

Supplementals

Supplemental Table S1. Percentage of amino acid similarity between CsLPEAT1a isoform variants (A) and CsLPEAT2 isoforms (B).

A	CsLPEAT1a.1	CsLPEAT1a.2
CsLPEAT1a.1		99.24%
CsLPEAT1a.2	99.24%	

B	CsLPEAT2a	CsLPEAT2b	CsLPEAT2c
CsLPEAT2a		98.17%	98.17%
CsLPEAT2b	98.17%		97.61%
CsLPEAT2c	97.61%	97.61%	

Supplemental Table S2. Primers used to amplify CsLPEAT genes, later cloned into the Gateway system. attB1 fragments of forward primers and attB2 fragments of reverse primers are underlined

Gene-of-Interest	Primer Role	Primer Sequence (5' to 3')
CsLPEAT1	forward	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> GTGTTTACCTTT TACGGCGAATCTA
	reverse	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> TCGTGTAATCAA ACATTTGGCTTTT
CsLPEAT2	forward	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> ACATTCGACCAC AAAGCAAAAAGGC
	reverse	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> AAAGTTTGCTCA GAACTAGAGG

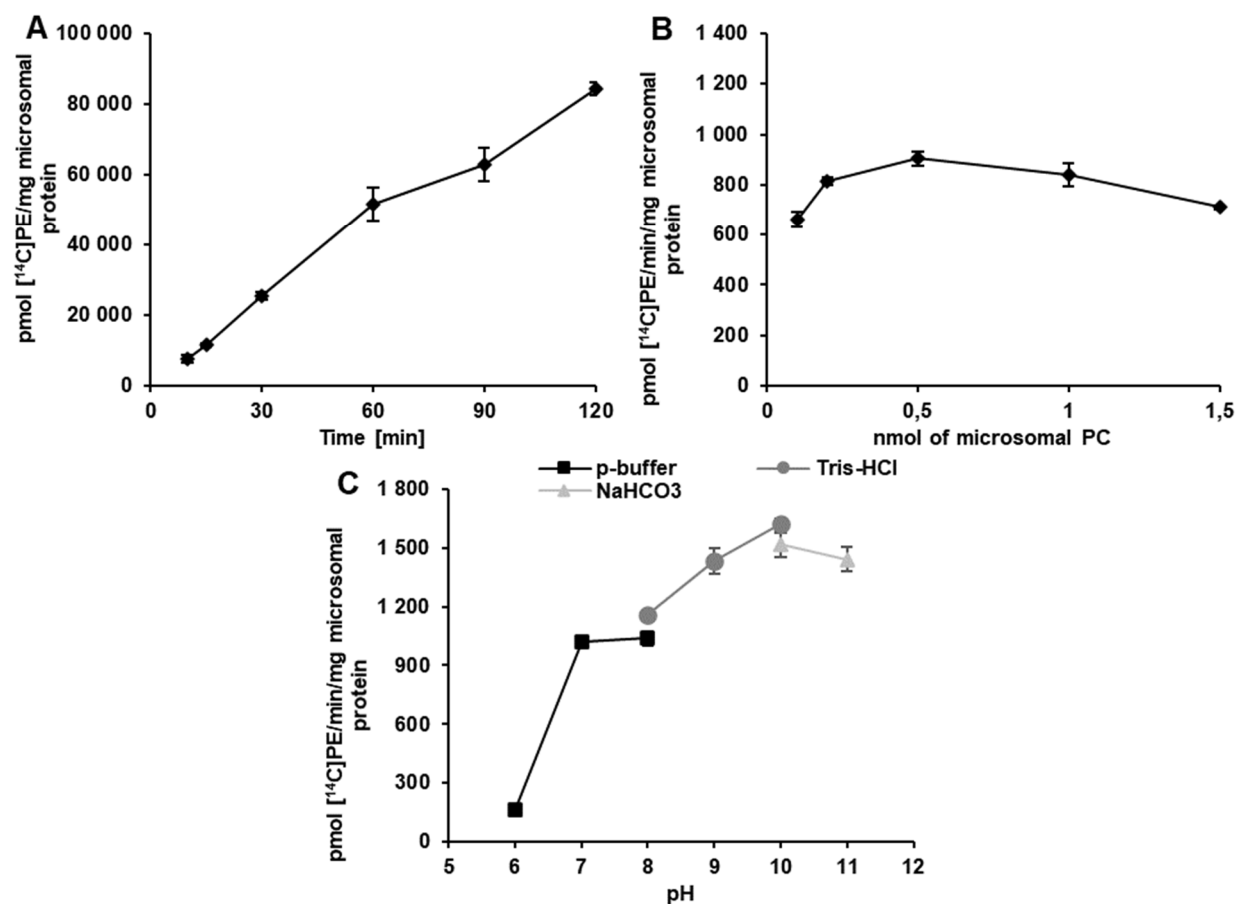
Supplemental Table S3. Primers used in RT-qPCR analysis.

Gene-of-Interest	Reference Sequence	Primer Role	Primer Sequence (5' to 3')
CsACT2	XM 010489499.1	forward	ACAATTTCCCGCTCTGCTGTTGTG
CsACT2	XM 010489499.1	reverse	AGGGTTTCTCTCTTCCACATGCCA
CsTIP41	XM 019233743.1	forward	TTGGCGAGAATGCATTAGTCCTT
CsTIP41	XM 019233743.1	reverse	ATTGTCGGCCAGTTCATCTTCATA
CsLPEAT1a	XM 010431575.2	forward	GACGACGACGACGAGAAA

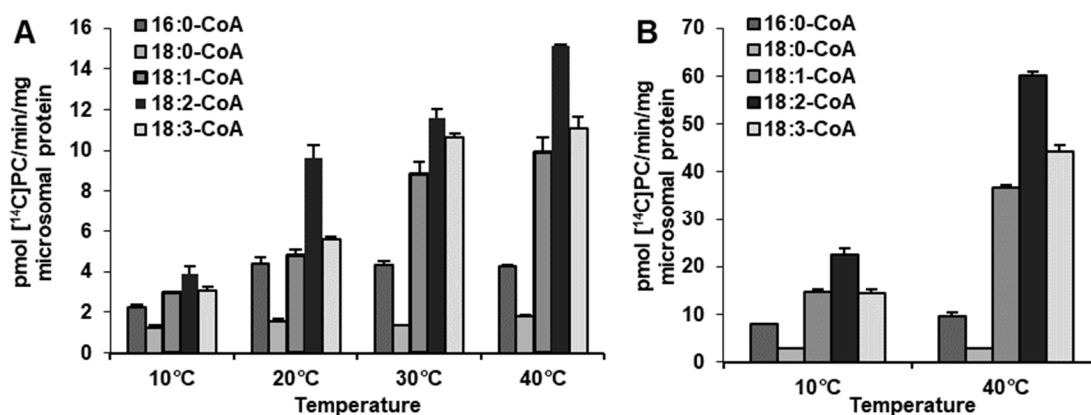
CsLPEAT1a	XM_010431575.2	reverse	TAAATGAAAGAAAGGGCTTCAG
CsLPEAT1b	XM_010419341.2	forward	AAAGCTGAAATCTTCACGG
CsLPEAT1b	XM_010419341.2	reverse	CAAAGAAAGAAAGGGGCTT
CsLPEAT1c	XM_010474544.2	forward	CTAGAGACGACGAGGAGGAG
CsLPEAT1c	XM_010474544.2	reverse	CTCTGATTCCATTGTTCTTCTTCTA
CsLPEAT2a	XM_010519887.1	forward	AAGCAAAAAGGCTGTGTCAATC
CsLPEAT2a	XM_010519887.1	reverse	TTCTGGTTGATCGACGGATGG
CsLPEAT2b	XM_010509827.2	forward	TGATCCATCATCAACCAGAG
CsLPEAT2b	XM_010509827.2	reverse	AAGAACAGGAGGCTCTGT
CsLPEAT2c	XM_010508235.2	forward	AGCAAAAAGGCTGTGAGTAC
CsLPEAT2c	XM_010508235.2	reverse	TGGATGATTGATGATGGATCAAC

Supplemental Table S4. List of reference sequences used for sequence alignments and evolutionary analyses.

Gene Denotation	Reference Sequence	Species of Origin
CsLPEAT1b	XM_010419341.2	<i>Camelina sativa</i>
CsLPEAT1c	XM_010474544.2	<i>Camelina sativa</i>
<i>At</i> LPEAT1	AT1G80950.1	<i>Arabidopsis thaliana</i>
<i>At</i> LPEAT1	XP_020889625.1	<i>Arabidopsis lyrata</i>
<i>Bn</i> LPEAT1.1	XP_013702411.1	<i>Brassica napus</i>
<i>Bn</i> LPEAT1.2	XP_013648598.1	<i>Brassica napus</i>
<i>Bo</i> LPEAT1	XP_013591940.1	<i>Brassica oleracea</i>
<i>Br</i> LPEAT1	XP_009104503.2	<i>Brassica rapa</i>
<i>Es</i> LPEAT1	XP_006389853.1	<i>Eutrema salsugineum</i>
<i>Rs</i> LPEAT1	XP_018457802.1	<i>Raphanus sativus</i>
CsLPEAT2a	XM_010519887.1	<i>Camelina sativa</i>
CsLPEAT2b	XM_010509827.2	<i>Camelina sativa</i>
CsLPEAT2c	XM_010508235.2	<i>Camelina sativa</i>
<i>At</i> LPEAT2	AT2G45670.1	<i>Arabidopsis thaliana</i>
<i>At</i> LPEAT2	XP_002882025.1	<i>Arabidopsis lyrata</i>
<i>Bn</i> LPEAT2.1	XP_013734815.1	<i>Brassica napus</i>
<i>Bn</i> LPEAT2.2	XP_022571765.1	<i>Brassica napus</i>
<i>Bo</i> LPEAT2	XP_013631253.1	<i>Brassica oleracea</i>
<i>Br</i> LPEAT2	XP_009133738.1	<i>Brassica rapa</i>
<i>Es</i> LPEAT2	XP_006397746.1	<i>Eutrema salsugineum</i>
<i>Rs</i> LPEAT2	XP_018484721.1	<i>Raphanus sativus</i>



Supplemental Figure S1. Effects of various factors on the activity of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs) of *Camelina sativa* *in vivo* leaves. A – time dependency; B – microsomal content dependency; C - pH dependency. Mean values and SD are presented (data from at least three independent assays).



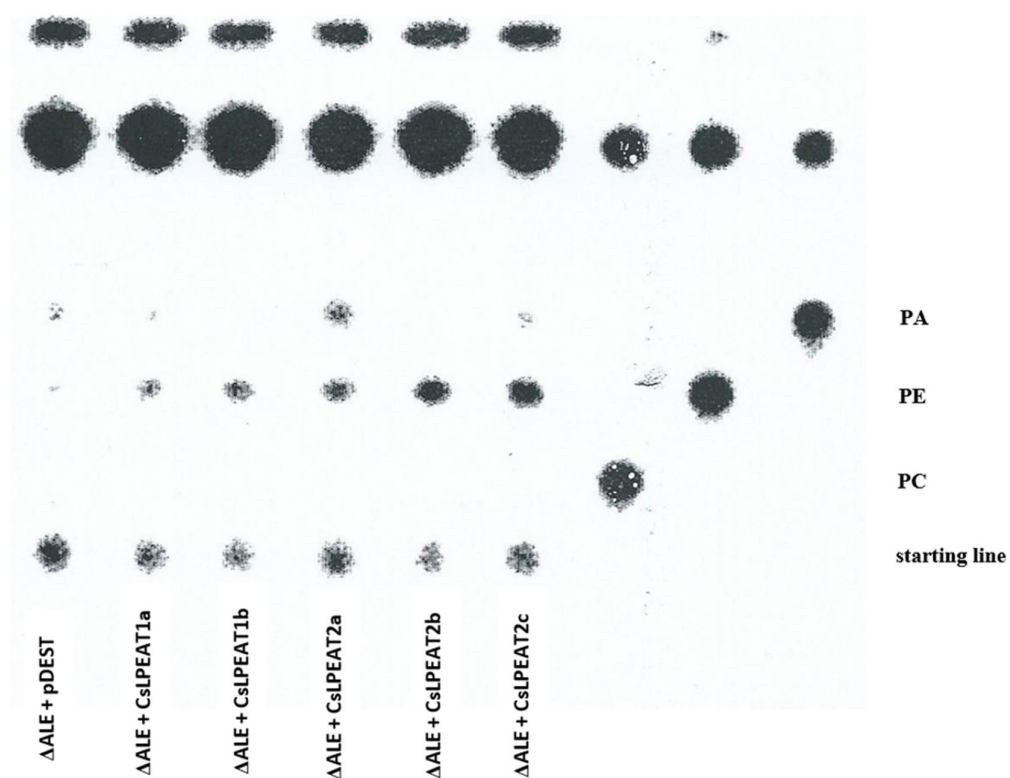
Supplemental Figure S2. Activity of acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) present in microsomal fraction of *Camelina sativa* tissues towards five various acyl-CoAs at different temperatures. (A – *in vivo* leaves; B – *in vitro* roots). Mean values and SD are presented (data from at least three independent assays).

