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Supplementary Material

Involvement of OpsLTP1 from *Opuntia streptacantha* in abiotic stress adaptation and lipid metabolism

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Fig. S1

A

1 GGGGCATCAAAACAACTTCAAACCCCTACCACTCTTTATATATACGTTCTTCCCGTCTAGCTGTAAAACCAACCAGAATATTACCAACCC
M A S S A V V K L A C A V F L C M V V A A P Y A E A A I T
91 AGAATGGCTAGCTCAGCGGTTGTTAAGTTAGCTTGCGCTGTTTTCCTATGCATGGTTGTTGCTGCACCCTATGCAGAAGCGGCCATAACC
61 C G A V A Q N L A P C I T F L K A G G A P S A A C C A G V K
181 TGTGGCGCAGTGGCACAGAACCTCGCCCCATGCATAACGTTCTTGAAGGCTGGGGGTGCACCATCAGCTGCCTGTTGCGCCGGGGTTAAG
91 K L V S M A T T S A D R K T A C G C L K Q T A G T I P G L N
271 AAGCTTGTGAGCATGGCTACCA***CCTCGGCCGATCGGAAAAC***TGCTTGTGGGTGCCTCAAACAAA***CTGCTGGTACTATTCCAGGTC***TTAAC
121 Y G N A A A L P G K C G T S V P Y P I S P N T D C S K V N *
361 TACGGAAATGCTGCTGCTCTGCCTGGCAAATGTGGCACTAGTGTGCCTTACCCCATTAGCCCTAACACCGACTGCTCCAAGGTGAACTAG

451 ATGGATCGGATGACTATATGACAAGGGAGAGAACGCATGGAG**T**AAATAATGAAATGAGTCATGAGGGGACTACTTTATCCCCATCACTAT

541 CATATAGTTTCTCTCCATGAACTTGTGTACTCTTATGCTTGTTGTGTTTTCTGTTTTAGGATAATTATCTCTGTACTGTAATTTCTTCTT

631 ATGAAGATGGTATTACATATTATTAAGAAAAAAAAAAAAAAAAAAAAAAAAAA

B

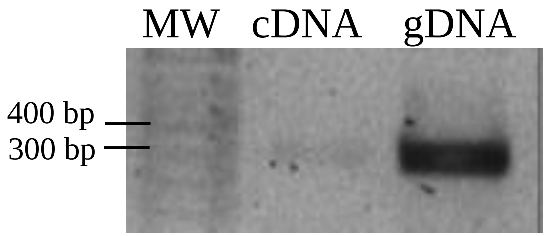


Fig. S1. Sequence analysis and genomic characterization of *OpsLTP1* gene. A) ORF and predicted amino acid sequence of *Opuntia streptacantha* lipid transfer protein 1 (OpsLTP1). The qRT-PCR primers are shown in bold, italics, and underlined letters. Polyadenylation signals are enclosed in rectangles and stop codon is indicated with an asterisk. **B)** ORF amplification of *OpsLTP1* using cDNA and genomic DNA isolated from *O. streptacantha* cladodes. MW, molecular weight; gDNA, genomic DNA.

