SUPPORTING INFORMATION

Loading of malonyl-CoA onto tandem acetyl carrier protein domains of polyunsaturated fatty acid synthases

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Material included: Supplementary Figures S1 to S9

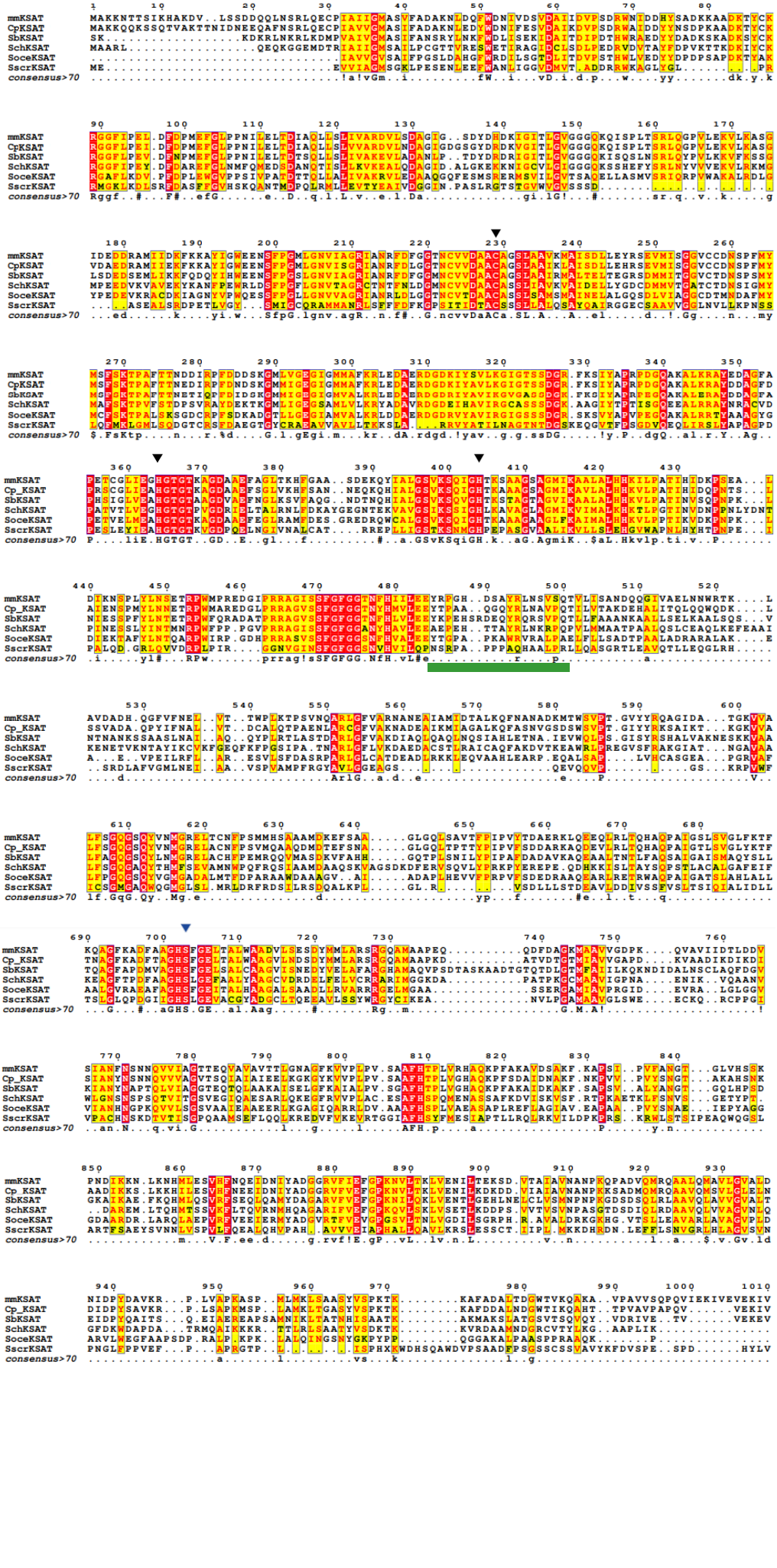


Figure S1. Multiple sequence alignment of KS-AT domains. Alignment of representative KS-AT protein regions from Pfa and FAS systems. Sequences correspond to PfaA (mmKSAT) from *Moritella marina* (Acc. no. Q9RA21), PfaA (Cp_KSAT) from *Colwellia psychrerythraea* (Acc. no. Q47ZG8), PfaA (SbKSAT) from Shewanella baltica (Acc. no. A9KUH8), PFA1 (SchKSAT) from *Schizochytrium* ATCC 20888 (Acc. no. AAK72879), pfa2 (SoceKSAT) from *Sorangium cellulosum* (Acc. no. A9EPF7) and mFAS (SscrKSAT) from Sus scrofa (Acc. no. A5YV76). Identical residues are shown in white on a red background, while similar residues are shown in red. The unstructured loop that connects both domains is marked with a green line. The positions of the catalytic residues of both domains have been marked with black (KS) and blue (AT) arrows, respectively.

