

## Peer Review File

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### Reviewer A:

The paper entitled " Histone acylations as a mechanism for regulation of intestinal epithelial cells " is well written. It covers an important scientific subject. My suggestions to improve the manuscript are as follows:

Reply: We thank this reviewer for the constructive and insightful suggestions that improved the manuscript.

The subject needs to be comprehensively discussed. For example, the role of gut microbiota and the immune system in this matter is neglected.

Reply: We agreed with the reviewer and improved the sections about the role of gut microbiota and the immune system.

Different epigenetic changes as methylation, propylation, butyrylation, et., are suggested to be discussed more detailed.

Reply: We agreed with the reviewer and improved the discussion about different epigenetic changes. An additional figure was made to illustrate the various lysine acylations (Figure 1).

The number of figures needs to be more, and there is no table.

Reply: We add 3 more figures to the review.

The references with 2022 and 2023 are just a few. New findings in this field need to be covered.

Reply: We have integrated more recent articles into our text, as suggested by reviewer 1.

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### Reviewer B:

In this review manuscript, authors highlighted histone acylation by acetylation, crotonylation, butyrylation, beta-hydroxybutyration modulating intestinal epithelial proliferation and differentiation, adaptation to diet and microbiota stimuli, preventing inflammation and maintaining circadian clock. The involvement of short chain fatty acids, especially acetic acid, propionic acid and butyric acid, the gut microbiota fermented products of non-digested fiber, in activating histone acylase and inhibiting histone deacylase were also discussed. The Figure summarized the findings related to histone acylation and deacylation as epigenetic mechanisms that regulate intestinal epithelial homeostasis pretty well. The manuscript was well written.

Reply: We thank this reviewer for revising our manuscript.

Only two minor errors need correction;

In the Abstract (Page 2), the sentence "...that regulate cell transcription" in line #25 should be changed as "...that regulate gene transcription";

In page 4, the sentence "...that difficult the access of transcription factors" in line# #67 need re-written.

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Reply: We changed the sentence in line #25 to "...that regulate gene transcription", as recommended. The sentence "...that difficult the access of transcription factors" in page 4, line #67 was also changed to "[...] that difficult the binding of transcription factors to DNA [...]".

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**Reviewer C:**

This review is fine and I think the focus on histone acylations and intestinal epithelial cells is ok. The figure is nice, too. They may consider one additional figure (given the focus on acylations) that illustrates the various lysine acylations, so a nonspecialist can see, e.g. what is acetylation, propionylation, what is the difference between crotonylation and butyrylation etc.

Reply: We thank this reviewer for the constructive and insightful suggestions that improved the manuscript. An additional figure was made to illustrate the various lysine acylations (Figure 1).

The authors may consider strengthening integration and conceptional aspects, such as the idea that SCFA-HDACs-histone modifications-gene expression axis may function as a rheostat to sense microbiota in the gut.

Reply: We agreed with the reviewer and improved the conceptual aspects appointed. We summarized it in figure 4.

A weakness of this review is the almost absence of the discussion of readers of histone acylations (proteins with bromodomains, YEATS-domain etc), given that these are likely affectors of these modifications. This could also be summarised in an additional figure.

Reply: We agreed with the reviewer and improved the discussion of readers of histone acylations. We summarized it in figure 2.

Given the topic, they may consider integration of this very recent paper:

Intestinal epithelial HDAC3 and MHC class II coordinate microbiota-specific immunity.

Eshleman EM, Shao TY, Woo V, Rice T, Engleman L, Didriksen BJ, Whitt J, Haslam DB, Way SS, Alenghat T.J Clin Invest. 2023 Feb 15;133(4):e162190. doi: 10.1172/JCI162190.

PMID: 36602872

And maybe also these two:

Lactic Acid-Producing Probiotic *Saccharomyces cerevisiae* Attenuates Ulcerative Colitis via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota.

Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, He Q, Liang H, Xin X, Wang L, Lou C, Cao X, Chen X, Li B, Wang B, Zhao J. *Front Immunol.* 2021 Nov 24;12:777665. doi: 10.3389/fimmu.2021.777665. eCollection 2021. PMID: 34899735

Epigenetic master regulators HDAC1 and HDAC5 control pathobiont Enterobacteria colonization in ileal mucosa of Crohn's disease patients.

Chervy M, Sivignon A, Dambrine F, Buisson A, Sauvanet P, Godfraind C, Allez M, Le Bourhis L, The Remind Group, Barnich N, Denizot J.

*Gut Microbes.* 2022

Reply: Thanks for the suggestions. We have integrated more recent articles into our text, including two of those suggested by reviewer 3. The article “Lactic Acid-Producing Probiotic *Saccharomyces cerevisiae* Attenuates Ulcerative Colitis via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota” was not integrated because it explores the epigenetic profile of macrophages, deviating from the theme of our review on intestinal epithelial cells.

When they describe research showing that class I HDACs are also decrotonylases and debutyrylases, they also should cite the Fellows et al. *Nat Com* 2018 paper in this context (their reference Nr 60).

Reply: We apologize for the absence of Fellows et al. (2018) reference. We have revised our citations and added Fellows' paper in this context.

Other comments:

Page 4, line 67: This statement seems out of place, transcription factors do not necessarily bind histones, rather DNA.

Main body' should not be a subheading. Please, find something more meaningful.

Page 7, line 153: I do not understand this sentence, as those below.

Page 7, Line 157: Strategies that affect its on ISCs impact on their proliferation: loss of MPC increases proliferation which the opposite response is observed in cells overexpressing it (49).

on: Its what? I do not understand this...

Page 8, Line 162 Collectively, these reports show the importance of epigenetic mechanisms for the development and maintenance of epithelium.

It seems like so far the focus was on histone acetylation, so better say: “..the importance of histone acetylation...”

Page 8, line 181: Collectively, these reports show the importance of epigenetic mechanisms for the development and maintenance of epithelium.

It seems like so far the focus was on histone acetylation, so better say: “..the importance of histone acetylation...”

Page 11, line 248: define 'Zeitgeber time'.

Last sentence of review should be reformulated, I guess it means 'specificity of function of each acylation' or similar.

I made additional corrections and suggestions directly in the text, with track changes.

Reply: We improved our manuscript according to the corrections and suggestions listed above.

For the comment on page 4, line 67: we modified the sentence to "[...] that difficult the binding of transcription factors to DNA [...]"

We removed 'Main body' as a subheading.

For the comment on page 7, line 153: we modified the sentence to "[...] and seems to be regulated by histone acetylation. [...]"

For the comment on page 8, line 157: we modified the sentence to "[...] Strategies that affect its expression on ISCs, also impact cell proliferation. The loss of MPC increases proliferation, while the opposite response is observed in cells overexpressing it (46). [...]"

For the comment on page 8, line 162: we changed the term "epigenetic mechanisms" to "histone acetylation".

For the comment on page 8, line 181: we added the references suggested for this statement.

For the comment on page 11, line 248: we defined 'Zeitgeber time' as "[...] a representation of the diurnal cycle (ZT0 for lights on and ZT12 for lights off) [...]"

We reformulated the last sentence of the review as reviewer 3 suggested.