

Supplemental Materials for

Myeloid Signature Reveals Immune Contexture and Predicts The Prognosis of Hepatocellular Carcinoma

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Supplemental Materials Inventory

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Supplemental Methods

Patient Information

The primary cohort included 488 patients from the Sun Yat-sen University Cancer Center (SYSUCC) who were randomly divided into a primary training subset ($n = 244$) and a primary test subset ($n = 244$). An independent internal validation cohort (341 patients from the SYSUCC) and an external validation cohort (combined from two separate cohorts, which included 94 and 254 patients from the Taizhou Hospital of Zhejiang Province [THZP] and Fudan University Zhongshan Hospital [FUZH], respectively) were also enrolled. The inclusion criteria used for patient enrollment from the consecutive cohorts were as follows: no anticancer therapies; no diagnosis or history of any other concurrent malignancies; no concurrent autoimmune diseases, HIV or syphilis; and available follow-up data. The exclusion criteria were Child-Pugh C liver function and evidence of hepatic decompensation, which included refractory ascites, esophageal or gastric variceal bleeding, or hepatic encephalopathy. The clinicopathological characteristics of the patients in the four cohorts are summarized in **Supplemental Table 2**.

Curative resection for HCC was performed with the following intraoperative goals: (1) the complete removal of all tumor nodules, (2) a resection margin of at least 1 cm, and (3) a lack of tumor cells on the cut surface based on histological examinations. Intraoperative ultrasound and postsurgical contrast-enhanced computed tomography (CT) were routinely used to verify the complete resection of HCC. Patients were postoperatively followed up with regular surveillance at 2- to 4-month intervals. Serum alpha-fetoprotein (AFP) levels (≤ 25 ng/mL was considered AFP negative), abdominal ultrasonography, and chest radiography were performed for surveillance for recurrence. Further examinations, including CT, hepatic angiography, and biopsies (when necessary), were performed when tumor recurrence or metastasis was suspected. Patients with confirmed recurrence received further treatments, including a second surgical resection, transcatheter arterial chemoembolization, radiofrequency ablation, percutaneous ethanol injection or sorafenib treatment.

A cohort of 51 patients who had recurrent disease after hepatectomy for HCC at SYSUCC between January 2002 and March 2016 were additionally recruited. A daily dose of 800 mg sorafenib was prescribed after the diagnosis of tumor recurrence. Inclusion criteria for this cohort were as follows: (1) histologically confirmed HCC and previous hepatectomy; (2) tissue sections available for IHC staining; (3) recurrent HCC confirmed based on at least two imaging technologies (e.g., hepatic ultrasound together with CT and/or magnetic resonance imaging); (4) complete medical records and precise follow-up data; (5) sorafenib therapy after HCC recurrence; and (6) no second treatment, (e.g., transcatheter arterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection) before confirmed disease progression. Patients who received sorafenib before recurrence and/or metastasis were excluded from the study. Response to sorafenib treatment was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). Therapeutic efficacy after confirmed progression and a second treatment was not taken into account as response to sorafenib.

Specimens and Tissue Microarray (TMA) Construction

Formalin-fixed paraffin-embedded tissue specimens were used to construct TMAs, as previously described (1). Briefly, hematoxylin and eosin-stained slides were reviewed by pathologists who were blinded to the patients' clinical characteristics and outcomes. TMAs were constructed using two separate 1.0-mm tissue cores taken from regions of adjacent liver (≥ 2 cm from the edge of the tumor) and the intratumoral regions (a total of four punches for each specimen). TMAs containing the tissue cores were then cut into 5- μ m sections for IHC staining.

Multiplexed Immunofluorescence Staining

To investigate heterogeneity among myeloid cells in the TME from different MRS groups, we performed multiplexed immunofluorescence staining with a TSA Fluorescence Kit (Panovue, China), as previously described (2). Different primary antibodies were sequentially applied, followed by incubation with a horseradish peroxidase (HRP)-conjugated secondary antibody and tyramide signal amplification (TSA). Details regarding the antibodies used for IHC and immunofluorescence

staining are provided in **Supplemental Table 16**. The slides were microwave heat-treated after each TSA procedure. Nuclei were stained with DAPI after all antigens had been labeled.

Isolation and culture of blood cells and preparation of culture supernatants from hepatoma cells

Human neutrophils and monocytes were isolated from peripheral blood of healthy donors, as previously described (3, 4). The purified neutrophils were cultured for 24 hours in DMEM containing 10% human AB serum, supplemented with 15% culture supernatants from hepatoma cells (TSN) as indicated. For monocyte-derived macrophages, CD14⁺ monocytes were cultured in DMEM containing 10% human AB serum for 7 days to allow differentiation into macrophages. After differentiation, cells were treated with medium or 15% TSN for 3 days. Cell phenotypes were then examined with flow cytometry. Human hepatoma HepG2 cells were obtained from the American Type Culture Collection. QGY-7701 cells were from Shanghai Institute of Biochemistry and Cell Biology. All cells were tested for mycoplasma contamination using a single-step PCR method, and were maintained in DMEM medium supplemented with 10% FBS (10099-141, Gibco, USA). TSN was prepared by plating 5×10^6 tumor cells in 10 mL of complete DMEM medium in 100-mm dishes for 24 hours and thereafter changing the medium to complete medium supplemented with 10% human AB serum. After the incubation, the media were harvested, centrifuged to remove cells, and stored in aliquots at -80°C.

Flow Cytometry

Flow cytometry was performed as previously described (4, 5). Details regarding the antibodies used for flow cytometry are provided in **Supplemental Table 17**. Data were acquired using a Gallios or a CytoFLEX S flow cytometer (Beckman Coulter, USA) and analyzed with FlowJo Software (BD, USA).

Total RNA Isolation and Gene Expression Microarray Assay

RNA samples from 21 HCC tumors (MRS^{low}, $n = 10$; MRS^{high}, $n = 11$; **Supplemental Figure 17A** and **Supplemental Table 11**) were subjected to a gene expression microarray assay. A total of 50–100 mg of tumor tissue was ground with a mortar and pestle in liquid nitrogen and homogenized in TRIzol Reagent (Ambion, USA). Total RNA was extracted according to the manufacturer's instructions and quantified with a Nanodrop Instrument (Thermo Scientific, USA). Total RNA was checked for the RIN to inspect RNA integrity using an Agilent Bioanalyzer 2100 (Agilent Technologies, USA). Qualified total RNA was further purified using a RNeasy mini kit (QIAGEN, Germany) and a RNase-Free DNase Set (QIAGEN, Germany). Total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies, USA), following the manufacturer's instructions. Labeled cRNA was purified with a RNeasy mini kit (QIAGEN, Germany) and hybridized onto Agilent whole human genome oligonucleotide microarrays containing 58341 different oligonucleotide probes (SurePrint G3 Human Gene Expression 8 × 60 K Microarray Kit, Agilent Technologies, USA). Slides were scanned using an Agilent Microarray Scanner (Agilent Technologies, US) with default settings.

Gene Expression and Functional Enrichment Analyses

The gene expression microarray data of 21 HCC samples were pre-processed using the R package *limma* (6). Batch effects removal and data normalization was performed based on the surrogate variables analysis (SVA) method (7). We used the R package *sva* to identify and estimate surrogate variables for known and unknown sources of variation in gene expression microarray experiments and then performed data normalization using the *ExpressionNormalizationWorkflow* package. Differential expression analysis was then performed with the *limma* package. Function enrichment analyses, including the gene ontology and gene set enrichment analyses, were performed using the R package *clusterProfiler* (8). Gene sets used for gene set enrichment analysis are provided in **Supplemental Table 12**.

TCGA-LIHC Data Analysis

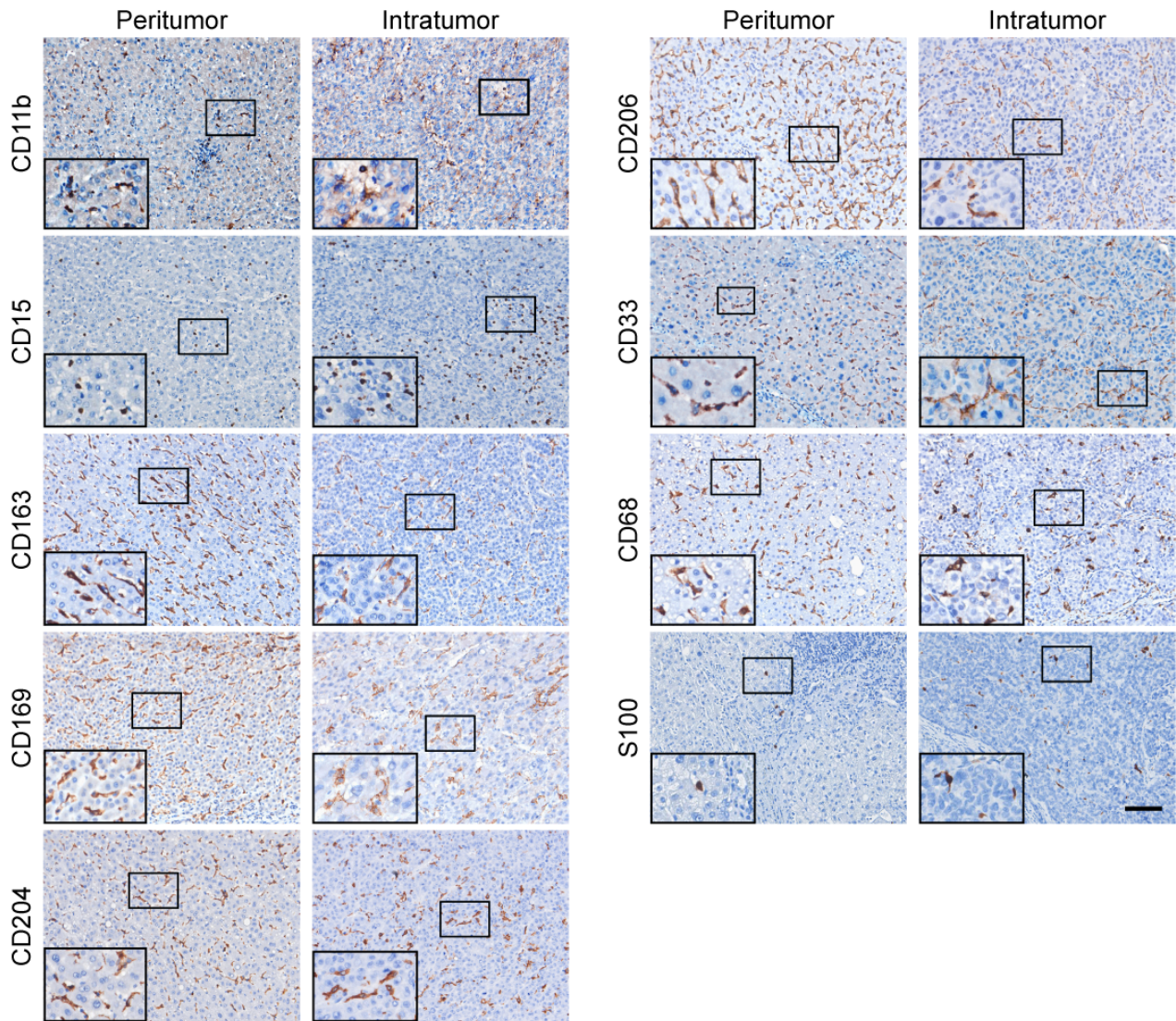
Transcriptome profiling data from the TCGA-LIHC project and corresponding metadata were downloaded (on December 4, 2018), preprocessed, normalized, and filtered using the R package *TCGAbiolinks* (9). Single-sample gene set enrichment analysis was performed using a non-parametric unsupervised method, called gene set variation analysis (GSVA) with the *GSVA* package. GSVA performs a change in coordinate systems, transforming the data from a gene by sample matrix to a gene set by sample matrix, thereby allowing for the evaluation of pathway enrichment for each sample. The *monocle* package and the Monocle 2 algorithm (10) was used to draw a 'climb trajectory' of MRS estimates across the TCGA-LIHC samples. The up-regulated and down-regulated genes identified with gene expression microarrays (**Supplemental Table 11**) served as marker genes that guide the 'semi-supervised' machine learning progress, as Monocle could augment these markers with other related genes. In this way, the 371 HCCs from the TCGA-LIHC project were ordered in 'pseudotime' based on transcriptional similarities identified by the Monocle 2 algorithm (**Figure 7A**).

