

Support information

Impact of titanium dioxide nanoparticles on intestinal community in the 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis mice and the intervention effect of vitamin E

Yanjun Gao, Tingyu Li, Shumin Duan, Lizhi Lv, Yuan Li, Lin Xu, Yun Wang

Y Gao¹, T Li^{2,3}, S Duan¹, L Lv¹, Y Li¹, L Xu¹, Dr. Y Wang¹

¹ Department of Occupational and Environmental Health Sciences School of Public Health Peking University Beijing 100191, China

² Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of sciences, Kunming 650201, China

³ College of Life Science, Northwest University, No. 1 Taibai North Road, Xi'an 710069, China

Table S1. The criteria for DAI scoring

Score	Criteria		
	Weight Loss	Stool consistency	Hemoccult
0	None	Well form pellets	Negative
1	1-5%	Soft stool	+
2	6-10%	Loose stool	++
3	11-15%	-	+++
4	>15%	Diarrhoea	++++/Gross bleeding

Table S2. The criteria for colon mucosal damage index(CMDI) assessment

Features and grades	Score
No damage	0
Hyperaemia; no ulcers	1
Hyperaemia and thickening of the bowel wall; no ulcers	2
One ulcer without thickening of the bowel wall	3
Two or more sites of ulceration/inflammation	4
Two or more major sites of ulceration and inflammation or one site of ulceration/inflammation extended >1 cm along the length of the colon	5
If damage covered >2 cm along the length of the colon, the score was increased by 1 for each additional cm of involvement	6–10

Table S3. The criteria for histological examination of colon

Features	Grades	Score
absence of goblet cell and crypt	slight absence of goblet cell	1
	severe absence of goblet cell	2
	slight absence of crypt	3
	severe absence of crypt	4
degree of inflammatory cell infiltration	reached the basal layer of crypt	1
	reached the mucosal muscle layer	2
	penetrated deeply into the mucosal muscle layer	3
	reached the submucosa	4

Table S4 Further evaluation of the statistical significance of the spatial separation among the different groups in PCoA plots using AMOVA analysis (*P*-value)

<i>P</i> -value	CT	TiO ₂	TNBS	TNBS+TiO ₂	TNBS+VE	TNBS+TiO ₂ +VE
CT	-					
TiO ₂	0.139	-				
TNBS	0.029*	0.064	-			
TNBS+TiO ₂	0.055	0.034*	0.013*	-		
TNBS+VE	0.214	0.072	0.089	0.188	-	
TNBS+TiO ₂ +VE	0.018*	0.030*	0.012*	0.170	0.353	-

*: *P*-value less than 0.05

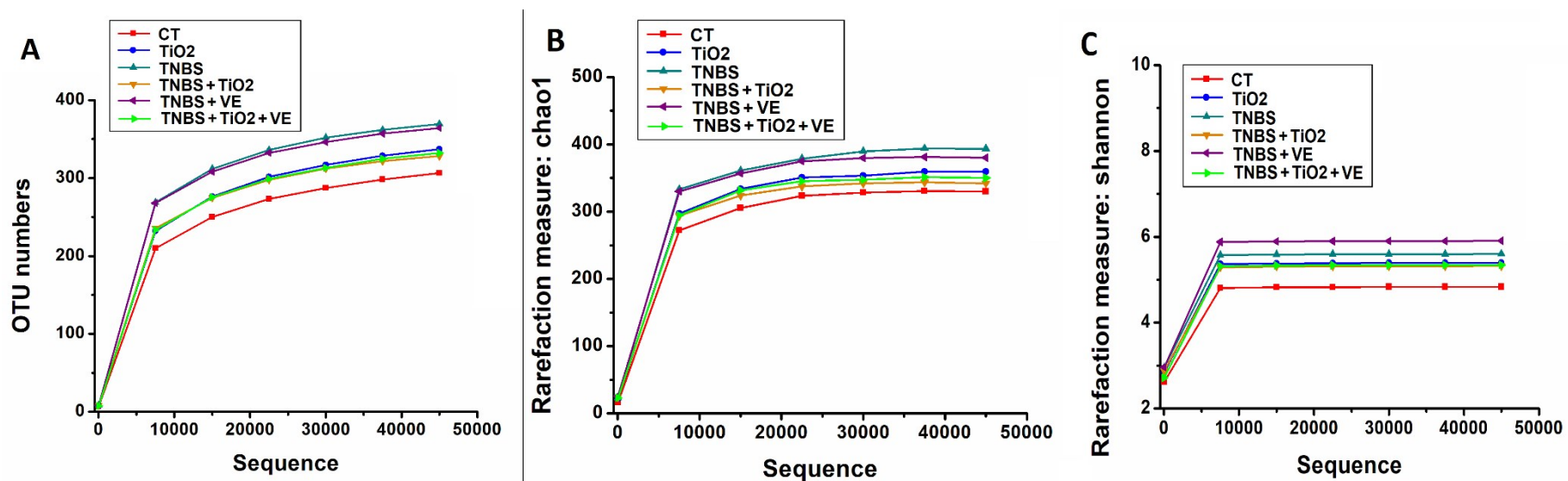


Figure S1 Rarefaction curves calculated for total OTUs abundance (A), Chao1 index (B) and Shannon index (C). The plateau exhibited in the rarefaction curves indicated that the sequencing depth was good enough to recover the vast majority of bacterial species in the samples.

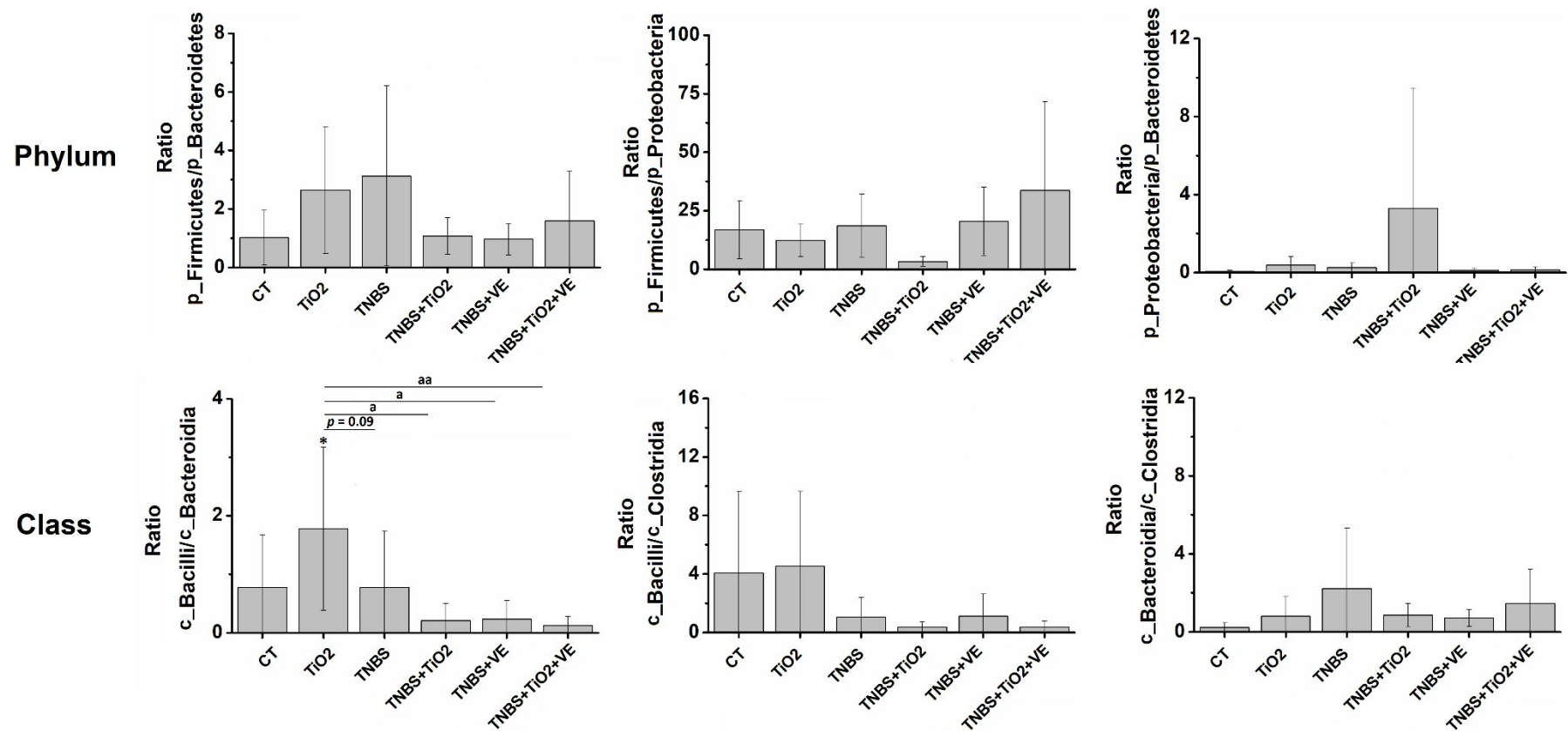


Figure S2 Ratio of relative abundance between top 3 abundant bacteria at the phylum (A) and class (B) levels in mice stool samples after the colitis inducement and TiO₂-NPs or vitamin E administration. Significant difference was found between the CT group and the experimental groups (* $p < 0.05$). Significant difference was also found between the experimental groups (^{aa} $p < 0.01$, ^a $p < 0.05$).

