# A missense variant in human perilipin 2 (*PLIN2* Ser251Pro) reduces hepatic steatosis in mice

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Table of contents

**Supplementary materials:**

***Animal experiment***

Experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. All efforts were made to treat animals with humane care and minimize their discomfort. Mice were kept in barrier facilities (12-hour light/12-hour dark cycle) with *ad libitum* food and water, unless stated. Littermate controls were utilized.

*Adfp*D2–3 mice were provided by Dr. Palczewski. *Adfp*D2–3 mice lack exons 2 and 3 of the *Adfp* gene but do have mRNA expression of an unstable short-form *Adfp* (1). We crossed these mice from Balb/c to C57B6/J mice for more than ten generations. We called these mice *Plin2* KO-mice (1).

***Sphingolipid and ceramide analyses***

Liver tissue was homogenized in a RIPA buffer (50 mM Tris (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1% v/v NP-40, 0.5% w/v sodium deoxycholate, 0.1% v/v SDS, 50 mM sodium fluoride). Protein concentrations were determined by BCA assay. Liver homogenate and plasma samples were analyzed by high performance liquid chromatography-tandem mass spectrometry for ceramide content at the Lipidomics Shared Resource at the Medical University of South Carolina. Hepatic ceramide data were normalized to total protein levels.

***Lipidomic***

*Tissue preparation for lipids extraction*

Sections of approximately 10 mg of frozen tissues were cut on a tile kept in dry ice with a new blade kept in dry ice. The tissue was added to a microcentrifuge tube with 0.6 mL 80% methanol (MeOH) and 20 µL on internal standard mix (1:1, SPLASH® LIPIDOMIX #330707 and Ceramide/Sphingoid Internal Standard Mixture I #LM6002, both from Avanti Polar Lipids, Alablaster, AL) and kept in dry ice. Samples were omogenised by pulse sonication (30 half-second pulse on ice) and incubated on ice for 20 min for metabolites extraction. Each tube was then vortexed 3x 30 seconds each. The tissue homogenates were then moved to a 10 mL glass borosilicate tube with a screw cap. The remaining tissue in the microcentrifuge tubes was rinsed into the glass tube with 0.5 mL MeOH. In each of the tubes, 5 mL methyl tert-butyl ether (MTBE) were added and then tubes were shaken vigorously for 30 min. In each tube, 1.2 mL water was added and then tubes vortexed for 30 sec each. Centrifugation for 10 min @ 1000xg created two phases. The top clear phase was moved to a clean glass Pyrex tube and dried down under

nitrogen. 100 µL MTBE/MeOH=1/3 (v/v) was used to re-suspend the residue. The sample was spun down at 10, 000 x g for 10 min at 4°C and only the top 50 µL were transferred to a HPLC vial for LC-MS analysis. A pooled sample was created by mixing 10 µL of each re-suspended sample. 2 µL injections were made.

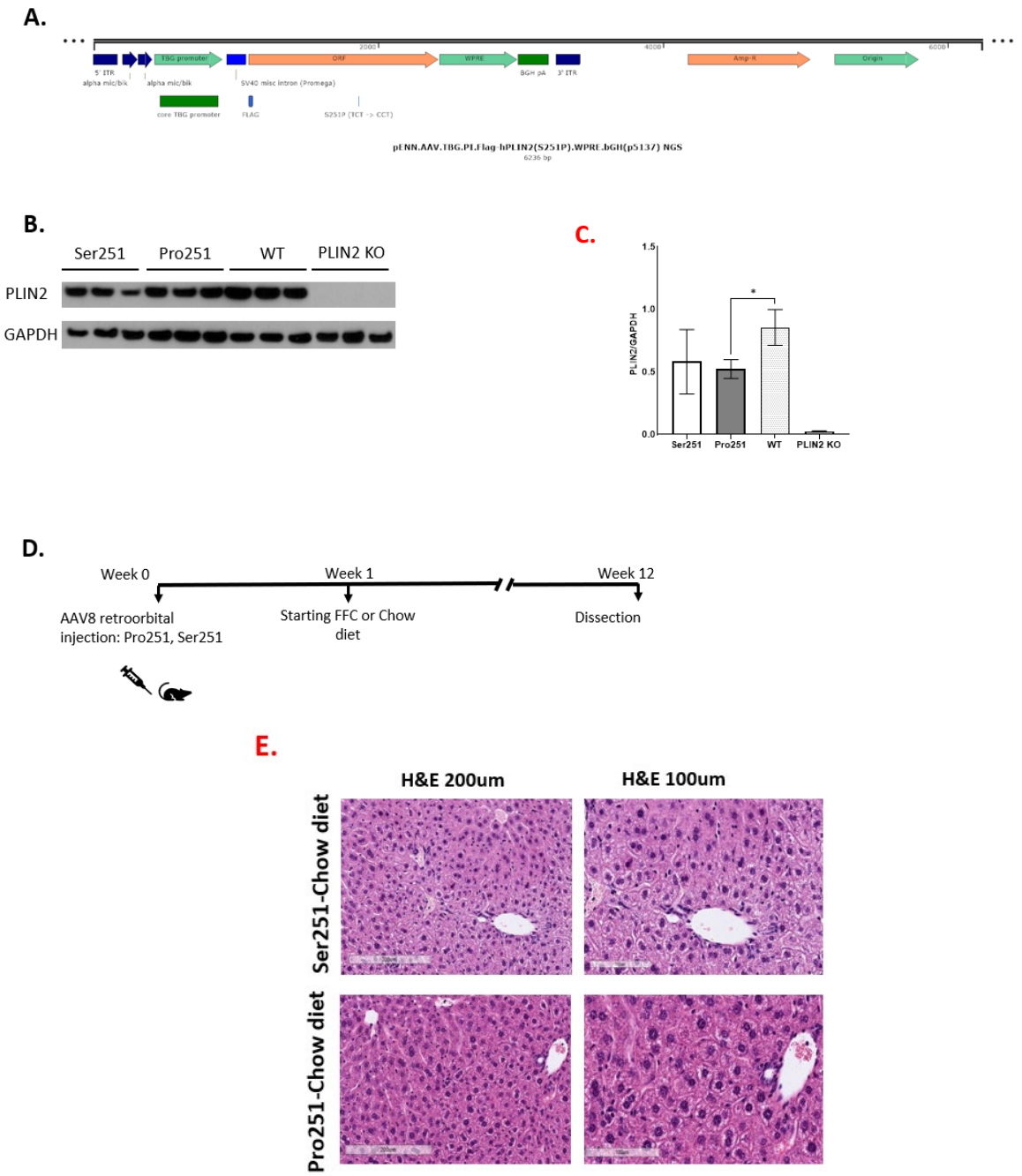
*Liquid chromatography high resolution -mass spectrometry (LC-HRMS) for lipids.*

Metabolites were separated using a Ascentis Express C18, 2.1 × 150 mm 2.7µm column (Sigma-Aldrich, St. Louis, MO) on an UltiMate 3000 HPLC system. The metabolites were eluted on a 0.4 m/min flow-rate gradient using Solvent A (4:6 v/v water:acetonitrile, 0.1% formic acid, 10 mM ammonium formate) and Solvent B (1:9 v/v acetonitrile:isopropanol, 0.1% formic acid, 10 mM ammonium formate). The gradient was as follows: 10% B at 0 min, 10% B at 1 min, 40% B at 4 min, 75% B at 12 min, 99% B at 21 min, 99% B at 24 min, 10% B at 24.5 min, 10% at 30 min. Separations were performed at 55 °C.

