Distinct effects of milk-derived and fermented dairy protein on gut microbiota and cardiometabolic markers in diet-induced obese mice.

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Online Supplementary Material

Supplementary Data

Supplemental Table 1: Chemical composition of MP, FMP and YP lyophilized products.

	MP (Milk Product)	FMP (Fermented Milk Product)	YP (greek Yogurt Product)
Total solids (TS, %)	96.1 ± 0.75	96.5 ± 0.15	94.4 ± 0.21
Total nitrogen (TN, %)	9.27 ± 0.07	9.20 ± 0.03	9.03 ± 0.07
Total nitrogen protein (TNP, %)[1]	59.1 ± 0.42	58.7 ± 0.19	57.6 ± 0.42
Non-nitrogen protein (NPN, %)	0.19 ± 0.00	0.20 ± 0.01	0.36 ± 0.01
True protein (TP, %)[2]	57.9	57.4	55.3
NPN/TN ratio	0.02	0.02	0.04
Lactose (%)	27.8 ± 0.14	12.8 ± 0.02	3.23 ± 0.14
Galactose (%)	<LD[3]	4.58 ± 0.01	10.9 ± 0.44
Fat (%)	1.11 ± 0.04	1.18 ± 0.03	1.15 ± 0.02
Cholesterol (mg/100g)	39.5 ± 1.41	38.6 ± 1.05	38.5 ± 0.56
Ash (%)	7.95 ± 0.01	7.68 ± 0.12	7.65 ± 0.03
Organic acids (mg/g) :			
Acetic	<LD	<LD	<LD
Citric	16.5 ± 0.11	12.3 ± 0.11	15.1 ± 0.55
Lactic	<LD	218 ± 1.15	119.1 ± 4.63
Cations (g/100g) :			
Ca	2.03 ± 0.05	1.92 ± 0.05	1.95 ± 0.02
Na	0.20 ± 0.01	0.35 ± 0.01	0.32 ± 0.00
K	1.06 ± 0.05	1.05 ± 0.05	1.02± 0.04
Mg	0.11 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
Anions (g/100g) :			
P/PO_4^{-3}	1.24 ± 0.06	1.45 ± 0.02	1.44 ± 0.02
Cl	0.34 ± 0.02	0.25± 0.01	0.30 ± 0.01
S/SO_4^{-2}	0.09 ± 0.00	0.03 ± 0.00	0.03 ± 0.00

[1]Values are mean ± standard deviation (n≥ 3)

[2]TNP = TN x 6,38

[3]TP = (TN-NPN) x 6,38. TP values are used for the calculation of NPN

[4]<LD : values below the limit of detection.

[5]LD was 0.1 % for galactose and 1 mg/g for organic acids.

