We have experience in applying pseudotime trajectory analysis using Slingshot [1] to elucidate the development pathways of cells in the human prefrontal cortex. Our analysis leveraged UMAP coordinates to model cell differentiation pathways, beginning with the cluster associated with L2/3 IT cell type. By incorporating labels derived from our annotation pipeline, we were able to construct a minimum spanning tree and smooth it through principal curve analysis, effectively capturing the dynamic progression of cells along developmental trajectories. Moreover, differential gene expression analysis was conducted using the tradeSeq package [2], where a generalized additive model fitted to the pseudotime trajectories allowed us to identify key genes, such as SEMA6A and SOX6, showing significant expression changes across cortical layers. Figure 1 illustrates the results of cell trajectory analysis across four subclasses of excitatory neurons within the human prefrontal cortex in our lab's recent publication [3].

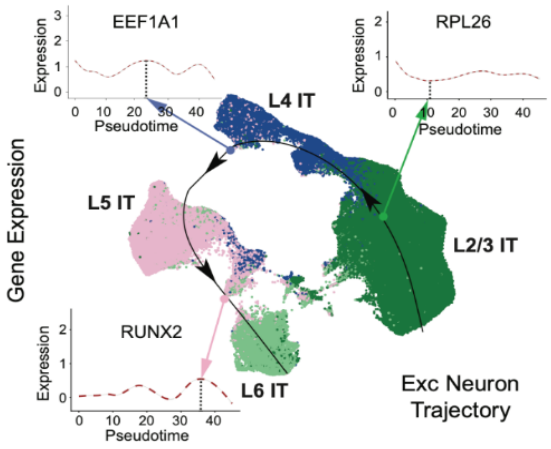

Figure 1. UMAP plot illustrating the predicted trajectory of excitatory IT neurons moving through cortical layers from L2/3 to L6 within the prefrontal cortex.

In our preliminary analysis, we utilized snATAC-seq and snRNA-seq data from healthy individuals to predict regulatory links between TFs and their target genes, identifying both proximal and distal regulatory interactions. Using a combination of SCENIC and scGRNom pipelines, we successfully constructed cell-type-specific GRNs that captured key regulatory elements across the brain cell types.

In our previous work, we developed an intercellular communication network by integrating publicly accessible ligand-receptor pair data with snRNA-seq. We identified distinct broad communication patterns among excitatory, inhibitory, and glial cell types, with significant alterations in these patterns observed in patients with neuropsychiatric disorders such as schizophrenia and bipolar disorder. Specifically, we noted that excitatory neurons showed reduced incoming signaling in schizophrenia, whereas inhibitory neurons exhibited increased signaling.

1. Street, K., et al., *Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics.* BMC Genomics, 2018. **19**(1): p. 477.
2. Van den Berge, K., et al., *Trajectory-based differential expression analysis for single-cell sequencing data.* Nat Commun, 2020. **11**(1): p. 1201.
3. Emani, P.S., et al., *Single-cell genomics and regulatory networks for 388 human brains.* bioRxiv, 2024.