

Dobutamine suppresses YAP1 signaling to disrupt tumor-stroma crosstalk in hepatocellular carcinoma

Prachi Sharma

Amity University

Pallavi Chakraborty

Shiv Nadar Institution of Eminence

Shaikh Maryam Ghufran

University of Alabama at Birmingham

Bornika Roy

Amity University

Basundhara Das

Amity University

Jaydeb Pal

Amity University

Shrishty Sakshi

University of Delhi

Shivani Jaiswal

Amity University

Rudra Nayan Das

Shiv Nadar Institution of Eminence

Shinjinee Sengupta

Amity University

Sampa Ghose

All India Institute of Medical Sciences (AIIMS)

Subhrajit Biswas

subhrajit.biswas9@gmail.com

Amity University

Research Article

Keywords:

Posted Date: October 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11112779/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Hepatocellular carcinoma progression is heavily driven by the tumor microenvironment, yet the specific functional contribution of stromal YAP1 signaling to paracrine cancer progression remains incompletely understood. We investigated the clinical and biological significance of stromal YAP1 signaling in HCC and evaluated the therapeutic potential of its pharmacological suppression using dobutamine.

Methods

YAP1 expression and clinical correlations were evaluated in TCGA-LIHC and paired human HCC tissues (n=92), alongside single-cell RNA sequencing datasets (GSE149614 and GSE189903). Human hepatic stellate cells (LX2), endothelial cells (HUVEC), and Hep3B liver cancer cells were used for functional assays. Proliferation, cell cycle distribution, migration, 3D spheroid growth, and Matrigel tube formation were evaluated following dobutamine treatment. Paracrine tumor-stroma communication was assessed using conditioned media crosstalk models. An *in vivo* zebrafish (*Danio rerio*) Hep3B xenograft model was established to quantify tumor burden, host survival, and target gene expression.

Results

Single cell transcriptomics and human tissue analyses revealed that active YAP1 signaling is concentrated within cancer-associated fibroblasts and endothelial cells, where reduced inhibitory YAP1 Ser127 phosphorylation significantly correlated with aggressive, unencapsulated human HCCs. Dobutamine induce YAP-Ser127 phosphorylation, promoted cytoplasmic YAP1 retention, and reduced expression of YAP-responsive genes (CTGF, CYR61, ANKRD1) in LX2 and HUVECs without cytotoxicity. This attenuated stellate cell activation markers (COL1A1, FN1, α SMA) and impaired endothelial tube formation alongside reduced VEGFR2 and Ang-2 expression. Furthermore, conditioned media from dobutamine-treated stromal cells significantly reduced Hep3B proliferation, migration, 3-D spheroid growth, and epithelial-mesenchymal transition (N-cadherin, Vimentin) by enhancing pYAP-Ser127 levels in recipient cancer cells. In zebrafish xenografts, dobutamine significantly improved host survival (62% vs. 25%, $p < 0.05$), reduced tumor coverage area, and suppressed downstream YAP1 targets (*ccn2a*, *cyr61*) and vascular markers (*acta2*, *vegfc*).

Conclusions

Pharmacological suppression of stromal YAP1 signaling by dobutamine effectively attenuates stromal activation, angiogenesis, and paracrine tumor-stroma crosstalk, providing a therapeutic strategy to target the HCC microenvironment.

Introduction

Hepatocellular carcinoma (HCC) constitutes approximately 75-85% of primary liver cancers and remains a leading cause of cancer-related mortality. GLOBOCAN 2024 estimates, approximately 843,000 new liver cancer cases and 732,000 deaths globally, highlighting the continued clinical burden of this disease [1]. Although resection and transplantation can provide curative treatment for early stage disease, recurrence is common, partly due to the tumor microenvironment (TME) [2]. The TME includes immune and stromal populations, including hepatic stellate cells (HSCs), and endothelial cells that support tumor growth, invasion, angiogenesis, and therapeutic resistance [3]. Despite advances in systemic therapies, including, multikinase and immune checkpoint inhibitors, treatment benefit remains limited in advanced HCC [4,5], highlighting the tumor-stroma axis as a potential therapeutic target.

Activated HSCs are key components of the tumor-supportive HCC microenvironment [6,7]. Following liver injury, quiescent HSCs acquire a myofibroblast-like phenotype and promote tumor progression through the release of growth factors, matrix-remodeling enzymes, and extracellular matrix (ECM) proteins [8–11]. They also contribute to an immunosuppressive TME [12,13]. Transforming growth factor- β (TGF- β) is a key mediator of HSC–HCC crosstalk, promoting fibrosis and HSC activation while enhancing tumor cell proliferation, invasion, and progression [14–16]. Although targeting stromal mediators such as TGF β , CTGF, TIMP-1, PDGF can attenuate these effects [17–20], inhibition of individual mediators may not sufficiently disrupt the broader stromal program. Endothelial cells provide an additional tumor-supportive compartment, with VEGF/VEGFR2 (vascular endothelial growth factor/vascular endothelial growth factor receptor 2) signaling driving angiogenesis and vascular remodeling that facilitate HCC growth and dissemination [21–24]. Thus targeting regulatory pathways that coordinate fibrogenic and endothelial stromal compartments may provide a broader strategy for disrupting HCC TME.

Yes-associated protein (YAP), the major transcriptional effector of the Hippo signaling, is an established regulator of tumorigenesis, metastasis, and therapeutic resistance [25,26]. Hippo kinases MST1/2 and LATS1/2 regulate YAP activity, with phosphorylation at Ser127 promoting 14-3-3 binding and cytoplasmic sequestration, thereby limiting YAP-TEAD dependent transcription [27–31]. In HCC, aberrant YAP activation promotes proliferation, survival, and epithelial-mesenchymal transition (EMT) [30,32,33]. Importantly, YAP also regulates stromal biology. In cancer-associated fibroblasts (CAFs), YAP1 activation promotes expression of pro-fibrotic and ECM-associated genes, including COL1A1, LOX, and ACTA2, whereas in endothelial cells it contributes to vascular remodeling and angiogenic responses [34,35]. However, the role of stromal YAP signaling in coordinating tumor-stroma communication in HCC remains incompletely defined.

Evidence from other tumor types suggest that stromal YAP represents a therapeutically relevant target. YAP inhibition in colorectal and pancreatic cancers can suppress fibroblasts or stellate cell activation and attenuate paracrine signaling that supports tumor progression [36,37]. However, the commonly used YAP-TEAD inhibitor Verteporfin has reported YAP-independent effects, limiting its specificity [38,39]. Drug repurposing therefore offers a promising strategy for identifying alternative pharmacological approaches to modulate YAP signaling [40,41].

Dobutamine is a clinically established sympathomimetic agent that activates β_1 , β_2 , and α_1 -adrenoceptors [42,43]. Although primarily used for cardiovascular indications, previous studies have suggested that dobutamine can inhibit YAP nuclear localization and exerts antitumor effects in other cancer models [44,45]. However, its effect on tumor-stroma crosstalk and stromal YAP signaling in HCC has not been examined.

Here, we examined the effects of dobutamine on YAP signaling and tumor-supportive functions in human hepatic stellate cell line (LX2) and human umbilical vein endothelial cells (HUVEC). Using LX2 and HUVECs, we assessed stromal proliferation, migration, activation, and angiogenic responses and investigated YAP Ser127 phosphorylation and localization as potential mechanisms. We further examined whether dobutamine-treated stromal cells altered the malignant properties and YAP signaling of HCC cells through paracrine communication. Finally, using a zebrafish xenograft model [46], we evaluated the *in vivo* effect of dobutamine on HCC tumor growth and dissemination. Together, these studies investigate stromal YAP signaling as a potential therapeutic target for disrupting tumor-stroma crosstalk in HCC.

Materials and methods

Publicly available transcriptomic data analysis

Normalized gene expression data for HCC and normal liver tissues were obtained from The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) datasets. Transcript abundance values expressed as transcripts per million (TPM) were used to compare YAP1 expression between normal liver tissues (n=50) and primary HCC tumors (n=371).

For stromal cell association analyses, publicly available human liver RNA-sequencing datasets were analyzed using fragments per kilobase of transcript per million mapped reads (FPKM)-normalized expression values, which were log₂-transformed prior to analysis. Correlation analyses were performed using markers of hepatic stellate cell activation and fibrosis (LOX, PDGFRB, COL3A1, SPARC and COL1A1) and endothelial/angiogenic markers (KDR, LYVE1 and FLT1).

Cell culture

Hepatocellular cell line (Hep3B) was obtained from the Institute of Liver and Biliary Sciences, New Delhi, India. HCC cells were regularly cultured in Dulbecco Modified Eagle Medium (DMEM) which was reconstituted and supplemented with 10% FBS., 100U/ml of penicillin and 100µg/ml of streptomycin (100U/ml Penstrep, Himedia).

Human activated HSCs cell line LX2 (a kind gift from Professor Scott L Friedman, Icahn School of Medicine, USA) were routinely cultured in high glucose DMEM supplemented with 2% (v/v) FBS. Human Umbilical Vein Endothelial Cells (HUVECs) were obtained from Indian Institute of Technology, New Delhi and propagated in ready to use Endothelial Cells Growth Media (ECGS, Sigma, # 211-500). All

experiments were conducted using HUVECs between 2 to 6 passages. All cells were grown at 37°C with 5% CO₂ in humidified atmosphere.

Collection of conditioned medium (CM)

Stromal cells were seeded at the density of 1×10^6 cells per 100mm dishes. As they reach 70% confluency, the cells were treated with dobutamine for 24h. Following treatment, cells were washed twice with sterile PBS followed by addition of 5ml of serum free media and overnight incubation for CM collection. The CM was collected from both treated and untreated dishes and centrifuged for 10min at 2000 rcf to remove cellular debris and dead cells. The resulting supernatant was filtered through a 0.22µm syringe filter (Millipore) to remove residual cellular contaminants. The filtered CM was aliquoted and stored at -80°C deep freezer (Thermo Fisher Scientific) until further use.

Co-culture

Hep3B cell line was seeded at 1×10^6 cells/well in 6-well plate and cultured in DMEM containing 10% (v/v) FBS (#RM1112, Himedia, India) at 37°C with 5% CO₂. At 70-80% confluency, cells were incubated for 48h with a 1:1 mixture of serum free Hep3B media and stromal cells' (LX2 and HUVEC).

MTT cell viability assay

Cell viability was determined using an MTT assay (HiMedia, #TC191). Cells were seeded in 96-well plates at 1×10^4 cells/well in DMEM supplemented with 2% FBS and allowed to attach for 24 h and 48 h. Cells were subsequently exposed to the indicated experimental conditions for an additional 24 h. MTT reagent was added to a final concentration of 0.5mg/ml and incubated for 4 h at 37°C. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570nm using a Multiskan multifunction microplate reader (Thermo Scientific, #51119100). Cell viability was expressed relative to the untreated control. All experiments were performed in triplicate.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was reverse transcribed using iScript cDNA synthesis kit (Biorad, #1708891) according to the manufacturer's instructions. The resulting cDNA was diluted 1:5 and subjected to quantitative PCR using SYBR Green PCR Master Mix (Applied Biosystems, #A25742). Amplification was performed under the following conditions: 95°C for 30 s, 52.9°C for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 10 min. ACTB (β-actin) was used as the internal reference gene. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Primer sequences were designed using Primer3Plus and are listed in **Supplementary Table S1, S2**.