Fig. S2

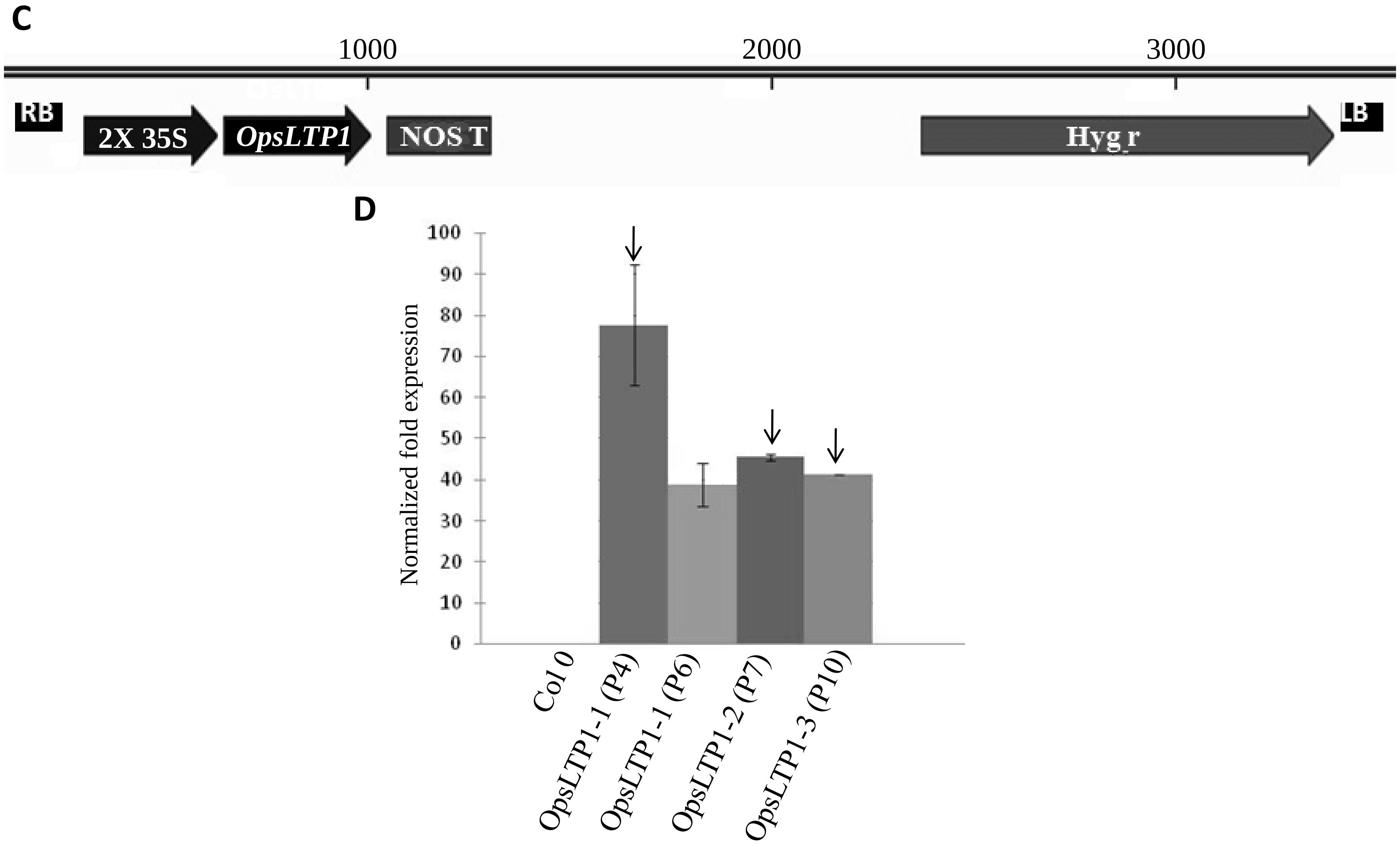
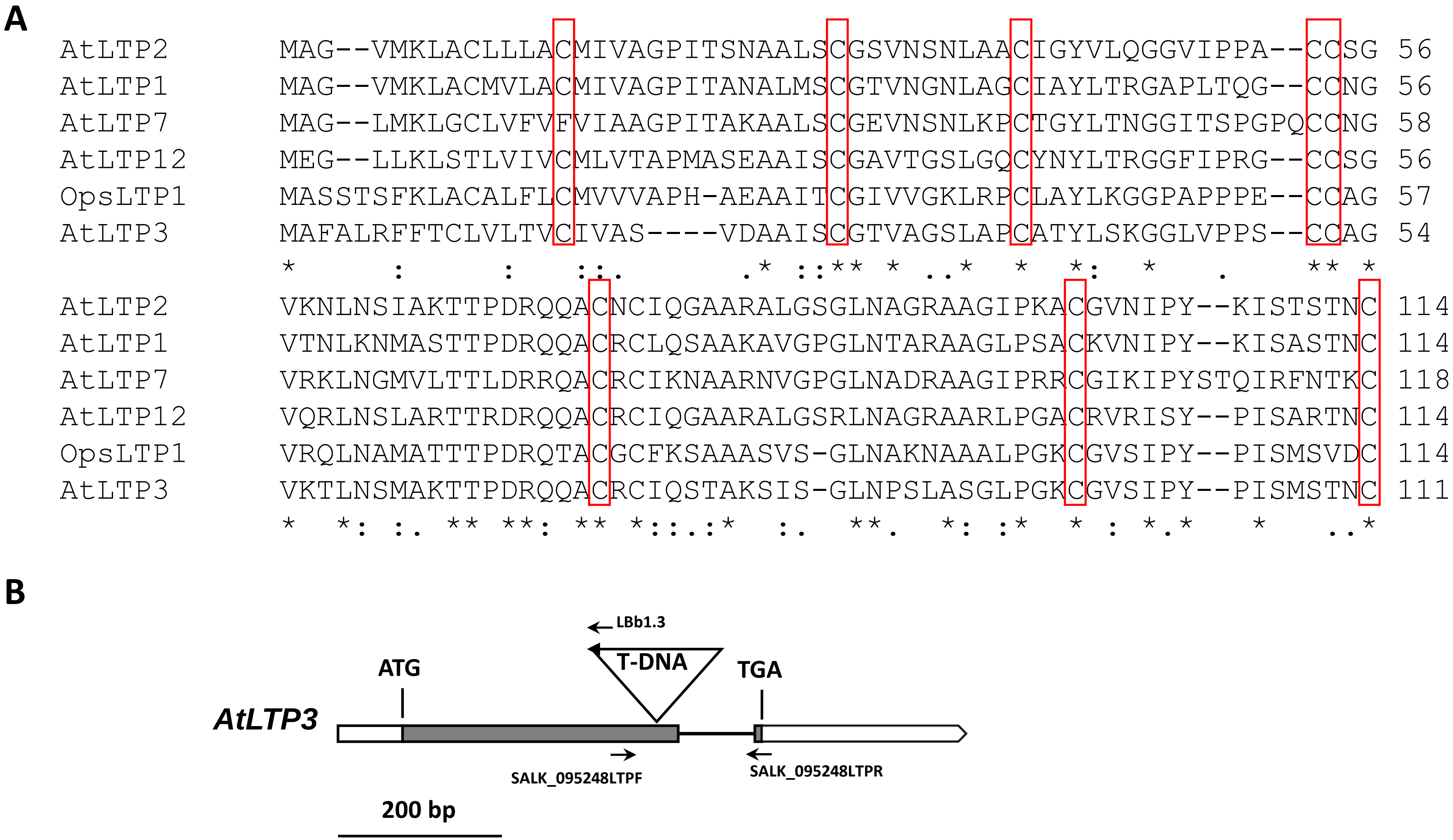


