
Figures and figure supplements

Maternal diet-induced obesity during pregnancy alters lipid supply to mouse E18.5 fetuses and changes the cardiac tissue lipidome in a sex-dependent manner

Lucas C Pantaleao et al

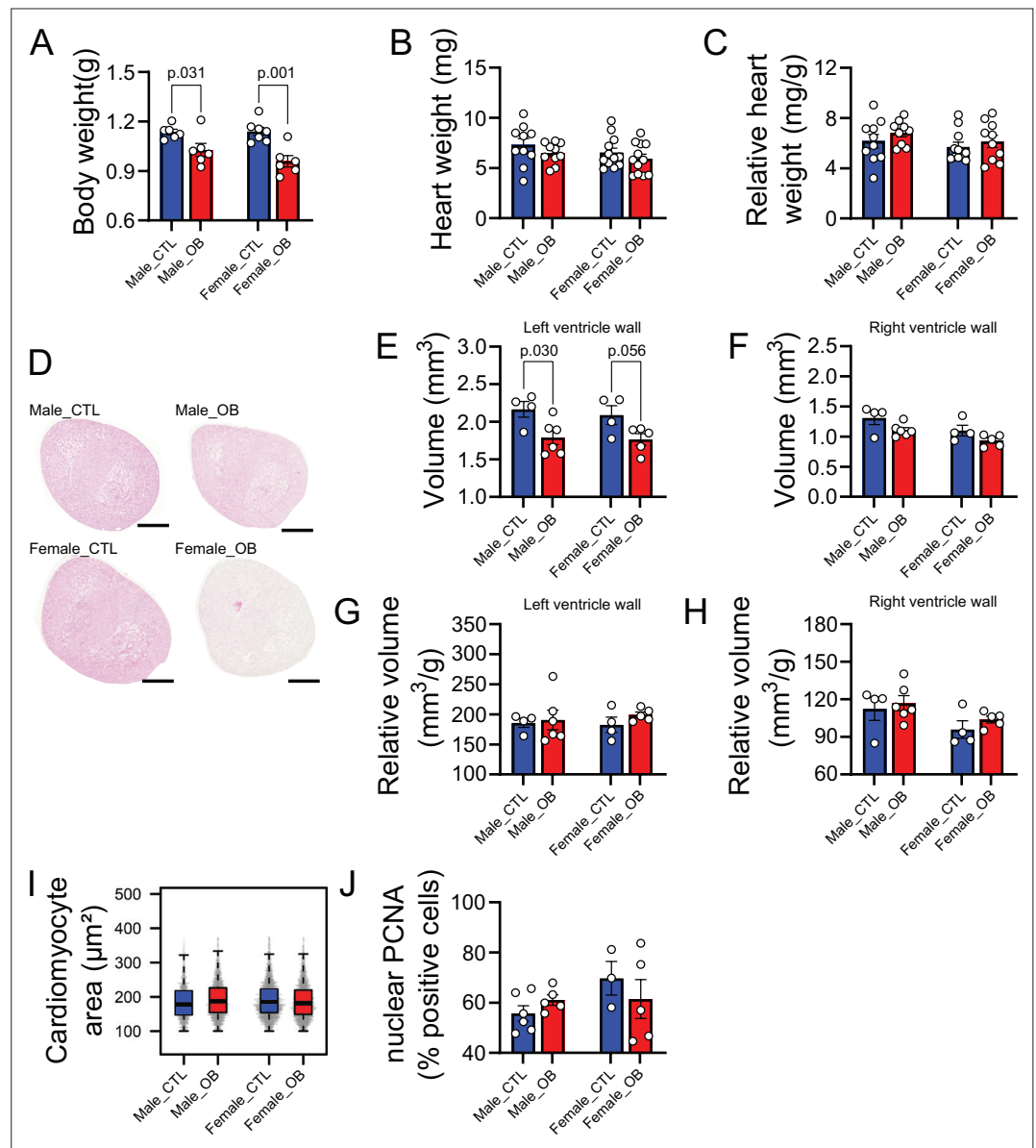


Figure 1. Fetal characteristics at gestational day 18.5. **(A)** Body weight of male and female fetuses from healthy control (CTL) and obese (OB) mouse dams at gestational day 18.5. Male CTL n = 6, male OB n = 6, female CTL n = 7, female OB n = 6. **(B–C)** Heart weight and heart weight/litter average body weight ratio of male and female fetuses from CTL and OB dams at gestational day 18.5. Male CTL n = 10, male OB n = 10, female CTL n = 12, female OB n = 12. **(D)** Histological sections stained with eosin of male and female fetuses from CTL and OB dams at gestational day 18.5 used in cardiac stereology (see also **Figure 1—figure supplement 1**). Scale bar indicates 500 μ m. **(E–H)** Left and right ventricular wall volume and ventricular wall volume/body weight ratio of male and female fetuses from healthy control (CTL) and obese (OB) mouse dams at gestational day 18.5. Male CTL n = 4, male OB n = 6, female CTL n = 4, female OB n = 5. **(I)** Boxplot showing median and quartiles of cardiomyocyte area distribution in male and female fetuses from CTL and OB dams at gestational day 18.5. Under the boxplot a beeswarm plot shows individual area of each cardiomyocyte analysed. Male CTL n = 6, male OB n = 7, female CTL n = 6, female OB n = 6 (see **Figure 1—figure supplement 2** for a representative image of stained cardiomyocytes). **(J)** Nuclear PCNA-positive cells percentage in hearts of male and female fetuses from CTL and OB dams at gestational day 18.5. Male CTL n = 6, male OB n = 5, female CTL n = 3, female OB n = 5. In panels A–H and J, p-values were calculated by Student t-test. In panel I, p-values were calculated using linear mixed-effects model followed by Tukey's *post-hoc* test.

