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Title	Prebiotic supplementation in frail older people affects specific gut microbiota taxa but not global diversity
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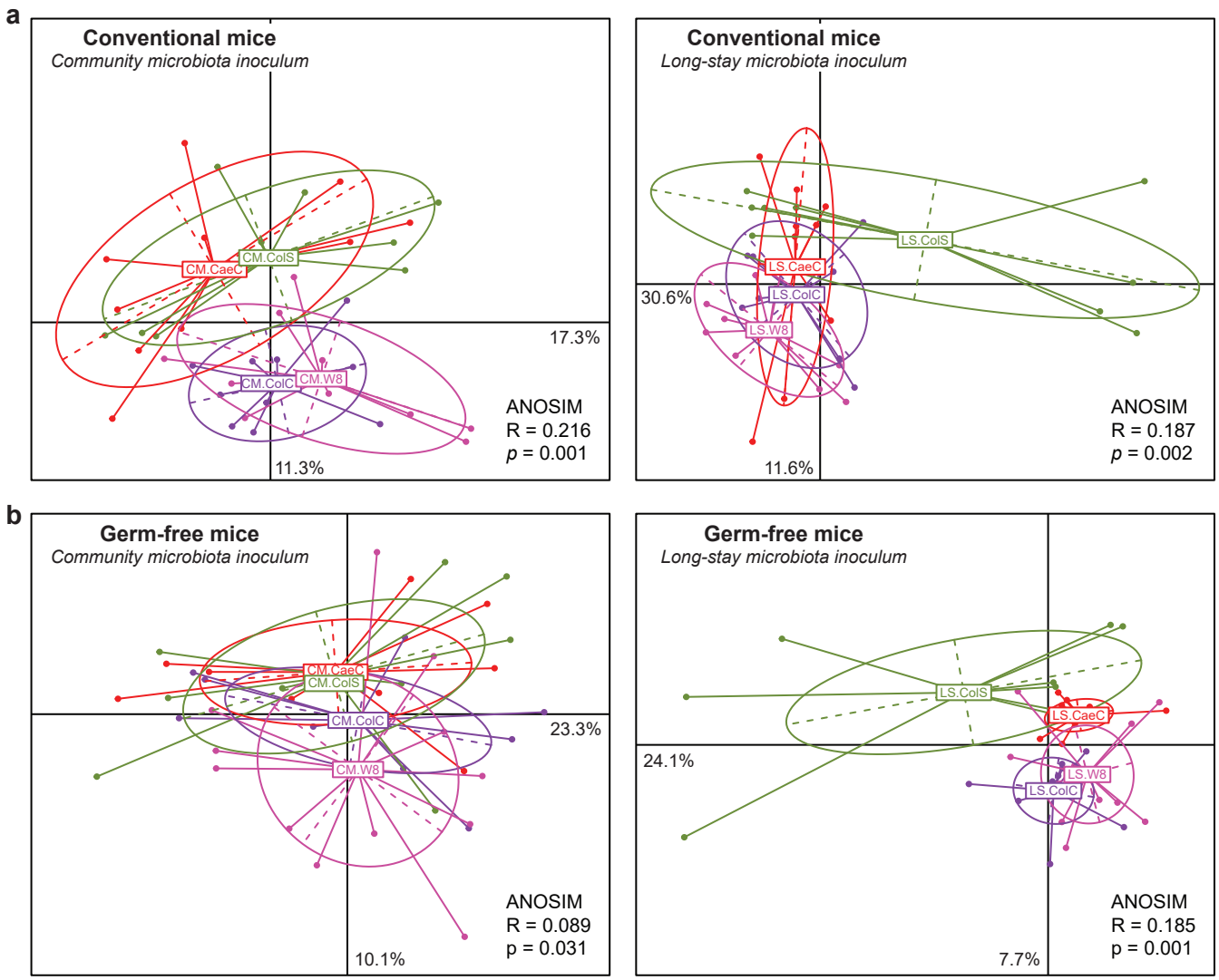


Figure S1. Principle coordinates analysis plots comparing bacterial community structure between faeces, colonic scrapings, colonic contents and caecal contents at week 8 based on Spearman distances. (**a**) Samples from conventional mice cross over design. (**b**) Samples from germ-free mice cross over design. The significant differences between groups were calculated by analysis of similarity (ANOSIM) tests. Abbreviations: CM, Community; LS, Long-stay; W8, week 8 (feaces); ColS, colonic scraping; ColC, colonic content; CaeC, caecal content.

