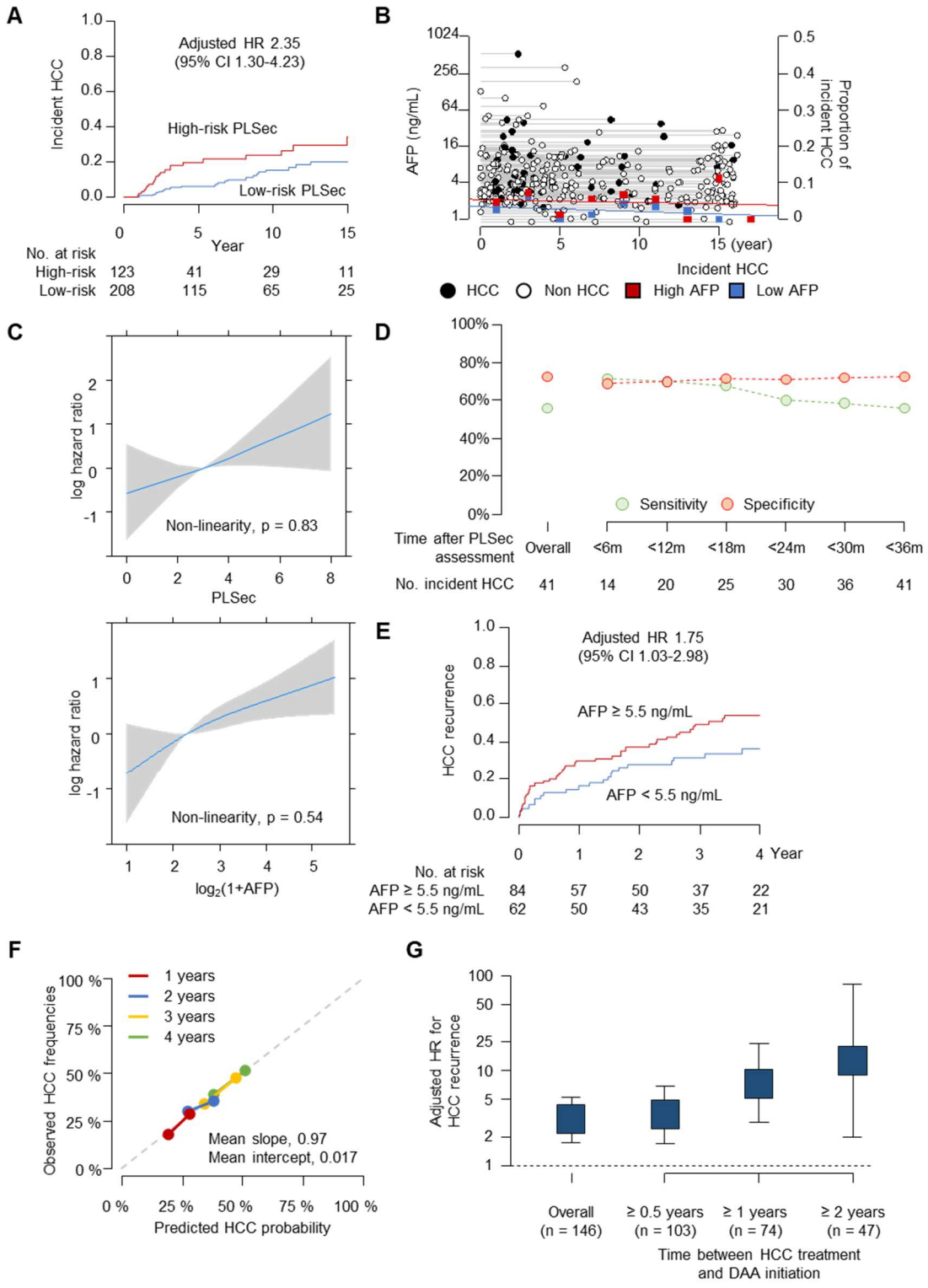



**Figure S1.** Prognostic associations of PLSec-AFP, PLSec, and AFP in validation set 1-3, Related to Figure 2-4.

(A) Association of PLSec with incident HCC in validate set 1. (B) Associations of AFP with time to HCC development in individual patients in validation set 1. Closed and open circles indicate HCC development and censored observation, respectively. Gray horizontal lines indicate duration of clinical follow-up. Red and blue squares indicate proportion of incident HCC every 2 years in patients with high (≥ 5 ng/mL) and low (< 5 ng/mL) AFP at enrollment, respectively. Proportion of HCC incidence was consistent over time in both AFP-high and low groups and not associated with AFP levels ($p = 0.76$ and 0.46 by Pearson correlation test, respectively). (C) Log-transformed hazard ratio of incident HCC related to PLSec (upper panel) and AFP (lower panel) in validation set 1. Gray shadows indicate 95% confidence interval. (D) Sensitivity and specificity of high-risk PLSec-AFP were stable over time in validation set 2. (E) Association of high AFP with HCC recurrence. High AFP was defined as ≥ 5.5 ng/mL, a cut-off frequently used in the context of post-SVR HCC risk prediction in validation set 3. (F) Calibration plot of high AFP at various time points in validation set 3. (G) Association of high-risk PLSec-AFP with HCC recurrence according to time between HCC treatment and DAA initiation in validation set 3.

PLSec, prognostic liver secretome signature; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; DAA, direct-acting antivirals.

