Table S1. PERMANOVA and PERMDISP results for beta-diversity analysis of gut microbiota across the five experimental groups.

Ordination method	Distance metric	PERMANOVA R^2	PERMANOVA P-value	PERMDISP P-value
PCoA	Bray–Curtis	0.699	0.001	0.091
PCA	Euclidean	0.686	0.001	0.803

Note. PERMANOVA (permutational multivariate analysis of variance) tests whether centroids differ between groups; PERMDISP (multivariate homogeneity of groups dispersions) tests whether within-group dispersion differs between groups. Significant PERMANOVA but non-significant PERMDISP indicates that observed group separation is driven by true differences in community composition rather than unequal dispersion. PCoA, principal coordinates analysis; PCA, principal component analysis.

Table S2. Detailed PERMDISP analysis for homogeneity of multivariate dispersions (Bray–Curtis distance, PCoA).

Source	Df (between, within)	F value	Pr (>F)
Treatment group	4, 25	3.21	0.091

Note. PERMDISP was performed on Bray–Curtis distances with 999 permutations. The non-significant result (P = 0.091) confirms that the significant PERMANOVA result is not an artifact of unequal within-group dispersion across the five groups. Df, degrees of freedom.

Table S3. Sensitivity analyses for cage-level confounding: ordinary ANOVA versus linear mixed-effects models with cage as a random intercept.

Outcome	n/group	ANOVA p (unadjusted)	lmer p (cage-adjusted)	Cage variance	ICC	Control vs OVA	OVA vs PP0208	OVA vs HK-PP0208	OVA vs EV
Nose rubbing (count)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001
Sneezing (count)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001
Spleen index (mg/10 g BW)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001
Serum IL-4 (pg/mL)	12	<0.001	<0.001	1.1×10^{-8}	≈ 0	<0.001	<0.001	<0.001	<0.001
Serum IL-10 (pg/mL)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001
Serum IFN-γ (pg/mL)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001
Serum total IgE (μg/mL)	12	<0.001	≈ 0 (singular)	—	—	0.003	0.002	<0.001	0.014
Splenic $CD4^+CD25^+FOXP3^+$ Treg (%)	12	<0.001	≈ 0 (singular)	—	—	<0.001	<0.001	<0.001	<0.001

Note. For each outcome, ordinary one-way ANOVA across the five treatment groups is shown alongside the minimum fixed-effect p-value from a linear mixed-effects model fitted with treatment group as a fixed effect and home cage as a random intercept (lme4::lmer in R). For all outcomes except IL-4, the estimated cage-level variance was at the boundary (zero), and the model was singular, indicating negligible within-cage correlation; ICC (intraclass correlation coefficient) was therefore not estimable. For IL-4, the cage variance was 1.1×10^{-8}, corresponding to an

ICC ≈ 0. In all cases, group-level significance after cage adjustment was identical to the unadjusted ANOVA, confirming that the reported between-group differences are not attributable to cage pseudoreplication. Pairwise p-values shown are two-sample t-tests between the indicated groups. HK-PP0208, heat-killed L. helveticus PP0208; EV, extracellular vesicles derived from L. helveticus PP0208.

Table S4. Annotation confidence and MS/MS spectral information for the eight key differential metabolites identified in untargeted fecal metabolomics.

Metabolite	Metabolite ID	Ionization mode	MSI annotation level	Fragmentation score	m/z	RT (min)
Propionic acid	metab_14796	neg	Level B(i)	47.6	72.0810	1.05
Spermidine	metab_13029	pos	Level B(i)	91.3	146.1649	0.44
Urocanic acid	metab_6125	pos	Level B(i)	99.0	130.0497	0.65
L-DOPA	metab_9523	pos	Level B(i)	86.0	178.0507	0.86
Farnesyl acetone	metab_4123	pos	Level B(i)	61.4	267.1974	0.39
Lpe(O-18:0)	metab_23526	neg	Level B(i)	37.4	451.2185	—
Lpe(O-20:0)	metab_23235	neg	Level B(i)	85.5	481.2785	—
PeO-18:0/0:0	metab_58462	pos	Level B(i)	45.6	468.4397	—

Note. All eight metabolites were annotated at MSI level 2 (putative identification) based on spectral library matching against KEGG and HMDB, with exact mass tolerance ±5 ppm and retention-time matching. Fragmentation scores range from 37.4 to 99.0 (higher scores indicate stronger spectral match). Ionization modes: neg, negative mode; pos, positive mode. RT, retention time; —, not detected/eluted within the analytical gradient. Lpg, lysophosphatidylglycerol; Pe, phosphatidylethanolamine-related ether lipid.

