Supplementary Information for

# Screening microbially produced Δ9-tetrahydrocannabinol using a yeast biosensor workflow

William M. Shaw[1,2,3,4], Yunfeng Zhang[5], Xinyu Lu[3,4], Ahmad S. Khalil[1,2,6], Graham Ladds[7], Xiaozhou Luo[5] and Tom Ellis[3,4*]

[1]Biological Design Center, Boston University, Boston, MA 02215, USA

[2]Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA

[3]Department of Bioengineering Imperial College London, London, SW7 2AZ, UK

[4]Imperial College Centre for Synthetic Biology, Imperial College London, London, SW7 2AZ, UK

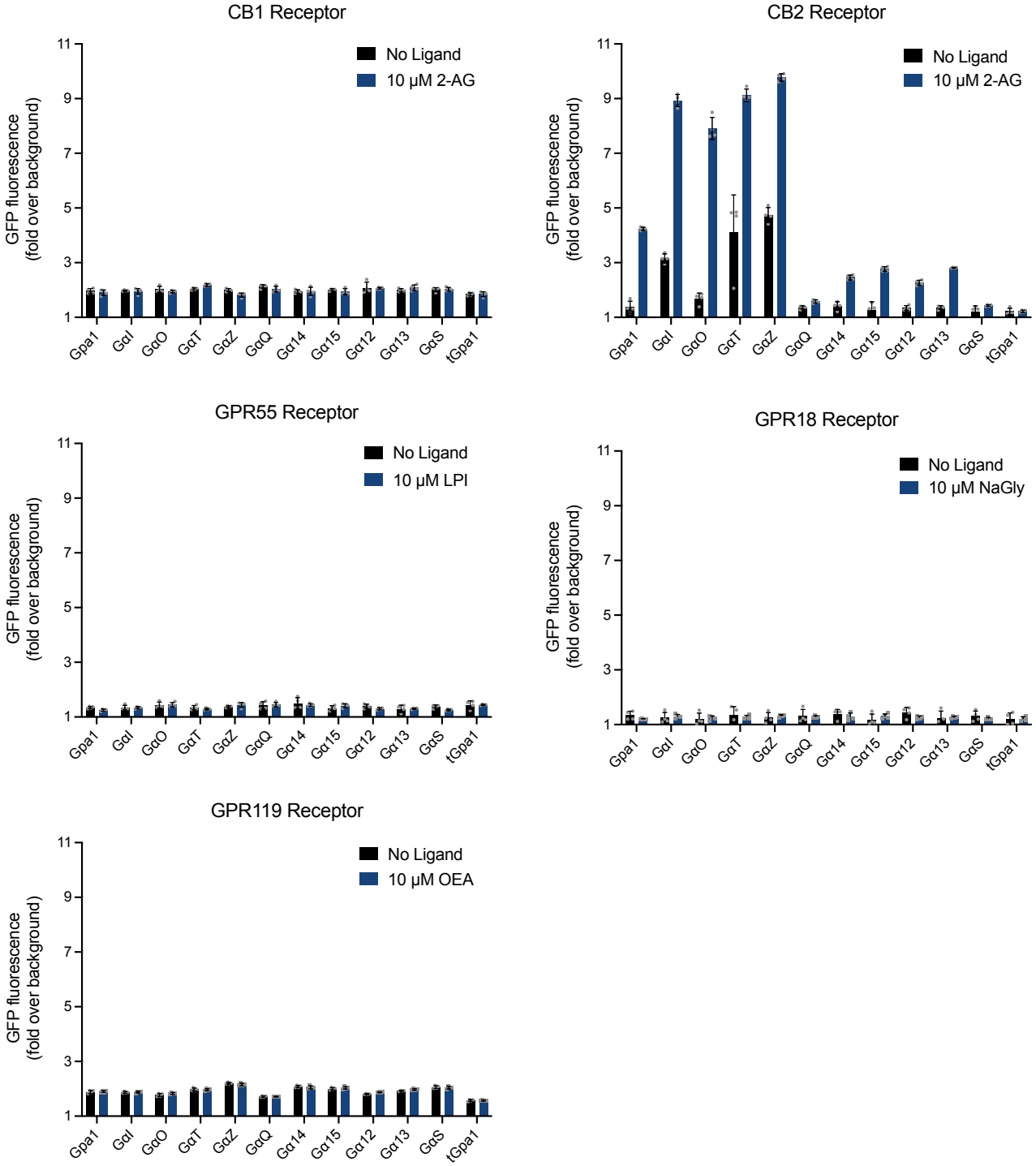
[5]Center for Synthetic Biochemistry, Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, 518055, China

[6]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA

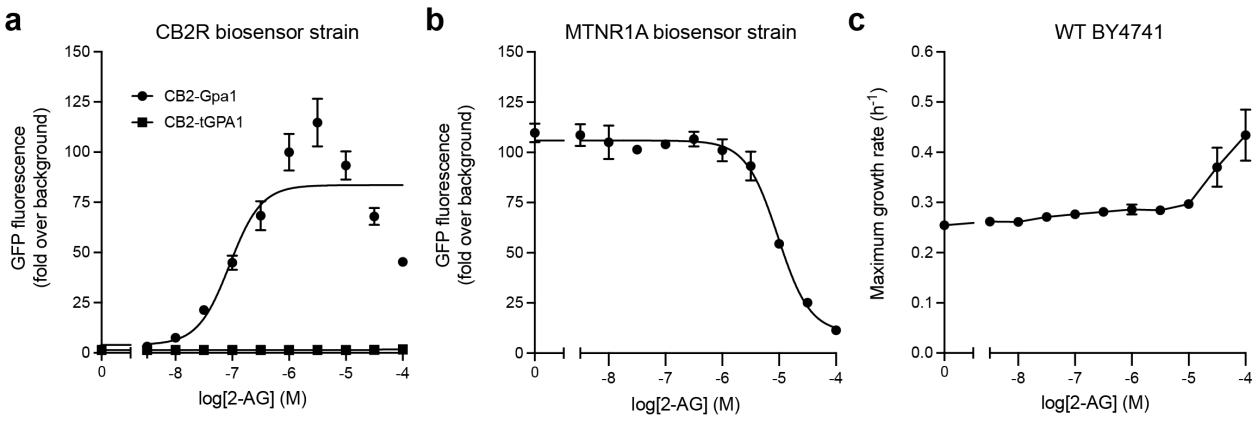
[7]Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, UK

