

# Liver-specific deletion of the *Plpp3* gene alters plasma lipid composition and worsens atherosclerosis in apoE<sup>-/-</sup> mice

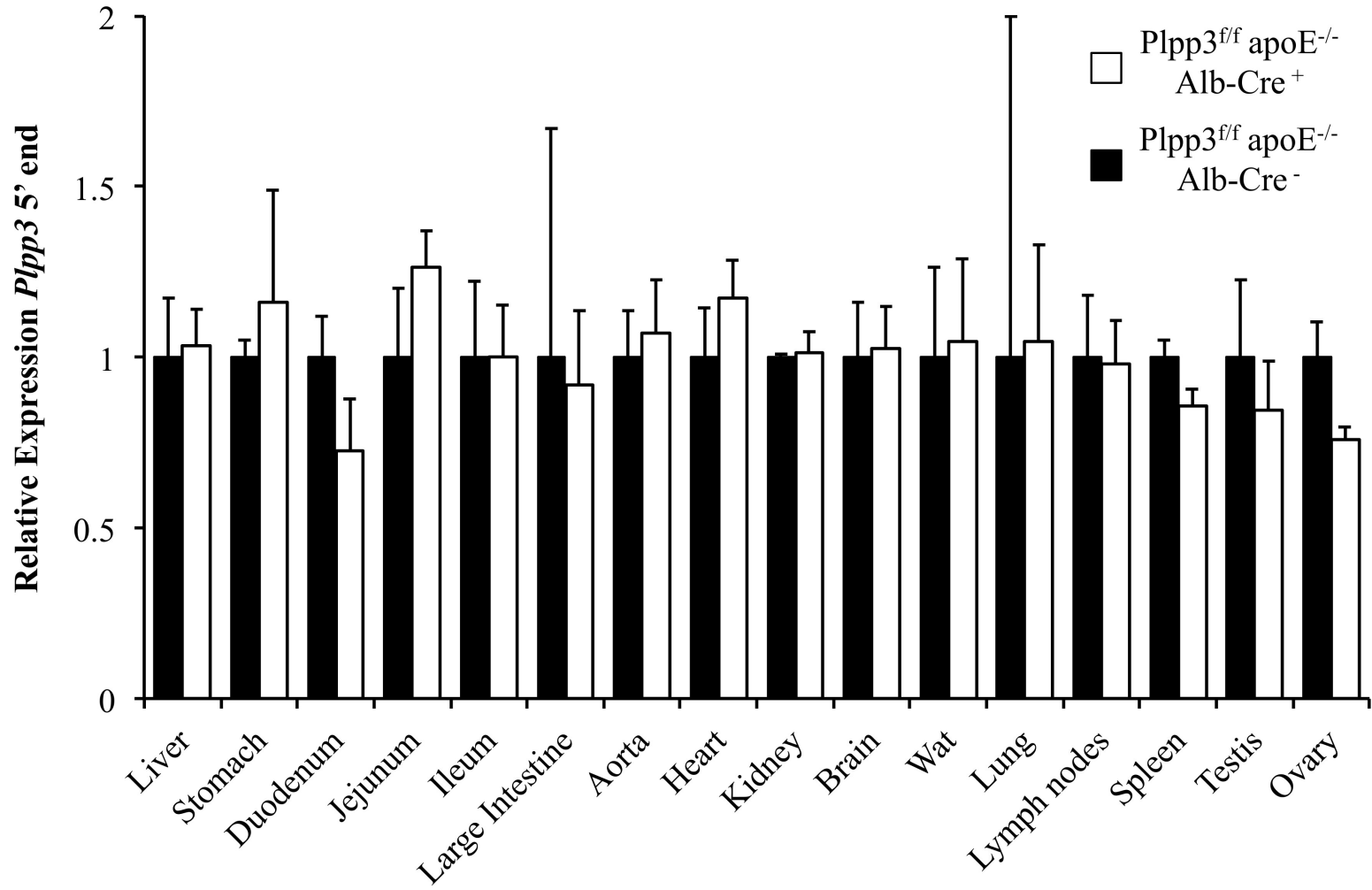
