Diet Alters Fecal Microbiota Transplantation Efficiency in Germ-Free Mice

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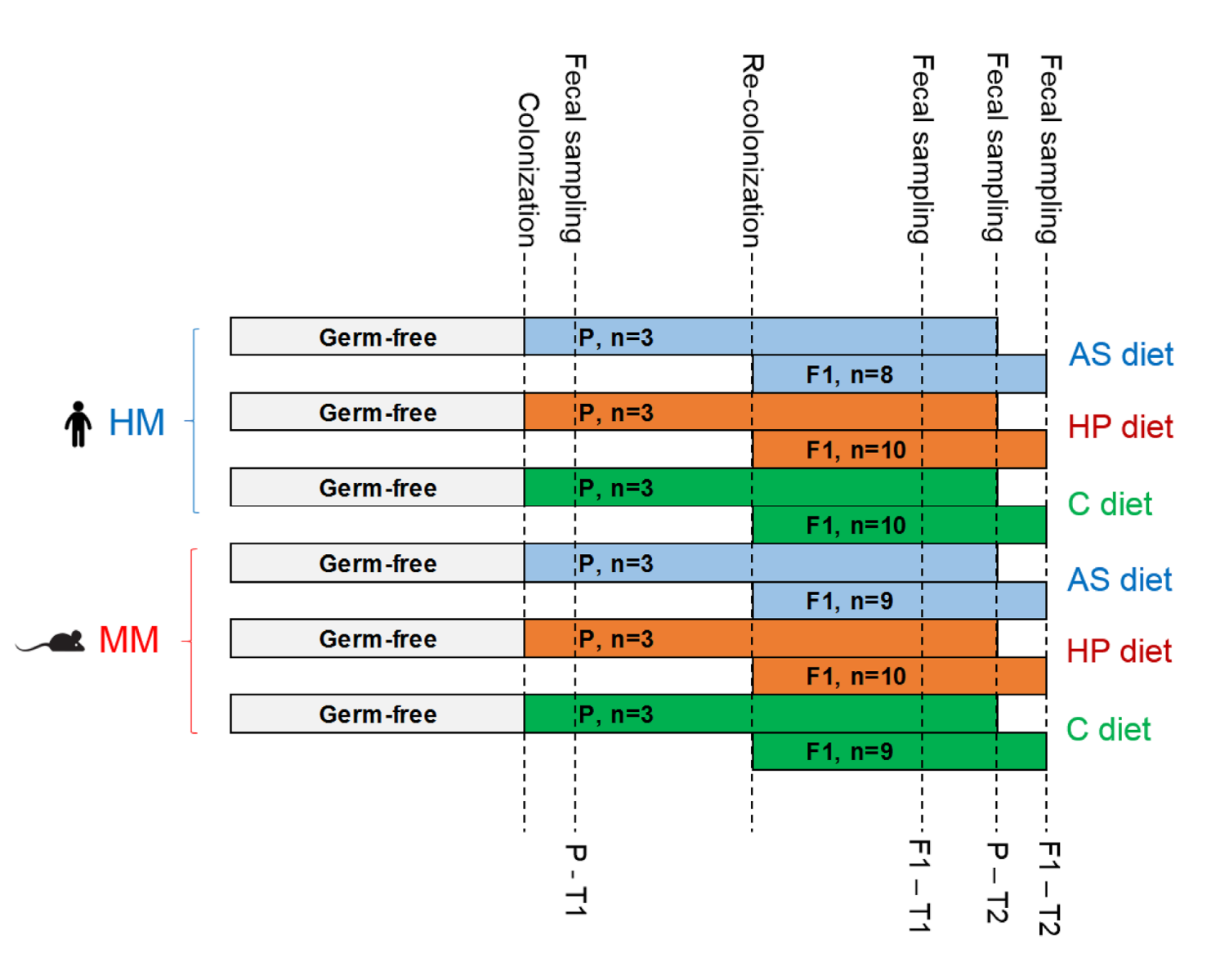
ABSTRACT

Fecal microbiota transplantation (FMT) in germ-free mice is a common approach in microbiome research and drug testing. However, FMT with complex, human microbiotas in mice does not fully capture the microbial community of the donor, nor does it stimulate the murine immune system in the same way as a murine microbiota. It was hypothesized that diet affects the efficiency of a human microbiota to establish in recipient germ-free mice and subsequent immune responses. Therefore, it was tested whether custom diets with altered fat content or fat/protein sources (soybean oil vs. milk fat or soy protein vs. casein), compared to a standard rodent chow, would improve human microbiota establishment and immune system characteristics.

