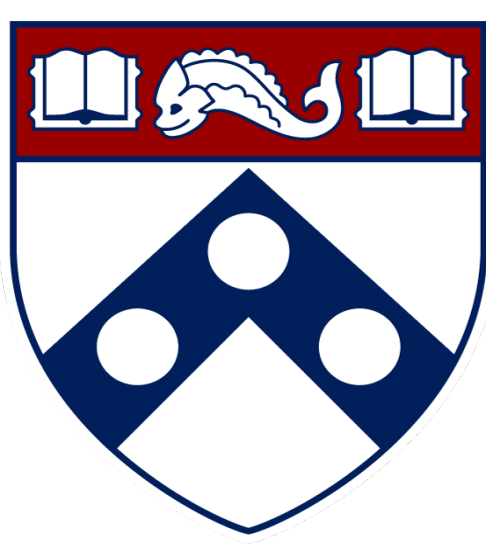




Identification of a novel candidate causal variant for Alzheimer's Disease, rs7149638, regulating FERMT2 expression

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Introduction

- ❖ Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia.
- ❖ While AD has a strong genetic component, only ~25% of cases can be attributed with certainty to known loci—APP, PSEN1/2 (early-onset familial AD), or the APOE4 risk allele.
- ❖ The genetic basis of the more common late-onset AD remains largely unresolved.
- ❖ Genome wide association studies (GWAS) have identified over 70 loci associated with AD risk.
- ❖ Because of linkage disequilibrium and long-range genomic interactions GWAS cannot pinpoint true causal variants and their effector genes.
- ❖ Functional follow-up studies post-GWAS are essential to bridge this gap.

Variant-to-gene mapping in AD

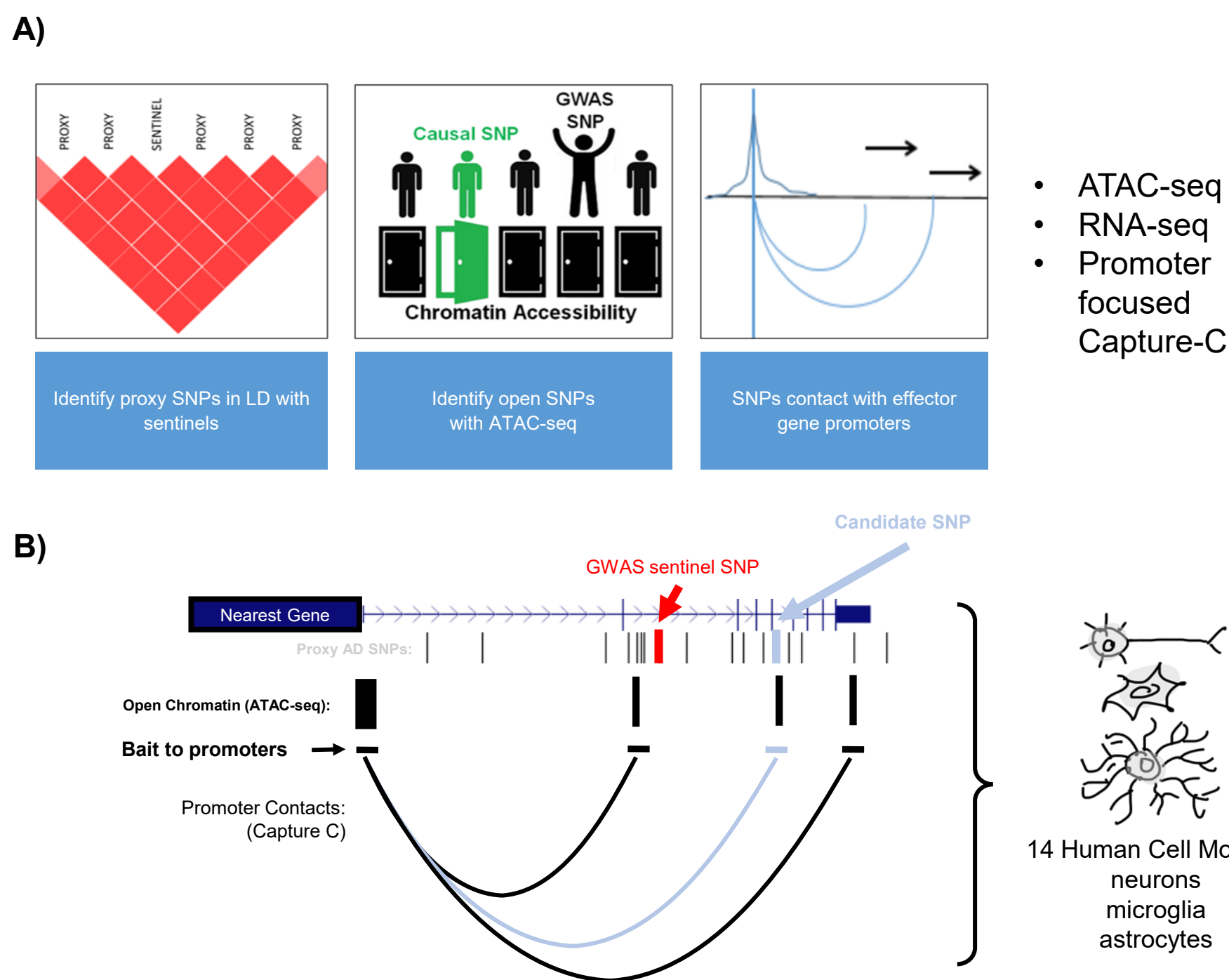


Fig 1. Combining GWAS data with multi-omics to identify AD candidate variant-gene pairs. **A)** The schematic of our variant-to-gene mapping strategy. **B)** Mapping of AD regulatory single nucleotide polymorphisms (SNPs) to target genes using promoter focused Capture-C in different brain cell types.

Single-cell CRISPR interference screening

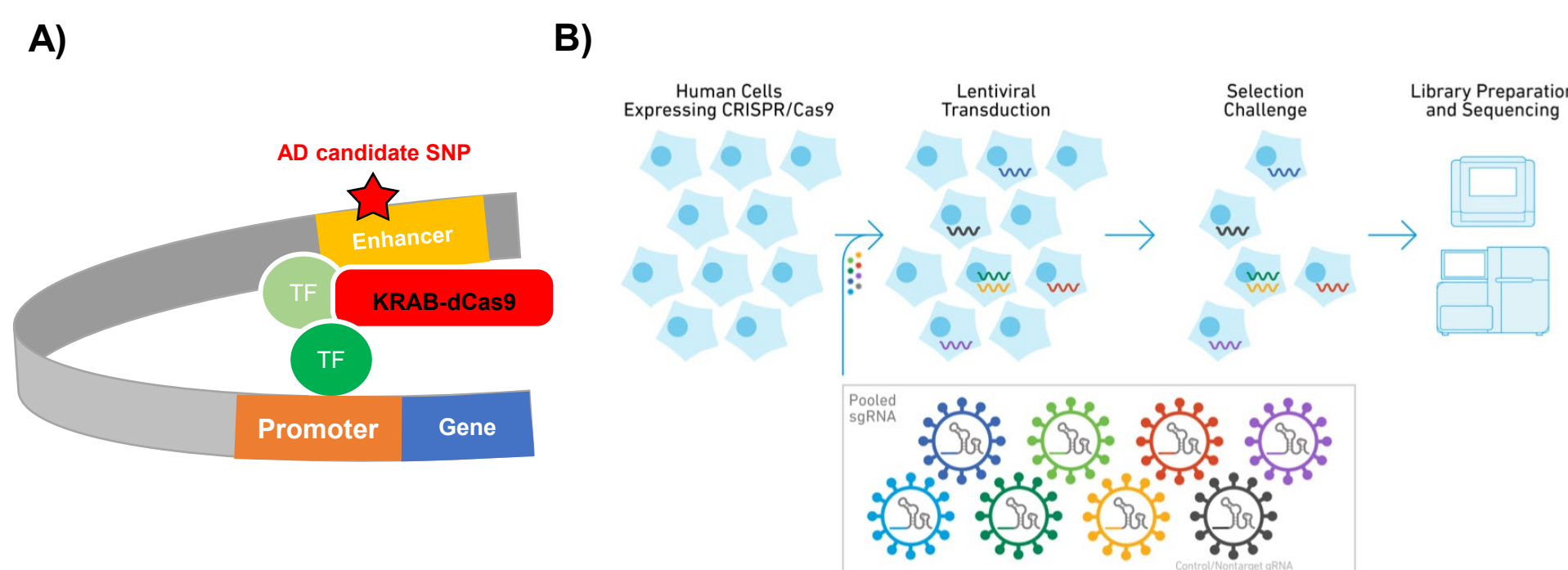


Fig 2. Perturbation of candidate enhancer regions harboring AD-risk SNPs with a KRAB repressor in a single-cell CRISPRi screen in the human microglial cell line, HMC3. **A)** Long-range contact between a putative enhancer region carrying an AD candidate SNP and the promoter of its effector gene. **B)** Work-flow of our CRISPRi screen with scRNA-seq readout (from 10X).

