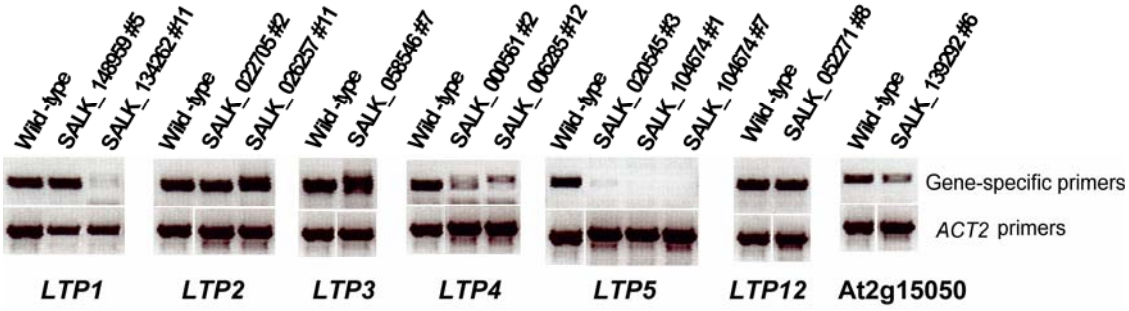
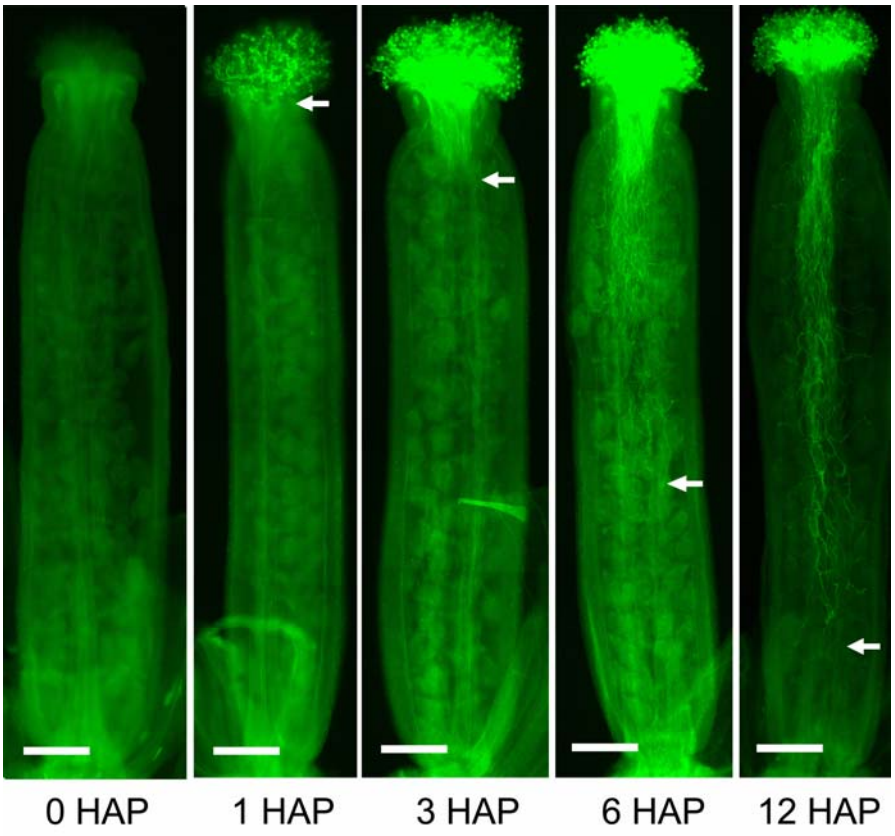
Supplemental Data. Chae et al. (2009). A gain-of-function mutation of *Arabidopsis* Lipid Transfer Protein 5 disturbs pollen tube tip growth and fertilization.

Supplemental Figure 1. A Genome-Wide Search for SCA-like *Arabidopsis* LTPs. *Arabidopsis* proteins, annotated as 'lipid transfer protein' or 'putative LTP', were obtained from the TAIR data bank (Altschul et al., 1990). An unrooted neighbor-joining tree of 107 *Arabidopsis* LTPs or putative LTPs, lily SCA1 (Q9SW93), and maize LTP (P19656) was generated to identify the SCA-like LTP cluster (Figure 1A). Arrows indicate the three other LTPs that were previously studied for their functions using a genetic approach: Defective in induced resistance 1 (DIR1) (Maldonado et al., 2002), azelaic acid induced 1 (AZI1) (Jung et al., 2009), and glycosylphosphatidylinositol-anchored LTP 1 (LTPG1) (DeBono et al., 2009).