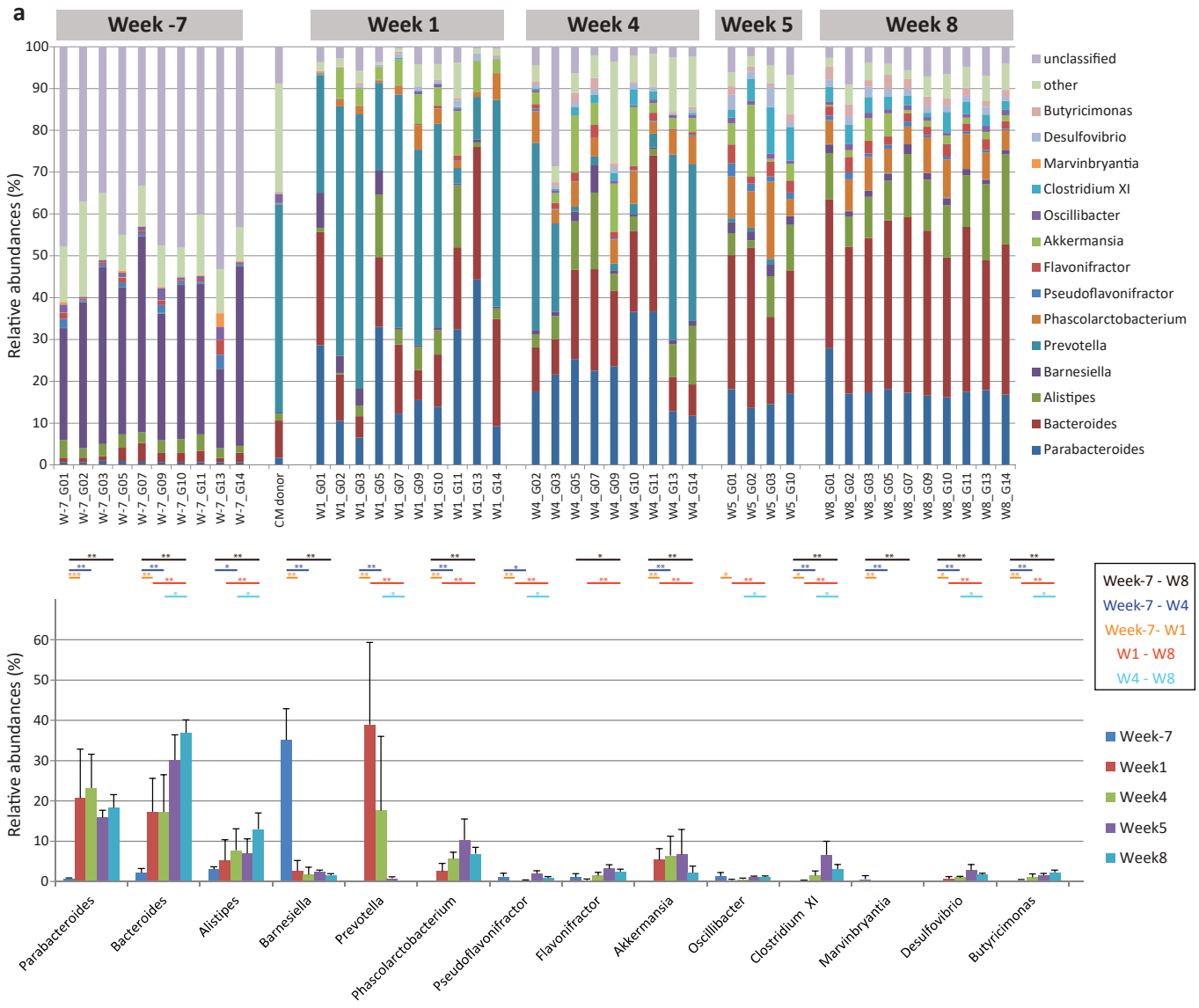

a
Week -7
Week 1
Week 4
Week 5
Week 8
Relative abundances (%)
unclassified
other
Butyricimonas
Desulfovibrio
Marvinbryantia
Clostridium XI
Oscillibacter
Akkermansia
Flavonifractor
Pseudoflavonifractor
Phascolarctobacterium
Prevotella
Barnesiella
Alistipes
Bacteroides
Parabacteroides
W-7_G01
W-7_G02
W-7_G03
W-7_G05
W-7_G07
W-7_G09
W-7_G10
W-7_G11
W-7_G13
W-7_G14
CM donor
W1_G01
W1_G02
W1_G03
W1_G05
W1_G07
W1_G09
W1_G10
W1_G11
W1_G13
W1_G14
W4_G02
W4_G03
W4_G05
W4_G07
W4_G09
W4_G10
W4_G11
W4_G13
W4_G14
W5_G01
W5_G02
W5_G03
W5_G10
W8_G01
W8_G02
W8_G03
W8_G05
W8_G07
W8_G09
W8_G10
W8_G11
W8_G13
W8_G14
Week-7 - W8
Week-7 - W4
Week-7- W1
W1 - W8
W4 - W8
Week-7
Week1
Week4
Week5
Week8
Parabacteroides
Bacteroides
Alistipes
Barnesiella
Prevotella
Phascolarctobacterium
Pseudoflavonifractor
Flavonifractor
Akkermansia
Oscillibacter
Clostridium XI
Marvinbryantia
Desulfovibrio
Butyricimonas

Figure S2a.

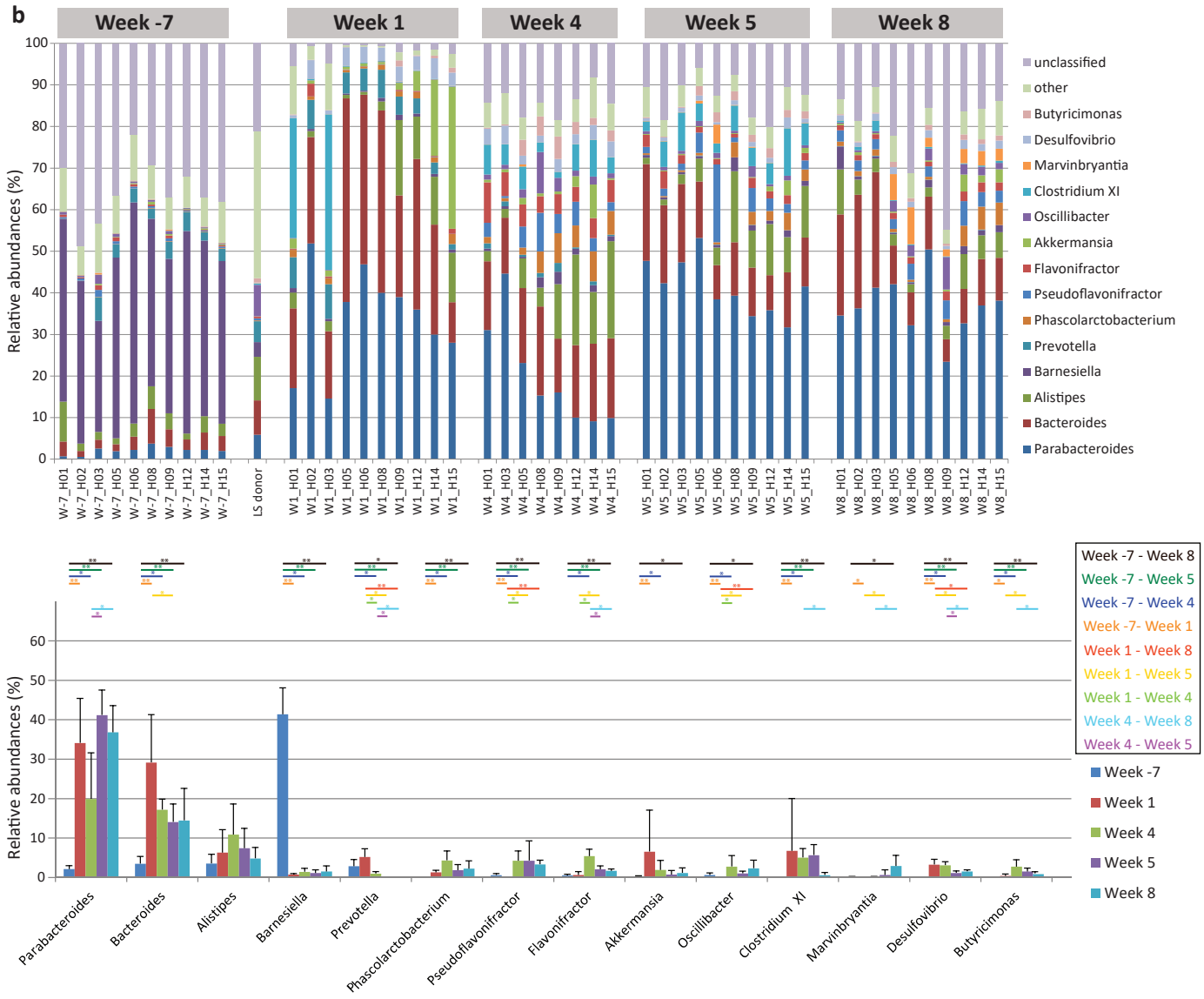


Figure S2. Gut microbiota composition at the genus level of conventional mice inoculated with a community microbiota (**a**) or with a long-stay microbiota (**b**), grouped by time points. *P*-values were calculated using Wilcoxon signed-rank tests and were corrected for the multiple comparison using the Benjamini–Hochberg adjustment, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Abbreviations: CM donor, healthy community subject; LS donor, frail long-stay subject; and Week -7 (week minus 7), murine microbiota before antibiotic treatment.