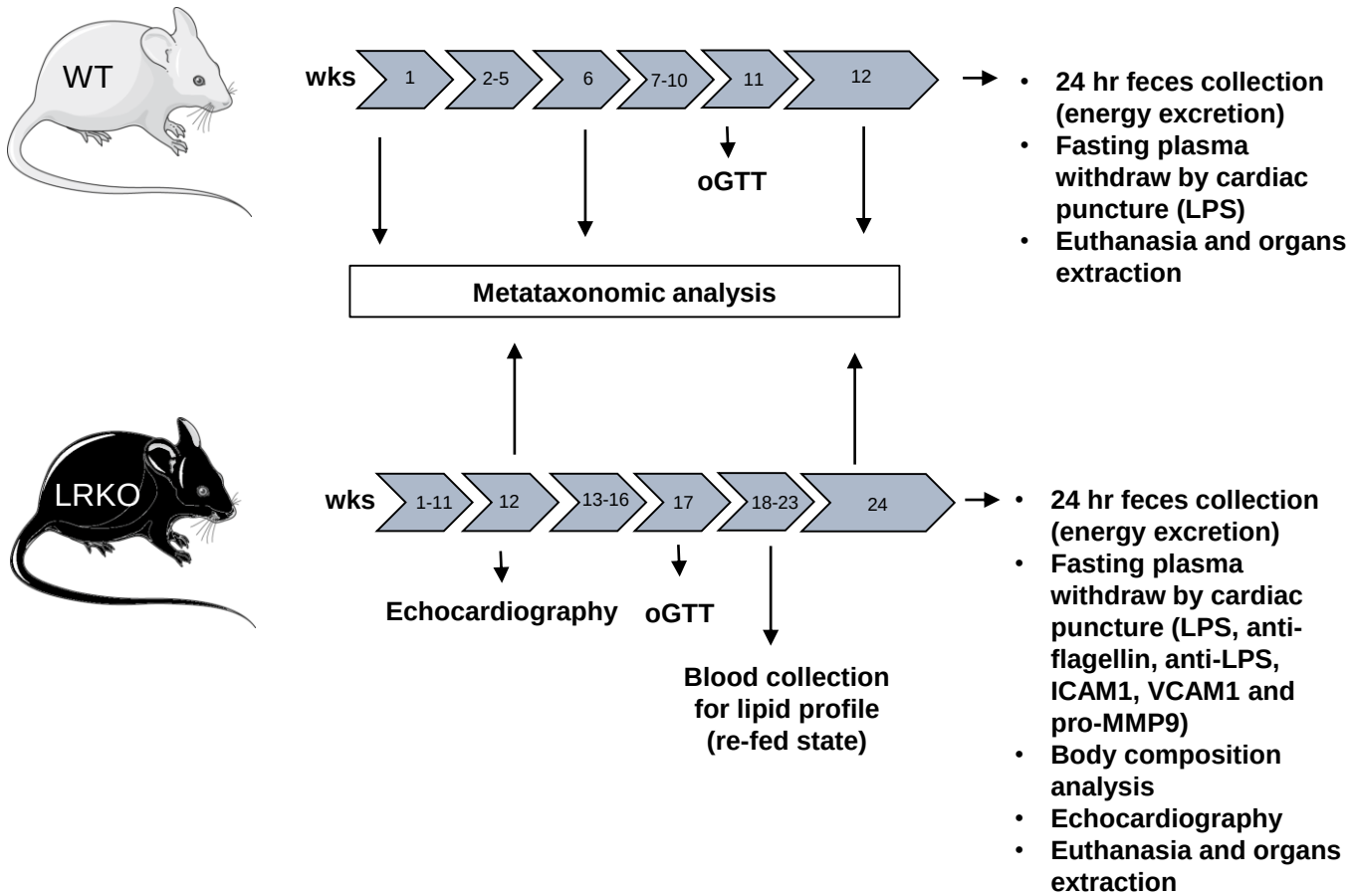
Supplementary Data

Supplemental Figure 1

Experimental diets

Non-dairy protein	Milk product	Fermented milk product	Yogurt product
↓	↓	↓	↓
No replacement	HFHS with 50% of protein replacement with milk product	HFHS with 50% of protein replacement with fermented milk product	HFHS with 50% of protein replacement with yogurt product
↓	↓	↓	↓
NDP	**MP**	**FMP**	**YP**

Experimental design

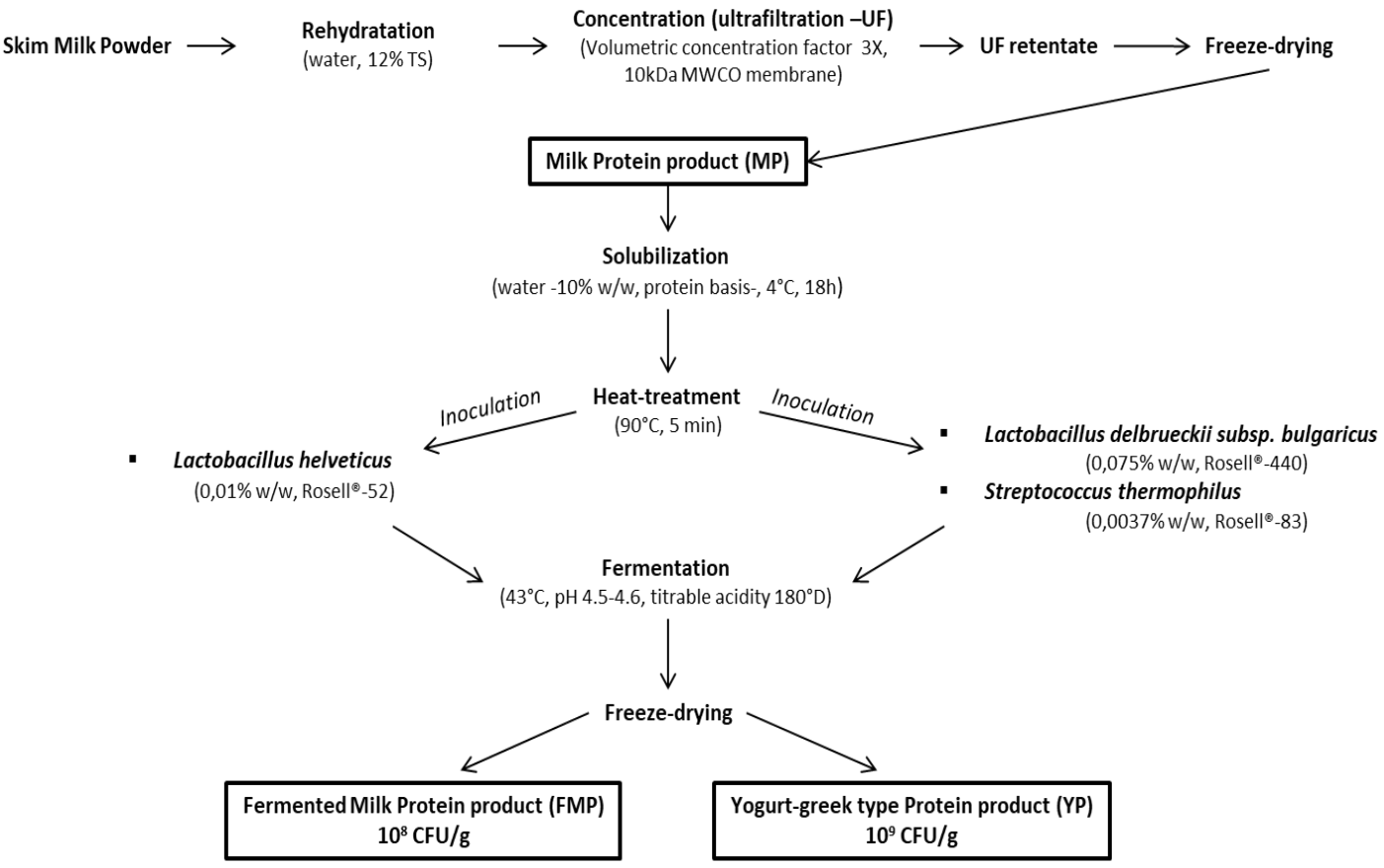


Supplemental Figure 1. Experimental study design.

* Illustrations were obtained on https://smart.servier.com, published by LES LABORATOIRES SERVIER, SAS.

