Figure S1 Structural analysis of rumen microbial communities **(A)** Core-pan gene dilution curves;

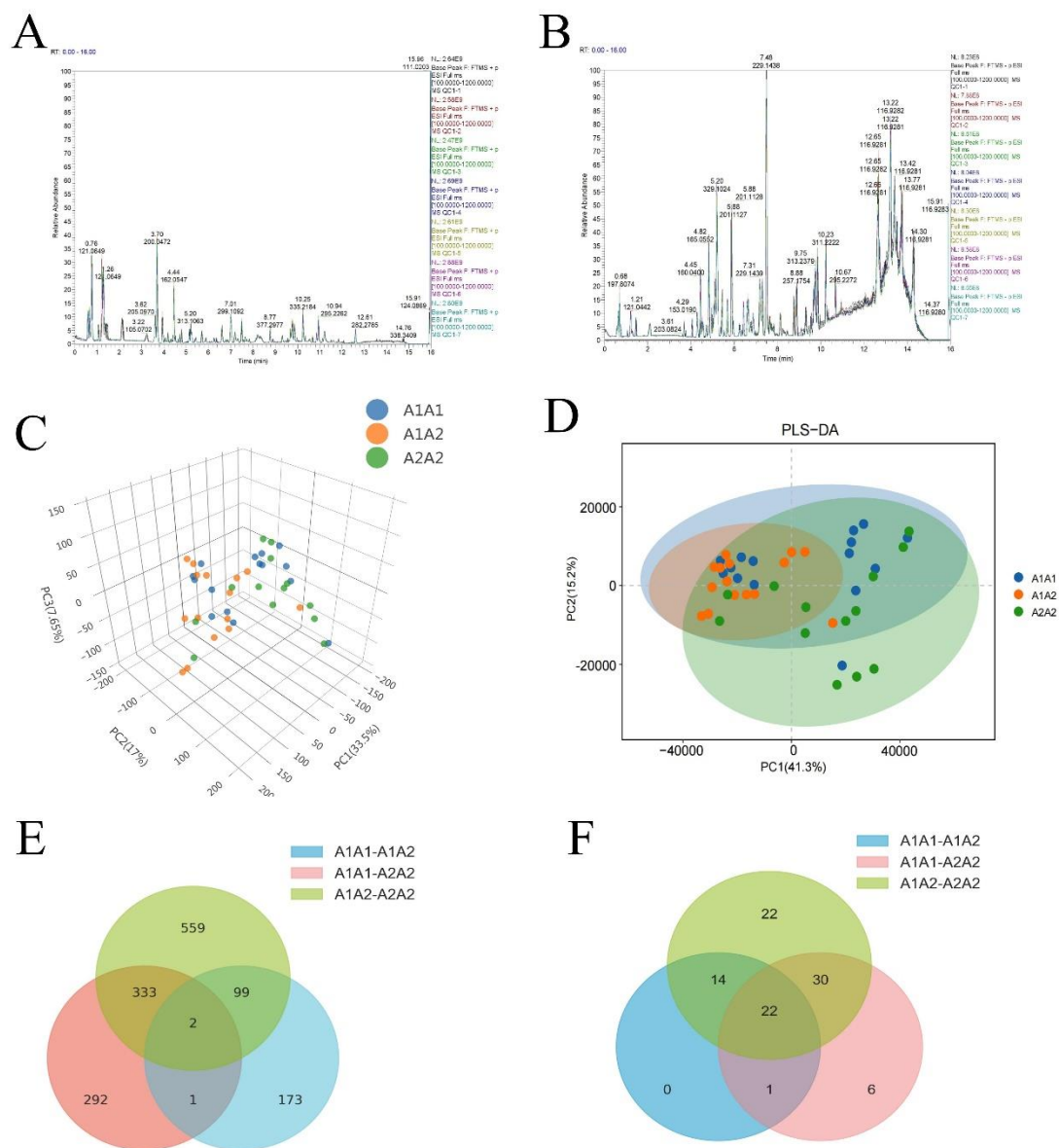**(B)** Venn diagram of genes among the three groups;**(C)** PCA analysis; **(D)** Anosim analysis graphs.

