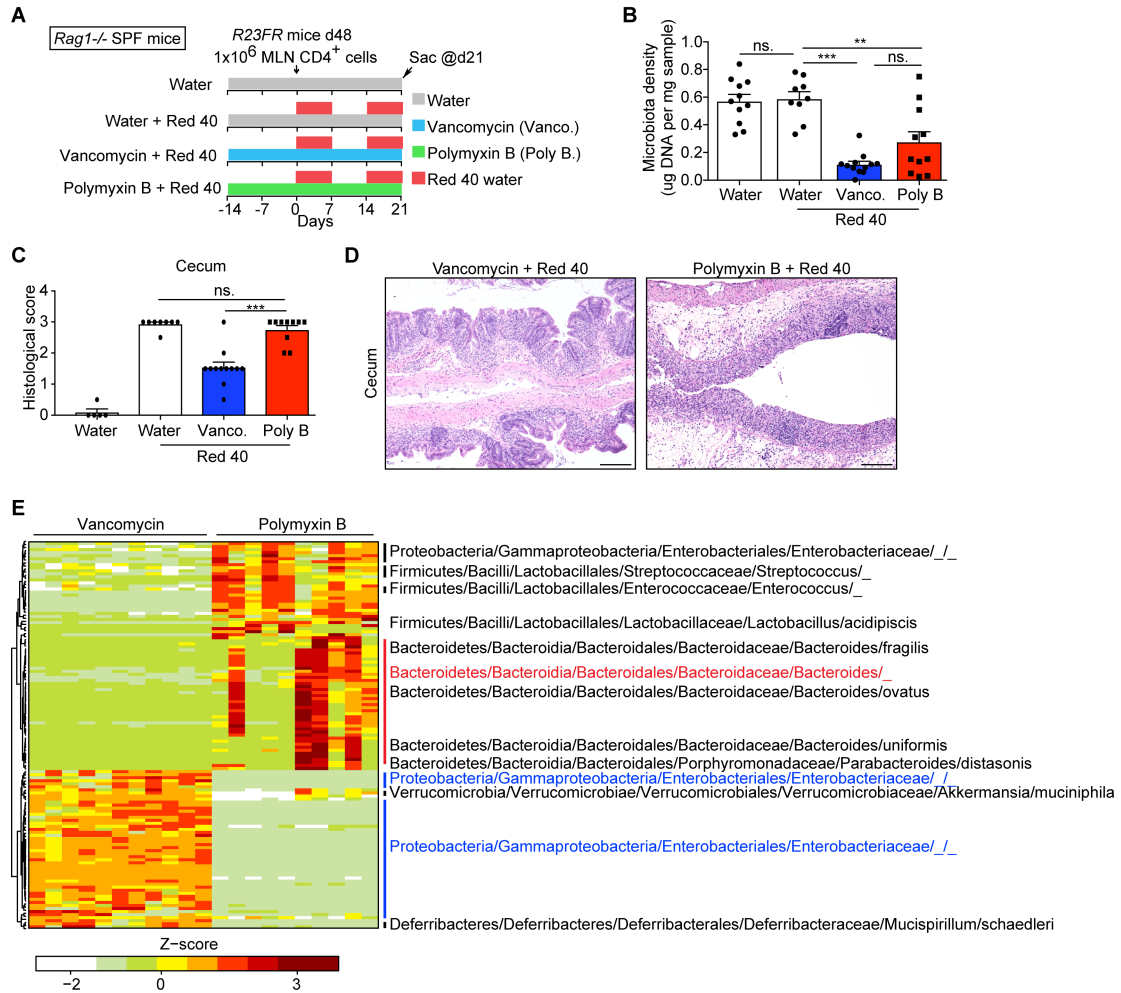


**Fig S1. Red 40 is colitogenic component in custom diet 2019(TD 160647), Related to Figure 1.**

(A) Experimental scheme. The diet 2019 (TD 160647) was solubilized with ethanol and then with water. The colitogenic activities of these fractions were tested by feeding them

