Appropriate exercise level attenuates gut dysbiosis and valeric acid increase to improve neuroplasticity and cognitive function after surgery in mice

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KEY RESOURCES TABLE

REAGENT or RESOURCE	RESOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-PSD95	Abcam	Cat#GR326885-1
Rabbit polyclonal anti- Synapsin 1	Abcam	Cat#GR3275672-2
alpha-Tubulin rabbit Ab	cell signaling Technology	Cat#2144S
Rabbit monoclonal anti-C3 antibody	Abcam	Cat#GR3273564-7
Goat monoclonal anti-GFAP antibody	Millipore	Cat#GR3313543-1
Rat monoclonal anti-C3ar antibody	Hycult Biotech	Cat#HM1123-27428M0819-A
Rabbit monoclonal anti-Iba-1 antibody	Wako Chemical Co.	Cat#019-19741
Rat monoclonal anti-BrdU antibody	Abcam	Cat#GR3289293-1
Donkey anti-goat IgG antibody conjugated with Alexa Fluor 488	Invitrogen	Cat#1463163
Donkey anti-rabbit IgG antibody conjugated with Alexa Fluor 594	Invitrogen	Cat#1987293
Donkey anti-rat IgG antibody conjugated with Alexa Fluor 594	Invitrogen	Cat#1979379
Donkey anti-rabbit IgG antibody conjugated with Alexa Fluor 647	Invitrogen	Cat#1826679?
Hoechst 33342	Thermo Scientific	Cat#U12842162
Drugs		
Amoxicillin/Clavulanic acid	Sigma-Aldrich	Cat#SM800607
Metronidazole	Sigma-Aldrich	Cat#M3761
Cefazolin	West-Ward	Cat#319033.1

Valeric sodium	Toronto Research Chemicals	Cat#V091420
Critical Commercial Assays		
Nylon cell strainer	Fisher Scientific	Cat#08-771-2
RNeasy Micro kit	QIAGEN	Cat#74004
5X All in-One MasterMix	Applied Biological Materials	Cat#G485
NovaSeq 6000 S4 Reagent kit	Illumina	Cat#20012866
DNA isolation kit	QIAGEN	Cat#12855-100
AMPure XP magnetic beads	Beckman Coulter	Cat#A63881
Nextera XT Index Kit	Illumina	Cat#FC-131-2001
Qubit dsDNA HS assay kit	Thermo Scientific	Cat#Q33230
FD Rapid GolgiStain kit	FD NeuroTechnologies	Cat#PK401
Mouse IL-1β ELISA kits	R&D SYSTEM	Cat#SMLB00C
Mouse IL-6 ELISA kits	R&D SYSTEM	Cat#SM6000B
Mouse C3 ELISA kits	Abcam	Cat#ab263884
Mouse GDNF ELISA kits	Biosensis	Cat#BEK-2229-2P
Chemicals		
Recombinant mouse GDNF	Abcam	Cat#GR2862-22
C3ar-agonist	cayman chemical	Cat#21683
C3ar-antagonist	Sigma-Aldrich	Cat#559410
BrdU	Sigma-Aldrich	Cat#APN17133-1-1
9-chloromethylanthracene	TCI America	Cat#: A5502
18-Crown-6	Sigma-Aldrich	Cat#: 80833
2-Ethylbutyric acid	Millipore Sigma	Cat# 109959
10×RIPA buffer	Thermo Scientific	Cat#89901
Protease inhibitor cocktail	Sigma-Aldrich	Cat#SRE0055
Tetramethylammonium hydroxide	TCI America	Cat# T0138

Figure legends for supplemental figures

Fig. S1. *Exercise altered gut microbiota.* Nine-week old male mice were with or without exercise for 4 weeks. Presentation of α diversity is in panels A and B. Presentation of β diversity is in panel C. Bacterial abundance is presented in panel D. n = 16 for all panels. C-S: control mouse samples harvested before surgery, E-ES: exercise mouse samples harvested before surgery.

Fig. S2. *Exercise stabilized gut microbiota after surgery.* Nine-week old male mice with or without exercise for 4 weeks were subjected to left carotid artery exposure (surgery) under isoflurane anesthesia. Presentation of α diversity is in panels A to D. Presentation of β diversity is in panels E and F. n = 8 for all panels. S: surgery mouse samples harvested just before surgery, SP3: surgery mouse samples harvested on post-surgery day 3, SP7: surgery mouse samples harvested on post-surgery day 7, ES: exercise mouse samples harvested just before surgery, ESP3: exercise mouse samples harvested on post-surgery day 3, ESP7: exercise mouse samples harvested on post-surgery day 7.

Fig. S3. *Antibiotics reduced gut microbiota.* Nine-week old male mice were treated with antibiotics to eliminate their native gut microbiota. A: bacterial DNA concentrations in feces. Presentation of α diversity is in panels B to D. Presentation of β diversity is in panels E and F. Bacterial abundance is presented in panel G. n = 8 for all panels. C; control, Anti: antibiotics.

Fig. S4. *Transplantation with feces from exercise mice altered gut microbiota.* Nine-week old mice were treated with antibiotics to eliminate their native gut microbiota and then transplanted with feces from exercise mice (ET) or control mice (CT). Presentation of α diversity is in panels A and B. Presentation of β diversity is in panel C. Bacterial abundance is presented in panel D. n = 8 for all panels.

Fig. S5. *Successful establishment of transplanted gut microbiota.* Nine-week old male mice received transplantation of feces from control mice or mice with surgery. Presentation of α diversity is in panels A, B and C. Presentation of β diversity is in panels D and E. Bacterial abundance is presented in panel F. n = 10. CT: control mouse received transplantation of feces from control mice, ST: control mouse received transplantation of feces from surgery mice.

Fig. S6. *Transplantation of gut microbiota from surgery mice induced learning and memory dysfunction.* Nine-week old mice received antibiotic treatment for 7 days (Antibiotic) or transplantation of feces from control mice (Trans-Control) (panels A to C). In another experiment, nine-week old male mice received transplantation of feces from control mice (Trans-Control) or mice with surgery (Trans-Surgery) (panels D to F). A and D: training sessions of Barnes maze test. B and E: memory assessment of Barnes maze test. C and F: novel object recognition test. Results in panels A and D are mean ± S.D. and results in panels B, C, E and F are median ± interquartile range with presentation of value of individual mouse (n = 11 – 12 for panels A to C, = 16 – 17 for panels D to F).

Fig. S7. *Changes of short chain fatty acids (SCFAs) under various experimental conditions.* Blood and feces were harvested from mice 2 weeks after fecal transplantation (for panels A and D), 7 days after surgery (panel B) or 4 weeks after the onset of exercise protocol (panel C). Results are mean ± S.D. (panels A, C, D) with presentation of value of individual mouse or median ± interquartile range (panel B) with presentation of value of individual mouse (n = 7 for all panels). Trans-Control: mice transplanted with feces from control mice, Trans-Exe: mice transplanted with feces from exercise mice, Exe: exercise, Sur: surgery, NS: normal saline, Val: valeric acid.

Fig. S8. *Intracerebroventricular injection of small dose of valeric acid failed to induce learning and memory dysfunction*. Thirteen-week old male mice received intracerebroventricular injection of normal saline (NS) or valeric acid (Val). A: training sessions of Barnes maze test. B: memory assessment of Barnes maze test. C: novel object recognition test. Results in panel A are mean ± S.D. and results in panels B and C are median ± interquartile range with presentation of value of individual mouse (n = 14 – 15).

Fig. S9. *Exercise via regulating gut microbiota and blood valeric acid concentrations attenuated surgery-induced immune and inflammatory responses.* Hippocampus of 9-week old mice with or without 4-week exercise was harvested at various times after surgery for immunostaining or ELISA. A: representative Iba-1 and C3ar immunostaining images of hippocampus harvested 48 h after surgery. B and C: quantification of Iba-1 and C3ar immunostaining of hippocampus harvested 48 h after surgery. D and E: quantification of C3. F and G: IL-1β quantified by ELISA. H and I: IL-6 quantified by ELISA. Results are mean ± S.D. with presentation of value of individual mouse (n = 6 for panels B and C, = 9 for panels D to F and H, = 10 for panels G and I). Trans-Control: mice transplanted with feces from control mice, Trans-Exe: mice transplanted with feces from exercise mice, Exe: exercise, Sur: surgery, NS: normal saline, Val: valeric acid, post: post-surgery.

Fig. S10. *Exercise via regulating gut microbiota and blood valeric acid concentrations attenuated surgery-induced decrease of brain cell genesis and dendritic arborization.* Brain of 9-week old mice with or without 4-week exercise was harvested 19 days after surgery for immunostaining or Golgi staining. A: representative GFAP and BrdU immunostaining images of hippocampus. B and C: quantification of GFAP and BrdU positively stained cells in the hippocampus. D and E: representative Golgi staining images of hippocampus. F and G: quantification of intersections among the dendritic branches and spine density in the hippocampus. Results are mean ± S.D. with presentation of value of individual mouse (n = 6 for panel B, = 8 for other panels). Trans-Control: mice transplanted with feces from control mice, Trans-Exe: mice transplanted with feces from exercise mice, Exe: exercise, Sur: surgery, NS: normal saline, Val: valeric acid.

Fig. S11. *Exercise attenuated surgery-induced reduction of PSD95 and synapsin 1*. Hippocampus was harvested 2 days after surgery for Western blotting. A and B: representative Western blotting images. C and D: quantification of PSD95 and synapsin 1. Result in panel C is median ± interquartile range with presentation of value of individual mouse and result in panel D is mean ± S.D. with presentation of value of individual mouse (n = 8 for all panels). Trans-Control: mice transplanted with feces from control mice, Trans-Exe: mice transplanted with feces from exercise mice, Exe: exercise, Sur: surgery, Syn-1: synapsin 1.

Fig. S12. *Exercise stabilized gut microbiota of mice with surgery in old mice.* Nineteen-month old male mice with or without exercise for 4 weeks were subjected to left carotid artery exposure (surgery) under isoflurane anesthesia. Presentation of α diversity is in panels A, B, D, E, G, H, J and K. Presentation of β diversity is in panels C, F, I and L. Bacterial abundance is presented in panels M and N. n = 16 for panels A, B, C and M, and = 8 for other panels. OC-OS: old control mouse samples harvested before surgery, OE-OES: old exercise mouse samples harvested before surgery, OS: old surgery mouse samples harvested just before surgery, OSP: old surgery mouse samples harvested on post-surgery day 7, OES: old exercise mouse samples harvested before surgery, OESP: old exercise mouse samples harvested on post-surgery day 7.

Fig. S13. *Exercise attenuated surgery-induced reduction of PSD95 and synapsin 1 in old mice.* Hippocampus was harvested 2 days after surgery for Western blotting. A: representative Western blotting images. B: quantification of PSD95 and synapsin 1. Results are mean ± S.D.

with presentation of value of individual mouse (n = 8). Exe: exercise, Sur: surgery, Syn-1: synapsin 1.

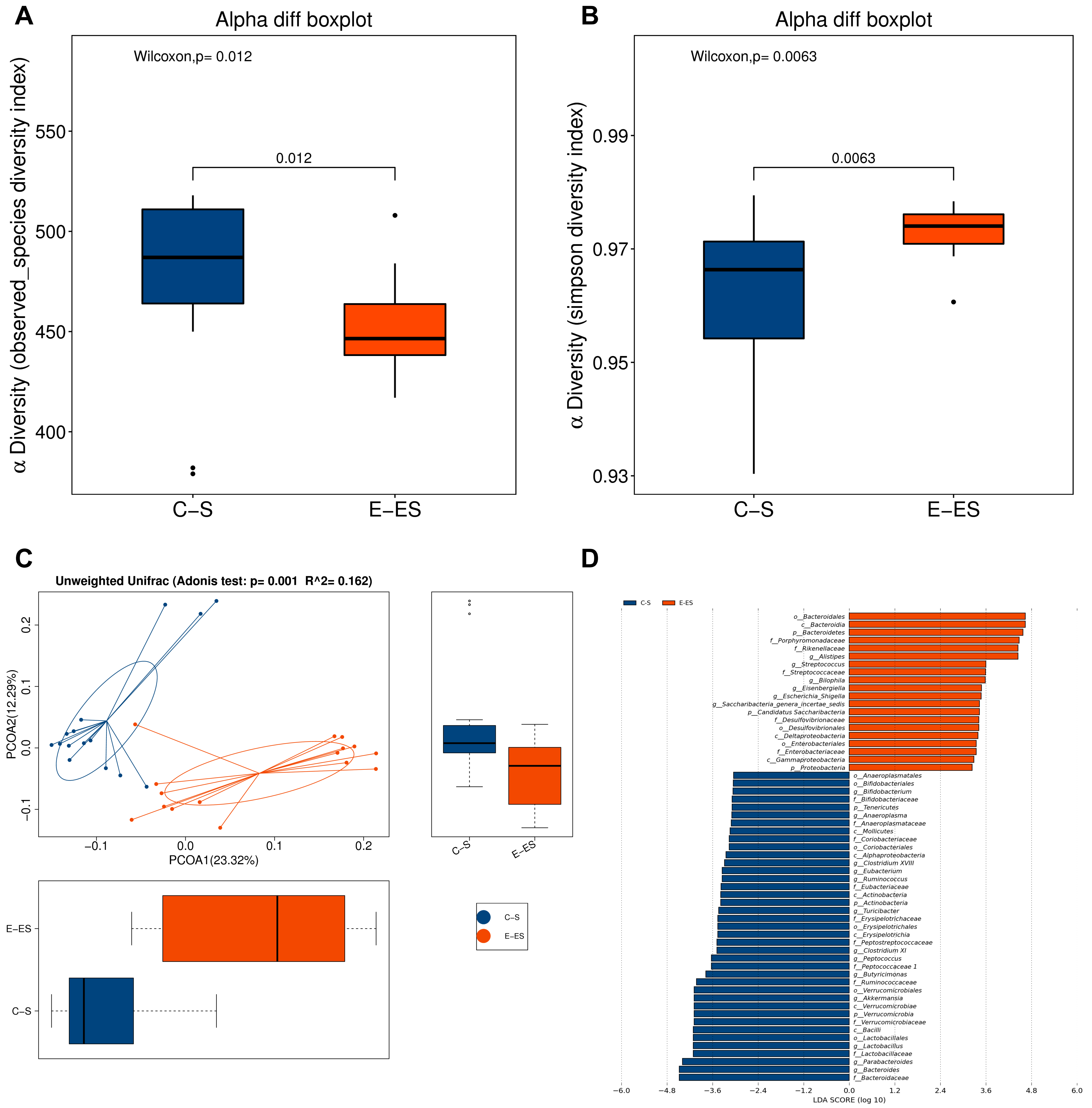
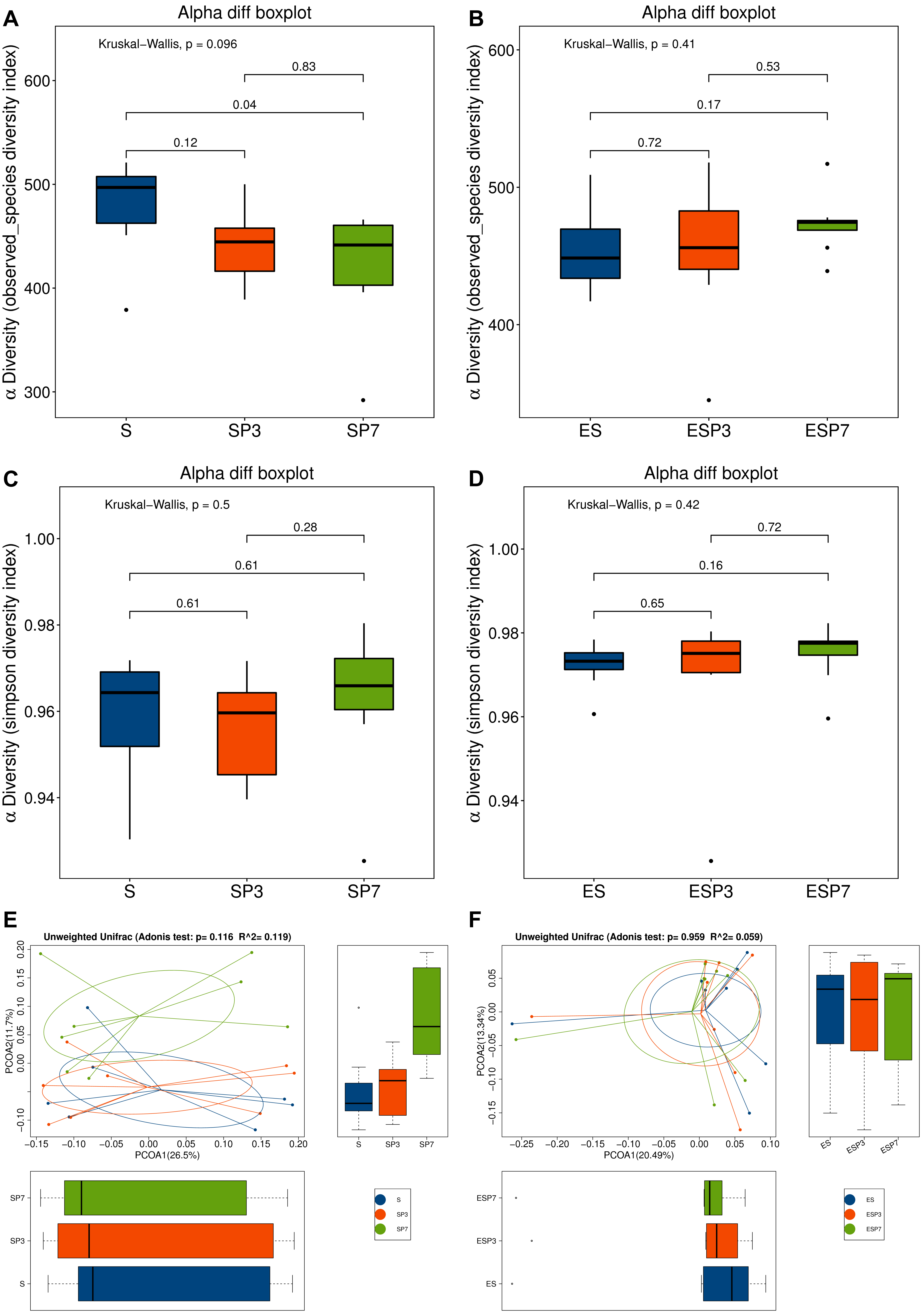

A
Alpha diff boxplot
Wilcoxon,p= 0.012
0.012
α Diversity (observed_species diversity index)
550
500
450
400
C−S
E−ES
B
Alpha diff boxplot
Wilcoxon,p= 0.0063
0.0063
α Diversity (simpson diversity index)
0.99
0.97
0.95
0.93
C−S
E−ES
C
Unweighted Unifrac (Adonis test: p= 0.001 R^2= 0.162)
PCOA2(12.29%)
PCOA1(23.32%)
−0.1
0.0
0.1
0.2
C−S
E−ES
D
C-S
E-ES
o__Bacteroidales
c__Bacteroidia
p__Bacteroidetes
f__Porphyromonadaceae
f__Rikenellaceae
g__Alistipes
g__Streptococcus
f__Streptococcaceae
g__Bilophila
g__Eisenbergiella
g__Escherichia_Shigella
g__Saccharibacteria_genera_incertae_sedis
p__Candidatus Saccharibacteria
f__Desulfovibrionaceae
o__Desulfovibrionales
c__Deltaproteobacteria
o__Enterobacteriales
f__Enterobacteriaceae
c__Gammaproteobacteria
p__Proteobacteria
o__Anaeroplasmatales
o__Bifidobacteriales
g__Bifidobacterium
f__Bifidobacteriaceae
p__Tenericutes
g__Anaeroplasma
f__Anaeroplasmataceae
c__Mollicutes
f__Coriobacteriaceae
o__Coriobacteriales
c__Alphaproteobacteria
g__Clostridium XVIII
g__Eubacterium
g__Ruminococcus
f__Eubacteriaceae
c__Actinobacteria
p__Actinobacteria
g__Turicibacter
f__Erysipelotrichaceae
o__Erysipelotrichales
c__Erysipelotrichia
f__Peptostreptococcaceae
g__Clostridium XI
g__Peptococcus
f__Peptococcaceae 1
g__Butyricimonas
f__Ruminococcaceae
o__Verrucomicrobiales
g__Akkermansia
c__Verrucomicrobiae
p__Verrucomicrobia
f__Verrucomicrobiaceae
c__Bacilli
o__Lactobacillales
g__Lactobacillus
f__Lactobacillaceae
g__Parabacteroides
g__Bacteroides
f__Bacteroidaceae
−6.0
−4.8
−3.6
−2.4
−1.2
0.0
1.2
2.4
3.6
4.8
6.0
LDA SCORE (log 10)

Fig. S1



A
Alpha diff boxplot
Kruskal−Wallis, p = 0.096
α Diversity (observed_species diversity index)
0.83
0.04
0.12
S
SP3
SP7
B
Alpha diff boxplot
Kruskal−Wallis, p = 0.41
0.53
0.17
0.72
ES
ESP3
ESP7
C
Alpha diff boxplot
Kruskal−Wallis, p = 0.5
α Diversity (simpson diversity index)
0.28
0.61
0.61
D
Alpha diff boxplot
Kruskal−Wallis, p = 0.42
0.72
0.16
0.65
E
Unweighted Unifrac (Adonis test: p= 0.116 R^2= 0.119)
PCOA1(26.5%)
PCOA2(11.7%)
F
Unweighted Unifrac (Adonis test: p= 0.959 R^2= 0.059)
PCOA1(20.49%)
PCOA2(13.34%)

Fig. S2

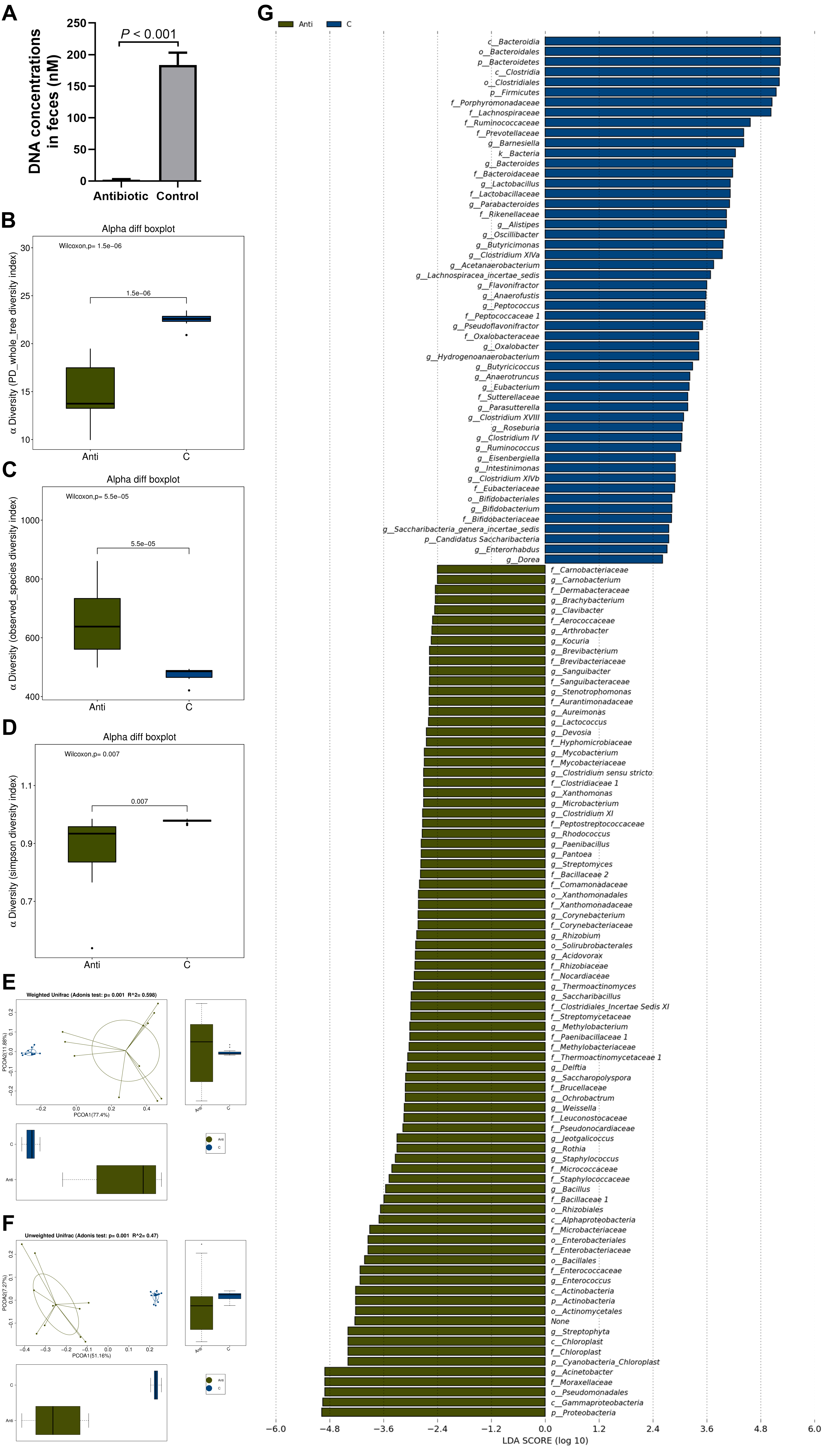

A
DNA concentrations in feces (nM)
P < 0.001
Antibiotic
Control
B
Alpha diff boxplot
Wilcoxon,p= 1.5e−06
1.5e−06
α Diversity (PD_whole_tree diversity index)
Anti
C
C
Alpha diff boxplot
Wilcoxon,p= 5.5e−05
5.5e−05
α Diversity (observed_species diversity index)
Anti
C
D
Alpha diff boxplot
Wilcoxon,p= 0.007
0.007
α Diversity (simpson diversity index)
Anti
C
E
Weighted Unifrac (Adonis test: p= 0.001 R^2= 0.598)
PCOA1(77.4%)
PCOA2(11.88%)
F
Unweighted Unifrac (Adonis test: p= 0.001 R^2= 0.47)
PCOA1(51.16%)
PCOA2(7.27%)
G
Anti
C
c__Bacteroidia
o__Bacteroidales
p__Bacteroidetes
c__Clostridia
o__Clostridiales
p__Firmicutes
f__Porphyromonadaceae
f__Lachnospiraceae
f__Ruminococcaceae
f__Prevotellaceae
g__Barnesiella
k__Bacteria
g__Bacteroides
f__Bacteroidaceae
g__Lactobacillus
f__Lactobacillaceae
g__Parabacteroides
f__Rikenellaceae
g__Alistipes
g__Oscillibacter
g__Butyricimonas
g__Clostridium XlVa
g__Acetanaerobacterium
g__Lachnospiracea_incertae_sedis
g__Flavonifractor
g__Anaerofustis
g__Peptococcus
f__Peptococcaceae 1
g__Pseudoflavonifractor
f__Oxalobacteraceae
g__Oxalobacter
g__Hydrogenoanaerobacterium
g__Butyricicoccus
g__Anaerotruncus
g__Eubacterium
f__Sutterellaceae
g__Parasutterella
g__Clostridium XVIII
g__Roseburia
g__Clostridium IV
g__Ruminococcus
g__Eisenbergiella
g__Intestinimonas
g__Clostridium XlVb
f__Eubacteriaceae
o__Bifidobacteriales
g__Bifidobacterium
f__Bifidobacteriaceae
g__Saccharibacteria_genera_incertae_sedis
p__Candidatus Saccharibacteria
g__Enterorhabdus
g__Dorea
f__Carnobacteriaceae
g__Carnobacterium
f__Dermabacteraceae
g__Brachybacterium
g__Clavibacter
f__Aerococcaceae
g__Arthrobacter
g__Kocuria
g__Brevibacterium
f__Brevibacteriaceae
g__Sanguibacter
f__Sanguibacteraceae
g__Stenotrophomonas
f__Aurantimonadaceae
g__Aureimonas
g__Lactococcus
g__Devosia
f__Hyphomicrobiaceae
g__Mycobacterium
f__Mycobacteriaceae
g__Clostridium sensu stricto
f__Clostridiaceae 1
g__Xanthomonas
g__Microbacterium
g__Clostridium XI
f__Peptostreptococcaceae
g__Rhodococcus
g__Paenibacillus
g__Pantoea
g__Streptomyces
f__Bacillaceae 2
f__Comamonadaceae
o__Xanthomonadales
f__Xanthomonadaceae
g__Corynebacterium
f__Corynebacteriaceae
g__Rhizobium
o__Solirubrobacterales
g__Acidovorax
f__Rhizobiaceae
f__Nocardiaceae
g__Thermoactinomyces
g__Saccharibacillus
f__Clostridiales_Incertae Sedis XI
f__Streptomycetaceae
g__Methylobacterium
f__Paenibacillaceae 1
f__Methylobacteriaceae
f__Thermoactinomycetaceae 1
g__Delftia
g__Saccharopolyspora
f__Brucellaceae
g__Ochrobactrum
g__Weissella
f__Leuconostocaceae
f__Pseudonocardiaceae
g__Jeotgalicoccus
g__Rothia
g__Staphylococcus
f__Micrococcaceae
f__Staphylococcaceae
g__Bacillus
f__Bacillaceae 1
o__Rhizobiales
c__Alphaproteobacteria
f__Microbacteriaceae
o__Enterobacteriales
f__Enterobacteriaceae
o__Bacillales
f__Enterococcaceae
g__Enterococcus
c__Actinobacteria
p__Actinobacteria
o__Actinomycetales
None
g__Streptophyta
c__Chloroplast
f__Chloroplast
p__Cyanobacteria_Chloroplast
g__Acinetobacter
f__Moraxellaceae
o__Pseudomonadales
c__Gammaproteobacteria
p__Proteobacteria
LDA SCORE (log 10)

