Dr. Lian-Sheng Ma
Editorial Office Director, World Journal of Gastroenterology

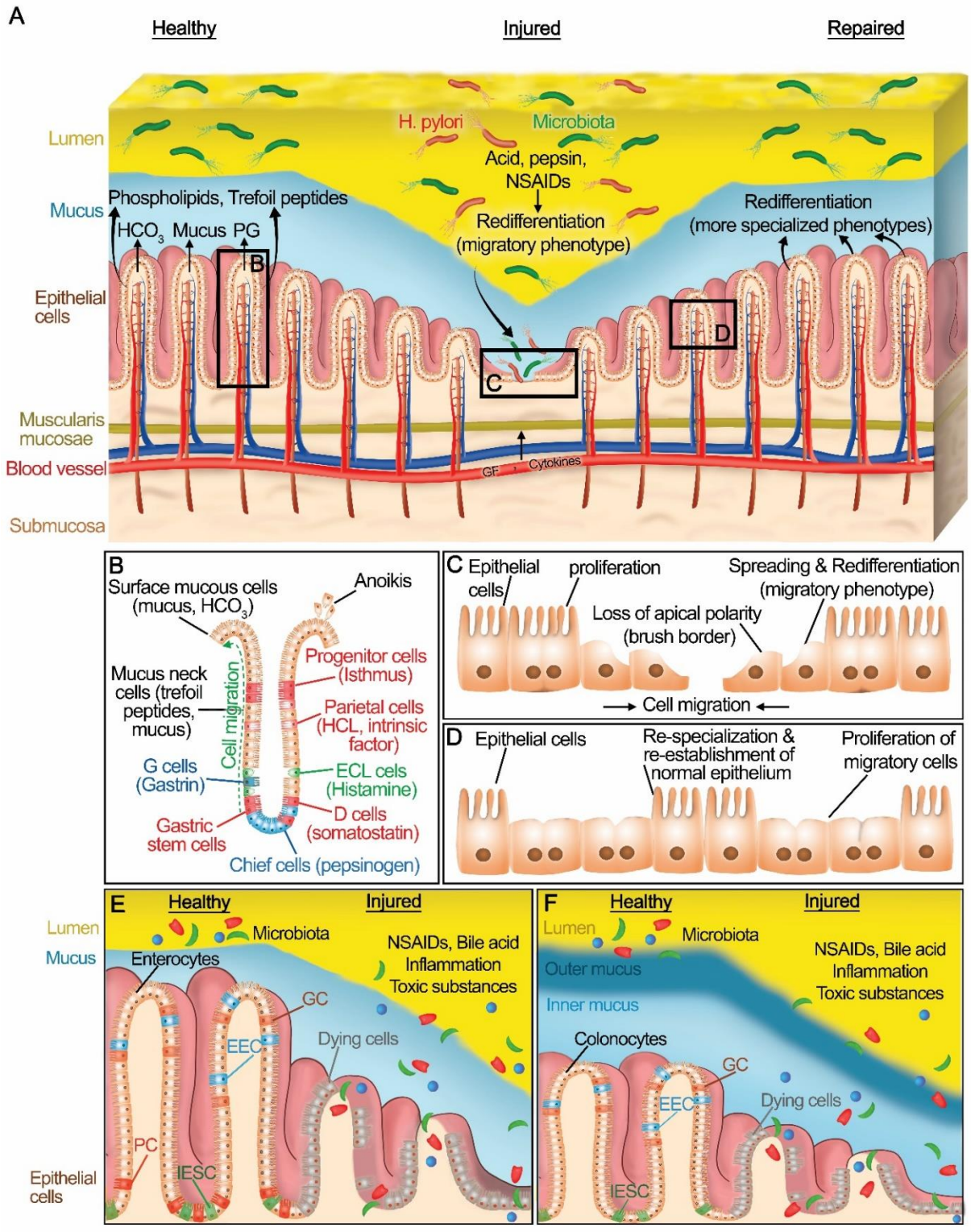
Dear Dr. Ma:

We thank you for giving us the opportunity to address the comments and concerns of the reviewers. We also thank the reviewers for their careful reading and valuable comments on the manuscript. Below, we address the comments of each reviewer in a point-by-point fashion. We have revised the manuscript to address their concerns and have highlighted green every change we made.

Comments from Reviewer 1

1. *In some parts of the review the facts and data are given as accepted truth representative for the whole gastro-intestinal system. The authors should make the attempt to give the data more precise to illustrate the loco-regional high diversity of molecular/ cellular wound-healing and the underlying mechanisms along the GI-tract.*

   Response
   We have updated Figure 1 by changing the Fig 1 title to "Normal gastrointestinal homeostasis, injury, and healing", changing the Fig 1A description to "Structure of gastric epithelium in healthy, injured, and repaired states" and adding diagrams that apply to the small intestine and large intestine in new components of figure 1. Also, we have distinguished "the gut" by either stating "upper GI vs lower GI" or "stomach, small intestine or large intestine/colon" (highlighted green) throughout the manuscript.


A
Healthy
Injured
Repaired
Lumen
Mucus
Epithelial cells
Muscularis mucosae
Blood vessel
Submucosa
H. pylori
Microbiota
Acid, pepsin, NSAIDs
Redifferentiation (migratory phenotype)
Phospholipids, Trefoil peptides
HCO3 Mucus PG
Redifferentiation (more specialized phenotypes)
B
C
D
GF
Cytokines
B
Surface mucous cells (mucus, HCO3)
Anoikis
Mucus neck cells (trefoil peptides, mucus)
Cell migration
Progenitor cells (Isthmus)
Parietal cells (HCL, intrinsic factor)
G cells (Gastrin)
ECL cels (Histamine)
D cells (somatostatin)
Gastric stem cells
Chief cells (pepsinogen)
C
Epithelial cells
proliferation
Spreading & Redifferentiation (migratory phenotype)
Loss of apical polarity (brush border)
Cell migration
D
Epithelial cells
Re-specialization & re-establishment of normal epithelium
Proliferation of migratory cells
E
Healthy
Injured
Lumen
Mucus
Epithelial cells
Microbiota
Enterocytes
NSAIDs, Bile acid
Inflammation
Toxic substances
GC
EEC
Dying cells
PC
IESC
F
Healthy
Injured
Lumen
Microbiota
Outer mucus
Inner mucus
NSAIDs, Bile acid
Inflammation
Toxic substances
Colonocytes
GC
EEC
Dying cells
IESC

(Starts from page 6, paragraph 1, line 1 in the manuscript)

Figure 1 Normal gastrointestinal homeostasis, injury, and healing.

A) Structure of gastric epithelium in healthy, injured, and repaired states. A healthy gastric barrier is essential to maintain gastric homeostasis. In a healthy state, there is an equilibrium between gastric injury and mucosal healing. An excess of destructive factors such as acid, pepsin, NSAIDs, and *H. pylori* leads to gastric barrier disruption. These noxious agents then diffuse deeper into the mucosa and create wounds. Epithelial cells at the edge of the injury redifferentiate to a migratory phenotype and collectively migrate as a sheet to close the wound. After successful restitution, the migrated cells redifferentiate to more specialized phenotypes. (HCO_3: bicarbonate, *H. pylori*: Helicobacter pylori, PG: prostaglandins, NSAIDs: Nonsteroidal anti-inflammatory drugs)

B) A diagram depicting the structure and cell types of gastric epithelium. (ECL cells: Enterochromaffin-like cells, HCO_3: bicarbonate)

C) In the injured state, epithelial cells at the edge of the wound spread and redifferentiate to a migratory phenotype, losing their classical apical brush border and assuming a more squamous morphology. Then, they migrate as a sheet to cover the injured area, with cells at the front of the migrating sheet transmitting traction forces to cells farther back via cell-cell contacts. Epithelial cells behind these migrating cells subsequently proliferate to provide more cells to fully cover larger wounds.

D) Cells that have migrated across the defect may themselves then proliferate once the barrier has been reformed. In addition, following migration and proliferation, the migrated cells redifferentiate back to more specialized phenotypes.

E) Structure of small intestinal epithelium in healthy and injured states. (PC: Paneth cells, IESC: Intestinal epithelial stem cells, EEC: Enteroendocrine cells, GC: Goblet cells, NSAIDs: Nonsteroidal anti-inflammatory drugs)

F) Structure of large intestinal epithelium in healthy and injured states. A healthy intestinal barrier is essential to maintain intestinal homeostasis. In the healthy state, there is an equilibrium between intestinal injury and mucosal healing. An excess of destructive factors such as NSAIDs, inflammation, bile acid, and toxic luminal substances leads to

intestinal barrier disruption. These noxious agents then diffuse deeper into the mucosa and create wounds. Epithelial cells at the edge of the injury follow the processes described in the figure legends for in Fig 1C and Fig 1D. (IESC: Intestinal epithelial stem cells, EEC: Enteroendocrine cells, GC: Goblet cells, NSAIDs: Nonsteroidal anti-inflammatory drugs)