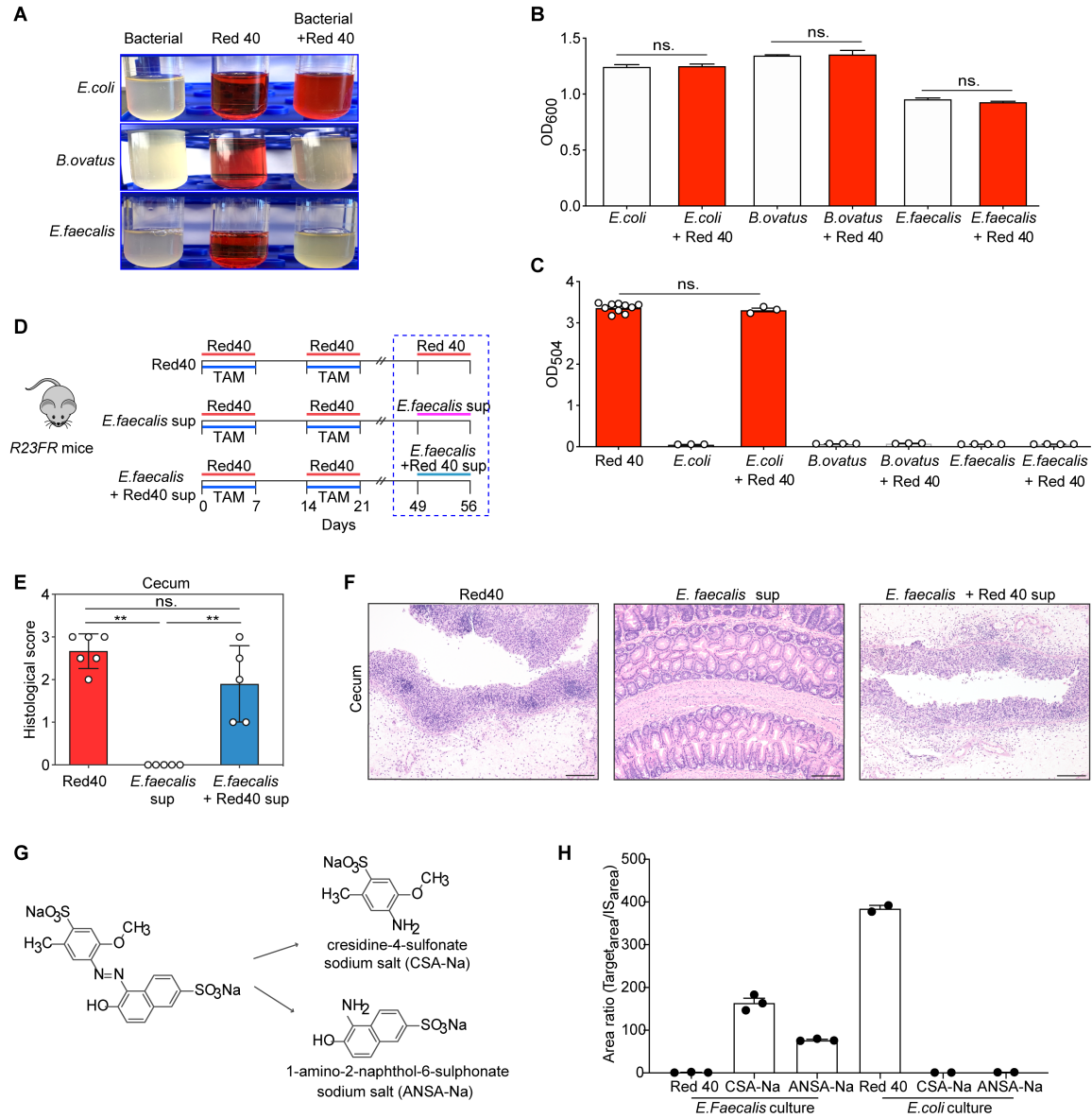
to *R23FR* mice in remission (d48, after diet 2019 TAM treatment). **(B)** Fecal lipocalin-2 (Lcn-2) levels were measured by ELISA at day 56. **(C,D)** Histologic scores **(C)** and representative H&E-stained sections **(D)** of the cecum of experimental mice described in Fig. S1A. **(E)** Experimental scheme. Diet 5053 (grey), diet 2019 (Red 40) (TD. 160647), diet 2019 (grey) (TD. 130833), diet 2019 (grey) plus 0.025% Red 40 in drinking water, and diet 5053 (grey) plus 0.025% Red 40 in drinking water were feed to *R23FR* mice in remission (d48, after diet 2019 TAM treatment). **(F)** Fecal Lcn-2 levels were measured by ELISA at day 56. **(G,H)** Histologic scores **(G)** and representative H&E-stained sections **(H)** of the cecum of experimental mice described in Fig. S1E. Scale bars, 50  $\mu$ m. In **(B)** **(C)** **(F)** and **(G)**, each dot indicates an individual mouse. Error bars indicate SEM. ns  $p > 0.05$ , \*\*\*  $p < 0.001$ , by nonparametric Mann-Whitney test.