METHODS & MATERIALS



Study design

Germ-free parental (P) mice were colonized with human microbiota (HM) or mouse microbiota (MM) inoculum at six weeks of age and received animal source (AS) diet, human profile (HP) diet, or control (C) diet. When F1 pups were born, the microbiota colonization was reinforced by applying the inoculum to the abdomen of the lactating dams. Fecal samples for 16S rRNA gene sequencing analysis were sampled at two time points (T1, T2) for P and F1, respectively:

P-T1 = one week post-colonization, or seven weeks of age
P-T2 = ten weeks post-colonization, or 16 weeks of age
F1-T1 = four weeks of age; F1-T2 = six weeks of age

Macronutrient composition of study diets

The control (C) diet was Altromin #1320, and the Animal Source (AS) and Human Profile (HP) diets were based on the control diet recipe.

Macronutrient Composition	AS Diet (Animal Source)			HP Diet (Human Profile)			C Diet (Control)		
	Source	kcal%	gm%	Source	kcal%	gm%	Source	kcal%	gm%
Carbohydrates	Soy, corn, wheat	64	46.1	Soy, corn, wheat	55	48.3	Soy, corn, wheat	64	46.4
Proteins	Caseins, soy	24	19.2	Soy	20	19.1	Soy	24	19.2
Fats	Milk fat	12	4.3	Soybean oil	25	10.5	Soybean oil	12	4.3
Vitamins, Minerals	Calcium carbonate, dicalcium phosphate, premixed vitamins, premixed minerals, trace elements								

Fraction of shared OTUs between donor inoculum and recipient mice

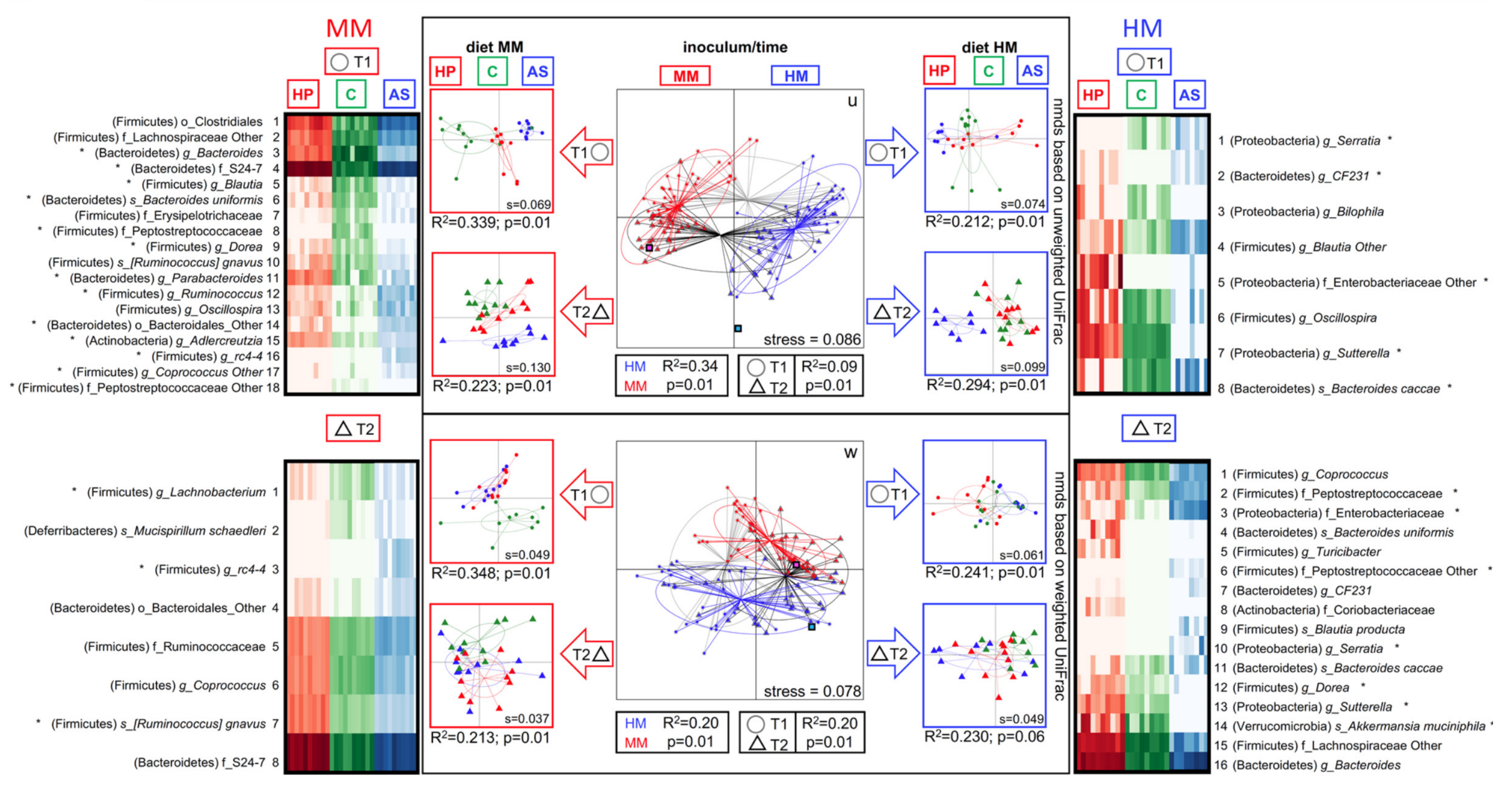
	Human Microbiota			Mouse Microbiota		
Inoculum	100%			100%		
P-T1	42%±16	50%±23	37%±11	68%±7^{b}	37%±5	37%±4
P-T2	48%±0	48%±4	38%±2	70%±10	63%±6	62%±4
F1-T1	30%±6	37%±12	33%±3	65%±3^{c}	55%±6	53%±7
F1-T2	30%±4	40%±9^{a}	36%±4	68%±8$^{(d)}$	61%±6	65%±6
Diet	↑ AS	↑ HP	↑ C	↑ AS	↑ HP	↑ C

100%
70%
50%
40%
30%

P-T1/T2 = parent (breeder) generation at 7/16 weeks old
F1-T1/T2 = F1 pups at 4/6 weeks old
a. HP vs. AS: p = 0.008, CI = [0.0242; 0.1731]
b. AS vs. C/HP: p = 0.001, CI = [-0.4460; -0.1741]
c. AS vs. C: p<0.0001, CI = [-0.1887; -0.0584]; AS vs. HP: p = 0.001, CI = [-0.1701; -0.0398]
d. AS vs. HP: p = 0.056, CI = [-0,1506; 0,0017]

Effect of diet and age on gut microbiota composition of mice born with human or mouse microbiota

Differences in the gut microbiota composition of F1 mice due to: time (four weeks of age = T1; six weeks of age = T2), diets (HP, C, AS), and inoculum (MM, HM). NMDS plots based on unweighted (u) and weighted (w) UniFrac distance matrices show clear separation according to the inoculum source (MM = red; MM = blue), and time (T1 = circle; T2 triangle). The effect of diet (HP, C, and AS respectively: red, green, and blue) on microbial composition was further examined within each inoculum (red and blue arrows) and each time point (T1 = circle; T2 triangle) for which individual NMDS plots were generated. The heatmaps depict taxa selected with ANCOM (at p = 0.1) to be different between the diets at each time point for both MM and HM. Significant differences (ANCOM p<0.05) are marked with stars. Adonis results (R2 and p values) are presented below each NMDS plot. Donor inoculum samples of MM and HM are marked with magenta and blue squares respectively. S values represent the stress of each NMDS ordination.



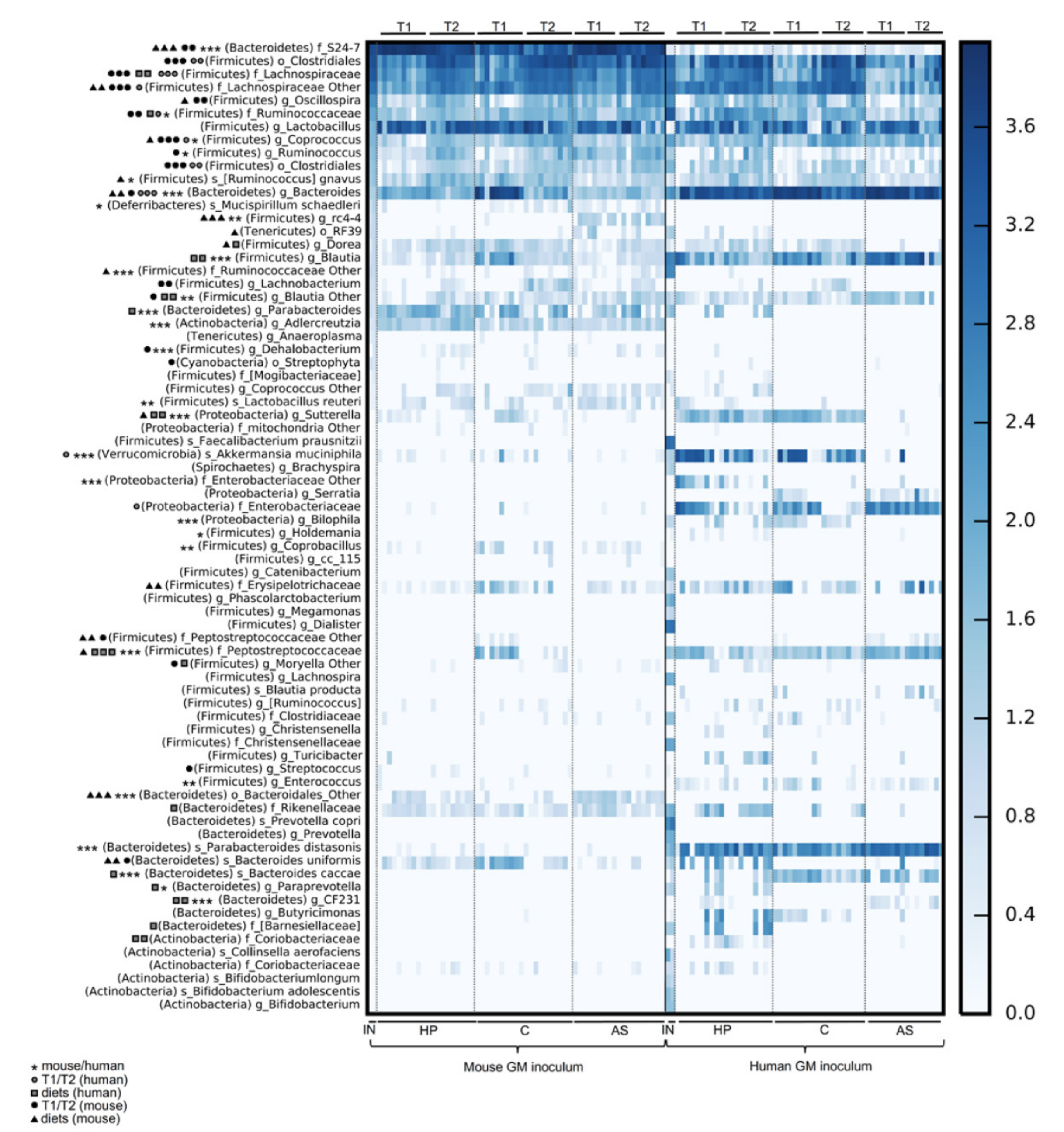
T1 = four weeks of age
T2 = six weeks of age

