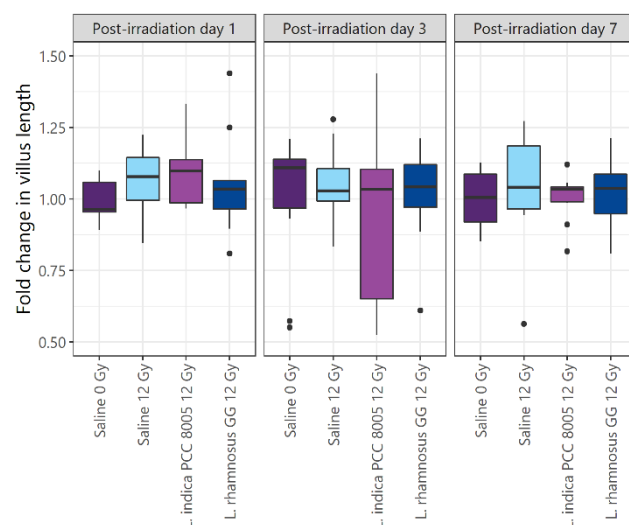*Supplementary Materials*



**Figure S1.** Boxplots showing the fold changes in mucosal parameter villus length following pelvic (sham-)irradiation for all different experimental groups, *n* = 10 per group. Statistical differences were assessed by linear modelling.
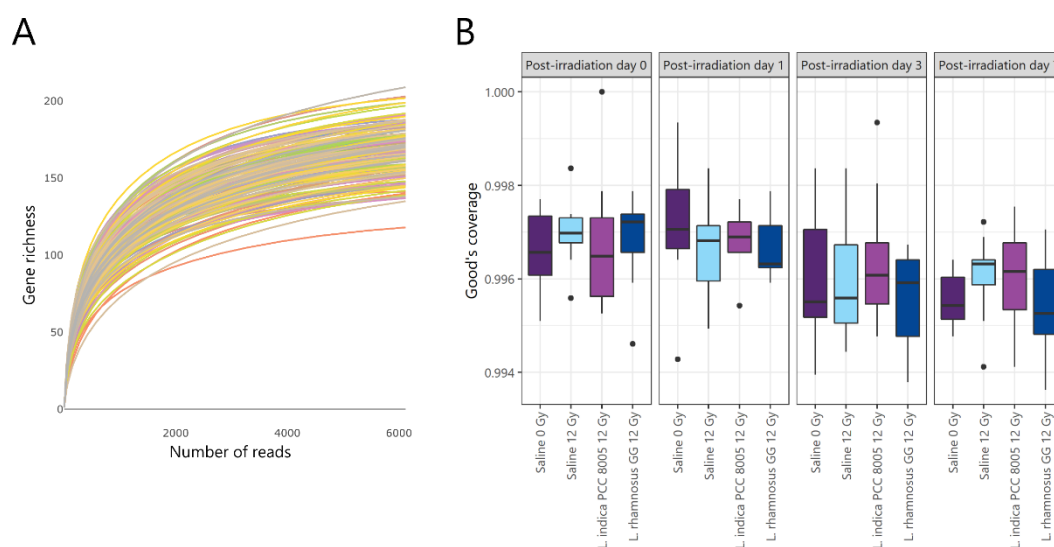


