Author’s response to reviews

Title: SBF-1 inhibits contact hypersensitivity in mice through down-regulation of T-cell-mediated responses

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Response to Reviewers’ Comments
All changes have been marked by using yellow highlighting in the revised manuscript.

Editor Comments:
1. The authors relabeled the “Model” group as “Vehicle” in Fig. 4. Please also do this for Fig. 5.
   Response: Thank you for your advice. The correct figure labeled with “Vehicle” have been provided in the revised manuscript (Fig. 5).

2. Item #7 from the previous “Reviewer 1” was misinterpreted. The question was meant to ask why the vehicle for the PCl changed from sensitization (EtOH) to challenge (OO).
   Response: In our experiments, mice were sensitized by applying PCl to the shaved skin on their abdomens and challenged by applying PCl to the right ear. PCl is slightly soluble in ethanol. However, absolute ethanol is reported to induce various cutaneous changes, which may affect the measurement of ear swelling. Therefore, vehicle was changed to olive oil in challenge.

3. The authors clarified the statistics used (Item #9.f. from Reviewer 1 of the previous review) to indicate that one-way ANOVA was used for the statistics, however Fig. 4 states that Student’s t test was used. Please clarify.
   Response: Thank you for your comments. The correct description has been added to the Figure Legends section of the revised manuscript (Page 21, line 2).

4. In the Methods section, the PCR section should be titled “Semiquantitative PCR”, since real-time
PCR was not used in this study.
Response: Thank you for your comments. The correct description has been added to the Methods section of the revised manuscript (Page 7, line 14).

5. Please indicate the source (company) of the cytokine ELISA kits.
Response: The source of the cytokine ELISA kits is in Reagents section of the manuscript (Page 5, lines 20-21).

Minor comment
1. Recommend the following with respect to indicating that PCl is synonymous with picryl chloride. Since readers may be confused by reference to “picryl chloride (TNCB)” in the abstract, I suggest removing TNCB in this section (Page 2, line 8) and instead edit in the Results section (page 12, lines 6 – 7) as follows:
“In order to assess the immunosuppressive property of SBF-1 in vivo, contact dermatitis was induced in BALB/c mice with picryl chloride (also referred to as trinitrochlorobenzene, TNCB).”
Response: Thank you for your advice. We have followed your suggestion to move TNCB to the Results section of revised manuscript (Page 12, lines 7-8).

2. I agree with Reviewer #2 that IL-2 is not, generally-speaking, considered a pro-inflammatory cytokine and this language should be avoided.
Response: Thank you for your comments. We have changed the statement to “T cell cytokines” in the revised manuscript (Page 2, line 15; Page 11, lines 12, 19-20; Page 20, line 3).

English language revisions
To assist with English language and grammar issues, I recommend the following revisions to improve readability:
1. Change: “Flow cytometry analysis was performed for the activation and apoptosis.” (p. 2, lines 6 -7) to “Flow cytometry analysis was performed to assess T cell activation and apoptosis.”
Response: Thank you for your comments. The statement has been changed to “Flow cytometry analysis was performed to assess T cell activation and apoptosis” in revised manuscript (Page 2, lines 6-7).

2. Change: “On the other hand, the proinflammatory cytokines, including IL-2 and IFN-γ were down-regulated by SBF-1 in a dose-dependent manner.” (p. 2, lines 15-16) to “In addition, SBF-1 also downregulated the induction of the T cell cytokines, IL-2 and IFN-γ in a dose-dependent manner.”
Response: Thank you for your comments. The statement has been changed to “In addition, SBF-1 also downregulated the induction of the T cell cytokines, IL-2 and IFN-γ in a dose-dependent manner” in revised manuscript (Page 2, lines 15-16).

3. Change (p. 4, lines 5-8): “However, because of their nonselective inhibition on non-activated T lymphocytes and activated T lymphocytes, most of these agents have reported to cause severe side effects including immunosuppression and the increased risk of infection. Until today, there is an urgent need for effective immunosuppressive agents with high selectivity and low toxicity.” to “However, because of their nonselective inhibition on non-activated T lymphocytes and activated T lymphocytes, most of these agents are reported to cause severe side effects including immunosuppression and the increased risk of infection. Thus, there is an urgent need for effective immunosuppressive agents with high selectivity and low toxicity.”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 4, lines 6 and 8).
4. Change (p.6, lines 16-18): “After 4 h of additional incubation, remove the culture media, 200 μl DMSO was added to dissolve the crystals. The absorption values at 570 nm were determined” to “After 4 h of additional incubation, the culture media was removed and 200 μl DMSO was added to dissolve the crystals. The absorption values at 570 nm were determined using a microplate reader.”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 6, lines 16-18).

5. The following phrase is unclear (p.9, line 13): “3: the affection of other side of the ears.” Is leukocyte infiltration or epidermal injury being assessed (or both)? Perhaps: “3: inflammation to the ventral side of the ear.”?
Response: Thank you for your comments. The statement has been changed to “3: inflammation to the ventral side of the ear” in revised manuscript (Page 9, line 13).

6. On p. 10 line, I suggest changing “On the other hand,…” to “In addition,…”
Response: Thank you for your comments. The statement has been changed to “In addition” in revised manuscript (Page 10, line 6).

7. Change (p. 10 lines 18 – 20): “Previous study showed that CsA greatly affected cell viability both in activated and non-activated T cells [16]. These results suggest that the immunosuppressive activity of SBF-1 was not caused by cytotoxicity, which is distinguished” to “Previous study showed that CsA greatly decreased cell viability both in activated and non-activated T cells [16]. These results suggest that, in contrast to CsA, the immunosuppressive activity of SBF-1 is not caused by cytotoxicity of immune cells.
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 10, lines 18-20).

8. Change (p. 12, line 22 – p. 13, line 1) “… are highly required” to “are needed”
Response: Thank you for your comments. The statement has been changed to “needed” in revised manuscript (Page 13, line 1).

9. Change (p. 13, lines 11-14) “In addition, it should be noticed that SBF-1 did not affect survival of non-activated T cells at the doses mentioned above, indicating that this compound had selectivity to degree (Fig. 1f and g)” to “In addition, it is notable that SBF-1 did not affect survival of non-activated T cells at the doses mentioned above, indicating that this compound had selectivity for activated T cells to some degree (Fig. 1f and g)”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 13, lines 13-14).

10. Suggest changing (p.13, lines 18 – 19): “The effects of SBF-1 on T cells activation should be implicated by inhibition of related signaling pathways” to “Thus, these results suggest that SBF-1 likely inhibits signaling pathways related to T cell activation.”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 13, lines 19-20).

11. Change (p.14, lines 9 – 11): “Therefore, we believed that the major effect of SBF-1 is on inhibition of T cell activation and then suppression of cytokines production” to “Overall, the data suggest that the major effect of SBF-1 is on inhibition of T cell activation causing suppression of cytokine production.”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page
14, lines 10-12).

12. Change (p. 14, line 12) “was” to “is”
Response: Thank you for your comments. The statement has been changed to “is” in revised manuscript (Page 14, line 13).

13. Change (p.14, lines 13 – 14): “We found that, when intraperitoneal injected in the induction phase of CHS, SBF-1 significantly suppressed the…” to “We found that SBF-1, when administered by intraperitoneal injection in the induction phase of CHS, significantly suppressed the…”
Response: Thank you for your comments. The statement has been changed in revised manuscript (Page 14, lines 14-16).