Peer Review File

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Reviewer A

Comment 1: Is "colorectoscopy" another name given for "colonoscopy" in certain countries. I am unfamiliar with that term.

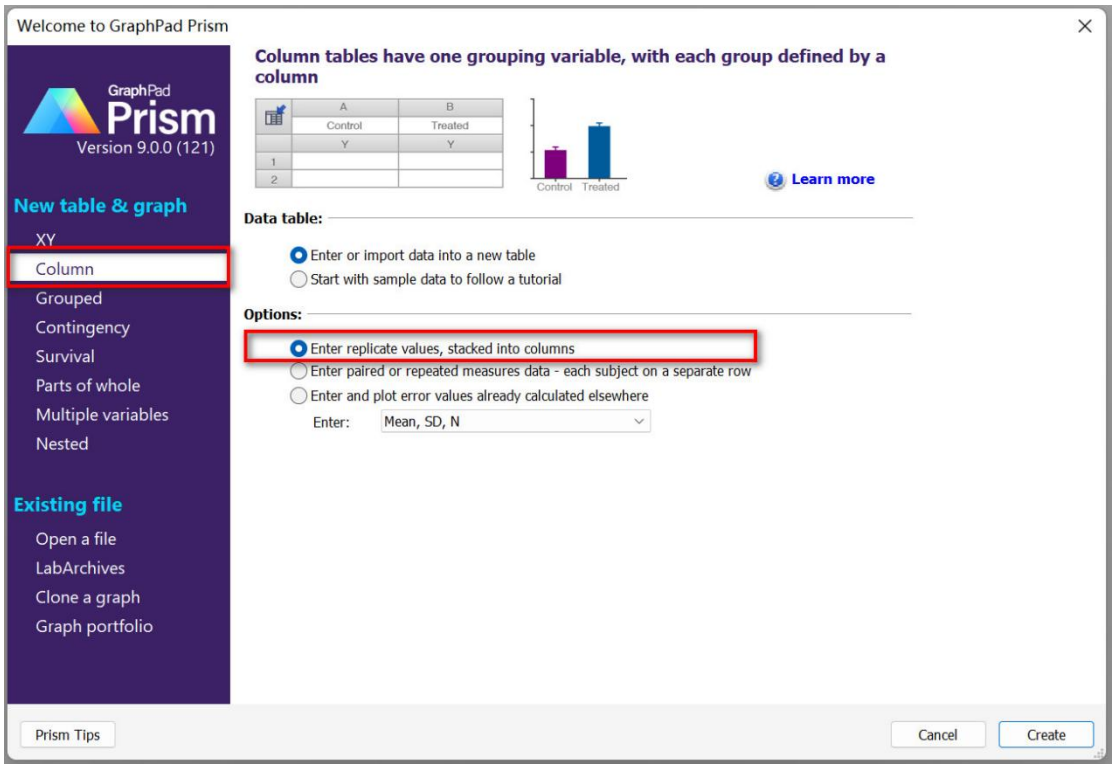
Reply 1: Thank you for your careful review. Indeed, both terms are capable of indicating the examination of the colon. Currently, the term "colonoscopy" is used to represent the majority of colonoscopies. Therefore, to avoid misunderstanding, we have changed "colorectoscopy" to "colonoscopy" in the manuscript.

Changes in the text: We have modified our text as advised (see Page 3, line 63).

