**Peer Review File**

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**Reviewer A**

**Comment 1:** In LPS-activated BMDCs the inhibition of PRMT5 seems not to affect oxygen consumption or mitochondrial acidification although affecting iNOS message but not relative Nitrite concentration. This section of the data does not seem very convincing.

**Reply 1:** The nitrite concentration was analyzed by using the Total Nitric Oxide Assay Kit (Beyotime, China). It is an indirect way to quantify NO expression by measuring the nitrite, and some reagents in the kit are unstable and need careful handling. The results we obtained each time were quite different; as you can see in Figure 3J, the SEM are higher than those in other figures. Perhaps this is why the data seem unconvincing.

**Changes in the text:** None.

**Comment 2:** The in vivo data appear to confirm the anti-inflammatory activity of the inhibitor of PRMT5 when preemptively injected intrapalatally (in 40 micro-Liter volume?). The volume of the injections seems enormous compared to the limited soft tissue in the murine palate.

**Reply 2:** The injection was performed at three sites on the papillae side of the second molar. Also, not all liquid can be injected into the gingiva each time because of the thin gingival epithelial. So, the amount in each site is around 10 μl at maximum each time. We have added the detailed information to the Methods section.

**Changes in the text:** Page 12, line 256.

**Comment 3:** The manuscript seems to attribute al lot of the activity of the PRMT5 produced in CD11c+ cells and therefore to its inhibitor EPZ015666 on DCs. This assumption in vivo does not take into account that the expression of PRMT5 occurs primarily in the B cell compartment and not as much in the DC or Macrophage compartment.

**Reply 3:** Thank you for your insightful comment. It is known that in vivo studies are more complicated than in vitro studies. As you mentioned here and in Comment 5, other sources (such as B cells and endothelial cells) may also be involved in this process. However, in our study, we focused only on DCs due to their well-studied association with periodontitis.
**Changes in the text:** None.

**Comment 4:** In addition, the assessment of the message and PRMT5 protein is not proven to co-localize to CD11c positive cells (described in methods but not in results). A co-localization experiment is critical particularly with CD11c in the gingival tissue and in the cervical LN.

**Reply 4:** We apologize for the ambiguous text. CD11c or PRMT5 antibody was applied to lymph node slices or gingiva tissue slices individually, not applied together for colocalization. We have modified the text in the Methods section to be clearer.
In the first part of the in vivo study (Figure 4), we planned to clarify the possible association of PRMT5 with periodontitis. In the second part of the in vivo study (Figure 5), we focused only on the migration of DCs, which indicates the DC immune response.
**Changes in the text:** Page 14, line 294-296.

**Comment 5:** The in vivo effects of the inhibition of PRMT5 cannot be entirely attributed to the PRMT5 expressed by DCs but the author should consider other sources of PRMT5 for example CD105+ endothelial cells or 175 B lymphoblasts that may be present in the gingival tissue and NALT that is dissected from the murine maxilla. The method of gingival dissection is not well described and it may well be containing portions of not all components of the murine NALT.
**Reply 5:** In the in vitro study, we attribute the effects of the inhibition of PRMT5 to the PRMT5 expressed by DCs. But in the in vivo study, we showed only what we observed and made a reasonable connection between PRMT5 inhibition and periodontitis. We explain that the results indicate the involvement of DCs in this process. We thank the reviewer for this insightful comment and hope to find out the involvement of B cells and endothelial cells in our future study.

The excision of the gingiva was performed according to a protocol called “Isolation, Processing and Analysis of Murine Gingival Cells”. The protocol outlines a technique for precisely isolating murine gingival tissue for periodontal disease study by only isolating the gingival tissue that surrounds the three molars. We have added the information in the Methods section.

**Changes in the text:** Page 12, line 263-2.

**Comment 6:** There are no black dotted lines in Figure 5C. Also, the bone loss in the positive control group (Liga) appears free of inflammatory infiltrate and therefore attributable to an anatomical variation and not to inflammation.

**Reply 6:** We have enlarged the black dotted lines for better visualization.

In the positive control group (Liga), we observed the disappearance of mucosal epithelium, the formation of ulcers, the proliferation of granulation, and infiltration of numerous inflammatory cells. However, in the experiment group (Liga+EPZ), only proliferation of small vessels and infiltration of small numbers of inflammatory cells were observed. Thus, the bone loss in the Liga group was attributable to inflammation caused by ligation. We have added a black square to indicate the area in the figure and added this information in the Results section.

