

# Oral exposure to environmental pollutant benzo[a]pyrene impacts the intestinal epithelium and induces gut microbial shifts in murine model.

## Supplementary Information

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† These authors contributed equally to this work.

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**Table S1.** Richness (OTU number, Chao1) and diversity (Shannon, Phylogenetic Diversity) values for fecal and mucosa-associated bacteria of different mouse groups (control, vehicle, BaP). The values are given as the means  $\pm$  SD. The operational taxonomic units (OTUs) were defined with 97% sequence identity. Calculations were made based on rarefied OTU tables at 20,500 and 5,800 sequences for fecal and mucosal samples, respectively.

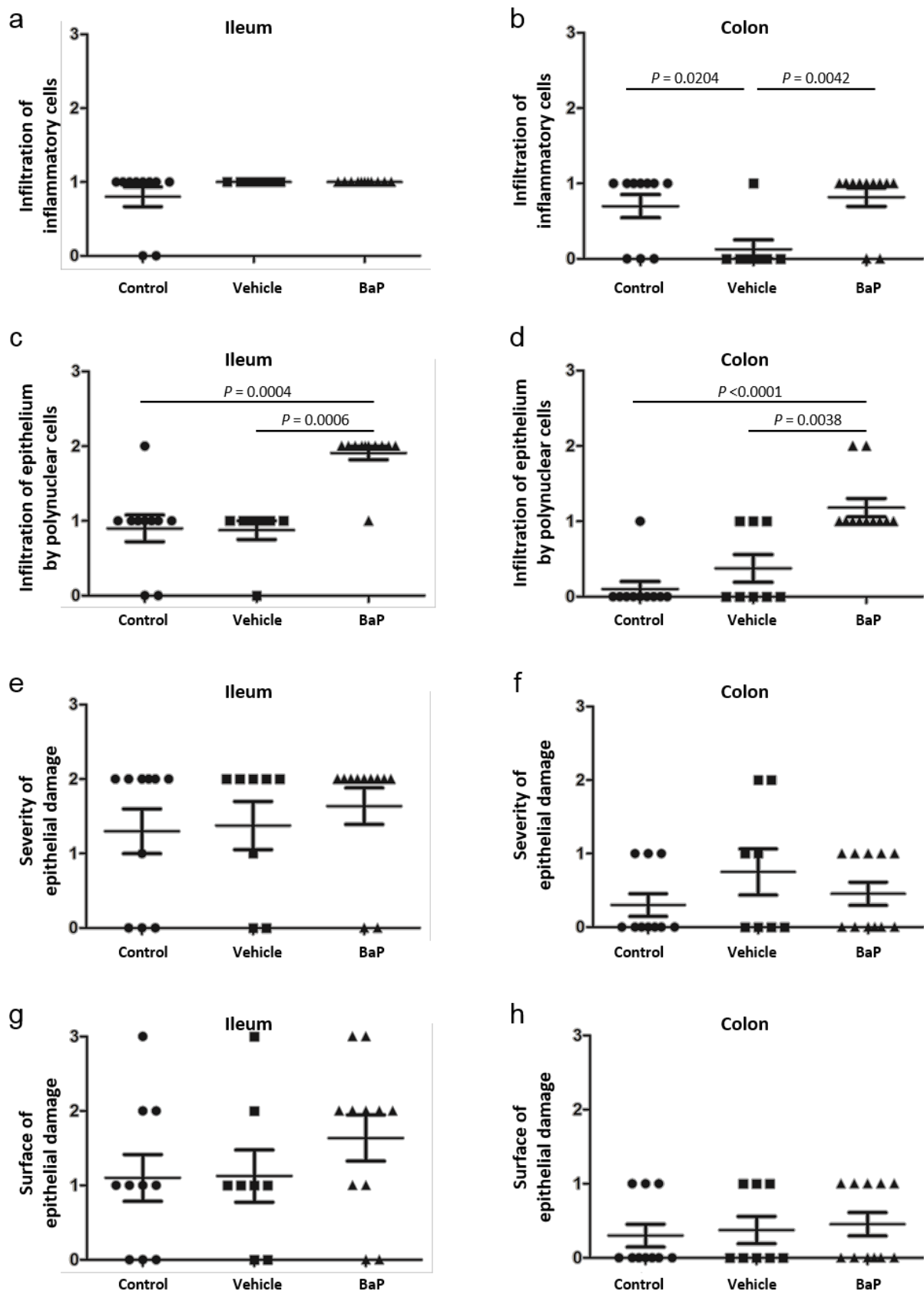
Index	Faecal microbiota						Mucosal microbiota		
	T0	T1	T7	T14	T21	T27	Ileum	Proximal colon	Distal colon
<b>OTUs</b>									
Control	4 174 $\pm$ 246	3 866 $\pm$ 583	4 403 $\pm$ 82	4 036 $\pm$ 530	4 244 $\pm$ 162	4 344 $\pm$ 96	1 067 $\pm$ 193	1 107 $\pm$ 36	1 155 $\pm$ 288
Vehicle	4 042 $\pm$ 290	4 050 $\pm$ 138	4 183 $\pm$ 440	4 073 $\pm$ 198	4 222 $\pm$ 269	4 139 $\pm$ 177	906 $\pm$ 300	985 $\pm$ 196	918 $\pm$ 58
BaP	4 081 $\pm$ 219	3 830 $\pm$ 269	4 090 $\pm$ 417	4 401 $\pm$ 110	4 312 $\pm$ 209	3 988 $\pm$ 187	912 $\pm$ 225	1 224 $\pm$ 167	978 $\pm$ 42
<b>Chao1</b>									
