

# Cell-type associations with phenotypes highlight roles of gut-wall cells in disease

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## Background

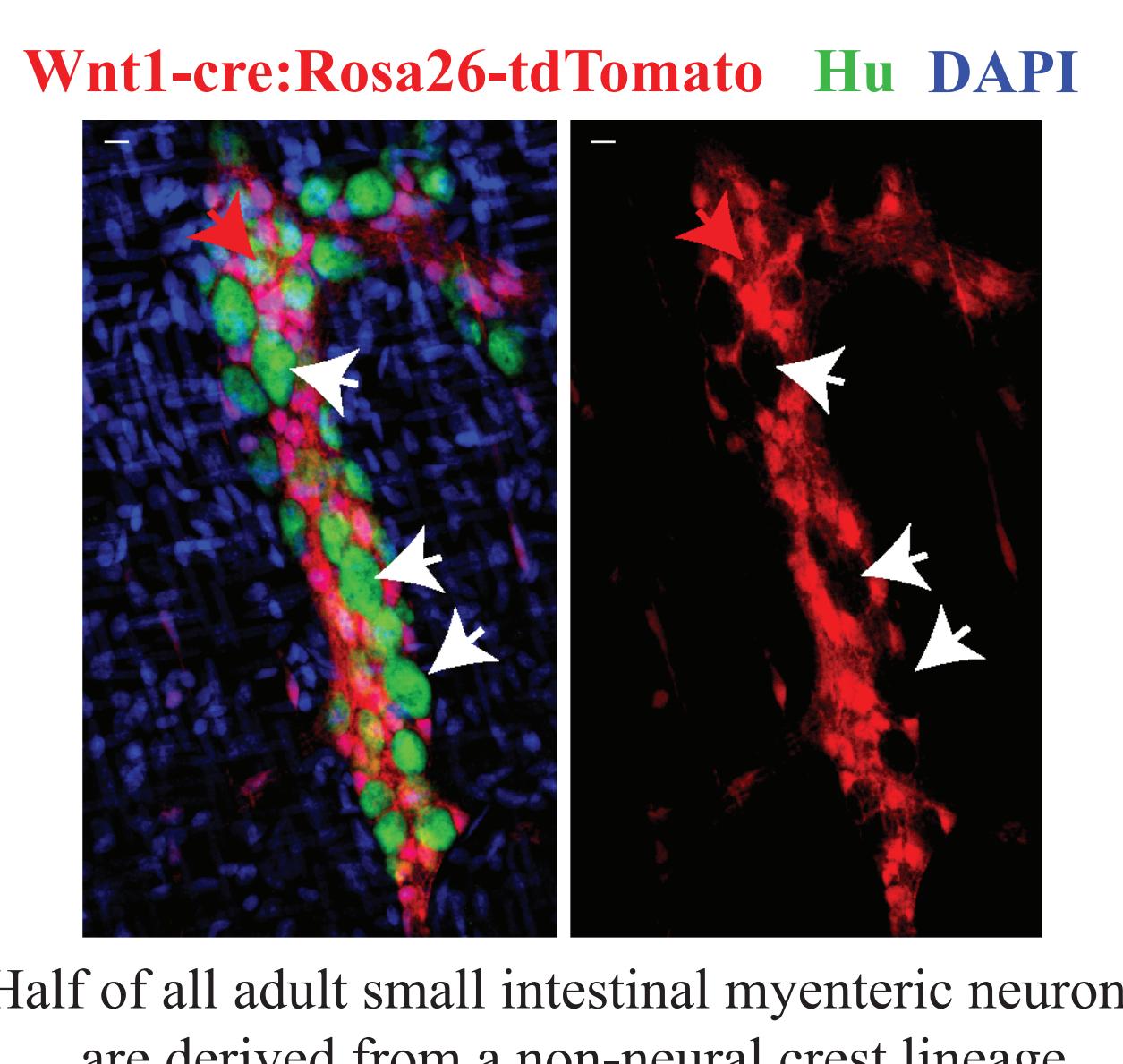
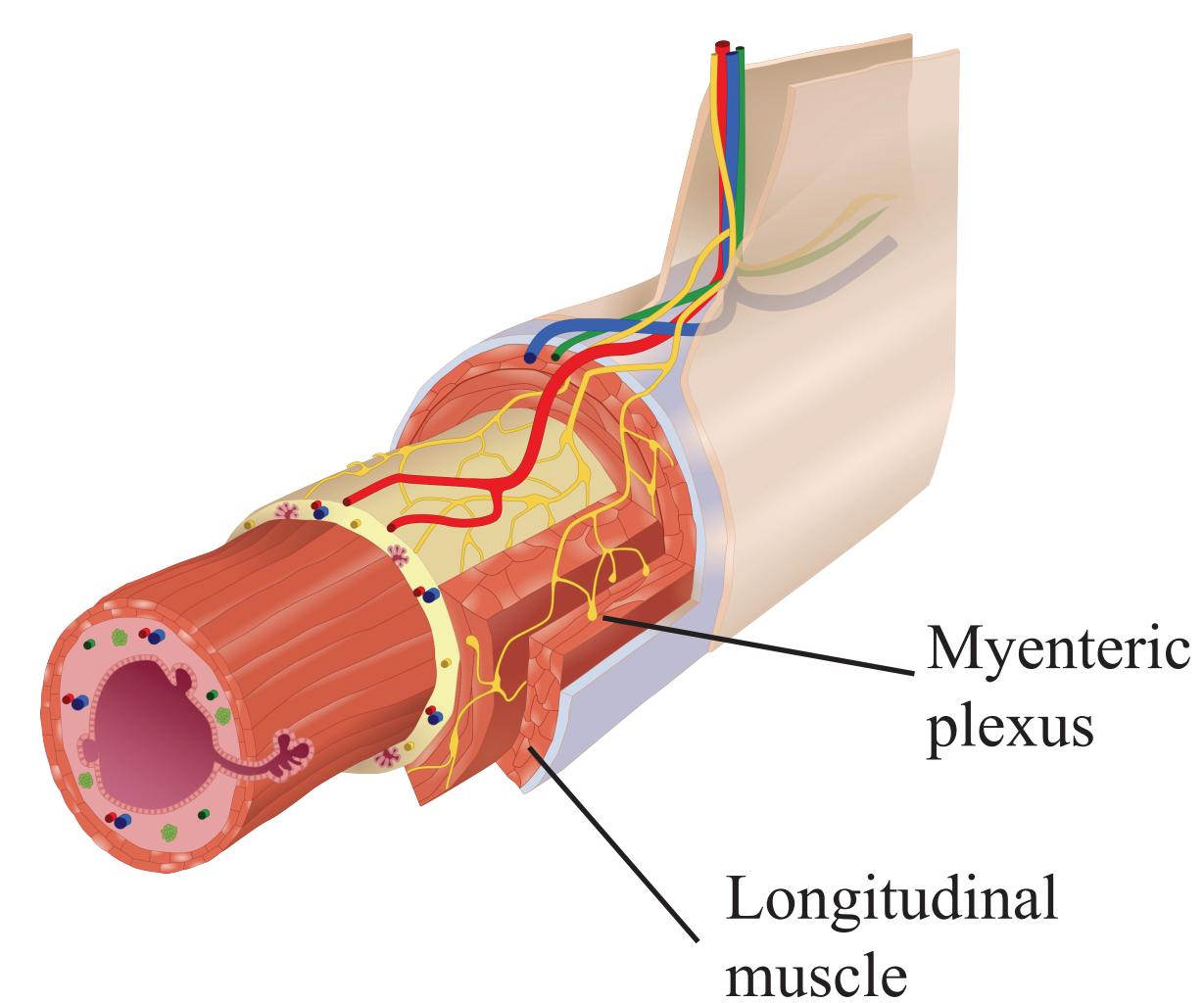
- The enteric nervous system (ENS) regulates critical gut functions including digestion, intestinal motility, and immune defense, amongst others
- While previously thought to be of exclusively neural-crest origin, we have recently identified a novel population of ganglionic cells that contribute to the enteric nervous system (mesoderm-derived enteric neurons, or MENs)
- The specific role of MENs in maintaining GI function is currently not well understood
- Alterations in proportions of neurons of these two different lineages is associated with changes in gut function and disease**
- Whether gut disease-associated genes are specifically expressed by MENs is not known

