Rebollo et al., Identification and quantification of transposable element transcripts using Long-Read RNA-seq in Drosophila germline tissues.
– revised manuscript

The authors have put substantial effort in revising the manuscript, which, in my opinion, is now significantly improved. They have addressed sufficiently all my comments, especially by clarifying the issue of single- vs multi-mapping TE reads and by softening their conclusions on the biological significance of the non-replicated data. Thus, I maintain my feeling, that the strength of the manuscript lays in the technology used and the developed analysis tools. These, largely thanks to the comments of the other two reviewers, should now be much more reproducible and merit their sharing with the community.

Below, I have suggested a few last modifications:

2, 252, 266, 303 and throughout: For clarity, in a final version of the manuscript, the authors should better avoid using “long-read RNA-seq” and replace with “long-read cDNA-seq”, in order to avoid any confusion with ONT direct RNA sequencing approaches.

258: The statement “between ~1 to ~3 million reads per tissue” when there are only two samples in total (one per tissue) is misleading. Please rewrite.

Fig 1A and S10-11: I strongly feel that the transcript length bias, which is well explained in the text, is very important for this study and for others that might want to perform similar type of analysis. Thus, graphs from figures S10 and S11 should be moved to the main fig 1 (either in addition to or replacing the panel 1A).

288: Replace “to ensure” with “to check if”.

294: Move “(as suggested by the cDNA profile, Figure S1)” at the end of the sentence.

349: Please add a caution note reminding the reader that definite conclusions on the difference between sexes would require replicating the results.

371: Suggestion: replace “single-copy” with “copy-specific”.

393: Should be: “unambiguously mapping”

Fig 3B: The y-axis range for Pogo element graphs is too high. Add: “Each dot represents a unique genomic copy”.

487: Should be: “ONT long-read sequencing detects”

488: Please add that, knowing the poor recovery of long transcripts, expression of longer TEs copies might be underestimated. This statement, present in lines 510-512, can be moved up.

501: Please change to “may unvail” (in regard to lack of replicates, frequent low read support and more extensive splicing analysis).

544: Should be: “only one or two”

602: Should be: “to specific copies of transposable elements”.

616: Should be: “retrotransposed”

610: Should be: “TE transcripts are spliced”

614: Please add: “While our results suggest that TE splicing could be prevalent, additional studies with biological replicates, high sequencing coverage and mechanistic insights into the splicing machinery will be needed to confirm our observations.” Or a similar statement.