

Probiotic interventions promote metabolic health in high fat-fed hamsters in association with gut microbiota and endocannabinoidome alterations

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Table S1. Proportion of variance explained (R^2) by group-wise PERMANOVA tests using Bray-Curtis beta diversity distances of gut microbiota composition of the ileum and caecum.¹

Ileum	LFLC-Milk	HFHC-Milk	HFHC-La	HFHC-Lp
HFHC-Milk	0.19 *	—	—	—
HFHC-La	0.14	0.18 *	—	—
HFHC-Lp	0.21 **	0.09	0.16 **	—
HFHC-LaLp	0.14 **	0.17 *	0.07	0.17 **
Caecum	LFLC-Milk	HFHC-Milk	HFHC-La	HFHC-Lp
HFHC-Milk	0.16 *	—	—	—
HFHC-La	0.18 *	0.14 *	—	—
HFHC-Lp	0.17 *	0.07	0.17 *	—
HFHC-LaLp	0.21 **	0.18 **	0.08	0.2 **

¹ Benjamini and Hochberg FDR-corrected P -values; * $P < 0.05$; ** $P < 0.01$.

Table S2. Bacterial genera significantly modified by probiotic interventions.¹

	KW	LFLC-Milk HFHC-Milk	vsHFHC-La HFHC-Milk	vsHFHC-Lp HFHC-Milk	vsHFHC-LaLp HFHC-Milk	vs
Ileum						
<i>Pseudoramibacter</i>	<0.001	0.002	0.02	–	0.02	
<i>Lactococcus</i>	<0.001	0.01	–	–	–	
<i>Megasphaera</i>	0.005	–	–	0.09	–	
<i>Lactobacillus</i>	0.005	–	0.04	–	0.004	
<i>Mucispirillum</i>	0.03	0.03	–	0.1	–	
<i>Peptostreptococcus</i>	0.04	0.05	–	–	–	
<i>Streptococcus</i>	0.04	0.08	–	–	–	
<i>Hungatella</i>	0.06	0.003	0.04	0.01	0.003	
<i>Terrisporobacter</i>	0.07	0.01	0.06	0.07	0.002	
<i>Blautia</i>	0.08	0.003	0.05	0.03	0.003	
<i>Allobaculum</i>	0.08	–	0.02	0.05	0.05	
<i>Helicobacter</i>	0.08	–	–	–	–	
<i>Coprobacter</i>	0.1	–	0.08	–	0.02	
Caecum						
<i>Pseudoramibacter</i>	<0.001	–	0.04	0.05	0.09	
<i>Clostridium_IV</i>	<0.001	–	<0.001	0.11	–	
<i>Desulfobaculum</i>	0.002	0.06	0.06	0.02	–	
<i>Clostridium_XIVb</i>	0.006	0.08	0.03	0.03	0.08	
<i>Romboutsia</i>	0.006	0.003	<0.001	<0.001	0.003	
<i>Parabacteroides</i>	0.01	<0.001	0.06	<0.001	–	
<i>Coprobacter</i>	0.01	–	0.01	0.01	–	
<i>Turicibacter</i>	0.02	–	0.01	0.01	0.01	
<i>Helicobacter</i>	0.04	–	0.09	–	–	
<i>Acetatifactor</i>	0.04	0.03	0.08	0.01	–	
<i>Bifidobacterium</i>	0.05	0.04	0.06	0.02	0.01	
<i>Gemmiger</i>	0.06	–	0.03	0.001	–	
<i>Hungatella</i>	0.06	0.10	0.01	0.004	0.02	
<i>Eubacterium</i>	0.06	–	–	0.09	–	
<i>Blautia</i>	0.08	–	0.06	0.03	0.01	
<i>Olsenella</i>	0.08	–	–	–	0.05	
<i>Clostridium_s.s.</i>	0.09	0.05	0.02	0.002	0.05	
<i>Allobaculum</i>	0.1					

¹ Only taxa with Kruskal-Wallis $P \leq 0.1$ (KW) are included in the table; – : not significant.

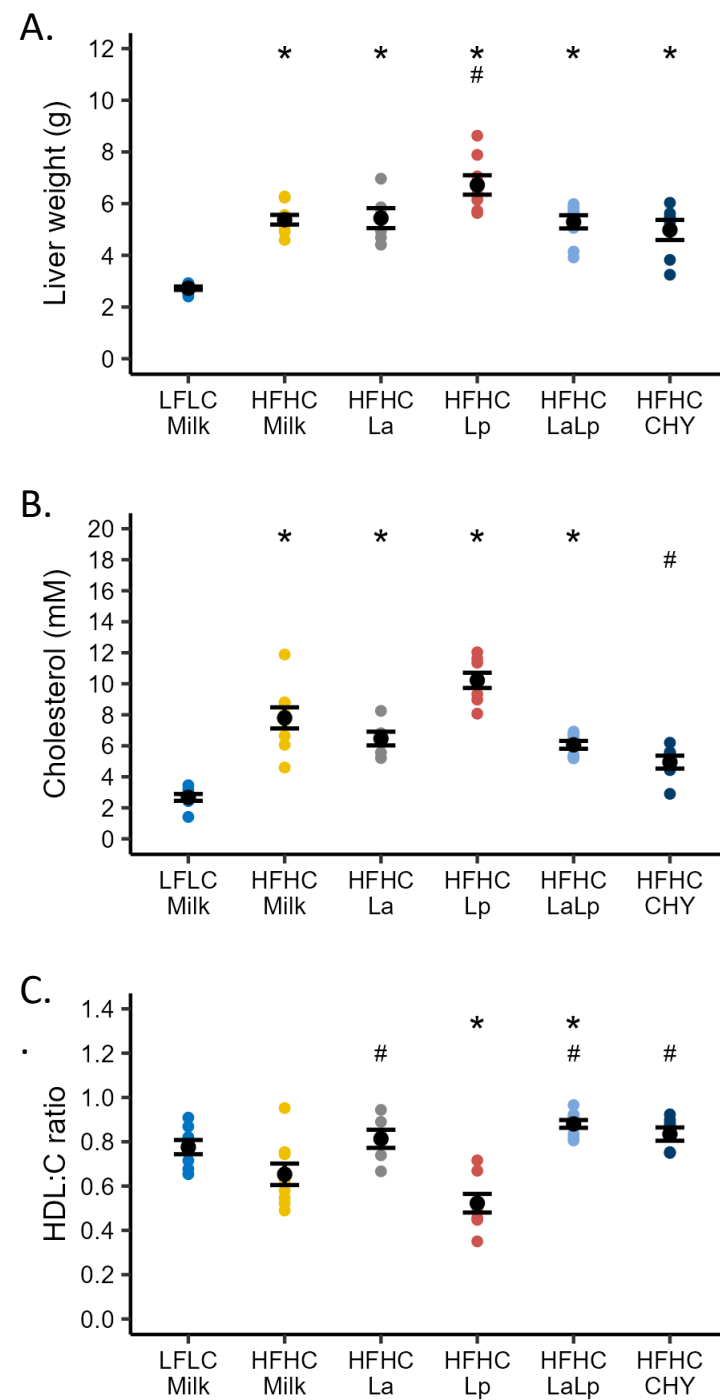


Figure S1. Probiotic interventions improve metabolic parameters in hypercholesterolemic and hypertriglyceridemic hamsters. (A) Liver weight, (B) non-HDL cholesterol and (C) HDL cholesterol to total cholesterol ratio at Week 6 are illustrated with mean $\pm$ SEM. All groups: $n=9$ except for HFHC CHY: $n=7$. One-way ANOVA and Tukey post-hoc. * $P<0.05$ vs compared to LFLC Milk; # $P<0.05$ vs HFHC Milk.

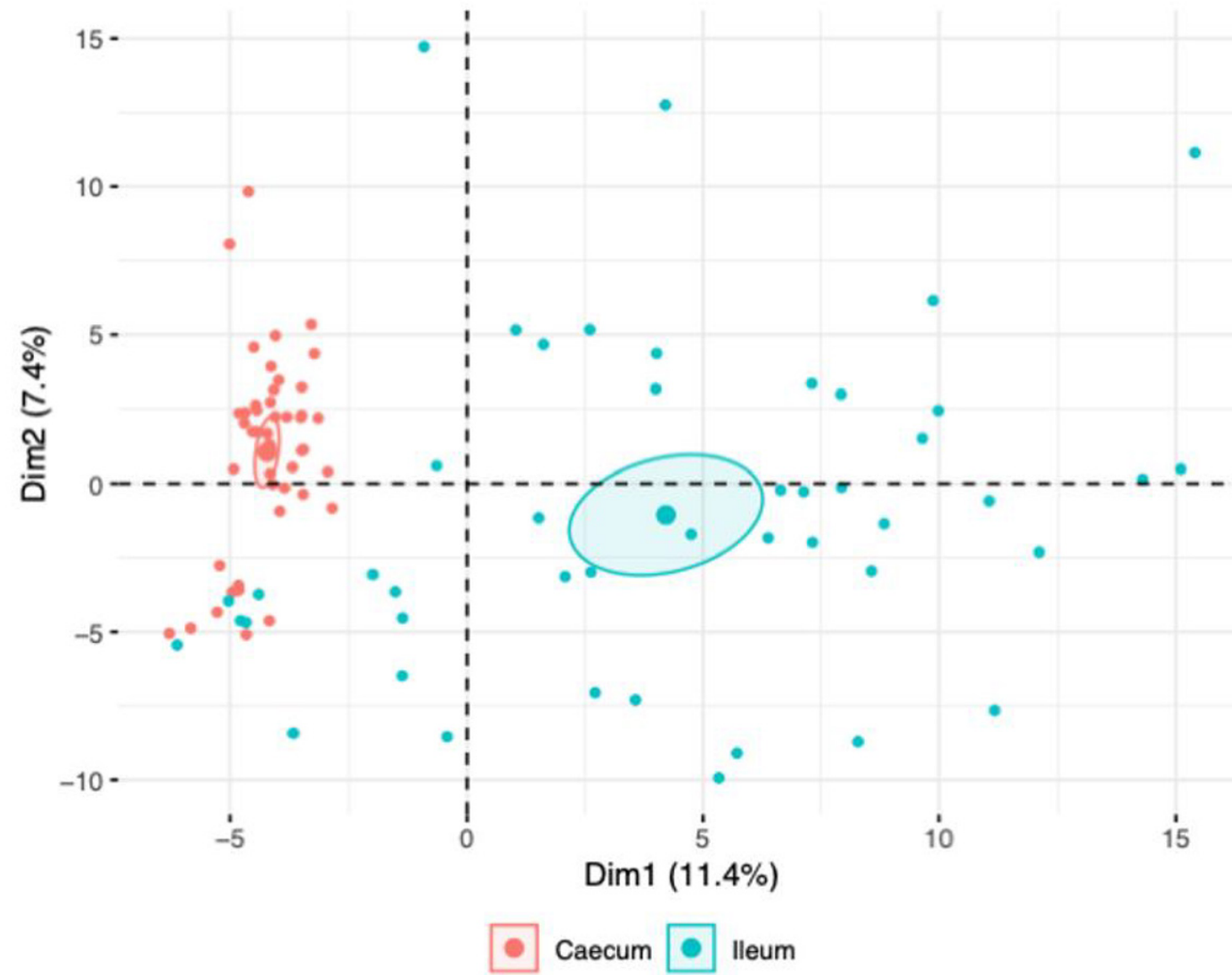


Figure S2. Principal component analysis (PCA) of microbiota composition in the ileum and caecum. Data are shown with confidence interval ellipses (95%).

Figure S3. Relative abundance plots of gut bacterial families in the ileum (top) and caecum (down) for each intervention group. Relative abundances of gut bacterial families in each individual according to intervention groups. Only bacterial families with a mean relative abundance greater than 1% were included. For ileum LFLC-Milk: n=7, HFHC-Milk: n=9, HFHC-La: n=6, HFHC-Lp: n=8, HFHC-LaLp: n=8) and for caecum (LFLC-Milk: n=8, HFHC-Milk: n=8, HFHC-La: n=6, HFHC-Lp: n=8, HFHC-LaLp: n=8).

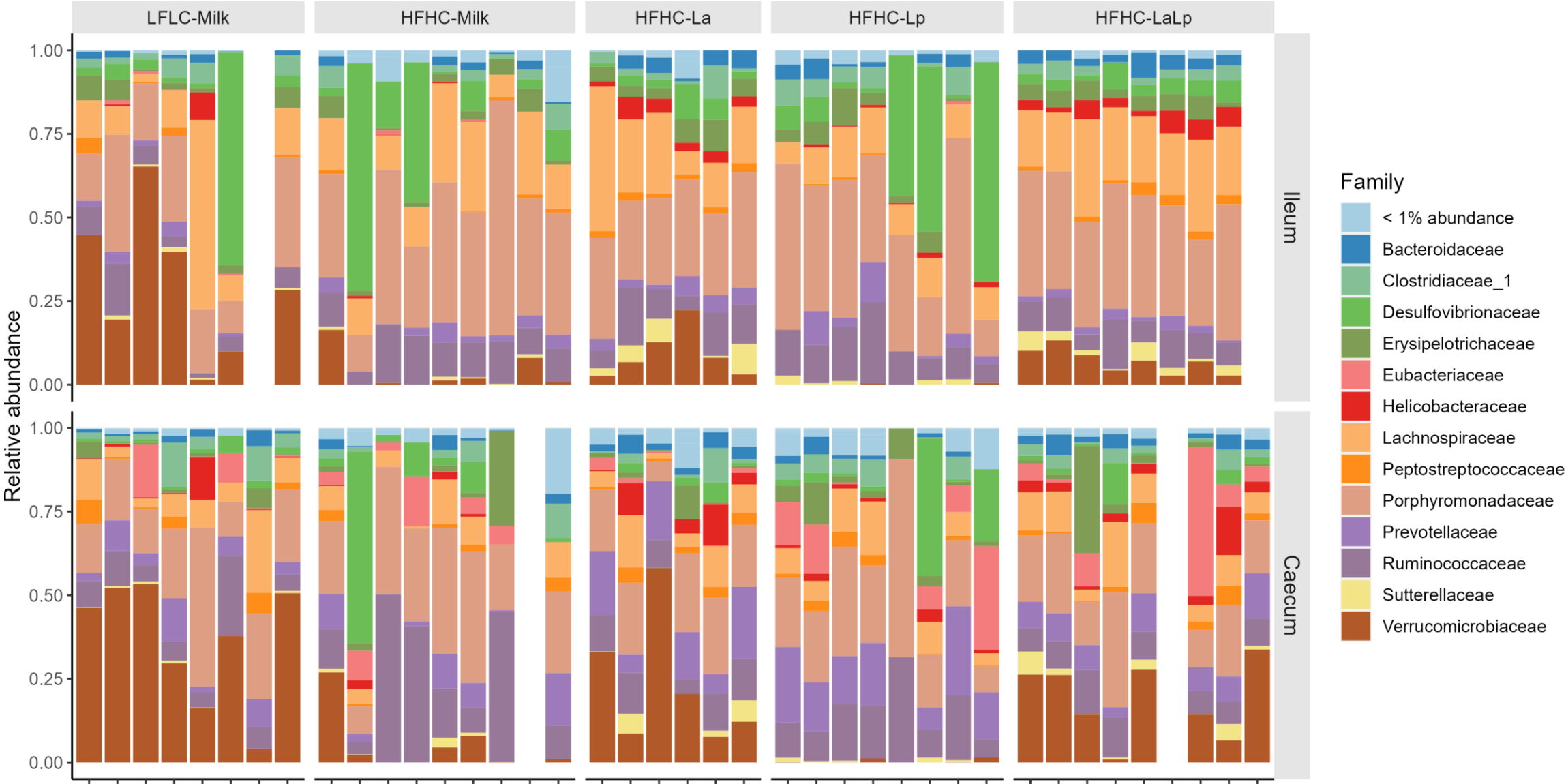
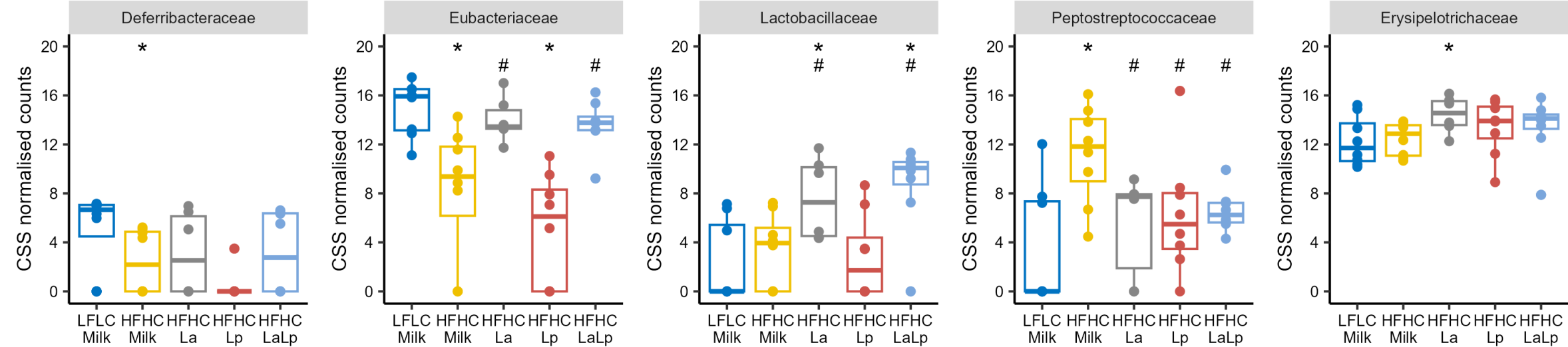


