Supplementary Material

Supplementary Figure 1: Food intake.

(A, B, C) Food intake of Lipistase- or vehicle-treated LDLrKO, LDLrKO-PPARαKO, and APOEKO mice monitored over a period of 8 months.

Supplementary Figure 2: Effect of Lipistase on 3T3-L1 cell differentiation.

(A) Quantification of oil red O assay of 3T3L1 differentiated cells (day 5) treated with the PPARγ ligand Rosiglitazone (Rosi, 1 µM), the PPARα ligand WY-14 643 (WY, 1 µM) and Lipistase (Lip, 160 pg/mL). * $p < 0.05$. **(B)** Lipistase effect on mRNA expression levels of genes encoding adipose-secreted proteins (Leptin, Adiponectin, Resistin and Adipsin), a lipogenic transcription factor (SREBP-1c) and lipogenic markers (ACC1, FAS and ADFP) during the differentiation of 3T3-L1 cells at the indicated days.

Supplementary Figure 3: Effect of Lipistase on the levels of hepatic total cholesterol, cholesterol esters, and triglycerides.

The levels of the abovementioned lipids were measured in LDLrKO and LDLrKO-PPARαKO after a 10 month-treatment with Lipistase.

Supplementary Figure 4: Comparative mRNA expression levels of genes involved in fatty acid uptake, fatty acid catabolism and lipogenesis in muscle, adipose tissue, and liver.

mRNA expression levels of markers for fatty acid uptake, fatty acid catabolism, and lipogenesis measured in LDLrKO and LDLrKO-PPARαKO after a 10 month treatment with Lipistase.

Supplementary Figure 5: Lipistase increases oxygen consumption in APOEKO mice.

Oxygen consumption measured after a 10 month-treatment with Lipistase (*$p < 0.05$; nAPOEKO = 4 per group).

Supplementary Figure 6. Athero-protective effects of Lipistase in APOEKO mice. **(A)** Oil red O staining of aortic roots from mice treated with Lipistase or vehicle (Control) starting at 3 weeks of age for 3 months (left panel). **(B)** Oil red O stained aortic roots dissected from offspring of Lipistase- or vehicle- (Control) treated mice. These offsprings were also treated after weaning for 3 months. The representative images (left panels) show plaque area in aortic roots.

Supplementary Figure 7: Lipistase reduced postprandial hypertriglyceridemia. Progression of plasma triglyceride levels within 5 hours after administration of 300 µL of soy oil by gavage (Time 0) to mice fasted for 12 hours after a 3 month Lipistase or vehicle (Control) treatment.

Supplementary Figure 8: Fertility and viability. **(A & B)** Number of alive or dead pups at 3 months post-weaning after Lipistase or vehicle (Control) treated parent mice before conception, mothers during gestation and lactation, and pups after weaning.

Supplementary Table 1: **Kown beneficial effects of Lipistase components on lipid metabolism.**

Lipistase components	**Proven actions on lipid metabolism**
zinc	antioxidant activity [1], vascular protection [2]
iron	tissue oxygenation [3], cardiac protection [4]
selenium enriched yeast	anti-atherosclerosis [3, 5]
vitamin B3	oxidative metabolism [6]
vitamins B9, B6, B12, E and F	anti-atherosclerosis [6]
magnesium	anti-atherosclerosis [7]

fish oil	plasma lipid lowering via lipid catabolism promotion [8], anti-atherosclerosis [9-11]
evening primrose oil	anti-inflammatory activity [12], vascular protection [13]
rapeseed oil	plasma lipid lowering [14] via lipid catabolism promotion in liver [15, 16]
olive oil	antioxidant activity [17]
grapeseed oil	activation of mitochondrial oxidation in skeletal muscle [18]
Fucus vesiculosus	anti-obesity [19-21], anti-inflammatory activity [22, 23], plasma lipid lowering via lipid absorption inhibition [24], antioxidant activity [25-27]
Chondrus crispus, Palmaria palmata, Crithmum maritimum	antioxidant activity [28-31]
Vitis vinifera	anti-atherosclerosis [32-37], antioxidant activity [38-42], plasma lipid lowering via lipid absorption inhibition [43, 44]
Allium sativum	plasma lipid lowering [45-47], via hepatic lipogenesis inhibition [48, 49], antioxidant activity [50], anti-atherosclerosis [51-53]

1. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. *Free Radic Biol Med* 1990;**8**:281-291.

2. Pearce LL, Wasserloos K, St Croix CM, Gandley R, Levitan ES, Pitt BR. Metallothionein, nitric oxide and zinc homeostasis in vascular endothelial cells. *J Nutr* 2000;**130**:1467S-1470S.

3. Reilly C. The Nutritional Trace Metals. Blackwell Publishing Ltd, 2004.

4. Lauffer RB. Iron stores and the international variation in mortality from coronary artery disease. *Med Hypotheses* 1991;**35**:96-102.

5. Cinemre H, Bilir C, Gokosmanoglu F, Kadakal F. Anti-Saccharomyces cerevisiae antibodies in acute myocardial infarction. *J Investig Med* 2007;**55**:444-449.