Nomogram Construction

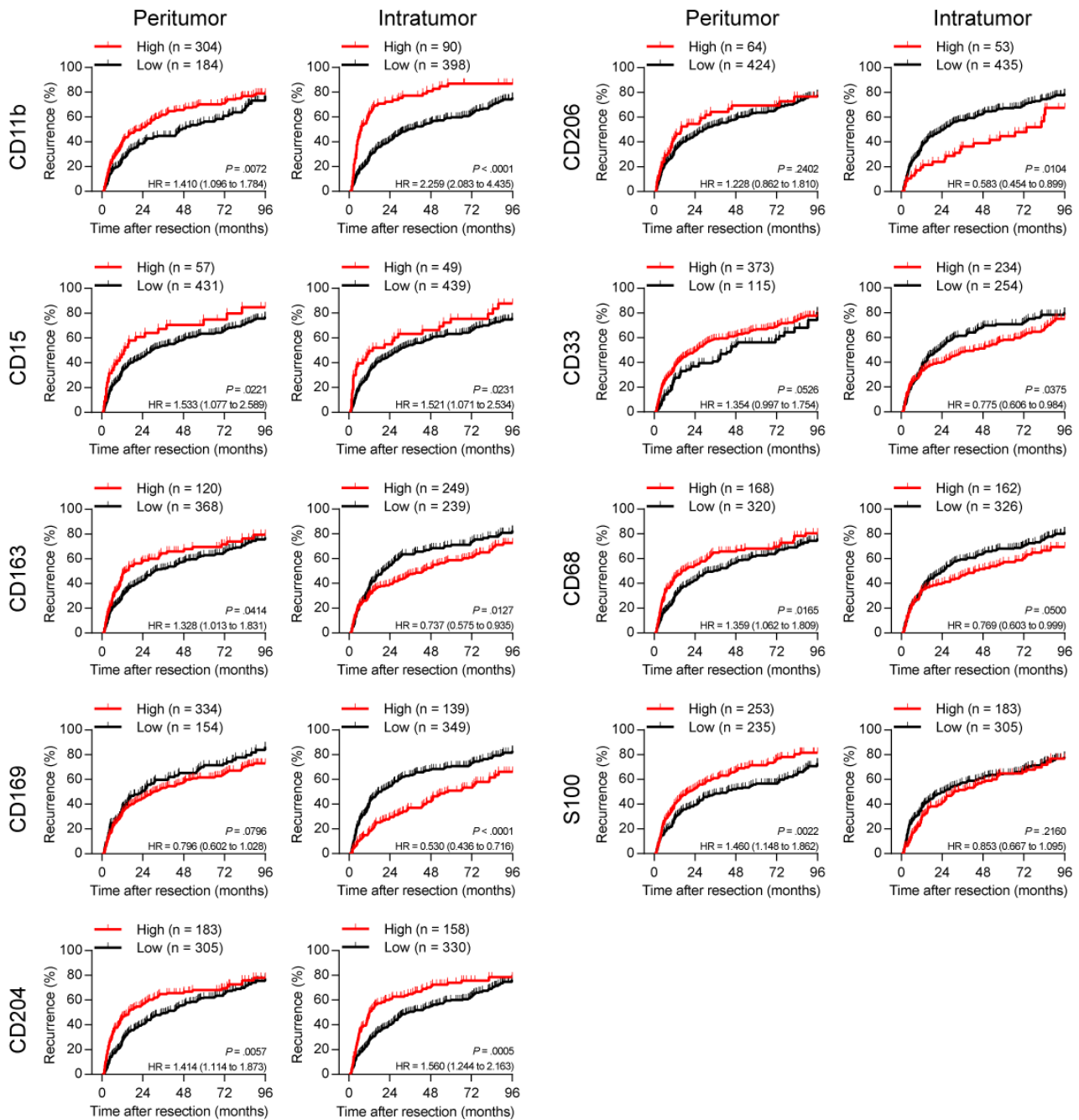
To exemplify the potential use of the MRS in clinical decisions, we constructed two nomograms to predict HCC recurrence and overall patient survival after resection surgery (**Figure 8, A and B**). To do so, we put a panel of prognosis-related parameters into a primary Cox proportional hazards regression model, which includes MRS, age and gender of the patient, level of AFP and ALT in blood, pathological stage, Child–Pugh score, tumor size (diameter), tumor number (singular or multiple), presence of vascular invasion, presence of cirrhosis, presence of tumor capsule, presence of HBV or HCV infection, and the ALBI stage. The fitting model was constructed, examined, and simplified using the R package *rms*. The final fitting model was built on parameters selected using a backward step-down method (with total residual Akaike information criterion as the stopping rule) to identify the variables that remained in the final model. These nomograms were validated in multiple cohorts to obtain unbiased estimates of model performance (discrimination, calibration, and clinical usefulness). The R package *survivalROC* (11) was used for time-dependent receiver

operating characteristic (ROC) analysis. Area under curve (AUC) of the ROC curves were estimated with 1000 × bootstrap resampling for each parameter. Decision curve analysis (DCA) is a method for assessing whether nomogram-assisted decisions may improve patient outcomes (12). DCA estimates clinical usefulness of prediction models on the basis of the threshold probability (i.e., the probability that triggers a medical intervention, equating to the probability at which the harm of a false-positive intervention exceeds the harm of a false-negative non-intervention).

