**SUPPLEMENTAL MATERIAL**

**SILAC-based characterization of plasma-derived extracellular vesicles in patients undergoing partial hepatectomy**

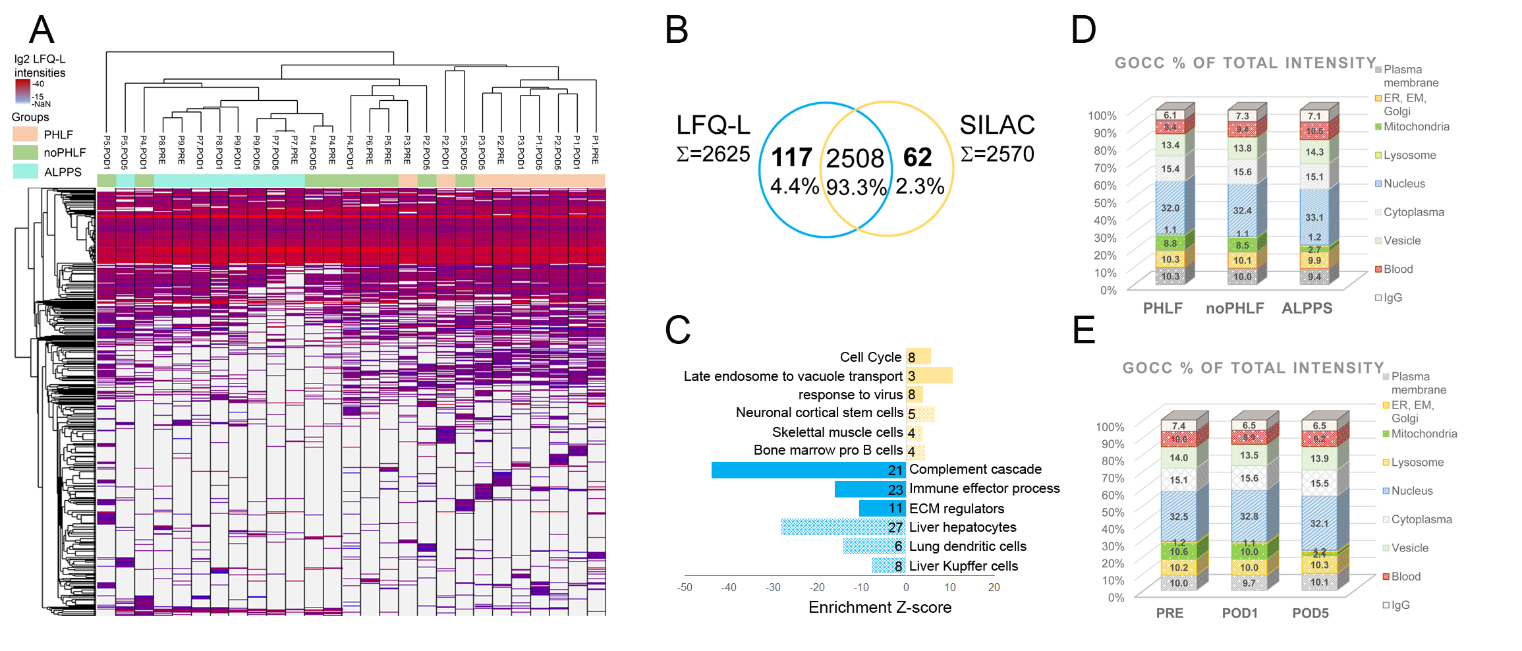
Ulrike Resch1,2**\***, Hubert Hackl3, David Pereyra4, Jonas Santol4, Laura Brunnthaler1, Joel Probst4, Anna Sofie Jankoschek4, Monika Aiad4, Hendrik Nolte2, Marcus Krueger2,Patrick Starlinger34,5, Alice Assinger1**\***