6. McDowell LR. Vitamins in Animal and Human Nutrition. Iowa State University Press, 2000.

7. Ashmead. Intestinal absorption of metal ions. Thomas Publisher, 1985.

8. Rustan AC, Christiansen EN, Drevon CA. Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed omega-3 and omega-6 fatty acids. *Biochem J* 1992;**283 (Pt 2)**:333-339.

9. Connor WE, DeFrancesco CA, Connor SL. N-3 fatty acids from fish oil. Effects on plasma lipoproteins and hypertriglyceridemic patients. *Ann N Y Acad Sci* 1993;**683**:16-34.

10. Lopez D, Moller M, Denicola A, Casos K, Rubbo H, Ruiz-Sanz JI *et al.* Long-chain n-3 polyunsaturated fatty acid from fish oil modulates aortic nitric oxide and tocopherol status in the rat. *Br J Nutr* 2008;**100**:767-775.

11. Margioris AN. Fatty acids and postprandial inflammation. *Curr Opin Clin Nutr Metab Care* 2009;**12**:129-137.

12. Yoshimoto-Furuie K, Yoshimoto K, Tanaka T, Saima S, Kikuchi Y, Shay J *et al.* Effects of oral supplementation with evening primrose oil for six weeks on plasma essential fatty acids and uremic skin symptoms in hemodialysis patients. *Nephron* 1999;**81**:151-159.

13. Jack AM, Keegan A, Cotter MA, Cameron NE. Effects of diabetes and evening primrose oil treatment on responses of aorta, corpus cavernosum and mesenteric vasculature in rats. *Life Sci* 2002;**71**:1863-1877.

14. Gulesserian T, Widhalm K. Effect of a rapeseed oil substituting diet on serum lipids and lipoproteins in children and adolescents with familial hypercholesterolemia. *J Am Coll Nutr* 2002;**21**:103-108.

15. Christiansen RZ, Christiansen EN, Bremer J. The stimulation of erucate metabolism in isolated rat hepatocytes by rapeseed oil and hydrogenated marine oil-containing diets. *Biochim Biophys Acta* 1979;**573**:417-429.

16. Murata M, Ide T, Hara K. Reciprocal responses to dietary diacylglycerol of hepatic enzymes of fatty acid synthesis and oxidation in the rat. *Br J Nutr* 1997;**77**:107-121.

17. Visioli F, Bellomo G, Montedoro G, Galli C. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* 1995;**117**:25-32.

18. Chainier F, Roussel D, Georges B, Meister R, Rouanet JL, Duchamp C *et al.* Cold acclimation or grapeseed oil feeding affects phospholipid composition and mitochondrial function in duckling skeletal muscle. *Lipids* 2000;**35**:1099-1106.

19. Bocanegra A, Bastida S, Benedi J, Rodenas S, Sanchez-Muniz FJ. Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J Med Food* 2009;**12**:236-258.

20. Maeda H, Tsukui T, Sashima T, Hosokawa M, Miyashita K. Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. *Asia Pac J Clin Nutr* 2008;**17 Suppl 1**:196-199.

21. Moro CO, Basile G. Obesity and medicinal plants. *Fitoterapia* 2000;**71 Suppl 1**:S73-82.

22. Cardoso ML, Xavier CA, Bezerra MB, Paiva AO, Carvalho MF, Benevides NM *et al.* Assessment of Zymosan-Induced Leukocyte Influx in a Rat Model using Sulfated Polysaccharides. *Planta Med* 2009.

23. Patankar MS, Oehninger S, Barnett T, Williams RL, Clark GF. A revised structure for fucoidan may explain some of its biological activities. *J Biol Chem* 1993;**268**:21770-21776.

24. Ikeda I, Tanaka K, Sugano M, Vahouny GV, Gallo LL. Inhibition of cholesterol absorption in rats by plant sterols. *J Lipid Res* 1988;**29**:1573-1582.

25. Lee S, Lee YS, Jung SH, Kang SS, Shin KH. Anti-oxidant activities of fucosterol from the marine algae Pelvetia siliquosa. *Arch Pharm Res* 2003;**26**:719-722.

26. Ruperez P, Ahrazem O, Leal JA. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed Fucus vesiculosus. *J Agric Food Chem* 2002;**50**:840-845.

27. Zaragoza MC, Lopez D, M PS, Poquet M, Perez J, Puig-Parellada P *et al.* Toxicity and antioxidant activity in vitro and in vivo of two Fucus vesiculosus extracts. *J Agric Food Chem* 2008;**56**:7773-7780.

28. Matsuhiro B, Urzua CC. Heterogeneity of carrageenans from Chondrus crispus. *Phytochemistry* 1992;**31**:531-534.

29. Meot-Duros L, Magne C. Antioxidant activity and phenol content of Crithmum maritimum L. leaves. *Plant Physiol Biochem* 2009;**47**:37-41.

30. Yuan H, Song J, Zhang W, Li X, Li N, Gao X. Antioxidant activity and cytoprotective effect of kappa-carrageenan oligosaccharides and their different derivatives. *Bioorg Med Chem Lett* 2006;**16**:1329-1334.

31. Yuan YV, Carrington MF, Walsh NA. Extracts from dulse (Palmaria palmata) are effective antioxidants and inhibitors of cell proliferation in vitro. *Food Chem Toxicol* 2005;**43**:1073-1081.

32. Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. *Br J Pharmacol* 1997;**120**:1053-1058.

33. Diebolt M, Bucher B, Andriantsitohaina R. Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. *Hypertension* 2001;**38**:159-165.

34. Ekshyyan VP, Hebert VY, Khandelwal A, Dugas TR. Resveratrol inhibits rat aortic vascular smooth muscle cell proliferation via estrogen receptor dependent nitric oxide production. *J Cardiovasc Pharmacol* 2007;**50**:83-93.

35. Lee B, Moon SK. Resveratrol inhibits TNF-alpha-induced proliferation and matrix metalloproteinase expression in human vascular smooth muscle cells. *J Nutr* 2005;**135**:2767-2773.

36. Leifert WR, Abeywardena MY. Cardioprotective actions of grape polyphenols. *Nutr Res* 2008;**28**:729-737.

37. Perez-Jimenez J, Saura-Calixto F. Grape products and cardiovascular disease risk factors. *Nutr Res Rev* 2008;**21**:158-173.

38. Cestaro B, Simonetti P, Cervato G, Brusamolino A, Gatti P, Testolin G. Red wine effects on peroxidation indexes of rat plasma and erythrocytes. *Int J Food Sci Nutr* 1996;**47**:181-189.

39. Goni I, Brenes A, Centeno C, Viveros A, Saura-Calixto F, Rebole A *et al.* Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poult Sci* 2007;**86**:508-516.

40. Rodrigo R, Rivera G, Orellana M, Araya J, Bosco C. Rat kidney antioxidant response to long-term exposure to flavonol rich red wine. *Life Sci* 2002;**71**:2881-2895.

41. Roig R, Cascon E, Arola L, Blade C, Salvado MJ. Moderate red wine consumption protects the rat against oxidation in vivo. *Life Sci* 1999;**64**:1517-1524.

42. Simonetti P, Ciappellano S, Gardana C, Bramati L, Pietta P. Procyanidins from Vitis vinifera seeds: in vivo effects on oxidative stress. *J Agric Food Chem* 2002;**50**:6217-6221.

43. Pal S, Naissides M, Mamo J. Polyphenolics and fat absorption. *Int J Obes Relat Metab Disord* 2004;**28**:324-326.

44. Tebib K, Besancon P, Rouanet JM. Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. *J Nutr* 1994;**124**:2451-2457.

45. Kojuri J, Vosoughi AR, Akrami M. Effects of anethum graveolens and garlic on lipid profile in hyperlipidemic patients. *Lipids Health Dis* 2007;**6**:5.

46. Sobenin IA, Andrianova IV, Demidova ON, Gorchakova T, Orekhov AN. Lipid-lowering effects of time-released garlic powder tablets in double-blinded placebo-controlled randomized study. *J Atheroscler Thromb* 2008;**15**:334-338.

47. Yeh YY, Lin, R. I., Yeh, S. M. & Evens, S. Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. In: T. OHOTTJWST, ed. Food Factors for Cancer Prevention. Tokyo: Springer, 1997:226-230.

48. Yeh YY, Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. *J Nutr* 2001;**131**:989S-993S.

49. Yeh YY, Yeh SM. Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* 1994;**29**:189-193.

50. Ide N, Lau BH. Garlic compounds minimize intracellular oxidative stress and inhibit nuclear factor-kappa b activation. *J Nutr* 2001;**131**:1020S-1026S.

51. Campbell JH, Efendy JL, Smith NJ, Campbell GR. Molecular basis by which garlic suppresses atherosclerosis. *J Nutr* 2001;**131**:1006S-1009S.

