

Engineering metazoan fatty acid synthase to control chain length applied in yeast

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Supplementary Methods

Synthesis of decanoyl-CoA ester

Synthesis of C10-ACP

Supplementary Tables

Supplementary Table 1: Table of TR domains screened for this work. Given is the internal number, the enzyme class, the molecular weight, the type of reduction and their native organism.

Supplementary Table2: Cloning strategies and primers for plasmids created in this study. Supplementary Table2: Cloning strategies and primers for plasmids created in this study.

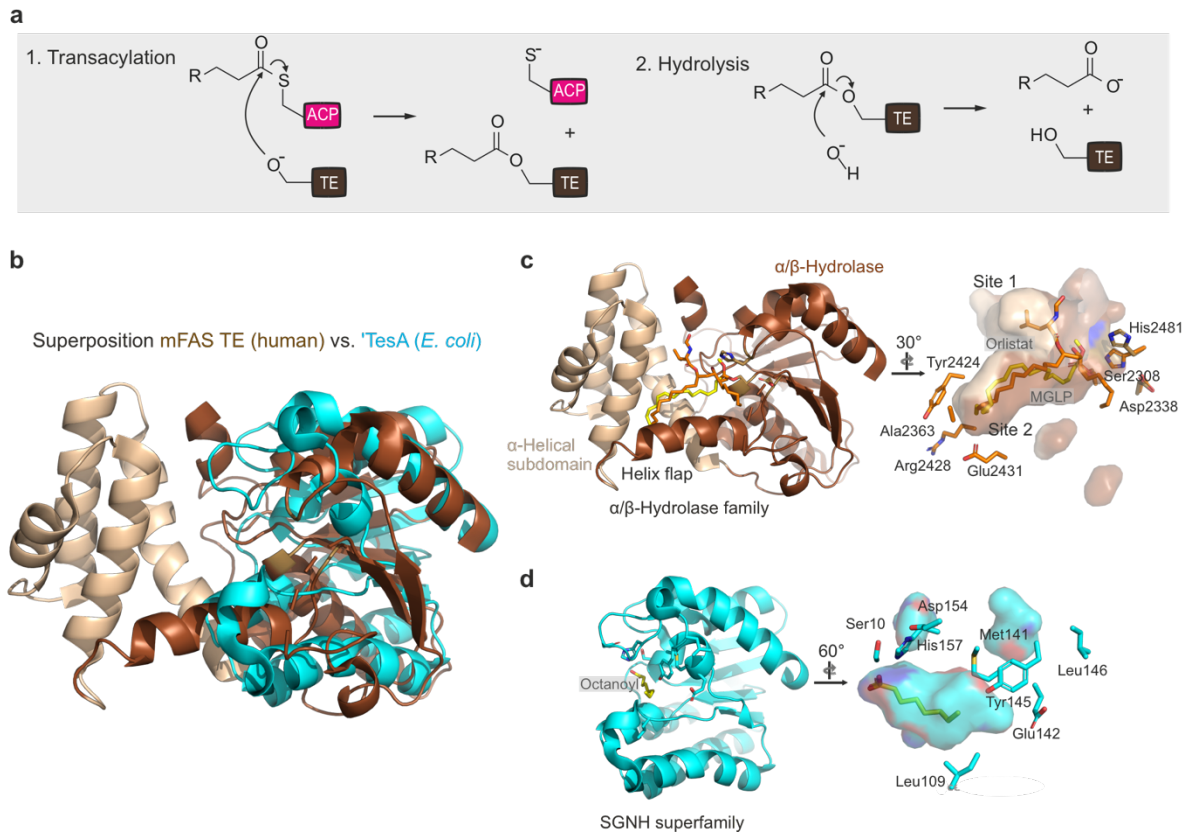
Supplementary Table 3: Cloning strategies and primers for expression cassettes created in this study.

Supplementary Table 4: Table of strains and their genotypes used in this study.

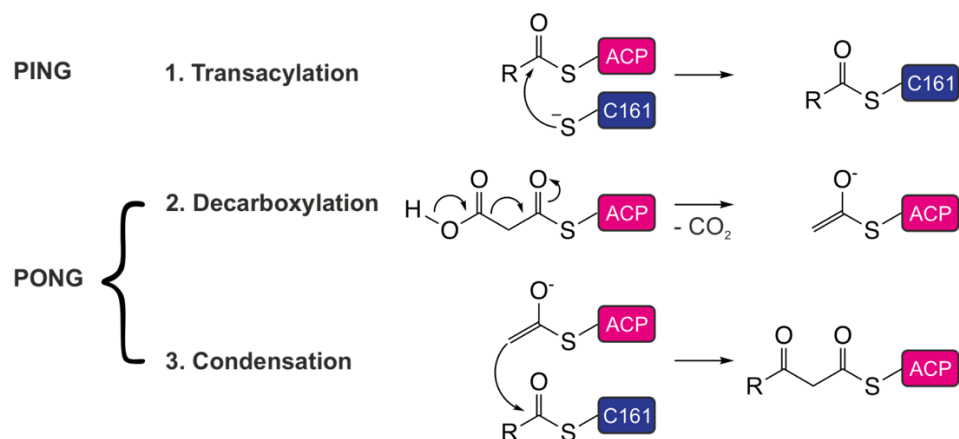
Supplementary Table 5: Amino acid and DNA sequences of the constructs used in the FA chain length experiments.

Supplementary Table 6: Amino acid and DNA sequences of the reductase domains screened for their capability to use C10-mACP as substrates.

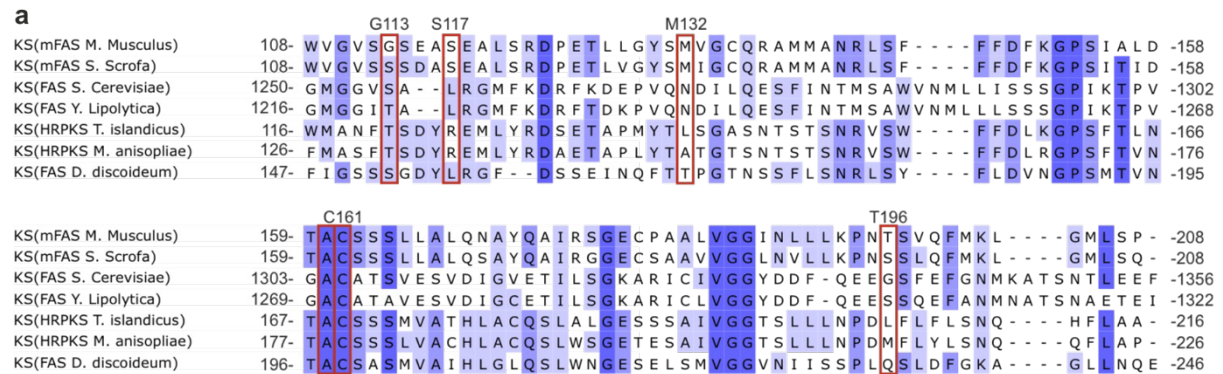
Supplementary Table 7: Amino acid and DNA sequences of the constructs used in the fatty aldehydes/alcohol experiments.



Supplementary Figure 2: TE-mediated hydrolysis and structural comparison of mFAS TE domain vs. *E. coli* TesA. (a) The ACP-bound acyl moiety is transacylated onto the active serine of the TE domain. The acyl chain is then released by hydrolysis. (b) Superposition of mFAS TE (human) (PDB ID: 3tjm) colored in brown and *E. coli* TesA (PDB ID: 1u8u) in cyan, both in cartoon representation. (c) Structure of the mFAS TE (human) with a covalently bound methyl γ -linolenylphosphonate (MGLP, PDB ID: 3tjm) in yellow and bound orlistat (PDB ID: 2px6) in orange. The TE domain consists of an α/β -hydrolase fold and an inserted helical subdomain, depicted in light brown for clarity. Active site and binding site residues are highlighted in stick representation. The binding pocket surface shows the two binding sites, both at the interface to the subdomains. Binding site 2 is occupied by acyl chains of ligands orlistat and MGLP, of which MGLP causes the structuring of an amphiphilic α -helix, termed “helix flap”. Amino acid residues, suggested to be involved in chain length specificity, are highlighted in stick representation (for orlistat bound structure). (d) TesA adopts a typical α/β -hydrolase fold in which a hydrophobic groove is formed at the protein surface. The absence of a lid or capping subdomain contributes to its broader substrate specificity. The binding pocket surface is shown with an octanoyl ligand (in green), and residues, mutated in this study, are in stick representation (PDB ID: 1u8u).



Supplementary Figure 3: Schematic depiction of the KS Ping-Pong mechanism. In the Ping step, the ACP-bound acyl is transacylated onto the active cysteine of the KS domain. The Pong step begins with the decarboxylation of a malonyl unit, which was previously loaded onto the free ACP (after Ping step) via the MAT. The resulting enolate attacks the cysteine-bound acyl chain and releasing the β -ketoacyl as ACP-bound moiety from the KS.

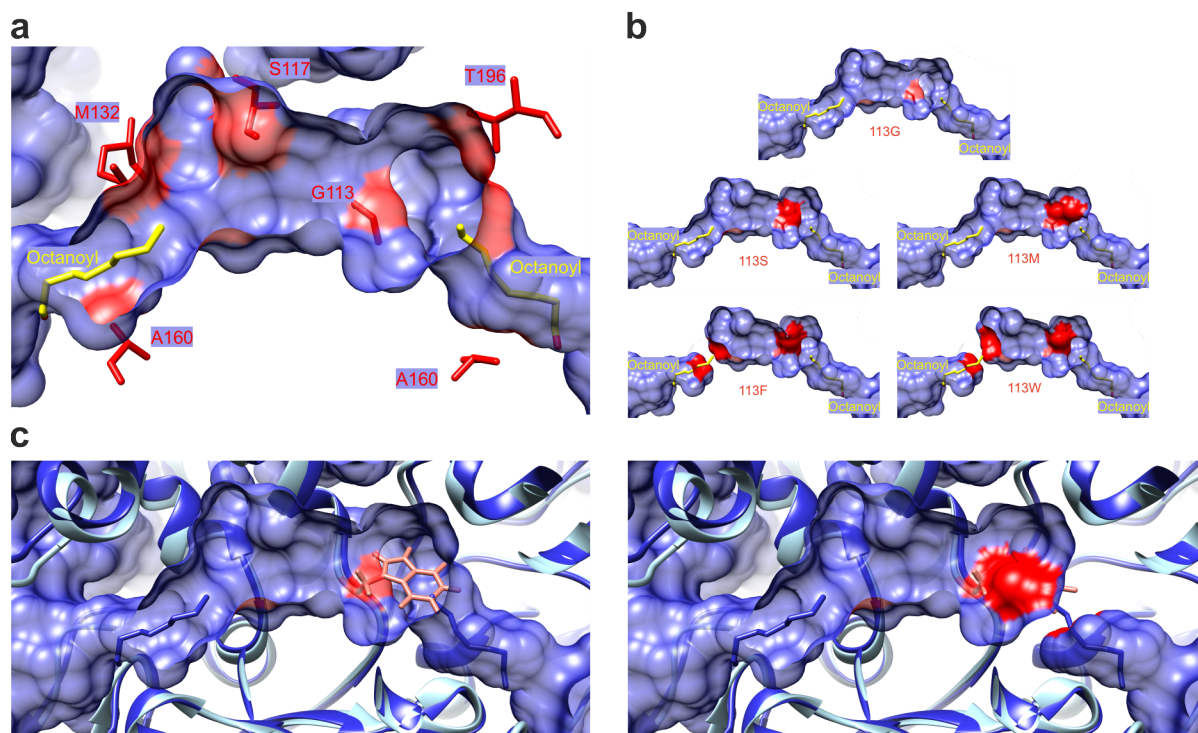


b

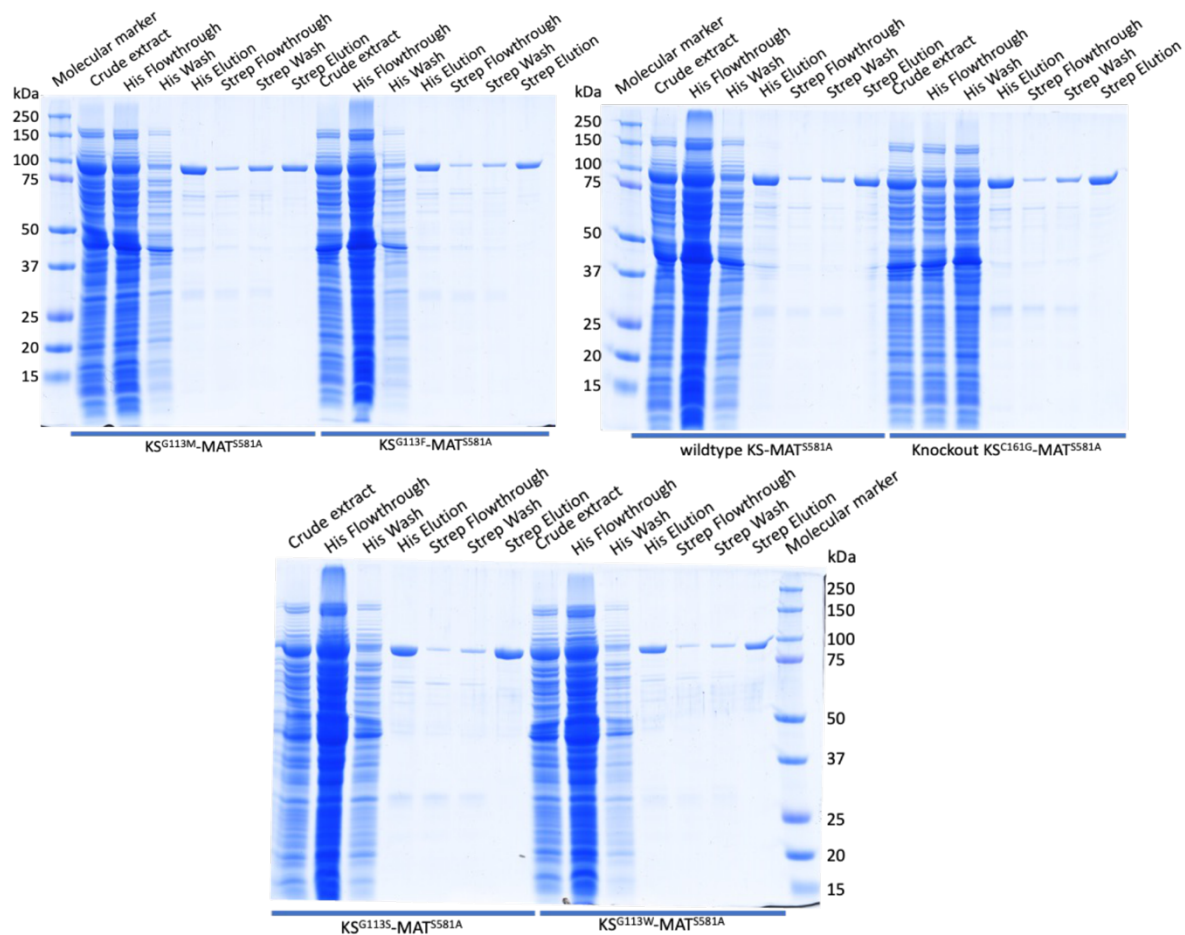
	KS(mFAS M. Musculus)	KS(mFAS S. Scrofa)	KS(FAS S. Cerevisiae)	KS(FAS Y. Lipolytica)	KS(HRPKS T. islandicus)	KS(HRPKS M. anisopliae)	KS(FAS D. discoideum)
KS(mFAS M. Musculus)	100%	87%	20%	12%	36%	38%	35%
KS(mFAS S. Scrofa)	89%	100%	21%	13%	38%	38%	35%
KS(FAS S. Cerevisiae)	13%	13%	100%	36%	13%	12%	14%
KS(FAS Y. Lipolytica)	17%	18%	78%	100%	18%	16%	16%
KS(HRPKS T. islandicus)	34%	35%	19%	12%	100%	70%	35%
KS(HRPKS M. anisopliae)	36%	35%	18%	11%	70%	100%	36%
KS(FAS D. discoideum)	31%	31%	19%	11%	33%	34%	100%

Legend: 10% 25% 50% 70% 90%

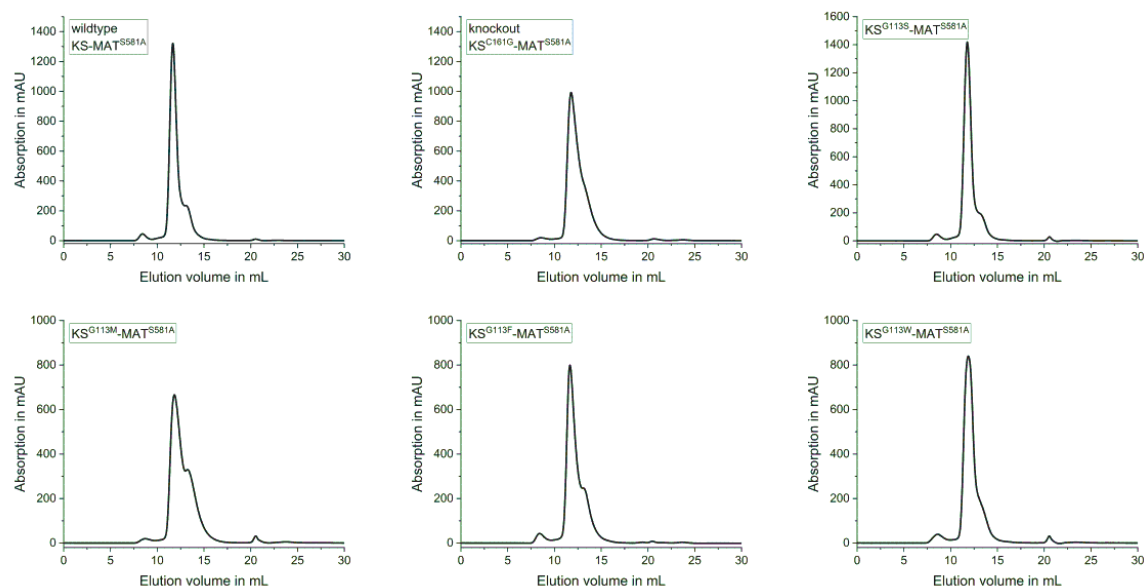
Supplementary Figure 4: Comparison of selected KS sequences. (a) Sequence alignment. Mutated positions are marked in red and annotated according to murine mFAS numbering. *Mus musculus* ([P19096](#)), *Sus scrofa* ([A5YV76](#)), *Saccharomyces cerevisiae* ([P19097](#)), *Yarrowia lipolytica* ([A0A371C916](#)), *Talaromyces islandicus* ([A0A0U1MAK2](#)), *Metarhizium anisopliae* ([A0A0D9NM54](#)), *Dictyostelium discoideum* ([Q54FI3](#)). (b) Similarity of KS sequences used in the alignment above. (Created with UGENE²)



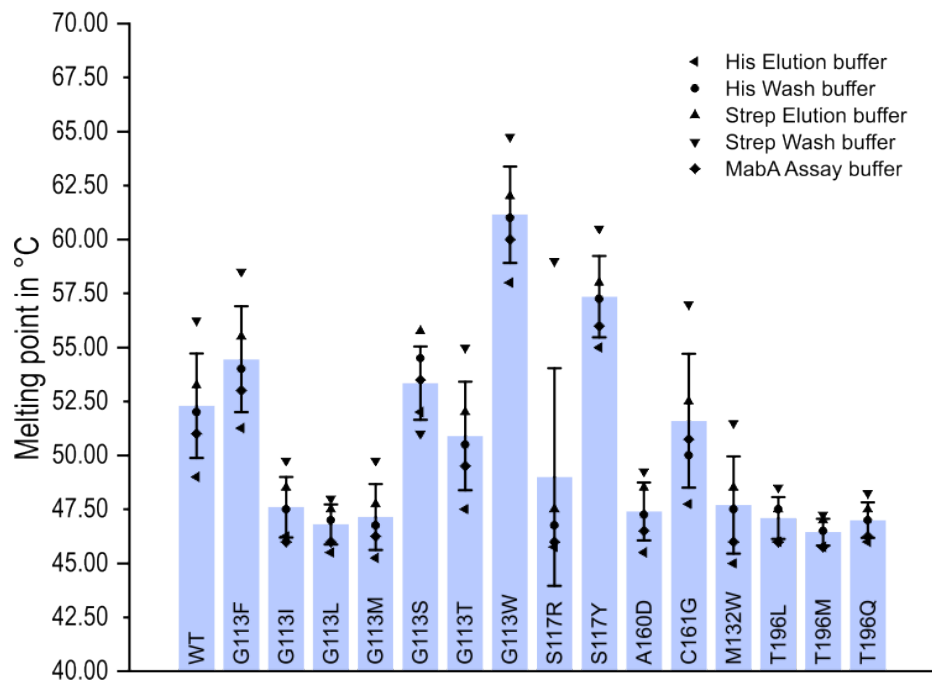
Supplementary Figure 5: The KS binding tunnel. (a) Depiction of the KS binding tunnel surface from octanoyl bound structure (PDB IDs: 6rop). Octanoyl chains are colored yellow, residues mutated in the KS mutant screen are colored red. (b) Surface depiction of the binding pocket of the mouse KS with an octanoyl chain bound to the active site serine (PDB IDs: 6rop). Position 113 highlighted in red. Residue 113 was exchanged using the rotamers tool from chimera and by selecting the most probable positioning. (c) Computational analysis of the sterically most demanding mutation (G113W) in the KS binding pocket. Overlay of the octanoyl-bound crystal structure with tryptophane in position of G113 for clarity in dark blue (PDB: 6ROP) with an energy optimized G113W mutant KS (light blue). Zoom onto tryptophane replacing G113. The two binding tunnels (dark blue KS_WT with C8 and light blue energy optimized G113W mutant without substrate) are depicted as surfaces calculated with chimera. This shows the steric hindrance a growing acyl chain encountered in the G113W mutant. Energy optimization of the G113W mutant was done with Cyrus Bench (ref). The RMSD value between the backbones of the crystal structure and the energy optimized structure is 0.701 Å.



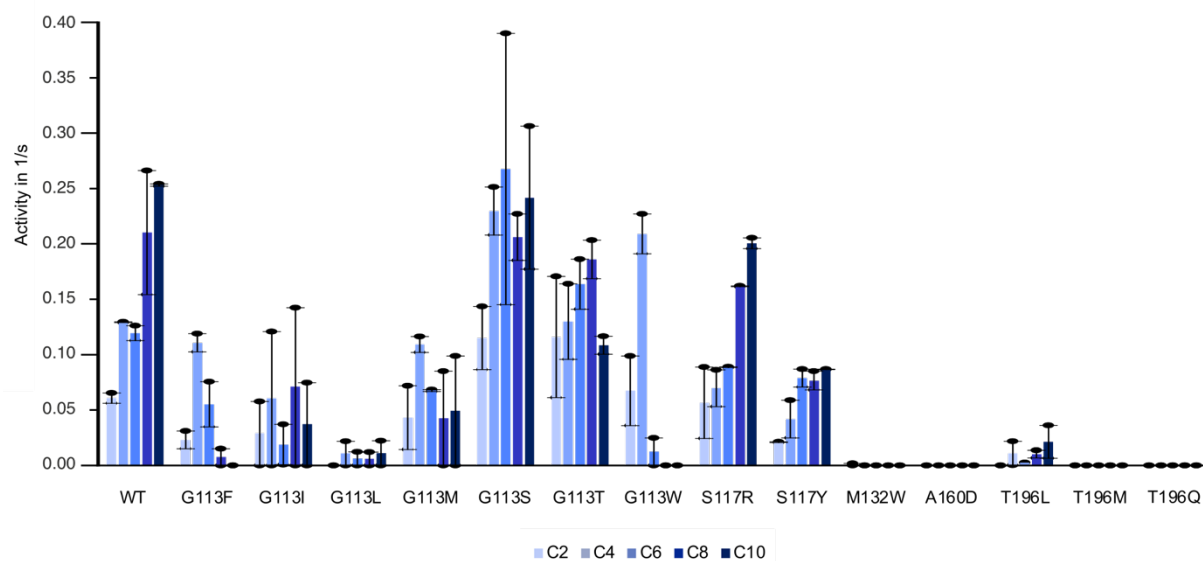
Supplementary Figure 6: Representative SDS-PAGE analysis of the KS^{G113X}-MAT^{S581A} didomain constructs (X refers to M, S, F and W, wildtype for comparison) recombinantly produced in *E. coli* for the detailed enzyme kinetic analysis of the KS. Apparent molecular weights of target proteins agree with calculated molecular weight of 97 kDa.



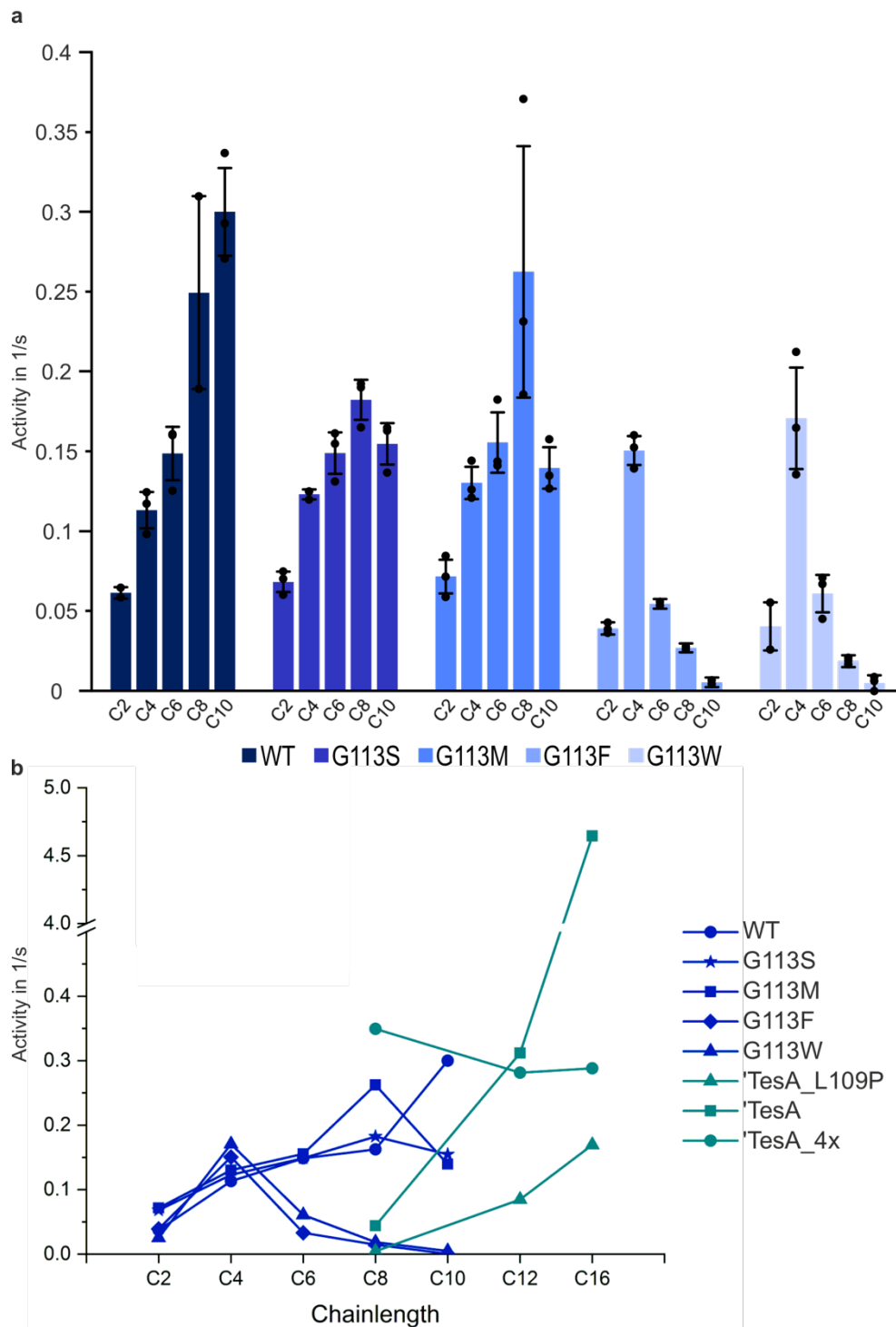
Supplementary Figure 7: Representative size-exclusion chromatograms of KS^{G113X}-MAT^{S581A} mutants expressed for the detailed kinetic analysis of the KS. All chromatograms show a prominent peak at 12 mL elution volume, in line with the preferential dimeric state of the didomain construct, and a lower peak/shoulder at 13 mL of the monomeric species. Fractions of dimeric proteins were collected and used for further experiments.



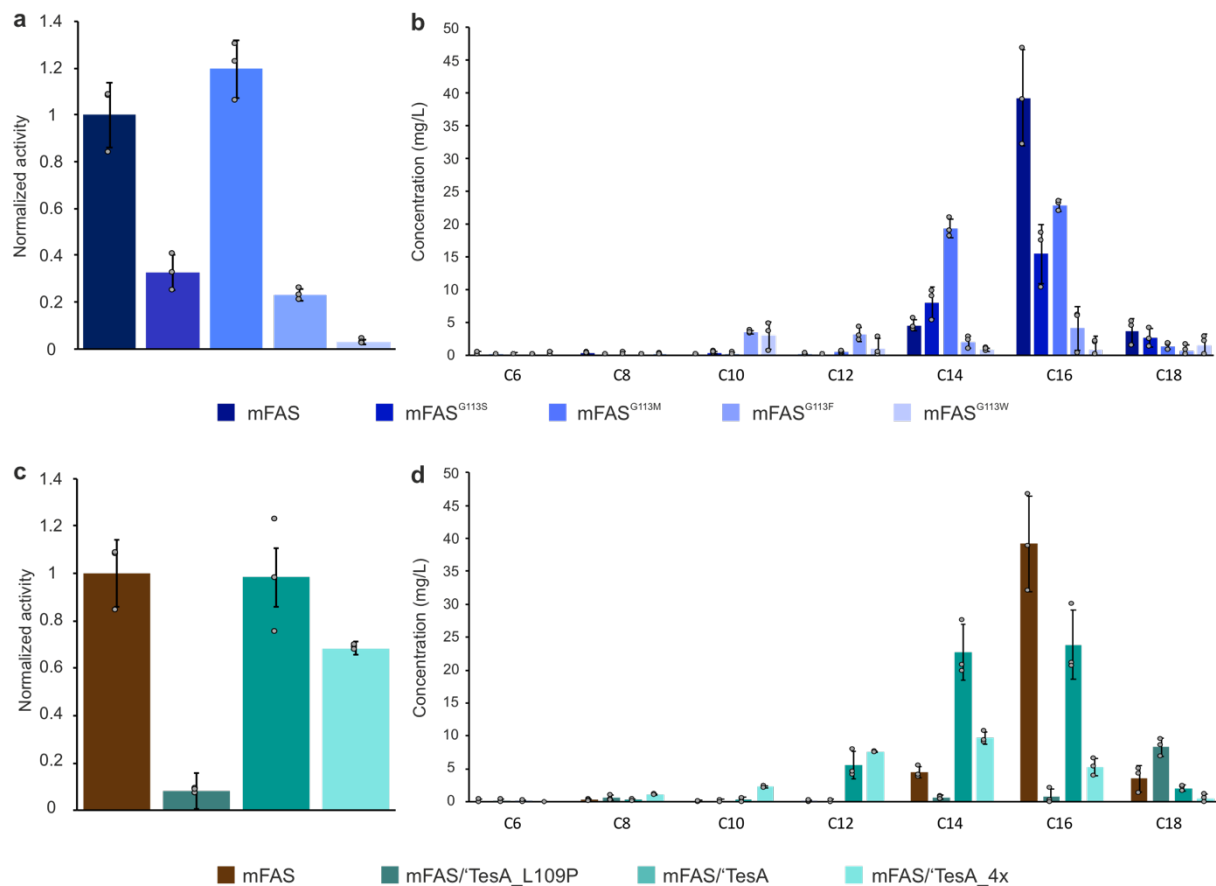
Supplementary Figure 8: Thermal shift assay of KS^{G113X}-MAT^{S581A} mutants, as well as other KS mutants, recorded in in five different buffers (His Wash, His Elution, Strep Wash, Strep Elution, and MabA assay buffer). Data is represented as mean +/- SD of the five different buffers.



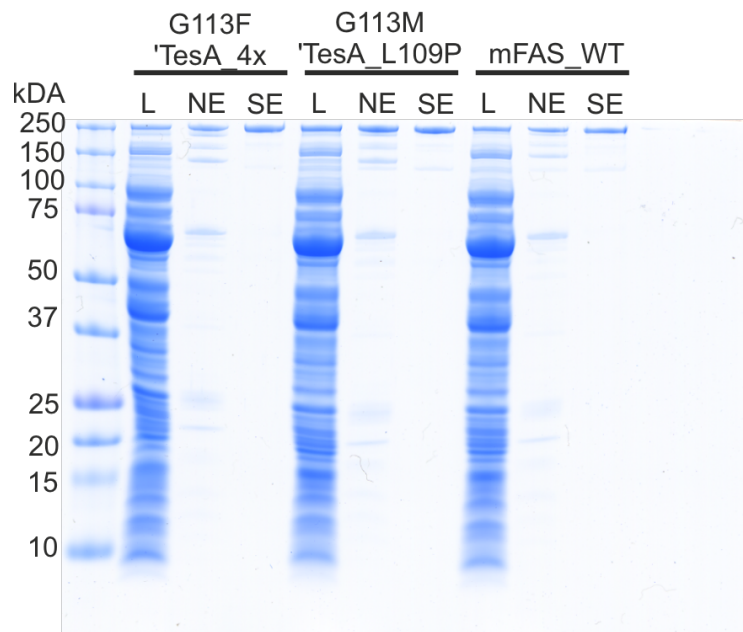
Supplementary Figure 9: Screening of KS mutants. The initial reaction velocity was determined at $v_{\max}$ of 20 μM Mal-ACP in technical duplicates of the wildtype and 16 mutants for acetyl- (C2), butyryl- (C4), hexanoyl- (C6), octanoyl- (C8) and decanoyl- (C10) ACP using the MabA assay. Error bars indicate the propagated error of standard deviations of blank measurement (KS knockout) and positive measurement.



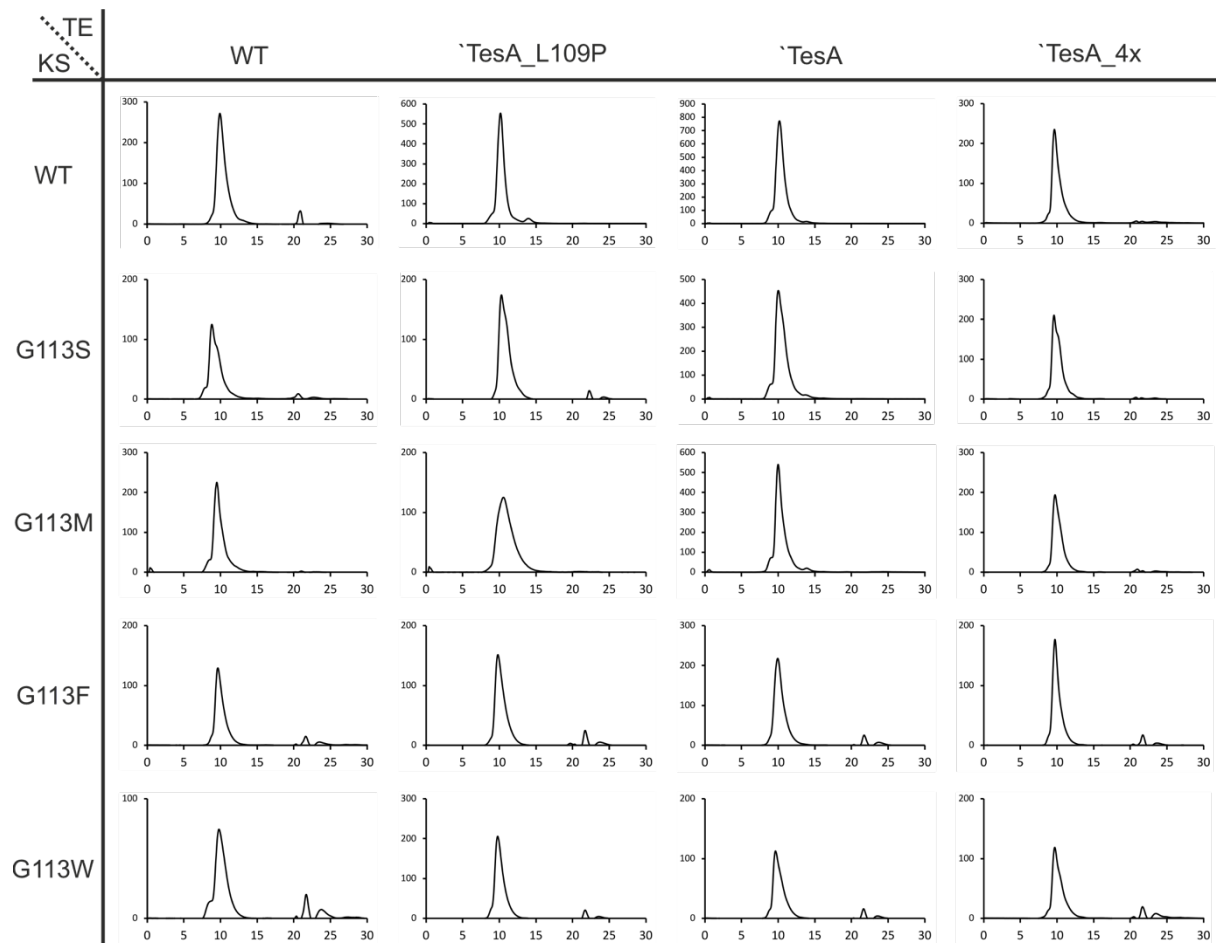
Supplementary Figure 10: KS elongation rates. (a) KS elongation activity of the WT and four G113 point mutations for chain lengths between C2 and C10. The rates represent the mean of three biological replicates and are given in 1/s. The error bars reflect the error propagation of blank (knockout) and positive (mutant) measurements standard deviation. (b) Apparent rates for the substrate octanoyl-ACP of five KS variants (blue) and three 'TesA variants (turquoise) in 1/s. Data for the KS mutations was recorded in this study, data for the 'TesA variants is from Deng et al.³



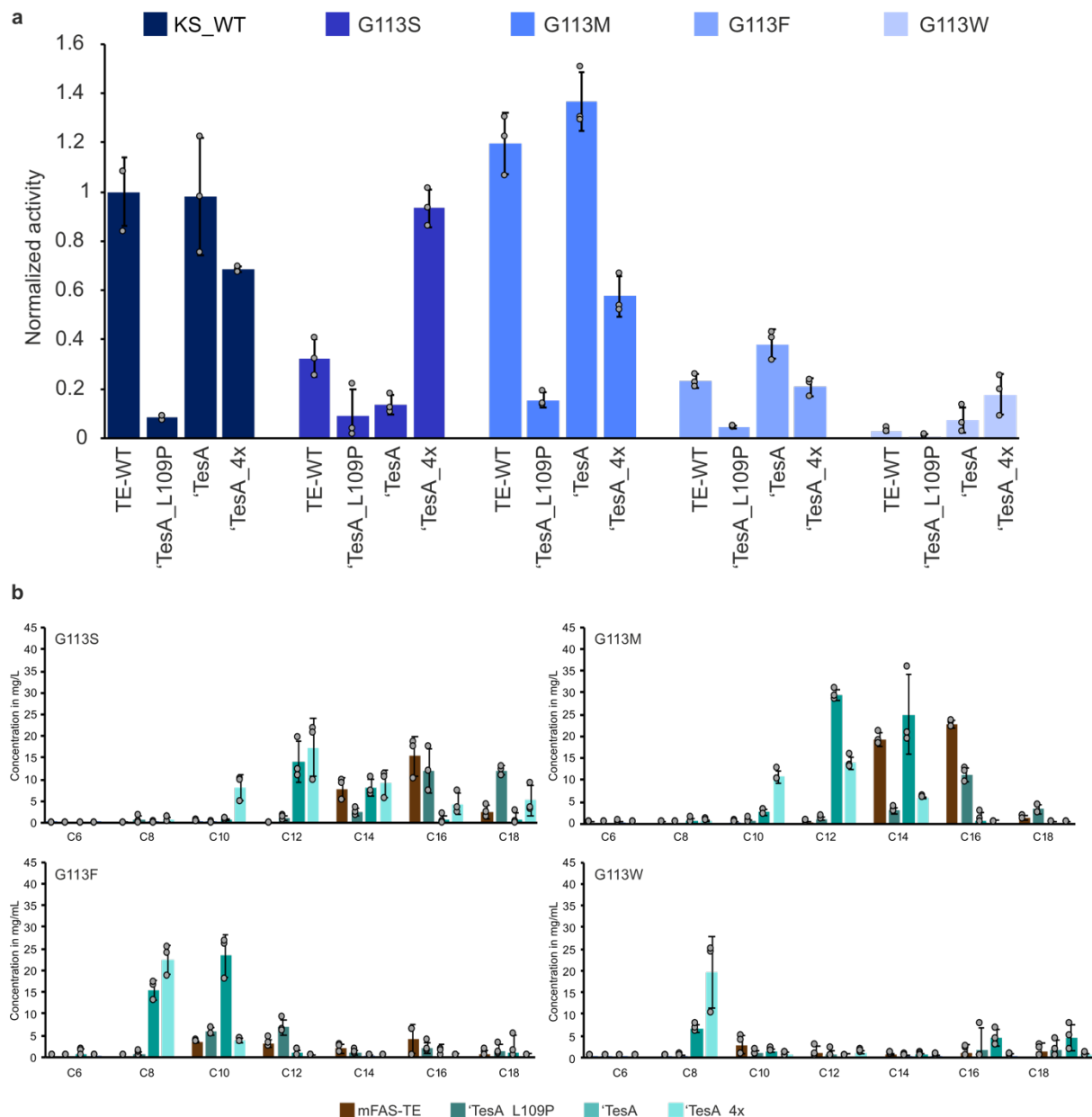
Supplementary Figure 11: *In vitro* assays of mFAS KS-mutants and mFAS/TesA hybrids. (a) Normalized activity of five mFAS mutated in KS at position 113 (data collected in biological triplicates with three technical replicates each). NADPH decrease was monitored via its fluorescence (excited 348-320 nm, detected 470-420 nm) over time. Activities of variants were normalized to WT activity. This figure panel is also shown in Figure 2e, and here included for clarity. (b) FAs were produced *in vitro* by incubation of the enzyme, Ac-CoA, Mal-CoA and NADPH overnight and measured via gas chromatography. This is an accompanying figure to Figure 2f, where FA concentrations are sorted by mFAS variants. (c/d) Normalized activity of different mFAS/TesA hybrids mutated in 'TesA; including wildtype mFAS (mFAS_WT) as reference. Data collection as described for (a and b) in biological triplicates with three technical replicates each. Data presented as the mean $\pm$ SD of three biological replicates. Figure panels c and d are accompanying figures to Figure 3a and b.