Control	15 082.4 $\pm$ 1 451.3	14 354.3 $\pm$ 2 086.6	15 960.8 $\pm$ 466.8	15 162.3 $\pm$ 1 618.9	15 426.6 $\pm$ 770.1	15 831.8 $\pm$ 516.4	4 345.3 $\pm$ 756.5	4 433.6 $\pm$ 590.8	4 585.1 $\pm$ 1 155.7
Vehicle	15 245.6 $\pm$ 1 082.1	14 575.3 $\pm$ 728.5	15 413.1 $\pm$ 1 499.9	15 158.3 $\pm$ 936.9	15 655.8 $\pm$ 1 232.2	15 287.8 $\pm$ 834.0	3 708.0 $\pm$ 1 643.8	3 932.3 $\pm$ 829.1	3 900.3 $\pm$ 619.4
BaP	14 455.9 $\pm$ 1 130.9	13 946.5 $\pm$ 953.9	14 389.2 $\pm$ 1 410.2	15 940.5 $\pm$ 406.0	16 139.6 $\pm$ 826.0	14 343.1 $\pm$ 983.6	3 534.4 $\pm$ 940.9	5 054.4 $\pm$ 958.1	3 867.7 $\pm$ 303.4
<b>Shannon</b>									
Control	8.52 $\pm$ 0.25	8.44 $\pm$ 0.35	8.87 $\pm$ 0.10	8.65 $\pm$ 0.36	8.57 $\pm$ 0.19	8.71 $\pm$ 0.11	7.07 $\pm$ 0.54	7.61 $\pm$ 0.14	7.58 $\pm$ 0.45
Vehicle	8.57 $\pm$ 0.18	8.49 $\pm$ 0.14	8.55 $\pm$ 0.37	8.36 $\pm$ 0.24	8.48 $\pm$ 0.33	8.45 $\pm$ 0.26	6.05 $\pm$ 1.09	6.97 $\pm$ 0.59	7.06 $\pm$ 0.20
BaP	8.35 $\pm$ 0.25	8.05 $\pm$ 0.18	8.65 $\pm$ 0.31	8.84 $\pm$ 0.11	8.68 $\pm$ 0.18	8.35 $\pm$ 0.22	6.35 $\pm$ 0.93	7.65 $\pm$ 0.47	7.31 $\pm$ 0.25
<b>PD</b>									
Control	365.9 $\pm$ 18.2	348.9 $\pm$ 38.7	378.8 $\pm$ 8.1	358.3 $\pm$ 34.9	372.7 $\pm$ 8.2	378.4 $\pm$ 5.2	122.8 $\pm$ 15.8	125.6 $\pm$ 3.2	130.5 $\pm$ 24.4
Vehicle	360.6 $\pm$ 15.5	355.2 $\pm$ 8.7	364.6 $\pm$ 25.0	361.7 $\pm$ 13.6	368.1 $\pm$ 15.1	364.2 $\pm$ 12.3	108.1 $\pm$ 24.2	116.3 $\pm$ 15.5	109.9 $\pm$ 4.1
BaP	360.5 $\pm$ 13.4	343.7 $\pm$ 18.5	360.9 $\pm$ 25.7	379.9 $\pm$ 7.5	375.7 $\pm$ 12.6	356.3 $\pm$ 10.1	108.4 $\pm$ 18.4	133.9 $\pm$ 12.9	115.8 $\pm$ 3.2



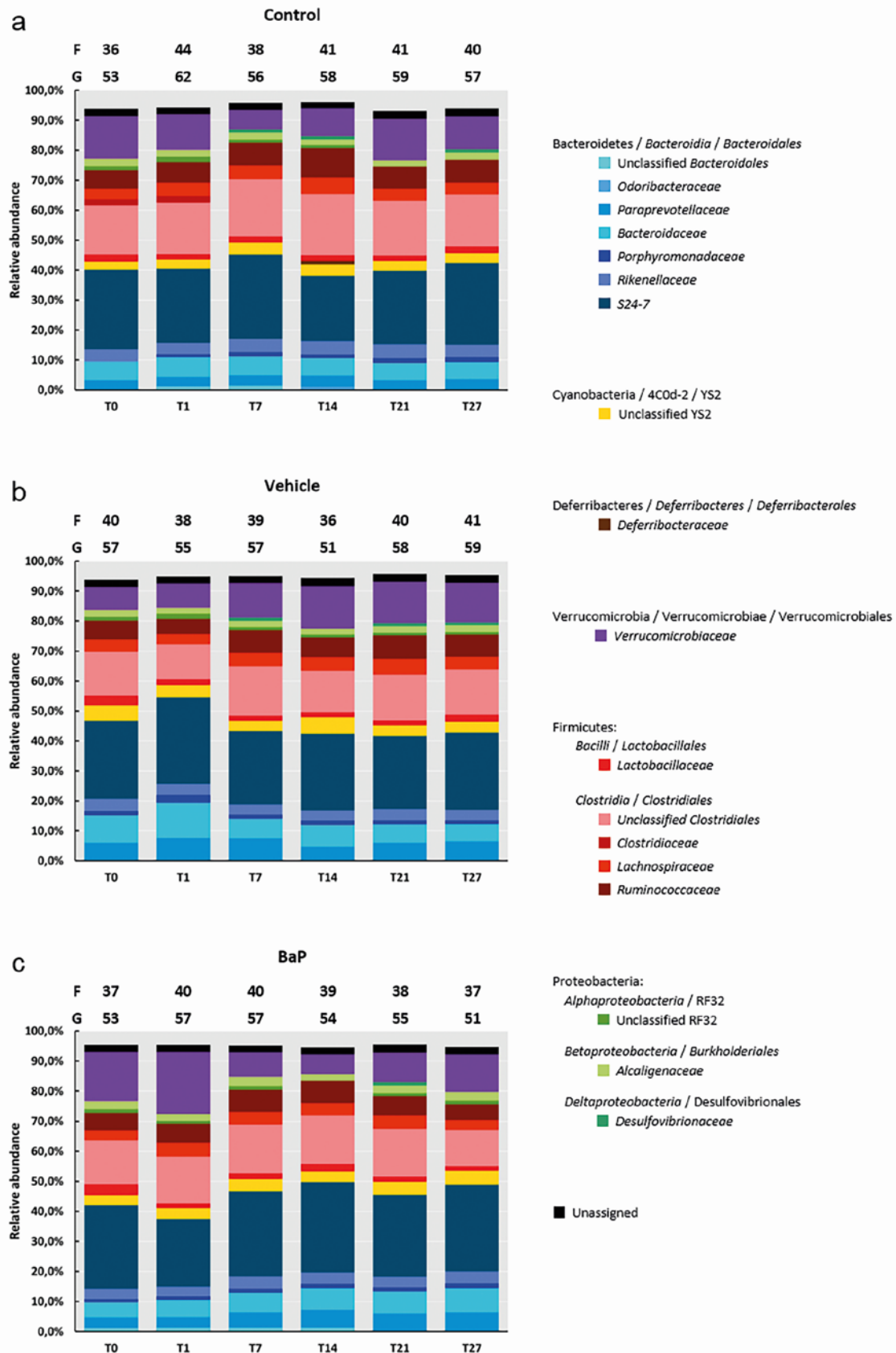


**Table S4:** Histological scoring parameters. The total inflammation score was determined by the sum of each criterion and could reach a maximum of 12.

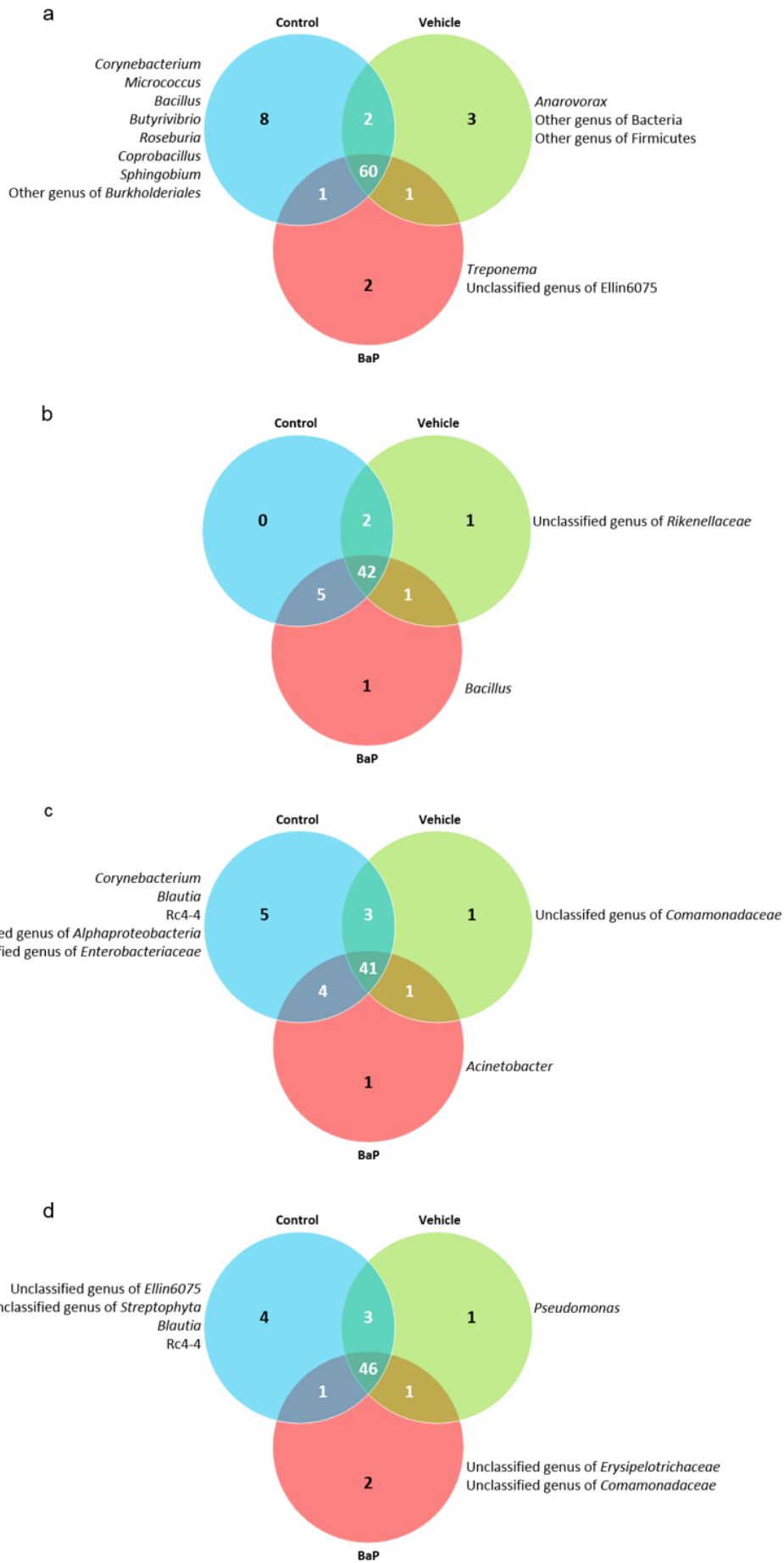
Score	0	1	2	3
Infiltration of inflammatory cells (x10 magnification)	Rare inflammatory cells in the lamina propria	Increased number of inflammatory cells, including neutrophils in the lamina propria	Confluence of inflammatory cells extending into submucosa	Transmural extension of the inflammatory cell infiltrate
Infiltration of epithelium by polynuclear cells (x20 magnification)	No infiltration	Few inside the crypt	Lot of inside the crypt	Cryptic abscess
Severity of epithelial damage	Absence of mucosal damage	Lymphoepithelial lesions	Mucosal erosion/ulceration	Extensive mucosal damage and extension through deeper structure of the bowel wall
Surface of epithelial damage	Normal	Focal	Multifocal	Wide



**Fig S1.** The score of each criterion used in the combined histological scoring of inflammation for the ileum and colon of mice. (a-b) Infiltration of inflammatory cells, (c-d) infiltration of epithelium by polynuclear cells, (e-f) severity of epithelium damage and (g-h) surface of epithelium damage of mouse ileum and colon orally exposed to sterile physiological saline (control), sunflower seed oil (vehicle) or benzo[a]pyrene (BaP). Each symbol represents an individual mouse. The values are represented with mean  $\pm$  SEM and p-values are derived from a Mann-Whitney test.

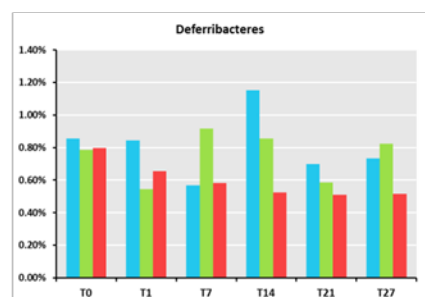
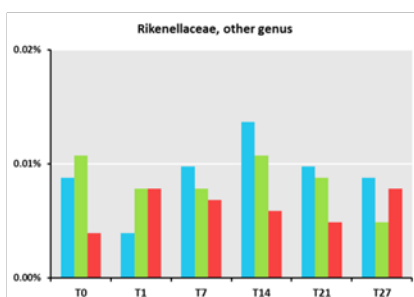
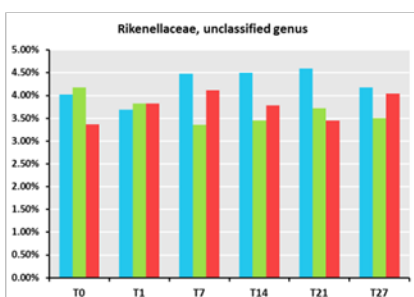
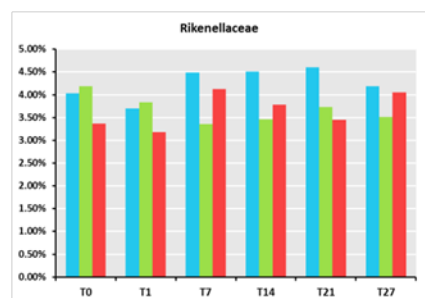
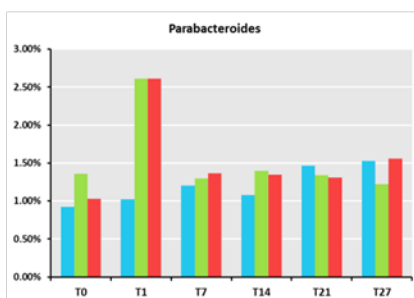
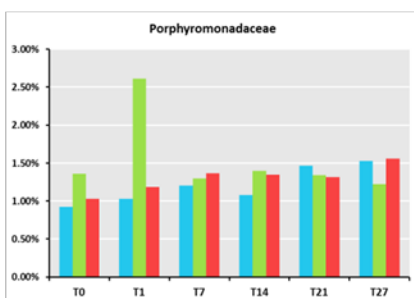
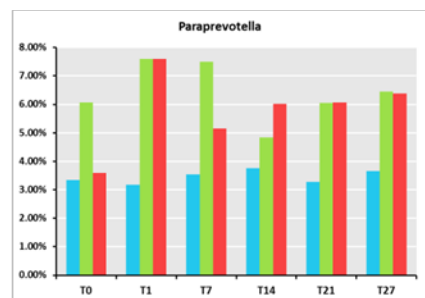
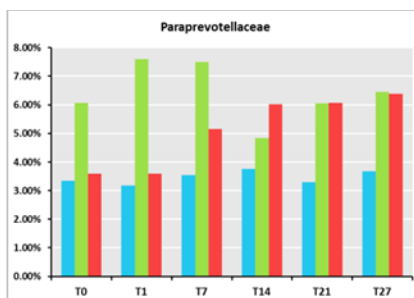
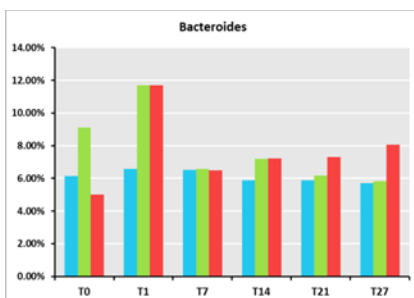
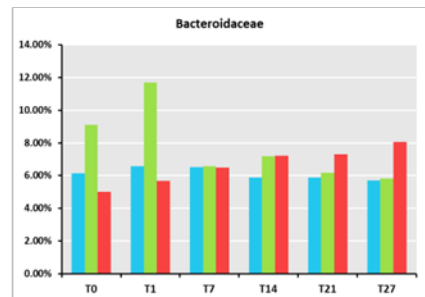
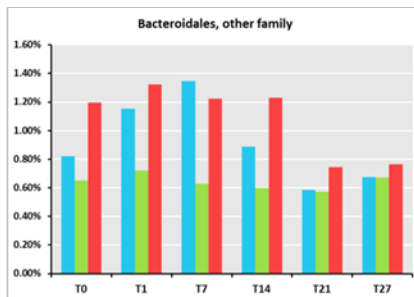
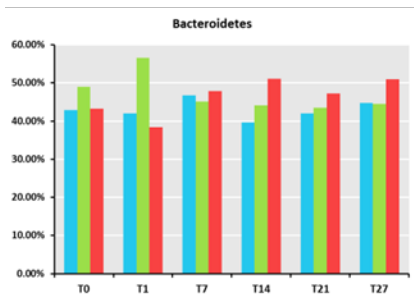
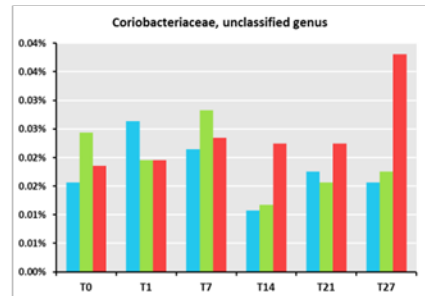
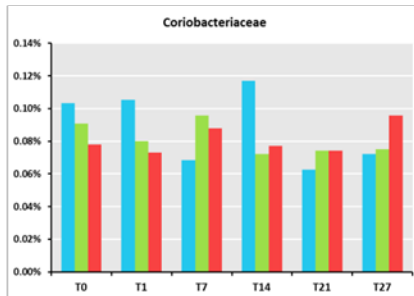
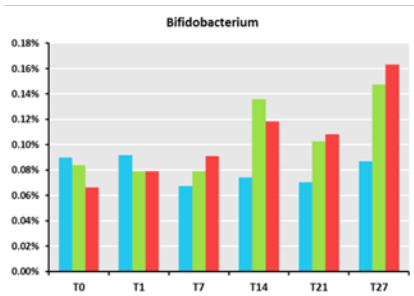
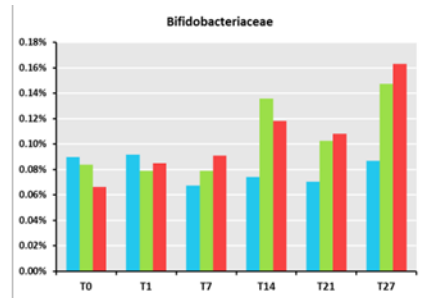
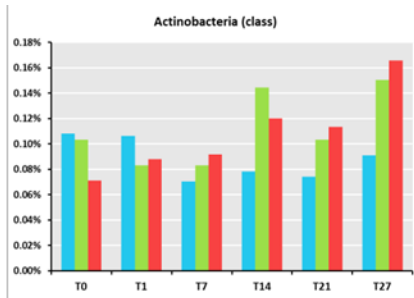
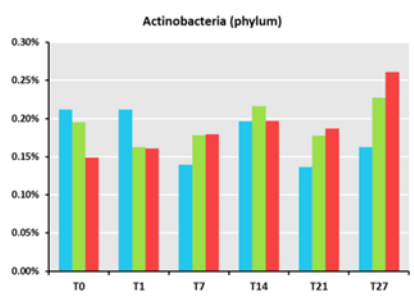


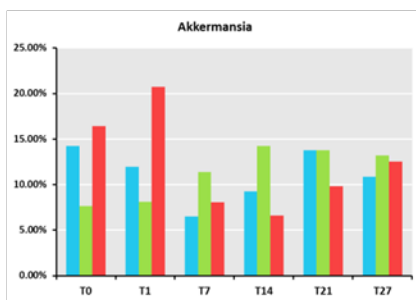
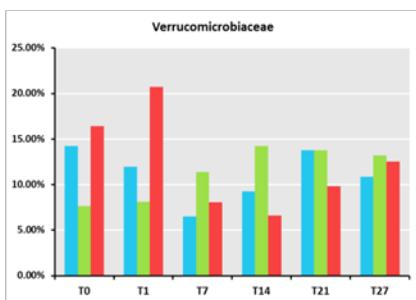
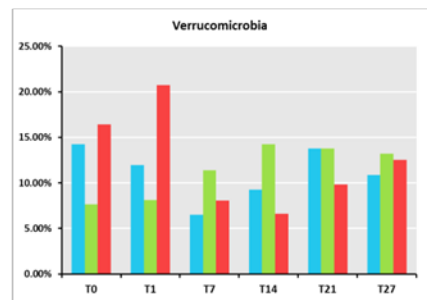
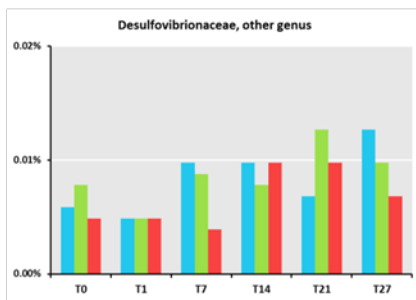
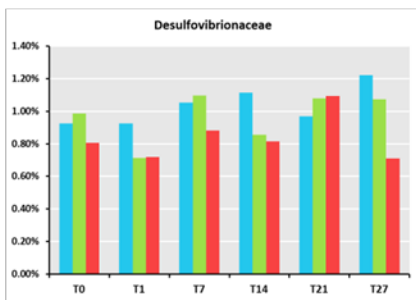
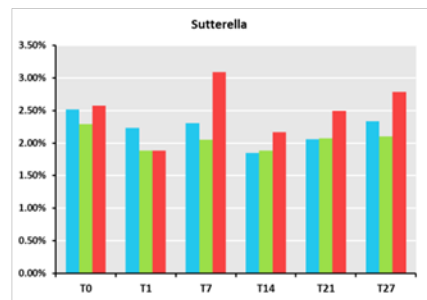
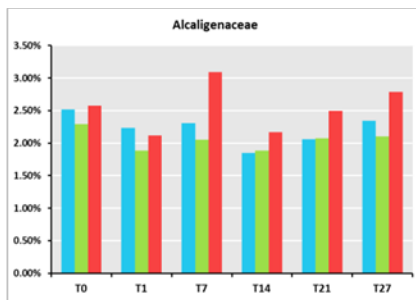
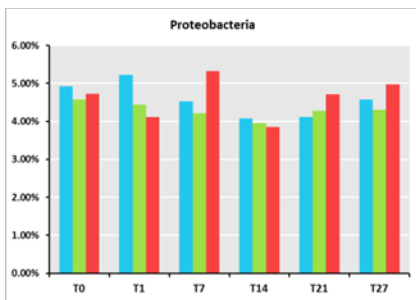
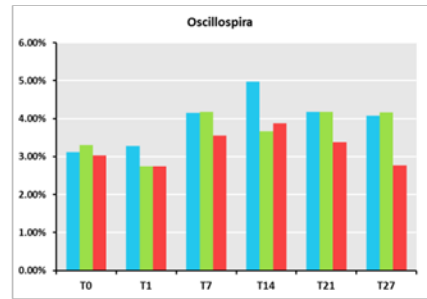
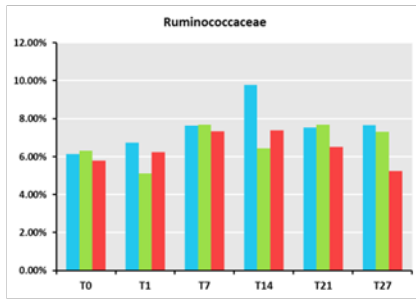
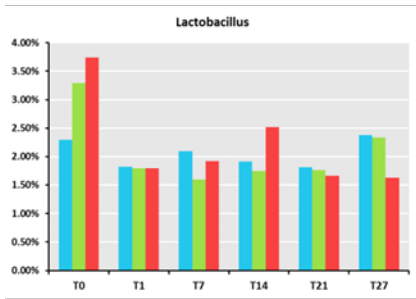
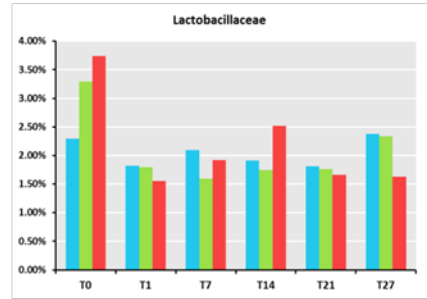
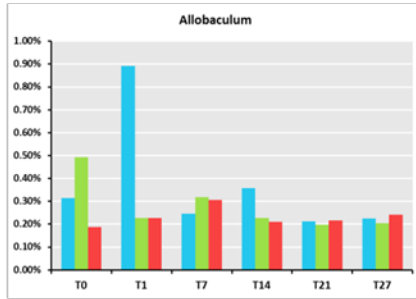
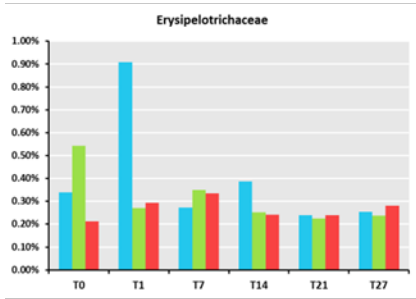
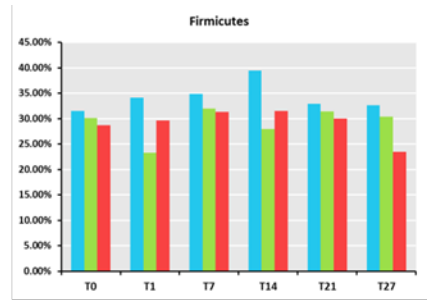
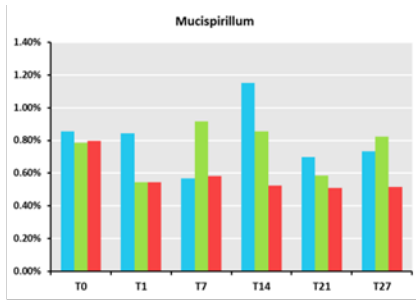
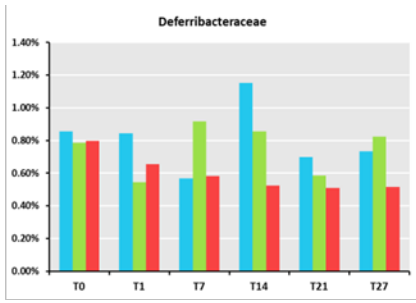
**Fig S2.** Faecal microbiota composition profiles at the family level in mice for control (a), vehicle (b) and BaP-treated groups (c) revealed by 16S rRNA gene sequencing. Bar plots are presented by group. Only the dominant bacterial taxa (relative abundance >1%) are represented. Families within the same bacterial phylum are indicated using different shades of the same color. The numbers above the profiles correspond to the total numbers of bacterial families (F, first line) and genera (G, second line) observed for each stool sample.



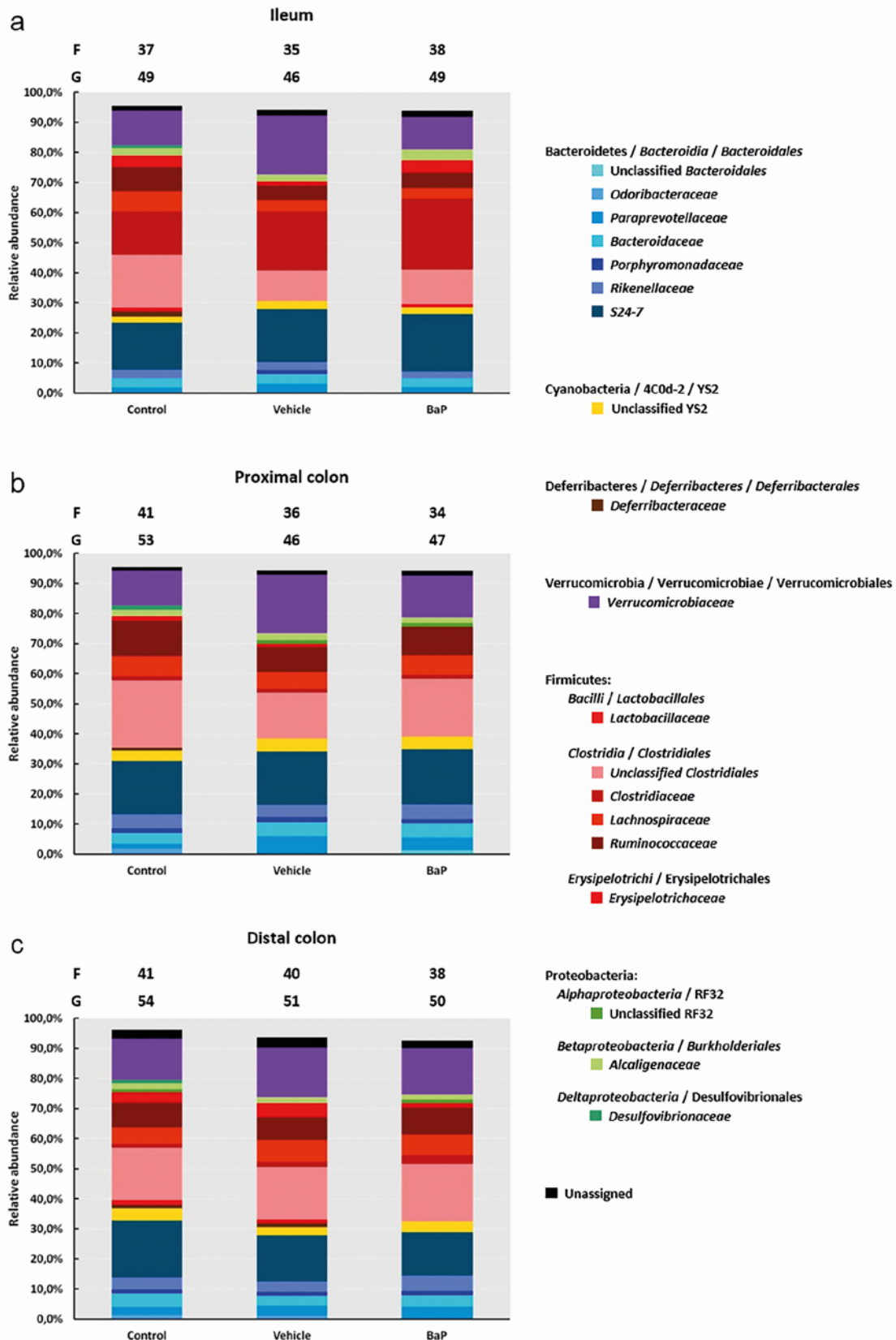
**Fig S3.** Shared and specific bacterial genera detected in control, vehicle and BaP-treated groups for faecal (a) and mucosa-associated microbiota in the ileum (b), proximal colon (c) and distal colon (d). Specific bacterial genera are listed next to the circle corresponding to the group in which they were detected.



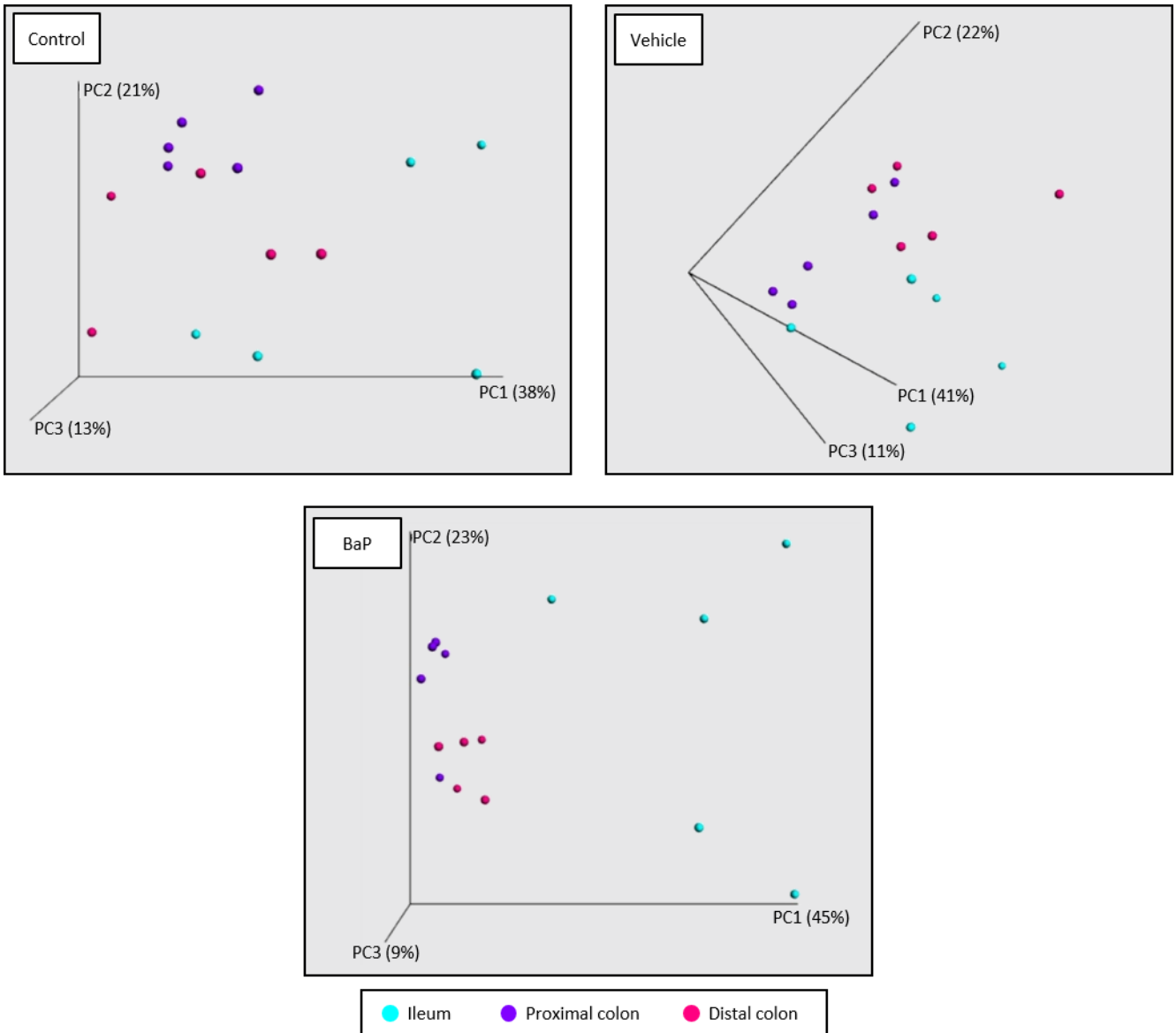




**Fig S4.** Temporal variations (from T0 to T27) of interesting taxa relative abundance in faecal microbiota in mice for control (blue), vehicle (green) and BaP-treated groups (red) revealed by 16S rRNA gene sequencing. Bar plots are presented by taxa.



**Fig S5.** Mucosa-associated microbiota composition profiles at the family level in the ileum (a), proximal colon (b) and distal colon (c) in mice revealed by 16S rRNA gene sequencing. Bar plots are presented by organ localization. Only the dominant bacterial taxa (relative abundance >1%) are represented. Families within the same bacterial phylum are indicated by different shades of the same color. The numbers above the profiles correspond to the total numbers of bacterial families (F, first line) and genera (G, second line) observed for each tissue sample.



**Fig S6.** Mucosa-associated bacteria of the ileum, proximal colon and distal colon for control, vehicle and BaP-treated mice differentiated by principal coordinate analysis (PCoA) on a weighted UniFrac distance matrix. The ileum, proximal and distal colon were represented in blue, purple and pink respectively.