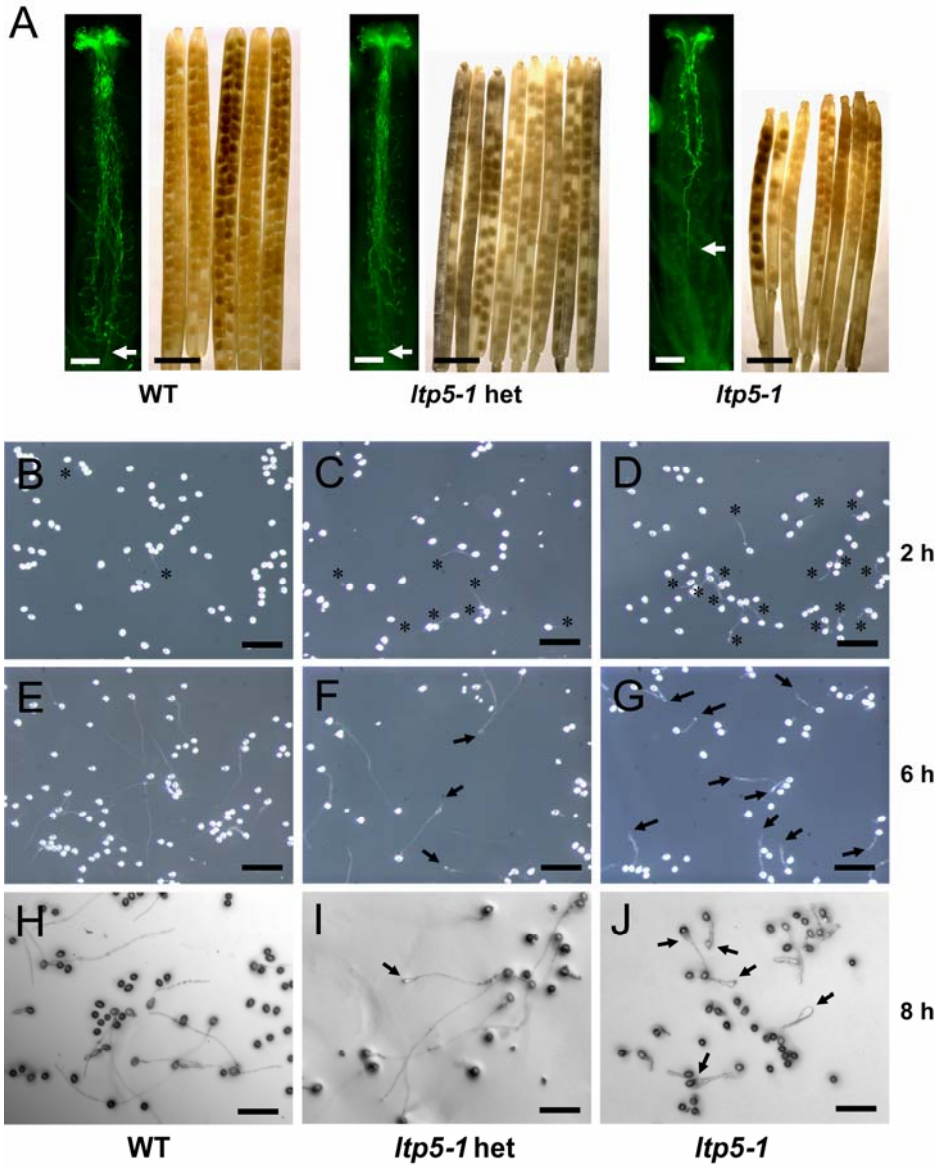


Supplemental Figure 2. Gene Expression Levels in T-DNA Insertion Lines of *SCA*-like *Arabidopsis LTP*s.

Homozygous T-DNA alleles for *SCA*-like *LTP*s, obtained by PCR-based genotyping (see Supplemental Table 1 online), were evaluated for gene expression by RT-PCR analysis (three replicates for alleles of *LTP5* and two for others) using the gene-specific primer sets (see Supplemental Table 3 online). PCRs were performed in 35 cycles.



