Supplementary Information for: Uncovering the hidden antibiotic potential of Cannabis

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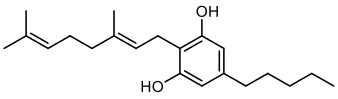
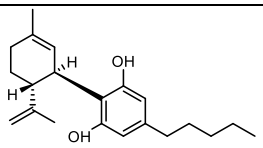
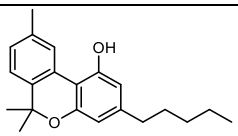
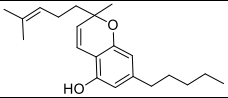
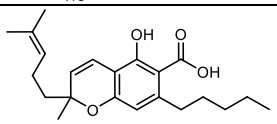
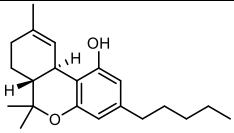
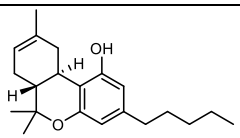
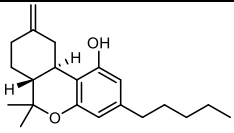
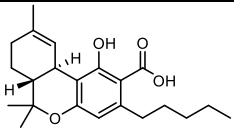
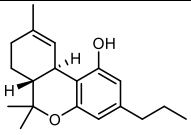
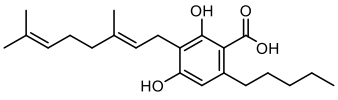
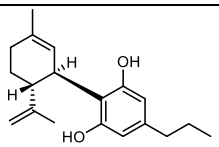
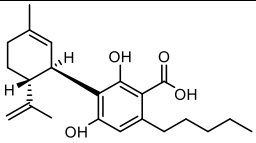
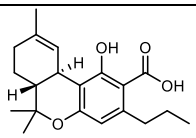
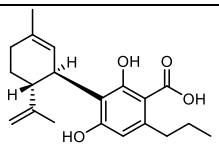
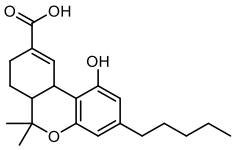
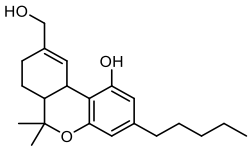
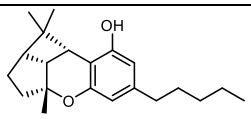
Supplementary Table 1. Antimicrobial activity of cannabinoid analogs against MRSA USA300

Entry	Compound Name (Abbreviation)		Structure	MIC (μg/mL)
1	cannabigerol	(CBG)		2
2	cannabidiol	(CBD)		2
3	cannabinol	(CBN)		2
4	cannabichromene	(CBC)		8
5	cannabichromenic acid	(CBCA)		2
6	(-)Δ^9-tetrahydrocannabinol	(THC)		2
7	(-)Δ^8-tetrahydrocannabinol	(-Δ8THC)		2
8	exo-tetrahydrocannabinol	(exo-THC)		2
9	Δ^9-tetrahydrocannabinolic acid A	(THCAA)		4
10	Δ^9-tetrahydrocannabivarin	(THCV)		4

11	cannabigerolic acid	(CBGA)		4
12	cannabidivarin	(CBDV)		8
13	cannabidiolic acid	(CBDA)		16
14	tetrahydrocannabivarinic acid	(THCVA)		16
15	cannabidivarinic acid	(CBDVA)		32
16	(±) 11-nor-9-carboxy-Δ^9-THC			>32
17	(±) 11-hydroxy-Δ^9-THC			>32
18	cannabicyclol	(CBL)		>32

Supplementary Table 2. MRSA transposon mutants sensitized to sub-lethal concentrations of CBG.

