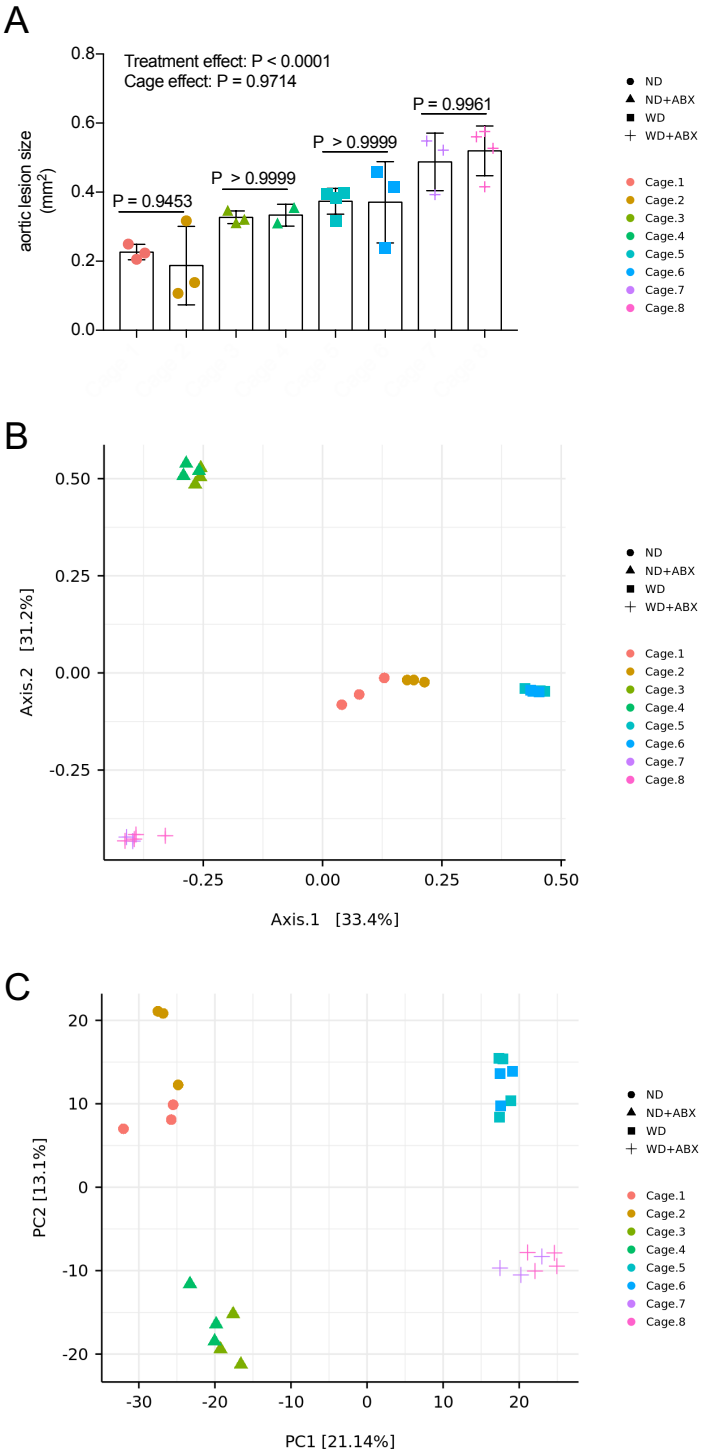
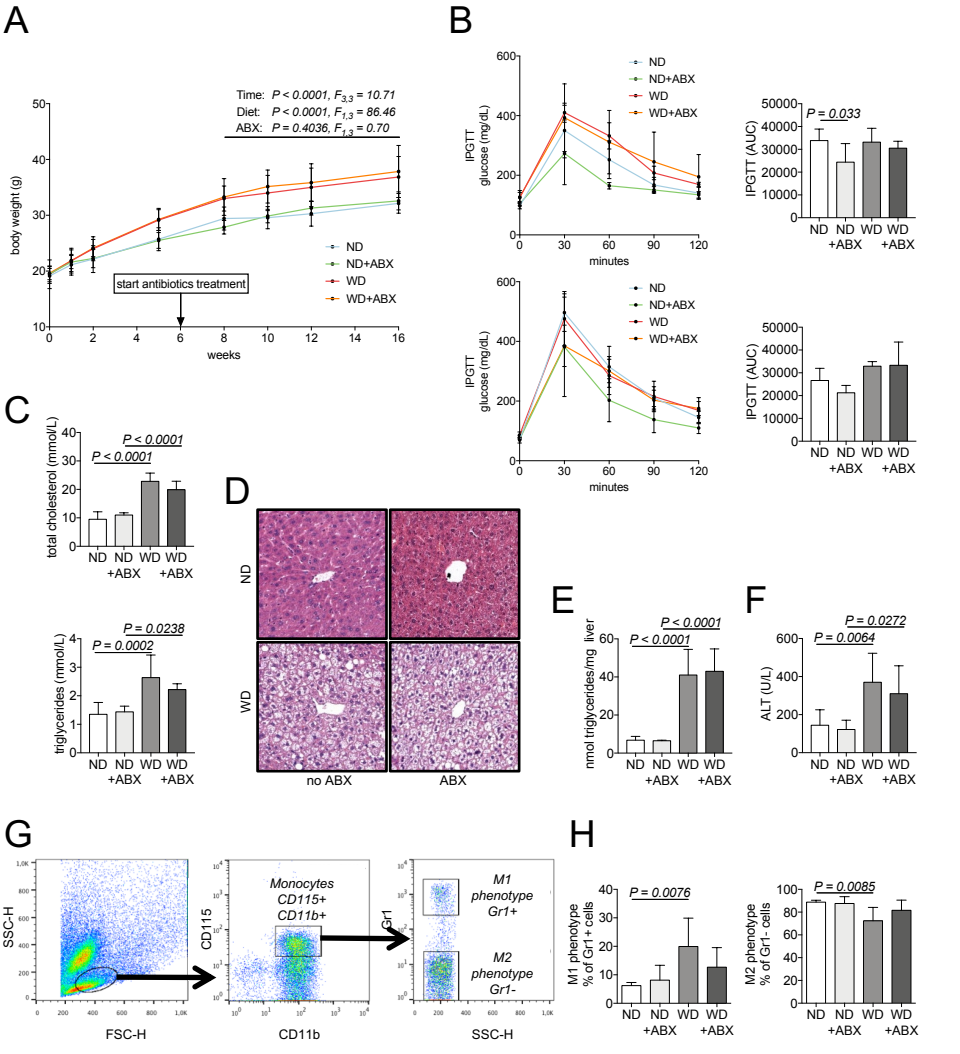
