# Supplementary Material

# Novel Iterative Genome Mining and Engineering of a Bifunctional KvVDH Enable Selective Production of Furan Carboxylic Acids from High-Concentration 5-Hydroxymethylfurfural

Yanan Cui,[a,b] Jie Zhang,[a,b] Xijun Xing,[a,b] Hanwen Zhang,[a,b] Yutong Chen,[a,b] Liqiang Fan,[a,b] Xu Li,[a,b] Yongjun Qiu,[a,b] Chen Deng *[a,b] and Liming Zhao *[a,b,c]

[a.] *State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

[b.] *Shanghai Collaborative Innovation Center for Biomanufacturing Technology (SCICBT), Shanghai 200237, China*

[c.] *Shanghai Frontiers Science Center of Optogenetic Techniques for Cell Metabolism, School of Pharmacy, East China University of Science and Technology, 130 Mei Long Road, Shanghai 200237, China*

***Correspondence**

Liming Zhao

Chen Deng

School of Biotechnology, East China University of Science and Technology, Shanghai 200237. China

Tel./Fax: +86 21 64250829

E-mail address: zhaoliming@ecust.edu.cn

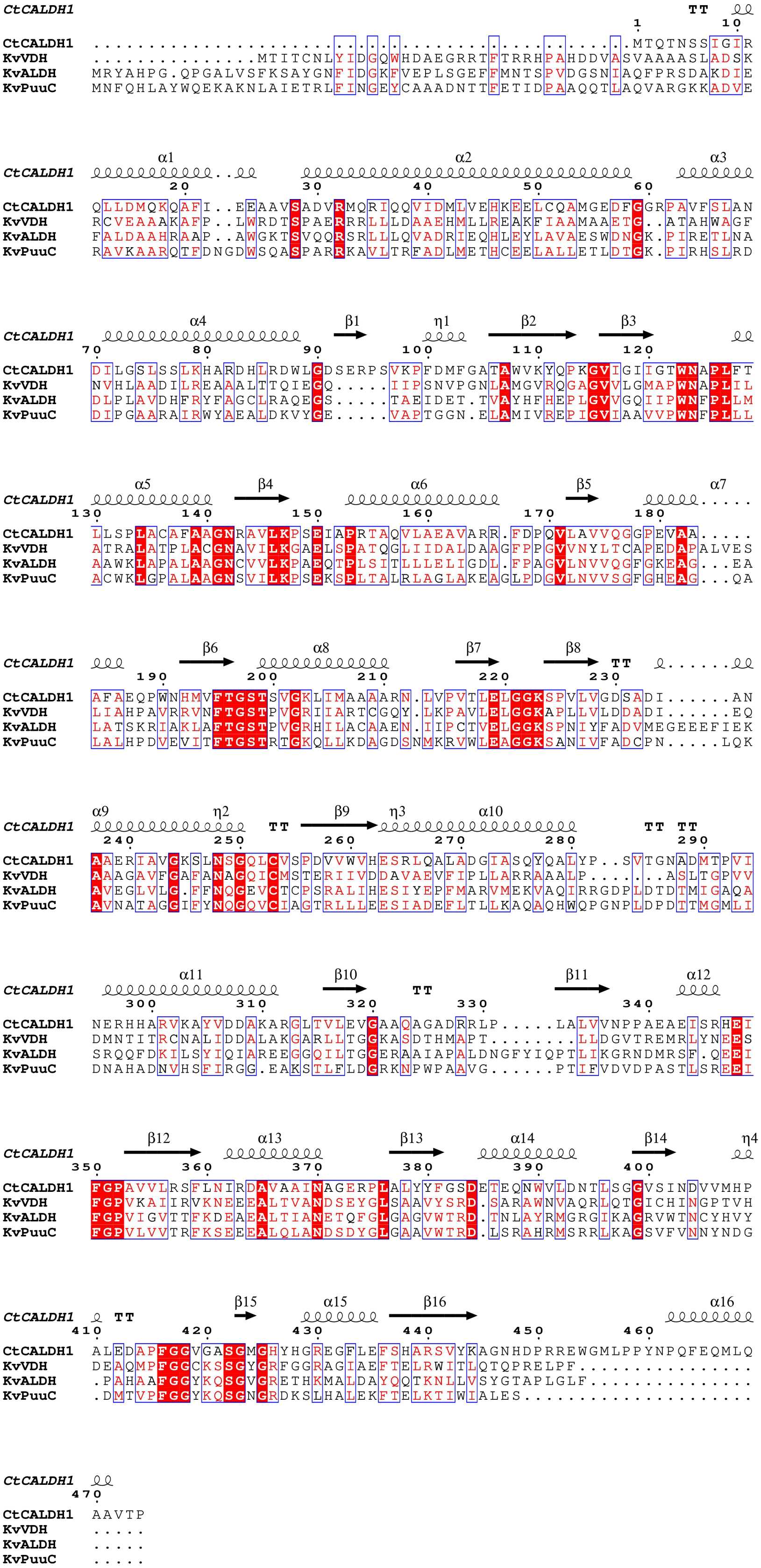
E-mail address: chendeng@ecust.edu.cn

# Contents

## Supplementary Figure

## Supplementary Table

# Supplementary Figure



```
CtCALDH1  .................................................MTQTNSSIGIR
KvVDH     ...............MTITCNLYIDGQWHDAEGRRTFTRRHPAHDDVASVAAAASLADSK
KvALDH    MRYAHPG.QPGALVSFKSAYGNFIDGKFVEPLSGEFFMNTSPVDGSNIAQFPRSDAKDIE
KvPuuC    MNFQHLAYWQEKAKNLAIETRLFINGEYCAAADNTTFETIDPAAQQTLAQVARGKKADVE

CtCALDH1  QLLDMQKQAFI..EEAAVSADVRMQRIQQVIDMLVEHKEELCQAMGEDFGGRPAVFSLAN
KvVDH     RCVEAAAKAFP..LWRDTSPAERRRLLLDAAEHMLLREAKFIAAMAAETG..ATAHWAGF
KvALDH    FALDAAHRAAP..AWGKTSVQQRSRLLLQVADRIEQHLEYLAVAESWDNGK.PIRETLNA
KvPuuC    RAVKAARQTFDNGDWSQASPARRKAVLTRFADLMETHCEELALLETLDTGK.PIRHSLRD

CtCALDH1  DILGSLSSLKHARDHLRDWLGDSERPSVKPFDMFGATAWVKYQPKGVIGIIGTWNAPLFT
KvVDH     NVHLAADILREAAALTTQIEGQ.....IIPSNVPGNLAMGVRQGAGVVLGMAPWNAPLIL
KvALDH    DLPLAVDHFRYFAGCLRAQEGS.....TAEIDET.TVAYHFHEPLGVVGQIIPWNFPLLM
KvPuuC    DIPGAARAIRWYAEALDKVYGE.....VAPTGGN.ELAMIVREPIGVIAAVVPWNFPLLL

CtCALDH1  LLSPLACAFAAGNRAVLKPSEIAPRTAQVLAEAVARR.FDPQVLAVVQGGPEVAA.....
KvVDH     ATRALATPLACGNAVILKGAELSPATQGLIIDALDAAGFPPGVVNYLTCAPEDAPALVES
KvALDH    AAWKLAPALAAGNCVVLKPAEQTPLSITLLLELIGDL.FPAGVLNVVQGFGKEAG...EA
KvPuuC    ACWKLGPALAAGNSVILKPSEKSPLTALRLAGLAKEAGLPDGVLNVVSGFGHEAG...QA

CtCALDH1  AFAEQPWNHMVFTGSTSVGKLIMAAAARN.LVPVTLELGGKSPVLVGDSADI......AN
KvVDH     LIAHPAVRRVNFTGSTPVGRIIARTCGQY.LKPAVLELGGKAPLLVLDDADI......EQ
KvALDH    LATSKRIAKLAFTGSTPVGRHILACAAEN.IIPCTVELGGKSPNIYFADVMEGEEEFIEK
KvPuuC    LALHPDVEVITFTGSTRTGKQLLKDAGDSNMKRVWLEAGGKSANIVFADCPN.....LQK

CtCALDH1  AAERIAVGKSLNSGQLCVSPDVVWVHESRLQALADGIASQYQALYP..SVTGNADMTPVI
KvVDH     AAAGAVFGAFANAGQICMSTERIIVDDAVAEVFIPLLARRAAALP.......ASLTGPVV
KvALDH    AVEGLVLG.FFNQGEVCTCPSRALIHESIYEPFMARVMEKVAQIRRGDPLDTDTMIGAQA
KvPuuC    AVNATAGGIFYNQGQVCIAGTRLLLEESIADEFLTLLKAQAQHWQPGNPLDPDTTMGMLI

CtCALDH1  NERHHARVKAYVDDAKARGLTVLEVGAAQAGADRRLP.....LALVVNPPAEAEISRHEI
KvVDH     DMNTITRCNALIDDALAKGARLLTGGKASDTHMAPT........LLDGVTREMRLYNEES
KvALDH    SRQQFDKILSYIQIAREEGGQILTGGERAAIAPALDNGFYIQPTLIKGRNDMRSF.QEEI
KvPuuC    DNAHADNVHSFIRGG.EAKSTLFLDGRKNPWPAAVG......PTIFVDVDPASTLSREEI

CtCALDH1  FGPAVVLRSFLNIRDAVAAINAGERPLALYYFGSDETEQNWVLDNTLSGGVSINDVVMHP
KvVDH     FGPVKAIIRVKNEEEALTVANDSEYGLSAAVYSRD.SARAWNVAQRLQTGICHINGPTVH
KvALDH    FGPVIGVTTFKDEAEALTIANETQFGLGAGVWTRD.TNLAYRMGRGIKAGRVWTNCYHVY
KvPuuC    FGPVLVVTRFKSEEEALQLANDSDYGLGAAVWTRD.LSRAHRMSRRLKAGSVFVNNYNDG

CtCALDH1  ALEDAPFGGVGASGMGHYHGREGFLEFSHARSVYKAGNHDPRREWGMLPPYNPQFEQMLQ
KvVDH     DEAQMPFGGCKSSGYGRFGGRAGIAEFTELRWITLQTQPRELPF................
KvALDH    .PAHAAFGGYKQSGVGRETHKMALDAYQQTKNLLVSYGTAPLGLF...............
KvPuuC    .DMTVPFGGYKQSGNGRDKSLHALEKFTELKTIWIALES.....................

CtCALDH1  AAVTP
KvVDH     .....
KvALDH    .....
KvPuuC    .....
```

Fig. S1. Protein sequence alignment of CtCALDH1 with KvPuuC, KvVDH and KvALDH from *Klebsiella variicola*.

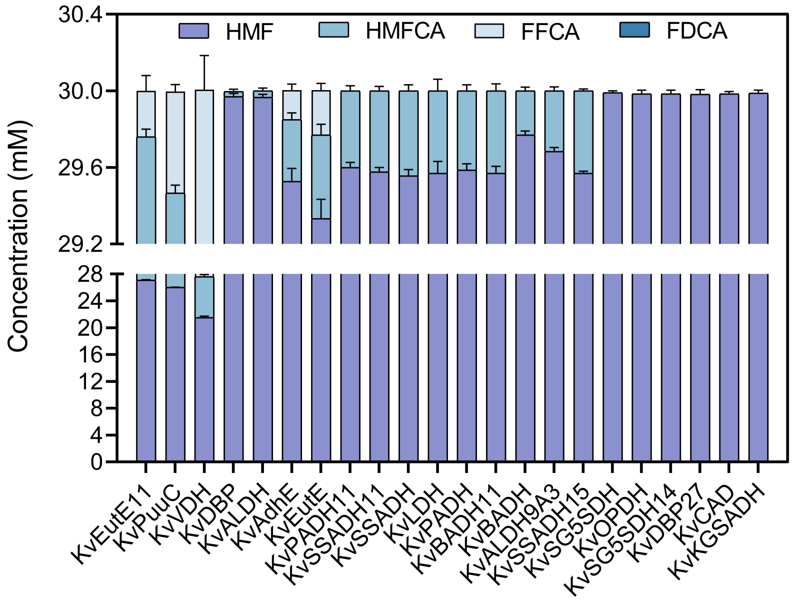


Fig. S2. Distribution of catalytic products of HMF in crude *E. coli* cell lysates expressing the enzyme gene from *Klebsiella variicola*. The reaction system contained 10 mM $NAD^+$, 30 mM HMF and 50 mM $NaH_2PO_4$-$Na_2HPO_4$ buffer (pH 7.0). The reaction was conducted at 30 °C for 24 h.

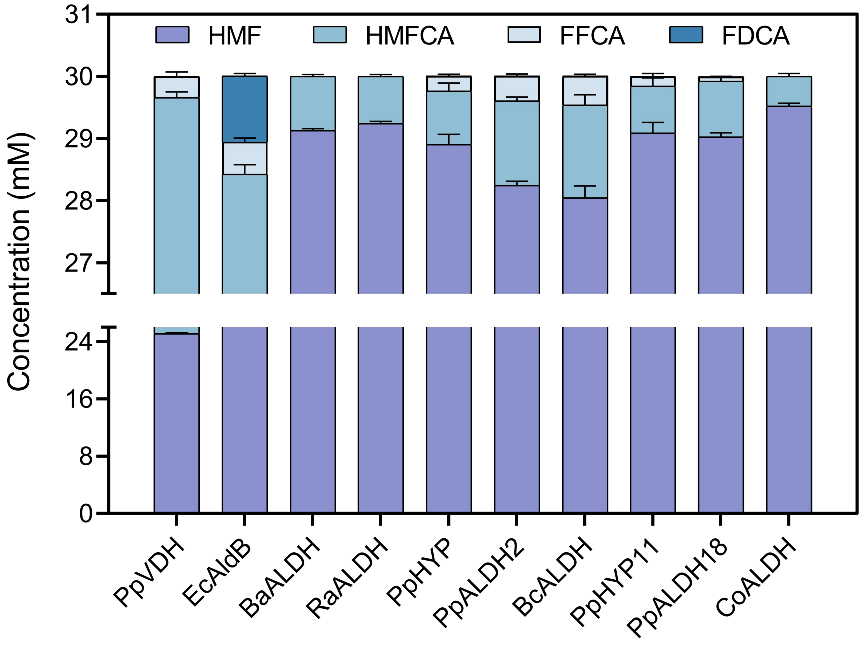


Fig. S3. Distribution of catalytic products of HMF in crude *E. coli* cell lysates expressing the enzyme gene including PpVDH, EcAldB, BaALDH, RaALDH, PpHYP, PpALDH2, BcALDH, PpHYP11, PpALDH18 and CoALDH. The reaction system contained 10 mM $NAD^+$, 30 mM HMF and 50 mM $NaH_2PO_4$-$Na_2HPO_4$ buffer (pH 7.0). The reaction was conducted at 30 °C for 24 h.

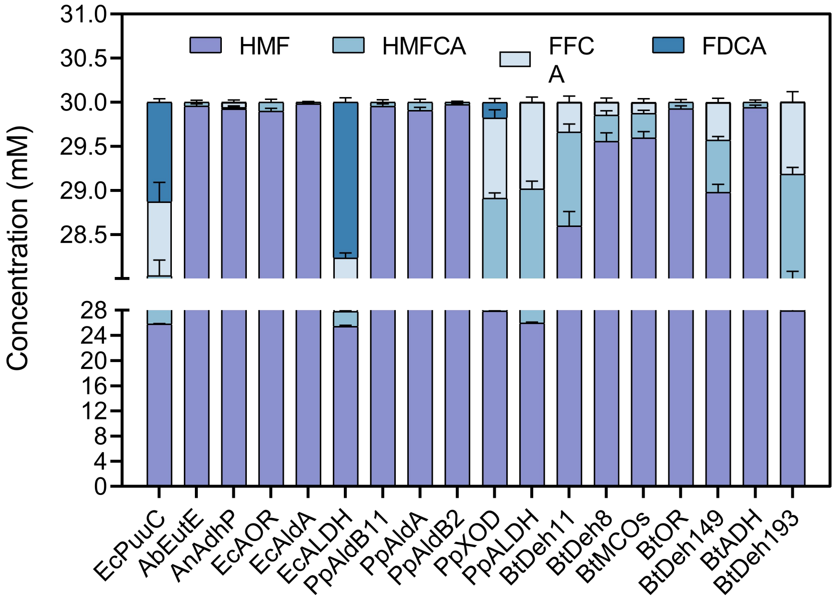


Fig. S4. Distribution of catalytic products of HMF in crude *E. coli* cell lysates expressing the enzyme gene including EcPuuC, AbEutE, AnAdhP, EcAOR, EcAldA, EcALDH, PpAldB11, PpAldA, PpAldB2, PpXOD, PpALDH, BtDeh11, BtDeh8, BtMCOs, BtOR, BtDeh149, BtADH and BtDeh193. The reaction system contained 10 mM $NAD^+$, 30 mM HMF and 50 mM $NaH_2PO_4$-$Na_2HPO_4$ buffer (pH 7.0). The reaction was conducted at 30 °C for 24 h.

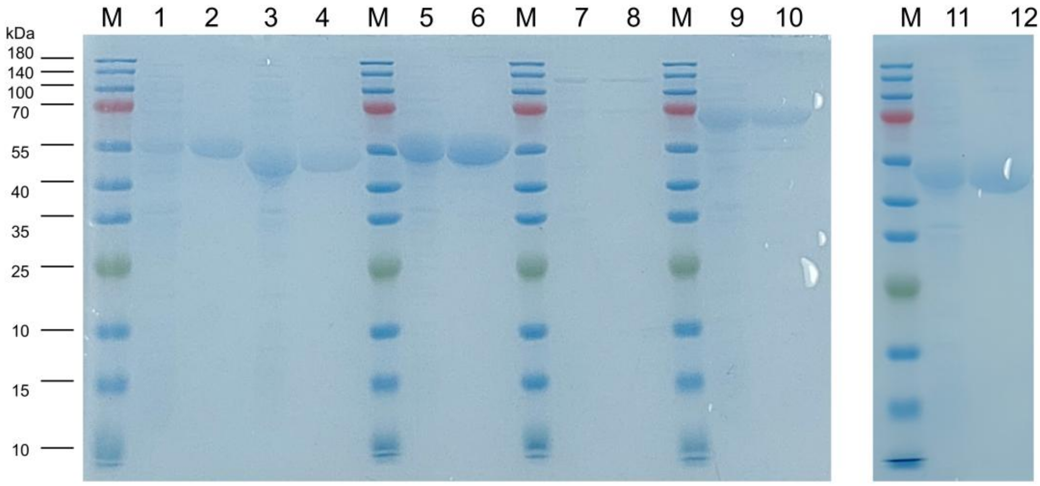


Fig. S5. SDS-PAGE analysis of PpALDH， PpVDH， EcPuuC， EcAldB， EcALDH and PpXOD. Lane M, protein marker; lane 1,the supernatant of EcPuuC; lane 2, the purified enzyme of EcPuuC; lane 3,the supernatant of PpALDH; lane 4, the purified enzyme of PpALDH; lane 5,the supernatant of EcAldB; lane 6, the purified enzyme of EcAldB; lane 7,the supernatant of PpXOD; lane 8, the purified enzyme of PpXOD; lane 9,the supernatant of EcALDH; lane 10, the purified enzyme of EcALDH; lane 11,the supernatant of PpVDH; lane 12, the purified enzyme of PpVDH.

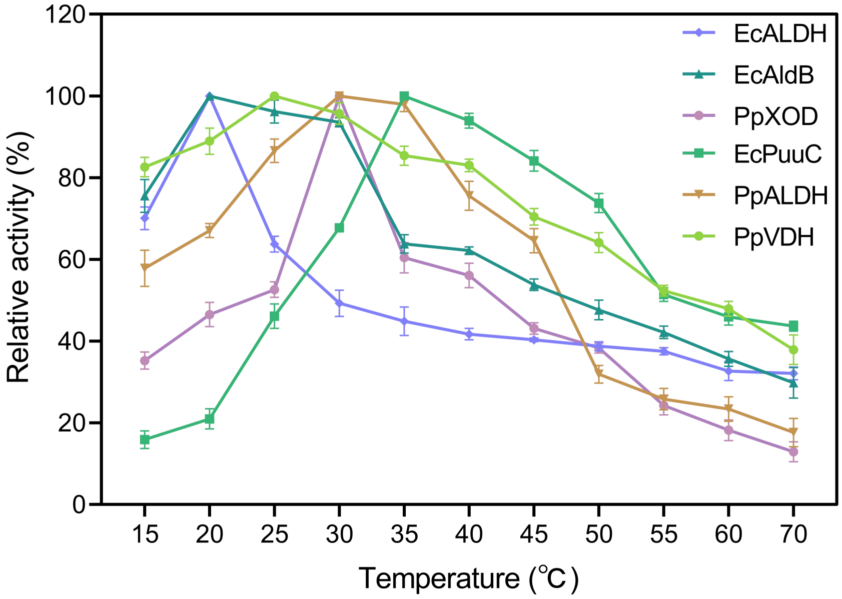


Fig. S6. Optimal reaction temperatures for KvVDH, PpALDH, PpVDH, EcPuuC, EcAldB, EcALDH and PpXOD. Enzyme activity at the optimal temperature was considered as 100%.

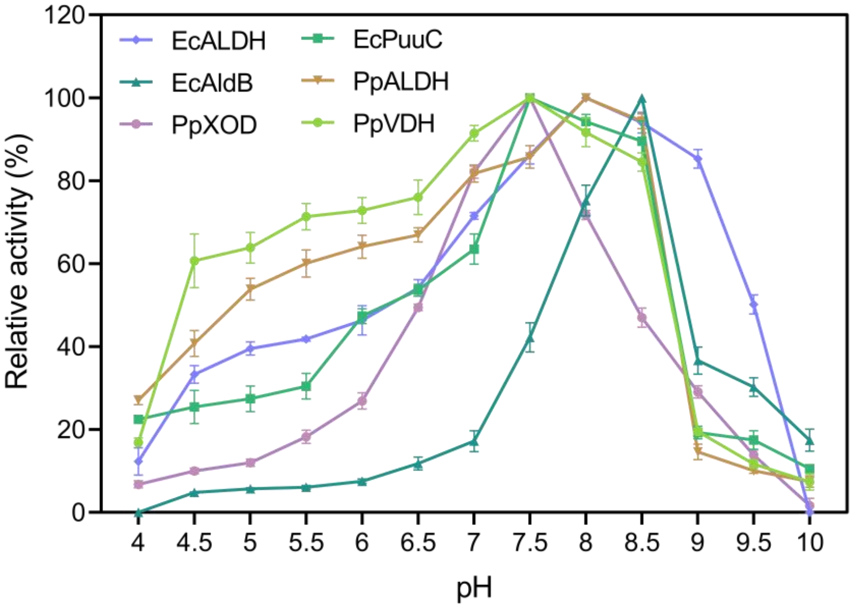


Fig. S7. Optimal reaction pH for KvVDH, PpALDH, PpVDH, EcPuuC, EcAldB, EcALDH and PpXOD. Enzyme activity at the optimal pH was considered as 100%.

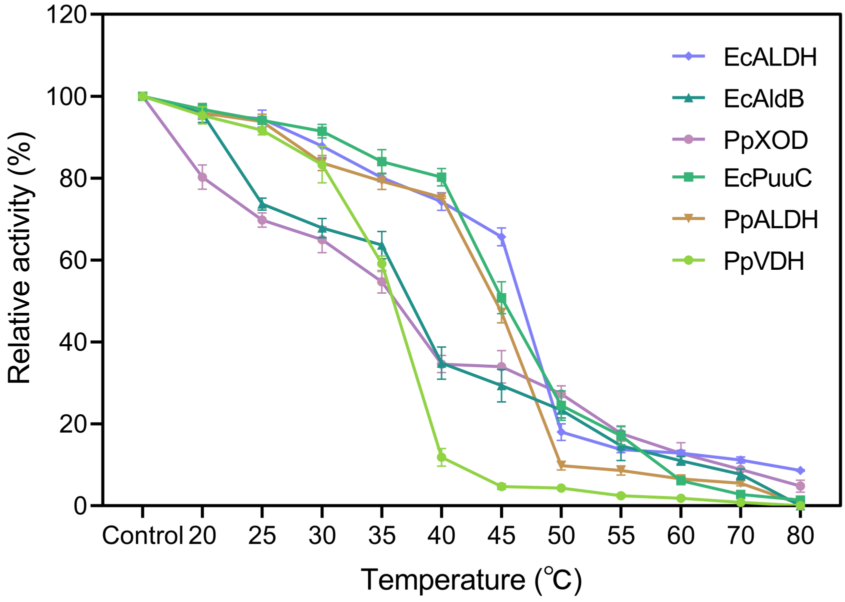


Fig. S8. Relative activity of KvVDH, PpALDH, PpVDH, EcPuuC, EcAldB, EcALDH and PpXOD after incubation at 20 – 80 °C for 1 h. The initial enzyme activity prior to incubation was considered as 100%.

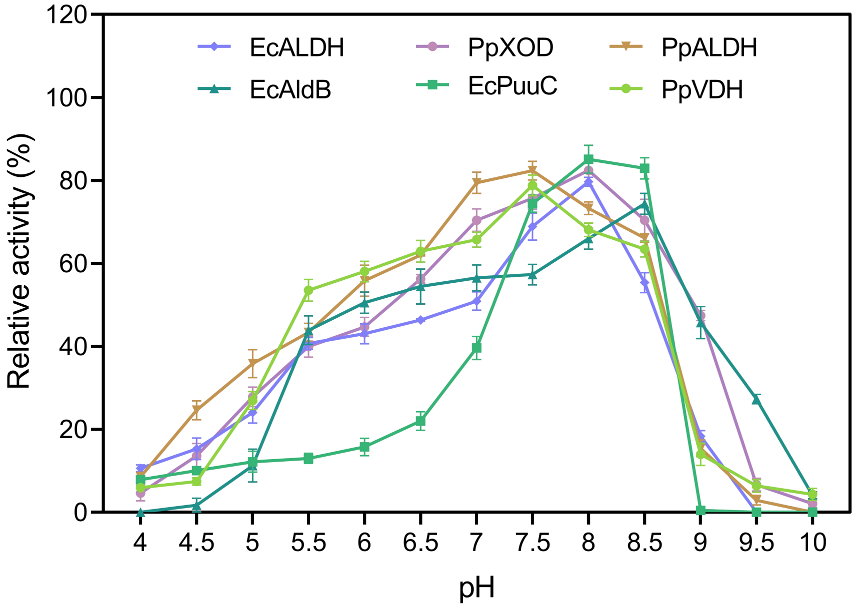


Fig. S9. The relative activity of KvVDH, PpALDH, PpVDH, EcPuuC, EcAldB, EcALDH and PpXOD after incubation at pH 4.0 – 10.0 for 1 h. The initial enzyme activity without incubation was considered as 100%.

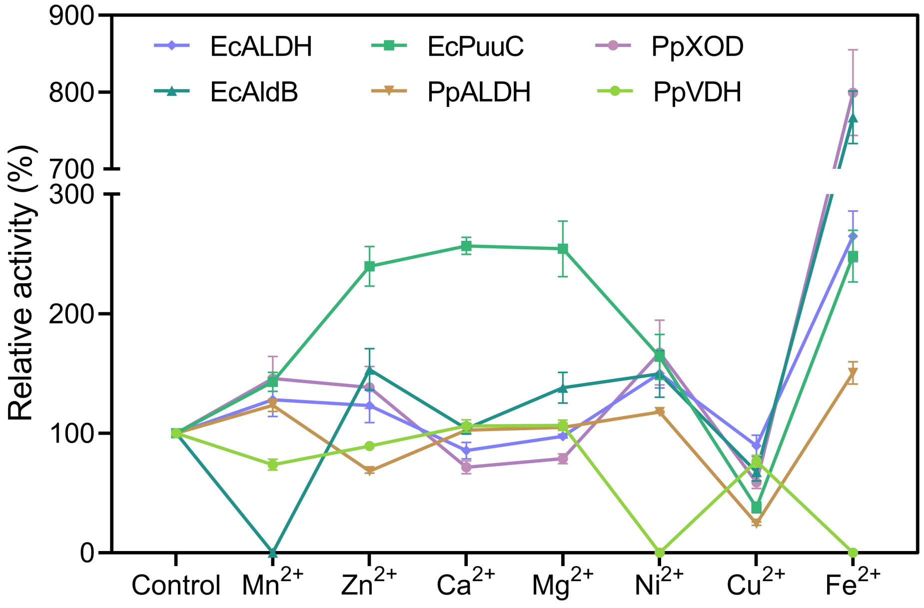
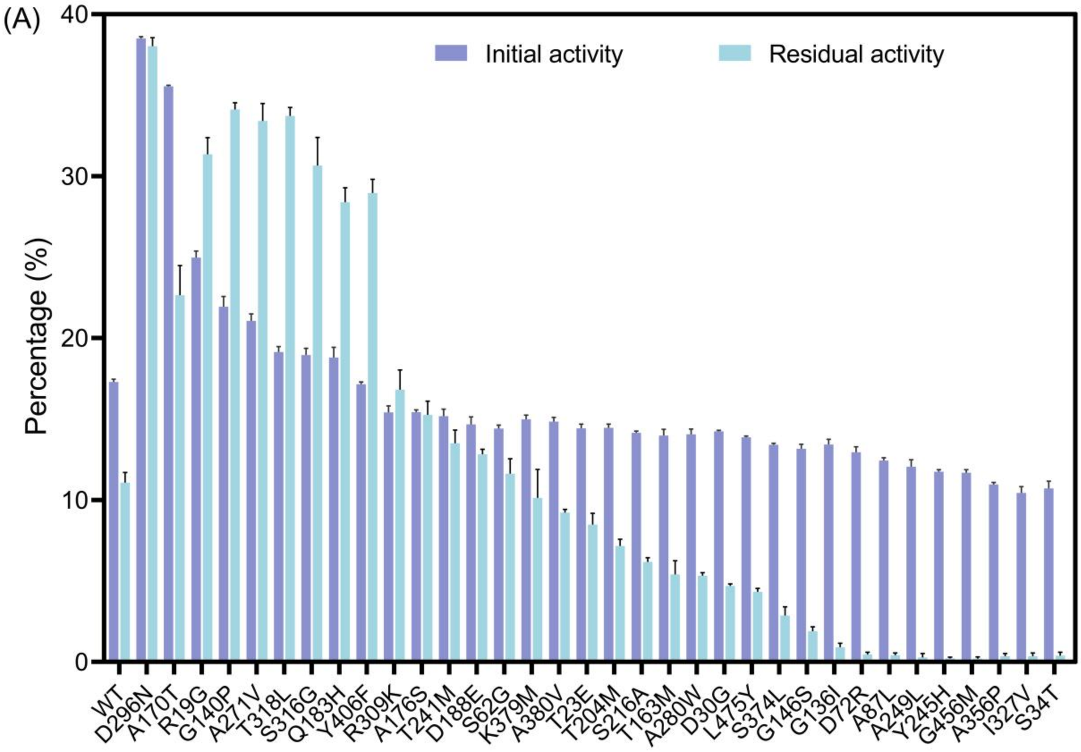


Fig. S10. Effect of different metal ion additions on the enzymatic activities of KvVDH, PpALDH, PpVDH, EcPuuC, EcAldB, EcALDH and PpXOD. Enzyme activity without metal ion addition was considered as 100%.



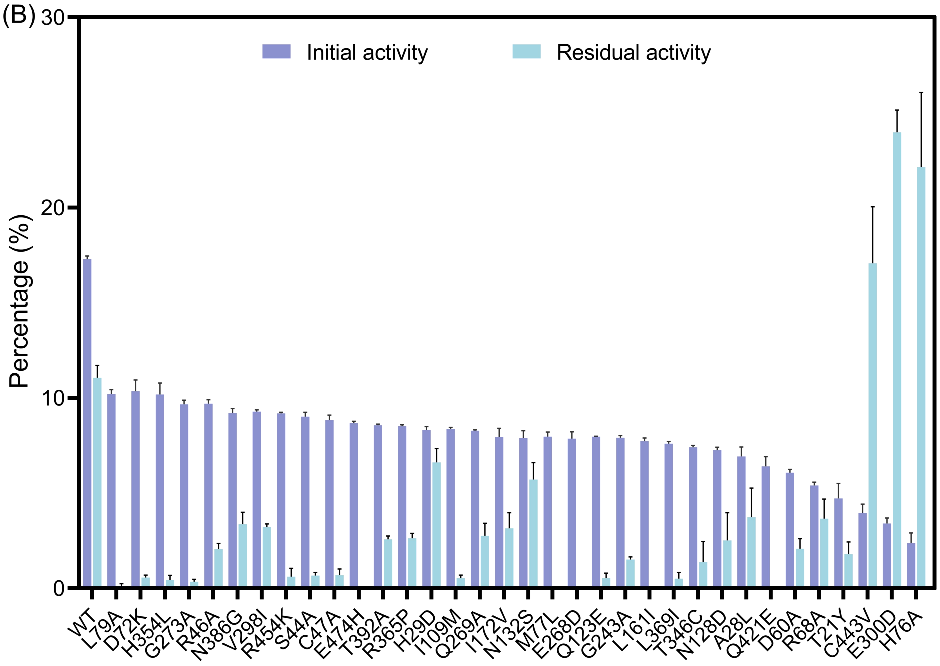


Fig. S11. Initial and residual activity of single variants predicted by FireProt and Consensus Finder. Initial activity was defined as the conversion rate of HMF by unincubated recombinase. Residual activity was defined as the ratio of HMF conversion rate by recombinase after incubation at 40 °C for 60 min to the conversion rate by unincubated recombinase. WT KvVDH was set as the control group.

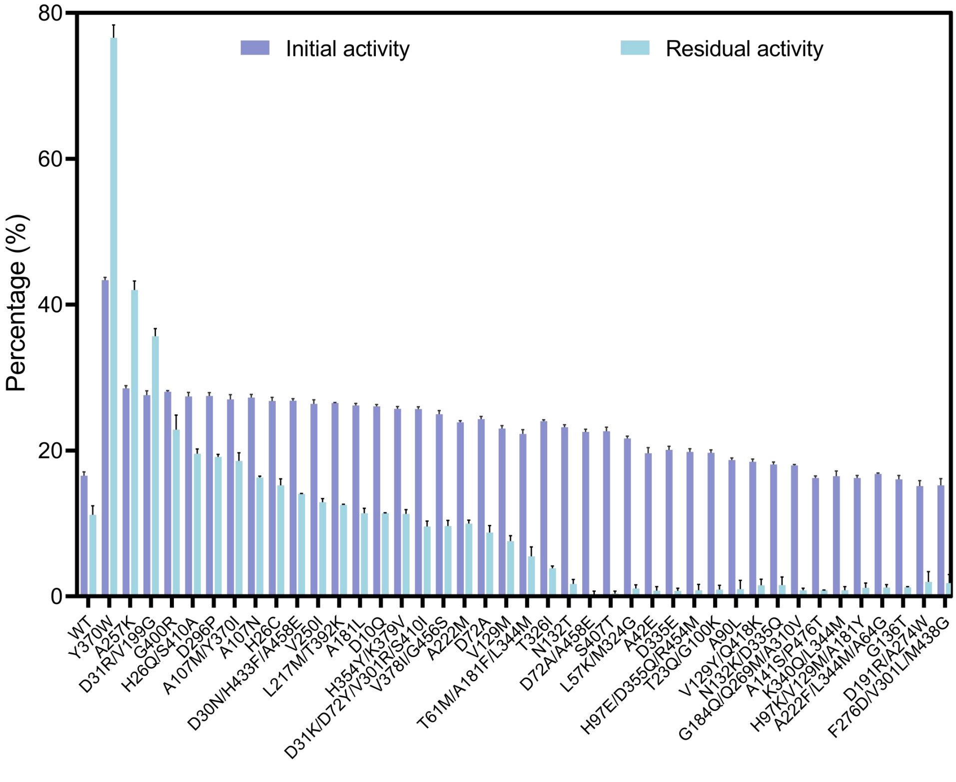


Fig. S12. Initial activity and residual activity of variants selected by random mutation screening. WT KvVDH and Y370W were set as the control group.

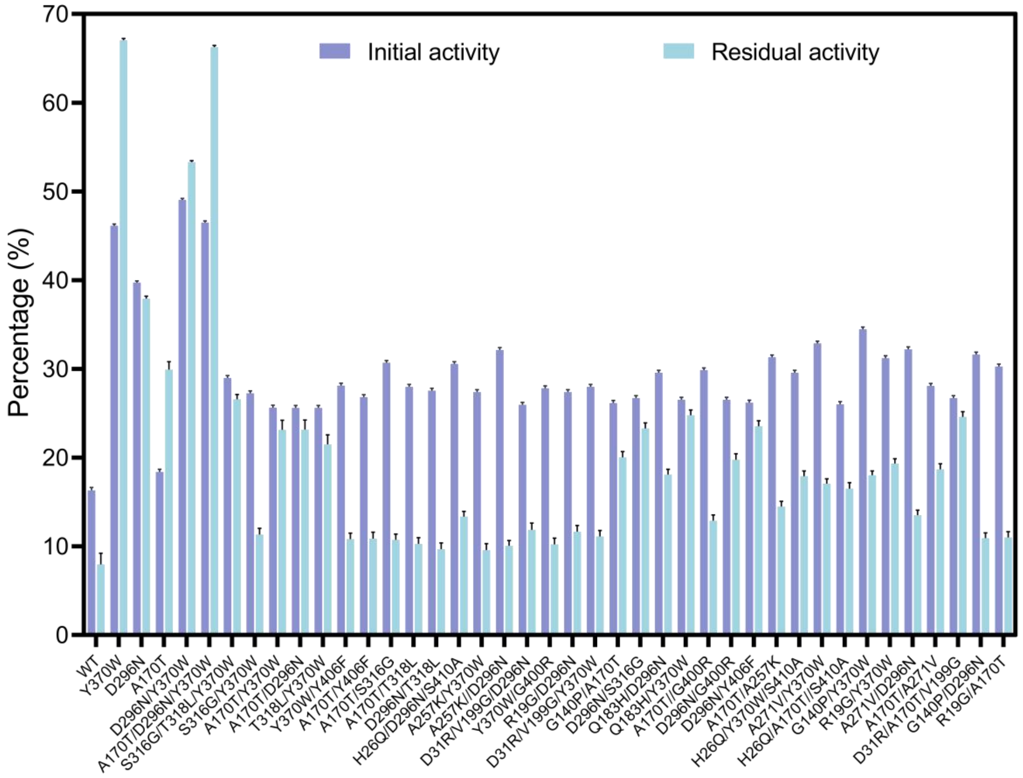


Fig. S13. Initial activity and residual activity of combinatorial variants derived from computational design and random mutagenesis screening. WT KvVDH and Y370W were set as controls.

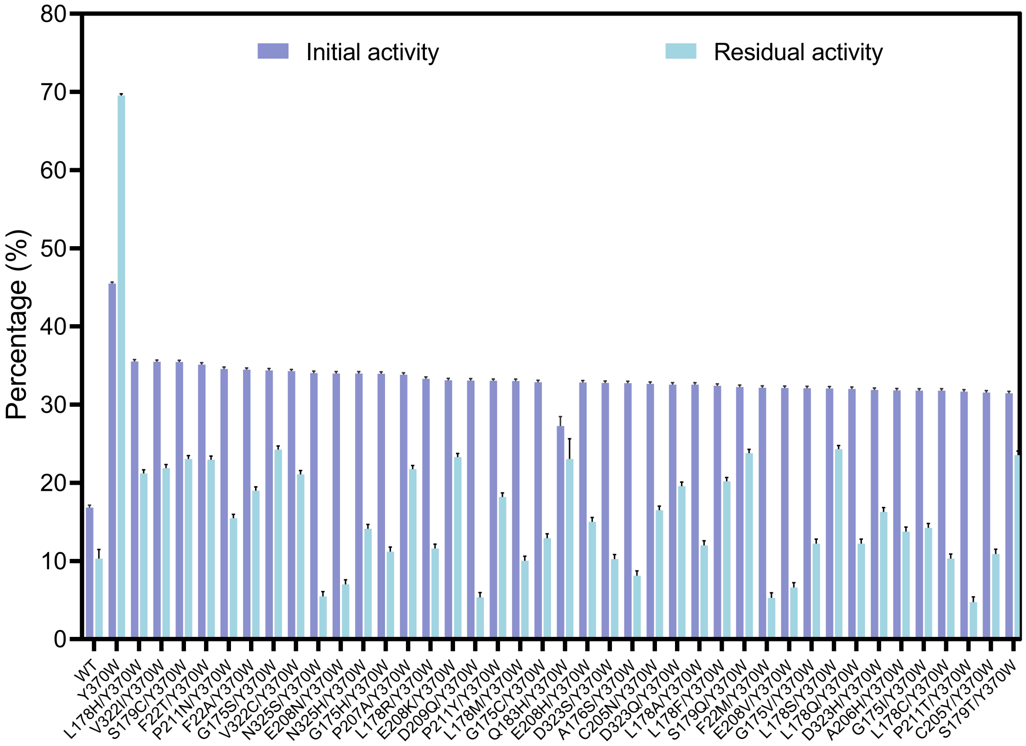


Fig. S14. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

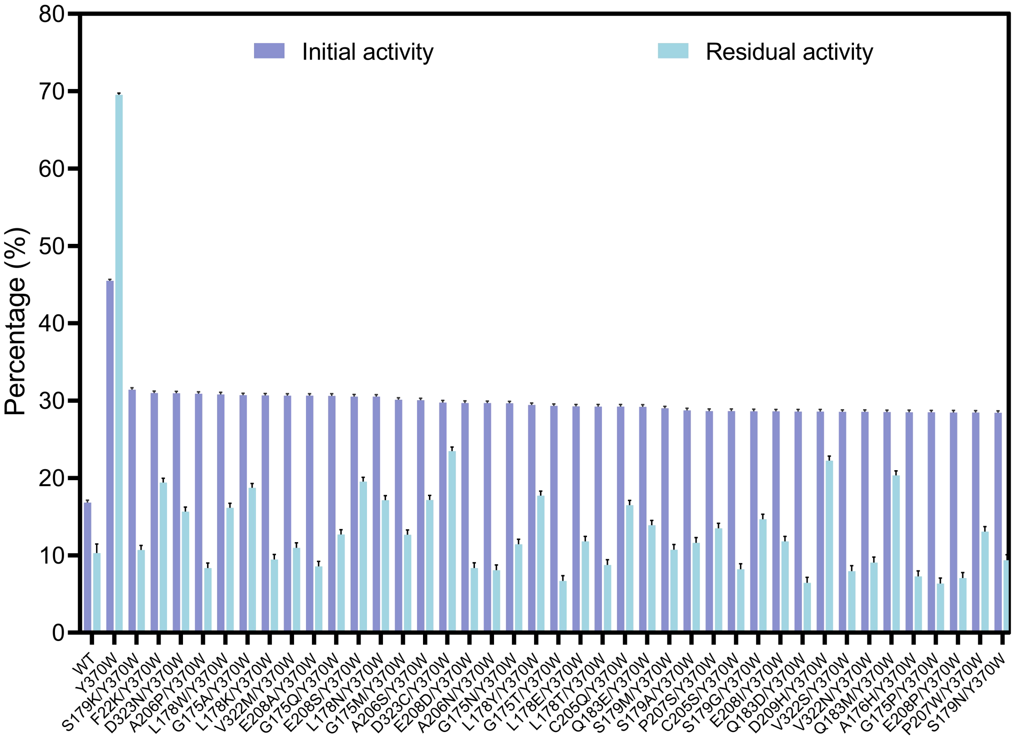


Fig. S15. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

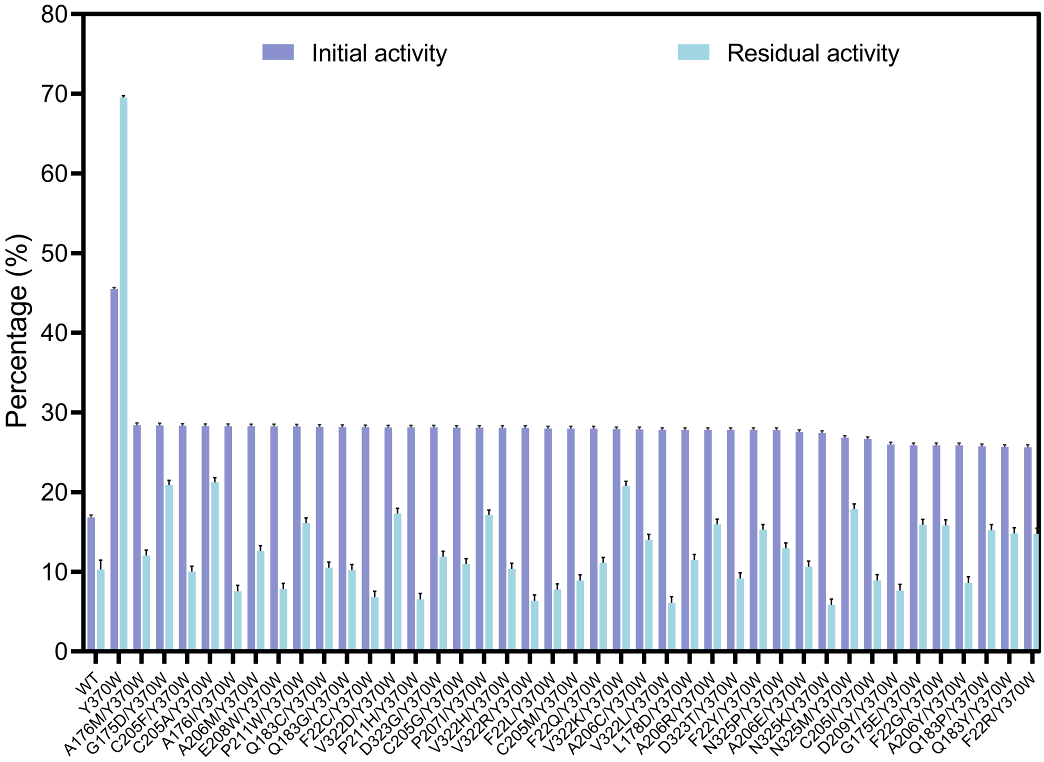


Fig. S16. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

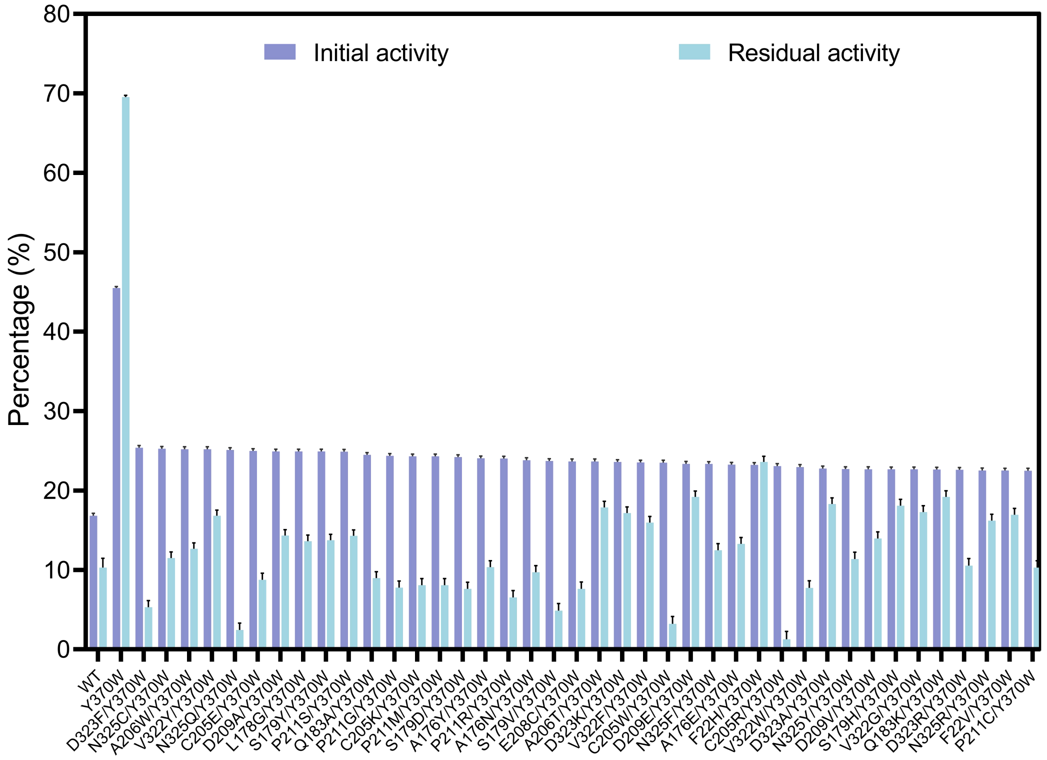


Fig. S17. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

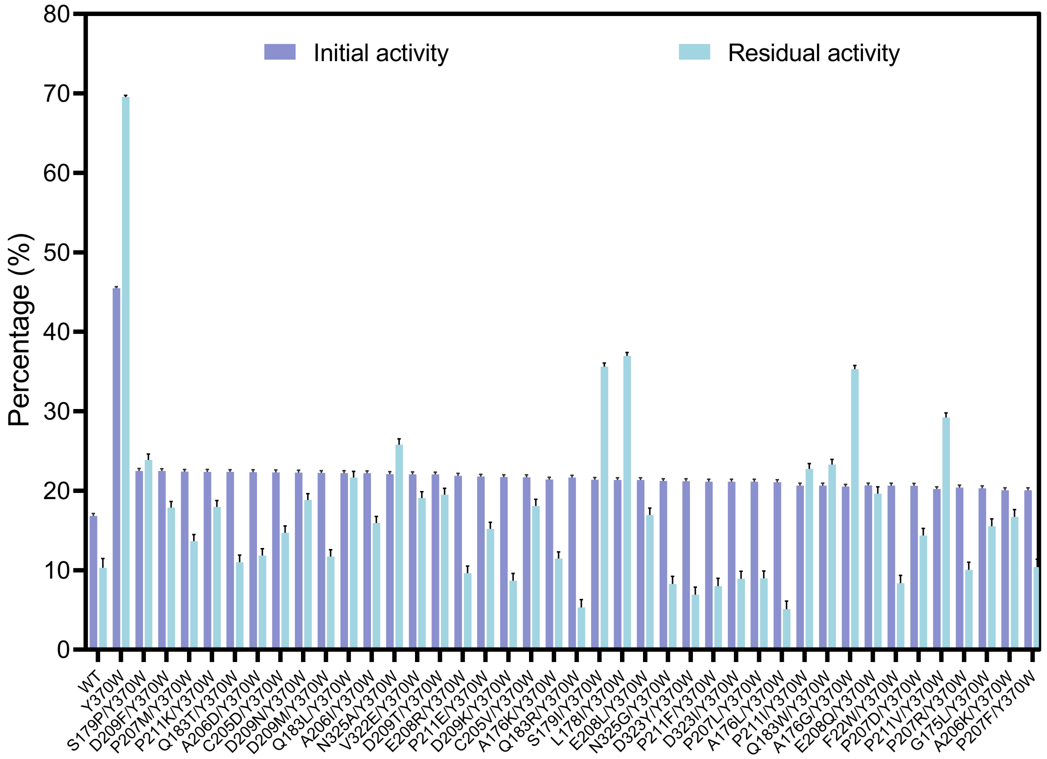


Fig. S18. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.。

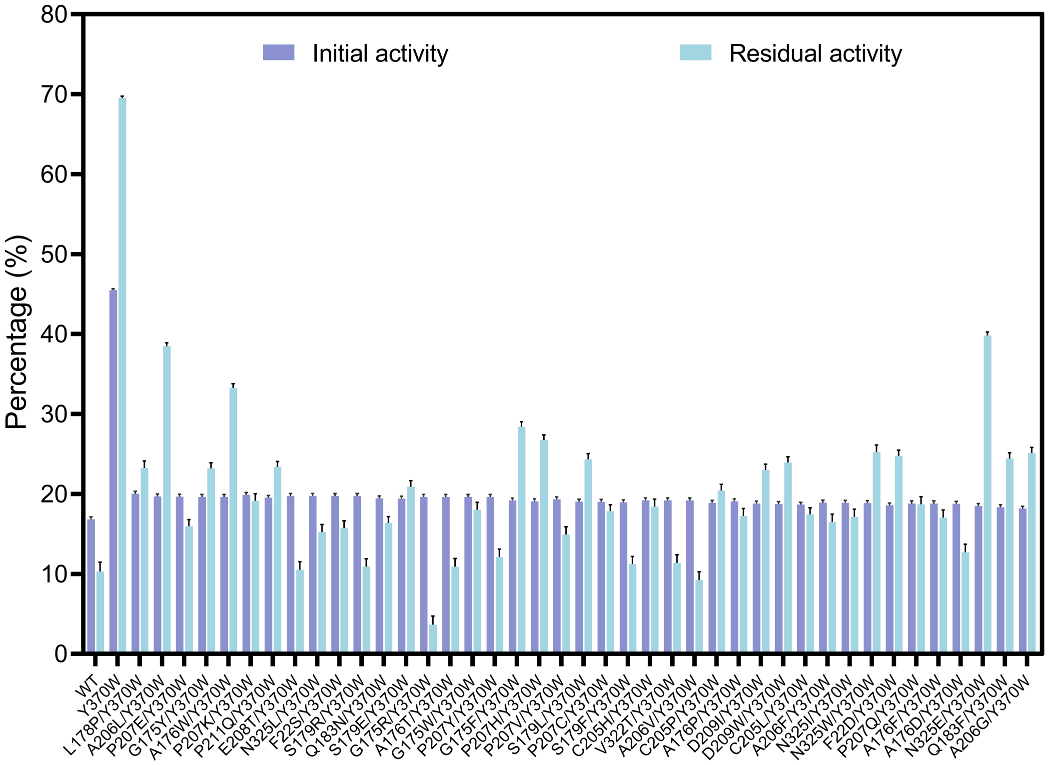


Fig. S19. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

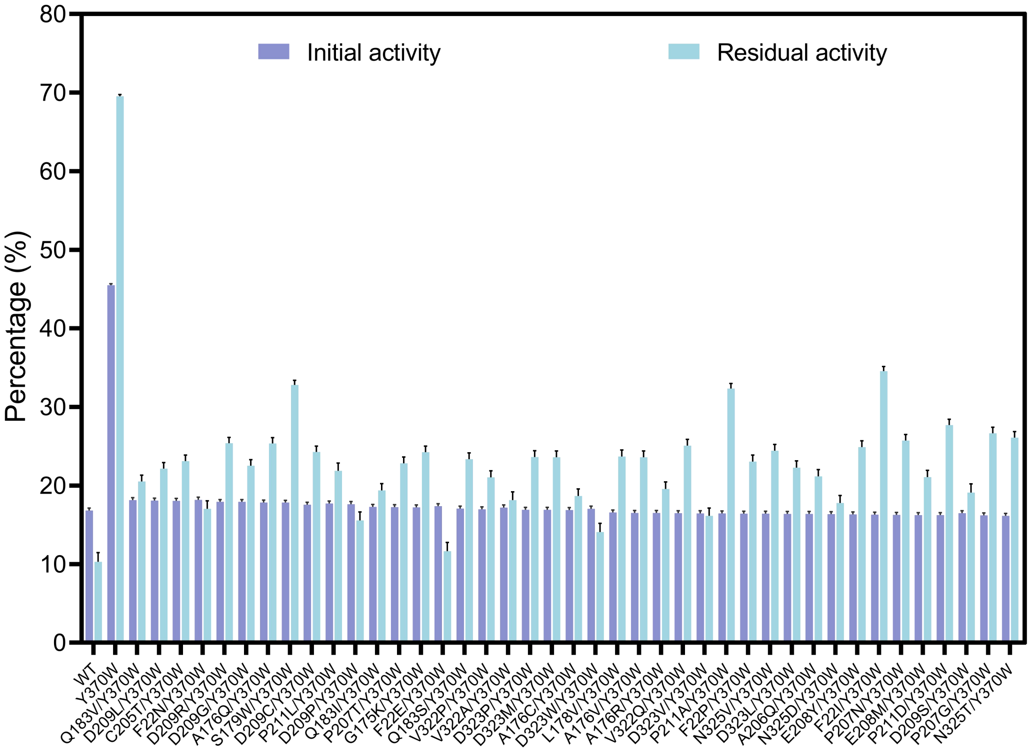


Fig. S20. Site saturation mutagenesis assessment of the Y370W variant, including initial activity and residual activity. WT KvVDH and Y370W were set as control groups.

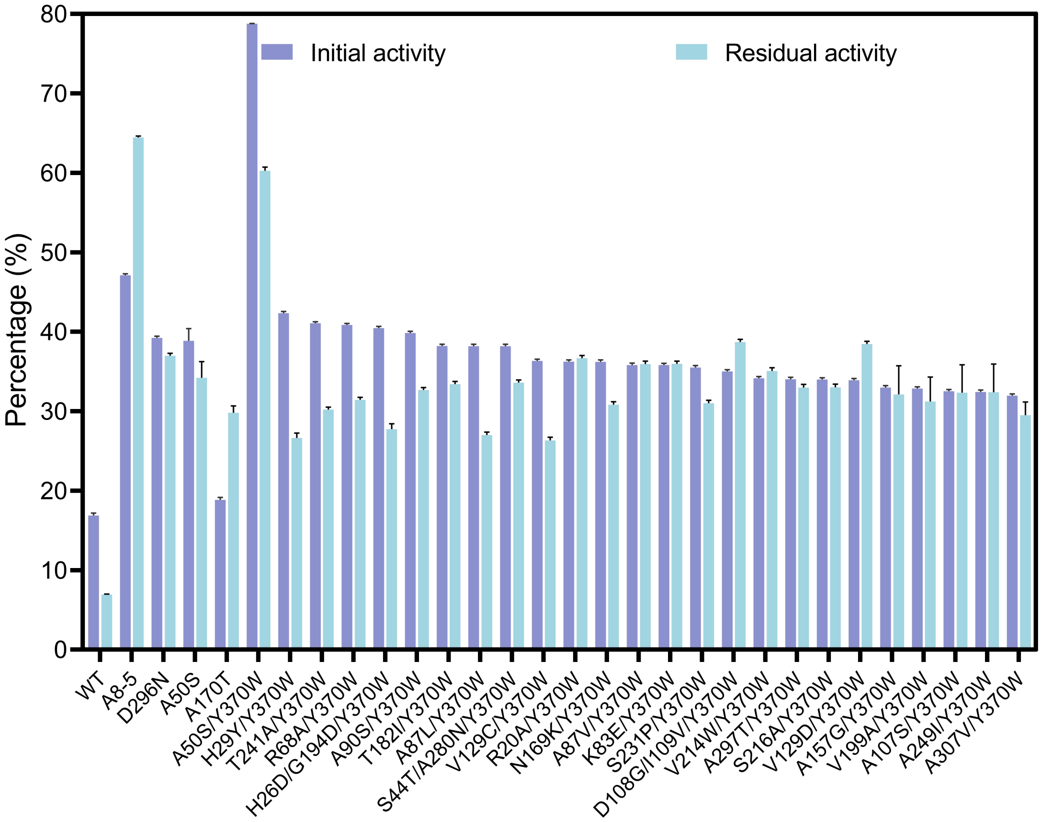


Fig. S21. Evaluation of region-random mutagenesis for Y370W, including initial and residual activity. WT KvVDH, A50S and Y370W were set as controls.

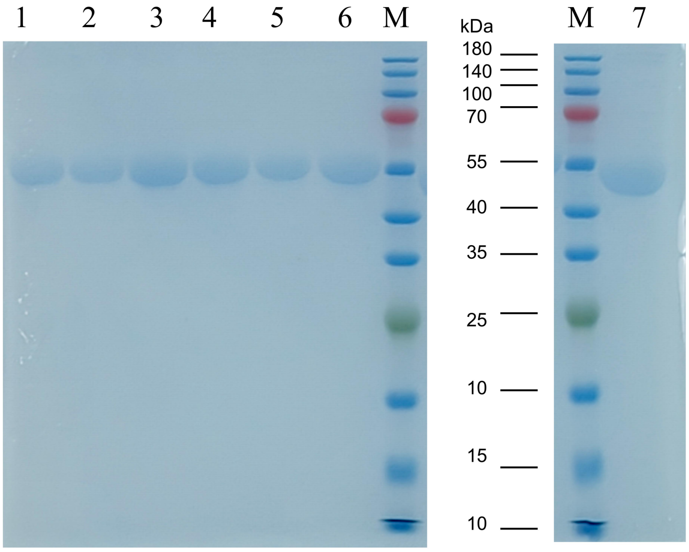


Fig. S22. SDS-PAGE analysis of the mutants of KvVDH. Lane M, protein marker; lane 1, the purified enzyme of WT KvVDH; lane 2, the purified enzyme of D296N; lane 3, the purified enzyme of Y370W; lane 4, the purified enzyme of M1 (D296N/Y370W); lane 5, the purified enzyme of M2 (A170T/D296N/Y370W); lane 6, the purified enzyme of M3 (A50S/Y370W); lane 7, the purified enzyme of NOX .

