

Spatial Transcriptomic Profiling of Human Hypothalamic Sex Differences: Ventromedial and Arcuate Hypothalamic Nuclei

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The Tuberal Hypothalamus: A Social, Metabolic, and Endocrine Node

The tuberal hypothalamus, which includes the ventromedial hypothalamus (VMH) and arcuate (ARC) nuclei, has been characterized extensively in rodents for their roles in controlling social and appetitive behavior as well as metabolism. Several functions of both ARC and VMH have been shown to be sex-differential at molecular, physiologic, and behavioral levels in adult rodent, largely mediated through neuron populations expressing gonadal hormone receptors including estrogen receptor *Esr1* (1). Hypothalamic functions are summarized in **Figure 1** below.

To address the lack of modern transcriptomic data from metabolically healthy and reproductive-age adult hypothalamus of both sexes, we performed spatial transcriptomics (10x Visium platform) on postmortem tuberal hypothalamus (**Figure 2**). We used these results to select 100 genes with specific, sex-differential, or spatially variable gene expression in ARC/VMH for single-molecule profiling with *in situ* sequencing with the 10x Xenium platform. Tissue was from neurotypical, metabolically healthy young-to middle-aged donors (27-48 years old) in a design powered to detect transcriptomic sex differences (N=6 female samples per platform from the same 4 female donors; N=4 (Visium) or 7 (Xenium) male samples from the same 4 male donors). We identified major spatial divisions of VMH and ARC, novel human-specific features marking these areas, and investigate sex differences within these domains and their subdivisions.

Analyses were performed in R 4.3.1 using the following packages: *SpatialExperiment*, *scater*, *scran*, *nnSVG*, *Harmony*, *BayesSpace*, *SpatialLIBD*, *dreamlet*, and *fgsea* (utilizing gene sets provided in mSigDB 2023, release #2). Visualizations were created using *SpatialLIBD*, *ggplot2*, and *Biorender*.

