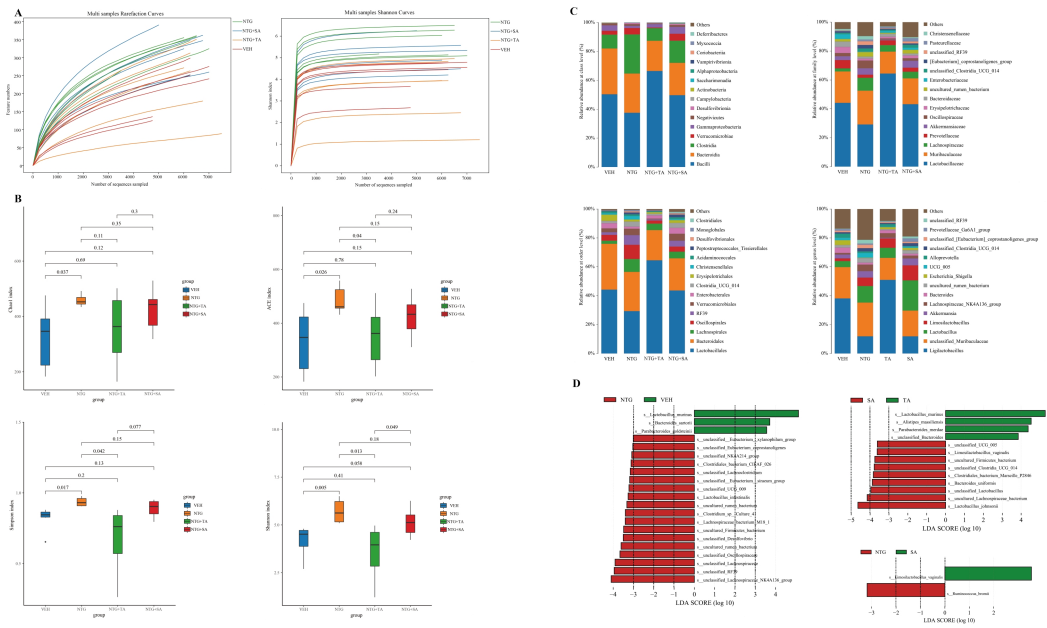




Supplementary Figure 1. Diversity and compositional changes of the gut microbiota. (A) The rarefaction curve and Shannon Index curve; (B) Alpha-diversity indexes, including Chao1, ACE, Simpson index, and Shannon Index; (C) The composition of the gut microbiota at class, family, order, and genus levels; (D) An LefSe analysis was conducted to compare the NTG group with the VEH group, the NTG+SA group with the NTG+TA group, and the NTG group with the NTG+SA group.

Supplementary Table 1. Primer sequences information.

Primer name	The sequence (5' to 3')
S100a4-F	TCCACCTTCCACACATACTCAG
S100a4-R	TTCATTGTCCCTGTTGCTGT
Vamp8-F	CTGCCTTGGGTGGAACAGA
Vamp8-R	TTGTTTCGGAGGTGGTCCAG
GAPDH-F	CCTGGAGAAACCTGCCAAGTA
GAPDH-R	TCATACCAGGAAATGAGCTTGAC

Note: F, forward primer; R, reverse primer; Gapdh was used as the reference gene.

Supplementary Table 2. Statistical comparison of mechanical withdrawal threshold and tail-flick latency across all groups at baseline and post-treatment time points (days 1-9).