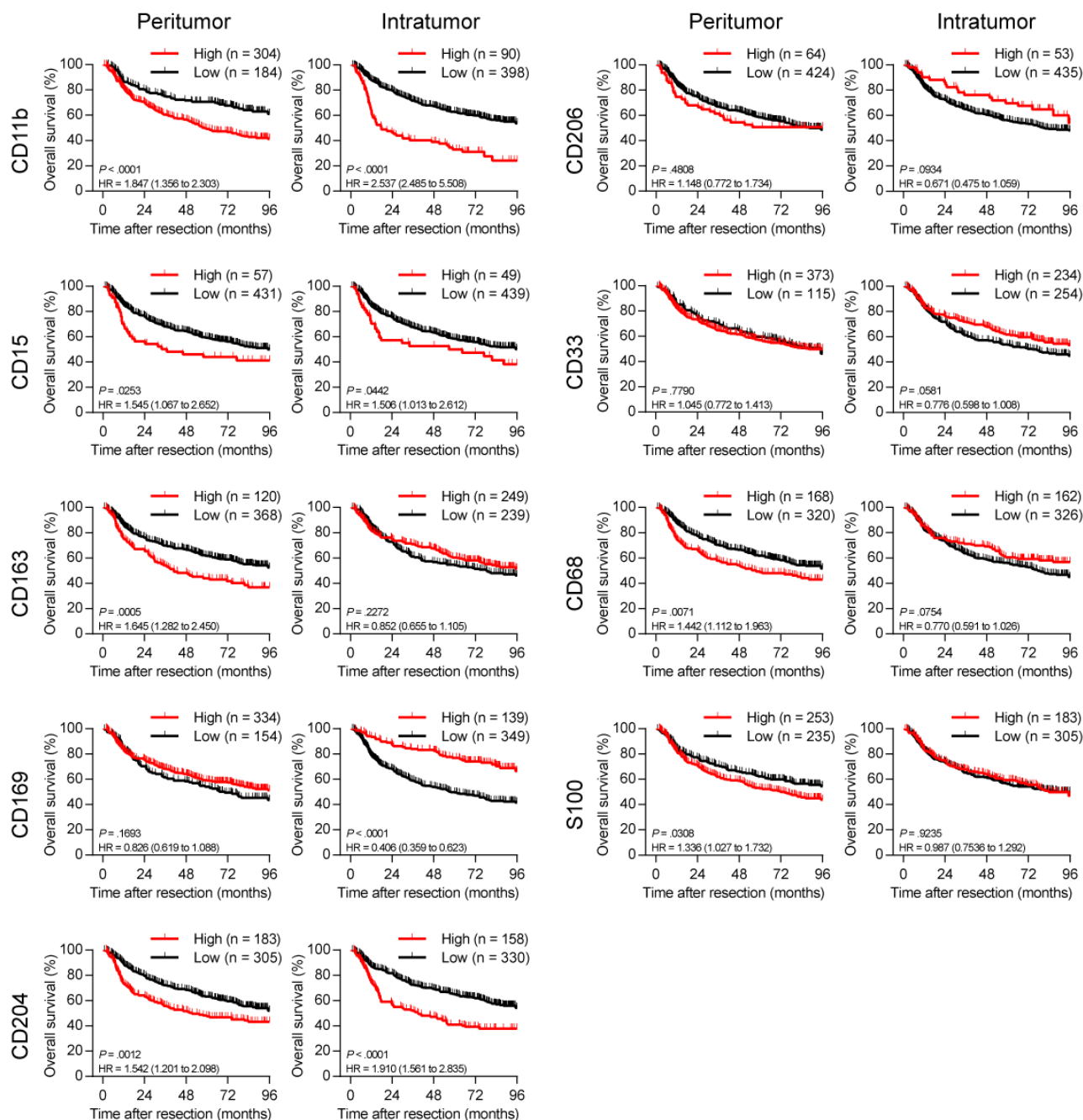
Supplemental Figures



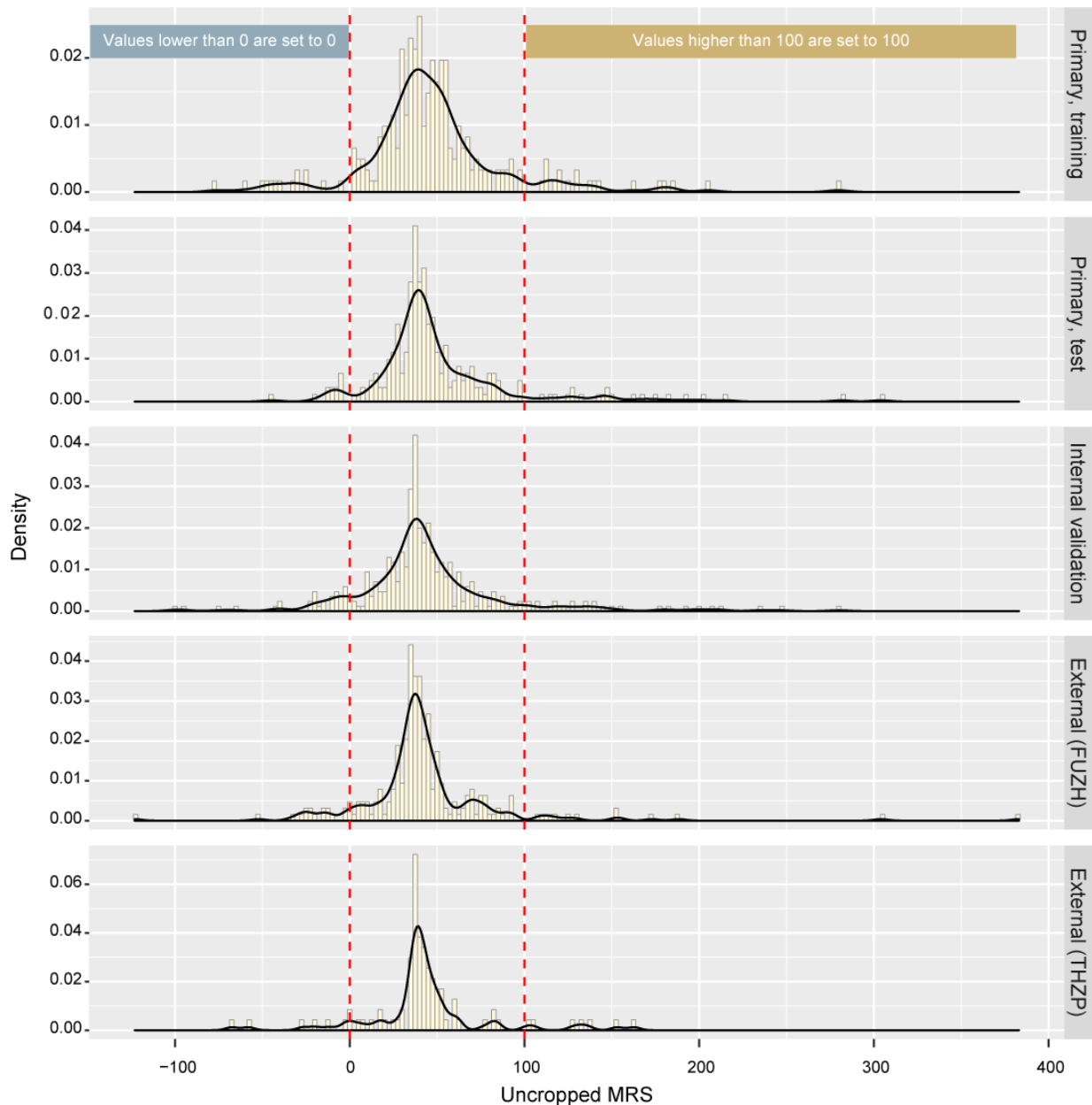
Supplemental Figure 1. IHC assessment of the 18 myeloid features in HCC. IHC staining for nine myeloid markers in peritumor liver and intratumor tissues of HCC. Representative images of the histological detection are shown. Scale bar, 100 μ m.



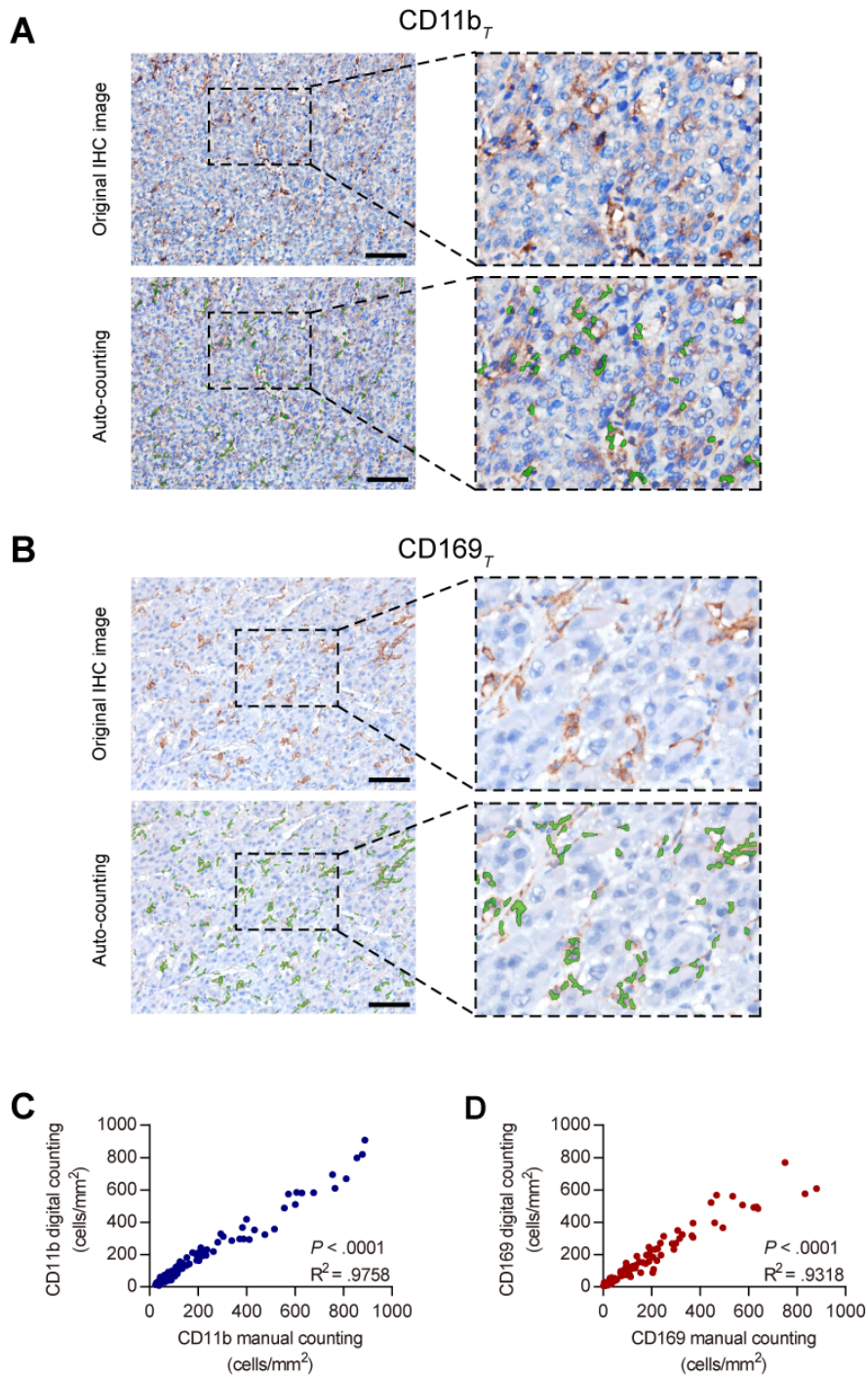
Supplemental Figure 2. The prognostic relevance of the 18 myeloid features to postsurgery HCC recurrence. Kaplan-Meier survival analysis of time to recurrence (TTR) in the primary cohort of HCC patients according to expression of the 18 myeloid features. Cutoffs were determined using the X-tile program, similar to that described in **Supplemental Figure 7**. *P* values were calculated using log-rank tests.



Supplemental Figure 3. The prognostic relevance of the 18 myeloid features to the postsurgery overall survival of HCC patients. Kaplan-Meier survival analysis of overall survival (OS) in the primary cohort of HCC patients according to expression of the 18 myeloid features. Cutoffs were determined using the X-tile program, similar to that described in **Supplemental Figure 7**. *P* values were calculated using the log-rank tests.

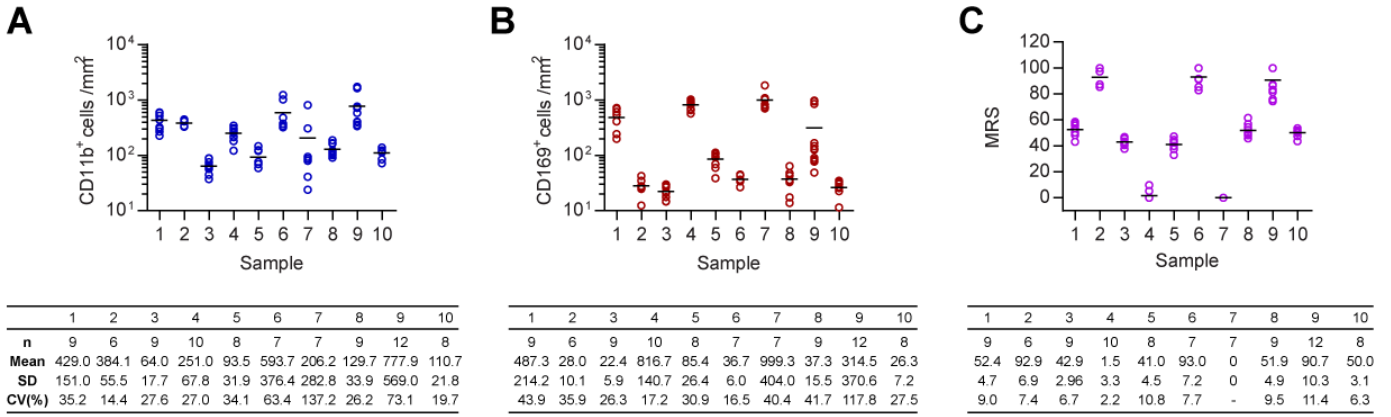


Supplemental Figure 4. The distribution of uncropped MRS values. The Lasso Cox regression model was built as indicated in **Figure 2**. The penalized coefficients are 0.000965 and -0.000636 for CD11b_T and CD169_T, respectively. The cord formula, $0.000965 \times \text{CD11b}_T - 0.000636 \times \text{CD169}_T$, was multiplied by the constant 166.67, and 35 was added to fit in a distribution mainly in the range from 0–100, resulting in the final scoring formula as follows: $\text{MRS} = 0.161 \times \text{CD11b}_T - 0.106 \times \text{CD169}_T + 35$. The distribution of the uncropped MRS values is shown. To facilitate further analysis, an MRS lower than 0 was assigned the value of 0, and an MRS higher than 100 was assigned the value of 100, as indicated in this figure.

