

SUPPLEMENTARY MATERIALS

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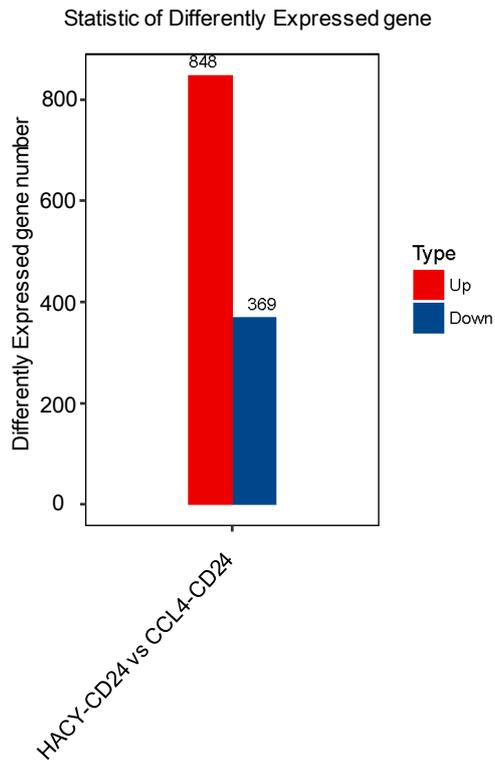
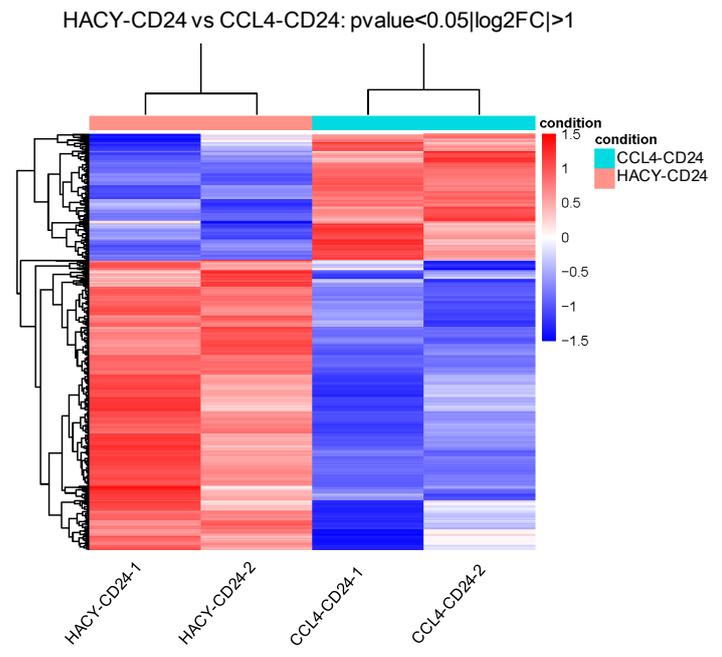
Supplementary methods

Western blot

The protein extracts were submitted to western blotting standard protocol. Briefly, livers tissues were dissected with 300 μ l of ice cold lysis buffer rapidly in tube and homogenized with an electric homogenizer, then centrifuged for 20 min at 12,000 rpm at 4°C in a micro centrifuge. Protein concentrations were measured using a Pierce™ BCA Protein Assay Kit (Both from Thermo scientific). Then proteins were subjected to electrophoresis on 8–10% Bis-Tris protein gels and transferred to nitrocellulose membranes (GE healthcare), which were incubated with the primary antibodies, followed by a fluorescently conjugated secondary antibody. The fluorescence density on nitrocellulose membranes was measured on Odyssey CLx Western Blot Detection System (LI-COR Biosciences).

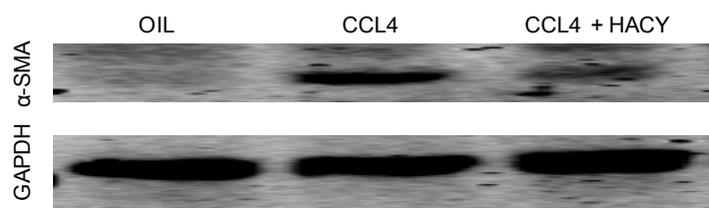
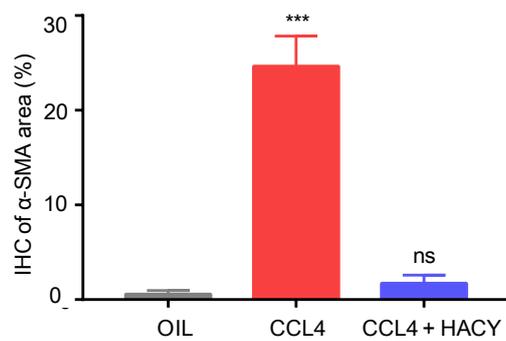
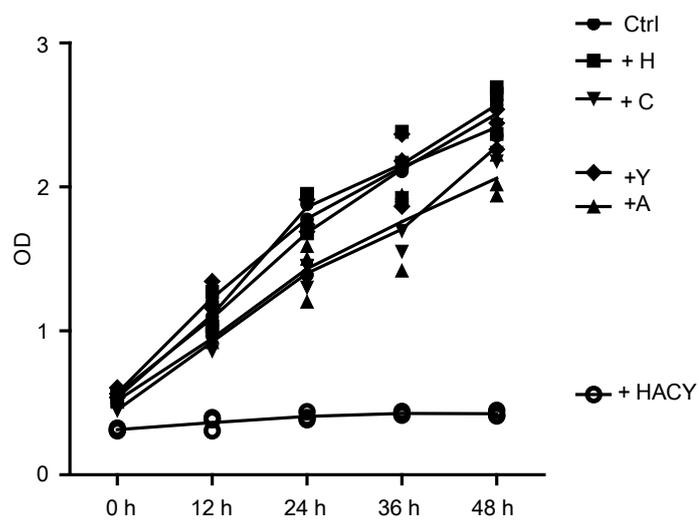
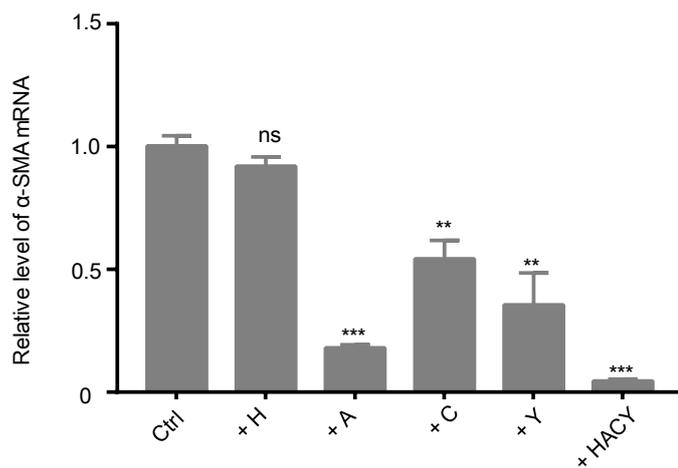
Quantitative real-time PCR (qPCR)

Total RNA of cells was extracted using TRIZOL reagent (Invitrogen) according to the manufacturer's protocols. Real-time PCR analyses were performed using a LightCycler® 96 Real-Time PCR System (Roche) and SYBR Green PCR kit (Roche). Gene transcription was evaluated using the $\Delta\Delta$ Ct method normalized to the housekeeping gene actin beta (ACTB). Primer sequences are respectively listed in Table S3.

A**B**

Supplementary Figure 1

(A, B) mRNA sequencing was performed to compare global gene expression profiles. Figure depicts a comparison of up- and down-regulated genes ($p < 0.05$ and $|\log_2FC| > 1$) between HACY-CD24 cells and CCL4-CD24 cells. Heat maps for the expression of genes related to hepatic function in CD24⁺ progenitor cells.

A**B****C****D**

Supplementary Figure 2

(A) The protein levels of α -SMA from models of liver fibrosis with or without HACY treatment at 40 days were detected by western blotting. GAPDH was used as an internal control.

(B) Quantification of the α -SMA levels shown in A, as determined by Image J software

(C) Growth curves of mHSCs with HGF, Y-27632, A-83-01, CHIR99021, or HACY, at different time points, as determined by CCK-8 assays. Ctrl represents a blank control.