52. Lau BH. Suppression of LDL oxidation by garlic. *J Nutr* 2001;**131**:985S-988S.

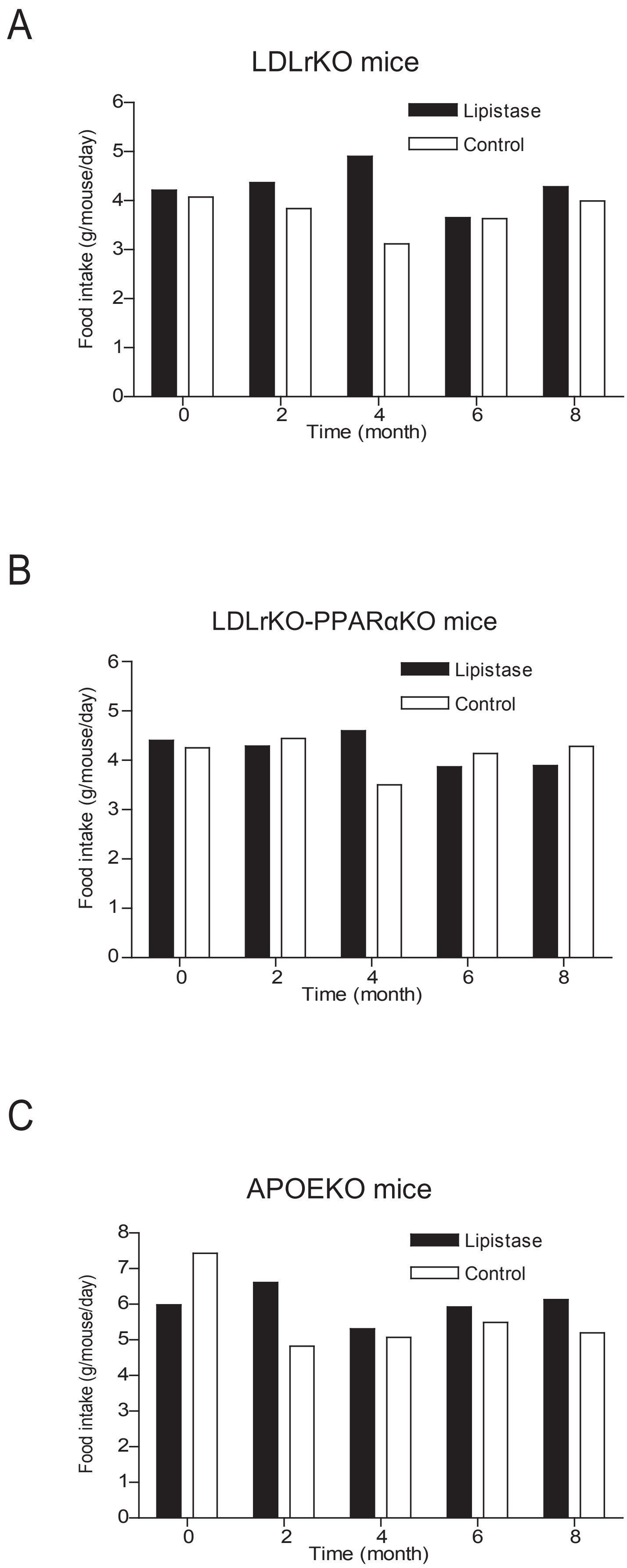
53. Slowing K, Ganado P, Sanz M, Ruiz E, Tejerina T. Study of garlic extracts and fractions on cholesterol plasma levels and vascular reactivity in cholesterol-fed rats. *J Nutr* 2001;**131**:994S-999S.

Supplementary Table 2: Primers used for real-time RT-qPCR

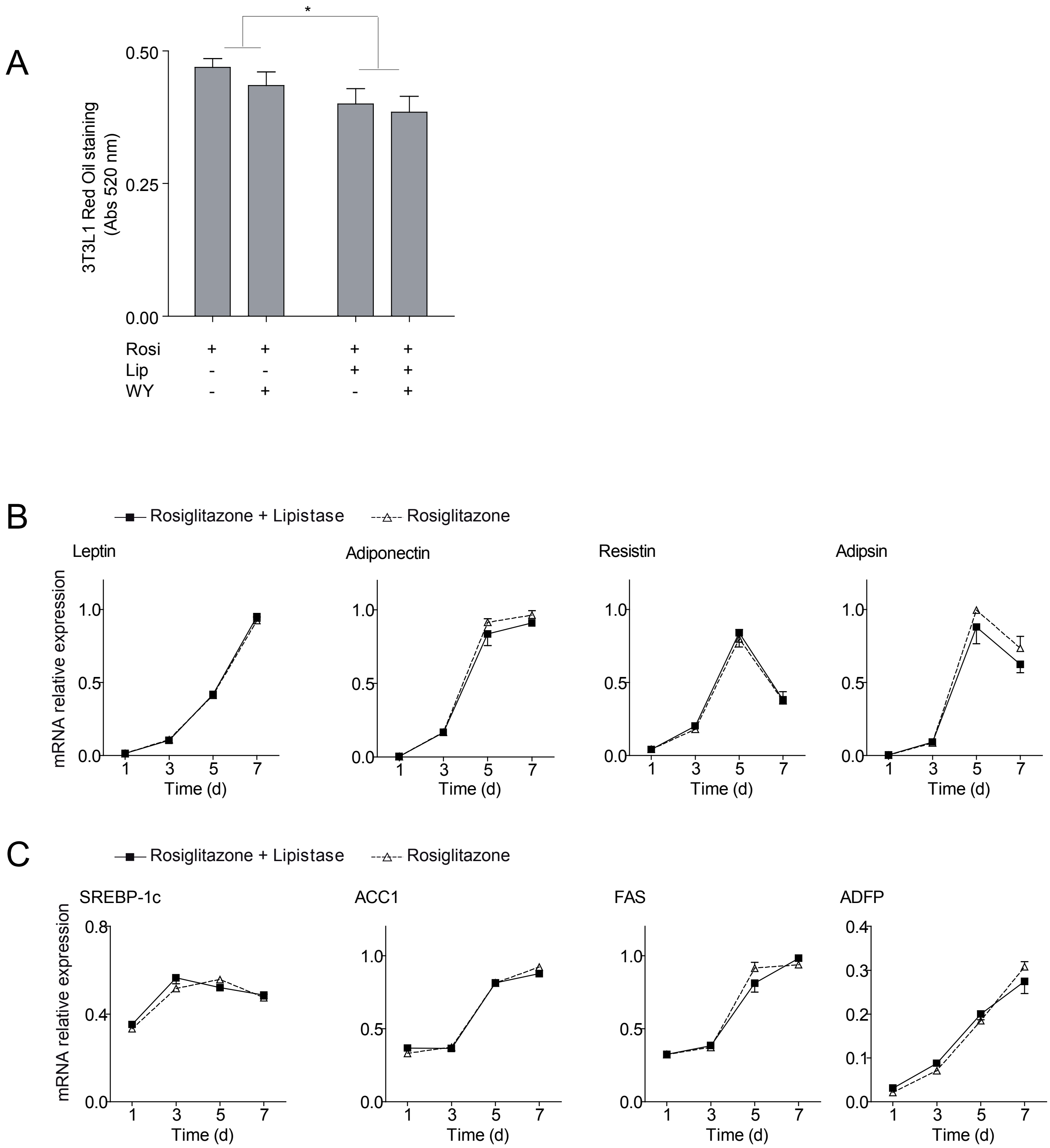
Gene	**Forward** (3’-5’)	**Reverse** (3’-5’)
CD36	CAT.NO. QT01058253 (Qiagen quantitect)	
ACOX1	CAT.NO. QT01775851 (Qiagen quantitect)	
BFE	TGTTCTTGGCTTGGGAACG	TCCCCACCCTTGCAAAAG
UCP3	CAT.NO. QT00115339 (Qiagen quantitect)	
TNFα	CAT.NO. QT00104006 (Qiagen quantitect)	
MCP-1	CAT.NO. QT00167832 (Qiagen quantitect)	
MIP1-α	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
SREBP-1c	CAGCTCAGAGCCGTGGTGA	TTGATAGAAGACCGGTAGCGC