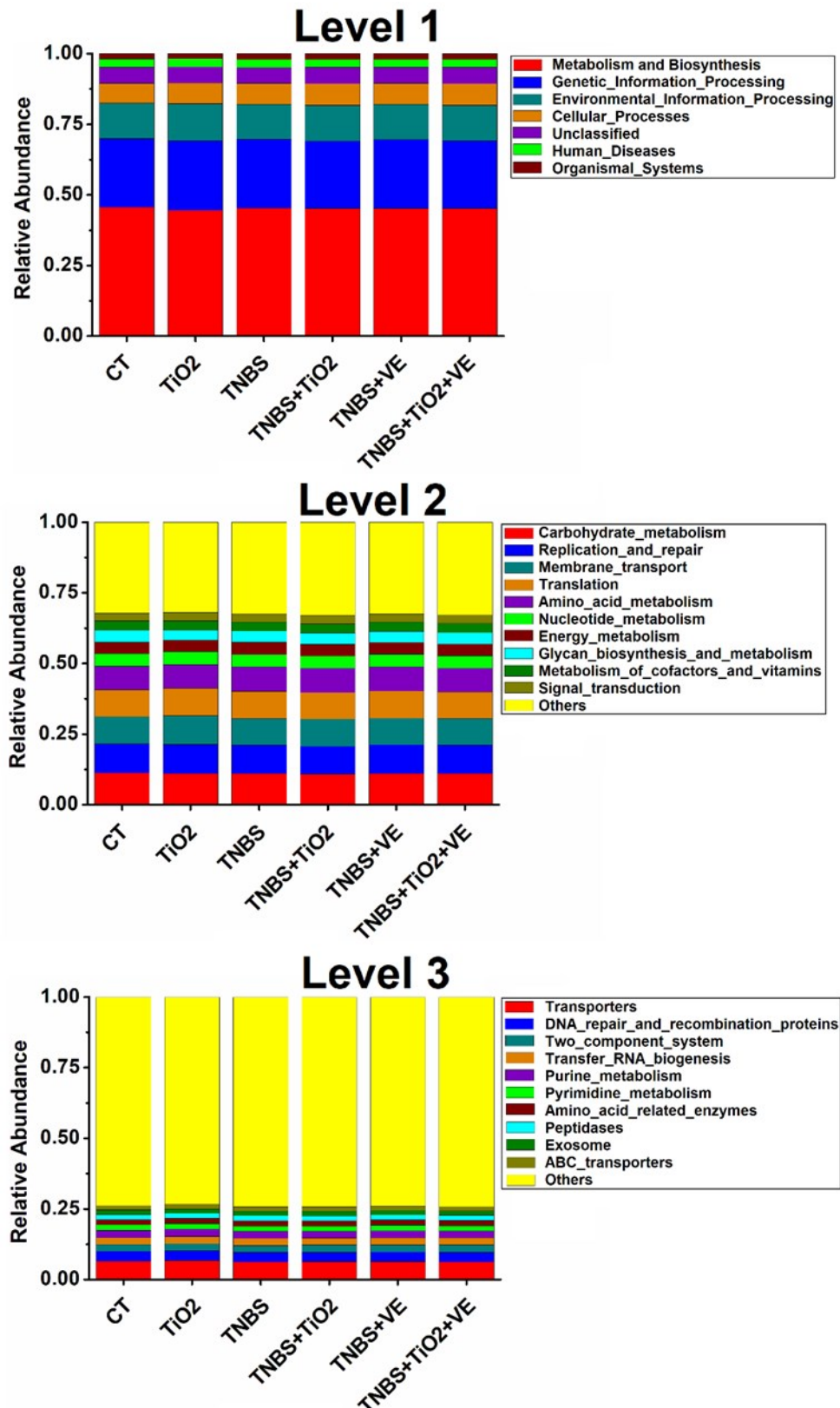


Figure S3 Top ten relative abundances of bacteria function at the Level 1, Level 2 and Level 3 in mice stool samples after the colitis inducement and TiO₂-NPs or vitamin E administration.

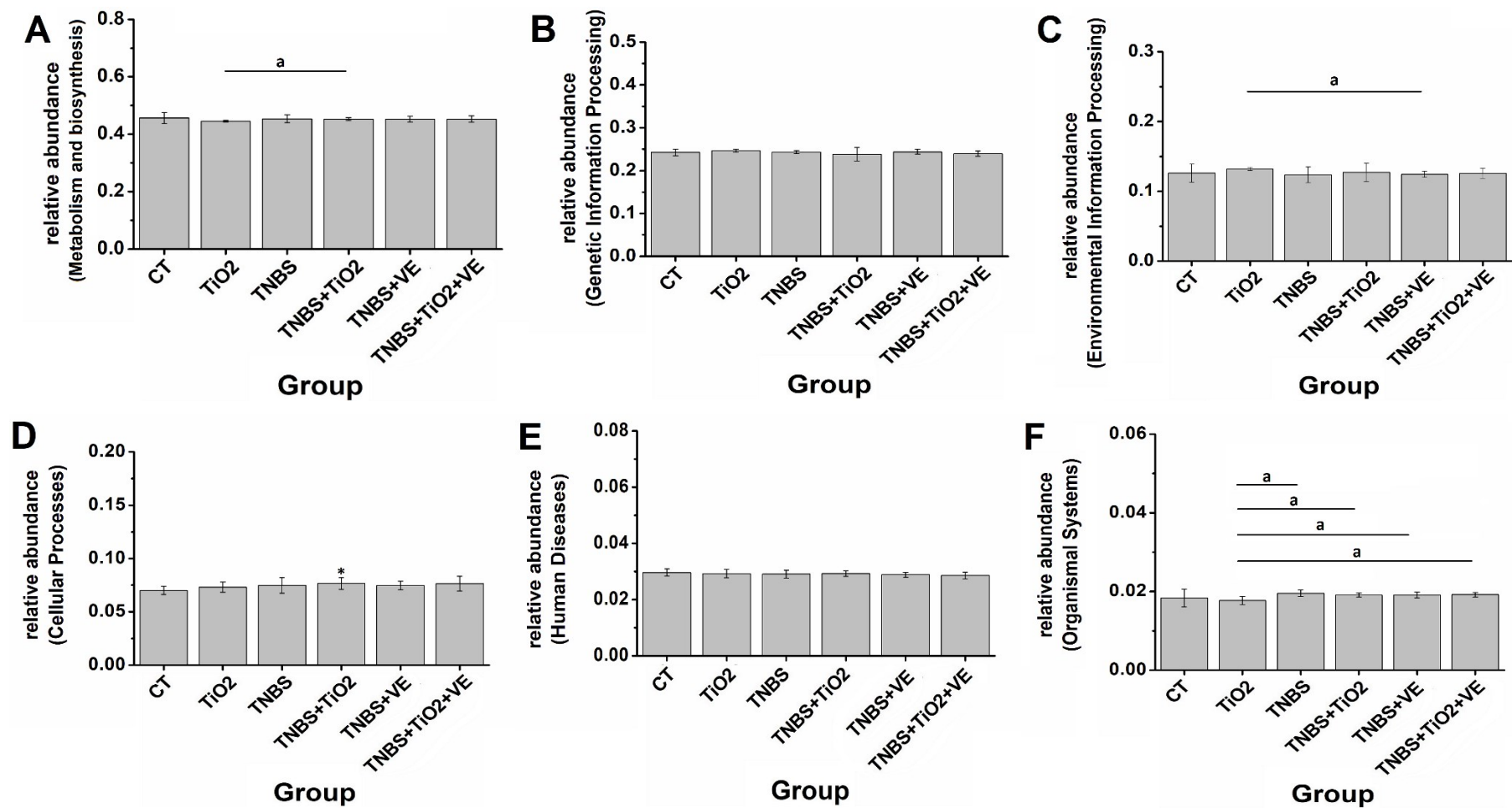
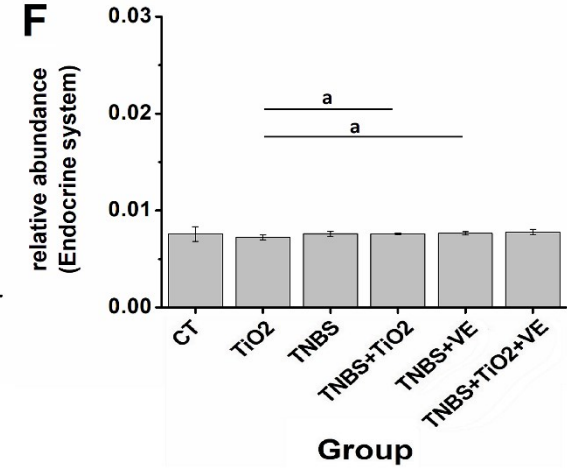
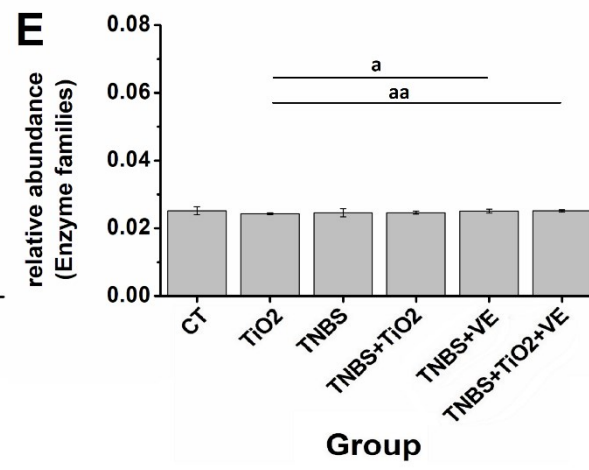
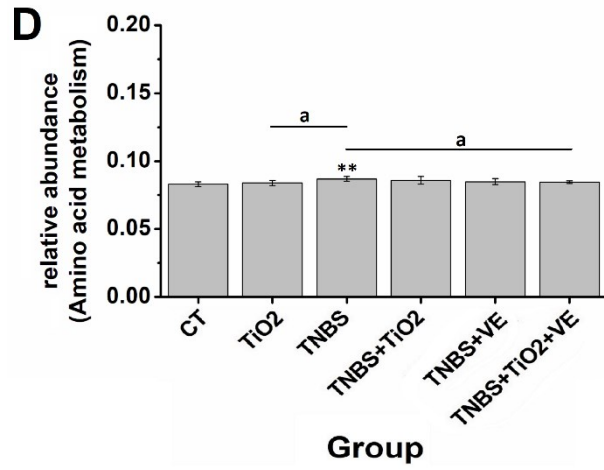
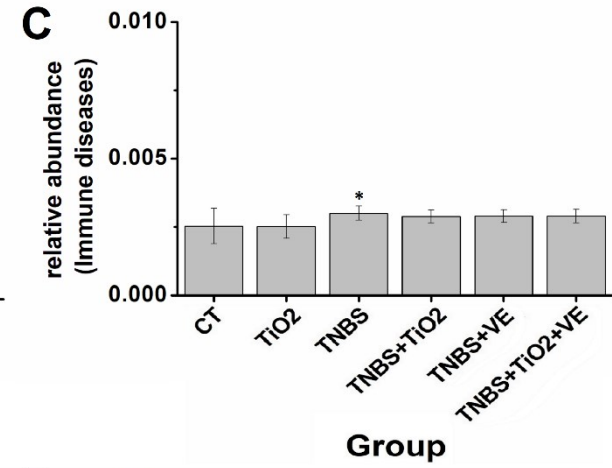
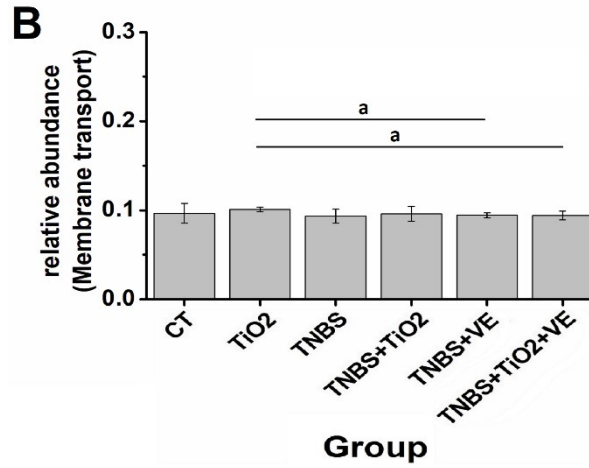
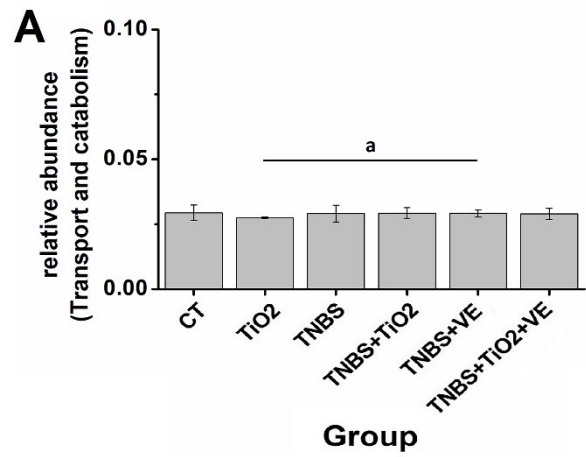


