

# Biallelic human genetic background defines brain immune environment for neuroinflammation

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## Introduction

- CHRFAM7A*, a human restricted fusion gene between *CHRNA7* and *ULK4*, is expressed in over 99% of the human population and detected in the nervous and immune systems.
- CHRFAM7A* protein incorporates into  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR), alters  $Ca^{2+}$  dynamics, turns on Rac1 and leads to actin cytoskeleton GOF. The inverted *CHRFAM7A* allele exerts genetic epistasis on *ULK4* and leads to microtubule cytoskeleton GOF.

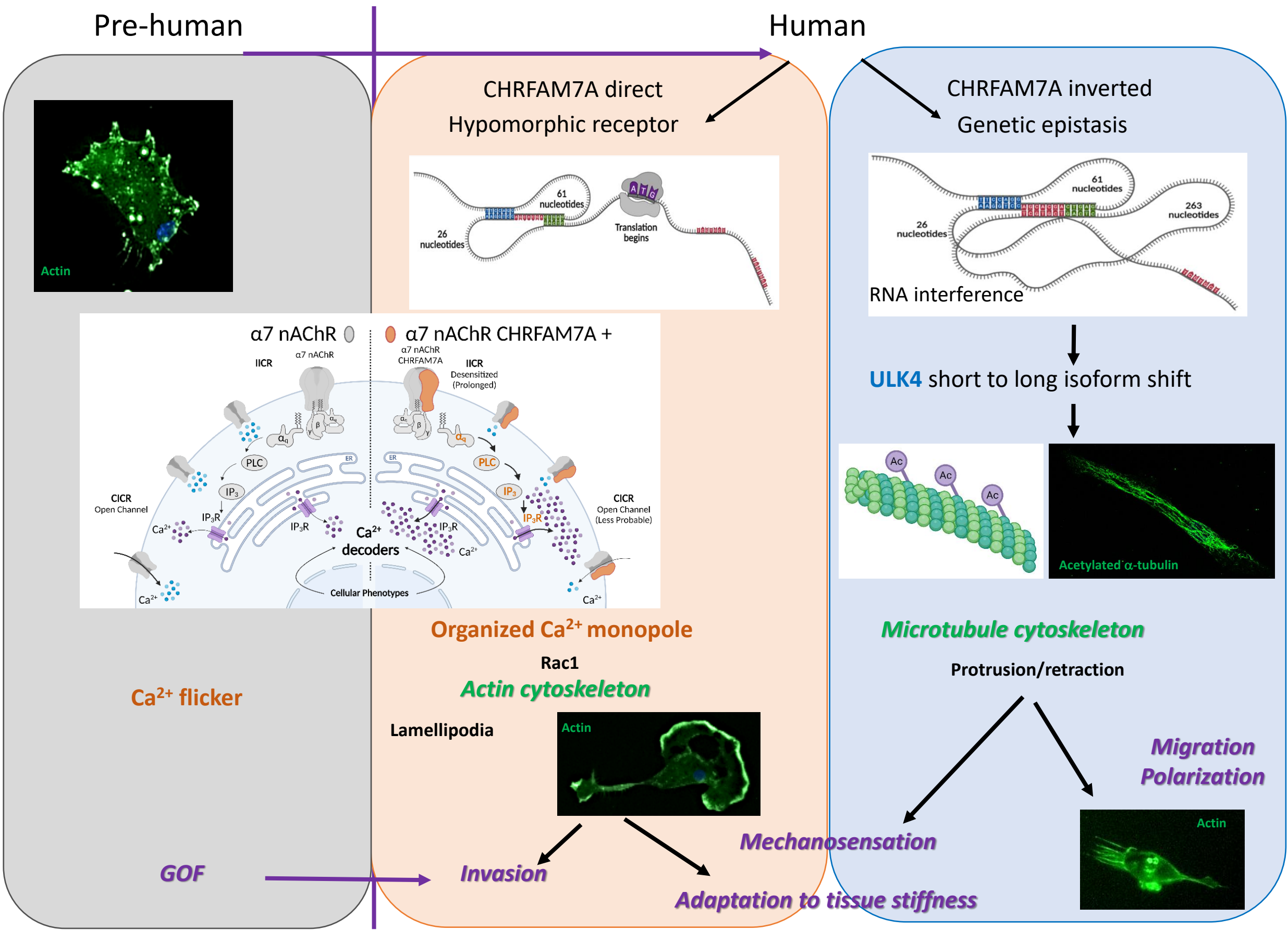


**Figure 1.** Schematic of the proposed model indicating allelic *CHRFAM7A* cytoskeleton GOF.

## Methods

### Human brain RNAseq

- 198 human brain post mortem cognitive normal donors from the ROSMAP cohort were used for RNAseq and TaqMan. DNA was genotyped by dual locus specific assays. RNAseq data was obtained from ROSMAP. A count-based GLM tool (DESeq2) is used to detect at least a significant difference between pairwise GT status using covariates age, sex and PMI. Gene-signature was queried using msigDB's C2CP and C5GO.