Fig. S3

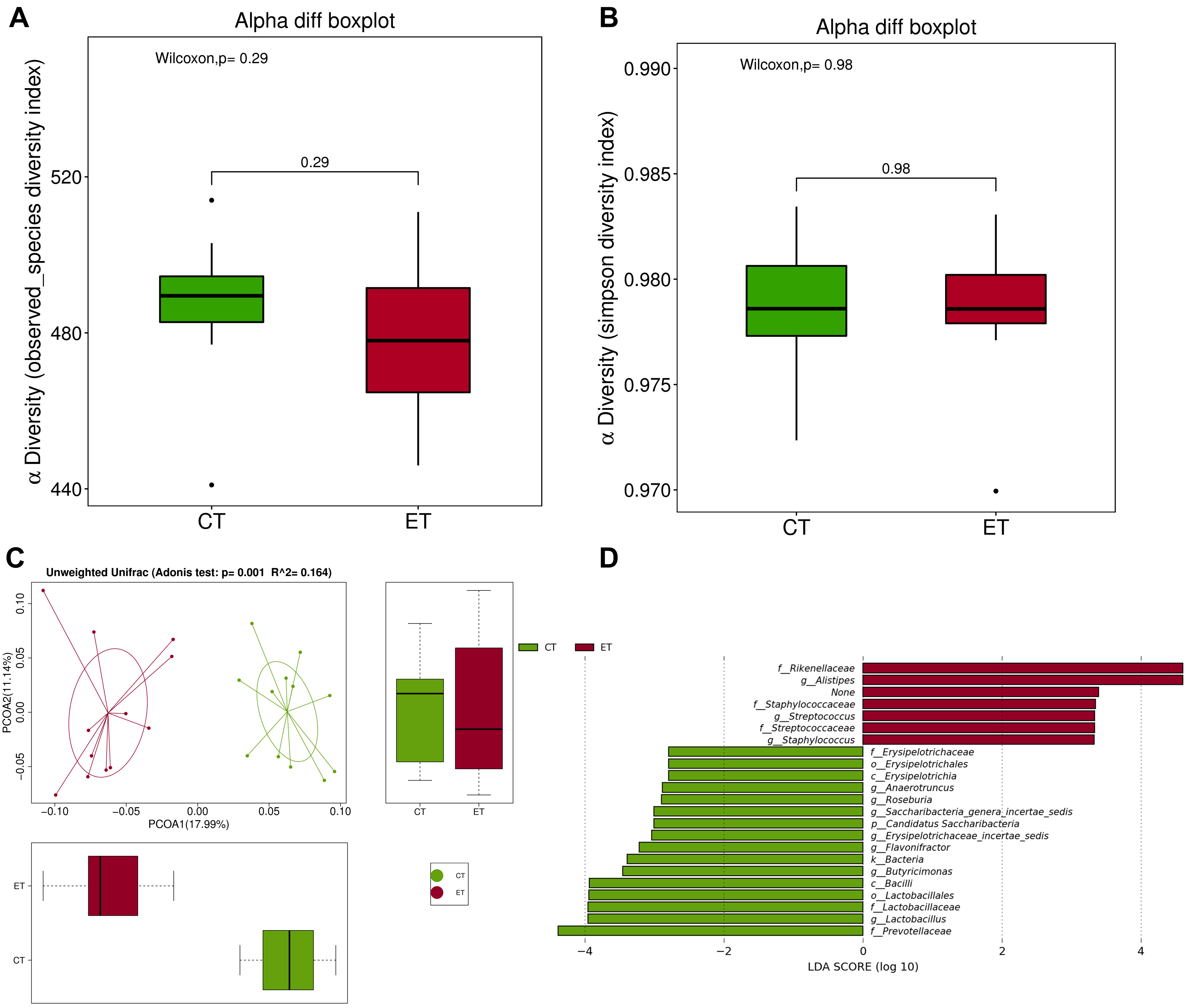

A
Alpha diff boxplot
Wilcoxon,p= 0.29
0.29
α Diversity (observed_species diversity index)
520
480
440
CT
ET
B
Alpha diff boxplot
Wilcoxon,p= 0.98
0.98
α Diversity (simpson diversity index)
0.990
0.985
0.980
0.975
0.970
CT
ET
C
Unweighted Unifrac (Adonis test: p= 0.001 R^2= 0.164)
PCOA2(11.14%)
PCOA1(17.99%)
CT
ET
D
CT
ET
f__Rikenellaceae
g__Alistipes
None
f__Staphylococcaceae
g__Streptococcus
f__Streptococcaceae
g__Staphylococcus
f__Erysipelotrichaceae
o__Erysipelotrichales
c__Erysipelotrichia
g__Anaerotruncus
g__Roseburia
g__Saccharibacteria_genera_incertae_sedis
p__Candidatus Saccharibacteria
g__Erysipelotrichaceae_incertae_sedis
g__Flavonifractor
k__Bacteria
g__Butyricimonas
c__Bacilli
o__Lactobacillales
f__Lactobacillaceae
g__Lactobacillus
f__Prevotellaceae
LDA SCORE (log 10)

