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Supplementary Materials

Gut microbiota prevents small intestinal tumor formation due to bile acids in gnotobiotic mice

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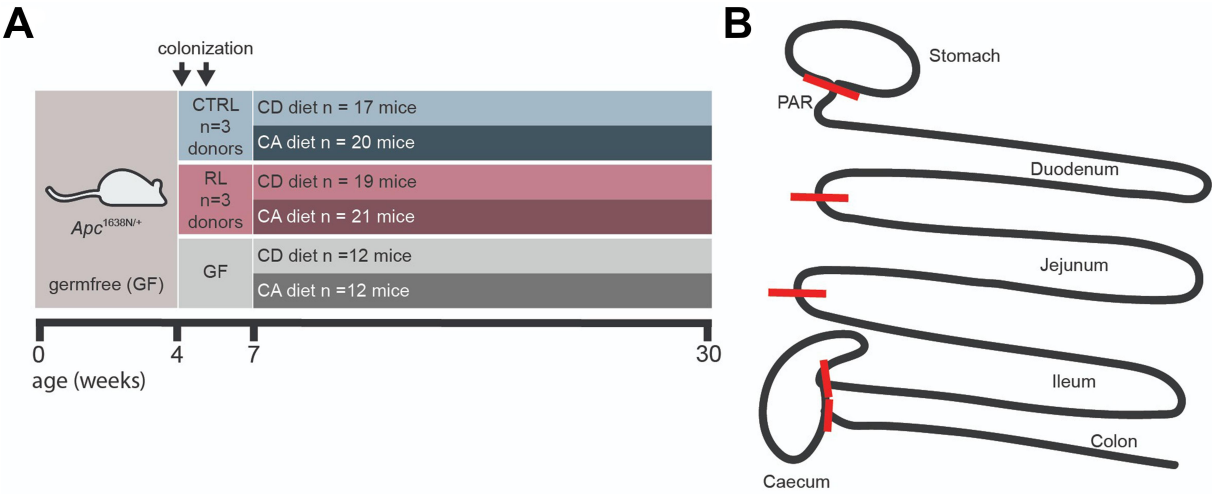
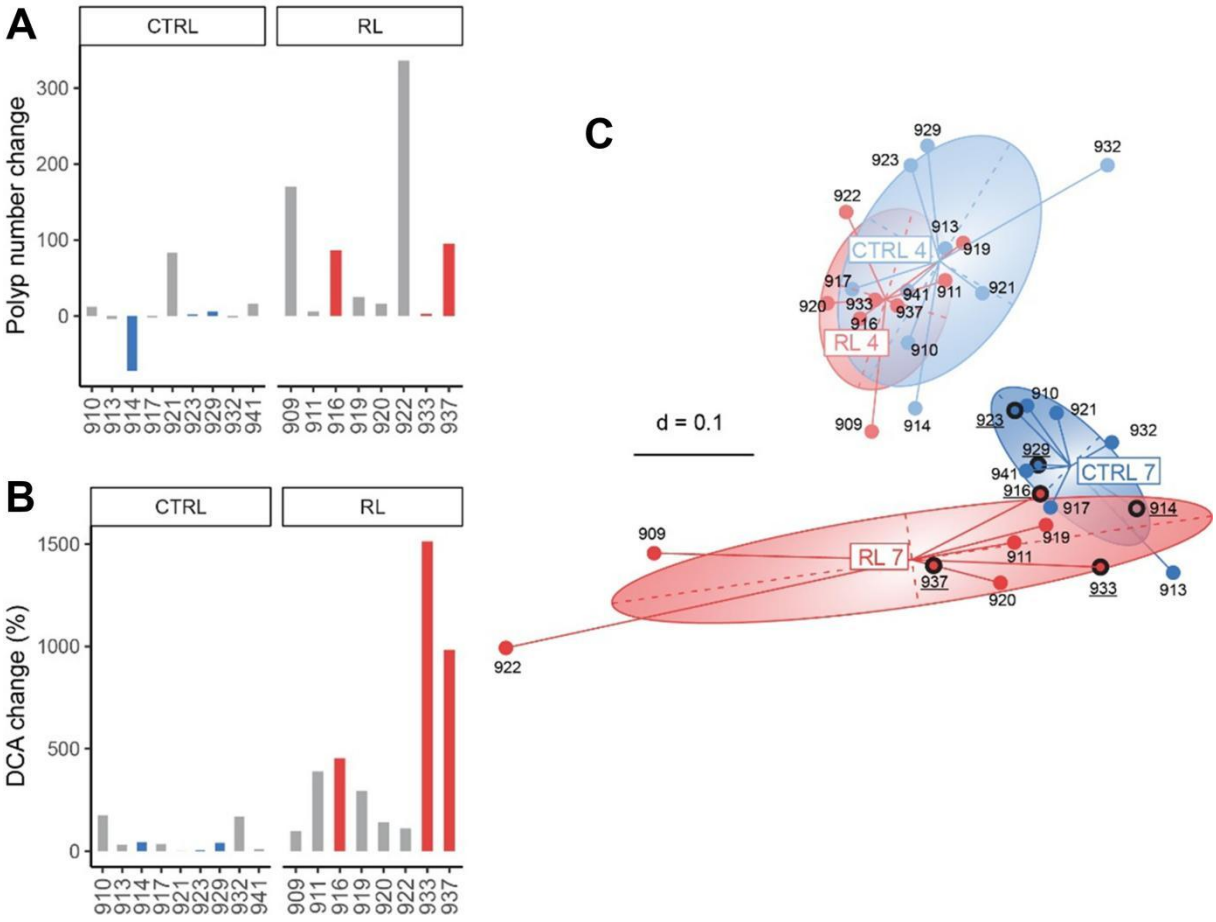
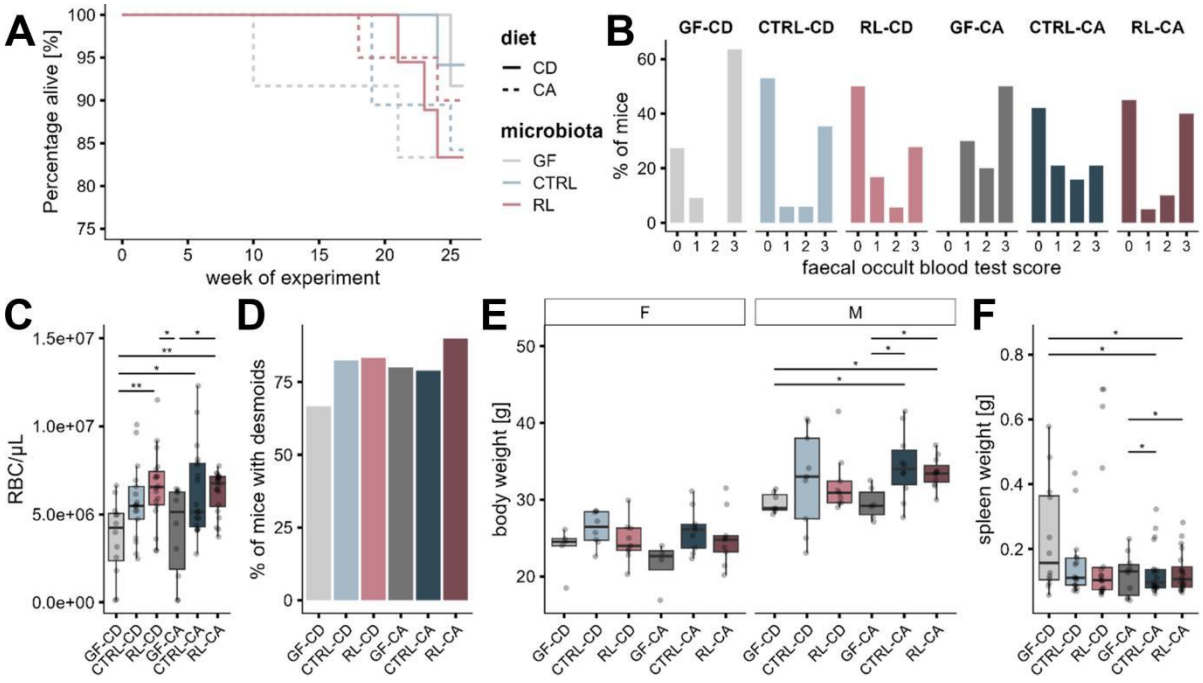


Supplementary Figure 1. Experimental design and sampling information. (A) Simplified scheme of the experimental design, including timeline and number of donor pigs and recipient mice; (B) Gut regions as defined for cutting during sampling.

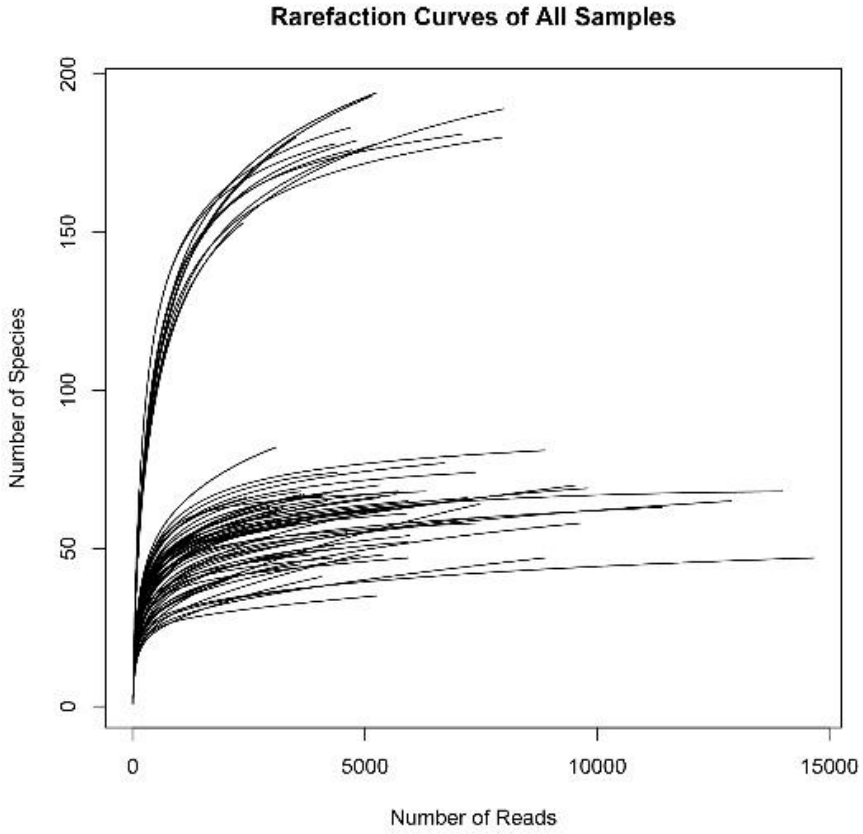


Supplementary Figure 2. Information on the selected pigs that served as donors for fecal microbiota transfer (n = 3 for each diet). (A) Change in polyp number in the distal colon; (B) Change in fecal DCA levels over the feeding period (7 months vs. 4 months); (C) Microbiota profiles, shown as NMDS plots based on Generalized UniFrac distances. The pigs selected for FMT are marked in color (a and b) or with underscore and black circles (C). Colors: blue = pigs fed the control diet (CTRL); red = pigs fed the diet supplemented with red meat and lard (RL). Light colors indicate the 4-month time point (before intervention). Dark colors indicate the 7-month time point (after intervention).

