Supplementary Information

Infection of specific strains of *Streptococcus mutans*, oral bacteria, confers a risk of ulcerative colitis

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Supplementary information includes:
Supplementary Tables S1 to S3, Supplementary Figures S1 to S6.

Supplementary Tables

Supplementary Table S1. Summary of IBD patients.

Total number (Male:Female)		98 (59:39)
Age (mean ± SD)		44.51 ± 16.33
Diagnosis (Severity)	UC	98 (mild: 48, moderate: 7, remission: 43)
Duration of disease (mean ± SD)		7.64 ± 6.44 years
Extent	UC	entire colon: 33 left hemicolon: 43 rectum: 19 unknown: 3

Oral samples were collected from multiple institutes and hospitals. UC, ulcerative colitis; SD, standard deviation.

Supplementary Table S2. Analysis of *S. mutans*-serotype and *cnm* in IBD patients.

NO	gender	age	duration of disease	severity	extent	*S. mutans*	serotype	*Cnm*
1	M	34	2y	moderate	entire colon	+	*c*	−
2	M	24	2y	moderate	left hemicolon	+	*c*	−
3	F	23	15y	mild	left hemicolon	+	*c*	−
4	M	35	13y	mild	left hemicolon	−		
5	M	31	10y	mild	rectum	−		
6	M	42	5y	mild	rectum	+	*c*	+
7	M	58	10y	mild	left hemicolon	+	*c*	−
8	M	64	14y	moderate	left hemicolon	+	*k*	+
9	F	71	10y	mild	left hemicolon	+	*k*	−
10	M	39	2y	moderate	left hemicolon	+	*c*	−
11	F	55	23y	moderate	left hemicolon	+	*c*	−
12	M	75	1y	remission	entire colon	+	*c*	−
13	M	60	7y	remission	entire colon	+	*f*	+
14	M	27	1y	remission	left hemicolon	+	*c*	−
15	F	54	4y	remission	rectum	+	*c*	−
16	M	69	2m	mild	left hemicolon	+	*c*	−
17	M	33	4m	moderate	entire colon	+	*c*	−
18	M	19	4m	mild	entire colon	+	*k*	−
19	F	77	8m	remission	entire colon	+	*c*	−
20	M	69	1y	mild	N/A	−		
21	F	47	21y	remission	left hemicolon	+	*f*	+
22	F	47	13y	remission	entire colon	+	*f*	+
23	F	52	17y	moderate	left hemicolon	+	*c*	+
24	M	58	3m	mild	rectum	+	*c*	−
25	F	72	9y	mild	rectum	+	*c*	−
26	F	79	24y	mild	rectum	−		
27	M	36	9y	remission	entire colon	+	*e*	−
28	F	32	11y	remission	rectum	−		
29	M	49	19y	remission	left hemicolon	−		
30	F	43	10y	remission	rectum	−		
31	M	70	2m	mild	left hemicolon	+	*c*	−
32	F	64	2y	mild	left hemicolon	+	*k*	−
33	F	64	12y	mild	rectum	−		
34	F	62	3y	mild	whole	+	*c*	−
35	M	27	7y	mild	left hemicolon	−		
36	M	36	4y	mild	rectum	−		
37	F	33	12y	mild	entire colon	+	*f*	+

