Reviewer's report

**Title:** Therapeutic Potential of Reprogramming the Wnt Pathway in Human Embryonal Carcinoma Cells

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**Reviewer:** Raymond Habas

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This interesting manuscript examines Wnt signaling components and the reprogramming of this pathway during differentiation of human EC cells. The authors find clear evidence for activation of canonical Wnt signaling during RA-induced differentiation monitored by a reporter-based assay or knockdown of POU5F1. A number of Wnt ligands were found to be up-regulated and some repressed along with Frizzled receptors and other components of the Wnt pathway. Overall this is an interesting study and one that will likely be of interest to BMC Cancer readers. I however have a few concerns that I would like the authors to address prior to acceptance of this manuscript.

- For their studies in Figure 1, I would like the authors to show evidence of the efficacy of the knockdown of POU5F1. This is an important conclusion and it would be useful to know the levels of knockdown achieved, preferably by examining the POU5F1 protein.

- In Figure 1, to further clarify this important observation that canonical Wnt signaling is active, I would like the authors to show this using an independent approach such as examining the nuclear levels of #-cat or levels of active #-cat using the ABC antibody.

- In Figure 3, the Western blot shown the efficacy of knockdown of Fz5 and Fz7 lacks an important control lane namely un-transfected cells. Furthermore it is not clear why the Fz7 overexpressed positive control band runs higher that the band in there other lanes, can the authors address this?

- In the supplemental Figure 5, it is unclear why the Wnt5a-2 siRNA that resulted in the mid-expression levels of Wnt5a gave the strongest effect on colony formation?

- As the authors observe a strong effect on colony formation likely mediated by non-canonical Wnt signaling, I would like the authors to examine some aspect of the activation level of this pathway such as JNK activation or a similar assay to add weight to this observation.

**Level of interest:** An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.