*Corresponding author: Tom Ellis t.ellis@imperial.ac.uk

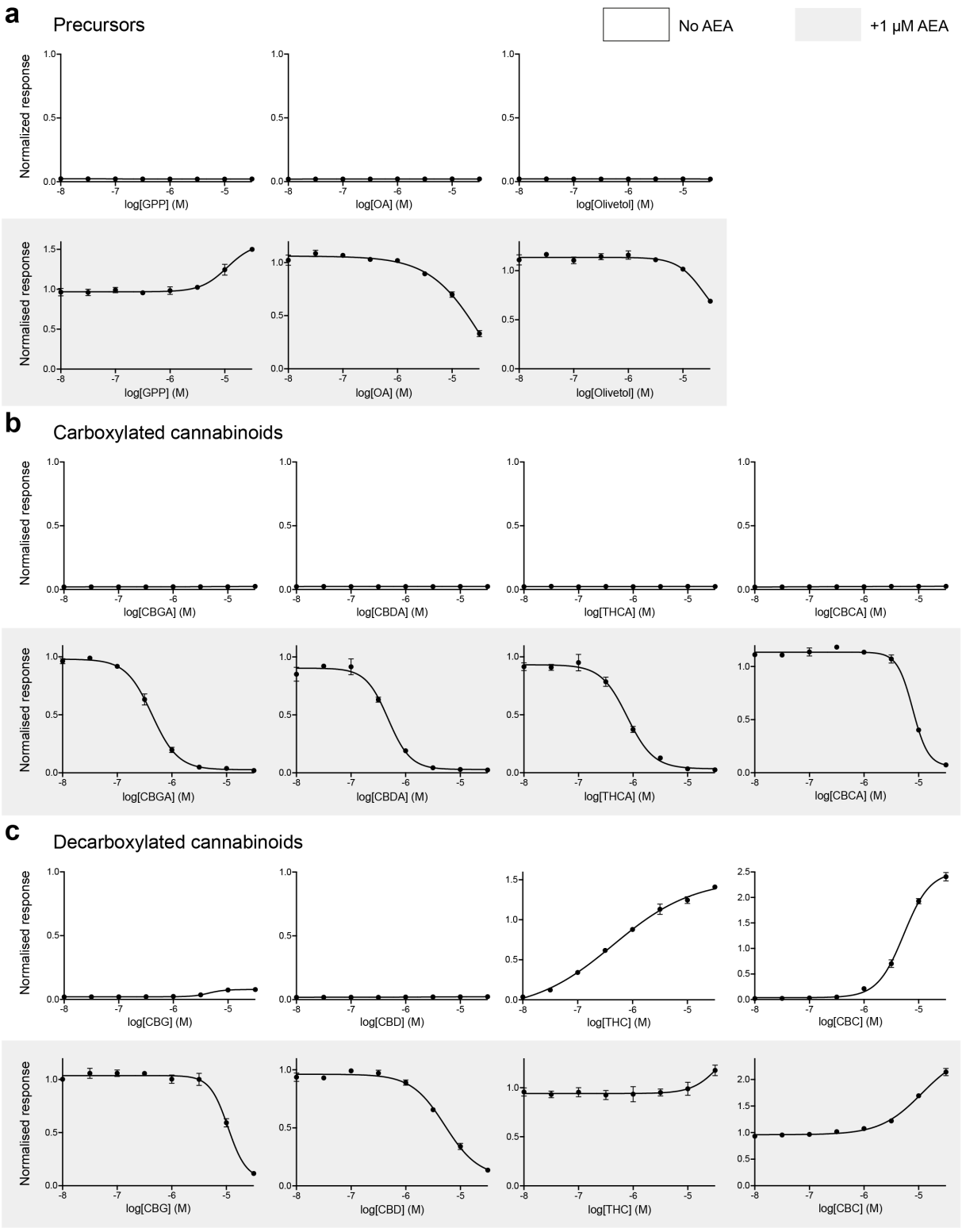
**This Supplementary Information includes:**



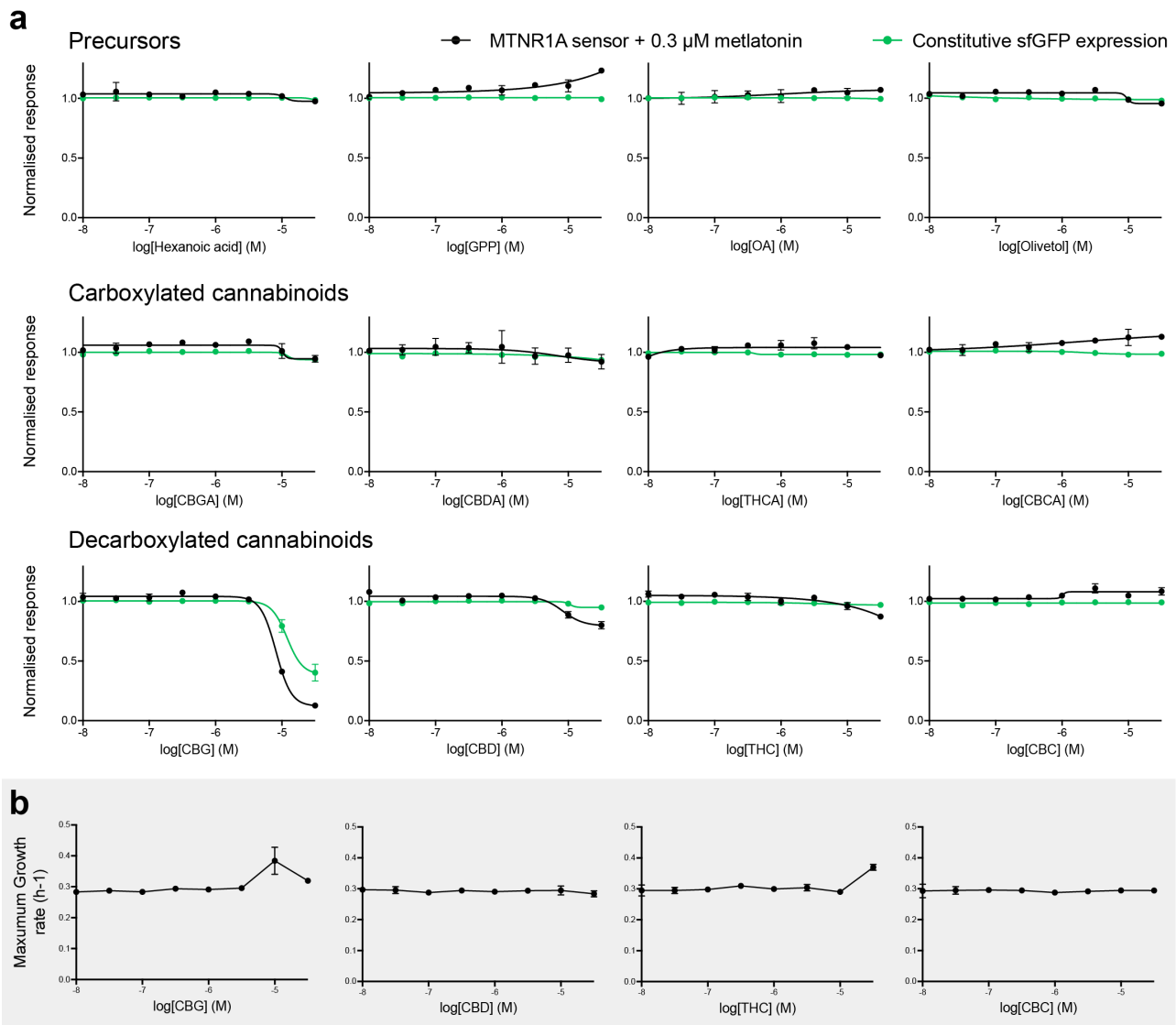

**Supplementary Fig. 1. Screening the cannabinoid GPCR/Gα variant strains against endogenous agonists.** ON/OFF response of the 60 GPCR/Gα strains to 10 µM of the endogenous agonists 2-AG (CB1R and CB2R), NaGly (GPR18), LPI (GPR55), and OEA (GPR119). Experimental measurements are GFP levels per cell as determined by a plate reader and shown as the mean ± SD from four biological replicates. Note that the small but observable differences in GFP expression after addition of ligand in CB1R and GPR18 yeast disappear upon normalising for receptor-independent changes in **Fig 1c**, which are also seen in the tGpa1 control.