Supplementary Data

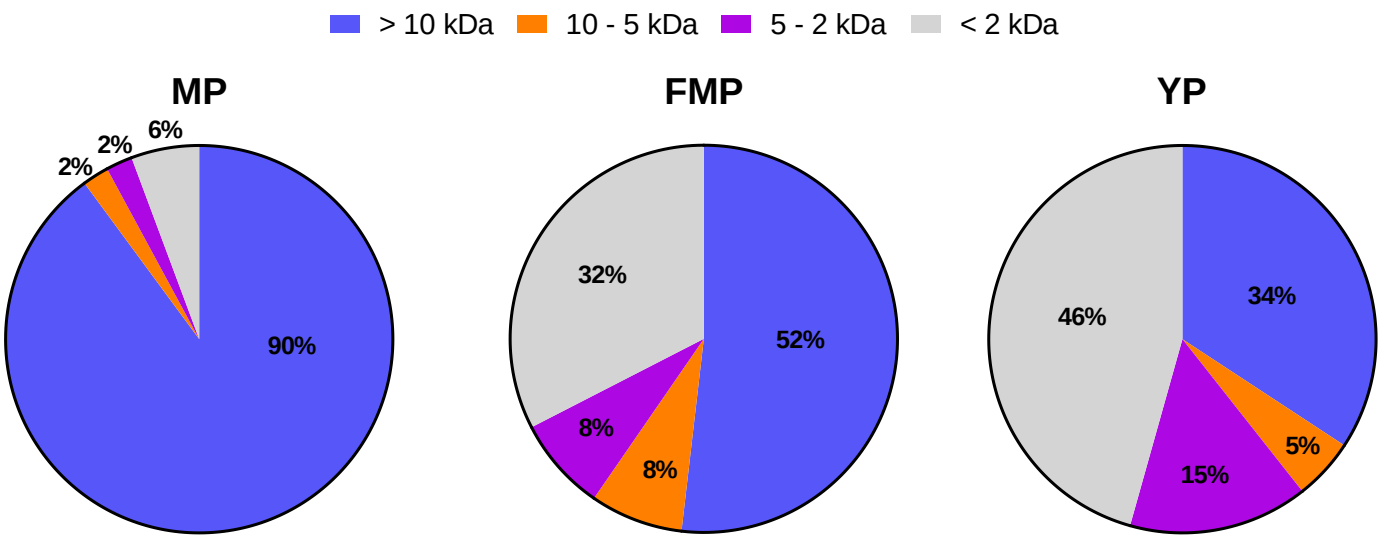
Supplemental Figure 2



Supplemental Figure 2. Detailed formulation process of the fermented dairy products. CFU: Colony-forming unit.

Supplementary Data

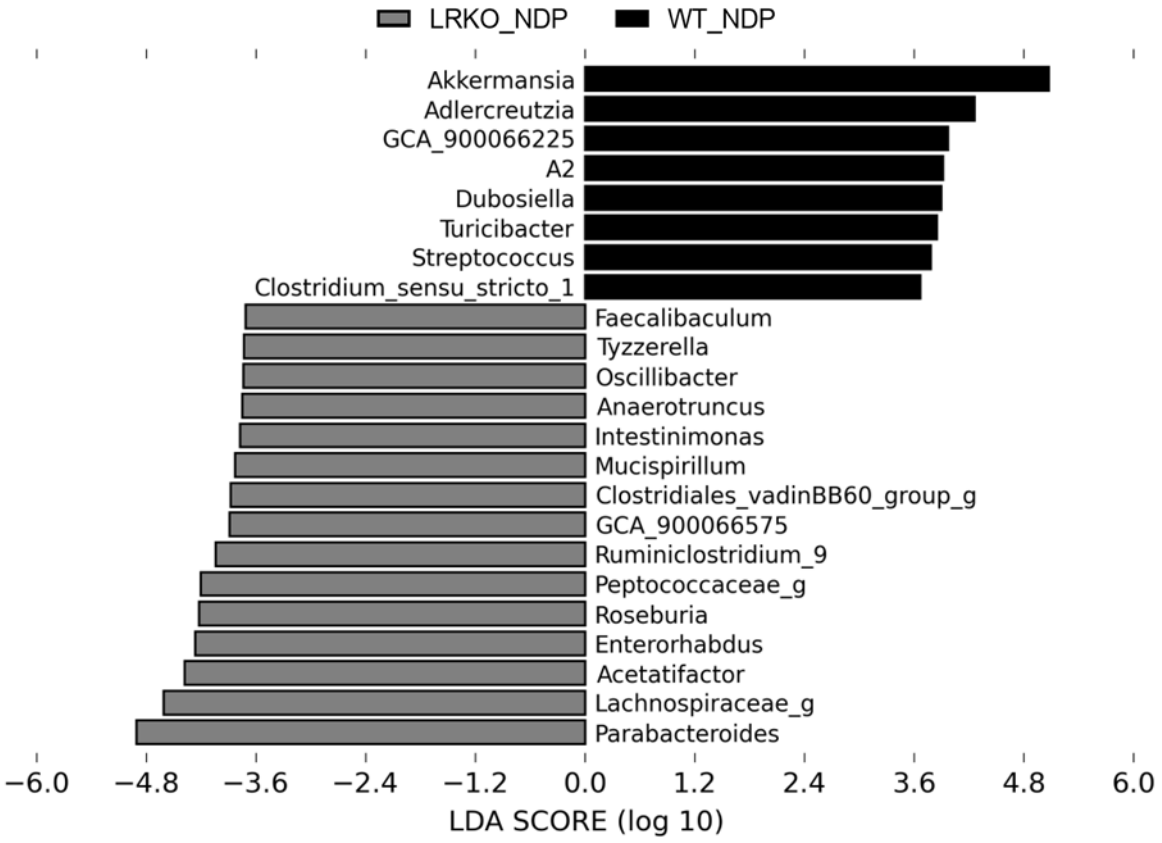
Supplemental Figure 3



Supplemental Figure 3. Molecular weight (Da) distribution of MP, FMP and YP products peptides. Molecular weight distribution profile of protein/peptide components of dairy products. FMP: fermented milk product; MP: milk product; YP: yogurt product.

