

Supplementary Information for

Trifluoroacetate mediated reduction of atherosclerosis in mice

Wei Tang¹, Audrey S. Black¹, Romana Moench¹, Katayoon Marzban¹, Juan Antonio Raygoza Garay¹, James J. Zheng¹, Louis Conway¹, Antonio F. M. Pinto², Christopher G. Parker¹, Alan Saghatelian², Luke J. Leman¹, and M. Reza Ghadiri^{1,3*}

*Corresponding author. Email: ghadiri@scripps.edu

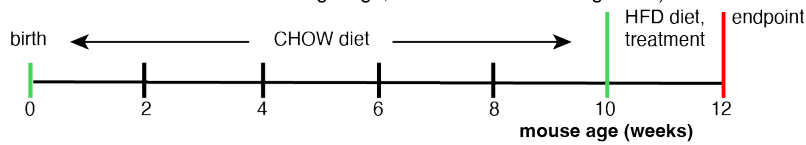
The file includes:

Supplementary Figures 1 to 34

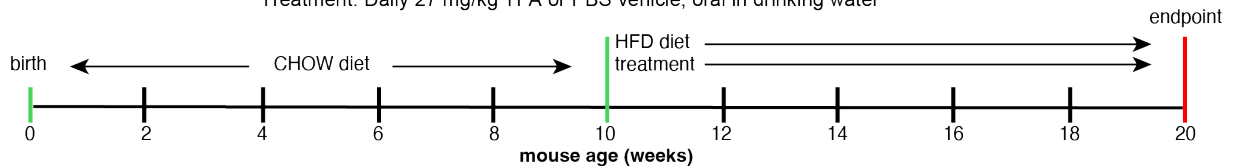
Supplementary Table 1

Ldlr^{-/-} mice

2-wk duration treatment Mice: LDLR^{-/-}, female or male, 4–9 mice per group
Diets: HFD = TD.94059 Atherogenic Diet (37% fat, 1.25% cholesterol)
CHOW = LabDiet 5053 (13% fat)
Treatment: variable daily TFA dose (typically 27 mg/kg) and route of administration (i.p. injection, oral gavage, or ad libitum in drinking water)



10-wk duration treatment Mice: LDLR^{-/-}, female, 8 mice per group
Diets: HFD = TD.94059 Atherogenic Diet (37% fat, 1.25% cholesterol)
CHOW = LabDiet 5053 (13% fat)
Treatment: Daily 27 mg/kg TFA or PBS vehicle, oral in drinking water



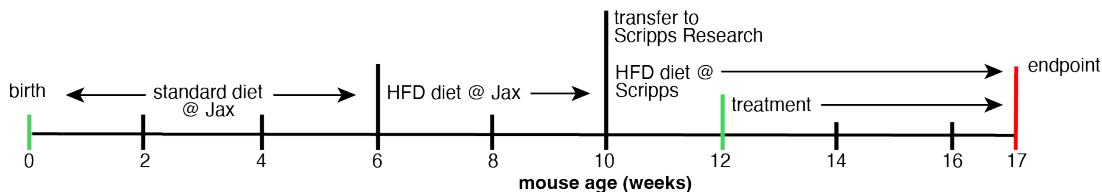
ApoE^{-/-} mice

10-wk duration treatment Mice: apoE^{-/-}, female or male, 10 mice per group
Diets: HFD = TD.88137 Adjusted Calories Diet (42% fat, 0.2% cholesterol)
CHOW = LabDiet 5053 (13% fat)
Treatment: Daily 27 mg/kg TFA or PBS vehicle, oral in drinking water



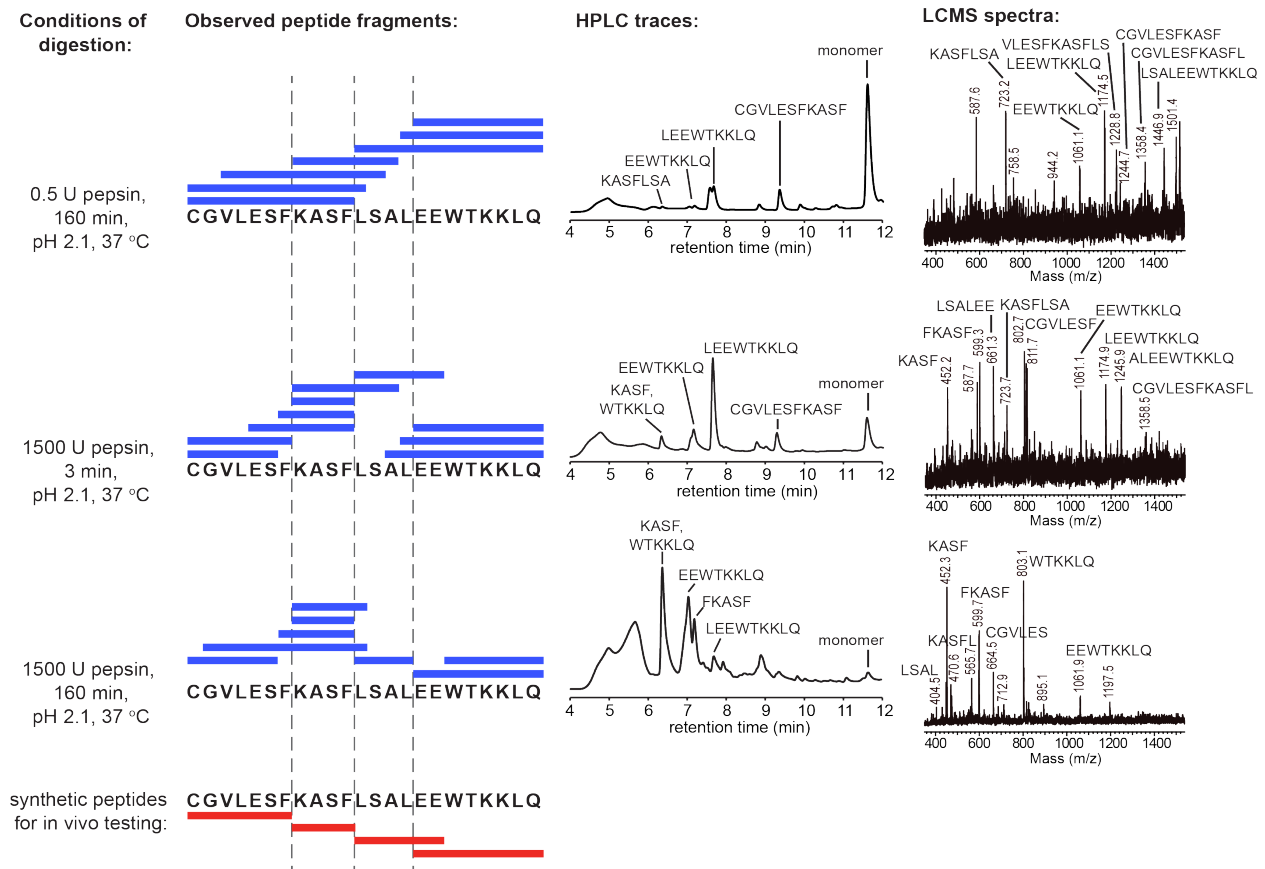
wild-type C57Bl/6J mice

5-wk duration treatment Mice: male C57BL/6J DIO (stock # 380050) or C57BL/6J DIO control mice (stock # 380056), 10 mice/group
Diets: HFD = Research Diets D12492 (60% fat)
CHOW = Research Diets D12450J (10% fat)
Treatment: Daily 18 mg/kg TFA or PBS vehicle, oral gavage



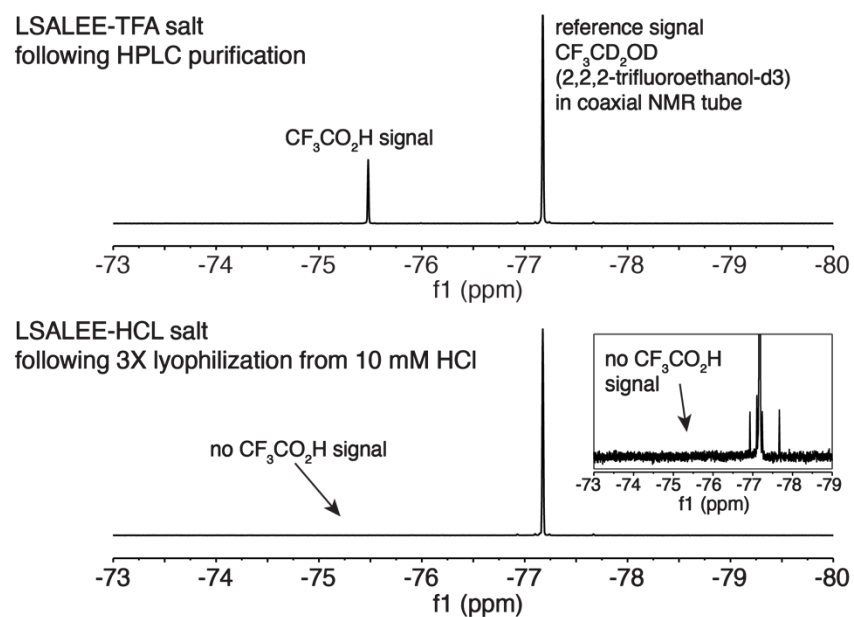
Supplementary Figure 1.

Schedules of feeding and dosing for the described studies.



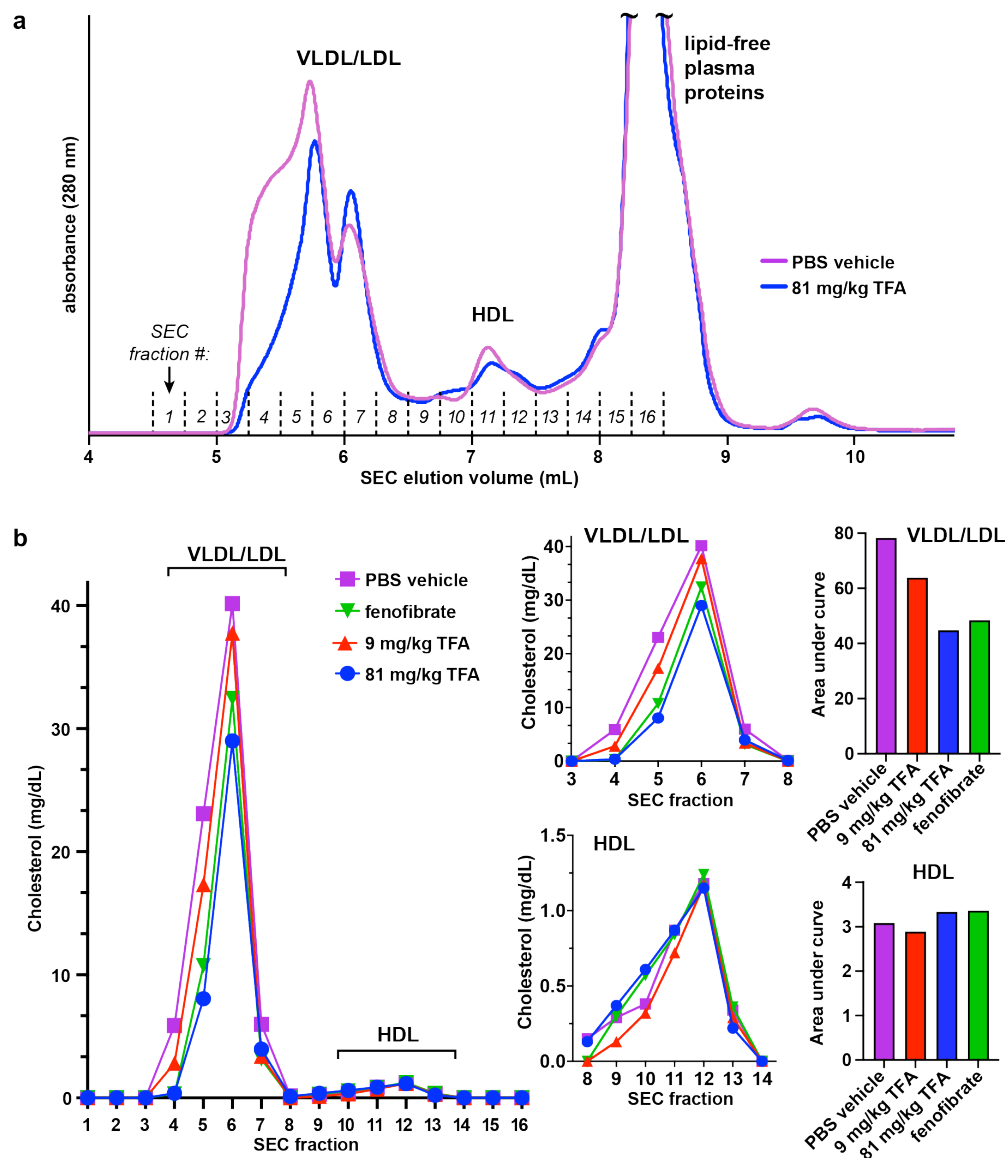
Supplementary Figure 2.

To determine which peptide fragments might be produced following oral administration of the 23-residue parent apoA-I mimetic peptide, *in vitro* digestions with pepsin were carried out at pH 2, 37° C. The three digestions involved increasing enzyme activity to approximate early, middle, and late peptide fragments that might be formed *in vivo*. The digestion reactions were quenched using pepstatin, and the digests were analyzed by LCMS analysis. Based on our analysis of the observed peptide fragments produced in the digests (blue bars), we chose four peptide fragments to synthesize for follow-up *in vivo* testing (red bars). The Cys residue in the parent peptide was alkylated with iodoacetamide, resulting in a residue mass of 161.04 Da. It should be noted that fragments containing a Trp residue will have a higher extinction coefficient than those without Trp, resulting in higher UV absorbance in HPLC traces.



