Response to Reviewers

**Reviewer 1**

1-1) I would still recommend tempering statements based on Np1-Fc treatment, since the authors indicate that Np1-Fc is not as effective at Np-1/VEGF inhibition. For example, the final sentence of the results section, which states, "knockdown of VEGF signaling by two distinct methods…suggesting that there are different cell responses to loss of VEGF", may not be accurate, since the authors propose that there are technical differences between the methods. For the same reason, the statement in the preceding paragraph, "We did find that trailer Np1-Fc neural crest cell molecular profiles were most similar to trailer control neural crest cells", should probably be qualified, since no change in gene expression might just be attributable to inefficient inhibition.

*Response: We have made the suggested changes.*

**Reviewer 2**

2-1) The requested movies were added, but the presentation order of movies 1-5 is illogical. Why identify movies 1 and 2 as such when they are the last to be discussed? The presentation order should be altered.

*Response: Done.*

2-2) The coverage of Np-1 siRNA versus Np1-Fc injection is better but not fully resolved. I agree that diffusion of the Fc could affect the response, but are the authors not concerned that blocking signaling of the factor that is supposed to induce lead response is resulting in upregulation of invasive lead gene expression, including genes that were induced by VEGF in vivo and in culture?

*Response: We appreciate the Reviewer’s comment and have added a sentence to clarify this in the text. Briefly, our interpretation of the upregulation of invasive genes in lead neural crest cells transfected with Np1 siRNA is that cells may respond to other signals for guidance information, perhaps through Np2 or VEGFR2. With Np1-Fc, no invasive genes were upregulated.*

2-3) p.21: "These results indicated that VEGF is not required for proper migration of the trailer cell population," which is consistent with the data. But on p. 23: "We conclude that microenvironmental signals, including VEGF, impact the lead and trailer neural crest cell identity." This sentence is not resolved as indicated in the rebuttal. It is too general (only VEGF was tested), and normally VEGF does not affect the trailer cells. Please select a more precise wording.

*Response: We agree and have appropriately changed the text on pg. 23.*

2-4) Figure 3 legend: based upon the Venn diagram that was added, I think it should be indicated that all genes listed were significantly changed, not downregulated as stated, since genes in green are upregulated and then unchanged. Figure 5: legend, G, H, the distinction between between control and control \* needs to be explained. Red is not included in the key at the end.

*Response: We thank the Reviewer for this suggestion and have clarified both figure legends. Briefly, all genes listed were initially downregulated upon exposure to VEGF. No genes were upregulated in this diagram upon initial exposure to VEGF. The color coding is then indicating responses during the removal or readdition. In Fig. 5 legend, we have clarified the controls.*