Dear Dr. Dsouza:

We would like to thank the reviewers for the thoughtful review and constructive comments regarding our manuscript. Our response to each reviewer and editor is detailed below in a point-by-point fashion; our changes to the manuscript are highlighted in blue.

**Comments from Peer-Reviewers:**   
**Reviewer #1:**  
**Manuscript Summary:**  
Authors have elegantly presented a viral transduction as alternative and efficient method for the gene modification. They also described a protocol to generate frozen sections from intact organoids for IHC.

- We appreciate this reviewer’s positive comments.   
  
**Reviewer #2:**  
**Manuscript Summary:**  
The manuscript by Xian and colleagues provides a detailed protocol to modify gene expression in intestinal organoids in 3D cultures. The method is based upon viral transduction coupled with magnetic nanoparticles and application of a magnetic field to increase transduction efficiency.  
Intestinal 3D cultures are currently considered as a major tool to study intestinal stem cells in an integrated context. Several protocols have described methods to modify gene expression in organoids, but their efficiency remains low. CRISPR/Cas9-based genome editing has also been proposed as a key tool to engineer isolated intestinal stem cells - essentially to introduce point mutations-, but transfection of these cells remains a challenging issue. Thus, this new technology of viral transduction, nanoparticles and magnetic field appears of great interest when considering altering the expression of a gene by gain-of-function/loss-of-function approaches.  
In summary, the article is clear, well written and more importantly, the methodology excellently detailed.  
I have no further comments.

--We appreciate this reviewer’s positive comments  
  
**Reviewer #3:**  
**Manuscript Summary:**  
The manuscript describes a protocol for viral-mediated transduction of organoids in order to genetically manipulate their DNA. This protocol is well written and very easy to follow. More importantly, it will allow other laboratories to use this technique for their own in vitro studies of intestinal organoids.  
We appreciate this reviewer’s positive comments.

**Major Concerns:**  
No major concerns  
  
**Minor Concerns:**  
It might be a good idea to show original pictures as well as a cartoon of crypt and villus  
The GFP in Figure 4B is slightly out of focus.

- The GFP in Figure 4B appears slightly out of focus because GFP is expressed in a 3D plane whereas the image is taken as a 2D plane. Thus, some GFP that will be out of focus.   
  
**Reviewer #4:**  
**Manuscript Summary:**  
In the manuscript "Genetic Engineering of Intestinal Organoids via Magnetic Nanoparticle Transduction of Viral Vectors for Cryosectioning and Molecular Analysis", the authors describe the process of preparing for and culturing intestinal crypts for transduction and downstream applications. They do a thorough job of describing the techniques used and present a clear protocol for others to follow. If the authors could address the following very minor issues, the manuscript would be of interest to JoVE.  
  
**Major Concerns:**  
None  
  
**Minor Concerns:**  
1. For how long can the concentrated viral particles be stored at -80C? (Section 2.4 step 5)

- Concentrated particles can be stored for 6 months to a year. We added this to the text at 2.4.5 to clarify this important point.  
2. What is the recommended timeframe between steps 3 and 4?

- Transductions can be performed directly on isolated crypt cells prior to forming organoids or once organoids have been established and passaged. While we have not formally tested whether the time that organoids are cultured affects the transduction efficiency, we have performed transductions in organoids generated after several days or several weeks. We added a new step (4.1) to clarify this important point. We also edited step 5.1 to indicate that isolated crypt cells can also be transduced.  
3. For step 8, are cultures disrupted in the same manner as used for viral transduction?

- Step 8 does not require disruption of cultures.   
4. Figure 1a and 1b would be clearer with labels for the cells or a short description. Table 1 is hard to follow.

-We appreciate the suggestion and added labels as well as a short description with images of crypts and villi. Table 1 was also been revised.

**Response to the JoVE Editors**

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

1) Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

a) Examples NOT imperative tense: “To isolate crypts from the small intestine, mice are first humanely sacrificed according...”;” Approximately 24 h after seeding, the crypts will organize into small, round cystic shapes”; Lines 225-227 etc.

- The text was revised to the imperative tense.

• Please ensure that the manuscript title best reflects the filmable content (i.e. the portions you highlight).

-Yes, the title reflects the filmable content.  
  
• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more details to the following protocol steps (please note that this is guide, and not a complete list). Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.**There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

1) Line 113: Please cite a reference for the plasmid use.

- We now cite a references for the plasmid used.

2) Line 142: Mention culture temperature and duration.

- Culture temperature and duration were added.  
3) Line 167: Mention euthanasia method.

- Euthanasia method was added.  
4) Line 170: Mention dissection tools used.

- Dissection tools were added.  
5) Line 259: Mention incubation temperature.

- Incubation temperature was added.   
6) Section 8: More details are needed for fluorescence microscope, and flow cytometry, RT PCR (mention primers, and cycle conditions).

- Section 8 is a validation step for gene expression and/or protein levels. Therefore, investigators should use their own optimized protocols to validate their gene or protein of interest.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

- The protocol has been formatted accordingly.   
  
• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.  
  
The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.  
The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.  
Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.  
Notes cannot be filmed and should be excluded from highlighting.  
The protocol has been formatted accordingly.   
  
• **Results:** Please add at least one paragraph of results text that explains your representative results in the context of the technique you describe; i.e. how do these results show the technique, suggestions about how to analyze the outcome etc. This text should be written in paragraph form under a "Representative Results" heading and should refer to all of the results figures. You may include the figure captions under this heading but the captions and figure text must be separate entities.

- A paragraph of the results text was added to the Representative Results section.  
  
• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

The discussion was modified to include the following:

1) Modification and troubleshooting of the protocol

2) Limitations

3) Significance of methods with respect to existing methods

4) Additional future applications

5) Critical steps   
  
• **Figure/Tables:**  
1) Fig 1, Fig 4A, B, C: Please expand the legends to adequately describe the figures, e.g. what do the colors indicate? Please discuss the significance.  
2) Fig 4A, C: Please provide scale bars, and define them in the figure legend.

We have addressed these issues.   
  
• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are OPTI- MEM, Falcon 2054, lipofectamine 2000, Amicon Centrifugal Filter, Amicon filter, Eppendorf, Thermo fisher, (Boekel Scientific 260250 Orbitron Rotator II), matrigel, pipetman, ViroMag, MF10000, OZBIOSCIENCES, m Addgene, Glutamax

1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

- Commercial names were eliminated but could be found by checking the indicated references or Materials Tables if needed.   
2) Please check Table 1, and Figure 2 as well

- Corrected as noted above.

• **Table of Materials:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as animals strains, microscope, etc

- Table of materials have been updated accordingly.  
  
• Please define all abbreviations at first use (e.g. DTT, ENR, etc)

- Abbreviations were defined at first use.  
  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

- All SI units have been formatted.  
  
• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

-We did not use any published figure for this manuscript.