Materials and Methods

Chemicals and reagents

Antibiotics, analytical grade glycerol, isopropyl β-D-1-thiogalactopyranoside (IPTG), sodium chloride, sodium ascorbate, glucose, and iron sulfate heptahydrate, HPLC grade acetonitrile, methanol, trifluoracetic acid (TFA), and acetic acid were purchased from Merck (USA). Restriction enzymes, Phusion High Fidelity DNA Polymerase, NEBuilder HiFi DNA Assembly, *E. coli* 5-alpha competent cells and *E. coli* BL21(DE3) competent cells (an *E. coli* B strain derivative) were purchased from New England BioLabs Inc., USA. Yeast extract and tryptone for medium preparation were purchased from Oxiod, Thermo Scientific, UK. Water for all the experiments was purified to a resistivity of ≥ 18.2 MΩ.cm. Thebaine, oripavine, codeine and morphine and partially processed crude poppy extract (obtained from a manufacturing line run using established optimized extraction methods[1]) were provided by Sun Pharmaceutical Industries Australia Pty Ltd.

Plasmids and strains

Plasmids used in this study are listed in Supplementary Table 1, primers and synthetic sequences are listed in Supplementary Table 8 and 9. Synthetic gBlock sequences were codon optimized for expression in *E. coli* B strains and ordered from Integrated DNA Technologies (IDT, USA). Sufficient vector homology (~20 bp) for assembly by NEBuilder HiFi DNA Assembly was designed into the gBlocks to speed up construct creation. The T7 polymerase expression vector pET24b-6H-MBP (Merck, USA) was used to control expression of the genes of interest. Each open reading frame (ORF) was *N*-terminally fused to a six-histidine tag (6H) and a maltose binding protein (MBP). In addition, the ORFs were under the transcriptional control of the T7 promoter and the T7 terminator. *E. coli* 5-alpha competent cells were used for plasmid cloning and maintenance, while *E. coli* BL21(DE3) competent cells were used for protein expression and biotransformation. Assembled constructs were verified by sequencing (Australian Genome Research Facility, Australia) using primers UM13/UM03 (see Supplementary Table 8).

The CODM.2 (UniProtKB: D4N502) (referred to here as WT CODM for ease of comparison) expression plasmid employing *E. coli* codon usage, pGWKS100, was created by assembling the

1.1 kb CODM gBlock (UMg4) into the BamHI- and XhoI-linearized pET24b-6H-MBP backbone using NEBuilder.

Several of the expression vectors (pGWKS101, 119-126, see Supplementary Table 1) were created using the same procedure; the wild type residue was replaced with the appropriate residue by PCR amplification (see Supplementary Table 8) and then cloned into the linearized pET24b-6H-MBP expression vector using NEBuilder. For example, to construct the expression vector, pGWKS101, of the endogenous CODM E259K variant (NCBI Reference Sequence: XP_026416234.1) the glutamic acid (E) residue present within the CODM WT isoform was replaced with a lysine (K) residue by PCR amplification (UM15/UM05 and UM06/UM18) and the two fragments assembled into linearized pET24b-6H-MBP via NEBuilder.

The remaining expression vectors (pGWSK131-133) (see Supplementary Table 1) were created by assembling the respective 1.1 kb ORF gBlocks (UMg8-26) (see Supplementary Table 9) into the linearized pET24b-6H-MBP backbone using NEBuilder.

Cell culture and protein expression

Cells were cultured overnight at 37°C in Lysogeny Broth medium (0.5% yeast extract, 1% tryptone and 1% NaCl) supplemented with kanamycin (50 μg/mL). 1 mL of the overnight cell culture was inoculated into conical flask that contained 50 mL 2-YT medium (1% yeast extract, 1.6% tryptone and 0.5% NaCl), supplemented with kanamycin (50 μg/mL) and 1 mL glycerol. Protein expression was induced by IPTG addition (0.1 mM) when OD600 (optical density measured at 600 nm) reached 0.4-0.8 and continued for 22 hours at 18°C. Following protein expression cells pellets were collected, by centrifugation at 3,000 g for 15 minutes, and resuspended in 15% glycerol to an OD600 of 100 for biotransformation.

Whole cell biotransformation

Biotransformation reactions were conducted in a 20 mL sample volume consisting of the indicated alkaloid (1 mM thebaine, 1 mM codeine or thebaine crude poppy extract), 100 mM phosphate buffer (pH 6.0), 0.5% w/w glucose, 10 μM iron(II) sulfate ($FeSO_4$) and 10 mM sodium ascorbate and *E. coli* cells with an OD600 of 10. The reaction was conducted at 24°C with shaking at 220

rpm. Samples were taken regularly at different time intervals. Samples collected were centrifuged, at 16,000 g for 7 minutes, and the supernatant was analyzed by liquid chromatography-mass spectrometer for alkaloid quantification. Three independent replicates were conducted for CODM mutant biotransformations on pure thebaine and codeine, and three for thebaine crude extract biotransformations.

Protein expression analysis

The soluble protein produced by protein expression was determined by SDS-PAGE analysis using the BugBuster™ protocol provided by the manufacturer. Briefly, the soluble protein fraction was liberated from frozen cell pellets, where the OD600 was 1, by resuspension in BugBuster™ protein extraction reagent containing benzonase (25 units/mL of BugBuster™ reagent) (Merck, Germany). The insoluble cell debris and soluble protein containing supernatant were then separated by centrifugation at 16,000 g for 20 minutes and 4°C. The soluble protein fractions were run on a pre-cast Bolt 8% Bis-Tris Plus Gel (ThermoFisher Scientific, USA) in MOPS running buffer at 120 V for 60 minutes. Protein size was estimated using the Precision Plus protein™ Kaleidoscope™ Prestained Protein Standard (Bio-Rad Laboratories, USA). Band intensity (less background intensity and normalized to the 75 kDa molecular weight band) was calculated using the Image Lab software (Bio-Rad Laboratories, USA). Soluble protein expression was determined for each of the CODM mutant assays independent replicates.

Statistical analysis

Minitab® was employed for statistical analysis. A general linear model ANOVA (analysis of variance) was used to determine statistical significance with a P-value of 0.05. Dunnett's method was used to created 95% confidence intervals for comparison against the control strain.

