

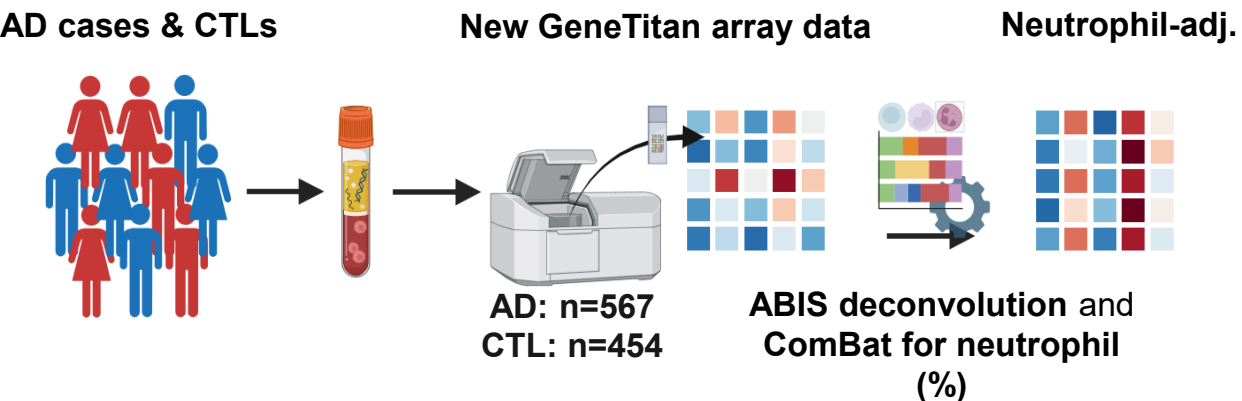
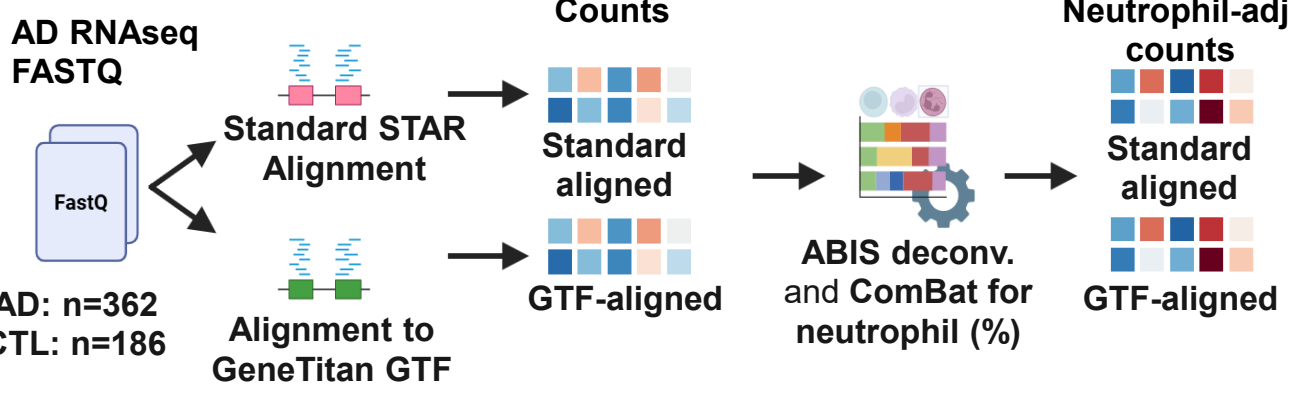
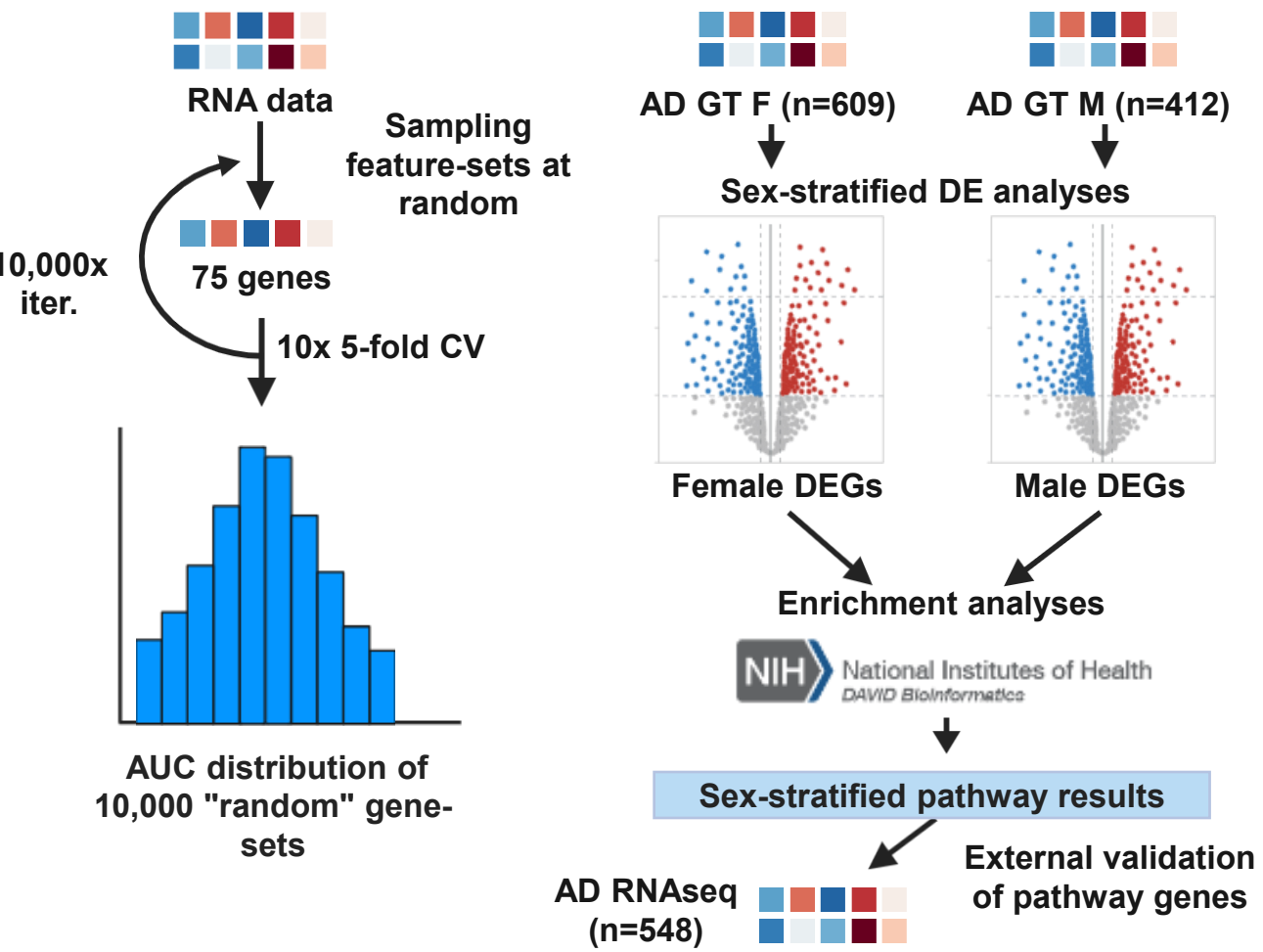
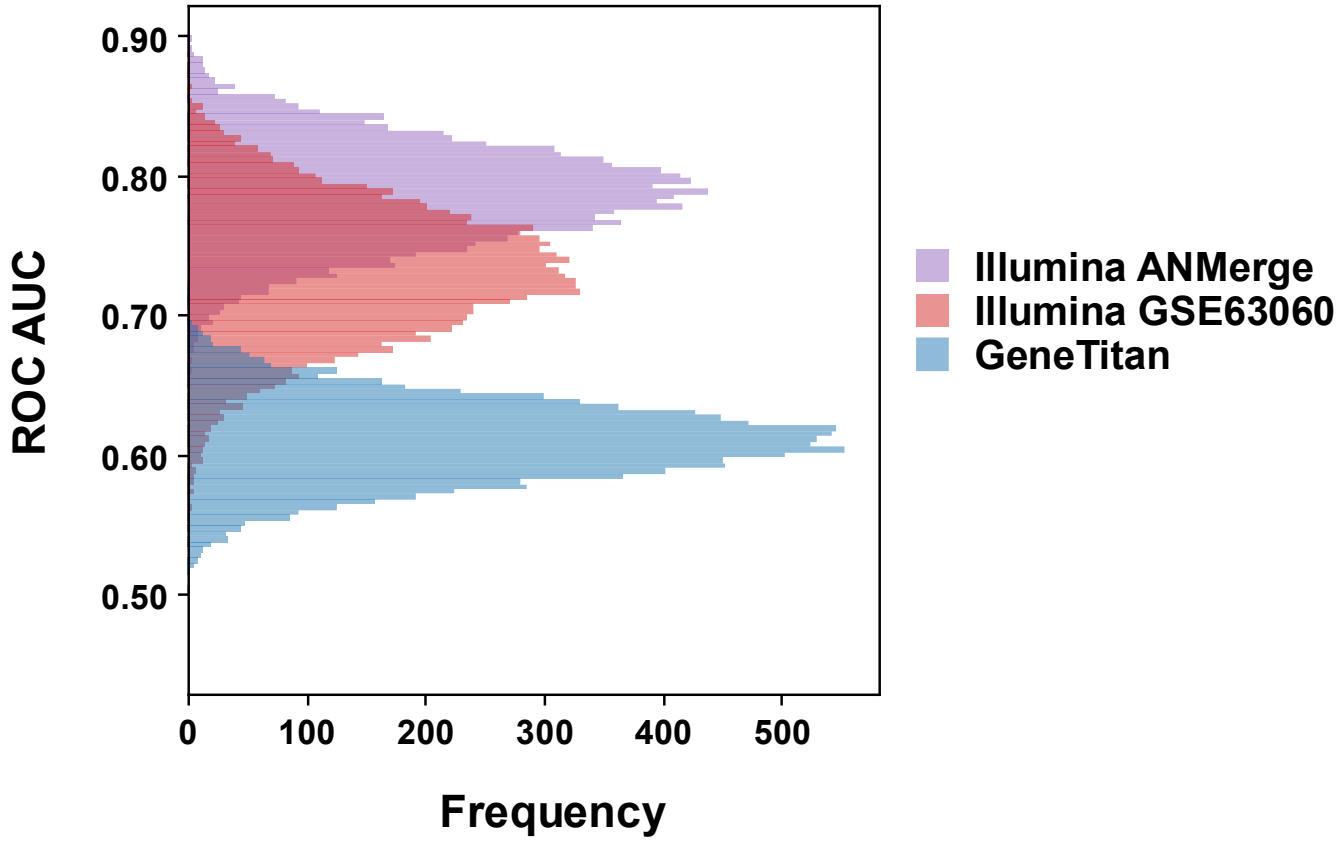
