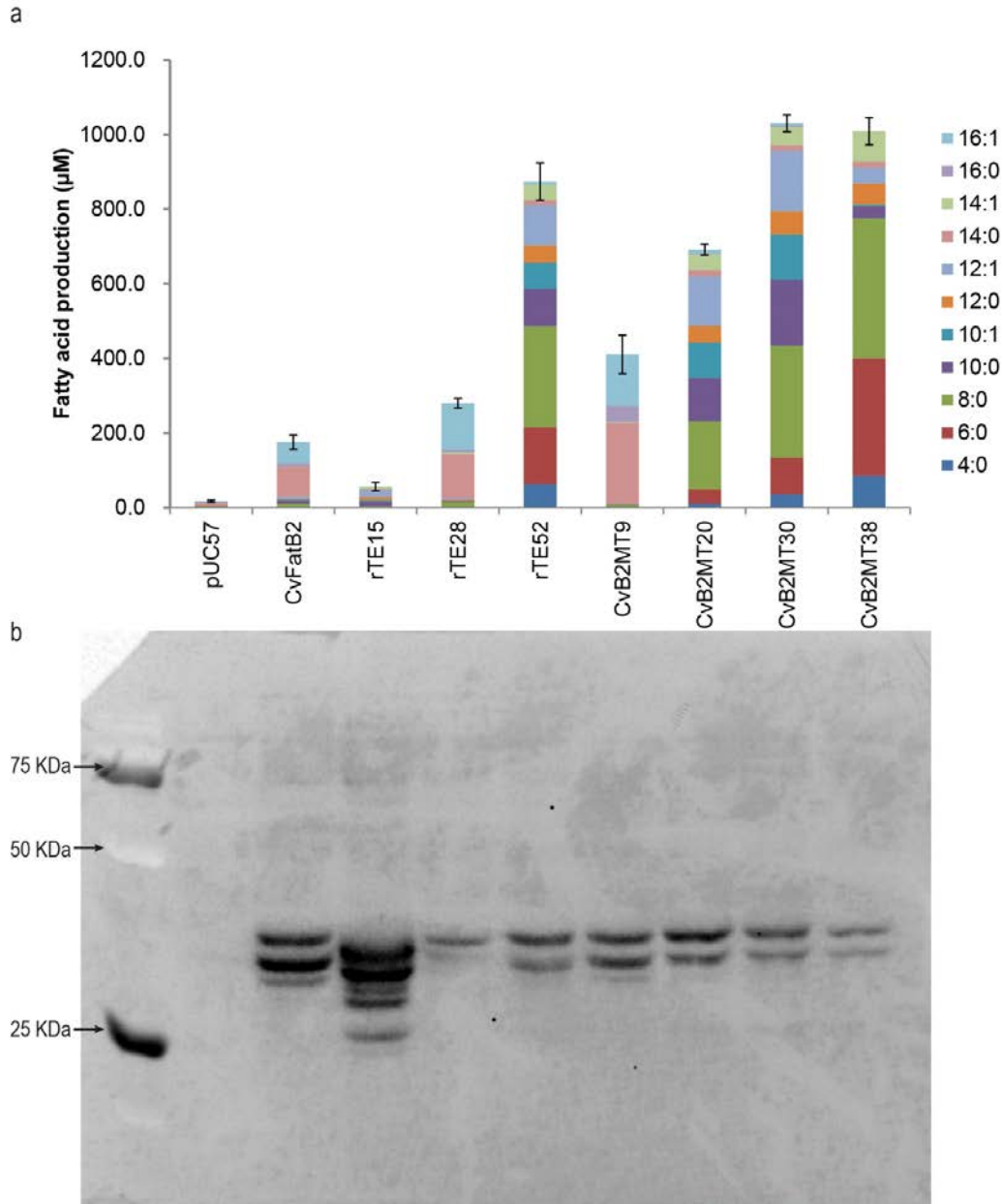


Supplementary Information

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

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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 continued

