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Supplemental Information

Genetic Regulation of Plasma Lipid Species and Their Association with Metabolic Phenotypes

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Supplementary Figures and Figure Legends

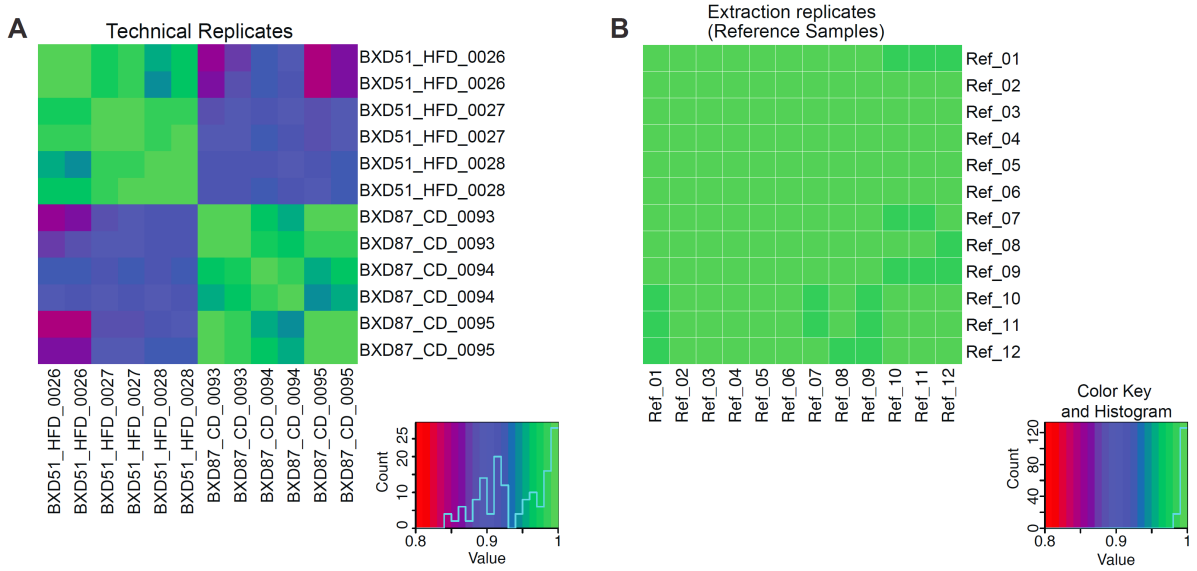

Figure S1. Quality assessment of MS measurements and reproducibility. Related to Figure 1. (**A**) Heatmap of correlations between technical replicates (from separate runs) of six plasma samples showing high Spearman correlation between the replicates vs. between the strains. (**B**) Heatmap of extraction replicates of the same liver sample extracted and run in 12 batches, equivalent to the 12 batches of the BXD samples.