Fig. S2. Generation of *OpsLTP1* overexpressing *Arabidopsis* plants. A) Schematic diagram of *OpsLTP1* cloning in pMDC32 binary vector used to generate *OpsLTP1* overexpressing transgenic plants. **B)** Relative quantification of *OpsLTP1* in overexpressing transgenic lines. Col-0 was used as a control. Expression data was normalized with the housekeeping gen *AtEF1*. Transgenic lines selected for further analysis are indicated by arrows; *n*=3.

Fig. S3



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AtLTP2      MAG--VMKLACLLLACMIVAGPITSNAALSCGSVNSNLAACIGYVLQGGVIPPA--CCSG 56
AtLTP1      MAG--VMKLACMVLACMIVAGPITANALMSCGTVNGNLAGCIAYLTRGAPLTQG--CCNG 56
AtLTP7      MAG--LMKLGCLVFVFVIAAGPITAKAALSCGEVNSNLKPCTGYLTNGGITSPGPQCCNG 58
AtLTP12     MEG--LLKLSTLVIVCMLVTAPMASEAAISCGAVTGSLGQCYNYLTRGGFIPRG--CCSG 56
OpsLTP1     MASSTSFKLACALFLCMVVVAPH-AEAAITCGIVVGKLRPCLAYLKGGPAPPPE--CCAG 57
AtLTP3      MAFALRFFTCLVLTVCIVAS----VDAAISCGTVAGSLAPCATYLSKGGLVPPS--CCAG 54
            *     :     :   ::.      .* ::** * ..*  *  *:  *   .    ** *

AtLTP2      VKNLNSIAKTTPDRQQACNCIQGAARALGSGLNAGRAAGIPKACGVNIPY--KISTSTNC 114
AtLTP1      VTNLKNMASTTPDRQQACRCLQSAAKAVGPGLNTARAAGLPSACKVNIPY--KISASTNC 114
AtLTP7      VRKLNGMVLTTLDRRQACRCIKNAARNVGPGLNADRAAGIPRRCGIKIPYSTQIRFNTKC 118
AtLTP12     VQRLNSLARTTRDRQQACRCIQGAARALGSRLNAGRAARLPGACRVRISY--PISARTNC 114
OpsLTP1     VRQLNAMATTTPDRQTACGCFKSAAASVS-GLNAKNAAALPGKCGVSIPY--PISMSVDC 114
AtLTP3      VKTLNSMAKTTPDRQQACRCIQSTAKSIS-GLNPSLASGLPGKCGVSIPY--PISMSTNC 111
            *  *: :. ** **: ** *::.:*  :.  **.  *: :*  * : *.*   *   ..*
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Fig. S3