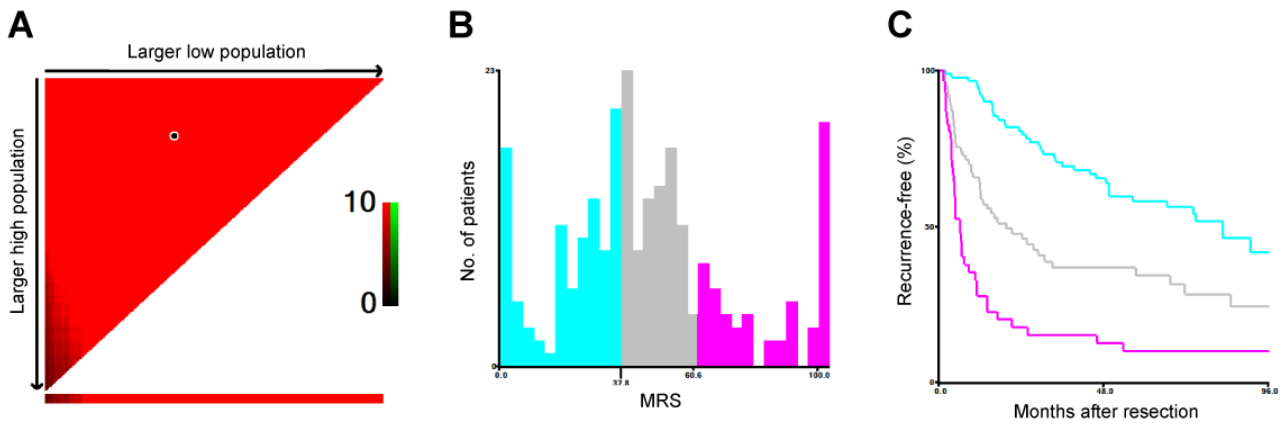


Supplemental Figure 5. Standardized image analysis and automatic cell counting. (A–B)

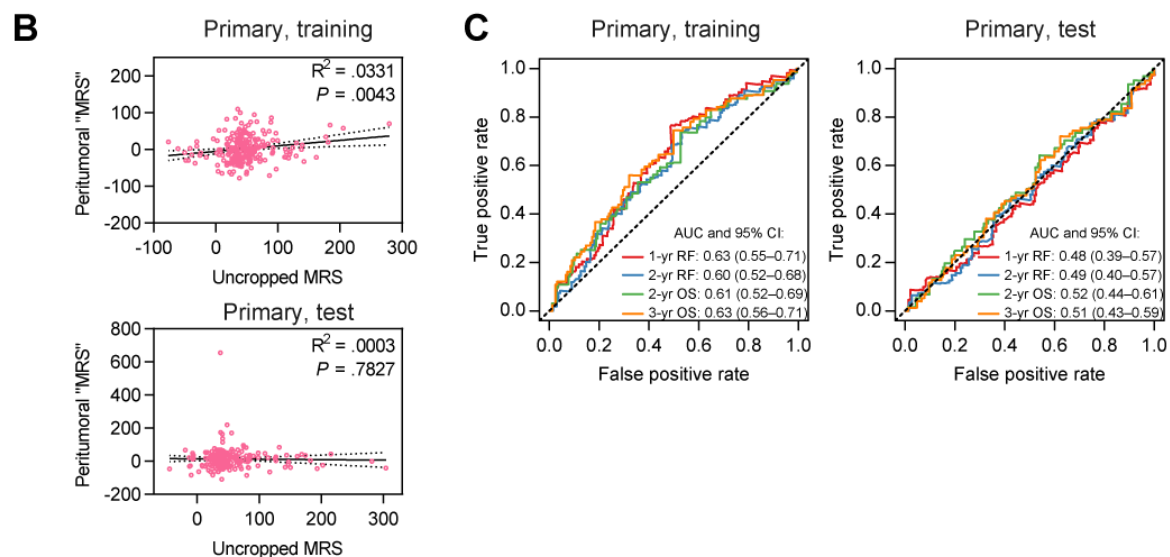
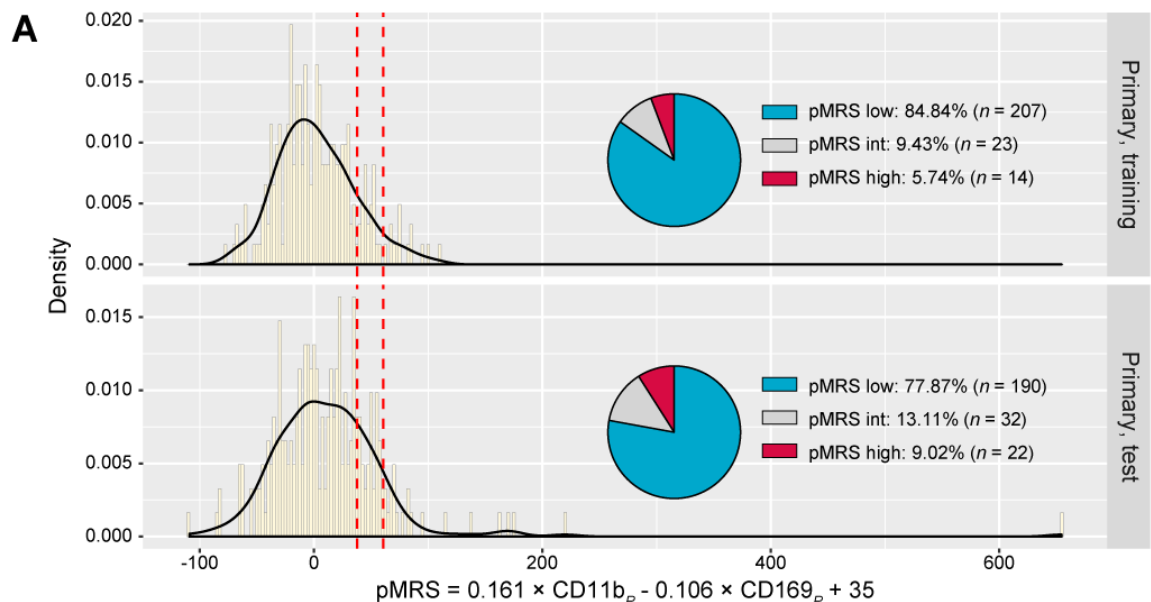
Representative examples of CD11b and CD169 immunostaining in intratumor tissues are shown as original IHC images. Stained cells represented in green with InForm Cell Analysis 2.2 (Perkin-Elmer, USA) are shown as “Auto-counting”. Subscript “T”, intratumor tissue. Scale bar, 100 μ m. (C–D) Correlation analysis of manual and digital counting of cells positive for CD11b and CD169. The R^2 and P values from results of Pearson correlation analyses are shown.



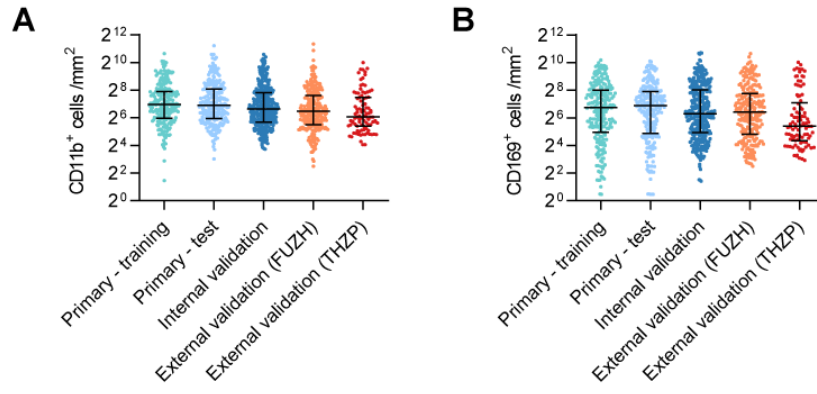
Supplemental Figure 6. Assessing the variance of CD11b_T, CD169_T, and MRS values. A separate set of 10 HCC patients who underwent curative resection at the SYSUCC was included to evaluate the reproducibility of TMA core-based IHC features. At least six blocks from the intratumoral area of each tumor node were collected. The numbers of CD11b⁺ cells and CD169⁺ cells and the values of the MRS were determined. The mean, standard deviation (SD) and coefficients of variation (CV) of values estimated based on different blocks of the same tumor node are shown.



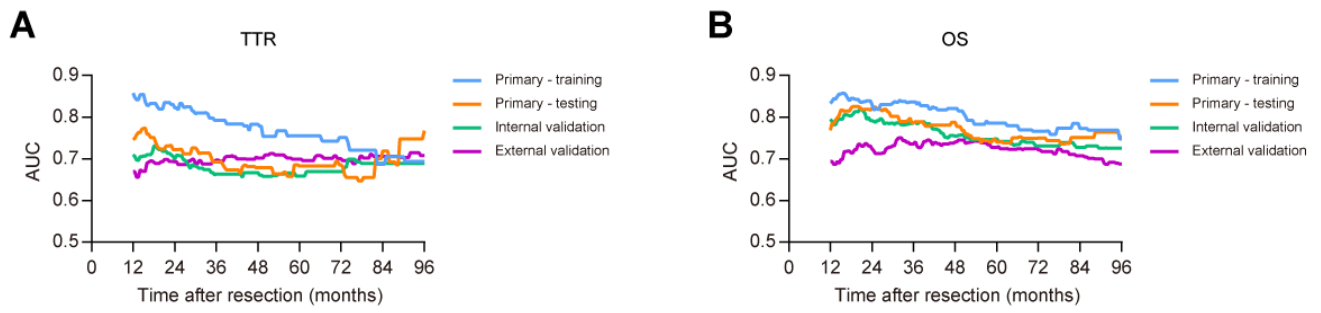
Supplemental Figure 7. The optimum cutoff values of the MRS were determined using the X-tile program. (A) The X-tile plot shows the χ^2 log-rank values produced when dividing the training cohort with two cut-points, producing subsets of patients with low, intermediate, and high MRSs (13). The X-axis represents all potential cutoff points from low to high (left to right) that define a subset with low MRS; the Y-axis represents cut-points from high to low (top to bottom) that define a subset with high MRS. Coloration of the plot represents the strength of the association at each division. Red represents a positive association between the MRS and recurrence; green represents an inverse association. The chosen cutoff point is highlighted by the black/white circle. **(B)** Distribution of the MRS in the training cohort. The cutoff point is shown on a histogram for the training cohort. **(C)** The discriminatory power of the three subgroups of patients divided according to the cutoff values determined in **(A)** is shown as a Kaplan-Meier plot.



