

Supplementary materials

Supplementary Table 1 Four most abundant phyla for human and mouse samples.

Species	Diet	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria
Human	Not modified	60.4 (1.3)	29.7 (1.4)	5.3 (0.6)	3.8 (0.6)
Mouse	0.2% Cellulose	48.0 (1.1) ***	36.5 (1.1) **	11.2 (0.9) *	1.3 (0.1) ^{ns}
Mouse	Psyllium	28.0 (0.8) ***	44.4 (0.8) ***	9.4 (0.6) ^{ns}	16.0 (1.0) ***
Mouse	Psyllium+ RS	32.6 (1.2) ***	46.6 (1.1) ***	6.7 (0.5) ^{ns}	12.6 (1.0) ***
Mouse	Psyllium+ Inulin	22.3 (0.9) ***	38.1 (0.8) ***	31.0 (0.9) ***	7.3 (0.4) ^{ns}

Data are presented as mean and SEM (%), n=15 for the mice samples and n=76 for the human samples.

Analysed by two-way ANOVA with Tukey's *post hoc* test.

Asterisks indicate different from human at the same column: *P<0.05, **P<0.01, ***P<0.001.

Supplementary Table 2 Bacteria taxa enriched in cancer patients with low and high faecal SCFAs.

Concentrations of three major gut microbiota produced SCFAs including acetate, propionate and butyrate (mM)	Low		High	
	Average	SEM	Average	SEM
f_Bacteroidaceae g_Bacteroides s_Bacteroides_coprophilus	0.09	0.09	1.26	0.62
f_Lachnospiraceae g_Agathobacter	1.75	0.34	2.98	0.45
f_Lactobacillaceae g_Lactobacillus s_Lactobacillus_johnsonii	0.00	0.00	0.81	0.52
f_Lachnospiraceae g_Agathobacter _	1.15	0.24	2.08	0.33
f_Tannerellaceae g_Parabacteroides s_Parabacteroides_merdae	0.31	0.08	0.82	0.19
f_Lachnospiraceae g_Dorea	0.86	0.10	1.23	0.12
f_Prevotellaceae g_Prevotellaceae_NK3B31_group	0.00	0.00	0.30	0.20
f_Lachnospiraceae g_Dorea s_uncultured_bacterium	0.32	0.05	0.58	0.09
f_Lactobacillaceae g_Lactobacillus s_Lactobacillus_ruminis	0.00	0.00	0.23	0.16
f_Clostridia_vadinBB60_group g_Clostridia_vadinBB60_group s_uncultured_bacterium	0.23	0.13	0.05	0.02
f_Erysipelotrichaceae g_Turicibacter	0.42	0.26	0.28	0.10
f_Lachnospiraceae g_Frisingicoccus s_uncultured_organism	0.00	0.00	0.00	0.00
f_Izemoplasmatales g_Izemoplasmatales s_uncultured_organism	0.29	0.15	0.01	0.00
f_Oscillospiraceae g_UCG_002 _	1.14	0.24	0.63	0.09
f_Oscillospiraceae g_UCG_002	2.36	0.38	1.28	0.18
f_Rikenellaceae g_Alistipes _	1.71	0.55	0.44	0.26
f_Oscillospiraceae	4.73	0.52	3.07	0.39
f_Streptococcaceae	4.09	1.64	1.76	0.72
f_Streptococcaceae g_Streptococcus	4.09	1.64	1.76	0.72

Median of the three SCFAs combined was the cut off between high and low levels.

Supplementary Table 3 Four most abundant phyla of three serial human faecal samples prepared at 24-hour intervals for 72-hours.

Sample	Processing time (Hours)	Firmicutes (%)	Bacteroidetes (%)	Actinobacteria (%)	Proteobacteria (%)
S1	0	74.9	9.9	15.1	0.2
	24	71.1	10.9	17.8	0.2
	48	72.1	8.5	19.3	0.1
	72	72.1	6.3	21.5	0.1
S2	0	47.5	44.6	6.4	1.5
	24	45.3	46.3	7.0	1.4
	48	45.1	45.6	7.8	1.5
	72	43.7	47.8	7.2	1.3
S3	0	74.9	24.1	0.3	0.7
	24	75.8	21.0	1.2	1.9
	48	81.5	16.7	0.6	1.2
	72	81.9	15.9	0.8	1.5

Supplementary Table 4 Rodent diets without corn starch used in the study with varying levels of cellulose, psyllium, psyllium plus resistant starch, or inulin per 4000 kcal.

	Low fibre		High fibre (Psyllium)		High fibre (Psyllium+RS)		High fibre (Psyllium+Inulin)	
	2 gm Cellulose/4000kcal		50 gm Psyllium/4000kcal		50 gm Psyllium + 100 gm RS/4000kcal		50 gm Psyllium + 100 gm Inulin/4000kcal	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	21	20	21	20	19	20	19	20
Carbohydrate	67	64	63	64	65	64	56	59
Fat	7	16	7	16	7	16	7	16
Total		100		100		100		95
kcal/gm	4.20		4.05		3.84		3.81	
Ingredient	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein	200	800	200	800	200	800	200	800
L-Cysteine	3	12	3	12	3	12	3	12
Dextrose, Monohydrate	397.49	1590	384.99	1540	322.69	1291	347.49	1390
Hi-Maize Corn Starch 260 (56% RS)	0	0	0	0	179	501	0	0
Maltodextrin 10	132	528	132	528	132	528	132	528
Sucrose	100	400	100	400	100	400	100	400
Cellulose, BM200	2	0	100	0	0	0	0	0
Inulin (Orafti HP)	0	0	0	0	0	0	100	150
Psyllium (AEP Colloids)	0	0	50	50	50	50	50	50
Soybean oil	70	630	70	630	70	630	70	630
t-Butylhydroquinone	0.014	0	0.014	0	0.014	0	0.014	0
Mineral mix S10022G	35	0	35	0	35	0	35	0
Vitamin mix V10037	10	40	10	40	10	40	10	40
Choline bitartrate	2.5	0	2.5	0	2.5	0	2.5	0
Total	952	4000	987.5	4000	1041.21	4000	1031.25	4000
Cellulose (gm/kg diet)	2.1		0		0		0	
Psyllium (gm/kg diet)	0		50.6		48		47.6	
Resistant starch (gm/kg diet)	0		0		96		0	
Inulin (gm/kg diet)	0		0		0		100	

The content of resistant starch is based on the CoA of Hi-Maize Corn Starch, which has 62.8% TDF-dry basis and 11.7% moisture.

Supplementary Table 5 Definitions of immune cell populations based on expression of cell surface markers.

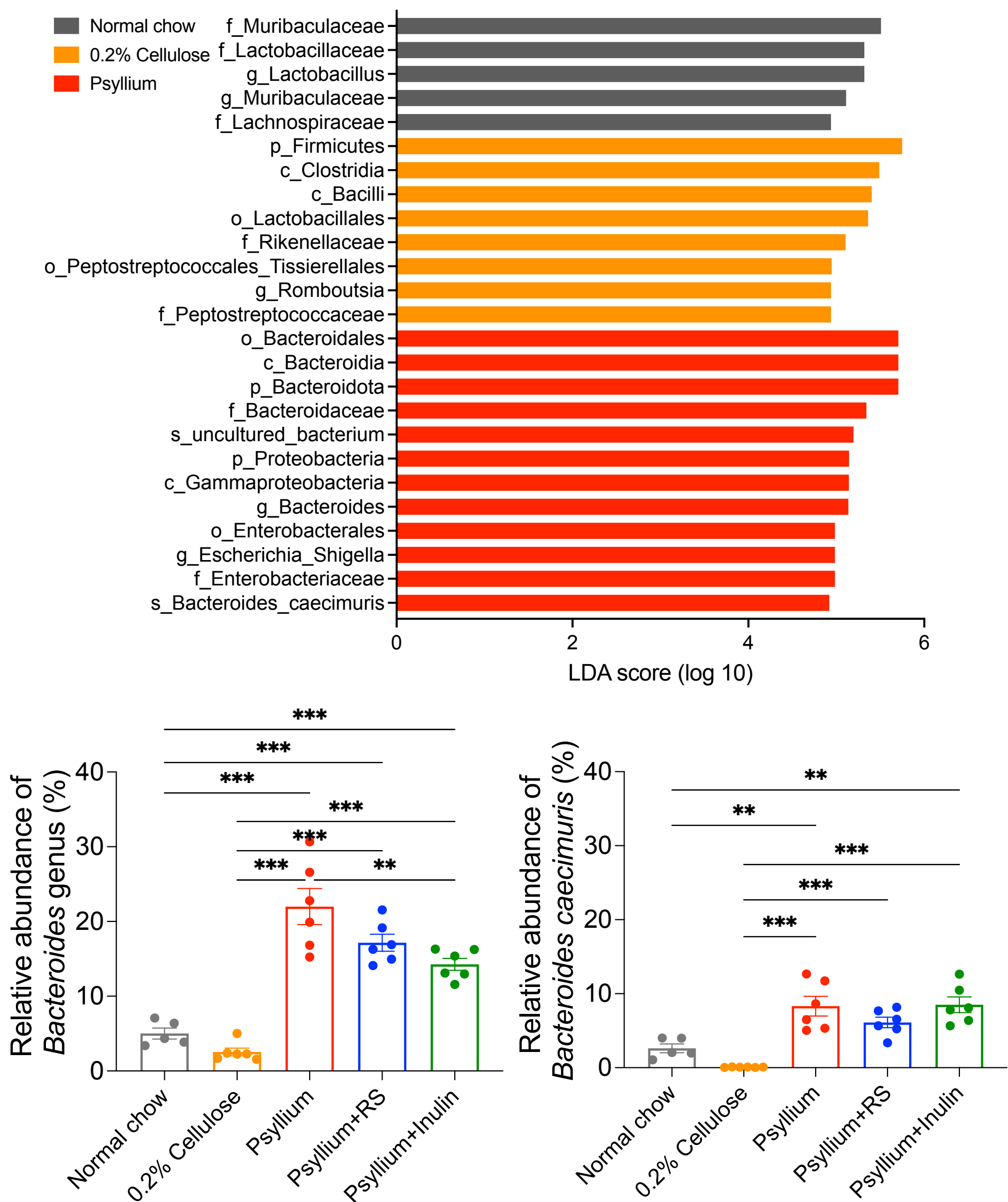
Immune cell population	Cell surface markers
Total leukocytes	CD45 ⁺
T lymphocytes	CD45 ⁺ CD3 ⁺
CD8 T cells	CD45 ⁺ CD3 ⁺ CD8 ⁺
CD4 T cells	CD45 ⁺ CD3 ⁺ CD4 ⁺
B lymphocytes	CD45 ⁺ CD19 ⁺
Natural killer (NK) cells	CD45 ⁺ CD49b ⁺
Macrophages	CD45 ⁺ F4/80 ⁺
Dendritic cells (DC)	CD45 ⁺ CD11c ⁺
Immature myeloid cells	CD45 ⁺ Gr-1 ⁺ CD11b ⁺

Supplementary Table 6 Antibody titrations and catalogue numbers.

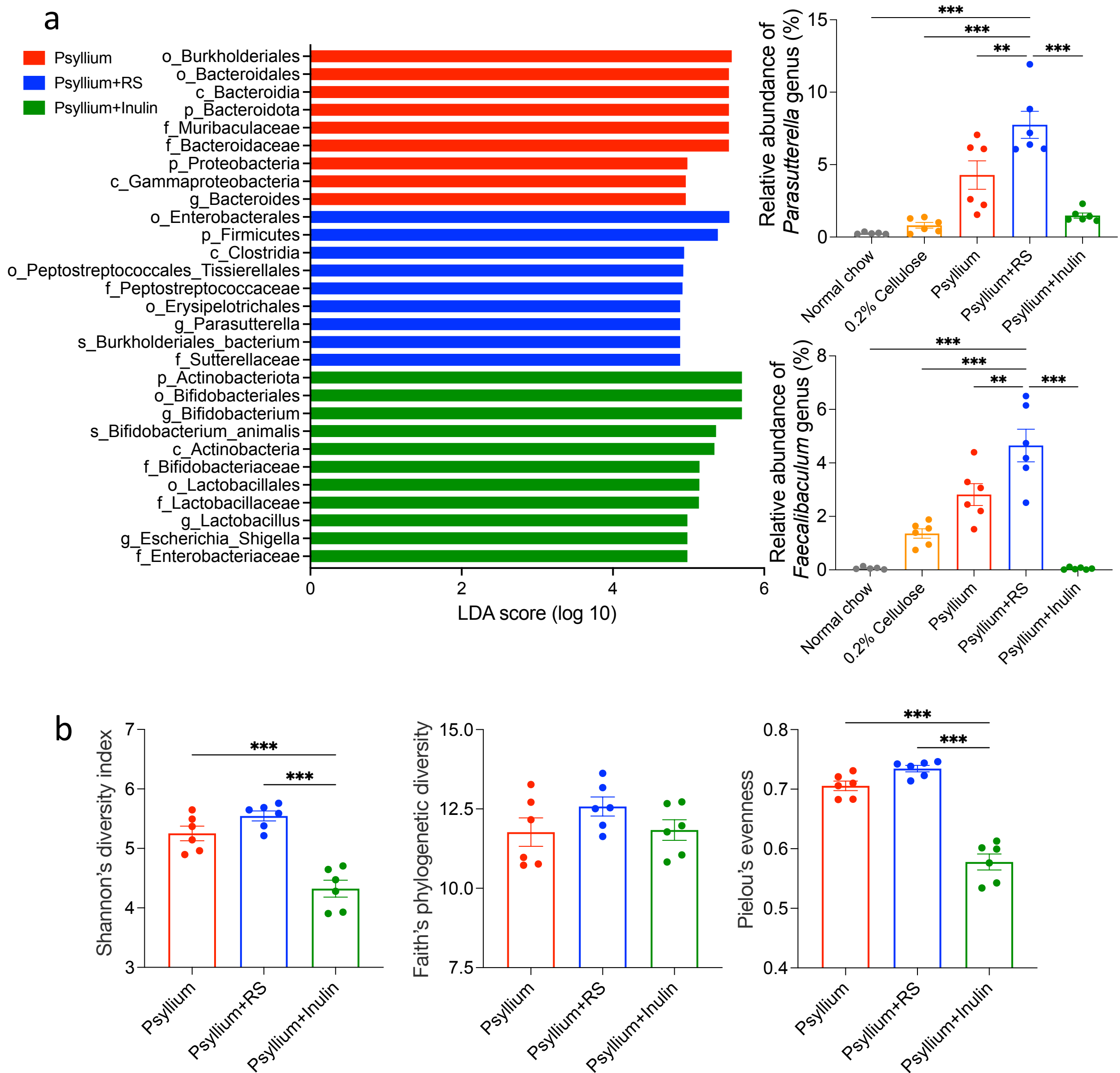
Fluorophore	Myeloid panel	Lymphoid panel
416/451	Fixable Violet Dead Cell Stain Kit (1:650; L34963)	
AF700	CD45 (1:40; eBioscience 56-0451-82)	
APC	Ly6G (1:650; eBioscience 17-5931-82)	CD49b (1:80; Cat #17-5971-82)
PE-Cy7	CD11b (1:10000; Cat #25-0112-82)	CD3 (1:40; eBioscience 25-0031-81)
FITC	CD11c (1:800; Cat #11-0114-82)	CD8 (1:160; eBioscience 11-0081-82)
PE	F4/8b (1:650; eBioscience 12-4801-82)	CD19 (1:650; Cat #12-0193-82)
PerCP-Cy5.5	Gr-1 (1:650; Cat #45-5931-80)	CD4 (1:400; Cat #45-0042-82)

Supplementary Table 7 Effect of technical factors on sample composition (Bray-Curtis)

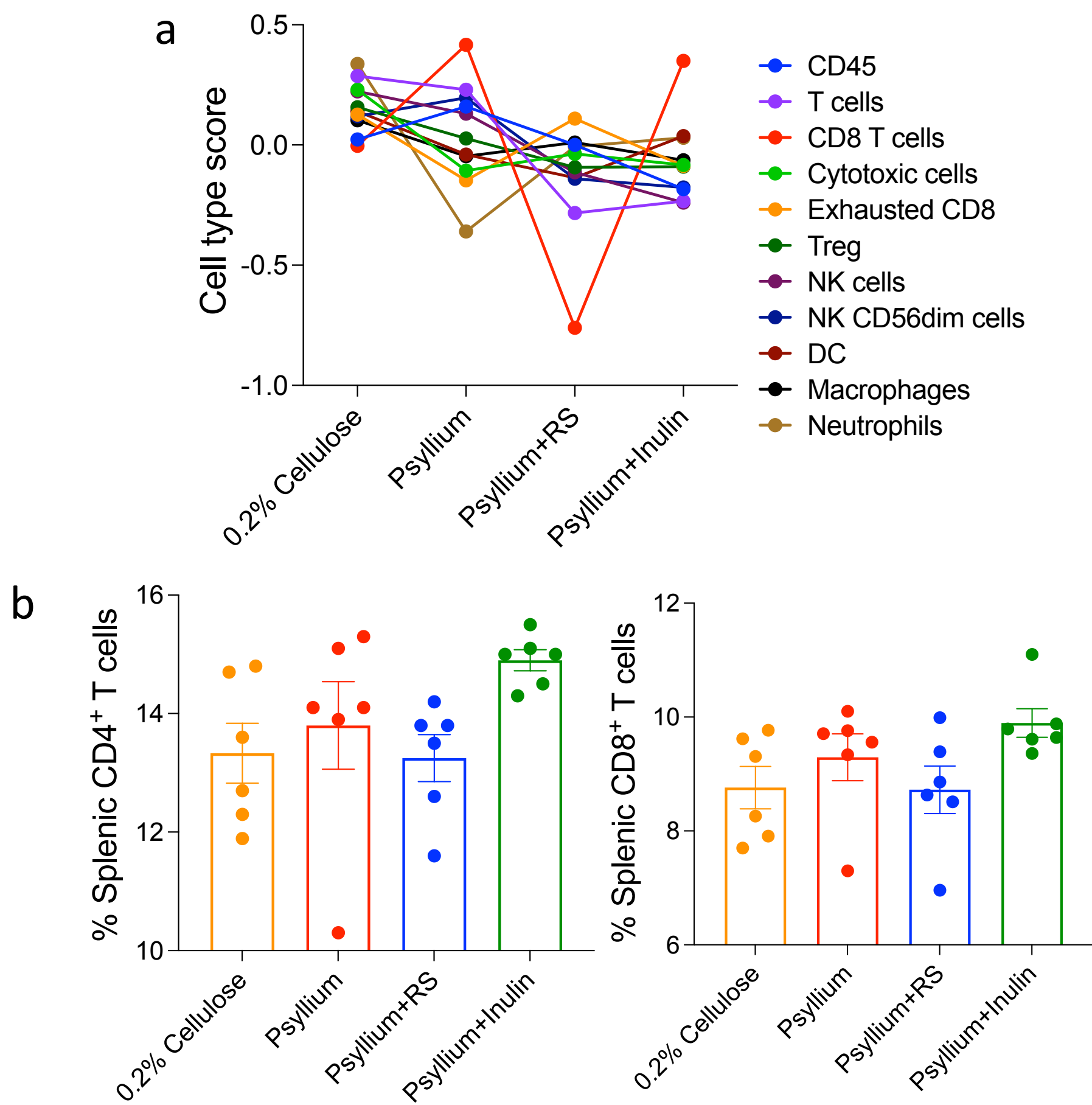
Factor	R2	Pr(>F)	
Date of processing	0.73281	0.028	*
Sex	0.01584	0.17	
Date of DNA extraction	0.17497	0.626	
Days before DNA extraction	0.01336	0.876	
Extraction kit	0.01444	0.53	



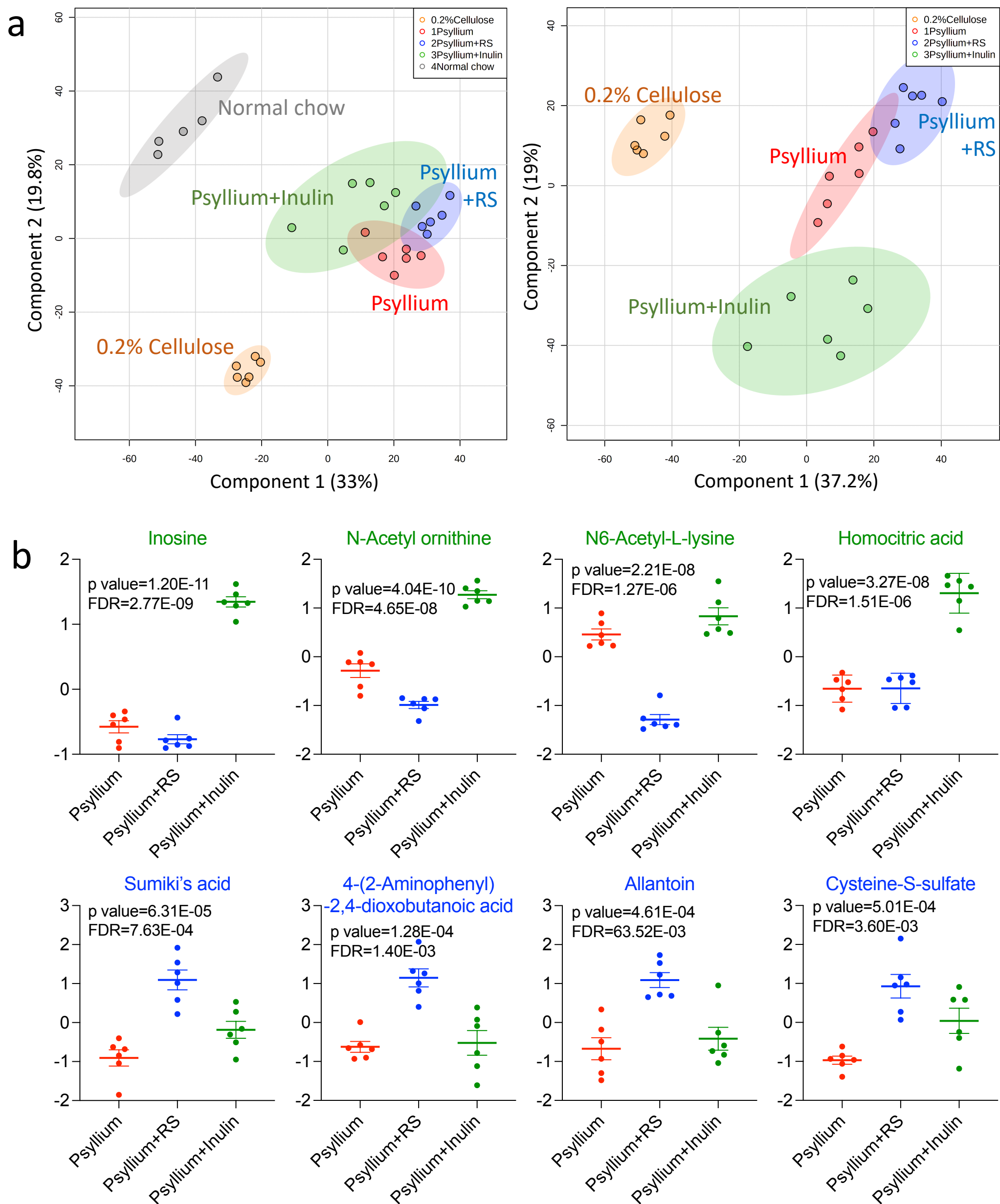
Supplementary Fig. 1 Differences in bacterial components in the normal chow, 0.2% cellulose and psyllium groups. Linear discriminant analysis (LDA) scores computed for differentially abundant taxa in the microbiomes of normal chow (grey), 0.2% cellulose (orange) and psyllium (red). Length indicates the effect size associated with a taxon. Relative abundances of *Bacteroides* genus and *Bacteroides caecimuris* of different dietary groups compared with psyllium by ANOVA. Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



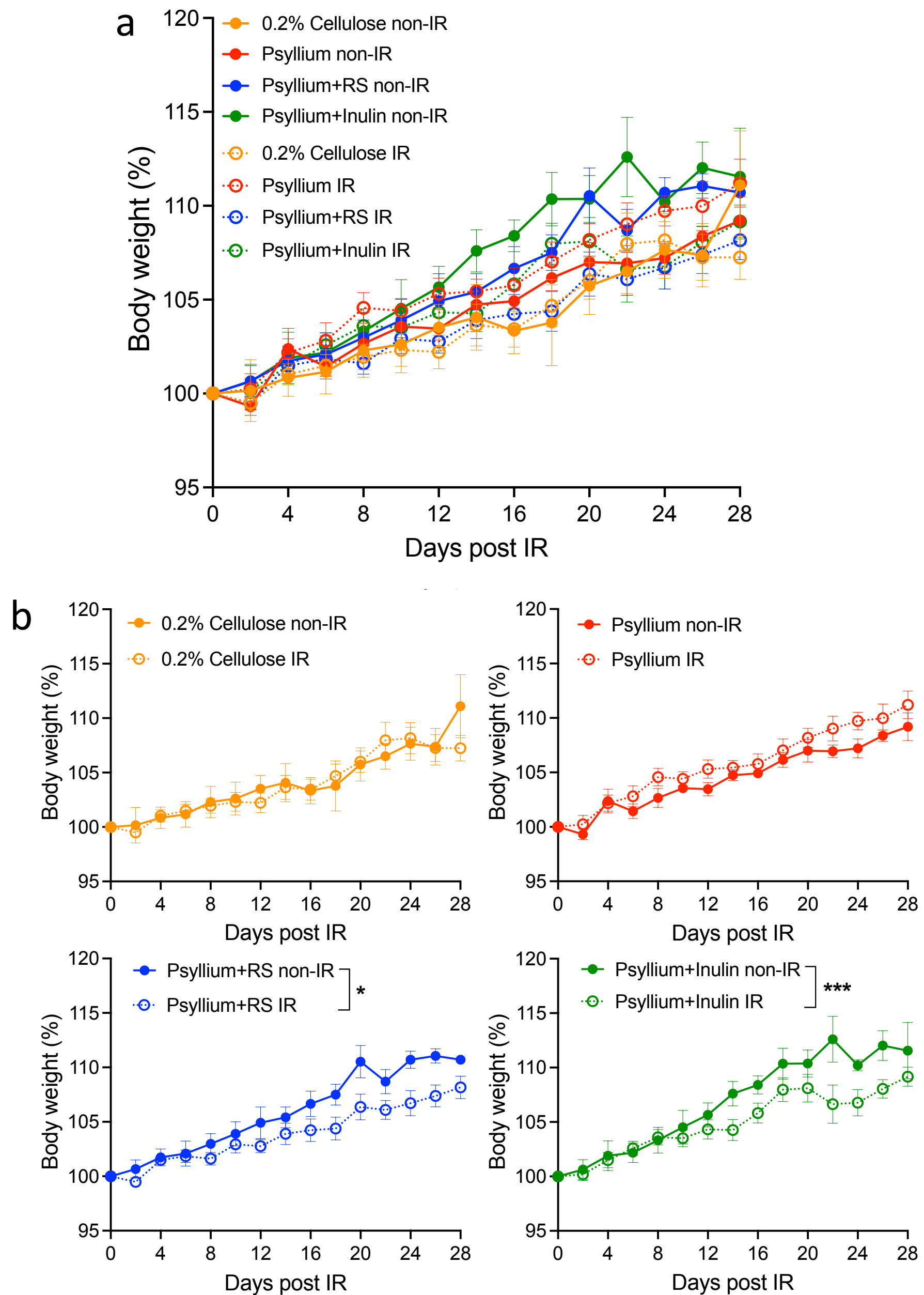
Supplementary Fig. 2 Differences in bacterial components in the psyllium, psyllium plus RS and psyllium plus inulin groups. (a) Linear discriminant analysis (LDA) scores computed for differentially abundant taxa in the microbiomes of psyllium (red), psyllium plus RS (blue) and psyllium plus inulin (green). Length indicates the effect size associated with a taxon. Relative abundances of *Parasutterella* genus and *Faecalibaculum* genus of different dietary groups compared with psyllium plus RS by ANOVA. (b) Alpha diversity using Shannon's diversity index (a measure of community richness), Faith's phylogenetic diversity (a measure of community richness that incorporates phylogenetic difference between species) and Pielou's evenness (a measure of community evenness) of faecal microbiotas. One-way ANOVA with Dunnett's multiple comparison test was used to compare the means of different dietary groups. Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



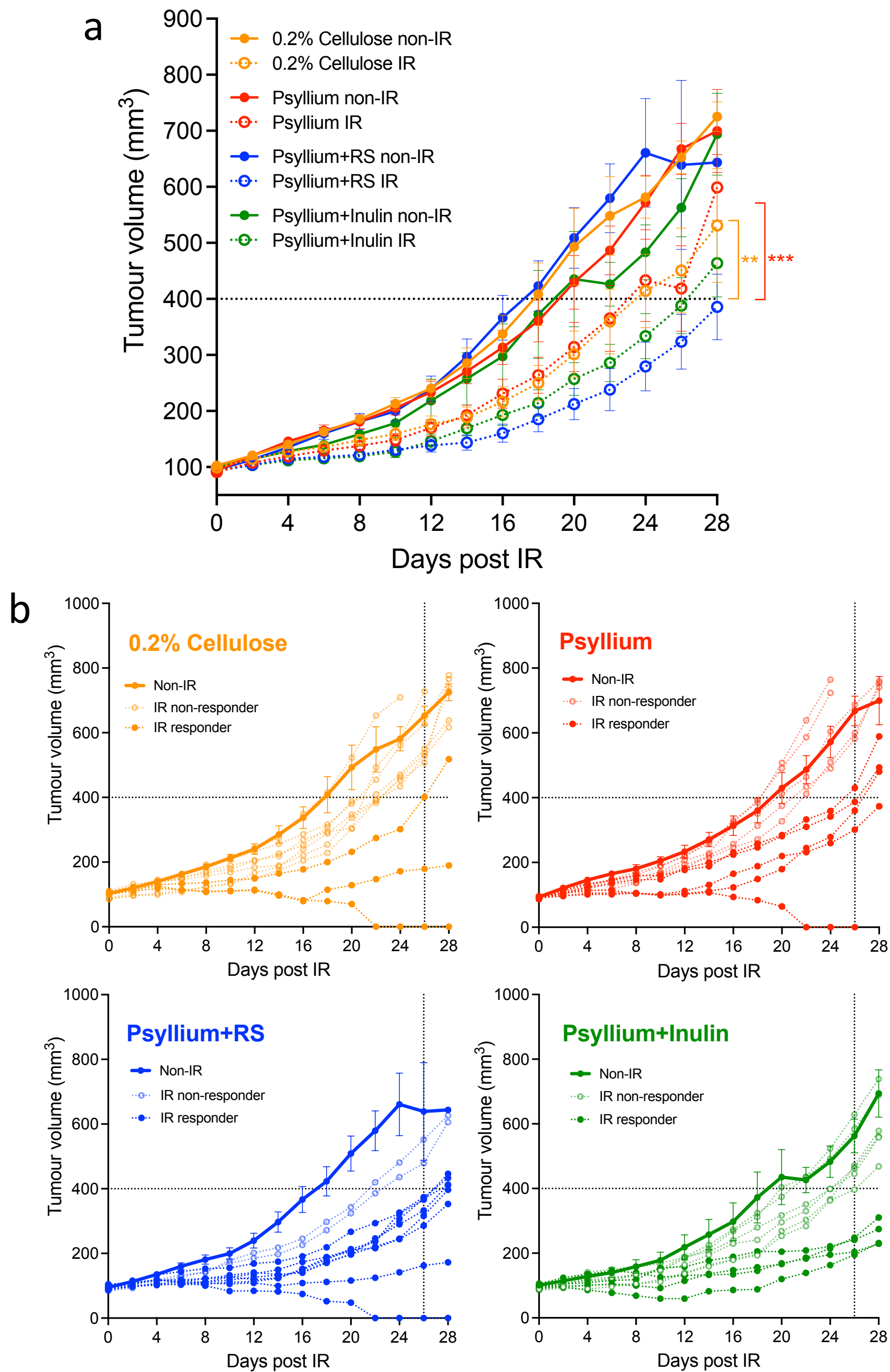
Supplementary Fig. 3 Local tumour and systemic immune responses in all dietary groups. (a) Overall immune cell profiling evaluated on a NanoString platform. (b) Percentages of helper T and cytotoxic T cells measured by flow cytometry analysis. One-way ANOVA with Dunett's multiple comparison test was used to compare the means of different dietary groups. Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



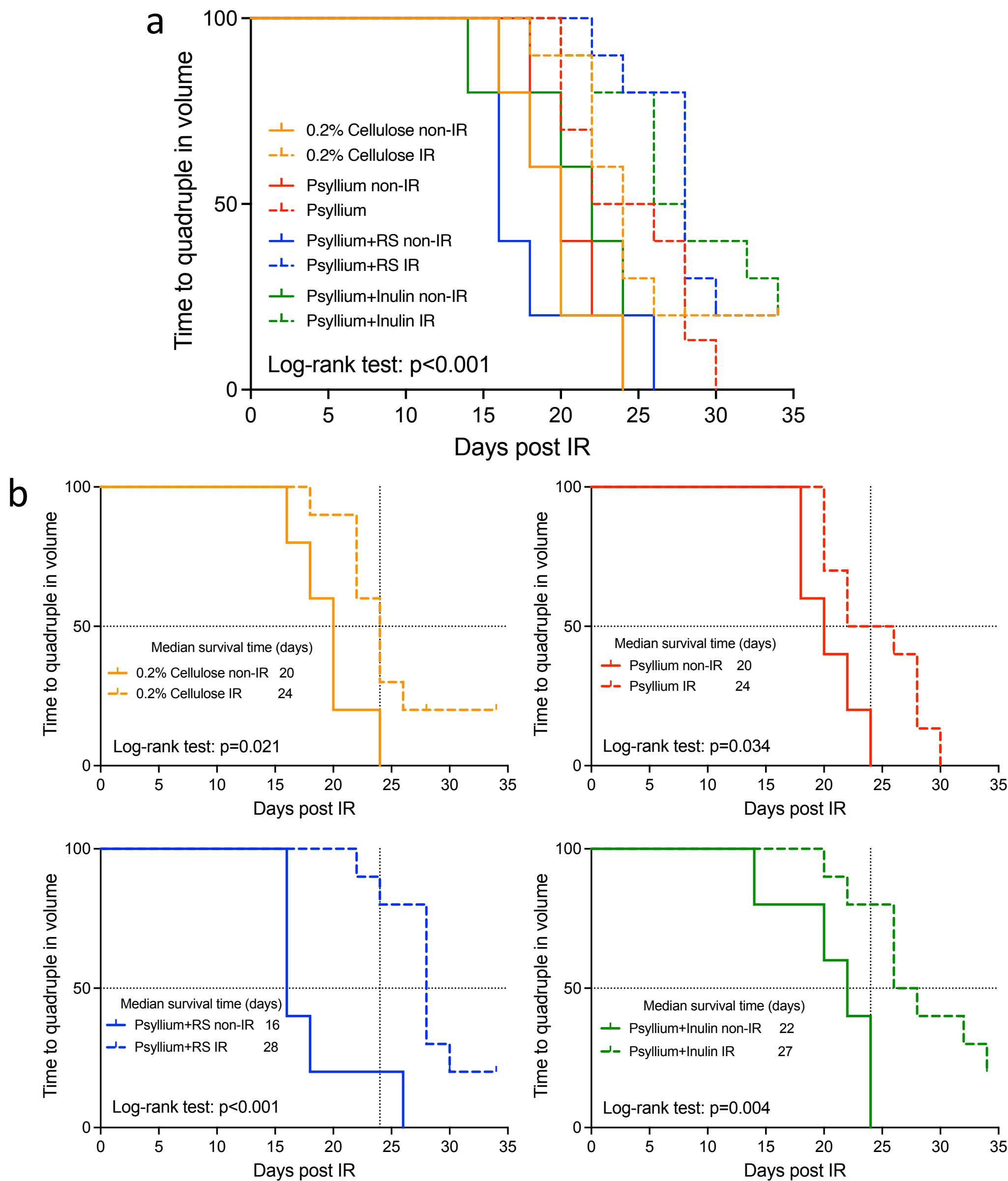
Supplementary Fig. 4 Discovery metabolomics analysis of caecal contents in all dietary groups. (a) Principal component analysis for caecal metabolites of different dietary groups. The left panel included the normal chow group, while the right panel removed it to obtain a better resolution for 0.2% cellulose and all psyllium-containing diets groups. (b) The top four metabolites which had the lowest p values in ANOVA test in each dietary group and their relative levels among different dietary groups. Data is mean $\pm$ SEM.



Supplementary Fig. 5 Body weight changes of non-IR and IR cohorts of each dietary groups for the mice that did not receive IR or following IR. Body weight curves of (a) all and (b) single dietary groups were shown. Slopes of body weight curves were calculated by linear regression and compared by ANOVA test and Dunnett's multiple comparison test. Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