ACC1	ATTGGGCACCCCAGAGCTA	CCCGCTCCTTCAACTTGCT
FAS	CCTCTGATCAGTGGCCTCCTC	GGATTCGGGAATACAAGTGGC
SCD1	AGATCTCCAGTTCTTACACGACCAC	GACGGATGTCTTCTTCCAGGTG
Elovl3	CAT.NO. QT00115675 (Qiagen quantitect)	
DGAT1	CGTGGGCGACGGCTACT	GAAACCACTGTCTGAGCTGAACA
DGAT2	GCCCGCAGCGAAAACA	GTCTTGGAGGGCTGAGAGGAT
ADFP	CTACGACGACACCGAT	CATTGCGGAATACGGAG
PPARγ2	GCCCACGAACTTCGGAATC	TGCGAGTGGTCTTCCATCAC
CEBPα	GAGCTGAGTGAGGCTCTCATTCT	TGGGAGGCAGACGAAAAAAC
LPL	AGTGGCCGAGAGCGAGAAC	CCACCTCCGTGTAAATCAAGAAG
aP2	CCGCAGACGACAGGAAGG	AGGGCCCCGCCATCT
Leptin	AACCCTCATCAAGACCATTGTCA	CCTCTGCTTGGCGGATACC
Adiponectin	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Resistin	GCTGCTGCCAAGGCTGAT	TCTCCTTCCACCATGTAGTTTCC
Adipsin	GCCTGATGTCCTGCATCAACT	GCGCAGATTGCAGGTTGTC
Cyclo (housekeeping)	TTTGACTTGCGGGCATTT	GGACGCTCTCCTGAGCTACAGA
36B4 (housekeeping)	ACCTCCTTCTTCCAGGCTTT	CCCACCTTGTCTCCAGTCTTT