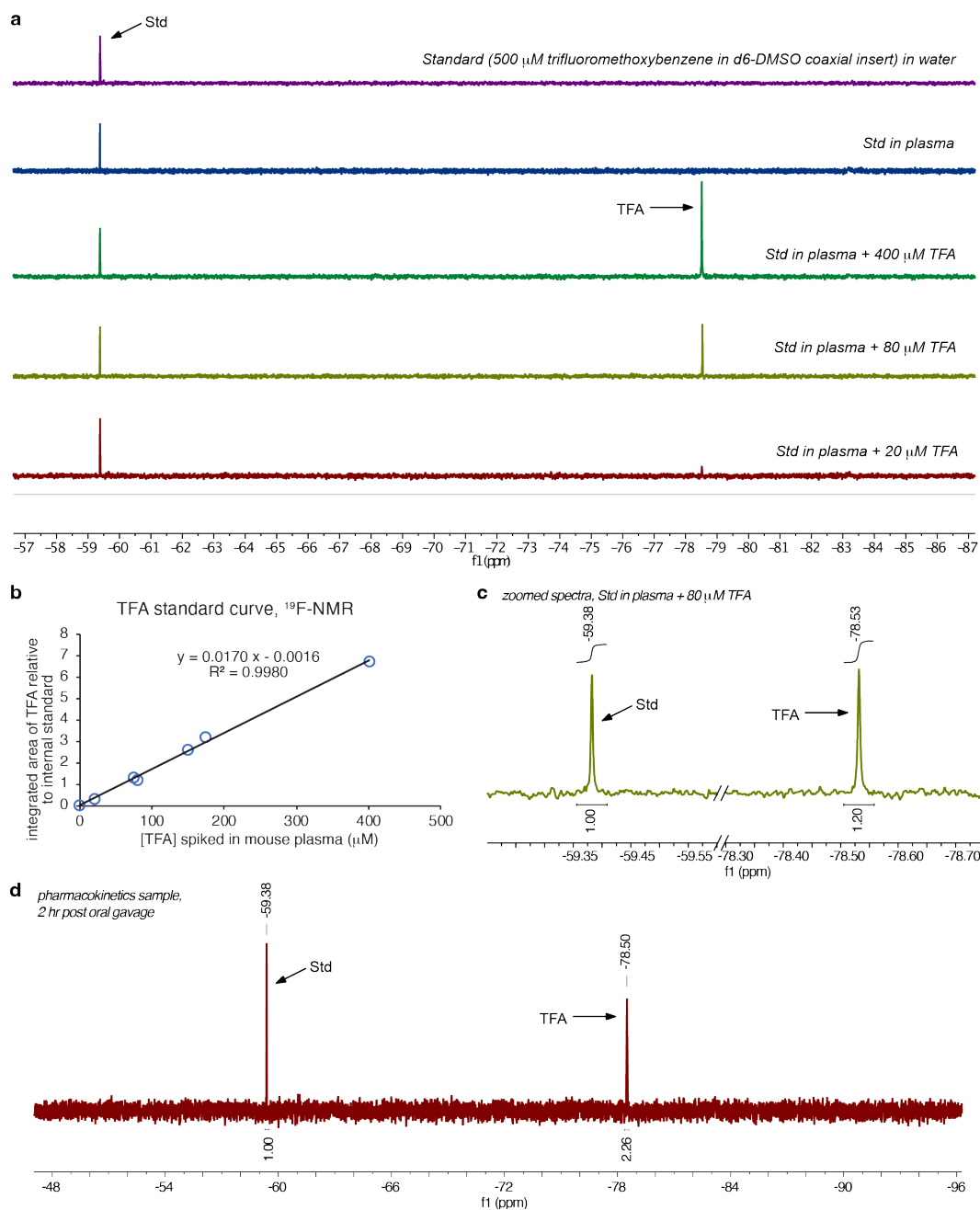
Supplementary Figure 3.

¹⁹F-NMR analysis confirming complete removal of TFA in hexapeptide LSALEE.



Supplementary Figure 4.

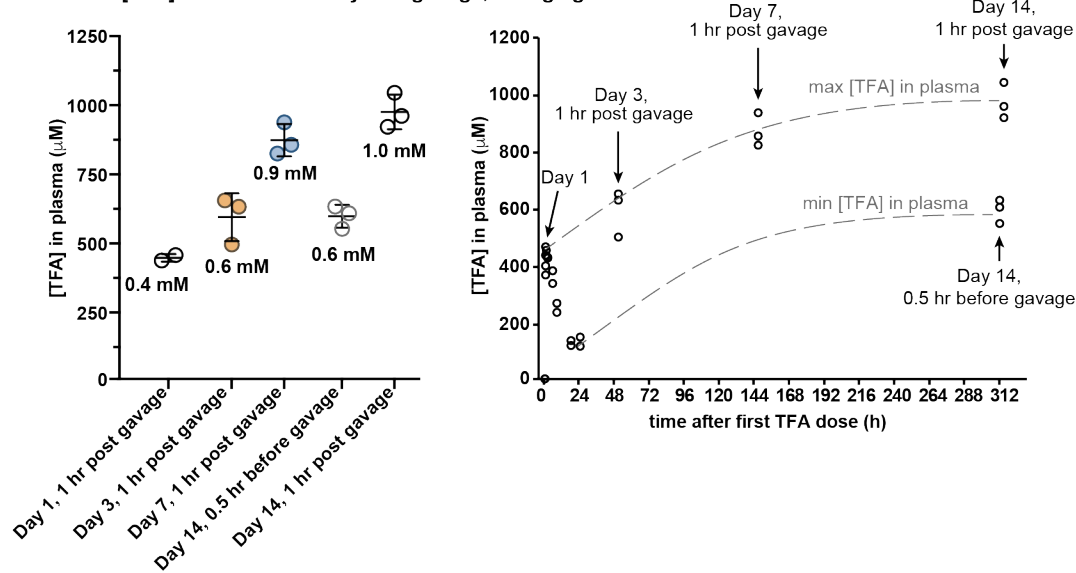
Plasma from three randomly chosen mice per group was pooled and fractionated via SEC to analyze the lipoprotein profile. **a)** The SEC 280 nm absorbance traces are shown for the PBS vehicle group and the 81 mg/kg TFA group. **b)** Cholesterol levels found in the SEC fractions. Zoomed traces for the cholesterol measurements in VLDL/LDL region and HDL region are shown, along with area under curve values on the right. The experiment was repeated twice independently with similar results.



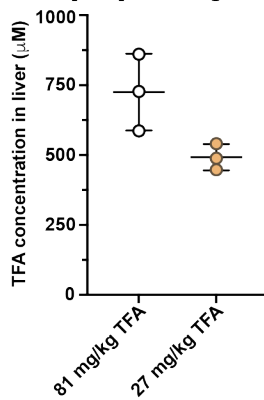
Supplementary Figure 5.

Development of the ^{19}F -NMR assay to measure TFA concentration in plasma of treated mice. Plasma samples were diluted to 0.5 mL total volume and placed in an NMR tube along with a coaxial tube insert containing 500 μ M trifluoromethoxybenzene in d6-DMSO as an internal concentration standard. **a)** ^{19}F -NMR spectra of plasma from untreated mice spiked with known concentrations of TFA, used to generate a standard curve. **b)** The standard curve for measuring TFA in plasma samples from TFA-treated mice. **c)** Zoomed ^{19}F -NMR spectra of the peaks for Std and TFA in the plasma sample spiked with 80 μ M TFA. **d)** ^{19}F -NMR spectra of plasma drawn from a mouse two hours after oral gavage with a 27 mg/kg dose of TFA.

a Plasma [TFA] for two-week daily oral gavage, 27 mg/kg TFA

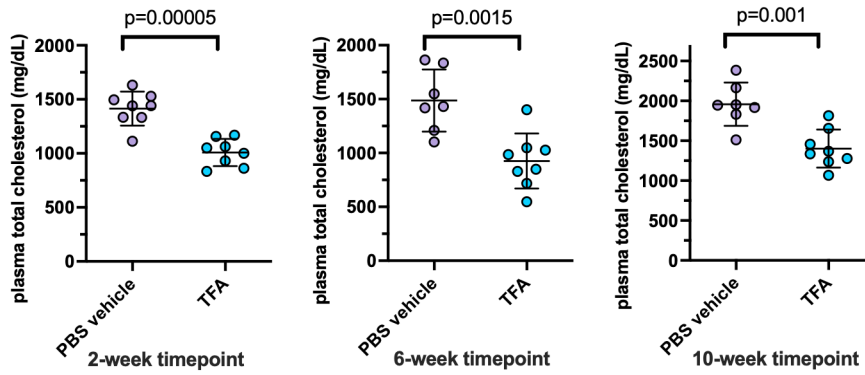


b Liver [TFA] following two-week daily oral gavage



Supplementary Figure 6.

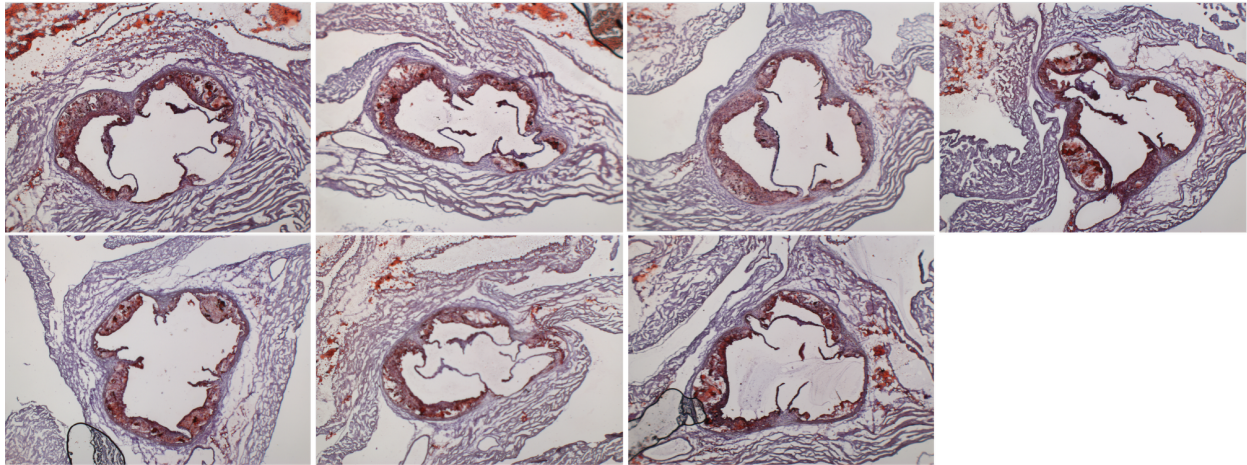
Measured concentrations of TFA in plasma and liver tissue during two-week studies involving daily oral gavage of TFA to *Ldlr*^{-/-} mice. **(a)** For each timepoint from 0.25 h to 25 h, blood samples were taken from *n*=2 animals; for later timepoints, blood samples were taken from *n*=3 animals. On the left, the individual datapoints are shown along with mean ± SD. The graph on the right shows data combined from two separate PK studies: the single-dose pharmacokinetic study (shown as data for “Day 1”) and the data from the two-week study for day 3, day 7, and day 14. The dotted gray curves are estimates of the average maximum and minimum TFA concentrations, based on the observations during the study. **(b)** Livers were harvested on day 15, between 25-29 hours following the final administration of TFA to male *Ldlr*^{-/-} mice on day 14. For each datapoint, ~80 mg liver tissue from *n*=3 mice was separately homogenized in 0.6 mL lysis buffer and the concentration of TFA in the lysate was measured by ¹⁹F-NMR using an internal concentration standard. To back-calculate the concentration of TFA in the original liver tissue, we assumed that the liver tissue was composed entirely of water (no solid tissue); the actual TFA concentration within liver cells would therefore be somewhat higher than the values reported, because liver is only approximately 70-75% water in reality.



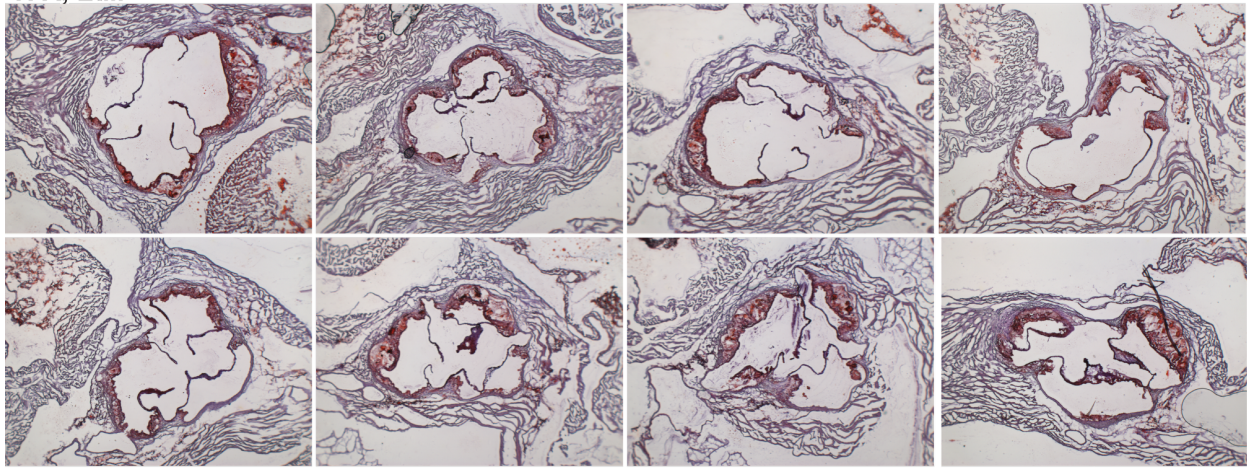
Supplementary Figure 7.

Plasma cholesterol levels in female *Ldlr*^{-/-} mice throughout a 10-week anti-atherosclerosis study. TFA was administered treatment *ad libitum* in the drinking water (27 mg/kg/day). The data are shown as mean $\pm$ SD. *p* values were determined by unpaired two-tailed Student's *t*-test comparing the experimental group to PBS vehicle group. Sample sizes were: TFA at each timepoint (n=8 mice), PBS vehicle at 2-week timepoint (n=8 mice), PBS vehicle at 6-week and 10-week timepoints (n=7 mice).

