Active-site loop variations adjust activity and selectivity of the cumene dioxygenase

Supplementary Information

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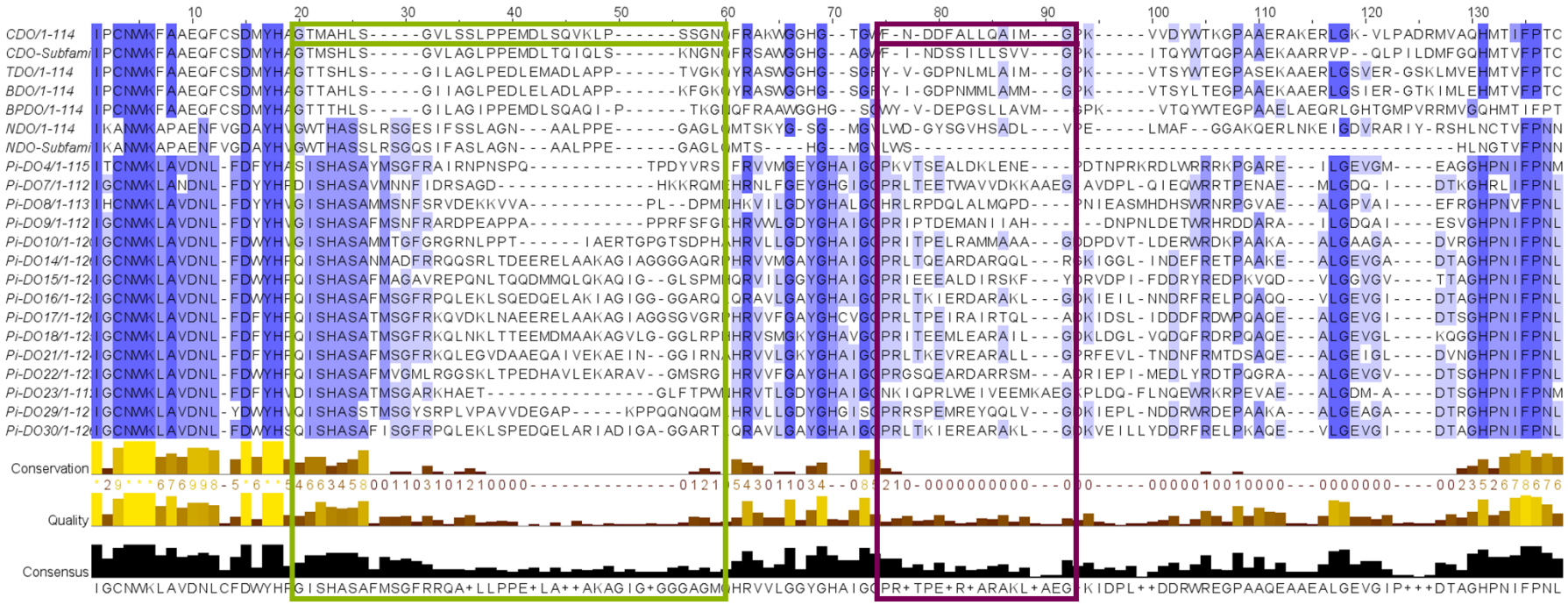
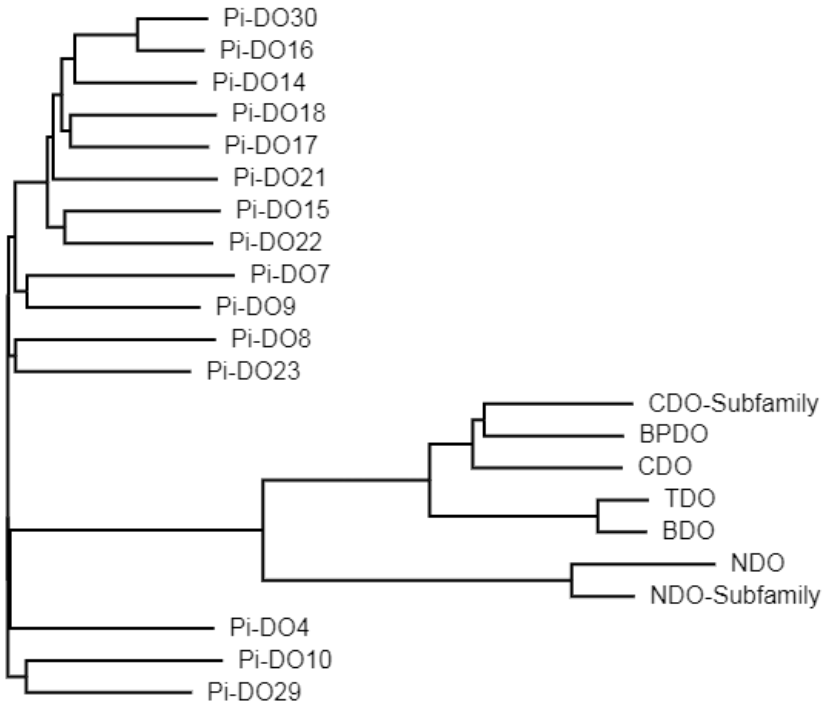
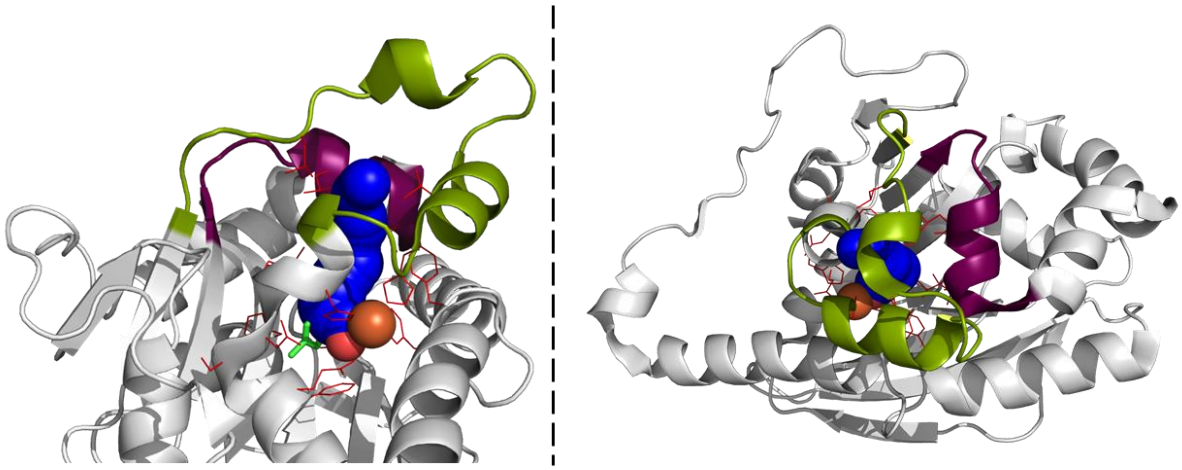


Supplementary Fig. 1: Excerpt of the sequence alignment of α-subunits of the CDO from *Pseudomonas fluorescens* IP01 with the CDO subfamily, biphenyl dioxygenase (BPDO) from *Paraburkholderia xenovorans* (LB400), toluene dioxygenase (TDO) from *Pseudomonas putida* F1, benzene dioxygenase (BDO) from *Pseudomonas putida* ML2, naphthalene dioxygenase (NDO) from *Pseudomonas* sp. NCIB 9816-4, the NDO subfamily and 15 α-subunits from the genome of *Phenylobacterium immobile* E (Pi-DO). The degree of conservation is shown from low (white) to high (blue) as well as in the conservation line. The alignment quality, consensus sequence and occupancy are shown in the bottom lines. Loop 1 (green), loop 2 (purple) and the corresponding residues of the other ROs are highlighted by frames in the corresponding colors.



Supplementary Fig. 2: Phylogenetic tree of the aligned α-subunits of the oxygenases of CDO, CDO subfamily, BPDO, TDO, BDO, NDO, NDO-subfamily and from *Phenylobacterium immobile* E (Pi-DO).

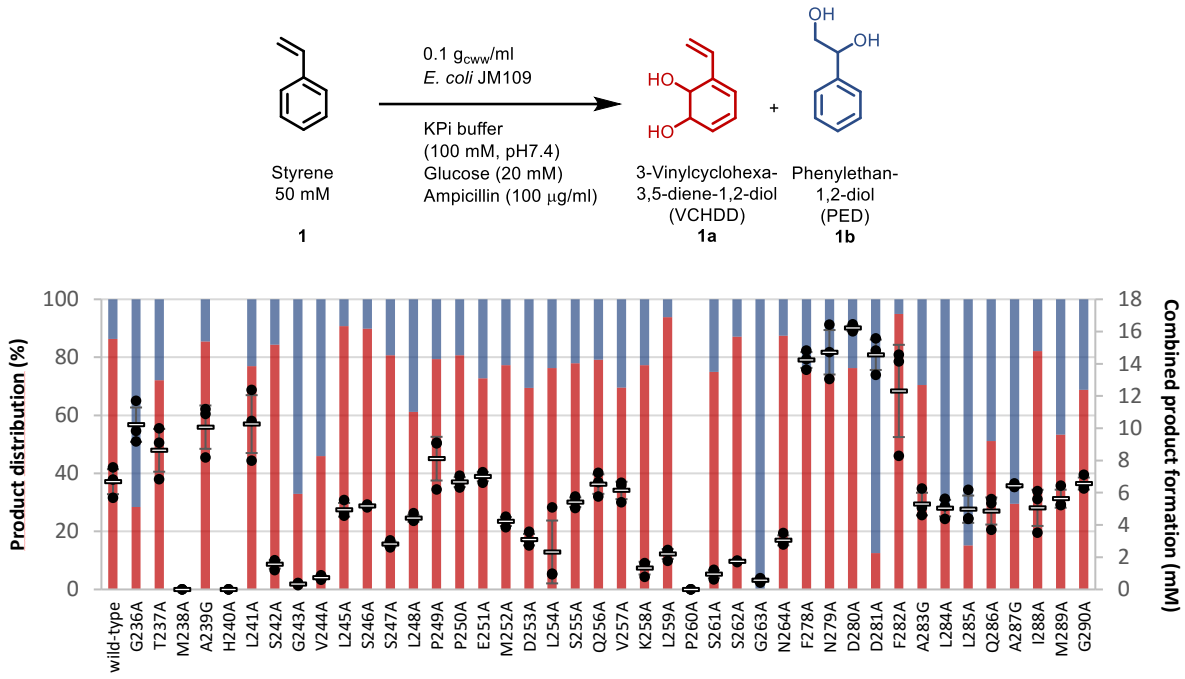


Supplementary Fig. 3: CAVER analysis of the crystal structure of the α-subunit of the CDO oxygenase from *Pseudomonas fluorescens* IP01 (PDB entry 1wql). Side view (left structure) and top view (right structure) of loop 1 (green) and loop 2 (purple) in the crystal structure of one α-subunit (grey) of the CDO oxygenase (PDB entry 1wql). The simulated tunnel (blue), the catalytically active iron (orange sphere), molecular oxygen (red spheres) and active site residues (red sticks) are shown as well as the docked substrate *R*-limonene (green sticks). Iron was selected as starting point with a tunnel radius of 1.2 Å.

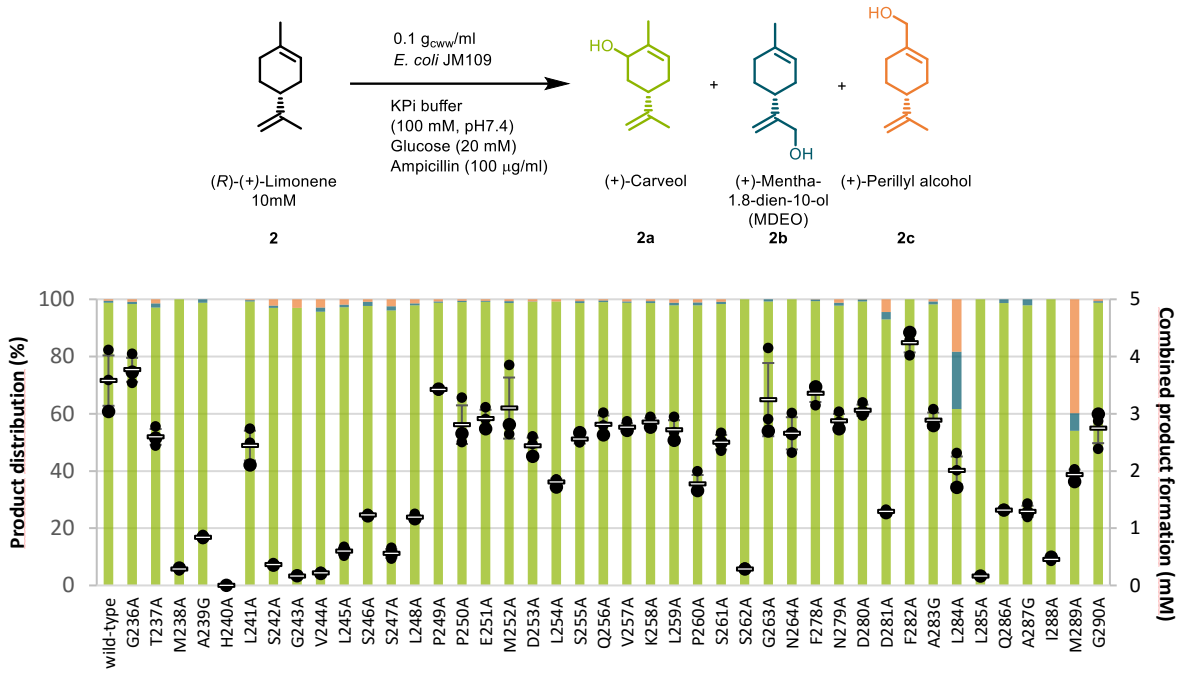
Supplementary Table 1: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the Wild-type and the variants obtained from the alanine scan of the CDO. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.56 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
G236A	10.22 ± 1.07	28.4	71.6	3.77 ± 0.21	98.3	0.9	0.8	0.87 ± 0.03	91.7	8.3
T237A	8.63 ± 1.32	72.1	27.9	2.60 ± 0.14	97.1	1.4	1.5	0.90 ± 0.02	94.9	5.1
M238A	0 ± 0	0.0	0.0	0.29 ± 0.02	100.0	0.0	0.0	0 ± 0	0.0	0.0
A239G	10.07 ± 1.35	85.5	14.5	0.84 ± 0.03	98.8	1.2	0.0	0.13 ± 0.01	89.2	10.8
H240A	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
L241A	10.26 ± 1.80	77.0	23.0	2.44 ± 0.26	99.3	0.4	0.4	1.41 ± 0.08	92.9	7.1
S242A	1.56 ± 0.26	84.4	15.6	0.37 ± 0.01	97.0	0.8	2.2	0.15 ± 0.01	92.3	7.7
G243A	0.33 ± 0.06	32.9	67.1	0.17 ± 0.00	97.0	0.0	3.0	0.07 ± 0.00	100.0	0.0
V244A	0.73 ± 0.11	45.9	54.1	0.22 ± 0.01	95.7	1.4	2.9	0.09 ± 0.01	89.9	10.1
L245A	4.94 ± 0.43	90.7	9.3	0.60 ± 0.06	97.3	0.9	1.9	0.16 ± 0.01	93.5	6.5
S246A	5.17 ± 0.07	89.9	10.1	1.23 ± 0.02	97.6	1.5	0.9	0.17 ± 0.01	93.7	6.3
S247A	2.81 ± 0.17	80.7	19.3	0.56 ± 0.08	96.1	1.5	2.4	0.15 ± 0.00	93.6	6.4
L248A	4.42 ± 0.21	61.1	38.9	1.19 ± 0.04	97.9	0.6	1.5	0.69 ± 0.06	92.7	7.3
P249A	8.11 ± 1.36	79.4	20.6	3.42 ± 0.02	98.8	0.5	0.7	0.79 ± 0.00	93.4	6.6
P250A	6.66 ± 0.30	80.8	19.2	2.81 ± 0.34	99.0	0.4	0.5	0.89 ± 0.02	93.1	6.9
E251A	7.00 ± 0.29	72.7	27.3	2.92 ± 0.15	99.2	0.3	0.6	0.68 ± 0.02	93.1	6.9
M252A	4.22 ± 0.28	77.3	22.7	3.10 ± 0.53	98.7	0.7	0.7	0.66 ± 0.02	93.2	6.8
D253A	3.09 ± 0.35	69.5	30.5	2.44 ± 0.14	99.1	0.2	0.7	0.41 ± 0.03	95.4	4.6
L254A	2.32 ± 1.95	76.3	23.7	1.81 ± 0.06	99.2	0.0	0.8	0.57 ± 0.04	95.1	4.9

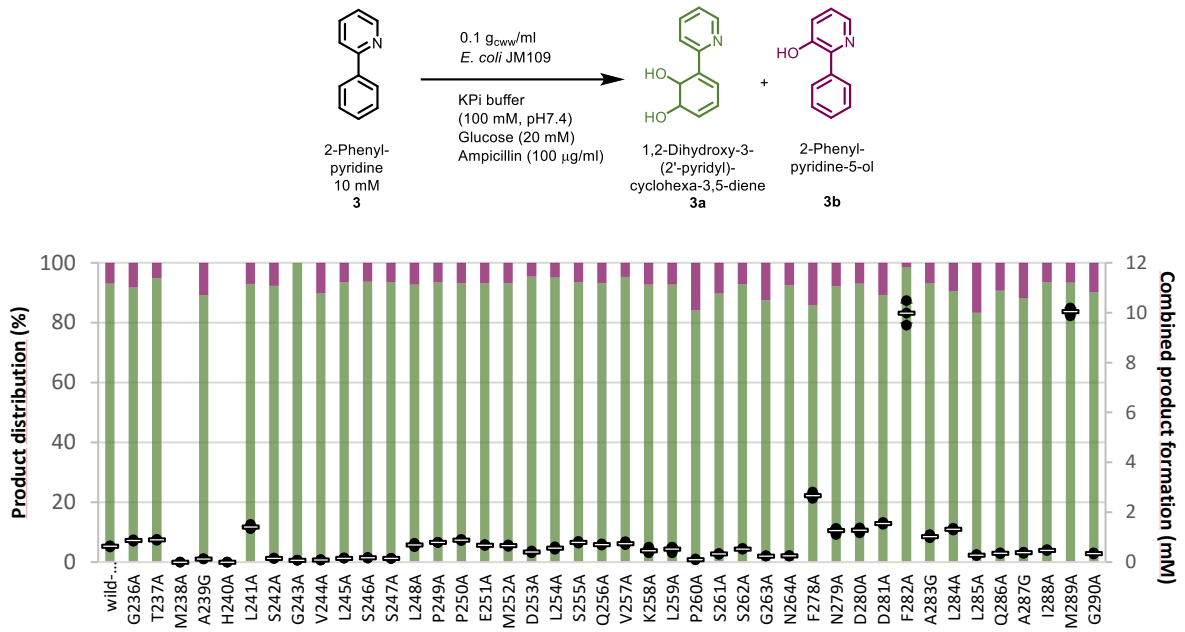
S255A	5.41 ± 0.29	77.9	22.1	2.56 ± 0.08	98.7	0.6	0.6	0.80 ± 0.03	93.5	6.5
Q256A	6.53 ± 0.60	79.1	20.9	2.81 ± 0.16	99.0	0.6	0.4	0.71 ± 0.02	93.2	6.8
V257A	6.14 ± 0.54	69.6	30.4	2.77 ± 0.07	98.8	0.4	0.8	0.74 ± 0.05	95.3	4.7
K258A	1.32 ± 0.40	77.3	22.7	2.85 ± 0.07	98.7	0.6	0.7	0.46 ± 0.10	92.7	7.3
L259A	2.20 ± 0.31	93.8	6.2	2.72 ± 0.17	97.9	0.8	1.2	0.53 ± 0.11	92.8	7.2
P260A	0 ± 0	0.0	0.0	1.78 ± 0.15	97.9	1.0	1.1	0.11 ± 0.01	84.0	16.0
S261A	0.94 ± 0.25	74.9	25.1	2.50 ± 0.13	98.3	0.8	0.9	0.33 ± 0.02	89.7	10.3
S262A	1.73 ± 0.04	87.2	12.8	0.29 ± 0.01	100.0	0.0	0.0	0.53 ± 0.01	92.9	7.1
G263A	0.56 ± 0.11	0.0	100.0	3.25 ± 0.64	99.3	0.7	0.0	0.25 ± 0.00	87.5	12.5
N264A	3.04 ± 0.31	87.4	12.6	2.66 ± 0.28	100.0	0.0	0.0	0.25 ± 0.01	92.5	7.5
F278A	14.24 ± 0.49	75.8	24.2	3.36 ± 0.15	99.3	0.5	0.1	2.67 ± 0.11	85.8	14.2
N279A	14.71 ± 1.38	73.7	26.3	2.88 ± 0.12	97.8	1.0	1.2	1.26 ± 0.11	92.2	7.8
D280A	16.21 ± 0.18	76.3	23.7	3.06 ± 0.10	99.2	0.7	0.1	1.28 ± 0.09	93.1	6.9
D281A	14.56 ± 0.94	12.6	87.4	1.29 ± 0.02	93.0	2.6	4.4	1.56 ± 0.03	89.2	10.8
F282A	12.31 ± 2.86	95.0	5.0	4.24 ± 0.17	100.0	0.0	0.0	9.99 ± 0.40	98.6	1.4
A283G	5.30 ± 0.70	70.5	29.5	2.89 ± 0.13	98.2	0.9	0.8	1.02 ± 0.06	93.1	6.9
L284A	5.04 ± 0.51	27.9	72.1	2.01 ± 0.24	61.6	20.1	18.3	1.32 ± 0.04	90.5	9.5
L285A	4.98 ± 0.85	15.2	84.8	0.16 ± 0.00	100.0	0.0	0.0	0.28 ± 0.02	83.3	16.7
Q286A	4.87 ± 0.84	51.1	48.9	1.32 ± 0.01	98.7	1.3	0.0	0.36 ± 0.02	90.7	9.3
A287G	6.42 ± 0.11	29.5	70.5	1.29 ± 0.10	97.8	2.2	0.0	0.38 ± 0.02	88.2	11.8
I288A	5.06 ± 1.12	82.2	17.8	0.45 ± 0.03	100.0	0.0	0.0	0.48 ± 0.01	93.5	6.5
M289A	5.63 ± 0.56	53.3	46.7	1.94 ± 0.09	54.1	6.2	39.8	10.05 ± 0.13	93.3	6.7
G290A	6.57 ± 0.37	68.8	31.2	2.75 ± 0.26	98.8	0.6	0.6	0.35 ± 0.02	90.1	9.9



Supplementary Fig. 4: Product distribution and formation of the two products 3-vinylcyclohexa-3,5-diene-1,2-diol 1a (VCHDD, red, bottom bars) and phenylethan-1,2-diol 1b (PED, blue, top bars) for the biotransformation of styrene with the wild-type and variants obtained from the alanine scan of loop 1 and 2 of the CDO. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.