1. **Supplemental Table**
2. **Supplmentary Figures**
3. **Description to supplemental data**
4. **Supplementary Table**

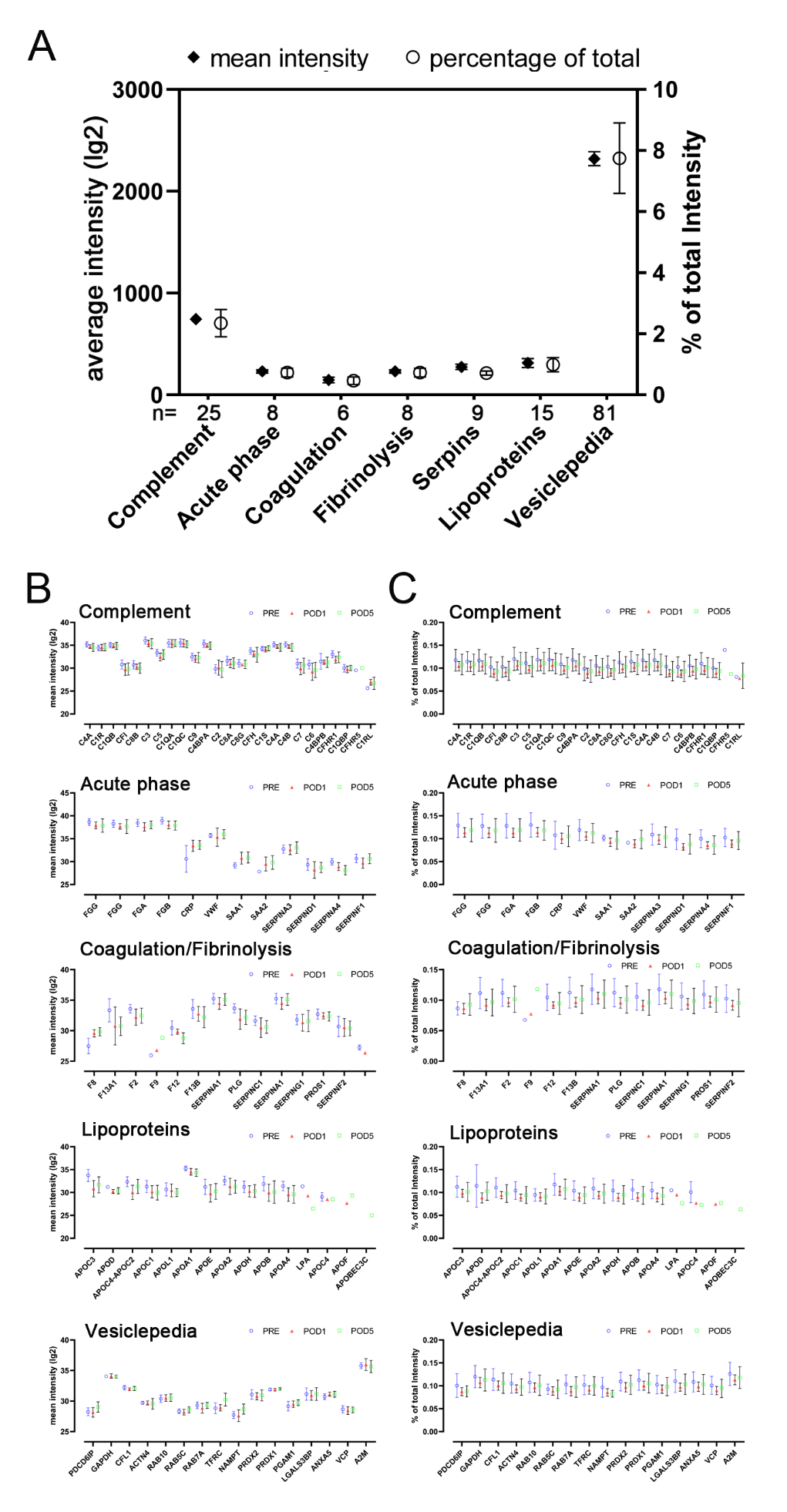
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | | **Cohort (n=9)** | **PHLF Cohort (n=3)** | **no PHLF Cohort (n=3)** | **ALPPS Cohort (n=3)** | **Missing values** |
| Sex | Male | 7 (77.8%) | 3 (100%) | 2 (66.6%) | 2 (66.6%) |  |
| Female | 2 (22.2%) | 0 (0.0%) | 1 (33.3%) | 1 (33.3%) |  |
| Age (y, range) |  | 64.9 (50.0-78.6) | 65.3 (56.5-76.0) | 58.6 (50.0-69.6) | 70.9 (56.5-78.6) |  |
| Hepatic resection | Minor (< 3 segments) | 2 (22.2%) | 0 (0.0%) | 0 (0.0%) | 2 (66.6%) |  |
| Major  (≥ 3 segments) | 7 (77.8%) | 3 (100.0%) | 3 (100.0%) | 1 (33.3%) |  |
| **Hepatic comorbidities\*** | |  |  |  |  |  |
|  | Steatosis (%) | 14.0 (0.0-60.0%) | 33.0 (0.0-60.0%) | 8 (0.0-20.0%) | 0 (0.0-0.0%) |  |
|  | Steatohepatitis | 3 (33.3%) | 2 (66.6%) | 1 (33.3%) | 0 (0.0) |  |
|  | Fibrosis | 6 (66.6%) | 3 (100.0%) | 2 (66.6%) | 1 (33.3%) | 2 (22.2%) |
|  | CASH | 3 (33.3%) | 2 (66.6%) | 1 (33.3%) | 0 (0.0) | 1 (2.1%) |
| **Preoperative parameters** | |  |  |  |  |  |
|  | PDR (%) | 20.5 (15.0-30.0) | 18.6 (17.7-19.4) | 23.7 (18.0-30.0) | 15.0 (15.0-15.0) | 3 (33.3%) |
|  | Platelet counts (×103/µL) | 221 (178-267) | 199 (178-206) | 237 (234-239) | 233 (201-267) | 1 (11.1%) |
|  | SB (mg/dL) mean (range) | 0.8 (0.3 – 2.9) | 1.5 (0.4 – 2.9) | 0.5 (0.3-0.6) | 0.4 (0.3-0.6) | 1 (11.1%) |
|  | PT (%) mean (range) | 98  (45 – 120) | 76 (45 – 98) | 108 (106-110) | 114 (109-120) | 1 (11.1%) |
