Supporting Information

Theabrownin Derived from Pu-erh Tea Enhances Intestinal Barrier Integrity and Microbiota Balance via the ST6GalNAc6–STn–MUC2 Axis in Inflammation

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1. SUPPLEMENTARY METHODS

1.1 LC-MS analysis of TB.

UPLC parameter: Agilgent 1290 UPLC (Agilgent, California, USA) with waters BEH C18 column (1.7 μm, 2.1 × 100 mm) was used. TB were diluted with distilled water and filtered through a 0.45 μm syringe filter. Mobile phases were 0.1% formic acid-water (A) and acetonitrile (B). The linear gradient programs were set as 0-8 min, 95-5% A; 8-14 min, 5-95% A. Sample injection volume of 5 μL; Flow rate of 0.3 mL/min and column oven temperature of 40 ℃.

Mass spectrometry: Agilgent QTOF 6550 (Agilgent, California, USA). MS conditions of positive ion mode: source voltage of -3.2 kV, sheath gas temperature of 350 ℃, and sheath gas flow of 12L/min. The primary mass spectrometry scan range is 50-1000 m/z. Traditional Chinese medicine database (TCM-databse.cdb) is used for ingredient matching.

1.2 Justification of dosage selection.

According to the US FDA's guidelines for oral dose conversion between human and mouse, the human equivalent dose is calculated using Equation 1, where the conversion factor (W) is 0.11. The human equivalent dose of TB is calculated to be 6.6 g/60kg.

$$human\ oral\ dose\ (mg/kg) = W \times mouse\ oral\ dose\ (mg/kg) \quad (1)$$

2. SUPPLEMENTARY FIGURES

Table S1 Phenolic compounds identified in TB by UPLC-MS.

Figure S1. Inhibition of ST6GalNAc6 abrogated the protective effect of TB against colitis in mice. Daily variations in body weight (A) and DAI score after inhibitor treatment (B) (n=10); Representative images (C) and length statistical analysis of colon (D) (n=10); Representative microscope images of colon section beneath H&E staining (E) and AB-PAS (F) (n=6). Scale bars: 100 μm (F, upper panel), 20 μm (F, lower panel). Each data point represents the average of per treatment and time point. Values that do not share a common letter are significantly different at $p < 0.05$

Figure S2. Inhibition of ST6GalNAc6 attenuated the suppressive effect of TB on STn expression. Immunofluorescence results of STn (A) (n=6); Relative expression of ST6GalNAc 6 (B) (n=6). Scale bars: 100 μm (A, upper panel), 20 μm (A, zoom). Values not sharing a common letter are significantly different at $p < 0.05$.

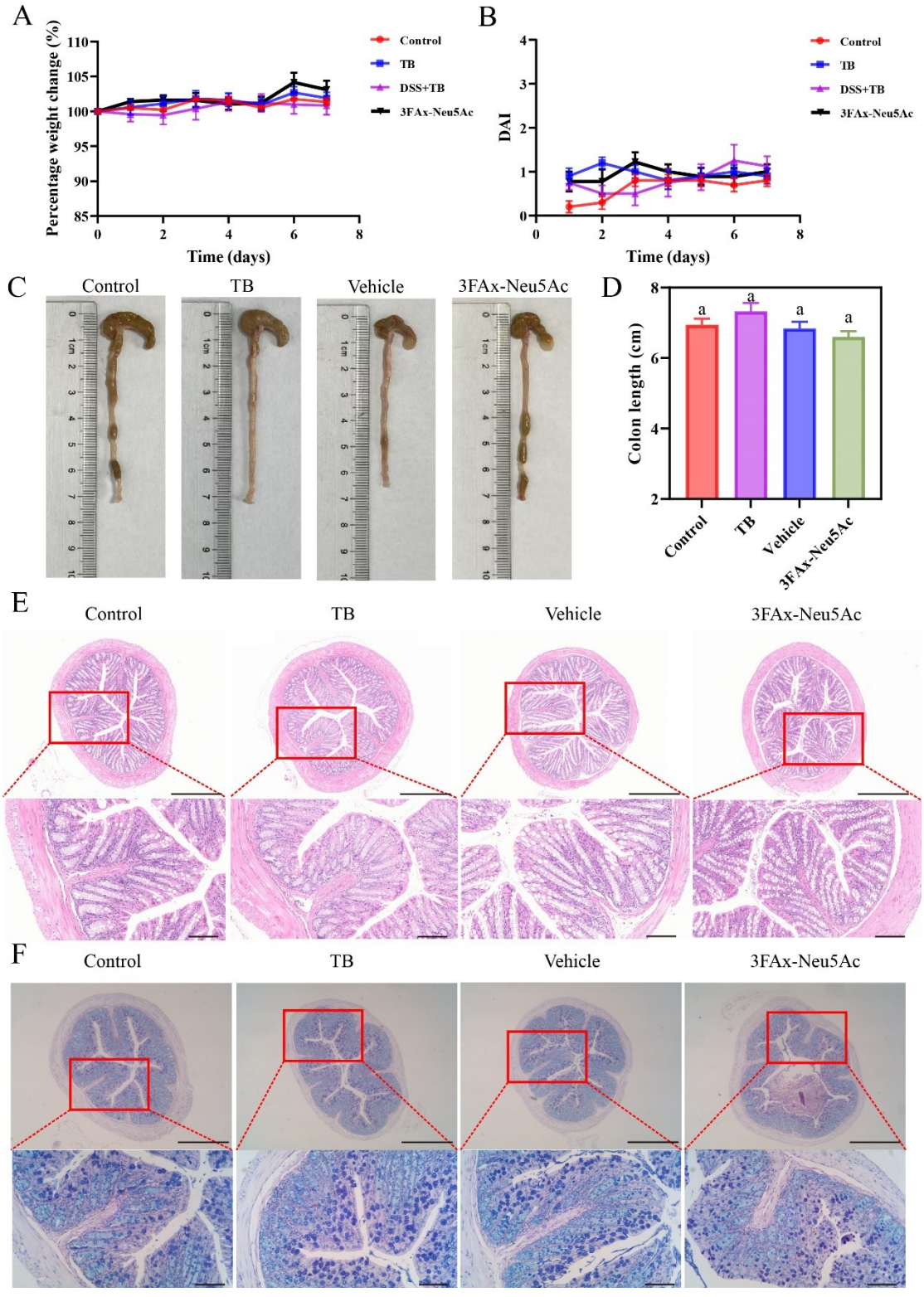
53 **Table S1** Phenolic compounds identified in TB by UPLC-MS.

