

Familial Alzheimer's Disease Mutations Drive an Enhanced Interferon Response in iPSC-Derived Microglia

Abstract

Autosomal dominant Alzheimer's disease (ADAD), caused by highly penetrant mutations in APP, PSEN1, and PSEN2, provides a unique model for defining the earliest pathogenic events in Alzheimer's disease. While ADAD has traditionally been viewed through a neuron-centric lens driven by altered amyloid processing, emerging evidence from genetic, biomarker, and functional studies implicates microglia as key regulators of disease onset and progression. However, it remains unclear how ADAD-causing mutations directly or indirectly alter microglial states, functions, and responses to pathological substrates. Induced pluripotent stem cells (iPSCs) derived from twenty-two donors carrying heterozygous ADAD mutations from the Dominantly Inherited Alzheimer Disease Network (DIAN), and wild-type, related controls were differentiated into microglia via hematopoietic progenitor intermediates. After microglial maturation, bulk RNA-sequencing was performed, with reads aligned, annotated, and quality-controlled prior to downstream analysis. Differential gene expression was assessed using DESeq2, and significantly enriched biological processes were identified through pathway enrichment analysis. Connectivity Map (CMap) analysis was then used to connect the resulting disease-associated expression signature to candidate perturbagens and functional pathways. Differential expression analysis revealed 202 differentially expressed genes between ADAD mutation carriers and non-carrier controls. The ADAD microglia signature revealed a transcriptional shift toward an activated microglial state. Differential genes were enriched in pathways associated with type I interferon signaling, antiviral responses, innate immune regulation, and cytokine/adaptive immunity. To identify compounds that may reverse this ADAD-associated microglia signature, we integrated the ADAD microglia transcriptomic data with Connectivity Map perturbation profiles. This analysis identified perturbagens concordant with the mutation-driven state, including pathways linked to neuroinflammation such as PI3K/mTOR and SYK signaling, both known regulators of pro-inflammatory cytokine production. Together, these findings suggest that ADAD mutations may drive a primed interferon-responsive microglial state, consistent with a microglia-mediated mechanism of increased neuroinflammation and disease pathogenesis in AD.