Fig. S4

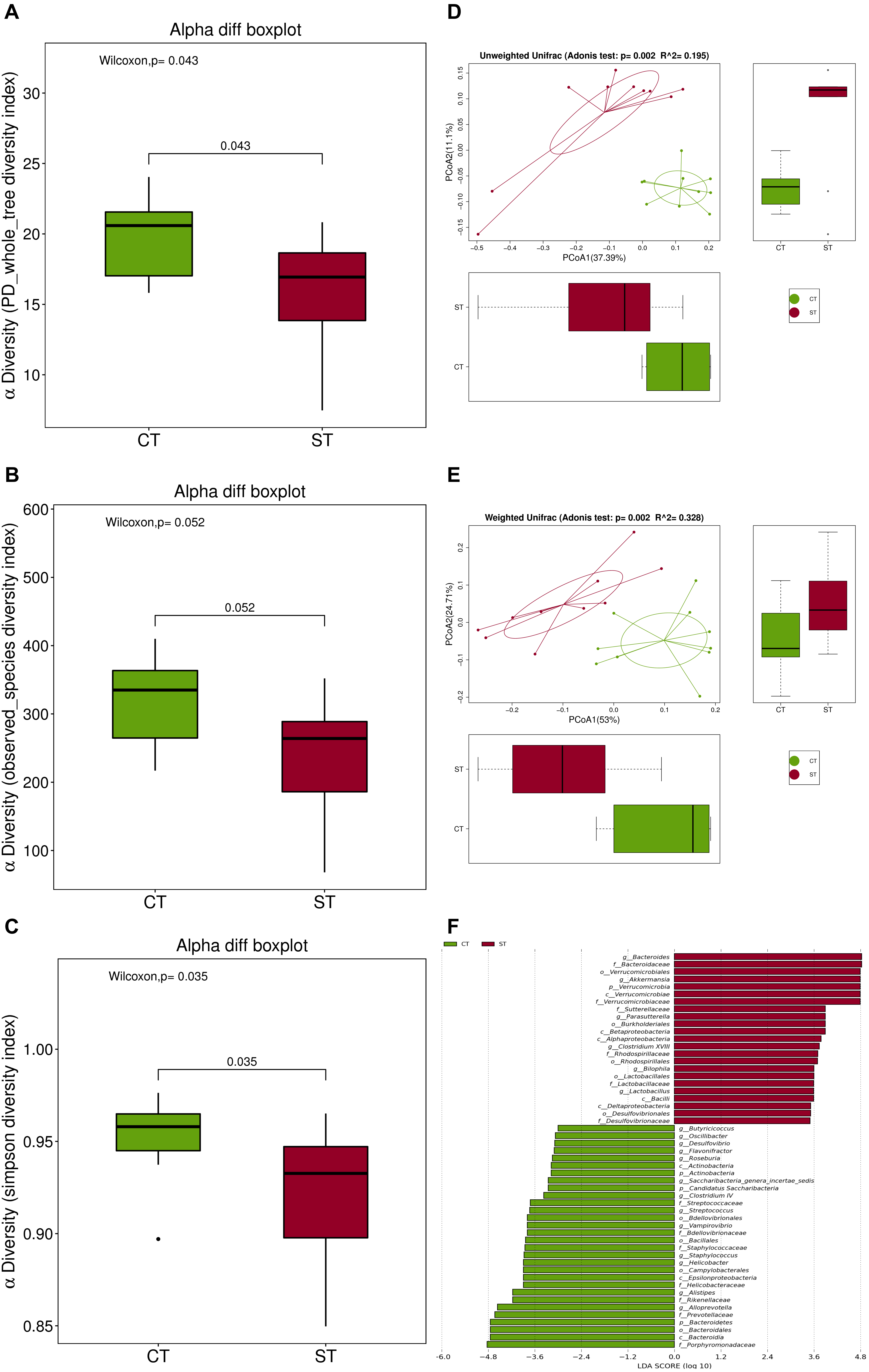

A
Alpha diff boxplot
Wilcoxon,p= 0.043
0.043
α Diversity (PD_whole_tree diversity index)
CT
ST
B
Alpha diff boxplot
Wilcoxon,p= 0.052
0.052
α Diversity (observed_species diversity index)
CT
ST
C
Alpha diff boxplot
Wilcoxon,p= 0.035
0.035
α Diversity (simpson diversity index)
CT
ST
D
Unweighted Unifrac (Adonis test: p= 0.002 R^2= 0.195)
PCoA1(37.39%)
PCoA2(11.1%)
CT
ST
E
Weighted Unifrac (Adonis test: p= 0.002 R^2= 0.328)
PCoA1(53%)
PCoA2(24.71%)
CT
ST
F
CT
ST
g__Bacteroides
f__Bacteroidaceae
o__Verrucomicrobiales
g__Akkermansia
p__Verrucomicrobia
c__Verrucomicrobiae
f__Verrucomicrobiaceae
f__Sutterellaceae
g__Parasutterella
o__Burkholderiales
c__Betaproteobacteria
c__Alphaproteobacteria
g__Clostridium XVIII
f__Rhodospirillaceae
o__Rhodospirillales
g__Bilophila
o__Lactobacillales
f__Lactobacillaceae
g__Lactobacillus
c__Bacilli
c__Deltaproteobacteria
o__Desulfovibrionales
f__Desulfovibrionaceae
g__Butyricicoccus
g__Oscillibacter
g__Desulfovibrio
g__Flavonifractor
g__Roseburia
c__Actinobacteria
p__Actinobacteria
g__Saccharibacteria_genera_incertae_sedis
p__Candidatus Saccharibacteria
g__Clostridium IV
f__Streptococcaceae
g__Streptococcus
o__Bdellovibrionales
g__Vampirovibrio
f__Bdellovibrionaceae
o__Bacillales
f__Staphylococcaceae
g__Staphylococcus
g__Helicobacter
o__Campylobacterales
c__Epsilonproteobacteria
f__Helicobacteraceae
g__Alistipes
f__Rikenellaceae
g__Alloprevotella
f__Prevotellaceae
p__Bacteroidetes
o__Bacteroidales
c__Bacteroidia
f__Porphyromonadaceae
LDA SCORE (log 10)

Fig. S5

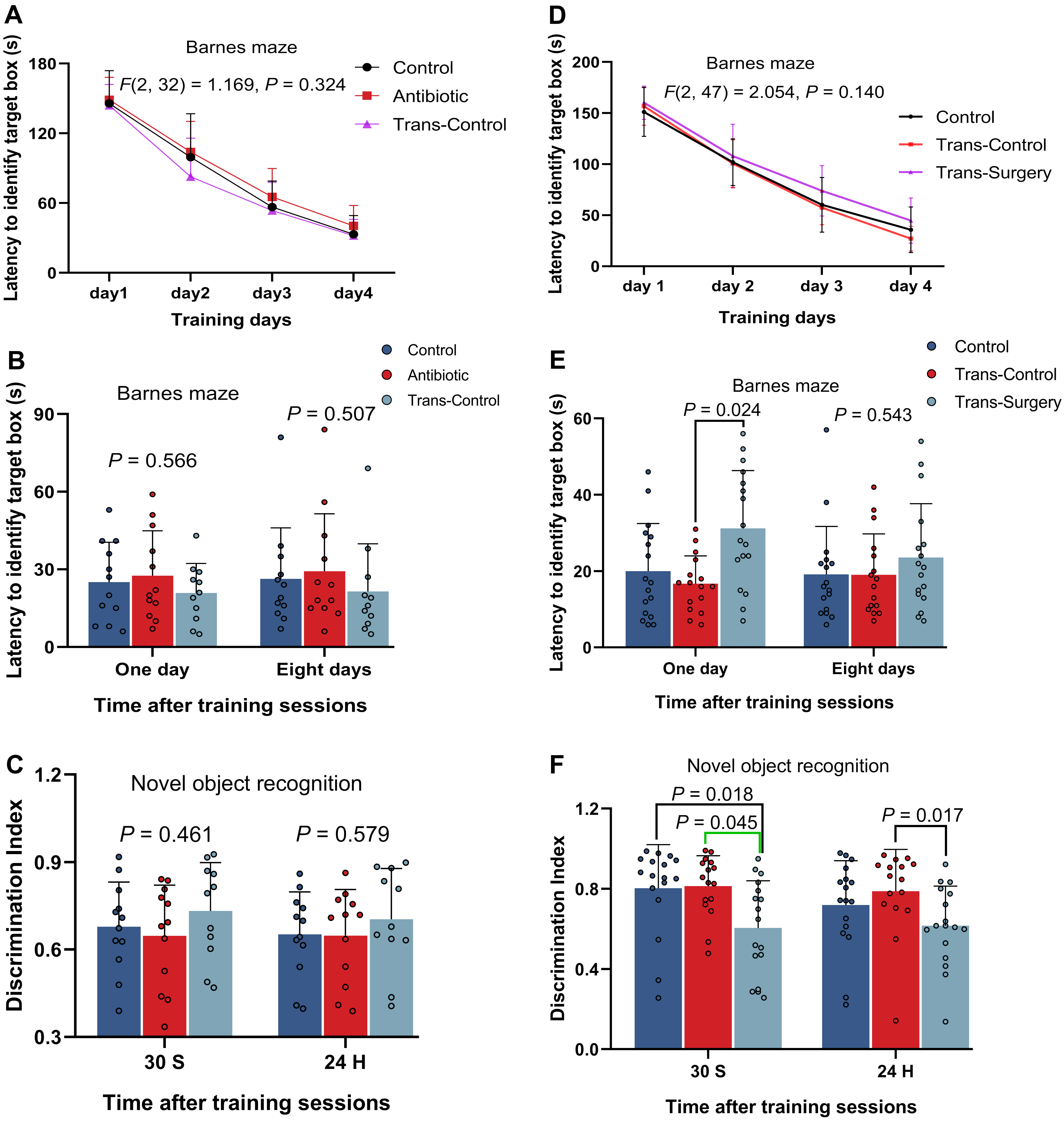
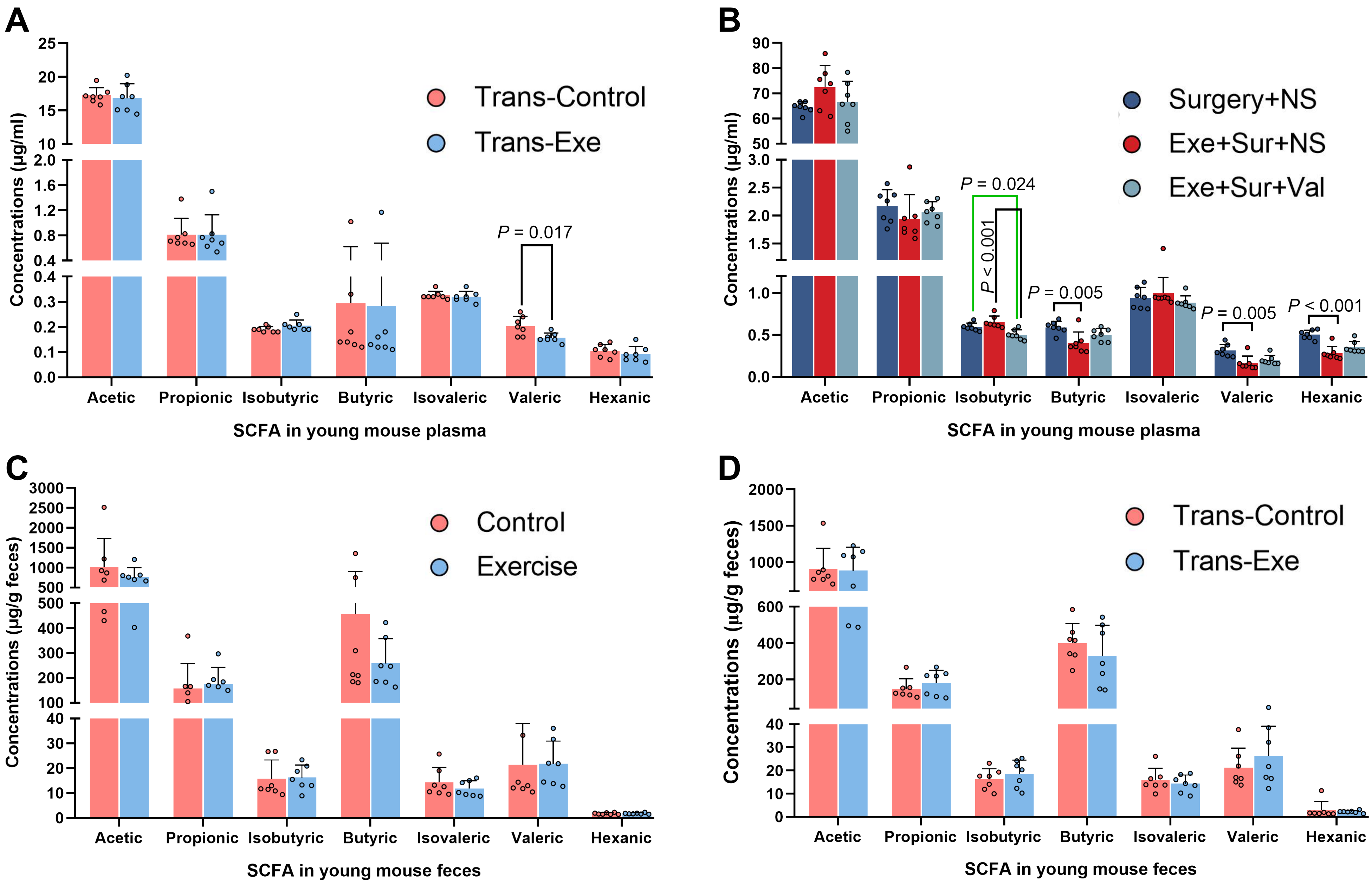

