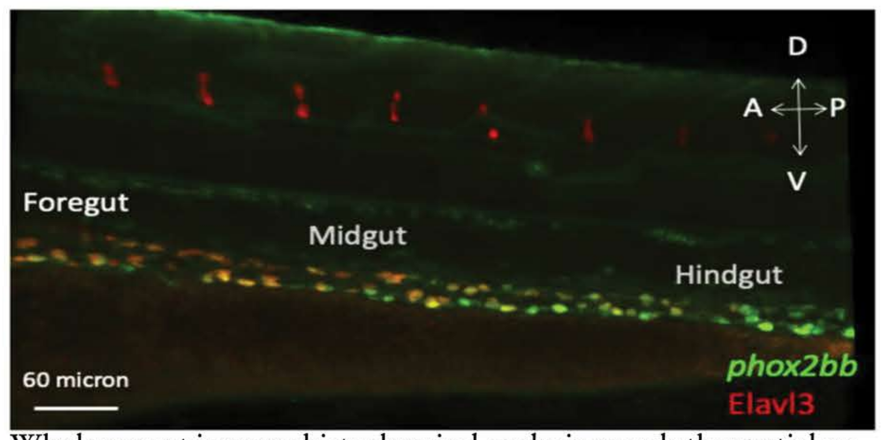
Role of BMP signaling during zebrafish enteric neural crest development

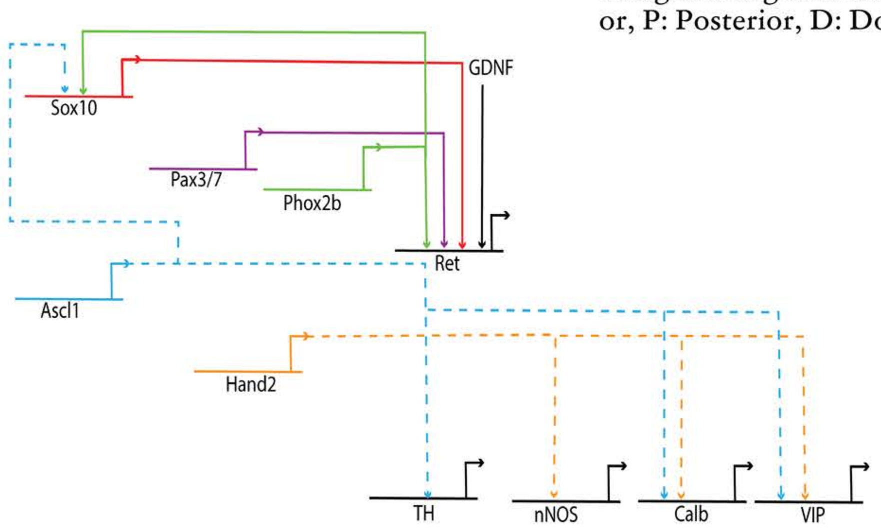
Joshua A. Moore[1,2], Mignote Tadesse[1], Alexis Barnett[1] and Rosa A. Uribe[1,2]
1. Department of Biosciences, Rice University, Houston, Texas
2. Biochemistry and Cell Biology Program, Rice University, Houston, Texas

1. Enteric Nervous System

1. The vertebrate enteric nervous system (ENS) consists of a series of interconnected ganglia within the muscle walls of the gut and is largely responsible for coordinating peristalsis, water balance, and regulation of hormonal secretions.