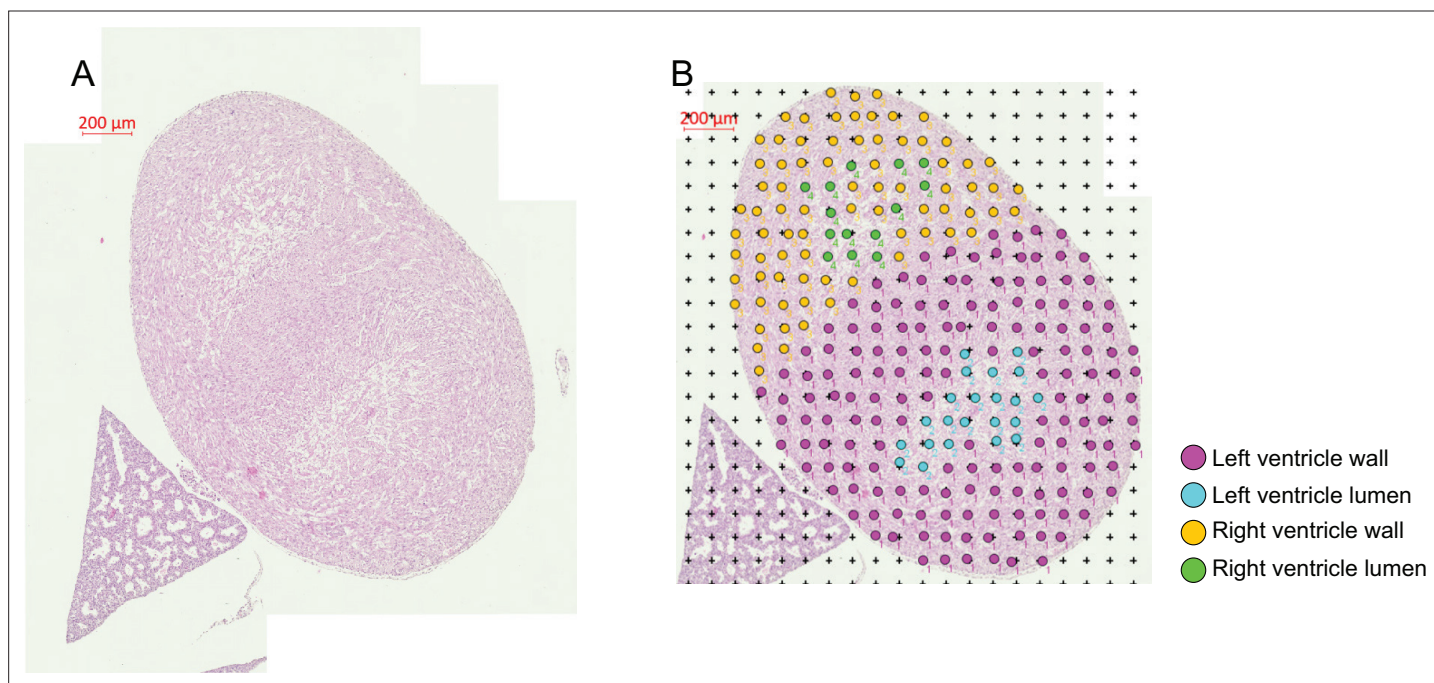


Figure 1—figure supplement 1. (A–B) Representative images of fetal torso prior (A) and after (B) blinded identification of cardiac structures by a trained histologist.

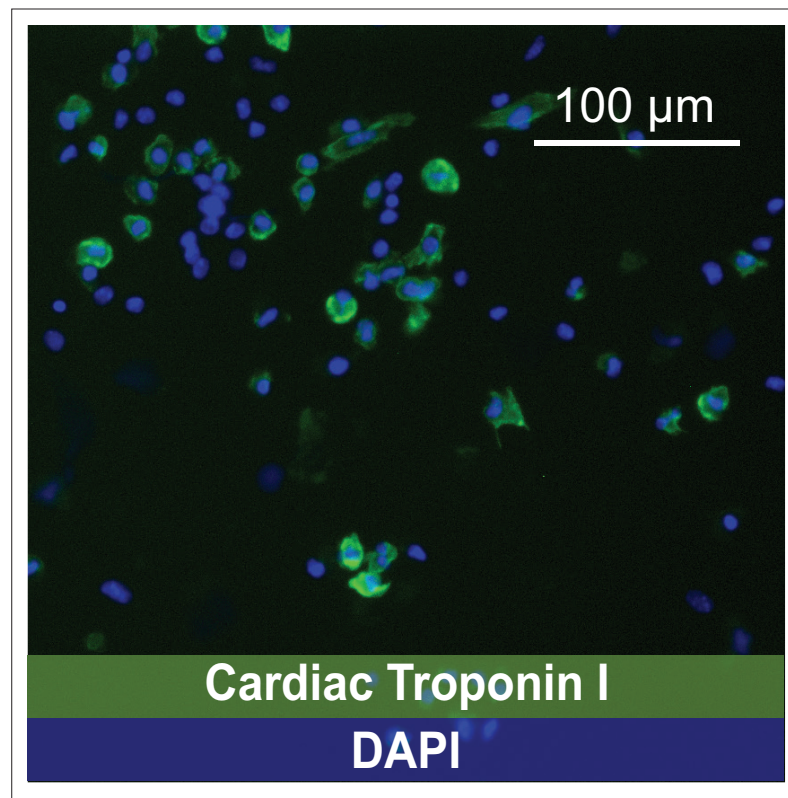


Figure 1—figure supplement 2. Representative image of immunofluorescence identifying cardiomyocytes using a mAb anti-Cardiac Troponin I IgG followed by incubation with an Alexa Fluor 488-conjugated secondary IgG.