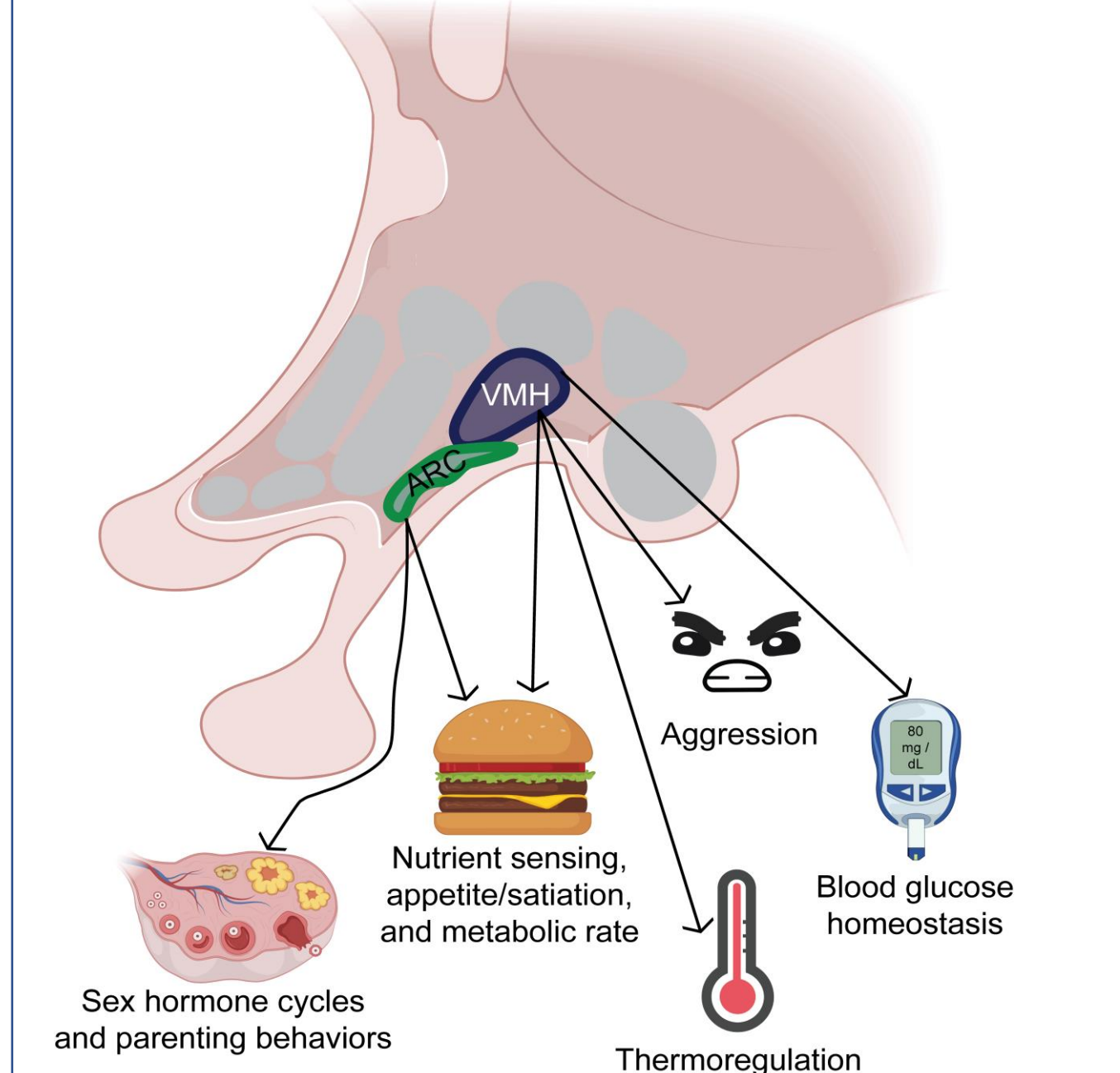


Figure 1. Functions of the VMH and ARC characterized in rodent literature to date. ARC and VMH cooperatively regulate LH and GnRH via the kisspeptin (KISS1) system (2); the VMH regulates physiologic and behavioral responses to blood glucose, and ARC regulates appetite/satiety more broadly (3); and rodent VMH populations have sex-differentiated roles in gating aggressive behavior (4).

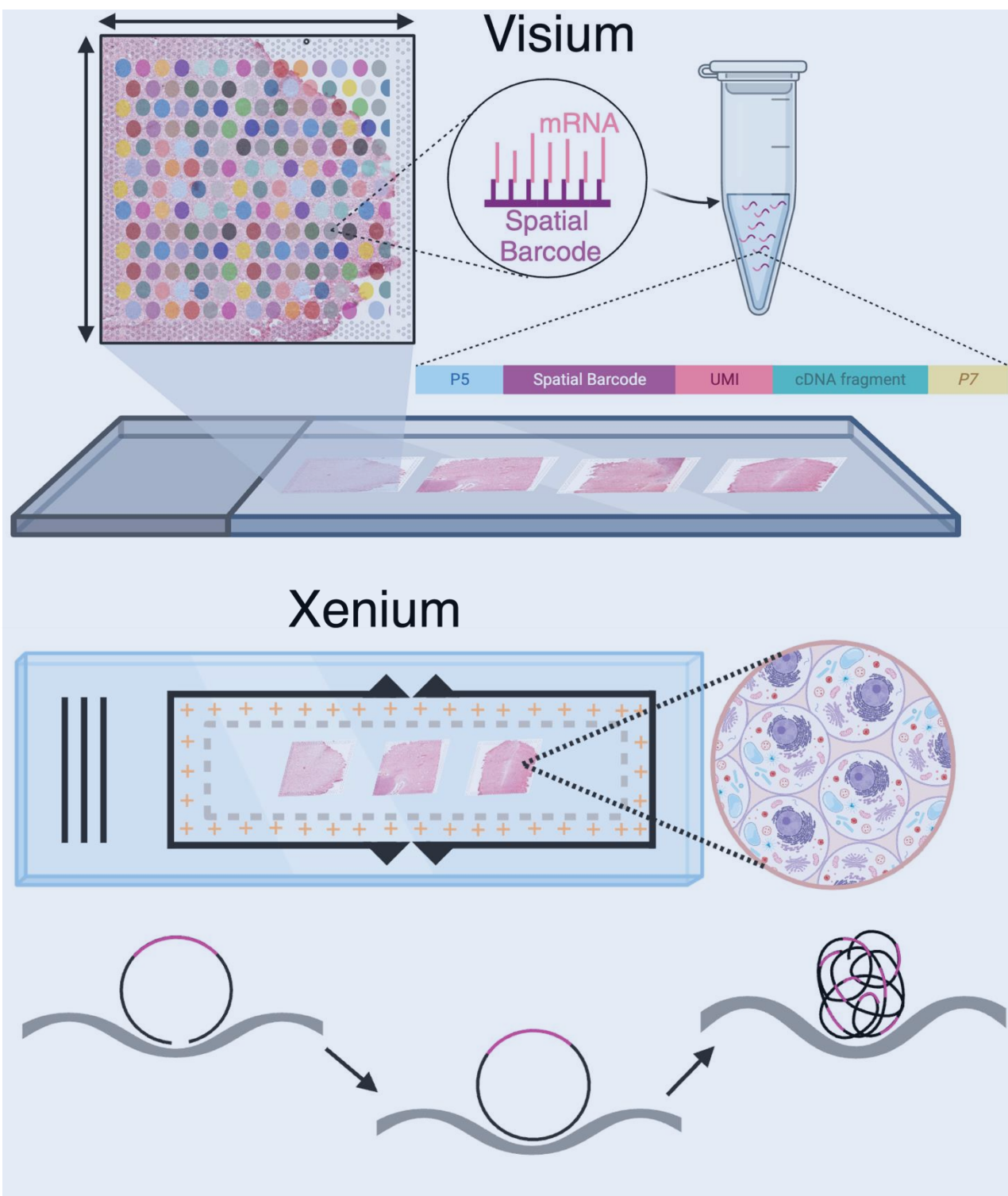


Figure 2. Overview of 10x Genomics' Visium and Xenium platforms. Visium: Fresh-frozen tissue is mounted onto slides with pre-linked sequencing primers, which encode the spot of origin for each RNA. Xenium uses probes against RNAs of interest and rolling-circle amplification *in situ* to mark transcript locations. Nucleotide-fluorescent protein hybrids bind segments of amplified products, producing microscope-detected signals over several rounds to encode an RNA's identity.