## Objectives

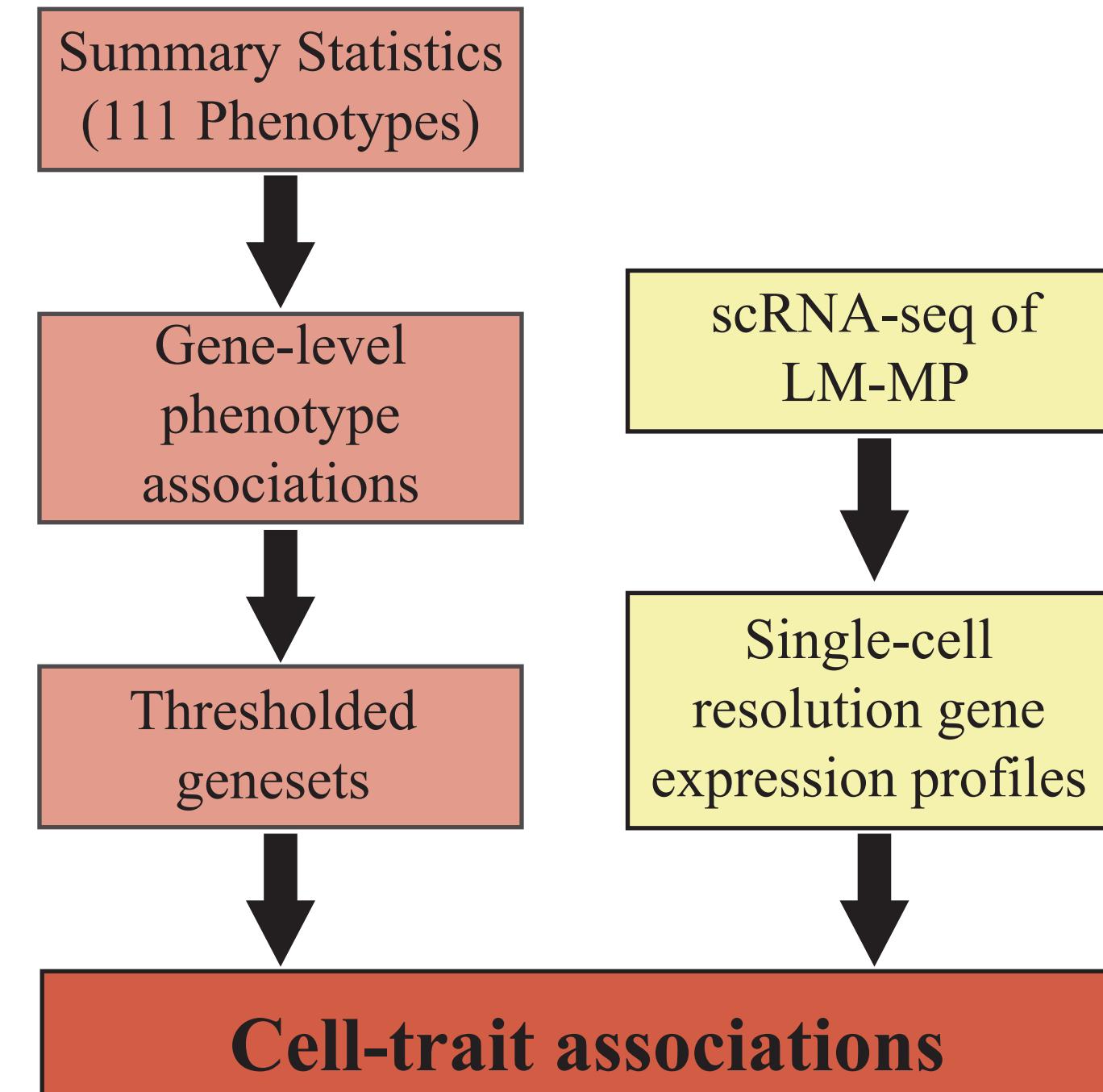
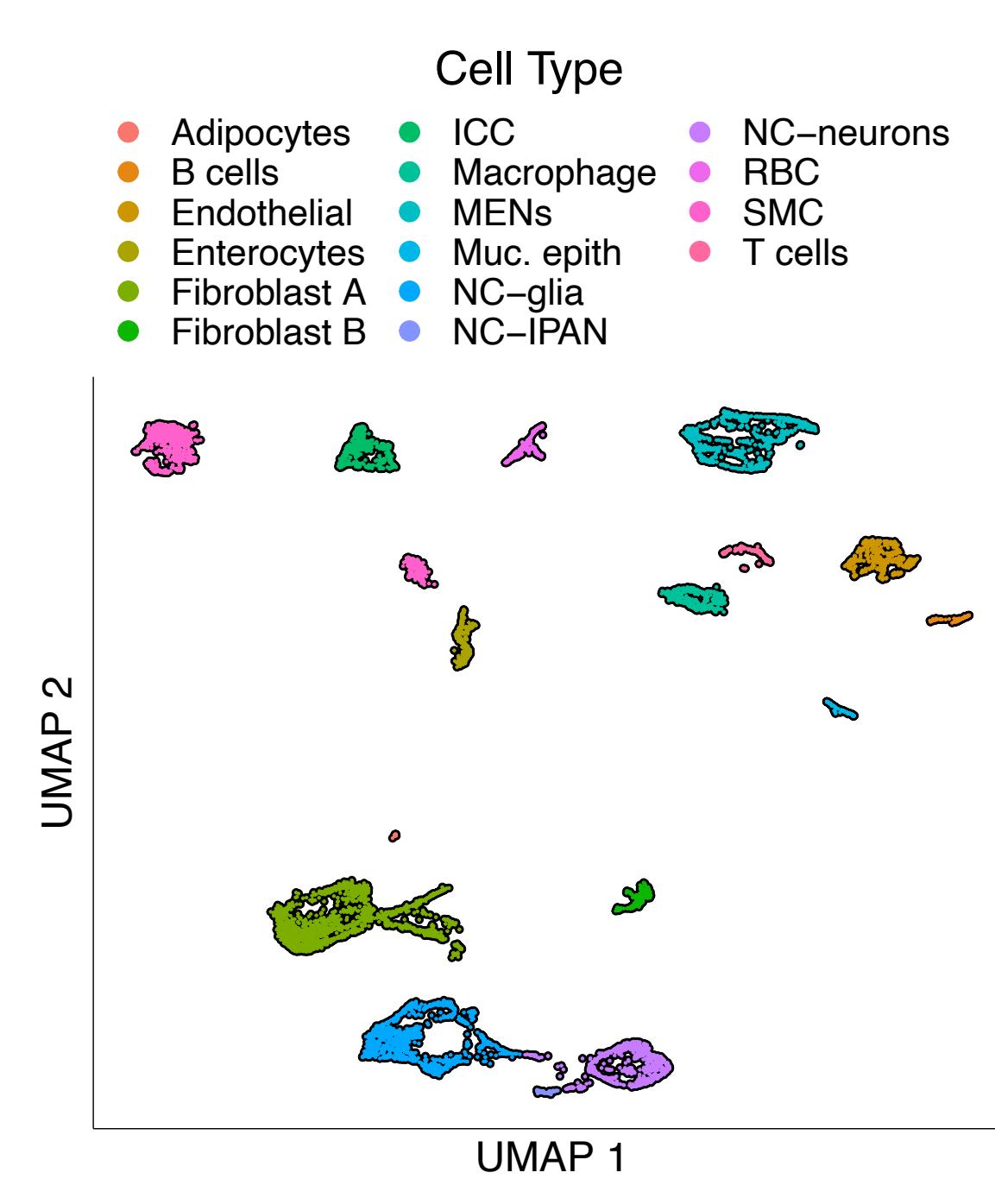
- Characterize the role of the distinct enteric neuronal lineages in health and disease
- Utilize publicly-available GWAS to construct phenotype-relevant gene sets
- Integrate these gene sets with single-cell RNA expression to **highlight associations between cell state and biological (disease) pathways**

## Methods

- Single-cell RNA-sequencing was used in mice to generate gene expression profiles for ENS-relevant cell types in the longitudinal muscle and myenteric plexus of the small intestine (P10, P20, P60)
- To screen cell types in the ENS for association with diverse phenotypes, GWAS summary statistics were collected for 111 phenotypes (104 from UKBB, 7 from various independent studies)
- MAGMA was used to generate gene-level associations with each trait (MAGMA-pval, 10kb windows), and gene sets were constructed from top scoring genes (FDR < 0.05; 100-500 gene constraint)
- Cells were queried for their expression of phenotype-relevant gene homologs via Single Cell Disease Risk Score (scDRS), yielding cell-trait associations



Half of all adult small intestinal myenteric neurons are derived from a non-neural crest lineage



## Results

- Associations of B cells and macrophages with a variety of hematological traits and immune-involved diseases were recovered
- We discover putative uncharacterized associations for MENs with rheumatoid factor serum levels and instances of hernia

## Conclusions & Limitations

- Integration of phenotype-associated genetic loci with expression data suggests implicated cell types, which will inform future experiments in disease-relevant contexts
- Future work will utilize expression networks to prioritize candidate genes and validate via smFISH
- False negatives may arise due to
  - underpowered GWAS or inaccurate classifications (e.g. ICD codes, self-reported measures)
  - profiling of cells from a young, healthy mouse, rather than in a aged or disease context

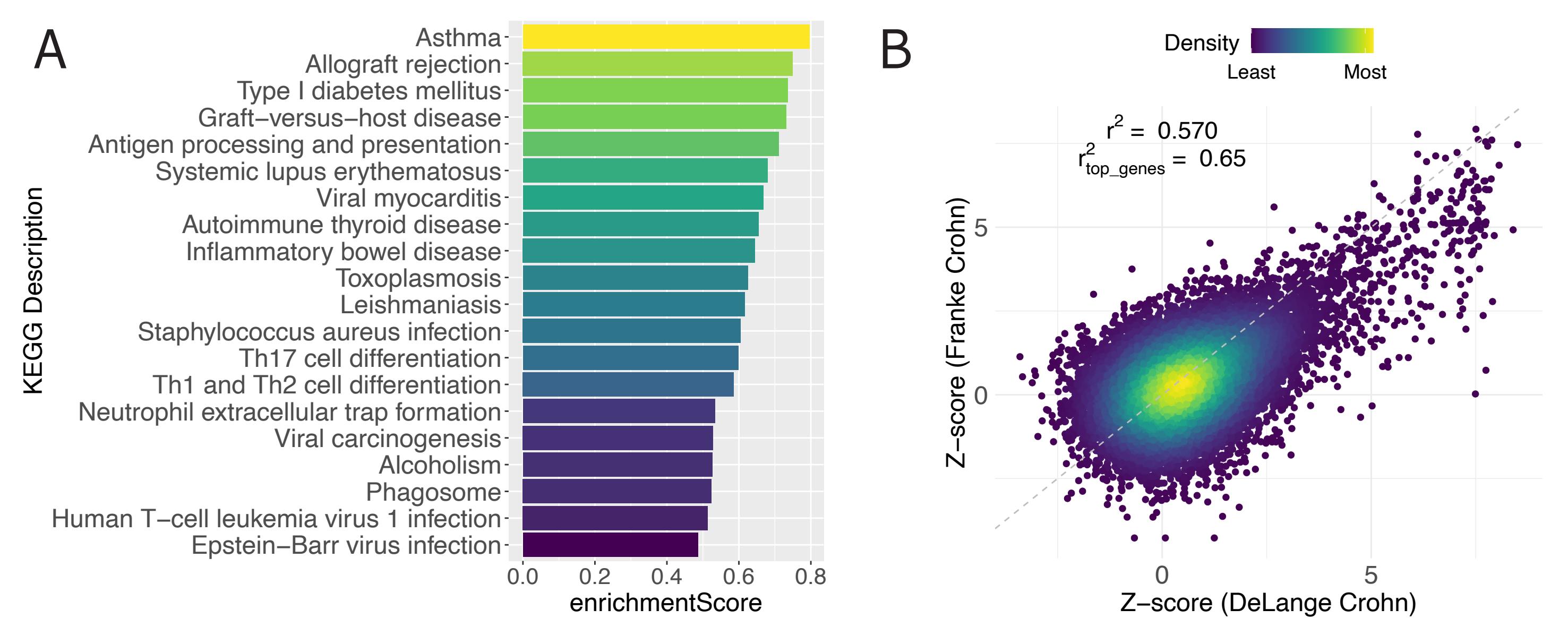


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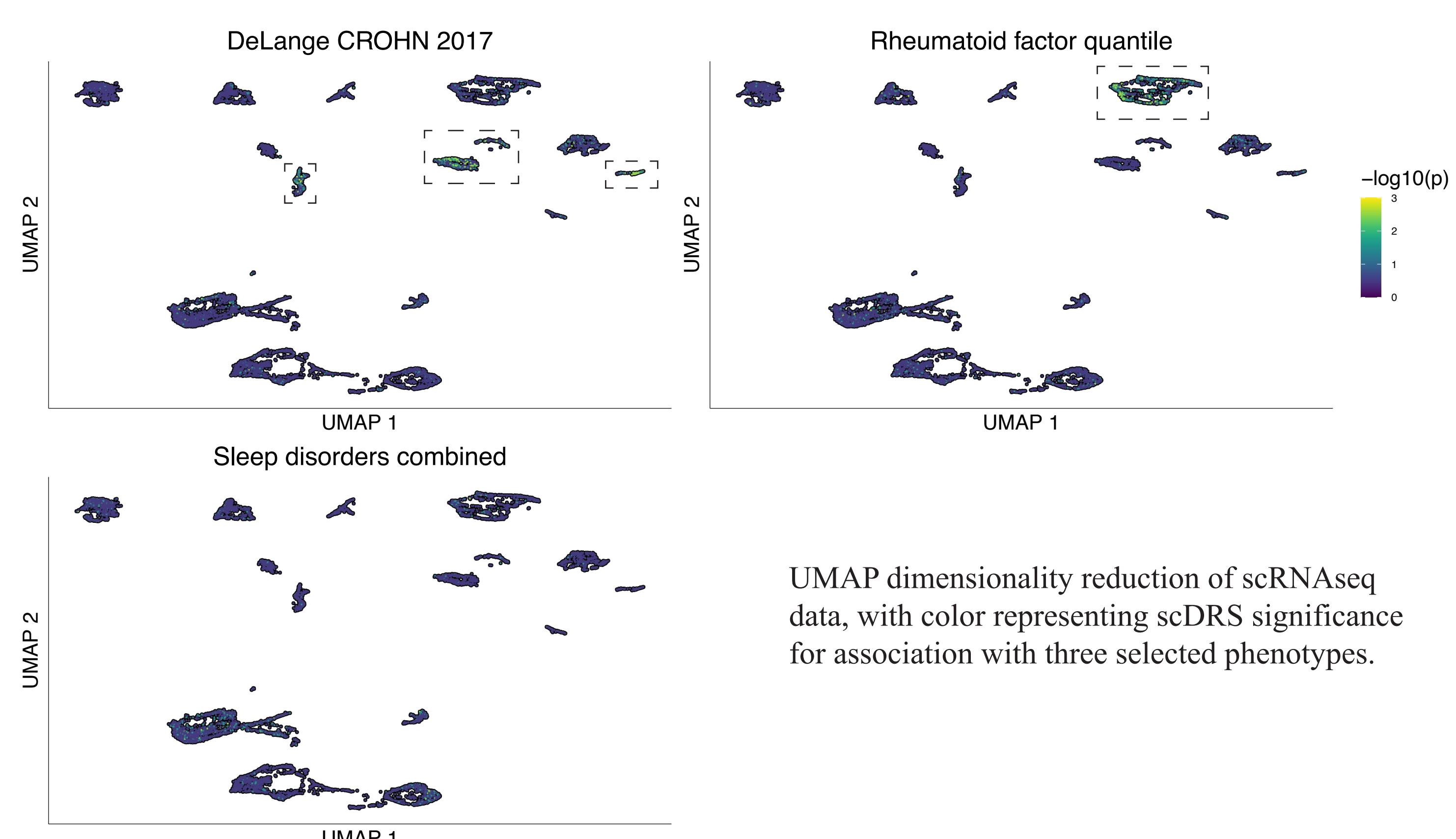
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jaredslosberg.github.io

## Acknowledgments

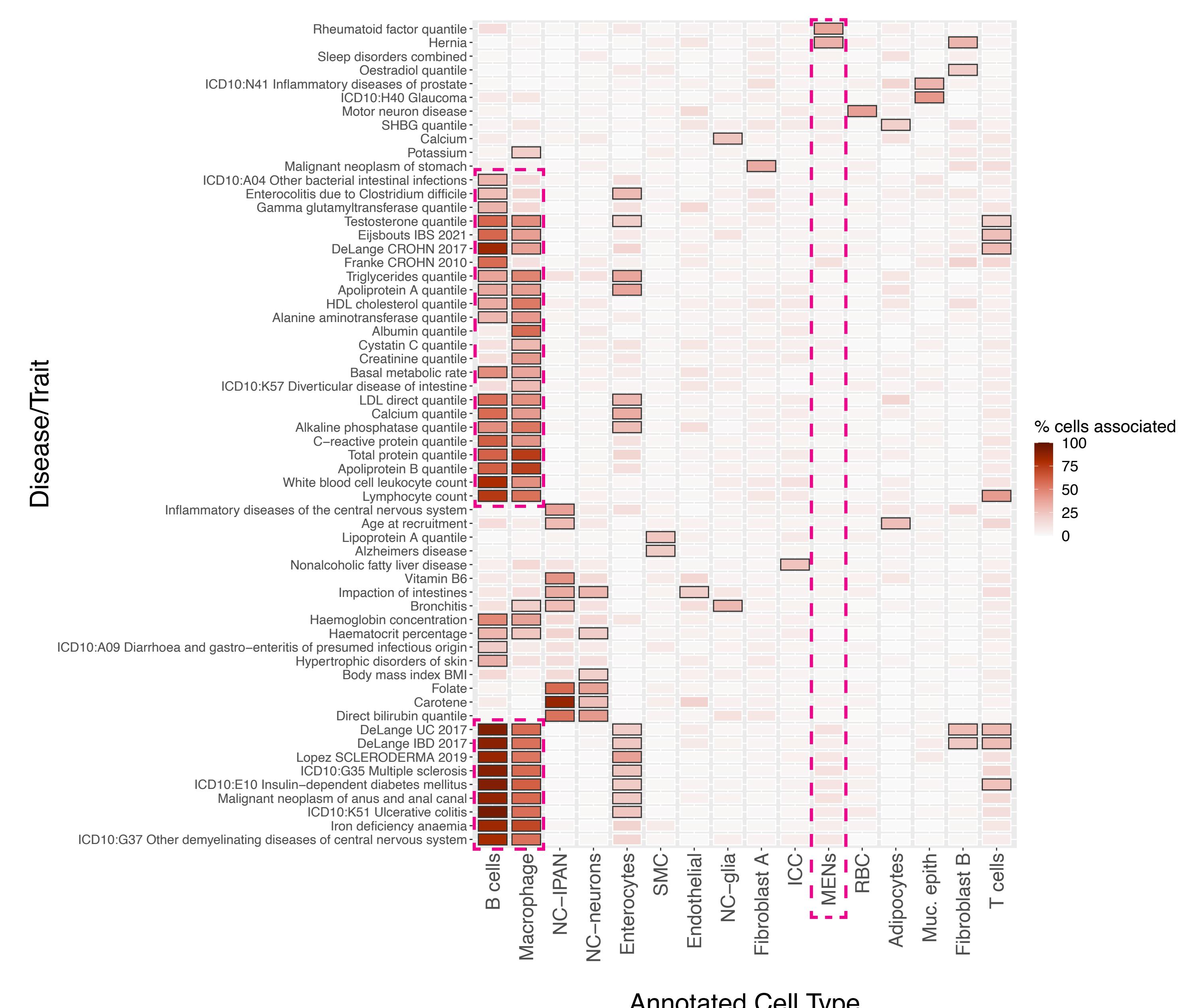
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Quality control metrics for gene-level statistical associations. A) Gene set enrichment analysis. Gene sets for Crohn's (DeLange) were tested against KEGG terms, with top 20 enriched terms shown. B) Comparison of 17,984 MAGMA gene-level scores for two Crohn's GWAS with independent cohorts. Correlation for "top genes" was calculated on 314 genes shared between thresholded genesets.



UMAP dimensionality reduction of scRNAseq data, with color representing scDRS significance for association with three selected phenotypes.



Associations between 16 cell types and 60 filtered phenotypes for which at least one positive association was discovered. Tile color reflects the percent of cells within a cluster that passed a nominal threshold of 5% FPR for each phenotype. Tiles are outlined in black if 20% of the cells in that cluster are associated. Pink dashed boxes highlight two cell-trait clusters involving myeloid/lymphoid cells and blood/immune-dysregulated phenotypes, as well as the association scores for MENs.

## References

- Kulkarni, S. et al. (2020) Neural crest-derived neurons are replaced by a newly identified mesodermal lineage in the post-natal and aging enteric nervous system [Preprint]. BioRxiv. <https://doi.org/10.1101/2020.08.25.262832>
- de Leeuw, C. A. et al (2015). MAGMA: Generalized Gene-Set Analysis of GWAS Data. PLOS Computational Biology, 11(4), e1004219. <https://doi.org/10.1371/journal.pcbi.1004219>
- Zhang, M. J., Hou, K., et al (2022). Polygenic enrichment distinguishes disease associations of individual cells in single-cell RNA-seq data. Nature Genetics, 54(10), 1572–1580. <https://doi.org/10.1038/s41588-022-01167-z>