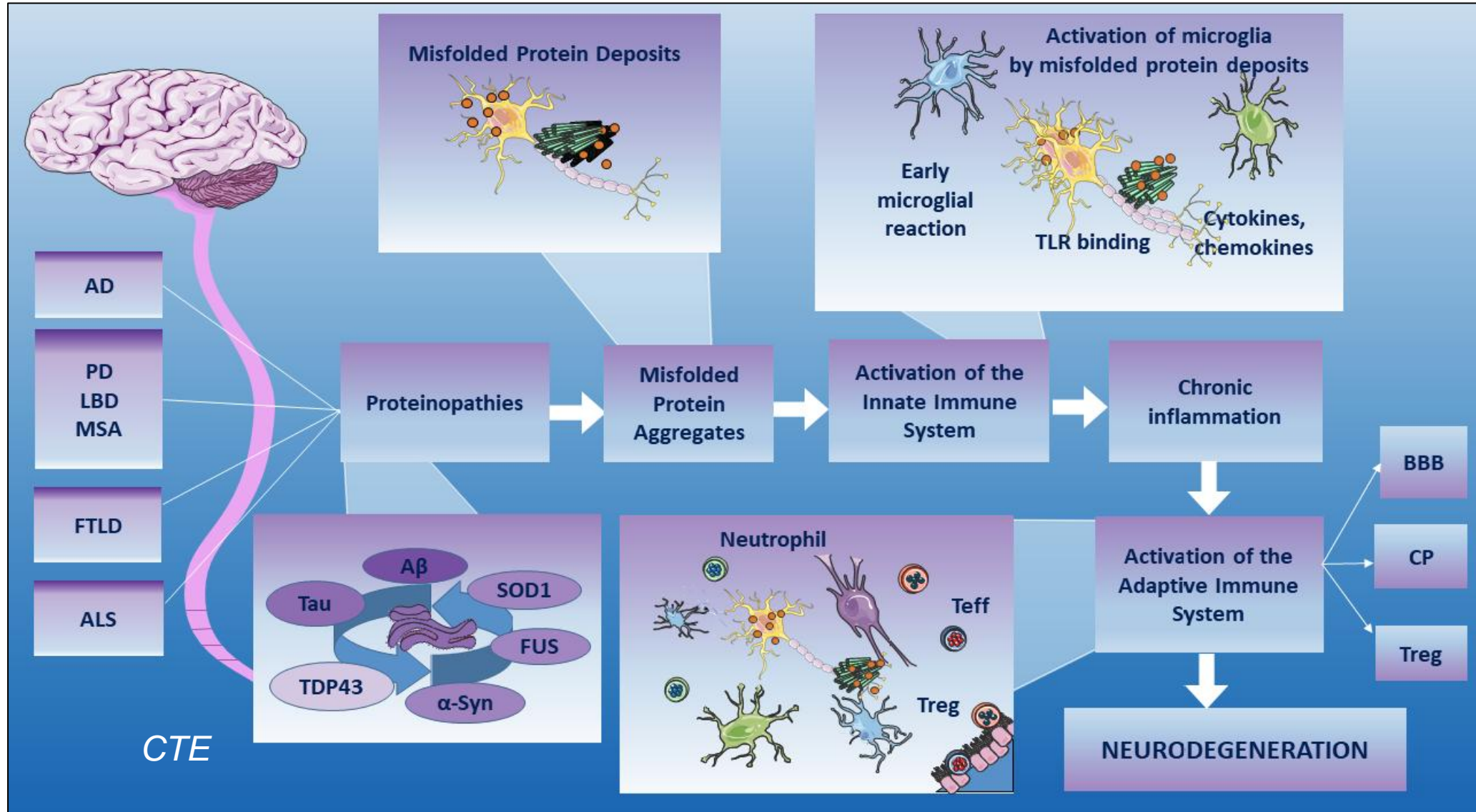


Neuroinflammatory Markers in Former Athletes with Repetitive Head Impacts

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INFLAMMATION & NEURODEGENERATION





Repetitive Head Impacts (RHI): A Key Risk Factor for CTE

- *RHI occurs in contact sports and military settings.*
 - *It's the strongest environmental risk factor for chronic traumatic encephalopathy (CTE).*
 - *CTE has no in vivo diagnosis — it's only confirmed post-mortem.*
 - *Neuroinflammation may be an early and persistent feature in RHI and CTE pathology.*
-

Repetitive Head Impacts (RHI): A Key Risk Factor for CTE

- *Post-mortem studies show microglial activation and astrogliosis in CTE brains.*
- *Post-mortem analysis of athletes revealed CTE pathology in 11 cases, highlighting the complexity and presence of multiple neuropathological changes.*

Case Nr	CTE	PSP	AGD	CBD	GGT	PART	AD	CAA	LBD	MND	FTLD-TDP	LATE-NC	NIHBD	Nr of pathologies	Nr of proteins
1														3	1
2														4	2
3														3	3
4														5	4
5														3	1
6														1	1
7														5	4
8														6	3
9														3	3
10														5	4
11														4	3
12														5	2

Tau
 A β and Tau
 A β α Syn
 TDP-43
 Other

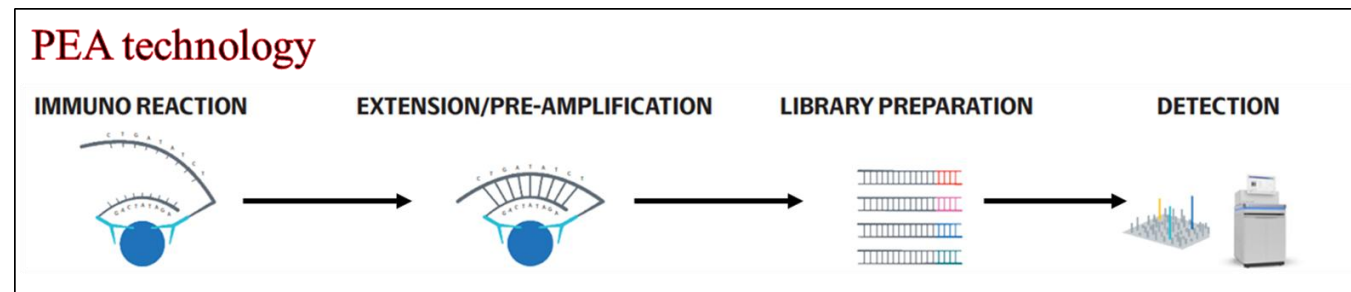
Methodology – LIQUID BIOPSY

Patient cohort and CSF collection

- CSF was collected from participants using ADNI protocol

Inflammatory marker measurement obtained using Proximity Extension Assay (PEA) technology from OLINK

- Highly sensitive and specific multiplex immunoassay PEA technology with Olink® Explore Inflammation I and II panels (<http://www.olink.com>): quantification of **737 inflammatory markers** in the CSF
- Normalized Protein eXpression (NPX) values for proteins were obtained as read-out