### Primary Human Macrophages

- Peripheral blood was obtained from 70 adults with informed consent, and peripheral blood mononuclear cells were isolated by density gradient centrifugation using SepMate™ PBMC Isolation Tubes and LymphoPrep (1.077 g/ml-1; both Stem Cell Technologies, Vancouver, Canada). Cells were attached and cultured in RPMI-1640 medium supplemented with 10% FBS and MCSF (100 ng/ml).

### NFκB translocation and quantification

- Human primary MΦ were treated with TLR agonists (LPS, imiquimod (IMI), PAM2CSK4.). The cells were fixed with 4% PFA and immunocytochemistry (ICC) was performed with the anti-p65 (RelA) antibody. Confocal images were obtained using ECHO microscope (20x objective).
- Manders' colocalization coefficient:** Operator and fluorescent intensity independent high throughput (5 images, >100 cells for each condition) NFκB translocation quantification was performed using the JACoP plugin in ImageJ. Manders' coefficient was calculated (M1) to quantify red signal (p65) overlapping blue signal (DAPI). With cytoplasmic to nuclear translocation of NFκB Manders' coefficient increases.

### TaqMan

- RNA was isolated in parallel experiments and TaqMan assays for IL-6 and CXCL10 were performed.

### ELISA

- The level of IL-6 was measured in the supernatants of primary human macrophages by Human IL-6 ELISA Kit (Thermo Fisher Scientific).

