

**Computational redesign of acyl-ACP thioesterase with improved selectivity towards  
medium-chain fatty acids**

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## Supporting Information

### *Computational and experimental $\Delta\Delta G$ calculations*

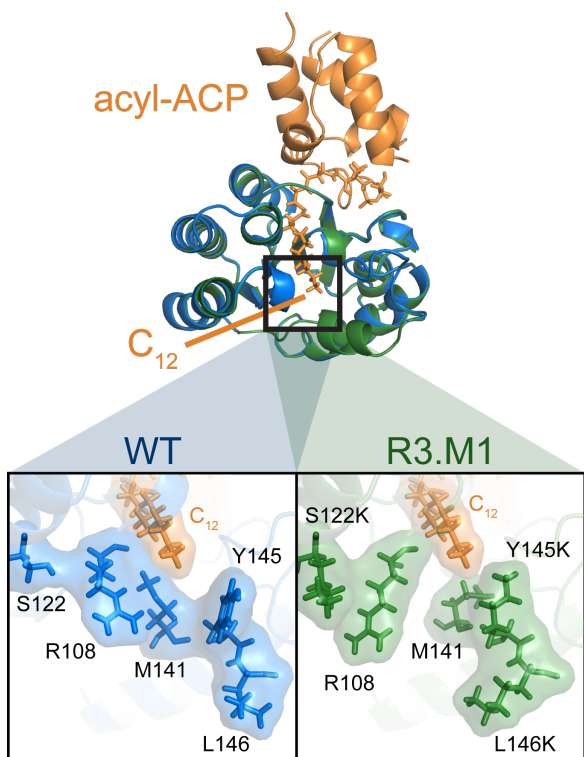
Using Michaelis-Menten kinetics, experimental  $\Delta G$  values were estimated from total FFA production levels. Correlations were found between  $\Delta\Delta G_{\text{comp}}$  and  $\Delta\Delta G_{\text{exp}}$  for C<sub>14</sub> in the R3 results and C<sub>12</sub> in the R4 results (primary constraint for each round). We used the largest cluster of mutants with similar yields (similar  $k_{\text{cat}}$  values,  $p < 0.05$ ) for each round. Apparent  $k_{\text{cat}}$  values were estimated to be proportional to the total amount of FFA produced since data was collected after the same time period (i.e., number of turnovers per 24 hours). The largest R3 cluster included eight variants (M1-M4 and M6-M9), and the largest R4 cluster included six variants (M1, M8, M13, M14, M17, and M18). These clusters represent the data points presented in **Figure S5**. Since the value of  $k_{\text{cat}}[E]_{0t}$  was approximated, we performed a sensitivity analysis by varying the initial value by  $\pm 50\%$ . The Pearson correlation coefficient for the R3 data was  $0.6 \pm 0.2$  and  $0.43 \pm 0.07$  for the R4 data. The correlation coefficients (after rounding) did not change for the sensitivity analysis, indicating that the value of  $k_{\text{cat}}[E]_{0t}$  did not strongly affect the correlation coefficients.

The correlations between  $\Delta\Delta G_{\text{comp}}$  and  $\Delta\Delta G_{\text{exp}}$  for R3 and R4 results are presented in **Figure S5**. Linear regression of the R3 and R4 data can be fitted to Equations S1 and S2, respectively.

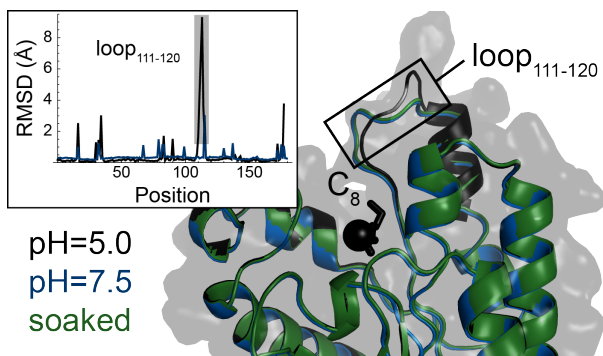
$$\Delta\Delta G_{\text{exp}} = 0.0011 \Delta\Delta G_{\text{comp}} - 0.11 \quad (\text{S1})$$

$$\Delta\Delta G_{\text{exp}} = 0.000038 \Delta\Delta G_{\text{comp}} + 0.0043 \quad (\text{S2})$$

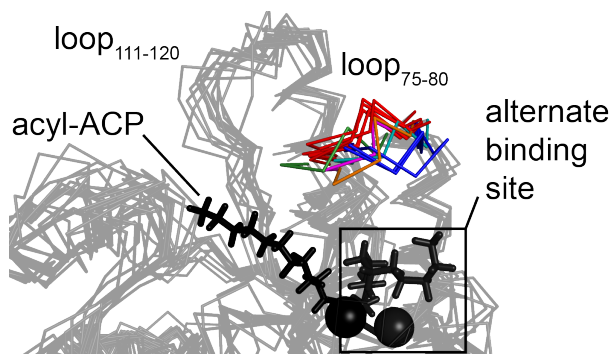
It is worthwhile mentioning that both Equations S1 and S2 are expected to have a slope of 1 and a y-intercept of 0. However, the slope of the regressions are not equal to 1 because calculated energies using molecular mechanics are not quantitatively meaningful so they will not match the true Gibbs free energies<sup>1</sup>. If Equations S1 and S2 are rescaled such that their slopes are equal to 1, the y-intercepts become -101.4 kJ/mol and 112.0 kJ/mol.



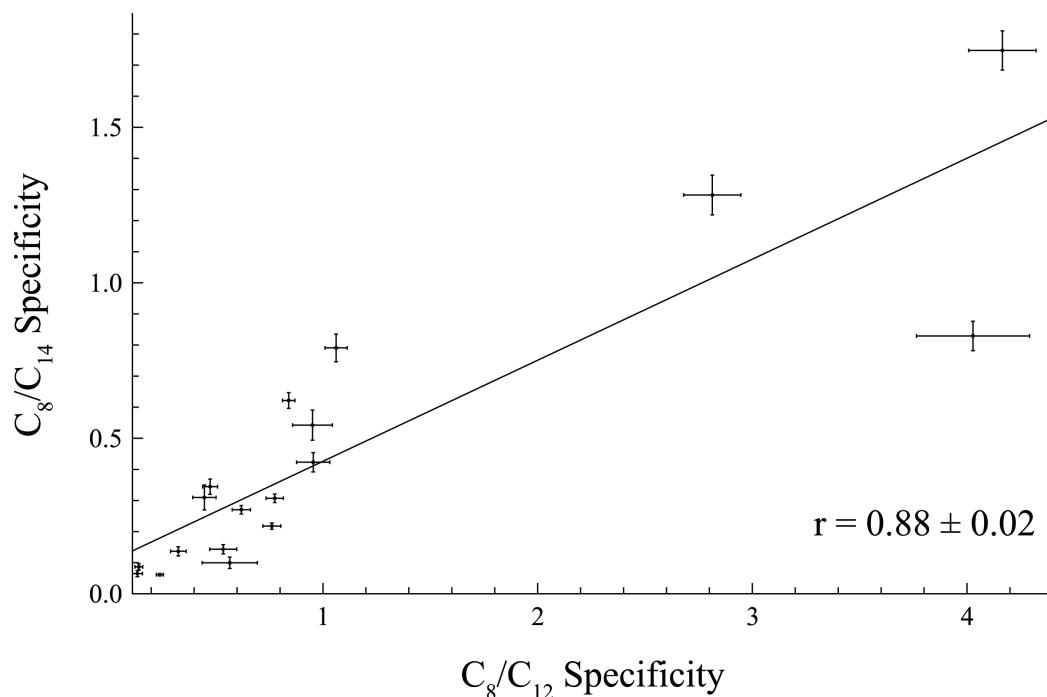
**Figure S1. IPRO-derived structure of R3.M1 bound to dodecanoyl-ACP.** The quaternary structure of the complex is shown at top with proteins shown as cartoons. Acyl-ACP is orange and the dodecanoyl moiety (C<sub>12</sub>) is depicted as sticks. The inset contrasts the binding sites of WT (blue) and R3.M1 (green). Mutated positions in R3.M1 (122, 145, and 146) are shown as sticks with a transparent surface. Two other nearby residues that show substantial movements are also formatted as sticks with transparent surfaces.



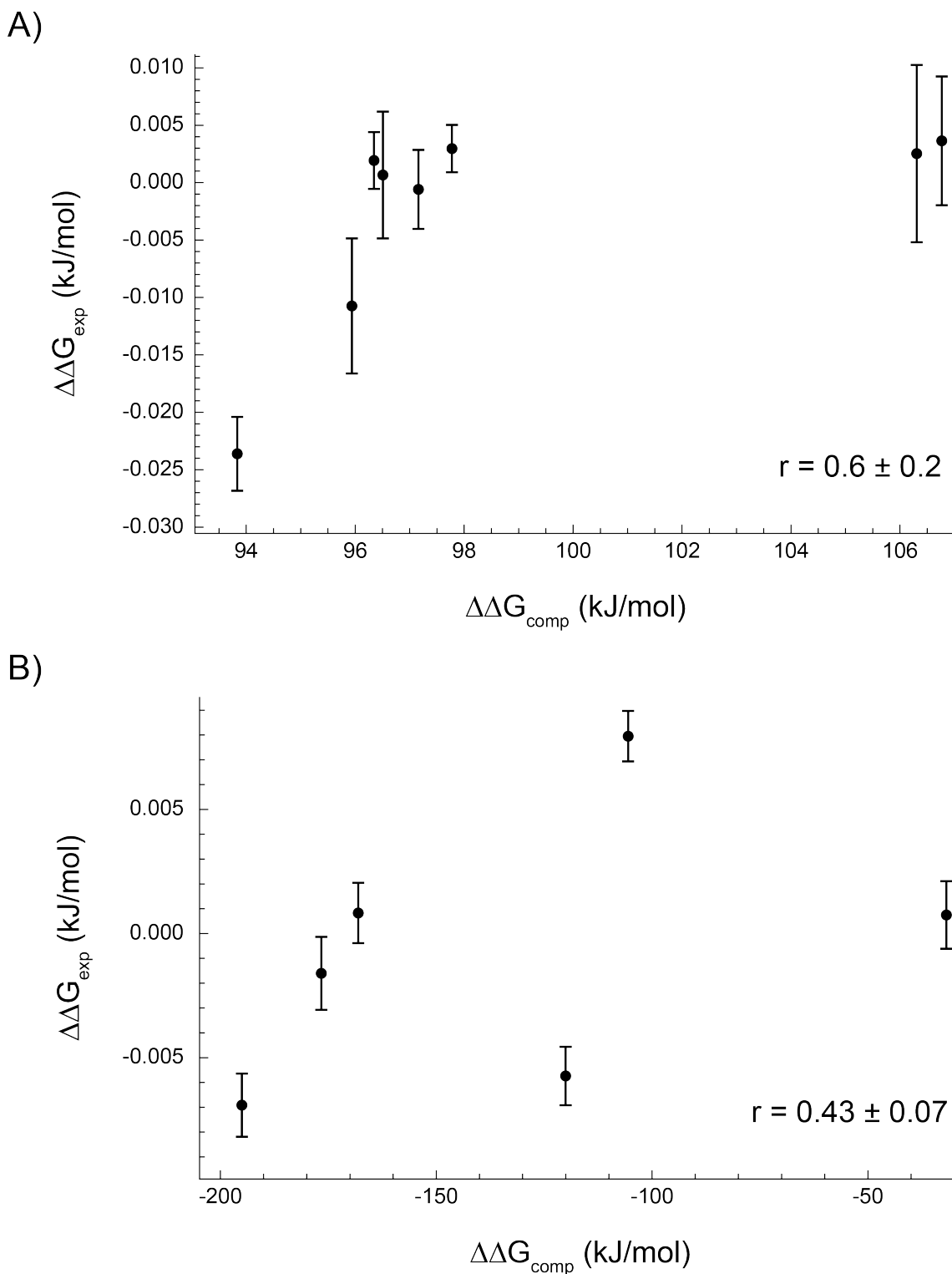
**Figure S2. Crystallized R3.M4 at three different pH conditions.** Crystallized R3.M4 under three different pH conditions is shown bound to octanoic acid ( $C_8$ ). R3.M4 is shown as a cartoon and colored black (pH 5.0), blue (pH 7.5), or green (crystallized at pH 5.0 and buffered at pH 7.5). **The carbonyl oxygen the FFA is shown as a sphere.** The loop with the largest conformational change between the three structures is labeled ( $loop_{111-120}$ ). The inset plots the RMSD of R3.M4 at pH 5.0 (black) and pH 7.5 (blue) to the soaked structure against the residue position number, quantifying the regions with the largest changes. Position 112 in the pH 5.0 structure is gray because it was not solved for the crystal and was instead modeled.



**Figure S3. Structures of bound R3.M1, R3.M4, and WT enzymes from MD simulations.** Each of the variants was shown as a black ribbon. The critical switch loop found between positions 75 and 80 was colored based on clustering of different conformations. Crystallized WT:C<sub>8</sub> published by Lo *et al.*, crystallized WT:C<sub>8</sub> from this study, crystallized R3.M4:C<sub>8</sub> (pH 5.0) from this study, WT:C<sub>12</sub> from MD, WT:C<sub>14</sub> from MD, and R3.M1:C<sub>12</sub> from MD assume a similar conformation and are colored red. Unbound ‘TesA, an unbound L109P variant (that abolishes switch loop movement), and a bound L109P variant from previous crystallography experiments assume a similar conformation and are colored blue (from Lo *et al.*). R3.M4:C<sub>8</sub> from MD, R3.M4:C<sub>12</sub> from MD, R3.M1:C<sub>14</sub>, and WT:C<sub>8</sub> are colored green, cyan, orange, and pink, respectively. **The carbonyl oxygen atoms of the acyl-ACP structures are depicted as spheres.**



**Figure S4. Correlation between R4  $C_8/C_{12}$  and  $C_8/C_{14}$  molar composition.** The strong correlation ( $r=0.88\pm 0.02$ ) between these values supports the concept that reduced binding site volume for  $C_{12}$  binding furthermore eliminates binding to  $C_{14}$  due to its longer aliphatic chain. The slope for the linear regression is less than one, which could imply an alternative binding mode for  $C_{14}$  in addition to the conformation assumed for  $C_{12}$  binding. Error bars are standard deviations in mole fractions propagated from **Table S1**.



