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SUPPLEMENTAL DATA

FDPS links tumor progression, phosphoproteomic reprogramming, and therapeutic vulnerability in hepatocellular carcinoma

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Full article is available at the following link: FDPS links tumor progression, phosphoproteomic reprogramming, and therapeutic vulnerability in hepatocellular carcinoma

Table S1. Sequences of small interfering RNAs targeting FDPS specifically

Sequences	Sense (5'-3')	Antisense (5'-3')
Homo-309	CCAGAAUUCAGAUGUUUAUTT	AUAAACAUCUGAAUUCUGGTT
Homo-785	GUUCCUAUCAGACUGAGAUTT	AUCUCAGUCUGAUAGGAACTT
Homo-939	GUACAUGGCAGGAAUUGAUTT	AUCAAUUCCUGCCAUGUACTT

Abbreviation: FDPS, farnesyl diphosphate synthase.

Table S2. Primer sequences and thermal cycling conditions

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC
FDPS	AGCACTTCTCCCAGATCGTTAG	GAATGCTACTACCACCGTCAAAC
Revers transcription	25°C (10s), followed by 42°C (15min) and 85°C (5s)	
qRT-PCR	94°C (30s), followed by 45 cycles of 94°C (5s) and 60°C (30s)	

Abbreviations: FDPS, farnesyl diphosphate synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR, quantitative real-time polymerase chain reaction.

Table S3. Antibodies used for Western blot analysis

Trade name	Manufacturer	Cat.No.	Dilution
FDPS Polyclonal antibody	Proteintech	16129-1-AP	1:1000
human BCL2 Polyclonal antibody	Proteintech	12789-1-AP	1:2000
Caspase 9/P53 Polyclonal antibody	Proteintech	10380-1-AP	1:1000

Phospho-Caspase 9 (Ser196) Polyclonal antibody	Proteintech	28794-1-AP	1:1000
Cleaved Caspase 3 Polyclonal antibody	Proteintech	25128-1-AP	1:1000
mTOR Antibody [C15M14]	Selleck	F0169	1:1000
Phospho-mTOR (Ser2448) Antibody [H1M6]	Selleck	F0158	1:1000
Beta Actin Polyclonal antibody	Proteintech	20536-1-AP	1:5000
Alpha Tubulin Polyclonal antibody	Proteintech	11224-1-AP	1:5000
Multi-rAb^{TM} HRP-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L)	Proteintech	RGAR001	1:10000

Abbreviations: BCL2, B-cell lymphoma 2; Cat. No., catalog number; FDPS, farnesyl diphosphate synthase; H+L, heavy and light chains; HRP, horseradish peroxidase; mTOR, mechanistic target of rapamycin; p53, tumor protein p53; Ser, serine.

Table S4. Number of subjects at risk in the *in vivo* pamidronate experiment

Time (days)	0	10	14	18	22	23
PBS	10	10	10	9	6	5
PAM	10	10	10	10	10	9

Abbreviations: PAM, pamidronate; PBS, phosphate-buffered saline.