## Results

### 1. Top hits and pathways linked to AD

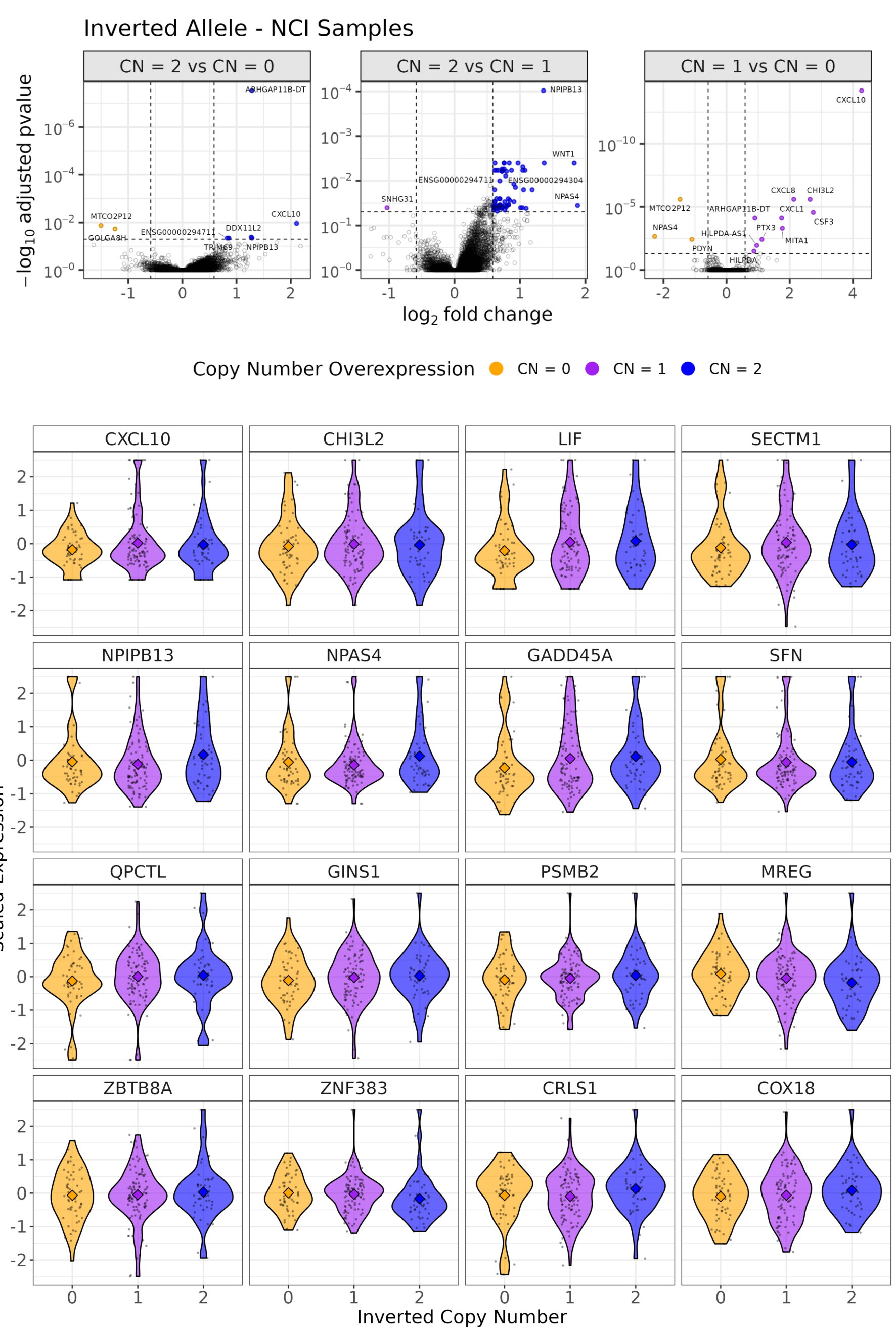


**Figure 2.** Vulcano plots demonstrating direct, inverted and heterozygous pairwise comparisons. Violin plots demonstrate gene expression differences for top hits. Lollipop graph demonstrate the ying-yang of the pairwise comparisons. In humans null is rare thus findings are relative differences between the genotypes.

### 2. CHRFAM7A alleles define distinct signatures in aging brain

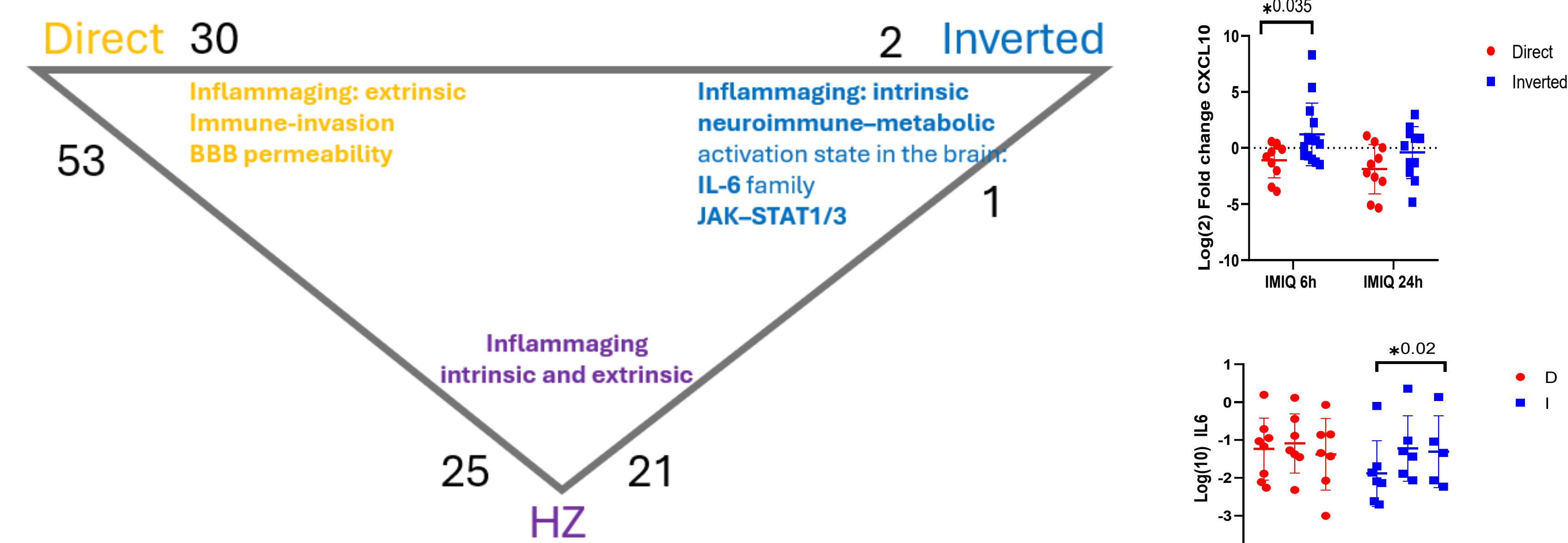


**Figure 3.** Distinct aging signatures are associated with the direct and inverted allele; the heterozygous mechanism is the combined extrinsic and intrinsic inflammaging.