**Figure S5.** Correlation between experimentally estimated and computationally calculated  $\Delta\Delta G$  for (A) R3 and (B) R4. Experimental  $\Delta\Delta G$  values were estimated using the product compositions from Table S1 (see Supporting Information). Binding was measured between the enzyme and myristoyl-ACP for R3 and measured between the enzyme and lauroyl-ACP for R4 (i.e., the primary computational constraint). Error bars indicate experimental error, but calculated values are replicable and have no associated error.

## Tables

**Table S1. FFA production profiles for various thioesterases expressed in *E. coli*.** This table provides the mole fractions of various FFAs and total FFA in the cell lysate for enzymes considered in this study as well as those found in literature. Computationally-predicted mutants are named Rx.My, rationally-designed mutants are named Rx.RDy, and randomly-generated mutants are labeled RM.My, where x is the Round number and y is the Mutant number. Any mutant labeled in this manner derives from this study. Heterologously-expressed and overexpressed thioesterases in *E. coli* are named using their GenBank accession label and engineered mutants from literature (beginning with “CvB2MT”, “rTE”, “TEGm” or “UcFatB”) are named as they are in the original source. “nd” indicates that the value was not determined or provided.

Name	Organism	8:0	10:0	12:0	12:1	14:0	14:1	16:0	16:1	18:0	18:1	Total FFA ( $\mu\text{M}$ )	Notes	Source
Control	<i>E. coli</i>	2.4 $\pm$ 0.3	0.6 $\pm$ 0.2	3.6 $\pm$ 0.8	0.6 $\pm$ 0.9	17 $\pm$ 2	0.51 $\pm$ 0.06	57 $\pm$ 6	12 $\pm$ 2	1.3 $\pm$ 0.1	4.5 $\pm$ 0.6	300 $\pm$ 20	-	-
WT	<i>E. coli</i>	5.9 $\pm$ 0.3	1.09 $\pm$ 0.06	20 $\pm$ 1	7.5 $\pm$ 0.5	29 $\pm$ 2	7.1 $\pm$ 0.4	14.2 $\pm$ 0.8	11.6 $\pm$ 0.8	0.22 $\pm$ 0.01	2.1 $\pm$ 0.1	1900 $\pm$ 50	-	-
R1.M1	<i>E. coli</i>	19 $\pm$ 2	6.0 $\pm$ 0.5	12.1 $\pm$ 0.7	31 $\pm$ 2	4.4 $\pm$ 0.3	14.9 $\pm$ 0.7	4.7 $\pm$ 0.7	6.4 $\pm$ 0.8	1.52 $\pm$ 0.10	0.11 $\pm$ 0.01	4300 $\pm$ 100	-	-
R1.M2	<i>E. coli</i>	13 $\pm$ 2	2.2 $\pm$ 0.5	5.8 $\pm$ 0.5	2.0 $\pm$ 0.1	28 $\pm$ 2	3.7 $\pm$ 0.3	27 $\pm$ 2	8.5 $\pm$ 0.6	0.9 $\pm$ 0.2	4.1 $\pm$ 0.5	650 $\pm$ 30	-	-
R1.M3	<i>E. coli</i>	5 $\pm$ 4	1 $\pm$ 2	3 $\pm$ 2	3 $\pm$ 2	11 $\pm$ 2	0.0 $\pm$ 0.0	60 $\pm$ 10	2.5 $\pm$ 0.5	1.9 $\pm$ 0.4	4 $\pm$ 1	190 $\pm$ 40	-	-
R1.M4	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	10 $\pm$ 10	3 $\pm$ 6	12 $\pm$ 2	3 $\pm$ 1	64 $\pm$ 8	3.2 $\pm$ 0.7	2.5 $\pm$ 0.5	3.5 $\pm$ 0.7	230 $\pm$ 30	-	-
R1.M5	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	3 $\pm$ 5	2 $\pm$ 3	13 $\pm$ 3	2 $\pm$ 3	70 $\pm$ 6	4 $\pm$ 1	2.3 $\pm$ 0.3	4.3 $\pm$ 0.6	160 $\pm$ 20	-	-
R1.M6	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	10 $\pm$ 10	4 $\pm$ 6	14 $\pm$ 5	2 $\pm$ 4	60 $\pm$ 10	4 $\pm$ 2	2.3 $\pm$ 0.5	4 $\pm$ 1	180 $\pm$ 30	-	-
R1.M7	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	2 $\pm$ 3	0.0 $\pm$ 0.0	13 $\pm$ 2	3 $\pm$ 3	72 $\pm$ 5	3.5 $\pm$ 0.8	2.4 $\pm$ 0.5	4.2 $\pm$ 0.8	170 $\pm$ 20	-	-
R1.M8	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1 $\pm$ 2	0.0 $\pm$ 0.0	13 $\pm$ 4	1 $\pm$ 2	74 $\pm$ 5	3.3 $\pm$ 0.8	2.5 $\pm$ 0.7	4 $\pm$ 1	170 $\pm$ 30	-	-
R1.M9	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	5 $\pm$ 4	2 $\pm$ 3	14 $\pm$ 2	2 $\pm$ 3	69 $\pm$ 4	3.0 $\pm$ 0.4	2.6 $\pm$ 0.5	4.1 $\pm$ 0.3	180 $\pm$ 10	-	-
R1.M10	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	4 $\pm$ 3	0.0 $\pm$ 0.0	13 $\pm$ 1	1 $\pm$ 2	72 $\pm$ 3	3.3 $\pm$ 0.2	2.4 $\pm$ 0.2	4.8 $\pm$ 0.9	164 $\pm$ 8	-	-
R1.M11	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	3 $\pm$ 3	0.0 $\pm$ 0.0	14 $\pm$ 3	0.0 $\pm$ 0.0	73 $\pm$ 4	4 $\pm$ 1	2.6 $\pm$ 0.5	3.4 $\pm$ 0.7	180 $\pm$ 20	-	-
R1.M12	<i>E. coli</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	4 $\pm$ 3	2 $\pm$ 3	13 $\pm$ 2	3 $\pm$ 3	69 $\pm$ 4	2.9 $\pm$ 0.3	2.5 $\pm$ 0.3	4.0 $\pm$ 0.6	160 $\pm$ 10	-	-
R2.M1	<i>E. coli</i>	7 $\pm$ 1	0.0 $\pm$ 0.0	1.2 $\pm$ 0.1	0.0 $\pm$ 0.0	12 $\pm$ 1	0.7 $\pm$ 0.1	47 $\pm$ 2	13.6 $\pm$ 0.7	0.7 $\pm$ 0.0	4.2 $\pm$ 0.3	510 $\pm$ 20	-	-
R2.M2	<i>E. coli</i>	7.3 $\pm$ 0.4	0.7 $\pm$ 0.0	1.4 $\pm$ 0.1	0.0 $\pm$ 0.0	14.7 $\pm$ 0.7	0.9 $\pm$ 0.0	53.8 $\pm$ 0.9	15.7 $\pm$ 0.8	0.8 $\pm$ 0.0	4.7 $\pm$ 0.2	447 $\pm$ 8	-	-
R2.M3	<i>E. coli</i>	8 $\pm$ 1	0.0 $\pm$ 0.0	1.4 $\pm$ 0.1	0.0 $\pm$ 0.0	11.8 $\pm$ 0.4	0.7 $\pm$ 0.0	47 $\pm$ 1	14.6 $\pm$ 0.7	0.8 $\pm$ 0.0	4.1 $\pm$ 0.3	480 $\pm$ 10	-	-
R2.M4	<i>E. coli</i>	6.3 $\pm$ 0.7	0.6 $\pm$ 0.0	1.3 $\pm$ 0.1	0.0 $\pm$ 0.0	12.4 $\pm$ 0.8	0.7 $\pm$ 0.1	47 $\pm$ 2	14 $\pm$ 1	0.4 $\pm$ 0.0	3.7 $\pm$ 0.4	520 $\pm$ 20	-	-
R2.M5	<i>E. coli</i>	5.9 $\pm$ 0.4	2.4 $\pm$ 0.2	12.9 $\pm$ 0.8	6.1 $\pm$ 0.4	38 $\pm$ 2	6.4 $\pm$ 0.5	8.4 $\pm$ 0.6	17 $\pm$ 2	0.1 $\pm$ 0.0	1.8 $\pm$ 0.2	3500 $\pm$ 100	-	-
R2.M6	<i>E. coli</i>	1.2 $\pm$ 0.2	0.0 $\pm$ 0.0	1.3 $\pm$ 0.1	0.0 $\pm$ 0.0	11.6 $\pm$ 0.6	0.7 $\pm$ 0.0	49 $\pm$ 2	15.3 $\pm$ 0.9	0.7 $\pm$ 0.1	4.0 $\pm$ 0.3	500 $\pm$ 10	-	-
R2.M7	<i>E. coli</i>	1.3 $\pm$ 0.1	0.0 $\pm$ 0.0	1.4 $\pm$ 0.0	0.0 $\pm$ 0.0	12.4 $\pm$ 0.5	0.7 $\pm$ 0.0	48 $\pm$ 1	16.2 $\pm$ 0.4	0.8 $\pm$ 0.0	4.1 $\pm$ 0.2	510 $\pm$ 10	-	-
R2.M8	<i>E. coli</i>	1.5 $\pm$ 0.1	0.0 $\pm$ 0.0	1.6 $\pm$ 0.1	0.0 $\pm$ 0.0	15 $\pm$ 1	0.9 $\pm$ 0.1	57 $\pm$ 2	19 $\pm$ 1	0.8 $\pm$ 0.0	4.9 $\pm$ 0.5	440 $\pm$ 20	-	-
R2.M9	<i>E. coli</i>	1.0 $\pm$ 0.1	0.0 $\pm$ 0.0	1.2 $\pm$ 0.1	0.0 $\pm$ 0.0	11.0 $\pm$ 0.7	0.6 $\pm$ 0.0	48 $\pm$ 1	15.4 $\pm$ 0.5	0.8 $\pm$ 0.0	4.1 $\pm$ 0.3	420 $\pm$ 10	-	-
R2.M10	<i>E. coli</i>	1.2 $\pm$ 0.2	0.8 $\pm$ 0.1	1.5 $\pm$ 0.2	0.0 $\pm$ 0.0	14 $\pm$ 2	0.8 $\pm$ 0.1	56 $\pm$ 3	20 $\pm$ 3	0.9 $\pm$ 0.1	4.7 $\pm$ 0.4	380 $\pm$ 30	-	-
R2.M11	<i>E. coli</i>	1.4 $\pm$ 0.1	0.0 $\pm$ 0.0	1.6 $\pm$ 0.1	0.0 $\pm$ 0.0	14.3 $\pm$ 0.9	0.8 $\pm$ 0.0	57 $\pm$ 1	18.5 $\pm$ 0.8	1.0 $\pm$ 0.0	5.1 $\pm$ 0.2	355 $\pm$ 10	-	-
R2.M12	<i>E. coli</i>	1.3 $\pm$ 0.2	0.0 $\pm$ 0.0	1.6 $\pm$ 0.1	0.0 $\pm$ 0.0	13.1 $\pm$ 0.8	0.8 $\pm$ 0.0	57.1 $\pm$ 0.9	18.9 $\pm$ 0.5	2.0 $\pm$ 0.1	5.2 $\pm$ 0.2	343 $\pm$ 7	-	-