A
Barnes maze
F(2, 32) = 1.169, P = 0.324
Control
Antibiotic
Trans-Control
Latency to identify target box (s)
180
120
60
0
day1
day2
day3
day4
Training days
D
Barnes maze
F(2, 47) = 2.054, P = 0.140
Control
Trans-Control
Trans-Surgery
Latency to identify target box (s)
200
150
100
50
0
day 1
day 2
day 3
day 4
Training days
B
Barnes maze
Control
Antibiotic
Trans-Control
P = 0.566
P = 0.507
Latency to identify target box (s)
90
60
30
0
One day
Eight days
Time after training sessions
E
Barnes maze
Control
Trans-Control
Trans-Surgery
P = 0.024
P = 0.543
Latency to identify target box (s)
60
40
20
0
One day
Eight days
Time after training sessions
C
Novel object recognition
P = 0.461
P = 0.579
Discrimination Index
1.2
0.9
0.6
0.3
30 S
24 H
Time after training sessions
F
Novel object recognition
P = 0.018
P = 0.045
P = 0.017
Discrimination Index
1.2
0.8
0.4
0.0
30 S
24 H
Time after training sessions

Fig. S6



A
Trans-Control
Trans-Exe
Concentrations (μg/ml)
P = 0.017
Acetic
Propionic
Isobutyric
Butyric
Isovaleric
Valeric
Hexanic
SCFA in young mouse plasma
B
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
Concentrations (μg/ml)
P = 0.024
P < 0.001
P = 0.005
P = 0.005
P < 0.001
SCFA in young mouse plasma
C
Control
Exercise
Concentrations (μg/g feces)
SCFA in young mouse feces
D
Trans-Control
Trans-Exe
Concentrations (μg/g feces)
SCFA in young mouse feces

Fig. S7

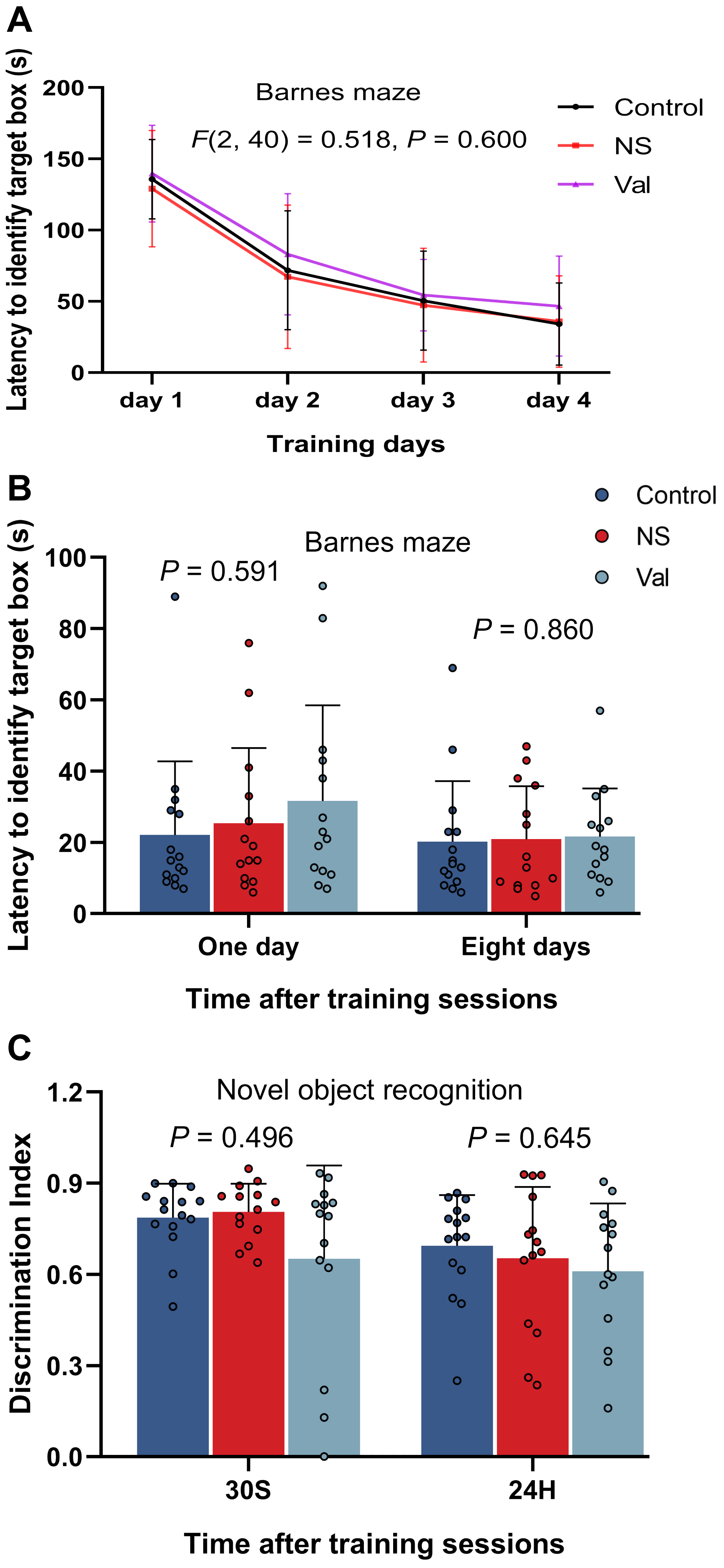

A
Barnes maze
F(2, 40) = 0.518, P = 0.600
Control
NS
Val
Latency to identify target box (s)
200
150
100
50
0
day 1
day 2
day 3
day 4
Training days
B
Control
NS
Val
Barnes maze
P = 0.591
P = 0.860
Latency to identify target box (s)
100
80
60
40
20
0
One day
Eight days
Time after training sessions
C
Novel object recognition
P = 0.496
P = 0.645
Discrimination Index
1.2
0.9
0.6
0.3
0.0
30S
24H
Time after training sessions

Fig. S8

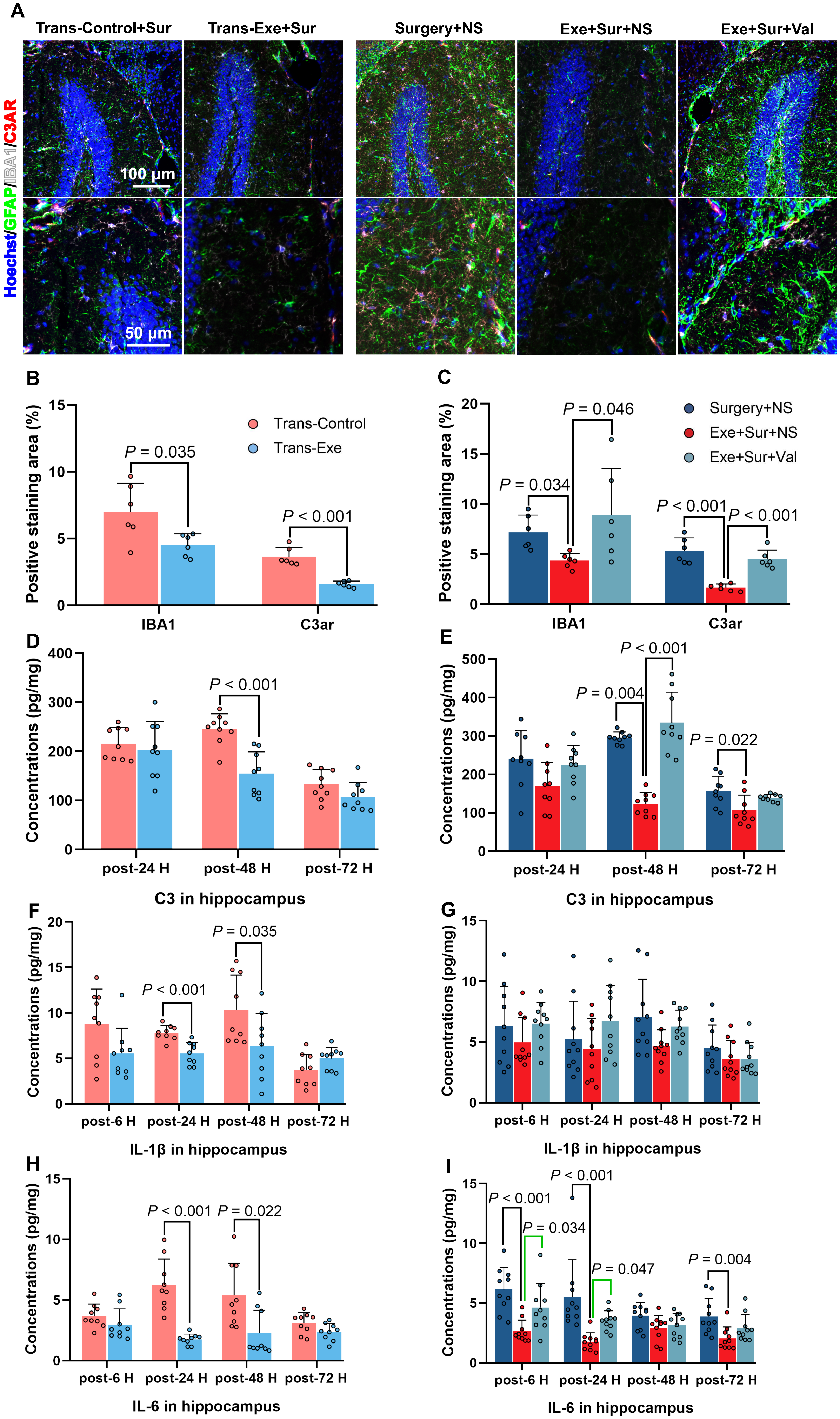

A
Trans-Control+Sur
Trans-Exe+Sur
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
Hoechst/GFAP/IBA1/C3AR
100 μm
50 μm
B
Positive staining area (%)
Trans-Control
Trans-Exe
P = 0.035
P < 0.001
IBA1
C3ar
C
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
P = 0.046
P = 0.034
P < 0.001
P < 0.001
D
Concentrations (pg/mg)
P < 0.001
post-24 H
post-48 H
post-72 H
C3 in hippocampus
E
P < 0.001
P = 0.004
P = 0.022
C3 in hippocampus
F
P = 0.035
P < 0.001
post-6 H
IL-1β in hippocampus
G
IL-1β in hippocampus
H
P < 0.001
P = 0.022
IL-6 in hippocampus
I
P < 0.001
P < 0.001
P = 0.034
P = 0.047
P = 0.004
IL-6 in hippocampus

Fig. S9

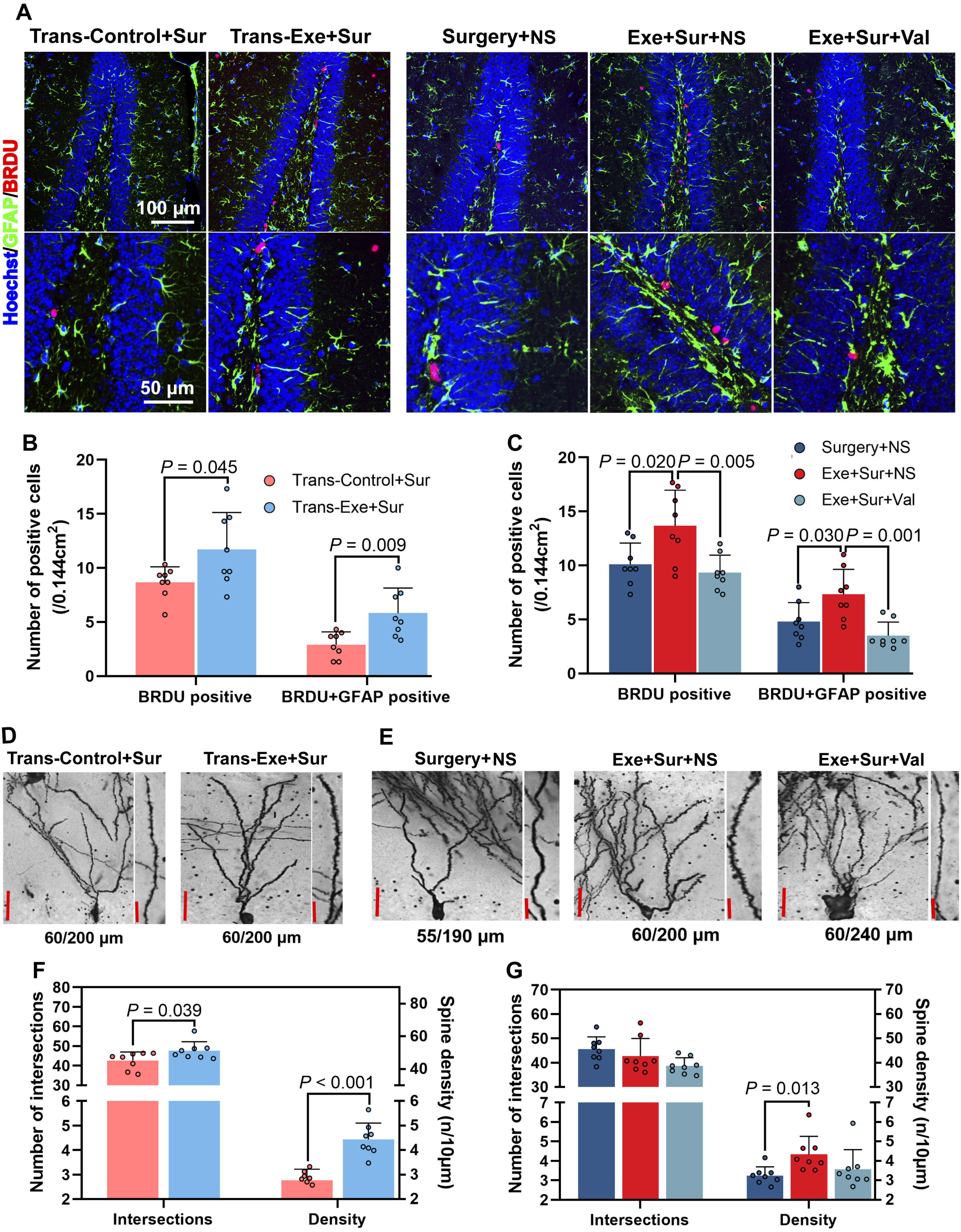
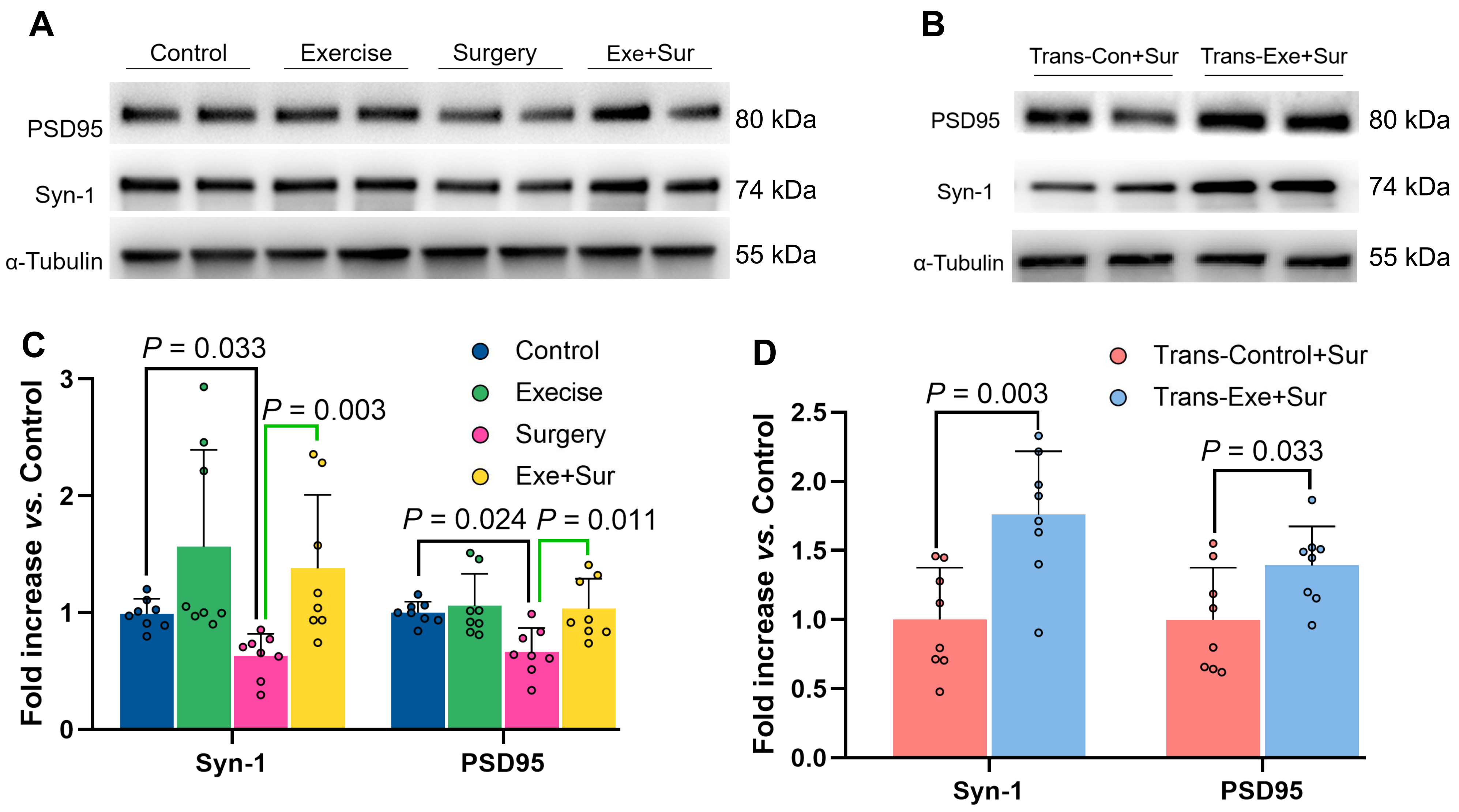

A
Trans-Control+Sur
Trans-Exe+Sur
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
Hoechst/GFAP/BRDU
100 μm
50 μm
B
Number of positive cells (/0.144cm²)
P = 0.045
P = 0.009
Trans-Control+Sur
Trans-Exe+Sur
BRDU positive
BRDU+GFAP positive
C
P = 0.020
P = 0.005
P = 0.030
P = 0.001
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
BRDU positive
BRDU+GFAP positive
D
Trans-Control+Sur
Trans-Exe+Sur
60/200 μm
60/200 μm
E
Surgery+NS
Exe+Sur+NS
Exe+Sur+Val
55/190 μm
60/200 μm
60/240 μm
F
Number of intersections
Spine density (n/10μm)
P = 0.039
P < 0.001
Intersections
Density
G
Number of intersections
Spine density (n/10μm)
P = 0.013
Intersections
Density

Fig. S10



A
Control
Exercise
Surgery
Exe+Sur
PSD95
Syn-1
α-Tubulin
80 kDa
74 kDa
55 kDa
B
Trans-Con+Sur
Trans-Exe+Sur
PSD95
Syn-1
α-Tubulin
80 kDa
74 kDa
55 kDa
C
P = 0.033
P = 0.003
P = 0.024
P = 0.011
Control
Execise
Surgery
Exe+Sur
Fold increase vs. Control
0
1
2
3
Syn-1
PSD95
D
P = 0.003
P = 0.033
Trans-Control+Sur
Trans-Exe+Sur
Fold increase vs. Control
0.0
0.5
1.0
1.5
2.0
2.5
Syn-1
PSD95

Fig. S11

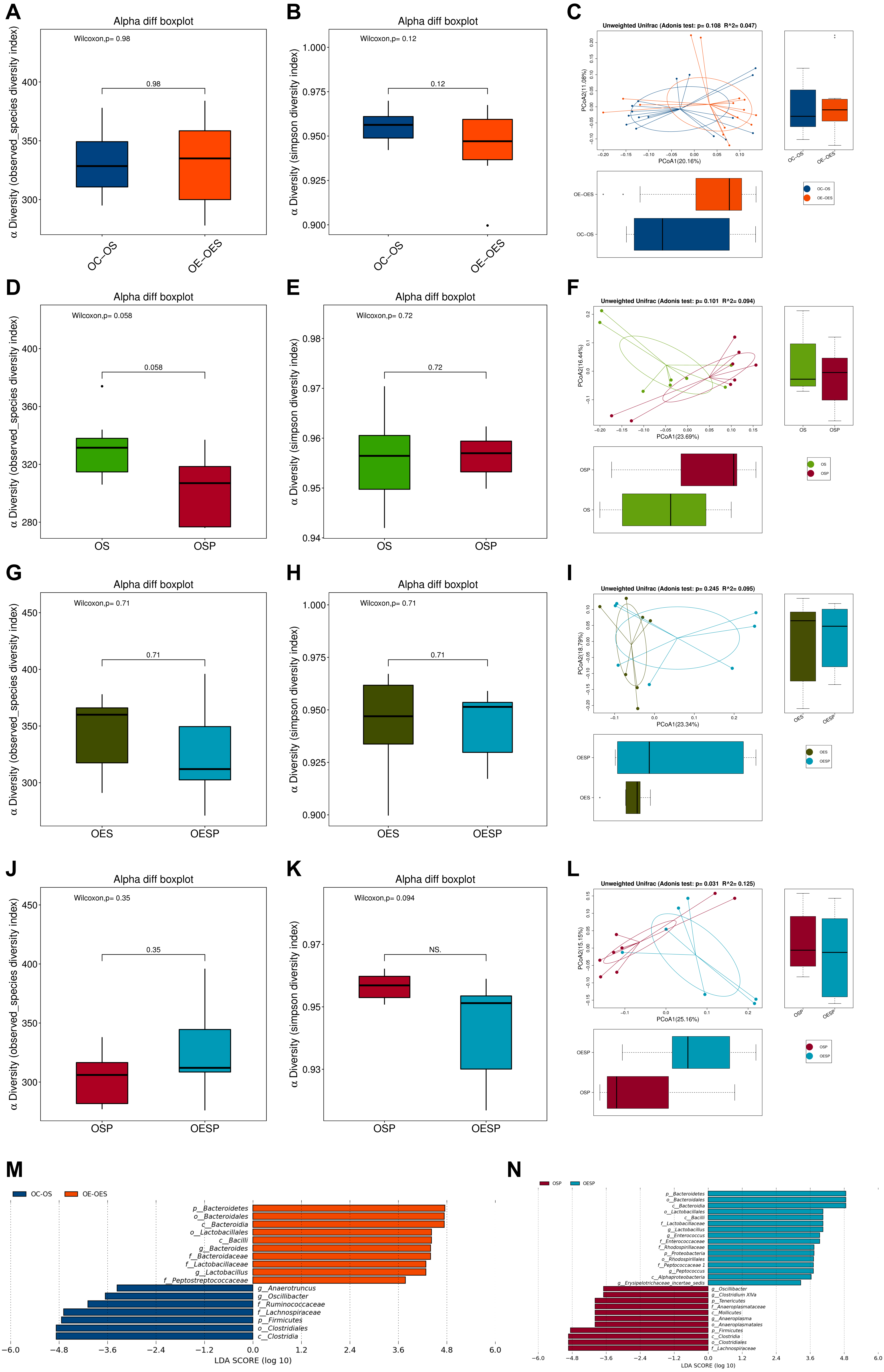
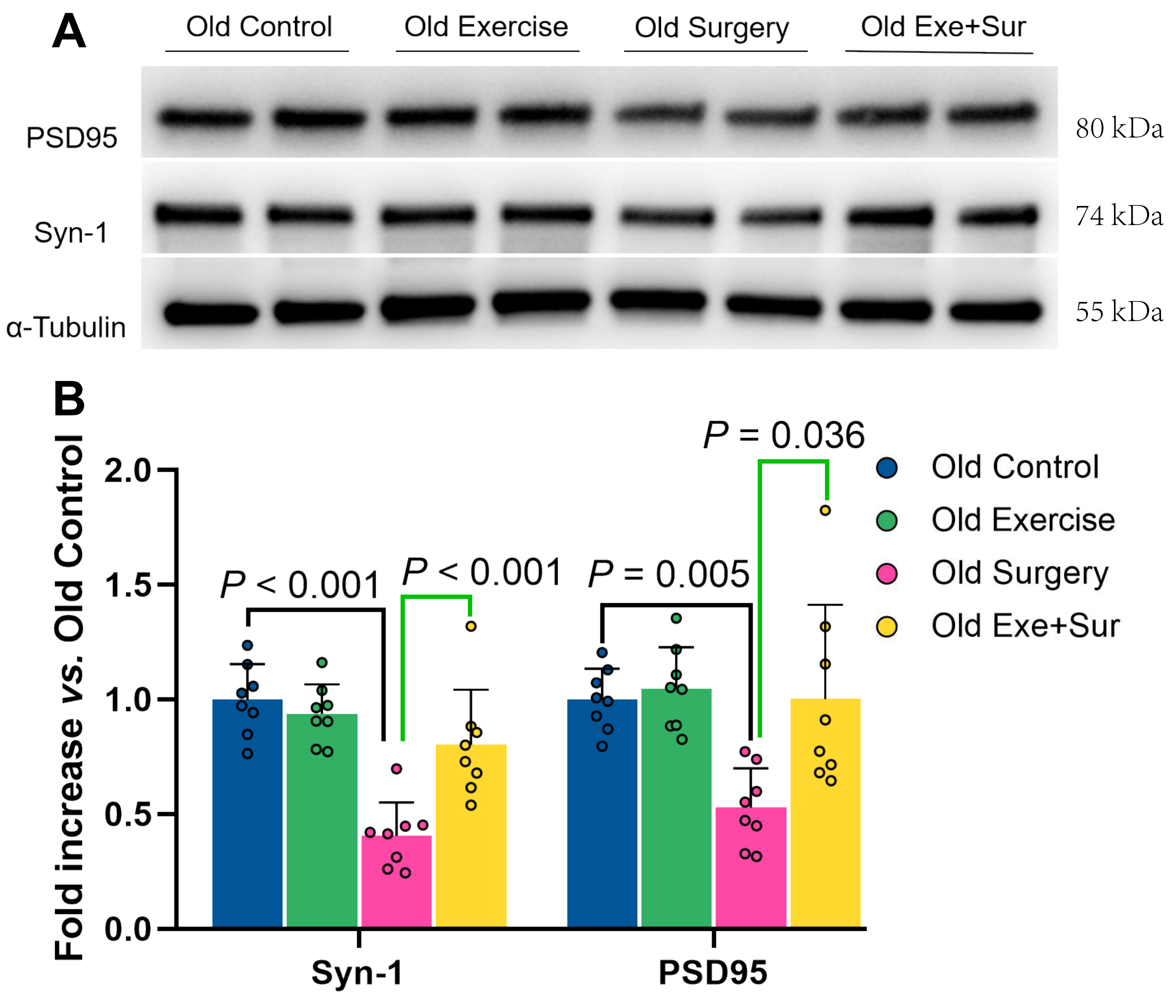

A
Alpha diff boxplot
Wilcoxon,p= 0.98
0.98
α Diversity (observed_species diversity index)
OC–OS
OE–OES
B
Alpha diff boxplot
Wilcoxon,p= 0.12
0.12
α Diversity (simpson diversity index)
C
Unweighted Unifrac (Adonis test: p= 0.108 R^2= 0.047)
PCoA1(20.16%)
PCoA2(11.08%)
D
Alpha diff boxplot
Wilcoxon,p= 0.058
0.058
OS
OSP
E
Alpha diff boxplot
Wilcoxon,p= 0.72
0.72
F
Unweighted Unifrac (Adonis test: p= 0.101 R^2= 0.094)
PCoA1(23.69%)
PCoA2(16.44%)
G
Alpha diff boxplot
Wilcoxon,p= 0.71
0.71
OES
OESP
H
Alpha diff boxplot
Wilcoxon,p= 0.71
0.71
I
Unweighted Unifrac (Adonis test: p= 0.245 R^2= 0.095)
PCoA1(23.34%)
PCoA2(18.79%)
J
Alpha diff boxplot
Wilcoxon,p= 0.35
0.35
K
Alpha diff boxplot
Wilcoxon,p= 0.094
NS.
L
Unweighted Unifrac (Adonis test: p= 0.031 R^2= 0.125)
PCoA1(25.16%)
PCoA2(15.15%)
M
OC-OS
OE-OES
p__Bacteroidetes
o__Bacteroidales
c__Bacteroidia
o__Lactobacillales
c__Bacilli
g__Bacteroides
f__Bacteroidaceae
f__Lactobacillaceae
g__Lactobacillus
f__Peptostreptococcaceae
g__Anaerotruncus
g__Oscillibacter
f__Ruminococcaceae
f__Lachnospiraceae
p__Firmicutes
o__Clostridiales
c__Clostridia
LDA SCORE (log 10)
N
OSP
OESP
p__Bacteroidetes
o__Bacteroidales
c__Bacteroidia
o__Lactobacillales
c__Bacilli
f__Lactobacillaceae
g__Lactobacillus
g__Enterococcus
f__Enterococcaceae
f__Rhodospirillaceae
p__Proteobacteria
o__Rhodospirillales
f__Peptococcaceae 1
g__Peptococcus
c__Alphaproteobacteria
g__Erysipelotrichaceae_incertae_sedis
g__Oscillibacter
g__Clostridium XIVa
p__Tenericutes
f__Anaeroplasmataceae
c__Mollicutes
g__Anaeroplasma
o__Anaeroplasmatales
p__Firmicutes
c__Clostridia
o__Clostridiales
f__Lachnospiraceae
LDA SCORE (log 10)

Fig. S12



A
Old Control
Old Exercise
Old Surgery
Old Exe+Sur
PSD95
Syn-1
α-Tubulin
80 kDa
74 kDa
55 kDa
B
Fold increase vs. Old Control
2.0
1.5
1.0
0.5
0.0
P < 0.001
P < 0.001
P = 0.005
P = 0.036
Syn-1
PSD95
Old Control
Old Exercise
Old Surgery
Old Exe+Sur

Fig. S13