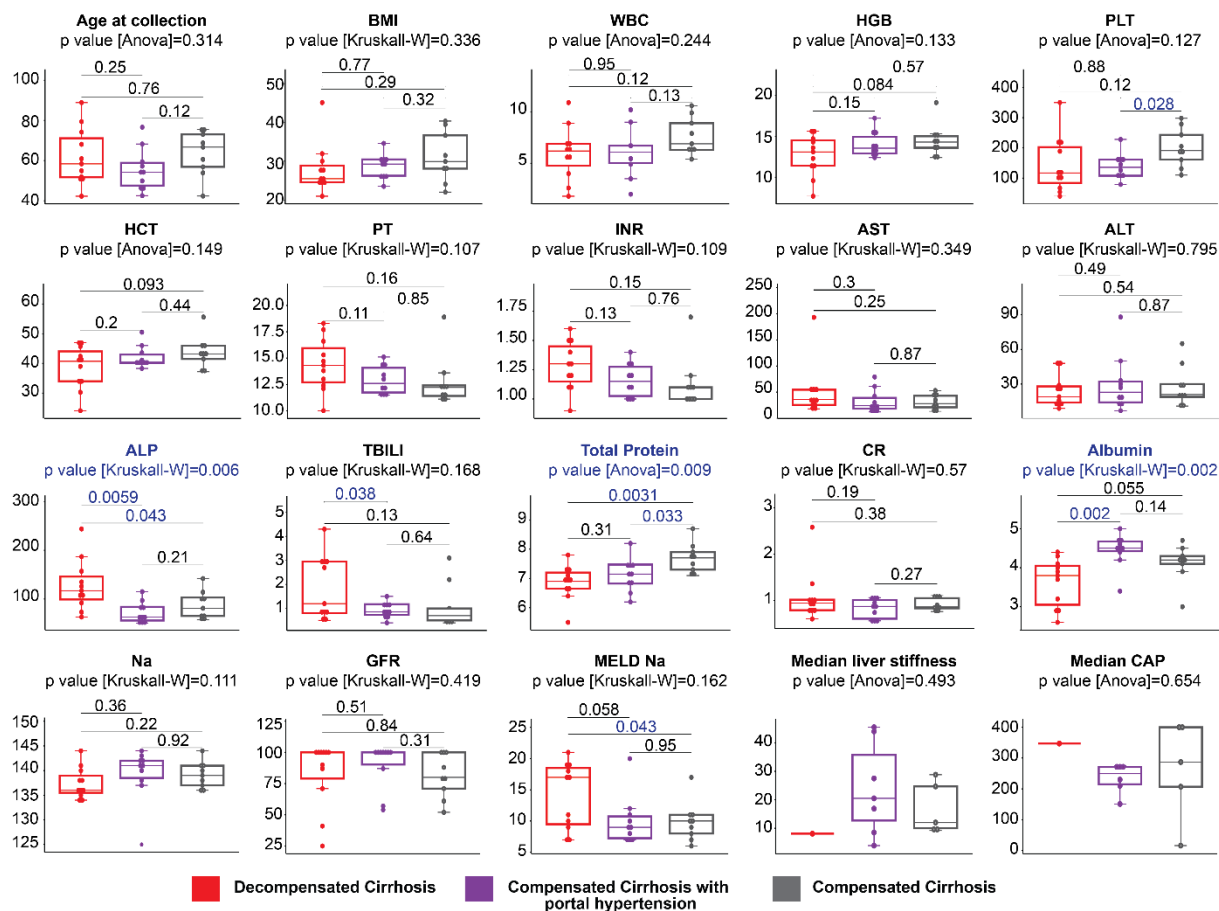
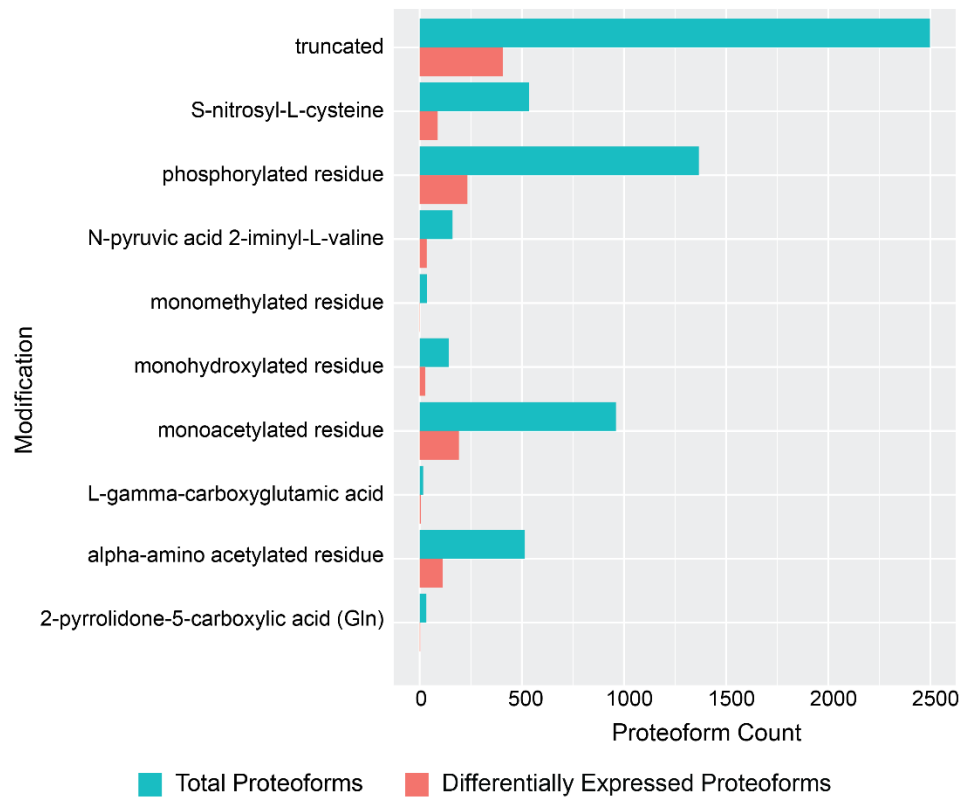


