Supplementary Materials

A novel phenazine analog, CPUL1, suppresses autophagic flux and proliferation in hepatocellular carcinoma: insight from integrated transcriptomic and metabolomic analysis

Jiaqin Chen[1,†], Dong Feng[2,†], Yuanyuan Lu[1,†], Yanjun Zhang[1], Hanxiang Jiang[1], Man Yuan[3], Yifan Xu[3], Jianjun Zou[4, 5], Yubing Zhu[4, 5], Jingjing Zhang[4, 5], Chun Ge[4, 5,*], Ying Wang[1,*]

[1]*School of Life Science and Technology, China Pharmaceutical University, Nanjing 211198, China*

[2]*Nanjing Southern Pharmaceutical Technology Co., Ltd., Nanjing 211100, China*

[3]*Department of Clinical Pharmacy, School of Basic Medicine & Clinical Pharmacy, China Pharmaceutical University, Nanjing 210009, China*

[4]*Department of Pharmacy, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China*

[5]*Department of Clinical Pharmacology, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China*

[*]Correspondence:

Chun Ge (gechun901020@163.com), Ying Wang (waying@cpu.edu.cn)

[†]These authors have contributed equally to this work.

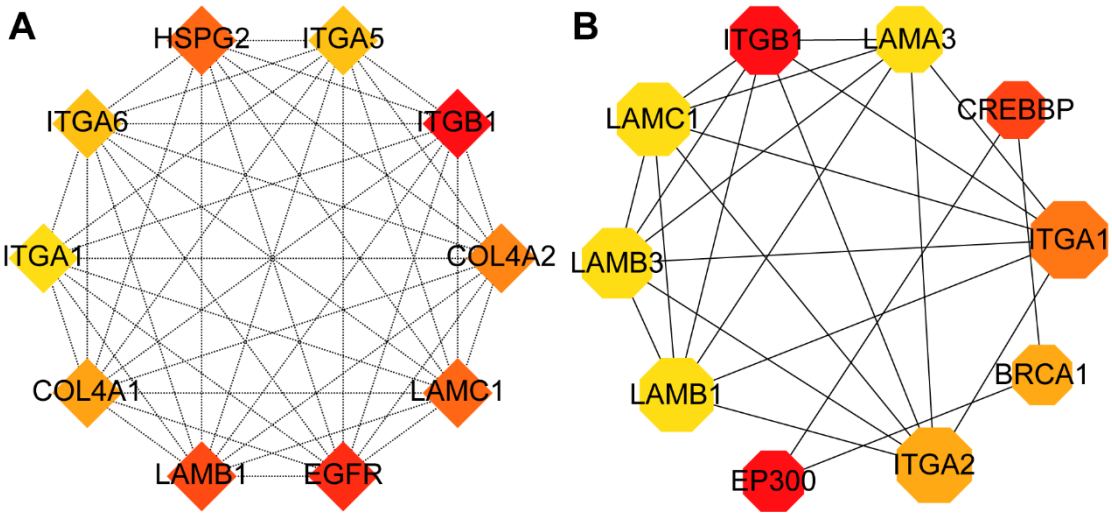

Figure S1 The top-10 hub gene network. This network was calculated using the degree algorithm of the Cytohubba plug-in. (A) The hub gene expression in 6h- CPUL1-treated and control. (B) The hub gene expression in 48h- CPUL1-treated and control.