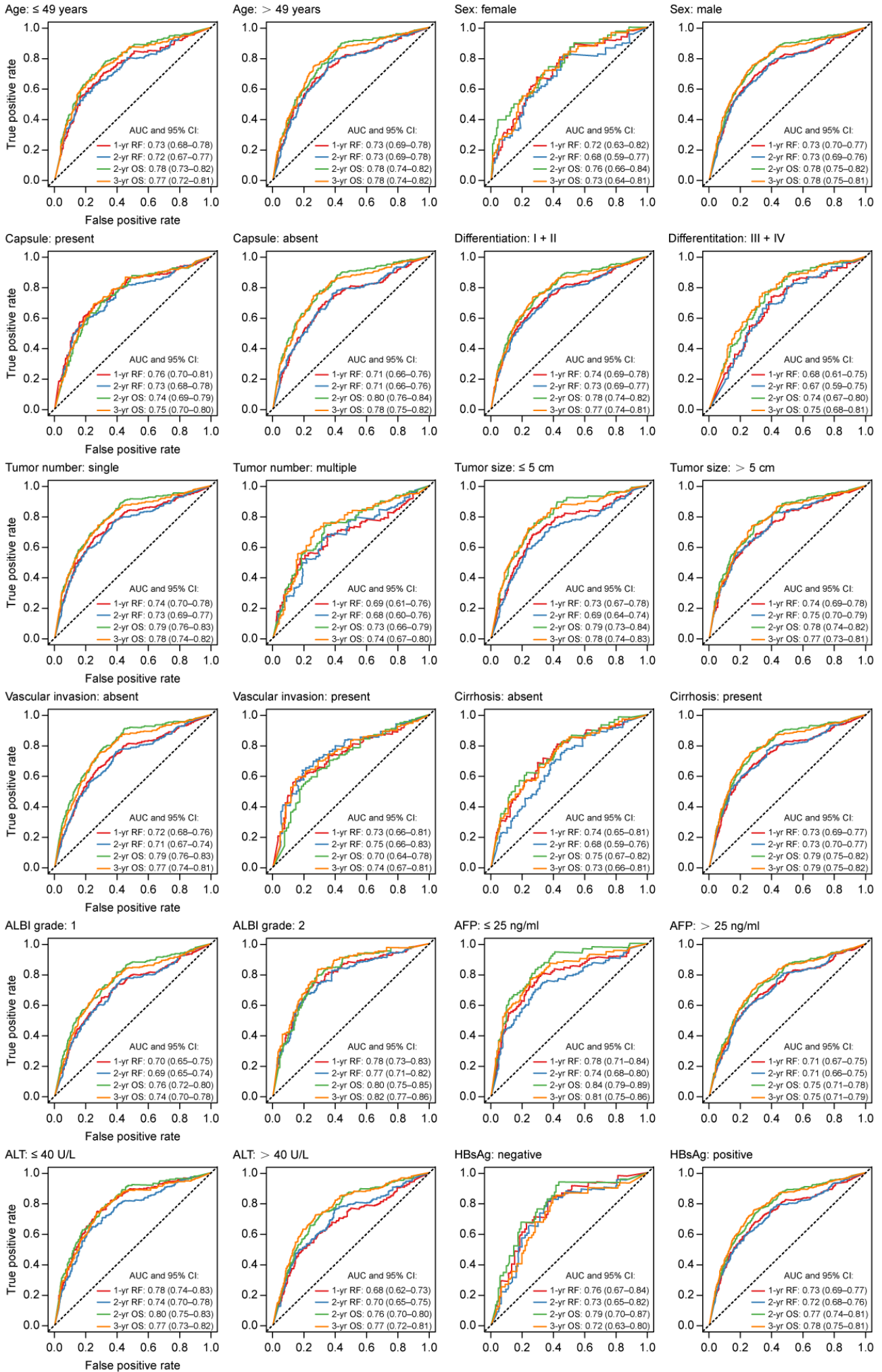
Supplemental Figure 8. The distribution of peritumoral MRS (pMRS) values. The peritumoral "MRS" (referred to as pMRS) was calculated using a similar formula of the MRS as follows: $pMRS = 0.161 \times CD11b_p - 0.106 \times CD169_p + 35$. **(A)** The distribution of the pMRS values in the primary-training and primary-test cohorts are shown. The red dash lines indicate the first and second cut-off point of the MRS: 37.9 and 60.6. The vast majority of the pMRS values are under the cutoff of 37.9. **(B)** Correlation between MRS and pMRS. The R^2 and P values from results of Pearson correlation analyses are shown. **(C)** ROC analysis of the prognostic relevance of the pMRS to postsurgery recurrence and survival. The 95% confidence intervals (95% CI) of the area under curve (AUC) was calculated using 1000 \times bootstrap estimates. AUC, area under the ROC curve; RF, recurrence free; ROC curve, receiver operator characteristic curve.



Supplemental Figure 9. Quantification of CD11b_T and CD169_T densities in each cohort. (A) The distribution of intratumoral CD11b⁺ cell densities in each cohort. Error bar, median and IQR. (B) The distribution of intratumoral CD169⁺ cell densities in each cohort. Error bar, median and IQR.



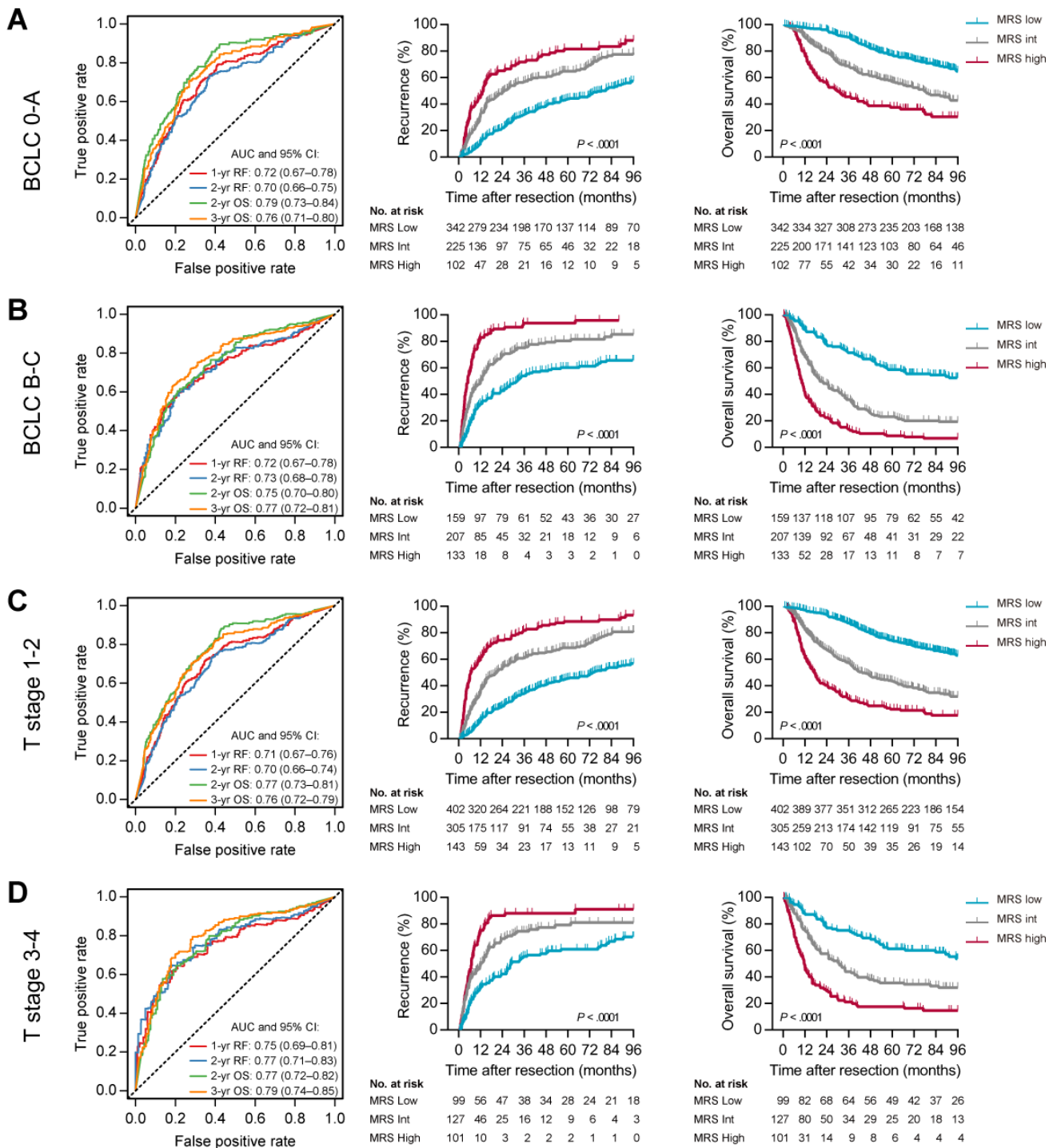
Supplemental Figure 10. Area under the curve (AUC) of time-dependent ROC curves for the MRS in the four cohorts. (A and B) AUC values of ROC curve (Y-axis) for MRS to predict TTR (A) and OS (B) at different time points (X-axis).



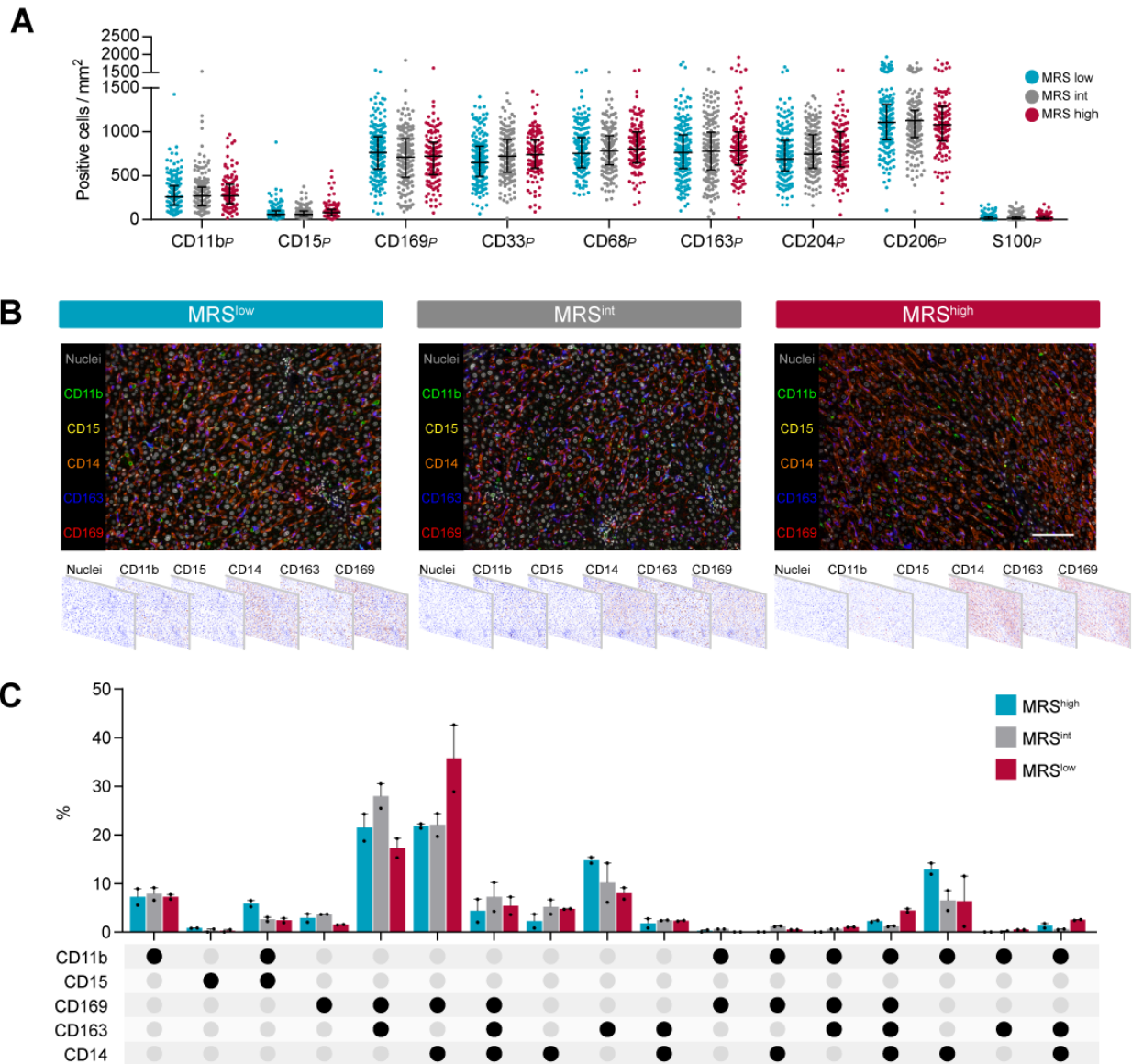
Supplemental Figure 11. ROC analysis of the relevance of the continuous MRS values to HCC prognosis in the entire cohort stratified by clinicopathological risk factors. Patients in the entire cohort were divided into subgroups according to indicated clinicopathological risk factors. The relevance of the MRS to 1-year recurrence, 2-year recurrence, 2-year OS, and 3-year OS was analyzed. The 95% confidence intervals (95% CI) of the area under curve (AUC) was calculated using 1000 bootstrap estimates. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin grade; ALT, alanine aminotransferase; HBsAg, surface antigen of the hepatitis B virus.

