Supplemental Information

YAP1 Inhibition in HUVECs Is Associated with Released Exosomes and Increased Hepatocarcinoma Invasion and Metastasis

Yan Li, Xiaodong Zhang, Qianqian Zheng, Yijun Zhang, Yingbo Ma, Chen Zhu, Liang Yang, Xueqiang Peng, Qi Wang, Biao Wang, Xin Meng, Hangyu Li, and Jingang Liu
**Supplement Information**

**Material and methods**

**Immunohistochemical staining (IHC)**

4 μm-thick sections were treated with 0.3% endogenous peroxidase blocking solution for 20 minutes. Sections were treated sequentially with normal goat serum for 20 minutes and incubated with anti-YAP1 and anti-CD31 antibody at a dilution of 1:100. SP staining kit and DAB kit were supplied by Maixin-Bio Co., Fuzhou, China. Samples were analyzed by brightfield microscopy (Olympus, Tokyo, Japan).

**RNA isolation and quantitative real-time PCR**

RNA was isolated using TRIzol reagent (Invitrogen). cDNA was synthesized from 5 μg total RNA by reverse transcription according to the manufacturer’s instructions (Promega, Beijing, China). mRNA levels were measured by quantitative real-time PCR on a Light Cycler 480. Amplification was carried out in a 20 μl volume for 40 cycles and the product was detected using SYBR Green.

**Western blot**

The extracted proteins were separated by 12% SDS-PAGE, then transferred to polyvinylidene fluoride membranes. After incubating with 5% BSA, the membranes were probed with antibodies overnight at 4°C and then incubated with HRP-labeled secondary antibodies. Relative expression was normalized to β-actin or GAPDH using
Image J software.

Cell proliferation assay

CCK-8 assay was used to detect cell viability after transfection and after treatment with siYAP1 or verteporfin. Briefly, 3×10^3 cells were seeded in a 96-well plate, then cultured in medium containing 5% FBS. After 24, 48, and 72 hours, the absorbance values were measured at 450 nm. Each experiment was repeated in triplicate.

Migration and Invasion

For the migration assay, 3 × 10^4 cells were seeded into the upper chamber of a transwell plate in 200 μl of serum-free media, while the lower chamber was filled with 600 μl of media supplemented with 20% FBS. For the invasion assay, 3 × 10^4 cells were seeded into the top chambers, which had been pre-coated with Matrigel, and the lower chamber was filled with 600 μl of media supplemented with 20% FBS. After incubation for 24 h, migrated/invaded cells in the lower compartment were fixed and stained with 0.1% crystal violet. Cells were quantified using Image J. The experiments were repeated at least in triplicate.

Electron Microscopy

Sample Cells were removed and fixed in 5% glutaraldehyde (protected from light) in 0.1 M phosphate buffer at 4 °C. The isolated exosomes (20-40μ) heavy suspension droplets on the special copper mesh of electron microscope, then performed them on
negative stain of 20μl 2% phosphotungstic acid for 10min. The samples were analyzed using H-7650 electron microscope at 100KV.
Figure legends

Fig.S1 The YAP1 expression is positively correlated with CD31, SPHK1, SPHK2, and VEGF angiogenic factors by GEPIA. X axis showed the expression of YAP. Y axis showed the expression of angiogenic factors.

Fig.S2 Activated YAP1 promotes proliferation, migration and angiogenesis of vascular endothelial cells. A Proliferation assay of HUVECs with transfecting YAP5SA via CCK8 detection. B Transwell migration assay of HUVECs after transfected YAP5SA 24 h. C Matrigel capillary tube formation of HUVECs when transfected YAP5SA. *P < 0.05; **P < 0.01.
**Fig.S3** Fluorescence microscopy images of PKH67-labeled Exosomes and hepatoma cells. Microscopy images of Hep3B and Huh-7 cells treated for 6 h with the indicated PKH67-labeled EVs, to evaluate EV up-take.

**Fig.S4** Bioanalysis of MALAT1 in hepatocellular carcinoma. A GSEA results showed metastasis signatures enriched in hepatocellular carcinoma generated from TCGA and categorized into subgroups according to their median MALAT1 expression. 

**B-C** The OS and DFS of MALAT1 by GEPIA. 

**D-E** Transwell was used to detect the migration ability of Hep3B and Huh7 cells after MALAT1 knockdown by transfected MALAT1 siRNAs. **P < 0.01; ***P < 0.001.

**Fig.S5** Transwell was used to detect invasion and migration of exosomes on Hep3B and Huh-7 cells when ERK1/2 activity was inhibited by PD98059. **P < 0.01; #: no significance.
| Primers     | sequence                                      |
|------------|-----------------------------------------------|
| YAP1       | 5'-AGAATCAGTCAGAGTGCTC-3'                    |
|            | 5'-CGCAGCCTCTCTCTCTCC-3'                     |
| MALAT1     | 5'-CGCAGCGAGAGTGTCGCTC-3'                    |
|            | 5'-TTCTGTGTTATGCGCTGTGTG-3'                  |
| TUG1       | 5'-CAACCATTAGGAGCCCTG-3'                     |
|            | 5'-GCTTTACTGAGGTGCAATT-3'                    |
| LINC00965  | 5'-GCACAGCTTAGGGTCATGT-3'                    |
|            | 5'-CGATGAGCAAGAAAGCAACT-3'                   |
| NBR2       | 5'-AGTTTTGTCCTCCACCTTGTG-3'                  |
|            | 5'-AGCAGCTCTCCAGTGCTCTG-3'                   |
| LINC00998  | 5'-AGACAATGCGCCCAAGAGAAA-3'                  |
|            | 5'-GACACAGGCGCAACAACAAA-3'                   |
| LINC00471  | 5'-CACAAAGGCTGACAGGCAAT-3'                   |
|            | 5'-GCAGAGCGAGGTATGAGGC-3'                    |
| LINC00471  | 5'-GACAGCGGATCGACATCAT-3'                    |
|            | 5'-AGACTCAGGCTGATGGGTA-3'                    |
| DLEU2      | 5'-TGCGCAACTTCAGGCTTGTG-3'                   |
|            | 5'-TGGAACAGGCAAACCTCTGGG-3'                  |
| LINC01128  | 5'-GTCTGGTTGTGTCCTGCTTG-3'                   |
|            | 5'-GACGAGGTTGTGCCCTGAGAG-3'                  |
| LINC00652  | 5'-CAGCTAGTGTCAGTCTAAT-3'                    |
|            | 5'-GGGCTTAAAGCAGTCAAG-3'                     |
| LINC01341  | 5'-GAACCTGTCTACAGTGCTC-3'                    |
|            | 5'-AATGCCAGCGTAAAGTGAC-3'                    |
| GAS5       | 5'-AACTTGCTGGGACACAGCTTA-3'                  |
|            | 5'-GCACCTCAGGGCTTGAGG-3'                     |
| LINC00152  | 5'-CAACAGTGATTCGCGCATT-3'                    |
|            | 5'-GCAAGCGAGGTTATGAGGC-3'                    |
| LINC00341  | 5'-GGCCCTACAGGAGTGCTAGC-3'                   |
|            | 5'-TCCCTCATCTCCAGAGGTT-3'                    |
| LINC00889  | 5'-GAAGTGTCGCTGACGGGTAG-3'                   |
|            | 5'-CTCCTTTACTGAGGTGCGTG-3'                   |
| 18S        | 5'-GCAGAAATCCAGGGCAGTACT-3'                  |
|            | 5'-TCTTTCAGTGCTCCAGT-3'                     |
### Table S2

| Name       | Supplier     | Cat No.     |
|------------|--------------|-------------|
| Anti-YAP1  | CST          | 14074s      |
| Anti-CD31  | Immunoway    | YM6277      |
| Anti-CD63  | GeneText     | #GTX28219   |
| Anti-CD81  | ProteinTech  | 66866-1-Ig  |
| Anti-MMP2  | CST          | 40994S      |
| Anti-MMP9  | CST          | 13667S      |
| Anti-VAMP3 | Novus        | NBP2-76983  |
| Anti-VAMP8 | Abcam        | ab76021     |
| syntaxin 4 | Lifespan     | LS-B9936    |
| Anti-STXBP5| Biorbyt      | orb448533   |
| Anti-SNAP23| Abcam        | ab3340      |
| Anti-p-ERK1/2| CST      | 9101S      |
| Anti-ERK1/2 | CST          | 4695S      |
| Anti- GAPDH| ProteinTech  | 60004-1-Ig  |
| Anti- β-actin| ProteinTech| 60008-1-Ig  |

Table S1 Primers

Table S2 Anti-bodies