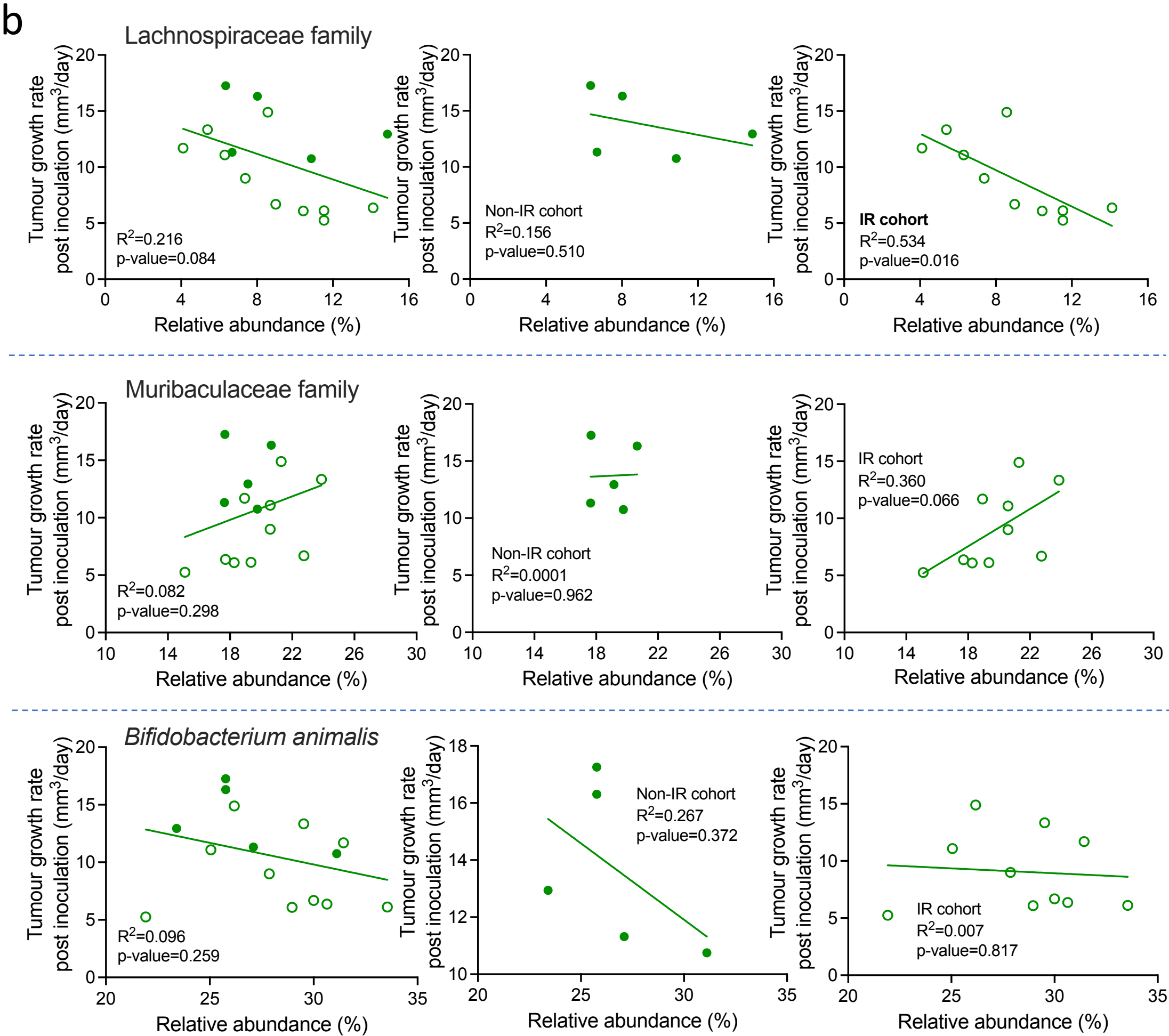
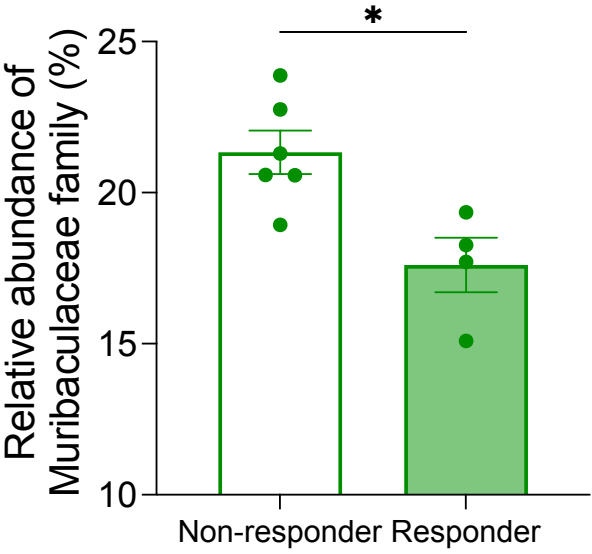
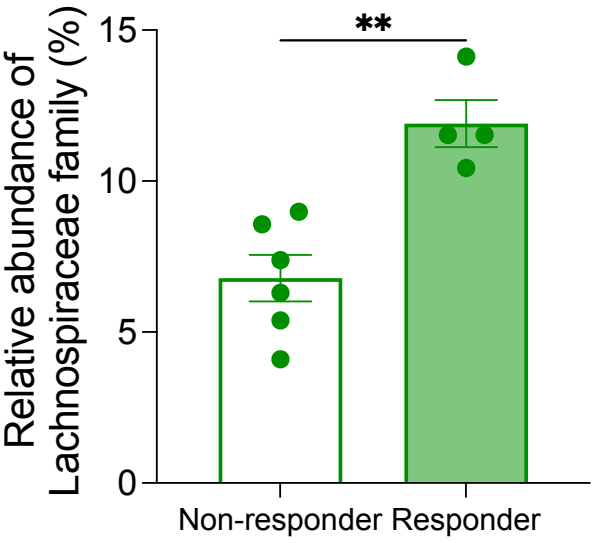
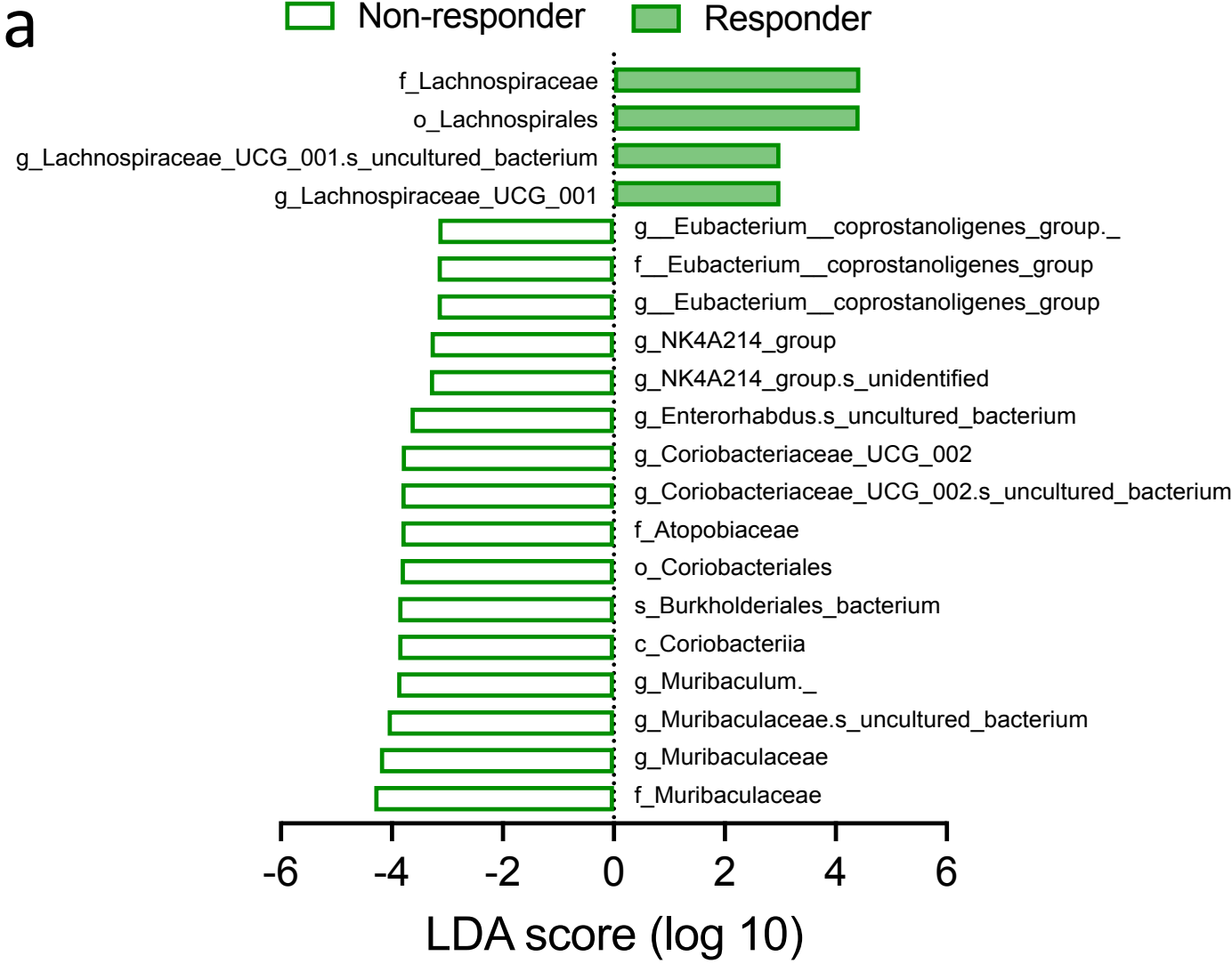


Supplementary Fig. 6 Psyllium plus RS radiosensitised UPPL1591 bladder cancer cell allografts. Treatment of irradiated (6 Gy) UPPL1591 allograft with 0.2% cellulose, psyllium, psyllium plus RS or inulin (n=5 for non-IR and n=10 for IR in each group). (a) The overall growth curves of non-irradiated and irradiated mice were plotted in solid and dotted lines, respectively. In panel (b), solid lines were mean of tumour growth curves of non-IR mice and dotted lines were individual growth curves of IR mice. Day 0 represents the day when tumours reached 80-100 mm³ and received IR. Data shown as mean and standard error. Slopes of tumour curves were calculated by linear regression to represent tumour growth rates and compared by ANOVA test and Dunnett's multiple comparison test. Data are presented as mean $\pm$ SEM. *p<0.05; **p<0.01; ***p<0.001.

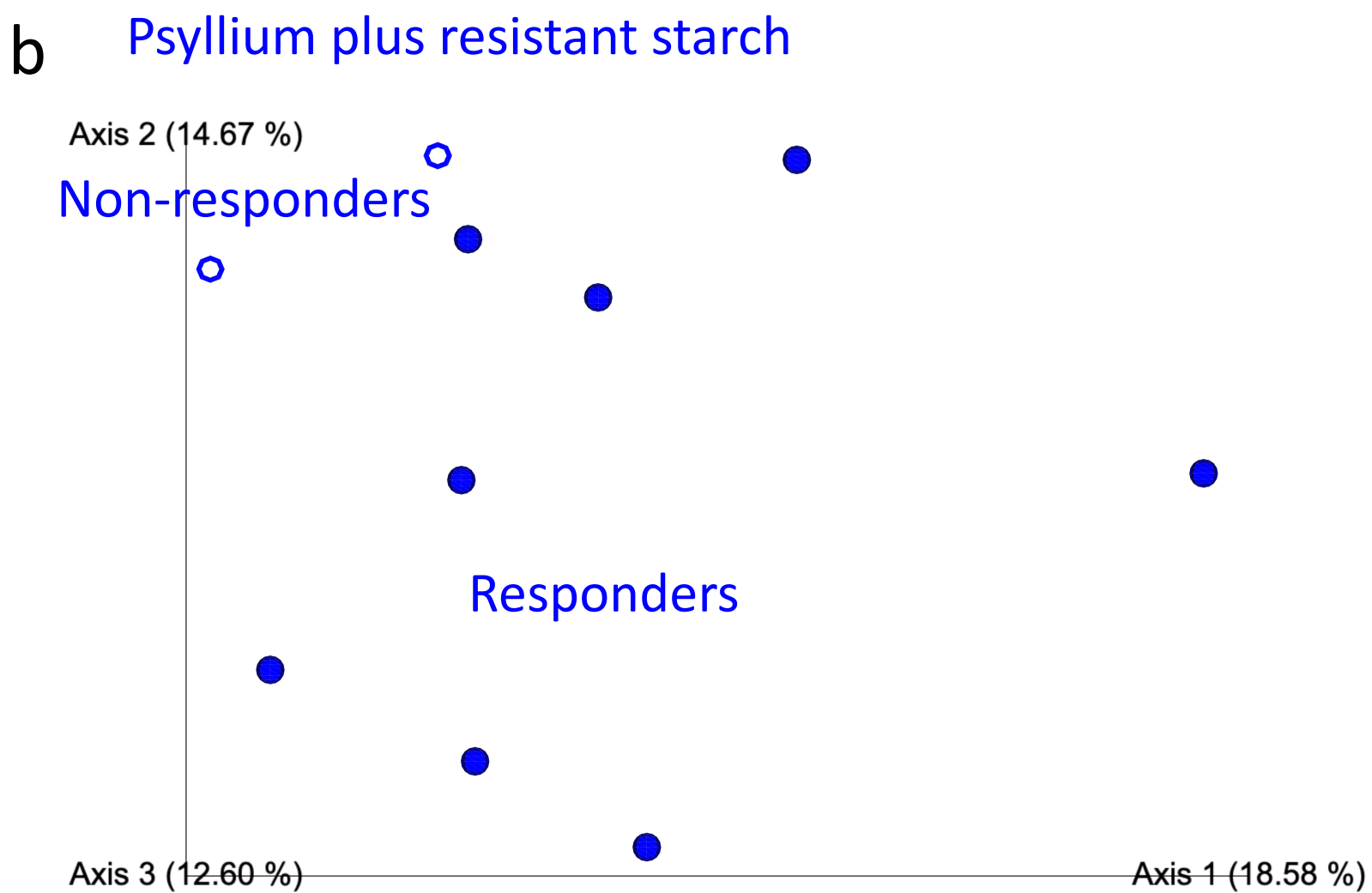
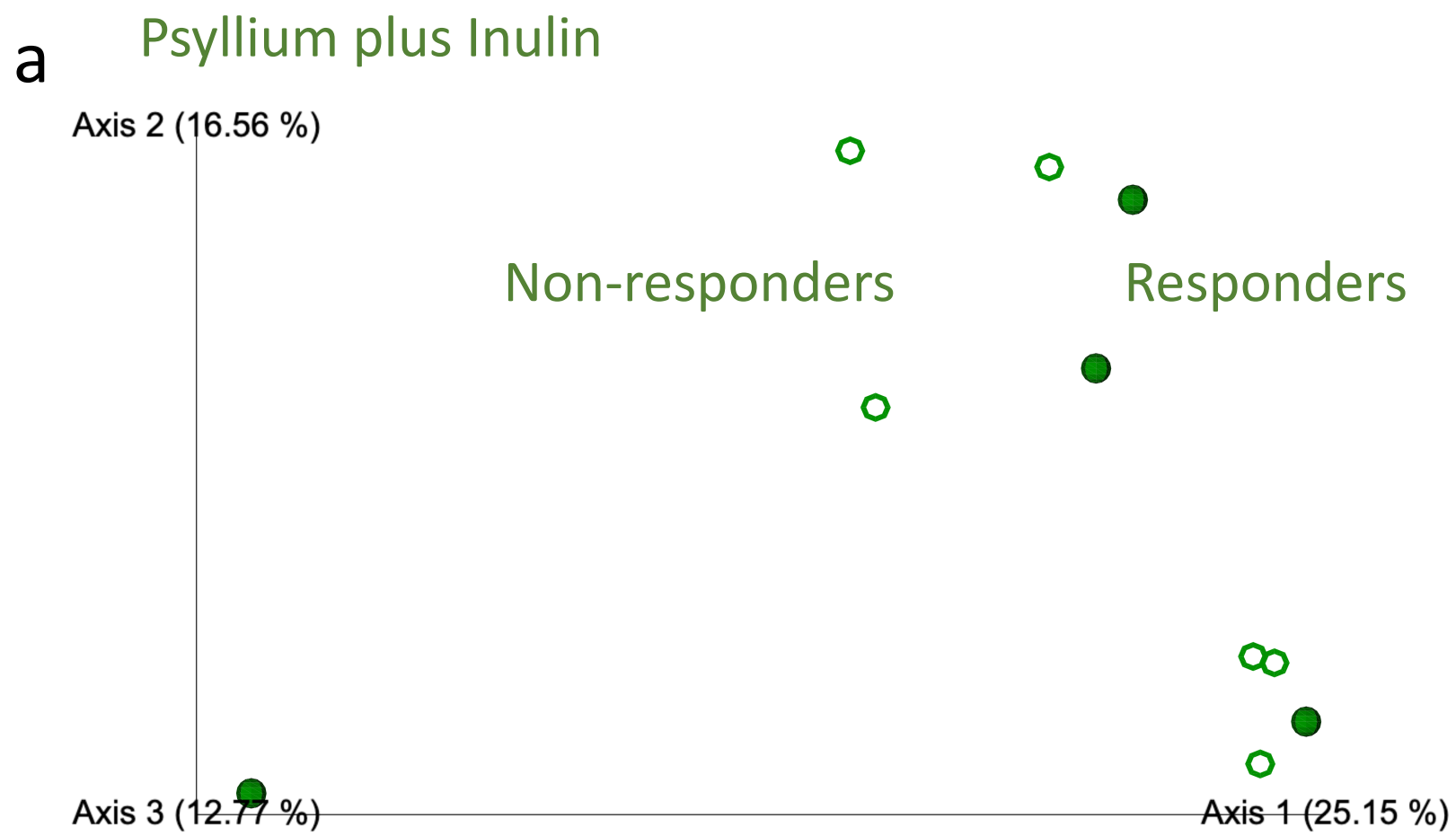


Supplementary Fig. 7 Survival analysis of tumour-bearing mice without and with IR in different dietary groups. (a) Overall and (b) individual Kaplan–Meier survival curve of mice with UPPL1591 allografts showing plots of time to quadruple in tumour volume.

Psyllium+Inulin IR cohort

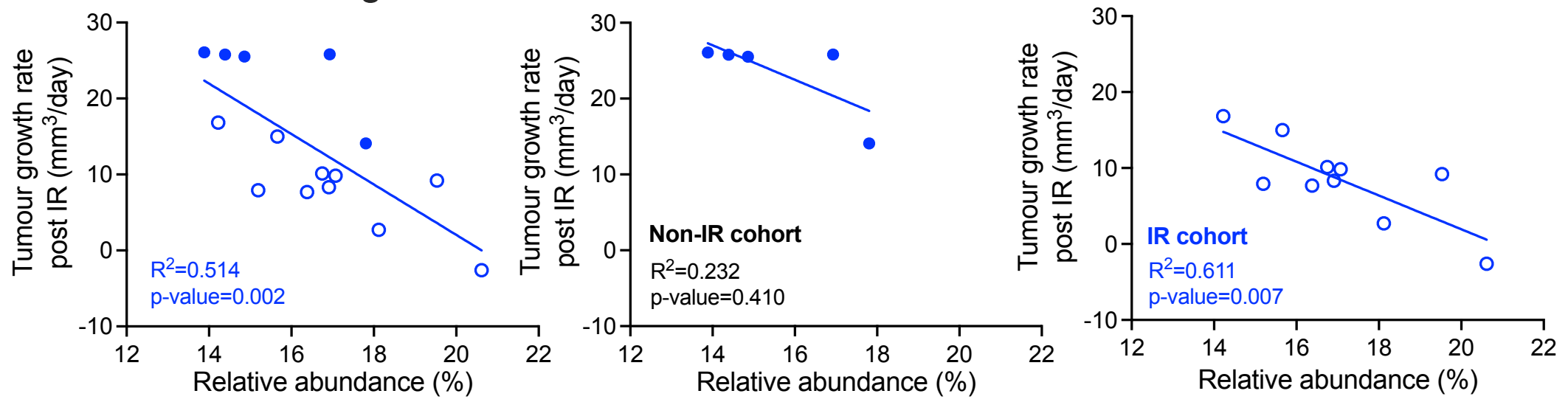


Supplementary Fig. 8 Differences in bacterial components in responders and non-responders in the psyllium plus inulin group. (a) Linear discriminant analysis (LDA) scores computed for differentially abundant taxa in the microbiomes of responders and non-responders. The alpha value was 0.05 for Kruskal-Wallis test and length of bar indicates the effect size associated with a taxon. (b) Correlation between the *Lachnospiraceae* family, *Muribaculaceae* family and *Bifidobacterium animalis* versus the tumour growth in non-IR and IR cohorts of psyllium plus inulin. Tumour curve slopes were calculated by linear regression to represent tumour growth rates.

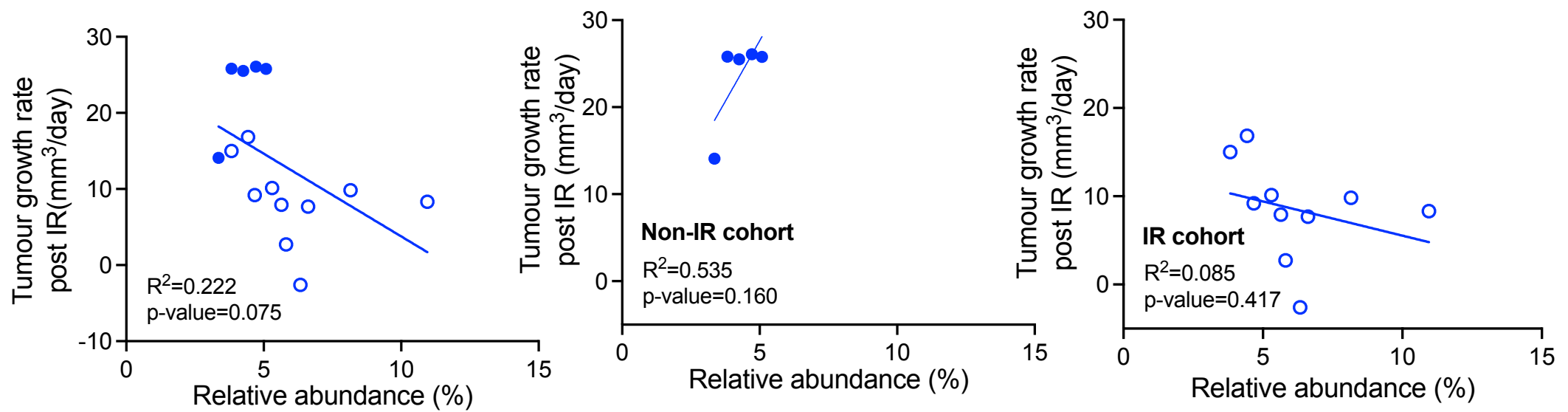


Supplementary Fig. 9 Principal coordinate analysis using Jaccard distance of faecal microbiota in the IR cohorts of (a) psyllium plus inulin or (b) psyllium plus RS. Faecal samples were collected when tumours reached 700 mm³ (n=10/IR cohort in each dietary group).