Supplemental Figure 12. Kaplan-Meier survival analysis of time to recurrence (TTR) in the entire cohort of HCC patients according to the MRS classifier, as stratified by clinicopathological risk factors. Patients in the entire cohort were divided into subgroups according to indicated clinicopathological risk factors. *P* values were calculated using the log-rank test for trend. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin grade; ALT, alanine aminotransferase; HBsAg, surface antigen of the hepatitis B virus.

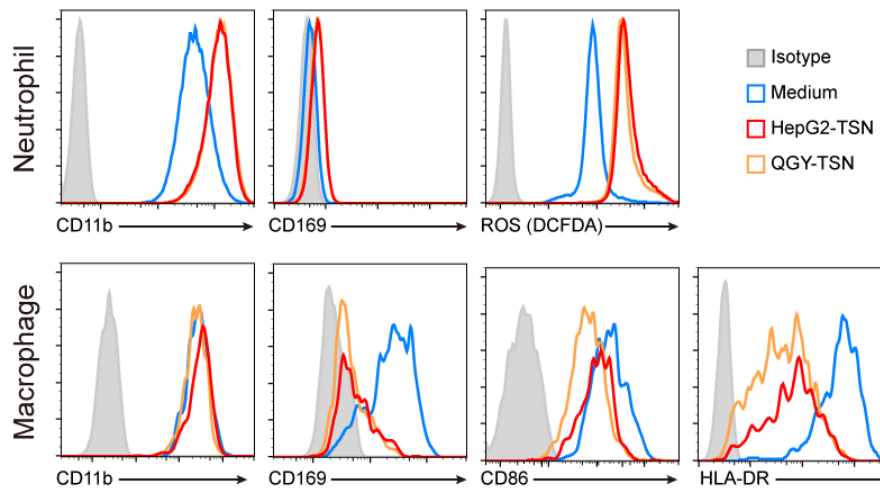
Supplemental Figure 13. Kaplan-Meier survival analysis of overall survival (OS) in the entire cohort of HCC patients according to the MRS classifier, stratified by clinicopathological risk factors. Patients in the entire cohort were divided into subgroups according to indicated clinicopathological risk factors. *P* values were calculated using the log-rank test for trend. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin grade; ALT, alanine aminotransferase; HBsAg, surface antigen of the hepatitis B virus.



Supplemental Figure 14. ROC and Kaplan-Meier survival analyses of TTR and OS according to the MRS in subgroups of HCC patients, stratified by pathological staging. (A and B) Patients in the entire cohort were divided into subgroups according to BCLC staging. (C and D) Patients in the entire cohort were divided into subgroups according to T staging (AJCC). The performance of MRS in predicting TTR and OS in subgroups was assessed with ROC and Kaplan-Meier analyses. The 95% CI of the AUC was calculated using 1000 bootstrap estimates. *P* values were calculated using the log-rank test for trend.

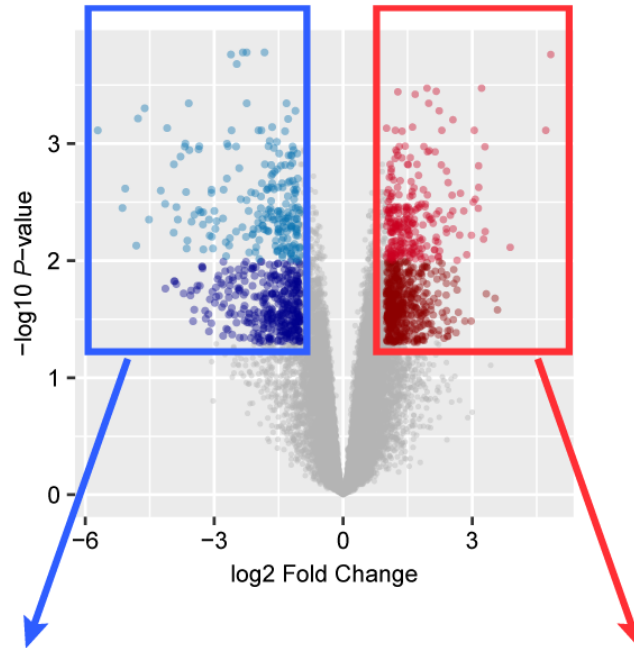
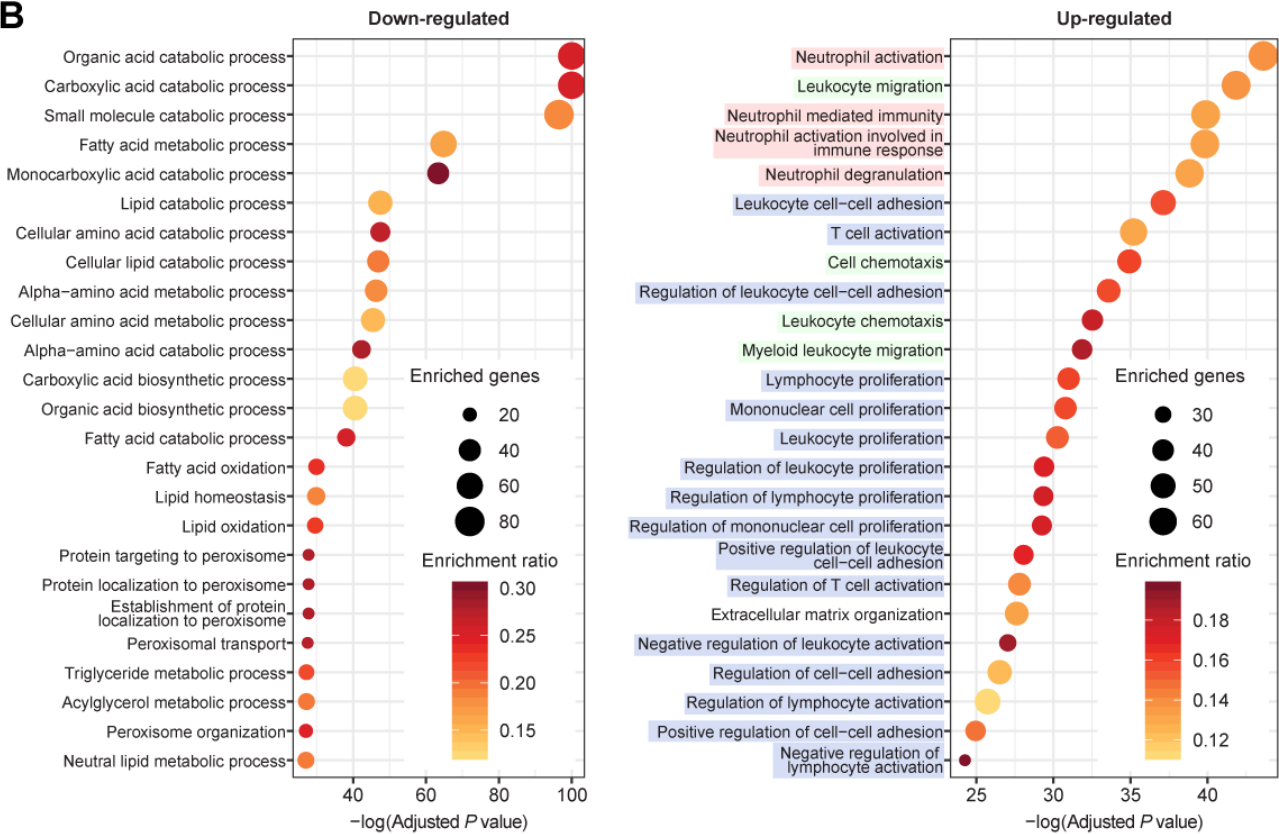


Supplemental Figure 15. The composition of the myeloid cell contexture in the peritumoral liver tissue of HCC. (A) Densities of myeloid subsets in peritumoral (P) area of tumors with low, intermediate and high MRS estimates. Error bar, median and IQR. * $P < 0.05$; ns, not significant (two-way ANOVA followed by Tukey's test). **(B)** Representative multiplexed IF images show that the myeloid cell subtype composition of the peritumoral tissue is similar in different MRS subgroups. Scale bar, 100 μm . **(C)** Different compositions of myeloid contextures in MRS^{low} ($n = 2$), MRS^{int} ($n = 2$), and MRS^{high} ($n = 2$) tissues. Data are shown in an UpSet plot. Each row corresponds to one myeloid marker, and each column (a subgroup including three bars) corresponds to a subset of cells with the indicated pattern of marker co-expression. Circles are either light-gray, indicating that this subset was negatively/marginally stained for that marker, or black, showing this subset expressed that marker. Error bar, mean and SEM.



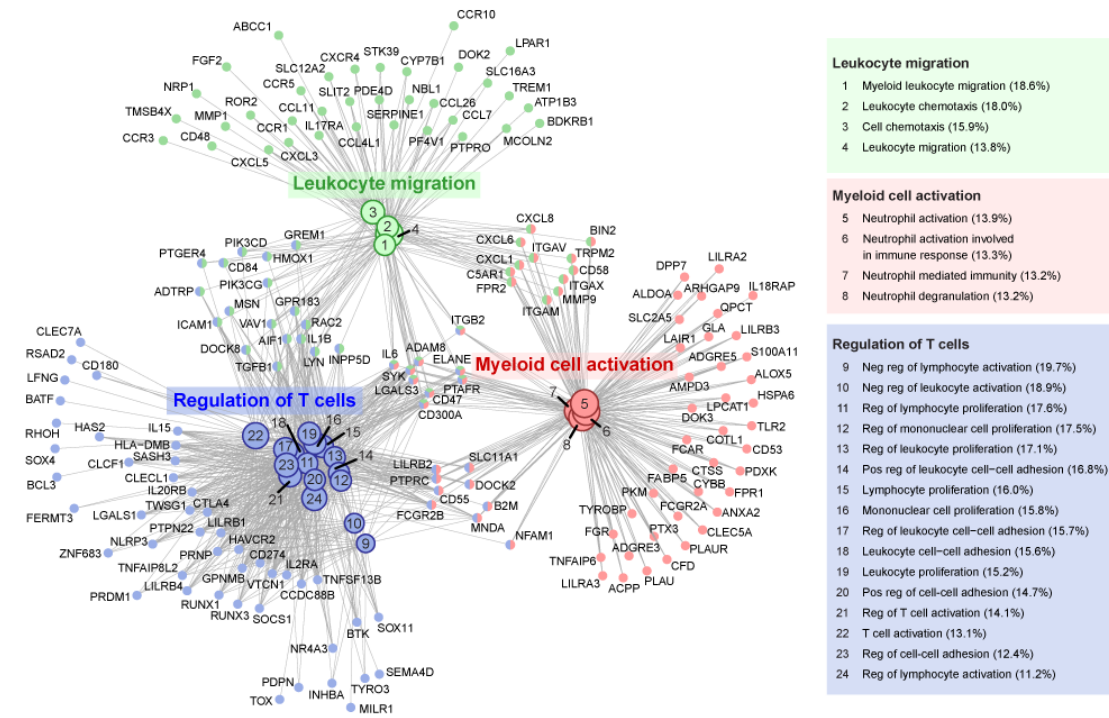
Supplemental Figure 16. The impact of tumor cell culture supernatant on myeloid cell phenotype.