|  | AP (U/L) mean (range) | 96 (64 – 128) | 67 (64 – 68) | 108 (90 – 126) | 117 (98-128) | 1 (11.1%) |
|  | GGT (U/L) mean (range) | 68 (33– 112) | 51 (33-74) | 74 (36 – 112) | 81 (66-88) | 1 (11.1%) |
|  | AST (U/L) mean (range) | 42 (25 – 71) | 57 (43 – 71) | 37 (27 – 46) | 25 (25-25) | 4  (44.4%) |
|  | ALT (U/L) mean (range) | 38 (20 – 81) | 54 (30 – 81) | 39 (37 – 41) | 22 (20-25) | 1 (11.1%) |
|  | Albumin (g/L) mean (range) | 41.8 (37.3 – 47.2) | 42.3 (37.3 – 47.2) | 40.8 (40.8-40.8) | n.a. | 6 (66.6%) |
| **Morbidity** | |  |  |  |  |  |
|  | No morbidity | 5 (55.5%) | 3 (100.0%) | 2 (66.6%) | 0 (0.0%) |  |
|  | Grade I | 1 (11.1%) | 0 (0.0%) | 1 (33.3%) | 0 (0.0%) |  |
|  | Grade II | 2 (22.2%) | 0 (0.0%) | 0 (0.0%) | 2 (66.6%) |  |
|  | Grade III | 1 (11.1%) | 0 (0.0%) | 0 (0.0%) | 1 (33.3%) |  |
|  | Grade IV | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |  |
|  | Grade V | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |  |
| **Postoperative stay** | |  |  |  |  |  |
|  | ICU (days) | 1.0 (0.0-2.0) | 1.0 (1.0-2.0) | 1.0 (0.0-1.0) | 1 (0.0-2.0) |  |
|  | Total hospitalization (d) | 11 (7-22) | 8 (6-10) | 9 (7-14) | 22 (7-22) |  |
| **PHLF ISGLS** | |  |  |  |  |  |
|  | no PHLF | 6 (66.6%) | 0 (0.0%) | 3 (100.0%) | 3 (100.0%) |  |
|  | Grade A | 1 (11.1%) | 1 (33.3%) | 0 (0.0%) | 0 (0.0%) |  |
|  | Grade B | 2 (22.2%) | 2 (66.6%) | 0 (0.0%) | 0 (0.0%) |  |
|  | Grade C | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |  |

