Supporting Table 1. Clinicopathologic features between the two groups of patients with HCV-high and HCV-low loads used in this study.

		High (no.=9)	Low (no.=8)	*P*
HCV RNA (mean±SE)		69,910±8,729	64±17	0.00008
Male / female		5 / 4	8 / 0	
Age (range)		68.9 (60-77)	62.5 (50-70)	
Stage	I	2	2	
	II	7	5	
	IIIA	0	1	
Liver function (mean±SE)				
ICG-R15 (%)	(<10)	14.1±2.54	12.4±2.18	
Alb (g/dL)	(6.7-8.3)	4.02±0.16	3.99±0.21	
AST (IU/L)	(8-38)	62.1±19.7	37.6±6.04	
ALT (IU/L)	(40-44)	52.0±14.2	42.8±13.6	
T.bil (mg/dL)	(0.3-1.2)	0.79±0.17	0.59±0.09	
Fibrosis	F1	3	3	
	F2	1	4	
	F3	4	1	
	F4	0	0	
HCV genotype				
	1b	7	4	
	1b+2a	2	0	
	2a	0	3	
	2b	0	1	

The HCV RNA levels are indicated as an arbitrary unit, the viral copy number per 50 ng of total liver RNA normalized to the 18S rRNA quantity: High, HCV-high load (>30000 units of HCV RNA); Low, HCV-low load (<300 units of HCV RNA). The fibrosis score was determined according to the New Inuyama Classification.[1] HCV genotyping was performed as described previously.[2] HCV RNA, age, ICG-15 (indocyanin green retention at 15 min), Alb (serum albumin), AST (serum aspartate aminotransferase), ALT (serum alanine aminotransferase) and T.bil (total bilirubin) were analyzed by Mann-Whitney U test. Sex and stage were analyzed by Fisher's exact probability test. Fibrosis and HCV genotype were analyzed by the χ^2 test. Only HCV RNA was significantly different, and other variables were not. There was also no significant correlation between viral loads and all clinicopathologic variables according to a simple logistic regression analysis. To exclude the possible involvement of difference in sex ratio, we performed the same analyses of all variables using only male samples, a 5-to-8 comparison. No significant difference was observed among all of the clinicopathologic variables, but the TMPRSS2 gene and the 13 other genes were significantly different.

Supporting Table 2. Clinicopathologic features between two groups of patients with HCV-high and HCV-low loads used in the comprehensive analysis of gene expression.

Chronic hepatitis without liver cirrhosis

		High (no.=5)	Low (no.=5)	*P*
HCV RNA (mean±SE)		74,665±14,625	71±20	0.009
Male / female		4 / 1	5 / 0	
Age (range)		66.6 (62-71)	65.2 (58-71)	
Stage	I	2	2	
	II	3	3	
	IIIA	0	0	
Liver function (mean±SE)				
ICG-R15 (%)	(<10)	12.4±2.38	8.90±2.25	
Alb (g/dL)	(6.7-8.3)	4.02±0.24	4.18±0.27	
AST (IU/L)	(8-38)	51.8±10.8	35.0±4.92	
ALT (IU/L)	(40-44)	45.8±8.75	39.8±13.5	
T.bil (mg/dL)	(0.3-1.2)	0.66±0.13	0.58±0.13	
Fibrosis	F1	2	1	
	F2	0	3	
	F3	3	0	
	F4	0	(1)	
HCV genotype				
	1b	4	5	
	1b+2a	1	0	
	2a	0	0	
	2b	0	0	

		Liver cirrhosis		
		High (no.=7)	Low (no.=3)	*P*
HCV RNA (mean±SE)		140,287±59,501	97±64	0.017
Male / female		5 / 2	3 / 0	
Age (range)		63.3 (54-69)	65.7 (56-73)	
Stage	I	6	2	
	II	1	1	
	IIIA	0	0	
Liver function (mean±SE)				
ICG-R15 (%)	(<10)	28.1±7.61	28.3±8.36	
Alb (g/dL)	(6.7-8.3)	3.84±0.11	4.00±0.42	
AST (IU/L)	(8-38)	54.1±4.7	53.0±11.1	
ALT (IU/L)	(40-44)	47.9±7.40	48.0±12.86	
T.bil (mg/dL)	(0.3-1.2)	0.94±0.14	0.81±0.08	
Fibrosis	F1	0	0	
	F2	0	0	
	F3	(2)	0	
	F4	5	3	
HCV genotype				
	1b	6	2	
	1b+2a	1	0	
	2a	0	1	
	2b	0	0	

The number of the fibrosis score in parentheses indicates a discrepancy between F3 or F4 determined by two pathologists. The statistical analyses were performed as described in Supporting Table 1, and none of the variables, except for HCV RNA, were significantly different between the two groups.

Supporting Table 3. One hundred thirty genes differentially expressed in the livers with high vs. low HCV loads.

No.	Accession No.	Gene symbol	Fold change
Chronic hepatitis liver without liver cirrhosis			
1	NM_002122	*HLA-DQA1*	4.17
2	NM_003733	*OASL*	3.87
3	NM_005909	*MAP1B*	3.30
4	NM_005656	*TMPRSS2*	3.29
5	NM_002993	*CXCL6*	3.21
6	NM_003311	*PHLDA2*	3.09
7	AI763378	*EHF*	3.07
8	BC042028	---	2.99
9	BE675995	*SPEC2*	2.99
10	AK025180	---	2.97
11	BC089425	*LOC129607*	2.92
12	NM_003068	*SNAI2*	2.88
13	NM_006403	*NEDD9*	2.84
14	NM_001549	*IFIT3/ IFIT4*	2.83
15	NM_002353	*TACSTD2*	2.79
16	NM_001001887	*IFIT1*	2.78
17	NM_002354	*TACSTD1*	2.75
18	NM_006417	*IFI44*	2.72
19	NM_001565	*CXCL10*	2.61
20	NM_002303	*LEPR*	2.52
21	BF724558		2.52
22	NM_152878	*MAFF*	2.48
23	NM_004030	*IRF7*	2.47
24	NM_003749	*IRS2*	2.37
25	NM_017631	*FLJ20035*	2.37
26	AV699047	---	2.37
27	AI475680	---	2.34
28	AA937109	*FNBP1*	2.32
29	AW954199		2.32
30	AK025967	---	2.31
31	NM_016323	*HERC5*	2.28
32	NM_139266	*STAT1*	2.27
33	NM_002581	*PAPPA*	2.27
34	NM_005242	*F2RL1*	2.26
35	BX647703	---	2.24
36	AK021801	---	2.23
37	AK001125	---	2.23
38	AV733347	*LOC56902*	2.22
39	NM_004420	*DUSP8*	2.21
40	AK026659	---	2.21
41	NM_033260	*FOXQ1*	2.20
42	NM_005139	*ANXA3*	2.19
43	BE671038	*LRRC16*	2.19
44	BF590263	*CSPG2*	2.17
45	NM_018362	*LIN7C*	2.16
46	NM_004117	*FKBP5*	2.15
47	NM_003045	*SLC7A1*	2.13
48	AK074440	---	2.13

No.	Accession No.	Gene symbol	Fold change
continued			
67	AK096893		-6.03
68	NM_198462	*FLJ46154*	-4.14
69	NM_021614	*KCNN2*	-3.65
70	NM_000567	*CRP*	-3.53
71	NM_000450	*SELE*	-3.45
72	BF514098		-3.44
73	AI580142		-3.24
74	N80145		-3.23
75	M27830		-3.08
76	AW975324		-2.97
77	AA018404		-2.81
78	AK022897		-2.53
79	NM_003633	*ENC1*	-2.37
80	NM_213589	*RAPH1*	-2.34
81	AL049437		-2.31
82	NM_001187	*BAGE*	-2.30
83	BC022380	*FLJ00310*	-2.20
84	AI650260		-2.13
85	AK000674	*LOC134145*	-2.09
86	AB019490	*RABGAP1L*	-2.04
87	BF511381	*HMGA2*	-2.01
Chronic hepatitis liver with liver cirrhosis			
1	BC020750	*SDS*	8.09
2	NM_005101	*G1P2*	3.58
3	NM_003733	*OASL*	3.55
4	NM_001300	*KLF6*	3.30
5	NM_024786	*ZDHHC11*	3.20
6	AK093529	---	3.08
7	N55072	---	3.03
8	NM_006887	*ZFP36L2*	2.67
9	NM_015675	*GADD45B*	2.45
10	BC020765		2.45
11	NM_005542	*INSIG1*	2.36
12	NM_032527	*ZGPAT*	2.32
13	NM_002462	*MX1*	2.27
14	NM_001065	*TNFRSF1A*	2.27
15	BF528646		2.22
16	AW612461	---	2.10
17	NM_001001924	*MTSG1*	2.07
18	NM_005243	*EWSR1*	2.06
19	NM_018584	*CaMKIINalpha*	2.06
20	NM_000505	*F12*	2.03
21	NM_002616	*PER1*	2.02
22	U62733	*CPT1B*	2.02
23	NM_030582	*COL18A1*	2.01
24	NM_005178	*BCL3*	2.00
25	AA777349	---	2.00
26	BC006435	---	2.00

49	NM_004864	*GDF15*	2.12	27	NM_019063	*EML4*	2.00
50	AV715309	---	2.11	28	NM_002432	*MNDA*	-2.83
51	NM_005257	*GATA6*	2.10	29	NM_016613	*DKFZp586G0123/ SLC25A24*	-2.44
52	AK091504	*SFX3*	2.10	30	NM_197947	*CLECSF12*	-2.33
53	NM_001122	*ADFP*	2.10	31	AL833097		-2.31
54	NM_030674	*SLC38A1*	2.09	32	AI741439	*SLC8A1*	-2.29
55	NM_004867	*ITM2A*	2.07	33	NM_016613	*DKFZp434L142*	-2.25
56	NM_001547	*IFIT2*	2.07	34	NM_007115	*TNFAIP6*	-2.24
57	NM_004244	*CD163*	2.03	35	BG231961		-2.22
58	NM_032964	*CCL15*	2.03	36	AI799128		-2.14
59	NM_016114	*ASB1*	2.02	37	BF438173	*FST*	-2.11
60	AL049435	---	2.02	38	NM_003916	*AP1S2*	-2.10
61	NM_025074	*FRAS1*	2.01	39	AI743207		-2.07
62	U73936	*JAG1*	2.01	40	AI732988		-2.04
63	NM_015066	*TRIM35*	2.01	41	NM_020117	*LARS*	-2.02
64	AK000850		2.01	42	AI743123		-2.02
65	AW297731		2.01	43	NM_015173	*TBC1D1*	-2.02
66	AI806747	---	2.00	44	AI949827	*NFE2L3*	-2.01

A total of 130 genes differentially expressed more than 2 fold between the two groups of liver samples containing HCV-high and HCV-low viral loads were identified as described in Supporting Fig. 1. Eighty-seven genes were from 5-to-5 comparisons of patients with chronic hepatitis without liver cirrhosis, and 44 genes were from 7-to-3 comparisons of patients with chronic hepatitis with liver cirrhosis (Supporting Table 2). *OASL* with gray shading overlaps in the two comparisons. Positive and negative values of the fold change indicate up-regulation and down-regulation, respectively, in the livers with high HCV loads compared with those with low HCV loads.

Supporting Table 4. Primer sequences

Experiment	Accession No.	Gene symbol	Primer sequence (5'-3')[a] forward (upper) / reverse (lower)
HCV quantitation		HCV type 1b	GACCAAGCTCAAACTCACTC GCACGAGACAGGCTGTGATA
HCV genotyping		(1st PCR)	CGCGCGACDAGGAAGACTTC ATGTACCCCATGAGGTCGGC
		(2nd PCR)	AGGAAGACTTCSGAGCGRTC TGCCTTGGGGATAGGCTGAC (1a) GAGCCATCCTGCCCACCCCA (1b) CCAAGAGGGACGGGAACCTC (2a) ACCCTCGTTTCCGTACAGAG (2b)
Internal control	NM_002046.4	18S rRNA	AAACGGCTACCACATCCAAG CCTCCAATGGATCCTCGTTA
		GAPDH	GGTCGGAGTCAACGGATTTG GGATCTCGCTCCTGGAAGAT
		RPL34	GCACCAAAATCTGCATGTG GCCCTGCTGACATGTTTCTT
Validation	NM_002122	HLA-DQA1	CACCCGGCTACCTAATTCC CCCTGGATGAAAGATGGAAA
	NM_003733	OASL	CGTGGCAGAAGGGTACAGAT AAGGGTTCACGATGAGGTTG
	NM_005909	MAP1B	CTGGATGACATCAGCAATGG AGGGGTTCGTGTTGTCTTTG
	NM_005656	TMPRSS2[b]	CACTGTGCATCACCTTGACC ACACACCGATTCTCGTCCTC
	NM_005656	TMPRSS2[c]	ATGGCTTTGAACTCAGGGTC TTAGCCGTCTGCCCTCATTT
	NM_002993	CXCL6	TGTTTACGCGTTACGCTGAG GACAAACTTGCTTCCCGTTC
	NM_003068	SNAI2	CTTTTTCTTGCCCTCACTGC ACAGCAGCCAGATTCCTCAT
	NM_006403	NEDD9	AAGCCCTCTCAGAGCCTACC GCGTTGAGAAGGGAAATGAA
	NM_001549	IFIT3	GAACATGCTGACCAAGCAGA CAGTTGTGTCCACCCTTCCT

NM_002353	TACSTD2	ACCTCCAAGTGTCTGCTGCT
		GTCGTAGAGGCCATCGTTGT
NM_001565	CXCL10	ACCGTACGCTGTACCTGCAT
		TCTTGATGGCCTTCGATTCT
NM_002303	LEPR	TCCCATATCTGAGCCCAAAG
		CTGCTTTCACACTGGATGGA
BF724558		TGCCACTCTTCAAAGGCTTC
		GACCCGGAGAGCTGTTTCTT
NM_152878	MAFF	AAACCTGGGTGTCCTCACTG
		CCATCCGTGTCACCTTCTCT
NM_017631	DDX60	GGTCAAGACCTGATGGGAGA
		TTTGCCTTGGTATCCTCTGG
AV699047	LOC100422737	GCTGGGATGACAAGGAAGAC
		ATTCTGCCCCACCCTAAAAC
AK021801		AGCCAGTTTGCAGTCAGTGTT
		GAGGCAACATGAACCAGGAG
NM_018362	LIN7C	ACAGAAGAGGGCCTTGGATT
		CCCCCATGTCTATCAGCAAT
U73936	JAG1	GACTCATCAGCCGTGTCTCA
		TGGGGAACACTCACACTCAA
AK000850		GGGTAGAAGCCGTCATGTGT
		AGCAATCTGCTTTTGGCACT
N80145		CACCTTGGATGACGAAACAA
		GAGTTTCTGGGAAGGCAAAA
AB019490	RABGAP1L	TCCAAGCTGCGAAAAACTCT
		TTCAAAGGGACACCAGGAAG
NM_006887	ZFP36L2	GTGCAAGTACGGCGAAAAGT
		AGCCGATGGTATGAAAGGTG
NM_005542	INSIG1	CCTATCTTTGGGCCTTTGGT
		TGACGCCTCCTGAGAAAAAT
NM_001065	TNFRSF1A	GTGCCTACCCCAGATTGAGA
		TGTCGATTTCCCACAAACAA
NM_003916	AP1S2	GTTTCTTTTGGGAGGGGAAG
		ACACTACGTGGGGTTTCAGC
NM_020240	CDC42SE2	CGGCGGATTGACAGAAGTAT
		ACCTCCATAACCTCCCTTGG
NM_016613	FAM198B	TTATCCAGCACCTGTGGTCA
		CTACAGCAGGGAGCACCTTC

	AI741439	SLC8A1	TCTTGCATTGGTCGATTTCA GCTGTGCACAATACACACAAAA	
	NM_013386	SLC25A24	TTATCCAGCACCTGTGGTCA CTACAGCAGGGAGCACCTTC	
Transmembrane serine protease	NM_002569.2	FURIN	Hs00965485_g1	TaqMan gene expression assay[e]
	NM_002151.2	HPN	Hs01056332_m1	
	NM_005656	TMPRSS2[d]	Hs01120965_m1	
	NM_153609.2	TMPRSS6	Hs00542184_m1	
	NM_182973.1	TMPRSS9	Hs01572421_m1	
	NM_021978.3	ST14	Hs01058386_m1	
	NM_024022.2	TMPRSS3	Hs00225101_m1	
	NM_006587.2	CORIN	Hs00198141_m1	
	NM_019894.3	TMPRSS4	Hs00212669_m1	
	NM_004262.2	HAT	Hs00975370_m1	
Control		GAPDH	Hs02758991_g1	

[a] The primer sequences for SYBR green qPCR were designed by primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

[b, c] SYBR green qPCR of TMPRSS2 mRNA was performed using primer pair b for human liver (Fig. 1A) and primer pair c for TMPRSS2-expressing cell clones (Fig. 5A and Fig. 6A).

[d] TaqMan gene expression assay (Life Technologies) was used for qPCR of TMPRSS2 mRNA in Huh7-25-CD81 cells (Fig. 4A) and human liver (Supporting Fig. 4) infected with or without HCV.

[e] Assay IDs (Life Technologies) used are shown.

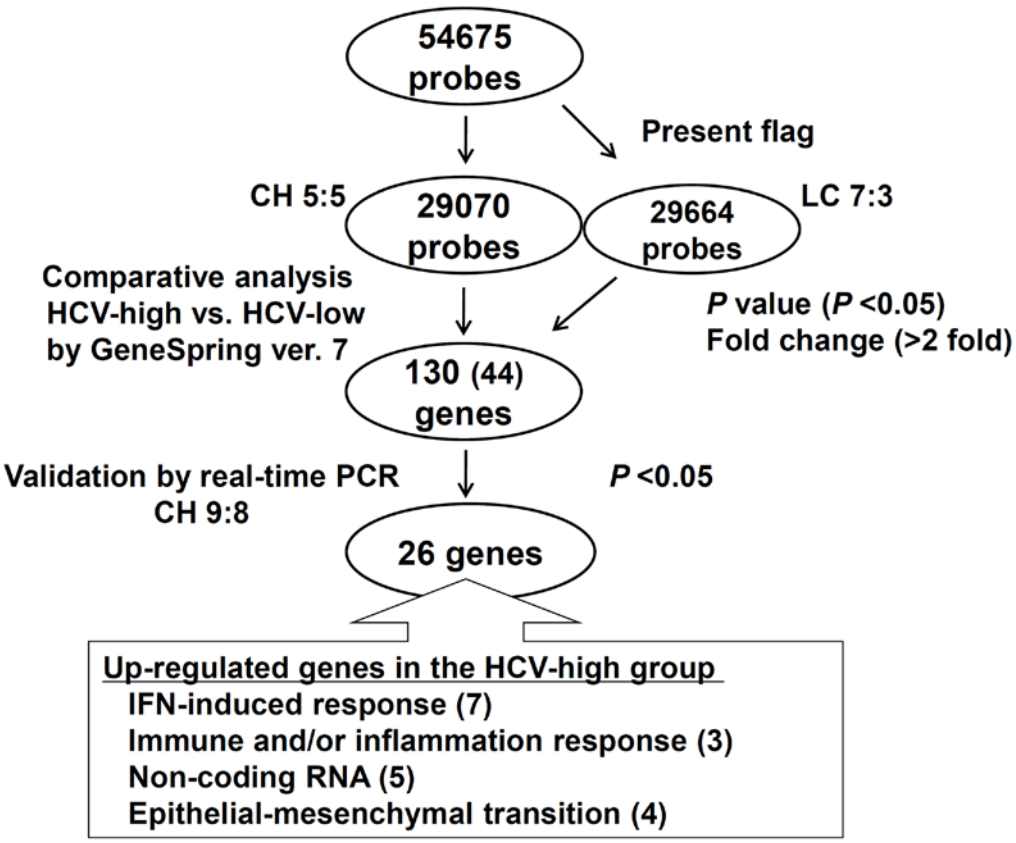
Supporting Table 5. Twenty-six up-regulated genes in the livers of the HCV-high group

Gene symbol	Gene title	Fold change	*P*		Function
HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1	9.28	0.002	ISG	Immune response
OASL	2'-5'-Oligoadenylate synthetase-like	7.27	0.0003	ISG	Anti-HCV replication
SNAI2	snail family zinc finger 2	7.03	0.004	EMT	E-cadherin repressor, anti-apoptosis
(AK021801)		4.63	0.021	ncR	Intron of BICC1 (bicaudal C homolog 1)
NEDD9	Neural precursor cell expressed, developmentally down-regulated 9	3.84	0.006	EMT	Focal adhesion
AP1S2	adaptor-related protein complex 1, sigma 2 subunit	3.66	0.011		Vesicle traffic
(AK000850)		3.36	0.004	ncR EMT	Intron of NEDD9
TMPRSS2	**Transmembrane protease, serine 2**	**3.31**	**0.002**		**Proteolysis**
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1	3.11	0.011		Na^{+}/Ca^{2+} exchanger
MAP1B	Microtubule-associated protein 1B	2.94	0.008		Microtubule stabilization
LOC100422737		2.93	0.008	ncR	Long non-coding RNA
MAFF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	2.82	0.002		Cellular stress response
SLC25A24	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 24	2.73	0.046		Calcium-dependent mitochondrial solute carrier
DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	2.72	0.002	ISG	Anti-viral helicase
(N80145)		2.69	0.027	ncR	EST
LEPR	Leptin receptor	2.68	0.015		Liver fibrosis
CXCL10	Chemokine (C-X-C motif) ligand 10	2.68	0.021	ISG	Immune response
ZFP36L2	ZFP36 ring finger protein-like 2	2.65	0.002		RNA-binding protein, erythroid self-renewal
(BF724558)		2.63	0.0006	ncR	EST

JAG1	Jagged 1	2.59	0.027	ISG EMT	Notch 1 ligand
TACSTD2	Tumor-associated calcium signal transducer 2	2.58	0.006		Cell surface Ca signal transducer
IFIT3	Interferon-induced protein with tetratricopeptide repeats	2.47	0.027	ISG	TBK1 activation-mediated IRF3 phosphorylation
KLF6	Kruppel-like factor 6	2.29	0.008	ISG	Tumor suppressor, carcinogenesis
LIN7C	Lin-7 homolog C (C. elegans)	2.19	0.036		Cell-cell adhesion, tumor suppressor
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	2.07	0.027		Inflammation, apoptosis
FAM198B	Family with sequence similarity 198, member B	1.98	0.046		unknown

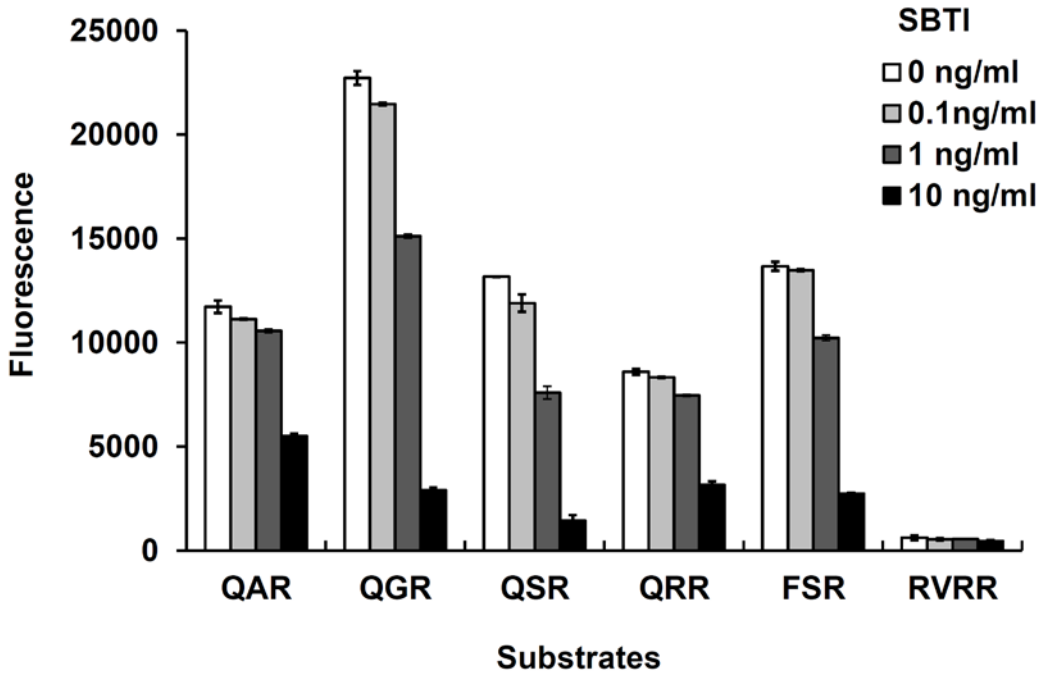
Twenty-six genes differentially expressed between the two groups of liver samples containing HCV-high and HCV-low viral loads were identified and validated as described in Supporting Fig. 1. The Mann-Whitney U test was performed to analyze the two-group comparisons, and *P* values (<0.05) are shown. A name in parentheses in the gene symbol column indicates the accession number of the gene. Six genes with gray shading overlap with previous studies that compared HCV-negative and -positive livers.[3-7] ISG, IFN-stimulating genes listed in Interferome v2.01[8]; EMT, epithelial mesenchymal transition; ncR, non-coding RNA; EST, expression sequence tag.

Supporting Figures

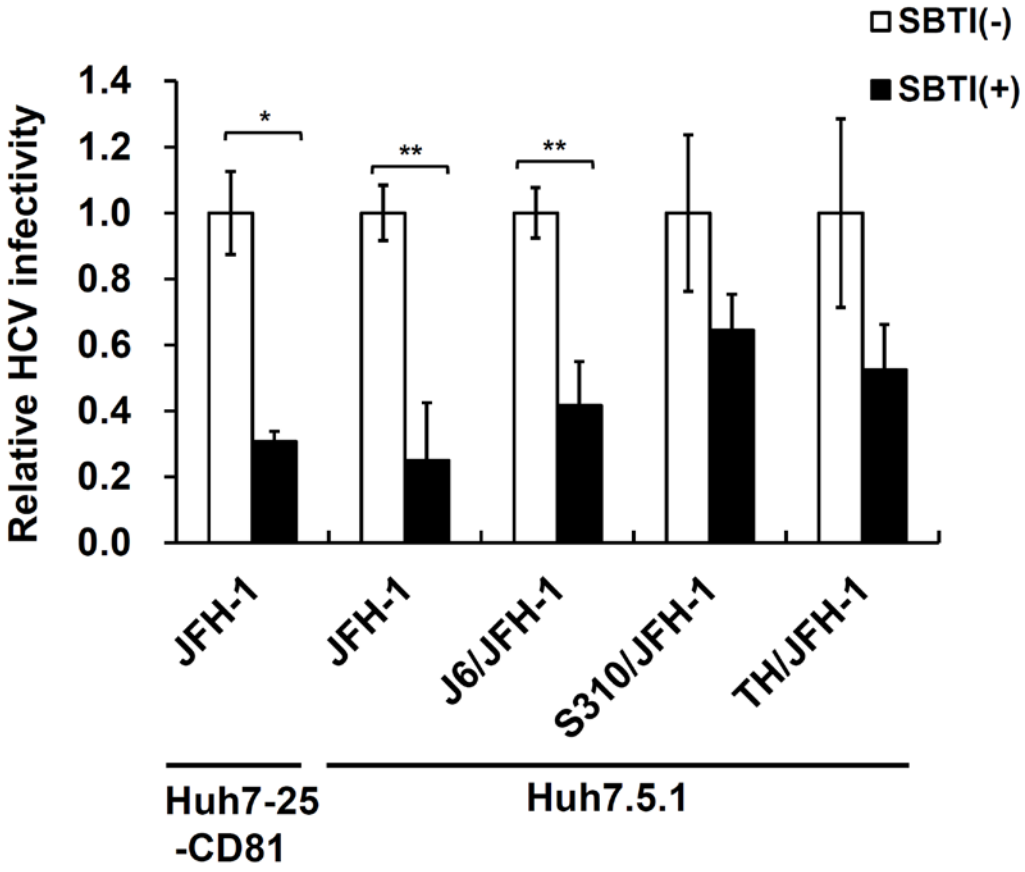


Supporting Fig. 1. Summary of comparative study of comprehensive gene expression between two groups of chronic hepatitis livers with HCV-high (n=5) and HCV-low (n=5) viral loads. We also performed a comparative gene expression study as a reference between two groups of cirrhotic livers with HCV-high (n=7) and HCV-low (n=3) viral loads. Gene expression in the 20 human liver tissues was analyzed using the Human Genome U133 Plus 2.0 array, which contains 54,675 probe sets (Affymetrix). Gene expression call (present/absent) was calculated from raw data with GeneChip Operating Software 1.0 (GCOS, Affymetrix). Then, we compared the gene expression profiles statistically with Gene Spring version 7 (Agilent Technologies, Santa

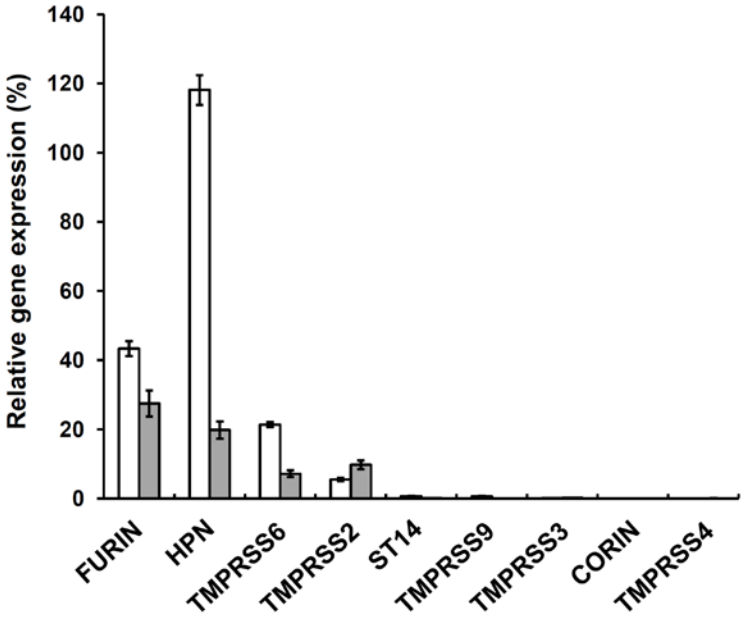
Clara, CA). At first, the output data were normalized per chip and per gene, and then, all 54,675 probes were filtered with at least one present flag in 10 samples each. We obtained 29,070 and 29,664 probes as targets for comparison, respectively. After filtering probes that were significantly differentially expressed by more than 2 fold between the two groups (P<0.05 by Student's T test, Welch's T test and the parametric analysis based on cross-gene error model), we obtained 130 genes as candidate differentially expressed genes (Supporting Table 3); 87 and 44 genes were from each comparison, and one gene, OASL, overlapped. After validation of the representative probes using qPCR in 9-to-8 comparisons of chronic hepatitis livers without liver cirrhosis, 26 genes were identified as up-regulated in the HCV-high group of chronic hepatitis (Supporting Table 5). The qPCR of gene expression was normalized against 18S rRNA, which was the most consistently expressed gene in all patients' samples**,** compared to GAPDH and ribosomal protein L34 (RPL34) mRNAs. One half of the genes are ISGs, or related to the immune response, inflammation and EMT. Five noncoding RNAs of unknown function were also present in the up-regulated genes in the HCV-high group (Supporting Table 5). CH, chronic hepatitis without liver cirrhosis; LC, liver cirrhosis.