PBS vehicle, *Ldlr*^{-/-}

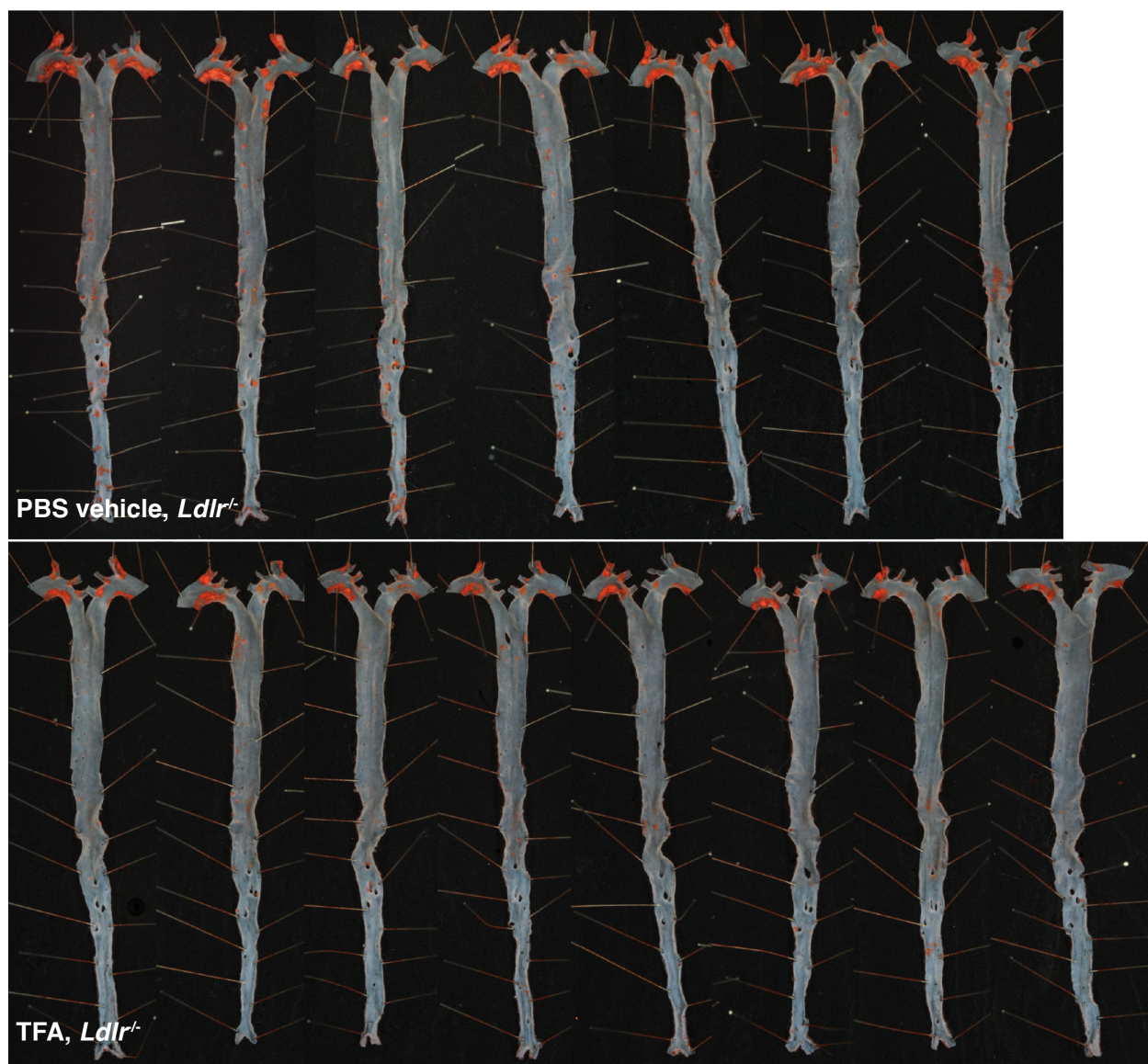


TFA, *Ldlr*^{-/-}



Supplementary Figure 8.

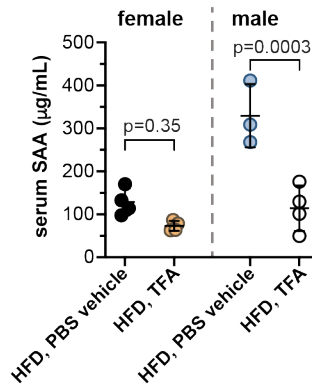
Aortic sinus cross-sections from TFA- and PBS vehicle-treated HFD-fed *Ldlr*^{-/-} mice (female) following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.



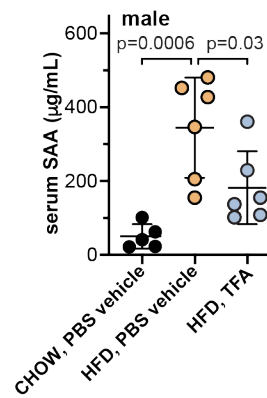
Supplementary Figure 9.

Aortas from TFA- and PBS vehicle-treated HFD-fed *Ldlr*^{-/-} mice (female) following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.

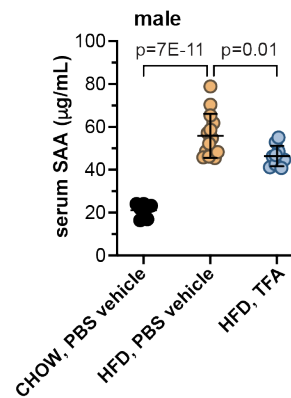
Ldlr^{-/-} mice



Apoe^{-/-} mice

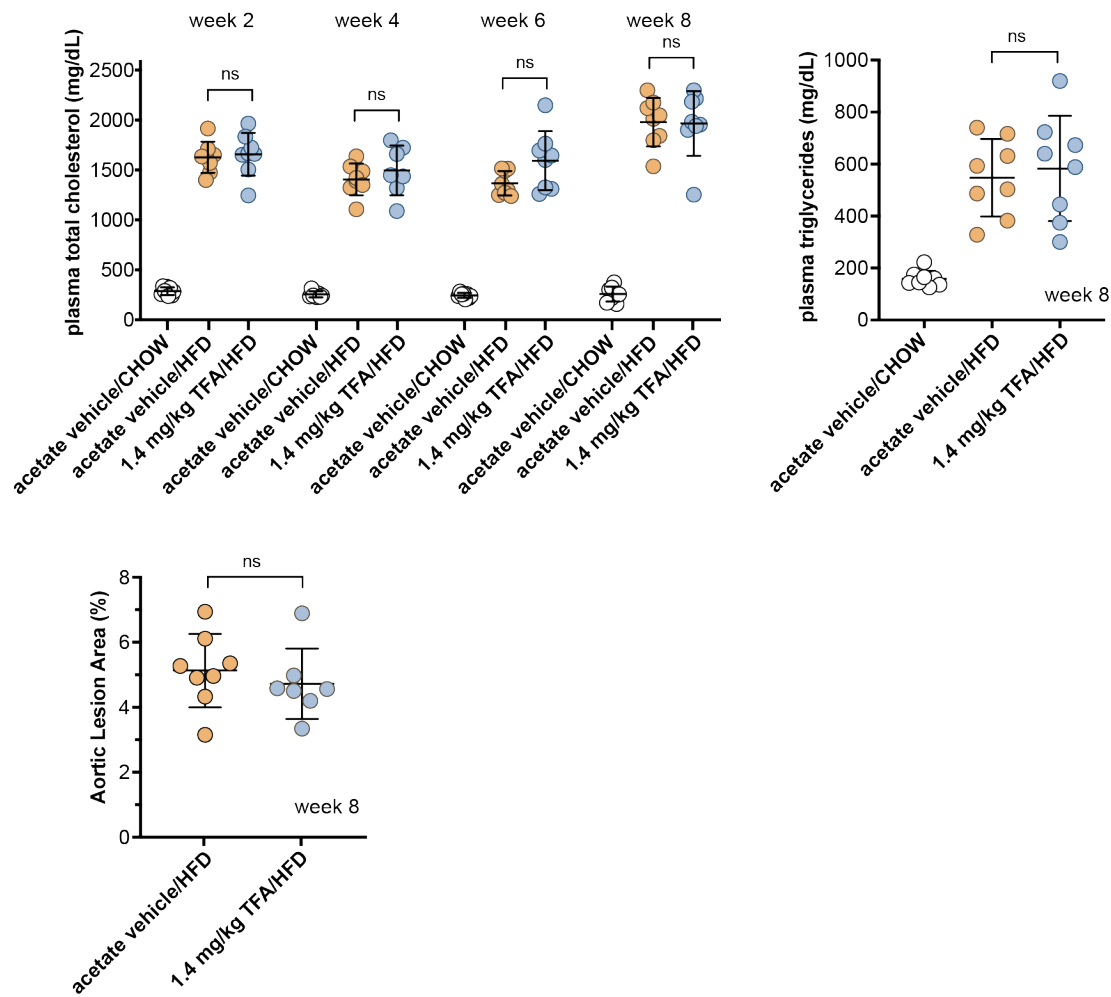


wild-type C57BL/6J mice



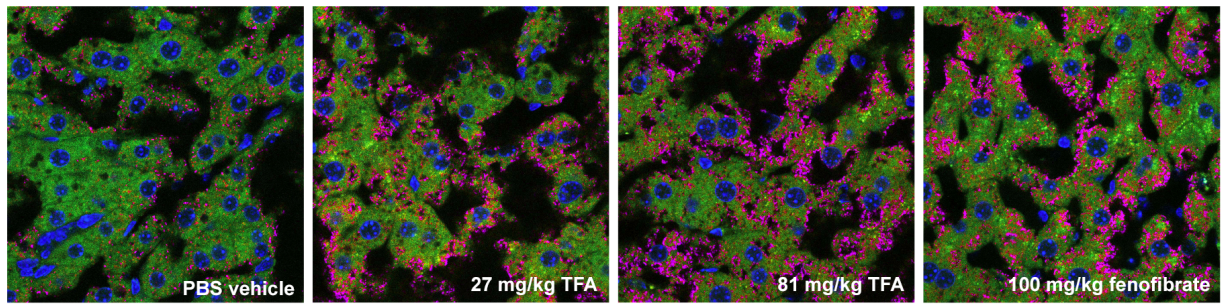
Supplementary Figure 10.

Serum SAA levels were lowered in *Ldlr*^{-/-}, *Apoe*^{-/-}, and wild-type C57BL/6J mice following TFA treatment. For *Ldlr*^{-/-} mice, plasma was taken following two weeks of a daily i.p. injection of TFA (27 mg/kg/day). For wild-type C57BL/6J mice (male), plasma was taken following four weeks of a daily oral gavage of TFA (18 mg/kg/day). The data are shown as mean ± SD. *p* values were determined by one-way ANOVA, post hoc Dunnett's multiple comparison test comparing the experimental group to HFD PBS vehicle group. *Ldlr*^{-/-} mice sample sizes were: male HFD, PBS vehicle (n=3 mice); other groups (n=4 mice). *Apoe*^{-/-} mice sample sizes were: CHOW, PBS vehicle (n=5 mice), other groups (n=6 mice). C57BL/6J sample sizes were: CHOW, PBS vehicle (n=8 mice); HFD, PBS vehicle (n=14 mice); HFD, TFA (n=10 mice).



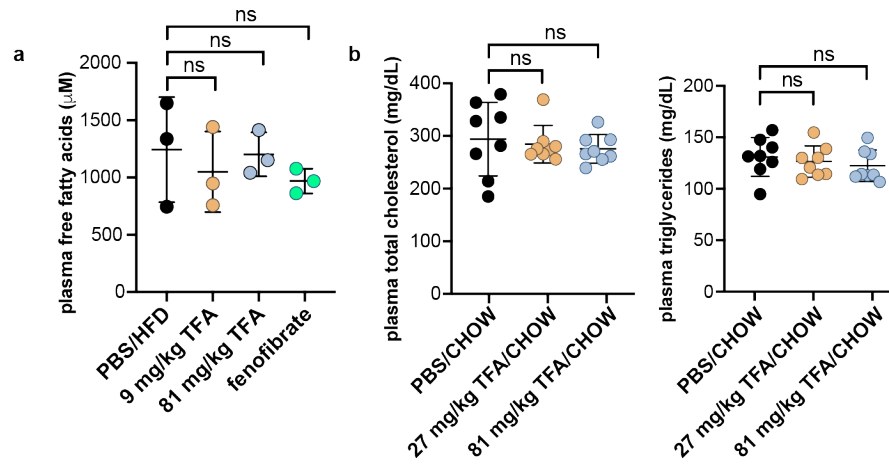
Supplementary Figure 11.

In an 8-week atheroprotection study using a low dose of TFA (1.4 mg/kg/day) with an i.p. route of administration in HFD-fed female *Ldlr*^{-/-} mice, TFA treatment did not cause changes in plasma cholesterol or triglyceride levels, nor did it reduce the development of aortic lesions. The vehicle in this study was 10 mM acetate buffer, pH 4. The data are shown as mean ± SD. *p* values were determined by one-way ANOVA comparing the experimental group to PBS vehicle group; ns, not significantly different. Samples sizes were: cholesterol panel and triglycerides panel, n=8 mice for all groups; aortic lesion panel, n=8 mice for acetate vehicle/HFD group and n=7 mice for 1.4 mg/kg TFA/HFD group.