Supplementary Data

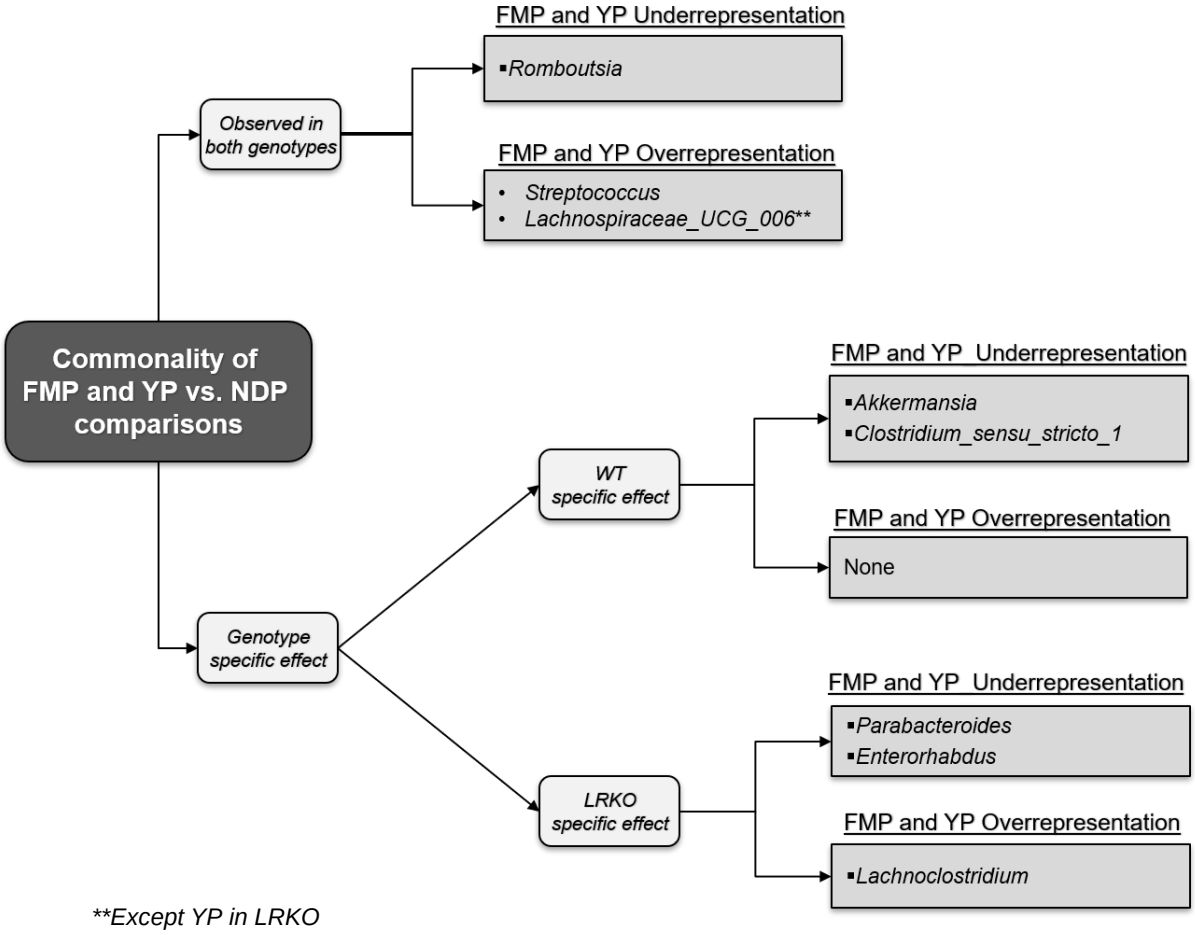
Supplemental Figure 4



Supplemental Figure 4. Genotype comparisons on taxa relative abundances. Related to Figures 1-2. Linear discriminant analysis with effect size (LEfSe) analysis of bacterial taxa present in WT *versus* LRKOB100 mice at week 12. NDP: non-dairy protein.