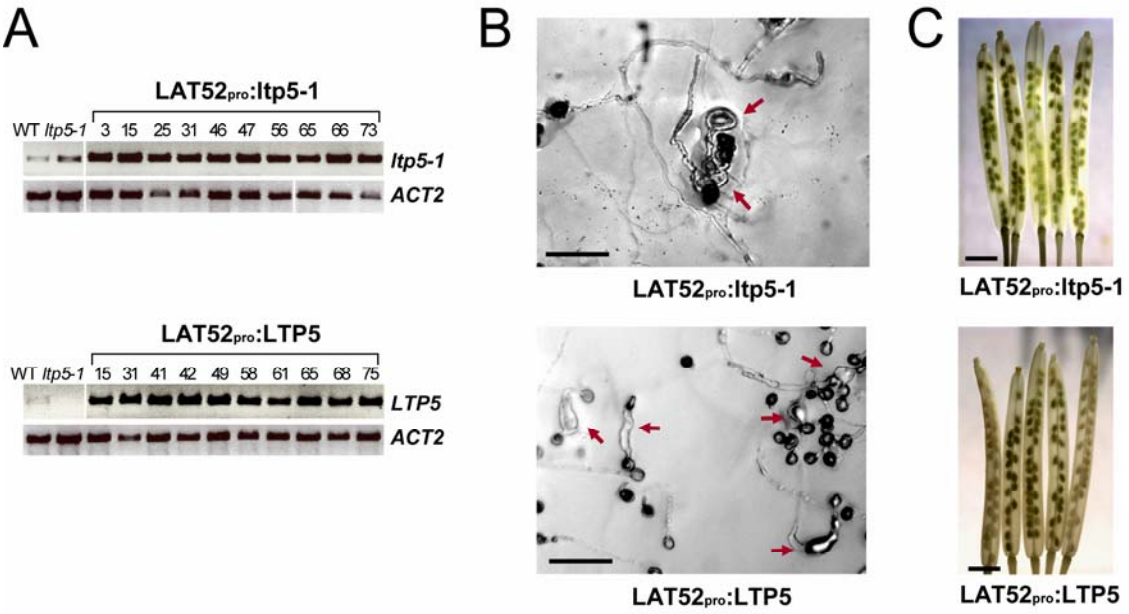
Supplemental Figure 3. *In Vivo Arabidopsis* Pollen Tube Growth.
About five wild-type flowers at stage 12 (Smyth et al., 1990) per each time course (0 to 12 hours) were emasculated a day before hand pollination. Pollen tube growth was visualized by aniline blue staining. Arrows indicate the growing pollen tube front. HAP, hours after pollination. Bar = 200μm.



Supplemental Figure 4. Heterozygous *ltp5-1* Showed Disturbed Pistil Function in Seed Formation and Abnormal *In Vitro* Pollen Tube Morphology.

(A) *In vivo* pollen tube growth and siliques. Flowers at stage 14 (Smyth et al., 1990) were stained with aniline blue to visualize *in vivo* pollen tube growth. Arrows indicate the pollen tube front. Bar = 200μm. Mature siliques were decolorized with 100% EtOH to examine seed set. Bar = 2mm.

(B to **J)** *In vitro* pollen tube growth assay. Pollen tube growth and tip morphology were examined at 2 **(B** to **D)**, 6 **(E** to **G)**, and 8 **(H** to **J)** hours after germination, respectively. Stars indicate precociously germinating pollen tubes. Arrows indicate the abnormally swollen pollen tube tips of *ltp5-1* het (~16%, *n* = 80) and *ltp5-1* (59%, *n* = 106). Bar = 100μm.

