

Supplementary material

Fig. S1

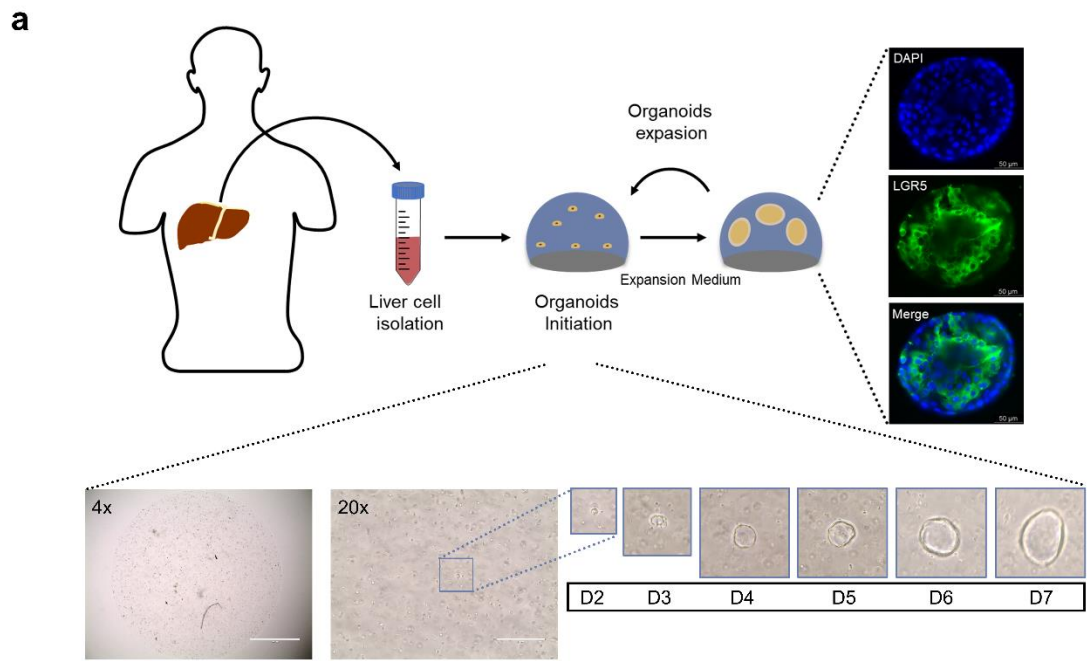
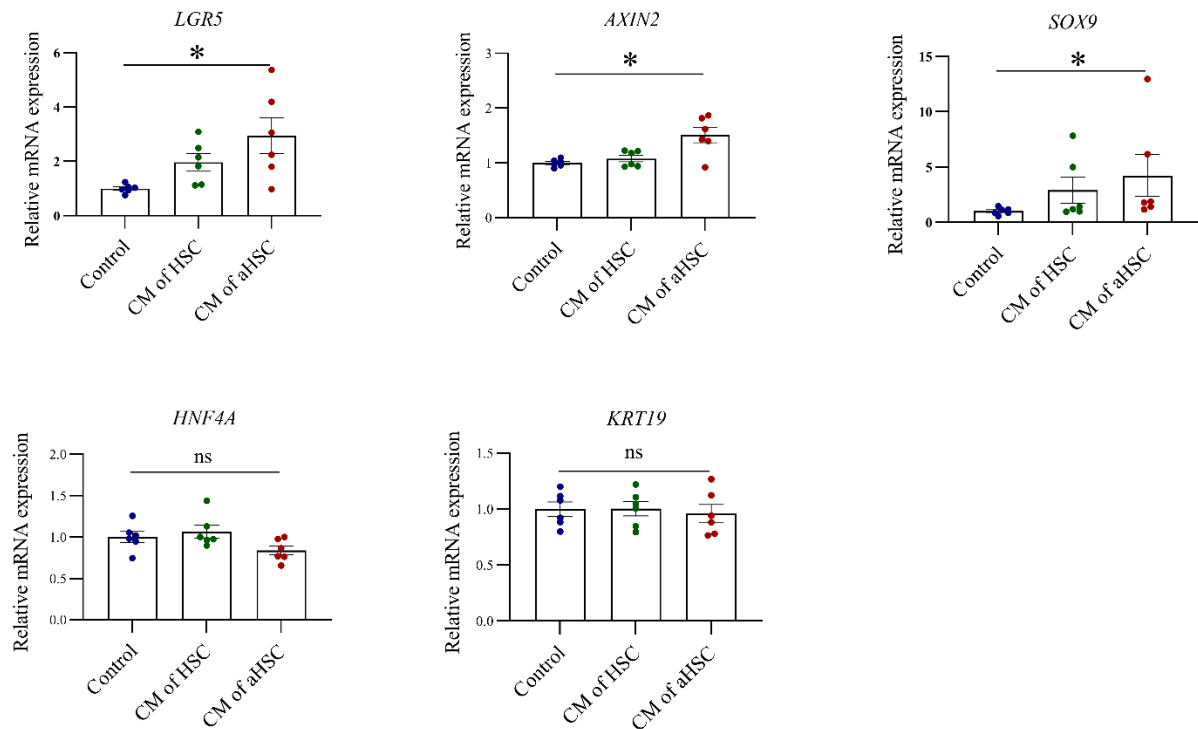