	(105)	105	110	120	130	140	156	Section 3
AEM72523 CvFatB2 (80)	TF	I	NQLPDWSMLLA	AITTA	FLAAEKQWMMLDR	KPKRLDML	EDP	FGLGRVVD
AEM72524 CvFatB3 (85)	TF	IS	QLPDWSMLLS	VAITTVF	VAAEKQWTMLDR	SKRPDLV	VEPE	-----VQD
AAC49180 CpFatB2 (79)	TF	I	NQLPVDWSMLLS	AVTTVFG	VAAEKQWPMLDR	SKRPDML	VEPL	GLVDRIYD
AAB51525 GmFatB1 (72)	TF	I	NQLPDWSMLLA	AITTVFL	AAEKQWMMLD	WKP	RRPDML	IDTFGLGRIVQD
EER87824 SbFatB1 (89)	TF	Y	NQLPDWSMLLA	AVTTI	FLAAEKQWTLLD	WKP	KKPDML	VDTFGFGRIIQD
EER88593 SbFatB2 (86)	TF	Y	NQLPDWSMLLA	AVTTI	FLAAEKQWTMLD	WKP	RRPDML	TDTFGFGRIIHD
AEM72519 CnFatB1 (89)	TF	Y	NQLPDWSVLLAA	AVTTI	FLAAEKQWTLLD	WKP	RRPDML	TDAFSLGKIVQD
AAD42220 EgFatB (89)	TF	Y	NQLPDWSVLLAA	AVTTI	FLAAEKQWTLLD	WKP	RRPDML	TGAFSLGKIVQD
AEM72520 CnFatB2 (90)	TF	Y	NQLPDWSVLLAA	AVTTI	FLAAEKQWTLLD	WKP	RRPDML	ADAFGLGKIVQD
AAG43857 IgFatB1 (100)	TF	Y	NQLPDWSVLLAA	AVTTI	FLAAEKQWTLLD	WKR	GGPDML	TDAFGLGKIITEN
AAG43858 IgFatB2 (98)	TF	Y	NQLPDWSVLLAA	AVTTI	FLAAEKQWTLLD	WKR	GGPDMLS	DAFGLPKIITEN
EDQ65090 PpFatB (19)	-----A	ILAA	IAGVLA	AEENQ	RRHDKT	-----E	VPV	DVFRQGLIVES
EER96252 SbFatB3 (53)	-----N	GAAAV	ADVRL	VPA	PPAS	VEG	-----D	DGGDAFRLGKIVVEG
AAC48990 ChFatB1 (83)	TF	I	NQLPDWSMLLA	AITTVFL	AAEKQWMMLD	WKP	KRPDML	VDPFGLGSIVQD
AEM72521 CnFatB3 (83)	TS	Y	NQLPDWSMLLA	AIRTI	FSAAEKQWTLLD	SKR	GADAV	ADASGVGKMKVKN
AAB71731 UaFatB1 (44)	TF	I	NQLPDWSMLLA	AITTVFL	AAEKQWMMLD	WKP	KRPDML	VDPFGLGRIVQD
AAC49269 ChFatB2 (84)	TF	L	HQLPDWSRLI	TAITTVF	VK-S	KRPDM	HDR	SKRPDMLVDSFGLVESTVQD
CAB60830 CfFatB3 (84)	A	F	L	NQLPDWSMLL	TAITTVF	VAAEKQWTMLD	R	SKRPDMLVDSVGLKSIIVRD
AAC49784 CwFatB2 (85)	TF	L	NQLPDWSRLI	RTAITTVF	VAAEKQETRL	R	SKRPDML	VDFWFGSETIVQD
AAC49179 CpFatB1 (85)	A	F	N	QLPDWSMLL	TAITTVF	VAF	EKR	WTMDFDRSKRPNMLMDSFGLERIVQD
AEM72522 CvFatB1 (84)	A	F	L	NQLPDWSMLL	TAITTVF	VAAEKQWTMLD	R	SKRPDMLVDSVGLKSIIVRD
	(157)	157	170	180	190	208	Section 4	
AEM72523 CvFatB2 (132)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETVMNHLQ	ETALNHVKTAGL	LSNDG	FGFRTP
AEM72524 CvFatB3 (132)	GV	SFRQSE	FS	I	RSYEIGVDR	TASIE	ETLMNIFQ	ETSLNHCKSLGGLINDGFGFRTP
AAC49180 CpFatB2 (131)	GV	SFRQSE	FS	I	RSYEIGADRTASIE	ETLMNMFQ	ETSLNHCKIIGL	INDGFGFRTP
AAB51525 GmFatB1 (124)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETVMNHLQ	ETALNHVKTAGL	LLGDG	FGGSTP
EER87824 SbFatB1 (141)	GLVFRQNE	LS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVKTAGL	LLGDG	FGGATP
EER88593 SbFatB2 (138)	GLMFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVKTAGL	LLGDG	FGGSTP
AEM72519 CnFatB1 (141)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVRN	AGLLGDG	FGGATP
AAD42220 EgFatB (141)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVRN	AGLLGDG	FGGATP
AEM72520 CnFatB2 (142)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVKS	SAGLMG	DGFGATP
AAG43857 IgFatB1 (152)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVR	CAGLLGN	GFGSTP
AAG43858 IgFatB2 (150)	GILYRQKE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVK	CAGLLGN	GFGSTP
EDQ65090 PpFatB (56)	RLVY	GT	EV	I	RSYEIGADRTASIE	ETVMNHFQ	ETALNHVWMS	GLAGDGF
EER96252 SbFatB3 (90)	TLVYRQCE	EV	I	RSYEIGPDR	TATMETLMNHLQ	ETALNHVMCS	GLAGDGF	GGATL
AAC48990 ChFatB1 (135)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETVMNHLQ	ETALNHVKI	AGLSNDG	FGFRTP
AEM72521 CnFatB3 (135)	GLVFRQNE	FS	I	RSYEIGVDR	SRASVEALMNHFQ	ETSLNHCKCIGL	MHGG	FGCTP
AAB71731 UaFatB1 (96)	GLVFRQNE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETALNHVKS	SVGLLED	DGLGSTR
AAC49269 ChFatB2 (135)	GLVFRQSE	FS	I	RSYEIGTDR	TASIE	ETLMNHLQ	ETSLNHCKSTG	ILLDGGFRIL
CAB60830 CfFatB3 (136)	GLVSRQSE	FL	I	RSYEIGADRTASIE	ETLMNHLQ	ETSLNHCKSLG	LLINDG	FGFRTP
AAC49784 CwFatB2 (137)	GLVFRERE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETSLNHCKSV	GLLINDG	FGFRTP
AAC49179 CpFatB1 (137)	GLVFRQSE	FS	I	RSYEICADRTASIE	ETVMNHVQ	ETSLNQCKSI	GLLDG	FGGRSP
AEM72522 CvFatB1 (136)	GLVSRHSE	FS	I	RSYEIGADRTASIE	ETLMNHLQ	ETSLNHCKSLG	LHNDG	FGFRTP