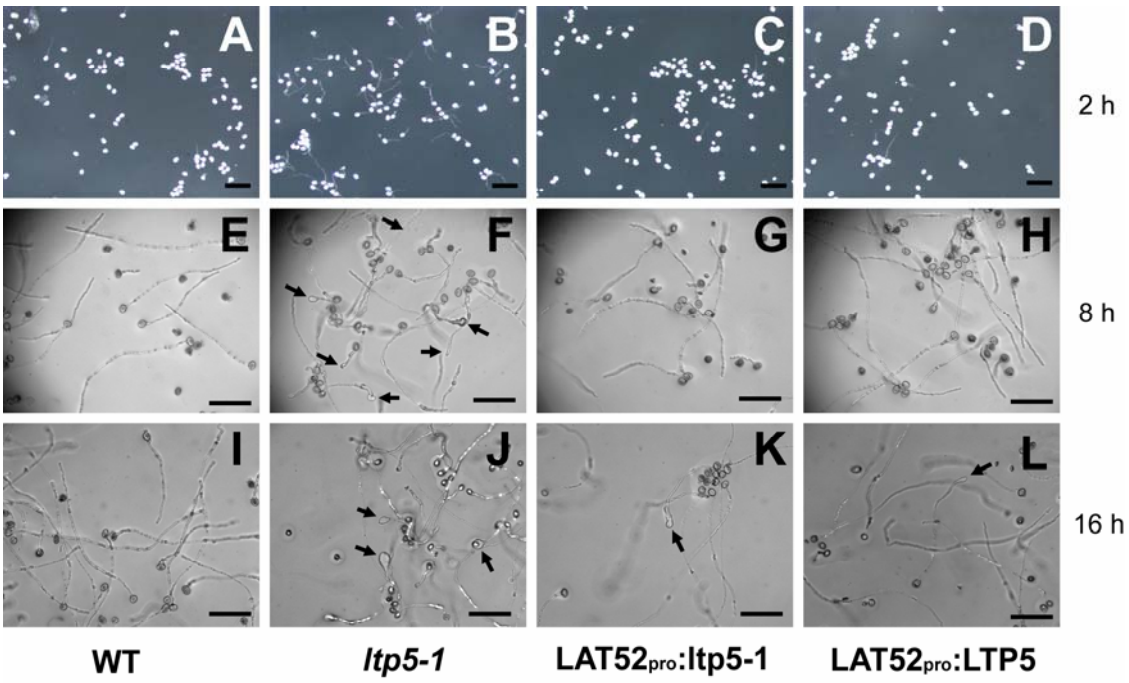


Supplemental Figure 5. Pollen-Targeted *ltp5-1* or *LTP5* Overexpression Lines at T1 Generation Resulted in Abnormal Pollen Tube Tip Morphology *In Vitro* and a Defect in Seed Formation.

(A) RT-PCR analysis in two replicates for the transgene expression in LAT52$_{pro}$:ltp5-1 or LAT52$_{pro}$:LTP5 plants. Total RNAs were purified from the inflorescence of ten randomly selected plants. The primer set, LTP5-5B and LTP5m-3K in Figure 1B, was used to amplify *ltp5-1* transcripts. The LTP5 gene-specific primer set, LTP5-5 and LTP5-3 in Figure 1B, was used to amplify *LTP5* transcripts. Wild-type and *ltp5-1* plants were used as gene expression controls. PCRs were performed in 30 cycles. *ACT2* levels were examined as the PCR control.

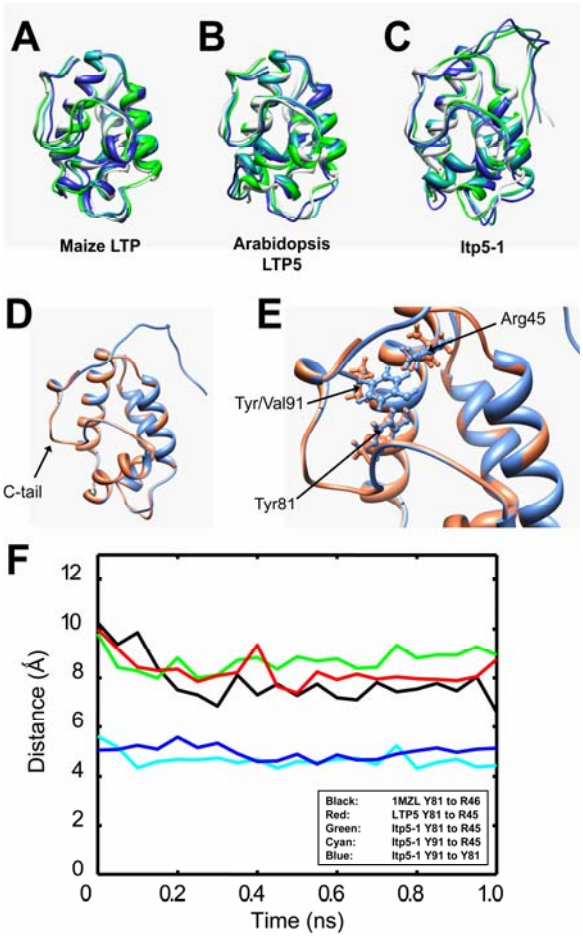
(B) *In vitro* pollen tube growth assay of LAT52$_{pro}$:ltp5-1 or LAT52$_{pro}$:LTP5 plants. Both LAT52$_{pro}$:ltp5-1 and LAT52$_{pro}$:LTP5 pollen displayed severely swollen pollen tube tips in 8 hours (Arrows). Bar = 100μm.