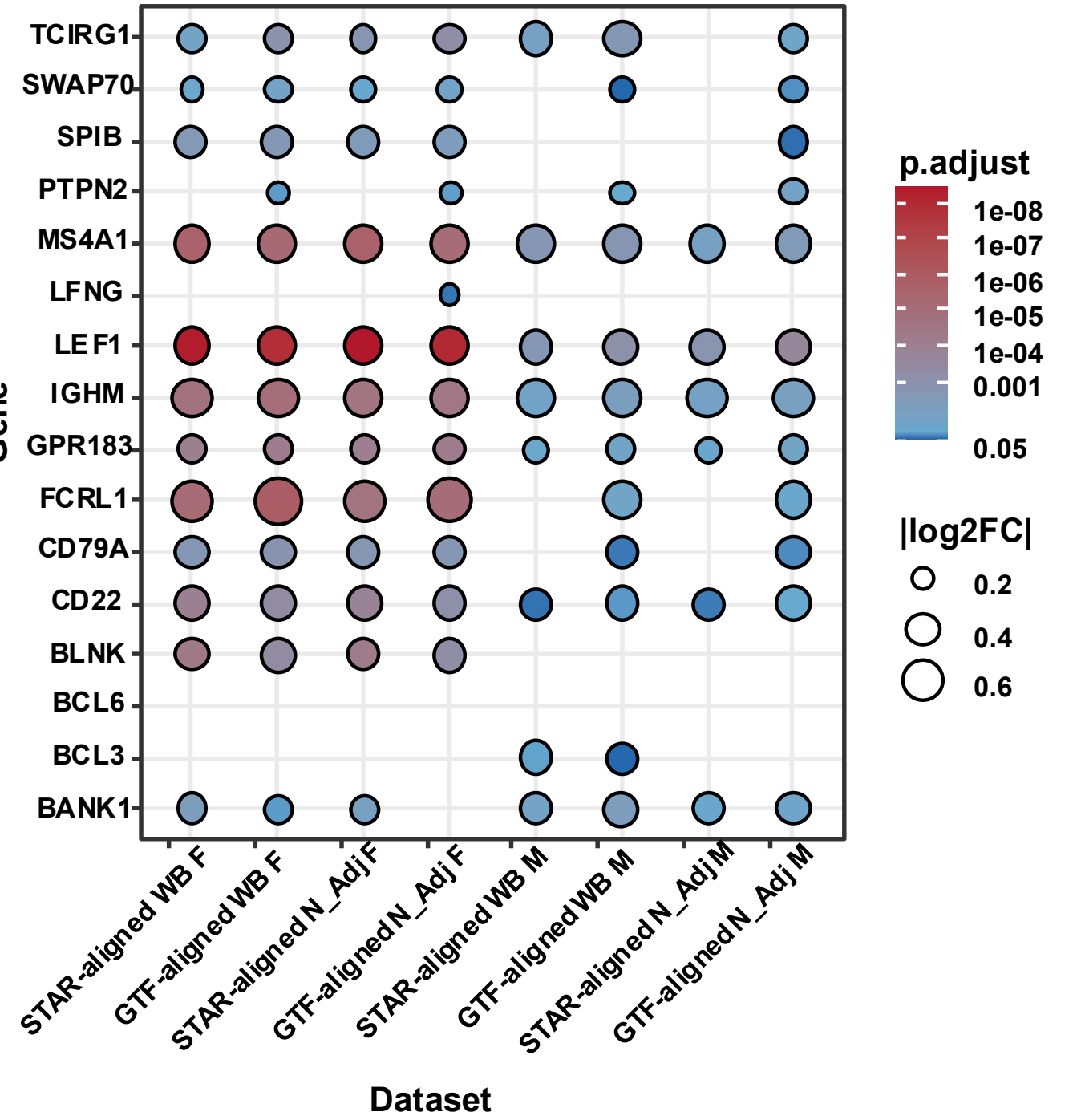
Sex-stratified Blood Transcriptomics Highlights Immune Dysregulation In Alzheimer’s Disease

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Neutrophil-adjusted blood transcriptomics reveal a robust B-cell–driven immune signature shared by women and men with Alzheimer’s disease, while mitochondrial pathway changes appear composition-dependent.