Figure S2 The TIC plot in **(A)** Positive and **(B)** Negative ion mode**(C)** PCA of the three metabolomes; **(D)** PLS-DA of the three metabolomes; **(E)** Wayne plots of the three differential metabolites; **(F)** Wayne plots of the three differential metabolic pathways.
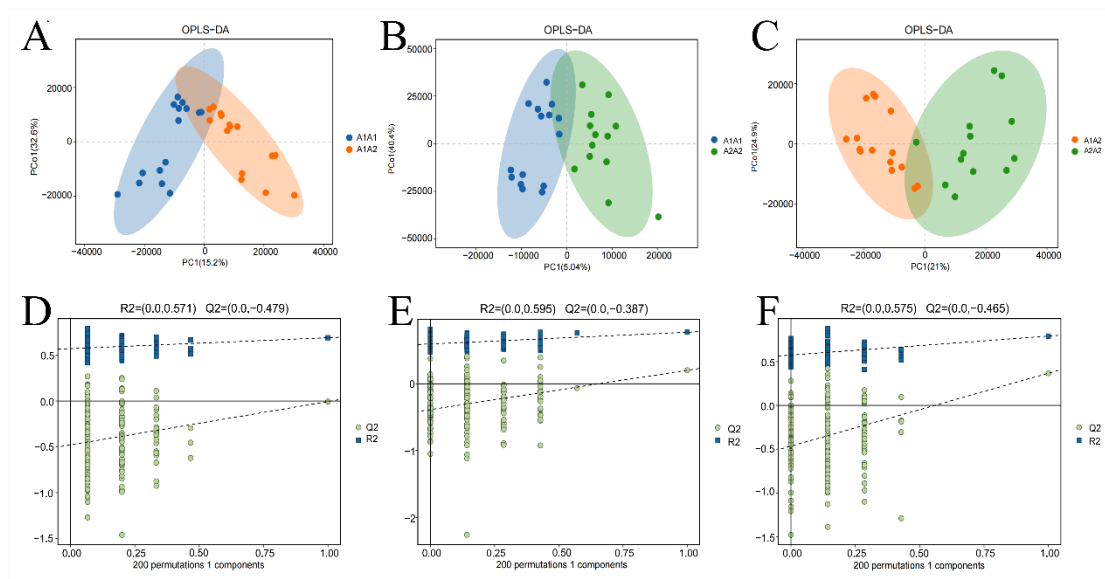
Figure S3 **(A-C)** OPLS-DA of rumen metabolites for groups A1A1, A1A2, and A2A2; **(D-F)** OPLS-DA replacement test plots **(**Further replacement tests of the OPLS-DA model for positive and negative ion pattern data showed that the model was valid for evaluation).

A

Group
A1A1
A1A2

2,8-Quinolinediol
Kuhlmanniquinol
4-Hydroxy-5,7,4'-trimethoxyflavan
3-(4-Hydroxyphenyl)-3,5,6,8-tetrahydro-2H-chromene-4,7-dione
2S-hydroxy-3-(10Z-tetradecenoyloxy)-propanoic acid
ascr#18
2,3-Dinor-TXB2
(Z)-5-[(2R,3S,4S)-4-Hydroxy-2-[(E)-3-hydroxyoct-1-enyl]oxan-3-yl]pent-3-enoic acid
Tupichinol A
Dodecanedioic acid
Aflatoxin B1 dialcohol
Glyzarin
oscr#24
Farnesyl Thiosalicylic Acid
Cinncassiol A
2,3-dinor-PGE1
(10S,13S,16R)-d14-9-PhytoF[9S,12S]
oscr#19
Proline betaine
Pelargonidin 3-sophoroside
Pelargonidin 3,5-di-(6-acetylglucoside)
N1,N10-Dicoumaroylspermidine
(10E,12Z)-(9S)-9-Hydroperoxyoctadeca-10,12-dienoic acid
alpha-Dimorphecolic acid
9(S)-HOTrE
Punicic acid
3-Deazaneplanocin A
Ricinoleic acid
Adenine
3alpha-Hydroxy-5beta-chola-7,9(11)-dien-24-oic Acid
Cholic acid
8,9-Epoxyeicosatrienoic acid
3'-AZIDO-2',3'-DIDEOXYURIDINE
19-methyl-heneicosanoic acid
Ptilosteroid B
MG(16:0/0:0/0:0)
4-Chloro-L-phenylalanine
Thiabendazole
Dibutyl phthalate
Phthalic acid
Pyridazine-3,6-diol
bhas#32
pyridine-3,4-diol
(S)-Methyl 2,5-diamino-5-oxopentanoate
C16 Sphinganine
N,N-dimethyl-Safingol
Armillaripin
beta-D-fructose 2,6-bisphosphate
1alpha-hydroxy-24-(dimethylphosphoryl)-25,26,27-trinorvitamin D3
1-(11Z-docosenoyl)-glycero-3-phosphate