(C) Siliques of both LAT52$_{pro}$:ltp5-1 and LAT52$_{pro}$:LTP5 plants were small in size and were shown to harbor small numbers of seeds. Bar = 2mm.



Supplemental Figure 6. *In Vitro* Pollen Tube Growth of $LAT52_{pro}$:ltp5-1 or $LAT52_{pro}$:LTP5 at T3 Generation.

Pollen of transgenic plants, which contain the transgene in a homozygous and single copy, were germinated on solid medium for 2 **(A** to **D)**, 8 **(E** to **H)**, and 16 hours **(I** to **L)**, respectively. Neither $LAT52_{pro}$:ltp5-1 **(C)** nor $LAT52_{pro}$:LTP5 **(D)** showed precocious pollen germination at 2 hours, unlike *ltp5-1* **(B)**. In addition, they did not show abnormal tube tip morphology until 16 hours of the germination **(K** and **L)**. Arrows indicate the abnormally swollen pollen tube tips. Bar = 100μm.



Supplemental Figure 7. Comparison of Molecular Dynamics (MD) Snapshots for Maize LTP, *Arabidopsis* LTP5 and ltp5-1.
(A to **C)** Superposition of ribbon representations of the initial structures (0 ns – no MD) and three MD snapshots (50 ps, 500 ps, and 1 ns) for **(A)** maize LTP, **(B)** *Arabidopsis* LTP5, and **(C)** ltp5-1. The coloring scheme is as follows: white for 0 ns, green for 50 ps, blue-green for 500 ps, and blue for 1 ns.
(D and **E)** Comparison of LTP5 and ltp5-1 structures without MD simulation. **(D)** Superposition of backbone ribbon representations of LTP5 and ltp5-1. **(E)** Tyr91 in ltp5-1, replacing Val91 in LTP5, is inserted in between the two conserved residues, Tyr 81 and Arg 45. The coloring scheme is as follows: Pink for LTP5 and cyan for ltp5-1.
(F) Side chain distances during the 1 ns MD simulation of ltp5-1 between residues Tyr81 and Arg45 with residue Tyr91 in ltp5-1, replacing Val91 of LTP5. For comparison, side chain distances for the Tyr81-Arg45 (Arg46 in maize numbering) pairs of LTP5, ltp5-1 and maize LTP are also shown from their respective 1 ns MD trajectories. Distances in the 4 – 6 Å range indicate the presence of π-cation interaction for the pair Tyr91-Arg45 and π-staking interaction for the pair Tyr91-Tyr81. The remaining distances, involving Tyr81-Arg45/46, are outside possible interaction range, although there is a tendency for maize Tyr81 and Arg46 to get closer towards the end of the MD trajectory. The coloring scheme is black for maize LTP Tyr81-Arg46, red for LTP5 Tyr81-Arg45, green for ltp5-1 Tyr81-Arg45, cyan for ltp5-1 Tyr91-Arg45, and blue for ltp5-1 Tyr91-Tyr81. All distances were measured using the CZ atom for arginine and the centroid, or geometric center, of the six carbons of the benzene ring for tyrosine.

Supplemental Table 1. PCR-based Genotyping Analysis for SALK T-DNA Insertion Alleles of *SCA*-like *Arabidopsis LTP*s.

LTPs (Gene locus)	SALK Number: T-DNA insertion	Genotype (I.D.[#])
LTP1 (At2g38540)	SALK_148959: 1kb-Promotor	-/- (5,10,11)
LTP1 (At2g38540)	SALK_134262: 1kb-Promotor	-/- (8,11); -/+ (2,6,7)
LTP2 (At2g38530)	SALK_022705: 3'-UTR[¶]	-/- (1,2,3,4,5,8,10)
LTP2 (At2g38530)	SALK_026257: 1kb-Promotor	-/- (11)
LTP3 (At5g59320)	SALK_058546: 1kb-Promotor	-/- (7); -/+ (5)
LTP4 (At5g59310)	SALK_000561: 1kb-Promotor	-/- (1,2,6,7)
LTP4 (At5g59310)	SALK_006285: 1kb-Promotor	-/- (4, 12)
LTP5 (At3g51600)	SALK_020545: Intron	-/- (3, 7)
LTP5 (At3g51600)	SALK_104674: Intron	-/- (1, 7)
LTP (At2g15050)	SALK_139292: 5'-UTR[¶]	-/- (6)
LTP12 (At3g51590)	SALK_052271: Intron	-/- (8, 12); -/+ (4)

#, Identification number of a specific plant used in the experiment.