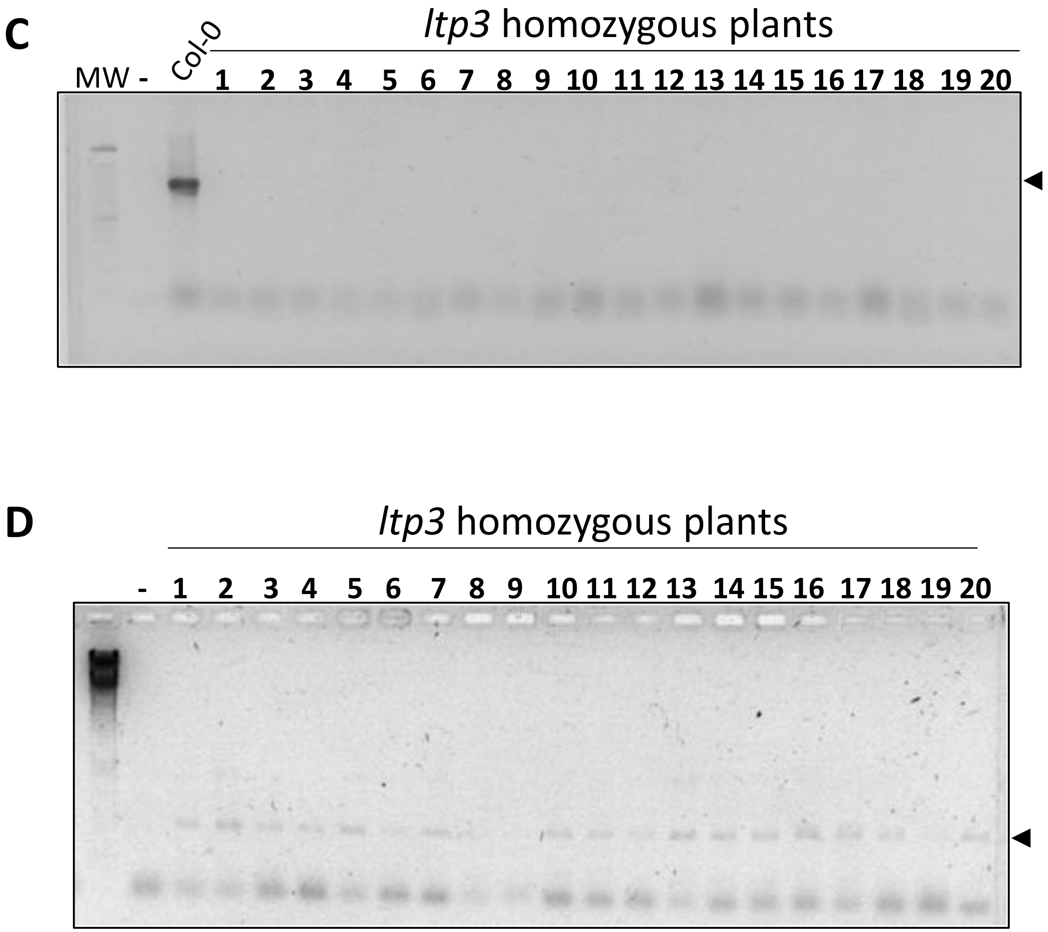

Fig. S3. Comparative analysis of OpsLTP1 and homolog *Arabidopsis thaliana* LTPs and selection of *ltp3* homozygous mutants. A) Multiple sequence alignment. Red rectangles indicate the eight conserved cysteines. **B)** Schematic representation of the T-DNA insertion in *AtLTP3* gene from *ltp3* mutant plants. T-DNA insertion is represented by an inverted triangle and primers used for amplification are indicated by black arrows. The grey boxes represent exons and a line indicates the unique intron of *AtLTP3* gene. **C)** Amplification of a 1024 bp product lacking of the T-DNA insertion in the *AtLTP3* gene; thus, only Col-0 plants amplified the expected product. **D)** Amplification of a 500 bp product indicating the T-DNA insertion in *ltp3* mutant plants. MW, molecular weight; -, negative control.