fAD mutations are heterogeneous

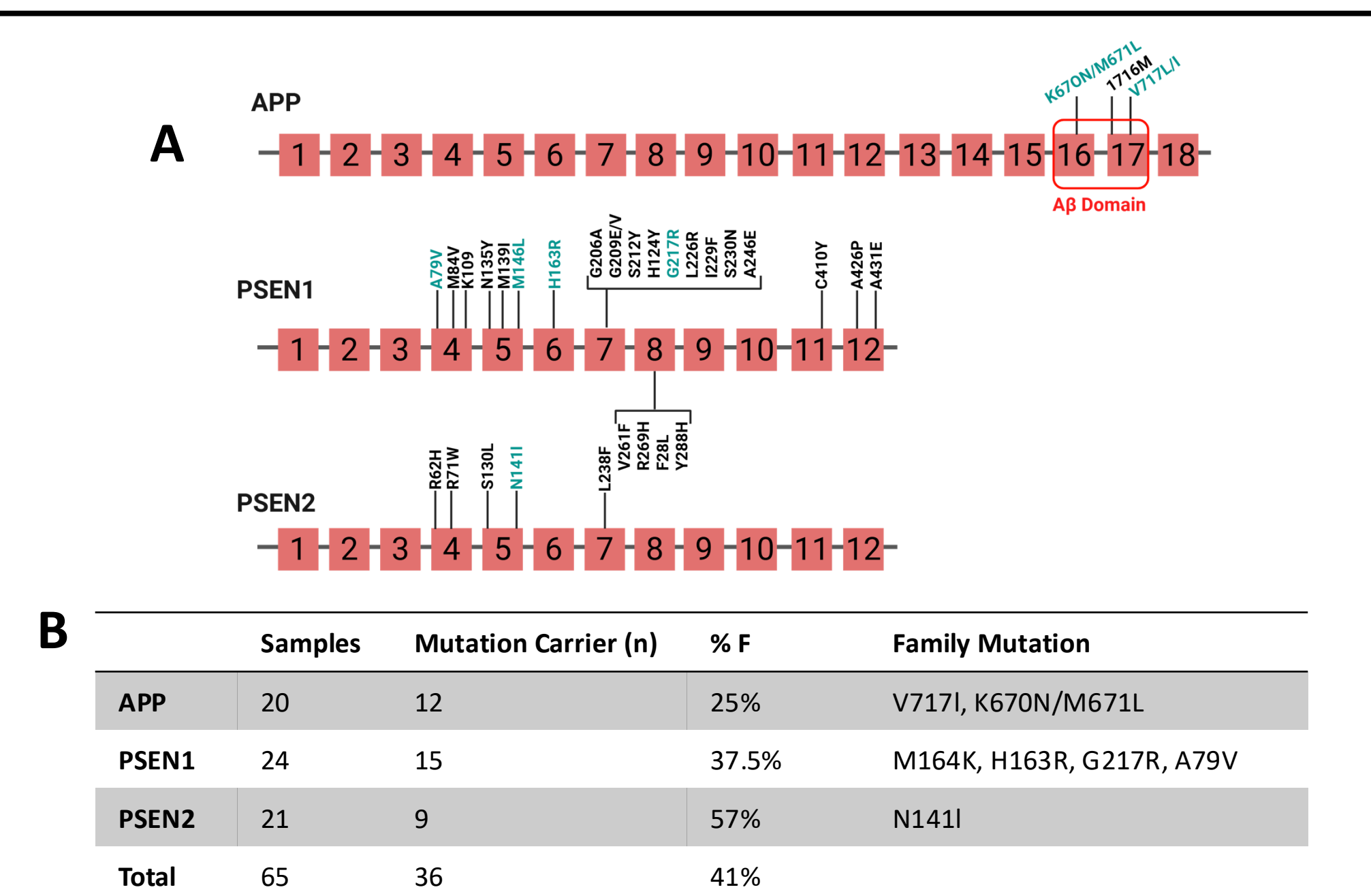
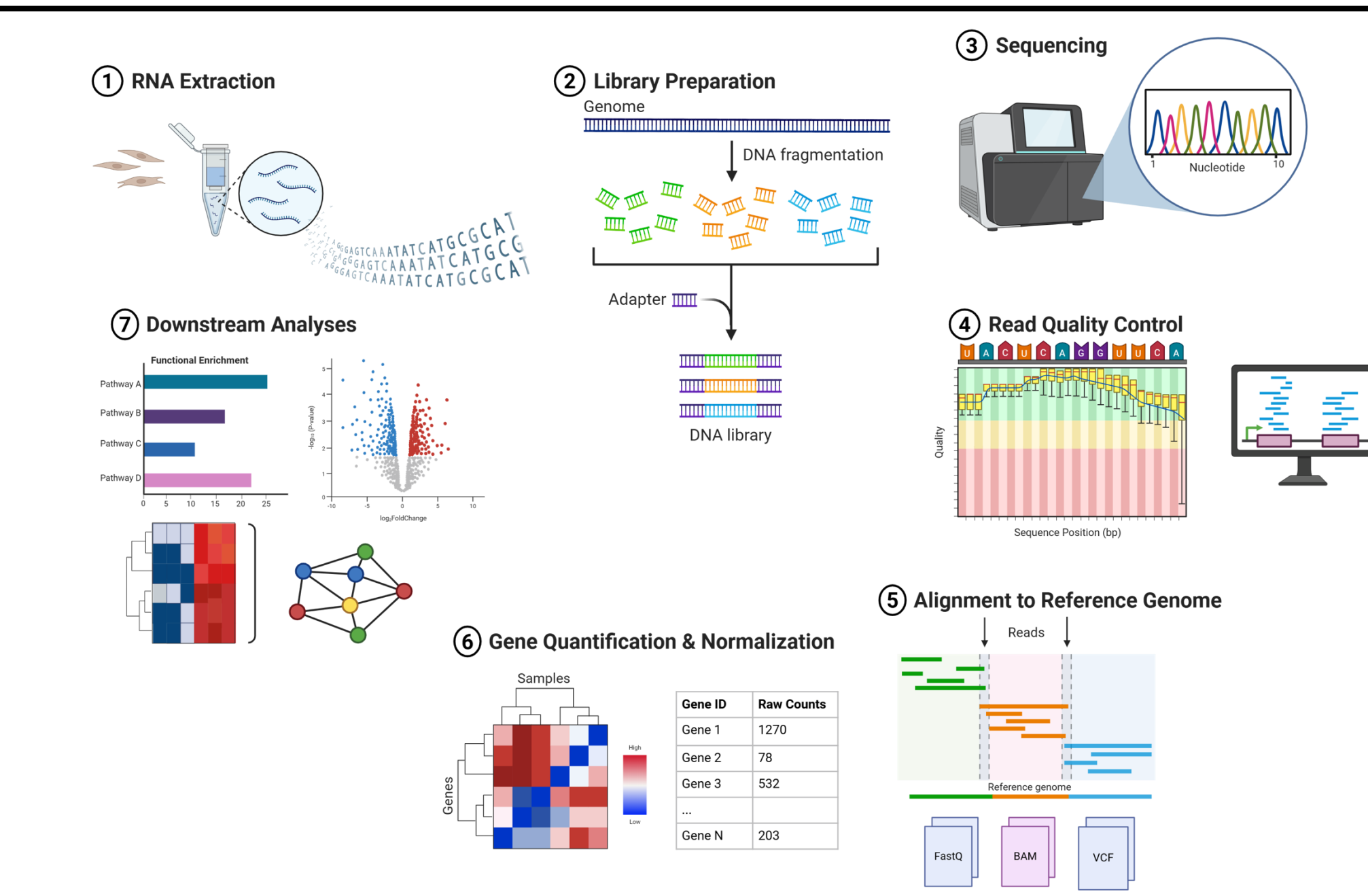


