

Identification of Protein Abundance Changes in Hepatocellular Carcinoma Tissues Using PCT-SWATH

PCT-SWATH检测肝癌组织蛋白质丰度变化

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Background of thyroid nodules



Hepatocellular carcinoma is the **fifth frequent malignancy** worldwide and ranks as the third leading cause of cancer-related mortality[1].

In China, HCC leads to **more than 300 000 deaths every year** [2].

Currently three FDA approved serum biomarkers and CT have low sensitivity and specificity[3]. Better biomarkers are needed for HCC.

[1] H. B. El-Serag, K. L.

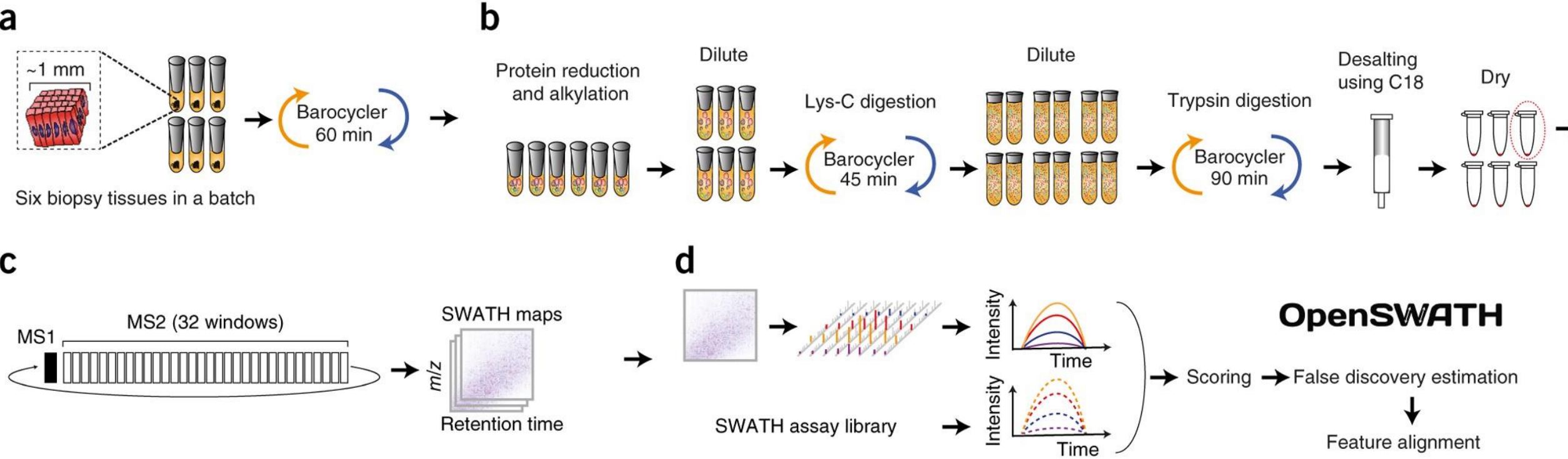
Rudolph, *Gastroenterology* 2007

[2] T. Yau, V. Y. Tang, T. J. Yao, S. T. Fan, C. M. Lo, R.

T. Poon, *Gastroenterology* 2014

[3] European Association For The Study Of The Liver, European Organisation For Research and Treatment of Cancer, *J. Hepatol.* 2012

PCT-SWATH



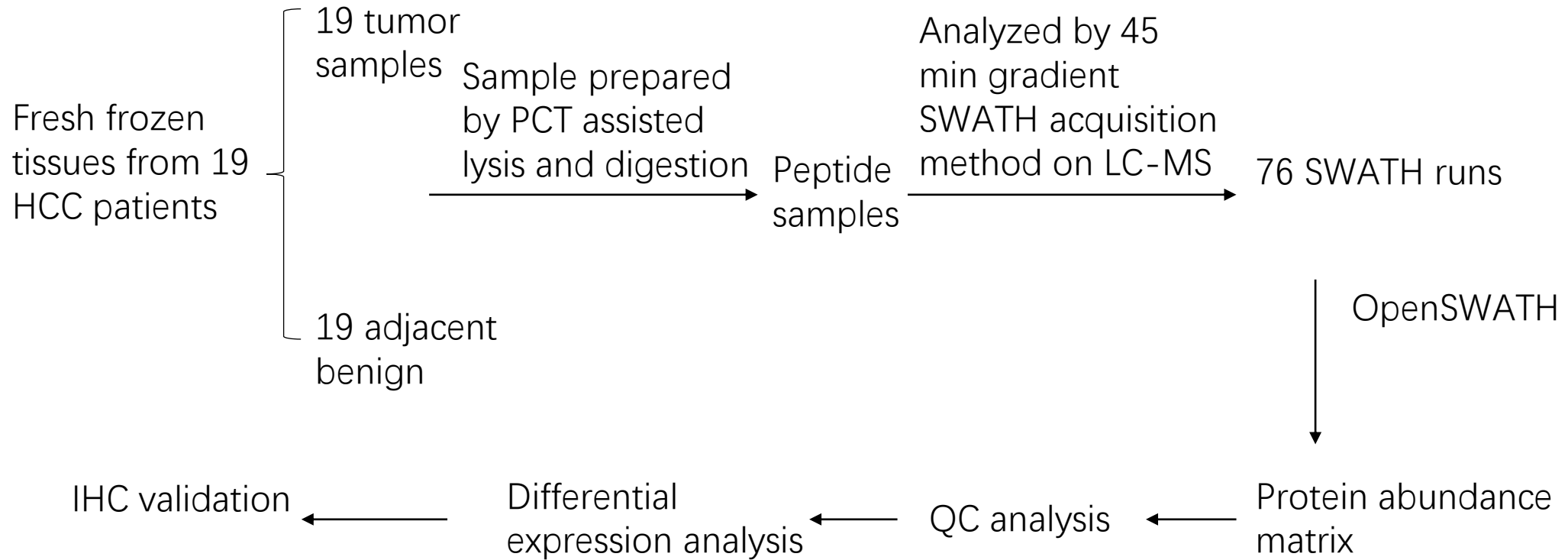
T. Guo, P. Kouvonen, C. et al. *Nat Med.* 2015