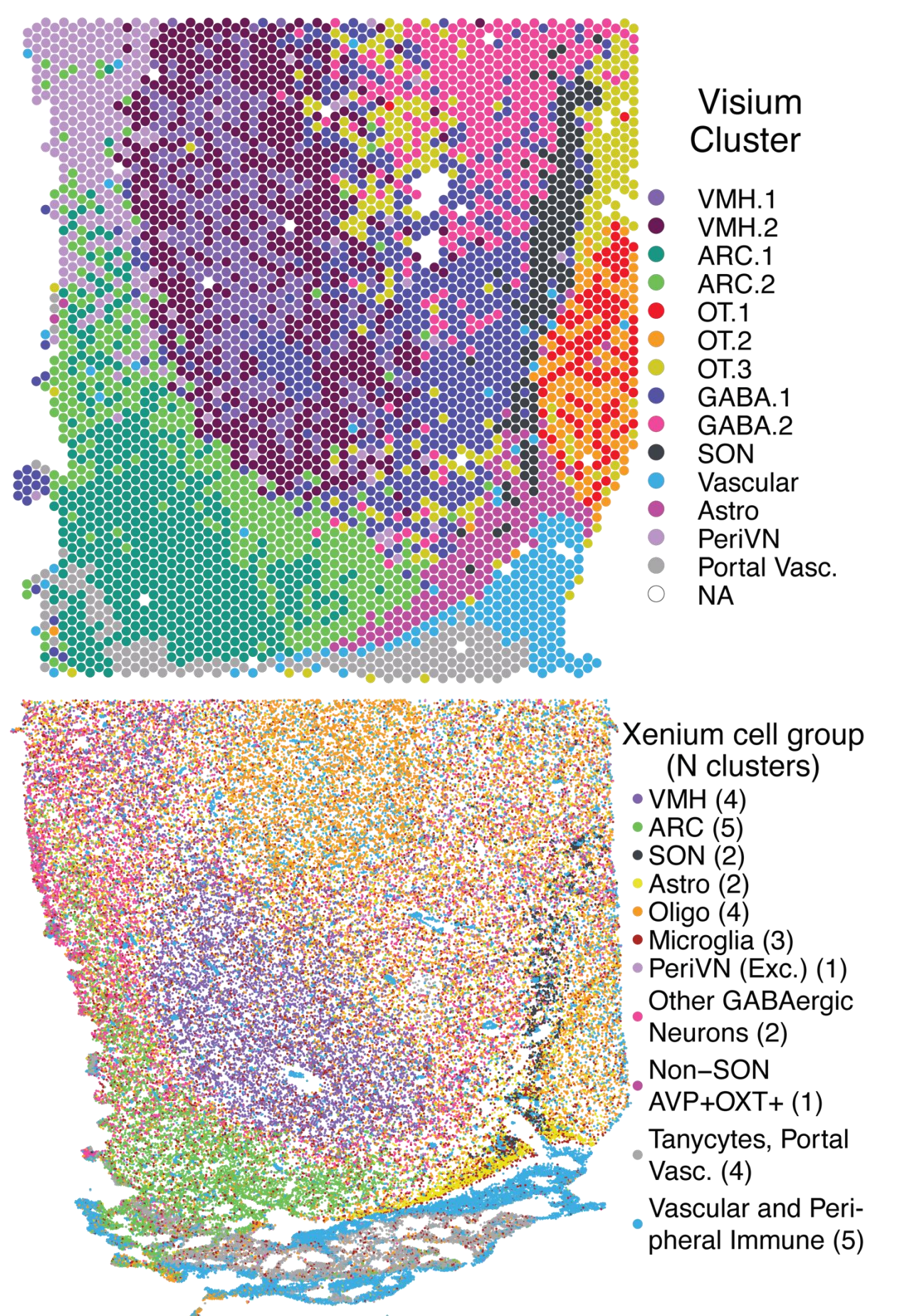


Figure 3. Expression clusters in Visium- (top) or Xenium- (bottom) analyzed tissue from one donor. Visium spatial clustering with *BayesSpace* and 15 clusters; Xenium non-spatial clustering with *Banksy* and Leiden clustering, yielding 41 clusters grouped here by major cell type.