For the HRMS analysis, a recently calibrated QE Exactive-HF mass spectrometer (Thermo Fisher Scientific) was used in positive ion mode with an HESI source. The operating conditions were: spray voltage at 3.5 kV; capillary temperature at 285°C; auxiliary temperature 370°C; tube lens 45. Nitrogen was used as the sheath gas at 45 units, the auxiliary gas at 10 units and sweep gas was 2 units. The same MS conditions were used in negative ionization mode, but with a spray voltage at 3.2 kV. Control extraction blanks were made in the same way using just the solvents instead of the tissue homogenate. The control blanks were used for the exclusion list with a threshold feature intensity set at 1e10^5. Untargeted analysis and targeted peak integration was conducted using LipidsSearch 4.2 (Thermo Fisher Scientific) as described by Wang et al ( DOI: 10.4155/bio-2021-0098).

An external mass calibration was performed using the standard calibration mixture approximately every three days. All samples were analyzed in a randomized order in full scan MS that alternated with MS2 of top 20, with HCD scans at 30, 45 or 60 eV. Full scan resolution was set to 120,000 in the scan range between m/z 250–1800. The pool sample was run every 15 samples. Lipids quantification was done from the full scan data. The areas were normalized based on the amount of the internal standard added for each class (as found in the Table x1). All amounts were normalized to the original tissue weight.

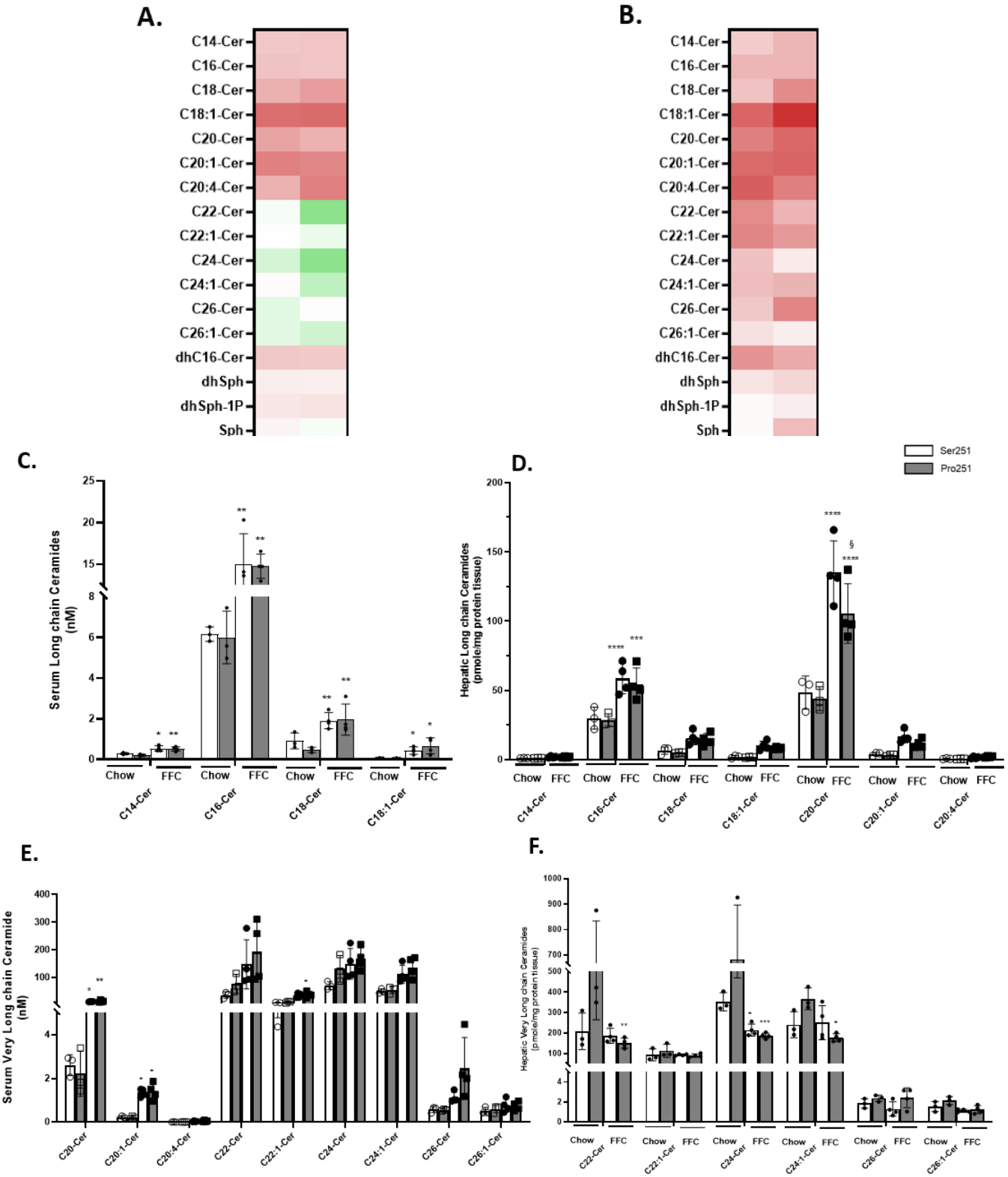
***Fig. S1***

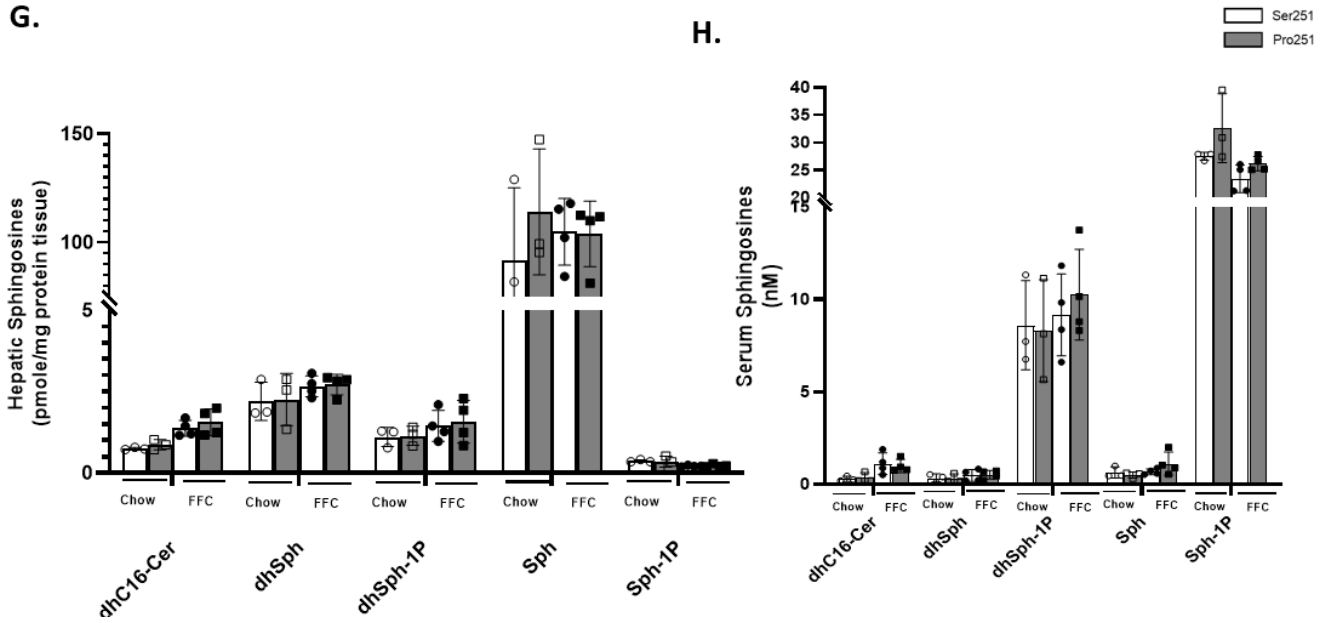



Generation of a mouse model of *Plin2*-Pro251 in hepatocytes and experimental design. Map of the *Plin2*-Pro251 plasmid (A), hepatic PLIN2 protein and mRNA expression (B-C), study design (D). Liver histology for the Pro251 and Ser251 groups on chow diet (E). PLIN2 relative protein

levels by Western Blotting were normalized to GAPDH (B and C). Statistical analyses were performed using a two-tailed unpaired *t*-test or one-way ANOVA with Tukey's posthoc test. *Indicates statistical difference between diets, *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.0001. §Indicates statistical difference between genotypes, §P < 0.05; §§P < 0.005; §§§P < 0.0005; §§§§P < 0.0001.