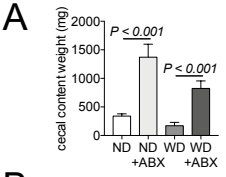
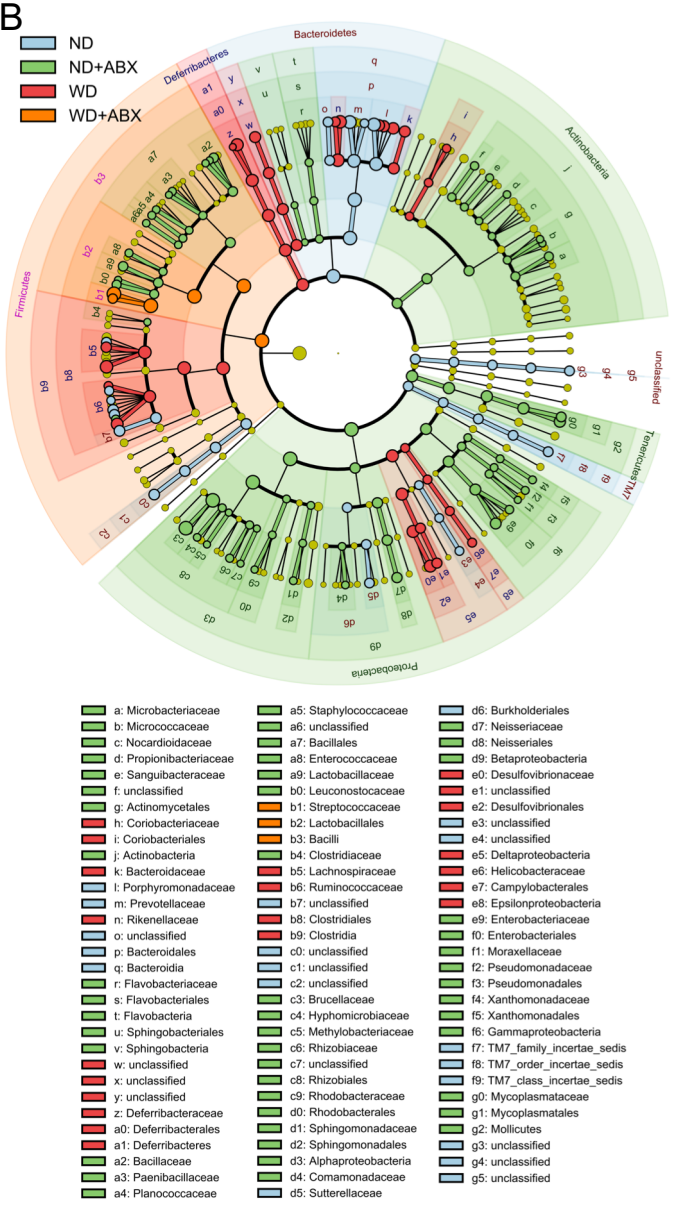