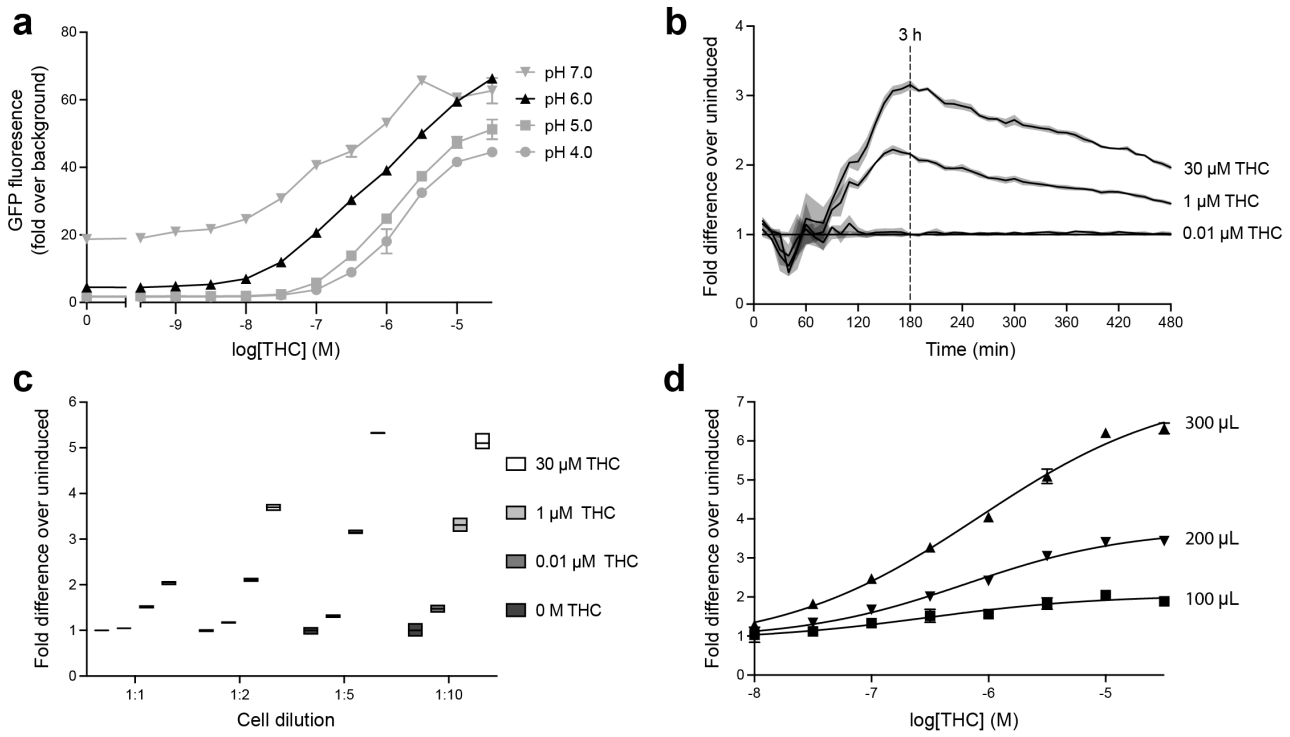

**Supplementary Fig. 2. Receptor independent effects of 2-AG. a**, 2-AG dose-response curves with the CB2R/Gpa1 biosensor (circles) and the CB2R/tGpa1 control (squares). **b**, 2-AG dose-response curves with a MTNR1A biosensor strain from Shaw et al. at the half maximal-effective concentration ($EC_{50}$) of melatonin (0.3 μM). Receptor-independent effect on reporter output seen above 1 μM 2-AG, possibly explaining the non-sigmoidal dose-response curve in **a**. Experimental measurements are GFP levels per cell determined by flow cytometry and shown as the mean ± SD from four biological replicates. Curves were fitted using GraphPad Prism variable slope (four parameter) nonlinear regression fit. **c**, Maximum growth rate of wildtype BY4741 yeast over a range of 2-AG concentrations. Interestingly, an increase in growth rate is seen above 1 μM 2-AG. Measurements are maximum growth rate calculated from growth at exponential phase in YPD and shown as mean ± SD from four biological replicates for a and b, and eight biological replicates for c.