B

Group
A1A1
A2A2

7-Epi-12-hydroxyjasmonic acid
Gibberellin A59
oscr#7
Noralfentanil
(2R,3R)-2-Aminooctadecane-1,3-diol
bhos#42
Dimethindene
Dehydromatricaric acid
4-(2-Aminophenyl)-2,4-dioxobutanoic acid
Thonzylamine
2-(3-Hydroxy-1H-indol-2-yl)acetic acid
Americanol A
3-(3-Hydroxyphenyl)propanoic acid
Quercetin 3-O-sophoroside
Glycyrrhetol
Adenosine
Schisanhenol
Baohuoside I
Acetophenazine
9H-Purine-9-butanoic acid, 6-amino-alpha-hydroxy-, methyl ester
PE-Cer(d14:2(4E,6E)/16:0(2OH))
Momorcharaside B
LysoPE(15:0/0:0)
True blue
Inosine
Glucose pyruvate lactate
LysoPE(0:0/16:0)
LysoPE(0:0/15:0)
LysoPE(14:0/0:0)
Ceranapril
Linagliptin
24-Nor-5beta-cholane-3alpha,7alpha,12alpha-triol
Estriol-16-Glucuronide
Methyl helianthenoate A glucoside
5,8,12-Trihydroxy-9-octadecenoic acid
N-(m-Methoxybenzyl)hexadecanamide
Guanosine
FAHFA(16:0/14-O-18:0)
PG(5-iso PGF2VII-13:0)
Cibaric acid
5-Megastigmen-7-yne-3,9-diol 3-glucoside
Foretinib
PG(18:3(9Z,12Z,15Z)/0:0)
LPIM2(16:0/0:0)
Astemizole
Adrenic acid
Daidzin
Oleoylethanolamide