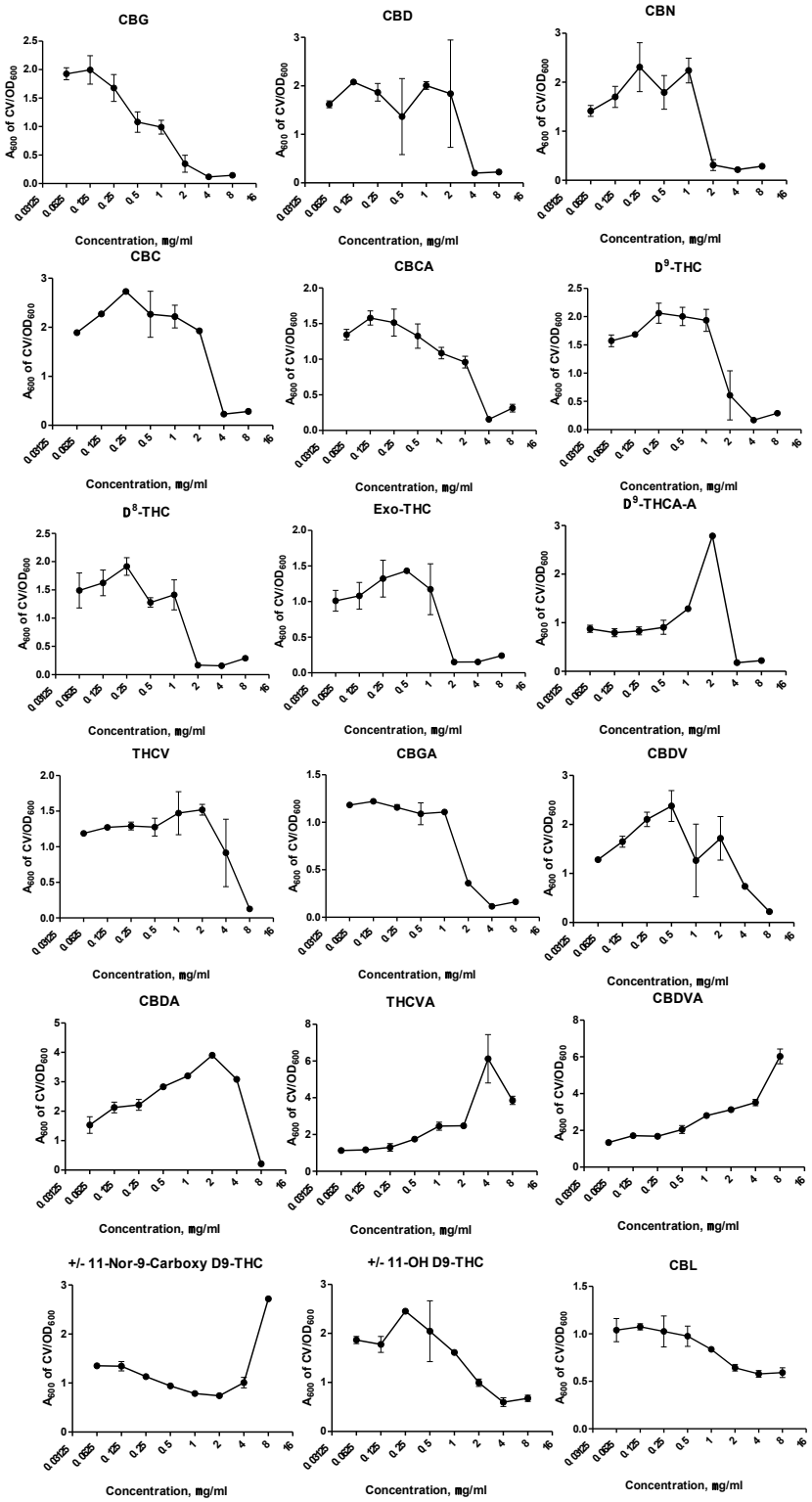
Gene	Function	Transposon
aroC	chorismate synthase	SAUSA300_1357
	putative endoribonuclease L-PSP	SAUSA300_0474
estA	tributyrin esterase	SAUSA300_2564
-	hypothetical protein	SAUSA300_0553
-	BioY family protein	SAUSA300_2233
atpA	ATP synthase F1, alpha subunit	SAUSA300_2060
	conserved hypothetical protein	SAUSA300_1780
graS	sensor histidine kinase	SAUSA300_0646
tcaB	teicoplanin resistance associated membrane protein TcaB	SAUSA300_2301
-	hypothetical protein	SAUSA300_2330
	conserved hypothetical protein	SAUSA300_0199
	accessory secretory protein Asp1	SAUSA300_2587
	putative hemolysin III	SAUSA300_2129
-	hypothetical protein (pyrazinamidase/nicotinamidase pncA)	SAUSA300_1899
	conserved hypothetical protein	SAUSA300_2212
thiE	thiamine-phosphate pyrophosphorylase	SAUSA300_2047
	conserved hypothetical protein	SAUSA300_0847
cap5B	capsular polysaccharide biosynthesis protein Cap5B	SAUSA300_0153
qoxC	quinol oxidase, subunit III	SAUSA300_0961
ribBA	riboflavin biosynthesis protein	SAUSA300_1713
sdhA	succinate dehydrogenase, flavoprotein subunit	SAUSA300_1047
hemB	delta-aminolevulinic acid dehydratase	SAUSA300_1615
	conserved hypothetical protein	SAUSA300_1294
-	TENA/THI-4 family protein	SAUSA300_2050
topB	DNA topoisomerase III	SAUSA300_2208
-	pyruvate ferredoxin oxidoreductase, alpha subunit	SAUSA300_1182
-	pyridoxal biosynthesis lyase PdxS	SAUSA300_0504
sdhB	succinate dehydrogenase iron-sulfur subunit	SAUSA300_1048
sgtB	monofunctional glycosyltransferase	SAUSA300_1855
	glycosyl transferase, group 1 family protein	SAUSA300_0550
	putative membrane protein	SAUSA300_0917
bioD	dethiobiotin synthase	SAUSA300_2373
mqo	malate:quinone oxidoreductase	SAUSA300_2312
	cation efflux family protein	SAUSA300_2099
	putative lipase/esterase	SAUSA300_0641
-	drug transporter	SAUSA300_2451
sucD	succinyl-CoA synthetase subunit alpha	SAUSA300_1139
lspA	lipoprotein signal peptidase	SAUSA300_1089
pckA	phosphoenolpyruvate carboxykinase	SAUSA300_1731
msrA	methionine sulfoxide reductase A	SAUSA300_1256
-	PTS system, galactitol-specific enzyme II, B component	SAUSA300_0240

