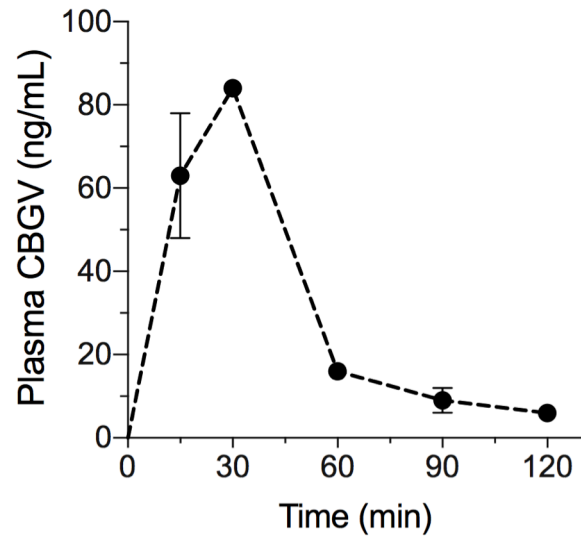## Cannabigerolic acid, a major biosynthetic precursor molecule in cannabis, exhibits divergent effects on seizures in mouse models of epilepsy

L.L. Anderson, M. Heblinski, N. L. Absalom, N.A. Hawkins, M. Bowen, M.J. Benson, F. Zhang, D. Bahceci, P.T. Doohan, M. Chebib, I.S. McGregor, J.A. Kearney and J.C. Arnold
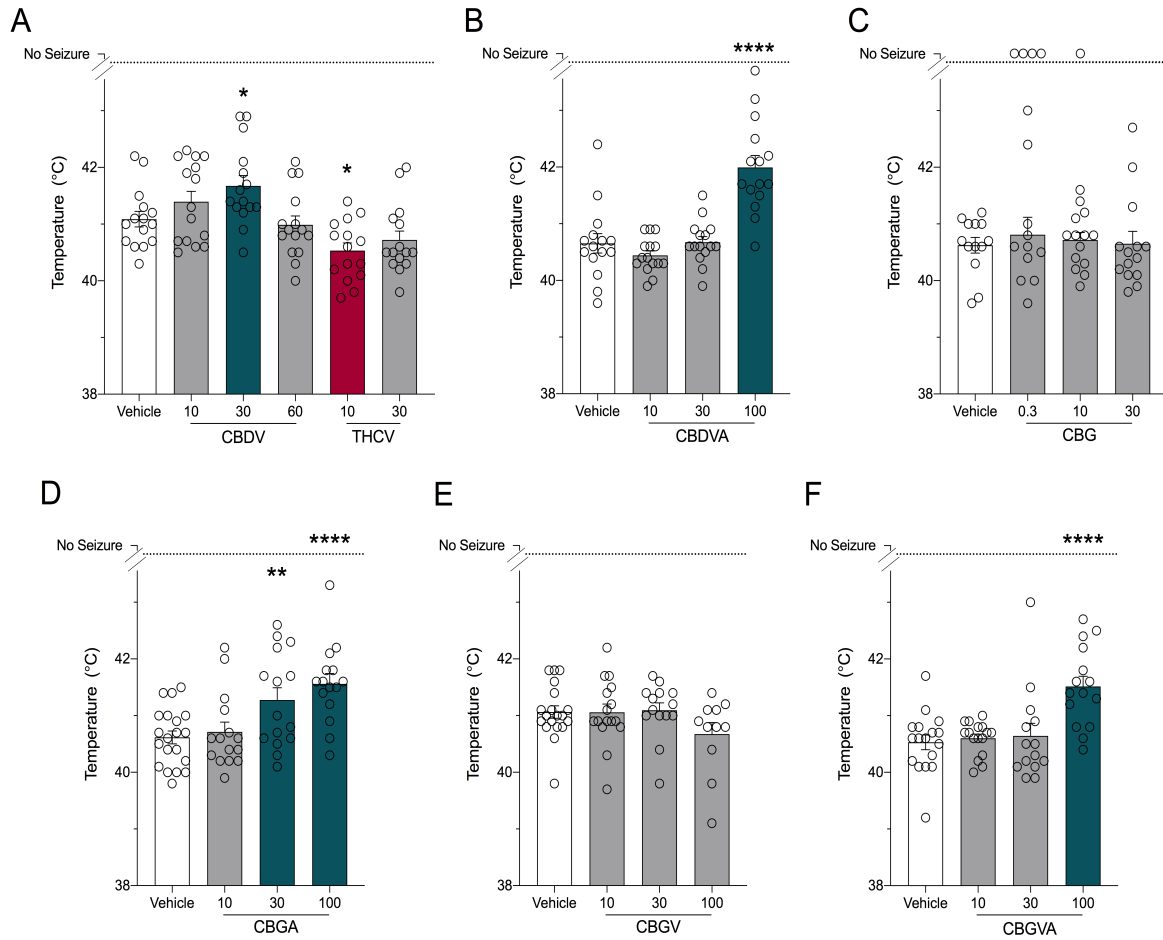
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**This PDF file includes:**