Whole mount immunohistochemical analysis reveals the spatial expression of phox2bb and Elavl3 markers within enteric neurons along the length of the developing zebrafish gut at 72 hpf. A: Anterior, P: Posterior, D: Dorsal, V: Ventral



Enteric gene regulatory network (Modified from Martik and Bronner 2017)

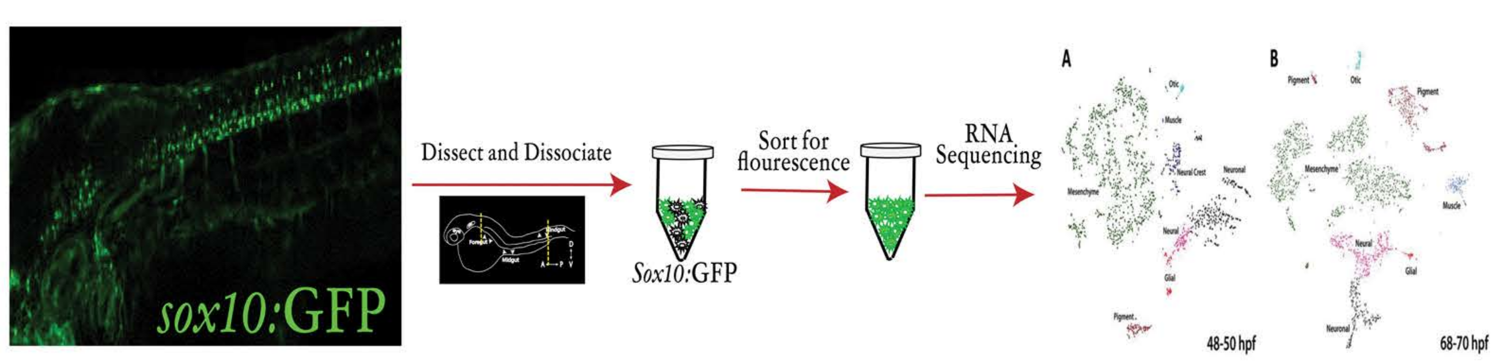
2. Complex and combinatorial nature of the signaling mechanisms involved in the establishment of the ENS, it has been difficult to tease apart major driving forces of many of the defects involved in aberrant ganglion formation in the gut.

2. What signals drive ENS formation?

To better elucidate and expand the gene regulatory network that regulates the formation of the enteric nervous system from the vagal nerual crest pool we utilized single cell sequencing analysis, chemical attenuation, hybridization chain reaction and immunohistochemical analysis to test our central hypothesis: **Bone Morphogenic Protein Signaling modulates enteric nerual crest colonization of the zebrafish (*Danio rerio*) gut tube.**