**Table 1: Plasma cohort patient characteristics:** Hepatic comorbities\*, all patients received Neoadjuvant chemotherapy; y, years; d, days; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CASH, chemotherapy-induced acute steatohepatitis; CCC, cholangiocellular carcinoma; GGT, gamma-glutamyl transpeptidase; HCC, hepatocellular carcinoma; ICU, intensive care unit; ISGLS, International Study Group of Liver Surgery; mCRC, metastatic colorectal cancer; PHLF, post-hepatectomy liver failure; PDR, plasma disappearance rate; PT, prothrombin time; SB, serum bilirubin.

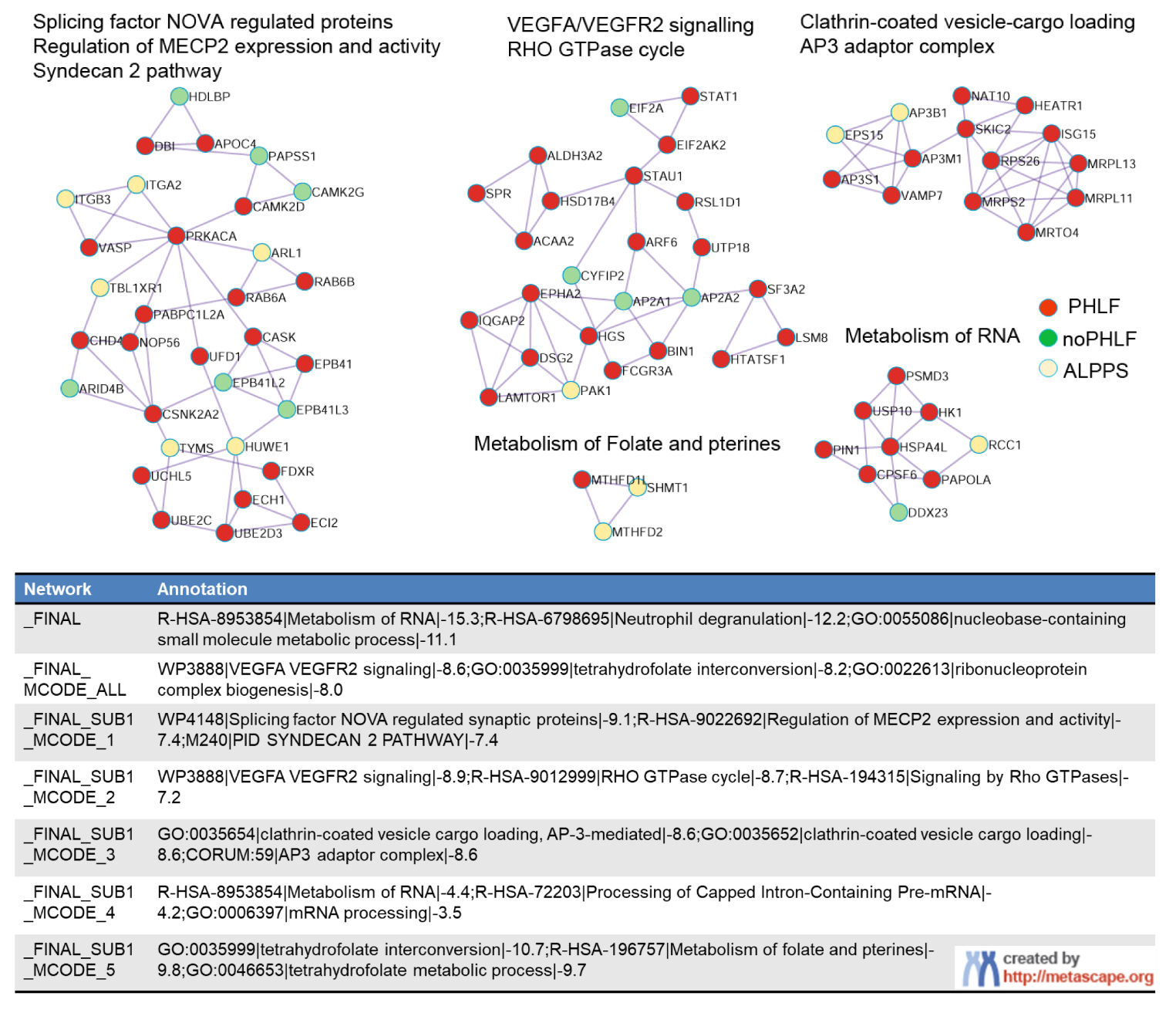
1. **Supplementary Figures**

****

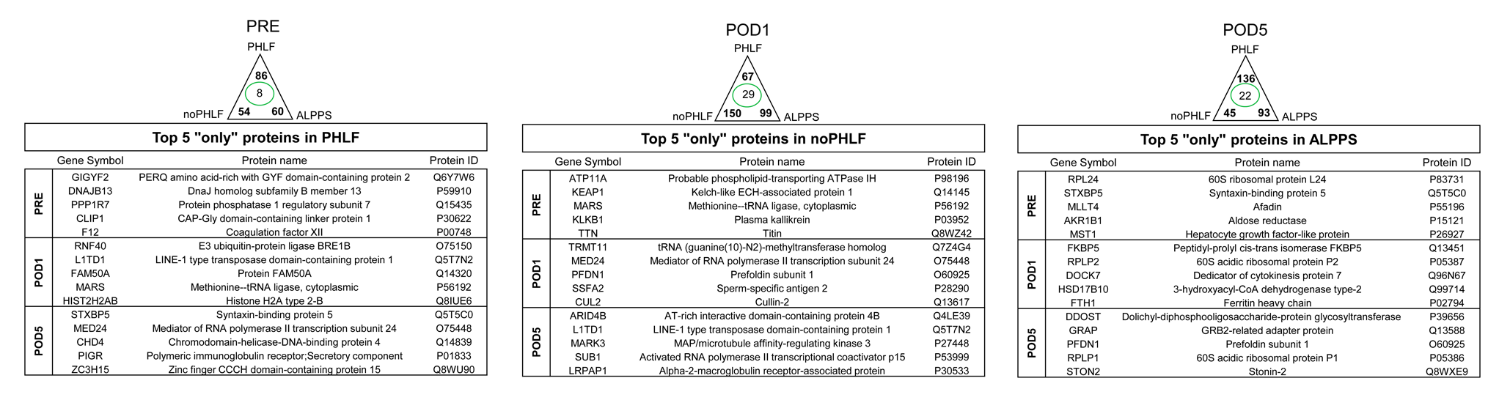
**Supplementary Figure 1: Qualitative comparison of LFQ-L and SILAC identifications to capture plasma specific proteins: A)** Heatmap of hierarchical clustered samples (average linked Euclidian distance on log2 transformed LFQ-L intensities, missing values in light grey) to visualize data completeness across sample groups PHLF, noPHLF and ALPPS as indicated. **B)** Venn diagram of common and unique proteins identified on the basis of LFQ-L and SILAC-ratios, **C)** Enriched Gene Ontology and cell type signatures of 117 LFQ-L (blue) or 62 SILAC-only proteins (yellow) with respective protein numbers denoted. **D)** Overall GOCC categorization of all quantified proteins and their relative contribution to the sum intensity (LFQ-L) in sample groups “outcome” (PHLF, noPHLF and ALPPS) or “time” (PRE, POD1, POD5). Corresponding source data can be found in Suppl. Data 1.