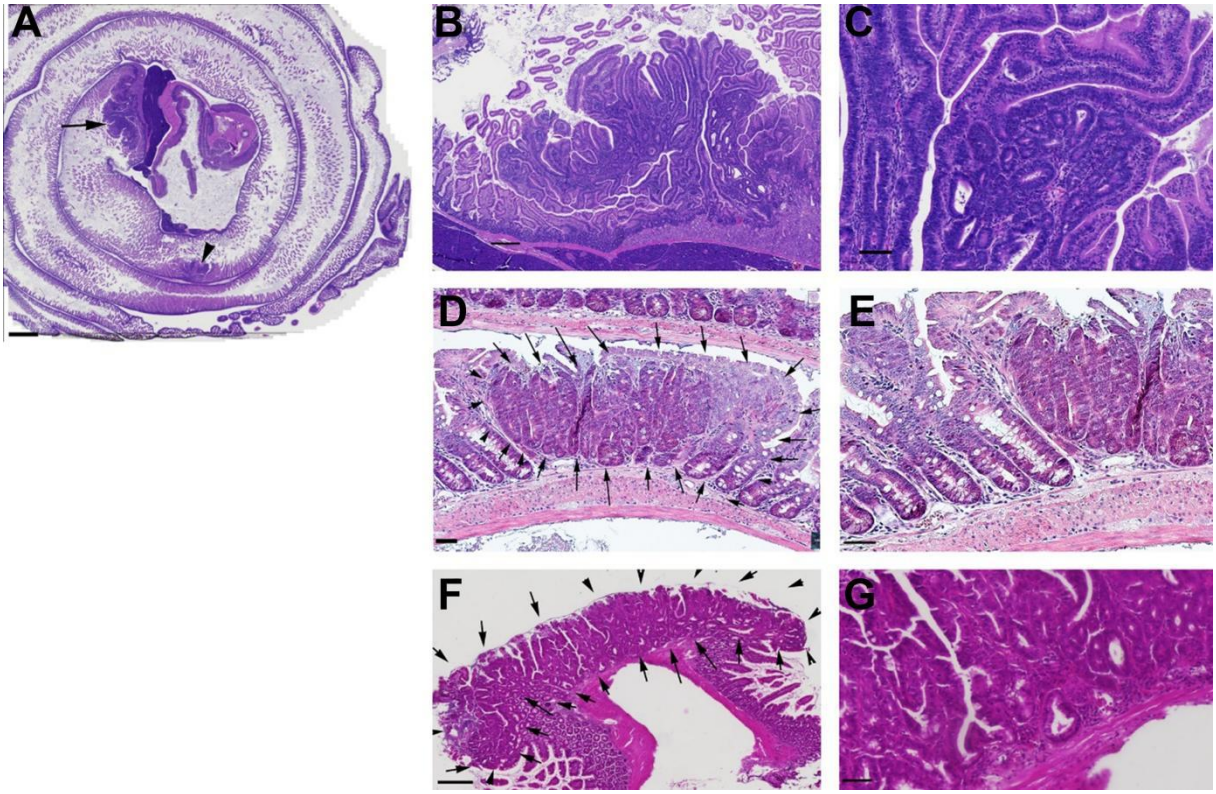


Supplementary Figure 3. Additional sampling data. (A) Kaplan-Meier survival curves (log-rank test, $P = 0.9$); (B) Percentage of mice assigned to the different fecal occult blood test scores; (C) Red blood cell (RBC) count; (D) Percentage of mice with desmoids; (E) Body weight over time; (F) Spleen weight. Statistics in (C): Kruskal-Wallis followed by Dunn's multiple comparisons with Benjamini-Hochberg adjustment (*P.adj < 0.05; **P.adj < 0.01). GF, germfree; CTRL, recipient mice colonized with the microbiota from donor pigs fed a control diet; RL= recipient mice colonized with the microbiota from donor pigs fed an experimental diet enriched in red meat and lard; CD, control diet (recipient mice); CA, control diet supplemented with the primary bile acid cholic acid (recipient mice).

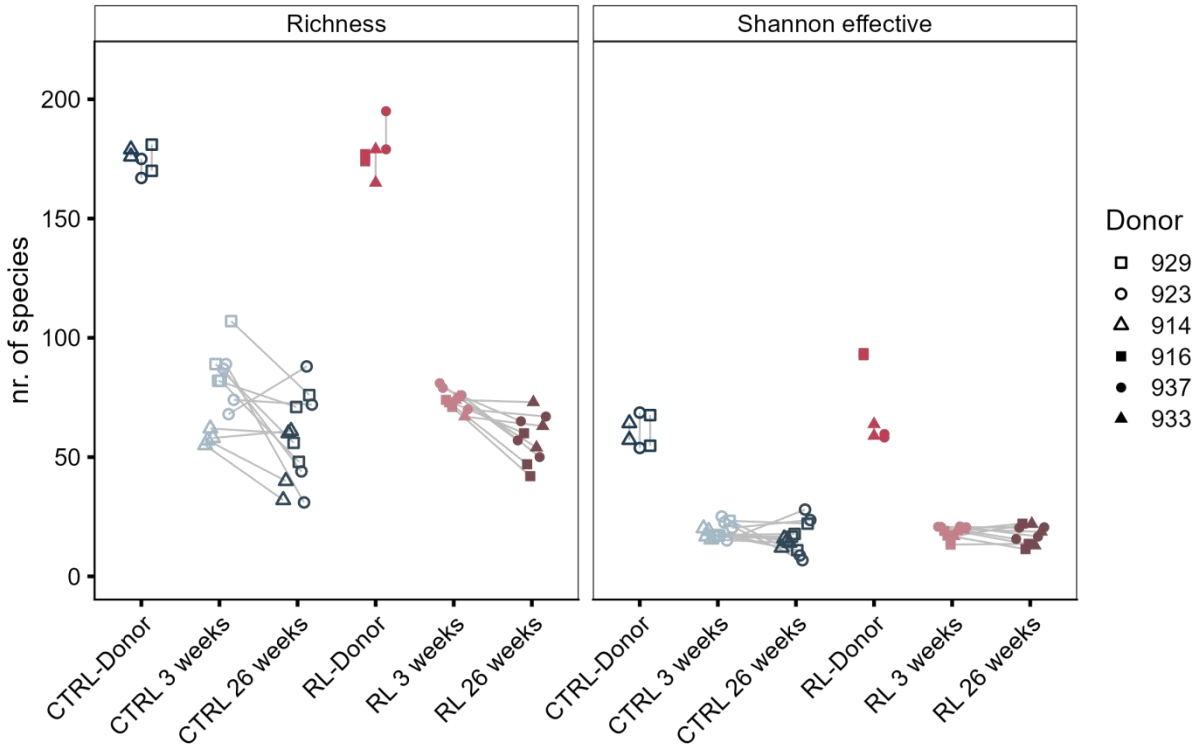
Supplementary Figure 4. Arbitrary scoring scheme for Fecal Occult Blood (FOB) tests.