Supplementary Figure S1. Analysis of cage effect in the mouse model.
(A) Analysis of cage effect on atherosclerotic lesion size. ND: normal diet, WD: Western diet, ABX: antibiotics treatment. n = 2 – 4 per group. Analysis by two-way ANOVA with Tukey's post hoc test. Data are the mean ± S.D.
(B) Analysis of cage effect on cecal microbiome composition by principal coordinate analysis. n = 3 – 4 per group.
(C) Analysis of cage effect on serum metabolome by principal component analysis. n = 3 – 4 per group.



Supplementary Figure S2. Metabolic phenotyping of ApoE -/- mice subjected to antibiotics and different diets.
(A) Body weight of mice over time. ND: normal diet, WD: Western diet, ABX: antibiotics treatment. P- and F-values by 3-way ANOVA. n = 6 – 7 per group.
(B) Intraperitoneal glucose tolerance test (IPGTT) 2 (upper graph) and 8 weeks (lower graph) after initiation of antibiotics treatment. Area under the curve (AUC) analysis of IPGTT analyzed by two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group.
(C) Analysis of serum cholesterol and triglycerides in the fasted state prior to sacrifice. Two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group.
(D) Micrographs of liver sections stained with hematoxylin and eosin stain.
(E) Analysis of triglyceride content of the liver. Two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group.
(F) Analysis of serum alanine aminotransferase (ALT) in the fasted state prior to sacrifice. Two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group.
(G) Flow cytometry gating strategy to evaluate M1/M2 ratio of circulating monocytes.
(H) Quantification of M1 and M2 circulating monocytes. Two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group.
All data are the mean ± S.D.



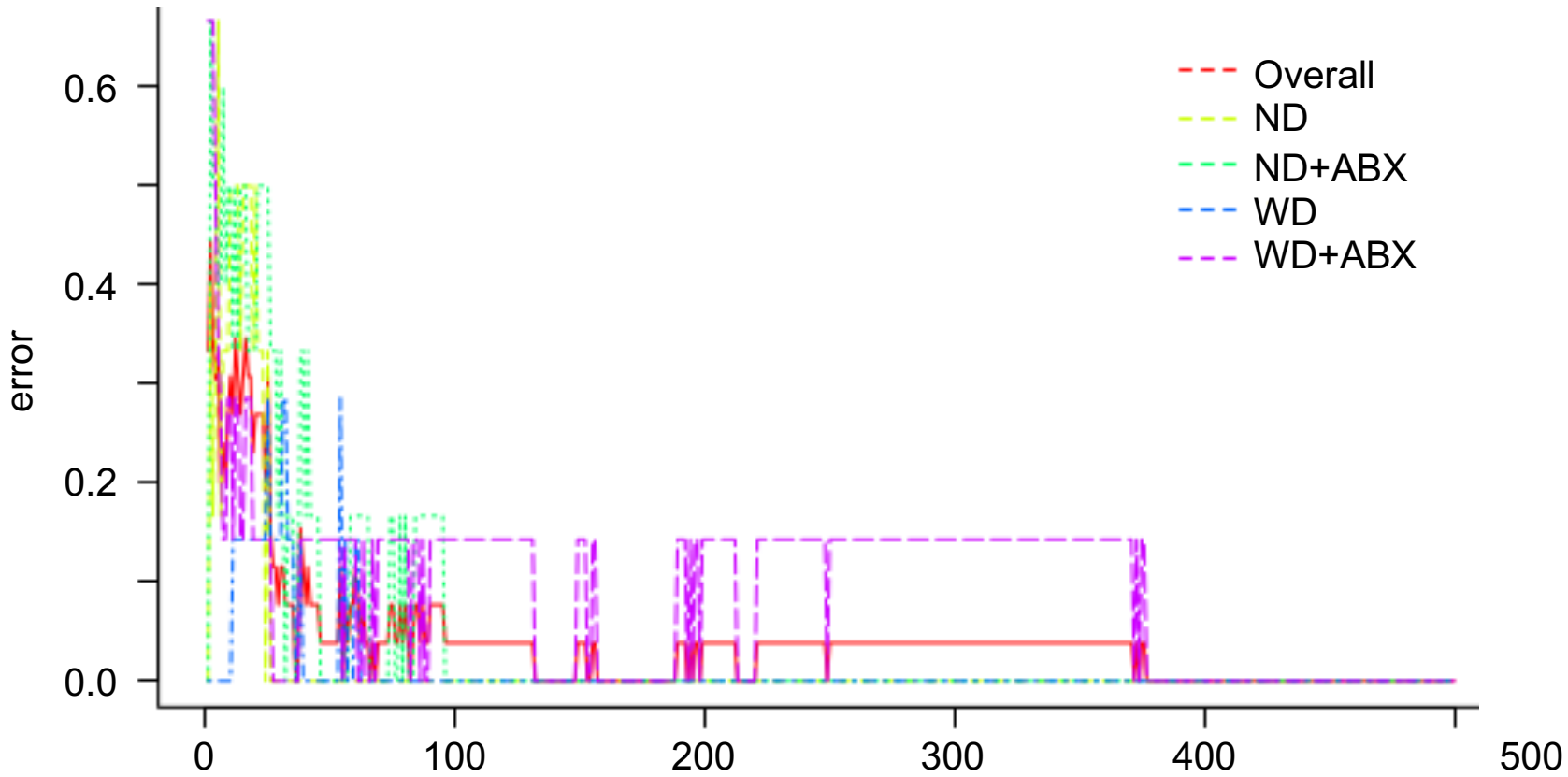


a: Microbacteriaceae
b: Micrococcaceae
c: Nocardioidaceae
d: Propionibacteriaceae
e: Sanguibacteraceae
f: unclassified
g: Actinomycetales
h: Coriobacteriaceae
i: Coriobacteriales
j: Actinobacteria
k: Bacteroidaceae
l: Porphyromonadaceae
m: Prevotellaceae
n: Rikenellaceae
o: unclassified
p: Bacteroidales
q: Bacteroidia
r: Flavobacteriaceae
s: Flavobacteriales
t: Flavobacteria
u: Sphingobacteriales
v: Sphingobacteria
w: unclassified
x: unclassified
y: unclassified
z: Deferribacteraceae
a0: Deferribacterales
a1: Deferribacteres
a2: Bacillaceae
a3: Paenibacillaceae
a4: Planococcaceae
a5: Staphylococcaceae
a6: unclassified
a7: Bacillales
a8: Enterococcaceae
a9: Lactobacillaceae
b0: Leuconostocaceae
b1: Streptococcaceae
b2: Lactobacillales
b3: Bacilli
b4: Clostridiaceae
b5: Lachnospiraceae
b6: Ruminococcaceae
b7: unclassified
b8: Clostridiales
b9: Clostridia
c0: unclassified
c1: unclassified
c2: unclassified
c3: Brucellaceae
c4: Hyphomicrobiaceae
c5: Methylobacteriaceae
c6: Rhizobiaceae
c7: unclassified
c8: Rhizobiales
c9: Rhodobacteraceae
d0: Rhodobacterales
d1: Sphingomonadaceae
d2: Sphingomonadales
d3: Alphaproteobacteria
d4: Comamonadaceae
d5: Sutterellaceae
d6: Burkholderiales