**Table S1.** Univariable and multivariable analyses in validation set 1 (multi-etiology cirrhosis cohort), Related to Figure 2.

| | | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| Variable | No. (%) | HR (95% CI) | p value | HR (95% CI) | p value |
| PLSec | | | | | |
| As continuous | – | 1.25 (1.06 – 1.48) | 0.009 | 1.19 (1.01 – 1.41) | 0.04 |
| High risk defined as ≥ 4 | 123 (37%) | 2.33 (1.30 – 4.18) | 0.004 | | |
| Age | – | 1.02 (0.99 – 1.06) | 0.24 | | |
| Sex male | 195 (59%) | 1.31 (0.71 – 2.40) | 0.39 | | |
| Race/ethnicity (vs. black) | | | | | |
| White | 311 (94%) | 0.25 (0.06 – 1.06) | 0.06 | | |
| Hispanic | 8 (2%) | $4.70\times10^{-8}$ (0 – Inf) | 1.00 | | |
| Others | 3 (1%) | 0.94 (0.08 – 10.5) | 0.96 | | |
| Etiology | | | | | |
| HCV | 123 (37%) | 1.38 (0.77 – 2.46) | 0.28 | | |
| HBV | 13 (4%) | 0.39 (0.05 – 2.87) | 0.36 | | |
| ARLD | 60 (18%) | 1.14 (0.55 – 2.37) | 0.72 | | |
| NAFLD | 20 (6%) | 0.87 (0.27 – 2.81) | 0.81 | | |
| Cryptogenic | 39 (12%) | 0.83 (0.30 – 2.31) | 0.71 | | |
| Others | 76 (23%) | 0.77 (0.36 – 1.65) | 0.50 | | |
| Obesity | 140 (42%) | 0.76 (0.42 – 1.38) | 0.37 | | |
| Active hazardous alcohol drinking | 34 (11%) | 0.45 (0.11 – 1.88) | 0.28 | | |
| Diabetes | 76 (23%) | 0.60 (0.27 – 1.35) | 0.22 | | |
| Child-Pugh class B or C (vs. A) | 204 (63%) | 1.51 (0.82 – 2.77) | 0.19 | | |
| FIB-4 ≥ 3.25 (vs. < 3.25) | 225 (69%) | 1.84 (0.93 – 3.64) | 0.08 | | |
| Platelet count ($\times10^3/\mu L$) | | | | | |
| As continuous | – | 0.998 (0.993 – 1.003) | 0.40 | | |
| < $140\times10^3/\mu L$ | 250 (76%) | 1.20 (0.61 – 2.38) | 0.60 | | |
| < $100\times10^3/\mu L$ | 176 (54%) | 1.29 (0.71 – 2.34) | 0.40 | | |
| < $80\times10^3/\mu L$ | 118 (36%) | 1.56 (0.87 – 2.81) | 0.14 | | |
| AST (IU/L) | | | | | |
| As continuous | – | 1.003 (0.999 – 1.008) | 0.11 | | |
| > 40 IU/L | 255 (77%) | 1.52 (0.70 – 3.27) | 0.29 | | |
| > 100 IU/L | 70 (21%) | 1.48 (0.75 – 2.93) | 0.26 | | |
| ALT (IU/L) | | | | | |
| As continuous | – | 1.003 (0.997 – 1.009) | 0.32 | | |
| > 40 IU/L | 216 (65%) | 0.95 (0.52 – 1.76) | 0.88 | | |
| > 100 IU/L | 58 (18%) | 1.48 (0.75 – 2.93) | 0.26 | | |
| AFP | | | | | |
| As continuous | – | 1.004 (1.001 – 1.009) | 0.008 | | |

| | | | | | |
|---|---|---|---|---|---|
| As continuous (log-transformed)* | – | 1.38 (1.14 – 1.67) | <0.001 | 1.38 (1.14 – 1.68) | 0.001 |
| ≥ 5 ng/mL | 130 (40%) | 1.64 (0.90 – 2.96) | 0.10 | | |
| ≥ 10 ng/mL | 62 (19%) | 2.44 (1.31 – 4.57) | 0.005 | | |
| ≥ 20 ng/mL | 22 (7%) | 3.05 (1.35 – 6.87) | 0.007 | | |

*AFP was log-transformed as $\log_2(1+AFP)$.

PLSec, prognostic liver secretome signature; HR, hazard ratio; CI, confidence interval; HCV, hepatitis C virus; HBV, hepatitis B virus; ARLD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; ALT, alanine transaminase; AST, aspartate transaminase; AFP, alpha-fetoprotein.

**Table S2.** Test for proportional hazards assumption in validation set 1, Related to Figure 2.

| Variable | Chi-square | p value |
|---|---|---|
| PLSec-AFP score | <0.01 | 0.95 |
| PLSec-AFP (≥ 1.66) | 1.46 | 0.23 |
| PLSec | 0.58 | 0.45 |
| PLSec (≥ 4) | 1.13 | 0.29 |
| $\log_2$(1+AFP) (as continuous) | 0.84 | 0.36 |
| AFP ≥ 5 ng/mL | 0.27 | 0.60 |

Proportional hazards assumption is tested based on scaled Schoenfeld residuals using cox.zph() function in survival R package.

PLSec, prognostic liver secretome signature; AFP, alpha-fetoprotein.

