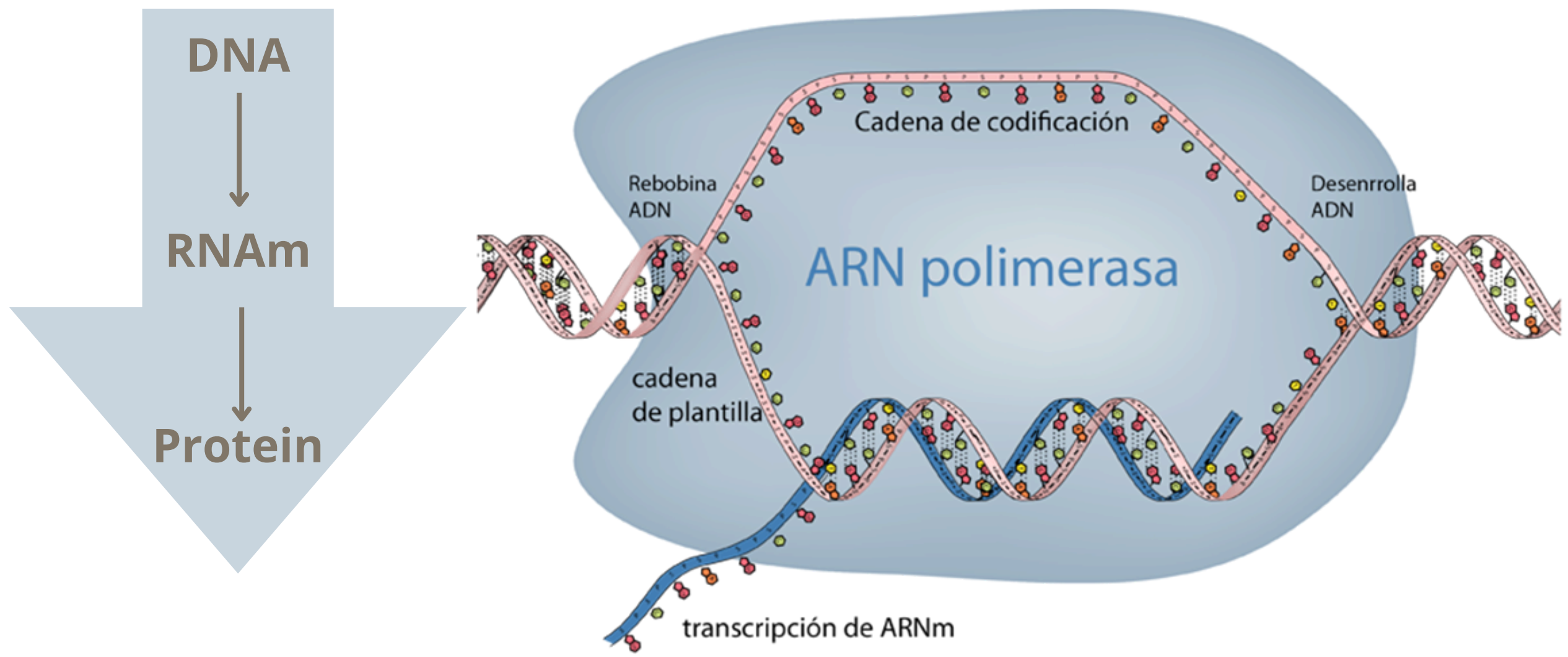


INTRODUCTION

The intestinal epithelium contains tuft cells, a rare cell type (<0.5%) with chemosensory and immune functions. Their role is not fully understood, but they may secrete IL-25 and produce acetylcholine. Single-cell RNA sequencing (scRNA-seq) allows detailed analysis of their gene expression and functional diversity.



NCBI Gene database – Gene ID: 53603 (<https://www.ncbi.nlm.nih.gov/gene/53603>)

OBJECTIVES

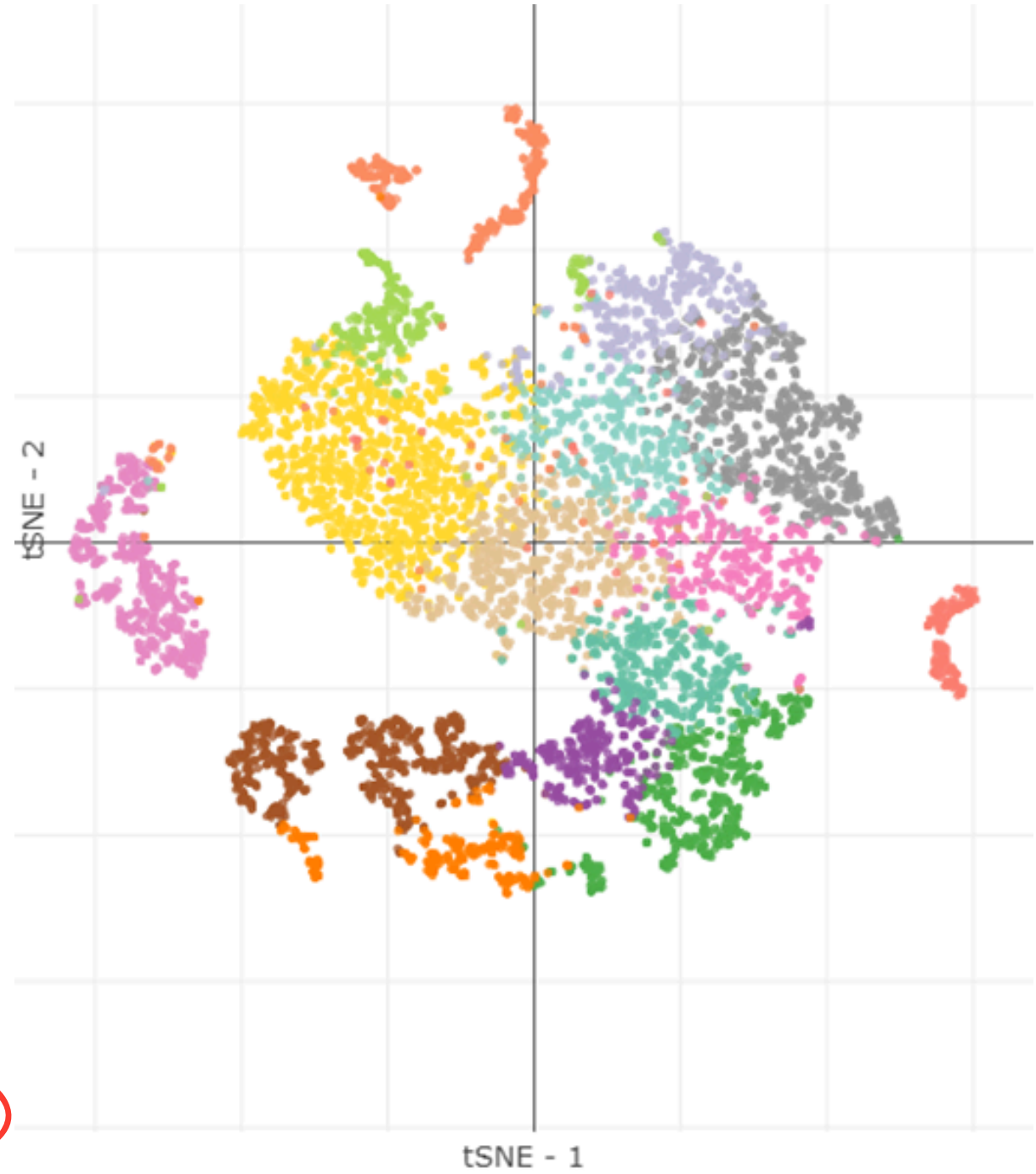
- To characterize the gene expression profile of intestinal tuft cells using scRNA-seq.
- To investigate non-neuronal cholinergic signaling via acetylcholine synthesis.
- To explore alternative immunomodulatory pathways beyond IL-25 signaling.

MATERIALS AND METHODS

Dataset	SCP44 (Small Intestinal Epithelium), from Broad Institute
Species	Mouse (n=6)
Technique	3' scRNA-seq
Analysis	Gene expression visualized using t-SNE and dot plots
Genes analyzed	identity markers (<i>Avil</i> , <i>Dclk1</i> , <i>Sucnr1</i>), cholinergic (<i>Chat</i> , <i>Slc</i> family) and immune-related (<i>Il25</i> , <i>Ptgs1</i> , <i>Alox5</i> , <i>Tslp</i>).

Note : scRNA-seq reveals mRNA expression, but does not confirm protein production. Results should be interpreted cautiously and ideally validated with proteomic or immunodetection methods.

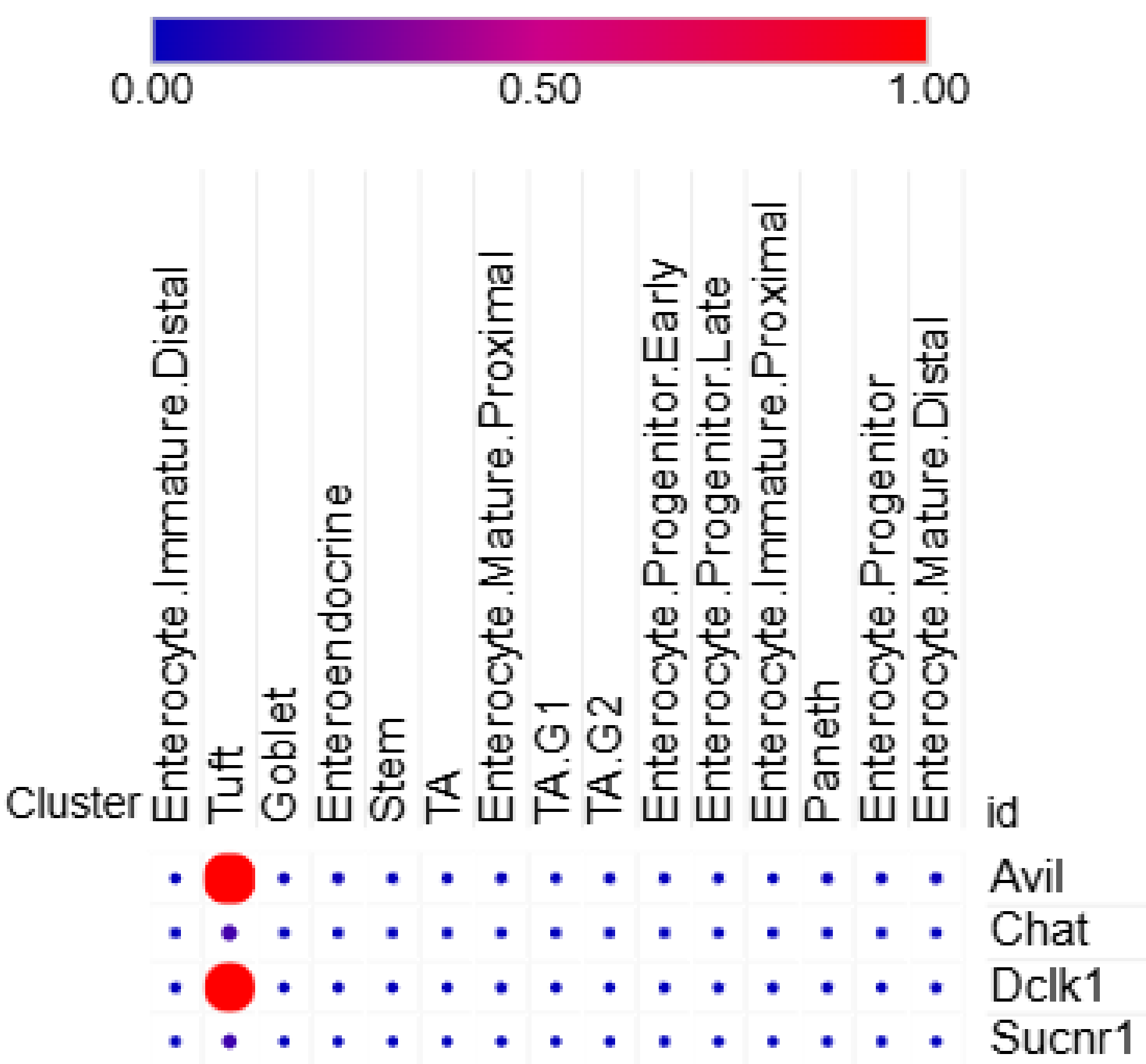
Enterocyte.Immature.Di...	512
Enterocyte.Immature.Pr...	297
Enterocyte.Mature.Disti...	241
Enterocyte.Mature.Proxi...	581
Enterocyte.Progenitor	356
Enterocyte.Progenitor.E...	829
Enterocyte.Progenitor.Late	404
Enteroendocrine	310
Goblet	510
Paneth	260
Stem	1267
TA	665
TA.G1	408
TA.G2	410
Tuft	166



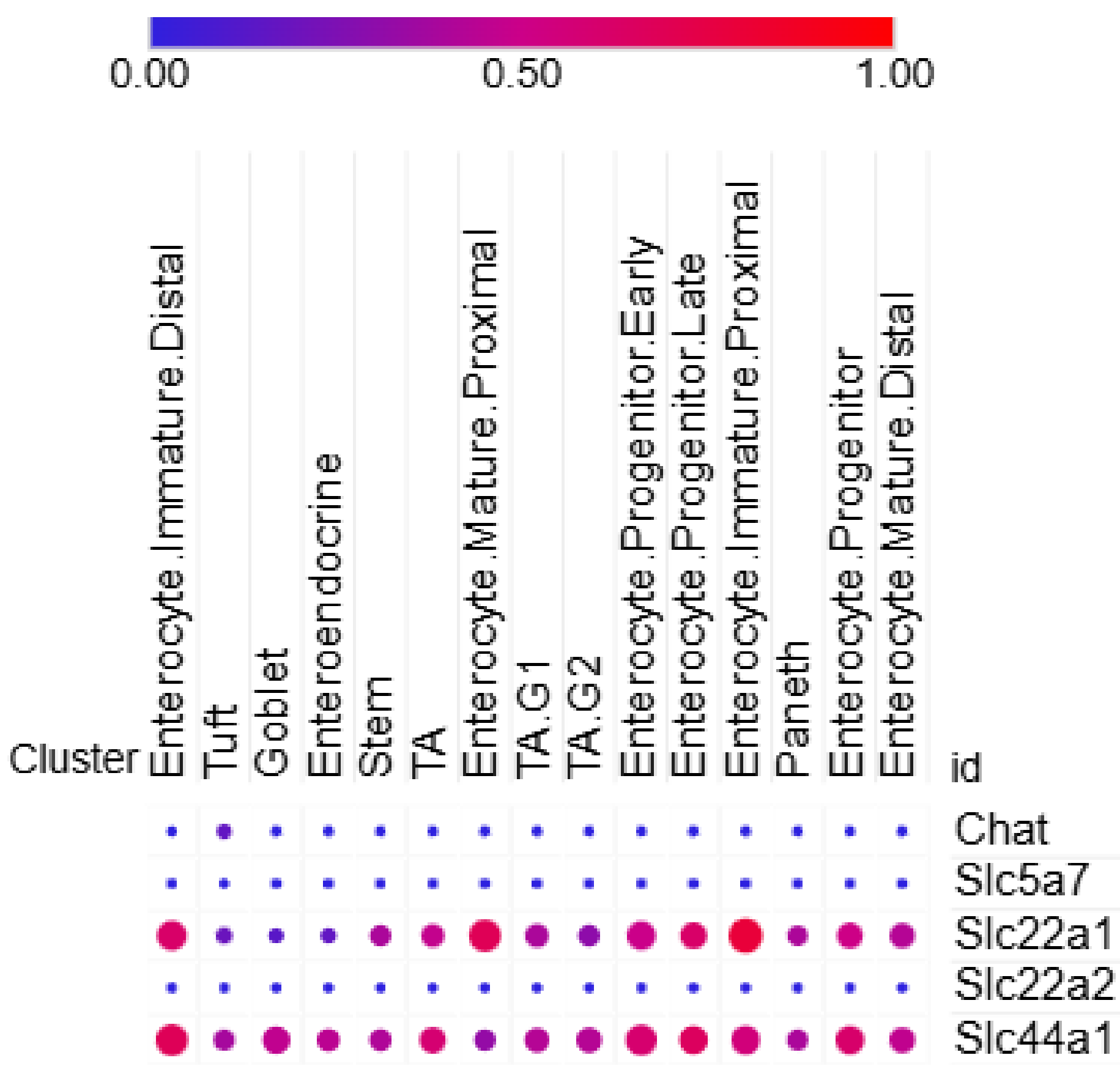
Source: Broad Institute – Single Cell Portal, SCP44: Small Intestinal Epithelium (https://singlecell.broadinstitute.org/single_cell/study/SCP44)

RESULTS

1. Tuft Cell Identity

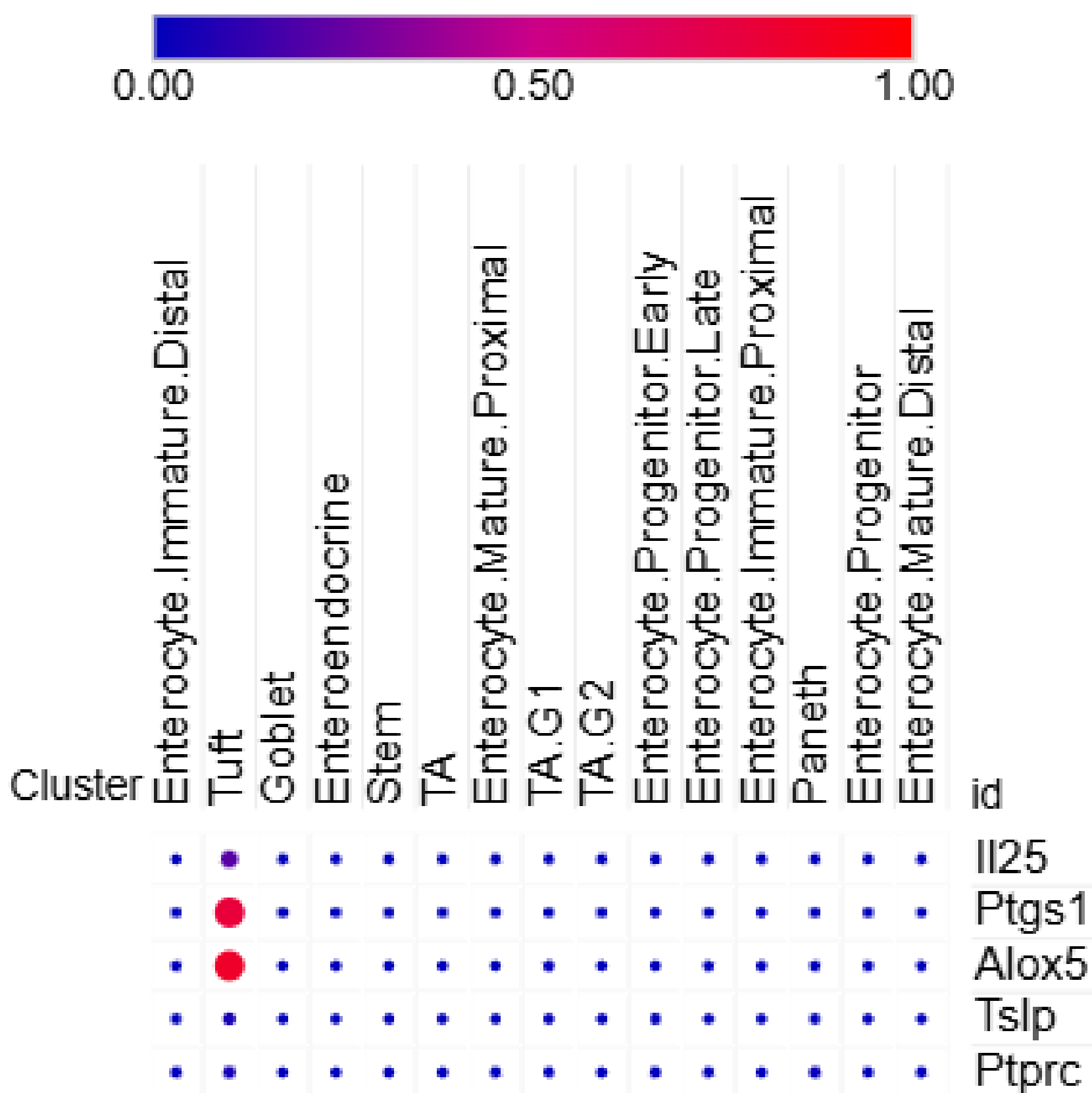


2. Acetylcholine Pathway



Data and plots from: Broad Institute – SCP44: Small Intestinal Epithelium (accessed via Single Cell Portal).

3. Alternative Immune Functions



EXPLORE THE INDIVIDUAL TRANSCRIPTOMIC PROFILE OF TUFT CELLS



By scanning this QR code, you will access the interactive heatmap from the SCP44-Small Intestinal Epithelium dataset on the Single Cell Portal (Broad Institute), where you can visualize gene expression data for our genes of interest at single-cell resolution.

Each cell in the heatmap represents an individual intestinal epithelial cell from mouse organized by cell type. The last columns correspond to tuft cells. Blue indicates no expression, while red indicates the highest relative expression level.

CONCLUSIONS

- Single-cell transcriptomics of intestinal tuft cells validated known markers (*Avil*, *Dclk1*, *Sucnr1*), although none showed absolute expression. A combination of markers is recommended for accurate identification.
- Chat*, was exclusively expressed in a tuft cell subset, confirming their ability to synthesize acetylcholine. However, none of the analyzed choline transporters seem to explain choline uptake, suggesting the existence of uncharacterized mechanisms.
- Tuft cells may contribute to immune regulation via IL-25-independent pathways, particularly through eicosanoid biosynthesis, indicating a constitutive and heterogeneous immunomodulatory potential.
- These findings were obtained under physiological (non-inflamed) conditions. In pathological contexts such as infection, tuft cell numbers and functions are expected to change significantly.