38	M	40	12y	mild	left hemicolon	+	*c*	−
39	M	47	2y	mild	rectum	−		
40	M	44	19y	mild	entire colon	+	*f*	−
41	M	39	4y	mild	entire colon	−		
42	M	32	11y	mild	left hemicolon	+	*c*	−
43	M	46	5y	mild	left hemicolon	+	*f*	−
44	M	72	3y	mild	entire colon	+	*c*	−
45	F	62	6y	mild	rectum	−		
46	F	28	9y	mild	left hemicolon	−		
47	M	32	7y	mild	left hemicolon	+	*f*	+
48	M	83	2y	mild	left hemicolon	+	*c*	−
49	F	74	9y	mild	left hemicolon	+	*k*	−
50	M	36	3y	mild	entire colon	+	*c*	−
51	F	36	2y	mild	entire colon	−		
52	M	22	3y	mild	left hemicolon	+	*c*	−
53	M	51	3y	mild	entire colon	−		
54	M	70	24y	mild	left hemicolon	−		
55	M	31	1y	mild	left hemicolon	−		
56	M	29	7y	mild	rectum	+	*c*	−
57	F	44	7y	mild	left hemicolon	+	*c*	−
58	F	44	5y	mild	left hemicolon	−		
59	M	59	2y	mild	left hemicolon	−		
60	M	39	3y	mild	rectum	−		
61	F	61	7y	mild	N/A	+	*c*	−
62	M	47	3y	mild	rectum	−		
63	M	37	3y	mild	left hemicolon	−		
64	M	56	7y	mild	left hemicolon	+	*k*	+
65	F	51	18y	mild	entire colon	+	*k*	+
66	F	37	3y	mild	rectum	−		
67	M	21	4y	remission	entire colon	−		
68	F	35	8y	mild	left hemicolon	−		
69	M	28	9y	remission	entire colon	+	*c*	−
70	F	28	1y	remission	left hemicolon	−		
71	F	42	13y	mild	left hemicolon	−		
72	M	22	2y	remission	rectum	−		
73	M	51	36y	remission	entire colon	−		
74	F	54	9y	remission	entire colon	−		
75	M	31	2y	remission	entire colon	−		
76	M	28	14y	mild	entire colon	−		
77	F	31	2y	remission	left hemicolon	+	*c*	+
78	M	57	8y	remission	entire colon	−		
79	M	31	7y	remission	entire colon	+	*c*	−

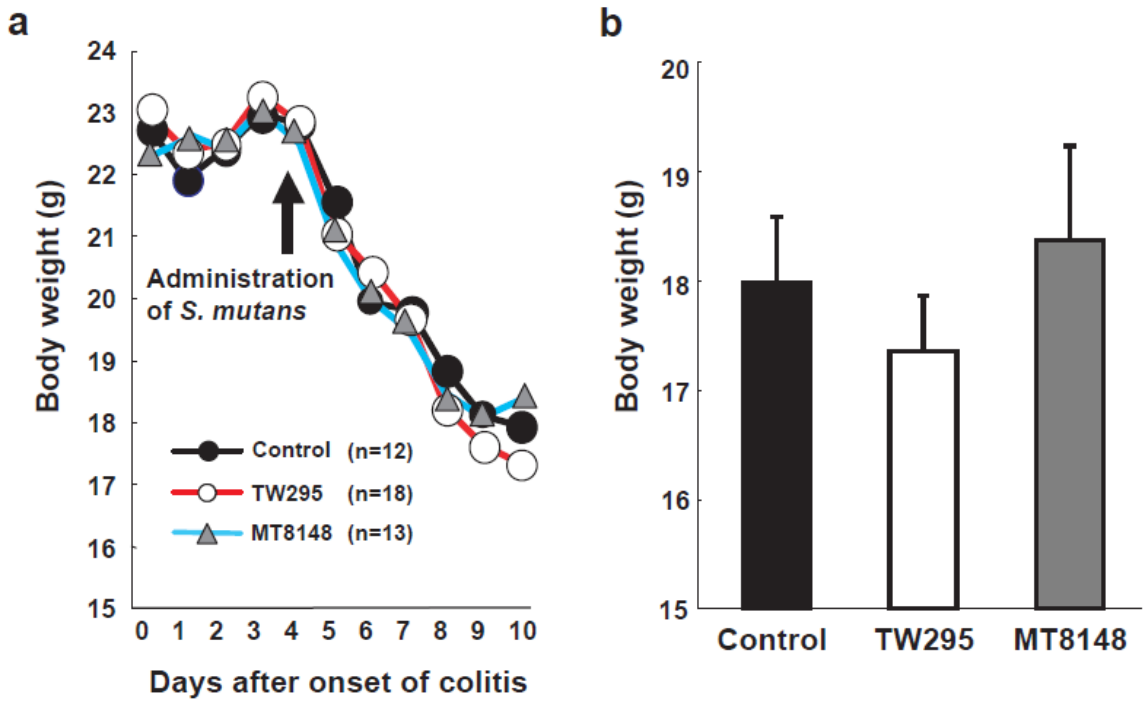
80	F	28	12y	remission	entire colon	−		
81	F	23	14y	remission	entire colon	+	*c*	−
82	M	26	10y	remission	left hemicolon	−		
83	F	38	11y	remission	rectum	−		
84	M	47	12y	remission	entire colon	+	*c*	−
85	M	68	1y	mild	entire colon	−		
86	F	63	3y	mild	left hemicolon	+	*c*	−
87	F	35	5y	mild	entire colon	+	*c*	−
88	F	39	15y	remission	entire colon	+	*c*	−
89	M	24	4y	remission	entire colon	+	*c*	−
90	M	27	7y	remission	left hemicolon	−		
91	M	36	3y	mild	entire colon	+	*c*	−
92	F	67	6y	remission	entire colon	+	*c*	−
93	M	30	2y	remission	left hemicolon	+	*c*	−
94	M	37	8y	remission	left hemicolon	+	*c*	−
95	F	24	5y	remission	rectum	−		
96	F	48	8y	remission	left hemicolon	−		
97	M	35	15y	remission	left hemicolon	+	*c*	−
98	M	29	1y	mild	left hemicolon	−		

