**Academic Review Feedback – lncRNAs and HIV Viral Latency Grant**

In this grant proposal, the research team seeks to gain additional insight into the functional importance and mechanistic roles that lncRNAs play as regulators of HIV viral latency and reactivation in CD4+ T cells in the context of T cell activation in an effort to provide foundational knowledge to facilitate the future eradication of this retroviral reservoir. Specifically, the authors performed a screen in which they identified the lncRNA CYTOR as a potential regulator of the establishment or persistence of HIV latency, prompting a series of proposed experiments aimed at elucidating the direct and indirect regulatory roles of this lncRNA in this clinically relevant context. Overall this proposed project seems fairly strong, although some reorganization of certain sections may be warranted to streamline the text further. I think that the scientific flow of the proposed studies is logical, and they are of interest to me as an immunologist and former member of a viral immunity-focused lab, and it may be a competitive submission (provided it fits well with the scope of the call for funding/target funding body).

Below are comments/feedback on the individual sections of this manuscript that may further improve this proposal:

Introduction:

* If possible, consider providing some discussion of the potential clinical implications of elucidating the importance of lncRNAs in the context of HIV latency. This may be particularly valuable given that you discuss other strategies that have been unsuccessful in the past such as the Shock-and-Kill technique.

Preliminary data:

* Is it expected to see viral reactivation in such a small fraction of the T cells following PMA/ionomycin stimulation? I’m not familiar with this particular cell line, but P/I are very strong stimulants, so some clarification of the dose level used would be helpful as a reference guideline.
* Other sources seem to capitalize CYTOR and I have done so in the text. I recommend updating your Figures accordingly.
* For Figure 1D, I assume the primary cells are not HIV-infected. It’s possible that reviewers will question whether similar result replication would be observed in primary HIV-infected CD4+ T cells, although I know such experiments would be challenging to perform. Stressing this limitation may be of value.
* A bit more discussion of the mechanisms whereby CYTOR has previously been reported to influence tumor cell malignancy or other functions (at the mechanistic level) would be beneficial to give less familiar readers a clearer sense for lncRNA mechanisms of action.
* You should indicate the statistical tests used for all comparisons, and should also make sure that your bars indicating significance are long enough and properly aligned for all Figures to make the comparisons very clear.
* Some of your Figure legends contain inconsistencies in things like capitalization and spacing (over expression vs. Overexpression, etc.) – carefully review them all for consistency.
* You should use consistent color schemes for overexpression and knockdown data (right now you switch between blue, green, and red which makes your Figures less intuitive)
* I would suggest including an abbreviated table for your MS results of CYTOR interactors.
* Figure 4A doesn’t really serve any purpose at the point in the grant it is included.
* Possibly due to the editing of the text, several of the Figures align somewhat strangely with the surrounding text – before final submission, quickly check and fix these issues (i.e. Figures 4 and 5 have misalignment between the Figure and text boxes).

Objectives and Specific Aims:

* For each Aim, I think it may be beneficial to provide a specific but short hypothesis statement to focus your experimental goals.
* For Aim 1, your text is fairly general and repeats some of the text from the above sections. I would suggest rewriting this to provide a more specific and detailed overview of the actual experimental protocols and comparisons that will be made in SA1, as you do for SA2.
* I don’t think it makes sense to introduce a model Figure at the end of SA3 as you currently do – instead, I would either introduce it at the start of the specific aims section, or I would move it elsewhere in the grant.

Significance and Innovation:

* Some parts of this section are a bit redundant from other parts of this study, including some nearly identical sentences. While this cannot be fully avoided, I would suggest trimming out some of the most redundant parts of this section as suggested in the comments provided in the text.
* You note that you identified many lncRNAs differentially expressed in activated HIV-infected T cells, but they are otherwise largely not discussed in this study. Perhaps consider discussing alternative lncRNA targets as potential foci for alternative or follow-up experiments to expand the scope of these results with a bit more specificity, either in this section or a different part of the manuscript? A few sentences would likely suffice, particularly if any of the other targets are likely to exhibit similar roles based on prior literature citations or your preliminary work.

Research Approach:

* T cells from four donors may not be sufficient to control for donor-to-donor variability - have you done any preliminary experiments confirming that this is a reasonable number of donors to use to ensure that CYTOR expression isn’t highly variable?
  + In addition, clarification regarding donor composition (healthy young donors? How will cells be isolated – positive or negative enrichment?) is needed.
* Much of the text in Aim 1b feels redundant with the CRISPR section in your preliminary data section – consider largely merging the two to cut down on such repetition (perhaps by moving the CRISPR data to this section).
* For HIV patient experiments, I think some additional discussion of the numbers of patients that will be included and whether there are any restrictions on patient exclusion/inclusion that will be imposed for this study.
* In Aim 2A you mention CYTOR transitioning between subcellular compartments, but this is not really something you have discussed at any prior point in the grant proposal, making this somewhat unexpected/possibly confusing here. Consider more clearly laying the groundwork for this.
* Your “Expected outcomes, potential pitfalls, and alternative strategies” sections need to actually describe your Expected outcomes – they do not do so very effectively at present