Identification of rs7149638-FERMT2 as a candidate causal variant-effector gene pair for AD

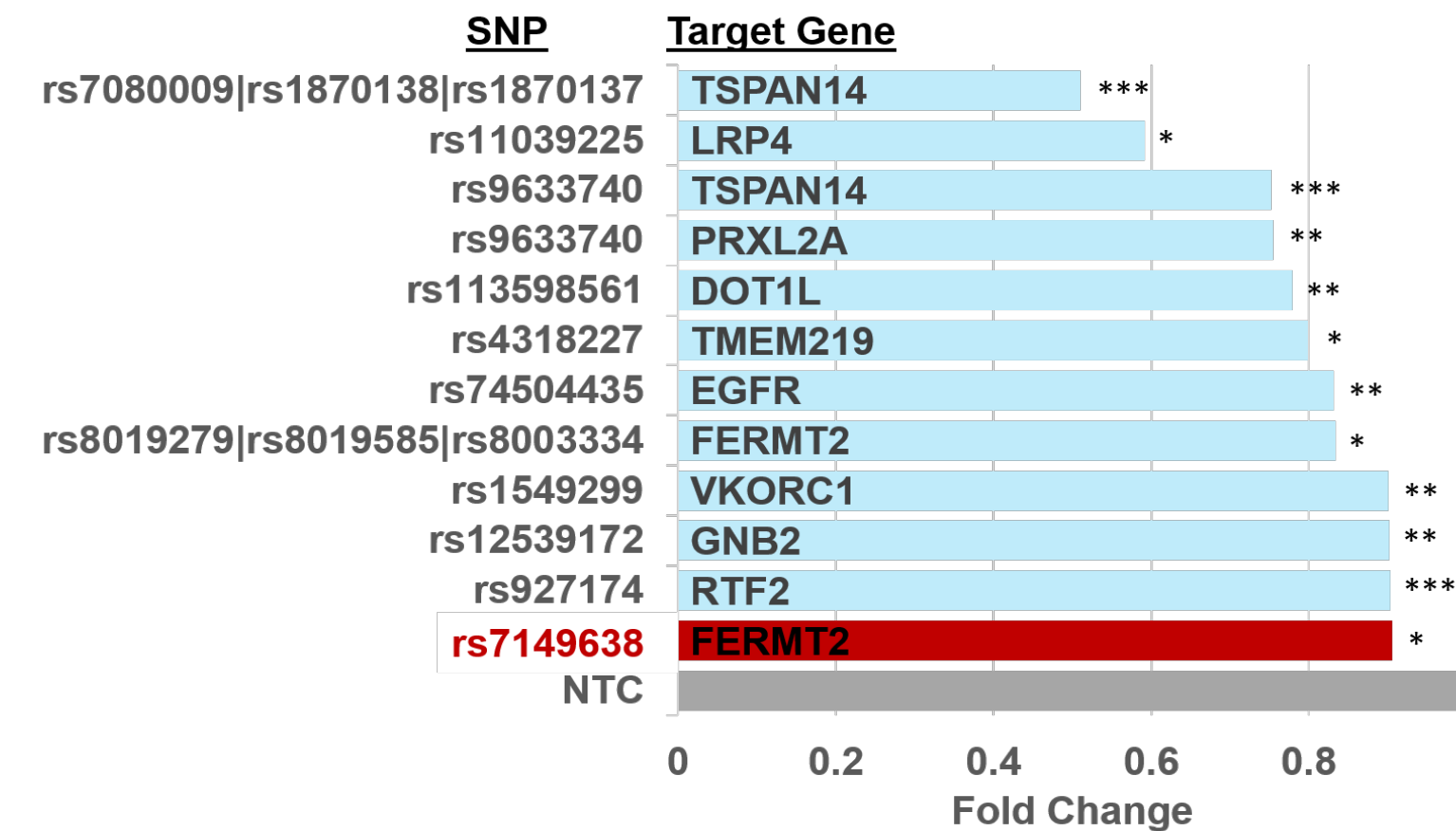


Fig 3. rs7149638-FERMT2 is one of the top hits from our CRISPRi screen. Targeting the SNP rs7149638 with dCas9-KRAB in the human microglial cell line HMC3 resulted in ~20% reduction in FERMT2 expression. Corrected p-values: *, p<0.1; **, p<0.05; ***, p<0.005. [1]

