

Response to the reviewer's comments

point-by-point response

Reviewer 2

1. The manuscript needs to be carefully checked. For example: Both colorectal carcinoma (CC) and colorectal cancer (CRC) appear in the text and need to be unified. In addition, the abbreviations that have been defined in the article can be used directly after appearing, and there is no need to list them again.

Response: We agree with the reviewers and have checked and revised the relevant descriptions in the text.

2. In Figure 8 I can clearly see that in the immunofluorescence staining, the red and green markers are different markers because their staining parts are different. However, in Figure 9, it seems that the red and green parts overlap greatly. Why is this?

Response: Thanks for the kindly reminder of the reviewers, we think that the reason for this may be the difference of cell types (293T and SW480), as well as the difference of endogenous expression of GFP and immunofluorescence detection. The purpose of our experiment is to determine the co-localization, we believe that these differences do not affect the results of co-localization.

3. Fig 2D,E and Fig 6B which should be replaced by new one is too difficult to distinguish;

Response: We agree with the reviewers and have replaced the original images with high resolution and enlarged images.

4. The words in brackets in Figure 9A and C are GFP, and the words in brackets in Figure 9G are green. Is there an error?

Response: Thanks for the kindly reminder from the reviewers. It's really a mistake of annotation. We have made a new annotation and correction.

5. The discussion part can be more comprehensive, authors could discuss the limitations of the current research or the regrets so far and what kind of follow-up research can be carried out in the future.

Response: We agree with the reviewers and in the last paragraph, we have discussed the limitations of the current research or the regrets so far and what kind of follow-up research could be carried out in the future.

References:

1. Sun B, Zheng YL. Simultaneous Quantification of Multiple Alternatively Spliced mRNA Transcripts Using Droplet Digital PCR. *Methods Mol Biol.* 2018;1768:387-400.
2. Wang H, Wang H, Duan X, Sun Y, Wang X, Li Z. Highly sensitive and multiplexed quantification of mRNA splice variants by the direct ligation of DNA probes at the exon junction and universal PCR amplification. *Chem Sci.* 2017 May 1;8(5):3635-3640.
3. Wan J. Antisense-mediated exon skipping to shift alternative splicing to treat cancer. *Methods Mol Biol.* 2012;867:201-8.
4. Maruyama R, Yokota T. Creation of DMD Muscle Cell Model Using CRISPR-Cas9 Genome Editing to Test the Efficacy of Antisense-Mediated Exon Skipping. *Methods Mol Biol.* 2018;1828:165-171.

Reviewer 1

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth most common cause of cancer death. Despite treatment advances for CRC over the past decades, novel molecular therapeutic strategies are required to generate informative biomarkers and identify new targets. In this study, authors generated PCR primers capable of specific detection of the ABI1-SiL isoform. Furthermore, compared with ABI1-p65, expression of ABI1-SiL is significantly decreased in colorectal cancer tissues and cell lines, and over expression of ABI1-SiL represses migration and adhesion in SW480 colon cancer cells. Based on their findings, both ABI1-p65 and ABI1-SiL are able to interact and co-localize with WAVE2 and ABI1-p65 in SW480 cells. Authors are commended for their extensive and well carried out study and offering a putative biomarker and novel therapeutic target for colon cancer.

Response: There are no specific comments. Thank you.

Reviewer 3

The article with the title “ABI1 splice isoform-L (ABI1-SiL) plays an anti-oncogenic role in colorectal carcinoma through interactions with WAVE2 and full-length ABI1” is in generally well done. Title: Appropriate. It reflects the main content of the research. Authorship: Is correct. Institutions: are correct ORCID number is correct Authors contribution is correct Abstract. Is a structured abstract according to the required format. In 288 words authors showed a summary of the content of the manuscript. Key words: 5 that reflect the content of the study. Core Tip: In 71 words author reflect properly aspects that should call attention to the readers Background: It is a basic study with a

high importance for the clinical practice. Colorectal cancer is a common disease and important cause of cancer-related mortality. **Method:** Authors made the detailed description of the investigations. **Results:** Authors demonstrated that overexpression of ABI1-SiL in SW480 cells significantly increases the cell surface area and inhibits the adhesive and migration properties of the cells, but does not alter their invasive capacity. **Discussion:** Authors made a detailed and informative discussion of the results. **Illustrations:** They show 9 figures with their corresponding legend. All figures are showing clearly making and adequate support of the results. **Biostatistics:** This work met the requirements of biostatistics. **References:** Authors cited properly actualized references of high interest for their propose in introduction and discussion **Organization of the study:** It was properly organized **Research method reporting.** As a basic study it have been reported according with the corresponding guidelines **Comments to the author.** In this manuscript authors confirmed the hypotheses that ABI1-SiL may have anti-oncogenic roles by competitively binding to WAVE2, and directly interacting with phosphorylated and non-phosphorylated ABI1-p65, functioning as a dominant-negative molecule towards ABI1-p65. This was recognized by authors as a point of recommendation for the future.

Response: There are no specific comments. Thank you.