HM = human microbiota
MM = mouse microbiota

AS = animal source diet
HP = human profile diet

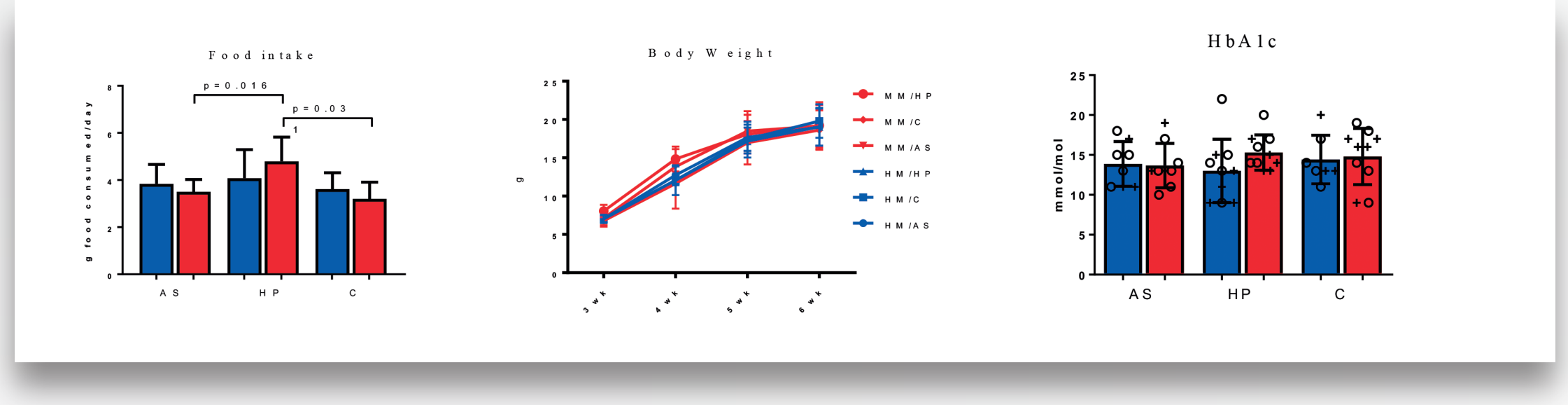
C = control diet

Overview of gut microbiota composition of mice born with a human or mouse microbiota and fed three different diets