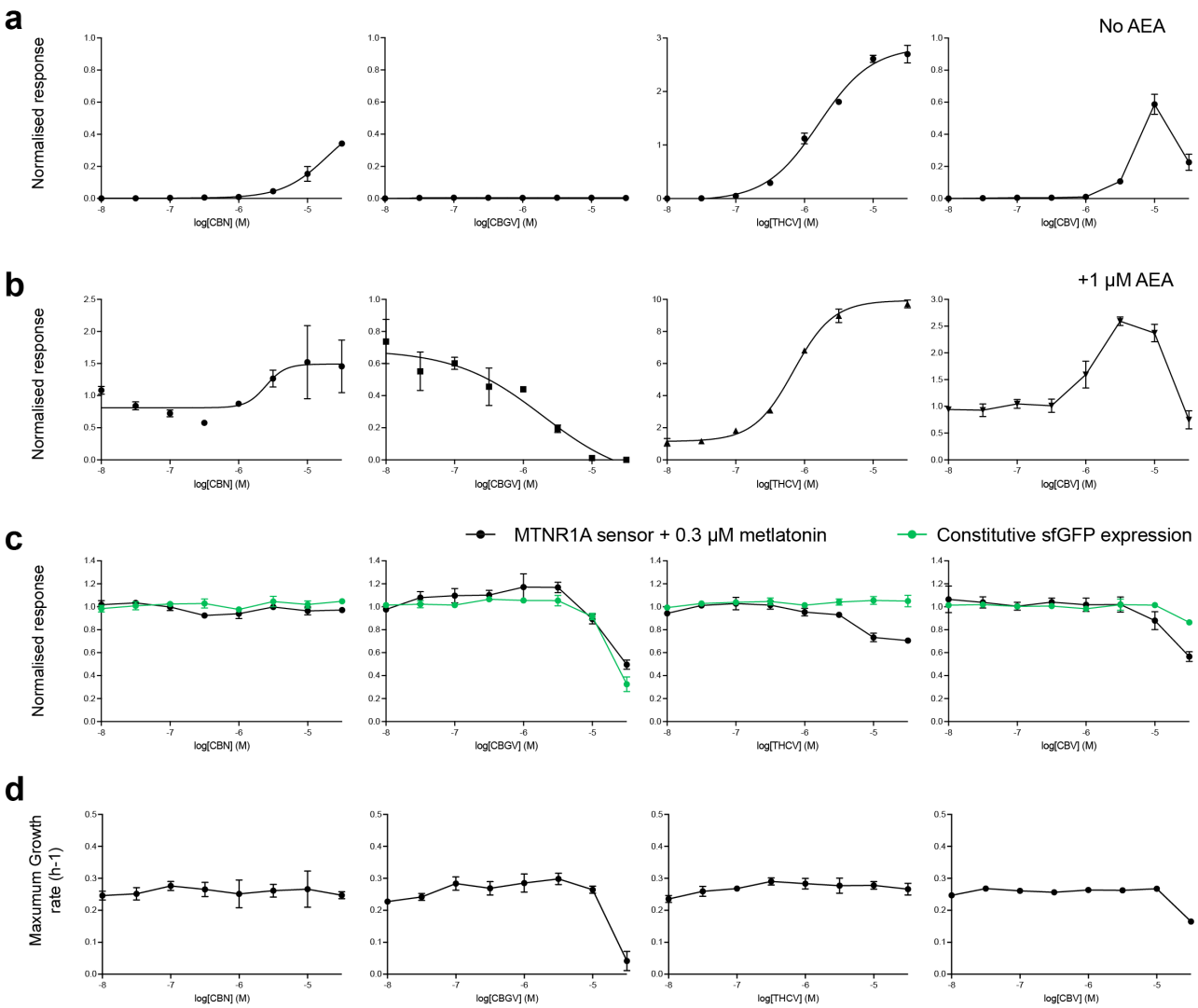

**Supplementary Fig. 3. CB2 biosensor dose-response curves with cannabinoids and their precursors. a**, Dose-response curves of the cannabinoid biosynthesis precursors in the presence (grey shade) and absence (no shade) of 1 μM AEA**. b**, Dose-response curves of the cannabinoid acids in the presence (grey shade) and absence (no shade) of 1 μM AEA**. c**, Dose-response curves of the decarboxylated cannabinoids in the presence (grey shade) and absence (no shade) of 1 μM AEA**.** Experimental measurements are GFP levels per cell determined by flow cytometry and shown as the mean ± SD from three biological replicates. All data are normalised to the no ligand (0) and 1 μM AEA (1) CB2 biosensor response. Curves were fitted using GraphPad Prism variable slope (four parameter) nonlinear regression fit.



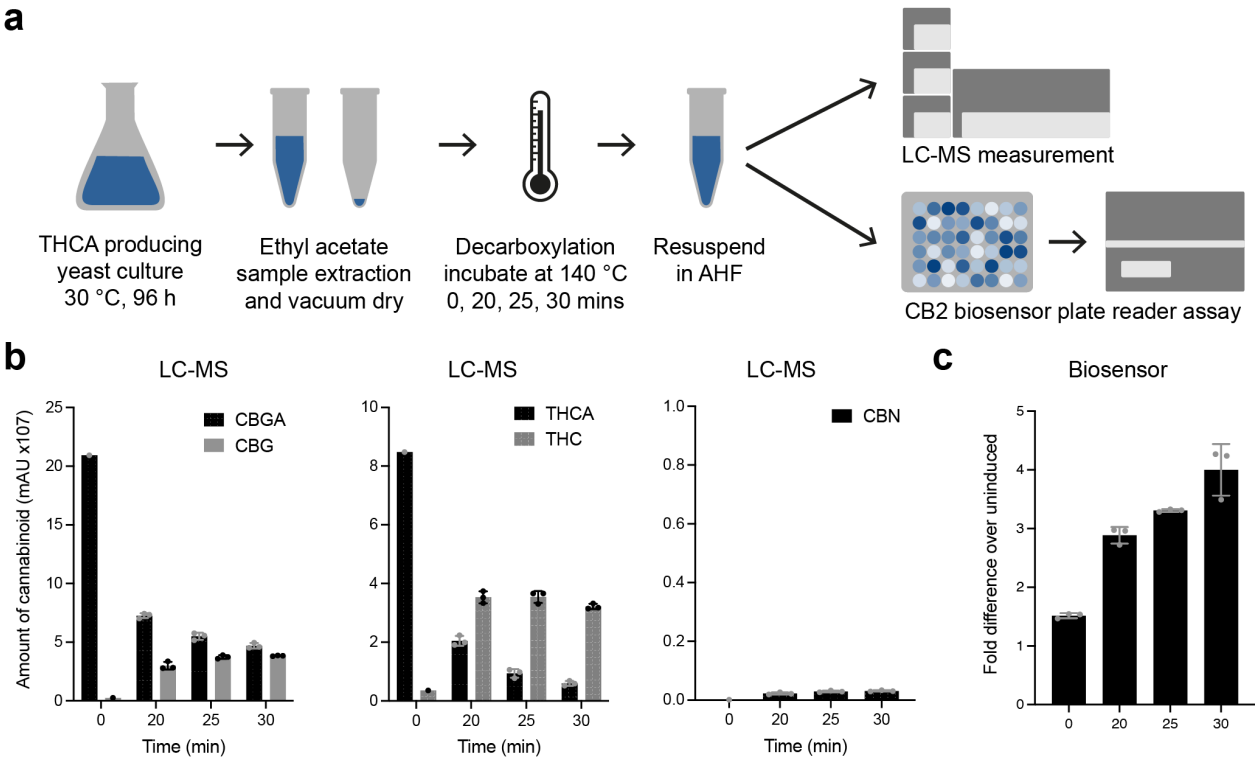

**Supplementary Fig. 4. Receptor-independent effects of cannabinoids and precursors. a**, Dose-response of the MTNR1A melatonin biosensor (yWS1544, from Shaw et al.) and constitutive GFP expression (BY4741 *pTDH3*-sfGFP-*tTDH1*-*URA3*, this study) to the various cannabinoids and precursors. Black circles and curves are the MTNR1A response at the half maximal-effective concentration ($EC_{50}$) of melatonin (0.3 µM). Green circles and curve are constitutive GFP expression. Experimental measurements are GFP levels per cell as determined by flow cytometry and shown as the mean ± SD from three biological replicates. All data are normalised to CB2 biosensor response without cannabinoid (1) and untransformed cells (0), and curves were fitted using GraphPad Prism variable slope (four parameter) nonlinear regression fit. **b**, Maximum growth rate of wildtype BY4741 yeast over a range of cannabinoid concentrations. Measurements are maximum growth rate calculated from growth at exponential phase in YPD and shown as mean ± SD from two biological replicates.




**Supplementary Fig. 5. CB2 biosensor plate reader assay optimisation. a**, THC dose-response curves with the CB2 biosensor at pH 4 (circles), pH 5 (squares), pH 6 (up triangles), and pH 7 (down triangles). CB2 biosensor behaviour at pH 6 (black) displayed low leak, high sensitivity, and a wide operational range, and so was chosen as the pH for final biosensor assay. Experimental measurements are GFP level per cell as determined by flow cytometry and shown as the mean ± SD from three biological replicates. **b**, Time course of GFP signal after induction with THC. Maximum signal over background (no induction) was seen after 3 h, and so was chosen for the final biosensor assay. **c**, THC dose-response of the CB2 biosensor at different cell dilutions. Diluting cells by resuspending a saturated culture in 5 x fresh media produced the highest signal with lowest error, and so was chosen for the final biosensor assay. **d**, THC dose-response curves of the CB2 biosensor, transferring 100, 200, or 300 µL for measurement in the plate reader. Experimental measurements are GFP level per cell as determined on a plate reader and shown as the mean ± SD from three biological replicates.




**Supplementary Fig. 6. Properties of the oxidised and variant cannabinoids, cannabinol (CBN), cannabigerovarin (CBGV), Δ9-tetrahydrocannabivarin (THCV), and cannabivarin (CBV). a**, Dose-response curves of cannabinoids with the CB2 biosensor in the absence of AEA. Data normalised to the 30 µM THC response (1). **b**, Dose-response curves of cannabinoids with the CB2 biosensor in the presence of 1 µM AEA. Data normalised to the 1 µM AEA response (1). **c**, Dose-response curves of cannabinoids in the absence of AEA with the MTNR1A biosensor in the presence of 0.3 µM melatonin (black) and constitutive GFP expression (green). Data normalised to the no cannabinoid response (1). Experimental measurements are GFP level per cell determined by flow cytometry and shown as the mean ± SD from three biological replicates. Curves were fitted using GraphPad Prism variable slope (four parameter) nonlinear regression fit for CBN, CBGV, and THCV. **d**, Maximum growth rate of wildtype BY4741 yeast over a range of variant cannabinoid concentrations. Measurements are maximum growth rate calculated from growth at exponential phase in YPD and shown as mean ± SD from three biological replicates.




