

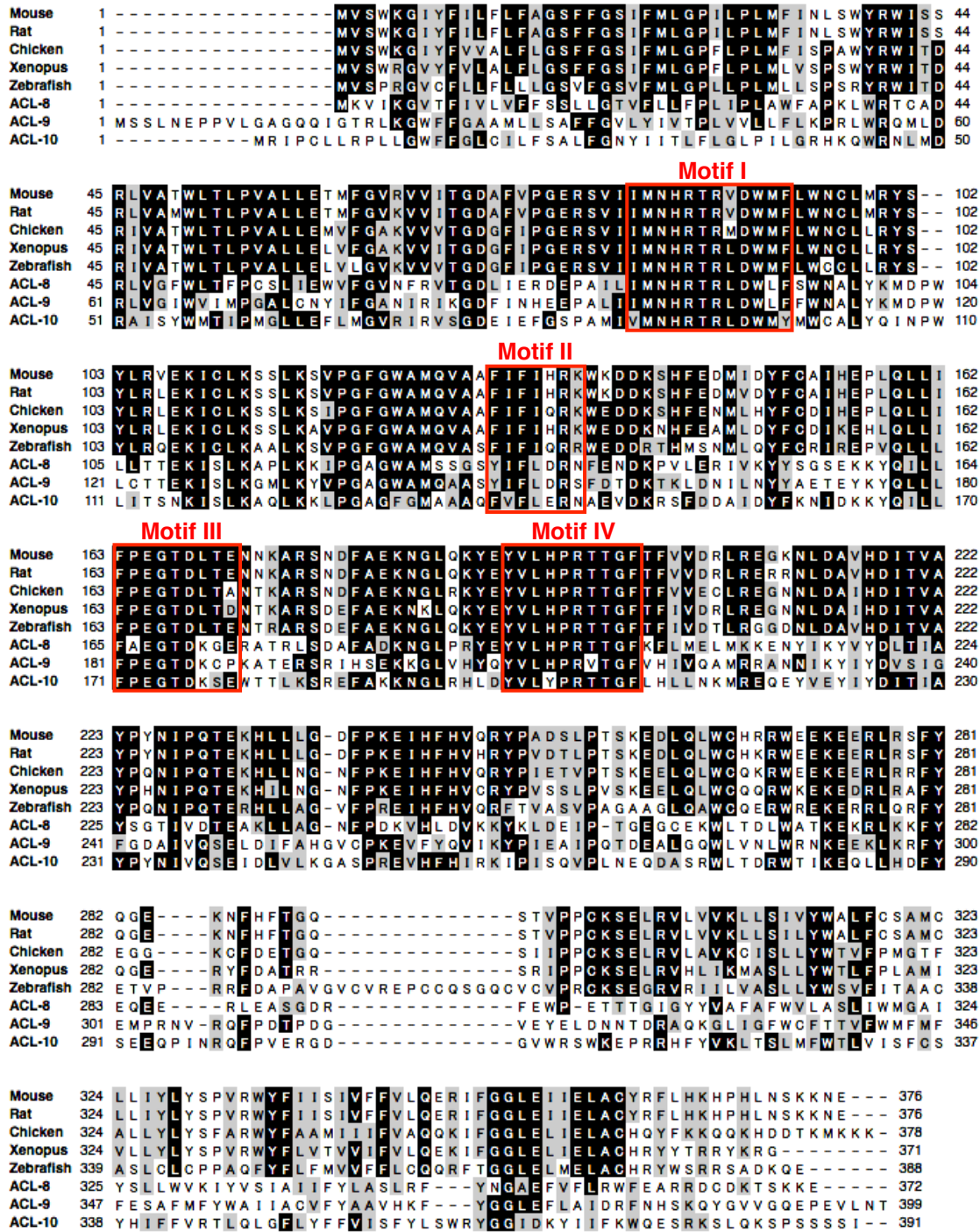
**Supplementary Table 1. AGPAT family**

Human	<i>C. elegans</i> homologue
GPAT1	<i>acl-6</i>
GPAT2	<i>acl-6</i>
GPAT3	<i>acl-4, acl-5</i>
GPAT4	<i>acl-4, acl-5</i>
AGPAT1	<i>acl-1, acl-2</i>
AGPAT2	<i>acl-1, acl-2</i>
DHAPAT	<i>acl-7</i>
LYCAT/LCLAT1/ALCAT1	<i>acl-8, acl-9, acl-10</i>
LPGAT1	<i>acl-12, acl-13, acl-14</i>
AGPAT5	<i>acl-11</i>
Tafazzin	<i>acl-3</i>
AGPAT3	-
AGPAT4	-
LPCAT1	-
LPCAT2/LysoPAFAT	-
LPEAT2	-