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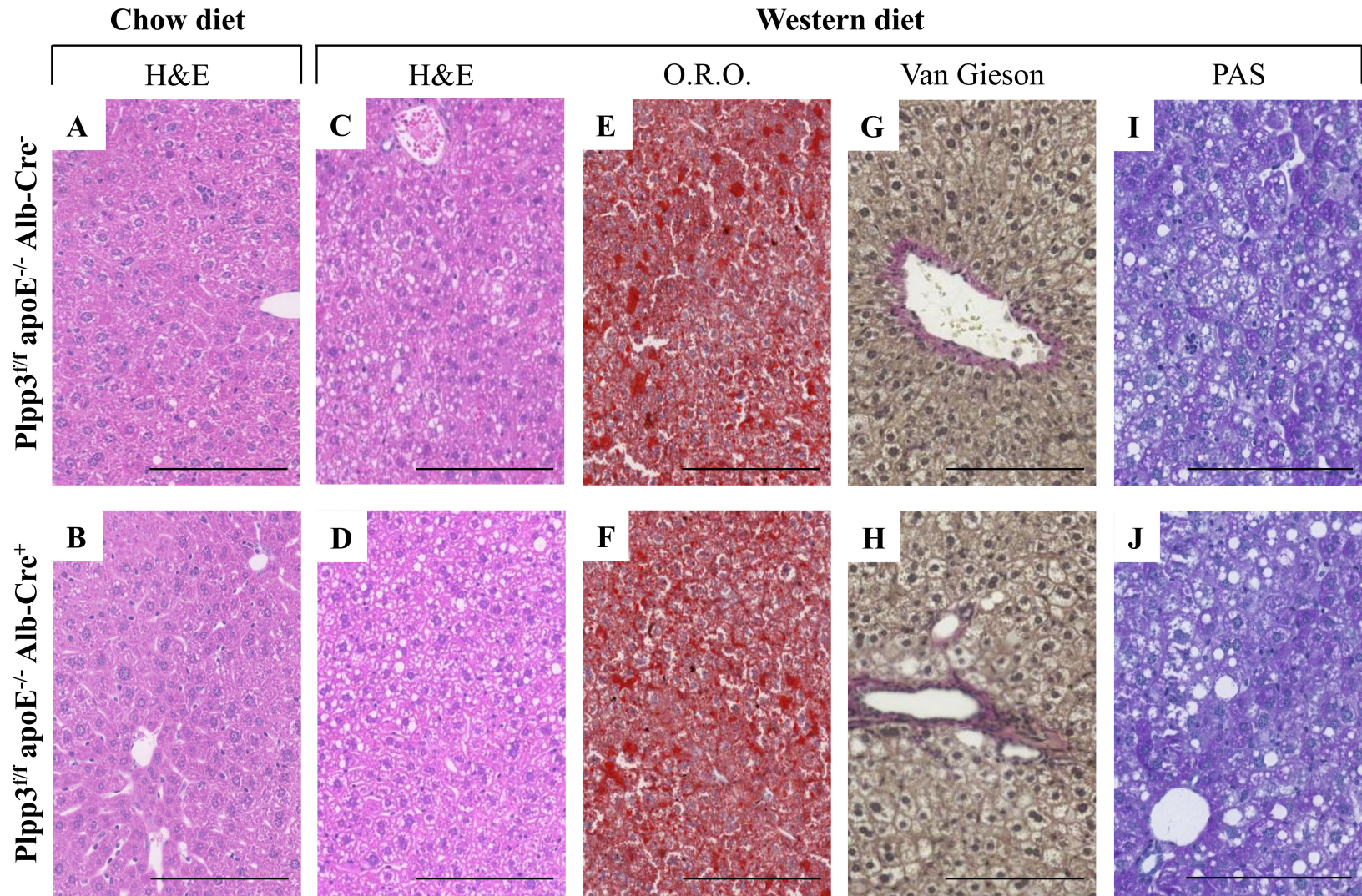
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## Supplementary Figure 1