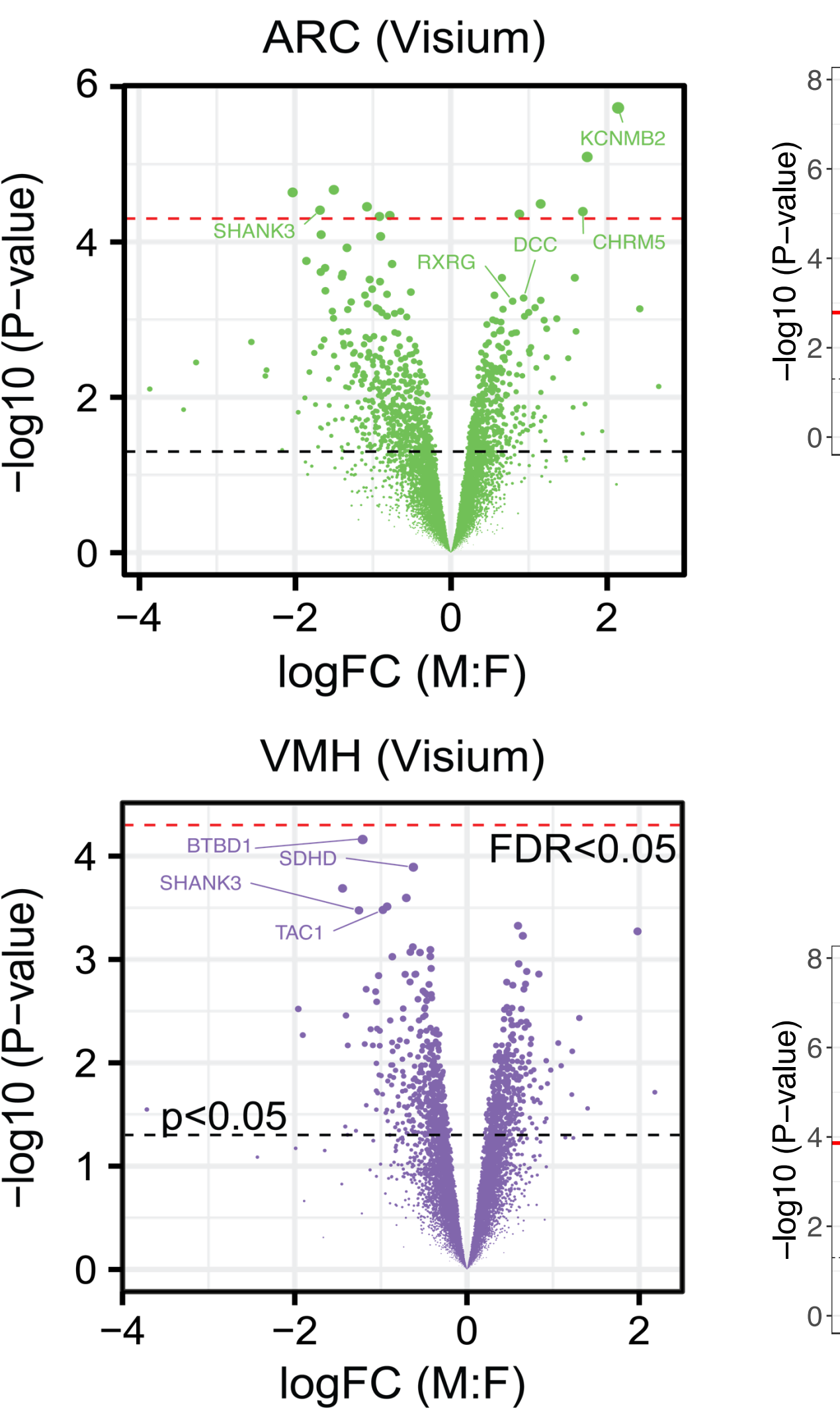


Figure 7. Sex-differential expression (DE) in Visium domains and Xenium VMH/ARC cell clusters. Red line: FDR<0.05; black line $p < 0.05$.