*acl-1~14* ; AGPAT family members in *C. elegans*. At least 16 genes belong to the AGPAT family in human. AGPAT3, AGPAT4, LPCAT1, LPCAT2/LysoPAFAT and LPEAT2 are not conserved in *C. elegans*. GPAT, glycerol-3-phosphate acyltransferase; AGPAT, 1-acylglycerol-3-phosphate *O*-acyltransferase; DHAPAT, dihydroxyacetonephosphate acyltransferase; LYCAT and LCLAT, lysocardiolipin acyltransferase; ALCAT, acyl-CoA:lysocardiolipin acyltransferase; LPGAT, lysoPG acyltransferase; LPCAT, lysoPC acyltransferase; PAF, platelet-activating factor; LPEAT, lysoPE acyltransferase.

# Supplementary Figure 1

A

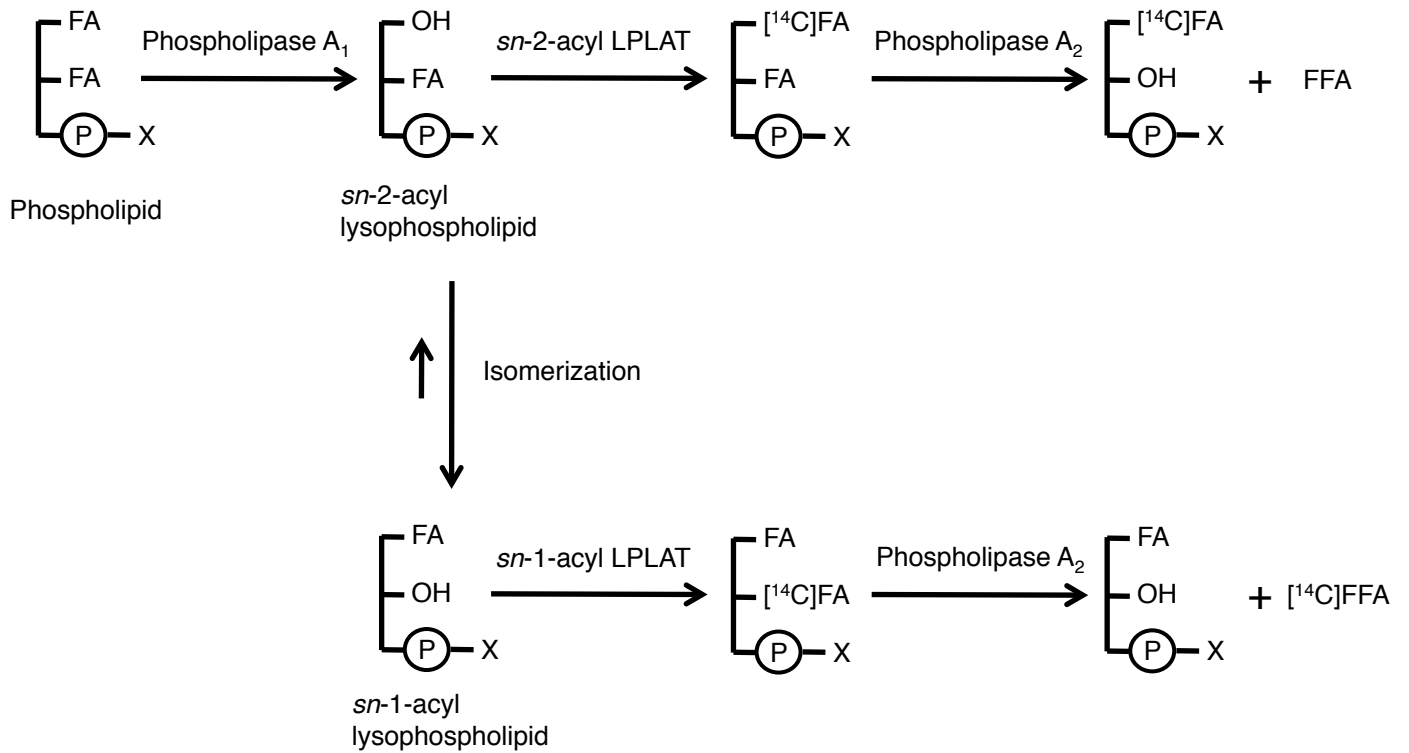


B

	Motif I	Motif II	Motif III	Motif IV
Mouse GPAT1	<sup>227</sup> LPVHRSHIDYLL	<sup>273</sup> GFFIRRR	<sup>313</sup> FLEGTRSRS	<sup>347</sup> ILVIPVGISY
Mouse AGPAT1	<sup>98</sup> VSNHQSSLDLLG	<sup>141</sup> IIFIDRK	<sup>173</sup> FPEGTRNHN	<sup>200</sup> VPIIPVMSS
Mouse LYCAT	<sup>82</sup> IMNHRTRVDWMF	<sup>130</sup> FIFIHRK	<sup>163</sup> FPEGTDLTE	<sup>191</sup> YVLHPRTTGF
Rat LYCAT	<sup>82</sup> IMNHRTRVDWMF	<sup>130</sup> FIFIHRK	<sup>163</sup> FPEGTDLTE	<sup>191</sup> YVLHPRTTGF
Chicken LYCAT	<sup>82</sup> IMNHRTRMDWMF	<sup>130</sup> FIFIQRK	<sup>163</sup> FPEGTDLTA	<sup>191</sup> YVLHPRTTGF
Xenopus LYCAT	<sup>82</sup> IMNHRTRLDWMF	<sup>130</sup> FIFIHRK	<sup>163</sup> FPEGTDLTD	<sup>191</sup> YVLHPRTTGF
Zebrafish LYCAT	<sup>82</sup> IMNHRTRLDWMF	<sup>130</sup> FIFIQRR	<sup>163</sup> FPEGTDLTE	<sup>191</sup> YVLHPRTTGF