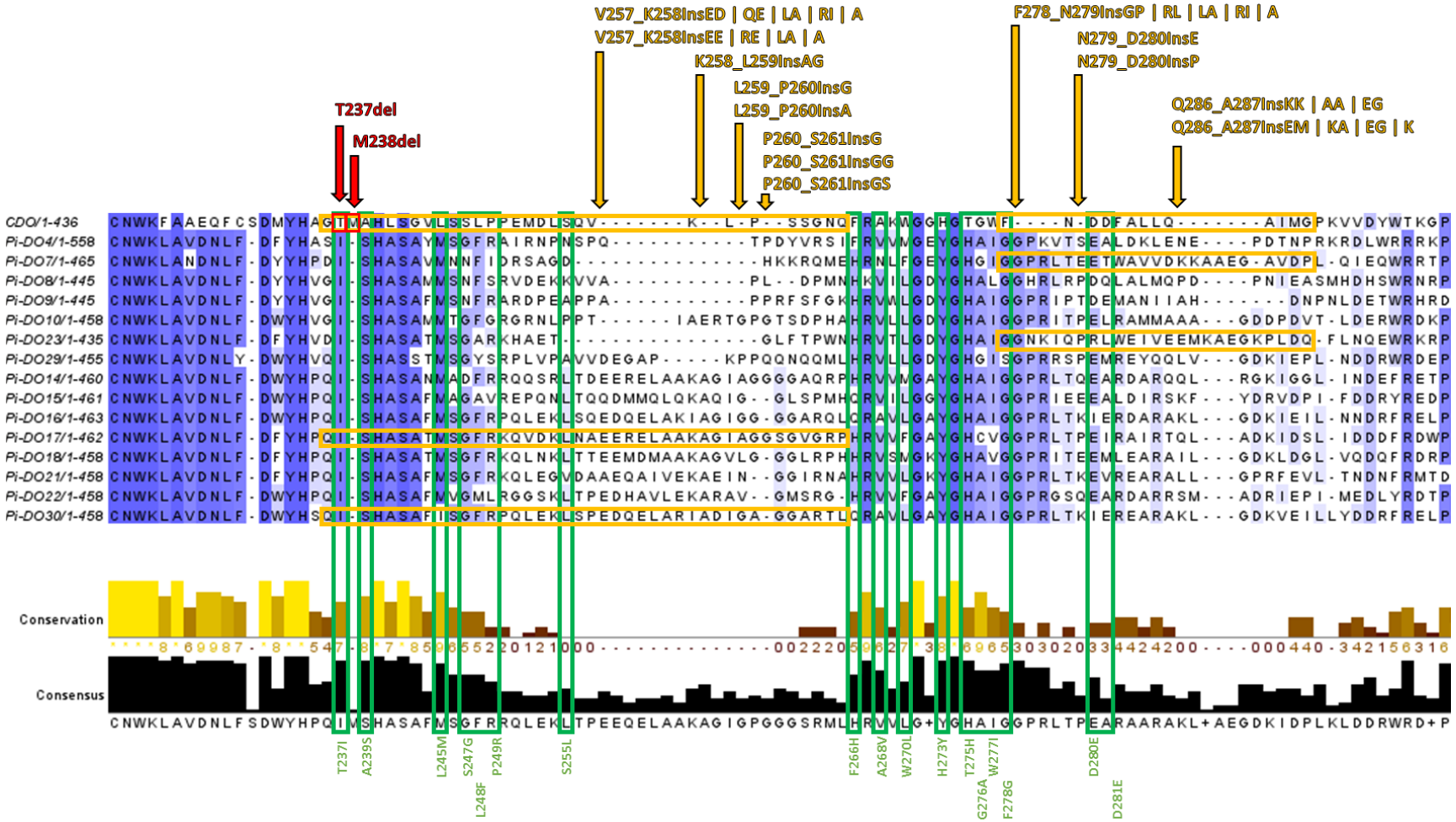
Supplementary Fig. 5: Product distribution and formation of the products (+)-carveol 2a (green, bottom bars), (+)-mentha-1.8-dien-10-ol 2b (MDEO, blue, middle bars) and (+)-perillyl alcohol 2c (orange, top bars) for the biotransformation of (*R*)-limonene 2 with the wild-type and variants obtained from the alanine scan of loop 1 and 2 of the CDO. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.



Supplementary Fig. 6: Product distribution of the products 1,2-dihydroxy-3-(2'-pyridyl)cyclohexa-3,5-diene 3a (green, bottom bars) and 2-phenylpyridine-5-ol 3b (purple, top bars) for the biotransformation of 2-phenylpyridine 3 with the wild-type and variants obtained from the alanine scan of loop 1 and 2 of the CDO. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.

Supplementary Table 2: Conversion of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the Wild-type and selected saturation variants. Biotransformations were performed in technical triplicates and standard deviation (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	**Selectivity (%)**		**Pf (mM)**	**Selectivity (%)**			**Pf (mM)**	**Selectivity (%)**	
		1a	**1b**		**2a**	**2b**	**2c**		**3a**	**3b**
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
F278V	0.24 ± 0.07	100.0	0.0	5.31 ± 0.79	100.0	0.0	0.0	1.57 ± 0.04	94.1	5.9
F282V	1.31 ± 0.06	69.4	30.6	5.15 ± 0.28	100.0	0.0	0.0	1.28 ± 0.02	97.0	3.0
F282T	0.05 ± 0.00	100.0	0.0	2.78 ± 0.24	100.0	0.0	0.0	9.82 ± 0.20	97.3	2.7
L284G	0.31 ± 0.08	84.7	15.3	2.08 ± 0.19	30.8	19.4	49.7	0.74 ± 0.04	91.4	8.6
Q286F	0.65 ± 0.05	67.5	32.5	1.86 ± 0.16	100.0	0.0	0.0	0.40 ± 0.05	93.8	6.2
I288S	0.02 ± 0.03	41.6	58.4	1.31 ± 0.41	69.9	3.5	26.5	1.11 ± 0.03	93.3	6.7
I288T	0.10 ± 0.01	73.9	26.1	2.14 ± 0.20	96.4	0.1	3.4	1.96 ± 0.02	93.6	6.4
M289K	0.09 ± 0.01	0.0	100.0	0.06 ± 0.00	100.0	0.0	0.0	0.15 ± 0.01	86.7	13.3



Supplementary Fig. 7: Excerpt of the sequence alignment of CDO with α-subunits of the oxygenases from *Phenylobacterium immobile* E and variant selection for the adaption library. Single point mutations are indicated in green, single point deletions in red. Introduced sequence-alignment based insertions are highlighted in yellow, the loops 1 and 2 of the CDO together with the longest corresponding loops from oxygenases originated from *Phenylobacterium immobile* E are framed in yellow. Slashes in the variant designation represent stepwise insertions

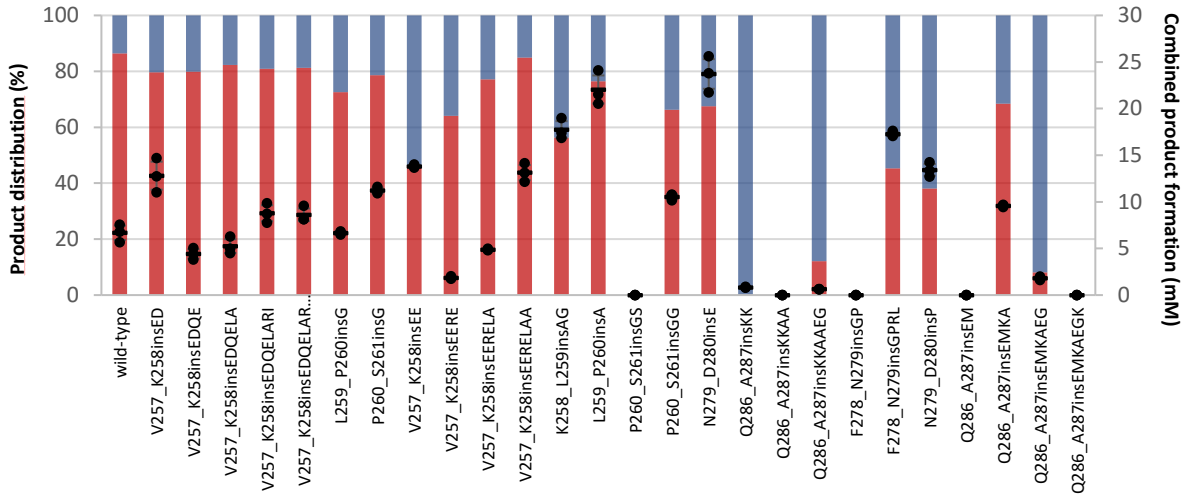
Supplementary Table 3: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type, and deletion and single-point mutation variants based on loops in oxygenases from *Phenylobacterium immobile* E. Biotransformations were performed in technical triplicates and standard deviation (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
T237del	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
M238del	0 ± 0	0.0	0.0	0.12 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
T237I	7.99 ± 0.13	78.5	21.5	0.44 ± 0.03	100.0	0.0	0.0	0.06 ± 0.01	100.0	0.0
A239S	6.89 ± 0.21	69.8	30.2	0.86 ± 0.02	97.4	0.8	1.8	0.55 ± 0.02	91.0	9.0
L245M	0 ± 0	0.0	0.0	0.10 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
S247G	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
L248F	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
P249R	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
S255L	5.35 ± 0.10	71.8	28.2	0.58 ± 0.02	96.7	2.3	1.0	0.05 ± 0.01	81.4	18.6
F266H	8.14 ± 0.42	75.8	24.2	1.38 ± 0.02	98.0	0.9	1.1	0.11 ± 0.01	86.6	13.4
A268V	4.18 ± 0.07	73.6	26.4	0.84 ± 0.06	97.4	1.9	0.7	0 ± 0	0.0	0.0
W270L	2.52 ± 0.12	84.4	15.6	2.74 ± 0.41	99.1	0.4	0.5	0.22 ± 0.02	92.4	7.6
H273Y	0 ± 0	0.0	0.0	0.14 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
T275H	1.46 ± 0.07	84.4	15.6	1.39 ± 0.02	99.2	0.4	0.5	0.51 ± 0.01	95.4	4.6
G276A	0 ± 0	0.0	0.0	1.22 ± 0.06	100.0	0.0	0.0	0.19 ± 0.01	92.1	7.9
W277I	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
F278G	0.93 ± 0.13	75.5	24.5	2.12 ± 0.05	96.7	0.9	2.4	0.49 ± 0.02	86.2	13.8
D280E	2.24 ± 0.04	89.1	10.9	3.21 ± 0.18	99.5	0.5	0.0	0.37 ± 0.01	93.4	6.6
D281E	14.25 ± 0.49	55.9	44.1	0.64 ± 0.03	100.0	0.0	0.0	0.07 ± 0.01	90.9	9.1

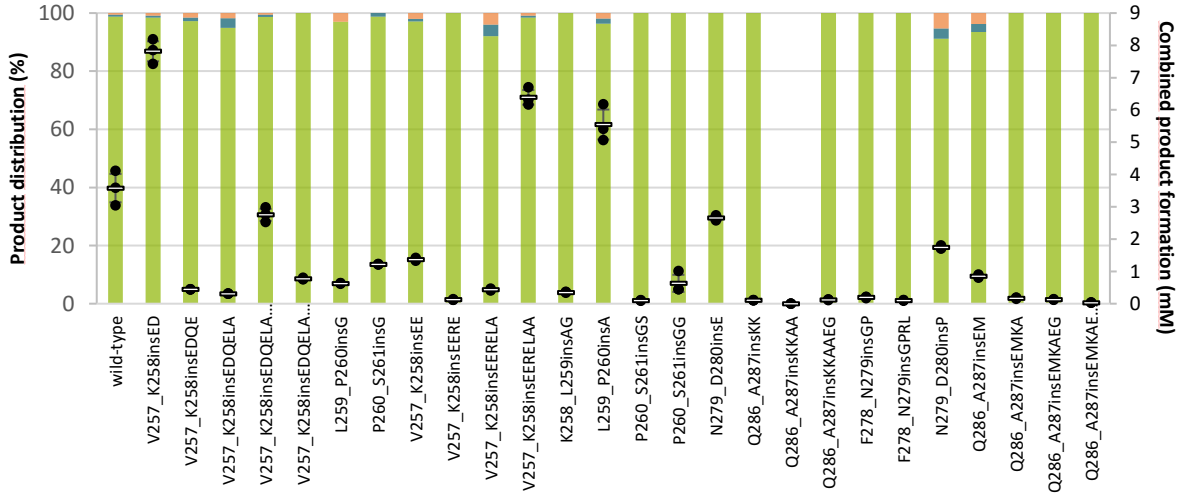
Supplementary Table 4: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type and insertion variants based on loops in oxygenases from *Phenylobacterium immobile* E. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
V257_K258 insED	12.81 ± 1.50	79.7	20.3	7.81 ± 0.31	98.4	0.7	0.9	0.64 ± 0.05	92.8	7.2
V257_K258 insEDQE	4.42 ± 0.50	79.9	20.1	0.44 ± 0.01	97.3	1.2	1.5	0.08 ± 0.01	89.8	10.2
V257_K258 insEDQELA	5.25 ± 0.74	82.3	17.7	0.314 ± 0.00	95.0	3.2	1.8	0.09 ± 0.01	90.1	9.9
V257_K258 insEDQELARI	8.78293 ± 0.86	81.0	19.0	2.75 ± 0.19	98.6	0.8	0.6	0.42 ± 0.02	94.9	5.1
V257_K258 insEDQELARIA	8.61 ± 0.68	81.3	18.7	0.77 ± 0.02	100.0	0.0	0.0	0.38 ± 0.12	94.7	5.3
L259_P260 insG	6.68 ± 0.15	72.6	27.4	0.62 ± 0.01	97.0	0.0	3.0	0.12 ± 0.00	91.2	8.8
P260_S261 insG	11.23 ± 0.29	78.6	21.4	1.22 ± 0.01	98.7	1.3	0.0	0.10 ± 0.01	92.5	7.5
V257_K258 insEE	13.81 ± 0.15	46.5	53.5	1.37 ± 0.04	97.2	0.9	2.0	0.10 ± 0.01	91.7	8.3
V257_K258 insEERE	1.86 ± 0.11	64.0	36.0	0.12 ± 0.01	100.0	0.0	0.0	0 ± 0	0.0	0.0
V257_K258 insEERELA	4.88 ± 0.07	77.2	22.8	0.43 ± 0.03	92.1	3.9	4.0	0.12 ± 0.01	90.2	9.8
V257_K258 insEERELAA	13.14 ± 0.82	84.9	15.1	6.39 ± 0.23	98.4	0.7	1.0	1.97 ± 0.13	96.2	3.8
K258_L259 insAG	17.75 ± 0.91	56.3	43.7	0.35 ± 0.01	100.0	0.0	0.0	0.26 ± 0.01	91.7	8.3
L259_P260 insA	22.06 ± 1.51	76.4	23.6	5.55 ± 0.46	96.3	1.7	1.9	1.05 ± 0.07	92.5	7.5
P260_S261 insGS	0 ± 0	0.0	0.0	0.092 ± 0.01	100.0	0.0	0.0	0 ± 0	0.0	0.0
P260_S261 insGG	10.55 ± 0.27	66.2	33.8	0.63 ± 0.27	100.0	0.0	0.0	0.07 ± 0.01	92.3	7.7
N279_D280 insE	23.72 ± 1.59	67.5	32.5	2.65 ± 0.07	100.0	0.0	0.0	1.03 ± 0.10	91.9	8.1
Q286_A287 insKK	0.83 ± 0.05	0.0	100.0	0.10 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
Q286_A287 insKKAA	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
Q286_A287 insKKAAEG	0.64 ± 0.02	12.2	87.8	0.12 ± 0.01	100.0	0.0	0.0	0 ± 0	0.0	0.0

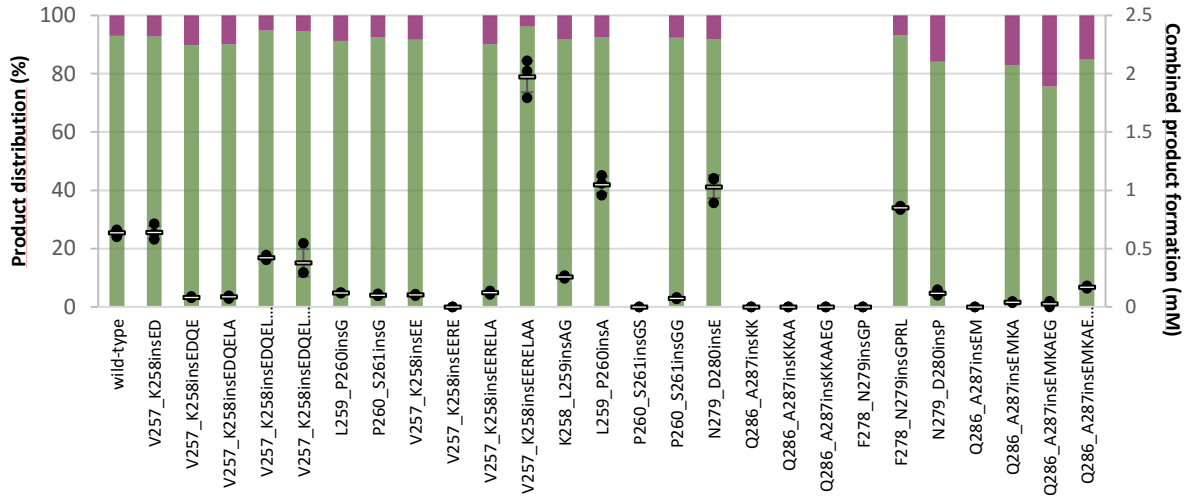
F278_N279 insGP	0 ± 0	0.0	0.0	0.19 ± 0.02	100.0	0.0	0.0	0 ± 0	0.0	0.0
F278_N279 insGPRL	17.28 ± 0.23	45.3	54.7	0.10 ± 0.01	100.0	0.0	0.0	0.85 ± 0.01	93.2	6.8
N279_D280 insP	13.437 ± 0.63	38.1	61.9	1.74 ± 0.04	91.2	3.6	5.3	0.12 ± 0.02	84.1	15.9
Q286_A287 insEM	0 ± 0	0.0	0.0	0.85 ± 0.04	93.5	2.7	3.8	0 ± 0	0.0	0.0
Q286_A287 insEMKA	9.59 ± 0.10	68.4	31.6	0.16 ± 0.02	100.0	0.0	0.0	0.04 ± 0.00	82.8	17.2
Q286_A287 insEMKAEG	1.81 ± 0.14	8.2	91.8	0.13 ± 0.01	100.0	0.0	0.0	0.03 ± 0.02	75.7	24.3
Q286_A287 insEMKAEGK	0 ± 0	0.0	0.0	0.03 ± 0.00	100.0	0.0	0.0	0.17 ± 0.01	84.8	15.2



Supplementary Fig. 8: Product distribution and formation of the two products 3-vinylcyclohexa-3,5-diene-1,2-diol 1a (VCHDD, red, bottom bars) and phenylethan-1,2-diol 1b (PED, blue, top bars) for the biotransformation of styrene with the wild-type and insertion variants based on loops in oxygenases from *Phenylobacterium immobile* E. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.