**Table S3**. Association of high-risk PLSec-AFP with incident HCC after various adjustment, Related to Figure 2-4.

| | Unadjusted | | Model 1 | | Model 2 | | Model 3 | | Model 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cohort | HR or OR | 95% CI | Adjusted HR or OR | 95% CI | Adjusted HR or OR | 95% CI | Adjusted HR or OR | 95% CI | Adjusted HR or OR | 95% CI |
| Validation set 1 (n=331): Cirrhosis with mixed etiology (prospective–retrospective cohort) | | | | | | | | | | |
| Overall | 2.96 | (1.64-5.35) | 3.01 | (1.64-5.51) | 2.90 | (1.61-5.25) | 2.65 | (1.43-4.91) | 2.71 | (1.44-5.12) |
| Validation set 2 (n=41:123): Resolved HCV hapatitis/cirrhosis (nested case-control series) | | | | | | | | | | |
| Overall | 3.85 | (1.70-8.71) | 3.80 | (1.66-8.66) | 3.92 | (1.70-9.03) | 3.91 | (1.70-8.95) | 3.95 | (1.67-9.35) |
| Cirrhosis | 3.15 | (1.31-7.62) | 3.12 | (1.27-7.65) | 3.13 | (1.26-7.74) | 3.22 | (1.31-7.91) | 3.09 | (1.21-7.87) |
| Validation set 3 (n=146): Resolved HCV hepatitis/cirrhosis after HCC therapies (prospective–retrospective cohort) | | | | | | | | | | |
| Overall | 2.90 | (1.78-4.74) | 3.08 | (1.78-5.31) | 2.58 | (1.54-4.34) | 3.03 | (1.76-5.22) | 2.79 | (1.52-5.12) |
| Cirrhosis | 3.00 | (1.74-5.16) | 3.44 | (1.86-6.36) | 2.61 | (1.48-4.63) | 3.10 | (1.73-5.58) | 3.10 | (1.58-6.05) |

PLSec-AFP of ≥ 1.66 was defined as high-risk.

In validation set 2, ORs were adjusted for the following variables in each model with conditioning on the pairs of cases and the matched controls.

Model 1, age (as continuous), sex, obesity, diabetes, and active hazardous alcohol drinking in validation set 1 and 3 and obesity, diabetes, and active hazardous alcohol drinking in validation set 2.

Model 2, Child-Pugh class (A vs. B or C).

Model 3, FIB-4 index (≥3.25 vs.<3.25).

Model 4, All variables.

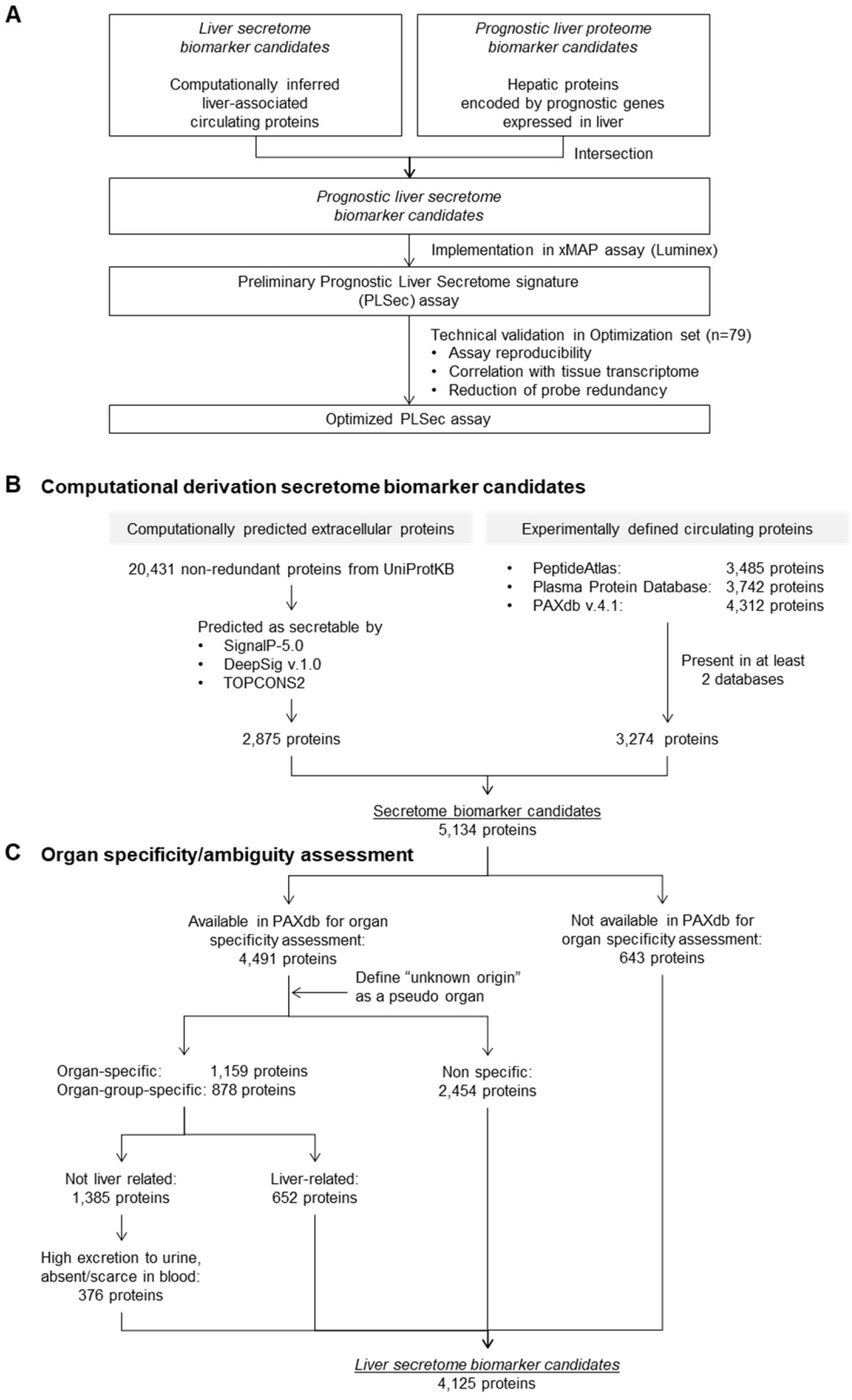
PLSec, prognostic liver secretome signature; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; HR, hazard ratio; OR, odds ratio; CI, confidence interval; HCV, hepatitis C virus.