Among 98 IBD patients, 56 were *S. mutans* positive (57.14%). The detection frequency of the CBP-encoding *cnm* gene expressing *S. mutans* in the IBD patients was always confirmed by isolation and culture of the individual strains. Each isolation and identification of the *cnm*-positive or negative status was repeated multiple times. Various serotypes of *S. mutans* in patients were also investigated.

Supplementary Table S3. Primer information.

Gene Name	Primer Set	PCR Product Size (bp)
GAPDH	5'-GCACAGTCAAGGCCGAGAAT-3'	151
	5'-GCCTTCTCCATGGTGGTGAA-3'	
mouse TNF-α	5'-CCTGTAGCCCACGTCGTAGC-3'	374
	5'-TTGACCTCAGCGCTGAGTTG-3'	
mouse IL-6	5'- GAGGATACCACTCCCAACAGACC-3'	141
	5'- AAGTGCATCATCGTTGTTCATACA-3'	
mouse IFN-γ	5'-AGCTCTGAGACAATGAACGC-3'	504
	5'-GGACAATCTCTTCCCCACCC-3'	
human IFN-γ	5'-TCGGTAACTGACTTGAATGTCCA-3'	100
	5'-TCCTTTTTCGCTTCCCTGTTTT-3'	

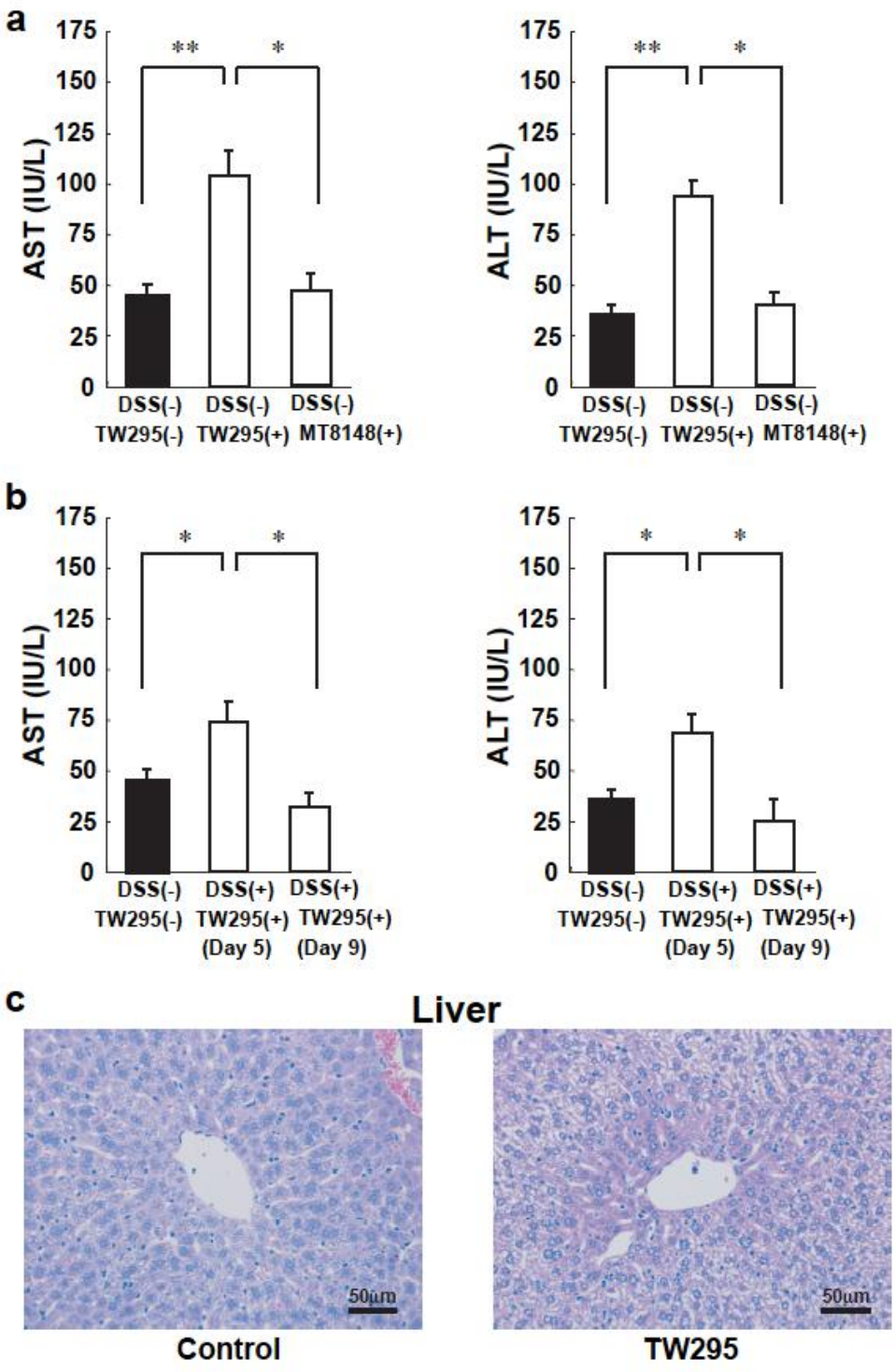
Supplementary Figures



Supplementary Figure S1. Aggravation of mouse colitis by TW295, a specific strain of *S. mutans*.

(a): Change in body weight up to day 10. Each point represents the mean value from 12-18 different animals.

(b): The body weights of each group on day 15. Each column represents the mean ± standard error (SEM) from 12-18 different animals.

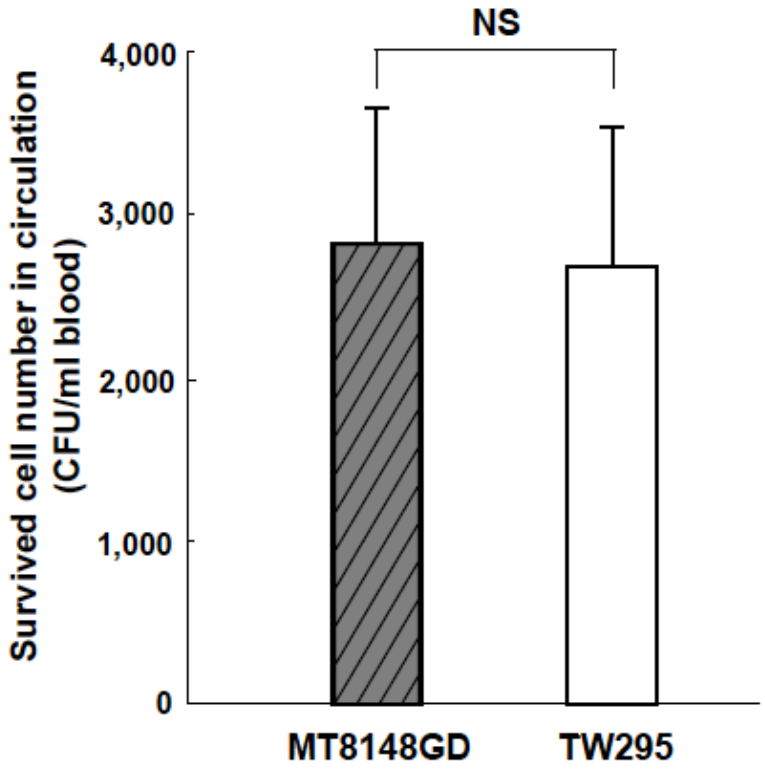


Supplementary Figure S2. Effect of TW295 on the liver is transient.

