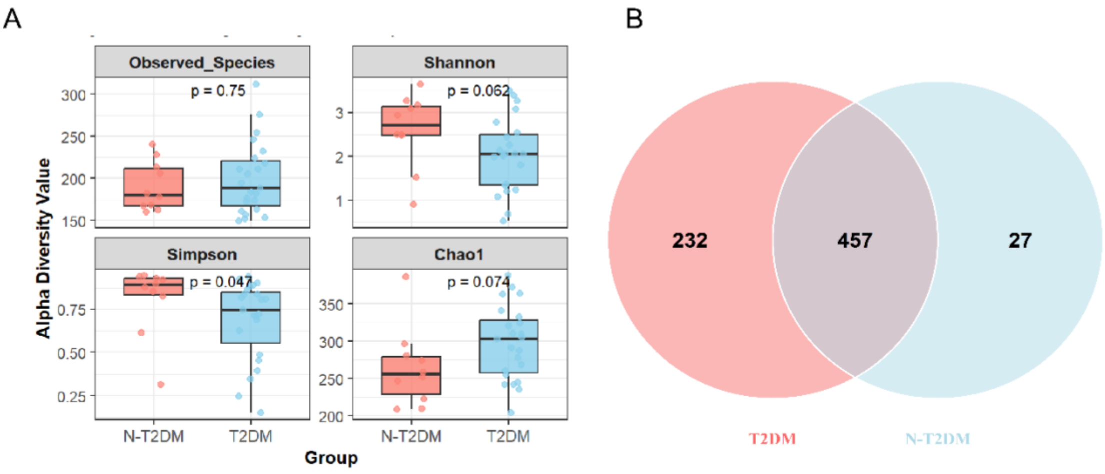

Figure S1. Gut microbial diversity is reduced in T2DM patients.

(A) Alpha-diversity comparison between non-diabetic controls (N-T2DM, n=10) and T2DM patients (T2DM, n=23), measured by the Simpson index. (B) Venn diagram illustrating the unique and shared Operational Taxonomic Units (OTUs) between the N-T2DM and T2DM groups.

Data are presented as mean ± SEM (or as box plots showing median and interquartile range). Statistical analysis was performed using Mann-Whitney U test. *P < 0.05**.**

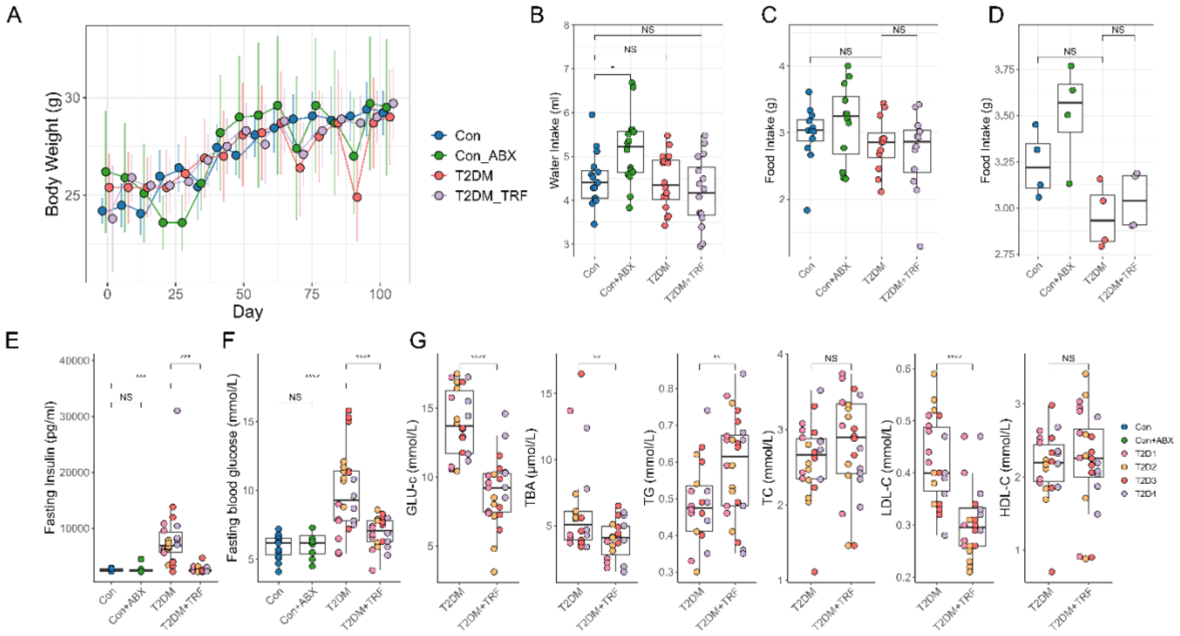

Figure S2. Metabolic parameters of T2DM-humanized mice under TRF intervention. (A) Weekly body weight changes across all experimental groups (Con, Con+ABX, T2DM, and T2DM+TRF). (B) Average weekly water intake. (C) Average weekly food intake. (D) Average weekly food intake during the active (dark) phase (ZT12-ZT20) for T2DM and T2DM+TRF groups. (E) Fasting plasma glucose levels. (F) Fasting serum insulin levels. (G) Serum biochemical analysis panel comparing T2DM and T2DM+TRF groups, showing levels of Glucose, Total Bile Acids (TBA), Triglycerides (TG), Total Cholesterol (TC), LDL-C, and HDL-C.

Data are presented as mean ± SEM. Groups for (A-F) are: Con (n=12), Con+ABX (n=8), T2DM (n=24), and T2DM+TRF (n=24). Group (G) compares T2DM (n=24) and T2DM+TRF (n=24). Statistical significance for all pairwise comparisons was determined by the Mann-Whitney U test. (for B-G). *P < 0.05, **P < 0.01***P < 0.001.

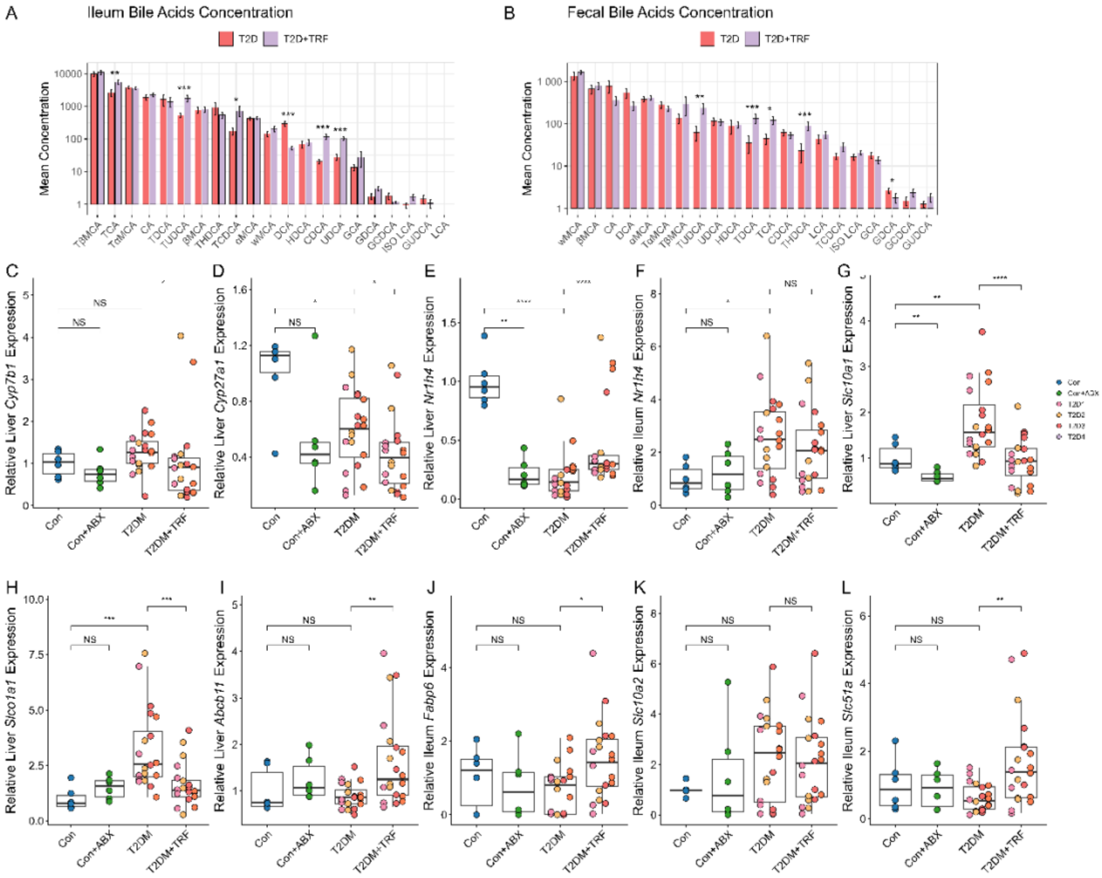

Figure S3. TRF comprehensively remodels bile acid profiles, synthesis, signaling, and transport in T2DM-humanized mice.