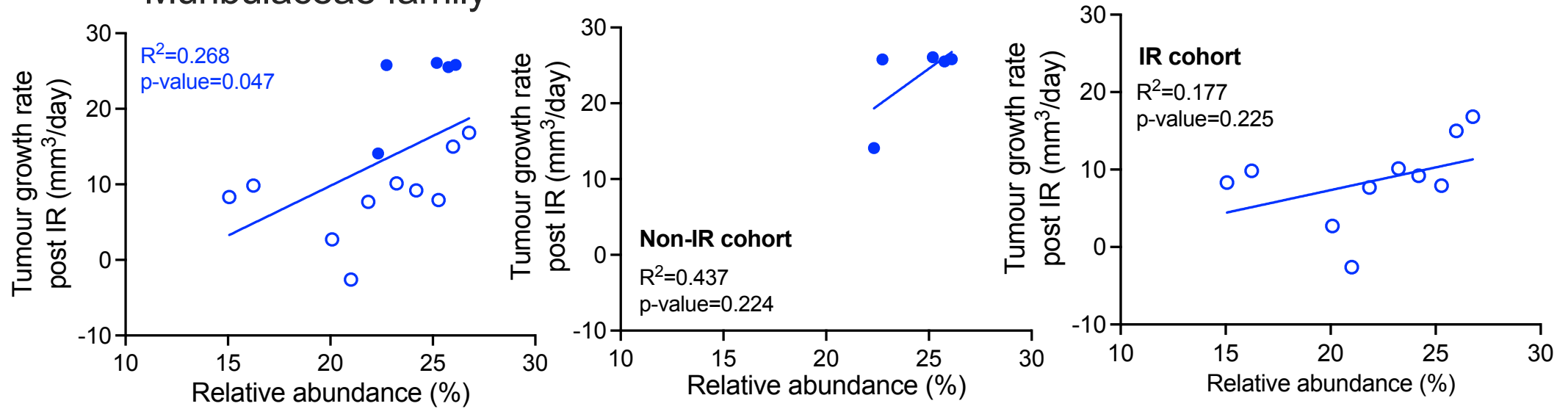
Bacteroides genus



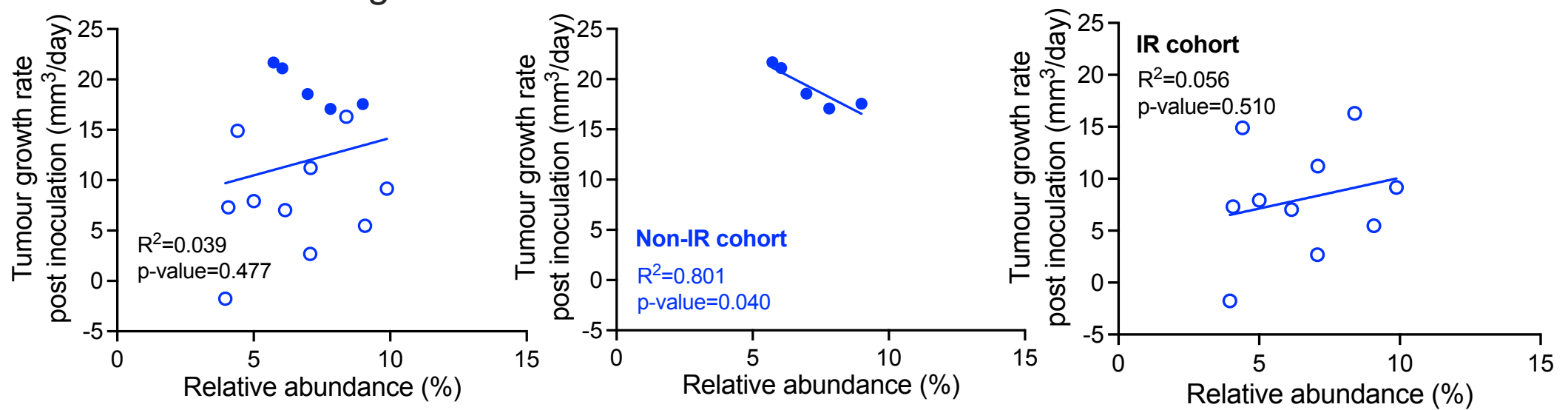
Peptostreptococcaceae family



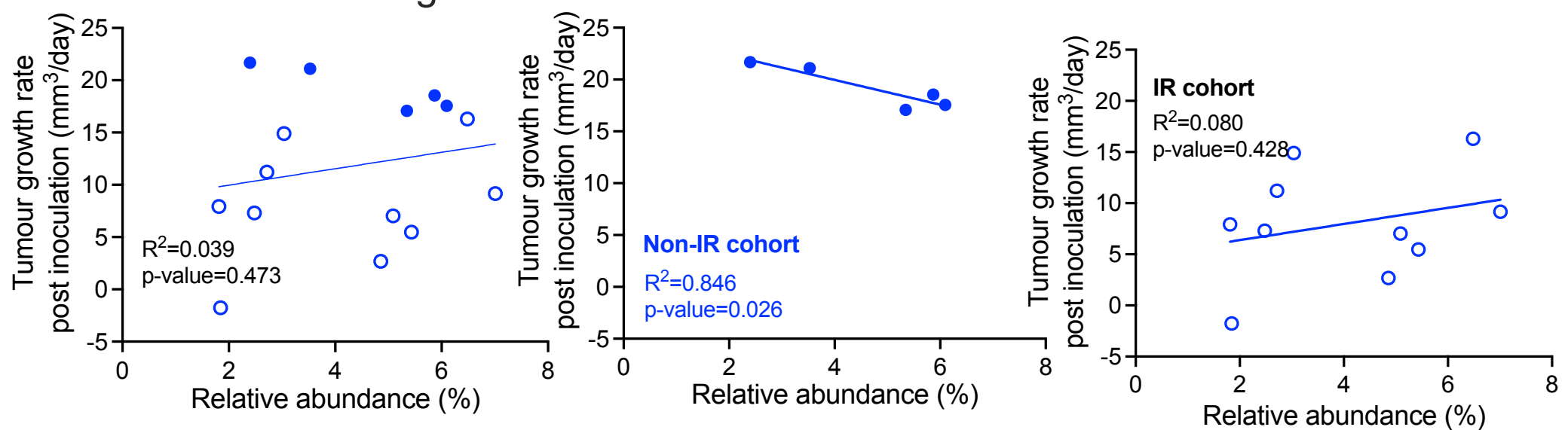
Muribulaceae family



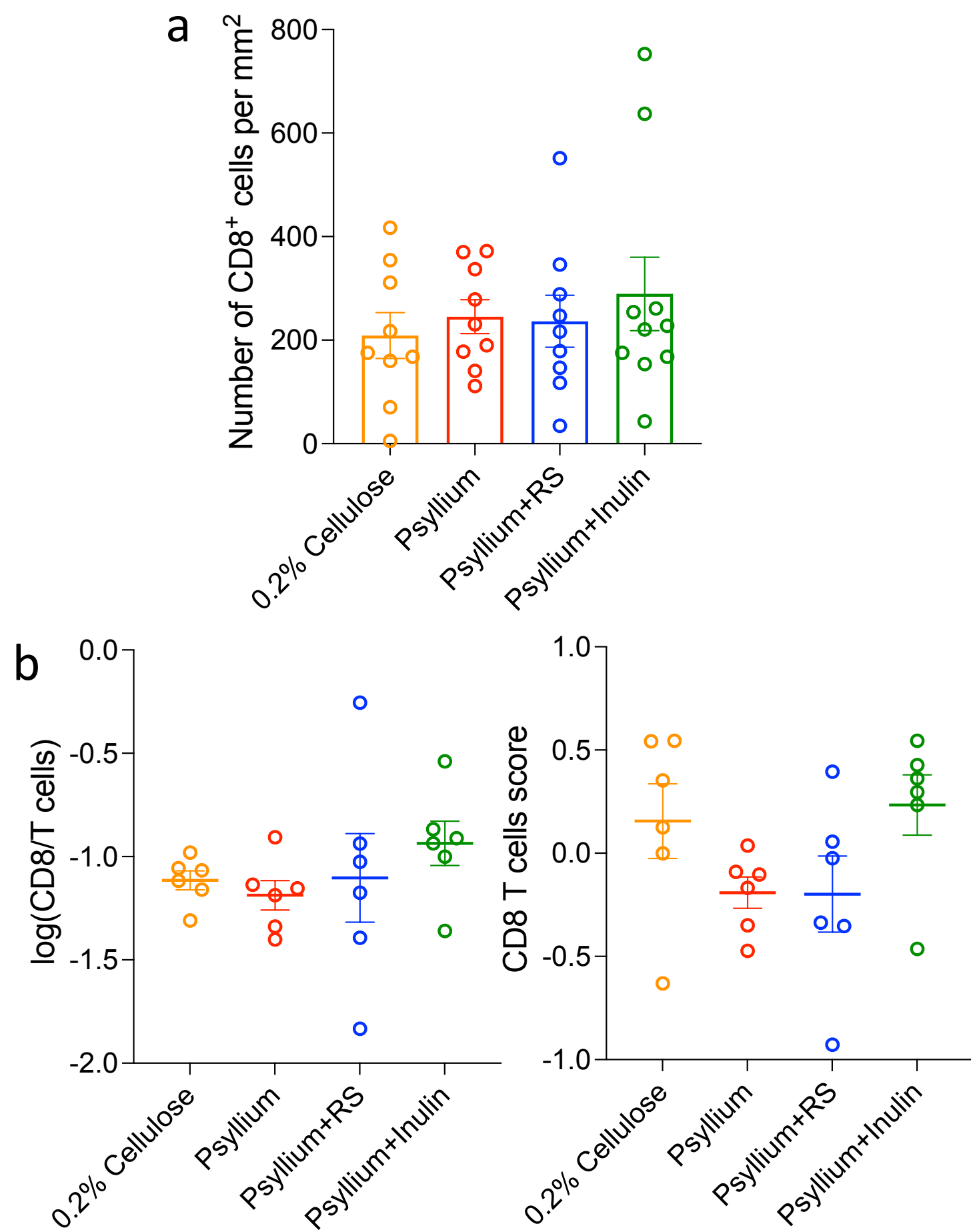
Parasutterella genus



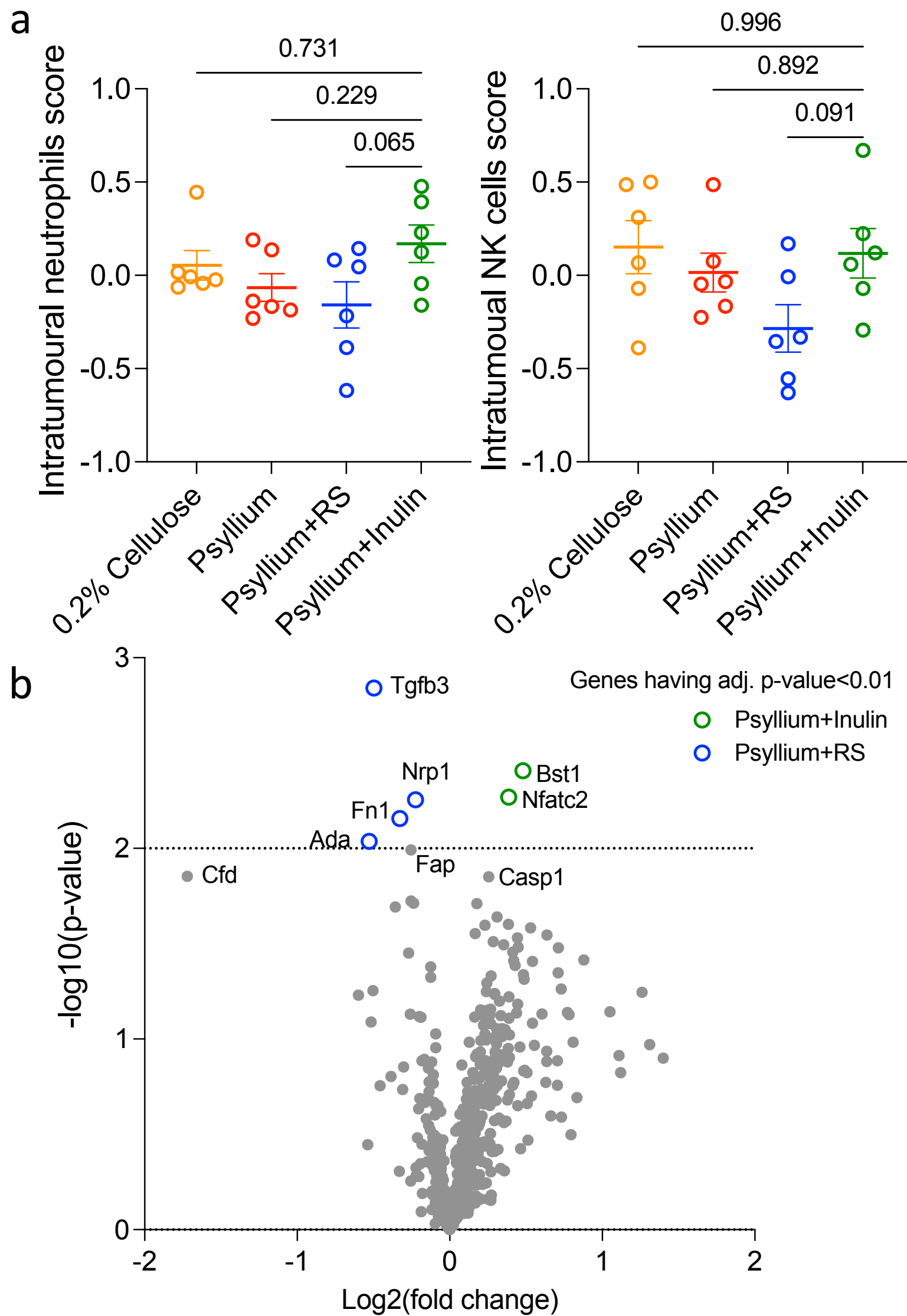
Faecalibaculum genus



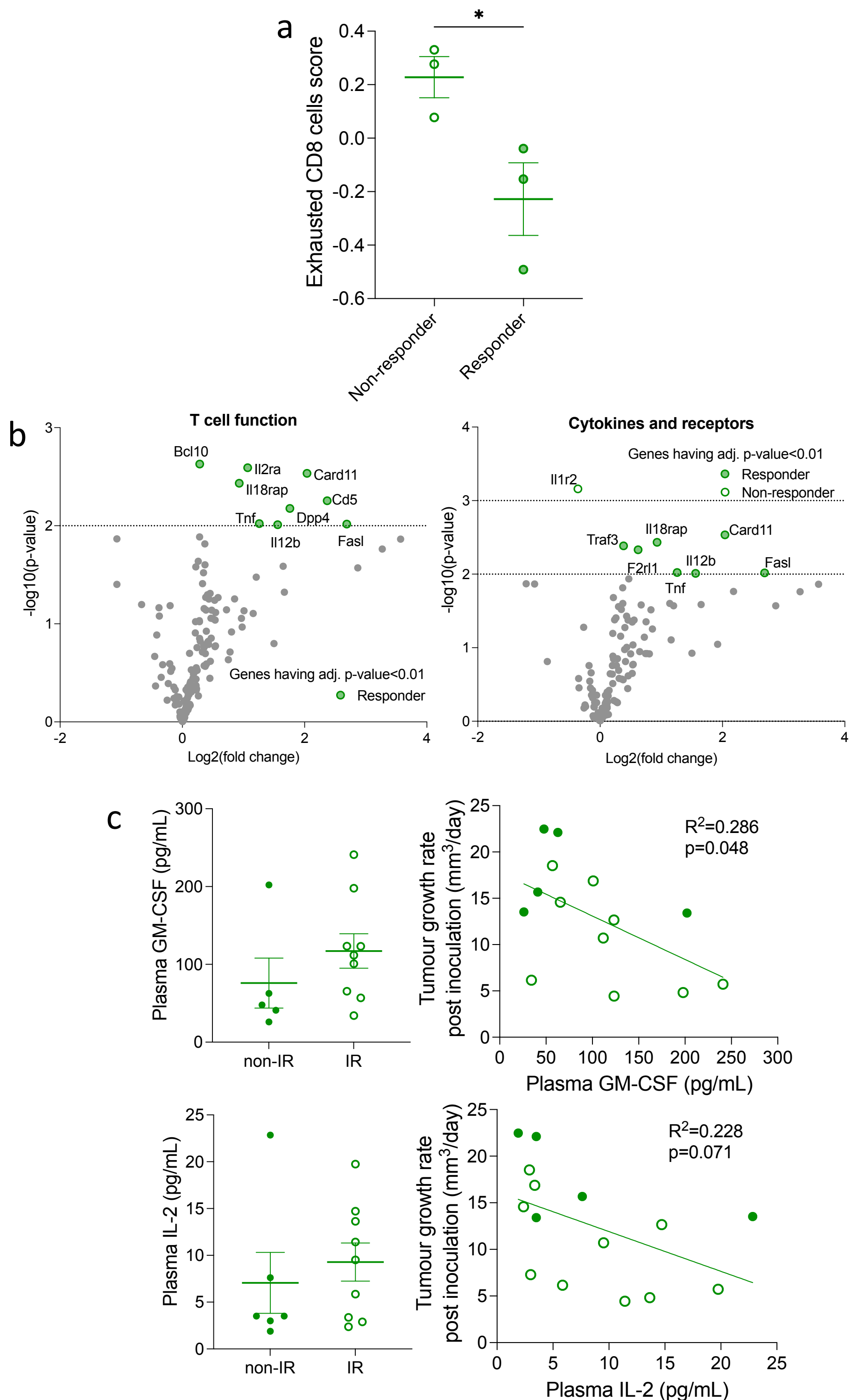
Supplementary Fig. 10 Correlation between the gut microbiota versus the tumour growth in non-IR and IR cohorts of psyllium plus RS. Tumour curve slopes were calculated by linear regression to represent tumour growth rates.



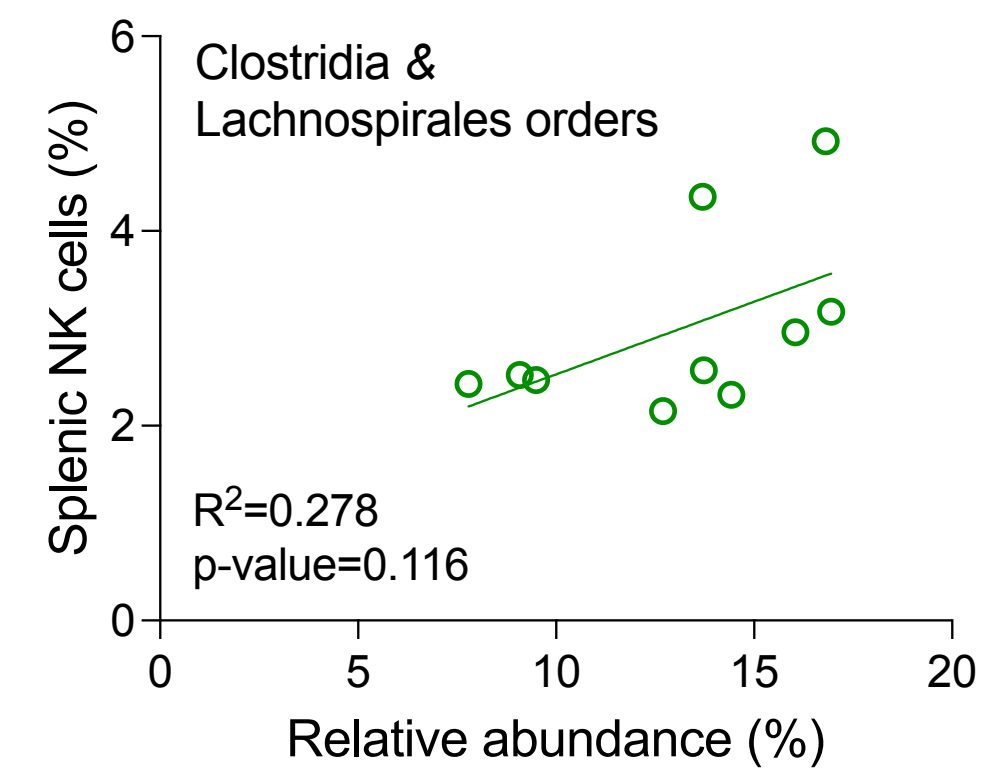
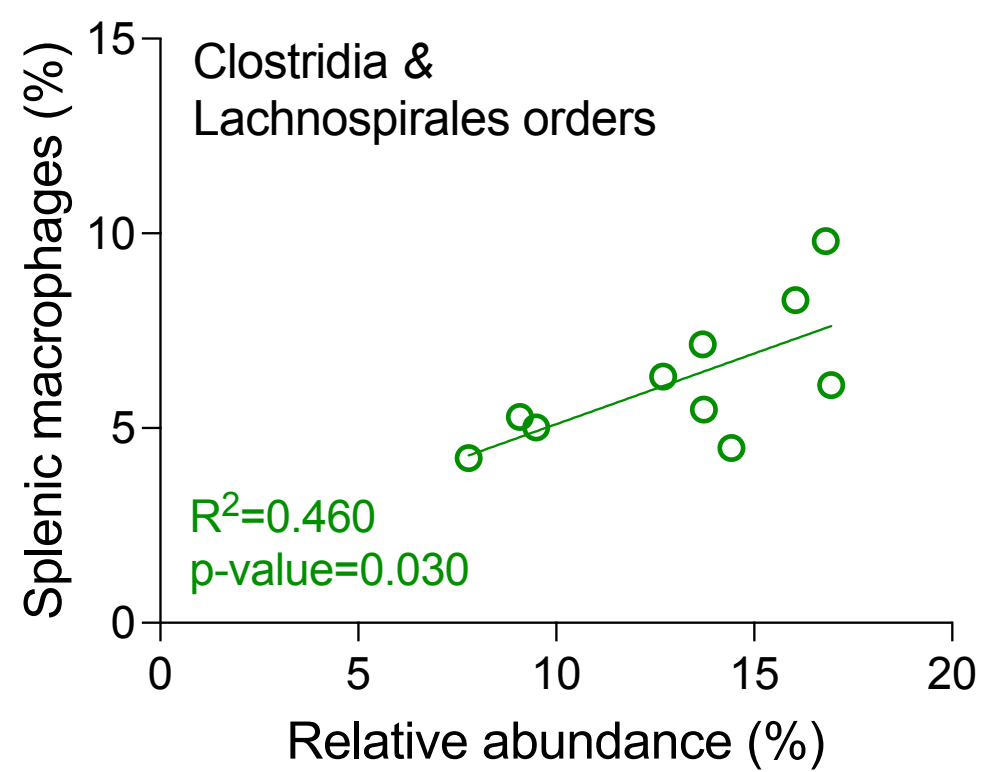
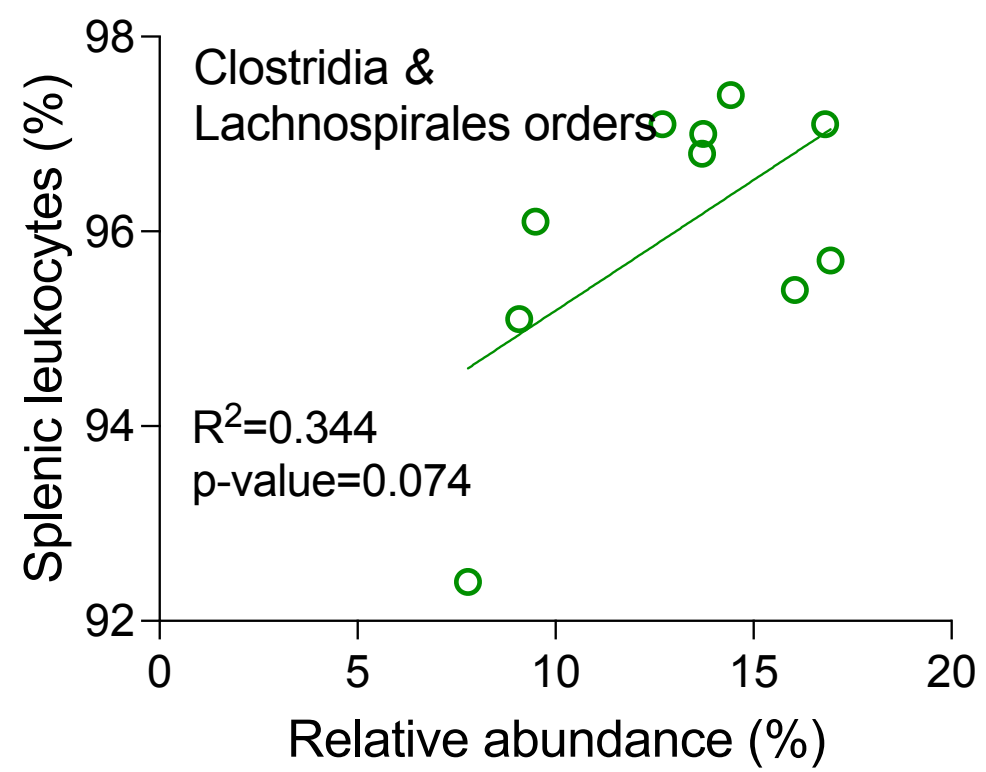
Supplementary Fig. 11 Local tumour cytotoxic T cells in the IR cohorts of all dietary groups. (a) IHC staining to assess the numbers of cytotoxic T cells in the irradiated tumours (n=10/group). (b) NanoString analysis of CD8⁺ cells and their ratio over T cells to assess the populations of cytotoxic T cells in the irradiated tumours (n=6/group). The expression of each immune cell's marker genes was normalised by a reference gene set and cell scores were calculated as the log₂(average of normalised gene expression). Data shown as mean and standard error.