Figure S4 The relative abundance of functional annotation (level 1) in mice stool samples after the colitis induction and TiO₂-NPs or vitamin E administration. Significant difference was found between the CT group and the experimental groups (* $p < 0.05$). Significant difference was also found between the experimental groups (^a $p < 0.05$).



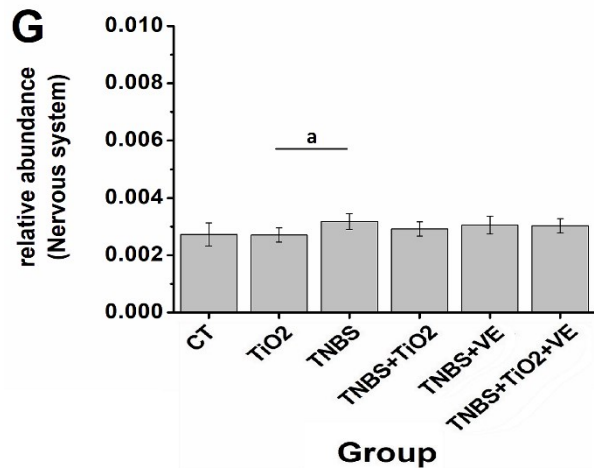
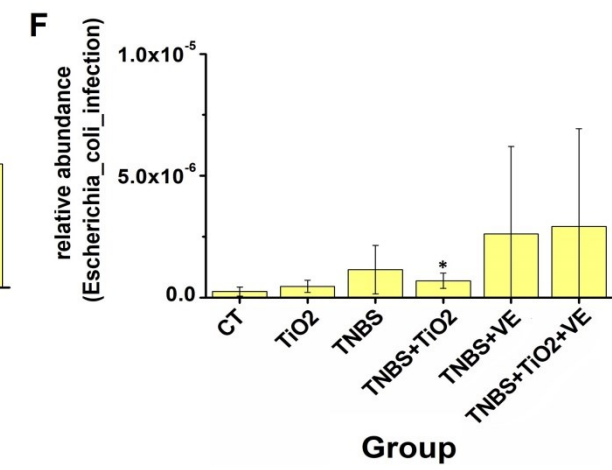
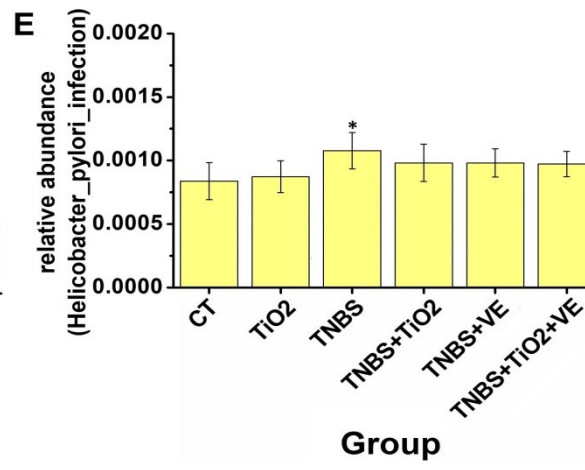
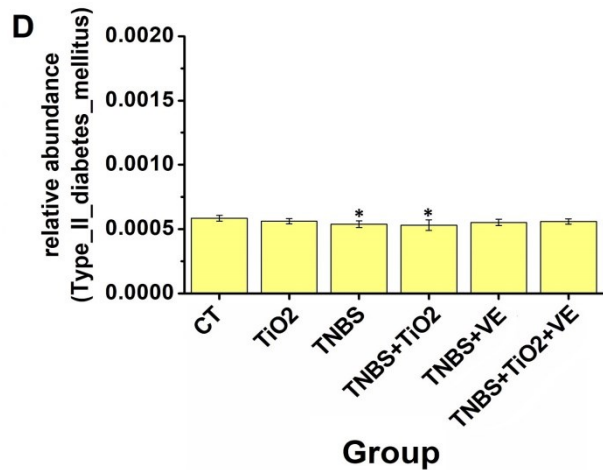
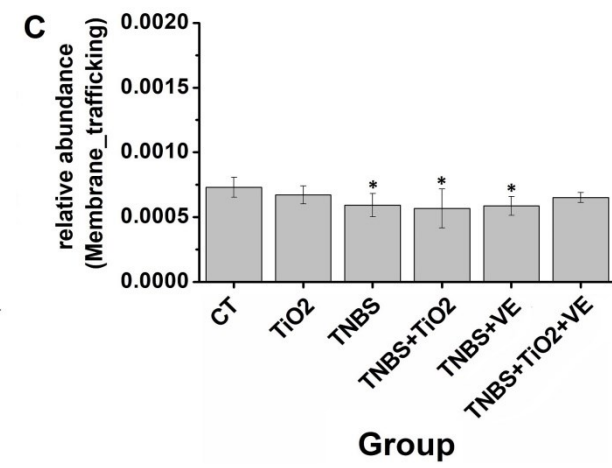