*Fig. S2*

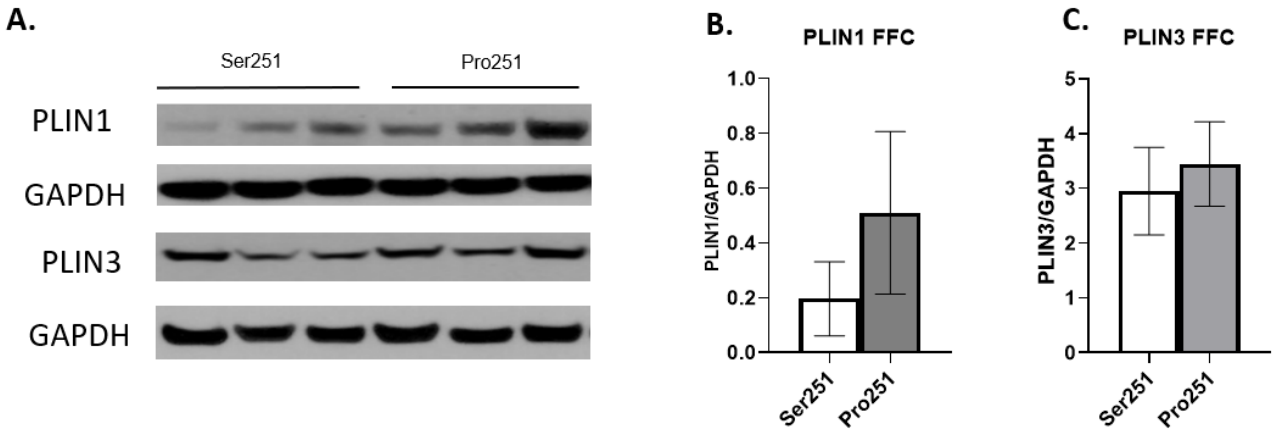

A.
B.
C14-Cer
C16-Cer
C18-Cer
C18:1-Cer
C20-Cer
C20:1-Cer
C20:4-Cer
C22-Cer
C22:1-Cer
C24-Cer
C24:1-Cer
C26-Cer
C26:1-Cer
dhC16-Cer
dhSph
dhSph-1P
Sph
C.
Serum Long chain Ceramides (nM)
D.
Hepatic Long chain Ceramides (pmole/mg protein tissue)
Ser251
Pro251
Chow
FFC
E.
Serum Very Long chain Ceramide (nM)
F.
Hepatic Very Long chain Ceramides (pmole/mg protein tissue)




In the heatmap, the color code indicates the log2 of the ratio between means of the groups for an individual ceramide. A more intense red color indicates a greater increase of absolute concentration of the individual ceramide in the liver (A) and serum (B). Serum long chain ceramides (C), hepatic long chain ceramides (D), serum very long chain ceramides (E), hepatic very long chain ceramides (F), hepatic sphingolipids (G) and serum sphingolipids (H). Statistical analyses were performed using a two-tailed unpaired *t*-test or one-way ANOVA with Tukey's posthoc test. *Indicates statistical difference between diets, *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.0001. §Indicates statistical difference between genotypes, §P < 0.05; §§P < 0.005; §§§P < 0.0005; §§§§P < 0.0001.

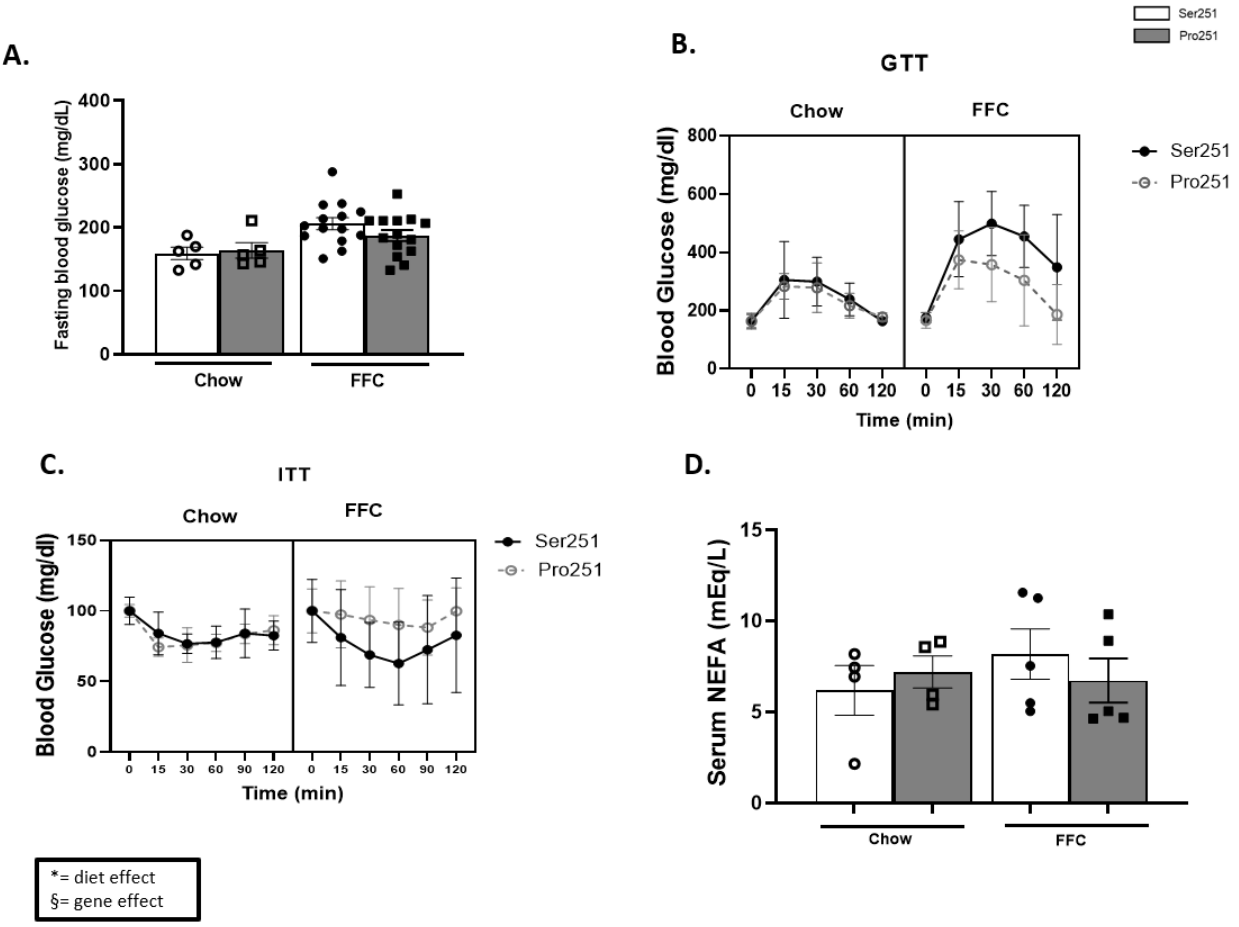
***Fig. S3***

PLIN1, PLIN3 relative protein levels by Western Blotting were normalized to GAPDH (B and C).



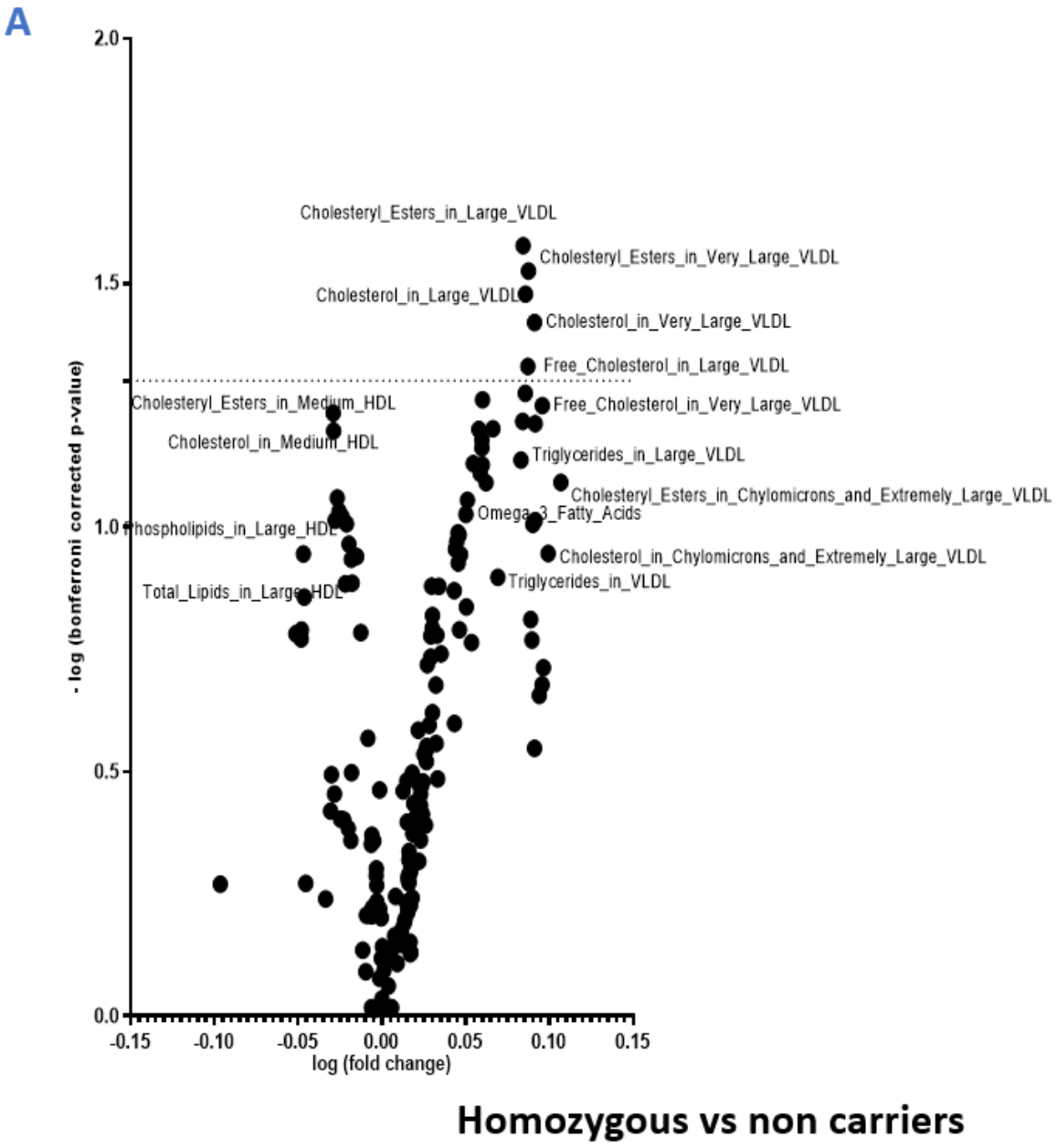

Statistical analyses were performed using a two-tailed unpaired *t*-test or one-way ANOVA with Tukey's posthoc test. *Indicates statistical difference between diets, *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.0001. §Indicates statistical difference between genotypes, §P < 0.05; §§P < 0.005; §§§P < 0.0005; §§§§P < 0.0001.

***Fig. S4***

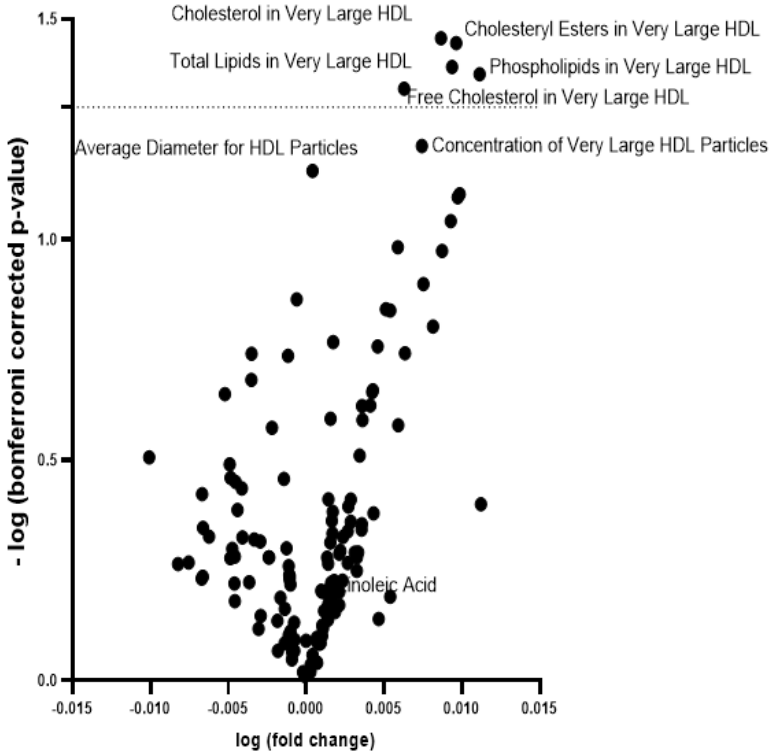



Fasting blood glucose (A), GTT (B), ITT (C), and serum NEFA (D). Statistical analyses were performed using a two-tailed unpaired *t*-test or one-way ANOVA with Tukey's posthoc test. *Indicates statistical difference between diets, *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.0001. §Indicates statistical difference between genotypes, §P < 0.05; §§P < 0.005; §§§P < 0.0005; §§§§P < 0.0001.

***Fig. S5.***

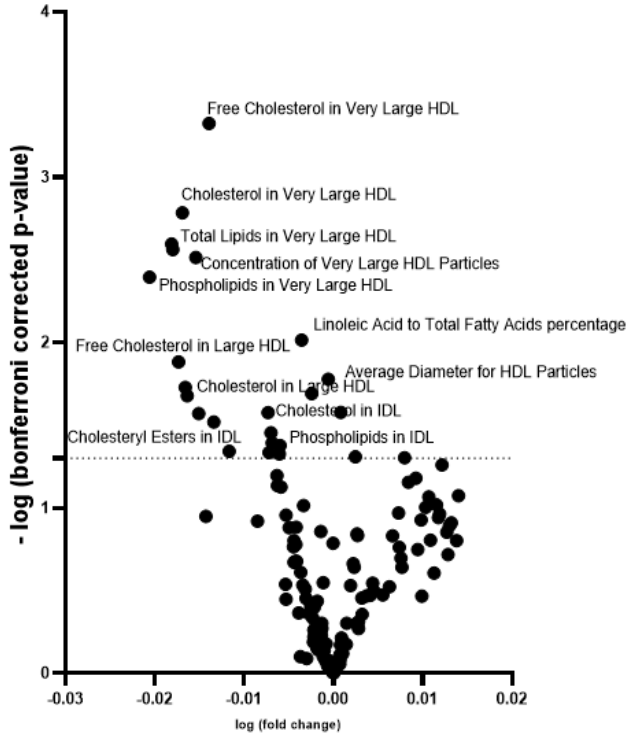

A
2.0
1.5
1.0
0.5
0.0
- log (bonferroni corrected p-value)
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
log (fold change)
Cholesteryl_Esters_in_Large_VLDL
Cholesteryl_Esters_in_Very_Large_VLDL
Cholesterol_in_Large_VLDL
Cholesterol_in_Very_Large_VLDL
Free_Cholesterol_in_Large_VLDL
Cholesteryl_Esters_in_Medium_HDL
Free_Cholesterol_in_Very_Large_VLDL
Cholesterol_in_Medium_HDL
Triglycerides_in_Large_VLDL
Cholesteryl_Esters_in_Chylomicrons_and_Extremely_Large_VLDL
Omega_3_Fatty_Acids
Phospholipids_in_Large_HDL
Cholesterol_in_Chylomicrons_and_Extremely_Large_VLDL
Triglycerides_in_VLDL
Total_Lipids_in_Large_HDL
Homozygous vs non carriers

B.




Male homozygous vs non carriers

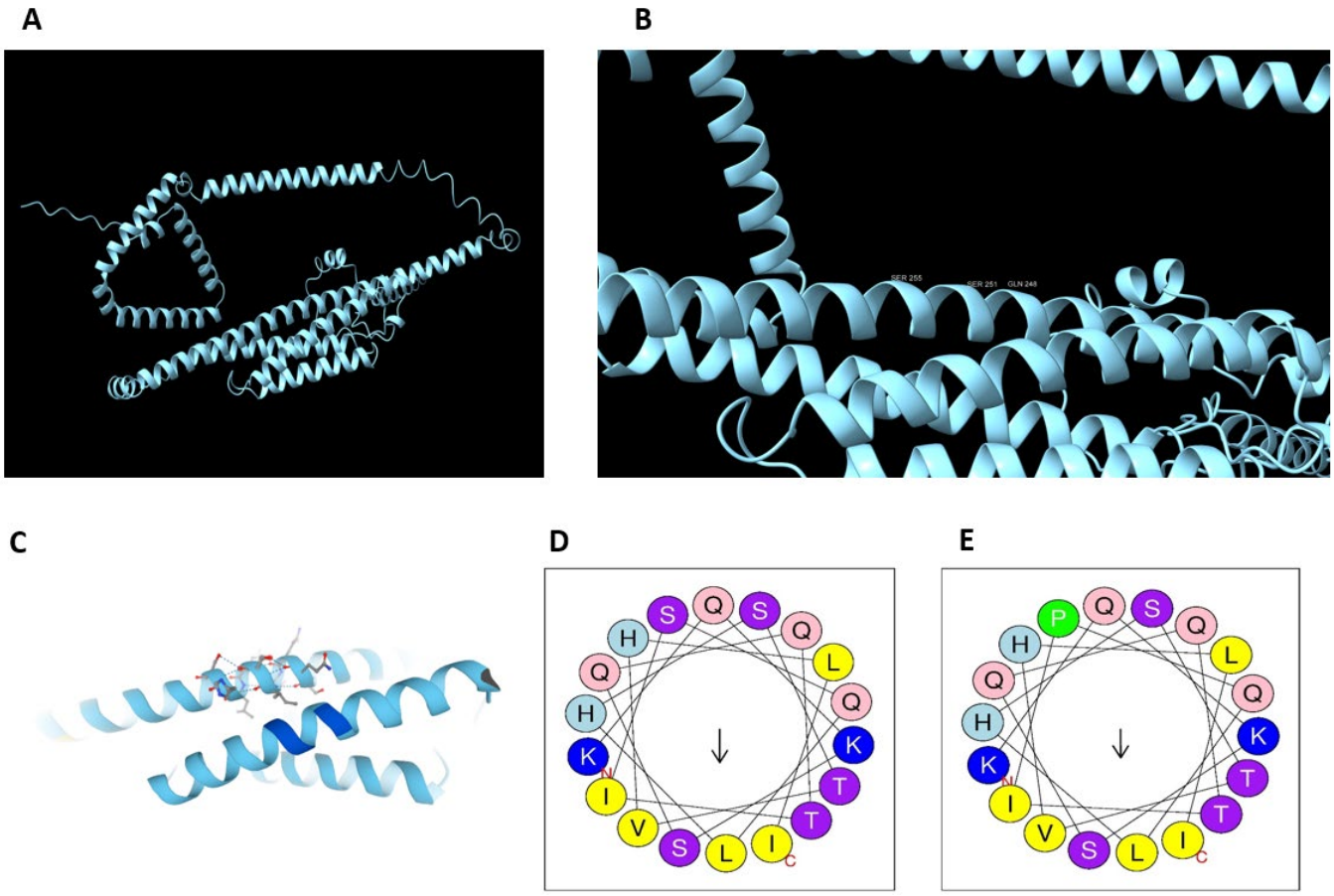
C.




Female homozygous vs non carriers

Volcano plot for the lipidomic parameters derived from the UKB database (A), volcano plot for the lipidomic parameters in male (B), volcano plot for the lipidomic parameters in female (C). The x-axis displays the log fold change, comparison between Homozygous Pro251 carriers and non-carriers. The dotted line marks the Bonferroni significant.

***Fig. S6***




Structure of human perilipin 2 (437 amino acids) and predicted by AlphaFold (A); Expanded view of PLIN2 structure showing that S251 is situated in the 4-helix bundle domain and in the middle of a long helix that spans residues from T225 to L281 (B); Further expansion of structure to show hydrogen bonding interactions of S251 (C). Helical wheel of residues spanning from K243 (labeled with a red N) to I260 (labeled with a red C) in wild-type human PLIN2 (D); Helical wheel of residues spanning from K243 to I260 in the S251P variant of human PLIN2 (E).

## *Table S1*

**Change in mice liver triglyceride species in both chow and FFC diet groups. Comparison between *Pro251* and *Ser251*.**

| | Chow diet | | FFC diet | |
|---|---|---|---|---|
| **Triglycerides** | **Log2 (Fold change)** | **P value** | **Log2 (Fold change)** | **P value** |
| TG(12:0e_6:0_20:4) | -0.4 | 0.252 | -1.62 | 0.128 |
| TG(12:1e_6:0_22:6) | -0.4 | 0.302 | -1.43 | 0.155 |
| TG(14:0_18:2_18:3) | -0.9 | 0.132 | -1.95 | 0.077 |
| TG(14:0_22:6_22:6) | -1.2 | 0.005 | -1.03 | 0.085 |
| TG(15:0_16:0_18:1) | -1.4 | 0.094 | -1.60 | 0.077 |
| TG(15:0_16:1_18:1) | -0.5 | 0.245 | -2.14 | 0.056 |
| TG(15:0_16:1_18:2) | -1.0 | 0.126 | -2.07 | 0.066 |
| TG(15:0_18:1_18:2) | -1.0 | 0.063 | -1.91 | 0.109 |
| TG(15:0_18:1_20:5) | -1.6 | 0.205 | -2.14 | 0.236 |
| TG(15:0_18:1_22:6) | -1.0 | 0.034 | -0.90 | 0.205 |
| TG(15:0_18:2_18:2) | -0.8 | 0.047 | -1.81 | 0.116 |
| TG(15:0_18:2_18:3) | -1.0 | 0.113 | -0.89 | 0.608 |
| TG(15:0_18:2_20:5) | -1.1 | 0.220 | -2.37 | 0.105 |
| TG(15:0_18:2_20:5) | -0.4 | 0.499 | -1.55 | 0.222 |
| TG(15:0_18:2_22:6) | -0.8 | 0.031 | -1.19 | 0.109 |
| TG(15:0_22:6_22:6) | -1.2 | 0.006 | -0.83 | 0.202 |
| TG(16:0_10:0_18:2) | -1.1 | 0.046 | -1.63 | 0.184 |
| TG(16:0_12:1_18:1) | -0.5 | 0.359 | -2.29 | 0.056 |
| TG(16:0_12:1_18:2) | -0.1 | 0.771 | -1.79 | 0.164 |
| TG(16:0_14:0_16:0) | -1.0 | 0.337 | -1.94 | 0.066 |
| TG(16:0_14:0_18:1) | -1.3 | 0.178 | -1.99 | 0.046 |
| TG(16:0_14:0_18:2) | -0.5 | 0.536 | -2.20 | 0.072 |
| TG(16:0_14:0_22:6) | -1.2 | 0.028 | -1.59 | 0.050 |
| TG(16:0_14:2_22:6) | -1.5 | 0.158 | -1.99 | 0.096 |
| TG(16:0_16:0_16:0) | -1.2 | 0.165 | -1.56 | 0.055 |
| TG(16:0_16:0_17:0) | -1.1 | 0.141 | -0.78 | 0.213 |
| TG(16:0_16:0_18:1) | -1.3 | 0.067 | -1.90 | 0.094 |
| TG(16:0_16:0_24:0) | -0.8 | 0.314 | -0.39 | 0.415 |
| TG(16:0_16:1_16:1) | -0.6 | 0.469 | -2.26 | 0.067 |
| TG(16:0_16:1_18:1) | -1.2 | 0.134 | -2.11 | 0.149 |
| TG(16:0_16:1_18:3) | -1.2 | 0.176 | -1.94 | 0.089 |
| TG(16:0_17:0_18:1) | -1.3 | 0.060 | -1.42 | 0.120 |
| TG(16:0_17:1_18:1) | -1.5 | 0.069 | -1.60 | 0.092 |
| TG(16:0_18:1_18:1) | -1.9 | 0.008 | 0.78 | 0.593 |

| | | | | |
|---|---|---|---|---|
| TG(16:0_18:1_18:1) | -1.2 | 0.033 | -1.65 | 0.100 |
| TG(16:0_18:1_18:1) | -0.8 | 0.386 | 1.90 | 0.467 |
| TG(16:0_18:1_18:2) | -0.2 | 0.581 | -2.88 | 0.301 |
| TG(16:0_18:1_19:0) | -0.9 | 0.186 | -1.49 | 0.090 |
| TG(16:0_18:1_20:4) | -1.1 | 0.040 | -1.13 | 0.051 |
| TG(16:0_18:1_21:0) | -1.5 | 0.173 | -1.11 | 0.096 |
| TG(16:0_18:1_22:0) | -0.9 | 0.217 | -0.83 | 0.227 |
| TG(16:0_18:1_22:6) | -0.9 | 0.013 | -1.24 | 0.111 |
| TG(16:0_18:1_23:0) | -0.7 | 0.340 | -0.63 | 0.287 |
| TG(16:0_18:1_24:0) | -0.7 | 0.339 | -0.72 | 0.346 |
| TG(16:0_18:2_18:3) | 0.1 | 0.860 | -3.25 | 0.215 |
| TG(16:0_18:3_22:6) | 0.1 | 0.899 | -1.91 | 0.055 |
| TG(16:0_20:5_22:6) | -0.9 | 0.030 | -1.47 | 0.078 |
| TG(16:0_22:6_22:6) | -1.4 | 0.026 | -1.16 | 0.103 |
| TG(16:0_6:0_18:1) | -0.5 | 0.643 | -1.36 | 0.221 |
| TG(16:1_12:1_18:1) | -1.0 | 0.106 | -1.87 | 0.113 |
| TG(16:1_14:0_18:2) | -1.4 | 0.081 | -2.41 | 0.053 |
| TG(16:1_14:1_18:2) | -0.3 | 0.355 | -2.20 | 0.059 |
| TG(16:1_16:1_18:1) | -1.0 | 0.112 | -1.91 | 0.150 |
| TG(16:1_18:1_18:1) | -0.9 | 0.030 | -1.65 | 0.065 |
| TG(16:1_18:1_18:2) | -1.1 | 0.008 | -1.32 | 0.361 |
| TG(16:1_18:2_18:2) | 2.4 | 0.001 | -1.11 | 0.190 |
| TG(16:1_18:2_18:2) | -1.3 | 0.027 | -2.29 | 0.090 |
| TG(16:1_18:2_18:3) | -0.8 | 0.060 | -1.63 | 0.111 |
| TG(16:1_18:2_18:3) | 0.2 | 0.780 | -1.53 | 0.225 |
| TG(16:1_18:3_18:3) | -0.9 | 0.053 | -4.11 | 0.051 |
| TG(17:0_18:1_18:1) | -0.8 | 0.110 | -5.65 | 0.137 |
| TG(17:0_18:1_20:4) | -1.1 | 0.017 | -1.21 | 0.062 |
| TG(17:0_18:1_20:5) | -1.1 | 0.059 | -1.62 | 0.083 |
| TG(17:0_18:1_22:5) | -0.9 | 0.055 | 0.21 | 0.795 |
| TG(17:0_18:1_22:6) | -0.8 | 0.006 | 0.11 | 0.787 |
| TG(17:0_20:5_20:5) | -1.5 | 0.022 | -1.36 | 0.071 |
| TG(17:0_22:6_22:6) | -0.9 | 0.006 | -2.57 | 0.089 |
| TG(18:0_16:0_16:0) | -1.4 | 0.152 | -1.32 | 0.046 |
| TG(18:0_16:0_17:0) | -1.0 | 0.137 | -0.87 | 0.102 |
| TG(18:0_16:0_18:1) | -1.3 | 0.055 | -1.48 | 0.076 |
| TG(18:0_18:0_18:0) | -0.8 | 0.294 | -0.69 | 0.285 |
| TG(18:0_18:0_20:4) | -2.0 | 0.015 | -1.09 | 0.059 |
| TG(18:0_18:1_18:1) | -1.5 | 0.015 | -1.63 | 0.098 |
| TG(18:0_18:1_22:6) | -1.2 | 0.001 | -1.13 | 0.173 |
| TG(18:0_20:4_22:6) | -0.8 | 0.008 | -0.26 | 0.692 |
| TG(18:0_22:6_22:6) | -0.8 | 0.070 | -1.46 | 0.004 |