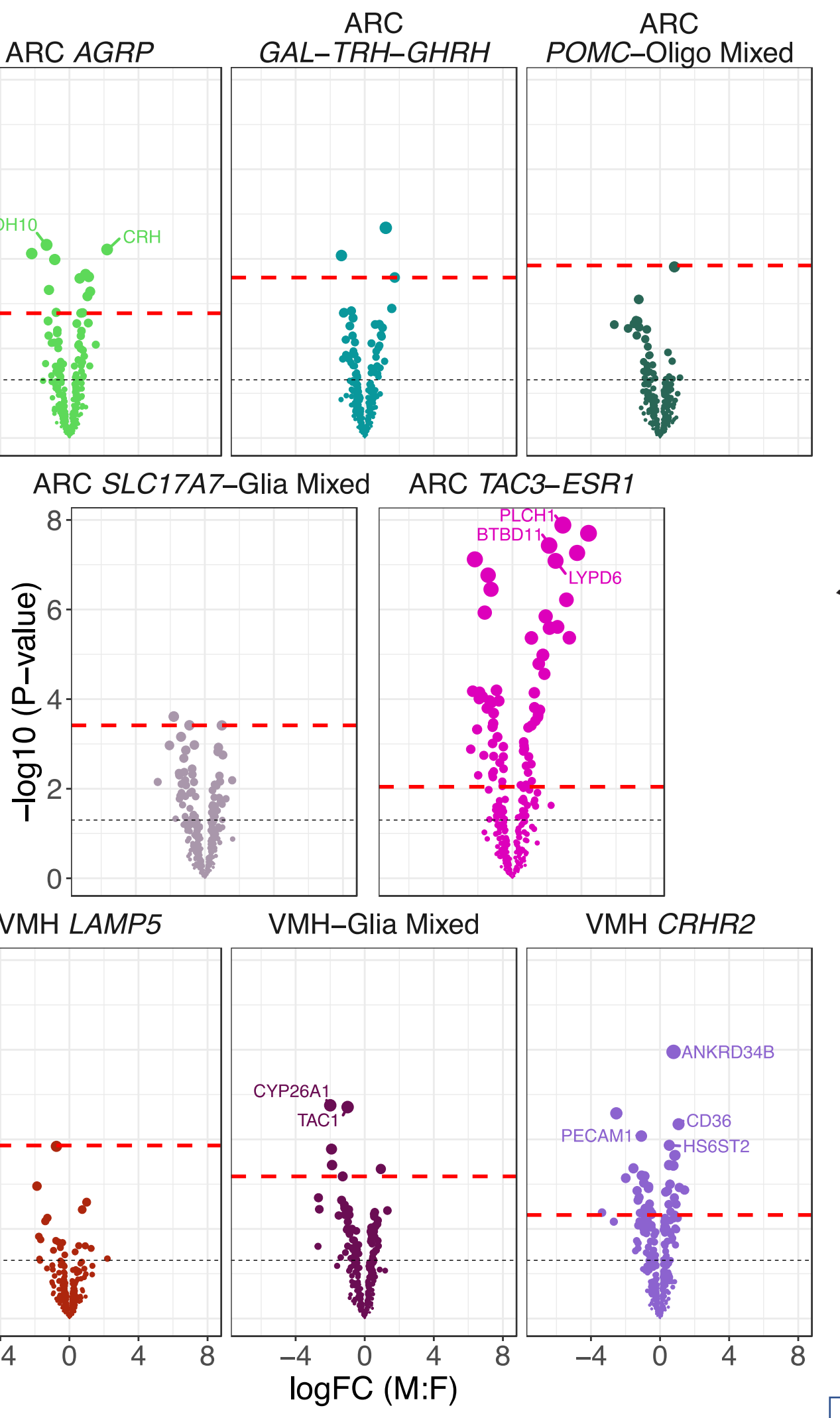


Figure 8. Attribution of sex DE to cell types with Xenium. Pseudobulk per sample. Bold: DE at FDR<0.05.

