

Convergent Proteomic Signatures Across Human Neuronal Models of Alzheimer's Disease

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BACKGROUND

Alzheimer's disease is characterized by the accumulation of core pathologies, including amyloid beta (A β) plaques and neurofibrillary tangles composed of hyperphosphorylated tau. However, synaptic dysfunction and neuronal loss are the pathological features that most closely track with cognitive decline¹. Human iPSC-derived neurons (iNeurons) offer a scalable platform to model these processes, but the disease relevance of these in vitro systems remains unclear. We combined functional and proteomic profiling of iNeurons to determine the extent to which these models reflect in vivo disease states.

METHODS

Human iPSC-derived neurons were perturbed using AD-relevant stressors - oligomeric A β (oA β) or tau (oTau) - and genetic risk modeling (CRISPR-engineered APP Swedish mutations) to generate disease-relevant neuronal states. Neuronal network activity was assessed using multielectrode array (MEA) recordings, and molecular responses were profiled using DIA-based quantitative proteomics of neuronal lysates. Proteomic signatures were benchmarked against AD reference profiles from postmortem brain proteomics². Analyses emphasized biodomain² and subdomain³ level concordance with postmortem brain proteomics to identify conserved disease-aligned programs and assess the extent to which in vitro neuronal states recapitulate in vivo AD biology.

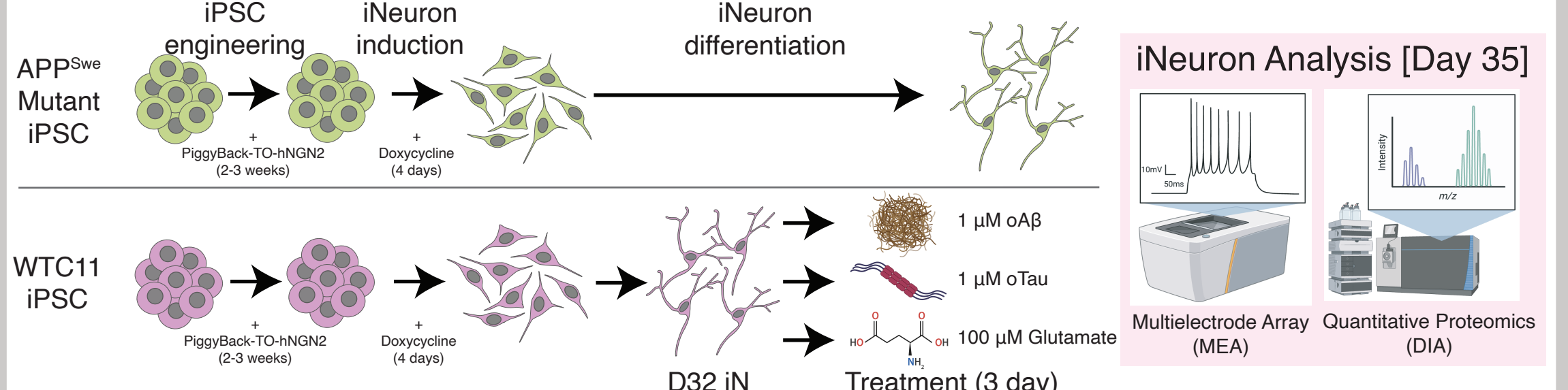


Figure 1. Schematic showing iPSC derived neuron experimental design

Mutant APP, oA β , and oTau affect iNeuron Activity

A Wild Type (WTC11) and APP^{SWE} Mutant MEA recordings showing burst frequency and duration over time (s).

B Quantification of features from data in A. (C) Quantification of mean firing rate following 24h treatment with 100 μ M glutamate or vehicle. (D) Quantification of MEA data from iNeurons before (0 hr) and 24h after treatment with oA β or oTau.

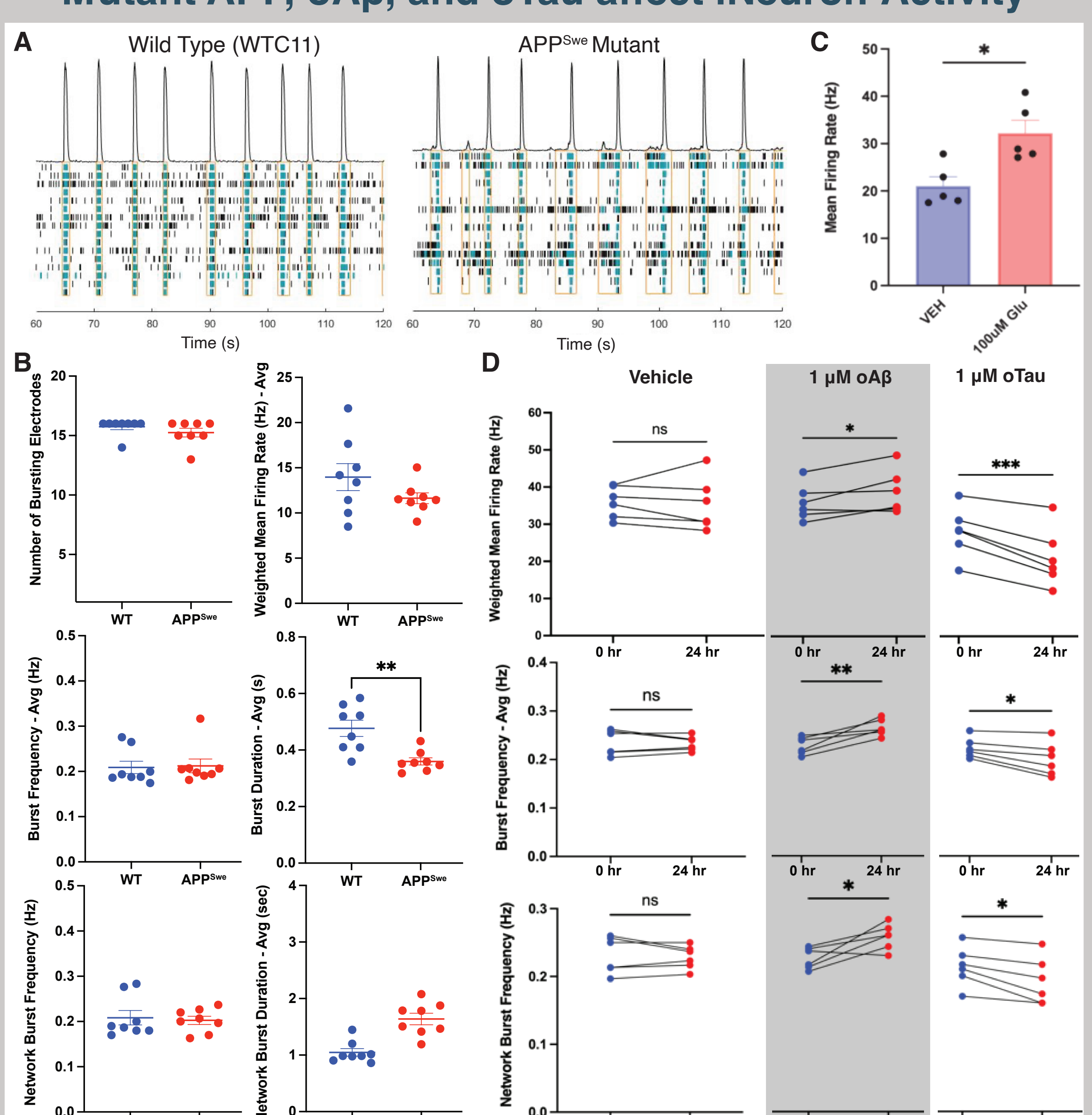


Figure 2. (A) Representative raster plots of MEA recordings from 4-week-old wild type (WTC11) or mutant (APP^{SWE}) lines. (B) Quantification of features from data in A. (C) Quantification of mean firing rate following 24h treatment with 100 μ M glutamate or vehicle. (D) Quantification of MEA data from iNeurons before (0 hr) and 24h after treatment with oA β or oTau.

Convergent Proteomic Responses

A Number of significantly differentially abundant proteins (adjusted p-value ≤ 0.05) in each experimental group. (B) Number of overlapping significant up- ($\uparrow$) and down-regulated ($\downarrow$) proteins between experimental groups. Points show significant overlaps by Fisher's exact test, with size showing the odds ratio and fill color showing the $-\log_{10}$ p-value. (C) Volcano plots showing the $\log_2$ fold change (x-axis) and $-\log_{10}$ adjusted p-value for each protein, while the size of each point shows the number of peptides quantified for each protein. Significantly differentially abundant proteins (adj p ≤ 0.05) are plotted in red and DE proteins that overlap across conditions (e.g. from 3B) are labelled.

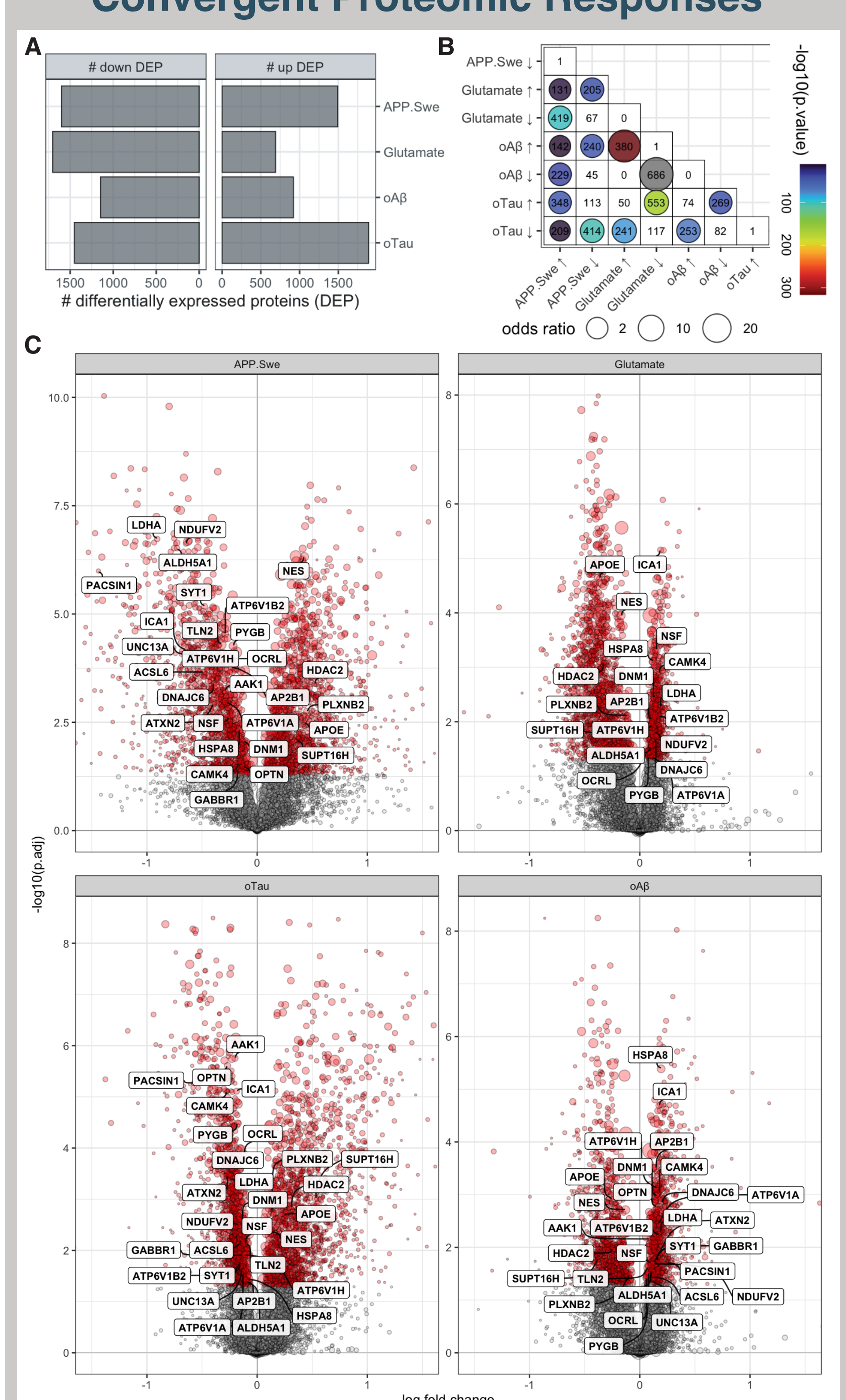


Figure 3. (A) Number of significantly differentially abundant proteins (adjusted p-value ≤ 0.05) in each experimental group. (B) Number of overlapping significant up- ($\uparrow$) and down-regulated ($\downarrow$) proteins between experimental groups. Points show significant overlaps by Fisher's exact test, with size showing the odds ratio and fill color showing the $-\log_{10}$ p-value. (C) Volcano plots showing the $\log_2$ fold change (x-axis) and $-\log_{10}$ adjusted p-value for each protein, while the size of each point shows the number of peptides quantified for each protein. Significantly differentially abundant proteins (adj p ≤ 0.05) are plotted in red and DE proteins that overlap across conditions (e.g. from 3B) are labelled.

CONCLUSIONS

- AD-relevant stressors alter iNeuron network activity and protein abundance in condition-specific patterns**
 - APP^{SWE}, oA β , and oTau produce distinct effects on firing rate, burst frequency, and network burst duration measured by MEA
 - Each stressor drives hundreds of differentially abundant proteins, with APP^{SWE} and oTau producing the largest proteomic response
- Proteomic signatures converge across stressor conditions, identifying a shared AD-associated neuronal program**
 - Significant overlap of proteins up in oA β or Glutamate and down in oTau or APP^{SWE}, and vice versa
 - These effects mirror MEA results, which group oA β and glutamate as having opposite effects of oTau
- iNeuron proteomic signatures align with human postmortem AD brain proteomics across multiple biodomains**
 - GSEA results echo convergence at the protein level, showing subdomains that are similarly affected across conditions
 - Multi-contrast enrichment identifies subdomains where iNeuron proteomics are concordant with postmortem AD brain proteomics
 - Protein-level rank-rank analysis reveals the specific proteins driving pathway-level concordance, including in the *trans-synaptic signaling* subdomain, which provides testable mechanistic interpretation of differences in MEA network activity
 - Several of the overlapping significantly differentially expressed proteins are present in these subdomains
- These data validate stressor treated iNeurons as a disease-relevant platform for target validation experiments**

Concordance with Human AD Brain Proteomics

A Gene set enrichment analysis (GSEA) results, using protein signed $-\log_{10}$ p-value as a ranking statistic, mapped to AD subdomains³. Each point represents a gene ontology (GO) term contained within a subdomain, grouped by biodomains. Position along the x-axis corresponds to the normalized enrichment score (NES) and the size of each point corresponds to the $-\log_{10}$ adjusted p-value. (B) Multi-contrast concordance analysis⁴ of iNeuron proteomics versus the human AMP-AD proteomics across select subdomains; fill = concordance direction and magnitude (signed s.dist), size = joint effect size, black outline = FDR ≤ 0.05 . (C) Protein-level rank positions in the iNeuron $\times$ AMP-AD space for the highlighted gene sets; background = 2D kernel density, labeled proteins = high-density hotspots (black) and overlapping DEP (red), as in Figs 3B & 3C.

