nature portfolio

Peer Review File

Specific catalytically impaired DDX3X mutants form sexually dimorphic hollow condensates

Editorial Note: This manuscript has been previously reviewed at another journal. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed my concerns from the last round of review. Overall, the work shows that several DDX3X mutants with defects in the ATPase/RNA binding cycle tend to form 'hollow' condensates in test cell lines and can localize with proteins involved in signaling pathways. As these mutants were chosen because of links to disease, the work raises the possibility that similar condensates are formed in organisms and may contribute to diseases. In addition, DDX3Y is found to enhance dynamics less effectively than DDX3X, which may contribute to sex biases.

With regard to my previous point on the fluorescence anisotropy experiments measuring RNA release, I appreciate the authors inclusion of raw, un-normalized binding data in their response, and in line with their suggestion in the response, I think these data should be included in the manuscript in some form. Simply put, it is critical to know that each of the mutants was largely RNA-bound at the start of the experimental time course, as the experimental signal depends on release of the bound RNA. The point that that the mutants that form hollow condensates retain bound RNA is made clear in the non-normalized data.

On a related point, the authors do not seem to strongly interpret the absence of any detectable decrease in bound RNA for the longer ssRNA or the duplex for any DDX3X variant (p. 8). Is this lack of a decrease because these RNA species bind more tightly, and under the conditions of the experiment any released RNA is rapidly re-bound? As it stands, it is unclear why the authors make the general conclusion that the mutants have decreased RNA release rates, when that behavior is only observed for one of three RNA species tested.

Minor point

On p. 8, it would be more helpful to report the ATPase results as a rate constant by dividing the reaction rate (1 μM/min for WT or 0.2 μM/min for mutants) by the DDX3X concentrations.

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We are happy to have addressed your concerns! We have replaced the normalized data in Figure 2f and Extended data Figures 2 d and 2e with the non-normalized as requested.

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In our concurrent preprint (**citation 20**), we explain in more detail why we interpret short strand release as a measure of activity. Briefly, in that study, we found that the addition of ATP primarily triggered the release of the short RNA strand and that the short strand release of DDX3X was faster than that of DDX3X, which correlates with their ATPase activities and RNA unwinding rates as measured by ensemble assays.

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We have done so, please see **page 8**