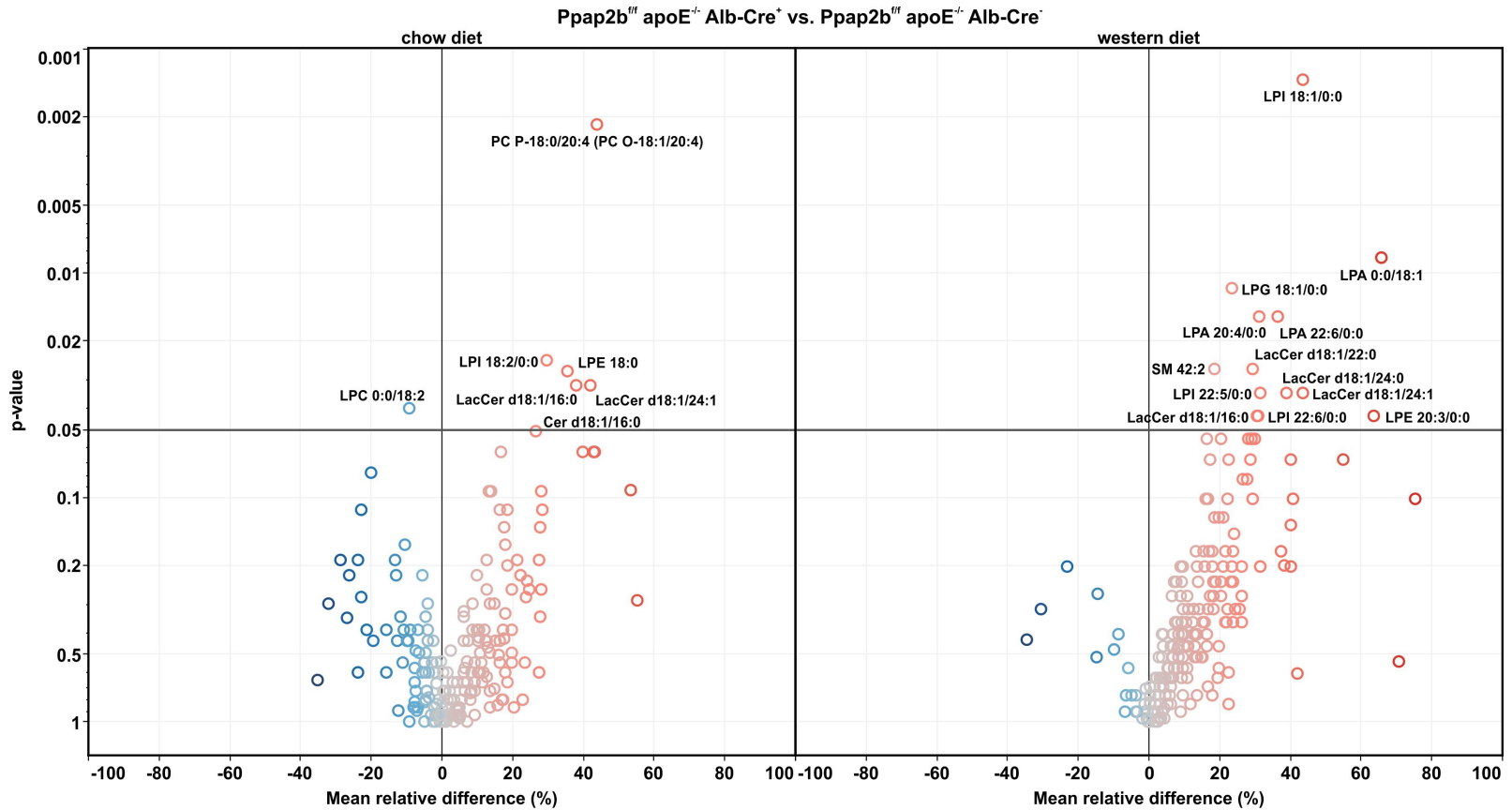
**Supplementary Figure 1. Quantitative expression of the *Plpp3* mRNA 5' end.** Expression levels of *Plpp3* mRNA are shown, quantified by qPCR targeting the region upstream of the Cre recombinase-mediated floxed exons excision site. Values were normalized to the expression of the transcript in each of the tissues from *Plpp3*<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup> mice. (n=6 for liver, n=3 for all the other tissues, p>0.05 by Wilcoxon rank-sum test). Wat = white adipose tissue.