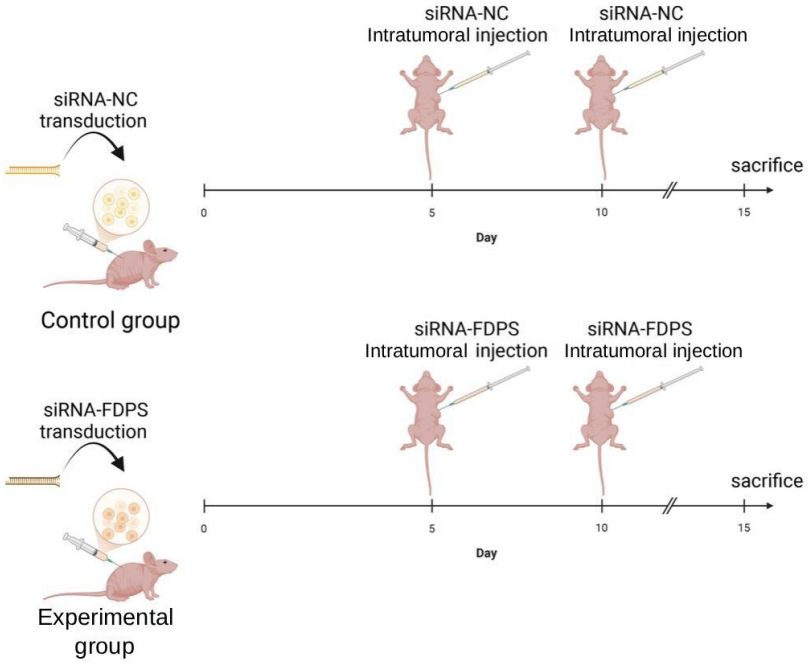


Figure S1. Experimental design and timeline of the nude mouse xenograft assay. Six-week-old male nude mice were randomly assigned to control and experimental groups (n = 8 per group). Subcutaneous xenografts were established on day 0 using Huh-7 cells transduced with siRNA-NC in the control group and Huh-7 cells transduced with siRNA-FDPS in the experimental group. To maintain gene-silencing efficiency *in vivo*, mice received intratumoral injections of the corresponding siRNA on days 5 and 10 after cell inoculation (5 nmol/20 g body weight). All animals were sacrificed on day 15 for tumor harvest and subsequent analyses. **Abbreviations**: FDPS, farnesyl diphosphate synthase; siRNA-FDPS, small interfering RNA targeting FDPS; siRNA-NC, negative control small interfering RNA.

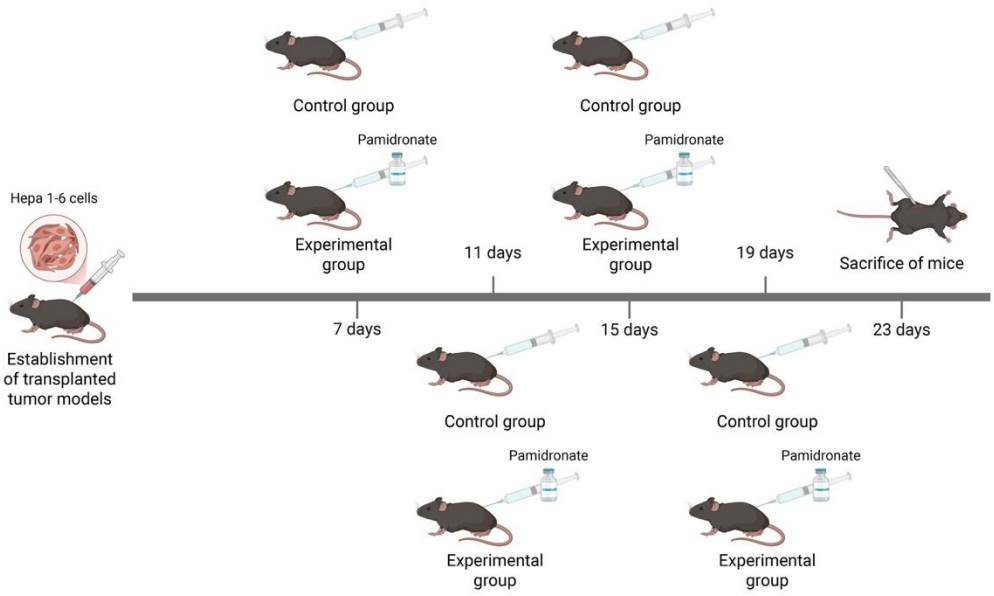


Figure S2. Experimental design and timeline of the *in vivo* pamidronate treatment assay. Subcutaneous transplanted tumor models were established in six-week-old male C57BL/6 mice by inoculation of Hepa1-6 cells (1×10^6 cells/mL, 0.1 mL per mouse) into the lower left flank on day 0. Mice were then randomly assigned to a control group or a pamidronate-treated group (n = 10 per group). Starting on day 7 after tumor cell inoculation, the experimental group received intraperitoneal pamidronate (5 mg/kg dissolved in PBS), whereas the control group received an equal volume of PBS, with treatments administered on days 7, 11, 15, and 19. All mice were sacrificed on day 23 for tumor harvest and subsequent analyses. **Abbreviation**: PBS, phosphate-buffered saline.

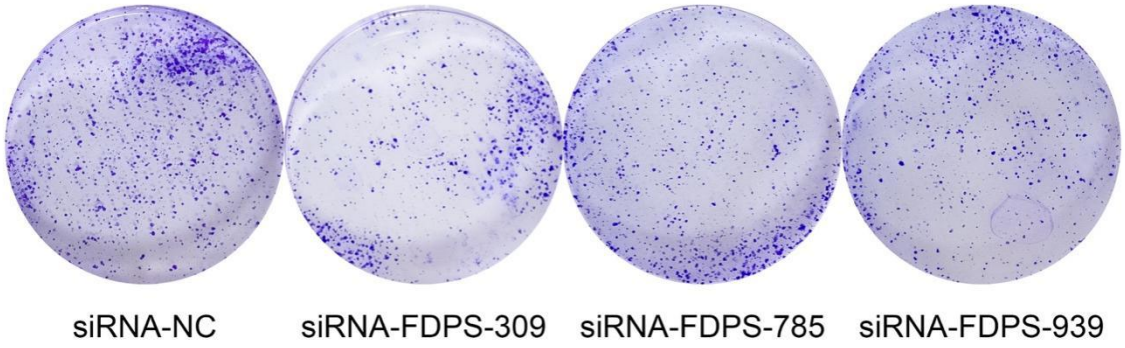


Figure S3. Three independent FDPS-targeting siRNAs exert consistent inhibitory effects on clonogenic growth in Huh-7 cells. Representative colony formation images of Huh-7 cells transfected with siRNA-NC or three different FDPS-targeting small interfering RNAs (siRNA-FDPS-309, siRNA-FDPS-785, and siRNA-FDPS-939). Compared with the negative control, all three FDPS-targeting siRNAs reduced colony formation, indicating a consistent inhibitory effect of FDPS knockdown on the proliferative/clonogenic capacity of Huh-7 cells and supporting the selection of Homo-785 for subsequent experiments. **Abbreviations**: FDPS, farnesyl diphosphate synthase; siRNA-FDPS, small interfering RNA targeting FDPS; siRNA-NC, negative control small interfering RNA.

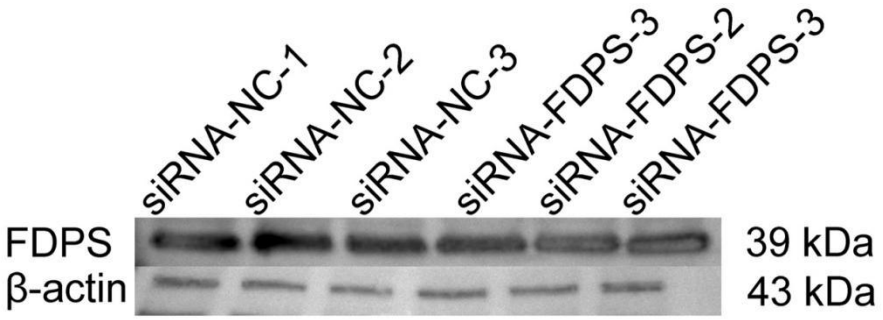


Figure S4. Representative Western blot analysis of FDPS expression in Huh-7 xenograft tumor tissues on day 15. Tumor tissues harvested from nude mice in the siRNA-NC and siRNA-FDPS groups were analyzed by Western blot to assess whether FDPS silencing was maintained *in vivo*. Compared with the control group, tumors from the siRNA-FDPS group showed reduced FDPS protein expression, confirming sustained knockdown after intratumoral administration and supporting the xenograft growth results shown in Figure 3. β-actin was used as the loading control. **Abbreviations**: FDPS, farnesyl diphosphate synthase; siRNA-FDPS, small interfering RNA targeting FDPS; siRNA-NC, negative control small interfering RNA.

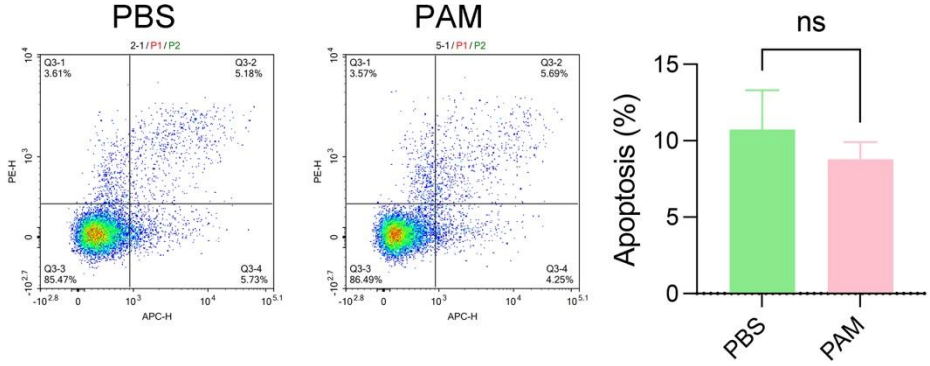


Figure S5. Pamidronate does not significantly alter apoptosis in Huh-7 cells. Representative Annexin V-APC/PI flow cytometry plots and quantitative analysis of apoptotic cells in Huh-7 cells treated with PAM or PBS control. In contrast to genetic FDPS knockdown, pamidronate treatment did not produce a significant change in the apoptotic rate, suggesting that the pro-apoptotic effect associated with FDPS depletion may not be fully mediated through its catalytic product. ns, not significant. Abbreviations: APC, allophycocyanin; PAM, pamidronate; PBS, phosphate-buffered saline; PI, propidium iodide.