

PROTEOMIC ASSESSMENT OF CSF BIOMARKERS OF NEURODEGENERATION FROM A MINIMALLY INVASIVE AND SERIAL CSF COLLECTION TECHNIQUE IN MICE

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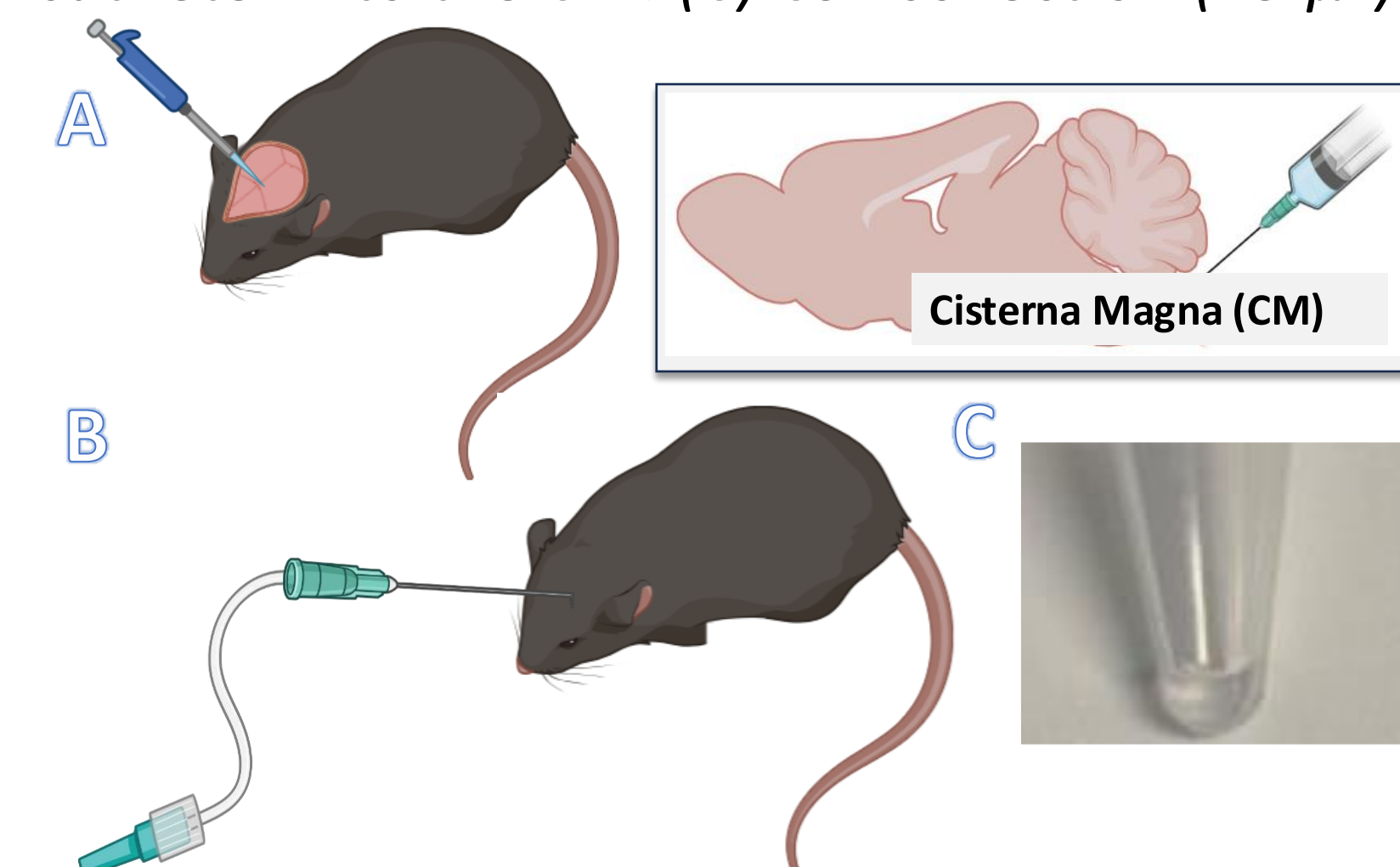
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Introduction

- CSF collection in mice is technically challenging and often requires invasive surgery to expose the cisterna magna (CM)
- Existing methods limit sampling to 1–2 timepoints per mouse
- We developed a minimally invasive Non-Surgical (Non-Sx) method for serial CSF collection
- This builds on prior work (Han et al., 2023; Loubopoulos et al., 2025)
- Serial sampling enables longitudinal analysis and improves translational relevance

Non-Surgical vs Surgical CSF collection

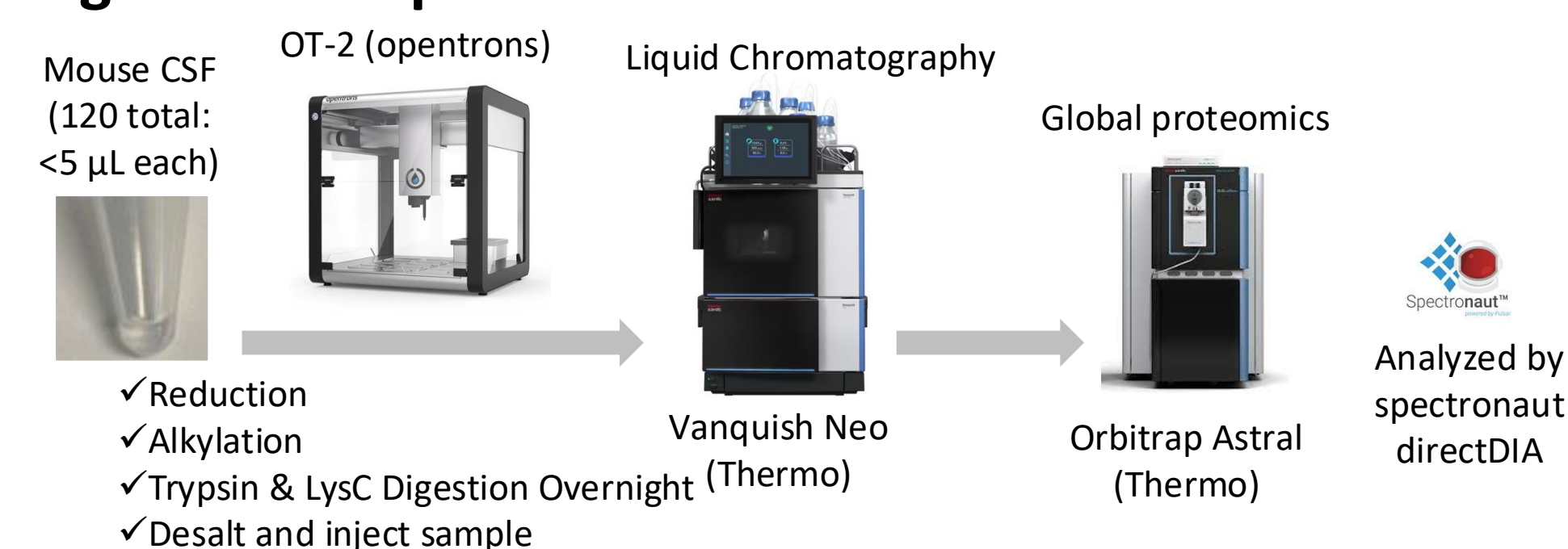
Figure 1. CSF collection methods. (A) Surgical exposure of the CM. (B) Non-Sx insertion of a ~2 mm bent needle-catheter into the CM. (C) CSF collection (~5 μ L).



- The Non-Sx CSF collection method enables rapid, repeated CSF collection up to twice weekly, with volumes (<5 μ L)
- No adverse effects or weight loss observed
- Suitable for terminal collection with comparable success
- Reduced risk of blood contamination

CSF proteomics workflow

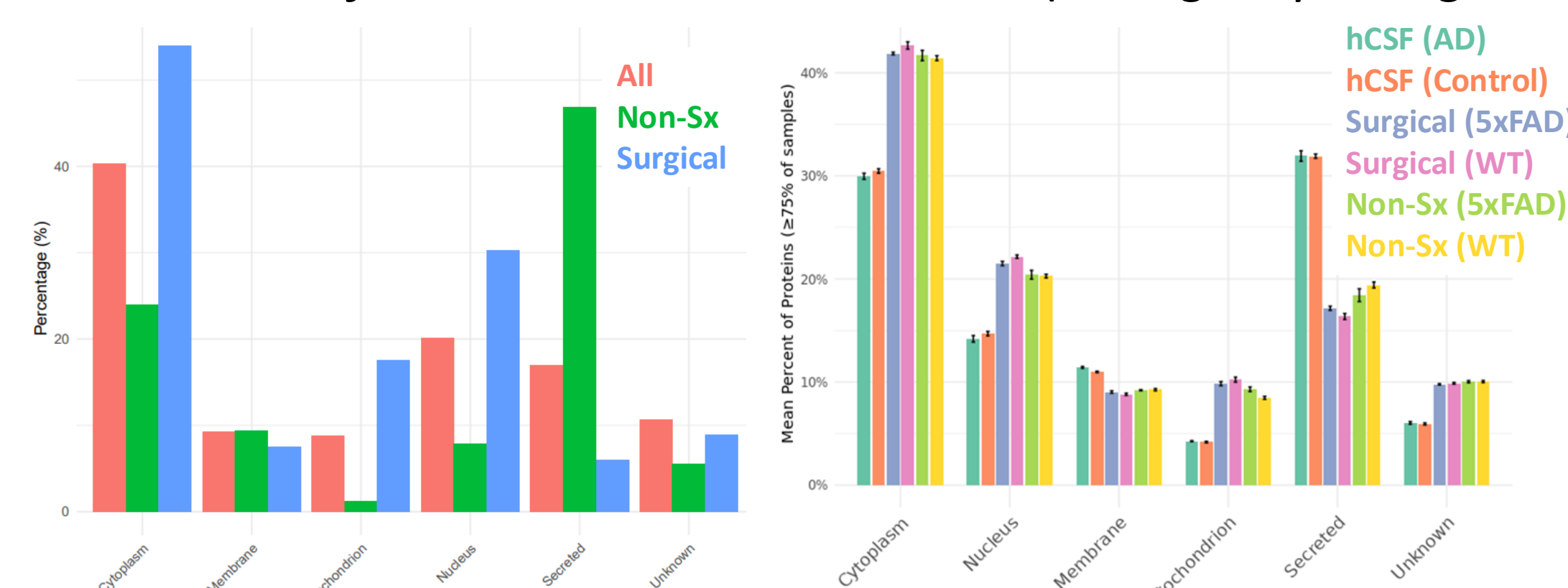
Figure 2. CSF proteomics workflow



- CSF with visible or suspected blood contamination were excluded from the analysis