Fig. S4

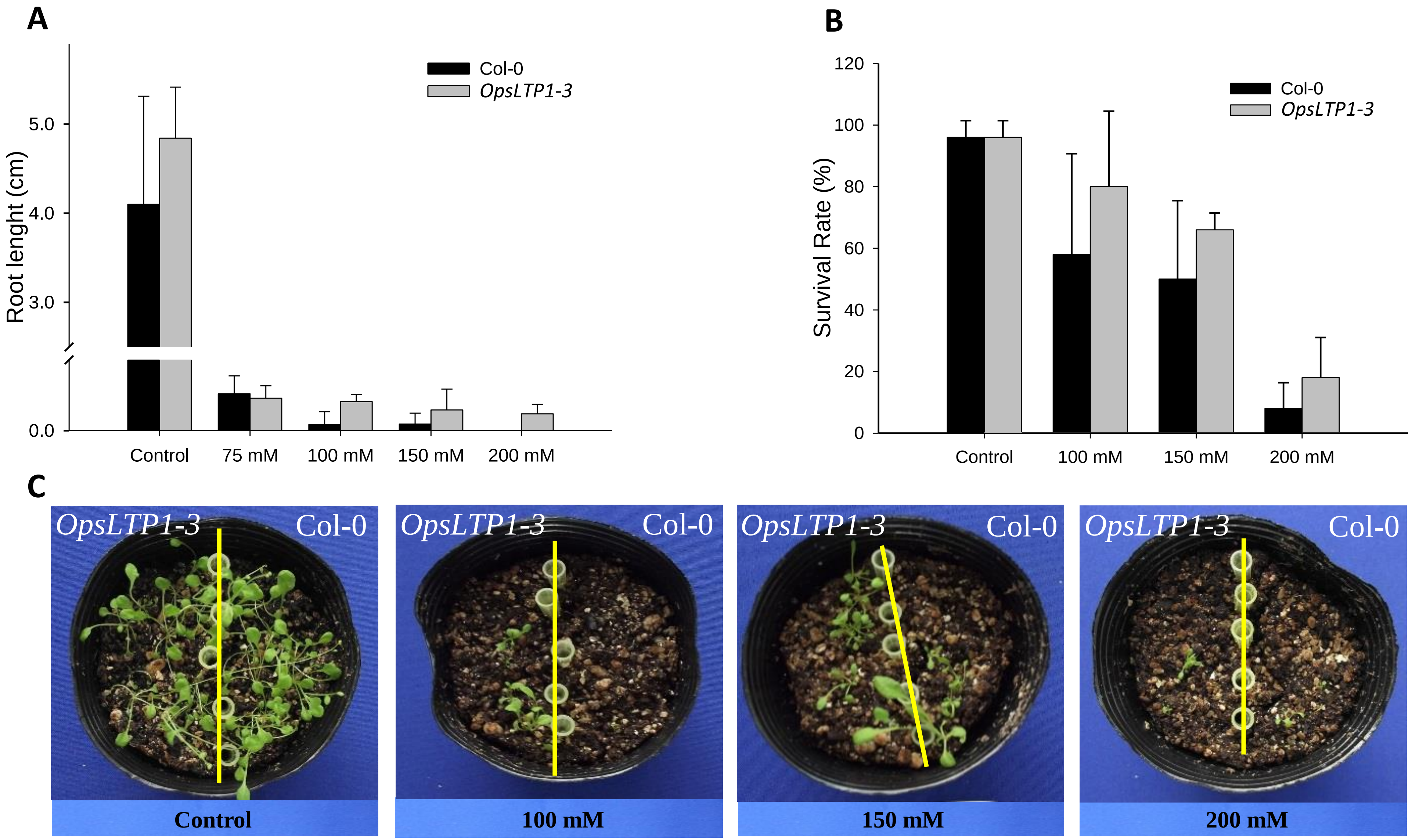

Fig. S4. Effects of salt treatments in *OpsLTP1-3* overexpressing line on germination and seedling recovery. A) Root length under different salt treatments on germination. **B)** Survival rate, and **C)** growth of *OpsLTP1* overexpressing seedlings after salt treatment recovery in soil pots.