**Supplementary Fig. 7. Decarboxylation of THCA producing yeast cell extract. a**, Workflow for extracting and decarboxylating acid cannabinoids from yeast and converting them into their non-acid forms and assessment of incubation time at 140 °C by LC-MS and the CB2 biosensor assay. AHF, acetonitrile/H2O/formic acid (80/20/0.05%). **b**, Relative amounts of all cannabinoids detected in cell extract after incubation at 140 °C over time. Experimental measurements are relative amounts as determined by LC-MS and shown as mean ± SD from three technical replicates. **c**, CB2 biosensor response to yeast THCA producer extract after heating at 140 °C for different lengths of time. Data was normalised uninduced CB2 biosensor response (1). Experimental measurements are GFP level per cell as determined on a plate reader and shown as the mean ± SD from three biological replicates.

**Note to readers**: Different microbial systems, biosynthetic pathways and culture conditions may lead to different ratios of cannabinoids. In this case, the variant cannabinoids are not present, due to the lack of native olivetolic acid biosynthesis. It is recommended that users should recharacterize the optimal time of incubation at 140 °C before undertaking new screening efforts, as this time may between experimental systems. This optimisation can be done using the CB2 biosensor and selecting the time that leads to peak biosensor induction from a control strain that is known to produce THCA.

**Supplementary Table 1. THCAS mutant strains in the yS231 library.** Colour scale of LC-MS data set to lowest (white) and highest (blue) relative amounts of THC, as a visual aid.

