Supporting Information

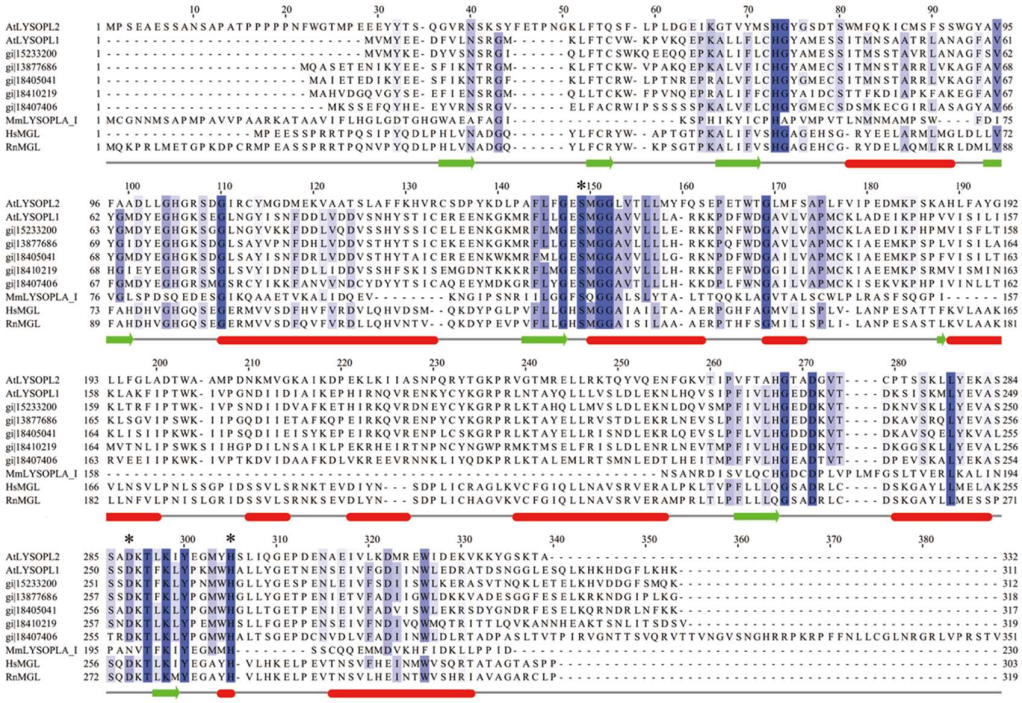

Figure S-1. Structure-based alignment using software Jalview version 1.6. Structure-based alignment of AtLYSOPL2, AtLYSOPL1, AtLYSOPL1-like proteins (gi15233200, gi13877686, gi18405041, gi18410219, gi18407406), *M. musculus* LysoPLA I, *H. sapiens* MGL, and *R. norvegicus* MGL. Identical amino acid residues are marked in dark blue, homologous residues in light blue, and gaps as dashes; α-helices (red lines) and β-strands (green lines) are indicated below the sequences. The conserved catalytic residues (Ser-147, Asp-268 and His-298) are marked with asterisks.