Basal hind paw mechanical threshold	MD	95.00% CI	Adjusted <i>P</i> Value
Day1			
NTG vs. VEH	-0.01111	-2.573 to 2.551	>0.9999
NTG+TA vs. NTG	0.6167	-1.885 to 3.119	>0.9999
NTG+SA vs. NTG	-0.5222	-3.096 to 2.052	>0.9999
NTG+SA vs. NTG+TA	-1.139	-3.250 to 0.9718	0.431
Day3			
NTG vs. VEH	-1.478	-3.014 to 0.05846	0.0584
NTG+TA vs. NTG	1.528	-0.2328 to 3.288	0.0874
NTG+SA vs. NTG	-0.7389	-3.410 to 1.932	>0.9999
NTG+SA vs. NTG+TA	-2.267	-4.602 to 0.06847	0.0564
Day5			
NTG vs. VEH	-4.328	-5.884 to -2.771	0.0005
NTG+TA vs. NTG	3.406	0.1562 to 6.655	0.0413
NTG+SA vs. NTG	0.8556	-1.472 to 3.184	>0.9999
NTG+SA vs. NTG+TA	-2.55	-6.107 to 1.007	0.1755
Day7			
NTG vs. VEH	-7.783	-11.15 to -4.420	0.0012
NTG+TA vs. NTG	5.461	2.419 to 8.503	0.0038
NTG+SA vs. NTG	0.65	-4.277 to 5.577	>0.9999
NTG+SA vs. NTG+TA	-4.811	-8.485 to -1.138	0.016
Day9			
NTG vs. VEH	-12.57	-14.94 to -10.20	<0.0001
NTG+TA vs. NTG	11.69	9.158 to 14.22	<0.0001
NTG+SA vs. NTG	1.353	-0.8449 to 3.550	0.2905
NTG+SA vs. NTG+TA	-10.34	-12.57 to -8.103	<0.0001
Post-treatment hind paw mechanical threshold	MD	95.00% CI of diff.	Adjusted <i>P</i> Value
Day1			
NTG vs. VEH	-1.633	-3.853 to 0.5866	0.1603
NTG+TA vs. NTG	0.8833	-0.1378 to 1.904	0.0885
NTG+SA vs. NTG	-0.5667	-2.554 to 1.421	>0.9999
NTG+SA vs. NTG+TA	-1.45	-3.279 to 0.3794	0.1227
Day3			
NTG vs. VEH	-7.201	-8.982 to -5.421	<0.0001
NTG+TA vs. NTG	5.394	3.373 to 7.416	0.0006
NTG+SA vs. NTG	1.861	-1.329 to 5.051	0.3428

NTG+SA vs. NTG+TA	-3.533	-8.131 to 1.064	0.1373
Day5			
NTG vs. VEH	-8.917	-13.22 to -4.616	0.0019
NTG+TA vs. NTG	6.378	3.954 to 8.801	0.0006
NTG+SA vs. NTG	0.9889	-4.910 to 6.888	>0.9999
NTG+SA vs. NTG+TA	-5.389	-9.490 to -1.288	0.0157
Day7			
NTG vs. VEH	-12.89	-15.56 to -10.23	<0.0001
NTG+TA vs. NTG	9.839	7.699 to 11.98	<0.0001
NTG+SA vs. NTG	1.028	-1.661 to 3.717	>0.9999
NTG+SA vs. NTG+TA	-8.811	-11.21 to -6.412	0.0001
Day9			
NTG vs. VEH	-15.39	-17.17 to -13.61	<0.0001
NTG+TA vs. NTG	13.78	9.004 to 18.56	0.0004
NTG+SA vs. NTG	0.3611	-2.887 to 3.609	>0.9999
NTG+SA vs. NTG+TA	-13.42	-15.98 to -10.86	<0.0001
Basal tail-flick latency	MD	95.00% CI of diff.	Adjusted P Value
Day1			
NTG vs. VEH	0.005555	-0.5212 to 0.5323	>0.9999
NTG+TA vs. NTG	-0.1	-1.090 to 0.8898	>0.9999
NTG+SA vs. NTG	-0.07222	-0.9912 to 0.8468	>0.9999
NTG+SA vs. NTG+TA	0.02778	-0.4504 to 0.5060	>0.9999
Day3			
NTG vs. VEH	-0.8944	-1.402 to -0.3868	0.0042
NTG+TA vs. NTG	0.4111	-0.4495 to 1.272	0.5996
NTG+SA vs. NTG	-0.03333	-1.025 to 0.9585	>0.9999
NTG+SA vs. NTG+TA	-0.4444	-1.464 to 0.5754	0.752
Day5			
NTG vs. VEH	-1.8	-2.538 to -1.062	0.0009
NTG+TA vs. NTG	1.256	0.3626 to 2.149	0.0117
NTG+SA vs. NTG	0.3222	-0.7475 to 1.392	>0.9999
NTG+SA vs. NTG+TA	-0.9333	-1.996 to 0.1291	0.0834
Day7			
NTG vs. VEH	-1.856	-2.393 to -1.319	0.0002
NTG+TA vs. NTG	1.117	0.3852 to 1.848	0.008
NTG+SA vs. NTG	-0.2333	-0.9290 to 0.4623	>0.9999
NTG+SA vs. NTG+TA	-1.35	-2.115 to -0.5850	0.0041
Day9			
NTG vs. VEH	-2.217	-2.987 to -1.447	0.0004
NTG+TA vs. NTG	1.478	0.7949 to 2.161	0.0016
NTG+SA vs. NTG	-0.03333	-0.5608 to 0.4942	>0.9999
NTG+SA vs. NTG+TA	-1.511	-2.191 to -0.8311	0.0014
Post-treatment tail-flick latency	MD	95.00% CI of diff.	Adjusted