**Fig S2. Bacteria enriched in the polymyxin B-treated mice associated with Red 40 induced colitis, Related to Figure 4.**

(A) Schematic representation of the strategy for identifying bacteria associated with Red 40 induced colitis from commensal flora by using antibiotic treatment. (B) Fecal microbiota density in SPF mice treated with antibiotics at d21 in Fig S2A. (C-D) Histologic scores (C) and representative H&E-stained sections (D) of the cecum of experimental mice. Scale bars, 50µm. In (B) and (C), each dot indicates an individual mouse. Data are shown as mean± SEM, ns p >0.05, \*\* p<0.01, \*\*\* p<0.001 by nonparametric Mann-Whitney test. (E) Fecal microbiome analyses of vancomycin or polymyxin B treated mice at d21 in Fig S2A. Pearson hierarchical clustering of the

abundance profiles of 129 OTUs that were significantly different between vancomycin treated mice and polymyxin B treated mice (ANOVA  $Q < 0.05$  [FDR]; Tukey  $P < 0.05$ ; fold  $> 4$ ). Each column represents a different mouse.



**Fig S3. *B. ovatus* and *E. faecalis* but not *E. coli* can metabolize Red 40 in vitro,**

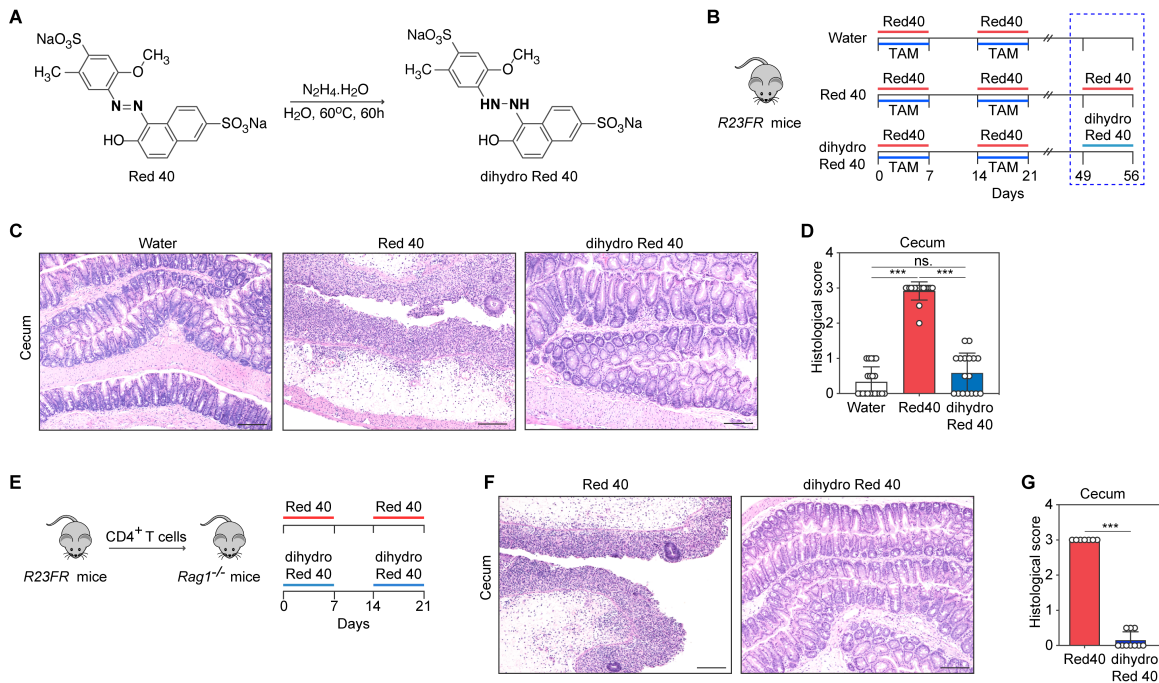
**Related to Figure 5.**

(A) Macroscopic view of *E. coli*, *B. ovatus* and *E. faecalis* culture with Red 40 (0.25g/L) in vitro. (B) Optical density (OD<sub>600nm</sub>) of bacterial culture with and without Red 40. Notice that Red 40 did not affect bacterial growth. Each dot indicates an individual tube of culture bacteria. Error bars indicate SEM. (C) The optical density (OD<sub>504nm</sub>) of Red 40 (indicating of decoloring of Red 40) in the culture supernatant after Red 40 culture with and without bacteria. Notice that both *B. ovatus* and *E. faecalis* but not *E. coli* can

metabolize Red 40. Each dot indicates an individual tube of culture bacteria. Error bars indicate SEM. Ns,  $p > 0.05$ .

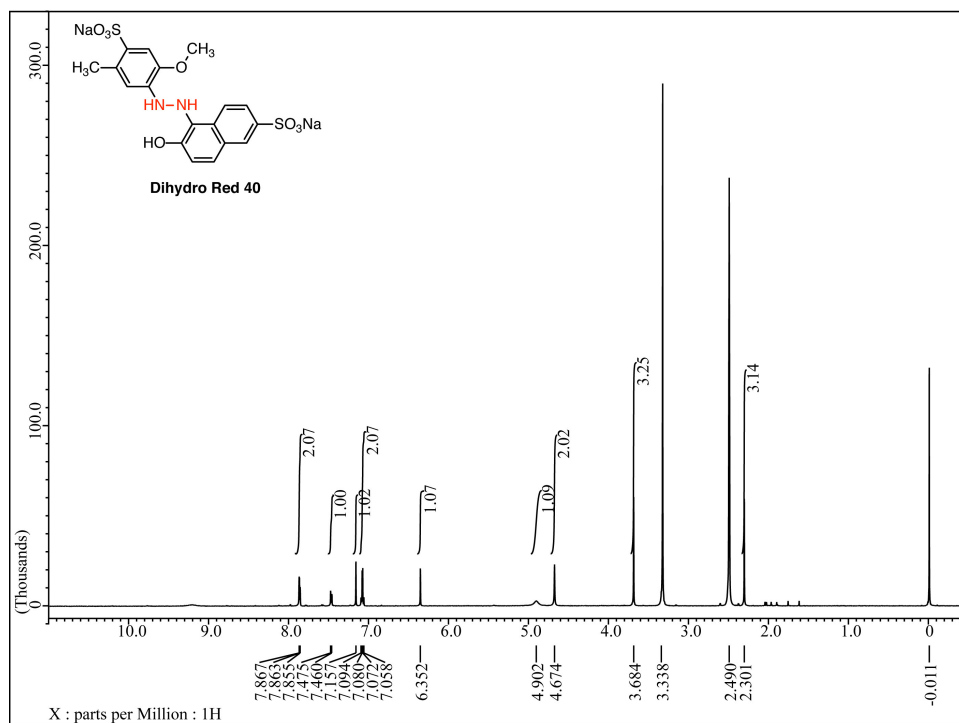
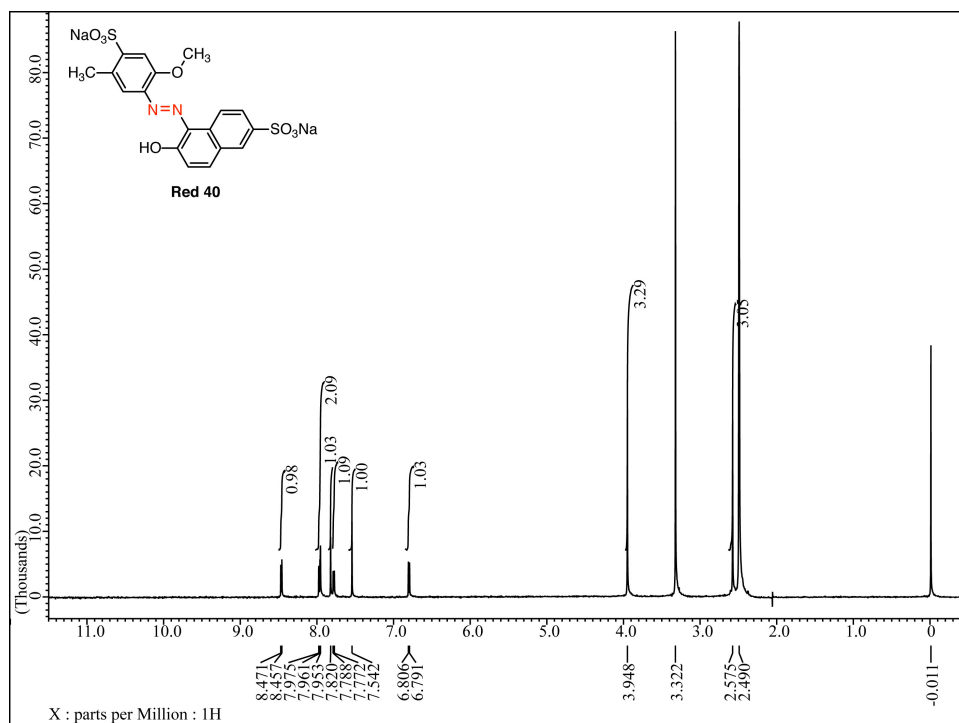
**(D)** Experiments designed to test colitogenic components in *E. faecalis* culture with Red 40. *R23FR* mice were treated with TAM in the food to induce IL-23 expression and treated with 0.025% Red 40 (0.25g/L) in drinking water for 2 cycles. From day 49, groups of remission animals were treated with supernatants from *E. faecalis* culture with Red 40 (Fig. S3A) or with supernatants from *E. faecalis* culture alone (Fig. S3A) for 7 days. Mice were sacrificed for histological analyses at day 56. **(E,F)** Histologic scores **(E)** and representative H&E-stained sections **(F)** of the cecum of experimental mice described in Fig. S3D. In **(E)**, each dot indicates an individual mouse. Error bars indicate SEM. Scale bars in **(F)**, 50 $\mu$ m.