R2.M13	<i>E. coli</i>	1.1±0.3	0.0±0.0	1.4±0.3	0.0±0.0	9±3	0.6±0.2	62±8	11±5	0.7±0.7	4±2	480±90	-	-
R2.M14	<i>E. coli</i>	1.8±0.2	0.9±0.1	2.6±0.3	0.0±0.0	15±1	1.1±0.1	44±2	15±1	0.9±0.1	4.1±0.4	460±20	-	-
R2.RD1	<i>E. coli</i>	2.9±0.2	2.8±0.2	20.5±0.9	1.7±0.1	31.2±0.7	7.8±0.3	17.1±0.7	13.2±0.5	0.1±0.0	2.0±0.1	2100±30	-	-
R2.RD2	<i>E. coli</i>	1.2±0.0	0.8±0.0	1.5±0.1	0.0±0.0	12.2±0.3	0.7±0.0	46.4±0.9	15.4±0.6	0.8±0.0	4.5±0.1	380±8	-	-
R2.RD3	<i>E. coli</i>	1.4±0.1	0.9±0.0	1.9±0.1	0.0±0.0	16±1	0.8±0.0	55.7±0.9	17.8±0.6	1.0±0.0	4.7±0.4	336±6	-	-
R2.RD4	<i>E. coli</i>	1.2±0.1	0.7±0.0	1.4±0.1	0.0±0.0	12.7±0.8	0.6±0.0	46±2	15±1	0.8±0.1	4.1±0.3	420±10	-	-
R2.RD5	<i>E. coli</i>	2.4±0.1	0.9±0.1	2.2±0.1	0.0±0.0	14±1	1.3±0.1	44±2	15±1	0.8±0.1	3.9±0.2	430±10	-	-
R3.M1	<i>E. coli</i>	15±4	6±2	35±7	14±4	14±4	5±2	6±2	3.8±0.8	0.1±0.0	1.4±0.4	3500±400	-	-
R3.M2	<i>E. coli</i>	26±5	3.2±0.7	13±3	9±2	21±4	4.4±0.8	11±3	11±2	0.1±0.0	1.6±0.5	4100±400	-	-
R3.M3	<i>E. coli</i>	25±6	3±1	19±5	5±1	23±7	4±1	12±4	7±2	0.2±0.1	3±1	2300±300	-	-
R3.M4	<i>E. coli</i>	50±3	3.9±0.5	9±1	5.6±0.7	13±1	1.8±0.2	8.9±0.7	5.8±0.5	0.1±0.0	1.7±0.1	4300±300	-	-
R3.M5	<i>E. coli</i>	3±1	0.7±0.6	2.5±0.5	0.0±0.0	17±3	0.9±0.2	53±5	13±2	7±7	3.7±0.5	380±40	-	-
R3.M6	<i>E. coli</i>	9.4±0.8	1.4±0.1	11.9±0.8	4.3±0.3	36±2	6.3±0.6	12.2±0.9	16±1	0.2±0.0	2.5±0.2	3400±100	-	-
R3.M7	<i>E. coli</i>	20±9	6±3	29±10	11±5	16±7	4±2	7±3	5±2	0.1±0.1	1.7±0.8	3100±600	-	-
R3.M8	<i>E. coli</i>	16±2	6.1±0.9	33±4	14±2	13±2	5.4±0.9	7±1	4.1±0.9	0.1±0.0	1.8±0.4	3900±200	-	-
R3.M9	<i>E. coli</i>	21±3	4.7±0.4	22±2	7.7±0.8	21±2	4.1±0.4	11±2	6.3±0.9	0.2±0.0	2.9±0.4	2500±100	-	-
R3.M10	<i>E. coli</i>	3±1	1.0±0.3	1.8±0.6	0.0±0.0	15±5	0.8±0.3	58±9	15±5	1.0±0.3	4±2	390±70	-	-
R3.RD1	<i>E. coli</i>	20±20	4±3	20±10	7±4	20±30	4±5	10±10	10±10	0.2±0.2	2±4	1300±600	-	-
R3.RD2	<i>E. coli</i>	19.2±0.4	4.8±0.1	20.5±0.6	7.3±0.2	24.7±0.6	4.3±0.1	11±1	6.5±0.3	0.2±0.0	2.0±0.1	1980±30	-	-
R3.RD3	<i>E. coli</i>	10±3	3±1	35±9	13±5	18±6	6±3	7±3	6±2	0.1±0.0	1.5±0.6	3300±500	-	-
R3.RD4	<i>E. coli</i>	13±1	2.1±0.1	22±1	7.3±0.3	29±2	6.9±0.4	9±1	7.9±0.6	0.1±0.0	1.8±0.2	4600±100	-	-
R3.RD5	<i>E. coli</i>	21±4	5±1	21±4	8±2	25±5	5±1	8±2	6±2	0.1±0.0	1.8±0.5	2700±300	-	-
R4.M1	<i>E. coli</i>	10.6±0.4	2.6±0.2	13.0±0.9	4.1±0.4	34±1	5.2±0.5	14.3±0.4	11.5±0.7	0.0±0.0	2.2±0.1	2850±60	-	-
R4.M2	<i>E. coli</i>	23±1	4.1±0.2	12.3±0.3	9.0±0.3	24±1	4.2±0.1	10.6±0.7	11.5±0.6	0.0±0.0	1.3±0.1	3430±70	-	-
R4.M3	<i>E. coli</i>	3.1±0.2	0.9±0.0	9.1±0.3	3.8±0.1	43.8±0.9	7.1±0.2	8.9±0.3	19.7±0.7	0.0±0.0	2.1±0.1	3370±60	-	-
R4.M4	<i>E. coli</i>	6.8±0.6	1.2±0.1	8.8±0.9	3.9±0.4	42±3	6.2±0.6	8.4±0.8	19±2	0.0±0.0	1.8±0.3	4700±200	-	-
R4.M5	<i>E. coli</i>	11±1	3.0±0.2	15±1	8.9±0.6	30±3	4.3±0.5	11±1	14±2	0.0±0.0	1.8±0.3	4700±200	-	-
R4.M6	<i>E. coli</i>	6.0±0.6	2.3±0.2	12.5±0.8	5.9±0.4	38±2	6.5±0.5	8.3±0.6	17±1	0.0±0.0	2.0±0.2	3800±100	-	-
R4.M7	<i>E. coli</i>	2.7±0.4	1.2±0.1	17±2	2.7±0.4	37±2	4.9±0.6	17.6±0.9	11.1±0.4	0.0±0.0	2.9±0.1	2200±70	-	-
R4.M8	<i>E. coli</i>	12.0±0.8	2.4±0.1	18.6±0.7	6.7±0.3	29.7±0.9	5.2±0.2	13.2±0.6	10.2±0.4	0.0±0.0	1.9±0.1	2890±50	-	-
R4.M9	<i>E. coli</i>	3.5±0.4	1.0±0.0	20±1	4.7±0.5	35.9±0.9	4.8±0.3	15.7±0.9	9.5±0.3	0.0±0.0	2.6±0.2	2550±60	-	-
R4.M10	<i>E. coli</i>	10.7±0.4	2.6±0.1	8.3±0.3	5.5±0.4	28.3±0.8	6.6±0.3	17.9±0.5	15.7±0.4	0.0±0.0	2.0±0.1	2280±30	-	-
R4.M11	<i>E. coli</i>	4.3±0.7	0.0±0.0	3.4±0.8	4.2±0.9	15±2	28±3	4.6±0.3	30±2	0.0±0.0	2.9±0.3	1110±60	-	-
R4.M12	<i>E. coli</i>	14.5±0.5	0.0±0.0	3.6±0.2	0.0±0.0	17.5±0.8	0.0±0.0	35.9±0.7	12.8±0.3	0.0±0.0	3.3±0.1	660±10	-	-
R4.M13	<i>E. coli</i>	37.9±0.8	3.1±0.1	4.7±0.2	4.4±0.2	17.0±0.6	4.7±0.2	13.4±0.6	12.2±0.3	0.0±0.0	1.5±0.1	2830±40	-	-
R4.M14	<i>E. coli</i>	20.0±0.5	2.6±0.1	15.3±0.4	8.5±0.4	26±1	6.1±0.3	6.5±0.3	12.7±0.8	0.0±0.0	1.1±0.1	2910±50	-	-
R4.M15	<i>E. coli</i>	33.2±0.7	3.6±0.2	6.0±0.4	5.8±0.3	20±1	5.8±0.4	10.9±0.4	12.0±0.9	0.0±0.0	1.2±0.1	3490±70	-	-
R4.M16	<i>E. coli</i>	9.6±0.4	1.5±0.1	8.9±0.4	3.7±0.2	39.3±0.9	4.9±0.2	11.5±0.3	16.5±0.6	0.0±0.0	2.0±0.1	4000±70	-	-

R4.M17	<i>E. coli</i>	18±1	2.0±0.2	10.9±0.9	7.5±0.5	26±1	6.8±0.5	13.3±0.4	12.8±0.7	0.0±0.0	1.6±0.1	3050±80	-	-
R4.M18	<i>E. coli</i>	15±1	1.7±0.1	9.2±0.6	6.2±0.3	28.0±0.9	6.8±0.3	14.4±0.5	14.9±0.5	0.0±0.0	1.7±0.1	3150±60	-	-
R4.RD1	<i>E. coli</i>	18±2	2.6±0.1	8.7±0.4	6.7±0.3	29±1	4.1±0.2	12.7±0.8	16.2±0.8	0.0±0.0	1.7±0.1	3230±90	-	-
R4.RD2	<i>E. coli</i>	35±1	5.2±0.1	16.4±0.4	12.7±0.3	16.8±0.3	3.5±0.1	3.9±0.2	5.5±0.2	0.0±0.0	0.8±0.1	3800±60	-	-
R4.RD3	<i>E. coli</i>	39±1	5.0±0.2	10.8±0.5	8.4±0.2	19.5±0.7	3.5±0.1	6.1±0.5	7.2±0.4	0.0±0.0	1.0±0.1	3500±60	-	-
R4.RD4	<i>E. coli</i>	22±2	3.0±0.2	7.9±0.6	6.6±0.4	21±2	5.0±0.3	17±1	13.5±0.9	0.0±0.0	2.0±0.2	2690±90	-	-
R4.RD5	<i>E. coli</i>	34±2	5.6±0.3	7.7±0.3	7.7±0.3	15.4±0.5	4.3±0.1	12.6±0.6	9.3±0.3	0.0±0.0	1.5±0.1	2910±90	-	-
R4.RD6	<i>E. coli</i>	29.5±0.6	4.5±0.2	7.2±0.3	6.7±0.4	16.9±0.4	4.1±0.2	15.7±0.6	10.2±0.7	0.0±0.0	1.8±0.1	2400±30	-	-
R4.RD7	<i>E. coli</i>	3.4±0.4	1.6±0.2	13.3±0.5	0.8±0.1	12±1	1.1±0.2	10.0±0.4	43±1	0.7±0.0	3.7±0.4	540±10	-	-
R4.RD8	<i>E. coli</i>	9.1±0.6	2.5±0.1	21±1	6.5±0.3	31±2	5.0±0.3	11±1	12±1	0.0±0.0	2.0±0.3	4100±200	-	-
R4.RD9	<i>E. coli</i>	6.5±0.2	2.0±0.0	21.4±0.4	11.9±0.3	27.9±0.5	6.6±0.2	8.4±0.2	11.8±0.5	0.0±0.0	1.2±0.0	2420±30	-	-
R4.RD10	<i>E. coli</i>	4.5±0.2	1.3±0.0	27.1±0.6	10.6±0.2	28.3±0.8	8.4±0.2	6.6±0.3	9.8±0.4	0.0±0.0	1.2±0.1	2570±40	-	-
R4.RD11	<i>E. coli</i>	4.8±0.6	0.0±0.0	26±3	10±1	26±2	7.5±0.8	15±1	10.3±0.7	0.0±0.0	1.6±0.1	1670±80	-	-
RM.M1	<i>E. coli</i>	4.5	1.6	15.2	4.4	47.7	6.1	15.8	0.2	0.1	3.2	3100	a	-
RM.M2	<i>E. coli</i>	3.9	1.2	21.0	8.4	34.6	8.2	17.9	0.3	0.2	2.3	1700	a	-
RM.M3	<i>E. coli</i>	2.6	0.7	17.9	5.8	41.0	7.2	19.7	0.2	0.2	2.8	2500	a	-
RM.M4	<i>E. coli</i>	1.4	0.6	15.0	2.8	54.7	3.4	13.1	0.3	0.4	5.4	1100	a	-
RM.M5	<i>E. coli</i>	4.0	2.4	3.8	nd	21.9	nd	5.2	nd	2.1	3.2	120	a	-
RM.M6	<i>E. coli</i>	4.9	1.3	10.9	3.7	49.4	6.4	19.1	0.2	0.1	2.8	3100	a	-
RM.M7	<i>E. coli</i>	2.5	0.7	16.8	5.5	42.2	6.2	20.7	0.2	0.2	2.9	2300	a	-
RM.M8	<i>E. coli</i>	3.9	1.0	18.6	7.2	35.9	8.1	19.4	0.3	0.3	3.1	1500	a	-
RM.M9	<i>E. coli</i>	3.4	2.3	4.2	nd	24.6	nd	7.6	nd	1.8	2.6	130	a	-
RM.M10	<i>E. coli</i>	2.9	1.0	19.0	7.5	35.0	8.1	20.0	0.6	0.3	3.2	1200	a	-
RM.M11	<i>E. coli</i>	4.1	2.4	3.9	nd	23.8	nd	5.2	1.8	1.8	2.9	120	a	-
RM.M12	<i>E. coli</i>	5.5	1.2	18.6	3.5	48.9	3.7	12.7	0.1	0.2	4.4	3300	a	-
RM.M13	<i>E. coli</i>	3.7	nd	3.9	nd	32.0	nd	5.1	1.7	1.8	2.6	140	a	-
RM.M14	<i>E. coli</i>	2.6	1.1	19.9	8.4	33.2	8.3	17.2	0.8	0.4	2.7	850	a	-
RM.M15	<i>E. coli</i>	3.2	0.6	11.8	5.0	44.4	11.0	20.4	0.2	0.2	2.0	3300	a	-
RM.M16	<i>E. coli</i>	3.2	1.1	19.5	7.7	35.2	8.0	18.2	0.5	0.4	2.7	1100	a	-
RM.M17	<i>E. coli</i>	3.0	0.9	15.6	5.8	41.0	7.5	20.6	0.4	0.2	2.6	1600	a	-
RM.M18	<i>E. coli</i>	3.2	1.1	19.1	7.3	36.5	8.3	18.1	0.4	0.4	2.6	1200	a	-
RM.M19	<i>E. coli</i>	3.4	1.0	19.3	7.3	35.2	7.5	19.9	0.6	0.4	3.2	1300	a	-
RM.M20	<i>E. coli</i>	3.1	0.9	18.3	8.1	38.1	8.6	18.4	0.2	0.2	2.0	1600	a	-
RM.M21	<i>E. coli</i>	2.5	0.7	16.6	3.6	49.3	5.5	15.9	0.1	0.2	4.2	3200	a	-
RM.M22	<i>E. coli</i>	1.4	0.7	11.0	0.5	66.6	1.4	8.7	0.4	0.4	5.6	780	a	-
RM.M23	<i>E. coli</i>	4.6	2.7	4.1	nd	23.2	nd	6.9	nd	2.0	3.0	120	a	-
RM.M24	<i>E. coli</i>	3.8	1.6	7.5	nd	41.8	1.1	6.3	1.1	1.2	1.5	200	a	-
RM.M25	<i>E. coli</i>	2.7	0.9	23.4	4.1	45.9	4.4	14.2	0.1	0.1	2.9	3800	a	-

RM.M26	<i>E. coli</i>	3.9	2.5	3.6	nd	20.3	nd	6.2	nd	2.3	3.1	110	a	-
RM.M27	<i>E. coli</i>	3.4	0.8	19.1	4.8	46.6	6.9	13.7	0.1	0.1	3.5	3600	a	-
RM.M28	<i>E. coli</i>	3.9	2.4	3.1	nd	18.9	nd	6.2	1.8	2.2	3.0	110	a	-
RM.M29	<i>E. coli</i>	21.9	1.7	16.3	2.7	38.0	3.0	11.3	0.2	0.2	3.8	2500	a	-
RM.M30	<i>E. coli</i>	3.5	0.8	17.9	7.0	39.8	7.4	19.0	0.2	0.2	2.2	2000	a	-
RM.M31	<i>E. coli</i>	5.9	1.6	7.9	2.1	43.9	6.3	13.0	0.5	1.0	4.1	400	a	-
RM.M32	<i>E. coli</i>	2.9	0.9	17.6	6.3	40.3	7.0	19.1	0.5	0.3	2.6	1700	a	-
RM.M33	<i>E. coli</i>	4.9	2.3	4.0	nd	21.6	nd	6.5	nd	1.9	2.7	120	a	-
RM.M34	<i>E. coli</i>	3.1	1.0	20.9	7.8	36.4	8.2	17.8	0.2	0.3	2.2	1600	a	-
RM.M35	<i>E. coli</i>	3.5	1.0	16.4	7.5	38.1	7.4	18.0	0.6	0.5	2.7	920	a	-
RM.M36	<i>E. coli</i>	3.2	1.1	21.7	7.4	38.9	6.6	17.8	0.1	0.1	1.9	2500	a	-
RM.M37	<i>E. coli</i>	3.4	1.2	20.5	7.7	35.1	7.9	16.9	0.5	0.4	2.5	1000	a	-
RM.M38	<i>E. coli</i>	2.2	0.8	19.1	5.3	43.0	6.2	18.9	0.2	0.2	3.0	2700	a	-
RM.M39	<i>E. coli</i>	4.9	2.3	31.6	12.4	28.1	6.9	10.4	0.3	0.2	1.9	2100	a	-
RM.M40	<i>E. coli</i>	2.5	0.8	19.1	3.9	48.3	5.0	15.8	0.1	0.1	3.5	3500	a	-
RM.M41	<i>E. coli</i>	2.2	0.7	18.6	5.7	43.8	7.3	17.7	0.3	0.2	2.6	3200	a	-
RM.M42	<i>E. coli</i>	5.0	1.1	9.2	4.7	42.8	15.5	18.7	0.2	0.2	1.4	3500	a	-
RM.M43	<i>E. coli</i>	3.5	1.1	20.0	7.8	35.4	8.6	16.8	0.5	0.4	2.4	960	a	-
RM.M44	<i>E. coli</i>	3.2	1.0	20.0	7.6	37.3	8.2	17.7	0.3	0.3	2.3	1500	a	-
RM.M45	<i>E. coli</i>	4.3	2.4	3.9	nd	21.7	nd	6.7	1.8	2.3	2.7	120	a	-
RM.M46	<i>E. coli</i>	4.6	2.6	3.9	nd	22.0	nd	6.5	nd	2.0	2.7	110	a	-
RM.M47	<i>E. coli</i>	1.4	0.7	10.4	0.5	63.6	1.2	8.0	0.6	0.5	6.9	540	a	-
RM.M48	<i>E. coli</i>	1.9	0.7	18.5	5.4	43.6	7.0	18.6	0.3	0.2	2.7	2700	a	-
RM.M49	<i>E. coli</i>	2.6	1.5	8.8	nd	51.2	1.1	6.5	nd	1.4	1.5	210	a	-
RM.M50	<i>E. coli</i>	4.5	2.4	3.6	nd	21.4	nd	6.3	nd	2.0	2.6	120	a	-
RM.M51	<i>E. coli</i>	4.5	2.4	3.7	nd	21.8	nd	6.3	nd	2.1	2.7	120	a	-
RM.M52	<i>E. coli</i>	3.1	1.1	19.3	7.2	37.5	7.7	19.2	0.2	0.3	2.4	1400	a	-
RM.M53	<i>E. coli</i>	3.1	1.4	18.4	7.4	32.9	8.1	17.2	0.4	0.6	3.5	550	a	-
RM.M54	<i>E. coli</i>	3.9	2.1	3.9	nd	30.3	nd	6.7	nd	1.7	2.4	130	a	-
RM.M55	<i>E. coli</i>	2.9	1.1	20.6	8.0	35.3	8.1	17.6	0.5	0.4	2.5	1100	a	-
RM.M56	<i>E. coli</i>	4.3	1.6	20.1	7.8	27.1	7.0	16.9	0.4	0.7	4.0	540	a	-
RM.M57	<i>E. coli</i>	4.4	3.7	23.0	19.3	27.9	5.5	12.9	0.1	0.2	1.6	1900	a	-
RM.M58	<i>E. coli</i>	2.8	1.3	21.9	8.5	33.0	7.6	17.6	0.3	0.5	2.8	930	a	-
RM.M59	<i>E. coli</i>	4.3	1.1	12.7	5.1	42.8	7.7	21.4	0.2	0.2	2.3	1600	a	-
RM.M60	<i>E. coli</i>	2.8	0.9	23.0	6.8	38.5	6.6	17.5	0.3	0.1	2.4	2900	a	-
RM.M61	<i>E. coli</i>	4.3	1.6	20.2	8.2	29.8	7.4	15.6	0.6	0.7	3.7	570	a	-
AAC49179	<i>C. palustris</i>	97.5±0.2	0.9±0.1	0.6±0.1	0.2±0.0	0.2±0.1	0.2±0.0	nd	0.1±0.1	nd	nd	710±50	-	2
AAB71731	<i>U. americana</i>	44±3	23±1	3.7±0.4	7.9±0.9	9.8±0.8	1.5±0.2	1.4±0.1	4.4±0.7	nd	nd	1100±60	-	2

AAG43857	<i>I. germanica</i>	3.3±0.4	0.5±0.1	0.7±0.1	1.0±0.1	30±3	0.3±0.1	20±3	44±4	nd	nd	260±20	-	<sup>2</sup>
AAG43858	<i>I. germanica</i>	8±4	nd	1±1	nd	30±10	nd	20±10	40±20	nd	nd	15±5	-	<sup>2</sup>
EER87824	<i>S. bicolor</i>	4.8±0.7	0.2±0.0	1.6±0.3	0.4±0.1	46±5	nd	13±3	35±6	nd	nd	130±10	-	<sup>2</sup>
EER88593	<i>S. bicolor</i>	6±1	0.7±0.1	3.2±0.5	0.6±0.3	45±5	3.3±0.9	11±2	31±4	nd	nd	91±8	-	<sup>2</sup>
AEM72519	<i>C. nucifera</i>	14±3	1.1±0.3	1.4±0.3	1.1±0.1	44±5	0.4±0.3	6±1	31±5	nd	nd	130±10	-	<sup>2</sup>
AEM72520	<i>C. nucifera</i>	1.7±0.2	0.1±0.0	1.0±0.2	0.3±0.1	36±3	0.7±0.1	16±2	44±3	nd	nd	570±30	-	<sup>2</sup>
AEM72521	<i>C. nucifera</i>	11.1±0.8	1.2±0.1	34±2	6.1±0.6	14±2	23±3	2±1	9±3	nd	nd	200±10	-	<sup>2</sup>
AEM72522	<i>C. viscosissima</i>	52±6	26±5	7±2	6±2	7±2	0.9±0.6	nd	nd	nd	nd	79±10	-	<sup>2</sup>
AEM72523	<i>C. viscosissima</i>	4±1	0.5±0.2	1.0±0.3	0.9±0.4	47±2	0.7±0.5	26±2	19±2	nd	nd	249±9	-	<sup>2</sup>
AEM72524	<i>C. viscosissima</i>	7±5	5±3	2±2	2±2	84±6	nd	nd	nd	nd	nd	19±2	-	<sup>2</sup>
AAD42220	<i>E. guineensis</i>	14±4	1.9±0.8	2±1	2±1	47±5	3±3	nd	26±5	nd	nd	37±4	-	<sup>2</sup>
EDQ65090	<i>P. patens</i>	9±1	0.4±0.1	0.4±0.1	0.2±0.0	42±4	0.2±0.1	16±3	32±4	nd	nd	380±30	-	<sup>2</sup>
EER96252	<i>S. bicolor</i>	5.8±0.9	1.8±0.8	1.2±0.3	1.1±0.4	34±3	0.9±0.2	18±3	37±3	nd	nd	180±10	-	<sup>2</sup>
EES11622	<i>S. bicolor</i>	5±3	nd	nd	nd	50±10	nd	15±8	29±9	nd	nd	9±2	-	<sup>2</sup>
EEH52851	<i>M. pusilla</i>	4±2	nd	0±1	0.6±0.1	65±4	8±4	nd	23±3	nd	nd	16±2	-	<sup>2</sup>
ACL08376	<i>D. vulgaris</i>	29±1	3.5±0.2	7.9±0.3	24±1	6.0±0.4	24±1	1.2±0.4	2.6±0.3	nd	nd	330±9	-	<sup>2</sup>
CAH09236	<i>B. fragilis</i>	20±2	2.7±0.2	3.6±0.4	19±1	5.1±0.3	25±1	2.2±0.3	5.4±0.4	nd	nd	215±6	-	<sup>2</sup>
ABR43801	<i>P. distasonis</i>	18±5	6.3±0.4	16±1	9.3±0.8	21±2	27±2	nd	nd	nd	nd	70±4	-	<sup>2</sup>
AAO77182	<i>B. thetaiotaomicron</i>	13.4±0.8	2.1±0.2	4.6±0.7	16.7±0.9	6±1	26±1	nd	2.6±0.3	nd	nd	60±3	-	<sup>2</sup>
ABG82470	<i>C. perfringens</i>	70±4	3.0±0.5	nd	1.1±0.2	nd	9±2	nd	nd	nd	nd	72±10	-	<sup>2</sup>
EEG55387	<i>C. asparagiforme</i>	26±6	6±1	7±2	1.6±0.8	35±9	18±6	nd	1.3±0.9	nd	nd	26±4	-	<sup>2</sup>
EET61113	<i>M. formatexigens</i>	31.8±0.3	5.1±0.1	4.3±0.2	8.9±0.5	1.9±0.2	10.5±0.2	0.4±0.2	1.2±0.1	nd	nd	381±3	-	<sup>2</sup>
EDV77528	<i>G. sp.</i>	9±4	2±1	7±3	30±10	11±3	32±8	0±1	2±1	nd	nd	60±10	-	<sup>2</sup>
BAH81730	<i>S. dysgalactiae</i>	30±1	5.0±0.2	5.7±0.3	13.5±0.7	4.4±0.3	20±1	0.3±0.1	3.3±0.1	nd	nd	620±10	-	<sup>2</sup>
ABJ63754	<i>L. brevis</i>	55.5±0.7	2.6±0.1	3.8±0.1	7.9±0.2	1.9±0.1	6.3±0.2	nd	0.7±0.1	nd	nd	710±10	-	<sup>2</sup>
CAD63310	<i>L. plantarum</i>	68.0±0.8	1.2±0.1	2.8±0.2	4.6±0.2	1.9±0.1	6.9±0.4	nd	0.5±0.1	nd	nd	440±10	-	<sup>2</sup>
EEL82564	<i>A. tetradus</i>	87±2	2.2±0.3	1.1±0.2	2.8±0.5	1.2±0.2	3.0±0.5	0.1±0.1	0.7±0.1	nd	nd	1400±100	-	<sup>2</sup>
CAE80300	<i>B. bacteriovorus</i>	37±3	3.3±0.5	6.7±0.7	7.6±0.6	8.2±0.7	28±2	1.6±0.2	6.6±0.5	nd	nd	330±20	-	<sup>2</sup>
ABN54268	<i>R. thermocellum</i>	8.4±0.4	4.5±0.2	2.7±0.2	7.9±0.4	9.8±0.8	60±1	0.8±0.7	4.7±0.4	nd	nd	98±3	-	<sup>2</sup>
Q9SJE2	<i>A. thaliana</i>	nd	nd	nd	nd	19.4	nd	53.0	14.9	1.0	10.9	100	a,c	<sup>3</sup>
AAX51637	<i>M. longifolia</i>	nd	nd	nd	nd	7±4	nd	66±5	15±2	10±3	nd	nd	b,c,d	<sup>4</sup>
AAX51636	<i>D. butyracea</i>	nd	nd	nd	nd	17.8±0.6	nd	71±4	0.6±0.1	2.6±0.1	5.2±0.4	nd	b	<sup>5</sup>
AHF72806	<i>L. communis</i>	nd	6±2	17±6	nd	16±2	nd	17±2	20±3	1.5±0.2	15±2	nd	b	<sup>6</sup>
EST75919	<i>E. coli</i>	2.7	0.8	12.8	2.2	49.5	2.6	11.9	11.9	0.9	4.0	1400	a,b	<sup>7</sup>
AAC49151	<i>C. camphorum</i>	nd	nd	5.8	0.6	27.6	12.4	24.2	16.9	1.2	11.3	1600	a	<sup>8</sup>
Q41635	<i>U. californica</i>	nd	nd	63.2	13.7	3.0	10.2	1.6	4.3	nd	4.0	1000	a,b	<sup>9</sup>
ADB79567	<i>A. hypogaea L.</i>	nd	nd	4±1	nd	8±2	nd	45±3	14±3	2.3±0.3	5.8±0.9	nd	b	<sup>10</sup>
AAX51636	<i>D. butyracea</i>	nd	nd	8.3	nd	6.8	nd	43.6	5.1	29.7	6.5	580±40	a,b	<sup>11</sup>

