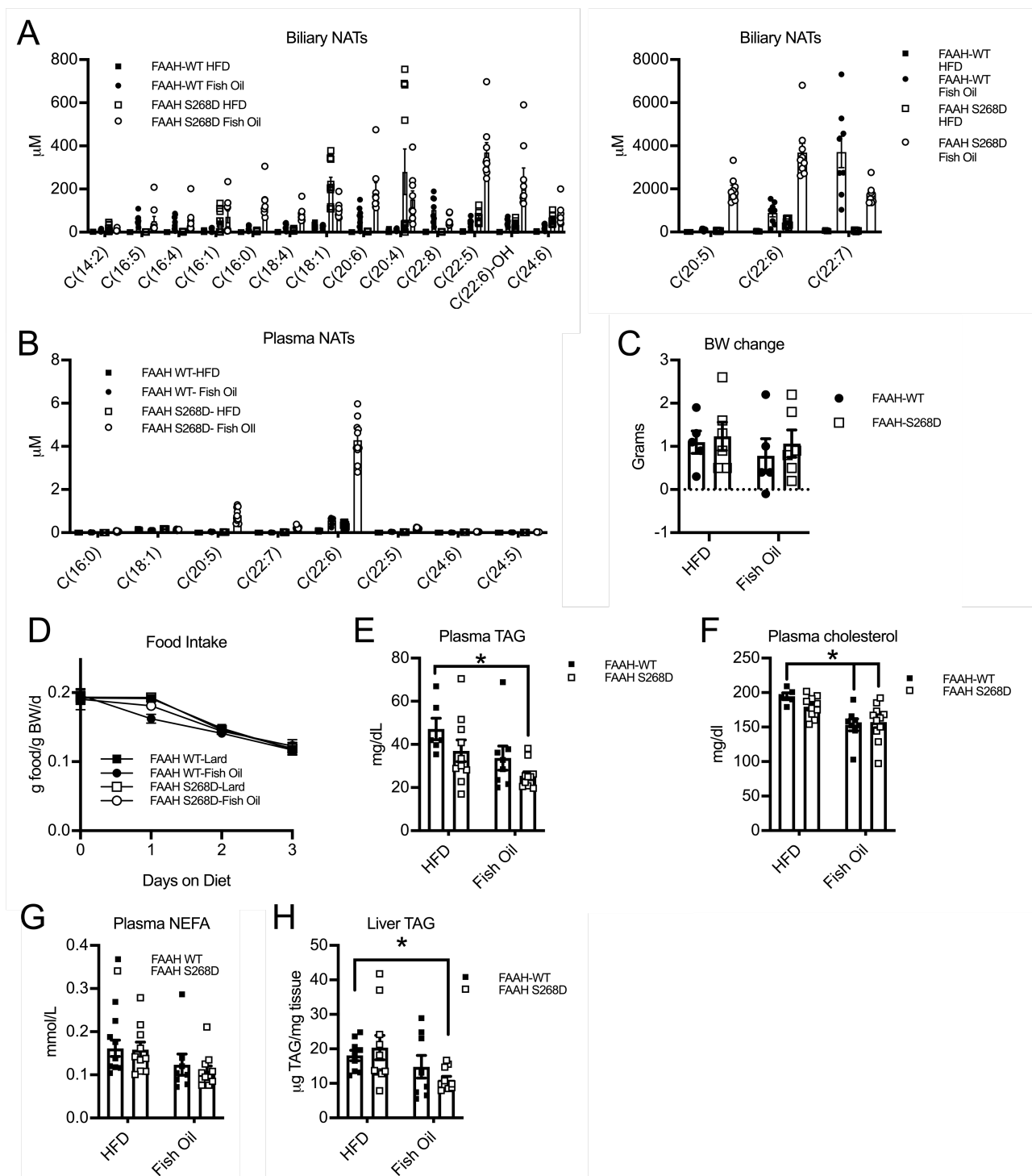


Supplemental material

Product characterization of synthesis of C22:6 NAT and C20:4 NAT

C22:6 NAT (Sodium 2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethane-1-sulfonate): The dried product produced NMR spectra consistent with sodium 2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethane-1-sulfonate. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.92 (t, 3H, J=7.5 Hz, Me), 2.04 (quint, 2H, J=7.3 Hz, CH₂-Me), 2.08 (t, 2H, J=7.5 Hz, CH₂), 2.22–2.27 (m, 2H, CH₂), 2.58 (t, 2H, J=7.4 Hz, CH₂), 2.76–2.83 (m, 10H, CH₂), 3.29–3.34 (m, 2H, CH₂), 5.27–5.39 (m, 12H, CH₂), 7.77 (br t, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆), δ: 14.54, 20.47, 23.48, 25.55, 25.59, 25.64, 25.75 (2C), 35.78, 35.88, 51.05, 127.37, 128.12, 128.19, 128.27, 128.32 (3C), 128.49, 128.50, 128.54, 129.30, 131.97, 171.53. Purified product was obtained in 86% yield from acid. Direct infusion of the product by positive ion mode mass spectrometry revealed expected m/z ratios: 436.1 [M+H]⁺, 458.1 [M+Na]⁺.

C20:4 NAT (Sodium 2-((5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenamido)ethane-1-sulfonate): The dried product produced NMR spectra consistent with sodium 2-((5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenamido)ethane-1-sulfonate. ¹H NMR (400 MHz, methanol-*d*₄), δ: 0.90 (t, 3H, J=6.8 Hz, Me), 1.29–1.37 (m, 6H, (CH₂)₃Me), 1.67 (quint, 2H, J=7.5 Hz, CH₂), 2.04–2.14 (m, 4H, CH₂), 2.20 (t, 2H, J=7.6 Hz, CH₂), 2.80–2.86 (m, 6H, CH₂), 2.95 (t, 2H, J=6.9 Hz, CH₂), 3.58 (t, 2H, J=6.9 Hz, CH₂), 5.33–5.48 (m, 8H, CH₂), 7.90 (br s, NH). ¹³C NMR (100 MHz, methanol-*d*₄), δ: 13.00, 22.19, 25.12 (3C), 25.36, 26.30, 26.75, 29.03, 31.22, 35.15, 35.24, 50.03, 127.34, 127.48, 127.72, 127.78, 128.03, 128.32, 128.74, 129.76, 174.43. Purified product was obtained in 87% yield from acid. Direct infusion of the product by positive ion mode mass spectrometry revealed expected m/z ratios: 412.0 [M+H]⁺, 434.1 [M+Na]⁺.



Supplementary Fig. 1. Effects of fish oil feeding after 3 days. Male FAAH WT and FAAH S268D mice were fed a 45% fat diet +/- 20% kcal from fish oil for 3 d. A) Biliary NATs (n=8-10). B) Plasma NATs (n=9-11). C) Body weight change after 3 d of diet (n=5-6). D) Food intake (n=5-6). E-G) Plasma lipids after 3 d of diet (n=6-11). H) Liver TAG after 3 d of diet (n=8-11). Data presented as mean \pm SEM. *p-value<0.05 compared to vehicle using FDR-corrected t tests (A,B), 2-way ANOVA (C, E-H) or Bonferroni-corrected t tests (D).