**(G)** Azo-reduction of Red 40 yields the compounds cresidine-4-sulfonate sodium salt (CSA-Na) and 1-amino-2-naphthol-6-sulphonate sodium salt (ANSA-Na). **(H)** The area ratio of target to trypan blue (internal standard, IS). The metabolic analysis of Red 40, CSA-Na and ANSA-Na in the culture supernatant of *E. faecalis* and *E.coli* cultured with 0.25g/L Red 40. Each dot indicates an individual sample. Error bars indicate SEM.



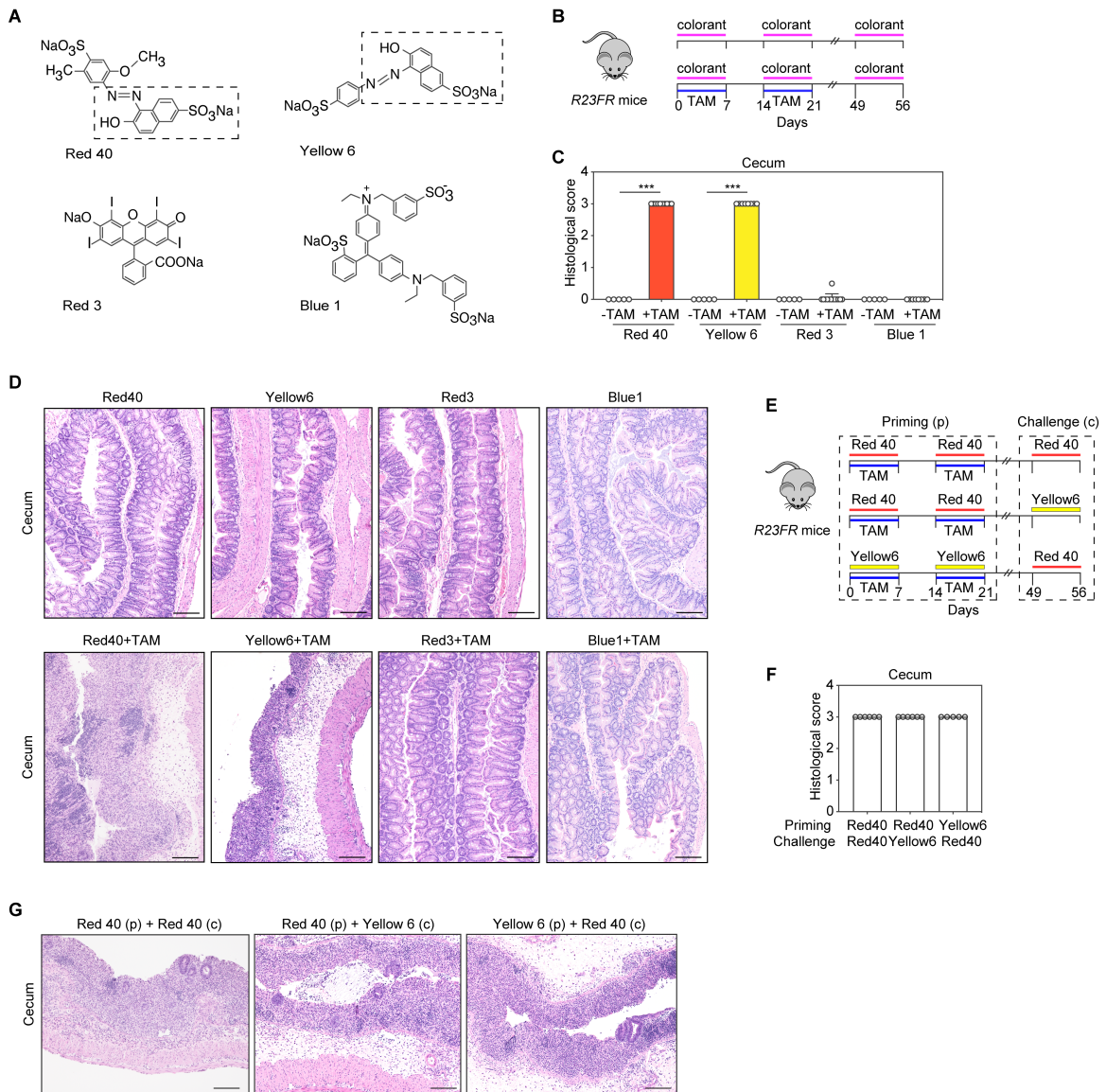
**Fig S4. Inactivation of the azo bond significantly reduces the colitogenic properties of Red 40, Related to Figure 5.**

**(A)** Preparation of dihydro Red 40 from Red 40. **(B)** Experimental scheme. *R23FR* mice were treated with TAM in the food to induce IL-23 expression and treated with 0.025% Red 40 (0.25g/L) in drinking water for 2 cycles. At d49, animals were treated with Red 40 in drinking water (0.25g/L) or with dihydro Red 40 in drinking water (0.25g/L) for 7 days. Mice were sacrificed for histological analyses at day 56. **(C,D)** Representative H&E-stained sections **(C)** and histologic scores **(D)** of the cecum of experimental mice described in Fig. S4B. **(E)** Experiments designed to test Red 40 and dihydro Red 40 in *Rag1*<sup>-/-</sup> mice transferred with *R23FR* CD4<sup>+</sup> T cells. **(F,G)** Representative H&E-stained sections **(F)** and histologic scores **(G)** of the cecum of experimental mice at d21 described in Fig. S4E. Scale bars, 50 $\mu$ m. In **(D)** and **(G)**, each dot indicates an individual mouse. Error bars indicate SEM. ns p > 0.05, \*\*\* p < 0.001, by nonparametric Mann-Whitney test.



**Fig S5.**  $^1\text{H}$  NMR spectra of the Red 40 and Dihydro Red 40 in  $\text{DMSO-}d_6$ , Related to STAR Methods.





**Fig S6. Role of other food colorants on the development of colitis in *R23FR* mice,**

**Related to Figure 6.**

(A) Chemical structure of Red 40, Red 3, Yellow 6 and Blue 1. The dotted line rectangles indicate the moiety of ANSA-Na in Red 40 and Yellow 6. (B) Experimental scheme. Group of animals were treated with TAM in the food to induce IL-23 expression and treated with 0.025% indicated food colorants (0.25g/L) in drinking water. Control groups of *R23FR* mice were treated with 0.025% indicated food colorants in drinking

water alone. **(C,D)** Histologic scores **(C)** and representative H&E-stained sections **(D)** of the cecum of experimental mice at day 56 described in Fig. S6B.