Statistical analysis

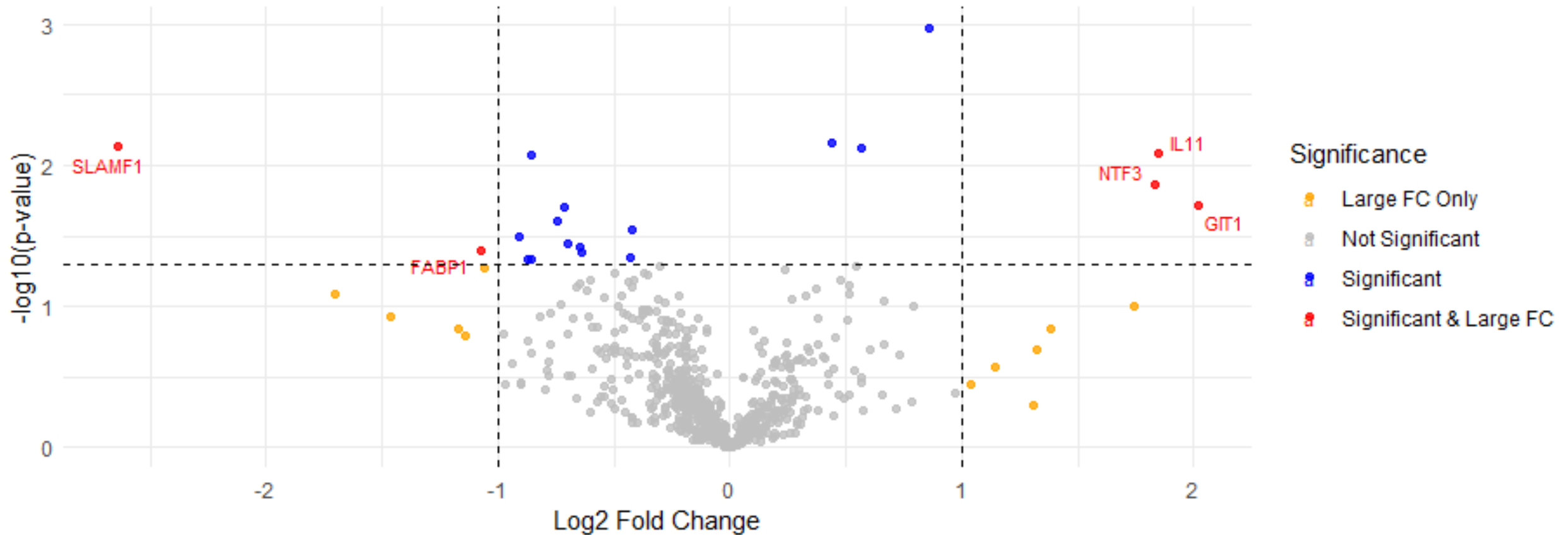
- Multiple linear regression analyses were performed and comparison of mean NPX values were done using ANOVA F-tests. FDR correction done for multiple testing ($q < 0.05$: significant)

Statistical Overview

-
- **Linear Modeling (limma):** Differential expression analysis, adjusted for Age, Sex, and PlateID, with FDR correction.
 - **Non-parametric Testing:** Mann–Whitney U test used when normality assumptions were not met.
 - **Linear Regression:** Modeling NPX with CSF NfL, adjusted for Age and PlateID.
 - **Pearson's Correlation:** Associations with NPX, FDR corrected.

Cohort				
	HC (n = 26) (Mean ± SD)		RHI (n = 35) (Mean ± SD)	
Age (years)	63.9 ±8.5		55.4 ±13.8	(p<0.05)
Sex (M/F)	14/12		34M/1F	
Memory function score	NA		-0.23 ±1.2	
Executive function score	NA		0.2 ± 0.8	
Number of concussions	NA		8.9 ± 8.2	
White matter volume (mm ³)	NA		505,305.1 ± 48831.9	
Hippocampal volume (mm ³)	NA		8,359.6 ±855.2	
CSF NfL (pg/mL)	NA		960.3 ±847.3	

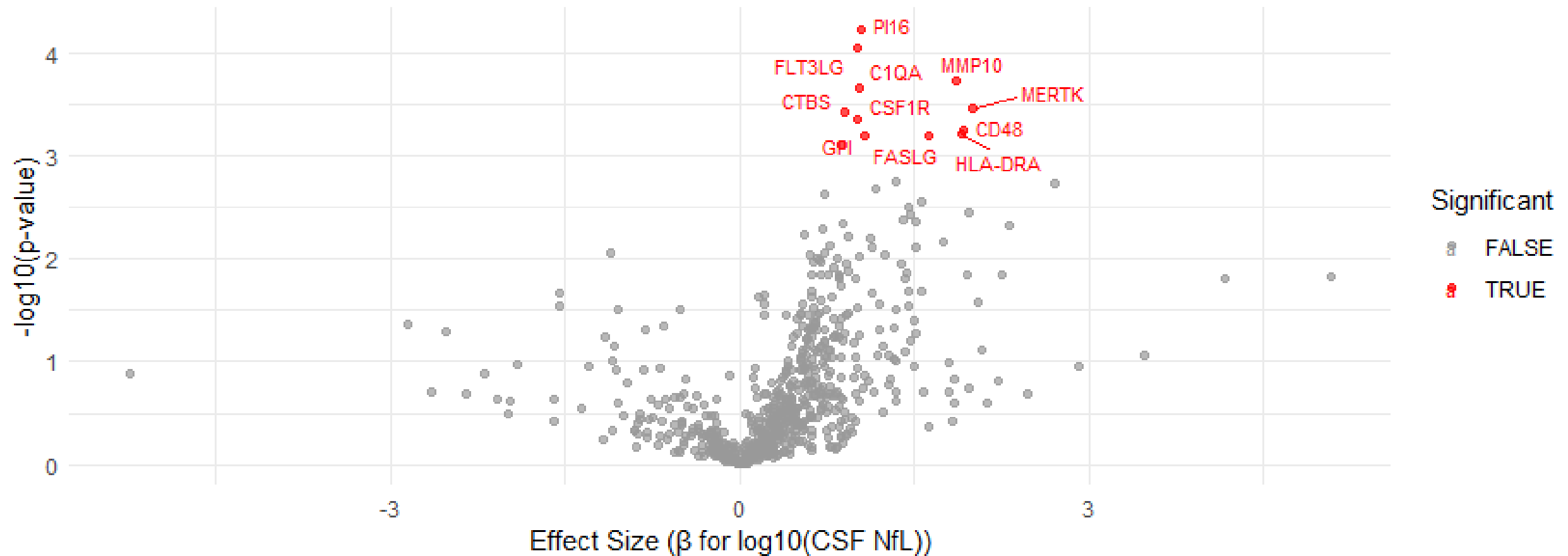
Differential Protein Expression: RHI vs Healthy Controls



