

FDFT1 Acts as a Negative Regulator of Autophagy by Modulating AMPK–ULK1 Signaling in Hepatocellular Carcinoma Cells

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Abstract

Autophagy is a conserved catabolic process that degrades proteins and damaged organelles to maintain cellular homeostasis, and its role in cancer depends on stage and context. Farnesyl-diphosphate farnesyltransferase 1 (FDFT1) is an essential enzyme in the sterol branch of the mevalonate pathway, but its functions in hepatocellular carcinoma (HCC) and in the regulation of autophagy remain poorly understood. In this study, we show that FDFT1 acts as a negative regulator of autophagy in HCC cells. Loss of FDFT1 led to increased autophagosome formation and fusion with lysosomes, whereas its overexpression suppressed both basal and induced autophagy. These changes were associated with AMPK–ULK1 signaling, suggesting that FDFT1 influences a central pathway controlling autophagy. Our findings connect cholesterol metabolism with autophagy regulation and tumor growth, highlighting FDFT1 as a potential prognostic marker and therapeutic target in liver cancer.

Key Words: AMPK/ULK1, Autophagy, FDFT1, Liver cancer, Cholesterol

INTRODUCTION

Liver cancer is a growing global health concern. In 2020, about 906,000 new cases and 830,000 deaths were reported, making it the sixth most common cancer and the third leading cause of cancer-related death worldwide (Llovet *et al.*, 2021; Sung *et al.*, 2021). Hepatocellular carcinoma (HCC), the predominant form of liver cancer, arises in the context of chronic liver diseases and is associated with poor prognosis. Among the metabolic factors implicated in HCC, cholesterol has attracted increasing attention. Epidemiological studies have reported an inverse relationship between serum cholesterol levels and liver cancer incidence (Ding *et al.*, 2019; Zhou and Sun, 2021). Furthermore, low cholesterol levels correlate with poor postoperative outcomes in HCC patients, suggesting that dysregulated cholesterol metabolism contributes to liver cancer progression (Gao *et al.*, 2017; Jiang *et al.*, 2016; Lee *et al.*, 2016; Wang *et al.*, 2014).

Cholesterol homeostasis is controlled by dietary intake,

cellular efflux, and *de novo* synthesis through the mevalonate (MVA) pathway. Among these, the MVA pathway is of particular interest in cancer biology and other disease contexts, as many of its intermediates are indispensable for tumor cell growth and survival (Ding *et al.*, 2019; Hart *et al.*, 2015; Khan *et al.*, 2025). Among the enzymes involved in cholesterol biosynthesis, farnesyl-diphosphate farnesyltransferase 1 (FDFT1) plays a critical role by directing the metabolite farnesyl pyrophosphate (FPP) toward the sterol branch of the mevalonate (MVA) pathway. FDFT1 has been reported to act either as an oncogene or as a tumor suppressor, depending on the cancer type (Ha and Lee, 2020). For instance, it promotes metastasis and proliferation in lung cancer (Yang *et al.*, 2020), whereas reduced expression of FDFT1 has been associated with poor prognosis in gastric and colorectal cancers (Weng *et al.*, 2020; Zhao *et al.*, 2021). Moreover, it has been recently reported that FDFT1 knockdown can delay HCC progression by modulating the HNF4A/ALDOB/AKT1 signaling axis, highlighting the oncogenic role of FDFT1 in HCC development (Cai *et al.*, 2025).

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Autophagy is a conserved catabolic process that degrades long-lived proteins and damaged organelles to maintain cellular homeostasis (Lee *et al.*, 2024). The role of autophagy in cancer can vary depending on tumor stage and cellular context (Mathew *et al.*, 2007). For example, in HCC, autophagy can either protect against malignant transformation by promoting apoptosis or, conversely, support tumor progression by sustaining metabolic adaptation under hypoxic and nutrient-deprived conditions (Hashemi *et al.*, 2023). Importantly, cholesterol metabolism and autophagy are closely interconnected. Previous studies have shown that statin treatment or cholesterol depletion enhances autophagy gene expression and promotes autophagic activity (Shapira *et al.*, 2021; Wang *et al.*, 2015). These findings raise the possibility that FDFT1, as a pivotal enzyme in the sterol branch of the MVA pathway, may indirectly regulate autophagy by altering cholesterol flux. However, the direct role of FDFT1 in autophagy regulation has not been elucidated.

In this study, we sought to investigate whether FDFT1 regulates autophagy in HCC cells and to delineate the molecular mechanisms involved. Our results demonstrate that FDFT1 suppresses autophagic flux by inhibiting autophagosome formation and autophagosome-lysosome fusion, at least in part through modulation of the AMPK–ULK1 signaling pathway.

MATERIALS AND METHODS

Cell culture

Human liver cancer cell lines (HepG2 and Huh7) were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM (Welgene, Cat. LM 011-01) or RPMI 1640 (Welgene, Cat. LM 001-05) supplemented with 10% heat-inactivated FBS (Welgene, Gold Serum, Cat. S 001-07) and 1% penicillin–streptomycin (Welgene, 100×, Cat. LS 202-02) for routine maintenance and all experiments, except for those related to migration, invasion, and wound healing. Cells were maintained in a humidified incubator at 37°C with 5% CO₂. Chloroquine (CQ; C6628-25G, Sigma-Aldrich, USA) was dissolved in sterile water and used at a final concentration of 50 μM, and rapamycin (#9904, Cell Signaling Technology) was dissolved in DMSO and used at a final concentration of 100 nM to inhibit and induce autophagy, respectively. The final DMSO concentration in culture media did not exceed 0.1%, and an equivalent volume of vehicle was added to control wells.