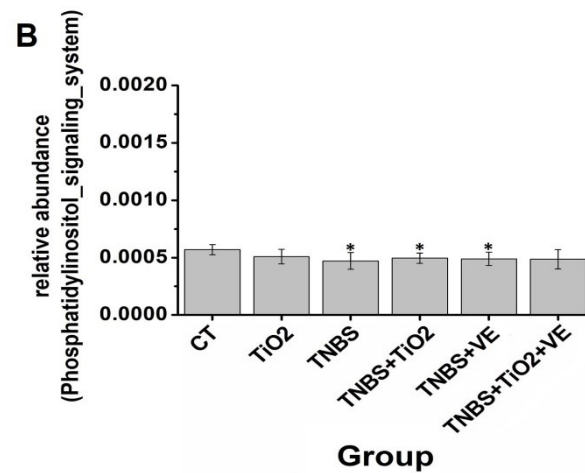
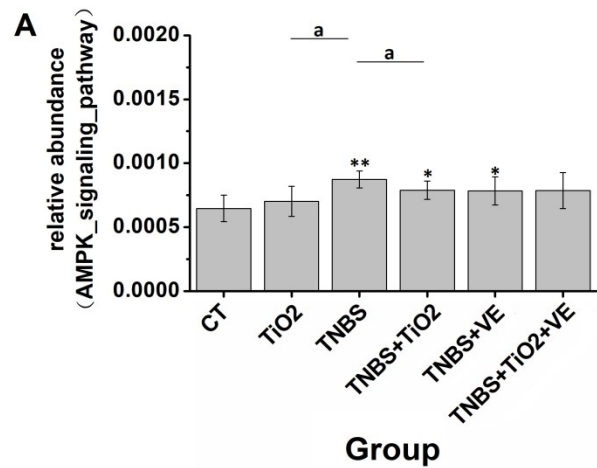
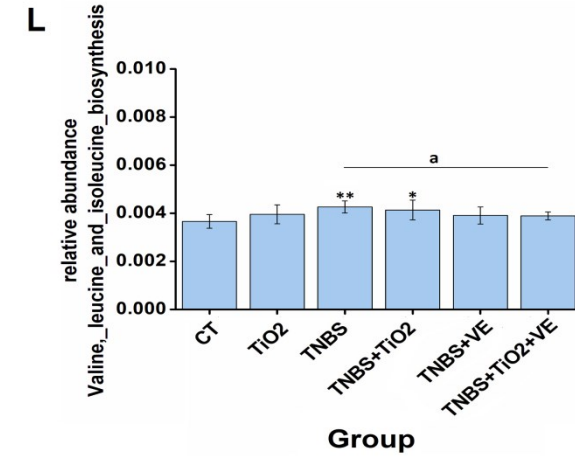
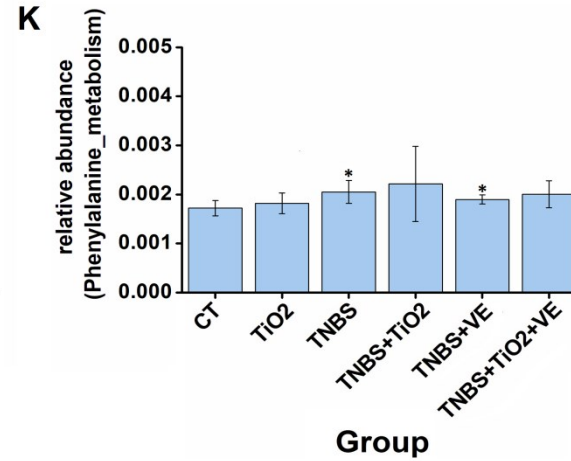
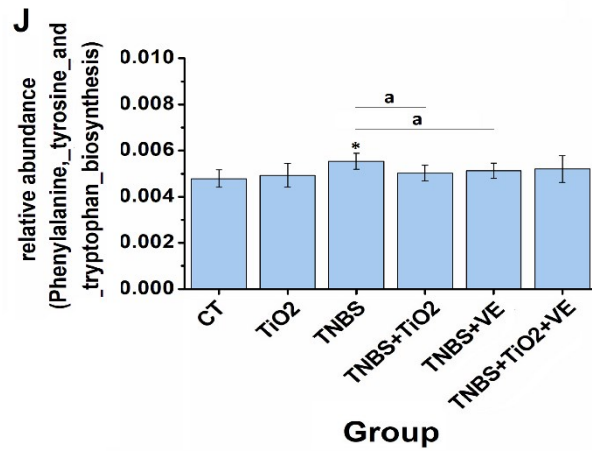
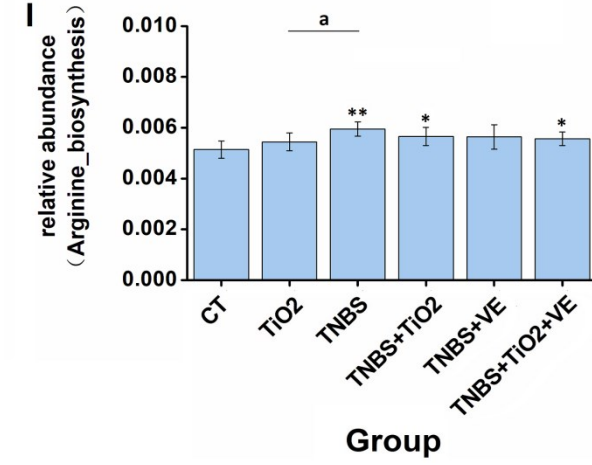
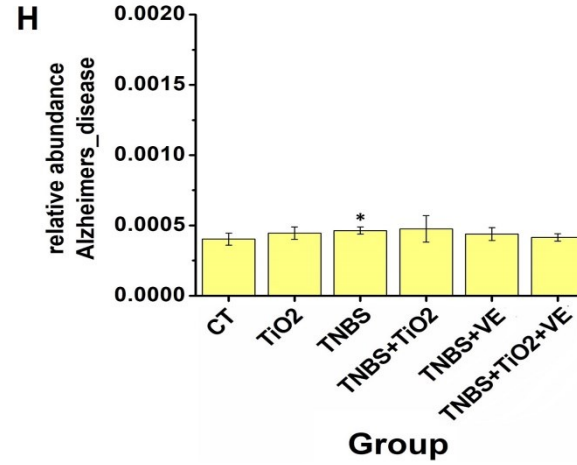
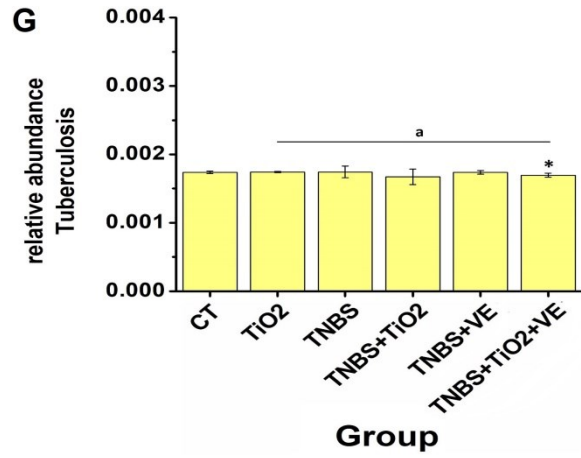
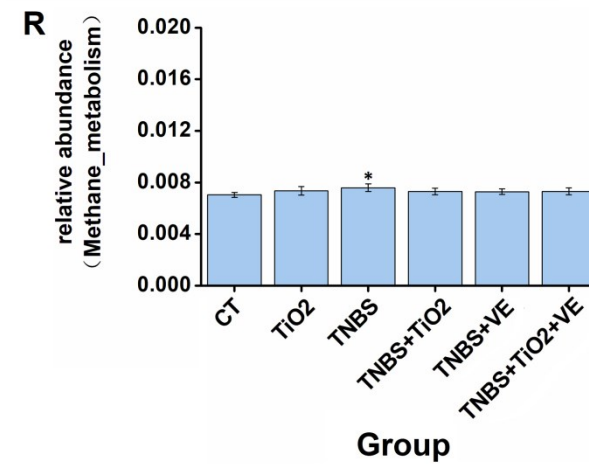
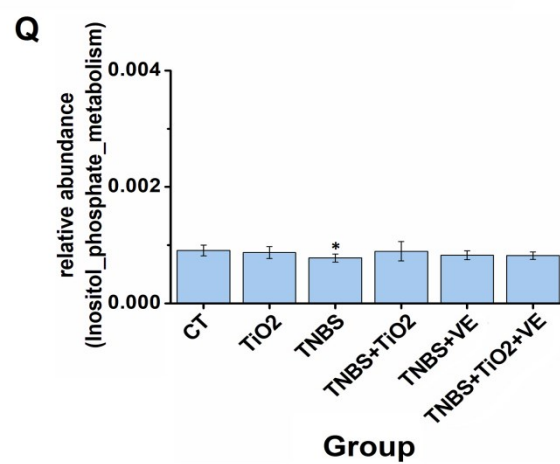
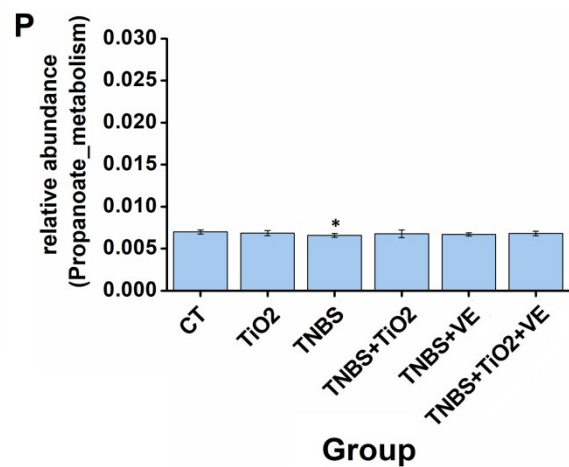
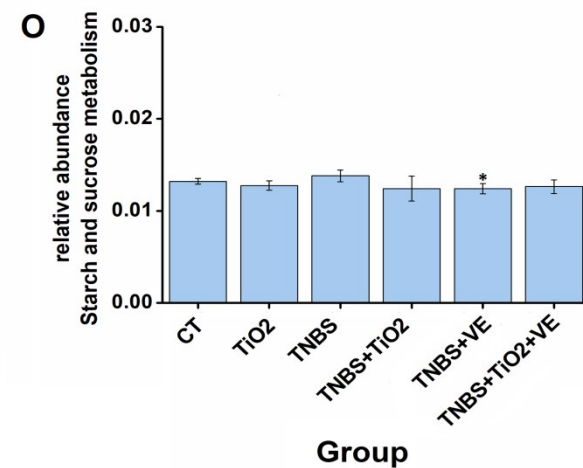
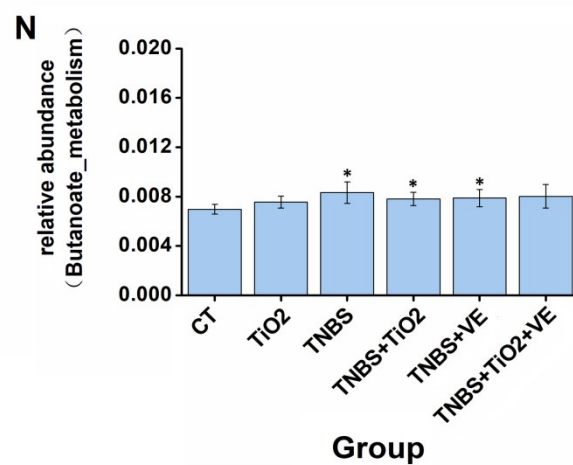
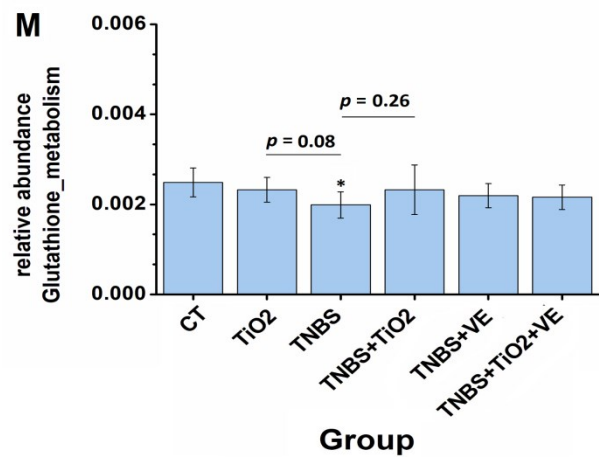


