

SUPPLEMENTAL MATERIAL

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

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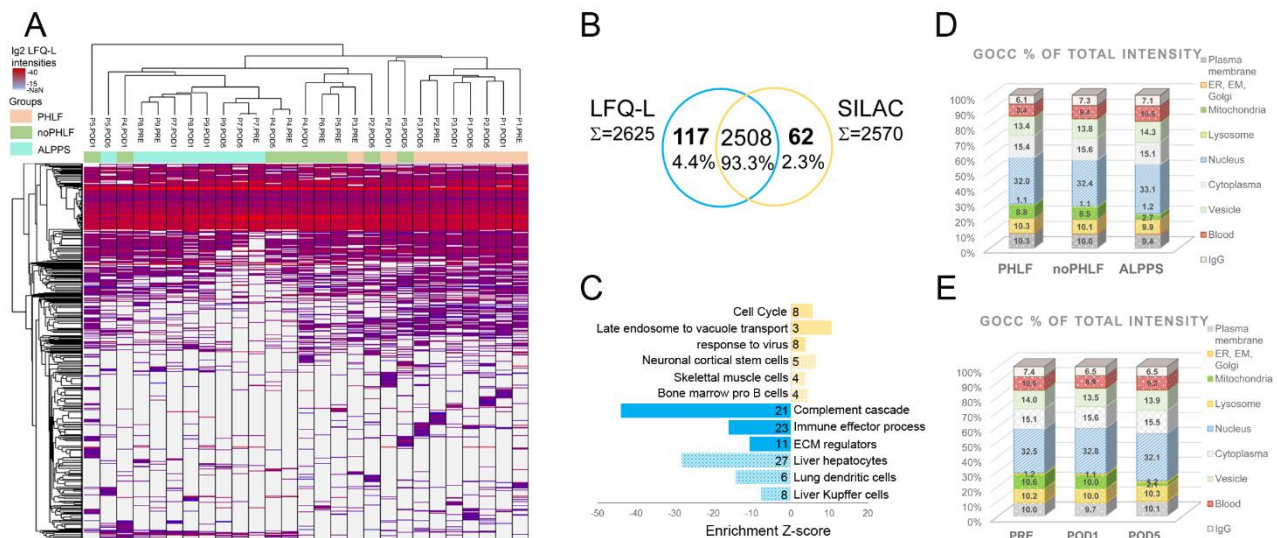
- 1) Supplemental Table**
- 2) Supplementary Figures**
- 3) Description to supplemental data**

1) Supplementary Table S1

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 grade (mean)	1	1	1	1	2 (22.2%)
	CASH	3 (33.3%)	2 (66.6%)	1 (33.3%)	0 (0.0)	1 (2.1%)
Etiology of underlying liver disease						
	No underlying liver disease	6	1	2	3	
	MetALD	3	2	1	0	
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 (×10 ³ /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%)	

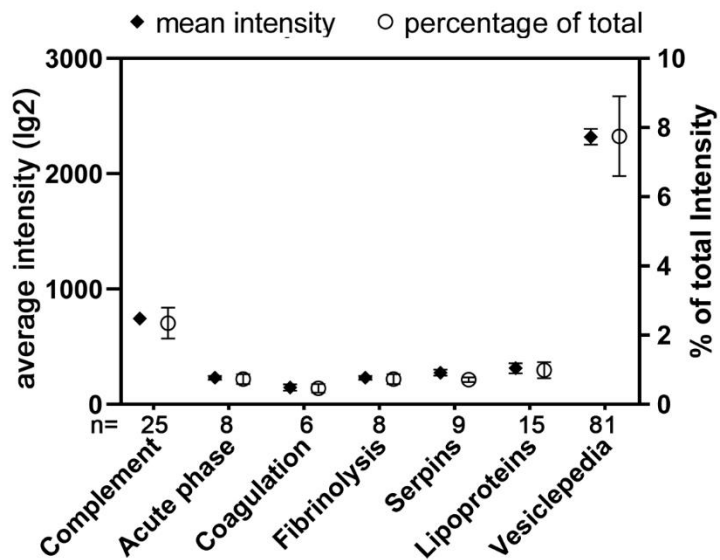
2) Supplementary Figures



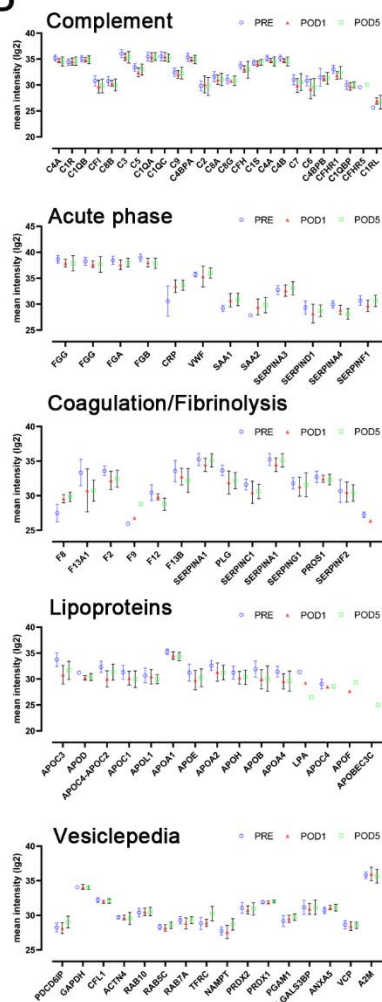
Supplementary Figure 1: Qualitative comparison of LFQ-L and SILAC identifications to capture plasma specific proteins: **A)** Heatmap of hierarchical clustered samples (average linked Euclidian distance on log2 transformed LFQ-L intensities, missing values in light grey) to visualize data completeness 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” (before surgery, 1 and 5 days after PHx). Corresponding source data can be found in Suppl. Data 1.