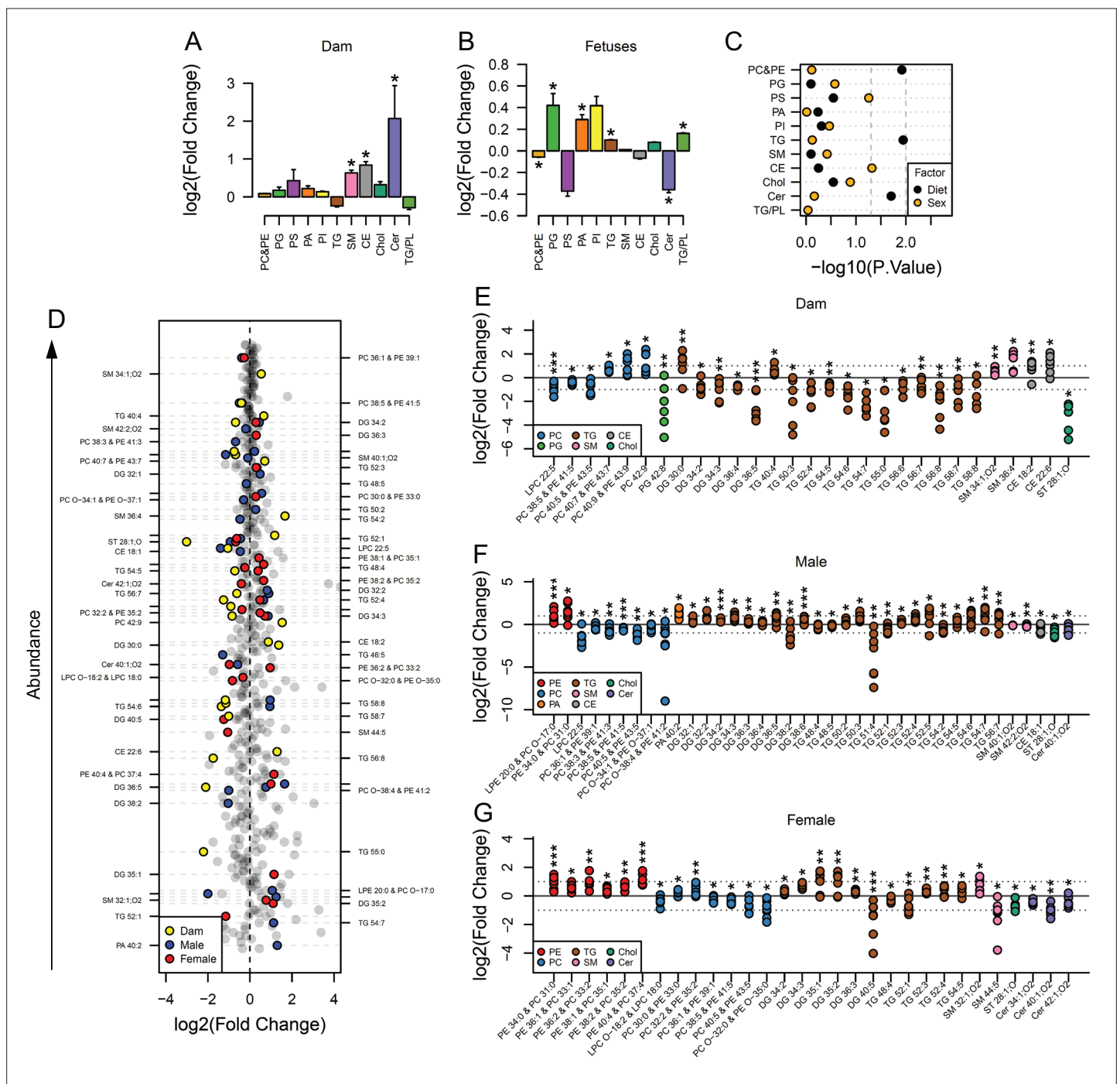


Figure 2. Maternal and fetal serum lipidome measured by direct infusion mass spectrometry. (A–B) Relative changes in serum lipid classes abundance in obese dams (A), obese fetuses (B). Values are mean + SE. * $p < 0.05$ calculated by Student t-test or Mann-Whitney test. (C) Influence of maternal diet and sex on fetal serum lipid classes abundance as calculated by factorial ANOVA. (D) Regulation of maternal and fetal serum lipid species ranked according to their abundance. Coloured dots represent statistically regulated species as calculated by univariate Student t-test ($p < 0.05$) and PLS-DA VIP (vip score > 1) in maternal or fetal OB serum compared to CTL. (E–G) Serum levels of regulated lipids from obese dams (E) and from male (F) and female (G) fetuses of obese dams at gestational day 18.5. Each dot represents a result from one obese fetus' serum relative to the average of results for individual lipids in the control group (straight line). Dam CTL $n = 9$, dam OB $n = 6$, male fetuses CTL $n = 10$, male fetuses OB $n = 8$, female fetuses CTL $n = 10$, female fetuses OB $n = 7$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by Student t-test. In figures A–C: PE, phosphatidylethanolamines/odd chain phosphatidylcholines; PC, phosphatidylcholines/odd-chain phosphatidylethanolamines; PG, phosphatidylglycerols; PS, phosphatidylserines; PA, phosphatidic acids; PI, phosphatidylinositols; TG, monoglycerides, diglycerides and triglycerides; SM, sphingomyelins; CE, cholesteryl esters; Cer, ceramides; PL, phospholipids. In figures D–G, other isobaric lipids can contribute to these signals (**Supplementary file 1**). See also **Figure 2—figure supplement 1** and **Figure 2—figure supplement 2**.

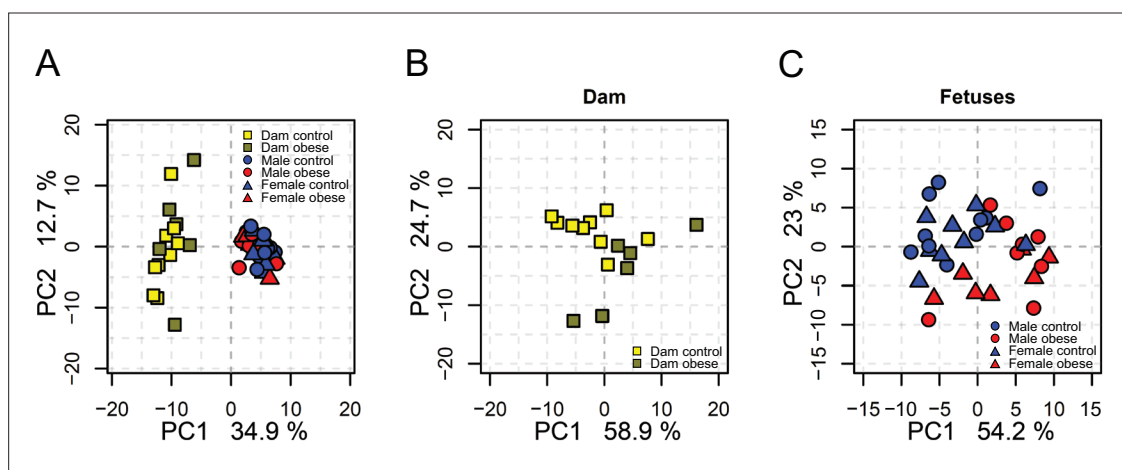
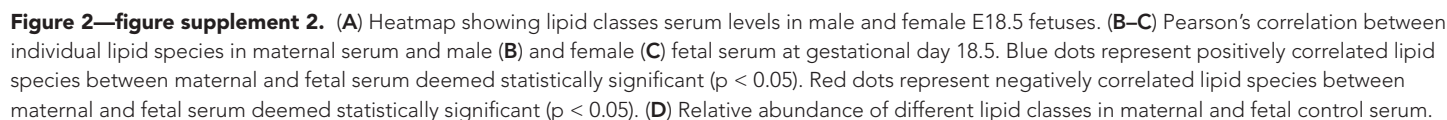


Figure 2—figure supplement 1. (A) PCA plots showing the PC1 and PC2 scores for individual dam and fetal serum lipidomes at gestational day 18.5. (B–C) PCA plots showing the PC1 and PC2 scores for individual dam (B) or fetal (C) serum lipidomes.