Supplementary Data

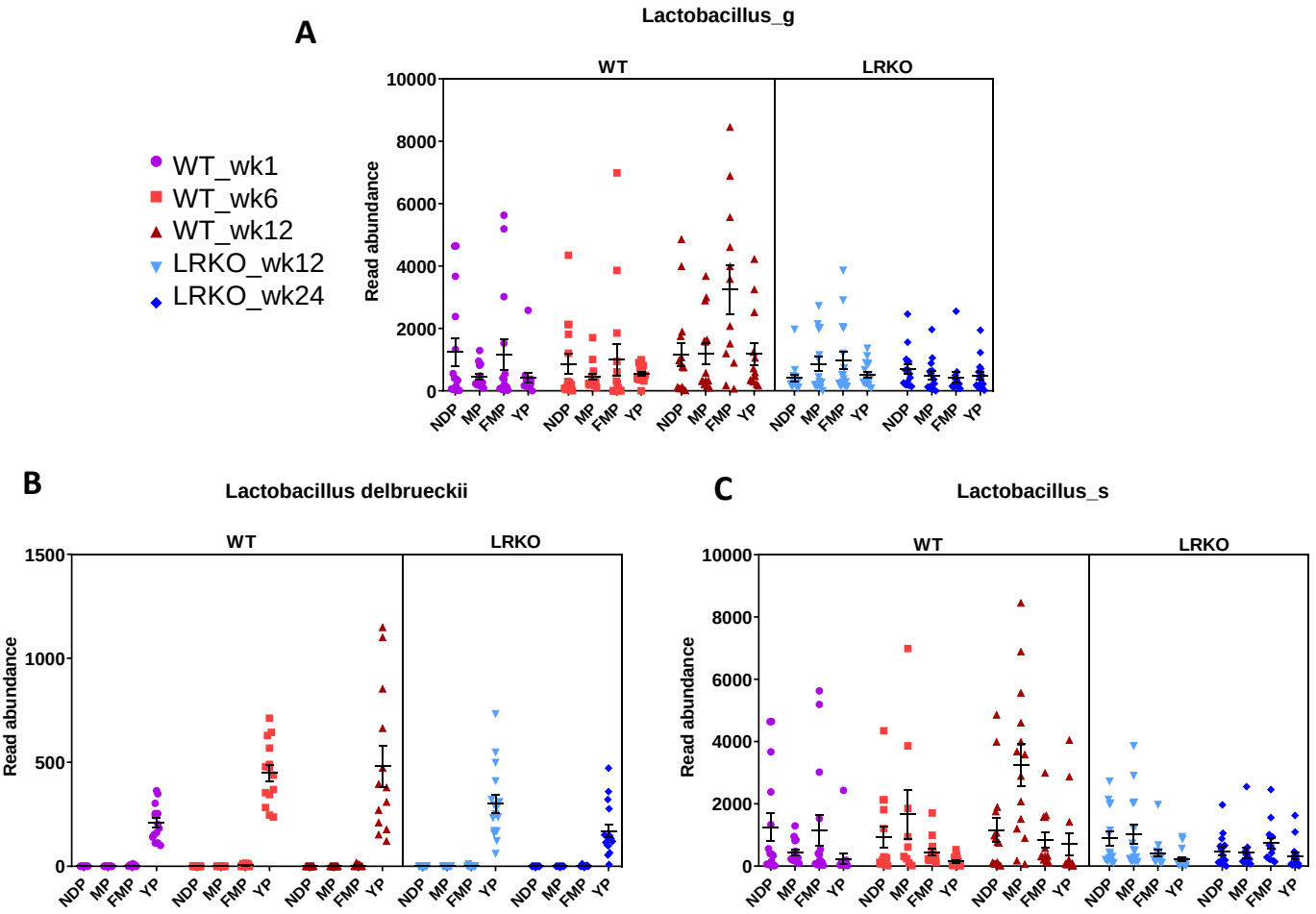
Supplemental Figure 5



Supplemental Figure 5. Fermented dairy profile: Effect of FMP and YP in fecal metataxonomic abundances compared to NDP, related to Figure 2. FMP: fermented milk product; NDP: non-dairy protein; YP: yogurt product.

Supplementary Data

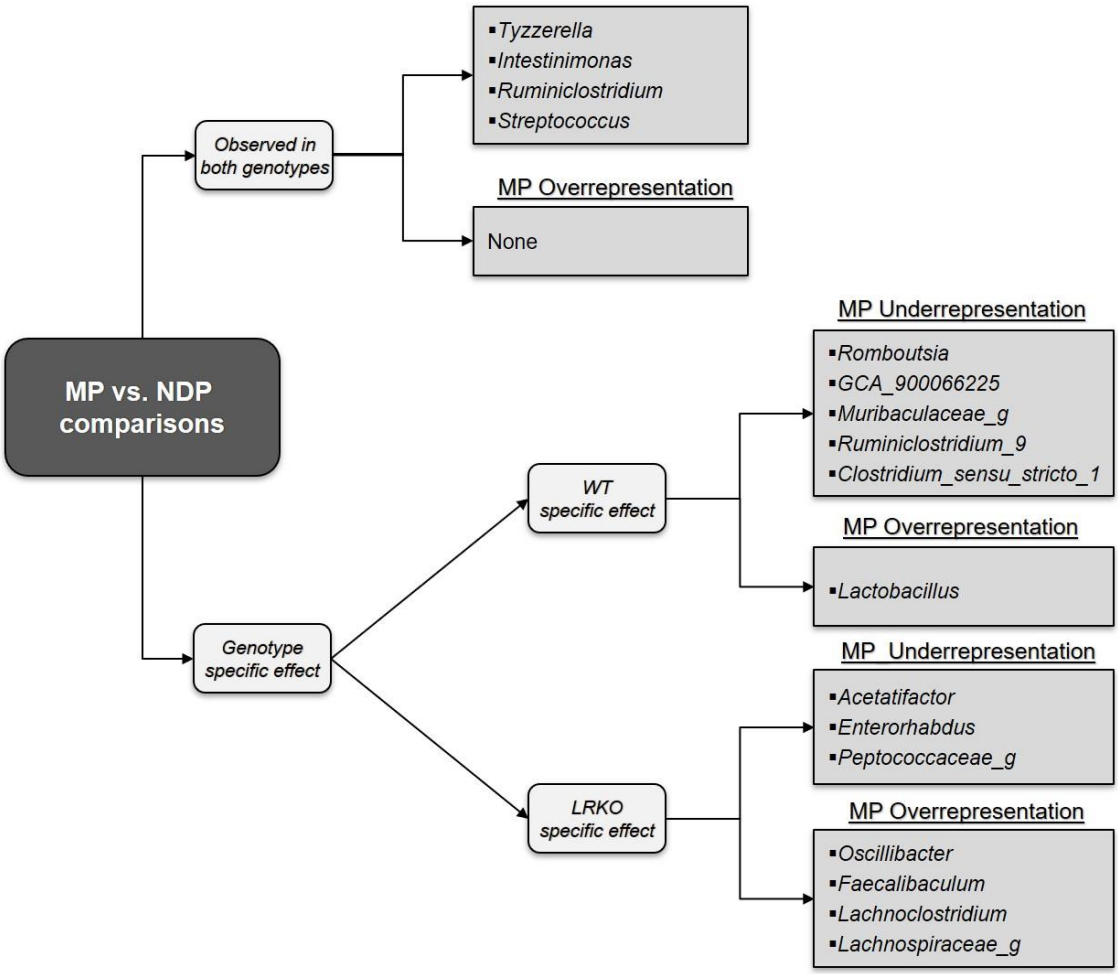
Supplemental Figure 6



Supplemental Figure 6. Effect of dairy products consumption in *Lactobacillus* species, related to Figures 1-3. Read abundance of (**A**) *Lactobacillus* genus and (**B**) *Lactobacillus delbrueckii* and (**C**) unknown *Lactobacillus* species. Values are mean ± SEM. FMP: fermented milk product; MP: milk product; NDP: non-dairy protein; YP: yogurt product.

