

# Review of: "Chronically Elevated O-GlcNAcylation Limits Nitric Oxide Production and Deregulates Specific Pro-Inflammatory Cytokines"

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**Potential competing interests:** The author(s) declared that no potential competing interests exist.

This manuscript has addressed O-GlcNAcylation regulates macrophage inflammation by modulating iNOS activity. The authors further describes that increased-O-GlcNAc increases cytokine production of macrophage. The authors conclude that balanced O-GlcNAc cycling is important for normal macrophage inflammation. Although this manuscript attempts to describe the importance of iNOS O-GlcNAcylation in macrophage inflammation, the data is too preliminary to support that O-GlcNAc changes of iNOS modulate iNOS function or inflammation.

1. Author used trypan blue as a method to check the cell viability results (Figure 1E). This method is strongly influenced by the subjectivity of the researcher. Therefore, the author should do additional experiments to quantify cell viability.
2. In Figure 3, iNOS transcription level is not changed in OGA KO BMDM but NO level decreased. Based in this result, the authors concluded that "This finding suggests that elevated O-GlcNAc acted to inhibit iNOS function." in page 5. However, it requires further evidences, since NO level can be affected by SOD activity or NO substrate level. Therefore, the authors need to show if iNOS activity will be changed in OGA KO BMDMs.
3. In Figure 3, OGA KO- and NIL-induced NO decrease and IL-6 increase will be changed by OGT inhibition or other O-GlcNAc inhibitors?
4. Have the authors tried iNOS IP and western blotting with RL-2 or OGT or IP with OGT and western blotting with iNOS? The data is not clear enough if iNOS O-GlcNAc level is changed in OGA KO. Moreover, the RL-2 level of MO and M1 WT is look similar in WGA sample (input looks different). Please check does the iNOS O-GlcNAcylation level and activity was changed by pharmacological OGA inhibition.
5. The n number of figure 4 is not sufficient. It requires to add more n numbers.
6. The significance of figure 4B should be indicated.
7. Please explain why there is more lysate in the second lane compared to first lane with decrease in iNOS O-GlcNAcylation in Figure 4C.
8. Figures are not labeled properly. Please correct. (Ex. Figure3, 4)
9. Effect of O-GlcNAc changes on iNOS expression is affected by glucose dose. Have the authors checked that glucose utilization is different between WT and KO mice.