Supplementary Fig. 9: Product distribution and formation of the products (+)-carveol 2a (green, bottom bars), (+)-mentha-1.8-dien-10-ol 2b (MDEO, blue, middle bars) and (+)-perillyl alcohol 2c (orange, top bars) for the biotransformation of (*R*)-limonene 2 with the wild-type and insertion variants based on loops in oxygenases from *Phenylobacterium immobile* E. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.



Supplementary Fig. 10: Product distribution and formation of the products 1,2-dihydroxy-3-(2'-pyridyl)cyclohexa-3,5-diene 3a (green, bottom bars) and 2-phenylpyridine-5-ol 3b (purple, top bars) for the biotransformation of 2-phenylpyridine 3 with the wild-type and insertion variants based on loops in oxygenases from in *Phenylobacterium immobile* E. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Source data are provided as a Source Data file.

Supplementary Table 5: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type and deletion variants. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	**Selectivity (%)**		**Pf (mM)**	**Selectivity (%)**			**Pf (mM)**	**Selectivity (%)**	
		1a	**1b**		**2a**	**2b**	**2c**		**3a**	**3b**
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
E251del	13.14 ± 2.74	84.5	15.5	3.38 ± 0.21	97.2	1.3	1.5	2.18± 0.21	94.7	5.3
P250_M252 del	12.57 ± 2.20	82.4	17.6	2.01 ± 0.16	96.3	1.6	2.1	2.74 ± 0.10	94.2	5.8
L248_L254 del	0 ± 0	0.0	0.0	0.17 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
V257del	13.36 ± 0.54	77.1	22.9	2.57 ± 0.16	96.6	1.2	2.1	2.49 ± 0.049	93.6	6.4
Q256_K258 del	15.26 ± 0.56	44.1	55.9	3.68 ± 0.16	97.4	1.1	1.6	1.96 ± 0.04	91.5	8.5
L254_P260 del	0 ± 0	0.0	0.0	0.04 ± 0.00	100.0	0.0	0.0	0 ± 0	0.0	0.0
A283del	15.82 ± 1.99	89.2	10.8	8.92 ± 0.80	98.2	0.8	1.0	10.16 ± 0.47	98.7	1.3
F282_L284 del	19.93 ± 1.97	6.3	93.7	0.60 ± 0.02	100.0	0.0	0.0	3.68 ± 0.11	93.0	7.0
D280_Q286 del	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0

Supplementary Table 6: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type and insertion variants after V257 based on LILI. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

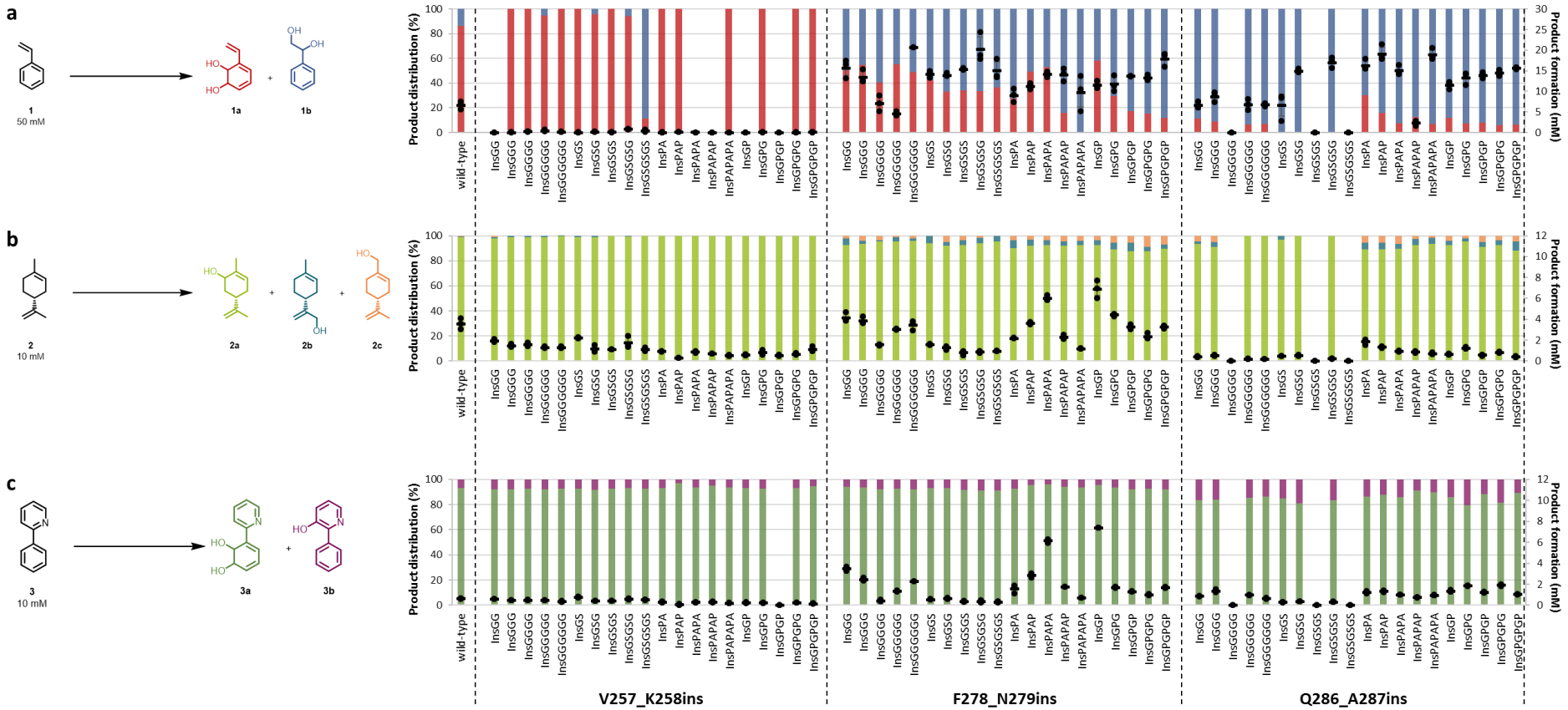
	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
V257_K258 insGG	0 ± 0	0.0	0.0	1.96 ± 0.11	97.6	1.2	1.2	0.61 ± 0.00	92.2	7.8
V257_K258 insGGG	0.07 ± 0.01	100.0	0.0	1.49 ± 0.11	98.8	1.2	0.0	0.48 ± 0.01	92.1	7.9
V257_K258 insGGGG	0.22 ± 0.07	100.0	0.0	1.61 ± 0.14	98.9	1.1	0.0	0.52 ± 0.02	92.5	7.5
V257_K258 insGGGGG	0.49 ± 0.22	94.8	5.2	1.32 ± 0.08	98.6	1.4	0.0	0.51 ± 0.01	92.1	7.9
V257_K258 insGGGGGG	0.17 ± 0.09	100.0	0.0	1.32 ± 0.08	99.8	0.2	0.0	0.37 ± 0.03	92.8	7.2
V257_K258 insGS	0.05 ± 0.02	100.0	0.0	2.22 ± 0.04	98.8	1.2	0.0	0.77 ± 0.05	92.6	7.4
V257_K258 insGSG	0.22 ± 0.10	95.6	4.4	1.19 ± 0.27	98.8	1.2	0.0	0.41 ± 0.01	91.6	8.4
V257_K258 insGSGS	0.09 ± 0.03	100.0	0.0	1.13 ± 0.01	100.0	0.0	0.0	0.43 ± 0.02	92.7	7.3
V257_K258 insGSGSG	0.87 ± 0.11	94.1	5.9	1.75 ± 0.47	99.4	0.6	0.0	0.58 ± 0.05	93.1	6.9
V257_K258 insGSGSGS	0.41 ± 0.29	11.3	88.7	1.126 ± 0.16	100.0	0.0	0.0	0.52 ± 0.03	92.5	7.5
V257_K258 insPA	0.03 ± 0.04	100.0	0.0	0.95 ± 0.04	100.0	0.0	0.0	0.31 ± 0.03	92.9	7.1
V257_K258 insPAP	0.10 ± 0.02	100.0	0.0	0.33 ± 0.02	100.0	0.0	0.0	0.08 ± 0.05	97.0	3.0
V257_K258 insPAPA	0 ± 0	0.0	0.0	0.88 ± 0.07	100.0	0.0	0.0	0.27 ± 0.02	93.7	6.3
V257_K258 insPAPAP	0 ± 0	0.0	0.0	0.73 ± 0.01	100.0	0.0	0.0	0.31 ± 0.04	94.9	5.1
V257_K258 insPAPAPA	0.01 ± 0.02	100.0	0.0	0.55 ± 0.06	100.0	0.0	0.0	0.22 ± 0.02	93.5	6.5
V257_K258 insGP	0 ± 0	0.0	0.0	0.58 ± 0.04	100.0	0.0	0.0	0.25 ± 0.05	93.2	6.8
V257_K258 insGPG	0.10 ± 0.04	100.0	0.0	0.84 ± 0.18	100.0	0.0	0.0	0.24 ± 0.01	92.5	7.5
V257_K258 insGPGP	0 ± 0	0.0	0.0	0.58 ± 0.04	100.0	0.0	0.0	0 ± 0	0.0	0.0
V257_K258 insGPGPG	0.07 ± 0.02	100.0	0.0	0.67 ± 0.07	100.0	0.0	0.0	0.27 ± 0.01	93.1	6.9
V257_K258 insGPGPGP	0.09 ± 0.02	100.0	0.0	1.15 ± 0.20	100.0	0.0	0.0	0.17 ± 0.02	94.4	5.6

Supplementary Table 7: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type and insertion variants after F278 based on LILI. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
F278_N279 insGG	15.74 ± 1.85	50.2	49.8	3.73 ± 0.54	92.7	4.9	2.4	3.49 ± 0.16	94.1	5.9
F278_N279 insGGG	13.44 ± 1.33	55.0	45.0	4.18 ± 0.37	93.3	2.7	4.0	2.48 ± 0.11	93.5	6.5
F278_N279 insGGGG	7.07 ± 1.56	40.4	59.6	3.87 ± 0.30	95.2	1.3	3.5	0.45 ± 0.08	92.3	7.7
F278_N279 insGGGGG	4.59 ± 0.46	55.4	44.6	1.58 ± 0.06	95.5	3.1	1.4	1.37 ± 0.06	92.5	7.5
F278_N279 insGGGGGG	20.69 ± 0.03	49.0	51.0	3.05 ± 0.05	95.7	2.4	2.0	2.31 ± 0.00	92.4	7.6
F278_N279 insGS	14.23 ± 0.67	42.5	57.5	3.45 ± 0.38	94.0	6.0	0.0	0.57 ± 0.04	93.0	7.0
F278_N279 insGSG	13.97 ± 0.33	33.2	66.8	1.60 ± 0.05	91.9	3.2	4.9	0.64 ± 0.04	93.3	6.7
F278_N279 insGSGS	15.41 ± 0.18	34.1	65.9	1.30 ± 0.16	92.5	4.0	3.5	0.39 ± 0.05	91.8	8.2
F278_N279 insGSGSG	20.31 ± 2.89	33.5	66.5	0.82 ± 0.17	94.1	4.4	1.5	0.39 ± 0.08	91.0	9.0
F278_N279 insGSGSGS	15.09 ± 2.03	36.4	63.6	0.90 ± 0.08	95.6	4.4	0.0	0.32 ± 0.04	91.0	9.0
F278_N279 insPA	9.04 ± 1.31	27.8	72.2	0.97 ± 0.04	90.2	6.3	3.6	1.60± 0.32	92.8	7.2
F278_N279 insPAP	11.29 ± 0.61	49.4	50.6	2.21 ± 0.03	92.1	4.6	3.3	2.86 ± 0.16	95.5	4.5
F278_N279 insPAPA	14.23 ± 0.54	52.9	47.1	3.66 ± 0.11	92.7	3.7	3.7	6.16 ± 0.14	96.0	4.0
F278_N279 insPAPAP	14.11 ± 1.25	15.8	84.2	6.03 ± 0.22	92.2	3.4	4.4	1.76 ± 0.01	94.0	6.0
F278_N279 insPAPAPA	9.82 ± 3.56	0.0	100.0	2.29 ± 0.19	92.5	3.5	4.0	0.73 ± 0.00	93.9	6.1
F278_N279 insGP	11.55 ± 0.73	58.2	41.8	1.21 ± 0.03	92.3	4.1	3.7	7.42 ± 0.00	95.6	4.4
F278_N279 insGPG	11.76 ± 1.59	29.4	70.6	6.92 ± 0.68	89.0	5.2	5.7	1.72 ± 0.06	93.5	6.5
F278_N279 insGPGP	13.71 ± 0.08	17.3	82.7	4.44 ± 0.12	87.6	6.8	5.6	1.31 ± 0.04	92.2	7.8
F278_N279 insGPGPG	13.31 ± 0.53	15.4	84.6	3.28 ± 0.19	87.6	3.6	8.8	1.02 ± 0.06	92.5	7.5
F278_N279 insGPGPGP	17.94 ± 1.41	11.6	88.4	2.37 ± 0.23	89.4	3.5	7.1	1.68 ± 0.05	92.2	7.8

Supplementary Table 8: Conversions of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the wild-type and insertion variants after Q286 based on LILI. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Pf: combined product formation in mM. Regioselectivity in %. Source data are provided as a Source Data file.

	Pf (mM)	Selectivity (%)		Pf (mM)	Selectivity (%)			Pf (mM)	Selectivity (%)	
		1a	1b		2a	2b	2c		3a	3b
Wild-type	6.68 ± 0.77	86.4	13.6	3.58 ± 0.44	98.8	0.7	0.5	0.64 ± 0.03	93.0	7.0
Q286_A287 insGG	6.61 ± 0.70	11.2	88.8	0.45 ± 0.04	93.5	1.9	4.6	0.89 ± 0.02	83.6	16.4
Q286_A287 insGGG	8.74 ± 0.97	9.1	90.9	0.57 ± 0.05	91.2	3.6	5.2	1.38 ± 0.10	83.9	16.1
Q286_A287 insGGGG	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
Q286_A287 insGGGGG	6.84 ± 1.08	6.2	93.8	0.23 ± 0.01	100.0	0.0	0.0	0.99 ± 0.03	85.4	14.6
Q286_A287 insGGGGGG	6.75 ± 0.26	7.1	92.9	0.19 ± 0.01	100.0	0.0	0.0	0.67 ± 0.04	86.1	13.9
Q286_A287 insGS	6.65 ± 2.69	0.0	100.0	0.48 ± 0.01	96.8	3.2	0.0	0.30 ± 0.01	84.9	15.1
Q286_A287 insGSG	15.00 ± 0.38	0.0	100.0	0.56 ± 0.05	100.0	0.0	0.0	0.36 ± 0.01	80.9	19.1
Q286_A287 insGSGS	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
Q286_A287 insGSGSG	17.07 ± 1.03	0.0	100.0	0.25 ± 0.02	100.0	0.0	0.0	0.34 ± 0.01	83.4	16.6
Q286_A287 insGSGSGS	0 ± 0	0.0	0.0	0 ± 0	0.0	0.0	0.0	0 ± 0	0.0	0.0
Q286_A287 insPA	16.32 ± 1.13	30.2	69.8	1.87 ± 0.22	89.0	5.5	5.5	1.26 ± 0.08	86.4	13.6
Q286_A287 insPAP	19.14 ± 1.6	15.6	84.4	1.35 ± 0.08	89.1	5.4	5.5	1.34 ± 0.07	87.8	12.2
Q286_A287 insPAPA	15.10 ± 0.94	7.5	92.5	0.96 ± 0.04	89.5	4.2	6.3	1.01 ± 0.01	85.8	14.2
Q286_A287 insPAPAP	2.39 ± 0.50	12.2	87.8	0.88 ± 0.05	92.4	5.1	2.5	0.78 ± 0.03	91.2	8.8
Q286_A287 insPAPAPA	18.93 ± 1.19	7.1	92.9	0.73 ± 0.078	93.2	4.9	1.9	0.96 ± 0.02	89.6	10.4
Q286_A287 insGP	11.52 ± 0.79	11.8	88.2	0.66 ± 0.05	92.4	3.4	4.2	1.37 ± 0.07	86.0	14.0
Q286_A287 insGPG	13.35 ± 1.26	7.5	92.5	1.23 ± 0.08	95.6	2.4	2.0	1.86 ± 0.03	79.8	20.2
Q286_A287 insGPGP	13.94 ± 0.61	7.9	92.1	0.59 ± 0.05	91.1	3.7	5.1	1.23 ± 0.05	88.5	11.5
Q286_A287 insGPGPG	14.52± 0.61	5.8	94.2	0.86 ± 0.04	92.7	3.8	3.4	1.92 ± 0.08	81.4	18.6
Q286_A287 insGPGPGP	15.67 ± 0.24	6.5	93.5	0.42 ± 0.03	88.1	7.0	4.8	1.04 ± 0.01	89.5	10.5



Supplementary Fig. 11: Biotransformation results with variants derived from the LILI approach. a, Product formation and distribution of the products **1a** (red, bottom bars) and **1b** (blue, top bars) obtained during the biotransformation of **1** with the wild-type and variants of the LILI library. **b**, Product formation and distribution of the products **2a** (green, bottom bars), **2b** (blue, middle bars) and **2c** (orange, top bars) obtained during the biotransformation of **2** with the wild-type and variants of the LILI library. **c**, Product formation and distribution of the products **3a** (green, bottom bars) and **3b** (purple, top bars) obtained during the biotransformation of **3** with the wild-type and variants of the LILI library. The reaction conditions as mentioned in Fig. 2 were applied, the substrate concentrations are indicated in the reaction equation. The reactions were performed in technical triplicates (black dots) with average values (horizontal bar) and standard deviations (calculated using Excel version 2016) indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.

Supplementary Table 9: Different wild-types and active-site variants known from literature compared to selected loop variants from this study. t.w.: this work. n.d.: not determined. Source data are provided as a Source Data file.

			Product formation (%)	Selectivity (%)			Annotation	Source
				1a	1b			
Styrene 1	CDO wild-type		13.4 ± 1.55	86.4	13.6 (*ee* 18.4 ± 1.1 *R*-**1b**)		50 mM substrate 0.1 g_{cww}/ml *E. coli*	t.w.
	Literature	CDO wild-type (*Pseudomonas fluorescens* IP01)	71 ± 6	99.7	0.3 (*ee* 43 ± 3 *R*-**1b**)		10 mM substrate, 0.2 g_{cww}/ml *E. coli*	[1]
		NDO wild-type (*Pseudomonas sp.* NCIB 9816-4)	74 ± 3	0	>99 (*ee* 78 ± 1 *R*-**1b**)		10 mM substrate, 0.2 g_{cww}/ml *E. coli*	[2]
		TDO wild-type (*Pseudomonas. putida* F1)	9	85	15 (*ee* >99 *R*-**1b**)		10 mM substrate	[3]
	Active-site	CDO_M232A	97 ± 10	8	92 (*ee* 95 ± 1 *R*-**1b**)		10 mM substrate, 0.2 g_{cww}/ml *E. coli*	[1]
		NDO_H295A	92 ± 2	<1	>99 (*ee* 79 ± 1 *R*-**1b**)		10 mM substrate, 0.2 g_{cww}/ml *E. coli*	[2]
		TDO_T365N	14	24	76 (*ee* >99 *R*-**1b**)		10 mM substrate,	[3]
	Loop	CDO_N279_D280insE	47.4 ± 3.2	67.5	32.5 (*ee* 11.4 ± 5.8 *R*-**1b**)		50 mM substrate 0.1 g_{cww}/ml *E. coli*	t.w.
		CDO_F282A	24.6 ± 5.7	95.0	5.0 (*ee* 72.7 ± 0.6 *R*-**1b**)		50 mM substrate 0.1 g_{cww}/ml *E. coli*	t.w.
		CDO_Q286_A287insGSGSG	34.1 ± 2.1	<1	>99 (*ee* 6.4 ± 0.2 *R*-**1b**)		50 mM substrate 0.1 g_{cww}/ml *E. coli*	t.w.
		CDO_F282_L284del	39.9 ± 4.0	6.3	93.7 (*ee* 1.4 ± 0.6 *R*-**1b**)		50 mM substrate 0.1 g_{cww}/ml *E. coli*	t.w.
				2a	**2b**	**2c**		
(*R*)-Limonene 2	CDO wild-type		35.8 ± 4.4	98.8 (*ee* 99.6 ± 0.1 1*R*,5*S*-**2a**)	0.7	0.5	0.1 g_{cww}/ml *E. coli*	t.w.
	Literature	CDO wild-type (*Pseudomonas fluorescens* IP01)	46 ± 10	> 95 (*ee* > 98 ± 0.1 1*R*,5*S*-**2a**)	<5	0	0.2 g_{cww}/ml *E. coli*	[1]
		CDO wild-type (*Pseudomonas putida S1*)	40	>95 (*ee* >98 1*R*,5*S*-**2a**)	0	0	OD_{600} = 3.0 6.2 mM substrate	[4]
		NDO wild-type (*Pseudomonas sp.* NCIB 9816-4)	n.d.	92	8	0	0.2 g_{cww}/ml *E. coli*	[2]
	Active-site	CDO_M232A	>99	95 (*ee* > 98 1*R*,5*S*-**2a**)	5	0	0.2 g_{cww}/ml *E. coli*	[1]
		NDO_H295A_V260A	213% of wild-type	93 (*ee* 62 1*R*,5*S*-**2a**)	7	0	0.2 g_{cww}/ml *E. coli*	[2]
	Loop	CDO_A283del	89.2 ± 8.0	98.2 (*ee* 62 1*R*,5*S*-**2a**)	0.8	1.0	0.1 g_{cww}/ml *E. coli*	t.w.
		CDO_L284G	20.8 ± 1.9	30.8 (*ee* >99 1*R*,5*S*-**2a**)	19.4	49.7	0.1 g_{cww}/ml *E. coli*	t.w.
		CDO_M289A	19.4 ± 0.9	54.1 (*ee* >99 1*R*,5*S*-**2a**)	6.2	39.8	0.1 g_{cww}/ml *E. coli*	t.w.

				3a	3b			
2-Phenyl-pyridine 3	**CDO wild-type**		6.4 ± 0.3	93.0 (*ee* >99 1*S*, 2*R*-**3a**)	7.0			t.w.
	Literature	**TDO wild-type (*Pseudomonas putida* UV4)**	1% yield	100 (*ee* >98 1*S*, 2*R*-**3a**)				[5]
		NDO wild-type (*Pseudomonas* sp. NCIB 9816-4)	25% yield	≥95			≤ 5% *cis*-3,4-dihydrodiol (*ee* >98 1*S*, 2*R*) was formed	[5]
		BPDO wild-type (*Sphingomomas yanoikuyae* (B8/36))	59% yield	≥95				[5]
	Loop	**CDO_A283del**	>99	98.7 (*ee* >99 1*S*, 2*R*-**3a**)	1.3			t.w.
		CDO_F282A	99.9 ± 4.0	98.6 (*ee* >99 1*S*, 2*R*-**3a**)	1.4			t.w.
		CDO_Q286_A287insGPG	18.6 ± 0.3	79.8 (*ee* >99 1*S*, 2*R*-**3a**)	20.2			t.w.

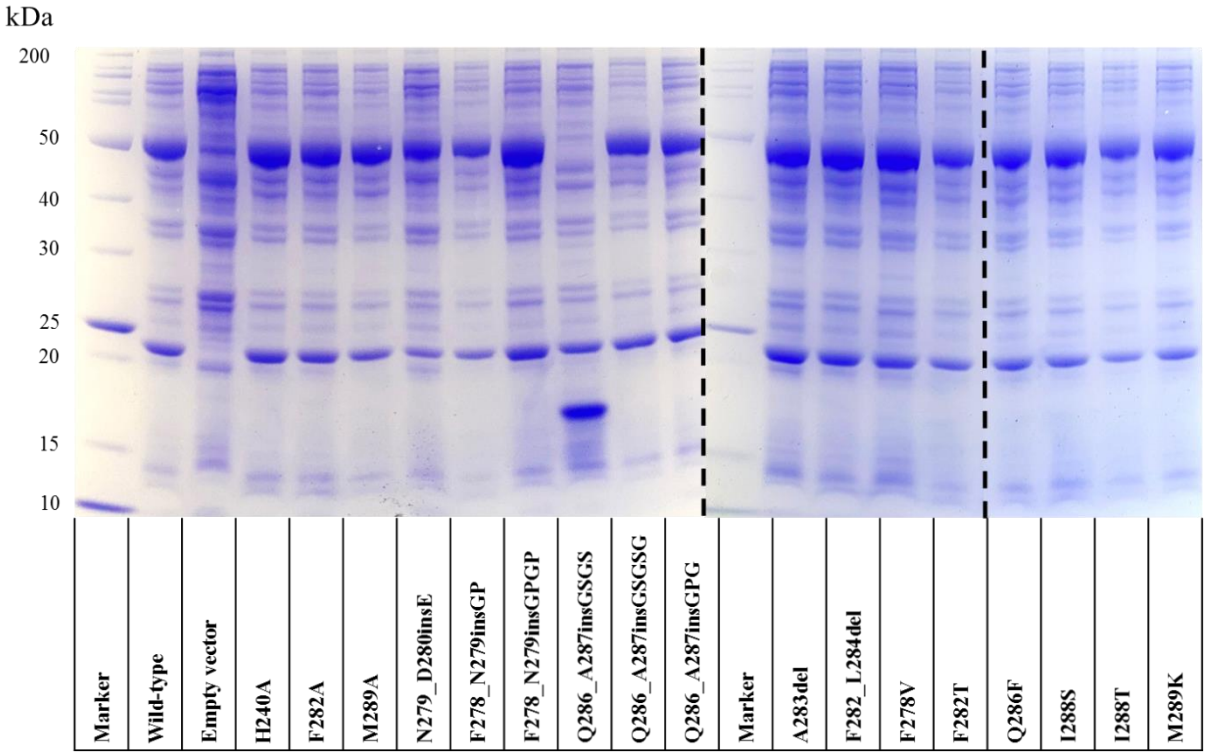
Supplementary Table 10: Enantiomeric excesses of the biotransformations of styrene 1, (*R*)-limonene 2 and 2-phenylpydridine 3 with the selected variants from Supplementary Table S19, and saturation and deletion variants. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Enantiomeric excess in %. n.d.: not determined. -: not detected. Source data are provided as a Source Data file.

	Product (%)		
Variant	**Phenylethan-1,2-diol** **1b**	**Carveol** **2a**	**1,2-Dihydroxy-3-(2'pyridyl)-cyclohexa-3,5-diene** **3a**
Wild-type	18.43 ± 1.08 (*R*)-**1b**	99.73 ± 0.13 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
F282A	72.72 ± 0.63 (*R*)-**1b**	n.d.	>99 % (+)-(1*S*,2*R*)-**3a**
M289A	n.d.	99.97 ± 0.05 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
N279_D280insE	11.42 ± 5.84 (*R*)-**1b**	n.d.	>99 % (+)-(1*S*,2*R*)-**3a**
A283del	42.00 ± 0.59 (*R*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
F282_L284del	1.49 ± 0.15 (*R*)-**1b**	93.30 ± 0.72 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
F278V	41.32 ± 6.64 (*R*)-**1b**	99.99 ± 0.01 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
F282V	69.83 ± 3.61 (*R*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
F282T	-	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
L284G	26.03 ± 1.08 (*R*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
Q286F	15.83 ± 0.23 (*R*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
I288S	15.56 ± 0.70 (*S*)-**1b**	99.76 ± 0.02 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
I288T	1.17 ± 0.83 (*S*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**
M289K	23.97 ± 0.44 (*R*)-**1b**	>99.9 (1*R*,5*S*)-**2a**	>99 % (+)-(1*S*,2*R*)-**3a**

The enantiomeric excess of **3a** was determined by comparison to two in-house standards of (+)-(1*S*,2*R*)-**3a**. The configuration was determined by optical rotation.[5] Multiple columns and methods were applied, which all reveal only one enantiomer.

Supplementary Table 11: Diastereomeric excesses of the biotransformations (*R*)-limonene 2 with the selected variants from Supplementary Supplementary Table 10, saturation and deletion variants. Biotransformations were performed in technical triplicates and standard deviations (calculated using Excel version 2016) are indicated. Diastereomeric excess in %. Source data are provided as a Source Data file.

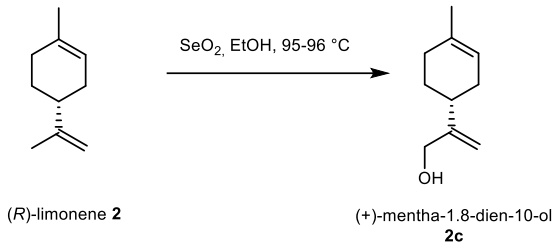
Variant	Carveol **2a**
Wild-type	94.70 ± 0.37 (1*R*,5*S*)-**2a**
M289A	97.05 ± 0.26 (1*R*,5*S*)-**2a**
A283del	87.27 ± 1.14 (1*R*,5*S*)-**2a**
F282_L284del	98.41 ± 0.02 (1*R*,5*S*)-**2a**
F278V	55.10 ± 2.53 (1*R*,5*S*)-**2a**
F282V	99.14 ± 0.01 (1*R*,5*S*)-**2a**
F282T	99.41 ± 0.20 (1*R*,5*S*)-**2a**
L284G	38.14 ± 1.81 (1*R*,5*S*)-**2a**
Q286F	98.11 ± 0.08 (1*R*,5*S*)-**2a**
I288S	90.23 ± 1.53 (1*R*,5*S*)-**2a**
I288T	99.12 ± 0.06 (1*R*,5*S*)-**2a**
M289K	88.02 ± 0.82 (1*R*,5*S*)-**2a**



Supplementary Fig. 12: SDS-PAGE analysis of the empty vector, expressed wild-type and selected variants of the CDO. The analysis of the expression levels was performed in a RunBlue 12% Bis-Tris Gel from Expedeon (Abcam, Berlin, Germany) with PageRuler™ Unstained Protein Ladder (Thermo Scientific, Waltham, USA) as marker. Whole cell samples were standardized to OD_{600} = 4. The bands of the α-subunit (52 kDa) and β-subunit (23 kDa) are clearly visible, except for the α-subunit of Q286_A287insGSGS. The bands of the reductase (44 kDa) and ferredoxin (12 kDa) were not observed. Source data are provided as a Source Data file. Dashed lines represent different gels and vertically sliced image. SDS-PAGE analysis was performed independently 15 times for the wildtype with comparable results. Other variants were analyzed *via* SDS-PAGE with the wildtype as positive control at least once and repeated when preparation errors occurred.

All obtained variants were analyzed *via* non-native SDS-PAGE of the whole cells as shown in Supplementary Fig. S12. All variants, also these which showed no activity for any of the tested variants, were expressed. No native SDS-PAGE was observed, so no conclusions about the solubility or correct folding could be drawn.

Supplementary Note 1: Synthesis of (+)-mentha-1.8-dien-10-ol (2b)

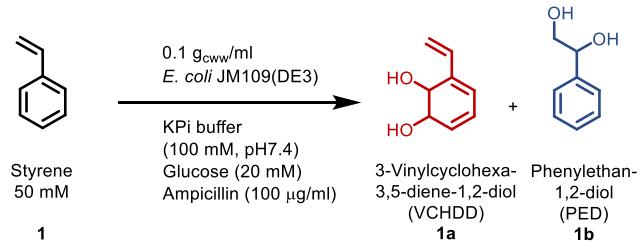


The synthesis of (+)-mentha-1.8-dien-10-ol **2b** was performed according to Thomas and Bucher.[6]

Yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$,): δ 1.43-1.56 (2 H, m), 1.66 (3 H, s), 1.78-2.23 (6 H, m), 4.18 (2 H, d, *J* 5.7), 4.91 (1 H, s), 5.04-5.07 (1 H, m), 5.40 (1 H, br s); ^{13}C NMR (500 MHz $CDCl_3$,): δ 23.48, 28.18, 30.53, 31.36, 36.91, 65.19, 107.83, 120.45, 133.85, 153.67. MS (GC, EI): m/z (%) =153 (1, M^+), 152 (11.8, M), 134 (47), 121 (17), 119 (91), 107 (14), 106 (100), 105 (39), 94 (34), 93 (61), 92 (46), 91 (82), 84 (25), 83 (16), 79 (80), 77 (42), 68 (60), 67 (72), 65 (16)

GC-MS and NMR analysis revealed contaminations with formed perillyl alcohol.

Supplementary Note 2: Determination of product formation, distribution, enantiomeric excess and diastereomeric excess



Supplementary Fig. 13: Conversion of styrene to 3-vinylcyclohexa-3,5-diene-1,2-diol 1a and 1-phenylethan-1,2-diol 1b

3-vinylcyclohexa-3,5-diene-1,2-diol (1a)

MS (GC, EI) m/z (%) = 139 (1.7, M+), 138 (19, M), 120 (94), 109 (21), 92 (39), 91 (100), 81 (19), 79 (23), 77 (24), 65 (17)

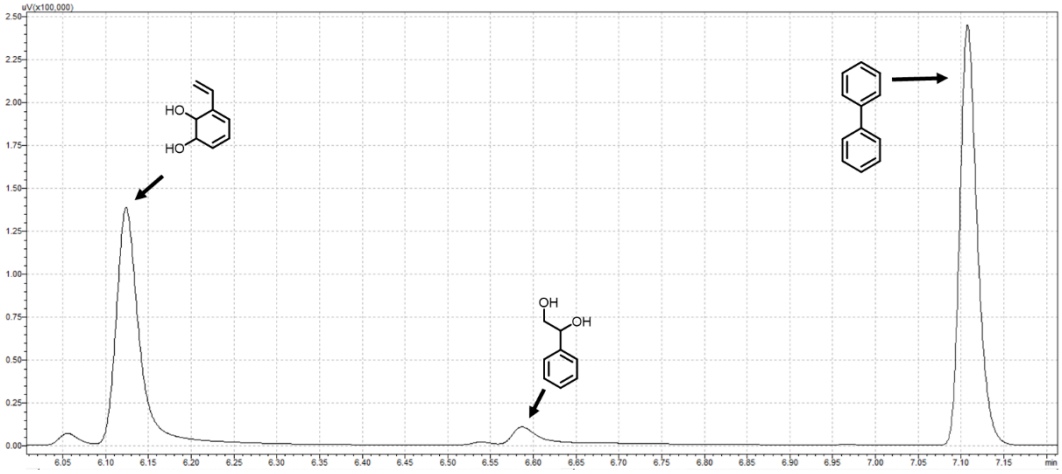
1-phenyl-1,2-ethanediol (1b)

Rieske non-heme iron oxygenase (RO)-catalyzed biotransformations

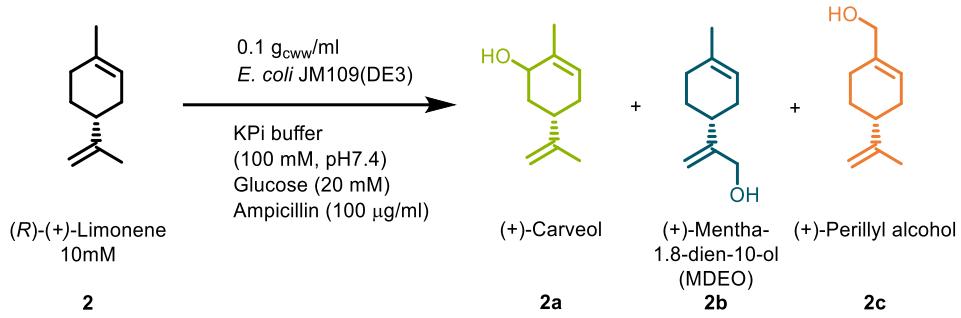
MS (GC, EI) m/z (%) = 139 (0.8, M^+), 138 (9, M), 108 (9), 107 (100), 91 (4), 79 (48), 77 (28)

Standard Sigma Aldrich

MS (GC, EI) m/z (%) = 139 (2, M^+), 138 (23, M), 108 (22), 107 (100), 91 (10), 79 (100), 77 (77)



Supplementary Fig. 14: GC-FID chromatogram of CDO wild-type catalyzed biotransformation of styrene 1.



Supplementary Fig. 15: Conversion of (*R*)-(+)-limonene 2 to (+)-carveol 2a, (+)-mentha-1.8-dien-10-ol 2b and (+)-perillyl alcohol 2c.

(+)-carveol 2a

Rieske non-heme iron oxygenase (RO)-catalyzed biotransformation

MS (GC, EI) m/z (%) = 153 (1.2, M^+), 152 (11, M), 137 (11), 119 (11), 109 (100), 108 (13), 95 (13), 94 (4), 93 (11), 92 (5), 91 (15), 84 (47), 83 (25), 81 (10), 80 (8), 79 (10), 69 (17), 55 (18)

Standard Sigma-Aldrich

MS (GC, EI) m/z (%) = 153 (0.3, M+), 152 (3, M), 137 (68), 119 (34), 109 (77), 108 (12), 95 (24), 94 (25), 93 (27), 92 (17), 91 (24), 84 (100), 83 (44), 81 (25), 80 (25), 79 (24), 69 (36), 55 (31)

(+)-mentha-1.8-dien-10-ol 2b

Rieske non-heme iron oxygenase (RO)-catalyzed biotransformation

MS (GC, EI) m/z (%) = 153 (5, M^+), 152 (18, M), 134 (63), 121 (27), 119 (100), 107 (22), 106 (79), 105 (50), 95 (29), 94 (49), 93 (80), 92 (54), 91 (82), 84 (40), 79 (95), 77 (42), 68 (95), 67 (98), 55 (49)

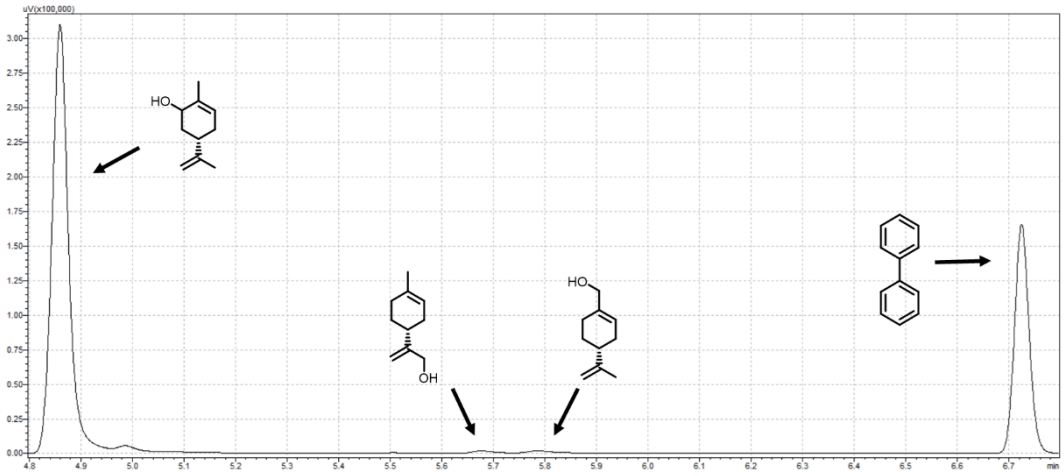
(+)-perillyl alcohol 2c

Rieske non-heme iron oxygenase (RO)-catalyzed biotransformation

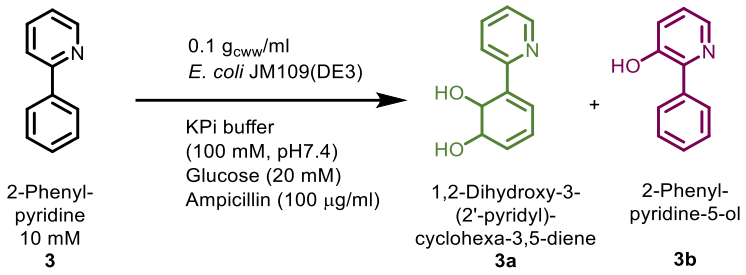
MS (GC, EI) m/z (%) = 153 (1.6, M^+), 152 (9, M), 121 (83), 119 (31), 109 (26), 108 (28), 107 (37), 106 (18), 105 (22), 95 (38), 94 (52), 93 (95), 91 (53), 81 (37), 79 (100), 77 (30), 68 (90), 67 (80), 55 (44)

Standard Sigma-Aldrich

MS (GC, EI) m/z (%) = 153 (2, M+), 152 (18, M), 121 (87), 119 (48), 109 (35), 108 (33), 107 (14), 106 (28), 105 (28), 95 (18), 94 (18), 93 (90), 91 (61), 81 (29), 79 (100), 77 (32), 68 (86), 67 (77), 55 (39)



Supplementary Fig. 16: GC-FID chromatogram of CDO wild-type catalyzed biotransformation of (*R*)-(+)-limonene 2.



Supplementary Fig. 17: Conversion of 2-phenylpyridine 3 to 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a and 2-phenyl-pyridine-5-ol 3b.