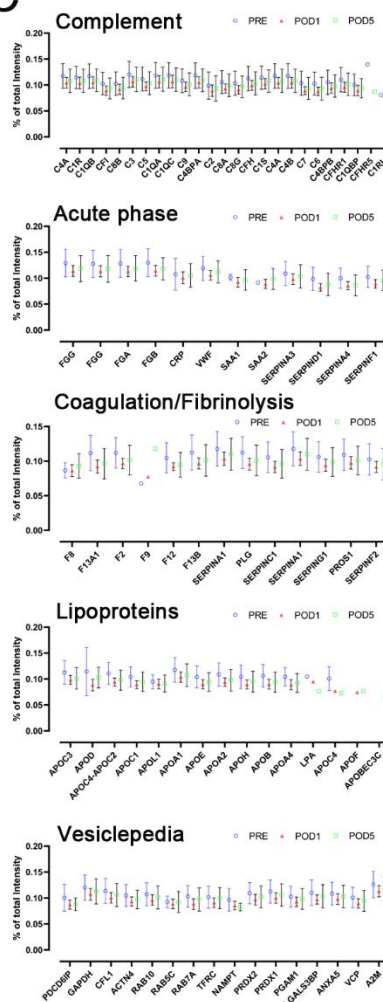
A



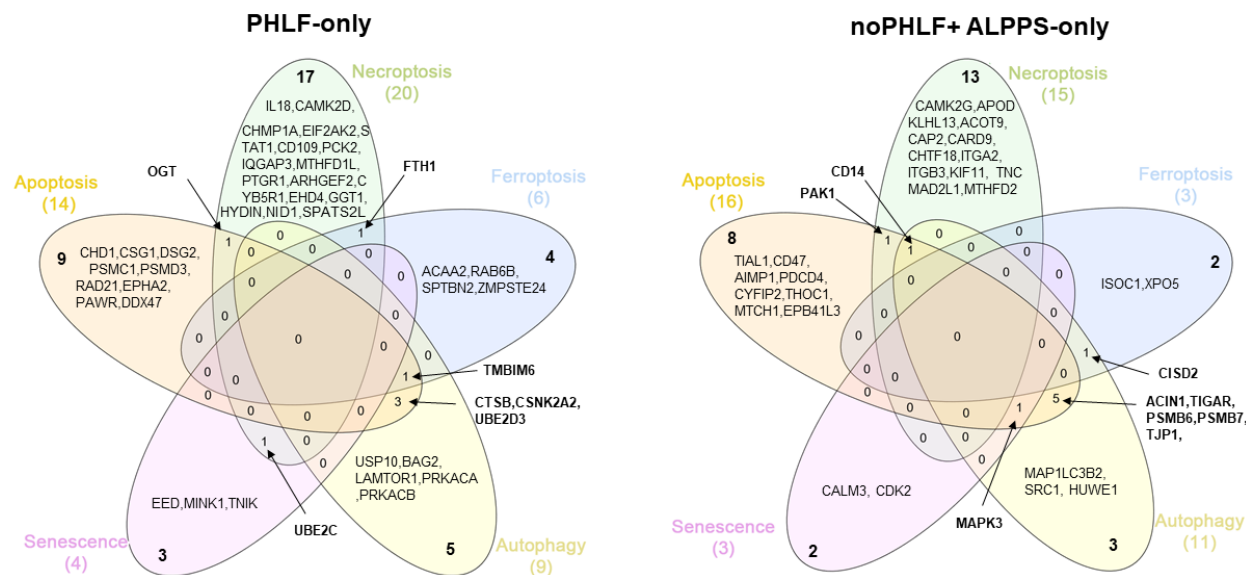
B



C

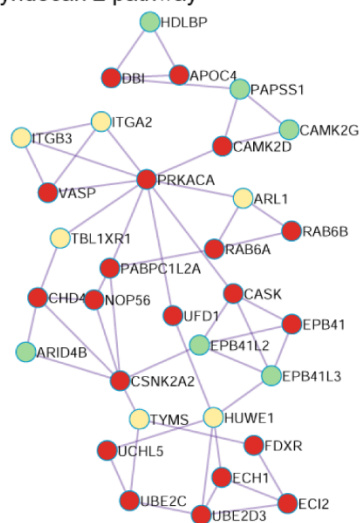


Supplementary Figure 2: Blood- and Vesiclepedia proteins identified and quantified in plasma EVs: Cumulative **(A)** and individual **(B, C)** abundance of blood (complement, acute phase, coagulation fibrinolysis, serpins (serine-protease inhibitor family members), lipoproteins and selected EV-proteins (Vesiclepedia) quantified in EV-samples at three different time points (before surgery, on postoperative day 1 and 5) 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.

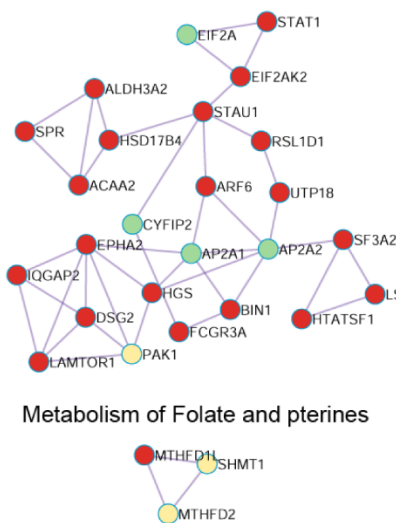


Supplementary Figure 3: EV-proteins with functions in stress response and homeostasis. Venn diagrams of proteins with functions in cell death (apoptosis, necroptosis and ferroptosis), auto(mito)phagy and senescence identified in 255 PHLF or 198 noPHLF + ALPPS only identified at least 3 times in outcome groups across all three time points (before surgery, on postoperative day 1 and 5).

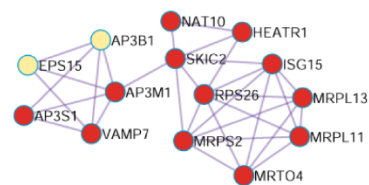
Splicing factor NOVA regulated proteins
Regulation of MECP2 expression and activity
Syndecan 2 pathway



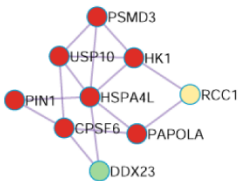
VEGFA/VEGFR2 signalling
RHO GTPase cycle