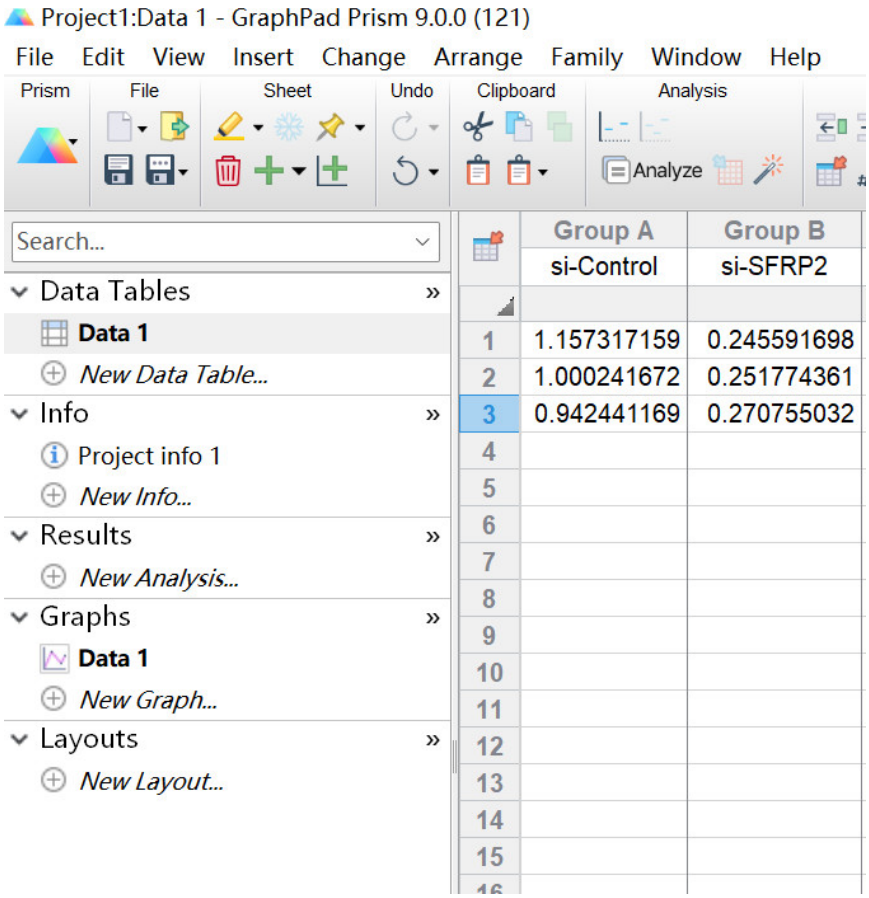
Comment 2: The error bars presented - for example - in the HCT-116 data, were those SD or SEM? It should be SD.

Reply 2: Thank you for your thought-provoking comments and valuable suggestions. The error bars in the HCT-116 data in our manuscript represent SD. In the 'statistical analysis' paragraph of the methods section (see Page 7, line 176), we state that we used GraphPad Prism 9.0 software for the statistical calculations, and in the results presentation section we chose SD. For example, for the PCR results, after we entered the 2-ΔΔCt values for three replicate experiments, we chose SD to represent the error bars. Please see the graphical illustration below:

First, open Graphpad prism 9 and select Column.

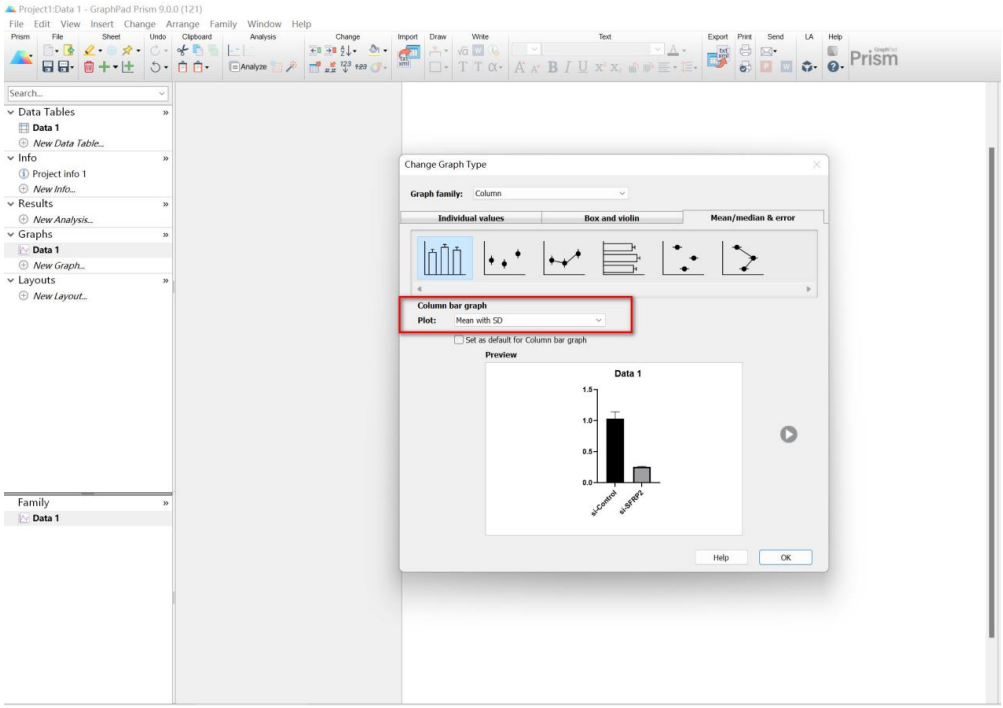


Then, copy the 2-ΔΔCt data from the qPCR of HCT116 and paste it into the data tables.



