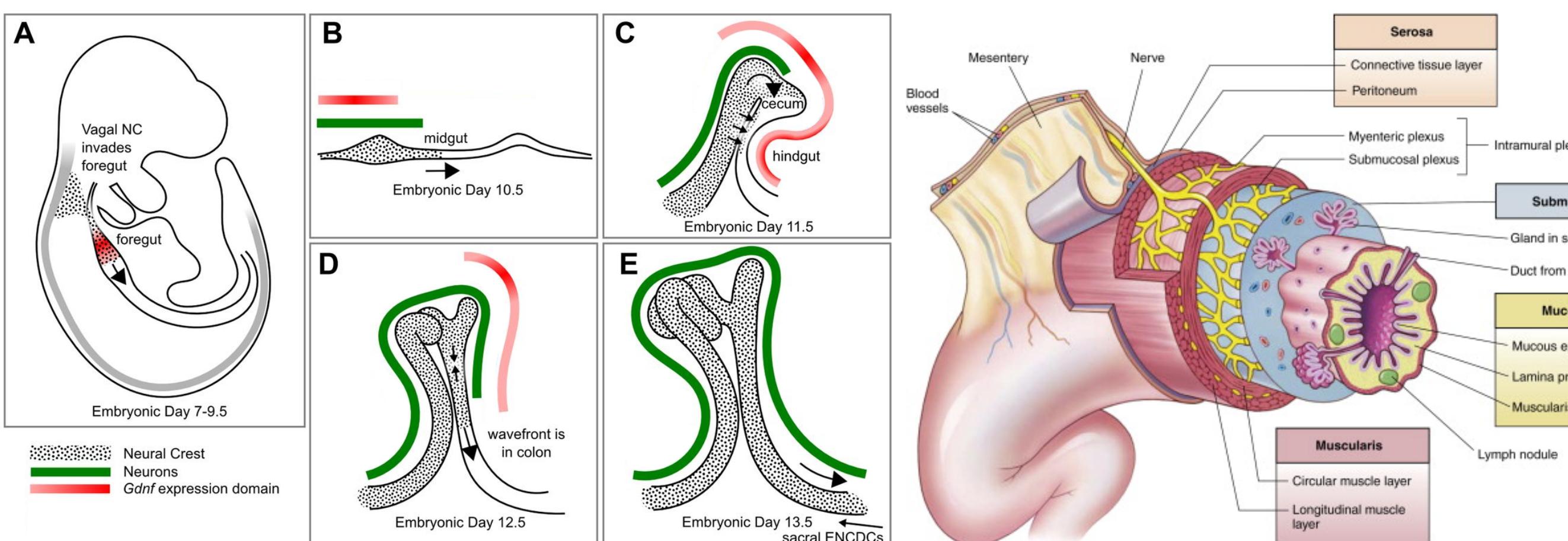


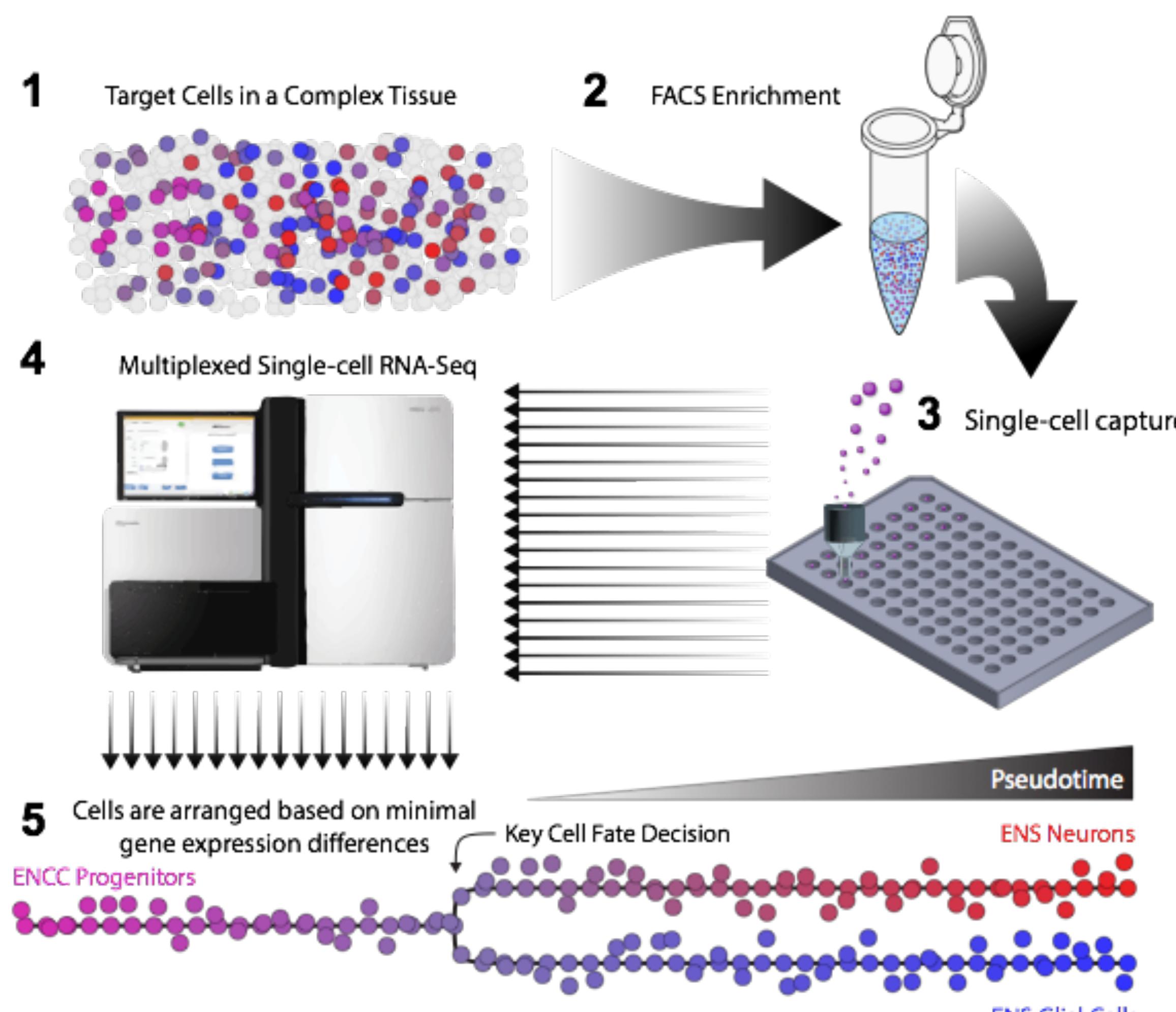
Background



In the mouse, invasion of vagal neural crest cells into the gut tube initiates the development of the enteric nervous system (ENS) in early embryogenesis. These neural-crest-derived cells, referred to as enteric neural crest cells (ENCC), migrate caudally along the developing gut. As they migrate, ENCC differentiate to form the ganglia of the myenteric and submucosal plexuses, which together constitute the ENS. To maintain the progenitor pool ENCC must also continuously proliferate. *Ret* is the key player in both the migration and proliferation of ENCC. The wave of ENCC that migrates through the gut is preceded by expression of glial derived neurotrophic factor (*Gdnf*), the product of which is a ligand of RET. RET is also known to be involved in cell cycle progression and has been linked to multiple cancers, indicating a role in promoting proliferation.

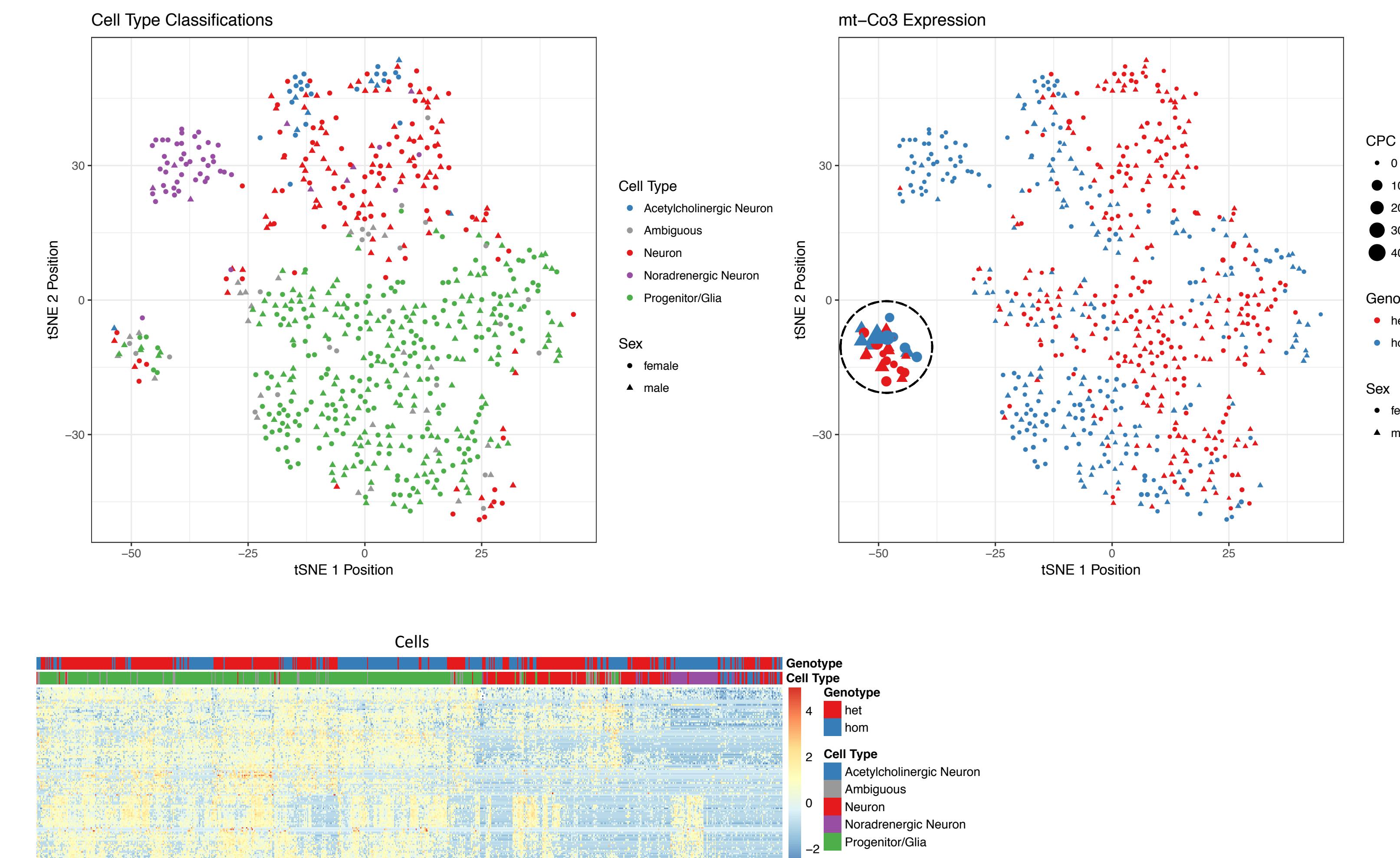
Defects in *RET* lead to defects in the formation of the ENS: mutations in *RET* and other key genes in the *RET* gene regulatory network have been linked to Hirschsprung disease (HSCR), also known as aganglionic megacolon. HSCR is characterized by the absence of ganglia in the affected portion of the gut, which results in non-functional, aganglionic segments of colon that must be surgically resected. The mechanism by which defects in *RET* lead to the absence of ganglia has not yet been discerned. In our work we are investigating the effects of loss of function of *Ret* on the transcriptional trajectories of would-be *Ret*-expressing cells.

Experimental Outline



Our model system is a transgenic mouse line with cyan fluorescent protein (CFP) cDNA followed by a poly(A) signal sequence inserted into the first exon of *Ret*. CFP is expressed under the *Ret* promoter, but transcription halts after the poly(A) sequence. RNA-sequencing was performed on single cells (scRNA-seq) from E12.5 mouse gut, isolated using fluorescence-activated cell sorting (FACS) based on the expression of cyan fluorescent protein (CFP). Data were analyzed using the Monocle framework in R.

Identification of Cell Types

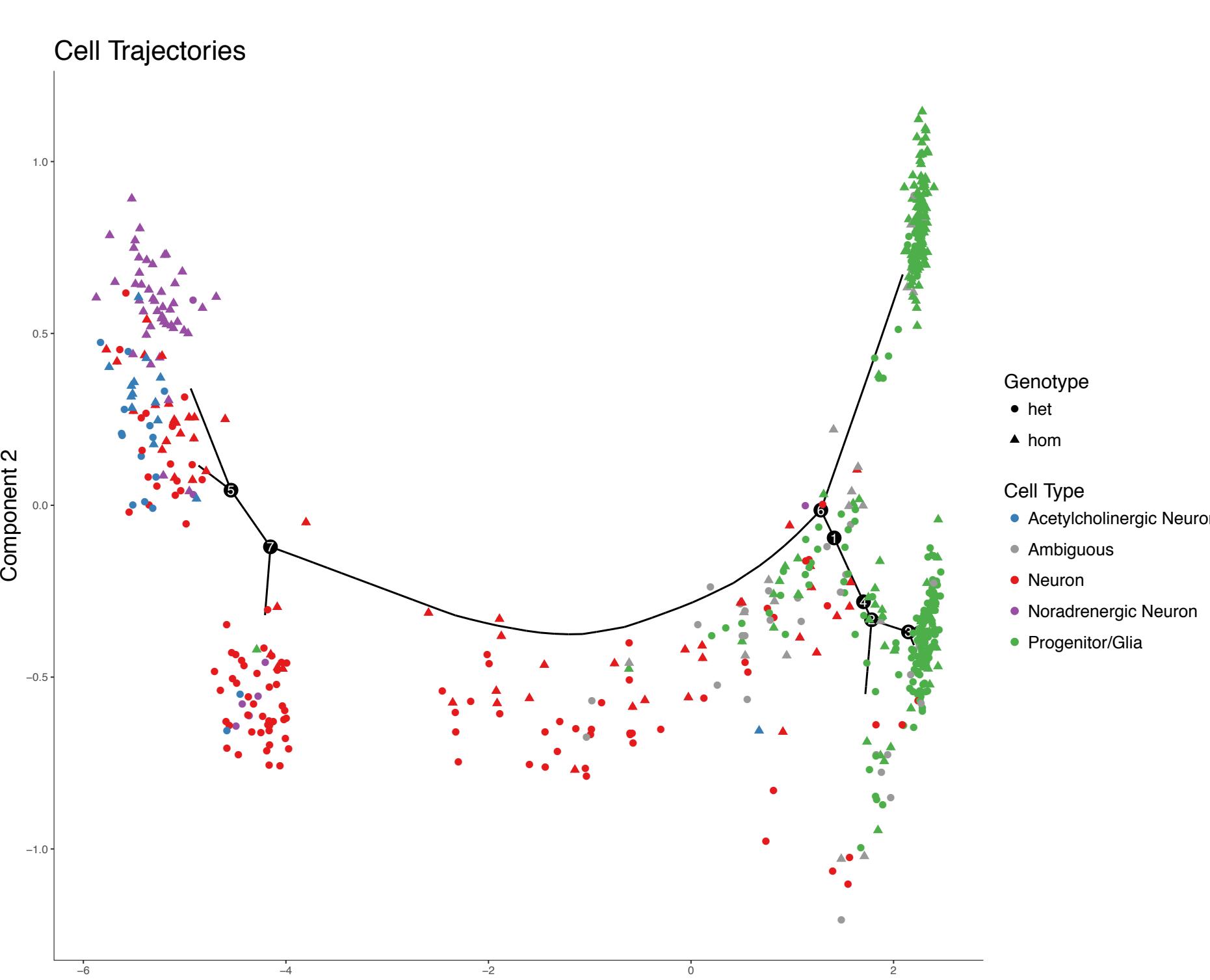


661 cells are shown above after dimensionality reduction through t-stochastic neighbor embedding (tSNE), a dimensionality reduction method. Cells are classified by expression of key marker genes, such as: *Ache* for acetylcholinergic neurons (*Ache* neurons), *Dbh* for noradrenergic neurons, *Snap25* for neurons that do not fall into either of the aforementioned subtypes, and *Sox10* for progenitors/glia. One population of cells, circled in the above figure, does not cluster according to cell type. These cells highly express *mt-Co2* and *mt-Co3* (shown above) as well as several other mitochondrial genes. These cells were likely under oxidative stress from the dissociation and sorting process and therefore cluster together as a dying cell population.

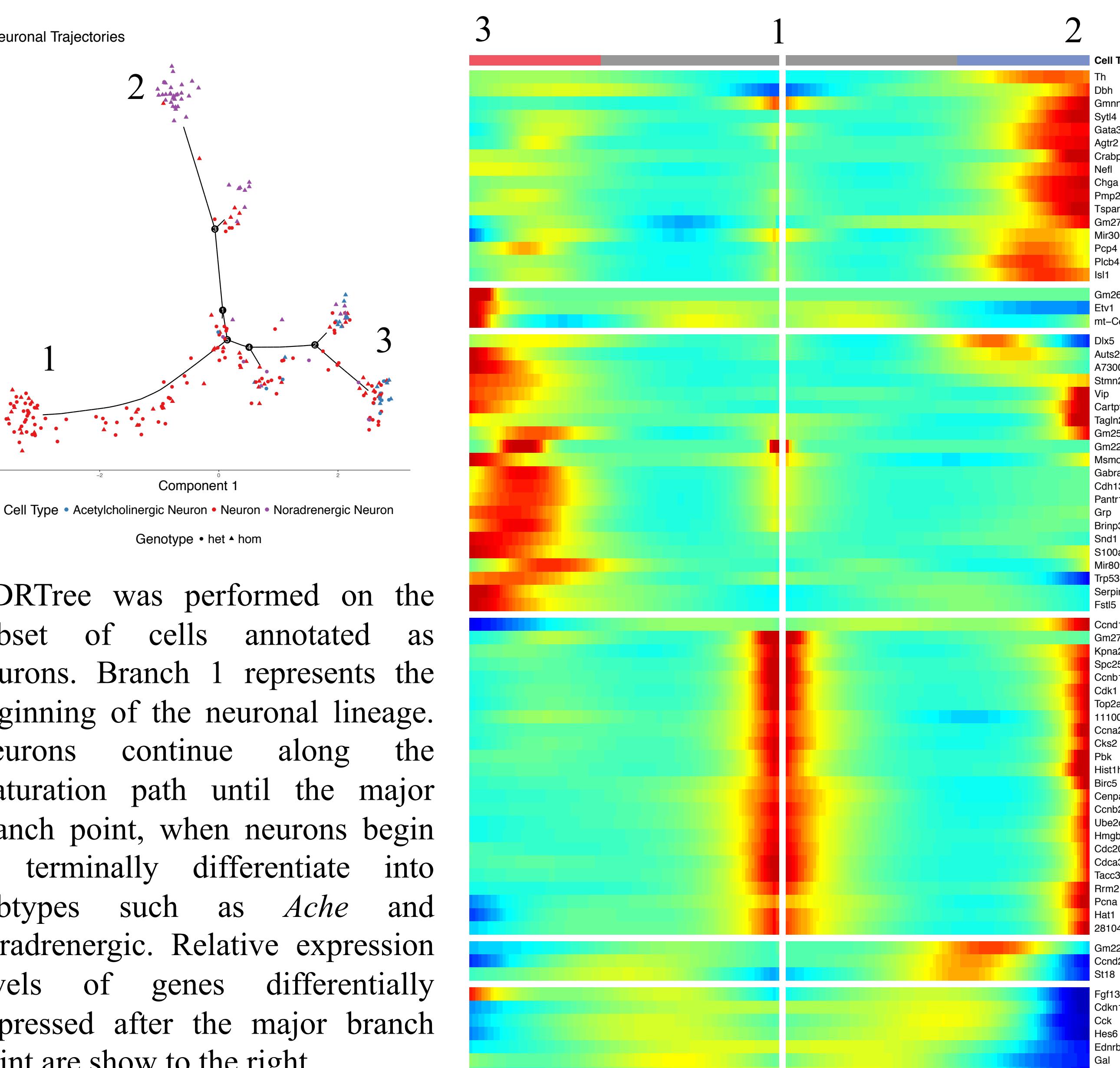
To the left, the expression profiles of 618 genes differentially expressed (FDR 1×10^{-15}) with respect to cell type and genotype are shown.

Lineage Reconstruction

Using discriminative dimensionality reduction with trees (DDRTree) the cells can be visualized along a pseudotemporal trajectory, which represents their progress along development. As seen to the right, the cells annotated as progenitor/glial cells transition into an uncommitted neuronal population, which later terminally differentiates into either *Ache* neurons or noradrenergic neurons. The proximity of two cells in the tree gives an indication of their similarity. More cells are annotated as glial than neuronal, but the tight clustering of the glial cells in comparison to the neurons indicates the glial population exhibits less variability. Additionally, the major branch points for both glia and neurons appear to be genotype driven.



Genotypic Bias in Neuronal Trajectories



Major Findings and Future Directions

Major findings:

- At least two major cell types express *Ret* in the developing mammalian gut and are easily distinguished by their expression profiles
 - Ret* null cells are further along the pseudotime trajectory than their heterozygous counterparts, indicating they may have begun their differentiation earlier
 - Homozygous *Ret* LOF leads to differential gene expression in neurons, including higher proportions of terminally differentiated neurons and enrichment of *Ache* neurons in female *Ret* null mice

future directions:

- In situ* validation of the cell populations and their differences across sex and genotype using single-molecule RNA fluorescence *in situ* hybridization
Repeat the scRNA-seq experiment with other time points during mouse ENS development
Measure proliferation rates of *Ret*-expressing cells during ENS development to determine if cells from null embryos are differentiating early

