Capturing ENS cell and gene clusters in a Cat's Cradle

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Gastrointestinal physiology relies on the enteric nervous system (ENS), which coordinates diverse functions through integrated interactions with multiple cell lineages. Miscommunication between those cellular systems has adverse effects on intestinal health leading to pathology. Recent advances in our understanding of ENS development, function and dysfunction have relied on analysis of single cell (SC) data sets.

In a typical Seurat analysis of a SC data set we analyse a gene expression matrix where the rows are genes and columns are cells. The Louvain algorithm then clusters cells into cell types and tools such as UMAP and tSNE allow us to see these cell clusters spread out in two dimensions. In a novel analysis, we transpose this expression matrix allowing us to cluster genes and visualise these gene clusters in two dimensions. These gene clusters reveal sets of genes cooperating to carry out particular functions. In addition, we are able to query the relationship between gene clusters and cell clusters and display this as a bipartite graph. This allows us to investigate which cell clusters up-regulate which gene clusters thereby revealing common functionality between distinct cell types.

To date we have analysed two SC data sets and annotated the resulting gene clusters by hand. In both cases these have resulted in a mix of gene clusters which represent functions we recognize (e.g., cell cycle, innate immune activation, glia / neurogenic genes, etc.) and gene sets whose function we have not yet determined. Interestingly, gene sets of known function also contain genes not previously assigned to this function, thereby generating (perhaps) testable hypotheses. As a positive control, we note that Ifng response genes cluster very tightly in one of our gene UMAPs.

Examining up-regulation of gene clusters in cell clusters reveals relationships between particular cell clusters, e.g., our TCells1 and TCells2 clusters share upregulation of our gene cluster 11, while our gene cluster 9 is upregulated in only one of these and neither of these is upregulated in our TCells3, thus validating our choices in cell-clustering.

\* These people are all stars!

† These people are all cross with the last author.