Figure 1. Genetic landscape and demographic characteristics of the autosomal dominant Alzheimer's disease (ADAD) cohort. A. Schematic of APP, PSEN1, and PSEN2 exonic structures indicating the relative positions of reported familial Alzheimer's disease mutations. Cyan labels denote variants selected for analyses. B. Summary of cohort demographics.

Leveraging bulk RNA-Seq to profile the ADAD transcriptome



Establishing iPSC-derived microglia to model ADAD mutations

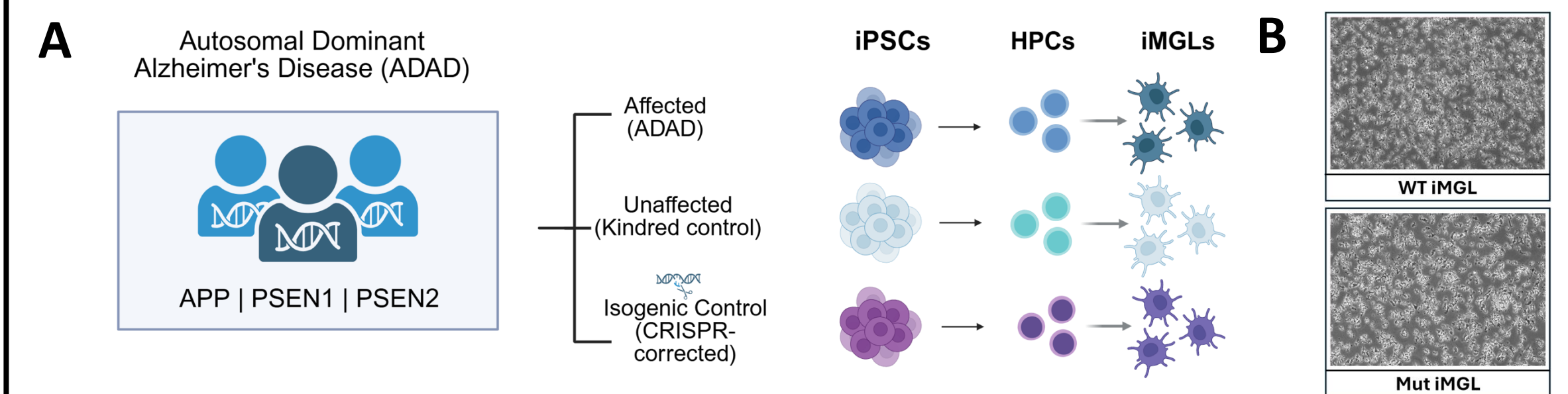


Figure 2. Developing iMGLs from ADAD cohort. A. Schematic workflow showing the generation of induced microglia-like cells (iMGLs) from three donor cohorts representing ADAD mutations. B. Representative phase-contrast images demonstrating late-stage differentiation morphology of wild-type (WT iMGL) and mutant (Mut iMGL) microglial lines.

ADAD mutations converge on shared differentially expressed genes

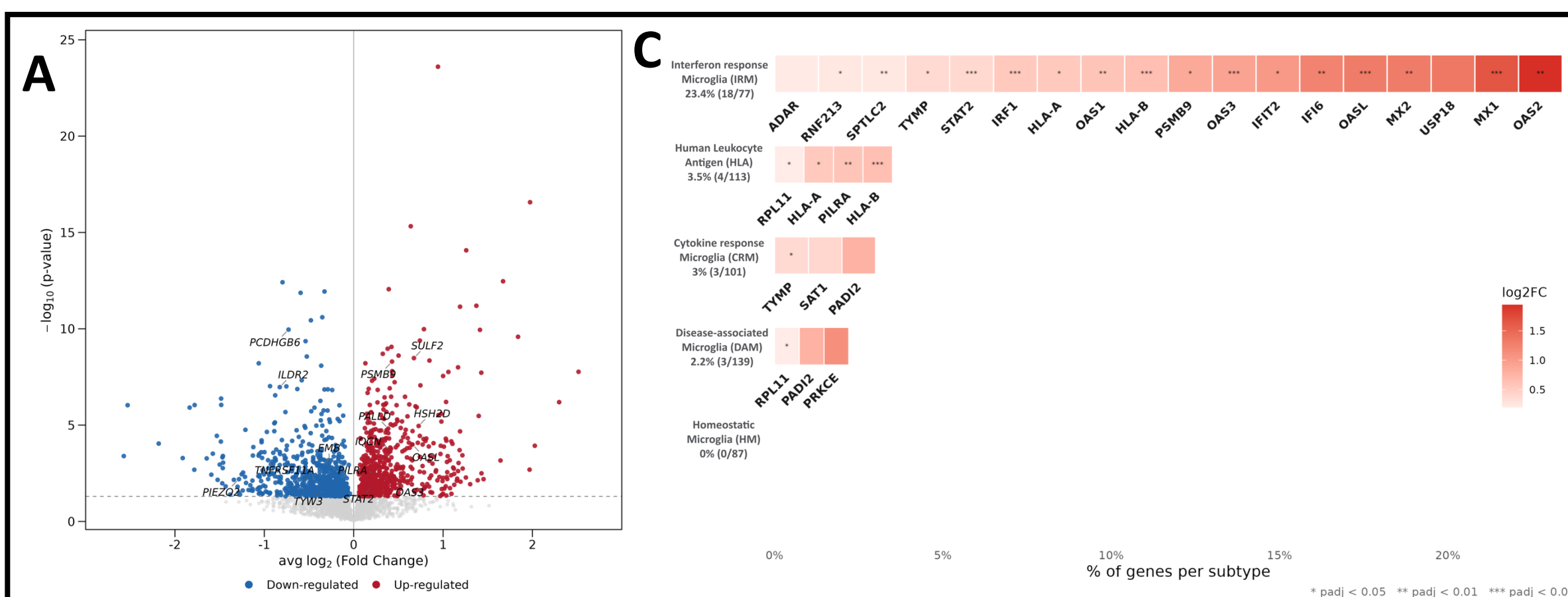


Figure 3. DESeq2 analyses reveal a highly concordant transcriptomic signature across ADAD mutations. A. Volcano plot depicting the overall distribution of differential expression highlighting key up-regulated (red) and down-regulated (blue) genes. B. Venn diagram showing the overlap of differentially expressed genes (DEGs; pvalue < 0.05) from APP, PSEN1, and PSEN2 mutant iMGLs compared to controls. 202 concordant genes are shared across all three genotypes. C. Microglia subtype annotation and enrichment profile of the shared ADAD DEGs. Percentages indicate the proportion of genes mapping to specific microglial states. Asterisks denote Benjamini-Hochberg (BH) adjusted significance levels.

