The manuscript titled “Hipk is required for JAK/STAT activity and promotes hemocyte-derived tumorigenesis” aims to determine if there is a role for Hipk in JAK/STAT signaling. The novelty of this paper lies in the fact that hipk has been reported to have a role in several different pathways, but not yet in JAK/STAT signaling. The authors used several techniques to show that hipk overexpression in flies causes pigmented masses derived from hemocytes. They also showed that loss of hipk improves tumorigenesis outcomes in flies with the hop Tum-1 mutation, a mutation previously reported to be associated with increased JAK/STAT signaling. Lastly, the paper shows that hipk activates JAK/STAT signaling downstream of upd and interacts with Stat92E, the transcription factor of the pathway in flies. Collectively, the paper provides strong evidence to support the claim that hipk has a role in JAK/STAT signaling and should be further investigated as a potential therapeutic target for hemocyte derived human cancers. However, to help the reader better interpret the data, some clarifications and experiments could be addressed.

Clarifications that would aid to data interpretation:

1) On the pre-print website BioRXiv, the supplemental data was not available. It would be helpful if the supplemental data was included to allow the reader to further understand the experiments and conclusions, adding to the strength of the data in the body of the paper.

2) In Figure 1C, P35 treatment resulted in tumors that seem smaller than the hipk overexpressed animals that were not treated with P35. We have two questions regarding this experiment - Why do you think this happens? Do these results suggest that P35 affects tumor formation?

3) Figure 2E nicely shows the rescue effect of hipk in hop Tum-1 mutants. To make the data clearer, it would be relevant to see significance values in the graph or individual data points from these experiments.

4) In Figure 3, the authors do not state what RFP represents. As a reader it was difficult to look at the figure and understand the data without the meaning of RFP. What is the nature of the RFP construct? Does it represent loss of hipk? The addition of this information will significantly increase the reader’s ability to understand the data in the figure. Furthermore, it would also be helpful to the reader if the authors could quantify the fluorescence in the images to allow easier comprehension of the data.
Experiments that would aid to data interpretation:

1) In Figure 1G-H, the authors compared hemocyte amounts. To contribute to the conclusions of this experiment, it would be helpful to see an additional experiment where proliferation markers were tested. This way, it would be clear that the hemocytes are in fact proliferating more versus more cells differentiating into hemocytes. Also, is there a potential of the hemocyte clusters to form tumors? Are there any other characteristics of the hemocyte clusters that are indicative of early tumor formation? Could hemocyte clusters form structures that look like melanotic tumors?

2) Throughout the paper, it would be helpful to have mRNA and/or protein levels of target genes of the JAK/STAT pathway to further convince the reader of the involvement of Hipk in the JAK/STAT signaling pathway.

3) Finally, we were unsure about the role of Hipk in the JAK/STAT signaling pathway when compared to Hop - Do the authors hypothesize that hop and hip are acting in parallel to regulate JAK/STAT signaling or that one is acting downstream of the other?