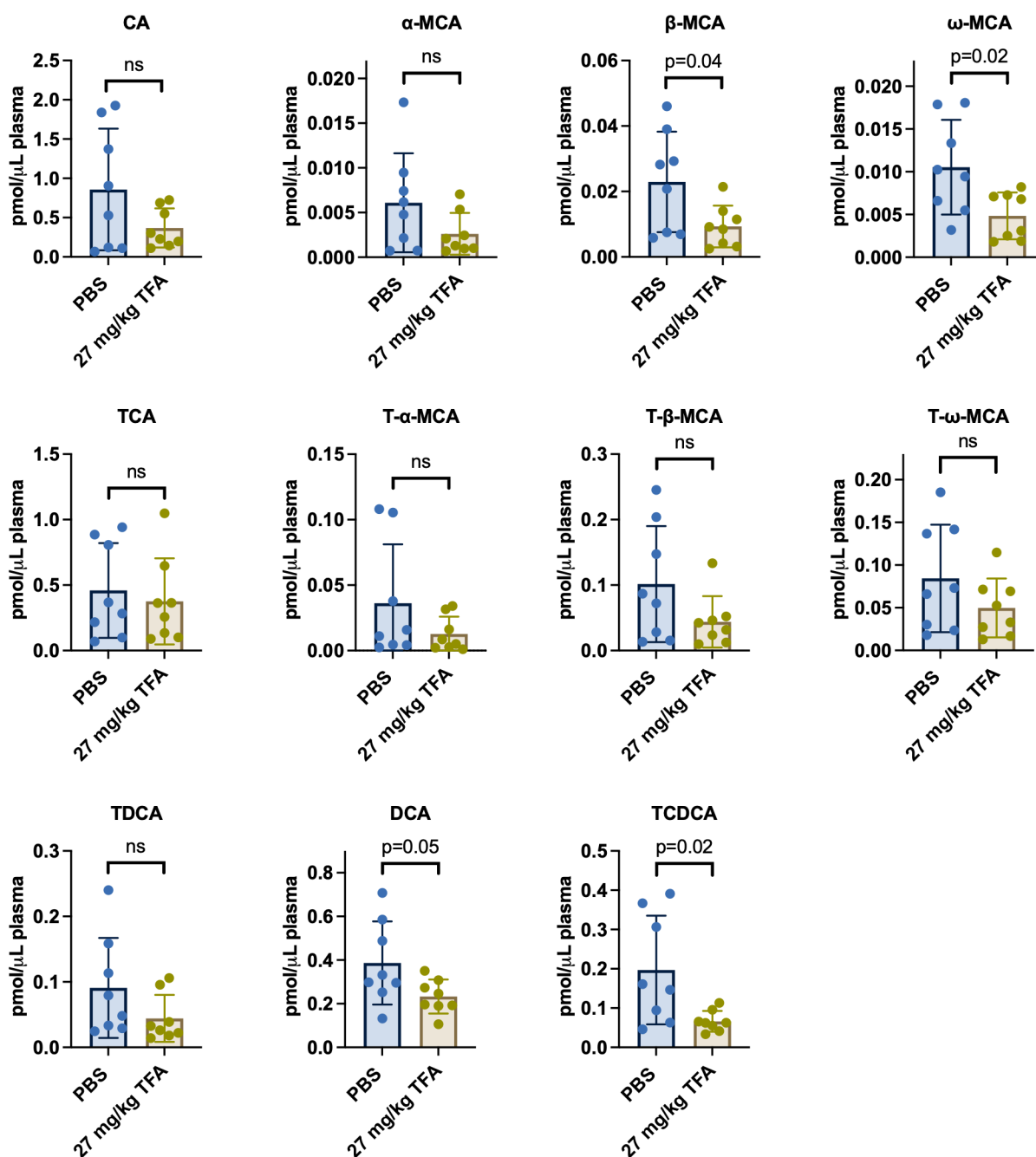
Supplementary Figure 12.

Confocal microscopy at 60x magnification of liver cells from the TFA- or vehicle-treated *Ldlr*^{-/-} animals, stained magenta for PMP70, a marker of peroxisome proliferation. Magenta, AF568 stain for PMP70; blue, DAPI stain for nuclei; green, autofluorescence. Images are representative of five biological replicates per group.



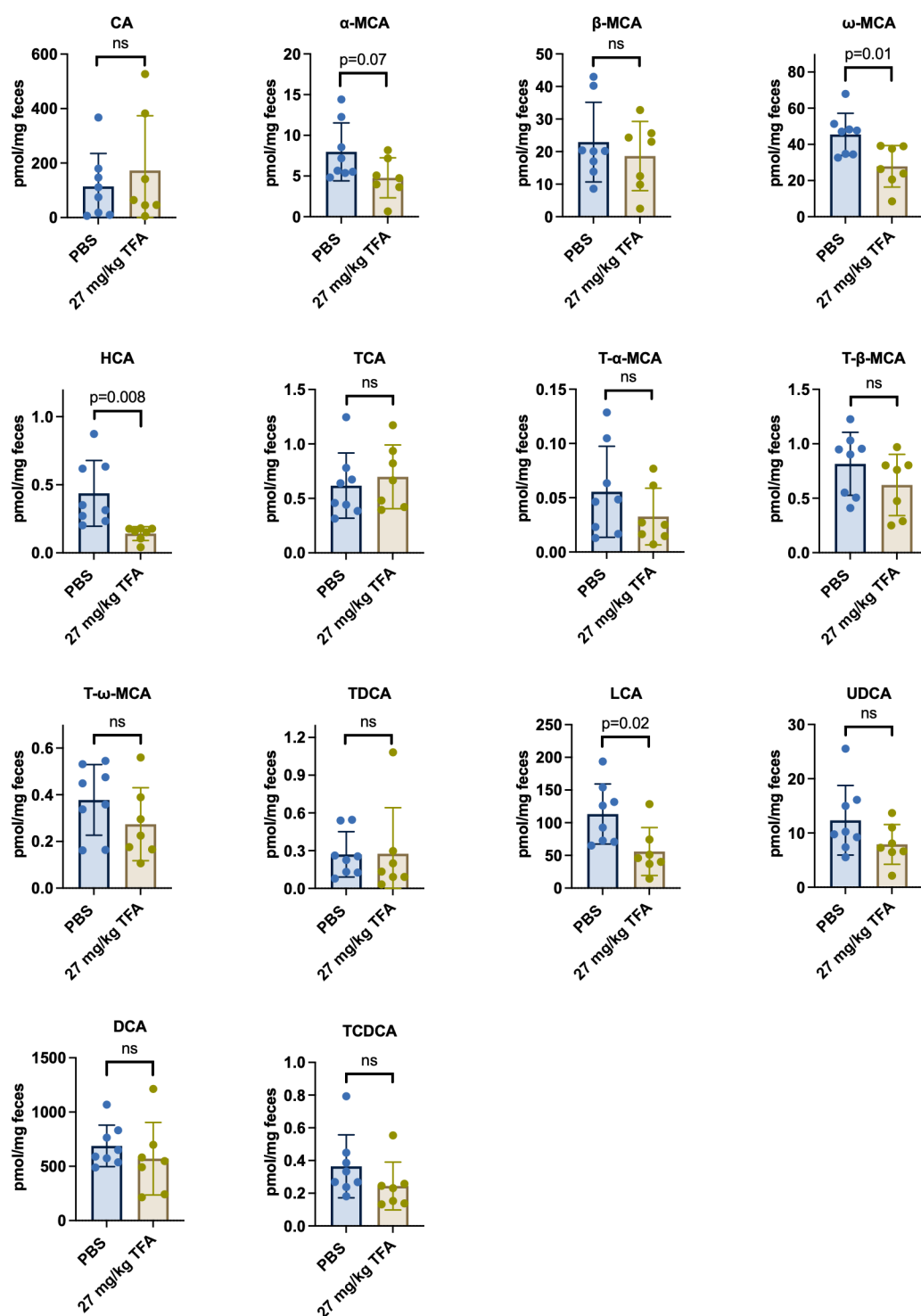
Supplementary Figure 13.

(a) No significant changes were observed in plasma free fatty acid levels of female *Ldlr*^{-/-} mice fed a HFD when treated with TFA for two weeks by daily oral gavage. **(b)** No changes were observed in triglyceride or cholesterol levels of male *Ldlr*^{-/-} mice fed a CHOW diet when treated with TFA for two weeks by daily oral gavage. The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA, post hoc Dunnett's multiple comparison test comparing the experimental groups to PBS vehicle group; ns, not significantly different. Sample numbers were: n=3 mice/group for all groups in panel **a**; n=8 mice/group for all groups in panel **b**.



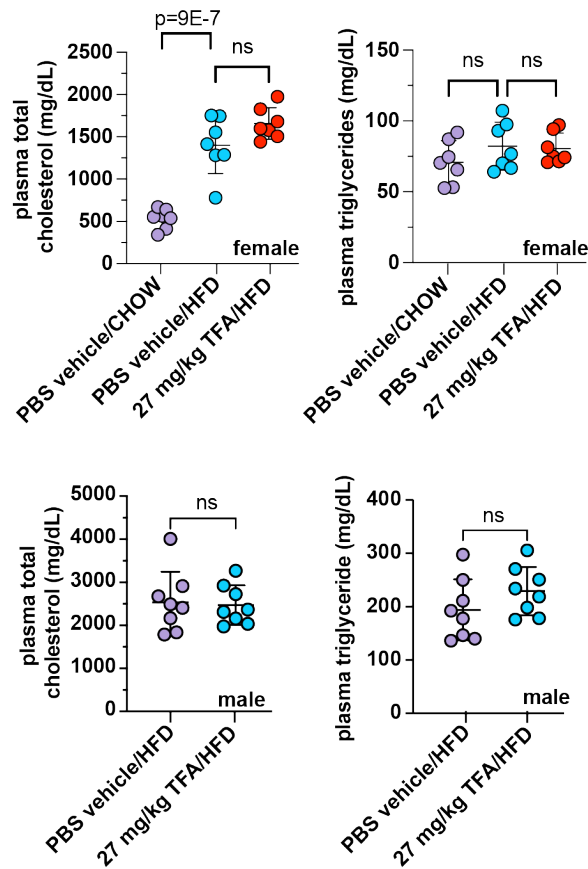
Supplementary Figure 14.

Observed bile acid levels in the plasma of male LDL-null mice (n=8 mice per group) following 2-week administration of TFA (27 mg/kg/day) or PBS vehicle in the drinking water. Bile acid levels were determined by targeted mass spectrometry. The data are shown as mean $\pm$ SD. *p* values were determined by unpaired two-tailed Student's *t*-test comparing the experimental group to PBS vehicle group; ns, not significantly different.



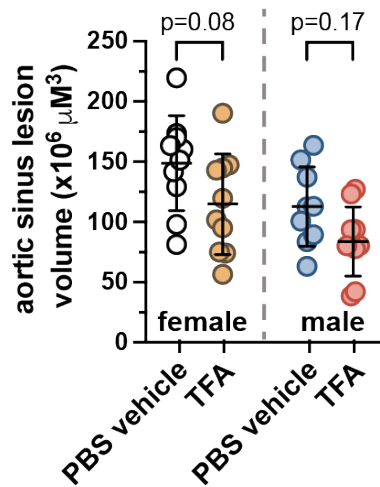
Supplementary Figure 15.

Observed bile acid levels in the feces of male LDL-null mice (n=8 mice for PBS vehicle group, n=7 mice for TFA group) following 2-week administration of TFA (27 mg/kg/day) or PBS vehicle in the drinking water. Bile acid levels were determined by targeted mass spectrometry. The data are shown as mean $\pm$ SD. *p* values were determined by unpaired two-tailed Student's *t*-test comparing the experimental group to PBS vehicle group; ns, not significantly different.



Supplementary Figure 16.

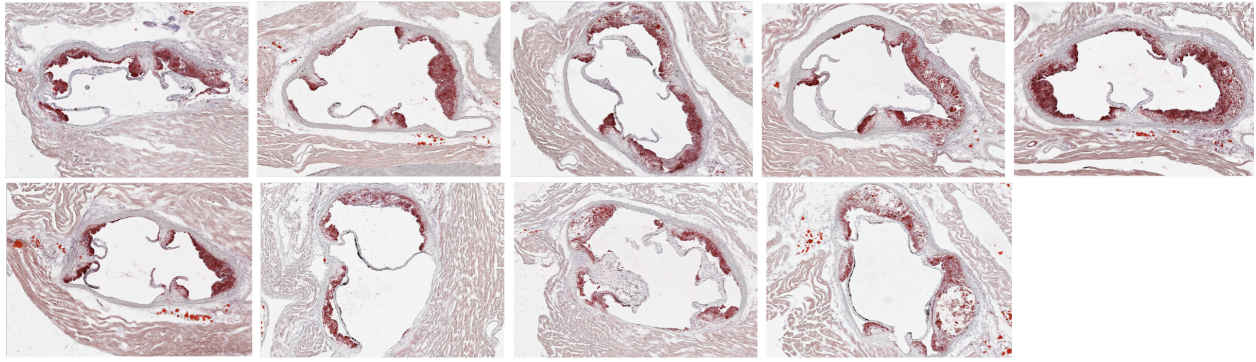
No changes were observed in triglyceride or cholesterol levels of *Apoe*^{-/-} mice following 2-week TFA treatment (*ad libitum* in drinking water). The data are shown as mean $\pm$ SD. *p* values for the female studies were determined by one-way ANOVA, post hoc Sidak's multiple comparison test comparing the experimental group to PBS vehicle/HFD group; *p* values for the male studies were determined by unpaired two-tailed Student's *t*-test; ns, not significantly different. Sample numbers were: *n*=7 mice for all groups in female studies, *n*=8 mice for all groups in male studies.



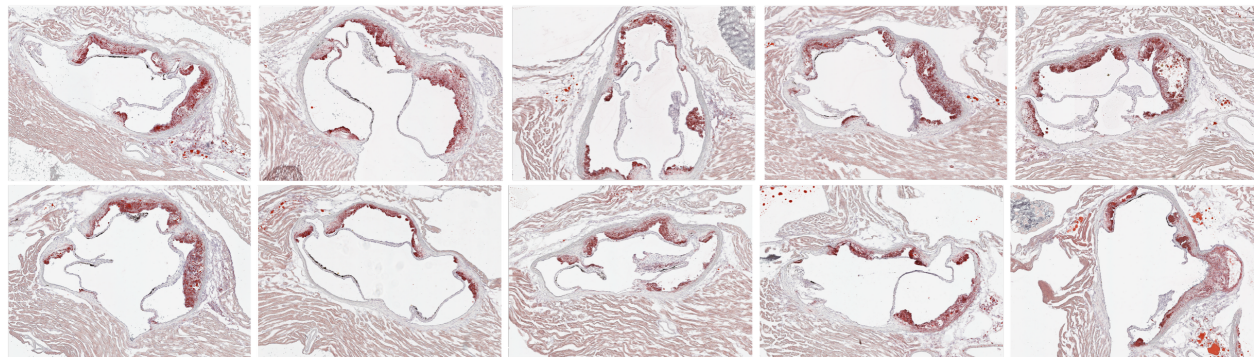
Supplementary Figure 17.