Objectives

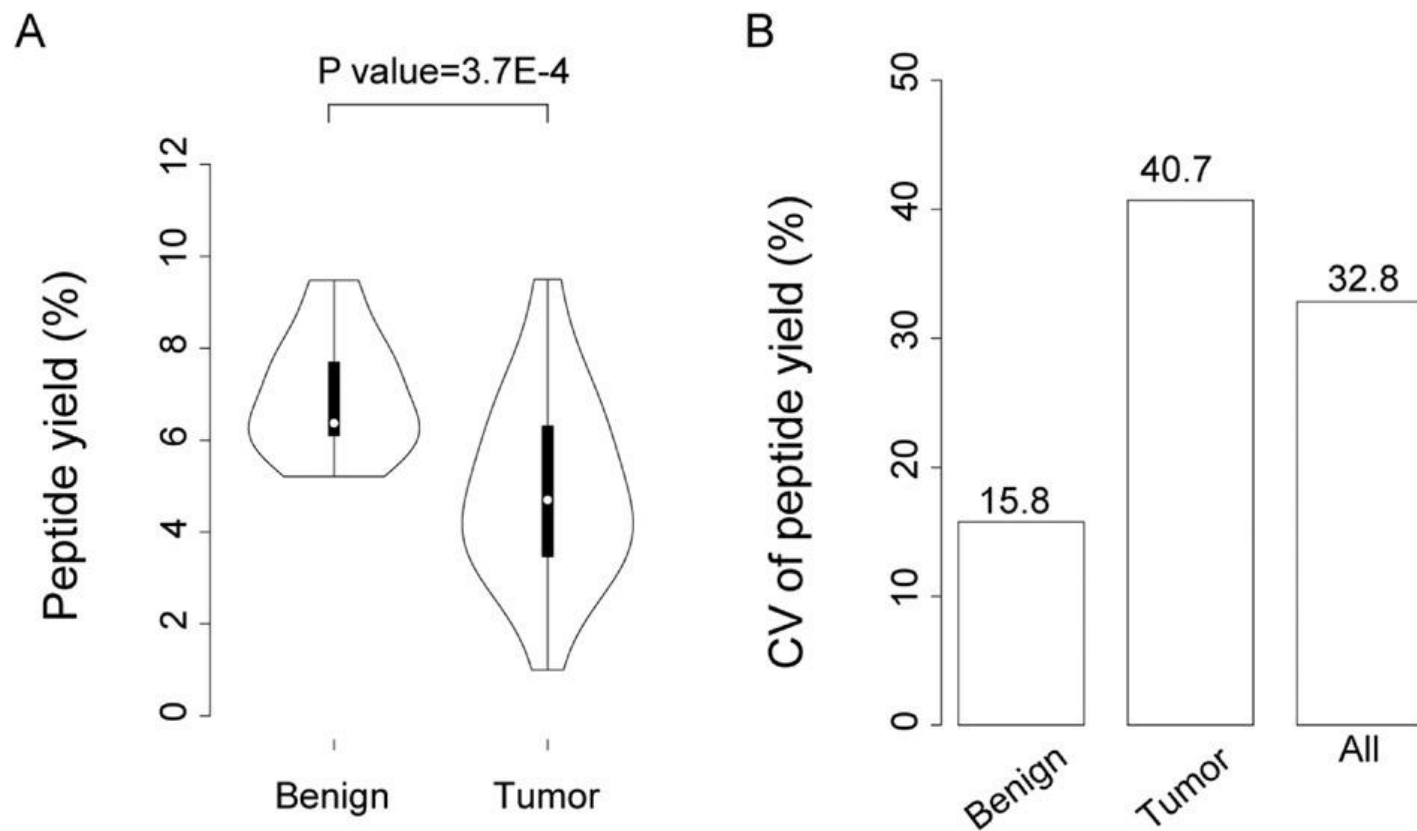
Combining PCT and shorter gradient of SWATH to improve throughput while keep the reproducibility and enough proteome depth of proteomic analysis of clinical tissue biopsies.

Find potential protein biomarkers of HCC.