ADAD iMGLs enrich pathways related to interferon signaling

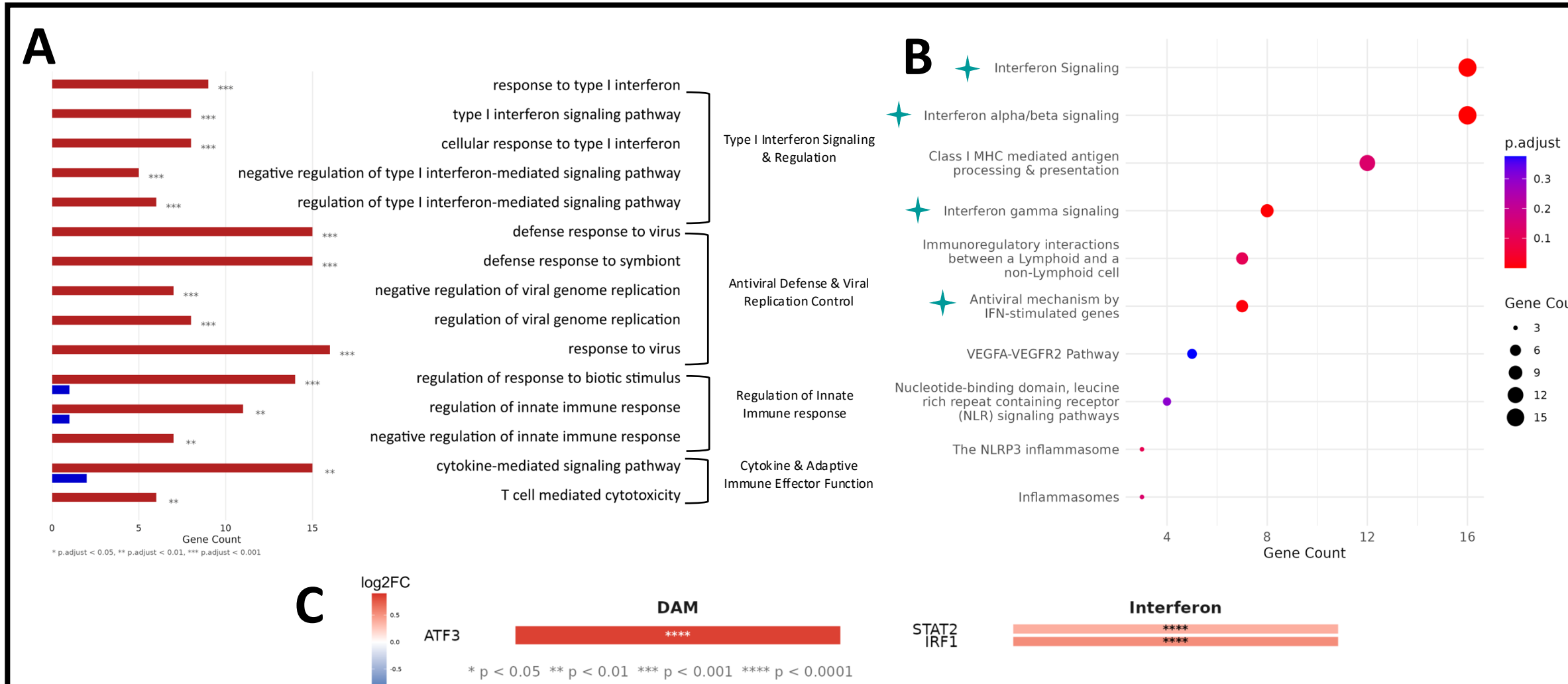


Figure 4. Pathway and transcriptional profiling reveal upregulation of interferon signaling in ADAD iMGLs. A. Bar plot displaying the top 15 (padj < 0.05) enriched Gene Ontology: Biological Processes (GO:BP) terms categorized into functional clusters: Type I interferon signaling and regulation (n = 5), antiviral defense and viral replication control (n = 5), regulation of innate immune response (n = 3), and cytokine and adaptive immune effector function (n = 2). B. Dot plot of REACTOME pathway enrichment analysis highlighting significant (padj < 0.05) activation of interferon cascades, HLA class I antigen presentation, and inflammasome pathways. Cyan asterisks highlight pathways of interest. C. Differential expression profiling of key transcription factors categorized by functional networks: Disease-Associated Microglia (DAM) and interferon response. Asterisks denote Benjamini-Hochberg (BH) adjusted significance levels.