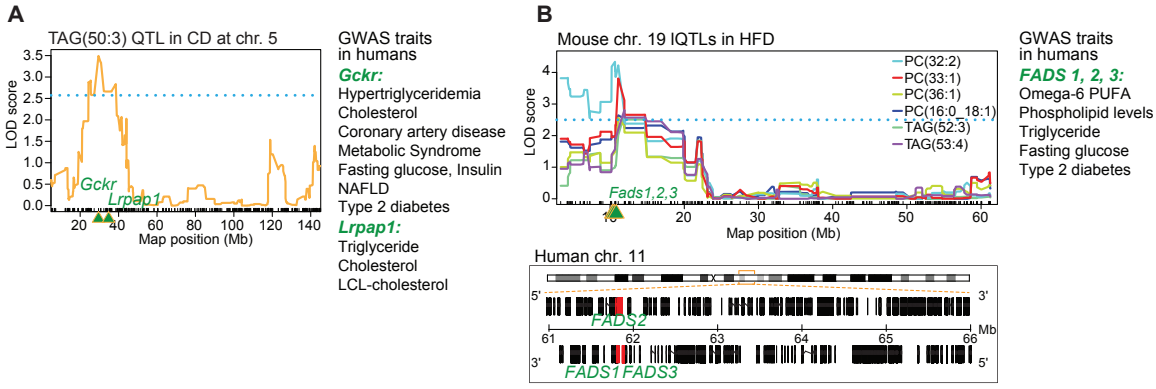

Figure S2. lQTLs harboring genes associated with abnormal lipid metabolism in human GWAS. Related to Figure 3. (**A**) QTL locus for TAG(50:3) enclosing the human GWAS gene: *Gckr* and *Lrpap1.* (**B**) QTL hotspot locus for the 6 lipid species on mouse Chr19 enclosing the GWAS genes: *Fads 1, 2, 3*. The syntenic region on human Chr11 is shown below. The genomic location of the genes is shown in green in the positive stand for FADS2 and in the negative strand for FADS1 and FADS3. The GWAS phenotypes associated with the genes is indicated on the right. Blue dotted line represents the suggestive QTL threshold.