(a): Effect of bacterial administration on ALT and AST. Vehicle, TW295, or MT8148 was administered to the mice without DSS administration. Twenty-four hours after the administration of bacteria, blood samples were collected, and ALT and AST values were measured and expressed as IU/L. Each column represents the mean ± SEM from 4-6 different animals. Statistical significance was determined using Bonferroni's method after analysis of variance (ANOVA). * $p<0.05$; ** $p<0.01$, respectively.

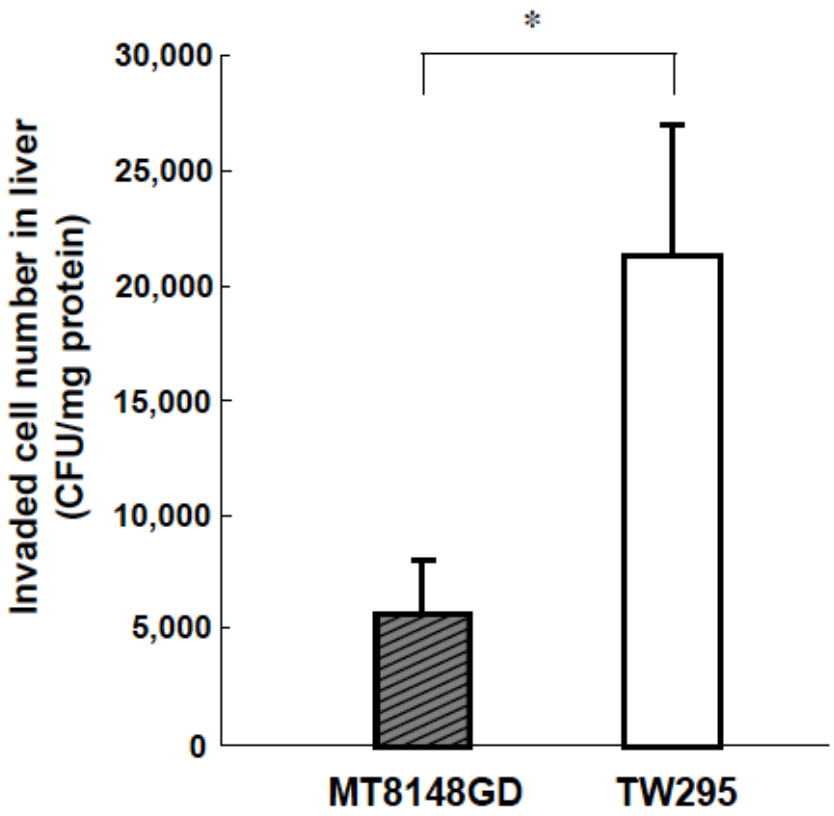
(b): Transient increase in ALT and AST after the administration of TW295. Twenty-four (as day 5) and 120 (as day 9) hours after the administration of TW295 under treatment with DSS, blood samples were collected, and ALT and AST values were measured. Each column represents the mean ± SEM from 3-5 different animals. The increases in ALT and AST values produced by administration of TW295 were a transient response. *$p<0.05$.

(c): Typical microscopic images of the liver in control and TW295-infected mice on day 10. No marked changes between control and TW295-infected mice were observed.