			<i>P</i> Value
Day1			
NTG vs. VEH	-0.6722	-1.425 to 0.08029	0.0782
NTG+TA vs. NTG	0.1056	-0.3587 to 0.5698	>0.9999
NTG+SA vs. NTG	-0.04444	-1.247 to 1.158	>0.9999
NTG+SA vs. NTG+TA	-0.15	-1.025 to 0.7251	>0.9999
Day3			
NTG vs. VEH	-1.622	-2.117 to -1.127	0.0002
NTG+TA vs. NTG	0.9389	0.2419 to 1.636	0.0141
NTG+SA vs. NTG	-0.1444	-0.7228 to 0.4339	>0.9999
NTG+SA vs. NTG+TA	-1.083	-2.173 to 0.005936	0.0511
Day5			
NTG vs. VEH	-2.161	-3.046 to -1.276	0.0009
NTG+TA vs. NTG	1.167	0.2854 to 2.048	0.0152
NTG+SA vs. NTG	-0.1222	-1.009 to 0.7641	>0.9999
NTG+SA vs. NTG+TA	-1.289	-2.115 to -0.4627	0.0073
Day7			
NTG vs. VEH	-2.294	-2.884 to -1.705	<0.0001
NTG+TA vs. NTG	1.05	0.07508 to 2.025	0.0369
NTG+SA vs. NTG	-0.2444	-0.5996 to 0.1107	0.2018
NTG+SA vs. NTG+TA	-1.294	-2.095 to -0.4944	0.0062
Day9			
NTG vs. VEH	-2.511	-2.851 to -2.171	<0.0001
NTG+TA vs. NTG	1.744	0.7949 to 2.694	0.0034
NTG+SA vs. NTG	-0.2278	-0.8849 to 0.4293	>0.9999
NTG+SA vs. NTG+TA	-1.972	-3.052 to -0.8929	0.0035

Note: For hind paw mechanical threshold and tail-flick latency data, two-way ANOVA was performed, and Bonferroni's post-hoc test was used to control for multiple comparisons.

Supplementary Table 3. Statistical comparison of proinflammatory cytokine (TNF- α , IL-1 β , IL-6) levels among four groups.

TNF- α	MD	95.00% CI	Adjusted <i>P</i> Value
NTG vs. VEH	24.58	12.22 to 36.93	0.0001
NTG+TA vs. NTG	-22.39	-34.75 to -10.04	0.0003
NTG+SA vs. NTG	-5.38	-17.73 to 6.974	0.6225
NTG+SA vs. NTG+TA	17.01	4.660 to 29.37	0.005
IL-1 β	MD	95.00% CI of diff.	Adjusted <i>P</i> Value
NTG vs. VEH	3.165	1.091 to 5.239	0.002
NTG+TA vs. NTG	-2.893	-4.967 to -0.8192	0.0045

NTG+SA vs. NTG	-0.9867	-3.061 to 1.087	0.5546
NTG+SA vs. NTG+TA	1.907	-0.1675 to 3.981	0.0786
IL-6	MD	95.00% CI of diff.	Adjusted P Value
NTG vs. VEH	12.17	6.569 to 17.77	<0.0001
NTG+TA vs. NTG	-8.113	-13.72 to -2.511	0.0032
NTG+SA vs. NTG	-2.832	-8.434 to 2.771	0.5052
NTG+SA vs. NTG+TA	5.282	-0.3211 to 10.88	0.0691

Note: For ELISA data of TNF- α , IL-1 β , and IL-6, inter-group differences were analyzed using one-way ANOVA followed by Tukey's post-hoc test.

Supplementary Table 4. Statistical comparison of *S100a4* and *Vamp8* expression levels among four groups.