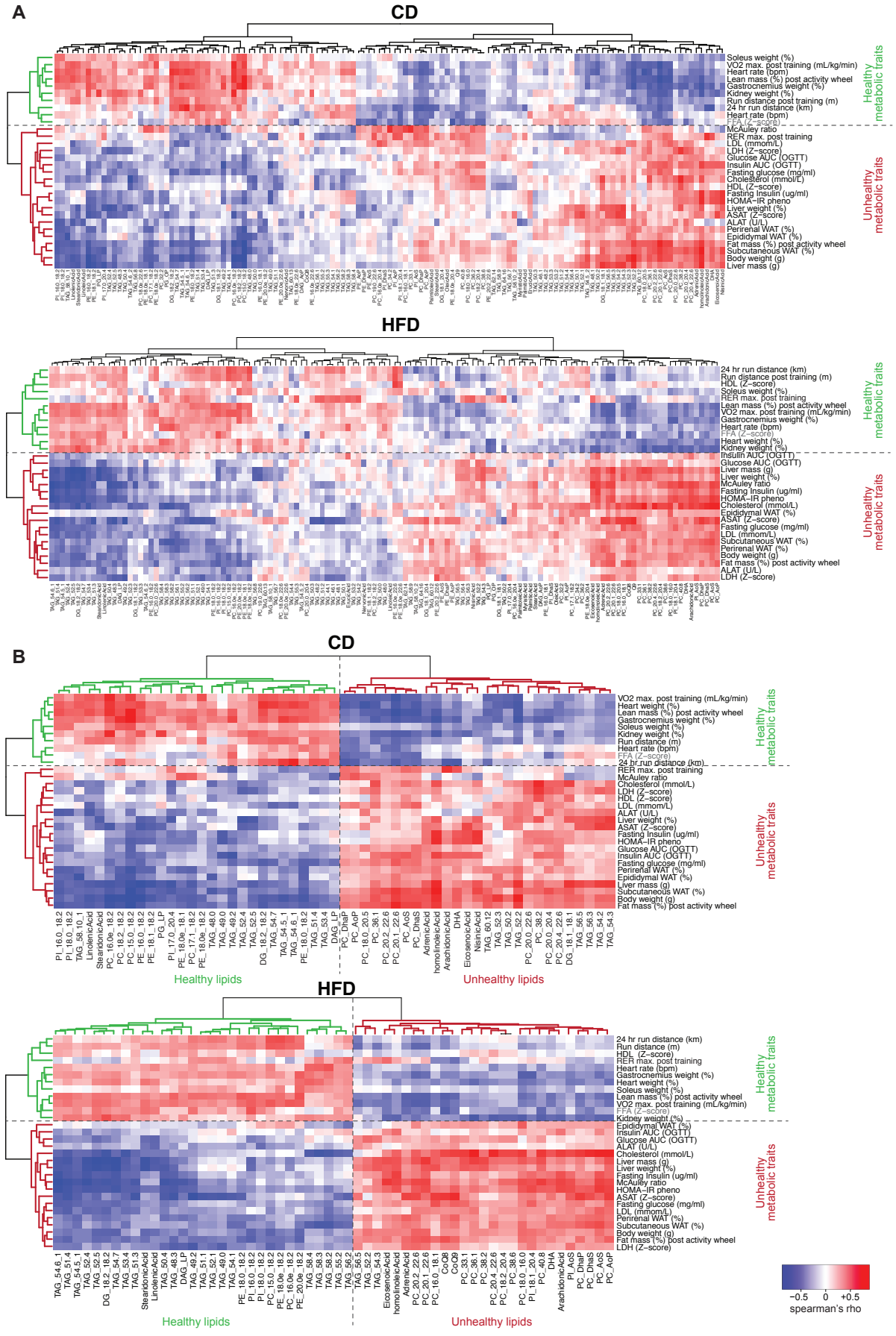
A
CD
Soleus weight (%)
VO2 max. post training (mL/kg/min)
Heart rate (bpm)
Lean mass (%) post activity wheel
Gastrocnemius weight (%)
Kidney weight (%)
Run distance post training (m)
24 hr run distance (km)
Heart rate (bpm)
FFA (Z−score)
McAuley ratio
RER max. post training
LDL (mmom/L)
LDH (Z−score)
Glucose AUC (OGTT)
Insulin AUC (OGTT)
Fasting glucose (mg/ml)
Cholesterol (mmol/L)
HDL (Z−score)
Fasting Insulin (ug/ml)
HOMA−IR pheno
Liver weight (%)
ASAT (Z−score)
ALAT (U/L)
Perirenal WAT (%)
Epididymal WAT (%)
Fat mass (%) post activity wheel
Subcutaneous WAT (%)
Body weight (g)
Liver mass (g)
Healthy metabolic traits
Unhealthy metabolic traits
HFD
24 hr run distance (km)
Run distance post training (m)
HDL (Z−score)
Soleus weight (%)
RER max. post training
Lean mass (%) post activity wheel
VO2 max. post training (mL/kg/min)
Gastrocnemius weight (%)
Heart rate (bpm)
FFA (Z−score)
Heart weight (%)
Kidney weight (%)
Insulin AUC (OGTT)
Glucose AUC (OGTT)
Liver mass (g)
Liver weight (%)
McAuley ratio
Fasting Insulin (ug/ml)
HOMA−IR pheno
Cholesterol (mmol/L)
Epididymal WAT (%)
ASAT (Z−score)
Fasting glucose (mg/ml)
LDL (mmom/L)
Subcutaneous WAT (%)
Perirenal WAT (%)
Body weight (g)
Fat mass (%) post activity wheel
ALAT (U/L)
LDH (Z−score)
Healthy metabolic traits
Unhealthy metabolic traits
B
CD
VO2 max. post training (mL/kg/min)
Heart weight (%)
Lean mass (%) post activity wheel
Gastrocnemius weight (%)
Soleus weight (%)
Kidney weight (%)
Run distance (m)
Heart rate (bpm)
FFA (Z−score)
24 hr run distance (km)
RER max. post training
McAuley ratio
Cholesterol (mmol/L)
LDH (Z−score)
HDL (Z−score)
LDL (mmom/L)
ALAT (U/L)
Liver weight (%)
ASAT (Z−score)
Fasting Insulin (ug/ml)
HOMA−IR pheno
Glucose AUC (OGTT)
Insulin AUC (OGTT)
Fasting glucose (mg/ml)
Perirenal WAT (%)
Epididymal WAT (%)
Liver mass (g)
Subcutaneous WAT (%)
Body weight (g)
Fat mass (%) post activity wheel
Healthy metabolic traits