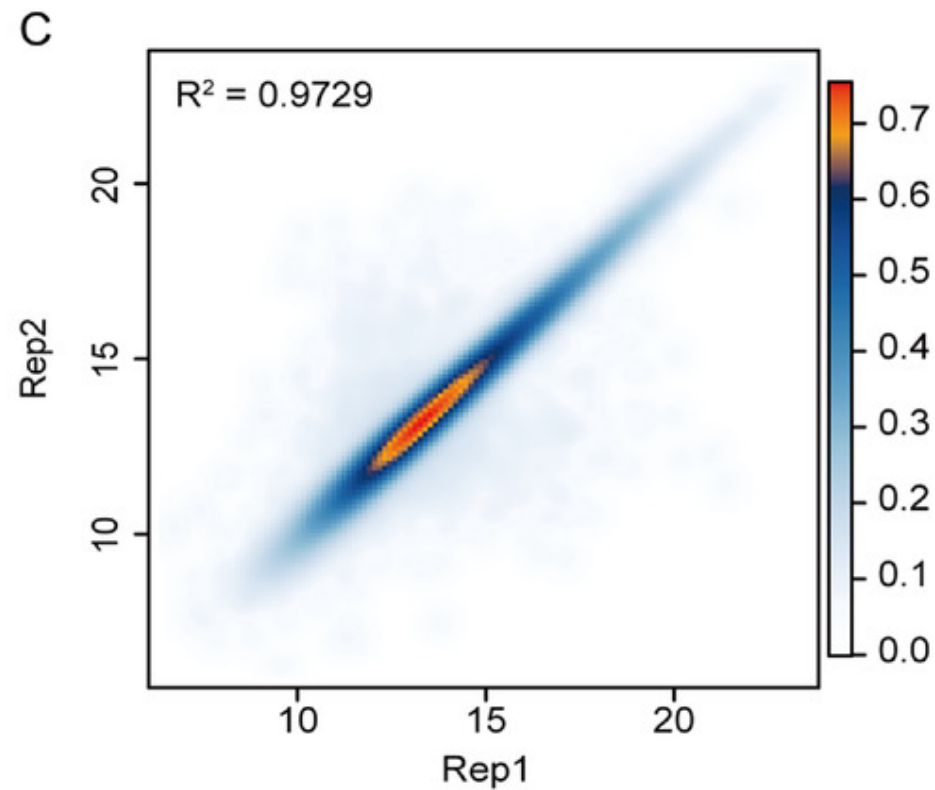
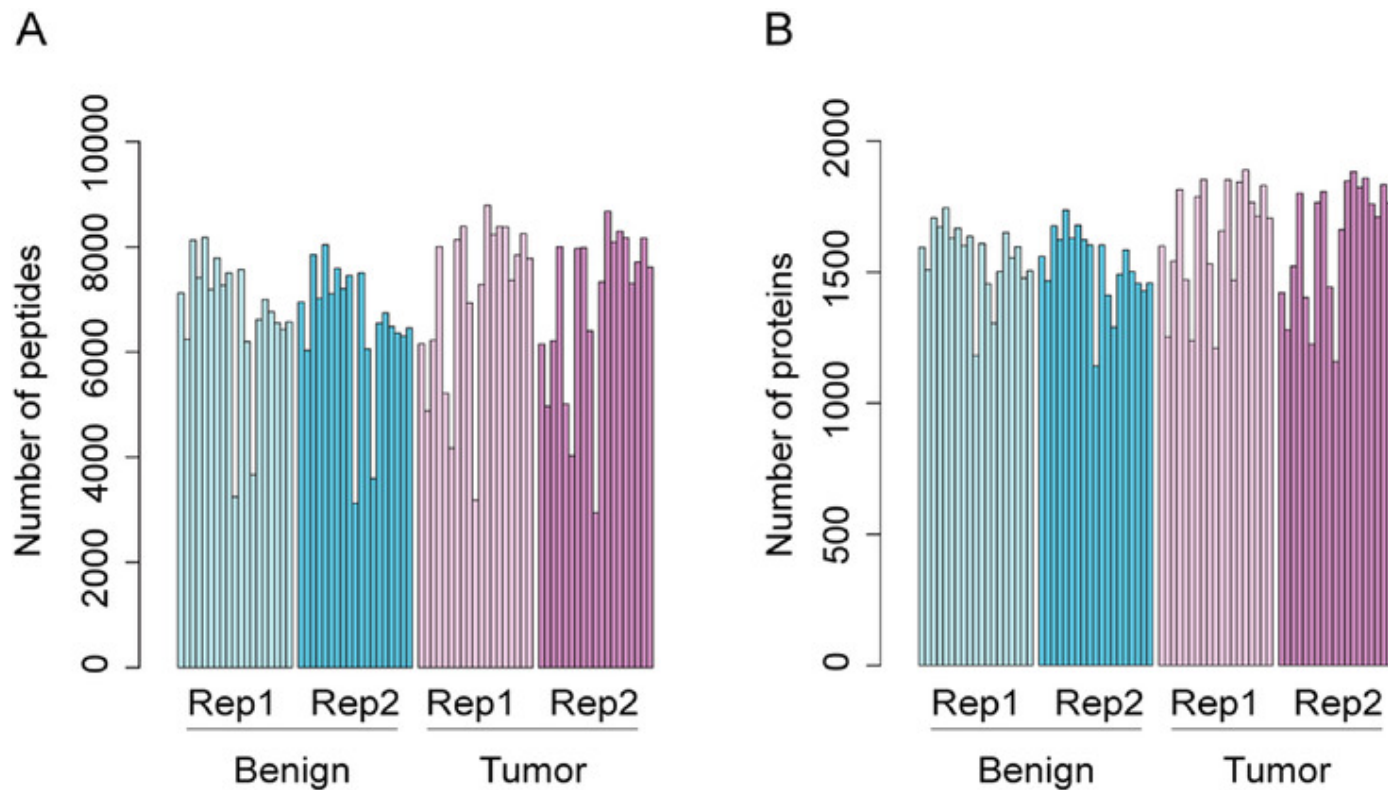
Study Design