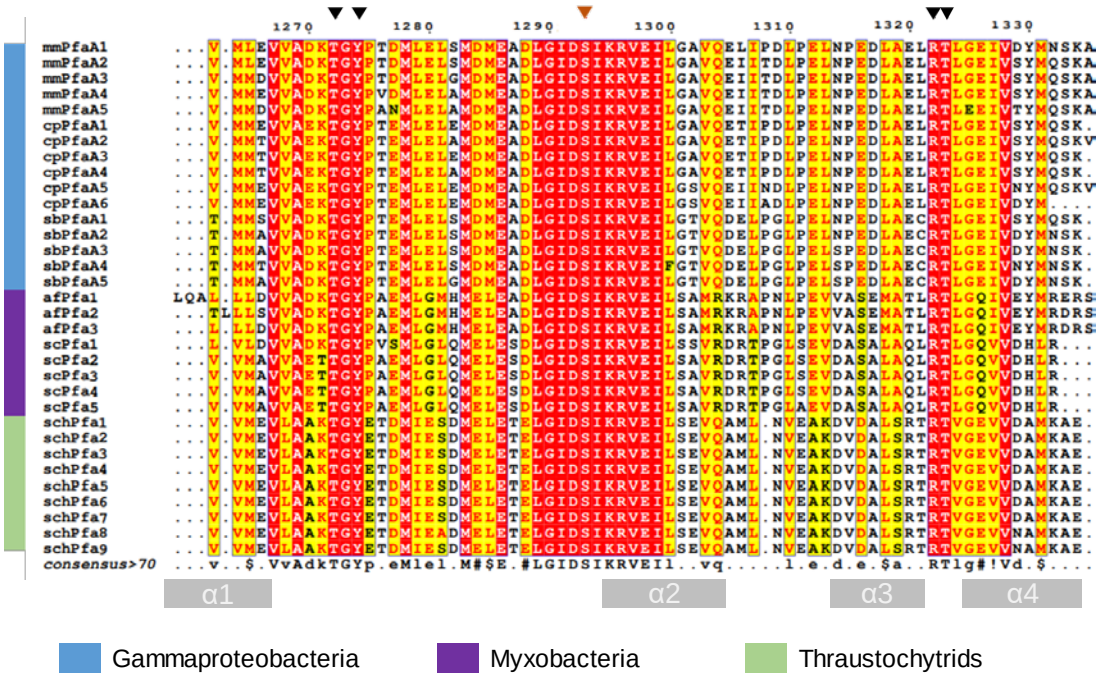


Figure S2. Multiple sequence alignment of representative tandem ACPs individual domains of PfaA-like proteins. The alignment of the tandem ACP individual domains from PfaA-like proteins is shown. Sequences correspond to PfaA (mmPfaA1, mmPfaA2, mmPfaA3, mmPfaA4, mmPfaA5) from *Moritella marina* (Acc. no. Q9RA21), PfaA (cpPfaA1, cpPfaA2, cpPfaA3, cpPfaA4, cpPfaA5, cpPfaA6) from *Colwellia psychrerythraea* (Acc. no. Q47ZG8), PfaA (sbPfaA1, sbPfaA2, sbPfaA3, sbPfaA4, sbPfaA5) from *Shewanella baltica* (Acc. no. A9KUH8), pfa2 (abPfa1, abPfa2, abPfa3) from *Aetherobacter fasciculatus* (Acc. no. A0A076Q2L6), pfa2 (scPfa1, scPfa2, scPfa3, scPfa4, scPfa5) from *Sorangium cellulosum* (Acc. no. A9EPF7) and PFA1 (schPfa1, schPfa2, schPfa3, schPfa4, schPfa5, schPfa6, schPfa7, schPfa8, schPfa9) from *Schizochytrium* ATCC 20888 (Acc. no. AAK72879). The position of the selected residues for the alanine screening of mmPfaA1 is marked with black arrows and the serine active site with a red arrow. Identical residues are shown in white on a red background, while similar residues are shown in red. The type of organism is indicated by color bars. Position of α-helices is represented with grey boxes at the bottom of the alignment.

