

Supplementary Materials

Harnessing a Novel P450 Fatty Acid Decarboxylase from *Macrococcus caseolyticus* for Microbial Biosynthesis of Odd Chain Terminal Alkenes

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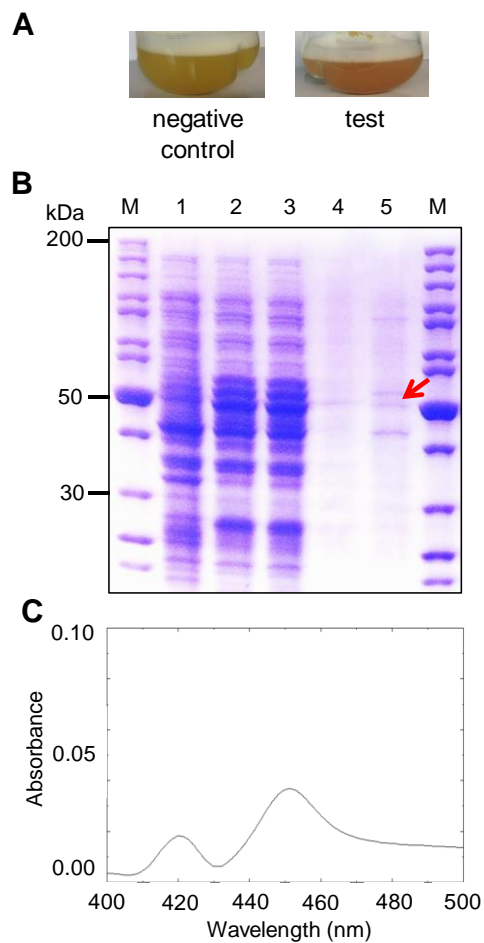
#Current address: Pfizer Inc, Kalamazoo, MI

Running title: Harnessing OleT_{MC} for terminal alkene synthesis in *E. coli*

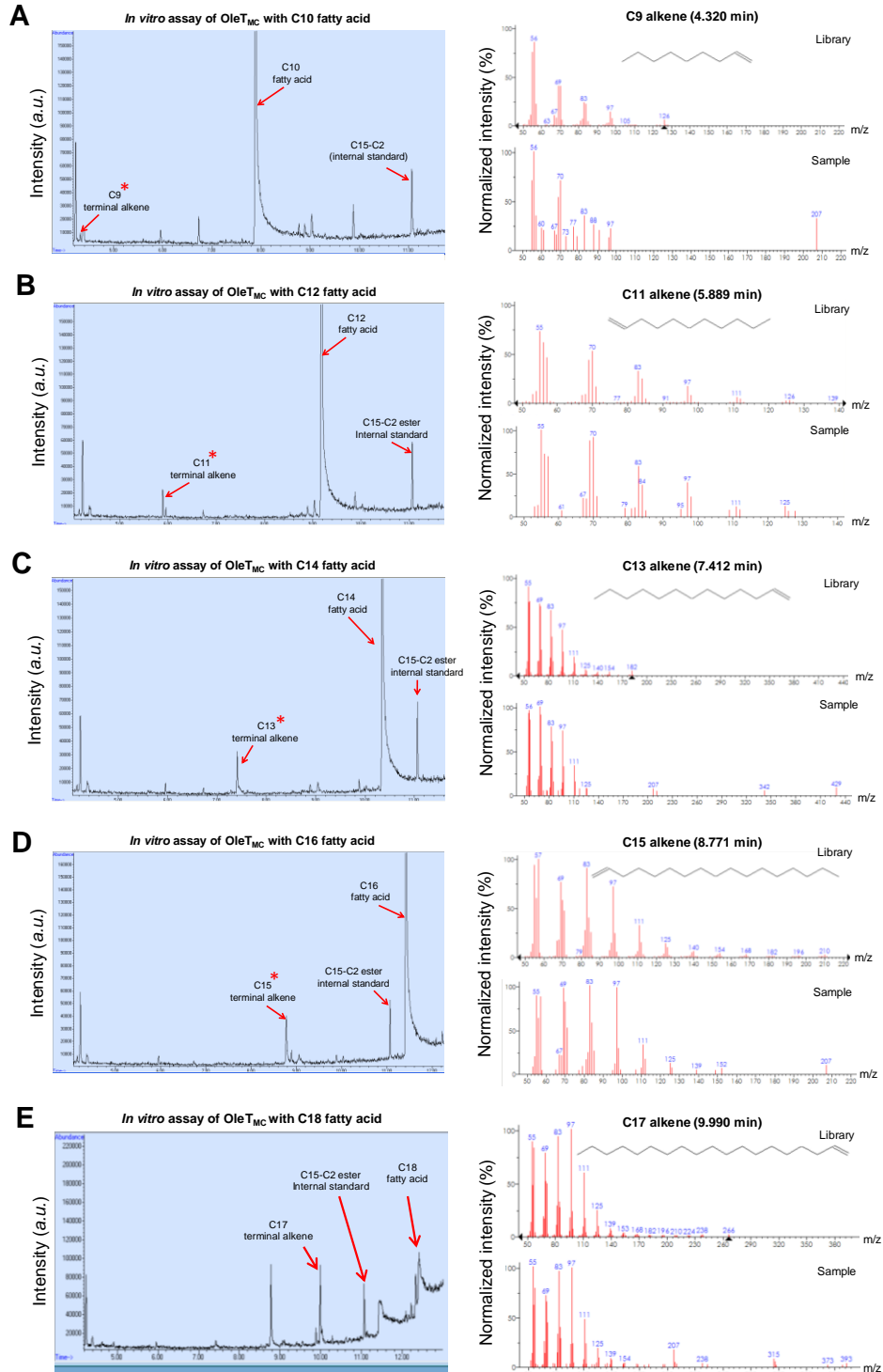
Supplementary Figure S1. Alignment of OleT_{JE} with the CYP152 P450 enzyme family. The sequences were aligned via MUSCLE and sequence identities were calculated by BLASTp using OleT_{JE} as a template.

