

Enhancing plasma proteome profiling through cost-effective, robust and high-throughput workflows based on hyper-porous magnetic beads

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INTRODUCTION

- Mag-Net™** (Wu *et al.*, 2023 DOI:10.1101/ 2023.06.10.544439) is a high-throughput, cost-effective method for deep plasma profiling that utilizes the unique features of hyper-porous MagReSyn® technology:
 - High capacity and porosity beads allows for enrichment of membrane vesicles
 - The strong magnetic moment enables rapid handling of beads, and ease of automation on a variety of magnetic bead handling stations and liquid handling stations
- We have optimized the workflows to fit on a single microplate in a KingFisher Duo system, and further allowing for reduction of plasticware and associated chemicals when using other automation systems
- We present **Mag-Net™ HP** (High Performance)
 - After an exhaustive optimization of Mag-Net™, we are presenting an enhanced method for the enrichment of vesicles from plasma, with comparative data for proteome profiling
- We developed and apply an in-house R-script for the automated performance assessment of Mag-Net™

METHODS

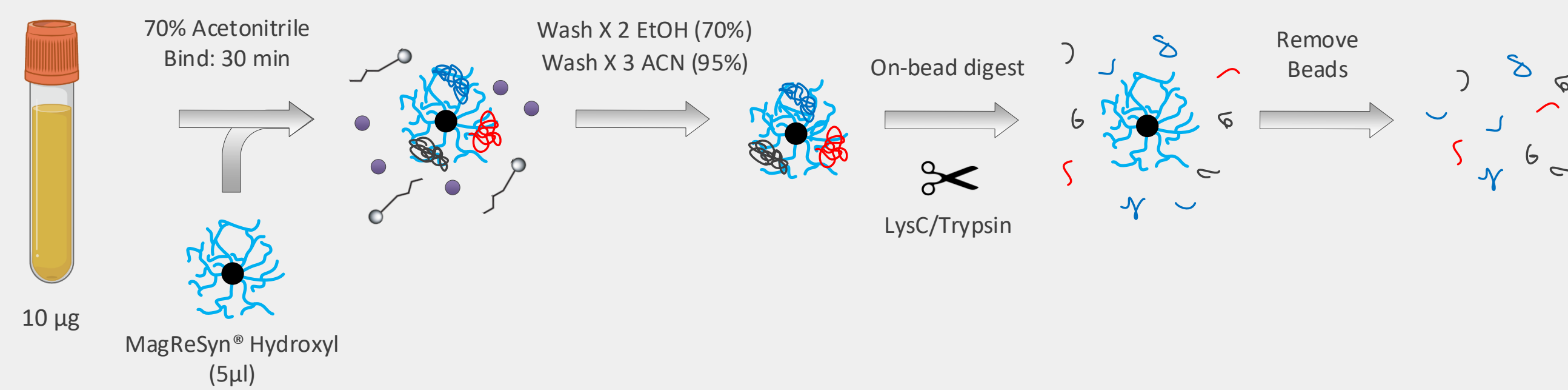
- All workflows were semi-automated using KingFisher™ Flex or Duo (.bdz method files available on request)
- A:** Raw Plasma clean-up workflow
- B:** The general outline of the **Mag-Net™** workflow as described by Wu *et al.* in 2023
- C:** Optimized workflow with reduced processing steps
- D:** Optimized Mag-Net™ HP workflow, using 5 µl of bead formulation and 5 µl of plasma
- All data was derived from plasma from a single healthy donor

PLASMA PREPARATION

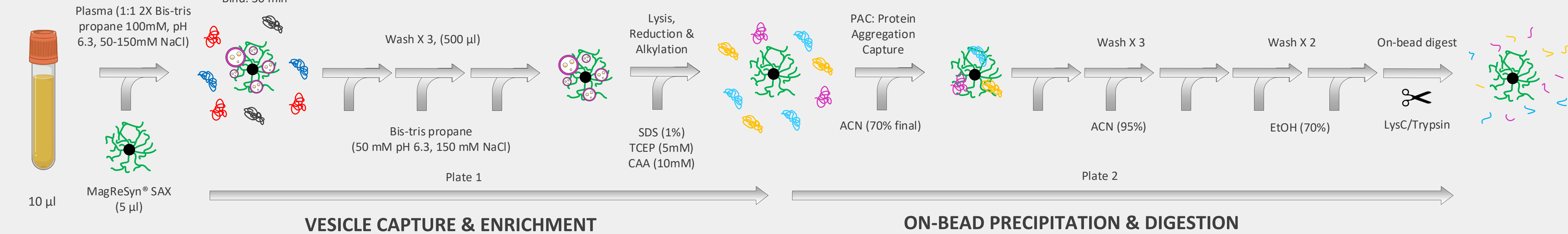
Plasma for Mag-Net™ was processed validated using plasma collected based on the Early Detection Research Network (EDRN) Standard Operating Procedure for collection of EDTA plasma, from a single healthy donor