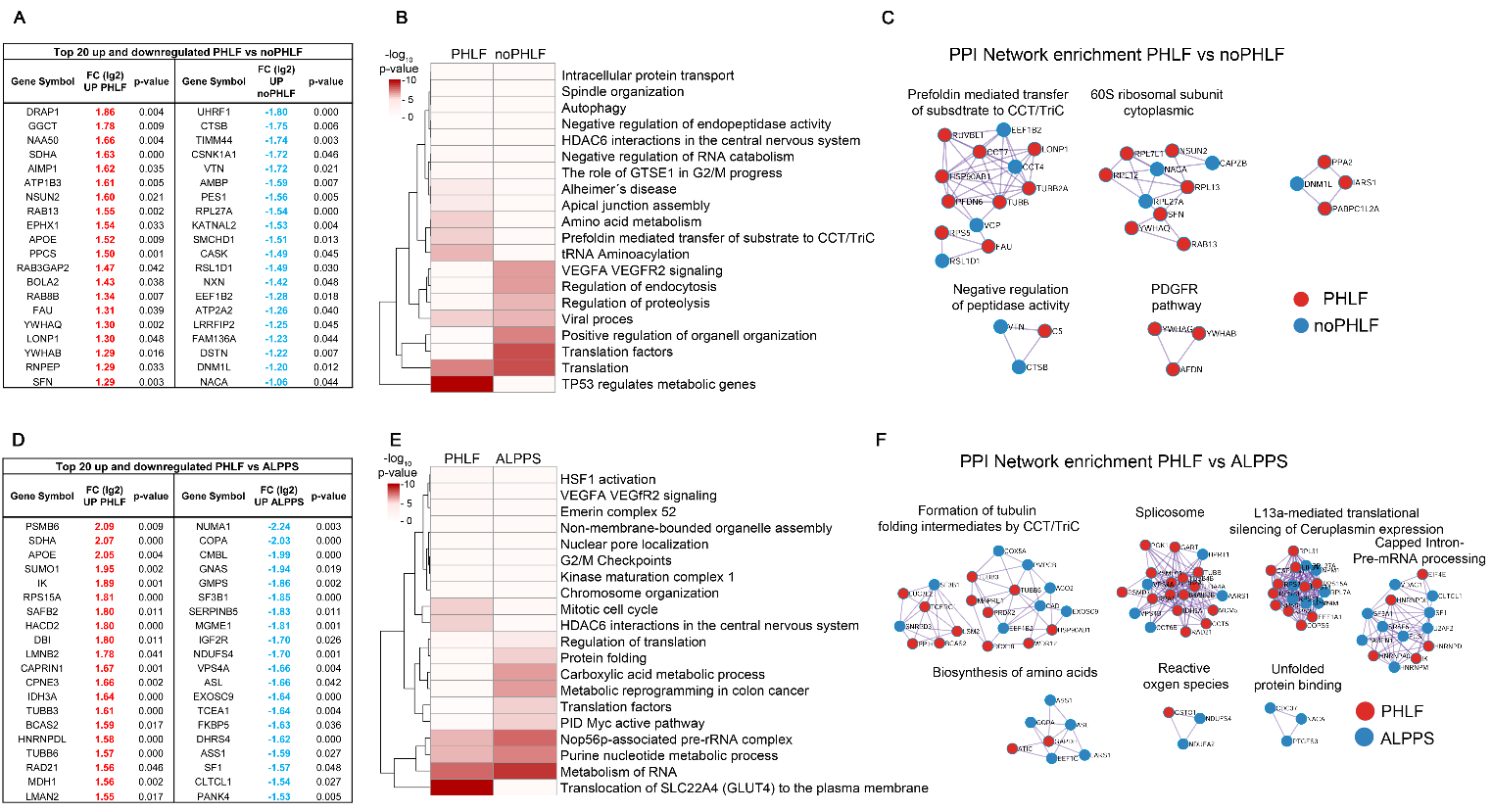
**Supplementary Figure 2: Blood- and Vesiclepedia proteins identified and quantified in plasma EVs:** Cumulative **(A)** and individual **(B, C)** abundance of blood (complement, acute phase, coagulation fibrinolysis, serpins (serine-protease inhibitor family members), lipoproteins and selected EV-proteins (Vesiclepedia) quantified in PRE, POD1 and POD5 EV-samples with respective protein counts in each category denoted on the x-axis. Data are expressed as mean±sd log2-transformed LFQ-L intensity values **(B)** or percentage of total intensities **(C)** in merged outcome groups. Corresponding source data can be found in Suppl. Data 1.