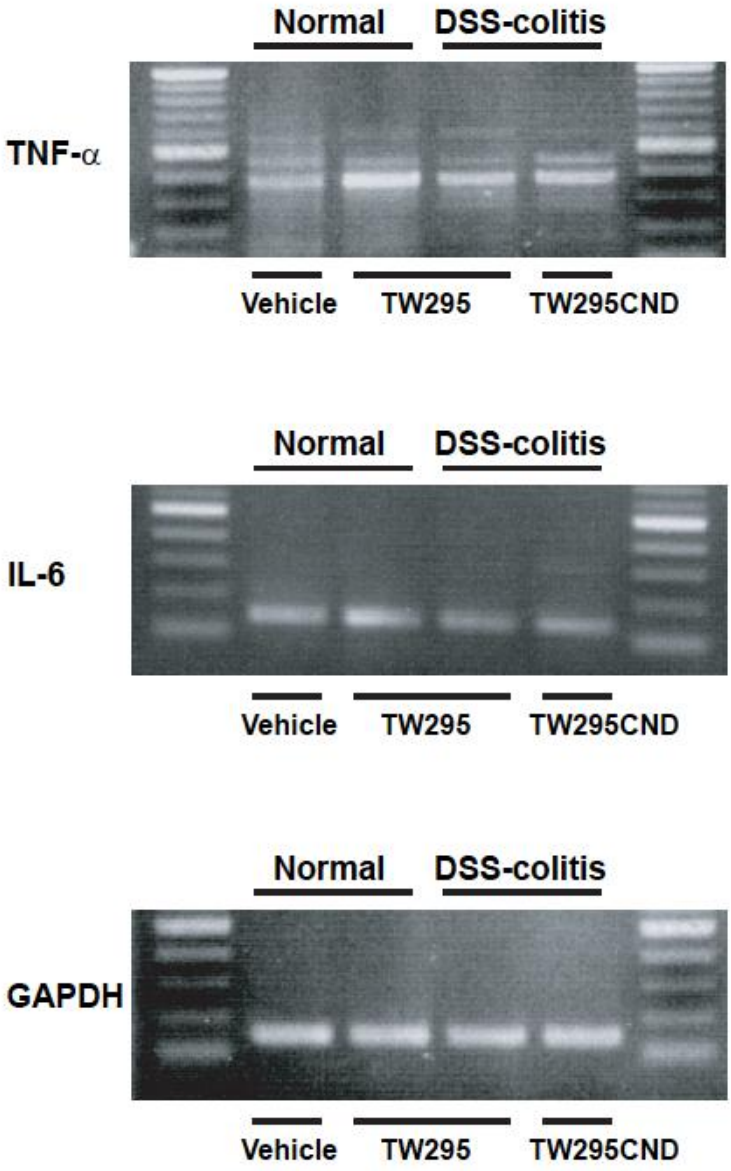
Supplementary Figure S3. Survivability of MT8148GD in blood circulation.

Survivability of MT8148GD, the isogenic mutant strain of MT8148 with a glucose defection in the side chain, was investigated in vivo colitis mice. Briefly, 30 min after the administration of MT8148GD or TW295 ($1x10^7$ CFU) in colitis mice, blood samples were collected. The samples (100 μl) were inoculated onto MSB agar plates and anaerobically incubated according to the method described in Method section. The viable cell numbers were counted and survived cell numbers are expressed as CFU/ml blood. Each column represents the mean ± SEM from 3-4 different animals. Statistical significance was determined using Student's t-test. NS; no significant (p=0.9425).



