

Review of: "The Tudor Domain-Containing Protein, Kotsubu (CG9925), Localizes to the Nuage and Functions in piRNA Biogenesis in *D. melanogaster*"

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In order to illustrate the piRNA biogenesis, Li et al. identified and characterized one of the *Drosophila* Tudor domain-containing protein-1 (Tdrd1) orthologs, named it Kotsubu (Kots, CG9925). Kots localizes to the Nuage in the germline cells as other piRNA components, it also exhibits dynamic localizations during spermatogenesis. Loss of Kots cause a moderate reduction in piRNAs and derepression of TEs from both male and female flies. Overall, this decent work describes canonical methods to explore the functions of Kots in germline.

There are several concerns regarding this study listed in below:

1. Since the authors address Kots as a Tudor domain-containing protein, it did not provide any functional details related to these Tudor domains. In Handler et al. paper ([10.1038/emboj.2011.308](https://doi.org/10.1038/emboj.2011.308)), they identify the core Tudor domain features including CG9925, it would be nice to see the biological relevance due to different truncations or disruption of each Tudor domain.
2. The author implies Kots interacts with both Ago3 and Aub, and might even involves in the homotypic ping-pong cycle, it would be interesting to know whether these interactions depending on arginine methylation or not? Do these interactions pattern partially interpret the observed Kots-based piRNA abundance rather than ping-pong signature or piRNA diversity regulation?
3. It seems Kots has more severe defects in testis than in ovary, it would be interesting to dissect Kots functions mainly in testis, especially its role during spermatogenesis.
4. The authors showed piRNA abundance in Kots mutant were more affected in tests than in ovary, but the ping-pong signature was unaltered in testes. It's not clear by just simple explanation of alternative mechanism of piRNA biogenesis in the testes. It would be nice to address in which step does Kots affect piRNA processing, so the study could provide hints of molecular mechanism and future direction.