| | | | | |
|---|---|---|---|---|
| TG(18:0_22:6_22:6) | -1.4 | 0.229 | -0.92 | 0.185 |
| TG(18:0e_12:4_12:4) | -0.1 | 0.763 | -1.40 | 0.310 |
| TG(18:1_14:0_14:0) | -1.1 | 0.225 | -2.19 | 0.049 |
| TG(18:1_17:1_18:1) | -1.2 | 0.015 | -1.53 | 0.205 |
| TG(18:1_17:1_18:2) | -1.6 | 0.057 | -1.71 | 0.126 |
| TG(18:1_17:1_22:5) | -0.5 | 0.491 | -1.45 | 0.258 |
| TG(18:1_17:1_22:6) | -2.0 | 0.043 | -1.13 | 0.092 |
| TG(18:1_18:1_18:1) | -1.5 | 0.008 | -1.84 | 0.087 |
| TG(18:1_18:1_18:1) | 0.4 | 0.728 | -2.58 | 0.358 |
| TG(18:1_18:1_18:2) | -1.0 | 0.001 | -1.62 | 0.164 |
| TG(18:1_18:1_18:2) | -0.9 | 0.361 | -3.10 | 0.300 |
| TG(18:1_18:1_18:3) | -0.9 | 0.003 | -1.50 | 0.229 |
| TG(18:1_18:1_20:3) | -0.9 | 0.004 | -0.77 | 0.330 |
| TG(18:1_18:1_20:3) | -1.5 | 0.035 | -1.36 | 0.053 |
| TG(18:1_18:1_20:4) | -1.1 | 0.025 | -1.76 | 0.071 |
| TG(18:1_18:1_20:4) | -1.9 | 0.074 | -1.11 | 0.254 |
| TG(18:1_18:1_20:5) | -1.0 | 0.009 | -1.37 | 0.138 |
| TG(18:1_18:1_21:0) | -1.1 | 0.124 | -0.72 | 0.347 |
| TG(18:1_18:1_21:1) | -1.2 | 0.051 | -1.71 | 0.059 |
| TG(18:1_18:1_22:0) | -1.5 | 0.078 | -1.04 | 0.225 |
| TG(18:1_18:1_22:1) | -1.6 | 0.148 | -1.37 | 0.117 |
| TG(18:1_18:1_22:3) | 0.1 | 0.785 | -1.61 | 0.001 |
| TG(18:1_18:1_22:4) | -1.2 | 0.008 | -1.34 | 0.065 |
| TG(18:1_18:1_22:5) | -1.4 | 0.008 | -1.51 | 0.106 |
| TG(18:1_18:1_22:6) | -1.9 | 0.036 | -1.16 | 0.221 |
| TG(18:1_18:1_23:0) | -0.8 | 0.273 | -0.44 | 0.450 |
| TG(18:1_18:1_23:1) | -0.7 | 0.455 | -0.92 | 0.232 |
| TG(18:1_18:1_24:1) | -1.6 | 0.050 | -1.23 | 0.217 |
| TG(18:1_18:2_18:3) | -0.5 | 0.412 | -1.04 | 0.197 |
| TG(18:1_18:2_20:4) | -1.0 | 0.008 | -1.61 | 0.171 |
| TG(18:1_18:2_20:4) | 0.1 | 0.828 | -1.30 | 0.118 |
| TG(18:1_18:2_20:5) | -0.4 | 0.431 | -1.13 | 0.191 |
| TG(18:1_18:2_21:1) | -1.3 | 0.012 | -1.59 | 0.111 |
| TG(18:1_18:2_22:1) | -1.6 | 0.024 | -1.72 | 0.141 |
| TG(18:1_18:2_22:5) | -1.5 | 0.000 | -1.04 | 0.130 |
| TG(18:1_18:2_22:6) | -1.0 | 0.001 | -1.26 | 0.156 |
| TG(18:1_18:2_24:1) | -1.2 | 0.058 | -1.07 | 0.249 |
| TG(18:1_20:3_22:6) | -1.2 | 0.019 | -1.22 | 0.079 |
| TG(18:1_20:4_22:6) | -1.3 | 0.002 | -0.78 | 0.171 |
| TG(18:1_20:5_22:6) | -1.1 | 0.013 | -0.85 | 0.205 |
| TG(18:1_22:0_22:6) | -0.8 | 0.157 | 0.80 | 0.359 |
| TG(18:1_22:1_22:6) | -1.4 | 0.024 | 0.90 | 0.464 |

| | | | | |
|---|---|---|---|---|
| TG(18:1_22:5_22:5) | -0.7 | 0.363 | -0.92 | 0.060 |
| TG(18:1_22:5_22:6) | -1.4 | 0.006 | -0.31 | 0.629 |
| TG(18:1_22:6_22:6) | -1.2 | 0.001 | -0.78 | 0.223 |
| TG(18:2_17:1_18:2) | -0.9 | 0.033 | -1.64 | 0.151 |
| TG(18:2_17:1_18:2) | 0.9 | 0.327 | -1.66 | 0.016 |
| TG(18:2_17:1_20:4) | -0.9 | 0.072 | -1.88 | 0.110 |
| TG(18:2_17:1_22:6) | -0.9 | 0.033 | -1.22 | 0.119 |
| TG(18:2_18:2_18:2) | -1.1 | 0.000 | -1.81 | 0.219 |
| TG(18:2_18:2_20:4) | -0.8 | 0.007 | -1.08 | 0.172 |
| TG(18:2_18:2_22:6) | -1.0 | 0.002 | -1.19 | 0.067 |
| TG(18:2_18:2_22:6) | -0.9 | 0.007 | -1.05 | 0.256 |
| TG(18:2_22:6_22:6) | -1.0 | 0.014 | -0.71 | 0.347 |
| TG(18:2_22:6_22:6) | -0.8 | 0.143 | -0.67 | 0.536 |
| TG(18:2+5O_16:0_16:0) | -0.7 | 0.008 | -1.98 | 0.103 |
| TG(18:3_17:1_18:2) | -0.9 | 0.154 | -1.48 | 0.094 |
| TG(18:3_18:2_18:2) | -1.2 | 0.161 | -3.45 | 0.324 |
| TG(18:3_18:2_18:2) | 0.5 | 0.455 | -2.06 | 0.241 |
| TG(18:3_18:2_18:3) | -0.7 | 0.036 | -1.50 | 0.276 |
| TG(18:3_18:2_18:3) | -0.3 | 0.593 | -2.34 | 0.074 |
| TG(18:3_18:2_20:5) | -0.7 | 0.046 | -1.49 | 0.140 |
| TG(18:3_18:2_22:6) | -0.6 | 0.042 | -1.38 | 0.192 |
| TG(18:3_18:3_18:3) | -0.5 | 0.381 | -1.47 | 0.114 |
| TG(18:3_22:6_22:6) | -0.8 | 0.018 | -0.58 | 0.396 |
| TG(18:3+5O_16:0_16:0) | -0.7 | 0.011 | -1.82 | 0.203 |
| TG(18:4_16:1_16:1) | -0.9 | 0.188 | -1.97 | 0.066 |
| TG(18:4_16:1_18:2) | -0.2 | 0.818 | -1.37 | 0.080 |
| TG(18:4_18:1_18:1) | -1.2 | 0.006 | -1.41 | 0.096 |
| TG(19:0_18:1_18:1) | -1.3 | 0.057 | -1.29 | 0.110 |
| TG(19:0_18:1_20:4) | -1.0 | 0.025 | -0.20 | 0.710 |
| TG(19:1_16:0_18:1) | -1.5 | 0.037 | -1.37 | 0.102 |
| TG(19:1_18:1_18:1) | -2.4 | 0.083 | -1.55 | 0.089 |
| TG(19:1_18:1_18:2) | -1.2 | 0.004 | -1.49 | 0.110 |
| TG(19:1_18:2_18:2) | -1.0 | 0.185 | -1.68 | 0.113 |
| TG(19:1_18:2_22:6) | -1.4 | 0.004 | -0.82 | 0.319 |
| TG(19:1_20:5_20:5) | -1.5 | 0.029 | -1.01 | 0.168 |
| TG(20:0_16:0_16:0) | -1.2 | 0.196 | -0.75 | 0.140 |
| TG(20:0_16:0_18:1) | -1.2 | 0.159 | -1.29 | 0.136 |
| TG(20:0_18:1_18:1) | -1.7 | 0.031 | -1.32 | 0.140 |
| TG(20:0_18:1_22:6) | -1.0 | 0.021 | -0.74 | 0.344 |
| TG(20:1_18:1_18:1) | -3.6 | 0.004 | -1.70 | 0.098 |
| TG(20:1_18:1_18:1) | -0.7 | 0.124 | 1.57 | 0.099 |
| TG(20:1_18:1_18:2) | -1.0 | 0.003 | -0.20 | 0.875 |