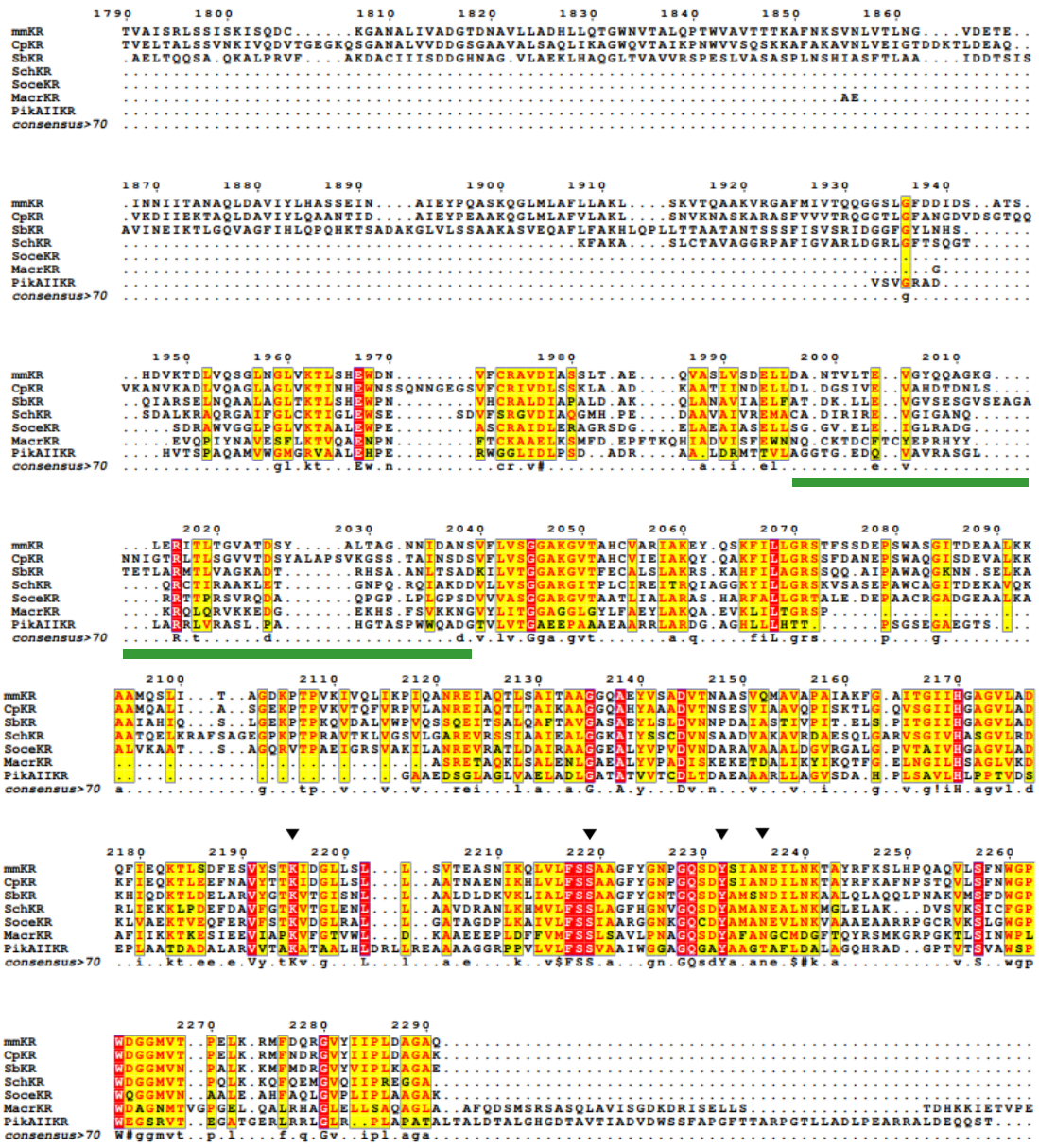


Figure S3 Multiple sequence alignment of KR'-KR domains. Alignment of representative KR'-KR protein regions from Pfa and PKS system. Sequences correspond to PfaA (mmKR) from *Moritella marina* (Acc. no. Q9RA21), PfaA (CpKR) from *Colwellia psychrerythraea* (Acc. no. Q47ZG8), PfaA (SbKR) from Shewanella baltica (Acc. no. A9KUH8), PFA1 (SchKR) from *Schizochytrium* ATCC 20888 (Acc. no. AAK72879), Macrolactin PKS (MacrKR) from *Bacillus amyloliquefaciens* (Acc. no. Q1RS63) and PikaII module (PikAIIKR) from *Streptomyces venezuelae* (Acc. no. Q9ZGI4). Identical residues are shown in white on a red background, while similar residues are shown in red. The unstructured loop that connect both KR domains was marked with a green line. The positions of the catalytic residues of the KR domains have been marked with black arrows.

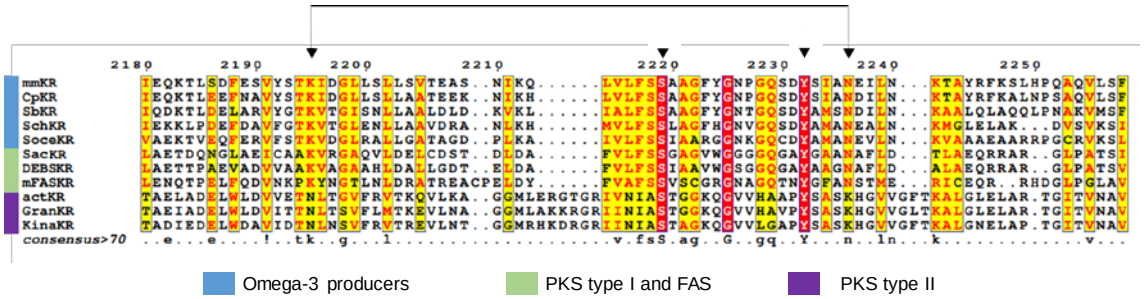


Figure S4. Multiple sequence alignment showing the swapped KR K/N residues wihin PfaA homologous proteins. The alignment of the KR active site region responsible