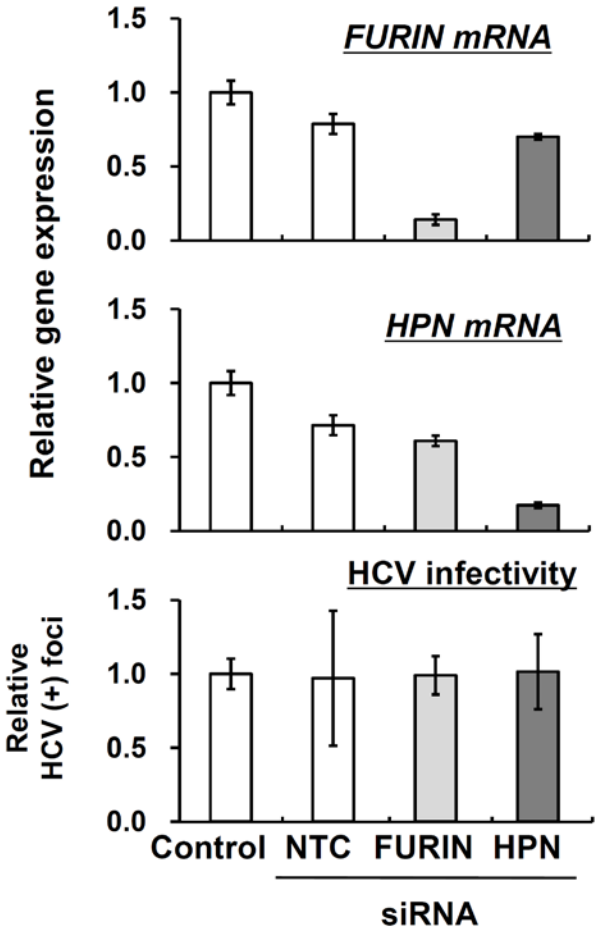
Supporting Fig. 2. Assessment of the inhibition of trypsin by SBTI using synthetic peptides as substrates. The reaction mixture of 100 μM peptide substrate, 10 ng/mL of trypsin and various concentrations (0, 0.1, 1, 10ng/mL) of SBTI in 200 μL Opti-MEM were prepared in 96-well black plates. Immediately after the trypsin was added, the fluorescence released was measured every 15 minutes for 3 hours at 28ºC. The enzyme activity was linearly increased in a time-dependent manner and in a dose-dependent manner in the previous experiment (data not shown). The fluorescence was released for 60 minutes. Trypsin targeted five peptides except for RVRR, and SBTI inhibited all cleavage activity in a dose-dependent manner. The inhibition rate was slightly variable among substrate peptides.



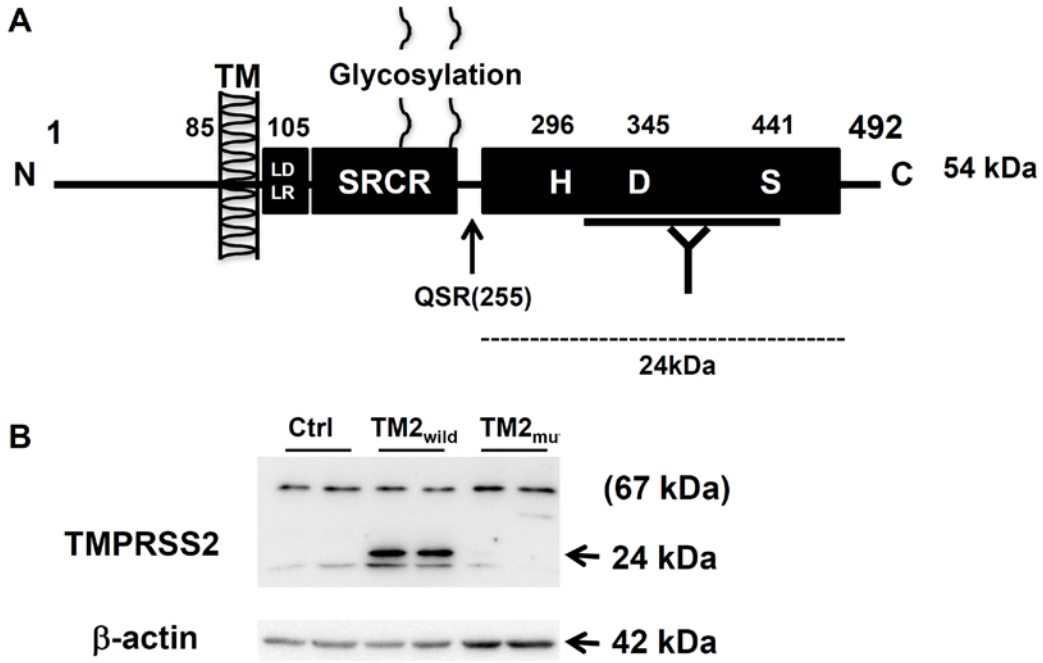
Supporting Fig. 3. Infectivity of various HCVcc in the presence of SBTI. Huh7.5.1 cells ($4x10^4$) pre-cultured for 1 day, were inoculated with 300 focus-forming units of various HCVcc in the presence of 1 mg/mL SBTI. After 2 hours, the inoculum was removed and the cells were cultured in fresh complete medium for 2 days. The HCV infectivity was determined as described in Fig. 2, and the control experiment in the absence of SBTI was expressed as 1.0. The experiment was performed in triplicate, and the result is expressed as the mean ± SD. J6/JFH1[9], S310/JFH1[10] and TH/JFH1[11] were chimeric, JFH-1 HCVcc-containing structural genes of genotype 2a, 3a and 1b, respectively. * and **, $P<0.05$ and $P<0.01$ by the Student's t-test, respectively.