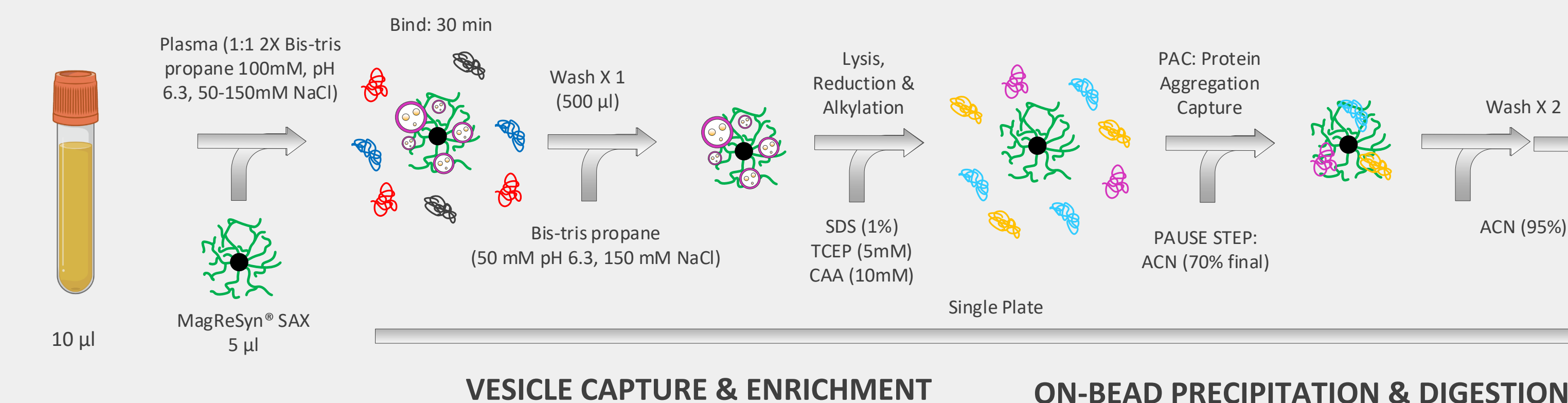
A: RAW PLASMA CLEAN-UP WORKFLOW



B: ORIGINAL MAG-NET™ WORKFLOW

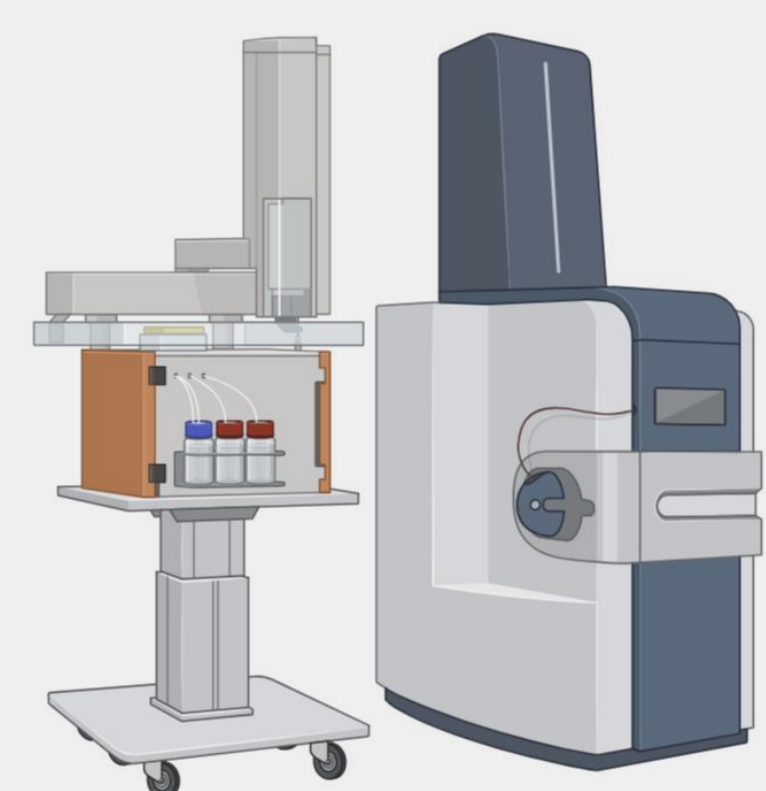


C: STREAMLINED MAG-NET™ WORKFLOW



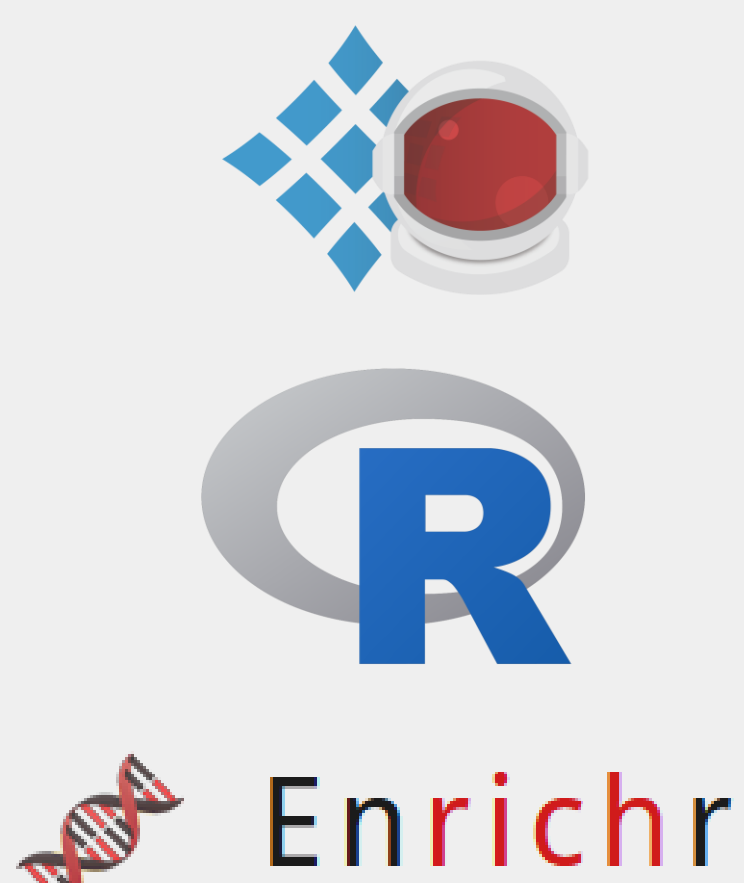
LC MS ANALYSIS

Equivalent amounts of digests were loaded on an Evotip, analysis using Evosep One 40 or 60 