| Mutant strain | Codon at AA 415 | Amino acid change | Note | LC-MS THC amount (mAU x$10^7$) | Biosensor read 1 (arb. units) | Biosensor read 2 (arb. units) | Biosensor average (arb. units) |
|---|---|---|---|---|---|---|---|
| yS231-1 | GTA>TAT | V415Y | | 2.59 | 1089 | 1113 | 1101 |
| yS231-3 | GTA>ATG | V415M | | 4.44 | 1058 | 1079 | 1069 |
| yS231-4 | GTA>TTT | V415F | | 3.13 | 1134 | 1130 | 1132 |
| yS231-5 | GTA>AAT | V415N | | 3.70 | 1132 | 1086 | 1109 |
| yS231-6 | GTA>TAT | V415Y | | 2.24 | 948 | 923 | 935 |
| yS231-7 | GTA>TAG | V415* | *, stop codon | 1.89 | 889 | 871 | 880 |
| yS231-8 | GTA>AAG | V415K | | 2.52 | 1014 | 1014 | 1014 |
| yS231-9 | GTA>AGT | V415S | | 2.42 | 961 | 961 | 961 |
| yS231-10 | GTA>CTG | V415L | | 2.63 | 940 | 942 | 941 |
| yS231-11 | GTA>CTG | V415L | | 3.50 | 1174 | 1136 | 1155 |
| yS231-12 | GTA>TAT | V415Y | | 1.73 | 856 | 823 | 840 |
| yS231-13 | GTA>ACT | V415T | | 2.73 | 1009 | 980 | 994 |
| yS231-14 | GTA>CGT | V415R | | 1.91 | 819 | 811 | 815 |
| yS231-15 | GTA>CAT | V415H | | 1.84 | 808 | 799 | 804 |
| yS231-16 | GTA>TAT | V415Y | | 1.67 | 959 | 931 | 945 |
| yS231-17 | GTA>TGT | V415C | | 2.28 | 925 | 888 | 906 |
| yS231-18 | GTA>CTG | V415L | | 3.66 | 1240 | 1237 | 1239 |
| yS231-19 | GTA>TGT | V415C | | 3.66 | 1399 | 1436 | 1418 |
| yS231-20 | GTA>GTA | V415V | Wild type | 3.98 | 1425 | 1340 | 1383 |
| yS231-21 | GTA>TCG | V415S | | 1.94 | 1060 | 1083 | 1072 |
| yS231-22 | GTA>CAT | V415H | | 1.62 | 862 | 888 | 875 |
| yS231-23 | GTA>CCT | V415P | | 1.33 | 781 | 812 | 797 |
| yS231-24 | GTA>CCT | V415P | | 2.18 | 1048 | 1085 | 1066 |
| yS231-25 | GTA>ACT | V415T | | 2.97 | 1639 | 1592 | 1616 |
| yS231-26 | GTA>ATT | V415I | | 3.03 | 1676 | 1573 | 1625 |
| yS231-28 | GTA>CCT | V415P | | 1.68 | 1327 | 1322 | 1325 |
| yS231-29 | GTA>CGT | V415R | | 1.78 | 1493 | 1423 | 1458 |
| yS231-30 | GTA>GAG | V415E | | 2.06 | 1440 | 1488 | 1464 |
| yS231-31 | GTA>TAG | V415* | *, stop codon | 2.11 | 1398 | 1390 | 1394 |
| yS231-33 | GTA>GCT | V415A | | 2.91 | 1629 | 1725 | 1677 |
| yS231-34 | GTA>AAT | V415N | | 1.67 | 1193 | 1154 | 1174 |
| yS231-35 | GTA>AAT | V415N | | 1.75 | 1195 | 1173 | 1184 |
| yS231-36 | GTA>ACG | V415T | | 2.46 | 1537 | 1511 | 1524 |
| yS231-37 | GTA>ACT | V415T | | 3.39 | 1773 | 1697 | 1735 |
| yS231-39 | GTA>CGT | V415R | | 2.00 | 1234 | 1179 | 1207 |
| yS231-40 | GTA>CAT | V415H | | 1.97 | 1420 | 1313 | 1367 |
| yS231-42 | GTA>CCT | V415P | | 2.10 | 1208 | 1181 | 1194 |
| yS231-43 | GTA>CAG | V415Q | | 2.11 | 1383 | 1378 | 1381 |
| yS231-44 | GTA>ACT | V415T | | 2.00 | 1462 | 1445 | 1454 |
| yS231-45 | GTA>TTT | V415F | | 1.91 | 1829 | 1763 | 1796 |
| yS231-46 | GTA>ACG[a] | V415T | [a], AAG>TAG at K457* | 2.06 | 1433 | 1366 | 1399 |
| yS231-48 | GTA>GTG | V415V | Synonymous codon | 3.78 | 1824 | 1745 | 1784 |
| yS231-49 | GTA>ACT | V415T | | 3.09 | 1497 | 1540 | 1518 |
| yS231-51 | GTA>TAT | V415Y | | 1.95 | 1300 | 1366 | 1333 |
| yS231-52 | GTA>CAT | V415H | | 2.29 | 1592 | 1620 | 1606 |
| yS231-53 | GTA>AAT | V415N | | 2.16 | 1177 | 1213 | 1195 |
| yS231-54 | GTA>CCG | V415P | | 1.46 | 844 | 852 | 848 |
| yS231-55 | GTA>AAT | V415N | | 1.03 | 839 | 835 | 837 |
| yS231-56 | GTA>TAT[b] | V415Y | [b], AAC>GAC at N514D | 1.47 | 820 | 827 | 824 |
| yS231-57 | GTA>AAT | V415N | | 1.42 | 848 | 882 | 865 |
| yS231-58 | GTA>GTA | V415V | Wild type | 3.21 | 1169 | 1168 | 1169 |
| yS231-59 | GTA>CTG | V415L | | 2.07 | 900 | 883 | 892 |
| yS231-61 | GTA>GTT | V415V | Synonymous codon | 1.58 | 851 | 839 | 845 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| yS231-62 | GTA>CAG | V415Q | | 0.98 | 816 | 805 | 810 |
| yS231-63 | GTA>CCT | V415P | | 1.56 | 939 | 914 | 926 |
| yS231-64 | GTA>AAG | V415K | | 2.27 | 920 | 875 | 897 |
| yS231-65 | GTA>ACT | V415T | | 3.00 | 1085 | 1066 | 1075 |
| yS231-68 | GTA>TGG | V415W | | 1.28 | 820 | 796 | 808 |
| yS231-69 | GTA>TTG | V415L | | 1.96 | 891 | 909 | 900 |
| yS231-70 | GTA>AAG | V415K | | 1.60 | 864 | 835 | 849 |
| yS231-71 | GTA>GCG | V415A | | 2.22 | 929 | 905 | 917 |
| yS231-72 | GTA>CTG | V415L | | 1.86 | 900 | 894 | 897 |
| yS231-73 | GTA>ATG | V415M | | 1.46 | 1030 | 1008 | 1019 |
| yS231-74 | GTA>GCT | V415A | | 1.96 | 1089 | 1100 | 1094 |
| yS231-75 | GTA>AGG | V415R | | 1.35 | 825 | 843 | 834 |
| yS231-76 | GTA>AAT | V415N | | 1.57 | 838 | 832 | 835 |
| yS231-77 | GTA>TAT | V415Y | | 1.71 | 887 | 909 | 898 |
| yS231-78 | GTA>ACT | V415T | | 2.06 | 907 | 948 | 928 |
| yS231-79 | GTA>CAG | V415Q | | 1.46 | 858 | 887 | 873 |
| yS231-80 | GTA>CCT | V415P | | 1.38 | 839 | 853 | 846 |
| yS231-81 | GTA>GAT | V415D | | 1.80 | 868 | 873 | 870 |
| yS231-82 | GTA>TAT | V415Y | | 1.46 | 852 | 861 | 856 |
| yS231-83 | GTA>TTG | V415L | | 2.08 | 939 | 974 | 957 |
| yS231-84 | GTA>GCT | V415A | | 2.67 | 1090 | 1054 | 1072 |
| yS231-85 | GTA>GCT | V415A | | 2.63 | 1099 | 1114 | 1106 |
| yS231-87 | GTA>ACT | V415T | | 2.58 | 1032 | 1028 | 1030 |
| yS231-88 | GTA>TAG | V415* | *, stop codon | 1.69 | 1035 | 1015 | 1025 |
| yS231-89 | GTA>TAG | V415* | *, stop codon | 1.31 | 813 | 810 | 812 |
| yS231-90 | GTA>CAT | V415H | | 1.53 | 847 | 838 | 843 |
| yS231-91 | GTA>TCT | V415S | | 1.81 | 850 | 859 | 855 |
| yS231-92 | GTA>ACT | V415T | | 1.41 | 807 | 821 | 814 |
| yS231-93 | GTA>GTA | V415V | Wild type | 2.95 | 1115 | 1221 | 1168 |
| yS231-94 | GTA>ATT | V415I | | 1.56 | 824 | 805 | 814 |
| yS231-95 | GTA>CCG | V415P | | 1.21 | 812 | 822 | 817 |
| yS231-96 | GTA>AGT | V415S | | 1.25 | 840 | 823 | 832 |
| yS231-97 | GTA>ATG | V415M | | 4.89 | 1568 | 1595 | 1582 |
| yS231-98 | GTA>TGT | V415C | | 3.14 | 892 | 936 | 914 |
| yS231-99 | GTA>TGT | V415C | | 4.52 | 1252 | 1298 | 1275 |
| yS231-100 | GTA>CAG | V415Q | | 1.83 | 867 | 872 | 870 |
| yS231-101 | GTA>GCG | V415A | | 2.30 | 931 | 987 | 959 |
| yS231-102 | GTA>GTT | V415V | Synonymous codon | 2.48 | 885 | 929 | 907 |
| yS231-103 | GTA>CTG | V415L | | 3.40 | 949 | 1007 | 978 |
| yS231-104 | GTA>TGG | V415W | | 4.59 | 1721 | 1749 | 1735 |
| yS231-105 | GTA>GAG | V415E | | 3.90 | 1015 | 1049 | 1032 |
| yS231-106 | GTA>TCG | V415S | | 1.21 | 858 | 850 | 854 |
| yS231-107 | GTA>ACG | V415T | | 2.67 | 1033 | 1019 | 1026 |
| yS231-108 | GTA>CAG | V415Q | | 1.45 | 848 | 845 | 846 |
| yS231-109 | GTA>ACG | V415T | | 2.52 | 984 | 947 | 966 |
| yS231-110 | GTA>GTA | V415V | Wild type | 5.25 | 1792 | 1756 | 1774 |
| yS231-111 | GTA>TAG | V415* | *, stop codon | 2.37 | 950 | 939 | 945 |
| yS231-112 | GTA>GTA | V415V | Wild type | 2.96 | 1032 | 1011 | 1022 |
| yS231-113 | GTA>TTG | V415L | | 1.53 | 886 | 884 | 885 |
| yS231-114 | GTA>CAT | V415H | | 1.27 | 849 | 849 | 849 |
| yS231-115 | GTA>AAG | V415K | | 2.00 | 957 | 941 | 949 |
| yS231-116 | GTA>TCG | V415S | | 1.22 | 814 | 839 | 827 |
| yS231-118 | GTA>ATG | V415M | | 1.91 | 872 | 880 | 876 |
| yS231-119 | GTA>AAG | V415K | | 1.66 | 855 | 881 | 868 |
| yS231-120 | GTA>TTG | V415L | | 2.03 | 879 | 910 | 895 |

**Supplementary Table 2. Ligands used in this study.**

| Ligand | Abbreviation | Solvent | Supplier | Product code |
|---|---|---|---|---|
| 2-Arachidonoylglycerol | 2-AG | Acetonitrile | Sigma | A8973 |
| Anandamide | AEA | Ethanol | Fluorochem | M05752 |
| *N*-Arachidonylglycine | NaGly | Ethanol | ABCAM | ab120346 |
| L-α-Lysophosphatidylinositol | LPI | DMSO | Sigma | L7635 |
| Oleoylethanolamine | OEA | Ethanol | Sigma | O0383 |
| Hexanoic acid | Hexanoic acid | Methanol | Sigma | 153745 |
| Geranyl pyrophosphate | GPP | Methanol | Sigma | G6772-1VL |
| Divarinic acid | DA | Methanol | TRC | D494463 |
| Olivetolic acid | OA | Methanol | TRC | O533005 |
| Olivetol | Olivetol | Methanol | Sigma | 152633 |
| Cannabigerolic acid | CBGA | Acetonitrile | Sigma | C-142-1ML |
| Cannabidiolic acid | CBDA | Acetonitrile | Sigma | C-144-1ML |
| $\Delta^9$-Tetrahydrocannabinolic acid | THCA | Acetonitrile | Sigma | T-093-1ML |
| Cannabichromenic acid | CBCA | Acetonitrile | Sigma | C-150-1ML |
| Cannabigerol | CBG | Methanol | Sigma | C-141-1ML |
| Cannabidiol | CBD | Methanol | Sigma | C-045-1ML |
| $\Delta^9$-Tetrahydrocannabinol | THC | Methanol | Sigma | T4764-1ML |
| Cannabichromene | CBC | Methanol | Sigma | C-143-1ML |
| Cannabinol | CBN | Methanol | Sigma | C-046-1ML |
| Cannabigerovarin | CBGV | Methanol | Sigma | C-227-1ML |
| Tetrahydrocannabivarin | THCV | Methanol | Sigma | T-094-1ML |
| Cannabivarin | CBV | Methanol | Sigma | C-225-1ML |

**Supplementary Table 3. DNA sequences used in this study.** All DNA sequences are in the Yeast MoClo Toolkit (YTK) format as a type 3-4a part using the pYTK001 backbone (CamR-ColE1, not shown). BsaI recognition site (grey highlight), BsaI overhang (bold), open reading frame (underlined), start codon (green highlight), stop codon (red highlight).

| Name | Insert sequence |
|---|---|
| **pWS2692**<br><br>**CB1R** | GGTCTCATATGAAGTCTATTTTGGACGGTTTGGCTGATACCACCTTCAGAACTATTACTACCGATTTG<br>TTGTACGTCGGTTCCAACGATATTCAGTACGAAGATATTAAGGGTGACATGGCTTCTAAGTTGGGTT<br>ACTTTCCTCAAAAGTTCCCATTGACATCTTTCAGAGGTTCTCCATTCCAAGAAAAAATGACTGCTGGT<br>GATAACCCACAATTGGTTCCAGCTGATCAAGTTAACATTACCGAGTTCTACAACAAGTCCCTGTCCTC<br>ATTCAAAGAAAACGAAGAAAACATTCAGTGCGGCGAAAACTTCATGGATATTGAATGTTTCATGGTCT<br>TGAACCCATCTCAACAATTGGCTATTGCTGTTTTGTCTTTGACCTTGGGTACTTTCACTGTCTTGGAA<br>AACTTGTTGGTTTTGTGCGTTATCTTGCACTCCAGATCTTTGAGATGTAGACCATCCTACCATTTCATT<br>GGTTCTTTGGCTGTTGCAGATTTGTTGGGTTCTGTTATTTTCGTCTACTCCTTCATCGATTTCCACGTT<br>TTCCATAGAAAGGACTCCAGAAACGTTTTCTTGTTCAAGTTAGGTGGTGTTACTGCTTCTTTCACTGC<br>TTCTGTTGGTTCCTTGTTTTTGACTGCTATTGACAGGTACATCTCCATCCATAGACCATTGGCTTACA<br>AGAGAATAGTTACTAGACCAAAGGCTGTTGTTGCTTTCTGTTTGATGTGGACTATTGCTATCGTTATT<br>GCAGTCTTGCCATTGCTAGGTTGGAATTGTGAAAAGTTGCAATCCGTTTGCTCCGATATTTTCCCACA<br>TATTGACGAAACCTACCTGATGTTTTGGATTGGTGTTACCTCTGTCTTGTTGTTGTTTATCGTTTACGC<br>CTACATGTACATTTTGTGGAAGGCTCATTCTCATGCCGTTAGAATGATTCAAAGAGGTACTCAGAAGT<br>CGATCATCATCCATACTTCAGAAGATGGTAAGGTTCAAGTCACTAGACCAGATCAAGCTAGAATGGA<br>TATTAGATTGGCTAAGACCTTGGTCTTGATCTTGGTTGTTTTGATTATTTGCTGGGGTCCTTTGTTGG<br>CCATTATGGTTTATGATGTTTTCGGCAAGATGAACAAGCTGATTAAGACTGTTTTCGCCTTCTGTTCT<br>ATGCTGTGTTTGTTGAACTCTACTGTCAACCCAATTATCTACGCCTTGAGATCTAAGGATTTGAGACA<br>TGCCTTTAGGTCTATGTTCCCATCTTGTGAAGGTACTGCTCAACCATTGGATAATTCTATGGGTGATT<br>CTGACTGCTTGCACAAACATGCTAACAATGCTGCTTCAGTTCATAGAGCTGCTGAATCTTGTATTAAG<br>TCCACCGTTAAGATTGCCAAGGTTACCATGTCTGTTTCTACTGATACTTCTGCTGAAGCCTTGTAACT<br>CGAG**TGGC**TGAGACC |
| **pWS2693**<br><br>**CB2R** | GGTCTCATATGGAAGAGTGTTGGGTTACTGAAATTGCCAACGGTTCTAAAGACGGTTTGGATTCTAA<br>TCCCATGAAGGACTACATGATTTTGTCTGGTCCACAAAAAACTGCTGTTGCTGTTTTGTGTACTTTGT<br>TGGGTTTGTTGTCTGCCTTGGAAAACGTTGCTGTCCTGTACTTGATTCTGTCCTCTCATCAATTGAGA<br>AGAAAGCCCTCTTACTTGTTCATTGGTTCTTTGGCTGGTGCTGATTTTTTGGCTTCTGTTGTTTTTGCT<br>TGCTCCTTCGTTAACTTCCATGTTTTCCATGGTGTTGATTCCAAGGCTGTTTTCTTGTTGAAGATTGG<br>TTCTGTCACTATGACTTTCACTGCTTCTGTAGGTTCTTTGTTGTTGACCGCTATTGATAGATACCTGT<br>GTTTGAGATATCCACCATCTTACAAGGCTTTGTTGACTAGAGGTAGAGCTTTGGTTACTTTGGGTATT<br>ATGTGGGTTTTGTCCGCTTTGGTTTCTTACTTGCCATTGATGGGTTGGACTTGTTGTCCAAGACCATG<br>TTCTGAATTATTCCCATTGATCCCAAACGACTACTTGTTGAGTTGGTTGTTGTTTATCGCCTTCTTGTT<br>CTCCGGTATTATCTACACTTATGGTCACGTTTTGTGGAAGGCTCATCAACACGTTGCTTCATTATCTG<br>GTCATCAAGATAGACAAGTTCCAGGTATGGCTAGAATGAGATTGGATGTTAGATTGGCTAAGACCTT<br>GGGTTTAGTTTTGGCCGTTTTGTTGATTTGTTGGTTCCCAGTTTTGGCTTTGATGGCTCATTCTTTAG<br>CTACTACCTTGTCCGATCAAGTTAAGAAGGCTTTTGCTTTCTGTTCCATGTTGTGCTTGATCAACTCT<br>ATGGTTAACCCAGTTATCTACGCTTTGAGATCCGGTGAAATTAGATCTTCTGCTCATCATTGCTTGGC<br>CCATTGGAAAAAATGTGTTAGAGGTTTGGGTTCCGAAGCAAAAGAAGAAGCTCCAAGATCTTCAGTT<br>ACAGAAACTGAAGCTGATGGTAAGATTACTCCATGGCCAGATTCTAGAGATTTGGATTTGTCTGATTG<br>TTAACTCGAG**TGGC**TGAGACC |
| **pWS2694**<br><br>**GPR55** | GGTCTCATATGTCTCAACAAAACACTTCTGGTGATTGCTTGTTCGACGGTGTTAACGAATTGATGAAA<br>ACCTTGCAATTCGCCGTTCATATTCCAACTTTTGTCTTGGGTTTGCTGTTGAACTTGTTGGCTATTCA<br>CGGTTTCTCCACGTTCTTGAAGAATAGATGGCCAGATTATGCTGCTACCTCTATCTACATGATTAACT<br>TGGCTGTTTTCGACCTGTTGTTGGTTTTGTCTTTGCCATTCAAAATGGTCTTGTCCCAAGTCCAATCT<br>CCATTTCCATCTTTGTGTACTTTGGTCGAATGCCTGTACTTCGTTTCTATGTACGGTTCTGTTTTCACC<br>ATCTGCTTCATCTCTATGGATAGATTCTTGGCCATTAGATACCCACTGTTGGTTTCTCATTTGAGATC<br>CCCAAGAAAGATTTTCGGTATTTGTTGCACCATCTGGGTTTTAGTTTGGACTGGTTCTATTCCCATCT<br>ACTCATTTCATGGTAAGGTCGAGAAGTACATGTGCTTCCATAATATGTCTGATGATACCTGGTCTGCC<br>AAGGTGTTTTTTCCATTGGAAGTTTTCGGTTTCTTGCTGCCAATGGGTATTATGGGTTTTTGTTGCTC<br>CAGATCCATCCATATTTTGTTGGGTAGAAGAGATCATACCCAAGATTGGGTTCAACAAAAGGCTTGC<br>ATATACTCTATTGCTGCTTCTTTGGCTGTGTTCGTTGTTTCATTTTTGCCAGTTCACTTGGGCTTTTTC<br>TTGCAATTTTTGGTGAGGAACTCCTTCATCGTTGAATGTAGAGCTAAGCAGTCTATCTCCTTCTTCTT<br>GCAACTGTCTATGTGCTTCTCTAACGTTAACTGTTGCTTGGATGTTTTCTGCTACTACTTCGTCATCA<br>AAGAATTCAGGATGAACATCAGAGCCCATAGACCATCTAGAGTTCAATTGGTTTTACAAGACACCAC<br>CATCTCTAGAGGTTAACTCGAG**TGGC**TGAGACC |