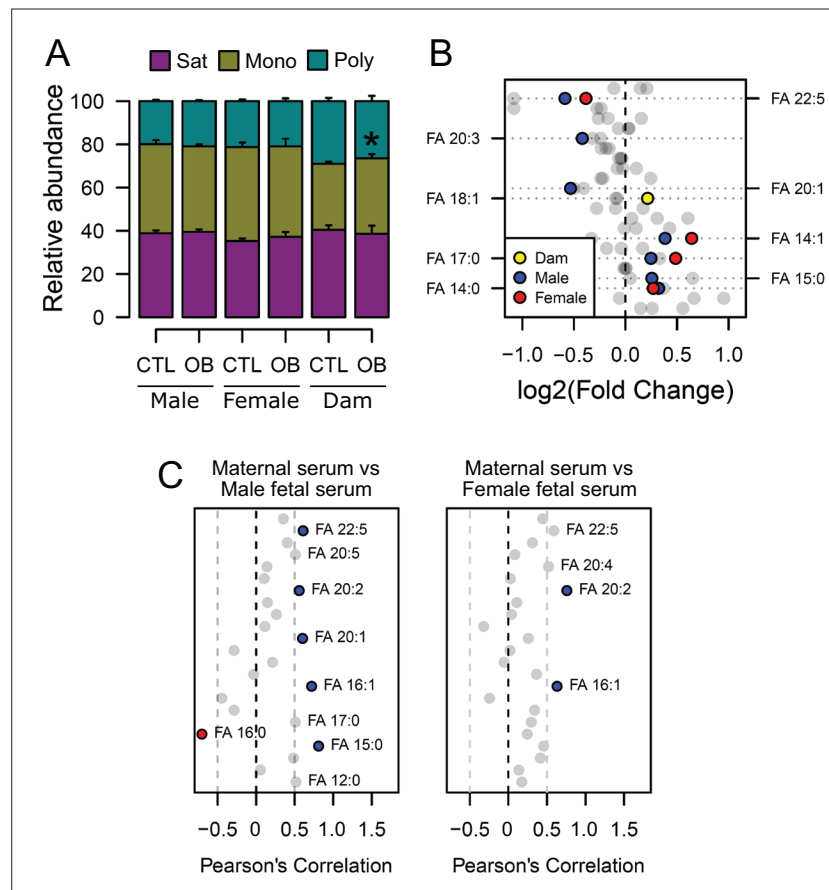


Figure 3. Fatty acid composition of serum phospholipids measured by direct infusion mass spectrometry using in-source CID fragmentation. **(A)** Grouped saturated, monounsaturated and polyunsaturated fatty acids content in maternal, male and female fetal serum at gestational day 18.5. Values are mean + SE. **(B)** Regulation of maternal and fetal serum fatty acids. Coloured dots represent statistically regulated fatty acids as calculated by univariate Student t-test or Mann-Whitney test ($p < 0.05$) in maternal or fetal OB serum compared to CTL. **(C)** Pearson's correlation between maternal serum fatty acids and the same fatty acids detected in the fetal serum. Blue and red dots represent species with significant positive and negative association ($p < 0.05$). Dam CTL $n = 8$, dam OB $n = 6$, male fetuses CTL $n = 10$, male fetuses OB $n = 8$, female fetuses CTL $n = 8$, female fetuses OB $n = 6$. See also **Figure 3—figure supplement 1** and **Figure 3—figure supplement 2**.

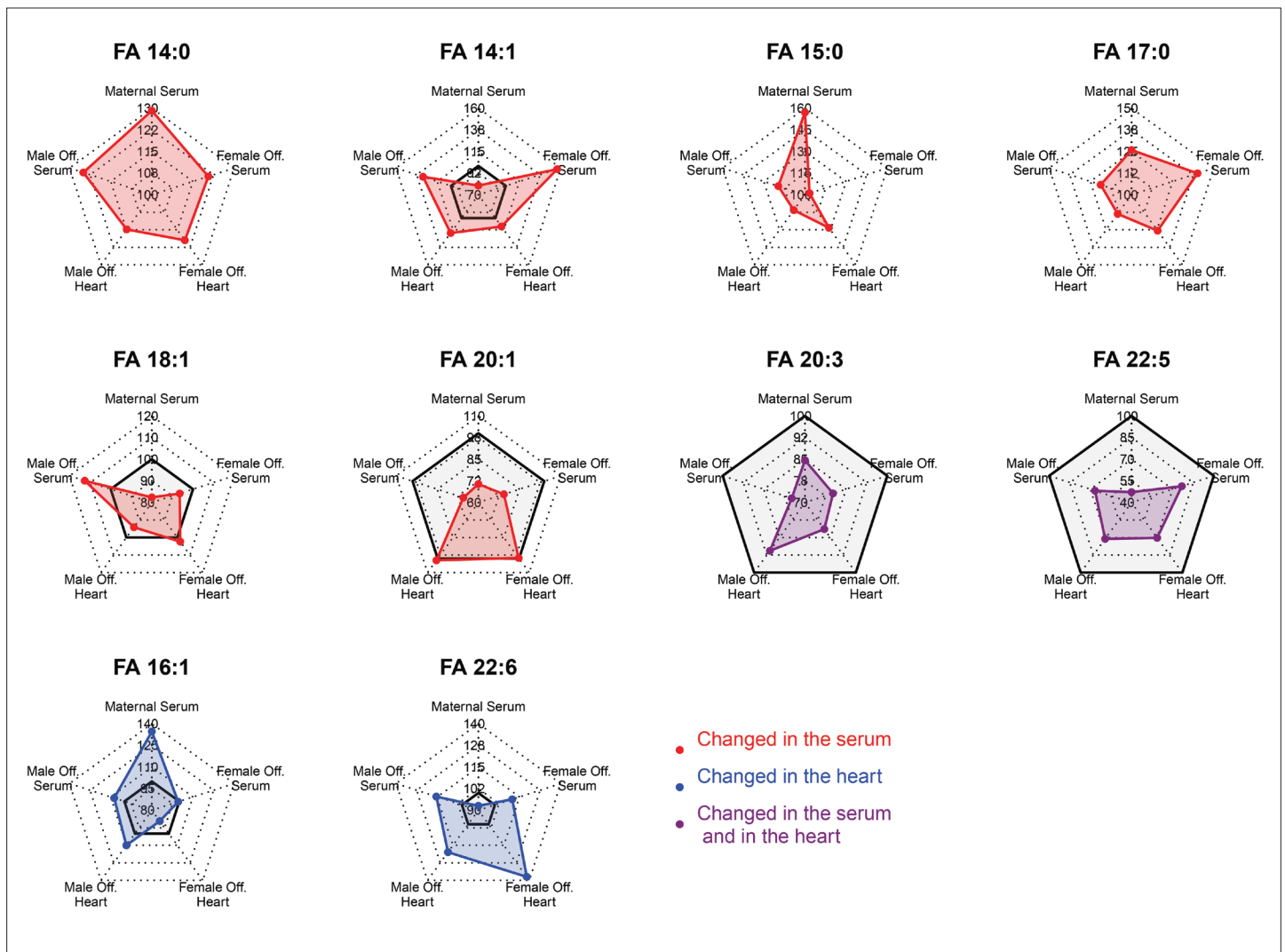


Figure 3—figure supplement 1. Radar plots showing regulation of fatty acids statistically changed in the serum or in the heart of fetuses from obese dams in different compartments. Gray-shaded area indicates negative changes.