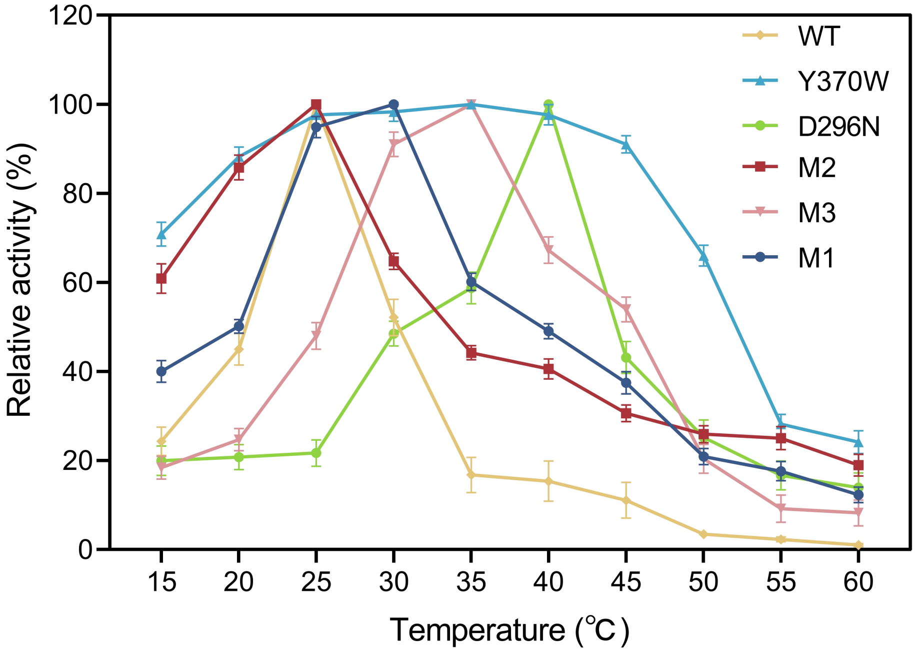


Fig. S23. Optimal reaction temperature for KvVDH and its variants. Enzyme activity at the optimal temperature is considered 100%.

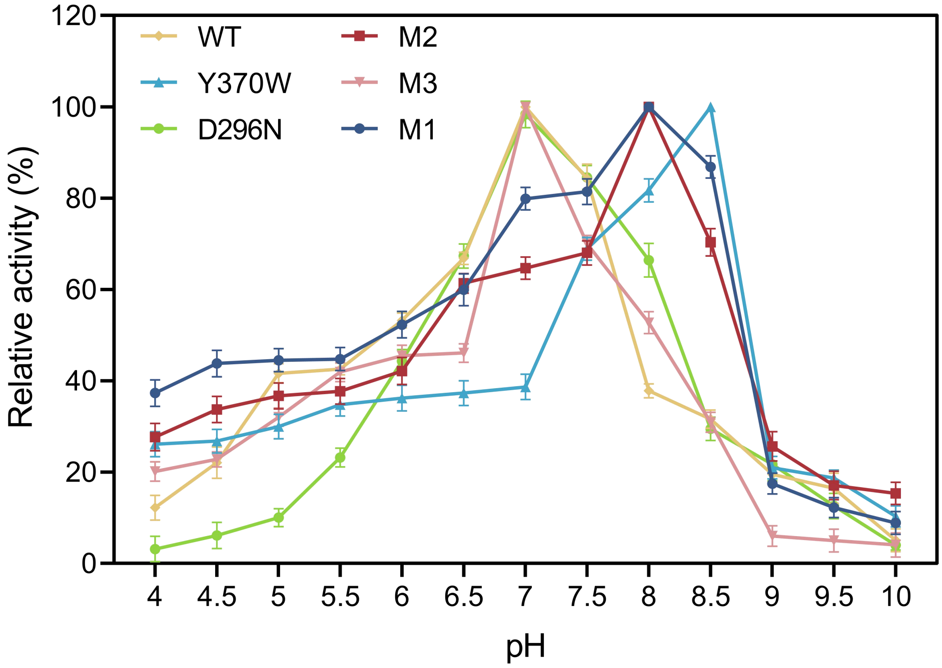


Fig. S24. Optimum reaction pH for KvVDH and its variants. Enzyme activity at the optimum pH was considered as 100%.

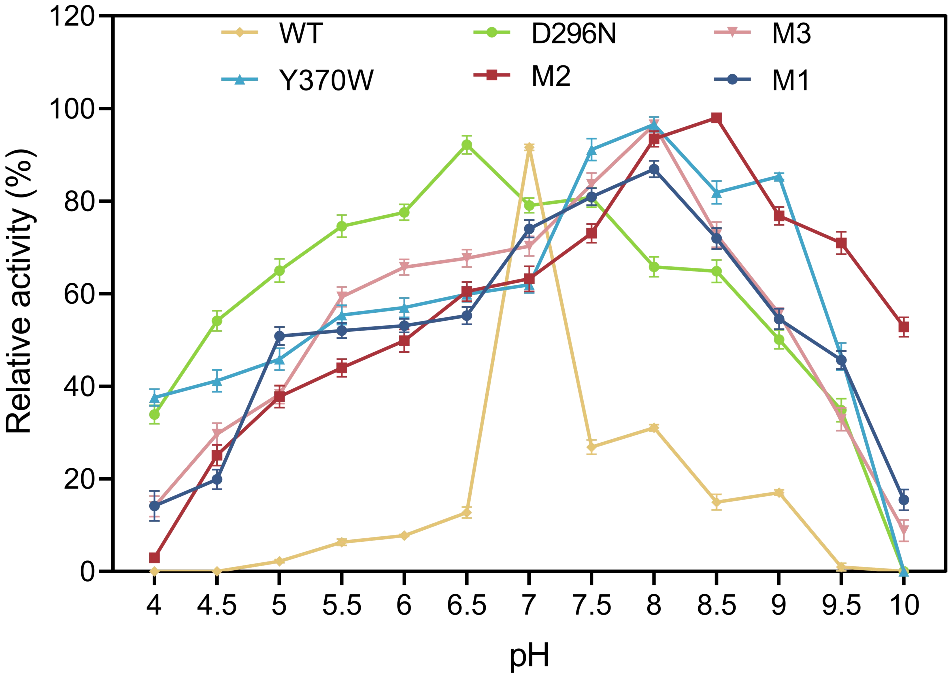


Fig. S25. The relative activity of KvVDH and its variants after incubation at pH 4.0–10.0 for 1 h. The initial enzyme activity without incubation was considered as 100%.

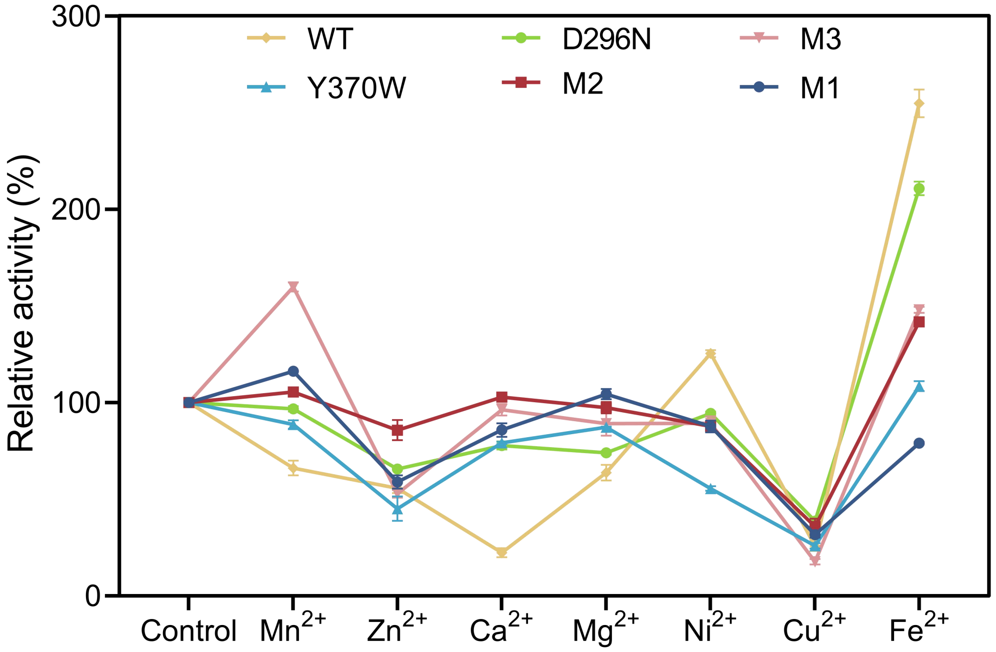


Fig. S26. Effect of different metal ion additions on the enzymatic activities of KvVDH and its variants. Enzyme activity without metal ion addition was considered as 100%.

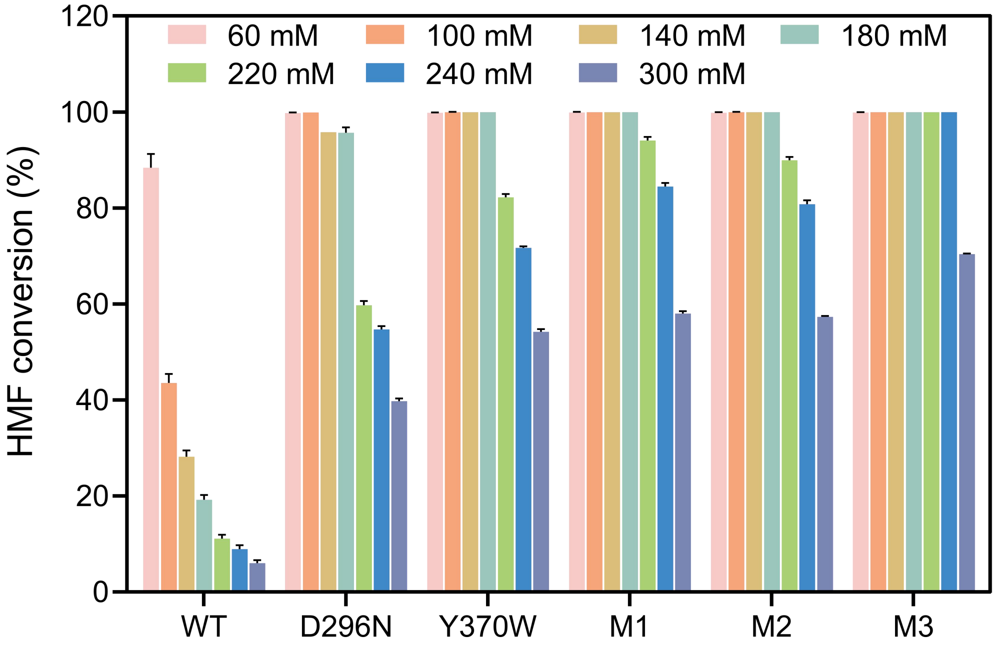


Fig. S27. HMF conversion rates after 12 h of reaction of *E. coli* cells containing WT KvVDH and various mutants in the presence of 60–300 mM HMF.

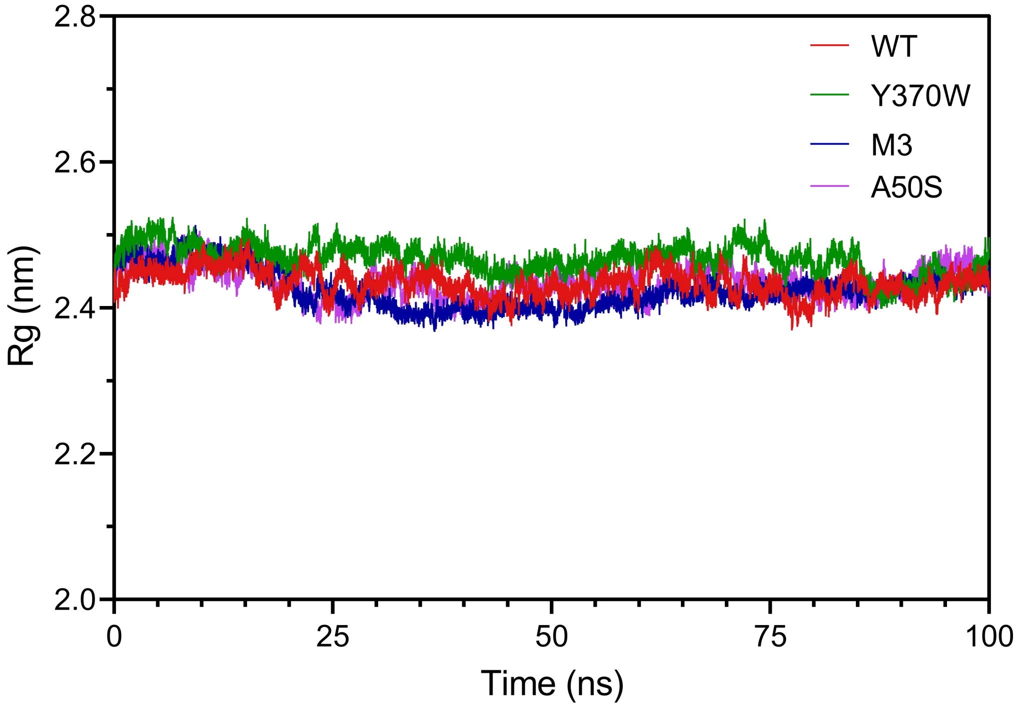


Fig. S28. Radius of gyration (Rg) of WT KvVDH, Y370W, A50S and M3 (A50S/Y370W) complexes with ligands during 100 ns MD simulations.

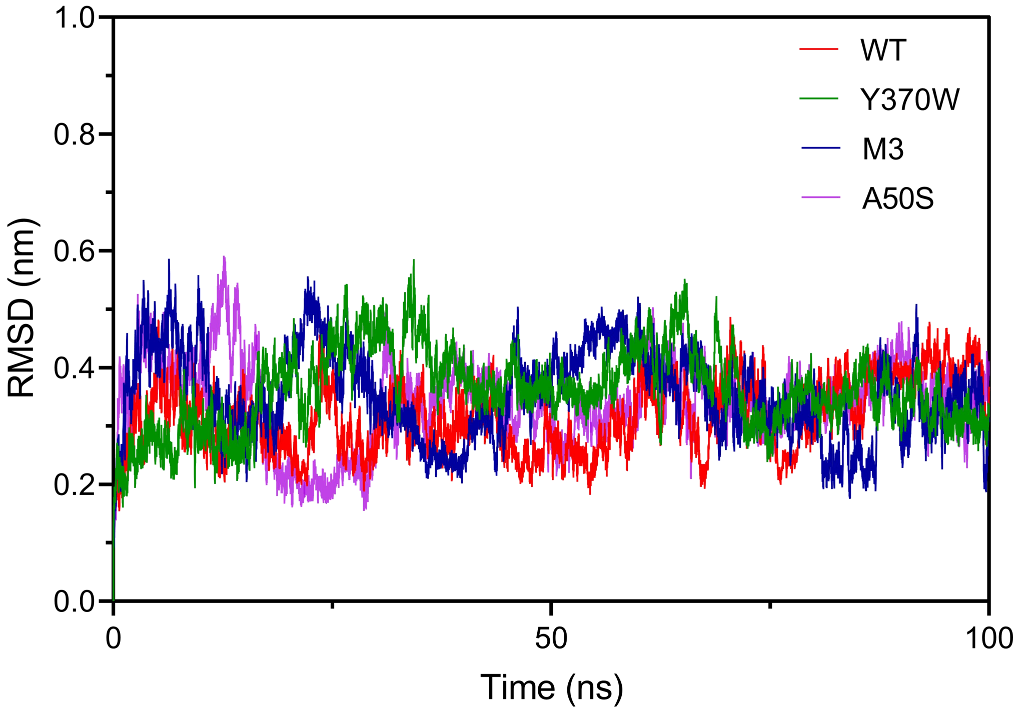


Fig. S29. Root mean square deviation (RMSD) of WT KvVDH, Y370W, A50S and M3 (A50S/Y370W) complexes with ligands during 100 ns MD simulations.

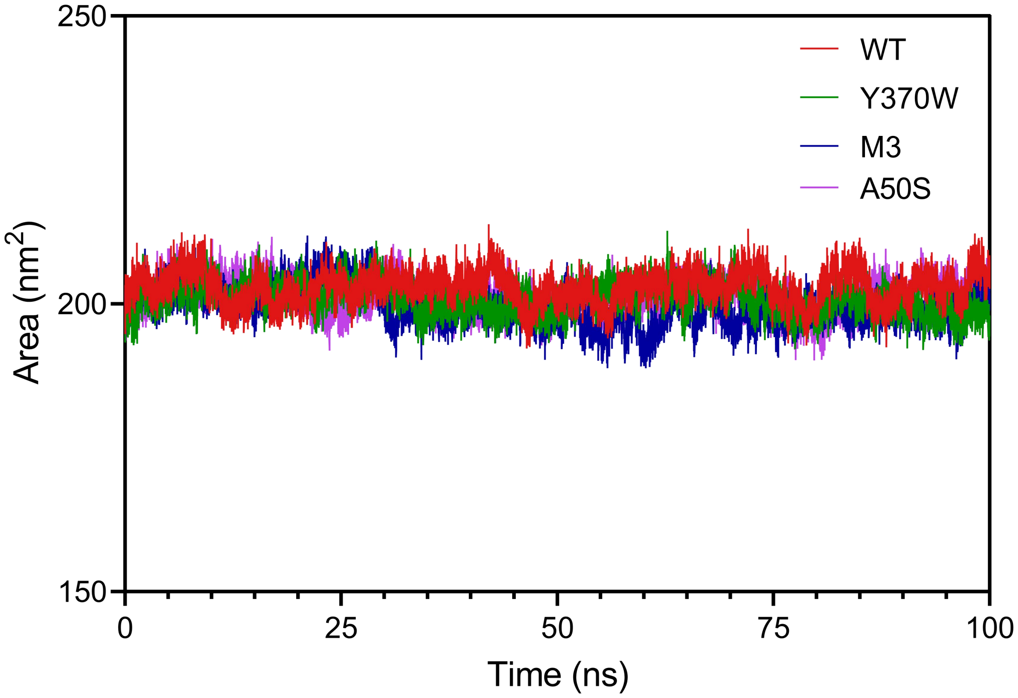


Fig. S30. Solvent-accessible surface area (SASA) of WT KvVDH, Y370W, A50S and M3 (A50S/Y370W) complexes with ligands during 100 ns MD simulations.

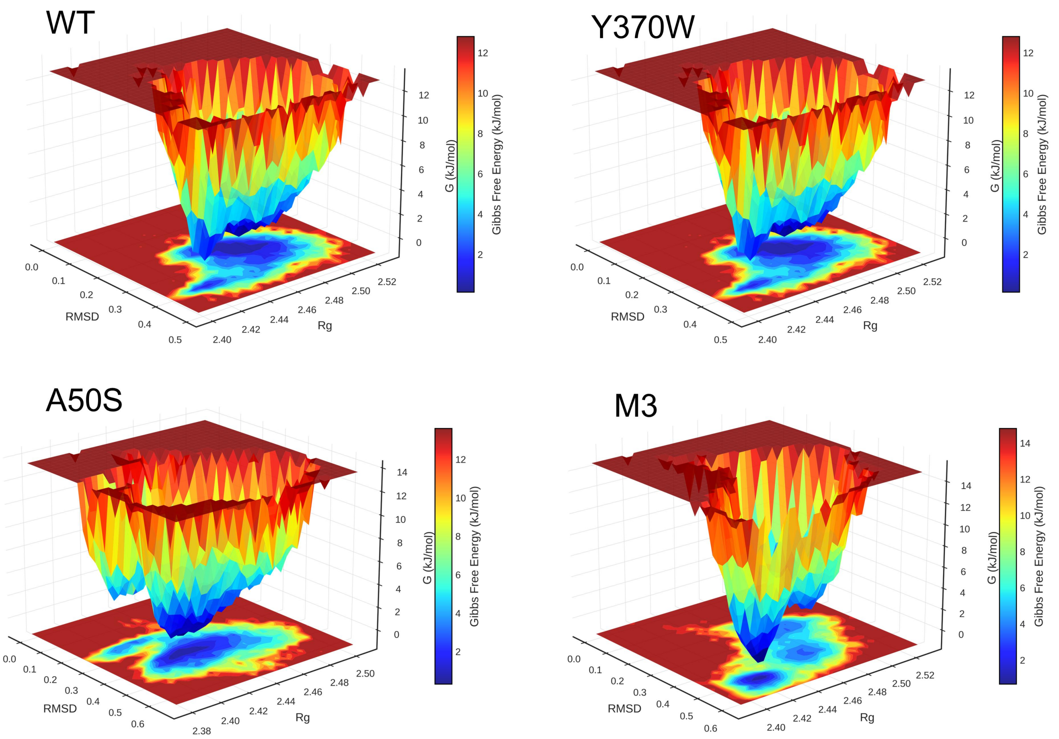


Fig. S31. Free energy landscape of WT KvVDH, Y370W, A50S and M3 (A50S/Y370W) complexes with HMF and NAD+. Energy ranged from high to low, represented in red and blue, respectively.

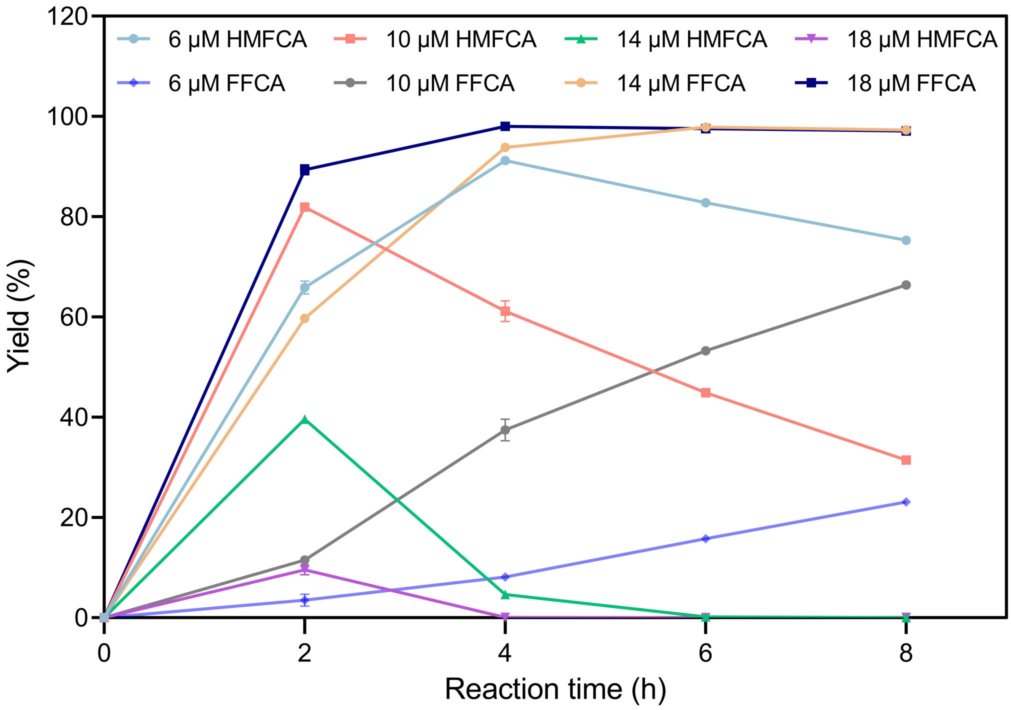


Fig. S32. Time course of HFMCA and FFCA synthesis catalyzed by purified M3 (A50S/Y370W) at different loading levels (6 μM, 10 μM, 14 μM and 18 μM) in the presence of 60 mM HMF.

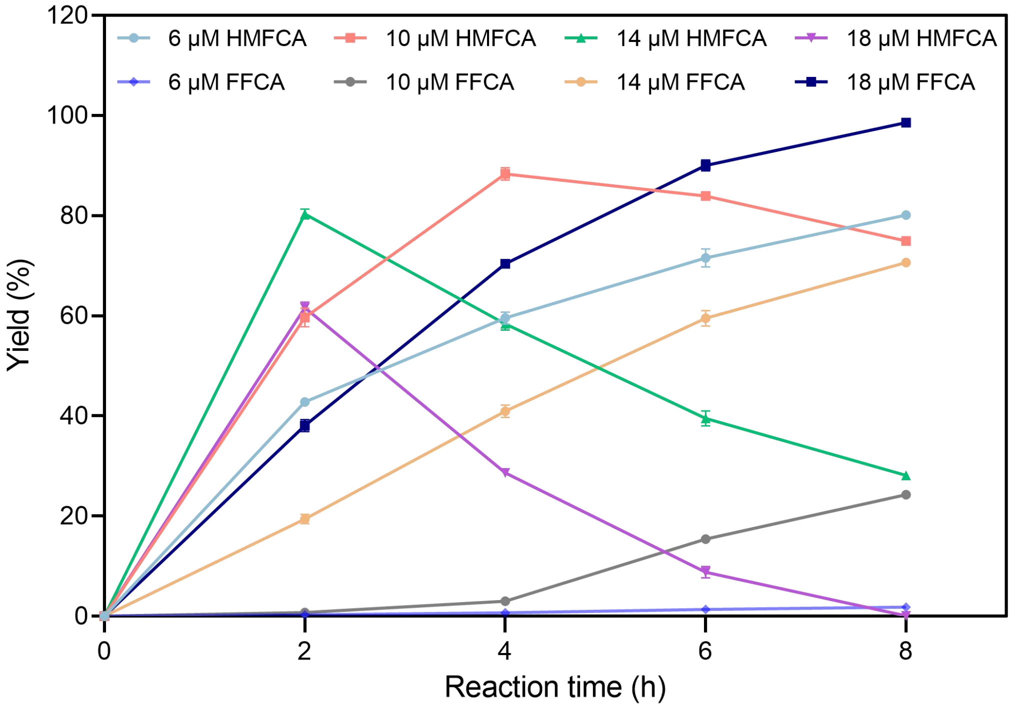


Fig. S33. Time course of HFMCA and FFCA synthesis catalyzed by purified M3 (A50S/Y370W) at different loading levels (6 μM, 10 μM, 14 μM and 18 μM) in the presence of 90 mM HMF.

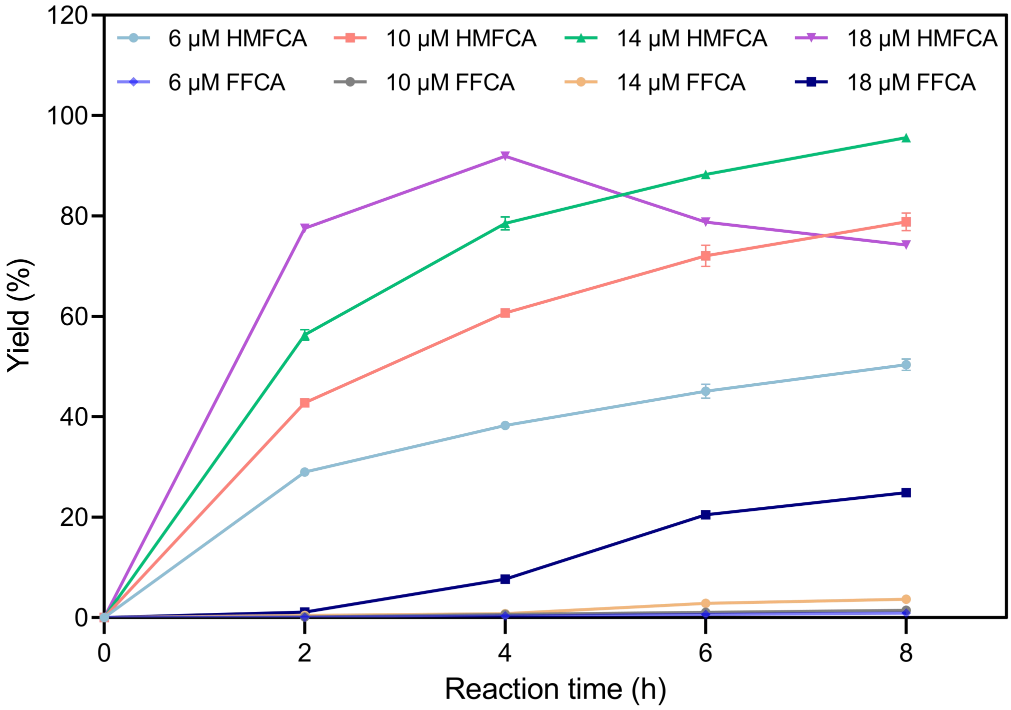


Fig. S34. Time course of HFMCA and FFCA synthesis catalyzed by purified M3 (A50S/Y370W) at different loading levels (6 μM, 10 μM, 14 μM and 18 μM) in the presence of 120 mM HMF.

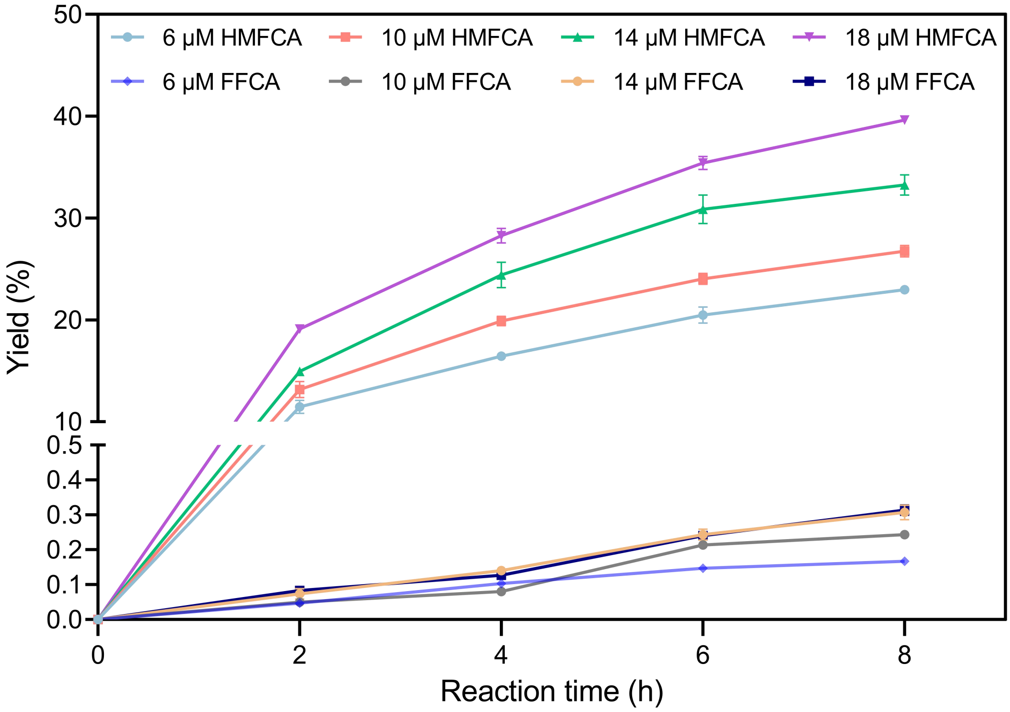


Fig. S35. Time course of HFMCA and FFCA synthesis catalyzed by purified M3 (A50S/Y370W) at different loading levels (6 μM, 10 μM, 14 μM and 18 μM) in the presence of 180 mM HMF.

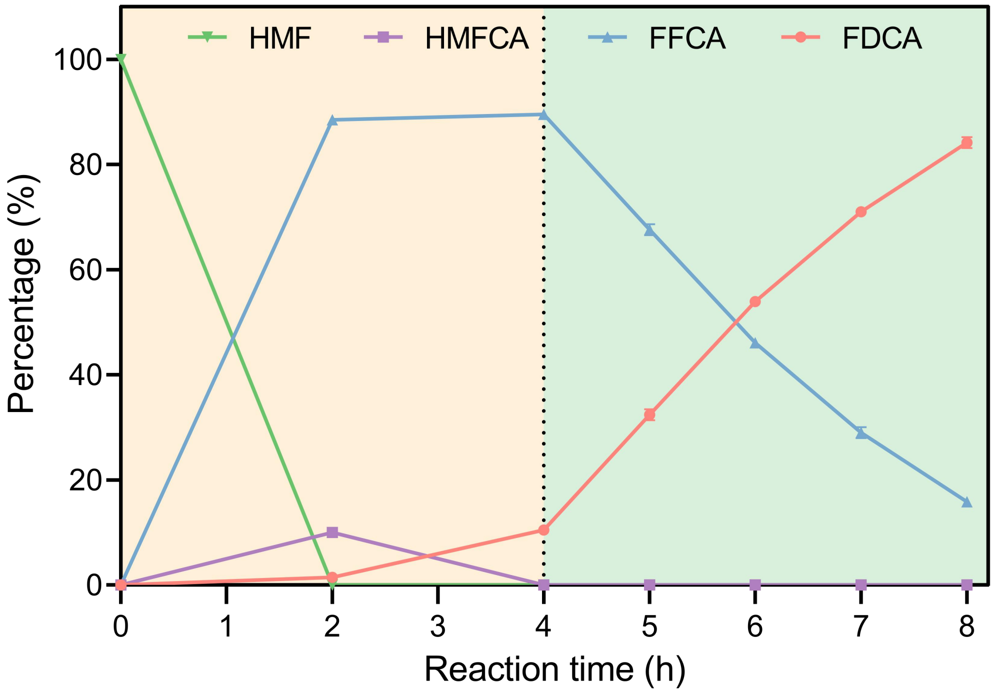


Fig. S36. A one-pot two-step enzyme cascade system for the selective catalytic oxidation of HMF to FDCA. The reaction system contained 30 mM HMF, 10 μM M3 (A50S/Y370W), 40 μM EcAldB and 20 μM NOX.

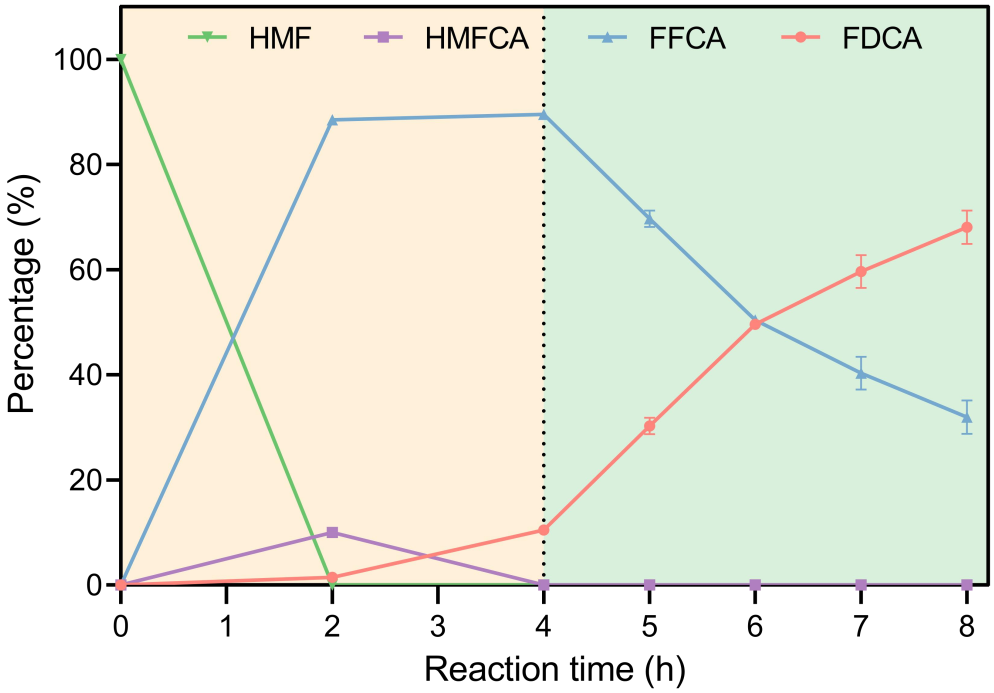


Fig. S37. A one-pot two-step enzyme cascade system for the selective catalytic oxidation of HMF to FDCA. The reaction system contained 30 mM HMF, 10 μM M3 (A50S/Y370W), 40 μM EcPuuC and 20 μM NOX.

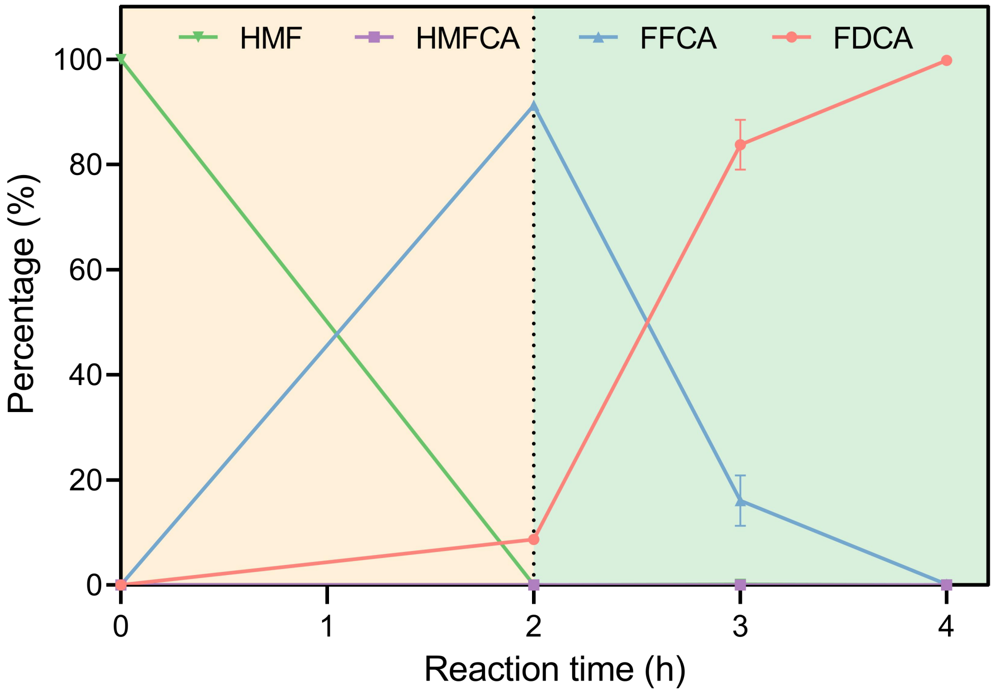


Fig. S38. One-pot two-step enzyme cascade system for the selective catalytic oxidation of HMF to FDCA. The reaction system contained 30 mM HMF, 10 μM M3 (A50S/Y370W), 80 μM EcALDH and 20 μM NOX.

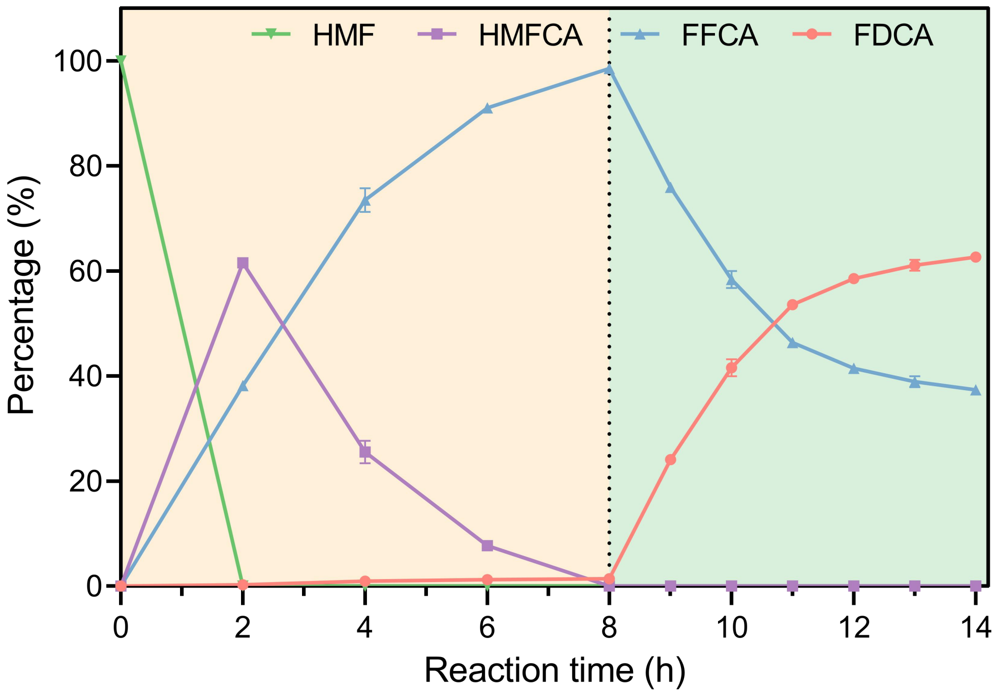


Fig. S39. One-pot two-step enzyme cascade system for the selective catalytic oxidation of HMF to FDCA. The reaction system contained 90 mM HMF, 18 μM M3 (A50S/Y370W), 80 μM EcALDH and 20 μM

NOX.