Performance of PCT-assisted peptide preparation

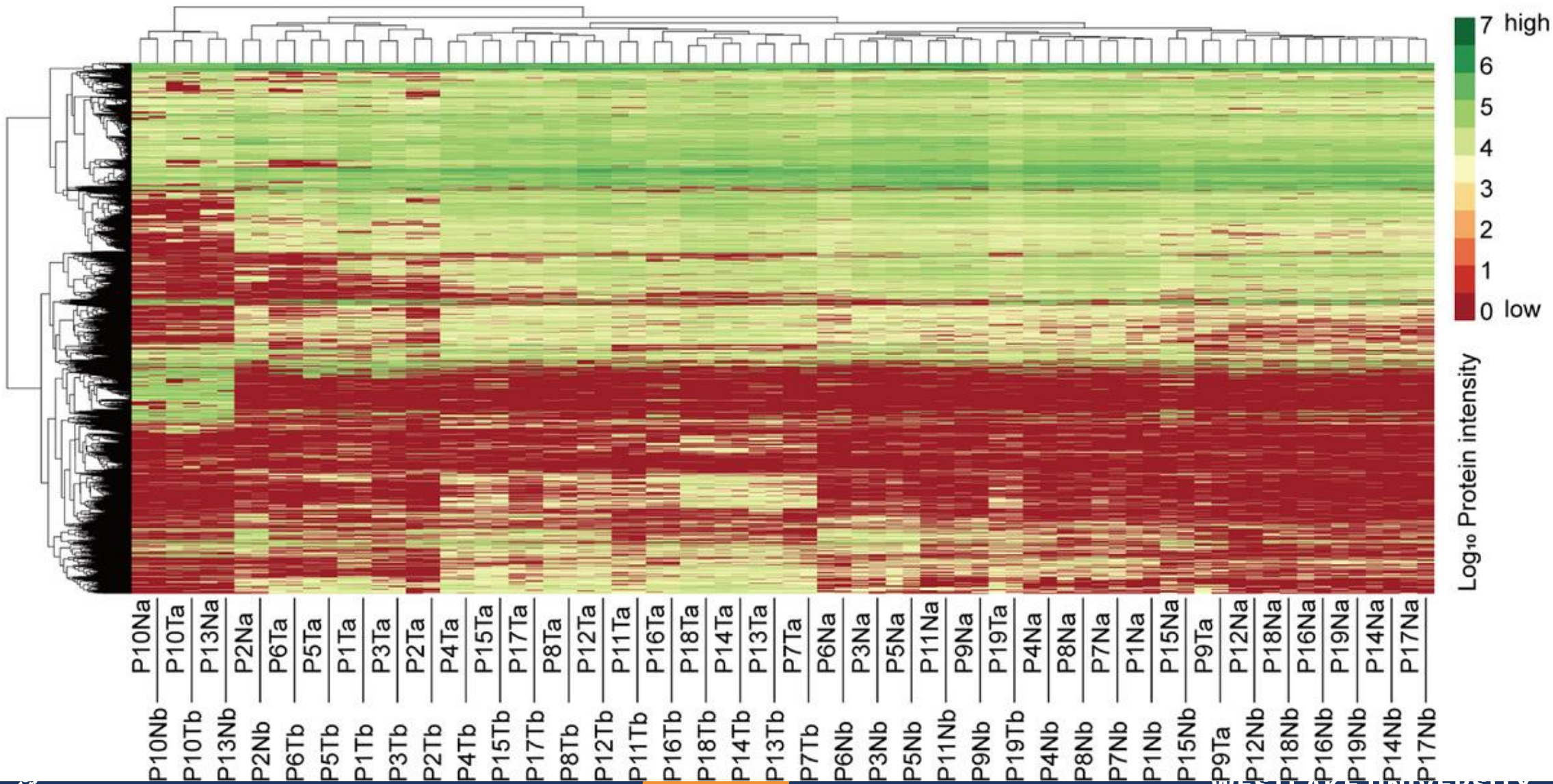


Performance of PCT-assisted peptide preparation

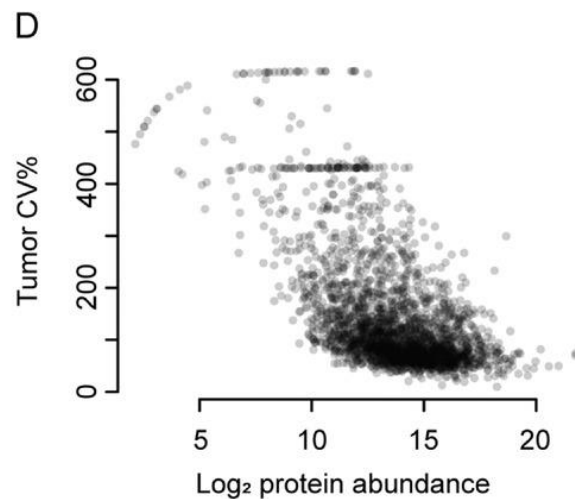
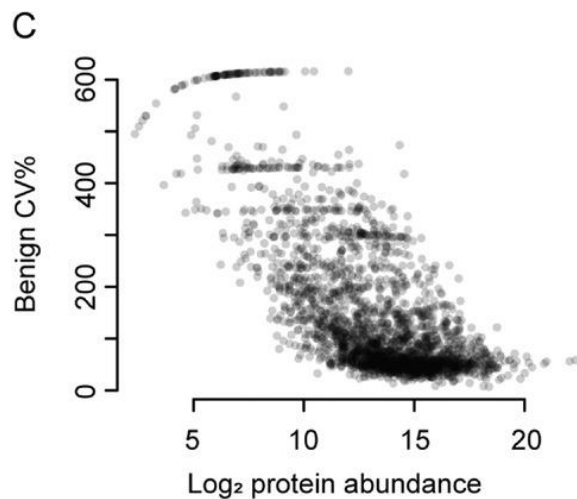
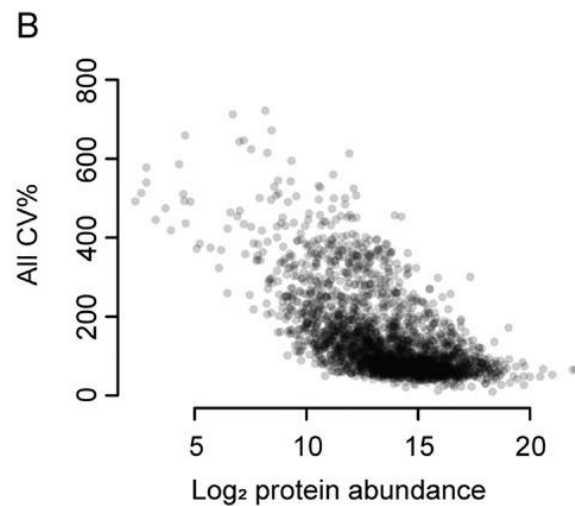
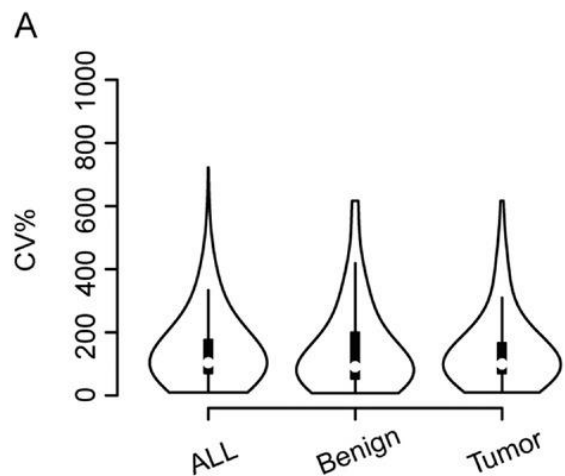


Heatmap overview of protein abundance patterns in 76 samples

D

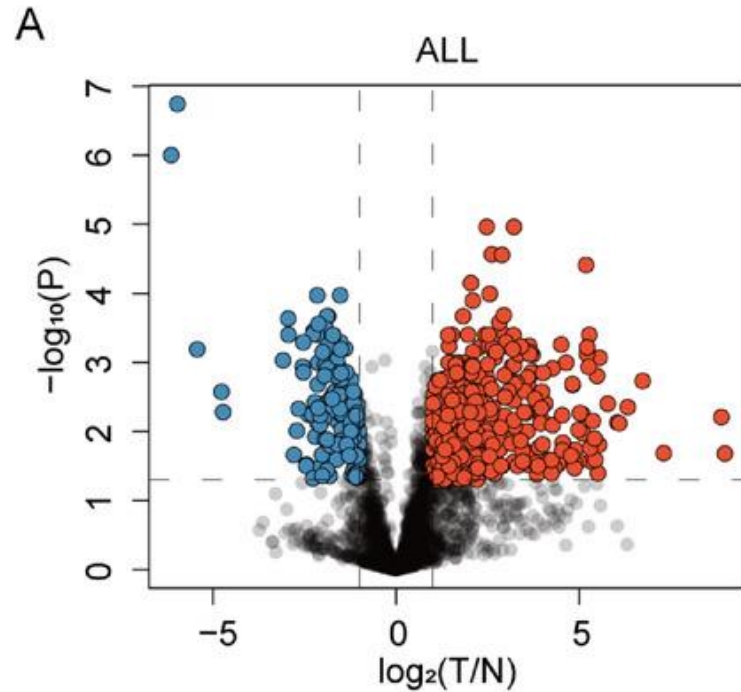


Variability of protein expression in liver tissues

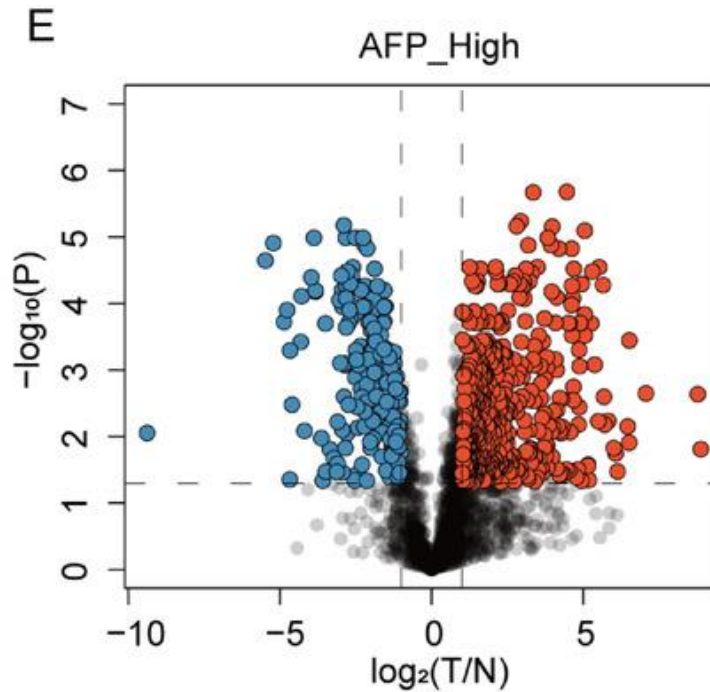


Tumor tissues have a slightly higher CV than benign

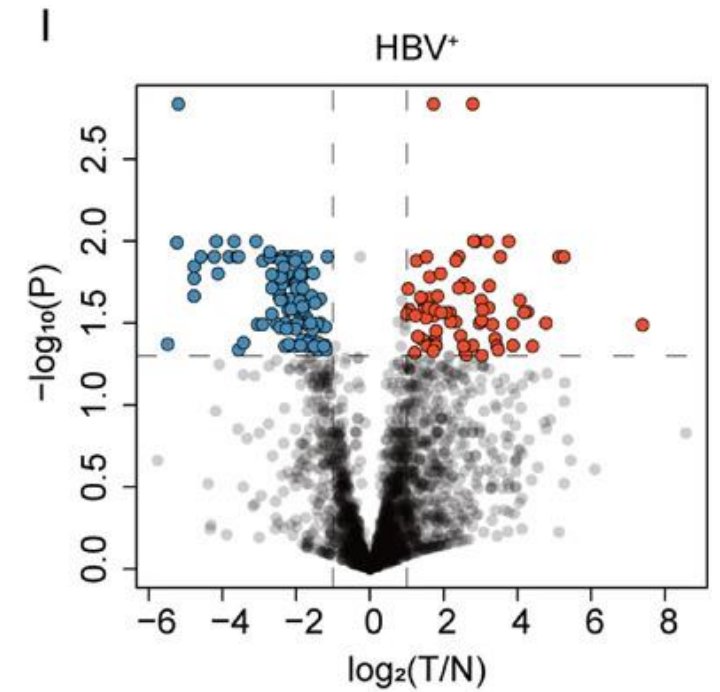
Differential regulated proteins in HCC



541 dysregulated proteins in all



419 up-regulated
192 down-regulated



106 up-regulated
75 down-regulated

All proteins found regulated in HCC

A

Up-regulated proteins and pathways

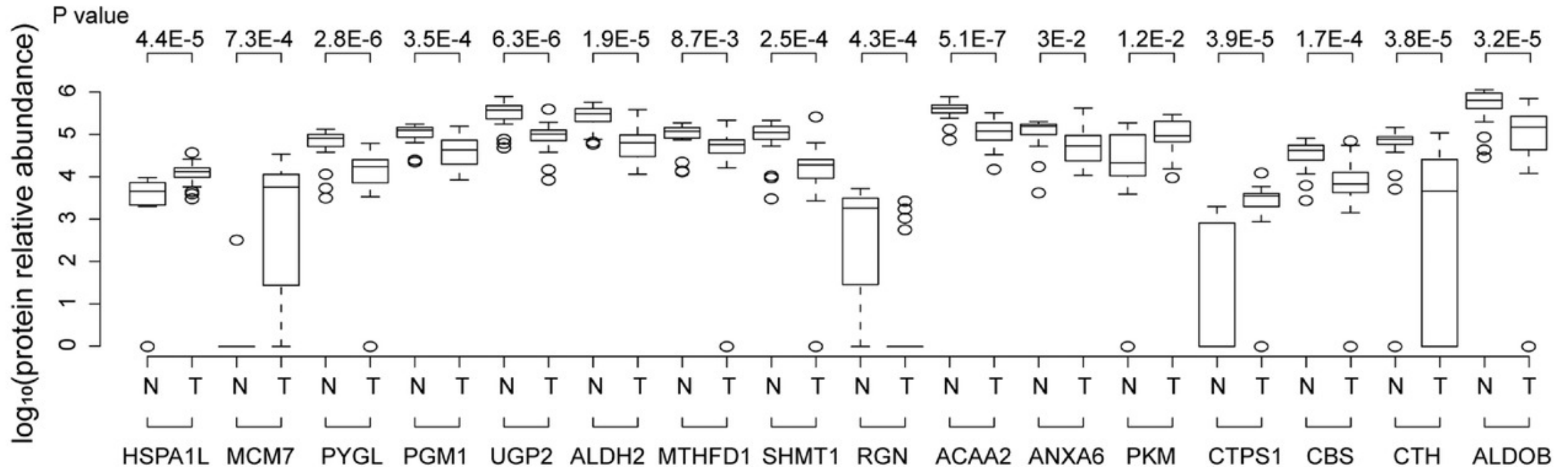
Down-regulated proteins and pathways

DNA replication MCM7 SSRP1	Translation regulation EIF3F/EIF3H EIF4H HSPB1 MTIF2 DDX3X	Cell-cell adhesion&Cell-cell adherens junction OLA1 AHSA1 RAB1A ABCF3 DDX3X CTNNB1 NC1 PAK2 GCN1 EIF4H HSP90AB1 HSPA1A HSPA8 PKM	Oncogene family RAB1A Pathways in cancer CTNNB1 HSP90AB1 PKM
DNA repair RUVBL2 NONO SMC1A SSRP1	Posttranslational modification PSMC1 AHSA1	Stress response GCN1 AHSA1 HSP90AB1 HSPA1L HSPA1A HSPA4 HSPA8 HSPB1 DDX3X	ATPase activity ABCF3 DDX3X OLA1 RUVBL2 HSPA1A HSPA8 PSMC1
Transcription initiation and elongation ROLRC SSRP1 POLR1C PSMC1	Protein processing in ER HSP90AB1 HSPA1A HSPA1L HSPA8	MAPK signaling pathway HSPA1A/HSPA1L HSPA8 HSPB1 PAK2	Apoptosis PSMC1 EIF3F EIF3H DDX3X CTNNB1 PAK2 RUVBL2
Transcription regulation RUVBL2 BTF3 CTNNB1 HSPA8 NONO NCL DDX3X GCN1	Cell proliferation SMC1A RCC1 CTNNB1 PAK2 EIF3F/ EIF3H	Wnt signaling pathway DDX3X CTNNB1 MARK1	Antigen processing & presentation HSP90AB1 HSPA1A HSPA1L HSPA4 HSPA8 HSPB1
mRNA maturation PABPC1 NONO DDX3X HSPA8	Mitochondria transcription transcription MRPL12	Tumor necrosis factor-mediated signaling pathway HSPB1 HSPA1A PSMC1	Innate Immune response NONO DDX3X CTNNB1
Regulation of mRNA stability HSPA1A HSPA8 HSPB1 PABPC1 PSMC1	Mitochondria Translation MRPL11 MRPL12 MRPL19 MRPL37 MRPL41 MRPL49 MRPL53 MRPS23 MTIF2		
Translation initiation EIF3F/EIF3H EIF4H PABPC1 RPL8 RPS7 NARS			

Amino-acid biosynthesis CTH CBS MTHFD1 OTC ALDOB SHMT1	Antibiotics biosynthesis UGP2 ACAT1 ACAA2 ACSS2 ALDH1B1 ALDH2 ALDOB CTH CBS FDPS OTC PGM1 RGN SHMT1
Pyruvate metabolism ACAT1 ACSS2 ALDH1B1 ALDH2	Fatty acid metabolism ACAA2 ACAT1
Carbon metabolism ACAT1 ACSS2 ALDOB RGN SHMT1	Lipid biosynthesis FDPS
Terpenoid backbone biosynthesis ACAT1 FDPS ALDH1B1 Glycolysis/ Gluconeogenesis	Fatty acid degradation ACAT1 ACAA2 ALDH1B1 ALDH2
ACSS2 ALDH2 ALDOB PGM1	Ethanol oxidation ACSS2 ALDH1B1 ALDH2
Sucrose metabolism UGP2 PGMM1 PYGL	Pentose phosphate pathway ALDOB PGM1 RGN
	Glycogen biosynthetic process UGP2 PGM1

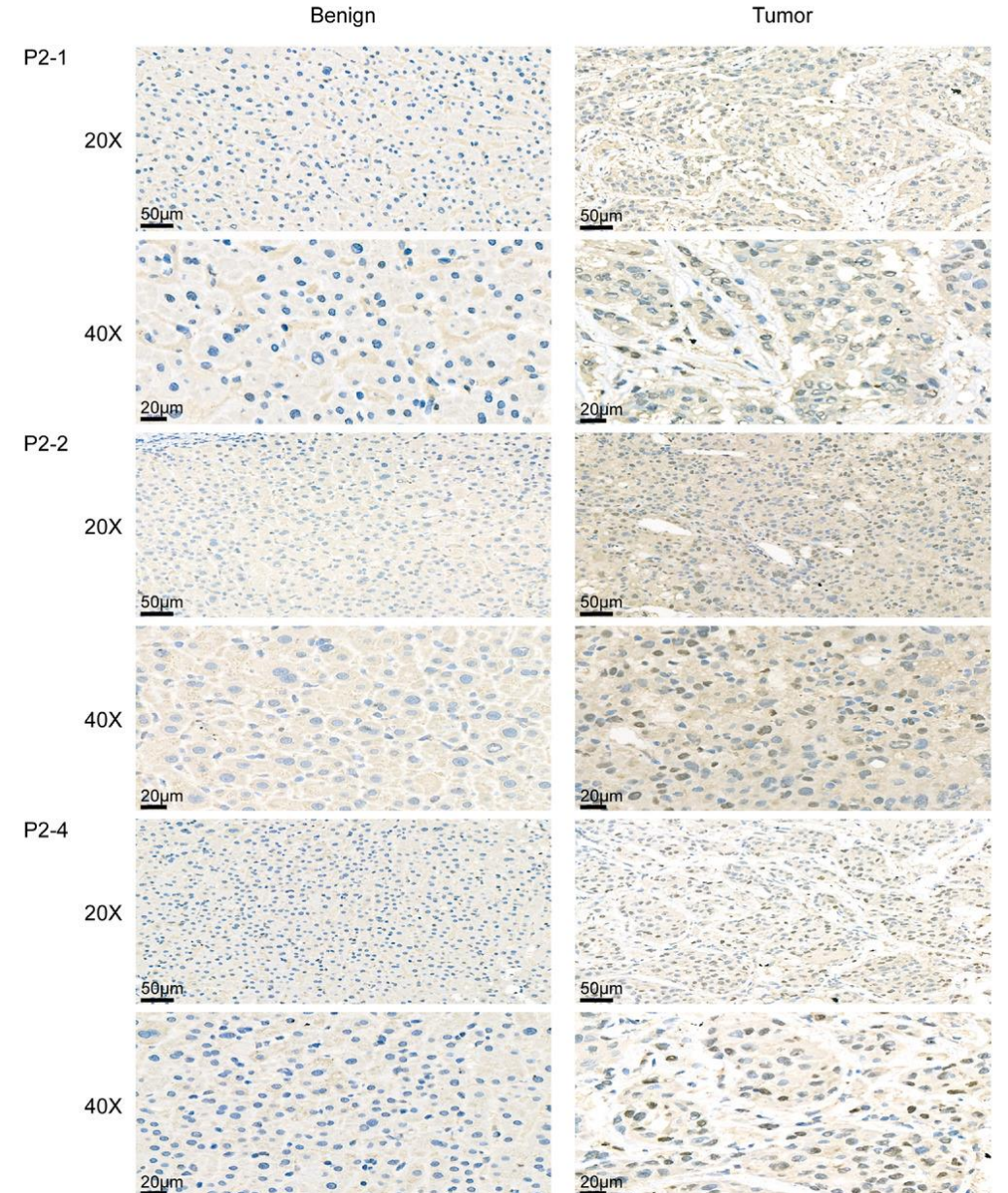
16 selected proteins significantly regulated in HCC tumors

B



Detection of MCM7 in HCC Samples Using IHC

MCM7 IHC staining to three additional patients validated the upregulation of MCM7 in liver tumors



Take-home messages

- In conclusion, we report an optimized PCT–SWATH workflow enabling analysis of clinical tissue specimens with increased sample throughput without compromise of quantitative accuracy and proteomic coverage.
- Our study identified a few regulated proteins in this HCC cohort. Proteins with increased abundance are mainly related to mass production, oncogenic signaling, and immunity, whereas metabolic proteins are shown with lower expression.
- We identified 16 dysregulated proteins of reported clinical relevance to HCC. Upregulation of MCM7 in tumor tissue samples was observed using IHC in additional patients.
- The study demonstrates that the PCT–SWATH methodology has potential to be practically applied in clinical research to analyze tissue samples in high throughput.

Proteomics Clinical Applications

Research Article |  Full Access

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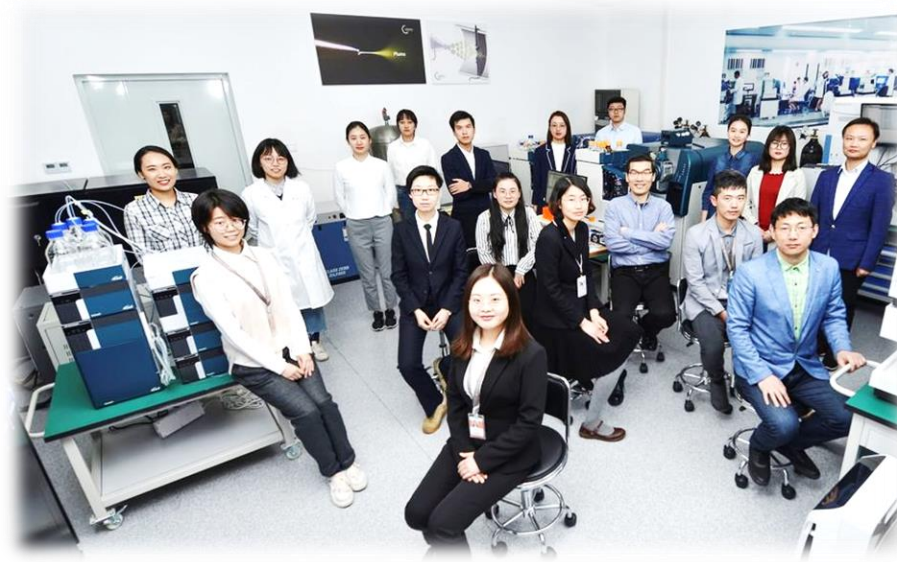
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THANK YOU

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