## Supplementary Figure 2



**Supplementary Figure 2. Liver histology of chow and Western-diet fed mice.** Representative photomicrographs of H&E staining of chow-fed *Plpp3<sup>ff</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup>* and *Plpp3<sup>ff</sup> apoE<sup>-/-</sup> Alb-Cre<sup>+</sup>* mice (A, B). H&E (C, D), Oil Red O (E, F), Van Gieson, (G, H) and PAS (I, J) staining of liver sections from Western-fed *Plpp3<sup>ff</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup>* and *Plpp3<sup>ff</sup> apoE<sup>-/-</sup> Alb-Cre<sup>+</sup>* mice. Bar length = 200  $\mu$ m.

# Supplementary Figure 3



**Supplementary Figure 3. Volcano plot highlighting the most significant changes in the levels of plasma lipids caused by hepatic *Ppp3* deletion in mice fed a chow or Western diet.** Data are expressed as the percentage of the mean relative difference; n=8-10, Wilcoxon rank-sum test. In the figures, the red colour refers to increased concentrations and the blue colour refers to decreased concentrations in *Ppp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>+</sup>* vs *Ppp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup>* mice. Cer d18:1 = Ceramide d18:1; LacCer = Lactosylceramide; LPA = Lysophosphatidic acid; LPC = Lysophosphatidylcholine; LPE = Lysophosphatidylethanolamine; LPG = Lysophosphatidylglycerol; LPI = Lysophosphatidylinositol; PC O = Phosphatidylcholine; SM = Sphingomyelin.

**Supplementary Table 1. Effects of diet on plasma lipid classes in Plpp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup> and Plpp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>+</sup> mice.**