Supplementary Figure 2 continued

	Section 5													
(209)	209	220	230	240	250	260								
AEM72523 CvFatB2 (184)	EMYKRDLI	WVVA	AKMQVMV	NRYP	TWGD	TVEVN	TWVA	AKS	GKNG	MRRD	WLI	SD	CH	
AEM72524 CvFatB3 (184)	EMCKRDLI	WVVT	KMQI	EVNRY	PTWGD	TIEVT	TWVSE	SGKN	GMSR	DWLI	SD	CH		
AAC49180 CpFatB2 (183)	EMCKRDLI	WVVT	KMQI	EVNRY	PTWGD	TIEVN	TWVSA	SGKH	GMGR	DWLI	SD	CH		
AAB51525 GmFatB1 (176)	EMSKRNLI	WVVT	KMQV	EVDRYP	TWGD	VVQVD	TWVSA	SGKN	GMRD	WLI	RD	GN		
EER87824 SbFatB1 (193)	EMSKRNLI	WVVS	KIQ	LIVE	QYPS	WGD	DMVQ	VD	TWVAA	AGKN	GMRD	WH	VDYN	
EER88593 SbFatB2 (190)	EMSKRNLI	FVVVS	QMQA	IVERYP	PCWGD	TVEVD	TWVSA	NGKN	GMRD	WH	IR	LSI		
AEM72519 CnFatB1 (193)	EMSKRNLI	WVVT	KMQV	LVEH	YPS	WGD	VVEVD	TWVG	AS	SGKN	GMRD	WH	VDYR	
AAD42220 EgFatB (193)	EMSKRNLI	WVVT	KMQV	LVEH	YPS	WGD	VVEVD	TWVG	AS	SGKN	GMRD	WH	VDYR	
AEM72520 CnFatB2 (194)	EMSKRNLI	WVVT	KMRV	LIERYP	SWGD	VVEVD	TWVG	PT	GKN	GMRD	WH	VDHR		
AAG43857 IgFatB1 (204)	EMSKRNLI	WVVT	KMQV	LVEH	YPS	WGD	VVEVD	TWAG	GS	SGKN	GMRD	WH	VDSC	
AAG43858 IgFatB2 (202)	EMSKMNLI	WVVT	KMQV	LVEH	YPS	WGD	VVEVD	TWAA	AS	SGKN	GMRD	WH	VDWC	
EDQ65090 PpFatB (108)	AMSCNLI	WVVT	RMQV	HVE	QYPA	WGN	NIVEM	D	TWVA	AS	SGKN	GMRD	WH	VDYK
EER96252 SbFatB3 (142)	QMSLRK	LIVV	TRINI	QV	DKYS	RWGD	VVEI	D	TWVA	SS	SGKN	GMRD	WH	VDRN
AAC48990 ChFatB1 (187)	EMYKRDLI	WVVA	AKMQVMV	NRYP	TWGD	TVEVN	TWVA	AKS	GKNG	MRRD	WLI	SD	CH	
AEM72521 CnFatB3 (187)	EMTRRNLI	WVVA	KMLV	HVERYP	PWGD	VVQ	INTW	LSS	SGKN	GMRD	WH	VDCC		
AAB71731 UaFatB1 (148)	EMSLRNLI	WVVT	KMQV	AVDRYP	TWGD	EVQV	SSWAT	A	IGKN	GMR	REW	IV	TDFR	
AAC49269 ChFatB2 (187)	EMCKRDLI	WVVI	KMQI	KVNRYP	PAWGD	TVEI	INTR	FR	RLGK	IGMGR	DWLI	SD	CH	
CAB60830 ClFatB3 (188)	GMCKNDLI	WVLT	KMQI	IMVNRYP	PTWGD	TVEI	INTW	FS	QSGK	IGMAS	DWLI	SD	CH	
AAC49784 CwFatB2 (189)	EMCTRDLI	WVLT	KMQI	IVNRY	PTWGD	TVEI	INSW	FS	QSGK	IGMGR	EWLI	SD	CH	
AAC49179 CpFatB1 (189)	EMCKRDLI	WVVT	KMQI	IMVNRYP	PTWGD	TIEV	STW	L	QSGK	IGMGR	DWLI	SD	CH	
AEM72522 CvFatB1 (188)	GMCKNDLI	WVLT	KMQI	IMVNRYP	PTWGD	TVEI	INTW	FS	QSGK	IGMAS	DWLI	SD	CH	

