**Response to Comments**

•Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

Thank you for the excellent comments as we attempt to perfect the manuscript.

•The Short Abstract should clearly summarize the nature and aims of the protocol.

The short abstract has been edited to better summarize the aims and nature of the protocol.

•Scattered grammar issues should be addressed: -Line 33: "at firs" -1.3: "Apply a smooth thick layer of hair removal and leave in place for 3 minutes." Missing a word, probably hair removal cream? -The first sentence of step 3.2 is not written in imperative tense; it could become a Note after 3.1, or could be re-written to use imperative tense as part of 3.2. -Line 102: should be a space between 1 and cm. -In the Note following 4.1, units should not be crashed. -Step 8.1 and 9.2: Should be "perform" rather than "preform." -Step 9.1: Degree symbol is incorrectly crashed with the temperature number.

The various grammatical errors have been corrected.

•Regarding formatting, a protocol section should have at least three steps. For instance, it is not necessary to create a separate section for euthanasia, when this "section" consists of a single step. We recommend combining all operative sections under one heading or, at the least, combining pre-infusing and post-infusion sections.

All protocol sections have been edited to streamline the protocol.

•Additional detail is required: -1.1: Please specify means of administration (one assumes a nose cone?). -1.2: Please specify type of microscope (one assumes a dissecting microscope). -9.2: Section using what?

These details have been added.

•If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

The figures are original.

•JoVE reference format requires that the DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

The DOIs have been included when available.

•IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

**Reviewers' comments:**

**Reviewer #1:** *Manuscript Summary:* This is a great paper from a lab with top expertise in this technology.  *Major Concerns:* None.  *Minor Concerns:* None.  *Additional Comments to Authors:* N/A

Thank you for the excellent feedback.

**Reviewer #2:** *Manuscript Summary:* In this manuscript entitled "Pancreatic Duct Infusion: A Effective and Selective Method of Drug and Viral Delivery" by Joseph Fusco et al., the authors describe in detail a standardized protocol to infuse solutions to the pancreas through the pancreatic duct. The paper is clearly written, providing the necessary level of detail for the readers to replicate it. Critical steps and limitations are identified and described. Some points could be clarified:

*Major Concerns:*

1. In the Protocol Section, step 4. Infusion, 4.1- it would be beneficial to provide a range of adequate infusion volumes. What is the minimum and maximal volume that should/can be infused into the pancreas to balance maximum efficiency with the least tissue disturbance?

This range has been added to the step.

2. Protocol section, step 9. Sample Preparation 9.1- should the liver be added to the list of collected control tissues? It is mentioned in line 153 as a control but not mentioned during the collection step. Also, the liver is one of the organs most at risk of unspecific delivery and should be consistently evaluated for specificity of the procedure.

The liver was added as an additional control tissue.

*Minor Concerns:*

1. The title should read: Pancreatic Duct Infusion: AN effective method…. vs. A effective method…..

The title has been changed.

2. Line 146- the word "realized" in "the desired effect should be realized in the pancreas" should be replaced by a more adequate and clear concept. Perhaps "evident", "clear"?

The wording has been edited.

*Additional Comments to Authors:* N/A

Thank you for the thoughtful feedback.

**Reviewer #3:** *Manuscript Summary:* A surgical procedure for the delivery of agents to the mouse pancreas is described. The agent used in this example is an adeno-associated viral vector expressing GFP. The procedure is described simply and effectively with attention to suitable methods to prevent infusion into the liver and other non-targeted organs. Key points are appropriately emphasized, especially the technical challenge of the surgical procedure and the likelihood of eliciting variable degrees of inflammation from the procedure.

*Major Concerns:* No major concerns other than suggesting that the authors provide an estimate of the success rate (measured as exclusive delivery to the pancreas) that might be expected for the novice, well-trained and expert practitioner.

The success rate has been included.

*Minor Concerns:* Typographic issues identified by line and some general minor concerns: 33 - "firs" should be "first" (Also in front page abstract) 134, 141 - "preform" should be "perform" 201 - "The have" should be "They have" or preferably, "The authors have" 70 - The reference to "vet ointment" can be understood by context but should be replaced with a more specific compound. The compound should be added to the list of materials. In many places, a space is needed between the value and unit of measure (e.g, 10uL, 5mg/kg, ...)

These typographical errors have been edited.

*Additional Comments to Authors:* N/A

Thank you for the thoughtful feedback.