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Supplemental Information

S119N Mutation of the E3 Ubiquitin Ligase SPOP Suppresses SLC7A1 Degradation to Regulate Hepatoblastoma Progression

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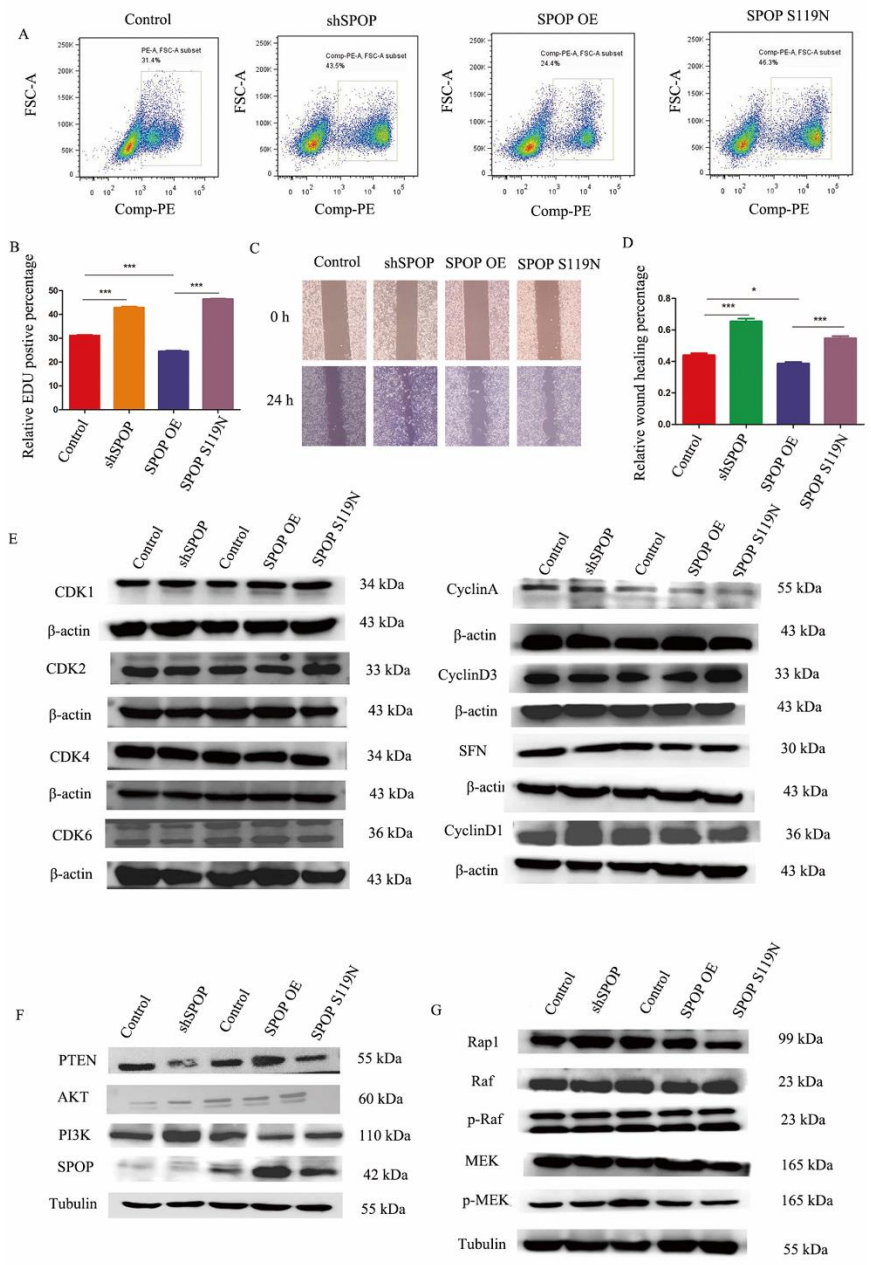

Figure S1. Impact on other phenotypes and signaling pathways.

(A and B) Relative EDU-positive percentage shown in the control, shSPOP, SPOP OE, or SPOP S119N group in HepG2 cells. (C and D) Relative wound-healing percentage shown after 0 and 24 h in HepG2 cells. (E) Western blot analysis for cell cycle proteins showed no difference in the control, shSPOP, SPOP OE, or SPOP S119N groups in HepG2 cells. (F) PI3K/Akt signaling pathway was activated by SPOP knockdown and S119N mutation, but inhibited by SPOP over-expression. (G) Rap/Raf/MEK signaling pathway showed no difference in the control, shSPOP, SPOP OE, or SPOP S119N groups in HepG2 cells.