d7: Neisseriaceae
d8: Neisseriales
d9: Betaproteobacteria
e0: Desulfovibrionaceae
e1: unclassified
e2: Desulfovibrionales
e3: unclassified
e4: unclassified
e5: Deltaproteobacteria
e6: Helicobacteraceae
e7: Campylobacterales
e8: Epsilonproteobacteria
e9: Enterobacteriaceae
f0: Enterobacteriales
f1: Moraxellaceae
f2: Pseudomonadaceae
f3: Pseudomonadales
f4: Xanthomonadaceae
f5: Xanthomonadales
f6: Gammaproteobacteria
f7: TM7_family_incertae_sedis
f8: TM7_order_incertae_sedis
f9: TM7_class_incertae_sedis
g0: Mycoplasmataceae
g1: Mycoplasmatales
g2: Mollicutes
g3: unclassified
g4: unclassified
g5: unclassified

Supplementary Figure S3. Cecal content weight and group-specific changes in cecal microbiome composition in the mouse model.

(A) Cecal content weight. Two-way ANOVA with Tukey's post hoc test. n = 6 – 7 per group. ND: normal diet, WD: Western diet, ABX: antibiotics treatment. Data are the mean ± S.D.

(B) LEfSe (Linear discriminant analysis effect size) to identify microbial communities at the phylum, class, order, family, and genus levels that interacted consistently with our treatments. Taxa enriched for each group indicated by a positive linear discriminant analysis (LDA) score are highlighted in color. n = 6 – 7 per group.