(D) α -SMA expression in primary mHSCs with HGF, Y-27632, A-83-01, CHIR99021, or HACY on day 3 was determined by qRT-PCR analysis.

For B, D, the results are shown as mean \pm s.d. of three independent experiments, **p < 0.01, ***p < 0.001, ns represents no significance

Supplementary table1: Age and gender of the 7 patients with clinically diagnosed liver fibrosis.

| Patients Number | Age | Gender |
|------------------------|------------|---------------|
| 1 | 78 | male |
| 2 | 51 | male |
| 3 | 75 | male |
| 4 | 56 | male |
| 5 | 50 | male |
| 6 | 57 | male |
| 7 | 67 | male |

Supplementary table 2: Age and gender of the 5 patients with clinically diagnosed hepatic hemangioma.

| Patients Number | Age | Gender |
|------------------------|------------|---------------|
| 1 | 49 | male |
| 2 | 50 | male |
| 3 | 63 | male |
| 4 | 61 | male |
| 5 | 32 | female |

Supplementary table 3: Primer list.

| Gene | Forward sequence 5' ->3' | Reverse sequence 5' ->3' |
|---------|--------------------------|---------------------------|
| CD24 | GTTCACCCGTTCCCGGTAA | CCCCCTCTGGTGGTAGCGTAA |
| CK19 | GTTTCAGTACGCATITGGGTCAG | GAGGACGAGGTCACGAAGC |
| Hnf4a | ATGCGACTCTCTAAAACCCTTG | ACCTTCAGATGGGGACGIGT |
| G6pc | CGACTCGCTATCTCCAAGTGA | GGGCGTIGTCCAAACAGAAT |
| Tat | TGCTGGATGTTCGCGTCAATA | CGGCTTCACCTTCATGTIGTC |
| Ttr | CTGCTGTAGACGTGGCTGTAA | CTTCCAGTACGATTIGGTGTCC |
| Cps1 | TACCCGGAAGCACTIACGTAT | GCCAGCCAGTGGTATAGTCATT |
| Cyp1a2 | AGTACATCTCCTIAGCCCCAG | GGGTCCGGGTGGATTCTTC |
| Cyp3a11 | CCTGGGTGCTCCTIAGCAATC | CAAGGAGAGGCCGTTIGACCA |
| CD133 | ACTGGGGCTGTGTGGAAAG | GCATTGAAGGTATCTTGGGTCTC |
| Epcam | CTGGCGTCTAAATGCTTGGC | CCTTGTCCGGTCTTCGGACTC |
| Sox9 | AGTACCCGCATCTGCACAAC | ACGAAGGGTCTTCTCGCT |
| Alb | CAAGAGTGAGATCGCCCATCG | TIACCTCTGCAC'AAATTGGCA |
| Actin | ATGCCACAGGATTCATACCCAAG | CTCTAGACTTTCGAGCAGGAGATGG |
| GAPDH | GGCATGGACTGTGGTCATGAG | TGCACCACCAACTGCTIAGC |

| | | |
|----------------------------------|---------------------------------|---------------------------------|
| α-SMA | CCCAGACATCAGGGAGTAATGG | TCATCGGATACTTCAGCGTCA |
| COLLα1 | ATCGGTCATGCTCTCTCCAAACCA | ACTGCAACATGGAGACAGGTCAGA |
| Desmin | GTTCAGACTTGACTCAGGCAG | TCTCGCAGGTGTAGGACTGG |
| Nestin | CCCTGAAGTCGAGGAGCTG | CTGCTGCACCTTAAGCGA |
| GFAP | TCTCGAATGACTCTCCACTC | AAGCTCCGCCTGGTAGACAT |
| ACTB | GCCTCGCTGTCCACCTTCC | TGCTGTACCTTACCGTTC |
| H-CD24 | CTCTTACCCACCGCAGATTIATTC | AGAGTGAGACCACGAAGAGAC |
| H-Alb | GAGACCAGAGGTIGATGTGATG | AGTTCGGGGCATAAAAAGTAAG |
| H-α-SMA | AAAAGACAGCTACGTGGGTGA | GCCATGTTCATCGGGTACTTC |