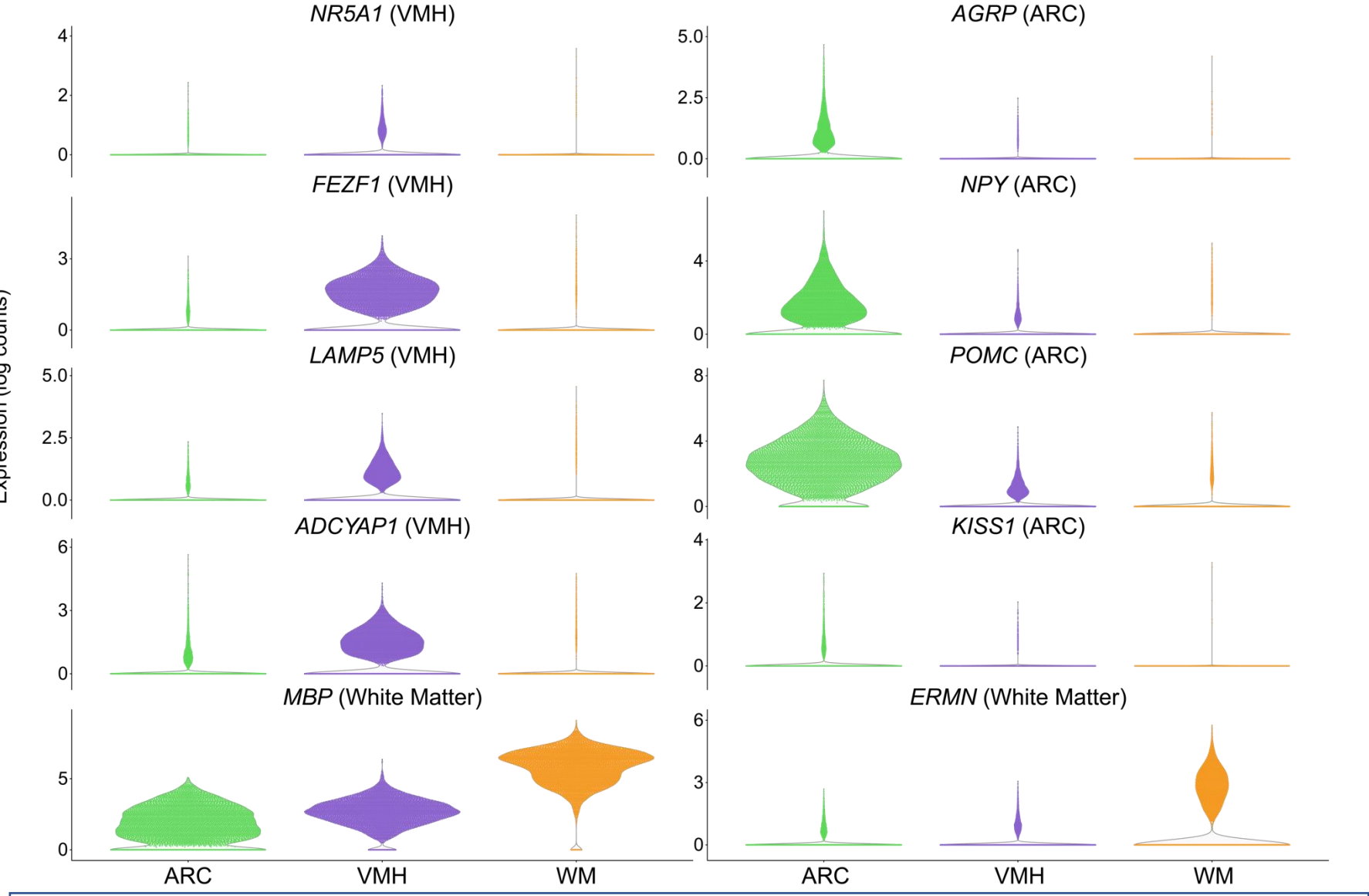


Figure 4. Known and novel marker genes enriched in VMH and ARC.

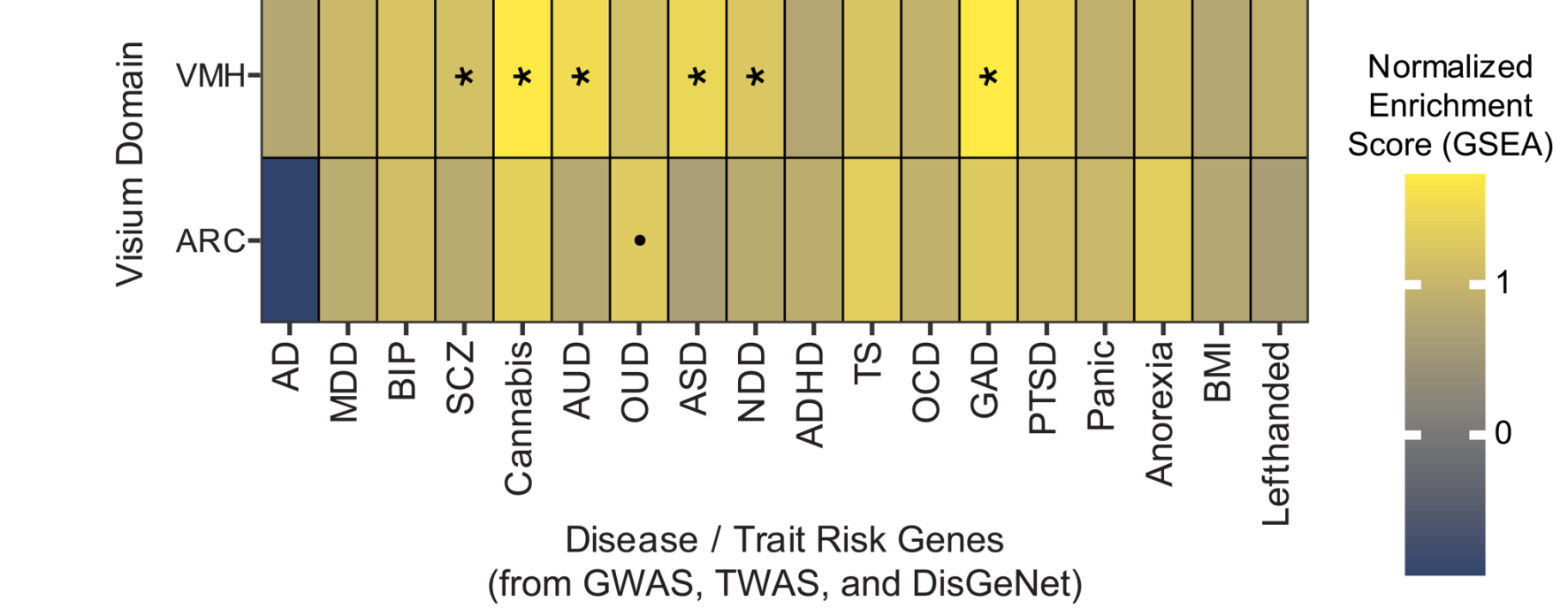


Figure 5. Genes associated with SCZ, substance use disorders, ASDs / neurodevelopmental disorders, and anxiety are enriched for VMH marker-like expression patterns. GSEA analysis. Gene sets collated from GWAS, TWAS literature, plus curated genes in DisGeNet. * FDR<0.05 ; * uncorrected $p < 0.05$.