## Supplementary Figures



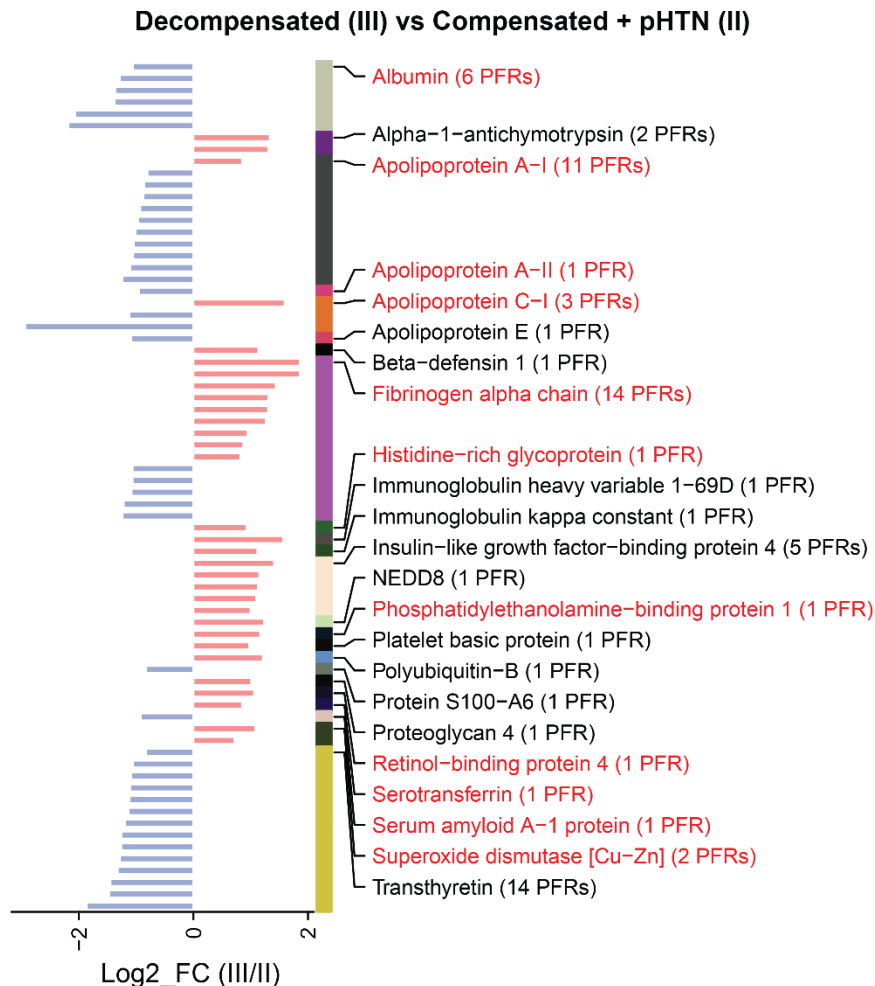
**Figure S1. Clinical parameters of patients enrolled in this study.** Clinical parameters shown in blue are statistically significant different among groups. Significance ( $p$  value  $< 0.05$ ) was calculated by Anova or Kruskal-Wallis (Kruskall-W) tests, depending on the data distribution determined by Shapiro test. Abbreviations: BMI (body mass index), WBC (white blood cell count), HGB (hemoglobin), PLT (platelets), HCT (hematocrit), PT (prothrombin time), INR (international normalized ratio), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), TBILI (total bilirubin), CR (serum creatinine), Na (Sodium), GFR (glomerular filtration rate), MELD-Na (Model for End Stage Liver Disease Sodium), CAP (controlled attenuation parameter).

## Proteoform identification in liver cirrhosis progression



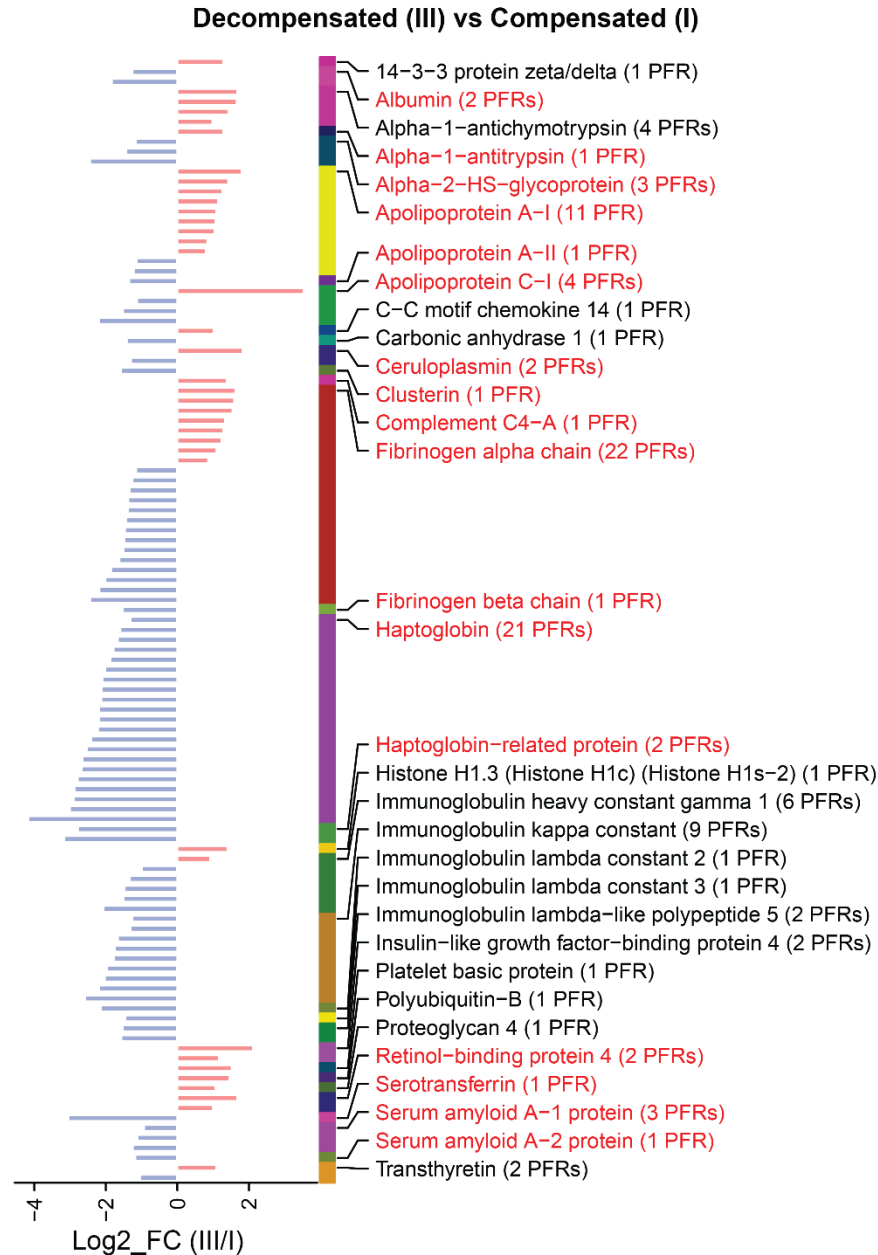
**Figure S2. T Common proteoform modifications detected in the TDP analysis.** The number of truncations and top-10 most common post-translational modifications identified in the total and differentially expressed proteoforms (DEPs) captured in the discovery LC-MS/MS TDP analysis.

## Proteoform identification in liver cirrhosis progression



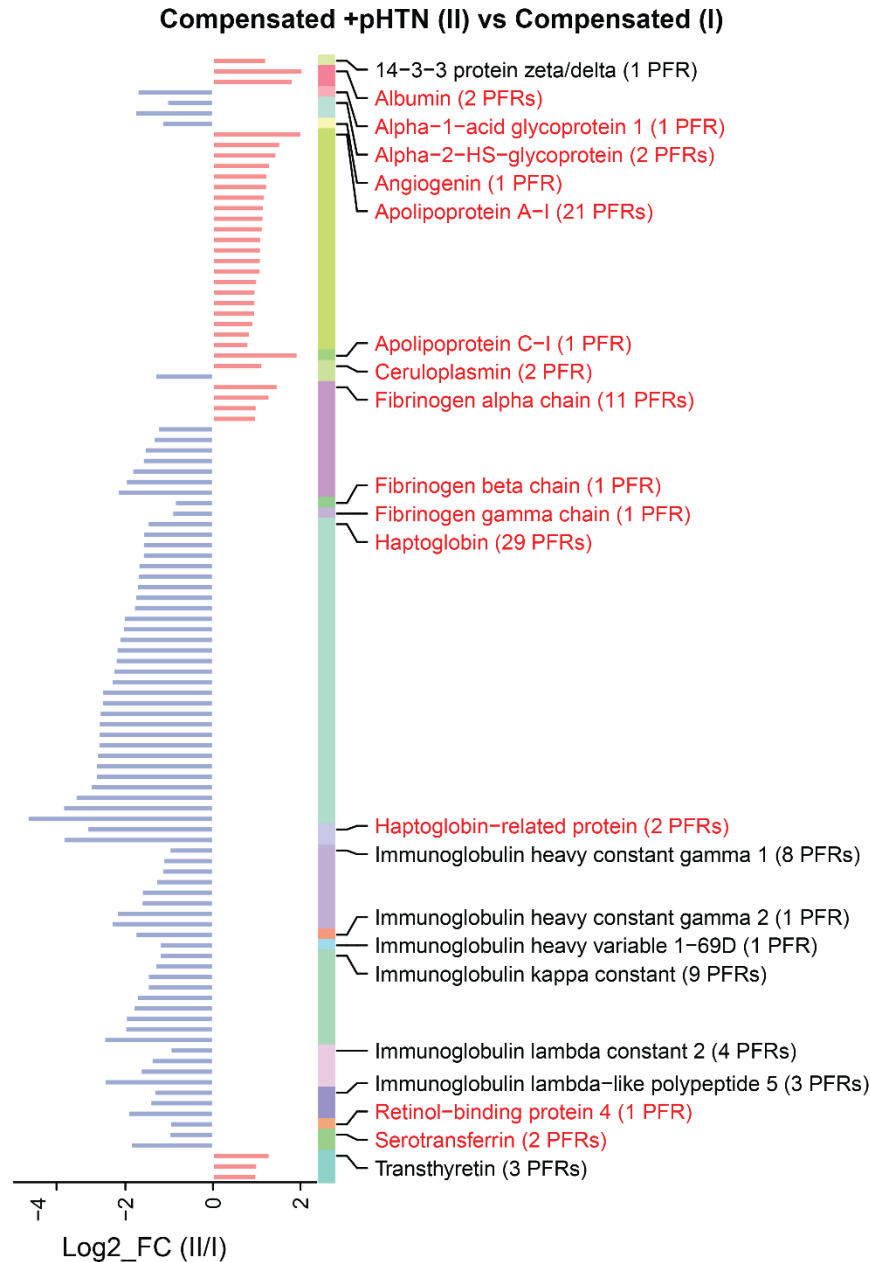
**Figure S3. Differentially expressed proteoforms (DEPs) from decompensated (III) vs compensated with portal hypertension (+ pHTN) (II) patients.** DEPs are grouped by proteins, and then ordered by fold change. The protein and number of DEPs derived from that protein are shown to figure right. For example, fibrinogen alpha chain had 14 DEPs identified. Proteins highlighted in red are enriched in liver at a transcriptional level. Abbreviations: PFR (proteoform).