Supplementary Fig. 12 Local tumour immune responses in the IR cohorts of all dietary groups and. (a) NanoString analysis of neutrophils and NK cells in the irradiated tumours (n=10/group). (b) Differential immune-related gene expression between psyllium plus RS and psyllium plus inulin groups (n=6/group). The result was partitioned by psyllium plus RS. A negative log₂(fold change) indicates the gene overexpressed in psyllium plus RS group and a positive value indicates the gene overexpressed in psyllium plus inulin group.

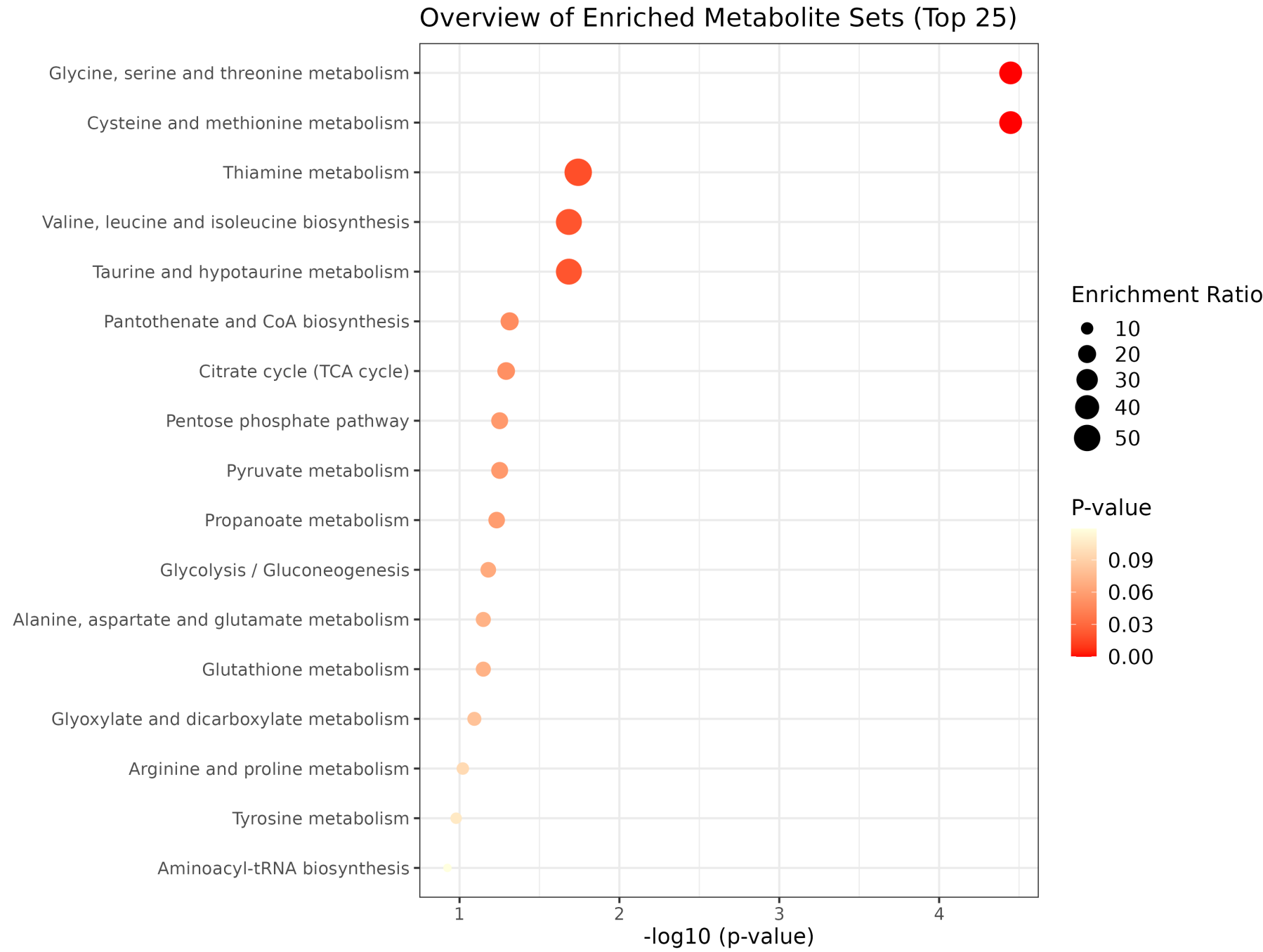


Supplementary Fig. 13 Local tumour and systemic immunity in psyllium plus inulin stratified by tumour response and IR. (a) NanoString analysis of exhausted CD8 cells and (b) gene sets of pathways of cytokines and receptors and T cell function on tumours in responders and non-responders in psyllium plus inulin groups (n=3/group). (c) Levels of plasma Th1 cytokines, GM-CSF and IL-2, stratified by IR. Correlations of these cytokines versus tumour growth rates in psyllium plus inulin group. Data are presented as mean $\pm$ SEM. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

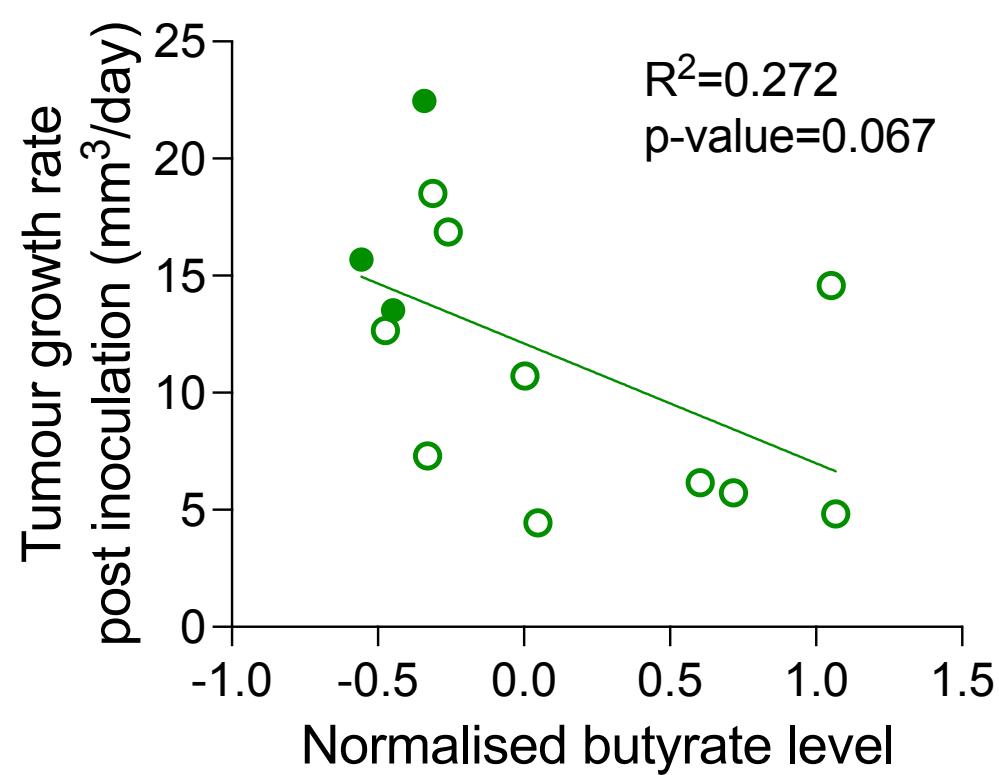
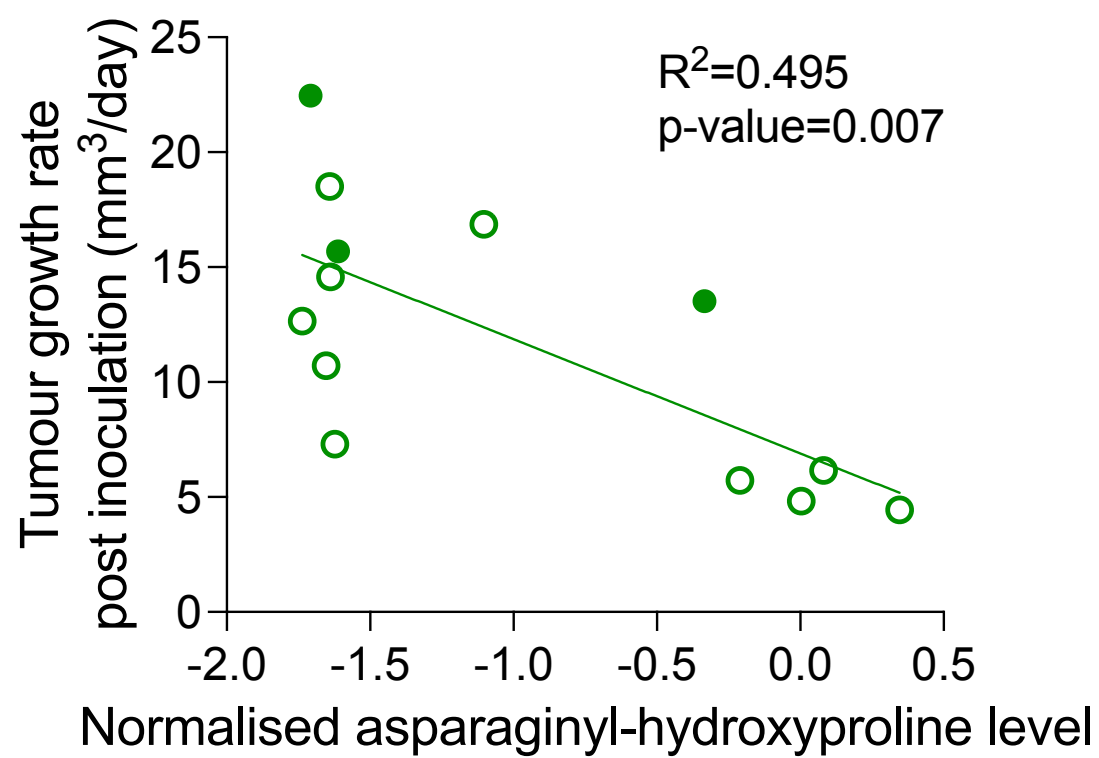
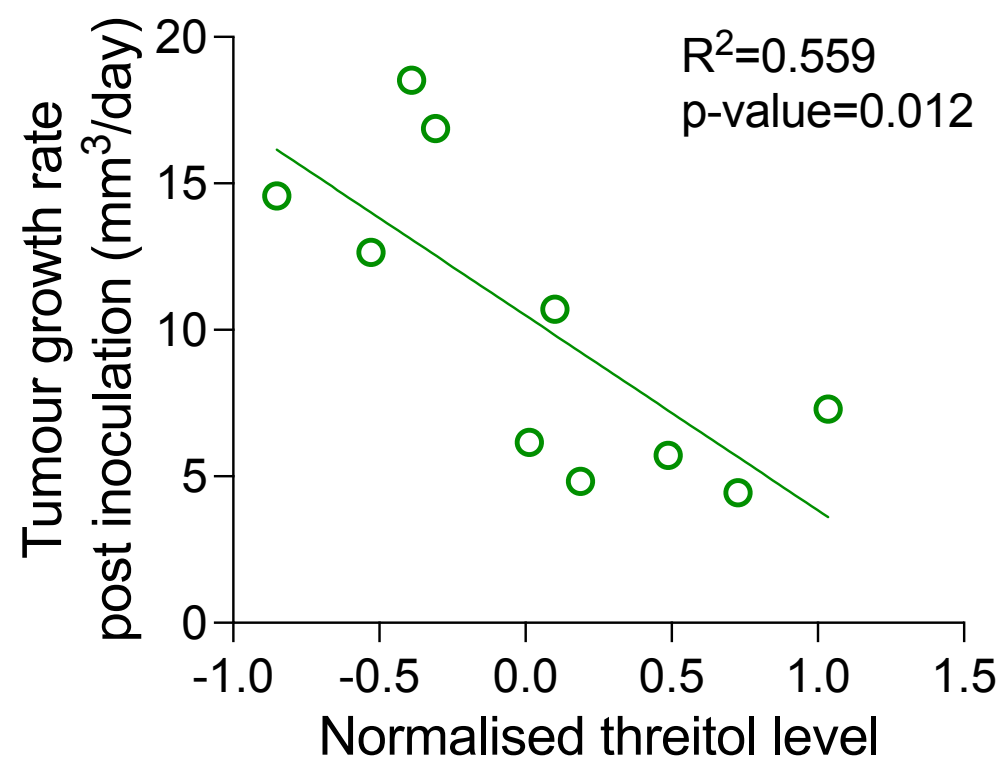


Supplementary Fig. 14 Correlations between the Clostridia and Lachnospirales orders and the populations of splenic leukocytes, macrophages, and natural killer cells in the IR cohorts of psyllium plus inulin group.

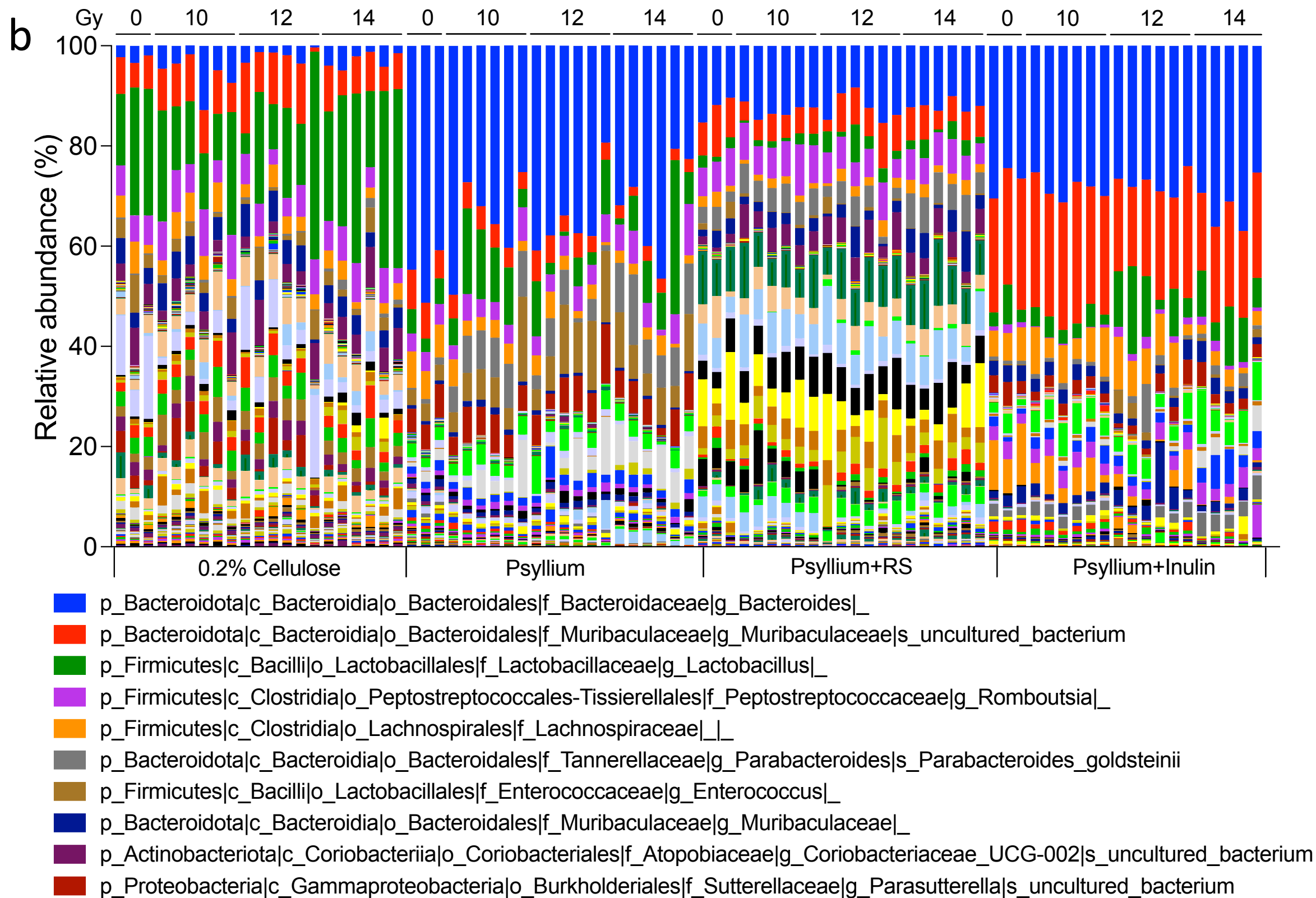
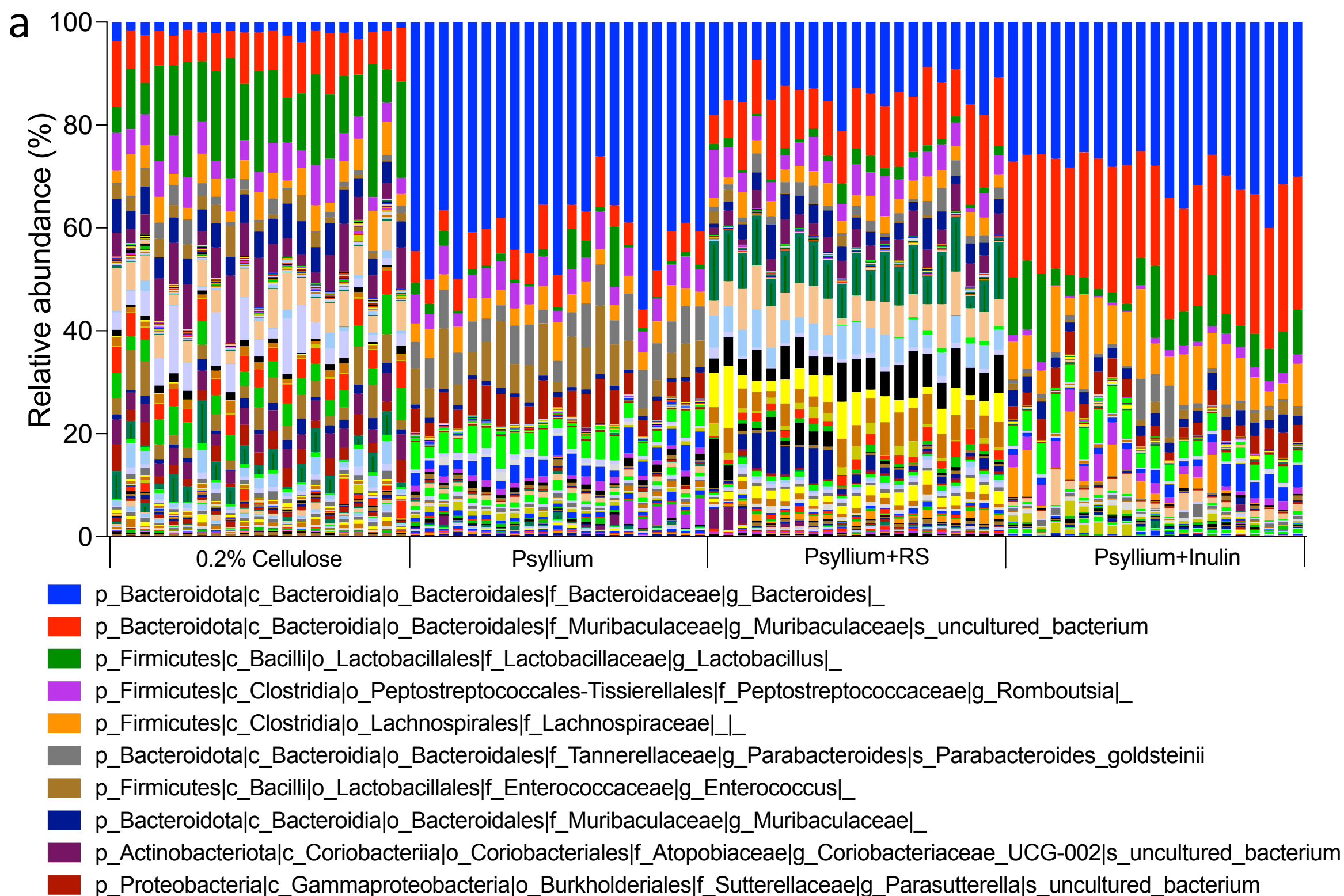
	Slope	R squared	P value
Cysteine	0.100	0.719	0.002
Deoxyribose	0.058	0.657	0.005
Malic acid	0.106	0.612	0.008
Caffeic acid	0.087	0.545	0.015
Isoferulic acid	-0.062	0.541	0.015
Pyruvic acid	0.053	0.435	0.038
2-Ketobutyric acid	0.077	0.399	0.050



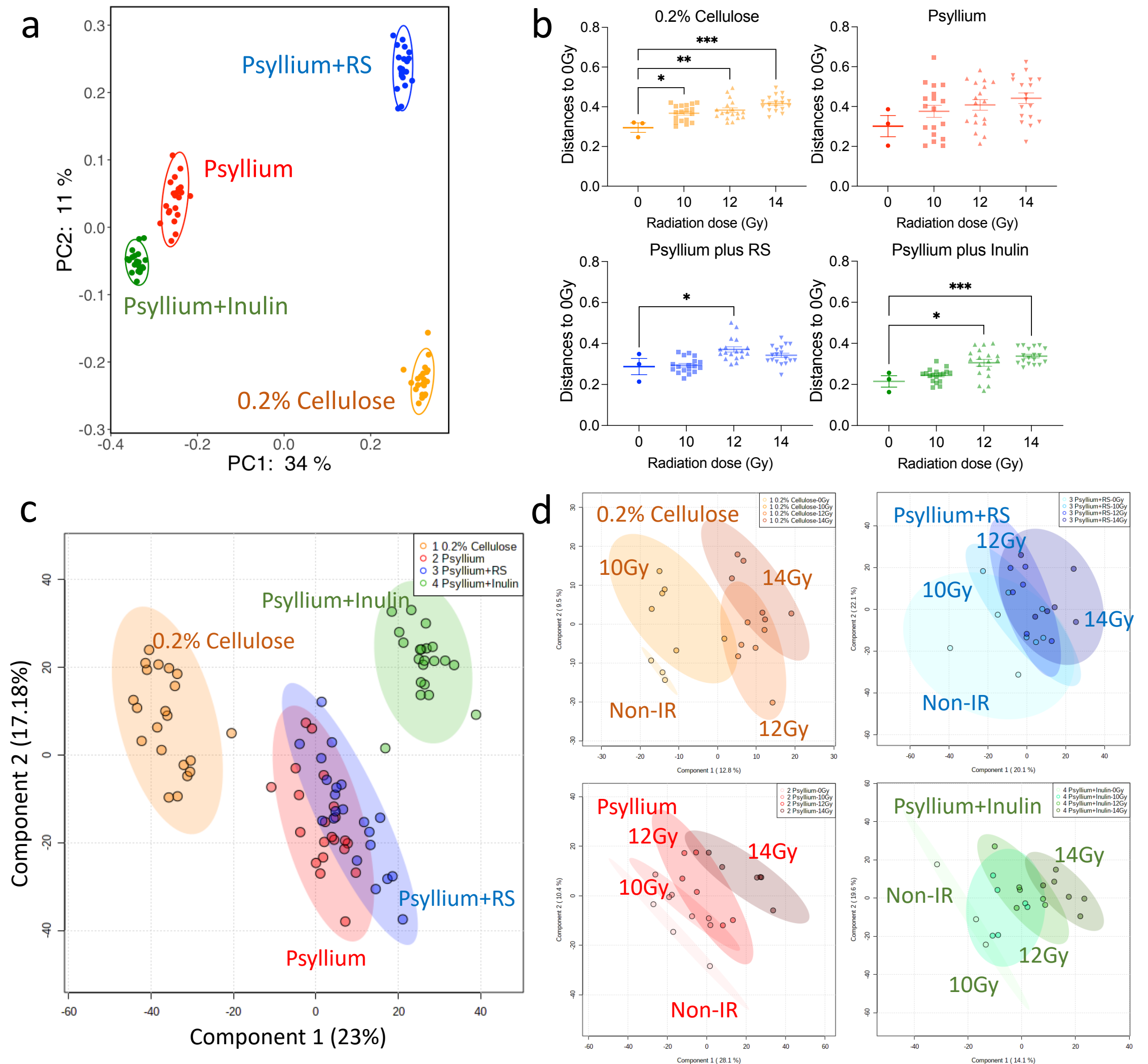
Supplementary Fig. 15 Metabolites and KEGG pathway that were associated with tumour growth in mice fed with psyllium plus RS. The top table listed the metabolites that positively (no colour) or negatively (blue) correlated to tumour growth in psyllium plus RS group. The figure below showed KEGG pathways enrichment analysis of metabolites that positively correlated to the tumour growth in psyllium plus RS. The intensity of colour denotes the P-value and the dot size denotes the enrichment ratio.



Supplementary Fig. 16 Correlations between the caecal threitol, asparaginyI-hydroxyproline and butyrate levels versus the tumour growth rate in IR cohort with or without non-IR cohort in the psyllium plus inulin group.

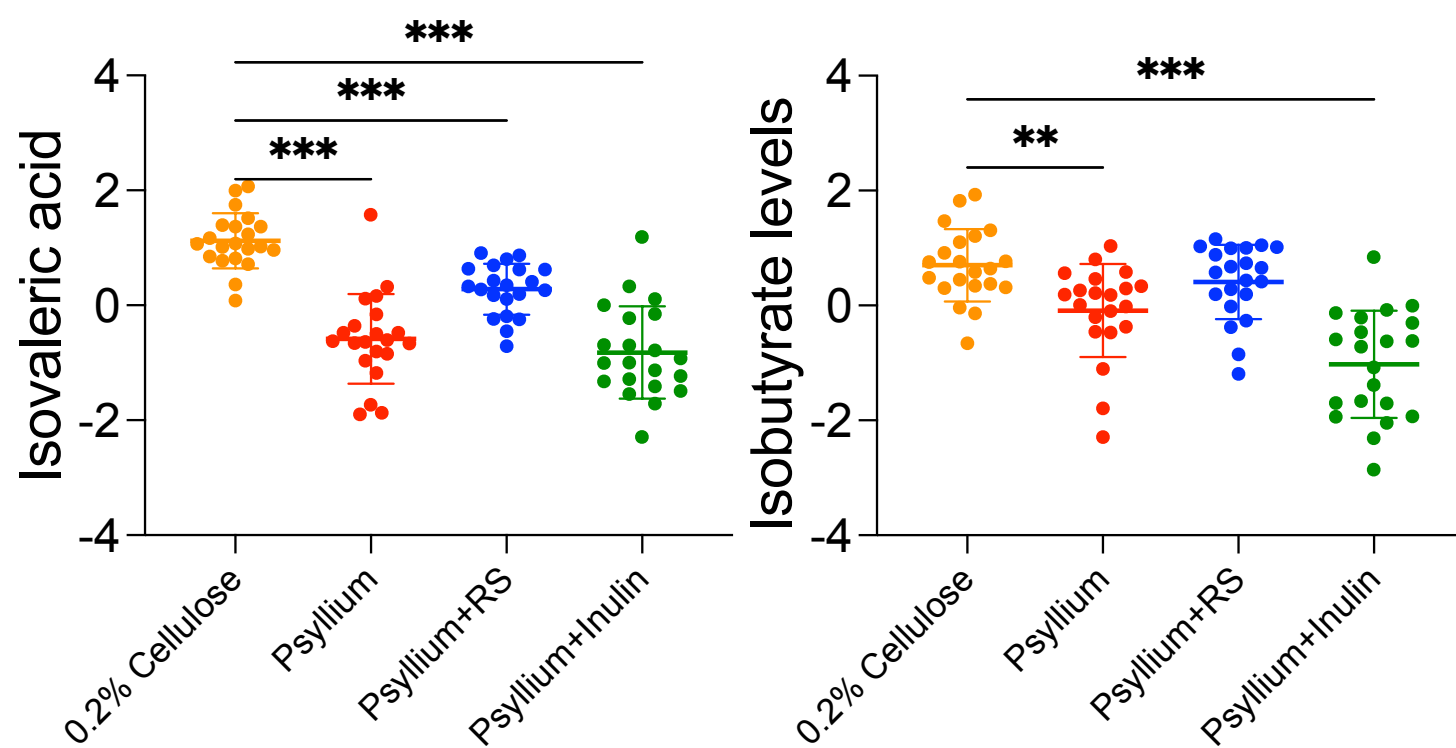


Supplementary Fig. 17 Phylogenetic composition of faecal microbiota before and after irradiation in the acute toxicity experiment. Faecal samples were collected (a) pre-IR and (b) 3.75 days post-IR (n=3/non-IR cohort, n=6/IR cohort in each IR doses and dietary group). The samples are presented in the same order in the top and bottom panels.

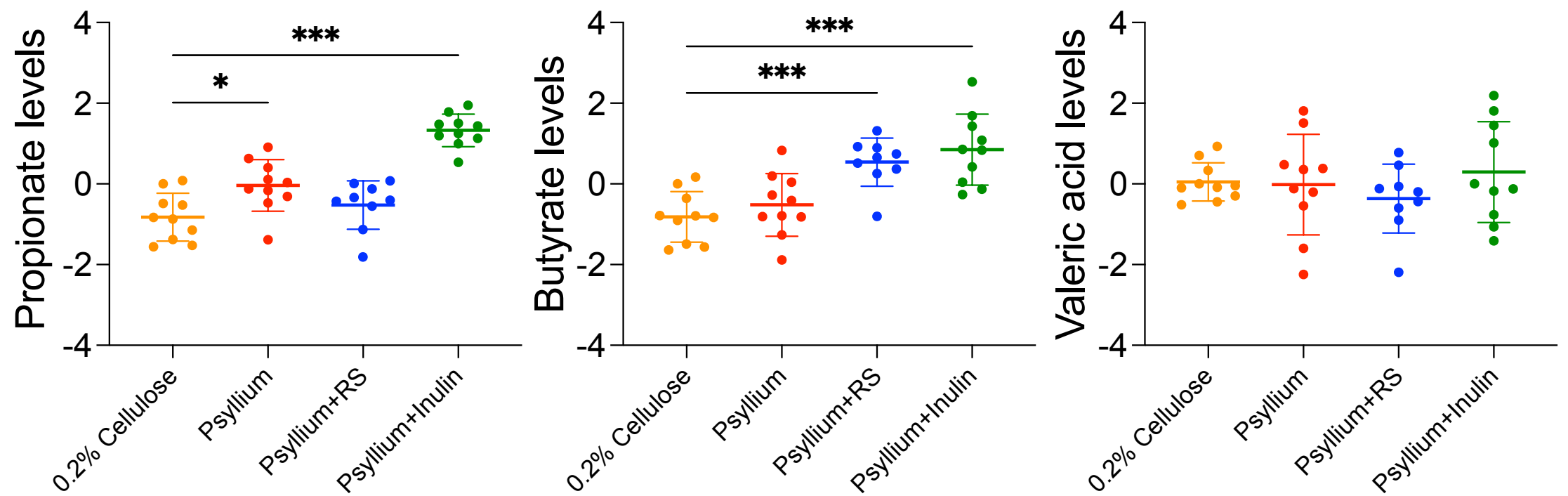


Supplementary Fig. 18 Beta diversity of the gut microbiota and the metabolites profile in non-tumour-bearing mice after 3-week modified diet and 3.75 days after SARRP IR. (a) Principal coordinate analysis of faecal microbiota using Bray-Curtis dissimilarity. (b) Distances of gut microbiota in irradiated mice compared to non-IR controls in each dietary groups. (c) Principal component analysis of metabolites profile in all and each dietary groups. (d) Distances of metabolites profile in irradiated mice compared to non-IR controls in each dietary groups. The intensity of colour reflects the IR dose.

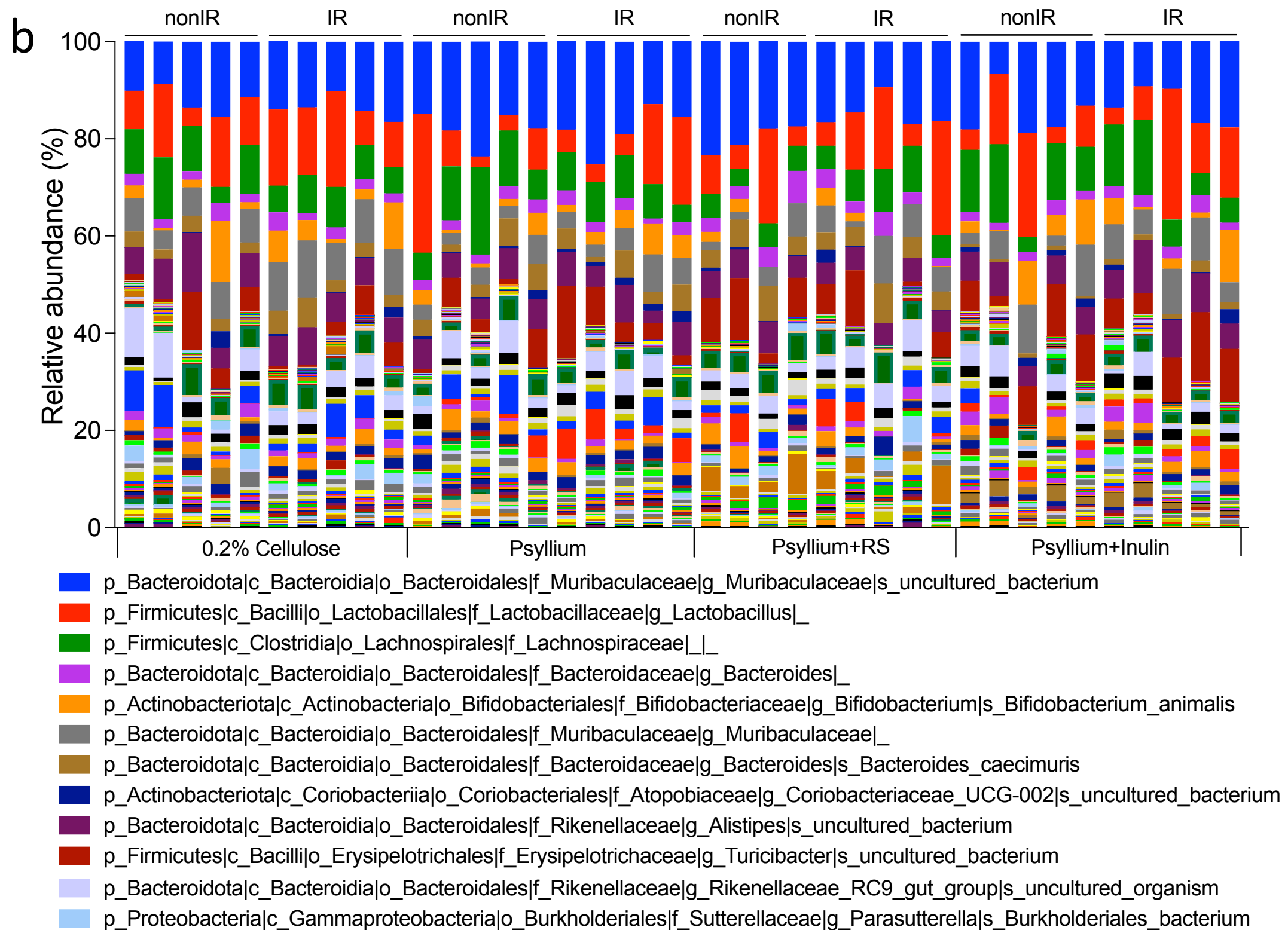
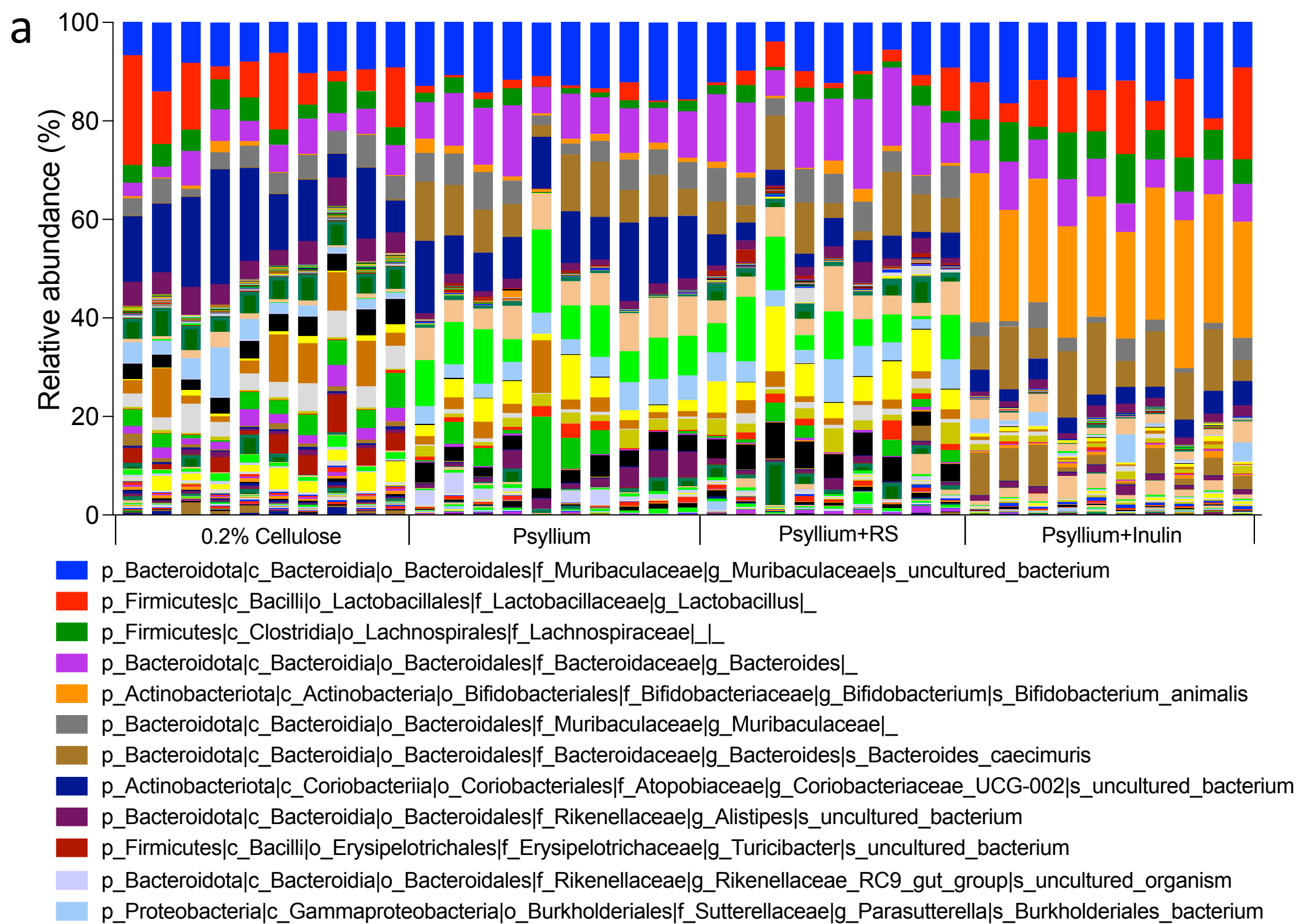
a Acute toxicity experiment



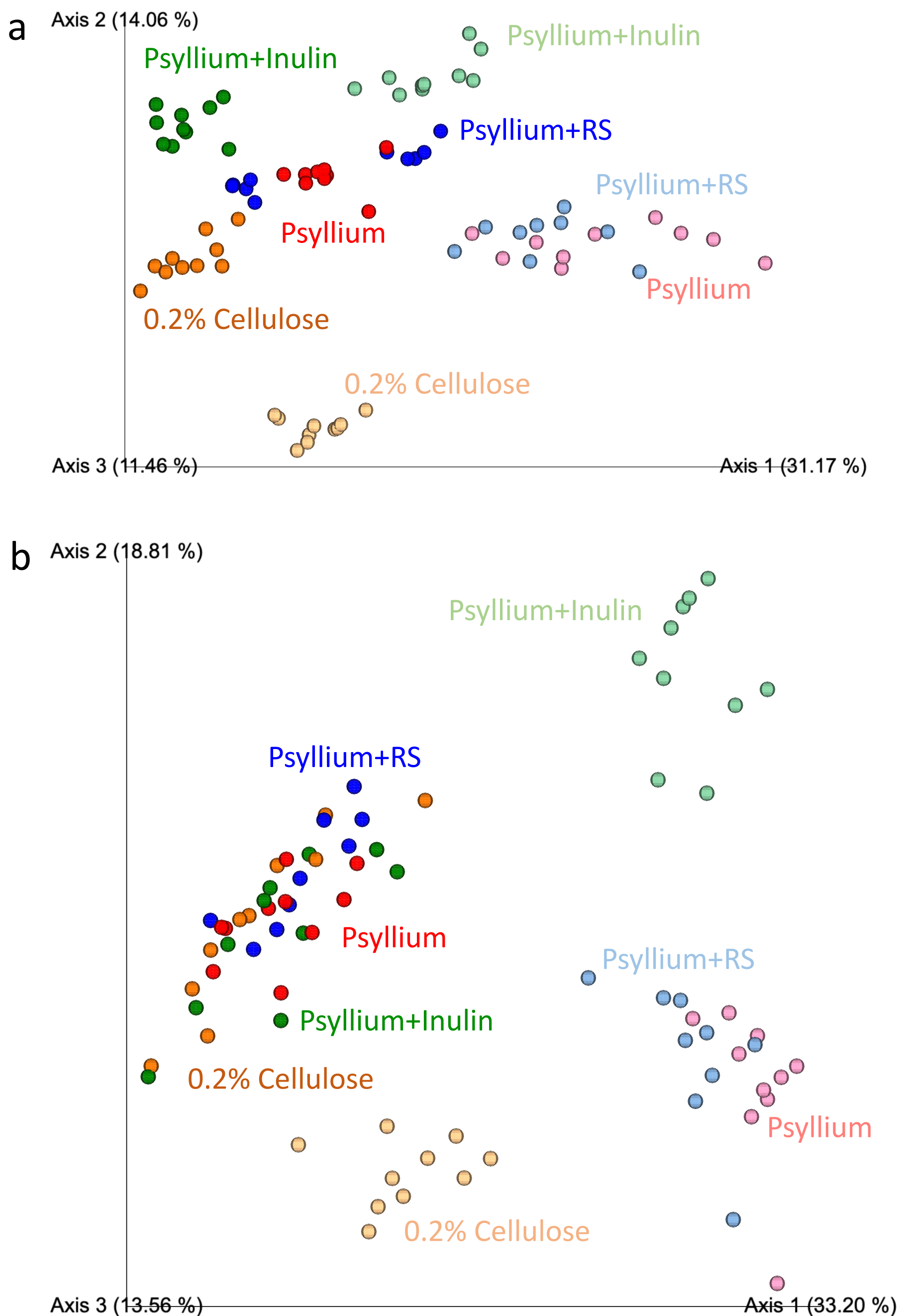
b Late toxicity experiment



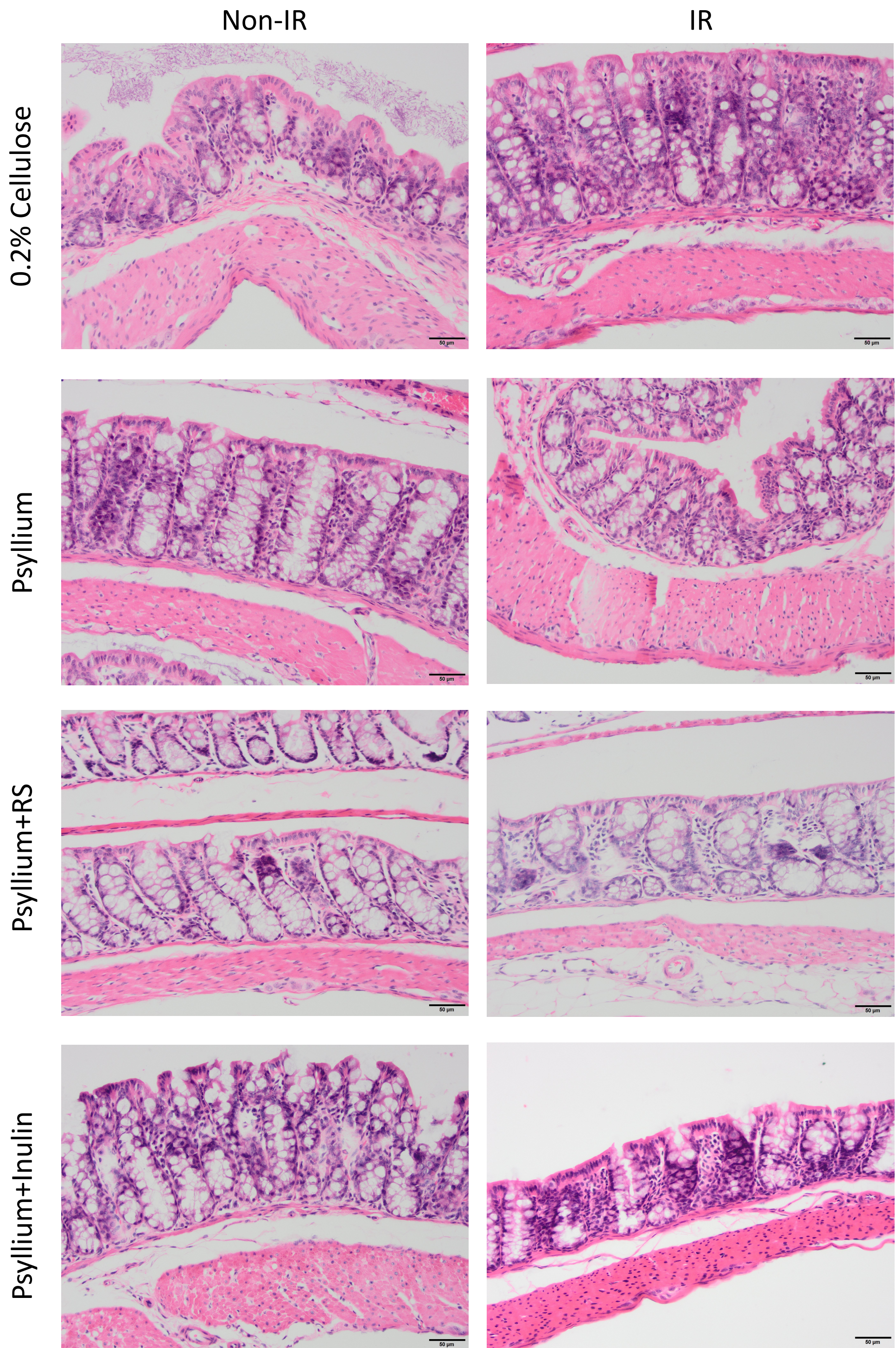
Supplementary Fig. 19 Caecal SCFAs in non-tumour-bearing mice after 3-week modified diet in acute toxicity and late toxicity experiments. Relative levels of (a) isovaleric acid and isobutyrate in the acute toxicity experiment, and (b) propionate, butyrate and valeric acid in the late toxicity experiment.



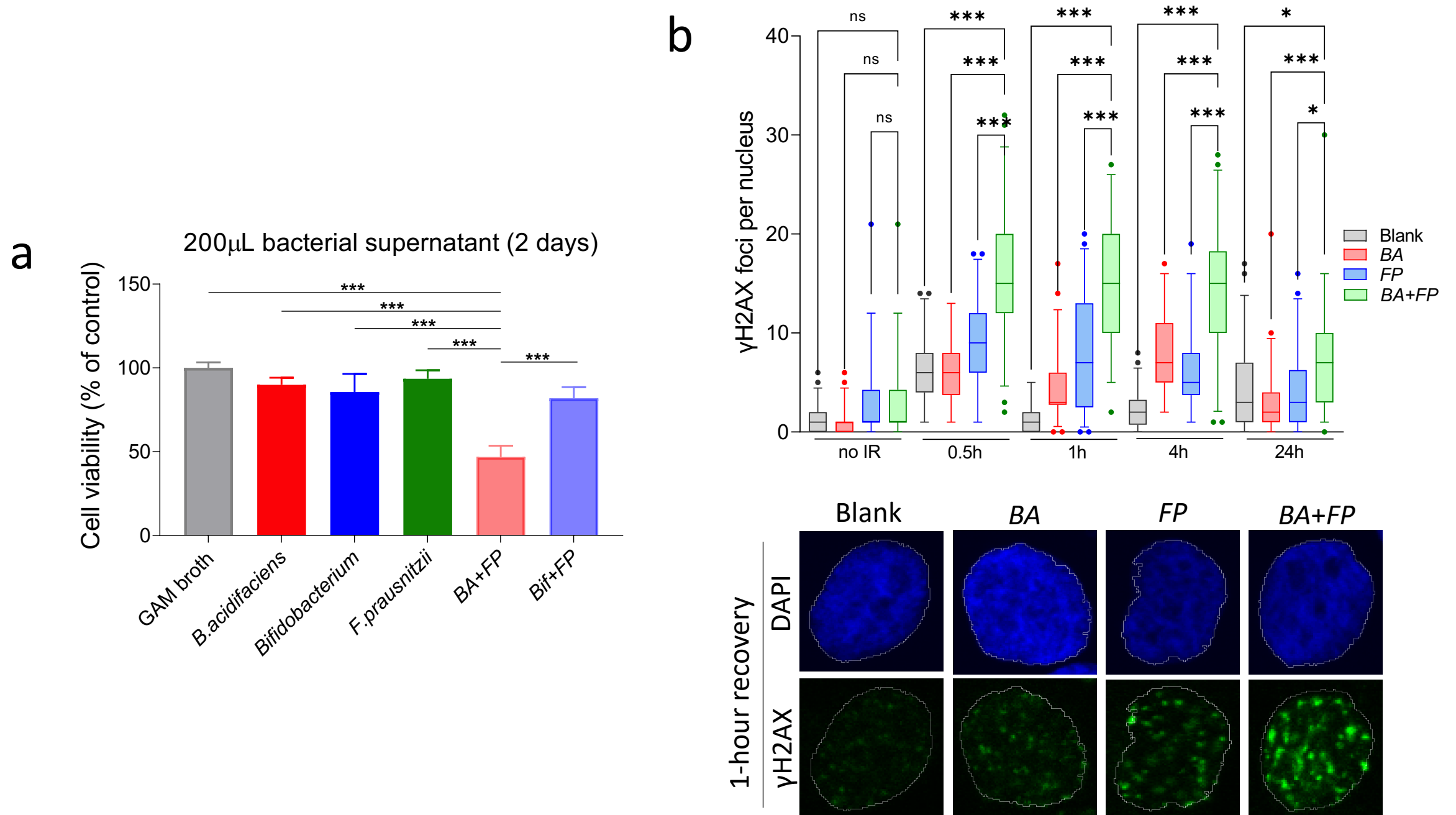
Supplementary Fig. 20 Phylogenetic composition of faecal microbiota before and after irradiation in the late toxicity experiment. Faecal samples were collected (a) 9 weeks post modified diets (n=5/non-IR cohort, n=5/IR cohort in each dietary group) and (b) 5 weeks after changing back to normal chow. The samples were in the same order in the top and bottom panels.



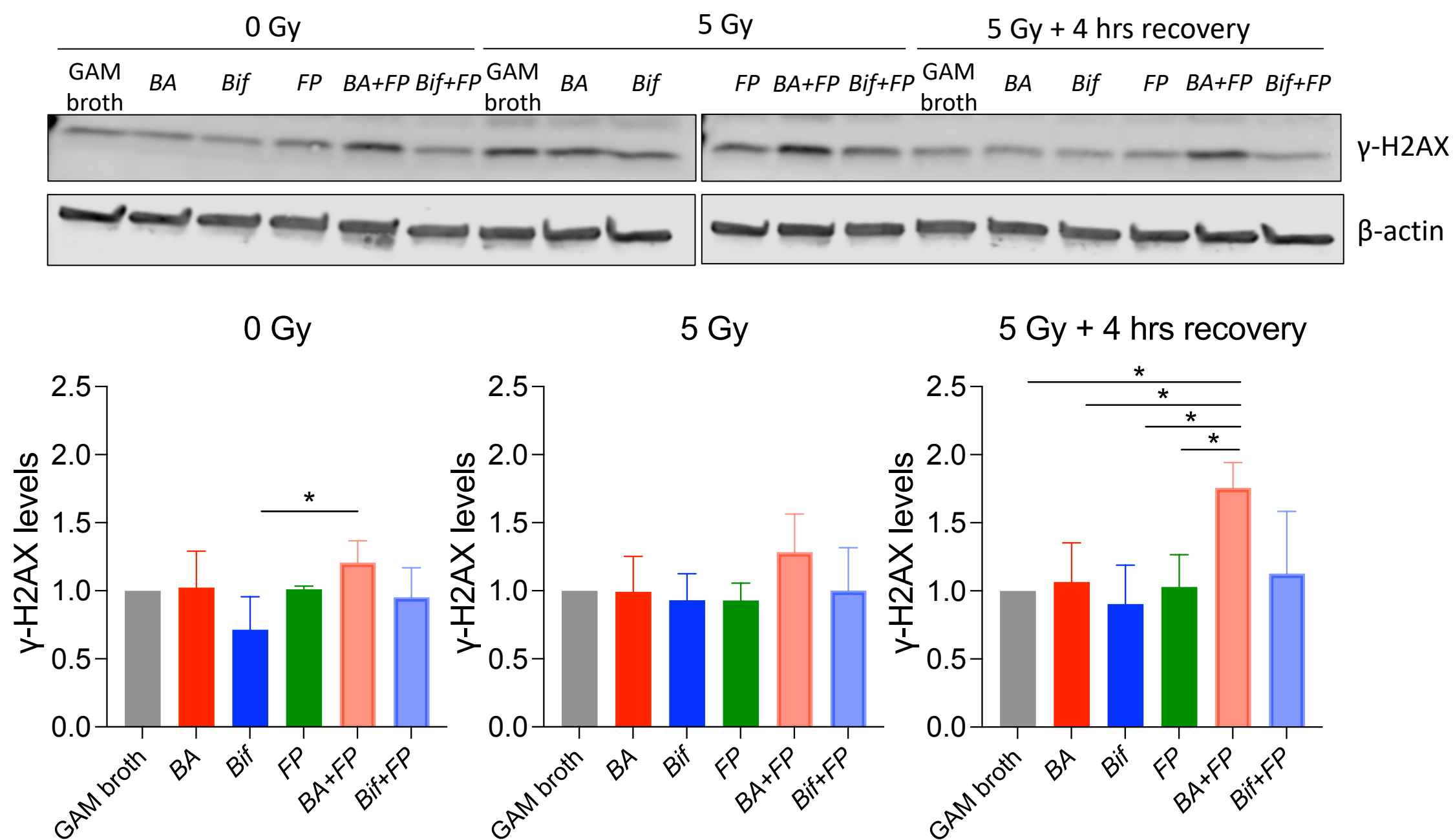
Supplementary Fig. 21 Beta diversity of faecal microbiota in the late toxicity experiment. Principal coordinate analysis using (a) unweighted and (b) weighted UniFrac of faecal microbiotas (n=5/non-IR cohort, n=5/IR cohort in each dietary group) that were collected 9 weeks post modified diets (light colours) and 5 weeks after changing back to normal chow (dark colours).



Supplementary Fig. 22 Representative images of mouse large intestine sections in non-tumour-bearing mice after 22-week modified diet with or without SARRP IR. The presence of inflammation and fibrosis was assessed at 20-weeks post-irradiation of all dietary groups on ‘Swiss rolls’ sections that were stained by Haematoxylin & eosin. The images were taken at x200 magnification and the bar = 50 μm.

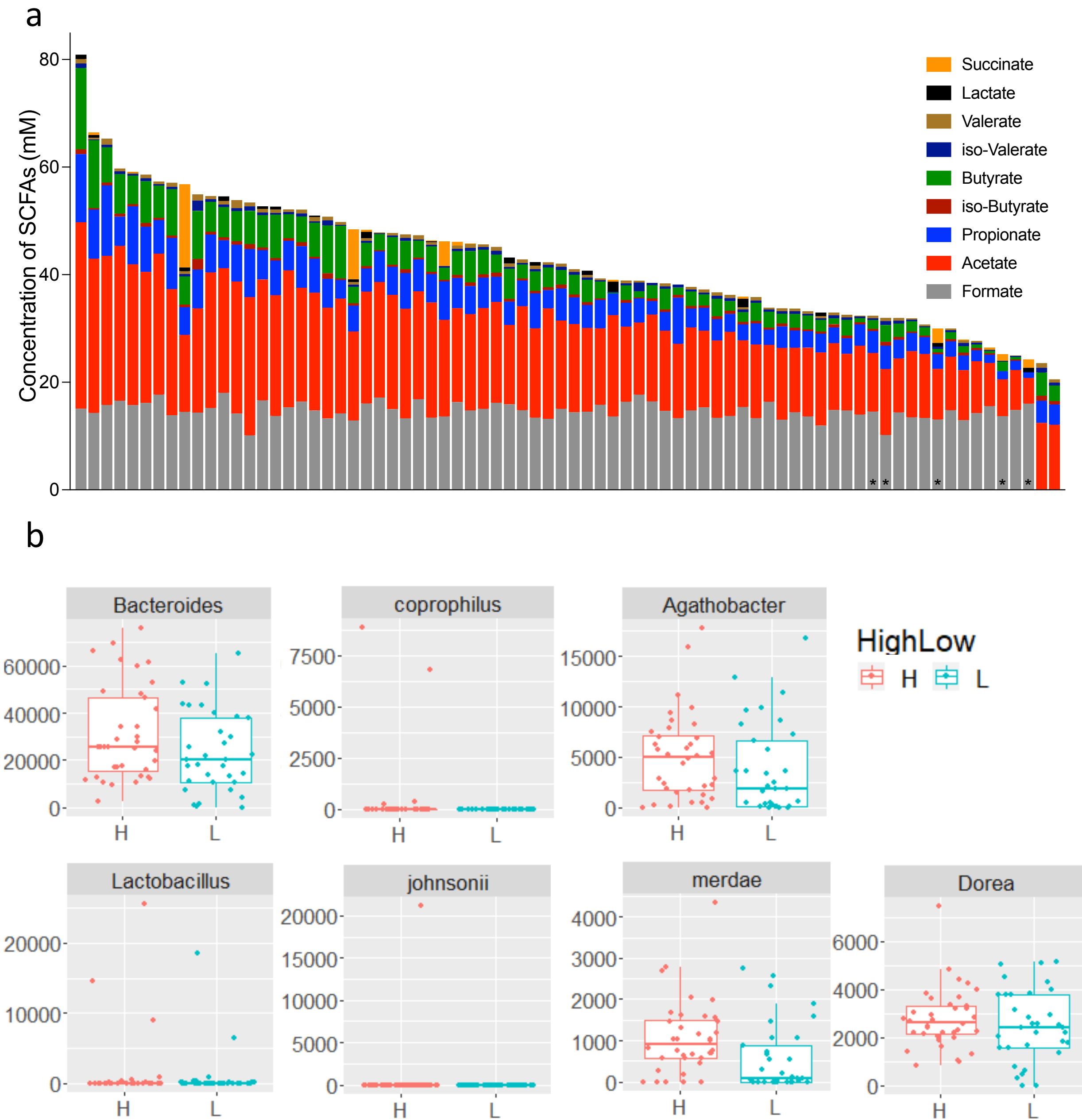


Supplementary Fig. 23 BA+FP increased cytotoxic responses and DNA damage in T24 bladder cancer cells. (a) The cell survival of T24 cells treated with 200 μL of GAM broth or bacterial supernatants, in 500 μL of medium for 2 days (N=3). (b) Immunofluorescence microscopy analysis of γ-H2AX levels (N=3) of T24 cells treated with 100 μL bacterial supernatants in 2 mL of medium for 24 hours. DNA damage was evaluated after treating with 2 Gy ionising radiation. pHs of GAM broth and bacterial supernatants were all neutralised to 7.2. BA+FP denotes the co-culture of *B.acidifaciens* and *F.prausnitzii*, while Bif+FP denotes the co-culture of *Bifidobacterium* and *F.prausnitzii*. Data are presented as mean ± SD. *p<0.05; **p<0.01; ***p<0.001.

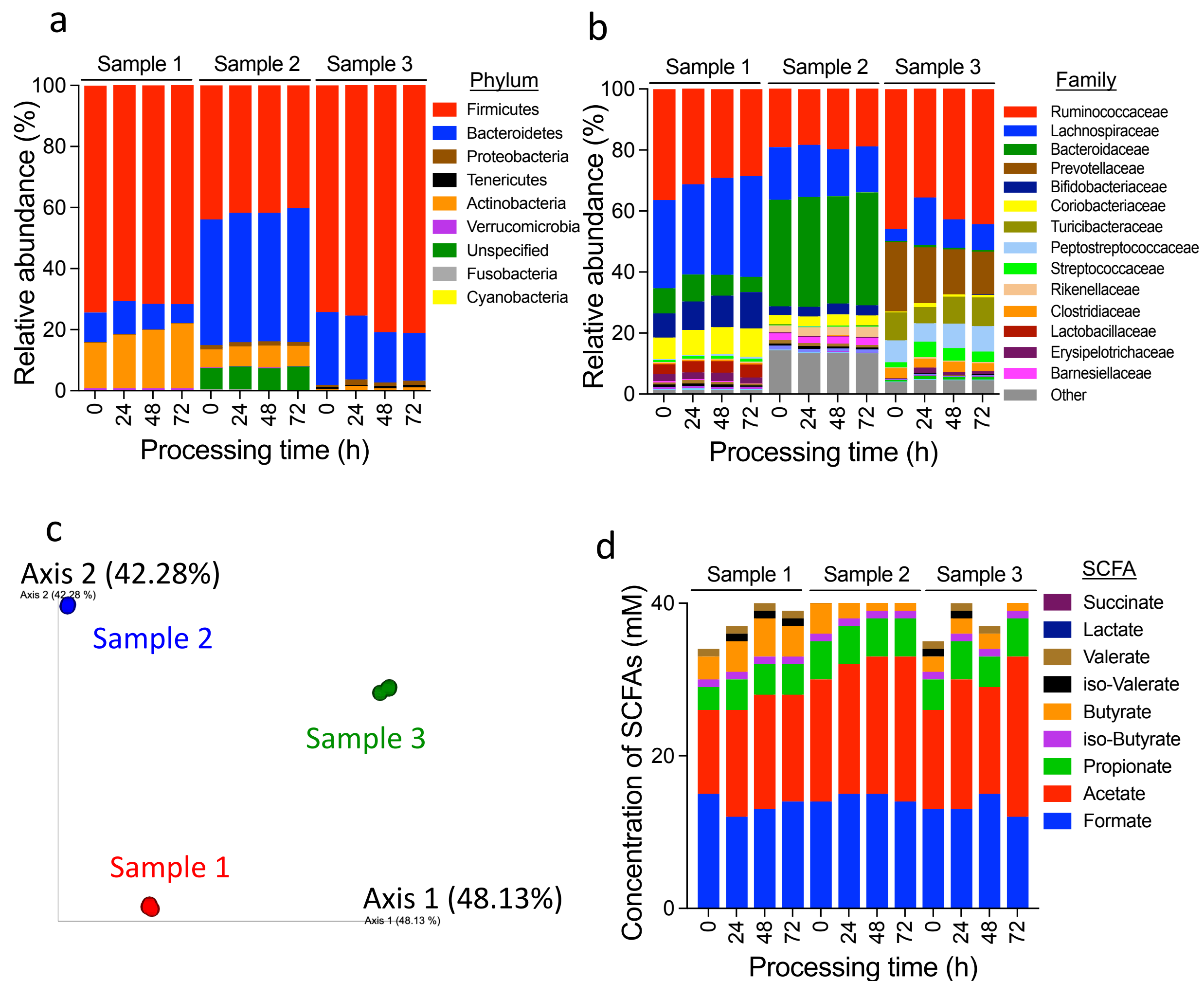


Supplementary Fig. 24 BA+FP increased histone acetylation levels and DNA damage in bladder cancer cells.

Western blot analysis of γ-H2AX level (N=3) of RT112 cells treated with different bacterial supernatants in 6-well plates. DNA damage was evaluated after treating with 5 Gy ionising radiation. The samples derived from the same experiment and the blots were processed in parallel. pHs of GAM broth and all bacterial supernatants were neutralised to 7.2. BA+FP denotes the co-culture of *B. acidifaciens* and *F. prausnitzii*, while Bif+FP denotes the co-culture of *B. animalis* and *F. prausnitzii*. Data are presented as mean ± SD. *p<0.05; **p<0.01; ***p<0.001.



Supplementary Fig. 25 Production of SCFAs in pelvic cancer patients. (a) Concentrations of SCFAs in cancer patients. Asterisk indicates right sided colon samples. (b) Abundance of taxa found to be significantly associated with SCFA. Samples are grouped by high vs low faecal acetate, propionate and butyrate. The median of these three SCFAs combined was the cut off between high and low levels. Each dot is an individual sample.



Supplementary Fig. 26 Comparison of bacterial and SCFA relative abundances between human samples

processed serially showed similar profiles between the different processing times: 0, 24, 48 and 72 hours. (a)

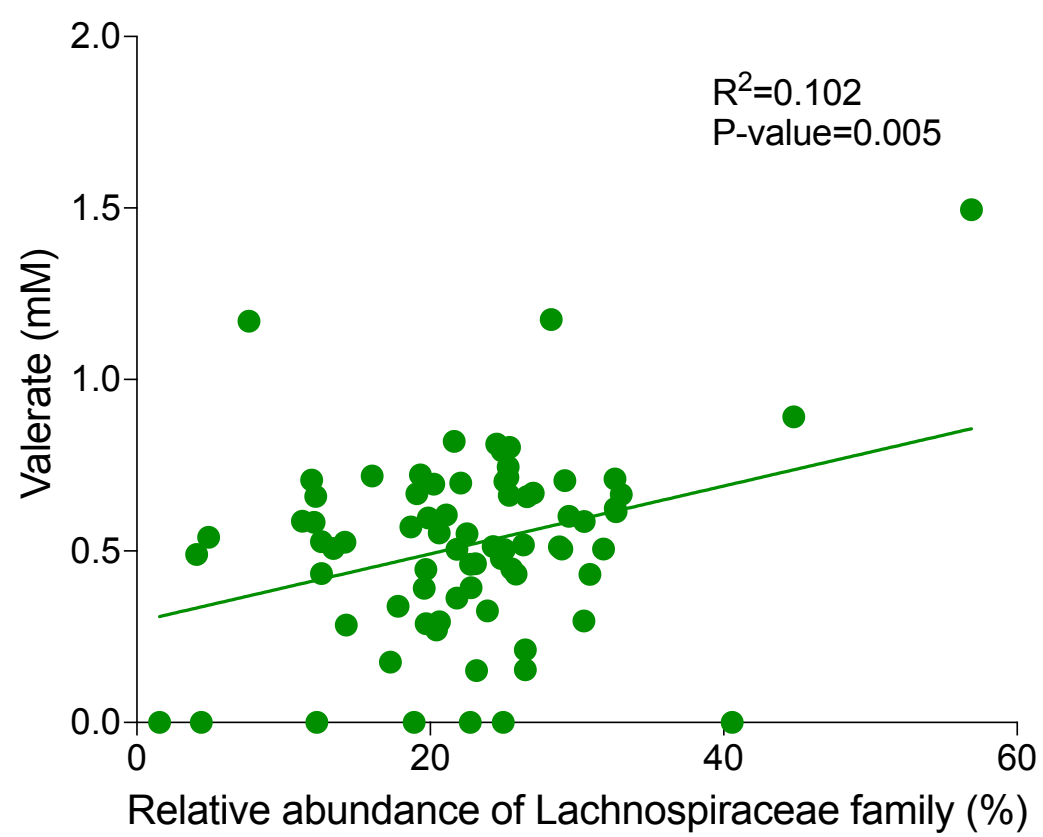
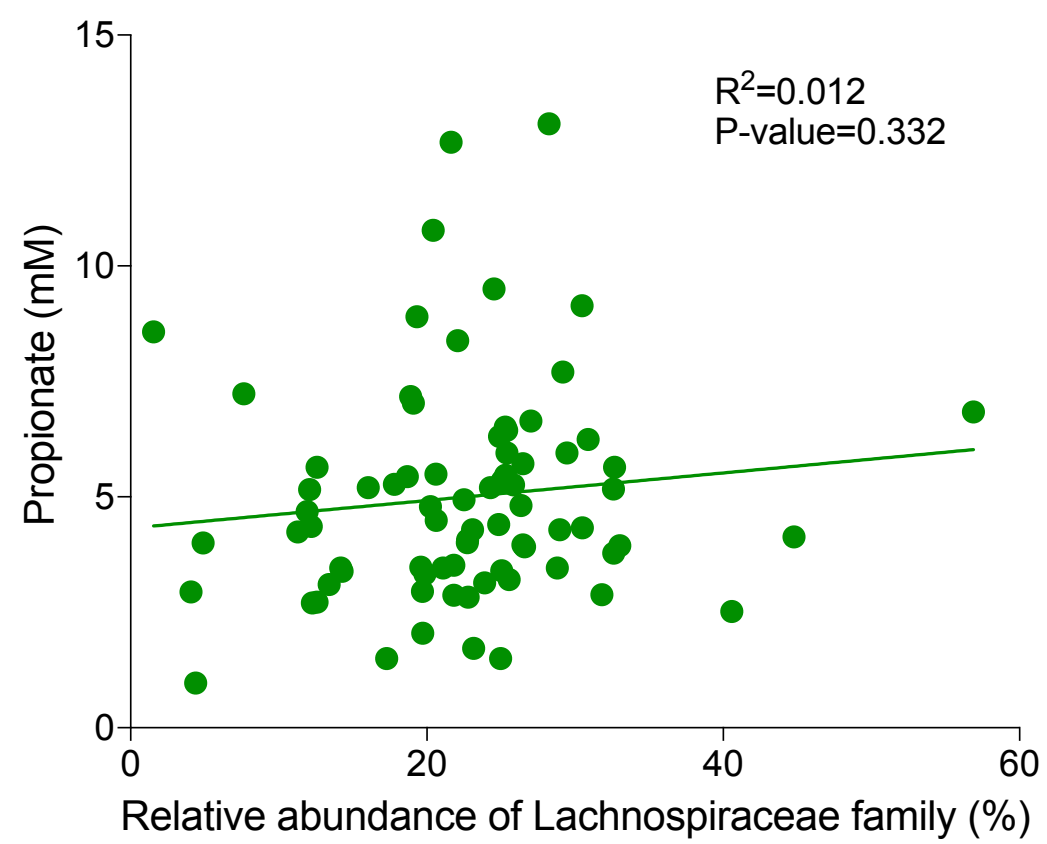
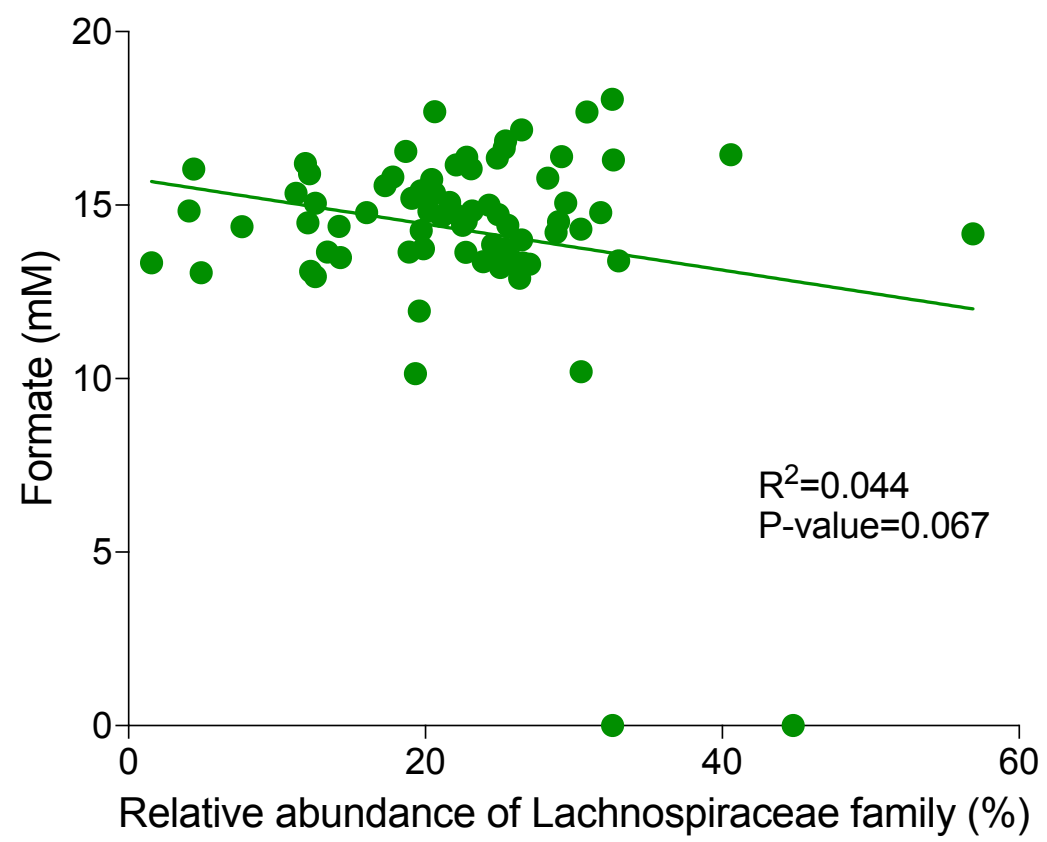
Relative abundances of bacteria at the phylum level for the serial samples. (b) Relative abundances of the 15 most

abundant bacteria at the family level for the serial samples. (c) Principal coordinate analysis of the serial samples. P

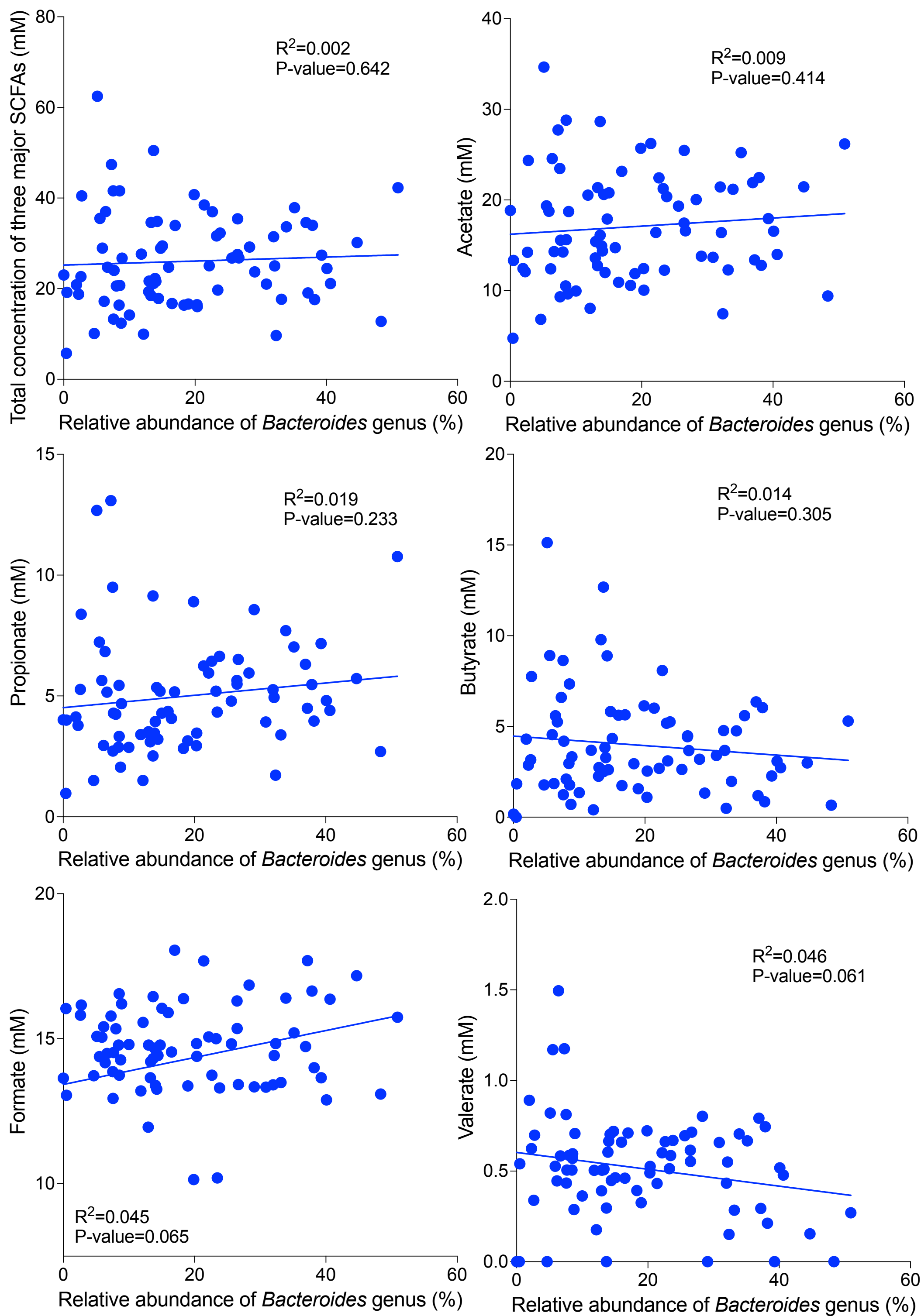
value was 0.001 for PERMANOVA test. (d) SCFA concentrations for the serial samples. Among all SCFAs, only the

concentration of butyrate was lower in sample 3 (p-value=0.002 vs. sample 1 and =0.01 vs. sample 2) despite a

significant difference seen in gut microbiota profiles between individuals (p=0.001).



Supplementary Fig. 27 Correlations between Lachnospiraceae family and faecal SCFAs levels in cancer patients.



Supplementary Fig. 28 Correlations between *Bacteroides* genus and faecal SCFAs levels in cancer patients.