Supplementary Table 3. Bacterial strains and plasmids used in this study.

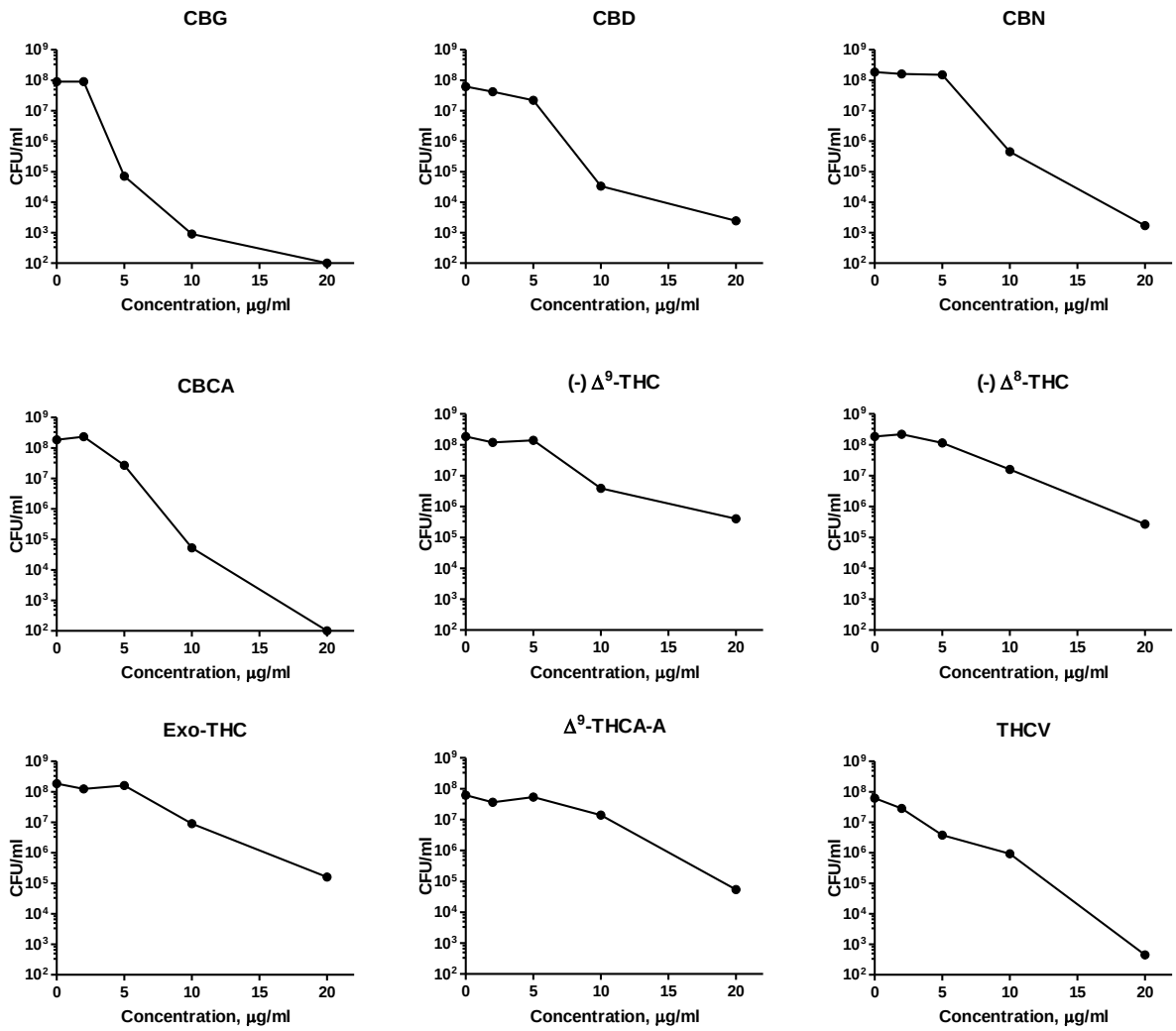
Bacterial Strain	Description	Reference
S. aureus		
USA300	USA300 LAC isolated in 2002 from a skin and soft tissue infection of an inmate in the Los Angeles County Jail in California, USA.; hypervirulent community-associated MRSA; cured of antibiotic resistance plasmid; also known as JE2; parent of the NTML.	Laboratory stock*
NTML	Nebraska Transposon Mutant Library Screening Array; 1920 *S. aureus* subsp. aureus USA300 JE2, transposon (Tn) mutants arrayed in five 384-well microtiter plates. Ery^R	NARSA*
B. subtilis 168		BGSC**
CRISPRi collection	*B. subtilis* CRISPRi essential gene knockdown strain collection	[1]
P. aeruginosa	Clinical isolate	
PAO1		[2]
E. coli	$lacZ^+Y^-I^-$, *E. coli* with constitutive expression of β-galactosidase but lacking the lactose permease	
ML35	ML35 with periplasmic β-lactamase	[3]
ML35pBR322		This study
K-12 BW25113		Lab stock
A. baumannii		
ATCC19606	*A. baumannii* lacking lipooligosaccharides (LOS)	ATCC
ATCC19606-LOS^-		[4]

