Supplemental Materials for

In-depth Profiling and Quantification of the Lysine Acetylome in Hepatocellular Carcinoma with a Trapped Ion Mobility Mass Spectrometer

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Other Supplemental Materials for this manuscript include:

Tables S3 to S8

Table S1. List of the specimens and the clinical information.

Patient ID	Gender	Age (years)	Diagnose	Laennec Stage	Specimens
HG_H1	M	56	HCC	F4C	Tumor, NAT
HG_H10	M	42	HCC	IV	Tumor, NAT
HG_H2	M	63	HCC	F4C	Tumor, NAT
HG_H3	M	48	HCC	IV	Tumor, NAT
HG_H4	M	53	HCC	F4C	Tumor, NAT
HG_H6	M	69	HCC	F4A	Tumor, NAT
LC4	M	53	HCC	IV	Tumor, NAT
LC6	M	46	HCC	NA	Tumor, NAT

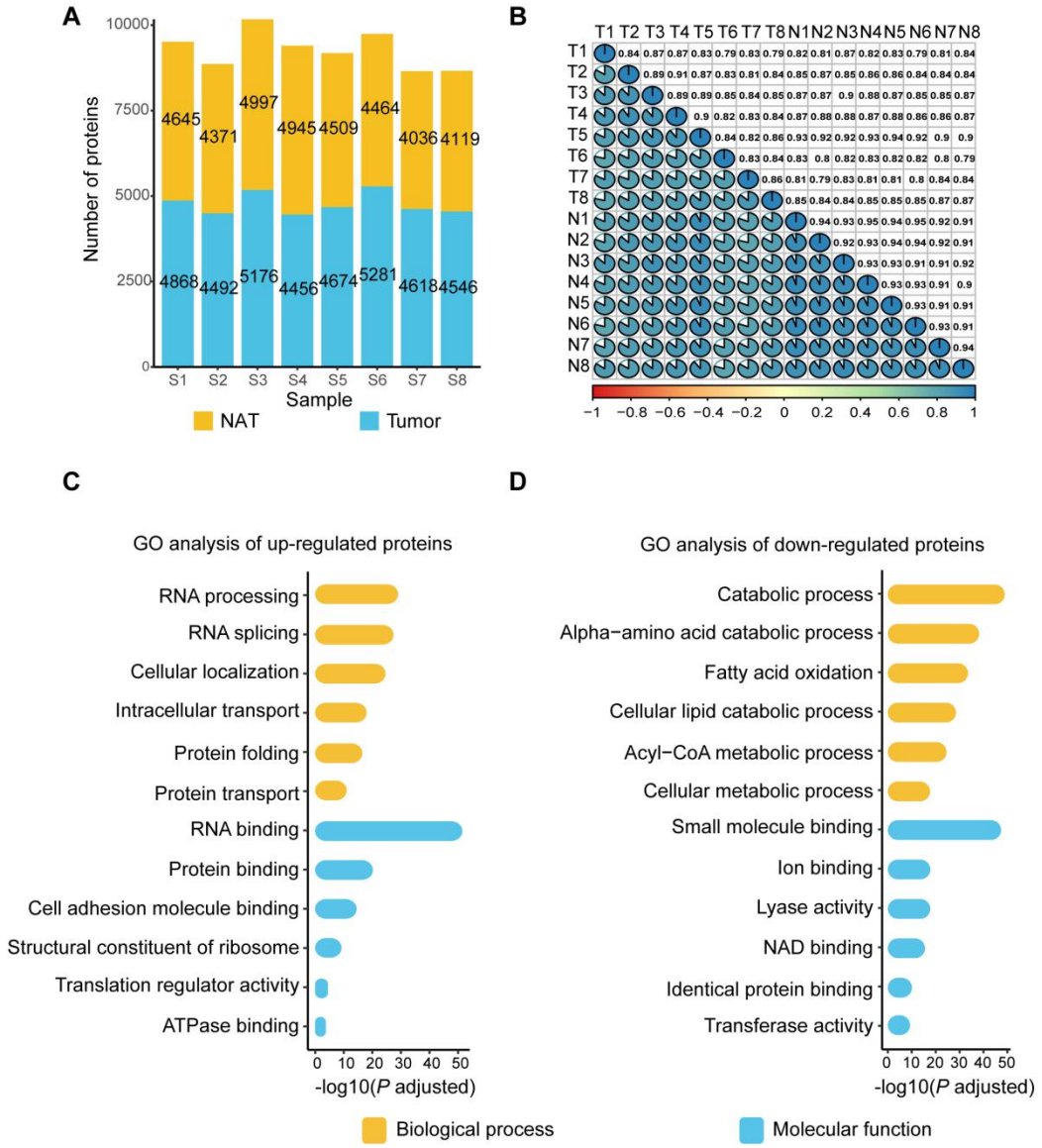
NA, non-available; F, female; M, male; NAT, Normal adjacent tissue.

Table S2. Sequences of the DNA primers used for qRT-PCR and siRNAs for SIRT2 knockdown.

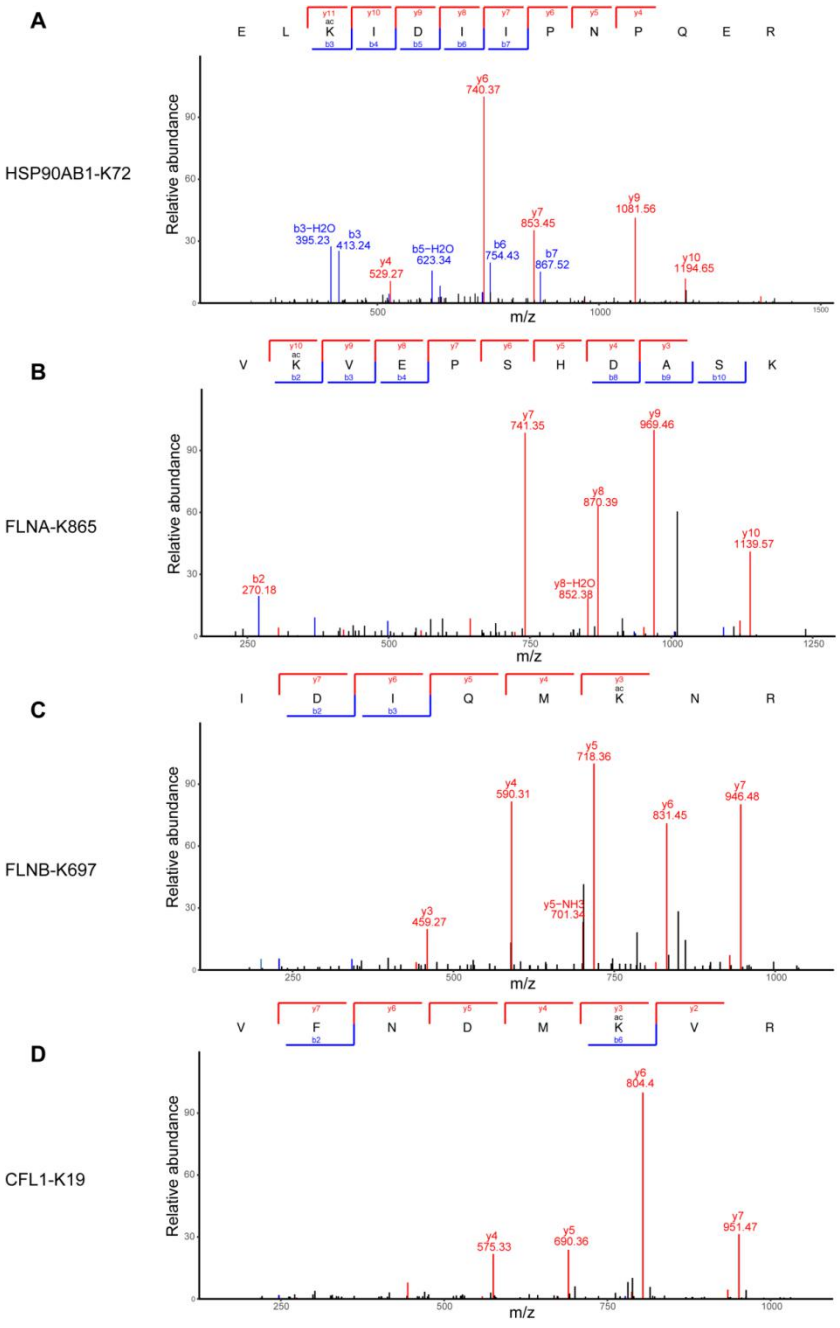
Name	Sequence (5'-3')
GAPDH	Forward: GGTGAAGGTCGGAGTCAACG Reverse: CAAAGTTGTCATGGATGACC
SIRT2	Forward: GGCATACAGCAGTAAACACAAC Reverse: CTAGCTATGATCCTAACCCAAG
siSIRT2-1 *	Sense: GCCAACCAUCUGUCACUACUUTT Antisense: AAGUAGUGACAGAUGGUUGGCTT
siSIRT2-2	Sense: CCUGCUCAUCAACCAGGAGAATT Antisense: UUCUCCUUGUUGAUGAGCAGGTT
siSIRT2-3	Sense: CCUGUGGCUAAGUAAACCAUATT Antisense: UAUGGUUUACUUAGCCACAGGTT

* Selected for the cellular experiments.

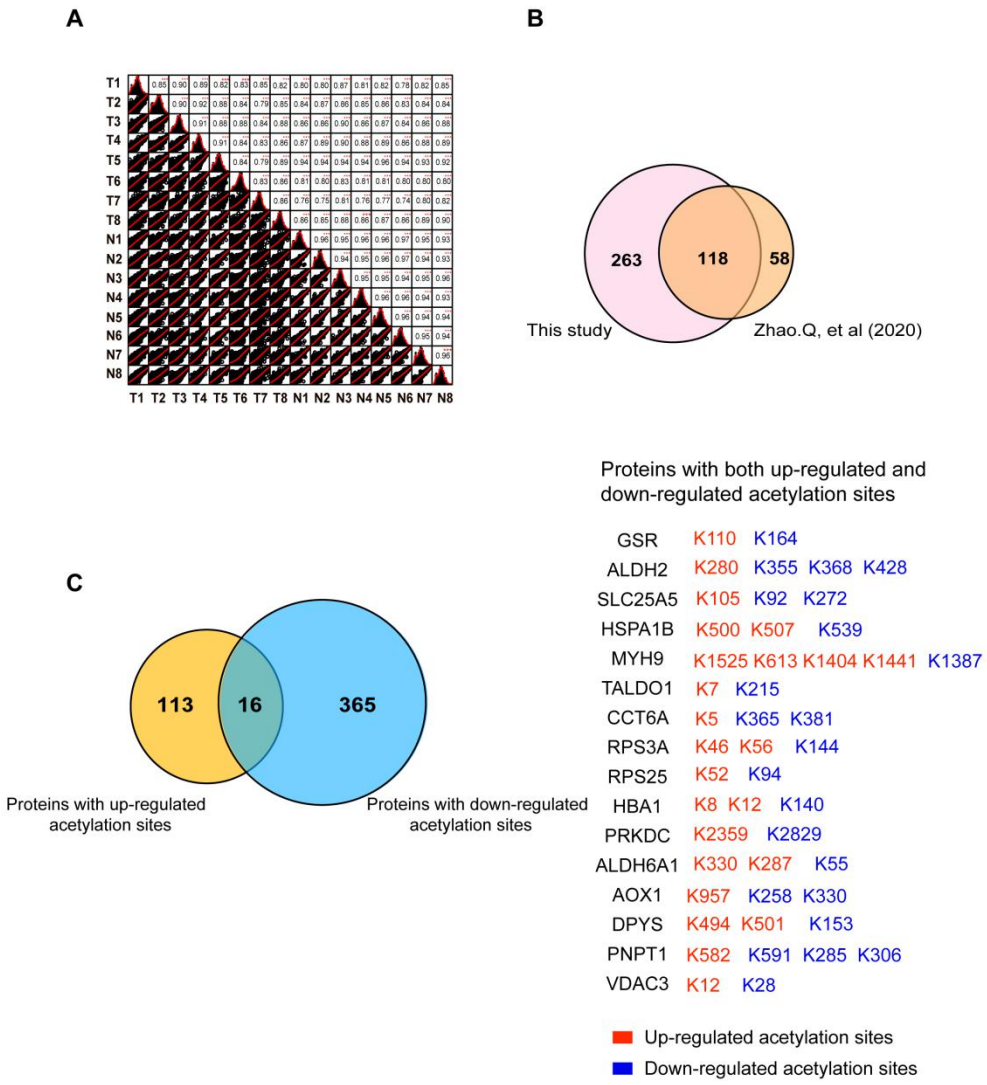
Supplemental Figures



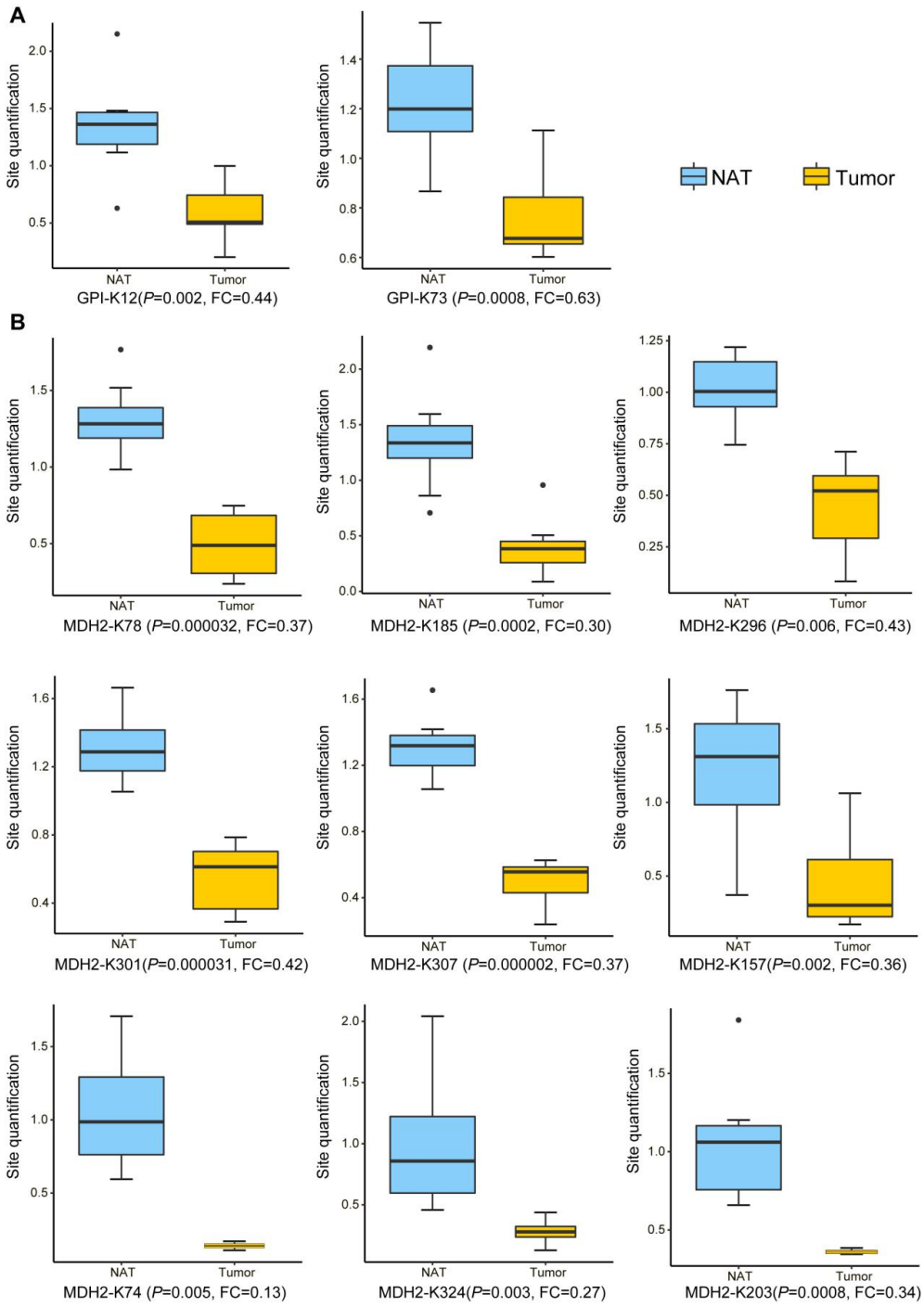
Supplemental Figure S1. Quantitative proteomics analysis of the HCC tumors and NATs. **A**, Numbers of proteins identified in tumors and NATs from individual patients. **B**, Correlation analysis of protein intensities between samples. Pearson correlation analysis was conducted. Biological process and molecular function analysis of the up-regulated (**C**) and down-regulated (**D**) proteins. The analysis was conducted by g:Profiler (https://biit.cs.ut.ee/gprofiler/gost).



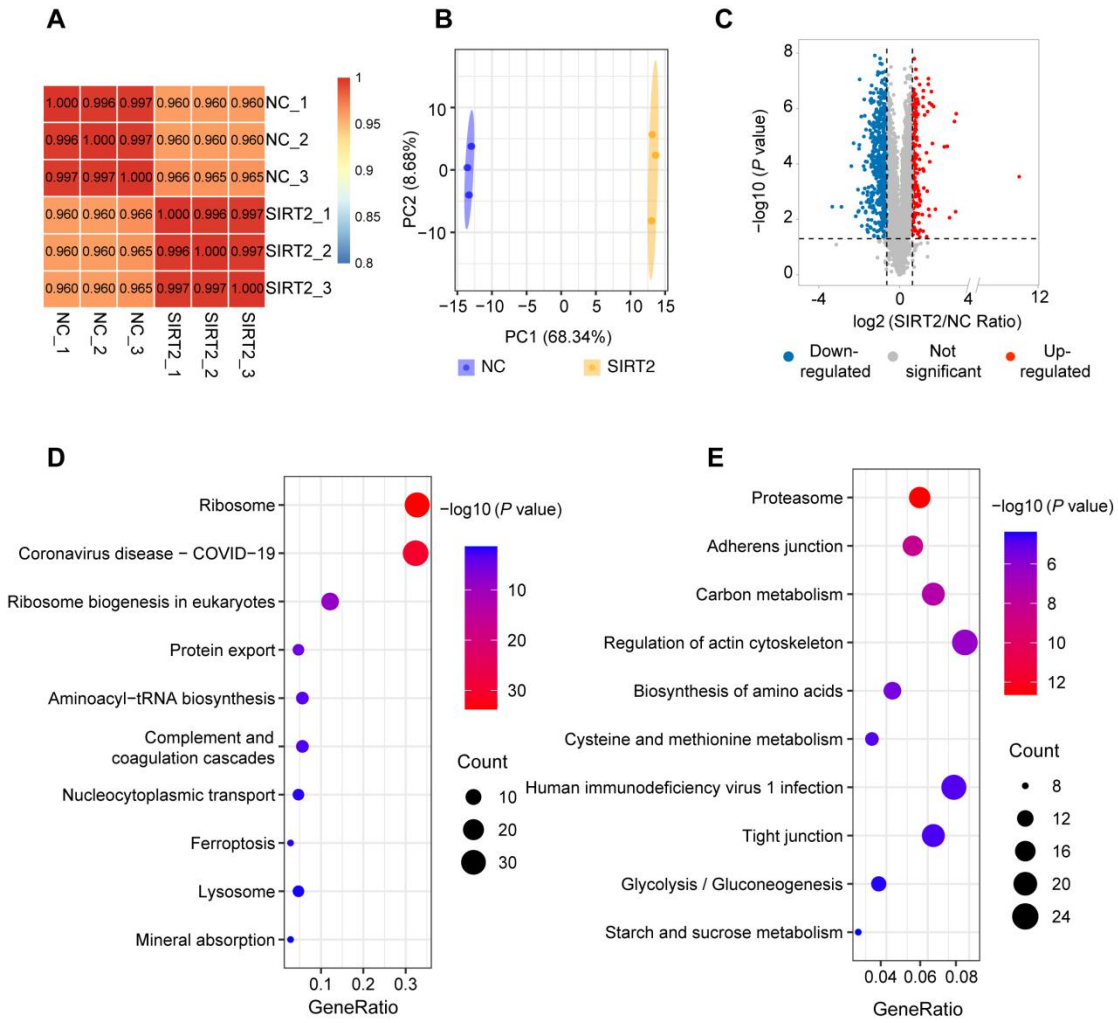
Supplemental Figure S2. MS/MS spectra of selected tumor-specific acetylpeptides. **A**, HSP90AB1-K72; **B**, FLNA-K865; **C**, FLNB-K697; **D**, CFL1-K19.



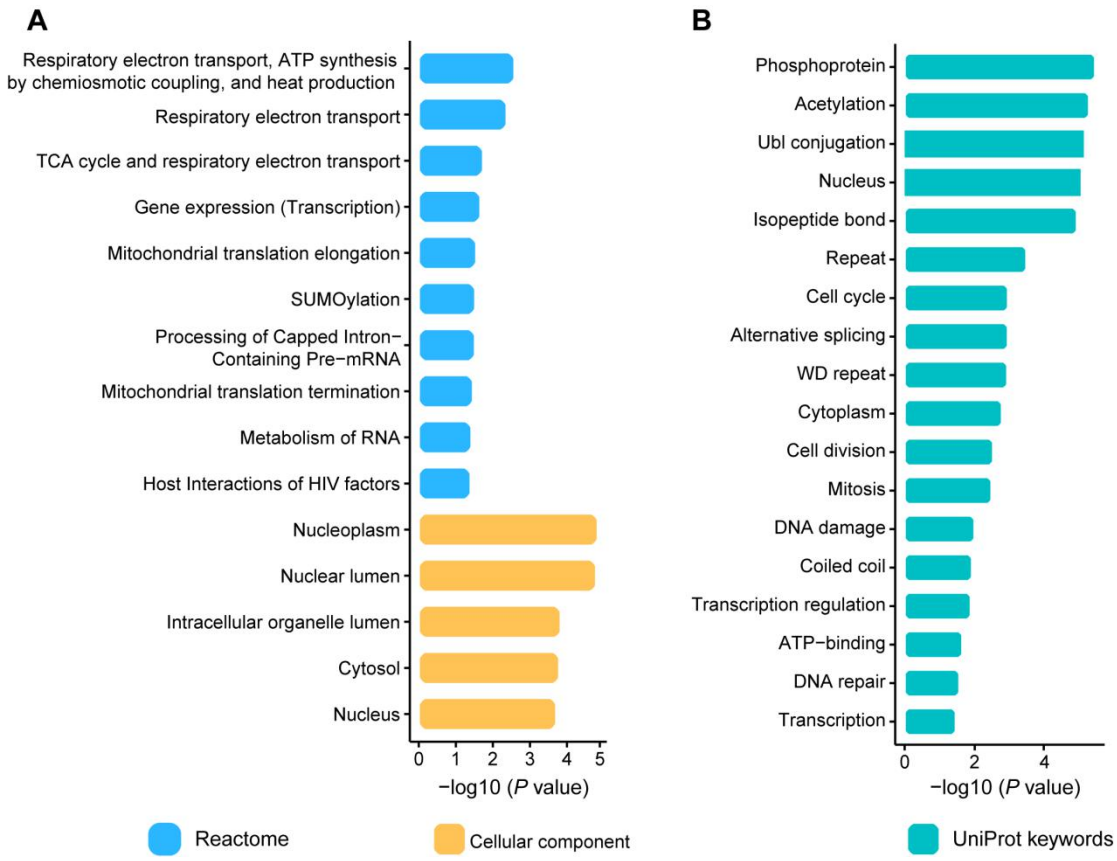
Supplemental Figure S3. Identification and quantification of the K-acetylome in HCC. **A**, Correlation analysis of K-acetylpeptide intensities between samples. Pearson correlation analysis was conducted. **B**, Comparison of our results and a previous report of HCC acetylproteome study (26). **C**, Proteins containing both up-regulated and down-regulated K-acetylation sites.



Supplemental Figure S4. Proteins with K-acetylation sites down-regulated in tumors. **A,** Quantification of the two K-acetylation sites in GPI. **B**, Quantification of the nine K-acetylation sites in MDH2.



Supplemental Figure S5. Quantitative analysis of the proteome changes in response to SIRT2 overexpression in Huh7 cells. **A,** Correlation analysis of protein intensities between samples. Pearson correlation analysis was conducted. **B,** Principal component analysis visualization of protein expression in SIRT2-overexpressing cells and NC cells. **C**, Volcano plot illustrating differentially expressed proteins (fold change>1.5, $P < 0.05$, by two-sided Student's *t*-test). **D** and **E**, KEGG pathway enrichment analysis of the up-regulated (C) and down-regulated (D) proteins. The analysis was conducted by clusterProfiler. (version: 3.16.0).



Supplemental Figure S6. Functional analysis of the down-regulated K-acetylation sites induced by SIRT2 overexpression. **A**, Reactome pathway and cellular component enrichment analysis of the proteins containing down-regulated K-acetylation sites in SIRT2-overexpressing cells. **B**, UniProt keywords significantly associated with the down-regulated K-acetylation sites in SIRT2-overexpressing cells. The analysis was conducted by AGOTOOL (https://agotool.org/) using the abundance-corrected proteome as the control for statistical analysis (P<0.05 as cutoff).