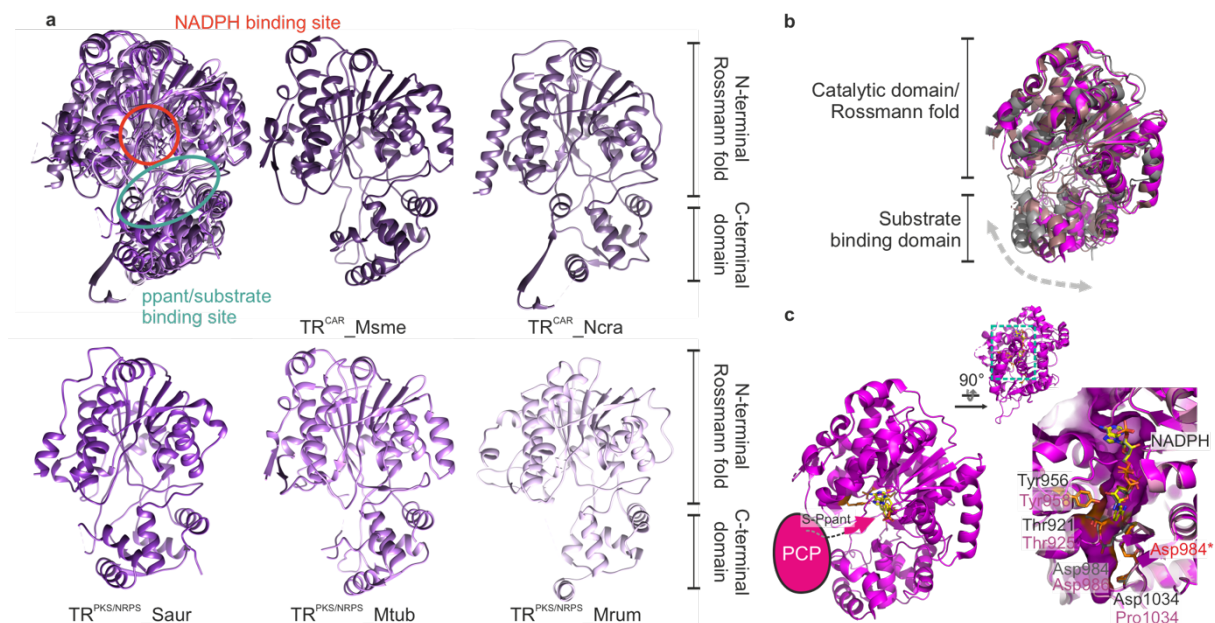
Supplementary Figure 12: Representative analysis of protein purity by SDS-PAGE for selected mFAS/'TesA constructs. A 10% Schagger-gel was used. Samples are lysate (L), elution from the nickel-chelating column (NE) and elution from the STREP-tactin column (SE). Apparent molecular weights of target proteins agree with calculated molecular weight of 270 kDa. Proteins are pure after the STREP column, as judged from SE lanes.



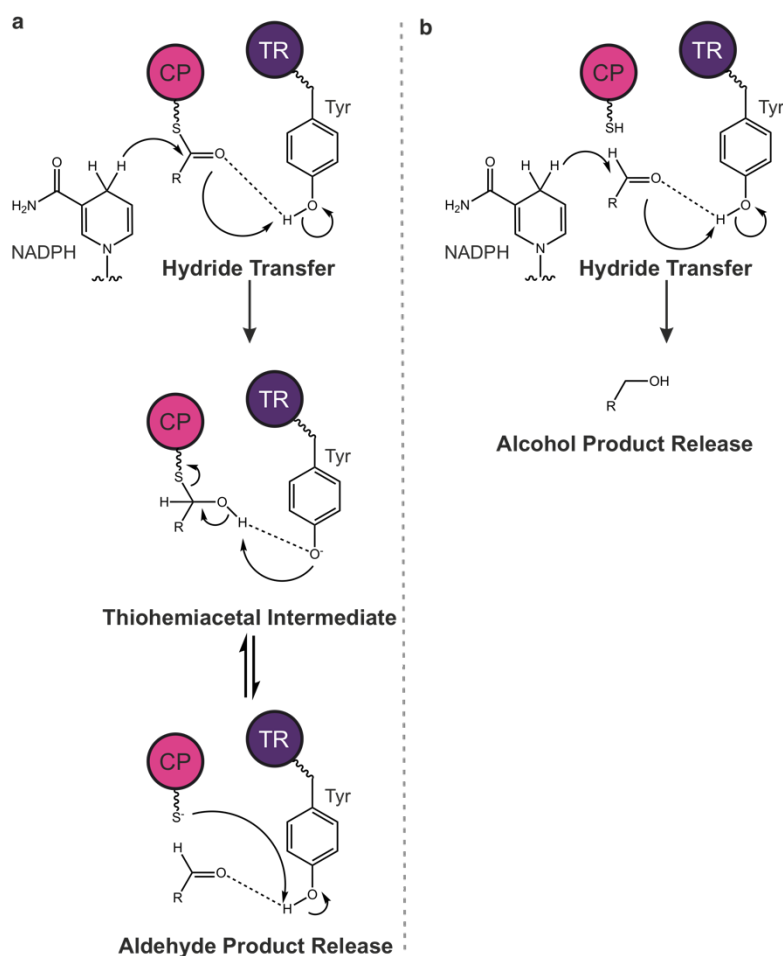
Supplementary Figure 13: Analysis of protein purity by SEC for the 20 mFAS/TesA constructs used in this study. X-axis shows the elution volume in mL, Y-axis shows the 280 nm absorption in mAU. Proteins elute as dimers mainly, with varying amounts of monomeric species. Just proteins in dimeric state were used for in vitro experiments.



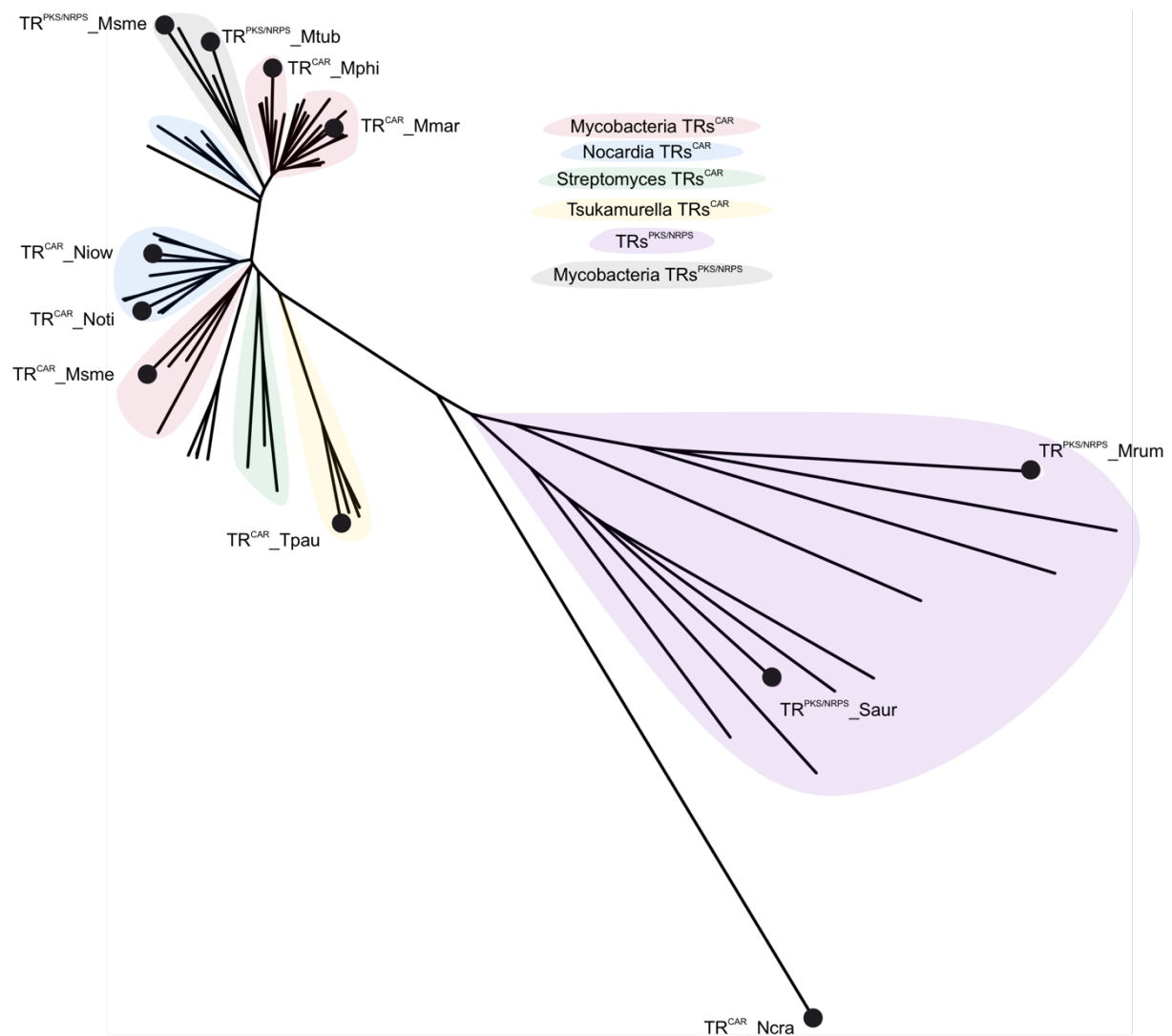
Supplementary Figure 14: Normalized activity and product spectra of mFAS-hybrids. (a) Normalized activity of mFAS variant harboring KS mutated to G113S, G113M, G113F or G113W (or non-mutated WT KS) combined and one of the three 'TesA variants 'TesA, 'TesA_L109P or 'TesA_4x (or mFAS TE mTE). Data are normalized to WT activity (373 nmol/min/mg protein), and were collected in biological triplicates with three technical replicates each. This is an accompanying figure to Figure 3a. (b) Product distributions of mFAS-hybrids grouped by KS mutants (data collected in biological triplicates with three technical replicates each). FAs were produced in vitro by incubation of the enzyme, Ac-CoA, Mal-CoA and NADPH overnight and measured via gas chromatography. Data presented as the mean $\pm$ SD of three biological replicates. This is an accompanying figure to Figure 3b.



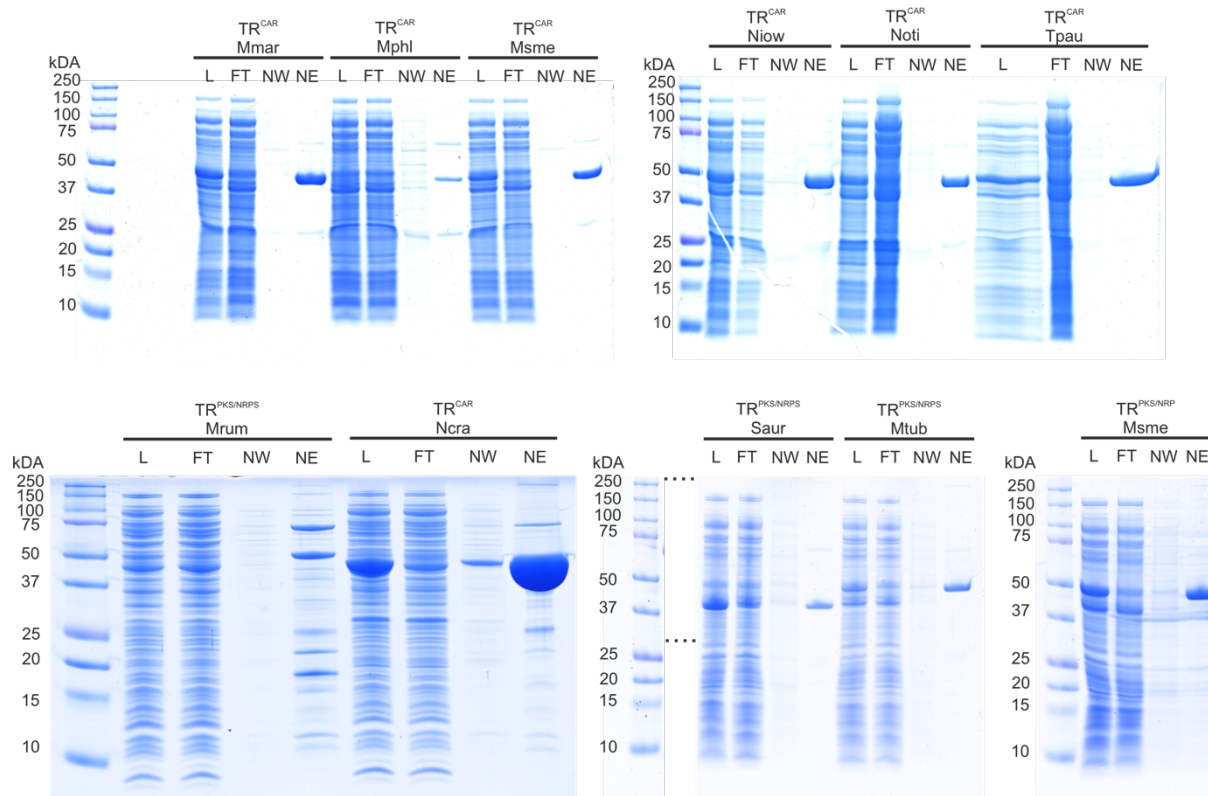
Supplementary Figure 15: Thioreductase domains used in mFAS/TR engineering. (a) Structures of TR domains used in this study for which X-ray structures are available. Overlay of the five structures (top left) showing a well conserved Rossmann-fold with its NADPH binding site and a more divergent C-terminal domain responsible for substrate recognition. PDB ID codes are: 5MSO (TR^{CAR}_Mmar), 8AEP (TR^{CAR}_Ncra), 4W4T (TR^{PKS/NRPS}_Saur), 4DQV (TR^{PKS/NRPS}_Mtub) and 6VTZ (TR^{PKS/NRPS}_Mrum). Thioreductase domains used in mFAS/TR engineering. (b) Superposition of TR^{CAR} from *Mycobacterium marinum* (magenta, PDB ID: 5msu; 60% sequence identical to *M. smegmatis* TR^{CAR}), and TRs^{PKS/NRPS} from *M. tuberculosis* (purple, PDB ID: 4u5q, 55%) and *Stigmatella aurantiaca* (grey, PDB ID: 4u7w, 30%). The overall conserved fold shows rigid body like conformational variability between the catalytic domain and the substrate binding domain that assists substrate binding (grey arrow). (c) *M. marinum* TR^{CAR} (PDB ID: 5msu) with selected residues in the active site and binding tunnel highlighted in stick representation. Zoom in active site reveals conformational dynamics of the loop carrying Asp984 (*M. marinum* numbering; *M. smegmatis* number attached in purple) switching between the active on-state (Asp984*) and the inactive off-state. Active site and binding site residues are highlighted in stick representation. Bound NADPH is shown in yellow.



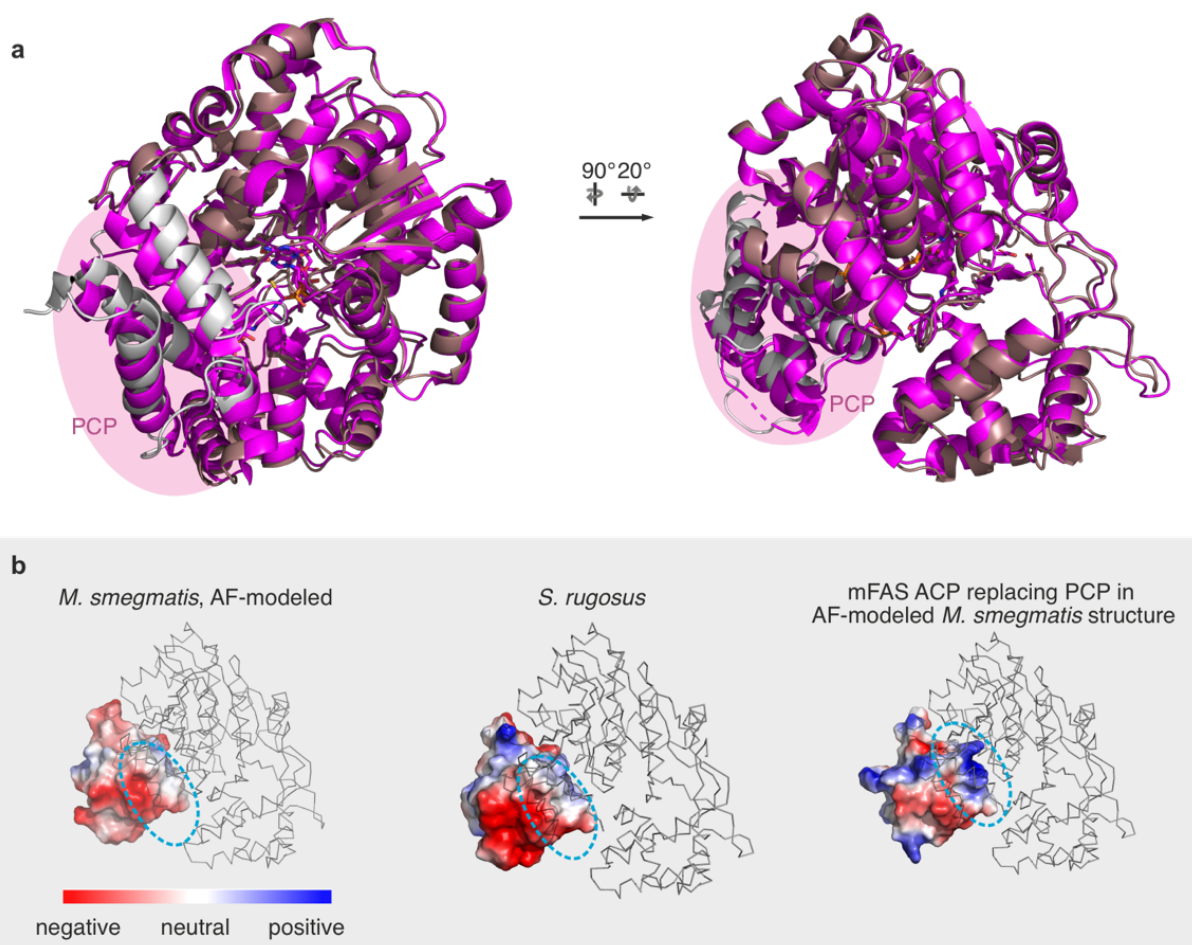
Supplementary Figure 16: Mechanism of TR-mediated reduction and product release. (a) (First) 2-electron reduction. Carrier protein (CP) bound substrate is reduced to the corresponding aldehyde using one NADPH as hydride-donor. (b) Second 2-electron reduction. Formed aldehyde can be reduced to alcohol by a second equivalent of NADPH. Figure adapted from Mullaney et al.⁴



Supplementary Figure 17: Phylogenetic tree of TRs^{CAR} and TRs^{PKS/NRPS}. Sequences from the TR^{CAR}s were used from Finnigan et al.⁵ and sequences from TRs^{PKS/NRPS} were retrieved from Uniprot and Genbank. Sequence alignment was done using aMAFFT, and the tree was calculated using BLOSUM62. Groups of TRs were colored and annotated. TR^{CAR}-Ncra poses as outlier since it is a fungal TR.



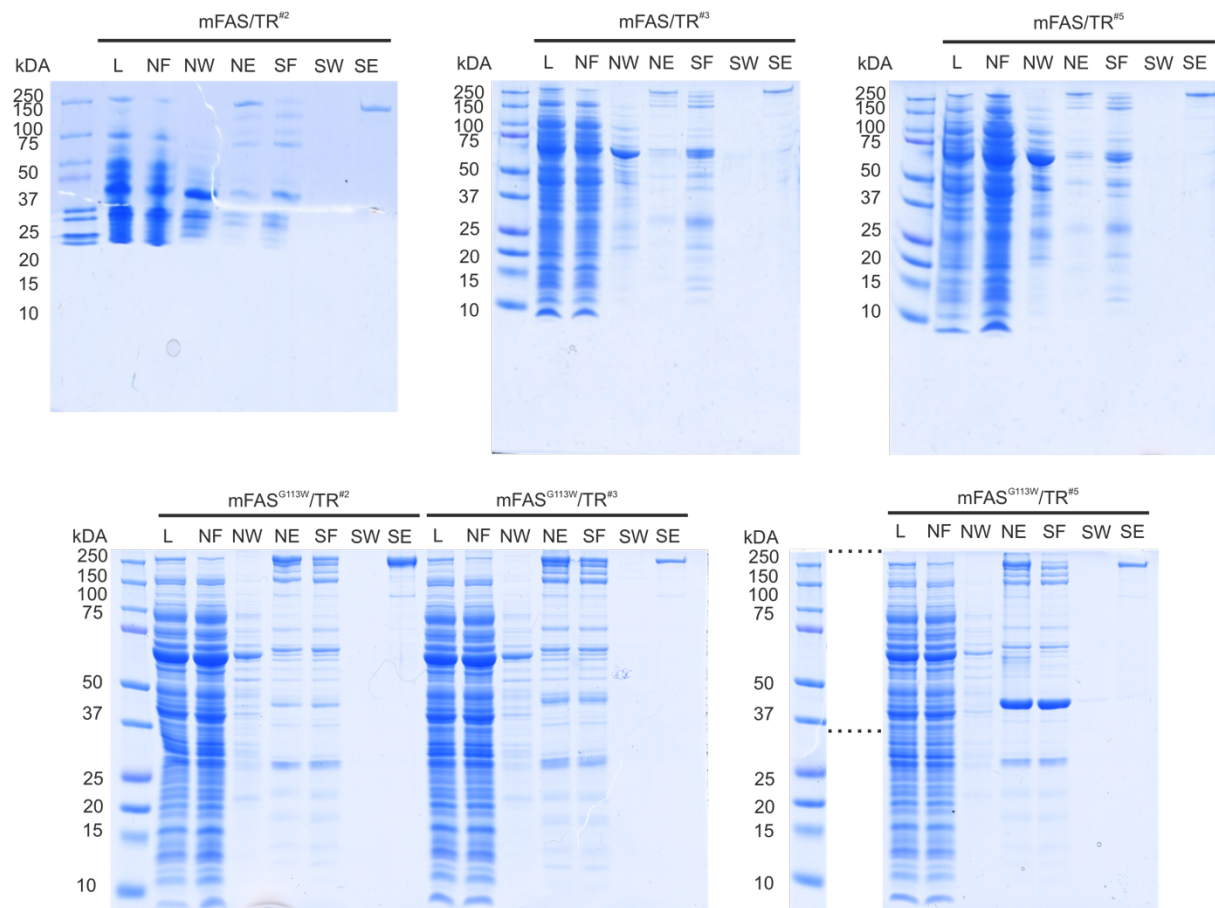
Supplementary Figure 18: SDS-PAGE of TR-domains. A 10% Schagger-gel was used. Samples are lysate (L), flowthrough (FT), wash (NW) and elution from the nickel-column (NE). Apparent molecular weights of target proteins agree with the calculated weights. All proteins are pure after the nickel-chelating column, as judged from NE lanes.



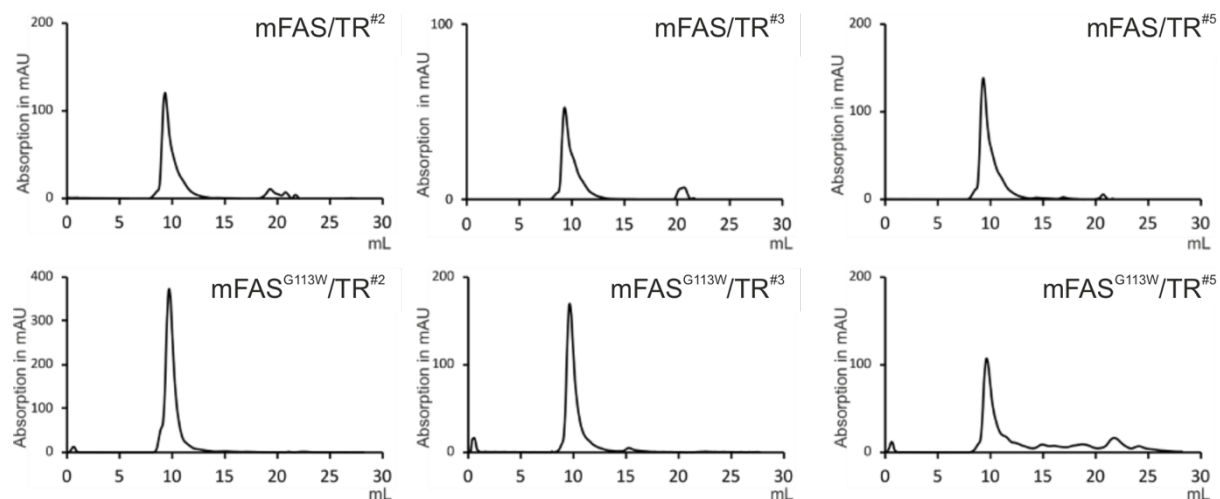
Supplementary Figure 19: Interaction of carrier protein with TR^{CAR}. **a** PCP-TR^{CAR} from *Segniliparus rugosus* with docked PCP and phosphopantetheine extending into the binding pocket (PDB ID: 5msv) in superposition with the *M. smegmatis* PCP-TR^{CAR} AlphaFold3 model. Structures align with rmsd of 0.85 Å. **b** Vacuum electrostatics indicated for PCP of modeled *M. smegmatis* PCP-TR^{CAR} (right/top), for *S. rugosus* PCP-TR^{CAR} (middle) and mFAS ACP modeled to the PCP atomic coordinates of *M. smegmatis* PCP-TR^{CAR} AlphaFold3 model with Modeller (bottom). Different surface potentials indicate a potential mismatch in polarity between the mFAS ACP and the TR interface and suboptimal electrostatic complementarity.

	ACP α 4	10	20	30	TE α 1
mFAS	MSSKTDSATDTTA	- - -	PKSRSDTSLKQNQLNLSTL	-	
mFAS_R2	MSSKTDSAAATARRPGDGRPTFAGVHGDDAAEVHARD				
mFAS_R3	MSSK	- - -	AAAAQRTAGDRRPSFTTVHGADATEIRASE		
mFAS_R5	MSSKTDSAAASERDSG	-	SRPTAATVHGDDGL	-	LRADD
					TR- α 1/ β 1

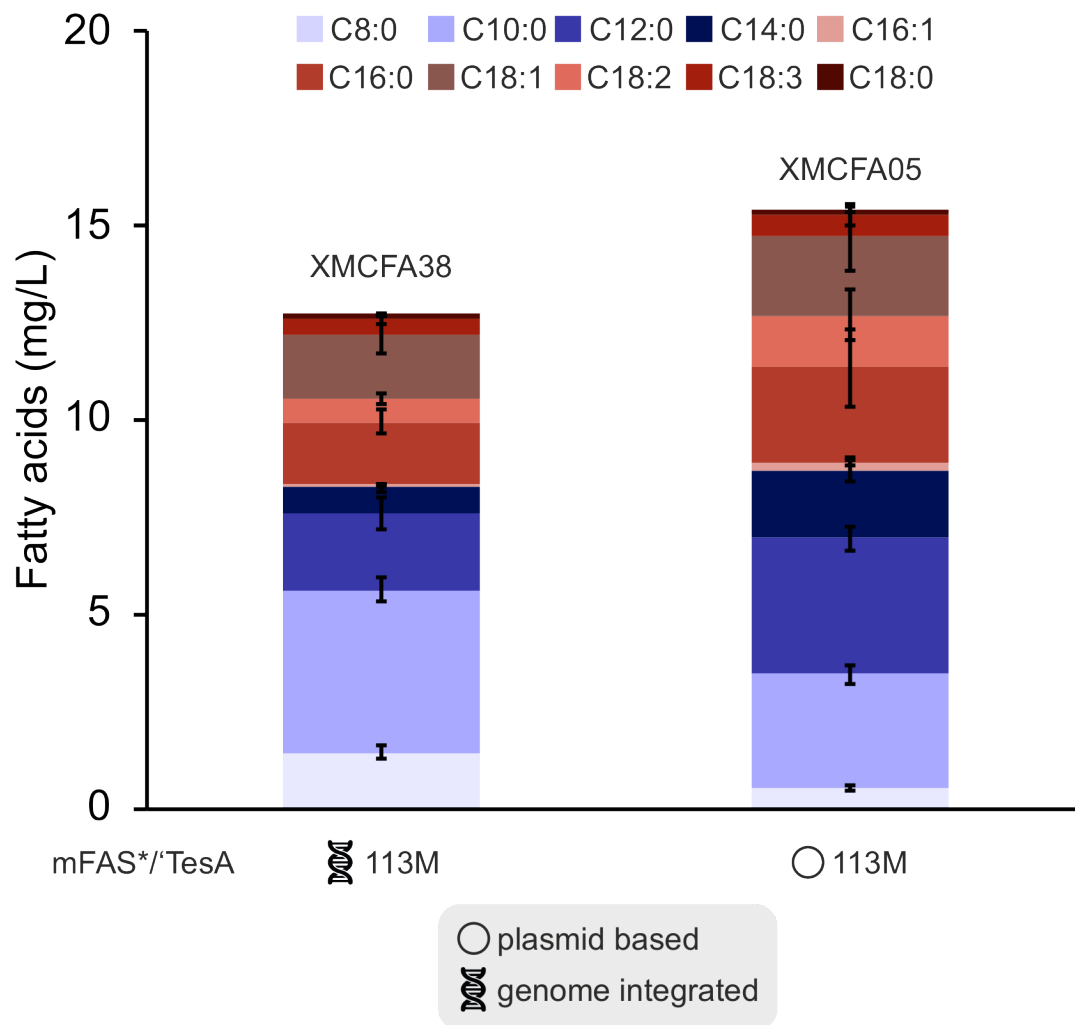
Supplementary Figure 20: Sequence alignment of linkers in mFAS_WT and mFAS/TR hybrids. Alignment was done using MAFFT webserver with default settings. C-terminal structural element of mFAS ACP (pink), N-terminal structural element of mFAS TE (brown) and N-terminal structural element TRs (purple) are highlighted. Linker lengths are 25 (mFAS), 29 (mFAS/TR^{#2}), 26 (mFAS/TR^{#3}) and 27 (mFAS/TR^{#5}).



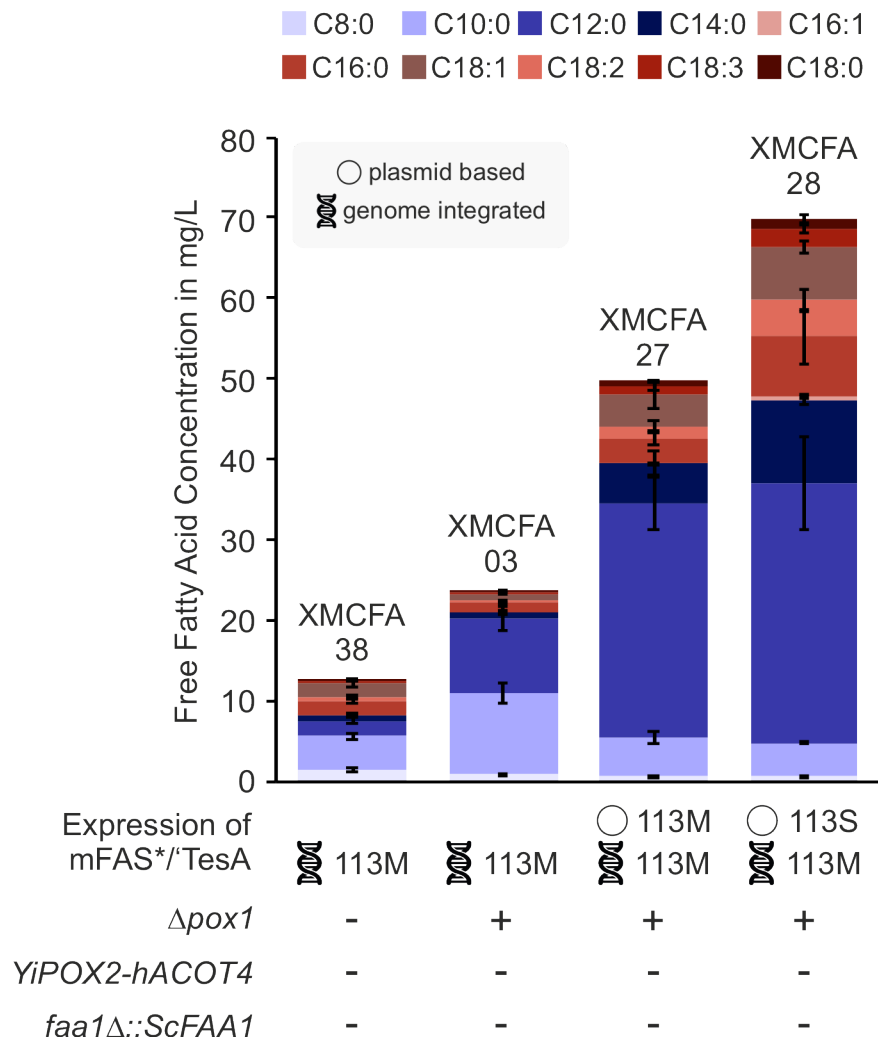
Supplementary Figure 21: SDS-PAGE for mFAS/TR hybrids. A 10% Schagger-gel was used. Samples are lysate (L), flowthrough (NF), wash (NW) and elution from the nickel-column (NE) and flowthrough (SF), wash (SW) and elution from the strep-column (SE). Apparent molecular weights of target proteins agree with the calculated weights of about 270 kDa. All proteins are pure after the STREP-tactin column, as judged from SE lanes.