1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a

Rieske non-heme iron oxygenase (RO)-catalyzed biotransformations

MS (LC, ESI, positive) m/z (%) = 191 (M^+, 12), 190 (100, M), 189 (58), 173 (4), 172 (35)

Standard from in-house library, derived from an upscaling of biotransformation of 2-phenylpyridine with TDO Wild-type

MS (LC, ESI, positive) m/z (%) = 191 (M^+, 6), 190 (100, M), 173 (6), 172 (48)

Brown solid; $[\alpha]_D$ +161 (*c* 1.1, MeOH); ^{1}H NMR δ ($CDCl_3$, 500 MHz), 4.50 (1 H, m, H-1), 4.93 (1 H, d, $J_{1,2}$ 9.7, H-2), 6.18 (2 H, m, H-5, H-6), 6.65 (1 H, d, $J_{4,5}$ 5.4, H-4), 6.89 (1 H, dd, $J_{4',3'}$ 9.9, $J_{4',5'}$ 2.5, H-4'), 7.51 (1 H, d, $J_{3',4'}$ 5.4, H-3'), 7.6 (1 H, d, $J_{5',4'}$ 8, H-5'), 8.51 (1 H, d, $J_{6',5'}$ 4.6, H-6'); ^{13}C NMR δ (500 MHz, $CDCl_3$) 67.26, 69.24, 119.98, 122.13, 123.58, 124.82, 131.44, 136.21, 136.97, 148.19, 157.33; [5]

2-phenyl-pyridine-5-ol 3b

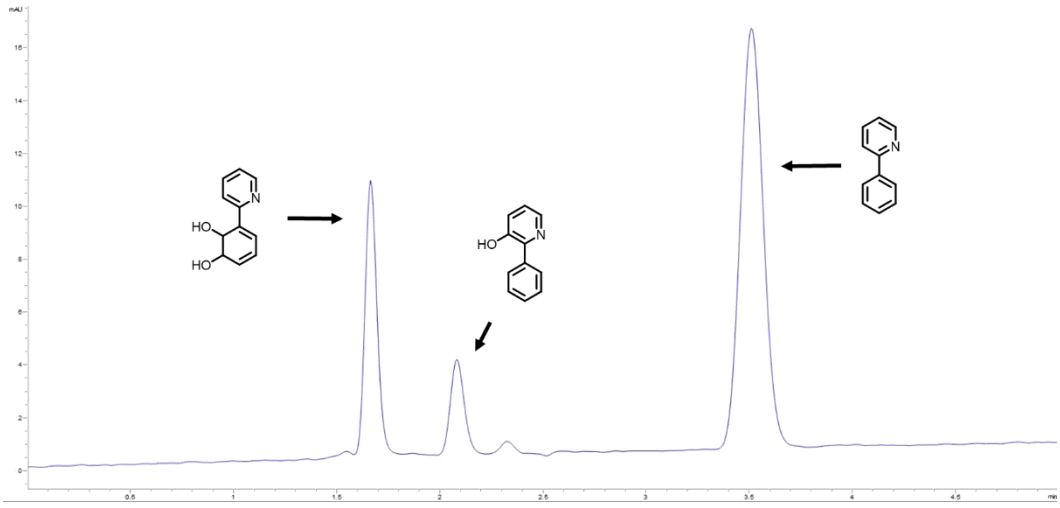
Rieske non-heme iron oxygenase (RO)-catalyzed biotransformations

MS (LC, ESI, positive) m/z (%) = 173 (12, M^+), 172 (100, M)

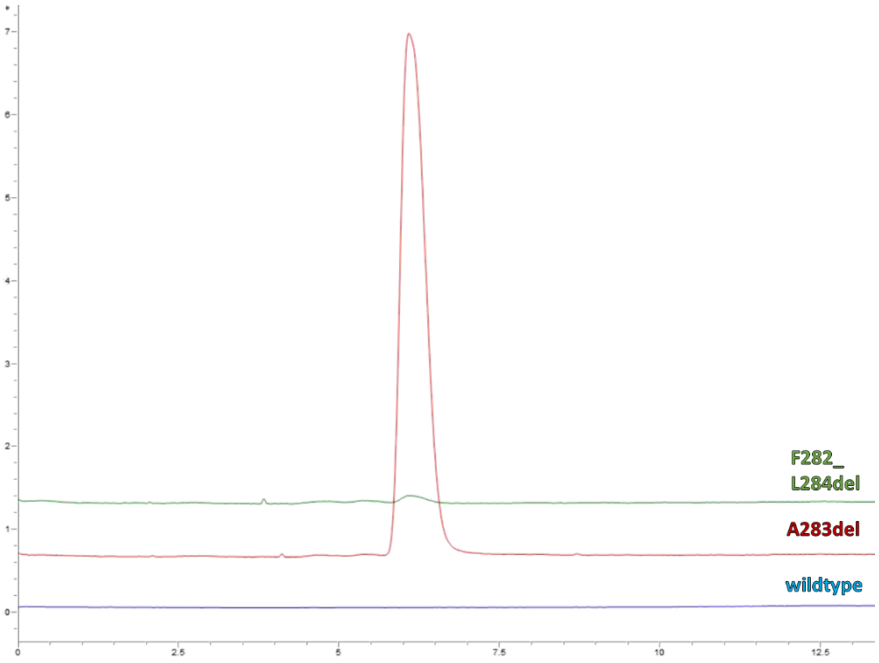
Standard Fluorochem

MS (LC, ESI, positive) m/z (%) = 173 (13, M^+), 172 (100, M)

MS (GC, EI) m/z (%) = 172 (5.6, M^+), 171 (52, M), 170 (100), 169 (2), 144 (1), 143 (7), 142 (7), 141 (3), 140 (2), 117 (6), 116 (9), 115 (27)



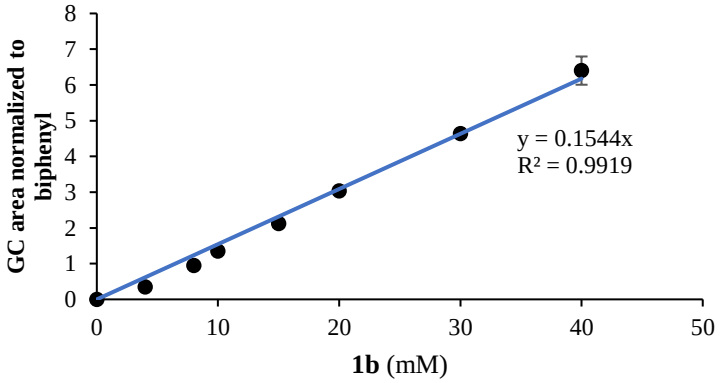
Supplementary Fig. 18: DAD chromatogram of CDO wild-type catalyzed biotransformation of 2-phenylpyridine at a wavelength of 210 nm.



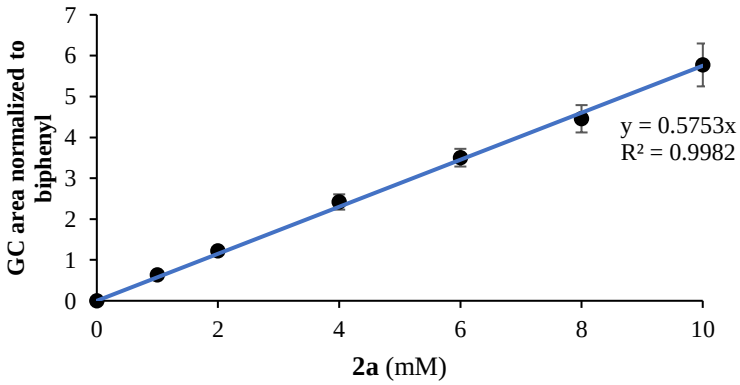
Supplementary Fig. 19: LC-MS spectrum of the biotransformation of phenazone in SIM-mode (m/z 222). Comparison between the wild-type (blue, bottom line) with no product formation, F282_L284del (green, top line) with product traces and A283del (red, middle line) with clear product formation.

Product formations and distribution

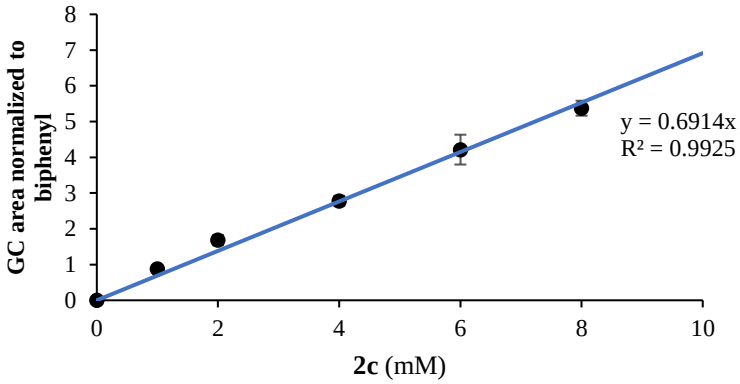
The quantification of the different products was performed with biphenyl as internal standard under the same conditions as the corresponding biotransformations. 1 M DMSO stocks of the corresponding products were applied for standard solutions, incubated, extracted and analyzed. LC/MS analysis of the in-house standard of 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene (**3a**) showed traces of 2-phenyl-pyridine-5-ol (**3b)**. Based on the corresponding calibration curve, the amount of 2-phenyl-pyridine-5-ol was subtracted from the used concentration of 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene standard to ensure correct concentrations.



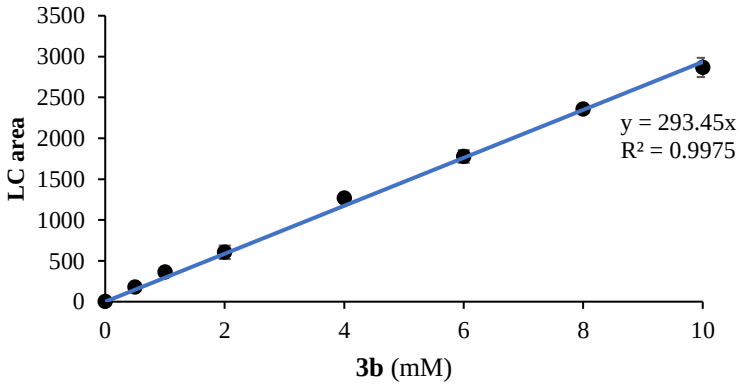
Supplementary Fig. 20. Calibration curve of 1-phenyl-1,2-ethandiol (1b). Data acquisition was performed in technical triplicates per concentration with average values and standard deviations (calculated using Excel version 2016) are indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.



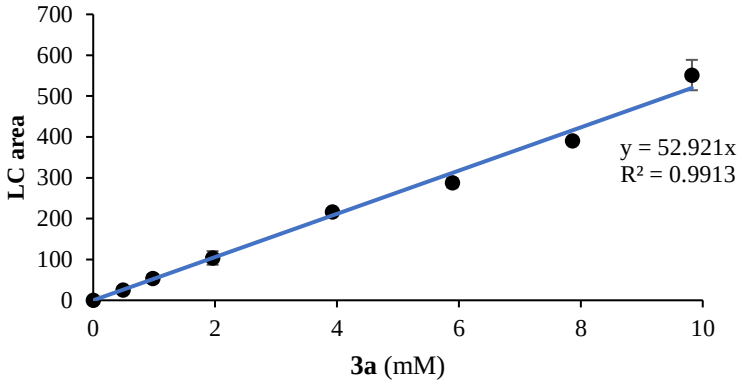
Supplementary Fig. 21. Calibration curve of the mixture of isomers of (-)-carveol (2a). Data acquisition was performed in technical triplicates per concentration with average values and standard deviations (calculated using Excel version 2016) are indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.



Supplementary Fig. 22. Calibration curve of (+)-perillyl alcohol (2c). Data acquisition was performed in technical triplicates per concentration with average values and standard deviations (calculated using Excel version 2016) are indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.



Supplementary Fig. 23. Calibration curve of 2-phenyl-pyridine-5-ol (3b). Data acquisition was performed in technical triplicates per concentration with average values and standard deviations (calculated using Excel version 2016) are indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.



Supplementary Fig. 24. Calibration curve of 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene (3a). Data acquisition was performed in technical triplicates per concentration with average values and standard deviations (calculated using Excel version 2016) are indicated. Error bars may be covered by markers. Source data are provided as a Source Data file.

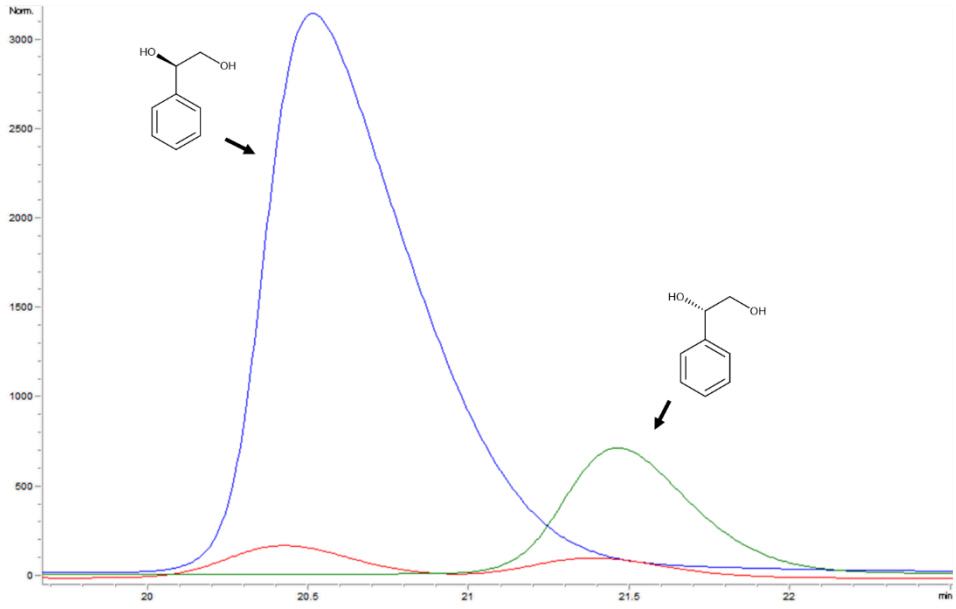
The product 3-vinylcyclohexa-3,5-diene-1,2-diol **1a** polymerizes when concentrated in vacuo, so the synthesis of a standard for quantification is not possible. Also, the synthesis of (+)-mentha-1.8-dien-10-ol **2b** yielded a standard with impurities with perillyl alcohol, despite repeated column chromatography. To ensure correct quantification, we used the relative response factor to determine the concentrations in reference to a standard.[7,8] For **1a**, we used **1b** as standard, while **2c** was applied as reference standard for **2b**. The RF values were calculated from the effective carbon numbers (ECN) of the respective compound (ECN_X) in relation the the ECN of the standard (ECN_{STD}) with equation (1).

$$RF = \frac{ECN_X}{ECN_{STD}} \quad (1)$$

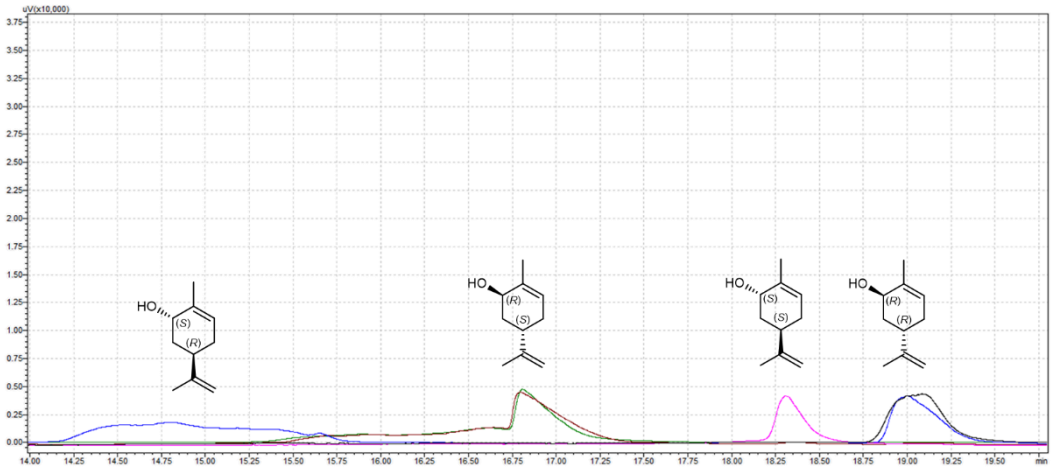
Calibration curves were determined by the quantification of the analytes by external calibration and normalization by the calculated RF values (Supplementary Table 12).

Supplementary Table 12: Calculated ECN and RF values.

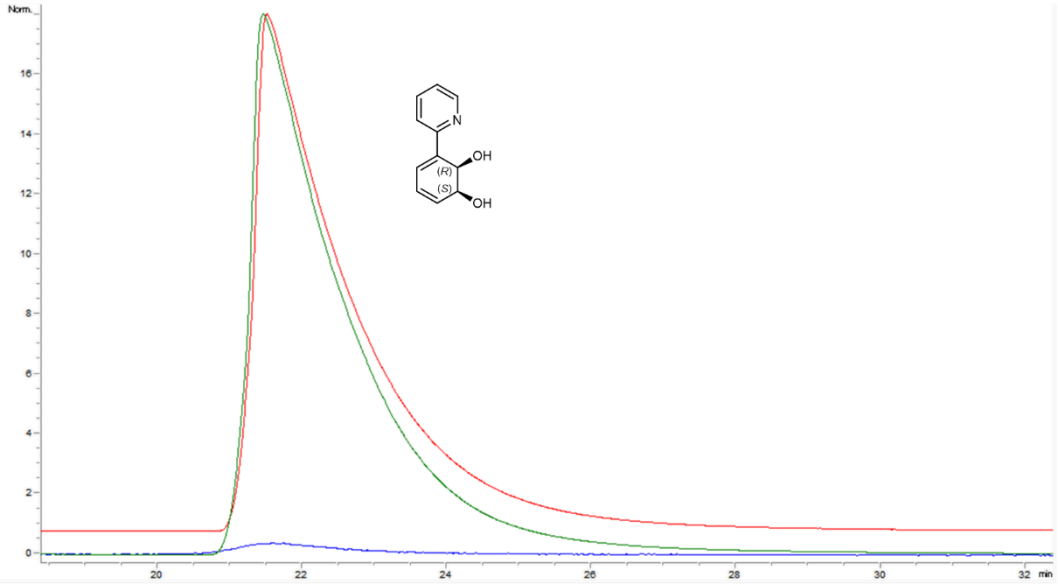
Compound	ECN	RF
1a	6.2	0.919
1b	6.5	
2b	9.3	1
2c	9.3	



Supplementary Fig. 25: Chiral HPLC analysis of 1-phenyl-1,2-ethandiol 1b enantiomers of the commercially available standards of (*R*)-1b (blue, Sigma Aldrich, 99 % optical purity), (*S*)-1b (green, Sigma Aldrich, 99 % optical purity) and the RO-catalyzed biotransformation of 1 (red).

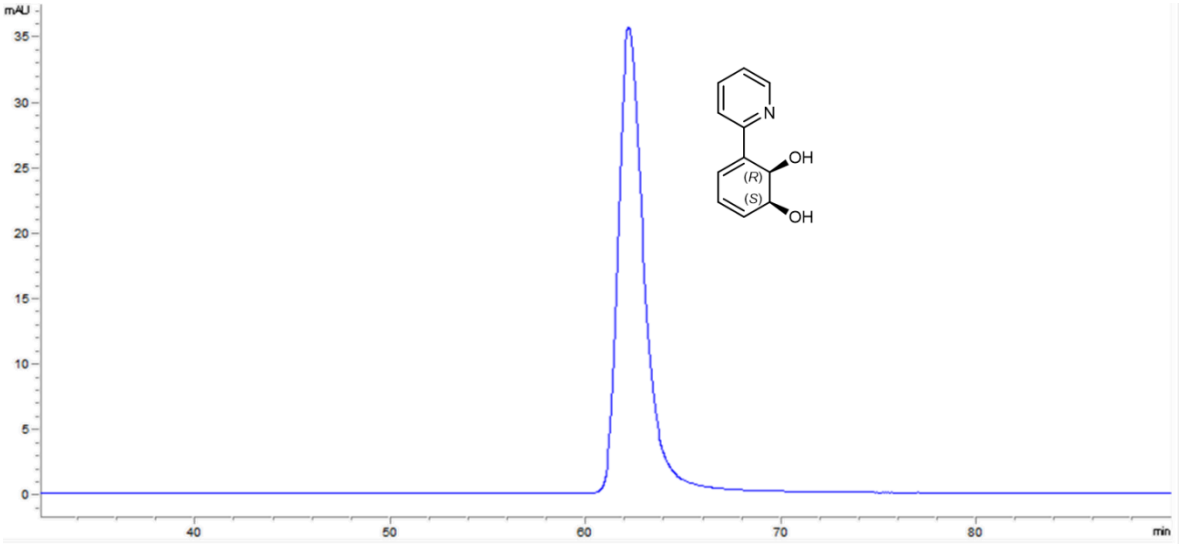


Supplementary Fig. 26: Chiral GC-FID analysis of carveol 2a enantiomers. Commercially available isomer mixture of (-)-carveol (black, Sigma Aldrich, 97 %) containing (1*S*,5*R*)-**2a** and (1*R*,5*R*)-**2a**. (1*S*,5*S*)-carveol (pink) from our in-house library, synthesis according to Bermejo *et al.*.[1,9] (1*R*,5*R*)-carveol (blue) from our in-house library, synthesis according to Dhulut *et al.*.[1,10] (1*R*,5*S*)-carveol (green) derived from CDO_M232A-catalyzed biotransformation of **1**.[1] (1*R*,5*S*)-carveol (red) derived from CDO wild-type-catalyzed biotransformation of **1**.