Western blot analysis

Western blot analysis was performed as described previously (Rho *et al.*, 2025). Cells (4×10⁵/well) were seeded into 6-well culture plates and incubated overnight before transfection with FDFT1 siRNA, control siRNA (siCon), FDFT1 expression vector, empty vector (E.V.), or other indicated treatments. After treatment, cells were collected and lysed in 1× RIPA buffer (R4100-010, GenDEPOT) supplemented with phosphatase inhibitor cocktail (100×, P3200-001, GenDEPOT) and protease inhibitor cocktail (100×, P3100-001, GenDEPOT) for 30 min on ice. Lysates were centrifuged at 14,000×g for 15 min at 4°C, and the supernatants were collected and stored at –80°C until use. Protein concentrations were determined in duplicate using the Pierce Coomassie (Bradford) Protein Assay Kit (Cat. 23200, Thermo Fisher Scientific).

SDS-PAGE and immunoblotting were performed using standard methods with the following primary antibodies: FDFT1 (ab195046, Abcam); LC3A/B (#12741, CST); β-actin (sc-47778, SCBT); GAPDH (sc-32233, SCBT); TOM20 (#42406, CST); AMPKα (#2532, CST); phospho-AMPKα Thr172 (#2535, CST); phospho-ULK1 Ser317 (#12753, CST). HRP-conjugated goat anti-rabbit IgG (S A002-500, GenDEPOT) and m-IgGκ BP-HRP (sc-516102, SCBT) were used as secondary antibodies.

Plasmid and small-interfering RNA

FDFT1 cDNA (Flag-FDFT1-constructed plasmid) and the empty control plasmid (Flag-constructed plasmid) were obtained from the Korean Cell Bank. FDFT1 siRNA (h) (sc-61610, Santa Cruz Biotechnology) and its control siRNA were purchased from Santa Cruz Biotechnology. The FDFT1 siRNA (h) is a pool of three different siRNA duplexes: sc-61610A (sense: CACUUUGGCUGCCUGUUAUtt, antisense: AUAA-CAGGCAGCCAAAGUGtt), sc-61610B (sense: CCAUCUAC-CUGUCGUUUGUtt, antisense: ACAACGACAGGUAGAUG-Gtt), and sc-61610C (sense: GUAUGAUCCUGGCUAGAAUtt, antisense: AUUCUAGCCAGGAUCAUActt). Transfections were performed using Lipofectamine™ 3000 and P3000™ reagents (Thermo Scientific) according to the manufacturer's protocol in penicillin/streptomycin-free medium. HepG2 and Huh7 cells were seeded and cultured overnight at 37°C to reach 75–80% confluence before transfection. Six hours after transfection, the medium was replaced with fresh culture medium, and the cells were subsequently used for downstream experiments. Transfection efficiency was confirmed by western blotting.

Autophagic flux assay using adenoviral mCherry–GFP–LC3 construct

HepG2 and Huh7 cells transfected with siRNA or FDFT1 plasmids were seeded at densities of 1×10⁵ and 2×10⁵ cells/well, respectively, on sterile glass coverslips in 12-well plates and incubated overnight. Cells were then infected with adenovirus encoding mCherry–GFP–LC3 (Ad-mCherry–GFP–LC3), which was a kind gift from Professor Kim Sang Gun (Dongguk University) (Kim *et al.*, 2021), in DMEM containing 10% FBS. After 6–8 h, the medium was replaced with fresh culture medium. After 24 h, cells were washed with DPBS, fixed with 4% (w/v) paraformaldehyde for 15 min at room temperature, and mounted using antifade medium containing DAPI (VECTASHIELD® Hardset, Vector Laboratories). Images were acquired with a confocal laser scanning microscope (K1-Fluo) using 60× or 100× oil-immersion objectives. Autophagosomes were identified as mCherry+GFP+ puncta (yellow), and autolysosomes as mCherry+GFP– puncta (red only).

Immunofluorescence analysis

HepG2 and Huh7 cells transfected with siRNA or vector plasmids were seeded at densities of 1×10⁵ and 2×10⁵ cells/well, respectively, on sterile glass coverslips placed in 12-well plates and incubated for 36 h. Following experimental treatments, cells were briefly washed with DPBS and fixed in 4% (w/v) paraformaldehyde for 10 min at room temperature. Cells were permeabilized with 0.1% Triton X-100 in 3% BSA for 15 min, blocked in 3% BSA in DPBS for 30 min, and washed three times with DPBS. Primary antibodies, including the LAMP1 antibody (1:200; Transduction Laboratories, Lexington,

ton, Kentucky, USA), were diluted in 0.1% Triton X-100 in 3% BSA and incubated with cells overnight at 4°C. After washing with DPBS, cells were incubated for 1 h at room temperature with the following secondary antibodies: Alexa Fluor® 594 goat anti-mouse IgG (A11005) and Alexa Fluor™ 488 donkey anti-rabbit IgG (A21206). Cells were washed, mounted onto slides with DAPI-containing antifade mounting medium (VECTA-SHIELD® Hardset with DAPI, Vector Laboratories), and imaged using a confocal laser scanning microscope (K1-Fluo).

Statistical analysis

Data are presented as mean ± SEM, as indicated in the figure legends, from three independent experiments unless otherwise specified. Differences between groups were analyzed using two-tailed unpaired Student's t-test, or Two-way Anova with $p < 0.05$ considered statistically significant.