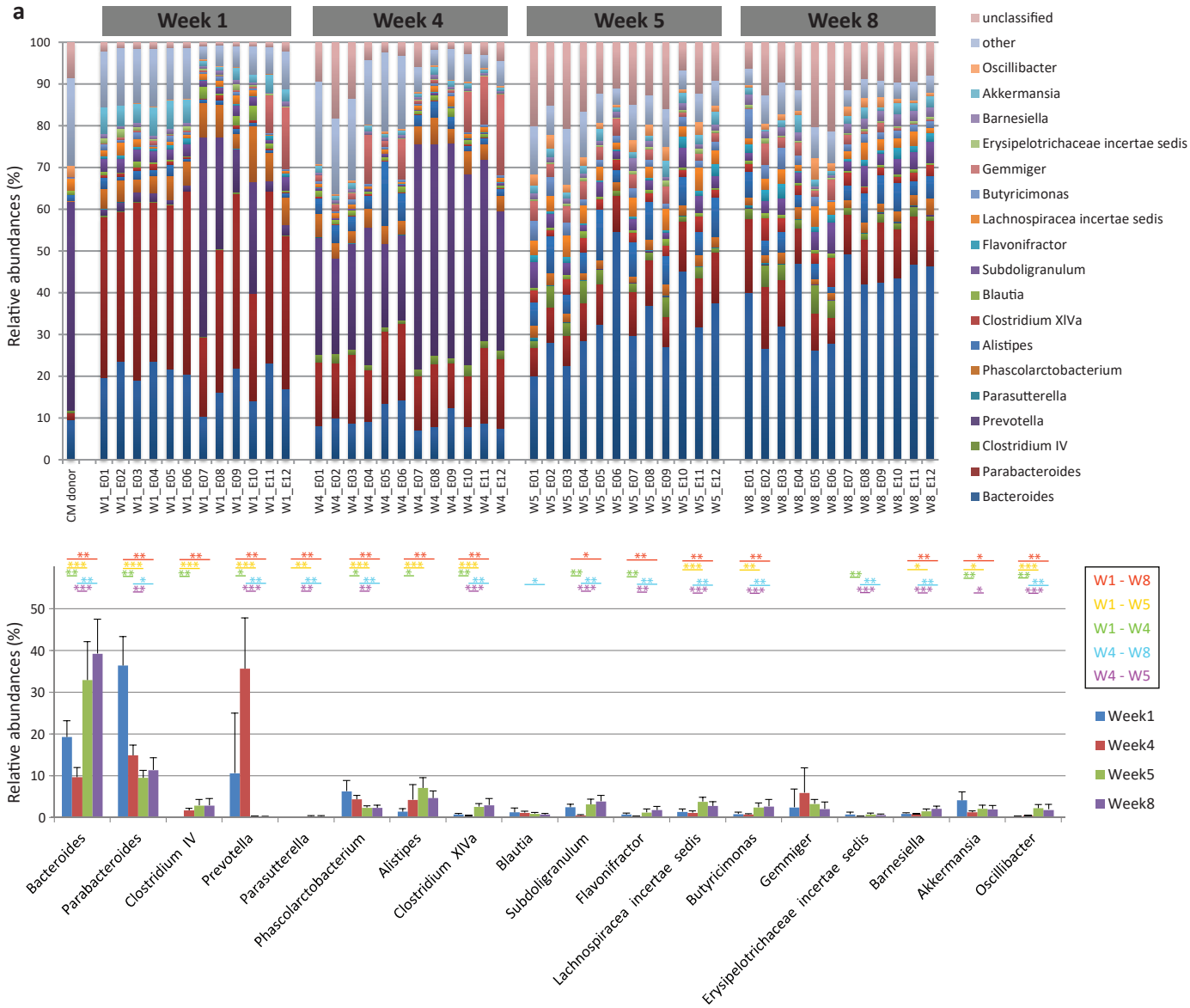

a
Week 1
Week 4
Week 5
Week 8
Relative abundances (%)
unclassified
other
Oscillibacter
Akkermansia
Barnesiella
Erysipelotrichaceae incertae sedis
Gemmiger
Butyricimonas
Lachnospiracea incertae sedis
Flavonifractor
Subdoligranulum
Blautia
Clostridium XlVa
Alistipes
Phascolarctobacterium
Parasutterella
Prevotella
Clostridium IV
Parabacteroides
Bacteroides
CM donor
W1_E01
W1_E02
W1_E03
W1_E04
W1_E05
W1_E06
W1_E07
W1_E08
W1_E09
W1_E10
W1_E11
W1_E12
W4_E01
W4_E02
W4_E03
W4_E04
W4_E05
W4_E06
W4_E07
W4_E08
W4_E09
W4_E10
W4_E11
W4_E12
W5_E01
W5_E02
W5_E03
W5_E04
W5_E05
W5_E06
W5_E07
W5_E08
W5_E09
W5_E10
W5_E11
W5_E12
W8_E01
W8_E02
W8_E03
W8_E04
W8_E05
W8_E06
W8_E07
W8_E08
W8_E09
W8_E10
W8_E11
W8_E12
W1 - W8
W1 - W5
W1 - W4
W4 - W8
W4 - W5
Week1
Week4
Week5
Week8
Bacteroides
Parabacteroides
Clostridium IV
Prevotella
Parasutterella
Phascolarctobacterium
Alistipes
Clostridium XlVa
Blautia
Subdoligranulum
Flavonifractor
Lachnospiracea incertae sedis
Butyricimonas
Gemmiger
Erysipelotrichaceae incertae sedis
Barnesiella
Akkermansia
Oscillibacter

Figure S3a.

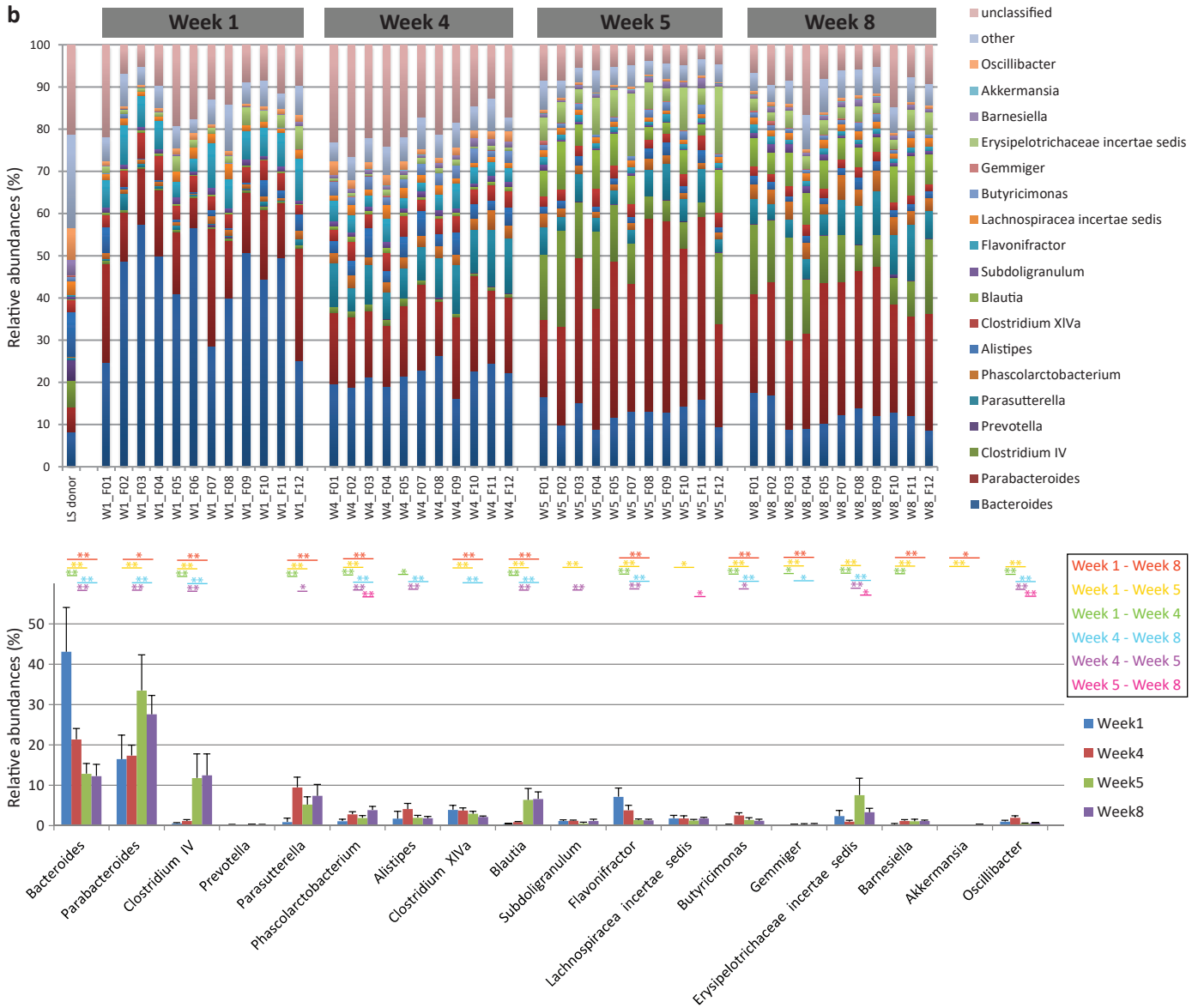


Figure S3. Gut microbiota composition at the genus level of germ-free mice inoculated with a community microbiota (**a**) or with a long-stay microbiota (**b**), grouped by time points. *P*-values were calculated using Wilcoxon signed-rank tests and were corrected for the multiple comparison using the Benjamini–Hochberg adjustment, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: CM donor, healthy community subject; LS donor, frail long-stay subject.