Clathrin-coated vesicle-cargo loading
AP3 adaptor complex



Metabolism of RNA



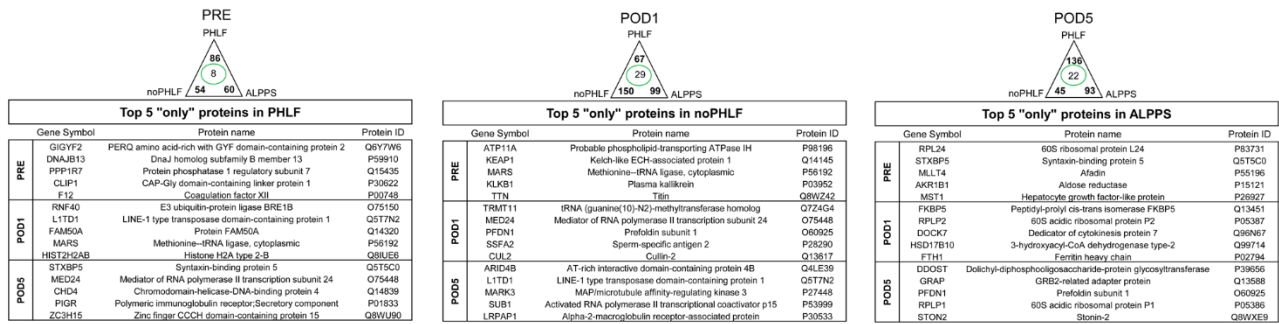
Metabolism of Folate and pterines



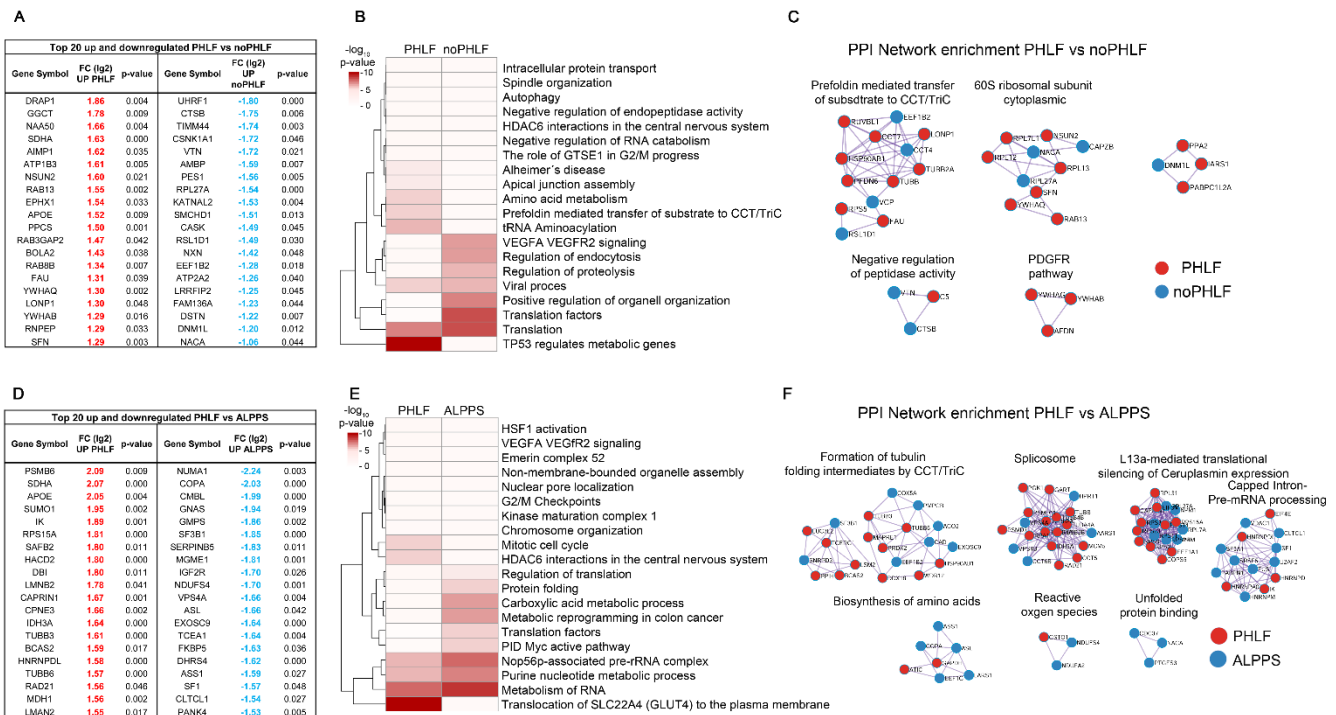
Network	Annotation
_FINAL	R-HSA-8953854 Metabolism of RNA -15.3;R-HSA-6798695 Neutrophil degranulation -12.2;GO:0055086 nucleobase-containing small molecule metabolic process -11.1
_FINAL_MCODE_ALL	WP3888 VEGFA VEGFR2 signaling -8.6;GO:0035999 tetrahydrofolate interconversion -8.2;GO:0022613 ribonucleoprotein complex biogenesis -8.0
_FINAL_SUB1_MCODE_1	WP4148 Splicing factor NOVA regulated synaptic proteins -9.1;R-HSA-9022692 Regulation of MECP2 expression and activity -7.4;M240 PID SYNDECAN 2 PATHWAY -7.4
_FINAL_SUB1_MCODE_2	WP3888 VEGFA VEGFR2 signaling -8.9;R-HSA-9012999 RHO GTPase cycle -8.7;R-HSA-194315 Signaling by Rho GTPases -7.2
_FINAL_SUB1_MCODE_3	GO:0035654 clathrin-coated vesicle cargo loading, AP-3-mediated -8.6;GO:0035652 clathrin-coated vesicle cargo loading -8.6;CORUM:59 AP3 adaptor complex -8.6
_FINAL_SUB1_MCODE_4	R-HSA-8953854 Metabolism of RNA -4.4;R-HSA-72203 Processing of Capped Intron-Containing Pre-mRNA -4.2;GO:0006397 mRNA processing -3.5
_FINAL_SUB1_MCODE_5	GO:0035999 tetrahydrofolate interconversion -10.7;R-HSA-196757 Metabolism of folate and pterines -9.8;GO:0046653 tetrahydrofolate metabolic process -9.7