We have inserted more information (highlighted green) into the "Drivers of mucosal injury" section that now addresses this issue. Our text now states:

(Starts from page 11, paragraph 1, line 1 in the manuscript)

NSAIDs injure the upper GI mucosa mainly by cyclooxygenase (COX)-1 inhibition, resulting in a decrease in prostaglandins, mucus, and bicarbonate secretion. Moreover, NSAIDs also alter another important component of mucosal defense, the gastric microcirculatory system. Upon irritation, the gastric mucosa normally increases blood flow to remove any toxins, bacterial products, or back-diffusing acid. Impairment of this hyperemic reaction increases the vulnerability of gastric mucosa to damage[38]. Inhibition of prostaglandins, potent vasodilators, by NSAIDs leads to an increase in vascular tone and thus reduces gastric mucosal blood flow[39], consequently, increases ischemic tissue damage and exacerbating the mucosal injury[40]. NSAIDs may also induce local gastric mucosal injuries independent of prostaglandin deficiency[41]. NSAIDs may lyse phospholipids from mucosal epithelial cells and may increase mucosal permeability, which then allows mucosal exposure to luminal aggressive factors such as bacteria and gastric acid[42].

The molecular and cellular mechanisms of NSAID-induced lower GI mucosa are clearly distinct from NSAID-induced upper GI injuries[42,43]. As in the stomach, NSAIDs may inhibit COX-1 and contribute to mucosal damage. However, unlike gastric injury, the bile acid and intestinal microbiota play a crucial role in the pathophysiology of NSAID-induced intestinal injury[42,44]. NSAIDs and gut microbiota have complex and dynamic interactions. The gut microbiota can alter the efficacy and toxicity of NSAIDs either directly by biotransforming them into metabolites or indirectly by altering the host

metabolism (e.g., interfering with hepatic function)[45]. On the other hand, NSAIDs themselves can directly change the composition and function of the gut microbiota or indirectly by altering the physiological functions of the host[45]. For instance, NSAIDs alter the intestinal microbiome by increasing the total number of bacteria and the proportion of gram-negative bacteria, which seems to be linked to the activation of toll-like receptor (TLR) 4 that increases inflammation and contributes to an intestinal injury [46–48].

NSAIDs make complexes with bile acids by glucuronidation in the liver. This interaction alters the stability and structure of bile acids and potentiates bile acid toxicity in the lower GI tract[42]. These NSAID-bile acid complexes are secreted into the duodenum and subsequently reabsorbed back in the ileum via the enterohepatic circulation. Within the intestinal lumen, particularly, in the colon, conjugated primary bile acids are deconjugated into more toxic secondary bile acids, mainly by the gram-positive bacteria[49]. There is cross-talk between the microbiome and the bile acids because bile acids can control the composition of the intestinal microbiome, which in turn regulates the composition and size of the bile acid pool[50,51]. Alteration in the colonic microbiota may cause a shift towards to generation of more toxic secondary bile acids, which eventually increase intestinal permeability, particularly in the colon, bacterial translocation, and mucosal inflammation[52–54].

2. *NSAIDs are discussed as important substances for mucosal injury. In a short paragraph the morphological overlap of NSAID induced injuries with ischemic triggered tissue damage should be addressed and loco-regional differences of molecular mechanisms should be introduced to the reader.*

Response

We have inserted more information (highlighted green) into the "Drivers of mucosal injury" section that now addresses this issue. Our text now states:

(Starts from page 11, paragraph 1, line 1 in the manuscript)

NSAIDs injure the upper GI mucosa mainly by cyclooxygenase (COX)-1 inhibition, resulting in a decrease in prostaglandins, mucus, and bicarbonate secretion. Moreover,

NSAIDs also alter another important component of mucosal defense, the gastric microcirculatory system. Upon irritation, the gastric mucosa normally increases blood flow to remove any toxins, bacterial products, or back-diffusing acid. Impairment of this hyperemic reaction increases the vulnerability of gastric mucosa to damage[38]. Inhibition of prostaglandins, potent vasodilators, by NSAIDs leads to an increase in vascular tone and thus reduces gastric mucosal blood flow[39], consequently, increases ischemic tissue damage and exacerbating the mucosal injury[40]. NSAIDs may also induce local gastric mucosal injuries independent of prostaglandin deficiency[41]. NSAIDs may lyse phospholipids from mucosal epithelial cells and may increase mucosal permeability, which then allows mucosal exposure to luminal aggressive factors such as bacteria and gastric acid[42].

The molecular and cellular mechanisms of NSAID-induced lower GI mucosa are clearly distinct from NSAID-induced upper GI injuries[42,43]. As in the stomach, NSAIDs may inhibit COX-1 and contribute to mucosal damage. However, unlike gastric injury, the bile acid and intestinal microbiota play a crucial role in the pathophysiology of NSAID-induced intestinal injury[42,44]. NSAIDs and gut microbiota have complex and dynamic interactions. The gut microbiota can alter the efficacy and toxicity of NSAIDs either directly by biotransforming them into metabolites or indirectly by altering the host metabolism (e.g., interfering with hepatic function)[45]. On the other hand, NSAIDs themselves can directly change the composition and function of the gut microbiota or indirectly by altering the physiological functions of the host[45]. For instance, NSAIDs alter the intestinal microbiome by increasing the total number of bacteria and the proportion of gram-negative bacteria, which seems to be linked to the activation of toll-like receptor (TLR) 4 that increases inflammation and contributes to an intestinal injury [46–48].

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circulation. Within the intestinal lumen, particularly, in the colon, conjugated primary bile acids are deconjugated into more toxic secondary bile acids, mainly by the gram-positive bacteria[49]. There is cross-talk between the microbiome and the bile acids because bile acids can control the composition of the intestinal microbiome, which in turn regulates the composition and size of the bile acid pool[50,51]. Alteration in the colonic microbiota may cause a shift towards to generation of more toxic secondary bile acids, which eventually increase intestinal permeability, particularly in the colon, bacterial translocation, and mucosal inflammation[52–54].

3. *Bile acids are addressed as molecules with tissue damage capacity. Additional information is necessary concerning the heterogeneity of bile acids and their divers tissue effects in the GI-tract.*

Response

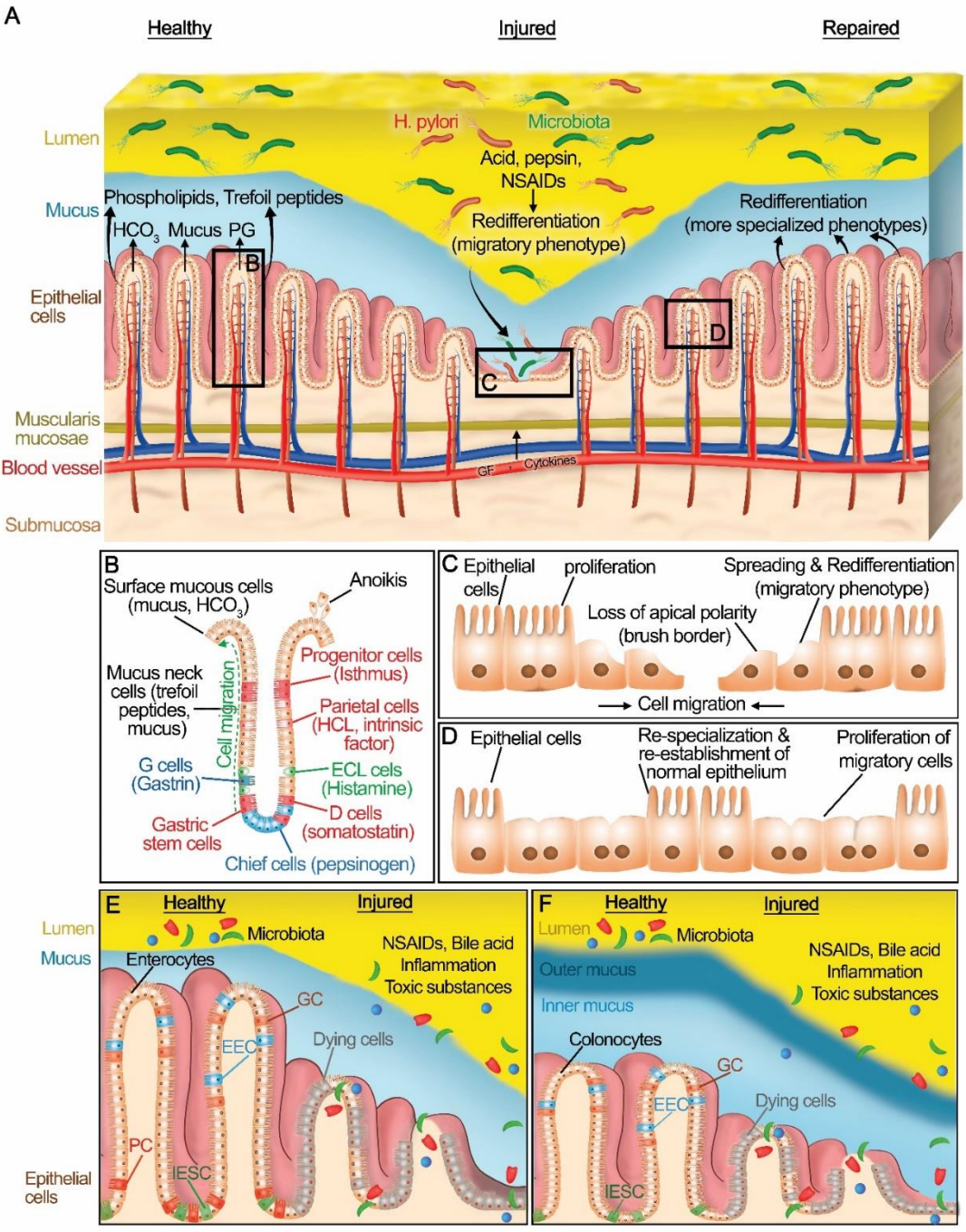
We have inserted more information (highlighted green) into the "Drivers of mucosal injury" section that now addresses this issue. Our text now states:

(Starts from page 12, paragraph 1, line 1 in the manuscript)

NSAIDs make complexes with bile acids by glucuronidation in the liver. This interaction alters the stability and structure of bile acids and potentiates bile acid toxicity in the lower GI tract[42]. These NSAID-bile acid complexes are secreted into the duodenum and subsequently reabsorbed back in the ileum via the enterohepatic circulation. Within the intestinal lumen, particularly, in the colon, conjugated primary bile acids are deconjugated into more toxic secondary bile acids, mainly by the gram-positive bacteria[49]. There is cross-talk between the microbiome and the bile acids because bile acids can control the composition of the intestinal microbiome, which in turn regulates the composition and size of the bile acid pool[50,51]. Alteration in the colonic microbiota may cause a shift towards to generation of more toxic secondary bile acids, which eventually increase intestinal permeability, particularly in the colon, bacterial translocation, and mucosal inflammation[52–54].

4. *Figure 1: the scheme addresses mucosal healing in the stomach. The information "gut" is misleading. Alternative an additional scheme is necessary addressing mucosal healing in the small/ large gut.*

Response



(Starts from page 6, paragraph 1, line 1)

Figure 1 Normal gastrointestinal homeostasis, injury, and healing.

A) Structure of gastric epithelium in healthy, injured, and repaired states. A healthy gastric barrier is essential to maintain gastric homeostasis. In a healthy state, there is an equilibrium between gastric injury and mucosal healing. An excess of destructive factors such as acid, pepsin, NSAIDs, and *H. pylori* leads to gastric barrier disruption. These noxious agents then diffuse deeper into the mucosa and create wounds. Epithelial cells at the edge of the injury redifferentiate to a migratory phenotype and collectively migrate as a sheet to close the wound. After successful restitution, the migrated cells redifferentiate to more specialized phenotypes. (HCO_3: bicarbonate, *H. pylori*: Helicobacter pylori, PG: prostaglandins, NSAIDs: Nonsteroidal anti-inflammatory drugs)

B) A diagram depicting the structure and cell types of gastric epithelium. (ECL cells: Enterochromaffin-like cells, HCO_3: bicarbonate)

C) In the injured state, epithelial cells at the edge of the wound spread and redifferentiate to a migratory phenotype, losing their classical apical brush border and assuming a more squamous morphology. Then, they migrate as a sheet to cover the injured area, with cells at the front of the migrating sheet transmitting traction forces to cells farther back via cell-cell contacts. Epithelial cells behind these migrating cells subsequently proliferate to provide more cells to fully cover larger wounds.

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F) Structure of large intestinal epithelium in healthy and injured states. A healthy intestinal barrier is essential to maintain intestinal homeostasis. In the healthy state, there is an equilibrium between intestinal injury and mucosal healing. An excess of destructive factors such as NSAIDs, inflammation, bile acid, and toxic luminal substances leads to intestinal barrier disruption. These noxious agents then diffuse deeper into the mucosa

and create wounds. Epithelial cells at the edge of the injury follow the processes described in the figure legends for in Fig 1C and Fig 1D. (IESC: Intestinal epithelial stem cells, EEC: Enteroendocrine cells, GC: Goblet cells, NSAIDs: Nonsteroidal anti-inflammatory drugs)

Comments from Reviewer 2

1. *The title of the article does not fully correspond to the content of the article. instead of "Gut" I recommend to use "gastrointestinal mucosa".*

   Response
   We agree with the reviewer. We have changed the title to "Gastrointestinal mucosal homeostasis, injury, and healing: new therapeutic targets".