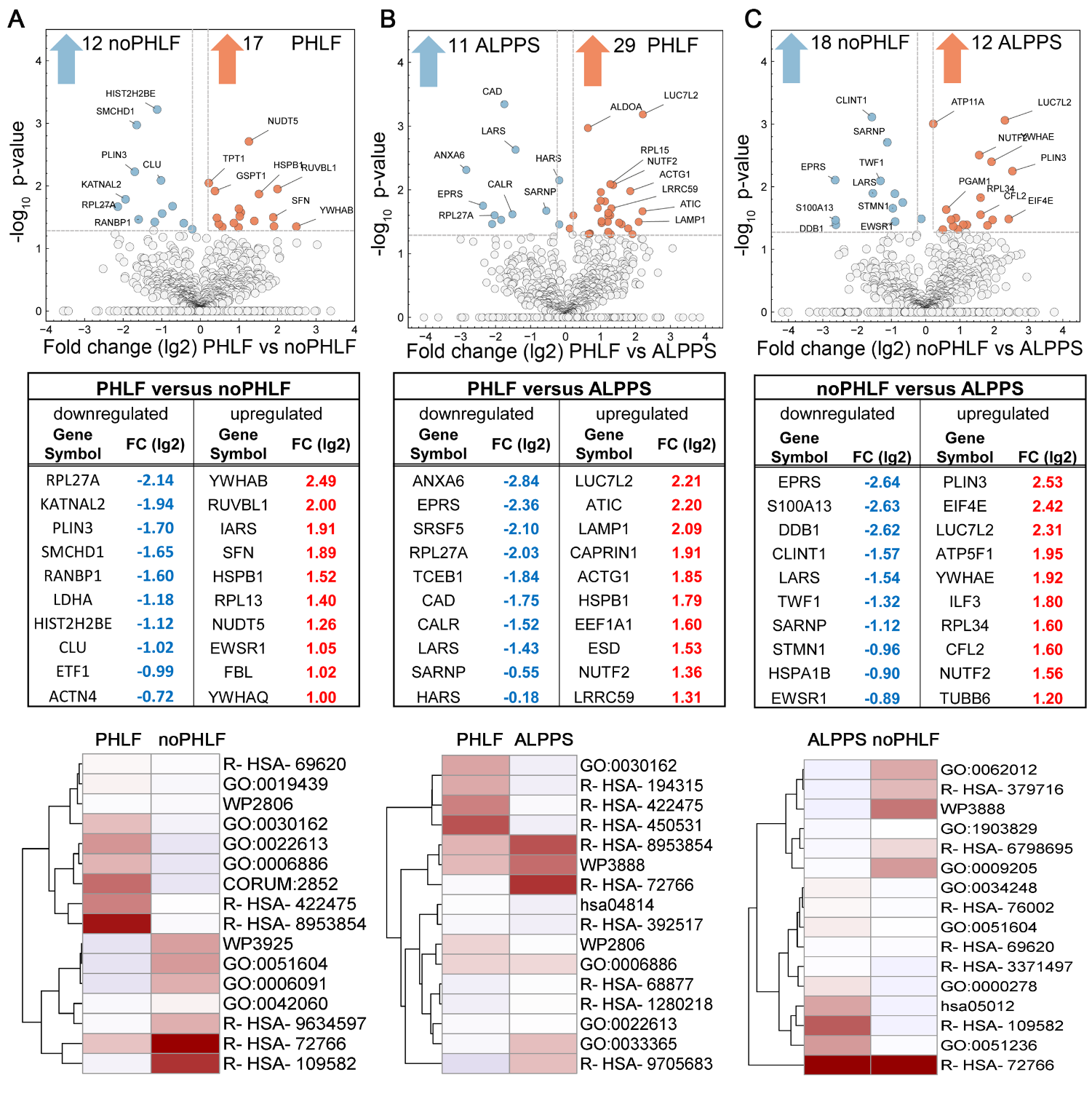
**Supplementary Figure 3: Enriched protein-protein-interaction (PPI) network modules in EVs: A)** Combined PPI network module enrichment analysis of proteins identified only in PHLF (225), noPHLF (88) or ALPPS (70) independent of sampling time after filtering LFQ-L identifications for at least 3 valid values as depicted in Venn diagram Fig. 2C. **B)** Enrichment-term-table with corresponding p-values (log10) on overall (\_FINAL\_MCODE\_ALL) and sub-network modules (\_FINAL\_SUB1\_MCODE 1-5). Analysis was performed in Metascore using the “multiple gene list”-option (Suppl. Data 2) with default settings and networks were color coded in Cytoscape.