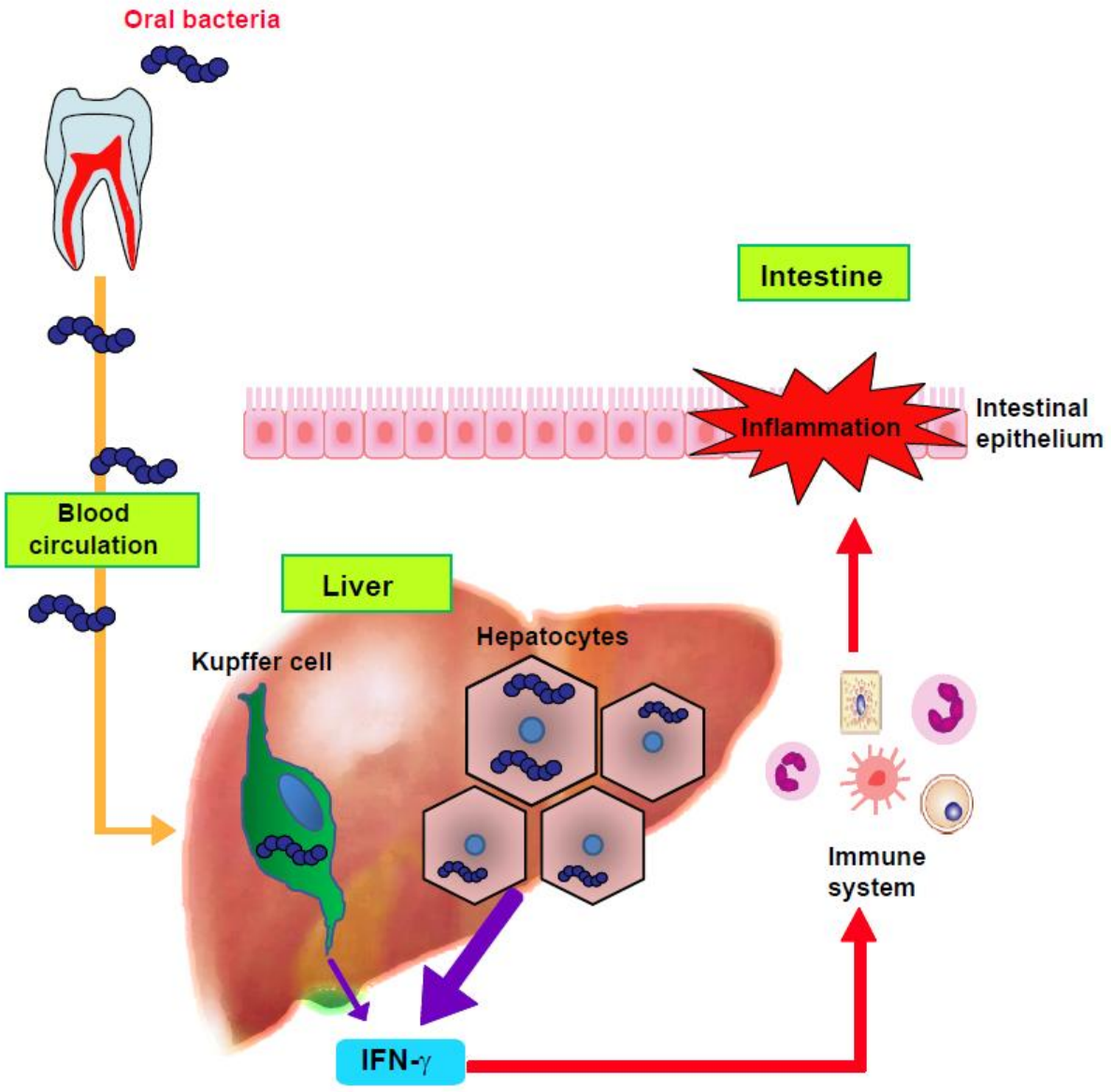
Supplementary Figure S4. Localization of MT8148GD in liver tissues.

Localization of MT8148GD, the isogenic mutant strain of MT8148 with a glucose defection in the side chain, in liver tissues was investigated in vivo colitis mice. Briefly, 180 min after the administration of MT8148GD or TW295 ($1x10^7$ CFU) in colitis mice, liver samples were collected. The samples were minced and squeezed, then were inoculated onto MSB agar plates and anaerobically incubated according to the method described in Method section. The viable cell numbers were counted and invaded cell numbers in liver are expressed as CFU/mg protein. Each column represents the mean ± SEM from 3-6 different animals. Statistical significance was determined using Student's t-test. *; statistically significant (p=0.0175).



Supplementary Figure S5. Effect of TW295 on the expression of inflammatory cytokines.

Expression of TNF-α and IL-6 in liver tissues after the administration of TW295 or TW295CND to mice both in DSS-induced colitis and normal controls. GAPDH is an internal standard for PCR.



Supplementary Figure S6. Hypothetical mechanism of oral bacteria-mediated aggravation of colitis