Conserved gene and pathway signature across human brain datasets

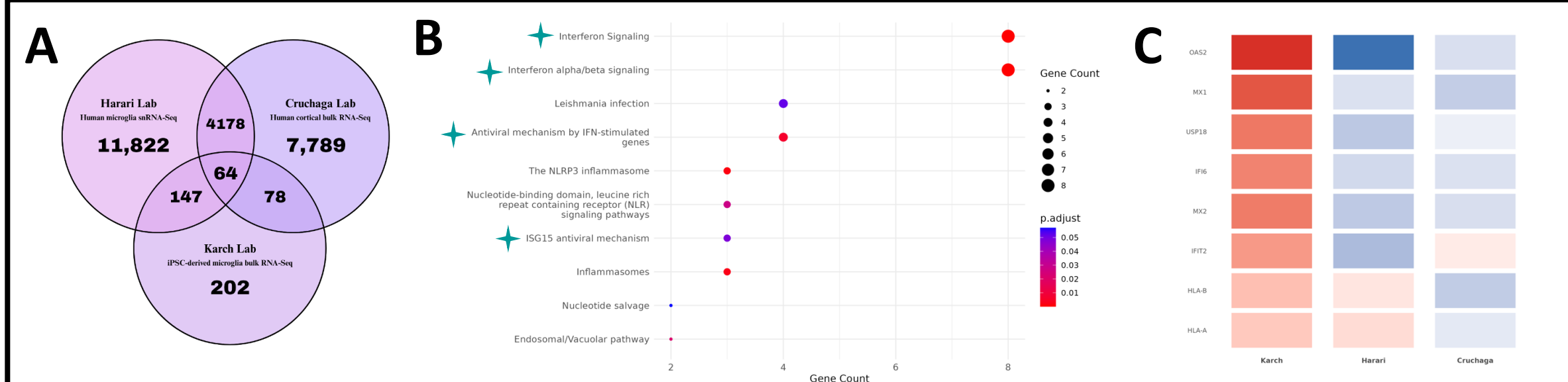


Figure 5. ADAD iMGL Signature recapitulated using other methods. A. Venn diagram showing the overlap of differentially expressed genes (DEGs; pvalue < 0.05) between Brase et al. microglia snRNA-Seq, the Cruchaga Lab's Cortical Bulk RNA-Seq, and this dataset (Karch). B. REACTOME pathway analysis for 64 overlapped genes (padj < 0.05), pathways of interest denoted with cyan asterisk. C. Heatmap of genes present in pathways of interest across each of the datasets (Red = up-regulated; Blue = down-regulated).