	Section 6												
(261)	261	270	280	290	300	312							
AEM72523 CvFatB2 (236)	TGEILTRAS	SVVMMN	NKTR	KLSKI	PDEV	REIE	PHFV	SAP	--	VIED	DD	-R	
AEM72524 CvFatB3 (236)	SGEILIRAT	SVWAMN	NKTR	RLSKI	PDEV	QEI	VPYF	VDS	SAP	--	VIED	-D	-R
AAC49180 CpFatB2 (235)	TGEILIRAT	SVWAMN	NKTR	RLSKI	PDEV	QEI	EPFV	SAP	--	VIVD	-D	-R	
AAB51525 GmFatB1 (228)	TGETLIRAS	SVVMMN	NKTR	RLSKI	PDEV	REI	GSYF	VNS	DP	--	VVEE	DG	-R
EER87824 SbFatB1 (245)	SGRTLIRAS	SVVMMN	NKTR	RLSK	MPDEV	RAE	IGPY	FNGRS	--	AITD	EQS	-E	
EER88593 SbFatB2 (242)	TGHTILKAT	SKWMMN	NKTR	KLARI	PDEV	TEI	EPYF	FERS	--	AIVD	EDN	-R	
AEM72519 CnFatB1 (245)	TGQTLIRAT	SVVMMN	NKTR	KLSK	MPDEV	RAE	IGPY	FVEHA	--	AIVD	EDS	-R	
AAD42220 EgFatB (245)	TGQTLIRAT	SVVMMN	NKTR	KLSK	MPDEV	RAE	IGPY	FMEHA	--	AIVD	EDS	-R	
AEM72520 CnFatB2 (246)	SGQTLIRAT	SVVMMN	NKTR	KLSK	VPEV	RAE	IGPY	FVERA	--	AIVD	EDS	-R	
AAG43857 IgFatB1 (256)	TGQTIMRAS	SNWMMN	NKTR	KLSK	FPEV	RAE	IRPY	FMDRV	--	PLID	EDN	-R	
AAG43858 IgFatB2 (254)	TGQTIMRAS	SNWMMN	NQTR	RLSK	FPEV	RAE	IEPY	FME	RA	--	PVID	EDN	-R
EDQ65090 PpFatB (160)	SGQTLARAT	SVVMMN	NKTR	KLSK	MPDEV	RAE	ISPY	FLE	RFA	IKDE	ED	MT	-Q
EER96252 SbFatB3 (194)	TKNMIARAT	SNWMMN	RETR	RLSKI	PDEV	QEV	LP	FYLE	RS	IIA	ADAT	GSGR	
AAC48990 ChFatB1 (239)	TGEILTRAS	SVVMMN	NKTR	RLSKI	PDEV	RNE	IEPH	FVDS	PP	--	VIED	DD	-R
AEM72521 CnFatB3 (239)	TGLPIMRGT	SVVMMN	NKTR	RLSK	LPEV	RAE	ITP	FFSER	D	--	AVLD	ING	-R
AAB71731 UaFatB1 (200)	TGETLLRAT	SVVMMN	NKTR	RLSKI	PDEV	WHE	IGP	SFL	DA	PP	LP	TVED	DG
AAC49269 ChFatB2 (239)	TGEILVRAT	SAYAMN	NKTR	RLSK	LPEV	HQE	IVPL	FVDS	P	---	VIED	SD	-L
CAB60830 ClFatB3 (240)	TGEILIRAT	SVWAMN	NKTR	RF	SRLPEV	QEL	TPH	FVDS	PH	--	VIED	ND	-Q
AAC49784 CwFatB2 (241)	TGEILVRAT	SAWAMN	NKTR	RF	SKLPEV	QEL	IAPH	FVDA	PP	--	VIED	ND	-R
AAC49179 CpFatB1 (241)	TGEILVRAT	SVYAMN	NKTR	RF	SKLPEV	QEL	FAPH	FLDS	PP	--	AIED	ND	-G
AEM72522 CvFatB1 (240)	TGEILIRAT	SVWAMN	NKTR	RF	SRLPEV	QEL	TPH	FVDS	PH	--	VIED	ND	-Q