Freshly isolate peripheral blood CD15⁺ neutrophils and CD14⁺ monocyte-derived macrophages were stimulated with HCC tumor cell culture supernatant (TSN) or control medium. Cell phenotypes were then examined with flow cytometry. ROS, reactive oxygen species.

A**B**

Supplemental Figure 17. Differential gene expression analysis and GO annotation. (A) The volcano plot shows P values adjusted by the Benjamini–Hochberg (BH) method and fold changes in gene expression (MRS^{high} vs MRS^{low}). (B) The 25 top GO terms enriched in downregulated (left) and upregulated (right) genes are shown. Immune-related GO terms are shaded with different colors according to the subgroups of activities, as described in **Supplemental Figure 18A**.

A

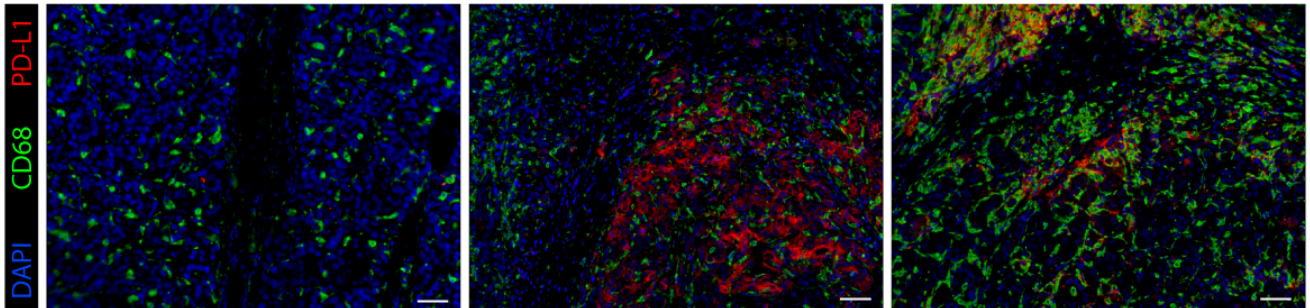


B

PD-L1 < 5% in both macrophages and tumor cells

PD-L1 < 5% in macrophages, PD-L1 ≥ 5% in tumor cells

PD-L1 ≥ 5% in macrophages, PD-L1 < 5% in tumor cells



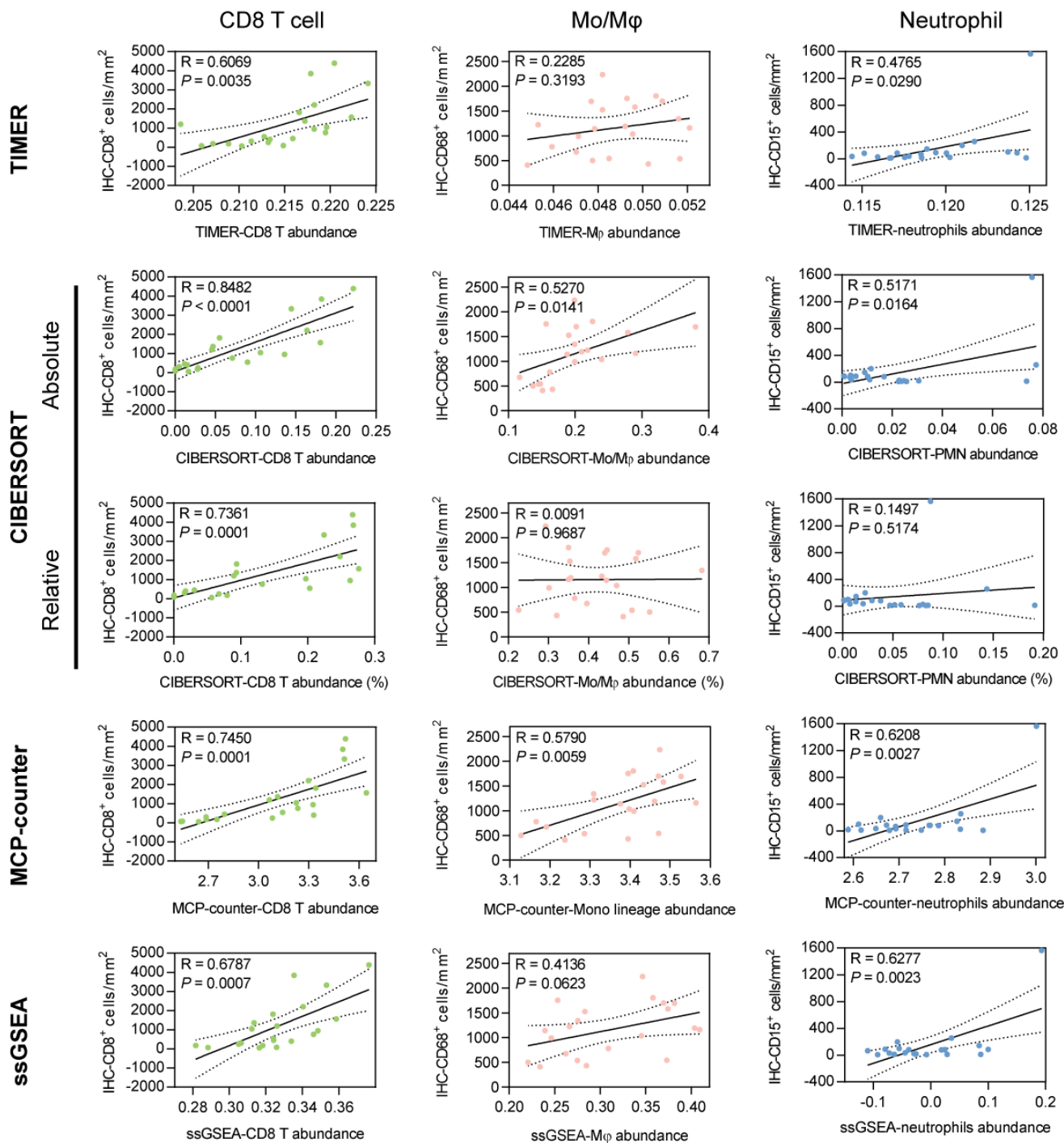
C

No. (% of row)	PD-L1 < 5% macrophages	PD-L1 ≥ 5% macrophages	Total	<i>P</i>
MRS low	127 (86%)	21 (14%)	148	0.0033
MRS int	142 (87%)	22 (13%)	164	(χ^2 test)
MRS high	70 (71%)	28 (29%)	98	0.0075
Total	339	71	410	(χ^2 test for trend)

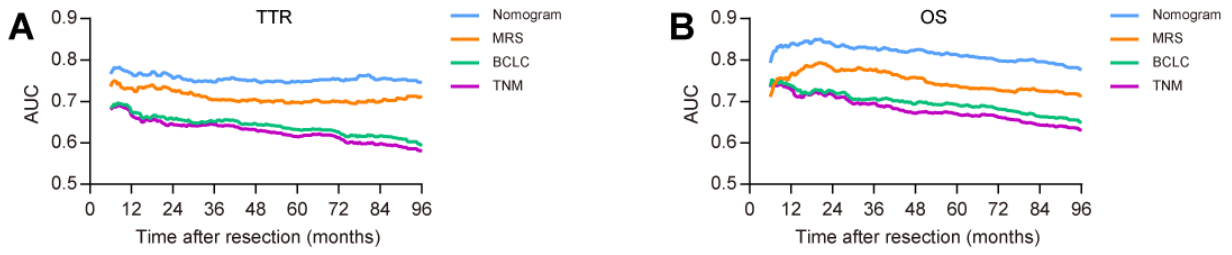
No. (% of row)	PD-L1 < 5% tumor cells	PD-L1 ≥ 5% tumor cells	Total	<i>P</i>
MRS low	123 (83%)	25 (17%)	148	0.0001
MRS int	155 (95%)	9 (5%)	164	(χ^2 test)
MRS high	75 (77%)	23 (23%)	98	0.3555
Total	353	57	410	(χ^2 test for trend)

Supplemental Figure 18. A high MRS indicates a more suppressive immune microenvironment.

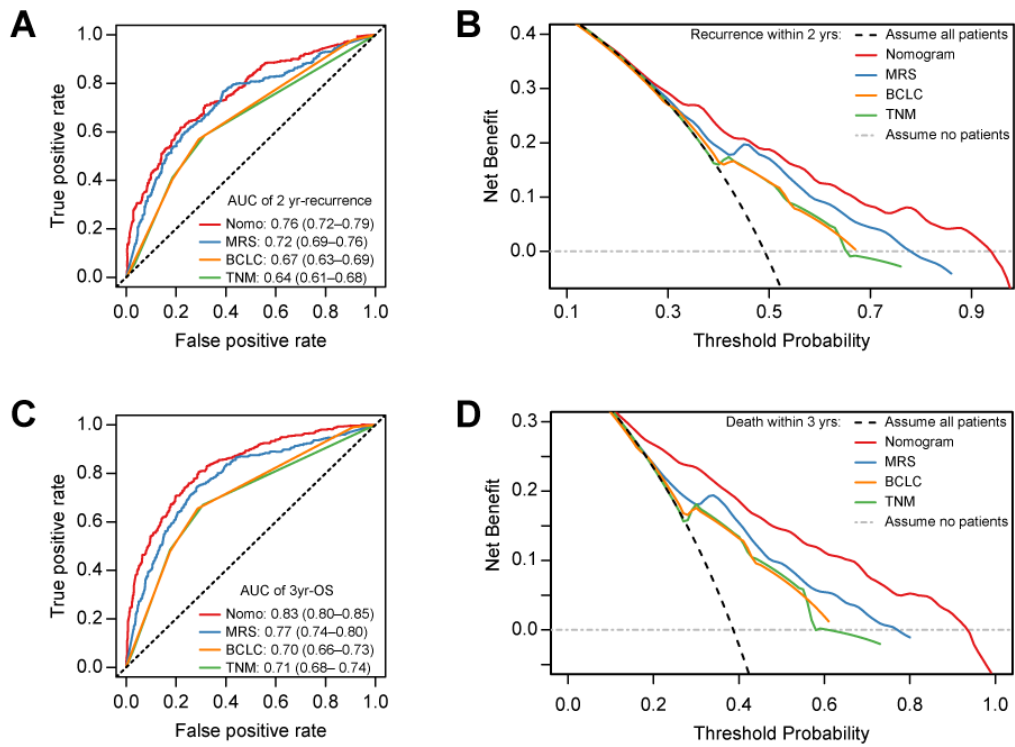
(A) The gene-concept network of top enriched immune-related GO terms and genes involved in MRS^{high} HCCs, corresponding to **Supplemental Figure 17B**. (B) Paraffin-embedded HCC tumor tissue sections were subjected to TSA-IHC staining using anti-human CD68 and anti-human PD-L1 antibodies. Images were obtained using the Vectra System and analyzed by InForm image analysis software. Representative images are shown. Scale bar, 50 μ m. (C) Correlation between the MRS grouping and PD-L1 expression on tumor cells (left) or macrophages (right) was examined with χ^2 tests.



