Answering Reviewers

To dear editor and reviewers:

Thank you for reviewing my article.

Now, we will respond to the reviewer/editorial comments and queries as follows below:

Reviewer #1:

Authors reported good written paper with clear aim and interesting results. the main issue of the study was following: IBD was well associated with increased circRNA_103516 due to dysregulation in hsa-miR-19b-1-5p. Authors believe that circRNA_103516 can be a diagnostic biomarker of IBD. Although this is good written paper, I see that there are several items need to be explained.

1. **Section "Introduction." Authors should give a little bit more evidence regarding molecular targets of circRNA_103516 in IBD.**

We add this section to "Introduction" in page 6----

In this study, we focus on circRNA_103516, which is located at chr3:171969049-172028671 and spliced from *FNDC3B*. It was reported that *FNDC3B* might play a role in the epithelial-to-mesenchymal transition (EMT) and activates several cancer pathways, including phosphoinositide 3-kinase (PI3-kinase) /Akt, retinoblastoma 1 (Rb1) and Transforming Growth Factor (TGFβ) signalling[13]. Our bioinformatics analysis showed that hsa_circRNA_103516 is predicted to harbour hsa-miR-147b, hsa-miR-19b-1-5p, hsa-miR-134-3p, hsa-miR-576-5p, and hsa-miR-493-5p. Among them, miR-19b was found to be decreased in the serum and intestinal tissue of IBD patients[14]. Thus, in the present study, we explore the link between circRNA_103516 and hsa-miR-19b-1-5p in PBMCs from IBD patients and determine possible correlations with the clinical phenotypes of CD and UC.