ACL-8	<sup>82</sup> IMNHRTRLDWLF	<sup>132</sup> YIFLDRN	<sup>165</sup> FAEGTDKGE	<sup>193</sup> YVLHPRTTGF
ACL-9	<sup>98</sup> IMNHRTRLDWLF	<sup>148</sup> YIFLDRS	<sup>181</sup> FPEGTDKCP	<sup>209</sup> YVLHPRVTGF
ACL-10	<sup>88</sup> VMNHRTRLDWMY	<sup>138</sup> FVFLERN	<sup>171</sup> FPEGTDKSE	<sup>200</sup> YVLYPRTTGF
LYCAT consensus	XMNHRTRXDWF	XIFXXRX	FXEGTDXXX	YVLHPRTTGF

**Supplementary Figure 1.** Alignment of the LYCAT/ACL-8, -9, -10 subfamily members in AGPAT family. (A) Alignment of *C. elegans* ACL-8, ACL-9, ACL-10 and the closest homologues (LYCAT) from mouse, rat, chicken, xenopus and zebrafish. Identical amino acids are shown on a black background and similar amino acids are on a grey background. The four conserved AGPAT motifs (motif I-IV) are boxed. Accession numbers for the sequences used were as follows: mouse: NP\_001074540; rat: XP\_343021, chicken: NP\_001026210; xenopus: NP\_001135517, zebrafish: NP\_998435; ACL-9: NP\_504644; ACL-10: NP\_505971. The amino acid sequence of ACL-8 was determined as described previously (12). (B) Analysis of conserved amino acids of LYCAT proteins in the AGPAT motifs. Red amino acids show consensus motifs that define AGPAT family. Blue amino acids are “LYCAT signature amino acids”, which are highly conserved in LYCAT proteins of various species, but not among other AGPAT family members, such as GPAT1 or AGPAT1. Numbers refer to amino acid residue position within each protein sequence. Accession number.: mouse GPAT1; NP\_032175, mouse AGPAT1; NP\_001156851.