(A) Individual bile acid levels in the ileum. (B) Individual bile acid levels in the feces. (C) Relative liver mRNA expression of the alternative bile acid synthesis enzyme *Cyp7b1*. (D) Relative liver mRNA expression of the alternative bile acid synthesis enzyme *Cyp27a1*. (E) Relative liver mRNA expression of the nuclear receptor *Fxr* (*Nr1h4*). (F) Relative ileum mRNA expression of the nuclear receptor *Fxr* (*Nr1h4*). (G) Relative liver mRNA expression of key bile acid transporter *Slc10a1* (*NTCP*). (H) Relative liver mRNA expression of key bile acid transporter *Slco1a2* (*OATP1A2*). (I) Relative liver mRNA expression of key bile acid transporter *Abcb11* (*BSEP*). (J) Relative ileum mRNA expression of key bile acid transporter *Slc10a2* (*ASBT*). (K) Relative ileum mRNA expression of key bile acid binder *Fabp6* (*I-BABP*). (L) Relative ileum mRNA expression of key bile acid transporter *Slc51a* (*OSTα*).

Data are presented as mean ± SEM. Groups are: Con (n=6), Con+ABX (n=6), T2DM (n=20), and T2DM+TRF (n=20). Statistical significance for all pairwise comparisons was determined by the Mann-Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001.