Figure S4. Diet and probiotic interventions modify the relative abundance of specific bacterial families in the microbiota of the ileum and the caecum. Relative abundances of selected gut bacterial families of interest in response to the interventions in the (A) ileum and the (B) caecum. Plots detail the log CSS-normalised bacterial counts for each intervention groups. Boxes extend from first to third quartile, horizontal lines indicate median, and whiskers extend to the maximal and minimal values within 1.5 times the interquartile range (IQR) from Q1 and Q3. For ileum LFLC-Milk: n=7, HFHC-Milk: n=9, HFHC-La: n=6, HFHC-Lp: n=8, HFHC-LaLp: n=8) and for caecum (LFLC-Milk: n=8, HFHC-Milk: n=8, HFHC-La: n=6, HFHC-Lp: n=8, HFHC-LaLp: n=8). Dunnet's post-hoc. * $P<0.05$ vs compared to LFLC Milk; # $P<0.05$ vs HFHC Milk.

A.



B.

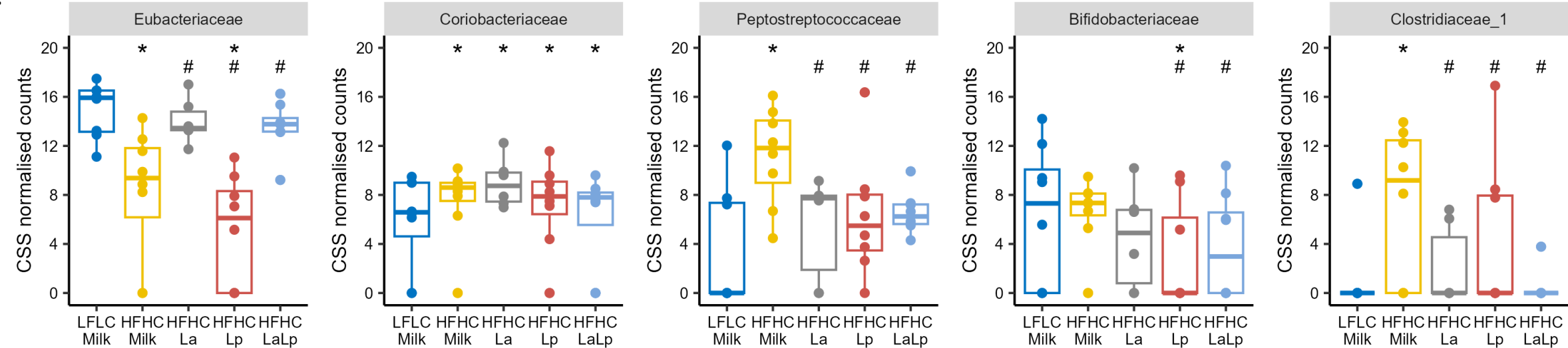
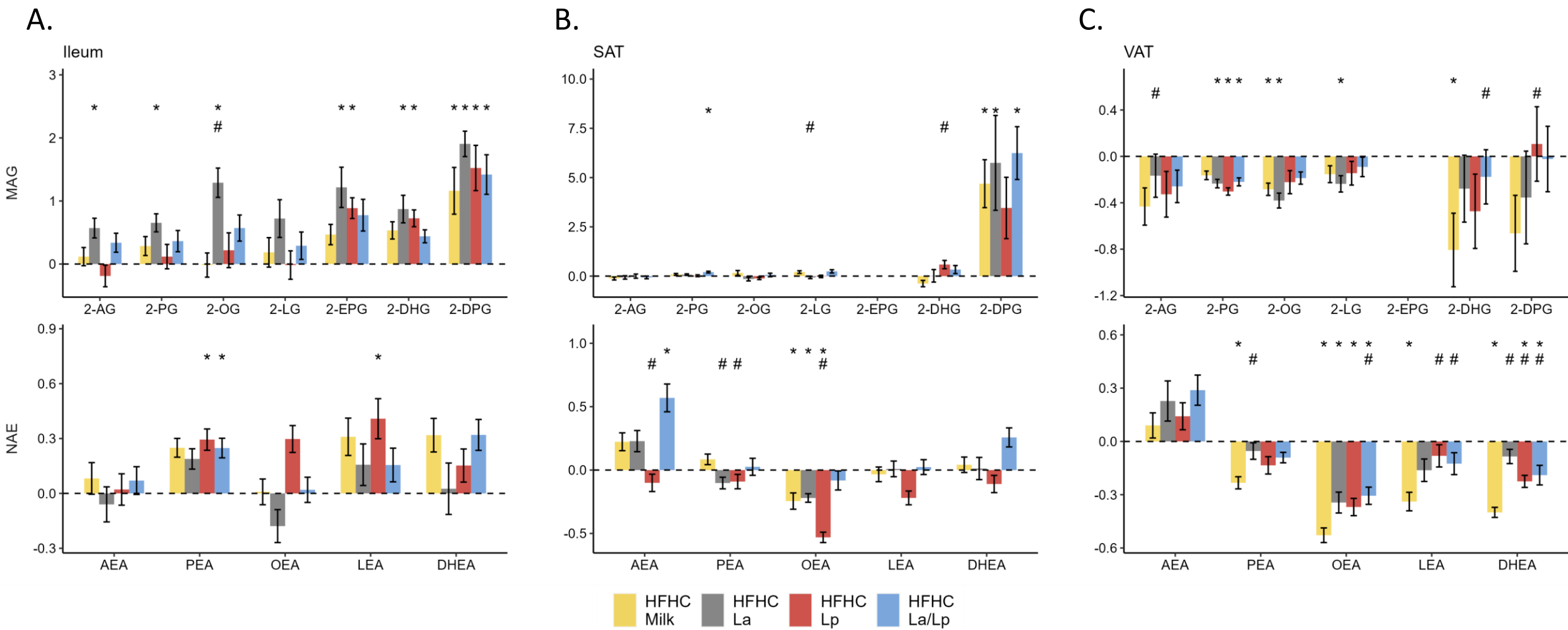


Figure S5. Probiotic interventions reverse diet-induced alterations in tissue levels of eCBome mediators. Relative changes in NAE and 2-MAG levels in (A) ileum, (B) inguinal adipose tissue (SAT), and (C) epididymal adipose tissue (VAT) compared to LFLC Milk hamsters. All groups: n=9 except for LFLC Milk and HFHC Lp: n=8, and for HFHC La: n=6. One-way ANOVA and Tukey post-hoc. * $P<0.05$ vs compared to LFLC Milk; # $P<0.05$ vs HFHC Milk.