Aortic sinus atherosclerotic lesions were reduced, albeit not to a statistically significant degree, by daily administration of TFA (27 mg/kg) in the drinking water for 10 weeks in HFD-fed *ApoE*^{-/-} mice. The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA, post hoc Sidak's multiple comparison test comparing the experimental group to PBS vehicle group. Sample numbers were: female PBS vehicle (n=10 mice), female TFA (n=10 mice), male PBS vehicle (n=9 mice), male TFA (n=10 mice).

PBS vehicle, male *Apoe*^{-/-}

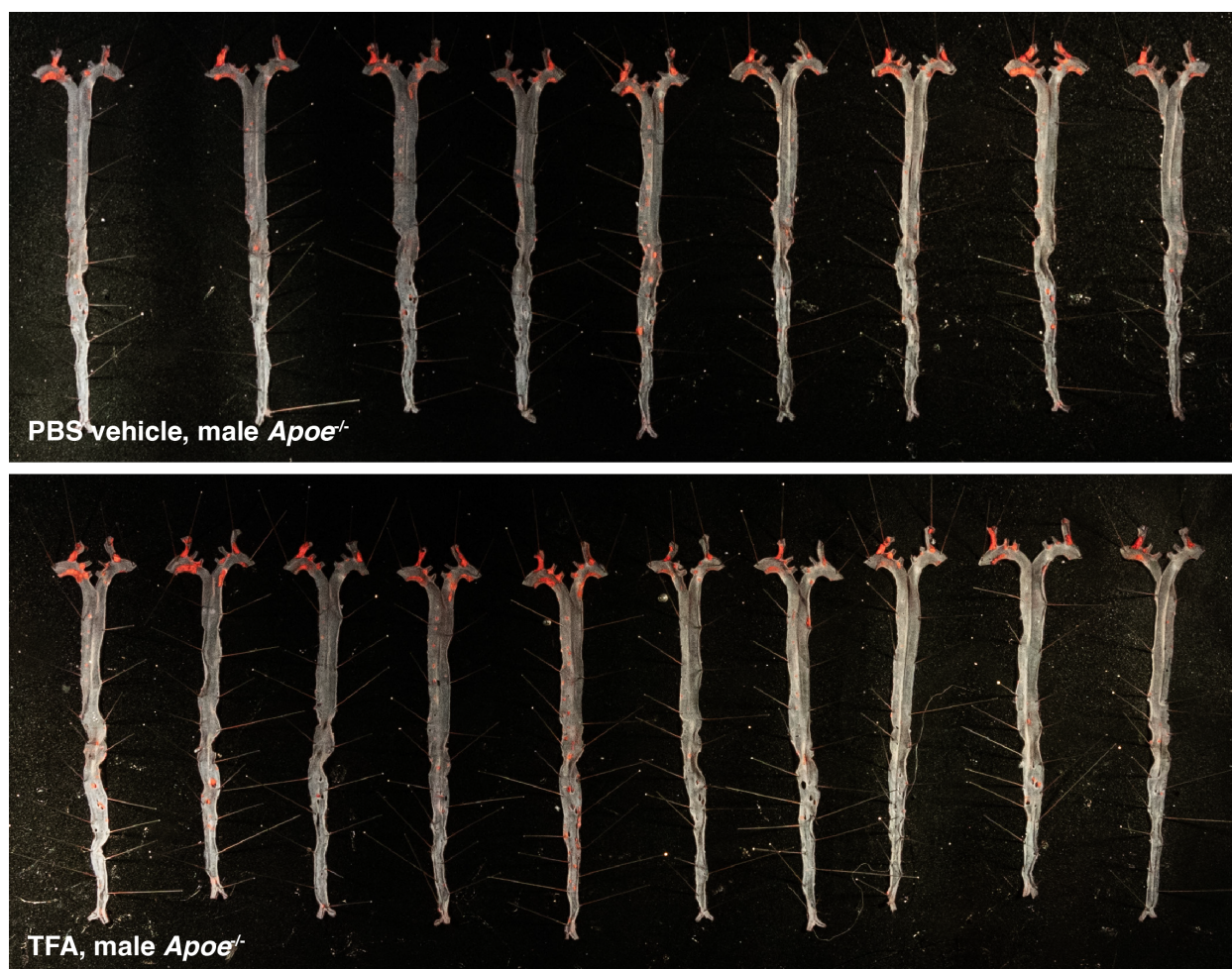


27 mg/kg TFA, male *Apoe*^{-/-}



Supplementary Figure 18.

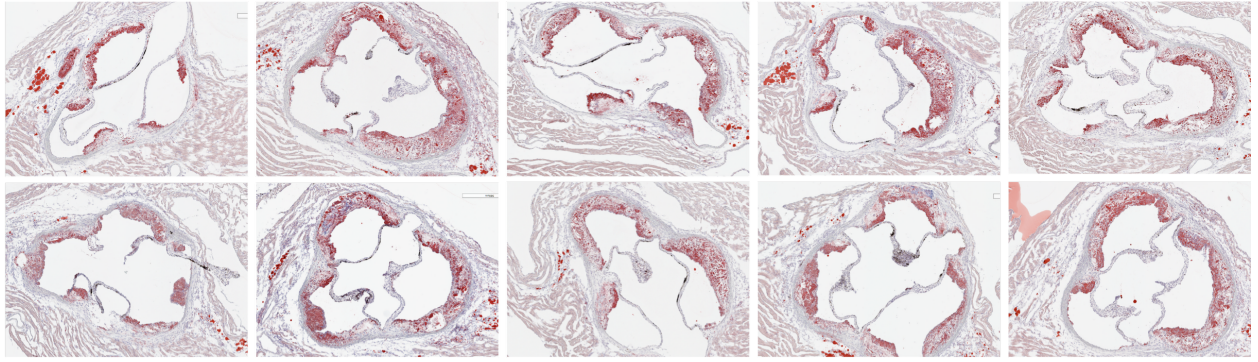
Aortic sinus cross-sections from male TFA- and PBS vehicle-treated HFD-fed *Apoe*^{-/-} mice following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.



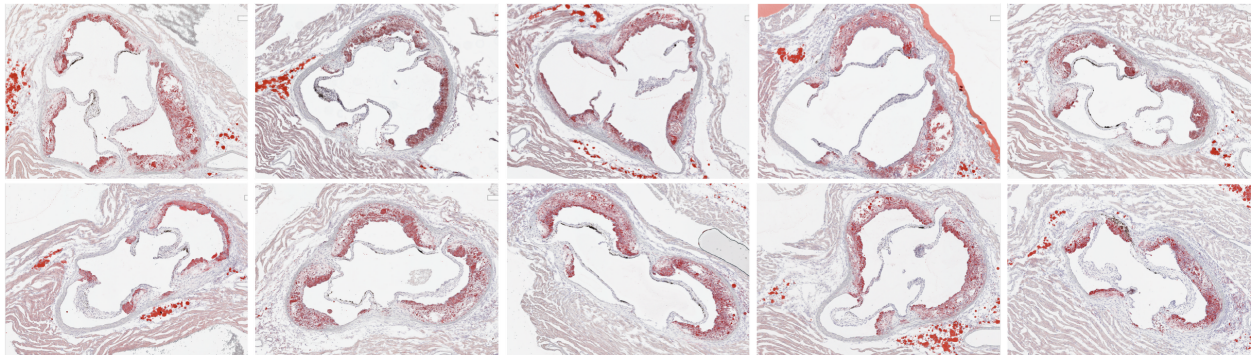
Supplementary Figure 19.

Aortas from male TFA- and PBS vehicle-treated HFD-fed *Apoe*^{-/-} mice following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.

PBS vehicle, female *Apoe*^{-/-}

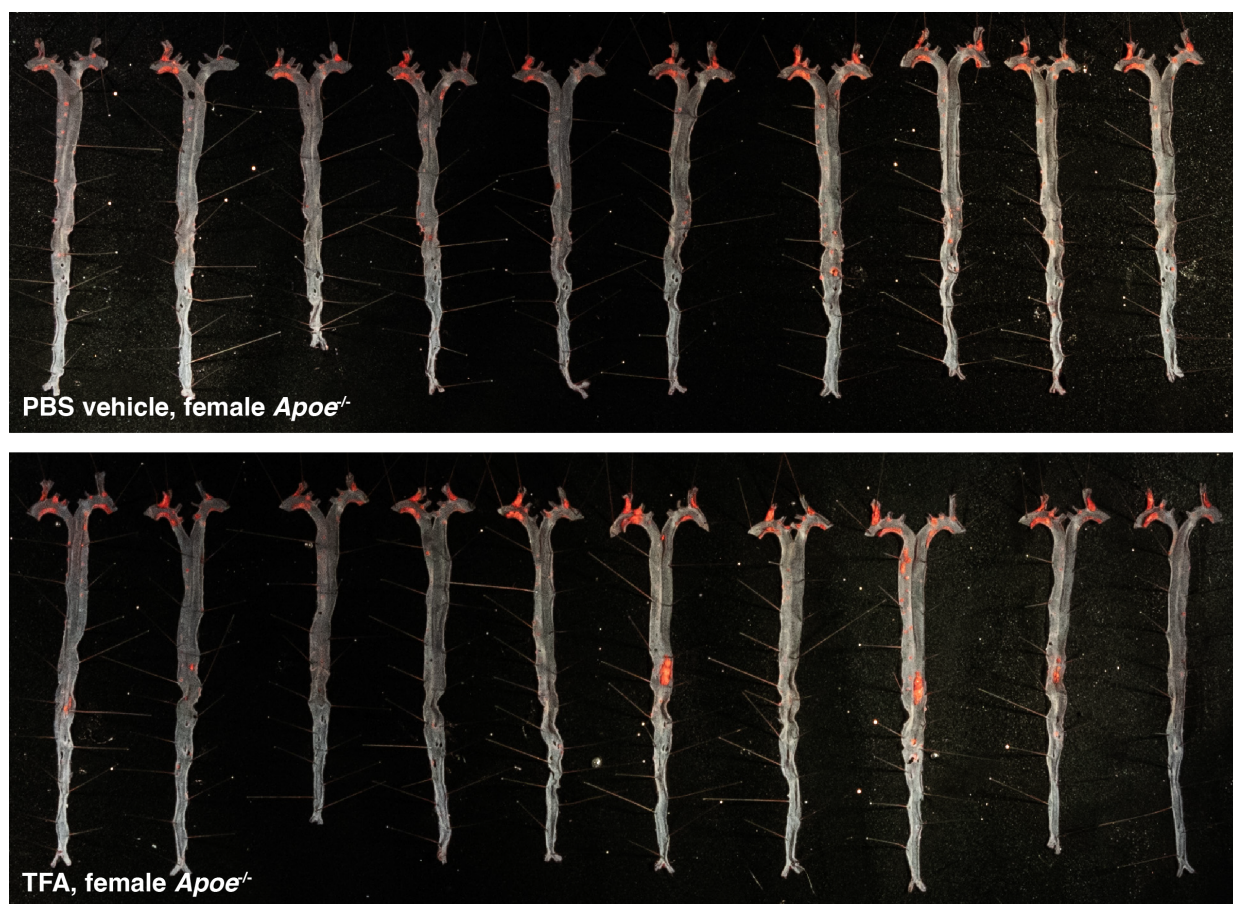


27 mg/kg TFA, female *Apoe*^{-/-}



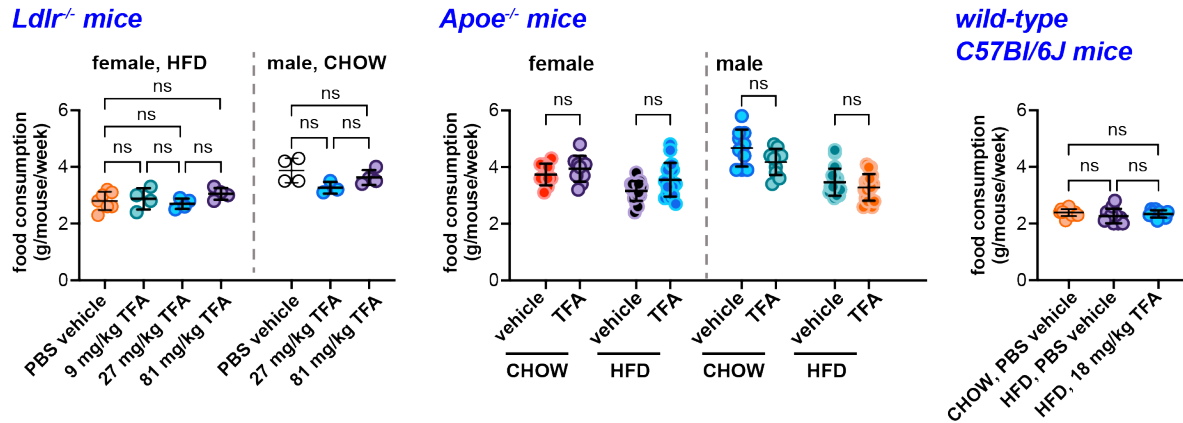
Supplementary Figure 20.

Aortic sinus cross-sections from female TFA- and PBS vehicle-treated HFD-fed *Apoe*^{-/-} mice following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.