3. Experimental Design

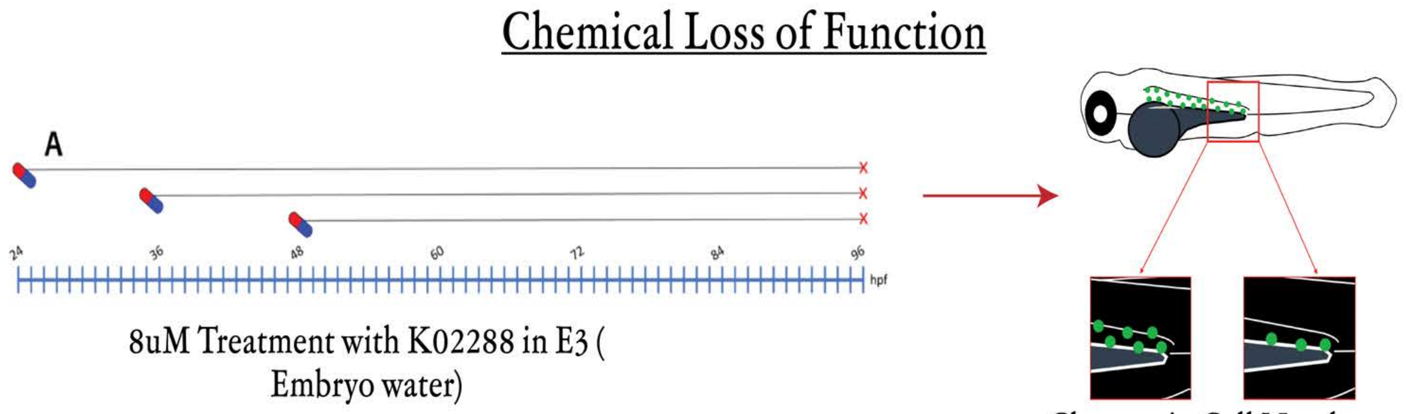
Single Cell Analysis



100 Embryos at each timepoint

tsne plots and other analysis

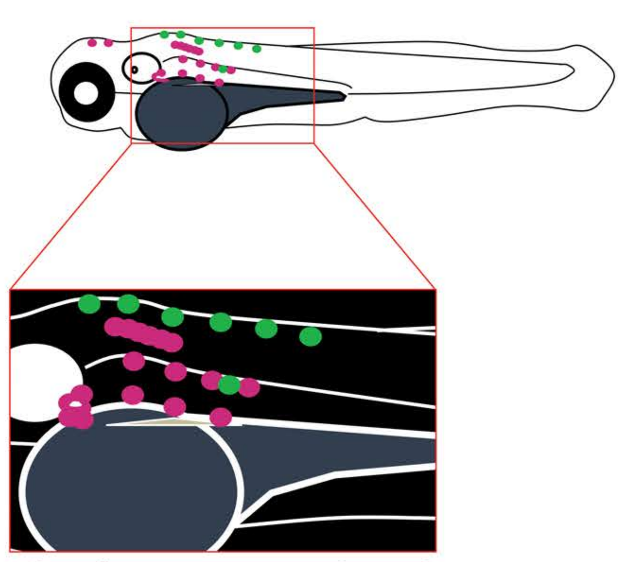
Chemical Loss of Function



8uM Treatment with K02288 in E3 (Embryo water)

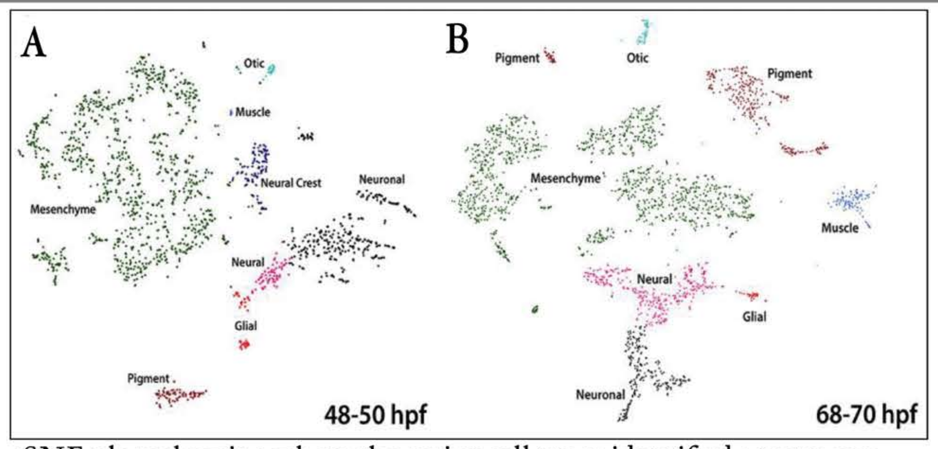
Changes in Cell Number
Assay fish for ENS defects

HCR and Immunohistochemical Analysis



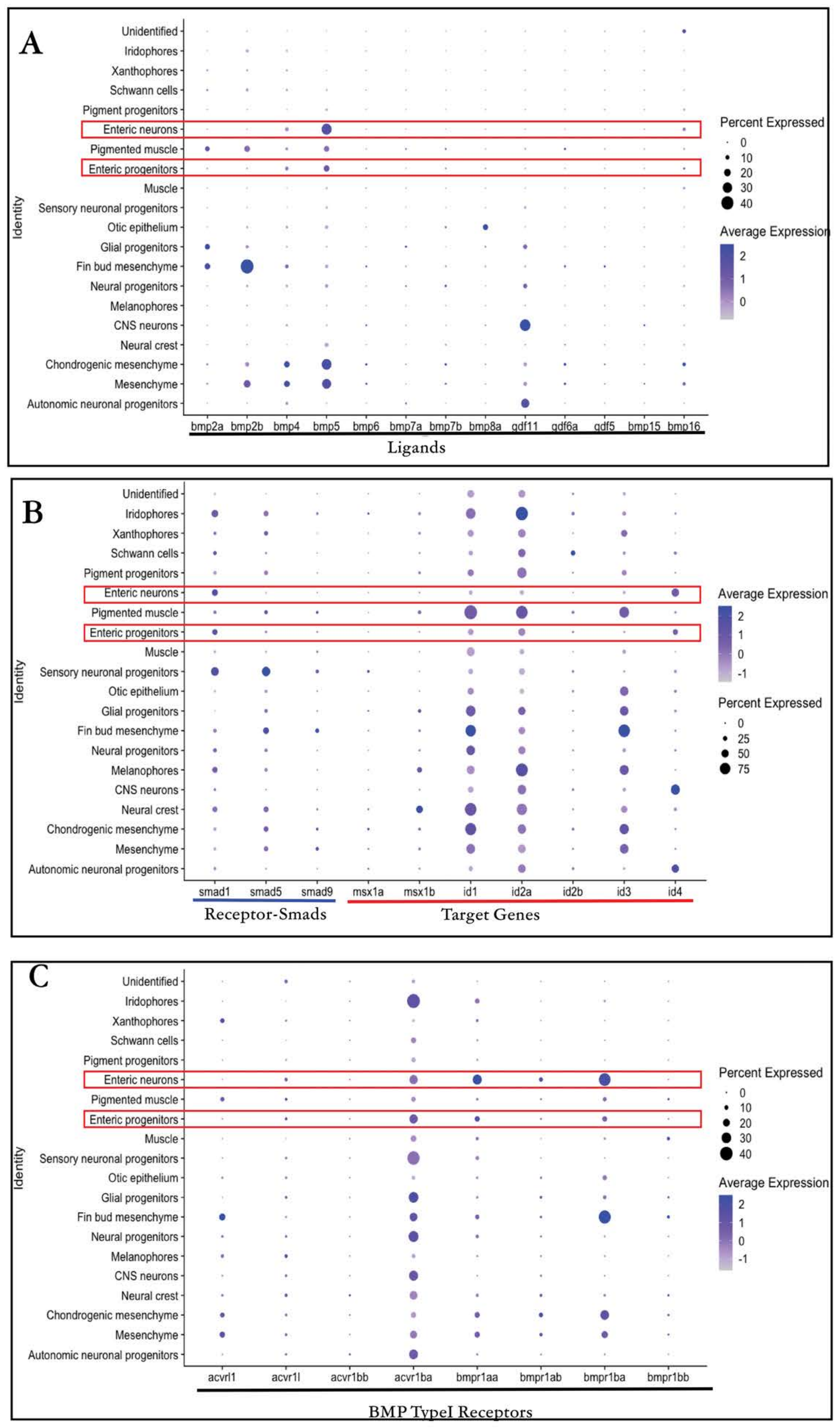
Identify co-expression of neural crest

4. Identification of Neural Crest and Neural Crest Derivatives



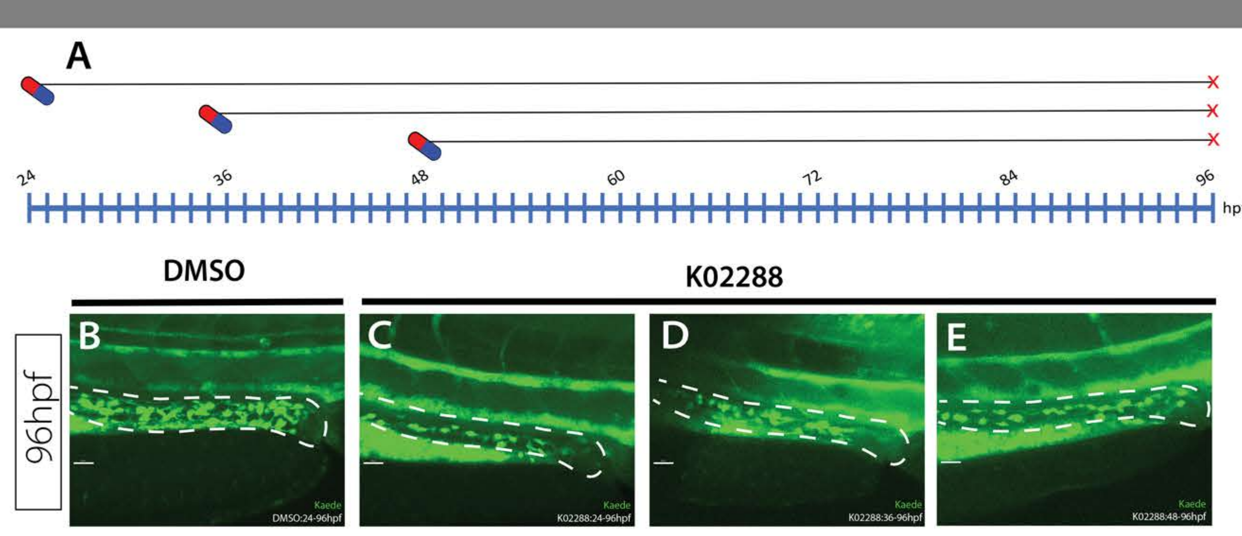
tSNE plots showing where the major cell types identified among sox10:GFP+ cells (Howard et al., 2021) arrange in the (A) 48-50 hpf and (B) 68-70 hpf datasets. Each dot represents a single sox10:GFP+ cell, color-coded by major cell type annotation.

5. BMP ligands, receptors, R-Smads and gene targets differentially expressed in Neural Crest derivatives



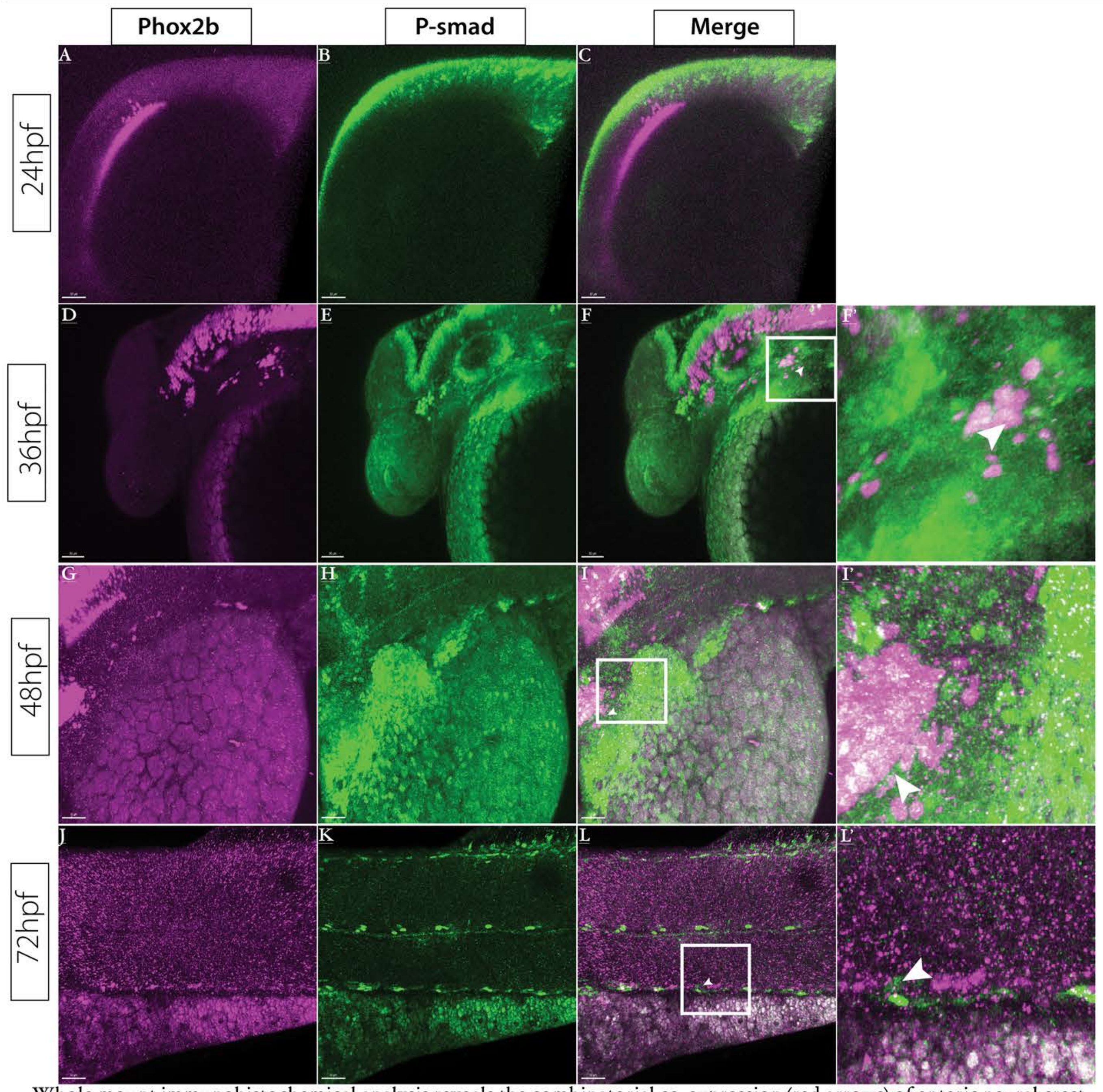
Dotplots showing differntialt expression of BMP pathway members identified among sox10:GFP+ cells across combined data from 48-50 hpf and 68-70 hpf datasets (Howard et al., 2021) . We indentfied the (A) Ligands, (B) Receptor Smads and Target genes, and (C) BMP type I receptors. Dot size denotes precentage of population expressing gene of interest. Color intensity denotes level of expresion compared to the average.