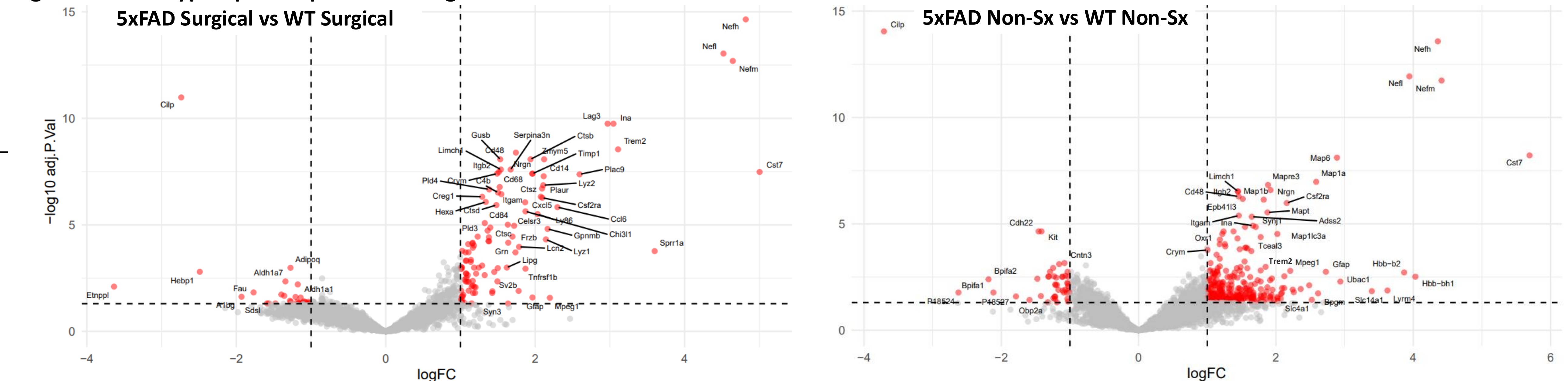
Results

Figure 3. Subcellular localization of the mouse CSF proteome comparing methods (A) and with human CSF (B). Mouse CSF: female 5xFAD and WT mice (n=9/group; Surgical and Non-Sx). Human CSF: AD and control (n=15/group).



- CSF is an extracellular fluid: high levels of secreted proteins are expected, while $\uparrow$ intracellular proteins suggest contamination
- Non-Sx: $\downarrow$ cytoplasmic/mitochondrial/nuclear proteins, $\uparrow$ secreted proteins (**Figure 3A**)
- Surgical: $\uparrow$ cytoplasmic/mitochondrial proteins, with $\downarrow$ secreted proteins (**Figure 3A**)
- Mouse and human CSF showed distinct subcellular localization profiles (**Figure 3B**).

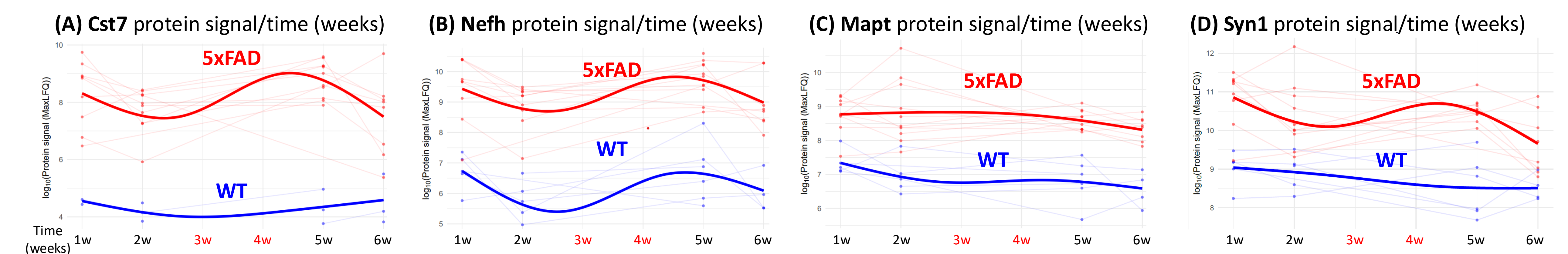
Figure 4. Genotype-specific protein changes across CSF collection methods.



- The Non-Sx method identified more genotype-specific proteins; only 33 increased and 2 decreased proteins overlapped between methods (**Figure 4**)

Figure 5. Genotype- and time-specific differentially expressed proteins over time.

Mouse CSF: Female 5xFAD and WT mice; 6 weekly collections, 4 analyzed by proteomics (n=6/group).



- Cst7, Nefh, Syn1, and Nefm were increased in 5xFAD vs WT (FDR <0.05); Mapt was nominally significant (FDR: 0.075) (**Figure 5**)
- 313 proteins showed time-dependent changes, including CD68; no proteins showed a genotype $\times$ time interaction

Conclusions

CSF is obtainable and quantifiable using a minimally invasive technique which:

- Was well-tolerated for survival collections;
- Has enhanced sensitivity to biomarkers of interest; and;
- Has improved translatability to larger animal models and human studies based on collection methodology and proteomics data

References: 1. Han, JR, et al. "A Minimally-Invasive Method for Serial Cerebrospinal Fluid Collection and Injection in Rodents with High Survival Rates." *Biomedicine* 11, no. 6 (2023): 1609.
2. Loubopoulos, A, et al. "An Improved Method for Sampling and Quantitative Protein Analytics of Cerebrospinal Fluid of Individual Mice." *Molecular & Cellular Proteomics: MCP* 24, no. 5 (2025): 100958.

Acknowledgments/Disclosures

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