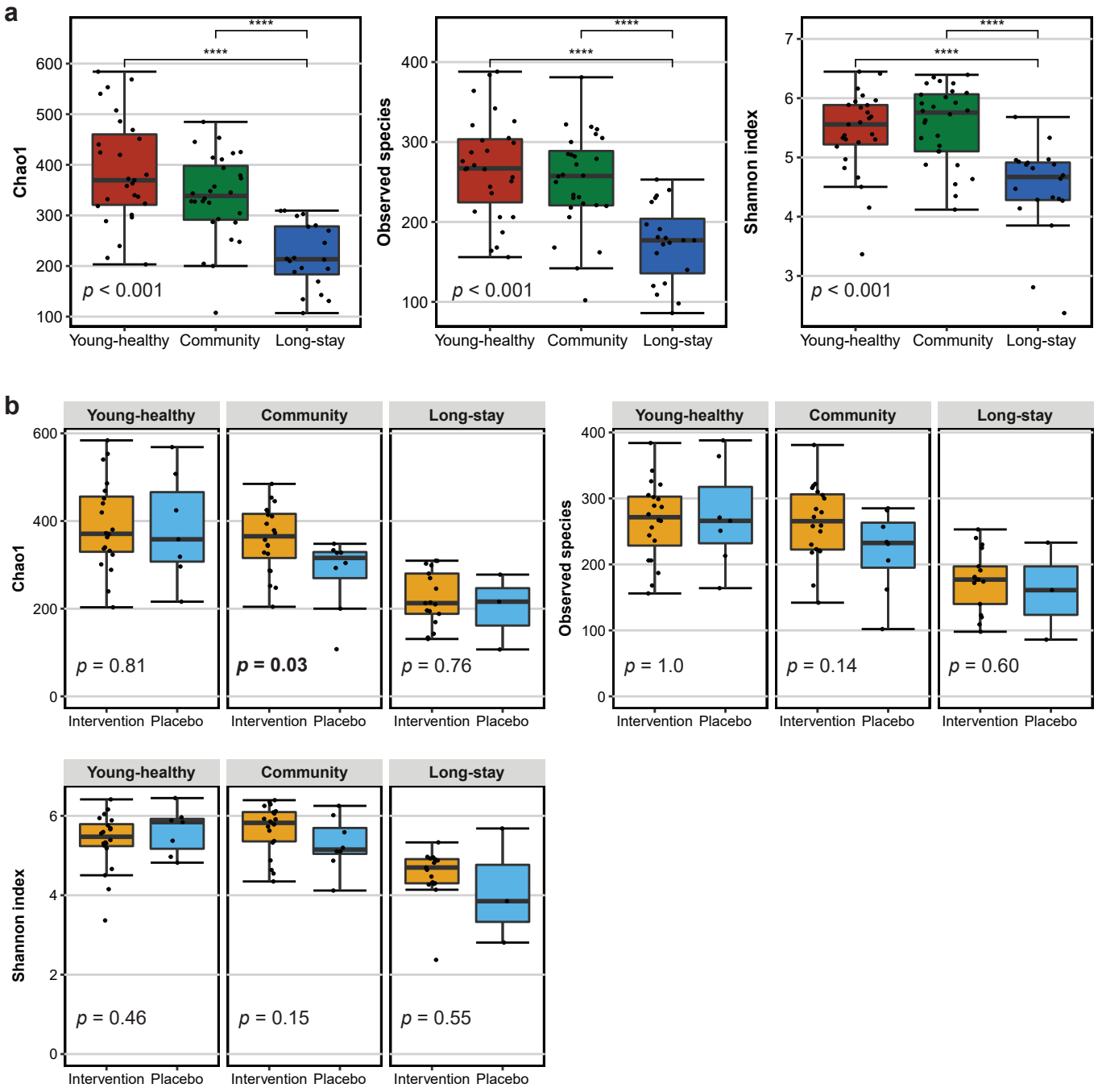


Figure S4. Alpha diversity comparisons of the human gut microbiome at baseline (T0), grouped by residence location (**a**) or residence location and treatment group (**b**). *P*-values were calculated using Kruskal-Wallis test for for three independent groups and Mann-Whitney U tests for unpaired groups, ***$p < 0.001$.

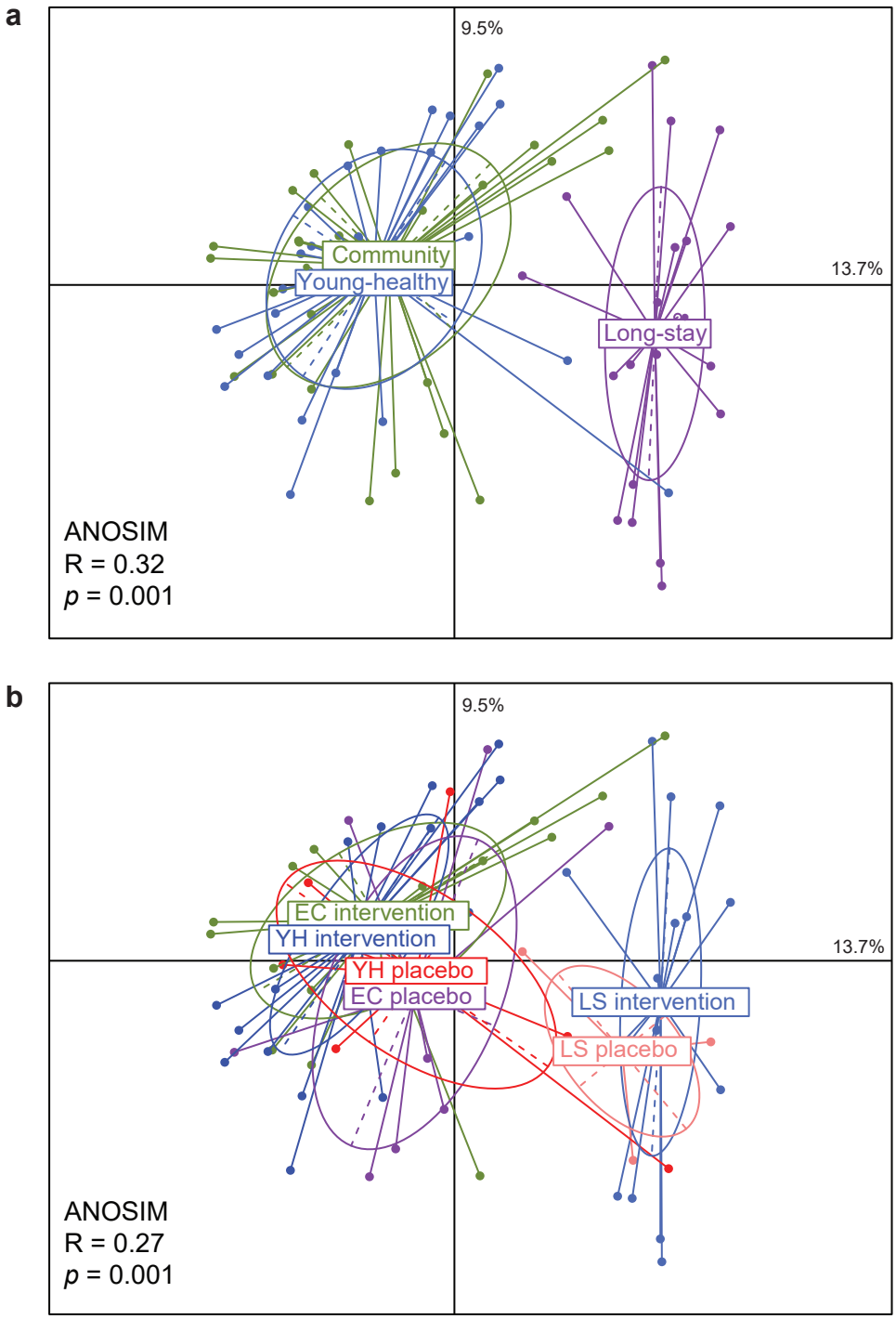


Figure S5. Beta diversity comparisons based on Bray-Curtis distances of the human gut microbiome at baseline (T0), grouped by residence location (**a**) or residence location and treatment group (**b**). The significant differences between groups were calculated by ANOSIM tests. Abbreviations: Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay.