for catalysis in omega-3 synthases in comparison with PKS and FAS systems is shown. Sequences correspond to PfaA (mmKR) from *Moritella marina* (Acc. no. Q9RA21), PfaA (CpKR) from *Colwellia psychrerythraea* (Acc. no. Q47ZG8), PfaA (SbKR) from *Shewanella baltica* (Acc. no. A9KUH8), PFA1 (SchKR) from *Schizochytrium* ATCC 20888 (Acc. no. AAK72879), Pfa2 (SoceKR) from *Sorangium cellulosum* (Acc. no. A9EPF7), SpnC PKS (SacKR) from *Saccharopolyspora spinosa* (Acc. no. Q9ALM4), DEBS (DEBSKR) from *Streptomyces hygroscopicus* (Acc. no. AQW50878), mammalian FAS (mFASKR) from *Sus scrofa* (Acc. no. A5YV76), Actinorhodin PKS (actKR) from *Streptomyces coelicolor* (Acc. no. P16544), Granaticin PKS (GranKR) from *Streptomyces violaceoruber* (Acc. no. P16542) and Kinamycin PKS (KinaKR) from *Streptomyces sp. ERV7* (Acc. no. AAO65349). Identical residues are shown in white on a red background, while similar residues are shown in red. The position of the catalytic motifs is marked with black stars, and K/N swapping is represented with two arrows connecting both residues.