created by
<http://metascape.org>

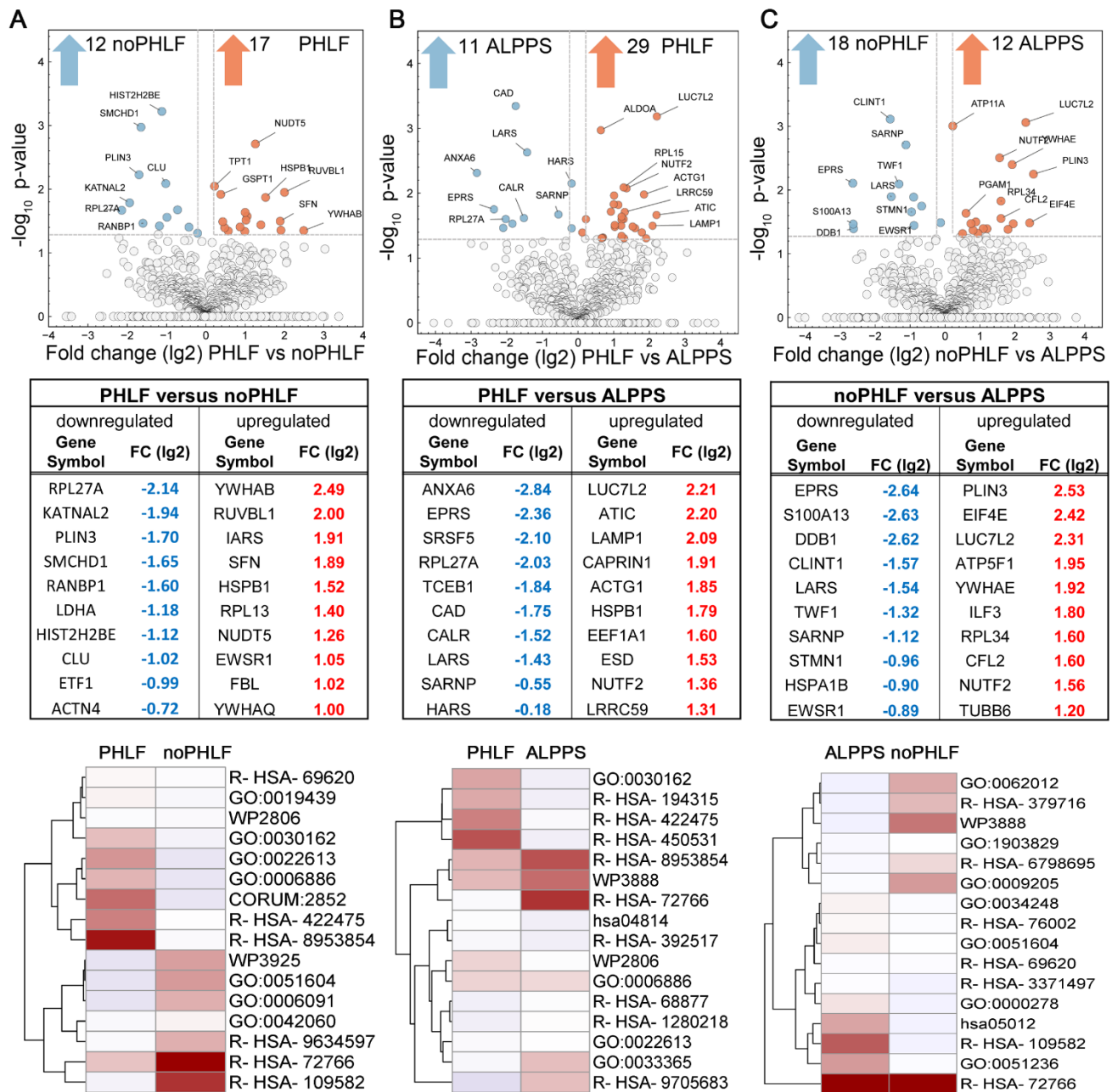
Supplementary Figure 4: Enriched protein-protein-interaction (PPI) network modules in EVs: **A)** Combined PPI network module enrichment analysis of proteins identified only in PHLF (225), noPHLF (88) or ALPPS (70) independent of sampling time after filtering LFQ-L identifications for at least 3 valid 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.