| | |
|---|---|
| **pWS2695**<br><br>**GPR18** | GGTCTCATATGATTACCTTGAACAATCAAGATCAGCCCGTTCCATTCAATTCTTCTCATCCAGACGAG<br>TACAAGATTGCTGCTTTGGTTTTCTACTCCTGCATTTTCATTATCGGCTTGTTCGTTAACATTACCGCT<br>TTGTGGGTTTTCTCTTGTACCACCAAAAAGAGAACTACCGTTACCATCTACATGATGAATGTTGCCTT<br>GGTTGACCTGATTTTCATCATGACTTTGCCATTCAGGATGTTCTACTACGCTAAAGATGAATGGCCTT<br>TCGGTGAATACTTCTGCCAAATTTTGGGTGCTTTGACTGTTTTCTACCCATCTATTGCTTTGTGGTTG<br>TTGGCTTTCATTTCTGCTGATAGATATATGGCCATCGTTCAACCTAAATACGCCAAAGAGTTGAAGAA<br>CACTTGTAAGGCTGTTTTGGCTTGTGTTGGTGTTTGGATTATGACTTTGACTACTACTACCCCTCTGC<br>TGTTGTTGTACAAAGATCCAGATAAGGATTCTACTCCAGCTACCTGTTTGAAGATTTCCGATATTATC<br>TACTTGAAGGCCGTTAACGTTTTGAACTTGACTAGATTGACCTTCTTCTTCTTGATCCCCTTGTTCATT<br>ATGATCGGTTGCTACTTGGTTATCATCCACAATTTGTTGCATGGTAGGACCTCTAAATTGAAGCCAAA<br>GGTCAAAGAAAAGTCCATCAGAATCATCATCACCTTGTTGGTTCAAGTTTTGGTTTGCTTCATGCCAT<br>TCCATATTTGCTTCGCTTTCTTGATGTTAGGTACTGGTGAAAACTCTTACAATCCATGGGGTGCTTTT<br>ACTACCTTCTTGATGAATTTGTCTACCTGCTTGGATGTCATCCTGTACTACATCGTTTCTAAGCAATTC<br>CAAGCCAGAGTTATCTCCGTTATGTTGTACAGAAACTACCTGAGGTCTATGCGTAGAAAGTCTTTTAG<br>ATCAGGTTCCTTGAGGTCCTTGTCCAACATTAACTCTGAAATGCTGTAACTCGAG**TGGC**TGAGACC |
| **pWS2696**<br><br>**GPR119** | GGTCTCATATGGAATCTTCATTTTCTTTCGGTGTTATCTTGGCTGTTTTGGCCTCTTTGATTATTGCTA<br>CCAATACCTTGGTTGCTGTTGCCGTTTTGTTGTTGATTCATAAGAACGACGGTGTCTCTTTGTGCTTC<br>ACTTTGAATTTGGCTGTTGCTGATACCTTGATCGGTGTTGCTATTTCTGGTTTGTTGACCGATCAATT<br>GTCATCTCCATCAAGACCAACTCAAAAGACCTTGTGTTCTTTGAGAATGGCTTTCGTTACTTCTTCAG<br>CTGCTGCTTCTGTTTTGACCGTTATGTTGATTACCTTCGATAGATACTTGGCCATCAAGCAACCATTC<br>AGATACTTGAAGATTATGTCCGGTTTTGTTGCTGGTGCTTGTATTGCTGGTTTATGGTTGGTTTCTTA<br>CTTGATTGGCTTTTTGCCATTGGGTATTCCAATGTTCCAACAAACTGCTTACAAGGGTCAATGTTCTT<br>TCTTCGCTGTTTTCCATCCACATTTCGTTTTGACCTTGTCTTGCGTTGGTTTTTTTCCAGCTATGCTGT<br>TGTTCGTTTTCTTCTACTGTGACATGTTGAAGATCGCCTCTATGCATTCCCAACAGATTAGAAAAATG<br>GAACATGCTGGTGCAATGGCTGGTGGTTATAGATCACCAAGAACTCCATCTGATTTCAAGGCTTTGA<br>GAACTGTTTCAGTGTTGATTGGTTCTTTCGCTTTGTCTTGGACTCCATTCTTGATTACTGGTATCGTTC<br>AAGTTGCTTGTCAAGAATGTCACTTGTACTTGGTCTTGGAAAGATACTTGTGGTTGTTAGGTGTTGGC<br>AACTCTTTGTTGAATCCATTGATCTATGCCTACTGGCAGAAAGAAGTCAGATTGCAGTTGTATCATAT<br>GGCCTTGGGTGTTAAGAAGGTCTTGACATCTTTCCTGTTGTTCTTGTCTGCTAGAAATTGTGGTCCA<br>GAAAGACCAAGAGAATCTTCTTGTCATATCGTCACCATCTCCAGCTCTGAATTTGATGGTTAACTCGA<br>G**TGGC**TGAGACC |

**Supplementary Note 1. Gating strategy for flow cytometry.**

Yeast cells were gated for singlets using FSC-H vs FSC-A and to remove background noise. No other Gating was performed on global yeast population. > 10,000 events were collected and analysed within the singlets gate for each measurement.