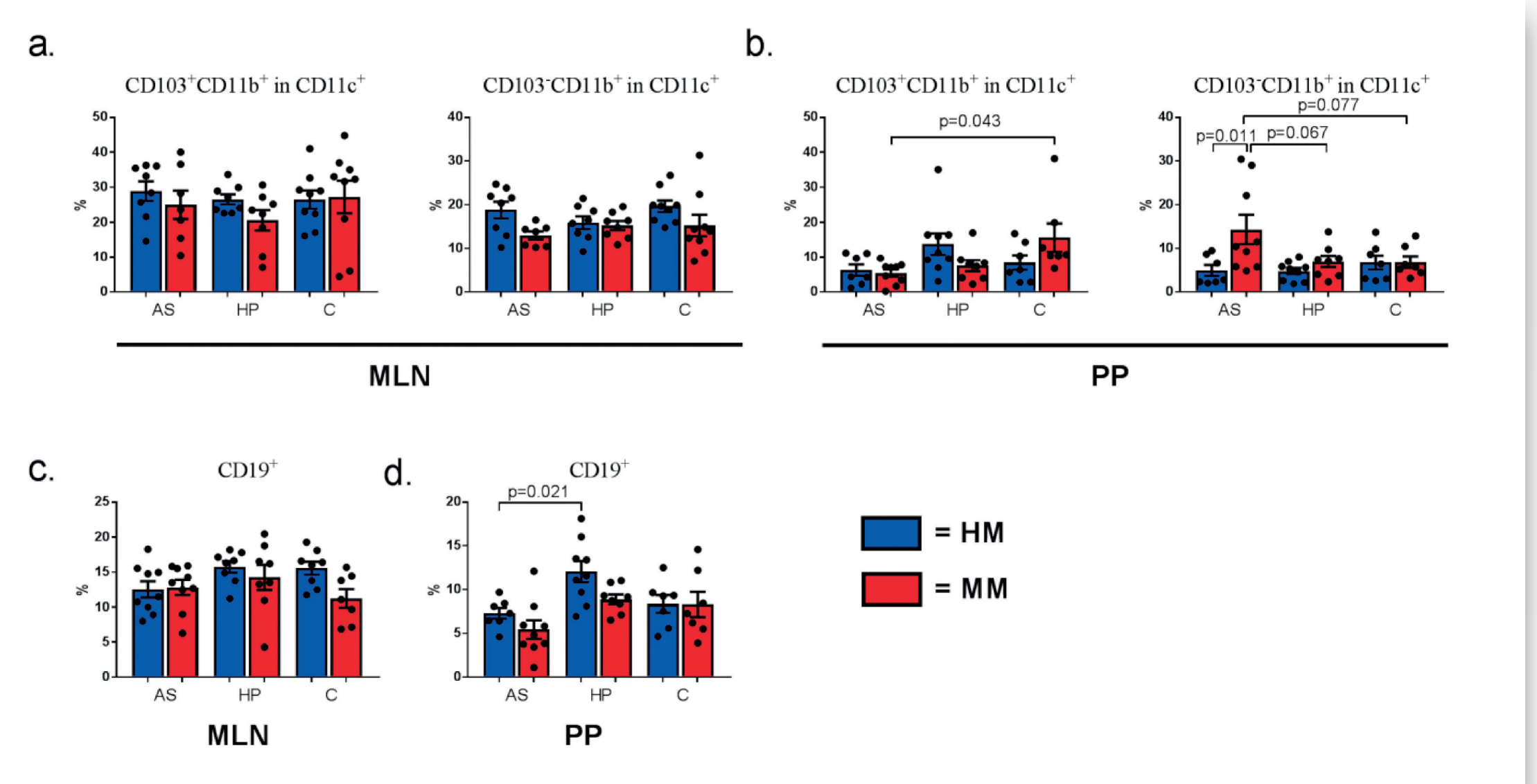
Symbols next to the taxonomic label indicate the level of significance of different abundances:
1 symbol: p<0.05,
2 symbols: p<0.01, and
3 symbols: p<0.001;
FDR corrected p

T1 = four weeks of age
T2 = six weeks of age
IN = inoculum
AS = animal source diet
HP = human profile diet
C = control diet
GM = gut microbiota



Increased food intake but unaltered body weight and glucose control with study diets