Western blot analysis

Cells were lysed using RIPA buffer supplemented with protease inhibitor (Roche, #11697498001) and phosphatase inhibitor cocktail (Roche, #4906845001). Cells were incubated on ice with intermittent

vortexing and centrifuged at 12,000g for 30 min at 4°C. Protein concentrations were determined by the Bradford assay (Thermo Fisher Scientific, #20279). Equal amounts of protein (30µg) were separated on 8-12% SDS-polyacrylamide gels and transferred onto PVDF membranes (Bio-Rad, #1620177). Membranes were blocked in 5% skimmed milk or Bovine serum albumin (BSA) in 1X TBST for 1 h and incubated overnight at 4°C with primary antibodies, followed by HRP-conjugated secondary antibodies for 2 h at room temperature. Immunoreactive bands were visualized using Pierce ECL substrate (Thermo Fisher Scientific, #34094). Densitometric analyses were performed using ImageJ software, and protein expression levels were normalized to GAPDH. Antibody details are provided in **Supplementary Table S3**.

Immunofluorescence

Cells were cultured on gelatin-coated coverslips (Corning, #2850-18) in 12-well plates. Following treatment with dobutamine for 24 h, cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature and permeabilized in blocking buffer containing 5% BSA and 0.3% Tritonx-100 for 1 h. Cells were subsequently incubated with primary antibodies overnight at 4°C in a humidified chamber. After washing with PBST, cells were incubated with fluorophore-conjugated secondary antibodies for 2 h at room temperature in the dark. Nuclei were counterstained with DAPI, and coverslips were mounted using Fluoroshield (Sigma-Aldrich, #F6182-20ML). Fluorescence images were acquired using a confocal laser scanning microscope (Leica TCS SP8). Antibody details are provided in **Supplementary Table S3**.

Scratch wound migration assay

Cells were seeded in 12-well plates at a density of 1×10^5 cells per well and grown to confluence. A linear wound was generated using a sterile 200µl pipette tip, and detached cells were removed by washing with PBS. Images were acquired immediately after scratching (0h) using an inverted microscope.

Cells were subsequently incubated under different conditions for 24 h. Wound closure was monitored by imaging the same fields at 24 h and 48 h. Migration was quantified using ImageJ software and expressed as the percentage of wound closure relative to the initial wound area. Four randomly selected fields were analyzed per well.

Transwell migration assay

Cell migration was assessed using Boyden chambers (24-well transwell inserts with 8µm pore size polycarbonate membrane). LX2 (1×10^5 cells) or HUVEC cells (5×10^4) were suspended in 200µl serum-free medium and seeded into the upper chamber, while 600µl complete growth medium was added to the lower chamber as a chemoattractant. After 24 h, non-migrated cells were removed from the upper surface of the membrane using a cotton swab. Cells that migrated to the lower surface were fixed with 20% methanol, stained with 0.25% crystal violet, and observed under a light microscope. Quantification was done using ImageJ software.

For a separate experiment, Hep3B cells (1×10^5) suspended in CM (diluted 1:1 with DMEM) derived from dobutamine treated or untreated LX2 cells or HUVEC cells were seeded in the upper chamber. DMEM supplemented with 10% FBS was plated on the lower chambers and migration was assessed.

Tube formation assay

The angiogenic potential of HUVECs was assessed using a Matrigel-based tube formation assay. Briefly, 96-well plates were coated with Matrigel and incubated at 37°C for 30 min to allow polymerization. HUVECs (1.5×10^4 cells/well) were seeded onto the Matrigel-coated wells in endothelial growth medium (ECGS, Sigma, # 211-500) containing dobutamine and vehicle control. Tubelike structures were visualized after 16h using a phase-contrast microscope. Quantitative analysis of angiogenic parameters, including the number of nodes, meshes, segments, and total tube length, was performed using ImageJ software. All experiments were conducted in triplicate.

Spheroid Formation Assay

Hep3B cells were cultured in DMEM/F12 spheroid medium under non-adherent conditions until compact primary spheroids formed. Primary spheroids were collected, allowed to settle, and washed twice with PBS. For secondary spheroid formation, primary spheroids were dissociated with accutase by gently pipetting and intermittent tapping until a single-cell suspension was obtained. Accutase activity was quenched with complete medium, followed by centrifugation and PBS washing. Cells were resuspended in spheroid medium, and 5,000 cells were seeded per well under non-adherent conditions. Secondary spheroid formation was monitored at the indicated time points, and spheroid morphology and size were assessed using ImageJ/Fiji.

Zebrafish xenograft and drug treatment

The Assam wildtype (ASWT) strain of *Danio rerio* was maintained under standard laboratory conditions at 28°C. Xenotransplantation was performed using previously established protocol [47,48]. At 2 days post-fertilization (2dpf), dechorionated embryos were used for xenografting. Hep3B cells were labelled with CM-Dil (Invitrogen) and approximately 200 cells were microinjected into the perivitelline space of each embryo using a micromanipulator under a stereomicroscope. Injected embryos were maintained at 34°C in E3 medium.

At 12h post-injection (12hpi), xenografted larvae were randomly assigned to vehicle control (0.1%DMSO) and dobutamine-treated (50µM) groups and exposed for 18-24 h. Tumor formation and metastatic dissemination were assessed using fluorescent stereomicroscope (Nikon AX R). Tumor coverage was quantified by measuring fluorescent tumor area using ImageJ software [49].

For gene expression analysis, total RNA was isolated from pooled whole xenografted larvae (n=30 per group) using TRIzol reagent, followed by RT-qPCR analysis of *yap1*, *ccn2a* (*ctgf*, *yap1* downstream target), *acta2*, *vegfaa*, and *vegfc* (angiogenesis marker). Gene expression levels were normalized to *gapdh*.

Statistical analysis

Data are presented as mean $\pm$ SEM or mean $\pm$ SD as deemed appropriate from at least three independent experiments. Statistical analyses were performed using GraphPad Prism 10. Comparison between two groups was conducted using unpaired two-tailed Student's t-tests, whereas comparisons involving multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. For experiments involving two independent variables, including wound-healing assays and dose-response studies, two-way ANOVA followed by Tukey's multiple-comparison test was used. Differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$; **ns**, not significant.

Results

Integrated bioinformatics and functional analyses identify stromal YAP1 signaling as a critical regulator of hepatocellular carcinoma progression

To investigate the role of YAP1 signaling in HCC, we first analyzed *YAP1* expression in the TCGA-LIHC cohort. *YAP1* expression was elevated in HCC compared with normal liver and increased with advancing tumor grade, consistent with its established association between YAP1 signaling and HCC progression (**Fig. 1A-B**). Because YAP1 activity is primarily regulated post-translationally, we next examined YAP Ser127 phosphorylation in paired human HCC samples. Reduced tumor Ser127 phosphorylation was observed in 37 of 92 patients (40.2%). Using progressively stringent thresholds, 18 patients (19.6%) showed a >1.25 -fold reduction, 1 patient (13%) showed a >1.41 -fold reduction, and 3 patients (3.3%) showed a >2 -fold reduction in tumor Ser127 phosphorylation relative to matched non-tumor tissue (**Supplementary Table S4; Figure 1C**). The frequency of reduced Ser127 phosphorylation was broadly consistent with previous reports describing low Ser127 phosphorylation in a subset of human HCCs [50]. Importantly, infiltrative tumors with an incomplete tumor capsule exhibited significantly lower Ser127 phosphorylation than encapsulated tumors (Wilcoxon $p=0.003$), linking loss of this inhibitory phosphorylation to an aggressive HCC phenotype (**Fig. 1D**).

We next examined the cellular context of YAP1 signaling using publicly available single-cell RNA sequencing datasets (GSE149614 and GSE189903). Cancer-associated fibroblasts (CAFs) and endothelial cells showed higher YAP1 target activity than other major HCC cell populations, with CTGF and CYR61 predominantly expressed in fibroblast and endothelial populations (**Fig. 1E-F**). Consistently, across human liver transcriptomic datasets, *YAP1* expression showed positive correlations with stromal/ECM-associated genes, including *LOX*, *PDGFRB*, *COL3A1*, *SPARC*, and *COL1A1*, and endothelial-associated genes including *KDR* (*VEGFR2*), *LYVE1*, and *FLT1* (*VEGFR1*) (**Fig. 1G; Supplementary Figure S1**).

Collectively, these findings highlight the relevance of YAP1 signaling in the stromal and vascular compartments of HCC and provide a rationale for targeting YAP Ser127 phosphorylation as a potential therapeutic strategy.

Dobutamine exhibits anti-proliferative and anti-migratory effects in stromal cells

Previous studies have reported antitumor effects of dobutamine associated with inhibition of YAP1 nuclear localization and transcriptional activity, supporting its use as a pharmacological modulator of YAP1 signaling [44,51]. To assess whether dobutamine similarly affects stromal cells, LX2 and HUVECs were treated with increasing concentrations of dobutamine (10-160 μ M) for 24 and 48 h. Dobutamine showed limited cytotoxicity within the tested range with IC₅₀ values of **148.3 $\pm$ 10 μ M** and **73.04 $\pm$ 10 μ M** in LX2 and **452.6 μ M** and **108.5 μ M** in HUVECs at 24 and 48h, respectively (Fig. 2A-C). The concentration used subsequent experiments (40 μ M) was substantially below these IC₅₀ values.

We next assessed the effects of dobutamine on stromal cell proliferation and migration. Dobutamine significantly reduced fluorescence intensity of Ki67 in both HUVECs and LX2 cells and increased G0/G1-phase populations with a concomitant decrease in S-phase cells, indicating cell-cycle arrest (Fig. 2D, E; Supplementary Figure S2). Dobutamine also markedly impaired stromal cell migration. In LX2 cells dobutamine reduced wound closure from ~65% to ~23% under basal conditions and from ~98% following TGF β stimulation to ~6% after dobutamine treatment at 48 h (Fig. 2F, H). HUVEC wound closure was similarly reduced to ~49% (Fig 2I, K). Transwell assays confirmed these findings, showing significantly fewer migrating LX2 and HUVEC cells following dobutamine treatment (Fig. G, H, J and K). Collectively, these findings demonstrate that dobutamine restrains stromal cell proliferation and migration while exhibiting minimal cytotoxicity, supporting its potential role in modulating tumor-associated stromal functions.