Role of FERMT2 in AD pathogenesis

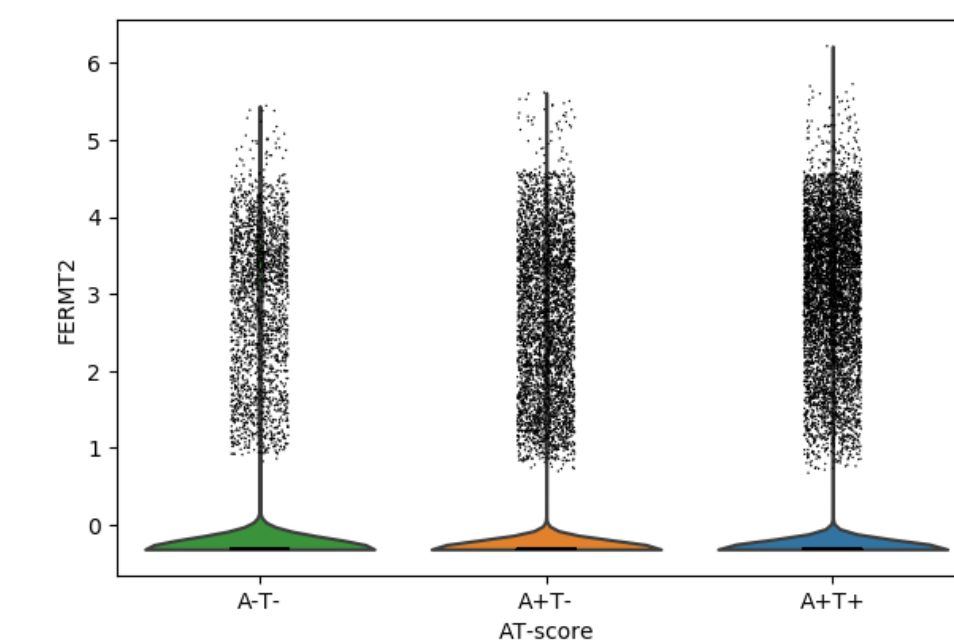


Fig 4. FERMT2 decreases with AT-neuropathology score. Differential gene expression analysis of snRNA-seq from post-mortem brain tissue derived from AD cases shows that *FERMT2* levels decreased with Amyloid-Tau score (AT score), with A+T+ samples having significantly lower *FERMT2* levels as compared to A-T- samples. A+ denotes the presence of amyloid; T+ denotes the presence of tau tangles (scored by clinical and genotype data). *Data courtesy of Edward Lee's lab.*

Data from previous literature:

- ❖ [2] Knock-down of *FERMT2* in HEK293T cells and rat primary neurons *increased* levels of APP and APP-derived amyloid beta (A β) fragments.
- ❖ [3] Knock down of *FERMT2* in iNeurons *reduced* levels of A β and phosphorylated tau levels.

Research Question: What is the direction of effect of *FERMT2* on AD pathology and how does the novel AD SNP rs7149638 regulate *FERMT2* expression?