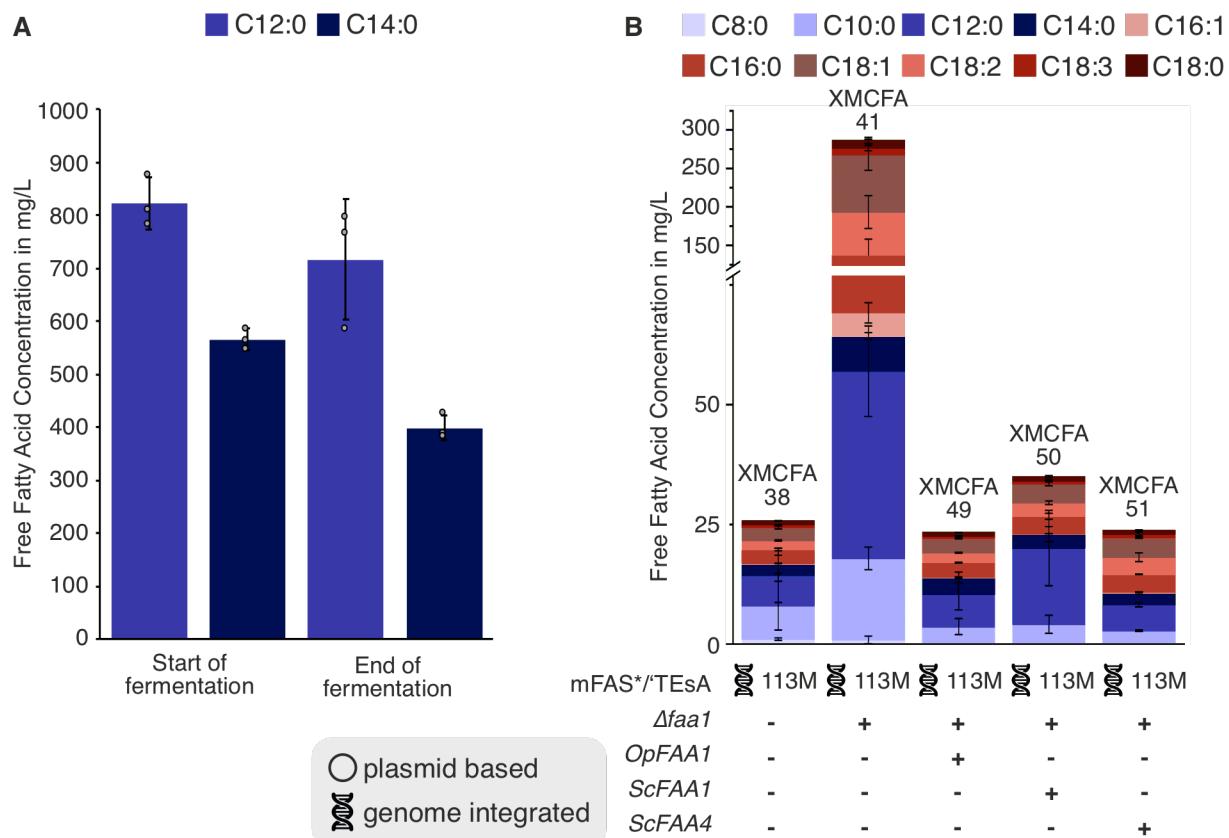
Supplementary Figure 22: Analysis of protein purity by SEC for the mFAS/TR hybrids. All proteins are pure, and mainly in dimeric form. If there was a shoulder present – indicating the monomer – only dimeric fractions were used.



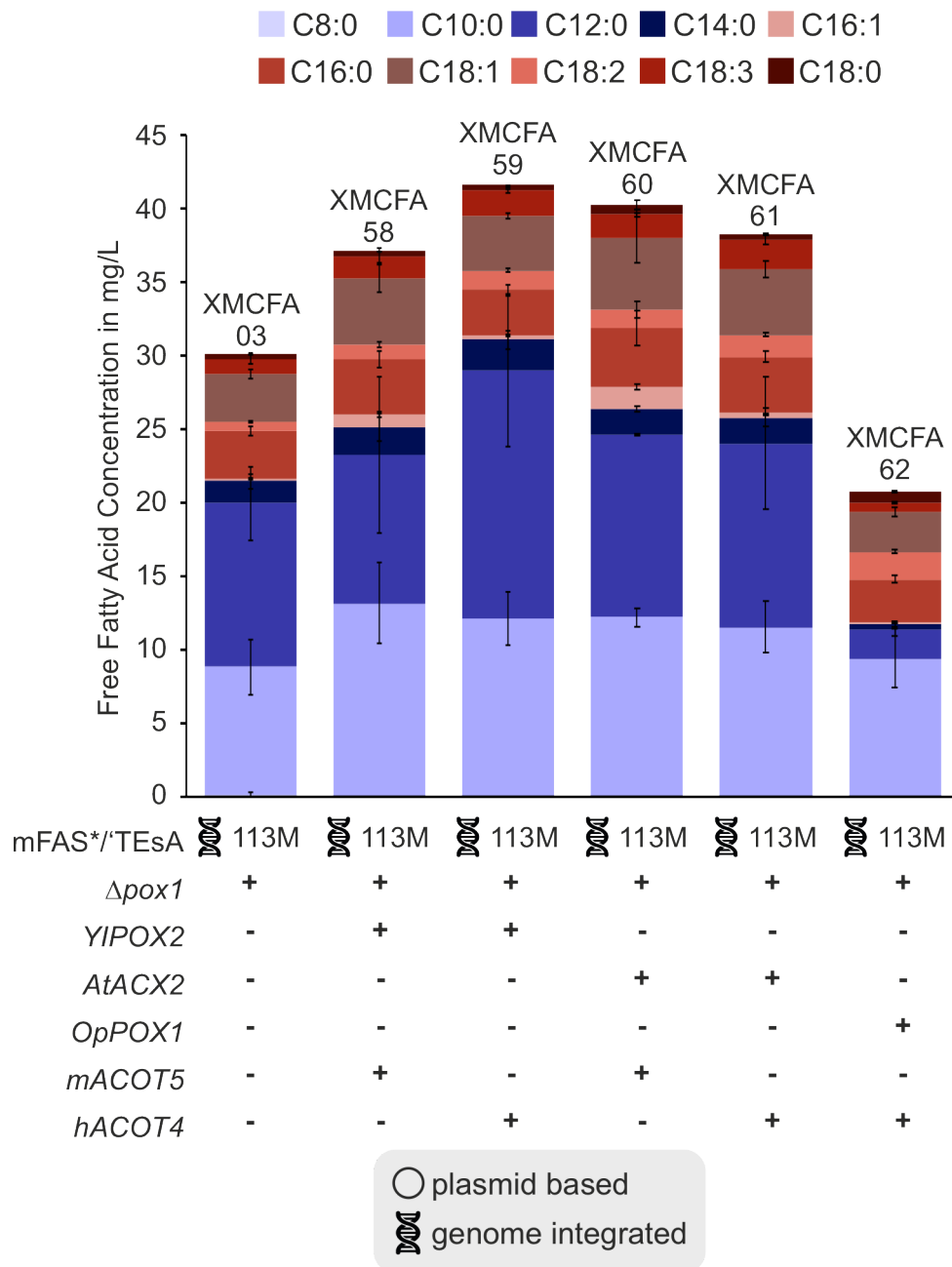
Supplementary Figure 23: Production of free FAs by using mFAS^{G113M}/TesA hybrids with different overexpression forms. Production of free FAs by a *O. polymorpha* strain with mFAS^{G113M}/TesA hybrid. mFAS^{G113M}/TesA hybrid was encoded from plasmid (XMCFA05) and genome (XMCFA38), respectively. *O. polymorpha* strains were cultivated in minimal medium containing 20 g/L glucose. All data presented as the mean $\pm$ SD of three yeast clones.



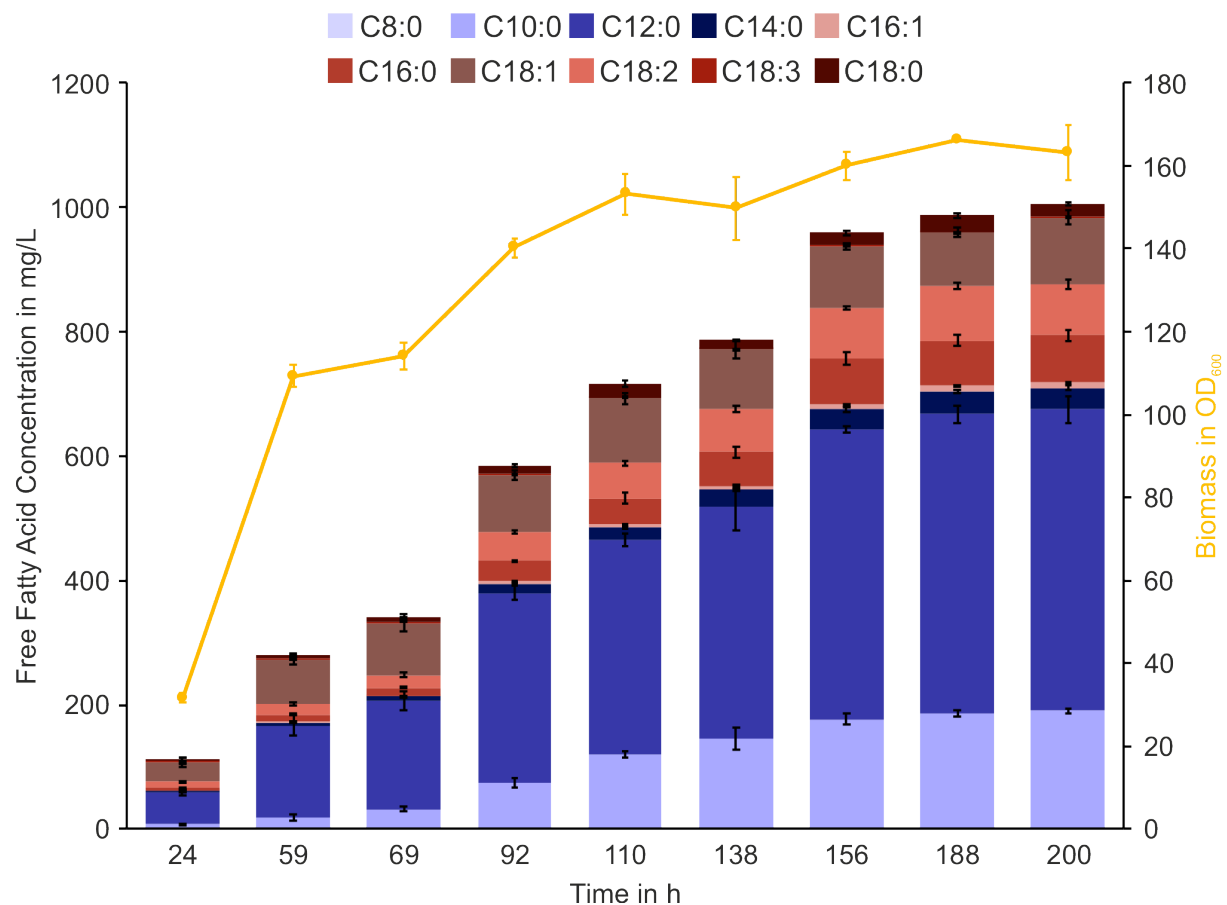
Supplementary Figure 24: Blocking β -oxidation for production of free fatty acids by *O. polymorpha* strains with mFASG113M/TesA integrated in the genome. The latter three strains carry a POX1 knockout to reduce loss of FAs due to β -oxidation, and the latter two strains (XMCFA27 and XMCFA28) have increased copy of mFAS hybrids through additional plasmid-based expression of mFASG113S/TesA and mFASG113M/TesA, respectively. All data presented as the mean $\pm$ SD of three yeast clones.



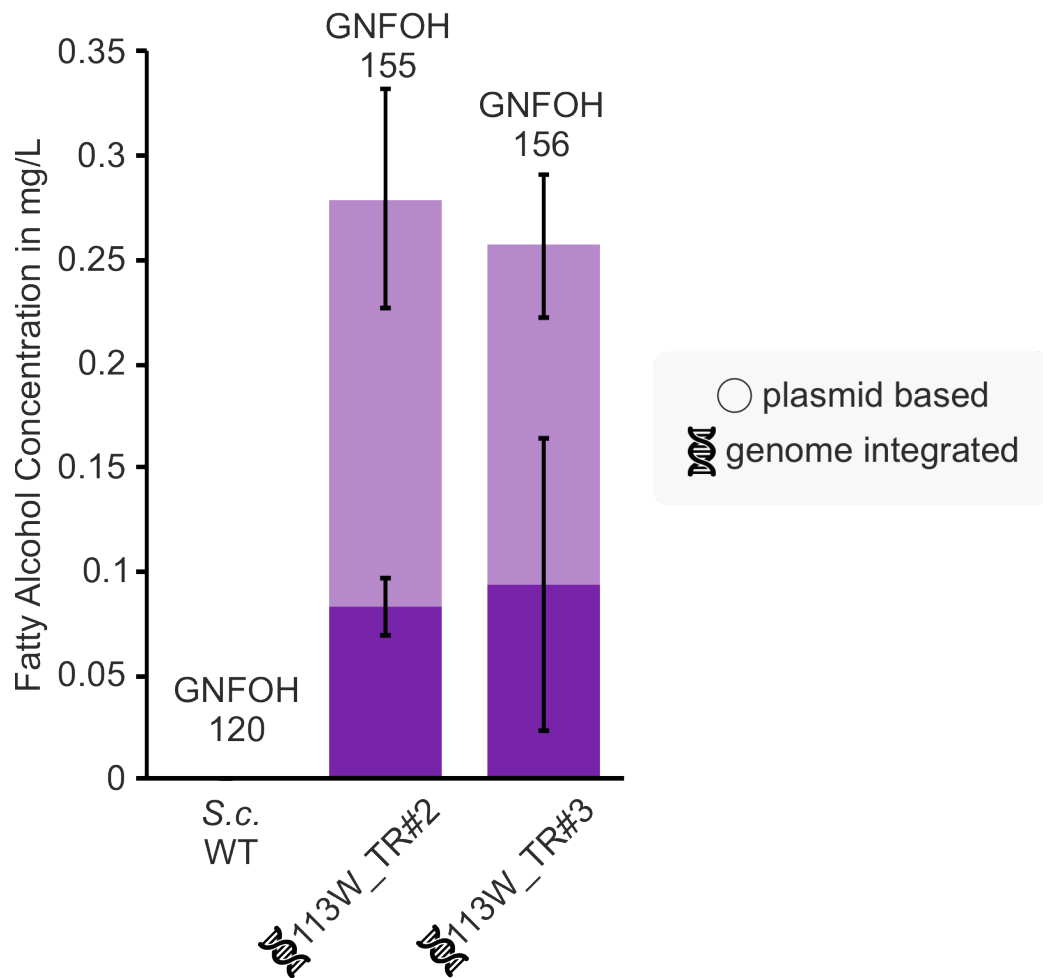
Supplementary Figure 25: Screening long-chain fatty acyl-CoA synthases. a Consumption of C12/C14 FAs by wildtype *O. polymorpha* strain. **b** Replacing endogenous *FAA1* with other fatty acyl-CoA synthase genes from *S. cerevisiae* (*ScFAA1* and *ScFAA4*) in engineered *O. polymorpha* strain with genome integrated mFAS^{G113M}/T_{ES}A. The latter four strains carry a *FAA1* knockout to reduce loss of FAs due to β -oxidation, with three strains harboring different fatty acyl-CoA synthases through genome-based expression of *OpFAA1*, *ScFAA1* and *ScFAA4*, respectively. *O. polymorpha* strains were cultivated in minimal medium containing 20 g/L glucose. All data presented as the mean $\pm$ SD of three yeast clones.



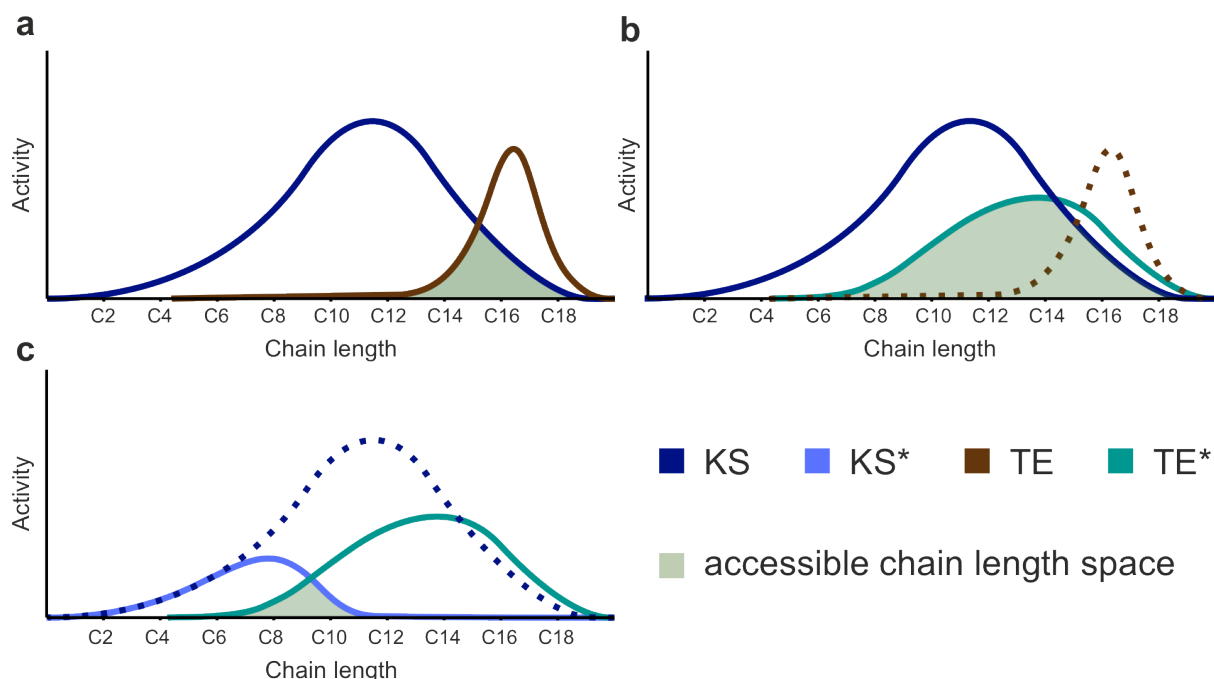
Supplementary Figure 26: Optimizing the combination of various fatty acyl-CoA oxidases and thioesterases. The *O. polymorpha* strains all harbor the mFAS^{G113M}/TEsA encoding gene integrated in the genome and the POX1 gene knockout. The latter five strains have introduced in genome different combinations of genes encoding fatty acyl-CoA oxidase (YIPOX2, AtACX2 and OpPOX1) and thioesterase (mACOT5 and hACOT4) through genome-based expression. *O. polymorpha* strains were cultivated in minimal medium containing 20 g/L glucose. All data presented as the mean $\pm$ SD of three yeast clones.



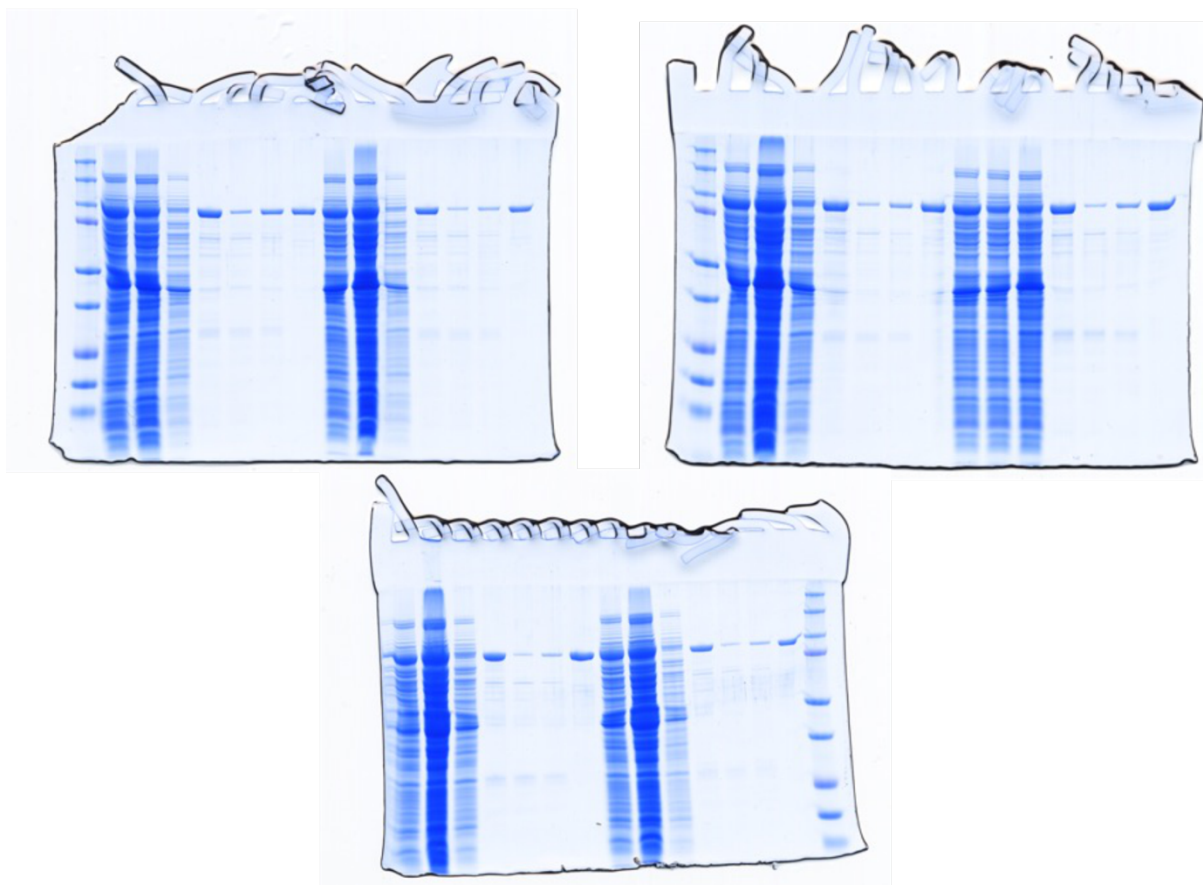
Supplementary Figure 27: Fed-batch fermentation of strain XMCFA69 in 1.5 L bioreactors. Depicted are the FFA fractions and the biomass at different timepoints. All data is the mean from three biological replicates and the depicted errors are the corresponding SD.



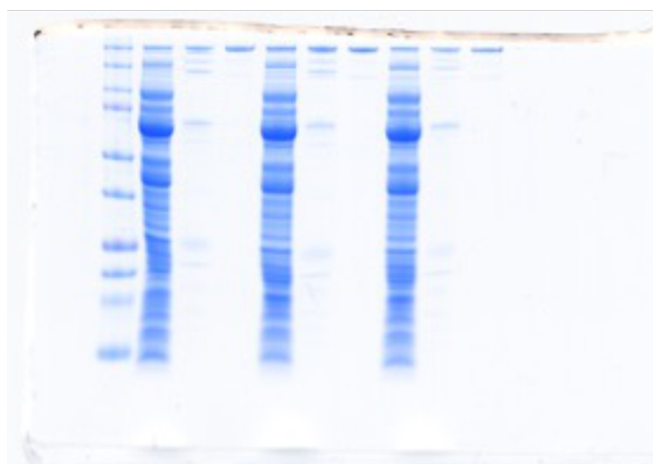
Supplementary Figure 28: Production of fatty alcohols by *S. cerevisiae* strains. The strains express the hybrids mFAS mFASG113W/TR#2 and mFAS mFASG113W/TR#3, respectively. O. p. and S. c. strains were cultivated in minimal medium containing 20 g/L glucose. All data presented as the mean $\pm$ SD of three yeast clones.



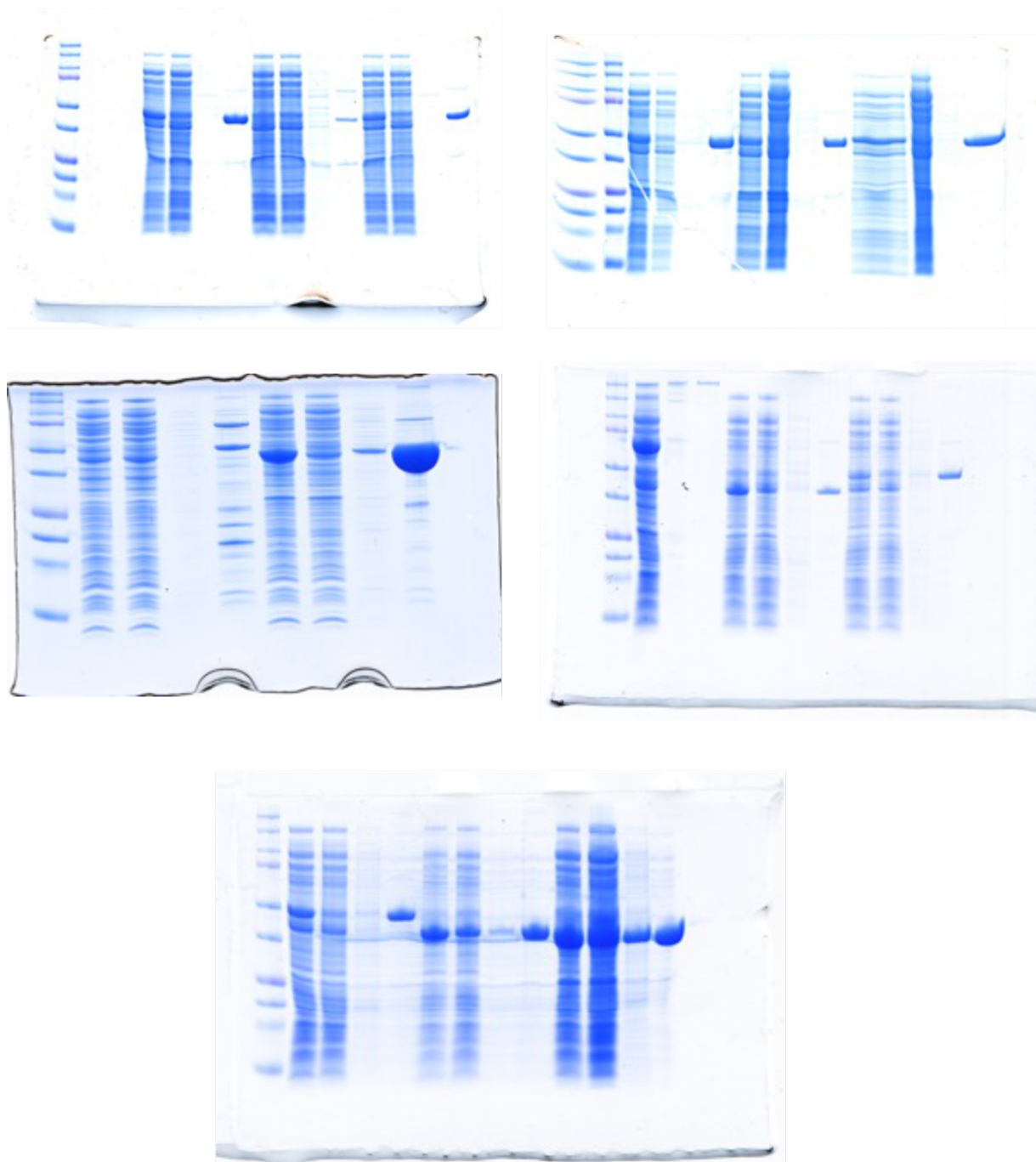
Supplementary Figure 29: Conceptual representation of the putative kinetic behavior of the interplay between KS and TE. Graphs display estimated activities of KS and TE domains for saturated acyl-ACP across different chain lengths, which will also be referred to as specificity in the following. The intersection reflects the product output spectrum. **(a)** Scheme illustrates the kinetic scenario of wildtype mFAS with its domains KS and TE. KS (dark blue) and TE (brown) reach similar peak activities, although at different chain length; KS at C12 and TE at C16. At chain length of C12, the KS-mediated elongation is dominant, while the low activity of TE at C12 prevents the TE to intercept chain extension. There is an inverse situation at C16, meaning that the KS activity is decreased at increased TE activity. Here, the TE outcompetes chain extension leading to C16-FA as main product. The competition for the acyl chain shifts further in favor of the termination reaction by the TE domain for C18 chain length, reflecting the long chain length limit of the wildtype mFAS **(b)** A thioesterase domain (termed TE*) with termination activity at C14 and generally broadened specificity replaces the native mFAS TE. The domains KS and TE share activity for a broad range of chain length; thus they are able to both act on a range of acyl-ACPs, leading to a broadened spectrum. This scenario reflects the generalist mFAS/TesA **(c)** The generalist mFAS/TesA' exhibits susceptibility to the output chain length when modulated by KS kinetics. The chain length output spectra are shifted towards shorter chains, when the KS of the generalist mFAS/TesA is engineered to decrease elongation activities for chains longer chains (indicated by KS*). Since elongation is hampered at longer chain length, the intersection is narrowed and product output shifted to FAs of shorter chain lengths. In this work, mFAS/TesA with KS mutations G113S/F/W exhibit shifted FA output, with the situation depicted in **(c)** illustrating variant mFAS^{G113W}/TesA.



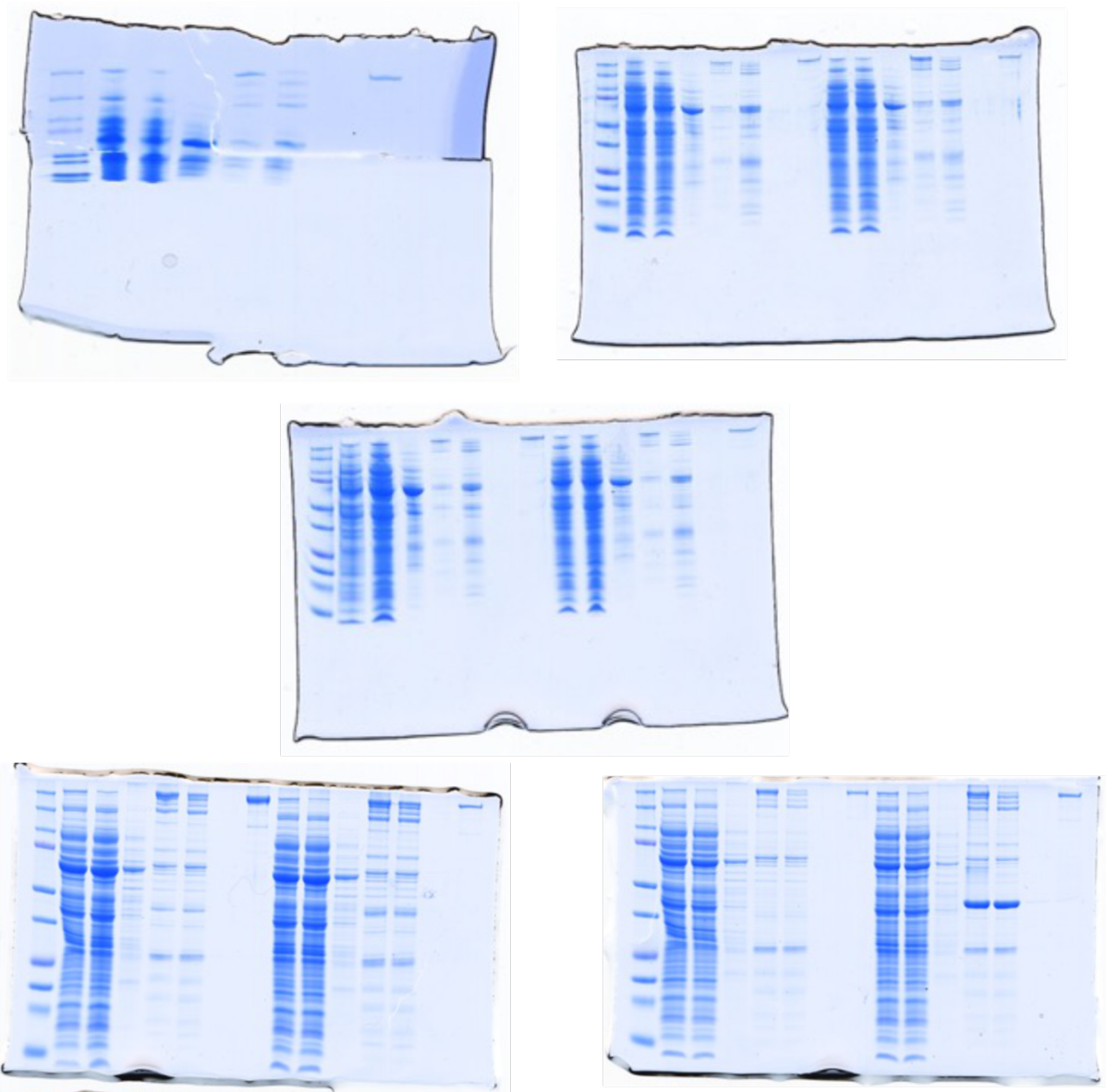
Supplementary Figure 30: Uncropped SDS-Page gels from supplementary figure 6.



Supplementary Figure 31: Uncropped SDS-Page gels from supplementary figure 6.



Supplementary Figure 32: Uncropped SDS-Page gels from supplementary figure 18.



Supplementary Figure 33: Uncropped SDS-Page gels from supplementary figure 23.

Supplementary Methods:

Synthesis of decanoyl-CoA ester

Decanoyl-CoA ester was prepared as done by *Gusenda et al.*³⁴

Shortly, the synthesis was performed under argon-atmosphere. 202 mg decanoic acid (1.17 mmol, 6 eq) was dissolved in 6 mL abs. THF and cooled to 0 °C. 90 µL ethyl chloroformate (1.16 mmol, 5.9 eq) and 160 µL triethylamine (TEA) (1.15 mmol, 5.9 eq) were added to the solution and stirred for 45 min at 0 °C. The reaction mixture was transferred to a 2 mL Eppendorf-tube and centrifuged for 5 min at 20000 x g. The supernatant was transferred to a solution of 150 mg CoA (0.195 mmol, 1 eq) in 6 mL 0.1 M NaHCO₃ and stirred for 1 h at RT. The solution was divided into two parts and each fraction was poured into 25 mL -20 °C cold acetone. The mixtures were centrifuged for 5 min at 10000 x g and the supernatant was discarded. The crude product was dried under reduced pressure and stored at -20 °C until further purification.

Isolation of the product was performed using a reverse phase (RP) liquid chromatography (LC) column with C18-RP silica gel (part. size 48-65) from Thermo Scientific and MeOH/H₂O 1:1 as eluent. The collected fractions were combined and the organic solvent removed under reduced pressure.

Yield: 45% as white solid.

Synthesis of C10-ACP

C10-ACP was biosynthesized as done by *Gusenda et al.*³⁴

Shortly, SFP, C10-CoA and apo-ACP were mixed in 5 mL buffer (50 mM Tris, 10 mM MgCl₂, pH 7.5) to final concentrations of: SFP (15 µM, 0.05 eq), C10-CoA (1500 µM, 5 eq) and apo-ACP (300 µM, 1 eq). The reaction mixture was incubated for 10 min at 37 °C and 300 x g and the reaction progress was checked via HPLC analysis. Therefore, 5 µL sample were mixed with 25 µL 0.1% TFA solution and injected to the Discovery® BIO Wide Pore C5 LC-column from Supelco and eluted with a H₂O + 0.1%TFA/ACN + 0.1%TFA gradient (20-98 % in 16 min). The reaction mixture was poured onto a Strep Tactin XT column, washed with 3 CV Strep-Wash buffer and eluted with 2.5 CV Strep-Elution buffer. The elution fraction was rebuffed to ACP-buffer (50 mM potassium phosphate, 200 mM KCl, 1 mM EDTA, 10% (v/v) glycerol, pH 7.0) and concentrated using Amicon® Ultra Centrifugal filters from Merck with a 10 kDa cut-off.

Lists:

Supplementary Table 2: Table of TR domains screened for this work. Given is the internal number, the enzyme class, the molecular weight, the type of reduction and their native organism.

Short form	Plasmid number	Enzyme class	Molecular weight in kDa	Type [e ⁻]	Organism
#1	pAR388	CAR	50.9	2	<i>Mycobacterium marinum</i>
#2	pAR389	CAR	51.0	2	<i>Mycobacterium phlei</i>
#3	pAR390	CAR	50.5	2	<i>Mycobacterium smegmatis</i>
#4	pAR391	CAR	50.6	2	<i>Nocardia iowensis</i>
#5	pAR392	CAR	50.7	2	<i>Nocardia otitidiscaviarum</i>
#6	pAR393	CAR	50.4	2	<i>Tsukamurella paurometabola</i>
#7	pMMH12	CAR	46.6	2/4	<i>Neurospora crassa</i>
#8	pAR394	PKS/NRPS	44.8	4	<i>Stigmatella aurantiaca</i>
#9	pAR395	PKS/NRPS	51.6	4	<i>Mycobacterium tuberculosis</i>
#10	pAR396	PKS/NRPS	52.8	4	<i>Mycobacterium smegmatis</i>
#11	pMMH05	NRPS	48.8	2/4	<i>Methanobrevibacter ruminantium</i>

Supplementary Table3: Cloning strategies and primers (purchased from Sigma Aldrich) for plasmids created in this study. Plasmids were constructed using In-Fusion Cloning (Takara). The DNA encoding for the TR domains was amplified from their genomic DNA and introduced into a pET-22b (+) vector. Other plasmids were derived from plasmids prepared by us and collaboration partners in earlier projects.