RESULTS

Knockdown of FDFT1 promotes autophagic flux

Given that cholesterol levels influence autophagy (Cheng *et al.*, 2006; Shapira *et al.*, 2021), we examined whether FDFT1, a key enzyme in the MVA pathway for cholesterol biosynthesis, contributes to autophagy regulation in liver cancer cells. To assess the impact of FDFT1 on autophagosome formation, LC3-II levels were measured by western blotting in HepG2 and Huh7 cells transfected with FDFT1 siRNA. Notably, FDFT1 knockdown increased endogenous LC3-II levels in both cell lines compared to control cells (Fig. 1A, 1B). An increase in LC3-II levels can reflect either enhanced autophagic flux or impaired degradation. To distinguish between these possibilities, we treated cells with chloroquine (CQ), which inhibits autophagosome-lysosome fusion and thus blocks LC3-II degradation (Ding and Yin, 2009). Upon CQ treatment, increased autophagosome formation results in greater accumulation of LC3-II, whereas reduced autophagic degradation does not cause an additional increase beyond CQ's effect

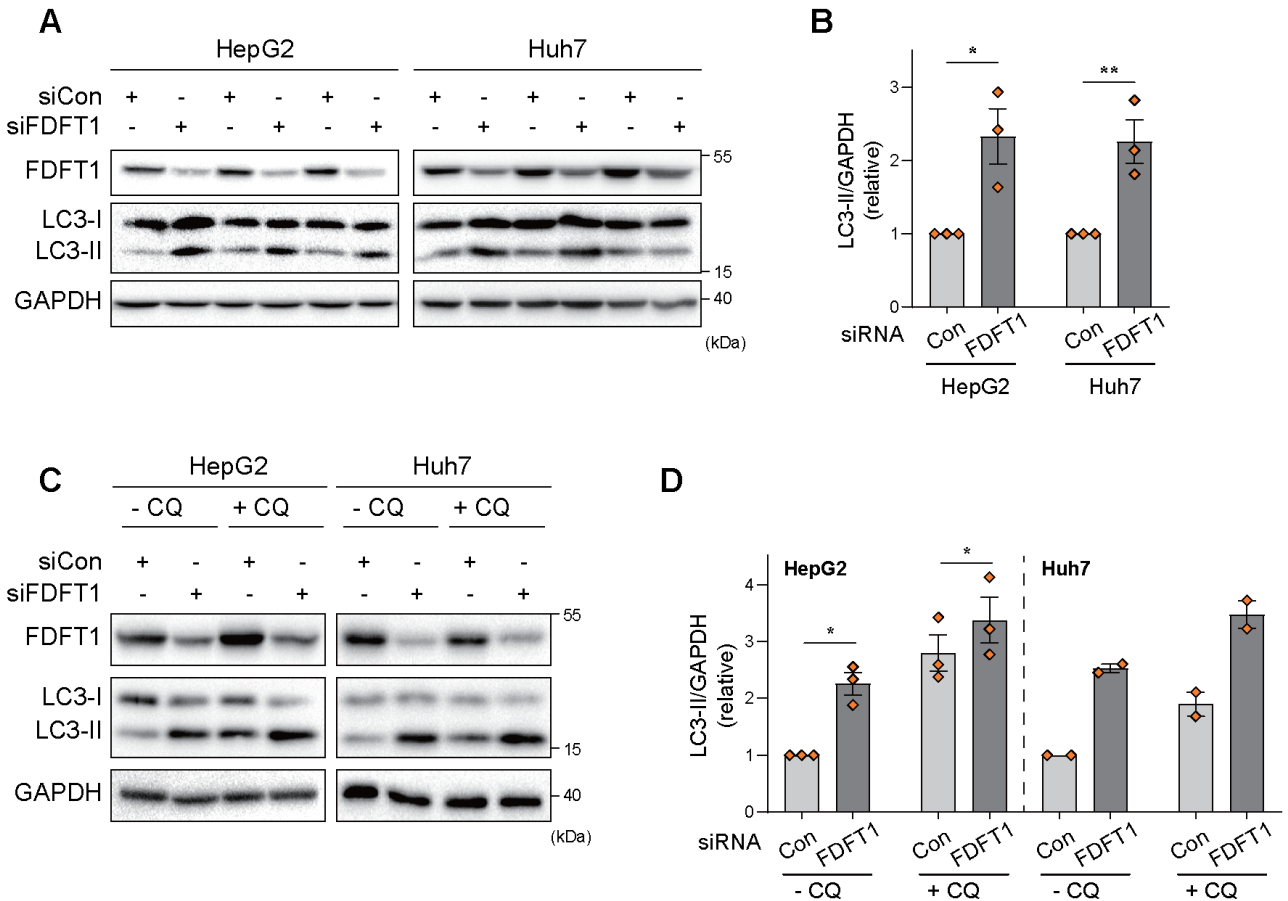


Fig. 1. Knockdown of FDFT1 promotes autophagic flux in HCC cells. (A) Western blot analysis showing protein levels of FDFT1, LC3-I, and LC3-II in HepG2 and Huh7 cells transfected with control siRNA (siCon) or FDFT1 siRNA (siFDFT1). (B) Quantification of LC3-II levels relative to GAPDH in (A), normalized to the corresponding control cells; data from three independent experiments are plotted (error bars, SEM). (C) Western blot analysis of FDFT1, LC3-I, and LC3-II in the same cell lines transfected with siCon or siFDFT1, with or without chloroquine (CQ, 50 μ M) treatment. (D) Quantification of LC3-II levels relative to GAPDH in (C), normalized to the corresponding control cells; data from three (HepG2) and two (Huh7) independent experiments are plotted (error bars, SEM). The positions of molecular-mass markers (in kDa) are indicated to the right of the blots. * $p < 0.05$, ** $p < 0.01$ using a two-tailed Student's t-test.

