Supplementary Information

Circulating let-7 levels in plasma and extracellular vesicles correlate with hepatic fibrosis progression in chronic hepatitis C

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Supporting Material and Methods

Study Design for the Anti-Hepatitis C Virus Antibody Positive Blood Donor Cohort .
Volunteer blood donors from the Greater Chesapeake and Potomac Region of the
American Red Cross and from Department of Transfusion Medicine of the National
Institutes of Health, who tested anti-hepatitis C virus (HCV) positive on replicate testing
were enrolled beginning in August 1990. The aim of this cohort is to follow-up these
unselected subjects and assess 1) the proportion of HCV-infected individuals who
become chronic carriers, 2) the risk factors for HCV acquisition, 3) the long-term
outcomes based on findings of hepatic histological evaluations and 4) the relationship of
hepatic fibrosis with a variety of demographic, virologic, serologic, biochemical and
host genetic factors. Both age at infection and duration of HCV infection were
extrapolated from the reported date of probable exposure, specifically, blood transfusion
prior to 1991, the first year of injection drug use, or the date of a well-defined
needlestick exposure. For these objectives, chronic hepatitis C (CHC) patients were
recommended to receive liver biopsies approximately every five years until they receive
antiviral therapies. Further information of this cohort was described previously.(1, 2)

Collection of Samples and Clinical Data. Plasma and liver tissue samples and clinical
data were obtained at the initial liver biopsy from 1992 to 2003, and at the repeat biopsy
from 1998 to 2010. Of 190 plasma samples studied, 63 were available on the same date
as the liver biopsy, 66 within 3 months of the biopsy date and 61 were obtained between
3 and 12 months of the biopsy. Formalin-fixed paraffin-embedded (FFPE) specimens of
liver tissues were available for only a subset of patients: only 6 of 43 cases where a
specimen was available at the initial biopsy had a paired specimen at the repeat biopsy,
and additional 37 specimens were available only at the repeat biopsy.

Preparation for RNA. For microarray analysis, we extracted total RNA from 400 μL of plasma in each subject using two RNeasy MinElute spin columns (Qiagen, Hilden, Germany), without adding synthetic *Caenorhabditis elegans* (cel)-miR-39 miRNA mimic: miReasy Serum/Plasma Spike-In Control (Qiagen). Total RNA was eluted from each column with 30 μL of nuclease-free water, and then the solutions of the same subject were put together. We used 200 ng of the total RNA derived from plasma, which were quantified by spectrophotometer as described below, for the labeling of microarray analysis. However, we should note that it is generally hard to quantify accurate concentrations of total RNA including miRNA extracted from serum or plasma samples by spectrophotometer because the levels are low. For quantitative real-time polymerase chain reaction (qRT-PCR) assay, we extracted total RNA from 200 μL of plasma for each subject, in which 5.6×10^8 copies of cel-miR-39 was added as spike-in RNA for later normalization; then total RNA was eluted from each column with 60 μL of nuclease-free water. Extracellular vesicles (EVs) including exosomes were purified from 200 μL of plasma, and 5.6×10^8 copies of synthetic cel-miR-39 miRNA mimic as spike-in control were added, followed by total RNA extraction using exoRNeasy Serum/Plasma Midi Kit (Qiagen) according to the manufacturer's instructions. Total RNA was eluted from each column with 60 μL of nuclease-free water. For RNA extraction from liver tissues, we recut FFPE specimens, each section 10 μm thick, and obtained 5-10 slides (total 50-100 μm), followed by total RNA extraction using miRNeasy FFPE Kit (Qiagen) according to the manufacturer's instructions. Total RNA was eluted from each column with 60 μL of nuclease-free water. The quality and integrity of RNA were assessed with the RNA 6000 Nano Assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The concentration of total RNA was quantified by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

qRT-PCR for miRNA Analysis. The assay IDs of TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA) are as follows: hsa-miR-122-5p (assay ID 002245); hsa-let-7a-5p (assay ID 000377); hsa-let-7c-5p (assay ID 000379); hsa-let-7d-5p (assay ID 002283); has-miR-16-5p (assay ID 000391); has-miR-92a-3p (assay ID 000431); has-miR-486-5p (assay ID 001278); has-miR-20a-5p (assay ID 000580); has-miR-155-5p (assay ID 002623); RNU6B (assay ID 001093); and cel-miR-39-3p (assay ID 000200). One micro-liter of total RNA extracted from plasma, 3 µL from EVs and outside EVs fraction (non-EVs), and 10 ng from liver tissues were respectively subjected to reverse transcription with TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) and the respective TaqMan MicroRNA Assay reagents for target genes in a total volume of 15 µL reaction mixture, followed by qRT-PCR in a total volume of 20 µL reaction mixture according to the manufacture's protocol. Amplification was carried out as follows: 95°C for 10 min, 45 cycles of 95°C for15 s and 60°C for 60 s. All reactions were carried out in triplicate.

Laboratory and Histological Tests. Hematologic and blood chemistry tests were carried out using standard assays. Quantitative HCV RNA levels were measured by the COBAS Amplicor HCV Monitor Test, version 2.0 (Roche, Branchburg, NJ), and HCV genotyping was performed by the INNO-LiPA 2.0 assay (Innogenetics, Ghent, Belgium). All liver biopsy specimens were read by the same expert hepatopathologist without knowledge of the patients' clinical history. A biopsy specimen was considered adequate if it contained >10 portal tracts. The extent of liver fibrosis was scored from 0 to 6, using the Ishak fibrosis scale (0: no fibrosis; 1–2: portal fibrotic expansion; 3–4: bridging fibrosis; and 5–6: cirrhosis).(3) Necrosis and inflammation were graded using a modification of the histologic activity index (HAI) on a scale of 0–18.(4, 5) Histological fibrosis indexes: Fibrosis-4 (FIB-4) index and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) score, were calculated as previously described:

FIB-4 = (age [year] $\times$ AST [IU/L]) / (platelet count [10^9/L] $\times\sqrt{}$ALT [IU/L]), APRI = ((AST / ULN) / platelet count [10^9/L]) $\times$ 100.(6, 7) Fibrosis progression rate (FPR) between paired liver biopsies was calculated as follow: (Ishak score at the repeat biopsy - Ishak score at the initial biopsy + 1) / duration (year).

SNP Genotyping. Genotypes of single nucleotide polymorphisms (SNPs) within or near *IFNL4*: rs12979860, *PNPLA3*: rs738409, *MERTK*: rs4374383, *TULP1*: rs9380516, *RNF7*: rs16851720 and *HLA* class II: rs910049 and rs3135363, which were previously reported to be associated with hepatic fibrosis progression or inflammatory activities in CHC,(8-11) were determined according to the manufacturer's instructions using TaqMan SNP Genotyping Assay (Applied Biosystems, Carlsbad, CA).

Bioinformatic Analysis of miRNAs: Target Prediction. TargetScan was used to predict target genes of let-7 (http://www.targetscan.org/). Pathway analysis of the target genes of let-7 was carried out using Ingenuity Pathway Analysis (Qiagen).

Statistical Analysis. The one-way analysis of variance (ANOVA) was used to determine the statistical significances in microarray signal intensities among the different groups. Categorical variables were compared between groups by the chi-square test, and non-categorical variables by Mann–Whitney *U*-test. Pearson's correlation coefficient (r) was used for the assessment of correlation between continuous variables, while Spearman's rank correlation coefficient (rho) was used for that between continuous and categorical variables. The receiver operating characteristic (ROC) curve analyses were carried out and the area under the curve (AUC) was calculated to evaluate feasibility of using the selected miRNA levels as markers for discriminating significant hepatic fibrosis. Multivariate logistic regression analyses were performed to evaluate whether the selected miRNA levels were independently

associated with significant hepatic fibrosis. Changes in miRNA expression levels between paired liver biopsies were compared by the paired *t*-test. $P < 0.05$ was considered significant in all tests. Statistical analyses were performed using R (version 3.1.1) and EZR (version 1.27) that is based on R and R commander.(12)

Supporting Table 1. Clinical characteristics of subjects in the discovery set

	HC (n=16)	mild CH (n=16)	severe CH (n=16)	*P*-value
Gender, male / female	8 / 8	8 / 8	8 / 8	N.S.
Age, years	39 (35 - 48)	40 (37 - 45)	43 (40 - 50)	N.S.
Age at infection, years[*]	N.A.	19 (15 - 21)	23 (20 - 24)	N.S.
Duration of infection, year[*]	N.A.	22 (21 - 23)	20 (15 - 24)	N.S.
Race, Caucasian / African American/ Others	N.A.	13 / 2 / 1	12 / 4 / 0	N.S.
Platelet count, $\times 10^9$/L	N.A.	241 (210 - 273)	181 (159 - 216)	0.006
AST, IU/L	N.A.	27 (22 - 29)	57 (50 - 70)	< 0.001
ALT, IU/L	N.A.	34 (27 - 36)	81 (56 - 117)	< 0.001
ALP, IU/L	N.A.	92 (59 - 104)	79 (71 - 112)	N.S.
γ-GTP, IU/mL	N.A.	32 (24 - 73)	57 (35 - 123)	N.S.
Total bilirubin, mg/dL	N.A.	0.5 (0.5 - 0.6)	0.7 (0.4 - 0.8)	N.S.
Albumin, g/dL	N.A.	4.2 (3.9 - 4.4)	4.1 (3.9 - 4.4)	N.S.
AFP, ng/mL	N.A.	2.5 (1.8 - 3.7)	7.2 (3.9 - 11.0)	< 0.001
APRI	N.A.	0.29 (0.24 - 0.43)	0.96 (0.64 - 1.36)	< 0.001
FIB-4	N.A.	0.78 (0.71 - 1.04)	1.70 (1.21 - 2.00)	< 0.001
HCV RNA	N.A.	6.88 (6.40 - 7.38)	6.27 (5.98 - 6.65)	N.S.
Ishak score, 0 / 1 / 2 / 3 / 4 / 5 / 6	N.A.	16 / 0 / 0 / 0 / 0 / 0 / 0	0 / 0 / 0 / 7 / 5 / 2 / 2	< 0.001
HAI score , ≤7 / ≥8	N.A.	12 / 4	4 / 12	0.005
HCV genotype, 1 / 2 / 3 / 1+2 / N.D.	N.A.	13 / 1 / 0 / 1 / 1	13 / 2 / 1 / 0 / 0	N.S.

Data are expressed as number for categorical data or the median (first-third quantiles) for non-categorical data. Data of HC were obtained at drawing plasma sample, and those of mild CH and severe CH were obtained at initial liver biopsy. [*]Data were available in 18 patients: mild CH (n=7) and severe CH (n=11), respectively. *P*-values were calculated by the chi-square test for categorical variables and by Mann-Whitney *U*-test for non-categorical variables. HC, healthy control; CH, chronic hepatitis; N.S., not significant; N.A., not applicable; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; AFP,

alpha-fetoprotein; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; HAI, histologic activity index; N.D., not determined.

Supporting Table 2. Differentially expressed microRNAs in plasma between chronic hepatitis C patients and healthy individuals

| Transcript ID | log$_2$ (average normalized signal intensity) | | Fold change | P-value[*] |
	HC (n=16)	CHC (n=32)		
hsa-miR-574-5p	1.53811	7.83847	78.813	8.59E-16
hsa-miR-4793-3p	3.81314	9.0345	37.3068	7.83E-14
hsa-miR-4440	3.98656	8.73963	26.9659	3.98E-11
hsa-miR-619-5p	3.44194	8.18001	26.6869	1.22E-12
hsa-miR-1825	2.12504	6.8081	25.6886	4.64E-12
hsa-miR-3613-3p	4.64235	9.18903	23.3715	1.71E-07
hsa-miR-6877-3p	1.45336	5.98305	23.0979	3.75E-18
hsa-miR-4668-5p	5.02968	9.45634	21.5058	1.01E-07
hsa-miR-3148	1.47105	5.89046	21.398	8.07E-10
hsa-miR-1281	3.95584	8.20281	18.9874	3.91E-13
hsa-miR-4507	3.8725	8.03343	17.8881	4.65E-19
hsa-miR-4484	6.60386	10.6941	17.0328	1.27E-15
hsa-miR-3178	3.76518	7.67276	15.0071	1.47E-06
hsa-miR-3620-5p	4.59343	8.44242	14.4099	2.06E-21
hsa-miR-4763-3p	4.71118	8.51722	13.9872	8.11E-18
hsa-miR-3201	1.25956	5.02397	13.5894	7.17E-07
hsa-miR-6850-5p	4.57673	8.1201	11.659	1.52E-11
hsa-miR-6803-5p	6.03979	9.55955	11.4697	6.21E-26
hsa-miR-4701-3p	2.35563	5.86095	11.3555	5.80E-06
hsa-miR-297	3.08178	6.58696	11.3544	1.73E-06
hsa-miR-8084	1.31994	4.80513	11.1982	6.05E-06
hsa-miR-6875-3p	1.09922	4.53302	10.8064	7.96E-09
hsa-miR-1910-5p	1.30578	4.73607	10.78	3.38E-11
hsa-miR-4516	5.79574	9.1217	10.028	1.48E-16
hsa-miR-3197	1.58444	4.89867	9.94677	2.76E-05
hsa-miR-6089	7.96295	11.1631	9.19073	1.14E-20
hsa-miR-6732-5p	6.53585	9.71035	9.02857	1.09E-17
hsa-miR-8069	6.8599	9.96491	8.60401	2.12E-21
hsa-miR-6821-5p	4.28892	7.36171	8.41401	1.14E-10
hsa-mir-6800	5.23788	8.25946	8.12056	1.57E-18
hsa-miR-3656	6.38344	9.36574	7.90247	5.95E-13
hsa-miR-1228-5p	5.23409	8.16575	7.62988	5.46E-08

hsa-miR-2861	6.36421	9.29259	7.61254	3.88E-19
hsa-miR-1268a	3.12626	6.04981	7.58711	4.87E-06
hsa-miR-1469	5.88443	8.79237	7.50543	1.11E-16
hsa-miR-6090	8.10281	10.9608	7.25008	2.61E-17
hsa-miR-1268b	3.28894	6.14321	7.23138	7.23E-06
hsa-miR-638	7.10966	9.93631	7.09424	3.40E-22
hsa-miR-4463	4.1219	6.94776	7.09037	2.94E-06
hsa-miR-6791-5p	5.27929	8.08536	6.99377	8.37E-10
hsa-miR-6869-5p	6.89085	9.64416	6.74261	1.34E-13
hsa-miR-4466	7.21508	9.95096	6.66165	2.97E-16
hsa-miR-3940-5p	5.32997	8.05856	6.62807	3.90E-09
hsa-miR-4632-5p	3.37864	6.08024	6.50524	9.76E-07
hsa-miR-4687-3p	5.55406	8.24981	6.47888	3.05E-09
hsa-miR-6756-5p	3.90806	6.59326	6.43172	7.22E-08
hsa-miR-4651	3.82043	6.48812	6.35411	1.39E-07
hsa-miR-940	2.44446	5.08983	6.25658	1.26E-07
hsa-miR-3665	8.09714	10.7373	6.23395	2.95E-19
hsa-miR-6724-5p	6.09162	8.71905	6.17923	7.14E-12
hsa-miR-4497	6.89303	9.51701	6.16448	1.02E-08
hsa-miR-4436b-5p	1.39316	3.99987	6.09114	4.36E-07
hsa-miR-4707-5p	4.79674	7.39801	6.06821	1.53E-13
hsa-miR-195-3p	1.36041	3.93559	5.95943	4.35E-06
hsa-miR-762	6.48147	8.99613	5.71462	1.03E-09
hsa-miR-7114-5p	1.38485	3.8819	5.64532	1.37E-05
hsa-miR-595	1.66136	4.13556	5.55661	2.70E-04
hsa-miR-4787-5p	7.66834	10.1263	5.4943	4.02E-19
hsa-miR-5787	7.16588	9.62174	5.4864	2.45E-15
hsa-miR-6800-5p	5.15008	7.59943	5.46169	6.60E-08
hsa-miR-4459	3.10007	5.53442	5.4052	2.74E-04
hsa-miR-4767	1.44674	3.87352	5.37695	4.26E-07
hsa-miR-6088	7.22384	9.61854	5.25866	2.31E-08
hsa-miR-6087	7.85178	10.2455	5.25503	2.99E-14
hsa-miR-7108-5p	5.62021	7.99002	5.16874	4.60E-08
hsa-miR-4539	2.00688	4.36958	5.14332	1.28E-05
hsa-miR-4745-5p	5.02439	7.38653	5.14132	7.14E-06
hsa-miR-6794-5p	3.99657	6.35197	5.11734	1.24E-06

hsa-miR-1237-5p	6.33661	8.6543	4.98534	5.09E-06
hsa-miR-6125	7.19293	9.50589	4.96902	9.18E-14
hsa-miR-6729-5p	7.18195	9.49069	4.9545	7.99E-15
hsa-miR-1587	2.46682	4.77164	4.94105	3.55E-06
hsa-miR-4281	5.17721	7.47561	4.91915	7.35E-05
hsa-miR-3128	1.08941	3.36359	4.83723	2.47E-04
hsa-miR-6727-5p	6.70974	8.98288	4.83375	3.03E-14
hsa-miR-3960	8.31368	10.5809	4.81406	9.14E-17
hsa-miR-4532	7.39453	9.63928	4.73955	5.15E-07
hsa-miR-6749-5p	3.18311	5.41862	4.70928	3.93E-05
hsa-miR-6752-5p	5.51601	7.74076	4.67428	2.68E-06
hsa-miR-1915-3p	6.9758	9.18315	4.61826	2.84E-06
hsa-miR-6798-5p	3.52427	5.72801	4.60672	2.78E-04
hsa-miR-191-3p	1.35479	3.5217	4.49059	2.25E-07
hsa-miR-4717-3p	1.98413	4.14923	4.48497	8.50E-05
hsa-miR-4488	6.60979	8.76644	4.45879	2.50E-07
hsa-miR-6775-5p	3.42924	5.58216	4.44725	1.62E-04
hsa-miR-7704	7.86212	10.0114	4.43593	4.96E-16
hsa-miR-933	1.35351	3.50275	4.43593	1.03E-08
hsa-miR-4689	2.43815	4.58681	4.43417	5.74E-04
hsa-miR-6722-3p	3.65283	5.80025	4.43036	5.07E-04
hsa-miR-5571-5p	1.05961	3.15776	4.28159	1.22E-06
hsa-miR-3663-3p	1.50266	3.58523	4.23559	9.72E-05
hsa-miR-1909-5p	1.33082	3.40577	4.21329	1.99E-06
hsa-miR-3185	3.14562	5.21333	4.19221	9.07E-07
hsa-miR-6743-5p	4.57246	6.60601	4.09412	2.88E-06
hsa-miR-663a	4.50521	6.53497	4.08339	2.71E-05
hsa-miR-6824-5p	1.54971	3.51583	3.90715	1.26E-03
hsa-miR-548x-3p	1.46921	3.42855	3.88886	8.13E-06
hsa-miR-4487	3.5324	5.46494	3.81726	1.89E-08
hsa-miR-1263	1.93123	3.85132	3.78447	2.83E-05
hsa-miR-548a-3p	3.08083	4.98867	3.75246	1.60E-04
hsa-miR-4734	4.43798	6.33593	3.72682	1.82E-04
hsa-miR-548ae	1.61041	3.50376	3.71496	5.70E-05
hsa-miR-6753-3p	1.06064	2.94164	3.6833	8.08E-06
hsa-miR-3613-5p	3.67443	5.55393	3.67948	2.88E-04

hsa-miR-4270	3.5981	5.42183	3.53995	2.74E-03
hsa-miR-6786-5p	7.02072	8.80075	3.43434	4.66E-05
hsa-miR-8075	6.59436	8.37357	3.43237	6.12E-07
hsa-miR-548ac	2.45149	4.22952	3.42957	4.00E-04
hsa-miR-4710	1.56425	3.27618	3.27598	3.57E-04
hsa-miR-4508	6.41128	8.11423	3.25565	1.94E-05
hsa-miR-937-5p	3.26773	4.96995	3.254	3.60E-05
hsa-miR-328-5p	4.15566	5.85264	3.24221	4.94E-03
hsa-miR-4758-5p	3.37513	5.05635	3.20699	1.06E-03
hsa-let-7f-1-3p	1.15292	2.81788	3.17106	7.21E-06
hsa-miR-6765-5p	4.85101	6.49457	3.12436	2.89E-03
hsa-miR-6858-5p	4.43079	6.07314	3.12175	6.12E-04
hsa-miR-1207-5p	3.51814	5.14652	3.09164	1.93E-03
hsa-miR-6503-3p	1.05569	2.64917	3.01775	1.20E-06
hsa-miR-1323	1.33747	2.92583	3.00709	3.14E-05
hsa-miR-4797-5p	1.31122	2.88238	2.97144	1.20E-04
hsa-miR-149-3p	5.98903	7.54351	2.93727	1.49E-03
hsa-miR-6799-5p	2.47978	3.99538	2.8592	5.70E-03
hsa-miR-1225-5p	1.91534	3.41547	2.82867	2.65E-03
hsa-miR-548aj-3p	1.61624	3.09144	2.78022	2.32E-04
hsa-miR-4462	1.68297	3.14808	2.76083	6.25E-03
hsa-miR-6741-5p	1.89946	3.3606	2.75324	5.11E-03
hsa-miR-1909-3p	2.48283	3.93122	2.72904	8.25E-03
hsa-miR-548aq-3p	1.20517	2.64915	2.72072	4.33E-05
hsa-miR-6789-5p	4.72161	6.15896	2.70823	2.12E-03
hsa-miR-6865-3p	1.09169	2.52192	2.6949	1.06E-04
hsa-miR-6754-3p	1.02844	2.45123	2.68104	5.85E-06
hsa-miR-4685-3p	1.29282	2.7152	2.68027	1.63E-04
hsa-miR-3196	6.28905	7.70291	2.66449	1.95E-03
hsa-miR-4455	1.09036	2.46657	2.59586	1.62E-03
hsa-miR-4490	1.29535	2.66474	2.58362	1.15E-04
hsa-miR-4423-3p	1.1622	2.52454	2.57103	2.62E-03
hsa-miR-4720-5p	1.88079	3.24134	2.56783	1.17E-04
hsa-miR-1306-3p	1.56918	2.92965	2.56769	6.38E-03
hsa-miR-1972	1.25249	2.60792	2.55875	4.64E-04
hsa-miR-1298-3p	1.79581	3.14664	2.55059	4.87E-04

hsa-miR-1227-5p	4.13276	5.47992	2.5441	6.44E-03
hsa-miR-4695-5p	3.12967	4.44457	2.48785	3.52E-03
hsa-miR-6069	1.36509	2.66153	2.45623	4.22E-06
hsa-miR-4310	1.09397	2.38371	2.44484	5.87E-05
hsa-miR-16-2-3p	1.35726	2.63584	2.42599	4.98E-06
hsa-miR-1272	1.4178	2.69374	2.42157	8.15E-05
hsa-miR-1228-3p	2.22788	3.50343	2.4209	1.29E-03
hsa-miR-6860	1.65036	2.92399	2.41771	3.08E-03
hsa-miR-3944-3p	1.47186	2.74133	2.41073	1.35E-03
hsa-miR-6736-5p	1.84339	3.10944	2.40503	5.45E-04
hsa-miR-8085	1.18969	2.43603	2.37238	1.58E-03
hsa-miR-5001-5p	5.14025	6.37369	2.35126	1.92E-04
hsa-miR-939-5p	2.30536	3.53454	2.34435	8.64E-03
hsa-miR-603	1.31325	2.50663	2.28688	6.12E-04
hsa-miR-3663-5p	1.04835	2.23304	2.27315	2.22E-03
hsa-miR-548u	1.33601	2.50532	2.24905	2.59E-04
hsa-miR-3910	1.16323	2.31802	2.22653	1.32E-04
hsa-miR-6772-3p	1.19227	2.34463	2.22278	7.87E-04
hsa-miR-346	1.01258	2.15622	2.20938	8.94E-06
hsa-miR-5681a	1.03265	2.16032	2.18506	6.37E-06
hsa-miR-4538	1.1106	2.23797	2.1846	2.25E-03
hsa-miR-1538	0.986042	2.11279	2.18366	9.13E-04
hsa-miR-4708-5p	1.04089	2.16336	2.1772	1.43E-06
hsa-miR-4725-5p	1.37746	2.49934	2.17631	1.25E-04
hsa-miR-4690-5p	2.23648	3.35766	2.17526	2.88E-03
hsa-miR-4706	3.41865	4.5174	2.14169	4.79E-03
hsa-miR-7109-3p	1.11609	2.18756	2.10158	1.13E-04
hsa-miR-6886-3p	1.20349	2.27289	2.09856	3.23E-04
hsa-let-7b-3p	1.21415	2.27804	2.09057	7.37E-05
hsa-miR-202-5p	1.34419	2.39767	2.07553	3.47E-04
hsa-miR-548ap-3p	1.407	2.44515	2.05359	1.18E-03
hsa-miR-548z	1.50664	2.52337	2.02333	1.18E-03
hsa-miR-548h-3p	1.50664	2.52337	2.02333	1.18E-03
hsa-miR-7852-3p	1.13283	2.14588	2.01817	3.61E-05
hsa-miR-6800-3p	3.05986	4.07136	2.01601	8.69E-03
hsa-miR-628-5p	1.81117	2.81832	2.00993	2.72E-03

hsa-miR-487a-5p	1.91413	0.911697	-2.00337	4.47E-08
hsa-miR-425-3p	3.74453	2.73085	-2.01906	3.43E-03
hsa-miR-2277-5p	3.05314	2.03621	-2.02361	7.68E-05
hsa-miR-4317	1.81273	0.795666	-2.0238	5.78E-14
hsa-miR-210-3p	2.34765	1.32191	-2.03601	2.46E-06
hsa-miR-1273h-5p	2.40377	1.35346	-2.07097	1.41E-06
hsa-miR-378c	2.42281	1.32724	-2.13697	4.69E-07
hsa-miR-193a-5p	4.26702	3.168	-2.14209	3.26E-03
hsa-miR-4306	2.68238	1.55916	-2.17834	3.48E-06
hsa-miR-7977	2.54217	1.41008	-2.19177	5.31E-09
hsa-miR-18b-5p	2.1435	1.00445	-2.20236	2.49E-09
hsa-miR-146b-5p	2.44215	1.29915	-2.2084	6.40E-07
hsa-miR-345-5p	2.41849	1.19065	-2.34216	1.92E-07
hsa-miR-30b-5p	2.2374	1.00899	-2.34309	1.37E-08
hsa-miR-4467	4.54576	3.30647	-2.36083	7.19E-03
hsa-miR-370-3p	2.27665	0.996037	-2.42942	2.25E-08
hsa-miR-30a-5p	2.66718	1.38338	-2.43479	3.06E-06
hsa-miR-1273g-3p	8.65411	7.34989	-2.46951	1.52E-03
hsa-let-7b-5p	10.7291	9.41565	-2.48532	1.50E-06
hsa-miR-543	2.30593	0.987283	-2.49433	1.73E-08
hsa-miR-148b-3p	2.3162	0.94514	-2.58661	4.21E-12
hsa-miR-877-5p	3.93223	2.55518	-2.59737	8.19E-09
hsa-miR-431-5p	2.31747	0.911747	-2.64951	2.85E-10
hsa-miR-324-3p	3.95169	2.5124	-2.71187	4.29E-04
hsa-miR-1260b	3.11578	1.66598	-2.73169	4.34E-13
hsa-miR-3615	3.21186	1.73498	-2.78346	1.89E-08
hsa-miR-485-3p	2.62746	1.14257	-2.79895	5.20E-10
hsa-miR-574-3p	4.67419	3.14444	-2.88736	2.32E-04
hsa-miR-4284	2.82803	1.2697	-2.94514	5.52E-12
hsa-miR-654-5p	2.50474	0.943363	-2.95135	2.24E-10
hsa-miR-132-3p	2.98246	1.37331	-3.05071	8.40E-12
hsa-miR-143-3p	2.67409	1.06282	-3.05521	1.09E-09
hsa-miR-532-5p	3.43514	1.75816	-3.19758	6.81E-05
hsa-miR-15a-5p	2.89941	1.21102	-3.22296	1.47E-09
hsa-miR-6852-5p	2.61693	0.920318	-3.24139	6.56E-13
hsa-miR-181d-5p	2.77639	1.07383	-3.25479	2.39E-15

hsa-miR-93-3p	3.10113	1.38816	-3.27835	1.57E-08
hsa-miR-29a-3p	3.22897	1.49708	-3.32162	5.35E-11
hsa-miR-766-3p	3.94947	2.20097	-3.36009	1.06E-10
hsa-miR-5100	3.53097	1.76889	-3.39186	7.81E-08
hsa-miR-185-5p	8.31401	6.52872	-3.44686	4.24E-04
hsa-miR-324-5p	4.02188	2.20537	-3.52226	1.59E-06
hsa-miR-494-3p	3.17379	1.35107	-3.53747	4.23E-11
hsa-miR-379-5p	2.76993	0.94517	-3.54248	1.09E-12
hsa-miR-191-5p	9.5739	7.72991	-3.59003	2.49E-04
hsa-miR-21-5p	2.90477	1.0195	-3.69422	1.92E-12
hsa-miR-378a-3p	3.95862	2.07123	-3.69965	7.75E-06
hsa-miR-320c	11.1779	9.19324	-3.95767	3.05E-08
hsa-miR-4446-3p	3.25769	1.23428	-4.06543	9.76E-19
hsa-miR-128-3p	3.08425	1.04439	-4.11205	7.16E-16
hsa-miR-181b-5p	3.82705	1.75918	-4.19267	7.41E-09
hsa-miR-320b	11.3151	9.24441	-4.201	9.61E-07
hsa-miR-485-5p	3.20569	1.12999	-4.21548	4.79E-13
hsa-miR-320a	11.6138	9.50947	-4.30005	2.12E-09
hsa-miR-199a-5p	3.21486	1.10087	-4.32889	3.35E-15
hsa-miR-342-3p	6.80371	4.67591	-4.37052	8.43E-04
hsa-miR-4454	5.64049	3.50869	-4.38263	3.34E-06
hsa-miR-130b-3p	4.91613	2.72181	-4.57673	2.13E-07
hsa-miR-339-5p	3.4407	1.21886	-4.66488	9.53E-16
hsa-miR-28-5p	3.29051	1.03061	-4.78958	1.70E-17
hsa-miR-200c-3p	3.44439	1.16664	-4.84921	1.37E-16
hsa-miR-130a-3p	4.47957	2.20147	-4.85041	3.95E-09
hsa-miR-342-5p	3.59208	1.30262	-4.88876	4.97E-12
hsa-miR-93-5p	8.82198	6.53099	-4.89391	3.49E-05
hsa-miR-1307-3p	5.11249	2.81441	-4.91803	3.44E-08
hsa-miR-140-3p	5.83642	3.51609	-4.99443	3.67E-04
hsa-miR-421	3.44556	1.1075	-5.05622	9.30E-14
hsa-miR-425-5p	7.61558	5.26077	-5.11529	3.30E-04
hsa-miR-664a-5p	3.5432	1.1772	-5.15511	6.95E-18
hsa-miR-339-3p	3.69121	1.31574	-5.18904	1.03E-13
hsa-miR-30c-5p	3.69579	1.30228	-5.25433	8.40E-15
hsa-miR-2110	4.52382	2.08056	-5.43872	4.69E-12

hsa-miR-1973	3.53109	1.08462	-5.45079	6.35E-16
hsa-miR-487b-3p	3.62635	1.16748	-5.49787	4.82E-16
hsa-miR-139-3p	3.70699	1.2313	-5.56234	2.31E-19
hsa-miR-330-3p	3.60045	1.10559	-5.63674	8.30E-23
hsa-miR-505-5p	3.71514	1.10195	-6.11856	8.73E-16
hsa-miR-423-5p	8.05264	5.41566	-6.22027	1.92E-06
hsa-miR-486-3p	4.77346	2.0831	-6.45473	1.16E-08
hsa-miR-155-5p	4.06587	1.35694	-6.53837	1.76E-14
hsa-miR-628-3p	3.85935	1.0899	-6.8185	2.69E-18
hsa-miR-25-3p	6.86632	3.98899	-7.34788	1.88E-05
hsa-miR-23a-3p	10.8132	7.91299	-7.46559	7.41E-08
hsa-miR-145-5p	4.70445	1.75677	-7.71507	2.01E-12
hsa-miR-24-3p	10.3113	7.35192	-7.77794	5.05E-08
hsa-miR-17-5p	8.85984	5.87497	-7.91652	7.84E-07
hsa-miR-625-5p	4.01302	1.01785	-7.97322	2.41E-15
hsa-miR-22-3p	7.62118	4.59614	-8.14007	1.28E-05
hsa-miR-20b-5p	5.18895	2.12511	-8.36195	4.53E-09
hsa-miR-197-3p	5.28695	2.17471	-8.64724	4.99E-19
hsa-miR-629-5p	4.52407	1.38644	-8.80082	4.29E-19
hsa-miR-181a-5p	5.80458	2.62175	-9.08085	2.30E-09
hsa-miR-23a-5p	4.69014	1.50051	-9.12378	1.44E-25
hsa-miR-484	4.51765	1.30159	-9.29251	1.06E-23
hsa-miR-152-3p	4.21569	0.96654	-9.50804	3.35E-27
hsa-miR-99b-5p	5.21776	1.93963	-9.70097	4.31E-17
hsa-miR-20a-5p	7.56035	4.27659	-9.73891	1.52E-07
hsa-miR-26a-5p	9.15117	5.85976	-9.7907	1.31E-07
hsa-miR-106b-3p	5.15679	1.86529	-9.79132	4.73E-12
hsa-miR-103a-3p	9.5133	6.21722	-9.82241	1.40E-06
hsa-miR-106a-5p	8.68853	5.38895	-9.8463	1.38E-07
hsa-miR-127-3p	5.0326	1.72051	-9.93203	1.07E-11
hsa-let-7d-3p	4.5085	1.1373	-10.3474	1.06E-23
hsa-miR-4429	7.85534	4.43497	-10.7062	4.99E-10
hsa-let-7f-5p	4.52073	1.07519	-10.8946	9.82E-17
hsa-miR-107	9.02843	5.56494	-11.031	2.50E-08
hsa-miR-28-3p	4.81978	1.35061	-11.0745	1.27E-21
hsa-miR-106b-5p	6.10376	2.56808	-11.597	4.83E-10

hsa-miR-30d-5p	6.27065	2.72032	-11.7154	2.03E-11
hsa-miR-18a-5p	5.29943	1.74778	-11.726	2.99E-15
hsa-miR-320d	9.60528	6.00078	-12.1636	1.98E-09
hsa-miR-139-5p	4.89113	1.2482	-12.4919	1.60E-20
hsa-miR-151b	4.85664	1.14972	-13.0585	4.25E-23
hsa-miR-1301-3p	5.25067	1.52703	-13.2108	4.51E-22
hsa-miR-328-3p	4.97625	1.24327	-13.2966	2.66E-31
hsa-miR-433-3p	4.748	1.00991	-13.3438	1.41E-21
hsa-miR-223-3p	5.61399	1.78782	-14.1839	1.08E-16
hsa-miR-125a-5p	6.12957	2.22314	-14.9953	1.67E-15
hsa-miR-23b-3p	8.44038	4.50528	-15.2961	1.01E-10
hsa-let-7c-5p	9.34825	5.26999	-16.892	8.80E-10
hsa-let-7g-5p	5.22692	1.14422	-16.944	3.66E-24
hsa-miR-15b-5p	6.26687	2.15125	-17.3351	1.02E-16
hsa-miR-27b-3p	5.33589	1.14441	-18.271	3.20E-28
hsa-let-7d-5p	9.12999	4.81046	-19.9669	1.67E-13
hsa-miR-222-3p	7.21395	2.88671	-20.0737	9.88E-14
hsa-miR-27a-3p	6.05905	1.7275	-20.1338	1.83E-19
hsa-miR-126-3p	7.70348	3.33569	-20.6461	7.68E-15
hsa-miR-134-5p	6.00878	1.4825	-23.0433	8.37E-22
hsa-miR-744-5p	7.54613	3.01767	-23.0783	3.18E-15
hsa-miR-423-3p	6.57152	2.01001	-23.613	1.58E-19
hsa-miR-320e	6.94591	2.33805	-24.384	3.61E-20
hsa-miR-409-3p	6.44061	1.77721	-25.3408	3.68E-23
hsa-miR-382-5p	6.15836	1.47109	-25.7636	6.65E-24
hsa-miR-199a-3p	5.94475	1.23986	-26.0805	4.45E-33
hsa-miR-199b-3p	5.94475	1.23986	-26.0805	4.45E-33
hsa-miR-652-3p	7.3503	2.56507	-27.5738	1.51E-14
hsa-miR-4485	6.06409	1.27548	-27.6385	4.39E-30
hsa-let-7e-5p	6.43541	1.57826	-28.9832	6.40E-25
hsa-miR-361-5p	7.92495	3.06576	-29.0245	5.92E-15
hsa-let-7i-5p	7.6474	2.65115	-31.9169	5.98E-16
hsa-miR-151a-5p	8.21729	3.14888	-33.5539	2.71E-14
hsa-miR-584-5p	7.24772	2.00138	-37.9582	6.11E-24
hsa-miR-432-5p	6.99072	1.72922	-38.3591	1.37E-17
hsa-miR-151a-3p	7.75542	2.41812	-40.4287	6.05E-17

hsa-miR-146a-5p	8.40952	2.80184	-48.7619	4.08E-19
hsa-miR-221-3p	8.93936	3.1176	-56.562	2.87E-16
hsa-let-7a-5p	8.82716	2.82106	-64.271	4.65E-19

176 microRNAs were up-regulated and 147 were down-regulated in chronic hepatitis C (CHC) compared to healthy control (HC). They satisfied the following conditions: fold change < -2 or > 2; P-value < 0.05 by the one-way analysis of variance (ANOVA); P-value < 0.05 by the false discovery rate. [*]P-values by the one-way ANOVA.

Supporting Table 3. Clinical characteristics of chronic hepatitis C patients with or
without significant hepatic fibrosis: Ishak score 3-6 at initial liver biopsy

	Ishak score		
	0 - 2 (n = 106)[*]	3 - 6 (n = 24)[†]	*P*-value
Gender, male / female	54 / 52	13 / 11	N.S.
Age at initial biopsy, years	40 (36 - 46)	44 (40 - 50)	0.022
Age at infection, years[‡]	20 (16 -23)	23 (20 - 25)	N.S.
Duration of infection, year[‡]	22 (17 - 28)	21 (14 - 25)	N.S.
Race, Caucasian / African American/ Others	91 / 13 / 2	20 / 4 / 0	N.S.
Platelet count, $\times 10^9$/L	231 (198 - 264)	179 (155 - 227)	< 0.001
AST, IU/L	33 (27 - 46)	57 (43 - 79)	< 0.001
ALT, IU/L	50 (36 - 70)	74 (53 - 114)	0.006
ALP, IU/L	71 (59 - 90)	76 (66 - 100)	N.S.
γ-GTP, IU/mL	32 (22 - 74)	50 (33 - 104)	0.041
Total bilirubin, mg/dL	0.6 (0.5 - 0.7)	0.7 (0.5 - 0.8)	N.S.
Albumin, g/dL	4.3 (4.0 - 4.5)	4.3 (3.9 - 4.5)	N.S.
AFP, ng/mL	2.9 (1.9 - 4.0)	4.7 (2.7 - 9.9)	0.002
APRI	0.48 (0.34 - 0.64)	0.92 (0.64 - 1.36)	< 0.001
FIB-4	0.92 (0.70 - 1.15)	1.61 (1.19 - 2.40)	< 0.001
HCV RNA, log copies/mL	6.59 (6.18 - 7.17)	6.27 (6.03 - 6.70)	N.S.
HAI score, ≤7 / ≥8	61 / 45	6 / 18	0.004
HCV genotype, 1 / 2 / 3 / 1+2 / N.D.	81 / 17 / 2 / 3 / 3	18 / 3 / 2 / 0 / 1	N.S.
SNP genotype			
rs12979860: *IFNL4*, CC / CT / TT	32 / 53 / 21	6 / 15 / 3	N.S.
rs738409: *PNPLA3*, CC / CG / GG	54 / 48 / 4	16 / 8 / 0	N.S.
rs4374383: *MERTK*, AA / AG / GG	19 / 50 / 37	2 / 14 / 8	N.S.
rs9380516: *TULP1*, CC / CT / TT	69 / 33 / 4	18 / 6 / 0	N.S.
rs16851720: *RNF7*, AA / AC / CC / N.D.	50 / 24 / 3 / 29	5 / 10 / 0 / 9	N.S.
rs910049: *HLA* class II, GG / GA / AA / N.D.	48 / 35 / 9 / 14	14 / 7 / 0 / 3	N.S.
rs3135363: *HLA* class II, TT / TC / CC	61 / 36 / 9	15 / 7 /2	N.S.

Data are expressed as number for categorical data or the median (first-third quartiles)
for non-categorical data. [*]Ishak 0 (n=43); 1 (n=39); 2 (n=24). [†] Ishak 3 (n=13); 4 (n=7);
5 (n=2); 6 (n=2). [‡]Data were available in 90 patients: Ishak 0-2 (n=72) and 3-6 (n=18),

respectively. *P*-values were calculated by the chi-square test for categorical variables and by Mann-Whitney *U*-test for non-categorical variables. N.S., not significant; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; AFP, alpha-fetoprotein; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; HAI, histologic activity index; N.D., not determined; SNP, single nucleotide polymorphism.

Supporting Table 4. Logistic regression analysis of factors associated with significant hepatic fibrosis: Ishak score 3-6 at initial liver biopsy

Variables	Odds ratio (95% CI)	*P*-value
Analysis 1		
Age	1.050 (0.990 - 1.120)	0.102
APRI	1.830 (1.050 - 3.200)	0.033
$\log_2$ (let-7a-5p/cel-miR-39)	0.549 (0.363 - 0.830)	0.004
Analysis 2		
Age	1.060 (0.995 - 1.120)	0.073
APRI	1.850 (1.050 - 3.280)	0.034
$\log_2$ (let-7c-5p/cel-miR-39)	0.601 (0.395 - 0.915)	0.017
Analysis 3		
Age	1.040 (0.980 - 1.110)	0.190
APRI	1.990 (1.130 - 3.490)	0.017
Log_2 (let-7d-5p/cel-miR-39)	0.541 (0.371 - 0.788)	0.001
Analysis 4		
FIB-4	1.570 (0.960 - 2.570)	0.072
$\log_2$ (let-7a-5p/cel-miR-39)	0.563 (0.372 - 0.850)	0.006
Analysis 5		
FIB-4	1.690 (1.020 - 2.800)	0.041
Log_2 (let-7c-5p/cel-miR-39)	0.615 (0.407 0.929)	0.021
Analysis 6		
FIB-4	1.630 (1.020 - 2.600)	0.043
$\log_2$ (let-7d-5p/cel-miR-39)	0.549 (0.378 - 0.797)	0.002

Different variables were selected in each analysis.

CI, confidence interval; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4.

Supporting Table 5. Area under the receiver operating characteristic curve for the combination of each let-7 and APRI or FIB-4 index

Combination	AUC (95% CI)
let-7a-5p + APRI	0.814 (0.720 – 0.907)
let-7a-5p + FIB-4	0.821 (0.726 – 0.917)
let-7c-5p + APRI	0.784 (0.680 – 0.888)
let-7c-5p + FIB-4	0.799 (0.698 – 0.899)
let-7d-5p + APRI	0.820 (0.732 – 0.907)
let-7d-5p + FIB-4	0.825 (0.737 – 0.914)

AUC, area under the curve; CI, confidence interval; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4.

Supporting Table 6. Clinical characteristics of chronic hepatitis C patients who received paired liver biopsies

	n = 60
Gender, male / female	30 / 30
Age at initial biopsy, years	40 (36 - 44)
Age at infection, years[*]	21 (19 -23)
Duration of infection, year[*]	19 (16 - 23)
Race, Caucasian / African American/ Others	49 / 10 / 1
Platelet count, $\times 10^9$/L	213 (185 - 252)
AST, IU/L	35 (27 - 54)
ALT, IU/L	52 (36 - 68)
ALP, IU/L	72 (58 - 96)
γ-GTP, IU/mL	39 (24 - 78)
Total bilirubin, mg/dL	0.6 (0.5 - 0.8)
Albumin, g/dL	4.2 (4.0 - 4.5)
AFP, ng/mL	2.9 (2.1 - 4.2)
APRI	0.50 (0.30 - 0.70)
FIB-4	0.93 (0.73 - 1.41)
HCV RNA, log copies/mL	6.61 (6.15 - 7.16)
Ishak score, 0 / 1 / 2 / 3 / 4 / 5 / 6	24 / 18 / 8 / 4 / 4 / 2 / 0
HAI score, ≤7 / ≥8	33 / 27
HCV genotype, 1 / 2 / 3 / 1+2 / N.D.	46 / 10 / 1 / 2 / 1

Data indicate at the initial liver biopsy. Data are expressed as number for categorical data or the median (first-third quartiles) for non-categorical data. *Data were available in 36 patients. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; AFP, alpha-fetoprotein; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; HAI, histologic activity index; N.D., not determined.

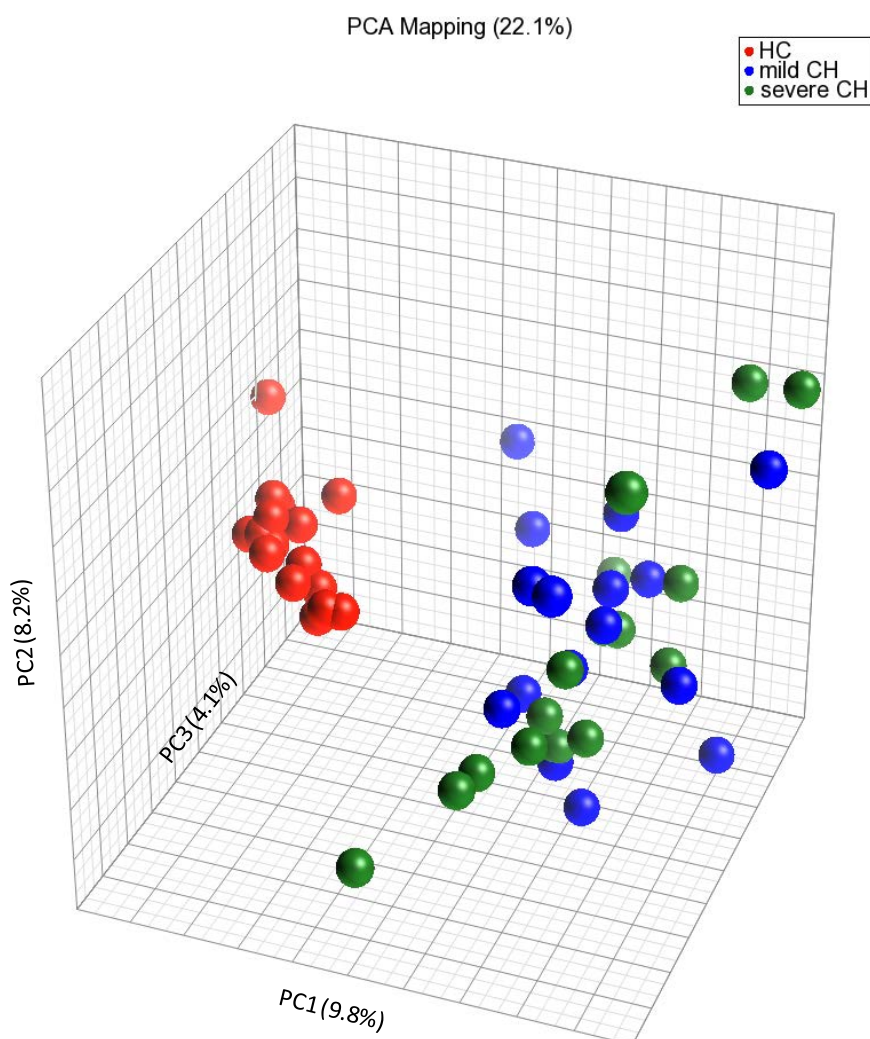Supporting Table 7. Clinical characteristics of chronic hepatitis C patients analyzed microRNA expression in liver tissues

	Initial biopsy (n = 43)	Repeat biopsy (n = 43)
Gender, male / female	22 / 21	22 / 21
Age at biopsy, years	44 (37 - 50)	46 (42 - 51)
Race, Caucasian / African American/ Others	34 / 8 / 1	33 / 9 / 1
Platelet count, $\times 10^9$/L	230 (189 - 265)	230 (189 - 265)
AST, IU/L	40 (31 - 62)	44 (30 - 61)
ALT, IU/L	54 (38 - 107)	51 (35 - 88)
APRI	0.49 (0.36 - 0.89)	0.55 (0.41 - 0.93)
FIB-4	1.04 (0.73 - 1.59)	1.21 (0.96 - 1.73)
Ishak score, 0 / 1 / 2 / 3 / 4 / 5 / 6	11 / 16 / 7 / 4 / 3 / 0 / 2	9 / 13 / 11 / 6 / 2 / 2 / 0
HAI score, ≤7 / ≥8 / N.D.	23 / 20 / 0	27 / 15 / 1
HCV genotype, 1 / 2 / 3 / 1+2 / N.D.	33 / 4 / 2 / 1 / 3	33 / 6 / 1 / 2 / 1

Formalin-fixed paraffin-embedded (FFPE) specimens of liver tissues were available for only a subset of patients: only 6 of 43 cases where a specimen was available at the initial biopsy had a paired specimen at the repeat biopsy, and additional 37 specimens were available only at the repeat biopsy. Therefore, total 86 FFPE samples used for microRNA expression analysis in liver tissue do not reflect repeat biopsies in the 43 patients. Data indicate at the initial or the repeat liver biopsy in each group. Data are expressed as number for categorical data or the median (first-third quartiles) for non-categorical data. AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; HAI, histologic activity index; N.D., not determined.

Supporting Table 8. Top five canonical pathways related to target genes of let-7 using Ingenuity Pathway Analysis

Top Canonical Pathways	*P*-value[*]	Overlap[†]	Molecules[‡]
Hepatic Fibrosis / Hepatic Stellate Cell Activation	4.82E-06	13.7 % (25/183)	SMAD2, COL5A2, COL4A1, TGFBR1, COL4A5, COL4A6, IL10, IL6R, COL9A3, COL4A2, IL6, PDGFB, FAS, COL15A1, COL1A2, COL1A1, IGF1, EDN1, IGF1R, COL24A1, COL11A1, TNFRSF1B, CCR7, FASLG, COL3A1
PPAR/RXR Activation	3.11E-05	12.8 % (23/179)	PPARA, SMAD2, TGFBR1, NRAS, PRKAB2, TGFBR3, IKBKE, IL6, ACVR2B, ACVR1B, NCOA3, ADCY9, GHR, PRKAA2, NCOR1, MEF2C, CHUK, INSR, ADIPOR2, MAP4K4, ACVR1C, ACVR2A, PPARGC1A
Molecular Mechanisms of Cancer	3.73E-05	10.1 % (37/365)	GAB2, TGFBR1, FZD3, ARHGEF7, TAB2, CCND1, MAPK11, FAS, E2F6, RB1, PAK1, NLK, GNAT1, E2F5, HIPK2, FASLG, E2F2, CDC25A, TP53, SMAD2, PMAIP1, AKT2, NRAS, CASP3, ARHGEF15, RALB, CDK6, MAPK9, BCL2L1, ADCY9, CCND2, CBL, FZD4, RASGRP1, CDKN1A, WNT1, GNAL
Chronic Myeloid Leukemia Signaling	5.49E-05	16.1 % (15/93)	TP53, GAB2, AKT2, NRAS, TGFBR1, CDK6, IKBKE, CCND1, BCL2L1, RB1, E2F6, CDKN1A, E2F5, CHUK, E2F2
STAT3 Pathway	5.93E-05	17.8 % (13/73)	GHR, TGFBR1, NRAS, NTRK3, TGFBR3, CDKN1A, IGF1R, MAPK9, SOCS4, INSR, SOCS7, MAPK11, CDC25A

[*]*P*-values were calculated by the right-tailed Fisher exact test.

[†]Overlap ratios were calculated as follow: (number of target genes of let-7) / (total number of reference genes) in each canonical pathway.

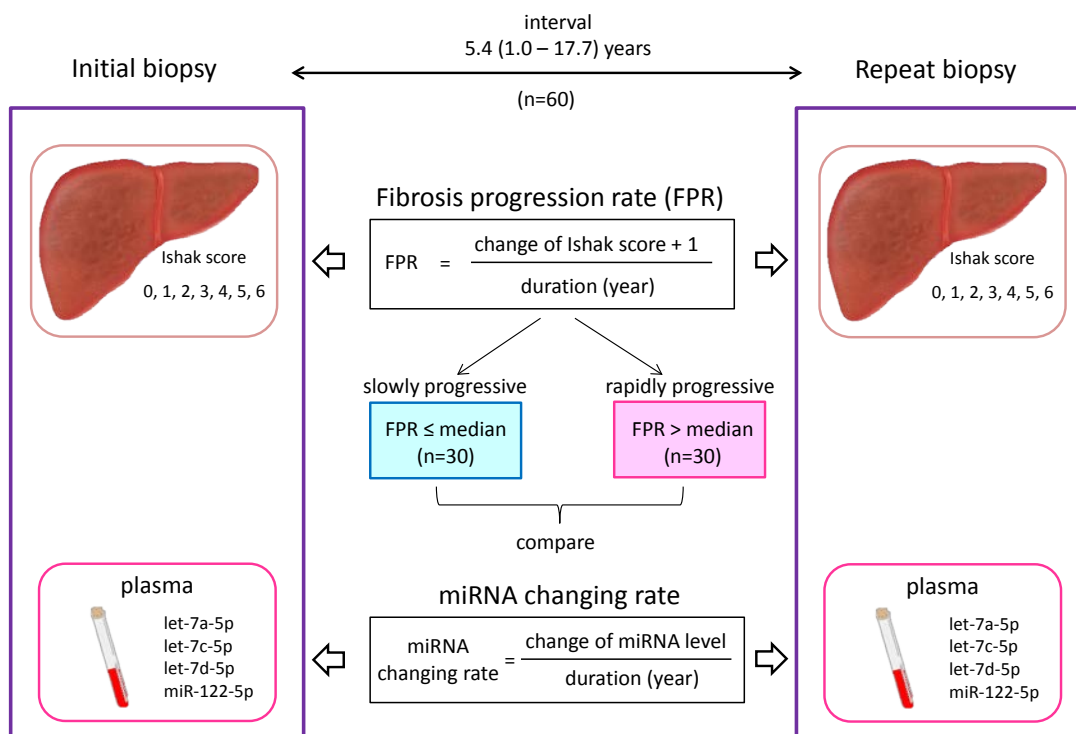[‡]Molecules represent target genes of let-7 in each canonical pathway.

Supporting Fig. 1. Principal component analysis showing the relations among the three groups resulting from comprehensive microRNA profile in plasma by microarray. The analysis was based on the result of the normalized signal intensities of probes for human microRNAs. The accumulative contribution rate was 22.1%. Each principal component contribution rate is shown in each axis. PCA, Principal component analysis; HC, healthy control; CH, chronic hepatitis; PC, principal component.
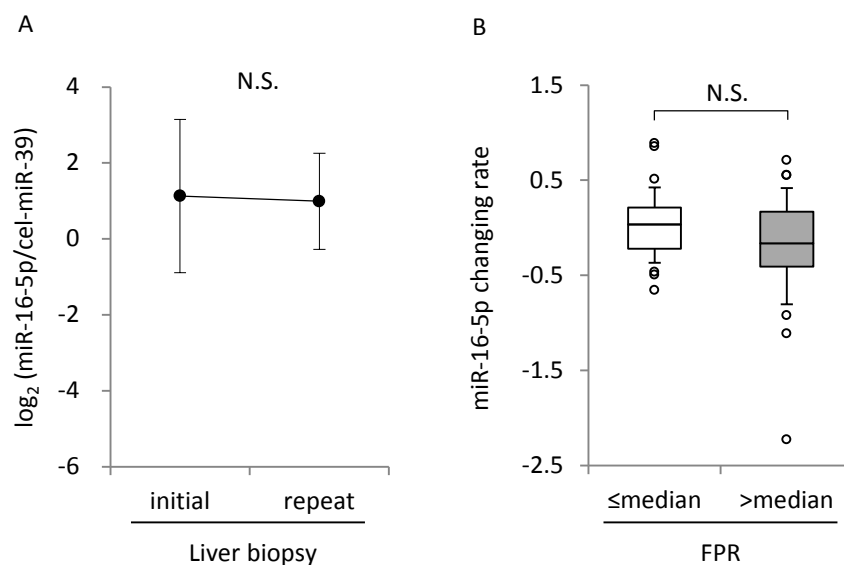
A

B

Supporting Fig. 2. Candidate endogenous reference microRNAs based on the microarray results. A) Top two sole candidate endogenous reference miRNAs. We sorted all the miRNAs analyzed by microarray in the discovery set: HC (n=16); mild CH (n=16); and severe CH (n=16), using the normalized signal intensities according to the following conditions: 1) average in all cases $\geq 5 \log_2$; 2) standard deviation in all cases < 2; 3) no significant difference among the three groups by the one-way analysis of variance and Mann-Whitney U-test ($P > 0.1$). B) Of the miRNAs with average signal intensity in all cases $\geq 5 \log_2$, miR-92-3p and miR-486-5p were top two miRNAs with less standard deviation. However, these miRNAs expression levels were significantly different among the three groups, but no difference in average of them. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. HC, healthy control; CH, chronic hepatitis.
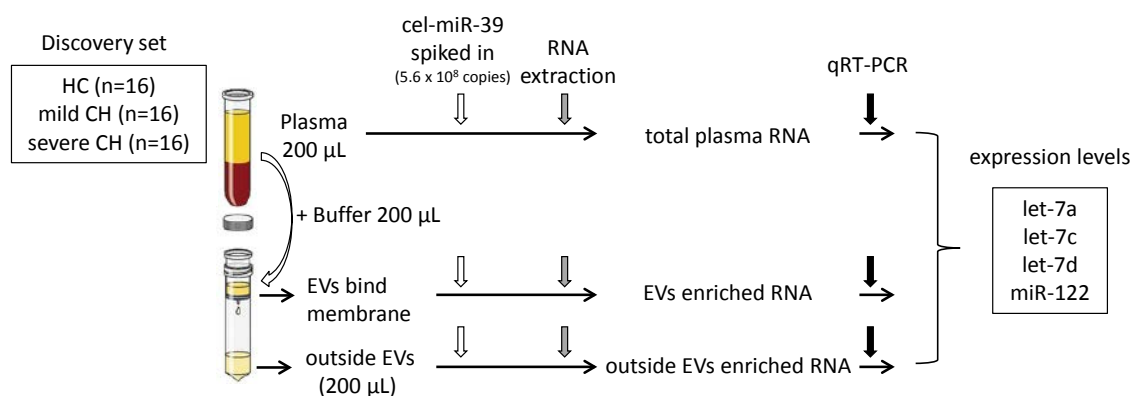
Supporting Fig. 3. Correlations of microRNA levels in plasma between microarray and qRT-PCR using different microRNA as a reference gene for normalization in the discovery set (n=48) by Peason's correlation coefficient (r). The expression levels of each miRNA by qRT-PCR were normalized to those of spike-in cel-miR-39 (A), endogenous miR-16-5p (B), and the average of endogenous miR-92a-3p and miR-468-5p (C), respectively. qRT-PCR, quantitative real-time polymerase chain reaction.
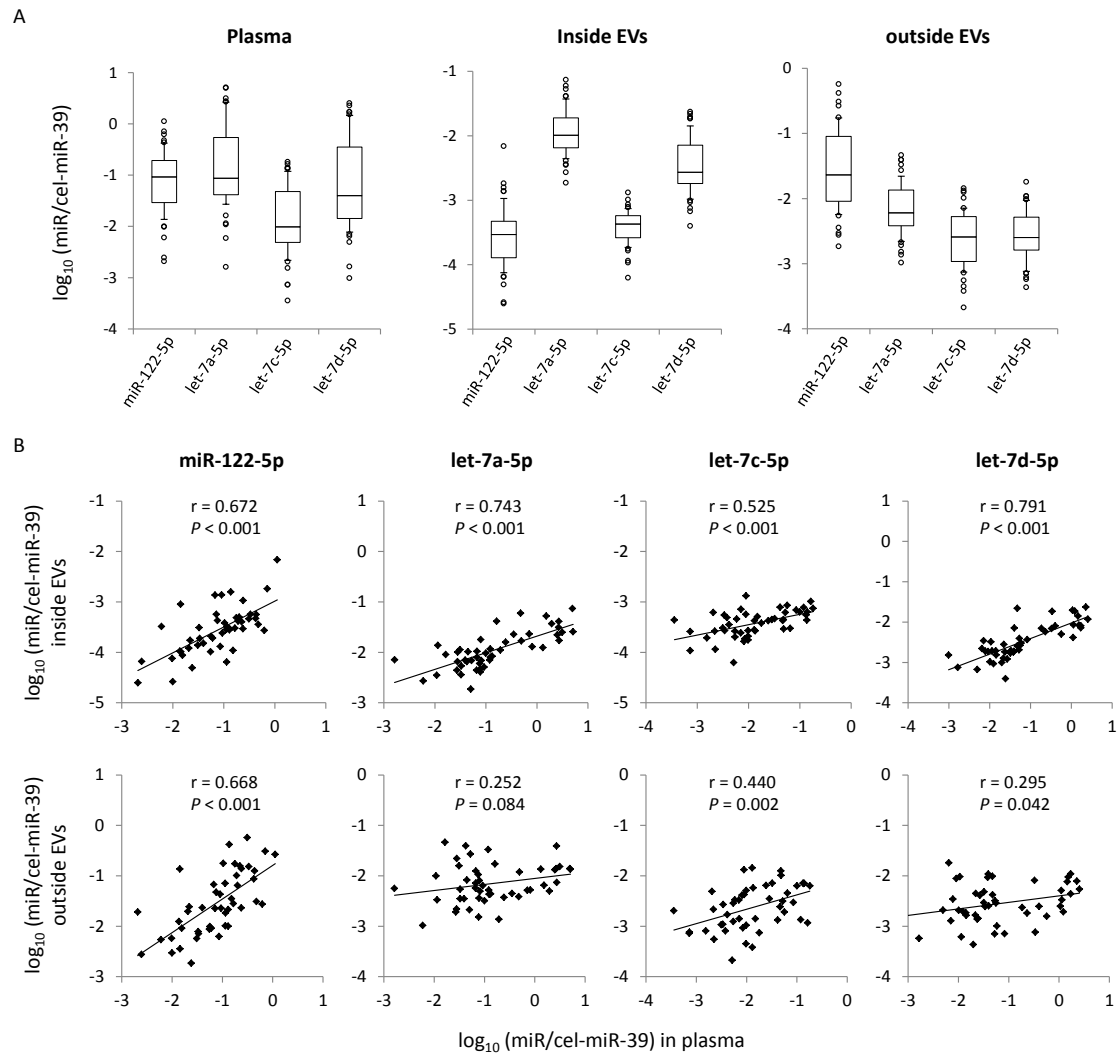
interval
5.4 (1.0 – 17.7) years
(n=60)

Initial biopsy

Ishak score
0, 1, 2, 3, 4, 5, 6

Fibrosis progression rate (FPR)

$$FPR = \frac{\text{change of Ishak score} + 1}{\text{duration (year)}}$$

slowly progressive

FPR ≤ median
(n=30)

rapidly progressive

FPR > median
(n=30)

compare

Repeat biopsy

Ishak score
0, 1, 2, 3, 4, 5, 6

plasma

let-7a-5p
let-7c-5p
let-7d-5p
miR-122-5p

miRNA changing rate

$$\text{miRNA changing rate} = \frac{\text{change of miRNA level}}{\text{duration (year)}}$$

plasma

let-7a-5p
let-7c-5p
let-7d-5p
miR-122-5p

Supporting Fig. 4. Scheme for the study of longitudinal circulating microRNA analysis in chronic hepatitis C patients who received paired liver biopsies. We calculated fibrosis progression rates (FPR) of the 60 patients between the paired biopsies, and divided into the two groups according to the median FPR: slowly fibrosis progressive group with ≤ median FPR and rapidly fibrosis progressive group > median FPR, and compared the changing rates of these miRNAs levels between the two groups. FPR and miRNA changing rate were calculated as follows: FPR = (Ishak score at the repeat biopsy - Ishak score at the initial biopsy + 1) / duration (year); miRNA chnaging rate = [$\log_2$ (miRNA / cel-miR-39) at the repeat biopy - $\log_2$ (miRNA / cel-miR-39) at the initial biopsy] / duration (year).
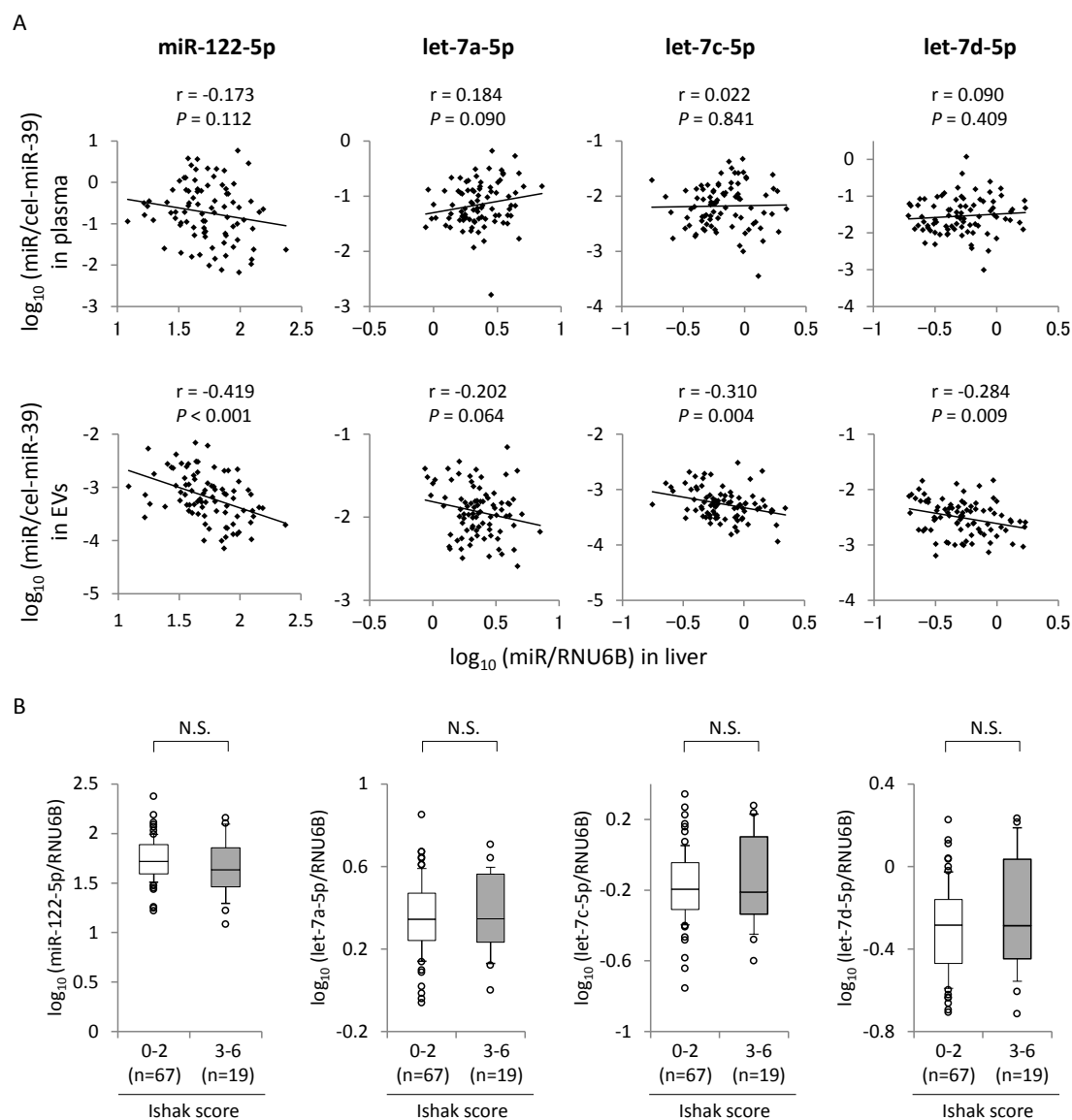
Supporting Fig. 5. Association between changes in miR-16 levels in plasma and hepatic fibrosis progression in longitudinal analysis. A) Changes in miR-16-5p expression levels between paired liver biopsies (n = 60). Data represent mean ± standard deviation. *P*-values were calculated by the paired *t*-test. B) We divided the 60 patients into two groups according to the median fibrosis progression rates (FPR) between the paired biopsies, and compared the changing rates of miR-16-5p levels between the two groups. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. *P*-values were calculated by Mann-Whitney *U*-test. N.S., not significant.
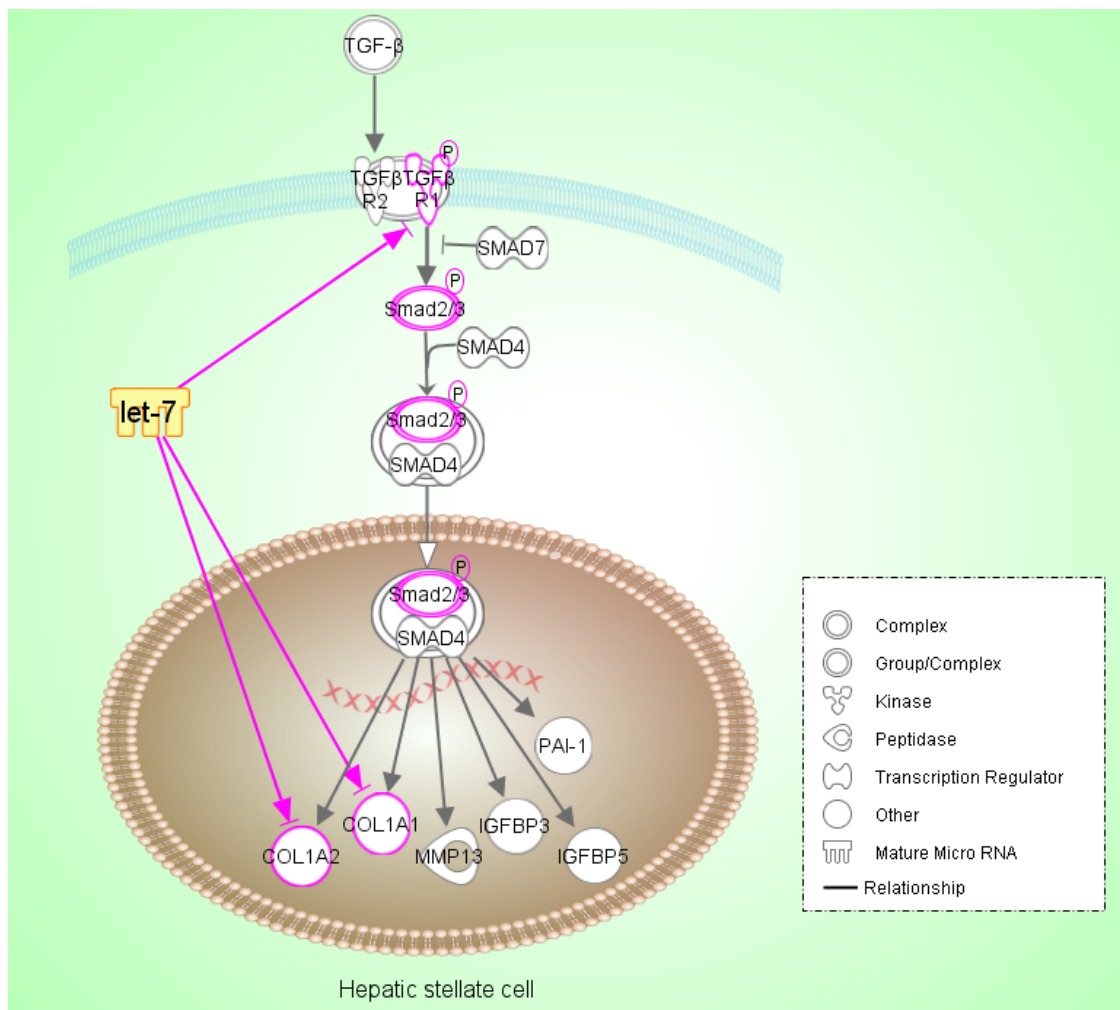
Supporting Fig. 6. Scheme for microRNA expression analysis inside or outside extracellular vesicles in the discovery set: HC (n=16); mild CH (n=16); and severe CH (n=16). The detailed methods of total RNA extraction from plasma and extracellular vesicles (EVs) are described in Supporting Material and Methods. In the process of purification of EVs, we recovered the flow-through as an outside EVs fraction in plasma, and added 5.6×10^8 copies of synthetic cel-miR-39 miRNA mimic to 200 µL of the outside EVs fraction as spike-in control, followed by total RNA extraction using miRNeasy Serum/Plasma Kit (Qiagen). Then, we measured the expression levels of let-7s and miR-122 in each fraction by qRT-PCR. HC, healthy control; CH, chronic hepatitis; EVs, extracellular vesicles; qRT-PCR, quantitative real-time polymerase chain reaction.

Supporting Fig. 7. Expression analysis of let-7s and miR-122 inside or outside extracellular vesicles in the discovery set (n=48). A) Expression levels of let-7s and miR-122 in plasma, inside EVs and outside EVs. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. B) Correlations between let-7s and miR-122 levels in plasma and those inside or outside extracellular vesicles using Pearson's correlation coefficient (r). EVs, extracellular vesicles.

Supporting Fig. 8. Expression analysis of let-7s and miR-122 in liver tissue. Eighty-six liver tissue samples (see Supporting Table 7) and their corresponding plasma and extracellular vesicles were analyzed. A) Correlations of let-7s and miR-122 levels between liver tissue and plasma or extracellular vesicles using Peason's correlation coefficient (r). B) Expression levels of let-7s and miR-122 in liver tissue in patients with or without significant fibrosis (Ishak score 3-6 at the initial or the repeat liver biopsy). Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. *P*-values were calculated by Mann-Whitney *U*-test. EVs, extracellular vesicles; N.S., not significant.

Supporting Fig. 9. Scheme for the early signaling events in hepatic stellate cells. Estimated let-7 target genes by TargetScan are represented in magenta. Of them, previous studies have shown that let-7 indeed targeted *TGFBR1*, *COL1A1* and *COL1A2*(shown with an arrow).(13-17)

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