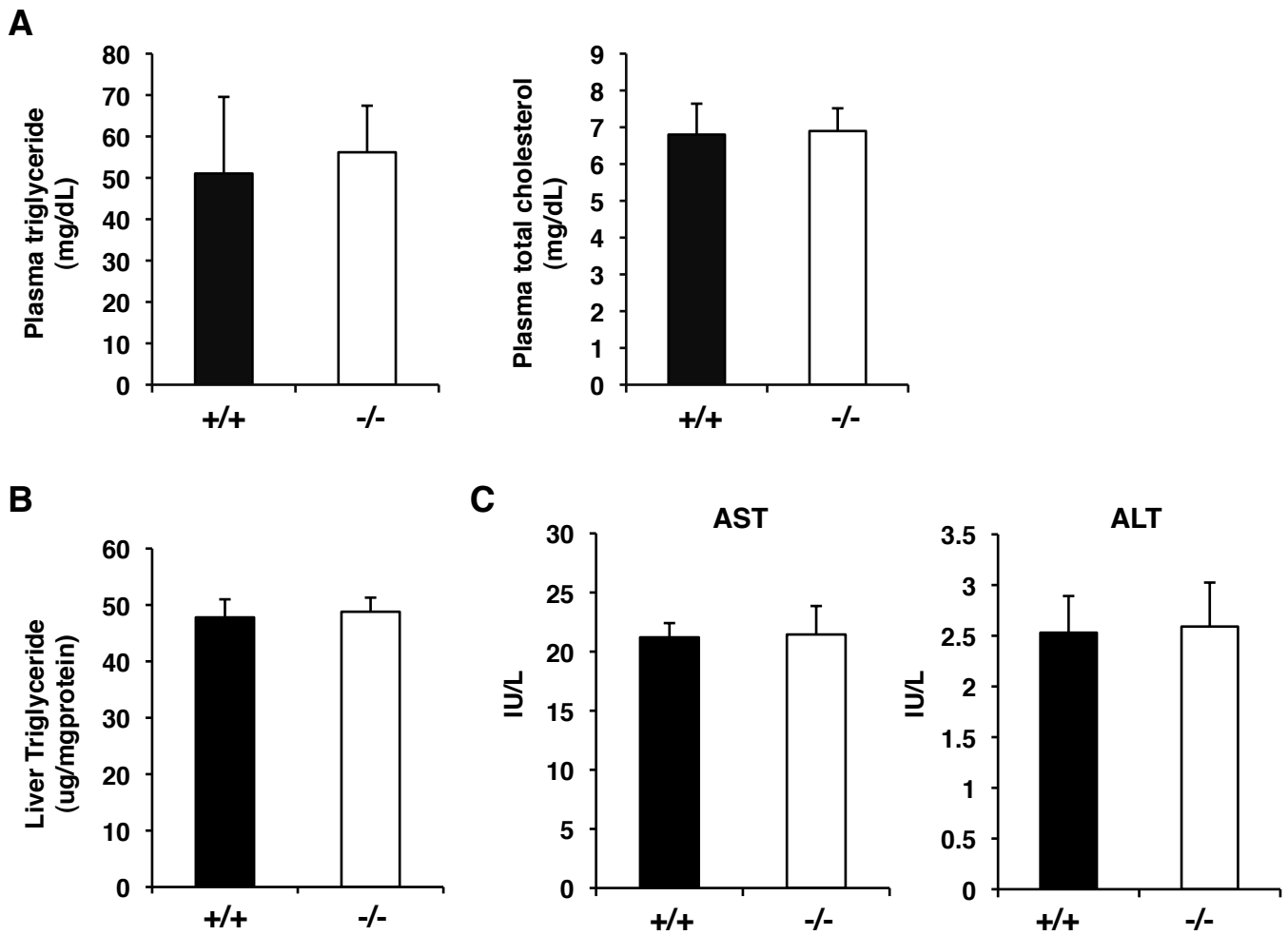
## Supplementary Figure 2



**Supplementary Figure 2.** A scheme of the procedure for determining *sn*-2-acyl LPLAT activity.

After the *in vitro* acyltransferase assay, the lipids were extracted and separated by TLC as described in *Materials and Methods*. The resulting Phospholipid fractions were re-extracted from the TLC plates and treated with phospholipase A<sub>2</sub>. The distribution of radioactivity among the reaction products (free fatty acid and lysophospholipid) was assessed following TLC. X; polar head group, LPLAT; lysophospholipid acyltransferase, FA; fatty acid, FFA; free fatty acid.

## Supplementary Figure 3



**Supplementary Figure 3.** Plasma and liver lipid levels, serum AST and ALT levels were not altered in LYCAT<sup>-/-</sup> mice. Plasma triglyceride and total cholesterol (A), hepatic triglyceride (B), serum AST and ALT (C) levels in LYCAT<sup>+/+</sup> and LYCAT<sup>-/-</sup> mice were determined. LYCAT<sup>+/+</sup>, closed bars; LYCAT<sup>-/-</sup>, open bars. Data represent the mean  $\pm$  SEM of triplicate measurements.