

MULTIMODAL EPIGENETIC PROFILING OF cfDNA IN ALZHEIMER'S DISEASE REVEALS METHYLATION, FRAGMENTOMIC AND TISSUE OF ORIGIN ALTERATIONS

P2-03

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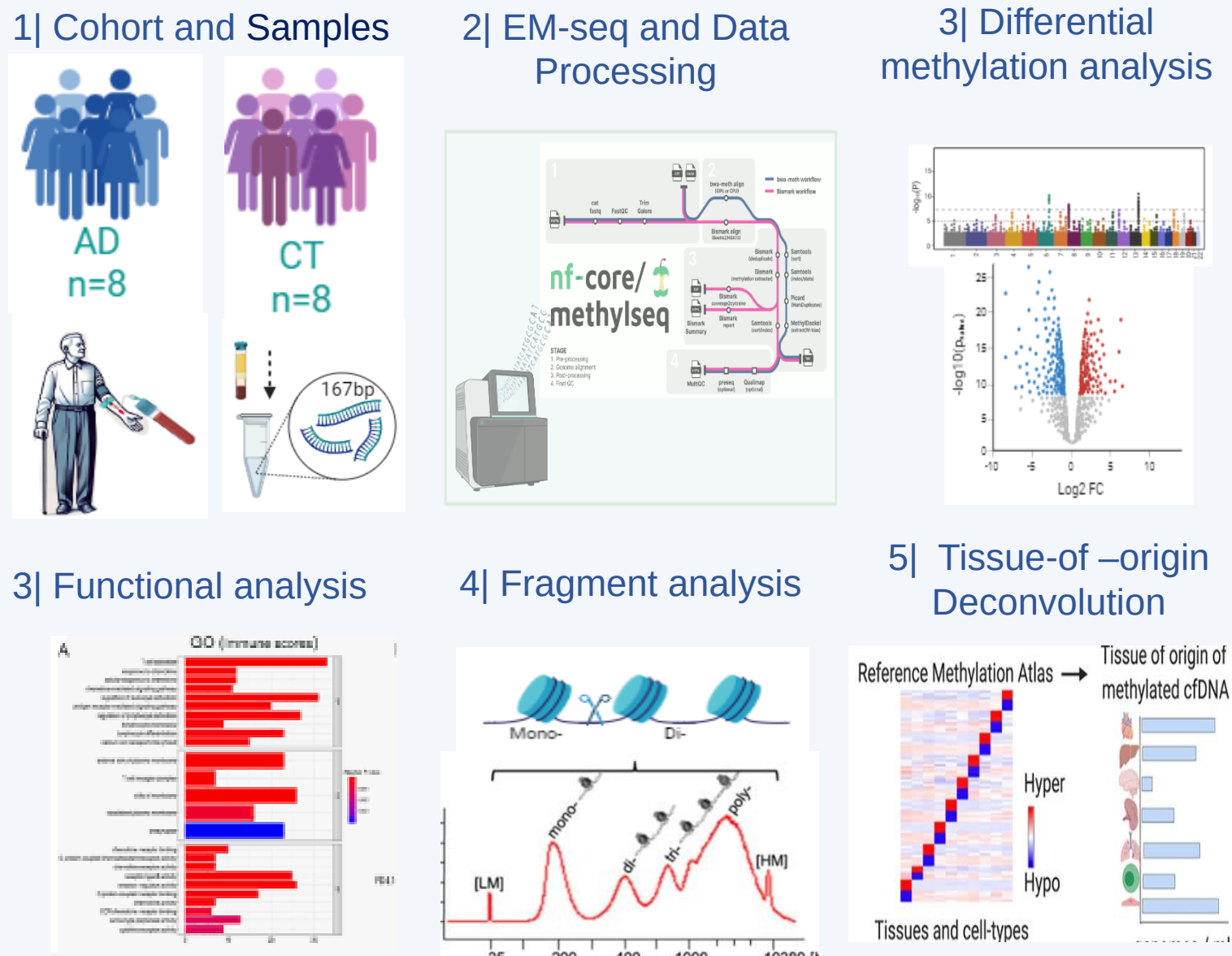
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INTRODUCTION

In recent years, new Alzheimer's disease (AD) DNA methylation variants have been reported in genes biologically relevant in human brain tissue^(1,2,3,4). However, this AD-specific epigenetic information remains locked in the brain tissue. Liquid biopsy provides access to organ-specific information by means of cell-free DNA (cfDNA), offering a promising source of biomarkers, reflecting both epigenetic states and nucleosomal fragmentation patterns of the tissue^(5,6,7). Here, we integrated differential DNA methylation analysis (DMA), fragmentomic profiling, and fragment based deconvolution to investigate cfDNA alterations associated with AD.