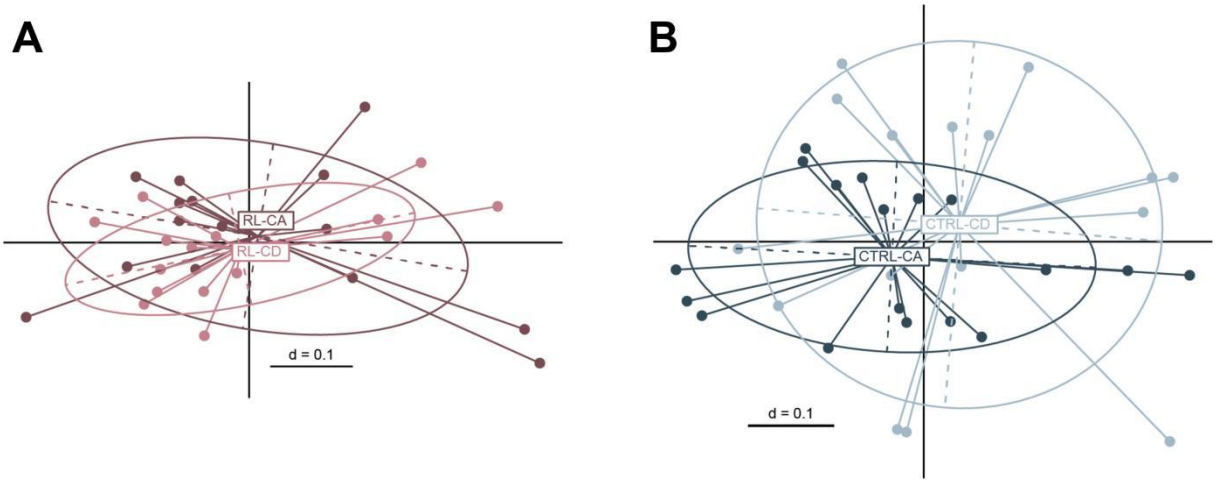
Supplementary Figure 5. Rarefaction curves of donor samples (top lines) ad recipient mice (bottom lines).



Supplementary Figure 6. Exemplary HE staining of tumors shown in Figure 1. (A) Overview (Swiss roll) of gut tissue from a germfree $Apc^{1638N/+}$ mouse (female, 7 months of age) fed autoclaved standard chow diet; bar = 1 mm; arrow shows a tumor in the periampullar region (PAR); arrowhead shows a tumor in the duodenum. (B, C) adenoma in the PAR of a germfree mouse fed the CA diet; (B) Overview, bar = 250 µm; note the presence of pancreatic tissue at the bottom; (C) high power field of view of dysplastic area, bar = 50 µm; (D, E) Adenoma in the jejunum of a gnotobiotic mouse (CTRL microbiota) fed the CA diet; d Overview, arrows denote the outline of the lesion, bar = 250 µm. e High power field of view, bar = 50 µm; (F, G) adenoma with low grade dysplasia in the duodenum of germfree mouse fed the CTRL diet. (D) Overview, bar = 250 µm. (E) High power field of view, bar = 50 µm.



Supplementary Figure 7. Richness (left plot) and Shannon effective counts (right plot) over time in donor samples (pig stool), mouse feces 3 weeks after colonization (light colors), and cecal content at the end of the experiment, i.e., 36 weeks after colonization (dark colors). The donors are indicated with different symbols. Colors: blue, CTRL microbiota; red, RL microbiota. Samples from the same mouse/donor are connected with grey lines.



Supplementary Figure 8. Pairwise comparisons of microbiota structure, shown as NMDS plots. (A) Comparison of diet effect within RL-recipient mice; PERMANOVA, *P*.adj = 0.057. (B) Comparison of diet effect within CTRL-recipient mice; *P*.adj = 0.017.

Supplementary Table 1. Published species/strains with a bai operon

Species	Strain	Accession nr.	Ref.
Clostridium scindens	ATCC35704	ABFY02000057	[1]
Clostridium [Extibacter] hylemonae	TN-271	AB023972	[2]
Clostridium leptum	DSM753	ABCB02000019	[1]
Clostridium sordelli	ATCC9714	NR_112173.1	[3]
Peptacetobacter hiranonis	TO-931	AB023970	[4,5]
Proteocatella sphenisci	PPP2	AF450134	[6]
Extibacter muris	DSM8650^T	KR364761	[7]

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