<i>S100a4</i>	MD	95.00% CI	Adjusted P Value
NTG vs. VEH	-0.3383	-0.4295 to -0.2471	<0.0001
NTG+TA vs. NTG	0.26	0.1688 to 0.3512	<0.0001
NTG+SA vs. NTG	0.01333	-0.07788 to 0.1045	0.9762
NTG+SA vs. NTG+TA	-0.2467	-0.3379 to -0.1555	<0.0001
<i>Vamp8</i>	MD	95.00% CI	Adjusted P Value
NTG vs. VEH	-0.2683	-0.3693 to -0.1674	<0.0001
NTG+TA vs. NTG	0.2017	0.1007 to 0.3026	<0.0001
NTG+SA vs. NTG	0.02833	-0.07262 to 0.1293	0.8601
NTG+SA vs. NTG+TA	-0.1733	-0.2743 to -0.07238	0.0006

Note: For RT-qPCR data, inter-group differences were analyzed using one-way ANOVA followed by Tukey's post-hoc test.

Supplementary Table 5. KEGG pathway enrichment analysis of candidate DEGs.

VEH vs NTG	KEGG Pathway	Gene ratio	Gene count	Nominal P value	Q value (adjusted P value)
	Protein digestion and absorption	8%	8	1.26E-05	0.002182359
	Proteoglycans in cancer	7%	7	0.005010 693	0.26845218

Gastric acid secretion	4%	4	0.006220 233	0.26845218
Neuroactive ligand-receptor interaction	9%	9	0.011288 697	0.355330665
Nitrogen metabolism	2%	2	0.014593 813	0.355330665
TGF-beta signaling pathway	4%	4	0.016319 778	0.355330665
Long-term depression	3%	3	0.019772 888	0.355330665
cGMP-PKG signaling pathway	5%	5	0.019888 067	0.355330665
Human T-cell leukemia virus 1 infection	8%	8	0.022722 397	0.355330665
Focal adhesion	6%	6	0.025517 358	0.355330665
Inflammatory bowel disease	5%	5	0.027679 185	0.355330665
ECM-receptor interaction	4%	4	0.028816 45	0.355330665
Platelet activation	4%	4	0.039616 612	0.412323546
Synaptic vesicle cycle	3%	3	0.040603 813	0.412323546
Th1 and Th2 cell differentiation	5%	5	0.044766 069	0.429335402

NTG vs NTG+TA	Pathway	Gene ratio	Gene count	Nominal <i>P</i> value	<i>Q</i> value (adjusted <i>P</i> value)
	GABAergic synapse	2.51%	13	0.0005026	0.039960165
	Complement and coagulation cascades	2.51%	13	0.00061193	0.039960165
	Axon guidance	3.87%	20	0.000789156	0.039960165
	cAMP signaling pathway	3.68%	19	0.008124436	0.159817684
	TGF-beta signaling pathway	2.13%	11	0.011801603	0.159817684
	Glutamatergic synapse	2.32%	12	0.012647785	0.159817684
	Neuroactive ligand-receptor interaction	5.61%	29	0.015725741	0.159817684

Inflammatory mediator regulation of TRP channels	2.13%	11	0.023908378	0.191109731
NOD-like receptor signaling pathway	3.29%	17	0.027163222	0.210547466
Vascular smooth muscle contraction	2.51%	13	0.030523561	0.21207035
cGMP-PKG signaling pathway	2.71%	14	0.031167257	0.21207035
Chemokine signaling pathway	2.90%	15	0.031505101	0.21207035
Serotonergic synapse	2.13%	11	0.041313598	0.239349022
Wnt signaling pathway	3.09%	16	0.042267676	0.239349022

Supplementary Table 6. Statistical comparison of fecal and plasma SCFA concentrations among four groups.