### 3. Validation in immune gain of function in human primary monocytes

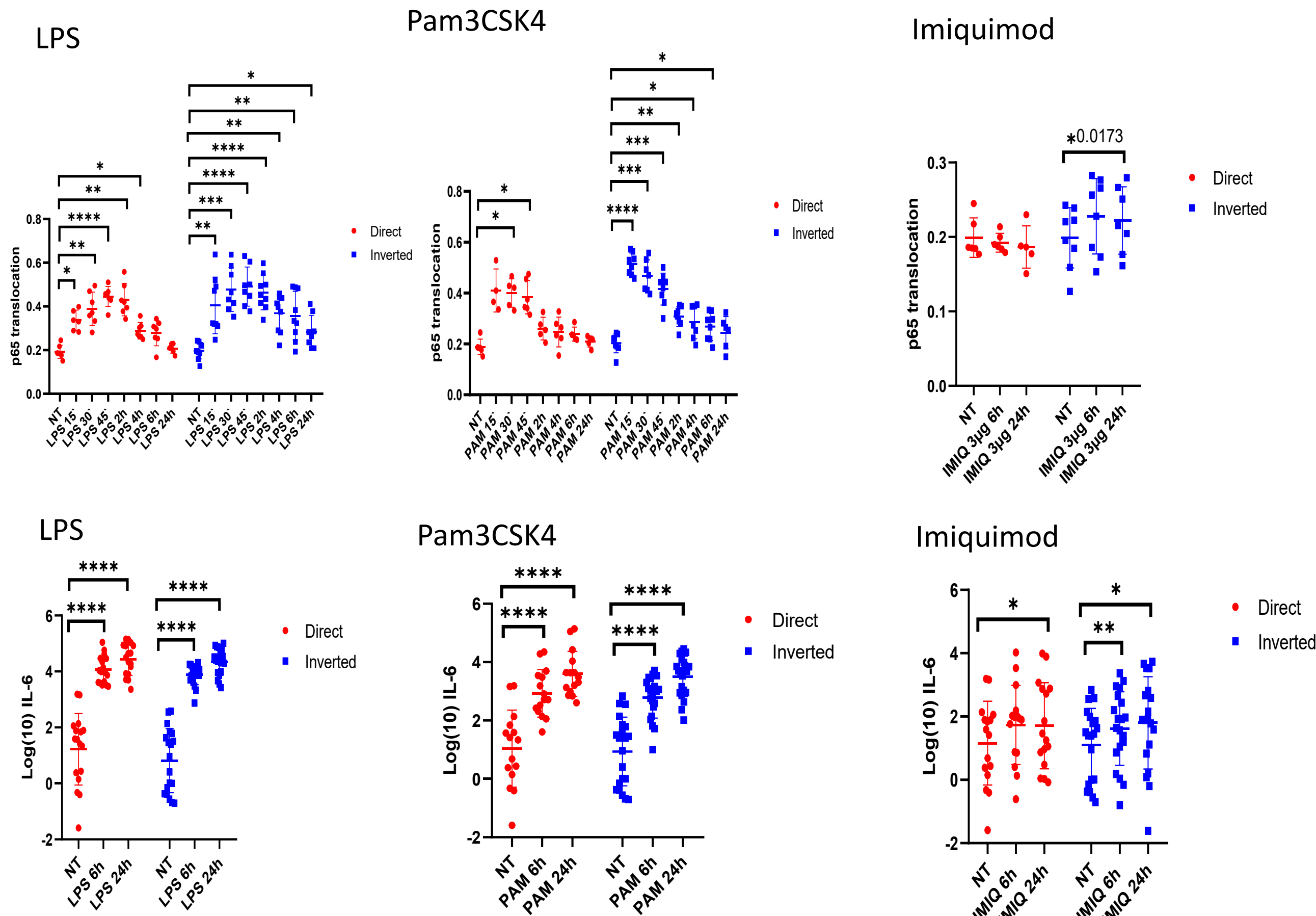


**Figure 4.** We previously demonstrated immune GOF was demonstrated in the direct monocytes differentiated from isogenic iPSC lines compared to null. The human data supports inverted allele associated immune GOF as well, albeit more towards Imiquimod suggesting viral selective pressure.

## Conclusions

- CHRFAM7A* alleles lead to binary cytoskeleton GOF
- In the human brain binary inflammaging signal is associated with the direct and inverted genotypes.
- In the direct brains ECM turnover, invasion and BBB breakdown are consistent with the actin cytoskeleton GOF.
- In the inverted brains neuro-metabolic-inflammatory signature suggest different mechanism.
- In the inverted brain neuroinflammation is mediated by *CXCL10* and IRM microglia.
- Human primary monocytes validated the enhanced immune response in inverted donors suggesting that both alleles are immune GOF.
- These data suggest that distinct anti-inflammatory strategies are needed for neuroinflammation on the *CHRFAM7A* direct and inverted genetic background.

## References

- Szigeti K, Ihnatovych I, Notari E, Dorn RP, Maly I, He M, et al. *CHRFAM7A diversifies human immune adaption through Ca(2+) signalling and actin cytoskeleton reorganization.* EBioMedicine. 2024;103:105093.
- Ihnatovych I, Saddler RA, Sule N, Szigeti K. Translational implications of *CHRFAM7A*, an elusive human-restricted fusion gene. Mol Psychiatry. 2024.
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