Figure S5 The relative abundance of functional annotation (level 2) in mice stool samples after the colitis inducement and TiO₂-NPs or vitamin E administration. Only difference with statistical significance was displayed. Significant difference was found between the CT group and the experimental groups (** $p < 0.01$, * $p < 0.05$). Significant difference was also found between the experimental groups (^{aa} $p < 0.01$, ^a $p < 0.05$).







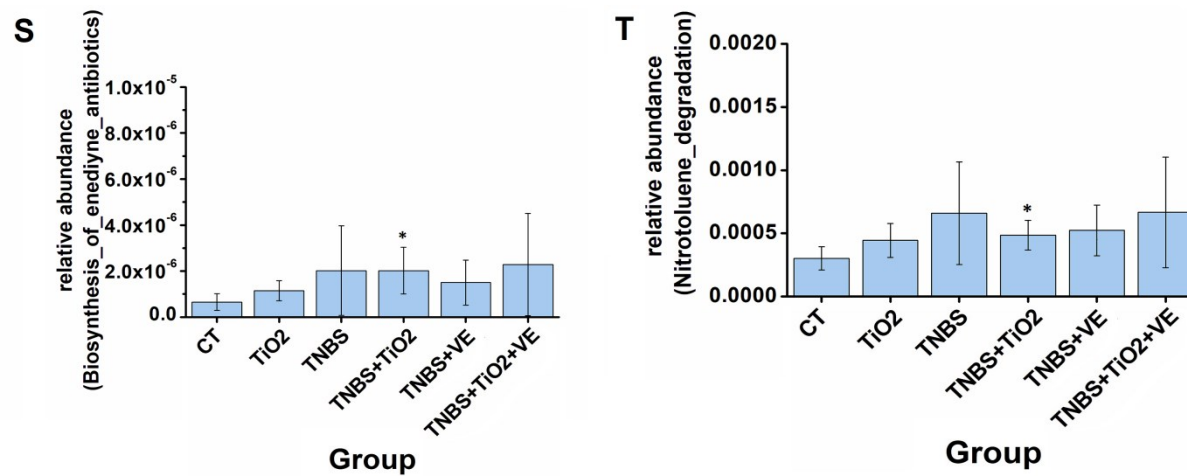
