1 **Extended methods**
2
3 **Mice**
4 *Rag1*$^{-/-}$ mice, *Foxp3*^{RFP}*Ifng*^{Katushka}*Il17a*^{GFP} reporter mice, *Il10*$^{-/-}$mice, *Mdr2*$^{-/-}$mice, *Il10*$^{-/-}$
5 *Mdr2*$^{-/-}$mice, and *Rag1*$^{-/-}$*Mdr2*$^{-/-}$ mice were bred and housed under specific pathogen-
6 free conditions (SPF) at the animal facility of the University Hospital Hamburg-
7 Eppendorf. *Il10*$^{-/-}$*Mdr2*$^{-/+}$ were crossed with each other in order to obtain *Il10*$^{-/-}$ and *Il10*$^{-}$
8 $^{/-}$*Mdr2*$^{-/-}$ littermates. These mice were then separated with respect to their genotype
9 after weaning. This way the mice could develop a specific microbiota dependent on
10 the genotype. *Il10*$^{-/-}$*Mdr2*$^{-/+}$ mice were bred with two different microbiomes, one that
11 does not induce spontaneous colitis (MB1) and a colitogenic microbiome (MB2),
12 containing *Helicobacter hepaticus*(24). Littermates were separated with respect to their
13 genotype. C57BL/6 wild-type mice were bred and housed under germ-free conditions
14 at the UKE, Hamburg. All animals were housed under a 12h dark/light cycle with an
15 ambient temperature of 22°C ± 1°C and 50% ± 5% relative humidity. Food and water
16 were provided *ad libitum*. Male and female 13 ±1 week old littermates were used for
17 all experiments. Animal experiments were approved by the local ethics committee
18 (N17/2012, N39/2021, N54/2022, N95/2023).
19
20 **Human studies**
21 Intestinal biopsies were taken from people undergoing colonoscopy at University
22 Hospital Hamburg-Eppendorf. We collected 2 paired biopsies at 4 sampling sites in the
23 colon and terminal ileum. One was used for RNA extraction while the other was used
24 for microbiota profiling. The biopsies were snap-frozen in liquid nitrogen directly after
25 colonoscopy and stored at −80°C until processing. For detailed patient information
26 (age, BMI, years of diagnosis, smoking, gender, IBD activity and medication) see
27 Wittek et al, 2023. Patients were included, if older than 18 years and without antibiotics
28 treatment for 6 months prior to endoscopy. Patients with infectious colitis, celiac
29 disease, or confirmed pregnancy were excluded. Disease severity was based on Mayo
30 score for UC (remission: 0-2, mild: 3-5, moderate: 6-10, severe 11-12 points) and
31 Harvey-Bradshaw index for CD (remission: 0-4, mild: 5-7, moderate: 8-16, severe >16
32 points). For comparison of IBD patients we assigned scores for disease severity
33 (remission: 0, mild: 1, moderate: 2, severe 3 points)(25). Human studies were
34 approved by the local ethical committee (Ethik Kommission der Ärztekammer Hamburg

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35 PV4444, PV7106). Patients or the public were not involved in the design, or conduct,
36 or reporting, or dissemination plans of our research.
37
38 **DDC-induced sclerosing cholangitis**
39 The chemically-induced model for experimental sclerosing cholangitis, 3,5-
40 diethoxycarbonyl-1,4-dihydrocollidine (DDC; Merk, Germany) was used, by adding 0.1
41 % DDC *w/w* to the diet. *Il10*$^{-/-}$ mice at 10–14-weeks old were fed for 8 days *ad libitum*
42 with a DDC diet. At day 8, mouse development of intestinal inflammation was assessed
43 by endoscopy. One day later, mice were sacrificed and organs were processed as
44 indicated.
45
46 **Transfer colitis**
47 Lymphocytes were isolated from the spleen and lymph nodes of
48 *Foxp3*^{RFP}*Il17a*^{Katushka}*Il10*^{GFP} reporter mice, and CD4^{+} T cells were pre-enriched using
49 MACS according to the manufacturer's instructions (Miltenyi Biotech, Bergisch-
50 Gladbach, Germany). Naïve CD4^{+} CD45RB^{high} *Foxp3*$^{RFP-}$ T cells were fluorescence
51 activated cell sorted after incubation with anti-mouse CD4-PacBlue and CD45RB-
52 AF647 fluorochrome-labeled antibodies (both Biolegend, London, England) using
53 theAria III device (BD Biosciences, Heidelberg, Germany). To induce colitis, $2x10^{4}$
54 Naïve CD4^{+} CD45RB^{high} *Foxp3*$^{RFP-}$ T cells were injected intraperitoneally into *Rag1*$^{-/-}$
55 and *Rag1*$^{-/-}$*Mdr2*$^{-/-}$ mice. Mice were monitored for development of intestinal
56 inflammation by weight loss and endoscopy.
57
58 **Anti-IL-10 receptor antibody-induced colitis**
59 Mice were each injected with 250 µg of anti-IL10 receptor-alpha (anti-IL10Ra; clone:
60 1B1, source: HHMI, R.A. Flavell) twice a week intraperitoneally. Colitis development
61 was monitored by weight loss and endoscopy. Thirteen days after the first injection,
62 mice were sacrificed and analyzed for pathological conditions of the intestine.
63
64 **DSS colitis**
65 Mice received drinking water supplemented with 2% DSS for 7 days, followed by 2
66 days of pure drinking water in the absence of DSS, to induce acute DSS colitis (DSS
67 m.w.: 36.000–50.000; MP Biomedicals, Illkirch, France). Colitis development was

