## **Supporting Information**

## Systematic Evaluation of the Use of Human Plasma and Serum for Mass-Spectrometry-Based Shotgun Proteomics

Jiayi Lan<sup>1</sup>, Antonio Núñez Galindo<sup>1</sup>, James Doecke<sup>2</sup>, Christopher Fowler<sup>3</sup>, Ralph N. Martins<sup>4, 5</sup>, Stephanie R. Rainey-Smith<sup>4</sup>, Ornella Cominetti<sup>1</sup> and Loïc Dayon<sup>1, \*</sup>

<sup>1</sup>Proteomics, Nestlé Institute of Health Sciences, 1015 Lausanne, Switzerland

<sup>2</sup>CSIRO Health and Biosecurity/Australian E-Health Research Centre, Brisbane, Queensland 4029, Australia

<sup>3</sup>The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3010, Australia

<sup>4</sup>Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia 6027, Australia

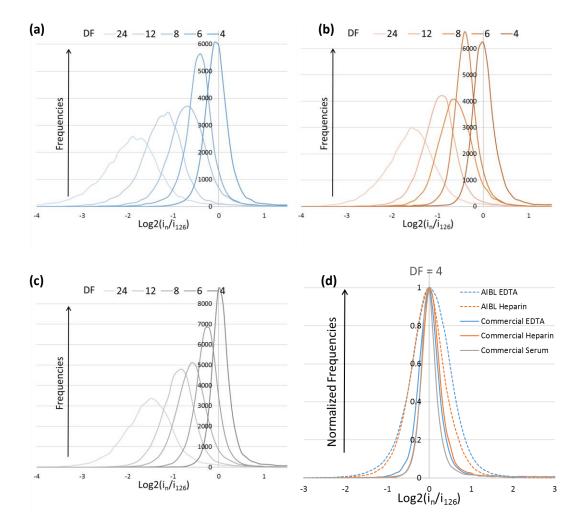
<sup>5</sup>Department of Biomedical Sciences, Macquarie University, New South Wales 2109, Australia

\*Tel: +41 21 632 6114; Fax: +41 21 632 6499

E-mail: loic.dayon@rd.nestle.com

## Table of contents of supporting information

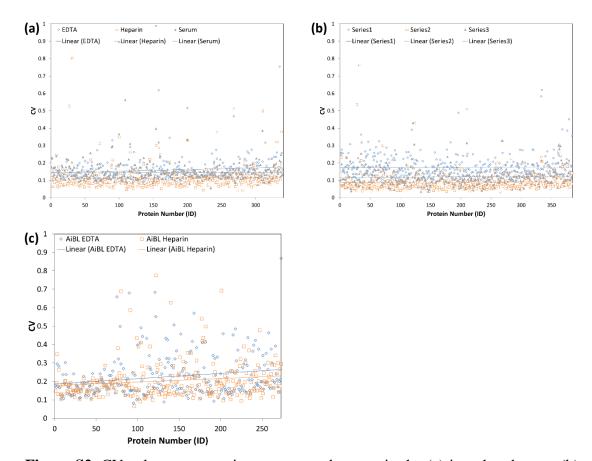
- 1. Log2( $i_n/i_{126}$ ) distribution of matched raw spectral data (**Figure S1**)
- 2. Descriptive statistics of the datasets (Table S1)
- 3. CV values per protein of interday, intraday and AIBL datasets (Figure S2)
- 4. Median CVs of interday and intraday datasets (Table S2)
- 5. Proteome coverage obtained with AIBL EDTA-plasma and heparin-plasma samples (Figure S3)
- 6. Scatter plot of the protein fold-change ratios in matched EDTA-plasma and heparinplasma samples from AIBL (**Figure S4**)



**Figure S1.** Log2( $i_n/i_{126}$ ) distribution of matched raw spectral data according to DF in (a) commercial EDTA-plasma, (b) commercial heparin-plasma, and (c) commercial serum samples. In (d), normalized distributions of matched raw spectral data (DF = 4) were compared between commercial samples and AIBL samples.

**Table S1.** Descriptive statistics of datasets: commercial EDTA-plasma with different DFs, commercial heparin-plasma with different DFs, commercial serum with different DFs, AIBL EDTA-plasma and AIBL heparin-plasma.