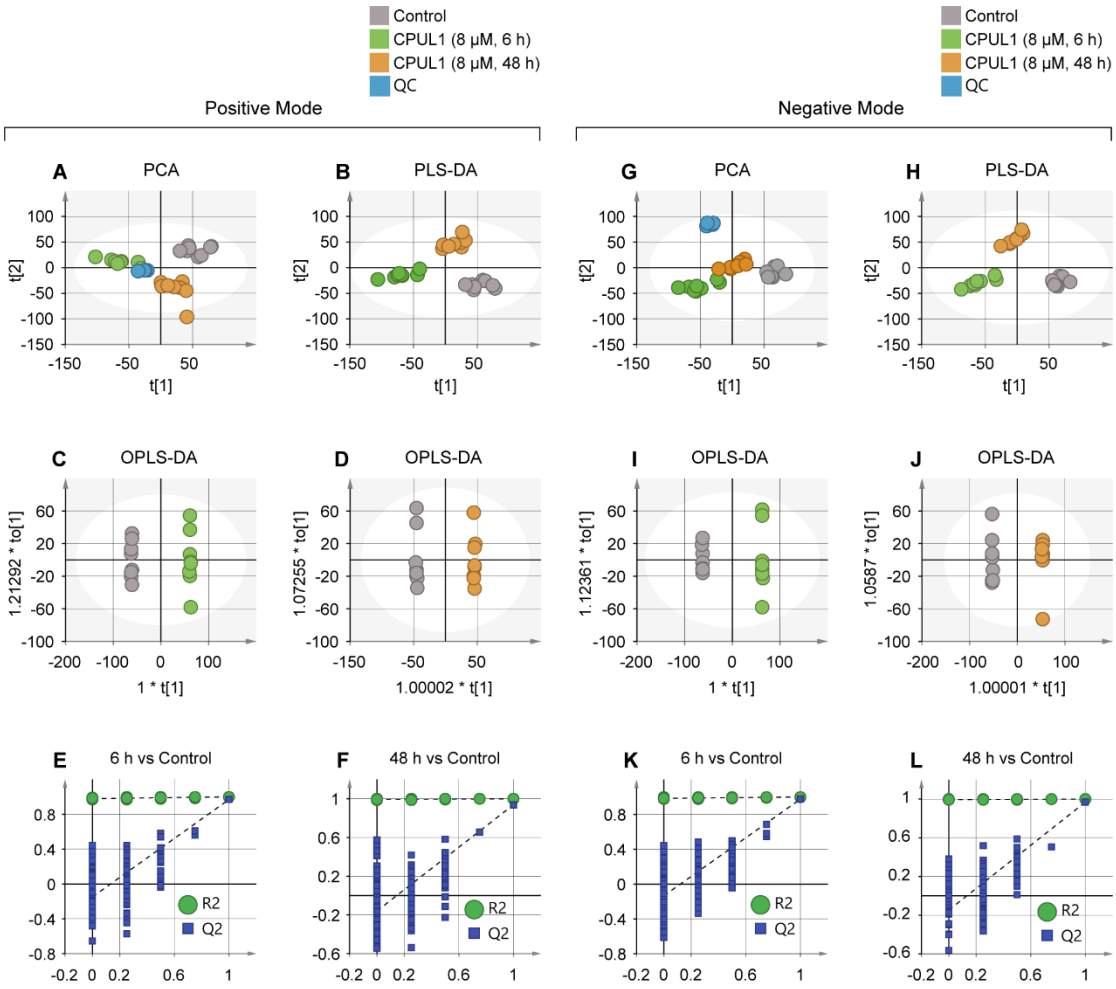

Figure S2 Metabolomic analysis of CPUL1-treated BEL-7402 cells. (A) Score plot from the PCA model classifying control, 6h-CPUL1-treated, 48h-CPUL1-treated, and QC groups in positive mode. (B) PLS-DA score plot of control and treated cells in positive mode. (C) OPLS-DA analysis between the control and the 6h-CPUL1-treated groups showed a good separation trend in positive mode. (D) OPLS-DA analysis between the control and the 48h-CPUL1-treated groups showed a good separation trend in positive mode. (E) Permutations plot analysis between the control and the 6h-CPUL1-treated groups showed the intersection of the Q2 regression line and the y-axis was less than zero in positive mode, indicating that the model was valid. (F) Permutations plot analysis between the control and the 48h-CPUL1-treated groups showed the intersection of the Q2 regression line and the y-axis was less than zero in

positive mode, indicating that the model was valid. (G) Score plot from the PCA model classifying control, 6h-CPUL1-treated, 48h-CPUL1-treated, and QC groups in negative mode. (H) PLS-DA score plot of control and treated cells in negative mode. (I) OPLS-DA analysis between the control and the 6h-CPUL1-treated groups showed a good separation trend in negative mode. (J) OPLS-DA analysis between the control and the 48h-CPUL1-treated groups showed a good separation trend in negative mode. (K) Permutations plot analysis between the control and the 6h-CPUL1-treated groups showed the intersection of the Q2 regression line and the y-axis was less than zero in negative mode, indicating that the model was valid. (L) Permutations plot analysis between the control and the 48h-CPUL1-treated groups showed the intersection of the Q2 regression line and the y-axis was less than zero in negative mode, indicating that the model was valid.