Fig. S1: The process of establishment of human liver organoids from healthy and cirrhotic liver tissues, and liver organoids were identified as LGR5 positive.

Fig S2

a



b

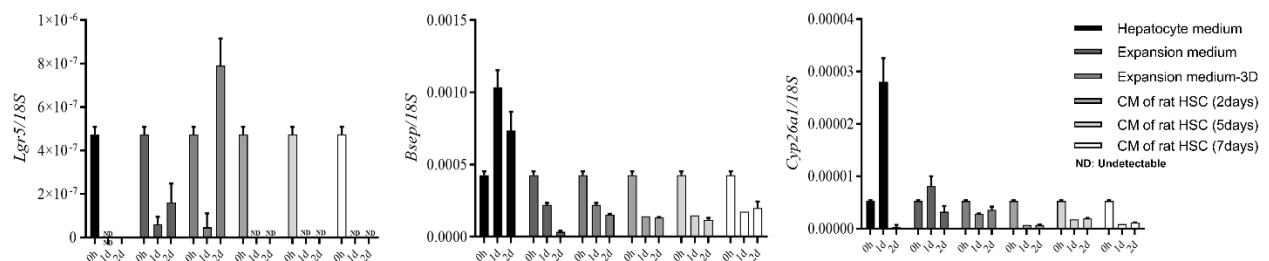


Fig. S2: Activated HSC promote growth of *ex vivo* cultured human tissue-derived liver organoids. A. qPCR-based analysis of human *LGR5*, *AXIN2*, *SOX9*, *HNF4A*, *KRT19* expression in organoids cultured with CM of HSC or activated HSC. Experiments were repeated at least 3 times. Data represent mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$ as compared to control, 1-way ANOVA. **B.** Activated HSC failed to initiate *LGR5* expression in rat primary hepatocytes. A, Rat primary hepatocytes and HSC were isolated from rat liver. HSC were cultured for 7 days for self-activation, and medium of HSC were collected on day 2, 5, 7 as condition medium. After 4 hours seeding, hepatocytes were treated with different medium: hepatocyte medium (control), organoids expansion medium, and condition medium from rat HSC. In Expansion medium-3D, hepatocytes were seeded in 3D-Matrigel and cultured with organoids expansion medium. The mRNA levels of *Lgr5* and mature hepatocyte markers such as *Cyp26a1* and *Bsep* were checked by qPCR.