Q9SQI3	<i>G. hirsutum</i>	nd	nd	0.1	nd	35.6	nd	21.3	36.6	1.4	4.9	3100±200	a,b	<sup>11</sup>
XP002515564	<i>R. communis</i>	nd	nd	0.1	nd	44.4	nd	17.0	32.9	1.2	4.4	9200±100	a,b	<sup>11</sup>
ABU96744	<i>J. curcas</i>	nd	nd	0.0	nd	44.4	nd	17.5	32.5	1.2	4.4	8700±200	a,b	<sup>11</sup>
WP004921669	<i>A. baylyi</i>	20±7	9±2	15±2	8.0±0.3	17±1	6±1	8±2	10±4	2.3±0.1	1.6±0.4	18000±2000	b	<sup>12</sup>
CAA57794	<i>A. brasilense</i>	nd	nd	nd	nd	5.6±0.3	nd	53±3	9.2±0.6	0.9±0.0	29±2	nd	b	<sup>13</sup>
EEC51251	<i>P. tricornutum</i>	nd	nd	nd	nd	2.8±0.2	nd	43.0±0.2	14.4±0.3	2.2±0.3	14.2±0.0	nd	b	<sup>14</sup>
AAL79361	<i>H. annuus L.</i>	nd	nd	nd	nd	nd	nd	39.9	3.6	21.6	10.9	nd	a	<sup>15</sup>
ADA64920	<i>L. lactis</i>	3.6±0.6	1.7±0.2	2.3±0.3	nd	4.6±0.6	nd	51±3	6.6±0.2	26±2	4.5±0.5	2800±100	-	<sup>16</sup>
AAC72882	<i>C. hookeriana</i>	93.5	2.2	0.9	0.2	0.0	0.1	1.2	0.0	nd	0.0	930	a	<sup>17</sup>
rTE3	<i>C. viscosissima</i>	42.5	26.6	4.1	9.5	1.4	1.6	1.3	0.8	nd	nd	510	a	<sup>17</sup>
rTE4	<i>C. viscosissima</i>	24.3	36.0	4.1	12.2	3.6	3.8	0.0	3.0	nd	nd	490	a	<sup>17</sup>
rTE8	<i>C. viscosissima</i>	17.9	10.2	3.4	5.3	31.6	6.4	0.0	17.9	nd	nd	87	a	<sup>17</sup>
rTE12	<i>C. viscosissima</i>	18.5	43.0	2.8	15.6	9.9	0.0	0.0	0.0	nd	nd	50	a	<sup>17</sup>
rTE15	<i>C. viscosissima</i>	4.1	21.0	14.7	34.3	5.1	10.0	0.0	0.0	nd	nd	56	a	<sup>17</sup>
rTE16	<i>C. viscosissima</i>	38.9	15.3	9.3	16.2	2.7	7.5	0.0	1.8	nd	nd	820	a	<sup>17</sup>
rTE20	<i>C. viscosissima</i>	16.1	19.9	12.4	24.3	2.8	13.2	0.0	2.2	nd	nd	390	a	<sup>17</sup>
rTE24	<i>C. viscosissima</i>	19.8	4.1	6.2	7.3	27.8	6.2	1.0	23.9	nd	nd	280	a	<sup>17</sup>
rTE28	<i>C. viscosissima</i>	4.8	1.0	1.3	1.7	41.4	1.8	2.3	44.8	nd	nd	280	a	<sup>17</sup>
rTE32	<i>C. viscosissima</i>	25.5	24.6	11.0	16.1	4.8	4.2	0.0	4.3	nd	nd	490	a	<sup>17</sup>
rTE36	<i>C. viscosissima</i>	6.9	18.5	13.1	17.6	11.7	17.4	0.0	9.2	nd	nd	190	a	<sup>17</sup>
rTE40	<i>C. viscosissima</i>	16.7	3.1	6.0	4.7	32.0	10.6	0.0	20.1	nd	nd	140	a	<sup>17</sup>
rTE44	<i>C. viscosissima</i>	6.8	1.7	1.3	1.1	46.4	5.8	0.0	31.3	nd	nd	47	a	<sup>17</sup>
rTE48	<i>C. viscosissima</i>	23.8	17.8	8.9	18.3	1.7	5.8	0.8	0.8	nd	nd	810	a	<sup>17</sup>
rTE51	<i>C. viscosissima</i>	0.0	20.9	2.4	30.0	21.8	2.1	0.0	0.0	nd	nd	6.4	a	<sup>17</sup>
rTE52	<i>C. viscosissima</i>	31.0	11.4	5.3	12.3	1.6	4.6	0.0	1.0	nd	nd	870	a	<sup>17</sup>
rTE56	<i>C. viscosissima</i>	24.9	5.6	7.5	8.2	26.2	4.6	0.8	14.8	nd	nd	410	a	<sup>17</sup>
rTE60	<i>C. viscosissima</i>	3.6	1.0	1.1	0.9	54.9	0.1	10.1	27.4	nd	nd	140	a	<sup>17</sup>
CvB2MT1	<i>C. viscosissima</i>	34.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	nd	nd	2.4	a	<sup>17</sup>
CvB2MT2	<i>C. viscosissima</i>	8.9	4.6	10.0	9.7	24.5	19.9	0.1	19.5	nd	nd	82	a	<sup>17</sup>
CvB2MT3	<i>C. viscosissima</i>	17.2	4.1	2.1	4.6	40.1	4.3	0.0	21.6	nd	nd	90	a	<sup>17</sup>
CvB2MT4	<i>C. viscosissima</i>	3.7	0.6	0.0	0.2	60.9	0.0	9.3	24.5	nd	nd	200	a	<sup>17</sup>
CvB2MT5	<i>C. viscosissima</i>	1.5	0.2	0.0	0.0	81.5	0.0	2.9	13.9	nd	nd	460	a	<sup>17</sup>
CvB2MT6	<i>C. viscosissima</i>	38.2	5.8	21.7	9.1	3.4	13.9	0.0	0.0	nd	nd	31	a	<sup>17</sup>
CvB2MT7	<i>C. viscosissima</i>	6.1	0.6	0.0	0.0	62.2	0.0	0.9	28.8	nd	nd	47	a	<sup>17</sup>
CvB2MT8	<i>C. viscosissima</i>	3.9	0.3	0.0	0.0	58.0	0.0	4.4	32.8	nd	nd	74	a	<sup>17</sup>
CvB2MT9	<i>C. viscosissima</i>	1.5	0.3	0.3	0.4	52.6	0.6	10.4	33.7	nd	nd	410	a	<sup>17</sup>
CvB2MT10	<i>C. viscosissima</i>	21.6	9.6	8.8	14.1	9.8	16.4	0.0	9.9	nd	nd	170	a	<sup>17</sup>
CvB2MT11	<i>C. viscosissima</i>	4.3	1.5	3.8	4.0	45.5	4.9	11.9	23.5	nd	nd	120	a	<sup>17</sup>

CvB2MT12	<i>C. viscosissima</i>	12.5	7.2	16.4	15.4	15.3	20.0	0.0	7.8	nd	nd	150	a	17
CvB2MT13	<i>C. viscosissima</i>	12.7	2.1	0.9	1.5	50.7	0.5	3.1	26.0	nd	nd	180	a	17
CvB2MT14	<i>C. viscosissima</i>	3.4	0.8	1.6	0.8	52.2	0.7	14.1	26.0	nd	nd	110	a	17
CvB2MT15	<i>C. viscosissima</i>	16.1	7.5	15.6	19.8	9.2	19.8	0.7	4.0	nd	nd	150	a	17
CvB2MT16	<i>C. viscosissima</i>	15.4	5.6	3.9	9.6	22.8	30.9	0.0	7.0	nd	nd	310	a	17
CvB2MT17	<i>C. viscosissima</i>	26.2	6.5	17.6	13.5	8.6	15.8	5.0	1.6	nd	nd	110	a	17
CvB2MT18	<i>C. viscosissima</i>	21.9	8.4	13.6	19.9	6.1	15.3	2.3	3.2	nd	nd	300	a	17
CvB2MT19	<i>C. viscosissima</i>	18.2	6.3	10.3	22.0	8.5	18.4	0.6	6.3	nd	nd	170	a	17
CvB2MT20	<i>C. viscosissima</i>	26.2	16.9	6.6	19.1	2.4	6.2	0.3	1.4	nd	nd	690	a	17
CvB2MT21	<i>C. viscosissima</i>	17.8	3.4	4.6	2.7	43.8	4.5	2.0	17.5	nd	nd	200	a	17
CvB2MT22	<i>C. viscosissima</i>	20.7	9.2	13.0	15.6	8.9	16.7	0.0	10.0	nd	nd	85	a	17
CvB2MT23	<i>C. viscosissima</i>	5.6	1.0	0.8	0.8	54.2	0.9	6.6	28.7	nd	nd	380	a	17
CvB2MT24	<i>C. viscosissima</i>	3.6	0.4	0.0	0.3	73.6	0.2	1.4	20.0	nd	nd	400	a	17
CvB2MT25	<i>C. viscosissima</i>	28.8	9.3	13.0	15.9	5.2	13.7	2.0	0.6	nd	nd	290	a	17
CvB2MT26	<i>C. viscosissima</i>	31.7	1.2	4.8	0.0	17.9	3.9	11.3	5.7	nd	nd	41	a	17
CvB2MT27	<i>C. viscosissima</i>	20.8	9.9	10.3	21.5	5.3	17.6	0.3	3.2	nd	nd	230	a	17
CvB2MT28	<i>C. viscosissima</i>	17.6	5.0	7.5	3.7	40.1	4.1	3.4	14.3	nd	nd	330	a	17
CvB2MT29	<i>C. viscosissima</i>	11.4	7.2	15.0	18.1	12.1	20.5	0.4	8.9	nd	nd	290	a	17
CvB2MT30	<i>C. viscosissima</i>	29.2	17.2	6.1	15.7	1.5	4.7	0.4	0.6	nd	nd	1000	a	17
CvB2MT31	<i>C. viscosissima</i>	17.9	5.9	9.0	19.6	12.1	17.3	0.7	8.1	nd	nd	390	a	17
CvB2MT32	<i>C. viscosissima</i>	21.5	7.3	4.8	14.4	9.5	30.6	0.0	3.4	nd	nd	330	a	17
CvB2MT33	<i>C. viscosissima</i>	27.2	7.9	9.6	20.4	5.1	12.5	0.0	3.6	nd	nd	570	a	17
CvB2MT34	<i>C. viscosissima</i>	31.8	9.6	9.2	18.4	2.8	11.2	0.3	0.4	nd	nd	460	a	17
CvB2MT35	<i>C. viscosissima</i>	43.1	2.5	3.6	4.1	3.0	8.8	0.0	0.1	nd	nd	120	a	17
CvB2MT36	<i>C. viscosissima</i>	40.3	0.0	0.0	0.0	2.4	0.0	0.0	0.0	nd	nd	47	a	17
CvB2MT37	<i>C. viscosissima</i>	40.2	3.4	2.8	1.0	1.1	2.0	0.0	0.2	nd	nd	330	a	17
CvB2MT38	<i>C. viscosissima</i>	37.2	3.1	5.7	4.2	1.6	8.1	0.0	0.0	nd	nd	1000	a	17
CvB2MT40	<i>C. viscosissima</i>	61.4	12.5	3.3	6.2	0.6	0.9	0.0	0.0	nd	nd	1100	a	17
CvB2MT41	<i>C. viscosissima</i>	36.2	3.8	1.4	0.7	0.0	0.0	0.0	0.0	nd	nd	93	a	17
CvB2MT42	<i>C. viscosissima</i>	27.0	15.0	6.9	18.9	2.6	6.1	1.5	1.1	nd	nd	820	a	17
CvB2MT43	<i>C. viscosissima</i>	4.1	1.2	1.5	1.4	47.0	0.7	11.5	31.0	nd	nd	170	a	17
CvB2MT44	<i>C. viscosissima</i>	22.7	23.6	7.9	24.2	1.8	4.0	0.0	0.2	nd	nd	710	a	17
CvB2MT45	<i>C. viscosissima</i>	22.5	15.8	13.1	24.4	2.6	11.4	0.0	0.0	nd	nd	570	a	17
CvB2MT47	<i>C. viscosissima</i>	18.5	12.0	17.9	17.5	1.4	4.5	22.6	0.0	nd	nd	190	a	17
CvB2MT48	<i>C. viscosissima</i>	13.7	38.6	6.7	17.5	0.0	3.9	0.2	0.0	nd	nd	140	a	17
TEGm157	Hybrid	12.0	0.7	4.0	0.5	30.9	4.6	19.1	19.2	nd	7.6	600	a	17
TEGm162	Hybrid	38.5	5.3	11.7	2.2	19.4	11.6	3.1	0.6	nd	1.5	1700	a	17
TEGm169	Hybrid	33.7	4.6	11.7	2.8	18.8	14.6	3.8	1.9	nd	2.7	1400	a	17