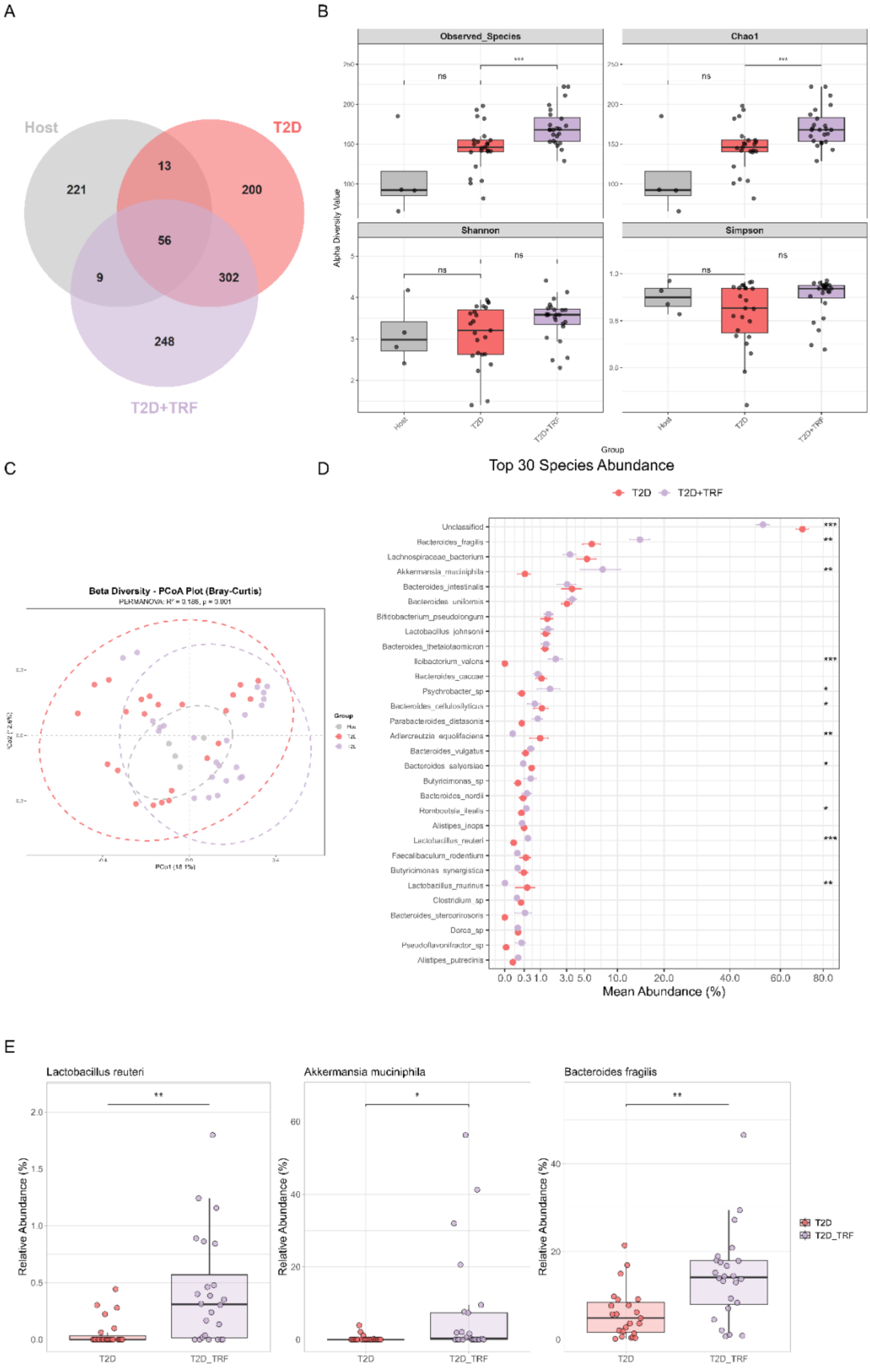

Figure S4. TRF alters microbial community membership, diversity, and specific species in T2DM-humanized mice.

(A) Venn diagram illustrating the unique and shared Amplicon Sequence Variants (ASVs) between T2DM (ad libitum, n=24) and T2DM+TRF (n=24) groups. (B) Comparison of microbial alpha-diversity, showing community richness (Observed ASVs and Chao1 index) and diversity (Shannon and Simpson indices) between the T2DM and T2DM+TRF groups. (C) Principal Coordinates Analysis (PCoA) plot of β-diversity (Bray-Curtis dissimilarity) comparing the community structure of the human donors (Host), the T2DM-humanized mice (T2D), and the TRF-treated T2D mice (T2D+TRF). (D) Dot plots showing the relative abundance of specific bacterial species in the T2DM and T2D+TRF groups. (E) Box plots comparing the relative abundance of key beneficial species, including *Lactobacillus reuteri*, *Akkermansia muciniphila*, and *Bacteroides fragilis*, showing their increase after TRF.

Data are presented as box plots (for B, E) or dot plots (for D) showing median and interquartile range (or mean ± SEM). Statistical analysis was performed Mann-Whitney U test (for B, D, E) and PERMANOVA (for C). *P < 0.05, **P < 0.01.