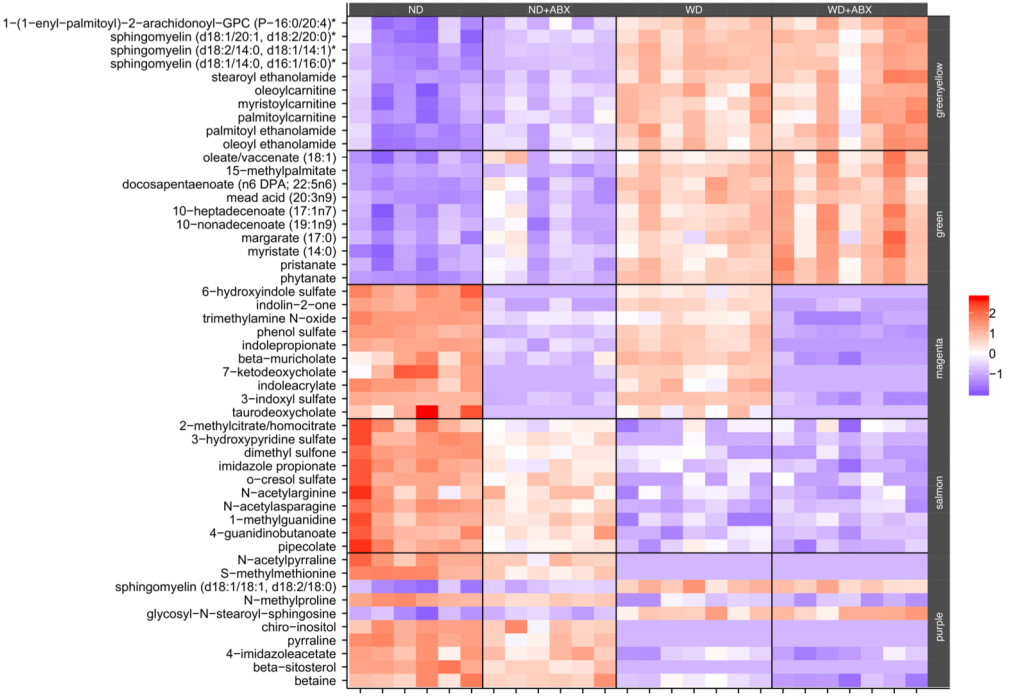


predicted group

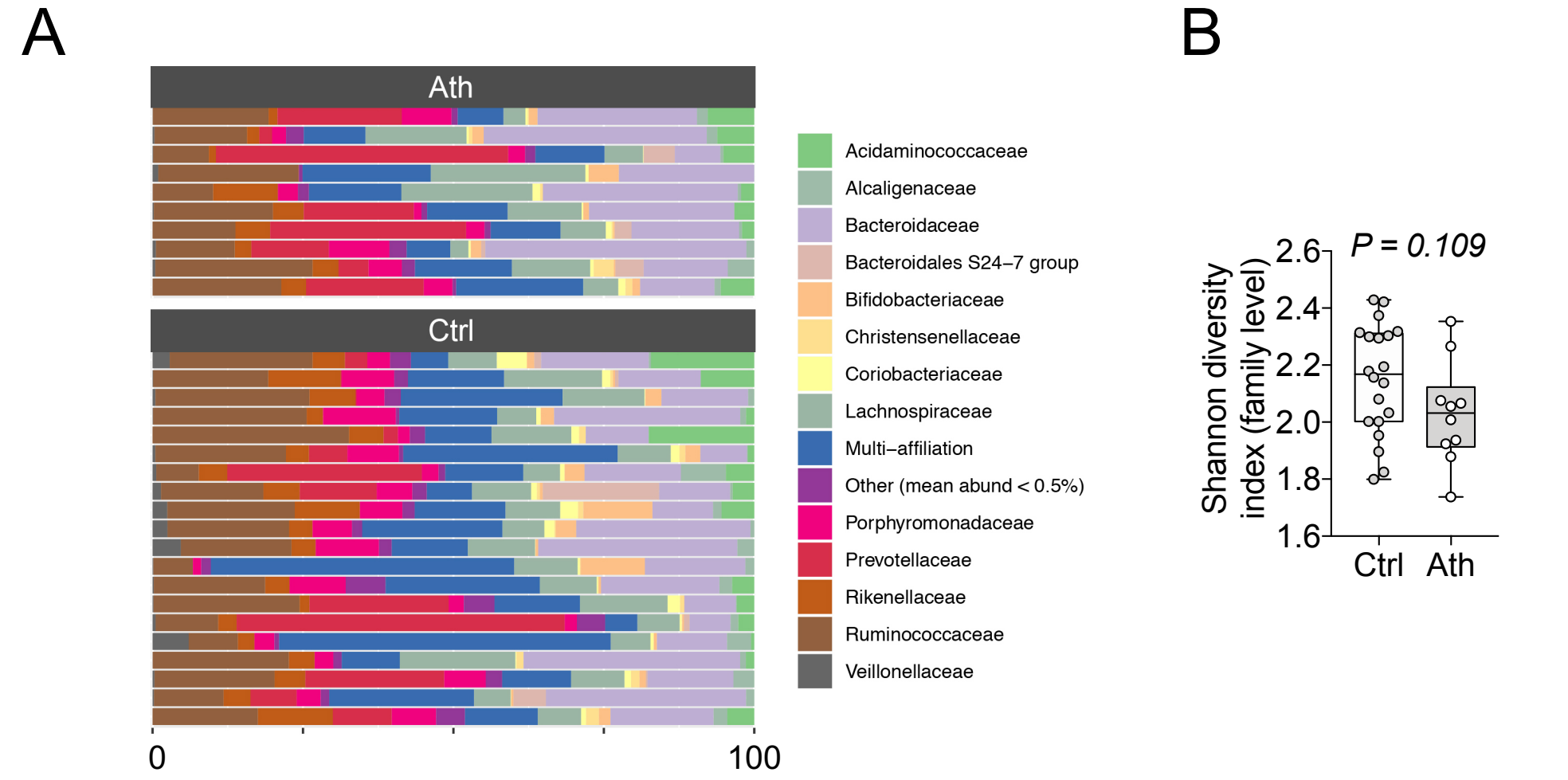
actual group	ND	ND +ABX	WD	WD +ABX	*Class error*
ND	6	0	0	0	*0.0*
ND+ABX	0	6	0	0	*0.0*
WD	0	0	7	0	*0.0*
WD+ABX	0	0	0	7	*0.0*

Out of the bag error = 0.0
Predictive accuracy = 100%

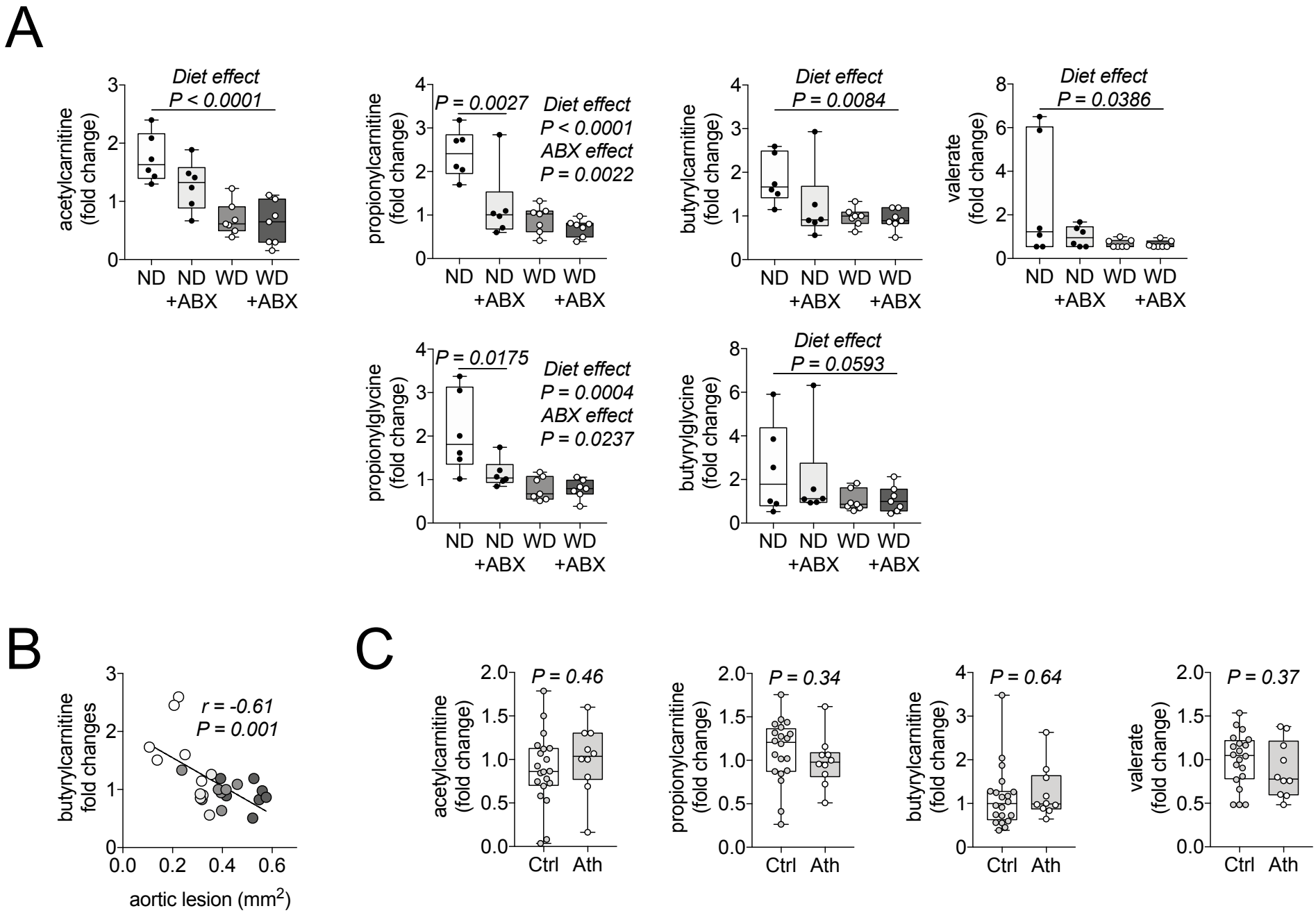
Supplementary Figure S4. Random Forest Claasification of mouse serum metabolomics.
Random Forest Classification tree and Random Forest confusion matrix of murine serum metabolomics shows 100% predictive accuracy in group assignment. ND: normal diet, WD: Western diet, ABX: antibiotics treatment. n = 6 – 7 per group.



Supplementary Figure S5. Top 10 ranked serum metabolites of metabolite WGCNA clusters in the mouse model.
Heatmap of top 10 ranked metabolites in each of the 5 metabolite clusters by weighted correlation network analysis of serum metabolomics that were subjected to phenotype-associated filtering. n = 6 – 7 per group.



Supplementary Figure S6. Analysis of fecal microbiota in the human cohort.
(A) Human fecal relative abundance on family level by 16S rRNA-targeted sequencing. Ath: patients with carotid atherosclerosis. Ctrl: control subjects. Ctrl group n = 20, Ath group n = 10.
(B) Human fecal bacterial *alpha* diversity by Shannon index. Boxplots: Center line: median; box limits: 25-75th percentiles; whiskers: min. to max. Data were analyzed via two-sided t-tests with Welch's correction.



Supplementary Figure S7. Analysis of short-chain fatty acid metabolism

(A) Short-chain fatty acid metabolites of mouse serum metabolomics. Data were analyzed by 2-way ANOVA with Tukey's posthoc test. Data are the mean ± S.D., n = 6 – 7 per group.. ND: normal diet, WD: Western diet, ABX: antibiotics treatment.

(B) Pearson correlation of mouse serum butyrylcarnitine levels to aortic lesion size, n = 5 – 7 per group.

(C) Short-chain fatty acid metabolites of human serum metabolomics. Data were analyzed by two-sided t-test with Welch's correction (Boxplots: Center line: median; box limits: 25-75th percentiles; whiskers: min. to max.). Ath: patients with carotid atherosclerosis. Ctrl: control subjects. Ctrl group n = 20, Ath group n = 10.