Unhealthy metabolic traits
PI_16:0_18:2
PI_18:0_18:2
TAG_58:10_1
LinolenicAcid
StearidonicAcid
PC_16:0e_18:2
PC_18:2_18:2
PC_15:0_18:2
PE_16:0_18:2
PE_18:1_18:2
PG_LP
PI_17:0_20:4
PE_18:0e_18:1
PC_17:1_18:2
PE_18:0e_18:2
TAG_48:0
TAG_49:2
TAG_52:4
TAG_52:5
DG_18:2_18:2
TAG_54:7
TAG_54:5_1
TAG_54:6_1
PE_18:0_18:2
TAG_51:4
TAG_53:4
DAG_LP
PC_DhaP
PC_AoP
PC_18:0_20:5
PC_38:1
PC_20:2_22:6
PC_20:1_22:6
PC_AoS
PC_DhaS
AdrenicAcid
homolinoleicAcid
ArachidonicAcid
DHA
EicosenoicAcid
NisinicAcid
TAG_60:12
TAG_52:3
TAG_50:2
TAG_52:2
PC_20:0_22:6
PC_38:2
PC_20:0_20:4
PC_20:4_22:6
DG_18:1_18:1
TAG_56:5
TAG_56:3
TAG_54:2
TAG_54:3
Healthy lipids
Unhealthy lipids
HFD
24 hr run distance (km)
Run distance (m)
HDL (Z−score)
RER max. post training
Heart rate (bpm)
Gastrocnemius weight (%)
Heart weight (%)
Soleus weight (%)
Lean mass (%) post activity wheel
VO2 max. post training (mL/kg/min)
FFA (Z−score)
Kidney weight (%)
Epididymal WAT (%)
Insulin AUC (OGTT)
Glucose AUC (OGTT)
ALAT (U/L)
Cholesterol (mmol/L)
Liver mass (g)
Liver weight (%)
Fasting Insulin (ug/ml)
McAuley ratio
HOMA−IR pheno
ASAT (Z−score)
Fasting glucose (mg/ml)
LDL (mmom/L)
Perirenal WAT (%)
Subcutaneous WAT (%)
Body weight (g)
Fat mass (%) post activity wheel
LDH (Z−score)
Healthy metabolic traits
Unhealthy metabolic traits
TAG_54:6_1
TAG_51:4
TAG_54:5_1
TAG_52:4
TAG_52:5
DG_18:2_18:2
TAG_54:7
TAG_53:4
TAG_51:3
StearidonicAcid
LinolenicAcid
TAG_51:5
TAG_48:3
DAG_LP
TAG_49:2
TAG_51:1
TAG_52:1
TAG_49:0
TAG_54:1
PE_18:0_18:2
PI_16:0_18:2
PI_18:0_18:2
PC_15:0_18:2
PC_16:0e_18:2
PC_18:0e_18:2
PE_20:0_18:2
TAG_58:4
TAG_58:3
TAG_58:2
TAG_55:2
TAG_56:2
TAG_56:5
TAG_52:2
TAG_54:3
EicosenoicAcid
homolinoleicAcid
AdrenicAcid
PC_20:2_22:6
PC_20:1_22:6
PC_16:0_18:1
CoQ8
CoQ9
PC_33:1
PC_36:1
PC_38:2
PC_20:4_22:6
PC_18:2_20:4
PC_38:6
PC_18:0_16:0
PI_18:1_20:4
PC_40:8
DHA
ArachidonicAcid
PI_AoS
PC_DhaP
PC_DhaS
PC_AoS
PC_AoP
Healthy lipids
Unhealthy lipids
−0.5
0
+0.5
spearman's rho

Figure S3. **Association of lipid species with metabolic traits. Related to Figure 4 and Table S6.** (**A and B**) Heatmap with an unsupervised hierarchical clustering of Spearman's correlation rho of (**A**) all the lipid species and metabolic traits in CD (top) and HFD (bottom); (**B**) select lipid species in each diet which show maximum number of significant correlation with metabolic traits (the lateral lipid species cluster of panel A). For panel B, the vertical green lipid cluster represents healthy lipids (specific and common in each diet), whereas the vertical red cluster represents the unhealthy lipids (specific and common in each diet). For A and B the horizontal green phenotype cluster represents healthy metabolic traits whereas the red cluster represents unhealthy metabolic traits. Red indicates positive correlation and blue negative correlation.

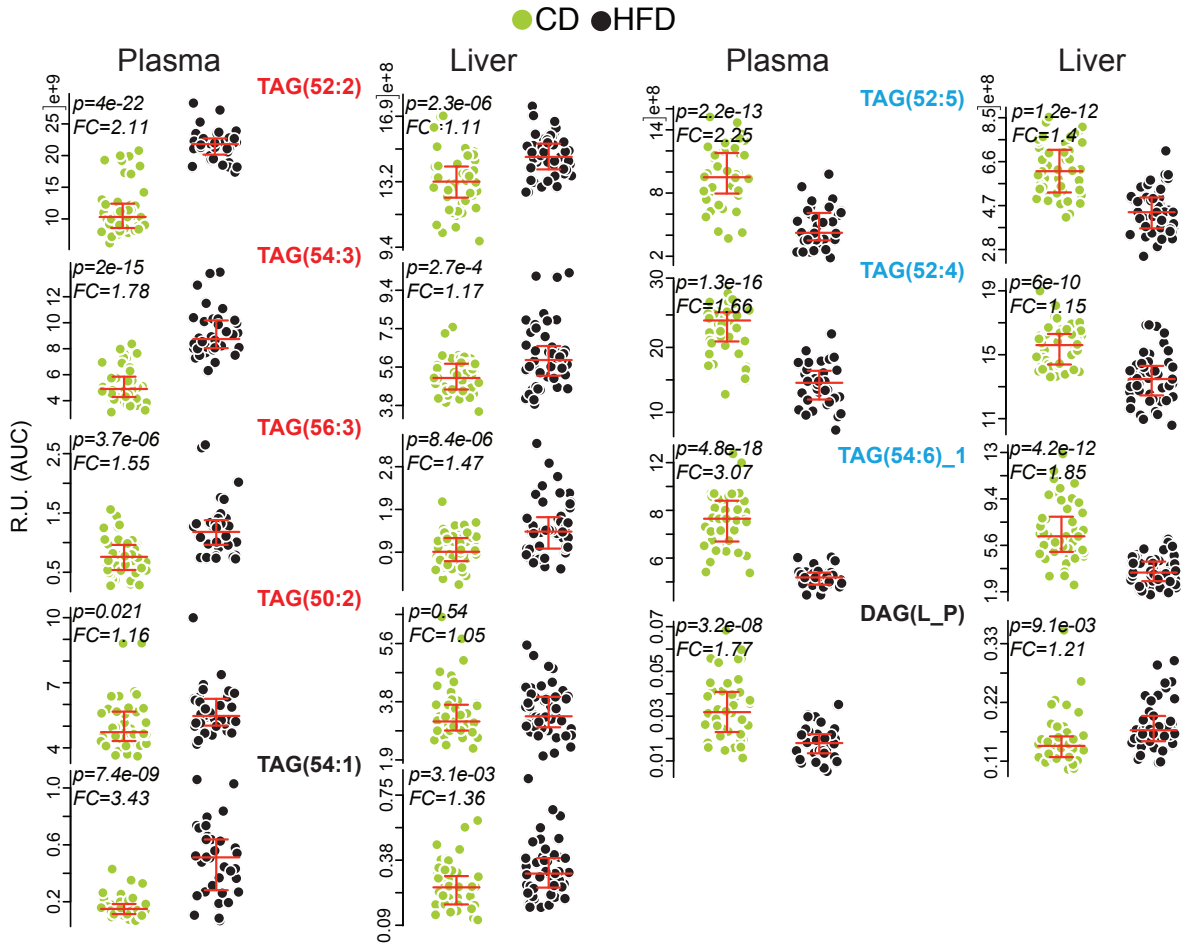

Figure S4. Plasma and liver levels of 9 TAG species having the most significant correlation between plasma and liver in both diets. Related to Figure 5 and Table S2. Dot plot showing the levels of 9 shortlisted lipids as potential lipid signatures of NAFLD in plasma and liver. Lipids in red font indicate pro-NAFLD signatures and those in blue font indicate anti-NAFLDsignatures. P-value < 0.05 by Welch's t-test was considered significant between diets.

