Reviewer# 02453616

Question 1: Why was circRNA 5139 chosen but why not 436 and 9285?

We chose candidate circRNA that was differential expressions on E16-E21 and had a certain trend. We first excluded rno_circ_0000436 because of its almost no differential expression on E16 in ARM group and control group. Indeed, rno_circ_0009285 had a good trend and statistical significance of differential expression, but the follow-up experiments showed that rno_circ_0009285 had no binding sites with the miR-324-3p targeting to Wnt5a, and we recently looking for another miRNA as the target of rno_circ_0009285 in the experiment. So in this study, we finally chose rno_circ_0005139 as the final candidate circRNA.

Question 2: Why was Wnt5a chosen?

Compared with Wnt2b and Wnt10, Wnt5a had been confirmed abnormal expression in the human, mouse and rat anorectal malformation. At the same time, Wnt5a played an essential role in the development of ARM, while the importance of Wnt2b and Wnt10b in ARM was still to be determined. The chose of Wnt5a was a more reliable basis to study the mechanism of circRNA targeting gene in this study.