Dobutamine inhibits YAP1 signaling by promoting Ser127 phosphorylation and reducing nuclear localization in stromal cells

YAP1 regulates HSC activation, ECM deposition, and angiogenic signaling within TME. β -adrenergic agonists such as dobutamine activate cAMP/ PKA signaling, leading to activation of Hippo pathway kinases LATS1/2, that phosphorylate YAP1 at Ser127 and promote its cytoplasmic sequestration [52]. To investigate whether dobutamine suppresses YAP signaling in stromal cells, we examined YAP1 transcriptional activity, phosphorylation status, and subcellular localization.

Dobutamine significantly reduced YAP1 and its target genes *CTGF*, *CYR61* and *ANKRD1* in both HUVECs and LX2 cells following 24 h of treatment (Fig. 3A, B). Western blot analysis revealed a significant increase in the pYAP Ser127/YAP ratio without changes in total YAP protein levels, following dobutamine treatment in both HUVEC and LX2 cells (Fig. 3C, D). Consistent with this, immunofluorescence demonstrated reduced nuclear or increased cytoplasmic YAP1 following dobutamine treatment (Fig. 3E, F). In LX2 cells, TGF- β 1 stimulation enhanced nuclear YAP1 localization, whereas co-treatment with dobutamine effectively prevented this response and restored cytoplasmic retention of YAP1.

Collectively, these results demonstrate that dobutamine increases inhibitory YAP-Ser127 phosphorylation and promotes cytoplasmic YAP1 localization, accompanied by reduced expression of YAP target genes in both LX2 and HUVEC cells.

Dobutamine suppresses hepatic stellate activation and endothelial angiogenic signaling

To determine whether dobutamine-mediated YAP1 inhibition alters stromal cell activation, LX2 and HUVECs were treated with dobutamine under basal or TGF β -stimulated conditions (**Fig. 4A, B**). In LX2 cells, dobutamine significantly reduced the expression of the HSC activation marker collagen1 type1 alpha 1 (*Col1A1*), fibronectin (*FN1*), and α -smooth muscle actin (α SMA) at both the transcript and protein levels, under basal and TGF- β stimulated conditions (**Fig. 4B, D; Supplementary Figure S3A**). Immunofluorescence further confirmed reduced α SMA and FN1 expression following dobutamine treatment, whereas TGF β -treated cells exhibited robust expression of these markers (**Fig. 4E, F**).

In HUVECs, dobutamine reduced angiogenesis-associated *VEGF*, *VEGFR1*, and *VEGFR2* transcripts (**Fig. 4A**) and reduced VEGFR2 and Ang-2 protein expression (**Fig. 4C**). Immunofluorescent cytochemistry analyses of VEGFR2 further supported these results (**Fig. 4G**). To determine whether dobutamine suppression of VEGFR2 and Ang-2 translated into functional anti-angiogenic activity, a matrigel based tube formation assay was performed. Dobutamine markedly impaired HUVEC tube formation, reducing tube length, nodes, segments, and meshes (**Fig. 4H; Supplementary Figure S3B-C**). To confirm that these responses were directly dependent on YAP1 signaling rather than off-target effects, we performed genetic loss-and gain-of-function experiments (**Supplementary Figure S4**). Specific siRNA-mediated silencing of YAP1 in LX2 and HUVEC cells phenocopied dobutamine treatment by significantly reducing COL1A1 and VEGFR2 expression. Conversely, ectopic overexpression of YAP1 rescued COL1A1 expression., demonstrating that dobutamine attenuates stellate activation and endothelial angiogenic signaling in a YAP- dependent manner.

Collectively, these findings demonstrate that dobutamine attenuates both HSC activation and endothelial angiogenic responses, thereby targeting key stromal compartments that promote HCC progression.

Pharmacological inhibition of stromal YAP1 disrupts tumor-stroma crosstalk and suppresses HCC aggressiveness

To determine whether stromal YAP affects HCC behavior, Hep3B cells were exposed to CM from untreated or dobutamine-treated LX2 and HUVECs. CM from untreated stromal cells enhanced migration and proliferation, whereas CM from dobutamine-treated cells markedly reduced wound closure and Ki67 positivity. (**Fig. 5A-C**). Similarly, CM from dobutamine-treated LX2 and HUVECs significantly reduced Hep3B spheroid growth at day 4 compared with CM from untreated stromal cells (**Fig. 5D-G**).

We next examined whether stromal YAP1 inhibition alters HCC signaling through paracrine mechanisms. CM derived from dobutamine-treated TGF β -activated LX2 cells and HUVECs reduced the expression of CTGF and CYR61 significantly at transcript level (**Fig. 5H**). Western blotting revealed that CM derived from dobutamine-treated stromal cells significantly increased Ser127 phosphorylation in Hep3B cells as compared with CM from untreated stromal cells (**Fig. 5I**). Consistent with increased Ser127 phosphorylation, CM from dobutamine-treated stromal cells reduced nuclear YAP and increased its cytoplasmic retention in Hep3B cells (**Fig. 5J**), indicating suppression of YAP1 activity through altered

stromal paracrine signaling. Direct effects of dobutamine on HCC cells were evaluated separately (**Supplementary Figure S5**).

We next examined whether these changes were associated with inhibition of EMT, the expression of mesenchymal markers was evaluated. CM from untreated stromal cells increased mesenchymal markers N-cadherin and Vimentin, whereas CM obtained from dobutamine-treated stromal cells significantly reduced their expression, as confirmed by Western blotting and N-cadherin immunofluorescence (**Fig. 5K-L**).

Collectively these findings suggest that pharmacological inhibition of stromal YAP1 alters tumor-stroma communication, resulted in reduced YAP1 transcriptional activity, and EMT-associated signaling in HCC cells and consequently limiting their migratory, proliferative, and three-dimensional growth potential.

Dobutamine suppresses tumor growth and YAP1 signaling in a zebrafish xenograft model

To evaluate *in vivo* antitumor efficacy of dobutamine, Dil-labelled Hep3B cells were xenotransplanted into the perivitelline space of 2-dpf zebrafish embryos. After 12h, embryos were treated with either DMSO (vehicle control) or 50 μ M dobutamine and analyzed 24h later (**Fig. 6A; Supplementary Table S5**).

The coverage of the xenotransplanted cells rapidly increased within 1.5 days after injection (**Fig. 6B, C**), with concomitant increase in the lethality. Dobutamine treatment significantly improved larval survival compared with vehicle-treated xenografts (62% vs. 25%, $P < 0.05$) (**Fig. 6D**). and markedly reduced the coverage area of Dil-labeled Hep3B xenografts (**Fig. 6E, E', F, and F'**). Quantitative analysis further confirmed a significant reduction in tumor area following dobutamine treatment, indicating effective inhibition of tumor growth (**Fig. 6G**).

To evaluate host specific gene expression in the zebrafish microenvironment without cross-reacting with human transcripts, qPCR was performed using zebrafish-specific primers. *yap1* transcript levels were unchanged across the experimental groups (**Fig. 6H**), consistent with the predominant post-translational regulation of YAP1 activity through phosphorylation and subcellular localization. In contrast, the YAP1 target genes *ccn2a* and *cyr61* were elevated in Hep3B-bearing larvae and significantly reduced following dobutamine treatment (**Fig. 6I, J**). These findings indicate that dobutamine suppresses YAP transcriptional activity without altering *yap* transcript abundance, supporting regulation at the post-translational level [53].

The expression of *acta2*, a major component of vascular smooth muscle cells, which is often associated with angiogenesis, was also significantly increased in xenografted larvae and was markedly reduced following dobutamine treatment (**Fig. 6K**). Assessing the expression of vascular endothelial growth factors (Vegfs), *vegfc* expression significantly decreased in dobutamine-treated xenografts compared with untreated tumor-bearing larvae (**Fig. 6L**). However, *vegfaa* expression showed no statistically significant difference among the experimental groups (**Fig. 6M**).

Collectively, these findings demonstrate that dobutamine suppresses tumor growth and improves survival *in vivo*. The reduction in proliferation, *yap* downstream targets *ccn2a*, and *cyr61*, and angiogenesis-associated markers are further in line with our *in vitro* data, supporting the therapeutic potential of dobutamine for targeting HCC.

Discussion

HCC is driven by complex interactions between malignant cells and the surrounding TME. In this study, we identify stromal YAP signaling as an important component of this interaction and investigate dobutamine as a pharmacological modulator of this pathway. YAP1, a TEAD-associated transcriptional co-activator, regulates tumor growth and metastasis and therefore emerged as a potential therapeutic target [54]. Although several compounds, including verteporfin, Super-TDU, fenamate, and narciclasine, have been developed to inhibit YAP-TEAD interactions, their therapeutic limitations highlight the need for alternative approaches that are crucial for YAP1 activity [55–58]. Our molecular docking showed that dobutamine could occupy a potential binding pocket within the YAP-TEAD interface, with predictive binding comparable to several clinically used HCC drugs, including sorafenib, regorafenib, lenvatinib, and cabozantinib (**Supplementary Figure S6**). While docking does not establish direct target engagement, these findings provide structural support for a potential relationship between dobutamine and YAP signaling. Previous studies have suggested that dobutamine increases YAP phosphorylation through β -adrenergic signaling and activation of LATS1/2, providing a plausible mechanism for YAP inhibition[59,60].

Patient-level transcriptomic analysis provides strong clinical relevance of YAP1 in HCC. Analysis of the TCGA-LIHC cohort demonstrated that *YAP1* expression was significantly elevated in primary HCC tumors compared with normal liver tissues (**Fig. 1A**), highlighting aberrant activation of YAP1 signaling during hepatocarcinogenesis. Beyond tumor cells alone, correlation analysis revealed that YAP1 expression was associated with stromal and endothelial gene signatures, including markers of hepatic stellate cell activation and fibrosis (LOX, PDGFRB, COL3A1, SPARC, and COL1A1) as well as endothelial and angiogenic signaling (KDR, LYVE1, and FLT1) (**Fig. 1G**). These findings indicate that YAP1 is associated with pro-fibrotic and pro-angiogenic features of the HCC TME. Importantly, our analysis of YAPSer127 phosphorylation further indicated that reduced inhibitory phosphorylation is associated with infiltrative tumor growth, supporting the relevance of post-translational YAP regulation in aggressive HCC.

Guided by these observations, we next explored the effects of dobutamine on stromal cells, a critical determinant of the tumor-microenvironment. Our findings indicated that dobutamine treatment reduced HSC activation markers, including α SMA, *Col1A1*, and *FN1*, and decreased endothelial markers associated with angiogenic signaling, including *ANGPT2*, and *VEGFR2*. These effects were accompanied by increased YAP Ser127 phosphorylation and reduced nuclear YAP localization, together with decreased expression of YAP target genes. Thus, dobutamine appears to restrain both fibrogenic and endothelial stromal phenotypes through modulation of YAP signaling (**Fig. 2-4**).