C

Group
A1A2
A2A2

Styrene
13,16-Epoxy-7,12-dihydroxy-9Z-octadecenoic acid
4-Hydroxycinnamic acid
(10S,13S,16R)-d14-9-PhytoF[9S,12S]
Aflatoxin B1 dialcohol
Dodecanedioic acid
5,8,12-Trihydroxy-9-octadecenoic acid
Phenylalanylproline
2,8-Quinolinediol
4-Hydroxy-5,7,4'-trimethoxyflavan
Kuhlmanniquinol
Quinaldine
Traumatic acid
3-(3-Hydroxyphenyl)propanoic acid
Azelaic acid
Americanol A
N,N-dimethyl-Safingol
C16 Sphinganine
Dibutyl phthalate
pyridine-3,4-diol
(S)-Methyl 2,5-diamino-5-oxopentanoate
7-dehydrocholesterol-d7
4-Chloro-L-phenylalanine
Thiabendazole
Ptilosteroid B
MG(16:0/0:0/0:0)
beta-D-fructose 2,6-bisphosphate
9S,11R,15S-trihydroxy-2,3-dinor-13E-prostaenoic acid-cyclo[8S,12R]
9S,10S,13R-trihydroxyoctadec-11E-enoic acid
Prostaglandin H2
17-Hydroxylinolenic acid
Adenine
(10E,12Z)-(9S)-9-Hydroperoxyoctadeca-10,12-dienoic acid
13(S)-Hydroperoxylinolenic acid
Peonidin 3-rhamnoside 5-glucoside
Pelargonidin 3-sophoroside
Pelargonidin 3,5-di-(6-acetylglucoside)
10-Oxo-11-octadecen-13-olide
Soyasaponin III
7,4'-Dihydroxyflavone
Apigenin
3-(3,4-Dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one
N1,N10-Dicoumaroylspermidine
3-Deazaneplanocin A
Ricinoleic acid
Calenduloside B
Soyasaponin II
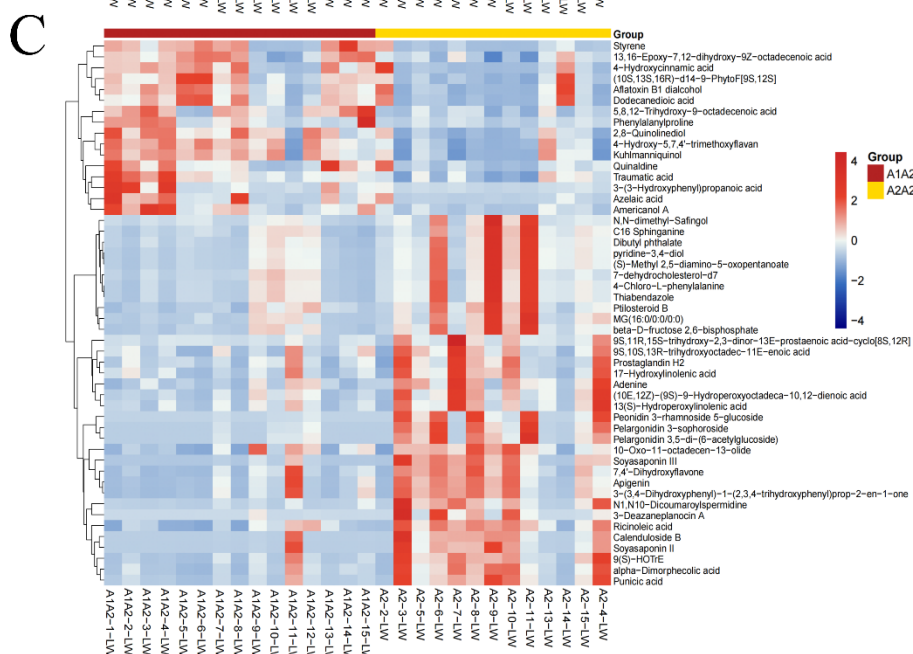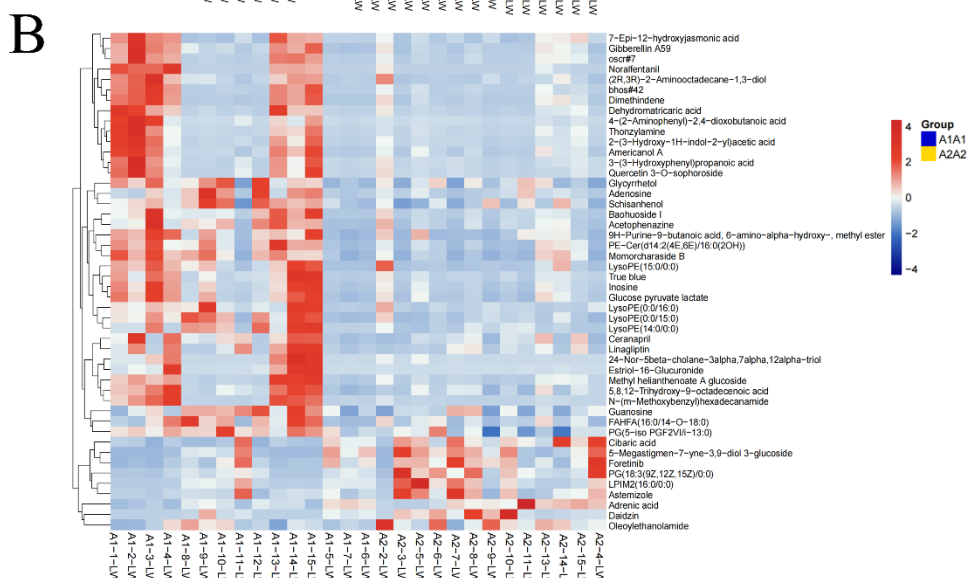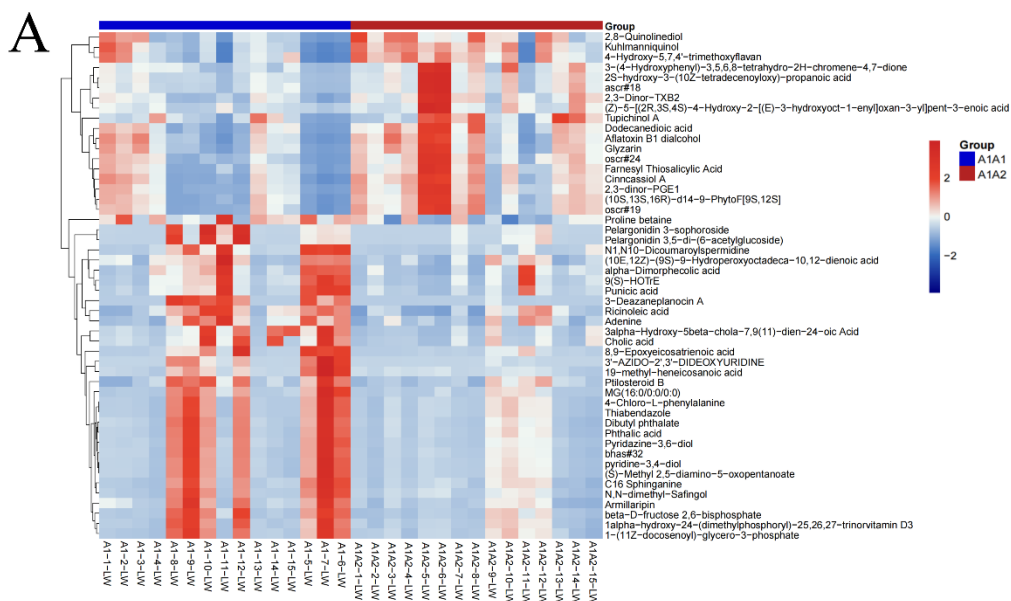9(S)-HOTrE
alpha-Dimorphecolic acid
Punicic acid