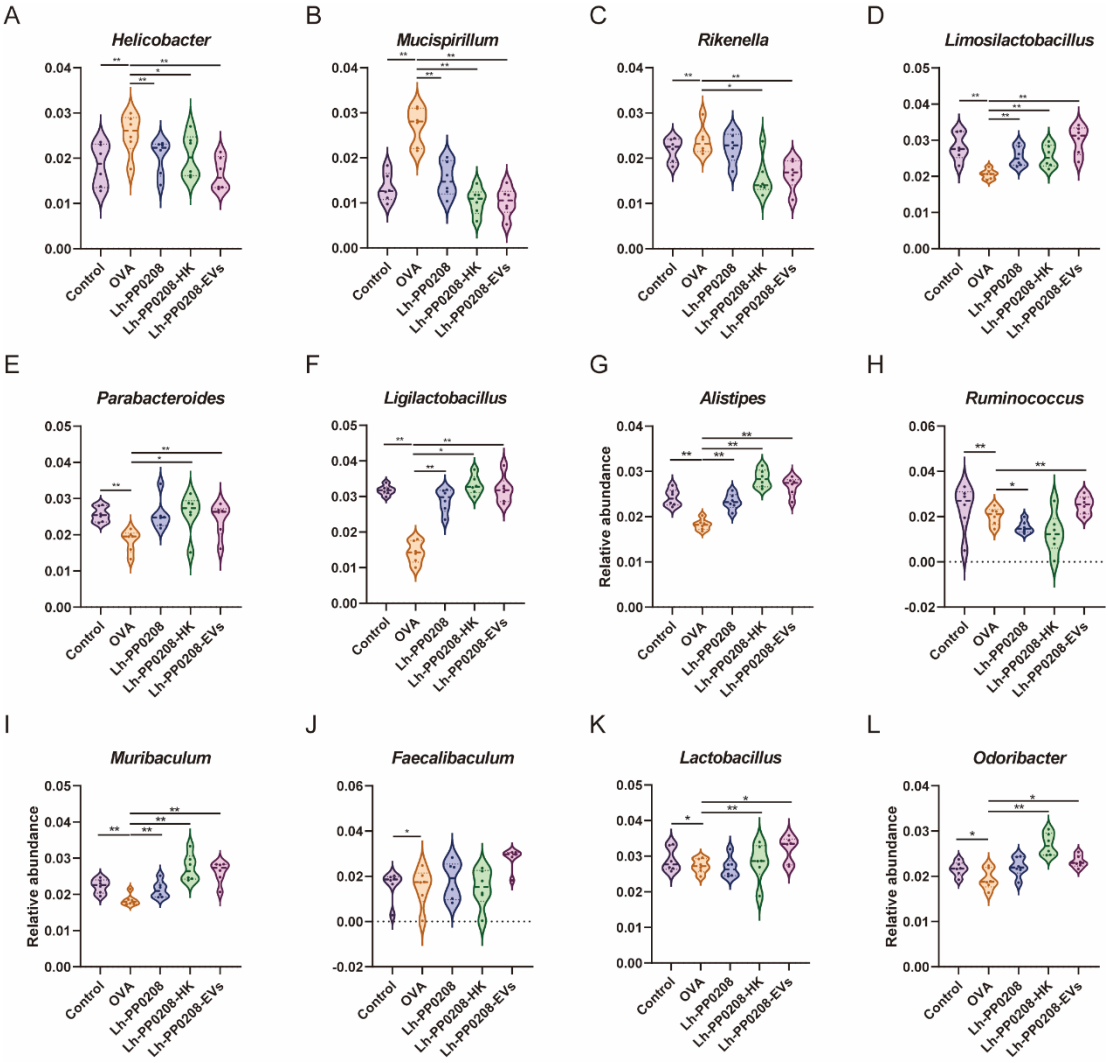

Figure S1. Violin plots showing quantitative comparisons of relative abundance for genera associated with allergic disease-related parameters (spleen index, behavioral symptoms, serum cytokines and IgE).