Fig. S5

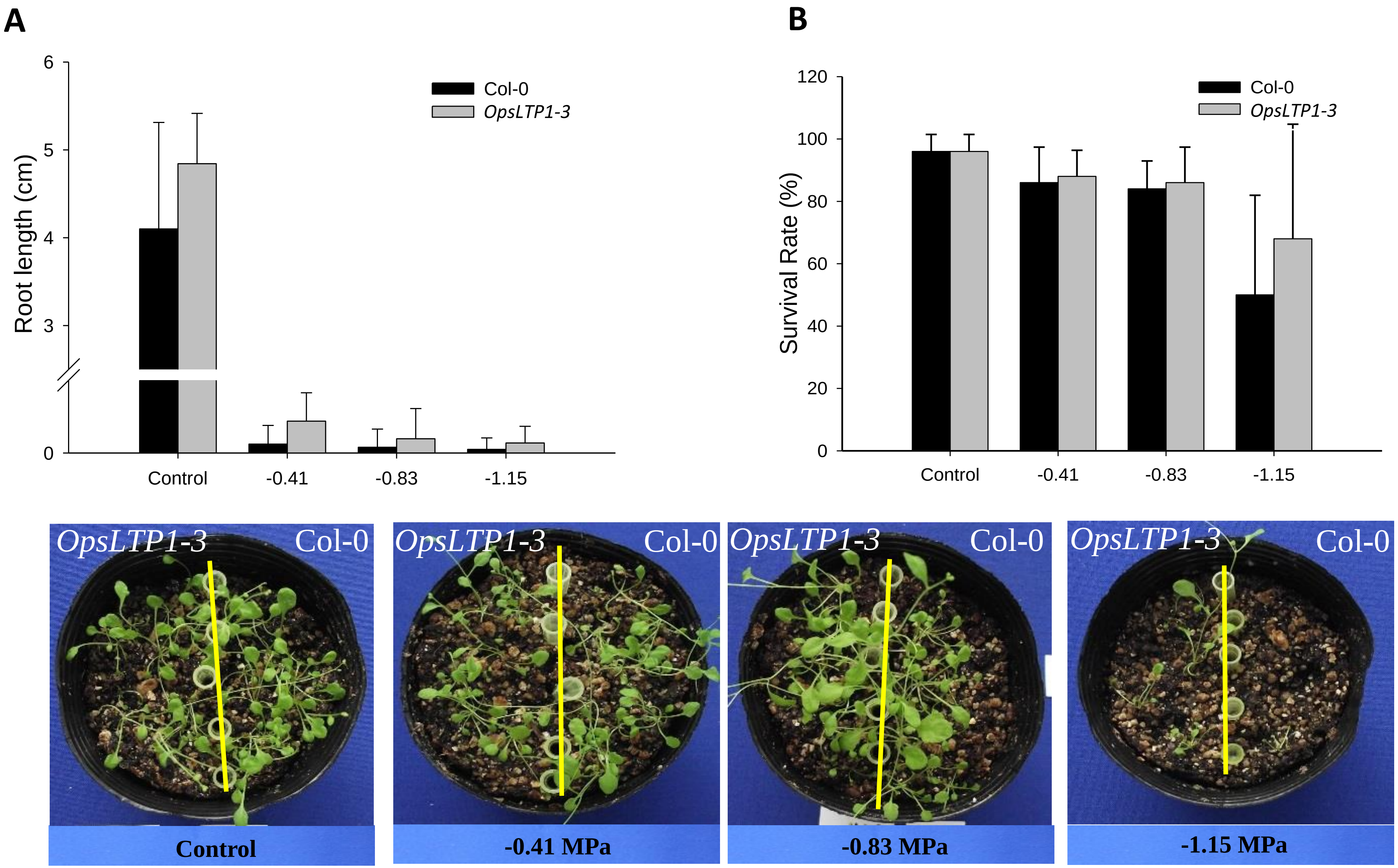

Fig. S5. Effects of osmotic treatments in *OpsLTP1-3* overexpressing line on germination and seedling recovery. A) Root length of seedlings germinated under different osmotic potentials over 15 days. **B)** Survival rate, and **C)** growth of *OpsLTP1* overexpressing seedlings after osmotic treatment recovery in soil pots.