Supplementary Data

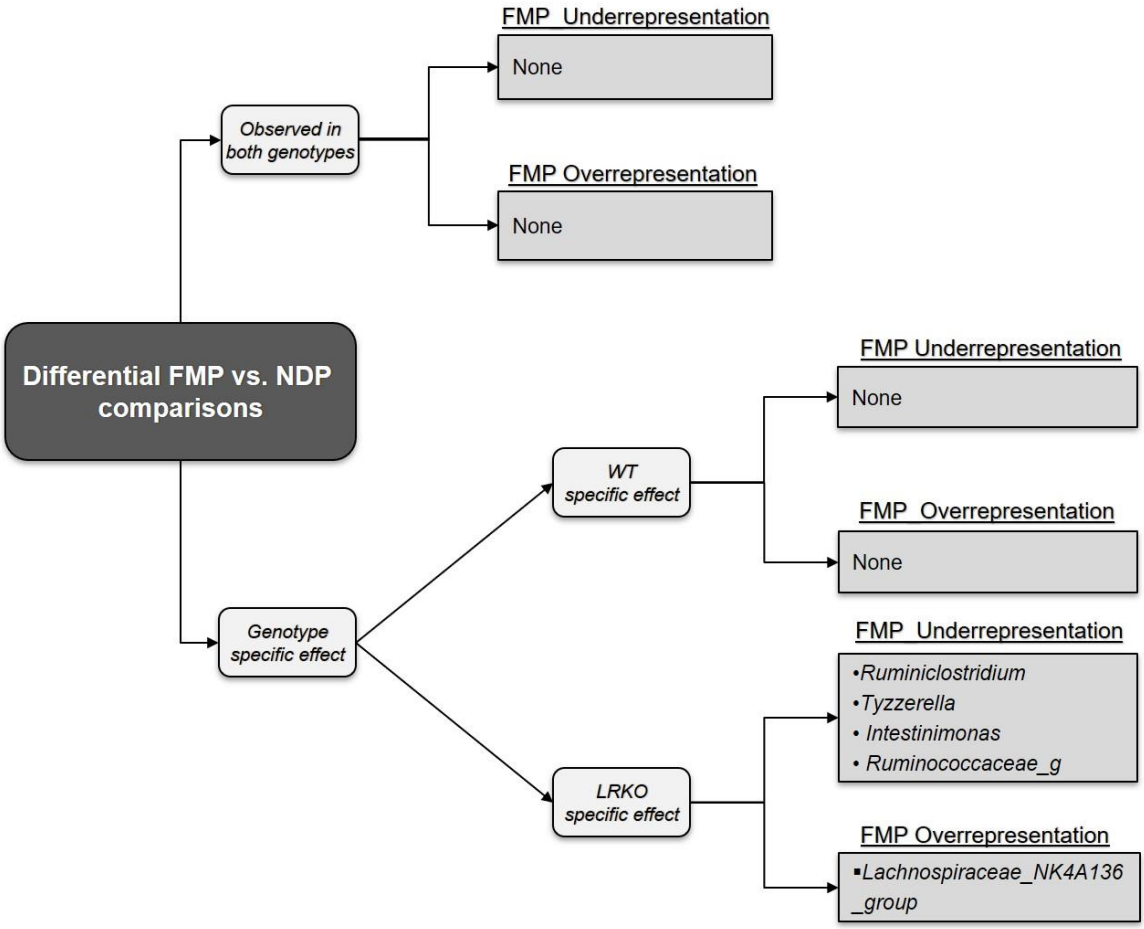
Supplemental Figure 7



Supplemental Figure 7. Exclusive effect of non-fermented MP in fecal metataxonomic abundances compared to NDP, related to Figure 2. MP: milk product; NDP: non-dairy protein.

Supplementary Data

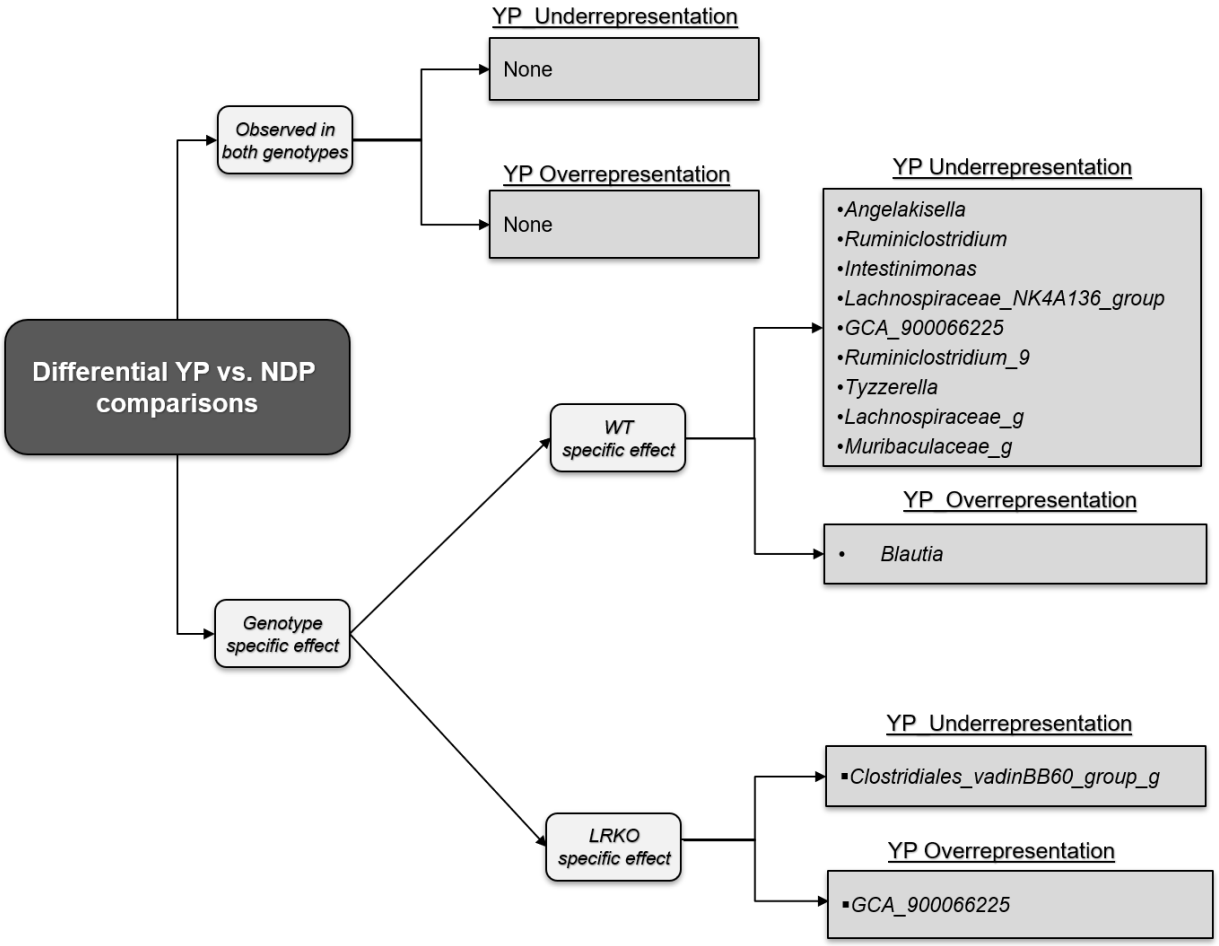
Supplemental Figure 8



Supplemental Figure 8. Exclusive effect of FMP in fecal metataxonomic abundances compared to NDP, related to Figure 2. FMP: fermented milk product; NDP: non-dairy protein.

Supplementary Data

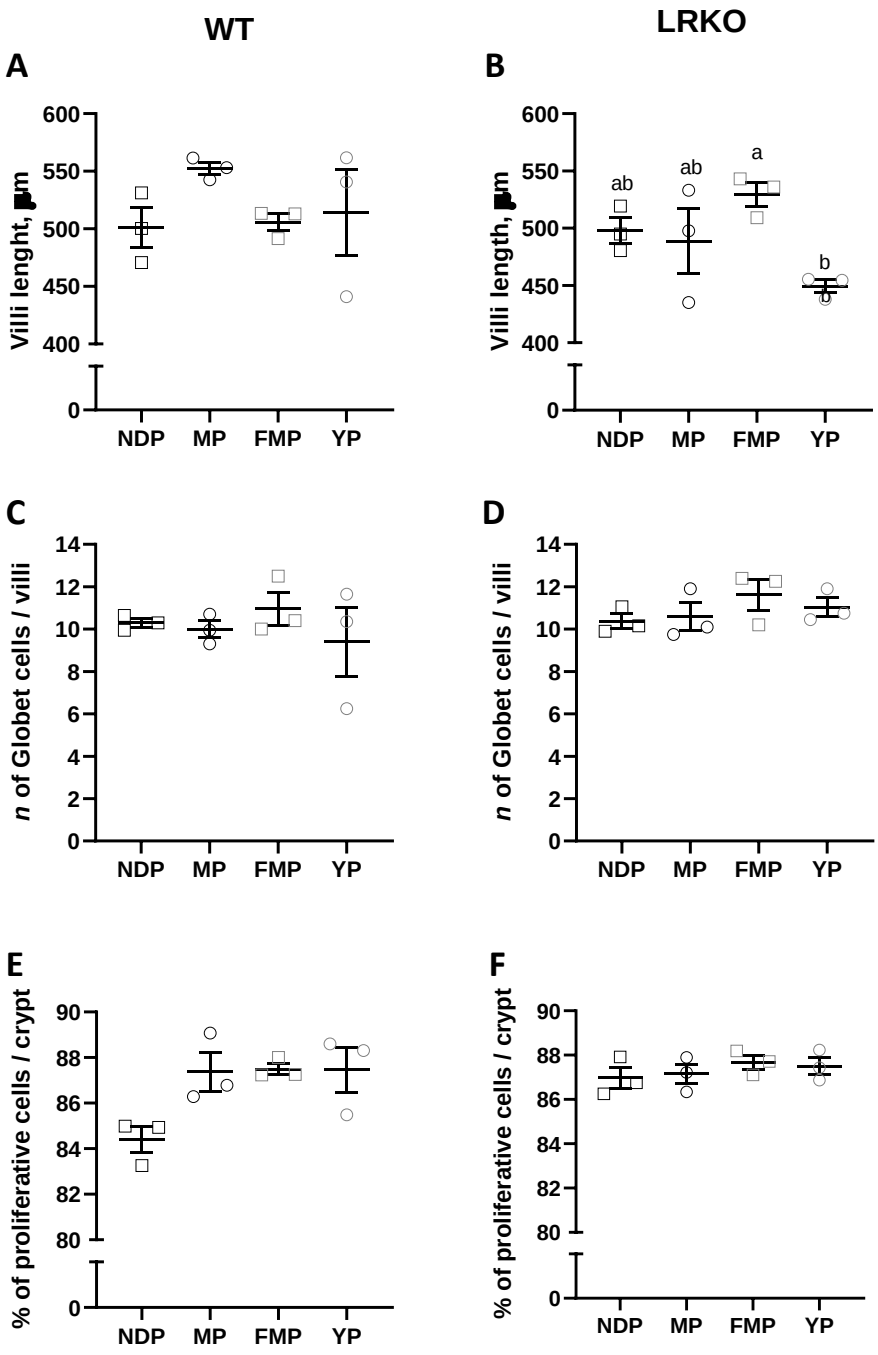
Supplemental Figure 9



Supplemental Figure 9. Exclusive effect of YP in fecal metataxonomic abundances compared to NDP, related to Figure 2. NDP: non-dairy protein; YP: yogurt product.

