**Resubmission of proposal # 1163/23**

Dear ISF committee members,

Please find the revised version of our proposal, “Uncovering the molecular mechanisms of parasite-host interactions during myxozoan infection of tilapia”. I have been highly encouraged by the positive recommendations and comments of the reviewers. All four reviewers agree that the project addresses an important biological question, namely host-parasite relationships and resistance mechanisms, using a well-suited model and state-of-the-art methodologies.

I am grateful for the reviewers’ thoughtful comments and suggestions, which resulted in a stronger and more comprehensive proposal. In the following point-by-point responses, we have addressed the specific concerns raised by the reviewers. The concerns relate to the study design, which we have changed to better describe the resistance of the Nile tilapia. Now, we also present robust and comprehensive preliminary results, including new proteomics profiling of the host and the parasite from the same infected tissue.

We are confident and enthusiastic that the enhanced proposal will provide comprehensive data, providing a mechanistic understanding of host-parasite interactions and the mode of action of the myxozoan. We envision our findings contributing to a broader understanding of the unknown mechanisms by which myxozoans modulate the host immune system. We hope you find the improved research plan and new data as exciting as we do and compelling enough to deserve ISF support.

Sincerely yours,

Tamar Lotan

**Point-by-point answers**

Referee 1

Referee 1 was very supportive of the proposal and had two comments to improve the study.

1. Collecting serum from the same individual fish from which other tissues will be taken. We agree with the referee, and we have addressed this point. Furthermore, this summer, we conducted a successful test for serum collection. The results are incorporated into the Preliminary Results section.
2. Increasing the number of biological replicates. We have accepted the referee’s comment and have increased the number of biological replicates to five. As demonstrated in our previously published study, this increase in replicates provides us with the statistical power needed to identify regulatory and signaling pathways. Additionally, we are sampling at several time points, including controls and three time points during infection, significantly enhancing the robustness of our analysis.

Referee 2

Referee 2 recommended granting the proposal. The referee noted some incomplete references, which we appreciate and have addressed in the revised proposal.

Referee 3

Referee 3 expressed strong support for the proposal and pointed out that the main challenge will be to combine the data for the final comprehensive analysis. We concur with the referee’s assessment. To address the challenge of analyzing multi-omics datasets, we will collaborate with the University of Haifa Bioinformatics Unit, which has accumulated significant expertise in analyzing complex biological datasets.

Referee 4

Referee 4 commented that the proposal is significant for advancing our understanding of Myxozoa biology, fish biology (particularly immunity), and the interactions between Myxozoa and fish. The referee also noted that the methodologies developed will enhance the strength and feasibility of the proposed research. However, the referee made several comments that need to be clarified.

1. In comment 1, the referee suggests collaboration with a fish expert. Over the years, we have consulted with the fish scientific community to design our studies better. We have now added a collaborative letter from Prof X, who is well-known in the field.
2. In comment 2, the referee commented that the significance of the proposal to aquaculture is somewhat limited to regions where blue tilapia and hybrid tilapia are cultivated. We fully concur with the reviewer’s assessment. However, it’s worth noting that the hybrid of blue and Nile tilapia is prevalent in China, which is the largest tilapia producer globally. Furthermore, gaining insight into the underlying molecular processes of resistance may illuminate these unknown fundamental processes.
3. The referee is unclear whether the omics data of Myxozoa will be obtained from the same gills used for the tilapia omics analysis. We apologize for any confusion, and in the revised proposal, we emphasize that the same tissue will be used for both parasite and host analyses, enabling the direct examination of host-pathogen interactions. Our recent publication titled “The molecular mechanisms employed by the parasite *Myxobolus bejeranoi* (Cnidaria: Myxozoa) from invasion through sporulation for successful proliferation in its fish host” also demonstrates the feasibility of the approach and provides data from the very onset of infection.
4. The referee suggests that the transcriptomic data may be limited based on our draft genome, and the parasite proteomics might be even more premature. We acknowledge that the draft genome we presented was relatively fragmented. Thus, we are currently re-sequencing the genome using PacBio long reads, which can be assembled more accurately and augment our existing sequence. However, our original proposal outlined our plan to assemble a de-novo transcriptome as an alternative strategy. Indeed, this approach proved highly successful. Data from the de-novo transcriptome assembly is sufficiently detailed to identify transcription factors, regulatory pathways, and signaling pathways, as demonstrated in our recent publication (Maor-Landaw et al., 2023). Furthermore, our preliminary proteomic data indicates that a myxozoan proteomic profile can be obtained using our de-novo transcriptome database, as shown in Preliminary results Fig. 6.
5. Unfortunately, we were not sufficiently detailed, and the terms’ susceptible’ and ‘resistance’ were not clearly defined. To address this, we added Preliminary results in Fig. 3A to illustrate that Nile tilapia is infected similarly to blue tilapia, exhibiting a similar parasite load after exposure to infection. However, at 30 days post-exposure in blue tilapia, the parasite continues to proliferate and forms cysts, whereas in Nile tilapia, the parasite level decreases. This difference demonstrates resistance to the infection by Nile tilapia.

The referee also recommends testing the response of Nile tilapia by qPCR to verify whether Nile tilapia indeed senses and reacts to the parasite. We have followed the referee’s suggestion and conducted experiments using two cytokines. The results demonstrate that in Nile tilapia, these markers are significantly upregulated after parasite infection, whereas in blue tilapia, there is no change compared to the control. The experiments show that Nile tilapia sense and react to the parasite, whereas the immune system is suppressed in blue tilapia. The results are presented in Preliminary Results, Figs. 3B and 3C.

1. The referee suggests that the disease may result from temperature conditions because Nile tilapia is more adapted to 30°C, whereas blue tilapia is more adapted to 25°C. However, our data does not support this hypothesis, as demonstrated in our response to Comment 5 above and our publication (Maor-Landaw et al., 2022), where we show that the parasite suppresses the immune response of blue tilapia. Furthermore, we conducted several experiments where fish were exposed to the parasite for 24 hours to one week and then transferred to 25°C containers. In all cases, Nile tilapia demonstrate resistance, even when cultivated at relatively colder temperatures. Conversely, despite being at its preferred temperature range, blue tilapia is not resistant to infection.