**Table S4.** Univariable analyses in validation set 2 and 3, Related to Figure 3 and 4.

| | Validaiton set 2* | | Validation set 3 | |
|---|---|---|---|---|
| Variable | No. (%) | OR (95% CI)** | No. (%) | HR (95% CI) |
| Age | - | - | - | 0.99 (0.96 – 1.02) |
| Male sex | - | - | 66 (45%) | 0.76 (0.46 – 1.24) |
| Cirrhosis | - | - | 117 (80%) | 0.98 (0.52 – 1.84) |
| Obesity | 40 (24%) | 0.95 (0.40 – 2.26) | 20 (14%) | 1.14 (0.57 – 2.33) |
| Active hazardous alcohol drinking | 12 (7%) | 2.79 (0.71 – 11.0) | 12 (9%) | 0.55 (0.20 – 1.53) |
| Diabetes | 23 (14%) | 0.71 (0.24 – 2.06) | 25 (18%) | 0.99 (0.52 – 1.90) |
| Child-Pugh class B or C (vs. A) | 144 (88%) | 1.36 (0.47 – 3.90) | 132 (90%) | 2.78 (1.44 – 5.35) |
| FIB-4 ≥ 3.25 (vs. < 3.25) | 113 (69%) | 1.17 (0.48 – 2.84) | 111 (76%) | 1.39 (0.74 – 2.61) |
| Platelet count ($\times 10^3/\mu L$) | | | | |
| As continuous | – | 0.99 (0.93 – 1.06) | - | 0.99 (0.95 – 1.03) |
| < $140\times 10^3/\mu L$ | 103 (63%) | 1.47 (0.65 – 3.36) | 101 (69%) | 0.99 (0.78 – 2.40) |
| < $100\times 10^3/\mu L$ | 60 (37%) | 1.33 (0.64 – 2.80) | 59 (40%) | 1.03 (0.63 – 1.69) |
| < $80\times 10^3/\mu L$ | 40 (24%) | 1.20 (0.52 – 2.75) | 37 (25%) | 1.13 (0.66 – 1.95) |
| ALT (IU/L) | | | | |
| As continuous | – | 1.02 (0.99 – 1.04) | - | 1.01 (0.998 – 1.03) |
| > 25 IU/L | 51 (31%) | 0.81 (0.36 – 1.83) | 31 (21%) | 1.53 (0.88 – 2.68) |
| > 40 IU/L | 13 (8%) | 1.41 (0.38 – 5.22) | 9 (6%) | 1.97 (0.84 – 4.57) |

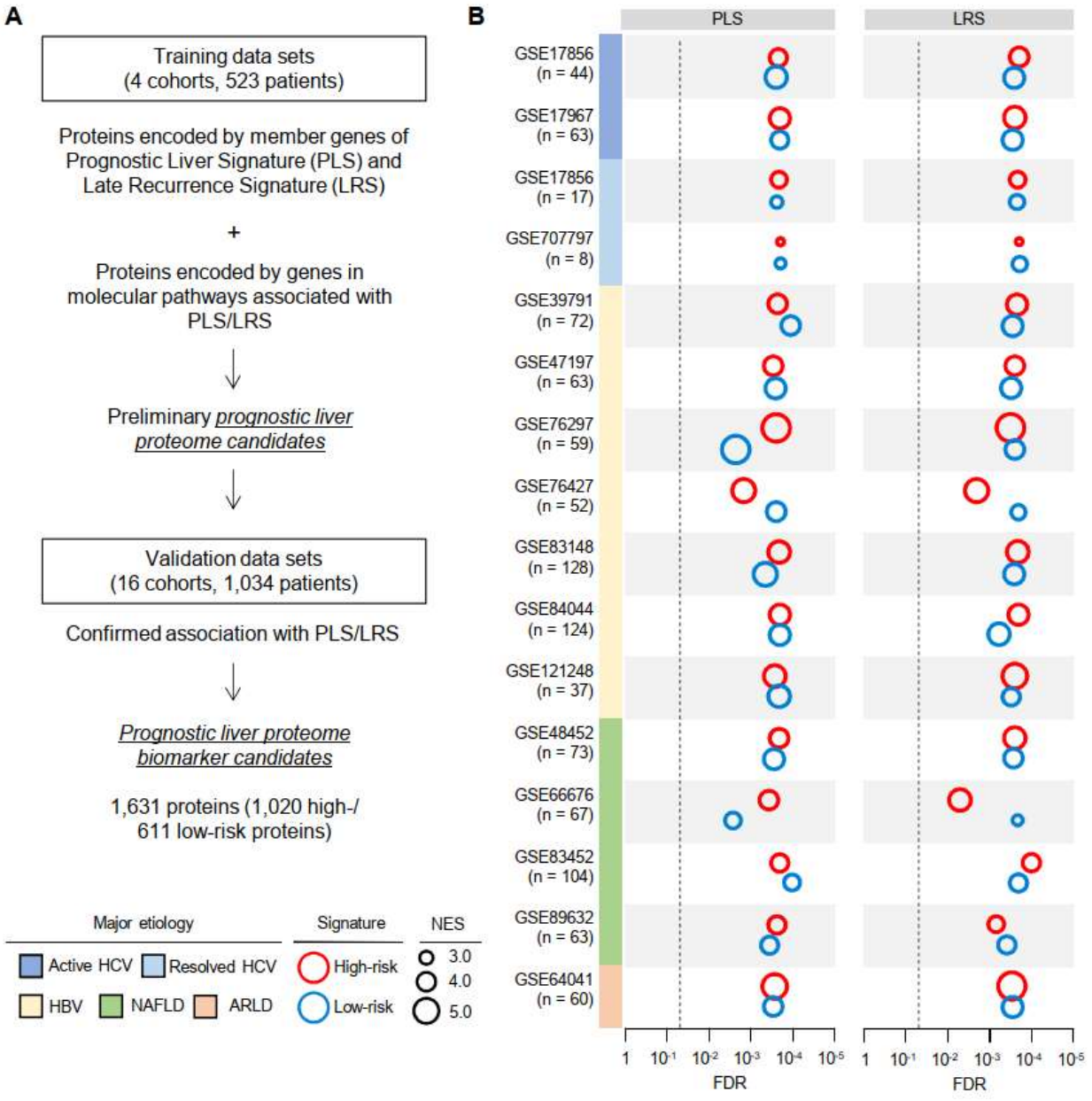
* ORs for age, sex, or cirrhosis were not available because these variables were used for matching.
** In validation set 2, ORs were calculated with conditioning on the pairs of cases and the matched controls.
OR, odds ratio; CI, confidence interval; HR, hazard ratio; FIB-4, fibrosis-4; ALT, alanine transaminase.

**Methods S1.** Workflow of Prognostic Liver Secretome signature (PLSec) derivation, Related to Figure 1 and STAR Methods.



(A) Overviwer of Prognostic Liver Secretome signature (PLSec) derivation (B) Workflow to derive secretome biomarker candidates. (C) Workflow of organ specificity/ambiguity assessment.

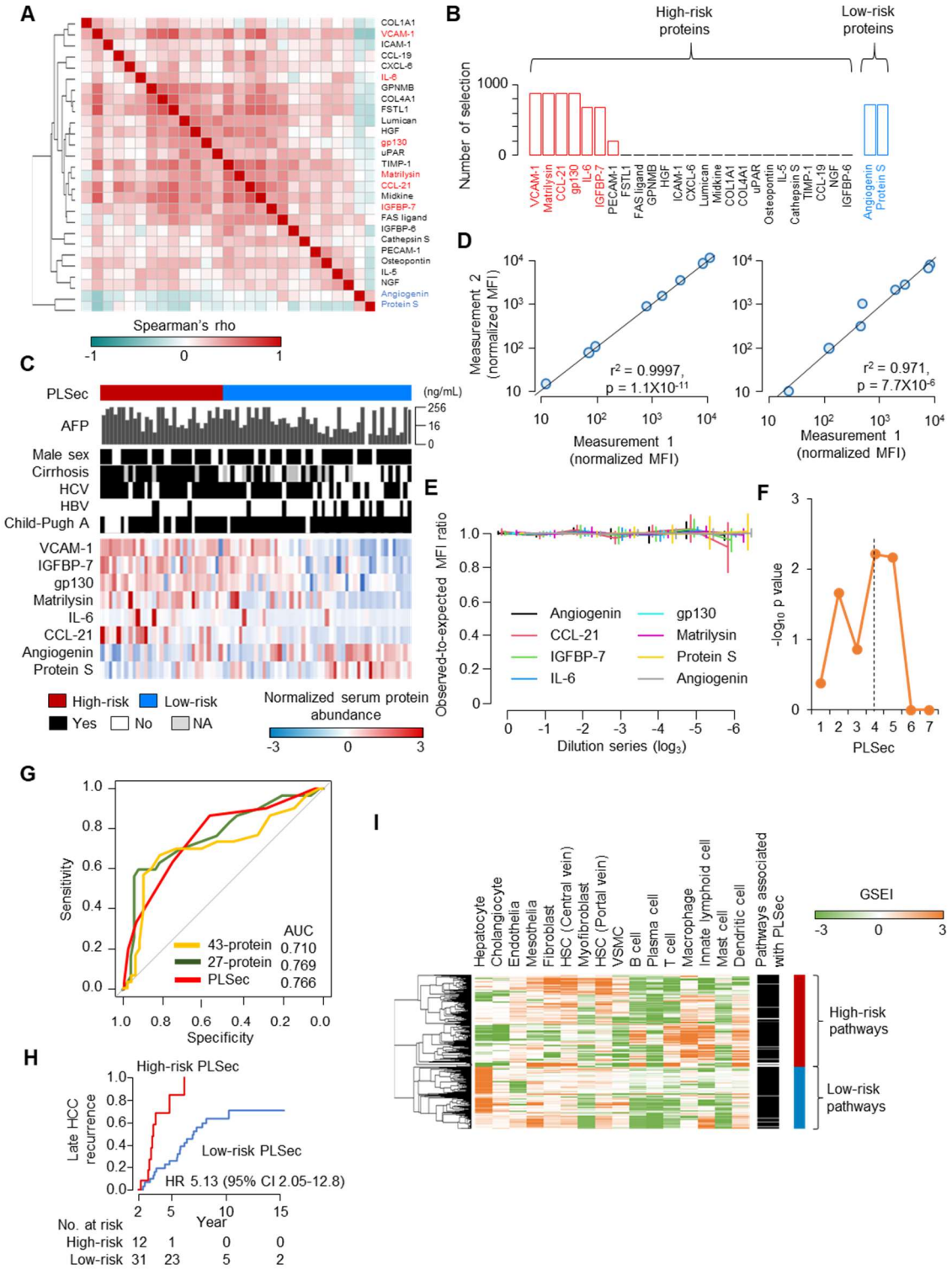
**Methods S2**. Computational derivation of *prognostic liver proteome biomarker candidates*, Related to STAR Methods.




(A) Workflow to derive *prognostic liver proteome biomarker candidates*. (B) Validation of *prognostic liver proteome signature* for association with tissue PLS/LRS status in the validation data sets (Table S1). Vertical dot lines indicate FDR of 0.05.

HCV, hepatitis C virus; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; ARLD, alcohol-related liver disease; PLS, prognostic liver signature; LRS, late recurrence signature; FDR, false discovery rate; NES, normalized enrichment score.

**Methods S3.** Optimization of PLSec protein panel, Related to STAR Methods.




(A) Correlations of abundance across the 27 proteins associated with tissue PLS/LRS status in the optimization set. Red- and blue-colored protein names indicate high- and low-risk proteins included in the final PLSec assay, respectively. (B) Number of selections for each protein as non-

redundant prognostic feature over the 1,000 iterative feature selections by LASSO. (C) Patten of the PLSec protein abundance and associated clinical variables in the optimization set (79 patients with chronic hepatitis or cirrhosis who had curative HCC resection). (D) Within-plate (left panel) and inter-plate/batch (right panel) reproducibility of PLSec between technical replicates. (E) Sensitivity of Luminex assay. Vertical bars indicate 95% confidence interval from 10 batches at each dilution. (F) Prognostic association of PLSec with late recurrence assessed by log-rank test according to PLSec cut-off values in the optimization set. (G) Capability of the 43-protein panel, the 27-protein panel, and the final 8-protein PLSec to predict tissue-transcriptome-signature-based risk classification. (H) Association of PLSec-based prognostic prediction with late HCC recurrence after curative surgical tumor resection. (I) Induction of the 466 PLS/LRS-associated pathways in single-cell genome-wide transcriptome profiles of human cirrhotic livers.

COL1A1, collagen type (I) alpha 1 chain; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; CCL-19, C-C motif chemokine 19; CXCL-6, C-X-C motif chemokine 6; IL-6, interleukin-6; GPNMB, transmembrane glycoprotein NMB; COL4A1, collagen alpha-1(IV) chain; FSTL1, follistatin-related protein 1; HGF, hepatocyte growth factor; uPAR, urokinase plasminogen activator surface receptor; TIMP-1, metalloproteinase inhibitor 1; CCL-21, C-C motif chemokine 21; IGFBP-7, insulin-like growth factor-binding protein 7; IGFBP-6, insulin-like growth factor-binding protein 6; PECAM-1, platelet endothelial cell adhesion molecule; IL-5, interleukin-5; NGF, beta-nerve growth factor; PLSec, prognostic liver secretome signature; AFP, alpha-fetoprotein; HCV, hepatitis C virus; HBV, hepatitis B virus; MFI, median fluorescent intensity; AUC, area under receiver operating characteristic curve; HR, hazard ratio; CI, confidence interval; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; VSMC, vascular smooth muscle cell; GSEI, gene signature enrichment index.

**Methods S4.** Cirrhosis/chronic hepatitis cohorts used for PLSec development, Related to STAR Mehotds.

| Authors | Year | Major etiology | Country | No. patients | Concomitant HCC, No. (%) | Tissue acquisition | Cirrhosis, No. (%) | Male sex, No. (%) | Age, median (IQR) | GEO accession |
|---|---|---|---|---|---|---|---|---|---|---|
| Training data sets | | | | | | | | | | |
| Hoshida *et al*. | 2013 | HCV | Italy | 216 | 0 (0%) | Liver biopsy | 216 (100%) | 116 (53%) | 59 (54-64) | GSE15654 |
| Roessler *et al*. | 2010 | HBV | China | 199 | 199 (100%) | Resection | 185 (93%) | 175 (88%) | 50 (44-58) | GSE14520 |
| Moylan *et al*. | 2014 | NAFLD | USA | 72 | 0 (0%) | Liver biopsy | 32 (44%)* | 25 (35%) | 51 (n.a.) | GSE49541 |
| Trepo et al. | 2017 | ARLD | Belgium | 36 | 0 (0%) | Liver biopsy | 36 (100%) | n.a. | n.a. | GSE94417 |
| Validation data sets | | | | | | | | | | |
| Tsuchiya et al. | 2010 | HCV | Japan | 44 | 44 (100%) | Resection | 22 (50%) | 32 (73%) | 66.4 ± 7.9** | GSE17856 |
| Archer et al. | 2009 | HCV | USA | 63 | 16 (25%) | n.a. | 63 (100%) | 52 (83%) | n.a. | GSE17967 |
| Meissner *et al*. | 2014 | SVR | USA | 17 | 0 (0%) | Liver biopsy | n.a. | n.a. | n.a. | GSE51699 |
| Meissner *et al*. | 2016 | SVR | USA | 8 | 0 (0%) | Liver biopsy | 3 (38%) | 7 (88%) | 55 (52-57) | GSE70779 |
| Kim *et al.* | 2014 | HBV | Korea | 72 | 72 (100%) | Resection | 36 (50%) | 58 (81%) | 57.5 (29-77) | GSE39791 |
| Halgand *et al.* | n.a. | HBV | France | 63 | n.a. | n.a. | n.a. | n.a. | n.a. | GSE47197 |
| Chaisaingmongkol *et al.* | 2017 | HBV | Thailand | 59 | 59 (100%) | Resection | n.a. | n.a. | n.a. | GSE76297 |
| Grinchuk *et al.* | 2018 | HBV/HCV | Singapore | 52 | 52 (100%) | Resection | n.a. | n.a. | n.a. | GSE76427 |
| Zhou *et al.* | 2017 | HBV | China | 128 | 0 (0%) | Liver biopsy | n.a. | n.a. | n.a. | GSE83148 |
| Wang et al. | 2017 | HBV | China | 124 | 0 (0%) | Liver biopsy | 10 (8%) | 88 (71%) | 40 (33-51) | GSE84044 |
| Hui et al. | 2007 | HBV | Singapore | 37 | 37 (100%) | Resection | n.a. | n.a. | n.a. | GSE121248 |
| Ahrens et al. | 2013 | NAFLD | Germany | 73 | 0 (0%) | Liver biopsy | 1 (1%) | 15 (21%) | 45 (38-51) | GSE48452 |
| Xanthakos et al. | 2015 | NAFLD | USA | 67 | 0 (0%) | Liver biopsy | 1 (2%) | 15 (22%) | 17 (15-18) | GSE66676 |
| Arendt et al. | 2015 | NAFLD | Canada | 63 | 0 (0%) | Liver biopsy | 4 (6%) | 27 (43%) | 41 (34-49) | GSE89632 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Francque et al. | 2017 | NASH | France | 104 | 0 (0%) | Liver biopsy | n.a. | 46 (44%) | 47 (35-54) | GSE83452 |
| Makowska et al. | 2016 | ARLD | Switzerland | 60 | 60 (100%) | Liver biopsy | 54 (90%) | 53 (88%) | 64 ± 12** | GSE64041 |

* Number (%) of advamced fibrosis, defined as F3 or F4, was shown.

** Mean ± standard deviation.

HCV; hepatitis C virus; HBV, hepatitis B virus; NAFLD, nonalcoholic fatty liver disease; ARLD, alcohol-related liver disease; SVR, sustained virologic response; IQR, interquartile range.

**Methods S5.** Prognostic Liver Signature (PLSec) protein panel and tested proteins in the optimization set, Related to STAR Mehotds.

| | | | | Correlations with PLS enrichment* | | | Correlations with LRS enrichment* | | |
|---|---|---|---|---|---|---|---|---|---|
| Protein name | Uniprot ID | Gene symbol | Anticipated prognostic association | Spearman's rho | p | FDR | Spearman's rho | p | FDR |
| **Vascular cell adhesion protein 1** | P19320 | *VCAM1* | High-risk | 0.40 | <0.001 | 0.003 | 0.37 | 0.001 | 0.005 |
| **Insulin-like growth factor-binding protein 7** | Q16270 | *IGFBP7* | High-risk | 0.31 | 0.005 | 0.02 | 0.38 | 0.001 | 0.005 |
| **gp130** | P40189 | *IL6ST* | High-risk | 0.24 | 0.04 | 0.09 | 0.21 | 0.06 | 0.14 |
| **Matrilysin** | P09237 | *MMP7* | High-risk | 0.41 | <0.001 | 0.002 | 0.26 | 0.02 | 0.06 |
| **Interleukin-6** | P05231 | *IL6* | High-risk | 0.18 | 0.12 | 0.20 | 0.19 | 0.09 | 0.18 |
| **C-C motif chemokine 21** | O00585 | *CCL21* | High-risk | 0.47 | <0.001 | <0.001 | 0.46 | <0.001 | 0.001 |
| **Protein S** | P07225 | *PROS1* | Low-risk | 0.40 | <0.001 | 0.001 | 0.38 | 0.001 | 0.001 |
| **Angiogenin** | P03950 | *ANG* | Low-risk | 0.21 | 0.06 | 0.06 | 0.25 | 0.03 | 0.03 |
| Follistatin-related protein 1 | Q12841 | *FSTL1* | High-risk | 0.44 | <0.001 | 0.001 | 0.35 | 0.002 | 0.008 |
| Hepatocyte growth factor | P14210 | *HGF* | High-risk | 0.38 | 0.001 | 0.004 | 0.44 | <0.001 | 0.001 |
| Metalloproteinase inhibitor 1 | P01033 | *TIMP1* | High-risk | 0.35 | 0.001 | 0.009 | 0.33 | 0.003 | 0.012 |
| Osteopontin | P10451 | *SPP1* | High-risk | 0.35 | 0.001 | 0.009 | 0.21 | 0.062 | 0.143 |
| Collagen alpha-1 (IV) chain | P02462 | *COL4A1* | High-risk | 0.34 | 0.002 | 0.010 | 0.31 | 0.005 | 0.018 |
| C-C motif chemokine 19 | Q99731 | *CCL19* | High-risk | 0.33 | 0.003 | 0.014 | 0.33 | 0.003 | 0.013 |
| Midkine | P21741 | *MDK* | High-risk | 0.32 | 0.004 | 0.016 | 0.39 | <0.001 | 0.003 |
| Lumican | P51884 | *LUM* | High-risk | 0.29 | 0.009 | 0.028 | 0.38 | 0.001 | 0.004 |
| Intercellular adhesion molecule 1 | P05362 | *ICAM1* | High-risk | 0.27 | 0.015 | 0.043 | 0.08 | 0.470 | 0.622 |
| Transmembrane glycoprotein NMB | Q14956 | *GPNMB* | High-risk | 0.26 | 0.021 | 0.053 | 0.20 | 0.079 | 0.165 |
| Interleukin-5 | P05113 | *IL5* | High-risk | 0.23 | 0.042 | 0.097 | 0.06 | 0.570 | 0.673 |
| Beta-nerve growth factor | P01138 | *NGF* | High-risk | 0.22 | 0.049 | 0.106 | 0.43 | <0.001 | 0.001 |
| Platelet endothelial cell adhesion molecule | P16284 | *PECAM1* | High-risk | 0.19 | 0.089 | 0.175 | 0.05 | 0.643 | 0.733 |
| Collagen alpha-1(I) chain | P02452 | *COL1A1* | High-risk | 0.19 | 0.097 | 0.175 | 0.16 | 0.167 | 0.274 |
| C-X-C motif chemokine 6 | P80162 | *CXCL6* | High-risk | 0.19 | 0.098 | 0.175 | 0.21 | 0.063 | 0.143 |
