Supplementary Information

Selective Targeting of Vascular Endothelial YAP Activity Blocks EndMT and Ameliorates Unilateral Ureteral Obstruction-Induced Kidney Fibrosis

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Supplementary information contains methods, three tables and one supplemental figure.

Methods: Cell lines and antibodies; Isolation of mouse kidney endothelial cells; RNA quantification and sequencing.

Supplementary Information Table S1: RNA sequencing data from kidney endothelial cells.

Supplementary Information Table S2: ShRNA targeting sequence.

Supplementary Information Table S3: All primers used for RT-PCR.

Supplementary Information Figure S1: Detecting the expression of renal TAZ in the mouse model of unilateral ureteral obstruction (UUO)

Methods

Cell lines and antibodies

Human umbilical vein endothelial cells (HUVECs) were purchased from iCell and cultured in primary endothelial cell culture system (PriMed-icell-002) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco). HEK293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified incubator with 5% CO2.

Anti-YAP1 (phosphoS127) (ab76252), goat anti-rat IgG (Alexa Fluor 555) (ab150158), goat anti-rabbit IgG (Alexa Fluor 488) antibodies were purchased from Abcam. Rabbit anti-YAP1 (D8H1X, 14074, western blot) was purchased from CST. Rabbit YAP1 polyclonal antibody (13584-1-AP, immunofluorescence), Rabbit TAZ polyclonal antibody (23306-1-AP), α-SMA polyclonal antibody (14395-1-AP), FSP1 (S100A4) polyclonal antibody (16105-1-AP)，Vimentin polyclonal antibody (10366-1-AP) were purchased from Proteintech. Rat anti-mouse CD31 antibody (MEC13.3, 553070) was purchased from BD Pharmingen. Goat anti-rabbit IgG secondary antibody (HRP) (31430) were purchased from Invitrogen.

Isolation of mouse kidney endothelial cells

The kidney tissue was treated as previously described, with some modifications [1]. For isolation of mouse kidney endothelial cells by magnetic beads, the suspensions were incubated with Dynabeads® Magnetic beads (Invitrogen) coated with rat anti-mouse CD31 antibodies for 1 h at 4°C with constant gentle tilting. Bead-bound cells were collected using a magnet: the free beads were washed away, while bead-bound cells representing kidney endothelial cells were retained.

RNA quantification and sequencing

Total cellular RNA was isolated with TRIzol reagent according to standard protocols. One-step qRT-PCR was performed with QuantiTect SYBR Green RT-PCR kit (Qiagen). The primers used for qRT-PCR are displayed in Supporting Information.

The total cellular RNA from mouse kidney endothelial cells was isolated with TRIzol reagent, and sent to Beijing Genomics Institute (BGI) for RNA-sequencing analysis. Sequenced reads were aligned to the Mus musculus reference genome (GRCm38.p5) with HISAT2 (version 2.1.0) [2], and the aligned reads were quantified to

obtain mRNA expression levels using HTSeq-count (version 0.9.1) [3]. DESeq2, a R/Bioconductor package, was used to identify differentially expressed genes across the samples[4].

Reference

[1] Ramachandran, P., Dobie, R., Wilson-Kanamori, J. R., Dora, E. F., Henderson, B. E. P., Luu, N. T., Portman, J. R., Matchett, K. P., Brice, M., Marwick, J. A., Taylor, R. S., Efremova, M., Vento-Tormo, R., Carragher, N. O., Kendall, T. J., Fallowfield, J. A., Harrison, E. M., Mole, D. J., Wigmore, S. J., Newsome, P. N., Weston, C. J., Iredale, J. P., Tacke, F., Pollard, J. W., Ponting, C. P., Marioni, J. C., Teichmann, S. A., and Henderson, N. C. (2019) Resolving the fibrotic niche of human liver cirrhosis at single-cell level, *Nature 575*, 512-518. Doi: 10.1038/s41586-019-1631-3.

[2] Kim, D., Langmead, B., and Salzberg, S. L. (2015) HISAT: a fast spliced aligner with low memory requirements, *Nature methods 12*, 357-360. Doi: 10.1038/nmeth.3317.

[3] Anders, S., Pyl, P. T., and Huber, W. (2015) HTSeq--a Python framework to work with high-throughput sequencing data, *Bioinformatics 31*, 166-169. Doi: 10.1093/bioinformatics/btu638.

[4] Love, M. I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome biology 15*, 550. Doi: 10.1186/s13059-014-0550-8.