**Supplementary Figure 4: Unique proteins in sampling time and outcome groups:** Intersections of “only” proteins identified a least twice at each timepoint (PRE, POD1, POD5) in respective outcome groups (PHLF, noPHLF or ALPPS). **B)** Top 5 proteins (ranked on the basis of LFQ-L abundance) for each time point in respective outcome groups. Complete lists are provided in Suppl. Data 2.



**Supplementary Figure 5: EV-protein cargo signatures discriminating PHLF from noPHLF and ALPPS: A-C**: Summary of pairwise comparison PHLF versus noPHLF-EVs. Top 20 up and downregulated EV-proteins (A), enriched GO-terms **(B)** and PPI Network modules **(C)** thereof. **D-F**: Summary of pairwise comparison PHLF versus ALPPS-EVs. Top 20 up and downregulated EV-proteins **(D)**, enriched GO-terms **(E)** and PPI Network modules **(F)**.

****

**Supplementary Figure 6: PREdictive outcome signatures:** Comparative statistical analysis of EV proteins before PHx (PRE) in different outcome groups. Volcano plots, top 10 regulated proteins and GO-pathway enrichments analysis (including significantly changed and “only” proteins) in PHLF versus noPHLF **(A)** or PHLF versus ALPPS **(B)** or noPHLF versus ALPPS **(C)**. GO-term-names of A and B are presented in Fig.4 and complete source data can be found in Suppl. Data 4.

1. **Description to supplementary data**

Supplementary Data 1:



Supplementary Data 2:



Supplementary Data 3:



Supplementary Data 4:

