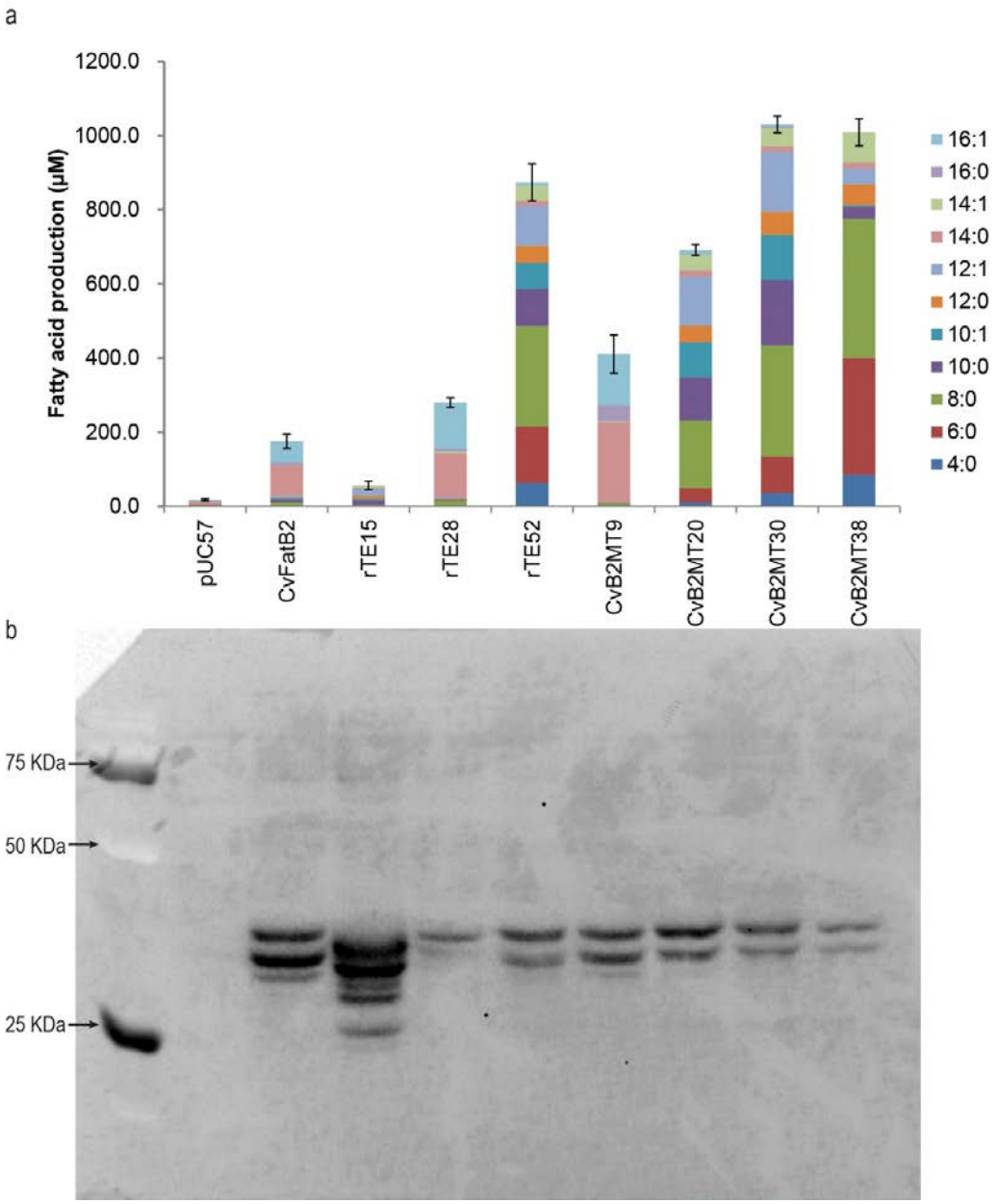
Supplementary Information

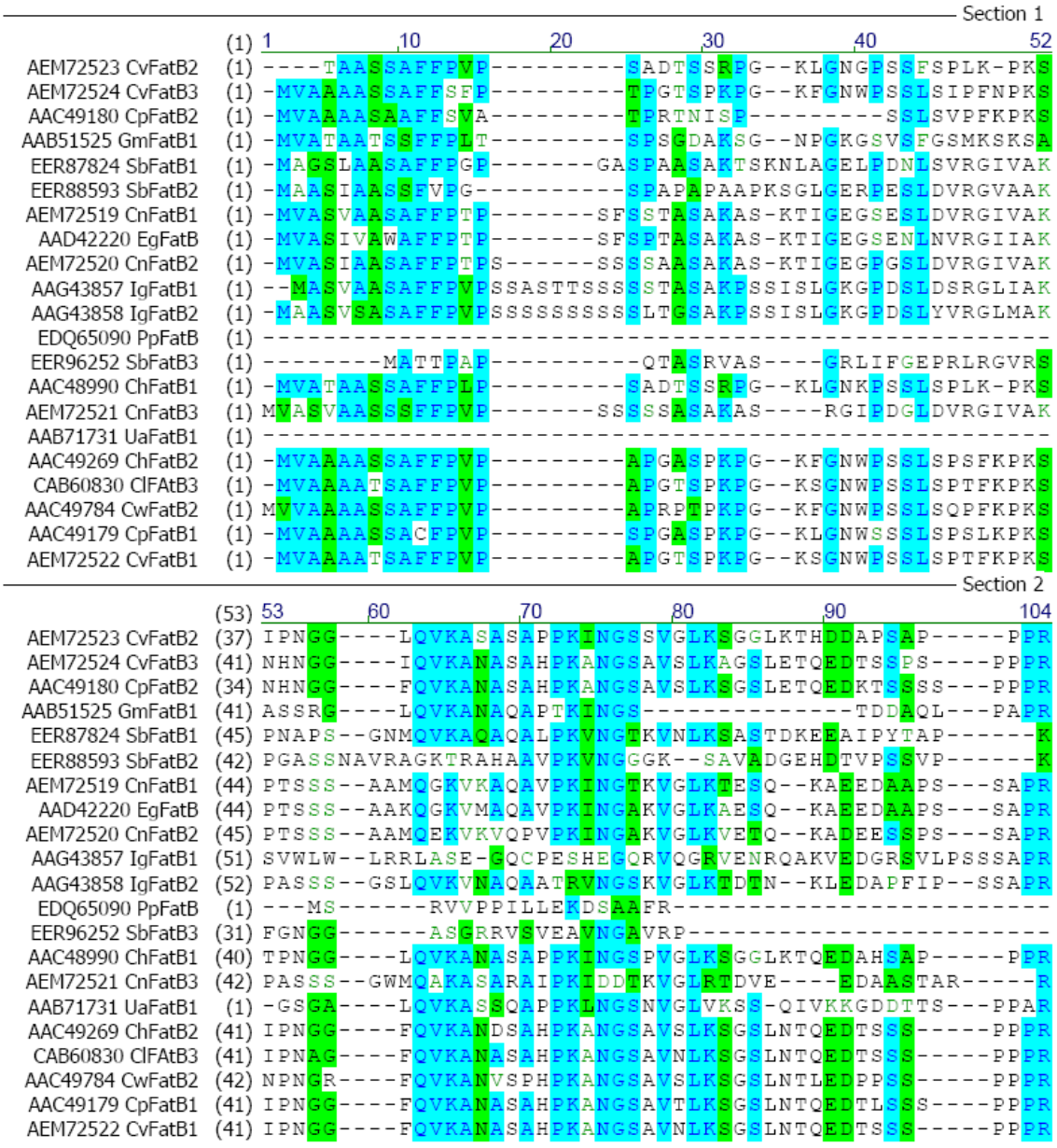
Two distinct domains contribute to the substrate acyl chain length selectivity of plant acyl-ACP thioesterase

Jing et al.



Supplementary Figure 1. Free FA titer and profile, and western blot analysis upon the expression of wild-type and seven representative CvFatB2 mutant enzymes in *E. coli* strains. Fatty acid titer is an average of 4 replicates, with error bars representing the standard error of the mean. The western blot analyses were conducted on 35 μg of soluble protein extracted from each *E. coli* strain, and CvFatB2 antibody was used to immunologically detect CvFatB2 variants. The multiple bands are probably the result of post-extraction proteolytic clipping of the expressed TEs. Because all the bands are immunologically recognized by our CvFatB2 antibody, we have integrated the signal from the entire collection of bands to compare the relative expression of the TE variants.

Supplementary Figure 2



Supplementary Figure 2 continued

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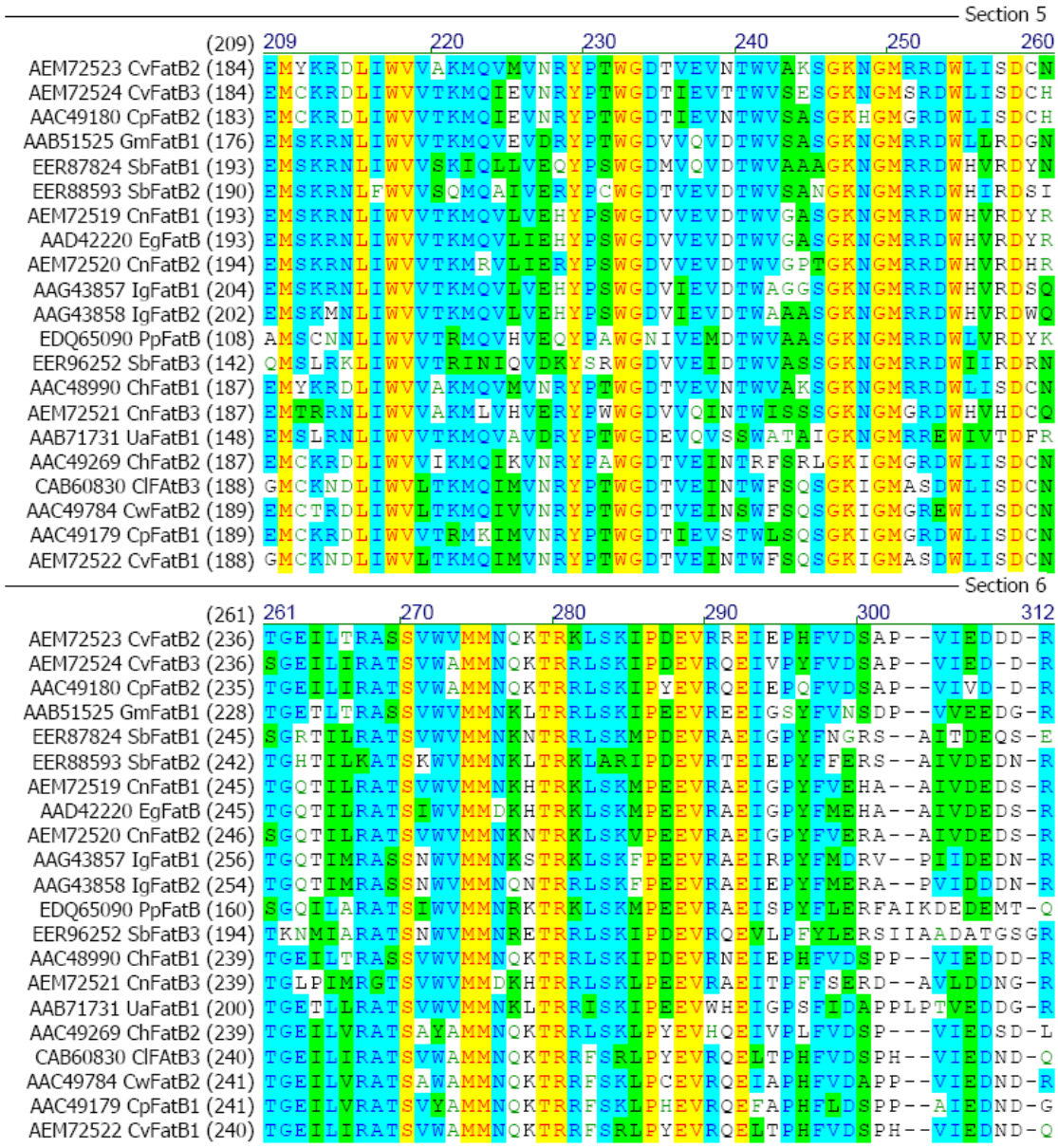
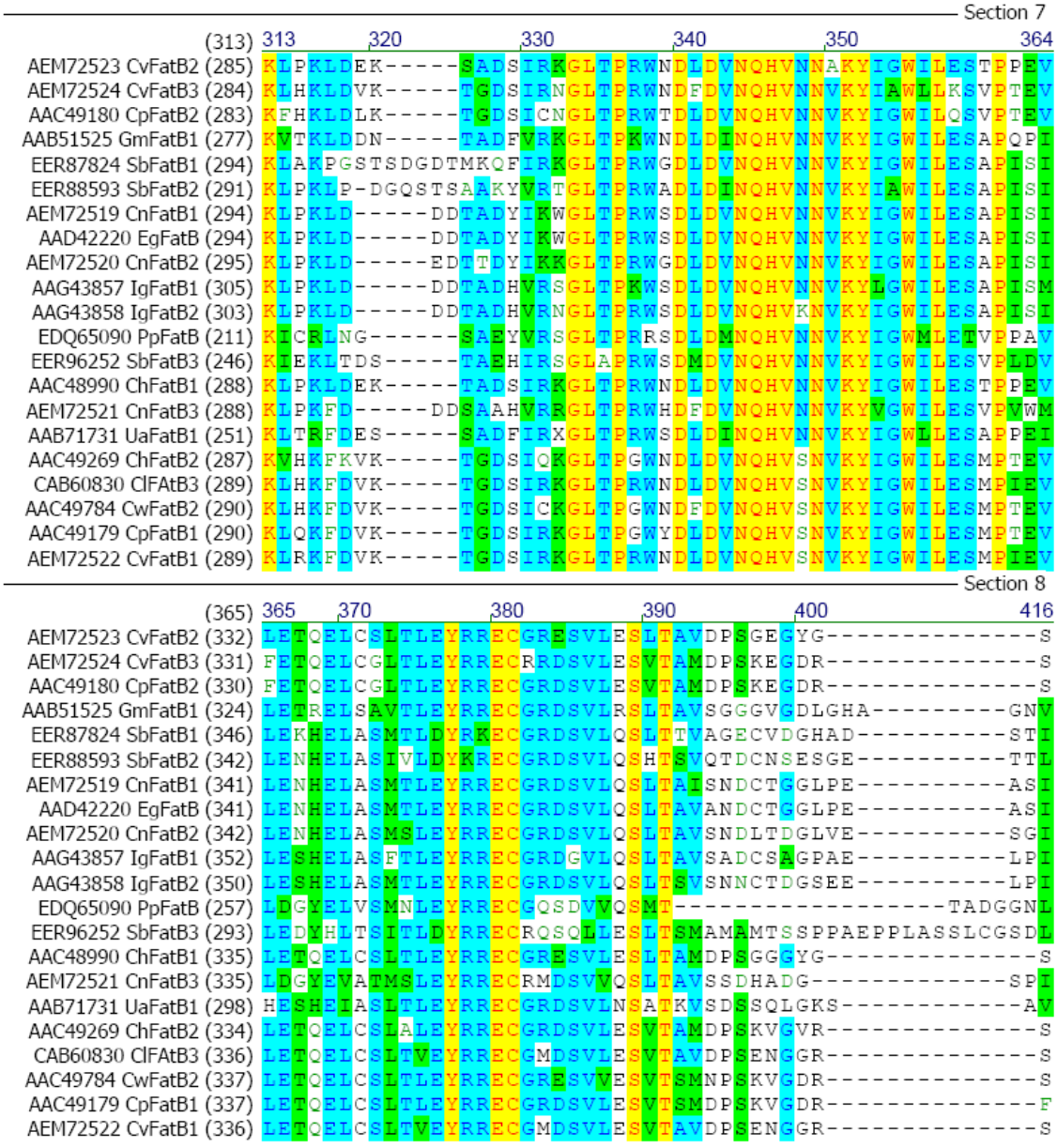
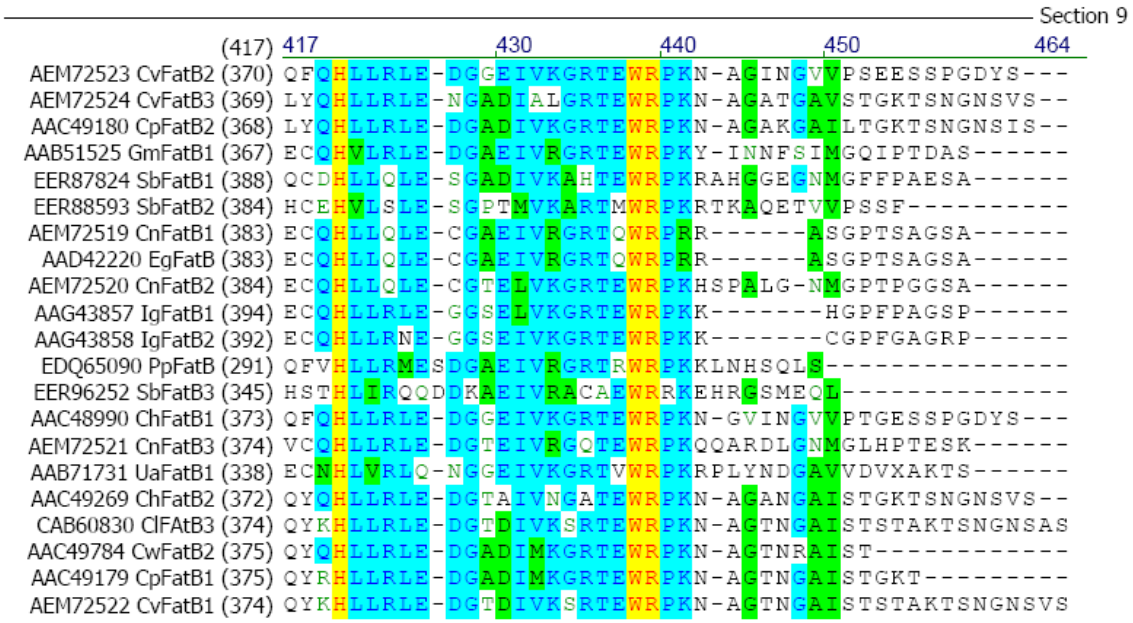


Supplementary Figure 2 continued



Supplementary Figure 2 continued



Supplementary Figure 2. Multiple sequence alignment of 21 functionally characterized plant acyl-ACP TEs. The alignment was constructed with Vector NTI - Alignment X, and the default settings identify identical residues (yellow highlighted), highly conserved residues (blue highlighted), weakly similar residues (green highlighted), and non-similar residues are not highlighted. Sequences are identified by their GenBank Accession Numbers, and source organism: Cv, *Cuphea viscosissima*; Cp, *Cuphea palustris*; Gm, *Garcinia mangostana*; Sb, *Sorghum bicolor*; Cn, *Cocos nucifera*; Eg, *Elaeis guineensis*; Ig, *Iris germanica*; Pp, *Physcomitrella patens*; Ch, *Cuphea hookeriana*; Ua, *Ulmus americana*; Cl, *Cuphea lanceolata*; Cw, *Cuphea wrightii*.

Supplementary Table 1. DNA primers used for the amplification of six overlapping fragments of CvFatB1 and CvFatB2 used in domain-swapping experiments

Domain-swapping fragment	Forward primers	Reverse primers
I	pUC57F (5'-CGGCTCGTATGTTGTGTGGAAT-3')	Cv-R2 (5'-GGTACGATCCGCGCCGATTTC-3')
II	Cv-F2 (5'-GAAATCGGCGCGGATCGTACC-3')	Cv-R3 (5'-CCAGGTCGGATAACGATTGAC-3')
III	Cv-F3 (5'-GTCAATCGTTATCCGACCTGG-3')	Cv-R4 (5'-CGGGTTTTCTGGTTCATCAT-3')
IV	Cv-F4 (5'-ATGATGAACCAGAAAACCCG-3')	Cv-R5 (5'-TCGTTCCAACGCGGCGTCAGACC-3')
V	Cv-F5 (5'-GGTCTGACGCCGCGTTGGAACGA-3')	Cv-R6 (5'-ATCTTCCAGACGCAGCAG-3')
VI	Cv-F6 (5'-CTGCTGCGTCTGGAAGAT-3')	pUC57R (5'-CTGCAAGGCGATTAAGTTGGGTAAC-3')

Supplementary Table 2. Comparison of the 15 residues that are predicted to affect substrate specificity of acyl-ACP TEs

	Fragments	II	III								IV					
	Residue Number	194[a]	213	217	218	219	223	226	227	248	257	260	268	276	282	289
Class I [b]	AEM72523 CvFatB2	V	V	V	A	K	N	R	R	V	L	I	I	A	D	L
	AEM72524 CvFatB3	V	V	V	S	E	N	S	R	A	L	I	I	A	D	L
	AAC49180 CpFatB2	V	V	V	S	A	H	G	R	A	L	I	I	A	D	L
	AAB51525 GmFatB1	V	V	V	S	A	N	R	R	V	L	I	I	D	D	L
	EER87824 SbFatB1	V	V	V	A	A	N	R	R	V	L	M	I	S	Q	P
	EER88593 SbFatB2	V	V	V	S	A	N	R	R	V	L	I	I	S	D	L
	AEM72519 CnFatB1	V	V	V	G	A	N	R	R	V	L	M	I	A	D	L
	AAD42220 EgFatB	V	V	V	G	A	N	R	R	V	L	M	I	A	D	L
	AEM72520 CnFatB2	V	V	V	G	P	N	R	R	V	L	V	I	A	D	L
	AAG43857 IgFatB1	V	V	A	G	G	N	R	R	V	L	F	I	V	D	L
	AAG43858 IgFatB2	V	V	A	A	A	N	R	R	V	L	F	I	A	D	L
	EDQ65090 PpFatB	V	M	V	A	A	N	R	R	V	L	M	I	F	M	L
	EER96252 SbFatB3	V	I	V	A	S	N	R	R	V	L	I	V	S	S	L
	AAC48990 ChFatB1	V	V	V	A	K	N	R	R	V	L	I	I	P	D	L
Class II and III	AEM72521 CnFatB3	V	I	I	S	S	N	G	R	V	L	L	I	D	N	F
	AAB71731 UaFatB1	V	V	A	T	A	N	R	R	V	I	I	I	P	D	F
	AAC49269 ChFatB2	V	I	F	S	R	I	G	R	A	L	L	I	P	S	F
	CAB60830 ClFatB3	L	I	F	S	Q	I	A	S	A	F	L	L	P	N	F
	AAC49784 CwFatB2	L	I	F	S	Q	I	G	R	A	F	L	I	P	N	F
	AAC49179 CpFatB1	V	V	L	S	Q	I	G	R	A	F	L	F	P	N	F
	AEM72522 CvFatB1	L	I	F	S	Q	I	A	S	A	F	L	L	P	N	F

a. Numbering of the residues are according to the sequence of CvFatB2 (GenBank Accession AEM72523.1), and their positions among the domain-swapping fragments are indicated; b. Each acyl-ACP TE is identified as either belonging to the FatB subclass I or II and III [1], and they are each identified by their GenBank Accession Numbers, and source organism: Cv, *Cuphea viscosissima*; Cp, *Cuphea palustris*; Gm, *Garcinia mangostana*; Sb, *Sorghum bicolor*; Cn, *Cocos nucifera*; Eg, *Elaeis guineensis*; Ig, *Iris germanica*; Pp, *Physcomitrella patens*; Ch, *Cuphea hookeriana*; Ua, *Ulmus americana*; Cl, *Cuphea lanceolata*; Cw, *Cuphea wrightii*.

Supplementary Table 3. FA composition and total free FA accumulation of *E. coli* strains expressing acyl-ACP TE mutants

Sample name	mol percentage (%)											Total free FAs (μM)
	4:0	6:0	8:0	10:0	10:1	12:0	12:1	14:0	14:1	16:0	16:1	
CvB2MT17	2.0	1.7	26.2	6.5	1.5	17.6	13.5	8.6	15.8	5.0	1.6	114±2
CvB2MT6	4.6	3.1	38.2	5.8	0.2	21.7	9.1	3.4	13.9	0.0	0.0	31±1
CvB2MT34	6.1	3.9	31.8	9.6	6.3	9.2	18.4	2.8	11.2	0.3	0.4	464±6
CvB2MT33	5.8	2.5	27.2	7.9	5.3	9.6	20.4	5.1	12.5	0.0	3.6	570±4
CvB2MT25	4.5	3.6	28.8	9.3	3.4	13.0	15.9	5.2	13.7	2.0	0.6	285±6
CvB2MT47	0.6	0.9	18.5	12.0	4.2	17.9	17.5	1.4	4.5	22.6	0.0	192±14
CvB2MT45	1.9	2.0	22.5	15.8	6.2	13.1	24.4	2.6	11.4	0.0	0.0	574±37
CvB2MT44	0.7	3.5	22.7	23.6	11.3	7.9	24.2	1.8	4.0	0.0	0.2	713±34
CvB2MT42	1.8	7.1	27.0	15.0	12.1	6.9	18.9	2.6	6.1	1.5	1.1	823±11
CvB2MT20	1.5	5.6	26.2	16.9	13.6	6.6	19.1	2.4	6.2	0.3	1.4	691±14
CvB2MT30	3.5	9.5	29.2	17.2	11.7	6.1	15.7	1.5	4.7	0.4	0.6	1031±22
CvB2MT48	0.1	1.2	13.7	38.6	18.1	6.7	17.5	0.0	3.9	0.2	0.0	137±4
CvB2MT40	1.8	4.8	61.4	12.5	8.5	3.3	6.2	0.6	0.9	0.0	0.0	1068±57
CvFatB1	0.0	3.5	45.3	26.1	8.9	3.9	9.6	1.1	0.9	0.0	0.6	542±32
CvB2MT29	2.4	1.5	11.4	7.2	2.5	15.0	18.1	12.1	20.5	0.4	8.9	287±12
CvB2MT12	2.2	1.6	12.5	7.2	1.6	16.4	15.4	15.3	20.0	0.0	7.8	149±4
CvB2MT2	1.0	0.7	8.9	4.6	1.1	10.0	9.7	24.5	19.9	0.1	19.5	82±8
CvB2MT32	3.1	1.3	21.5	7.3	3.9	4.8	14.4	9.5	30.6	0.0	3.4	331±11
CvB2MT16	2.2	0.9	15.4	5.6	1.6	3.9	9.6	22.8	30.9	0.0	7.0	308±19
CvB2MT31	3.4	2.2	17.9	5.9	3.7	9.0	19.6	12.1	17.3	0.7	8.1	393±7
CvB2MT19	4.4	2.1	18.2	6.3	2.8	10.3	22.0	8.5	18.4	0.6	6.3	168±6
CvB2MT15	4.0	2.1	16.1	7.5	1.2	15.6	19.8	9.2	19.8	0.7	4.0	150±6
CvB2MT27	3.3	2.0	20.8	9.9	5.8	10.3	21.5	5.3	17.6	0.3	3.2	230±5
CvB2MT18	4.2	1.9	21.9	8.4	3.1	13.6	19.9	6.1	15.3	2.3	3.2	300±8
CvB2MT22	2.9	1.6	20.7	9.2	1.5	13.0	15.6	8.9	16.7	0.0	10.0	85±6
CvB2MT10	3.0	2.2	21.6	9.6	4.6	8.8	14.1	9.8	16.4	0.0	9.9	171±13
CvB2MT38	8.5	31.1	37.2	3.1	0.6	5.7	4.2	1.6	8.1	0.0	0.0	1009±36
CvB2MT35	10.5	23.6	43.1	2.5	0.6	3.6	4.1	3.0	8.8	0.0	0.1	124±4
CvB2MT41	15.1	23.9	36.2	3.8	18.9	1.4	0.7	0.0	0.0	0.0	0.0	93±7
CvB2MT26	6.3	17.2	31.7	1.2	0.0	4.8	0.0	17.9	3.9	11.3	5.7	41±3
CvB2MT36	22.1	35.2	40.3	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	46±2
CvB2MT1	19.4	46.0	34.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4±0.8
CvB2MT37	8.9	40.1	40.2	3.4	0.3	2.8	1.0	1.1	2.0	0.0	0.2	333±12

CvB2MT43	0.4	0.7	4.1	1.2	0.5	1.5	1.4	47.0	0.7	11.5	31.0	174±11
CvB2MT9	0.1	0.1	1.5	0.3	0.1	0.3	0.4	52.6	0.6	10.4	33.7	411±51
CvB2MT14	0.0	0.4	3.4	0.8	0.0	1.6	0.8	52.2	0.7	14.1	26.0	108±1
CvB2MT11	0.1	0.4	4.3	1.5	0.2	3.8	4.0	45.5	4.9	11.9	23.5	120±8
CvB2MT13	1.0	0.8	12.7	2.1	0.7	0.9	1.5	50.7	0.5	3.1	26.0	183±6
CvFatB2	0.0	0.3	5.4	5.4	1.4	1.3	3.2	45.2	0.6	5.5	31.7	175±19
CvB2MT23	0.6	0.4	5.6	1.0	0.3	0.8	0.8	54.2	0.9	6.6	28.7	385±16
CvB2MT8	0.2	0.4	3.9	0.3	0.0	0.0	0.0	58.0	0.0	4.4	32.8	74±6
CvB2MT7	0.7	0.7	6.1	0.6	0.0	0.0	0.0	62.2	0.0	0.9	28.8	47±13
CvB2MT4	0.2	0.3	3.7	0.6	0.1	0.0	0.2	60.9	0.0	9.3	24.5	199±18
CvB2MT24	0.2	0.1	3.6	0.4	0.1	0.0	0.3	73.6	0.2	1.4	20.0	401±49
CvB2MT5	0.0	0.1	1.5	0.2	0.0	0.0	0.0	81.5	0.0	2.9	13.9	457±33
CvB2MT28	1.7	1.4	17.6	5.0	1.2	7.5	3.7	40.1	4.1	3.4	14.3	328±21
CvB2MT21	1.9	1.1	17.8	3.4	0.6	4.6	2.7	43.8	4.5	2.0	17.5	201±7
CvB2MT3	2.6	1.9	17.2	4.1	1.5	2.1	4.6	40.1	4.3	0.0	21.6	90±6

Green-shading identifies the major fatty acids produced by each strain expressing the mutant acyl-ACP TE enzyme.

Supplementary Reference

1. Jing, F. et al. Phylogenetic and experimental characterization of an acyl-ACP thioesterase family reveals significant diversity in enzymatic specificity and activity. *Bmc Biochem* **12**, 44 (2011).