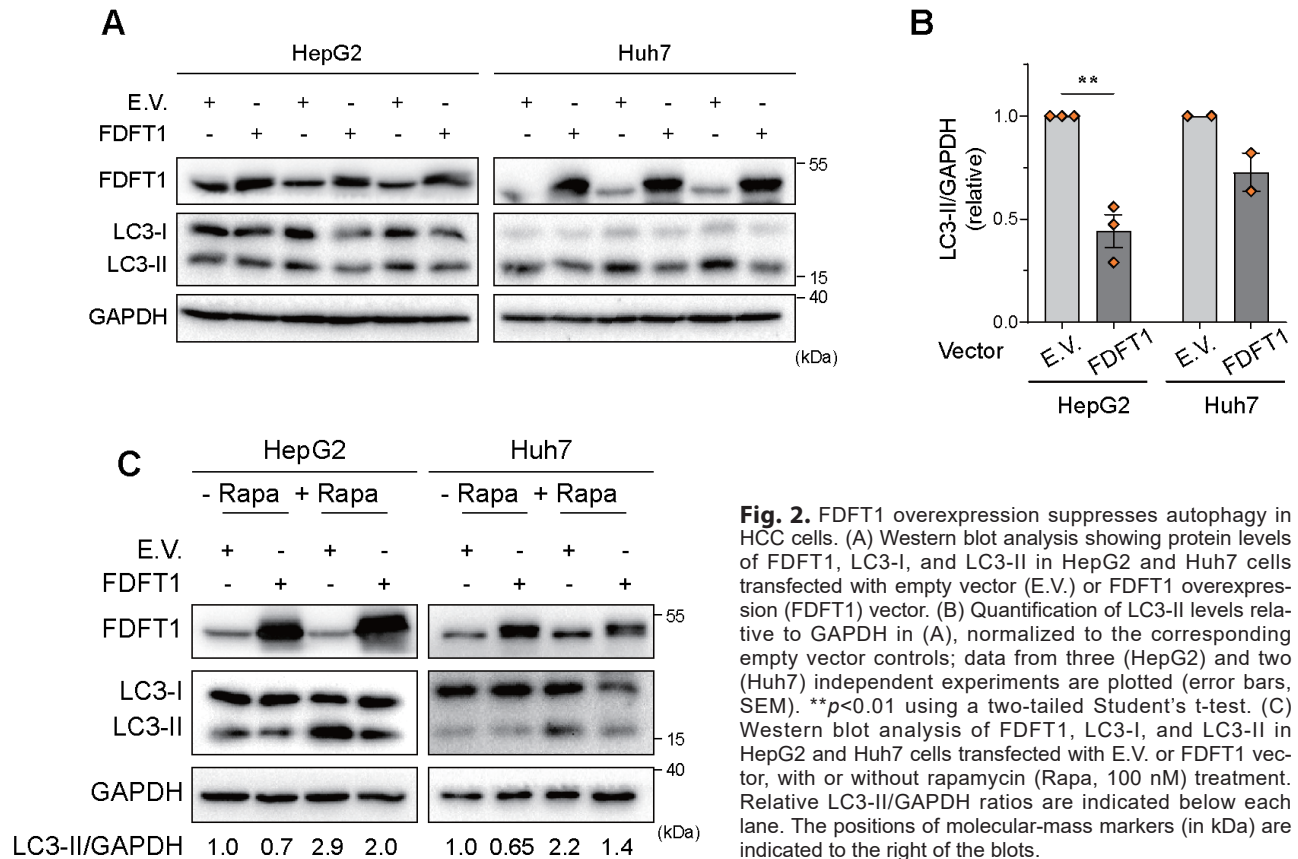


Fig. 2. FDFT1 overexpression suppresses autophagy in HCC cells. (A) Western blot analysis showing protein levels of FDFT1, LC3-I, and LC3-II in HepG2 and Huh7 cells transfected with empty vector (E.V.) or FDFT1 overexpression (FDFT1) vector. (B) Quantification of LC3-II levels relative to GAPDH in (A), normalized to the corresponding empty vector controls; data from three (HepG2) and two (Huh7) independent experiments are plotted (error bars, SEM). $**p < 0.01$ using a two-tailed Student's t-test. (C) Western blot analysis of FDFT1, LC3-I, and LC3-II in HepG2 and Huh7 cells transfected with E.V. or FDFT1 vector, with or without rapamycin (Rapa, 100 nM) treatment. Relative LC3-II/GAPDH ratios are indicated below each lane. The positions of molecular-mass markers (in kDa) are indicated to the right of the blots.

(Mizushima *et al.*, 2010). As shown in Fig. 1C and 1D, CQ treatment further increased LC3-II levels in both control and FDFT1 knockdown cells, indicating that FDFT1 knockdown promotes autophagic flux.

Overexpression of FDFT1 suppresses basal and rapamycin-induced autophagy

To complement the knockdown results, we next examined whether increased expression of FDFT1 alters autophagy in liver cancer cells. HepG2 and Huh7 cells transfected with an FDFT1 expression vector showed a clear reduction in basal LC3-II levels compared with empty vector controls (Fig. 2A, 2B). This result suggests that elevating FDFT1 expression is sufficient to suppress autophagic flux under steady-state conditions. We further tested the response to rapamycin, a well-established inducer of autophagy through mTOR inhibition. As expected, rapamycin treatment increased LC3-II accumulation in control cells. However, FDFT1 overexpression effectively suppressed rapamycin-induced LC3-II accumulation (Fig. 2C). These findings indicate that FDFT1 overexpression inhibits autophagy in both basal and rapamycin-treated conditions. Taken together, our observations suggest that FDFT1 functions as a negative regulator of autophagy in liver cancer cells.