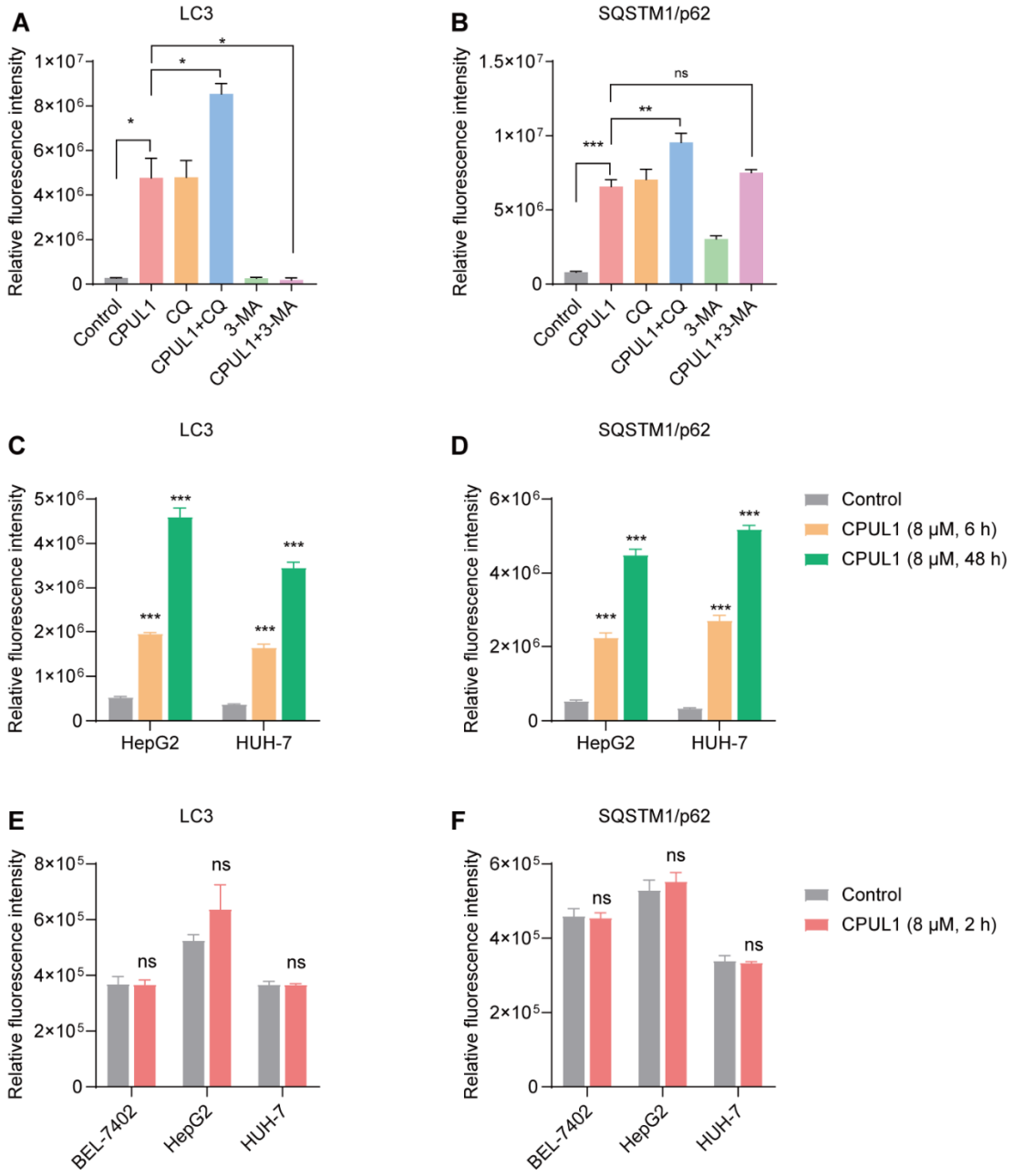

Figure S3 Quantitative analysis of fluorescent intensity of p62 and LC3 was performed to confirm the protein changes. (A, B) Fluorescence quantification of LC3 and p62 in BEL-7402 cells treated with CPUL1 (8 μM) and 3-MA (2 mM) or CQ (20 μM) for 48 h. (C, D) Fluorescence quantification of LC3 and p62 in HepG2 and HUH-7 cells treated with CPUL1 (8 μM) for 6 h or 48 h. (E, F) Fluorescence quanti-fication of LC3 and p62 in BEL-7402, HepG2 and HUH-7 cells treated with CPUL1 (8 μM) for 2 h.

Table S1. The summary of RNA sequencing data.

Samples	Raw reads (Mb)	Clean reads (Mb)	Q30%	GC%	Mapping rate
BEL-7402-1	40.79	40.44	94.69	52.60	92.22%
BEL-7402-2	36.59	36.30	94.42	52.65	93.03%
BEL-7402-3	41.87	41.56	94.49	52.78	92.71%
BEL-7402-6h-1	44.12	43.63	94.81	52.94	93.22%
BEL-7402-6h-2	41.23	40.90	94.52	53.26	93.01%
BEL-7402-6h-3	43.22	42.80	94.80	52.83	93.03%
BEL-7402-48h-1	47.02	46.71	94.83	53.29	93.37%
BEL-7402-48h-2	49.57	49.24	94.70	53.24	93.23%
BEL-7402-48h-3	38.61	38.29	95.01	53.31	93.46%