<i>At</i> LPEAT1	M E S E L K D L N S	N S N P P S S K E E	R P L L K S E S D L	A A A I E E L D K K	F A P Y A R T D L Y	G T M G L G P F P M	T E N I K L A V A L	V T L V P L R F L L	S M S L L L L Y Y I	90
<i>Cs</i> LPEAT1a.1	M E S E L K D M N S	N - - P P S S K E E	R P L L K S E S D L	A A A I E E L D K K	F A P Y A R T D L Y	G T M G L G P F P M	T E K I K L A V A M	V T L V P L R F L L	S M S L L L L Y Y I	88
<i>Cs</i> LPEAT1a.2	M E S E L K D M N S	N - - P P S S E E E	R P L L K S E S D L	A A A I E E L D K K	F A P Y A R T D L Y	G T M G L G P F P M	T E K I K L A V A M	V T L V P L R F L L	S M S L L L L Y Y I	88
<i>Cs</i> LPEAT1b	M E S E L K D M N S	N - - P P S S K E E	R P L L K S E S D L	A A A I E E L D K K	F A P Y A R T D L Y	G T M G L G P F P M	T E K I K L A V A M	V T L V P L R F L L	S M S L L L L Y Y I	88
<i>Cs</i> LPEAT1c	M E S E L K D M N S	N - - P P S S K E E	R P L L K S E S D L	A A A I E E L D K K	F A P Y A R T D L Y	G T M G L G P F P M	T E K I K L A V A M	V T L V P L R F L L	S M S L L L L Y Y I	88
<i>At</i> LPEAT1	I C R V F T L F S A	P Y R G P E E E E E	E G G V V F Q E D Y	A H M E G W K R T V	I V R S G R F L S R	V L L F V F G F Y W	I H E S C P D R D S	D M D S N P K T T S	T E I N Q K G E A A	180
<i>Cs</i> LPEAT1a.1	I C R L F T L F S A	P Y R G A E E E E E	E G G V V V Q E D Y	A H M R G W K R T V	I V S C G R F L S R	V L L F V F G F Y W	I H E T R L D R D S	L M D P N P K T T S	A E I S Q K G D A A	178
<i>Cs</i> LPEAT1a.2	I C R L F T L F S A	P Y R G A E E E E E	E G A V V V Q E D Y	A H M R G W K R T V	I V S C G R F L S R	V L L F V F G F Y W	I H E T R L D R D S	L M D P N P K T T S	A E I S Q K G D A A	178
<i>Cs</i> LPEAT1b	I C R L F T L F S A	P Y R G A E E E E E	E G A V V V Q E D Y	A H M R G W K R T V	I V S C G R F L S R	V L L F V F G F Y W	I H E T R L D R D S	L M D P N P K T T S	A E I S Q K G D A A	178
<i>Cs</i> LPEAT1c	I C R L F T L F S A	P Y R G A E E E E E	E G G V V V Q E D Y	A H M R G W K R T V	I V S C G R F L S R	V L L F V F G F Y W	I H E T R L D R D S	L M D P N P K T T S	V E I S Q K G D A A	178
		▽	▽	▽	▽					
<i>At</i> LPEAT1	T E E P E R P G A I	V S N H V S Y L D I	L Y H M S A S F P S	F V A K R S V G K L	P L V G L I S K C L	G C V Y V Q R E A K	S P D F K G V S G T	V N E R V R E A H S	N K S A P T I M L F	270
<i>Cs</i> LPEAT1a.1	T E E P E R P G A I	V S N H V S Y L D I	L Y H M S A S F P S	F V A K R S V G K L	P L V G L I S K C L	G C V Y V Q R E A K	S P D F K G V S G T	V N E R V R E A H R	N K S A P T I M L F	268
<i>Cs</i> LPEAT1a.2	T E E P E R P G A I	V S N H V S Y L D I	L Y H M S A S C P S	F V A K R S V G K L	P L V G L I S K C L	G C V Y V Q R E A K	S P D F K G V S G T	V N E R V R E A H R	N K S A P T I M L F	268
<i>Cs</i> LPEAT1b	T E E P E R P G A I	V S N H V S Y L D I	L Y H M S A S F P S	F V A K R S V G K L	P L V G L I S K C L	G C V Y V Q R E A K	S P D F K G V S G T	V N E R V R E A H R	N K S A P T I M L F	268
<i>Cs</i> LPEAT1c	T E E P E R P G A I	V S N H V S Y L D I	L Y H M S A S F P S	F V A K R S V G K L	P L V G L I S K C L	G C V Y V Q R E A K	S P D F K G V S G T	V N E R V R E A H R	N I S A P T I M L F	268
	▽	▽								
<i>At</i> LPEAT1	P E G T T T N G D Y	L L T F K T G A F L	A G T P V L P V I L	K Y P Y E R F S V A	W D T I S G A R H I	L F L I C Q V V N H	L E V I R I P V Y Y	P S Q E E K D D P K	L Y A S N V R R I M	360
<i>Cs</i> LPEAT1a.1	P E G T T T N G D Y	L L T F K T G A F L	A G T P V L P V I L	K Y P Y E R F S V A	W D T I S G A R H I	L F L I C Q V V N H	L E V I R I P V Y Y	P S Q E E K D D P K	L Y A S N V R R I M	358
<i>Cs</i> LPEAT1a.2	P E G T T T N G D Y	L L T F K T G A F L	A G T P V L P V I L	K Y P Y E R F S V A	W D T I S G A R H I	L F L I C Q V V N H	L E V I R I P V Y Y	P S Q E E K D D P K	L Y A S N V R R I M	358
<i>Cs</i> LPEAT1b	P E G T T T N G D Y	L L T F K T G A F L	A G T P V L P V I L	K Y P Y E R F S V A	W D T I S G A R H I	L F L I C Q V V N H	L E V I R I P V Y Y	P S Q E E K D D P K	L Y A S N V R R I M	358
<i>Cs</i> LPEAT1c	P E G T T T N G D Y	L L T F K T G A F L	A G T P V L P V I L	K Y P Y E R F S V A	W D T I S G A R H I	L F L I C Q V V N H	L E V I R I P V Y Y	P S Q E E K D D P K	L Y A S N V R R I M	358
<i>At</i> LPEAT1	A T E G N L I L S E	I G I S D K R I Y H	A T I N G N L S Q T	R D F H Q K E E						
<i>Cs</i> LPEAT1a.1A	A T E G N L I L S D	I G I G D K R I Y H	A T I N G N L S Q T	R D F H Q K E E						
<i>Cs</i> LPEAT1a.2A	A T E G N L I L S D	I G I G D K R I Y H	A T I N G N L S Q T	R D F H Q K E E						
<i>Cs</i> LPEAT1b	A T E G N L I L S D	I G I G D K R I Y H	A T I N G N L S Q T	R D F H Q K E E						
<i>Cs</i> LPEAT1c	A T E G N L I L S D	I G I G D K R I Y H	A T I N G N L S Q T	R D F H Q K E E						