LC-MS analysis of alkaloids

Quantification of alkaloids was performed using a Shimadzu LCMS-2020 liquid chromatograph mass spectrometer. Analysis was carried out on an Onyx Monolithic C18 column (100 x 4.6 mm, Phenomenex Australia Pty Ltd), with a linear gradient of 0-20% buffer B and at a flow rate increased from 1 mL/min to 2.5 mL/min over 10 min at 28°C [buffer A: 0.1% TFA in water; buffer B: 0.1% TFA in acetonitrile]. The detector wavelength was set at 285 nm with the reference

wavelength set at 360 nm. Alkaloid compounds in biotransformation samples were identified by comparing to alkaloid standards, referring to both retention time [morphine at 4.0 min, codeine at 6.8 min, oripavine at 7.5 min and thebaine at 10.1 min] and mass to charge ratio (*m/z*) [morphine 286, codeine 300, oripavine 298 and thebaine 312]. Shimadzu LabSolutions software was used to integrate the peak area for each compound to quantify the concentration of alkaloid in each sample, by referring to the peak area of alkaloid standard series with concentrations ranging from 25 µg/mL to 500 µg/mL. Samples were injected into the LC-MS with a 5 µL injection volume for analysis.

Supplementary figures and tables

Supplementary Table 1: *E. coli* strains corresponding plasmids used in this study

All strains were made in pET24b-6H-MBP

Strain	Plasmid	Genotype	Source
NA	pET24b-6H-MBP	T7 expression vector	Merck
WT CODM	pGWKS100	WT CODM (CODM.2) open reading frame (ORF) in pET24b-6H-MBP	This study
E259K	pGWKS101	CODM E259K (CODM.3) ORF in pET24b-6H-MBP	This study
E259D	pGWKS119	CODM E259D ORF in pET24b-6H-MBP	This study
E259H	pGWKS120	CODM E259H ORF in pET24b-6H-MBP	This study
E259Q	pGWKS121	CODM E259Q ORF in pET24b-6H-MBP	This study
E259A	pGWKS122	CODM E259A ORF in pET24b-6H-MBP	This study
E259S	pGWKS123	CODM E259S ORF in pET24b-6H-MBP	This study
E259G	pGWKS124	CODM E259G ORF in pET24b-6H-MBP	This study
R260T	pGWKS125	CODM R260T ORF in pET24b-6H-MBP	This study
E259G+R260T	pGWSK126	CODM E259G+R260T ORF in pET24b-6H-MBP	This study
R260K	pGWKS131	CODM R260K ORF in pET24b-6H-MBP	This study
E259D+R260K	pGWKS132	CODM E259D+R260K ORF in pET24b-6H-MBP	This study
E259G+R260K	pGWKS133	CODM E259G+R260K ORF in pET24b-6H-MBP	This study

Supplementary Table 2: Average oripavine and morphine yield of the *E. coli* strains expressing CODM mutants with varying amino acids at position 259. Biotransformation was assessed after four hours for oripavine or 30 minutes for morphine. Data are mean ± standard deviation of three independent replicates.

Strain	Oripavine yield (%)	Oripavine TSY (g/(L·h))	Morphine yield (%)	Morphine TSY (g/(L·h))
WT CODM	46 ± 2	$3.3\times10^{-2} \pm 1.6\times10^{-3}$	41 ± 6	$2.4\times10^{-1} \pm 4.0\times10^{-2}$
E259K	29 ± 2	$2.1\times10^{-2} \pm 1.1\times10^{-3}$	30± 3	$1.8\times10^{-1} \pm 2.2\times10^{-2}$
E259D	63 ± 2	$4.5\times10^{-2} \pm 2.4\times10^{-3}$	53 ± 7	$3.2\times10^{-1} \pm 5.6\times10^{-2}$
E259H	28 ± 1	$2.0\times10^{-2} \pm 6.7\times10^{-4}$	26 ± 3	$1.6\times10^{-1} \pm 2.1\times10^{-2}$
E259Q	30 ± 2	$2.2\times10^{-2} \pm 1.4\times10^{-3}$	28 ± 2	$1.6\times10^{-1} \pm 1.6\times10^{-2}$
E259A	22 ± 1	$1.6\times10^{-2} \pm 1.5\times10^{-3}$	22 ± 3	$1.3\times10^{-1} \pm 2.4\times10^{-2}$
E259S	25 ± 1	$1.8\times10^{-2} \pm 1.2\times10^{-3}$	26 ± 3	$1.5\times10^{-1} \pm 2.3\times10^{-2}$
E259G	58 ± 4	$4.1\times10^{-2} \pm 4.3\times10^{-3}$	51 ± 8	$3.0\times10^{-1} \pm 5.4\times10^{-2}$

Supplementary Table 3: Average oripavine and morphine yield of the *E. coli* strains expressing CODM mutants with varying amino acids at 259 or 260 or both amino acid residues. Biotransformation was assessed after four hours for oripavine or 30 minutes for morphine. Data are mean ± standard deviation of three independent replicates.

Strain	Oripavine yield (%)	Oripavine TSY (g/(L·h))	Morphine yield (%)	Morphine TSY (g/(L·h))
WT CODM	50 ± 1	$3.6\times10^{-2} \pm 2.1\times10^{-3}$	44 ± 8	$2.8\times10^{-1} \pm 4.4\times10^{-2}$
E259D	65 ± 1	$4.6\times10^{-2} \pm 6.6\times10^{-4}$	59 ± 1	$3.8\times10^{-1} \pm 1.5\times10^{-2}$
E259G	62 ± 3	$4.5\times10^{-2} \pm 1.5\times10^{-3}$	55 ± 2	$3.6\times10^{-1} \pm 1.7\times10^{-2}$
R260T	36 ± 1	$2.6\times10^{-2} \pm 9.8\times10^{-4}$	31 ± 2	$2.0\times10^{-1} \pm 1.2\times10^{-2}$
E259G+R260T	53 ± 3	$3.4\times10^{-2} \pm 3.0\times10^{-4}$	48 ± 3	$2.7\times10^{-1} \pm 2.3\times10^{-2}$
R260K	49 ± 1	$3.7\times10^{-2} \pm 1.8\times10^{-3}$	42 ± 5	$3.1\times10^{-1} \pm 1.2\times10^{-2}$
E259D+R260K	66 ± 2	$4.7\times10^{-2} \pm 1.2\times10^{-3}$	59 ± 2	$3.8\times10^{-1} \pm 6.5\times10^{-3}$
E259G+R260K	63 ± 2	$4.5\times10^{-2} \pm 6.2\times10^{-4}$	53 ± 3	$3.4\times10^{-1} \pm 1.8\times10^{-2}$

Supplementary Table 4: Oripavine yield for the *E. coli* strains expressing WT CODM or mutants E259D or E259G after select time points using thebaine as the substrate. Data are mean ± standard deviation of three independent replicates.

Time (h)	WT CODM yield (%)	WT CODM TSY (g/(L·h))	E259D yield (%)	E259D TSY (g/(L·h))	E259G yield (%)	E259G TSY (g/(L·h))
1	14 ± 1	$3.9\times10^{-2} \pm 2.2\times10^{-3}$	17 ± 1	$4.9\times10^{-2} \pm 1.4\times10^{-3}$	19 ± 1	$5.3\times10^{-2} \pm 3.0\times10^{-3}$
2	25 ± 2	$3.6\times10^{-2} \pm 2.5\times10^{-3}$	33 ± 1	$4.8\times10^{-2} \pm 1.4\times10^{-3}$	34 ± 1	$4.9\times10^{-2} \pm 2.4\times10^{-3}$
3	37 ± 3	$3.4\times10^{-2} \pm 2.5\times10^{-3}$	48 ± 1	$4.6\times10^{-2} \pm 1.9\times10^{-3}$	48 ± 2	$4.6\times10^{-2} \pm 2.2\times10^{-3}$
4	46 ± 3	$3.3\times10^{-2} \pm 2.2\times10^{-3}$	59 ± 1	$4.2\times10^{-2} \pm 1.7\times10^{-3}$	59 ± 2	$4.3\times10^{-2} \pm 1.2\times10^{-3}$
5	53 ± 3	$3.0\times10^{-2} \pm 1.9\times10^{-3}$	67 ± 1	$3.9\times10^{-2} \pm 1.2\times10^{-3}$	67 ± 2	$3.8\times10^{-2} \pm 1.3\times10^{-3}$
6	59 ± 3	$2.8\times10^{-2} \pm 1.7\times10^{-3}$	74 ± 1	$3.5\times10^{-2} \pm 1.5\times10^{-3}$	73 ± 3	$3.5\times10^{-2} \pm 1.3\times10^{-3}$
7	64 ± 2	$2.6\times10^{-2} \pm 1.0\times10^{-3}$	79 ± 2	$3.2\times10^{-2} \pm 9.7\times10^{-4}$	76 ± 3	$3.1\times10^{-2} \pm 1.0\times10^{-3}$
8	69 ± 4	$2.4\times10^{-2} \pm 1.6\times10^{-3}$	86 ± 3	$3.1\times10^{-2} \pm 1.7\times10^{-3}$	81 ± 1	$2.9\times10^{-2} \pm 6.0\times10^{-4}$
9	75 ± 4	$2.3\times10^{-2} \pm 1.4\times10^{-3}$	90 ± 4	$2.8\times10^{-2} \pm 1.4\times10^{-3}$	84 ± 2	$2.7\times10^{-2} \pm 8.5\times10^{-4}$
12	83 ± 4	$2.0\times10^{-2} \pm 1.0\times10^{-3}$	94 ± 5	$2.2\times10^{-2} \pm 1.1\times10^{-3}$	89 ± 3	$2.1\times10^{-2} \pm 1.0\times10^{-3}$
15	91 ± 2	$1.7\times10^{-2} \pm 2.2\times10^{-4}$	96 ± 6	$1.8\times10^{-2} \pm 4.4\times10^{-4}$	92 ± 0	$1.8\times10^{-2} \pm 4.8\times10^{-4}$
24	98 ± 2	$1.2\times10^{-2} \pm 1.5\times10^{-4}$	97 ± 4	$1.2\times10^{-2} \pm 5.8\times10^{-5}$	95 ± 1	$1.1\times10^{-2} \pm 2.1\times10^{-4}$

Supplementary Table 5: Morphine yield for the *E. coli* strains expressing WT CODM or mutants E259D or E259G after select time points using codeine as the substrate. Data are mean ± standard deviation of three independent replicates.

Time (h)	WT CODM yield (%)	WT CODM TSY (g/(L·h))	E259D yield (%)	E259D TSY (g/(L·h))	E259G yield (%)	E259G TSY (g/(L·h))
0.25	24 ± 2	$2.9\times10^{-1} \pm 2.4\times10^{-2}$	31 ± 2	$3.7\times10^{-1} \pm 3.1\times10^{-2}$	32 ± 2	$3.9\times10^{-1} \pm 3.9\times10^{-2}$
0.5	48 ± 4	$2.8\times10^{-1} \pm 2.3\times10^{-2}$	58 ± 3	$3.5\times10^{-1} \pm 2.1\times10^{-2}$	59 ± 2	$3.6\times10^{-1} \pm 1.8\times10^{-2}$
0.75	66 ± 5	$2.6\times10^{-1} \pm 1.9\times10^{-2}$	76 ± 4	$3.0\times10^{-1} \pm 1.8\times10^{-2}$	79 ± 4	$3.2\times10^{-1} \pm 5.4\times10^{-3}$
1	80 ± 5	$2.4\times10^{-1} \pm 1.4\times10^{-2}$	89 ± 3	$2.7\times10^{-1} \pm 8.9\times10^{-3}$	89 ± 1	$2.7\times10^{-1} \pm 7.3\times10^{-3}$
1.25	91 ± 4	$2.2\times10^{-1} \pm 9.9\times10^{-3}$	95 ± 1	$2.3\times10^{-1} \pm 4.4\times10^{-3}$	95 ± 1	$2.3\times10^{-1} \pm 5.9\times10^{-3}$
2	98 ± 3	$1.5\times10^{-1} \pm 5.3\times10^{-3}$	99 ± 2	$1.5\times10^{-1} \pm 4.4\times10^{-3}$	98 ± 4	$1.5\times10^{-1} \pm 2.8\times10^{-3}$
2.5	98 ± 4	$1.2\times10^{-1} \pm 5.0\times10^{-3}$	99 ± 2	$1.2\times10^{-1} \pm 4.2\times10^{-3}$	97 ± 5	$1.2\times10^{-1} \pm 4.0\times10^{-3}$

Supplementary Table 6: Oripavine time space yield (TSY) for *E. coli* strains expressing WT CODM or mutants E259D or E259G after select time points using raw poppy extract as the substrate. Data are mean ± standard deviation of three independent replicates.

Time (h)	WT CODM yield (%)	WT CODM TSY (g/(L·h))	E259D yield (%)	E259D TSY (g/(L·h))	E259G yield (%)	E259G TSY (g/(L·h))
1	7 ± 2	$2.0\times10^{-2} \pm 5.0\times10^{-3}$	11 ± 3	$3.3\times10^{-2} \pm 7.6\times10^{-3}$	12 ± 2	$3.4\times10^{-2} \pm 5.8\times10^{-3}$
2	17 ± 4	$2.4\times10^{-2} \pm 5.0\times10^{-3}$	25 ± 6	$3.6\times10^{-2} \pm 7.1\times10^{-3}$	26 ± 3	$3.7\times10^{-2} \pm 3.7\times10^{-3}$
3	25 ± 5	$2.4\times10^{-2} \pm 4.2\times10^{-3}$	37 ± 5	$3.6\times10^{-2} \pm 3.9\times10^{-3}$	40 ± 3	$3.8\times10^{-2} \pm 2.4\times10^{-3}$
4	32 ± 5	$2.3\times10^{-2} \pm 3.5\times10^{-3}$	45 ± 8	$3.3\times10^{-2} \pm 4.6\times10^{-3}$	51 ± 4	$3.7\times10^{-2} \pm 2.3\times10^{-3}$
5	37 ± 6	$2.2\times10^{-2} \pm 3.1\times10^{-3}$	50 ± 8	$2.9\times10^{-2} \pm 3.8\times10^{-3}$	59 ± 5	$3.4\times10^{-2} \pm 2.4\times10^{-3}$
6	41 ± 6	$2.0\times10^{-2} \pm 2.7\times10^{-3}$	54 ± 8	$2.6\times10^{-2} \pm 2.8\times10^{-3}$	64 ± 5	$3.1\times10^{-2} \pm 1.6\times10^{-3}$
7	42 ± 5	$1.7\times10^{-2} \pm 1.5\times10^{-3}$	55 ± 8	$2.3\times10^{-2} \pm 2.4\times10^{-3}$	68 ± 3	$2.8\times10^{-2} \pm 5.7\times10^{-3}$
8	46 ± 8	$1.7\times10^{-2} \pm 2.7\times10^{-3}$	58 ± 9	$2.1\times10^{-2} \pm 2.7\times10^{-3}$	75 ± 5	$2.7\times10^{-2} \pm 9.6\times10^{-4}$
9	46 ± 7	$1.5\times10^{-2} \pm 2.1\times10^{-3}$	57 ± 10	$1.8\times10^{-2} \pm 2.6\times10^{-3}$	73 ± 5	$2.4\times10^{-2} \pm 8.1\times10^{-4}$
12	46 ± 6	$1.1\times10^{-2} \pm 1.2\times10^{-3}$	58 ± 10	$1.4\times10^{-2} \pm 1.9\times10^{-3}$	74 ± 7	$1.8\times10^{-2} \pm 1.2\times10^{-3}$
15	46 ± 7	$9.0\times10^{-3} \pm 1.1\times10^{-3}$	60 ± 7	$1.1\times10^{-2} \pm 1.0\times10^{-3}$	75 ± 2	$1.4\times10^{-2} \pm 1.6\times10^{-4}$
24	48 ± 8	$5.8\times10^{-3} \pm 8.1\times10^{-4}$	57 ± 7	$6.9\times10^{-3} \pm 5.9\times10^{-4}$	76 ± 2	$9.1\times10^{-3} \pm 1.3\times10^{-4}$

Supplementary Table 7: Predicted change in WT CODM stability due to presence of the E259G or E259D mutations using the PremPS or INPS-Seq web servers. A negative value represents a stabilizing amino acid for PremPS and a positive value represents a stabilizing amino acid for INPS-Seq.

Stability prediction web server	E259G	E259D	Reference
PremPS	Stabilizing -0.71 kcal/mol	Stabilizing -0.38 kcal/mol	[2]
INPS-Seq	Stabilizing 0.59 kcal/mol	Destabilizing -0.02 kcal/mol	[3]

Supplementary Table 8: Primers used in this study.

The "Δ" primers are generic and thus were used as the upper primer (UM15) and lower primer (UM18) for all fragment 1 and 2 amplification, respectively.

Primer ID	Name	Sequence
UM13	Sequencing upper	GAAATCATGCCGAACATCCC
UM03	Sequencing lower	CCGGATATAGTTCCTCCTTTCAGC
UM05	CODM E259K fragment 1 lower	TCCACCGTTTTTCCTTCCGAAT
UM06	CODM E259K fragment 2 upper	GATTCGGAAGGAAAAACGGTGGATC
UM15	CODM E259Δ fragment 1 upper	CGGTCGTCAGACTGTCGATGA
UM18	CODM E259Δ fragment 2 lower	TTAGCAGCCGGATCTCAGTG
UM19	CODM E259D fragment 1 lower	TCCACCGGTCTTCCTTCCGAAT
UM20	CODM E259D fragment 2 upper	GATTCGGAAGGAAGACCGGTGGATC
UM21	CODM E259H fragment 1 lower	TCCACCGATGTTCCTTCCGAAT
UM22	CODM E259H fragment 2 upper	GATTCGGAAGGAACATCGGTGGATC
UM23	CODM E259Q fragment 1 lower	TCCACCGTTGTTCCTTCCGAAT
UM24	CODM E259Q fragment 2 upper	GATTCGGAAGGAACAACGGTGGATC
UM25	CODM E259A fragment 1 lower	TCCACCGTGCTTCCTTCCGAAT
UM26	CODM E259A fragment 2 upper	GATTCGGAAGGAAGCACGGTGGATC
UM27	CODM E259S fragment 1 lower	TCCACCGACTTTCCTTCCGAAT
UM28	CODM E259S fragment 2 upper	GATTCGGAAGGAAAGTCGGTGGATC
UM32	CODM E259G fragment 1 lower	TCCACCGACCTTCCTTCCGAAT
UM33	CODM E259G fragment 2 upper	GATTCGGAAGGAAGGTCGGTGGATC

Supplementary Table 9: Open Reading Frames.

All synthetic sequences were ordered from IDT, codon optimized for expression in *E. coli* B strains. Homology regions used for NEBuilder assembly are denoted by underlining.

Name	Sequence
CODM WT (UMg4)	CCTGAAAGACGCGCAGACTGGATCCATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCC AAGAGCTCGCCAAGTTGACCCTGGCCGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGT GCGTCAGTAACAGACGATGAAACGGTGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATT AGAGTTGGACAAGTTACACTCCGCTTGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAA TGGACAACATTAAGAGCGAAATCAAGGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGC GACTTTGAAGGGTTCGGTCAGCCTTATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTC GCTGCCACTTCACCTGCGCAAGCCGCACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGA GCAAGATGAAAAAACTGTCAACGGTGGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACT GACTTGTTCGAAGACGGGCTCCAAACGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCT TACAAGTCATAGTGACTTTTCTGGGTTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAG AGCGGTGGATCAGCATCAAACCGCTTCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGG ATCTATCGTAGTGTTGAGCACCGTGCAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAA ATTGGAATCCGAAATCGGTCCTATCTCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAG ACATTCTCAAGGAAAACCTTTCGCGCAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGACTCGAGCACCAC CACCACCACCACTGAGATCCGGCTGCTA
CODM E259K	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAAAACGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
CODM E259D	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAGACCGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
CODM E259H	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGAATTCGGAAGGAAGACCGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
CODM E259Q	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA

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CODM E259A	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAGCACGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
CODM E259S	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAAGTCGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
CODM E259G	ATGGAGACCCCAATCTTGATCAAACTTGGCAACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGC CGAGATCCCAAGTCGGTATACATGCACAGGTGAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGG TGCCGGTCATTGATTTGCAAAACTTATTAAGTCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCT TGCAAAGAGTGGGGCTTCTTTCAGCTTGTCAACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAA GGGCTTTTTTAACCTGCCGATGAATGAGAAGACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTT ATATTGAATCTGAGGATCAGCGCTTGGATTGGACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCG CACCTTTTTCCAGAACTTCCACTTCCGTTTCGCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGT GGTGTTTGAGATGTTAGAAAAGAGTTTGCAACTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAA CGATGCGCATGAATTACTATCCTCCATGTCCACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGG TTGACCATTTTACTCCAGTTAAACGAAGTGGAGGGGTTGCAGATTCGGAAGGAAGGTCGGTGGATCAGCATCAAACCGCT TCCAGACGCCTTTATTGTCAACGTAGGTGATATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTG CAGTCGTGAATAGTACCAAGGAGCGGCTTTCCATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATC TCTTCGTTGGTTACTCCTGAGACCCCTGCATTATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCG CAAACTTGATGGCAAGTCTTTCCTGGATTACATGCGCATGTGA
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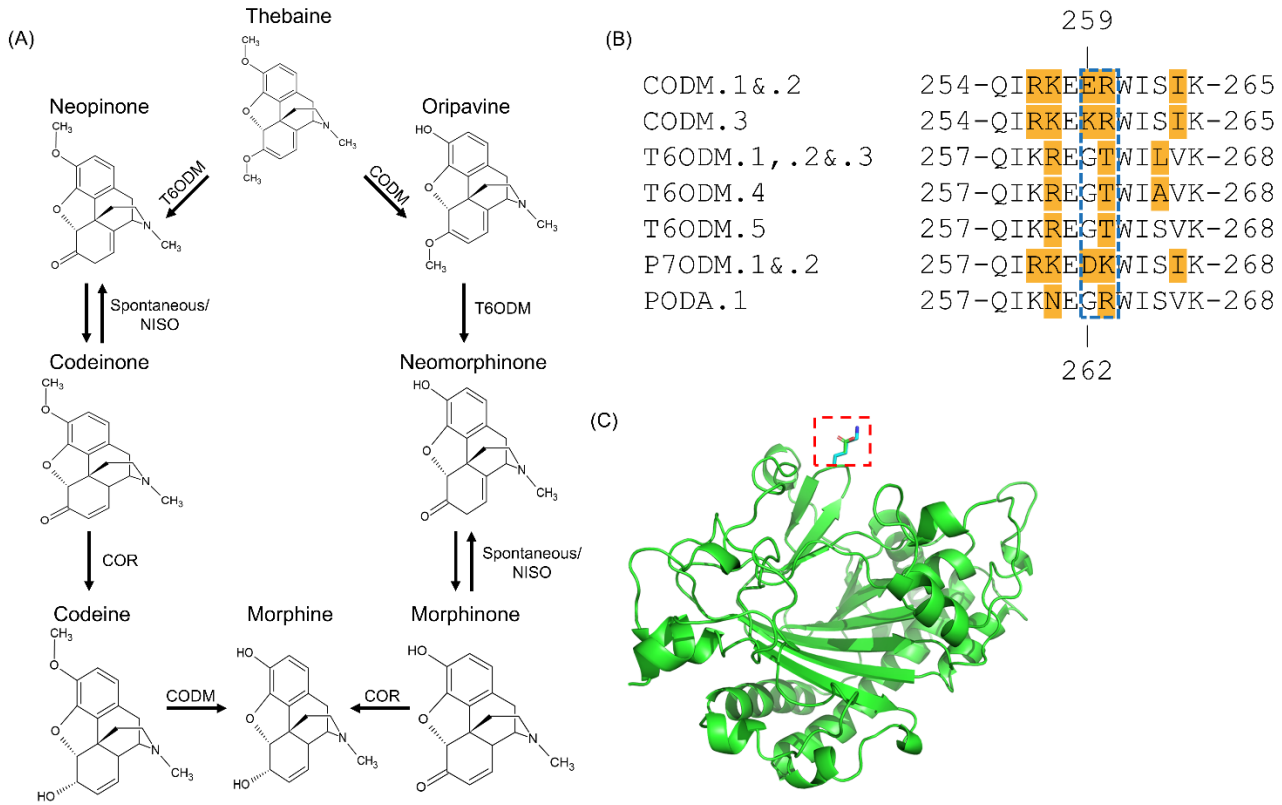
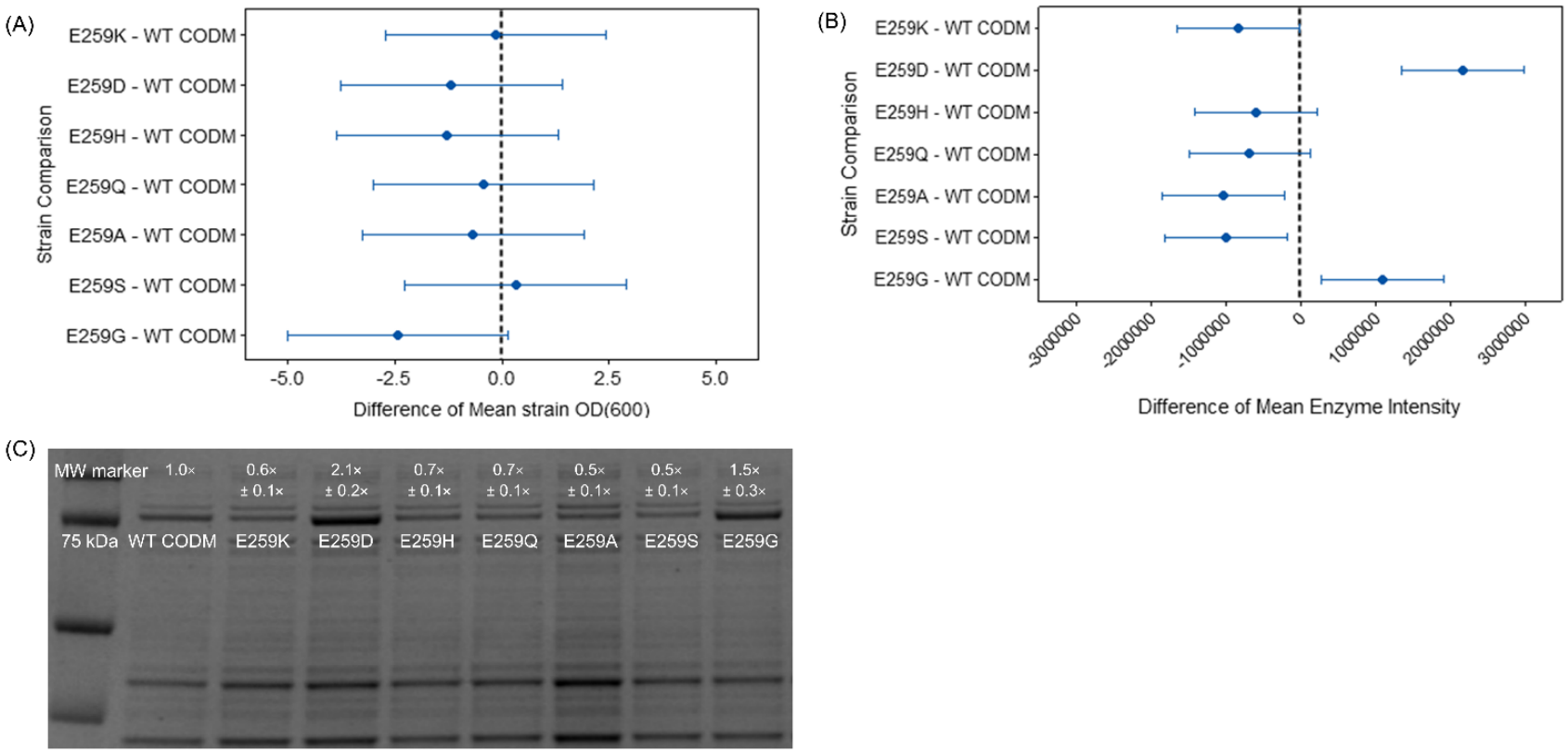
CODM R260K (UMg11)	CGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTGGATCCATGGAGACCCCAATCTTGATCAAACTTGGCA ACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGCCGAGATCCCAAGTCGGTATACATGCACAGGT GAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGGTGCCGGTCATTGATTTGCAAAACTTATTAAG TCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCTTGCAAAGAGTGGGGCTTCTTTCAGCTTGTCA ACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAAGGGCTTTTTTAACCTGCCGATGAATGAGAAG ACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTTATATTGAATCTGAGGATCAGCGCTTGGATTG GACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCGCACCTTTTTCCAGAACTTCCACTTCCGTTTC GCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGTGGTGTTTGAGATGTTAGAAAAGAGTTTGCAA CTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAACGATGCGCATGAATTACTATCCTCCATGTCC ACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGGTTGACCATTTTACTCCAGTTAAACGAAGTGG AGGGGTTGCAGATTCGGAAGGAAGAGaaaTGGATCAGCATCAAACCGCTTCCAGACGCCTTTATTGTCAACGTAGGTGAT ATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTGCAGTCGTGAATAGTACCAAGGAGCGGCTTTC CATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATCTCTTCGTTGGTTACTCCTGAGACCCCTGCAT TATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCGCAAACTTGATGGCAAGTCTTTCCTGGATTAC ATGCGCATGTGACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA
CODM E259D+R260K (UMg12)	CGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTGGATCCATGGAGACCCCAATCTTGATCAAACTTGGCA ACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGCCGAGATCCCAAGTCGGTATACATGCACAGGT GAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGGTGCCGGTCATTGATTTGCAAAACTTATTAAG TCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCTTGCAAAGAGTGGGGCTTCTTTCAGCTTGTCA ACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAAGGGCTTTTTTAACCTGCCGATGAATGAGAAG ACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTTATATTGAATCTGAGGATCAGCGCTTGGATTG GACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCGCACCTTTTTCCAGAACTTCCACTTCCGTTTC GCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGTGGTGTTTGAGATGTTAGAAAAGAGTTTGCAA CTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAACGATGCGCATGAATTACTATCCTCCATGTCC ACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGGTTGACCATTTTACTCCAGTTAAACGAAGTGG AGGGGTTGCAGATTCGGAAGGAAGACaaaTGGATCAGCATCAAACCGCTTCCAGACGCCTTTATTGTCAACGTAGGTGAT ATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTGCAGTCGTGAATAGTACCAAGGAGCGGCTTTC CATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATCTCTTCGTTGGTTACTCCTGAGACCCCTGCAT TATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCGCAAACTTGATGGCAAGTCTTTCCTGGATTAC ATGCGCATGTGACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA

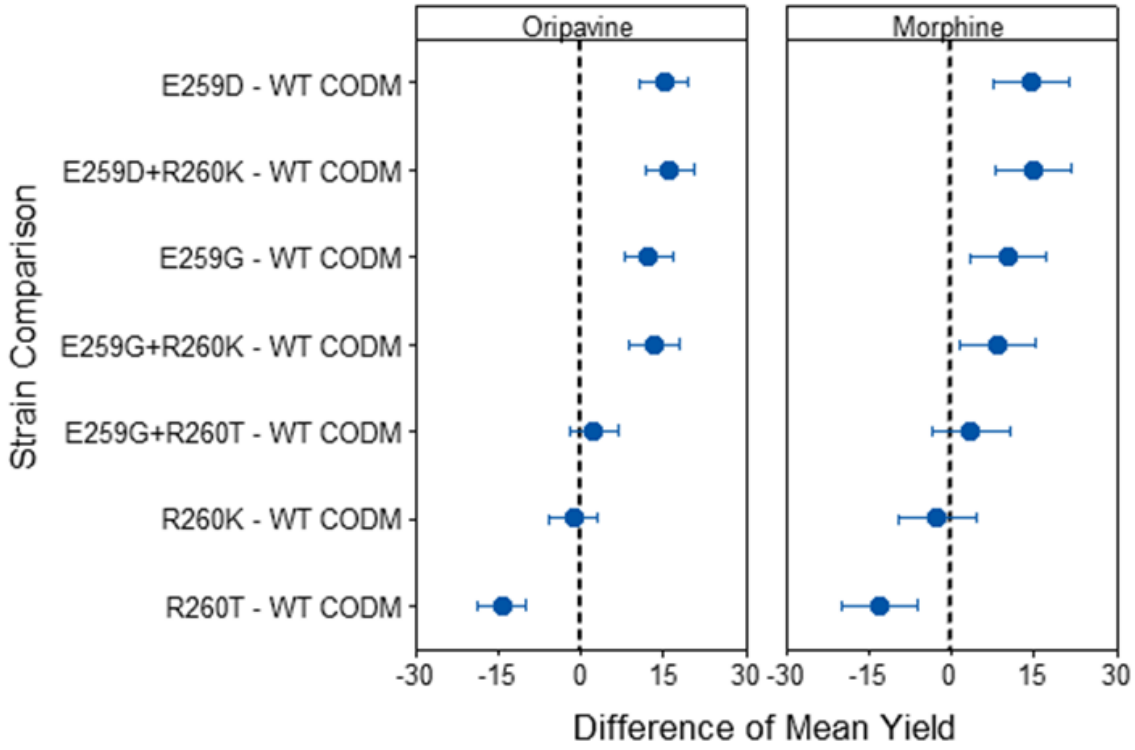
CODM E259G+R260K (UMg13)	CGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTGGATCCATGGAGACCCCAATCTTGATCAAACTTGGCA ACGGGCTCTCGATCCCTAGCGTCCAAGAGCTCGCCAAGTTGACCCTGGCCGAGATCCCAAGTCGGTATACATGCACAGGT GAGAGCCCACTTAACAACATCGGTGCGTCAGTAACAGACGATGAAACGGTGCCGGTCATTGATTTGCAAAACTTATTAAG TCCAGAGCCAGTAGTGGGGAAATTAGAGTTGGACAAGTTACACTCCGCTTGCAAAGAGTGGGGCTTCTTTCAGCTTGTCA ACCATGGCGTTGATGCCTTGTTAATGGACAACATTAAGAGCGAAATCAAGGGCTTTTTTAACCTGCCGATGAATGAGAAG ACCAAATACGGTCAGCAGGACGGCGACTTTGAAGGGTTCGGTCAGCCTTATATTGAATCTGAGGATCAGCGCTTGGATTG GACTGAGGTGTTCTCAATGCTCTCGCTGCCACTTCACCTGCGCAAGCCGCACCTTTTTCCAGAACTTCCACTTCCGTTTC GCGAGACCCTGGAGTCGTACTTGAGCAAGATGAAAAAACTGTCAACGGTGGTGTTTGAGATGTTAGAAAAGAGTTTGCAA CTTGTTGAGATTAAAGGTATGACTGACTTGTTCGAAGACGGGCTCCAAACGATGCGCATGAATTACTATCCTCCATGTCC ACGGCCTGAGTTGGTATTGGGTCTTACAAGTCATAGTGACTTTTCTGGGTTGACCATTTTACTCCAGTTAAACGAAGTGG AGGGGTTGCAGATTCGGAAGGAAggtaaaTGGATCAGCATCAAACCGCTTCCAGACGCCTTTATTGTCAACGTAGGTGAT ATTCTCGAAATTATGACCAACGGGATCTATCGTAGTGTTGAGCACCGTGCAGTCGTGAATAGTACCAAGGAGCGGCTTTC CATCGCCACATTCCATGACTCTAAATTGGAATCCGAAATCGGTCCTATCTCTTCGTTGGTTACTCCTGAGACCCCTGCAT TATTCAAGCGCGGGCGCTACGAAGACATTCTCAAGGAAAACCTTTCGCGCAAACTTGATGGCAAGTCTTTCCTGGATTAC ATGCGCATGTGACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA



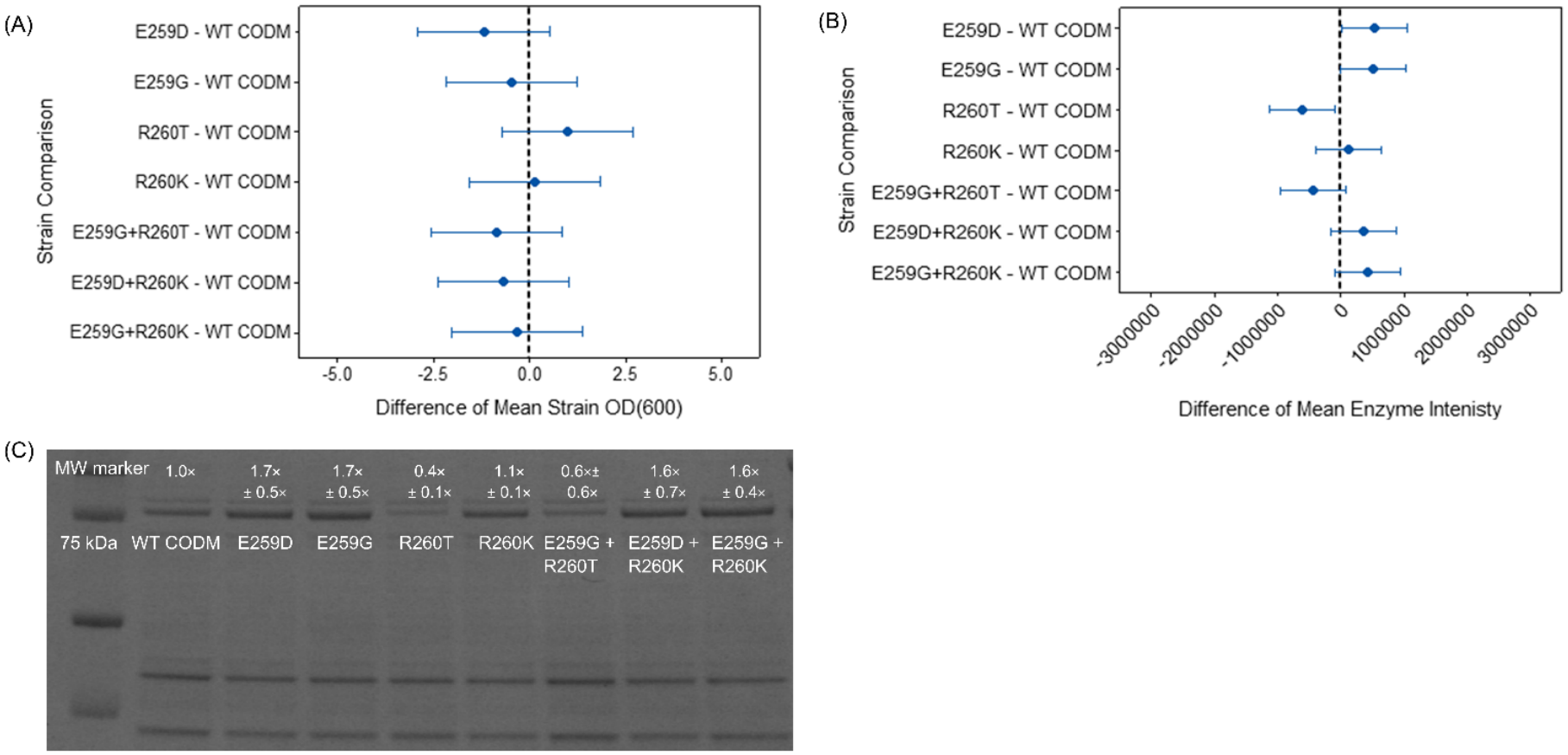
Supplementary Figure. 1 (A) Pathways in *P. somniferum* for the conversion of thebaine to morphine. (B) Sequence alignment of selected region of the three CODM variants (CODM.1,.2 and .3), the five T6ODM variants (T6ODM.1,.2,.3,.4 and .5), the two P7ODM variants (P7ODM.1 and .2) and the one PODA variant (PODA.1) found within *P. somniferum* HN1.[4] Non-identical residues are highlighted in orange. The navy box indicates the region of interest. T6ODM.1&.2 are identical at the protein level while T6ODM.3 differs by a single residue (A81S). P7ODM.1 &.2 differ by two residues: K315Q and K352N. Sequences were aligned using the MUSCLE methodology with SnapGene. (C) Homology model of *P. somniferum* CODM.1/.2 based on the crystal structure of *P. somniferum* T6ODM in complex with succinate (protein databank entry: 5O7Y). SWISS-MODEL was used for model creation. [5] The red box indicates the side chains of amino acid 259, where the single amino acid E259K difference between CODM.1/.2 (green) and CODM.3 (aqua) lies.



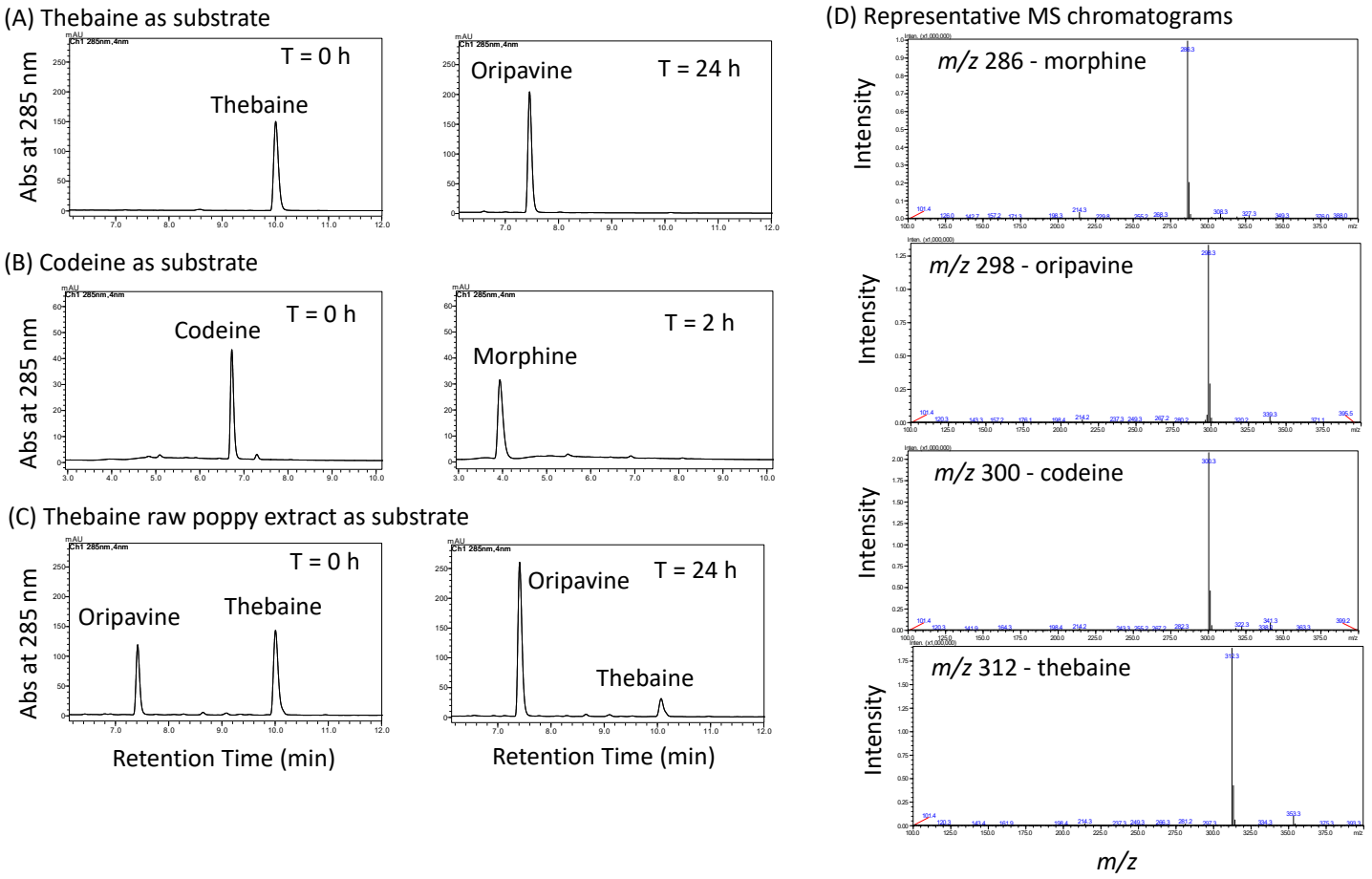
Supplementary Figure. 2 Mean difference in strain OD(600) (A) and mean difference in mutant enzyme intensity (B) of *E. coli* strains following expressing of CODM mutants with varying amino acids at position 259. Data are mean difference of three independent replicates ± 95% confidence interval. Representative SDS-PAGE (C) image the *E. coli* strains expressing CODM mutants with varying amino acids at position 259. Relative expression data are from semi-quantitative analysis of the SDS-PAGE image and are the mean ± standard deviation of three independent replicates.



Supplementary Figure. 3 Mean difference of oripavine and morphine yield produced by the *E. coli* strains expressing CODM mutants with varying amino acids at position 259. Biotransformations were conducted for 4 hours to assess oripavine production or 30 minutes to assess morphine production. Data are mean difference of three independent replicates ± 95% confidence interval.



Supplementary Figure. 4 Mean difference in strain OD(600) (A) and mean difference in mutant enzyme intensity (B) of *E. coli* strains following expressing of CODM mutants with varying amino acids at position 259 or 260 or both . Data are mean difference of three independent replicates ± 95% confidence interval. Representative SDS-PAGE (C) image the *E. coli* strains expressing CODM mutants with varying amino acids at position 259 or 260 or both. Relative expression data are from semi-quantitative analysis of the SDS-PAGE image and are the mean ± standard deviation of three independent replicates.



Supplementary Figure. 5 Representative HPLC chromatograms showing the presence of thebaine (retention time of 10.1 minutes) and oripavine (retention time of 7.5 minutes) or codeine (retention time of 6.8 minutes) and morphine (retention time of 4.0 minutes) at 285 nm when pure thebaine (A), pure codeine (B) or raw thebaine extract (C) are used as the feedstock for biotransformation at distinct time points. The presence of oripavine alone (A) or a mixture of oripavine and thebaine (C) is also shown after 24 hours of biotransformation for these feedstocks. Morphine is also shown as a product after 2 hours of biotransformation (B). (D) Representative MS chromatograms

showing the presence of morphine (*m/z* 286), oripavine (*m/z* 298), codeine (*m/z* 300) and thebaine (*m/z* 312) in the biotransformation reactions.

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