Supporting Figure 1. Pharmacokinetic analysis of CBGV. Concentration-time curve for CBGV in mouse plasma following a 1 mg/kg i.p. injection. Data are expressed as means ± SEM, with n = 4-5 per time point.

Supporting Figure 2. Hyperthermia-induced seizure screen. Temperature threshold for GTCS in individual mice treated acutely with (**A**) CBDV or THCV, (**B**) CBDVA, (**C**) CBG, (**D**) CBGA, (**E**) CBGV or (**F**) CBGVA. CBDV (30 mg/kg), CBDVA (100 mg/kg), CBGA (30 and 100 mg/kg) and CBGVA (100 mg/kg) treatments were anticonvulsant resulting in a significantly improved response to thermal seizure induction (blue-green bars); whereas, THCV (3 mg/kg) was proconvulsant and significantly lowered the thermal threshold (red bar). Error bars represent SEM, with n = 12-20 per group (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$; logrank Mantel-Cox).
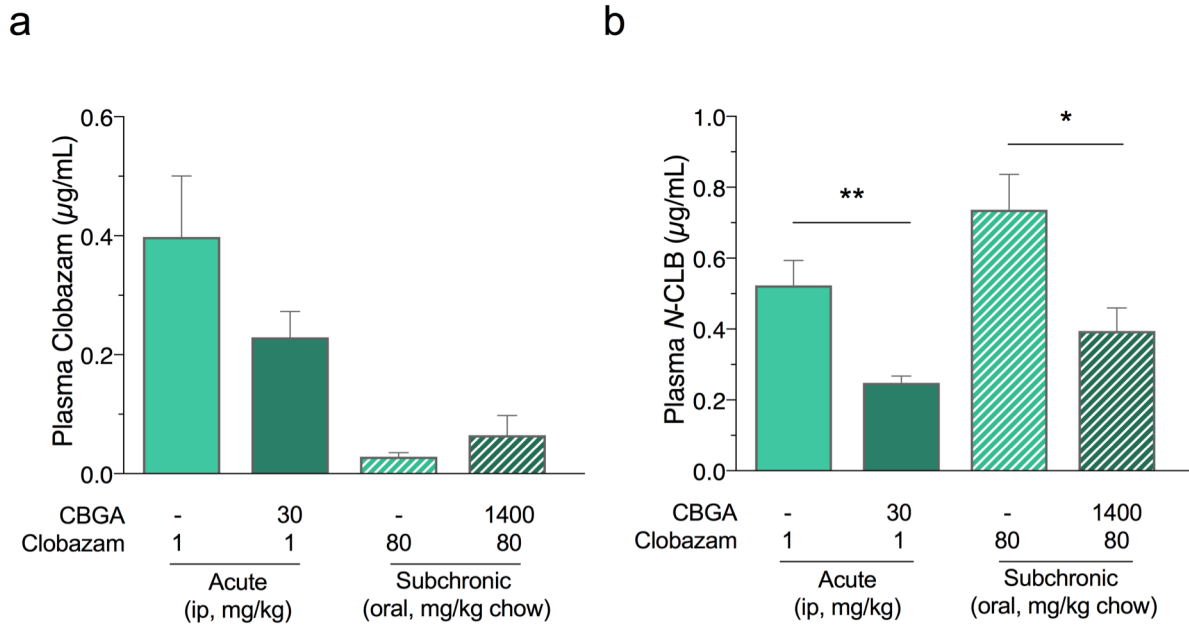
Supporting Figure 3. Pharmacokinetic interaction between CBGA and *N*-CLB, the active metabolite of clobazam. Average plasma concentrations of (**a**) clobazam and (**b**) *N*-CLB in *Scn1a*$^{+/-}$ mice from hyperthermia-induced seizure and spontaneous seizure experiments. Clobazam was administered acutely (solid bars) or subchronically (hatched bars) in the presence (dark green bars) and absence of CBGA (light green bars), with n = 5 per treatment. Significantly lower plasma *N*-CLB levels were observed following combination treatment of CBGA and clobazam (*$p < 0.05$, **$p < 0.01$; Student's t-test).
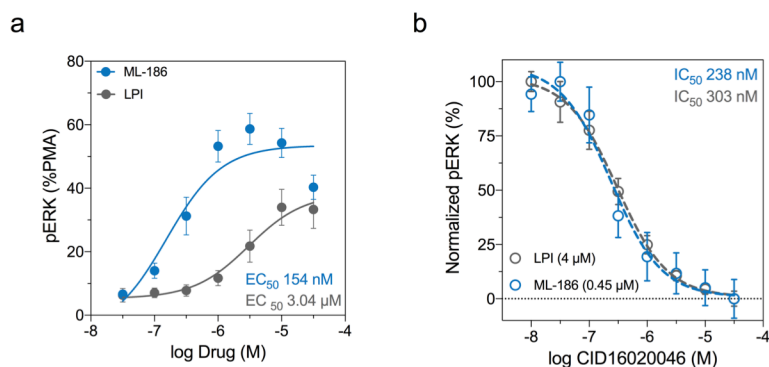