Supplementary Fig. 27: Chiral HPLC analysis of (1*S*,2*R*)-1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a with normal phase CHIRALPAK IB column. CDO wild-type (blue) and CDO_A283del (red) catalyzed biotransformation of **3**. In-house standard of (1*S*,2*R*)-3a (green).

We also evaluated a normal phase CHIRALPAK IC (250 mm x 4.6 mm, 5 µm particle size, Daicel (Europa) GmbH, Raunheim, Germany) for additional enantiomers of (1S,2R)-1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene. 6 µl sample were injected and the compounds were separated with 1.4 ml/min isocratic mobile phase of 90:10 n-hexane/isopropanol at 30 °C on the same normal phase HPLC-DAD system mentioned in the methods section. Analysis was performed using the DAD at a wavelength of 310 nm. Despite a length of 90 min and previously successful separation of enantiomers of similar products with this column, no additional peak was observed.



Supplementary Fig. 28: Chiral HPLC analysis of (1*S*,2*R*)-1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a with normal phase CHIRALPAK IC column. CDO wild-type (blue) and CDO_A283del (red) catalyzed biotransformation of **3**. In-house standard of (1*S*,2*R*)-3a (green).

Primers

Supplementary Table 13: Sequencing primers used in this study.

Name	Sequence (5‘ → 3‘)
pIP107D_seq1f	GATATGTACCATGCGGG
pIP107D_seq2f	CCAAAATGTACAGCTGTG
pIP107D_seq3f	GTGGATTTGCAGGTCGG
pIP107D_seq4f	GCCGCTGAAGATATATCCG
pIP107D_seq5f	GGTATCGCATGTGAGC

Supplementary Table 14: Site-directed mutagenesis primers used for the alanine scan.

Mutation	Name	Sequence (5‘ → 3‘)
G236A	G236A_fw	GATATGTACCATGCGGCAACGATGGCGCATCTT
	G236A_rv	GCGCCATCGTTGCCGCATGGTACATATC
T237A	T237A_fw	GCGGGAGCGATGGCGCATCTTTC
	T237A_rv	GAAAGATGCGCCATCGCTCCCGC
M238A	M238A_fw	GTACCATGCGGGAACGGCGGCGCATCTTTCAGGT
	M238A_rv	ACCTGAAAGATGCGCCGCCGTTCCCGCATGGTAC
A239G	A239G_fw	GGAACGATGGGCCATCTTTCAG
	A239G_rv	CTGAAAGATGGCCCATCGTTCC
H240A	H240A_fw	CATGCGGGAACGATGGCGGCTCTTTCAGGTGTATTGTC
	H240A_rv	GACAATACACCTGAAAGAGCCGCCATCGTTCCCGCATG
L241A	L241A_fw	GGACAATACACCTGAAGCATGCGCCATCGTTCCCG
	L241A_rv	CGGGAACGATGGCGCATGCTTCAGGTGTATTGTCC
D242A	D242A_fw	GGGAACGATGGCGCATCTTGCAGGTGTATTGTCCAGCCTCCCG
	D242A_rv	AAGATGCGCCATCGTTCCCGCATGGTACATATCGCTACAGAATTG
G243G	G243G_fw	GAACGATGGCGCATCTTTCAGCTGTATTGTCCAGCCTCCCGCC
	G243G_rv	CTGAAAGATGCGCCATCGTTCCCGCATGGTACATATCGCTACAGAAT
V244A	V244A_fw	GATGGCGCATCTTTCAGGTGCATTGTCCAGCCTCCCGCC
	V244A_rv	CACCTGAAAGATGCGCCATCGTTCCCGCATGGTACATATCGC
L245A	L245A_fw	GATGGCGCATCTTTCAGGTGTAGCGTCCAGCCTCCCGCCTG
	L245A_rv	TACACCTGAAAGATGCGCCATCGTTCCCGCATGGTACATATCGC
S246A	S246A_fw	GCGCATCTTTCAGGTGTATTGGCCAGCCTCCCGCCTGAAATG
	S246A_rv	CAATACACCTGAAAGATGCGCCATCGTTCCCGCATGGTACATATCG
S247A	S247A_fw	GCATCTTTCAGGTGTATTGTCCGCCCTCCCGCCTGAAATGGATTTGTC
	S247A_rv	GGACAATACACCTGAAAGATGCGCCATCGTTCCCGCATGGTAC
L248A	L248A_fw	GGTGTATTGTCCAGCGCCCCGCCTGAAATGGATT
	L248A_rv	AATCCATTTCAGGCGGGGCGCTGGACAATACACC
P249A	P249A_fw	GTATTGTCCAGCCTCGCGCCTGAAATGG
	P249A_rv	CCATTTCAGGCGCGAGGCTGGACAATAC
P250A	P250A_fw	TTGTCCAGCCTCCCGGCTGAAATGGATTTGTC
	P250A_rv	GACAAATCCATTTCAGCCGGGAGGCTGG
E251A	E251A_fw	CAGCCTCCCGCCTGCAATGGATTTGTCCC
	E251A_rv	GGGACAAATCCATTGCAGGCGGGAGGCTG
M252A	M252A_fw	CAGCCTCCCGCCTGAAGCGGATTTGTCCCAAGTAAAG
	M252A_rv	CTTTACTTGGGACAAATCCGCTTCAGGCGGGAGGCTG

D253A	D253A_fw	CCTCCCGCCTGAAATGGCTTTGTCCCAAGTAAAG
	D253A_rv	CTTTACTTGGGACAAAGCCATTTCAGGCGGGAGG
L254A	L254A_fw	CTCCCGCCTGAAATGGATGCGTCCCAAGTAAAGTTAC
	L254A_rv	GTAACTTTACTTGGGACGCATCCATTTCAGGCGGGAG
S255A	S255A_fw	CGCCTGAAATGGATTTGGCCCAAGTAAAGTTACCG
	S255A_rv	GTAACTTTACTTGGGCCAAATCCATTTCAGGCG
Q256A	Q256A_fw	CGCCTGAAATGGATTTGTCCGCAGTAAAGTTACCGTCAAGTG
	Q256A_rv	CACTTGACGGTAACTTTACTGCGGACAAATCCATTTCAGGCG
V257A	V257A_fw	GGATTTGTCCCAAGCAAAGTTACCGTCAAGTGG
	V257A_rv	CCACTTGACGGTAACTTTGCTTGGGACAAATCCATTTC
K258A	K258A_fw	GATTTGTCCCAAGTAGCGTTACCGTCAAGTG
	K258A_rv	CACTTGACGGTAACGCTACTTGGGACAAATC
L259A	L259A_fw	CCTGAAATGGATTTGTCCCAAGTAAAGGCACCGTCAAGTGGG
	L259A_rv	CCCACTTGACGGTGCCTTTACTTGGGACAAATCCATTTCAGG
P260A	P260A_fw	GATTTGTCCCAAGTAAAGTTAGCGTCAAGTGGG
	P260A_rv	CCCACTTGACGCTAACTTTACTTGGGACAAATC
S261A	S261A_fw	CAAGTAAAGTTACCGGCAAGTGGGAATCAG
	S261A_rv	CTGATTCCCACTTGCCGGTAACTTTACTTG
S262A	S262A_fw	CCAAGTAAAGTTACCGTCAGCTGGGAATCAGTTCC
	S262A_rv	GAACTGATTCCCAGCTGACGGTAACTTTACTTGGGAC
G263A	G263A_fw	GTTACCGTCAAGTGCGAATCAGTTCCGGGC
	G263A_rv	GCCCGGAACTGATTCGCACTTGACGGTAAC
N264A	N264A_fw	GTTACCGTCAAGTGGGGCTCAGTTCCGGGCTAAGTG
	N264A_rv	CACTTAGCCCGGAACTGAGCCCCACTTGACGGTAAC
F278A	F278A_fw	GACATGGGACCGGCTGGGCCAATGACGATTTCGCAC
	F278A_rv	GTGCGAAATCGTCATTGGCCCAGCCGGTCCCATGTC
N279A	N279A_fw	CATGGGACCGGCTGGTTCGCTGACGATTTCGCACTTC
	N279A_rv	GAAGTGCGAAATCGTCAGCGAACCAGCCGGTCCCATG
D280A	D280A_fw	CTGGTTCAATGCCGATTTCGCAC
	D280A_rv	GTGCGAAATCGGCATTGAACCAG
D281A	D281A_fw	CGGCTGGTTCAATGACGCTTTCGCACTTCTGCAAG
	D281A_rv	CTTGCAGAAGTGCGAAAGCGTCATTGAACCAGCCG
F282A	F282A_fw	CAATGACGATGCCGCACTTCTGC
	F282A_rv	GCAGAAGTGCGGCATCGTCATTG
A283G	A283G_fw	GTTCAATGACGATTTCGGACTTCTGCAAGCCATC
	A283G_rv	GATGGCTTGCAGAAGTCCGAAATCGTCATTGAAC
L284A	L284A_fw	GTTCAATGACGATTTCGCAGCTCTGCAAGCCATCATG
	L284A_rv	GATGGCTTGCAGAGCTGCGAAATCGTCATTGAACCAG
L285A	L285A_fw	GACCCATGATGGCTTGCGCAAGTGCGAAATCGTCATTG
	L285A_rv	CAATGACGATTTCGCACTTGCGCAAGCCATCATGGGTC
Q286A	Q286A_fw	GACGATTTCGCACTTCTGGCAGCCATCATGGGTCC
	Q286A_rv	GGACCCATGATGGCTGCCAGAAGTGCGAAATCGTC
A287G	A287G_fw	CGCACTTCTGCAAGCAATCATGGGTCCTAAGG
	A287G_rv	CCTTAGGACCCATGATTGCTTGCAGAAGTGCG
I288A	I288A_fw	CGCACTTCTGCAAGCCGCCATGGGTCCTAAGGTTG
	I288A_rv	CAACCTTAGGACCCATGGCGGCTTGCAGAAGTGCG
M289A	M289A_fw	CACTTCTGCAAGCCATCGCGGGTCCTAAGGTTGTCG
	M289A_rv	CGACAACCTTAGGACCCGCGATGGCTTGCAGAAGTG
G290A	G290A_fw	CTGCAAGCCATCATGGCTCCTAAGGTTGTCGATTAC
	G290A_rv	CGACAACCTTAGGAGCCATGATGGCTTGCAG

Supplementary Table 15: Site-directed mutagenesis primers used for saturation of selected loop positions

Mutation	Name	Sequence (5‘ → 3‘)
F278NNK	F278NNK_fw	CATGGGACCGGCTGGNNKAATGACGATTTCGCAC
	F278NNK_rv	GTGCGAAATCGTCATTMNNCCAGCCGGTCCCATG
D280NNK	D280NNK_fw	GACCGGCTGGTTCAATNNKGATTTCGCACTTCTG
	D280NNK_rv	CAGAAGTGCGAAATCMNNATTGAACCAGCCGGTC
D281NNK	D281NNK_fw	CGGCTGGTTCAATGACNNKTTCGCACTTCTGCAAG
	D281NNK_rv	CTTGCAGAAGTGCGAAMNNGTCATTGAACCAGCC
F282NNK	F282NNK_fw	CTGGTTCAATGACGATNNKGCACTTCTGCAAGCC
	F282NNK_rv	GGCTTGCAGAAGTGCMNNATCGTCATTGAACCAG
A283NNK	A283NNK_fw	GTTCAATGACGATTTCNNKCTTCTGCAAGCCATC
	A283NNK_rv	GATGGCTTGCAGAAGMNNGAAATCGTCATTGAAC
L284NNK	L284NNK_fw	CAATGACGATTTCGCANNKCTGCAAGCCATCATGG
	L284NNK_rv	CCATGATGGCTTGCAGMNNTGCGAAATCGTCATTG
L285NNK	L285NNK_fw	CAATGACGATTTCGCACTTNNKCAAGCCATCATGGGTC
	L285NNK_rv	GACCCATGATGGCTTGMNNAAGTGCGAAATCGTCATTG
Q286NNK	Q286NNK_fw	CGATTTCGCACTTCTGNNKGCCATCATGGGTCCTAAG
	Q286NNK_rv	CTTAGGACCCATGATGGCMNNCAGAAGTGCGAAATCG
A287NNK	A287NNK_fw	GATTTCGCACTTCTGCAANNKATCATGGGTCCTAAGGTTG
	A287NNK_rv	CAACCTTAGGACCCATGATMNNTTGCAGAAGTGCGAAATC
I288NNK	I288NNK_fw	CGCACTTCTGCAAGCCNNKATGGGTCCTAAGGTTGTC
	I288NNK_rv	GACAACCTTAGGACCCATMNNGGCTTGCAGAAGTGCG
M289NNK	M289NNK_fw	GCACTTCTGCAAGCCATCNNKGGTCCTAAGGTTGTCG
	M289NNK_rv	CGACAACCTTAGGACCMNNGATGGCTTGCAGAAGTGC

Supplementary Table 16: Site-directed mutagenesis primers used for the adaption library based on the sequence alignment with oxygenases from *Phenylobacterium immobile* E.

Mutation	Name	Sequence (5‘ → 3‘)
T237del	T237del_fw	GTACCATGCGGGAACGGCGCATCTTTCAGG
	T237del_rv	CCTGAAAGATGCGCCGTTCCCGCATGGTAC
M238del	M238del_fw	GTACCATGCGGGAATGGCGCATCTTTC
	M238del_rv	GAAAGATGCGCCATTCCCGCATGGTAC
T237I	T237I_fw	GATATGTACCATGCGGGAATAATGGCGCATC
	T237I_rv	GATGCGCCATTATTCCCGCATGGTACATATC
A239S	A239S_fw	CATGCGGGAACGATGTCGCATCTTTCAGGTG
	A239S_rv	CACCTGAAAGATGCGACATCGTTCCCGCATG
L245M	L245M_fw	CATCTTTCAGGTGTAATGTCCAGCCTCCCGC
	L245M_rv	GCGGGAGGCTGGACATTACACCTGAAAGATG
S247G	S247G_fw	CTTTCAGGTGTATTGTCCGGCCTCCCGCCTGAAATG
	S247G_rv	CATTTCAGGCGGGAGGCCGGACAATACACCTGAAAG
L248F	L248F_fw	GGTGTATTGTCCAGCTTCCCGCCTGAAATGGATTTG
	L248F_rv	CAAATCCATTTCAGGCGGGAAGCTGGACAATACACC
P249R	P249R_fw	GTGTATTGTCCAGCCTCCGTCCTGAAATGGATTTG
	P249R_rv	CAAATCCATTTCAGGACGGAGGCTGGACAATACAC
S255L	S255L_fw	CCTGAAATGGATTTGCTGCAAGTAAAGTTACCG
	S255L_rv	CGGTAACTTTACTTGCAGCAAATCCATTTCAGG
F266H	F266H_fw	GTCAAGTGGGAATCAGCACCGGGCTAAGTGGGGTG
	F266H_rv	CACCCCACTTAGCCCGGTGCTGATTCCCACTTGAC

A268V	A268V_fw	GAATCAGTTCCGGGTTAAGTGGGGTGGACATG
	A268V_rv	CATGTCCACCCCACTTAACCCGGAACTGATTC
W270L	W270L_fw	CAGTTCCGGGCTAAGCTGGGTGGACATGGGACC
	W270L_rv	GGTCCCATGTCCACCCAGCTTAGCCCGGAACTG
H273Y	H273Y_fw	GCTAAGTGGGGTGGATATGGGACCGGCTGGTTC
	H273Y_rv	GAACCAGCCGGTCCCATATCCACCCCACTTAGC
T275H	T275H_fw	GTGGGGTGGACATGGGCACGGCTGGTTCAATGAC
	T275H_rv	GTCATTGAACCAGCCGTGCCCATGTCCACCCCAC
G276A	G276A_fw	GGTGGACATGGGACCGCCTGGTTCAATGACGATTTC
	G276A_rv	GAAATCGTCATTGAACCAGGCGGTCCCATGTCCACC
W277I	W277I_fw	GGACATGGGACCGGCATTTTCAATGACGATTTC
	W277I_rv	GAAATCGTCATTGAAAATGCCGGTCCCATGTCC
F278G	F278G_fw	CATGGGACCGGCTGGGGCAATGACGATTTCGC
	F278G_rv	GCGAAATCGTCATTGCCCCAGCCGGTCCCATG
D280E	D280E_fw	GACCGGCTGGTTCAATGAAGATTTCGCACTTCTG
	D280E_rv	CAGAAGTGCGAAATCTTCATTGAACCAGCCGGTC
D281E	D281E_fw	GGCTGGTTCAATGACGAATTCGCACTTCTGCAAG
	D281E_rv	CTTGCAGAAGTGCGAATTCGTCATTGAACCAGCC

Supplementary Table 17: Site-directed insertion mutagenesis primers used for the adaption library based on the sequence alignment with oxygenases from Phenylobacterium immobile E.

Mutation	Name	Sequence (5‘ → 3‘)
V257_K258 insED	V257InsED_fw	GGATTTGTCCCAAGTAGAAGACAAGTTACCGTCAAGTGG
	V257InsED_rv	CCACTTGACGGTAACTTGTCTTCTACTTGGGACAAATCC
V257_K258 insEDQE	V257InsEDQE_fw	GGATTTGTCCCAAGTAGAAGACCAGGAAAAGTTACCGTCAAGTGG
	V257InsEDQE_rv	CCACTTGACGGTAACTTTTCCTGGTCTTCTACTTGGGACAAATCC
V257_K258 insEDQELA	V257InsEDQELA_fw	GGATTTGTCCCAAGTAGAAGACCAGGAACTGGCGAAGTTACCGTCAAGTGG
	V257InsEDQELA_rv	CCACTTGACGGTAACTTCGCCAGTTCCTGGTCTTCTACTTGGGACAAATCC
V257_K258 insEDQELARI	V257InsEDQELARI_fw	GGATTTGTCCCAAGTAGAAGACCAGGAACTGGCGCGTATTAAGTTACCGTCAAGTGG
	V257InsEDQELARI_rv	CCACTTGACGGTAACTTAATACGCGCCAGTTCCTGGTCTTCTACTTGGGACAAATCC
V257_K258 insEDQELARIA	V257InsEDQELARIA_fw	GGATTTGTCCCAAGTAGAAGACCAGGAACTGGCGCGTATTGCGAAGTTACCGTCAAGTGG
	V257InsEDQELARIA_rv	CCACTTGACGGTAACTTCGCAATACGCGCCAGTTCCTGGTCTTCTACTTGGGACAAATCC
L259_P260 insG	L259InsG_fw	GATTTGTCCCAAGTAAAGTTAGGCCCGTCAAGTGGGAATC
	L259InsG_rv	GATTCCCACTTGACGGGCCTAACTTTACTTGGGACAAATC
P260_S261 insG	L260InsG_fw	GTCCCAAGTAAAGTTACCGGGCTCAAGTGGGAATCAG
	L260InsG_rv	CTGATTCCCACTTGAGCCCGGTAACTTTACTTGGGAC
V257_K258 insEE	V257InsEE_fw	GGATTTGTCCCAAGTAGAAGAAAAGTTACCGTCAAGTGG
	V257InsEE_rv	CCACTTGACGGTAACTTTTCTTCTACTTGGGACAAATCC
V257_K258 insEERE	V257InsEERE_fw	GGATTTGTCCCAAGTAGAAGAACGCGAAAAGTTACCGTCAAGTGG
	V257InsEERE_rv	CCACTTGACGGTAACTTTTCGCGTTCTTCTACTTGGGACAAATCC