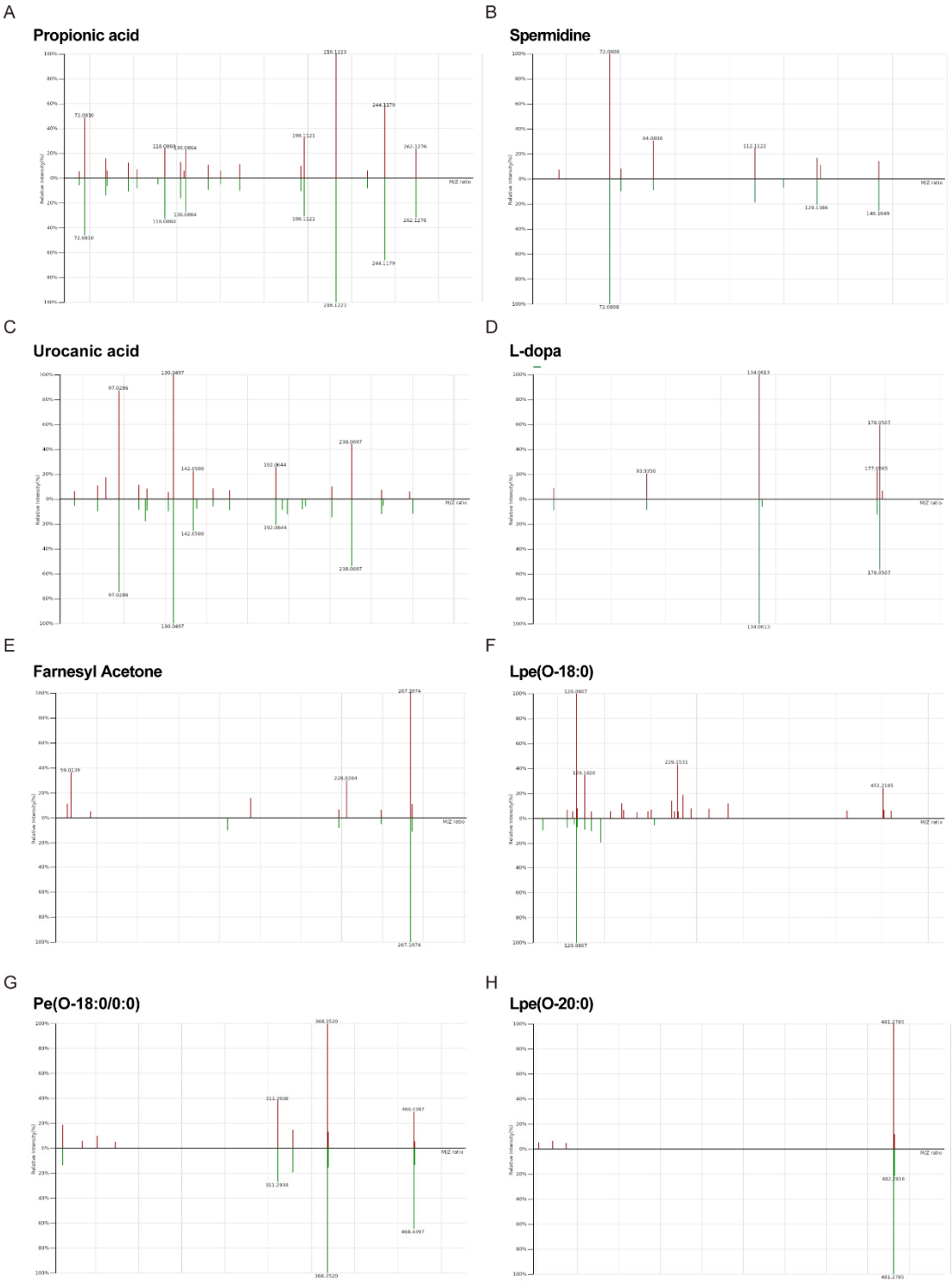

Figure S2. Representative MS/MS spectra of the eight key differential metabolites identified in untargeted fecal metabolomics.

Experimental MS/MS spectra (upward peaks) are compared against spectral library references (downward peaks) from KEGG and HMDB. Precursor ions and major fragment ions are annotated. (A) Propionic acid (m/z 73.0810, negative mode); (B) Spermidine (m/z 146.1649, positive mode); (C) Urocanic acid (m/z 139.0498, positive mode); (D) L-DOPA (m/z 130.0497, positive mode); (E) Farnesyl acetone (m/z 267.1974, positive mode); (F) Lpe(O-18:0) (m/z 451.2185, negative mode); (G) Pe(O-18:0/0:0) (m/z 468.4397, positive mode); (H) Lpe(O-20:0) (m/z 481.2785, negative mode). All metabolites were annotated at MSI level 2 with fragmentation scores ranging from 37.4 to 99.0 (see **Table S4** for details).