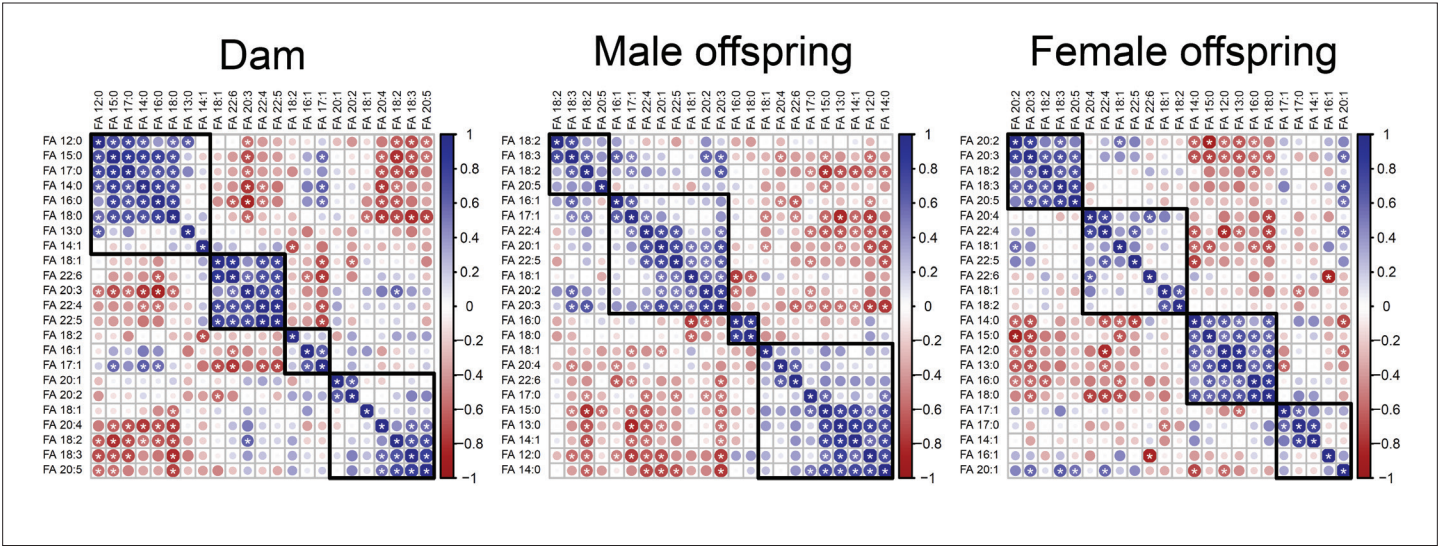


Figure 3—figure supplement 2. Correlation matrices showing Pearson's correlation between cardiac fatty acids in dams and fetuses. Fatty acids grouped following Euclidian clusterisation.

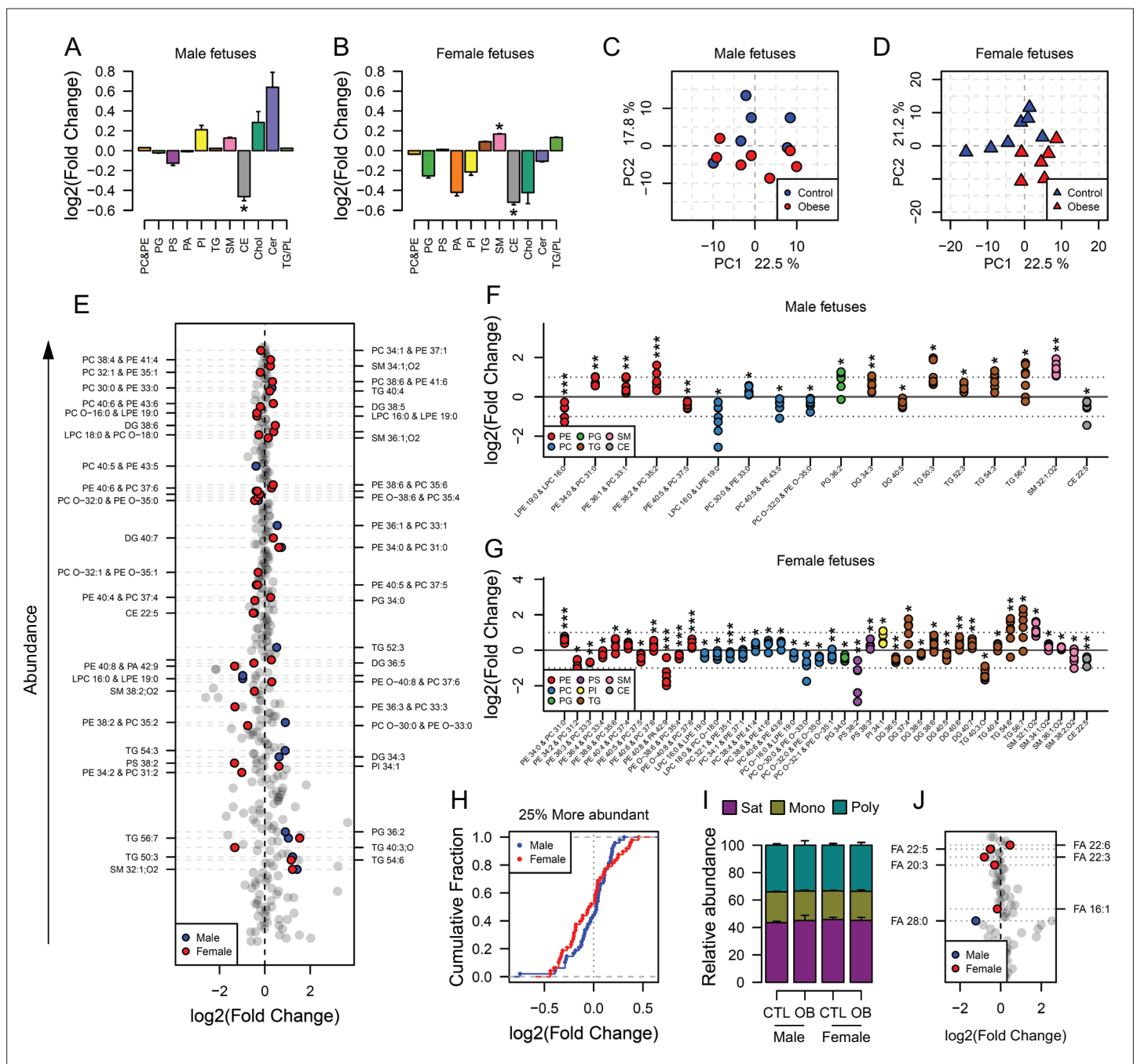


Figure 4. Maternal and fetal cardiac lipidome. (A–B) Relative changes in cardiac lipid classes in male (A) and female (B) fetuses from obese dams. Values are mean + SE. * $p < 0.05$ calculated by Student t-test or Mann-Whitney test. (C–D) PCA plots showing the PC1 and PC2 scores for individual male (C) and female (D) cardiac lipidomes. (E) Regulation of fetal cardiac lipid species ranked according to their abundance. Coloured dots represent statistically regulated species as calculated by univariate Student t-test ($p < 0.05$) and PLS-DA VIP (vip score > 1) in fetal OB hearts compared to CTL. (F–G) Cardiac levels of regulated lipids from male (F) and female (G) fetuses of obese dams at gestational day 18.5. Each dot represents a result from one obese heart, relative to the average of results for individual lipids in the control group (straight line). Male fetuses CTL $n = 6$, male fetuses OB $n = 7$, female fetuses CTL $n = 7$, female fetuses OB $n = 6$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by Student t-test. (H) Cumulative frequency of cardiac lipid species according to the log2 of the fold change in abundance between male and female fetuses from obese and control dams. (I) Grouped saturated, monounsaturated and polyunsaturated fatty acids content in male and female fetal hearts at gestational day 18.5. (J) Regulation of maternal and fetal serum fatty acids. Coloured dots represent statistically regulated fatty acids as calculated by univariate Student t-test or Mann-Whitney test ($p < 0.05$) in fetal OB hearts compared to CTL. Male fetuses CTL $n = 8$, male fetuses OB $n = 6$, female fetuses CTL $n = 7$, female fetuses OB $n = 7$. In figures A–B: PE, phosphatidylethanolamines/odd chain phosphatidylcholines; PC, phosphatidylcholines/odd-chain phosphatidylethanolamines; PC, phosphatidylcholines; PG, phosphatidylglycerols; PS, phosphatidylserines; PA, phosphatidic acids; PI, phosphatidylinositols; TG, monoglycerides,

