Responses to Reviewer's Comments

We are grateful to the reviewers for the constructive comments. We have made an attempt to revise the paper in accordance with the comments of the reviewers and feel that these revisions have greatly enhanced the quality of the manuscript.

Major added points

We performed several additional experiments to respond to the reviewer's request. The major points are follows: (a) we performed immunohistochemistry to show marked expression of IL-26 in patients with CD as compared to UC. (b) we performed immunohistochemistry of IL-26 receptors (IL-10R2 and IL-26R1) in the normal, active CD, inactive UC and in active CD in addition to active UC.

Reviewer #3 (Reviewer's code: 00503628)

1. The first figure demonstrates an elevated expression of IL-26 mRNA in biopsies of patients with active disease, which was significantly higher in patients with CD than UC. However, the results from figure 1 are not confirmed by the immunohistochemistry results (Figure 2A), which shows marked expression of IL-26 in the mucosa of UC patients.

   We agree with the reviewer's comment. We performed immunohistochemistry of IL-26 expression in patients with UC and CD. The number of IL-26 positive cells markedly increased in the inflamed mucosa of UC and CD patients as compared to in the normal mucosa. Moreover, the number of IL-26 positive cells more increased in the inflamed mucosa of CD patients as compared to in the inflamed mucosa of UC patients. This result confirmed the results from Figure 1. The results were shown in Figure 2A. These are escribed in result part (page 11, line 1).

2. The expression of IL-26 receptor (IL-20R1 and IL-10R2) in the normal, CD or inactive IBD.

   I appreciate the reviewer's suggestion. According to the suggestion, we performed the immunohistochemistry of IL-26 receptor (IL-20R1 and IL-10R2) expression in the normal mucosa, in the active mucosa from the CD patients, and in the inactive mucosa from CD and UC patients, in addition to in the active mucosa from UC patients. The results showed that colonic SEMFs (α-SMA positive cells) express the

IL-26 receptor in the normal mucosa, in the active/inactive mucosa of UC or CD patients. There was no difference in the expression level of IL-26 receptor in colonic SEMFs in all groups. These results were shown in Figure 3A. These are escribed in result part (page 11, line 14-16, 21-23).

3. The reason why SEMFs were focused in the expression of IL-26 receptor.

   As the reviewer suggested, SEMFs are minor population in the colon. However, as we described in the section of introduction, a number of studies revealed that human colonic SEMFs play important role in the pathogenesis in IBD. Moreover, there was no study about the function of IL-26 on SEMFs in the colon. Therefore, we focused on SEMFs as new target cells of IL-26.

Reviewer #4: (Reviewer's code: 03252972)

1. What is the reason of the increase of IL-26?

   As shown in Fig. 1, the expression of IL-26 is enhanced in the inflamed mucosa of UC and CD patients. Therefore, we preliminary investigated the induction of IL-26 in SEMFs using various kinds of inflammatory stimulators, such as IL-1β, Th1 cytokines, Th2 cytokines, and so on. However, the stimulation did not enhance the expression of IL-26 in SEMFs. We will perform more examination about the induction of IL-26 in SEMFs in the future as we speculated that some inflammatory stimulations are able to enhance the IL-26 expression in human colonic SEMFs.

2. Effect of inhibition of IL-26 or its pathway is not studied in the current paper.

   I appreciated the reviewer's comment.
   In the current study, we examined the effect of inhibitors of IL-26 signaling pathway in Figure 5.
   We found that IL-26 induced the expression of IL-6 and IL-8 by the activation of STAT1/3 and MAPKs/PI3K, leading to an activation of NF-κB and AP-1. Next, we showed that siRNA specific for STAT1 and STAT3 significantly suppressed IL-26-induced mRNA expression of IL-6 and IL-8. Moreover, we showed that the inhibitors of MAPKs and Akt also significantly suppressed IL-26-induced mRNA expression of IL-6 and IL-8. There results indicated that inhibition of IL-26

signaling pathway effectively suppressed the induction of inflammatory mediators by IL-26 in human colonic SEMFs.

3. What is the clinical importance or application of the findings of this study?

   I appreciated the reviewer's comments.
   In the current study, we showed that IL-26 was able to induce inflammatory mediators, IL-6 and IL-8, in human colonic SEMFs and that the expression of IL-26 was enhanced in IBD mucosa as compared to the normal mucosa. Therefore, we suggested that IL-26 contribute to the pathogenesis of inflammatory bowel disease by inducing the inflammatory mediators.
   For the clinical use, the investigation for the effect of IL-26 *in vivo* is required. However, as we mentioned in the introduction section, a murine IL-26 homologue has not been identified. Therefore, in the future, we will more precisely examine the effect of inhibitors of IL-26 or IL-26 signaling pathway *in vitro* using isolated cells from human samples in order to apply it to the clinical use.

We feel that the paper has been improved. We are grateful to the reviewer for these suggestions and for the time they devoted to reviewing the original manuscript.