

Supplementary Information

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

Prachi Sharma¹, Pallavi Chakraborty², Shaikh Maryam Ghufra³, Bornika Roy¹, Basundhara Das¹, Jaydeb Pal¹, Shrishty Sakshi⁴, Shivani Jaiswal¹, Rudra Nayan Das², Shinjinee Sengupta¹, Sampa Ghose⁵, Subhrajit Biswas¹ *

Contents:

Table S1: Quantitative PCR (qPCR) human primer nucleotide sequences

Gene Symbol	Nucleotide Sequence (5' to 3')	
α -Smooth Muscle Actin (α -SMA)	Forward	CAATGGCTCTGGGCTCTGTAA
	Reverse	ACCATCACCCCCTGATGTCT
Collagen Type I α (I) chain (COL1A1)	Forward	GACGAGACCAAGAACTGCCC
	Reverse	TTGGTCGGTGGGTGACTCT
Fibronectin (FN1)	Forward	TGGGCAACTCTGTCAACGAA
	Reverse	CCACTCATCTCCAACGGCAT
Yes Associated Protein (YAP)	Forward	GAGAGGAGCTGATGCCAAGTC
	Reverse	CGGTGCATGTGTCTCCTTAGA
Connective Tissue Growth Factor (CTGF)	Forward	GCCTCAATTTCTGAACACCATAGG
	Reverse	ACATTTCTACCTAGAAATCAGCCT
CYR61	Forward	ACCAAGGGGCTGGAATGCAA
	Reverse	ATAGTTCTTGGGGACACAGAGGAA
ANKRD1	Forward	CGAGAAACAACGAGAGGCAG
	Reverse	GAGCAGCCTTCAGAAACGTAG
ACTB	Forward	CGTCTTCCCCTCCATCGT
	Reverse	GGAATCCTTCTGACCCATGC

Table S2: Quantitative PCR (qPCR) zebrafish primer nucleotide sequences

Gene Symbol	Nucleotide Sequence (5' to 3')	
gapdh	Forward	GGCAAGCTTACTGGTATGGC
	Reverse	TGAGAGCAATACCAGCACCA
yap1	Forward	ACGGGTGGGAACAAGCTATT
	Reverse	GCTGGTTCATTGCGAAACGC
ccn2a	Forward	GATCGTAAAATGACCGGGCC
	Reverse	TTCTTAGCTTGGTGAGGCGA
cyr61	Forward	GCACTGATCTGCGGCATTTA
	Reverse	TGGTTTCCTCTAGCTGCCAA
vegfaa	Forward	TGGCTCCTGCAAATTCACACAA
	Reverse	ATCTTGGCTTTTCACATCTGCAA
vegfc	Forward	AAGGGCCCTAACAGAATGTC
	Reverse	TTTGAATGAAGGGTGTGAGG
acta2	Forward	CTGGACTGTGTAAGGCAGGC
	Reverse	CCTACCATCACTCCCTGATGTC

Table S3: Primary and secondary antibodies details

Target Protein	Company	Application	Dilution
α -SMA	CST, #19245	Western Blot, Immunofluorescence	1:1000
Collagen I	CST, #72026	Western Blot	1:1000
Fibronectin	Abcam, #ab2413	Western Blot, Immunofluorescence	1:2000
GAPDH	CST, #5174	Western Blot	1:2000
Ki67	CST, #9449	Immunofluorescence	1:200
YAP	Scbt, #sc-101199	Western Blot, Immunofluorescence	1:1000 1:100
pYAP	Invitrogen, #MA5-33207	Immunofluorescence	1:100
VEGFR2	CST, #2479S	WesternBlot, Immunofluorescence	1:1000 1:100
Ang-2	Biolegend, #682702	Western Blot	1:1000
Anti-rabbit HRP-conjugated secondary antibody	Jackson Immuno Research, #111-035-144	Western Blot	1:10,000
Anti-mouse HRP-conjugated secondary antibody	Jackson Immuno Research, #115-035-003	Western Blot	1:10,000
Alexa Fluor TM 488 goat anti-rabbit IgG (H+L)	Invitrogen, #A11008	Immunofluorescence	1:200
Alexa Fluor TM 568 goat anti-rabbit IgG (H+L)	Invitrogen, #A11011	Immunofluorescence	1:200
Alexa Fluor TM 594 goat anti-mouseIgG (H+L)	Invitrogen, #A11032	Immunofluorescence	1:200
Alexa Fluor TM 488 goat anti-mouse IgG (H+L)	Invitrogen, #A11029	Immunofluorescence	1:200

Table S4:

Definition of loss	Threshold (log2)	Patients	% of 92
Any reduction in tumor	less than 0	37	40.2
Greater than 1.19 fold	less than minus 0.25	19	20.7
Greater than 1.25 fold	less than minus 0.32	18	19.6
Greater than 1.41 fold	less than minus 0.50	12	13.0
Greater than 2 fold	less than minus 1.00	3	3.3

Supplementary Table S4: Proportions of patients showing reduced tumor YAP Ser127 phosphorylation relative to their own matched adjacent liver, at increasingly stringent thresholds.

Table S5:

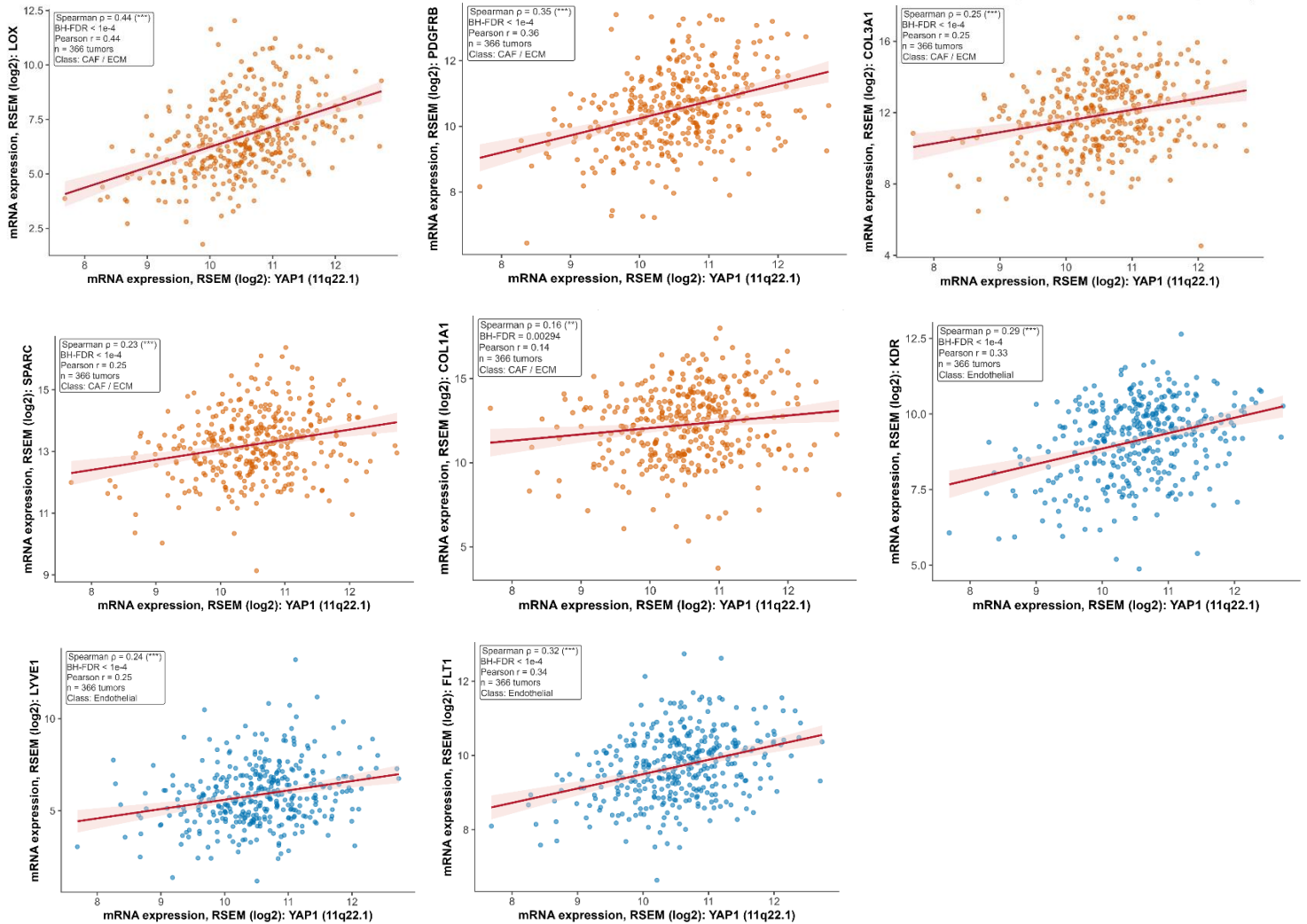
No. of fishes xenografted (2dpf)	No. of injected fishes in DMSO	No. of fishes surviving post injection in DMSO	No. of injected fishes in 50µM drug	No. of fishes surviving post injection in 50µM drug	Percentage surviving (2dpi) in DMSO	Percentage surviving (2dpi) in drug
100	12	2	15	7	16.66666667	46.66666667
100	8	0	9	4	0	44.44444444
100	13	4	13	9	30.76923077	69.23076923
100	24	6	24	15	30.76923077	75
100	20	10	20	15	25	62.5
100	26	8	20	15	50	75

Supplementary Table S5: Establishment and validation of the zebrafish xenograft model.

Summary of embryo survival following microinjection and subsequent treatment. The data demonstrate the feasibility and reproducibility of the zebrafish xenograft model used for subsequent assessment of tumor growth and dissemination.

Supplementary Figures with Legends

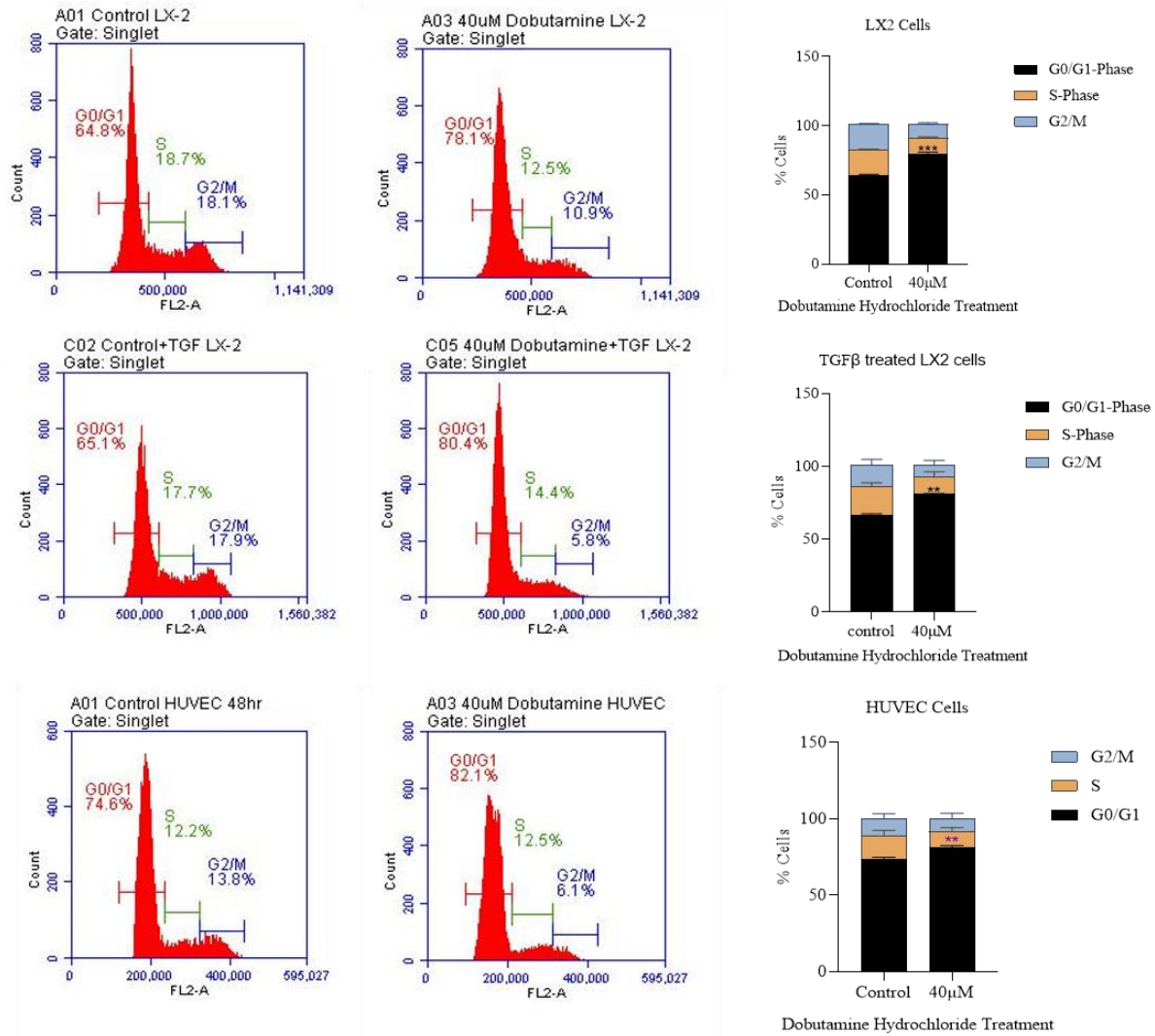
Figure S1.



Supplementary Fig. S1: Correlation of stromal and endothelial markers with YAP1 expression in HCC.

Scatterplot analysis showing the individual correlations between *YAP1* expression and *LOX*, *PDGFRB*, *COL3A1*, *SPARC*, *COL1A1*, *KDR*, *LYVE1*, and *FLT1* in HCC samples. Each dot represents an individual sample. Correlation coefficients and statistical significance are indicated within the respective plots.

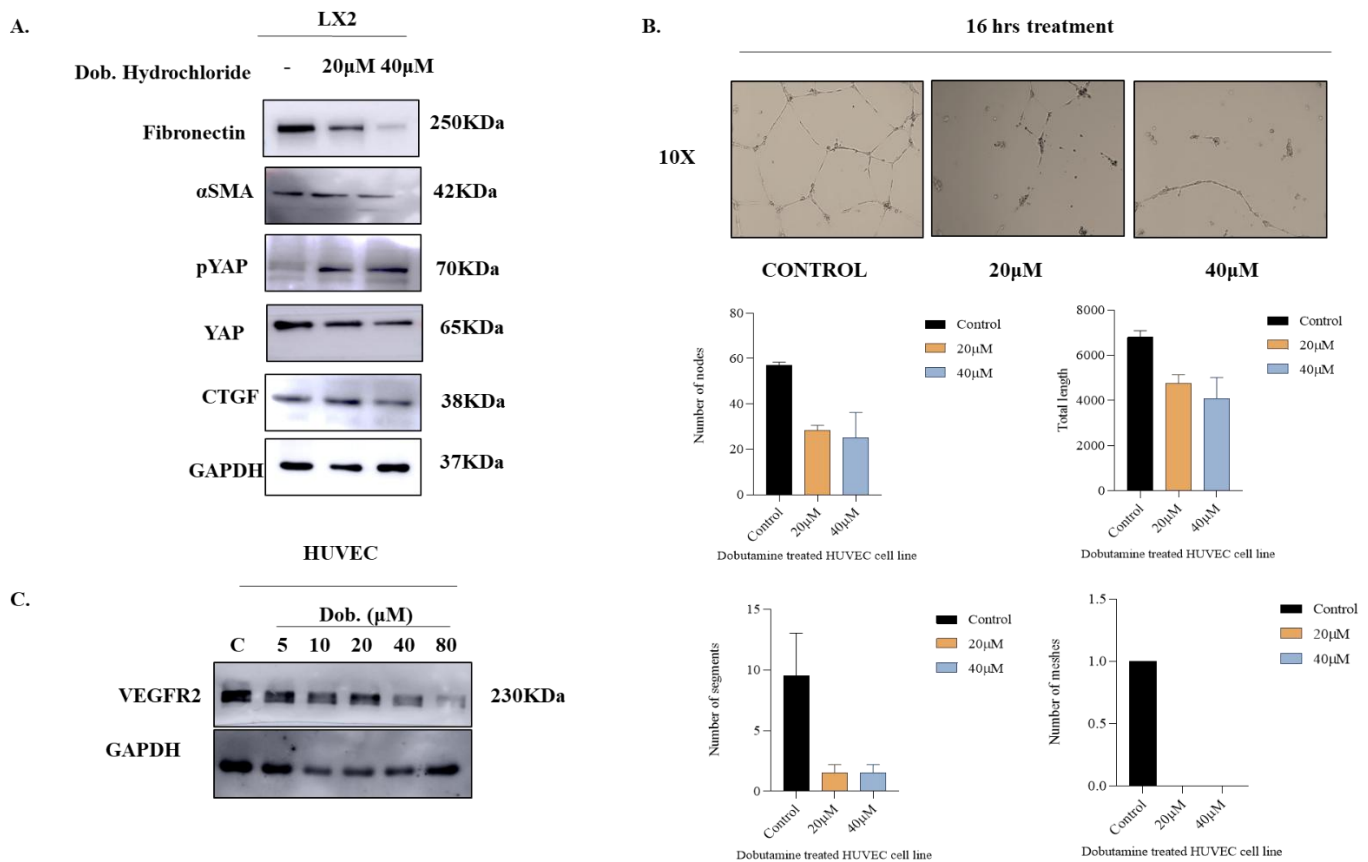
Figure S2.



Supplementary Figure S2: Dobutamine induces G0/G1 cell-cycle arrest in stromal cells.

(A) Flow cytometry histogram showing cell-cycle distribution of untreated and 40μM dobutamine treated LX2 cells after 24h. (B) Quantification of LX2 cells in G0/G1, S, and G2/M phases following dobutamine treatment. (low C) Flow cytometry histograms of TGFβ-stimulated LX2 cells treated with or without dobutamine for 24h. (D) quantification of cell cycle distribution in TGFβ-stimulated LX2 cells. (E) Flow cytometry histograms of untreated and dobutamine treated HUVEC cells after 48h. (F) Quantification of HUVEC cells in G0/G1, S, G2/M phases. Data are presented as mean±SEM from three independent experiments. Statistical significance was determined using two-way ANOVA followed by Tukey's multiple comparison test.

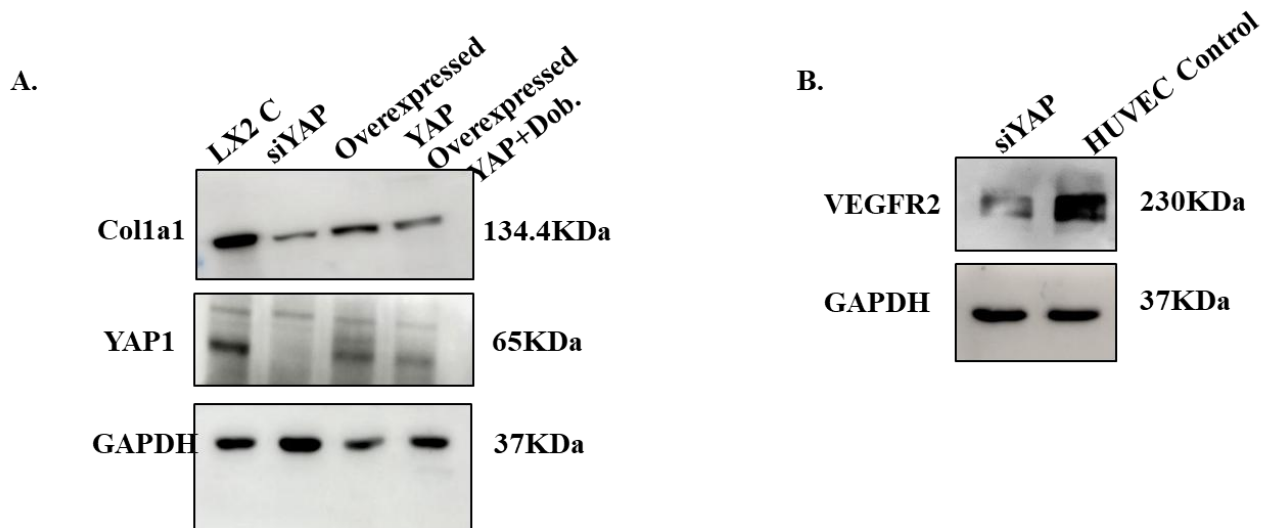
Figure S3.



Supplementary Figure S3: Additional characterization of the effects of dobutamine on stromal cells

(A) Representative Western blot analysis of Fibronectin1, collagen1 type1, alpha-SMA, YAP1, pYAP and CTGF in LX2 cells following dobutamine treatment, with GAPDH as loading control. (B) Representative images of Tube formation assay of HUVEC under control and dobutamine treatment (20μM, and 40μM). The imaging was done at 10X after 16h of treatment. Quantitative analysis of tube length, number of nodes, segments, and meshes was done using ImageJ/Fiji. (C) Western blot analysis of VEGFR2 expression in HUVEC cell line treated with increasing concentrations of dobutamine (0, 10, 20, 40, and 80μM) with GAPDH as a loading control.

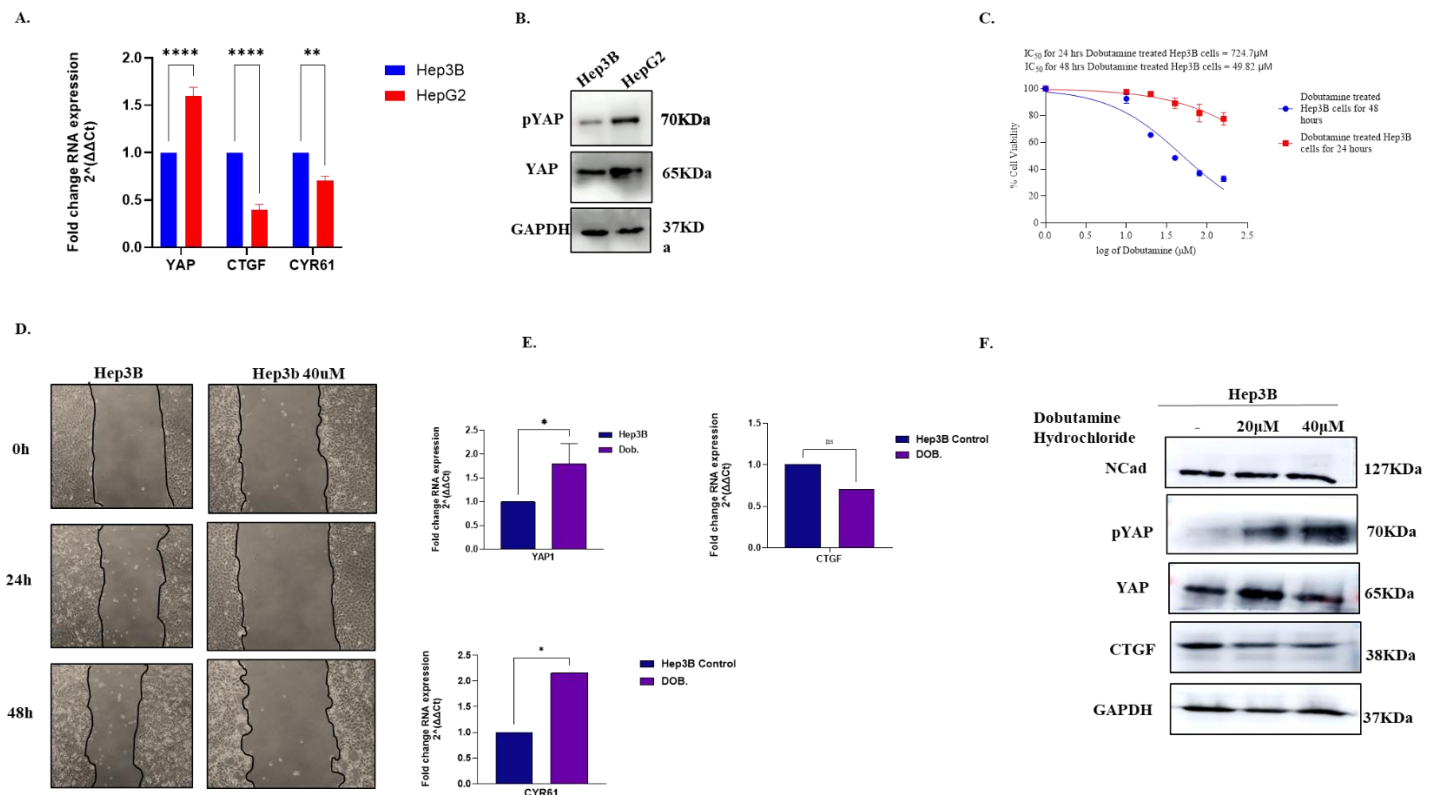
Figure S4.



Supplementary Figure S4: supportive validation of the contribution of YAP1 to stromal activation and endothelial signaling.

(A) Representative Western blot analysis of Col1A1 and YAP1 expression in LX2 cells following YAP1 silencing, YAP1 overexpression, and YAP1 overexpression in combination with dobutamine treatment. GAPDH was used as a loading control. (B) Representative Western blot analysis of VEGFR2 expression in HUVECs following YAP1 siRNA transfection. GAPDH was used as a loading control.

Figure S5.

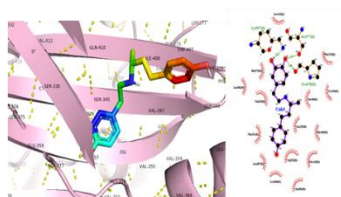


Supplementary Figure S5: Direct effects of dobutamine on HCC cells

(A) Relative mRNA expression of YAP, CTGF, and CYR61 in Hep3B and HpG2 cells. Gene expression was normalized to ACTBand calculated using $2^{-\Delta\Delta Ct}$ method (B) Western blot analysis of YAP and pYAP expression in Hep3B and HepG2 cells. (C) Effect of dobutamine on cell viability of Hep3B cells. (D) Representation scratch wound-healing images showing Hep3B migration under hep control and 40 μ M dobutamine treatment. (E) RT-qPCR analysis of YAP1, CTGF, and CYR61 in Hep3B cells. Gene expression was normalized to ACTB and calculated using $2^{-\Delta\Delta Ct}$ method. Data are presented as mean $\pm$ SEM from three independent experiments. Statistical significance was determined using an unpaired two-tailed Student's t-test. $P < 0.05$ was considered statistically significant. (F) Representative western blot showing the expression of N-cadherin, pYAP, YAP, and CTGF in Hep3B cells following direct treatment with 20 μ M and 40 μ M dobutamine. GAPDH was used as loading control.

Figure S6

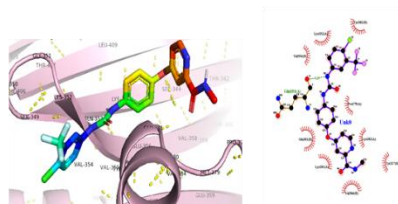
A. DOBUTAMINE



mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-9.2	0.000 0.000
2	-8.8	0.752 8.846
3	-8.3	22.049 25.945
4	-8.1	22.177 25.959
5	-8.1	1.500 2.246
6	-8.0	21.776 25.640
7	-7.7	1.477 2.071
8	-7.7	1.758 8.693
9	-7.6	22.477 24.485

Writing output ... done.

B. REGORAFENIB

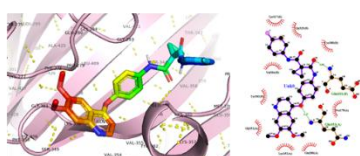


mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-9.5	0.000 0.000
2	-8.9	1.974 2.834
3	-8.8	17.197 19.199
4	-8.7	7.700 14.481
5	-8.7	5.099 10.973
6	-8.6	4.064 5.081
7	-8.6	5.171 10.578
8	-8.3	2.195 2.721
9	-8.3	7.911 9.772

Writing output ... done.

C:\Users\Bhaskar\Desktop\regorafenib>cd

C. SORAFENIB

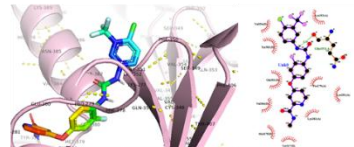


mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-9.0	0.000 0.000
2	-8.5	2.057 2.607
3	-8.4	6.049 11.036
4	-8.3	6.375 11.015
5	-8.3	6.302 10.572
6	-8.0	8.060 10.110
7	-7.9	27.000 29.960
8	-7.8	10.860 13.963
9	-7.8	27.349 29.511

Writing output ... done.

C:\Users\Bhaskar\Desktop\sorafenib>

D. LENVATINIB

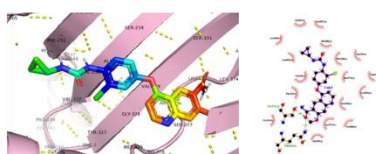


mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-10.7	0.000 0.000
2	-9.1	18.429 22.148
3	-8.8	18.164 20.149
4	-8.7	17.516 18.794
5	-8.4	19.060 21.239
6	-8.3	14.478 17.833
7	-8.0	18.245 21.579
8	-7.9	21.941 25.296
9	-7.9	17.886 21.880

Writing output ... done.

C:\Users\Bhaskar\Desktop\lenvatinib>

E. CABOZANTINIB



mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-9.2	0.000 0.000
2	-8.8	2.851 3.940
3	-8.5	1.394 1.948
4	-8.5	2.039 2.999
5	-8.4	2.318 2.637
6	-8.3	1.876 3.277
7	-7.6	3.360 4.620
8	-7.6	11.200 15.600
9	-7.5	4.705 9.638

Writing output ... done.

C:\Users\Bhaskar\Desktop\cabozantinib>

Supplementary Figure S6: In silico molecular docking analysis of dobutamine and clinically approved tyrosine kinase inhibitors with the YAP–TEAD complex.

Representative docking poses and binding affinity scores of dobutamine, regorafenib, sorafenib, lenvatinib, and cabozantinib against the YAP–TEAD complex. The predicted interactions indicate that dobutamine occupies a potential binding pocket within the YAP–TEAD interface with a favorable binding energy comparable to several approved HCC therapeutics. These computational results provide structural support for the observed inhibition of YAP signaling but require experimental validation.