2. *The first 10 pages describe in great detail the physiological and pathophysiological mechanisms of damage and protection of gastrointestinal mucosa, which are well known and do not require such detail, it is better to shorten this part and add more information about the molecular mechanisms.*

   Response
   According to the reviewer's suggestion, we have shortened the physiological and pathophysiological mechanisms of damage and protection of gastrointestinal mucosa (first ten pages) by about 20%. However, the science editor and reviewer #1 wanted us to explain loco-regional differences of molecular mechanism in NSAID-induced gastrointestinal injuries and healing, and heterogeneity of bile acids and their diverse tissue effects in the GI tract, therefore, the final length of the manuscript did not change much.

3. *It is recommended to elaborate more your reasoning about this problem. "However, if the wound extends into deeper layers such as the submucosa and muscularis, these must also be reconstructed for healing by processes beyond the scope of this review" - p.14, it is suggested to expand the review, or highlight that it is mainly about superficial defects of the gastrointestinal mucosa.*

   Response
   We have inserted an explanation (highlighted green) into the "Introduction" section to clarify the scope of this review that now addresses this issue. Our text now states:

(Starts from page 4, paragraph 1, line 6 in the manuscript)

This review focuses on mucosal injury and repair. Deeper injuries such as a deep

ulcer, trauma, fistula, or surgical transection and anastomotic healing all require a complex interaction among endothelial cells, fibroblasts, and other cell types to reconstitute the submucosal and muscular layers of the bowel wall. This is beyond the scope of the current review but has been previously reviewed[1–5]. Angiogenesis is critical to these efforts, and requires a complex interaction between endothelial cells, the extracellular matrix, growth factors and cytokines, and other cell types[6,7].

4. *It is necessary to update the literature review with recent studies of up to last 5 years.*

   Response

   According to the reviewer's suggestion, we have searched the most recent literature (last 5 years) and updated our references throughout the manuscript. Now, approximately 1/3 of the references are from the last 5 years.

Comments from Reviewer 3

1. *On page 12: "Although some authors describe the initial steps of this process as dedifferentiation, it is the firm opinion of the senior author that this should rather be considered a redifferentiation toward a migratory phenotype". This sentence has to be supported by data that go beyond pure morphology.*

   Response
   We agree with the reviewer. We have inserted a paragraph (highlighted green) into the "Mucosal healing processes" section to explain the idea of "restitution requires a migratory phenotypic redifferentiation" that now addresses this issue. Our text now states:

(Starts from page 14, paragraph 2, line 1 in the manuscript)

Restitution requires a phenotypic redifferentiation. Although some authors describe the initial steps of this process as dedifferentiation, it is the firm opinion of the senior author that this should rather be considered a redifferentiation toward a migratory phenotype. The gut epithelium normally consists of a monostratified layer of differentiated epithelial cells. At the edge of a mucosal wound, epithelial cells change their phenotype from differentiated columnar enterocytes or gastric cells to a migratory

phenotype. They lose their typical morphology and (for enterocytes and parietal cells) their microvilli[85], disassemble their apical specialized membrane components[86], flatten out and extend lamellipodia toward the defect. Such migrating cells adopt a squamous morphology with altered integrin[87–89] and cytoskeletal organization[73,85] and specialized cell signaling pathways[90–93] that adapt these cells toward motility(Figure 1C)[73,85,94–96] Moreover, it is worth noting that these signaling events are not only regulated by the activation of signaling proteins but also by the distribution and the amount of the signaling proteins within the migrating cells. For instance, both the actual amount of total FAK and the amount of active FAK decrease while the ratio of activated to total FAK increases both in vitro[94] and in vivo[92] as the epithelial cells shift to the migratory phenotype[73]. Similarly, both paxillin protein and tyrosine-phosphorylated paxillin decrease in migrating cells compare to static cells[94]. (Paxillin is an adapter protein critical to focal adhesion complex assembly and disassembly in response to various stimuli.)[97-100]. Total p38, ERK1, and ERK2 proteins do not show differences between migrating and static cells[94]. However, phosphorylated p38 increases, and phosphorylated ERK1 and ERK2 decreases in motile cells compared with nonmigrating cells[94].

Furthermore, the distribution of these signaling proteins also changes in migratory phenotype. In confluent cells, FAK localizes mainly in a perinuclear pattern while FAK appears explicitly at the cell borders contacting other cells in motile cells, with FAK immunoreactivity decreasing toward the migrating lamellipodia that face the wound edge[94]. In contrast to FAK, paxillin is localized at the lamellipodial edges in migrating cells[94]. The difference is more than semantic because considering these migratory cells as a specialized phenotype opens up the possibility for therapy to modulate that phenotype and thereby promote mucosal healing.

2. *The sentence on page 17 "A specific TFF receptor has not been described. Rather, the TFFs mediate epithelial restitution via the EGFR[146,147], CXC chemokine receptors (CXCR)[148,149], or other receptors." should be further detailed, since TFFs are identified as possible therapeutic agents.*

Response

We agree with the reviewer and have inserted a paragraph (highlighted green) into the "Regulators of mucosal healing and potential new therapeutic targets" section that now addresses this issue. Our text now states:

(Starts from page 19, paragraph 3, line 1 in the manuscript)

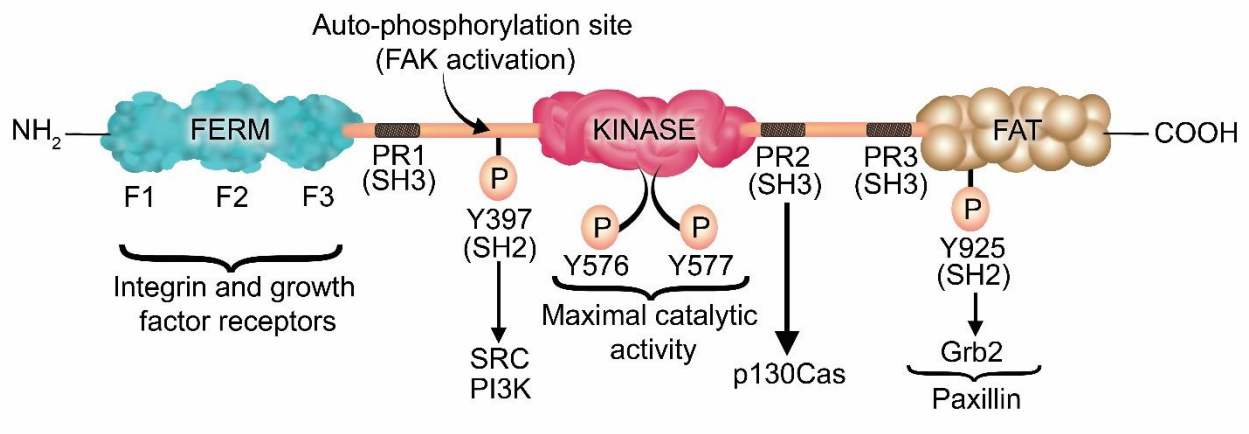
The TFFs are mostly distributed to the basolateral domain of gastric neck cells and parietal cells in the stomach, the Paneth cells in the small intestine, and the crypt cells in the colon[175]. TFF interactions and specific functions have been discussed in detail in a recent review[176]. A specific TFF receptor has not yet been described. However, some binding and functional studies propose potential TFF receptors that may influence epithelial restitution. TFFs have been reported to bind to transmembrane proteins such as the β1 integrin subunit, CRP-ductin, CXC chemokine receptor (CXCR) 4, CXCR7, proteinase-activated receptor (PAR) 2, PAR4, leucine-rich repeat and Immunoglobin-like domain-containing protein (LINGO) 2, LINGO3, and EGFR[177–181]. TFF3 enhances wound healing by activating EGFR and inducing MAPK[182] and PI3K/Akt signaling pathways *in vitro*[183] whereas TFF2 directly activates CXCR4 and enhances the phosphorylation of ERK1/2 and Akt in gastric epithelial cells[184]. Indeed, the CXCR4 antagonist AMD3100 blocks TFF2-dependent gastric epithelial repair[170]. TFFs, specifically TFF2 and TFF3, regulate epithelial motility via integrin-binding and activating focal adhesion kinase as well[175]. TFF2 also promotes cell migration via PAR4[185], while TFF3 activates PAR2[186]. Furthermore, TFF2 peptide may be required for optimum activity of EGFR and/or EGF signaling in the stomach because heparin-binding EGF and TGF-α do not induce EGFR activation in the stomachs of *Tff2* KO mice[177].