Fecal SCFAs	MD	95.00% CI	Adjusted P Value
Isobutyric acid			
NTG vs. VEH	9.803	-14.53 to 34.14	0.6774
NTG+TA vs. NTG	6.246	-18.09 to 30.58	0.8887
NTG+SA vs. NTG	4.047	-20.29 to 28.38	0.9658
NTG+SA vs. NTG+TA	-2.199	-26.54 to 22.14	0.9941
Propionic acid			
NTG vs. VEH	59.45	-261.3 to 380.2	0.9536
NTG+TA vs. NTG	-80.2	-400.9 to 240.5	0.8959
NTG+SA vs. NTG	68.76	-252.0 to 389.5	0.9309
NTG+SA vs. NTG+TA	149	-171.8 to 469.7	0.5736
Acetic acid			
NTG vs. VEH	678	-108.7 to 1465	0.1069
NTG+TA vs. NTG	-1028	-1815 to -241.4	0.0078
NTG+SA vs. NTG	140.4	-646.3 to 927.1	0.9582
NTG+SA vs. NTG+TA	1169	381.9 to 1955	0.0025
Butyric acid			
NTG vs. VEH	82.53	-186.2 to 351.2	0.8252
NTG+TA vs. NTG	41.87	-226.8 to 310.6	0.9715
NTG+SA vs. NTG	-56.25	-324.9 to 212.4	0.9352
NTG+SA vs. NTG+TA	-98.12	-366.8 to 170.6	0.7387
Isovaleric acid			
NTG vs. VEH	4.514	-13.73 to 22.76	0.8987
NTG+TA vs. NTG	7.913	-10.33 to 26.16	0.6255
NTG+SA vs. NTG	2.102	-16.14 to 20.35	0.9881

NTG+SA vs. NTG+TA	-5.811	-24.06 to 12.43	0.8093
Valeric acid			
NTG vs. VEH	-3.341	-29.90 to 23.22	0.9846
NTG+TA vs. NTG	-8.287	-34.84 to 18.27	0.8185
NTG+SA vs. NTG	-11.88	-38.44 to 14.67	0.6022
NTG+SA vs. NTG+TA	-3.597	-30.15 to 22.96	0.9809
Caproic acid			
NTG vs. VEH	-1.067	-4.715 to 2.582	0.8452
NTG+TA vs. NTG	-2.451	-6.100 to 1.197	0.2676
NTG+SA vs. NTG	-3.001	-6.649 to 0.6480	0.1311
NTG+SA vs. NTG+TA	-0.5493	-4.198 to 3.099	0.9742
Plasma SCFAs	MD	95.00% CI of diff.	Adjusted P Value
Isobutyric acid			
NTG vs. VEH	0.06041	0.02785 to 0.09298	0.0007
NTG+TA vs. NTG	-0.03376	-0.06633 to -0.001200	0.0414
NTG+SA vs. NTG	-0.01823	-0.05080 to 0.01433	0.3834
NTG+SA vs. NTG+TA	0.01553	-0.01703 to 0.04809	0.5136
Propionic acid			
NTG vs. VEH	0.1708	-0.03975 to 0.3813	0.1283
NTG+TA vs. NTG	-0.1351	-0.3457 to 0.07541	0.2763
NTG+SA vs. NTG	-0.1074	-0.3179 to 0.1032	0.4597
NTG+SA vs. NTG+TA	0.02778	-0.1828 to 0.2383	0.9787
Acetic acid			
NTG vs. VEH	-1.206	-9.573 to 7.161	0.9726
NTG+TA vs. NTG	-3.817	-12.18 to 4.550	0.5486
NTG+SA vs. NTG	-1.76	-10.13 to 6.607	0.9222
NTG+SA vs. NTG+TA	2.057	-6.310 to 10.42	0.8832
Butyric acid			
NTG vs. VEH	0.4876	-0.08267 to 1.058	0.1037
NTG+TA vs. NTG	-0.312	-0.8823 to 0.2582	0.4021
NTG+SA vs. NTG	-0.0493	-0.6196 to 0.5210	0.9938
NTG+SA vs. NTG+TA	0.2627	-0.3075 to 0.8330	0.541
Isovaleric acid			
NTG vs. VEH	0.02457	-0.009861 to 0.05900	0.2021
NTG+TA vs. NTG	-0.01044	-0.04488 to 0.02399	0.8049
NTG+SA vs. NTG	0.003325	-0.03111 to 0.03776	0.9914
NTG+SA vs. NTG+TA	0.01377	-0.02067 to 0.04820	0.6459
Valeric acid			
NTG vs. VEH	-0.000571	-0.01518 to 0.01404	0.9994
NTG+TA vs. NTG	0.002858	-0.01175 to 0.01747	0.936
NTG+SA vs. NTG	-0.006071	-0.02068 to 0.008538	0.6187
NTG+SA vs. NTG+TA	-0.008928	-0.02354 to 0.005680	0.3136
Caproic acid			

NTG vs. VEH	-0.001614	-0.01316 to 0.009930	0.9748
NTG+TA vs. NTG	-0.0003015	-0.01184 to 0.01124	0.9998
NTG+SA vs. NTG	0.005239	-0.006305 to 0.01678	0.5527
NTG+SA vs. NTG+TA	0.00554	-0.006003 to 0.01708	0.5086

Note: For fecal and plasma SCFAs levels data, inter-group differences were analyzed using one-way ANOVA followed by Tukey's post-hoc test.