Supporting Fig. 4. mRNA expression of transmembrane serine proteases in human liver tissues with and without HCV infection. The mRNA levels of nine transmembrane serine proteases were quantified by qPCR. The expression was normalized to GAPDH expression by a relative quantitation method. The relative gene expression was expressed as a percentage ratio of the expression of the target gene to that of GAPDH. Open bar, average of 4 samples of HCV-negative normal livers from patients with hepatic metastasis of colon cancer; closed bar, average of 4 samples of chronic hepatitis livers without liver cirrhosis containing HCV-high viral load; error bar, SE.



Supporting Fig. 5. HCV infectivity after knockdown of FURIN and HPN with siRNA. (Upper and middle panels) FURIN and HPN mRNA were quantified by qPCR 2 days after transfection of 20 nM of siRNA (ON-TARGETplus SMARTpool, Dharmacon). The gene expression was normalized to GAPDH expression as described in Fig. 4A. The relative gene expression was expressed as a ratio to the control experiment. (Lower panel) The cells**,** 2 days post-transfection with siRNA**,** were inoculated with HCVcc for 2 hours. Then, the HCV infectivity was determined as described in Fig. 2, and the relative infectivity is expressed as a ratio to the control experiment. The results of two experiments are expressed as the mean ± SD. Control, no transfection; NTC, non-targeting control siRNA.



Supporting Fig. 6. Immunoblotting of TMPRSS2 produced in TMPRSS2-wild type and TMPRSS2-mutant cell clones. (A) Structure of TMPRSS2 protein and the recognition site of the anti-TMPRSS2 antibody used. TMPRSS2 is a type II transmembrane serine protease and is thought to be activated by auto-cleavage at the C terminus of R255. QSR is the amino acid sequence of positions 3, 2 and 1 at the cleavage site. The antibody recognizes the C terminal active site (catalytic triad HDS) of trypsin-like serine protease. TM, transmembrane domain from aa 85 to aa 105; LDLR, low density lipoprotein receptor class A domain; SRCR, scavenger receptor cysteine-rich domain. (B) Immunoblotting of TMPRSS2. Fifty micrograms of protein extracted from the cells was subjected to sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis and blotted onto a polyvinylidene fluoride membrane. A protein band of 24 kDa was detected specifically in TMPRSS2-wild type clone cells, but not in TMPRSS2-mutant clone cells with 0.1 μg/ml of anti-TMPRSS2 rabbit IgG (ATLAS Antibodies,

Stockholm, Sweden) followed by 0.04 μg/ml of horse radish peroxidase-conjugated anti-rabbit IgG goat IgG Fab' (Immuno-Biological Laboratories, Gunma, Japan). The chemiluminescence was detected with an ImmunoStar LD reagent (Wako Pure Chemical Industries, Osaka, Japan) and a Light Capture (ATTO, Tokyo, Japan). A band of 67 kDa was nonspecific and TMPRSS2-mutant cells had no specific signal for TMPRSS2. Therefore, S441A is unreactive with the antibody, or non-cleaved TMPRSS2 is unreactive with the antibody. Beta-actin as a loading control was detected with 0.2 μg/ml of anti-β-actin mouse IgG (Abcam, Cambridge, UK), followed by 0.02 μg/ml of horse radish peroxidase-conjugated anti-mouse IgG goat IgG Fab' (Immuno-Biological Laboratories).

References

1. Ichida F, Tsuji T, Omata M, Ichida T, Inoue K, Kamimura T, et al. New Inuyama classification; new criteria for histological assessment of chronic hepatitis. Int Hepatol Commun 1996;6:112-119.
2. Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, et al. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 1992;73 (Pt 3):673-679.
3. Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. J Virol 2001;75:7059-7066.
4. Bigger CB, Guerra B, Brasky KM, Hubbard G, Beard MR, Luxon BA, et al. Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. J Virol 2004;78:13779-13792.
5. Bieche I, Asselah T, Laurendeau I, Vidaud D, Degot C, Paradis V, et al. Molecular profiling of early stage liver fibrosis in patients with chronic hepatitis C virus infection. Virology 2005;332:130-144.
6. Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. Hepatology 2005;42:702-710.
7. De Giorgi V, Monaco A, Worchech A, Tornesello M, Izzo F, Buonaguro L, et al. Gene profiling, biomarkers and pathways characterizing HCV-related hepatocellular carcinoma. J Transl Med 2009;7:85.
8. Rusinova I, Forster S, Yu S, Kannan A, Masse M, Cumming H, et al. Interferome v2.0: an updated database of annotated interferon-regulated genes. Nucleic Acids Res 2013;41:D1040-1046.
9. Date T, Kato T, Kato J, Takahashi H, Morikawa K, Akazawa D, et al. Novel

cell culture-adapted genotype 2a hepatitis C virus infectious clone. J Virol 2012;86:10805-10820.

10. Kim S, Date T, Yokokawa H, Kono T, Aizaki H, Maurel P, et al. Development of hepatitis C virus genotype 3a cell culture system. Hepatology 2014.
11. Takebe Y, Saucedo CJ, Lund G, Uenishi R, Hase S, Tsuchiura T, et al. Antiviral lectins from red and blue-green algae show potent in vitro and in vivo activity against hepatitis C virus. PLoS One 2013;8:e64449.