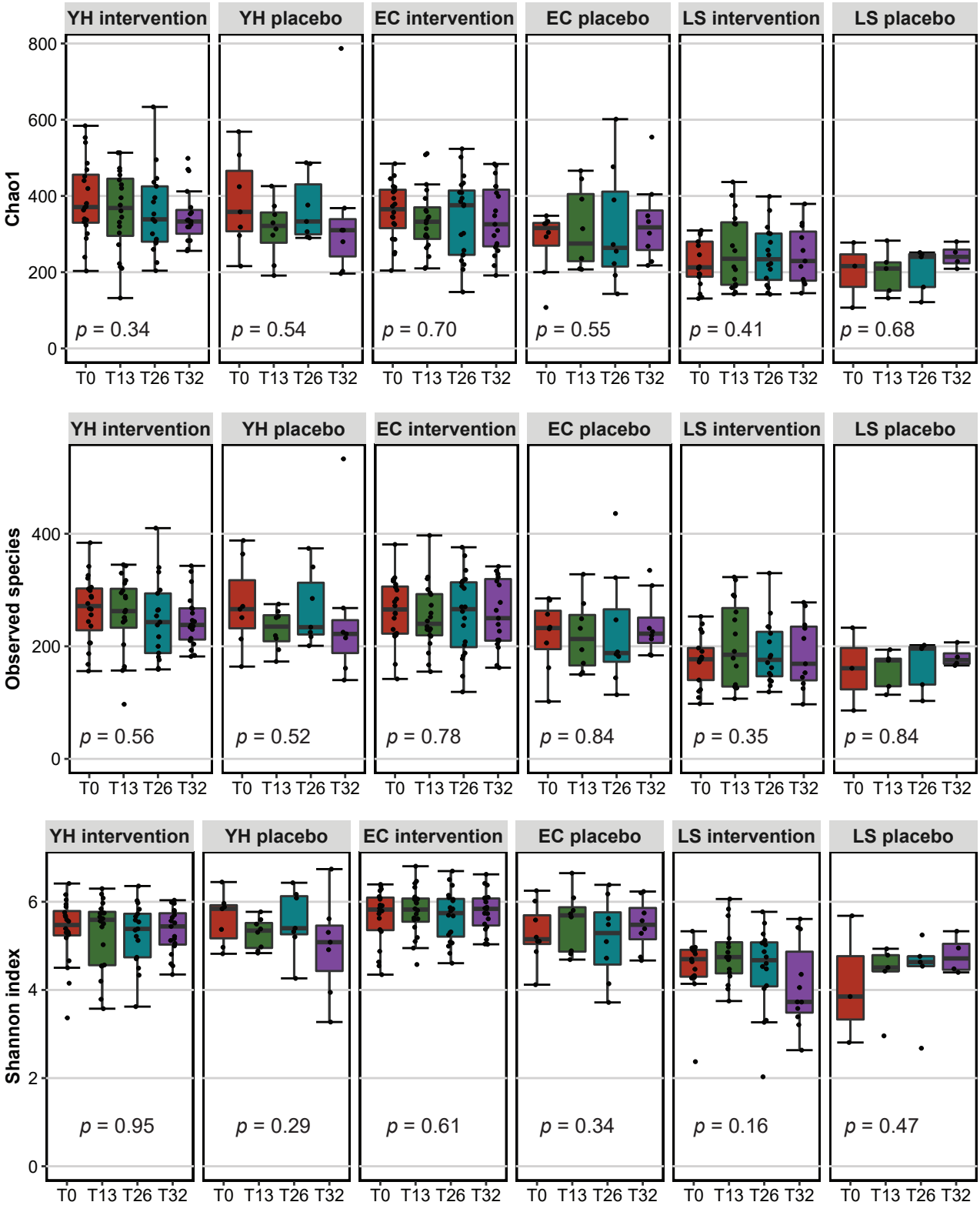


Figure S6. Boxplot of bacterial alpha-diversity indices for the 16S rRNA gene sequences between different time points in each treatment groups. *P*-values were calculated from linear mixed effects models. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay; T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).

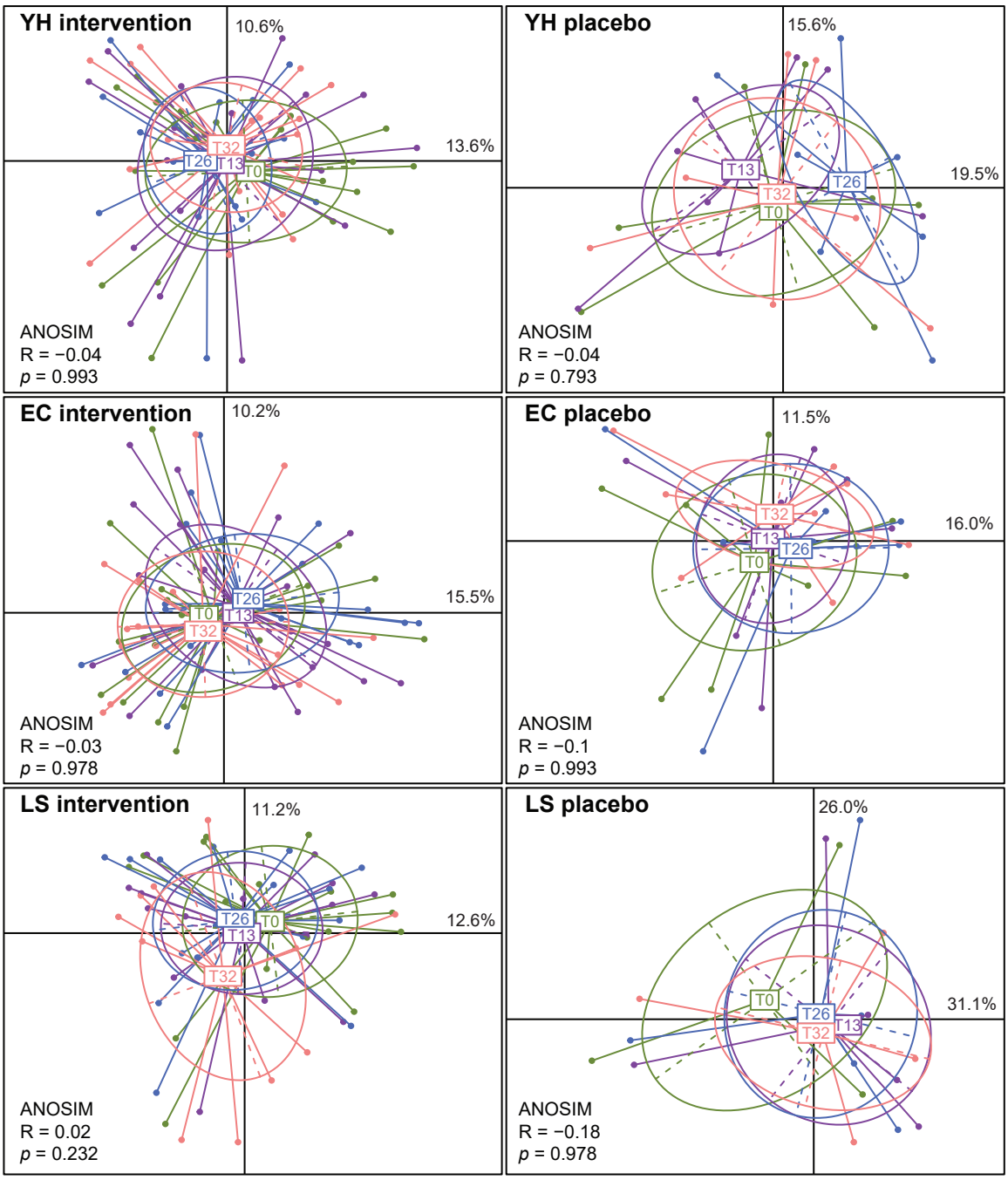


Figure S7. Principle Coordinates Analysis based on Bray-Curtis distances of partial sequences of bacterial 16S rRNA genes from human faecal samples of treatment groups, grouped by time points. The significant differences between groups were calculated by ANOSIM tests. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay; T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).

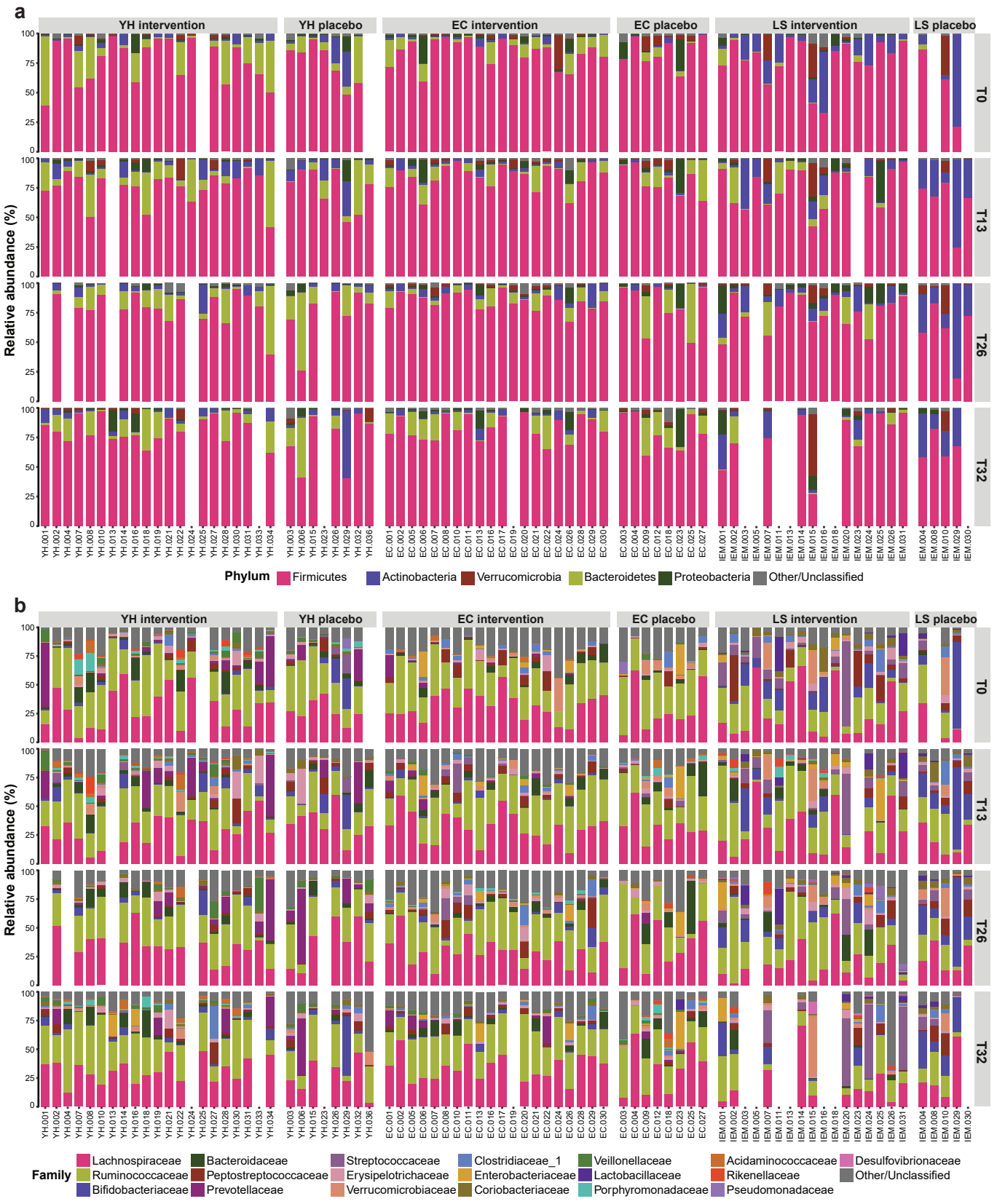


Figure S8. Relative abundance of faecal microbiota taxa at (**a**) phylum level, (**b**) family level in subjects, grouped according to treatment groups and time points. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay; T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).

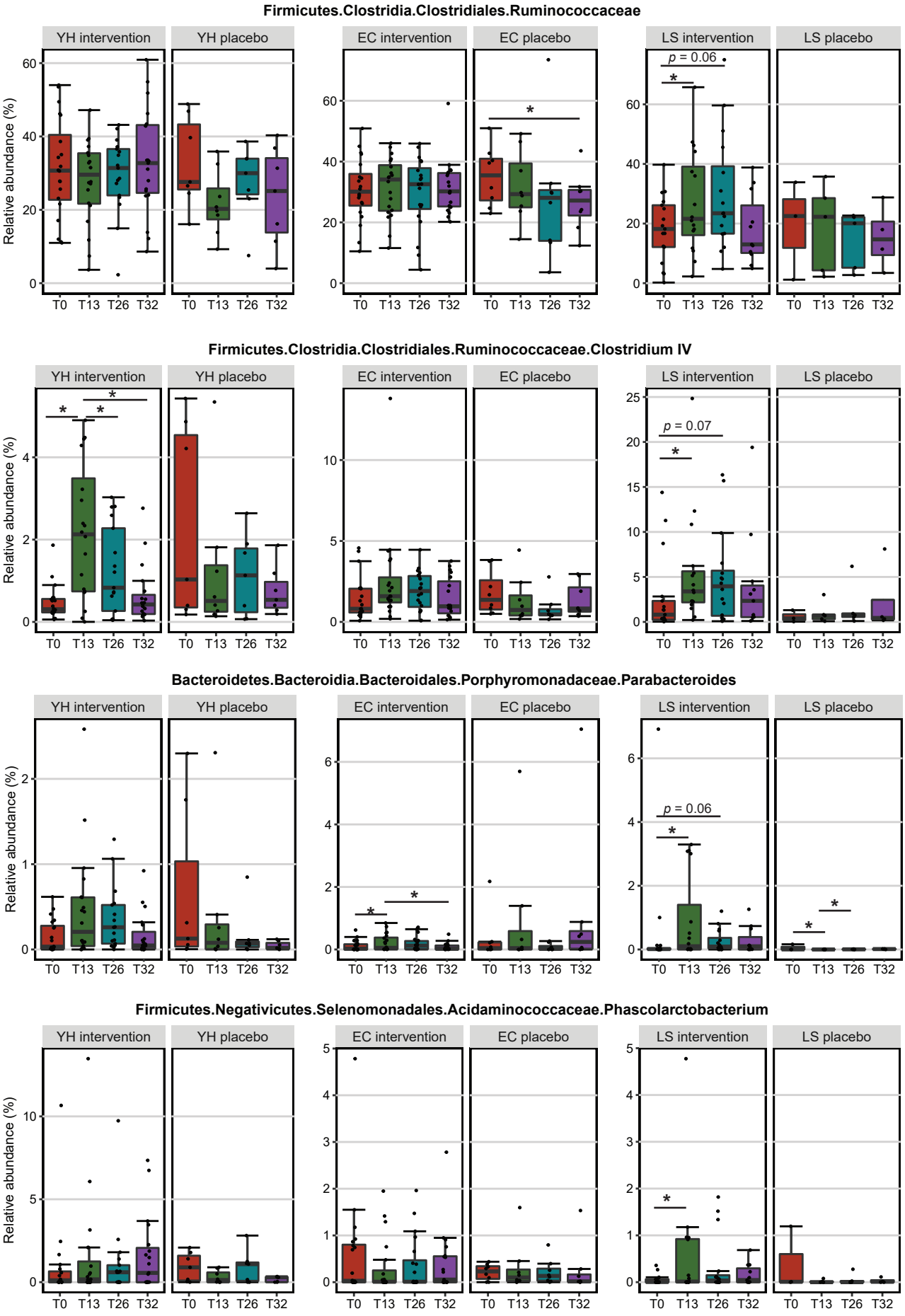
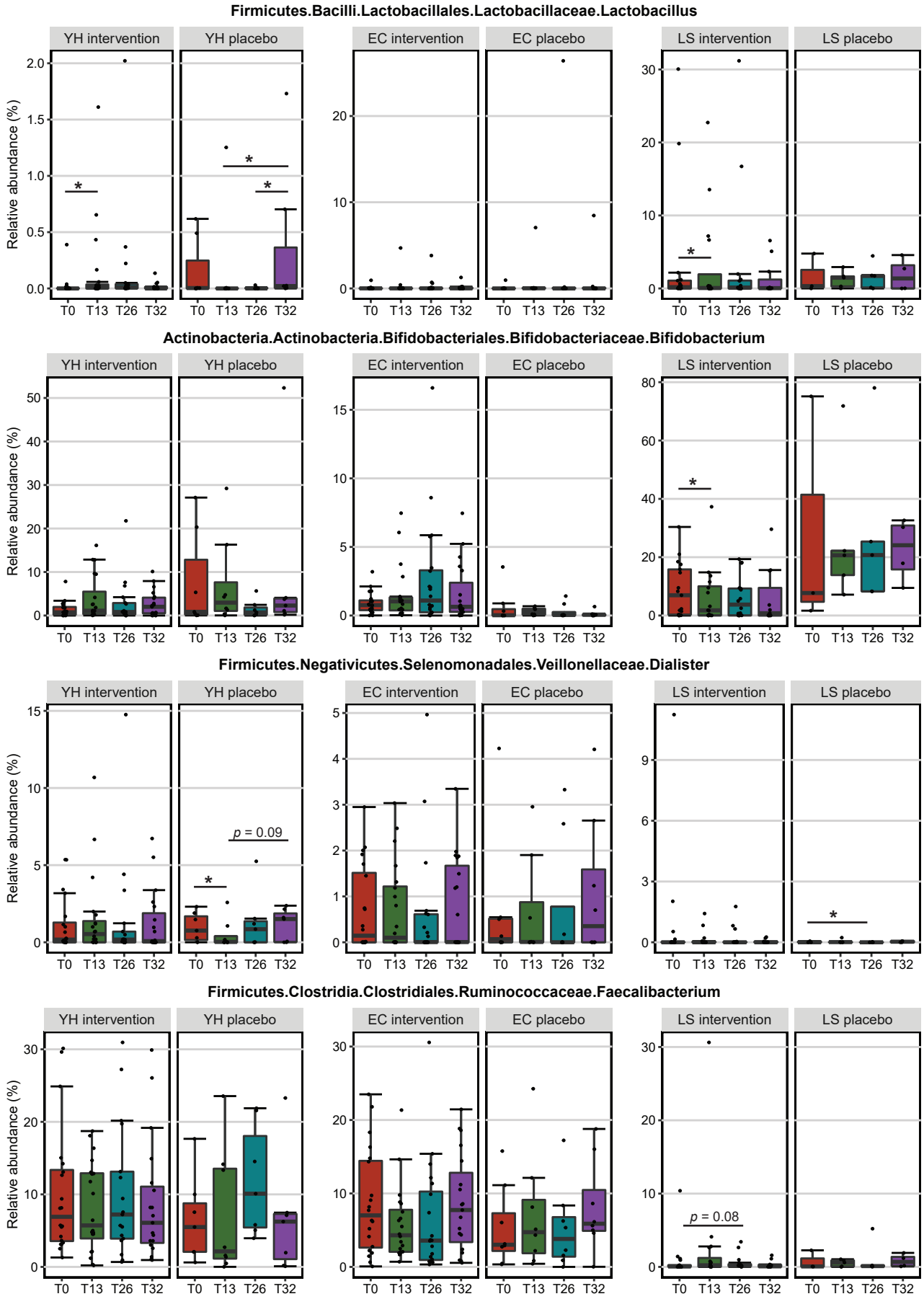

Firmicutes.Clostridia.Clostridiales.Ruminococcaceae
YH intervention
YH placebo
EC intervention
EC placebo
LS intervention
LS placebo
Relative abundance (%)
p = 0.06
T0 T13 T26 T32
Firmicutes.Clostridia.Clostridiales.Ruminococcaceae.Clostridium IV
p = 0.07
Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Parabacteroides
p = 0.06
Firmicutes.Negativicutes.Selenomonadales.Acidaminococcaceae.Phascolarctobacterium

Figure S9.



Supplementar **Figure S 9.** Box plot of relative abundances of the statistically significantly different taxa after treatment. Adjusted *p*-values were calculated by DESeq2 tests with Benjamini–Hochberg correction, *$p < 0.05$. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay; T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).

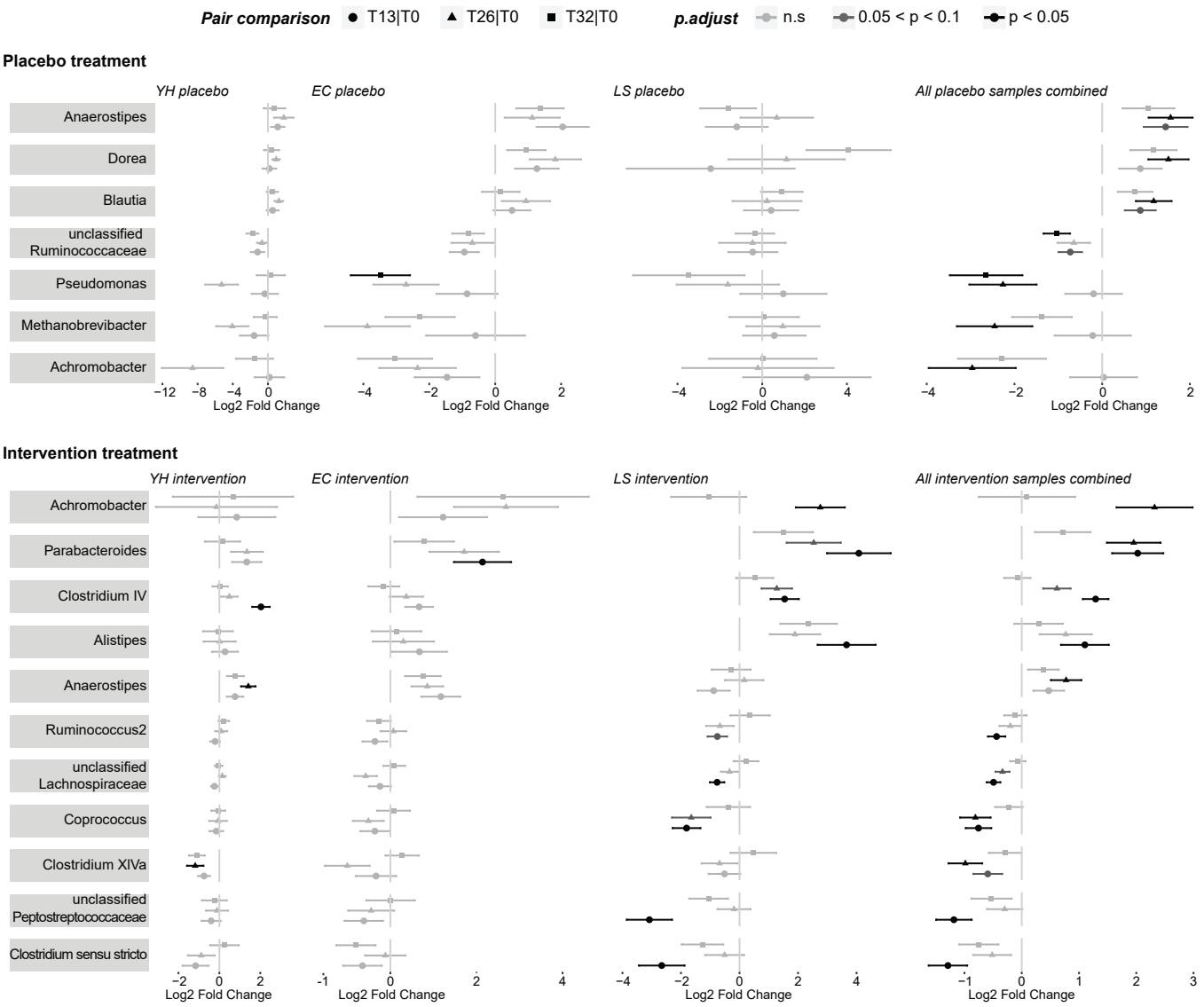


Figure S10. Genera that were significantly increased (log2 fold change > 0) or decrease (log2 fold change < 0) in the placebo/prebiotic intervention to baseline at T0 across all available intervention or placebo samples, compared with the results from the individual cohorts. Adjusted *p*-values were calculated by DESeq2 test with Benjamini–Hochberg correction. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay. T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).