Following these findings, we investigated whether dobutamine-treated stromal YAP inhibition influences HCC behavior through paracrine signaling. CM from dobutamine-treated LX2 and HUVECs reduced Hep3B migration and proliferation and attenuated EMT-associated features compared with CM from untreated stromal cells. Moreover, CM derived from dobutamine-treated stromal cells impaired Hep3B spheroid growth, resulting in reduced spheroid size. These findings indicate that stromal YAP inhibition alters the tumor-supportive secretory profile and limits the malignant properties of HCC cells (**Fig. 5**).

The *in vivo* relevance of these observations was further supported by zebrafish xenograft experiments, which demonstrated that dobutamine treatment significantly reduces tumor growth and xenotransplantation-associated mortality (**Fig. 6**). Consistent with our *in vitro* findings, the concomitant downregulation of YAP, its transcriptional targets, and the angiogenesis markers *vegfc* *in vivo* suggests that modulation of YAP signaling may contribute to the anti-tumor effects of dobutamine, supporting the translational relevance of our findings.

This study has several limitations. First, the use of LX2 and HUVEC cell lines may not fully recapitulate the heterogeneity of stromal populations in human HCC. Second, although the zebrafish xenograft model demonstrated the antitumor effects of dobutamine, it does not fully reproduce the complexity of the human HCC microenvironment. Future studies using primary patient-derived stromal cells, co-culture or organoid models, and mammalian *in vivo* models will be important to validate the therapeutic relevance of stromal YAP1 modulation by dobutamine.

In summary, this study integrates clinical transcriptomic analyses with mechanistic *in vitro* and *in vivo* experiments to establish YAP1 as a key regulator of stromal-vascular-tumor crosstalk in HCC. Our findings suggest that pharmacological modulation of YAP1 signaling using dobutamine not only suppresses stromal cell activation but also remodels the TME to limit cancer progression and metastatic spread. These results highlight the therapeutic potential of targeting YAP-dependent paracrine signaling and support further investigation into dobutamine based or YAP-focused strategies for the treatment of HCC.

Declarations

Supplementary information The online version contains supplementary material with links.

Ethics approval and consent to participate All zebrafish work was approved by the SNIoE Institutional Animal Ethics Committee (SNIoE/IAEC/2025/2/002)

Consent for publication Not Applicable

Data availability All the data can be obtained by contacting the corresponding author.

Competing interests The authors declare no competing interests.

Funding This work was supported by fellowships and grant supports from the Council of Scientific & Industrial Research (CSIR-09/915(0020)/2021-EMR-I to P. Sharma), Department of Biotechnology (DBT-102/IFD/SAN/3003/2017-2018 to S. Biswas) and Science and Engineering Research Board (SERB) (CRG/2018/003918 to S. Biswas and SERB- YSS/2015/000092 to S. Ghose) Indian Council of Medical Research (ICMR-2019-1306/SCR/ADHOC-BMS to S. Biswas), Anusandhan National Research Foundation (ANRF CRG/2023/001776) to S. Sengupta & ANRF-CRG/2023/006978 to S. Biswas).

Authors' contributions PS designed the study and wrote the manuscript. PC performed the xenograft transplantation experiment. MGS established and standardized the culture conditions for HUVEC cells and provided assistance with figure curation and correction. BD established and standardized the culture conditions for LX2 cells. BR critically revised the manuscript. JP contributed to the spheroid experiment. SJ and SS performed all bioinformatics and computational analyses. RD supervised the zebrafish *in vivo* experiments. S. Sengupta and SG assisted with proofreading and editing of the manuscript. SB conceptualized and supervised the study. All authors read and approved the final version of the manuscript.

Acknowledgements The authors would like to appreciate their laboratory members Jaydeb for helpful and constructive discussion. Authors are thankful to Manoj Kumar Gupta for flow cytometry based cell sorting using BD FASARIA III; grateful to Dr. W. Selvamurthy, prof. B.C. Das for encouraging and supporting our research at Amity University campus; thankful to confocal microscopy facility at Central Instrumentation Facility, Delhi University and All Indian Institute of Medical Sciences (AIIMS), Department of Science and Technology (DST) and FIST sponsored (SR/FST/LS-II/2017/115) Flow Cytometry Facility at Amity Institute of Molecular Medicine and Stem Cell Research (AIMMSCR) for experimentation. We thank the DST-FIST Confocal Microscope Facility (DST-FIST Grant No. SR/FST/LS-1/2017/59(c)), and the Center for Integrative and Translational Research (CITRES) at Shiv Nadar Institution of Eminence (SNIOE) for their assistance with zebrafish experiments.

References

1. Sung H, Filho AM, Laversanne M, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 34 cancers in 186 countries. *CA Cancer J Clin.* 2026;76:e70090. doi:10.3322/caac.70090 PubMed PMID: 42417444; PubMed Central PMCID: PMC13343830.
2. Llovet JM, De Baere T, Kulik L, Haber PK, Greten TF, Meyer T, et al. Locoregional therapies in the era of molecular and immune treatments for hepatocellular carcinoma. *Nature reviews Gastroenterology & hepatology.* 2021;18:293–313.
3. Hu M, Huang L. Strategies targeting tumor immune and stromal microenvironment and their clinical relevance. *Advanced Drug Delivery Reviews.* 2022;183:114137. doi:10.1016/j.addr.2022.114137
4. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers.* 2021;7:1–28. doi:10.1038/s41572-020-00240-3

5. Craig AJ, Von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. *Nature reviews Gastroenterology & hepatology*. 2020;17:139–52.
6. Puche JE, Saiman Y, Friedman SL. Hepatic Stellate Cells and Liver Fibrosis. In: Terjung R, editor. *Comprehensive Physiology* [Internet]. 1st ed. Wiley; 2013 [cited 2024 Oct 23]. p. 1473–92. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/cphy.c120035> doi:10.1002/cphy.c120035
7. Otranto M, Sarrazy V, Bonté F, Hinz B, Gabbiani G, Desmouliere A. The role of the myofibroblast in tumor stroma remodeling. *Cell adhesion & migration*. 2012;6:203–19.
8. Amann T, Bataille F, Spruss T, Mühlbauer M, Gäbele E, Schölmerich J, et al. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci*. 2009;100:646–53. doi:10.1111/j.1349-7006.2009.01087.x PubMed PMID: 19175606.
9. Carloni V, Luong TV, Rombouts K. Hepatic stellate cells and extracellular matrix in hepatocellular carcinoma: more complicated than ever. *Liver International*. 2014;34:834–43.
10. Coulouarn C, Corlu A, Glaise D, Guénon I, Thorgeirsson SS, Clément B. Hepatocyte–Stellate Cell Cross-Talk in the Liver Engenders a Permissive Inflammatory Microenvironment That Drives Progression in Hepatocellular Carcinoma. *Cancer Research*. 2012;72:2533–42. doi:10.1158/0008-5472.CAN-11-3317
11. Santamato A, Fransvea E, Dituri F, Caligiuri A, Quaranta M, Niimi T, et al. Hepatic stellate cells stimulate HCC cell migration via laminin-5 production. *Clinical science*. 2011;121:159–68.
12. Zhao W, Zhang L, Xu Y, Zhang Z, Ren G, Tang K, et al. Hepatic stellate cells promote tumor progression by enhancement of immunosuppressive cells in an orthotopic liver tumor mouse model. *Laboratory Investigation*. 2014;94:182–91. doi:10.1038/labinvest.2013.139
13. Zhao W, Zhang L, Yin Z, Su W, Ren G, Zhou C, et al. Activated hepatic stellate cells promote hepatocellular carcinoma development in immunocompetent mice. *International Journal of Cancer*. 2011;129:2651–61. doi:10.1002/ijc.25920
14. Nitta T, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. *Hepatology*. 2008;48:909–19. doi:10.1002/hep.22397
15. Giannelli G, Villa E, Lahn M. Transforming Growth Factor- β as a Therapeutic Target in Hepatocellular Carcinoma. *Cancer Research*. 2014;74:1890–4. doi:10.1158/0008-5472.CAN-14-0243
16. Dooley S, Weng H, Mertens PR. Hypotheses on the role of transforming growth factor- β in the onset and progression of hepatocellular carcinoma. *Digestive diseases*. 2009;27:93–101.
17. Mazzocca A, Fransvea E, Dituri F, Lupo L, Antonaci S, Giannelli G. Down-regulation of connective tissue growth factor by inhibition of transforming growth factor β blocks the tumor–stroma cross-talk and tumor progression in hepatocellular carcinoma. *Hepatology*. 2010;51:523–34.
18. Park SA, Kim MJ, Park SY, Kim JS, Lim W, Nam JS, et al. TIMP-1 mediates TGF- β -dependent crosstalk between hepatic stellate and cancer cells via FAK signaling. *Sci Rep*. 2015;5:16492. doi:10.1038/srep16492 PubMed PMID: 26549110; PubMed Central PMCID: PMC4637930.

19. Trefts E, Gannon M, Wasserman DH. The liver. *Current Biology*. 2017;27:R1147–51. doi:10.1016/j.cub.2017.09.019
20. Sharma S, Ghufuran SM, Aftab M, Bihari C, Ghose S, Biswas S. Survivin inhibition ameliorates liver fibrosis by inducing hepatic stellate cell senescence and depleting hepatic macrophage population. *Journal of Cell Communication and Signaling*. 2024;18:e12015.
21. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nature medicine*. 2000;6:389–95.
22. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature medicine*. 1995;1:27–30.
23. Torimura T, Sata M, Ueno T, Kin M, Tsuji R, Suzaku K, et al. Increased expression of vascular endothelial growth factor is associated with tumor progression in hepatocellular carcinoma. *Human Pathology*. 1998;29:986–91. doi:10.1016/S0046-8177(98)90205-2
24. Schoenleber SJ, Kurtz DM, Talwalkar JA, Roberts LR, Gores GJ. Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: systematic review and meta-analysis. *Br J Cancer*. 2009;100:1385–92. doi:10.1038/sj.bjc.6605017
25. Moroishi T, Hansen CG, Guan KL. The emerging roles of YAP and TAZ in cancer. *Nature reviews Cancer*. 2015;15:73. doi:10.1038/nrc3876 PubMed PMID: 25592648.
26. Sun T, Mao W, Peng H, Wang Q, Jiao L. YAP promotes sorafenib resistance in hepatocellular carcinoma by upregulating survivin. *Cell Oncol (Dordr)*. 2021;44:689–99. doi:10.1007/s13402-021-00595-z PubMed PMID: 33655469; PubMed Central PMCID: PMC12980756.
27. Zhao B, Li L, Lei Q, Guan KL. The Hippo–YAP pathway in organ size control and tumorigenesis: an updated version. *Genes Dev*. 2010;24:862–74. doi:10.1101/gad.1909210 PubMed PMID: 20439427.
28. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev*. 2007;21:2747–61. doi:10.1101/gad.1602907 PubMed PMID: 17974916.
29. Pan D. The hippo signaling pathway in development and cancer. *Dev Cell*. 2010;19:491–505. doi:10.1016/j.devcel.2010.09.011 PubMed PMID: 20951342; PubMed Central PMCID: PMC3124840.
30. Zhao B, Ye X, Yu J, Li L, Li W, Li S, et al. TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev*. 2008;22:1962–71. doi:10.1101/gad.1664408 PubMed PMID: 18579750; PubMed Central PMCID: PMC2492741.
31. Perra A, Kowalik MA, Ghiso E, Ledda-Columbano GM, Di Tommaso L, Angioni MM, et al. YAP activation is an early event and a potential therapeutic target in liver cancer development. *J Hepatol*. 2014;61:1088–96. doi:10.1016/j.jhep.2014.06.033 PubMed PMID: 25010260.
32. Oka T, Mazack V, Sudol M. Mst2 and Lats Kinases Regulate Apoptotic Function of Yes Kinase-associated Protein (YAP). *Journal of Biological Chemistry*. 2008;283:27534–46. doi:10.1074/jbc.M804380200
33. Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). *Genes Dev*. 2010;24:72–85. doi:10.1101/gad.1843810 PubMed PMID: 20048001; PubMed Central PMCID: PMC2802193.

