TOURO UNIVERSITY
CALIFORNIA
COLLEGE OF OSTEOPATHIC MEDICINE

TAMIRA ELUL, PH.D.
ASSOCIATE PROFESSOR
DEPARTMENT OF BASIC SCIENCES

May 17, 2019

Dear JoVE editor,

Thank you for considering our manuscript titled *"Microinjection of DNA into Eyebuds in Xenopus laevis Embryos, and Imaging of GFP Expressing Optic Axonal Arbors in Intact, Living Xenopus Tadpoles"* for publication in JoVE. We are grateful to the editors and reviewers for their thorough review and critique of the initial version our manuscript. In response to the comments made by the editor and reviewers, we made multiple changes to the manuscript (to text, figures, and table of materials) and uploaded additional files verifying copyright permission. In the attached pages, we explain our responses to all the specific comments of the editor and the reviewers.

Please let us know if you have any additional questions regarding our revised manuscript.

Sincerely,

Tamira Elul, Ph.D.

Associate Professor
Touro University California
Tamira.elul@tu.edu

Responses to Editorial comments:

1. Proofreading of the manuscript. The editor requested that we proofread our manuscript to ensure there are no spelling or grammar issues.
Response: As requested, we thoroughly checked our manuscript to eliminate all spelling or grammar issues.

2. Copyright Permission. The editor requested that we obtain copyright permission to reuse figures from previous publications, and that we cite the figures appropriately in the figure legends.
Response: We obtained and uploaded appropriate copyright permission to use figures from previous publications (Fig. 2A, Fig. 3, Fig. 4 in the revised manuscript). In addition, we have cited these figures appropriately in the figure legends stating, for example, "This Figure is modified from Jin et al…".

3. Revision of original lines 148-149. The editor requested that we revise lines 148-149 (now lines 160-161) to avoid textual overlap with previously published work.
Response: As requested, we revised these lines (now lines 160-161) to avoid overlap with other's previously published work. These lines originally read: "Previous studies have shown that co-lipofection of two plasmids into eyebuds of developing *Xenopus* embryos will result in their co-expression in single optic neurons at >90% frequency [9,10]. In the revised manuscript, these lines now state: " Studies have shown that lipofection of two plasmids into eyebuds of *Xenopus* embryos at these developmental stages will result in their co-expression in individual optic neurons [9,10]."

4. Commercial language. The editor requested that we remove all commercial language form our manuscript and use generic terms instead.
Response: We removed all commercial language from our manuscript. The manuscript now contains generic terms followed by reference to the Table of Materials.

5. Explanation of dejellying. The editor requested that we explain how to dejelly *Xenopus* embryos, including which culture conditions are used, $\%CO_2$, and temperature.
Response: In the revised manuscript, we include explicit and detailed instructions for how to dejelly *Xenopus* embryos, including culture conditions and temperature (Section 1.2). We also included a reference to a previous publication describing dejellying of *Xenopus* embryos.

6. Table of Materials The editor requested that we remove the embedded Table of Materials from the manuscript.
Response: As requested, we removed the table of materials from the manuscript. The table of materials is now submitted as an excel spreadsheet, separate from the manuscript.

7. Table of Materials: The editor requested that we sort the items in the table in alphabetical order according to the name of material/ equipment.
Response: We sorted the items in the table of materials in alphabetical order using the names of the material/equipment.

Responses to Reviewers' comments:

Reviewer #1:
Major Concerns:

1. A picture of the eye bud being injected
Reviewer # 1 requested that we include an additional picture of the eyebud to give a sense of scale.
Response: In the revised manuscript we added a figure (Fig. 2) that contains a schematic (A) and a photomicrograph (B) of a *Xenopus* embryo with the eyebud regions highlighted in red. As requested, this figure specifies where the DNA will be injected on the whole embryo, and also provides a sense of scale.

2. A picture of the well in which the embryo is placed for injection
Reviewer # 1 requested that we include a picture of the well in which the embryo is placed for injection.
Response: Embryos are not placed in a well for injection. Rather, embryos are placed in a 10 mm petri dish and held with forceps in the non-dominant hand for injection. We revised sections 4.2-4.3 (lines 214 -222) of the manuscript to more clearly state how the embryos are held during injection.

3. A picture of the micropipette before and after filling
Reviewer # 1 requested that we include a picture of the micropipette before and after filling.
Response: As requested, in the revised manuscript we included a picture of the micropipette before and after filling with DNA/DOTAP (Fig. 1). In the picture of the filled micropipette, we specifically indicate the boundary line between the DNA/DOTAP and mineral oil (Fig. 1B).

Minor Concerns:
1. Reviewer # 1 requested that we specify which version of Adobe Illustrator we used.
Response: We specified which version of Adobe Illustrator we used. As requested by the editor, the information regarding Adobe Illustrator was moved from the manuscript into the table of materials.

2. Reviewer # 1 asked why we highlighted sections 3 and 4 in yellow.
Response: JoVE instructions state that we should highlight specific sections of the protocol that we wish to have videorecorded. We chose to highlight the sections that describe preparation of micropipettes (Section 3) and microinjection of DNA/DOPTAP into eyebuds of *Xenopus* embryo (Section 4). These procedures are the most difficult to understand by reading a protocol, and accordingly, would benefit the most from videorecording.

Reviewer #2:

Reviewer # 2 did not have any specific critiques of our manuscript.