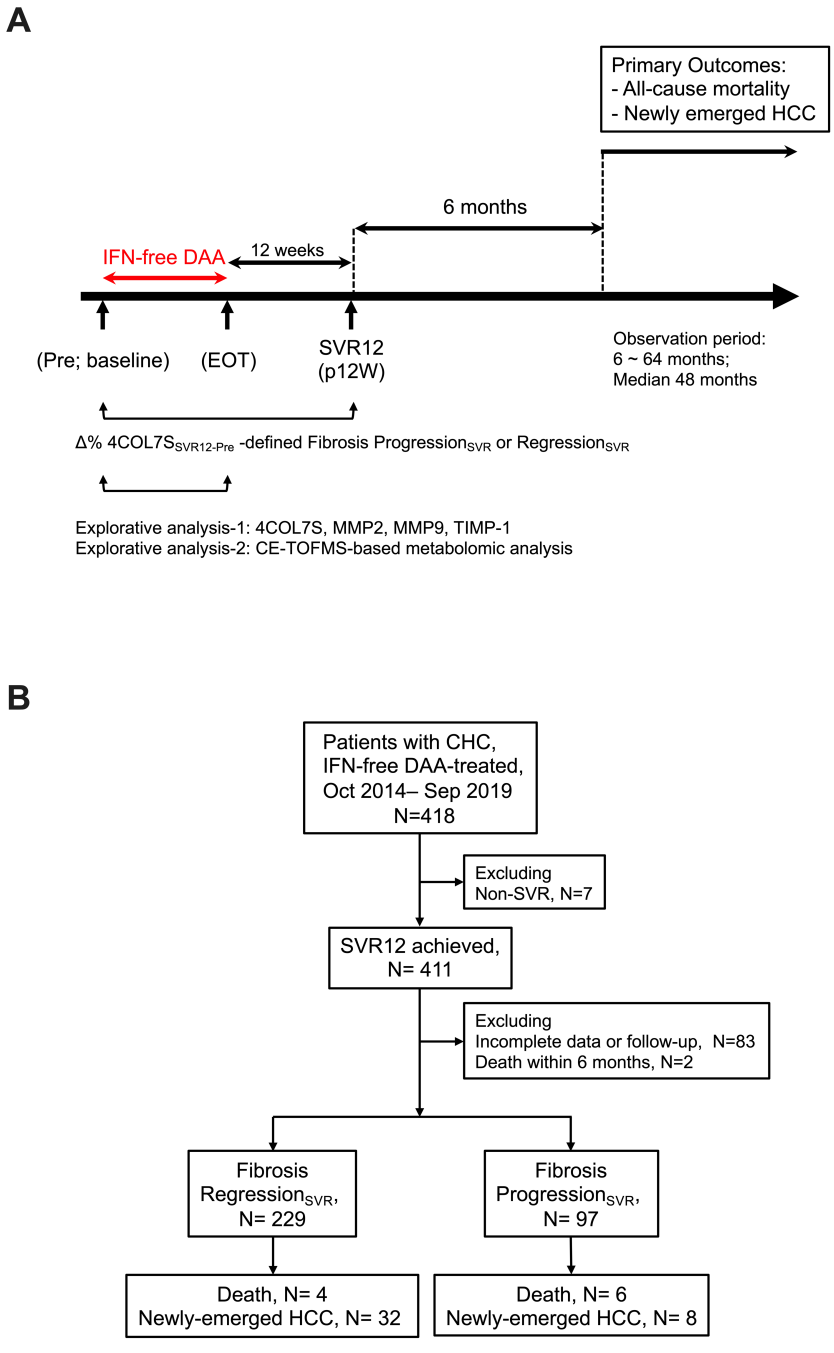
Supporting Information

Supporting Figures and Legends

S1 Fig.

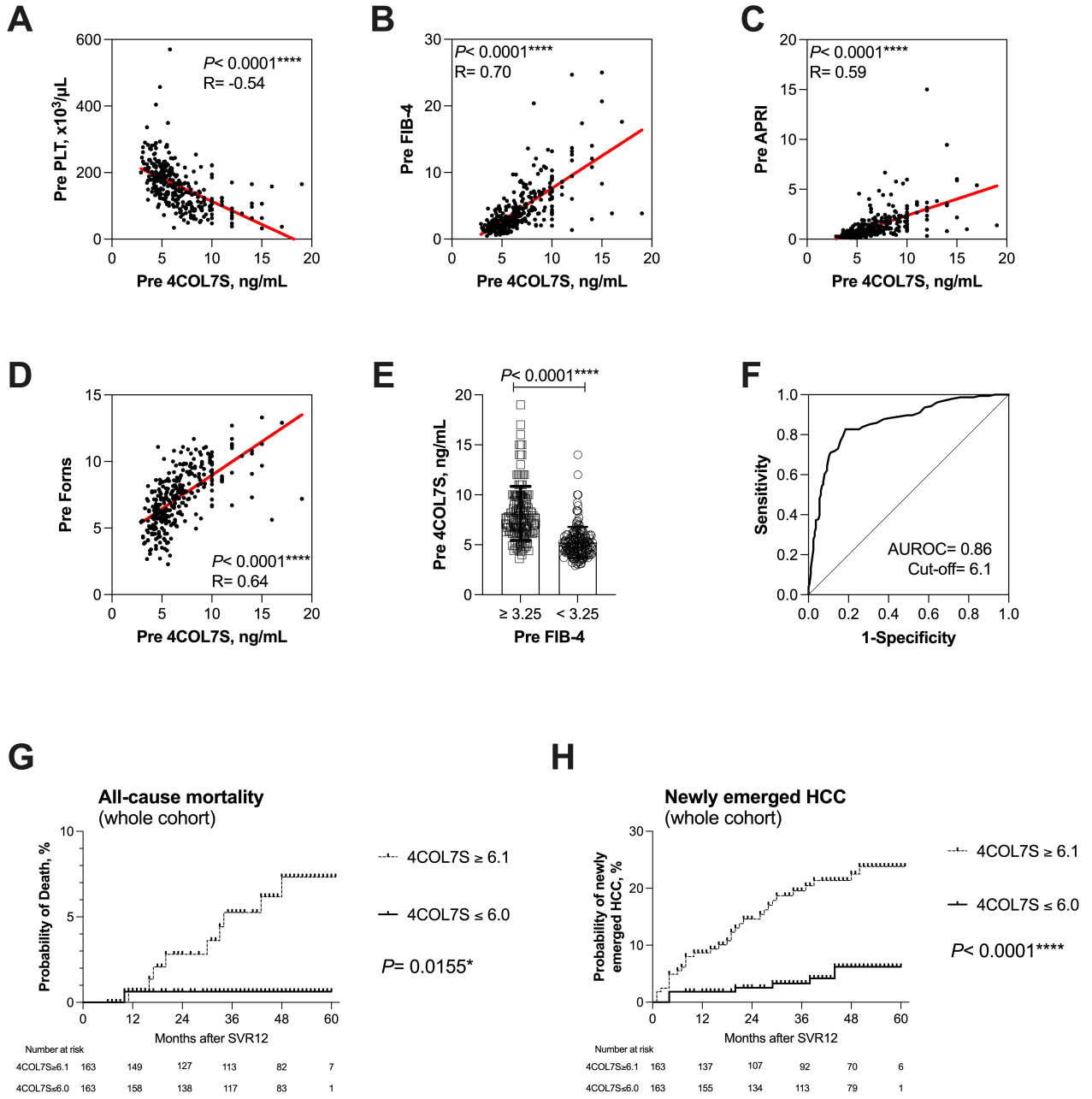


S1 Fig
Yamataka et al.

S1 Fig. Study design, sampling timepoints, and inclusion flow. The red arrow in panel A represents the duration of treatment with IFN-free direct antiviral agents.

Abbreviations: IFN, interferon; DAAs, direct antiviral agents; Pre, pre-treatment; EOT, end of treatment; p12w, 12 weeks after treatment; SVR12, sustained virological response at 12 weeks after the end of treatment; HCC, hepatocellular carcinoma; 4COL7S, type IV collagen 7S fragment; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

S2 Fig.

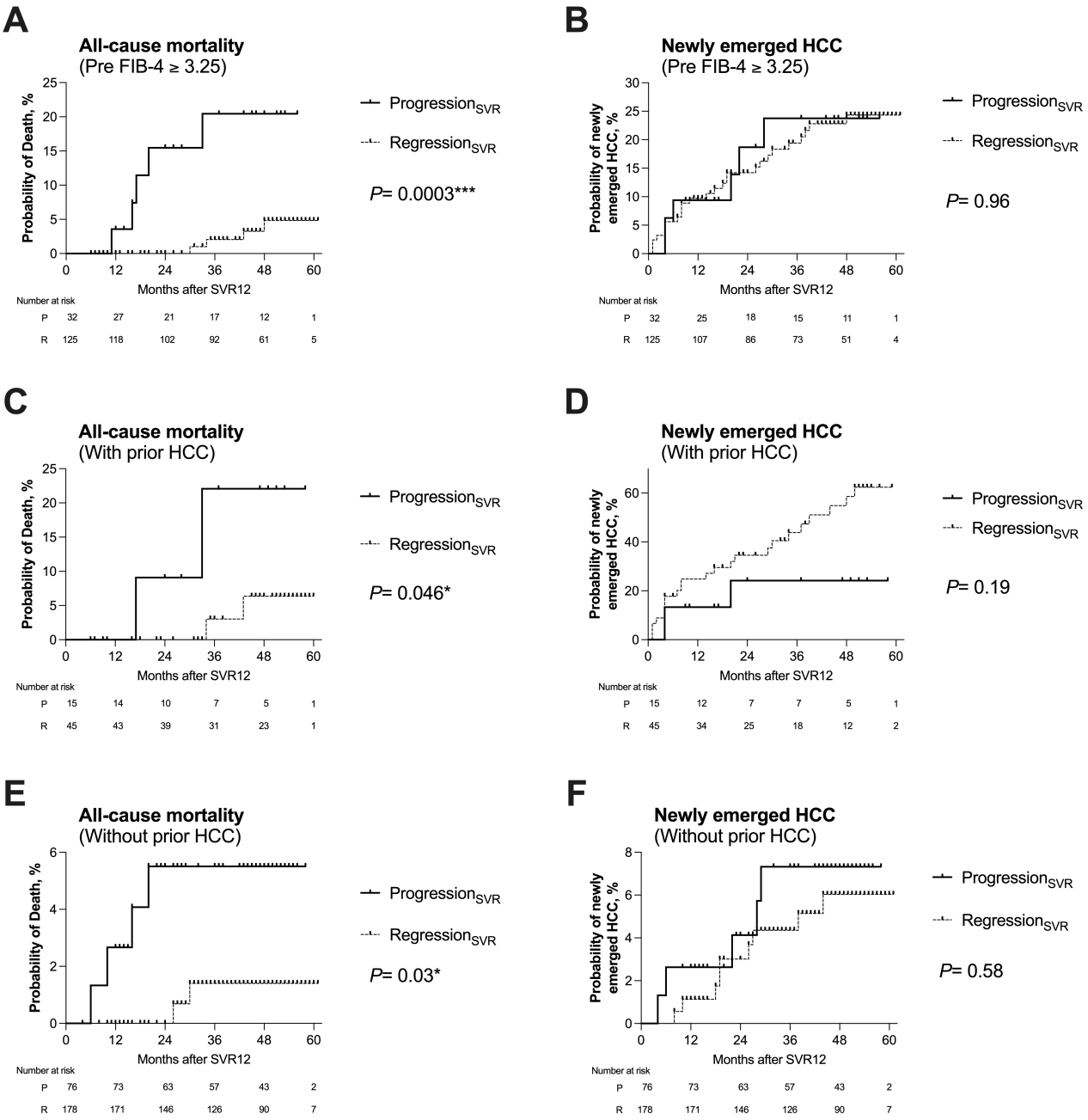


S2 Fig. Correlations between pre-treatment 4COL7S, non-invasive fibrosis indicators and post-SVR clinical outcomes in patients with chronic hepatitis C.

In 326 patients with CHC included in this study, correlations between pre-treatment serum levels of 4COL7S and platelet counts (A), FIB-4 (B), APRI (C), and Forns indices (D) are shown. (E) 4COL7S levels are compared based on stratifying patients with FIB-4 ≥ 3.25, which indicated advanced fibrosis. (F) An ROC analysis for serum levels of 4COL7S

diagnosing FIB-4 $\geq$ 3.25 is shown. A cutoff of 6.1 is yielded from the Youden index. (G, H) Kaplan-Meier analyses of all-cause mortality and new emergence of HCC post-SVR stratified by baseline 4COL7S. Data are presented as means with standard deviations. *P<0.05; ****P <0.0001. Abbreviations: SVR, sustained virological response; 4COL7S, type IV collagen 7S fragment; CHC, chronic hepatitis C; ROC, receiver operating characteristic; AUROC, area under the ROC curve.

S3 Fig.



S3 Fig. Kaplan-Meier analyses of all-cause mortality and new emergence of HCC post-SVR stratified by 4COL7S-defined fibrosis progression$_{SVR}$ and regression$_{SVR}$.

A comparison of 4COL7S-defined fibrosis progression$_{SVR}$ and regression$_{SVR}$, all-cause mortality (A, C, E), and accumulative frequency of newly emerged HCC (B, D, F) are evaluated in SVR12 in patients whose baseline FIB-4 indices $\geq$ 3.25 ng/mL (N= 157; A-B) or in patients with prior HCC (n= 60, C-D) or in those without (n= 254, E-F). *P < 0.05; ***P

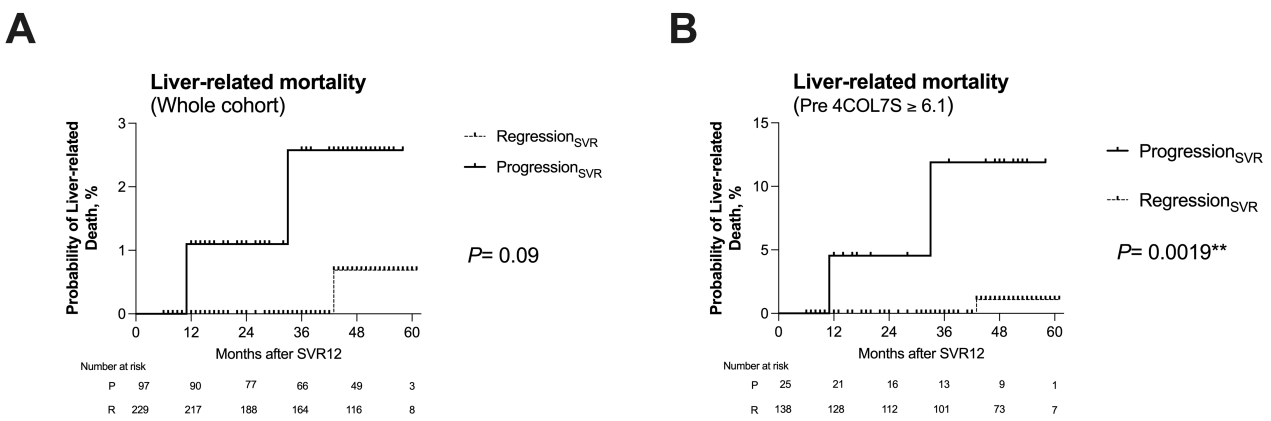
35 <0.001. Abbreviations: 4COL7S, type IV collagen 7S fragment; P, progression$_{SVR}$; R,

36 regression$_{SVR}$; SVR12, sustained virological response at 12 weeks after the end of treatment;

37 HCC, hepatocellular carcinoma

38

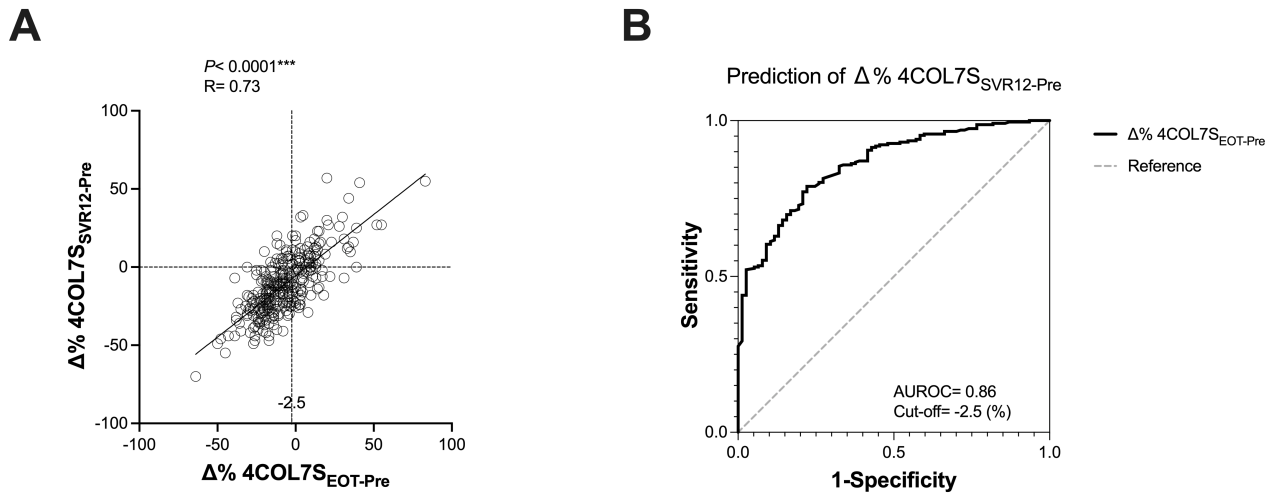
S4 Fig.



S4 Fig. Kaplan-Meier analyses of liver-related mortality post-SVR stratified based on 4COL7S-defined fibrosis progression$_{SVR}$ and regression$_{SVR}$.

A comparison of 4COL7S-defined fibrosis progression$_{SVR}$ and regression$_{SVR}$, liver-related mortality is evaluated from SVR12 in 326 IFN-free DAA-treated patients achieving SVR (whole cohort, A) or in those with baseline serum levels of 4COL7S $\geq$ 6.1 ng/mL (N= 163, B). $^{**}P < 0.01$. Abbreviations: 4COL7S, type IV collagen 7S fragment; P, progression$_{SVR}$; R, regression$_{SVR}$; SVR12, sustained virological response at 12 weeks after the end of treatment; IFN, interferon; DAAs, direct antiviral agents.

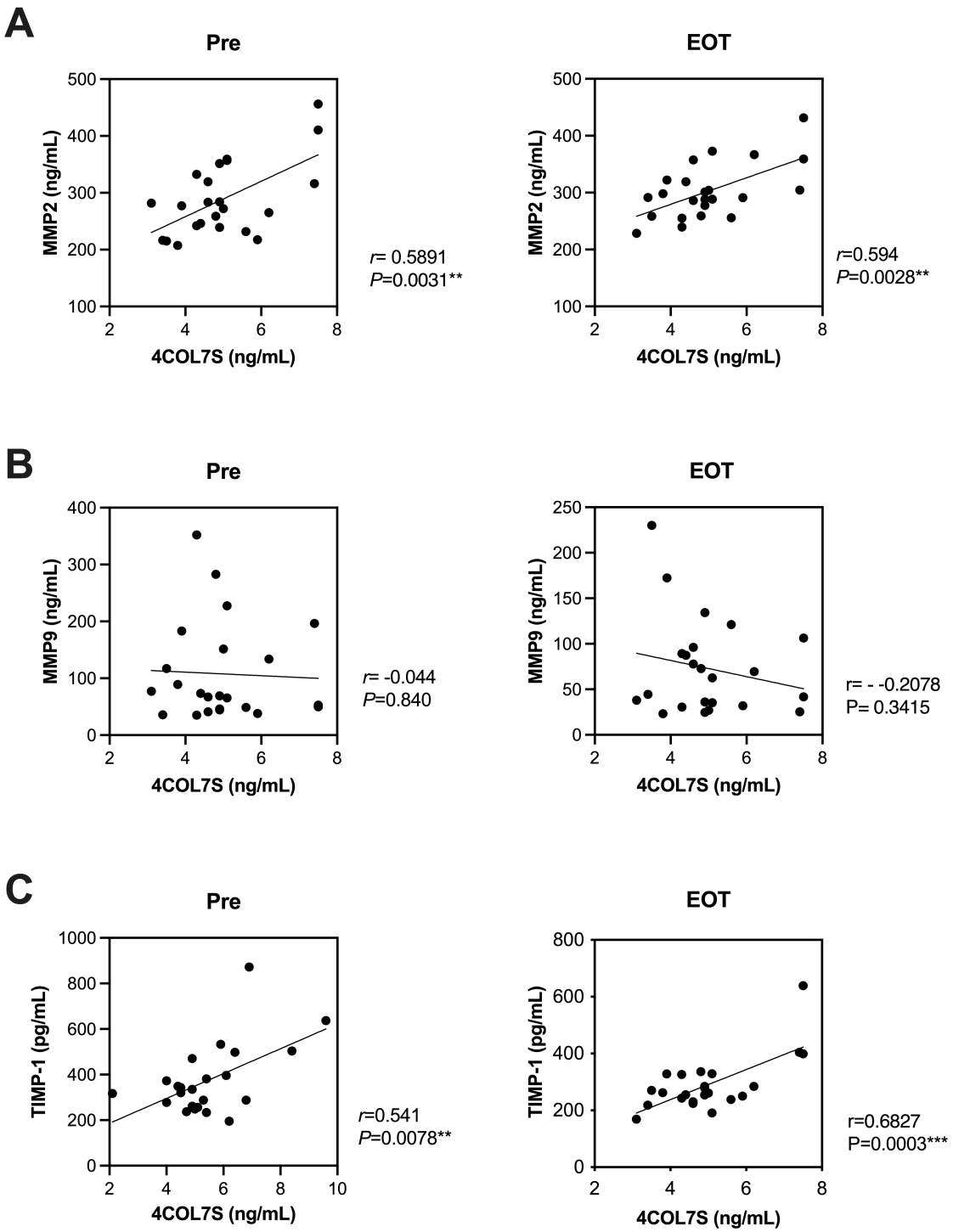
S5 Fig.



S5 Fig. Correlations and diagnostic ability between the change rates of 4COL7S at EOT and at SVR12.

In 326 IFN-free DAA-treated patients achieving SVR, the Spearman correlation analysis between the change rates of serum 4COL7S at EOT (Δ% $4COL7S_{EOT-Pre}$) and at SVR12 (Δ% $4COL7S_{SVR12-Pre}$) is shown (panel A). The diagnostic ability of Δ% $4COL7S_{EOT-Pre}$ for Δ% $4COL7S_{SVR12-Pre}$)≥0 by ROC analysis is shown (panel B). A cutoff of − 2.5% is obtained from the Youden index. Abbreviations: 4COL7S, type IV collagen 7S fragment; EOT, end of treatment; SVR12, sustained virological response at 12 weeks after the end of treatment; IFN, interferon; DAAs, direct antiviral agents; ROC, receiver operating characteristic

62 **S6 Fig.**



63

64 **S6 Fig. Correlations between serum 4COL7S and MMP2, MMP9, and TIMP-1 before**

65 **treatment and at EOT**

66 In the exploratory analysis involving 23 IFN-free DAA-treated patients achieving SVR,

67 correlations between serum 4COL7S and (A) MMP2, (B) MMP9, or (C) TIMP-1, at pre and

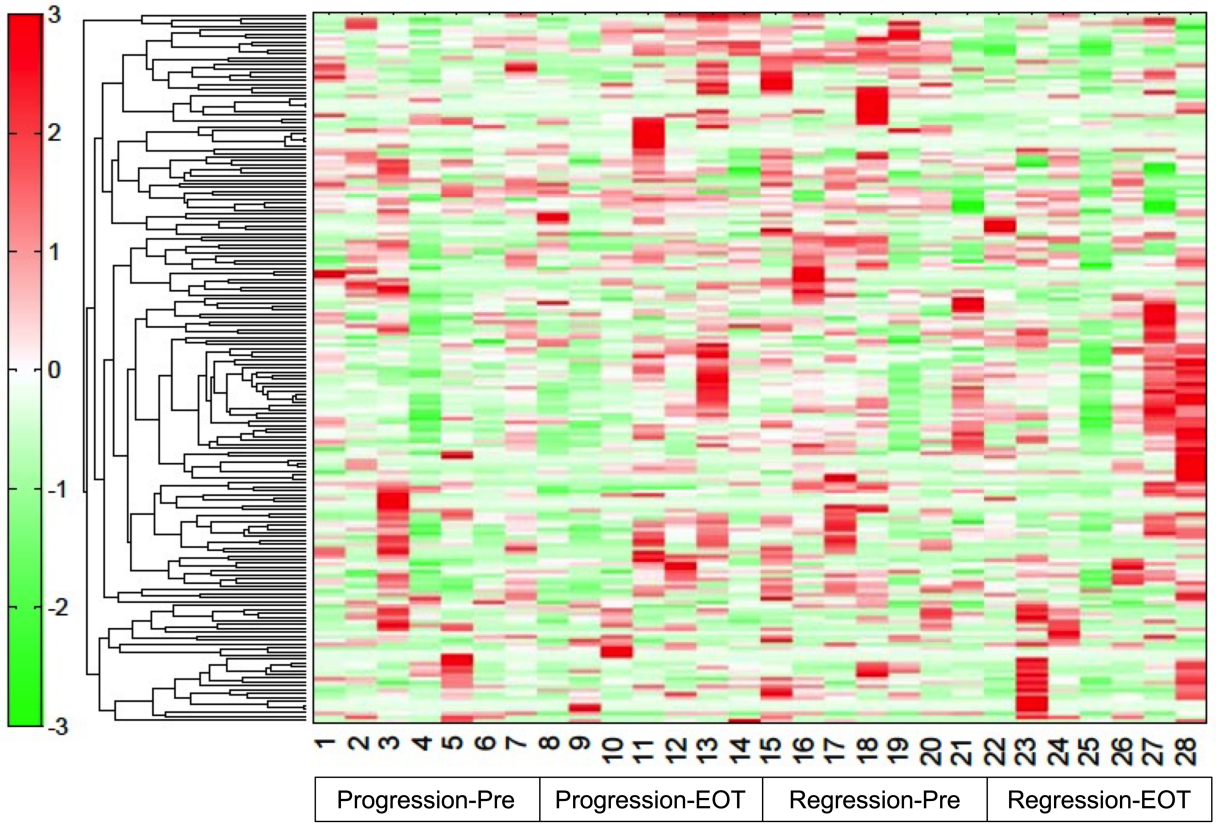
68 EOT, respectively, are shown. **$P < 0.01$; ***$P < 0.001$. Abbreviations: 4COL7S, type IV

69 collagen 7S fragment; EOT, end of treatment; IFN, interferon; DAAs, direct antiviral agents;

70 Pre, pre-treatment.

71

72 **S7 Fig.**



73

74 **S7 Fig. Hierarchical cluster analysis of metabolic patterns in exploratory analysis 2.**

75 Abbreviations: Pre, pre-treatment; EOT, end of treatment.

76

Supporting Tables S1-S4

S1 Table. Clinical features of censored deaths during observation

	Sex	Age	Months to death (from SVR12)	Prior HCC	Baseline Platelet, $\times 10^4/\mu L$	Pre 4COL7S, ng/mL	SVR12 4COL7S, ng/mL	4COL7S-defined P/R	Causes of death
1	M	91	34	Y	10.3	6.1	5.6	R	Cholangiocarcinoma
2	F	81	17	Y	6.7	6.9	7.5	P	Malignant Myeloma
3	M	53	11	N	9.0	8.3	7.5	P	HCC
4	M	57	30	N	7.4	10	8.9	R	CPA
5	F	79	20	N	13.9	6.3	7.1	P	Gastric cancer
6	F	79	43	Y	8.0	12	11	R	HCC
7	M	65	33	Y	6.4	7.9	12	P	HCC
8	F	78	48	N	9.2	8	8.4	R	Thyroid cancer
9	F	78	16	N	8.9	10	11	P	Interstitial pneumonitis
10	M	81	10	N	21.1	3.3	3.4	P	CPA

Abbreviations: M, male; F, female; SVR12, sustained virological response at week 12 after end of treatment; Y, yes; N, no; HCC, hepatocellular carcinoma; 4COL7S, type IV collagen 7S fragment; P, progression; R, regression; CPA, cardiopulmonary arrest.

S2 Table. Background clinical parameters stratified based on their fibrosis dynamics.

Baseline parameters	Regression$_{SVR}$†	Progression$_{SVR}$†	P
N	229	97	-
Sex (M/F), N (%)	82 (35.8) / 147 (64.2)	36 (37.1) / 61 (62.9)	0.90
Age, years	70.4± 12.0	70.3± 12.2	0.92
SOF-based DAAs, N (%)	147 (64.2)	62 (63.9)	1.00
Prior history of HCC, N (%)	45 (20.2)	15 (16.5)	0.53
Liver transplanted, N (%)	8 (3.5)	3 (3.1)	1.00
4COL7S, ng/mL	7.3± 2.6	5.2± 1.9	<0.0001****
AST, IU/L	59.9± 44.5	36.5± 19.7	<0.0001****
ALT, IU/L	57.8± 47.5	35.1± 28.2	<0.0001****
γGTP, IU/L	34 [23-59.5]	24 [16-38]	<0.0001****
Platelet, $\times 10^4$ /μL	15.2± 7.0	17.9± 5.9	0.0013**
FIB-4 index	5.30± 7.53	3.10± 2.58	0.0069**
APRI	1.75± 4.20	0.73± 0.68	0.0221*
Forns index	7.59± 2.21	6.59± 1.90	0.0002***

Data are shown as mean ±SD or median with interquartile range in brackets, as appropriate.

† 4COL7S-defined fibrosis progression$_{SVR}$ or regression$_{SVR}$: a change rate of serum 4COL7S from pre-treatment to SVR12≥ 0% is defined as fibrosis progression$_{SVR}$; and < 0% is defined as fibrosis regression$_{SVR}$.

Abbreviations: SOF, sofosbuvir; DAA, direct antiviral agents; HCC, hepatocellular carcinoma; 4COL7S, type IV collagen 7S fragment; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase.

S3 Table. Background parameters and clinical outcomes of study participants in exploratory analysis 1.

Parameters	Exploratory analysis 1
N	23
Background parameters	
Sex (M/F), N (%)	7 (30.4) / 16 (69.6)
Age, years	70 ± 8
SOF-based DAA, N (%)	18 (78.3)
Prior history of HCC, N (%)	3 (13.0)
Liver transplanted, N (%)	1 (4.3)
Non-invasive fibrosis parameters	
Pre 4COL7S, ng/mL	5.99 ± 1.5
EOT 4COL7S, ng/mL	5.56 ± 1.7
SVR12 4COL7S, ng/mL	5.20 ± 1.3
Clinical Outcomes	
Newly emerged HCC post SVR12, N (%)	2 (8.6)
All-cause mortality, N (%)	1 (4.3)

Data are shown as mean ±standard deviation.

Abbreviations: M, male; F, female; SOF, sofosbuvir; DAAs, direct antiviral agents; Pre, pre-treatment; 4COL7S, type IV collagen 7S fragment; EOT, end of treatment; HCC, hepatocellular carcinoma; SVR12, sustained virological response at week 12 after the end of treatment.

S4 Table. Detailed background parameters of study subject for exploratory analysis 2

Progression/ Regression†	Sex	Age, years	SOF-based	Prior HCC	Survival	Post-SVR HCC	Pre FIB-4	Pre eGFR§	F/U, months	Pre 4COL7S	EOT 4COL7S
Progression	M	80	Y	N	Y	N	1.77	53	27	4.7	5
Progression	F	82	Y	Y	Y	N	3.29	48	51	5.6	6.1
Progression	F	53	N	N	Y	N	1.87	87	45	4.2	4.8
Progression	F	40	Y	N	Y	N	1.20	67	50	4	4.6
Progression	F	48	Y	N	Y	N	1.18	54	54	4.2	4.4
Progression	F	74	Y	N	Y	N	2.25	63	48	4.2	5.6
Progression	F	82	Y	Y	Y	Y	6.78	54	28	7.0	7.6
Regression	F	60	Y	N	Y	N	2.15	82	32	8.4	7.4
Regression	F	60	Y	N	Y	N	4.98	75	15	6.4	4.9
Regression	F	74	Y	N	Y	N	4.42	67	52	6.3	6
Regression	M	67	Y	N	Y	N	2.70	59	48	4.7	4.1
Regression	M	73	Y	N	Y	N	1.61	65	52	4.9	4.3
Regression	F	42	Y	N	Y	N	1.35	70	49	5.3	4.3
Regression	F	56	Y	N	Y	N	3.26	63	49	5.3	4.8
Statistics (P)	1.00	0.56	1.00	0.46	1.00	1.00	0.64	0.20	0.95	0.12	0.60

† 4COL7S-defined fibrosis progression or regression: a change rate of serum 4COL7S from pre-treatment to EOT≥ 0% is defined as progression, and < 0% is defined as regression.

§ Unit: mL/min.1.73m^2.

Abbreviations: SOF, sofosbuvir; HCC, hepatocellular carcinoma; 4COL7S, type IV collagen 7S fragment; eGFR, estimated glomerular filtration rate; Pre, pre-treatment; EOT, end of treatment; SVR, sustained virological response.

111 **S5 Table. Datasets used for analysis (excel file)**

112