34. Yan W, Xiao G hui, Wang L juan, Zhou Y, Yang F, Mou K hou. CAFs activated by YAP1 upregulate cancer matrix stiffness to mediate hepatocellular carcinoma progression. *Journal of Translational Medicine*. 2025;23:450. doi:10.1186/s12967-025-06325-5
35. Thomann S, Weiler SME, Marquard S, Rose F, Ball CR, Tóth M, et al. YAP Orchestrates Heterotypic Endothelial Cell Communication via HGF/c-MET Signaling in Liver Tumorigenesis. *Cancer Research*. 2020;80:5502–14. doi:10.1158/0008-5472.CAN-20-0242
36. Naktubtim C, Payuhakrit W, Uttarawichien T, Hassametto A, Suwannalert P. YAP, a novel target regulates F-actin rearrangement-associated CAFs transformation and promotes colorectal cancer cell progression. *Biomedicine & Pharmacotherapy*. 2022;155:113757. doi:10.1016/j.biopha.2022.113757
37. Jiang Z, Zhou C, Cheng L, Yan B, Chen K, Chen X, et al. Inhibiting YAP expression suppresses pancreatic cancer progression by disrupting tumor-stromal interactions. *J Exp Clin Cancer Res*. 2018;37:69. doi:10.1186/s13046-018-0740-4 PubMed PMID: 29587800; PubMed Central PMCID: PMC5870346.
38. Zhang H, Ramakrishnan SK, Triner D, Centofanti B, Maitra D, Győrffy B, et al. Tumor-selective proteotoxicity of verteporfin inhibits colon cancer progression independently of YAP1. *Sci Signal*. 2015;8:ra98. doi:10.1126/scisignal.aac5418 PubMed PMID: 26443705; PubMed Central PMCID: PMC4818013.
39. Gibault F, Corvaisier M, Bailly F, Huet G, Melnyk P, Cotellet P. Non-photoinduced biological properties of verteporfin. *Current medicinal chemistry*. 2016;23:1171–84.
40. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug repurposing: progress, challenges and recommendations. *Nature reviews Drug discovery*. 2019;18:41–58.
41. Xia Y, Sun M, Huang H, Jin WL. Drug repurposing for cancer therapy. *Signal transduction and targeted therapy*. 2024;9:92.
42. Ruffolo RR. Review: The Pharmacology of Dobutamine. *The American Journal of the Medical Sciences*. 1987;294:244–8. doi:10.1097/00000441-198710000-00005
43. Vallet B, Dupuis B, Chopin C. Dobutamine: mechanisms of action and use in acute cardiovascular pathology. In: Vol. 40. 1991. p. 397–402.
44. Yin J, Dong Q, Zheng M, Xu X, Zou G, Ma G, et al. Antitumor activity of dobutamine on human osteosarcoma cells. *Oncology Letters*. 2016;11:3676–80. doi:10.3892/ol.2016.4479
45. Xie B, Xu Z, Yang G, Chen G, Li B, Hu L, et al. Antitumor effect of dobutamine on multiple myeloma via mitogen-activated protein kinase pathway in vitro. *Acta Biochimica et Biophysica Sinica*. 2016;1–3.
46. White R, Rose K, Zon L. Zebrafish cancer: the state of the art and the path forward. *Nat Rev Cancer*. 2013;13:624–36. doi:10.1038/nrc3589
47. Patowary A, Purkanti R, Singh M, Chauhan R, Singh AR, Swarnkar M, et al. A sequence-based variation map of zebrafish. *Zebrafish*. 2013;10:15–20.

48. Tonon F, Farra R, Zennaro C, Pozzato G, Truong N, Parisi S, et al. Xenograft Zebrafish Models for the Development of Novel Anti-Hepatocellular Carcinoma Molecules. *Pharmaceuticals (Basel)*. 2021;14:803. doi:10.3390/ph14080803 PubMed PMID: 34451900; PubMed Central PMCID: PMC8400454.
49. Hemlata, Murali N, Roy S, Joshi YC, Das RN, Hariharan A, et al. pH-responsive dual-drug-loaded bovine serum albumin nanoparticles for targeted cancer therapy. *Int J Biol Macromol*. 2026;365:152319. doi:10.1016/j.ijbiomac.2026.152319 PubMed PMID: 42070610.
50. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress the development of hepatocellular carcinoma through inactivation of the Yap1 oncogene. *Cancer Cell*. 2009;16:425–38. doi:10.1016/j.ccr.2009.09.026 PubMed PMID: 19878874; PubMed Central PMCID: PMC3023165.
51. Zheng HX, Wu LN, Xiao H, Du Q, Liang JF. Inhibitory effects of dobutamine on human gastric adenocarcinoma. *World Journal of Gastroenterology: WJG*. 2014;20:17092. doi:10.3748/wjg.v20.i45.17092 PubMed PMID: 25493021.
52. Zhao WB, Lu Q, Nguyen MN, Su Y, Ziemann M, Wang LN, et al. Stimulation of β -adrenoceptors up-regulates cardiac expression of galectin-3 and BIM through the Hippo signalling pathway. *Br J Pharmacol*. 2019;176:2465–81. doi:10.1111/bph.14674 PubMed PMID: 30932177; PubMed Central PMCID: PMC6592853.
53. Lin M, Zheng X, Yan J, Huang F, Chen Y, Ding R, et al. The RNF214-TEAD-YAP signaling axis promotes hepatocellular carcinoma progression via TEAD ubiquitylation. *Nat Commun*. 2024;15:4995. doi:10.1038/s41467-024-49045-y PubMed PMID: 38862474; PubMed Central PMCID: PMC11167002.
54. Piccolo S, Panciera T, Contessotto P, Cordenonsi M. YAP/TAZ as master regulators in cancer: modulation, function and therapeutic approaches. *Nat Cancer*. 2023;4:9–26. doi:10.1038/s43018-022-00473-z PubMed PMID: 36564601; PubMed Central PMCID: PMC7614914.
55. Sharma P, Ghufra SM, Roy B, Ghose S, Biswas S. Deciphering the signaling landscape of YAP in the tumor microenvironment—Insights into hepatocellular carcinoma progression. *FEBS letters*. 2026;600:1006–27.
56. Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, et al. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. *Genes Dev*. 2012;26:1300–5. doi:10.1101/gad.192856.112 PubMed PMID: 22677547; PubMed Central PMCID: PMC3387657.
57. Hau JC, Erdmann D, Mesrouze Y, Furet P, Fontana P, Zimmermann C, et al. The TEAD4-YAP/TAZ Protein-Protein Interaction: Expected Similarities and Unexpected Differences. *ChemBioChem*. 2013;14:1218–25. doi:10.1002/cbic.201300163
58. Kawamoto R, Nakano N, Ishikawa H, Tashiro E, Nagano W, Sano K, et al. Narciclasine is a novel YAP inhibitor that disturbs interaction between YAP and TEAD4. *BBA Advances*. 2021;1:100008. doi:10.1016/j.bbadv.2021.100008

59. Bao Y, Nakagawa K, Yang Z, Ikeda M, Withanage K, Ishigami-Yuasa M, et al. A cell-based assay to screen stimulators of the Hippo pathway reveals the inhibitory effect of dobutamine on the YAP-dependent gene transcription. *Journal of Biochemistry*. 2011;150:199–208. doi:10.1093/jb/mvr063

60. Elisi GM, Santucci M, D’Arca D, Lauriola A, Marverti G, Losi L, et al. Repurposing of Drugs Targeting YAP-TEAD Functions. *Cancers (Basel)*. 2018;10:329. doi:10.3390/cancers10090329 PubMed PMID: 30223434; PubMed Central PMCID: PMC6162436.

Figures

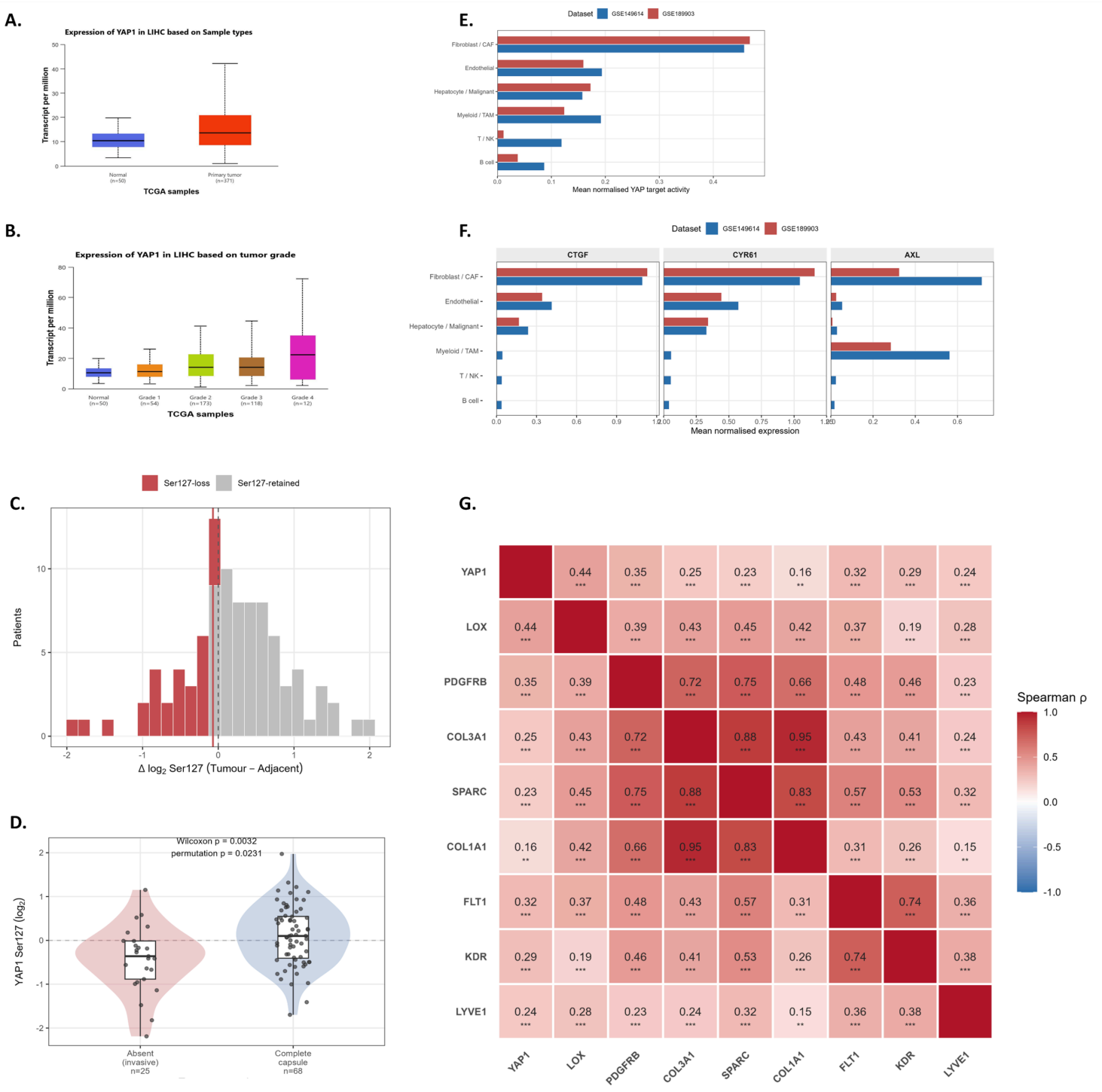


Figure 1

Bioinformatic analyses identify stromal cell-specific YAP activation associated with extracellular matrix remodeling and angiogenesis in hepatocellular carcinoma.

(A) Differential expression of YAP1 between normal liver tissues (n=50) and HCC samples (n=371) from the TCGA-LIHC cohort via UALCAN. Unpaired two sample T-test was employed to evaluate levels of significance in expression level between any two sample groups. ($P=1.62636570877339E-12$) **(B)** YAP1 expression according to histological tumor grade. **(C)** Frequency of reduced YAP Ser127 phosphorylation in paired human HCC samples. Reduced tumor Ser 127 phosphorylation was identified in 37 of 92 patients (40.2%); 18 patients (19.6%) showed a >1.25-fold reduction, 12 patients (13%) showed a >1.41-fold reduction, and 3 patients (3.3%) showed a >2-fold reduction relative to matched non-tumor tissue. **(D)** Association between YAP Ser127 phosphorylation and tumor capsule status/infiltrative growth. Tumors with an incomplete capsule and infiltrative growth exhibited significantly lower Ser127 phosphorylation than encapsulated tumors (Wilcoxon $p=0.003$). **(E)** YAP1 target activity across major HCC cell populations in publicly available datasets GSE149614 and GSE189903 respectively. Cancer associated fibroblasts and endothelial cells exhibited prominent YAP1 target activity. **(F)** Expression of canonical YAP target genes CTGF and CYR61 across HCC cell populations in the corresponding single-cell datasets. These genes were predominantly expressed in fibroblast and endothelial populations. **(G)** Correlation heat map showing association between *YAP1* expression and stromal/ECM-associated genes (LOX, PDGFRB, COL3A1, SPARC, and COL1A1) and endothelial-associated genes (KDR, LYVE1, and FLT1) in human liver transcriptomic datasets. Color intensity represents the strength of correlation, with statistical significance indicated as appropriate.

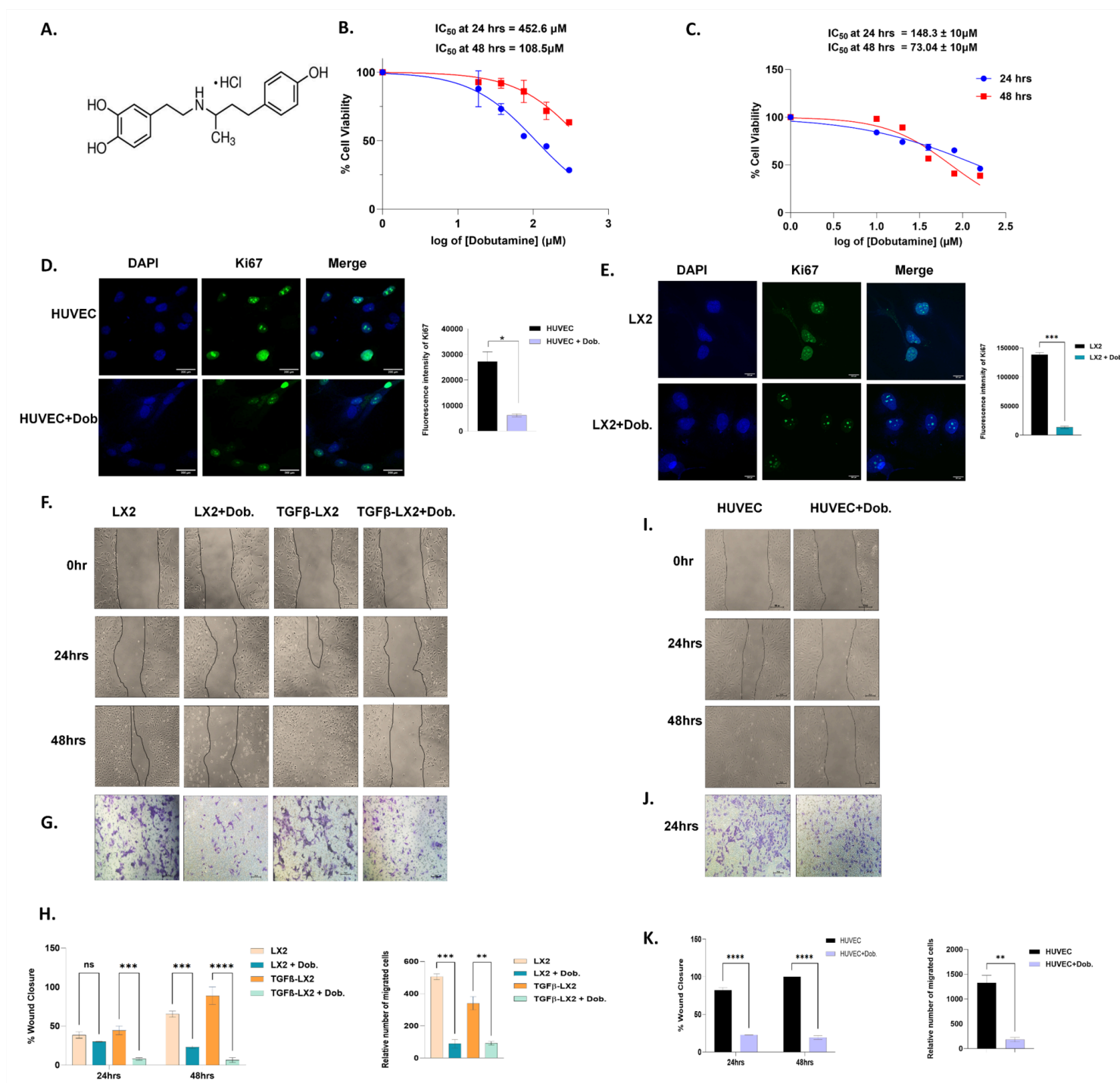


Figure 2

Dobutamine attenuates proliferation and migratory properties of hepatic stellate and endothelial cells

(A) Chemical structure of Dobutamine Hydrochloride. **(B–C)** Effect of Dobutamine on cell viability of HUVEC **(B)** and LX2 **(C)** cells assessed by the MTT assay. Cells were treated with increasing concentrations of Dobutamine (10, 20, 40, 80, and 160 μ M) for 24 h and 48 h. Cell viability was quantified as a percentage relative to untreated controls. Dose–response curves were generated and IC_{50} values calculated: HUVEC cells exhibited IC_{50} values of 452.6 μ M (24 h) and 108.5 μ M (48 h) whereas LX2 cells showed IC_{50} values of 148.3 $\pm$ 10 μ M (24 h) and 73.04 $\pm$ 10 μ M (48 h). Data represent mean $\pm$ SEM from

three independent experiments (n=3). **(D-E)** Representative immunofluorescence images of HUVEC and LX2 cells showing nuclei stained with DAPI (blue) and proliferative marker Ki67 (green). **(D)** Dobutamine treatment visibly reduces Ki67-positive nuclei in dobutamine-treated HUVEC cells compared with untreated controls. **(E)** Dobutamine treatment reduces Ki67 expression in LX2 cells, indicating suppression of proliferation and stromal activation. Scale bar = 200 μ m for all images. **(F-K)** Representative images of scratch wound healing and transwell migration assays for LX2 cells under different conditions: untreated control, dobutamine-treated, TGF β -activated, and combined dobutamine + TGF β treatment and HUVEC cells under two different conditions: untreated control, dobutamine-treated **(F)** Wound healing assay of LX2 cells images captured at 0, 24, and 48 hours using 40 μ M dobutamine hydrochloride, and **(G)** transwell migration assay images obtained after 24 hours. **(H)** statistical analysis of scratch wound healing and transwell assay respectively using two-way ANOVA, ($p < 0.0001$), **(I)** Wound healing assay of HUVEC cells images captured at 0, 24, and 48 hours using 40 μ M dobutamine hydrochloride, and **(J)** transwell migration assay images obtained after 24 hours. **(K)** Statistical analysis of scratch wound healing and transwell assay were performed using two-way ANOVA and one-way ANOVA respectively. Statistical significance is indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns, not significant.

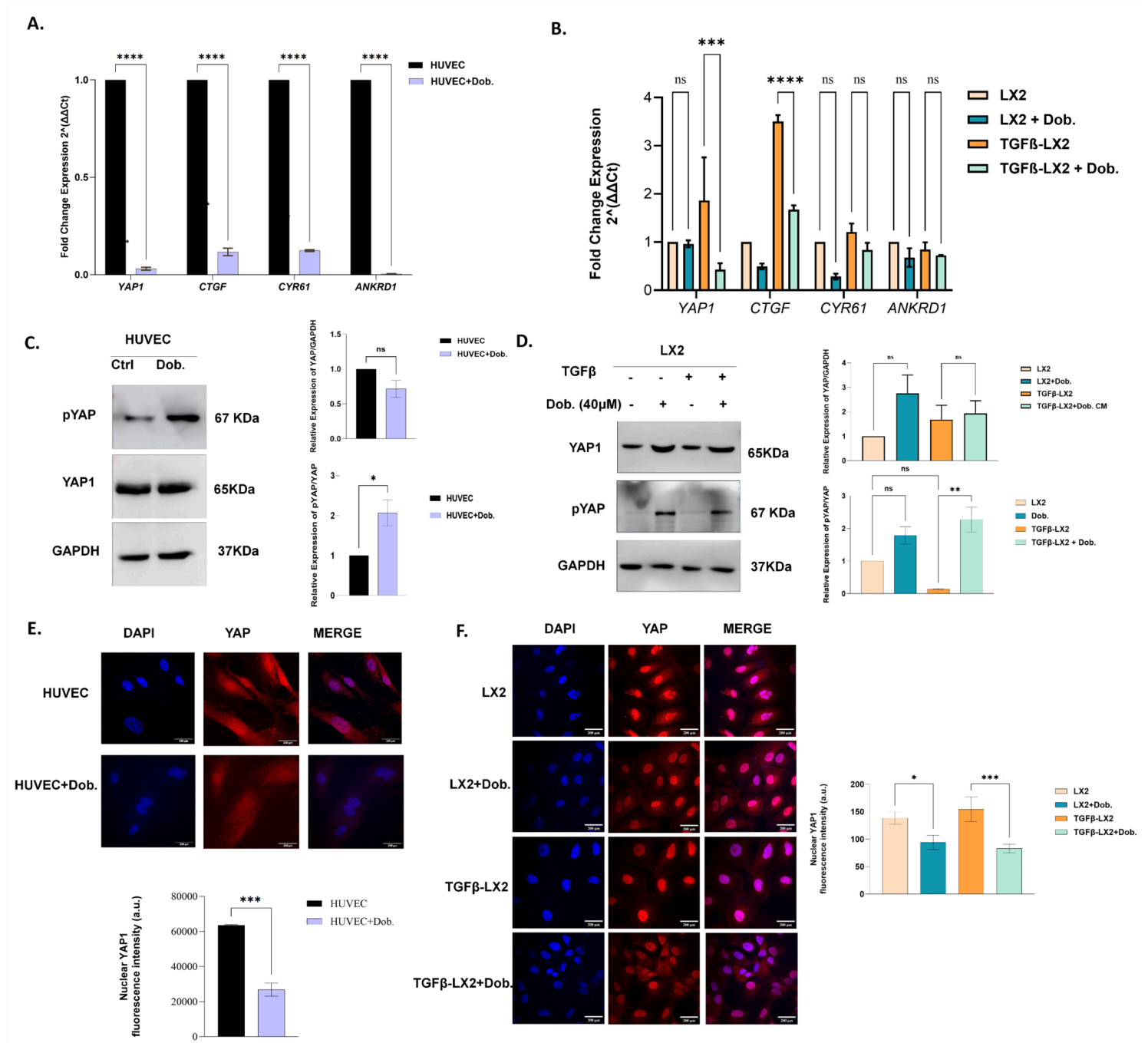


Figure 3

Suppressive effect of dobutamine in YAP signaling by promoting YAP Ser127 phosphorylation

(A–B) RT-qPCR analysis of YAP and its downstream target genes (CTGF, CYR61, and ANKRD1) in **(A)** HUVECs and **(B)** LX2 following treatment with Dobutamine (40 μ M) for 24 h. Relative mRNA expression levels was normalized to ACTB and calculated using the $2^{-\Delta\Delta Ct}$ method. Data are presented as mean $\pm$ SEM from three independent experiments ($n = 3$). Statistical analyses were performed using an unpaired two-tailed Student's *t*-test for panel A and one-way ANOVA followed by Tukey's multiple-comparison test for panel B. Statistical significance is indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns, not significant. **(C–D)** Representative Western blot analysis of total YAP and

phosphorylated YAP (p-YAP^{Ser127}) in **(C)** HUVECs and **(D)** LX2 cells following Dobutamine treatment, demonstrating increased Ser127 phosphorylation, consistent with YAP inactivation. Densitometric quantification of the pYAP/YAP ratio is shown. GAPDH was used as the loading control. Data are representative of three independent experiments. **(E)** Representative immunofluorescence images showing YAP subcellular localization in HUVECs under control and Dobutamine-treated conditions. Dobutamine significantly reduced nuclear accumulation of YAP and promoted cytoplasmic localization. **(F)** Representative immunofluorescence images of YAP localization in LX2 cells under control, Dobutamine-treated, TGF- β 1-stimulated, and combined TGF- β 1 + Dobutamine-treated conditions. TGF- β 1 stimulation increased nuclear YAP localization, whereas Dobutamine treatment markedly attenuated YAP nuclear accumulation even in activated LX2 cells, indicating suppression of YAP signaling. Nuclei were counterstained with DAPI (blue), and YAP is shown in red. Scale bar = 200 μ m. Data are presented as mean $\pm$ SD from three independent experiments (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparison test. *P < 0.05, **P < 0.01, ***P < 0.001; ns, not significant.

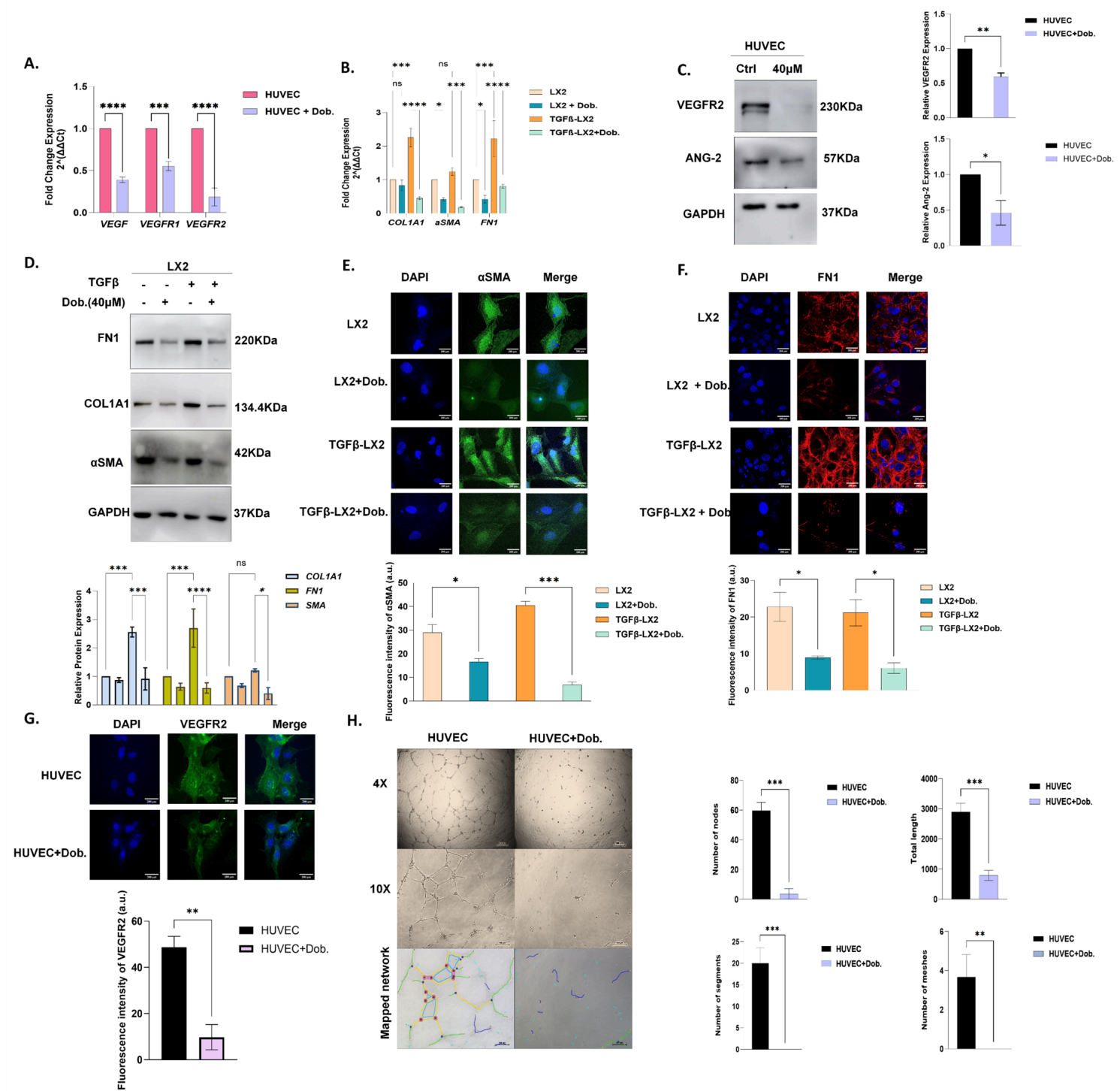


Figure 4

Hepatic stellate cell activation and endothelial angiogenic signaling are suppressed by dobutamine.

(A) RT-qPCR analysis of *VEGF*, *VEGFR1*, and *VEGFR2* expression in HUVECs following treatment with dobutamine (40 μ M) for 24 h. Relative gene expression was normalized to *ACTB* and calculated using the $2^{-\Delta\Delta C_t}$ method. **(B)** RT-qPCR analysis of the hepatic stellate cell activation markers *COL1A1*, *ACTA2* (α SMA), and *FN1* in LX2 cells cultured under control, dobutamine-treated, TGF- β 1-stimulated, or TGF- β 1 plus dobutamine-treated conditions. **(C)** Western blot analysis of VEGFR2 and ANG2 protein expression

in HUVECs following dobutamine treatment. Densitometric quantification of protein was normalized normalized to GAPDH. **(D)** Western blot analysis of COL1A1, FN1, and α SMA expression in LX2 cells under the indicated treatment conditions. Protein expression was normalized to GAPDH, and densitometric quantification is shown below. **(E)** Representative immunofluorescence images of α SMA (green) in LX2 cells cultured under control, dobutamine-treated, TGF- β 1-stimulated, and TGF- β 1 plus dobutamine-treated conditions. **(F)** Representative immunofluorescence images of FN1 (red) in LX2 cells under the indicated treatment conditions. **(G)** Representative immunofluorescence images of VEGFR2 (green) in HUVECs cultured in the absence or presence of dobutamine. **(H)** Effect of Dobutamine on *in vitro* tube formation assay. In HUVECs received 40 μ M Dobutamine, the lowest number of meshes, nodes, segments and tube length index was observed. Photography was done at 4x and 10x magnification after 16h. Gene expression data are presented as mean $\pm$ SEM, whereas protein and fluorescence quantification are presented as mean $\pm$ SD from three independent biological experiments (n = 3). Statistical analyses were performed using an unpaired two-tailed Student's *t*-test for comparisons between two groups and one-way ANOVA followed by Tukey's multiple-comparison test for experiments involving multiple treatment groups. Statistical significance is indicated as **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001; ns, not significant.

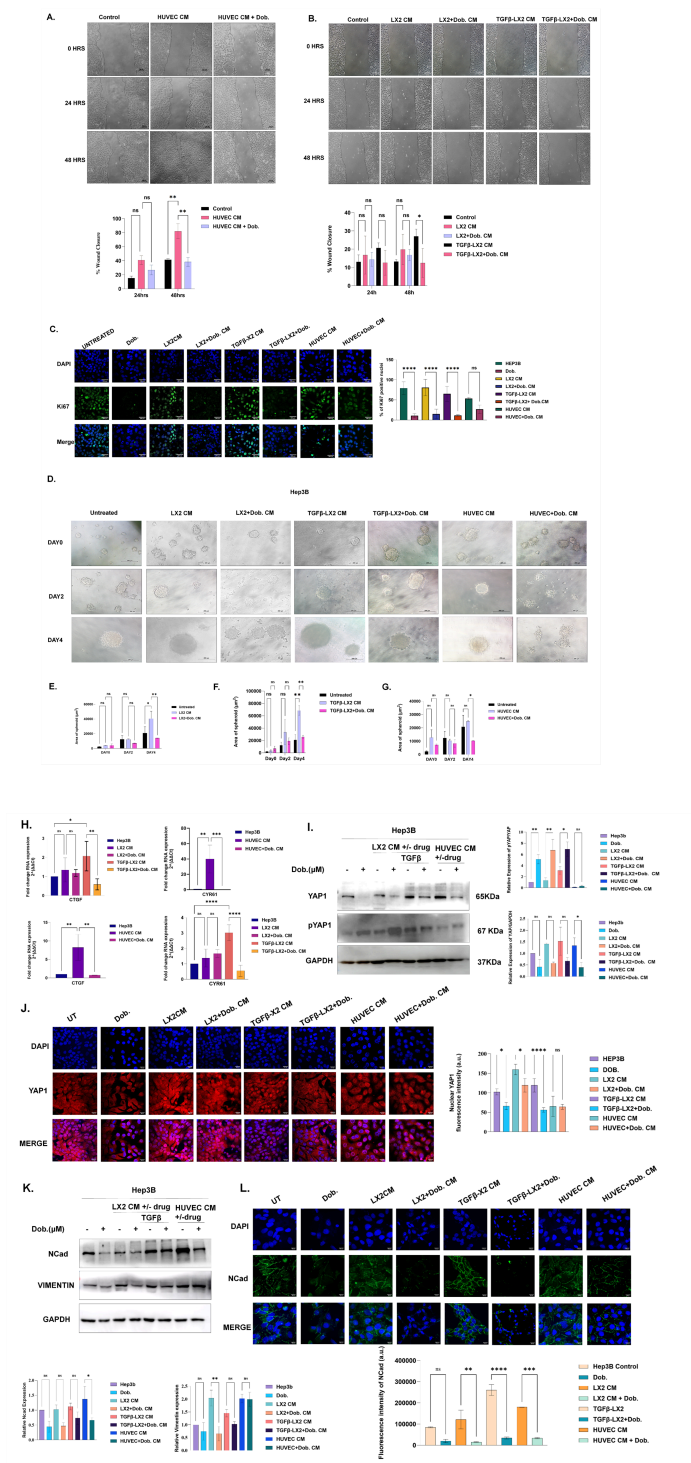


Figure 5

Conditioned media from dobutamine treated stromal cells alter Hep3B cell migration, proliferation, YAP1 signaling and EMT

CM were collected from untreated or dobutamine treated LX2 cells (with or without TGF- β stimulation) and HUVECs and subsequently applied to Hep3B cells. **(A, B)** Representative scratch wound-healing

images of Hep3B cells cultured in stromal cells treated or untreated CM. Images were acquired at 0, 24 and 48h. **(C)** Representative immunofluorescence images of ki67 expression in HEp3B cells after treatment with the indicated CM. nuclei were counterstained with DAPI (blue), and ki67 is shown in green. Quantification of mean ki67 fluorescence intensity is presented on the right **(D)** Representative bright-field images of Hep3B spheroids cultured with CM from untreated or dobutamine treated LX2 cells, TGF β -stimulated or dobutamine-treated TGF β stimulated X2 cells, and untreated or dobutamine-treated HUVECs at Day0, Day2, and Day4. **(E-G)** Quantification of spheroid area. Spheroid area was quantified using ImageJ. Data are presented as mean $\pm$ SEM from three independent experiments (n=3). **(H)** Representative RT-qPCR analysis of YAP downstream targets CTGF and CYR61 in Hep3B cells after treatment with the indicated CM **(I)** Representative western blot analysis of total YAP1 and phosphorylated YAP1(Ser127) in Hep3B cells treated with dobutamine alone or the indicated CM. GAPDH served as the loading control. Densitometric analysis of the pYAP/YAP ratio is shown. **(J)** Representative immunofluorescence images demonstrating YAP1 localization in Hep3B cells after treatment with the indicated CM. Nuclei were stained with DAPI (blue), and YAP1 is shown in red. Quantification of nuclear YAP1 fluorescence intensity is presented. **(K)** Representative Western blot analysis of the EMT markers N-cadherin and Vimentin in Hep3B cells following treatment with CM from indicated stromal cell groups. GAPDH served as the loading control. Corresponding densitometric quantification is shown below. **(L)** Representative immunofluorescence images of N-cadherin expression in Hep3B cells treated with the indicated CM. Nuclei were stained with DAPI (blue), N-cad is shown in green. Quantification of fluorescence intensity is shown below. Statistical significance was determined using one-way ANOVA for Western blot, immunofluorescence, and two-way ANOVA for wound-healing assays. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001; ns, not significant. Scale bar, 200 μ m

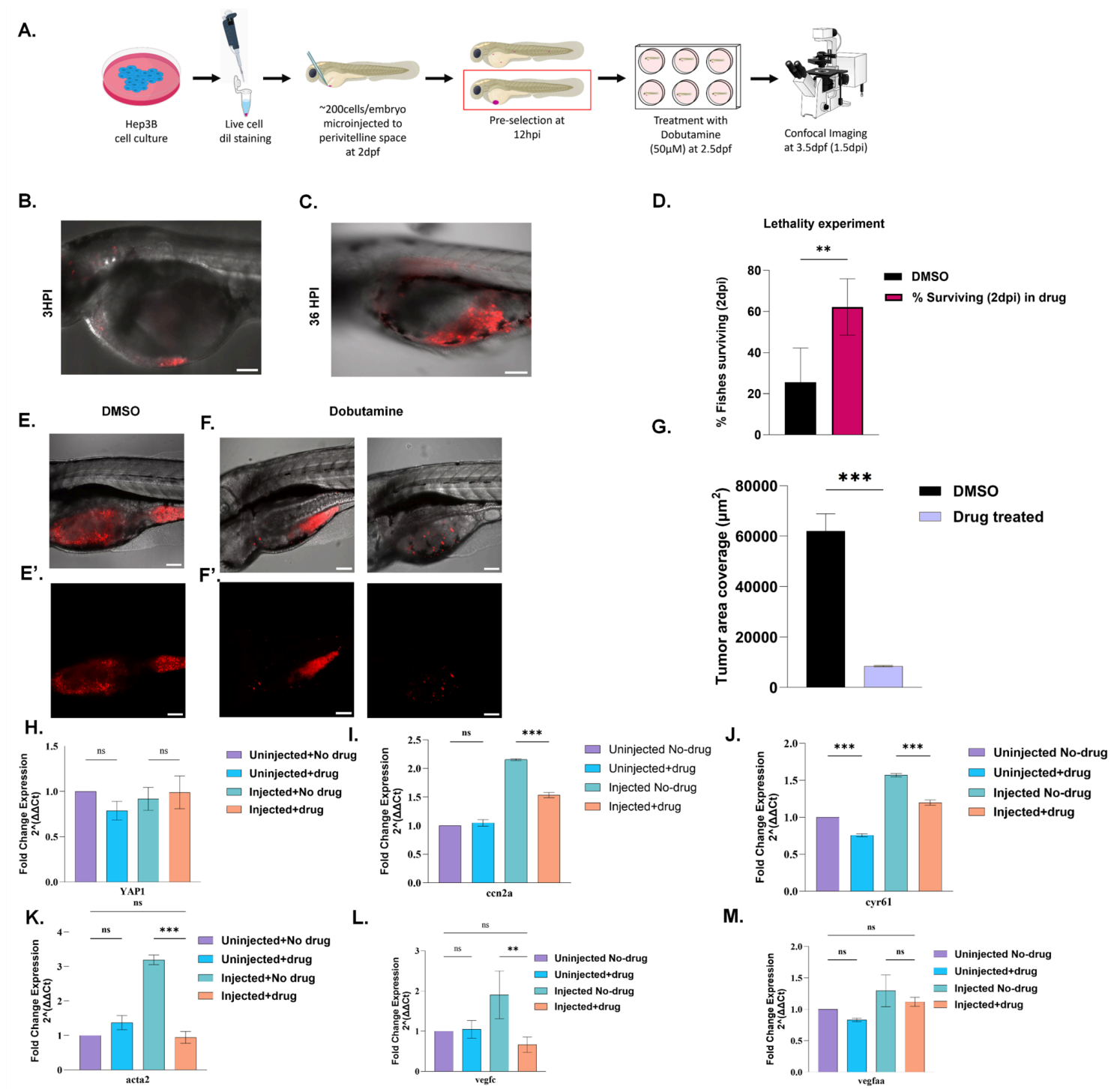


Figure 6

Dobutamine inhibits Hep3B xenograft progression and modulates YA1P-associated gene expression in zebrafish larvae.

(A) Summary of zebrafish xenograft survival data following transplantation of Dil-labeled Hep3B cells into the perivitelline space of zebrafish larvae at 2days post fertilization (dpf). Larvae were treated with vehicle control (DMSO) or 50 μM dobutamine, and survival was monitored at 1day post injection (dpi) and 2dpi. **(B, C)** Representative fluorescence images of zebrafish xenograft at 3hpi, and 36hpi respectively.

(D) Quantification of larval survival at 2dpi showing a significant increase in survival in the dobutamine-treated group compared with DMSO-treated controls. Data are presented as mean±SEM from three independent experiments. Statistical significance was determined using an unpaired Student's t-test ($P<0.05$). **(E,E',F,and F')** Representative brightfield and fluorescence images of zebrafish xenografts at 4dpf (2dpi) showing Dil-labelled Hep3B tumor cells in control and dobutamine-treated larvae. Dobutamine treatment markedly reduced tumor burden and dissemination compared with vehicle-treated control. Scale bars are indicated in the images. **(G)** Quantification of tumor area measured from fluorescent images using ImageJ software. Tumor burden was significantly reduced in dobutamine-treated larvae compared with DMSO controls. Data are presented as mean±SEM from three independent experiments and analyzed using an unpaired Student's t-test ($**P<0.001$). **(H-M)** RT-qPCR analysis of zebrafish xenografts to evaluate the effect of dobutamine treatment on YAP1, its downstream targets (*ccn2a*, *cyr61*), and tumor associated gene expression. Four experimental groups were included: uninjected-untreated larvae, uninjected-dobutamine-treated larvae, Hep3B-injected untreated larvae, and Hep3B-injected dobutamine-treated larvae.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [YAP1.Supplementary21.09.2026.pdf](#)
- [WesternDataORIGINALBLOTS.pdf](#)