Fig. S6

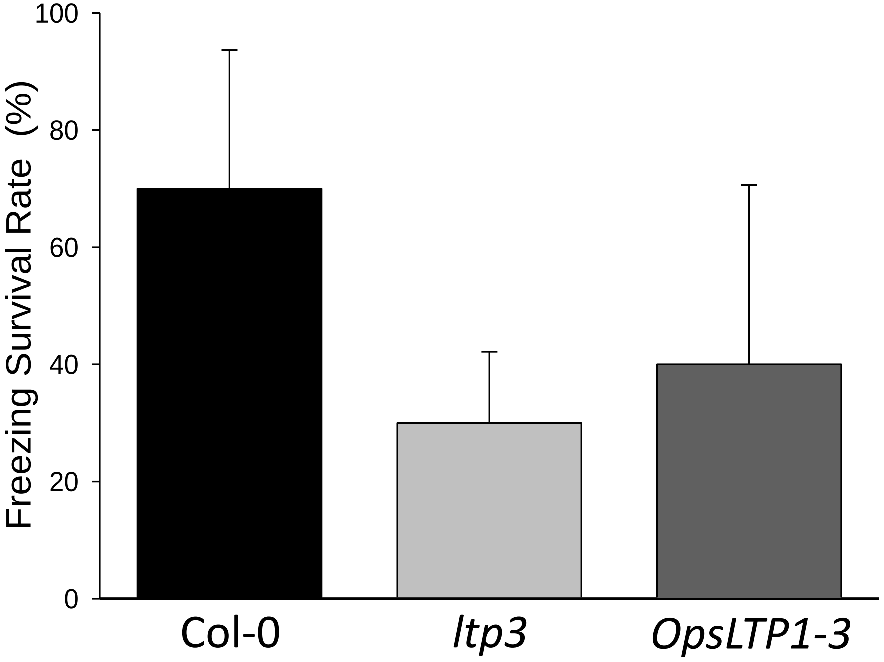

Fig. S6. Survival rates of *ltp3*, *OpsLTP1-3* and Col-0 seedlings under freezing stress. One-month seedlings were frozen 2 h at -20°C and recovered 10 days under optimal growth conditions.

Table S1. Primer sequences used in this study.

Primer name	Gen	Sequence	Use
OpsLTP-5	OpsLTP1	5´-CAGAATGGCTAGCTCAGCGGTT-3´	cDNA and genomic DNA amplification, cloning
OpsLTP-3	OpsLTP1	5’-CATGTAGTTCACCTTGGAGCAGTC-3’	cDNA and genomic DNA amplification, cloning
At5g60390EF1F	AtEF1	5´-CACCACTGGAGGTTTTGA GG-3´	qRT-PCR
At5g60390EF1R	AtEF1	5´-TGGAGTATTTGGGGGTGGT-3’	qRT-PCR
OpsLTPqPCR	OpsLTP1	5´-CCTCGGCCGATCGGAAAAC- 3´	qRT-PCR
OpsLTPqPCRrev	OpsLTP1	5´-GACCTGGAATAGTACCAGCAG-3´	qRT-PCR
SALK_095248LTPF	AtLTP3	5´-TCGATGCATAATCAAATCGTG-3´	ltp3 mutant verification
SALK_095248LTPR	AtLTP3	5´-GTTCAAACACAATGGCTTTCG-3´	ltp3 mutant verification
LBb1.3	T-DNA	5´-ATTTTGCCGATTTCGGAAC-3´	ltp3 mutant verification

Table S2. Identification of *OpsLTP1* orthologues in *Arabidopsis thaliana*.

Chromosome	Location	Locus	Gene	% identity
Chr 5	23928971-23929745 forward	At5g59320	AtLTP3	57
Chr 2	16128378-16129158 forward	At2g38530	AtLTP2	49
Chr 2	16130350..16131174 forward	At2g38540	AtLTP1	49
Chr 3	19135665..19136675 reverse	At3g51590	AtLTP12	47.2
Chr 2	6518843..6519360 forward	At2g15050	AtLTP7	43.6

Table S3. Fatty acid composition of triacylglycerols (storage neutral lipids) of wild-type Col-0, *ltp3* mutant *OpsLTP1* overexpressing *Arabidopsis* seeds. NFA, neutral fatty acids.