**Changes in the text:** Figure 5C; Page18, line 392-396; Page 25, line 554-555.

**Minor Comment 1-4:** “stimulation” is missing/ PKB/converted/ state this is first abbreviation.

**Reply 1-4:** We appreciate the reviewer’s attention to detail, and we have corrected these mistakes.

**Changes in the text:** Page 5, line 100; Page 6, lines 134, 136.

**Minor Comment 5:** What type of LPS was used? P.g-LPS may have been more relevant.

**Reply 5:** *E. coli* LPS was used. Our study initially aimed to identify the relationship between PRMT5 and DC activity in a general inflammation context; then, we later tested this finding in the ligatured model and observed an obvious anti-inflammatory effect and a change in DC migration. The reviewer made a very good point here, and

we are considering the use of *P.g*-LPS in a future study to further study periodontal inflammation.
**Changes in the text:** None.

**Minor Comment 6-9:** "Assessment of mitochondrial respiration and glycolysis" maybe a more appropriate title/ 2-deoxyglucose/ This is umclear/ not capital.

**Reply 6-9:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.
**Changes in the text:** Page 11, line 232, lines 237-238; Page 12, lines 248-249.

**Minor Comment 10:** 40ul seems a very big volume that may have crossed into the nasal cavity.

**Reply 10:** The injection was performed at three sites on the papillae side of the second molar. Additionally, not all liquid can be injected into the gingiva each time because of the thin gingival epithelium. Thus, the amount at each site is a maximum of approximately 10 μl each time, and it will not cross into the nasal cavity.
**Changes in the text:** None.

**Minor Comment 11:** Injection was done.

**Reply 11:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.
**Changes in the text:** Page 12, line 259.

**Minor Comment 12:** The excision of the gingiva was performed according to which protocol? The excision of the palatal gingiva without careful exclusion of NALT during dissection may provide data of lymphoid and myeloid cells present in NALT and not necessary of the gingiva.
**Reply 12:** The excision of the gingiva was performed according to a protocol called "Isolation, Processing and Analysis of Murine Gingival Cells". The protocol outlines a technique for precisely isolating murine gingival tissue for periodontal disease study by only isolating the gingival tissue that surrounds the three molars. We have added the information in the Methods section.

**Changes in the text:** Page 12, line 263-264.

**Minor Comment 13:** Was the tissue in RNAse inhibitors?
**Reply 13:** Yes, the tissue was subjected to RNAse inhibitors from the beginning.
**Changes in the text:** None.

**Minor Comment 14-15:** This statement is unclear/ The sentence should be reworded.
**Reply 14-15:** We apologize for the unclear sentence, and we have made modifications according to your suggestion.
**Changes in the text:** Page 13, line 282, line 287.

**Minor Comment 16**: The CD11c staining as well as the co-localization with the PRMT5 was not reported in the results.

**Reply 16:** Thank you for your question. Some of the text was ambiguous; PRMT5 was tested in gingival tissue, and CD11c was assessed in lymph nodes. They are not tested for colocalization. We have modified the text in the Methods section to improve clarity.
**Changes in the text:** Page 13, lines 294-396.

**Minor Comment 17:** What was the control staining? Preimmune rabbit? Absence of primary and only Anti-rabbit?
**Reply 17:** We apologize for the ambiguous text. We have modified this to improve clarity.
**Changes in the text:** Page 13, lines 294-295.

**Minor Comment 18:** where was 3$^{rd}$ molar measured?
**Reply 18:** Measurements were performed at 6 sites (including one site on the third molar) and added below Figure 5B.
**Changes in the text:** None.

**Minor Comment 19:** maxilla or mandible?

**Reply 19:** We apologize for the ambiguous text. Because we only put ligature around the maxillary molar, we only isolated the maxilla. We have modified the text to improve clarity.
**Changes in the text:** Page 17, line 376.

**Minor Comment 20:** It is unclear what the deviation for each bar represents. SEM or SD to inform about deviation out of several experiments? Deviation out of triplicate wells? What is the N of each bar?

**Reply 20:** SEM was used to inform the deviation, as mentioned in the Methods section (Page 6, line 269). In every experiment, we used triplicate wells in each group; however, the deviation was obtained from independent experiments. We have added the N in each figure.
**Changes in the text:** Each figure.