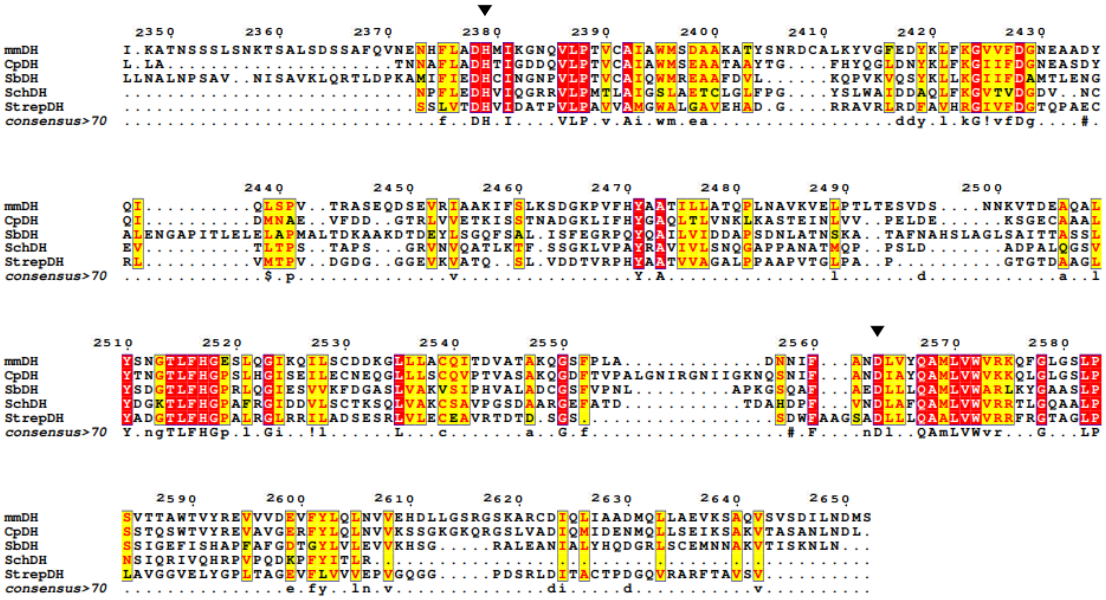


Figure S5. Multiple sequence alignment of DH domains. Alignment of representative DH protein regions from Pfa and PKS systems. Aligned sequences correspond to PfaA (mmDH) from *Moritella marina* (Acc. no. Q9RA21), PfaA (CpDH) from *Colwellia psychrerythraea* (Acc. no. Q47ZG8), PfaA (SbDH) from *Shewanella baltica* (Acc. no. A9KUH8), PFA1 (SchDH) from *Schizochytrium* ATCC 20888 (Acc. no. AAK72879) and a PKS type I (StrepDH) from *Streptomyces sp.* MUSC 93 (Acc. no. OIJ85280). Identical residues are shown in white on a red background, while similar residues are shown in red. The positions of the catalytic residues of the DH domain have been marked with black arrows.

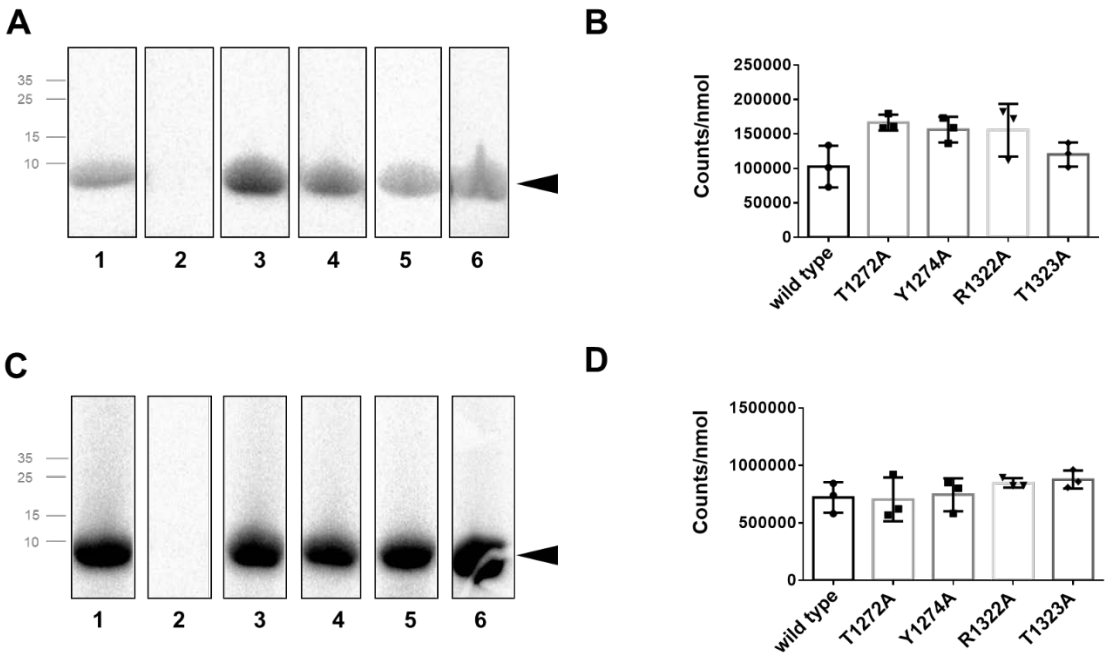
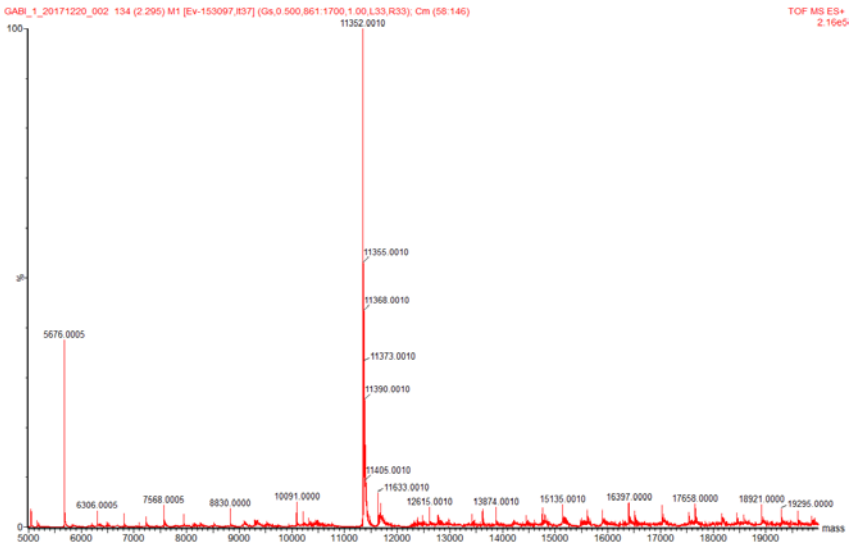