Supporting Figure 4. *In vitro* **characterization of GPR55 activity.** HEK293 cells expressing human GPR55 were used to characterize inhibitory and agonist activity of compounds. ERK phosphorylation (pERK) was used as a measure of GPR55 activation. (**a**) Concentration-response curves for activation of GPR55 by ML-186 (blue symbols) and LPI (gray symbols). Data are expressed as mean ± SEM normalized to 100 nM phorbol 12-myrisate 13-acetate (PMA) and fit to the Hill equation, with n = 3-4 per concentration. (**b**) Concentration-response for CID16020046 inhibition of GPR55 activation measured by ERK phosphorylation (pERK). GPR55 activation was measured following treatment with either LPI (gray symbols) or ML-186 (blue symbols) in the presence of varying concentrations of CID16020046 and expressed as a percentage of responses to ligand alone. Data are expressed as mean ± SEM, with n = 6 per group. Curves represent fit to a three-parameter log function and IC_{50} values are listed.
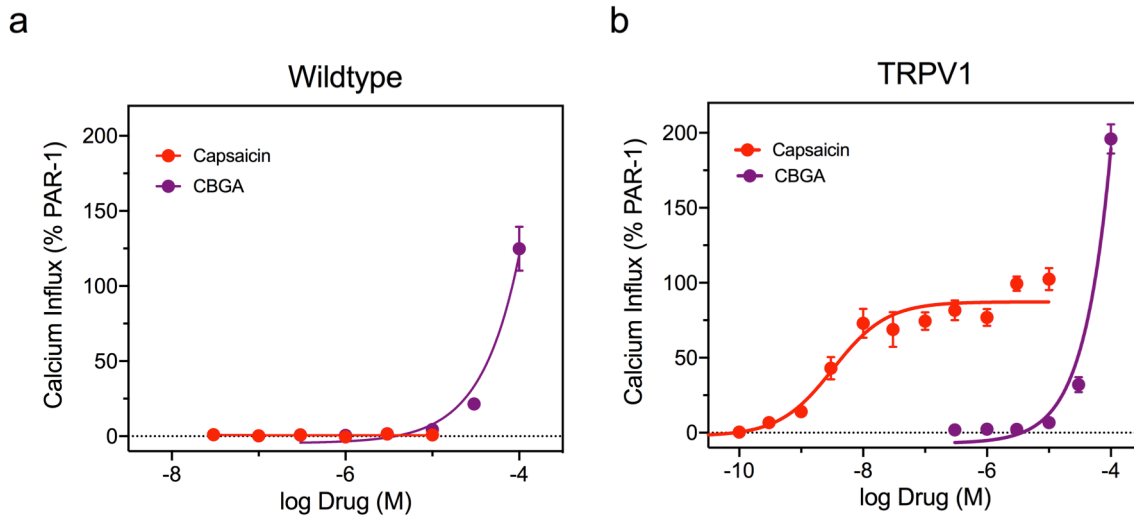
Supporting Figure 5. *In vitro* characterization of TRPV1 agonists. HEK293 cells expressing human TRPV1 were used to characterize agonist activity of capsaicin and CBGA. Changes in intracellular calcium were used as a measure of TRPV1 activation. Concentration-response curves for capsaicin (red) and CBGA (purple) in (**a**) wildtype and (**b**) TRPV1-expressing cells. Data are expressed as mean ± SEM normalized to PAR-1 and fit to the Hill equation, with n = 4-5 per group. The EC_{50} value of capsaicin for TRPV1 was 3.3 nM (95% Confidence Interval, 1.9 - 5.8 nM). Non-specific calcium influx was observed with CBGA treatment (30 and 100 µM).