Introduction	Methods	Results	Conclusions	References
Background Women have a higher lifetime risk of Alzheimer’s disease (AD) than men, but the underlying biological basis remains unclear. Previous AD blood transcriptomic studies are limited by technical bias, insufficient sample sizes for sex-stratified analyses, and inadequate adjustment for blood cell composition, complicating interpretation of transcriptomic findings. Challenges Technical confounding: Differences in laboratory processing (e.g. equipment, technician, time) introduce artefacts into gene expression, obscuring disease signals and complicating meta-analysis across multiple transcriptomic datasets. Biological confounding: Other health conditions (e.g. T2D, CVD) can influence blood cell composition [1], masking AD-specific gene changes. Contributions - We generated a large, batch-corrected GeneTitan whole-blood resource from the AddNeuroMed and Dementia Case Register clinical cohorts [2] and demonstrated that this dataset is technically robust. - In an analysis with an additional independent RNA-seq cohort, we assessed whether AD-associated peripheral transcriptomic signatures differ by sex and remain robust after adjustment for neutrophil content using ComBat [3].	Transcriptomic data were batch-corrected and adjusted for deconvoluted neutrophil fraction (estimated using absolute immune signal deconvolution [4]). Sex-stratified DE and pathway enrichment was performed on GeneTitan array data using samr, and ClusterProfiler using the DAVID database. Enriched genes were investigated in independent RNA-seq using DESeq2 New GeneTitan array data (n=1021, 567 AD, 454 CTL)  Griswold et al. RNA-seq (n=548, 362 AD, 186 CTL)  QC and Differential Expression analysis 	A)  We compared random classifier performance across existing AddNeuroMed/ANMerge and new GeneTitan data using 75-gene sets sampled at random. For each dataset, 10,000 logistic regression models produced AUC distributions; means closer to 0.5 indicate less bias. GeneTitan shows reduced bias and better suitability for classification analyses. B)  B-cell biology was altered in AD and appears consistent in men and women. Other pathways, including mitochondrial dysfunction, were not robustly detected as DE in blood transcriptomes.	Key findings - Sampling-at-random gene-set analyses showed reduced bias in the new GeneTitan data compared with legacy Illumina, supporting its use for sex-stratified blood transcriptomic analyses. - Neutrophil adjustment indicated that previously reported mitochondrial enrichment is composition-dependent, while revealing more robust immune signatures, particularly B-cell activation pathways. - B-cell pathway genes replicated in RNA-seq in both women and men, and updated analyses did not support significant sex-specific pathway differences, instead pointing to a shared peripheral B-cell–associated AD signal - Overall, differential expression in blood transcriptomics does not appear to capture sex differences in AD at this disease stage and may require earlier time points to clarify mechanisms underlying sex-biased onset. Future directions - Assess if sex differences in AD blood transcriptomes can be detected using co-expression network analysis - Model disease progression using longitudinal blood transcriptomic profiles to identify early sex-biased signals.	- Soehnlein O, Steffens S, Hidalgo A, et al. Neutrophils as protagonists and targets in chronic inflammation. <i>Nature Reviews Immunology</i> 2017 17:4 2017;17:248–61. https://doi.org/10.1038/nri.2017.10. - Lunnon K, Keohane A, Pidsley R, et al. Mitochondrial genes are altered in blood early in Alzheimer’s disease. <i>Neurobiol Aging</i> 2017;53:36–47. https://doi.org/10.1016/J.NEUROBIOLAGI.2016.12.029. - Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. <i>Biostatistics</i> 2007;8. https://doi.org/10.1093/biostatistics/kxj037. - Monaco G, Lee B, Xu W, et al. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. <i>Cell Rep.</i> 2019;26:1627–1640.e7. doi:10.1016/j.celrep.2019.01.041
Preprint  Scan for preprint, extended analyses and cohort details. 				