**Minor Comment 21:** What are the 4 groups? The legend is too small and cannot be visualized.
**Reply 21:** At the top of Figure 3G, the three compounds (oligo, FCCP, and rotenone/antimycin) target components of the electron transfer chain in the mitochondria. They were added to cell medium to measure ATP production, maximal respiration, and nonmitochondrial respiration, respectively.
At the top of Figure 3H, the first injection is a saturating concentration of glucose to measure the rate of glycolysis under basal conditions. The second injection (oligomycin) and third injection (2-DG) were added to reveal the cellular maximum glycolytic capacity and decrease in ECAR to confirm that the ECAR produced in the experiment is due to glycolysis. The legend has been enlarged for better visualization.
**Changes in the text:** Figure 4G-4H.

**Minor Comment 22:** There are no black dotted lines in the images 5C. Also the bone loss in the positive control group (Liga) appears free of inflammatory infiltrate and therefore attributable to an anatomical variation and not to inflammation.
**Reply 22:** We have enlarged the black dotted lines to improve visualization.

In the positive control group (Liga), we observed the disappearance of mucosal epithelium, the formation of ulcers, the proliferation of granulation, and the infiltration of numerous inflammatory cells. However, in the experimental group (Liga+EPZ), only the proliferation of small vessels and infiltration of small numbers of inflammatory cells were observed. Thus, the bone loss in the Liga group could be attributable to inflammation caused by ligation. We have added a black square in the figure and added this information to the Results section.

**Changes in the text:** Figure 5C; Page18, line 392-396; Page 25, line 554-555

**Minor Comment 23:** Flow cytometry panels should be labeled identify the groups.

**Reply 23:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.

**Changes in the text:** Figure 5F.

**Minor Comment 24:** PerCP-Cy5.5 is not described in the methods.

**Reply 24:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.

**Changes in the text:** Page 9, line 205.

**Reviewer B:**

**Comment 1:** The Results section should indicate the magnitude of the changes not just the statistical significance.

**Reply 1:** Thank you for your suggestion. We have made modifications in the Results section.

**Changes in the text:** The Results section.

**Comment 2:** The dose at which the inhibitor altered dendritic cell activity was generally greater than 10uM. However, the Ki for this inhibitor is 1000-fold less in the nM range. This discrepancy should be explained.

**Reply 2:** The Ki value and the concentration used in the cell study are two different entities. In the in vitro study, the higher concentration of EPZ helped it passively cross

the plasma membrane or enter the nucleus to combine with its target. This explains why the present study used micromolar doses of EPZ.

Many studies used EPZ from 5-10 μM and observed a good inhibition of SmD3 methylation and suppression of osteoclast differentiation in a concentration-dependent manner (Dong, Cellular Signalling, 2017; Chan et al., 2015); however, in the original inhibitor discovery paper, a higher concentration (50 μM) was also used to demonstrate the exquisite selective role of EPZ against a panel of 20 other protein methyltransferases (Chan et al., 2015).

Our study is the first investigation to test EPZ on DCs. The drug doses were set from extremely small amounts to 160 μM. The latter dose not only showed no toxicity to DC viability, but it also resulted in the most significant inhibition of proinflammatory cytokine production, rendering this the best concentration for use in further studies.

**Changes in the text:** None.

**Comment 3:** In addition, verification for PRMT5 dependence should be repeated in a few key experiments by use of siRNA, which can be done with primary DC.

**Reply 3:** Thank you for your suggestion. EPZ is a highly specific inhibitor of PRMT5 with no other targets reported to date. Because EPZ has been tested and demonstrated to provide good selective inhibition to PRMT5, through PRMT5 knockdown with siRNA/shRNA in a previous study (Dong, Cellular Signalling, 2017; Chan, Nature Chemical Biology, 2015; Christian J Braun, Cancer Cell, 2017), we did not consider this test in our study.

**Changes in the text:** None.

**Comment 4:** The maturation of DCs induced by LPS as measured by MHCII, CD80, and CD86 was extremely modest. Unless these experiments are repeated, the authors should focus on the effect of PRMT5 on changes in cytokine expression and indicate that measures of overall DC activation were difficult to assess due to the poor induction by LPS.

**Reply 4:** The experiments were repeated three times. Furthermore, the maturation of DCs induced by LPS as measured by MHCII, CD80, and CD86 was modest at 24 h,

but it became much more significant at 48 h. We have added the statistical analysis results in Figure 2.