Supplementary Figure 2 continued

Section 9

	(417)	417	430	440	450	464			
AEM72523 CvFatB2 (370)	QFQ	HLLRLE	DGGEIVKGRTEWRPKN	AG	INGV	PSEESSPGDYS---			
AEM72524 CvFatB3 (369)	LYQ	HLLRLE	NGADIALGRTEWRPKN	AG	ATGAV	STGKTSNGNSVS--			
AAC49180 CpFatB2 (368)	LYQ	HLLRLE	DGADIVKGRTEWRPKN	AG	AKGAL	LTGKTSNGNSIS--			
AAB51525 GmFatB1 (367)	ECQ	HLLRLE	DGAEIVRGRTEWRPKY	INN	F	SINGQIPTDAS-----			
EER87824 SbFatB1 (388)	QCD	HLLQLE	SGADIVKAHTEWRPKR	AH	G	GEGNMGFFPAESA-----			
EER88593 SbFatB2 (384)	HCE	HVLSLE	SGPTMVKARTMWRPKR	TK	Q	ETVVPSSF-----			
AEM72519 CnFatB1 (383)	ECQ	HLLQLE	CGAEIVRGRTEWRPKR	---	---	ASGPTSAGSA-----			
AAD42220 EgFatB (383)	ECQ	HLLQLE	CGAEIVRGRTEWRPKR	---	---	ASGPTSAGSA-----			
AEM72520 CnFatB2 (384)	ECQ	HLLQLE	CGTELVKGRTEWRPKH	S	P	HLG-NMGPTPGGSA-----			
AAG43857 IgFatB1 (394)	ECQ	HLLRLE	GGSELVKGRTEWRPKK	---	---	HGPFPPAGSP-----			
AAG43858 IgFatB2 (392)	ECQ	HLLRNE	GGSEIVKGRTEWRPKK	---	---	CGPFGAGRP-----			
EDQ65090 PpFatB (291)	QFV	HLLRME	DGAEIVRGRTRWRPKK	KL	N	HSQLS-----			
EER96252 SbFatB3 (345)	HST	HLE	RQQD	DK	A	EIVRACAEWRPK	EHRG	SMEQD	-----
AAC48990 ChFatB1 (373)	QFQ	HLLRLE	DGGEIVKGRTEWRPKN	G	V	INGV	PTGESSPGDYS---		
AEM72521 CnFatB3 (374)	VCQ	HLLRLE	DGTEIVRGRTEWRPK	Q	A	RDLGN	NGLHPTESK-----		
AAB71731 UaFatB1 (338)	ECN	HLLVRLQ	NGGEIVKGRTEWRPKR	P	L	YNDG	AVD	VXAKTS	-----
AAC49269 ChFatB2 (372)	QYQ	HLLRLE	DGTAIVNGATEWRPKN	AG	AN	GA	STGKTSNGNSVS--		
CAB60830 ClFatB3 (374)	QYK	HLLRLE	DGTDIVKSRTTEWRPKN	AG	T	NGA	IST	TAKTSNGNSAS	-----
AAC49784 CwFatB2 (375)	QYQ	HLLRLE	DGADIMKGRTEWRPKN	AG	T	NRA	IST	-----	
AAC49179 CpFatB1 (375)	QYR	HLLRLE	DGADIMKGRTEWRPKN	AG	T	NGA	IST	GKT	-----
AEM72522 CvFatB1 (374)	QYK	HLLRLE	DGTDIVKSRTTEWRPKN	AG	T	NGA	IST	TAKTSNGNSVS	-----

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'-CGGCTCGTATGTTGTGTGG AAT-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'-TCGTTCCAACGCGGGCGTCAGAC-3')
V	Cv-F5 (5'-GGTCTGACGCCGCGTTGGAACGA-3')	Cv-R6 (5'-ATCTTCCAGACGCAGCAG-3')
VI	Cv-F6 (5'-CTGCTGCGTCTGGAAGAT-3')	pUC57R (5'-CTGCAAGGCGATTAAGTTGGGT AAC-3')

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

	Fragments Residue Number	II	III								IV					
		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 III¹, 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 (μ M)
	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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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).