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diglycerides and triglycerides; SM, sphingomyelins; CE, cholesteryl esters; Cer, ceramides; PL, phospholipids. In figures E-G, other isobaric lipids can contribute to these signals (**Supplementary file 2**). See also **Figure 4—figure supplement 1** and **Figure 4—figure supplement 2**.

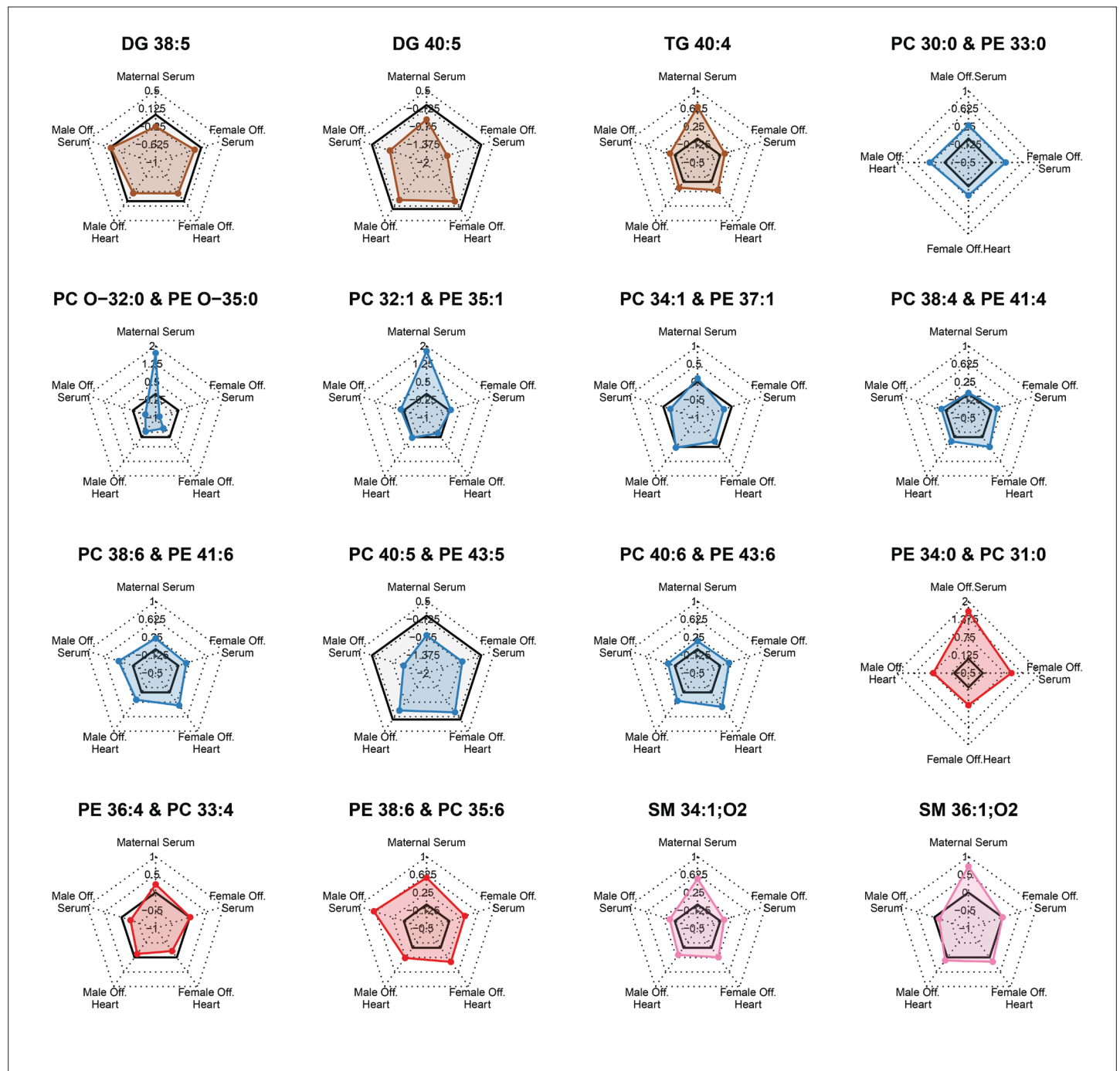


Figure 4—figure supplement 1. Radar plots showing regulation of most abundant statistically regulated lipids in the heart of fetuses from obese pregnancies in different compartments. Grey-shaded area indicates negative regulation.

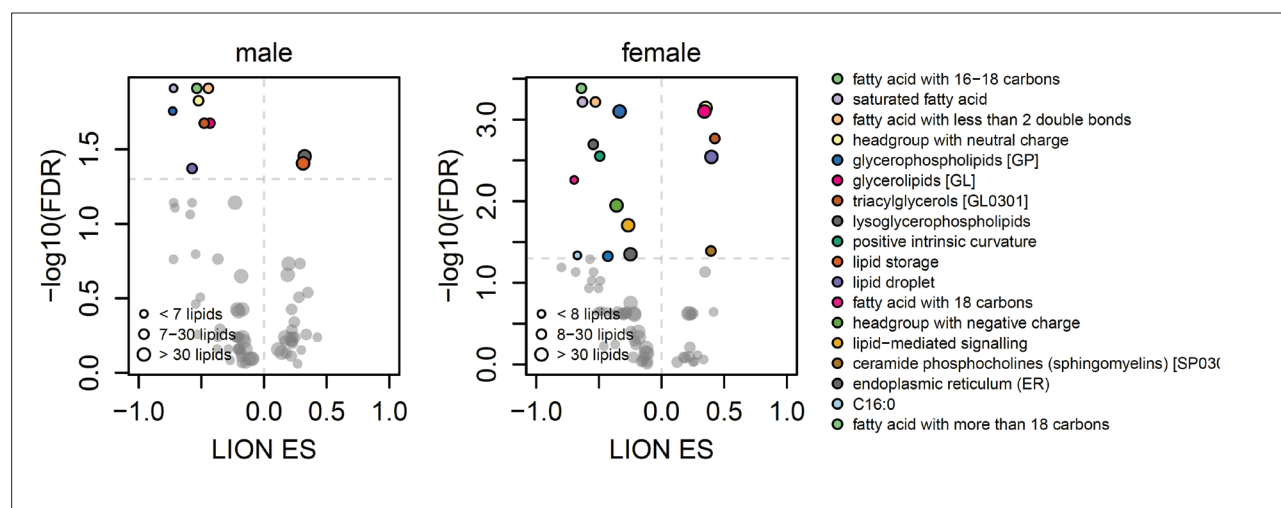


Figure 4—figure supplement 2. Scatterplots showing enrichment score (ES) and statistical significance of lipid ontology pathways from LION.

