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SUPPLEMENTARY INFORMATION

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Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

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Strain construction. DNA integrating sequences were constructed using a previously described method.[1] Manufacturer protocols and standard recombinant DNA procedures were followed for DNA purification (Qiagen), DNA amplification (New England Biolabs, Q5® High-Fidelity 2X Master Mix). All primers were designed using CASdesigner (https://casdesigner.jbei.org/). Briefly, DNA fragments to be integrated were PCR-amplified then co-transformed with a Cas9-based plasmid facilitating integration at the targeted locus. For transformations, a fresh overnight culture of target yeast was inoculated into 25 mL 2x-YPD in 250 mL shake flask at OD_{600nm} 0.2 and was incubated at 30°C and 200 RPM until the OD_{600nm} reached 1.0. Then, 5 mL of the culture was harvested by centrifugation for 5 min at 3000 x g, washed twice with half volume H_2O. The pellet was then resuspended with DNA fragments for integration (2 µg) and pCUT plasmid (0.25 µg) and then mixed with transformation mix (260 µL 50% PEG3350, 36 µL 1 M LiOAc, 10 µL ssDNA, 4 µL H_2O). The mixture was incubated at 42°C for 40 min and the pellet was collected by centrifugation for 1 min at 5000 x g. The pellets were then resuspended with 500 µL H_2O from which 100 µL was plated onto selective agar plates. The integration was validated by sequencing and the correct colony was used for downstream experiments after plasmid curing.

NphB expression, purification and *in vitro* assay. eCAN01 was grown in 2000 mL shake flasks containing 500 mL terrific broth medium and Carbenicillin at 37°C. Cultures were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside at OD_{600nm} 0.8 and incubated at 18°C overnight. Harvested cell pellets were resuspended in 20 mL lysis buffer (50 mM

NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 20 mM β-mercaptoethanol, 0.01% (v/v) Lyonase (Merck KGaA), Protease Inhibitor Cocktail V (Calbiochem, Merck KGaA), pH 8.0) and sonicated. Lysates were centrifuged (19,000 x g, 4°C, 20 min) and supernatant subjected to nickel-nitrilotriacetitc (Ni-NTA) acid 6xHis-tag mediated affinity chromatography (5 mL Ni-NTA resin = 1 column volume (cv)). Columns were equilibrated with 10 cvs equilibration buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 20 mM β-mercaptoethanol, pH 8.0). After loading, columns were washed with 30 cvs wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 50 mM imidazole, 20 mM beta-mercaptoethanol, pH 8.0). 2 cvs of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 300 mM imidazole, 20 mM β-mercaptoethanol, pH 8.0) were used to elute protein. Eluate fractions were concentrated with centrifuge filter units (molecular weight cutoff: 10 kDa, Amicon Ultra, Merck KGaA). Subsequently, 10% (v/v) glycerol was added. *In vitro* assays containing 5 mM GPP, 5 mM OA, 2.5 mM $MgCl_2$ and 1 mg/mL NphB were incubated for 24 h at room temperature and subsequently extracted as described above.

HIPT *in vitro* assay. yCAN21, yCAN22 and yCAN23 microsomes were prepared as described above, except cells were grown for 48 h in complete supplement mixture synthetic dropout medium (-URA) with 2% galactose and the resulting microsome pellets were resuspended in 4.5 mL buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM β-mercaptoethanol, pH 7.0). *In vitro* assays, containing 5 mM GPP and 5 mM OA, were incubated for 24 h at 30°C and subsequently extracted as described above.

REFERENCES

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