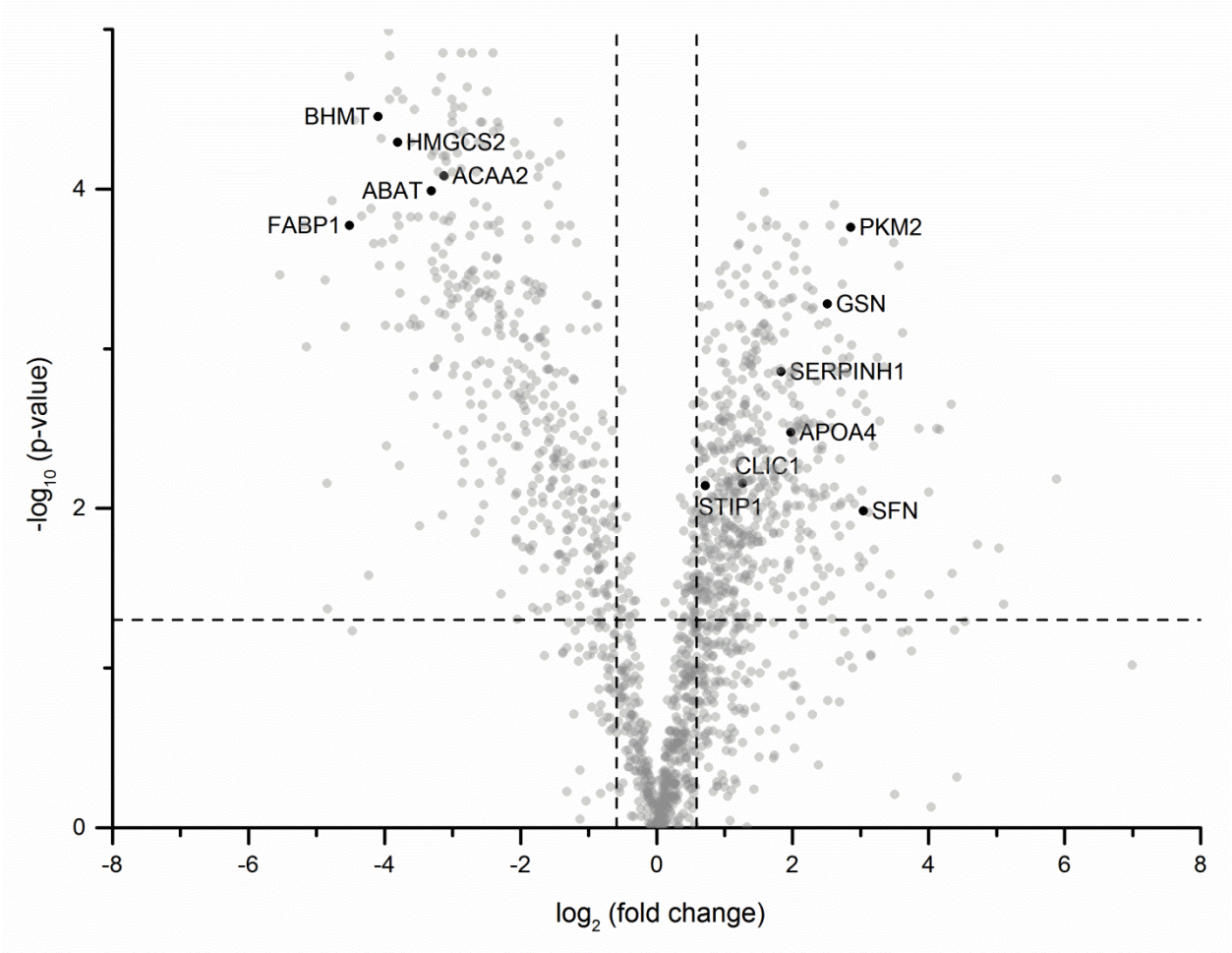
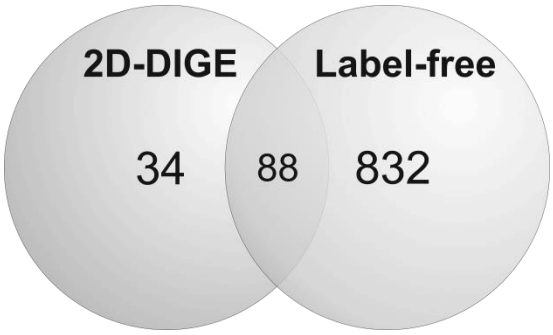
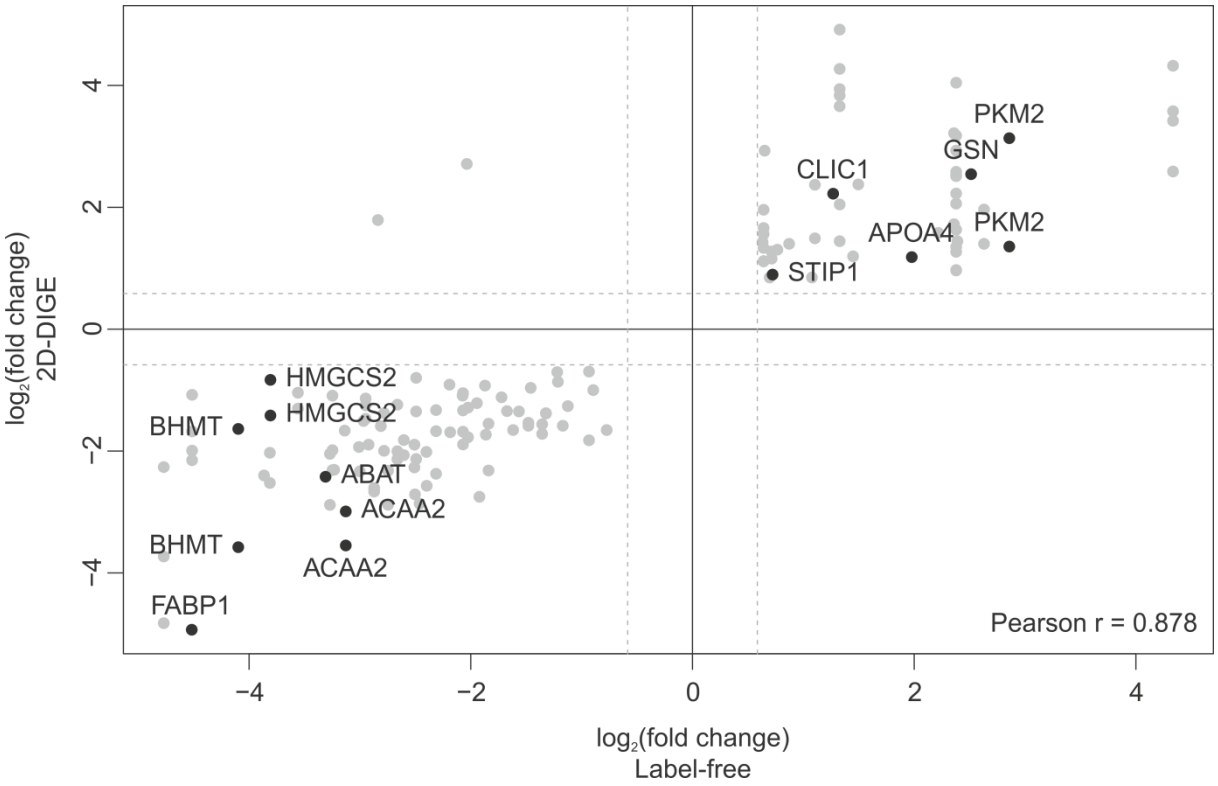
# Supplemental Data



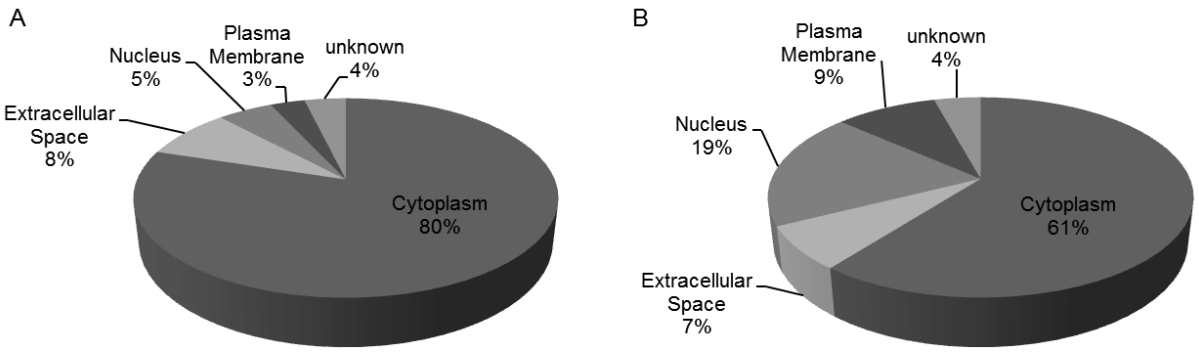

**Supplemental figure 1:** Volcano plot of all proteins identified in the label-free approach. Dashed lines indicate chosen cut-off values for the fold change (≥ 1.5) and the FDR adjusted p-value (≤ 0.05, for the method used for FDR adjustment see Benjamini and Hochberg, 1995). Proteins which were chosen for verification by immunohistochemistry are marked.



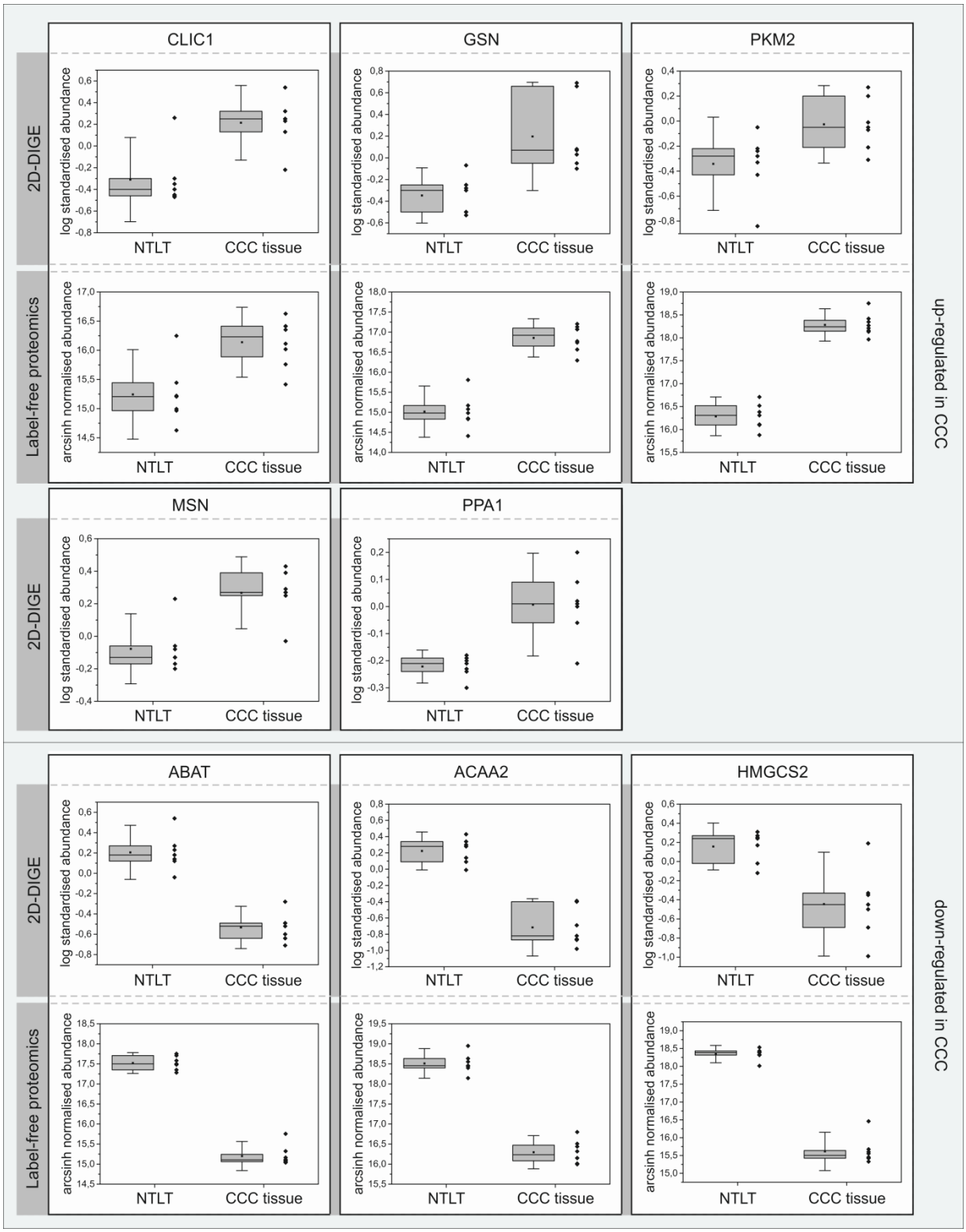

**Supplemental figure 2:** Venn diagram showing the numbers of differential proteins identified exclusively by 2D-DIGE, by label-free proteomics or by both methods. Filter criteria were set to fold change ≥ 1.5 and p-value ≤ 0.05 (FDR adjusted, Benjamini and Hochberg, 1995).



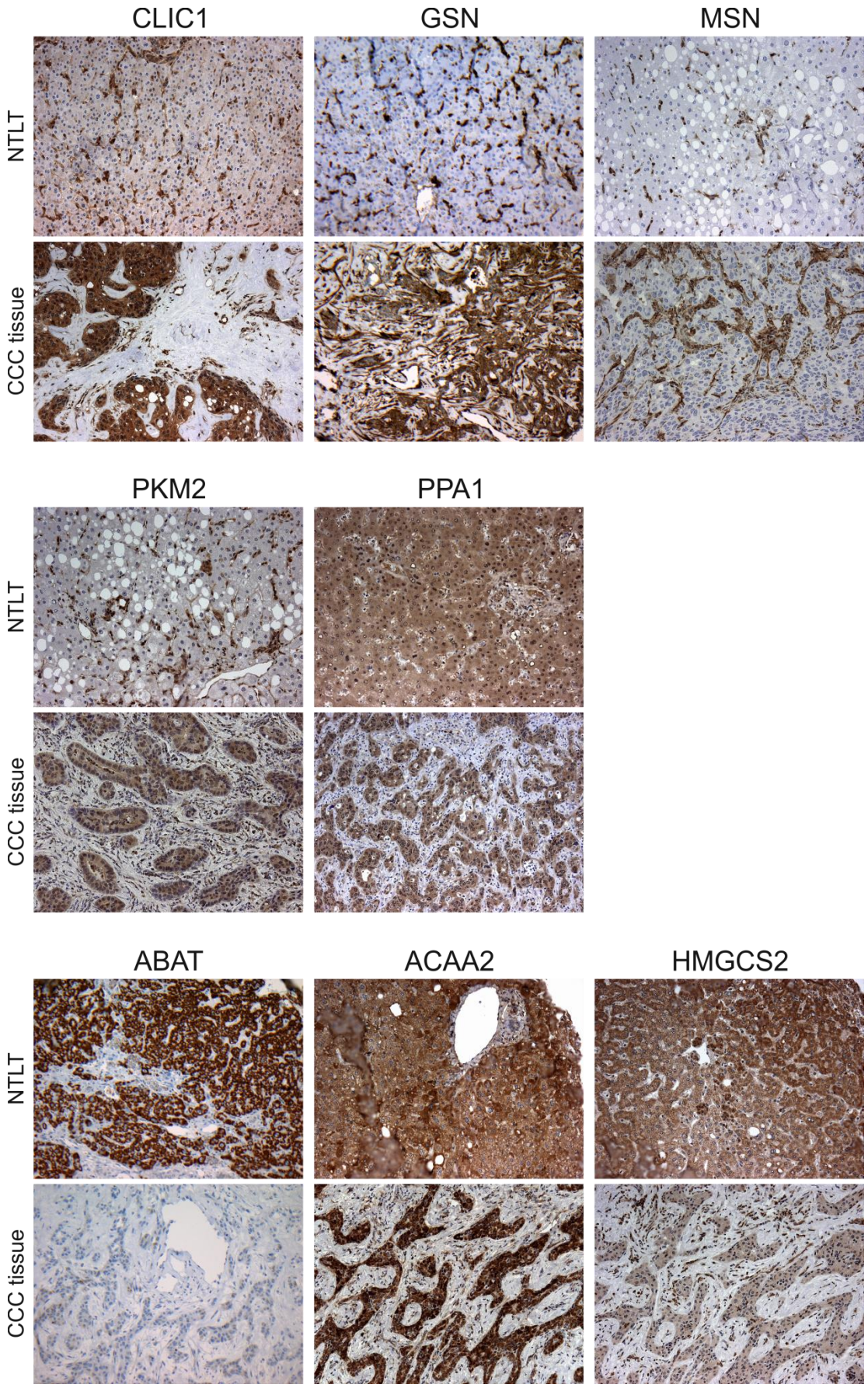

**Supplemental figure 3:** Scatter plot visualising the correlation between the fold changes obtained from the gel-based and the label-free approach. Multiple data points corresponding to the same protein represent different isoforms detected in the 2D-DIGE experiment.



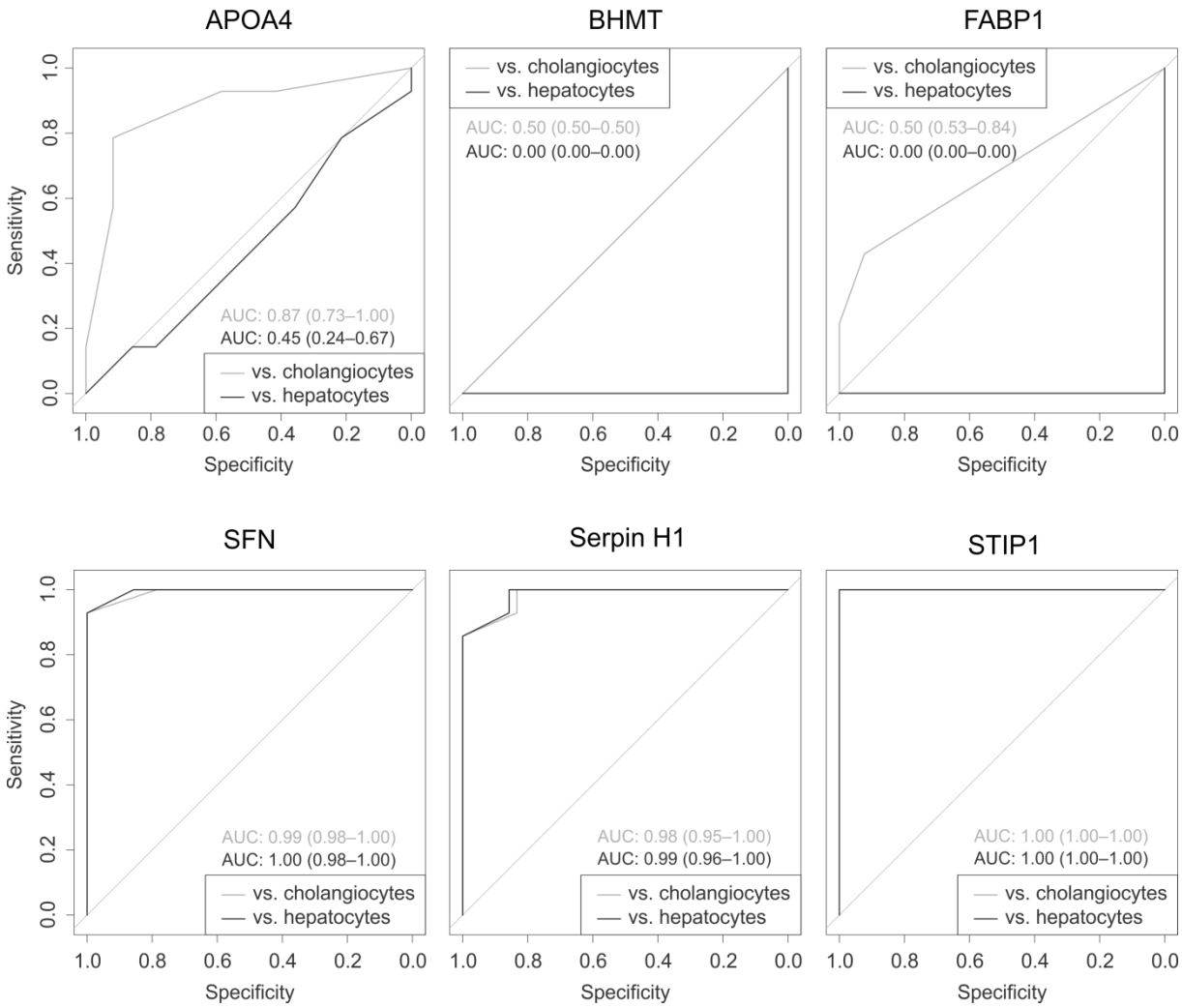

**Supplemental figure 4:** Localisations of differential proteins identified by 2D-DIGE (A) and label-free proteomics (B). Proteins were considered differential if the absolute fold change is at least 1.5 and the FDR adjusted p-value is below 0.05.




**Supplemental figure 5:** Regulation profiles of candidate proteins in the 2D-DIGE and the MS-based label-free experiments. Boxes represent 25th and 75th percentile, whiskers indicate the standard deviation, the median is shown as a horizontal line and the mean value as a square within the box. NTLT, non-tumorous liver tissue.




**Supplemental figure 6:** Verification of biomarker candidates by immunohistochemical staining of CCC tumour tissue and corresponding non-tumorous liver tissue (NTLT) from the same patient. Original magnification: x200.




**Supplemental figure 7:** Receiver operating characteristics curves for the candidate proteins APOA4, BHMT, FABP1, SFN, serpin H1 and STIP1 generated from the immuno-reactive scores gained in the immunohistochemical experiment using sample set 2 (n = 14). Area under the ROC curve (AUC) is given for each curve along with the corresponding 95% confidence interval. AUC values less than 0.5 indicate that observed IR scores in CCC are smaller in the respective comparison. If the direction is not considered important, these curves can be mirrored at the principal diagonal. For FABP1 (vs. hepatocytes) and BHMT (both comparisons), this would result in AUC values equal to one. Ideally, both ROC curves corresponding to the hepatocytes and cholangiocytes comparison show ROC curves above the identity and thus have AUC values greater than 0.5. Suppose, e.g., the cholangiocytes comparison yields AUC=1 and the other one yields AUC=0. That means, that all tumour samples show higher values than all the cholangiocyte samples and the group of hepatocytes shows values above those of the tumour samples (C < T < H).