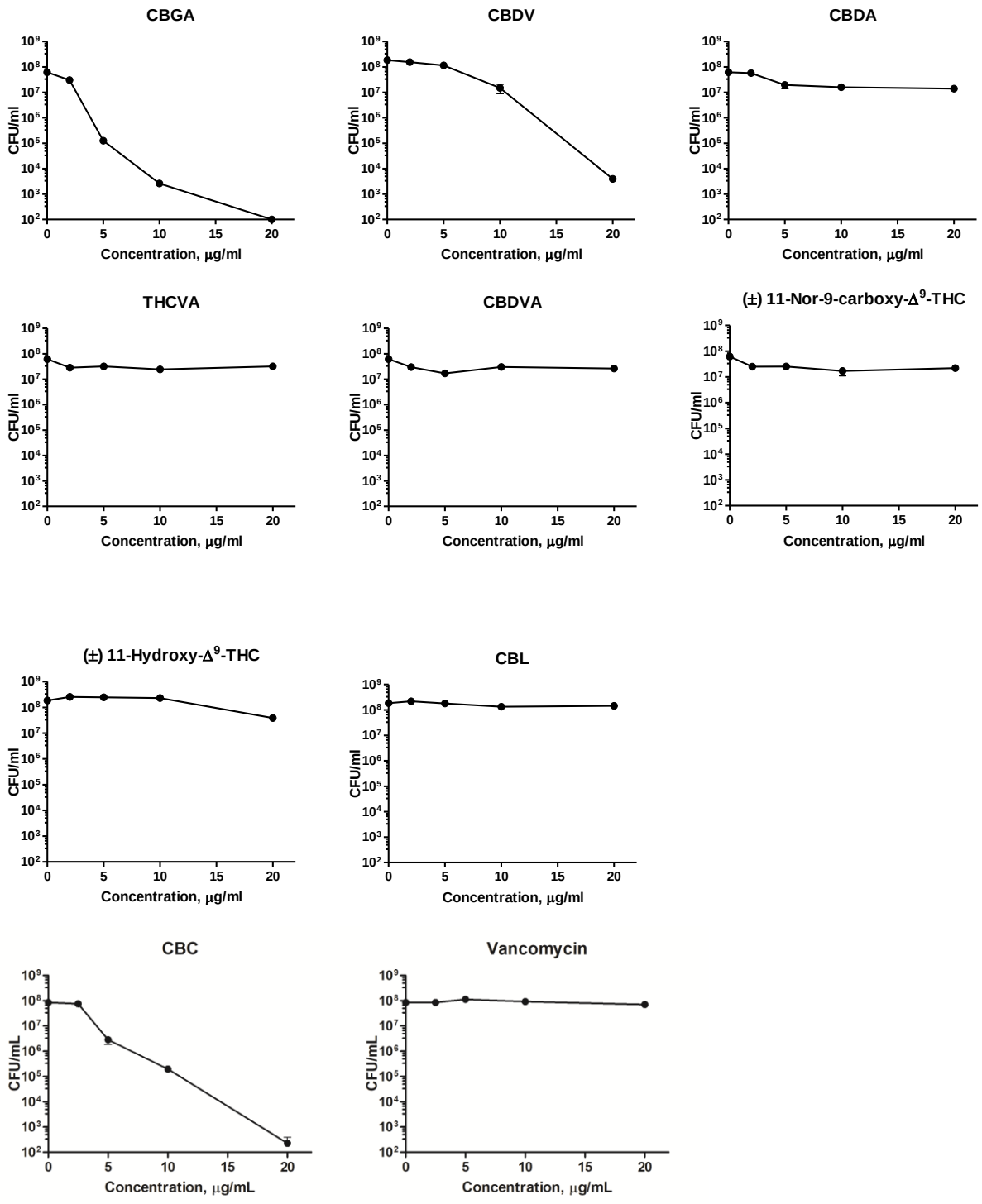
Bacterial Plasmid	Description	Reference
pBR322	Promoterless bioluminescent reporter plasmid encoding *luxABCDE*, Amp^R Cm^R	[5]

* Provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH.
** Bacillus Genetic Stock Center
Amp, ampicillin; Ery, erythromycin; Cm, chloramphenicol; Spec, spectinomycin; Strep, streptomycin
Strains and plasmids constructed in this study are available from the authors upon request.

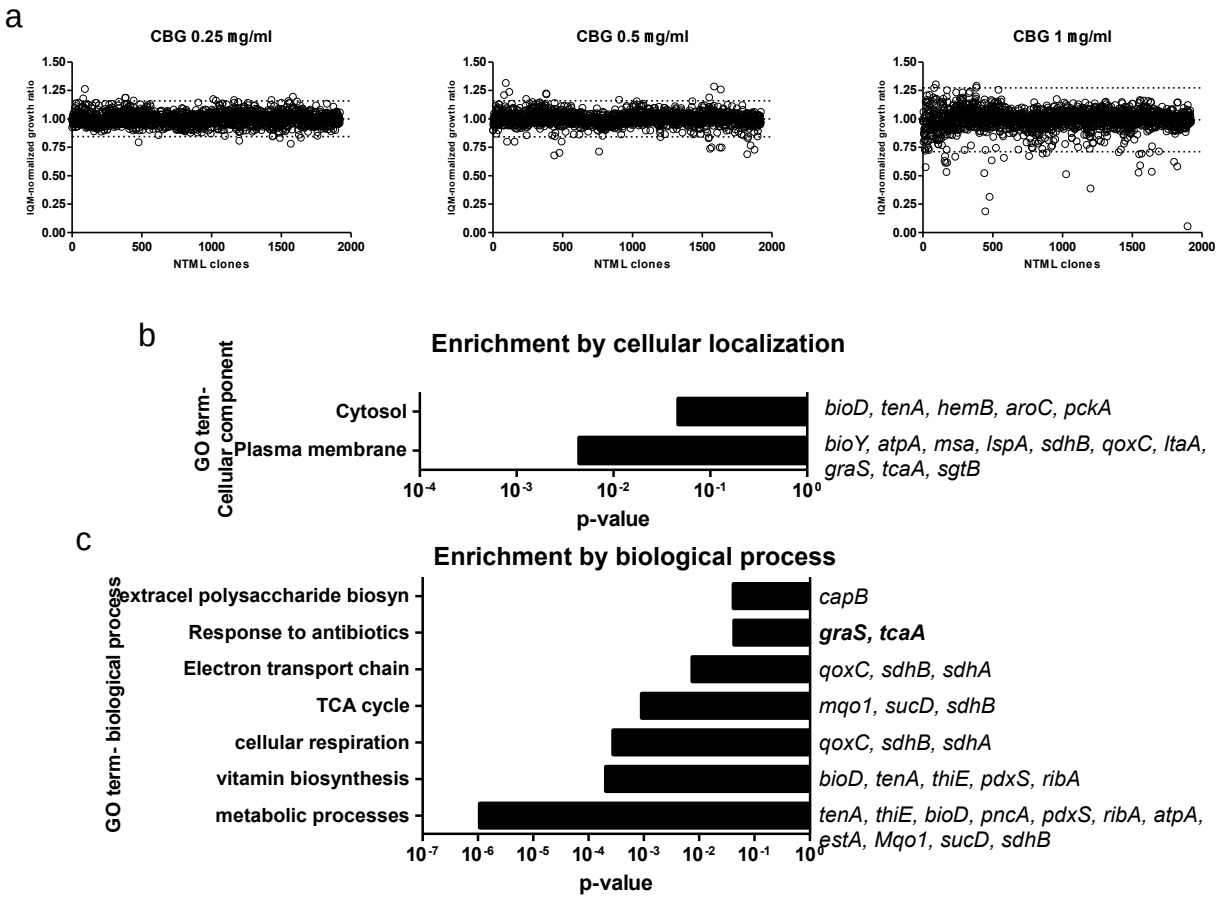


Supplementary Fig. 1. Effect of cannabinoids on biofilm formation of MRSA USA300. Shown is the effect of increasing concentrations of the cannabinoids on MRSA biofilm formation. The average A_{600nm} measurements of crystal violet stained biofilms normalized by the OD_{600} of planktonic cells are shown with error bars representing S.E.M. (n=4).

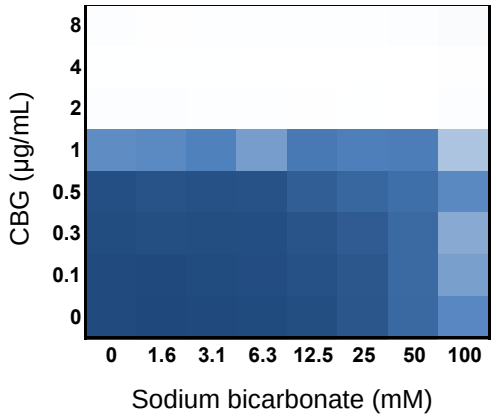

CBG
CBD
CBN
CBCA
(-) Δ9-THC
(-) Δ8-THC
Exo-THC
Δ9-THCA-A
THCV
CFU/ml
Concentration, µg/ml



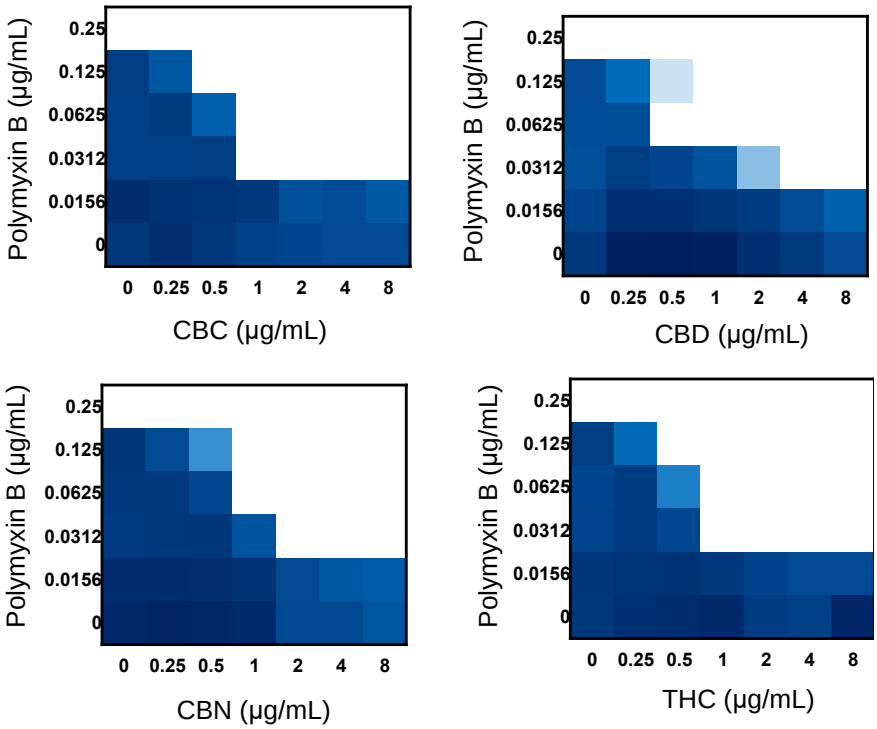
Supplementary Fig. 2. Killing of *S. aureus* USA300 persisters by various cannabinoids detected after 1 hour of treatment shown as mean ±S.E.M.