| Urokinase plasminogen activator surface receptor | Q03405 | *PLAUR* | High-risk | 0.18 | 0.110 | 0.189 | 0.17 | 0.142 | 0.253 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Insulin-like growth factor-binding protein 6 | P24592 | *IGFBP6* | High-risk | 0.16 | 0.161 | 0.245 | 0.03 | 0.803 | 0.844 |
| Granzyme A | P12544 | *GZMA* | High-risk | 0.15 | 0.179 | 0.263 | 0.29 | 0.010 | 0.033 |
| Fatty acid-binding protein, adipocyte | P15090 | *FABP4* | High-risk | 0.13 | 0.253 | 0.357 | 0.16 | 0.151 | 0.258 |
| Pro-epidermal growth factor | P01133 | *EGF* | High-risk | 0.12 | 0.298 | 0.405 | -0.02 | 0.891 | 0.914 |
| Tumor necrosis factor receptor superfamily member 6 | P25445 | *FAS* | High-risk | 0.12 | 0.313 | 0.405 | 0.23 | 0.046 | 0.126 |
| Endoglin | P17813 | *ENG* | High-risk | 0.11 | 0.319 | 0.405 | -0.07 | 0.558 | 0.673 |
| Cathepsin S | P25774 | *CTSS* | High-risk | 0.11 | 0.326 | 0.405 | 0.26 | 0.021 | 0.061 |
| Interferon gamma | P01579 | *IFNG* | High-risk | 0.10 | 0.389 | 0.465 | 0.05 | 0.679 | 0.752 |
| C-C motif chemokine 2 | P13500 | *CCL2* | High-risk | 0.07 | 0.562 | 0.601 | 0.01 | 0.923 | 0.923 |
| Thrombomodulin | P07204 | *THBD* | High-risk | 0.06 | 0.569 | 0.601 | 0.18 | 0.113 | 0.211 |
| Interleukin-2 | P60568 | *IL2* | High-risk | -0.02 | 0.873 | 0.873 | 0.04 | 0.720 | 0.777 |
| Matrix metalloproteinase-9 | P14780 | *MMP9* | High-risk | -0.04 | 0.730 | 0.749 | -0.06 | 0.574 | 0.673 |
| Growth-regulated alpha protein | P09341 | *CXCL1* | High-risk | -0.07 | 0.572 | 0.601 | 0.09 | 0.439 | 0.599 |
| Interleukin-7 | P13232 | *IL7* | High-risk | -0.08 | 0.481 | 0.547 | -0.09 | 0.418 | 0.591 |
| CD44 antigen | P16070 | *CD44* | High-risk | -0.10 | 0.397 | 0.465 | -0.10 | 0.359 | 0.546 |
| Vascular endothelial growth factor C | P49767 | *VEGFC* | High-risk | -0.16 | 0.160 | 0.245 | -0.10 | 0.360 | 0.546 |
| Platelet factor 4 | P02776 | *PF4* | High-risk | -0.19 | 0.093 | 0.175 | -0.10 | 0.391 | 0.573 |
| Platelet-derived growth factor D | Q9GZP0 | *PDGFD* | High-risk | -0.27 | 0.017 | 0.045 | -0.20 | 0.081 | 0.165 |
| Interleukin-18 | Q14116 | *IL18* | High-risk | -0.39 | 0.007 | 0.023 | -0.09 | 0.563 | 0.673 |

Bold proteins are included in the firnal PLSec panel.

* Correlations with signature enrichment with the same risk direction.

PLS, prognostic liver signature; LRS, late recurrence signature; FDR, false discovery rate.

**Methods S6.** Demographics of patients in the optimizaiton set, Related to STAR Mehotds.

| Variable | (n = 79) |
|---|---|
| Age, median (IQR), y | 58 (52-64) |
| Male sex, No. (%) | 62 (78%) |
| Etiology, No. (%): HCV/HBV/other | 58/16/5 (73%/20%/7%) |
| Advanced fibrosis, No. (%) | 58 (91%) |
| AST, median (IQR), IU/L | 42 (25-67) |
| Platelet count, median (IQR), $\times 10^3/\mu L$ | 134 (83-192) |
| AFP, median (IQR), ng/mL | 44 (12-218) |
| Time between HCC resection and serum collection, median (IQR),days | 92 (75-107) |
| Clinical follow-up time, median (IQR), y | 7.5 (3.5-9.7) |
| Tissue-based high-risk PLS, No. (%) | 30 (38%) |

Categorical variables are shown as n (%). Continuous variables are shown as median (IQR).

HCV, hepatitis C virus; HBV, hepatitis B virus; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; PLS, prognostic liver signature; IQR, interquartile range.