

## **SUPPLEMENTARY INFORMATION**

### **INTEGRATIVE miRNA AND GENE EXPRESSION PROFILING ANALYSIS OF HUMAN QUIESCENT HEPATIC STELLATE CELLS**

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**Supplementary Table 1.** Clinical characteristics of cadaveric liver donors

<b>liver ID</b>	<b>isolated cells</b>	<b>age</b>	<b>gender</b>	<b>ischemia time</b>	<b>Cause of brain death</b>
<b>L4</b>	qHSC and LSEC	12 years	female	16h30	Drowned
<b>L8</b>	qHSC and LSEC	1 days	male	4h40	Sudden death of unknown cause
<b>L10</b>	qHSC and LSEC	7 months	female	5h20	Sudden death of unknown cause
<b>L11</b>	qHSC and LSEC	7 days	male	4h25	Sudden death of unknown cause
<b>F3</b>	Hepatocytes	17 years	male	9h	Trauma
<b>F23</b>	Hepatocytes	16 years	female	<10h	Trauma

**Supplementary Table 2.** Characteristics of patients with cirrhosis and alcoholic hepatitis

<b>Validation Cohort (n=15)</b>	
<b>Characteristics</b>	<b>Median (25-75 IQR)</b>
Age (y)	54 (49-60)
Male n (%)	87
Alcohol intake (g/day)	100 (90-200)
<b>Laboratory and hemodynamic parameters</b>	
Hemoglobin (g/dL)	11.8 (10.7-13.5)
Leukocyte count x10 <sup>9</sup> /L	7.64 (4.3-11)
Platelet count x10 <sup>9</sup> /L	124 (84-208)
AST ( U/L)	127 (104-240)
ALT (U/L)	58 (14-67)
Serum albumin (g/dL)	29 (24-32.5)
Serum creatinine (mg/dL)	0.9 (0.6-1.3)
Serum bilirubin (mg/dL)	10 (1.7-19.6)
International normalized ratio	1.4 (1.2-1.8)
HVPG (mmHg)	17 (10-22)
<b>Alcoholic hepatitis severity</b>	
MELD score	21 (11-24)
ABIC score	7.9 (6.8-8.8)
Maddrey score	68.3 (62.8-82.5)

IQR, interquartile range; AST, aspartate aminotransferase level; ALT, alanine aminotransferase level; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; ABIC, age-bilirubin-INR-creatinine score.

**Supplementary Table 3.**List of primers used for gene expression analysis

<b>Gene Name</b>	<b>Forward sequence</b>	<b>Reverse sequence</b>
<b>GAPDH</b>	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
<b>ALBUMIN</b>	TGGCACAATGAAGTGGGTAA	CTGAGCAAAGGCAATCAACA
<b>PDGFR<math>\beta</math></b>	CCCTTATCATCCTCATCATGC	CCTTCCATCGGATCTCGTAA
<b>CD32b</b>	AGTGCCAGCATGGGCAGC	TGTCTCTTTCTGATGGCAAT
<b>F4/80</b>	GCCAGTTTGCACAGTTATAGTGA	GACTTCGGCATTAACTGGA
<b>ACTA2</b>	CTGTTCCAGCCATCCTTCAT	TCATGATGCTGTTGTAGGTGG
<b>COL1A1</b>	GACACAGAGTTTCAGTGG	CACCCTTAGCACCAACAG
<b>LOX</b>	CACAGTTGTCAACATTACC	CTGACCTTTAGGATATAGTTCCAG
<b>SPARCL1</b>	GGATTCAACTTCAATTTTTCTGC	AGAAGGTTGGTTGGATTACTTGA
<b>ATP1B2</b>	GAGGACGCACCAGTTTATGG	AACATGGCGGTGAGGAAC
<b>BTG2</b>	AGCGAGCAGAGGCTTAAGGT	CGGTAGGACACCTCATAGGG
<b>SPRY1</b>	GTGTGTTGGAAATCCACG GT	AAAGAAGGCTGCTGGATCAC
<b>PLAU</b>	TCACCACAACGACATTGCCTT	TGATCTCACAGCTTGTGCCAAA
<b>COL5A1</b>	CACAACTTGCCTGATGGAATAACA	GCAGGGTACAGCTGCTTGGT
<b>miRNA</b>	<b>primer sequence</b>	
<b>hsa-miR-192</b>	CTGACCTATGAATTGACAGCC	
<b>mmu-miR-192</b>	CTGACCTATGAATTGACAGCC	
<b>hsa-miR-139-5p</b>	TCTACAGTGACAGTGTCTCCAGT	
<b>hsa-miR-139-3p</b>	TGGAGACGCGGCCCTGTTGGAGT	
<b>hsa-miR-485-5p</b>	AAGACGGGAGGAAAGAAGGGAG	
<b>hsa-miR-142-3p</b>	TGTAGTGTTCCTACTTTATGGA	
<b>hsa-miR-375</b>	TTTGTTTCGTTCCGGCTCGCGTGA	
<b>hsa-miR-100</b>	AACCCGTAGATCCGAACCTGTG	
<b>hsa-miR-125b-1*</b>	ACGGGTTAGGCTCTTGGGAGCT	
<b>hsa-miR-132</b>	TAACAGTCTACAGCCATGGTCG	
<b>hsa-miR-21</b>	UAGCUUAUCAGACUGAUGUUGA	

**Supplementary Table 4.** Up-regulated miRNAs in human culture-activated compared to quiescent HSCs.

miRNA	FC (Q vs. A)	P value	Target genes
miR-100	112,49	$6 \cdot 10^{-7}$	CPNE5, BTG2, SPARCL1, PPAP2A, ABCG2
miR-99a	74,36	$1 \cdot 10^{-6}$	CPNE5, BTG2
miR-130b	11,38	$6,3 \cdot 10^{-5}$	ITGB4, ENTPD1
miR-625	7,93	0,0003	C7
miR-29a	68,21	0,0003	none
miR-222	37,30	0,0003	none
miR-28	6,39	0,0003	none
miR-365	8,56	0,0005	ST8SIA6, NR4A2, RASD1
miR-576-3p	15,96	0,0006	none
miR-106b	6,52	0,0006	ST8SIA6
miR-143	46,64	0,0006	TESC, ENTPD1
miR-22	30,56	0,0006	C7
miR-19b	4,58	0,0006	ITM2A
miR-224	16,59	0,0007	none
miR-125b	22,62	0,0007	none
miR-24	9,89	0,0007	none
miR-222*	134,47	0,0008	COL9A3
miR-362	14,83	0,0009	none
miR-374	7,52	0,0009	none
miR-517b	47,23	0,0010	LTBP4
miR-10b	10,13	0,0010	TESC, ITM2A
miR-34a*	42,93	0,0010	CCL19, SPP1
miR-27b	26,92	0,0011	SPARCL1
miR-130a	10,49	0,0011	none
miR-376a	28,77	0,0011	PPAP2A
miR-27a	34,84	0,0012	CCL19, SPARCL1
miR-589	9,28	0,0012	none
miR-193a-3p	237,84	0,0013	none
miR-210	99,97	0,0014	COL9A3
miR-34c	15,94	0,0014	none

**Supplementary Table 5.** Differentially expressed genes found targeted by a high number of deregulated miRNAs

mRNAs	FC.mRNA (Q vs. A)	miRNAs
ENTPD1	5,62	miR-130b, miR-143, miR-23a, miR-27b*, miR-335*, miR-337-5p, miR-539, miR-654-3p, miR-708, miR-758
SPARCL1	55,47	miR-100, miR-140-3p, miR-152, miR-154*, miR-199a-3p, miR-19b-1*, miR-27a, miR-27b, miR-301b, miR-655
VCAM1	13,98	miR-132, miR-181a, miR-22*, miR-340, miR-376c, miR-424, miR-487a, miR-671-3p, miR-935
ST8SIA6	14,01	let-7i*, miR-106b, miR-184, miR-31, miR-31*, miR-365, miR-616, miR-98
MTHFD1L	-9,43	miR-126*, miR-142-5p, miR-200a, miR-429, miR-522, miR-605, miR-638
PFKP	-16,73	miR-139-3p, miR-139-5p, miR-142-5p, miR-372, miR-523, miR-548b-5p, miR-628-5p
ACOT7	-4,97	miR-139-3p, miR-200a, miR-200b, miR-302c*, miR-375, miR-429
COL9A3	4,08	let-7b*, miR-210, miR-222*, miR-31*, miR-597, miR-598
DCBLD1	-4,11	miR-126, miR-139-3p, miR-302c*, miR-342-5p, miR-363, miR-885-5p
ITM2A	10,63	let-7a*, let-7b*, let-7i*, miR-10b, miR-19a, miR-19b
LOX	-67,33	miR-126*, miR-200a, miR-200b, miR-429, miR-548b-5p, miR-628-5p
PPAP2A	2,86	let-7g, miR-100, miR-181a-2*, miR-18b, miR-376a, miR-503
BTG2	23,21	miR-100, miR-18b, miR-21, miR-31, miR-99a
CCL19	5,52	miR-27a, miR-339-5p, miR-345, miR-34a*, miR-671-3p
DOCK7	-8,94	miR-126*, miR-135a, miR-142-5p, miR-223, miR-522
FAP	-26,34	miR-126, miR-135a, miR-200a, miR-375, miR-429
SPP1	21,36	miR-130b*, miR-181a, miR-34a*, miR-376c, miR-424
TESC	4,79	miR-10a, miR-10b, miR-143, miR-339-5p, miR-532-3p
WNK4	-7,06	miR-135a, miR-142-3p, miR-202, miR-342-5p, miR-639

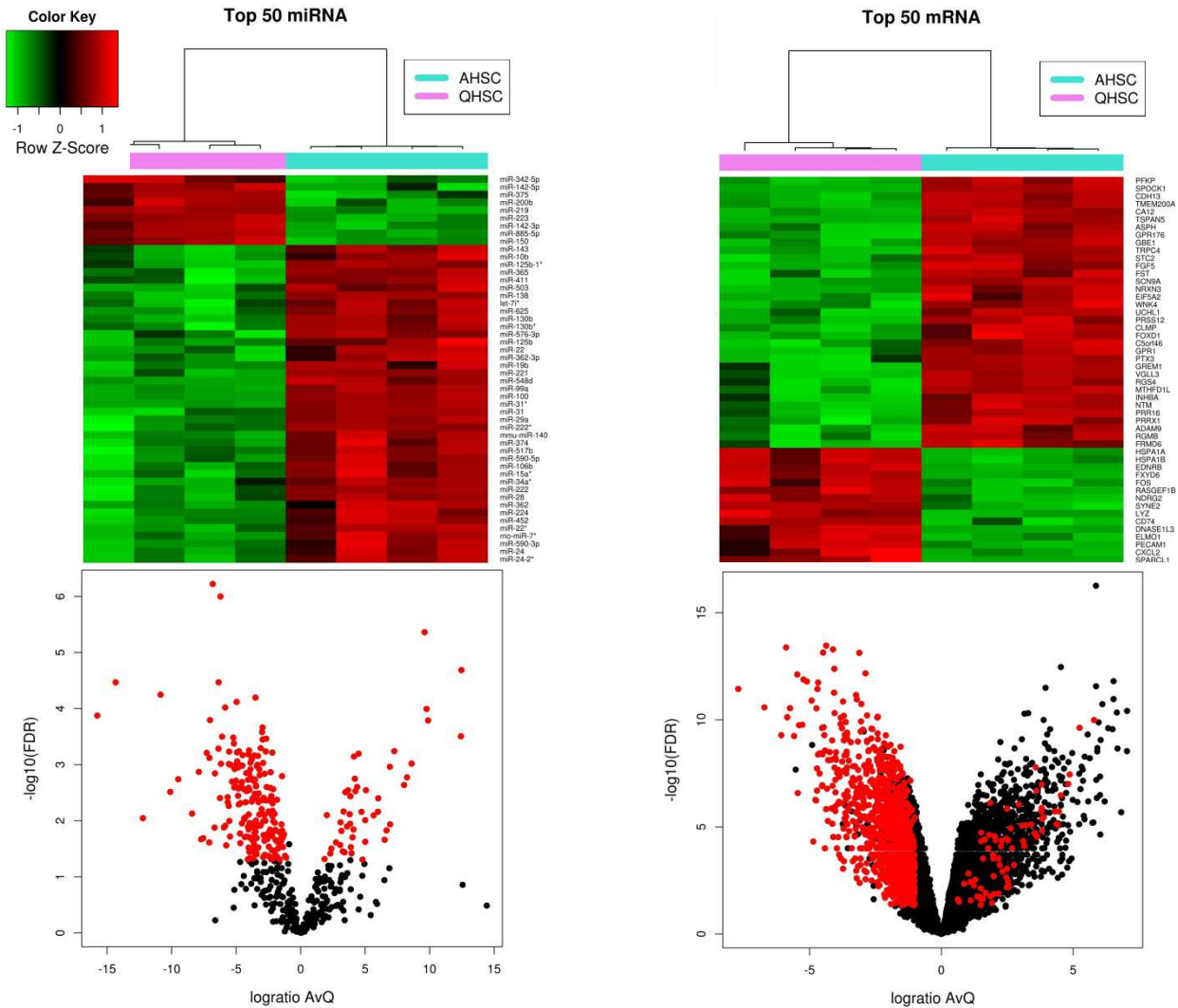
## FIGURE LEGENDS

**Supplementary Figure 1: Integrative analysis pipeline:** **(A)** Differential microRNA and mRNA expression analysis. Heatmaps represent the 50 most significant miRNAs and mRNAs together with the volcano plots of the differential expressed miRNAs and mRNAs. Red and black dots represent significant ( $FDR < 0.05$ ) and non-significant differential expressed genes/miRNAs, respectively **(B)** Correlation and intersection with Microcosm Database. The table shows the basic statistics for correlations between miRNA and target genes. Only those miRNA-mRNA pairs with Pearson Correlation Coefficient  $< 0$  and  $FDR < 0.05$  were considered and used for the intersection with Microcosm Database (8.7% of all potential correlations). Among all miRNA-target pairs predicted by MicroCosm (8463) only 9.16% present a negative and significant correlation and were considered for further analysis.

**Supplementary Figure 2: *In vitro* modulation of miRNA expression in human HSCs (LX2).** Reduction of miR-21 and miR-100 expression and up-regulation of miR-192 in LX2 cells was achieved by transfecting miR-21 antagomir (50nM), miR-100 antagomir (50nM) or miR-192 mimic (50nM), respectively (n=2) or control mimic/inhibitor. All expressions are relative to the solvent (blank).

# Supplementary figure 1

## A. Differential expression analysis



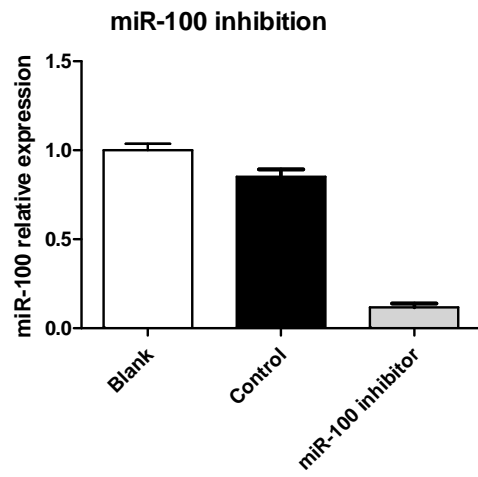
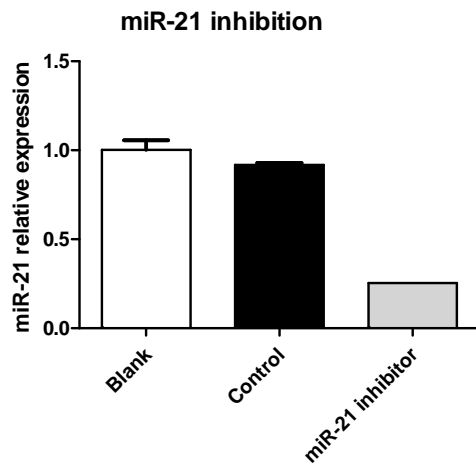
## B. Correlation and intersection with Microcosm Database

	Number	%
Differentially expressed miRNAs	259	
Differentially expressed mRNAs	1071	
Number of total correlations	276318	100
Total negative correlations	63032	22,81
Total negative correlations, $p < 0,05$	37130	13,43
Total negative correlations, $\text{FDR} < 0,05$	24031	8,70
Total targets predicted in MicroCosm	8463	100
Total negative correlated targets	1899	22,44
Total negative correlated targets, $p < 0,05$	1137	13,43
Total negative correlated targets, $\text{FDR} < 0,05$	775	9,16



## Supplementary Figure 2

A.



B.