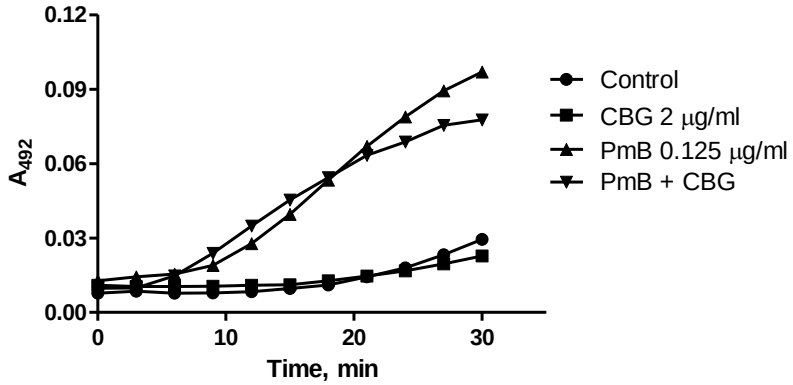
Supplementary Fig. 3. Chemical genomic studies using the Nebraska Transposon Mutant Library. **a**, Chemical genomic screen of the transposon library at various sub-lethal concentrations of CBG. **b**, Enrichment by cellular localization, whereby transposon mutants were classified based on gene ontology (GO). Enrichment was based on functional overrepresentation of the mutants resulting in sensitivity to CBG, using a Fisher's exact test to calculate p-value. **c**, Enrichment by biological processes, whereby transposon mutants were classified based on gene ontology (GO). Enrichment was based on functional overrepresentation of the mutants resulting in sensitivity to CBG, using a Fisher's exact test to calculate p-value.



Supplementary Fig. 4. Combination of CBG with sodium bicarbonate against MRSA USA300. The extent of inhibition is shown as a heat plot, such that the darkest blue color represents full bacterial growth.

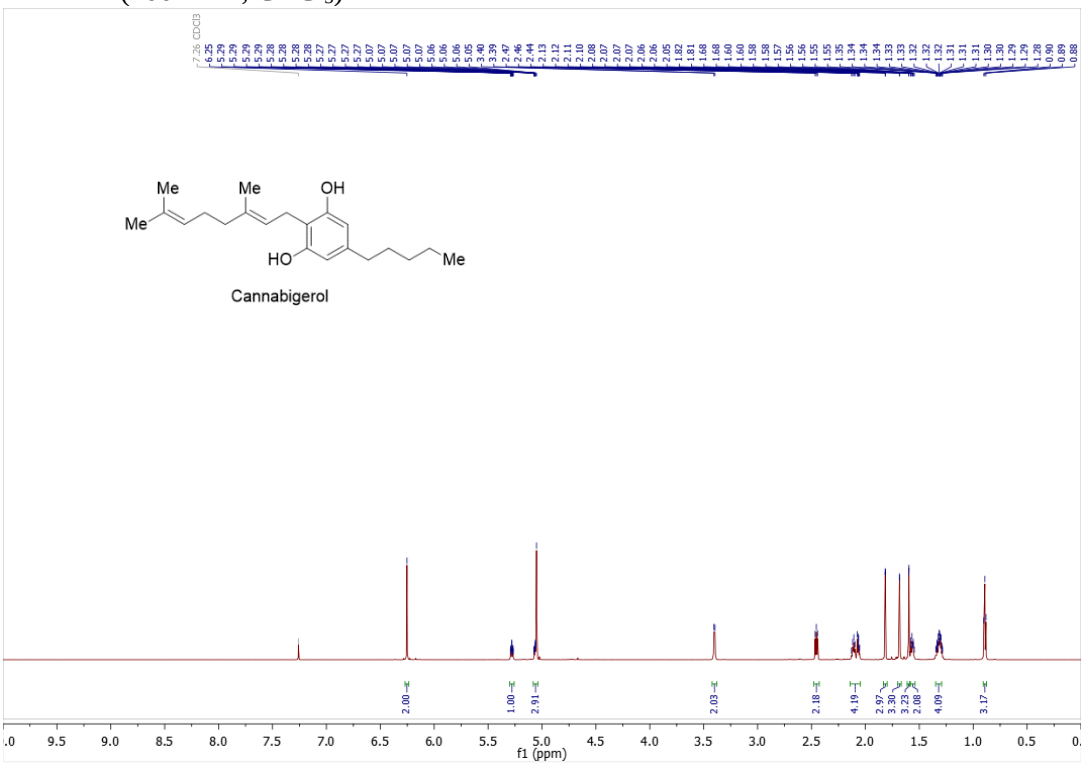


Supplementary Fig. 5. Checkerboard analysis of CBC, CBD, CBN and THC in combination with polymyxin B against *E. coli* (K-12 BW25113). The extent of inhibition is shown as a heat plot, such that the darkest blue color represents full bacterial growth.