Figure S4 **(G-I)** Differential hierarchical cluster analysis of the top 50 metabolites of rumen metabolites in groups A1A1, A1A2 and A2A2 (heat map).
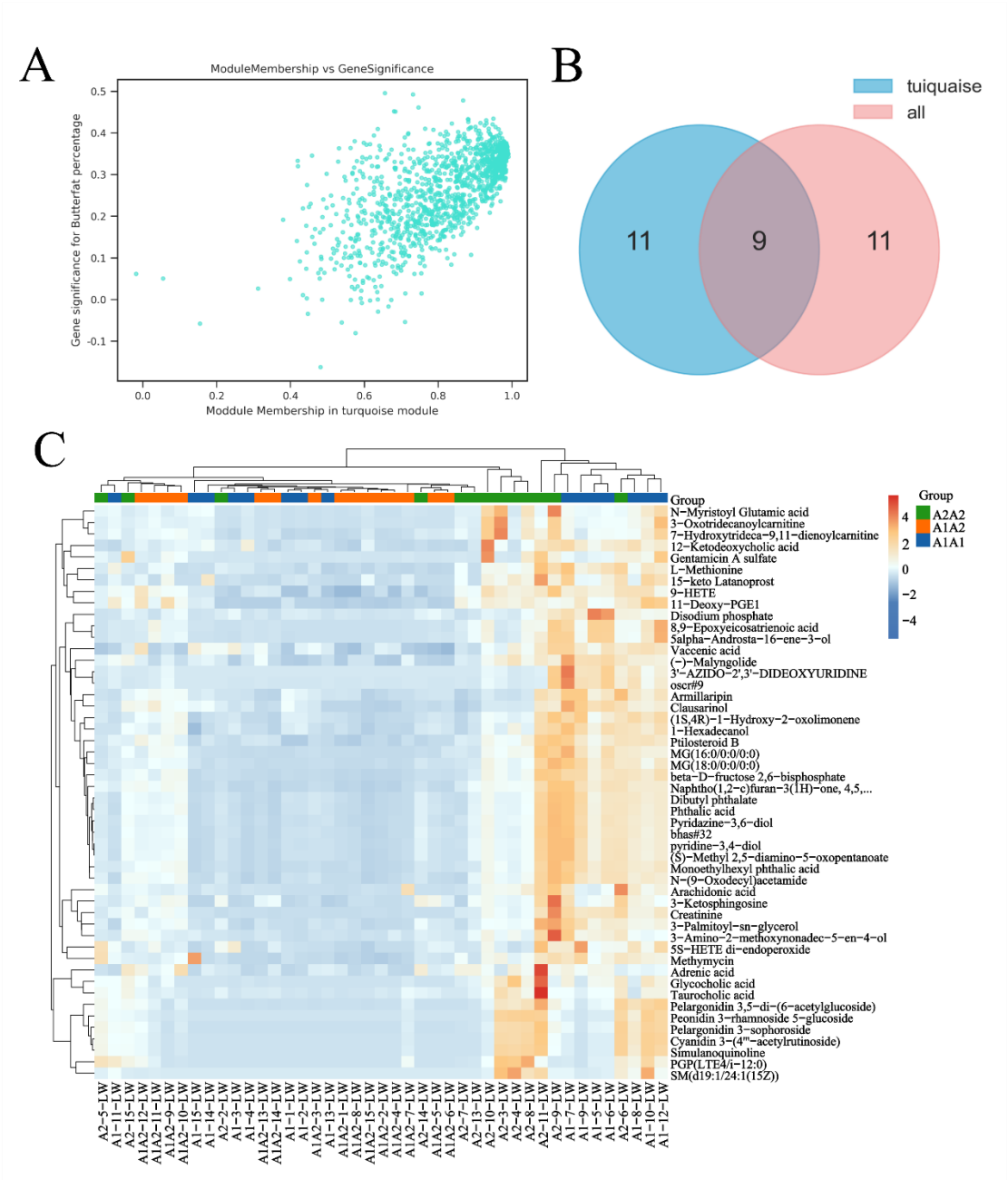

