Author's response to reviews

Title: Functional activation of proline-rich tyrosine kinase2 (PYK2) in peripheral blood mononuclear cells from patients with systemic lupus erythematosus

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Object: MS: 1131451551273331- Functional activation of proline-rich tyrosine kinase2 (PYK2) in peripheral blood mononuclear cells from patients with systemic lupus erythematosus.Dr Meiying Wang et al.

We appreciate your consideration of our manuscript, and we have reviewed the above manuscript according to the previous reviewer’s comments.

Reviewer #1

Quality of language

Would the manuscript benefit from being shortened? No

Does the manuscript require extensive language editing? No

1-- Fig 1. Although pyk2 is upregulated, the increase in p-pyk2 is less convincing and the ratio of p-pyk2/pyk2 might remain the same.

Done in page 12.

Yes, just as what the reviewer said, with PYK2’s upregulation, P-PYK2 is also increased and the ratio of P-PYK2/PYK2 remains the same. These results just indicated that PBMCs from SLE patients expressed more of both the total PYK2 protein and its activated/phosphorylated form.

2-- I was not convinced by the changes in Figure 2, indeed all four panels looked similar.

We have repeated the experiments and obtained these photos. Please look at Figure 2.

3-- Figure 3, again the ratio of p-pyk2/pyk2 should be presented, the change only in class IV is of interested. This may reflect the fact these patients are more active, it would be interesting to see the SLEDAI correlation data.

Done in page 13.

We have added the ratio of P-PYK2/PYK2 and analyzed the correlation between the ratio of p-PYK2/PYK2 and lupus nephritis, and no correlation was found for class IV lupus nephritis. Also, PYK2 activation did not show a correlation with the SLEDAI score.

4-- Figure 4, I was not convinced of the small differences presented in this figure, particularly with reference to the pyk-2 inhibitor.

Done in Figure 4.

We have increased the samples and repeated the experiments.

5-- I would make a similar comment about Figure 5 as well.

Done in Figure 5.

We have increased the samples and repeated the experiments.
Reviewer #2

Quality of language

Would the manuscript benefit from being shortened? No

Does the manuscript require extensive language editing? No

1--Lymphopenia is common in SLE. The authors have used crude PBMC and standardized for Actin. This means that they could theoretically just show more monocytic protein (rather than lymphocytic protein) in their blots of (active) SLE patients. This issue could be resolved by either re-blotting with markers for monocytes and/or T cells, or by demonstrating that equal amounts of monocytic and lymphocytic protein contain equal amounts of Pyk2 and pPyk2. PBMCs isolated from peripheral blood by Ficoll–Paque gradient centrifugation contain 90-95% lymphocyte, and PBMCs lysates proteins are mainly from lymphocyte protein. On the other hand, because of financial stress, we did not re-blot with markers for monocytes and/or T cells although perhaps I should have done. I hope these do not affect our interpretation of the result.

2--To close the chain of functional arguments, it would be important to demonstrate that PMA-activation indeed upregulates Pyk2 protein.

Done in page 13 and Figure 4

3--The Figure legends need to contain a few more details, such as the number of blots, for which Figure 1 is a representative example, or how the protein and p-Pyk2 levels depicted in Figure 3 are measured.

Done in Figure legends.