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68 monitored by endoscopy. The mice were sacrificed and analyzed for pathological
69 conditions of the intestine.
70
71 **Endoscopy**
72 Colonoscopy was performed at the indicated time points to monitor the severity of
73 intestinal inflammation as described before (Becker *et al.,* 2006) using the Coloview
74 System (Karl Storz, Germany). In brief, anesthetized mice were endoscopically scored
75 concerning 5 parameters: thickening of the colon, changes in vascular pattern,
76 granularity of the mucosal surface, stool consistency, and visible fibrin, each graded 1
77 to 3, resulting in an overall score between 0 (healthy) and 15 (severe colitis).
78
79 **Fecal microbiota transplantation**
80 For murine fecal transplantation, donor mice with a colitogenic microbiome (MB2) were
81 sacrificed and stool was collected from the colon, including the caecum, directly into
82 thioglycolate medium (Merck, Darmstadt, Germany). Pooled samples from a minimum
83 of 5 mice were smashed through a 70 µm cell strainer, frozen, and stored at -80°C.
84 Upon use, stool samples were thawed, transferred to BHI medium (Merck, Darmstadt,
85 Germany), and centrifuged for 10 min at 500g. The supernatant was resuspended in
86 BHI medium and immediately gavaged. Each mouse was gavaged with 200 ul of stool.
87 For human FMT, stool samples from people with IBD and PSC-IBD, coming to the
88 clinic for routine care appointments were collected and frozen in 20% glycerol and
89 processed for transfer into germ-free mice as described above.
90
91 **Taxonomic microbiota analysis**
92 Fresh stool samples of humans and mice were collected and immediately stored at
93 −20°C until analysis. DNA was extracted according to established protocols using a
94 method combining mechanical disruption (bead-beating) and phenol/chloroform-based
95 purification previously described (Turnbaugh *et al., 2009*). Briefly, a sample was
96 suspended in a solution containing 500 µL of extraction buffer (200 mM Tris, 20 mM
97 EDTA, and 200 mM NaCl [pH 8.0]), 200 µL of 20% SDS, 500 µL of
98 phenol:chloroform:isoamyl alcohol (24:24:1). Samples were homogenized twice with a
99 bead beater for 2 min. After precipitation of DNA, crude DNA extracts were
100 resuspended in Tris, EDTA (TE) buffer. Amplification of the V3-V4 region of the 16S

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101 rRNA gene was performed according to previously described protocols. Samples were
102 sequenced on an Illumina NovaSeq platform (PE250).
103 Microbiota profiling of adherent microbiota of human intestinal biopsies was carried out
104 as described (Wittek *et al.,* 2023). Briefly, DNA was extracted using the DNeasy Blood
105 & Tissue Kit, followed by amplification of variable regions V1 and V2 of the 16S rRNA
106 gene. PCR products were verified and quantified before pooling and sequencing on
107 the Illumina MiSeq v3 2x300bp. Demultiplexing after sequencing was based on 0
108 mismatches in the barcode sequences. We processed both data sets with the same
109 pipeline (dada2, pyhloseq, DESeq2) and reference data base (Silva). Where
110 sequencing occurred in two batches, we included the batch variable in the design
111 formula during DESeq2 analysis. Importantly, in both cases we sequenced all groups
112 to be compared with the same method.
113

114 **Transaminases**

115 To monitor liver damage, aspartate aminotransferase (ASAT) and alanine
116 aminotransferase (ALAT) were analyzed in blood serum at the Institute for
117 Experimental Immunology and Hepatology (UKE, Hamburg), using an automated
118 procedure (COBAS MIRA; Roche, Basel, Switzerland).
119

120 **Cell isolation**

121 Mice were sacrificed by CO_2 and O_2 and immediately perfused with 5mL PBS via the
122 left heart ventricle. Colons were harvested, rinsed in PBS, cut into small pieces, and
123 incubated in a buffer containing 1.5% DTT (AppliChem, Darmstadt, Germany) for 20
124 min at 37°C. The resulting cell suspension, including intraepithelial cells (IEL), was
125 collected. In a second step, lamina propria cells were isolated from the remaining
126 tissue using collagenase solution containing 100U/ml collagenase (Sigma-Aldrich,
127 Taufkirchen, Germany) and 1000U/ml Dnase I (AppliChem, Darmstadt, Germany).
128 Following a 45 min incubation step at 37°C, the content was smashed through a
129 100 µm cell strainer and pooled with IELs. Tissue homogenates were washed with
130 PBS + 1% FBS at 380g and 4°C for 10 min. Leukocytes were isolated using a Percoll
131 gradient (GE Healthcare, Uppsala, Sweden). After isolation, cells were processed as
132 indicated.
133

134 **Flow Cytometry**

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135 For surface staining, the cells were incubated with the following fluorochrome-
136 conjugated monoclonal antibodies: anti-CD45 (clone: 30F11), anti-CD3 (clone: 17A2),
137 anti-CD4 (clone: RM4-5), and anti-CD45RB (clone:C3 63-16A) in the presence of a
138 blocking anti-FcgR mAb (clone: 2.4G2) for 20 min at 4°C. Unless otherwise specified,
139 mAbs were purchased from Biolegend (London, England).
140 For intracellular Foxp3 expression, cell surface markers were stained as described
141 above, followed by fixation of bound antibodies with 4% formalin for 30 min and
142 permeabilization with 0.1% NP-40 for 4 min both at RT. For detection, cells were
143 incubated with the PE-conjugated Foxp3 mAb (clone: JES5-16E3, eBioscience)
144 overnight at 4°C. Cells were analyzed using a Fortessa flow cytometer (BD
145 Biosciences) and FlowJo software (Tree Star, Ashland, OR, USA).
146

147 **RNA extraction and Real-Time PCR analysis**

148 Total RNA was extracted from intestinal biopsies using Trizol Reagent (Invitrogen,
149 Waltham MA) and bead beating as previously described (Pelczar *et al.,* 2016). For
150 cDNA synthesis, we used the High-Capacity cDNA Reverse Transcription Kit
151 (Thermofisher) on 2mg of RNA per reaction, following the manufacturer's instructions.
152 For Real-time PCR (RT-PCR) analysis, TaqMan Fast Advanced Master Mix
153 (Thermofisher) was used. The following TaqMan Probes were used: *FOXP3*
154 (Hs01058534_m1) and *HPRT1* (Hs02800695_m1). Relative expression was
155 normalized to HPRT and calculated using the 2^{-DDCt} method. For significance testing,
156 we applied a linear mixed-effects model using the lme function in the nlme R package
157 (version 3.1) with the patient ID included as a random effect. This was followed by
158 post-hoc testing with Dunnett's multiple comparisons, using the glht function of the
159 multcomp R package.
160

161 **Immunohistochemistry**

162 Immunohistochemistry was performed on 5µm formalin fixed and paraffin embedded
163 sections of human colonic biopsies. Slides were deparaffinized and exposed to heat-
164 induced antigen retrieval for 5 minutes in an autoclave at 121°C in pH 7.8 wash buffer
165 (Dako, Glostrup, Denmark) and primary antibody specific for FOXP3 (dilution 1:72)
166 was applied. Bound antibody was then visualized using the EnVision Kit (Dako). All
167 sections were counterstained with hematoxylin. $FOXP3^{+}$ cells were counted in a

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168 blinded fashion from at least 5 areas of a given histological section and divided by the
169 total tissue surface obtained from the 5 areas.
170
171
172 **Microbiota data analysis**
173 16S sequencing reads from mouse stool samples were processed, aligned, and
174 quantified to the level of amplicon sequence variants (ASVs) using the dada2 (version
175 1.12.1) pipeline for paired reads (Callahan *et al.,* 2016). Forward and reverse reads
176 were trimmed to 220bp. Trimmed reads with more than 2 expected errors were
177 discarded. After merging forward and reverse reads, chimera removal was performed.
178 We kept all ASVs that were observed in at least 1% of all samples. Taxonomic
179 assignment up to the genus level was performed using the SILVA database from
180 September 2019 (v138) (Quast *et al.,* 2012).
181 Further analysis of ASV count tables was carried out using phyloseq (version 1.28.0)
182 (McMurdie *et al.,* 2013). For beta diversity plots, non-metric multidimensional scaling
183 (NMDS) was applied using Bray-Curtis dissimilarity. To find differentially abundant
184 ASVs between groups, DESeq2 (version 1.36) (Love *et al.,* 2014) was used using Wald
185 statistics and parametric fitting of dispersions, followed by log2 fold change shrinkage,
186 and adjustment for multiple testing. ASVs were then filtered to an adjusted p-value <
187 0.05, an absolute log2 fold change > 1, and being detected in at least 3 samples.
188 Microbiota profiling of adherent microbiota of human intestinal biopsies was carried out
189 as described in detail in Wittek *et al.,* 2023 following a similar pipeline as for the mouse
190 stool samples. Importantly, reads from individual ASVs were pooled on the genus level
191 to account for the reduced abundance of reads obtained from biopsies compared to
192 stool.
193
194 **Statistical analysis**
195 For detailed statistical analysis of microbiota data, see microbiota data analysis above.
196 Statistical analysis of all other data was performed with the GraphPad Prism® Software
197 (GraphPad Software, San Diego, CA, USA). Non-parametric two-sided Mann–Whitney
198 test was used. The significance level alpha was set to 0.05.
199

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