Supplementary Table 7. Exploratory correlations between gut microbial species and fecal SCFAs in the NTG+TA group

Microbial Species	SCFAs	Pearson r	P value
<i>Ruminococcus sp. WAL 17306</i>	Propionic acid	-0.860	0.028
<i>Bacteroides uniformis</i>	Propionic acid	-0.860	0.028
<i>Parabacteroides goldsteinii</i>	Acetic acid	-0.956	0.003
<i>Alistipes massiliensis</i>	Valeric acid	0.884	0.019
<i>Clostridiales bacterium Marseille P2846</i>	Valeric acid	0.820	0.046
<i>Clostridiales bacterium CIEAF 026</i>	Caproic acid	0.861	0.028
<i>Lactobacillus johnsonii</i>	Caproic acid	-0.836	0.038

Note: Pearson correlations with $|r| > 0.8$ and $P < 0.05$ were retained for exploratory interpretation.

P values were not adjusted for multiple comparisons. Positive r indicates a co-directional association; negative r indicates an inverse association.

Supplementary Table 8. Exploratory correlations between gut microbial species and TNC candidate DEGs in the NTG+TA group

Microbial Species	Gene	Pearson r	P value
<i>Parabacteroides goldsteinii</i>	<i>Foxo3</i>	-0.917	0.010
<i>Parabacteroides goldsteinii</i>	<i>Prkacb</i>	-0.828	0.042
<i>Parabacteroides merdae</i>	<i>Prkacb</i>	-0.825	0.043
<i>Ligilactobacillus murinus</i>	<i>Adcy7</i>	0.844	0.035
<i>Parabacteroides merdae</i>	<i>Adcy7</i>	-0.894	0.016
<i>Parabacteroides goldsteinii</i>	<i>Ccl19</i>	0.932	0.007
<i>Parabacteroides merdae</i>	<i>Ccl19</i>	0.942	0.005
<i>Lachnospiraceae bacterium M18-1</i>	<i>Ccr10</i>	0.831	0.040

Microbial Species	Gene	Pearson r	P value
<i>Parabacteroides merdae</i>	<i>Gng11</i>	0.956	0.003
<i>Ligilactobacillus murinus</i>	<i>Adcy9</i>	0.938	0.006
<i>Limosilactobacillus vaginalis</i>	<i>Adcy9</i>	0.814	0.049
<i>Ruminococcus bromii</i>	<i>Vav1</i>	-0.881	0.020
<i>Lactobacillus murinus</i>	<i>Adcy1</i>	-0.855	0.030
<i>Ligilactobacillus murinus</i>	<i>Adcy1</i>	0.921	0.009
<i>Parabacteroides goldsteinii</i>	<i>Vamp8</i>	0.914	0.0099
<i>Parabacteroides goldsteinii</i>	<i>Map2k4</i>	-0.897	0.015
<i>Ligilactobacillus murinus</i>	<i>Map2k4</i>	0.876	0.022
<i>Parabacteroides merdae</i>	<i>Map2k4</i>	-0.869	0.025
<i>Ligilactobacillus murinus</i>	<i>Traf3</i>	0.925	0.008
<i>Parabacteroides goldsteinii</i>	<i>S100a4</i>	0.878	0.021
<i>Parabacteroides merdae</i>	<i>Syng1</i>	-0.872	0.024
<i>Limosilactobacillus vaginalis</i>	<i>Irf7</i>	0.915	0.010
<i>Lachnospiraceae bacterium M18-1</i>	<i>Ctsk</i>	0.832	0.040
<i>Ruminococcus sp. WAL 17306</i>	<i>Ctsk</i>	0.921	0.009
<i>Bacteroides uniformis</i>	<i>Ctsk</i>	0.921	0.009

Note: Pearson correlations with $|r| > 0.8$ and $P < 0.05$ were retained for exploratory interpretation.

P values were not adjusted for multiple comparisons. Positive r indicates a co-directional association; negative r indicates an inverse association.