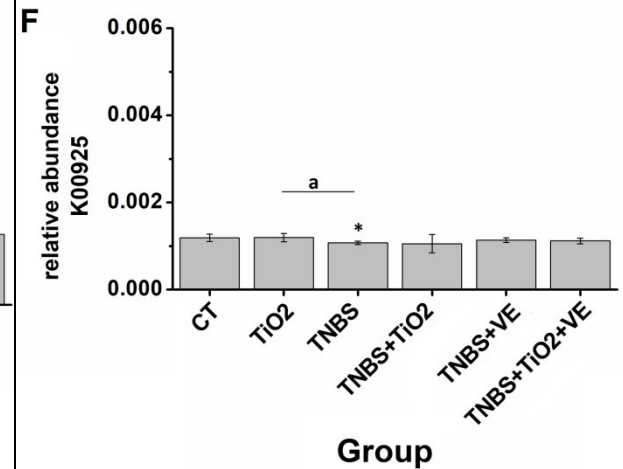
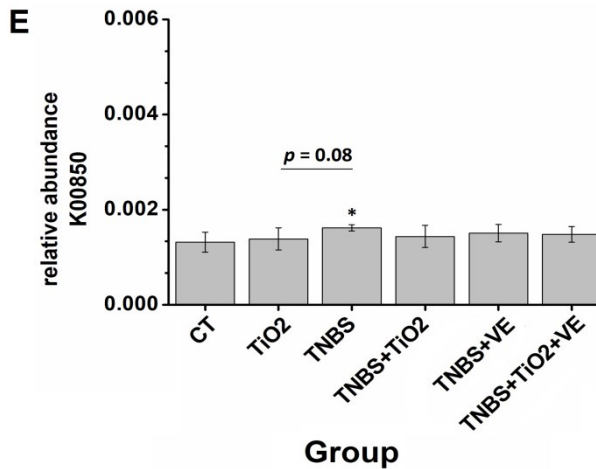
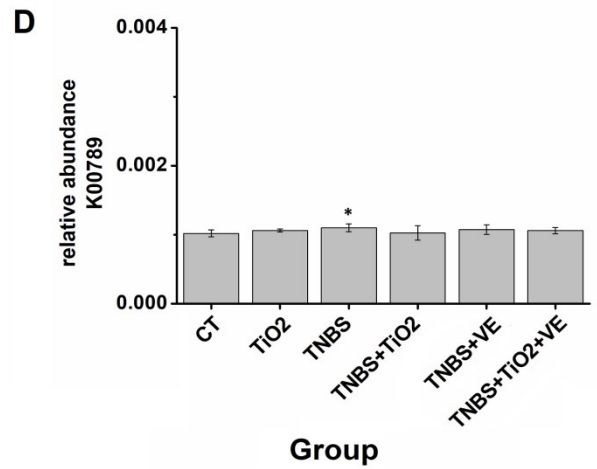
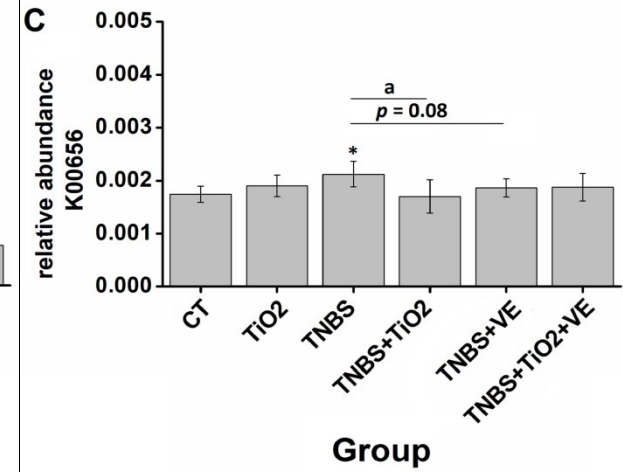
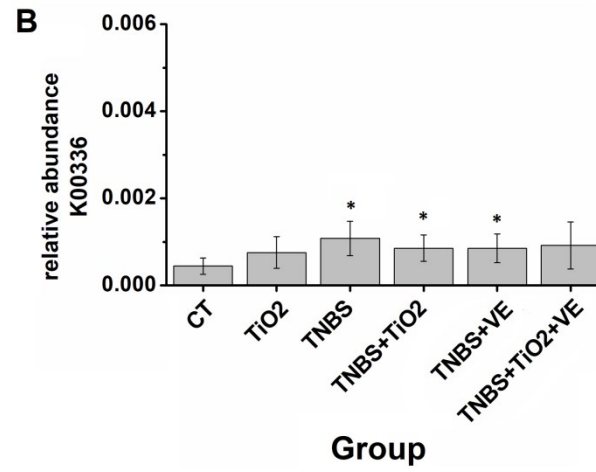
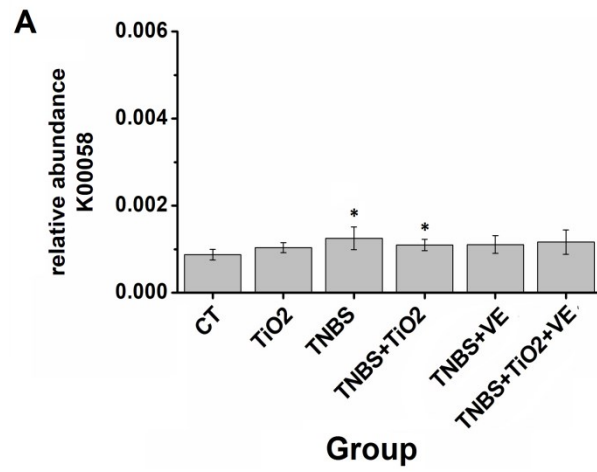
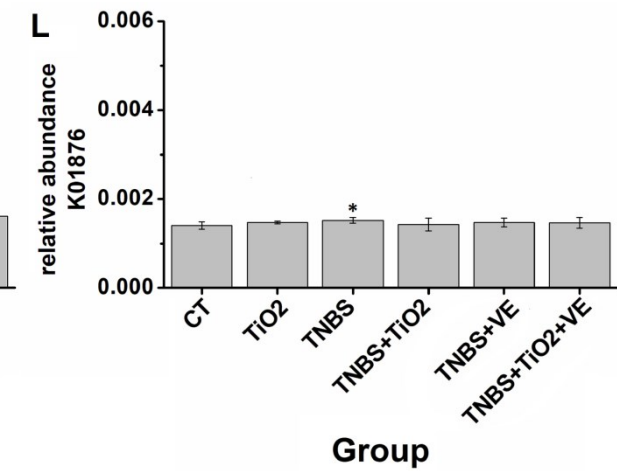
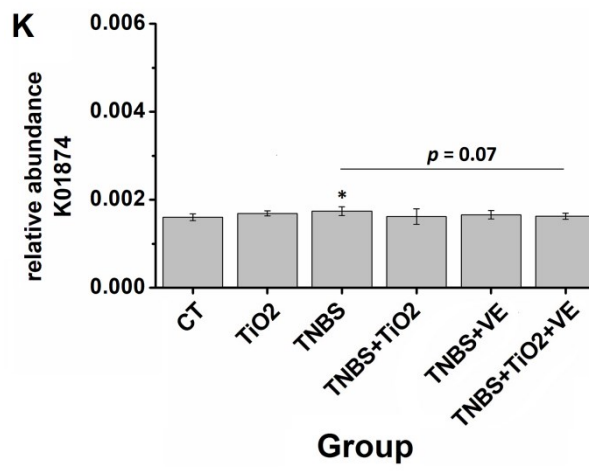
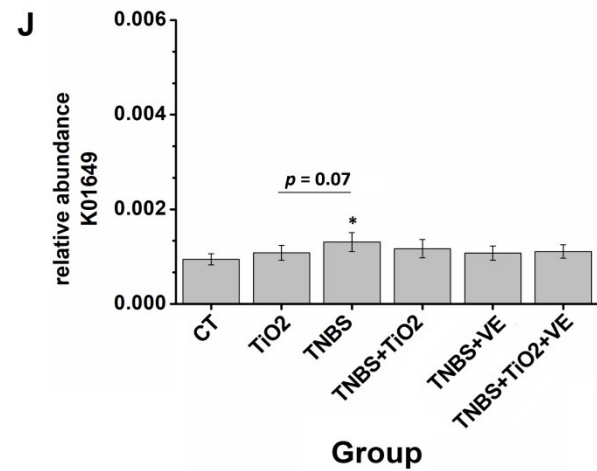
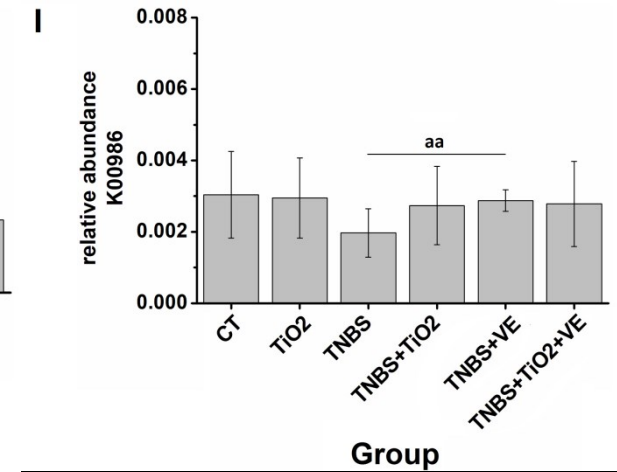
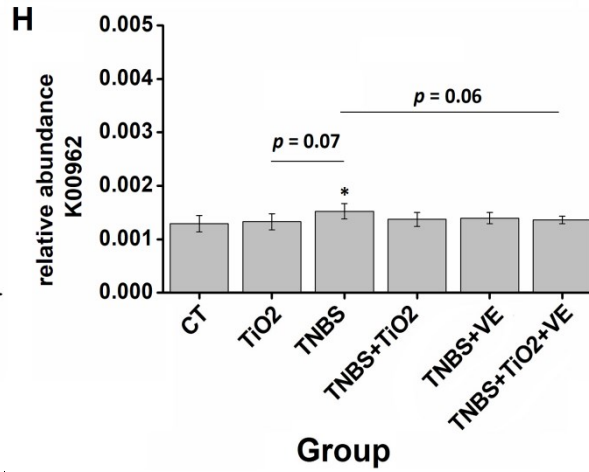
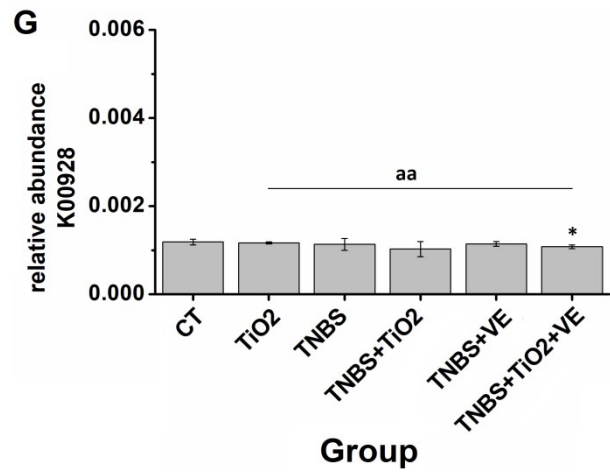