2. Flow chart with clear inclusion / non-inclusion criteria is required to be reported.

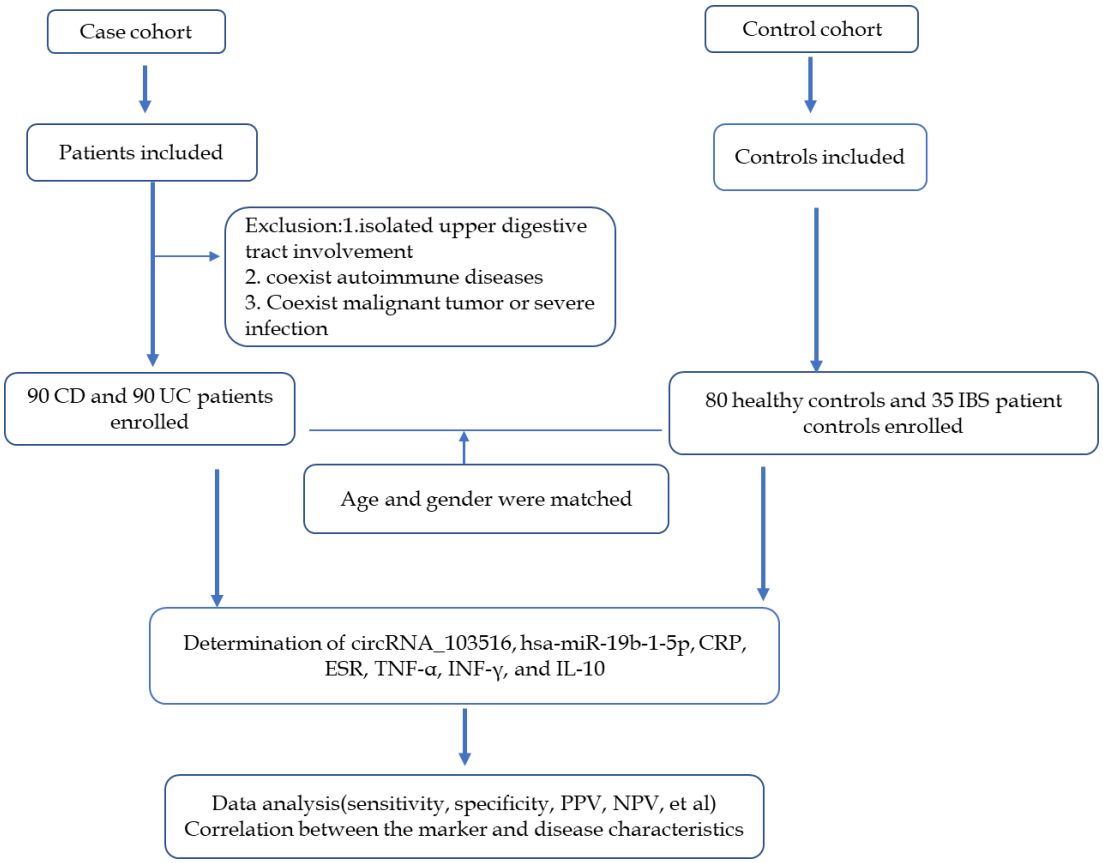

Figure1. Flow chart of patient selection in the study and main study procedures

3. Section Ethical declaration needs to be moved onto separate paragraph and it should be extended.

We add this section to" **Materials and methods**" ---showed in page 7.

Ethics statement

The present study was approved by the Ethics Committees of the Affiliated Suzhou Hospital of Nanjing Medical University (Jiangsu, China). All IBD patients and control subjects signed informed consent, in accordance with the relevant guidelines and regulations.

4. More information regarding study medication is required.

We supplemented the medication in Table 2, Table 5, Table 6. We now displayed some section as follows:

Table 2

Medications: n (%)		
5-ASA	80(88.9)	87(96.7)
Corticosteroids	50(11.7)	41(45.5)
Immunosuppressants	28(31.1)	14((15.6)

Anti- TNFα		13(14.4)		4(4.4)		
Surgery		4(4.4)		1(1.1)		

Table 5

Medications						
5-ASA	80	73.8%	1.309	0.340-5.035	0.696	NS
Corticosteroids	50	74.0%	2.329	0.959-5.655	0.062	NS
Immunosuppressants	28	67.9%	1.161	0.450-2.998	0.758	NS
Anti-TNFα	13	76.9%	1.905	0.483-7.505	0.357	NS
Surgery	4	75.0%	1.607	0.160-16.130	0.687	NS

Table 6.

Medications: n (%)						
5-ASA	87	57.5%	0.786	0.143-2.037	0.342	NS
Corticosteroids	41	70.7%	1.827	0.762-5.234	0.084	NS
Immunosuppressants	14	71.4%	1.234	0.673-3.238	0.705	NS
Anti-TNFα	4	50.0%	0.385	0.051-2.919	0.355	NS
Surgery	1	0.0%	0.000	0.000-	1.000	NS

5. Diagnostic accurancy, positive and negative predictive value, likelyhood ration should be calculated to discuss widely whether circRNA_103516 has to be a diagnostic marker.

We supplemented the diagnostic accurancy, positive and negative predictive value, likelyhood ration in Table 4. Please see the manuscript for details in page27.

Table4. Receiver-operating characteristic analysis of circRNA_103516 in peripheral blood mononuclear cells from inflammatory bowel disease

Groups	AUC (95%CI)	*P* value	Cutoff value	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	LR+	LR-	Diagnostic accurancy
CD VS HC	0.790(0.722-0.857)	<0.001	1.412	66.67(55.95%-76.26%)	78.75(68.17%-87.11%)	77.63%	67.02%	3.137	0.423	71.76%
UC VS HC	0.687(0.608-0.767)	<0.001	1.151	66.67(55.95%-76.26%)	62.50(50.96%-73.08%)	66.67%	62.50%	1.778	0.533	64.71%
CD VS UC	0.631(0.550-0.712)	0.002	1.963	54.44(43.6% - 64.98%)	68.89(58.26% to 78.23%)	55.05%	55.75%	1.750	0.661	59.41%

AUC: Area Under Curve; CD: Crohn's disease, UC: ulcerative colitis, HC: Healthy control, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio;

Reviewer #2:

it is a well written paper; however, a number of issues should be clarified:

1. **It is not clear on the Introduction section why the authors focused on circRNA_103516 and miR-19b-1-5p. They should give more information how they contribute to IBD**

We add this section to "Introduction" in page 6----

In this study, we focus on circRNA_103516, which is located at chr3:171969049-172028671 and spliced from *FNDC3B*. It was reported that *FNDC3B* might play a role in the epithelial-to-mesenchymal transition (EMT) and activates several cancer pathways, including phosphoinositide 3-kinase (PI3-kinase) /Akt, retinoblastoma 1 (Rb1) and Transforming Growth Factor (TGFβ) signalling[13]. Our bioinformatics analysis showed that hsa_circRNA_103516 is predicted to harbour hsa-miR-147b, hsa-miR-19b-1-5p, hsa-miR-134-3p, hsa-miR-576-5p, and hsa-miR-493-5p. Among them, miR-19b was found to be decreased in the serum and intestinal tissue of IBD patients[14]. Thus, in the present study, we explore the link between circRNA_103516 and hsa-miR-19b-1-5p in PBMCs from IBD patients and determine possible correlations with the clinical phenotypes of CD and UC.

2. It is not clear if the patients had active or inactive disease and if there is a difference at the expression levels between active and inactive

We displayed the expression level of circRNA_103516 in PMBC from IBD patients active or inactive(remission) stage in Figure4.

The expression level of circRNA_103516 was markedly increased, with values 2.614-fold and 1.953-fold higher in PBMCs from active CD and active UC, respectively ($P < 0.001$, $P < 0.001$, respectively). However, expression was only 1.639-fold and 1.319-fold higher in CD and UC patients who were in remission, respectively ($P < 0.001$, $P=0.024$) (**Figure 4A, B**), and circRNA_103516 levels in active CD and UC were higher than those in remittent CD and UC ($P=0.027$, $P=0.045$) (**Figure 4A, B**).

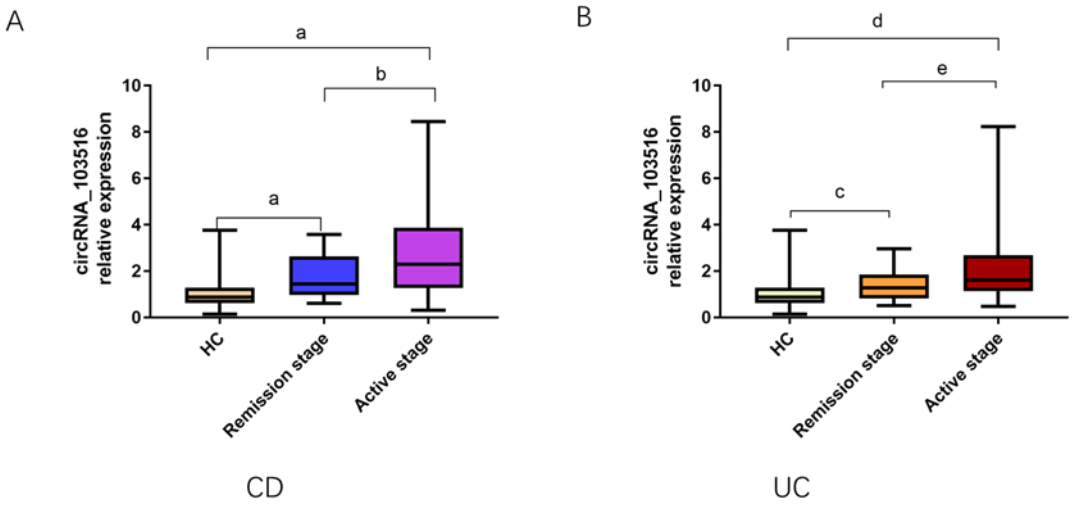

Figure4. The expression level of circRNA_103516 in peripheral blood mononuclear cells from inflammatory bowel disease patients at different stages. A, circRNA_103516 in CD patients at different stages. [a]$P < 0.001$ *vs* HC; [b]$P < 0.05$, Remission stage *vs* Active stage. B, circRNA_103516 in UC patients at different stage. [c]$P < 0.05$ *vs* HC and [d]$P < 0.001$ *vs* HC; [e]$P < 0.05$, Remission stage *vs* Active stage. $P < 0.05$ was considered statistically significant. CD: Crohn's disease, UC: ulcerative colitis.

3. It is not clear if the patients are naïve or under therapy since therapeutic approaches may influence the expression levels

We supplemented the medication in Table 2, Table 5, Table 6. We now displayed some section as follows:

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My manuscript language quality evaluation is B; Therefore, I have asked AJE website to revise and polish again, hoping to meet the requirements of reviewers. If experts have any questions, please contact me and I will revise again.