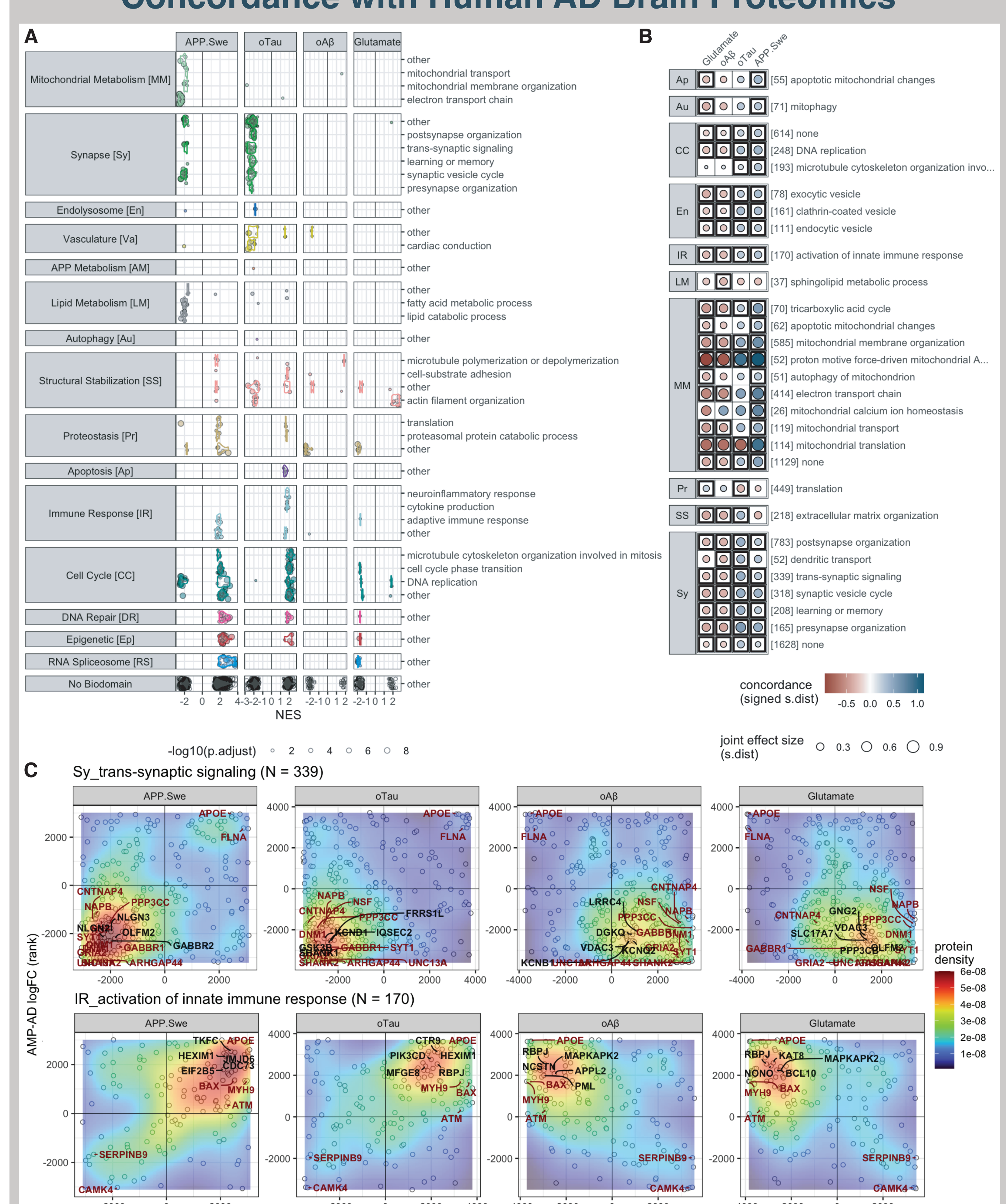


Figure 4. (A) Gene set enrichment analysis (GSEA) results, using protein signed $-\log_{10}$ p-value as a ranking statistic, mapped to AD subdomains³. Each point represents a gene ontology (GO) term contained within a subdomain, grouped by biodomains. Position along the x-axis corresponds to the normalized enrichment score (NES) and the size of each point corresponds to the $-\log_{10}$ adjusted p-value. (B) Multi-contrast concordance analysis⁴ of iNeuron proteomics versus the human AMP-AD proteomics across select subdomains; fill = concordance direction and magnitude (signed s.dist), size = joint effect size, black outline = FDR ≤ 0.05 . (C) Protein-level rank positions in the iNeuron $\times$ AMP-AD space for the highlighted gene sets; background = 2D kernel density, labeled proteins = high-density hotspots (black) and overlapping DEP (red), as in Figs 3B & 3C.

ACKNOWLEDGEMENTS

References:

- DeKosky & Scheff *Ann Neurol* (1990) doi: 10.1002/ana.410270502
- Cary *et al Alzheimers Dement* (2024) doi: 10.1002/trc2.12461
- Cary *et al Alzheimers Dement* (2026) doi: 10.1002/alz.71403
- Kaspi *et al BMC Genomics* (2020) doi: 10.1186/s12864-020-06856-9

For further information, including target packages, please see:
TREAT-AD: treatad.org
AD Knowledge portal: adknowledgeportal.synapse.org
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Interested in collaborating?
Reach us at treatad.info@sagebionetworks.org

TREAT-AD Resources:



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