Total lipids	Plpp3 <sup>f/f</sup> apoE <sup>-/-</sup> Alb-Cre <sup>-</sup>				Plpp3 <sup>f/f</sup> apoE <sup>-/-</sup> Alb-Cre <sup>+</sup>			
	Mean ± SEM (μmol/L)		Mean relative difference (%)	P-value	Mean ± SEM (μmol/L)		Mean relative difference (%)	P-value
	Western diet	Chow diet			Western diet	Chow diet		
CE	28131 ± 2238	509 ± 8156	<b>245%</b>	<b>8.2E-05</b>	32812 ± 2757	8491 ± 486	<b>286%</b>	<b>2.2E-05</b>
Cer d18:0	1.8 ± 0.1	0.02 ± 0.33	<b>462%</b>	<b>8.2E-05</b>	2.2 ± 0.2	0.34 ± 0.02	<b>541%</b>	<b>2.2E-05</b>
Cer d18:1	50.2 ± 6.9	1.4 ± 11.8	<b>327%</b>	<b>8.2E-05</b>	50.1 ± 3	9.9 ± 1.1	<b>407%</b>	<b>2.2E-05</b>
DAG	22.4 ± 2.1	0.5 ± 5.9	<b>280%</b>	<b>3.1E-04</b>	22.4 ± 1.2	5.7 ± 0.4	<b>293%</b>	<b>4.6E-05</b>
Gb3	3.8 ± 0.3	0.1 ± 1.1	<b>240%</b>	<b>8.2E-05</b>	4.6 ± 0.2	1.3 ± 0.1	<b>269%</b>	<b>2.2E-05</b>
Glc/GalCer	113.9 ± 4.8	2.2 ± 28.3	<b>303%</b>	<b>8.2E-05</b>	125.3 ± 5.8	28.4 ± 2.3	<b>341%</b>	<b>2.2E-05</b>
LPA	0.2 ± 0.01	0.01 ± 0.19	n.s.	<b>6.7E-01</b>	0.25 ± 0.02	0.2 ± 0.01	<b>28%</b>	<b>5.7E-03</b>
LPC	1058.9 ± 46.4	17.9 ± 584.4	<b>81%</b>	<b>8.2E-05</b>	1113.5 ± 28.9	582 ± 29.5	<b>91%</b>	<b>2.2E-05</b>
LPE	46.9 ± 3.5	2.0 ± 21.1	<b>122%</b>	<b>5.8E-04</b>	57.2 ± 4.7	23 ± 2.1	<b>148%</b>	<b>4.3E-05</b>
LPG	12.9 ± 3.6	4.1 ± 12.2	n.s.	<b>6.7E-01</b>	15.8 ± 4.4	9.5 ± 2.8	n.s.	<b>4.5E-01</b>
LPI	3.1 ± 0.2	0.1 ± 1.2	<b>161%</b>	<b>8.2E-05</b>	3.8 ± 0.2	1.3 ± 0.1	<b>190%</b>	<b>2.2E-05</b>
LSM	0.01 ± 0.001	0.0004 ± 0.0056	<b>119%</b>	<b>5.5E-03</b>	0.01 ± 0.001	0.0059 ± 0.0004	<b>130%</b>	<b>2.2E-05</b>
LacCer	15.9 ± 1.6	0.3 ± 3	<b>438%</b>	<b>8.2E-05</b>	21.5 ± 1.5	4.0 ± 0.3	<b>441%</b>	<b>2.2E-05</b>
PC	3573 ± 216	105 ± 1399	<b>155%</b>	<b>8.2E-05</b>	3742 ± 160	1328 ± 48	<b>182%</b>	<b>2.2E-05</b>
PC O	56.9 ± 5.2	1.3 ± 14.5	<b>291%</b>	<b>8.2E-05</b>	61.9 ± 2.3	17.2 ± 1.4	<b>260%</b>	<b>2.2E-05</b>
PE O	1.9 ± 0.3	0.3 ± 2.3	n.s.	<b>4.4E-01</b>	1.4 ± 0.2	2.3 ± 0.2	<b>-37%</b>	<b>1.7E-02</b>
PI	52.2 ± 3.2	2.3 ± 29.8	<b>75%</b>	<b>1.6E-04</b>	55.3 ± 2.5	29 ± 2.7	<b>91%</b>	<b>4.3E-05</b>
SM	981 ± 86	25 ± 378	<b>159%</b>	<b>1.6E-04</b>	1134 ± 47	401 ± 24	<b>183%</b>	<b>2.2E-05</b>

**Supplementary Table 1. Effects of diet on plasma lipid classes in Plpp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>-</sup> and Plpp3<sup>f/f</sup> apoE<sup>-/-</sup> Alb-Cre<sup>+</sup> mice.** Data are expressed as the mean  $\pm$  SEM; n=8-10, Wilcoxon rank-sum test. CE = Cholesteryl ester; Cer d18:0 = Ceramide d18:0; Cer d18:1 = Ceramide d18:1; DAG = Diacylglycerol; Gb3 = Globotriaosylceramide; Glc/GalCer = Glucosylceramide/Galactosylceramide; LPA = Lysophosphatidic acid; LPC = Lysophosphatidylcholine; LPE = Lysophosphatidylethanolamine; LPG = Lysophosphatidylglycerol; LPI = Lysophosphatidylinositol; LSM = Lysosphingomyelin; LacCer = Lactosylceramide; PC/PC O = Phosphatidylcholine; PE O = Phosphatidylethanolamine; PI = Phosphatidylinositol; SM = Sphingomyelin.