V257_K258 insEERELA	V257InsEERELA_fw	GGATTTGTCCCAAGTAGAAGAACGCGAACTGGCAAAGTTACCGTCAAGTGG
	V257InsEERELA_rv	CCACTTGACGGTAACTTTGCCAGTTCGCGTTCTTCTACTTGGGACAAATCC
V257_K258 insEERELAA	V257InsEERELAA_fw	GGATTTGTCCCAAGTAGAAGAACGCGAACTGGCGGCAAAGTTACCGTCAAGTGG
	V257InsEERELAA_rv	CCACTTGACGGTAACTTTGCCGCCAGTTCGCGTTCTTCTACTTGGGACAAATCC
K258_L259 insAG	K258InsAG_fw	GATTTGTCCCAAGTAAAGGCGGGCTTACCGTCAAGTGGG
	K258InsAG_rv	CCCACTTGACGGTAAGCCCGCCTTTACTTGGGACAAATC
L259_P260 insA	L259InsA_fw	GATTTGTCCCAAGTAAAGTTAGCGCCGTCAAGTGGGAATC
	L259InsA_rv	GATTCCCACTTGACGGCGCTAACTTTACTTGGGACAAATC
P260_S261 insGS	P260InsGS_fw	GTCCCAAGTAAAGTTACCGGGTAGCTCAAGTGGGAATCAG
	P260InsGS_rv	CTGATTCCCACTTGAGCTACCCGGTAACTTTACTTGGGAC
P260_S261 insGG	P260InsGG_fw	GTCCCAAGTAAAGTTACCGGGTGGTTCAAGTGGGAATCAG
	P260InsGG_rv	CTGATTCCCACTTGAACCACCCGGTAACTTTACTTGGGAC
N279_D280 insE	N279InsE_fw	GGGACCGGCTGGTTCAATAACGACGATTTCGCACTTC
	N279InsE_rv	GAAGTGCGAAATCGTCGTTATTGAACCAGCCGGTCCC
Q286_A287 insKK	Q286InsKK_fw	CGATTTCGCACTTCTGCAAAAAAAAGCCATCATGGGTCCTAAG
	Q286InsKK_rv	CTTAGGACCCATGATGGCTTTTTTTTGCAGAAGTGCGAAATCG
Q286_A287 insKKAA	Q286InsKKAA_fw	CGATTTCGCACTTCTGCAAAAAAAAGCGGCGGCCATCATGGGTCCTAAG
	Q286InsKKAA_rv	CTTAGGACCCATGATGGCCGCCGCTTTTTTTTGCAGAAGTGCGAAATCG
Q286_A287 insKKAAEG	Q286InsKKAAEG_fw	CGATTTCGCACTTCTGCAAAAAAAAGCGGCGGAAGGCGCCATCATGGGTCCTAAG
	Q286InsKKAAEG_rv	CTTAGGACCCATGATGGCGCCTTCCGCCGCTTTTTTTTGCAGAAGTGCGAAATCG
F278_N279 insGP	F278InsGP_fw	CATGGGACCGGCTGGTTCGGCCCAAATGACGATTTCGCAC
	F278InsGP_rv	GTGCGAAATCGTCATTTGGGCCGAACCAGCCGGTCCCATG
F278_N279 insGPRL	F278InsGPRL_fw	CATGGGACCGGCTGGTTCGGCCCACGTCTGAATGACGATTTCGCAC
	F278InsGPRL_rv	GTGCGAAATCGTCATTCAGACGTGGGCCGAACCAGCCGGTCCCATG
Q286_A287 insEM	N279InsP_fw	GGGACCGGCTGGTTCAATCCGGACGATTTCGCACTTC
	N279InsP_rv	GAAGTGCGAAATCGTCCGGATTGAACCAGCCGGTCCC
Q286_A287 insEM	Q286InsEM_fw	CGATTTCGCACTTCTGCAAGAAATGGCCATCATGGGTCCTAAG
	Q286InsEM_rv	CTTAGGACCCATGATGGCCATTTCTTGCAGAAGTGCGAAATCG
Q286_A287 insEMKA	Q286InsEMKA_fw	CGATTTCGCACTTCTGCAAGAAATGAAAGCCGCCATCATGGGTCCTAAG
	Q286InsEMKA_rv	CTTAGGACCCATGATGGCGGCTTTCATTTCTTGCAGAAGTGCGAAATCG
Q286_A287 insEMKAEG	Q286InsEMKAEG_fw	CGATTTCGCACTTCTGCAAGAAATGAAAGCCGAAGGCGCCATCATGGGTCCTAAG
	Q286InsEMKAEG_rv	CTTAGGACCCATGATGGCGCCTTCGGCTTTCATTTCTTGCAGAAGTGCGAAATCG
Q286_A287	Q286InsEMKAEGK_fw	CGATTTCGCACTTCTGCAAGAAATGAAAGCCGAAGGCAAAGCCATCATGGGTCCTAAG

insEMKAEGK	Q286InsEMKAEGK_rv	CTTAGGACCCATGATGGCTTTGCCTTCGGCTTTCATTTCTTGCAGAAGTGCGAAATCG

Supplementary Table 18: Site-directed mutagenesis primers used for the deletion variants. * = mutations incorporated by overlap PCR

Mutation	Name	Sequence (5‘ → 3‘)
E251del	P250DelE_fw	GTCCAGCCTCCCGCCTATGGATTTGTCCCAAGTAAAGTTACCG
	P250DelE_rv	AGGCGGGAGGCTGGACAATACACCTGAAAGATGCGCCATC
P250_M252del	P249DelPEM_fw	CAGGTGTATTGTCCAGCCTCCCGGATTTGTCCCAAGTAAAGTTACC
	P249DelPEM_rv	GGTAACTTTACTTGGGACAAATCCGGGAGGCTGGACAATACACCTG
L248_L254del *	S247DelLPPEMDL_fw	CGCATCTTTCAGGTGTATTGTCCAGCTCCCAAGTAAAGTTACCGTCAAGTGGG
	S247DelLPPEMDL_rv	GCTGGACAATACACCTGAAAGATGCGCCATCGTTCCCGCATGGTACATATC
V257del	Q256DelV_fw	GCCTGAAATGGATTTGTCCCAAAAGTTACCGTCAAGTGGGAATCAGTTC
	Q256DelV_rv	TTGGGACAAATCCATTTCAGGCGGGAGGCTGGACAATACACCTG
Q256_K258del	S255DelQVK_fw	CGCCTGAAATGGATTTGTCCTTACCGTCAAGTGGGAATCAGTTCCG
	S255DelQVK_rv	GGACAAATCCATTTCAGGCGGGAGGCTGGACAATACACCTGAAAG
L254_P260del *	D253DelLSQVKLP_fw	GCCTCCCGCCTGAAATGGATTCAAGTGGGAATCAGTTCCGGGCTAAG
	D253DelLSQVKLP_rv	ATCCATTTCAGGCGGGAGGCTGGACAATACACCTGAAAGATGCGCC
A283del	F282DelA_fw	GGCTGGTTCAATGACGATTTCCTTCTGCAAGCCATCATGGGTC
	F282DelA_rv	GAAATCGTCATTGAACCAGCCGGTCCCATGTCCACCCCAC
F282_L284del	D281DelFAL_fw	CCGGCTGGTTCAATGACGATCTGCAAGCCATCATGGGTCC
	D281DelFAL_rv	TCGTCATTGAACCAGCCGGTCCCATGTCCACCCCACTTAGC
D280_Q286del *	N279DelDDFALLQ_fw	CATGGGACCGGCTGGTTCAATGCCATCATGGGTCCTAAGGTTGTCG
	N279DelDDFALLQ_rv	ATTGAACCAGCCGGTCCCATGTCCACCCCACTTAGCCCGG

Supplementary Table 19: Site-directed mutagenesis primers used for the LILI library after position V257.

Mutation	Name	Sequence (5‘ → 3‘)
V257_K258 insGG	V257_K258insGG_fw	GGATTTGTCCCAAGTAGGTGGTAAGTTACCGTCAAGTGG
	V257_K258insGG_rv	CCACTTGACGGTAACTTACCACCTACTTGGGACAAATCC
V257_K258 insGGG	V257_K258insGGG_fw	GGATTTGTCCCAAGTAGGTGGTGGTAAGTTACCGTCAAGTGG
	V257_K258insGGG_rv	CCACTTGACGGTAACTTACCACCACCTACTTGGGACAAATCC
V257_K258 insGGGG	V257_K258insGGGG_fw	GGATTTGTCCCAAGTAGGTGGTGGTGGTAAGTTACCGTCAAGTGG
	V257_K258insGGGG_rv	CCACTTGACGGTAACTTACCACCACCACCTACTTGGGACAAATCC
V257_K258 insGGGGG	V257_K258insGGGGG_fw	GGATTTGTCCCAAGTAGGTGGTGGTGGTGGTAAGTTACCGTCAAGTG G
	V257_K258insGGGGG_rv	CCACTTGACGGTAACTTACCACCACCACCACCTACTTGGGACAAATC C
V257_K258 insGGGGGG	V257_K258insGGGGGG_fw	GGATTTGTCCCAAGTAGGTGGTGGTGGTGGTGGTAAGTTACCGTCAA GTGG
	V257_K258insGGGGGG_rv	CCACTTGACGGTAACTTACCACCACCACCACCACCTACTTGGGACAA ATCC
V257_K258 insGS	V257_K258insGS_fw	GGATTTGTCCCAAGTAGGTTCTAAGTTACCGTCAAGTGG
	V257_K258insGS_rv	CCACTTGACGGTAACTTAGAACCTACTTGGGACAAATCC
V257_K258 insGSG	V257_K258insGSG_fw	GGATTTGTCCCAAGTAGGTTCTGGTAAGTTACCGTCAAGTGG
	V257_K258insGSG_rv	CCACTTGACGGTAACTTAGAACCAGATACTTGGGACAAATCC
V257_K258 insGSGS	V257_K258insGSGS_fw	GGATTTGTCCCAAGTAGGTTCTGGTTCTAAGTTACCGTCAAGTGG
	V257_K258insGSGS_rv	CCACTTGACGGTAACTTAGAACCAGAACCTACTTGGGACAAATCC
V257_K258 insGSGSG	V257_K258insGSGSG_fw	GGATTTGTCCCAAGTAGGTTCTGGTTCTGGTAAGTTACCGTCAAGTG G
	V257_K258insGSGSG_rv	CCACTTGACGGTAACTTAGAACCAGAACCAGATACTTGGGACAAATC C
V257_K258 insGSGSGS	V257_K258insGSGSGS_fw	GGATTTGTCCCAAGTAGGTTCTGGTTCTGGTTCTAAGTTACCGTCAA GTGG
	V257_K258insGSGSGS_rv	CCACTTGACGGTAACTTAGAACCAGAACCAGAACCTACTTGGGACAA ATCC
V257_K258 insPA	V257_K258insPA_fw	GGATTTGTCCCAAGTACCGGCAAAGTTACCGTCAAGTGG
	V257_K258insPA_rv	CCACTTGACGGTAACTTTGCCGGTACTTGGGACAAATCC
V257_K258 insPAP	V257_K258insPAP_fw	GGATTTGTCCCAAGTACCGGCACCGAAGTTACCGTCAAGTGG
	V257_K258insPAP_rv	CCACTTGACGGTAACTTCGGTGCCGGTACTTGGGACAAATCC

V257_K258 insPAPA	V257_K258ins PAPA_fw	GGATTTGTCCCAAGTACCGGCACCGGCAAAGTTACCGTCAAGTGG
	V257_K258ins PAPA_rv	CCACTTGACGGTAACTTTGCCGGTGCCGGTACTTGGGACAAATCC
V257_K258 insPAPAP	V257_K258ins PAPAP_fw	GGATTTGTCCCAAGTACCGGCACCGGCACCGAAGTTACCGTCAAGTGG
	V257_K258ins PAPAP_rv	CCACTTGACGGTAACTTCGGTGCCGGTGCCGGTACTTGGGACAAATCC
V257_K258 insPAPAPA	V257_K258ins PAPAPA_fw	GGATTTGTCCCAAGTACCGGCACCGGCACCGGCAAAGTTACCGTCAAGTGG
	V257_K258ins PAPAPA_rv	CCACTTGACGGTAACTTTGCCGGTGCCGGTGCCGGTACTTGGGACAAATCC
V257_K258 insGP	V257_K258ins GP_fw	GGATTTGTCCCAAGTAGGTCCGAAGTTACCGTCAAGTGG
	V257_K258ins GP_rv	CCACTTGACGGTAACTTCGGACCTACTTGGGACAAATCC
V257_K258 insGPG	V257_K258ins GPG_fw	GGATTTGTCCCAAGTAGGTCCGGGTAAGTTACCGTCAAGTGG
	V257_K258ins GPG_rv	CCACTTGACGGTAACTTACCCGGACCTACTTGGGACAAATCC
V257_K258 insGPGP	V257_K258ins GPGP_fw	GGATTTGTCCCAAGTAGGTCCGGGTCCGAAGTTACCGTCAAGTGG
	V257_K258ins GPGP_rv	CCACTTGACGGTAACTTCGGACCCGGACCTACTTGGGACAAATCC
V257_K258 insGPGPG	V257_K258ins GPGPG_fw	GGATTTGTCCCAAGTAGGTCCGGGTCCGGGTAAGTTACCGTCAAGTGG
	V257_K258ins GPGPG_rv	CCACTTGACGGTAACTTACCCGGACCCGGACCTACTTGGGACAAATCC
V257_K258 insGPGPGP	V257_K258ins GPGPGP_fw	GGATTTGTCCCAAGTAGGTCCGGGTCCGGGTCCGAAGTTACCGTCAAGTGG
	V257_K258ins GPGPGP_rv	CCACTTGACGGTAACTTCGGACCCGGACCCGGACCTACTTGGGACAAATCC

Supplementary Table 20: Site-directed mutagenesis primers used for the LILI library after position F278.

Mutation	Name	Sequence (5‘ → 3‘)
F278_N279 insGG	F278_N279insGG_fw	GGACATGGGACCGGCTGGTTCGGTGGTAATGACGATTTCGCACTTC
	F278_N279insGG_rv	GAAGTGCGAAATCGTCATTACCACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGGG	F278_N279insGGG_fw	GGACATGGGACCGGCTGGTTCGGTGGTGGTAATGACGATTTCGCACTTC
	F278_N279insGGG_rv	GAAGTGCGAAATCGTCATTACCACCACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGGGG	F278_N279insGGGG_fw	GGACATGGGACCGGCTGGTTCGGTGGTGGTGGTAATGACGATTTCGCACTTC
	F278_N279insGGGG_rv	GAAGTGCGAAATCGTCATTACCACCACCACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGGGGG	F278_N279insGGGGG_fw	GGACATGGGACCGGCTGGTTCGGTGGTGGTGGTGGTAATGACGATTTCGCACTTC
	F278_N279insGGGGG_rv	GAAGTGCGAAATCGTCATTACCACCACCACCACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGGGGGG	F278_N279insGGGGGG_fw	GGACATGGGACCGGCTGGTTCGGTGGTGGTGGTGGTGGTAATGACGATTTCGCACTTC
	F278_N279insGGGGGG_rv	GAAGTGCGAAATCGTCATTACCACCACCACCACCACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGS	F278_N279insGS_fw	GGACATGGGACCGGCTGGTTCGGTTCTAATGACGATTTCGCACTTC
	F278_N279insGS_rv	GAAGTGCGAAATCGTCATTAGAACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGSG	F278_N279insGSG_fw	GGACATGGGACCGGCTGGTTCGGTTCTGGTAATGACGATTTCGCACTTC
	F278_N279insGSG_rv	GAAGTGCGAAATCGTCATTACCAGAACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGSGS	F278_N279insGSGS_fw	GGACATGGGACCGGCTGGTTCGGTTCTGGTTCTAATGACGATTTCGCACTTC
	F278_N279insGSGS_rv	GAAGTGCGAAATCGTCATTAGAACCAGAACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGSGSG	F278_N279insGSGSG_fw	GGACATGGGACCGGCTGGTTCGGTTCTGGTTCTGGTAATGACGATTTCGCACTTC
	F278_N279insGSGSG_rv	GAAGTGCGAAATCGTCATTACCAGAACCAGAACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGSGSGS	F278_N279insGSGSGS_fw	GGACATGGGACCGGCTGGTTCGGTTCTGGTTCTGGTTCTAATGACGATTTCGCACTTC
	F278_N279insGSGSGS_rv	GAAGTGCGAAATCGTCATTAGAACCAGAACCAGAACCGAACCAGCCGGTCCCATGTCC
F278_N279 insPA	F278_N279insPA_fw	GGACATGGGACCGGCTGGTTCCCGGCAAATGACGATTTCGCACTTC
	F278_N279insPA_rv	GAAGTGCGAAATCGTCATTTGCCGGGAACCAGCCGGTCCCATGTCC
F278_N279 insPAP	F278_N279insPAP_fw	GGACATGGGACCGGCTGGTTCCCGGCACCGAATGACGATTTCGCACTTC
	F278_N279insPAP_rv	GAAGTGCGAAATCGTCATTCGGTGCCGGGAACCAGCCGGTCCCATGTCC

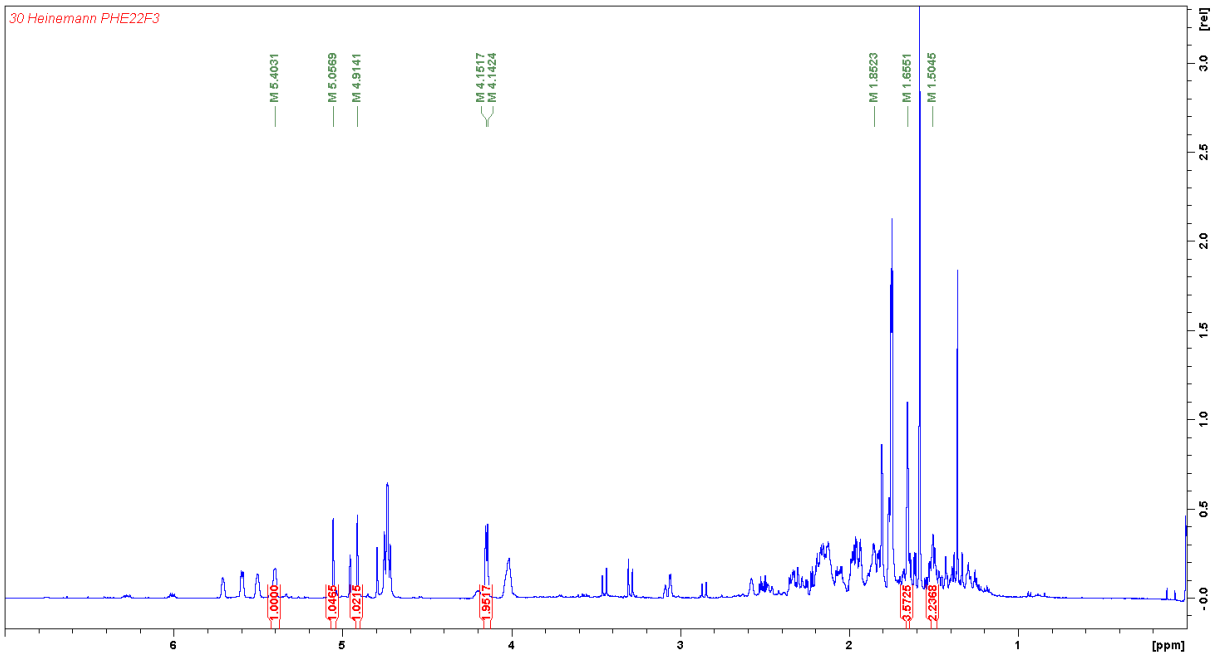
F278_N279 insPAPA	F278_N279ins PAPA_fw	GGACATGGGACCGGCTGGTTCCCGGCACCGGCAAATGACGATTTCGC ACTTC
	F278_N279ins PAPA_rv	GAAGTGCGAAATCGTCATTTGCCGGTGCCGGGAACCAGCCGGTCCCA TGTCC
F278_N279 insPAPAP	F278_N279ins PAPAP_fw	GGACATGGGACCGGCTGGTTCCCGGCACCGGCACCGAATGACGATTT CGCACTTC
	F278_N279ins PAPAP_rv	GAAGTGCGAAATCGTCATTCGGTGCCGGTGCCGGGAACCAGCCGGTC CCATGTCC
F278_N279 insPAPAP A	F278_N279ins PAPAPA_fw	GGACATGGGACCGGCTGGTTCCCGGCACCGGCACCGGCAAATGACGA TTTCGCACTTC
	F278_N279ins PAPAPA_rv	GAAGTGCGAAATCGTCATTTGCCGGTGCCGGTGCCGGGAACCAGCCG GTCCCATGTCC
F278_N279 insGP	F278_N279ins GP_fw	GGACATGGGACCGGCTGGTTCGGTCCGAATGACGATTTCGCACTTC
	F278_N279ins GP_rv	GAAGTGCGAAATCGTCATTCGGACCGAACCAGCCGGTCCCATGTCC
F278_N279 insGPG	F278_N279ins GPG_fw	GGACATGGGACCGGCTGGTTCGGTCCGGGTAATGACGATTTCGCACT TC
	F278_N279ins GPG_rv	GAAGTGCGAAATCGTCATTACCCGGACCGAACCAGCCGGTCCCATGT CC
F278_N279 insGPGP	F278_N279ins GPGP_fw	GGACATGGGACCGGCTGGTTCGGTCCGGGTCCGAATGACGATTTCGC ACTTC
	F278_N279ins GPGP_rv	GAAGTGCGAAATCGTCATTCGGACCCGGACCGAACCAGCCGGTCCCA TGTCC
F278_N279 insGPGPG	F278_N279ins GPGPG_fw	GGACATGGGACCGGCTGGTTCGGTCCGGGTCCGGGTAATGACGATTT CGCACTTC
	F278_N279ins GPGPG_rv	GAAGTGCGAAATCGTCATTACCCGGACCCGGACCGAACCAGCCGGTC CCATGTCC
F278_N279 insGPGPG P	F278_N279ins GPGPGP_fw	GGACATGGGACCGGCTGGTTCGGTCCGGGTCCGGGTCCGAATGACGA TTTCGCACTTC
	F278_N279ins GPGPGP_rv	GAAGTGCGAAATCGTCATTCGGACCCGGACCCGGACCGAACCAGCCG GTCCCATGTCC

Supplementary Table 21: Site-directed mutagenesis primers used for the LILI library after position Q286.