METHODS



The study included 16 subjects, 8 patients with AD and 8 age and sex-matched healthy controls. cfDNA was isolated from 2 mL plasma using QIAmp Circulating Nucleic Acid Kit (Qiagen). Subsequently, a genome-wide methylation analysis was executed using NEBNext Enzymatic Methyl-sequencing (EM-seq) (New England Biolabs) in the NovaSeq 6000 platform (Illumina). We performed DMA with DSS⁽⁸⁾, extracted fragmentomic features using FinaleToolkit⁽⁹⁾, and applied the UXM fragment-based framework to estimate tissue-of-origin contributions⁽¹⁰⁾.

RESULTS

Table1 | Differential Methylated Loci (DML) and Differential Methylated Regions (DMR)

	Methylation	DML		DMRs			
		No.	Genes	No.	Genes	DMR length (bp)	CpG counts
AD vs Control	Hypermethylated	20,626	6,363	35	37	143(51-356)	7 (4-65)
	Hypomethylated	40,275	9,361	235	226	162(51-4858)	5 (4-95)

Fig1. Genomic distribution and enrichment

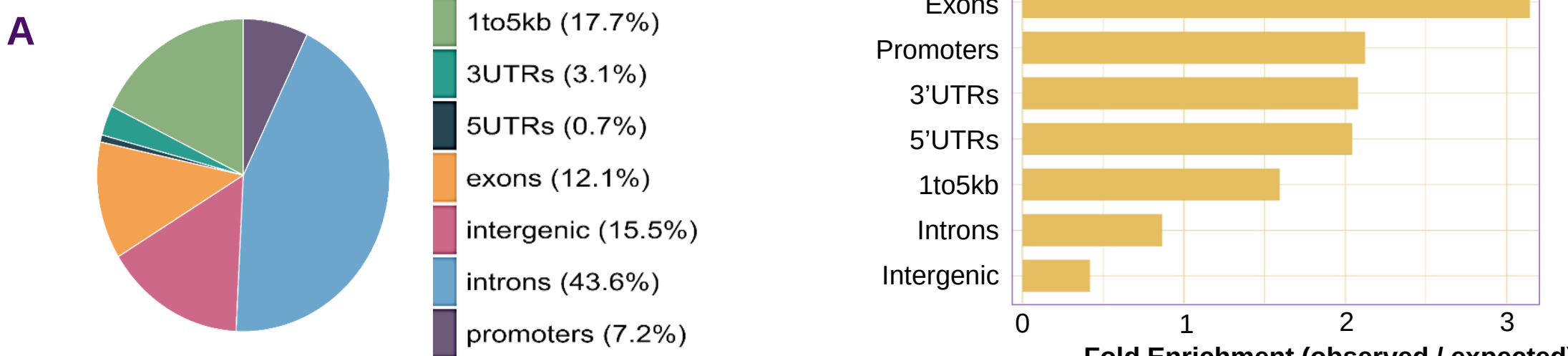


Fig1. A) DMLs distribution B) Enrichment of DMLs was observed in exons, promoters, 3'UTRs and 5'UTRs regions.

Fig3. Fragmentomic analysis

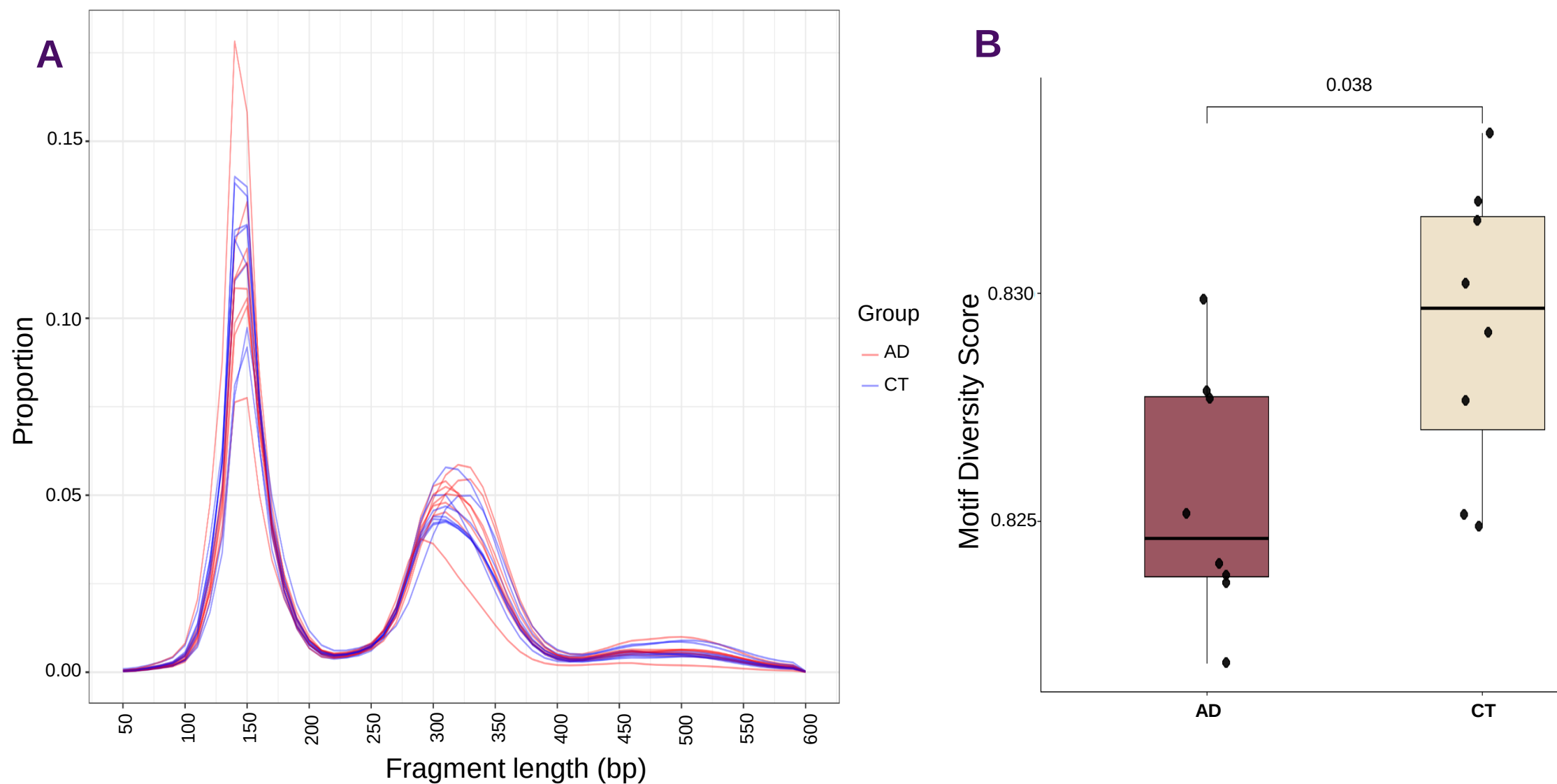


Fig 3. Fragment length profiling showed predominant peaks at ~150 bp and ~300 bp corresponding to mono- and dinucleosomes with no differences between groups. In contrast, end-motif diversity analysis showed a significant reduction in motif diversity score (MDS) in AD (p-value<0.05), indicating more uniform cfDNA cleavage patterns

Fig2. GO Functional analysis

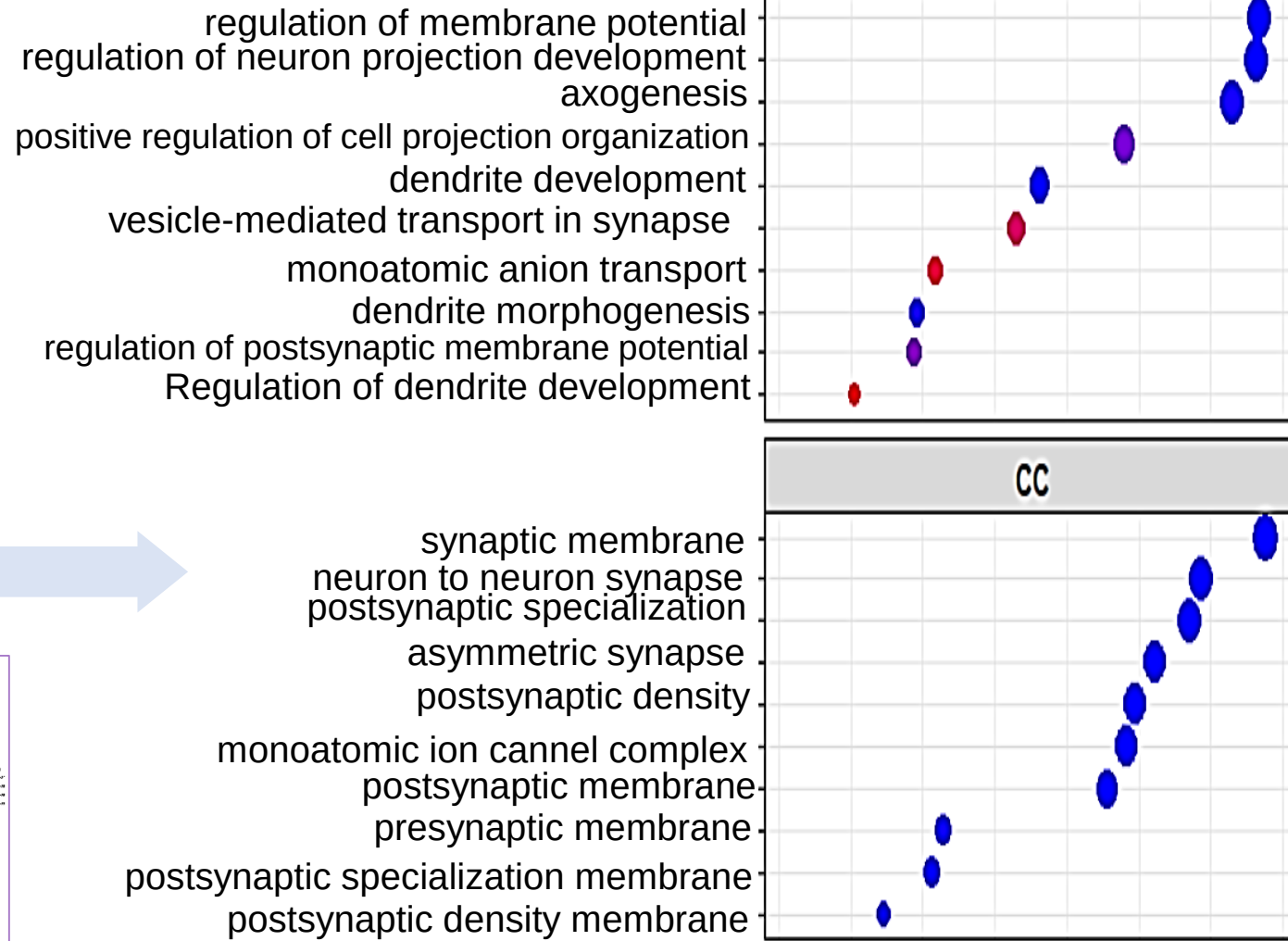


Fig 2. GO terms summary analysis revealed genes strongly associated with neurological biological processes such as neuron to neuron synapse signaling that are affected by AD.

Fig4. Tissue-of-origin deconvolution

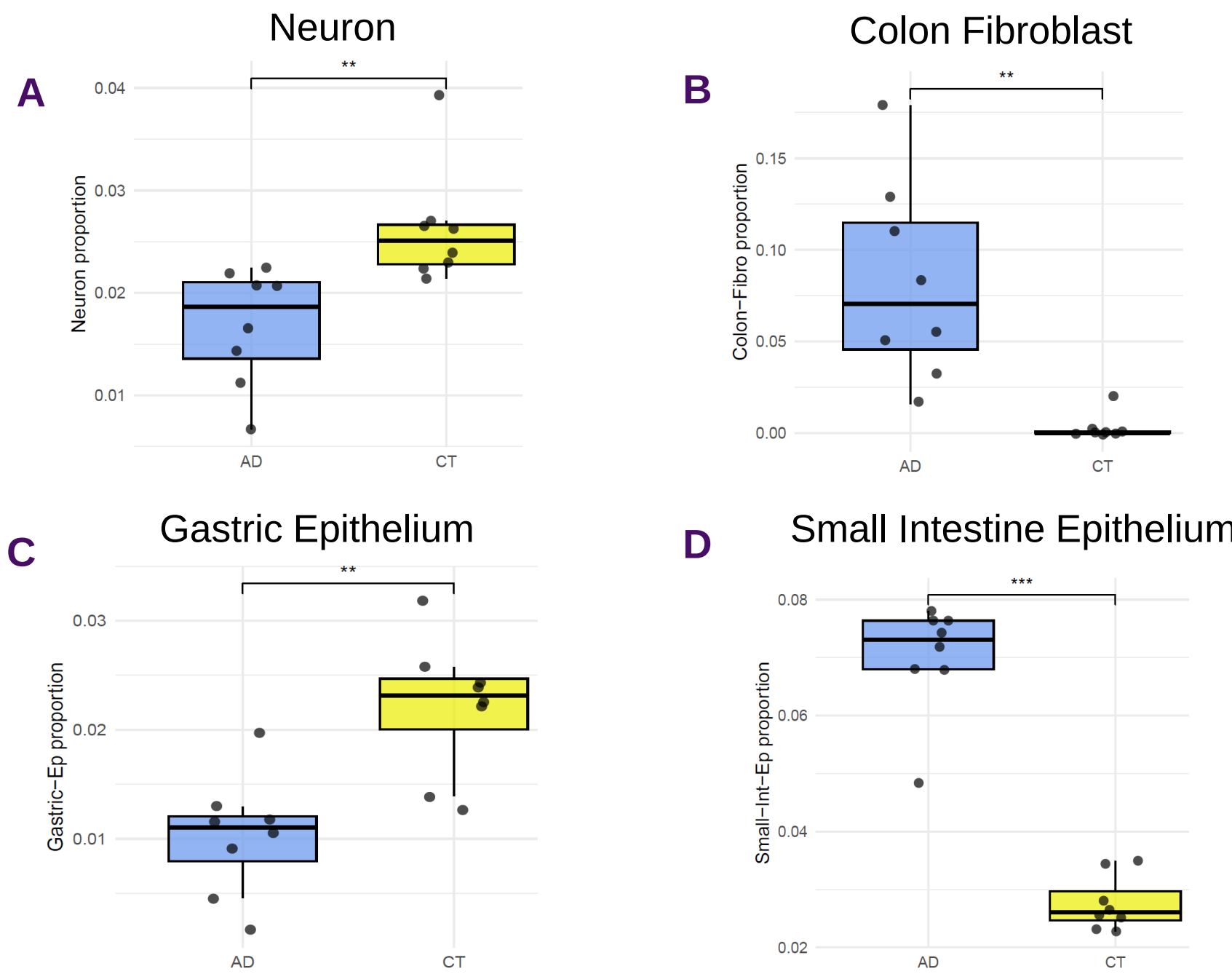


Fig 4. Deconvolution further identified significant shifts in tissue-of-origin contributions, with altered neuronal-derived cfDNA. Interestingly, we also observed an increased contribution of intestinal-associated fragments, a finding that converges with accumulating evidence implicating gut-brain axis perturbations in AD

CONCLUSIONS

Together, these multimodal cfDNA analyses uncover coordinated epigenetic, structural, and tissue-origin alterations in AD. The convergence of differential methylation, reduced end-motif diversity, and shifts in cfDNA cellular contributions highlights the capacity of plasma cfDNA to capture both central and peripheral biological processes underlying AD. These findings support the potential of liquid biopsy as a non-invasive approach to access AD-specific molecular information

REFERENCES



FUNDING

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CONTACT