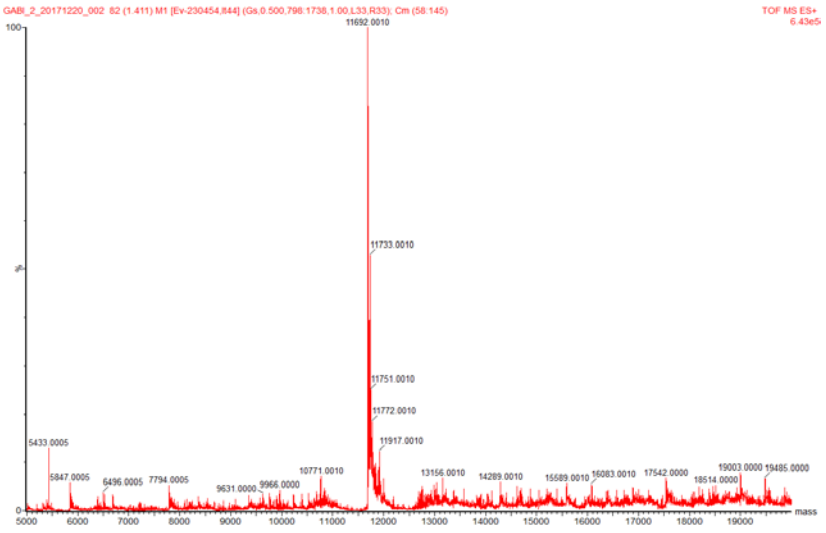
Figure S6. Alanine substitution screening for mmACP1. (A) Wild type mmACP1, the apo ACP form and the four mutants incubated with radiolabeled [14C]-malonyl-CoA analyzed by radio-SDS-PAGE. Lane 1: holo-mmACP1+Malonyl-CoA; 2: apo-mmACP1+Malonyl-CoA 3: holo-mmACP1(T1272A)+Malonyl-CoA; 4: holo-mmACP1(Y1274A)+Malonyl-CoA; 5: holo-mmACP1(R1322A)+Malonyl-CoA; 6: holo-mmACP1(T1323A)+Malonyl-CoA. (B) Quantification of the protein bands intensities of mmACP1 wild type and the mutants analyzed in A by densitometric scanning, normalized with molarity of each sample. (C) and (D) correspond to the same protein samples but incubated with mmKS-AT. Black arrows indicate the theoretical mmACP1 molecular weight.

A



apoACP

B



holoACP

Figure S7. High Definition Mass Spectrometry. High definition mass spectra of apo mmACP1 (A) and holo mmACP1 (B). The amount of desalted protein used for both experiments was 40 µg. MS spectra were manually acquired in the m/z range 500-1700. Default deconvolution parameters were used.