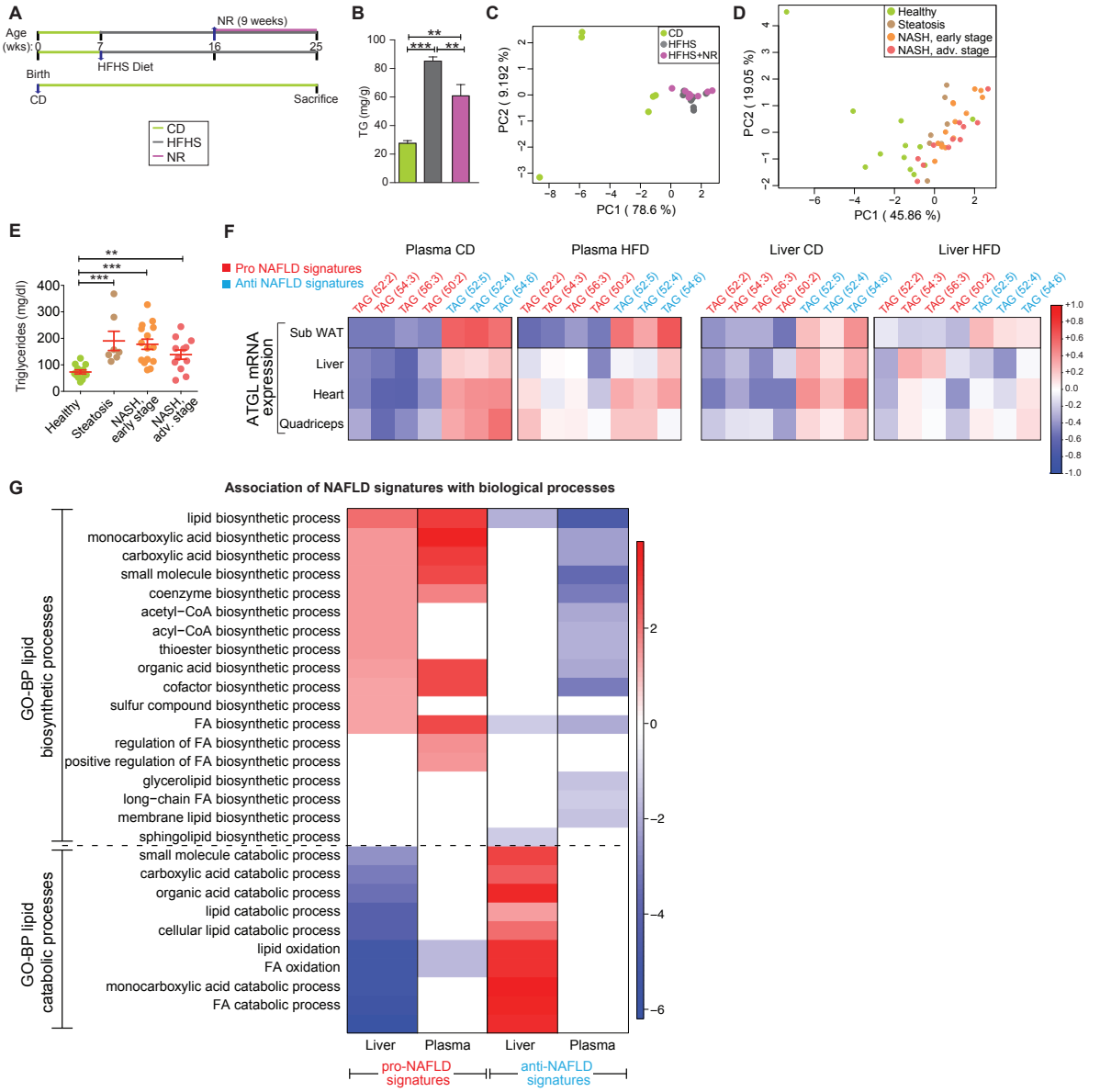

Figure S5. Assessment of TAG NAFLD signatures in mice and humans and their association with biosynthetic pathways. Related to Figure 6. (**A-C**) C57BL/6J mice: (**A**) Schematic illustration of the three experimental groups used for validation of the NAFLD signatures; mice on CD (green), mice on HFHS diet from 7-25 weeks (grey), mice starting HFHS diet at 7 weeks and treated with nicotinamide riboside (NR) 9 weeks after the start of HFHS diet until the end of the study (for 9 weeks - therapeutic intervention, magenta). (**B**) Liver total TAG concentration (normalized to liver weight). (**C**) Principal component analysis (PCA) of 55 TAG species shows clear separation of only the CD group on PC1. (**D and E**) Human subjects: (**D**) PCA of 55 TAG species measured in human plasma from healthy, steatosis and NASH patients. (**E**) Plasma total TAG levels in human samples. (**F and G**) BXDs: (**F**) Correlation matrix showing the Pearson correlation of *Atgl* mRNA expression in four different metabolic tissues with the pro- and anti-NAFLD TAG signatures in plasma (left) and liver (right) for both diets. (**G**) Gene ontology biological processes (GO BP) associated with

NAFLD TAG signatures. Pathway enrichment analysis was performed with the liver transcripts that significantly correlated (both positively and negatively) with the PC1 of pro- and anti-NAFLD signatures in liver and plasma. Red and blue cells represent the enriched pathways with the positively (scale bar: $\log_{10}$ p-value) and negatively (scale bar: $-\log_{10}$ p-value) correlated liver transcripts respectively. For B and E differences in mean TAG levels were compared using two-sample *t* tests. *p<0.05, **p<0.01, ***p<0.001.