Finally, find the Graphs and click on the name corresponding to the data above, and the graphs will appear. By selecting SD in the column bar, we get the graphs of the qPCR

results for HCT116 in the manuscript.



Comment 3: Did the RNA isolation remove genomic DNA?

Reply 3: Thank you for your thought-provoking comment. We use an RNA kit (Promega, Madison, USA) to isolate RNA, and we follow the manufacturer's instructions strictly. During the isolation process, there are specific steps to remove genomic DNA, as follows:

Prepare **DNase I incubation mix** by combining the following amounts of reagent, per sample, in the order listed:

Solution	**Volume**	×	**Number of Preps**	=	**Total**
Yellow Core Buffer	24µl				
$MnCl_2$, 0.09M	3µl				
DNase I	3µl				

Mix by gently pipetting; do not vortex. The volumes listed above make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare.

We re-examined the quality of the isolated RNA using a UV spectrophotometer again, and the results showed that the ratios of both A260/A280 and A260/A230 were between 1.8 and 2.2, confirming that there was almost no DNA residue in our isolated RNA samples (Figure R1 and R2).

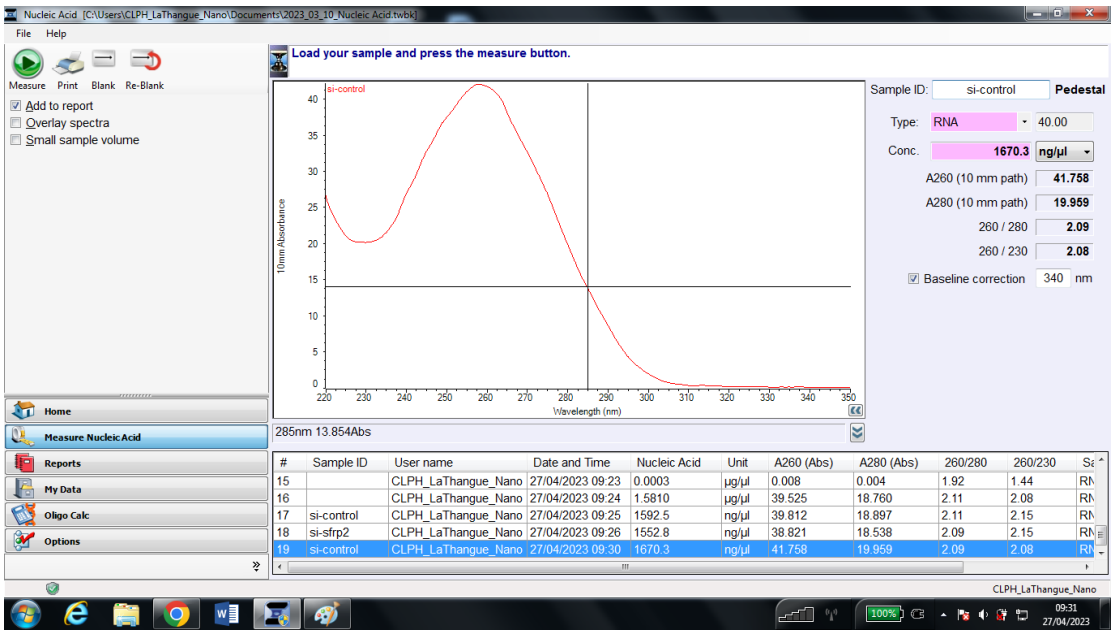


Figure R1: RNA test results of si-conctrol group.