FAME	Col-0	ltp3	OpsLTP1	F	p
16:0	12.3±2.9^{a}	10.1±1.8^{a}	9.9±0.7^{a}	1.70	0.273
18:0	4.6±0.4^{a}	3.3±0.1^{b}	3.2±0.5^{b}	15.95	0.007
18:1ω9	14.9±0.9^{a}	16.7±0.1^{a}	16.5±1.1^{a}	3.92	0.095
18:1ω7	1.4±0.2^{a}	1.6±0.04^{a}	1.6±0.1^{a}	1.79	0.260
18:2ω6	26.9±0.04^{a}	29.0±0.9^{ab}	30.6±1.0^{b}	24.3	0.003
18:3ω3	16.4±1.3^{a}	15.9±0.1^{a}	14.4±0.8^{a}	4.35	0.080
20:0	2.1±0.1^{a}	1.8±0.2^{ab}	1.4±0.1^{b}	9.58	0.019
20:1ω9	14.7±1.0^{a}	14.8±1.8^{a}	15.7±0.8^{a}	0.93	0.455
20:1ω7	1.4±0.1^{a}	1.6±0.1^{a}	1.7±0.2^{a}	3.02	0.138
20:2ω6	1.6±0.4^{a}	1.5±0.2^{a}	1.5±0.1^{a}	0.18	0.838
20:3ω3	0.4±0.2^{a}	0.5±0.02^{a}	0.3±0.1^{a}	1.78	0.261
22:1ω9	1.5±0.3^{a}	1.6±0.4^{a}	1.6±0.2^{a}	0.12	0.886
NFA (% dw)	5.1±0.6^{a}	5.0±1.0^{a}	5.8±0.6^{a}	4.63	0.073

Table S4. Fatty acid composition of tilacoid membrane galactoglycerolipids of wild-type Col-0, *ltp3* mutant *OpsLTP1* overexpressing *Arabidopsis* seeds. GFA, Galactoglycerolipid fatty acids.

FAME	Col-0	ltp3	OpsLTP1	F	p
16:0	34.2±4.9^{a}	17.4±10.0^{a}	22.6±7.1^{a}	5.7	0.051
16:2 ω6	2.4±0.1^{a}	0.6±0.5^{b}	1.4±0.7^{b}	10.64	0.016
18:0	20.0±3.4^{a}	5.6±2.6^{b}	11.4±5.4^{b}	9.95	0.018
18:1ω9	6.8±1.4^{a}	13.3±2.8^{b}	10.8±2.8^{b}	7.10	0.035
18:1ω7	1.2±0.1^{a}	2.0±0.1^{b}	1.9±0.5^{b}	6.60	0.040
18:2ω6	16.1±2.4^{a}	32.1±1.0^{b}	25.7±5.6^{b}	13.66	0.009
18:3ω3	8.3±1.3^{a}	13.3±4.2^{a}	10.2±2.3^{a}	2.83	0.098
20:0	1.2±0.3^{a}	1.2±0.8^{a}	1.3±0.3^{a}	0.60	0.941
20:1ω9	5.9±1.6^{a}	9.2±5.2^{a}	9.0±3.1^{a}	1.41	0.329
20:1ω7	0.4±0.3^{a}	0.9±1.1^{a}	1.0±0.5^{a}	1.35	0.341
20:2ω6	0.4±0.1^{a}	0.1±0.7^{a}	0.8±0.5^{a}	1.85	0.251
20:3ω3	0±0^{a}	0.2±0.1^{b}	0.3±0.1^{b}	11.26	0.014
22:1ω9	0.3±0.1^{a}	0.8±0.7^{a}	0.8±0.6^{a}	1.18	0.382
GFA (% dw)	0.2±0.1^{a}	0.5±0.2^{a}	0.4±0.3^{a}	0.83	0.489

Table S5. Fatty acid composition of cellular membrane phospholipids of wild-type Col-0, *ltp3* mutant *OpsLTP1* overexpressing *Arabidopsis* seeds. PFA, phospholipid fatty acids.

FAME	Col-0	ltp3	OpsLTP1	F	p
16:0	37.1±3.7^{a}	48.3±0.1^{b}	32.8±8.8^{a}	8.42	0.037
16:2 ω6	5.8±1.8^{a}	7.0±0.1^{a}	5.0±3.5^{a}	0.67	0.561
18:0	20.5±8.4^{a}	27.5±1.6^{a}	11.2±1.8^{a}	4.76	0.088
18:1ω9	6.3±2.3^{a}	2.8±0.1^{a}	10.5±4.9^{a}	5.55	0.070
18:1ω7					
18:2ω6	20.1±5.4^{a}	10.2±0.3^{b}	30.6±0.6^{c}	18.11	0.010
18:3ω3	4.7±2.0^{a}	0.8±0.1^{a}	3.7±2.6^{a}	3.87	0.116
20:0	0.7±0.3^{a}	0.8±0.1^{a}	0.7±0.3^{a}	0.38	0.708
20:1ω9	3.1±1.9^{a}	0.4±0.3^{a}	3.6±2.1^{a}	1.22	0.387
20:1ω7					
20:2ω6					
20:3ω3					
22:1ω9					
PFA (% dw)	0.06±0.02^{a}	0.02±0.003^{a}	0.05±0.01^{a}	6.20	0.060