**Figure S2.** An adequate depth of sequencing was reached to identify most diversity in the samples. (**A**) Rarefaction curve displaying gene richness as a function of the number of reads per sample. (**B**) Good's estimator of coverage as a measure of sample completeness.
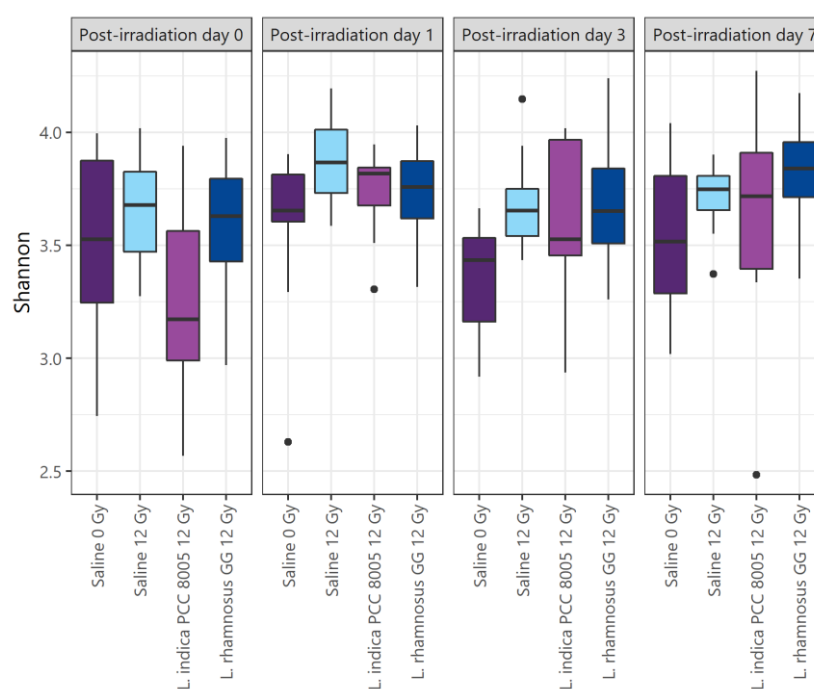
**Figure S3.** Changes in alpha diversity index Shannon, considering both richness and evenness, following (sham-)irradiation. Data are presented in boxplots, *n* = 10 per group. Time independent and –dependent differences were assessed by Mann Whitney U test (Bonferroni's post-hoc correction) and Wilcoxon signed rank test (Bonferroni's post-hoc correction), respectively.
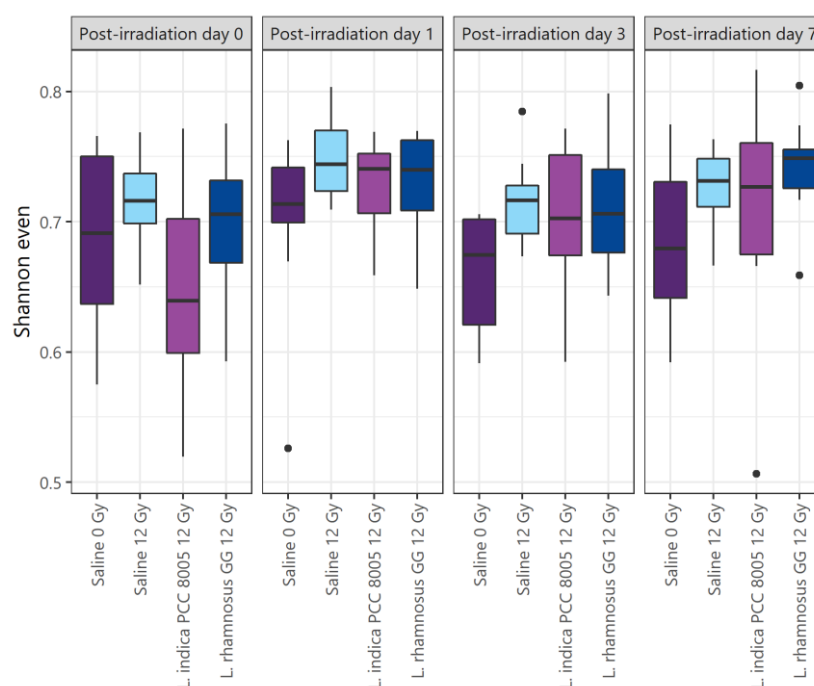