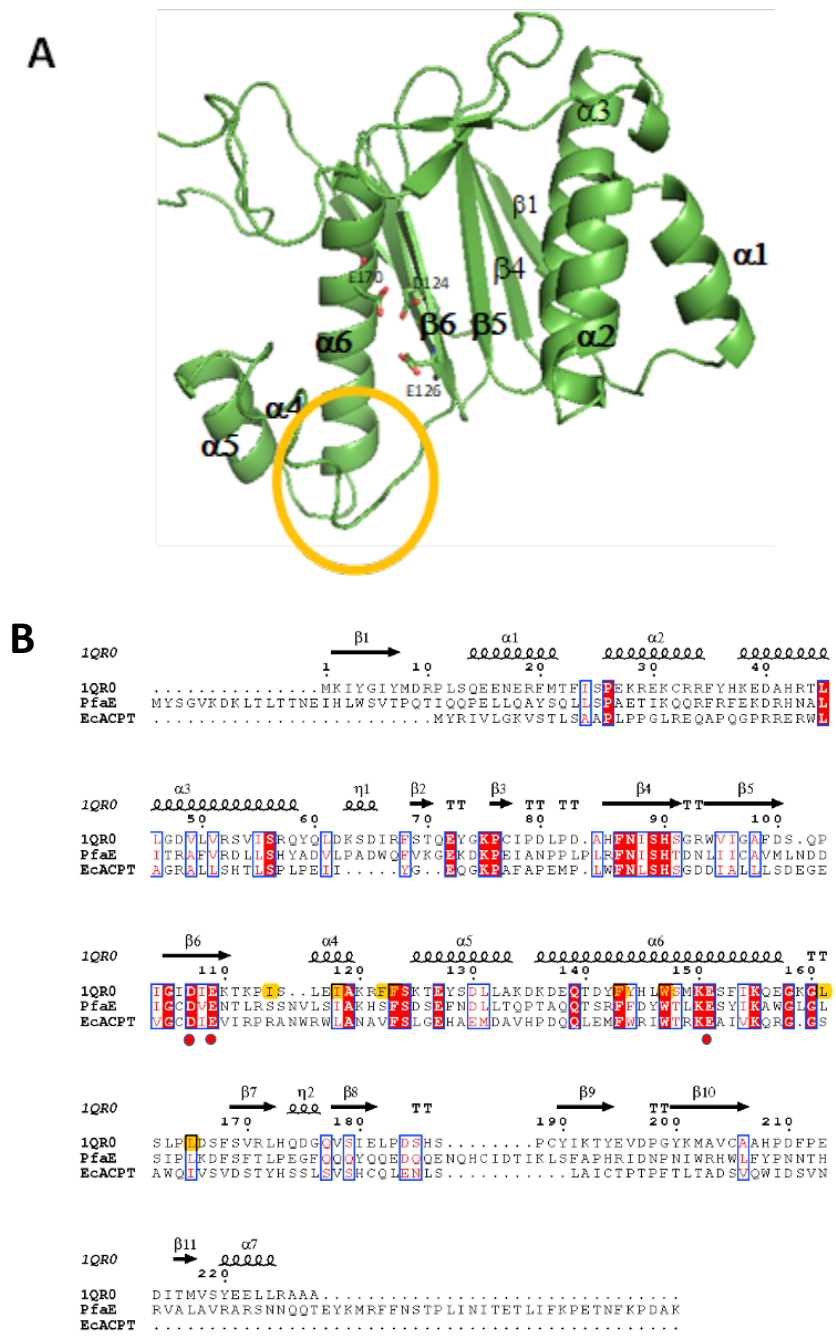


Figure S8. (A) Predicted structure of mmPfaE from *M. marina*. mmPfaE structure was modelled by Phyre2 using *Bacillus subtillis* Spf (1QR0) as template. Predicted catalytic residues D124, E126 and E170 are shown. The ACP-PfaE interaction zone is indicated with a yellow circle. (B) Alignment of mmPfaE with homologous proteins. The active site residues responsible for catalysis are highlighted with red dots and the residues responsible for ACP-PfaE interaction are marked in yellow.

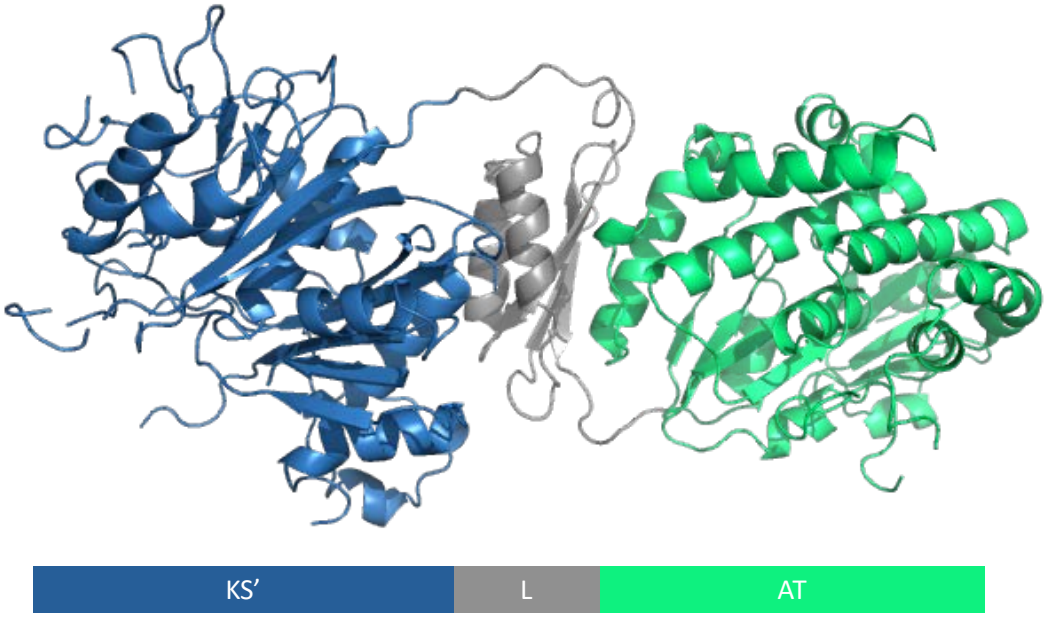


Figure S9. Predicted structure of mmPfaB from *M. marina*. A linker (grey) connects the pseudo keto synthase N-terminal domain (blue) and the acyl transferase domain (green).