## Proteoform identification in liver cirrhosis progression



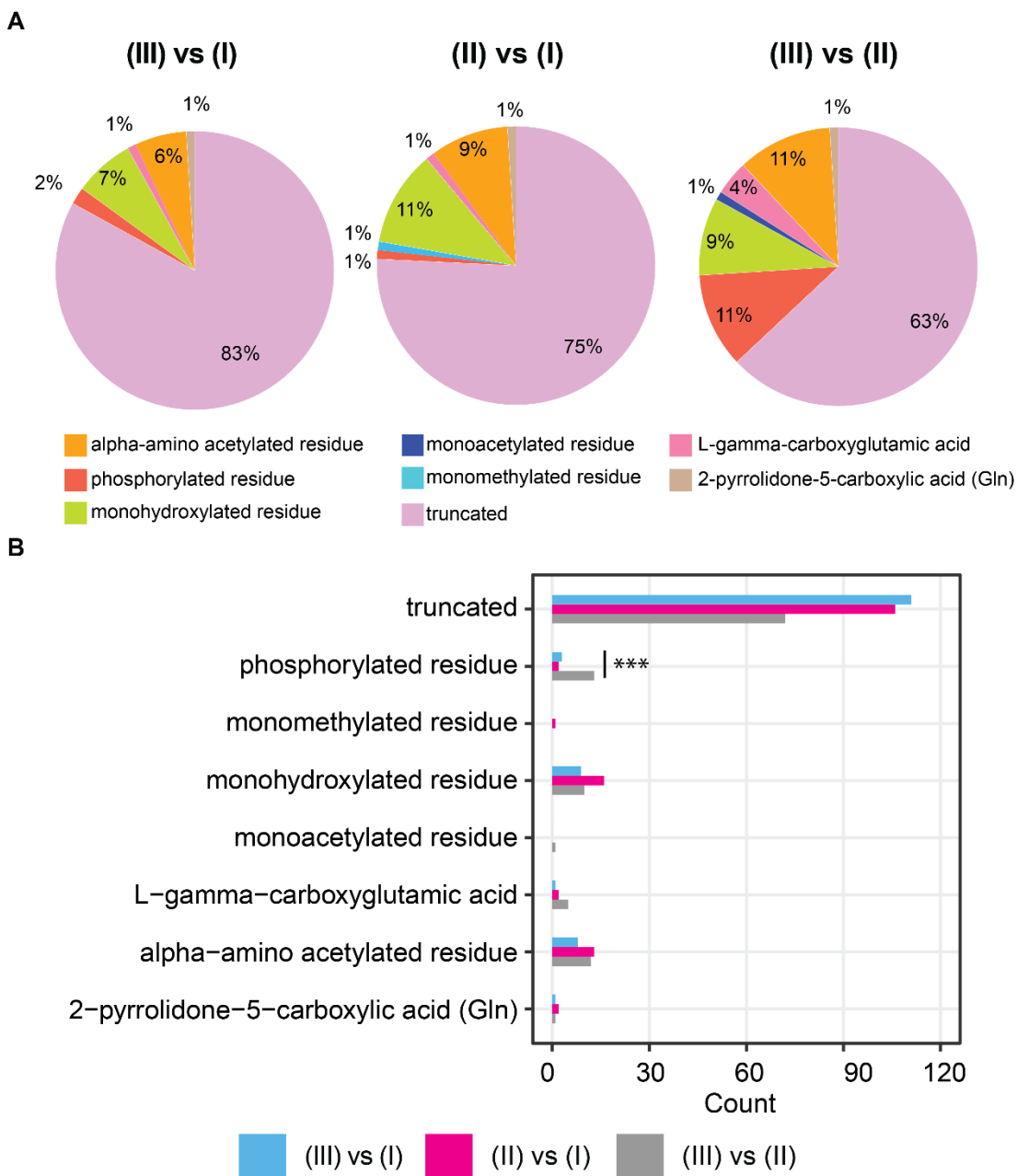
**Figure S4.** . Differentially expressed proteoforms (DEPs) from decompensated (III) vs compensated (I) patients. DEPs are grouped by proteins, and then ordered by fold change. The protein and number of DEPs derived from that protein are shown to figure right. For example, haptoglobin related protein had 2 DEPs identified. Proteins highlighted in red are enriched in liver at a transcriptional level. Abbreviations: PFR (proteoform).

## Proteoform identification in liver cirrhosis progression



**Figure S5. Differentially expressed proteoforms (DEPs) from compensated with portal hypertension (+ pHTN) (II) vs compensated (I) patients.** DEPs are grouped by proteins, and then ordered by fold change. The protein and number of DEPs derived from that protein are shown to figure right. For example, haptoglobin related protein had 2 DEPs identified. Proteins highlighted in red are enriched in liver at a transcriptional level. Abbreviations: PFR (proteoform).

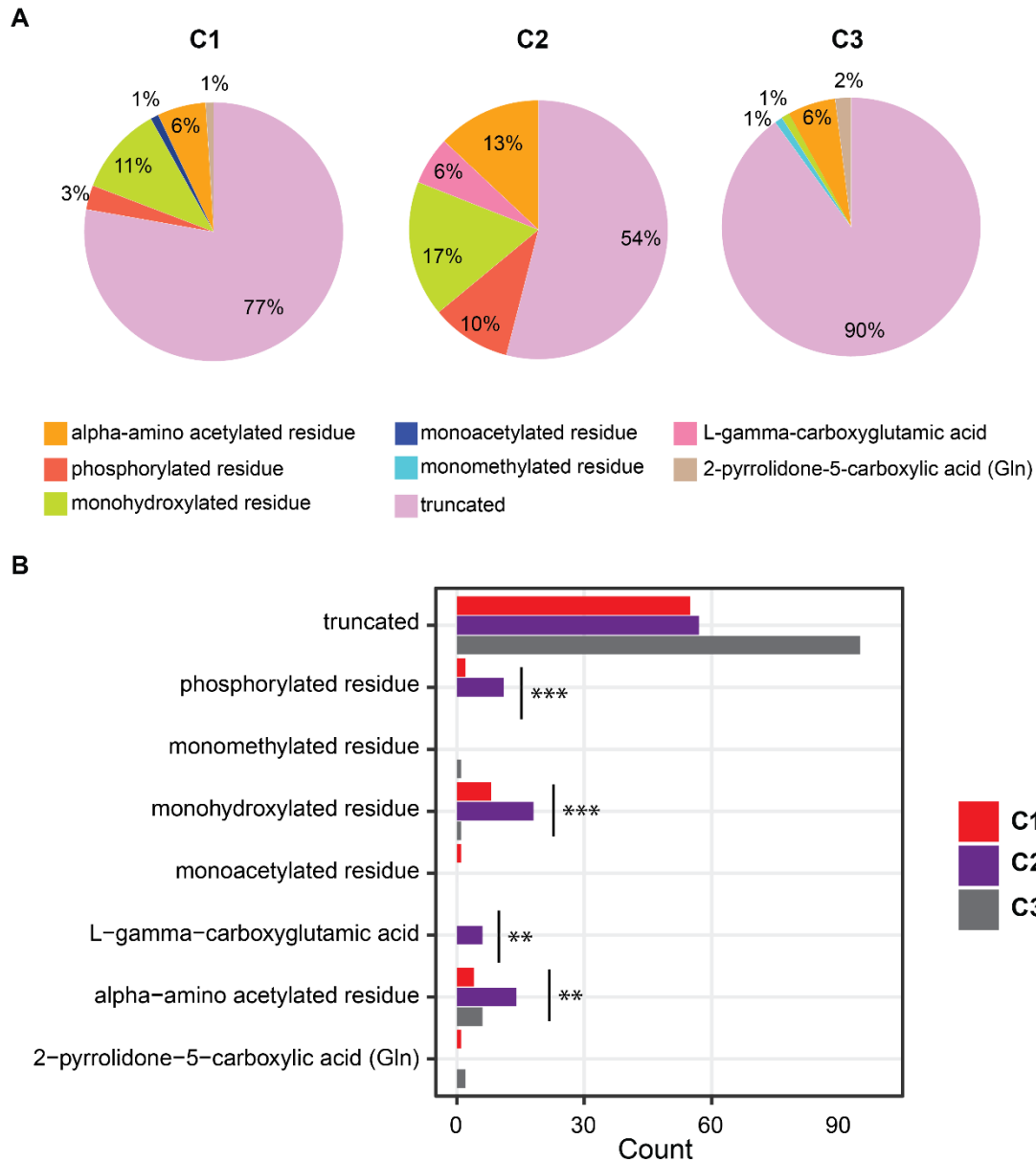
## Proteoform identification in liver cirrhosis progression



**Figure S6. Proteoform modifications in pairwise comparisons of cirrhosis stages. A)** Percentages and **B)** absolute number of differentially expressed proteoform (DEP) modifications identified in pairwise comparisons of Stage III vs I, II vs I, and III vs II. For example, 11% of

## Proteoform identification in liver cirrhosis progression

proteoforms differentially expressed between Stages III and II were phosphorylated residues (red portion of pie chart). Statistical significance was calculated with the Fisher exact test (adj.  $p$ -values: \*\*\*<0.001, \*\*<0.01, \*<0.05).



**Figure S7. Proteoform modifications compared across clusters. A) Percentages and B) Absolute number of differentially expressed proteoform (DEP) modifications identified in clusters 1 (C1), 2 (C2), and C3. For example, 90% of modifications to DEPs that clustered into C3 were**

## Proteoform identification in liver cirrhosis progression

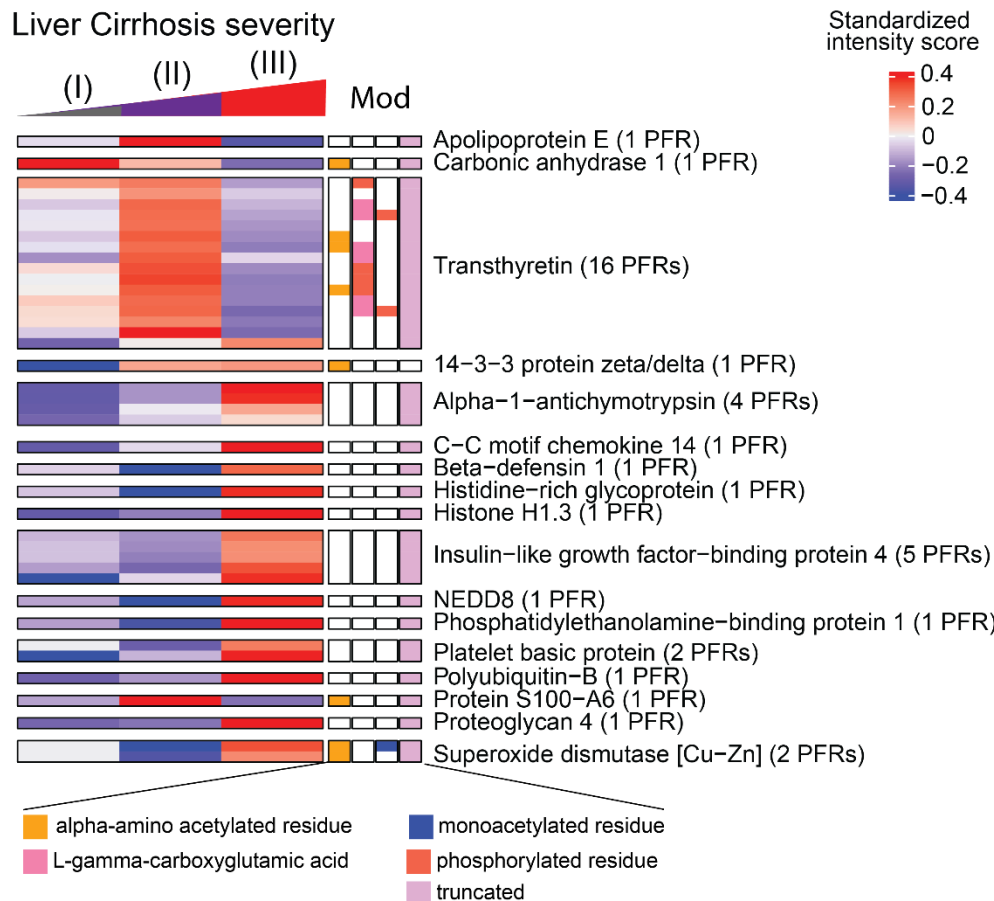
identified as truncations (light purple portion of the pie chart). Statistical significance was calculated with the Fisher exact test (adj. *p*-values: \*\*\*<0.001, \*\*<0.01, \*<0.05).

	Compensated cirrhosis (I)	Compensated cirrhosis + pHTN (II)	Decompensated cirrhosis (III)	
	3	2	1	<b>Cluster</b>
<b>Fibrinogen alpha chain (36 DEPs)</b>	<b>14</b>	<b>7</b>	<b>15</b>	<b>DEPs</b>
Truncations	14	7	15	<b>Mods</b>
PRF lenght	58	58.86	126	
Phosphorylation	-	1	3	
Mono hydroxylation	1	1	3	
<b>Apolipoprotein A-I (32 DEPs)</b>	-	<b>22</b>	<b>10</b>	<b>DEPs</b>
Truncations		22	9	<b>Mods</b>
α-amino acetylation		8	-	
Mono hydroxylation		25	6	
Mutations P27-->H		2	-	
<b>Haptoglobin (29 DEPs)</b>	<b>29</b>	-	-	<b>DEPs</b>
Isoforms	2			<b>Mods</b>
Truncations	29			
α-amino acetylation	4			
Mutations E71-->K N70-->D	10 2 8			

**Figure S8. Most common, liver-enriched differentially expressed proteoforms (DEPs) identified in TDP analysis.** DEPs relative to Fibrinogen alpha chain, Apolipoprotein A-I, and Haptoglobin are reported together with their modifications in the 3 clusters established in **Figure 2**. Proteins are shown with the number of DEPs in parentheses. Abbreviations: DEP (Differentially expressed proteoform), Mod (Modification).



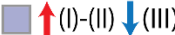
## Proteoform identification in liver cirrhosis progression



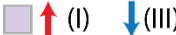
**Figure S9. Heatmaps of quantified proteoforms of proteins not enriched in the liver at transcriptional level.** Associated proteoform modifications are shown and defined by the figure legend. The proteins and number of identified proteoforms derived from each protein are shown to figure right. Abbreviations: PFR (proteoform), Mod (modification).

## Proteoform identification in liver cirrhosis progression

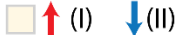
PFR	Protein	Uniprot ID	Sequence	Mutation	Phosphorylation	Mono hydroxylation	Half Cysteine	Methylation	Alpha amino acetylation
16559	Apolipoprotein C-I	P02654	29-83						
7510185	Albumin	P02768-3	25-277		S-82, 89		C-77, 86		
7033240	Apolipoprotein A-I	P02647	67-267			M-110,136			
7052923	Apolipoprotein A-I	P02647	35-267			M-110,136			
47927	Apolipoprotein A-II	P02652	19-100						
38719	Apolipoprotein C-I	P02654	27-83						
18702	Fibrinogen alpha chain	P02671	542-601						yes
2383017	Fibrinogen alpha chain	P02671	538-604						
6720044	Fibrinogen alpha chain	P02671	561-604						
6720045	Fibrinogen alpha chain	P02671	549-604						
6720672	Fibrinogen alpha chain	P02671	539-604						
7654834	Fibrinogen alpha chain	P02671	557-603						
74009	Serum amyloid A-1 protein	P0DJ18	20-122						
5435552	Serum amyloid A-1 protein	P0DJ18	21-122	G90-->D					yes
215903	Serum amyloid A-2 protein	P0DJ19	20-122	R89-->H					
6700458	Fibrinogen gamma chain	P02679	281-436	M410-->V					
6542874	Retinol-binding protein 4	P02753	19-194	A73-->T				R-139	
215854	Serotransferrin		636-698						



↑(I)-(II) ↓(III)



↑(I) ↓(III)



↑(I) ↓(II)

**Figure S10. Individual proteoforms upregulated in early-stage cirrhosis.** Differentially expressed proteoforms (DEPs) significantly upregulated in stages (I) and (II) and downregulated in stage (III) (dark purple), upregulated in stage (I) and downregulated in stage (III) (light purple), upregulated in stage (I) and downregulated in stage (II) (yellow). Each proteoform is shown with its unique proteoform number, protein name, Uniprot ID, amino acid sequence relative to the Uniprot ID, any relevant mutations, and presence or absence of post-translational modifications. Colors are based on the proteoform signatures created in **Figure 4**. Abbreviations: PFR (proteoform).

\*Note that half Cysteine could be artifact during the identification process.

## Proteoform identification in liver cirrhosis progression

PFR	Protein	Uniprot ID	Sequence	Mutation	Mono hydroxylation	Half Cysteine	Alpha amino acetylation	2-pyrrolidone-5-carboxylic acid
7233558	Albumin	P02768-3	25-325			C-77, 86, 325		
7493196	alpha-1acid glycoprotein 1	P02763	165-201					
18967	alpha-2HS glycoprotein	P02765	341 - 367					
56322	alpha-2HS glycoprotein	P02765	275 - 339					
74004	alpha-2HS glycoprotein	P02765	342 - 367					
5931629	Angiogenin	P03950	25-147	Q36-->L		C50,81,105,131		Q25
99695	Apolipoprotein C-I	P02654	29-83				yes	
7411348	Ceruloplasmin	P00450	20-58					
53707	Clusterin	P10909	414-449					
345982	Fibrinogen alpha chain	P02671	542-604		P-565			
6618516	Fibrinogen alpha chain	P02671	551-603					
6700442	Fibrinogen alpha chain	P02671	537-604					
6707159	Fibrinogen alpha chain	P02671	438-519			C-491		
6732079	Fibrinogen alpha chain	P02671	561-603					
6755154	Fibrinogen alpha chain	P02671	549-603					
6788241	Fibrinogen alpha chain	P02671	538-605					
7164381	Fibrinogen alpha chain	P02671	537-599					
7584008	Fibrinogen alpha chain	P02671	549-605					
7587565	Fibrinogen alpha chain	P02671	451-496					
73949	Haptoglobin	P00738-2	19-102					
73950	Haptoglobin	P00738-2	19-101					
73951	Haptoglobin	P00738-2	19-100					
73952	Haptoglobin	P00738-2	22-101					
73953	Haptoglobin	P00738-2	19-102				yes	
379773	Haptoglobin	P00738-1	104-160					
597885	Haptoglobin	P00738-2	18-101					
4927920	Haptoglobin	P00738-2	19-101			C-52, 86		
5055588	Haptoglobin	P00738-2	19-102	E71-->K			yes	
5055594	Haptoglobin	P00738-2	19-101				yes	
5269396	Haptoglobin	P00738-2	19-102	N70-->D		C-52		
5269397	Haptoglobin	P00738-2	19-101	N70-->D		C-90		
5269398	Haptoglobin	P00738-2	19-101	N70-->D		C-86		
5710193	Haptoglobin	P00738-2	19-101	N70-->D		C-52		
5865702	Haptoglobin	P00738-2	19-102	E71-->K		C-52,86	yes	
6372014	Haptoglobin	P00738-2	19-102	N70-->D		C-90		
6476920	Haptoglobin	P00738-1	78-160					
6720057	Haptoglobin	P00738-1	106-160					
6721265	Haptoglobin	P00738-1	78-161			C-86		
6889832	Haptoglobin	P00738-1	78-160			C-86		
7111729	Haptoglobin	P00738-2	22-101	N70-->D		C-90		
7488813	Haptoglobin	P00738-1	106-161					
7488879	Haptoglobin	P00738-2	17-101					
7591703	Haptoglobin	P00738-1	82-160			C-86,111		
7839836	Haptoglobin	P00738-2	22-101	N70-->D		C-52		
7839837	Haptoglobin	P00738-1	75-160			C-86		
7841882	Haptoglobin	P00738-2	19-100	N70-->D		C-52		
7841883	Haptoglobin	P00738-1	19-101			C-86		
7863012	Haptoglobin	P00738-1	97-160			C-111		

□ ↑ (I) ↓ (II)-(III)

**Figure S11. Individual proteoforms upregulated in Stage I disease.** Differentially expressed proteoforms (DEPs) significantly upregulated in stage (I) and downregulated in stages (II) and (III) (blue). Each proteoform is shown with its unique proteoform number, protein name, Uniprot ID, amino acid sequence relative to the Uniprot ID, any relevant mutations, and presence or absence of post-translational modifications. Colors are based on the proteoform signatures created in **Figure 4**. Abbreviations: PFR (proteoform).

\*Note that half Cysteine could be artifact during the identification process.

## Proteoform identification in liver cirrhosis progression

PFR	Protein	Uniprot ID	Sequence	Mutation	Phosphorylation	Mono hydroxylation	Half Cysteine	Alpha amino acetylation
7047846	Albumin	P02768-1	25-157		S-82, 89		C-77	
7841902	Albumin	P02768-1	25-288		S-82			
56323	Apolipoprotein A-I	P02647	19-267					yes
216392	Apolipoprotein A-I	P02647	25-267			M-110		
5052079	Apolipoprotein A-I	P02647	25-267			M-110		yes
5052090	Apolipoprotein A-I	P02647	25-266	P27-->H				yes
5508504	Apolipoprotein A-I	P02647	19-267			M-110,136		yes
6372372	Apolipoprotein A-I	P02647	23-267					
6468740	Apolipoprotein A-I	P02647	9-267			M-110		↓(I) ↑(II)
6472353	Apolipoprotein A-I	P02647	18-267			M-110,136		
6478105	Apolipoprotein A-I	P02647	12-267			M-110		
6481626	Apolipoprotein A-I	P02647	25-266			M-110		yes
6481628	Apolipoprotein A-I	P02647	25-266			M-110,136		yes
6799836	Apolipoprotein A-I	P02647	9-267			M-110,136		
6981680	Apolipoprotein A-I	P02647	25-266			M-136		yes
345984	Fibrinogen alpha chain	P02671	542-603			P-565		
5052085	Apolipoprotein A-I	P02647	25-267			M-136		
6472160	Apolipoprotein A-I	P02647	23-267			M-110,136		
6472193	Apolipoprotein A-I	P02647	5-267			M-110		
6563625	Apolipoprotein A-I	P02647	5-267					
6571467	Apolipoprotein A-I	P02647	30-267					
6686623	Apolipoprotein A-I	P02647	27-267					
6788235	Apolipoprotein A-I	P02647	6-267					
7013512	Apolipoprotein A-I	P02647	14-267					
7072437	Apolipoprotein A-I	P02647	29-267			M-110		
7497747	Apolipoprotein A-I	P02647	27-265			M-110		
4664283	Apolipoprotein C-I	P02654	13-83					↓(I) ↑(II) ↑(III)
597908	Ceruloplasmin	P00450	910-1065					
74946	Complement C4-A	P0C0L4	680-755					
53596	Fibrinogen alpha chain	P02671	576-629					
74881	Fibrinogen alpha chain	P02671	600-629					
367042	Fibrinogen alpha chain	P02671	548-629			P-565		
6700460	Fibrinogen alpha chain	P02671	459-629					
7811927	Fibrinogen alpha chain	P02671	592-628					
6678845	Retinol-binding protein 4	P02753	19-201				C-192	
6686230	Retinol-binding protein 4	P02753	19-201				C-88	
6372540	Alpha-1-antitrypsin	P001009	339-418					
6372550	Apolipoprotein A-I	P02647	21-267					
6481673	Apolipoprotein A-I	P02647	1-267			M-110		
597831	Fibrinogen alpha chain	P02671	548-629					↓(I) ↑(III)
6707163	Fibrinogen alpha chain	P02671	444-629					
7179072	Fibrinogen alpha chain	P02671	515-629					
7501917	Fibrinogen alpha chain	P02671	466-629		P-565	S-501		

**Figure S12. Individual proteoforms upregulated in late-stage cirrhosis.** Differentially expressed proteoforms (DEPs) significantly downregulated in stage (I) and upregulated in stage (II) (green), downregulated in stage (I) and upregulated in stages (II) and (III) (red), downregulated in stage (I) and upregulated in stage (III) (orange). Each proteoform is shown with its unique proteoform number, protein name and Uniprot ID, amino acid sequence relative to the Uniprot ID, and presence or absence of post-translational modifications. Colors are based on the proteoform signatures created in **Figure 4**. Abbreviations: PFR (proteoform).

\*Note that half Cysteine could be artifact