Figure S5 **(A)**Scatter plot of metabolites versus turquoise module members (MM);**(B)** MEturquoise metabolite set enrichment analysis (heatmap); **(C)** Wayne plots of MEturquoise top 20 differential metabolic pathways and top 20 differential metabolic pathways among the three groups.
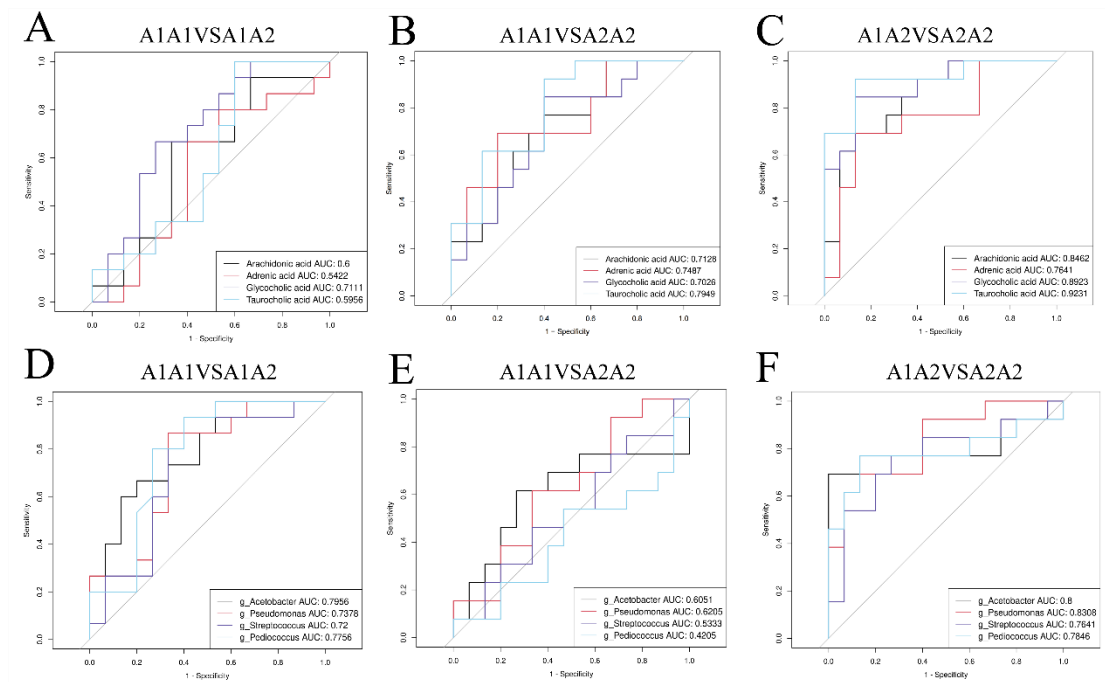
Figure S6 **(A-C)** ROC curves of AA, AdA, GCA, and TCA; **(D-F)** ROC curves of *g_Acetobacter,*

g_Pseudoxanthomonas, g_Streptococcus and *g_Pediococcus*.