SPD, diAPASEF short gradient method on Bruker timsTOF HT fitted with Pepsep column, or 40 SPD fitted with Ionopticks Aurora series column

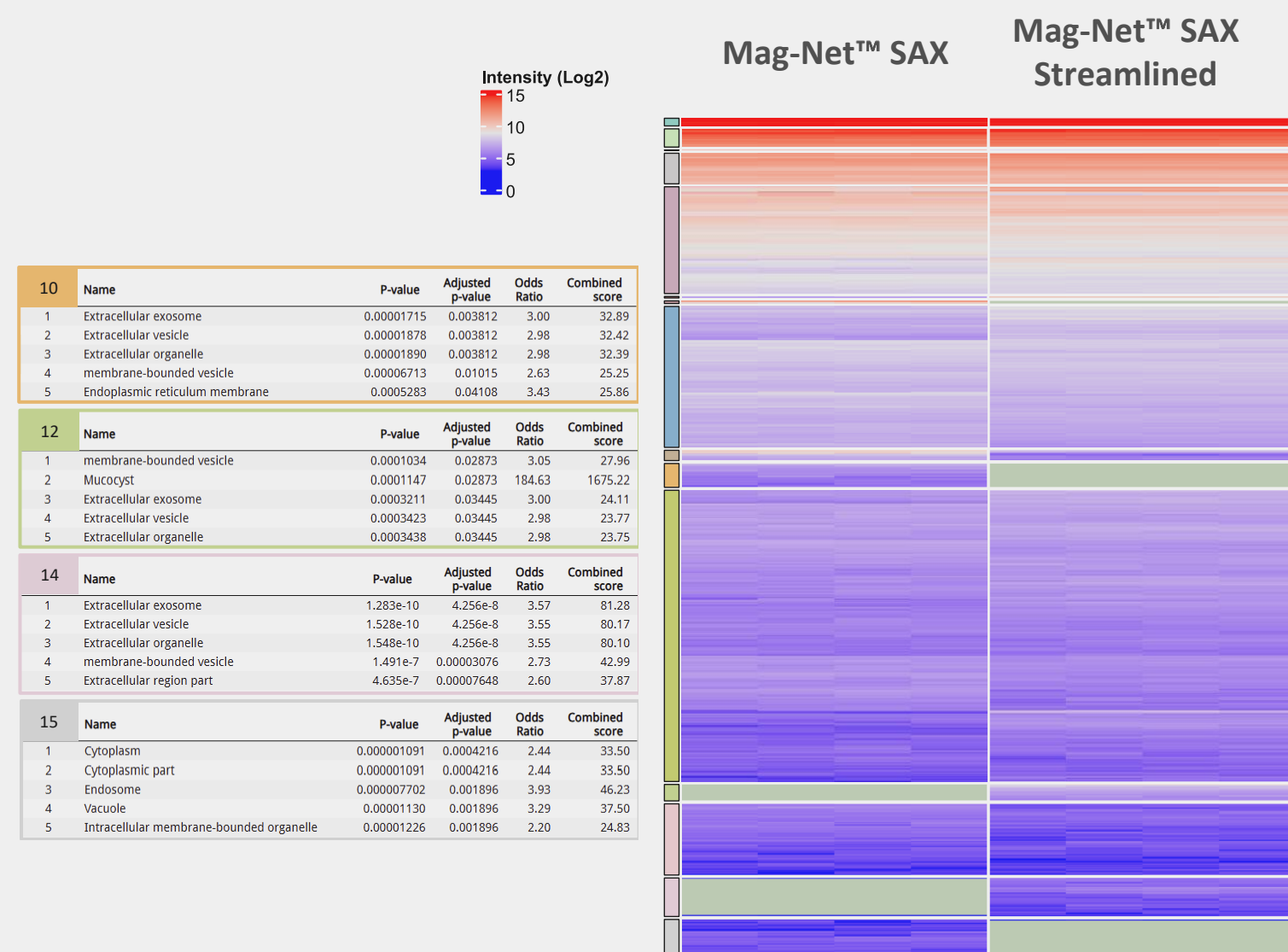


DATA ANALYSIS

Data was processed with Spectronaut (v19) using method evaluation mode, and analysis performed in R. GO analysis was performed with Enrichr.