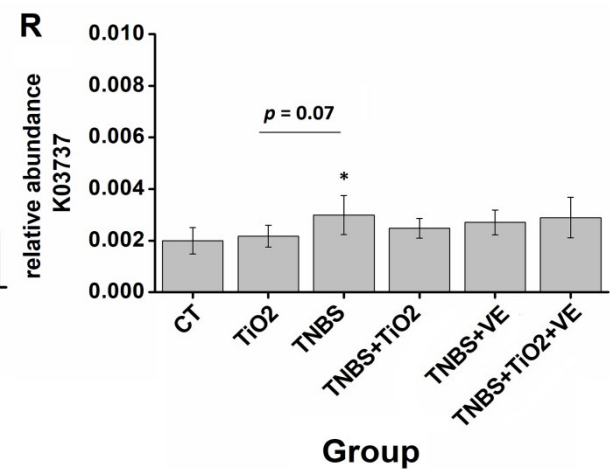
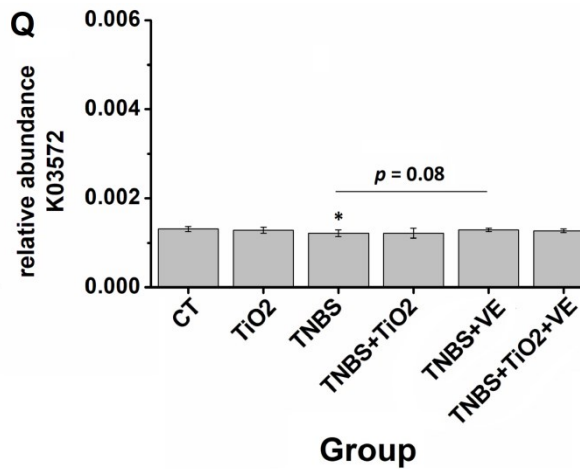
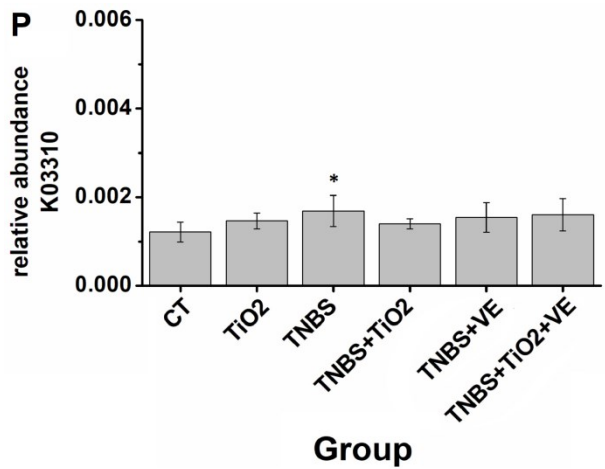
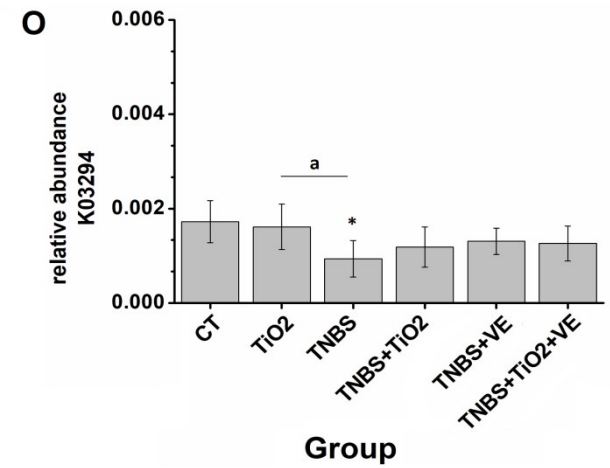
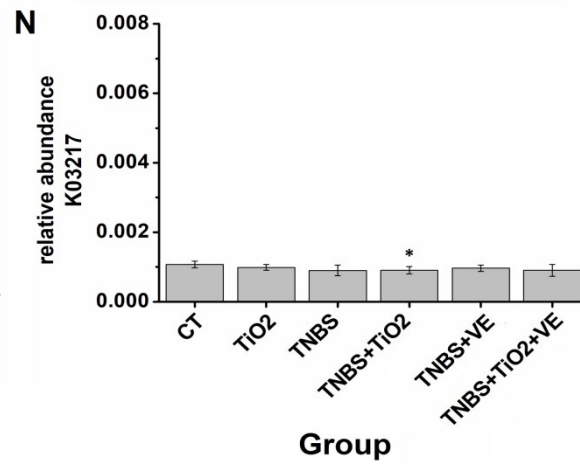
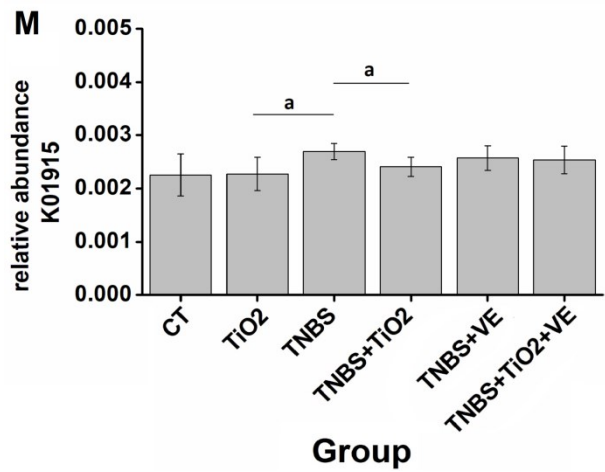
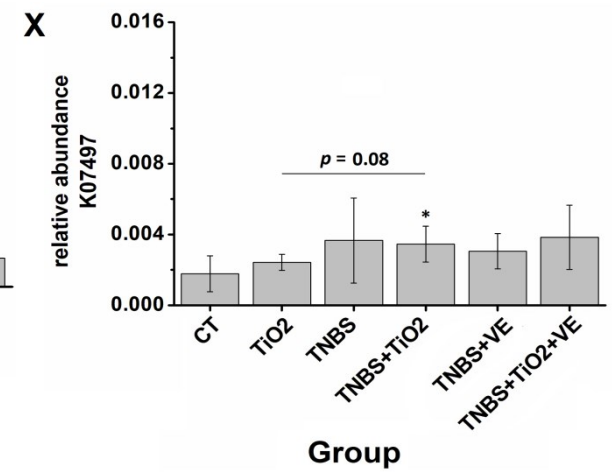
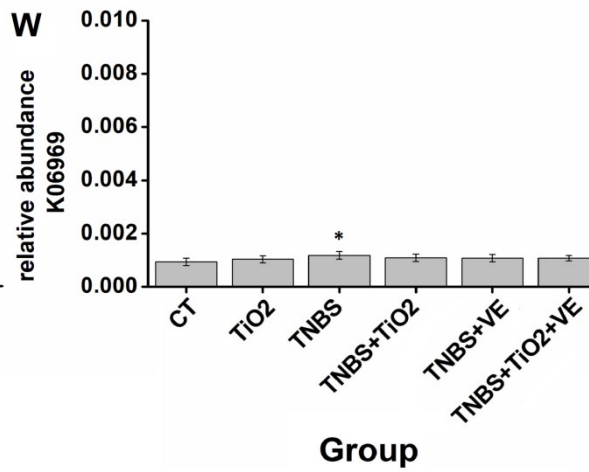
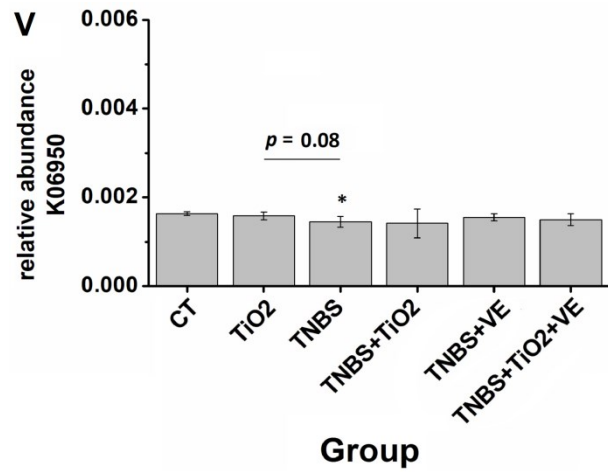
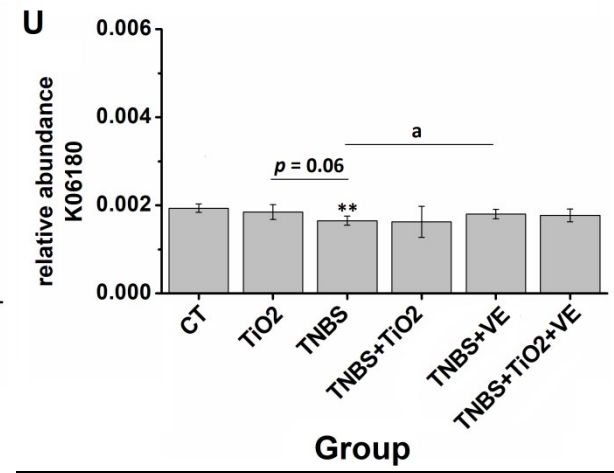
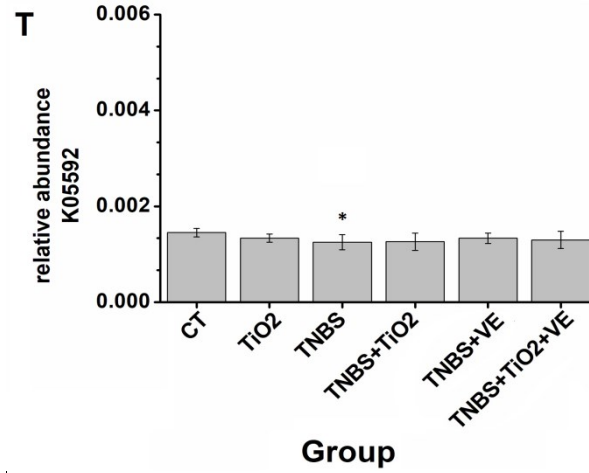
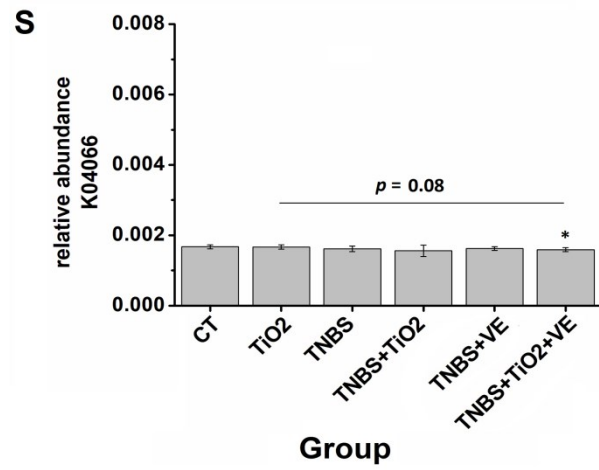