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'/Restriction Enzymes	Template
pAR388 (TR ^{CAR} _Mmar)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR38 8_I	PrAR648	CATATGGCGGCCGCGAGCTGCTAGAA AGCCAGGTTC	Mmar_CAR_ SFP(AKBoles)
			PrAR649	GATGAAGCTTACCTCCCAACAAACC CAACAATCTCAAGTC	
pAR389 (TR ^{CAR} _Mphi)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR38 9_I	PrAR650	CATATGGCGGCCGCAACCGCGCGC CGACCAG	Genomic DNA DSM: 43239
			PrAR651	GATGAAGCTTACCTCCGAGCAGCCC GAGCAGC	
pAR390 (TR ^{CAR} _Msme)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 0_I	PrAR652	CATATGGCGGCCGCGAGCGCAGCGC ACCGCG	Genomic DNA DSM: 43756
			PrAR653	GATGAAGCTTACCTCCGATCAGACC GAACTCACGC	
pAR391 (TR ^{CAR} _Niow)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 1_I	PrAR654	CATATGGCGGCCGCGAGCGGAACGC AACTCGGG	Genomic DNA DSM: 45107
			PrAR655	GATGAAGCTTACCTCCGAGCAGCTG AAGCAGTTCC	
pAR392 (TR ^{CAR} _Noti)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 2_I	PrAR656	CATATGGCGGCCGCAAGCGAACGC GATTCCGGC	Genomic DNA DSM: 43242
			PrAR657	GATGAAGCTTACCTCCGCCCTGAAT CAACCCAG	

pAR393 (TR ^{CAR} _Tpau)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 3_I	PrAR658	CATATGGCGGCCGCAAAGGCTCGAT CGGGTGGC	Genomic DNA DSM: 20162
			PrAR659	GATGAAGCTTACCTCCGTCGCGCGC ACCGGG	
pAR394 (TR ^{PKS/NRPS} _Saur)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 4_I	PrAR660	CATATGGCGGCCGCGAGCCGCGAAG ACAGGCTC	Genomic DNA DSM: 17044
			PrAR661	GATGAAGCTTACCTCCCCTCACCTC GGGTGCCTTG	
pAR395 (TR ^{PKS/NRPS} _Mtub)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 5_I	PrAR662	CATATGGCGGCCGCGACGAGATGCC CGACCGAC	Genomic DNA
			PrAR663	GATGAAGCTTACCTCCCAGCAGTCC GAGCAGTTGTAG	
pAR396 (TR ^{PKS/NRPS} _Msme)	InFusion	pAR3X X_V	PrAR646	GGAGGTAAGCTTCATCATCACCACC ACCACC	pAR18
			PrAR647	TGCGGCCGCCATATGTATATCTCCT TCTTAAAG	
		pAR39 6_I	PrAR664	CATATGGCGGCCGCGACACGACGCC GGTGAGCG	Genomic DNA DSM: 43756
			PrAR665	GATGAAGCTTACCTCCCAGCAGCCC GAGCAGCTG	
pAR401 (mFAS/TR ^{CAR} _Msme)	restriction enzyme	pAR40 1_V		Not1-HF, HindIII-HF	pAR387
		pAR40 1_I		Not1-HF, HindIII-HF	pAR390
pAR435 (mFAS/"TesA)	InFusion	pAR43 5_V	PrAR202	CTCGAGCATCATCACCACCAC	pAR18
			PrAR706	TTGGTTCTGCTTCAGAGACG	
		pAR43 5_I	PrAR729	CTGAAGCAGAACCAAGCCGCCGCA GCG	Genomic DNA <i>E. Coli</i> , selfmade
			PrAR730	GTGATGATGCTCGAGTGAGTCATGA TTACTAAAGGCTG	
pAR436 (mFAS/"TesA_ L109P)	InFusion	pAR43 6_1	PrAR729	CTGAAGCAGAACCAAGCCGCCGCA GCG	pAR435
			PrAR728	GTTTGCAGGCGGACGTATTTGCATT AACAAATGGTTC	

		pAR43 6_2	PrAR727	GCAAATACGTCCGCCTGCAAACATAT GGTCG	
			PrAR730	GTGATGATGCTCGAGTGAGTCATGA TTTACTAAAGGCTG	
pAR440 (mFAS ^{G113M})	InFusion	pAR44 0_1	PrAR321	TGGGTGGGTGTGAGTATKTCAGAGG CATCCGAGGCC	AR18
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pAR44 0_2	PrAR26	GAGGACCGAAGGAGCTAACC	AR18
			PrAR322	ACTCACACCCACCCAGACG	
pAR442 (mFAS ^{G113F})	InFusion	pAR44 2_1	PrAR323	TGGGTGGGTGTGAGTTWTTTCAGAG GCATCCGAGGCC	AR18
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pAR44 2_2	PrAR26	GAGGACCGAAGGAGCTAACC	AR18
			PrAR322	ACTCACACCCACCCAGACG	
pAR445 (mFAS ^{G113W})	InFusion	pAR44 5_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	AR18
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pAR44 5_2	PrAR26	GAGGACCGAAGGAGCTAACC	AR18
			PrAR322	ACTCACACCCACCCAGACG	
pDL007 (mFAS ^{G113M} /TesA)	InFusion	pDL007 _1	PrAR321	TGGGTGGGTGTGAGTATKTCAGAGG CATCCGAGGCC	pAR435
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL007 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR435
			PrAR322	ACTCACACCCACCCAGACG	
pDL008 (mFAS ^{G113F} /TesA)	InFusion	pDL008 _1	PrAR323	TGGGTGGGTGTGAGTTWTTTCAGAG GCATCCGAGGCC	pAR435
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL008 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR435
			PrAR322	ACTCACACCCACCCAGACG	
pDL010 (mFAS ^{G113M} /TesA _L109P)	InFusion	pDL010 _1	PrAR321	TGGGTGGGTGTGAGTATKTCAGAGG CATCCGAGGCC	pAR436
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL010 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR436
			PrAR322	ACTCACACCCACCCAGACG	
pDL011 (mFAS ^{G113F} /TesA _L109P)	InFusion	pDL011 _1	PrAR323	TGGGTGGGTGTGAGTTWTTTCAGAG GCATCCGAGGCC	pAR436
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL011 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR436
			PrAR322	ACTCACACCCACCCAGACG	
pDL013	InFusion		PrDL007	GCGACTCCTGCATTAGGAAG	pAR435

		pDL013 _1	PrDL014	GACCTCTTCCATAAAAAAGGGCAG	
		pDL013 _1	PrDL013	TTTATGGAAGAGGTCGGCAAGAAGC CACAATGGATGCAGGATG	pAR435
			PrDL008	CTTCCTAATGCAGGAGTCGC	
pDL017 (mFAS/"TesA_4x)	InFusion	pDL017 _1	PrDL15	CTGCTGCCCTTTTTTcTGGAtGAGGT CggCaagAAGC	pDL013
			PrDL10	AGTCAATTCAGGGTGGTGAATG	
		pDL017 _1	PrDL09	CACCTGAATTGACTCTCTTC	pDL013
			PrDL16	AAAAAAGGGCAGCAGCG	
pDL018 (mFAS ^{G113M} /"TesA_4x)	InFusion	pDL018 _1	PrAR321	TGGGTGGGTGTGAGTATKTCAGAGG CATCCGAGGCC	pDL017
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL018 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pDL017
			PrAR322	ACTCACACCCACCCAGACG	
pDL019 (mFAS ^{G113F} /"TesA_4x)	InFusion	pDL019 _1	PrAR323	TGGGTGGGTGTGAGTTWTTTCAGAG GCATCCGAGGCC	pDL017
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL019 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pDL017
			PrAR322	ACTCACACCCACCCAGACG	
pDL021 (mFAS ^{G113S} /"TesA)	InFusion	pDL021 _1	PrDL19	TGGGTGGGTGTGAGTTCGTCAGAG GCATCCGAGGCC	pAR435
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL021 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR435
			PrAR322	ACTCACACCCACCCAGACG	
pDL026 (mFAS ^{G113W} /"TesA)	InFusion	pDL026 _1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pAR435
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL026 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR435
			PrAR322	ACTCACACCCACCCAGACG	
pDL027 (mFAS ^{G113S})	InFusion	pDL027 _1	PrDL19	TGGGTGGGTGTGAGTTCGTCAGAG GCATCCGAGGCC	pAR18
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL027 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR18
			PrAR322	ACTCACACCCACCCAGACG	
pDL028 (mFAS ^{G113S} /"TesA_L109P)	InFusion	pDL028 _1	PrDL19	TGGGTGGGTGTGAGTTCGTCAGAG GCATCCGAGGCC	pAR436
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL028 _2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR436
			PrAR322	ACTCACACCCACCCAGACG	

pDL029 (mFAS ^{G113S} /TesA_4x)	InFusion	pDL029_1	PrDL19	TGGGTGGGTGTGAGTTCGTCAGAG GCATCCGAGGCC	pDL017
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL029_2	PrAR26	GAGGACCGAAGGAGCTAACC	pDL017
			PrAR322	ACTCACACCCACCCAGACG	
pDL030 (mFAS ^{G113W})	InFusion	pDL030_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pAR18
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL030_2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR18
			PrAR322	ACTCACACCCACCCAGACG	
pDL031 (mFAS ^{G113W} /TesA_L109P)	InFusion	pDL031_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	s
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL031_2	PrAR26	GAGGACCGAAGGAGCTAACC	pAR436
			PrAR322	ACTCACACCCACCCAGACG	
pDL032 (mFAS ^{G113W} /TesA_4x)	InFusion	pDL031_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pDL017
			PrAR27	GGTTAGCTCCTTCGGTCCTC	
		pDL031_2	PrAR26	GAGGACCGAAGGAGCTAACC	pDL017
			PrAR322	ACTCACACCCACCCAGACG	
pMMH01 (mFAS/TR ^{CAR} _Mphi)	InFusion	pMMH01_1	PrMMH01	CCGAAAGGAAGCTGAGTTGGC	pAR18
			PrMMH02	CGCGGTTGCGGCCGCCGAGTCAGT CTTGGAGGACATTC	
		pMMH01_2	PrMMH03	GCGGCCGCAACCGCG	pAR389
			PrMMH04	CTCAGCTTCCTTCGGGCTTTGTTA G	
pMMH02 (mFAS/TR ^{CAR} _Noti)	InFusion	pMMH02_1	PrMMH01	CCGAAAGGAAGCTGAGTTGGC	pAR18
			PrMMH05	TTCGCTTGCGGCCGCCGAGTCAGTC TTGGAGGACATTC	
		pMMH02_2	PrMMH06	GCGGCCGCAAGCGAAC	pAR392
			PrMMH04	CTCAGCTTCCTTCGGGCTTTGTTA G	
pMMH05 (TR ^{PKS/NRPS} _Mrum)	InFusion	pMMH05_V	PrMMH13	TTTGATTATTTGAAAAGCTTCATCA TCACCACCACC	pAR390
			PrMMH14	CTCTTCGCCAATGTCGGCCGCCATA TGTATATCTCCTTC	
		pMMH05_I	PrMMH11	GACATTGGCGAAGAGGACTTGG	Genomic DNA DSM: 1093

			PrMMH1 2	TTCAAAATAATCAAACCTATTCAAATA ATCAAACAATCG	
pMM07 (mFAS ^{G113W} TR ^{CAR} _Mphi)	InFusion	pMMH0 7_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pAR420
			PrMMH0 4	CTCAGCTTCCTTTCGGGCTTTGTTA G	
		pMMH0 7_2	PrAR322	ACTCACACCCACCCAGACG	pAR420
			PrMMH0 1	CCGAAAGGAAGCTGAGTTGGC	
pMMH08 (mFAS ^{G113W} TR ^{CAR} _Noti)	InFusion	pMMH0 8_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pAR422
			PrMMH0 4	CTCAGCTTCCTTTCGGGCTTTGTTA G	
		pMMH0 8_2	PrAR322	ACTCACACCCACCCAGACG	pAR422
			PrMMH0 1	CCGAAAGGAAGCTGAGTTGGC	
pMMH10 (mFAS ^{G113W} TR ^{CAR} _Msme)	InFusion	pMMH1 0_1	PrAR324	TGGGTGGGTGTGAGTTKGTCAGAG GCATCCGAGGCC	pAR401
			PrMMH0 4	CTCAGCTTCCTTTCGGGCTTTGTTA G	
		pMMH1 0_2	PrAR322	ACTCACACCCACCCAGACG	pAR401
			PrMMH0 1	CCGAAAGGAAGCTGAGTTGGC	
pMMH12 (TR ^{CAR} _Ncra)	InFusion	pMMH1 2_V	PrMMH3 1	AAGCTTCATCATCACCACCACC	pAR390
			PrMMH3 2	GGCCGCCATATGTATATCTCCTTC	
		pMMH1 2_I	PrMMH2 0	ATACATATGGCGGCCAGGGCACC CTGGACAATG	NcCAR_R_domain_Nt erm_10xHisTag (AK Winkler)
			PrMMH2 1	GTGATGATGAAGCTTAAAGTTCCATT GGCGACACCAG	
pHp-32	Gibson Assembl y	P5/P2	P5	TTTATGATGAGTAAATGATGTTTT ATTGTGTGAATAGGTG	pTaFAR1 (Ref. 4)
			P2	TTTATGATGAGTAAATGATGTTTT ATTGTGTGAATAGGTG	pTaFAR1 (Ref. 4)
		P565/P 530	P565	GCTCTTTAGTACAAAGATAATATAGA AACAAAATGTCTGCCGGCGCTGGCT C	pmFAS ^{G113M} _Op ^{OPT1}
			P530	CGATGGGGCAGGGTCCGATG	pmFAS ^{G113M} _Op ^{OPT1}
		P531/P 566	P531	CAGTGGAAACCCATCGGACCCTGCC CCATCGTCGTTGGGCGCCTTGGA CTG	pmFAS ^{G113M} _Op ^{OPT1}
			P566	CAATAAAAACATCATTTTACTCATCA TAAATTACGAGTCGTGGTTCACCAG TG	pmFAS ^{G113M} _Op ^{OPT1}

pHp-33	Gibson Assembly	P5/P537	P5	TTTATGATGAGTAAATGATGTTTT ATTGTGTGAATAGGTG	pHp-32
			P537	GACAGGGCCTCCGAGGCCTCCGAg aCGACACGCCCACCCACACGCCGG TG	pHp-32
		P569/P530	P569	GCGTGTGGGTGGGCGTGTCTtcTCG GAGGCCTCGGAGGCCC	pHp-32
			P530	CGATGGGGCAGGGTCCGATG	pHp-32
		P531/P566	P531	CAGTGAACCCATCGGACCCTGCC CCATCGTCGTTGGGCGCCTTGGA CTG	pHp-32
			P566	CAATAAAAACATCATTCTACTCATCA TAAATTACGAGTCGTGGTTCACCAG TG	pHp-32
pOpMCFA-01	Gibson Assembly	P5/079	P5	TTTATGATGAGTAAATGATGTTTT ATTGTGTGAATAGGTG	pHp-32
			079	CGACACGCCCACCCACACGC	pHp-32
		081/P530	081	GCGTGTGGGTGGGCGTGTCTcgTC GGAGGCCTCGGAGGC	pHp-32
			P530	CGATGGGGCAGGGTCCGATG	pHp-32
		P531/P566	P531	CAGTGAACCCATCGGACCCTGCC CCATCGTCGTTGGGCGCCTTGGA CTG	pHp-32
			P566	CAATAAAAACATCATTCTACTCATCA TAAATTACGAGTCGTGGTTCACCAG TG	pHp-32

Supplementary Table 3: Cloning strategies and primers for expression cassettes created in this study. Plasmids were constructed using either In-Fusion Cloning (Takara), Gibson Assembly, overlap extension polymerase chain reaction (OE-PCR) and restriction enzyme cloning and labeled accordingly in the tables.

Expression cassette	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'/Restriction Enzymes	Template
dOp-5-up	SOE-PCR	NS18up	p286	GGCCGAGAAGTATAACGTCAGACC	Genome of <i>O. polymorpha</i>
			p648	AGTCACACACCATATCCGAGCAG	
		P _{GAP} -mFAS ^G _{113M} -Te sA-up	p649	GGAAGCTCTGCTCGGATATGGTGTGTG ACTCCAATTATCATTAATAATCACTCAT GATCCCTGC	pHp-32
			p650	CGGTGTGAACCAGCAGGAAGC	
dOp-5-dn	SOE-PCR	mFAS ^G _{113M} -Te sA-dn-T _{ADH21}	p651	GCAAGCTGCTGTGGAAGGACAAC	pHp-32
			p652	GGCCCCGTCATTGTTTGAAAAACGCG AAACAGAATTATGTACTGATGCTGCTG GATAC	

		NS18dn	p653	TTTCGCGTTTTTTCAAACAATGACGGG	Genome of <i>O. polymorpha</i>
			p314	GCTGGATATGGCAGACAATAAATACTCAC	
dOpMCFA-07	SOE-PCR	NS2up	001	GAGAACGACTGACAACGTGCTAC	Genome of <i>O. polymorpha</i>
			002	AATAATTTATGGCTTCTGCGGAGAGAGTGCTGTAGTTGTGATTTTAATGCACACTTGAG	
		P _{ADH2-1-629}	003	CACTCTCTCCGCAGAAGCCATAAATTTTG	
			004	TTTAAATTGATTGATTGATTGATAGAAGGATGCCTAAG	
		SFP	054	TCCTTCTATCAATCAATCAATCAATTTAAATGAAGATTTACGGAATTTATATGGACCG	SFP-Op ^{opt1}
			055	TTACCTATTTACCTATTCAAATACCACCTTTATAAAAGCTCTTCGTACGAGACCA TTG	
		T _{FBA}	018	AGGTGGTATTTTGAATAGGTAAATAGGTAAATCAAC	Genome of <i>O. polymorpha</i>
			019	GCCTTCGGATATTTTCAGTTATGTGCGAC	
		NS2dw	020	CAACGTGACATAACTGAAATATCCGAAGGCGTTGCTGGTGCCATCTTTTGGC	
			021	CACTTCAAATTTGCTACCACTGAACTGG	
Donor SFP	SOE-PCR	IX-2up	P373(I X-2upF)	GGGGAAGTAAAGCACAAGGATTTGAAG	Genome of <i>S. cerevisiae</i>
			P431(GAL2 p-IX-2upR)	CCCCTGGTCCGTAAACCTCCTTGATTAGCCTTCCATCGGGATTACTAAGATCTCAAG	
		P _{GAL2}	P432(GAL2 p-F)	CTAATCCAAGGAGGTTTACGGACCAG	
			P429(GAL2 p-R)	TATGAAAGAATTATTTTTTTTATTATGTTAATCTTGTGTTTACTTAAC	
		SFP	P476(SFP-F)	ACATAATAAAAAAATAATTCTTTCATAATGAAGATTTACGGAATTTATATGGACCGCC	pCDF-1b-SFP
			P477(SFP-R)	TAAAAATCATAAATCATAAGAAATTCGCTTATAAAAGCTCTTCGTACGAGACCATTGTG	
		T _{ADH1}	P104	GCATATCTACAATTGGGTGAAATGGGG	Genome of <i>S. cerevisiae</i>
			P105	GCGAATTTCTTATGATTTATGATTTTTATT	
		IX-2dw	P394(ADH1t-IX-2dwF)	CGCTCCCCATTTACCCAATTGTAGATATGCCACTGCAGCTCTGCTTTGAAAAC	
			P382(I X-2dwR)	CCTGGTTTAGTTATCTGTAATGGTGGG	
Donor mFAS ^{G113W} _MphiR	SOE-PCR	X-3up	P351(X-3upF)	GCGCCTTTGATCCTTCCCC	Genome of <i>S. cerevisiae</i>
			P438(GAL2)	TCCCCTGGTCCGTAAACCTCCTTGATTAGGCCCGAGAAACGCAAATGC	

			p-X-3upR)		pMMH07
		P _{GAL2}	P432(GAL2 p-F)	CTAATCCAAGGAGGTTTACGGACCAG	
			P429(GAL2 p-R)	TATGAAAGAATTATTTTTTTTATTATGTT AATCTTGTGTTTACTTAAC	
		mFAS ^G _{113W_M} phiR up	P469(FAS1-F)	TAACATAATAAAAAAATAATTCTTTTCAT AATGTCCGAGGAGGTGGTGATAGCC	
			P470	CACTTTTCCGCTCACAATCAGATTG	
		mFAS ^G _{113W_M} phiR mid	P471	CACTGGCAATCTGATTGTGAGCG	
			P472	GGCCCATTTACCTGGAATGGC	
		mFAS ^G _{113W_M} phiR dw	P473	TCCCCTGATGCCATTCCAGG	
			P474	GTTGTGTGCTAGTGTCTCCCGTCTTCT GTTTAACCTCCGAGCAGCCCG	
		T _{PRM9}	P247(PRM9t -F)	ACAGAAGACGGGAGACACTAGC	Genome of <i>S. cerevisiae</i>
			P248(PRM9t -R)	ATTTTCAACATCGTATTTTCCGAAGCG	
		X-3dw	P404(PRM9t -X-3dwF)	AACGCTTCGGAAAATACGATGTTGAAA ATCCCCGCGTAAATACGCGG	
			P361(X-3dwR)	GCAGTAAATGCCGCGCTTAC	
Donor mFAS ^{G113W} _MsmeR	SOE-PCR	X-3up	P351(X-3upF)	GCGCCTTTGATCCTTCCCC	Genome of <i>S. cerevisiae</i>
			P438(GAL2 p-X-3upR)	TCCCCTGGTCCGTAAACCTCCTTGGAT TAGGCCCCGAGAAACGCAAATGC	
		P _{GAL2}	P432(GAL2 p-F)	CTAATCCAAGGAGGTTTACGGACCAG	
			P429(GAL2 p-R)	TATGAAAGAATTATTTTTTTTATTATGTT AATCTTGTGTTTACTTAAC	
		mFAS ^G _{113W_Ms} meR up	P469(FAS1-F)	TAACATAATAAAAAAATAATTCTTTTCAT AATGTCCGAGGAGGTGGTGATAGCC	pMMH10
			P470	CACTTTTCCGCTCACAATCAGATTG	
		mFAS ^G _{113W_Ms} meR mid	P471	CACTGGCAATCTGATTGTGAGCG	
			P472	GGCCCATTTACCTGGAATGGC	
			P473	TCCCCTGATGCCATTCCAGG	
			P475(FAS2-R)	GTTGTGTGCTAGTGTCTCCCGTCTTCT GTTTAACCTCCGATCAGACCGAACTC	
		T _{PRM9}	P247(PRM9t -F)	ACAGAAGACGGGAGACACTAGC	Genome of <i>S. cerevisiae</i>

		X-3dw	P404(PRM9t -X-3dwF)	AACGCTTCGGAAAAATACGATGTTGAAA ATCCCCGCGTAAATACGCGG	
			P361(X-3dwR)	GCAGTAAATGCCGCGCTTAC	
dOpMCFA-30(ScFAA1)/ dOpMCFA-31(ScFAA4)	SOE-PCR	4NS5up	040	GAGTCTCCGAGCGCAGACAAC	Genome of <i>O. polymorpha</i>
			181	CTAATTATGGCAACGGTGTCTTTAGGG CTACTCAGATATCGTCTCCAACGACCA C	
		P _{TEF1}	012	TAGCCCTAAAGACACCGTTGCC	
			011	TTTGTACGAATGTACTAATTTAAAGATC AGAAAAAATCTCATGTGG	
		ScFAA1/ ScFAA4	184/ 186	CTGATCTTTTAATTAGTACATTTCGTACA AAATGGTTGCTCAATATACCGTTCCAG/ CTGATCTTTTAATTAGTACATTTCGTACA AAATGACCGAACAATATTCGTTGCAG	Genome of <i>S. cerevisiae</i>
			185/ 187	CTTATTCAGTTAGCTAGCTGAGCTCGA GATATTAAGACGAACTATAAACGGCGT CAAC/ CTTATTCAGTTAGCTAGCTGAGCTCGA GATATTAAGTGTCTTTCTTTATAAACTCTT TCCACATCTGG	
		T _{YX212}	007	TATCTCGAGCTCAGCTAGCTAACTG	Genome of <i>O. polymorpha</i>
			022	CCATTCGCCATTCAGGCTGC	
		4NS5dw	044	CAACAGTTGCGCAGCCTGAATGGCGA ATGGCTCCTCTGGGCTGCTGTGGT	
			045	GTTGATCAAACGGGAATCCCC	
dOpMCFA-42	SOE-PCR	P _{FAA1}	265	GTACCGCTTAATGATCGAGGACTG	Genome of <i>O. polymorpha</i>
			267	AGTGATTTACGATCTGCAATAGTAGAA AATAGAGG	
		ScFAA1	263	TATTTTCTACTATTGCAGATCGTAAATC ACTATGGTTGCTCAATATACCGTTCCAG	Genome of <i>S. cerevisiae</i>
			264	GATCTTATGTGATACATACTTTGAAATC ATTAAGACGAACTATAAACGGCGTCAAC	
		T _{FAA1}	268	TGATTTCAAAGTATGTATCACATAAGAT CTAATAATAATCTACAG	Genome of <i>O. polymorpha</i>
			269	GTGCCGAAGTCATTGAGACAGTC	
donor YIPOX2-mACOT5	SOE-PCR	3NS3up	204	CCGCCTATTGACAAAATTCCGC	Genome of <i>O. polymorpha</i>
			219	CTAATTATGGCAACGGTGTCTTTAGGG CTACAAGAACAATAAAGCCAAATCCTT CAC	
		P _{TEF1}	012	TAGCCCTAAAGACACCGTTGCC	
			011	TTTGTACGAATGTACTAATTTAAAGATC AGAAAAAATCTCATGTGG	
		YIPOX2	220	CTGATCTTTTAATTAGTACATTTCGTACA AAATGAACCCAAACAACACCGG	pYIPOX2-Op ^{OPT1}
			221	CTTTGCCTGAGACAACTTAACTGCTGC AGAACCACCACTCCTCGTCCAGCTC GCAGATG	

		<i>mACO</i> <i>T5</i>	222	GGTGGTGGTTCTGCAGCAGTTAAGTTG TCTCAGGCAAAGGTGCCAACCGTGTCG CTGGAG	pmACOT5-Op ^{OPT1}
			223	CTCAAAAGCGGTATGTCCTTCCACGTC TCCTTACAGCTTGGCAGGCGATGG	
		<i>T_{AOX}</i>	205	GGAGACGTGGAAGGACATACCG	Genome of <i>O. polymorpha</i>
			206	CACTCGCTGGCCGGAACGCTCAAGTC GCTTCGCTGTGATGAAGCATACAACCT G	
		3NS3d w	207	GAAGCGACTTGAGCGTTCCG	
			208	GGGGTTCTTGTGTCAAGTGTATTGTAC	
donor YIPOX2-hACOT4 AtACX2-mACOT5 AtACX2-hACOT4 OpPOX1- mACOT5 (Based on donor YIPOX2-mACOT5, different fragments labeled with the same color are combined)		AtACX 2	226	CTGATCTTTTAATTAGTACATTTCGTACA AAATGGAGTCGAGAAGAGAGAAGAAC C	pAtACX2-Op ^{OPT1}
			227	CTTTGCCTGAGACAACTTAAGTCTGC AGAACCACCACCGAAGCCCACCACTTG GGTGATC	
		hACOT 4	224	GGTGGTGGTTCTGCAGCAGTTAAGTTG TCTCAGGCAAAGTCGGCCACCCTGATC CTGGAG	phACOT4-Op ^{OPT1}
			225	CTCAAAAGCGGTATGTCCTTCCACGTC TCCTTACAGCTTGGCAGGCGCGG	
		OpPOX 1	228	CTGATCTTTTAATTAGTACATTTCGTACA AAATGGACTCGACTATGCCAGAAGTG	Genome of <i>O. polymorpha</i>
			229	CTTTGCCTGAGACAACTTAAGTCTGC AGAACCACCACCTAATTTCGCAAAGT CTGGCTCGTC	

Supplementary Table 4: Table of strains and their genotypes used in this study.

Strain	Gene type	Resource
JQcr03L	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2</i>	Ref. 4
JQcr03L(SFP)	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}) =JQcr03L+NS2::(P_{ADH2-1-629}-SFP-T_{FBA})</i>	this study
XMCFA20	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); P_{GAP}-mFAS^{G113S}-T_{esA}-T_{ADH} =JQcr03L(SFP)+P_{GAP}-mFAS^{G113S}-T_{esA}-T_{ADH}</i>	this study
XMCFA05	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH} =JQcr03L(SFP)+P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}</i>	this study
XMCFA06	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); P_{GAP}-mFAS^{G113F}-T_{esA}-T_{ADH} =JQcr03L(SFP)+P_{GAP}-mFAS^{G113F}-T_{esA}-T_{ADH}</i>	this study
XMCFA38	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); NS18::(P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}) =JQcr03L(SFP)+NS18::(P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH})</i>	this study
XMCFA03	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); NS18::(P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}); pox1Δ =XMCFA38+pox1Δ</i>	this study
XMCFA41	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); NS18::(P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}); faa1Δ =XMCFA38+faa1Δ</i>	this study
XMCFA27	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); NS18::(P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}); pox1Δ; P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH} =XMCFA03+P_{GAP}-mFAS^{G113M}-T_{esA}-T_{ADH}</i>	this study
XMCFA28	<i>J MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-T_{URA3}); OpLEU2; NS2::(P_{ADH2-1-629}-SFP-T_{FBA}); NS18::(P_{GAP}-mFAS^{G113S}-T_{esA}-T_{ADH}); pox1Δ; P_{GAP}-mFAS^{G113S}-T_{esA}-T_{ADH} =XMCFA03+P_{GAP}-mFAS^{G113S}-T_{esA}-T_{ADH}</i>	this study
XMCFA50	<i>MATa; ura3Δ; T_{AOX1}::(P_{GAP}-hCAS9-T_{AOX1}); ku80Δ; NS5::(P_{TKL1}-ScSAE2-</i>	this study

	<i>T_{URA3}</i> ; <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>faa1Δ</i> ; <i>4NS5::</i> (<i>P_{TEF1-ScFAA1-T_{YX212}}</i>) = XMCFA41+4NS5:: (<i>P_{TEF1-ScFAA1-T_{YX212}}</i>)	
XMCFA51	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>faa1Δ</i> ; <i>4NS5::</i> (<i>P_{TEF1-ScFAA4-T_{YX212}}</i>) = XMCFA41+4NS5:: (<i>P_{TEF1-ScFAA4-T_{YX212}}</i>)	this study
XMCFA58	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-YIPOX2-mACOT5-T_{AOX}}</i>) = XMCFA03+3NS3:: (<i>P_{TEF1-YIPOX2-mACOT5-T_{AOX}}</i>)	this study
XMCFA59	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-YIPOX2-hACOT4-T_{AOX}}</i>) = XMCFA03+3NS3:: (<i>P_{TEF1-YIPOX2-hACOT4-T_{AOX}}</i>)	this study
XMCFA60	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-AtACX2-mACOT5-T_{AOX}}</i>) = XMCFA03+3NS3:: (<i>P_{TEF1-AtACX2-mACOT5-T_{AOX}}</i>)	this study
XMCFA61	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-AtACX2-hACOT4-T_{AOX}}</i>) = XMCFA03+3NS3:: (<i>3NS3::</i> (<i>P_{TEF1-AtACX2-hACOT4-T_{AOX}}</i>))	this study
XMCFA62	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-OpPOX1-hACOT4-T_{AOX}}</i>) = XMCFA03+3NS3:: (<i>3NS3::</i> (<i>P_{TEF1-OpPOX1-hACOT4-T_{AOX}}</i>))	this study
XMCFA65	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-YIPOX2-hACOT4-T_{AOX}}</i>); <i>FAA1::</i> ScFAA1 = XMCFA59+FAA1::ScFAA1	this study
XMCFA69	<i>MATa</i> ; <i>ura3Δ</i> ; <i>T_{AOX1}::</i> (<i>P_{GAP-hCAS9-T_{AOX1}}</i>); <i>ku80Δ</i> ; <i>NS5::</i> (<i>P_{TKL1-ScSAE2-T_{URA3}}</i>); <i>OpLEU2</i> ; <i>NS2::</i> (<i>P_{ADH2-1-629-SFP-T_{FBA}}</i>); <i>NS18::</i> (<i>P_{GAP-mFAS^{G113M}-T_{ES}A-T_{ADH}}</i>); <i>pox1Δ</i> ; <i>3NS3::</i> (<i>P_{TEF1-YIPOX2-hACOT4-T_{AOX}}</i>); <i>FAA1::</i> ScFAA1; <i>P_{GAP-mFAS^{G113S}-T_{ES}A-T_{ADH}}</i> = XMCFA65+ P_{GAP-mFAS^{G113S}-T_{ES}A-T_{ADH}}	this study
GNFOH120	<i>MATa</i> ; <i>MAL2-8c</i> ; <i>SUC2</i> ; <i>hfd1Δ</i> ; <i>gal80Δ</i> ; <i>gal1Δ</i> ; <i>gal7Δ</i> ; <i>his3Δ::</i> (<i>HIS3-T_{ENO2}</i>)+(P _{ADH2-PEX28-T_{HIS3}}); <i>ura3Δ::</i> (P _{TPH1-RtFAS1-T_{FBA1}})+(P _{TEF1-RtFAS2-T_{CYC1}})+ <i>amdSym</i> ; <i>X1-5::</i> P _{TEF1-Cas9-T_{CYC1}} ; <i>acc1::</i> P _{TEF1-ACC1} ; <i>pyc1::</i> P _{TEF1-PYC1} ; <i>XI-4::</i> (P _{TPH1-MPC1})+(P _{PGK1-MPC3-T_{DIT1}}); <i>X-2::</i> (P _{GAL1-AnACLa-T_{CYC1}})+(P _{GAL10-AnACLa-T_{ADH1}}); <i>gal10Δ::</i> (P _{TPH1-RtCIT1-T_{FBA1}})+(P _{TDH3-IDP2-T_{CYC1}})+(P _{TEF1-YHM2-T_{GAL1}}); <i>pgi1Δ::</i> (P _{COX9-PGI1})+(P _{TDH3-GND1-T_{CYC1}})+(P _{HXT7-TKL1-T_{TDH2}})+(P _{PGK1-TAL1-T_{ADH1}})+(P _{TEF1-ZWF1}); <i>idh2Δ::</i> P _{GSY1-IDH2} ; <i>XII-4::</i> (P _{ADH6-per1ADH5-T_{ADH1}})+(P _{ADH6-per2MaFAR1-T_{CYC1}}); <i>VII-2::</i> (T _{ADH1-per1ADH5-P_{GAL1,10-per2MaFAR1-T_{CYC1}}}); <i>VIII-2::</i> (P _{BDH2-per1ADH5-T_{ADH1}})+(P _{BDH2-per2MaFAR1-T_{CYC1}}); <i>XI-2::</i> (P _{HXT7-PXA1-T_{ADH1}})+(P _{HXT7-PXA2-T_{FBA1}}); <i>XI-6::</i> (T _{CPS1-per2PYC1-P_{GAL1,10-per2RtME-T_{PRM9}}}); <i>XI-8::</i> (T _{CPS1-IDP3-P_{GAL1,10-per2IDP2-T_{PRM9}}})	Ref. 5
GNFOH152	<i>MATa</i> ; <i>MAL2-8c</i> ; <i>SUC2</i> ; <i>hfd1Δ</i> ; <i>gal80Δ</i> ; <i>gal1Δ</i> ; <i>gal7Δ</i> ; <i>his3Δ::</i> (<i>HIS3-T_{ENO2}</i>)+(P _{ADH2-PEX28-T_{HIS3}}); <i>ura3Δ::</i> (P _{TPH1-RtFAS1-T_{FBA1}})+(P _{TEF1-RtFAS2-T_{CYC1}})+ <i>amdSym</i> ; <i>X1-5::</i> P _{TEF1-Cas9-T_{CYC1}} ; <i>acc1::</i> P _{TEF1-ACC1} ; <i>pyc1::</i> P _{TEF1-PYC1} ; <i>XI-4::</i> (P _{TPH1-MPC1})+(P _{PGK1-MPC3-T_{DIT1}}); <i>X-2::</i> (P _{GAL1-AnACLa-T_{CYC1}})+(P _{GAL10-AnACLa-T_{ADH1}}); <i>gal10Δ::</i> (P _{TPH1-RtCIT1-T_{FBA1}})+(P _{TDH3-IDP2-T_{CYC1}})+(P _{TEF1-YHM2-T_{GAL1}}); <i>pgi1Δ::</i> (P _{COX9-PGI1})+(P _{TDH3-GND1-T_{CYC1}})+(P _{HXT7-TKL1-T_{TDH2}})+(P _{PGK1-TAL1-T_{ADH1}})+(P _{TEF1-ZWF1}); <i>idh2Δ::</i> P _{GSY1-IDH2} ; <i>XII-4::</i> (P _{ADH6-per1ADH5-T_{ADH1}})+(P _{ADH6-per2MaFAR1-T_{CYC1}}); <i>VII-2::</i> (T _{ADH1-per1ADH5-P_{GAL1,10-per2MaFAR1-T_{CYC1}}}); <i>VIII-2::</i> (P _{BDH2-per1ADH5-T_{ADH1}})+(P _{BDH2-per2MaFAR1-T_{CYC1}}); <i>XI-2::</i> (P _{HXT7-PXA1-T_{ADH1}})+(P _{HXT7-PXA2-T_{FBA1}}); <i>XI-6::</i> (T _{CPS1-per2PYC1-P_{GAL1,10-per2RtME-T_{PRM9}}}); <i>XI-8::</i> (T _{CPS1-IDP3-P_{GAL1,10-per2IDP2-T_{PRM9}}}) <i>IX-2::</i> (P _{GAL2-SFP-T_{ADH1}}) = GNFOH120+ IX-2:: P_{GAL2-SFP-T_{ADH1}}	this study
GNFOH155	<i>MATa</i> ; <i>MAL2-8c</i> ; <i>SUC2</i> ; <i>hfd1Δ</i> ; <i>gal80Δ</i> ; <i>gal1Δ</i> ; <i>gal7Δ</i> ; <i>his3Δ::</i> (<i>HIS3-T_{ENO2}</i>)+(P _{ADH2-PEX28-T_{HIS3}}); <i>ura3Δ::</i> (P _{TPH1-RtFAS1-T_{FBA1}})+(P _{TEF1-RtFAS2-T_{CYC1}})+ <i>amdSym</i> ; <i>X1-5::</i> P _{TEF1-Cas9-T_{CYC1}} ; <i>acc1::</i> P _{TEF1-ACC1} ; <i>pyc1::</i> P _{TEF1-PYC1} ; <i>XI-4::</i> (P _{TPH1-MPC1})+(P _{PGK1-MPC3-T_{DIT1}}); <i>X-2::</i> (P _{GAL1-AnACLa-T_{CYC1}})+(P _{GAL10-AnACLa-T_{ADH1}}); <i>gal10Δ::</i> (P _{TPH1-RtCIT1-T_{FBA1}})+(P _{TDH3-IDP2-T_{CYC1}})+(P _{TEF1-YHM2-T_{GAL1}}); <i>pgi1Δ::</i> (P _{COX9-PGI1})+(P _{TDH3-GND1-T_{CYC1}})+(P _{HXT7-TKL1-T_{TDH2}})+(P _{PGK1-TAL1-T_{ADH1}})+(P _{TEF1-ZWF1}); <i>idh2Δ::</i> P _{GSY1-IDH2} ; <i>XII-4::</i> (P _{ADH6-per1ADH5-T_{ADH1}})+(P _{ADH6-per2MaFAR1-T_{CYC1}}); <i>VII-2::</i> (T _{ADH1-per1ADH5-P_{GAL1,10-per2MaFAR1-T_{CYC1}}}); <i>VIII-2::</i> (P _{BDH2-per1ADH5-T_{ADH1}})+(P _{BDH2-per2MaFAR1-T_{CYC1}}); <i>XI-2::</i> (P _{HXT7-PXA1-T_{ADH1}})+(P _{HXT7-PXA2-T_{FBA1}}); <i>XI-6::</i> (T _{CPS1-per2PYC1-P_{GAL1,10-per2RtME-T_{PRM9}}}); <i>XI-8::</i> (T _{CPS1-IDP3-P_{GAL1,10-per2IDP2-T_{PRM9}}})	this study

	T_{PRM9} IX-2::($P_{GAL2-SFP-T_{ADH1}}$); X-3:: ($P_{GAL2-mFAS^{G113W}-MphiR-T_{PRM9}}$) = GNFOH152+ X-3:: $P_{GAL2-mFAS^{G113W}-MphiR-T_{PRM9}}$	
GNFOH156	MATa; MAL2-8c; SUC2; hfd1Δ; gal80Δ; gal1Δ; gal7Δ; his3Δ::($HIS3-T_{ENO2}$)+(P_{ADH2}-PEX28-T_{HIS3}); ura3Δ::(P_{TPI1-RtFAS1-T_{FBA1}})+(P_{TEF1-RtFAS2-T_{CYC1}})+amdSym; X1-5::P_{TEF1-Cas9-T_{CYC1}}; acc1::P_{TEF1-ACC1}; pyc1::P_{TEF1-PYC1}; XI-4::(P_{TPI1-MPC1})+(P_{PGK1-MPC3-T_{DIT1}}); X-2::(P_{GAL1-AnACLa-T_{CYC1}})+(P_{GAL10-AnACLa-T_{ADH1}}); gal10Δ::(P_{TPI1-RtCIT1-T_{FBA1}})+(P_{TDH3-IDP2-T_{CYC1}})+(P_{TEF1-YHM2-T_{GAL1}}); pgi1Δ::(P_{COX9-PGI1})+(P_{TDH3-GND1-T_{CYC1}})+(P_{HXT7-TKL1-T_{TDH2}})+(P_{PGK1-TAL1-T_{ADH1}})+(P_{TEF1-ZWF1}); idh2Δ:: P_{GSY1-IDH2}; XII-4:: (P_{ADH6-per1ADH5-T_{ADH1}})+(P_{ADH6-per2MaFAR1-T_{CYC1}}); VII-2:: (T_{ADH1-per1ADH5-P_{GAL1,10-per2MaFAR1-T_{CYC1}}}); VIII-2::(P_{BDH2-per1ADH5-T_{ADH1}})+(P_{BDH2-per2MaFAR1-T_{CYC1}}); XI-2::(P_{HXT7-PXA1-T_{ADH1}})+(P_{HXT7-PXA2-T_{FBA1}}); XI-6::(T_{CPS1-per2PYC1-P_{GAL1,10-per2RtME-T_{PRM9}}}); XI-8::(T_{CPS1-IDP3-P_{GAL1,10-per2IDP2-T_{PRM9}}}); IX-2::($P_{GAL2-SFP-T_{ADH1}}$); X-3:: ($P_{GAL2-mFAS^{G113W}-MsmeR-T_{PRM9}}$) = GNFOH152+ X-3:: $P_{GAL2-mFAS^{G113W}-MsmeR-T_{PRM9}}$	this study

Supplementary Table 5: Amino acid and DNA sequences of the constructs used in the FA chain length experiments. Mutated positions and the performed mutations are highlighted.