6. Broad attenuation depletes enteric progenitors



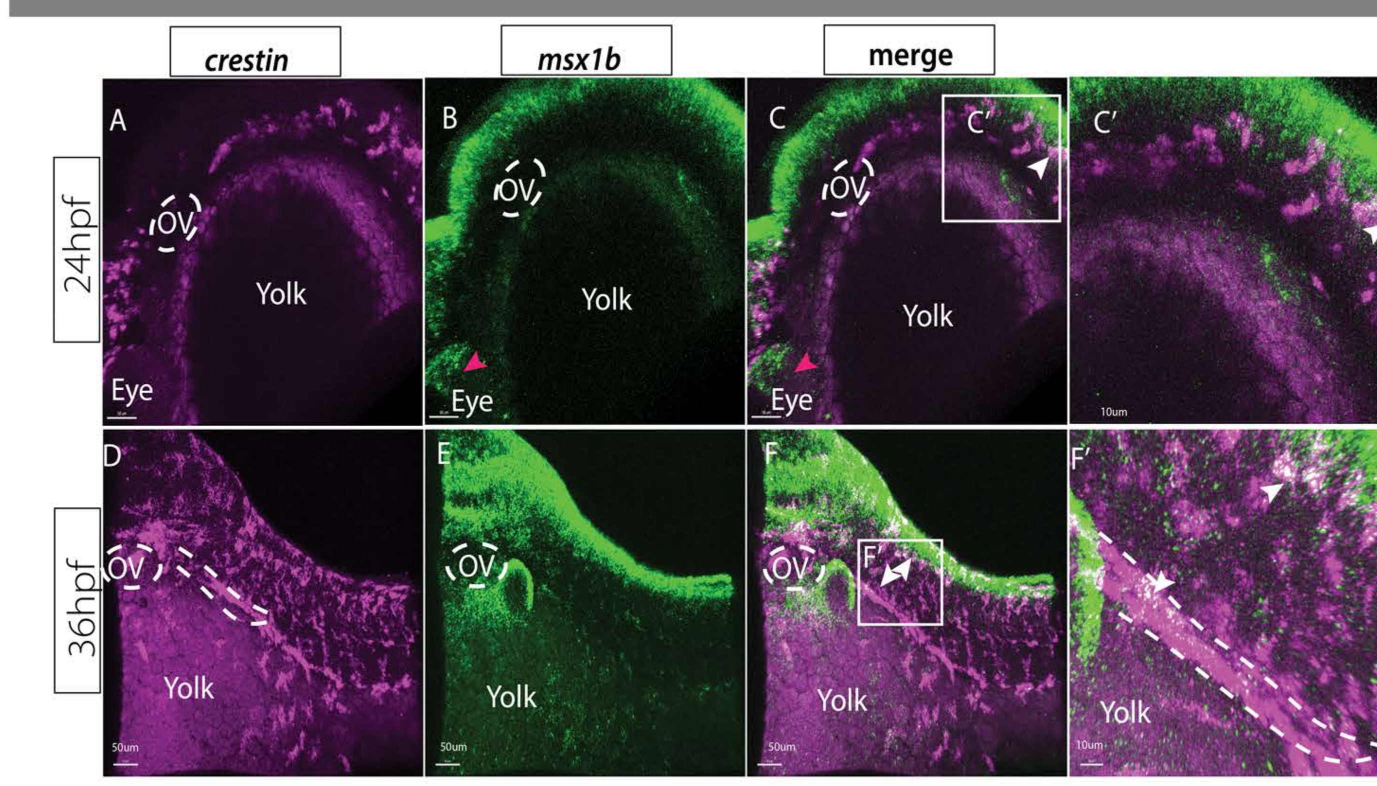
Schematic of K02288 mediated BMP inhibition paradigm (A). Confocal imaging of phox2bb:-Kaede+ embryos after 24-98 hpf DMSO control (B), or 24-98hpf (C), 36-98hpf (D) or 48-98hpf (E) 8um K02288 treatments. Reduced phox2bb:Kaede+ cells denote enteric progenitors, while dotted white line outlines distal gut tube. Anterior oriented to the left and dorsal to the top, in these images. Scale bar denotes 50 microns

7. BMP signaling is active during neural crest gut tube colonization



Whole mount immunohistochemical analysis reveals the combinatorial co-expression (red arrows) of enteric neural crest marker, Phox2b (magenta) and P-smad (green) at 36hpf and 48hpf (D-I). Smad mediated BMP signaling in phox2b+ progenitors lost by 72hpf (J-L). Anterior oriented to the left and dorsal to the top, in this image. Please define scale bar size here in the legend. Dotted oval denotes otic vesicle. Dotted lines out line gut tube. Scale bar denotes 50 microns. A: Anterior, P: Posterior, D: Dorsal, V:

8. *msx1b* expressed in migratory neural crest



(A-F) Whole mount HCR analysis reveals the combinatorial co-expression (C',F'-red arrows) of neural crest marker, crestin (magenta) and msx1b (green) at 24hpf (A-C') and 36hpf (D-F'). Anterior oriented to the left and dorsal to the top, in this image. Dashed lines outline the gut tube. Scale bar denotes 50 microns. A: Anterior, P: Posterior, D: Dorsal, V: Ventral

Acknoledgements

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development
Healthy pregnancies. Healthy children. Healthy and optimal lives.
F31HD104474

CPRIT

eLife

Howard AG 4th, Baker PA, Ibarra-García-Padilla R, Moore JA, Rivas LJ, Tallman JJ, Singleton EW, Westheimer JL, Corteguera JA, Uribe RA. An atlas of neural crest lineages along the posterior developing zebrafish at single-cell resolution. Elife. 2021 Feb 16;10:e60005. doi: 10.7554/eLife.60005. PMID: 33591267; PMCID: PMC7886338.