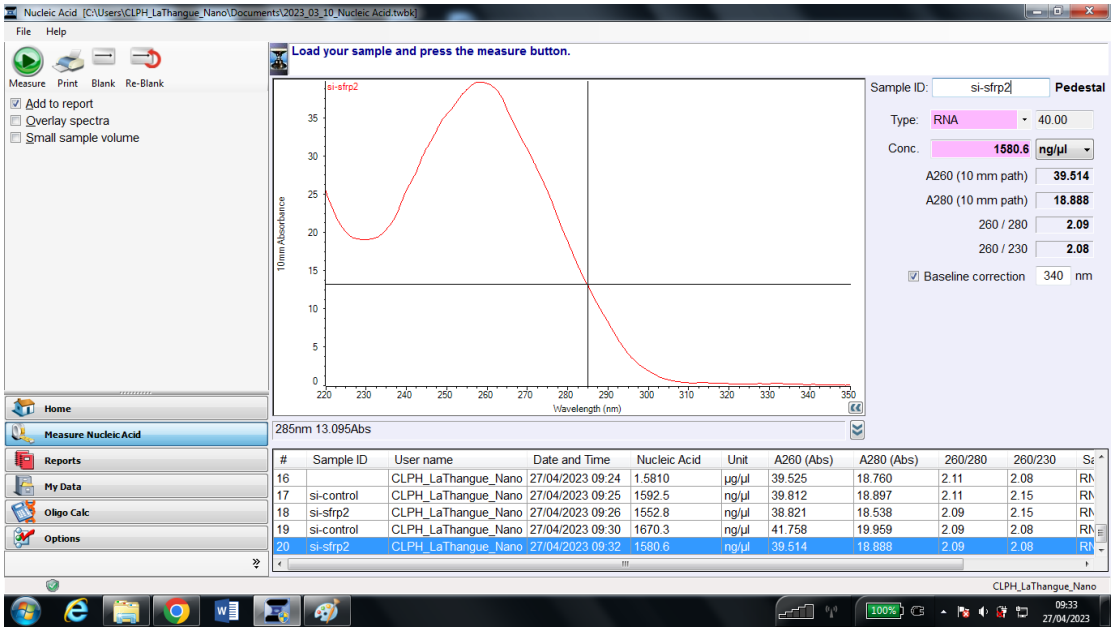


Figure R2: RNA test results of si-sfrp2 group.

Subsequently, we performed denaturing agarose gel electrophoresis, which also showed no DNA residues in our isolated RNA samples (Figure R3).

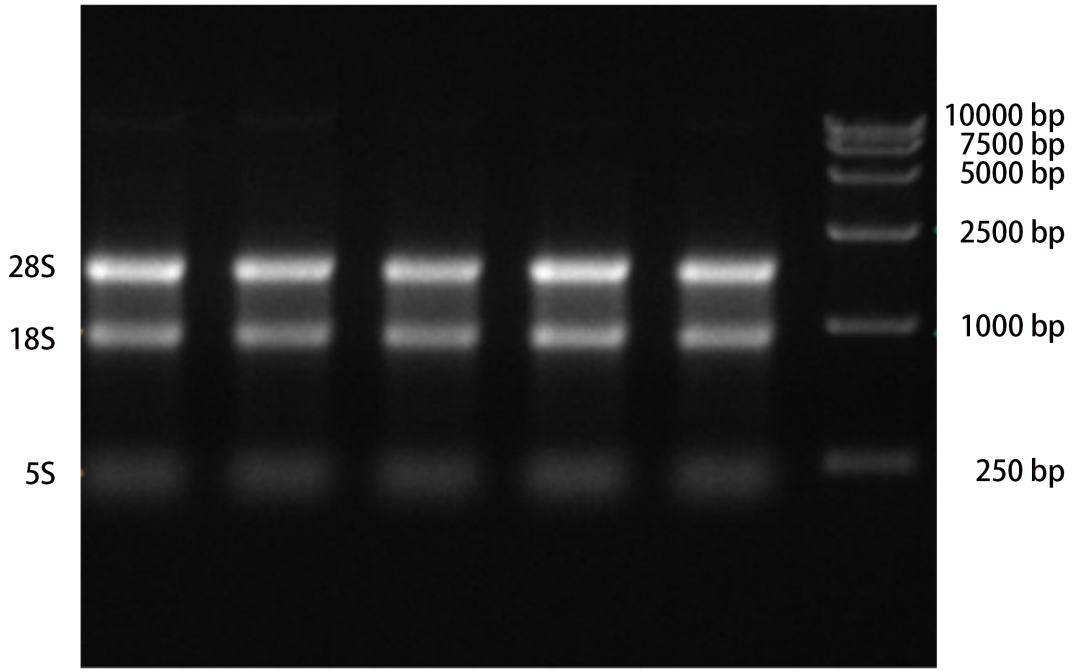


Figure R3: Denatured agarose gel electrophoresis result.