Construct	Sequence form start to stop codon
pAR18 (WT) pDL027 (G113S) pAR440 (G113M) pAR442 (G113F) pDL030 (G113W) (mouseFAS, Strep-and His- tagged) Position 113 highlighted	MSAWSHPQFEKGGGSGGSGSAWSHPQFEKGAGSEEVVIAGMSGKLPESENLQEFWANLIGGDMVTDDDR RWKAGLYGLPKRSGKLDLSKFDASFFGVHPKQAHTMDPQLRLLLEVSYEAIVDGGINPASLRGTNTGVVWGV SG/S/M/F/W SEASEALSRDPETLLGYSMVGCQRAMMANRLSFFDFKGPSIALDTACSSSLLALQNAQAI GECPAALVGGINLLKPNTSVQFMKLGMLSPDGTCSRFDSDSGSGYCRSEAVVAVLLTKKSLARRVYATILNAGT NTDGSKEQGVTFPSGEVQEQLICSLYQAGLAPESLEYIEAHGTGKVGDPQELNGITRSLCAFRQAPLLIGSTKS NMGHPEPASGLAALTAVLLSLEHGWWAPNLHFNPNPEIPALLDGRQLQVDRPLPVRGGNVGINSFGFGGSNV HVILQPNTRQAPAPTAHAALPHLLHASGRTEAVQDLEQGRQHSQDLAFVSMNLNDIAATPTAAMPFRGYTVL GVEGRVQEVQVSTNKRPLWFIGSGMGTQWRGMGLSLMRLDSFRESILRSEAVKPLGVKVSDDLSTDERTF DDIVHAFVSLTAIQIALIDLLTSVGLKPDGIHSLGEVACGYADGCLSQREAVLAAYWRGQCICKDAHLPPGSMA AVGLSWEECKQRCPAGVVPACHNSEDTVTISGPQAAVNEFVEQLKQEGVFAKEVRTGGLAFHSYFMEGIAPTL LQALKKVIREPRRSARWLSTSIPEAQWQSSLARTSSAEYNVNNLVSPVLFQEALWHIPEHAVVLEIAPHALLQ AVLKRGVKSSCTHPLMKRDHKNLEFFLTNLGKVHLTGINVPNALFPPEVPAPRGTPILSPHIKWDHSQTW DVPVAEDFPNGSSSSSATVYSIDASPEPDHYLVHDICIDGRVIFPGTGYLCLVWKTLARSLGLSLEETPVVFENV FHQATILPKTGTVALEVRLEASHAFVSDTGNLIVSGKVYLWEDPNSKLFDPHEVPTTPESASVSRLTQGEVY KELRLRGYDYGPFQFQICEATLEGEQKLLWKDNWVTFMDTMLQVSLGSSQSLQLPRTVTAIYIDPATHRQ KVYRLKEDTQVADVTTSRCLGITVSGGIHISRLQTATSSRRQEQVLVPTLEKFFVTPHMEAECLSESTALQKELQ LCKGLARALQTKATQQGLKAAMLGQEDPPQHGLPRLAAACQLQNLGNLQLELGEALAQRLLLPEDPLISGL LNSQALKACVDTALENLSTLKMKVAEVLAGEGHLYSRIPALLNTQPMQLLEYTATDRHPQALKDVQTKLQKH DVAQQQWNPSDPAPSSLGALDLLVCNCALATLGDPALALDNMVAALKEGGFLLVHTVLKGHALGETLACLPS VQPAPSLLSQEEWESLFSRKALHLVGLKRSFYGTALFLCRRAPQEKPIFLSVEDTSFQWVWDSLKSTLATSSSQP VWLTAMDCPTSGVGLVNLCKRKEPGGHRIRCILLNLSNTSHAPKLDPGSPQLQVLKHDLMNVYRDGAWG AFRHFQLEQDKPKEQTAHAFVNVLTRGDASIRWVSSPLKHTQPSSSGAQLCTVYYASLNFDRIMLATGKLS AIPGKWASRDCMLGMEFSGRDRRCRRVMGLVPAEGLATSVLLSSDFLWDVPSSWTLLEEAASVPVYTTAYYSL VVRGRIQRGETVLIHSGSGGVQAAISIALSLGCRVFTTVGSAEKRAYLQARFPQLDDTSFANSRDTSEFQHVLL HTGGKGVDLVNLSLAEEKLQASVRCLAQHGRFLEIGKFDLSNNHPLGMAIFLKNVTFHGLLDALFEEANDSW REVAALLKAGIRDGVVKPLKCTVFPKAQVEDAFRYMAQGGKHIGKVLVQVREEPEAVLPGAQPTLISAISKTC PAHKSIIITGGLGGFLELARWLVLRGARQLVLTSRSGIRTGYQAKHIREWRRQGIQVLVSTSNVSSLEGARALI AEATKLGPVGGVFNLAMVLRDAMLENQTPELFQDVNPKYNGTLNLDRTAREACPELDYFVAFSSVSCGRGN AGQTNYGANSTMERICEQRRHDGLPGLAVQWGAIGDVGIVLEAMGTNDTVIGGTLPQRISSCMEVLDLFLNQ PHAVLSSVFLAEKKAVAHGDDGTQRDLVKAVAHILGIRDLAGINLDSTLADLGLDSLGMGEVVRQILEREHDLVL PMREVRQLTLRLKLQEMSSKTDSDATDTTAPKSRSDTSLKQNLNLSTLLVNPEGPTLTQLNSVQSSERPLFLVHP IEGSTTVFHSLAAKLSVPTYGLQCTQAAPLDSIPNLAAYYIDCIKQVQPEGPYRIAGYSFGACVAFEMCSQLQAQQ GPAPTHNNLFLFDGSHTYVLAYTQSYRAKMTPGCEAAEAEALCFKIKQFLDVEHSKVL EALLPLKSLEDVAA SVDLITKSHHSLDRRELSFAAVSFYHKLRAADQYKPKAKYHGNVTLRAKTGGTYGEDLGADYNLSQVCDGKV SVHIEGDHRTLLEGSGLESINIHSLSAEPRVSVREGLEHHHHHHHHH
pAR18 (WT) pDL027 (G113S) pAR440 (G113M) pAR442 (G113F) pDL030 (G113W) (mouseFAS, Strep-and His- tagged) Position 113 highlighted	ATGAGCGCTTGAGCCATCCACAATTGAGAAGGGTGGAGGTCTGGCGGTGGATCGGGAGGTTACAGCGTGG AGCCACCCGAGTTCGAAAAAGCGCCGGATCCGAGGAGGTGGTGATAGCCGGTATGTCGGGGAAGTTGCCCG AGTCAGAGAACCTACAGGAGTCTGGGCCAACCTCATTGGTGGTGTGGACATGGTCACAGATGATGACAGGA GATGGAAGGCTGGGCTCTATGGATTACCAAGCGGTCTGGAAGCTGAAGGATCTCTCCAAGTTCGACGCCTC CTTTTGGGGTCCACCCCAAGCAGGCACACACAATGGACCCCAAGCTTCGGGCTGCTGTTGGAAGTCAGCTAT GAAGCAATTGTGGATGGAGGTATCAACCCAGCCTCACTCCGAGGAACGAACACTGGCGTCTGGGTGGGTGTGA GTGGT/TCG/ATG/TTT/TGG TACAGGCATCCGAGGCCCTTAGCAGAGATCCCGAGACGCTTCTGGGCTACA GCATGGTGGGCTGCCAGCGTGCAATGATGGCCAACCGGCTCTCTTTCTTCTTCGACTTCAAAGGACCAAGCAT TGCCCTGGACACAGCCTGCTCTCCAGCTTGTGGCACTACAGAATGCCTACCAGGCCATCCGTAGTGGGGAA TGCCCGCGGCCCTTGTGGGTGGGATCAACCTGCTCCTGAAGCCGAACACCTCTGTGCAGTTCATGAAGCTGG GCATGCTCAGCCCGACGGCACCTGCAGATCCTTTGATGATTCAGGGAGTGGATATTGTCGCTCTGAGGCTGT TGTAGCAGTTCTGCTGACTAAGAAGTCCCTGGCTCGGCGGTCTATGCCACGATTCTGAATGCCGACCAAT ACAGATGGCAGCAAGGAGCAAGGTGTAACATTCCTCTGGAGAAGTCCAAGAACAACCTATCTGCTCTCTGT ATCAGCCAGCTGGTCTGGCCCCGGAGTCGCTTGAGTATATTGAAGCCCATGGCACGGGCACCAAGTGGGTGA CCCCCAGGAAGTGAATGGCATTACTCGGTCCCTGTGCGCCTTCCGCCAGGCCCTCTGTTAATTGGCTCCACCA AATCCAACATGGGACACCCTGAGCCTGCCTCTGGGCTTGACGCCCTGACCAAGGTGCTGTTATCCCTGGAGCA TGGGGTCTGGGCCCCTAACCTGCACTTCCACAACCCCAACCTGAGATCCCAGCACTTCTTGATGGGCGGCTGC AGGTGGTTCGATAGGCCCCCTGCCTGTTCTGTGGTGGCAACGTGGGCATCAACTCATTTGGCTTCGGAGGCTCCA TGTTTCATGTATCTCCAGCCCAACACACGGCAGGCCCTGCGCCCACTGCACACAGCTGCCCCCTTCCCCATTG TGACGCCAGTGGACGCACCTTAGAGGCAGTGCAGGACCTGCTGGAACAGGCGCCGACGACAGCCAGGACCT GGCCTTTGTGAGCATGCTCAATGACATTGCGGCAACCCCTACAGCAGCCATGCCCTTCAGGGGTACACTGTG CTAGGTGTTGAGGGCCGTGTCCAAGAAGTGCAGCAAGTGTCCACCAACAAGCGCCCACTCTGGTTTACTGCT CAGGGATGGGACGCAGTGGCGCGGATGGGCTGAGCCTCATGCGCCTGGACAGCTTCGTGAGTCTATCCT CGCTCCGATGAGGCTGTGAAGCCGTGGGAGTGAAAGTGTGAGATCTGCTGTTGAGCACAGATGAGCGCACC TTTGATGACATCGTGCATGCCTTTGTGAGCCTCACTGCCATCCAGATTGCCCTCATCGACCTACTGACTTCTGT

Construct	Sequence form start to stop codon
	GGGACTGAAACCTGACGGCATCATTGGGCACTCCTTGGGAGAGGTTGCCTGTGGCTATGCAGATGGCTGTCTC TCCCAGAGAGAGGCTGTGCTTGCAGCTTACTGGCGAGGCCAGTGCATCAAAGATGCCACCTCCCGCCTGGAT CCATGGCAGCTGTTGGTTTGTCTGGGAGGAATGTAAACAGCGCTGCCCCGCTGGCGTGGTGCCTGCCTGCCA CAACTCTGAGGACACCGTGACCATCTCTGGACCTCAGGCTGCAGTGAATGAATTTGTGGAGCAGCTAAAGCAA GAAGGTGTGTTTGCCAAGGAGGTACGAACAGGAGGCCCTGGCTTTCCACTCCTACTTCATGGAAGGAATTGCCC CCACATTGCTGCAGGCTCTCAAGAAGGTGATCCGGGAACACGGCCGCGCTCGGCTCGATGGCTCAGCACCTC TATCCCTGAGGCCAGTGGCAGAGCAGCTGGCCCGCACATCTTCTGCCGAGTACAATGTCAACAACCTGGTG AGCCCTGTGCTCTTCCAGGAAGCACTGTGGCACATCCCTGAGCATGCCGTGGTGTGGAGATTGCGCCCCACG CACTGTTGCAGGCTGTCTGAAGCGAGGCGTGAAGTCCAGCTGCACCATCATTCCCTTGATGAAGAGGGATCA TAAAGATAACTTGGAGTTCTTTCTCACCAACCTTGGCAAGGTGCACCTCACAGGCATCAATGTCAACCCTAAC GCCTTGTTCACACCTGTGGAGTTCCCGGCTCCCGAGGGACTCCTCTCATCTCCCTCACATCAAGTGGGACCA CAGTCAGACTTGGGATGTCCCGTTGCTGAGGACTTCCCAAACGGCTCCAGCTCCTCCTGTACTACAGTCTAC AGCATCGACGCCAGTCTGAGTGCGCCGACCCTACCTGGTAGACCACTGCATTGACGGCCGGGTGCATCTTCCC TGGCACTGGTACCTGTGCCTGGTGTGAAGACACTGGCTCGCAGCCTGGGCTTGTCCCTAGAAGAGACCCCT GTGGTATTTGAGAATGTGTCGTTTCATCAGGCCACTATACTACCCAAGACAGGAACCGTGGCGCTGGAGGTGA GGCTGCTAGAGGCCTCCCATGCCTTTGAGGTGTCTGACACTGGCAATCTGATTGTGAGCGGAAAAGTGTACCT GTGGGAAGACCCGAACCTCAAGTTATTTCGACCACCCAGAAGTCCCAAACCCCCCTGAGTCTGCATCGGTCTCC CGCCTGACCCAGGGAGAAAGTATACAAGGAGCTGCGGCTGCGTGGCTATGATTATGGCCCTCAGTTCACAGGCA TCTGTGAGGCCACCCCTTGAAGGTGAACAAGGCAAGCTGCTCTGGAAGATAACTGGGTGACCTTCATGGACAC AATGCTGCAGGTATCCATTCTGGGTTCTAGCCAGCAGAGTCTACAGCTACCTAACCGGTGACCGCCATCTAT ATCGACCCCTGCCACCCACCGTCAGAAGGTGTACAGGCTGAAGGAGGACACTCAAGTGGCTGATGTGACAACGA GCCGCTGTCTGGGCATAACGGTCTCTGGTGGTATCCACATCTCAAGACTACAGACGACAGCAACCTCACGGCG GCAGCAAGAACAGCTGGTCCCCACCTTGGAAAAGTTCGTTTTACACCGCACATGGAGGCTGAGTGCCTGTCT GAGAGCACTGCCCTGCAGAAGGAGCTGCAACTGTGCAAGGGTCTGGCAGGGCTCTGCAGACCAAGGCCACCC AGCAAGGGCTGAAGGCGGCAATGCTTGGGCAAGAGGACCCTCCACAGCACGGGCTGCCTCGACTCCTGGCAGC TGCTTGCCAGTTGCAGCTCAACGGGAACCTGCAGCTGGAGCTGGGAGAAGCGCTGGCTCAAGAGAGGCTCCTG CTGCCAGAAGACCCCTCTGATCAGTGGCCTCCTCAACTCCCAGGCCCTCAAGGCCTGCGTAGACACAGCCCTGGA GAACTTGTCTACTCTCAAGATGAAGGTGGCAGAGGTGCTGGCTGGAGAAGGCCACTTGTATTTCCGAATCCCG GCACTGCTCAACACCCAGCCCATGCTACAACCTGGAATACACAGCCACCGACCGGCACCCCCAGGCCCTGAAGG ATGTTACAGACAACTGCAGCAGCATGATGTGGCGCAGGGCCAGTGAACCTTCCGACCTGCGCCACAGCAG CCTGGGTGCCCTTGACCTTCTGGTGTGCAACTGTGCATTAGCCACCCCTGGGGATCCAGCCTTGGCCCTGGAC AACATGGTAGCTGCCCTCAAGGAAGGTGGTTTCTGCTAGTGACACAGTGTCAAAGGACATGCCCTTGGGG AGACCTTGGCTGCCTACCTCTGAGGTGCAGCCTGCGCCAGCCTCCTAAGCCAGGAGGAGTGGGAGAGCCT GTTCTCGAGGAAGGCACTACACCTGGTGGGCTTAAAAGTCTTCTACGGTACTGCGCTGTTCTGTGCGCG CGAGCCATCCACAGGAGAAACCTATCTTCTGTCTGTGGAGGATACCAGCTTCCAGTGGGTGGACTCTCTGA AGAGCACTCTGGCCACGTCTCTCTCCACGCTGTGTGGCTAACGGCCATGGACTGCCCCACCTCGGGTGTGGTG GGTTTGGTGAATTGTCTCCGAAAAGAGCCGGGTGGACACCGGATTCCGGTGTATCCTGCTGTCCAACCTCAGCA ACACATCTCACGCCCCAAGTTGGACCTGGCTCTCCAGAGCTACAGCAGGTGCTAAAGCATGACCTCGTGAT GAACGTGTACCGGGACGGGGCTGGGTGCTTCCGTCACTTCCAGTTAGAGCAGGACAAGCCCAAGGAGCAG ACAGCGCATGCCTTTGTAAACGTCTCACCCGAGGGGACCTCGCCTCCATCCGTGGGTCTCCTCCCCCTGAA GCACACGCAGCCCTCGAGCTCAGGAGCACAGCTCTGCACTGTCTACTACGCCTCACTGAACTCCGAGACATC ATGCTGGCCACGGGCAAGCTGTCCCTGATGCCATTCCAGGTAATGGGCCAGCCGAGACTGCATGCTCGGCA TGGAGTTCTCAGGCCGGGATAGGTGTGGCCGGCGTGTGATGGGGCTGGTTCTGCAGAAGGCCTGGCCACCTC AGTCTGCTATCATCTGACTTCTCTGGGATGTACCCTCCAGCTGGACCCTGGAGGAGCGGCCCTGTGTCGCCG TCGTCTATAACCACTGCTTACTACTCGTTAGTGGTTCGCGGGCGCATCCAGCGTGGGGAGACCGTGTCTATCCA CTCAGGTTCAAGTGGTGTGGGCCAAGCGGCCATTTCCATTGCCCTCAGTCTGGGTGCGCGCTTTCACCACTG TGGGCTCTGCAGAGAAGCGAGCATACCTCCAGGCCAGGTTCCCTCAGCTTGATGACACCAGCTTTGCCAACTC GAGGGACACATCATTGAGCAGCACGTGTTACTGCACACAGGTGGCAAAGGGGTGACCTGGTCTCAACTCA CTGGCAGAAGAGAAGCTGCAGGCCAGTGTGCGGTGCTTGGCTCAGCATGGTCGCTTCTTAGAGATTGGCAAAT TTGATCTTTCTAACAACCCCTCTGGGCATGGCTATCTTCTTGAAGAACGTCACTTTCCATGGGATCCTGCT GGACGCCCTTTTGGAGGAGCCAATGACAGCTGGCGGGAGGTGGCGGCACCTCTGAAGGCTGGCATTCTGTGAT GGAGTCGTGAAGCCCCCTCAAGTGCACAGTGTTCCTCAAGGCCAGGTGGAAGATGCCTTCCGCTACATGGCTC AGGGGAAACACATTGGCAAAGTCTTGTCCAGGTACGGGAGGAGGAGCCTGAGGCTGTGCTGCCAGGGGCTCA GCCCCACCTGATTTCTGCCATCTCCAAGACCTTCTGCCAGCCCATAAAGATTACATCATCTGCTGGCCTAG GTGGCTTTGGCTGGAGCTGGCCCGGTGGCTCGTCTTCCGCGAGCCAGAGGCTTGTGCTGACTTCCCGATC TGGAATCCGCACCGGCTACCAAGCCAAGCACATTCGGGAGTGGAGACGCCAGGGCATCCAAGTGCTCGTGTCA ACAAGCAACGTGAGCTCACTGGAGGGGGCCCGTGCTCTCATCGCCGAAGCCACAAGCTGGGGCCCGTTGGGG GTGCTTCAACCTGGCCATGGTTTTGAGGGATGCCATGCTGGAGAACCAGACCCAGAGCTCTTCCAGGATGT CAACAAGCCCAAATAACAATGGCACCTGAACCTTGACAGGGCAACCCGGGAAGCTCGCCTGAGCTGGACTAC TTTGTGGCCTTCTCCTCTGTAAGCTGCGGGCGTGGTAATGCTGGCCAAACTAACACTACGGCTTCGCCAACTCTA CCATGGAGCGTATATGTGAACAGCGCAGGCACGATGGCCTCCAGGCCTTGGCGTGCAGTGGGGTGCATTGG TGACGTGGGCATTGTCTGGAAGCGATGGGCACCAATGACACAGTCACTGGAGGTACGCTGCCTCAGCGCATC TCCTCTGCATGGAGGTACTGGACCTTCTCTGAATCAGCCCCACGAGTCTGAGCAGCTTTGTGCTGGCAG

Construct	Sequence form start to stop codon
	AGAAGAAAGCTGTGGCCCATGGGGACGGGGACACCCAGAGGGATCTGGTGAAAGCTGTAGCACACATCCTAG GCATCCGAGACCTCGCAGGTATTAACCTGGACAGCACGCTGGCAGACCTCGGCCCTGGACTCGCTCATGGGTGT GGAAGTTCGTGAGATCCTGGAACGAGAACACGATCTGGTGCTGCCCATGCGTGAGGTGCGGCAGCTCACGCTG CGGAAACTTCAGGAAATGTCTCCAAGACTGACTCGGCTACTGACACGACAGCCCCAAGTCCAGGAGTGACA CGTCTCTGAAGCAGAACCAACTGAACCTGAGCACACTGCTGGTGAACCTGAGGGTCTACCTAATCCAGCT CAACTCGGTGACAGCTCTGAGCGGCTCTGTTCTTGTGCACCCATTGAGGGTCCACCACCGTGTTCACA GTCTGGCTGCCAAGCTCAGTGTGCCACCTACGGCTGCACTGACCCAAAGTCCCCCTGGATAGCATTCCG AACCTGGCTGCCTACTACATAGATTGCATCAAGCAAGTGCAGCCTGAGGGACCTACCGCATAGCTGGGTACT CATTTGGAGCCTGTGTAGCCTTCGAGATGTGCTCCAGCTGCAGGCCAGCAGGGCCAGCCCCGACCCACAAC AACCTCTTCTGTTTGACGGCTCACACACCTACGTGTTGGCCTACACCCAGAGTACCGGGCAAAGATGACCC CAGGCTGTGAAGCCGAGGCCGAGGCTGAGGCCTTATGCTTCTTCATAAAGCAGTTTCTTGATGTGGAACACAG CAAGGTGCTGGAGGCCCTGTGCCACTGAAGAGCCTGGAAGATCGGGTGGCTGCCTCCGTGGACCTTATCACT AAGAGTACCACAGCCTGGACGCCGAGAGCTGAGCTTTGCTGCCGTGTCTTCTACCACAAGCTCCGGGCAG CTGATCAGTATAAGCCCAAGCCAAGTACCATGGCAACGTGACACTGCTGCGTGCCAAGACAGGCGGCACCTA TGGCGAGGACTTGGGTGCTGACTACAACTCTCCAGGTGTGTGACGGGAAGGTGTCTGTGCACATCATTGAG GGTGACCACCGCACACTGCTGGAGGGCAGTGGCCTGGAATCCATCATCAACATCATCCATAGCTCCCTGGCTG AGCCACGAGTGAGTGTACGGGAGGGCCTCGAGCATCATCACCACCACCACCACCACTGA
pAR435 (mFAS ^{113S} /TesA) pAR436 (mFAS ^{113S} /TesA_L109P) pDL017 (mFAS ^{113S} /TesA_4x) pDL021 (mFAS ^{G113S} /TesA) pDL028 (mFAS ^{G113S} /TesA_L109P) pDL029 (mFAS ^{G113S} /TesA_4x) pDL007 (mFAS ^{G113M} /TesA) pDL010 (mFAS ^{G113M} /TesA_L109P) pDL018 (mFAS ^{G113M} /TesA_4x) pDL008 (mFAS ^{G113F} /TesA) pDL011 (mFAS ^{G113F} /TesA_L109P) pDL019 (mFAS ^{G113F} /TesA_4x) pDL026 (mFAS ^{G113W} /TesA) pDL031 (mFAS ^{G113W} /TesA_L109P) pDL032 (mFAS ^{G113W} /TesA_4x) (Strep _{mouse} FAS/TesA_H8) Position 113 Highlighted in red TesA_L109 highlighted in blue Positions for TesA4x highlighted in green	MSASWHPQFEKGGSGGGSGSAWHPQFEKGAGSEEVVIAGMSGKLPESENLQEFWANLIGVDMVTDDDR RWKAGLYGLPKRSGKLDLSKFDASFFGVHPKQAHTMDPQLRLLLEVSYEIVDGGINPASLRGTNTGVWVG SG/S/M/F/W SEASEALSRDPETLLGYSMVGCQRAMMANRLSFFDFKGPSIALDTACSSLLALQNAQAI RSGCPAALVGGINLLKPNSTSVQFMKLGMLSPDGTCSRFDSDSGSYCRSEAVVAVLLTKKSLARRVYATILNAGT NTDGSKEQGVTFPSGEVQEQLICSLYQAGLAPESLEYIEAHGTGKVGDPQELNGITRSLCAFRQAPLLIGSTKS NMGHPEPASGLAALTKVLLSLEHGVWAPNLHFNPNPEIPALLDGRQLQVDRPLPVRGGNVGINSFGFGGSNV HVILQPNTRQAPAPTAHAALPHLLHASGRTEAVQDLEQGRQHSQDLAFVSMNLNDIAATPTAAMPFRGYTVL GVEGRVQEVQVSTNKRPLWFICSGMGTQWRGMGLSLMRLDSFRESILRSDEAVKPLGVKVSDDLSTDERTF DDIVHAFVSLTAIQIALIDLLTSVGLKPDGHHLSLGEVACGYADGCLSQREAVLAAYWRGQCICKDAHLPPGSMA AVGLSWECKQRCPAGVVPACHNSEDVTISGPQAAVNEFVEQLKQEGVFAKEVRTGGALFHSYFMEGIAPTL LQALKKVIREPRPRSARWLSTISPEAQWQSSLARTSSAEYNNVNLVSPVLFQEALWHIPEHAVVLEIAPHALLQ AVLKRGVKSSCTIPLMKRDHKNLEFFLTNLGKVHLTGINVPNALFPPEVPAPRGTPPLISPHIKWDHSQTW DVPVAEDFPNGSSSSSATVYSIDASPEPDHYLVHCHIDGRVIFPGTYLCLVWKTLARSLGLSLEETPVVFENV FHQATILPKTGTVALEVRLLLEASHAFEVSDTGNLIVSGKVLWEDPNSKLFDPHEVPTPPESASVSRLTQGEVY KELRLRGYDYGPPQFGICEATLEGEQGLLWKNWVTFMDTMLQVSILGSSQSLQLPTRVTAIYIDPATHRQ KVYRLKEDTQVADVTTSRCLGITVSGGIIHISRLQTATSRRQEQVLPTLEKFVTPHMEAECLSESTALQKELQ LCKGLARALQTKATQQGLKAAMLGQEDPPQHGLPRLAAACQLQLNGNLQLELGEALAQERLLLPEPLISGL LNSQALKACVDTALENLSTLKMKVAEVLAGEGHLYSRIPALLNTQPMQLLEYTATDRHPQALKDVQTKLQKH DVAQQQWNPSDPAPSSLGALDLLVCNCALATLGDPALALDNMVAALKEGGFLLVHTVLKGHALGETLACLPSE VQPAPSLLSQEEWESLFSRKALHLVGLKRSFYGTALFLCRRAPQEKPIFLSVEDTSFQWVWDSLKSTLATSSSQP VWLTAMDCPTSGVVLNCLRKEPGGHRIRCILLSNLSNTSHAPKLDPGSPQLQVVKHDLVMNVYRDGAWG AFRHFQLEQDKPKEQTAHAFVNVLTGRDLASIRWVSSPLKHTQPSSGAQLCTVYYASLNFDRDIMLATGKLS PD AIPGKWASRDCMLGMEFSGRDRRCRRVMGLVPAEGLATSVLLSSDFLWDVPSSWTL EEAA SVPVVYTTAYYSL VVRGRIQRGETVLIHSGSGGVQAAISIALSLGCRVFTTVGSAEKRAYLQARFPQLDDTSFANSRDTSFQHVLL HTGGKGVDLVLNLSLAEKQLASVRCALQHGRLFLEIGKFDLSNNHPLGMAIFLKNVTFHGILLDALFEEANDSW REVAALLKAGIRDGVVKPLKCTVFPKAQVEDAFRYMAQGGKHIGKVLVQVREEEPEAVLPQAQPTLISAISKTC PAHKSYYITGGLGGFGLERARWLVRGAQRLVLTSRSGIRTGYQAKHIREWRRQGIQVLVSTSNVSSLEGARALI AEATKLGPVGGVFNLAMVLRDAMLENQTELPFQDVNPKPYNGTLNLD RATREACPELDYFVAFSSVSCGRGN AGQNTNYGFANSTMERICEQRRHDGLPLGAVQWGAIGDVGVLEAMGTNDTVIGGTL PQRISSCMEVLDLFLNQ PHAVFVLAEEKKAVAHGDDGTQRDLVKAVAHILGIRDLAGINLDSTLADLGLDSLMGVEVRQILEREHDLVLP MREVRLTLRLKQEMSSKTD SATDTTAPKSRSDTSLKQNQAAAADTLLILGDSLSAGYRMSASAAWPALLNDK WQSKTSVVNASISGDTSQGLARLPALLKQHQPRWVLVELGGNDGLRGFQPPQTEQLRQILQDVKAANAEP LLMQIRL/ P PANYGRRYNEAFSAIYPKLAKEFDVPLLPFF M/LE/DEVY/GL/KK PQWMQDDGIHPNRDAQPF IADWMAKQLQPLVNHDSLEHHHHHHHH
pAR435 (mFAS ^{113S} /TesA) pAR436 (mFAS ^{113S} /TesA_L109P)	ATGAGCGCTTGGAGCCATCCACAATTTGAGAAGGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCTCAGCGTGG AGCCACCCGAGTTCGAAAAAGCGCCGGATCCGAGGAGGTGGTGATAGCCGGTATGTGGGGAAAGTTGCCCGG AGTCAGAGAACCTACAGGAGTTCTGGGCCAACCTCATTGGTGGTGTGGACATGGTCACAGATGATGACAGGA GATGGAAGGCTGGGCTCTATGGATTACCCAAGCGGTCTGGAAAGCTGAAGGATCTCTCCAAGTTCGACGCCCTC

Construct	Sequence form start to stop codon
pDL017 (mFAS ^{+/} TesA_4x)	CTTTTGTGGGTGCCACCCCAAGCAGGCACACACAATGGACCCCAAGCTTCGGCTGCTGTGTGGAAGTCAGCTAT
pDL021 (mFAS ^{G113S} /TesA)	GAAGCAATTGTGGATGGAGGTATCAACCCAGCCTCACTCCGAGGAACGAACACTGGCGTCTGGGTGGGTGTGA
pDL028 (mFAS ^{G113S} /TesA_L10 9P)	GTGGT/TCG/ATG/TTT/TCGTCAGAGGCATCCGAGGCCCTTAGCAGAGATCCCGAGACGCTTCTGGGTACAC
pDL029 (mFAS ^{G113S} /TesA_4x)	GCATGGTGGGCTGCCAGCGTGAATGATGGCCAACCGGCTCTCTTTCTTCTTCGACTTCAAAGGACCAAGCAT
pDL007 (mFAS ^{G113M} /TesA)	TGCCCTGGACACAGCCTGCTCCTCCAGCTTGCTGGCACTACAGAATGCCTACCAGGCCATCCGTAGTGGGGAA
pDL010 (mFAS ^{G113M} /TesA_L1 09P)	TGCCCCGCGGCCCTTGTGGGTGGGATCAACCTGCTCCTGAAGCCGAACACCTCTGTGCAGTTCATGAAGCTGG
pDL018 (mFAS ^{G113M} /TesA_4x)	GCATGCTCAGCCCGACGGACCTGCAGATCCTTTGATGATTAGGGAGTGGATATTGTGCTCTGAGGCTGT
pDL008 (mFAS ^{G113F} /TesA)	TGTAGCAGTTCTGCTGACTAAGAAGTCCCTGGCTCGGCGGGTCTATGCCACGATTCTGAATGCCGGCACCAAT
pDL011 (mFAS ^{G113F} /TesA_L10 9P)	ACAGATGGCAGCAAGGAGCAAGGTGTAACATTCCCTCTGGAGAAGTCCAAGAACAACCTCATCTGCTCTCTGT
pDL019 (mFAS ^{G113M} /TesA_4x)	ATCAGCCAGCTGGTCTGGCCCCGGAGTTCGCTTGAGTATATTGAAGCCCATGGCACGGGCACCAAGGTGGGTGA
pDL026 (mFAS ^{G113W} /TesA)	CCCCCAGGAACTGAATGGCATTACTCGGTCCCTGTGCGCCTTCCGCCAGGCCCTCTGTAAATTGGCTCCACCA
pDL031 (mFAS ^{G113W} /TesA_L1 09P)	AATCCAACATGGGACACCTTGAGCCTGCCTCTGGGCTTGAGCCCTGACCAAGGTGCTGTTATCCCTGGAGCA
pDL032 (mFAS ^{G113W} /TesA_4x)	TGGGGTCTGGGCCCCTAACCTGCACCTCCACAACCCCAACCTGAGATCCAGCACTTCTTGATGGGCGGCTGC
(Strepl_mouseFAS/T esA_H8)	AGGTGGTTCGATAGGCCCTGCCTGTTCTGTTGGTGGCAACGTGGGCATCAACTATTGGCTTCGGAGGCTCCAA
Position 113 Highlighted in red TesA_L109 highlighted in blue Positions for TesA4x highlighted in green	TGTCACGCCAGTGGACGCACCTTAGAGGCAGTGCAGGACCTGCTGGAACAGGGCCCGCAGCACAGCCAGGACCT GGCCTTTGTGAGCATGCTCAATGACATTGCGGCAACCCCTACAGCAGCCATGCCCTTCAGGGGTTACACTGTG CTAGGTGTTGAGGGCCGTGTCCAAGAAGTGCAGCAAGTGTCCACCAACAAGCGCCCACTCTGGTTTCATCTGCT CAGGGATGGGCACGCAGTGGCGCGGGATGGGGCTGAGCCTCATGCGCCTGGACAGCTTCCGTGAGTCTATCCT GCGCTCCGATGAGGCTGTGAAGCCGTTGGGAGTGAAGTGTGAGTCTGCTGTTGAGCACAGATGAGCGCACCT TTTGATGACATCGTGCATGCCCTTTGTGAGCCTCACTGCCATCCAGATTGCCCTCATCGACCTACTGACTTCTGT GGGACTGAAACCTGACGGCATCATTGGGCACTCCTTGGGAGAGGTTGCCTGTGGCTATGCAGATGGCTGTCTC TCCCAGAGAGAGGCTGTGCTTGACGCTTACTGGCGAGGCCAGTGCATCAAAGATGCCACCTCCCGCCTGGAT CCATGGCAGCTGTTGGTTTGTCTGGGAGGAATGTAAACAGCGCTGCCCGCTGGCGTGGTGCCTGCCTGCCA CAACTCTGAGGACACCGTGACCATCTCTGGACCTCAGGCTGCAGTGAATGAATTTGTGGAGCAGCTAAAGCAA GAAGGTGTGTTTGCCAAGGAGGTACGAACAGGAGGCCCTGGCTTTCCACTCCTACTTCATGGAAGGAATTGCC CCACATTGCTGCAGGCTCTCAAGAAGGTGATCCGGGAACACGGCCGCGCTCGGCTCGATGGCTCAGCACCTC TATCCCTGAGGCCCAAGTGGCAGAGCAGCCTGGCCCGCACATCTTCTGCCGAGTACAATGTCAACAACCTGGTG AGCCCTGTGCTCTTCCAGGAAGCACTGTGGCACATCCCTGAGCATGCCGTGGTGTGCTGGAGATTGCGCCCCACG CACTGTTGCAGGCTGTCTGAAGCGAGGCGTGAAGTCCAGCTGCACCATCATTCCCTTGATGAAGAGGGATCA TAAAGATAAATTGGAGTTCTTTCTCACCAACCTTGGAAGGTTGCCAAGGTGCACCTCAGAGGCATCAATGTCAAC GCCTTGTTCCACCTGTGGAGTTCCCGGCTCCCGAGGGACTCCTCTCATCTCCCTCACATCAAGTGGGACCA CAGTCAGACTTGGGATGTCCCGTTGTCTGAGGACTTCCCAAACGGCTCCAGCTCCTCTCTGCTACAGTCTAC AGCATCGACGCCAGTCTGAGTGCAGCCGACCACTACCTGGTAGACCACTGCATTGACGGCCGGGTGCATCTTCCC TGGCACTGGCTACCTGTGCCTGGTGTGGAAGACACTGGCTCGCAGCTGGGCTTGTCCCTAGAAGAGACCCCT GTGGTATTTGAGAATGTGTCGTTTCATCAGGCCACTATACTACCCAAGACAGGAACCGTGGCGCTGGAGGTGA GGCTGCTAGAGGCCCTCCCATGCCTTTGAGGTGTCTGACACTGGCAATCTGATTGTGAGCGGAAAAGTGTACCT GTGGGAAGACCCGAACCTCAAGTTATTCGACCACCCAGAAGTCCCAACACCCCTGAGTCTGCATCGGTCTCC CGCCTGACCCAGGGAGAAGTATACAAGGAGTGGCGCTGCGTGGCTATGATTATGGCCCTCAGTTCAGGGGCA TCTGTGAGGCCACCCCTTGAAGGTGAACAAGGCAAGCTGCTCTGGAAGATAAAGTGGGTGACCTTCATGGACAC AATGTGCAGGTATCCATTCTGGGTCTAGCCAGCAGAGTCTACAGCTACCTACCCGTGTGACCGCCATCTAT ATCGACCCTGCCACCCACCGTCAGAAGGTGTACAGGCTGAAGGAGGACACTCAAGTGGCTGATGTGACAACGA GCCGTGTCTGGGCATAACGGTCTCTGGTGGTATCCACATCTCAAGACTACAGACGACAGCAACCTCACGGCG GCAGCAAGAACAGCTGGTCCCACTTGGAAAAGTTCGTTTTCACACCGCACATGGAGGCTGAGTGCCTGTCT GAGAGCACTGCCCTGCAGAAGGAGCTGCAACTGTGCAAGGGTCTGGCACGGGCTCTGCAGACCAAGGCCACCC AGCAAGGGCTGAAGGCGGAATGCTTGGGCAAGAGGACCCTCCACAGCACGGGCTGCCTCGACTCCTGGCAGC TGCTTGCCAGTTGCAGCTCAACGGGAACCTGCAGCTGGAGCTGGGAGAAGCGCTGGCTCAAGAGAGGCTCCTG CTGCCAGAAGACCCCTGTGATCAGTGGCCTCCTCAACTCCAGGCCCTCAAGGCCTGCGTAGACACAGCCCTGGA GAACCTGTCTACTCTCAAGATGAAGGTGGCAGAGGTGCTGGCTGGAGAAGGCCACTTGATTTCCCGAATCCCG GCACTGCTCAACACCCAGCCATGTCTACAACCTGGAATACACAGCCACCGACCGGCAACCCCAAGGCCCTGAAGG ATGTTTCAGACCAAACTGCAGCAGCATGATGTGGCGCAGGGCCAGTGAACCTTCCGACCTGCGCCAGCAG CCTGGGTGCCCTTGACCTTCTGGTGTGCAACTGTGCATTAGCCACCTGGGGATCCAGCCTTGGCCCTGGAC AACATGGTAGCTGCCCTCAAGGAAGGTGGTTTCTGCTAGTGACACAGTGTCAAAGGACATGCCCTTGGGG AGACCCTGGCCTGCCTACCTCTGAGGTGCAGCCTGCGCCAGCCTCCTAAGCCAGGAGGAGTGGGAGAGCCT GTTCTCGAGGAAGGCACTACACCTGGTGGGCTTAAAAGGTCCTTCTACGGTACTGCGCTGTCTGTGCGCG CGAGCCATCCACAGGAGAAACCTATCTTCTGTCTGTGGAGGATACCAGCTTCCAGTGGGTGGACTCTCTGA AGAGCACTCTGGCCACGTCCTCCTCCAGCCTGTGTGGCTAACGGCCATGGACTGCCCCACCTCGGGTGTGGTG GGTTTGGTGAATTGTCTCCGAAAAGAGCCGGGTGGACACCGGATTCCGTGTATCCTGCTGTCCAACCTCAGCA ACACATCTCAGCCCCCAAGTTGGACCTGGCTCTCCAGAGCTACAGCAGGTGCTAAAGCATGACCTCGTGAT GAACGTGTACCGGGACGGGGCTGGGTGCGCTTCCGTCACTTCCAGTTAGAGCAGGACCAAGCCCCAAGGACAG ACAGCGCATGCCCTTGTAAACGTCTCACCCGAGGGGACCTCGCCTCCATCCGTGGGTCTCTCCCCCTGAA GCACACGACGCCCTCGAGCTCAGGAGCACAGCTCTGCACTGTCTACTACGCCCTCACTGAACCTCCGAGACATC ATGCTGGCCACGGGCAAGCTGTCCCTGATGCCATTCCAGGTAATGGGCCACGGAGACTGCATGCTCGGCA