Summary of Pearson's Correlation Analysis

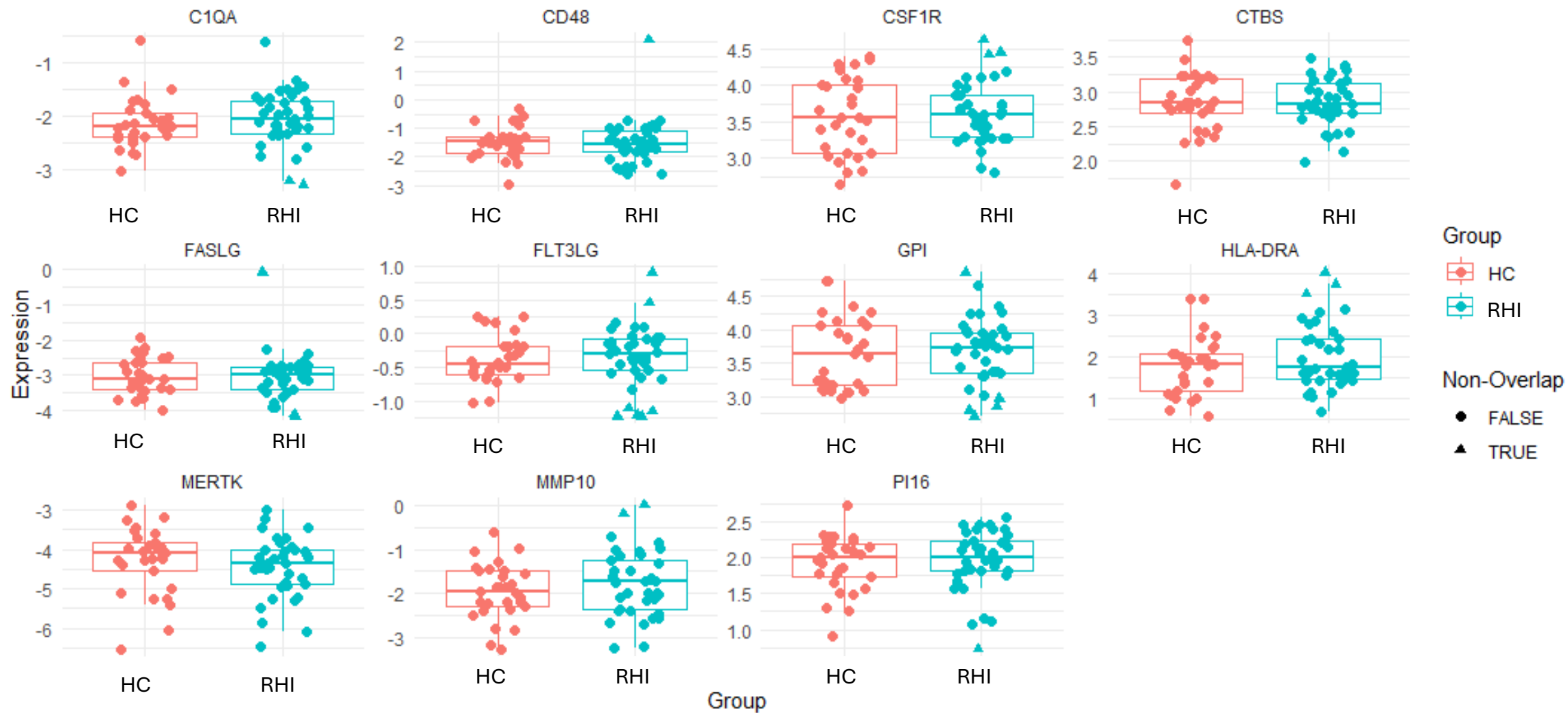
Variable	Markers with $p < 0.05$	FDR Significant
CSF NfL	298	231
Number of Concussions	12	0
Executive Function Score	43	0
Memory Function Score	20	0
Hippocampal Volume	112	0
White Matter Volume	80	0