Peak No.	Rt (min)	Mass	Formula	Diff (ppm)	Identification	Relative Content
1	0.54	180.07	$C_7H_8N_4O_2$	3.44	Theobromine	522.15
	0.57	88.016	$C_3H_4O_3$	-0.92	Pyroracemic acid	27.52
	0.59	174.05	$C_7H_{10}O_5$	-0.56	Shikimic acid	121.57
	2.15	170.02	$C_7H_6O_5$	-4.52	Gallic acid	43.00
2	2.25	274.08	$C_{15}H_{14}O_5$	-0.4	(-)-Epiafzelechin	584.88
3	2.35	290.08	$C_{15}H_{14}O_6$	3.84	(-)-Epicatechin	3214.53
4	2.39	236.06	$C_{12}H_{12}O_5$	-0.72	2-Methyl-5-carboxymethyl-7-hydroxychromanone	7.10
5	3.20	154.02	$C_7H_6O_4$	-2.55	3,5-Dihydroxybenzoic acid	920.76
6	3.24	460.15	$C_{20}H_{28}O_{12}$	0.32	Paeonolide	132.31
7	3.32	786.19	$C_{37}H_{38}O_{19}$	-1.72	Isorhamnetin 3-O-beta-(6"-E-p-coumaroyglucopyranoside)-7-O-beta-glucopyranoside	19.02
8	3.67	328.09	$C_{18}H_{16}O_6$	1.55	1,3,5,6-Tetrahydroxy-4-phenylxanthone	7.06
	3.72	122.04	$C_7H_6O_2$	-3.86	Benzoic acid	12.55
9	3.83	595.16	$C_{27}H_{31}O_{15}$	2.28	Pelargonidin-3,5-diglucoside	70.37
10	3.98	180.04	$C_9H_8O_4$	-1.3	Caffeic acid	248.77
11	4.19	594.15	$C_{27}H_{30}O_{15}$	-0.13	6,8-Bis(C-glucosyl)-apigenin	3116.64
12	4.34	226.12	$C_{12}H_{18}O_4$	-1.13	2-(1-Ethoxy-2-hydroxy)propyl-4-methoxyphenol	151.34
13	4.48	564.14	$C_{26}H_{28}O_{14}$	-0.29	5,7,4'-Trihydroxy-6-C-arabinoside-8-C-glucoside flavone	3535.38
14	4.57	433.11	$C_{21}H_{21}O_{10}$	-1.05	Pelargonidin-3-galactoside	152.79
15	4.57	434.11	$C_{21}H_{22}O_{10}$	-3.19	2-O-Caffeoyl arbutin	192.70
16	4.59	264.09	$C_{14}H_{16}O_5$	-0.85	1'-Acetoxyeugenol acetate	188.99
17	4.64	220.07	$C_{12}H_{12}O_4$	-1.09	Eugenitin	119.59
18	4.74	432.10	$C_{21}H_{20}O_{10}$	-0.24	3,4',5,7-Tetrahydroxyflavone-3-L-rhamnoside	8640.90
19	4.85	360.12	$C_{19}H_{20}O_7$	0.39	3,4-Dihydroxy-6,7,3',4'-tetramethoxyflavonol	76.84
20	4.88	772.20	$C_{33}H_{40}O_2$	-0.62	Kaempferol-3-diglucose-7-glucoside	406.20
21	5.03	610.15	$C_{27}H_{30}O_{16}$	0.67	Equisetrin	111.43
22	5.20	548.15	$C_{26}H_{28}O_{13}$	0.17	Apigenin bioside	230.87
23	5.20	756.21	$C_{33}H_{40}O_{20}$	0.48	Quercetin-3-O-[alpha-rhamnopyranosyl-(1-->4)-alpha-rhamnopyranosyl-(1-->6)-beta-glucopyranoside]	4533.27
24	5.33	740.21	$C_{33}H_{40}O_{19}$	-0.05	1-[(beta-D-Glucopyranosyl-(1-->3)-O-beta-D-glucopyranosyl-(1-->6)-O-beta-D-glucopyranosyl)oxy]-8-hyd	465.03
25	5.36	448.10	$C_{21}H_{20}O_{11}$	-0.65	3,3'4',5,7-Pentahydroxyvone-3-L-rhamnoside	494.59
26	5.85	252.09	$C_{13}H_{16}O_5$	-3.77	Eugenone	152.32
27	5.88	222.08	$C_{12}H_{14}O_4$	-5.18	2,3-Dihydro-5,7-dihydroxy-2,6,8-28trimethyl-4H-1-benzopyran-4-one	156.45
28	5.91	271.06	$C_{15}H_{11}O_5$	-0.26	Pelargonidin	291.19
29	6.25	312.12	$C_{15}H_{20}O_7$	3.31	3,4-Dihydroxy-allylbenzene-4-O-beta-D-glucopyranoside	1583.75
30	6.71	194.09	$C_{11}H_{14}O_3$	-1.47	2-Methoxy-4-(3-methoxy-1-propenyl)-phenol	1498.76
31	7.22	294.18	$C_{17}H_{26}O_4$	0.35	Embelin	2004.53

Table S1 (continued)

32	9.79	772.18	$C_{36}H_{36}O_{19}$	-2.12	Quercetin-3-O-beta-(6''-E-p-coumaroylglucopyranoside)-7-O-beta-gucopyranoside	56.31
33	10.48	224.10	$C_{12}H_{16}O_4$	-3.85	(Z)-4,5-Dihydro-6,7-cis-dihydroxy-3-butylidene phthalide	506.36
34	10.67	184.03	$C_8H_8O_5$	-6.02	3-Methoxygallic acid	145.69
35	13.57	268.16	$C_{15}H_{24}O_4$	2.44	1-(4-Hydroxy-3-methoxyphenyl)-3,5-octane-diol	7.71