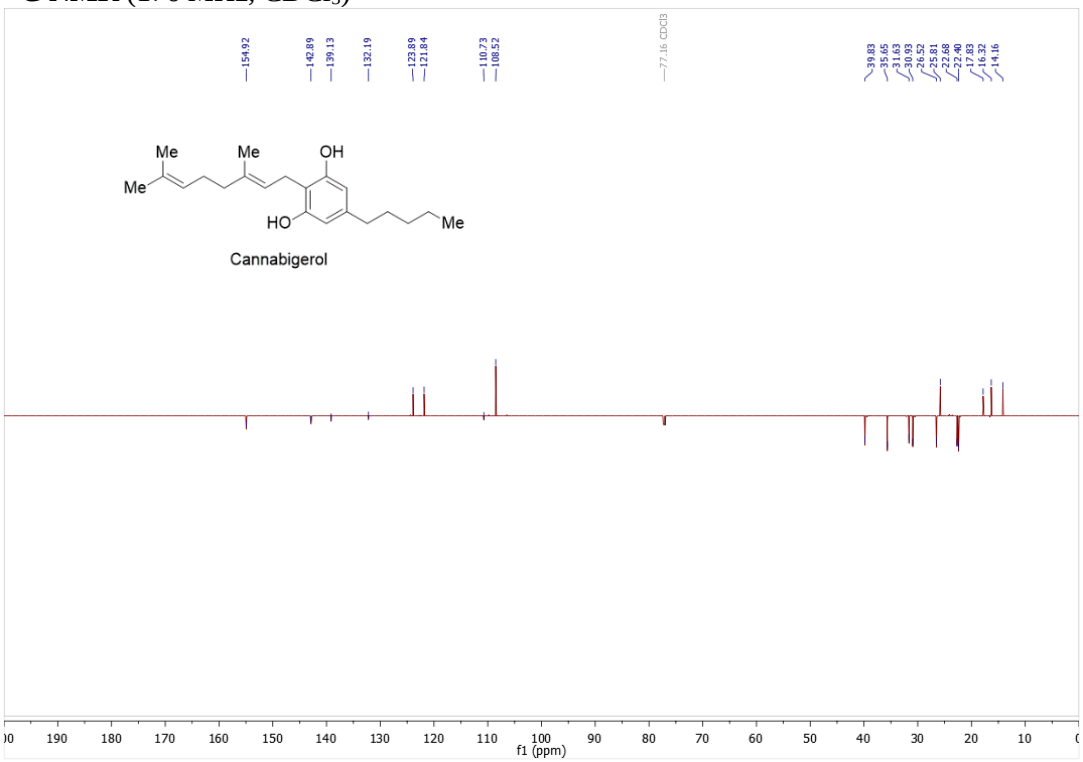


Supplementary Fig. 6. CBG is not active against the OM of *E. coli*, as measured by hydrolysis (absorbance at 492 nm) of nitrocefin upon permeation across the OM of an *E. coli* expressing a periplasmic β-lactamase.

^{1}H NMR (700 MHz, $CDCl_3$)



^{13}C NMR (176 MHz, $CDCl_3$)



Supplementary Fig. 7. Spectral data for CBG synthesis. This spectral data is consistent with previously reported literature. Choi, Young Hae, et al. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 15.6 (2004): 345-354.*

References

1. Peters, J. M.; Colavin, A.; Shi, H.; Czarny, T. L.; Larson, M. H.; Wong, S.; Hawkins, J. S.; Lu, C. H. S.; Koo, B. M.; Marta, E.; Shiver, A. L.; Whitehead, E. H.; Weissman, J. S.; Brown, E. D.; Qi, L. S.; Huang, K. C.; Gross, C. A., A Comprehensive, CRISPR-based Functional Analysis of Essential Genes in Bacteria. *Cell* **2016,** *165* (6), 1493-1506. DOI: 10.1016/j.cell.2016.05.003.
2. Holloway, B. W., Genetic recombination in *Pseudomonas aeruginosa*. *J Gen Microbiol* **1955,** *13* (3), 572-81.
3. Buttin, G.; Cohen, G. N.; Monod, J.; Rickenberg, H. V., Galactoside-permease of *Escherichia coli*. *Ann Inst Pasteur (Paris)* **1956,** *91* (6), 829-57.
4. Garcia-Quintanilla, M.; Pulido, M. R.; Pachon, J.; McConnell, M. J., Immunization with lipopolysaccharide-deficient whole cells provides protective immunity in an experimental mouse model of *Acinetobacter baumannii* infection. *PLoS One* **2014,** *9* (12), e114410. DOI: 10.1371/journal.pone.0114410.
5. Bolivar, F.; Rodriguez, R. L.; Greene, P. J.; Betlach, M. C.; Heyneker, H. L.; Boyer, H. W.; Crosa, J. H.; Falkow, S., Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **1977,** *2* (2), 95-113. DOI: 0378-1119(77)90000-2 [pii].