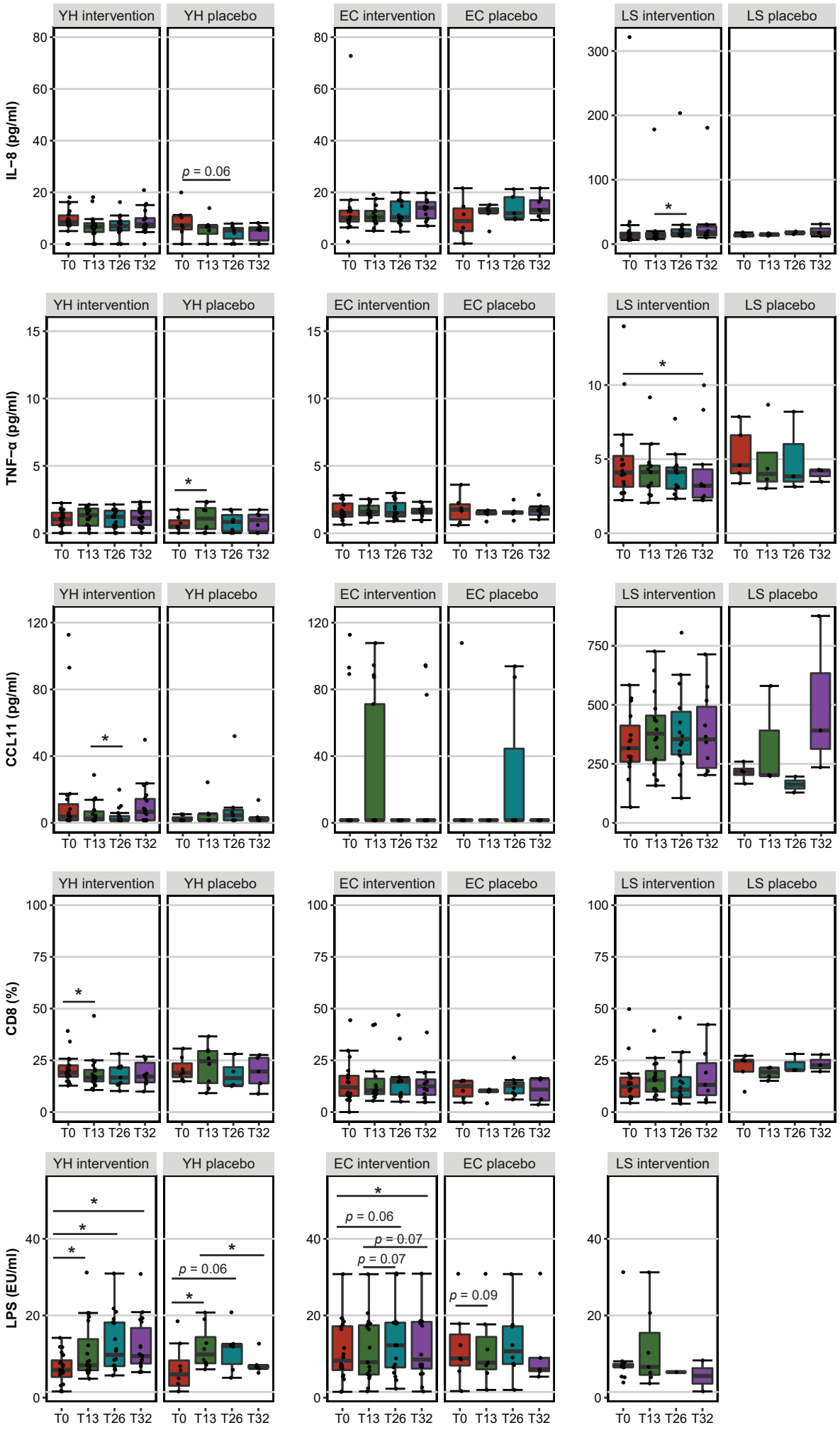

YH intervention
YH placebo
EC intervention
EC placebo
LS intervention
LS placebo
IL−8 (pg/ml)
TNF−α (pg/ml)
CCL11 (pg/ml)
CD8 (%)
LPS (EU/ml)
T0 T13 T26 T32
p = 0.06
p = 0.07
p = 0.09
*

Figure S11.

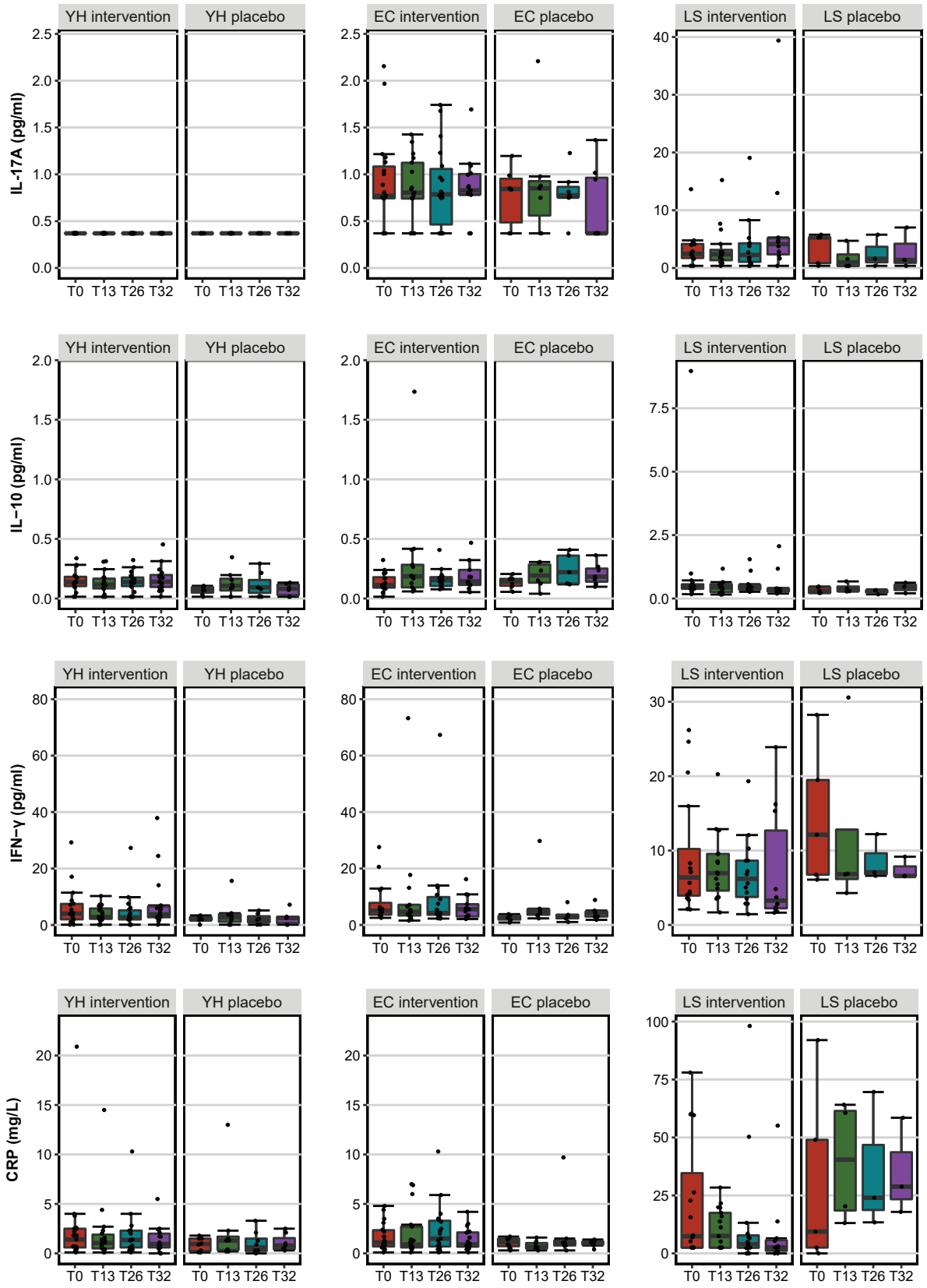


Figure S11. Immunological/inflammatory profiling between time points within treatment groups. *P*-values were calculated from Wilcoxon signed-rank tests, $*p < 0.05$. Abbreviations: YH, Young-healthy; CM, Community; LS, Long-stay; T0, baseline (week 0); T13, halfway (week 13); T26, end of study (week 26); and T32, six weeks after the cessation of treatment (week 32).