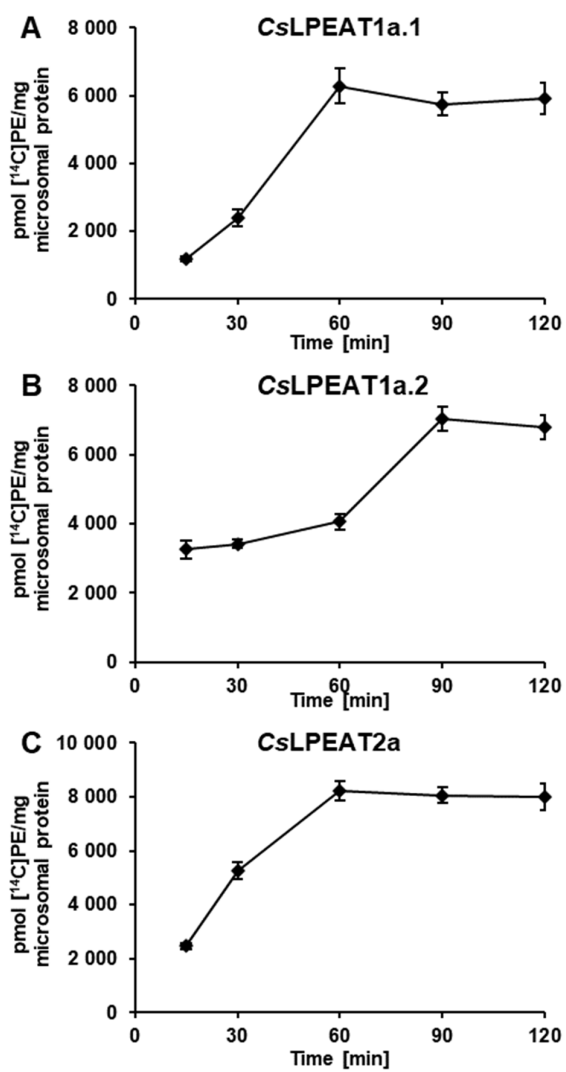
Supplementary Figure S3. Amino acid sequences' annotation of *Cs*LPEAT1 isoforms and *At*LPEAT1. LPE-binding site amino acids are denoted by golden arrows above.