**Changes in the text:** Figure 2.

**Comment 5:** The authors present no evidence that changes in Akt mediate glycolysis. Unless it is shown that Akt1 inhibition reverses the changes in glycolysis the data on Akt phosphorylation/Akt levels should be deleted and reference to Akt1 regulation deleted.

**Reply 5:** Thank you for your comments. We have put the data on Akt phosphorylation/Akt levels to the supplemental materials and deleted the connection between Akt and glycolysis, but only discussed AKT and its related pathways involved in cell activation and DC function.
discussed the phosphorylation/Akt levels and references to Akt1 regulation.
**Changes in the text:** Page 5, line 111; Page 8, lines 168-169; Page 10, lines 218-219; Page 17, line 367-369; Page 21, lines 460-462, lines 469-470; Page22, lines 496-497.

**Comment 6:** The immunohistochemistry to detect PRMT5 alone is not convincing as the background levels are relatively high. It is critical to show that their results are specific by inclusion of a control primary antibody, which should be added to the figure.
**Reply 6:** Thank you for your suggestion. We have added the control primary antibody result in the figure.
**Changes in the text:** Figure 4d.

**Comment 7**: The flow cytometry pattern from the lymph nodes is odd and not consistent with previous studies, particularly the MHCII negative cell numbers and double positive numbers. There is no indication that the events recorded are for live cells.
**Reply 7:** In the flow cytometry test, the events were gated to exclude dead cells and debris. Furthermore, the MHCII-negative cell numbers were affected by other cells around lymph nodes, and our study focused on the double-positive cell numbers, in accordance with previous studies that also gated DCs as CD11c+MHCII+ in murine lymph nodes and spleens (Xiao, The American Journal of Pathology, 2015; Wang;

IOVS, 2019). In case the ambiguous figure label has caused confusion, we have added the protein name and group name to clarify the figure.

**Changes in the text:** Figure 5

**Comment 8**: There is no cause-and-effect relationship established between the glycolytic pathways and changes in cytokine expression in DC and the Akt pathway. Any implied connection between them should be deleted from the Discussion and the rest of the manuscript.

**Reply 8:** Thank you for your comments. We have made modifications according to your suggestion.

**Changes in the text:** Page 5, line 111; Page 8, lines 168-169; Page 10, lines 218-219; Page 17, line 367-369; Page 21, lines 460-462, lines 469-470; Page22, lines 496-497.

**Comment 9**: The manuscript needs careful editing as the English usage needs to be improved.

**Reply 9:** The AJE English editing service have conducted the language polishing again.

**Comment 10**: Previous studies have examined DC function, particularly by the FOXO1 pathway, which has been investigated in experimental periodontitis. The Discussion would be enhanced by comparing the current results with those previously published on dendritic cells and periodontitis.

**Reply 10:** We thank the reviewer for this insightful comment. We have included the AKT results in the Supplemental results section and compared them with the related pathways (TREM-1 and FOXO1) involved in cell activation and DC function in the Discussion section.

**Changes in the text:** Page 21, lines 462-469.

**Comment 11**: The injection of PRMT5 inhibitor into the gingiva needs additional information. It is not possible to inject 40 μL in a single bolus in the mouse gingiva.

**Reply 11:** The injection was performed at three sites on the papillae side of the second molar. Additionally, not all liquid can be injected into the gingiva each time because of the thin gingival epithelial. Thus, the amount at each site is a maximum of

approximately 10 μl each time. We have added the detailed information to the Methods section.

**Changes in the text:** Page 12, line 256.

**Comment 12**: The methods state that gingival specimens were fixed in 10% paraformaldehyde. Do they mean 10% buffered formalin, which is equivalent to 4% paraformaldehyde?

**Reply 12:** According to the datasheet of the reagent (Leagene Bio; #DF0128), it contains 10% PFA, and the remaining contents are phosphates and deionized water.

**Changes in the text:** None.

**Comment 13**: The figure legends should clarify whether the experiments were performed in vitro or in vivo.

**Reply 13:** Thank you for your comments. We have made modifications according to your suggestion.

**Changes in the text:** The end of each figure legend.

**Comment 14**: The CD11c color is missing from the results section.

**Reply 14:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.

**Changes in the text:** Page 10, line 205; Figure 5F.

**Comment 15**: Figure 1 is labeled incorrectly.

**Reply 15:** We appreciate the reviewer's attention to detail, and we have corrected those mistakes.

**Changes in the text:** Figure 1D,1E.