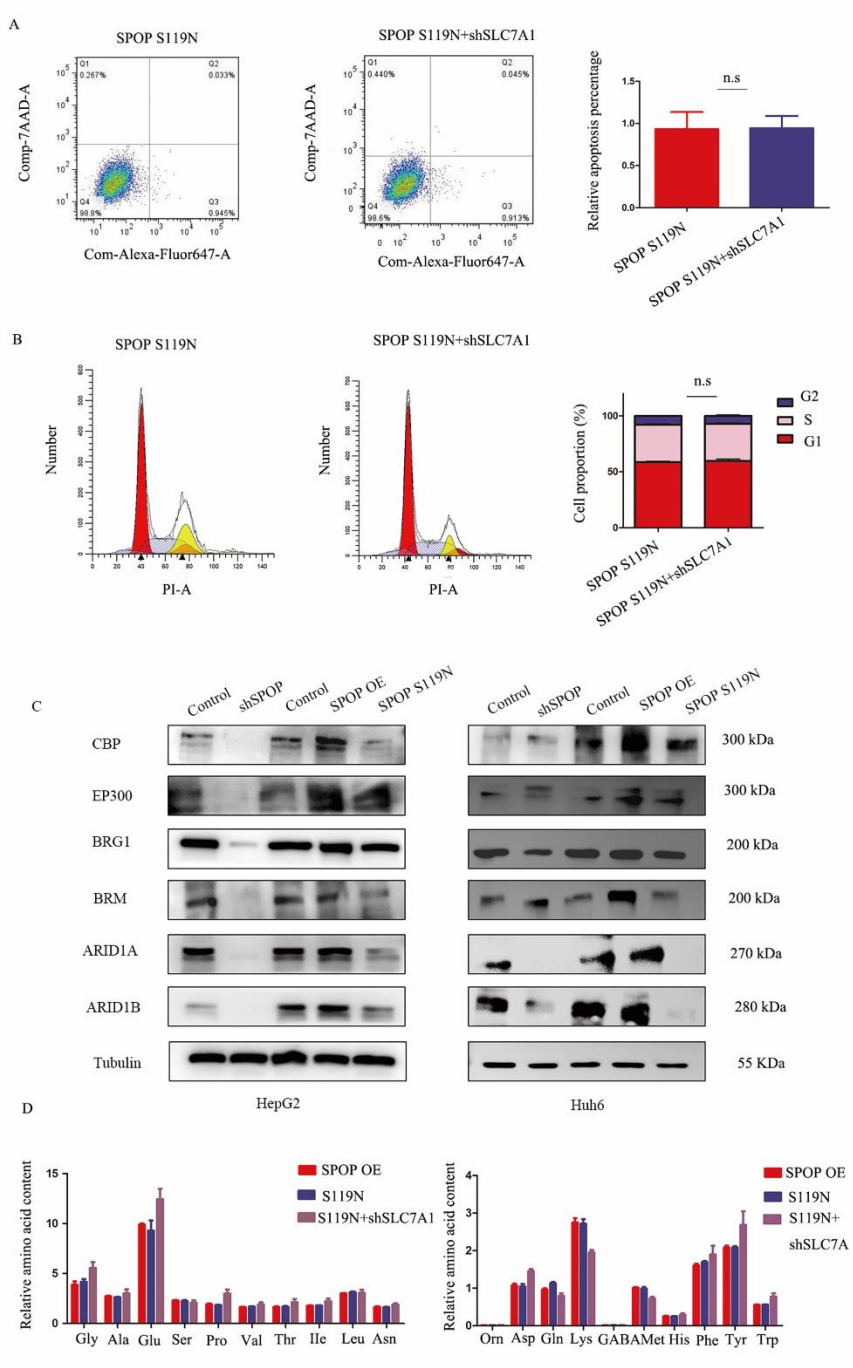

Figure S2. SPOP had an influence on SWI/SNF complex and arginine metabolism. (A) Representative results and statistical analysis of apoptosis by flow cytometry in SPOP S119N, and down-regulation of SLC7A1 in SPOP S119N of HepG2 cells. (B) Representative results and cell cycle analysis by flow cytometry in SPOP S119N, and down-regulation of SLC7A1 in SPOP S119N of HepG2 cells. (C) Western blot analysis showed SWI/SNF complex proteins decreased in SPOP knockdown and S119N mutation groups, but increased in the SPOP over-expressed groups.