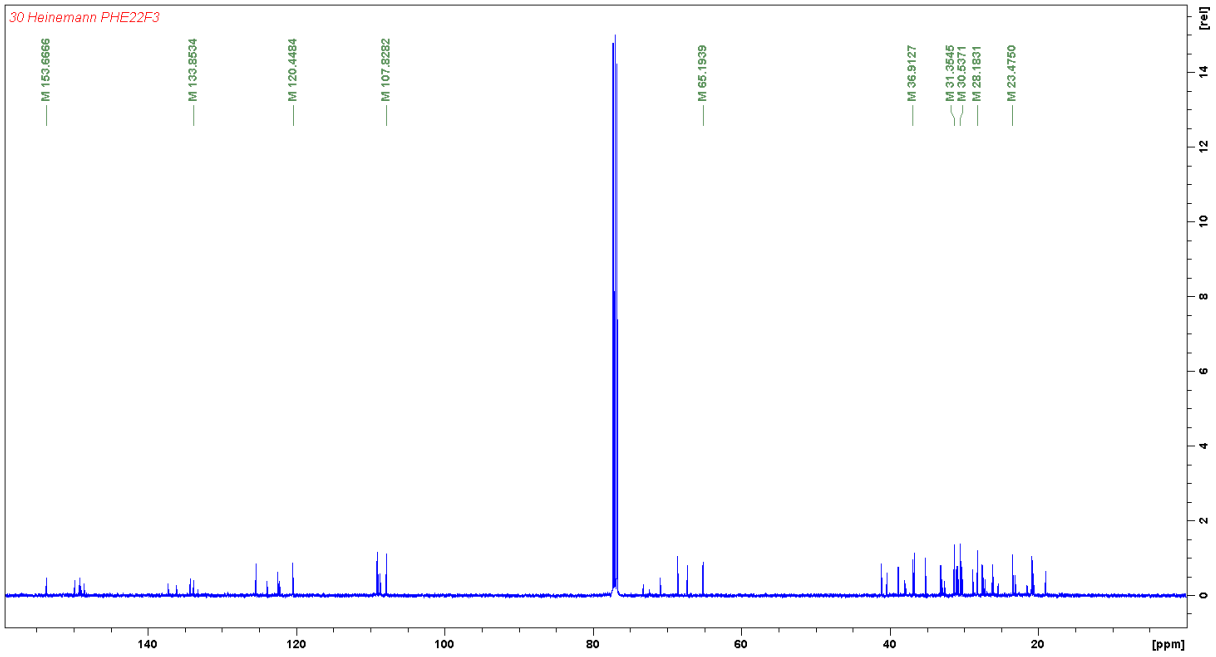
Mutation	Name	Sequence (5‘ → 3‘)
Q286_A287 insGG	Q286_A287insGG_fw	GACGATTTCGCACTTCTGCAAGGTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGG_rv	CCTTAGGACCCATGATGGCACCACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGGG	Q286_A287insGGG_fw	GACGATTTCGCACTTCTGCAAGGTGGTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGGG_rv	CCTTAGGACCCATGATGGCACCACCACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGGGG	Q286_A287insGGGG_fw	GACGATTTCGCACTTCTGCAAGGTGGTGGTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGGGG_rv	CCTTAGGACCCATGATGGCACCACCACCACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGGGGG	Q286_A287insGGGGG_fw	GACGATTTCGCACTTCTGCAAGGTGGTGGTGGTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGGGGG_rv	CCTTAGGACCCATGATGGCACCACCACCACCACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGGGGGG	Q286_A287insGGGGGG_fw	GACGATTTCGCACTTCTGCAAGGTGGTGGTGGTGGTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGGGGGG_rv	CCTTAGGACCCATGATGGCACCACCACCACCACCACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGS	Q286_A287insGS_fw	GACGATTTCGCACTTCTGCAAGGTTCTGCCATCATGGGTCCTAAGG
	Q286_A287insGS_rv	CCTTAGGACCCATGATGGCAGAACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGSG	Q286_A287insGSG_fw	GACGATTTCGCACTTCTGCAAGGTTCTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGSG_rv	CCTTAGGACCCATGATGGCACCAGAACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGSGS	Q286_A287insGSGS_fw	GACGATTTCGCACTTCTGCAAGGTTCTGGTTCTGCCATCATGGGTCCTAAGG
	Q286_A287insGSGS_rv	CCTTAGGACCCATGATGGCAGAACCAGAACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGSGSG	Q286_A287insGSGSG_fw	GACGATTTCGCACTTCTGCAAGGTTCTGGTTCTGGTGCCATCATGGGTCCTAAGG
	Q286_A287insGSGSG_rv	CCTTAGGACCCATGATGGCACCAGAACCAGAACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGSGSGS	Q286_A287insGSGSGS_fw	GACGATTTCGCACTTCTGCAAGGTTCTGGTTCTGGTTCTGCCATCATGGGTCCTAAGG
	Q286_A287insGSGSGS_rv	CCTTAGGACCCATGATGGCAGAACCAGAACCAGAACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insPA	Q286_A287insPA_fw	GACGATTTCGCACTTCTGCAACCGGCAGCCATCATGGGTCCTAAGG
	Q286_A287insPA_rv	CCTTAGGACCCATGATGGCTGCCGGTTGCAGAAGTGCGAAATCGTC
Q286_A287 insPAP	Q286_A287insPAP_fw	GACGATTTCGCACTTCTGCAACCGGCACCGGCCATCATGGGTCCTAAGG
	Q286_A287insPAP_rv	CCTTAGGACCCATGATGGCCGGTGCCGGTTGCAGAAGTGCGAAATCGTC

Q286_A287 insPAPA	Q286_A287ins PAPA_fw	GACGATTTCGCACTTCTGCAACCGGCACCGGCAGCCATCATGGGTCC TAAGG
	Q286_A287ins PAPA_rv	CCTTAGGACCCATGATGGCTGCCGGTGCCGGTTGCAGAAGTGCGAAA TCGTC
Q286_A287 insPAPAP	Q286_A287ins PAPAP_fw	GACGATTTCGCACTTCTGCAACCGGCACCGGCACCGGCCATCATGGG TCCTAAGG
	Q286_A287ins PAPAP_rv	CCTTAGGACCCATGATGGCCGGTGCCGGTGCCGGTTGCAGAAGTGCG AAATCGTC
Q286_A287 insPAPAP A	Q286_A287ins PAPAPA_fw	GACGATTTCGCACTTCTGCAACCGGCACCGGCACCGGCAGCCATCAT GGGTCCTAAGG
	Q286_A287ins PAPAPA_rv	CCTTAGGACCCATGATGGCTGCCGGTGCCGGTGCCGGTTGCAGAAGT GCGAAATCGTC
Q286_A287 insGP	Q286_A287ins GP_fw	GACGATTTCGCACTTCTGCAAGGTCCGGCCATCATGGGTCCTAAGG
	Q286_A287ins GP_rv	CCTTAGGACCCATGATGGCCGGACCTTGCAGAAGTGCGAAATCGTC
Q286_A287 insGPG	Q286_A287ins GPG_fw	GACGATTTCGCACTTCTGCAAGGTCCGGGTGCCATCATGGGTCCTAA GG
	Q286_A287ins GPG_rv	CCTTAGGACCCATGATGGCACCCGGACCTTGCAGAAGTGCGAAATCG TC
Q286_A287 insGPGP	Q286_A287ins GPGP_fw	GACGATTTCGCACTTCTGCAAGGTCCGGGTCCGGCCATCATGGGTCC TAAGG
	Q286_A287ins GPGP_rv	CCTTAGGACCCATGATGGCCGGACCCGGACCTTGCAGAAGTGCGAAA TCGTC
Q286_A287 insGPGPG	Q286_A287ins GPGPG_fw	GACGATTTCGCACTTCTGCAAGGTCCGGGTCCGGGTGCCATCATGGG TCCTAAGG
	Q286_A287ins GPGPG_rv	CCTTAGGACCCATGATGGCACCCGGACCCGGACCTTGCAGAAGTGCG AAATCGTC
Q286_A287 insGPGPG P	Q286_A287ins GPGPGP_fw	GACGATTTCGCACTTCTGCAAGGTCCGGGTCCGGGTCCGGCCATCAT GGGTCCTAAGG
	Q286_A287ins GPGPGP_rv	CCTTAGGACCCATGATGGCCGGACCCGGACCCGGACCTTGCAGAAGT GCGAAATCGTC

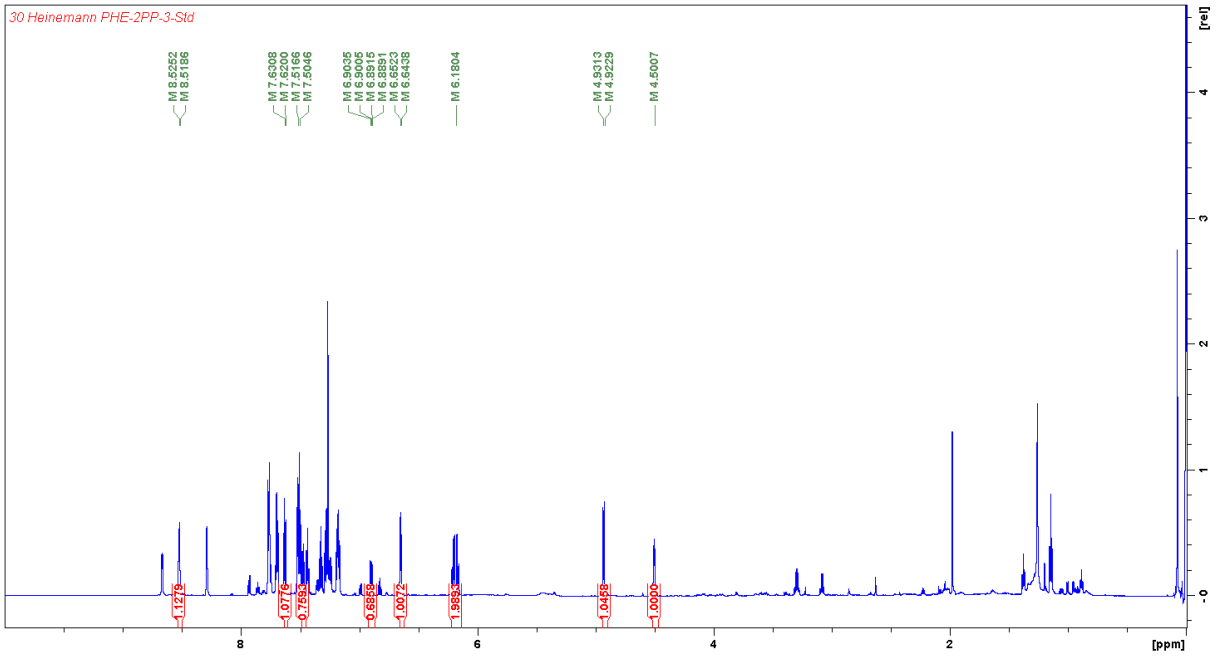
NMR spectra



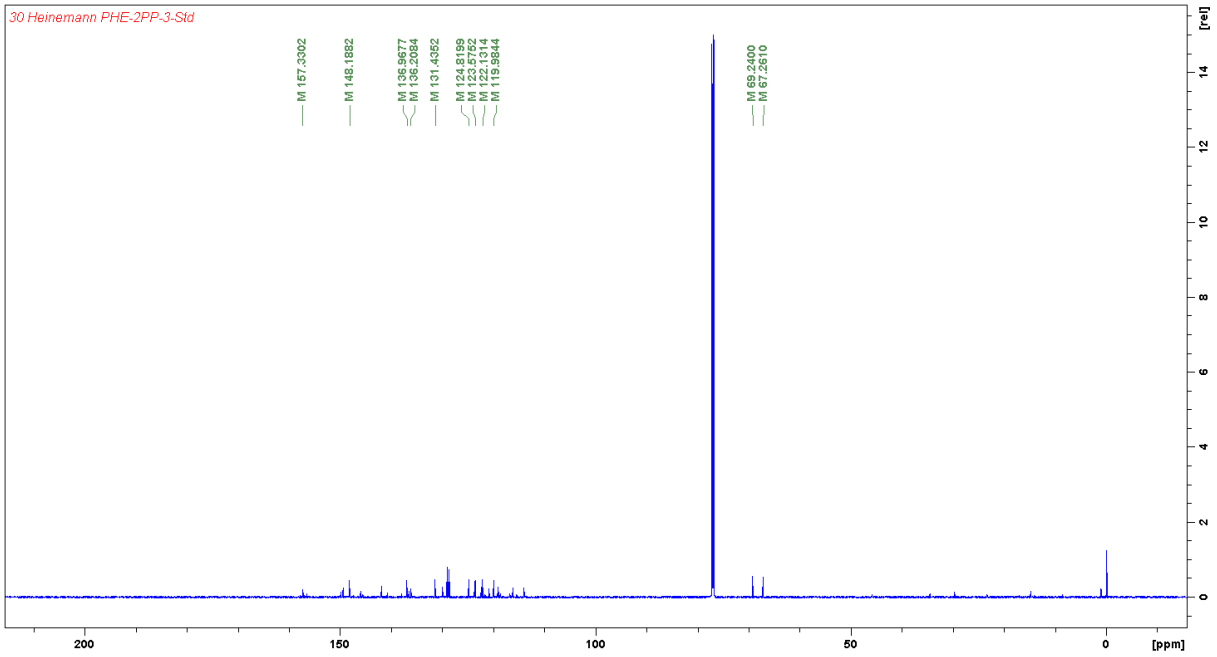
Supplementary Fig. 29: ^{1}H-NMR of the product of the synthesis of (+)-mentha-1.8-dien-10-ol 2b in $CDCl_3$



Supplementary Fig. 30: ^{13}C-NMR of the product of the synthesis of (+)-mentha-1.8-dien-10-ol 2b in $CDCl_3$



Supplementary Fig. 31: ^{1}H-NMR of the in-house standard of 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a.



Supplementary Fig. 32: ^{13}C-NMR of the in-house standard of 1,2-dihydroxy-3-(2'-pyridyl)-cyclohexa-3,5-diene 3a.

Sequence of the Cumene Dioxygenase from *Pseudomonas fluorescens* IP01

CDO gene cluster (GenBank: D37828.1)[11]

cumA1 (Oxygenase α-subunit) → cumA2 (Oxygenase β-subunit)→ cumA3 (Ferredoxin) → cumA4 (Reductase)

Overlapping sequence space between cumA3 and cumA4 is underlined.

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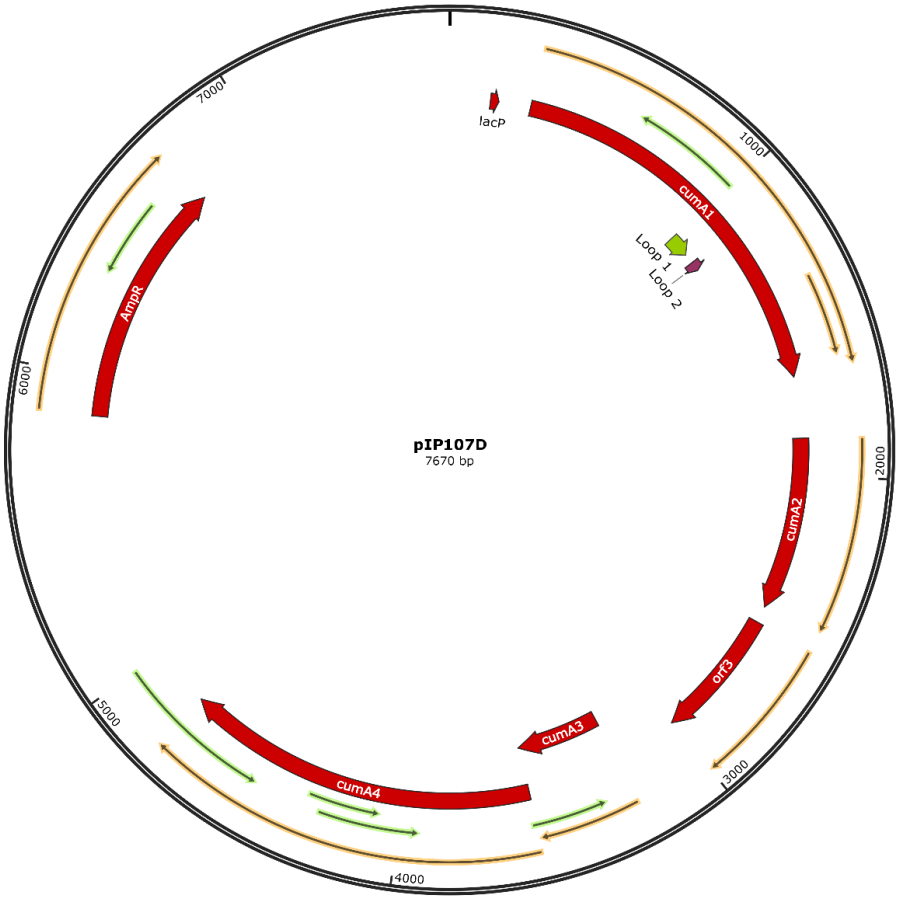

Fig. 33: Plasmid map of pIP107D.[1]

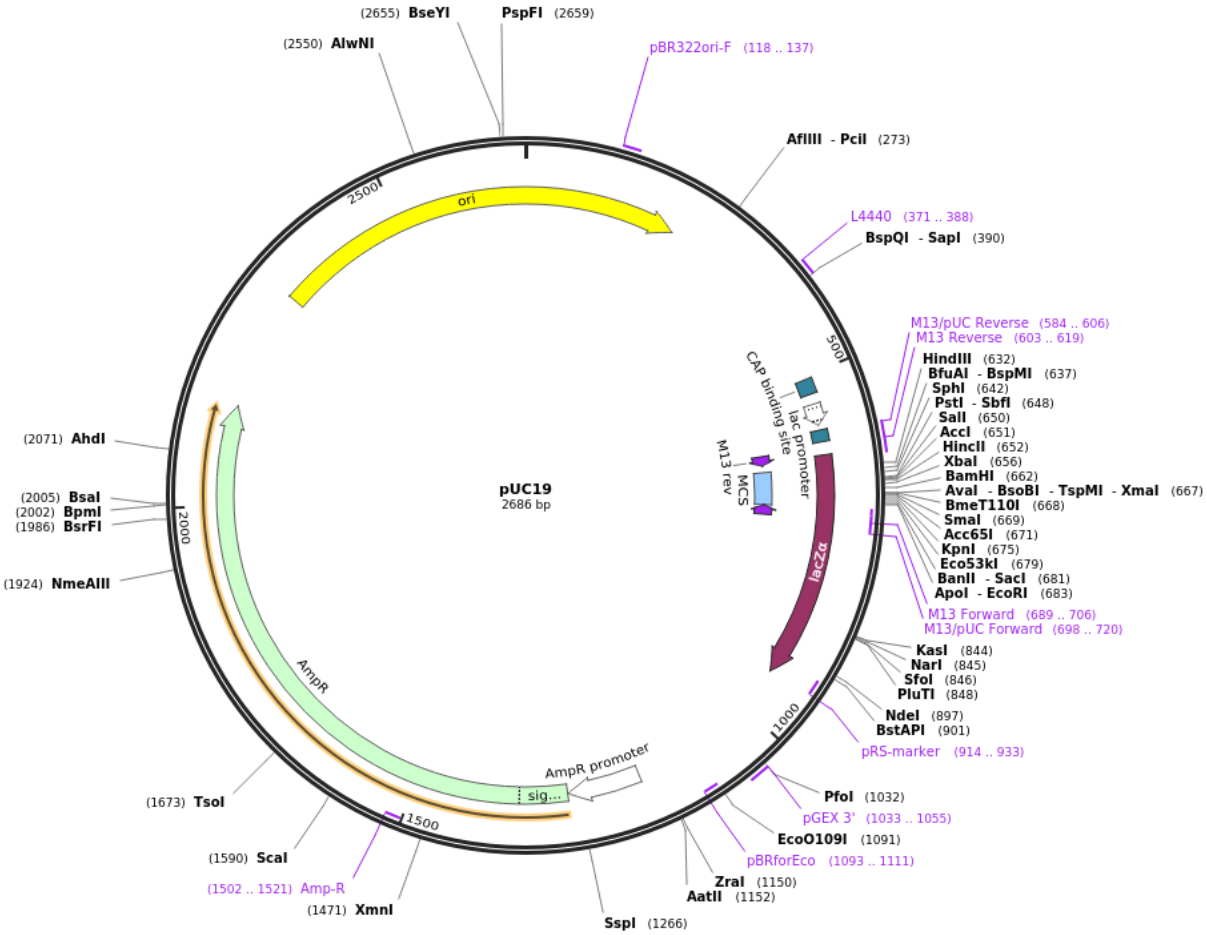

Fig. 34: Plasmid map of pUC19. (Source: https://www.addgene.org/50005, July 2020)

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