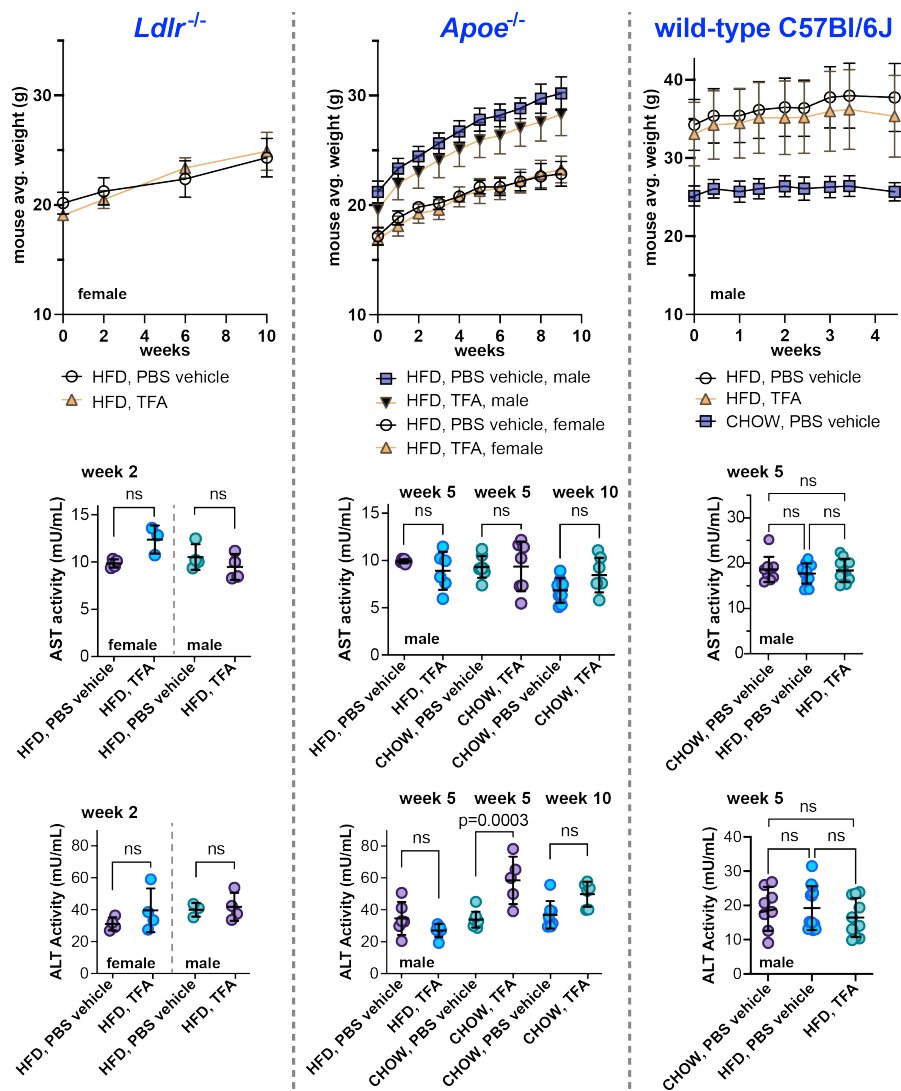
Supplementary Figure 21.

Aortas from female TFA- and PBS vehicle-treated HFD-fed *Ldlr*^{-/-} mice following 10-week treatment *ad libitum* in the drinking water (27 mg/kg/day). The lipid is stained red.



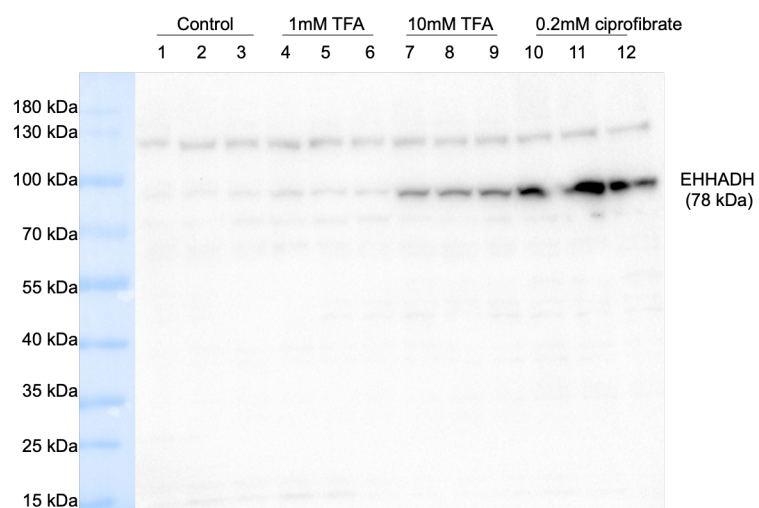
Supplementary Figure 22.

Food intake for the *in vivo* studies shown in Figure 4 of the manuscript. Food intake did not differ significantly between control animals vs. TFA-treated animals in any of the studies for *Ldlr*^{-/-}, *Apoe*^{-/-}, or wild-type C57BL/6J mice, ruling out differences in cholesterol or fat uptake as a source of phenotypic differences. Food intake in grams was recorded per cage on a weekly basis, from which the average weekly intake per mouse was calculated. Additional details about the diets are shown in Figure S1. The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA, post hoc Sidak's multiple comparison test. Sample numbers for female *Ldlr*^{-/-} mice studies were: PBS vehicle (n=7 weekly cage consumption recordings), 9 mg/kg TFA (n=4), 27 mg/kg TFA (n=4), 81 mg/kg TFA (n=4). Sample numbers for male *Ldlr*^{-/-} mice studies were: PBS vehicle (n=4 weekly cage consumption recordings), 27 mg/kg TFA (n=3), 81 mg/kg TFA (n=4). Sample numbers for *Apoe*^{-/-} mice studies fed CHOW diet were: n=10 weekly cage consumption recordings for all groups. Sample numbers for *Apoe*^{-/-} mice studies fed HFD diet were: n=18 weekly cage consumption recordings for all groups. Sample numbers for C57Bl/6J mice studies were: PBS vehicle/CHOW (n=14 weekly cage consumption recordings), PBS vehicle/HFD (n=11), 18 mg/kg TFA/HFD (n=10).



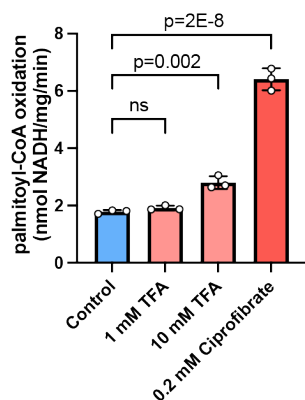
Supplementary Figure 23.

TFA treatment did not affect weight gain compared to vehicle-treated control animals over the course of multi-week studies, nor did TFA cause persistent changes in the plasma activity of liver enzymes alanine transaminase (ALT) or aspartate aminotransferase (AST). The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA comparing the experimental group to PBS vehicle group, post hoc Tukey's multiple comparison test; ns, not significantly different. Sample numbers for *Ldlr*^{-/-} weight: n=8 mice for both groups. Sample numbers for *Apoe*^{-/-} weight: n=10 mice for all groups except HFD/PBS vehicle/male (n=9 mice). Sample numbers for C57Bl/6J weight: n=10 mice for all groups except HFD/PBS vehicle (n=15). Sample numbers for *Ldlr*^{-/-} AST: n=4 mice for all groups except HFD/TFA/female (n=3). Sample numbers for *Apoe*^{-/-} AST: n=6 mice for 5 wk HFD/PBS vehicle and HFD/TFA; n=8 for 5wk and 10 wk CHOW/PBS vehicle, n=7 for 5wk and 10 wk CHOW/TFA. Sample numbers for C57Bl/6J AST: n=9 for CHOW/PBS vehicle, n=13 for HFD/PBS vehicle, n=10 for HFD/TFA. Sample numbers for *Ldlr*^{-/-} ALT: n=4 for all groups except HFD/PBS vehicle/male (n=3). Sample numbers for *Apoe*^{-/-} ALT: n=6 for 5 wk HFD/PBS vehicle and HFD/TFA; n=8 for 5wk and 10 wk CHOW/PBS vehicle, n=6 for 5wk and 10 wk CHOW/TFA. Sample numbers for C57Bl/6J ALT: n=8 for CHOW/PBS vehicle, n=13 for HFD/PBS vehicle, n=10 for HFD/TFA.



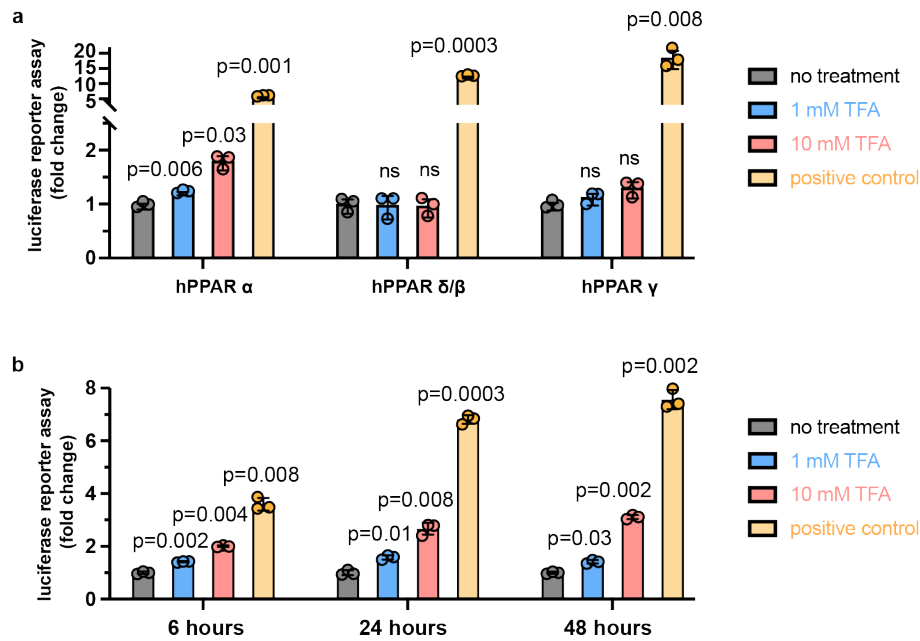
Supplementary Figure 24.

Western blot of rat FAO cell lysates showing that TFA treatment led to higher levels of EHHADH protein in the cells. The experiment was repeated twice independently with similar results.



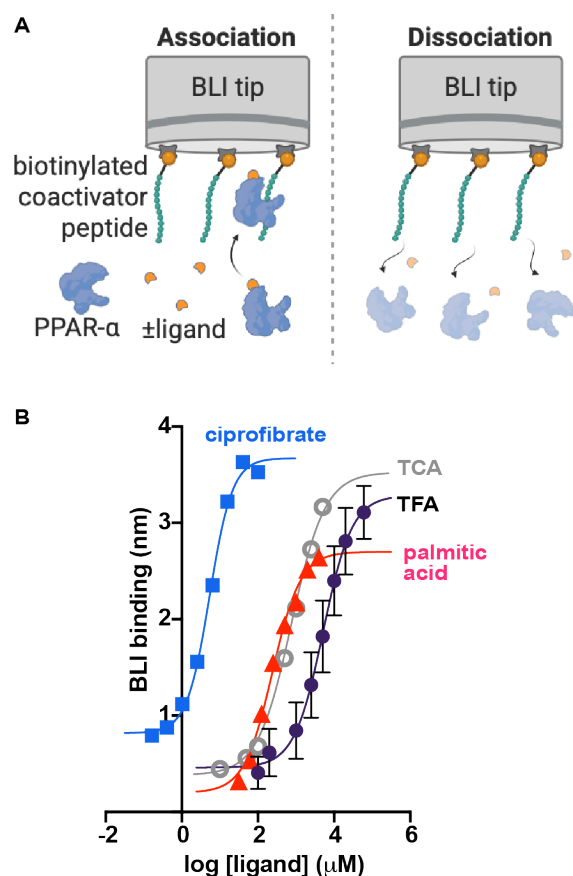
Supplementary Figure 25.

Palmitoyl-CoA oxidase activity in rat FAO liver cells following treatment with TFA or ciprofibrate. The data are shown as mean $\pm$ SD of technical triplicates. *p* values were determined by one-way ANOVA comparing the experimental group to PBS vehicle group, post hoc Sidak's multiple comparison test; ns, not significantly different. The experiment was repeated twice independently with similar results.



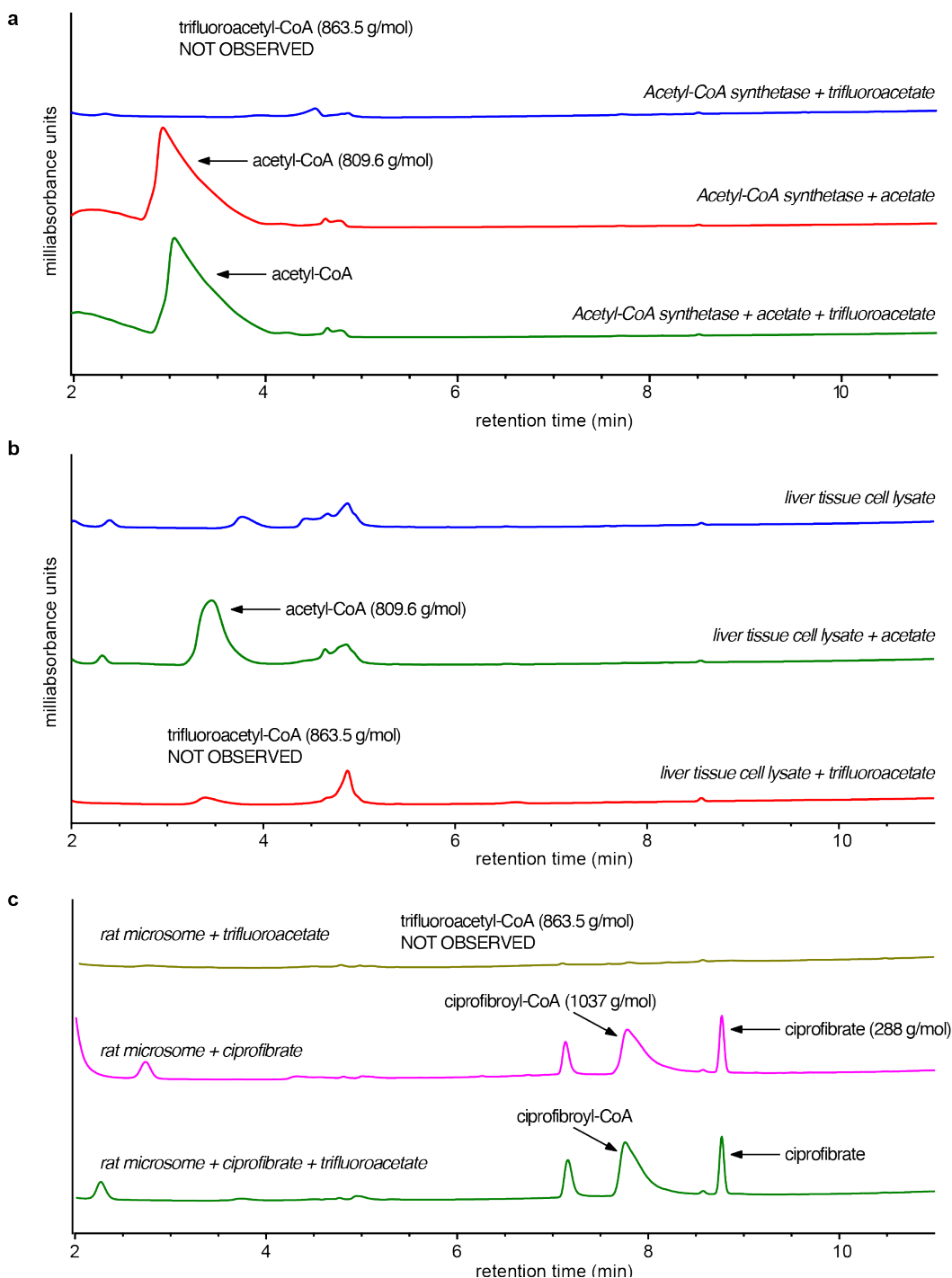
Supplementary Figure 26.

(a) Reporter assay in human HepG2 liver cells transfected with different human PPAR isoforms, in which luciferase expression is driven by activation of the PPAR response element. A different positive control was used for each PPAR isoform: 0.1 mM ciprofibrate for PPAR- α , 1 μ M GW501516 for PPAR- δ/β , and 10 μ M rosiglitazone for PPAR- γ . (b) Reporter assay in human HepG2 liver cells transfected with mouse PPAR- α , showing the effect of different TFA treatment times. The data are shown as mean $\pm$ SD for technical triplicates. *p* values were determined by one-way ANOVA comparing the experimental group to no treatment group; ns, not significantly different. The experiments were repeated twice independently with similar results.



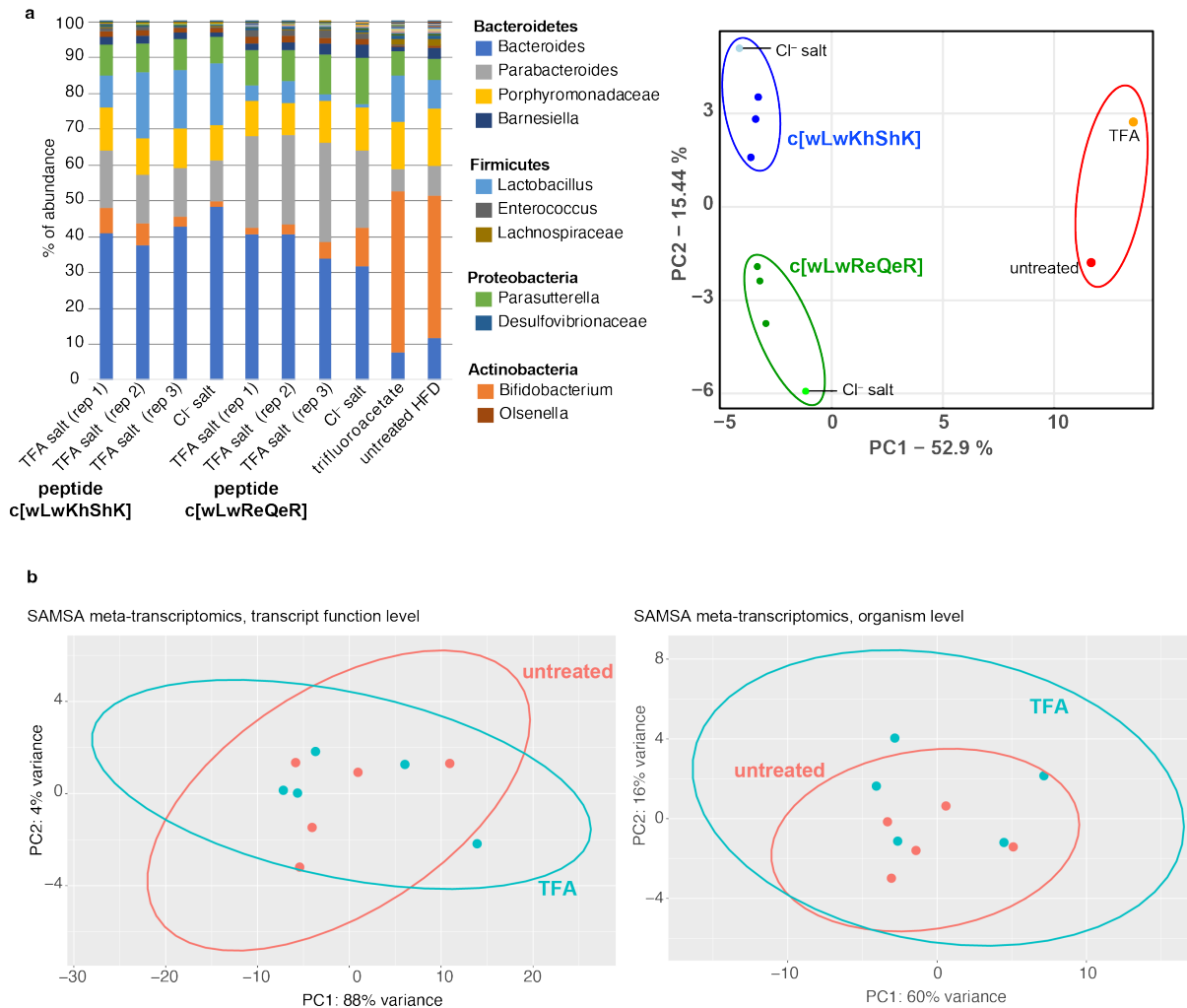
Supplementary Figure 27.

Overview of coactivator recruitment assay used to assess TFA binding to PPAR-α. (A) Schematic diagram of the BLI assay. Binding of ligands to PPAR-α causes a conformational change that promotes association of the PPAR-α/ligand complex to the LXXLL motif present in coactivator proteins. Accordingly, a biotinylated peptide containing the LXXLL motif was synthesized and adhered to a streptavidin-coated BLI tip so that BLI could be used to measure the EC₅₀ for putative ligands in promoting the binding of purified PPAR-α in solution to the LXXLL motif on the BLI tip. The sequence of the biotinylated coactivator peptide was: biotin-[PEG]-EAEPSLLKKLLLAPANTQ-NH₂. '[PEG]' refers to amino)ethoxy]ethoxy]acetic acid, incorporated using Fmoc-AEEA (CAS # 166108-71-0). Created in BioRender. Leman, L. (2026) <https://BioRender.com/boq8z5a> (B) Binding responses (maximum shift in the association phase) were plotted and fitted using nonlinear regression with a variable slope model to determine EC₅₀ values, using GraphPad Prism 11. The experiment was repeated twice independently with similar results. TCA = sodium trichloroacetate.



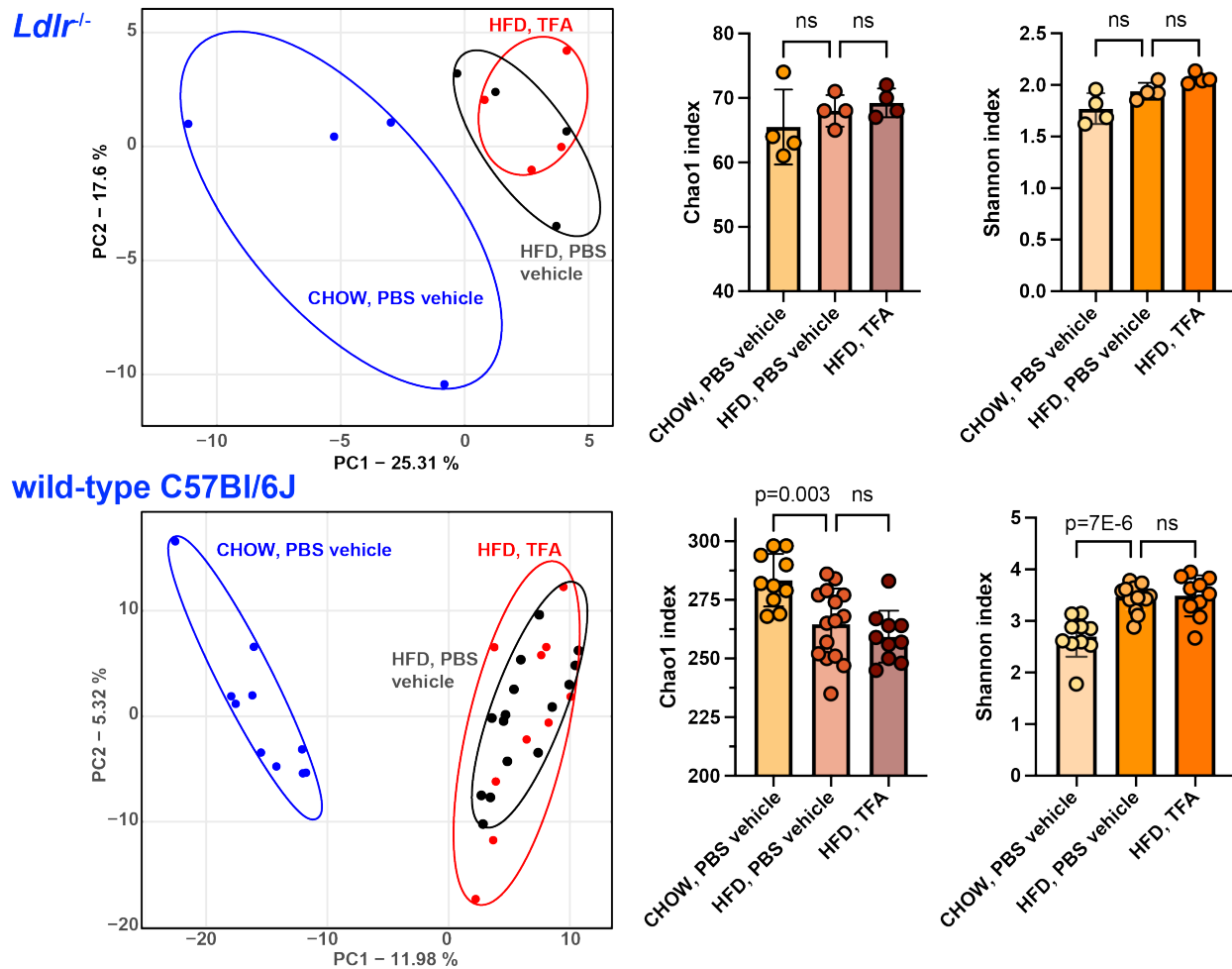
Supplementary Figure 28.

Biochemical experiments using purified acetyl-CoA synthetase (**a**), rat liver tissue lysate (**b**), or rat liver microsomes (**c**) suggest that trifluoroacetyl-CoA is not produced. Whereas acetyl-CoA and ciprofibril-CoA were observed under analogous conditions by LCMS analysis, trifluoroacetyl-CoA was not. Furthermore, the presence of TFA did not apparently affect the formation of acetyl-CoA or ciprofibril-CoA. The experiments were repeated at least twice independently with similar results.



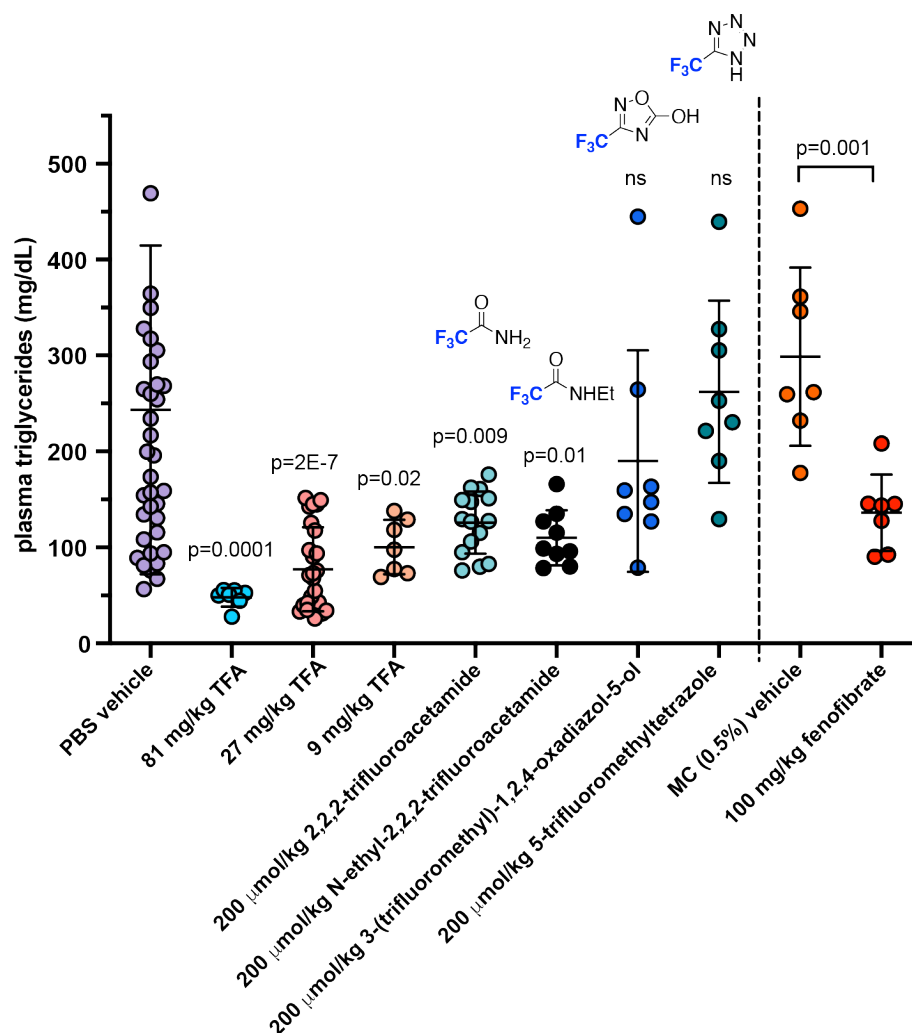
Supplementary Figure 29.

