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Temporal clinical, proteomic, histological and cellular immune responses of DSS-induced acute colitis

Nunes NS *et al*. Temporal analysis of DSS acute colitis

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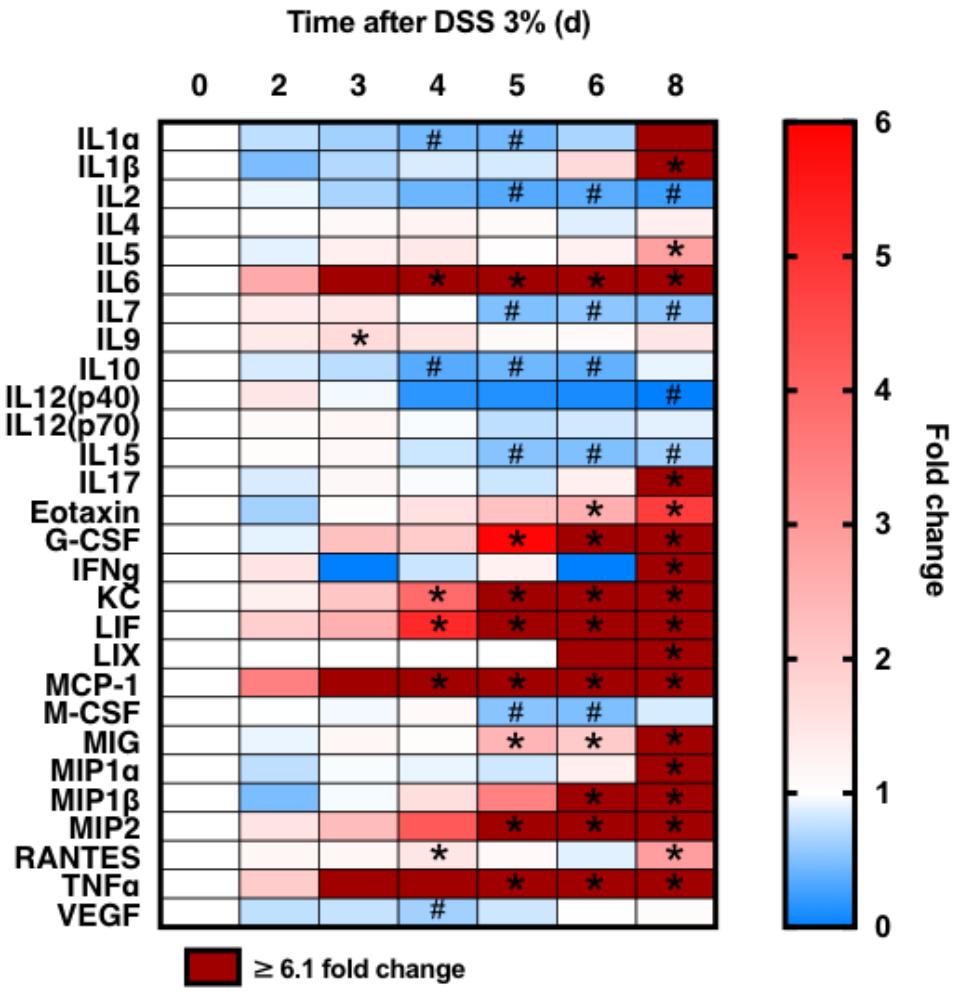
Answering Reviewers

Reviewer #1

We thank reviewer #1 for the comments and we have modified the manuscript and legends accordingly.

1) Due to the small sample size and large variations of data, the authors are highly suggested to conduct non-parametric statistical analysis and update the results and discussion sections accordingly. I guess this will change some of their findings dramatically.

   We appreciate the reviewer's suggestion and we have performed the non-parametric test Kruskal-Wallis for the proteomics analysis, which is the data with the highest variability in our manuscript. As observed below, the use of a non-parametric test has mostly added significance to a few time points/proteins. However, we feel that the ANOVA is a more conservative approach for this type of analysis, as published by our group before (*Proc Natl Acad Sci U S A*. 2017 Jan 3; 114(1): E75–E84), and that the differences seen in Kruskal-Wallis test do not change the final message of this paper.



2) The authors should discuss more about their finding of the discrepancy in clinical and histological/immunological outcomes after discontinuation of DSS at day 8.

We thank the reviewer for the suggestion and we have modified the discussion as follows (pages 13-14):

"Our data contradicts the previous report[9], in which animals started to improve clinically and histologically after DSS withdrawal. These differences between studies may reflect the influence of the microbiome and/or the animals' age, as previously reported in experimental DSS colitis[27,28]. In addition, it has been reported that UC patients in clinical and endoscopic based remission presenting with active histological inflammation possess a higher risk for clinical relapse[29,30]. In this way, our study may provide an understanding of the pathological and clinical response of severe human UC, with higher chances of relapsing and chronic disease. Since histological improvement could be seen as a new therapeutic approach and predictor of clinical relapse[31,32], the DSS

clinical and molecular time course may be useful for evaluating novel therapeutic approaches with the goal of clinical pathological complete remission."

Minor comments:

1) I guess "innate" is missing in the last line of "core tip" section.
   Yes, it is. We have corrected it in the manuscript.

2) Please provide the number of animals in the methods section more clearly.
   We have modified the methods section in order to make the number of animals clearer.

3) In figures, the reference group for comparing the values is not clear.
   We have modified the figure legends in order to make the comparisons clearer.

Reviewer #2

We thank reviewer #2 for the comments and we have modified the figures and legends accordingly.

1) Figure 4 and 8: The brown column means >6 but not presented in the scale bar.
   We have added the legend for the brown column in both figures, meaning ≥ 6.1 or ≥ 4.1 fold change, according to the respective scale.

2) Figure 5 to 7: It is confusing that data of IHC and flow cytometry are mixed.
   We have converged all the graphs from Colon, Spleen and MLN immune cell profile into one figure (Figure 5), so it is clearer to understand the pattern despite having IHC and flow cytometry mixed.

3) Figure 5 and 7: The photos of IHC are too small to recognize. Only a bar graph is enough.
   The photos of IHC and/or H&E from Spleen, MLN and Colon were reallocated as supplementary figures.

4) Figure 7: If MLN H&E staining are presented, the row and the column should be reversed as Figure 6. Figure 6 and 7 may be combined.
   Figures 6 and 7 were combined and reorganized as suggested by the reviewer.