Fig. S3

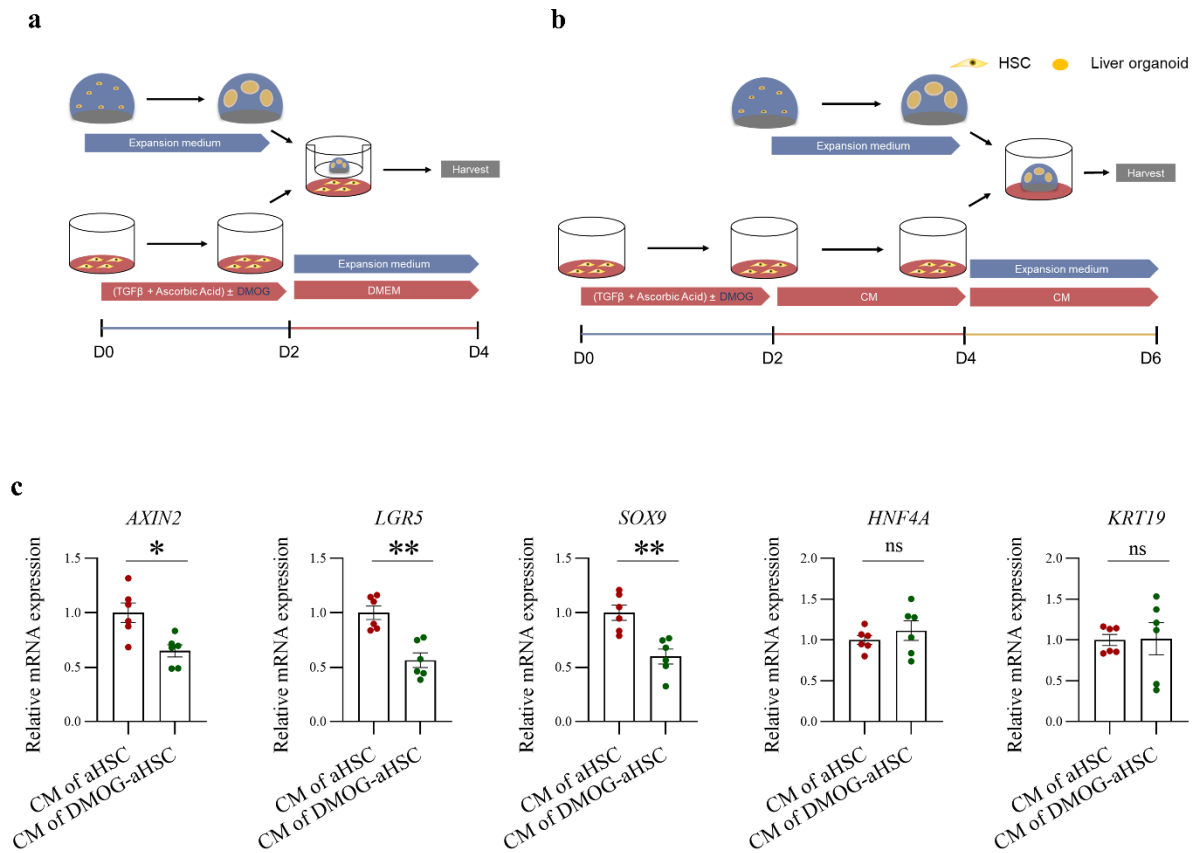


Fig. S3: *In vitro* reversal of HSC activation suppresses proliferation and LGR5 expression in co-cultured liver

organoids. **A**, the schematic experiments overview of human HSC pretreated with TGFβ1, ascorbic acid and DMOG and subsequently co-cultured with human liver organoids using transwell. **B**, the schematic overview of experiments of human liver organoids cultured with CM from human HSC activated by TGFβ1, ascorbic acid and DMOG. **C**, qPCR-based analysis of human *AXIN2*, *LGR5*, *SOX9*, *KRT19* and *HNF4A* expression in organoids cultured with CM of activated HSC treated with or without DMOG at day 2. Experiments were repeated at least 3 times. Data represent mean ± SEM. *p<0.05;