CD36: cluster of differentiation 36; ACOX1: peroxisomal acyl-coenzyme A oxidase 1; TNFα: tumor necrosis factor alpha; MCP-1: monocyte chemotactic protein-1; MIP1-α: macrophage inflammatory protein 1-alpha; SREBP-1c: sterol regulatory element-binding protein 1-c; FAS: fatty

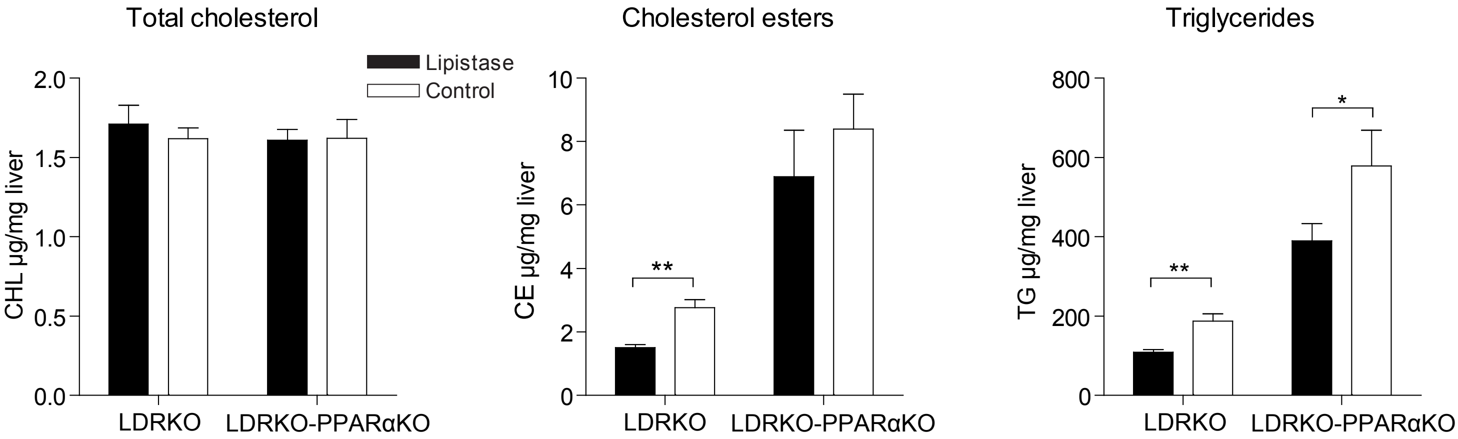
acid synthase; SCD1: stearoyl-CoA desaturase 1; Elovl3: elongation of very long chain fatty acids 3; DGAT1: diglyceride acyltransferase 1; DGAT2: diglyceride acyltransferase 2; ADFP: adipose differentiation-related protein; PPARγ2: peroxisome-proliferator activated receptor gamma 2; CEBPα: CCAAT/enhancer-binding protein alpha; LPL: lipoprotein lipase; aP2: adipocyte protein 2; Cyclo: cyclophilin B; 36B4: acidic ribosomal phosphoprotein P0.



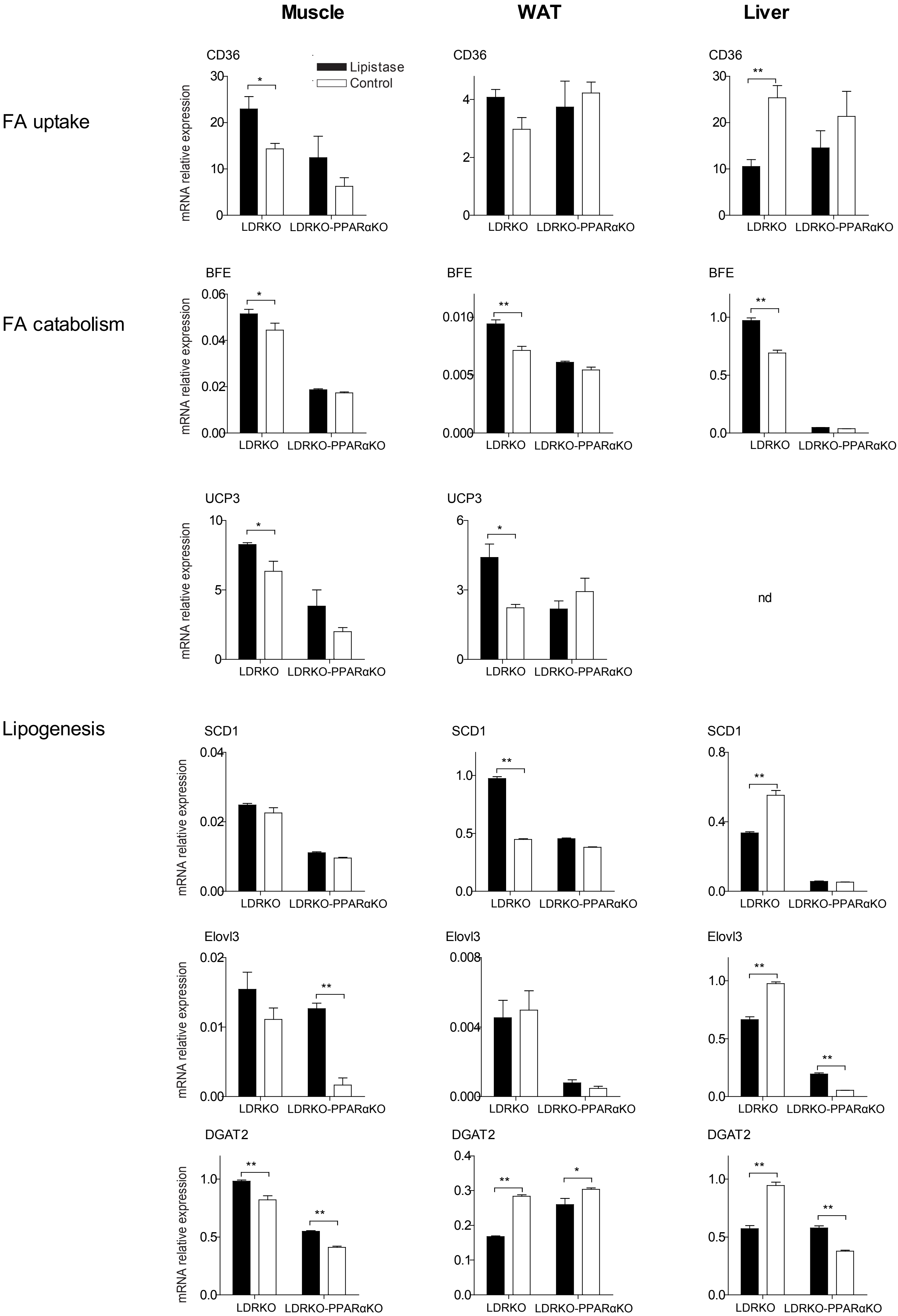
Supplementary Figure 1



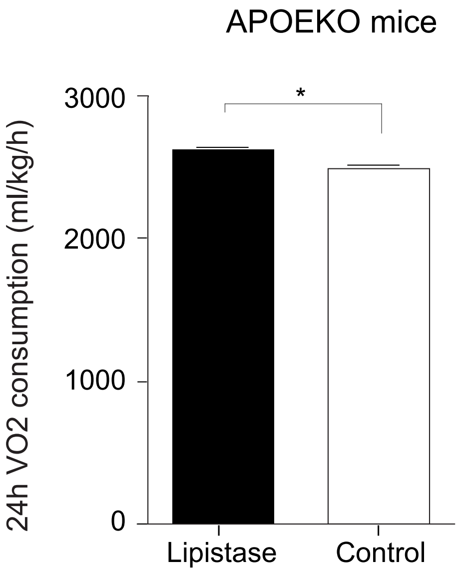
Supplementary Figure 2



Supplementary Figure 3

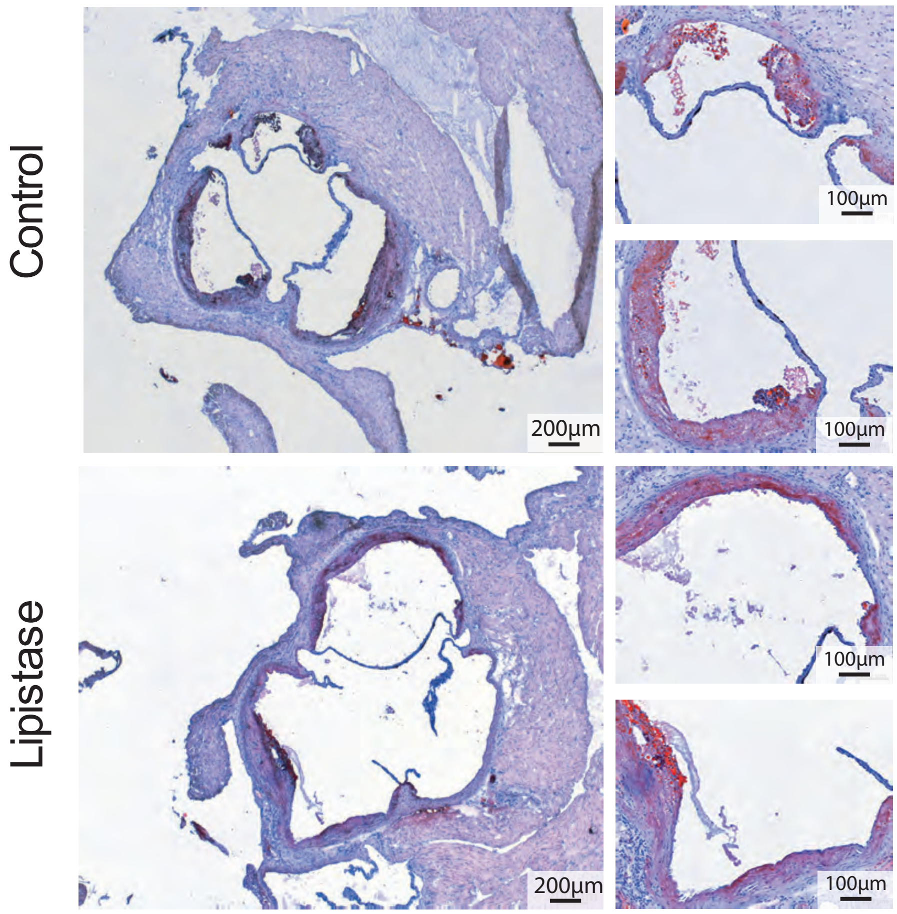


Supplementary Figure 4

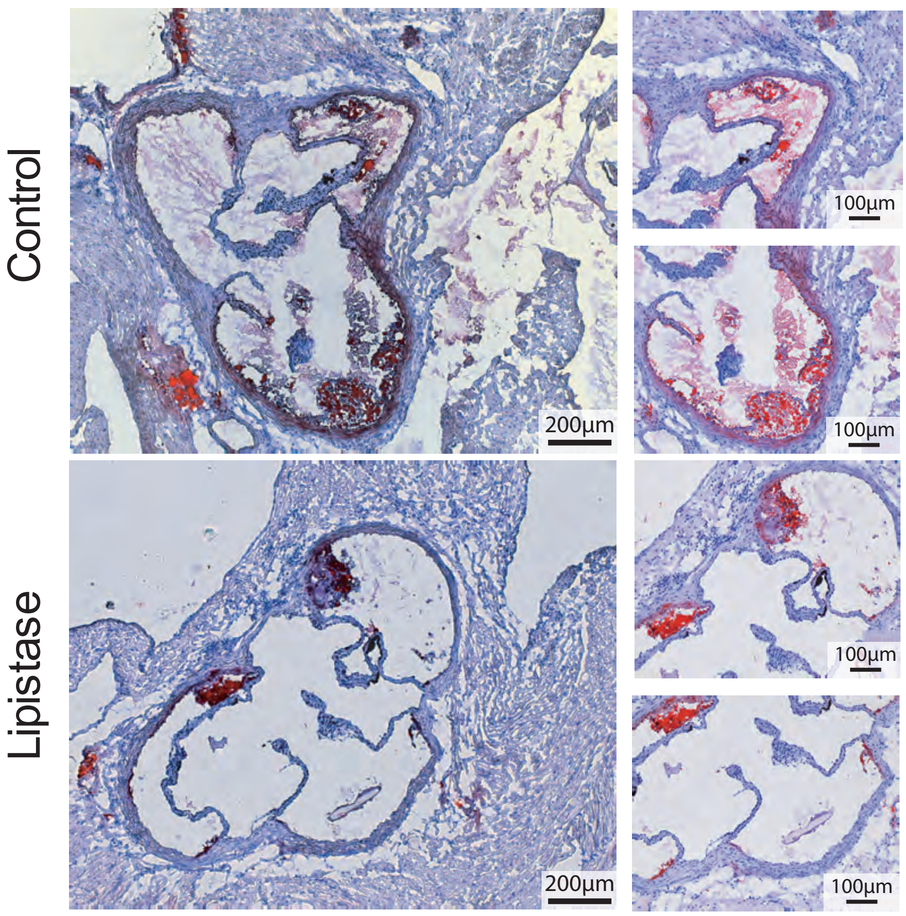


Supplementary Figure 5

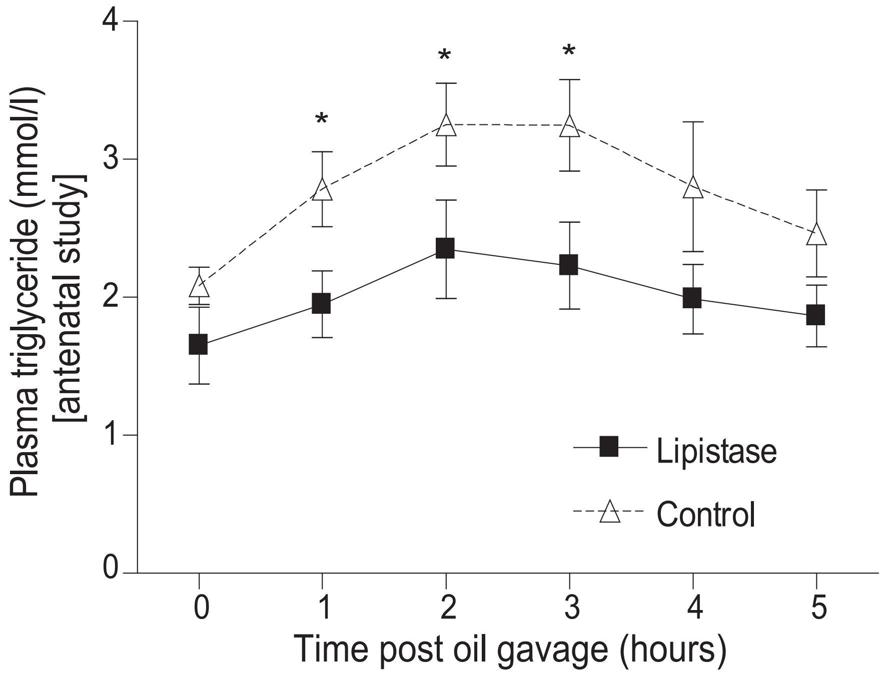
A



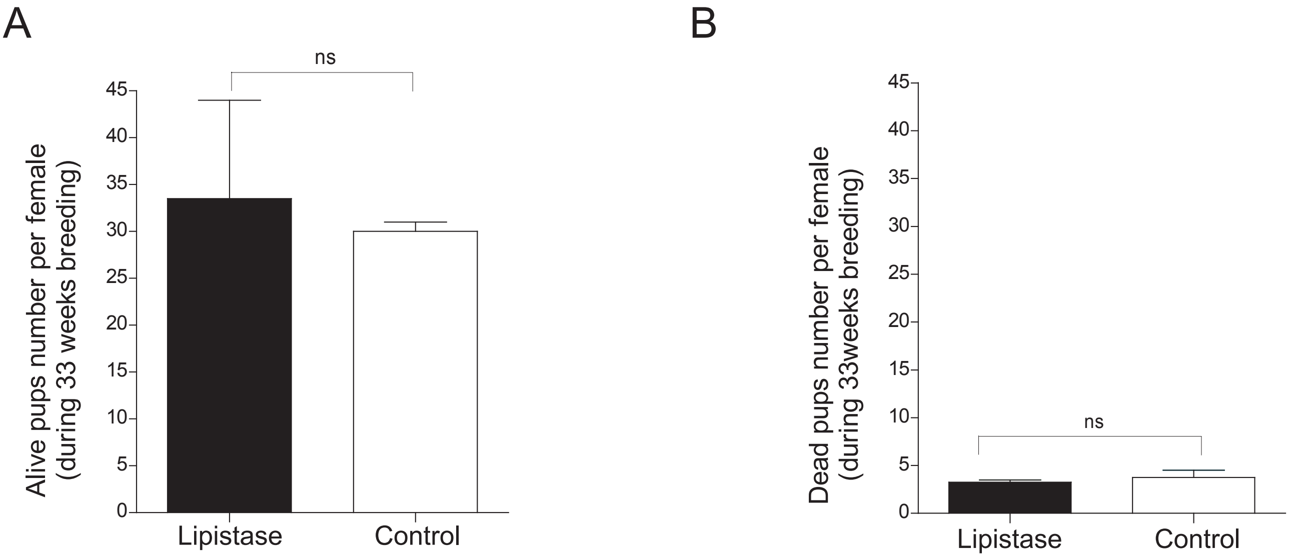
B



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8