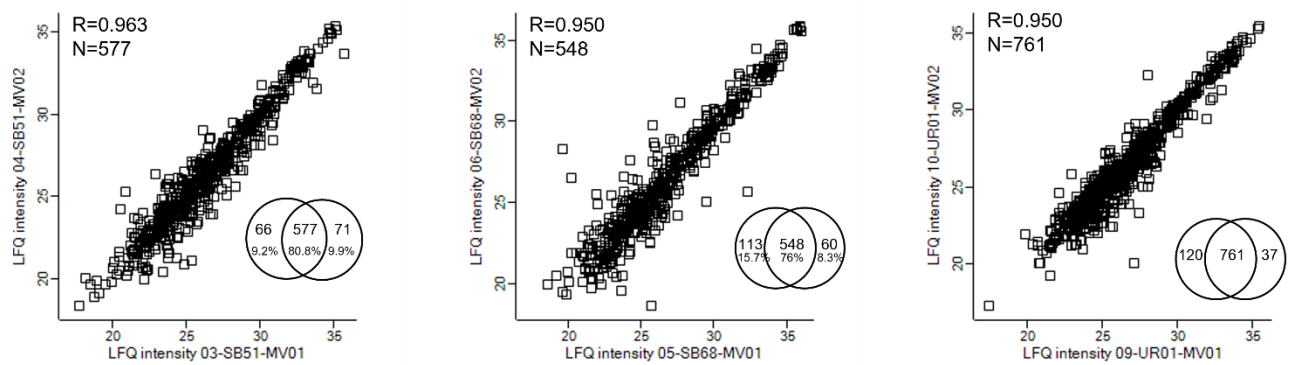
Supplementary Figure 5: Unique proteins in sampling time and outcome groups: Intersections of "only" proteins identified a least twice at each timepoint (prior to surgery, on postoperative day 1 and 5) in respective outcome groups (PHLF, noPHLF or ALPPS). Top 5 proteins (ranked on the basis of LFQ-L abundance) for each time point in respective outcome groups. Complete lists are provided in Suppl. Data 2.



Supplementary Figure 6: 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 7: PREdictive outcome signatures: prior to surgery: 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.



Supplementary Figure 8: Reproducibility of ultracentrifugation-based EV isolation: Plasma from 3 subjects (á 150µl in replica) were subjected to ultracentrifugation- based EV isolation and LC-MS/MS analysis as described in methods. Data were filtered for reverse, contaminants, LFQ intensity values lg2-transformed and replica plotted against each other. Venn diagram show identification overlaps and Pearson Correlation coefficient (R) on common proteins (N) inscribed in scatter plots.