| | F ⁷⁹ | H ⁸⁵ | R ²⁴⁵ | Identity (%) |
|---|-----------------|-----------------|------------------|--------------|
| 1. ADW41779.1_terminal_olefin-forming_fatty_acid_decarboxylase_Jeotgalicoccus_sp_ATCC_8456 | L[F]GKGA[H] | | [R]P | 100 |
| 2. BAA22987.1_fatty_acid_alpha-hydroxylase_Sphingomonas_paucimobilis | LLGQGGVQ | | RP | 37 |
| 3. NP_349922.1_hypothetical_protein_CA_C3330_Clostridium_acetobutylicum_ATCC_824 | LLGENAIQ | | RP | 40 |
| 4. NP_388092.1_fatty-acid_peroxygenase_Bacillus_subtilis_subsp_subtilis_str_168 | LVGVNAIQ | | RP | 42 |
| 5. WP_010897918.1_cytochrome_P450_Bacillus_halodurans | LVGENAIQ | | RP | 36 |
| 6. WP_011075937.1_cytochrome_P450_Corynebacterium_efficiens | L[F]GEGAV[H] | | [R]P | 32 |
| 7. WP_011247882.1_cytochrome_P450_Bacillus_clausii | LVGKGGVQ | | RP | 38 |
| 8. WP_011542827.1_cytochrome_P450_Sphingopyxis_alaskensis | LLVGGVQ | | RP | 36 |
| 9. WP_011578671.1_cytochrome_P450_Chelativorans_sp._BNC1 | LLVGGVQ | | RP | 37 |
| 10. WP_012117266.1_MULTISPECIES_cytochrome_P450_Bacillus_amyloliquefaciens_group | LVGTGAIQ | | RP | 42 |
| 11. WP_012200920.1_cytochrome_P450_Lachnoclostridium_phytofermentans | LVGVNAIQ | | RP | 38 |
| 12. WP_012399225.1_cytochrome_P450_Kocuria_rhizophila | L[F]GAGAV[H] | | [R]P | 31 |
| 13. WP_012960819.1_cytochrome_P450_Bacillus_pseudofirmus | LVGEDAIQ | | RP | 42 |
| 14. WP_013272538.1_cytochrome_P450_Clostridium_saccharolyticum | LVGVNAIQ | | RP | 38 |
| 15. WP_013288001.1_MULTISPECIES_cytochrome_P450_Micromonospora | LVGGVQ | | RP | 37 |
| 16. WP_015880780.1_cytochrome_P450_Exiguobacterium_sp._AT1b | LVGEGGVQ | | RP | 35 |
| 17. WP_041635889.1_cytochrome_P450_Macrocooccus_caseolyticus | L[F]GKGA[H] | | [R]P | 60 |
| 18. ZP_00964321.1_fatty_acid_alpha_hydroxylase_cytochrome_P450_Sulfitobacter_sp._NAS-14.1 | LVGEGGIQ | | RP | 34 |
| 19. ZP_01227146.1_acid_alpha_hydroxylase_cytochrome_P450_Aurantimonas_manganoxydans_SI85-9A1 | LVGKHGVQ | | RP | 35 |
| 20. ZP_01728364.1_fatty_acid_alpha_hydroxylase_cytochrome_P450_Cyanothece_sp._CCY0110 | LVGKGIQ | | RP | 37 |
| 21. ZP_02200540.1_Cytochrome_P450-like_protein_Methylobacterium_populi_BJ001 | LVQDHGSVM | | RP | 31 |
| 22. ZP_02617555.1_cytochrome_P450_Clostridium_botulinum_Bf | LVGENAIQ | | RP | 41 |
| 23. ZP_02994641.1_hypothetical_protein_CLOSPO_01760_Clostridium_sporogenes_ATCC_15579 | LVGENAIQ | | RP | 41 |
| 24. ZP_03227611.1_fatty_acid_alpha_hydroxylase_cytochrome_P450_Bacillus_coahuilensis_m4-4 | LVGEGGVQ | | RP | 40 |
| 25. ZP_03494465.1_cytochrome_P450_Alicyclobacillus_acidocaldarius_LAA1 | LVGENAIQ | | RP | 40 |
| 26. ZP_04432145.1_fatty_acid_alpha_hydroxylase_Bacillus_coagulans_36D1 | LVGENAIQ | | RP | 38 |
| 27. ZP_06401204.1_fatty_acid_alpha_hydroxylase_cytochrome_P450_Micromonospora_sp._L5 | LVGGVQ | | RP | 38 |
| 28. ZP_06874634.1_fatty_acid_beta-hydroxylating_cytochrome_P450_Bacillus_subtilis_subsp_spizizenii_ATCC_663 | LVGVNAIQ | | RP | 41 |
| 29. ZP_06884025.1_cytochrome_P450_Clostridium_lentocellum_DSM_5427 | LVGVNAIQ | | RP | 36 |