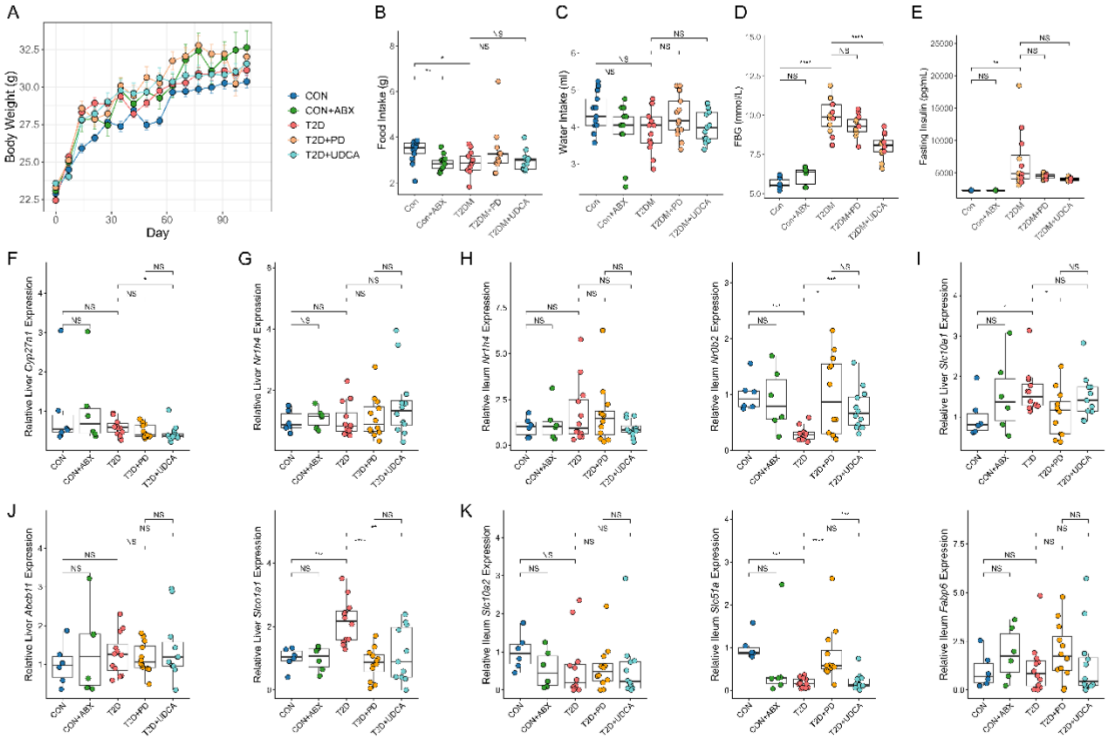

Figure S5. Metabolic and molecular profiling of *P. distasonis* and UDCA supplementation in T2DM-humanized mice.

(A) Weekly body weight changes. (B) Average weekly food intake. (C) Average weekly water intake. (D) Fasting plasma glucose levels. (E) Fasting serum insulin levels. (F) Relative liver mRNA expression of the alternative BA synthesis enzyme *Cyp27a1*. (G) Relative liver mRNA expression of the nuclear receptor *Fxr*. (H) Relative ileum mRNA expression of *Fxr* and *Shp*. (I) Relative liver mRNA expression of key BA transporters *Slc10a1* (*NTCP*). (J) Relative liver mRNA expression of key BA transporters (Slco1a2 (*OATP1A2*), and *Abcb11* (*BSEP*)) (K) Relative ileum mRNA expression of key BA transporters and binders (*Slc10a2* (*ASBT*), *Fabp6* (*I-BABP*), and *Slc51a* (*OSTα*)).

Data are presented as mean ± SEM. Groups are: Con (n=6), Con+ABX (n=6), T2DM (n=12), T2D+PD (n=12), and T2D+UDCA (n=12). Statistical significance for all pairwise comparisons was determined by the Mann-Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001.

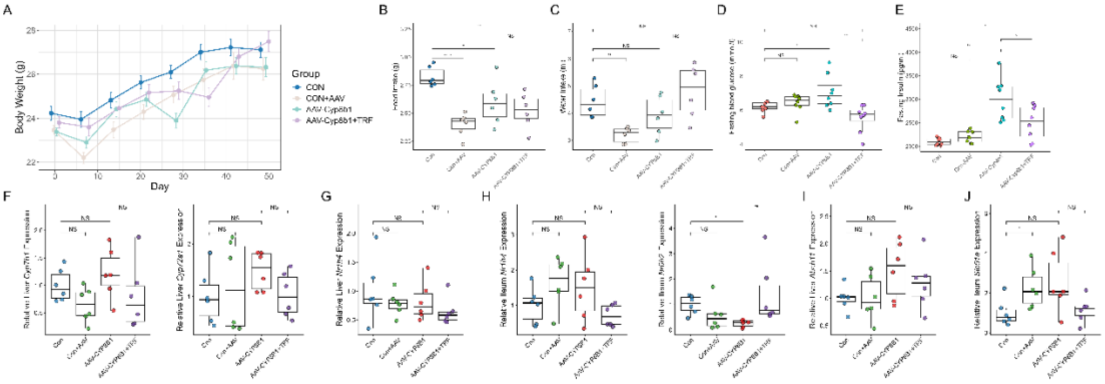

Figure S6. Metabolic and molecular profiling of AAV-Cyp8b1 overexpression and TRF intervention.