FDFT1 negatively regulates autophagosome generation and maturation

To further assess the role of FDFT1 in autophagy, we evaluated autophagosome formation by quantifying LC3 puncta, a

widely used marker for autophagosomes. FDFT1 knockdown increased puncta numbers compared with control cells (Fig. 3A, 3B, left). In contrast, FDFT1 overexpression led to a clear reduction in puncta formation (Fig. 3A, 3B, right). Since an increase in LC3 puncta does not necessarily indicate enhanced autophagic flux, we next examined autophagosome-lysosome fusion to determine whether the increase in LC3 dots was indeed accompanied by their delivery to lysosomes. The number of LC3 puncta colocalized with LAMP1 was increased in FDFT1-knockdown cells, indicating that enhanced autophagosome-lysosome fusion occurred (Fig. 3C, 3D, left). In contrast, FDFT1 overexpression significantly reduced LC3-LAMP1 colocalization compared with vector controls, indicating that FDFT1 suppressed autophagosome maturation (Fig. 3C, 3D, right).

We next examined whether FDFT1 affects autophagosome-lysosome fusion with a tandem fluorescently-tagged LC3 construct (mCherry-GFP-LC3), which emits both GFP and mCherry signals (yellow) in autophagosomes but only the mCherry signal in autolysosomes due to quenching of GFP in acidic environments (Kimura *et al.*, 2007). FDFT1 knockdown markedly increased both yellow and red-only puncta, while overexpression led to a significant reduction in their number (Fig. 3E-3L). These results suggest that FDFT1 negatively regulates autophagosome-lysosome fusion. Consistent with the Western blot data, CQ treatment increased the accumulation of yellow puncta in FDFT1 knockdown cells compared to controls, supporting the role of FDFT1 in autophagosome formation. Similarly, FDFT1 overexpression inhibited both au-

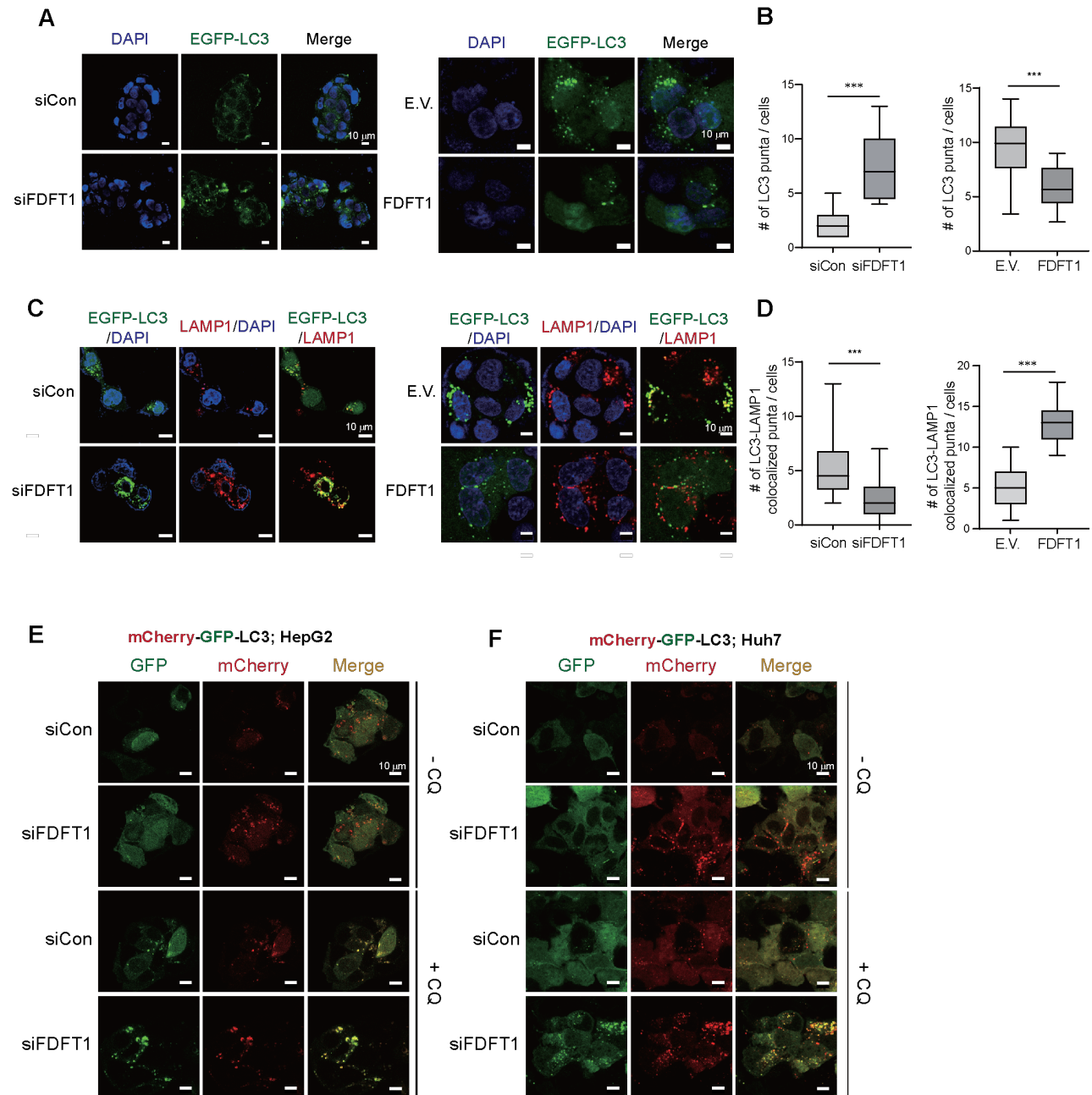


Fig. 3. FDFT1 negatively regulates autophagosome formation and autophagosome-lysosome fusion in HCC cells. (A) Representative images of HepG2 cells transfected with control siRNA (siCon), FDFT1 siRNA (siFDFT1), empty vector (E.V.), or FDFT1 expression vector and expressing EGFP-LC3 (kindly provided by Professor Sung Hoon Lee, Chung-Ang University). Nuclei were stained with DAPI (blue). Scale bars, 10 μ m. (B) Quantification of LC3 puncta per cell corresponding to panel (A). (C) Representative images showing the colocalization of EGFP-LC3 (green) and LAMP1 (red) in HepG2 cells. Nuclei were stained with DAPI (blue). Scale bars, 10 μ m. (D) Quantification of colocalized LC3-LAMP1 puncta per cell. (E, F) Representative fluorescence images of HepG2 (E) and Huh7 (F) cells transduced with mCherry-GFP-LC3, showing the effects of FDFT1 knockdown (siFDFT1) versus control (siCon) with or without chloroquine (CQ, 50 μ M). GFP (green), mCherry (red), and merged signals are shown. Scale bars, 10 μ m. (G, H) Quantification of autophagosomes (yellow puncta) and autolysosomes (red puncta) per cell, corresponding to the images in panels (E) and (F). (I, J) Representative mCherry-GFP-LC3 fluorescence images of HepG2 (I) and Huh7 (J) cells transfected with an empty vector (E.V.) or FDFT1 expression vector and treated with or without rapamycin (Rapa, 100 nM). GFP (green), mCherry (red), and merged signals are shown. Scale bars, 10 μ m. (K, L) Quantification of autophagosomes (yellow puncta) and autolysosomes (red puncta) per cell in panels (I) and (J). Data in (B, D) are box-whisker plots indicating the median, interquartile range, and whiskers extending to the minimum and maximum values; *** p <0.001. Data in (G, H, K, L) represent mean $\pm$ SEM from three independent experiments; ** p <0.01, *** p <0.001, and ns: non-significant using Two-way ANOVA with Tukey's multiple comparisons test.

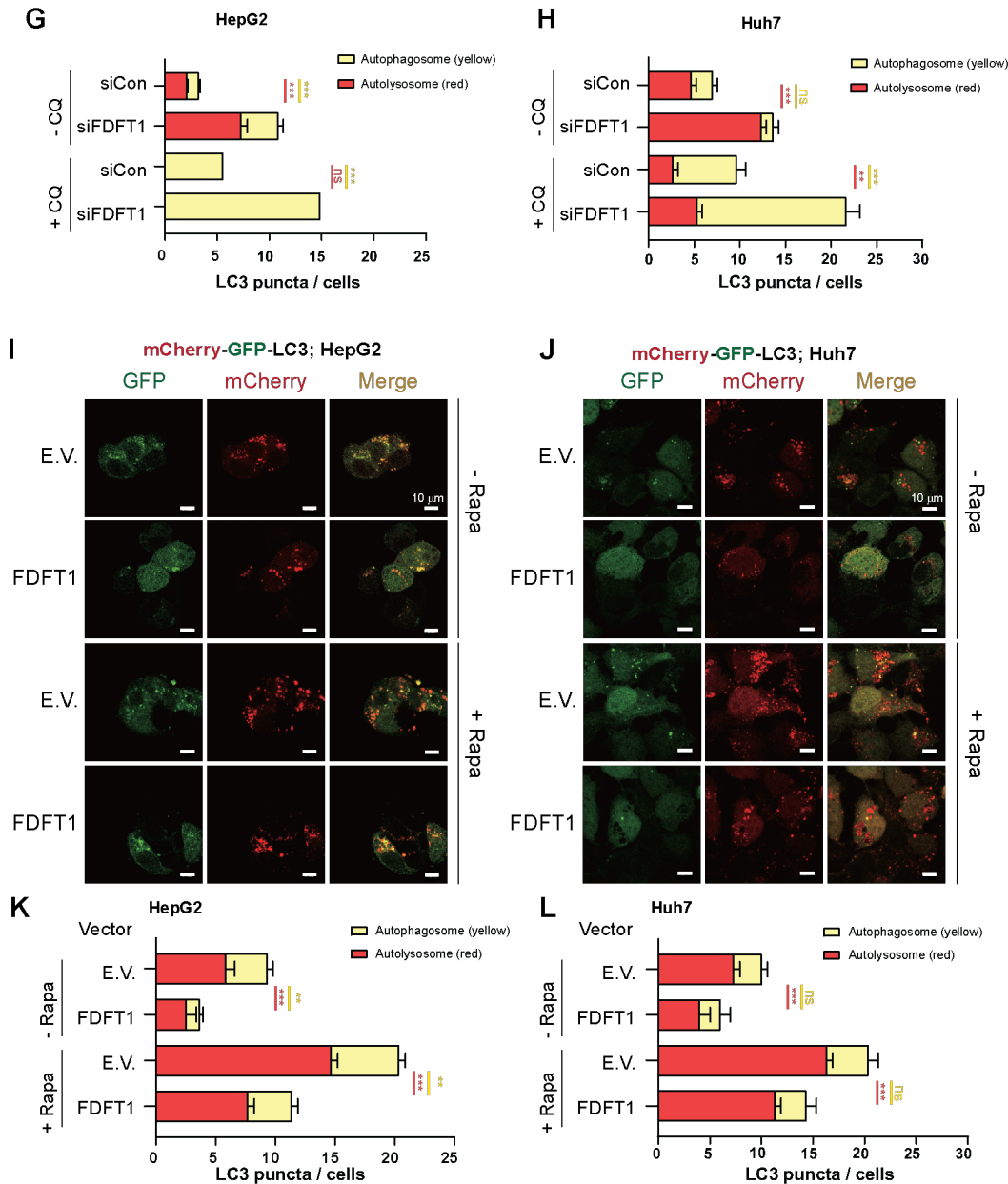


Fig. 3. Continued.

tophagosome generation and autophagosome–lysosome fusion under basal and rapamycin-treated conditions. Together, these findings indicate that FDFT1 suppresses autophagy in HCC cells by inhibiting autophagosome formation and modulating autophagosome–lysosome fusion.

FDFT1 modulates AMPK–ULK1 signaling axis

Because AMPK–ULK1 signaling is a major pathway that promotes autophagy initiation, we next tested whether FDFT1 influences this axis. Knockdown of FDFT1 increased phosphorylation of both AMPK and its downstream target ULK1 in HepG2 and Huh7 cells compared with control siRNA (Fig. 4A). In contrast, enforced expression of FDFT1 reduced phosphor-

ylation of AMPK and ULK1 relative to empty vector controls. These data suggest that FDFT1 suppresses autophagy, at least in part, by inhibiting the AMPK–ULK1 pathway.

DISCUSSION

In this study, we report that FDFT1 negatively regulates autophagy in HCC cells. Our results demonstrated that FDFT1 knockdown activated the AMPK–ULK1 signaling axis, thereby enhancing autophagosome generation and maturation. Previous studies have shown that inhibition of FDFT1 elevates intracellular levels of geranylgeranoic acid, which has been

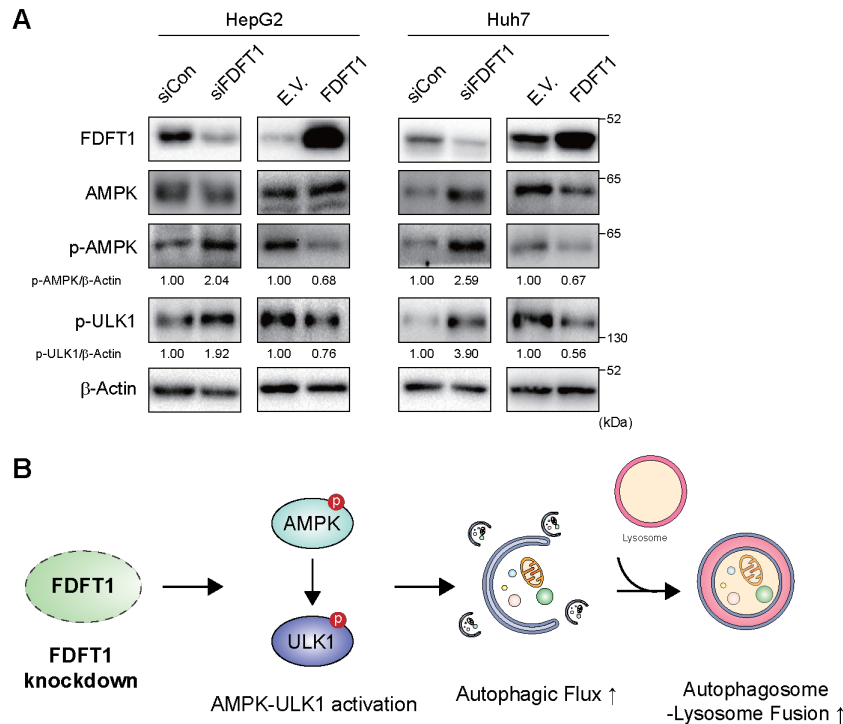


Fig. 4. FDFT1 negatively regulates AMPK–ULK1 signaling in HCC cells. (A) Western blot analysis of FDFT1, AMPK, phosphorylated AMPK (p-AMPK, Thr172), and phosphorylated ULK1 (p-ULK1, Ser317) in HepG2 and Huh7 cells transfected with control siRNA (siCon), FDFT1 siRNA (siFDFT1), empty vector (E.V.), or FDFT1 expression vector. β -Actin was used as a loading control. Relative phosphorylation levels (p-AMPK/ β -Actin, p-ULK1/ β -Actin) are indicated below each blot. The positions of molecular-mass markers (in kDa) are indicated to the right of the blots. (B) Proposed model for the role of FDFT1 in autophagy regulation. Knockdown of FDFT1 activates the AMPK–ULK1 pathway, leading to enhanced autophagic flux and increased autophagosome–lysosome fusion.

linked to mitochondrial ROS production and autophagy induction (Okamoto *et al.*, 2011; Shidoji and Tabata, 2019). Since ROS can promote autophagy through AMPK–ULK1 activation (Zhao *et al.*, 2025), it is possible that the effects we observed following FDFT1 knockdown are mediated, at least in part, by ROS signaling downstream of GGA accumulation. Although this remains to be experimentally validated, our findings suggest a plausible mechanism by which FDFT1 regulates autophagy. Based on these observations, we propose a working model illustrating the potential role of FDFT1 in controlling autophagy through AMPK–ULK1 signaling (Fig. 4B).

While our study reveals that FDFT1 functions as a negative regulator of autophagy in HCC, its role, as noted, is highly context-dependent. FDFT1 has been reported to act as an oncogene in several malignancies. For instance, in HCC, FDFT1 is highly expressed, strongly correlates with poor prognosis (Cai *et al.*, 2025), and promotes HCC progression by modulating cholesterol biosynthesis via the HNF4A/ALDOB/AKT1 (Cai *et al.*, 2025) and NeuroD1/KAT2A (Wu *et al.*, 2025) signaling axes. Indeed, genetic or pharmacological inhibition of FDFT1 can reduce HCC growth by regulating the TAZ pathway (Saito *et al.*, 2023). This oncogenic role is similarly observed in glioblastoma (GBM), where FDFT1 expression is elevated in GBM stem cells (GSCs) and maintains their stemness by activating the Akt pathway. Knockdown of FDFT1 in GBM not only inhibits proliferation but also enhances sensitivity to the chemotherapeutic agent temozolomide (Mo *et al.*, 2024). Furthermore, FDFT1 has been reported to promote oncogenesis

in tongue squamous cell carcinoma (Chattopadhyay and Mallick, 2023) and in certain breast cancer contexts (Chen *et al.*, 2024).

Conversely, FDFT1 functions as a tumor suppressor in several other cancer types. In gastric cancer, FDFT1 is downregulated, and its high expression correlates with a better prognosis for patients (Zhao *et al.*, 2021). In ovarian cancer, FDFT1 is also considered a tumor suppressor, where its forced expression retards cancer cell proliferation and invasion (Lu *et al.*, 2020). Notably, in bladder cancer, FDFT1 is identified as a tumor suppressor, and its reduced expression is directly linked to cisplatin resistance (Azhar *et al.*, 2024). Moreover, FDFT1's mechanism of action is intricately linked beyond autophagy to apoptosis and ferroptosis. In breast cancer cells, FDFT1 is the direct target of Artemisitene, which induces apoptosis by modulating the TNFR1/NF- κ B/NEDD4 pathway (Chen *et al.*, 2024). Interestingly, in colorectal cancer (Duan *et al.*, 2025) and some triple-negative breast cancers (Zeng *et al.*, 2025; Zhang *et al.*, 2025), FDFT1 has been reported to act as a pro-ferroptotic driver, promoting this tumor-suppressive cell death mechanism.

Given that FDFT1 is a key enzyme in the cholesterol biosynthetic pathway, another possible explanation is that the observed cellular effects reflect cholesterol-dependent mechanisms. Cholesterol has been reported to influence various processes such as autophagosome formation, lysosomal function, and membrane dynamics, all of which are relevant to autophagy (Barbero-Camps *et al.*, 2018; Jaishy and Abel,

2016; Ng *et al.*, 2022). Moreover, altered cholesterol levels have been shown to affect cancer progression through modulating redox balance, membrane fluidity, lipid raft integrity, and the localization of signaling proteins, thereby impacting cell survival and proliferation (Maja and Tyteca, 2022; Riscal *et al.*, 2019; Xiao *et al.*, 2023). In this regard, it is plausible that the phenotypic effects observed upon FDFT1 modulation may reflect cholesterol-dependent mechanisms. However, although FDFT1 is involved in cholesterol biosynthesis, it is not the rate-limiting step of the pathway (Ha and Lee, 2020). As such, overexpression of FDFT1 alone is unlikely to significantly alter cholesterol synthesis under physiological conditions, where upstream flux through the mevalonate pathway is tightly regulated. This suggests that the phenotypic effects of FDFT1 overexpression may not be solely attributable to changes in cholesterol levels. Instead, our findings raise the possibility that FDFT1 may exert functions independent of its enzymatic role. It would be interesting to investigate whether other functions of FDFT1 are involved. Future studies directly measuring intracellular cholesterol and GGA levels, as well as exploring the therapeutic implications of targeting FDFT1 in HCC, will be necessary to fully elucidate its role in autophagy regulation.

This complex, dual role of FDFT1 necessitates a clear, biomarker-driven strategy for patient stratification in clinical trials. For therapeutic approaches aiming to inhibit FDFT1, the primary cohort would be patients with high FDFT1-expressing tumors, such as HCC (Cai *et al.*, 2025) and GBM (Mo *et al.*, 2024). Selection biomarkers could include not only high FDFT1 mRNA/protein levels but also the activation of its downstream effectors, such as high phospho-AKT (Cai *et al.*, 2025; Mo *et al.*, 2024) or high TAZ expression (Saito *et al.*, 2023). In the context of our findings, low basal p-AMPK/p-ULK1 levels (Fig. 4A) or high expression of FDFT1-stabilizing proteins like EMC2 in TNBC (Dong *et al.*, 2025) could further refine this group. Conversely, for strategies aiming to restore FDFT1 function—perhaps to overcome chemoresistance—the target cohort would be patients with low FDFT1 expression. This group is exemplified by bladder cancer (Azhar *et al.*, 2024), ovarian cancer (Lu *et al.*, 2020), and gastric cancer (Zhao *et al.*, 2021). Key stratification biomarkers for this cohort would be the high expression of FDFT1-suppressing microRNAs, specifically miR-146b-5p (in bladder cancer) (Azhar *et al.*, 2024), miR-4425 (in ovarian cancer) (Lu *et al.*, 2020), and miR-216a-5p (in gastric cancer) (Zhao *et al.*, 2021).

Overall, our findings reveal a previously unrecognized role of FDFT1 as a negative regulator of autophagy in HCC cells. We did not directly test how this regulation affects cancer progression, but recent studies have shown that FDFT1 promotes HCC growth through the HNF4A/ALDOB/AKT1 signaling axis (Cai *et al.*, 2025). Based on our results, it is possible that FDFT1 contributes to tumor development not only through metabolic signaling but also by blocking autophagy. These insights highlight the broader importance of FDFT1 in liver cancer and support its potential as a therapeutic target and prognostic marker. Future studies will be needed to further clarify how FDFT1-mediated suppression of autophagy influences cancer progression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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