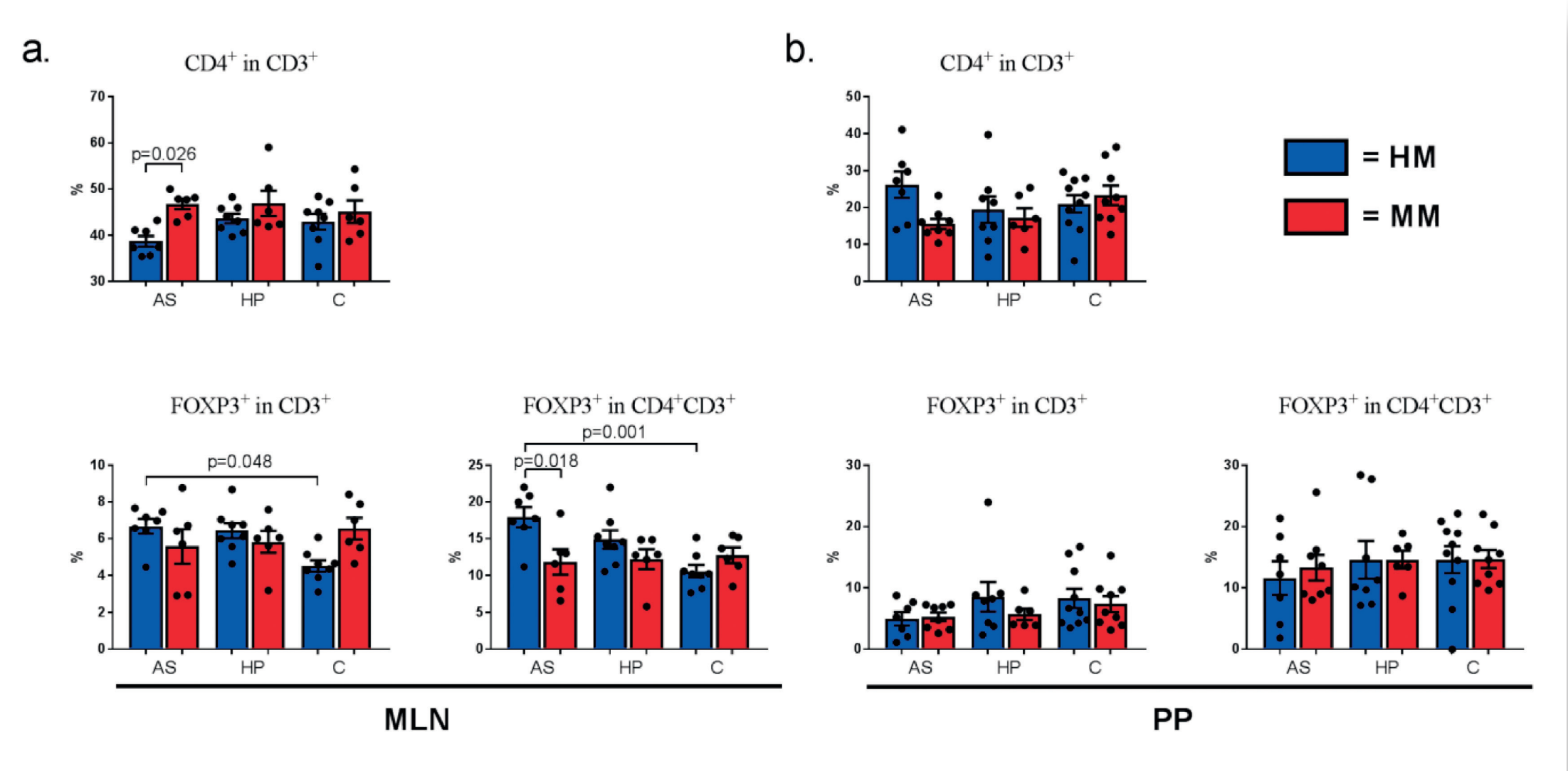
AS = animal source diet
HP = human profile diet
C = control diet
HM = human microbiota
MM = mouse microbiota



Dendritic and B cell populations

Cell populations were measured by flow cytometry at six weeks of age and analyzed by ANOVA with Sidak's multiple comparisons post-hoc test.

MLN = mesenteric lymph nodes
PP = Peyer's patches
Bars are SEM



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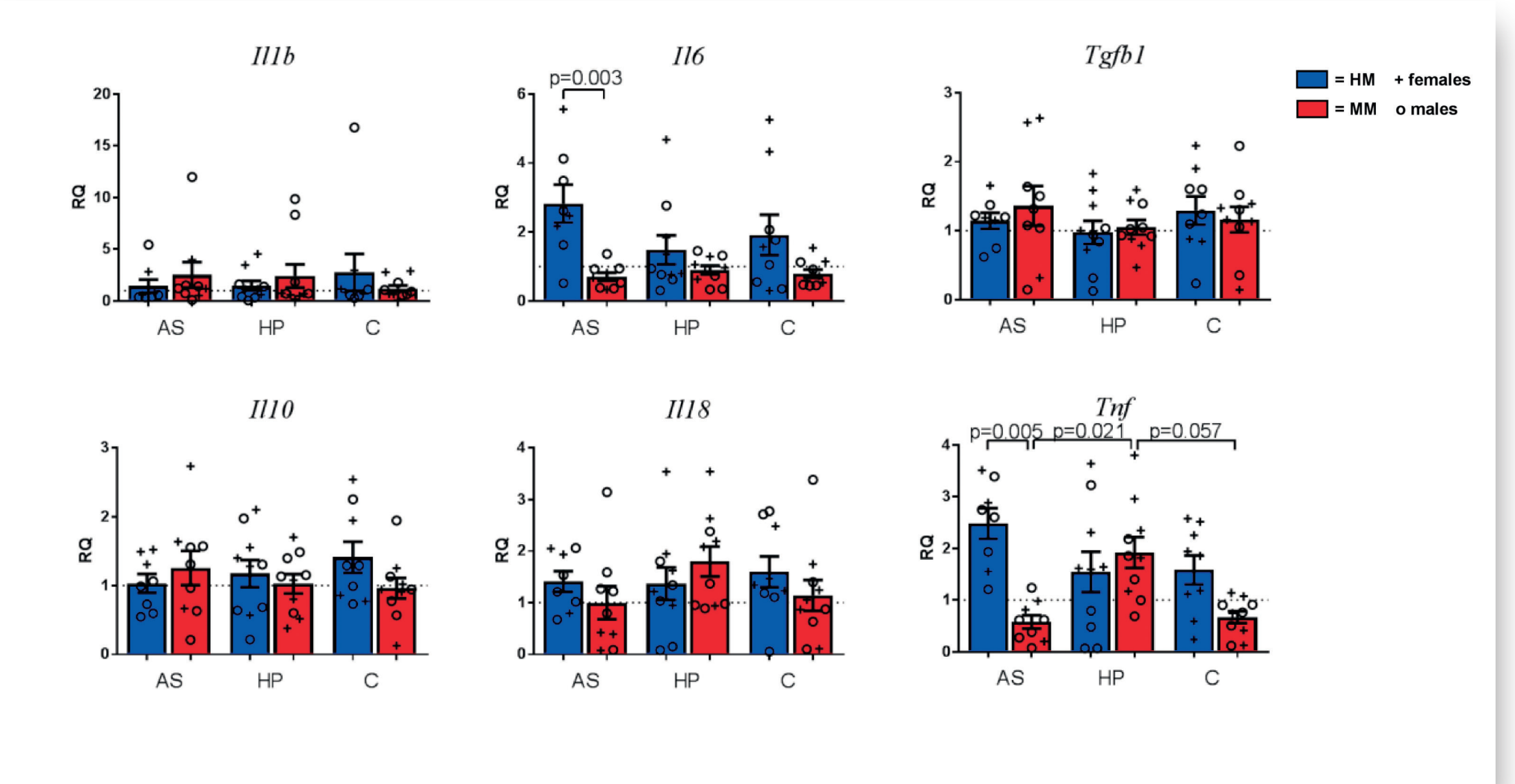

Colonic cytokine gene expression levels in F1 mice

mRNA levels were measured by qPCR, reported as the relative quantification (RQ) compared to the mean of mice with mouse microbiota (MM) on control (C) diet, and analyzed by one-way ANOVA with Tukey's multiple comparisons post-hoc test.

Bars are SEM

CONCLUSION

Altering the diet of mice transplanted with complex human fecal microbiotas is a promising approach for optimizing FMT efficiency in mice and for modulating subsequent immune system function. Coevolution between host and microbiota seems to play a role in the ability of the gut microbiota to respond to dietary changes. For mice with a mouse microbiota, qualitative properties of the diet were more important in shaping the microbiota and host immune system than quantitative macronutrient properties. Animal source diet altered the microbiota composition more than grain-based diet with increased fat. Further studies to optimize fat content and diet constituents are warranted in order to successfully model the human microbiota in mice and its effects on the immune system.

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