Construct	Sequence form start to stop codon
	TGGAGTTCTCAGGCCGGGATAGGTGTGGCCGGCGTGTGATGGGGCTGGTTCTCGCAGAAGGCCCTGGCCACCTC AGTCCTGCTATCATCTGACTTCCTCTGGGATGTACCCTCCAGCTGGACCCTGGAGGAGGCGGCCCTCTGTGCCCCG TCGTCTATACCACTGCTTACTACTCGTTAGTGGTTCGCGGGCGCATCCAGCGTGGGGAGACCGTGCTCATCCA CTCAGGTTTCAGGTGGTGTGGGCCAAGCGGCCATTTCCATTGCCCTCAGTCTGGGTGCCGCTTTCACCACTG TGGGCTCTGCAGAGAAGCGAGCATACCTCCAGGCCAGGTTCCCTCAGCTTGATGACACCAGCTTTGCCAACTC GAGGGACACATCATTTGAGCAGCACGTGTTACTGCACACAGGTGGCAAAGGGGTGCGACCTGGTCCTCAACTCA CTGGCAGAAGAGAAGCTGCAGGCCAGTGTGCGGTGCTTGGCTCAGCATGGTCGCTTCTTAGAGATTGGCAAAT TTGATCTTTCTAACAACCACCCTCTGGGCATGGCTATCTTCTTGAAGAACGTCACTTTCCATGGGATCCTGCT GGACGCCCTTTTTGAGGAGGCCAATGACAGCTGGCGGGAGGTGGCGGCACCTCTGAAGGCTGGCATTCTGTGAT GGAGTCGTGAAGCCCCTCAAGTGCACAGTGTTCCTCAAGGCCAGGTGGAAGATGCCTTCCGCTACATGGCTC AGGGGAAACACATTGGCAAAGTCCTTGTCCAGGTACGGGAGGAGGAGCCTGAGGCTGTGCTGCCAGGGGCTCA GCCCACCCTGATTTCTGCCATCTCCAAGACCTTCTGCCAGGCCATAAGAGTTACATCATCTAGTGGTGGCCTAG GTGGCTTTGGCCTGGAGCTGGCCCCGTGGCTCGTGCTTCGCGGAGCCCAGAGGCTTGTGTGACTTCCCGATC TGGAATCCGACCCGGCTACCAAGCCAAGCACATTCGGGAGTGAGACGCCAGGGCATCCAAGTGTCTGTGTCA ACAAGCAACGTGAGCTCACTGGAGGGGGCCCGTGCTCTCATCGCCGAAGCCACAAAGCTGGGGCCCGTTGGGG GTGTCTTCAACCTGGCCATGGTTTTGAGGGATGCCATGCTGGAGAACCAGACCCCAAGAGCTTCCAGGATGT CAACAAGCCCAATACAATGGCACCCCTGAACCTTGACAGGGCAACCCGGGAAGCCTGCCCTGAGCTGGACTAC TTTGTGGCCTTCTCCTCTGTAAGCTGCGGGCGTGGTAATGCTGGCCAACTAACTACGGCTTCGCCAACTCTA CCATGGAGCGTATATGTGAACAGCGCAGGCACGATGGCCTCCCAGGCCTTGCCGTGACGTGGGGTGGCATTGG TGACGTGGGCATTGTCTGGAAGCGATGGGCACCAATGACACAGTCATCGGAGGTACGCTGCCTCAGCGCATC TCCTCTGCATGGAGGTACTGGACCTCTTCTGAATCAGCCCCACGCAGTCCTGAGCAGCTTTGTGCTGGCAG AGAAGAAAGCTGTGGCCATGGGGACGGGGACACCCAGAGGGATCTGGTGAAAGCTGTAGCACACATCCTAG GCATCCGAGACCTCGCAGGTATTAACCTGGACAGCACGCTGGCAGACCTCGGCCTGGACTCGCTCATGGGTGT GGAAGTTCGTGATCCTGGAACGAGAACACGATCTGGTGTGCCATGCGTGAGGTGGCGCAGCTCAGCGTG CGGAACTTCAGGAAATGTCTCCAAGACTGACTCGGCTACTGACACGACAGCCCCAAGTCCAGGAGTGACA CGTCTCTGAAGCAGAACCAAGCCGCGCAGCGGACACGTTATTGATTCTGGGTGATAGCCTGAGCGCCGGGTA TCGAATGTCTGCCAGCGCGGCTGGCCTGCCTTGTGAATGATAAGTGGCAGAGTAAACGTCGGTAGTTAAT GCCAGCATCAGCGCGCACACCTCGCAACAAGGACTGGCGCGCCTTCCGGCTCTGCTGAAACAGCATCAGCCGC GTTGGGTGCTGGTTGAACTGGGCGGCAATGACGGTTTGGCTGGTTTTTCAGCCACAGCAAACCGAGCAAAACGCT GCGCCAGATTTTGCAGGATGTCAAAGCCGCCAACGCTGAACCATTTGTAATGCAAATACGTCTG/CCGCTGC AAACTATGGTCGCCGTATATAATGAAGCCTTTAGCGCCATTTACCCCAAACCTCGCCAAAGAGTTTGATGTTCCG CTGCTGCCCTTTTTTATG/CTGGA/GATGAGGTCTAC/GGCCTC/AAGCAAGCCACAATGGATGCAGGATGA CGGTATTATCCCAACCGCAGCGCCAGCGCTTTATTGCCGACTGGATGGCGAAGCAGTTGCAGCCTTTAGTA AATCATGACTCACTCGAGCATCATACCACCACCACCACCTGA
Codon-optimized sequence for <i>O.</i> <i>polymorpha</i> (mFAS ^{g113m} _Tesa)	ATGTCTGCCGGCGCTGGCTCGGAAGAGGTGGTGTATCGCCGGCATGTGGGCAAGCTGCCTGAGTCGGAGAACC TGCAAGAGTTCTGGGCCAACCTGATCGGCGCGTGGACATGGTGACCGACGATGACCGCAGATGGAAGCCCGG CCTGTATGGAAGTCCAAAGAGATCTGGCAAGTTGAAGGACCTGTGCAAGTTCGACGCGCTCGTTCTTCGGCGTG CACCACAAAGCAAGCCACACCATGGACCCACAGCTGAGACTGTTGCTGGAGGTGTGCTACGAGGCCATCGTGG ACGGCGGCATTAAACCTGCCTCGCTGAGAGGCACCAACACCGCGGTGTGGGTGGGCGTGTGATGTCGGAGGC CTCGGAGGCCCTGTGAGAGACCTTGAAGCCCTGCTGGGCTACTCGATGGTGGGCTGTGAGAGGCATGATG GCCAACAGACTGTGCTTCTTTTCGACTTCAAGGGCCCATCGATCGCCCTGGACACCGCTGCTGCTTTCGCT GCTGGCCCTGCAGAACGCTACCAAGCCATCAGATCGGGCGAGTGCCTGCGCCCTGGTGGGCGGCATCAACC TGTTGCTGAAGCCAAACACCTCGGTGCAGTTTATGAAGCTGGGCATGCTGTGCGCTGACGGCACCTGCAGATC GTTTCGACGACTCGGGCTCGGGCTACTGCAGATCGGAGGCCGTGGTGGCGGTGCTGCTGACCAAGAAGTCTGTTG GCTAGAAGAGTTTACGCTACCATCCTGAACCGCGGCACCAATACCGACGGCTCGAAGGAGCAAGGCGTGACCT TCCCATCGGGCGAGGTGCAAGAGCAGCTGATCTGCTGCTGTATCAGCCTGCGCGCTGGCCCTTGAGTCGCT GGAGTACATCGAGGCCACGGCACCGGCACCAAGTGGGCGACCCACAAGAGCTGAATGGCATCACGAGATCG CTGTGCGCCTTCAGACAAGCCCTTTGTTGATCGGCTCGACCAAGTCTAACATGGGCCACCCTGAGCCTGCCTC GGGCTTGGCCGCTTGACCAAGGTGCTGCTGTGCTGGAGCACGGAGTGTGGGCCCCAACCTGCACTTCCAC AACCCAAACCTGAGATCCCTGCCCTGCTGGACGGCAGACTGCAAGTGGTGACAGACCACTGCCTGTGAGAG GCGGCAACGTGGGCATCAACTCGTTTCGGCTTCGGCGGCTCGAACGTGCACGTGATCCTGCAGCCTAACACGAG ACAAGCCCTGCCCCAACCGCCACGCGCTCTGCCACACCTGCTGCACGCTCTGGCAGAACCTGGAGGGCCG TGCAAGACCTGCTGGAGCAAGGCAGACAGCACTCGCAAGACCTGGCTTTCGTGTGATGCTGAACGACATCGC CGCCACCCCTACCGCCGCTATGCCATTAGAGGCTACACCGTGTGGGCGTGGAGGGCAGAGTGAAGAGGTG CAGCAAGTGTGACCAACAAGAGACCACTGTGGTTTATCTGCTCGGCATGGGCACGCACTGGAGAGGCATGG GCCTGTGCTGATGAGACTGGACTCGTTTACAGAGTGCATCCTGAGATCGGACGAGGCGCTGAAGCCACTGGG CGTGAAGGTGTGCGACCTGTGCTGTGACCGACGAGAGAACCTTCGACGACATCGTGCACGCTTTCGTGTG CTGACGGCCATTAGATCGCCCTGATGACCTGCTGACCTCGGTGGGCTGAAGCCTGACGGCATCATCGGC ACTCGCTGGGCGAGGTTGCCTGCGGCTACGCCGACGGCTGCTTGTGCGAGAGAGGCGCTGCTGGCGCCTA CTGGAGAGGACAGTGATCAAGGACGCCCACTGCCACCTGGCTCGATGGCGCGCTGGGCTGTGCTGGGAG GAGTGAAGCAGAGATGCCCTGCGGCGTGGTGCCTGCCACAACCTCGGAGGACACCGTGACCATCTCGG GCCACAAGCCGCGTGAACGAGTTCTGTGGAGCAGCTGAAGCAAGAGGGCGTGTTCGCCAAAGAGGTGAGAAC CGGCGGCTGGCCTTCCACTCGTACTTTCATGGAGGGCATGCCCAACCTGCTGCAAGCCCTGAAGAAGGTG

Construct	Sequence form start to stop codon
	ATCAGAGAGCCAAGACCAAGATCGGCTAGATGGCTGTCGACCTCGATCCCTGAGGCTCAGTGGCAGTCGTCCG TGGCCCGCACCTCGTCGGCCGAGTACAACGTGAACAACCTGGTGTGCGCTGTGCTGTTCCAAGAGGCCCTGTG GCACATCCCTGAGCACGCCGTGGTGTGGAGATCGCCCCACACGCCCTGCTGCAAGCCGTGTTGAAGAGAGGC GTGAAGTCGTCGTGCACCATCATCCACTGATGAAGAGAGACCACAAGGACAACCTGGAGTCTTCCCTGACCA ACCTGGGCAAGGTGCACCTGACCGGCATCAACGTGAACCCAAACGCCCTGTTCCACCTGTGGAGTTCCTCTGCC CCAAGAGGCACCCCTTTGATCTCGCCACACATCAAGTGGGACCACTCGCAGACCTGGGACGTGCCTGTGGCCG AGGACTTCCAAACGGCTCGTCTTCGTCTTCGGCCACCGTGTACTCGATCGACGCCTCGCCTGAGTCGCCTGAC CACTACCTGGTGGACCACTGCATCGACGGCAGAGTGATCTTCCCTGGCACCGGTACCTGTGCCTGGTGTGGA AGACCCTGGCTAGATCGCTGGGCCCTGTCTCTGGAGGAGACCCCTGTGGTGTTCGAGAACGTGTGCTTCCACCA AGCCACCATCCTGCCAAGACCGGCACCGTGGCCCTGGAGGTGAGACTGCTGGAGGCCTCGCACGCCCTCGAG GTGTCGGACACCGGCAACCTGATCGTGTGCGGCAAGGTGTACCTGTGGGAGGACCCAAACTCGAAGCTGTTTCG ACCACCCTGAGGTGCCAACCCACCTGAGTCGGCCTCGGTGTGCGAGACTGACCCAAGGCGAGGTGTACAAGGA GCTGAGACTGAGAGGCTACGACTACGGCCACAGTTCCAAGGCATCTGCGAGGCCACCCCTGGAGGGCGAGCAA GGCAAGCTGCTGTGGAAGGACAACCTGGGTGACCTTCATGGACACCATGCTGCAAGTGTGATCCTGGGCTCGT CGCAGCAGTCGCTGCAGCTGCCAACGAGAGTGACCGCCATCTACATCGACCTGCCACCCATAGACAGAAGGT TTACAGACTGAAGGAGGACACCCAAGTGGCCGACGTGACCACCTCGAGATGCTGGGCATACCCGTGTGCGGC GGCATCCACATCTCGAGACTGCAGACCACCGCCACCTCGAGAAGACAACAAGAGCAAAGTGGTTCACCCCTGG AGAAGTTCGTGTTACCCCCACATGGAGGCCGAGTGCCTGTGCGAGTCGACCGCCCTGCAGAAGGAGCTGCA GCTGTGCAAGGGCCTGGCTAGAGCCCTGCAGACCAAGGCCACGCAGCAAGGCCTGAAGGCCGCCATGTGGGC CAAGAGGACCCACCACAGCACGGCCTGCCAAGACTGCTGGCCGCTGCCTGTGACGTGCAGCTGAACGGCAACC TGCAACTGGAGTTGGGCGAGGCCCTGGCCCAAGAGCGCCTGCTGTTGCCTGAGGACCCACTGATCTCGGGCCT GCTGAACTCGAAGCCTTGAAGGCCTGCGTGGACACCGCCCTGGAGAACCTGTGACCCCTGAAGATGAAGGTG GCCGAGGTGCTGGCCGGCGAGGGCCACCTGTACTCGAGAATCCCTGCCTTGTTGAATACGCAGCCAATGTGTC AGCTGGAGTACACGGCCACCGACAGACATCCACAAGCCCTGAAAGATGTGCAGACCAAGCTGCAGCAGCAGCA CGTGGCCCAAGGACAGTGAACCCATCGGACCCCTGCCCCATCGTCGTTGGGCGCCTTGGACTTGTGTTGTGTC AATTGCGCCCTGGCTACCCCTGGGCGACCCCTGCCCTGGCCCTGGACAACATGGTGGCCGCTTGAAGGAAGGCG GCTTCCTGCTGGTTACACCCGTGCTGAAGGGCCACGCCCTGGGAGAAACCTGGCCTGCTTGCCATCGGAGGT GCAGCCTGCCCCATCGCTGCTGTGCAAGAGGAGTGGGAGTCGCTGTTCTCGAGAAAGGCCCTGCACCTGGTG GGCCTGAAGAGATCGTTCTACGGCACCGCCCTGTTCTCTGTGCAAGAGCCATCCCAAGAGAAGCCAACTCT TCCTGTGCGTGGAGGACAGTCGTTTCAGTGGGTGACTCGTTGAAGTCGACCCCTGGCTACGTCGTCTTCGCA GCCTGTGTGGCTGACCGCCATGGACTGCCCAACCTCGGGCGTGGTGGGCTGAGTGAAGTGAAGGAG CCTGGCGGCCACAGAATCAGATGCATCCTGCTGTGCAACCTGTCTAACACCTCTCACGCCCAAGAGTGGACCC TGGCTCGCCTGAGCTGCAGCAAGTGTGAAGCAGACCTGGTGATGAACGTGTACAGAGACGGCGCCTGGGGC GCCTTCAGACACTTTCAGCTGGAGCAAGACAAGCCAAAGGAGCAGACCGCCACGCCCTTCGTGAACGTGCTGA CGAGAGGCGACCTGGCCTCGATCAGATGGGTGTGTCGCGCCACTGAAGCACACGCAGCCATCGTCTTCGGGGCGC TCAGCTGTGACCCGTGTACTACGCCTCGTGAACCTCAGAGACATCATGTGGCCACCGGCAAGCTGTGCGCT GACGCCATCCCTGGCAAGTGGGCCTCGAGAGACTGCATGTGGGCATGGAGTTCTCGGGCAGAGACAGATGCG GCAGAAGAGTGATGGGCCTGGTGCCTGCCGAAGGCCCTGGCCACCTCGGTGCTGCTGTGCTGACTTCTGTG GGACGTGCCATCGTCGTGGACCCTGGAGGAGGCGCCCTCGGTGCCTGTGGTGTACACCACCGCCTACTACTCGC TGGTGGTGAGAGGCAAGATTACAGAGGCGAGACCGCTGATCCACTCGGGCTGTGCGCGCTGGGCGCAAGC TGCCATCTCGATCGCCCTGTGCTGGGCTGCAGAGTGTTCACCACCGTGGGCTCGGCCGAGAAGAGAGCCTAC CTGCAAGCTAGATTCCACAGCTGGACGACACCTCGTTTGCTAATTGAGAGACACCTCGTTGCAACAACACG TGCTGCTGCACACCGGGCGGCAAGGGCGTGGACCTGGTGTGAACTCGTGGCCGAGGAGAAGCTGCAAGCCTC GGTGAGATGCCTGGCTCAGCACGGCAGATTCTGGAGATCGGCAAGTTCGACCTGTGCAACAATCACCCACTG GGCATGGCCATCTTCTGAAGAACGTGACCTTCCACGGCATCCTGCTGGACGCCCTGTTGAGAGAGGCCAACG ACTCGTGGAGAGAGGTGGCTGCCCTGCTGAAGGCCGAATCAGAGATGGCGTGGTGAAGCCACTGAAGTGCAC CGTGTTCCTCAAGGCCCAAGTGGAGGACGCCTTCAGATACATGGCCCAAGGCAAGCACATCGGCAAGGTGCTG GTGCAAGTGAGAGAGGAAGAGCCTGAGGCCGTGCTGCCTGGCGCTCAGCCAACCTGATCTCGGCCATCTCGA AGACCTTCTGCCCTGCCCACAAGTCGTACATCATCACGGCGGCTGGGCGGCTTCGGCCTGGAGCTGGCTAG ATGGCTGGTGTGAGAGGAGCTCAGAGACTGGTGTGACTCGAGATCGGGCATCAGAACCAGCTACCAAGCC AAGCACATCAGAGAGTGGAGAAGACAAGGCATCCAAGTGTGGTGTGACCTCGAATGTGTCGTCGCTGGAG GGCGCTAGAGCCCTGATCGCCGAGGCCACCAAGCTGGGCCCTGTGGGCGGCGTGTTCACCTGGCCATGGTTTC TGAGAGACGCCATGTGGAAGATCAGACCCCTGAGCTGTTCCAAGACGTGAACAAGCCAAAGTACAACGGCAC CCTGAACCTGGACAGAGCCACGAGAGAGGCCCTGCCCTGAGCTGGACTACTTCGTGGCCTTCTCGTCGGTGTG TGCGGCAGAGGCAACGCCGGACAGACCAACTACGGCTTCGCCAACTCGACCATGGAGAGAATCTGCGAGCAGC GCCGCCACGACGGACTGCCTGGCCTGGCTGTGCACTGGGCGCTATCGGCGACGTGGGCATCGTGTGGAGGC CATGGGCACCAACGACACCGTGATCGGCGGCACCCCTGCCACAGAGAATCTCGTCGTGATGGAGGTGCTGGAC CTGTTCTCTGAATCAGCCACACGCCGTGCTGTGCTCTTTGTGCTGGCCGAGAAGAAGGCTGTGGCTCACGGCG ACGGCGACACGCAGAGAGACCTGGTGAAGGCCGTGGCCCATATCTTGGGCATCAGAGACCTGGCCGCATCAA CCTGGACTCGACCCCTGGCCGATCTGGGCTTGGACTCGCTGATGGGCGTGGAGGTGAGACAAATCTGGAGAGA GAGCACGATCTGGTGTGCCAATGAGAGAGGTTAGACAGCTGACCCTGAGAAAGCTGCAAGAGATGTGTCG AAGACCGACTCGGCCACCGACACCACCGCCCCAAAGTCGAGATCGGACACCTCGCTGAAGCAGAACCAAGCCG CTGCCGCGACACCTGCTGATCTGGGCGACTCGCTGTGCGCGGCTACAGAATGTGCGCCTCGGCCGCTGG

Construct	Sequence form start to stop codon
	CCTGCCCTGCTGAACGACAAGTGGCAGTCGAAGACCTCGGTGGTGAACGCCTCGATCTCGGGCGACACCTCGC AGCAAGGCCTGGCTAGACTGCCTGCCTTGCTGAAGCAGCATCAGCCAAGATGGGTGCTGGTGGAGCTGGGCGG CAACGACGGCCTGAGAGGCTTTCAGCCACAGCAGACCGAGCAGACCCTGAGACAGATCCTGCAAGACGTGAAG GCCGCCAACGCCGAGCCACTGCTGATGCAGATCAGACTGCCTGCCAATTACGGCAGAAGATACAACGAGGCCT TCTCGGCCATTTACCCAAAGCTGGCCAAGGAGTTCGACGTGCCACTGCTGCCATTCTTCATGGAAGAGGTGTA CCTGAAGCCACAGTGGATGCAAGACGACGGCATCCACCCAAACAGAGACGCTCAGCCATTTCATCGCCGACTGG ATGGCCAAGCAGCTGCAGCCACTGGTGAACCACGACTCGTAA
Codon-optimized sequence for <i>O.</i> <i>polymorpha</i> (<i>SFP</i>)	ATGAAGATTTACGGAATTTATATGGACCGCCCGCTTTCACAGGAAGAAAATGAACGGTTCATGACTTTCATA TCACCTGAAAAACGGGAGAAATGCCGGAGATTTTATCATAAAGAAGATGCTCACCGCACCCCTGCTGGGAGAT GTGCTCGTTCGCTCAGTCATAAGCAGGCAGTATCAGTTGGACAAATCCGATATCCGCTTTAGCACGCAGGAAT ACGGGAAGCCGTGCATCCCTGATCTTCCCGACGCTCATTTC AACATTTCTCACTCCGGCCGCTGGGTCA TTGGT GCGTTTGATTACAGCCGATCGGCATAGATATCGAAAAAACGAAACCGATCAGCCTTGAGATCGCCAAGCGCT TCTTTTCAAAAACAGAGTACAGCGACCTTTTAGCAAAAGACAAGGACGAGCAGACAGACTATTTTATCATC TATGGTCAATGAAAGAAAGCTTTATCAAACAGGAAGGCAAAGGCTTATCGCTTCCGCTTGATTCCTTTTCAGT GCGCCTGCATCAGGACGGACAAGTATCCATTGAGCTTCCGGACAGCCATTCCCCATGCTATATCAAAACGTAT GAGGTCGATCCCGGTACAAAATGGCTGTATGCGCCGCACACCCTGATTTCCCCGAGGATATCACAATGGTCT CGTACGAAGAGCTTTTATAA

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Supplementary Table 4: Amino acid and DNA sequences of the reductase domains screened for their capability to use C10-mACP as substrates.

Construct	Sequence form start to stop codon
pAR388 (reductase domain of CAR from <i>Mycobacterium Marinum</i>)	MAAAAARKPGSSRPTFASVHGASNGQVTEVHAGDLSLDKFIDAATLAEAPRLPAANTQVRTVLLTGATGFLGRYL ALEWLERMDLVDGKLI CLVRAKSDTEARARLDKTFDSGDPELLAHYRALAGDHLEVL AGDKGEADLGLDRQWTWQ RLADTVDLIVDPAALVNHVLPYSQLFGPNALGTAELLRLALTSKIPYSYSTIGVADQIPPSAFTEDADIRVISATRA VDDSYANGYSNSKWAGEVLLREAHDL CGLPVAVFRCDMILADTTWAGQLNVPDMFTRMILSLAATGIAPGSFYEL AADGARQRAHYDGLPVEFIAEAI STLGAQSQDGFHTYHVMNPYDDGIGLDEFVDWLNESGCPQIRIADYGDWLQR FETALRALPDRQRHSSLPL LHNYRQPERPVRGSIAPTDRFRAAVQEAKIGPDKDIPHVGAPIIVKYVSDLRLLGLLG GKLHHHHHHHH
pAR388 (reductase domain of CAR from <i>Mycobacterium Marinum</i>)	ATGGCGGCCG CAGCTGCTAGAAAGCCAGGTTCTTCTAGACCAACCTTCGCTTCTGTCCACGGTGCTTCTAACGGTC AAGTCACCGAAGTTCACGCTGGTGACTTGTCTTGGACAAGTTCATCGATGCTGCTACCTTGGCTGAAGCTCCA GATTGCCAGCTGCTAACACCCAAGTTAGAACCGTTTGTGTGACCGGTGCTACCGGTTTCTTGGGTAGATACTTGG CTTTGGAATGGTTGGAAAGAATGGACTTGGTCGACGGTAAGTTGATCTGTTTGGTCAGAGCTAAGTCCGACACCG AAGCTAGAGCTAGATTGGACAAGACCTTCGACTCTGGTGACCCAGAATTGTTGGCTCACTACAGAGCTTGGCTG GTGACCACTTGAAGTTTTGGCTGGTGACAAGGGTGAAGCTGACTTGGGTTTGGACAGACAAACCTGGCAAAGAT TGGCTGACACCGTCGACTTGATCGTCGACCCAGCTGCTTTGGTCAACCACGTTTGGCATACTCTCAATTGTTCCG TCCAAACGCTTTGGGTACCGCTGAATTGTTGAGATTGGCTTTGACCTCCAAGATCAAGCCATACTCTTACACCTCT ACCATCGGTGTCGCTGACCAAATCCCACCATCTGCTTTCACCGAAGACGCTGACATCAGAGTCATCTCTGCTACCA GAGCTGTCGACGACTCTTACGCTAACGGTTACTCTAACTCTAAGTGGGCTGGTGAAGTTTTGTTGAGAGAAGCTC ACGACTTGTGTGGTTTGGCAGTTGCTGTTTTCAGATGTGACATGATCTTGGCTGACACCACCTGGGCTGGTCAAT TGAACGTTCCAGACATGTTACCAGAATGATCTTGTCTTTGGCTGCTACCGGTATCGCTCCAGGTTCTTTCTACGA ATTGGCTGCTGACGGTGCTAGACAAAGAGCTCACTACGACGGTTTGGCAGTGAATTCATCGCTGAAGCTATTTT TACTTTGGGTGCTCAATCTCAAGATGGTTTCCACACCTACCACGTTATGAACCCATACGACGACGGTATCGGTTT GGACGAATTCGTGCTGCTGTTGAACGAATCCGGTTGTCCAATCCAAAGAATCGTGACTACGGTGACTGGTTGCA AAGATTCGAAACCGCTTTGAGAGCTTTGCCAGATAGACAAAGACACTCTTCTTTGTTGCCATTGTTGCACAACTA CAGACAACCAGAAAGACCAGTCAGAGTTCTATCGCTCCAACCGATAGATTAGAGCTGCTGTTCAAGAAGCTAA GATCGGTCCAGACAAGGACATTCCACACGTCGGTGCTCCAATCATCGTTAAGTACGTCTCTGACTTGAGATTGTT GGGTTTGTGGGAGGTAAGCTTCATCATCACCACCACCACCAC
pAR389 (reductase domain of CAR from <i>Mycobacterium phlei</i>)	MAAATARRPGDGRPTFAGVHGDDAAEVHARDLTLDRFLDDATLAAATALPGPAPEIRTVLLTGATGFLGRYLALE WLERMAMVGGTLICLVRRDDAAARARLDQIFDSGDPELLRHRYELADRHLEVIAGDKSDAVLGLDRRTWQRLA DTVLDLIVDPAALVNHVLPYRELFGPNVVGTAELIRLALTGR LKPYLYTSTIAVGAGIAPGQFTEDADIRQISATRTLD DSYANGYATSKWAGEVLLREAHDL CGLPVAVFRCDMILADTSYAGQLNVPDMFTRLILSLVATGIAPLSFYELDAA GHRQRAHYDGLPVEFVAEAVSALGLDVAEDGGFATYHVMNPYDDGIGLDEFVDWLTGAGYPIERVNDYGTWTFQR FETAIRGLPERQRQASLLPLLHNYRQPPIRGSAAPTDRFRSAVQDAKIGPDKDIPHITPEVIVKYVTDLRLLGLLG GKLHHHHHHHH
pAR389 (reductase domain of CAR from <i>Mycobacterium phlei</i>)	ATGGCGGCCGCAACCGCGCGCCGACCAGGCGACGACGCGCCACCTTCGCGGGGTCCACGGCGACGACGCGCGCG AGGTGCACGCGCGCATCTGACCTGGACAGGTTCTCGACGACGCGACGCTGGCCGCGCGACCGCTCTGCCCGG TCCGGCGCGGAGATCCGCACGGTGCTGCTGACCGGGCGACCGGATTCTCGCGCGTACCTGGCGCTGGAGTGG CTGGAGCGGATGGCGATGGTCGGCGGCACCTGATCTGCCTGGTGCGCGGCGCGACGACGCGCGCGCGCGCGCG GGCTGGATCAGATCTTCGACTCCGCGCATCCGAAGTGTGCGCCACTACCGGGAGTTGGCCGACCGCCATCTCGA GGTCATCGCGGCGACAAGAGCGACGCCGCTCTCGGCTGGACCGCGGACCTGGCAGCGGTGGCCGATACCGTC GACCTGATCGTCGACCCCGCGCCCTGGTCAACCATGTGCTGCCGTACCGGGAGTTGTTGCGGCGCAACGTGGTCG GAACCGCGAGCTGATCCGTCTCGCGTCAACCGCAGGCTCAAGCCGTATCTGTACACCTCGACGATCGCTGTGGG CGCGGGGATTGCGCCCGGGCAGTTACCGAGGACGCCACATCCGGCAGATCAGCGCCACCCGGACACTCGATGAC AGCTACGCCAACGGCTACGCCACCAGCAAGTGGGCGGGCAGGTGCTGCTGCGGGAGGCACACGATCTGTGCGGCC TGCCGGTGGCGGTGTTCCGGTGCGACATGATCCTGGCCGACACAGCTACGCCGGTCAGCTCAACGTGCGCGACAT GTTACACCGGCTGATCCTGAGCCTGGTGCCACCGGGATCGCGCGCTGTGCTTCTACGAACCTCGACGCCGCGGG CACCGGCAGCGGGCGCACTACGACGGTCTGCCGGTGAGTTCTGTCGCGAGGCCGTCTCCGCACTCGGTCTCGACG TGGCCGAGGACGGCGGCTTCGCGACCTACCAGTGATGAACCCGTACGACGACGGCATCGGGCTCGACGAGTTCGT CGACTGGCTGACCGGGGCGGCTATCCGATCGAGCGGTCAACGACTACGGCACaTGGTTTCAGCGGTTTCGAGACC GCGATCCGCGGCTGCCCGAACGCGAGCGCAGCGCTCGCTGCTTCCGCTGCTGCACaCTACCAGCGGCGCGCAGC CGCGGATCCGCGGGTCGGCCGCCCGACCGATCGGTTCCGACGCGGGTGACGAGCGGAAGATCGGCCCGGACAA GGACATTCCGCACATACCCCGGAGGTGATCGTCAAGTACGTACCGATCTGCGGCTGCTCGGGCTGCTCGGAGGT AAGCTTCATCATCACCACCACCACCAC