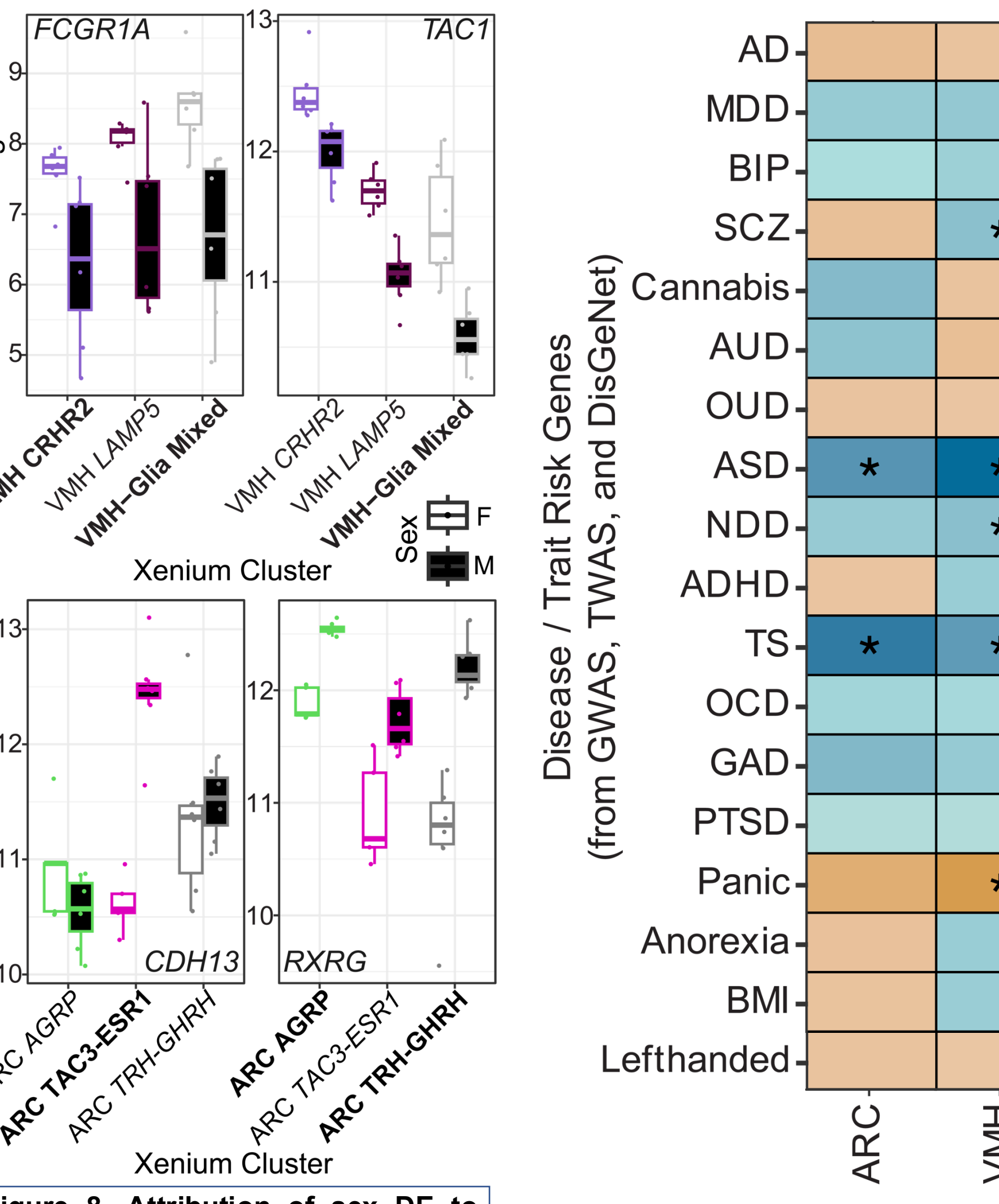


Figure 9. Genes with higher baseline male expression in VMH/ARC are enriched in ASD risk genes to a greater extent than NDD risk genes.