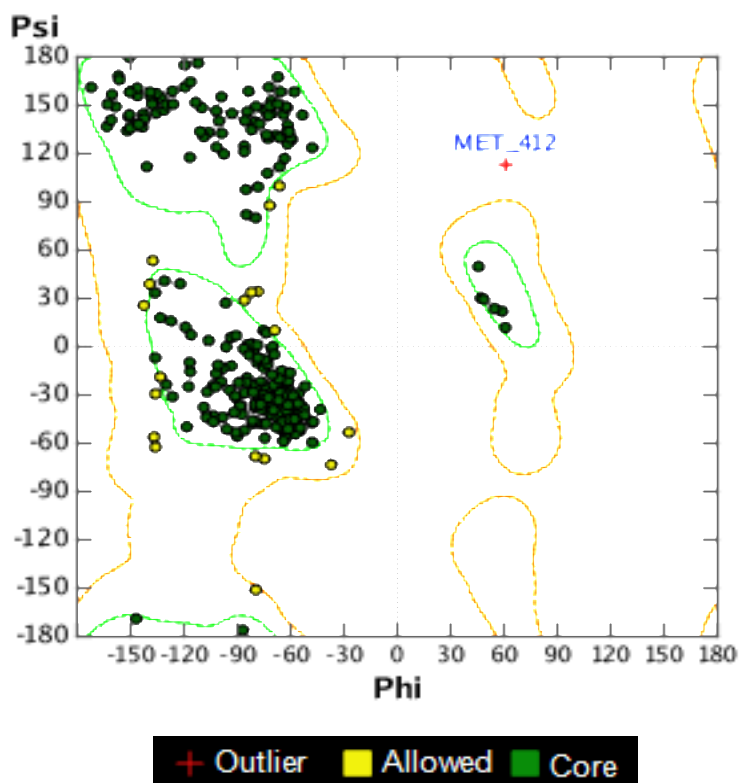
Supplementary Figure S2. Characterization of OleT_{MC}. **(A)** Reddish exhibition of BL21 (λ DE3)/pNN33 upon expressing OleT_{MC}. Negative control: cell culture of BL21 (λ DE3) without carrying OleT_{MC}. **(B)** SDS-PAGE analysis of the expressed OleT_{MC} in BL21 (λ DE3)/pNN33. Lane M, protein marker; lane 1, total cell extracts of un-induced cells; lane 2, total cell extracts of induced cells; lane 3, soluble fraction of induced cells; lane 4, insoluble fraction of induced cells; lane 5, Ni-NTA semi-purified fraction of induced cells. The arrow indicates the target protein OleT_{MC} with predicted mass of ~51 kDa. **(C)** Reduced carbon monoxide difference spectra of semi-purified OleT_{MC}



Supplementary Figure S3: GC/MS chromatograms for *in vitro* biosynthesis of terminal alkenes by OleT_{MC} using (A) C10:0 FA, (B) C12:0 FA, (C) C14:0, (D) C16:0 FA, and (E) C18:0.

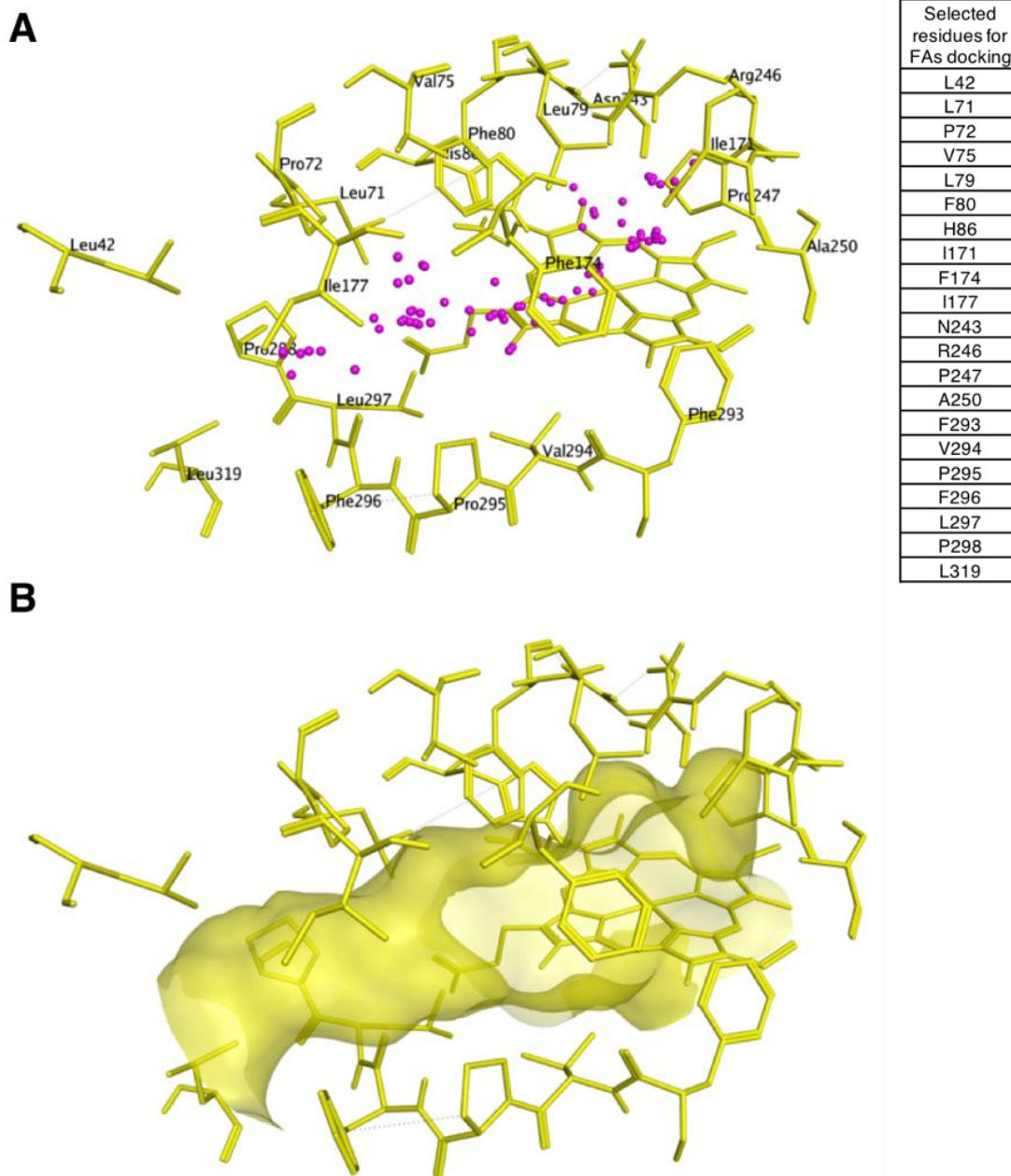


Supplementary Figure S4: Ramachandran plot of homology model of OleT_{MC}

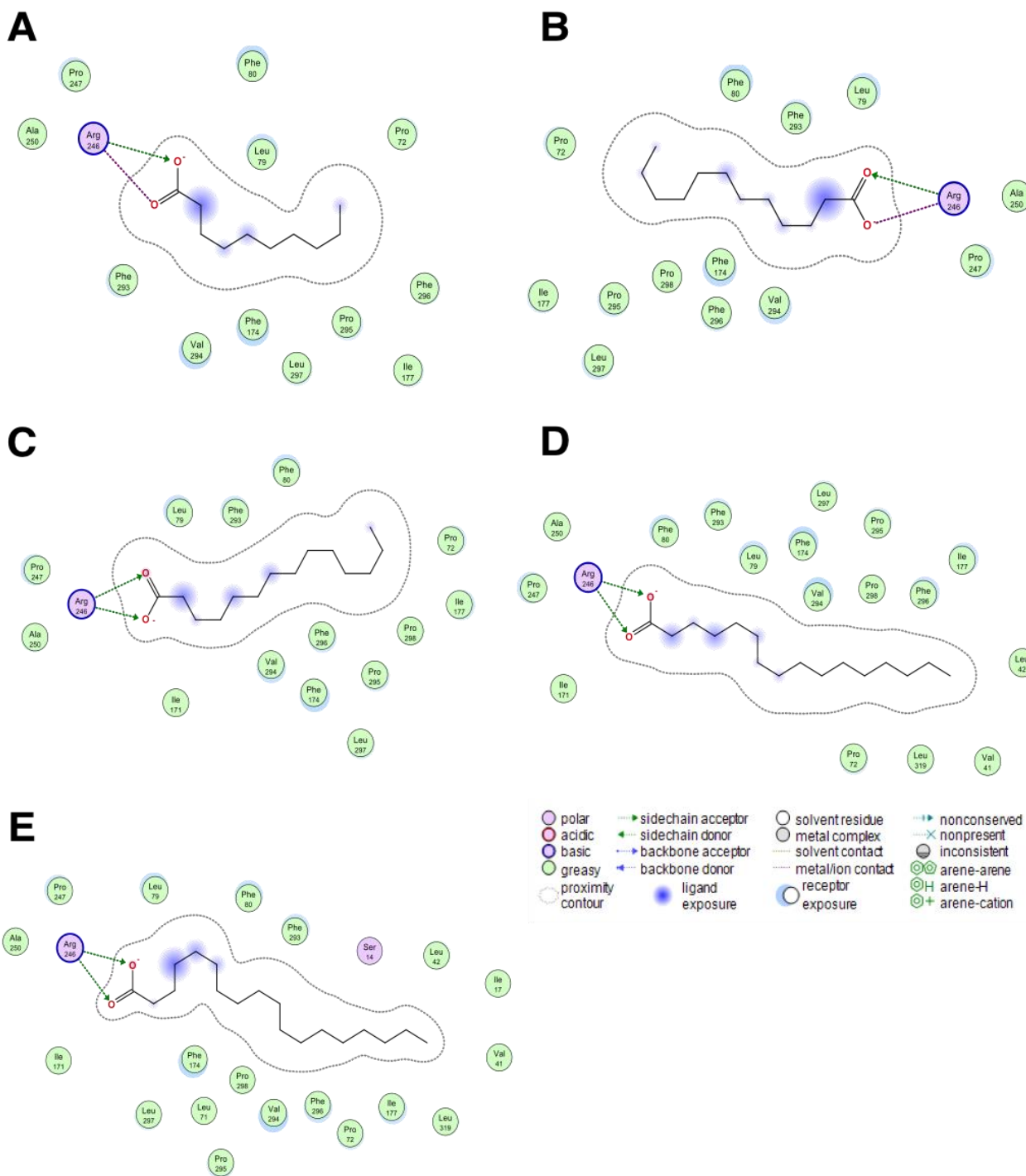


| # Outlier Region | Resides | Psi | Phi |
|------------------|---------|-------|-------|
| 1 | Pro295 | -56.5 | -85.7 |
| 2 | Met412 | 113.1 | 61.1 |

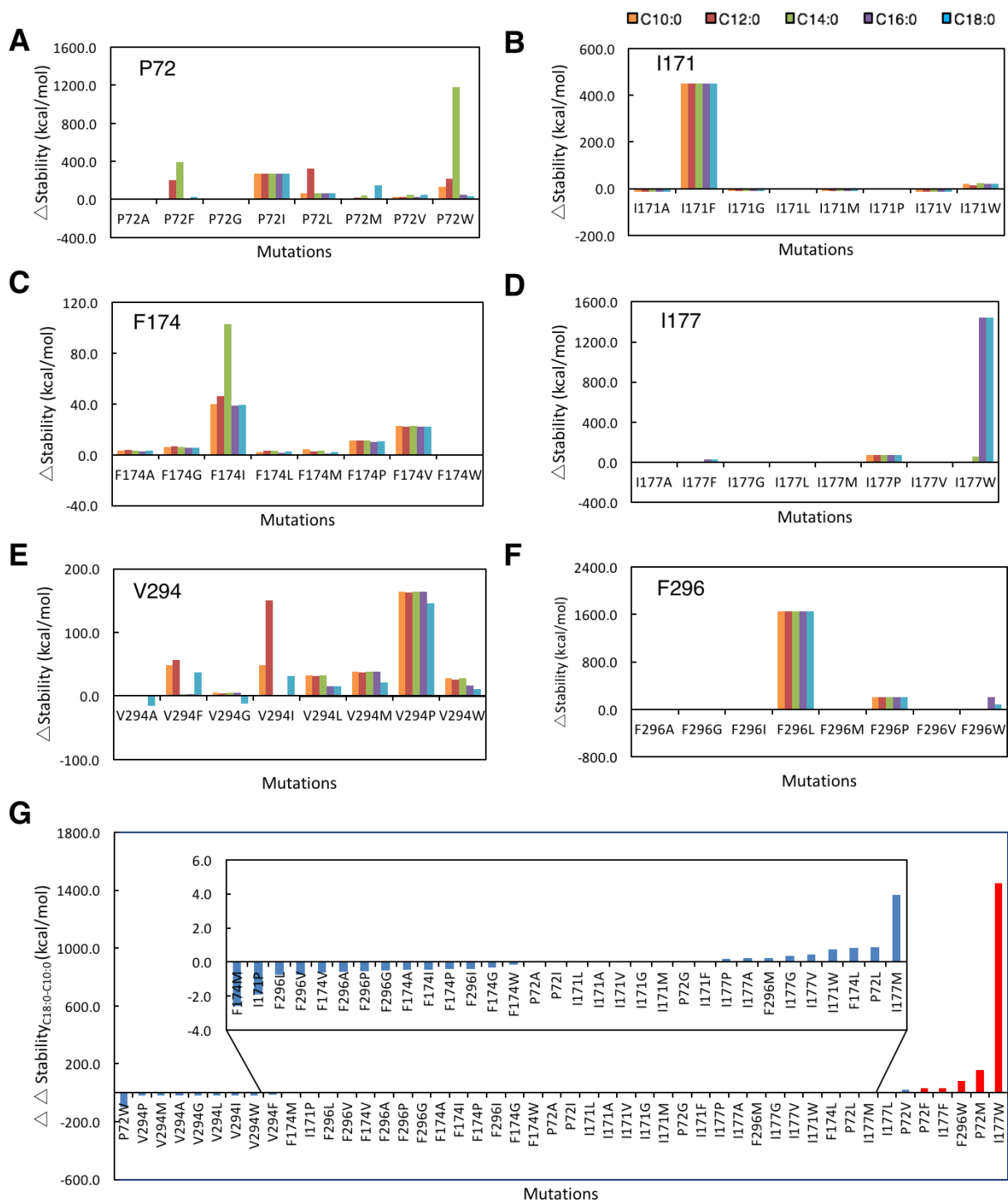
Supplementary Figure S5: (A) Selected residues for FAs docking (dummy atoms are in purple, potential FA binding site). (B) Molecular surface of potential FA binding site.



Supplementary Figure S6: 2D Ligand interaction diagrams for docking various FAs, including (A) C10:0, (B) C12:0, (C) C14:0, (D) C16:0, and (E) C18:0, into the homology model of OleT_{MC}.



Supplementary Figure S7: (A-F) Analysis of Δ Stability of the FA (C10:0-C18:0)-bound OleT_{MC} mutants **(G)** Δ Stability differences between the C18:0 FA and C10:0 FA-bound OleT_{MC} mutants.



Supplementary Figure S8: Normalized peak areas of saturated and unsaturated fatty acids from the GC/MS chromatograms for strains (A) EcNN101 and (B) EcNN201 during 48 h cell culturing.

The peak area of internal standard is used for normalizing the peak area of samples