Supporting Table 1. Parameters for LC-MS/MS Detection of Phytocannabinoids

Compound	Molecular weight	Parent > daughter ions (m/z)
CBDV	286.4	287.30 > 165.25
		287.30 > 123.20
CBDVA	330.4	331.10 > 191.05
		331.10 > 233.15
CBG	316.5	317.25 > 193.10
		317.25 > 123.25
CBGV	288.4	289.00 > 165.10
CBGVA	332.4.	333.10 > 315.20
CBGA	360.5	361.20 > 343.10
		361.20 > 219.10
THCV	286.4	287.30 > 165.20
		287.30 > 135.25

Supporting Table 2. Eurofins DiscoverX GPCR Screen

GPCR	Ligand
ADCYAP1R1	PACAP-27
ADORA3	2-Cl-IB-MECA
ADRA1B	Phenylephrine
ADRA2A	UK 14,304
ADRA2B	UK 14,304
ADRA2C	UK 14,304
ADRB1	Isoproterenol
ADRB2	Isoproterenol
AGTR1	Angiotensin II
AGTRL1	Apelin-13
AVPR1A	Vasopressin
AVPR1B	Vasopressin
AVPR2	Vasopressin
BDKRB1	LDA-Bradykinin
BDKRB2	Bradykinin
BRS3	TAPN-Bombesin
C3AR1	C3A Receptor Agonist (Short Fragment)
C5AR1	Complement C5a
C5L2	Complement C5a
CALCR	Calcitonin
CALCRL-RAMP1	beta CGRP
CALCRL-RAMP2	Adrenomedullin
CALCRL-RAMP3	Adrenomedullin
CALCR-RAMP2	Calcitonin
CALCR-RAMP3	Calcitonin
CCKAR	CCK-8
CCKBR	CCK-8
CCR10	CCL27
CCR1	CCL3
CCR2	CCL2
CCR3	CCL13
CCR4	CCL22
CCR5	CCL3
CCR6	CCL20
CCR7	CCL19
CCR8	CCL1
CCR9	CCL25
CHRM1	Acetylcholine
CHRM2	Acetylcholine
CHRM3	Acetylcholine
CHRM4	Acetylcholine
CHRM5	Acetylcholine
CMKLR1	Chemerin
CNR1	CP55940
CNR2	CP55940
CRHR1	Sauvagine
CRHR2	Sauvagine
CRTH2	PGD2
CX3CR1	Fractalkine
CXCR1	CXCL8
CXCR2	CXCL8