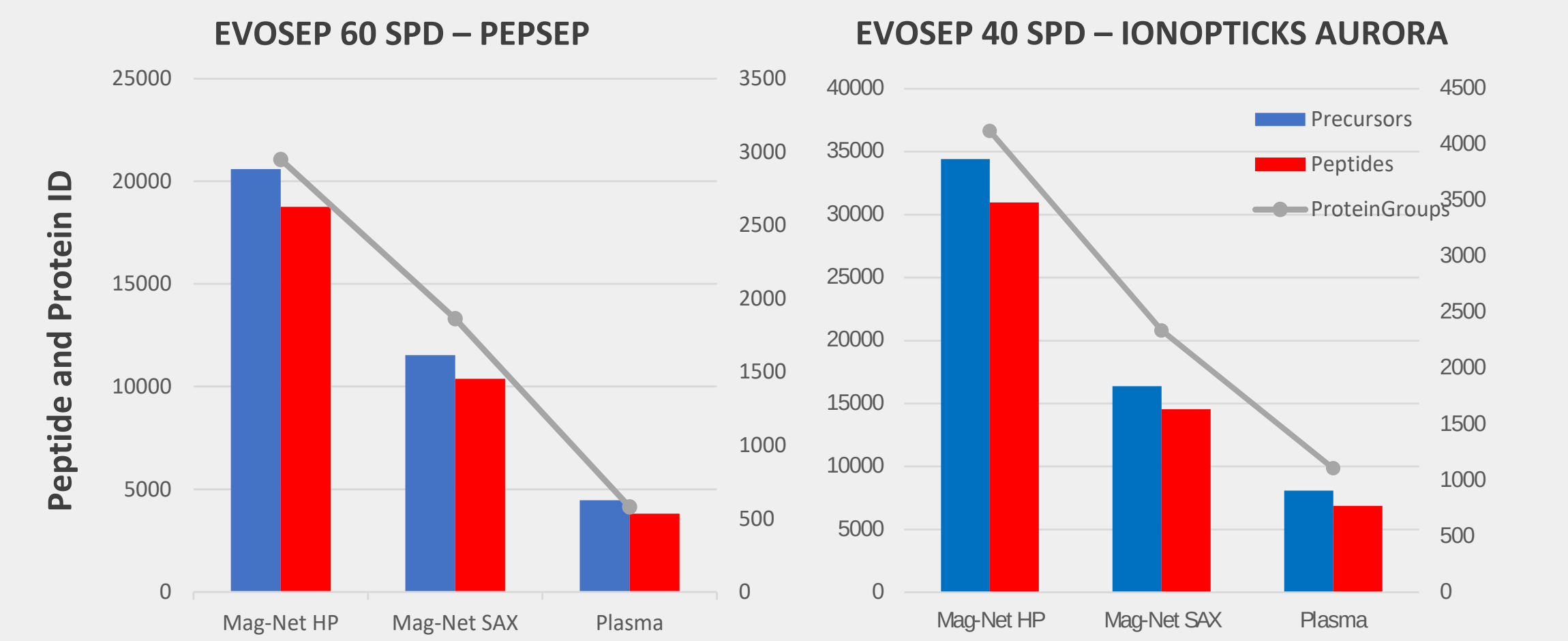
ACKNOWLEDGEMENTS



STREAMLINED WORKFLOW

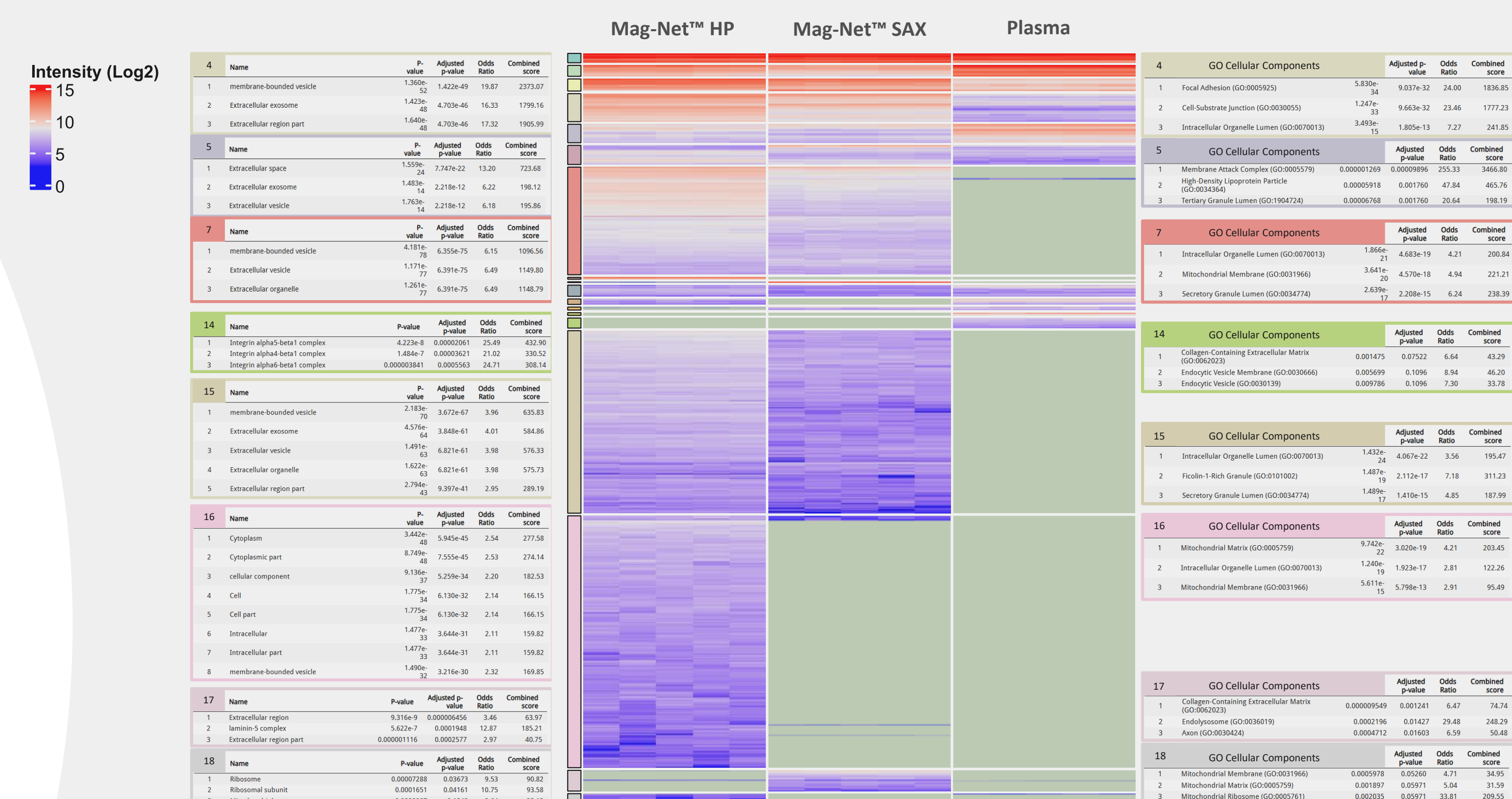
- Hierarchical clustering of proteins was queried for Jensen COMPARTMENTS using Enrichr. Some unique clusters were identified in each of the original Mag-Net™ SAX and streamlined workflows. The unique clusters were associated with alternate vesicle subsets, but were fairly small numbers of proteins.
- However, most vesicle markers from Vesiclepedia were not significantly affected (data not shown), but higher abundance of plasma proteins was observed.
- The streamlined workflow reduced sample processing time (by ~20%), halved the plasticware (one microplate instead of two), and saves on reagents (used primarily in the washing of the beads).

ENRICHMENT PROFILES: MAG-NET™ SAX vs HP

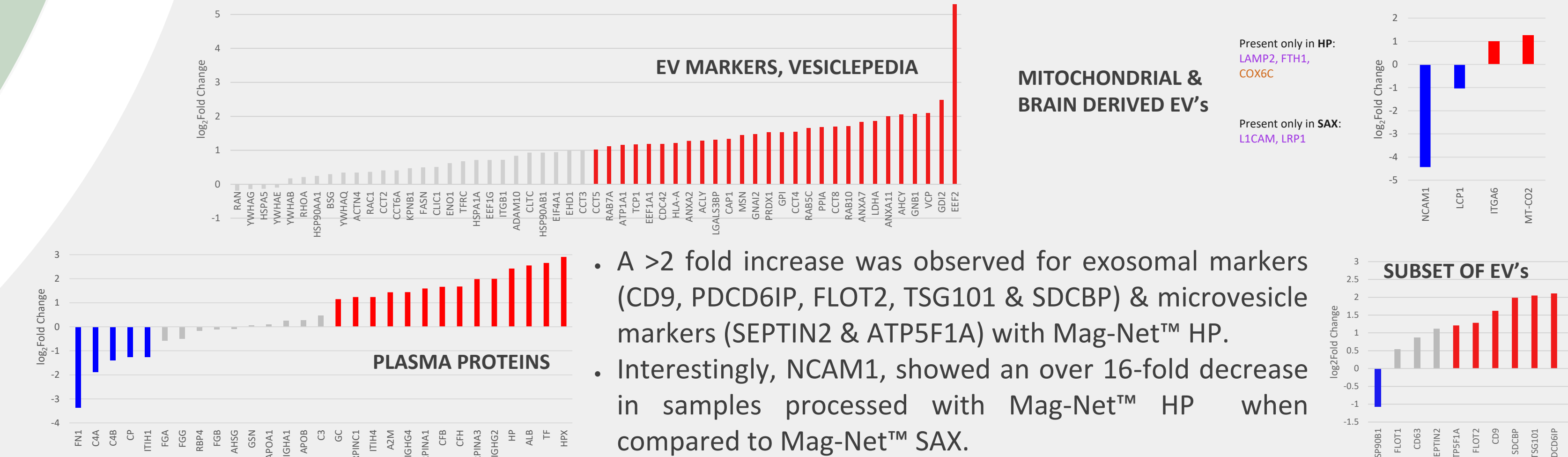


- Mag-Net™ HP displayed the highest plasma proteome coverage with a greater than 50 percent increase in protein group ID's compared to the original Mag-Net™ SAX workflow, and 1151 unique protein groups for the HP enrichments (60 SPD Pepsep column) and 1884 (40 SPD Ionopticks Aurora column).

JENSEN COMPARTMENT ANALYSIS, HP, SAX & PLASMA

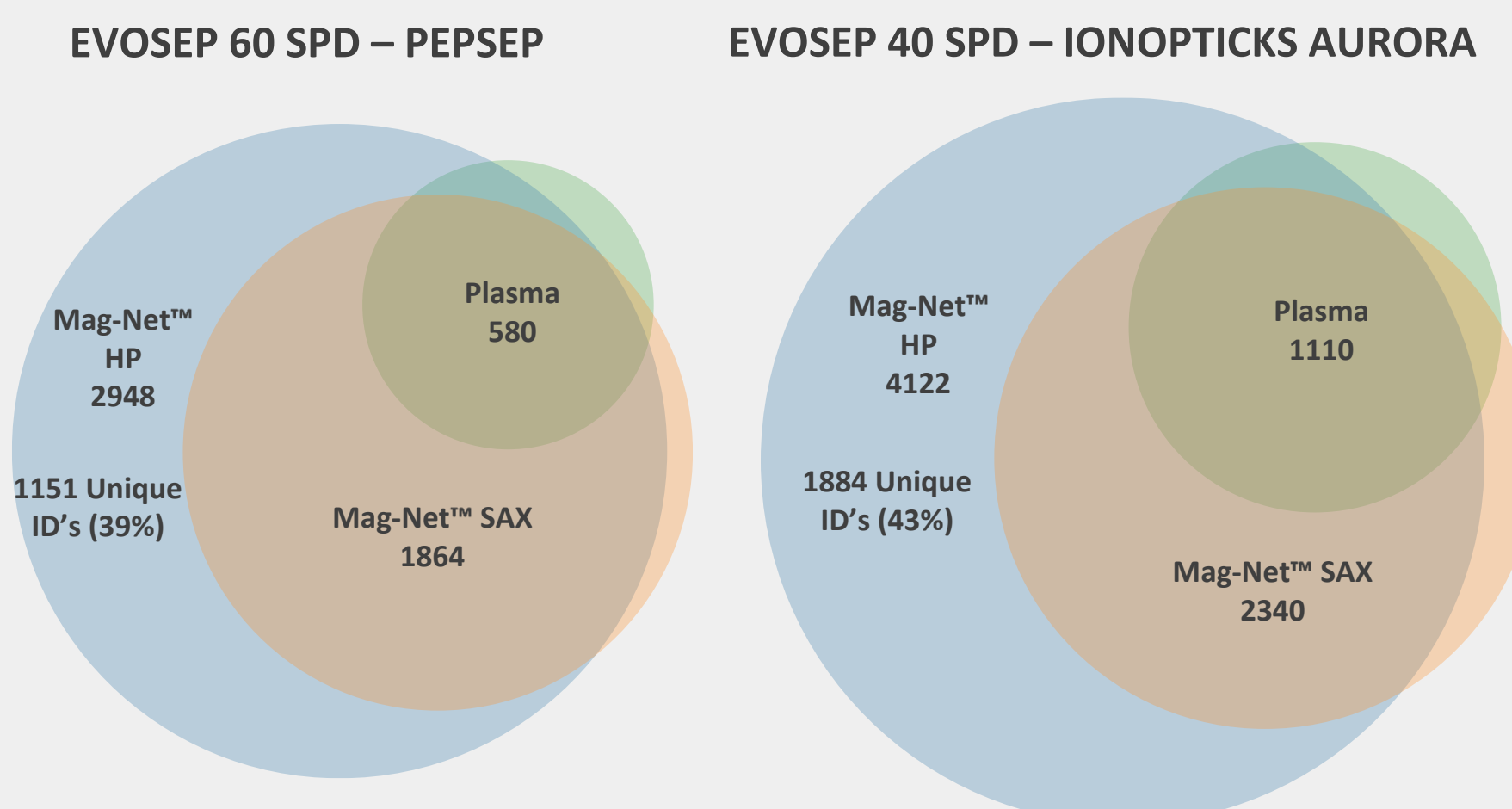


DIFFERENTIAL PROTEIN ABUNDANCE FOR SUBSETS

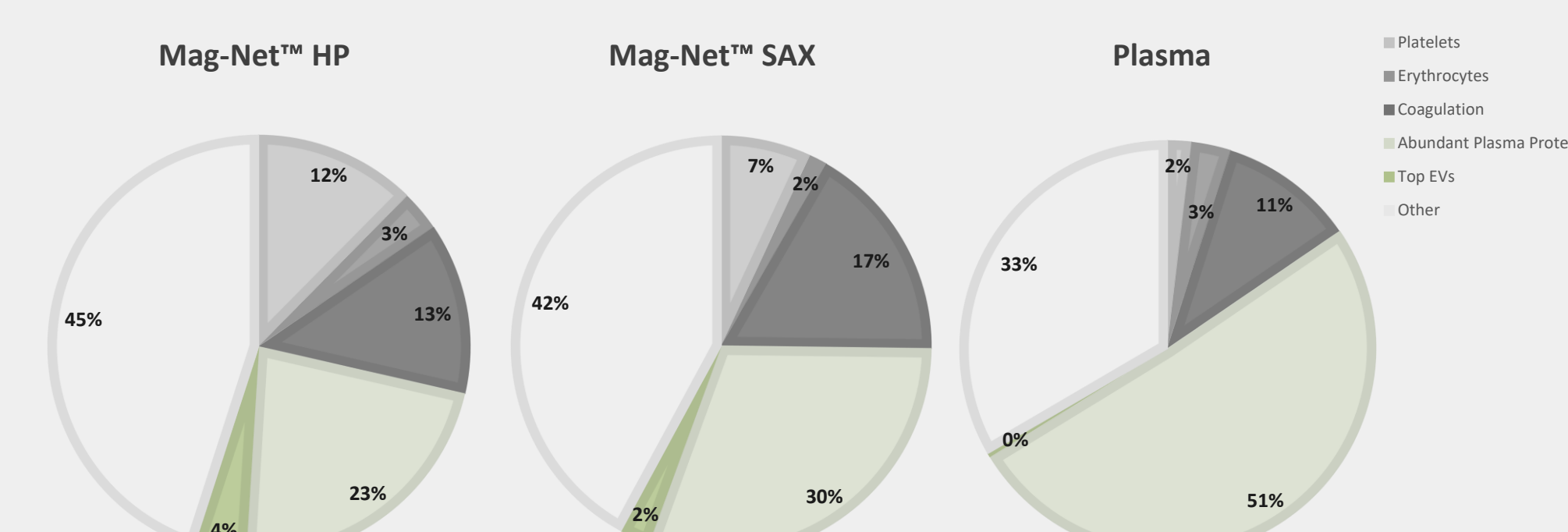


- A >2 fold increase was observed for exosomal markers (CD9, PDC61P, FLOT2, TSG101 & SDCBP) & microvesicle markers (SEPTIN2 & ATP5F1A) with Mag-Net™ HP.
- Interestingly, NCAM1, showed an over 16-fold decrease in samples processed with Mag-Net™ HP when compared to Mag-Net™ SAX.

RESULTS & DISCUSSION

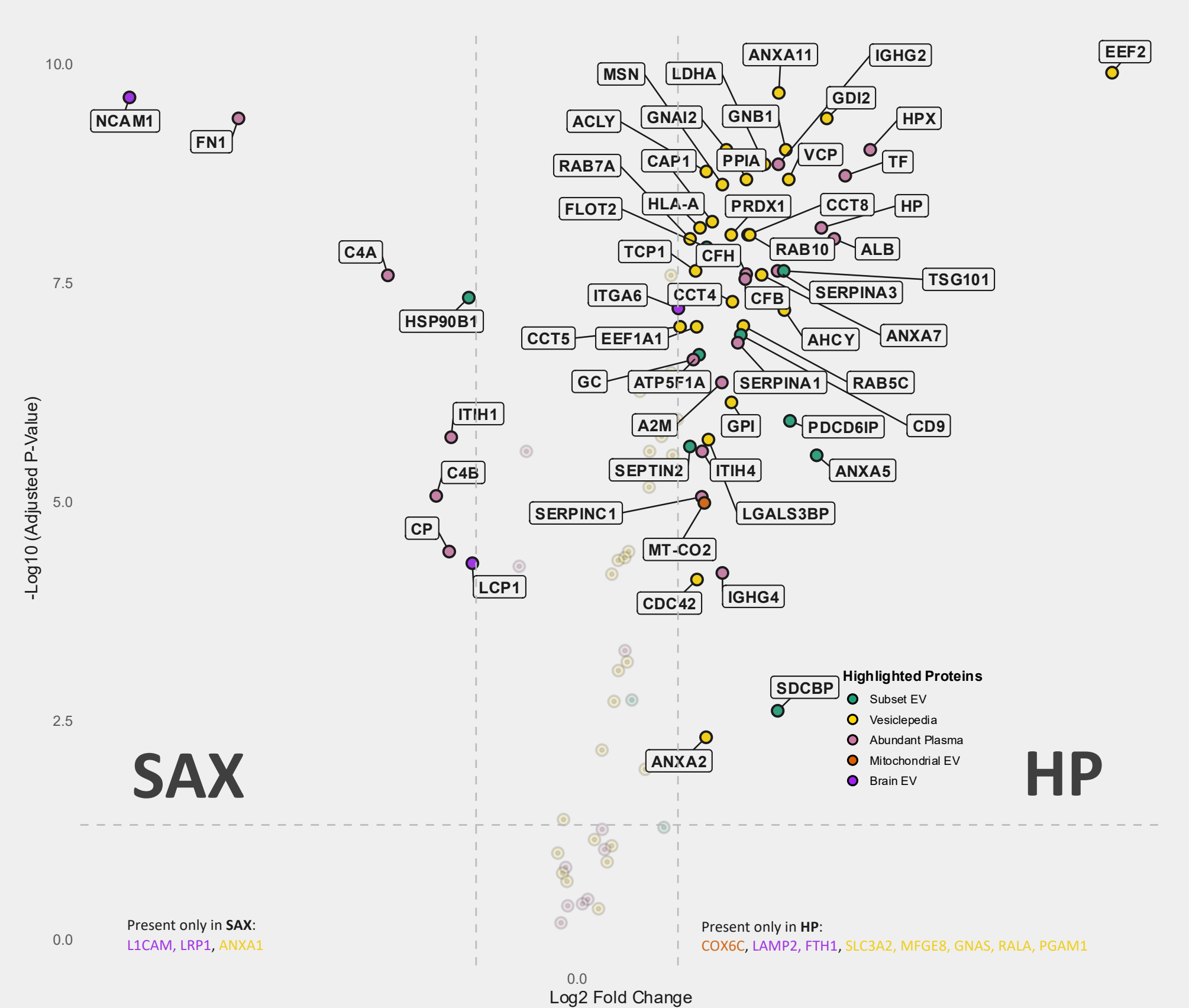


SIGNAL CONTRIBUTION FROM PLASMA



- Mag-Net™ HP and Mag-Net™ SAX workflows both showed a reduction in abundant plasma proteins with an increase in extracellular vesicle (EV)-related protein abundance when compared to plasma.
- The Mag-Net™ HP workflow doubled protein intensities of top EV-related proteins from Vesiclepedia, with a reduction in the total intensity of the top ranked abundant plasma proteins in comparison to Mag-Net™ SAX.

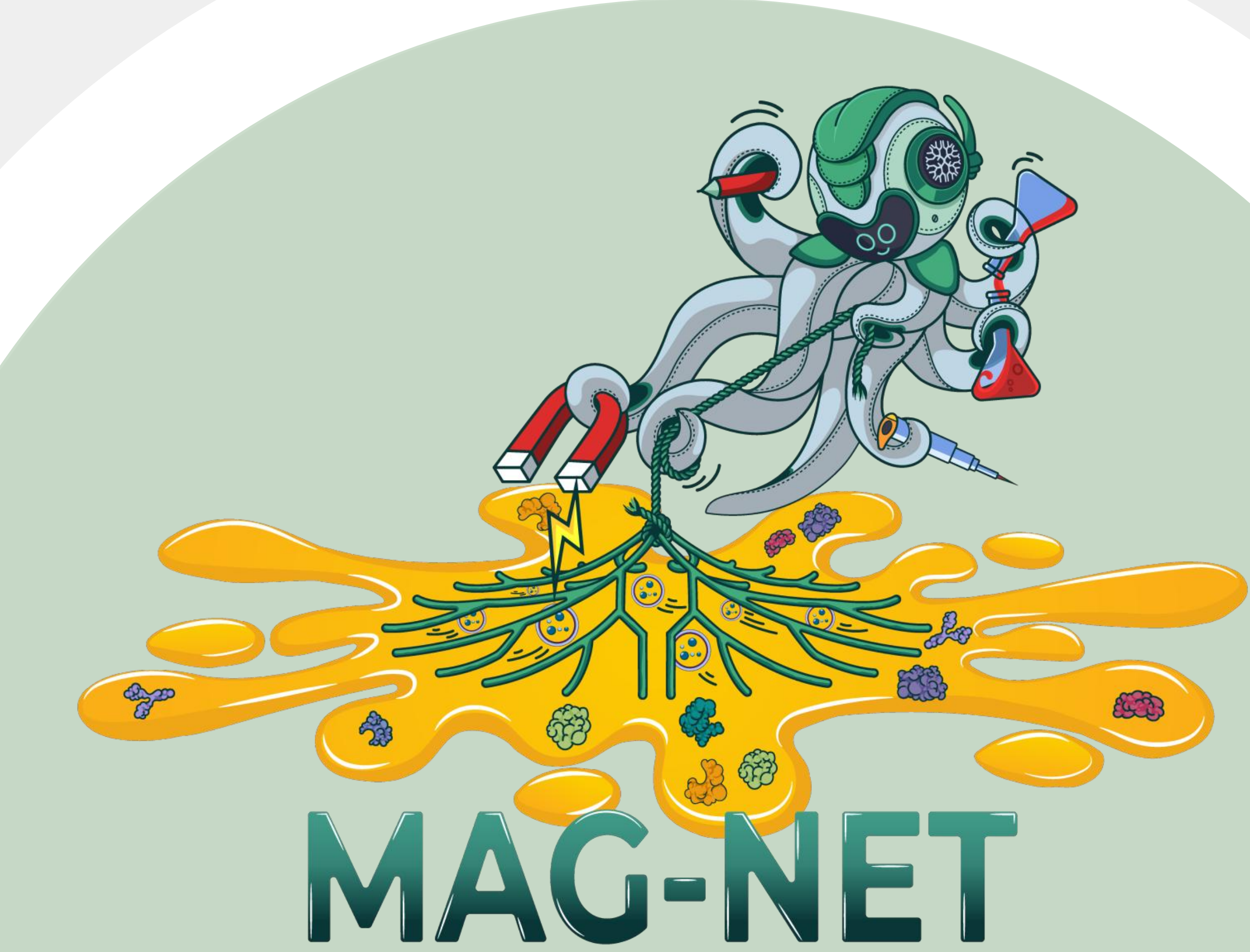
DISTRIBUTION OF PROTEIN ABUNDANCE



- Comparison of Mag-Net™ HP to the Mag-Net™ SAX workflow showed increased plasma proteome coverage with an overall improvement of more than 50% sample coverage, and increased abundance of EV markers from Vesiclepedia.

- GO compartment and cell component analysis showed increased coverage which was associated with enrichment of specific protein subsets not captured by the Mag-Net™ SAX, in particular mitochondrial proteins.

- Some markers such as NCAM1 (related to neuronally derived EV's) are however preferentially enriched by Mag-Net™ SAX, whereas mitochondrial EV markers such as MT-CO2 are enriched in Mag-Net™ HP.



MagReSyn® hyper-porous polymer beads enable capture of membrane vesicles for deep plasma profiling

Mag-Net™ HP shows over 2-fold enrichment in EV marker proteins over Mag-Net™ SAX

Mag-Net™ HP increases proteome coverage by over 50%, identifying several new GO clusters

The streamlined version of the protocol was used for the development of the automated sample processing of the Mag-Net™ HP Kit, buffers and conditions were performed as per the protocol supplied.

CONFLICT OF INTEREST DISCLOSURE
Justin Jordaan, Previn Naicker, Isak Gerber, Amy van Graan & Melissa Vorster are employed by ReSyn Biosciences, proprietors of MagReSyn® technology.

HEATMAPS OF PLASMA, EV & MITOCHONDRIAL DERIVED PROTEIN MARKERS