3. *Figure 3 should also include a panel showing the passages involved in FAK activation.*

Response

We agree with the reviewer. We have added a panel that shows FAK activation and another panel for maximal catalytic activity in Figure 3.

(Starts from page 29, paragraph 1, line 1 in the manuscript)



4. *A figure summarizing all the more promising new therapeutic approaches should be added.*

Response

According to the reviewer's suggestion, we have added a new figure. Figure 5 shows current and promising new therapeutic approaches.

(Starts from page 33, paragraph 1, line 1 in the manuscript)

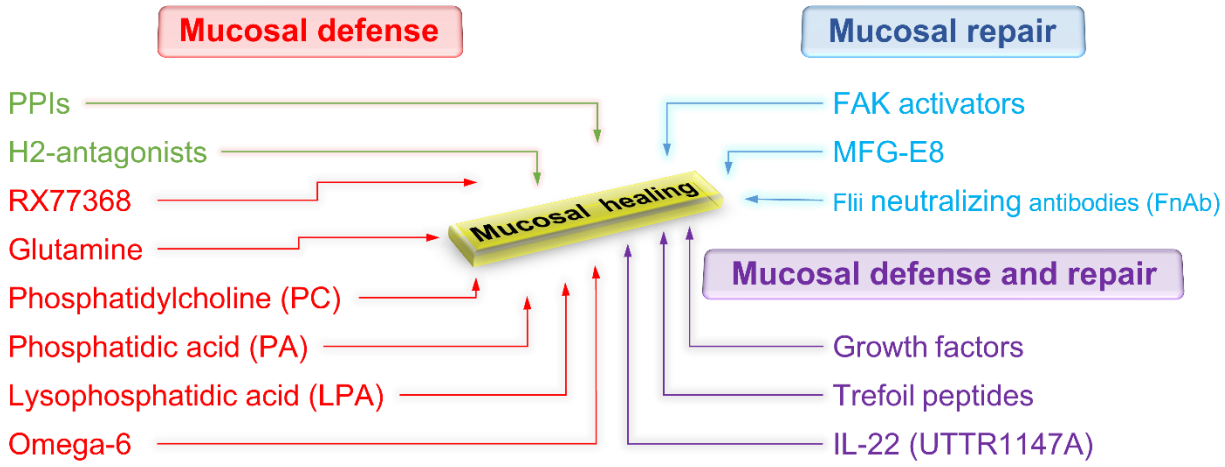


Figure 5 Current and promising new therapeutic approaches to gastrointestinal mucosal healing. Green represents currently available drugs. Red represents promising new therapeutic approaches that increase mucosal defense. Blue represents promising new therapeutic approaches that promote mucosal repair. Purple represents promising new therapeutic approaches that stimulate both mucosal defense and repair. PPIs: Proton pump inhibitors, H2-antagonists: Histamine-2 receptor antagonists, RX77368: The thyrotropin-releasing hormone analog, SCFAs: Short-chain fatty acids, FAK: Focal adhesion kinase, MFG-E8: Milk fat globule-epidermal growth factor 8, Flii: Flightless I.

5. *The sentence:" Thus, even though PPIs are still recommended to treat upper GI ulcers, their prophylactic use with NSAIDs to prevent upper GI injury is no longer recommended[42]." Should be modified, since guidelines recommend the use of PPI in patients with risk of peptic ulcer disease (DOI: 10.1136/gutjnl-2019-319300 and FDA guidelines).*

Response

According to the reviewer's suggestion, we have modified the mentioned sentence and highlighted green in the manuscript that now addresses this issue. Our text now states:

(Starts from page 12, paragraph 2, line 9 in the manuscript)

Thus, even though PPIs are still recommended to treat upper GI ulcers, their prophylactic use with NSAIDs to prevent upper GI injury is no longer recommended unless the patient has a moderate to high risk of peptic ulcer disease[55,56].

In summary, we believe that the manuscript has been greatly improved by the opportunity to revise it in response to the reviewers' critiques. We would again like to thank the reviewers for their careful reading and helpful suggestions and hope that the manuscript may now be considered for publication in *World Journal of Gastroenterology*. Please note that although the decision letter states that we should not have more than 3 references per journal, this is simply not realistic for this sort of comprehensive review with a very long reference list. We surmise that this instruction is intended to apply to research articles with much shorter reference lists, in which one journal should not be permitted to dominate the

reference list. That is not true here, and so we have left the reference list intact. We would welcome further guidance in this regard.

Best wishes,

Marc D. Basson, MD, PhD