pAR390 (reductase domain of CAR from <i>Mycobacterium smegmatis</i>)	MAAAAQRTAGDRRPSFTTVHGDADTEIRASELTLDKFIDAETLQAAPGLPKVTTEPRTVLLSGANGWLGRFLTQ WLERLAPVGGTLITIVRGRDDAAARARLTQAYDTPELSRRFAELADRHRLRVVAGDIGDPNLGLTPEIWHRLAAE VDLVVHPAALVNHVLPYRQLFGPNVVGTAIEVIKLALTERIKPVTYLSVSVAMGIPDFEEDGDIRTVSPVRPLDGGY ANGYGNKSWAGEVLLREAHDLCLGPLVATFRSDMILAHPRYRGQVNVPMFTRLLSLITGVAPRSFYIGDGERPR AHYPGLTVDFVAEAVTTLGAQQREGYVSYDVMPNHDDGISLDVFVDWLIRAGHPIDRVDDYDDWVRRFETALTA LPEKRRATVLP LLHAFRAPQAPLRGAPEPTEVFHAAVRTAKVGP GDIPHLDEALIDKYIRDLREFGLIGGKLHHH HHHHH
pAR390 (reductase domain of CAR from <i>Mycobacterium smegmatis</i>)	ATGGCGGCCGCAGCGCAGCGCACCGCGGGTGACCGCAGGCCGAGTTTCACCACCGTGACGGCGCGGACGCCACCG AGATCCGGGCGAGTGAGCTGACCTTGGACAAGTTTCATCGACGCCGAAACGCTCCaGGCCGCACCGGGTCTGCCCAA GGTCACCACCGAGCCACGGACGGTGTTGCTCTCGGGCGCCAACGGCTGGCTGGGCGCGTTCTCAGCTTGCACTGG CTGGAACGCTGGCACCTGTGCGGCGCACCTCATCACGATCGTGCGGGGCGCGACGACGCGCGCGGCCCGGCAC GGCTGACCCAGGCCTACGACACCGATCCCGAGTTGTCCGCGCGTTTCGCCGAGCTGGCCGACCGCCACCTGCGGGT GGTCGCGGTGACATCGGCGACCCGAATCTGGGCCTCACACCCGAGATCTGGCACCGGCTCGCCGCGGAGTGCAC CTGGTGGTGATCCGGCAGCGCTGGTCAACCACGTGCTCCCTACCGGCAGCTGTTCCGCCCCAACGTCGTGGGCA CGGCCGAGGTGATCAAGCTGGCCCTACCGAACGGATCAAGCCCGTCACGTACCTGTCCACCGTGTGCTGGCCAT GGGGATCCCGACTTCGAGGAGGACGGCGACATCCGGACCGTGAGCCCGGTGCGCCCGCTCGACGGCGGATAACGCC AACGGCTACGGCAACAGCAAGTGGGCGGGCAGGTGCTGCTGCGGGAGGCCACGATCTGTGCGGGTGCCTGTG GCGACGTTCCGCTCGGACATGATCTGGCGCATCCGCGCTACCGCGGTCAAGTCAACGTGCCAGACATGTTACGCG GACTCCTGTTGAGCCTCTTGATCACCGCGCTCGCGCGCGGTGCTTCTACATCGGAGACGGTGAGCGCCCGCGGGC GCACTACCCCGCCTGACGGTTCGATTTCTGTCGCCGAGGCGGTACGACGCTCGCGCGCGCAGCAGCGCGAGGGATAC GTGTCCTACGACGTGATGAACCCGCACGACGACGGGATCTCCCTGGATGTGTTCTGTTGACTGGCTGATCCGGGCG GGCCATCCGATCGACCGGGTTCGACGACTACGACGACTGGGTGCGTGGTTTCGAGACCGCGTTGACCGCGCTTCCCG AGAAGCGCGCGCACAGACCGTACTGCCGTGCTGCACGCGTTCCGCGCTCCGAGGACCGGTTGCGCGCGCGACCC CGAACCCACGGAGGTGTTCCACGCGCGGTGCGCACCGCGAAGGTGGGCGCGGAGACATCCCGCACCTCGACGAG GCGTGATCGACAAGTACATACCGGATCTGCGTGAGTTCGGTCTGATCGGAGGTAAGCTTCATCATCACCACCAC CACCACCAC
pAR391 (reductase domain of CAR from <i>Nocardia iowensis</i>)	MAAAAERNSGAKRPTFTSVHGGGSEIRAADLTLDKFIDARTLAAADSIPHAPVPAQTVLLTGANGYLGRFLCLEWL ERLDTGGTLICVVRGSDAAAARKRLDSAFDSGDPGLEHYQQLAARTLEVLADIGDPNLGLDDATWQRLAETV DLIVHPAALVNHVLPYPTQLFGPNVVGTAIEVRLAITARRKPVTYLSTVGVADQVDP AEYQEDSDVREMSAVRVVRE SYANGYGNKSWAGEVLLREAHDLCLGPLVAVFRSDMILAHRSYAGQLNVQDVFTRLILSVLATGIAPYSFYRTDADG NRQRAHYDGLPADFTAAAITALGIQATEGFRITYDVLNPNYDDGISLDEFVDWLVESGHPHQIRITDYSDFHFRFETAI RALPEKQRQASVLP LLDAYRNPAPVRGAILPAKEFQAQVQTAKIGPEQDIPHLASPLIDKYVSDLELLQLLGGKHLH HHHHHHH
pAR391 (reductase domain of CAR from <i>Nocardia iowensis</i>)	ATGGCGGCCGCAGCGGAACGCAACTCGGGCGCGAAGCGTCCCACCTTCACCTCGGTGCACGGCGGCGGTTCCGAGA TCCGCGCCCGCGATCTGACCTTCGACAAGTTTCATCGATGCCCGCACCTTGGCCGCGCGGACAGCATTCCGCACGC GCCGCTGCCAGCGCAGACGGTGCTGCTGACCGCGCGCAACGGCTACCTCGGCCGTTCTCTGCTGGATGGCTG GAGCGCTGGACAAGACGGGTGGCACGCTGATCTGCGTCTGCGCGGTAGTGACGCGCGCGCGGCCGCTAAACCG CTGGAAGTCCGCGTTTCGACAGCGCGGATCCCGGCTGCTCGAGCACTACCAGCAACTGGCCGACGACCGCTGGAAG TCTCTCGCGGTGATATCGGCGACCCGAATCTCGGTCTGGACGACGCGACTTGGCAGCGGTTGGCCGAAACCGTCA CCTGATCGTCCATCCCGCGCGTGGTCAACCACGTCTTCCCTACACCCAGCTGTTCCGCCCAATGTCGTGCGG ACCGCGGAAATCGTCCGTTGGCGATCACGGCGCGCGCAAGCCGTCACCTACCTGTGACCGTCCGAGTGGCGG ACGAGTTCGACCGCGCGGAGTATCAGGAGGACAGCGACGTCGCGGAGATGAGCGCGGTGCGCGTCTGCGCGAGA GTTACGCCAACGGCTACGGCAACAGCAAGTGGGCGGGGAGGTCTGCTGCGCGAAGCACACGATCTGTGTGGCT TGCCGGTTCGCGGTGTTCCGTTTCGGACATGATCTTGGCGCACAGCCGGTACGCGGGTCAAGTCCAGGACGCT GTTACCCCGGTGATCCTCAGCTGGTTCGCCACCGGCATCGCGCGTACTCGTTCTACCGAACCGACGCGGACGGC AACCGGACGCGGGCCACTATGACGCTTCCCGCGGACTTACGCGCGCGGCGATCACGCGCGCGGATCCGCGCTCGGATCCAAG CCACCGAAGCTTCCGACCTACGACGTGCTCAATCCGTACGACGATGGCATCTCCCTCGATGAATTCGTCACTG GCTCGTCAATCCGGCCACCGATCCAGCGCATCACCGACTACAGCGACTGGTTCCACCGTTTCGAGACGGCGATC CGCGCGTTCGCCGAAAAGCAACGCCAGGCTCGGTGCTGCGGTTGCTGGACGCTACCGCAACCCCTGCCGCGCG TCCGCGCGCGGATACTCCCGGCCAAGGAGTTCCAAGCGCGGTGCAACAGCCAAATCGGTCCGGAACAGGACA TCCCGCATTTGTCCGCGCACTGATCGATAAGTACGTACGCGATCTGGAAGTCTCAGCTGCTCGGAGGTAAGCT TCATCATCACCACCACCACCACCAC
pAR392 (reductase domain of CAR from <i>Nocardia otitidiscaviarum</i>)	MAAASERDSGRPTAATVHGDDGLLRADDLALEAFDPATLDAAAHLPSALEPPRTVLLTGANGYLGRFLALEWL QRLDVSGGTLICLIRGSDADSARRRLDAVFATGDPLEAHYRELAERRLRVLPGDIGEPNLGLREQDWRDLAETVD LIVHPAALVNHVLPYAQLFGPNVVGTAIEVIRLALTSRLKPVTYLSTVAVSAGIDPETFTEDGDIREISPVRRLLDDGYA NGYGNKSWAGEVLLRNAHDRFGLPVAVFRSDMILAHRSYAGQLNVPMFTRLLSVLATGLAPGSFHDHAHERH RAHYDGLPADFTAAAVTTLGSRVTSYETYDVLNPHDDGISLDTFVDWLEAGHPIDRIDDYAEWFARFDTALRAL PEHQHQHSLLP LLHAYRRPTPPLHGVALPAKHFAAVQQAALGPDGDIPHVTRELIEKYASDLRLGLIQGGGKLH HHHHHHH

pAR392 (reductase domain of CAR from <i>Nocardia otitidiscaviarum</i>)	ATGGCGGCCGCAAGCGAACGCGATTCCGGCAGTCGTCGACCGCCGCCACGGTGACGCGGACGACGGCCTGCTGC GGGCGGACGATCTCGCCCTGGAGGCATTCTCGACCCGGCGACCTGGACGCGGCCGCGCATCTGCCGAGCGCGCT GGAGCCGCCGCGCACGGTTCTGCTGACCGGCGCCAAACGGCTACCTCGGCCGCTTCTCGCCCTGGAATGGGTACAG CGCCTGGACGTCTCGGGCGGTACTCTGATCTGCCTGATCCGCGGCAGTGACGCCGACAGTGCTCGGCGCGCTCTCG ACGCGGTCTTCGCCACCGGTGATCCGGAGCTGGAAGCCCACTACCGCGAACTCGCCGAGCGCGGGTGCCTGTCTCT GCCCCGCGATATCGGTGAACCGAATCTCGGCCTGCGCGAACAGGATTGGCGCGATCTGGCCGAGACCGTGGACCT GATCGTGATCCGGCGGCCCTGGTCAACCATGTGCTGCCCTACGCCAGTTGTTTCGGCCCCAATGTTGTGCGTACC GCCGAGGTGATCCGTCTCGCGCTCACCTCCCGGCTGAAGCCGGTCACTACCTGTCCACCGTGGCGGTATCCGCGG GTATCGACCCGGAGACCTTCACCGAGGACGGCGATATCCGCGAGATCAGCCCCGTGCGCGGGTTCGACGACGGCTA CGCCAAATGGCTACGGCAACAGCAAATGGGCCGGCGAGGTGCTGTTGCGCAATGCGCAGACCGCTTCGGTCTCCCG GTGGCGGTCTTCCGCTCCGACATGATCTGGCCACAGCCGCTACGCCGACAGCTCAACGTGCCGACATGTTCA CCCCCTGTGCTGAGCGTGCTGGCGACCGGCTGGCCCCCGGCTCCTTCCACGATGCCACGGCGAGCGCCACCGC GCCACTACGACGGTCTCCAGCCGATTTACACGCCGCGGCCGTACCCACCTGGGCGAGCCGGGTGACCAAGTGGTT ACGAGACCTACGACGTGCTCAACCCGACGACGACGGGATCTCCCTGGACACCTTCGTGCACTGGCTCATCGAGGC GGGCCACCCCATCGACCGCATCGACGATTACGCCGAGTGGTTTCGCCGCTTCGACACCGCCCTGCGCGCCCTCCCG AACACCAACGCCAACACTCGCTGCTACCCCTGCTGCACGCCTACCGTCGCCCAACCCCTCCCTGCACGGAGTCGCC CTCCCCGCCAAGCACTTCGCGCGCGCGTACAGCAGCGGAAACTCGGCCCGGACGGGATATCCCGACGTGACGC GGGAGCTGATCGAGAAGTACGCGAGTGATCTGCGGTTACTGGGGTTGATTACGGGCGGAGGTAAGCTTCATCATC ACCACCACCACCAC
pAR393 (reductase domain of CAR from <i>Tsukamurella paurometabola</i>)	MAAAKARSGGVAAPTADSVHVGASVARATDLTLEKFIDPELLALAPTLPAATGEPNTVLLTGSTGYLGRFLLLDW LRRVAPHGGTVIALVRGADADDARRRVTAAGIDSDPDLTQEFTSLAEHLHLVIAGDFGSPALGLDDATWSDLAGR VDHVHCGALVNHVLPYDQLFGPNVVATGEVRLALTRRRKSVYDVSTVAVVPQDDGRVLVEDDDVRELGAERRI GADAYANGYAVSKWAGEVLLHEAADLADLPVVRFSRDMILAHRSRHFHQFNEVDQFTRLLLSIAETGLAPAFYTPD PSGHRPHYDGLPVDFTAETITLSAAGRSYRTFHVNLNANDDGVSLDSFVDWIAASGRSIERIDYDTWFARFEQA LQQLPDEARQRSVLP LLHAVREPAPAAGTSALSVDVFRGAVRETGVGPGDIPVLDRLALIEKYLRDFETAGWLAPGA RDGGKLHHHHHHHH
pAR393 (reductase domain of CAR from <i>Tsukamurella paurometabola</i>)	ATGGCGGCCGCAAAGGCTCGATCGGGTGCGCTCGCGGCACCGACCGCCGACTCGGTGCACGGCGTGGGTGCGAGCG TCGCCCGGGCCACCGACCTGACGCTGGAGAAATTTCATCGACCCCGAGCTCCTCGCGCTCGCGCCGACGCTTCCCGC GGCGACCGGTGAGCCGAACACCGTCTGCTACCGGATCCACCGGTACCTCGGCCGCTTCTGCTGCTGGACTGG TTGCGACGGGTGCTCCGCACGGCGGCACCGTGATCGCGCTGGTGCGCGCGCCGACGCGGACGATGCGCGACGCC GCGTCACGGCCGCGATCGGTGACTCGGATCCTGACCTGACACAAGAGTTACGTCACCTCGCGGAGCATCACCTCCA CGTGATCGCGGTGACTTCGGCAGCCCCGCACTCGGATCGACGATGCCACCTGGAGCGATCTCGCGGGCGAGTC GATCAGCTGGTGCACTGCGCGCGCGTCTGTCACCACTGCTGCCCTACGACCAACTGTTTCGGTCCCAATGTGGTG CCACCGCGAAGTGGTGCGACTCGCACTACCACGCGCCGCAAGTCCGTGGATTACGTCTCCACGGTGGCTGTGGT TCCGAGGATGACGGCCGCTCCTGGTTCGAGGACGACGATGTTTCGCGAGCTCGCGCCGAACCGCGCATCGGGCC GATGCCACGCGAACGGCTACGCCGTGAGCAAATGGGCGGCGAAGTGTGTTGCATGAGGACGCGACCTGGCG GACCTGCCGTGCGGGTGTTCGCTCCGATATGATCTTGGCGCACAGTCGATTCCACGGACAGTTCAACGAGGTGCG ACGAGTTCACCCGCTGCTCCTGAGTATCGCCGAGACCGGACTGGCGCGCGCTCGTTTACACGCCGGATCCGAG TGGACACCGCCGCACTACGACGGGTGCGGTGGACTTCACCGCCGAAGCGATCACCACGCTCAGCGCCGCGGGG CGTTTCGGGTACCGGACCTTCCACGTGCTCAACGCCAACGATGACGGCGTGAGCCTGGACAGCTTCGTGCACTGGA TCGCCGCTCGGGCCGAGCATCGAACGGATCGACGACTACGACACCTGGTTTCGCCCGGTTTCGAGCAGGCGCTCCA GCAGTCCCCGATGAGGCGCGCCAGCGGTGCGTCTGCCCTGCTGCACGCGGTGCGCGAGCCGGTCCGGCCGCGC GGGACCTCCCGCTGTGCGTGAGCCGTTCCGTGGTGCGGTGCGTGAGACCGGATAGGACCGGGGACATCCCG GTGCTCGATCGCGCCTGATCGAGAAGTACCTGCGCGACTTCGAGACCGCGGGTGGCTCGCGCCCGGTGCGCGCG ACGGAGGTAAGCTTCATCATCACCACCACCACCAC
pMMH12 (reductase domain of CAR from <i>Neurospora crassa</i>)	MAAQGTLDNEHHVMEALVEKYTRDLPKQKNPAPADEGQVVVITGTTGGIGSYLIDICSSSRVSKIICLNRSDEG KARQTASSSGRGLSTDFSKCEFYHADMSRADLGLPEVYSRLLSEVDRVIHNQWPVNFNIAVESFEPHIRGCRNLV DFSYKADKNVPIVFVSSIGTVDRWHEDEDRIVPEASLDDLALAGGYGQSKLVSSLIFDKAAEVSGVPTFVVRVQVA GPSSEKGYWKNQEWLPSIVASSAYLGVLPDSLGMQMTTIDWTPIEAIAKLLLEVSGVIDNVPLDKINGYFHVNPRT SWSALAPAVQEYYGDRIQKIVPLDEWLEALEKSQEKAEDVTRNPGIKLIDTYRTWSEGYKKGTFKVP LDMTRTKE YSKTMREMHAVTPELMKNWCQWQNFKLHHHHHHHH
pMMH12 (reductase domain of CAR from <i>Neurospora crassa</i>)	ATGGCGGCCAGGGCACCTGGACAATGAACATCACGTGATGGAAGCACTGGTTGAAAAATATACCCGTGATCTG CCGACGCCGAAACAGAACAAACCGGCTCCGGCGGACGAAGTCAAGTCGTGGTTATTACCCGTACACGGGTGGC ATCGGCTCCTACCTGATTGATATCTGCTCCTCATCGAGCCGCTGTCCAAAATTATCTGTCTGAATCGTTTCAGAAG ATGGCAAAGCGCGCCAGACGGCTCTAGTTCGGTCTGTGGCCTGAGCACCAGCTTTTCTAAATGCGAATTCTATCA TGACATATGAGCCGCGCTGACCTGGGTGCGGTCGGAAGTGACAGTCGTCTGCTGTCCGAAGTGGATCGCGT TATTTCATAACCACTGGCCGGTCAACTTTAATATTGCGGTGGAATCTTTTGAACCGCACATCCGTGGTTGTGCGAA TCTGGTGGATTTTAGTTATAAAGCCGACAAAACGTTCCGATTGTTTTCTGCTCATCGATCGGCACCGTCGATCG TTGGCAGGATGAAGACCGCATTTGTCCGGAAGCGTCACTGGATGACCTGTGCTGGCCGCGGTGGCTACGGCCA GTCCAAACTGGTGAGCTCTCTGATCTTTGATAAAGCAGCTGAAGTCTCAGGCGTGCCGACCGAAGTCGTGCGTGT TGGTCAGGTTGCGGGTCCGAGTTCGGAAGGTTATTGGAATAAACAAGAATGGCTGCCGTGATTCGCGATC

	ATCGGCTTACCTGGGTGTGCTGCCGGATAGCCTGGGCCAGATGACCACGATCGACTGGACGCCGATTGAAGCCATCGCAAAACTGCTGCTGGAAGTGTCTGGCGTTATTGATAACGTGCCGCTGGACAAAAATCAATGGTTATTTTCATGGCGTTAACCCGGAACGTACCAAGTTGGAGCGCTCTGGCCCCGGCAGTCCAGGAATATTACGGTGATCGCATTCAAAAAATCGTTCCGCTGGACGAATGGCTGGAAGCGCTGGAATAATCTCAGGAAAAAGCCGAAGATGTGACCCGTAACCCGGGCATTAACTGATCGACACCTATCGCACGTGGTCTGAAGGTTACAAGAAAGGCACGAAATTTGTTCCGCTGGAATGACCCGCACGAAAGAATATAGTAAACCATGCGTGAAATGCACGCTGTGACGCCGGAACGTGATGAAAACTGTGTGCGCAATGGAACTTTAAAGCTTCATCATCACACCACCACCACCAC
pAR394 (reductase domain of PKS/NRPS from <i>Stigmatella aurantiaca</i>)	MAAAAAKTGSLPAHDVTVEMEADAVLDAEIALGKALPPVTGALRTILLTGATGFLGAFLEELCRRTDARIYCLVR SKTEQEGMNRIRKNLESYSLWNEALAPRIVPVRGDIGQPLLGLSEKEFQRLSEEIDAIYHNGALVNFLYPYESMRAA NVLGTREILRLATRTRIKPLHYVSTVSVLPLGRKAPIREDEPLEGPSSLVGGYAQSKWVAEKLVREASRRGLPVTLR PGRVTGHSRTGAWNTDDLVCRTLKGCVRMVGAPSVDALDLTPVDYVSSAIVDLMSRPESIGQTYHLVNPQVFRA DEMWNYMRAFGYGLRVLVPYDQWLSELGSAASDSELGDLLMFLQVPPEDRSVGGPRMNVCDSGDTLKALGGTG TSCPSVDASLISTYLSLVHRGFLKAPEVRGGKLHHHHHHHH
pAR394 (reductase domain of PKS/NRPS from <i>Stigmatella aurantiaca</i>)	ATGGCGGCCGCAGCCGGAAGACAGGCTCGCTGCCAGCCACGATGTGACGGTGGAGATGGAAGCAGATGCTGTG CTGGATGCCGAGATCGCGCTGGGCAAGGCACTTCCGCCCCGTGACGGGGGCTTTGCGCACCATCTGCTGACCGGCG CCACGGGCTTCTCGGTGCTTTCTTGTGAGGAACTCTGCCGAGGACGACGCGCGCATTTATTGCTTGGTGCG CTCCAAGACAGAGCAGGAGGGGATGAACAGGATTCCGAAGAACCTGGAGAGCTACTCCTGTGGAACGAGGCTCT GGCGCTCGCATCGTTCCTGTCCGAGGAGATATTGGCCAACCGTTGTTGGGACTCTCGGAGAAGGAGTTCAGCG GCTCTCGGAAGAGATTGACGCCATTTATCACAATGGCGCGCTCGTCAATTTCTCTACCCCTACGAGTCCATGCCA GCGGCCAACGTGCTTGGCACGCGGGAGATCTCCGGCTGGCTACGCGGACGCGCATCAAACCGTTGACTACGTTT CGACTGTTTTCGGTATTGCCCTGGGACGAAAGGCTCCGATCCGCGAGGATGAGCCTTTGGAAGGGCCCTCAAGTT TGGTGGGAGGCTACGCACAGAGCAAGTGGGTTGCCGAGAAGCTTGTGAGAGAGGCTTCTCGGCGGGGCTCCCGG TGACCATCCTCCGGCCGGGACGGGTGACCGGCCACAGCCGACGCGGGGCTGGAACACGGACGATCTGGTGTGCAG GACCCTCAAGGGGTGCGTCCGGATGGGAGTGGCTCCAGCGTCGATGCGCTGCTTGACCTGACGCCCCGTCGATTAC GTCAGCAGTGCCATCGTGGATCTGTCCATGCGCCCGAGTCGATTGGCCAGACGTATCATCTCGTCAATCCGCACT TCGTGCGCGTGATGAGATGTGGAATTACATGCGAGCCTTTGGGTACGGATTGCGAGTCTCCCGTATGACCAAT GGCTTTCGGAATTGGGCTCCGCGGCATCGTCCGACAGCGAGCTGGGCGATCTTCTCATGTTCTCCAGAGGTTCC TCCAGAGGACCGGAGCGTTGGCGGACCGCGGATGGTGGTTTGCACAGTGGCGACACGCTGAAGGCCTTGGGAGG AACGGGAACGTCTCTGCCGTCGGTGGACGCCTCTTTGATTTCAACCTACCTCTCGTCTGCTCGTTACCGCGGCTTT CTCAAGGCACCCGAGGTGAGGGGAGGTAAGCTTCATCATCACACCACCACCACCAC
pAR395 (reductase domain of PKS/NRPS from <i>Mycobacterium tuberculosis</i>)	MAAADARPTSDPRLVSVHGDNPTEVHASDLDLDRFIDATLATAVNLPGPSPELRTVLLTGATGFLGRYLVLLEL RRLDVDGRILCLVRAEDEDARRRLEKTFDSGDPELLRHFKELAADRLEVAVAGDKSEPDGLDQPMWRRLAETVD LIVDSAAMVNAFPYHELFGPNVAGTAELIRIALTTKLKPFYVSTADVGAIEPSAFTEDADIRVISPTRTVDGGWA GGYGTSKWAGEVLLREANDLCALPVAVFRGMILADTSYAGQLNMSDWVTRMVLSLMATGIAPRSFYEPDSEGN RQRAHFDGLPVTFFVAEIAIVLGARVAGSSLAGFATYHVMNPHDDGIGLDEYVDWLIEAGYPIRRIDDFAEWLQRFE ASLGALPDRQRHSVLPMLLASNSQRLQPLKPTRGCSAPTDRFRAAVRAAKVGSKDNDPDIHVSAPTHINYVTNL QLLGLLGGKLHHHHHHHH
pAR395 (reductase domain of PKS/NRPS from <i>Mycobacterium tuberculosis</i>)	ATGGCGGCCGCACGAGATGCCGACCGACGAGATCCGCGCTTGGTGTCTGTGCACGGCGACAACCCACCGAGG TGATGCCAGCGACCTCACGCTGGACCGGTTATCGACGCCGACACGCTGGCCACCGCGCTCAACCTGCCGGGCCC GAGCCCCGAGCTACGGACGGTCTGCTGACGGGCGCGACGGGTTTCTCGGACGGTATCTGGTCTTGAATTGCTG CGGCGGCTGGACGTCGACGGCAGGCTGATCTGTTTGGTGGGGCGGAGTCCGACGAGGATGCGCGGCGTCTGTG GAGAAGACCTTCGATAGCGGTGACCCGGAATTGCTGCGGCACTTCAAGGAGCTTCCGCGCGACCGGCTGGAGGTC GTCGCAGGCGACAAGAGCGAACCCGACCTGGGCTGGACCAACCGATGTGGCGGCGGCTGGCCGAAACCGTGGAT TTGATTGTGATTCCGCGCGGATGGTCAACGCGTTTCCCTACCACGAATTGTTCCGGGCCAACGTCGCGGGCACCG CCGAGCTGATCCGAATCGCGCTTACCACCAAGCTCAAACCTTACCTACGTGTCAACCGCCGACGTGGGTGCTGC GATCGAGCCGTCGGCGTTACCCGAGGACGCCGACATCCGGGTAATCAGCCCCACCGCACCGTGCAGCGCGGCTGG GCTGGCGGCTACGGCACCAAGTAGGGCCGGTGAGGTGCTGCTGCGCGAGGCCAACGACCTGTGCGCGCTGCCGG TCGCGGTGTTTCTGCTGCGGGATGATCCTGGCCGACACAGCTATGCCGGACAGCTCAACATGTGCGACTGGGTAC CCGGATGGTGTGAGCTTGATGGCTACCGGCATCGCGCTCGTTCTGTTCTACGAACCGGACTCCGAGGGCAATCGG CAACGCGCACTTCGACGGGCTGCCAGTCACCTTCTGTTGCGGAGGCGATCGCGGTGCTGGGCGCGGGGTGGCCG GCTCATCGTTGGCGGGATTGCGACCTATCAGTGATGAACCCGACGACGACGGTATCGGGCTCGATGAGTATG TGGACTGGCTGATTGAGGCCGGCTACCCGATACGCGCATCGATGACTTTCGCGAGTGGTTGCAGCGGTTTGAGG CCAGCTGGGCGCTCTGCCGATCGGCAACGCCGGCACTCGGTGCTGCCGATGCTGTGGCGAGCAATCCCAGCG ATTGCGAGCCGCTTAAGCCGACCGGGGTGCTCCGCGCCGACCGACCGATTCCGTGCCGCGGTGCGAGCGGCGAAA GTCGGCTCCGACAAGGACAATCCAGACATCCGCGACGTGTCGGCGCGGACCATCATCACTACGTCCACCACTAC AACTGCTCGGACTGCTGGGAGGTAAGCTTCATCATCACACCACCACCACCAC

pAR396 (reductase domain of PKS/NRPS from <i>Mycobacterium smegmatis</i>)	MAAAHDAGERTDRVSFAAVHGSVDTEVHARDLTLDKFIDAPTLRTATTLPRPDGAVQTVLLTGATGFLGRYLLLE WLRQLRRVDDKVICLVRGKSDDEDARRRLEATFDTDPLLRKHFNELATERLQVVAGDKGQPNLGLDEQTWQRLAE SVDLIVDSAAAFVNSVLPYSELFGPNVVGTAELIRFALTSKLKPFNFVSTSDVGRQIEPSRFTEQADIRLVSATRKIEVG YANGYGNSKWAGEVLLREAHDHCLPVAVFRSGMIMVDPTYAGQLNVTDTVSRMVLIVATGVAPGSFYQRGDN GERQRAHFDGLPVDFAQAITKLGWQVARSVTDSTASGFETYHVMNPHDDGIGIDTYIDWLEAGYPIERIEDFGE WLQRFEAALQGLSDQQRQNSVLQMLTLLKQQAGELQPPVPTRGSFAPADRFQAAVRDANIGVEGEIPHVTVREVIV KYVTDLQLLGLLGGKLLHHHHHHHH
pAR396 (reductase domain of PKS/NRPS from <i>Mycobacterium smegmatis</i>)	ATGGCGGCCGCACACGACGCCGGTGAGCGGACCGACAGGGTCAGCTTCGACGCGGTGCACGGCAGCGACGTCACCG AGGTGCATGCGCGCGATCTGACACTGGACAAGTTTCATCGACGCTCCGACACTGCGGACCGCGACGACCTGCCCGG ACCCGACGGCGCGGTGCAGACGGTCTCTGACCGGGGCCACCGGATTCTAGGGCGCTACCTGCTGCTGGAATGG CTGAGGCAGTTGCGGCGTGTGCAGCACAAGGTGATCTGCCTGGTGCGCGGAAAGTCCGACGAGGACGCCCGCAGG CGCCTCGAGGCCACTTTCGACACAGATCCGTTGCTACGCAAGCATTTCAACGAACTGGCCACCGAGCGCCTGCAGG TCGTGCGGGCGACAAGGGACAACCGAACCTCGGGCTCGACGAACAGACCTGGCAGCGGCTGGCCGAAAGTGTCTG ACCTGATCGTCGATTGCGGCGCATTCGTCAACAGTGTGCTGCCCTACAGCGAACTGTTGCGACCCAACGTGGTGGG CACGGCCGAGCTGATCCGGTTCGCGCTGACCTCGAACTCAAGCCGTTCAACTTCGTGTGACGCTCGGACGTCGGC AGGCAGATCGAGCCGTCGCGGTTACCGAGCAGGCCGACATCCGGTTGGTCAGCGCGACCCGCAAGATCGAGGTC GGGTATGCCAACGGCTACGGCAACAGCAAGTGGGCAGGCGAGGTGCTGCTGCGCGAGGCCACGACCACTGCGGC CTGCCGGTGGCGGTGTTCCGGTCGGGCATGATCATGGTCGACCCGACCTACGCCGGGACGCTGAACGTCACGGACA CGGTGTGCGCATGGTGCTCAGCATCGTGCCACCGGTGTCGCACCCGGGTGCTTATCAGCGCGGTGACAACGG TGAGCGGCAGCGGGCACACTTCGACGGCTTGCCCGTCGACTTCGTGCGCCAGGCCATCACCAGCTCGGCTGGCAG GTCGCGAGGTGGTGACCGATTCCACCGCAAGCGGTTTCGAGACTTATCAGTGATGAACCCGATGACGACGGC ATCGGCATCGACACCTACATCGACTGGCTCATCGAGGCCGGATACCCGATCGAACGCGATCGAGGACTTCGGAGAG TGTTGTCAGCGGTTTGAAGCGGCCCTGCAGGGGCTGTCGGACCAACAGCGGCAGAACTCCGTGCTGCAGATGCTG ACGTTGCTCAAGCAGCAGCGGGGGAGCTGCAGCCACCAGTGCCCAACCGGGGCTGTTTGGCGCGGCAGACCGGT TCCAGGCCGCGGTCCGCGATGCGAACATCGGTGTGGAAGGCGAGATTCCGCATGTACGCGGGAGGTGTCATGTC AGTACGTGACCGATCTGCAGCTGCTCGGGTGTGCGGAGGTAAGCTTCATCATCACCACCACCACCACCAC
pMMH05 (reductase domain of NRPS from <i>Methanobreviba cter ruminantium</i>)	MAADIGEEDLDLTDIIKNYNYGEINELLQENTWENFFDGENLELGNVLLTGATGFLGIHILYEFIKSEEGKIYCMRLR KGKFDSCQERLIDVMNDYFDEDFDVLVGSRIPIEGDITEIDDFKQLEDEPIDTVINSAALVKHYTADDYIFRVNVDG VINGLKFAQTRNNIKYVQISTISVLSYSLNEEAYPNQEYDERTLYEQDLENKYVCSKFLAERAVLQAATKGLPVKI IRVGNLMSRYSDFVQKKNYDNTAFNLNIIKLGAMNPAMASEKVDMSQIDYVAKGILALSKTPEKSRVHFHMCN NHYISHRDIVDALNTYGYGIEVDFFEEKQIYEQNMENIQHITADFSIDDFDEEDDFEENVEIEQTVIDLHSLGFD WPEADEEYLKRLFDYLNKFDYFEKLHHHHHHHH
pMMH05 (reductase domain of NRPS from <i>Methanobreviba cter ruminantium</i>)	ATGGCGGCCGACATTGGCGAAGAGGACTTGGATTAACTGATGATATCATCAAAACTACAATTATGGCGAAATC AATGAGCTTCTCCAAGAGAATACATGGGAAAACCTCTTTGATGGTGAAATCTGGAATTGGGCAATGTCTATTG ACTGGAGCTACAGGATTTTATAGGAATCCATATCCTATATGAATTCATCAAAAGTGAGGAAGGAAAGATCTATTG TATGTTAAGGAAAGGCAAGTTTGATTCTGTGAGGAACGGTTGATTGATGTGATGAATGATTACTTTGATGAGG ATTTCACTGATCTCGTCCGCTCACGCATCATTTCAATCGAAGGGGACATACTGAAATCGATGATTTTAAACAGC TTGAGGATGAGCCTATTGATACGGTGATAAATTCGGCCGCACTTGTAAGCATTATACTGCCGATGATTATATCT TCAGGGTGAATGTGATGGAGTAATCAATGGACTTAAATTTGCCCAAAGTAAATAACATAAAATATGTACAG ATATCAACAATCAGCGTCTTTTCATCATACTCTTAAATGAAGAGGCATATCCGAATCAGGAATATGATGAAAGG ACATTATATTATGAGCAGGATCTAGAAAACAAATATGTATGCAGTAAGTTTTAGCTGAAAGGGCGGTTCTGCA AGCTGCAACTAAAGGGCTTCAGTAAAGATAATCAGAGTTGGAATCTTATGAGCCGTTATTCAGATGGTGTGTT CCAGAAAAATTATGATACAAATGCATTTTAAATAACATTAAACCATTAATAAATAGGAGCTATGAATCCTG CAATGGCTAGTGAAAAGGTTGACATGAGTCAAAATAGATTATGTTGCAAAGGGAATACTTGCAATGTCTAAGACT CCAGAGAAATCCAGGGTATTCATTGTATGAATAATCATTATATTTCCCATAGGGATATTGTTGATGCATTGAAT ACATACGGTTACGGAATTGAAGAAGTGGATTTCGAAGAATTCAAACAGATTACGAACAAAACATGAATGAGAA TATACAGGGTATAATAACTGCCGATTTTCAATTGATGACTTTGATGAGGAAGACGATTTTCGAGGAGAATGTGG AAATTGAACAGACTGTTGATATTCTTCATTGCTTAGGATTTGATTGGCCTGAAGCAGATGAAGAGTATCTTAAAC GATTGTTTGATTATTTGAATAAGTTTGATTATTTTGAAAAGCTTCATCATCACCACCACCACCACCAC

Supplementary Table 5: Amino acid and DNA sequences of the constructs used in the fatty aldehydes/alcohol experiments. Mutated positions and the performed mutations are highlighted.

Construct	Sequence form start to stop codon
pMMH01 (mouseFAS_ΔTE _CAR-TR_Mphi, Strep-and His- tagged) Position 113 highlighted	MSAWSHPQFEKGGGSGGSGSAWSHPQFEKGAGSEEVVIAGMSGKLPESENLQEFWANLIGGVDMVTDDDR RWKAGLYGLPKRSGKLDLSKFDASFFGVHPKQAHTMDPQLRLLLEVSYEAIVDGGINPASLRGTNTGVWVG SG/ W SEASEALSRDPETLLGYSMVGCQRAMMANRLSFFDFKGPSIALDTACSSSLLALQNAYQAIRSGECPAA LVGGINLLLKPNTSVQFMKLGMLSPDGTCSRFDSDSGSYCRSEAVVAVLLTKKSLARRVYATILNAGTNTDGSK EQGVTFPSGEVQEQLICSLYPAGLAPESLEYIEAHGTGTKVGDPELNGITRSLCAFRQAPLLIGSTKSNMGHP EPASGLAALTQVLLSLEHGVWAPNLHFHNPNEIPALLDGRQLQVDRPLPVRGGNVGINSFGFGGSSNVHVLQP NTRQAPAPTAHAALPHLLHASGRTLEAVQDLLEQGRQHSQDLAFVSMNLNDIAATPTAAMPFRGYTVLGVVEGR VQEVQQVSTNKRPLWFICSGMGTQWRGMGLSLMRLDSFRESILRSDEAVKPLGVKVSDDLSTDERTFDDIVH AFVSLTAIQIALIDLLTSVGLKPDGIHLSLGEVACGYADGCLSQREAVLAAYWRGQCIKDAHLPPGSMAAVGLS WEECKQRCPAGVVPACHNSEDVTISGPQAAVNEFVEQLKQEGVFAKEVRTGGLAHFSYFMEGIAPTLLQALK KVIREPRPRSARWLSTSIPEAQWQSSSLARTSSAEYNVNNLVSPVLFQEALWHIPEHAVVLEIAPHALLQAVLKR GVKSSCTIPLMKRDHKDNLFFLTNLGKVHLTGINVPNPALFPPVEFPAPRGTPPLISPHIKWDHSQTWDVPVA EDFPNGSSSSSATVYSIDASPESPDHYLDVHCIDGRVIFPGTGYLCLVWKTLLARSLGLSLEETPVVFENVFSHQAT ILPKTGTVALEVRLEASHAFEVSDTGNLIVSGKVYLWEDPNKSLFDHPEVPTPPESASVSRLTQGEVYKELRLR GYDYGPPQFQICEATLEGEQKLLWKDNWVTFMDTMLQVLSILGSSQSLQLPRTVTAIYIDPATHRQKVYRLK EDTQVADVTTSRCLGITVSGGIHISRLQTTATSRRQEQELVPTLEKFVFTPHMEAECLSESTALQKELQCKGLA RALQTKATQQLKAAMLGQEDPPQHGLPRLLAAACQLQLNGLQLLEGEALQERLLLPEPLISGLLNSQAL KACVDTALENLSTLKMKVAEVLAGEGHLSRIPALLNTQPMQLLEYTATDRHPQALKDVQTKLQQHDVAQGG WNPSDPAPSSLGALDLLVCNCALATLGDPALALDNMVAALKEGGFLLVHTVLKGHALGETLACLPEVQPPAPS LLSQEEWESLFSRKALHLVGLKRSFYGTALFLCRRRAIPQEKPIFLSVEDTSFQWVDSLKSTLATSSSQPVWLTA MDCPTSGVVGVLNCLRKEPGGHRIRCILLSNLSNTSHAPKLDPGSPELQVVLKHDLMNVYRDGAWGAFRHF QLEQDKPKEQTAHAFVNVLTTRGDLASIRWVSSPLKHTQPSSSGAQLCTVYYASLNFRDIMLATGKLSPAIPGK WASRDCMLGMEFSGRDRRCRRVMGLVPAEGLATSVLLSSDFLWDVPSSWTLLEEASVPVYTTAYYSLVVRG RIQRGETVLIHSGSGVGQAISIALSLGCRVFTTVGSAEKRAYLQARFPQLDDTSFANSRDTSFEQHVLHTGG KGVDLVLNSLAEELQASVRCLAQHGRFLEIGKFDLSNNHPLGMAIFLKNVTFHGILLDALFEEANDSWREVA ALLKAGIRDGVVPLKCTVFPKAQVEDAFRYMAQKGKIGKVLVQVREEPEAVLPGAQPTLISAISKTFCFAHK SYIITGGGFGLELARWLVLRGARQLVLTSRSGIRTGYQAKHIREWRRQGIQVLVSTSNVSSLEGARALIAEAT KLGPVGGVFNLAMVLRDAMLENQTPLEFQDVNPKYNGTLNLDRTREACPELDYFVAFSSVSCGRGNAGQT NYGFANSTMERICEQRRHDGLPGLAVQWGAIGDVGVLEAMGTNDTVIGGTLPRISSCMEVLDLFLNQPHAV LSSFVLAEEKAVAHGDDTQRDLVKAVAHILGIRDLAGINLDSTLADLGLDSLMGVEVRQILEREHDLVLP MREVRQLTLRKLQEMSSKTDAAATARRPGDGRPTFAGVHGDDAAEVHARDLTDRFLDDATLAAATALPGPAPE IRTVLLTGATGFLGRYLALEWLERMAMVGGTLLICLVGRDDAAARARLDQIFDSGDPPELLRHYRELADRHLEVI AGDKSDADLGLDRRTWQRLADTVDLIVDPAALNVHVLPHYRELFPGPNVVGTAELIRLALTGRLKPYLYTSTIAVG AGIAPGQFTEDADIRQISATRTLDDSYANGYATSKWAGEVLLREAHDLCLGPVAVFRCDMILADTSYAGQLNVP DMFTRLILSLVATGIAPLSFYELDAAGHRQRAHYDGLPVEFVAEAVSALGLDVAEDGGFATYHVMNPYDDGIGL DEFVDWLTGAGYPIEHVNDYGTWFRQFETAIRGLPERQQRASLLPLLSYQRPQPPIRGSAAPTDRFRSAVQD AKIGPDKDIPHITPEVIVKYVTDRLRLGLLGGKLHHHHHHH
pMMH01 (mouseFAS_ΔTE _CAR-TR_Mphi, Strep-and His- tagged) Position 113 highlighted	ATGAGCGCTTGGAGCCATCCACAATTGAGAAGGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTACAGCGTGG AGCCACCCGAGTTTCGAAAAAGCGCCGGATCCGAGGAGGTGGTGATAGCCGGTATGTCGGGGAAGTTGCCCG AGTCAGAGAACCTACAGGAGTTCTGGGCCAACCTCATTGGTGGTGTGGACATGGTCACAGATGATGACAGGA GATGGAAGGCTGGGCTCTATGGATTACCAAGCGGTCTGGAAGCTGAAGGATCTCTCCAAGTTCCGACGCCTC CTTTTGGGGTCCACCCCAAGCAGGCACACACAATGGACCCCAAGCTTCGGCTGCTGTTGGAAGTCAGCTAT GAAGCAATTGTGGATGGAGGTATCAACCCAGCCTCACTCCGAGGAACGAACACTGGCGTCTGGGTGGGTGTGA GT GGT / TGG TCAGAGGCATCCGAGGCCCTTAGCAGAGATCCCGAGACGCTTCTGGGTACAGCATGGTGGGCT GCCAGCGTGAATGATGGCAACCGGCTCTCTTTCTTCTCGACTTCAAAGGACCAAGCATTGCCCTGGACAC AGCCTGCTCCTCCAGCTTGCTGGCACTACAGAATGCCTACCAGGCCATCCGTAGTGGGAATGCCCGCGGCC TTGTGGGTGGGATCAACCTGCTCCTGAAGCCGAACACCTCTGTGCAGTTATGAAGCTGGGCATGCTCAGCCC GGACGGCACCTGCAGATCCTTTGATGATTAGGGAGTGGATATTGTCGCTCTGAGGCTGTTGTAGCAGTTCTG CTGACTAAGAAGTCCCTGGCTCGCGGGTCTATGCCACGATTCTGAATGCCGGCACCAATACAGATGGCAGCA AGGAGCAAGGTGTAACATTTCCCTCTGGAGAAGTCCAAGAACAACCTCATCTGCTCTCTGTATCAGCCAGCTGG TCTGCCCCGGAGTCGCTTGAATATTGAAGCCATGGCAGGGCACCAAGGTGGGTGACCCCAAGGAAGTGA AATGGCATTACTCGGTCCCTGTGCGCCTTCGCGCAGGCCCTCTGTTAATTGGCTCCACCAAAATCCAACATGGG ACACCTGAGCCTGCCTCTGGGCTTGACAGCCCTGACCAAGGTGCTGTTATCCCTGGAGCATGGGGTCTGGGCC CTAACCTGCACCTTCCACAACCCCAACCTGAGATCCAGCACTTCTTGATGGGCGGTGCAGGTGGTTCGATAG GCCCCCTGCCTGTTCTGGTGGCAACGTGGGCATCAACTCATTTGGCTTCGGAGGCTCAATGTTTCATGTCATC CTCCAGCCCAACACAGCGCAGGCCCTGCGCCACTGCACACGCTGCCCTTCCCATTTGCTGCACGCCAGTGG ACGCACCTTAGAGGCAGTGCAGGACCTGCTGGAACAGGGCCGCCAGCACAGCCAGGACCTGGCCTTTGTGAGC ATGCTCAATGACATTGCGGCAACCCCTACAGCAGCCATGCCCTTACAGGGTTACTGTGCTAGGTGTTGAGG