Supplementary Data

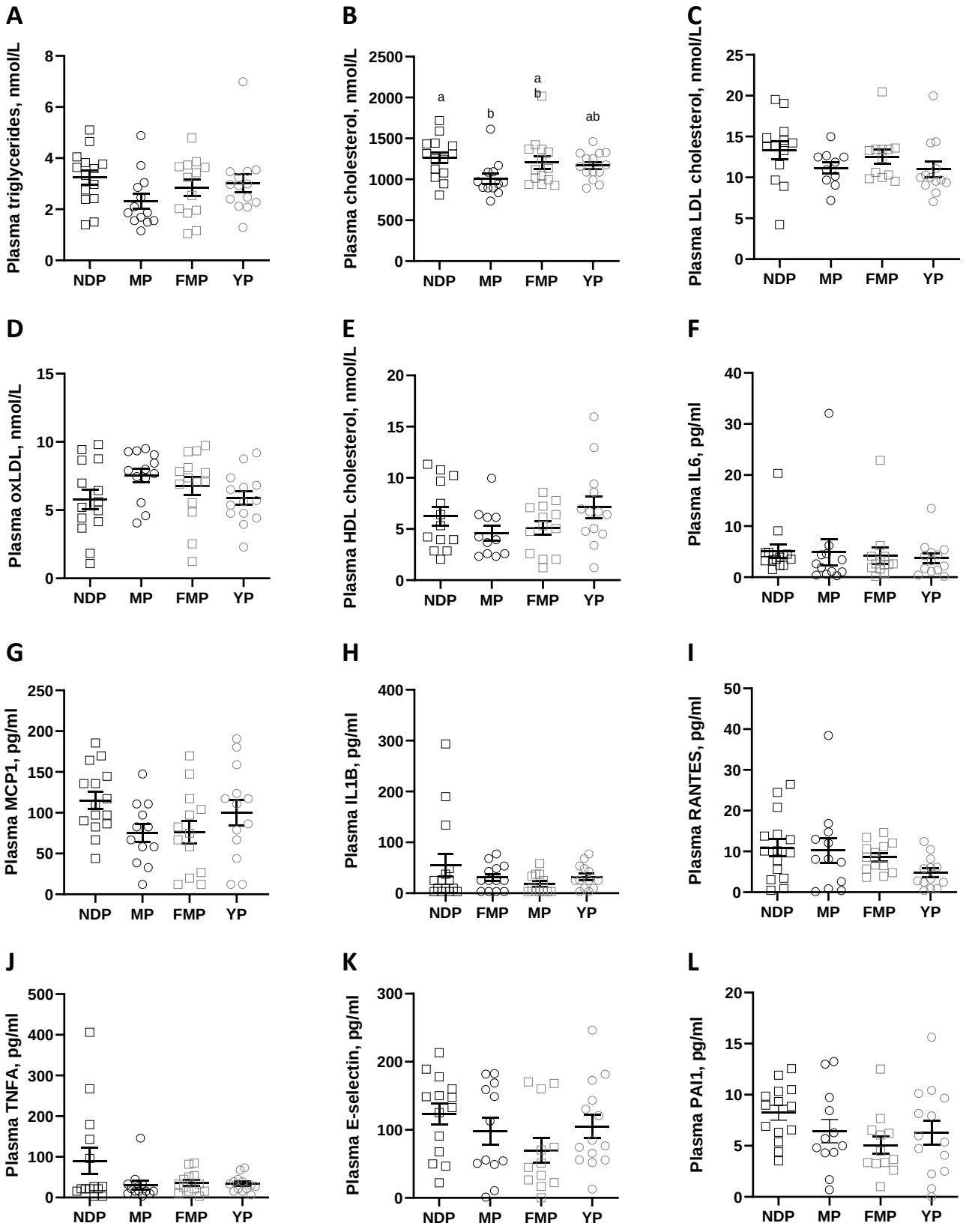
Supplemental Figure 10



Supplemental Figure 10. Effect of dairy products consumption in jejunal histology, related to Figure 1-3. Villi length, Goblet cells and cell proliferation were quantified in jejunum of WT (**A**, **C** and **E**, respectively) and LRKO (**B**, **D** and **F**, respectively) mice, n=3 (WT and LRKO). Values are mean ± SEM. All P values were determined by one-way ANOVA followed by Tukey's post hoc test. Within genotype, labeled means without a common letter differ, $P < 0.05$. FMP: fermented milk product; MP: milk product; NDP: non-dairy protein; YP: yogurt product.

Supplementary Data

Supplemental Figure 11



Supplemental Figure 11. Circulating lipoproteins, inflammatory markers and adhesion molecules in LRKO mice. Related to Figure 5. (**A**) Plasma triglycerides, (**B**) cholesterol, (**C**) LDL, (**D**) oxLDL, (**E**) HDL, (**F**) IL6, (**G**) MCP-1, (**H**) IL1B, (**I**) RANTES, (**J**) TNFA, (**K**) E-selectin, (**L**) PAI1, n=12-14. Data are expressed as mean ± SEM. All P values were determined by one-way ANOVA followed by Tukey post hoc test. Within genotype, labeled means without a common letter differ, $P < 0.05$. FMP: fermented milk product; MP: milk product; NDP: non-dairy protein; YP: yogurt product.