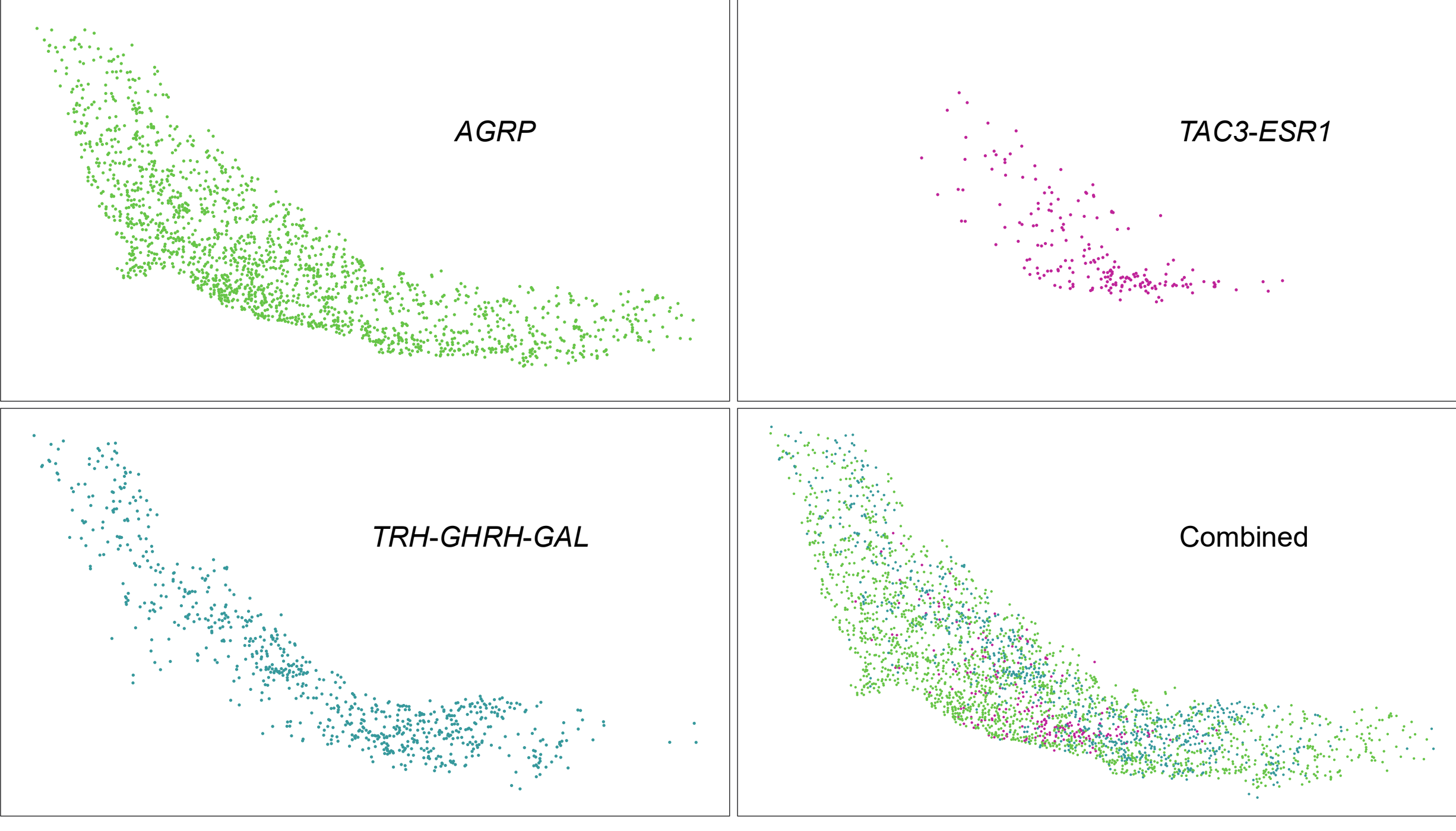


Figure 6. Xenium reveals spatial organization of neuron types in the human arcuate nucleus. Each of 3 key cell clusters of the 5 identified are plotted within the ARC 'domain'—the spatial field to which ARC neurons were restricted—of one sample.



Scan to read more and visualize the data!

Conclusions

- Spatial transcriptomic techniques are applicable to brain regions with dense-packed, complex anatomy, like hypothalamus.
- Hypothalamic gene expression defies patterns identified elsewhere in brain. *LAMP5*—an interneuron marker in other brain regions—marks excitatory VMH neurons.
- Sex-differential expression in adult human HYP is detectable even with small sample sizes.
- Across VMH- and ARC-specific populations, **sex effects are most marked in *TAC3-ESR1* ARC neurons.**
- Genes expressed more highly in **neurotypical male VMH** are **enriched for psychiatric disorder-associated genes.**

Acknowledgements and References

I would like to thank WCPG for allowing me to share this work; and the Martinowich, Maynard, Page, Collado, Hicks, and Hansen groups at Lieber and Johns Hopkins for their collaboration and consultation in the performing these assays and analyses. We all would like to acknowledge the brain donors and their families, without whom this work would not be possible. This work was funded by the Lieber Institute for Brain Development, 10x Genomics, and NIMH 5T32MH015330-44.

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