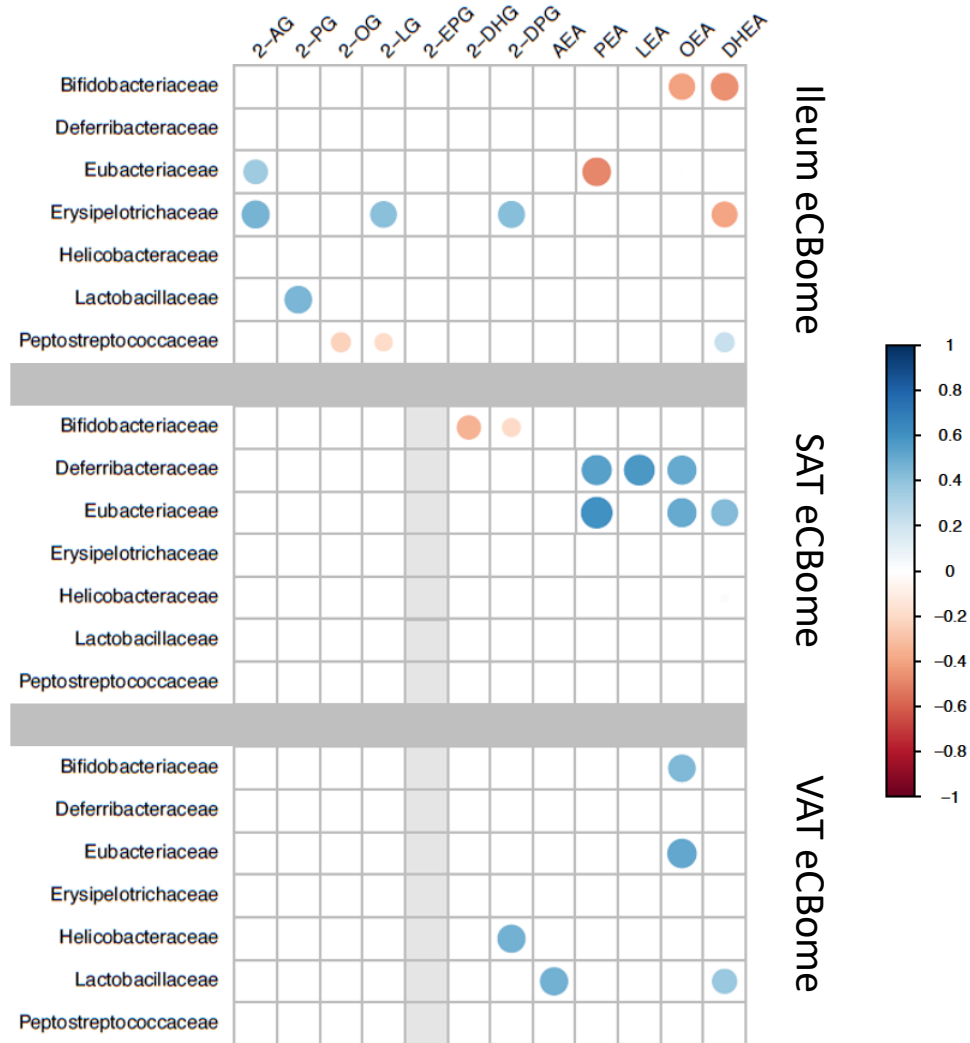


Figure S6. Changes in gut microbiota composition are associated with changes in 2 MAGs and NAEs. Heatmap illustrating significant Spearman rho correlation coefficients (n=38 per segments, $P<0.05$) between the relative abundance of selected bacterial families in the ileum and NAE or 2 MAG concentrations in the ileum, inguinal adipose tissue (SAT) and epididymal adipose tissue (VAT).