NfL Levels Correlations with Inflammatory Markers in RHI



Heterogeneity in RHI Expression Patterns

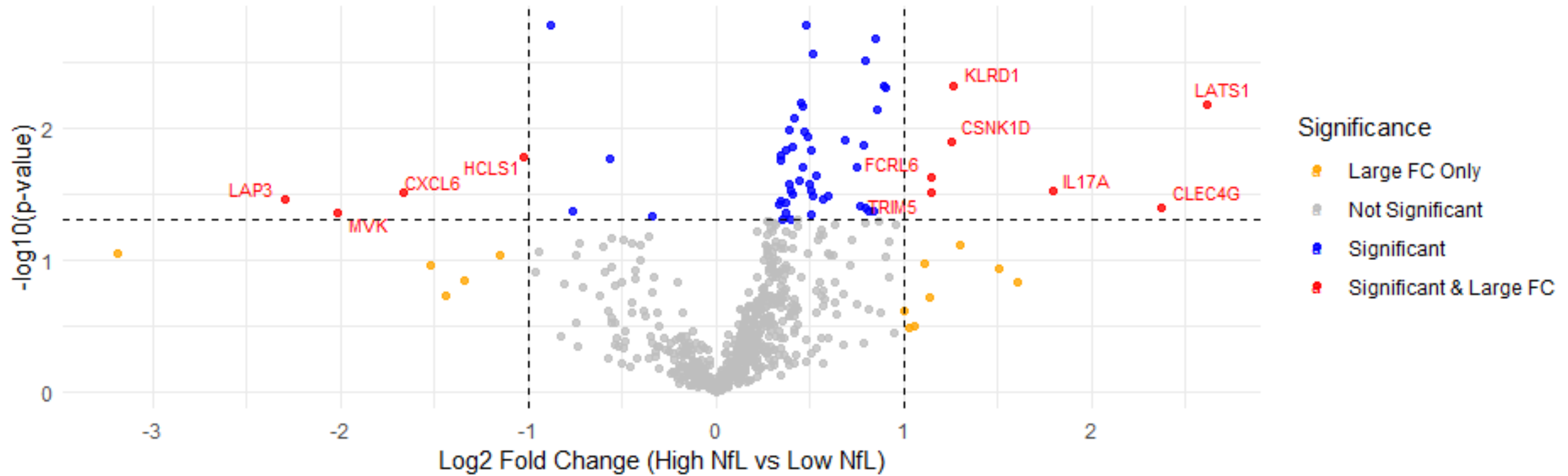
Non-overlapping CTE Individuals by Marker



High vs Low NfL Summary Table

	High NfL (N=17) (Mean ± SD)	Low NfL (N=18) (Mean ± SD)	P-value
Age	64.6 ± 9.5	46.7 ± 11.3	< 0.0001
Number of Concussions	8.1 ± 7.6	9.7 ± 8.7	0.7
cerebral white matter	491,537.0 ± 42,786.2	516,013.8 ± 50,529.6	0.2
HIPPOCAMPI	8,174.5 ± 675.1	8,503.6 ± 948.0	0.3
Executive Function Composite Score	0.025 ± 0.616	0.334 ± 0.901	0.3
Memory Function Composite Score	-0.166 ± 1.326	-0.272 ± 1.185	0.8
Mood/Behaviour Composite Score	0.104 ± 0.614	0.075 ± 0.625	1
CSF Nfl	1,537.96 ± 900.7	414.7 ± 129.8	< 0.0001

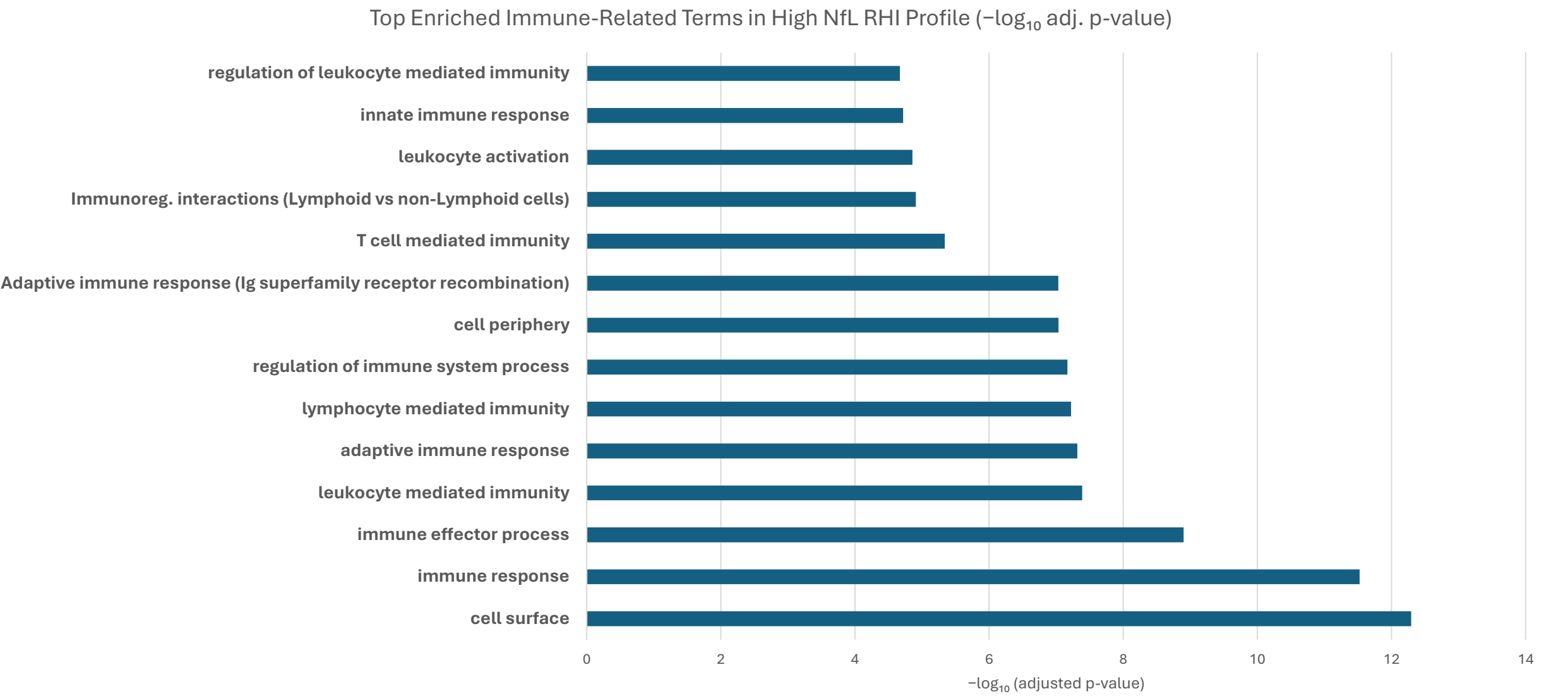
High vs. Low NfL RHI



Selection Criteria for Pathway Enrichment Analysis

- Upregulated in High NfL RHI individuals.
- RHI High NfL vs. Low NfL
- RHI High NfL vs. Healthy Controls
- Linear model of NPX $\sim \log(\text{CSF NfL})$

Pathway Analysis: Inflammatory Signature in High NfL RHI



Conclusion

- Repetitive Head Impacts (RHI) correlates with several inflammatory markers.
- High NfL RHI is associated with a strong immune-inflammatory signature:
- **Adaptive and innate immunity, including T cell activation.**

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