CXCR3	CXCL11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL12
DRD1	Dopamine
DRD2L	Dopamine
DRD2S	Dopamine
DRD3	Dopamine
DRD4	Dopamine
DRD5	Dopamine
EBI2	7a,25-Dihydroxycholesterol
EDG1	S-1-P
EDG3	S-1-P
EDG4	Oleoyl LPA
EDG5	S-1-P
EDG6	S-1-P
EDG7	Oleoyl LPA
EDNRA	Endothelin I
EDNRB	Endothelin 3
F2R	TFLLR-NH2
F2RL1	SLIGRL-NH2
F2RL3	AYPGKF-NH2
FFAR1	GW9508
FPR1	WKYMVm-NH2
FPRL1	WKYMVm-NH2
FSHR	FSH
GALR1	Galanin
GALR2	Galanin
GCGR	Glucagon
GHSR	Ghrelin
GIPR	GIP
GLP1R	Exendin-4
GLP2R	GLP II (1-33)
GPR1	Chemerin
GPR103	QRFP-26
GPR109A	Nicotinic Acid
GPR109B	3-Hydroxyoctanoic Acid
GPR119	Oleoyl Ethanolamide
GPR120	GW9508
GPR35	Zaprinast
GPR92	Oleoyl LPA
GRPR	GRP
HCRTR1	Orexin A
HCRTR2	Orexin A
HRH1	Histamine
HRH2	Histamine
HRH3	R-a methylhistamine
HRH4	Histamine
HTR1A	Serotonin
HTR1B	Serotonin
HTR1E	Serotonin
HTR1F	Serotonin
HTR2A	Serotonin

HTR2C	Serotonin
HTR5A	Serotonin
KISS1R	Kisspeptin-10
LHCGR	hCG
LTB4R	Leukotriene B4
MC1R	Melanotan II
MC3R	Melanotan II
MC4R	Melanotan II
MC5R	Melanotan II
MCHR1	MCH
MCHR2	MCH
MLNR	Motilin
MRGPRX1	BAM(8-22)
MRGPRX2	Cortistatin 14
MTNR1A	2-Iodomelatonin
NMBR	Neuromedin B
NMU1R	Neuromedin U-25
NPBWR1	Neuropeptide W23
NPBWR2	Neuropeptide W23
NPFFR1	RFRP-3
NPSR1b	Neuropeptide S
NPY1R	Peptide YY
NPY2R	Peptide YY
NTSR1	[Lys 8,9] Neurotensin
OPRD1	DADLE
OPRK1	Dynorphin A
OPRL1	Orphanin FQ
OPRM1	[Met] Enkephalin
OXER1	5-OxoETE
OXTR	Oxytocin
P2RY1	2-methylthio-ADP
P2RY11	ATP
P2RY12	2-methylthio-ADP
P2RY2	UTP
P2RY4	UTP
P2RY6	UTP
PPYR1	Pancreatic Polypeptide
PRLHR	PrRP-31
PROKR1	EG VEGF
PROKR2	EG VEGF
PTAFR	PAF
PTGER2	Prostaglandin E2
PTGER3	Prostaglandin E2
PTGER4	Prostaglandin E2
PTGFR	Cloprostenol
PTGIR	Beraprost
PTHR1	PTH(1-34)
PTHR2	TIP-39
RXFP3	Relaxin-3
SCTR	Secretin
SSTR1	Somatostatin 28
SSTR2	Somatostatin 28
SSTR3	Tyr-SST 14
SSTR5	Somatostatin 28

