Response to Reviewer 3 Comments

The authors thank the reviewers for their thoughtful comments, which have been addressed below. The comments and suggestions are included with responses to each written in red:

This is a report on the importance of TOR signaling pathway based on growth pattern of *Chlamydomonas reinhardtii*, changes in macromolecule composition, photosynthetic measurements, and changes in the reversibly oxidized cysteine thiol proteome after treatment with AZD8055. Authors interpreted that the global oxidative changes caused by TOR inhibition mirrored physiological modifications examined in this manuscript. I think that this manuscript demonstrates well that TOR plays a central role in regulating many aspects of cellular physiological states including photosynthesis through reversible thiol oxidation. Especially, authors showed the extensive analyses in processes of photosynthesis, some of which may be targets of TOR signaling. They provided the proteomic changes of redox-controlled proteins, which seemed to be further developed from their previous study and now were applied to TOR signaling pathway. Nevertheless, I feel that this study is preliminary to be published in the following senses and I hope authors to consider them seriously for contributing to communities studying the TOR signaling.

Major comments

Although this manuscript delivers many valuable analyses to readers, in my opinion, data do not clearly show how TOR signaling affects the many aspects of cellular physiology including photosynthesis. When TOR is inhibited by any means, it is quite expected that there will be serious changes or damages many of which may eventually lead to autophagy. In this sense, drastic changes in such and such physiology in Figures are expected although diverse techniques were used and extensive hypothetical description on how was made in Discussion. I think authors should have made efforts in answering the following questions and providing substantial data in, at least, a few cases that authors mentioned in Discussion.

- Which components of TOR signaling actually affect the physiological changes?
- Which specific step(s) of each metabolism or photosynthesis was(were) the target(s) of TOR signaling?

*Response: While finding the direct targets of TOR would be a useful and worthwhile endeavor, it is outside of the scope of this manuscript. The purpose of this study was to look at the extent of reversible oxidative signaling initiated through TOR via AZD8055-induced TOR inhibition. Furthermore, the direct targets of TOR have not all been identified in photosynthetic organisms (see Werth et al., 2019). By providing a blue print of the reversible oxidative signaling following TOR inhibition, future studies may be better poised to identify additional downstream substrates of TOR.*
Author mentioned the “direct” inhibition of photosynthesis in response to TOR inhibition (line 76). Does TOR kinase (or its downstream, e.g., S6K1) directly act on any step(s) of photosynthesis? Or did TOR inhibition result in initiating autophagy, minimizing the energy-consuming processes such as photosynthesis? I think authors need to provide more solid evidence on the “direct” connection between TOR signaling and photosynthesis to keep such comments.

Response: As the reviewer mentions, TOR certainly initiates autophagy (see Díaz-Troya et al., 2008). If the decreases in photosynthetic output were purely the result of autophagy, we would expect a subsequent decrease in chlorophyll concentration (Ishida et al., 2014). However, this was not the case: instead of chlorosing, the chlorophyll remained stagnant (Figure 2d), providing evidence that the change in cysteine oxidation was more likely the cause of decreased output. Additionally, it should be noted that while a previous study (Werth et al., 2019) determined several chloroplastic targets for the phosphorylation network following TOR inhibition, they do not include the most significant targets of cysteine oxidation, showing the regulation of these particular photosynthetic components to be a novel link to the TOR regulatory network. However, “direct” may imply a specific downstream target such as S6K1, as mentioned by the reviewer. To alleviate this confusion, “direct” was removed from lines 75 and 503. Lines 616 were rewritten to shift the focus to the regulation of photosynthesis rather than specific downstream targets of TOR.

Many other studies of TOR inhibition by diverse means other than AZD8055 have been published. There needs to be comparison between results in this manuscript and other TOR inhibition studies, and the some critical points of the comparsion needs to be mentioned. I hope there will be a few make-up experiments after authors went through the comparison, such as examining the level of gene expression or the protein for the deficient steps in AZD-treated algae authors mentioned.

Response: Previous TOR inhibition studies are cited throughout the manuscript. Much of our physiological data correlates well with previous studies - the one exception is total protein quantification, which has been addressed beginning in line 539. Gene expression has been previously studied upon TOR inhibition in Chlamydomonas (Kleessen et al., 2015, Ramundo et al., 2014), and both references have been added to the manuscript introduction (references 17 and 18). Both of these studies are done using rapamycin treatment rather than AZD8055. Ramundo et al. examined gene expression at longer time points (12+ hrs) while the time frame for changes in thiol oxidation are much shorter (measured in <60 min). Also, Kleessen et al used a wall-deficient strain of Chlamydomonas for gene expression analysis at 30 and 60 min, and we did not observe protein expression changes in our global proteomics data at the timepoints assayed for our experiment. Therefore, we believe it would be a stretch to make comparisons of the published gene expression data to our current redox proteomics data.

Minor comments
响应：我们已在图4的图注中添加内容，进一步解释OJIP参数。我们希望这将澄清有关缩略词的任何混淆。图的图注可以在补充材料部分找到。