In vitro analyses of TFA effects on gut bacteria. **a)** Fresh gut microbiome content was isolated by surgically removing cecum under anaerobic conditions from *Ldlr*^{-/-} mice that had been fed HFD for two weeks. The cecal contents were then mixed with growth media, distributed in 96-well plates, and incubated overnight in the presence of TFA (1 mM) or positive control gut bacteria remodeling cyclic peptides c[wLwReQeR] or c[wLwKhShK] (64 mM). The peptides were tested either as TFA salt (three replicates) or Cl⁻ salt. After the incubation, the composition of the gut microbiota community was assessed *en masse* by 16S rRNA sequencing of each well. As indicated by bacterial genera composition or Bray-Curtis beta diversity principal component analysis, the peptides had remodeled the gut bacteria community, whereas TFA did not appreciably remodel the community. **b)** To assess the possible effect of TFA on gut bacteria meta-transcriptomics, fresh microbiome content was isolated as described in part **a**. The cecal contents were mixed with growth media and incubated with TFA (1 mM) or mock buffer (n=5/group) for six hours. Total RNA was isolated. Library preparation and sequencing were carried out by Novogene. The data were analyzed using SAMSA2 on the transcript function level or organism level. No differences were observed between TFA treatment vs untreated samples.



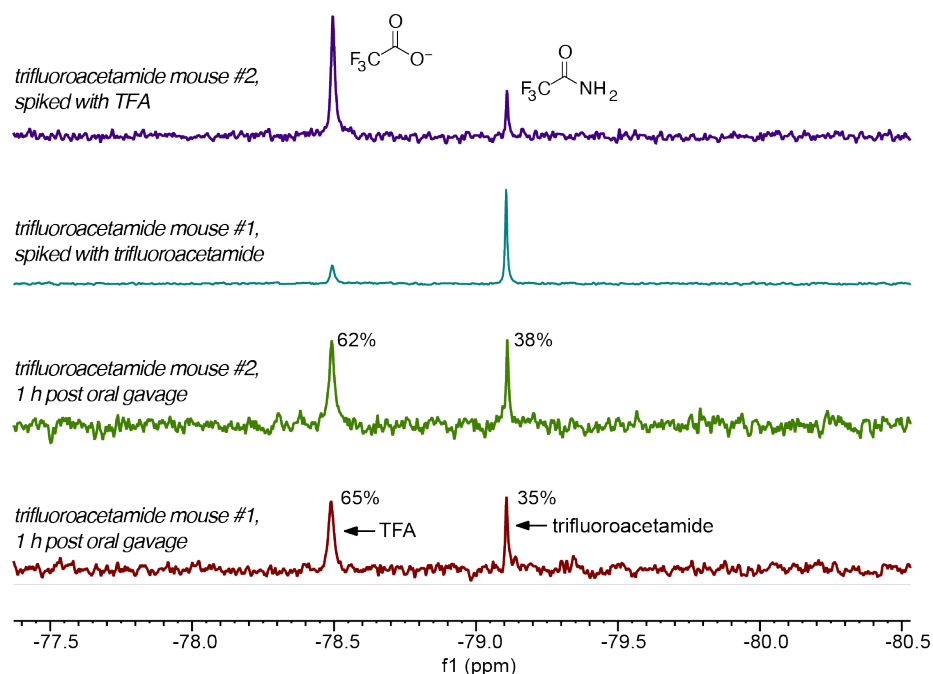
Supplementary Figure 30.

Analysis of 16S gut microbiome sequencing of fecal samples from PBS vehicle- or TFA-treated mice. Fecal samples were from *Ldlr*^{-/-} mice (2-week treatment, 27 mg/kg/day, n=4 mice /group) or wild-type C57BL/6J mice (4-week treatment; 27 mg/kg/day; n=10 mice /group for CHOW, PBS vehicle and HFD, TFA groups; n=15 mice /group for HFD, PBS vehicle group). On the left is shown a Bray-Curtis principal component analysis of beta diversity between samples. On the right, plots of alpha diversity are shown. The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA, post hoc Sidak's multiple comparison test; ns, not significantly different.



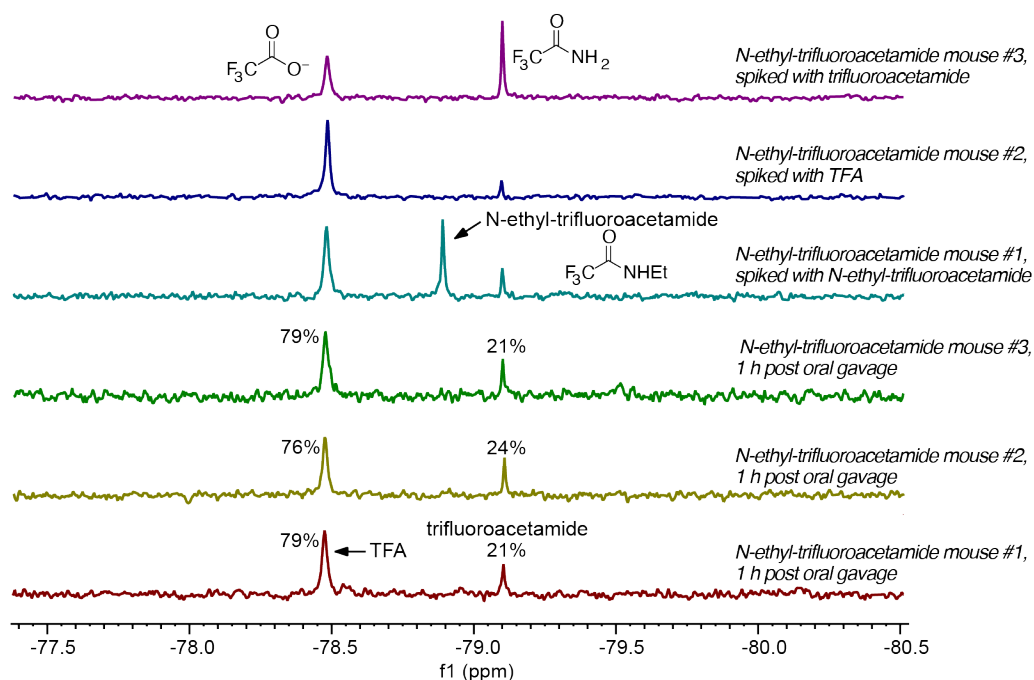
Supplementary Figure 31.

Reduction of plasma triglyceride levels by TFA and analogs in HFD-fed *Ldlr*^{-/-} mice. Each analog was administered by oral gavage for 2 weeks at the dose shown. The 200 μ mol dose used for each TFA analog is equivalent on a molar basis to a 27 mg/kg sodium TFA dose. The reductions in plasma triglyceride levels observed for N-ethyl-trifluoroacetamide and 2,2,2-trifluoroacetamide are likely due to the effects of TFA *in vivo* following oral administration. The data represent a combination of three independent vehicle-controlled studies for 27 mg/kg TFA, and a single study for the other chemicals, with each datapoint representing one mouse. The data are shown as mean $\pm$ SD. *p* values were determined by one-way ANOVA, post hoc Dunnett's multiple comparison test comparing the experimental groups to the corresponding vehicle group; ns, not significantly different. Sample numbers of mice in each group were: PBS vehicle (n=36 mice), 81 mg/kg TFA (n=7), 27 mg/kg TFA (n=26), 9 mg/kg TFA (n=7), 2,2,2-trifluoroacetamide (n=15), N-ethyl-2,2,2-trifluoroacetamide (n=9), 3-(trifluoromethyl)-1,2,4-oxadiazol-5-ol (n=8), 5-trifluoromethyltetrazole (n=8), MC vehicle (n=7), fenofibrate (n=7).



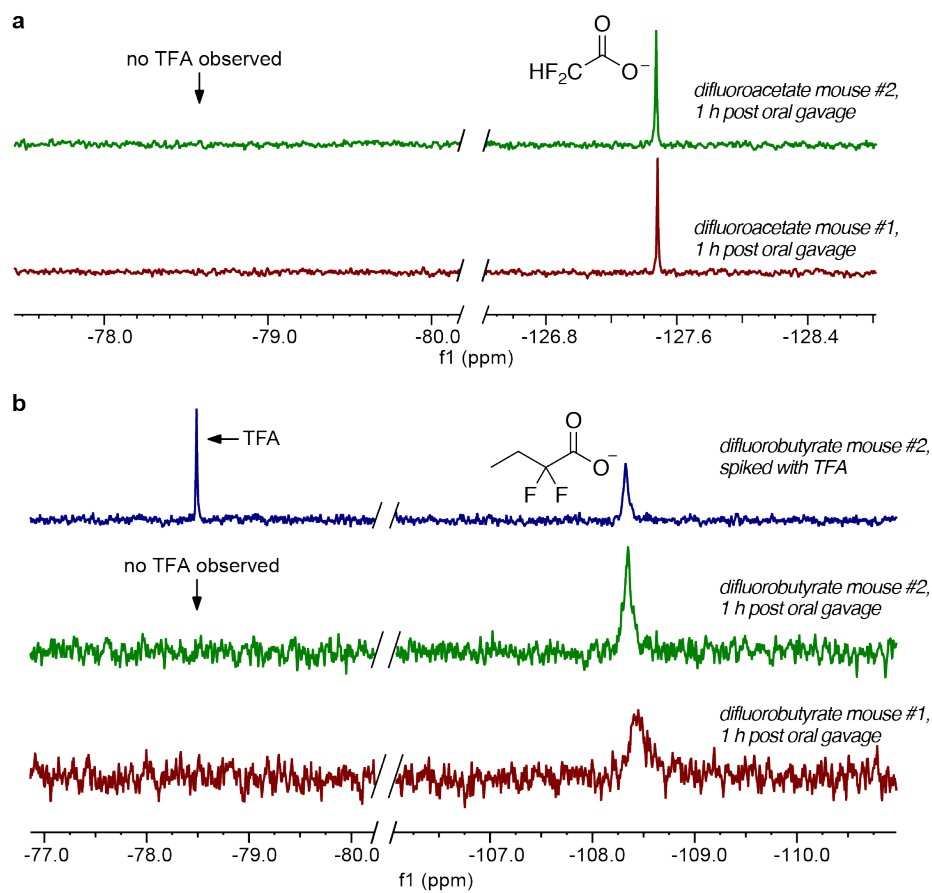
Supplementary Figure 32.

2,2,2-Trifluoroacetamide is converted into TFA *in vivo* following oral administration. Mice were gavaged with a 200 $\mu\text{mol/kg}$ dose of 2,2,2-trifluoroacetamide (equivalent dose to 27 mg/kg sodium TFA). After 1 h, blood was drawn and analyzed by ^{19}F -NMR. Around 64% of the trifluoroacetylated species in plasma was TFA. Spiking of the plasma with TFA or trifluoroacetamide confirmed the identity of the observed peaks.



Supplementary Figure 33.

N-Ethyl-trifluoroacetamide is converted into TFA and trifluoroacetamide *in vivo* following oral administration. Mice were gavaged with a 200 $\mu\text{mol/kg}$ dose of N-ethyl-trifluoroacetamide (equivalent dose to 27 mg/kg sodium TFA). After 1 h, blood was drawn and analyzed by ^{19}F -NMR. Around 78% of the trifluoroacetylated species in plasma was TFA, with the remainder being 2,2,2-trifluoroacetamide. None of the administered N-ethyl-trifluoroacetamide was observed at 1 h post gavage. Spiking of the plasma with TFA, N-ethyl-trifluoroacetamide, or trifluoroacetamide confirmed the identity of the observed peaks.



Supplementary Figure 34.

Difluoroacetate (**a**) and difluorobutyrate (**b**) are not appreciably converted into TFA over a period of 1 h *in vivo* following oral administration. Mice were gavaged with a 200 $\mu\text{mol/kg}$ dose of the compound (equivalent dose to 27 mg/kg sodium TFA). After 1 h, blood was drawn and analyzed by ^{19}F -NMR. No TFA was observed in either case at 1 h post gavage. Spiking of the plasma with TFA, difluoroacetate, or difluorobutyrate confirmed the identity of the observed peaks.

Primers	Sequence (5'to 3')
<i>For RT-qPCR</i>	
fEHHADH-F	TCAGTTGGCGTTCTTGGCTT
fEHHADH-R	GAAGCTTGGCCGTTCTGATG
fGAPDH-2ndF	GCCTGGAGAAACCTGCCAA
fGAPDH-2ndR	CACAGGAGACAACCTGGTCC
fACOX1-2ndF	CGTGCAGCCAGATTGGTAGA
fACOX1-2ndR	AACGACCACGTAGTGGCAAT
fACAA1-F	TCTACCACGGCTGGAACTC
fACAA1-R	ATAGGACCTCAGGACGCCAA
<i>For PPAR isoforms construction</i>	
CMVmPPAR- α -F	GTCGACCTGCAGGCATGCAAGCTTGCCACCATGGTGGACACAGAGA
CMVmPPAR- α -R	TTTCAGTTAGCCTCCCCAAGCTTCCATCTCAGGAAAGATCA
CMVmPPAR- δ/β -F	GTCGACCTGCAGGCATGCAAGCTTGCCACCATGGAACAGCCACAGGAG
CMVmPPAR- δ/β -R	TTTCAGTTAGCCTCCCCAAGCTTGGCTGCGGCCTTAGTACA
CMVmPPAR- γ -F	GTCGACCTGCAGGCATGCAAGCTTGCCACCATGGTTGACACAGAGATG
CMVmPPAR- γ -R	TTTCAGTTAGCCTCCCCAAGCTTGGGTGGGACTTTCCTGCTAA
<i>For mPPAR expression</i>	
28a-mPPAR- α -F	CACAGAGAACAGATTGGTGGTTGGGAACTTCAAGTGGACACAGAGAGCCCCAT
28a-mPPAR- α -R	TGGTGGTGGTGGTGCTCGAGTCAGTACATGTCTCTGTAGATCTCTTGC
<i>For CRISPR-Cas9 knock-out</i>	
fPPAR- α -gRNA1-F	CACCGACATCGAGTGTGCAATATG
fPPAR- α -gRNA1-R	AAACCATATTTCGACACTCGATGTC
fPPAR- α -gRNA2-F	CACCGCTGTGGAGGTCCCATAATA
fPPAR- α -gRNA2-R	AAACTATTATGGGACCTCCACAGC
fPPAR- α -VeriF	GCATGTATCACGACCCCTGT
fPPAR- α -VeriR	TACCTCACCTTCTCCCGAG

Supplementary Table 1.

Primers used in this study.