**Figure S4.** Changes in alpha diversity index Shannon even, considering solely evenness, following (sham-) irradiation. Data are presented in boxplots, *n* = 10 per group. Time independent and –dependent differences were assessed by Mann Whitney U test (Bonferroni's post-hoc correction) and Wilcoxon signed rank test (Bonferroni post-hoc correction), respectively.
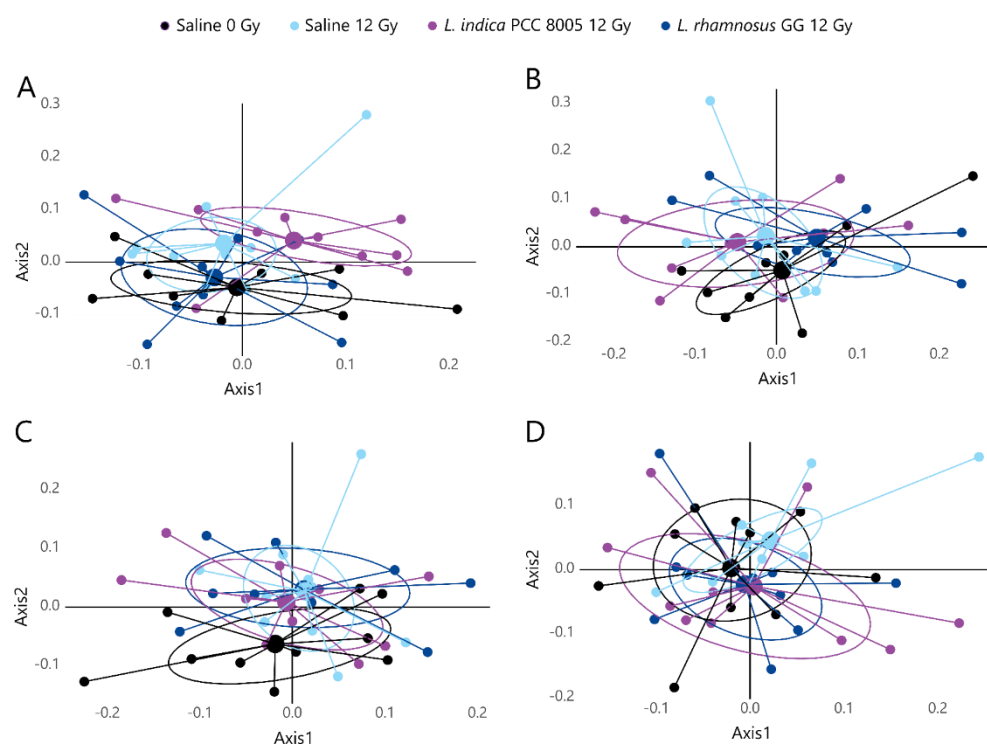
**Figure S5.** Unweighted UniFrac NMDS plots displaying inter-sample diversity among the different treatment groups at (**A**) post-irradiation day 0, (**B**) post-irradiation day 1, (**C**) post-irradiation day 3 and (**D**) post-irradiation day 7, $n \geq 9$ per group.



**Figure S6.** Heatmap representing the relative abundance of significant gut microbial dysbiosis markers detected in saline given mice following sham-irradiation as listed in Supplementary table 2, $n = 10$ per time point. PID = post-irradiation day.
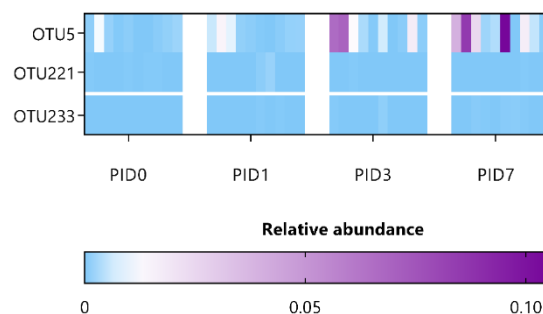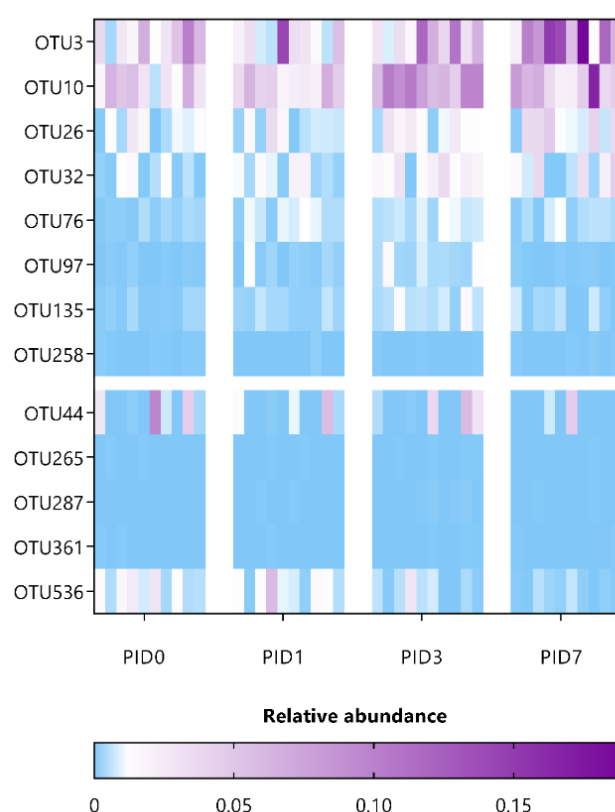
**Figure S7.** Heatmap representing the relative abundance of significant gut microbial dysbiosis markers detected in saline given mice following pelvic irradiation as listed in Supplementary table 3, *n* = 10 per time point. PID = post-irradiation day.

**Table S1.** *p* values (°*p* < 0.1, * *p* <0.05, ** *p* <0.01) of conditional unweighted UniFrac beta diversity analyses by AMOVA.

| | Unweighted Unifrac Beta Diversity |
|---|---|
| **PID0** | Overall *p* value: 0.015 * <br> *Post-hoc p* values: <br> • Saline 12 Gy vs Saline 0 Gy *p* = 0.244 <br> • *L. indica* PCC 8005 12 Gy vs Saline 0 Gy *p* = 0.054 ° <br> • *L. rhamnosus* GG 12 Gy vs Saline 0 Gy *p* = 0.16 <br> • *L. indica* PCC 8005 12 Gy vs Saline 12 Gy *p* = 0.179 <br> • *L. rhamnosus* GG 12 Gy vs Saline 12 Gy *p* = 0.125 <br> • *L. rhamnosus* GG 12 Gy vs *L. indica* PCC 8005 12 Gy *p* = 0.007 * |
| **PID1** | Overall *p* value: 0.235 |
| **PID3** | Overall *p* value: 0.408 |
| **PID7** | Overall *p* value: 0.008 * <br> *Post-hoc p* values: <br> • Saline 12 Gy vs Saline 0 Gy *p* = 0.077 ° <br> • *L. indica* PCC 8005 12 Gy vs Saline 0 Gy *p* = 0.015 * <br> • *L. rhamnosus* GG 12 Gy vs Saline 0 Gy *p* = 0.034 * <br> • *L. indica* PCC 8005 12 Gy vs Saline 12 Gy *p* = 0.279 <br> • *L. rhamnosus* GG 12 Gy vs Saline 12 Gy *p* = 0.132 <br> • *L. rhamnosus* GG 12 Gy vs *L. indica* PCC 8005 12 Gy *p* = 0.79 |

**Table S2.** Differential OTUs detected in saline given mice following sham-irradiation.

| Taxonomic Classification (Following Ribosomal Database Project) | ANCOM Biomarkers' Effect Size and W-Statistic | Highest NCBI Blast Hit (% Identity) |
|---|---|---|
| *Erysipelotrichaceae*_OTU5 | 1.47; *W* = 0.9 (PID3); 2.15; *W* = 0.9 (PID7) | *Faecalibaculum rodentium* ( > 97% identity) |
| *Coriobacteriaceae*_OTU221 | 1.06; *W* = 0.9 (PID1); 1.06; *W* = 0.9 (PID3); 1.06; *W* = 0.9 (PID7) | |
| *Firmicutes*_OTU233 | −1.27; *W* = 0.9 (PID7) | |

**Table S3.** Differential OTUs detected in saline given mice following pelvic irradiation.

| Taxonomic Classification (Following Ribosomal Database Project) | ANCOM Biomarkers' Effect Size and W-Statistic | Highest NCBI Blast Hit (% Identity) |
|---|---|---|
| *Lactobacillus*_OTU3 | 1.10; *W* = 0.9 (PID7) | *Lactobacillus taiwanensis* ( > 99% identity) |
| *Prevotella*_OTU10 | 1.16; *W* = 0.9 (PID3) | |
| *Bacteroides*_OTU26 | 1.10; *W* = 0.9 (PID7) | *Bacteroides stercorirosoris* ( > 98% identity) |
| *Porphyromonadaceae*_OTU32 | 1.04; *W* = 0.9 (PID1); 2.03; *W* = 0.9 (PID3); 1.51; *W* = 0.9 (PID7) | |
| *Porphyromonadaceae*_OTU76 | 1.08; *W* = 0.7 (PID3); 1.03; *W* = 0.7 (PID7) | |
| *Rhodospirillaceae*_OTU97 | 2.01; *W* = 0.8 (PID3) | |
| *Alistipes*_OTU135 | 1.90; *W* = 0.8 (PID3) | *Alistipes putredinis* (~97% identity) |
| *Clostridiales*_OTU258 | 1.09; *W* = 0.9 (PID1) | |
| *Lachnospiraceae*_OTU44 | −1.14; *W* = 0.9 (PID7) | |
| *Ruminococcaceae*_OTU265 | −1.16; *W* = 0.9 (PID3) | |
| *Bacteria*_OTU287 | −1.05; *W* = 0.9 (PID3) | |
| *Lachnospiraceae*_OTU361 | −1.12; *W* = 0.9 (PID3) | |
| *Lachnospiraceae*_OTU536 | −1.84; *W* = 0.8 (PID7) | |