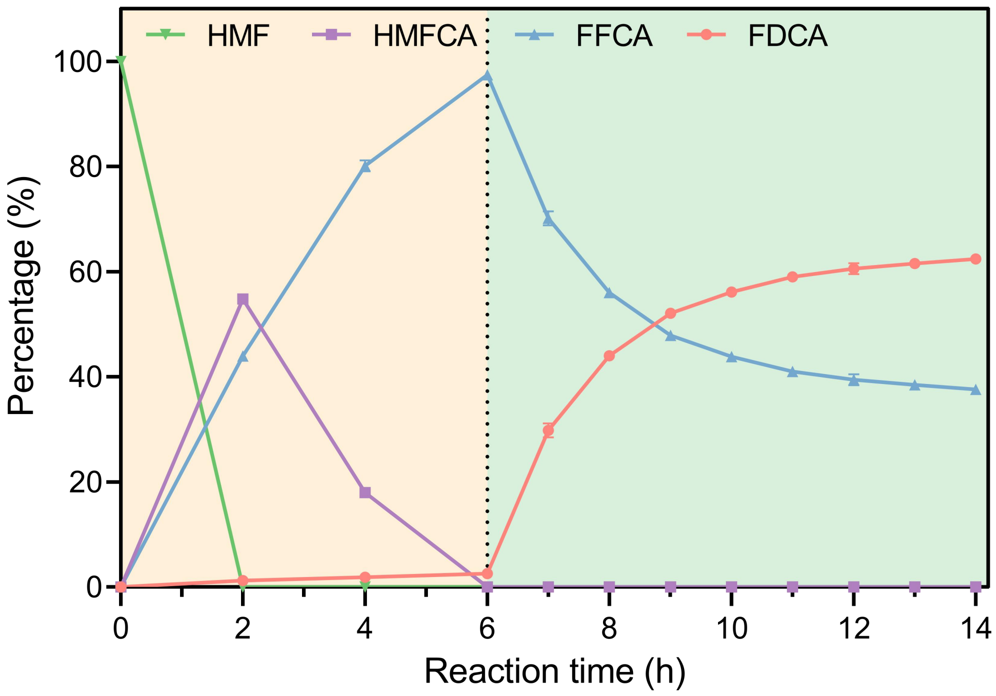


Fig. S40. One-pot two-step enzyme cascade system for the selective catalytic oxidation of HMF to

FDCA. The reaction system contained 120 mM HMF, 18 µM M3 (A50S/Y370W), 160 µM EcALDH and 20 µM NOX.

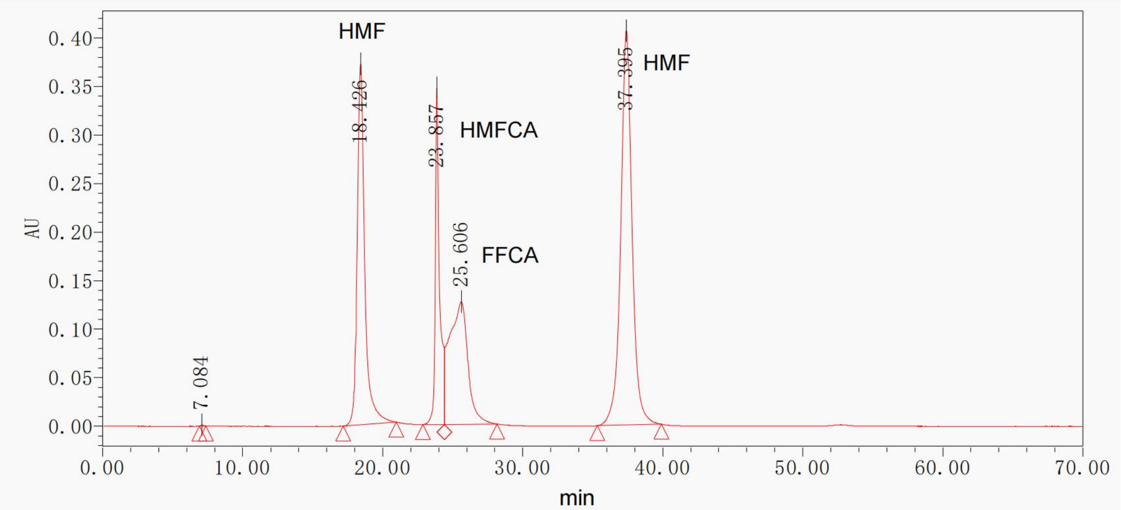


Fig. S41. HPLC Chromatograms of HMF, HMFCA, FFCA and FDCA.

## Supplementary Table

Table S1. Relevant sequence information used in this study.

| Enzyme | Original organism | NCBI protein accession nr | Codon optimization |
|---|---|---|---|
| CtVDH1 | *Comamonas testosteroni* | nr | no |
| KvEutE11 | *Klebsiella variicola* | nr | no |
| KvPuuC | *Klebsiella variicola* | nr | no |
| KvVDH | *Klebsiella variicola* | nr | no |
| KvALDH | *Klebsiella variicola* | nr | no |
| KvEutE | *Klebsiella variicola* | nr | no |
| KvSSADH11 | *Klebsiella variicola* | nr | no |
| KvSSADH | *Klebsiella variicola* | nr | no |
| KvLDH | *Klebsiella variicola* | nr | no |
| KvPADH | *Klebsiella variicola* | nr | no |
| KvBADH11 | *Klebsiella variicola* | nr | no |
| KvBADH | *Klebsiella variicola* | nr | no |
| KvALDH9A3 | *Klebsiella variicola* | nr | no |
| KvSSADH15 | *Klebsiella variicola* | nr | no |
| KvSG5SDH | *Klebsiella variicola* | nr | no |
| KvDBP | *Klebsiella variicola* | nr | no |
| KvOPDH | *Klebsiella variicola* | nr | no |
| KvSG5SDH14 | *Klebsiella variicola* | nr | no |
| KvDBP27 | *Klebsiella variicola* | nr | no |
| KvCAD | *Klebsiella variicola* | nr | no |
| KvKGSADH | *Klebsiella variicola* | nr | no |
| KvPADH11 | *Klebsiella variicola* | nr | no |
| KvAdhE | *Klebsiella variicola* | nr | no |
| BaALDH | *Bartonella apis* | WP_422536389.1 | yes |
| RaALDH | *Robbsia andropogonis* | WP_096349173.1 | yes |
| PpHYP | *Pseudomonadota bacterium* | MFM2 (A170T/D296N/Y370W)254457.1 | yes |
| PpALDH2 | *Pseudomonadota bacterium* | MBW4048360.1 | yes |
| BcALDH | *Burkholderia cepacia* | WP_175755202.1 | yes |
| PpHYP11 | *Pseudomonadota bacterium* | MFM2 (A170T/D296N/Y370W)341366.1 | yes |
| PpALDH18 | *Pseudomonadota bacterium* | MBS0453095.1 | yes |
| CoALDH | *Comamonas* | WP_182286085.1 | yes |
| BaALDH | *Bartonella apis* | WP_422536389.1 | yes |
| EcPuuC | *Escherichia coli* H386 | OSL13388.1 | yes |
| AbEutE | *Acinetobacter baylyi* ADP1 | AAP78946.1 | yes |
| EcAldB | *Escherichia coli* | WP_271711111.1 | yes |
| AnAdhP | *Aspergillus niger* | CAK96436.1 | yes |
| EcAOR | *Escherichia coli* | nr | yes |
| EcAldA | *Escherichia coli* | HBB8264191.1 | yes |
| EcALDH | *Escherichia coli* (strain K12) | WP_208484097.1 | yes |
| PpAldB11 | *Pseudomonas aeruginosa* | nr | yes |
| PpAldA | *Pseudomonas putida* KT2440 | AAN68099.1 | yes |
| PpAldB2 | *Pseudomonas putida* KT2440 | nr | yes |
| PpXOD | *Escherichia sp.* WS545 | WP_410235372.1 | yes |
| PpALDH | *Pseudomonas putida* KT2440 | nr | yes |
| PpVDH | *Pseudomonas sp. WHRI* 8519 | WP_349975037.1 | yes |
| BtDeh11 | *Bacillus tropicus* | nr | yes |
| BtDeh8 | *Bacillus tropicus* | nr | yes |
| BtMCOs | *Bacillus tropicus* | nr | yes |
| BtOR | *Bacillus tropicus* | nr | yes |
| BtDeh149 | *Bacillus tropicus* | nr | yes |
| BtADH | *Bacillus tropicus* | nr | yes |
| BtDeh193 | *Bacillus tropicus* | nr | yes |
| NOX | *Levilactobacillus brevis* | 5ER0_A | yes |

Table S2. Reaction systems in which HMF, FFF, HMFA, or FFA serve as substrates, respectively.

| Reaction results in | | | | | | | |
|---|---|---|---|---|---|---|---|
| Component | Final concentration | | | | | | |
| Substrate (HMF, FFF, HMFA, or FFA) | 10 mM | 10 mM | 10 mM | 10 mM | 10 mM | 10 mM | 10 mM |
| $NAD^+$ | 20 mM | 20 mM | 20 mM | 20 mM | 20 mM | 20 mM | 20 mM |
| KvVDH | 5 µM | - | - | - | - | - | - |
| PpVDH | - | 5 µM | - | - | - | - | - |
| EcPuuC | - | - | 5 µM | - | - | - | - |
| EcAldB | - | - | - | 5 µM | - | - | - |
| PpALDH | - | - | - | - | 5 µM | - | - |
| EcALDH | - | - | - | - | - | 5 µM | - |
| PpXOD | - | - | - | - | - | - | 5 µM |
| $MnCl_2 \cdot 4H_2O$ | - | - | 1 mM | - | 1 mM | 1 mM | 1 mM |
| $ZnCl_2$ | - | - | 1 mM | 1 mM | - | 1 mM | 1 mM |
| $CaCl_2$ | - | 1 mM | 1 mM | 1 mM | 1 mM | - | - |
| $MgCl_2 \cdot 6H_2O$ | - | 1 mM | 1 mM | 1 mM | 1 mM | - | - |
| $NiSO_4 \cdot 6H_2O$ | 1 mM | - | 1 mM | 1 mM | 1 mM | 1 mM | 1 mM |
| $CuSO_4 \cdot 5H_2O$ | - | - | - | - | - | - | - |
| $FeSO_4 \cdot 7H_2O$ | 1 mM | - | 1 mM | 1 mM | 1 mM | 1 mM | 1 mM |
| Reaction buffer | pH 8.0 | pH 7.5 | pH 7.5 | pH 8.5 | pH 8.0 | pH 8.0 | pH 7.5 |
| Reaction temperature | 30°C | 25°C | 35°C | 20°C | 30°C | 20°C | 30°C |

Table S3. Reaction systems catalyzed by M3 (A50S/Y370W) for HMF at different concentrations.

| Reaction results in | | | | |
|---|---|---|---|---|
| Component | Final concentration | | | |
| Substrate (HMF) | 60 mM、90 mM、120 mM or 180 mM | | | |
| $NAD^+$ | 10 mM | 10 mM | 10 mM | 10 mM |
| FAD | 0.2 mM | 0.2 mM | 0.2 mM | 0.2 mM |
| NOX | 20 µM | 20 µM | 20 µM | 20 µM |
| M3 (A50S/Y370W) | 6 µM | 10 µM | 14 µM | 18µM |
| $MnCl_2 \cdot 4H_2O$ | 1 mM | 1 mM | 1 mM | 1 mM |
| $FeSO_4 \cdot 7H_2O$ | 1 mM | 1 mM | 1 mM | 1 mM |
| Reaction buffer | pH 7.0 | pH 7.0 | pH 7.0 | pH 7.0 |
| Reaction temperature | 35°C | 35°C | 35°C | 35°C |

Table S4. Reaction compositions of reactions for HMF conversion to FDCA.

| Reaction results in | | | |
|---|---|---|---|
| Component | Final concentration | | |
| HMF | 30 mM | 30 mM | 30 mM |
| $NAD^+$ | 10 mM | 10 mM | 10 mM |
| FAD | 0.2 mM | 0.2 mM | 0.2 mM |
| NOX | 20 μM | 20 μM | 20 μM |
| M3 (A50S/Y370W) | 10 μM | 10 μM | 10 μM |
| $MnCl_2 \cdot 4H_2O$ | 1 mM | - | 1 mM |
| $FeSO_4 \cdot 7H_2O$ | 1 mM | 1 mM | 1 mM |
| EcPuuC | 40 μM | - | - |
| EcAldB | - | 40 μM | - |
| EcALDH | - | - | 40 μM |

Table S5. The thermostable single mutants predicted by PROSS and FireProt.

| Position | Residue | Mutation | Foldx | Rosetta | Conservation score | Correlation score | Majority | Ratio | B factor | Is BTC | Is Low Energy | Is High Energy | Is Low Combined | Is High Combined |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | R | G | -0.55 | null | 3 | 2.3 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 21 | T | Y | -2.87 | null | 5 | 1.13 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 23 | T | E | -0.14 | null | 3 | 1.84 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 28 | A | L | -0.3 | null | 3 | 2.73 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 29 | H | D | 0.05 | null | 4 | 3.23 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 30 | D | G | -1.92 | null | 4 | 1 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 34 | S | T | -1.37 | null | 6 | 1.03 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 44 | S | A | -0.74 | null | 5 | 1.13 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 46 | R | A | 0.34 | null | 2 | 2.27 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 47 | C | A | -1.83 | null | 5 | 0.72 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 60 | D | A | 0.23 | null | 3 | 1.42 | FALSE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 62 | S | G | 0.42 | null | 4 | 1.96 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 68 | R | A | 0.36 | null | 1 | 2.34 | FALSE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 72 | D | K | -1.47 | null | 4 | 1.64 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 72 | D | R | -2.06 | null | 4 | 1.64 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 76 | H | A | 0.49 | null | 2 | 3.12 | FALSE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 77 | M | L | 0.08 | null | 5 | 0.96 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 79 | L | A | 0.23 | null | 4 | 1.81 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 87 | A | I | -2.38 | null | 3 | 1.39 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 109 | I | M | -0.11 | null | 4 | 1.07 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 123 | Q | E | -0.01 | null | 5 | 0.66 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 128 | N | D | -0.03 | null | 6 | 1.72 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 132 | N | S | -0.38 | null | 4 | 1.65 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 136 | G | I | -2.21 | null | 5 | 1.51 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 140 | G | P | -2.56 | null | 5 | 0.23 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 146 | G | S | -2.64 | null | 5 | 0.68 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 161 | L | I | 0 | null | 5 | 1.45 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 163 | T | M | -2.4 | null | 4 | 0.96 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 170 | A | T | -0.15 | null | 6 | 0.62 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 172 | I | V | -0.54 | null | 6 | 0.54 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 176 | A | S | 0.38 | null | 6 | -0.18 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 183 | Q | H | -0.71 | null | 8 | -0.01 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 188 | D | E | -0.95 | null | 4 | 2.03 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 204 | T | M | -2.08 | null | 5 | 1.82 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 216 | S | A | -0.62 | null | 4 | 1.81 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 241 | T | M | -2.5 | null | 2 | 2.12 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 243 | G | A | -0.13 | null | 5 | 0.63 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 245 | Y | H | 0.19 | null | 5 | 1.7 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 249 | A | L | -2.07 | null | 4 | 0.61 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 268 | E | D | -0.02 | null | 5 | 0.36 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 269 | Q | A | -0.08 | null | 3 | 1.79 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 271 | A | V | -1.65 | null | 6 | 0.1 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 273 | G | A | -0.9 | null | 5 | 0.39 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 280 | A | W | -3.2 | null | 5 | 3.22 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 296 | D | N | -0.58 | null | 2 | 1.07 | FALSE | FALSE | null | FALSE | FALSE | FALSE | FALSE | FALSE |
| 298 | V | I | -0.87 | null | 6 | 0.99 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 300 | E | D | 0.4 | null | 5 | 0.48 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 309 | R | K | -0.17 | null | 6 | -0.06 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 316 | S | G | 0.12 | null | 5 | 0.01 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 318 | T | L | -2.97 | null | 5 | 0.02 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 327 | I | V | 0.18 | null | 4 | 2.67 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 346 | T | C | 0 | null | 4 | 2.81 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 354 | H | L | -0.99 | null | 4 | 2.17 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 356 | A | P | -1.85 | null | 3 | 3.24 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 365 | R | P | -0.23 | null | 4 | 1.08 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 369 | L | I | -0.05 | null | 5 | 0.83 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 374 | S | L | -2.39 | null | 5 | 1.24 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 379 | K | M | -3.57 | null | 5 | 1.92 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 380 | A | V | -2.42 | null | 3 | 1.88 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 386 | N | G | -2.12 | null | 4 | 1.69 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 392 | T | A | -0.6 | null | 4 | 2.44 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | FALSE |
| 406 | Y | F | -0.87 | null | 6 | -0.03 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 421 | Q | E | -0.15 | null | 5 | 1.13 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 443 | C | V | -0.56 | null | 5 | 0.65 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 454 | R | K | -0.05 | null | 4 | 0.6 | TRUE | FALSE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 456 | G | M | -2.33 | null | 5 | 1.04 | FALSE | FALSE | null | FALSE | FALSE | TRUE | FALSE | TRUE |
| 474 | E | H | 0.24 | null | 5 | 1 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |
| 475 | L | Y | 0.26 | null | 6 | 0.36 | TRUE | TRUE | null | TRUE | FALSE | FALSE | FALSE | TRUE |

Table S6. The specific activity of KvVDH and its mutants toward various substrates.[a]

| Enzymes | Specific activity (mU·mg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | HMF | HMFCA | FFCA | FDCA | Furfural | Furfuryl alcohol |
| PpVDH | 341.08 ± 14.34 | 27.08 ± 2.88 | 7.27 ± 1.13 | nd | 241.72 ± 16.07 | 77.48 ± 5.61 |
| EcPuuC | 353.65 ± 5.64 | 231.82 ± 1.47 | 59.49 ± 1.93 | nd | 200.58 ± 2.97 | 57.84 ± 7.20 |
| EcAldB | 183.12 ± 14.51 | 78.16 ± 6.08 | 58.74 ± 1.48 | nd | 68.85 ± 7.59 | 97.54 ± 13.15 |
| PpALDH | 410.07 ± 18.11 | 360.80 ± 4.72 | 163.84 ± 14.48 | 0.70 ± 0.48 | 51.17 ± 9.61 | 29.11 ± 2.26 |
| EcALDH | 72.27 ± 3.45 | 43.84 ± 0.48 | 101.81 ± 7.83 | nd | 115.00 ± 19.54 | 2.86 ± 5.02 |
| PpXOD | 26.91 ± 2.18 | 15.05 ± 1.28 | 11.20 ± 1.59 | nd | 20.37 ± 2.00 | nd |
| WT KvVDH | 873.08 ± 15.47 | 770.80 ± 2.25 | 61.63 ± 4.83 | nd | 740.41 ± 16.07 | 802.14 ± 6.02 |
| D296N | 2871.87 ± 44.41 | 571.76 ± 15.78 | 53.90 ± 0.18 | nd | 1282.92 ± 62.53 | 68.15 ± 5.16 |
| Y370W | 4121.64 ± 71.06 | 111.38 ± 5.48 | 104.79 ± 3.67 | nd | 3362.74 ± 48.77 | 660.18 ± 4.12 |
| M1 (D296N/Y370W) | 4557.26 ± 54.44 | 483.52 ± 14.44 | 162.22 ± 16.95 | nd | 543.52 ± 31.35 | 125.57 ± 5.88 |
| M2 (A170T/D296N/Y370W) | 4252.13 ± 96.73 | 3241.30 ± 249.42 | 100.82 ± 29.11 | nd | 4084.77 ± 28.34 | 703.59 ± 25.76 |
| M3 (A50S/Y370W) | 6340.5 ± 74.23 | 1727.31 ± 89.07 | 127.77 ± 2.82 | nd | 3305.67 ± 52.28 | 736.72 ± 34.46 |

[a]Activities were determined with purified enzymes and 10 mM HMF、HMFCA、FFCA、FDCA、furfural and furfuryl alcohol as substrates. Values represent the average of three individual measurements with corresponding standard deviation. Activities below detection limit are indicated (nd).

Table S7. The specific activity of KvVDH and its mutants toward various substrates.[a]

| Name | Use | Primer sequence [a] |
|---|---|---|
| pET-28a-Vector FWD | Linearization of the pET-28a Vector | 5'-GCGGCCGCTTTCGAATCTAGA-3' |
| pET-28a-Vector REV | Linearization of the pET-28a Vector | 5'-GAATTCCGGATCCATGGCGC-3' |
| KvPADH11 FWD | Genetic Cloning of KvPADH11 | 5'-TCCGGAATTCATGTCCACCTCTCAGATAGCGC-3' |
| KvPADH11 REV | Genetic Cloning of KvPADH11 | 5'-TCGAAAGCGGCCGCTTGGTTTCGCACCAGCCGT-3' |
| KvSSADH11 FWD | Genetic Cloning of KvSSADH11 | 5'-TCCGGAATTCATGCAACTCAACGATATGACACTGT-3' |
| KvSSADH11 REV | Genetic Cloning of KvSSADH11 | 5'-TCGAAAGCGGCCGCTTAAAGGCCAATACACATATATTTGATTTCTAAGTAATC-3' |
| KvSSADH FWD | Genetic Cloning of KvSSADH | 5'-TCCGGAATTCATGTCCGTGTTTCACTCAGATCTGT-3' |
| KvSSADH REV | Genetic Cloning of KvSSADH | 5'-GAAAGCGGCCGCTTACAACCCCTGGCAGAGATACTTCA-3' |
| KvLDH FWD | Genetic Cloning of KvLDH | 5'-TCCGGAATTCATGACAGCACCCGTTCAACAC-3' |
| KvLDH REV | Genetic Cloning of KvLDH | 5'-TCGAAAGCGGCCGCTCAGGCCTGCAGATAGACCAC-3' |
| KvPADH FWD | Genetic Cloning of KvPADH | 5'-TCCGGAATTCATGAGCGAGATAACCTTACTGGC-3' |
| KvPADH REV | Genetic Cloning of KvPADH | 5'-AAGCGGCCGCTTAATAGCGCACCATCACCGAC-3' |
| KvBADH11 FWD | Genetic Cloning of KvBADH11 | 5'-TCCGGAATTCATGAAAAAGATTAACCATTGGATCAACG-3' |
| KvBADH11 REV | Genetic Cloning of KvBADH11 | 5'-TCGAAAGCGGCCGCTCAGACTCCCCATTTCGGGAT-3' |
| KvBADH FWD | Genetic Cloning of KvBADH | 5'-TCCGGAATTCATGTCCCGAATGGCAGAAC-3' |
| KvBADH REV | Genetic Cloning of KvBADH | 5'-AAGCGGCCGCTTAAAATATGGACTGGAATTTACCCA-3' |
| KvALDH9A3 FWD | Genetic Cloning of KvALDH9A3 | 5'-TCCGGAATTCATGCAACACAACCTTTTGATAAACG-3' |
| KvALDH9A3 REV | Genetic Cloning of KvALDH9A3 | 5'-AGCGGCCGCTTAGCCATGCTTAACCATGACATGG-3' |
| KvSSADH15 FWD | Genetic Cloning of KvSSADH15 | 5'-ATCCGGAATTCATGGCCTATCAGACGGTGAA-3' |
| KvSSADH15 REV | Genetic Cloning of KvSSADH15 | 5'-AAGCGGCCGCTTAGCGTCGCACCACCAC-3' |
| KvSG5SDH FWD | Genetic Cloning of KvSG5SDH | 5'-TCCGGAATTCATGAGTCTGTGGATTAATGGCGAG-3' |
| KvSG5SDH REV | Genetic Cloning of KvSG5SDH | 5'-AAGCGGCCGCTCATGACGCCTCCCCGA-3' |
| KvDBP FWD | Genetic Cloning of KvDBP | 5'-TCCGGAATTCATGGGCACCACCACCAT-3' |
| KvDBP REV | Genetic Cloning of KvDBP | 5'-GCGGCCGCTTAGCCGATGGTCATCAGGCT-3' |
| KvOPDH FWD | Genetic Cloning of KvOPDH | 5'-ATCCGGAATTCATGACGATCACAGGAAACTTTATTG-3' |
| KvOPDH REV | Genetic Cloning of KvOPDH | 5'-AAGCGGCCGCTTAACCTAATGTCGGCATGCTG-3' |
| KvSG5SDH14 FWD | Genetic Cloning of KvSG5SDH14 | 5'-ATCCGGAATTCATGGCGGTAAAAGCACAGTTTA-3' |
| KvSG5SDH14 REV | Genetic Cloning of KvSG5SDH14 | 5'-AAGCGGCCGCTCAGAATGGCAGGCCCG-3' |
| KvDBP27 FWD | Genetic Cloning of KvDBP27 | 5'-ATCCGGAATTCATGCAGCAGTTAGCCAGCTAC-3' |
| KvDBP27 REV | Genetic Cloning of KvDBP27 | 5'-AAGCGGCCGCTTACGCCGGAAAATCCCCTTG-3' |
| KvCAD FWD | Genetic Cloning of KvCAD | 5'-TCCGGAATTCATGAGCCATGCGCGACC-3' |
| KvCAD REV | Genetic Cloning of KvCAD | 5'-AAGCGGCCGCTTAGCCCCTCAGCGCG-3' |
| KvKGSADH FWD | Genetic Cloning of KvKGSADH | 5'-TCCGGAATTCATGAATGCATCTCAGACCATGG-3' |
| KvKGSADH REV | Genetic Cloning of KvKGSADH | 5'-AAGCGGCCGCTCAATGAGAGGAGGAAATCGCTTC-3' |
| KvAdhE FWD | Genetic Cloning of KvAdhE | 5'-TCCGGAATTCATGGCTGTTACTAATATCGCTGAACTG-3' |
| KvAdhE REV | Genetic Cloning of KvAdhE | 5'-GAAAGCGGCCGCTTAAGCGGATTTCTTCGCTT-3' |
| KvEutE11 FWD | Genetic Cloning of KvEutE11 | 5'-TCCGGAATTCATGAATCAACAGGACATTGAACAGGTAGT-3' |
| KvEutE11 REV | Genetic Cloning of KvEutE11 | 5'-CGAAAGCGGCCGCTTATACGATTCGAAACGCATCCACCAG-3' |
| KvPuuC FWD | Genetic Cloning of KvPuuC | 5'-TCCGGAATTCATGAATTTTCAGCACCTGGCTTACTG-3' |

| | | |
|---|---|---|
| KvPuuC REV | Genetic Cloning of KvPuuC | 5'-TCGAAAGCGGCCGCTCAAGACTCCAGGGCAATCCAG-3' |
| KvVDH FWD | Genetic Cloning of KvVDH | 5'-TCCGGAATTCATGACAATCACCTGCAATCTGTACATTG-3' |
| KvVDH REV | Genetic Cloning of KvVDH | 5'-GAAAGCGGCCGCTCAGAAAGGGAGTTCGCGGG-3' |
| KvALDH FWD | Genetic Cloning of KvALDH | 5'-TCCGGAATTCATGCGTTATGCACATCCTGGC-3' |
| KvALDH REV | Genetic Cloning of KvALDH | 5'-TCGAAAGCGGCCGCTTAGAACAGACCCAGCGGCG-3' |
| KvEutE FWD | Genetic Cloning of KvEutE | 5'-TCCGGAATTCATGAATACAGCAGAACTGGAAACCCT-3' |
| KvEutE REV | Genetic Cloning of KvEutE | 5'-TCGAAAGCGGCCGCCTAGCGAATGGAAAAACCGTTGGTT-3' |
| PpVDH FWD | Genetic Cloning of PpVDH | 5'-GATCCGGAATTCATGCTGCAGGTGCCGCT-3' |
| PpVDH REV | Genetic Cloning of PpVDH | 5'-TCGAAAGCGGCCGCTTAAATCGGATAATGGCGCGGGC-3' |
| EcAldB FWD | Genetic Cloning of EcAldB | 5'-ATCCGGAATTCATGACCAATAATCCCCCTTCAGCA-3' |
| EcAldB REV | Genetic Cloning of EcAldB | 5'-GAAAGCGGCCGCTCAGAACAGCCCCAACGGT-3' |
| BaALDH FWD | Genetic Cloning of BaALDH | 5'-TCCGGAATTCATGGAAAACGTGCATCTGTTTATTGATGG-3' |
| BaALDH REV | Genetic Cloning of BaALDH | 5'-AAGCGGCCGCTTACAGCGGATAGCGGC-3' |
| RaALDH FWD | Genetic Cloning of RaALDH | 5'-TCCGGAATTCATGGTGTATGAAGTGAACCTGCTG-3' |
| RaALDH REV | Genetic Cloning of RaALDH | 5'-AAGCGGCCGCTTAAAACGGATAATGGCGCGG-3' |
| PpHYP FWD | Genetic Cloning of PpHYP | 5'-TCCGGAATTCATGAGCGATCTGAGCATGCTGAT-3' |
| PpHYP REV | Genetic Cloning of PpHYP | 5'-AAGCGGCCGCTTAAAACGGATAATGGCGCGGGGT-3' |
| PpALDH2 FWD | Genetic Cloning of PpALDH2 | 5'-ATCCGGAATTCATGAGCACCATTAGCATGCTGG-3' |
| PpALDH2 REV | Genetic Cloning of PpALDH2 | 5'-GAAAGCGGCCGCTTAAAACGGATAATGGCGCGGGGT-3' |
| BcALDH FWD | Genetic Cloning of BcALDH | 5'-ATCCGGAATTCATGAACCAGGTGACCATGCTGA-3' |
| BcALDH REV | Genetic Cloning of BcALDH | 5'-AAGCGGCCGCTTAAAACGGATAATGGCGCGG-3' |
| PpHYP11 FWD | Genetic Cloning of PpHYP11 | 5'-TCCGGAATTCATGACCACCATGACCATTCCGA-3' |
| PpHYP11 REV | Genetic Cloning of PpHYP11 | 5'-AAGCGGCCGCTTAAAACGGATAGCTGCGCGG-3' |
| PpALDH18 FWD | Genetic Cloning of PpALDH18 | 5'-ATCCGGAATTCATGAGCGCGATTAGCATGCT-3' |
| PpALDH18 REV | Genetic Cloning of PpALDH18 | 5'-AAGCGGCCGCTTAAAACGGATAATGGCGTTCGGT-3' |
| CoALDH FWD | Genetic Cloning of CoALDH | 5'-ATCCGGAATTCATGATTGAACAGAAAATGCTGATTGCGG-3' |
| CoALDH REV | Genetic Cloning of CoALDH | 5'-AAGCGGCCGCTTAAAACGGATAATGGCGTTCCGC-3' |
| EcPuuC FWD | Genetic Cloning of EcPuuC | 5'-CCATGGATCCGGAATTCATGAGCCCGGAAATTAACGGC-3' |
| EcPuuC REV | Genetic Cloning of EcPuuC | 5'-GAAAGCGGCCGCCGCTTCCAGGCTAATCCAAATGG-3' |
| AbEutE FWD | Genetic Cloning of AbEutE | 5'-CCATGGATCCGGAATTCATGCAGCATGTGCAGCTGCT-3' |
| AbEutE REV | Genetic Cloning of AbEutE | 5'-TCGAAAGCGGCCGCTTAAATCGGATAATGGCGCGGCTG-3' |
| EcAdhP FWD | Genetic Cloning of EcAdhP | 5'-CCATGGATCCGGAATTCATGGGCGCGACCAGC-3' |
| EcAdhP REV | Genetic Cloning of EcAdhP | 5'-TTCGAAAGCGGCCGCTTATTCAATTTTAATCGCCAGTTTGCCAAAATG-3' |
| EcAOR FWD | Genetic Cloning of EcAOR | 5'-CCGGAATTCATGGCTAACGGTTGGACAGGTAA-3' |
| EcAOR REV | Genetic Cloning of EcAOR | 5'-AAAGCGGCCGCTTACGCAGGCAGTAGATTGTGTGC-3' |
| EcAldA FWD | Genetic Cloning of EcAldA | 5'-TGGATCCGGAATTCATGAGCGTGCCGGTGC-3' |
| EcAldA REV | Genetic Cloning of EcAldA | 5'-GAAAGCGGCCGCTTAGCTCTGCAGATACACCACCTGG-3' |
| EcALDH FWD | Genetic Cloning of EcALDH | 5'-GGATCCGGAATTCATGAACTTTCATCATCTGGCGTATTGG-3' |
| EcALDH REV | Genetic Cloning of EcALDH | 5'-ATTCGAAAGCGGCCGCTTACGCTTCCAGGCTAATCCAAATG-3' |
| PpAldB11 FWD | Genetic Cloning of PpAldB11 | 5'-TCCGGAATTCATGCGTTATGCACATCCCGG-3' |
| PpAldB11 REV | Genetic Cloning of PpAldB11 | 5'-GAAAGCGGCCGCCTAGAAGAAGCCCAGCGGATTGAT-3' |
| PpAldA FWD | Genetic Cloning of PpAldA | 5'-CCATGGATCCGGAATTCATGAAAGATTCCAGCGATACCCATCC-3' |

|  |  |  |
|---|---|---|
| PpAldA REV | Genetic Cloning of PpAldA | 5'-TCGAAAGCGGCCGCTCAGAAGAGCCCGAGCGGTG-3' |
| PpAldB2 FWD | Genetic Cloning of PpAldB2 | 5'-CCATGGATCCGGAATTCATGATCTACGCACAACCCGGAAC-3' |
| PpAldB2 REV | Genetic Cloning of PpAldB2 | 5'-TCGAAAGCGGCCGCTTAGAAGAAGCCCAGCGGATTGATG-3' |
| PpXOD FWD | Genetic Cloning of PpXOD | 5'-ATGGATCCGGAATTCATGACCAACAACCCGCCGA-3' |
| PpXOD REV | Genetic Cloning of PpXOD | 5'-TCGAAAGCGGCCGCTTAAAACAGGCCCAGCGGTTTATCG-3' |
| PpALDH FWD | Genetic Cloning of PpALDH | 5'-GCCATGGATCCGGAATTCATGCATCCGATAGCTCCGCA-3' |
| PpALDH REV | Genetic Cloning of PpALDH | 5'-ATTCGAAAGCGGCCGCTCACTCGCCCTTGATACGCAC-3' |
| BtDeh11 FWD | Genetic Cloning of BtDeh11 | 5'-TCCGGAATTCATGTCAGTTCAGTACCGCGTG-3' |
| BtDeh11 REV | Genetic Cloning of BtDeh11 | 5'-CGAAAGCGGCCGCCTAGTCGTTTACGTAGAAGGTCTGCTT-' |
| BtDeh8 FWD | Genetic Cloning of BtDeh8 | 5'-TCCGGAATTCGTGACAAATGCAATTAACACCCTAAGCG-3' |
| BtDeh8 REV | Genetic Cloning of BtDeh8 | 5'-GAAAGCGGCCGCTCAGAGGCGGTTGCTGCGAGGTA-3' |
| BtMCOs FWD | Genetic Cloning of BtMCOs | 5'-TCCGGAATTCATGGATAGTCTTGATCTTCACTCCCG-3' |
| BtMCOs REV | Genetic Cloning of BtMCOs | 5'-TCGAAAGCGGCCGCTTAGGCCACCTCAGGCAGCCAA-3' |
| BtOR FWD | Genetic Cloning of BtOR | 5'-TCCGGAATTCATGGCAACTCTGAGAGCAGGATT-3' |
| BtOR REV | Genetic Cloning of BtOR | 5'-TCGAAAGCGGCCGCCTATAACCTTGCTACTTCGCCTTTATGC-3' |
| BtDeh149 FWD | Genetic Cloning of BtDeh149 | 5'-TCCGGAATTCATGTCAGTTCAGTACCGCGTG-3' |
| BtDeh149 REV | Genetic Cloning of BtDeh149 | 5'-GAAAGCGGCCGCCTAGTCGTTTACGTAGAAGGTCTGCTT-3' |
| BtADH FWD | Genetic Cloning of BtADH | 5'-TCCGGAATTCATGAAAACCCAAGCAGCAGTAGTT-3' |
| BtADH REV | Genetic Cloning of BtADH | 5'-TCGAAAGCGGCCGCCTAGAAAACGATAATTGGCTTAATCACTTCACC-3' |
| NOX FWD | Genetic Cloning of NOX | 5'-ATCCGGAATTCATGAAAGTCACAGTTGTTGGTTGTACAC-3' |
| NOX REV | Genetic Cloning of NOX | 5'-TTCGAAAGCGGCCGCTTAAGCGTTAACTGATTGGGCAACCT-3' |
| R19G FWD | Site-directed mutagenesis | 5'-GTAAAGGTGCGGCCGCCTTCGGCATCG-3' |
| R19G REV | Site-directed mutagenesis | 5'-CGATGCCGAAGGCGGCCGCACCTTTAC-3' |
| T21Y FWD | Site-directed mutagenesis | 5'-GTGGCGTCGGGTAAAATAGCGGCGGCCTTCGGCGGC-3' |
| T21Y REV | Site-directed mutagenesis | 5'-GCCGAAGGCCGCCGCTATTTTACCCGGCCAC-3' |
| T23E FWD | Site-directed mutagenesis | 5'-GGGTGGCGTCGCTCAAAGGTGCGGCGGCCTTC-3' |
| T23E REV | Site-directed mutagenesis | 5'-GAAGGCCGCCGCACCTTGAGCACGCCACCC-3' |
| A28L FWD | Site-directed mutagenesis | 5'-GCGACGTCGTCGTGTAGCGGGTGGCGTCGGG-3' |
| A28L REV | Site-directed mutagenesis | 5'-CCCGACGCCACCCGCTACACGGCGC-3' |
| H29D FWD | Site-directed mutagenesis | 5'-GACGTCGTCGTCGGCCGGGTGGC-3' |
| H29D REV | Site-directed mutagenesis | 5'-GCCACCCGGCCGACGACGACGTC-3' |
| D30G FWD | Site-directed mutagenesis | 5'-AGGCGACGTCGCCGTGGGCCGGG-3' |
| D30G REV | Site-directed mutagenesis | 5'-CCCGGCCCACGGCGACGTCGCCT-3' |
| S34T FWD | Site-directed mutagenesis | 5'-CGCAGCCACCGTGGCGACGTCGT-3' |
| S34T REV | Site-directed mutagenesis | 5'-ACGACGTCGCCACGGTGGCTGCG-3' |
| S44A FWD | Site-directed mutagenesis | 5'-CGACGCAGCGTTTGGCGTCGGCGAGACTGG-3' |
| S44A REV | Site-directed mutagenesis | 5'-CCAGTCTCGCCGACGCCAAACGCTGCGTCG-3' |
| R46A FWD | Site-directed mutagenesis | 5'-CTTCGACGCAGGCTTTGCTGTCGGCGAGACTGG-3' |
| R46A REV | Site-directed mutagenesis | 5'-CCAGTCTCGCCGACAGCAAAGCCTGCGTCGAAG-3' |
| C47A FWD | Site-directed mutagenesis | 5'-CGCGGCTTCGACGGCGCGTTTGCTGTCG-3' |
| C47A REV | Site-directed mutagenesis | 5'-CGACAGCAAACGCGCCGTCGAAGCCGCG-3' |

| | | |
|---|---|---|
| D60A FWD | Site-directed mutagenesis | 5'-GCCGGAGAGGTAGCACGCCACAGCG-3' |
| D60A REV | Site-directed mutagenesis | 5'-CGCTGTGGCGTGCTACCTCTCCGGC-3' |
| S62G FWD | Site-directed mutagenesis | 5'-GTCGTTCCGCCGGACCGGTATCACGCCACA-3' |
| S62G REV | Site-directed mutagenesis | 5'-TGTGGCGTGATACCGGTCCGGCGGAACGAC-3' |
| R68A FWD | Site-directed mutagenesis | 5'-CGTCGAGCAGTAAAGCGCGTCGTTCCGCCG-3' |
| R68A REV | Site-directed mutagenesis | 5'-CGGCGGAACGACGCGCTTTACTGCTCGACG-3' |
| D72K FWD | Site-directed mutagenesis | 5'-CATATGTTCCGCCGCCTTGAGCAGTAAACGGCG-3' |
| D72K REV | Site-directed mutagenesis | 5'-CGCCGTTTACTGCTCAAGGCGGCGGAACATATG-3' |
| D72R FWD | Site-directed mutagenesis | 5'-ATATGTTCCGCCGCGCGGAGCAGTAAACGGCG-3' |
| D72R REV | Site-directed mutagenesis | 5'-CGCCGTTTACTGCTCCGCGCGGCGGAACATAT-3' |
| H76A FWD | Site-directed mutagenesis | 5'-CCCGCAACAGCATAGCTTCCGCCGCGTCGA-3' |
| H76A REV | Site-directed mutagenesis | 5'-TCGACGCGGCGGAAGCTATGCTGTTGCGGG-3' |
| M77L FWD | Site-directed mutagenesis | 5'-CGCAACAGCAAATGTTCCGCCGCGTCG-3' |
| M77L REV | Site-directed mutagenesis | 5'-CGACGCGGCGGAACATTTGCTGTTGCG-3' |
| L79A FWD | Site-directed mutagenesis | 5'-AATTTAGCCTCCCGCGCCAGCATATGTTCCGCCGC-3' |
| L79A REV | Site-directed mutagenesis | 5'-GCGGCGGAACATATGCTGGCGCGGGAGGCTAAATT-3' |
| A87L FWD | Site-directed mutagenesis | 5'-CGGTCTCCGCCGCCATTAGGGCGATAAATTTAGCCTC-3' |
| A87L REV | Site-directed mutagenesis | 5'-GAGGCTAAATTTATCGCCCTAATGGCGGCGGAGACCG-3' |
| I109M FWD | Site-directed mutagenesis | 5'-GCTTCACGCAGCATATCGGCCGCCAGAT-3' |
| I109M REV | Site-directed mutagenesis | 5'-ATCTGGCGGCCGATATGCTGCGTGAAGC-3' |
| Q123E FWD | Site-directed mutagenesis | 5'-GCACGTTGGAAGGAATAATCTCCCCTTCAATTTGGGTGGTC-3' |
| Q123E REV | Site-directed mutagenesis | 5'-GACCACCCAAATTGAAGGGGAGATTATTCCTTCCAACGTGC-3' |
| N128D FWD | Site-directed mutagenesis | 5'-AGGTTGCCTGGCACGTCGGAAGGAATAATTTGCC-3' |
| N128D REV | Site-directed mutagenesis | 5'-GGCAAATTATTCCTTCCGACGTGCCAGGCAACCT-3' |
| N132S FWD | Site-directed mutagenesis | 5'-CATCGCCAGGCTGCCTGGCACGTTGGAA-3' |
| N132S REV | Site-directed mutagenesis | 5'-TTCCAACGTGCCAGGCAGCCTGGCGATG-3' |
| G136I FWD | Site-directed mutagenesis | 5'-CCCCTGGCGGACGATCATCGCCAGGTTG-3' |
| G136I REV | Site-directed mutagenesis | 5'-CAACCTGGCGATGATCGTCCGCCAGGGG-3' |
| G140P FWD | Site-directed mutagenesis | 5'-CCACCCCCGCCGGCTGGCGGACGC-3' |
| G140P REV | Site-directed mutagenesis | 5'-GCGTCCGCCAGCCGGCGGGGGTGG-3' |
| G146S FWD | Site-directed mutagenesis | 5'-ACGGCGCCATGCTCAGTACCACCCC-3' |
| G146S REV | Site-directed mutagenesis | 5'-GGGGTGGTACTGAGCATGGCGCCGT-3' |
| L161I FWD | Site-directed mutagenesis | 5'-CCAGCGGCGTTGCTATCGCCCGGGTAGCC-3' |
| L161I REV | Site-directed mutagenesis | 5'-GGCTACCCGGGCGATAGCAACGCCGCTGG-3' |
| T163M FWD | Site-directed mutagenesis | 5'-GCCAGCGGCATTGCCAGCGCCCGG-3' |
| T163M REV | Site-directed mutagenesis | 5'-CCGGGCGCTGGCAATGCCGCTGGC-3' |
| A170T FWD | Site-directed mutagenesis | 5'-CCCTTGAGGATCACCGTGTTGCCGCAGGCCA-3' |
| A170T REV | Site-directed mutagenesis | 5'-TGGCCTGCGGCAACACGGTGATCCTCAAGGG-3' |
| I172V FWD | Site-directed mutagenesis | 5'-GCGCCCTTGAGGACCACTGCGTTGCCG-3' |
| I172V REV | Site-directed mutagenesis | 5'-CGGCAACGCAGTGGTCCTCAAGGGCGC-3' |
| A176S FWD | Site-directed mutagenesis | 5'-GGCGACAGCTCCGAGCCCTTGAGGATC-3' |
| A176S REV | Site-directed mutagenesis | 5'-GATCCTCAAGGGCTCGGAGCTGTCGCC-3' |

| | | |
|---|---|---|
| Q183H FWD | Site-directed mutagenesis | 5'-GATAATCAGTCCATGAGTGGCCGGCGACAGC-3' |
| Q183H REV | Site-directed mutagenesis | 5'-GCTGTCGCCGGCCACTCATGGACTGATTATC-3' |
| D188E FWD | Site-directed mutagenesis | 5'-GCGTCAAGGGCCTCGATAATCAGTCCCTGA-3' |
| D188E REV | Site-directed mutagenesis | 5'-TCAGGGACTGATTATCGAGGCCCTTGACGC-3' |
| T204M FWD | Site-directed mutagenesis | 5'-TCAGGCGCACACATAAGATAGTTCACCACCCCCG-3' |
| T204M REV | Site-directed mutagenesis | 5'-CGGGGGTGGTGAACTATCTTATGTGTGCGCCTGA-3' |
| S216A FWD | Site-directed mutagenesis | 5'-CGATCAGCGCTTCCACCAGCGCCGG-3' |
| S216A REV | Site-directed mutagenesis | 5'-CCGGCGCTGGTGGAAGCGCTGATCG-3' |
| T241M FWD | Site-directed mutagenesis | 5'-ATTGCCCGCACATGCGGGCGATAATCCGCCC-3' |
| T241M REV | Site-directed mutagenesis | 5'-GGGCGGATTATCGCCCGCATGTGCGGGCAAT-3' |
| G243A FWD | Site-directed mutagenesis | 5'-GGTTTAAGATATTGCGCGCAGGTGCGGGCG-3' |
| G243A REV | Site-directed mutagenesis | 5'-CGCCCGCACCTGCGCGCAATATCTTAAACC-3' |
| Y245H FWD | Site-directed mutagenesis | 5'-CGCCGGTTTAAGATGTTGCCCGCAGGTGC-3' |
| Y245H REV | Site-directed mutagenesis | 5'-GCACCTGCGGGCAACATCTTAAACCGGCG-3' |
| A249L FWD | Site-directed mutagenesis | 5'-CCGCCGAGCTCCAGCACTAGCGGTTTAAGATATTGCCCG-3' |
| A249L REV | Site-directed mutagenesis | 5'-CGGGCAATATCTTAAACCGCTAGTGCTGGAGCTCGGCGG-3' |
| E268D FWD | Site-directed mutagenesis | 5'-CCGCCGCCGCTTGATCGATATCAGCATC-3' |
| E268D REV | Site-directed mutagenesis | 5'-GATGCTGATATCGATCAAGCGGCGGCGG-3' |
| Q269A FWD | Site-directed mutagenesis | 5'-CCCGCCGCCGCTGCCTCGATATCAGCATC-3' |
| Q269A REV | Site-directed mutagenesis | 5'-GATGCTGATATCGAGGCAGCGGCGGCGGG-3' |
| A271V FWD | Site-directed mutagenesis | 5'-CCGCGCCCGCCACCGCTTGCTCG-3' |
| A271V REV | Site-directed mutagenesis | 5'-CGAGCAAGCGGTGGCGGGCGCGG-3' |
| G273A FWD | Site-directed mutagenesis | 5'-CAAAAACCGCGGCCGCCGCCGCT-3' |
| G273A REV | Site-directed mutagenesis | 5'-AGCGGCGGCGGCCGCGGTTTTTG-3' |
| A280W FWD | Site-directed mutagenesis | 5'-GATCTGGCCGGCATTCCAGAAGGCGCCAAAAACC-3' |
| A280W REV | Site-directed mutagenesis | 5'-GGTTTTTGGCGCCTTCTGGAATGCCGGCCAGATC-3' |
| D296N FWD | Site-directed mutagenesis | 5'-CCTCCGCTACGGCGTTGTCGACAATAATGCG-3' |
| D296N REV | Site-directed mutagenesis | 5'-CGCATTATTGTCGACAACGCCGTAGCGGAGG-3' |
| V298I FWD | Site-directed mutagenesis | 5'-GATAAACACCTCCGCTATGGCGTCGTCGACAATAA-3' |
| V298I REV | Site-directed mutagenesis | 5'-TTATTGTCGACGACGCCATAGCGGAGGTGTTTATC-3' |
| E300D FWD | Site-directed mutagenesis | 5'-AGCGGGATAAACACATCCGCTACGGCGTC-3' |
| E300D REV | Site-directed mutagenesis | 5'-GACGCCGTAGCGGATGTGTTTATCCCGCT-3' |
| R309K FWD | Site-directed mutagenesis | 5'-GCAGGGCGGCGGCCTTCCTGGCCAGCAGC-3' |
| R309K REV | Site-directed mutagenesis | 5'-GCTGCTGGCCAGGAAGGCCGCCGCCCTGC-3' |
| S316G FWD | Site-directed mutagenesis | 5'-CCCCGTCAGGCCGGCGGGCAGGG-3' |
| S316G REV | Site-directed mutagenesis | 5'-CCCTGCCCGCCGGCCTGACGGGG-3' |
| T318L FWD | Site-directed mutagenesis | 5'-ATCAACCACCGGCCCTAGCAGGCTGGCGGGCAG-3' |
| T318L REV | Site-directed mutagenesis | 5'-CTGCCCGCCAGCCTGCTAGGGCCGGTGGTTGAT-3' |
| I327V FWD | Site-directed mutagenesis | 5'-GCGTTGCAGCGGGTGACGGTGTTCATATCAACC-3' |
| I327V REV | Site-directed mutagenesis | 5'-GGTTGATATGAACACCGTCACCCGCTGCAACGC-3' |
| T346C FWD | Site-directed mutagenesis | 5'-GCTTTACCACCGCATAGCAGGCGAGCGCCTTTG-3' |
| T346C REV | Site-directed mutagenesis | 5'-CAAAGGCGCTCGCCTGCTATGCGGTGGTAAAGC-3' |

| | | |
|---|---|---|
| H354L FWD | Site-directed mutagenesis | 5'-GTCGGCGCCATGAGGGTATCGCTGG-3' |
| H354L REV | Site-directed mutagenesis | 5'-CCAGCGATACCCTCATGGCGCCGAC-3' |
| A356P FWD | Site-directed mutagenesis | 5'-AGCAAGGTCGGCGGCATGTGGGTATCG-3' |
| A356P REV | Site-directed mutagenesis | 5'-CGATACCCACATGCCGCCGACCTTGCT-3' |
| R365P FWD | Site-directed mutagenesis | 5'-GCCGCATCTCGGGGGTCACGCCG-3' |
| R365P REV | Site-directed mutagenesis | 5'-CGGCGTGACCCCCGAGATGCGGC-3' |
| L369I FWD | Site-directed mutagenesis | 5'-AAGGACTCTTCGTTGTATATCCGCATCTCGCGGGTC-3' |
| L369I REV | Site-directed mutagenesis | 5'-GACCCGCGAGATGCGGATATACAACGAAGAGTCCTT-3' |
| S374L FWD | Site-directed mutagenesis | 5'-TTTACCGGGCCGAATAACTCTTCGTTGTACAGCCGCA-3' |
| S374L REV | Site-directed mutagenesis | 5'-TGCGGCTGTACAACGAAGAGTTATTCGGCCCGGTAAA-3' |
| K379M FWD | Site-directed mutagenesis | 5'-CCCGAATAATCGCCATTACCGGGCCGAAGGACTCTT-3' |
| K379M REV | Site-directed mutagenesis | 5'-AAGAGTCCTTCGGCCCGGTAATGGCGATTATTCGGG-3' |
| A380V FWD | Site-directed mutagenesis | 5'-TCTTAACCCGAATAATCACTTTTACCGGGCCGAAG-3' |
| A380V REV | Site-directed mutagenesis | 5'-CTTCGGCCCGGTAAAAGTGATTATTCGGGTTAAGA-3' |
| N386G FWD | Site-directed mutagenesis | 5'-CGTCAGCGCCTCTTCTTCACCCTTAACCCGAATAATCGCT-3' |
| N386G REV | Site-directed mutagenesis | 5'-AGCGATTATTCGGGTTAAGGGTGAAGAAGAGGCGCTGACG-3' |
| T392A FWD | Site-directed mutagenesis | 5'-CGTTGGCCACCGCCAGCGCCTCTTC-3' |
| T392A REV | Site-directed mutagenesis | 5'-GAAGAGGCGCTGGCGGTGGCCAACG-3' |
| Y406F FWD | Site-directed mutagenesis | 5'-CTGTCGCGGCTGAAGACCGCAGCGG-3' |
| Y406F REV | Site-directed mutagenesis | 5'-CCGCTGCGGTCTTCAGCCGCGACAG-3' |
| Q421E FWD | Site-directed mutagenesis | 5'-ATGGCAAATGCCCGTCTCCAGACGTTGCGCCAC-3' |
| Q421E REV | Site-directed mutagenesis | 5'-GTGGCGCAACGTCTGGAGACGGGCATTTGCCAT-3' |
| C443V FWD | Site-directed mutagenesis | 5'-CATAGCCCGAGGATTTGACGCCGCCAAACGGCATTT-3' |
| C443V REV | Site-directed mutagenesis | 5'-AAATGCCGTTTGGCGGCGTCAAATCCTCGGGCTATG-3' |
| R454K FWD | Site-directed mutagenesis | 5'-CGCGATGCCGGCCTTCCCCCCAAAGCGG-3' |
| R454K REV | Site-directed mutagenesis | 5'-CCGCTTTGGGGGGAAGGCCGGCATCGCG-3' |
| G456M FWD | Site-directed mutagenesis | 5'-GGTGAATTCCGCGATCATGGCCCGCCCCCCAAA-3' |
| G456M REV | Site-directed mutagenesis | 5'-TTTGGGGGGCGGGCCATGATCGCGGAATTCACC-3' |
| E474H FWD | Site-directed mutagenesis | 5'-CTCAGAAAGGGAGATGGCGGGGCTGGGTC-3' |
| E474H REV | Site-directed mutagenesis | 5'-GACCCAGCCCCGCCATCTCCCTTTCTGAG-3' |
| L475Y FWD | Site-directed mutagenesis | 5'-GCCGCTCAGAAAGGATATTCGCGGGGCTGGGTCTG-3' |
| L475Y REV | Site-directed mutagenesis | 5'-CAGACCCAGCCCCGCGAATATCCTTTCTGAGCGGC-3' |
| Y370W FWD | Site-directed mutagenesis | 5'-CGAAGGACTCTTCGTTCCACAGCCGCATCTCGCG-3' |
| Y370W REV | Site-directed mutagenesis | 5'-CGCGAGATGCGGCTGTGGAACGAAGAGTCCTTCG-3' |
| A257K FWD | Site-directed mutagenesis | 5'-CAGCAGCGGCTTTTTACCGCCGAGCTCCAGC-3' |
| A257K REV | Site-directed mutagenesis | 5'-GCTGGAGCTCGGCGGTAAAAAGCCGCTGCTG-3' |
| D31R FWD | Site-directed mutagenesis | 5'-ACCGAGGCGACGCGGTCGTGGGCCGG-3' |
| D31R REV | Site-directed mutagenesis | 5'-CCGGCCCACGACCGCGTCGCCTCGGT-3 |
| V199G FWD | Site-directed mutagenesis | 5'-GTAAGATAGTTCACCCCCCCCGGTGGGAAG-3' |
| V199G REV | Site-directed mutagenesis | 5'-CTTCCCACCGGGGGGGGTGAACTATCTTAC-3' |
| G400R FWD | Site-directed mutagenesis | 5'-CCGCAGCGGAAAGACGGTATTCACTGTCGTT-3' |
| G400R REV | Site-directed mutagenesis | 5'-AACGACAGTGAATACCGTCTTTCCGCTGCGG-3' |

|  |  |  |
|---|---|---|
| H26Q FWD | Site-directed mutagenesis | 5'-CCACGCTCTGGCGGCGTCGCGGCTGTAG-3' |
| H26Q REV | Site-directed mutagenesis | 5'-CTACAGCCGCGACGCCGCCAGAGCGTGG-3' |
| S410A FWD | Site-directed mutagenesis | 5'-CTTTACCCGACGCCAGCCGGCCCA-3' |
| S410A REV | Site-directed mutagenesis | 5'-TGGGCCGGCTGGCGTCGGGTAAAG-3' |
| F22 FWD | Site-saturation mutagenesis | 5'-CGCCGCACCNNKACCCGACGCCACCCG-3' |
| F22 REV | Site-saturation mutagenesis | 5'-TGGCGTCGGGTMNNGGTGCGGCGGCCTT-3' |
| G175 FWD | Site-saturation mutagenesis | 5'-ATCCTCAAGNNKGCGGAGCTGTCGCCG-3' |
| G175 REV | Site-saturation mutagenesis | 5'-ACAGCTCCGCMNNCTTGAGGATCACTGCGTTGCC-3' |
| A176 FWD | Site-saturation mutagenesis | 5'-ATCCTCAAGGGCNNKGAGCTGTCGCCGGCCA-3' |
| A176 REV | Site-saturation mutagenesis | 5'-CGACAGCTCMNNGCCCTTGAGGATCACTGCGTT-3' |
| L178 FWD | Site-saturation mutagenesis | 5'-AGGGCGCGGAGNNKTCGCCGGCCACTCAGG-3' |
| L178 REV | Site-saturation mutagenesis | 5'-TGGCCGGCGAMNNCTCCGCGCCCTTGAGGAT-3' |
| S179 FWD | Site-saturation mutagenesis | 5'-CGCGGAGCTGNNKCCGGCCACTCAGGGACT-3' |
| S179 REV | Site-saturation mutagenesis | 5'-GTGGCCGGMNNCAGCTCCGCGCCCTTGA-3' |
| Q183 FWD | Site-saturation mutagenesis | 5'-TGTCGCCGGCCACTNNKGGACTGATTATCGACGCCCTTG-3' |
| Q183 REV | Site-saturation mutagenesis | 5'-TAATCAGTCCMNNAGTGGCCGGCGACAGC-3' |
| C205 FWD | Site-saturation mutagenesis | 5'-ATCTTACCNNKGCGCCTGAAGATGCGC-3' |
| C205 REV | Site-saturation mutagenesis | 5'-CTTCAGGCGCMNNGGTAAGATAGTTCACCA-3' |
| A206 FWD | Site-saturation mutagenesis | 5'-TCTTACCTGTNNKCCTGAAGATGCGCCGG-3' |
| A206 REV | Site-saturation mutagenesis | 5'-TTCAGGMNNACAGGTAAGATAGTTCACCACC-3' |
| P207 FWD | Site-saturation mutagenesis | 5'-ACCTGTGCGNNKGAAGATGCGCCGGC-3' |
| P207 REV | Site-saturation mutagenesis | 5'-CGCCGGCGCATCTTCMNNCGCACAGGTAAGATAGTTCACCAC-3' |
| E208 FWD | Site-saturation mutagenesis | 5'-TGCGCCTNNKGATGCGCCGGCGCTGGTGGAAT-3' |
| E208 REV | Site-saturation mutagenesis | 5'-GCGCCGGCGCATCMNNAGGCGCACAGGTAAGATAGTTCA-3' |
| D209 FWD | Site-saturation mutagenesis | 5'-TGCGCCTGAANNKGCGCCGGCGCTGGTGGA-3' |
| D209 REV | Site-saturation mutagenesis | 5'-CGCCGGCGCMNNTTCAGGCGCACAGGTAAGATAGT-3' |
| P211 FWD | Site-saturation mutagenesis | 5'-CTGAAGATGCGNNKGCGCTGGTGGAATCGCT-3' |
| P211 REV | Site-saturation mutagenesis | 5'-TCCACCAGCGCMNNCGCATCTTCAGGCGCACAG-3' |
| V322 FWD | Site-saturation mutagenesis | 5'-ACGGGGCCGGTGNNKGATATGAACACCATCACCCGCTGCA-3' |
| V322 REV | Site-saturation mutagenesis | 5'-ATGGTGTTCATATCMNNCACCGGCCCCGTCAG-3' |
| D323 FWD | Site-saturation mutagenesis | 5'-GCCGGTGGTTNNKATGAACACCATCACCCGCTG-3' |
| D323 REV | Site-saturation mutagenesis | 5'-ATGGTGTTCATMNNAACCACCGGCCCCGTCA-3' |
| N325 FWD | Site-saturation mutagenesis | 5'-TTGATATGNNKACCATCACCCGCTGCAAC-3' |
| N325 REV | Site-saturation mutagenesis | 5'-GGTGATGGTMNNCATATCAACCACCGGCCCCGT-3' |

[a] N = A, C, G, T (equimolar amounts); K = G, T (equimolar amounts); M = A, C (equimolar amounts).