(A) Weekly body weight changes. (B) Average weekly food intake. (C) Average weekly water intake. (D) Fasting plasma glucose levels. (E) Fasting serum insulin levels. (F) Relative liver mRNA expression of alternative BA synthesis enzymes (*Cyp7b1* and *Cyp27a1*). (G) Relative liver mRNA expression of the nuclear receptor *Fxr*. (H) Relative ileum mRNA expression of *Fxr* and *Shp*. (I) Relative liver mRNA expression of the transporter *Abcb11* (BSEP). (J) Relative ileum mRNA expression of the transporter *Slc51a* (OSTα).

Data are presented as mean ± SEM. Groups are: Con (n=8), Con+AAV (AAV-Null) (n=8), AAV-CYP8B1 (n=8), and AAV-CYP8B1+TRF (n=8). Statistical significance for all pairwise comparisons was determined by the Mann-Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001.

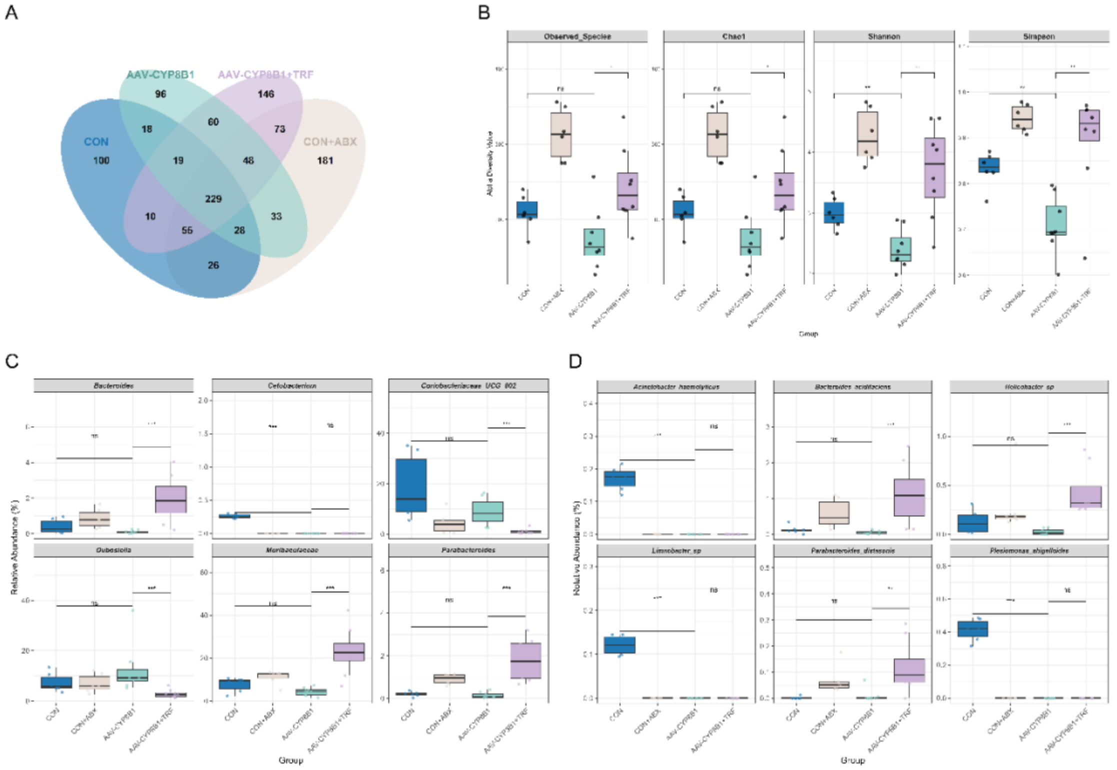

Figure S7. TRF alters microbial community membership and restores diversity in AAV-Cyp8b1 mice. Related to Figure 6.

(A) Venn diagram illustrating the unique and shared **Amplicon Sequence Variants (ASVs)** across the Control (Con), AAV-Null (Con+AAV), AAV-CYP8B1, and AAV-CYP8B1+TRF groups (n=8 per group). (B) Comparison of microbial alpha-diversity, showing community richness (e.g., Observed ASVs or Chao1 index) and diversity (Shannon and Simpson indices) across the four groups. (C) Relative abundance of representative microbial genera across the four groups. (D) Relative abundance of representative microbial species across the four groups.

Data are presented as box plots (for B) showing median and interquartile range. Statistical analysis (for B) was performed using one-way ANOVA with Tukey's post-hoc test. Statistical significance for all pairwise comparisons was determined by the Mann-Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001.