**p<0.01 as compared to control, unpaired Student's t-test.

Tab. S1

sample no	Ishak fibrosis score (0-5)				Metavir fibrosis score (0-4)				Knodell score (0, 1, 3, 4)				Percentage of LGR5+ cells	
	average				average				average					
67	3	3	4	3	2	2	3	2	3	3	3	3	3	4,59
70	4	4	4	4	3	3	3	3	3	3	3	3	3	4,39
80	6	5	5	5	4	4	4	4	4	4	4	4	4	3,75
82	6	6	6	6	4	4	4	4	4	4	4	4	4	3,18
86	6	6	6	6	4	4	4	4	4	4	4	4	4	5,50
99	2	2	3	2	1	1	2	1	1	1	3	2	1	2,12
109	1	0	0	0	1	0	0	0	1	0	0	0	0	1,89
119	2	2	1	2	1	1	1	1	1	1	1	1	1	1,67
122	0	2	1	1	0	1	1	1	0	1	1	1	1	2,27
132	0	0	0	0	0	0	0	0	0	0	0	0	0	1,07
133	3	1	3	2	2	1	1	1	3	1	1	2	1	2,50
151	0	0	0	0	0	0	0	0	0	0	0	0	0	2,97
154	1	0	1	1	1	0	1	1	1	0	1	1	1	1,62
158	6	6	6	6	4	4	4	4	4	4	4	4	4	6,56

Tab. S2

	Forward primer	Reverse primer	Probe Sequence	Species
<i>AXIN2</i>	CGGGAGCCACACCTTCT	TGGACACCTGCCAGTTTCTTT	CCAAGCCTGGCTCCAGAAGATCACAAA	Human
<i>KRT19</i>	CTGACCTGGCCTCCCACTT	TCGAAGGCTGAAGGAAGAG	CCCCTCAGCGTACTGATTTCTCCTCA	Human
<i>HNF4A</i>	CGTGCGGAAGAACCACATG	GCACTGGTTCCTCTTGCTTTGT	CGCACTGCCGGCTAAATCTGCA	Human
<i>LGR5</i>	TGTTTCAGGCTCAAGATGAACGT	AGCAGGTGTTACAGGGTTTG	CCCTTCATTCACTGTCAGTGTTCACCTTC	Human
<i>I8S</i>	CGGCTACCACATCCAAGGA	CCAATTACAGGGCCTCGAAA	CGCGCAAATTACCCACTCCCGA	Rat/human
<i>ACTA2</i>	CTGACTGACTACCTCATGAAGATCCT	CTTAATGTCAACGACGATTTC	CAGTTTCACCAACACGGCCGAG	Human
<i>COL1A1</i>	GGCCCAGAAGAACTGGTACATC	CCGCCATACTCGAACTGGAA	CCCCAAGGACAAGAGGCATGTCTG	Human
<i>CTGF</i>	TGTGTGACGAGCCCAAGGA	TCTGGGCCAAACGTGTCTTC	CCTGCCCTCGCGGCTTACCG	Human
<i>SOX9</i>	<i>SOX9</i> primers/probe set was purchased TaqMan Gene Expression Assays, ThermoFisher: Hs00165814_m1			Human
<i>Lgr5</i>	<i>Lgr5</i> primers/probe set was purchased TaqMan Gene Expression Assays, ThermoFisher: Rn01509661_m1			Rat
<i>Bsep</i>	CCAAGCTGCCAAGGATGCTA	CCTTCTCCAACAAGGGTGCTA	CATTATGGCCCTGCCGAGCA	Rat
<i>Cyp26a1</i>	GATGCGCGCTGGATAAG	CGCCTTCACGCCCGGTACA	GCAATCTCTTCTCTCTCCCAT	Rat

Tab. S3

Protein	Company	catalog number
KRT19	Abcam	ab52625
HNF4A	R&D	PP-H1415-00
LGR5	ProteinTech	21833-1-AP
SOX9	Sigma-Aldrich	AB5535
α -SMA	Sigma-Aldrich	A5228
Polyclonal Goat anti-rabbit immunoglobulins HRP	Dako	P0048
Goat anti-Rabbit Secondary Antibody, Alexa Fluor™ 488	Invitrogen	A-11008
Goat anti-Rabbit Secondary Antibody, Alexa Fluor™ 555	Invitrogen	A-21428
Goat anti-Mouse Secondary Antibody, Alexa Fluor™ 555	Invitrogen	A-21422
Goat anti-Mouse Secondary Antibody, Alexa Fluor™ 488	Invitrogen	A-32723

Tab. S1: Different fibrosis scores and the percentage of LGR5+ cells in liver tissues.

Tab. S2: The list of qPCR primers.

Tab. S3: The list of antibodies.