Construct	Sequence form start to stop codon
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Construct	Sequence form start to stop codon
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pMMH02 (mouseFAS_ΔTE _CAR-TR_Noti, Strep-and His- tagged) Position 113 highlighted	MSAWSHPQFEKGGSGGGSGSAWSHPQFEKGAGSEEVVIAGMSGKLPESENLQEFWANLIGVDMVTDDDR RWKAGLYGLPKRSGKLDLSKFDASFFGVHPKQAHMDPQLRLLLEVSYEAIVDGGINPASLRGTNTGVWVG SG/WSEASEALS RDPETLLGYSVMVGCQRAMMANRLSFFDFKGPSIALDTACSSLLALQNA YQAIRSGECPAA LVGGINLLKPNTSVQFMKLGMLSPDGTCSRFDSDSGSYCRSEAVVAVLLTKKSLARRVYATILNAGTNTD GSK EQGVTFPSGEVQEQLICSLYPAGLAPESLEYIEAHGTGTVGDPQELNGITRSLCAFRQAPLLIGSTKSNMGHP EPASGLAALTKVLLSLEHGVWAPNLHFHNPNEIPALLDGRQLVVDRLPLVRGGNVGINSFGFGGSGNVHVLQP NTRQAPAPTAHAALPHLLHASGRTLEAVQDLLEQGRQHSQDLAFVSMNDIAATPTAAMPFRGYTVLGV EGR VQEVQQVSTNKRPLWFICSGMGQTQWRGMGLSLMRLDSFRESILRSDEAVKPLGVKVS D L L L S T D E R T F D D I V H AFVSLTAIQIALIDL L L T S V G L K P D G I I G H S L G E V A C G Y A D G C L S Q R E A V L A A Y W R G C I K D A H L P P G S M A A V G L S WEECKQRCPAGVVPACHNSEDTVTISGPQAAVNEFVEQLKQEGVFAKEVRTGG L A F H S Y F M E G I A P T L L Q A L K KVIREPRPRSARWLSTISPEAQWQSSLARTSSAEYNVNNLVSPVLFQ E A L W H I P E H A V V L E I A P H A L L Q A V L K R GVKSSCTIIPLMKRDHKDNL E F L T N L G K V H L T G I N V N P N A L F P P V E F P A P R G T P L I S P H I K W D H S Q T W D V P V A EDFPNGSSSSATVYSIDASPESPDHYLV D H C I D G R V I F P G T G Y L C L V W K T L A R S L G L S L E T P V V F E N V S F H Q A T ILPKTGTVALEVRLL E A S H A F E V S D T G N L I V S G K V Y L W E D P N S K L F D H P E V P T P P E S A S V S R L T Q G E V Y K E L R L R GYDYGPFQFGICEATLEGEQGKLLWKDNWVT F M D T M L Q V S I L G S S Q S L Q L P T R V T A I Y I D P A T H R Q K V Y R L K EDTQVADVTTSRCLGITVSGGIHISRLQT T A T S R R Q Q E Q L V P T L E K F V F T P H M E A E C L S E S T A L Q K E L Q L C K G L A R A L Q T K A T Q Q G L K A A M L G Q E D P P Q H G L P R L L A A C Q L Q L N G N L Q L E L G E A L A Q E R L L L P E D P L I S G L L N S Q A L K A C V D T A L E N L S T L K M K V A E V L A G E G H L Y S R I P A L L N T Q P M L Q L E Y T A T D R H P Q A L K D V Q T K L Q Q H D V A Q Q W N P S D P A P S S L G A L D L L V C N C A L A T L G D P A L A L D N M V A A L K E G G F L L V H T V L K G H A L G E T L A C L P S E V Q P A P S L L S Q E E W E S L F S R K A L H L V L G K R S F Y G T A L F L C R R A I P Q E K P I F L S V E D T S F Q W V D S L K S T L A T S S S Q P V W L T A M D C P T S G V V G L V N C L R K E P G G H R I R C I L L S N L S N T S H A P K L D P G S P E L Q Q V L K H D L V M N V Y R D G A W G A F R H F Q L E Q D K P K E Q T A H A F V N V L T R G D L A S I R W V S S P L K H T Q P S S S G A Q L C T V Y Y A S L N F R D I M L A T G K L S P D A I P G K W A S R D C M L G M E F S G R D R C G R R V M G L V P A E G L A T S V L L S S D F L W D V P S S W T L E E A A S V P V V Y T T A Y S L V V R G R I Q R G E T V L I H S G S G V G Q A A I S I A L S L G C R V F T T V G S A E K R A Y L Q A R F P Q L D D T S F A N S R D T S F E Q H V L L H T G G K G V D L V L N S L A E E K L Q A S V R C L A Q H G R F L E I G K F D L S N N H P L G M A I F L K N V T F H G I L L D A L F E E A N D S W R E V A A L L K A G I R D G V V K P L K C T V F P K A Q V E D A F R Y M A Q G K H I G K V L V Q V R E E P E A V L P G A Q P T L I S A I S K T F C P A H K S Y I T G G L G G F G L E L A R W L V L R G A Q R L V L T S R S G I R T G Y Q A K H I R E W R R Q G I Q V L V S T S N V S S L E G A R A L I A E A T K L G P V G G V F N L A M V L R D A M L E N Q T P E L F Q D V N K P K Y N G T L N L D R A T R E A C P E L D Y F A F S S V S C G R G N A G Q T N Y G F A N S T M E R I C E Q R R H D G L P G L A V Q W G A I G D V G I V L E A M G T N D T V I G G T L P Q R I S S C M E V L D F L N Q P H A V L S S F V L A E K K A V A H G D G T Q R D L V K A V A H I L G I R D L A G I N L D S T L A D L G L D S L M G V E V R Q I L E R E H D L V L P M R E V R Q L T L R K L Q E M S S K T D S A A S E R D S G S R P T A A T V H G D D G L L R A D D L A L E A F L D P A T L D A A A H L P S A L E P P R T V L L T G A N G Y L G R F L A L E W L Q R L D V S G G T L I C L I R G S D A S A R R R L D A V F A T G D P E L A H Y R E L A E R R L R V L P G D I G E P N L G L R E Q D W R D L A E T V D L I V H P A A L V N H V L P Y A Q L F G P N V V G T A E V I R L A L T S R L K P V T Y L S T V A V S A G I D P E T F T E D G D I R E I S P V R R L D D G Y A N G Y G N S K W A G E V L L R N A H D R F G L P V A V F R S D M I L A H S R Y A G Q L N V P D M F T R L L S V L A T G L A P G S F H D A H G E R H R A H Y D G L P A D F T A A A V T T L G S R V T S G Y E T Y D V L N P H D D G I S L D T F V D W L I E A G H P I D R I D D Y A E W F A R F D T A L R A L P E H Q R Q H S L L P L L H A Y R R P T P L H G V A L P A K H F R A A V Q Q A K L

Construct	Sequence form start to stop codon
	GPDGDIPHVTRELIEKYASDLRLLLGLIQGGGKLHHHHHHHH
pMMH02 (mouseFAS_ΔTE _CAR-TR_Noti, Strep-and His- tagged) Position 113 highlighted	ATGAGCGCTTGGAGCCATCCACAATTTGAGAAGGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTACAGCGTGG AGCCACCCGAGTTTCGAAAAAGCGCCGGATCCGAGGAGGTGGTGATAGCCGGTATGTCGGGGAAGTTGCCCG AGTCAGAGAACCTACAGGAGTTCTGGGCCAACCTCATTGGTGGTGTGGACATGGTCACAGATGATGACAGGA GATGGAAGGCTGGGCTCTATGATTACCCAAGCGGTCTGGAAGCTGAAGGATCTCTCCAAGTTCGACGCCTC CTTTTTTGGGGTCCACCCCAAGCAGGCACACACAATGGACCCCCAGCTTCGGGTGCTGTTGGAAGTCAGTAT GAAGCAATTTGGGATGGAGGTATCAACCCAGCCTCACTCCGAGGAACGAACACTGGCGTCTGGGTGGGTGTGA GTGGT/TGGTCAGAGGCATCCGAGGCCCTTAGCAGAGATCCGAGACGCTTCTGGGTACAGCATGGTGGGCT GCCAGCGTGCAATGATGGCCAACGGCTCTCTTTCTTTCGACTTCAAAGGACCAAGCATTGCCCTGGACAC AGCCTGCTCCTCCAGCTTGGTGGCACTACAGAATGCCTACCAGGCCATCCGTAGTGGGGAATGCCCCGCGGCC TTGTGGGTGGGATCAACCTGCTCCTGAAGCCGAACACCTCTGTGCAGTTCATGAAGCTGGGCATGCTCAGCCC GGACGGCACCTGCAGATCCTTTGATGATTAGGGAGTGGATATTGTCGCTCTGAGGCTGTTGTAGCAGTTCTG CTGACTAAGAAGTCCCTGGCTCGGCGGGTCTATGCCACGATTCTGAATGCCGGCACCAATACAGATGGCAGCA AGGAGCAAGGTGTAACATTCCCTCTGGAGAAGTCCAAGAACAACCTCATCTGCTCTCTGTATCAGCCAGCTGG TCTGCCCCGGAGTCGCTTGAGTATATTGAAGCCCATGGCACGGGCACCAAGGTGGGTGACCCCCAGGAACGTG AATGGCATTACTCGGTCCCTGTGCGCCTTCCGCCAGGCCCTCTGTAAATTGGCTCCACCAAAATCCAACATGGG ACACCTGAGCCTGCCTCTGGGCTTGCAGCCCTGACCAAGGTGCTGTTATCCCTGGAGCATGGGCTCTGGGCC CTAACCTGCACCTCCACAACCCCAACCTGAGATCCAGCACTTCTTGATGGCGGGCTGCAGGTGGTCGATAG GCCCCGTGCTGTTGCTGGTGGAACGTGGGCATCAACTCATTGGCTTCGGAGGCTCCAATGTTTCATGTCATC CTCCAGCCCAACACACGGCAGGCCCTGCGCCCACTGCACACGCTGCCCTTCCCCATTTGCTGCACGCCAGTGG ACGCACCTTAGAGGCAGTGCAGGACCTGCTGGAACAGGGCCGCCAGCACAGCCAGGACCTGGCCTTTGTGAGC ATGCTCAATGACATTGCGGCAACCCCTACAGCAGCCATGCCCTTCAGGGGTTACACTGTGCTAGGTGTTGAGG GCCGTGTCCAAGAAGTGCAGCAAGTGTCCACCAACAAGCGCCCACTCTGGTTCATCTGCTCAGGGATGGGCAC GCAGTGGCGCGGGATGGGGCTGAGCCTCATGCGCCTGGACAGCTTCCGTGAGTCTATCCTGCGCTCCGATGAG GCTGTGAAGCCGTTGGGAGTGAAAGTGTGAGATCTGCTGTTGAGCACAGATGAGCGCACCTTTGATGACATCG TGCATGCCTTTGTGAGCCTCACTGCCATCCAGATTGCCCTCATCGACCTACTGACTTCTGTGGGACTGAAACC TGACGGCATCATTGGGCACTCCTTGGGAGAGGTTGCCTGTGGCTATGCAGATGGCTGTCTCTCCAGAGAGAG GCTGTGCTTGCAGCTTACTGGCGAGGCCAGTGCATCAAAGATGCCACCTCCCGCCTGGATCCATGGCAGCTG TTGGTTTGTCTGGGAGGAATGTAACACAGCGCTGCCCGCTGGCGTGGTGCTGCCTGCCACCAACTCTGAGGA CACCGTGACCATCTCTGGACCTCAGGCTGCAGTGAATGAATTTGTGGAGCAGCTAAAGCAAGAAGGTGTGTTT GCCAAGGAGGTACGAACAGGAGGCCTGGCTTTCACCTCCTACTTCATGGAAGGAATTGCCCCACATTGCTGC AGGCTCTCAAGAAGGTGATCCGGGAACACGGCCGCGCTCGGCTCGATGGCTCAGCACCTCTATCCCTGAGGC CCAGTGGCAGAGCAGCTGGCCCGCACATCTTCTGCCGAGTACAATGTCAACAACCTGGTGAGCCCTGTGCTC TTCCAGGAAGCACTGTGGCACATCCCTGAGCATGCCGTGGTGCTGAGATTGCGCCCCACGCACTGTTGAGG CTGTCTGAAGCGAGGCGTGAAGTCCAGCTGCACCATCATTCCCTTGATGAAGAGGGATCATAAAGATAACTT GGAGTTCTTTCTACCAACCTTGGCAAGGTGCACCTCACAGGCATCAATGTCAACCCTAACGCCCTTGTCCCAC CTGTGGAGTTCCCGGCTCCCGAGGGACTCCTCTCATCTCCCTCACATCAAGTGGGACCACAGTCAGACTTGG GATGTCCCGGTTGCTGAGGACTTCCCAAACGGCTCCAGCTCCTCTCTGCTACAGTCTACAGCATCGACGCCAG TCCTGAGTCGCCCGACCACTACCTGGTAGACCACTGCATTGACGGCCGGGTCACTTCTCCCTGGCACTGGCTACC TGTGCTGTTGTGGAAGACACTGGCTCGCAGCCTGGGCTTGTCCCTAGAAGAGACCCCTGTGGTATTTGAGAA TGTGTGTTTTATCAGGCCACTATACTACCAAGACAGGAACCGTGGCGCTGGAGGTGAGGTGCTAGAGGCC TCCCATGCCTTTGAGGTGTCTGACACTGGCAATCTGATTGTGAGCGGAAAAGTGTACCTGTGGGAAGACCCGA ACTCCAAGTTATTCGACCACCCAGAAGTCCCAACACCCCTGAGTCTGCATCGGTCTCCCGCCTGACCCAGGGA GAAGTATACAAGGAGCTGCGGCTGCGTGGCTATGATTATGGCCCTCAGTTCAGGGCATCTGTGAGGCCACCC TTGAAGGTGAACAAGGCAAGCTGCTCTGGAAGATAACTGGGTGACCTTCATGGACACAATGCTGCAGGTAT CCATTCTGGGTTCTAGCCAGCAGAGTCTACAGCTACCTACCCGTGTGACCGCCATCTATATCGACCTGCCACC CACCGTCAGAAGGTGTACAGGCTGAAGGAGGACACTCAAGTGGCTGATGTGACAACGAGCCGCTGTCTGGGCA TAACGCTCTCTGGTGGTATCCACATCTCAAGACTACAGACGACAGCAACCTCACGGCGGCAGCAAGAACAGCT GGTCCCCACCTTGGAAAAGTTCGTTTTCACACCGCACATGGAGGCTGAGTGCCTGTCTGAGAGCACTGCCTG CAGAAGGAGCTGCAACTGTGCAAGGGTCTGGCACGGGCTCTGCAGACCAAGGCCACCCAGCAAGGGCTGAAGG CGGCAATGCTTGGGCAAGAGGACCTCCACAGCACGGGCTGCCTCGACTCCTGGCAGTGTCTTGCAGTTGCA GCTCAACGGGAACCTGCAGCTGGAGCTGGGAGAAGCGCTGGCTCAAGAGAGGCTCCTGCTGCCAGAAGACCT CTGATCAGTGGCCTCCTCAACTCCAGGCCCTCAAGGCCCTGCGTAGACACAGCCCTGGAGAATTGTCTACTCT CAAGATGAAGGTGGCAGAGGTGCTGGCTGGAGAAGGCCAATTGTATTTCCGAATCCCGGCACTGCTCAACACC CAGCCCATGCTACAACCTGGAATACACAGCCACCGACCGGCACCCCGGCCCTGAAGGATGTTACAGACCAAC TGCAGCAGCATGATGTGGCGCAGGGCCAGTGAACCTTCCGACCTGCGCCACGAGCCTGGGTGCCCTTGA CCTTCTGGTGTGCACTGTGCATTAGCCACCTGGGGATCCAGCCTTGGCCCTGGACAACATGGTAGCTGCC CTAAGGAAGGTGGTTTCTCTGCTAGTGCACACAGTGTCTCAAAGGACATGCCCTTGGGGAGAGCCCTGGCCTGCC TACCCTCTGAGGTGCAGCCTGCGCCAGCCTCCTAAGCCAGGAGGTGGGAGAGCCTGTTCTCGAGGAAGGC ACTACACCTGGTGGGCTTAAAAGGTCCTTCTACGGTACTGCGCTGTTCTGTGCCGGCAGCCATCCACAG

Construct	Sequence form start to stop codon
	GAGAAACCTATCTTCTGTCTGTGGAGGATACCAGCTTCCAGTGGGTGGACTCTCTGAAGAGCACTCTGGCCA CGTCCTCCTCCCAGCCTGTGTGGCTAACGGCCATGGACTGCCCCACCTCGGGTGTGGTGGGTTTGGTGAATTG TCTCCGAAAAGAGCCGGGTGGACACCGGATTCCGGTGATCCTGCTGTCCAACCTCAGCAACACATCTCACGCC CCCAAGTTGGACCCTGGCTCTCCAGAGCTACAGCAGGTGCTAAAGCATGACCTCGTGATGAACGTGTACCGGG ACGGGGCCTGGGGTGCCTTCCGTCACCTTCCAGTTAGAGCAGGACAAGCCCAAGGAGCAGACAGCGCATGCCTT TGTAACGTCCTCACCCGAGGGGACCTCGCCTCCATCCGCTGGGTCTCTCCCCCTGAAGCACACGAGCCCT CGAGCTCAGGAGCACAGCTGTGACTGTCTACTACGCCTCACTGAACTCCGAGACATCATGCTGGCCACGGG CAAGCTGTCCCCTGATGCCATTCCAGGTAATGGGCCAGCCGAGACTGCATGCTCGGCATGGAGTTCTCAGGC CGGGATAGGTGTGGCCGGCGTGTATGGGGCTGGTTCTGCAGAAGGCCTGGCCACCTCAGTCTGCTATCAT CTGACTTCTCTGGGATGTACCCTCCAGCTGGACCCTGGAGGAGGCGGCTCTGTGCCCGTCTATACCACCT GCTTACTACTCGTTAGTGGTTTCGCGGGCGCATCCAGCGTGGGGAGACCGTGCTCATCCACTCAGGTTCAAGTG GTGTGGGCCAAGCGGCCATTTCATTGCCCTCAGTCTGGGCTGCCGCGTCTTCAACCACTGTGGGCTCTGCAGAG AAGCGAGCATACCTCCAGGCCAGGTTCCCTCAGCTTGATGACACCAGCTTTGCCAACTCGAGGGACACATCAT TTGAGCAGCAGTGTACTGCACACAGGTGGCAAAGGGGTCGACCTGGTCTCAACTCACTGGCAGAAGAGAA GCTGCAGGCCAGTGTGCGGTGCTTGGCTCAGCATGGTCGTTCTTAGAGATTGGCAAATTTGATCTTTCTAAC AACCACCTCTGGGCATGGCTATCTTCTGAAGAACGTCATTTCCATGGGATCCTGCTGGACGCCCTTTTGTG AGGAGGCCAATGACAGCTGGCGGGAGGTGGCGGCACTCCTGAAGGCTGGCATTCTGTGATGGAGTCTGTGAAGCC CCTCAAGTGACAGTGTTCCTCAAGGCCAGGTGGAAGATGCCTTCCGCTACATGGCTCAGGGGAAACACATT GGCAAAGTCTTGTCCAGGTACGGGAGGAGGAGCCTGAGGCTGTGCTGCCAGGGGCTCAGCCACCTGATT CTGCCATCTCCAAGACCTTCTGCCAGCCATAAGAGTTACATCATCACTGGTGGCTAGGTGGCTTTGGCCT GGAGCTGGCCCGGTGGCTCGTGTCTCGCGGAGCCAGAGGCTTGTGCTGACTTCCCGATCTGGAATCCGCAAC GGCTACCAAGCCAAGCACATTCCGGGAGTGGAGACGCCAGGGCATCCAAGTGTGCTGTAACAAGCAACGTGA GCTCACTGGAGGGGGCCGTCTCTCATCGCCGAAGCCACAAAGCTGGGGCCCGTTGGGGGTGTCTTCAACCT GGCCATGGTTTTGAGGGATGCCATGCTGGAGAACCAGACCCAGAGCTTTCAGGATGTCAACAAGCCAAA TACAATGGCACCCCTGAACCTTGACAGGGCAACCCGGGAAGCCTGCCCTGAGCTGGACTACTTTGTGGCCTTCT CCTCTGTAAGCTGCGGGCGTGGTAATGCTGGCCAACTAACTACGGCTTCGCCAACTCTACCATGGAGCGTAT ATGTGAACAGCGCAGGCACGATGGCCTCCCAGGCCTTGGCGTGCAGTGGGGTGCCATTGGTGACGTGGGCATT GTCCTGGAAGCGATGGGCACCAATGACACAGTCATCGGAGGTACGCTGCCTCAGCGCATCTCTCTGTCATGG AGGTACTGGACCTCTTCCTGAATCAGCCCCACGCAGTCTCTGAGCAGCTTTGTGCTGGCAGAGAAGAAAGCTGT GGCCATGGGGACGGGGACCCAGAGGGATCTGGTGAAGCTGTAGCACACATCTAGGCATCCGAGACCTC GCAGGTATTAACTGGACAGCACGCTGGCAGACCTCGGCCTGGACTCGCTCATGGGTGTGGAAGTTTCGTCAGA TCCTGGAACGAGAACACGATCTGGTGTGCCCATGCGTGAGGTGCGGCAGCTCACGCTGCGGAACTTCAGGA AATGTCTCCAAGACTGACTCGGCGGCCGAAGCGAATCCGGCAGTCTGCGACCGCGCCACGGTG CACGGCGACGACGGCCTGCTGCGGGCCGACGATCTCGCCCTGGAGGCATTCTCGACCGCGGACCCCTGGACGC GGCCGCGCATCTGCCGAGCGCGCTGGAGCCGCGCGCACGGTTCTGCTGACCGCGCCAACGGCTACCTCGGGC GCTTCTCGCCCTGGAATGGCTACAGCGCTGGACGTCTCGGGCGGTACTCTGATCTGCCTGATCCGCGGCAGT GACGCCGACAGTGTCTCGGCGCGCTCTCGACGCGGTCTTCGCCACCGGTGATCCGGAGCTGGAAGCCCACTACCG CGAATCGCCGAGCGGGCGGTGCGTGTCTGCCCGCGGATATCGGTGAACCGAATCTCGGCCTGCGGAAACAG GATTGGCGGATCTGGCCGAGACCGTGGACCTGATCGTCATCCGGCCGCCCTGGTCAACCATGTGCTGCCCT ACGCCAGTTGTTTCGCCCCCAATGTGTCGTTACCGCCGAGGTGATCCGCTCTCGCGCTGACCTCCCGGTGAAG CCGGTCACCTACCTGTCCACCGTGGCGGTATCCGCCGTATCGACCCGAGACCTTCAACGAGACGGCGATAT CCGCGAGATCAGCCCCGTGCGCCGCTCGACGACGGCTACGCCAATGGCTACGGCAACAGCAATGGGCCGGC GAGGTGCTGTTGCGCAATGCGCAGACCGCTTCGGTCTCCCGGTGGCGGTCTTCCGCTCCGACATGATCTGGC CCACAGCCGCTACGCCGACAGCTCAACGTGCCGACATGTTACCCGCTGCTGCTGAGCGTGTGCGGACCG GCCTGGCCCCCGGTCTCTTCCACGATGCCACGCGAGCGCCACCGCGCCCACTACGACCGTCTCCAGCCGAT TTCACCGCGCGCGCGTCAACACCTGGGCAGCCGGGTGACCACTGGTTACGAGACCTACGACGTGCTCAACCC GCACGACGACGGGATCTCCCTGGACACCTTCGTCGACTGGCTCATCGAGGCGGGCCACCCATCGACCGCATCG ACGATTACGCCGAGTGGTTTCGCCGCTTCGACACCGCCCTGCGCGCCCTCCCGAACCAACGCCAACACTCG CTGCTACCCCTGCTGCACGCTACCGTCGCCCCACCCCTCCCTGCACGGAGTGCCTTCCCGCAAGCACTTC CGCGCCCGGTACAGCAGGCGAACTCGGCCCGGACGGGATATCCCGCAGTGACGCGGGAGCTGATCGAGA AGTACGCGAGTGATCTGCGGTTACTGGGGTTGATTACGGGCGGAGGTAAGCTTCATCATCACCACCACCA CCAC
pAR401 (mouseFAS_ΔTE _CAR-TR_Msme, Strep-and His- tagged) Position 113 highlighted	MSAWSHPPQFEKGGGSGGSGSAWSHPQFEKGAGSEEVVIAGMSGKLPESENLQEFWANLIGGVDMVTDDDR RWKAGLYGLPKRSGKLDLSKFDASFFGVHPKQAHMTMDPQLRLLLEVSYEAIVDGGINPASLRGTNTGVWVG SG/ W SEASEALSRDPETLLGYSMVCGQRAMMANRLSFFFDKGPSIALDTACSSLLALQNAYQAIRSGECPAA LVGGINLLKPNSTSVQFMKLGMLSPDGTCSRFDSSGSGYCRSEAVVAVLLTKKSLARRVYATILNAGTNTDGSK EQGVTFPSGEVQEQLICSLYQAPGLAPESLEYIEAHGTGTVKGDPELNGITRSLCAFRQAPLLIGSTKSNMGP EPASGLAALTKVLLSLEHGVWAPNLHFHNPNEIPALLDGRQLQVDRPLPVRGGNVGINSFGFGGSGNVHILQP NTRQAPAPTAHAALPHLLHASGRTLEAVQDLLEQGRQHSQDLAFVSMNLNDIAATPTAAMPFRGYTVLGVGR VQEVQVSTNKRPLWFICSGMGTQWRGMGLSLMRLDSFRESILRSDEAVKPLGVKVSDDLSTDERTFDDIVH AFVSLTAIQIALIDLSTSVGLKPDGIHLSLEVACGYADGCLSQREAVLAAYWRGQCICKDAHLPPGSMAAVGLS WEECKQRCPAGVVPACHNSEDVTISGPQAAVNEFVEQLKQEGVFAKEVRTGGLAHFSYFMEGIAPTLLQALK KVIREPRPRSARWLSTSIPEAQWQSSLARTSSAEYNNVNLVSPVLFQEALWHIPEHAVVLEIAPHALLQAVLKR

Construct	Sequence form start to stop codon
	GVKSCTIPLMKRDHKDNLEFFLTNLGKVHLTGINVPNALFPPVEFPAPRGTP LISPHIKWDHSQTWDVPVA EDFPNGSSSSSATVYSIDASPESPDHYLVDHCIDGRVIFPGTGYLCLVWKT LARSLGLSLEETPVVFENVFSHQAT ILPKTGTVALEVR LLEASHAFEVSDTGNLIVSGKVYLWEDPNSKLFHDHPEVPTPPESASVSRLTQGEVYKELRLR GYDYGPPQFGICEATLEGEQGKLLWKDNWVTFMDTMLQVSILGSSQSLQLPTRVT AIYIDPATHRQKVYRLK EDTQVADVTTSRCLGITVSGGIHISRLQTTATSRRQEQELVPTLEKFVFTPHMEAECLSESTALQKELQLCKGLA RALQTKATQQGLKAAMLGQEDPPQHGLPRLLAAACQLQLNGNLQLELGEALAQERLLL PEDPLISGLLNSQAL KACVDTALENSTLKMKVAEVLAGEGHLYSRIPALLNTQPMLQLEYTATDRHPQALKDVT KLQQHDVAQGG WNPSDPAPSSSLGALDLLVCNCALATLGDPALALDNMVAALKEGGFLLVHTVLKGHALGETLACL PSEVQPAPS LLSQEEWESLFSRKALHLVGLKRSFYGTALFLCRRAPQEKFIFLSVEDTSFQWVDSLKSTLATSSSQPVWLTA MDCPTSGVVGLVNLCKRKEPGGHRIRCILLSNLSNTSHAPKLDPGSPELQVVLKHDLV MNVYRDGAWGAFRHF QLEQDKPKEQTAHAFVNVLT RGDLASIRWVSSPLKHTQPSSSGAQLCTVYASL NFRDIMLATGKLSPD AIPGK WASRDCMLGMEFSGRDRCGRRVMGLVPAEGLATSVLLSSDFLWDVPSSWTLEEAASVPVYTTAYYSLVVRG RIQRGETVLIHSGSGVGQA AISIALSLGCRVFTTVGSAEKRAYLQARFPQLDDTSFANSRDTSFEQHVL LHTGG KGVDLVLNSLAEELQASVRCLAQHGRFLEIGKFDLSNNHPLGMAIFLKNVTFHGILLDALFEEANDSWREVA ALLKAGIRDGVVKPLKCTVFPKAQVEDAFRYMAQKGK HIGKVLVQVREEEPAVLPGAQPTLISAISKTFCPAHK SYIITGGLGGFGL ELARWLVL RGAQRLVLT SRSGIRTGYQAKHIREWRRQGIQVLVSTSNVSSLEGARALIAEAT KLGPVGGVFNLAMVLRDAMLENQTPELFQDVNPKYNGTLNLD RATREACPELDYFVAFSSVSCGRGNAGQT NYGFANSTMERICEQRRHDGLPGLAVQWGAIGDVGIVLEAMGTNDTVIGGTLPQRISSCMEVLDLFLNQPHAV LSSFVLAEEKKAVAHGDDGTQRDLVKAVAHILGIRDLAGINLDSTLADLGLDSL MGVEVRQILERHDLVLP MRE VRQLTLRKLQEMSSKAAAAQRTAGDRRPSFTTVHGADATEIRASELTLDKFIDAETLRAAPGLPKVTTEPRTVL LSGANGWLGRFLTQLQWLERLAPVG GTLITIVRGRDDAAARARLTQAYDTPELSSRFEALADRHLRVVAGDIG DPNLGLTPEIWHRLAAEVDLVVHPAALVNHVLPYRQLFGPNVVGTA EVIKALALTERIKPVTYLS TVSVAMGIPD FEEDGDIRTVSPVRPLDGGYANGYGNSKWAGEVLLREAHDL CGLPVATFRSDMILAHPRYRGQVNV PDMFTR LLLSLLITGVAPRSFYIGDGERPRAHYPGLTVDFVAEAVTTLGAQQREGYVSYDV MNPHDDGISLDV FVDWLIR AGHPIDRVDDYDDWVRRFETALTALPEKRRAQTVLPLLHAFRAPQAPLRGAPEPTEVFHAAVRTAKVGP GDIP HLDEALIDKYIRDLREFLIGGK LHHHHHHHH
pAR401 (mouseFAS_ΔTE _CAR-TR_Msme, Strep-and His- tagged) Position 113 highlighted	ATGAGCGCTTGGAGCCATCCACAATTTGAGAAGGGTGGAGGTTCTGGCGGTGGATCGGGAGGTT CAGCGTGG AGCCACCCGAGTT CGAAAAAGCGCCGGATCCGAGGAGGTGGTGATAGCCGGTATGTCGGGGAAGTTGCCCG AGTCAGAGAACCTACAGGAGTTCTGGGCCAACCTCATTGGTGGTGTGGACATGGTCACAGATGATGACAGGA GATGGAAGGCTGGGCTCTATGGATTACCCAAGCGGTCTGGAAGCTGAAGGATCTCTCCAAGTTCCGACGCTC CTTTTGGGGTCCACCCCAAGCAGGCACACACAATGGACCCCAAGCTTCGGGTGCTGTTGGAAGTCAGCTAT GAAGCAATTGTGGATGGAGGTATCAACCCAGCCTCACTCCGAGGAACGAACACTGGCGTCTGGGTGGGTGTGA GT GGT/ TGG TCAGAGGCATCCGAGGCCCTTAGCAGAGATCCCAGACGCTTCTGGGTACAGCATGGTGGGCT GCCAGCGTGCAATGATGGCCAACCGCTCTCTTTCTTCTCGACTTCAAAGGACCAAGCATTGCCCTGGACAC AGCCTGCTCCTCCAGCTTGTGGCACTACAGAATGCCTACCAGGCCATCCGTAGTGGGGAATGCCCGCGGCC TTGTGGGTGGGATCAACCTGCTCTGAAGCCGAACACCTCTGTGCAGTT CATGAAGCTGGGCATGCTCAGCCC GGACGGCACCTGCAGATCCTTTGATGATT CAGGGAGTGGATATTGTCGCTCTGAGGCTGTTGTAGCAGTTCTG CTGACTAAGAAGTCCCTGGCTCGGCGGGTCTATGCCACGATTCTGAATGCCGGCACCAATACAGATGGCAGCA AGGAGCAAGGTGTAACATTCCCCTCTGGAGAAGTCCAAGAACAAC TATCTGCTCTCTGTATCAGCCAGCTGG TCTGGCCCCGAGTCGCTTGAGTATATTGAAGCCATGGCAGGGCACC AAGTGGGTGACCCCCAGGAATG AATGGCATTACTCGGTCCCTGTGCGCTTCCGCCAGGCCCTCTGTTAATTGGCTCCACAAATCCAACATGGG ACACCCTGAGCCTGCCTCTGGGCTTG CAGCCCTGACCAAGGTGCTGTTATCCCTGGAGCATGGGGTCTGGGCC CTAACCTGCACCTCCACAACCCCAACCTGAGATCCCAGCACTTCTTGATGGGCGGCTGCAGGTGGTCGATAG GCCCCTGCTGTTGCTGGTGGAACGTGGGCATCAACTCATTTGGCTTCGGAGGCTCCAATGTT CATGTCATC CTCCAGCCCAACACAGGCAGGCCCTGCGCCCACTGCACACGCTGCCCTTCCCCATTTGCTGCACGCCAGTGG ACGCACCTTAGAGGCAGTGCAGGACCTGCTGGAACAGGGCCGCCAGCAGCCAGGACCTGGCCTTTGTGAGC ATGCTCAATGACATTGCGGCAACCCCTACAGCAGCCATGCCCTTCAGGGGTTACTGTGCTAGGTGTTGAGG GCCGTGTCCAAGAAGTG CAGCAAGTGTCACCAACAAGCGCCCACTCTGGTTCATCTGCTCAGGGATGGGCAC GCAGTGGCGCGGGATGGGGCTGAGCCTCATGCGCTGGACAGCTTCCGTGAGTCTATCCTGCGCTCCGATGAG GCTGTGAAGCCGTTGGGAGTGAAAGTGCAGATCTGCTGTTGAGCACAGATGAGCGCACCTTTGATGACATCG TGCATGCCTTTGTGAGCCTCACTGCCATCCAGATTGCCCTCATCGACCTACTGACTTCTGTGGGACTGAAACC TGACGGCATCATTGGGCACTCCTTGGGAGAGGTTGCCTGTGGCTATGCAGATGGCTGTCTCTCCAGAGAGAG GCTGTGCTTGCAGCTTACTGGCGAGGCCAGTGCATCAAAGATGCCACCTCCCGCTGGATCCATGGCAGCTG TTGGTTTGCTCTGGGAGGAATGTAACAGCGCTGCCCCGCTGGCGTGGTGCCTGCCTGCCACAACCTCTGAGGA CACCGTGACCATCTCTGGACCTCAGGCTGCAGTGAATGAATTTGTGGAGCAGCTAAAGCAAGAAGGTGTGTTT GCCAAGGAGGTACGAACAGGAGGCCTGGCTTTCACCTCCTACTTCATGGAAGGAATTGCCCCACATTGCTGC AGGCTCTCAAGAAGGTGATCCGGGAACACGGCCGCGCTCGGCTCGATGGCTCAGCACCTCTATCCCTGAGGC CCAGTGGCAGAGCAGCCTGGCCGCACATCTTCTGCCGAGTACAATGTCAACAACCTGGTGAGCCCTGTGCTC TTCAGGAAGCACTGTGGCACATCCCTGAGCATGCCGTGGTGTGAGATTGCGCCCCACGCATAGTTTCAGG CTGCTCTGAAGCGAGGCGTGAAGTCCAGCTGCACCATCATTTCCCTTGATGAAGAGGATCAAAAGATAACTT GGAGTTCTTTCTACCAACCTTGGCAAGGTGCACCTCACAGGCATCAATGTCAACCTTAACGCCCTTGTCCAC CTGTGGAGTTCCCGCTCCCGAGGACTCCTCTCATCTCCCTCACATCAAGTGGGACCACAGTCAGACTTGG GATGTCCCGTTGCTGAGGACTTCCCAACGGCTCCAGCTCCTCTGCTACAGTCTACAGCATCGACGCCAG

Construct	Sequence form start to stop codon
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Construct	Sequence form start to stop codon
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