TACR1	Substance P
TACR2	Substance P
TACR3	Substance P
TBXA2R	I-BOP
TRHR	TRH
TSHR(L)	TSH
UTR2	Urotensin II
VIPR1	VIP
VIPR2	VIP
BAI1	PathHunter Detection Reagent
BAI2	PathHunter Detection Reagent
BAI3	PathHunter Detection Reagent
CCRL2	PathHunter Detection Reagent
DARC	PathHunter Detection Reagent
GHSR1B	Flash Detection Reagent
GPR101	Flash Detection Reagent
GPR107	PathHunter Detection Reagent
GPR12	Flash Detection Reagent
GPR123	PathHunter Detection Reagent
GPR132	PathHunter Detection Reagent
GPR135	PathHunter Detection Reagent
GPR137	PathHunter Detection Reagent
GPR139	PathHunter Detection Reagent
GPR141	PathHunter Detection Reagent
GPR142	Flash Detection Reagent
GPR143	PathHunter Detection Reagent
GPR146	PathHunter Detection Reagent
GPR148	Flash Detection Reagent
GPR149	PathHunter Detection Reagent
GPR15	PathHunter Detection Reagent
GPR150	Flash Detection Reagent
GPR151	PathHunter Detection Reagent
GPR152	PathHunter Detection Reagent
GPR157	PathHunter Detection Reagent
GPR161	PathHunter Detection Reagent
GPR162	PathHunter Detection Reagent
GPR17	PathHunter Detection Reagent
GPR171	Flash Detection Reagent
GPR173	PathHunter Detection Reagent
GPR176	PathHunter Detection Reagent
GPR18	Flash Detection Reagent
GPR182	PathHunter Detection Reagent
GPR20	PathHunter Detection Reagent
GPR23	PathHunter Detection Reagent
GPR25	Flash Detection Reagent
GPR26	Flash Detection Reagent
GPR27	PathHunter Detection Reagent
GPR3	PathHunter Detection Reagent
GPR30	Flash Detection Reagent
GPR31	PathHunter Detection Reagent
GPR32	PathHunter Detection Reagent
GPR37	PathHunter Detection Reagent
GPR37L1	PathHunter Detection Reagent
GPR39	PathHunter Detection Reagent

GPR4	Flash Detection Reagent
GPR45	PathHunter Detection Reagent
GPR50	PathHunter Detection Reagent
GPR52	Flash Detection Reagent
GPR55	PathHunter Detection Reagent
GPR6	PathHunter Detection Reagent
GPR61	PathHunter Detection Reagent
GPR65	PathHunter Detection Reagent
GPR75	PathHunter Detection Reagent
GPR78	Flash Detection Reagent
GPR79	Flash Detection Reagent
GPR83	PathHunter Detection Reagent
GPR84	PathHunter Detection Reagent
GPR85	Flash Detection Reagent
GPR88	PathHunter Detection Reagent
GPR22	PathHunter Detection Reagent
GPR97	PathHunter Detection Reagent
LGR4	PathHunter Detection Reagent
LGR5	Flash Detection Reagent
LGR6	PathHunter Detection Reagent
MRGPRD	PathHunter Detection Reagent
MRGPRE	PathHunter Detection Reagent
MRGPRF	PathHunter Detection Reagent
MRGPRX4	PathHunter Detection Reagent
OPN5	Flash Detection Reagent
OXGR1	PathHunter Detection Reagent
P2RY8	PathHunter Detection Reagent
TAAR5	Flash Detection Reagent

Supporting Table 3. Plasma cannabinoid concentrations in experimental *Scn1a*^{+/-} mice

Cannabinoid	Dose (mg/kg)	Plasma Concentration (µg/mL)
CBDV	10	562 ± 70 ng/mL
	30	3.1 ± 0.3
	60	4.0 ± 0.6
CBDVA	10	9 ± 2
	30	45 ± 6
	100	79 ± 10
CBG	0.3	2 ± 0 ng/mL
	10	92 ± 11 ng/mL
	30	1.1 ± 0.1
CBGV	10	807 ± 182 ng/mL
	30	1.5 ± 0.4
	100	7± 1
CBGVA	10	106 ± 16
	30	181 ± 18
	100	400 ± 23
CBGA	10	13 ± 1
	30	43 ± 3
	100	73 ± 10
THCV	3	81 ± 18 ng/mL
	10	352 ± 28 ng/mL

Supporting Table 4. Effect on GPR55 activity

	Treatment	EC_{50} (nM)	$pEC_{50} \pm SEM$	$E_{max} \pm SEM$ (%)
	Vehicle	266	6.57 ± 0.19	105 ± 9
CBGA	1 µM	680	6.16 ± 0.20	81 ± 8
(ML-186)	3 µM	323	6.49 ± 0.29	66 ± 9*
	10 µM	478	6.32 ± 0.55	5 ± 5**

Substrate listed in parantheses

EC_{50}, concentration of substrate at 50% maximal activity

E_{max}, maximal activity relative to 10 µM substrate (%)
*$p < 0.05$, **$p < 0.01$ compared to vehicle; Extra sum-of-squares F test