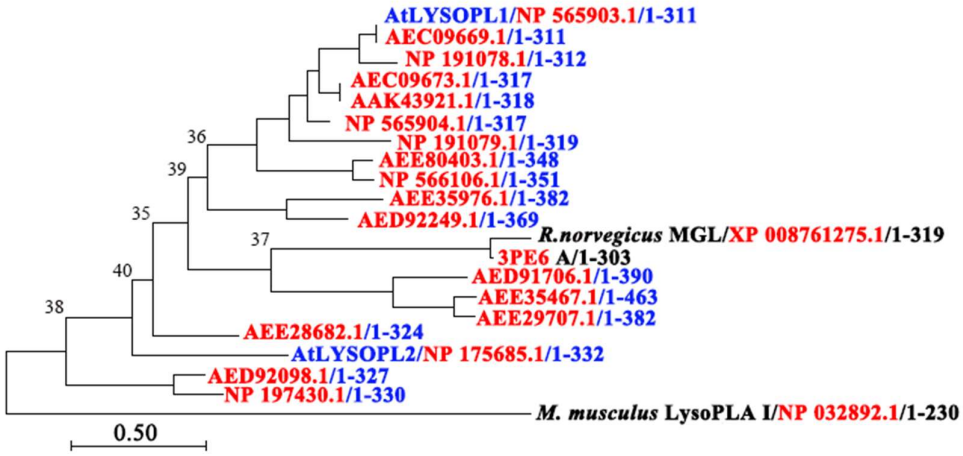

Figure S-2. Molecular phylogenetic analysis of LysoPL homologues. AtLYSOPL2, AtLYSOPL1, AtLYSOPL1-like proteins, *M. musculus* LysoPLA I, *H. sapiens* MGL, *R. norvegicus* MGL and *Arabidopsis* monoacylglycerol lipases (MAGL) were analyzed by the Maximum Likelihood method. The protein accession numbers are as follow: AtLYSOPL2 (NP_175685.1), AtLYSOPL1 (NP_565903.1), AtLYSOPL1-like proteins (NP_191078.1, AAK43921.1, NP_565904.1, NP_191079.1 and NP_566106.1), *M. musculus* LysoPLA I (NP_032892.1), *H. sapiens* MGL (3PE6_A), *R. norvegicus* MGL (XP_008761275.1) and *Arabidopsis* MAGL (AEE28682.1, AEE29707.1, AEE35467.1, AEE35976.1, AEC09669.1, NP_565904.1, AEC09673.1, NP_566106.1, NP_191078.1, NP_191079.1, AEE80403.1, AED91706.1, AED92098, AED92249.1 and NP_197430.1). Bootstrap values (> 35%) are recorded at the nodes. *Arabidopsis* proteins are denoted in blue, and protein accession numbers in red.

Reference

Jones, D. T., Taylor, W.R., and Thornton, J.M. (1992) The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* **8**, 275-82

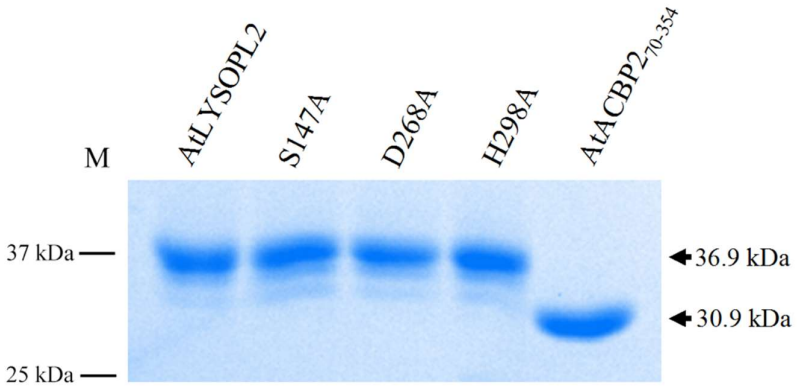

Figure S-3. SDS-polyacrylamide gel electrophoresis of the purified AtLYSOPL2 (wild type), AtLYSOPL2 mutant proteins (S147A, D268A, H298A) and AtACBP2$_{70-354}$. Eluted fractions of AtLYSOPL2 (wild type), AtLYSOPL2 (S147A), AtLYSOPL2 (D268A), AtLYSOPL2 (H298A) and AtACBP2$_{70-354}$ were collected. Arrows indicate homogeneity of the purified proteins. The predicted molecular weights of AtLYSOPL2 and AtACBP2$_{70-354}$ protein are 36.9 kDa and 30.9 kDa, respectively. M, protein marker.

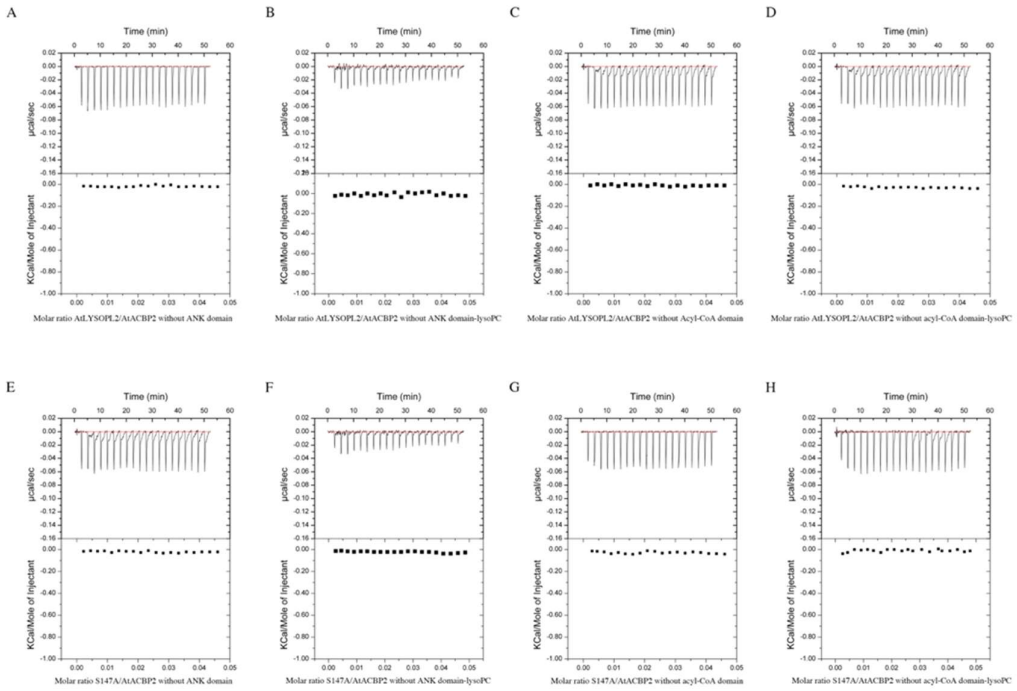

Figure S-4. ITC analysis of interactions of AtLYSOPL2 wild-type or its mutant (S147A) with AtACBP2$_{70-214}$ or AtACBP2$_{215-354}$ with/without lysoPC. (A-B) Wild-type AtLYSOPL2 with AtACBP2$_{70-214}$ alone (A) or complexed with lysoPC (B). AtACBP2$_{70-214}$ consists of the ACB domain lacking ankyrin repeats. (C-D) Wild-type AtLYSOPL2 with AtACBP2$_{215-354}$ alone (C) or complexed with lysoPC (D). (E-F) AtLYSOPL2 mutant (S147A) with AtACBP2$_{70-214}$ (E) or complexed with lysoPC (F). (G-H) AtLYSOPL2 mutant (S147A) with AtACBP2$_{215-354}$ alone (G) or complexed with lysoPC (H). AtACBP2$_{215-354}$ consists of the ANK domain lacking the ACB domain. Interactions were measured by titrating 20-30 µM AtLYSOPL2 wild-type or mutant proteins in the chamber with 300-400 µM AtACBP2 in the syringe. The values originate from Table 3.

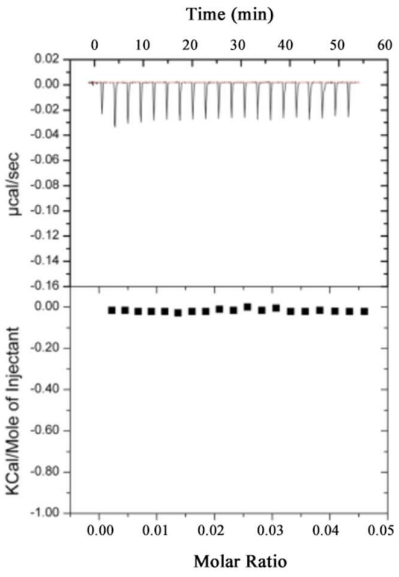

Figure S-5. ITC analysis of AtLYSOPL2 wild-type protein binding to lysoPC. ITC analysis of wild-type AtLYSOPL2 interactions with lysoPC. Interaction was measured by titrating 20-30 µM AtLYSOPL2 wild-type protein in the chamber with 600-800 µM lysoPC in the syringe. The values originate from Table 4.

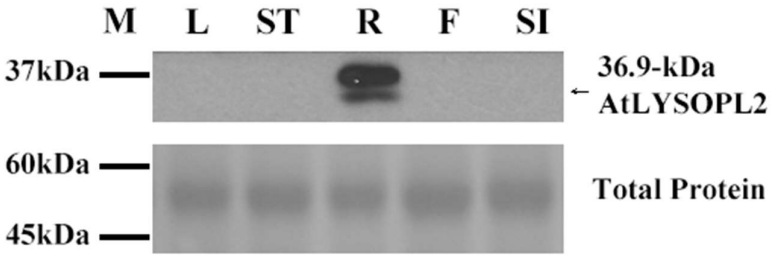

Figure S-6. Western blot analysis of AtLYSOPL2 expression in various tissues of *Arabidopsis*. Total proteins were extracted from leaves (L), stalks (ST), roots (R), flowers (F) and siliques (SI) of 5-week-old *Arabidopsis* and cross-reacted with anti-peptide antibodies against AtLYSOPL2. Bottom, gel stained with Coomassie Blue showing equal loading of protein amount in each lane. M, protein marker.

