List of Correction Made with Comments of the Reviewers

Dear editors,

Please find enclosed the revised version of our manuscript which title and Manuscript ID appeared below. We are very glad to have your view on our manuscript and have revised the manuscript and made correction including incorrect comments and conclusions, the English grammar, spelling and structure. We sincerely hope the manuscript is now acceptable and suitable for publication in your journal.

Yours sincerely

Dr.Chunlan Xu

Note: The line numbers and page numbers in the correction list are all in accordance with the original manuscript.

Manuscript ID: 37359

Title: Recombinant expressed vasoactive intestinal peptide analogue ameliorates TNBS-induced experimental colitis in rats

Changes made to the manuscript

Answers to the Reviewer 1

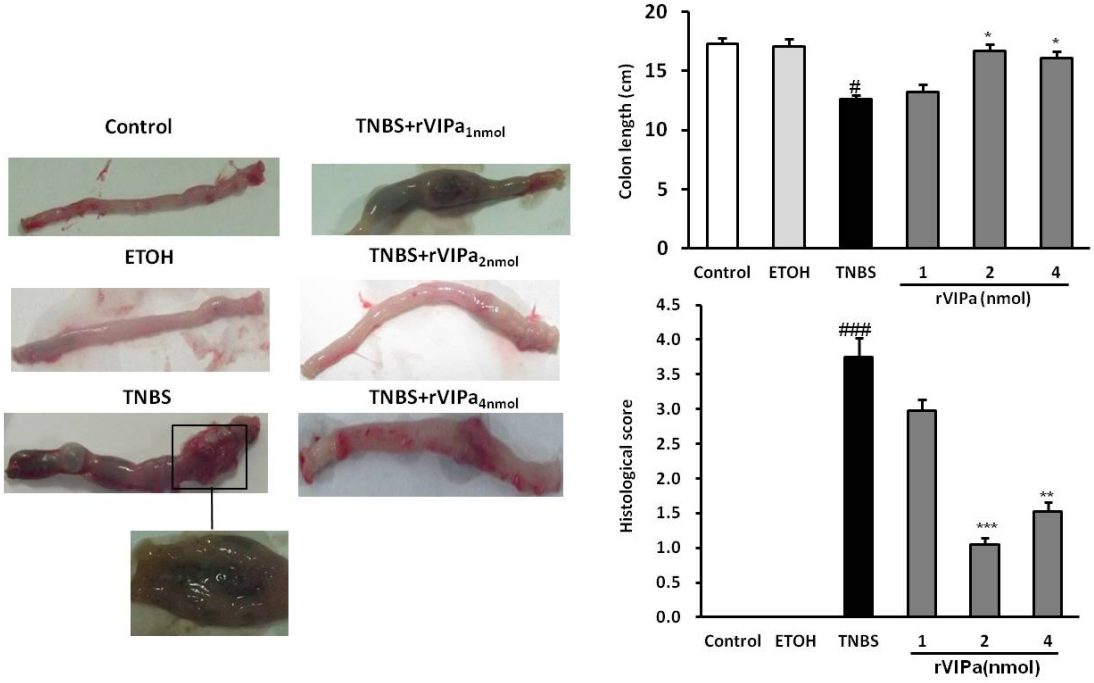
1. In the revised manuscript, we have corrected lines of flaws. For example, TNBS-induced ulcerative colitis have been changed to "TNBS-induced colitis"; Changed "microscopic assessment" to "Histological assessment", moreover, we improved the **Figure 2. Histological observation and evaluation of colon tissue** in the revised manuscript.
2. In addition, we have revised the manuscript and made correction including the English grammar, spelling and structure.

Answers to the Reviewer 2

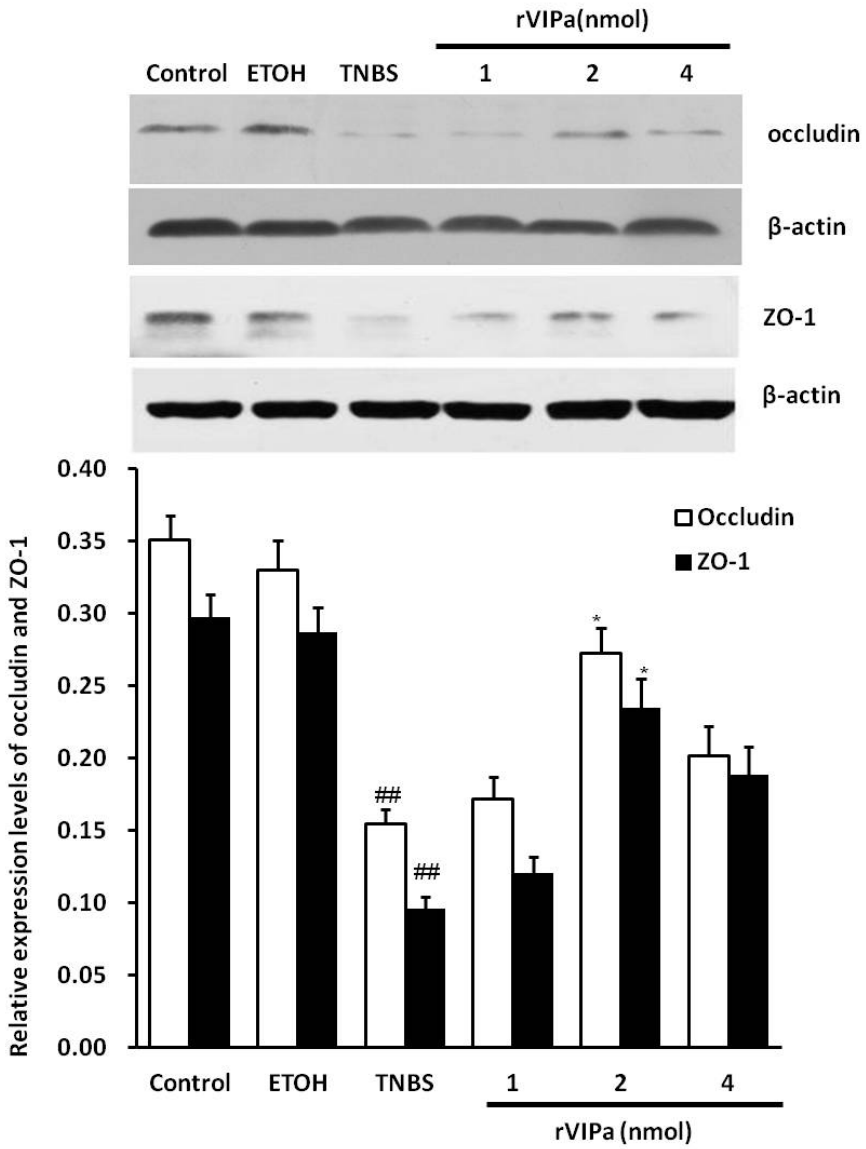
1. According to your suggestion, we have deleted "ulcerative" from the title. Moreover, TNBS-induced ulcerative colitis have been changed to "TNBS-induced colitis" throughout the manuscript.
2. In the section of discussion, we supplemented two sentences: Due to TNBS-induced colitis shares patholgy related to human CD. Therefore, TNBS-induced colitis is usually applied as a model simulating human CD.
3. **Material and methods:** the dose of TNBS-used has been supplemented. SD rats

from the TNBS-induced model groups were anesthetized and administered with 20 mg (in 250 μl of 50% ethanol) of TNBS via a 15 cm (diameter 2.0 mm) length of rubber infusion tube advanced 8 cm in the anus.

The data about the colon length has been given in the revised manuscript. As shown in the below **Figure 2.**



4. **Results:**Figure 5 the expression level of tight junction protein-ZO-1 was supplemented in the revised manuscript. As shown in the below **Figure 5.**



5. Discussion:

(1) Why they used TNBS as a model of ulcerative colitis?

In the revised manuscript, we supplemented the following information: An acute model of intestinal injury and inflammation based on the intrarectal administration of TNBS is known to induce an early production of inflammatory factors, followed by a Th1 response that resembles CD[22, 23].

[22]Morris G P, Beck P L, Herridge M S, Depew WT, Szewczuk M R, Wallace J L. Hapten-induced model of colonic inflammation and ulceration in the rat colon. Gastroenterology 1989; 96, 795–803.

[23]Neurath MF, Fuss I, Kelsall BL, Stuber W, Strober W. Antibodies to interleukin-12 abrogate established experimental colitis in mice. J. Exp. Med.

1995; 182, 1281–1290.

(2) Supplemented the reference: VIP restores immune tolerance by down-regulation of the inflammatory response and by induction of regulatory T cells[18].

Pozo D, Gonzalez-Rey E, Chorny A, Anderson P, Varela N, Delgado M. Tuning immune tolerance with vasoactive intestinal peptide: a new therapeutic approach for immune disorders. Peptides 2007; 28: 1833-1846.

(3) Discussed the data of VIPKO mice treated with DSS or DNBS.

Previous research showed that *Vip*$^{-/-}$ (VIPKO) mice VIPKO mice suffered impaired goblet cell development, leading to significantly reduced expression of mucin 2 (Muc2) and trefoil factor 3 (Tff3) as well as overt intestinal barrier dysfunction. These changes were associated with reduced expression of Cdx2, a transcription factor known to modulate cell proliferation, migration and differentiation [26]. Notably VIPKO mice also showed heightened susceptibility to DNBS and DSS-induced colitis, while treatment with VIP rescued the phenotype, protecting VIPKO mice against DSS-colitis[27].

[26] Coskun M, Troelsen JT, Nielsen OH. The role of CDX2 in intestinal homeostasis and inflammation. Biochimica et biophysica acta. 2011;1812(3):283–289. [DOI: 10.1016/j.bbadis.2010.11.008].

[27] Wu XJ, Conlin VS, Morampudi V, Ryz NR, Nasser Y, Bhinder G, Bergstrom KS, Yu HB, Waterhouse CC, Buchan AM, Popescu OE, Gibson WT, Waschek JA, Vallance BA, Jacobson K.Vasoactive intestinal polypeptide promotes intestinal barrier homeostasis and protection against colitis in mice. PloS One 2015; 10(5): e0125225. [DOI:10.1371/journal.pone.0125225]

(4) Page 12, line 6 The sentence have been improved: As the stimulation of TLR2 and -4 mediates the expression in a cascade of inflammation-related genes, the current study hypothesis was that TLRs could be involved in the physiopathological development of CD, and VIP could down-regulate, at least in part, the inflammatory response by modulating TLR expression.

Answers to the Reviewer 3

Thank you for your comments on our manuscript. You mentioned that the introduction is too long. In the revised manuscript, the induction has been simplified and improved.