Figure S1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DSS+TB
3FAx-Neu5Ac
B
DAI
Time (days)
Control
TB
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3FAx-Neu5Ac
C
Control
TB
Vehicle
3FAx-Neu5Ac
D
Colon length (cm)
a
a
a
a
Control
TB
Vehicle
3FAx-Neu5Ac
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Control
TB
Vehicle
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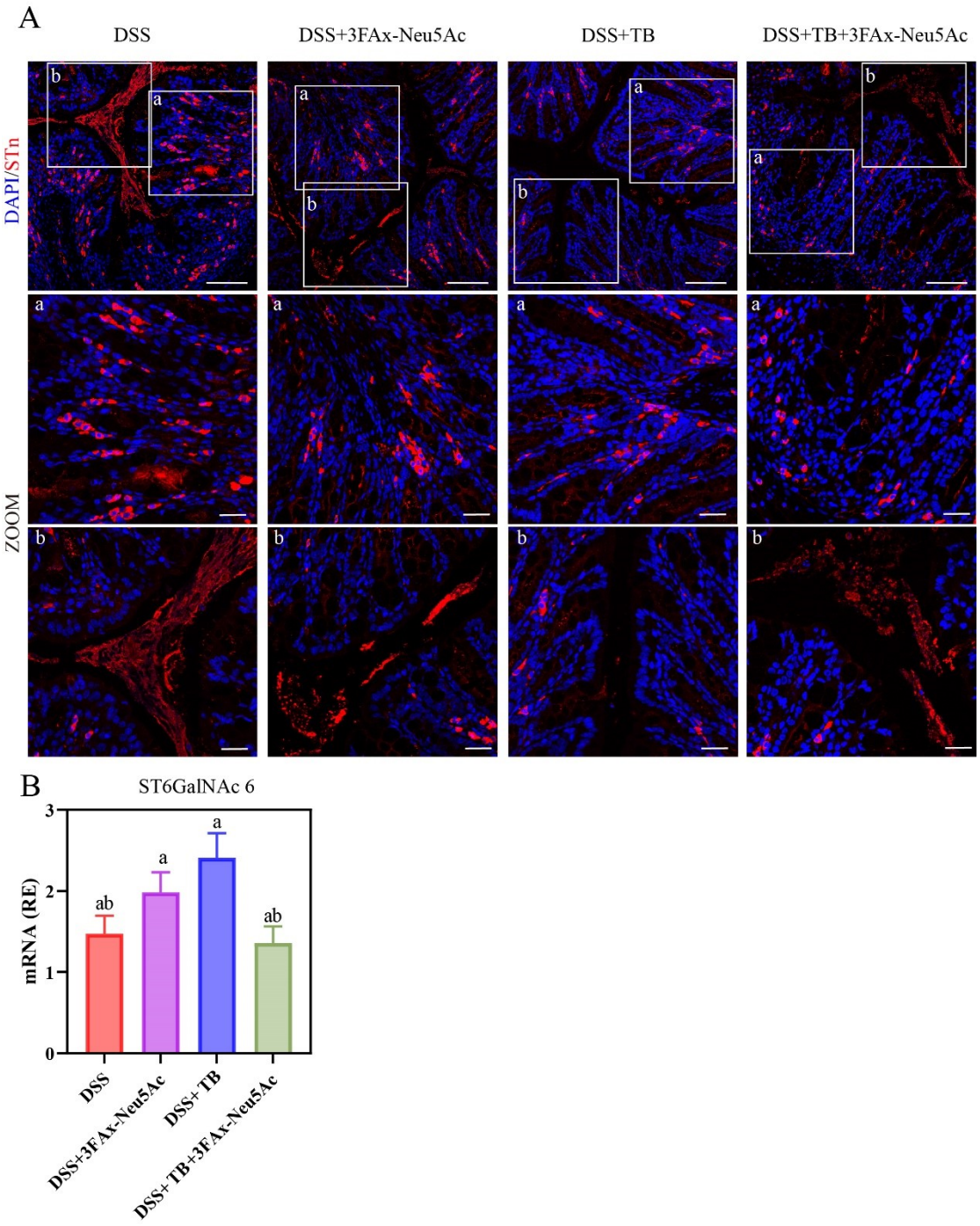
F
Control
TB
Vehicle
3FAx-Neu5Ac

Figure S2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DSS+TB
DSS+TB+3FAx-Neu5Ac
DAPI/STn
ZOOM
B
ST6GalNAc 6
mRNA (RE)
ab
a
a
ab
DSS
DSS+3FAx-Neu5Ac
DSS+TB
DSS+TB+3FAx-Neu5Ac