Validation of enhancer activity using luciferase assays

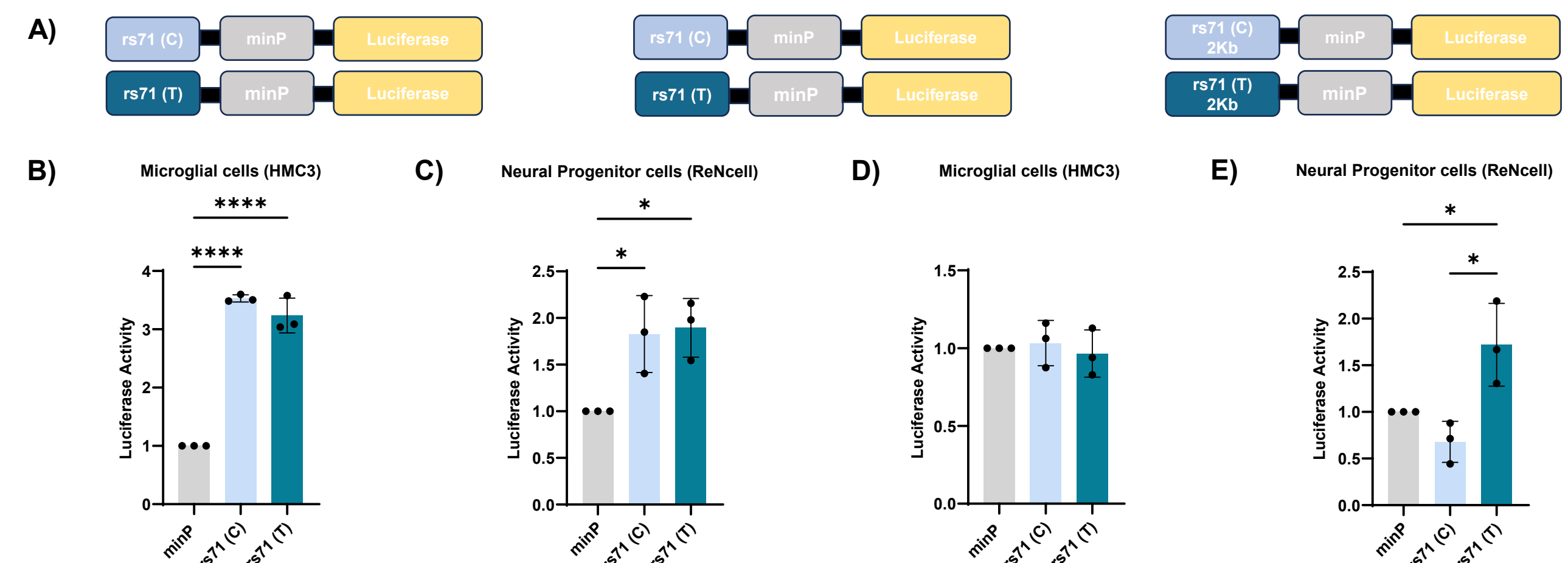


Fig 5. A regulatory region carrying AD SNP rs7149638 displays enhancer activity in microglial cells and neural progenitor cells. **A)** Constructs used in the luciferase reporter assay to measure regulatory activity of the region carrying rs7149638 by measuring changes in luciferase activity in the presence of only a minimal promoter vs minimal promoter and regulatory region. Significant enhancer activity observed for the non-risk allele **C** and risk allele **T** of rs7149638 in **B)** HMC3 cells and **C)** in a neural progenitor cell line - ReNcells. Expanding region carrying SNP to 2Kb resulted in **D)** no significant enhancer activity in HMC3 cells while **E)** in ReNcells a significant increase in enhancer activity for the risk allele **T** as compared to the non-risk allele **C** was observed indicating allele specific effects of the SNP. Significance via one-way ANOVA post-hoc Tukey multiple comparisons. *, p<0.05; ****, p<0.0001.

Conclusions

- ❖ Our variant-to-gene mapping framework integrates GWAS with multi-omics data (high-resolution promoter Capture-C, ATAC-seq, and RNA-seq) to identify candidate causal variants and effector genes.
- ❖ We then functionally validate them using pooled CRISPRi screens combined with scRNA-seq.
- ❖ Through this approach, we identified a novel SNP, rs7149638, as a candidate causal variant that regulates *FERMT2*, a gene previously implicated in APP processing.
- ❖ In our single-cell CRISPRi screen, perturbation of the rs7149638-linked region resulted in ~20% downregulation of *FERMT2* expression in HMC3, consistent with regulatory enhancer activity.
- ❖ To further investigate this locus, we conducted luciferase reporter assays using a 254 bp region encompassing rs7149638 in both HMC3 and ReNcell VM.
- ❖ In HMC3, the non-risk **C** allele and risk **T** allele drove a ~3-fold increase in luciferase activity, confirming enhancer activity in microglial cells.
- ❖ Similarly, in ReNcells, the non-risk **C** allele and risk **T** allele showed significant enhancer activity.
- ❖ Expanding the regulatory region to 2Kb, we observed allele-specific regulatory effects for the SNP, with risk allele **T** displaying significant enhancer effect as compared to the non-risk allele **C** in ReNcells while no such enhancer activity was observed for the 2Kb region in HMC3 cells.

Future directions: leveraging the 3D AD ReNcell model

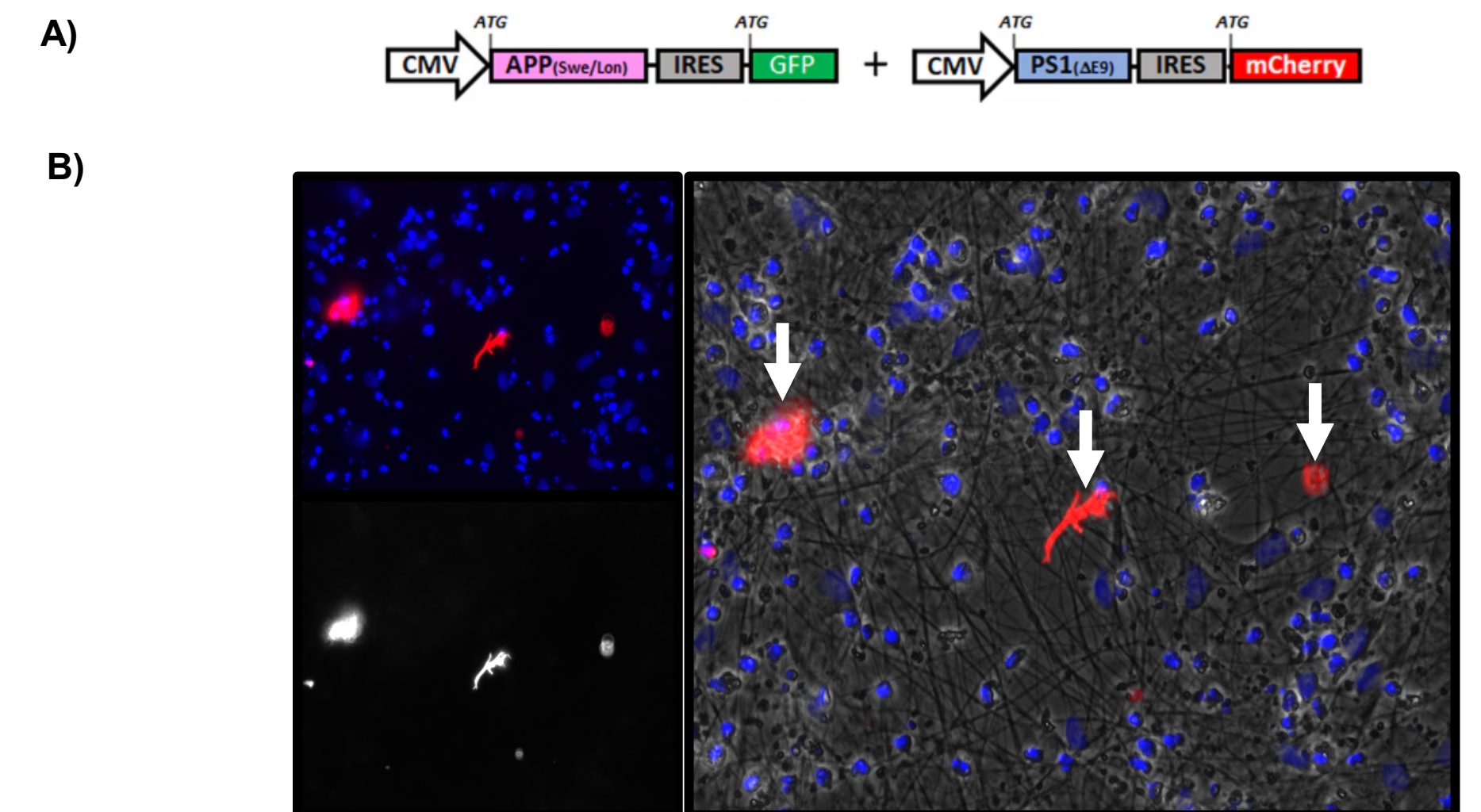


Fig 6. The ReNcell VM 3D model of AD recapitulates the A β plaque and tau tangle pathology by over-expressing APP and presenilin gene carrying AD mutations. **A)** Constructs carrying the APP (Swedish-London) and PSEN1 (Δ E9) familial mutations. **B)** Staining of 6-week differentiated FAD cells with anti-amyloid β 42 antibody shows the presence of amyloid β deposits indicated by the white arrows.

Future plans:

Modulating levels of *FERMT2* in the 3D FAD model to assess changes in A β plaques and tau tangles through overexpression and knock-down.

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