Supplementary Information Table S1: RNA sequencing data from kidney endothelial cells. (Separate document)

Supplementary Information Table S2: ShRNA targeting sequences are as following.

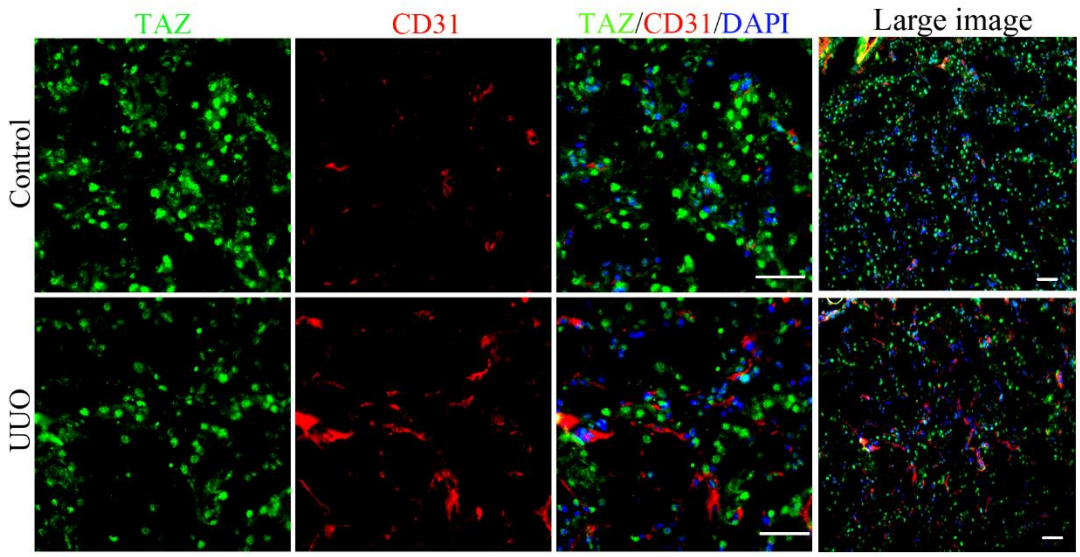
shNC	5'-CCGGTTCTCCGAACGTGTCACGTCTCGAGACGTGACACGTTCGGAGAATTTTT-3’
shF2RL1-1	5'-CCGGCGAAACCTCATCTCTACTAAACTCGAGTTTAGTAGAGATGAGGTTTCGTTTTT-3'
sh F2RL1-2	5'-CCGGGCTCTTTGTAATGTGCTTATTCTCGAGAATAAGCACATTACAAAGAGCTTTTT-3"
shF2RL1-3	5'-CCGGCCACTGTTAAGACCTCCTATTCTCGAGAATAGGAGGTCTTAACAGTGGTTTTT-3'
sh F2RL1-4	5'-CCGGCCTCTAAAGGAAGAAGCCTTACTCGAGTAAGGCTTCTTCCTTTAGAGGTTTTT-3'
shYAP	5'-CCGGCCCAGTTAAATGTTCACCAATCTCGAGATTGGTGAACATTTAACTGGGTTTTT-3'

Supplementary Information Table S3: All primers used for RT-PCR are as following.

GAPDH-realtime-F	5’-CCCACTCCTCCACCTTTGACG-3’
GAPDH- realtime-R	5’-CACCACCCTGTTGCTGTAGCCA-3’
F2RL1-realtime-F	5'- TGCTAGCAGCCTCTCTCTCC -3'
F2RL1-realtime-R	5'- CCAGTGAGGACAGATGCAGA -3'
IL1b-realtime-F	5'- CTTCGAGGCACAAGGCACAA -3'
IL1b-realtime-R	5'- TTCACTGGCGAGCTCAGGTA -3'
IL6-realtime-F	5'- CTCAATATTAGAGTCTCAACCCCCA -3'
IL6-realtime-R	5'- GAGAAGGCAACTGGACCGAA -3'
CXCL2-realtime-F	5'- TGTGACGGCAGGGAAATGTA -3'
CXCL2-realtime-R	5'- TCTGCTCTAACACAGAGGGAAAC -3'

Supplementary Figures

Supplementary Figure S1



Supplementary Figure S1.

Detecting the expression of renal TAZ in the mouse model of unilateral ureteral obstruction (UUO). Co-staining of UUO or control kidneys sections for TAZ and the vascular endothelial marker CD31.