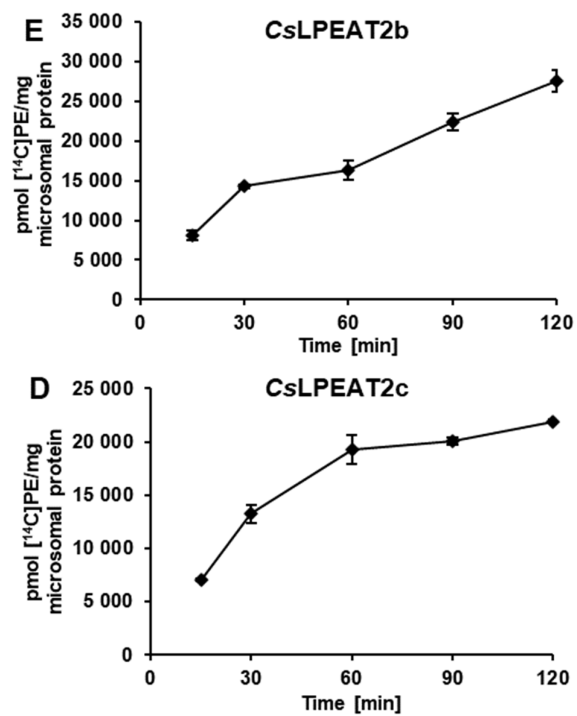
<i>At</i> LPEAT2	MADPDLSPL	IHHQSS-DQP	EVVISADDD	DD-----ESG	LNLLPAVVDP	RYSRGFEFDH	LN-PYGFLSE	SEPPVIGPTT	VDPFRNNTPG	83
<i>Cs</i> LPEAT2a	MADPDLSPL	IHHQPSVDQP	EVVISIGVDD	DDDDSSSTDG	LN-LPAAVGV	-YSRGFQFDH	RNNPYGFLSE	TEPPVIGPTT	VDPFRNNTPG	88
<i>Cs</i> LPEAT2b	MADPDLSPL	IHHQPSVDQP	EVVISIGVDD	DDDDSSSTDG	LNHLPAAVGV	-YSRGFQFDH	RNNPYGFLSE	TEPPVIGPTT	VDPFRNNTPG	84
<i>Cs</i> LPEAT2c	MADPDLSPL	IHHQSS-DQP	EVVISIGVDD	DDSSSSSTDG	LN-LPSAVGV	-VPRGFQFDH	CNNPYGFLSE	TEPPVIGPTT	VDPFRNNTPG	88
<i>At</i> LPEAT2	VSGLYEAKI	VICLPIALVR	LVLFAASTAV	GYLATKIALA	GWKDKENPMP	LWRCRIMWIT	RICTRCILFS	FGYQWIRRK	KPARREAP	173
<i>Cs</i> LPEAT2a	VFGLYEAKI	VICLPIALVR	LVLFGASTAV	GYLATKIALA	GWKDKENPMP	LWRCRIMWIT	RICTRCILFS	FGYQWIRRK	KPARREAP	178
<i>Cs</i> LPEAT2b	VFGLYEAKI	VICLPIALVR	LVLFGASTAV	GYLATKIALA	GWKDKENPMP	LWRCRIMWIT	RICTRCILFS	FGYQWIRRK	KPARREAP	174
<i>Cs</i> LPEAT2c	VFGLYEAKI	VICLPIALVR	LVLFGASTAV	GYLATKIALA	GWKDKENPMP	LWRCRIMWIT	RICTRCILFS	FGYQWIRRK	KPARREAP	178
<i>At</i> LPEAT2	VVSNHVSYLE	PIFYFYLESP	TIVASESHDS	LPFVGTTIRA	MQVIYVNRFS	QTSRKNAVHE	IKRKASCDRE	PRLLLFPEGT	TTNGKVLISF	263
<i>Cs</i> LPEAT2a	VVSNHVSYLE	PIFYFYLESP	TIVASESHDS	LPFVGTTIRA	MQVIYVNRFS	QTSRKNAVHE	IKRKASCDRE	PRLLLFPEGT	TTNGKVLISF	268
<i>Cs</i> LPEAT2b	VVSNHVSYLE	PIFYFYLESP	TIVASESHDS	LPFVGTTIRA	MQVIYVNRFS	QTSRKNAVHE	IKRKASCDRE	PRLLLFPEGT	TTNGKVLISF	264
<i>Cs</i> LPEAT2c	VVSNHVSYLE	PIFYFYLESP	TIVASESHDS	LPFVGTTIRA	MQVIYVNRFS	QTSRKNAVHE	IKRKASCDRE	PRLLLFPEGT	TTNGKVLISF	268
<i>At</i> LPEAT2	QLGAFIPGY	IQPVVRYPH	VHFDQSWGNI	SLTLMFRMF	TOFHNFEVE	YLPVIYPSEK	QKQNAVRLSQ	KTSHAATSE	NVVQTSHSFA	353
<i>Cs</i> LPEAT2a	QLGAFIPGY	IQPVVRYPH	VHFDQSWGNI	SLVLMFRMF	TOFHNFEVE	YLPVIYPSEK	QKQNAVRLSQ	KTSQAIAASL	NVVQTSHSYA	358
<i>Cs</i> LPEAT2b	QLGAFIPGY	IQPVVRYPH	VHFDQSWGNI	SLVLMFRMF	TOFHNFEVE	YLPVIYPSEK	QKQNAVRLSQ	KTSQAIAASL	NVVQTSHSYA	354
<i>Cs</i> LPEAT2c	QLGAFIPGY	IQPVVRYPH	VHFDQSWGNI	SLVLMFRMF	TOFHNFEVE	YLPVIYPSEK	QKQNAVRLSQ	KTSHAIAASL	NVVQTSHSYA	358
<i>At</i> LPEAT2	DLMLLNKATE	LKLENPSNYM	VEMARYESLF	HVSSLEATRE	LDTEVSMNPD	SSGRVRLHDF	LRGLKLKPCP	LSKRIFEEFD	VEKVGSITFK	443
<i>Cs</i> LPEAT2a	DLMLLNKATE	LKLENPSNYM	VEMAKYESLF	HISSELEATRE	LDTEVSMNPD	SSGRVRLHDF	LWGLRLKPCCT	LSKRIFEEFD	VEKVGSITFK	448
<i>Cs</i> LPEAT2b	DLMLLNKATE	LKLENPSNYM	VEMAKYESLF	HISSELEATRE	LDTEVSMNPD	SSGRVRLHDF	LWGLRLKPCCT	LSKRIFEEFD	VEKVGSITFK	444
<i>Cs</i> LPEAT2c	DLMLLNKATE	LKLENPSNYM	VEMAKYESLF	HISSELEATRE	LDTEVSMNPD	SSGRVRLHDF	LWGLRLKPCCT	LSKRIFEEFD	VEKVGSITFK	448
<i>At</i> LPEAT2	QFLFASGHVL	TQPLFQQTCE	LAFSHCDADG	DGYTTQELG	EALKNTIPNL	NKDEIRGMVH	LLDDDDQDERI	SQNDLLSCLR	RNPLLAFIFA	533
<i>Cs</i> LPEAT2a	QFLFASGHVL	TQPLFQQTCE	LVFESHCDRDG	DGYTTQELG	DAIKNTIPNL	NKADIQGTYN	LLDDDDQDERI	SQNDLLTCLR	RNPLLAFIFA	538
<i>Cs</i> LPEAT2b	QFLFASGHVL	TQPLFQQTCE	LVFESHCDRDG	DGYTTQELG	DAIKNTIPNL	NKAEIQGMVY	LLDDDDQDERI	SQNDLLTCLR	RNPLLAFIFA	534
<i>Cs</i> LPEAT2c	QFLFASGHVL	TQPLFQQTCE	LVFESHCDRDG	DGYTTQELG	DAIKNTIPNL	NKAEIQGMVY	LLDDDDQDERI	SQNDLLTCLR	RNPLLAFIFA	538
<i>At</i> LPEAT2	PDAPT									539
<i>Cs</i> LPEAT2a	PDAPT									544
<i>Cs</i> LPEAT2b	PDAPT									540
<i>Cs</i> LPEAT2c	PDAPT									544

Supplementary Figure S4. Amino acid sequences' annotation of *Cs*LPEAT2 isoforms and *At*LPEAT2. LPE-binding site amino acids are denoted by golden arrows above. Ca²⁺-binding protein domain is framed by a silver line.

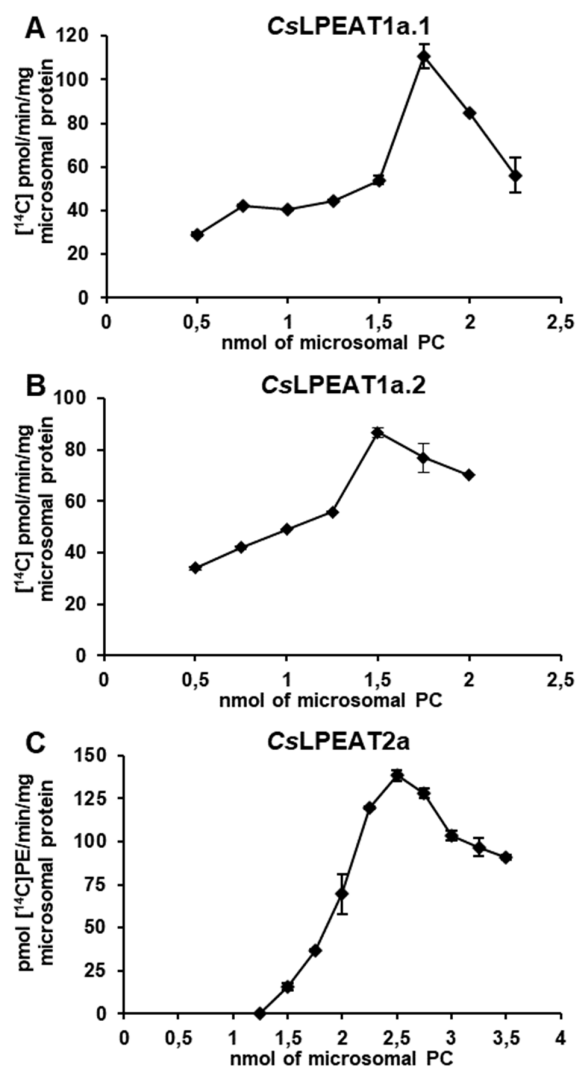


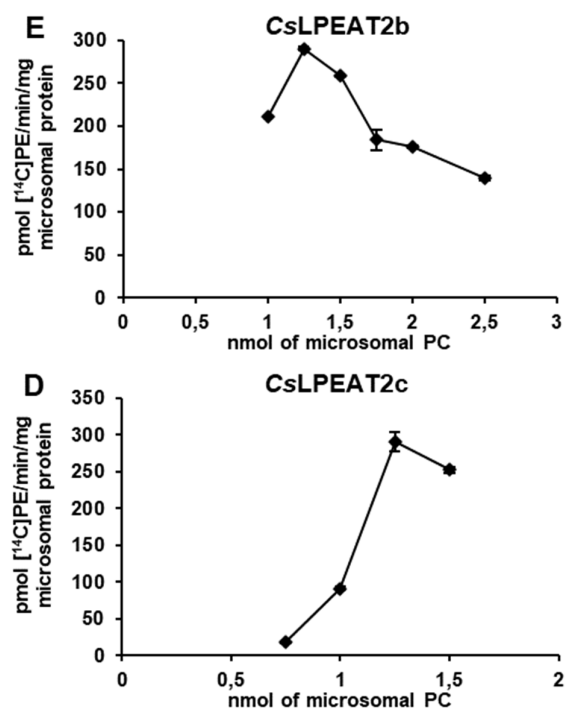
Supplemental Figure S5. Complementation of PE synthesis *via* LPEAT pathway in *S. cerevisiae* transformants overexpressed with cloned *CsLPEAT1a.1*, *CsLPEAT1a.2*, *CsLPEAT2a*, *CsLPEAT2b* and *CsLPEAT2c* isoforms. Figure presents the results of radioisotope assay analysed by Instant Imager, Packard Instrument Co. Assay was done with addition of 18:1-LPE and 18:1-CoA. The first line presents negative control (ΔALE strain) incapable to synthesize PE *via* LPEAT pathway. Radiolabeled standards of each phospholipids were obtained via enzymatic reaction conducted with wild strain BY4742.





Supplemental Figure S6. Effects of reaction time on the activity of various variants of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs). Mean values and SD are presented (data from at least three independent assays).





Supplemental Figure S7. Effects of amount of microsomal fraction on the activity of various isoforms of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs). Mean values and SD are presented (data from at least three independent assays).