Individual ADAD mutations elicit a shared interferon response

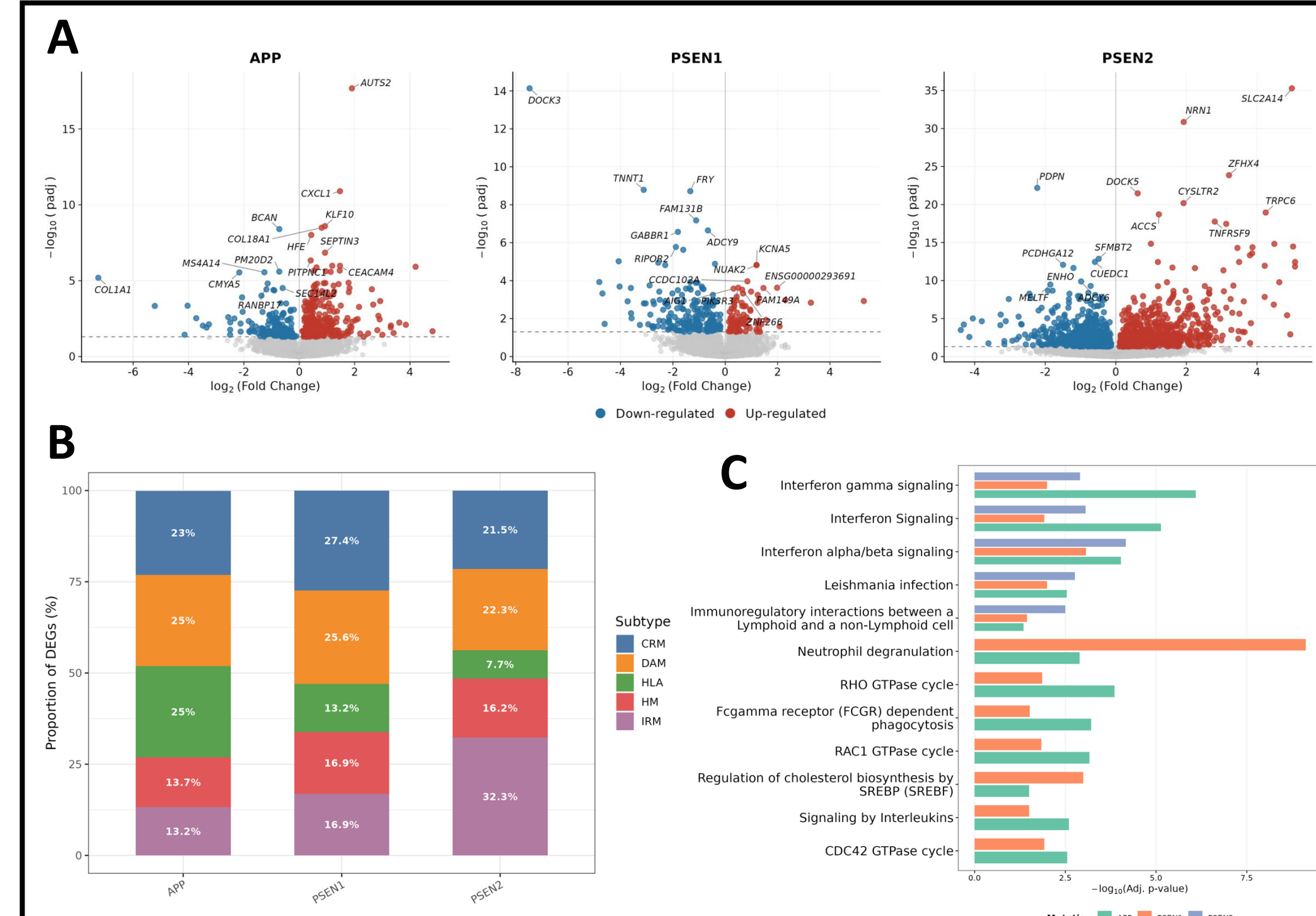


Figure 6. Gene mutation specific effects. A. Volcano plots showing differentially expressed genes (DEGs) independently for APP, PSEN1, and PSEN2 mutant iMGLs compared to controls. Significantly up-regulated transcripts are highlighted in red and down-regulated transcripts in blue. B. Stacked bar plot illustrating the percentage distribution of microglial functional subtypes (CRM, DAM, HLA, HM, IRM) across each specific mutation carrier group. C. Grouped horizontal bar plot depicting enriched REACTOME pathways (padj < 0.05) shared by more than one mutation, highlighting a convergent accentuation of interferon signaling cascades.

Connectivity Map identifies perturbagens to mimic or reverse ADAD profile

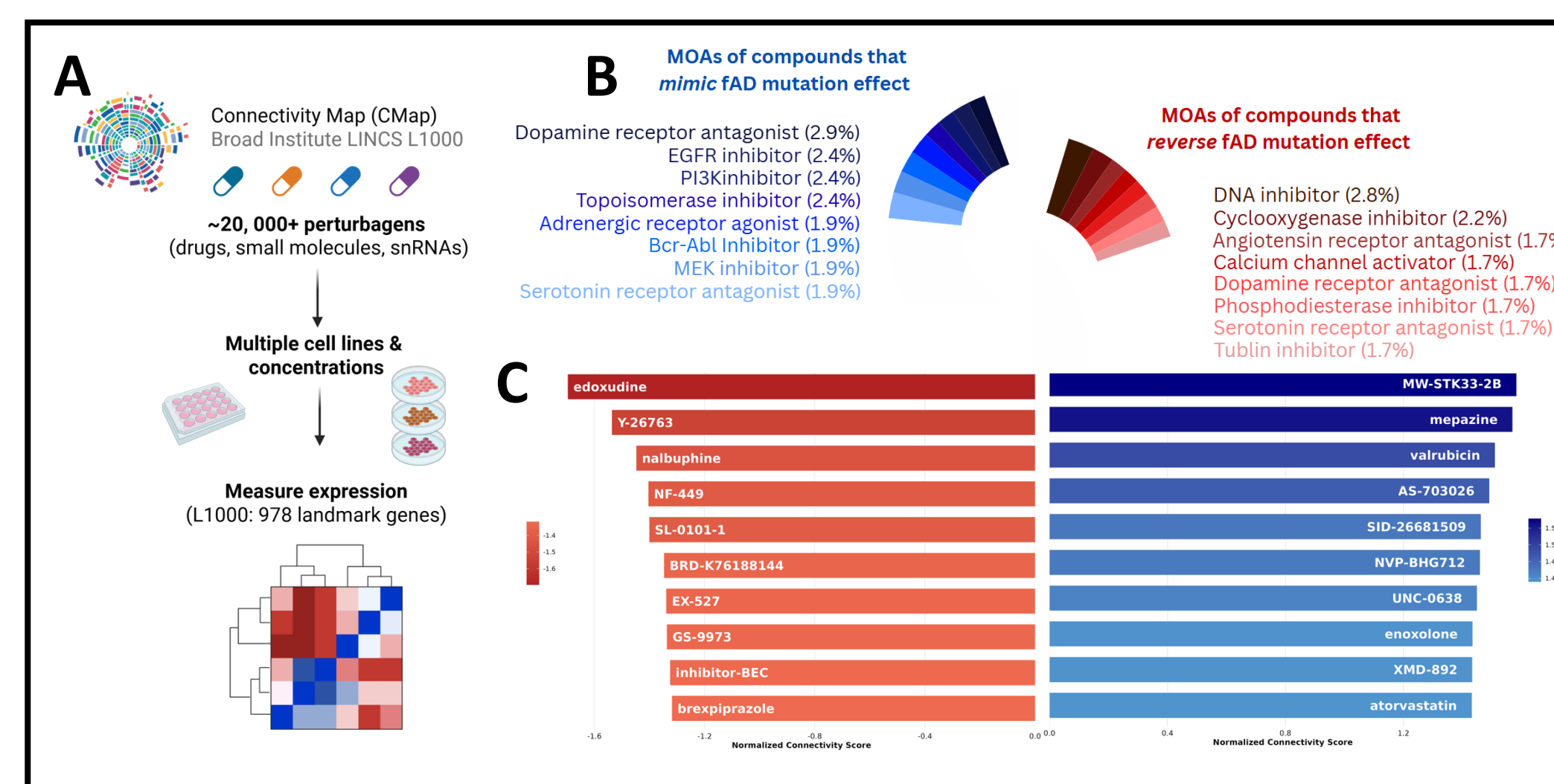
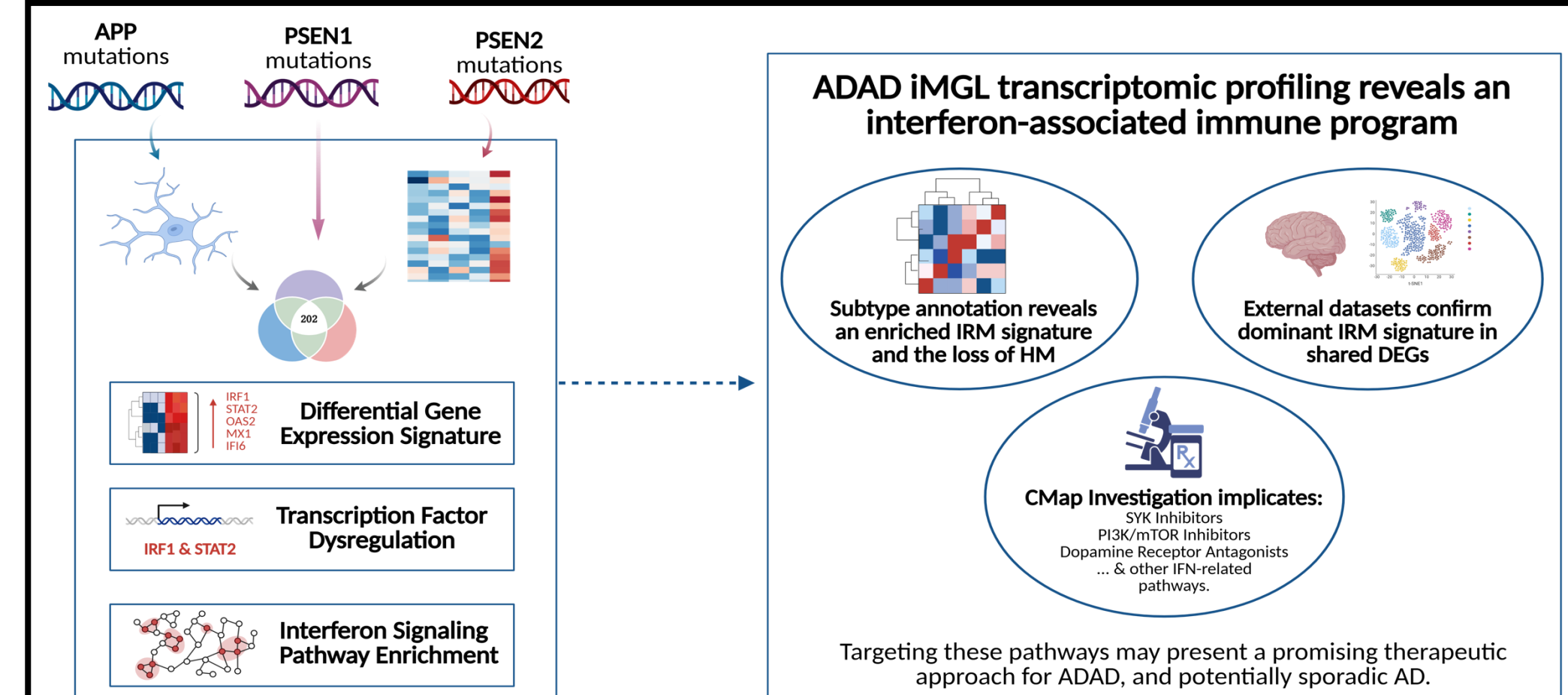


Figure 7. Connectivity Map reveals compounds that mimic or reverse ADAD transcriptional signature. A. Schematic of CMap pipeline, leveraging ~20,000 perturbagens across multiple cell lines to generate connectivity scores from 978 landmark genes. B. Fan charts showing the top MOAs of compounds that mimic and reverse the ADAD transcriptomic signature, queried against the CMap LINC L1000 reference database. C. Top individual perturburbations ranked by positive (blue; mimic) and negative (red; reverse) normalized connectivity scores, respectively.

Conclusions



Acknowledgments

Funding was provided by the NIH (U19-AG032438). We would like to thank the donors and their families for their altruism and contributions to this study, notably individuals who donated their fibroblasts to engineer these iPSC lines. Diagrams were created on BioRender. This work was supported by the Chan Zuckerberg Initiative (Computational Neurodegeneration Research Fellowship; 2027 Cohort)