**(E)** Experimental scheme. *R23FR* mice were treated with TAM in the food to induce IL-23 expression and treated with 0.025% Red 40 or Yellow 6 (0.25g/L) in drinking water for 2 cycles. From day 49, groups of remission animals were treated with Red 40 in drinking water (0.25g/L) or with Yellow 6 in drinking water (0.25g/L) for 7 days. Mice were sacrificed for histological analyses at day 56. **(F,G)** Histologic scores **(F)** and representative H&E-stained sections **(G)** of the cecum of experimental mice described in Fig. S6E.

Scale bars, 50 $\mu$ m. In **(C)** and **(F)**, each dot indicates an individual mouse. Error bars indicate SEM. \*\*\*  $p < 0.001$ , by nonparametric Mann-Whitney test.

**Table S1: Fecal microbiota composition of ex-GF *Rag1*<sup>-/-</sup> mice colonized with cultured library (#1001136). (Related to Figure 4H-J)**

Cultured Library	Colonized RAG GF mice #OTU ID # Constructed from biom file	mice #1	mice #2	mice #3	mice #4	mice #5	SUM of 5 mice	
<i>g_Bacteroides</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_	39.38	59.37	69.56	36.51	66.97	271.78	
<i>Escherichia coli</i>	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_ ;s_	41.23	15.10	20.33	53.45	11.96	142.07	
<i>Bacteroides ovatus</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_ovatus	8.46	10.10	2.46	1.18	4.48	26.66	
<i>Bacteroides uniformis</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_uniformis	1.42	4.18	1.60	1.95	9.67	18.83	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_	2.49	7.34	0.14	1.35	1.42	12.74	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_ ;s_	3.16	1.14	0.79	1.63	1.16	7.89	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_	0.46	0.82	1.65	0.85	2.16	5.94	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus	1.66	1.00	0.50	1.04	0.54	4.73	
<i>Enterococcus durans</i>	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus;s_	0.86	0.33	0.13	1.38	0.05	2.74	
<i>Enterococcus faecium</i>	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus;s_	0.86	0.33	0.13	1.38	0.05	2.74	
<i>Alistipes onderdonkii</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_ ;s_	0.12	0.06	1.62	0.06	0.47	2.33	
<i>Bacteroides fragilis</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_fragilis	0.23	0.31	0.41	0.20	0.28	1.41	
<i>Odoribacter sp</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Odoribacteraceae];g_Odoribacter;s_	0.06	0.00	0.53	0.02	0.37	0.97	
<i>Bifidobacterium longum</i>	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_longum	0.15	0.05	0.01	0.08	0.10	0.39	
<i>Bifidobacterium bifidum</i>	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_bifidum	0.05	0.01	0.16	0.07	0.02	0.32	
<i>Collinsella aerofaciens</i>	k_Bacteria;p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Collinsella;s_aerofaciens	0.06	0.04	0.00	0.06	0.04	0.21	
<i>Bacteroides caccae</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_caccae	0.00	0.01	0.06	0.00	0.11	0.19	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_ ;g_ ;s_	0.02	0.02	0.01	0.01	0.08	0.14	
<i>Eggerthella lenta</i>	k_Bacteria;p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Eggerthella;s_lenta	0.05	0.01	0.00	0.04	0.01	0.11	
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_ ;s_	0.04	0.02	0.01	0.02	0.01	0.08	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Dorea;s_	0.01	0.00	0.01	0.02	0.02	0.05	
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_ ;s_	0.01	0.02	0.01	0.00	0.01	0.05	
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_eggerthii	0.00	0.01	0.00	0.00	0.02	0.04	
<i>Bacteroides salyersiae</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_	39.38	59.37	69.56	36.51	66.97	271.78	Sequencing not deep enough, need to be further confirmed.
<i>Bacteroides stercoris</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_	39.38	59.37	69.56	36.51	66.97	271.78	
<i>Bacteroides thetaiotaomicron</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_	39.38	59.37	69.56	36.51	66.97	271.78	
<i>Bacteroides vulgatus</i>	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_	39.38	59.37	69.56	36.51	66.97	271.78	
<i>Bifidobacterium adolescentis</i>	Not found in the colonized RAG GF mice							
<i>Flavonifractor plautii</i>	Not found in the colonized RAG GF mice							
<i>Parabacteroides johnsonii</i>	Not found in the colonized RAG GF mice							

Note: Data represented here were filtered by min\_count\_fraction = 0.01% and min\_samples = 2.