Comment 4: Were there any no-RT negative controls for the RT-PCR to ensure that no left-over DNA was being amplified in the RT-PCR reactions?

Reply 4: Thank you for your thought-provoking comment. We set up a negative control at the time of reverse transcription. Specifically, in the reverse transcription assay, the negative control is performed without reverse transcriptase and the cDNA obtained from the reverse transcription step is subjected to subsequent fluorescent quantitative PCR. Our results showed that no amplification was detected, ensuring that no remaining DNA was amplified in the sample of the RT-PCR reaction.

Comment 5: In addition, the line used, HCT-116 is APC wild-type and beta-catenin mutant, while most CRCs are the opposite. That s something worth noting and that future experiments should use APC mutant lines.

Reply 5: Thank you for your thought-provoking comments and valuable suggestions. Indeed, approximately 80% of colorectal cancers are associated with mutations in the APC gene that result in elevated levels of β-catenin protein. Elevated levels of β-catenin and subsequent accumulation of the protein in the nucleus, where β-catenin activates numerous genes that drive cell proliferation and promote colorectal cancer growth and maintenance.

Due to the limitation of experimental conditions, we temporarily had only HCT116 cell at the time of conducting the experiments, which we are also aware of and point out in the discussion section. According to your suggestion, we will add APC mutant cell lines in future experiments for further validation.

Again, we would like to express our gratitude to you. Just because of your suggestions and comments that make it possible to substantially improve the quality of our manuscript in a short time. We have tried our best to revise the manuscript and address the comments, and hope that you will be satisfied with our revision.

Reviewer B

Comment 1: Figure 3
Please explain CRC and SFRPs in the legend.
Reply 1: We have added explanations about CRC and SFRPs in the legend (see Word version of Page 17, line 519-520).

Comment 2: Figure 4
Please explain FRZB, TPM, CRC and SFRP in the legend.
Reply 2: We have added explanations about FRZB, TPM, CRC and SFRP in the legend (see Word version of Page 17, line 523-525).

Comment 3: Figure 5
Please explain FRZB, TPM, CRC and SFRP in the legend.
Reply 3: We have added explanations about FRZB, TPM, CRC and SFRP in the legend (see Word version of Page 18, line 528-530).

Comment 4: Figure 4 and 5
Figures, tables and videos should be cited consecutively in the text and numbered in the order in which they are discussed. Figure 4A should be cited first, then Figure 4B, Figure 5A, Figure 5B etc. Please revise.
Reply 4: We have modified our text as advised (see Word version of Page 9, line 242-245, 219-220).

Comment 5: Figure 6
Please explain CRC and SFRP in the legend.
Reply 5: We have added explanations about CRC and SFRPs in the legend (see Word version of Page 19, line 535-536).

Comment 6: **Figure7**

Please explain CRC in the legend.

Reply 6: We have added explanations about CRC in the legend (see Word version of Page 19, line 541).

Comment 7: **Figure 8**

Please explain TPM and SFRP in the legend.

Reply 7: We have added explanations about TPM and SFRP in the legend (see Word version of Page 19, line 544).

Comment 8: **Figure 9**

Please explain KEGG, CRC and SFRP in the legend.

Reply 8: We have added explanations about KEGG, CRC and SFRP in the legend (see Word version of Page 19, line 552-554).

Comment 9: **Figure 10**

a) Please explain CCLE, CRC and SFRP in the legend.
b) Please provide the meaning of the symbol "*, **" in the legend.
c) Please provide the staining method of 10D in the legend.
d) Please provide the observational method of 10E in the legend.
e) Please provide the scale bar in the figure or magnification for 10E and 10F in the legend.

Reply 9: We have modified our text as advised (see Word version of Page 20, line 560-565).