Reviewer’s report

**Title:** Extracellular polysaccharides produced by Ganoderma formosanum stimulate macrophage activation via multiple pattern-recognition receptors

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**Reviewer:** Nien-Jung Chen

**Reviewer’s report:**

Ganoderma is a traditional Chinese medicine used for a long time with beneficial effect on modulating human immunity. However, the involved pharmacological mechanism mediated by Ganoderma treatment remains to be investigated. The authors previously isolated a polysaccharide fraction from G. formosanum (PS-F2) which shows immunostimulatory activities. In this report, they further investigated the molecular mechanisms involved in PS-F2-mediated macrophage stimulation, especially on the TNF-induction response. Their results suggested that Dectin-1, CR3 and TLR4 are the PRRs for the b-glucans in PS-F2 and mediate the activating signals, such as NF-kB and MAPKs, to control the induction of TNF. Moreover, by treating cells with specific inhibitor piceatannol, Syk was demonstrated involved in PS-F2/Dectin-1/CR3-mediated NF-kB and ERK, but was not involved in p38 and JNK activation which may be contributed by PS-F2/TLR4-mediated activation.

The questions posed are well defined and well-examined by appropriated methods. The data are well-presented with good quality. Most of the conclusions and the discussion are well balanced. However, there are minor points that should be discussed and also included in this report:

**Minor Essential Revisions**

1. Dectin-1 and CR3 are well-characterized in fungal PAMP recognition. In Figure 2A of this study, the authors used cells from C3H/HeJ, in comparison with cells from C3h/HeN, to demonstrate the involvement of TLR4 in PS-F2-mediated stimulation. However, the induction of TNF in the BMDMs derived from both types of mice are significantly lower (in pg/ml levels) than the results shown in RAW264.7 cells (in ng/ml levels). The authors should discuss the potential causes for this difference.

2. In addition, rather than TLR4, it has been suggested that TLR2 plays critical roles in mediating fungal stimulation. The role of TLR2 in PS-F2-mediated stimulation is not characterized in this study. It may be informative to compare the function of TL2 and TLR4 in PS-F2-stimulated RAW264.7 cells by using specific antagonistic antibodies for TL2 and TLR4.

3. In the signaling study of this research, the author use piceatannol as the specific Syk inhibitor to demonstrate the involvement of Syk in PS-F2-mediated signaling and responses. However, it is always a concern about the specificity and off-target effect when using a chemical blocker on signaling. It will more
convincing to include a negative control that shows piceatannol does not have any non-specific blocking effect on a Syk-independent signaling (such as poly I:C-TLR3 signal) in Figure 1E and Figure 4D.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests