Review of: "USP11 controls R-loops by regulating senataxin proteostasis"

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In this manuscript, the authors investigated the roles of USP11 and KEAP1 in regulating SETX ubiquitination and stabilization. In addition, they found that perturbed SETX stabilization causes R-loops accumulations and DNA double-strand. This study provides interesting mechanisms that link SETX stabilization to genomic instability regulated by USP11 and KEAP1. However, I think there are several weak points regarding physiological significance in vivo. Also, several data lack appropriate controls and images. My comments are as follows. (To avoid biased comments, I did not read the reviewer's comments and rebuttal letter.)

1. The authors address that R-loops are converted to double-strand breaks. On the other hand, it has also been reported that R-loops accumulate at the sites of double-strand break and contribute to their repairs. It seems that the physiological relevance between R-loop accumulation and double-strand breaks is not clear. Also, they did not provide in vivo data. I wished they could provide the detailed data, such as USP11 knockout mice and aged mice. Regarding these questions, I'm curious about how USP11 and KEAP1 are regulated in vivo, such as enzymatic activity and subcellular localization

2. The authors showed aging data using over-passaged HEK293 cells. HEK293 cells are not a better option for monitoring the effects of cellular senescence. I would like to suggest that they develop USP11 KO mice and isolate MEFs, followed by analyzing these data.

3. It seems that changes in the SETX ubiquitination/deubiquitination status look weak (e.g., Fig.4a Ubi-SETX, Fig.4c Ubi-eGFP-SETX1-667, Fig.4h K48, Fig.6d K48). Also, the band patterns do not look like ladders or smears (Fig.4a Ubi-SETX, Fig.6a Ubi-SETX, Fig.6d K48). The band pattern of ubiquitination looks weird (Fig.4e His, right lanes Ni PD). To discuss based on powerful data, it would be better to analyze the ubiquitination status using not only western blotting but also MS. Moreover, in vitro ubiquitination/deubiquitination assays are missing. I think they should have presented these data using recombinant proteins such as USP11 and Cul3Keap1.

4. Because overexpressed GFP proteins are frequently ubiquitinated in cells, the authors should have

shown negative controls such as empty vector and GFP expressed vector (Fig. 4c, 4e, 4h)

5. There are no image data for quantifying the number of S9.6 foci in almost all data.

6. The quantification methods have not been described. I'm wondering how the authors quantify the S9.6 foci?, what the criteria of the size and intensity are?, what kind of ImageJ functions were used?

Overall, this paper is very exciting and informative. On the other hand, I want to know about the detailed mechanisms of how USP11 and KEAP1 are regulated in vivo. It would be better to present in vivo data and discuss the physiological roles and molecular mechanisms.