Figure S6 The relative abundance of functional annotation (level 3) in mice stool samples after the colitis inducement and TiO₂-NPs or vitamin E administration. (A-C) functional annotation on information processing. (D-H) functional annotation on disease. (I-T) functional annotation on metabolism and biosynthesis. Significant difference was found between the CT group and the experimental groups (** $p < 0.01$, * $p < 0.05$). Significant difference was also found between the experimental groups (^{aa} $p < 0.01$, ^a $p < 0.05$).









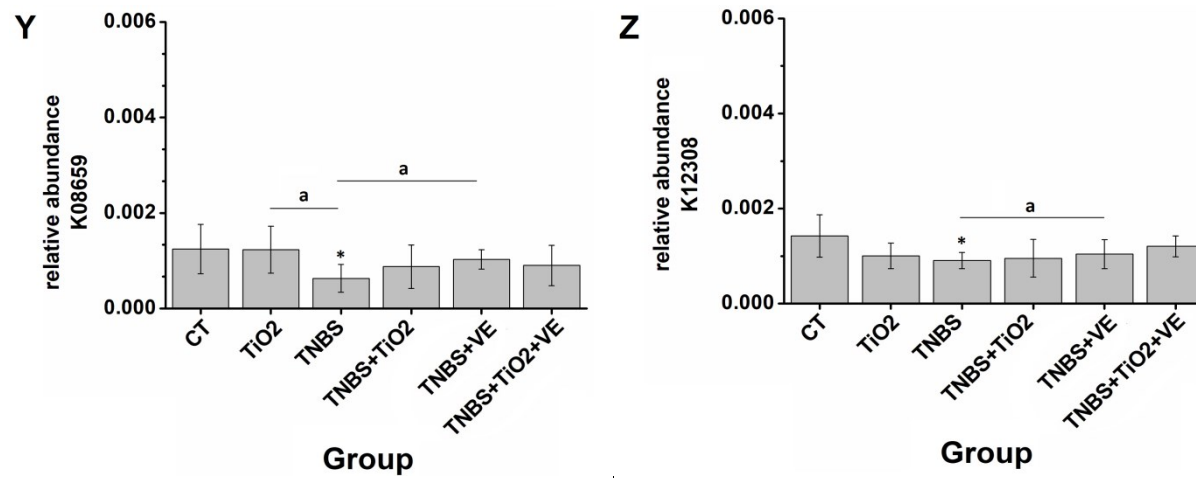
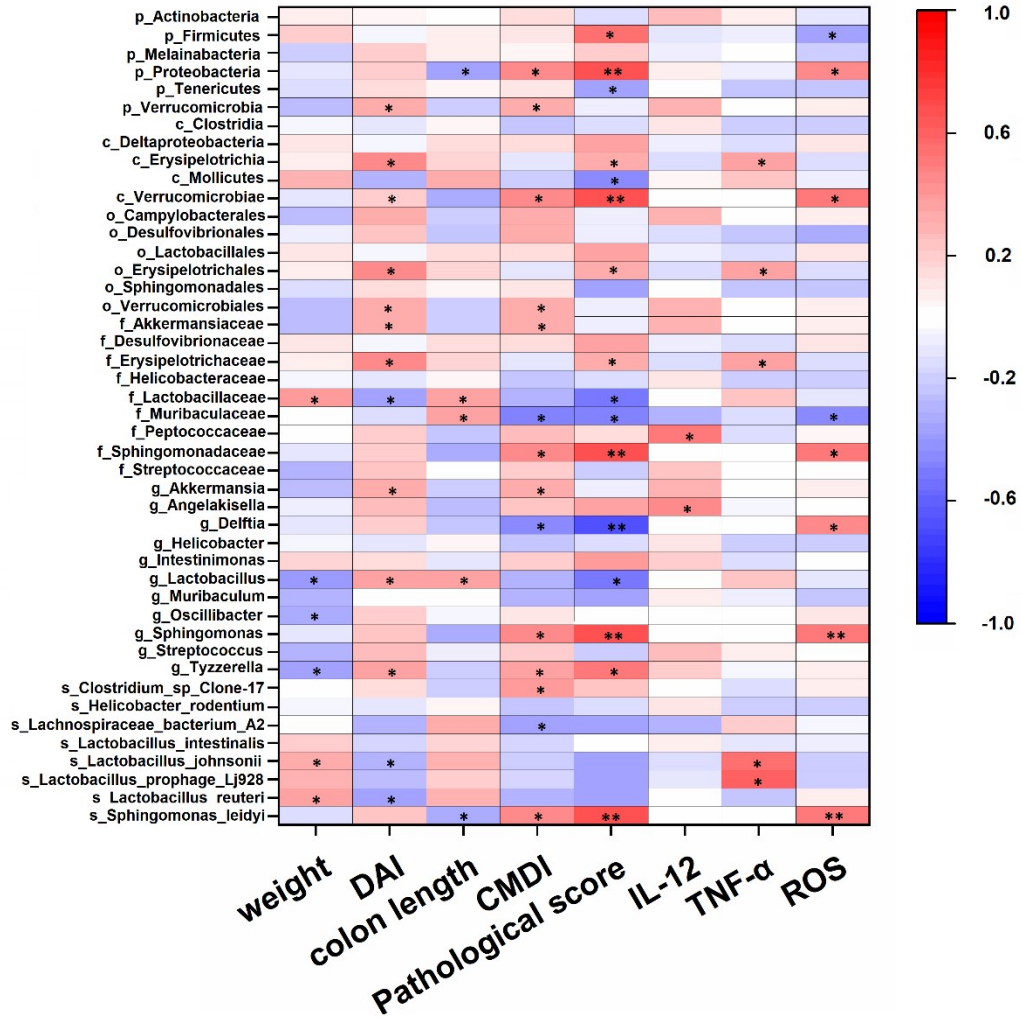
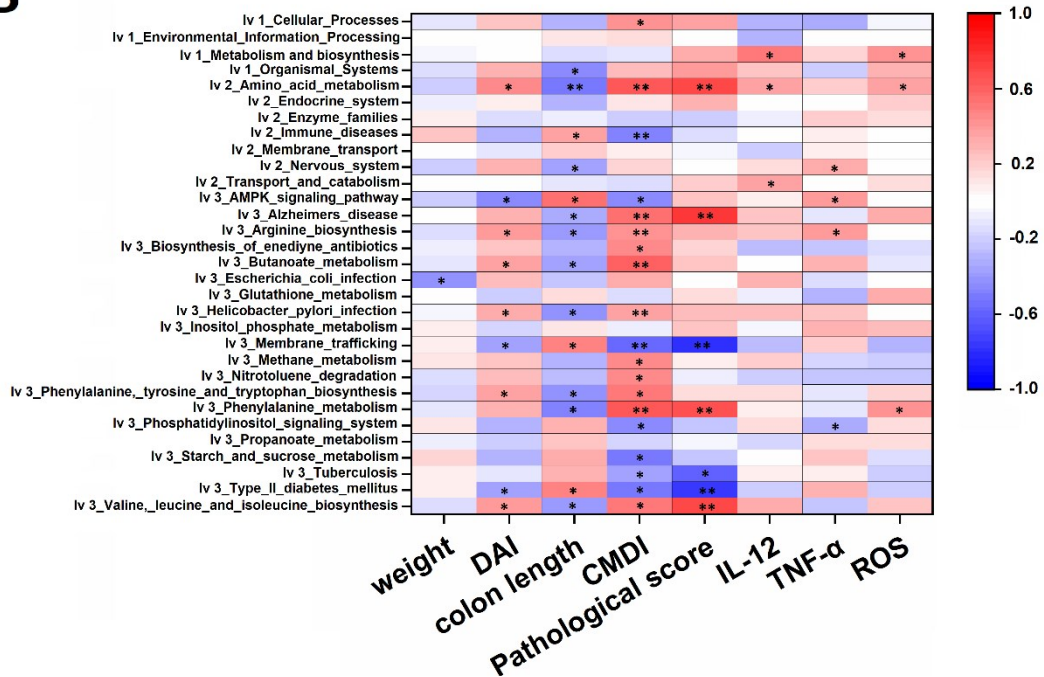


Figure S7 The relative abundance of functional annotation (level Ko) in mice stool samples after the colitis inducement and TiO₂-NPs or vitamin E administration. Significant difference was found between the CT group and the experimental groups (** $p < 0.01$, * $p < 0.05$). Significant difference was also found between the experimental groups (^{aa} $p < 0.01$, ^a $p < 0.05$).

A Correlation coefficient between taxonomics taxons and symptom indicators



B Correlation coefficient between functional taxons (lv 1-3) and symptom indicators



C Correlation coefficient between functional taxons (lv Ko) and symptom indicators

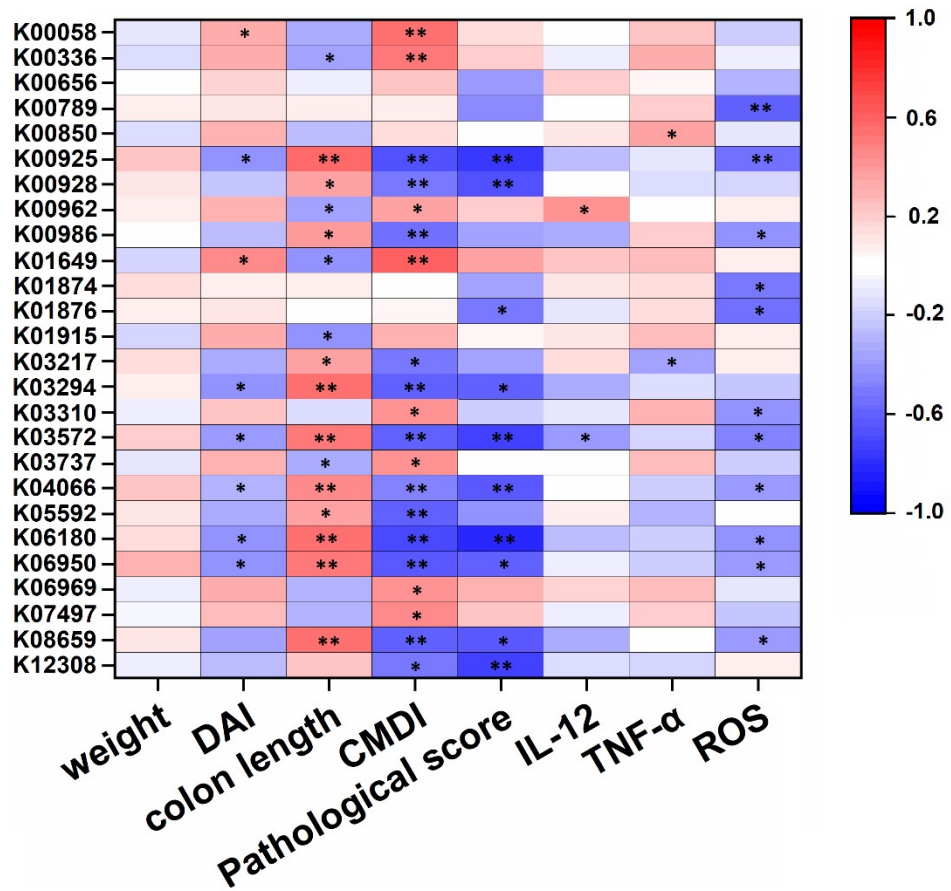


Figure S8 The correlation between colitis symptom indicators and taxonomics as well as functional profile. Correlation coefficient was statistically significant (** $p < 0.01$, * $p < 0.05$).