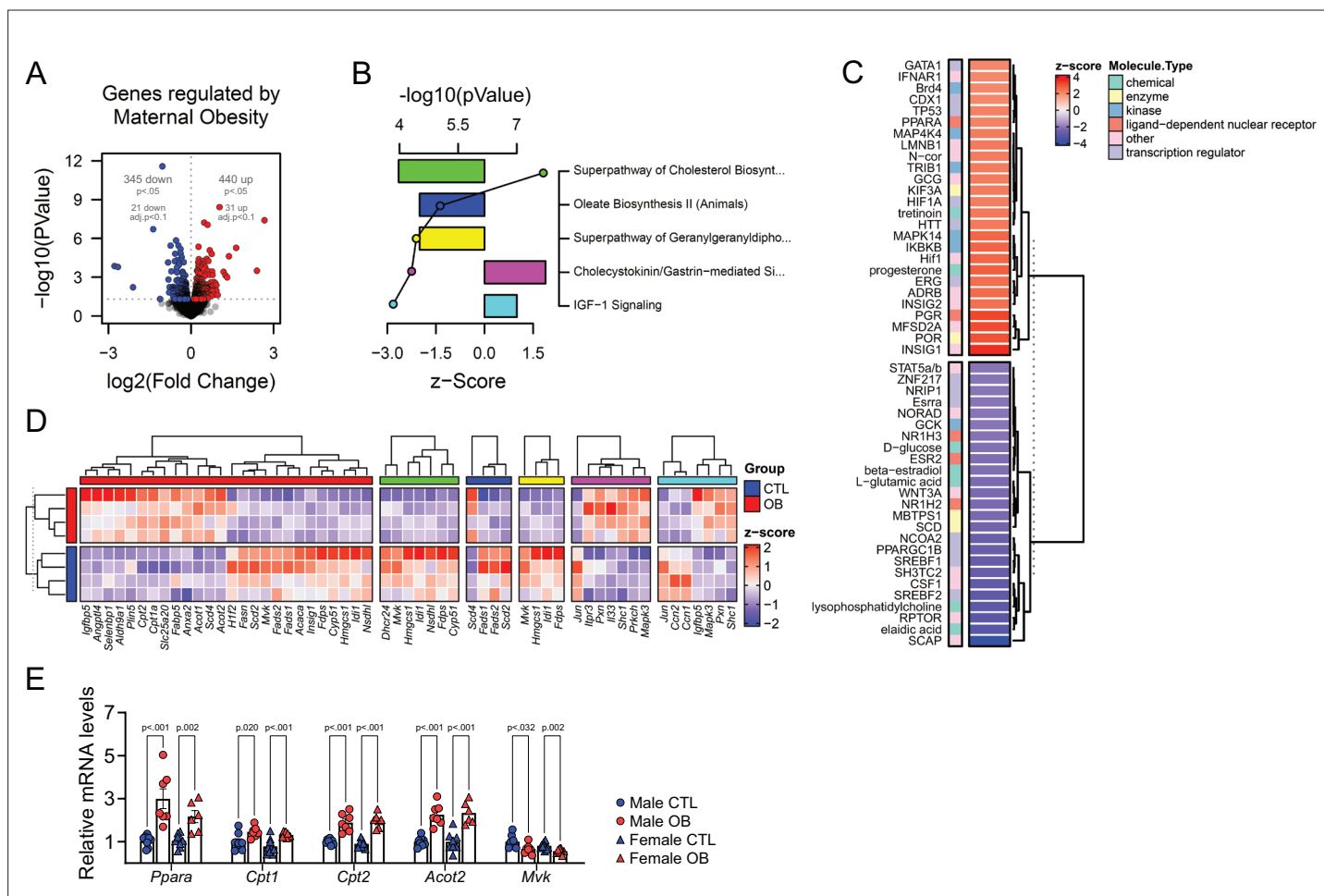


Figure 5. Fetal cardiac transcriptomics. (A) Volcano plot showing up and downregulated genes in the heart of obese fetuses. $p < 0.05$ and $\text{FDR} < 0.1$ by generalised linear models with quasi-likelihood tests. Full statistical data is available in **Figure 5—source data 1**. (B) Top five regulated Ingenuity Canonical Pathways predicted by analysis of cardiac transcriptome from male fetuses from obese dams compared to fetuses from control dams. A p-value cut-off of 0.01 calculated by likelihood-ratio test was used to select regulated genes included in the IPA analysis. Bars represent activation z-score per pathway; points represent p-value of enriched pathways estimated by IPA algorithm. (C) Activation z-score of top Ingenuity Upstream Regulators predicted by analysis of cardiac transcriptome from male fetuses from obese dams compared to fetuses from control dams. The complete lists of regulated IPA canonical pathways and IPA upstream regulators are available in **Figure 5—source data 2** and **Figure 5—source data 3**, respectively (D) Heatmap showing mRNA levels of genes regulated by PPAR-alpha activity (red bar), and genes mapped to 'Superpathway of Cholesterol Biosynthesis' (green bar), 'Oleate Biosynthesis II' (blue bar), 'Superpathway of Geranylgeranyldiphosphate Biosynthesis I' (yellow bar), 'Cholecystokinin/Gastrin-mediated Signalling' (pink bar) and 'IGF-1 Signalling' (light blue bar) Ingenuity Canonical Pathways in male E18.5 hearts as analysed by RNA Seq. CTL $n = 4$ and OB $n = 4$. (E) mRNA levels of selected markers of lipid metabolism in male and female fetal hearts. Male CTL $n = 8$, male OB $n = 8$, female CTL $n = 6$, female OB $n = 11$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Student t-test. Diet *** $p < 0.001$ by factorial ANOVA. Primer sequences are available in **Table 1**.