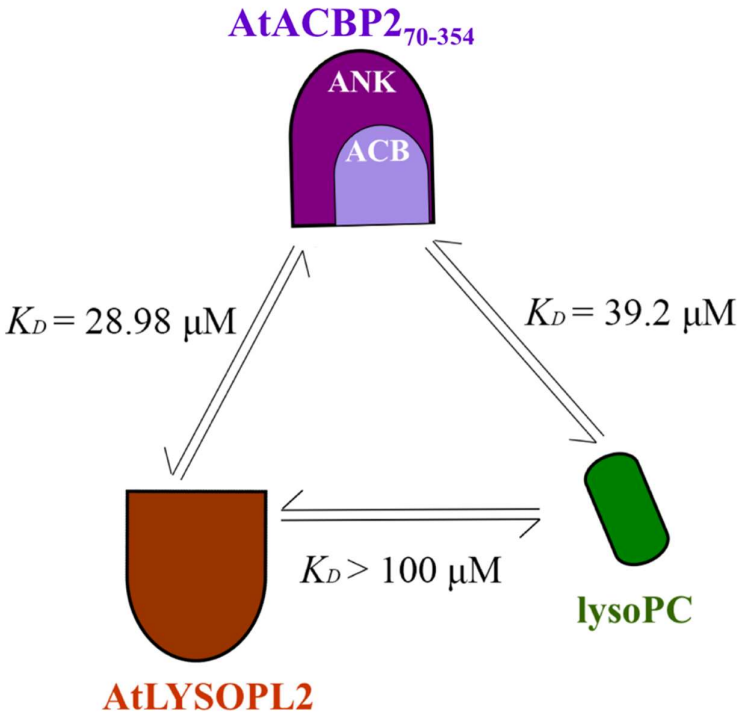

Figure S-7. Pictorial representation of the interactions between AtACBP2$_{70-354}$, AtLYSOPL2 and lysoPC. AtACBP2$_{70-354}$ binds to AtLYSOPL2 and lysoPC. LysoPC, green; ANK domain, purple; ACB domain, light purple; AtLYSOPL2, brown. The K_D values of AtACBP2$_{70-354}$ with AtLYSOPL2 and lysoPC were 28.98 µM and 39.2 µM, respectively. These values are displayed in Table 1, Table 2, Fig. 1 and Fig. 2. In comparison, the average K_D values for AtLYSOPL2 mutant proteins and lysoPC interaction were larger than 100 µM. These values are displayed in Table 4 and Fig. 6 .

Table S-1. Primers used in this study.

Description	Name (Orientation)	Sequences (5′ to 3′)
Primer pair to amplify the coding region of *AtLYSOPL2*	ML2040 (forward)	AAACC**CTGCAG**CATGCCGTCGGAAGC (*Pst*I)
	ML2041 (reverse)	AGT**CCATGG**TCAAGCGGTTTTAGATCC (*Nco*I)
Overlapping primer pair to introduce S1147A mutation	ML2298 (forward)	CCCAT*C*GCTTCACCAA
	ML2299 (reverse)	TTGGTGAAGC*G*ATGGG
Overlapping primer pair to introduce D268A mutation	ML2030 (forward)	CTCCAGCA*T*CC GCTGTC
	ML2031 (reverse)	GACAGCGG*A*TGCTGGAG
Overlapping primer pair to introduce H298A mutation	ML2032 (forward)	CAGCGA*GG*CATACATCC
	ML2033 (reverse)	GGATGTATG*CC*TCGCTG
Primer pair to amplify the coding region of *AtACBP2*	ML2037 (forward)	AA**ACCGGT**GGAGCGGATTCGCTTGTG (*Age*I)
	ML2039 (reverse)	AGT**AAGCTT**TTAGTCTGCCTGCTTTG (*Hind*III)
Primer pair to amplify the coding region of *AtACBP2*$_{70\text{-}214}$	ML3039 (forward)	AAACC**GGATCC**GCGGATTCGCTTGTG (*Bam*HI)
	ML3040 (reverse)	AGT**AAGCTT**TTAGGTTCCTCTTGAGTT (*Hind*III)
Primer pair to amplify the coding region of *AtACBP2*$_{215\text{-}354}$	ML3078 (forward)	ATAT**GAATTC**GGACCAGTTTTTAGCTC (*Eco*RI)
	ML2039 (reverse)	AGT**AAGCTT**TTAGTCTGCCTGCTTTG (*Hind*III)
Primers used to screen cloned plasmids	T7 (forward)	TAATACGACTCACTATAGGG

Restriction endonuclease sties (bracketed after each sequence) are bolded. Codons for the substituted amino acids are underlined with the altered nucleotides shown in italics.