TEGm171	Hybrid	17.6	0.8	6.4	3.1	26.2	5.9	12.1	23.0	nd	2.0	1100	a	<sup>17</sup>
TEGm173	Hybrid	2.8	0.4	3.5	0.5	35.9	3.1	12.0	36.2	nd	4.9	1200	a	<sup>17</sup>
TEGm181	Hybrid	3.7	0.7	4.8	0.5	35.5	4.6	15.1	29.2	nd	5.0	830	a	<sup>17</sup>
TEGm183	Hybrid	12.7	4.2	9.2	6.6	23.2	11.7	10.0	18.4	nd	2.4	1200	a	<sup>17</sup>
TEGm198	Hybrid	1.3	0.1	1.4	0.1	36.4	0.7	23.0	36.6	nd	0.0	710	a	<sup>17</sup>
TEGm200	Hybrid	1.8	0.4	4.7	3.7	19.2	8.3	16.6	41.5	nd	3.5	940	a	<sup>17</sup>
TEGm201	Hybrid	1.3	0.1	1.2	0.1	19.3	1.3	28.4	41.1	nd	7.0	590	a	<sup>17</sup>
TEGm202	Hybrid	2.8	0.3	4.0	0.8	30.1	2.9	17.9	36.4	nd	4.0	1000	a	<sup>17</sup>
TEGm203	Hybrid	0.6	0.1	2.4	0.0	45.1	2.2	24.1	19.6	nd	5.7	530	a	<sup>17</sup>
TEGm204	Hybrid	1.3	0.1	1.6	0.3	38.0	1.5	19.8	33.6	nd	3.4	1200	a	<sup>17</sup>
TEGm205	Hybrid	0.3	0.0	0.7	0.0	35.9	1.1	23.9	34.1	nd	3.9	890	a	<sup>17</sup>
TEGm215	Hybrid	9.0	0.7	5.6	1.7	30.6	6.5	15.2	24.3	nd	3.7	980	a	<sup>17</sup>
TEGm219	Hybrid	2.4	0.3	4.4	0.2	54.7	3.8	16.1	17.5	nd	0.0	530	a	<sup>17</sup>
TEGm245	Hybrid	1.0	0.0	0.7	0.0	32.1	0.0	27.7	38.5	nd	0.0	1100	a	<sup>17</sup>
TEGm250	Hybrid	20.0	4.3	3.8	2.0	19.8	3.5	19.1	17.1	nd	6.2	1600	a	<sup>17</sup>
TEGm258	Hybrid	50.4	6.4	4.7	1.8	9.1	1.3	10.2	1.9	nd	2.3	1600	a	<sup>17</sup>
TEGm288	Hybrid	30.9	5.5	9.8	8.5	12.9	7.1	12.1	8.3	nd	2.2	1300	a	<sup>17</sup>
TEGm413	Hybrid	14.8	6.6	13.6	7.6	14.2	5.9	14.7	16.9	nd	3.9	1100	a	<sup>17</sup>
TEGm419	Hybrid	13.2	7.6	13.7	12.2	15.2	8.0	8.9	15.2	nd	3.6	1400	a	<sup>17</sup>
TEGm492	Hybrid	2.5	1.5	8.6	0.0	21.9	2.8	25.7	24.0	nd	12.5	600	a	<sup>17</sup>
TEGm501	Hybrid	0.4	0.4	9.1	1.0	38.7	4.9	17.1	23.7	nd	4.6	870	a	<sup>17</sup>
TEGm520	Hybrid	2.4	3.1	8.8	10.3	24.0	15.0	11.4	20.6	nd	2.8	1000	a	<sup>17</sup>
TEGm546	Hybrid	1.3	5.2	21.1	7.1	22.1	11.7	14.9	12.2	nd	3.4	610	a	<sup>17</sup>
UcFatB-M1	<i>U. californica</i>	nd	nd	nd	nd	29±3	nd	24±3	32±2	10±1	5.3±0.8	140±10	b	<sup>18</sup>
UcFatB-M2	<i>U. californica</i>	nd	nd	nd	nd	33±4	nd	24±3	27±2	9.9±0.8	5.6±0.5	105±10	b	<sup>18</sup>
UcFatB-M3	<i>U. californica</i>	nd	nd	nd	nd	33±1	nd	32.7±0.6	21±1	8.4±0.4	5.8±0.4	111±4	b	<sup>18</sup>
UcFatB-M4	<i>U. californica</i>	nd	nd	nd	nd	15.4±0.6	nd	21±4	46±1	16±4	1.0±0.4	53±4	b	<sup>18</sup>
UcFatB-M5	<i>U. californica</i>	nd	nd	nd	nd	11.1±0.9	nd	42±6	6±2	41±4	0.4±0.4	24±2	b	<sup>18</sup>
UcFatB-M6	<i>U. californica</i>	nd	nd	nd	nd	12.9±0.9	nd	44±4	8.9±0.6	34±3	0.9±0.9	33±3	b	<sup>18</sup>
UcFatB-M7	<i>U. californica</i>	nd	nd	nd	nd	12±2	nd	45±6	10±1	30±4	2.6±0.5	38±4	b	<sup>18</sup>
UcFatB-M8	<i>U. californica</i>	nd	nd	nd	nd	12±2	nd	41±5	7±1	39±3	0.6±0.6	33±3	b	<sup>18</sup>

<sup>a</sup> Standard deviation not determined or provided; total FFA values with unknown standard deviation was reported to two significant figures

<sup>b</sup> Data approximated using image processing software

<sup>c</sup> Mole-based values not provided and incapable of being calculated; mass-based values used instead

<sup>d</sup> 16:0 and 16:1 fatty acid compositions combined

**Table S2. Sequences of all variants considered in this study.** Mutant names match the formatting provided for **Table S1**. “Changes” indicates the number of amino acid changes, or whether a frameshift or truncation occurred for the mutant. Amino acid substitutions are provided in the format *XyZ*, where *X* is the WT residue at position *y* and *Z* is the new amino acid. “stop” indicates a stop codon replaces the existing amino acid, resulting in a shorter peptide (a truncation). A frameshift indicates that a WT amino acid was eradicated and not replaced by a different residue.

Name	Changes	Mutations
R1.M1	1	Y145K
R1.M2	1	L11G
R1.M3	3	L11G, I107K, R108K
R1.M4	4	L11G, L76K, I107K, R108K
R1.M5	5	L11G, L76K, I107K, R108K, F139E
R1.M6	6	L11G, L76K, I107K, R108K, F139E, Y145R
R1.M7	6	L11G, L76K, I107K, R108K, F139E, Y145K
R1.M8	7	L11G, L76K, I107K, R108K, A111E, F139E, Y145R
R1.M9	5	I107K, R108K, A111E, F139E, Y145R
R1.M10	7	L11G, L76K, I107K, R108K, A111E, F139E, Y145K
R1.M11	5	I107K, R108K, A111E, F139E, Y145K
R1.M12	5	I107K, R108K, A111E, F139H, Y145K
R2.M1	3	G72F, F139W, Y145F
R2.M2	7	G72L, F139P, M141W, E142L, Y145F, G155A, I156L
R2.M3	5	L11W, G72F, F139W, E142P, Y145F
R2.M4	7	G72L, F139P, M141W, E142P, Y145P, G155W, I156L
R2.M5	2	E142P, Y145F
R2.M6	4	L11F, G72F, F139M, Y145F
R2.M7	4	G72F, Y145F, G155W, I156L
R2.M8	7	G72L, F139P, M141W, E142P, Y145P, G155A, I156L
R2.M9	5	L11F, G72F, F139W, E142P, Y145F
R2.M10	6	G72L, F139P, M141W, E142P, G155A, I156L
R2.M11	2	L11P, Y145F
R2.M12	4	L11F, G72F, F139W, Y145F
R2.M13	2	G72F, Y145F
R2.M14	3	G72A, E142P, Y145P
R2.RD1	1	G72A
R2.RD2	1	G72L
R2.RD3	1	L11P
R2.RD4	2	L11P, G72F
R2.RD5	1	L11W
R3.M1	3	S122K, Y145K, L146K



R3.M2	1	M141L
R3.M3	4	I107L, R108K, Y145K, L146K
R3.M4	3	M141L, Y145K, L146K
R3.M5	5	I107K, R108F, L109I, Y145K, L146K
R3.M6	1	R108K
R3.M7	2	Y145K, L146K
R3.M8	2	S122K, Y145K
R3.M9	3	S122L, Y145K, L146K
R3.M10	4	S122K, M141K, Y145K, L146K
R3.RD1	2	I107L, R108K
R3.RD2	3	I107K, R108F, L109I
R3.RD3	1	S122K
R3.RD4	1	S122L
R3.RD5	2	E142P, Y145K
R4.M1	3	I107M, R108F, E142P
R4.M2	3	M141L, E142P, Y145F
R4.M3	2	S122M, E142P
R4.M4	1	E142P
R4.M5	4	S122M, M141L, E142P, Y145F
R4.M6	2	E142P, Y145F
R4.M7	1	I107G
R4.M8	1	M141G
R4.M9	1	I107K
R4.M10	3	R108F, L109F, E142P
R4.M11	4	R108F, L109F, S122I, E142P
R4.M12	4	R108F, L109F, M141G, Y145F
R4.M13	5	R108F, L109F, S122M, Y145F, L146P
R4.M14	3	R108F, Y145F, L146P
R4.M15	3	R108F, L109F, Y145F
R4.M16	3	R108K, L109I, L146K
R4.M17	3	R108F, L109F, S122M
R4.M18	2	R108F, L109F
R4.RD1	2	M141L, E142P
R4.RD2	2	M141L, Y145K
R4.RD3	4	M141L, E142P, Y145K, L146K
R4.RD4	3	R108F, L109F, M141L
R4.RD5	6	R108F, L109F, M141L, E142P, Y145K, L146K
R4.RD6	5	R108F, L109F, M141L, Y145K, L146K
R4.RD7	1	H157A

R4.RD8	2	I107M, R108F
R4.RD9	1	R108F
R4.RD10	1	S122I
R4.RD11	1	S122M
RM.M1	2	S36T, P110T
RM.M2	1	L146I
RM.M3	2	A19S, A98P
RM.M4	3	Q49H, M105K, D161N
RM.M5	truncation	G8C, M105L, Y117stop
RM.M6	1	P110S
RM.M7	1	L57P
RM.M8	1	A123T
RM.M9	3	G72V, D74N, F79Y
RM.M10	1	Q93H
RM.M11	truncation	Q106stop
RM.M12	1	P158H
RM.M13	2	S41N, A162V
RM.M14	1	P148S
RM.M15	2	F121Y, P126L
RM.M16	1	R64H
RM.M17	2	P102A, A111V
RM.M18	2	F79Y, Q82H
RM.M19	1	L57Q
RM.M20	2	A22T, A129T
RM.M21	1	L136P
RM.M22	3	T3M, M17I, E69G
RM.M23	truncation	W33stop
RM.M24	frameshift	Q90single nucleotide deletion
RM.M25	2	G44C, A56T
RM.M26	frameshift	A25T, frameshift after K127
RM.M27	1	S12I
RM.M28	3	W23C, V68L, G75S
RM.M29	3	V38F, F139Y, Y145C
RM.M30	2	A22V, A171V
RM.M31	4	L109P, Y117N, A123V, K147E
RM.M32	2	N28K, A123V
RM.M33	frameshift	G8V frameshift resulting in stop codon at 10 <sup>th</sup> amino acid position
RM.M34	1	P55L
RM.M35	2	S41G, L92M

RM.M36	2	S47P, M170I
RM.M37	1	P135S
RM.M38	1	A25P
RM.M39	2	T35S, E142D
RM.M40	4	L92S, V95I, E101D, W169L
RM.M41	1	L92S
RM.M42	5	I42V, G75A, Q106R, A111G, P138S
RM.M43	1	L67M
RM.M44	1	A120V
RM.M45	truncation	E69stop
RM.M46	truncation	E119stop
RM.M47	1	S41N
RM.M48	1	L58R
RM.M49	truncation	A19T, W31stop
RM.M50	3	M105K, R115C, P135del
RM.M51	truncation	truncation one nucleotide after A40 resulting also in frameshift
RM.M52	2	M170I, A171V
RM.M53	1	W150L
RM.M54	2	A25T, S41I
RM.M55	2	P135T, D161N
RM.M56	1	<b>D2V</b>
RM.M57	2	A111T, M141I
RM.M58	1	G155D
RM.M59	1	R108C
RM.M60	1	T46I
RM.M61	1	E101V

**Table S3. Fatty acid production profiles for C<sub>12</sub>-specific random variants and their constituent point mutations.** This table provides the mole fractions of C<sub>8</sub>-C<sub>18</sub> FFAs and total FFA in the cell lysate for RM.M39 and RM.M57 (the two C<sub>12</sub>-specific RM variants) as well as the individual amino acid substitutions (see **Table S2**).

Name	Fatty Acid Composition (mol %)										Total FFA (μM)
	8:0	10:0	12:0	12:1	14:0	14:1	16:0	16:1	18:0	18:1	
WT	5.9±0.3	1.09±0.06	20±1	7.5±0.5	29±2	7.1±0.4	14.2±0.8	11.6±0.8	0.22±0.01	2.1±0.1	1900±50
T35S	2.7±0.9	0.9±0.1	16±4	6±2	28±7	6±2	21±7	17±2	0.3±0.1	2.3±0.4	1600±200
E142D	5.5±0.7	2.5±0.2	33±3	12±1	26±2	6.1±0.6	5.7±0.8	8.1±0.8	0.14±0.04	1.2±0.1	3000±100
RM.M39	6±2	2.5±0.4	32±6	12±2	26±6	6±1	6.4±0.6	8±2	0.10±0.01	1.2±0.3	2800±200
A111T	4±2	1.0±0.1	15±3	6±1	25±5	6±1	23±3	17±3	0.35±0.06	2.2±0.4	1140±80
M141I	4±2	2.5±0.3	17±4	14±3	21±4	3.8±0.9	20±2	14±2	0.32±0.04	2.2±0.3	1200±80
RM.M57	5±1	2.9±0.7	17±4	14±4	21±4	3.8±1.0	20±2	13±1	0.33±0.05	2.1±0.3	1400±100

**Table S4. ‘TesA residues sorted by distance from acyl-ACP for design position selection.** Design positions were selected from each round using the eight closest residues that were not excluded from consideration. The method used to sort by distance varied between R1-R2 and R3-R4. The sorted list is provided in the table. Residues are labeled by their WT amino acid followed by the position of the residue. Residues that were excluded from design position consideration were annotated. The distance used to sort the residues is provided within parentheses. Thus, the eight highest ranked residues that were not annotated (i.e., excluded) formed the set of design positions employed within IPRO.

<b>Rank</b>	<b>R1<sup>a</sup></b>	<b>R2<sup>a</sup></b>	<b>R3-R4<sup>b</sup></b>
1	Y145 (1.83 Å)	Y145 (1.83 Å)	P110 <sup>k</sup> (1.83 Å)
2	L109 <sup>f,h</sup> (2.70 Å)	L109 <sup>f</sup> (2.70 Å)	Y145 (2.70 Å)
3	P110 <sup>c</sup> (2.77 Å)	P110 <sup>c</sup> (2.77 Å)	R108 (2.77 Å)
4	R108 (3.02 Å)	R108 <sup>i</sup> (3.02 Å)	M141 (3.02 Å)
5	N73 <sup>d,g,h</sup> (3.08 Å)	N73 <sup>d,g</sup> (3.08 Å)	A111 <sup>l</sup> (3.08 Å)
6	S10 <sup>c,d,g</sup> (3.25 Å)	S10 <sup>c,d,g</sup> (3.25 Å)	L109 (3.25 Å)
7	G44 <sup>d,g,h</sup> (3.33 Å)	G44 <sup>d,g</sup> (3.33 Å)	I156 <sup>k</sup> (3.33 Å)
8	D9 <sup>g</sup> (3.47 Å)	D9 <sup>g</sup> (3.47 Å)	E142 (3.47 Å)
9	L11 (3.71 Å)	L11 (3.71 Å)	G155 <sup>k</sup> (3.71 Å)
10	A111 (3.72 Å)	A111 <sup>j</sup> (3.72 Å)	F139 <sup>k</sup> (3.72 Å)
11	G72 (3.81 Å)	G72 (3.81 Å)	N112 <sup>k,l</sup> (3.81 Å)
12	L76 (3.86 Å)	L76 <sup>j</sup> (3.86 Å)	Y113 <sup>k,l</sup> (3.86 Å)
13	H157 <sup>c,h</sup> (3.99 Å)	H157 <sup>c</sup> (3.99 Å)	V144 <sup>k</sup> (3.99 Å)
14	I156 <sup>h</sup> (4.21 Å)	I156 (4.21 Å)	N118 <sup>l</sup> (4.21 Å)
15	F139 (4.37 Å)	F139 (4.37 Å)	I107 (4.37 Å)
16	S43 <sup>g</sup> (4.37 Å)	S43 <sup>g</sup> (4.37 Å)	M151 <sup>k,l</sup> (4.37 Å)
17	D45 <sup>g</sup> (4.82 Å)	D45 <sup>g</sup> (4.82 Å)	L76 <sup>k</sup> (4.82 Å)
18	I107 (4.83 Å)	I107 <sup>i</sup> (4.83 Å)	G72 <sup>k</sup> (4.83 Å)
19	G155 (5.01 Å)	G155 (5.01 Å)	L146 (5.01 Å)
20	I42 <sup>g,h</sup> (5.14 Å)	I42 <sup>g</sup> (5.14 Å)	H157 <sup>k</sup> (5.14 Å)
21	E142 <sup>h</sup> (5.53 Å)	E142 (5.53 Å)	S122 (5.53 Å)
22	M141 (5.56 Å)	M141 (5.56 Å)	D154 <sup>k</sup> (5.56 Å)
23	E69 (5.62 Å)	E69 (5.62 Å)	P158 <sup>k</sup> (5.62 Å)
24	Y15 (5.96 Å)	Y15 (5.96 Å)	L11 <sup>k</sup> (5.96 Å)
25	N112 (5.98 Å)	N112 <sup>j</sup> (5.98 Å)	E143 (5.98 Å)
26	G71 (6.15 Å)	G71 (6.15 Å)	F121 <sup>k</sup> (6.15 Å)
27	M151 (6.22 Å)	M151 (6.22 Å)	G75 <sup>k</sup> (6.22 Å)
28	N118 (6.23 Å)	N118 <sup>j</sup> (6.23 Å)	F140 <sup>k</sup> (6.23 Å)
29	S12 (6.43 Å)	S12 (6.43 Å)	G114 <sup>k,l</sup> (6.43 Å)
30	F121 (6.66 Å)	F121 (6.66 Å)	Q106 (6.66 Å)
31	Y113 (6.67 Å)	Y113 <sup>j</sup> (6.67 Å)	P148 <sup>l</sup> (6.67 Å)

32	G75 (6.97 Å)	G75 <sup>j</sup> (6.97 Å)	Q152 <sup>k,l</sup> (6.97 Å)
33	G8 (6.99 Å)	G8 (6.99 Å)	W150 <sup>l</sup> (6.99 Å)
34	P158 <sup>c</sup> (7.02 Å)	P158 <sup>c</sup> (7.02 Å)	K147 <sup>l</sup> (7.02 Å)
35	D74 (7.06 Å)	D74 (7.06 Å)	Y117 <sup>k,l</sup> (7.06 Å)
36	T46 <sup>h</sup> (7.12 Å)	T46 (7.12 Å)	P138 <sup>k</sup> (7.12 Å)
37	G14 <sup>h</sup> (7.56 Å)	G14 (7.56 Å)	G71 <sup>k</sup> (7.56 Å)
38	A13 (7.56 Å)	A13 (7.56 Å)	R115 <sup>l</sup> (7.56 Å)
39	L70 <sup>h</sup> (7.67 Å)	L70 (7.67 Å)	N73 <sup>k</sup> (7.67 Å)
40	D154 <sup>c</sup> (7.87 Å)	D154 <sup>c</sup> (7.87 Å)	A162 <sup>k</sup> (7.87 Å)
41	S122 (8.22 Å)	S122 (8.22 Å)	S10 <sup>k</sup> (8.22 Å)
42	R77 <sup>h</sup> (8.22 Å)	R77 <sup>j</sup> (8.22 Å)	M105 (8.22 Å)
43	V144 (8.32 Å)	V144 (8.32 Å)	E119 <sup>l</sup> (8.32 Å)
44	Q49 (8.38 Å)	Q49 (8.38 Å)	Y15 <sup>k</sup> (8.38 Å)
45	Q106 (8.41 Å)	Q106 (8.41 Å)	Y125 <sup>k</sup> (8.41 Å)
46	F140 <sup>h</sup> (8.41 Å)	F140 (8.41 Å)	D9 <sup>k</sup> (8.41 Å)
47	S41 (8.51 Å)	S41 (8.51 Å)	D153 <sup>l</sup> (8.51 Å)
48	Y125 (8.55 Å)	Y125 (8.55 Å)	E69 <sup>k</sup> (8.55 Å)
49	L146 <sup>h</sup> (8.61 Å)	L146 (8.61 Å)	G78 <sup>k</sup> (8.61 Å)
50	E143 <sup>h</sup> (8.76 Å)	E143 (8.76 Å)	D74 <sup>k</sup> (8.76 Å)
51	M105 (9.31 Å)	M105 (9.31 Å)	I166 <sup>k</sup> (9.31 Å)
52	S47 (9.35 Å)	S47 (9.35 Å)	N159 <sup>k</sup> (9.35 Å)
53	R16 (9.41 Å)	R16 (9.41 Å)	R77 <sup>k</sup> (9.41 Å)
54	G114 <sup>h</sup> (9.51 Å)	G114 <sup>j</sup> (9.51 Å)	I124 (9.51 Å)
55	Q152 (9.75 Å)	Q152 (9.75 Å)	A120 (9.75 Å)
56	Y117 (9.76 Å)	Y117 <sup>j</sup> (9.76 Å)	Q149 <sup>l</sup> (9.76 Å)
57	R53 (9.87 Å)	R53 (9.87 Å)	A123 (9.87 Å)
58	W23 (10.13 Å)	W23 (10.13 Å)	L70 <sup>k</sup> (10.13 Å)
59	G78 (10.15 Å)	G78 <sup>j</sup> (10.15 Å)	L137 (10.15 Å)
60	E119 <sup>h</sup> (10.41 Å)	E119 <sup>j</sup> (10.41 Å)	S12 <sup>k</sup> (10.41 Å)
61	L7 <sup>h</sup> (10.46 Å)	L7 (10.46 Å)	P81 <sup>k</sup> (10.46 Å)
62	K147 <sup>h</sup> (10.68 Å)	K147 (10.68 Å)	R116 <sup>l</sup> (10.68 Å)
63	R115 <sup>h</sup> (10.78 Å)	R115 <sup>j</sup> (10.78 Å)	G44 <sup>k</sup> (10.78 Å)
64	G50 (10.89 Å)	G50 (10.89 Å)	W169 (10.89 Å)
65	T84 (11.10 Å)	T84 (11.10 Å)	G14 <sup>k</sup> (11.10 Å)
66	M17 (11.11 Å)	M17 (11.11 Å)	T84 (11.11 Å)
67	A40 <sup>h</sup> (11.16 Å)	A40 (11.16 Å)	F165 (11.16 Å)
68	Q163 (11.17 Å)	Q163 (11.17 Å)	F79 (11.17 Å)
69	W150 (11.31 Å)	W150 (11.31 Å)	G8 <sup>k</sup> (11.31 Å)
70	I166 (11.32 Å)	I166 (11.32 Å)	S43 <sup>k</sup> (11.32 Å)

71	D153 <sup>h</sup> (11.33 Å)	D153 (11.33 Å)	P126 (11.33 Å)
72	N39 (11.41 Å)	N39 (11.41 Å)	W23 <sup>k</sup> (11.41 Å)
73	N159 (11.42 Å)	N159 (11.42 Å)	L136 <sup>k</sup> (11.42 Å)
74	F79 (11.44 Å)	F79 <sup>j</sup> (11.44 Å)	S47 (11.44 Å)
75	A22 (11.51 Å)	A22 (11.51 Å)	Q163 <sup>k</sup> (11.51 Å)
76	V68 (11.54 Å)	V68 (11.54 Å)	T46 <sup>k</sup> (11.54 Å)
77	P148 <sup>e</sup> (11.59 Å)	P148 <sup>e</sup> (11.59 Å)	R16 <sup>l</sup> (11.59 Å)
78	P138 <sup>e</sup> (11.67 Å)	P138 <sup>e</sup> (11.67 Å)	M170 (11.67 Å)
79	Q48 (11.69 Å)	Q48 (11.69 Å)	D161 (11.69 Å)
80	I6 (11.72 Å)	I6 (11.72 Å)	L104 <sup>k</sup> (11.72 Å)
81	L88 (12.02 Å)	L88 (12.02 Å)	A13 <sup>k</sup> (12.02 Å)
82	P24 <sup>e</sup> (12.16 Å)	P24 <sup>e</sup> (12.16 Å)	D45 <sup>k</sup> (12.16 Å)
83	I124 (12.31 Å)	I124 (12.31 Å)	Q173 (12.31 Å)
84	R116 <sup>h</sup> (12.66 Å)	R116 <sup>i</sup> (12.66 Å)	S41 <sup>k</sup> (12.66 Å)
85	A123 <sup>h</sup> (12.68 Å)	A123 (12.68 Å)	A22 (12.68 Å)
86	A120 (12.69 Å)	A120 <sup>j</sup> (12.69 Å)	V68 (12.69 Å)
87	L137 (13.07 Å)	L137 (13.07 Å)	L88 <sup>k</sup> (13.07 Å)
88	L67 (13.19 Å)	L67 (13.19 Å)	R160 (13.19 Å)
89	L51 (13.27 Å)	L51 (13.27 Å)	M17 <sup>l</sup> (13.27 Å)
90	A162 (13.28 Å)	A162 (13.28 Å)	E85 (13.28 Å)
91	L104 (13.37 Å)	L104 (13.37 Å)	L67 (13.37 Å)
92	S18 <sup>h</sup> (13.41 Å)	S18 (13.41 Å)	A129 <sup>k</sup> (13.41 Å)
93	W169 (13.43 Å)	W169 (13.43 Å)	K127 (13.43 Å)
94	P81 <sup>e</sup> (13.54 Å)	P81 <sup>e</sup> (13.54 Å)	Q80 (13.54 Å)
95	A19 (13.56 Å)	A19 (13.56 Å)	A167 <sup>k</sup> (13.56 Å)
96	Q149 <sup>h</sup> (13.56 Å)	Q149 (13.56 Å)	L128 <sup>k</sup> (13.56 Å)
97	M170 (13.69 Å)	M170 (13.69 Å)	P24 <sup>k</sup> (13.69 Å)
98	I91 (13.76 Å)	I91 (13.76 Å)	I6 (13.76 Å)
99	R160 <sup>h</sup> (13.90 Å)	R160 (13.90 Å)	I42 (13.90 Å)
100	L136 (14.23 Å)	L136 (14.23 Å)	D168 (14.23 Å)
101	Q80 <sup>h</sup> (14.28 Å)	Q80 <sup>j</sup> (14.28 Å)	P164 (14.28 Å)
102	P126 <sup>e</sup> (14.42 Å)	P126 <sup>e</sup> (14.42 Å)	L7 <sup>k</sup> (14.42 Å)
103	A21 (14.45 Å)	A21 (14.45 Å)	L103 <sup>k</sup> (14.45 Å)
104	T87 (14.46 Å)	T87 (14.46 Å)	Q83 (14.46 Å)
105	E85 (14.64 Å)	E85 (14.64 Å)	T87 (14.64 Å)
106	A52 (15.04 Å)	A52 (15.04 Å)	K172 (15.04 Å)
107	L103 (15.26 Å)	L103 (15.26 Å)	G50 <sup>k</sup> (15.26 Å)
108	L57 (15.37 Å)	L57 (15.37 Å)	Q49 (15.37 Å)
109	Q83 (15.58 Å)	Q83 (15.58 Å)	Q82 (15.58 Å)