¶, Untranslated region

Supplemental Table 2. PCR primer sequences

PCR products	Forward primers	Reverse primers
PCR-based genotyping for T-DNA insertion alleles of *LTP5*		
1.5-kb for *LTP5*	674-5: TTGTACCAGCGAGGCCAGACAC	674-3: ACTGCATGTAGGGTCGATCGAC
1-kb for *T-DNA*	LBa1: TGGTTCACGTAGTGGGCCATCG	
RT-PCR analysis for *SCA*-like *Arabidopsis LTP*s (Gene-specific primers)		
LTP1	TAAGAGATCAATATGGCTGG	ATCATCTCACCGTTGCTAG
LTP2		AGTAGCTTCATTTGACCGTC
LTP3	CAAACACAATGGCTTTCG	AACGACGACGTAAGCTTC
LTP4		GATGTCGTTATTCCCCAC
LTP5	AAGATATGGAGGGACTCTTG	TTGATCACCTGACGGTGTTAC
LTP6	AAAGTAACATGAGATCTCTC	ATTTCTGCTTGTCTCACTG
LTP12	AATGGCGTTTACTCCGAAG	TCACTAGCCTCTTTCACAC
LTP (At2g15050)	CATTTACTGGAACTAAAGG	TTAACATATGTACGTGTTGC
RT-PCR analysis for the aberrant *LTP5* in *ltp5-1* plants		
Aberrant *LTP5*	LTP5-5B: GGATCCATGGAGGGACTCTTGAAG	LTP5m-3K: GGTACCTTAGGACCTCAAGTAAAAATG
LTP5 1st exon		LTP5C: GTTACAGTTGGTTCTGGC
PCR for plasmid constructs: LAT52$_{pro}$:LTP5, LAT52$_{pro}$:ltp5-1, and LTP5$_{pro}$:GUS		
LAT52 promoter	PLAT52-5R: GAATTCTATACCCCTTGGATAAGG	PLAT52-3B: GGATCCTTTAAATTGGAATTTTTTTTTTTCC
LTP5	LTP5-5B: GGATTCATGGAGGGACTCTTGAAG	LTP5-3Xb: TCTAGATCACCTGACGGTGTTACAG
ltp5-1		LTP5m-3Xb: TCTAGATTAGGACCTCAAGTAAAAATG
2-kb-upstream region of *LTP5*	PLTP5-5: GGGGACAAGTTTGTACAAAAAAGCAG GCTYYGTTTGTACATTATTCATATG	PLTP5-3: GGGGACCACTTTGTACAAGAAAGCTG GGTNATCTTAATTTTTTTTTTTTCTC

Supplemental Table 3. Transgenic Plants with Pollen-Specific Overexpression of *LTP5* or *ltp5-1* Gene in T1 Generation.

Phenotypes examined	No. of plants (%)	
	$LAT52_{pro}$:LTP5	**$LAT52_{pro}$:ltp5-1**
In vitro pollen growth assays	72	57
Abnormal pollen tube morphology	53 (73.6%)	49 (86%)
Examination of siliques	71	50
Small sized siliques (less than 1.3 cm)	20 (28.2%)	22 (44%)
Unfertilized eggs found in the silique	33 (46.5%)	34 (68%)

SUPPLEMENTAL DATA REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. **215,** 403-410.

DeBono, A., Yeats, T.H., Rose, J.K.C., Bird, D., Jetter, R., Kunst, L., and Samuelsa, L. (2009). Arabidopsis LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. Plant Cell **21,** 1230-1238.

Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., and Greenberg, J.T. (2009). Priming in systemic plant immunity. Science **324,** 89-91.

Maldonado, A.M., Doerner, P., Dixon, R.A., Lamb, C.J., and Cameron, R.K. (2002). A putative lipid transfer protein involved in systemic resistance signaling in Arabidopsis. Nature **419,** 399-403.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in Arabidopsis. Plant Cell **2,** 755-767.