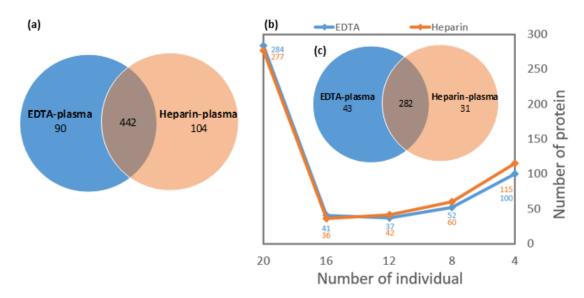
| DF                        | Expected ratio fold change | Expected log2<br>ratio fold<br>change | Median of log2 ratio fold change | Mean of log2<br>ratio fold<br>change | SD of log2<br>ratio fold<br>change | Lower 95% CI<br>of mean | Upper 95% CI<br>of mean | Kurtosis |  |  |
|---------------------------|----------------------------|---------------------------------------|----------------------------------|--------------------------------------|------------------------------------|-------------------------|-------------------------|----------|--|--|
| Commercial EDTA-plasma    |                            |                                       |                                  |                                      |                                    |                         |                         |          |  |  |
| 4                         | 1                          | 0                                     | -0.0979                          | -0.0241                              | 0.1949                             | -0.4062                 | 0.3579                  | 13.2962  |  |  |
| 6                         | 0.6667                     | -0.5850                               | -0.4740                          | -0.4731                              | 0.2009                             | -0.8668                 | -0.0794                 | 11.9852  |  |  |
| 8                         | 0.5                        | -1                                    | -0.7438                          | -0.7488                              | 0.2480                             | -1.2348                 | -0.2628                 | 5.7295   |  |  |
| 12                        | 0.3333                     | -1.5850                               | -1.2798                          | -1.2973                              | 0.2500                             | -1.7873                 | -0.8074                 | 5.1107   |  |  |
| 24                        | 0.1667                     | -2.5850                               | -1.9409                          | -1.9428                              | 0.2735                             | -2.4789                 | -1.4068                 | 2.7875   |  |  |
| Commercial heparin-plasma |                            |                                       |                                  |                                      |                                    |                         |                         |          |  |  |
| 4                         | 1                          | 0                                     | -0.0436                          | 0.0370                               | 0.1917                             | -0.3387                 | 0.4126                  | 14.1191  |  |  |
| 6                         | 0.6667                     | -0.5850                               | -0.4448                          | -0.4373                              | 0.1848                             | -0.7995                 | -0.0750                 | 14.3648  |  |  |
| 8                         | 0.5                        | -1                                    | -0.6674                          | -0.6612                              | 0.2399                             | -1.1315                 | -0.1910                 | 6.5827   |  |  |
| 12                        | 0.3333                     | -1.5850                               | -1.0056                          | -0.9986                              | 0.2312                             | -1.4518                 | -0.5455                 | 7.4635   |  |  |
| 24                        | 0.1667                     | -2.5850                               | -1.6249                          | -1.6372                              | 0.2609                             | -2.1485                 | -1.1258                 | 3.7380   |  |  |
|                           | Commercial serum           |                                       |                                  |                                      |                                    |                         |                         |          |  |  |
| 4                         | 1                          | 0                                     | -0.1023                          | -0.0118                              | 0.1700                             | -0.3450                 | 0.3214                  | 19.1040  |  |  |
| 6                         | 0.6667                     | -0.5850                               | -0.4068                          | -0.4067                              | 0.1989                             | -0.7966                 | -0.0168                 | 12.9393  |  |  |
| 8                         | 0.5                        | -1                                    | -0.7451                          | -0.7365                              | 0.2228                             | -1.1732                 | -0.2998                 | 8.1943   |  |  |
| 12                        | 0.3333                     | -1.5850                               | -1.0304                          | -1.0383                              | 0.2322                             | -1.4936                 | -0.5831                 | 7.4145   |  |  |
| 24                        | 0.1667                     | -2.5850                               | -1.6656                          | -1.6752                              | 0.2621                             | -2.1890                 | -1.1614                 | 3.8048   |  |  |
|                           | AIBL EDTA-plasma           |                                       |                                  |                                      |                                    |                         |                         |          |  |  |
| 4                         | 1                          | 0                                     | -0.1161                          | -0.1287                              | 0.3069                             | -0.7303                 | 0.4729                  | 1.1943   |  |  |
| AIBL heparin-plasma       |                            |                                       |                                  |                                      |                                    |                         |                         |          |  |  |
| 4                         | 1                          | 0                                     | -0.0827                          | -0.0871                              | 0.2857                             | -0.6472                 | 0.4729                  | 2.1827   |  |  |



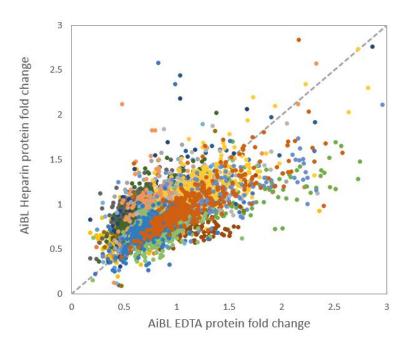
**Figure S2.** CV values per protein across sample types in the (a) interday dataset, (b) intraday dataset, and (c) AIBL dataset.

**Table S2.** Median CVs of protein abundance in interday and intraday repeatability study.

|             | EDTA-plasma | Heparin-plasma | Serum |
|-------------|-------------|----------------|-------|
| Interday CV | 14.1%       | 9.5%           | 13.4% |
| Intraday CV | 16.0%       | 7.3%           | 11.0% |



**Figure S3.** (a) Proteome coverage obtained in AIBL EDTA-plasma and heparin-plasma samples. (b) Reproducibility of IDs among 20 individuals. Proteins which were identified in more than 16 individuals were considered as consistent IDs. (c) Proteome coverage overlap between the consistent IDs in EDTA-plasma and heparin-plasma.



**Figure S4.** Scatter plot of the protein fold-change ratios measured in EDTA-plasma and heparin-plasma samples from AIBL. Each color represents an individual (i.e., n = 20), and each point represents given protein measured in the different sample types (i.e., EDTA-plasma versus heparin-plasma).