110	L128 (15.59 Å)	L128 (15.59 Å)	A21 (15.59 Å)
111	A25 (15.64 Å)	A25 (15.64 Å)	P135 (15.64 Å)
112	V38 <sup>h</sup> (15.71 Å)	V38 (15.71 Å)	L174 <sup>k</sup> (15.71 Å)
113	S20 <sup>h</sup> (15.81 Å)	S20 (15.81 Å)	Q48 (15.81 Å)
114	L5 (16.02 Å)	L5 (16.02 Å)	L27 <sup>k</sup> (16.02 Å)
115	A167 <sup>h</sup> (16.03 Å)	A167 (16.03 Å)	N39 <sup>k</sup> (16.03 Å)
116	Q82 <sup>h</sup> (16.04 Å)	Q82 (16.04 Å)	L26 <sup>k</sup> (16.04 Å)
117	L54 (16.11 Å)	L54 (16.11 Å)	I91 <sup>k</sup> (16.11 Å)
118	D161 (16.25 Å)	D161 (16.25 Å)	A171 (16.25 Å)
119	V66 (16.52 Å)	V66 (16.52 Å)	V134 <sup>l</sup> (16.52 Å)
120	F165 (16.57 Å)	F165 (16.57 Å)	K130 (16.57 Å)
121	L92 (16.80 Å)	L92 (16.80 Å)	A19 <sup>l</sup> (16.80 Å)
122	P164 <sup>e,h</sup> (16.88 Å)	P164 <sup>e</sup> (16.88 Å)	Q86 (16.88 Å)
123	Q173 (17.03 Å)	Q173 (17.03 Å)	L51 <sup>k</sup> (17.03 Å)
124	V37 (17.04 Å)	V37 (17.04 Å)	A25 <sup>k</sup> (17.04 Å)
125	Q86 <sup>h</sup> (17.06 Å)	Q86 (17.06 Å)	L92 (17.06 Å)
126	R89 (17.23 Å)	R89 (17.23 Å)	A40 <sup>k</sup> (17.23 Å)
127	L26 (17.38 Å)	L26 (17.38 Å)	P102 (17.38 Å)
128	P135 <sup>e</sup> (17.51 Å)	P135 <sup>e</sup> (17.51 Å)	R89 (17.51 Å)
129	K127 (17.53 Å)	K127 (17.53 Å)	V66 <sup>k</sup> (17.53 Å)
130	L27 (17.66 Å)	L27 (17.66 Å)	L177 (17.66 Å)
131	L174 (17.80 Å)	L174 (17.80 Å)	S18 <sup>l</sup> (17.80 Å)
132	L4 (18.13 Å)	L4 (18.13 Å)	E131 (18.13 Å)
133	A129 (18.20 Å)	A129 (18.20 Å)	V37 <sup>k</sup> (18.20 Å)
134	D168 (18.62 Å)	D168 (18.62 Å)	F132 <sup>l</sup> (18.62 Å)
135	P55 <sup>e</sup> (18.88 Å)	P55 <sup>e</sup> (18.88 Å)	L4 (18.88 Å)
136	A171 <sup>h</sup> (18.95 Å)	A171 (18.95 Å)	R53 <sup>k</sup> (18.95 Å)
137	V95 (19.03 Å)	V95 (19.03 Å)	L5 <sup>k</sup> (19.03 Å)
138	P102 <sup>e</sup> (19.14 Å)	P102 <sup>e</sup> (19.14 Å)	Q175 (19.14 Å)
139	Q90 (19.20 Å)	Q90 (19.20 Å)	L54 <sup>k</sup> (19.20 Å)
140	V134 (19.34 Å)	V134 (19.34 Å)	P176 (19.34 Å)
141	N28 (19.46 Å)	N28 (19.46 Å)	Q90 (19.46 Å)
142	H61 (19.60 Å)	H61 (19.60 Å)	S20 <sup>l</sup> (19.60 Å)
143	W65 (19.67 Å)	W65 (19.67 Å)	W65 (19.67 Å)
144	L177 (20.06 Å)	L177 (20.06 Å)	A52 (20.06 Å)
145	Q60 (20.07 Å)	Q60 (20.07 Å)	V95 (20.07 Å)
146	D94 (20.41 Å)	D94 (20.41 Å)	N28 (20.41 Å)
147	A56 (20.42 Å)	A56 (20.42 Å)	K30 <sup>l</sup> (20.42 Å)
148	L58 (20.50 Å)	L58 (20.50 Å)	V38 (20.50 Å)



149	K172 (20.68 Å)	K172 (20.68 Å)	V178 (20.68 Å)
150	K130 (20.74 Å)	K130 (20.74 Å)	D133 <sup>l</sup> (20.74 Å)
151	E101 (20.91 Å)	E101 (20.91 Å)	D29 <sup>l</sup> (20.91 Å)
152	W31 (20.95 Å)	W31 <sup>j</sup> (20.95 Å)	E101 (20.95 Å)
153	F132 (21.00 Å)	F132 (21.00 Å)	L57 (21.00 Å)
154	S36 <sup>h</sup> (21.31 Å)	S36 (21.31 Å)	W31 <sup>l</sup> (21.31 Å)
155	Q93 (21.32 Å)	Q93 (21.32 Å)	D94 (21.32 Å)
156	P63 <sup>e</sup> (21.42 Å)	P63 <sup>e</sup> (21.42 Å)	Q93 (21.42 Å)
157	D29 (21.54 Å)	D29 (21.54 Å)	P63 <sup>k</sup> (21.54 Å)
158	T3 (21.67 Å)	T3 (21.67 Å)	A100 <sup>k</sup> (21.67 Å)
159	E131 (21.97 Å)	E131 (21.97 Å)	T35 (21.97 Å)
160	K30 (22.18 Å)	K30 (22.18 Å)	K96 (22.18 Å)
161	Q175 (22.37 Å)	Q175 (22.37 Å)	H180 (22.37 Å)
162	V178 (22.79 Å)	V178 (22.79 Å)	T3 (22.79 Å)
163	A100 (23.19 Å)	A100 (23.19 Å)	L58 <sup>k</sup> (23.19 Å)
164	K59 (23.20 Å)	K59 (23.20 Å)	H61 <sup>k,l</sup> (23.20 Å)
165	D133 <sup>h</sup> (23.23 Å)	D133 (23.23 Å)	S36 (23.23 Å)
166	K96 (23.32 Å)	K96 (23.32 Å)	R64 (23.32 Å)
167	R64 (23.54 Å)	R64 (23.54 Å)	P55 <sup>k</sup> (23.54 Å)
168	P176 <sup>e</sup> (23.57 Å)	P176 <sup>e</sup> (23.57 Å)	A98 <sup>l</sup> (23.57 Å)
169	T35 (23.61 Å)	T35 <sup>j</sup> (23.61 Å)	A56 (23.61 Å)
170	Q62 <sup>h</sup> (24.10 Å)	Q62 (24.10 Å)	K34 <sup>l</sup> (24.10 Å)
171	A98 (24.21 Å)	A98 (24.21 Å)	Q32 <sup>l</sup> (24.21 Å)
172	D2 (24.93 Å)	D2 (24.93 Å)	D2 (24.93 Å)
173	H180 (25.52 Å)	H180 (25.52 Å)	A97 (25.52 Å)
174	A97 (25.70 Å)	A97 (25.70 Å)	Q60 <sup>l</sup> (25.70 Å)
175	Q32 (25.71 Å)	Q32 <sup>j</sup> (25.71 Å)	K59 (25.71 Å)
176	N99 <sup>h</sup> (26.55 Å)	N99 (26.55 Å)	N99 (26.55 Å)
177	K34 (27.24 Å)	K34 <sup>j</sup> (27.24 Å)	Q62 (27.24 Å)
178	A1 (27.76 Å)	A1 (27.76 Å)	A1 (27.76 Å)
179	S33 <sup>h</sup> (28.10 Å)	S33 <sup>j</sup> (28.10 Å)	S33 <sup>l</sup> (28.10 Å)

<sup>a</sup> Distance was calculated by finding the closest contact between the residue's heavy atoms and the acyl moiety carbon atoms of 'TesA:tetradecanoyl-ACP

<sup>b</sup> Distance was calculated between the residue's C $\beta$  atom (C $\alpha$  for glycine, C $\gamma$  for H180) and the  $\omega$ -1 atom of dodecanoyl-ACP

<sup>c</sup> Residue excluded because it is part of the 'TesA catalytic triad<sup>19</sup>

<sup>d</sup> Residue excluded because they form the 'TesA oxyanion hole<sup>19</sup>

<sup>e</sup> Residue excluded because legacy version of IPRO could not handle mutations from proline<sup>20</sup>

<sup>f</sup> Residue excluded because it was considered important for 'TesA function based on mutagenesis studies<sup>21</sup>

<sup>g</sup> Residue excluded because it was within 4.5 Å of the thioester sulfur atom

<sup>h</sup> Residue excluded because the C $\alpha$  atom was over 0.75 Å closer to the  $\omega$ -1 atom than the C $\beta$  atom (glycines not considered)

<sup>i</sup> Residue excluded based on R1 results that abolished 'TesA activity

<sup>j</sup> Residue excluded because it belongs to one of the three 'TesA flexible loops<sup>21</sup>

<sup>k</sup> Residue excluded because it has  $\geq 40\%$  sequence conservation in lysophospholipase L1-like subgroup alignment<sup>22</sup>

<sup>l</sup> Residue excluded because sequence alignment included at least one gap

**Table S5. Crystallography data collection statistics.** The data collection statistics for four structures (WT and R3.M4 under three different conditions) are provided.

	<b>WT</b>	<b>R3.M4 (pH 5.0)</b>	<b>R3.M4 (pH 7.5)</b>	<b>R3.M4 (pH 7.5 “soaked”)</b>
Resolution limits (Å)	50-1.65 (1.75-1.65) <sup>b</sup>	50-1.2 (1.3-1.2) <sup>b</sup>	50-1.15 (1.25-1.15) <sup>b</sup>	50-0.97 (1.07-0.97) <sup>b</sup>
Space Group	<i>P2</i> <sub>1</sub>	<i>P2</i> <sub>1</sub>	<i>P2</i> <sub>1</sub>	<i>P2</i> <sub>1</sub>
<b>Unit cell</b>				
<i>a</i> (Å)	40.9	40.7	40.6	40.5
<i>b</i> (Å)	82.1	55.2	55.3	55.0
<i>c</i> (Å)	53.9	42.3	42.2	42.0
β (°)	90.4	105.2	104.9	104.9
no. of independent reflections	42019 (6561)	56148 (11626)	62723 (12626)	102836 (24792)
completeness (%)	98.0 (94.9)	99.4 (97.3)	98.2 (91.8)	97.8 (92.8)
redundancy	3.7 (2.6)	3.7 (2.2)	2.3 (1.3)	4.1 (2.3)
avg <i>I</i> /avg σ( <i>I</i> )	10.6 (1.9)	13.2 (3.0)	11.0 (2.7)	11.8 (2.4)
<i>R</i> <sub>sym</sub> (%) <sup>a</sup>	6.8 (37.8)	5.6 (30.2)	4.5 (21.0)	6.0 (27.8)

$$^a R_{\text{sym}} = \left( \frac{\sum |\sum I - \bar{I}|}{\sum I} \right) \times 100$$

<sup>b</sup> Statistics for the highest resolution bin

**Table S6. Crystallography refinement statistics.** The refinement statistics for the crystallography experiments summarized in **Table S5** are provided.

	<b>WT</b>	<b>R3.M4 (pH 5.0)</b>	<b>R3.M4 (pH 7.5)</b>	<b>R3.M4 (pH 7.5 “soaked”)</b>
PDB code	5TIC	5TID	5TIE	5TIF
resolution limits (Å)	50-1.65	50-1.2	50-1.15	50-0.97
<i>R</i> -factor <sup>a</sup> (overall)%/ no. reflections	18.8/42019	15.9/56148	13.1/62723	12.3/102836
<i>R</i> -factor <sup>a</sup> (working)%/ no. reflections	18.6/39926	15.8/53269	12.9/59641	12.2/97699
<i>R</i> -factor <sup>a</sup> (free)%/ no. reflections	22.2/2093	17.7/2879	16.3/3082	13.7/5137
no. of protein atoms	2839	1448	1471	1542
no. of heteroatoms	347	344	391	449
<b>average B values</b>				
protein (Å <sup>2</sup> )	20.1	10.4	10.7	13.7
ligand (Å <sup>2</sup> )	-	21.5	20.6	27.6
solvent (Å <sup>2</sup> )	28.1	22.2	24.1	32.7
<b>weighted RMSDs from ideality</b>				
bond lengths (Å)	0.012	0.012	0.017	0.015
bond angles (°)	1.77	1.74	1.91	2.01
planar groups (Å)	0.008	0.009	0.010	0.010
<b>Ramachandran regions (%)<sup>b</sup></b>				
most favored	97.7	97.2	97.2	97.8
additionally allowed	2.3	2.8	2.8	2.2
generously allowed	0.0	0.0	0.0	0.0

$$^a R\text{-factor} = \left( \frac{\sum |F_o - F_c|}{\sum |F_o|} \right) \times 100$$

<sup>b</sup> Distribution of Ramachandran angles according to PROCHECK<sup>23</sup>

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