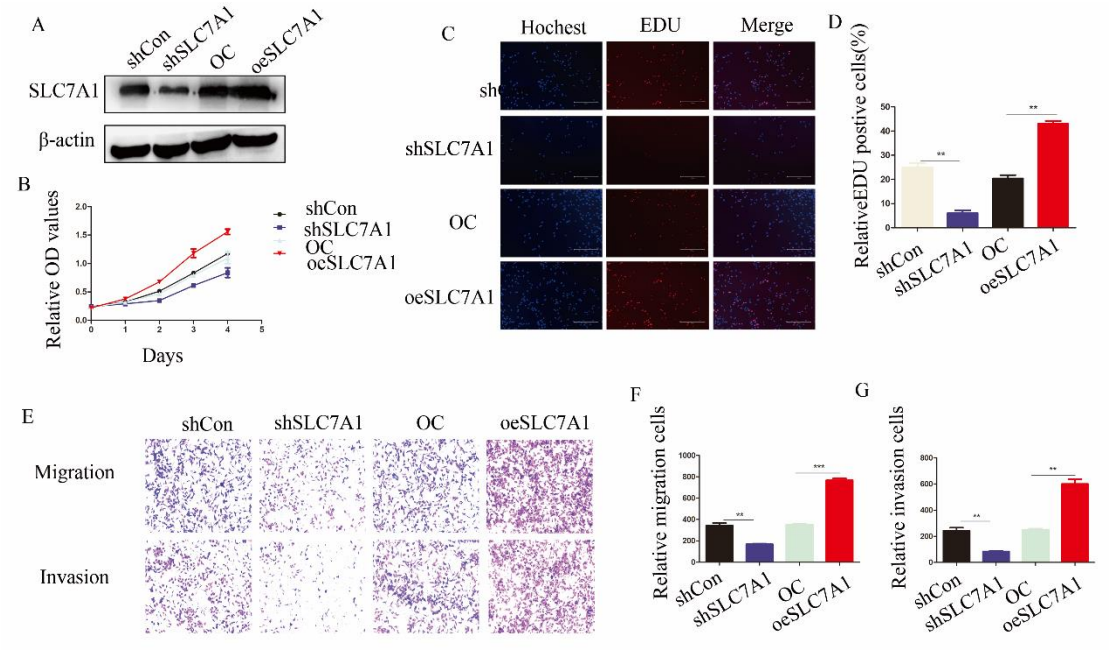

Figure S3. Down-regulation of SLC7A1 inhibited HepG2 cell proliferation, migration, and invasion.

(A) Western blot analysis showed down-regulation and up-regulation efficiency of SLC7A1. (B) CCK8 assay indicated that the silencing of SLC7A1 decreased cell proliferation, and the over-expression of SLC7A1 increased cell proliferation. (C and D) EDU assay results also showed that the knockdown of SLC7A1 inhibited cell proliferation, while the over-expression of SLC7A1 promoted cell proliferation. (E–G) Transwell assays showed that the down-regulation of SLC7A1 inhibited cell migration, invasion, and the over-expression of SLC7A1 promoted cell migration.

TableS1

Antibody information

Antibody	**Vendor**	**Catalog number**
SPOP	Proteintech	16750-1-AP
GAPDH	Proteintech	60004-1-Ig
β-Tubulin	Proteintech	10068-1-AP
β-Actin	Proteintech	60008-1-Ig
p21	CST	2947T
P27	CST	3686T
E-cadherin	CST	3195T
Vimentin	Santa Cruz	Sc-6206
N-cadherin	Proteintech	22018-1-AP
PI3K	Proteintech	20584-1-AP
AKT	CST	4691T
ERK	CST	4695T
p-ERK	CST	4370T
p38	CST	8690T
p-p38	CST	4511T
SLC7A1	Proteintech	14195-1-AP
CDK1	Proteintech	19532-1-AP
CDK2	Proteintech	0122-1-AP
CDK4	Proteintech	11026-1-AP
CDK6	Proteintech	14052-1-AP
CyclinA	CST	91500S
CyclinD1	Proteintech	60186-1-Ig
CyclinD3	Proteintech	26755-1-AP
SFN	Santa Cruz	Sc-166473
PTEN	Santa Cruz	Sc-7974
Rap1	Santa Cruz	Sc-28366
Raf	Santa Cruz	Sc-101504
p-Raf	Santa Cruz	Sc-271929
MEK	Santa Cruz	Sc-5294
p-MEK	Santa Cruz	Sc-166967
BRG1	Santa Cruz	Sc-374197
BRM	CST	11966T
CBP	CST	7389S
p300	CST	86377S
ARID1A	CST	12354S
ARID1B	CST	92964S