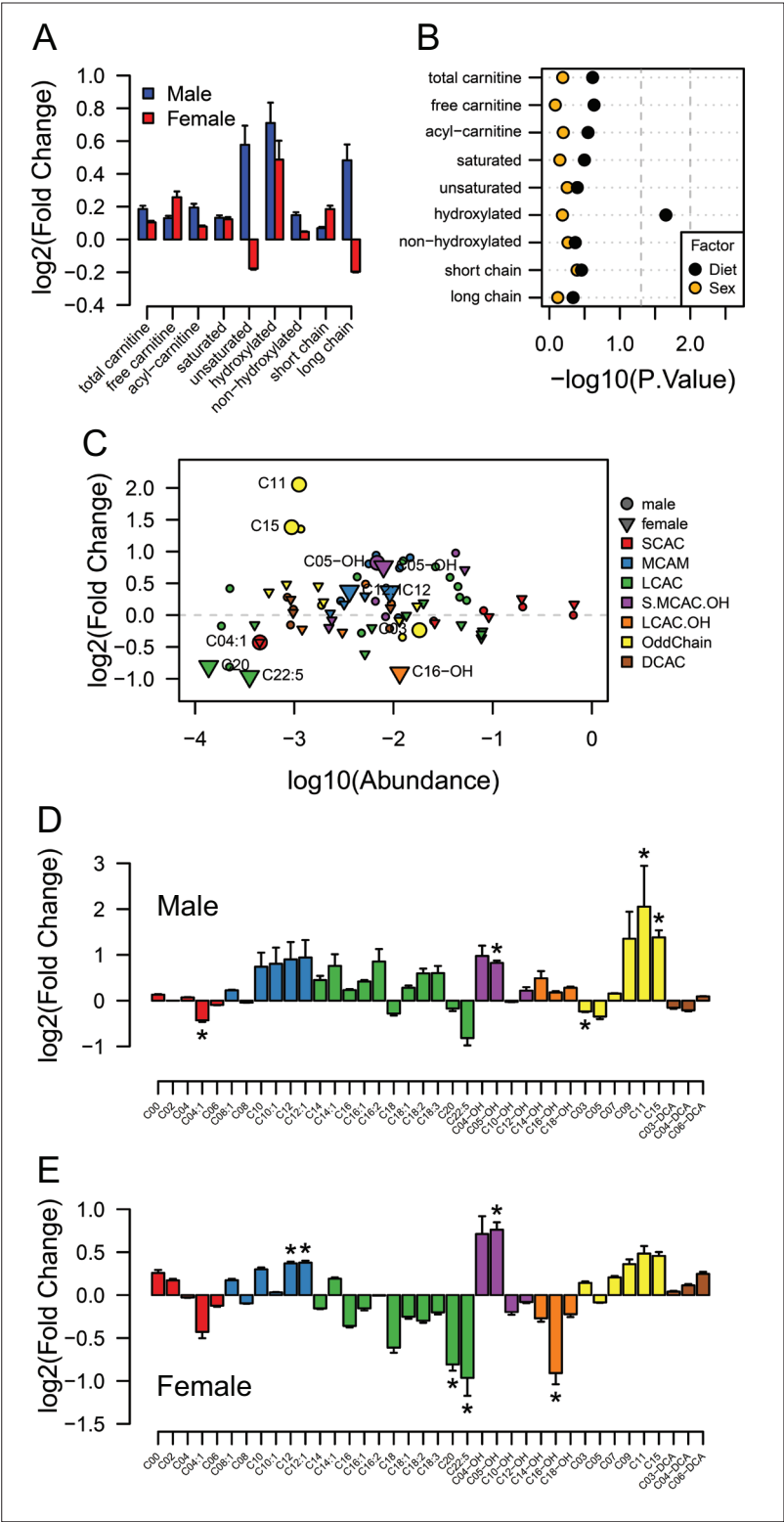


Figure 6. Acyl-carnitine levels in fetal hearts measured by LC-MS. **(A)** Relative changes in cardiac carnitine levels in male and female fetuses from obese dams. **(B)** Influence of maternal diet and sex on fetal cardiac carnitine classes levels as calculated by factorial ANOVA. **(C)** Relative fold change of individual acyl-carnitine levels in the heart of E18.5 fetuses from obese dams according to their abundance. Larger figures are acyl-carnitine species deemed as regulated with $p < 0.05$ by Student t-test or Mann-Whitney test. SCAC: small-chain acyl-carnitine; MCAC: medium-chain acyl-carnitine; LCAC: long-chain acyl-carnitine; S.MC.AC.OH: small- and medium-chain

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hydroxy acyl-carnitine, LCAC.OH: Long-chain hydroxy acyl-carnitine; Odd Chain: acyl-carnitines with an odd chain number; DCAC: dicarboxylic acyl-carnitines. **(D–E)** Individual acyl-carnitine species levels in male **(D)** and female **(E)** fetal hearts at 18.5 days of pregnancy. See **Supplementary file 3** for list of full names. * $p < 0.05$ by Student t-test or Mann-Whitney test. Male fetuses CTL $n = 7$, male fetuses OB $n = 7$, female fetuses CTL $n = 7$, female fetuses OB $n = 6$.

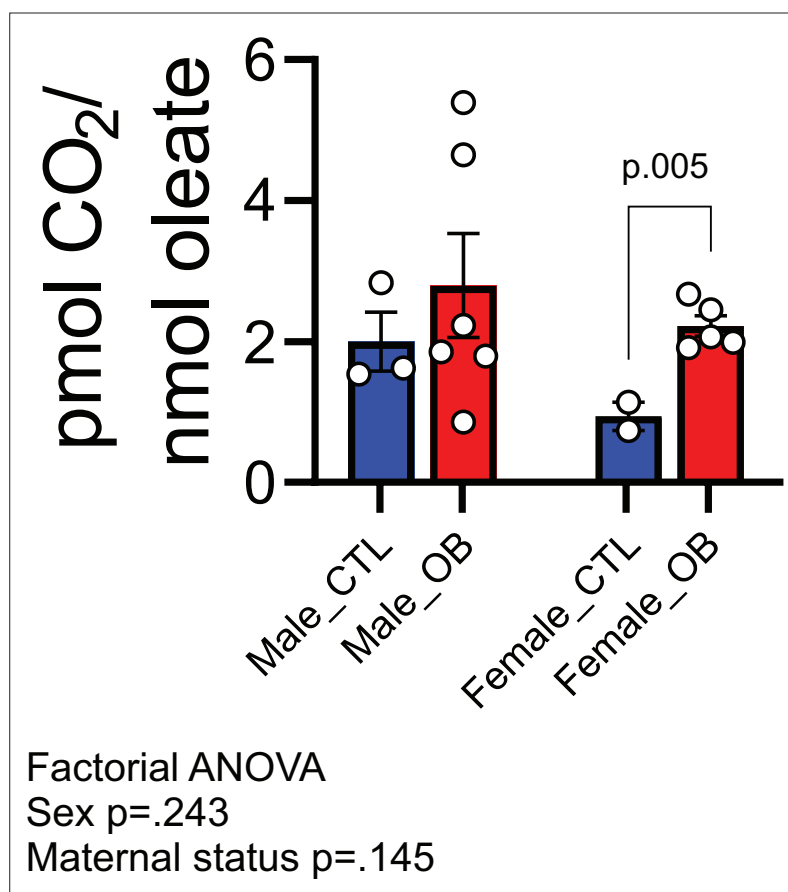


Figure 6—figure supplement 1. Estimated pmol [¹⁴C]-CO₂/ nmol [1-¹⁴C]-oleate produced by male and female fetal cardiomyocytes from CTL and OB dams at gestational day 18.5. Male CTL n = 3, male OB n = 6, female CTL n = 2, female OB n = 5.