| | | | | |
|---|---|---|---|---|
| TG(20:1_18:1_18:2) | -1.1 | 0.004 | -1.63 | 0.137 |
| TG(20:1_18:1_18:2) | -1.0 | 0.028 | -1.36 | 0.107 |
| TG(20:1_18:1_22:5) | -1.2 | 0.009 | -1.29 | 0.090 |
| TG(20:1_18:2_22:6) | -1.4 | 0.061 | -1.17 | 0.067 |
| TG(20:3_18:2_22:6) | -1.4 | 0.002 | -1.18 | 0.164 |
| TG(20:4e_18:1_18:1) | 0.7 | 0.120 | -1.26 | 0.245 |
| TG(20:5_17:1_18:2) | -2.0 | 0.017 | -1.47 | 0.007 |
| TG(20:5_18:2_18:2) | -0.9 | 0.023 | -1.49 | 0.130 |
| TG(20:5_18:2_20:5) | -0.5 | 0.254 | -1.50 | 0.252 |
| TG(20:5_18:2_22:5) | -0.9 | 0.014 | -1.29 | 0.206 |
| TG(20:5_18:2_22:6) | -0.5 | 0.183 | -1.25 | 0.257 |
| TG(22:1_18:2_22:6) | -0.3 | 0.518 | 1.58 | 0.050 |
| TG(22:5_17:1_18:2) | -1.1 | 0.004 | -2.00 | 0.117 |
| TG(22:5_18:2_18:2) | -1.3 | 0.001 | -0.92 | 0.184 |
| TG(22:5_18:2_22:6) | -1.2 | 0.001 | -1.01 | 0.381 |
| TG(22:6_17:1_22:6) | -0.9 | 0.005 | -2.65 | 0.337 |
| TG(22:6_17:1_22:6) | -0.9 | 0.073 | -0.77 | 0.486 |
| TG(24:0_18:2_18:2) | -0.3 | 0.448 | -0.35 | 0.563 |
| TG(28:0_10:4_18:2) | -0.5 | 0.117 | -1.95 | 0.200 |
| TG(30:0_18:1_18:1) | -1.6 | 0.184 | -0.51 | 0.521 |
| TG(4:0_16:0_16:0) | -1.1 | 0.380 | -0.68 | 0.505 |
| TG(4:0_16:0_18:1) | -1.0 | 0.439 | -0.55 | 0.628 |
| TG(4:0_16:0_18:2) | -0.9 | 0.441 | -0.64 | 0.684 |
| TG(4:0_18:1_18:2) | -1.2 | 0.378 | -0.88 | 0.464 |

Log2 fold change for each triglyceride (TG). Bonferroni corrected/adjusted p value, by dividing the original α-value (0.05) by the number of analyses on the dependent variable.

***Table S2***

***PLIN2 rs35568725* prevalence in the MVP population (Caucasic, African American, Hispanic)**

| Population | Reference | Alternative | MAF | Phenotype | Beta | SE | P |
|---|---|---|---|---|---|---|---|
| Trans-ethnic | A | G | 0.051 | ALT | 0.005 | 0.003 | 0.142 |
| White | A | G | 0.055 | ALT | 0.004 | 0.003 | 0.250 |
| Black | A | G | 0.013 | ALT | 0.014 | 0.013 | 0.276 |
| Hispanic | A | G | 0.028 | ALT | 0.006 | 0.015 | 0.690 |
| Asians | A | G | 0.020 | ALT | 0.031 | 0.052 | 0.559 |
| Trans-ethnic | A | G | 0.052 | NAFLD | 0.021 | 0.015 | 0.160 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| White | A | G | 0.055 | NAFLD | 0.031 | 0.016 | 0.051 |
| Black | A | G | 0.013 | NAFLD | -0.068 | 0.070 | 0.330 |
| Hispanic | A | G | 0.029 | NAFLD | -0.089 | 0.070 | 0.203 |
| Asians | A | G | 0.022 | NAFLD | 0.120 | 0.226 | 0.597 |

Additive logistic model corrected for age, sex, and the first ten ethnicity-specific principal components.

***Table S3***

**Association between PLIN2-Pro251 and type 2 diabetes, dyslipidemia, hypertension, and NAFLD in the MVP database (before Bonferroni correction)**

| Phenotype | Beta | SE | P | OR | 95% Low | 95% High |
|---|---|---|---|---|---|---|
| NAFLD | 0.003 | 0.003 | 0.25 | 1.00 | 0.99 | 1.01 |
| T2D | 0.06 | 0.02 | 0.002 | 1.07 | 1.02 | 1.11 |
| Cardiomyopathy | 0.07 | 0.03 | 0.016 | 1.08 | 1.01 | 1.15 |

Multivariable analysis adjusted for age, gender, and 5 PC principal components European Ancestry

***Table S4***

**Hepatic liver fat after adjustment for visceral adipose tissue:**

| Phenotype | Beta | SE | P-value |
|---|---|---|---|
| Liver volume | -2.812e-03 | 3.667e-03 | 0.443 |
| MRI-PDFF | -0.0973086 | 0.0732235 | 0.183 |
| Liver iron | 1.505257 | 0.866653 | 0.082 |

Multivariable analyses adjusted for age, sex, BMI, visceral adipose tissue, and PC1-10.

***Table S5***

**Magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) in BMI categories:**

**BMI: <18.5**

| Phenotype | Beta | SE | P-value |
|---|---|---|---|
| MRI-PDFF | 0.334 | 0.186 | 0.075 |
| Visceral adipose tissue | -0.007 | 0.160 | 0.962 |

Multivariable analyses adjusted for age, sex, and PC1-10.

**BMI: from 18.5 to 25**

| Phenotype | Beta | SE | P-value |
|---|---|---|---|
| MRI-PDFF | 0.032 | 0.067 | 0.630 |
| Visceral adipose tissue | -6.542e-17 | 2.791e-16 | 0.814 |

Multivariable analyses adjusted for age, sex, and PC1-10.

**BMI: from 25 to 30**

| Phenotype | Beta | SE | P-value |
|---|---|---|---|
| MRI-PDFF | -0.212 | 0.118 | 0.071 |
| Visceral adipose tissue | 1.840e-16 | 1.286e-16 | 0.152 |

Multivariable analyses adjusted for age, sex, and PC1-10.

**BMI: >30**

| Phenotype | Beta | SE | P-value |
|---|---|---|---|
| MRI-PDFF | -0.203 | 0.255 | 0.425 |
| Visceral adipose tissue | -1.494e-16 | 1.432e-16 | 0.296 |

Multivariable analyses adjusted for age, sex, and PC1-10.

***Supplementary reference***

1. Carr RM, Peralta G, Yin X, Ahima RS. Absence of perilipin 2 prevents hepatic steatosis, glucose intolerance and ceramide accumulation in alcohol-fed mice. PloS one. 2014;9(5):e97118.