Supplemental Figure 19. Correlation between bioinformatic quantitation of tumor-infiltrating immune cells and the results of *in situ* immunotyping. Transcriptomic profiling of 21 bulk HCC tissues with gene expression microarray was processed using different computational methods including TIMER (14), CIBERSORT (15) (using both the relative and absolute modes), MCP-counter (16) and single-cell gene-set enrichment analysis (ssGSEA) along with published human immune cell-specific gene sets (17). Data correlation was assessed by Pearson correlation tests.



Supplemental Figure 20. Area under the curve (AUC) of time-dependent ROC curves for the MRS, BCLC staging, and TNM staging in the entire cohort. (A and B) AUC values of the ROC curve analysis for the MRS, BCLC staging, and TNM staging to predict TTR (A) and OS (B) at different time points.



Supplemental Figure 21. The discrimination and clinical usefulness of MRS-based nomograms.

(A-B) Time-dependent ROC curve analysis (A) and the decision curve analysis (B) of the TTR nomogram predicting 2-year recurrence in the entire cohort, compared with the MRS, BCLC staging, and TNM staging. (C-D) Time-dependent ROC curve analysis (C) and the decision curve analysis (D) of the OS nomogram predicting 3-year survival in the entire cohort compared with the MRS, BCLC staging, and TNM staging. AUC, area under the curve. The AUC is indicated as the mean and 95% CI, estimated using 1000 × bootstrap resampling.

Supplemental Tables

Supplemental Table 1. Candidate Myeloid Markers

Markers	Myeloid subsets	References
CD11b, CD33	Common myeloid markers, widely expressed on MDSCs, monocytes, neutrophils, and subsets of macrophages	Gabrilovich and Nagaraj, et al. (2009)(18); Bronte, et al. (2016)(19); Okita, et al. (2014)(20); Cui, et al. (2013)(21)
CD68	Pan-macrophage marker	Holness & Simmons. (1993)(22); Ding, et al. (2009)(23); Kuang, et al. (2010)(24); Fan, et al. (2014)(25); Kong, et al. (2013)(26)
CD169	Anti-tumoral M ϕ marker associated with improved prognosis in HCC	Zhang, et al. (2016)(27); Li, et al. (2017)(28)
CD163	M2-macrophage associated marker	Jensen, et al. (2009)(29); Kong, et al. (2013)(26); Yeung, et al. (2015)(30)
CD204	M2-macrophage associated marker, correlated with poor outcomes in multiple types of cancer including HCC	Wang, et al. (2015)(31); Li, et al. (2017)(28); Hou, et al. (2014)(32)
CD206	M2-macrophage associated marker, indicating a poor prognosis in various malignancies including HCC	Shu, et al. (2016)(33); Dong, et al. (2016)(34); Tan-Garcia, et al. (2017)(35)
CD15	Neutrophils	Kuang, et al. (2011)(36); Li, et al. (2011)(37); Zhou, et al. (2012)(38); He, et al. (2016)(39)
S100	Dendritic cells	Ouyang, et al. (2016)(40)

Supplemental Table 2. Clinicopathological Characteristics of the HCC Patients in Each Cohort

Characteristics	Primary training		Primary test		Internal validation		External validation			
							THZP		FUZH	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total no. of patients	244		244		341		94		254	
Operation time	2006–2010				2001–2005		2006–2009		2004–2005	
Age, years										
Median	49		52		49		52		52	
Range	13–76		23–78		20–78		25–84		23–75	
Sex										
Male	215	88.11	217	88.93	306	89.74	78	82.98	206	81.1
Female	29	11.89	27	11.07	35	10.26	16	17.02	48	18.9
Tumor encapsulation										
Absent	134	54.92	140	57.38	189	55.43	40	42.55	142	55.91
Present	105	43.03	100	40.98	152	44.57	45	47.87	112	44.09
NA	5	2.05	4	1.64	—	—	9	9.57	—	—
Differentiation										
I–II	199	81.56	183	75.00	263	77.13	67	71.28	196	77.17
III–IV	45	18.44	58	23.77	70	20.53	27	28.72	55	21.65
NA	—	—	3	1.23	8	2.35	—	—	3	1.18
Tumor number										
Single	174	71.31	187	76.64	249	73.02	71	75.53	216	85.04
Multiple	70	28.69	57	23.36	92	26.98	14	14.89	38	14.96
NA	—	—	—	—	—	—	9	9.57	—	—
Tumor size										
≤ 5 cm	108	44.26	120	49.18	118	34.60	47	50.00	144	56.69
> 5 cm	136	55.74	124	50.82	223	65.40	46	48.94	110	43.31
NA	—	—	—	—	—	—	1	1.06	—	—
Vascular invasion										
Absent	214	87.70	218	89.34	291	85.34	58	61.70	164	64.57
Present	30	12.30	26	10.66	50	14.66	29	30.85	90	35.43
NA	—	—	—	—	—	—	7	7.45	—	—
BCLC stage										
0–A	150	61.48	161	65.98	154	45.16	54	57.45	150	59.06
B–C	94	38.52	83	34.02	187	54.84	31	32.98	104	40.94
NA	—	—	—	—	—	—	9	9.57	—	—
TNM stage										
I–II	166	68.03	181	74.18	188	55.13	86	91.49	208	81.89
III–IV	78	31.97	63	25.82	153	44.87	8	8.51	46	18.11

Supplemental Table 2 (continued). Clinicopathological Characteristics of the HCC Patients in Each Cohort

Characteristics	Primary training		Primary test		Internal validation		External validation			
							THZP		FUZH	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cirrhosis										
Absent	48	19.67	45	18.44	66	19.35	12	12.77	39	15.35
Present	193	79.10	194	79.51	275	80.65	77	81.91	215	84.65
NA	3	1.23	5	2.05	—	—	5	5.32	—	—
ALBI grade										
1	144	59.02	165	67.62	225	65.98	54	57.45	176	69.29
2	100	40.98	78	31.97	113	33.14	31	32.98	78	30.71
NA	—	—	1	0.41	3	0.88	9	9.57	—	—
Child-Pugh										
A	237	97.13	231	94.67	310	90.91	—	—	239	94.09
B	7	2.87	11	4.51	28	8.21	—	—	15	5.91
NA	—	—	2	0.82	3	0.88	94	100.00	—	—
AFP										
≤ 25 ng/mL	95	38.93	84	34.43	100	29.33	26	27.66	90	35.43
> 25 ng/mL	149	61.07	160	65.57	241	70.67	59	62.77	164	64.57
NA	—	—	—	—	—	—	9	9.57	—	—
ALT (U/L)										
≤ 40 U/L	135	55.33	142	58.20	166	48.68	41	43.62	133	52.36
> 40 U/L	109	44.67	102	41.80	175	51.32	44	46.81	121	47.64
NA	—	—	—	—	—	—	9	9.57	—	—
HBsAg										
Positive	220	90.16	217	88.93	294	86.22	72	76.60	210	82.68
Negative	24	9.84	27	11.07	47	13.78	13	13.83	42	16.54
NA	—	—	—	—	—	—	9	9.57	2	0.79
HCVAb										
Positive	1	0.41	4	1.64	5	1.47	1	1.06	3	1.18
Negative	243	99.59	240	98.36	336	98.53	82	87.23	248	97.64
NA	—	—	—	—	—	—	11	11.70	3	1.18
Follow-up (survival)										
Median (months)	59.35		55.48		36.3		48.47			
Range (months)	1–96		1.93–96		0.43–96		0.5–96			
Events (no.)	111		114		205		209			
Events (%)	45.49		46.72		60.12		60.06			
Follow-up (recurrence)										
Median (months)	15.27		11.48		11.97		26.27			
Range (months)	0.87–96		1–96		0.4–96		0.5–96			
Event (no.)	127		136		205		239			
Event (%)	52.05		55.74		60.12		68.68			

Abbreviations: ALBI grade, albumin–bilirubin grade; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; FUZH, Fudan University Zhongshan Hospital; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; NA, not available; THZP, Taizhou Hospital of Zhejiang Province.

Supplemental Table 3. Clinicopathological Characteristics of the Patients in the Primary Cohort

Characteristics	Primary-training		Primary-test		P*
	No.	%	No.	%	
Age, years					0.071 [†]
Median		49		52	
Range		13–76		23–78	
Sex					0.776
Male	215	88.11	217	88.93	
Female	29	11.89	27	11.07	
Tumor encapsulation					0.616
Absent	134	56.07	140	58.33	
Present	105	43.93	100	41.67	
NA	5	—	4	—	
Differentiation					0.130
I–II	199	81.56	183	75.93	
III–IV	45	18.44	58	24.07	
NA	—	—	3	—	
Tumor number					0.180
Single	174	71.31	187	76.64	
Multiple	70	28.69	57	23.36	
Tumor size					0.276
≤ 5 cm	108	44.26	120	49.18	
> 5 cm	136	55.74	124	50.82	
Vascular invasion					0.570
Absent	214	87.70	218	89.34	
Present	30	12.30	26	10.66	
BCLC stage classification					0.300
0–A	150	61.48	161	65.98	
B–C	94	38.52	83	34.02	
TNM stage					0.134
I–II	166	68.03	181	74.18	
III–IV	78	31.97	63	25.82	
Cirrhosis					0.763
Absent	48	19.67	45	18.44	
Present	193	79.10	194	79.51	
NA	3	1.23	5	2.05	
ALBI grade					0.042
1	144	59.02	165	67.90	
2	100	40.98	78	32.10	
NA	—	—	1	—	
Child-Pugh					0.326
A	237	97.13	231	95.45	
B	7	2.87	11	4.55	
NA	—	—	2	—	

Supplemental Table 3 (continued). Clinicopathological Characteristics of the Patients in the Primary Cohort

Characteristics	Primary-training		Primary-test		P*
	No.	%	No.	%	
AFP					0.301
≤ 25 ng/mL	95	38.93	84	34.43	
> 25 ng/mL	149	61.07	160	65.57	
ALT					0.522
≤ 40 U/L	135	55.33	142	58.20	
> 40 U/L	109	44.67	102	41.80	
HBsAg					0.657
Positive	220	90.16	217	88.93	
Negative	24	9.84	27	11.07	
HCVAb					0.372 ^{††}
Positive	1	0.41	4	1.64	
Negative	243	99.59	240	98.36	

*Calculated by Chi-Square test, unless otherwise indicated; [†]calculated by Mann-Whitney U test; ^{††}calculated by Fisher's exact test.

Abbreviations: ALBI grade, albumin–bilirubin grade; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BCLC stage classification, Barcelona Clinic Liver Cancer stage; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; NA, not available.

Supplemental Table 4. Correlation Between the Clinicopathological Characteristics and MRS Grouping in the Primary Cohort

Characteristics	Primary training cohort						<i>P</i> *	Primary test cohort						<i>P</i> *
	MRS ^{low} (<i>n</i> = 95)		MRS ^{int} (<i>n</i> = 92)		MRS ^{high} (<i>n</i> = 57)			MRS ^{low} (<i>n</i> = 96)		MRS ^{int} (<i>n</i> = 90)		MRS ^{high} (<i>n</i> = 58)		
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
Age, years							0.912 [†]							0.001 [†]
Median	48		48		47			55		47		51		
Range	20–76		13–74		24–73			31–78		23–74		23–71		
Sex							0.182							0.525
Male	83	87.37	78	84.78	54	94.74		88	91.67	79	87.78	50	86.21	
Female	12	12.63	14	15.22	3	5.26		8	8.33	11	12.22	8	13.79	
Tumor encapsulation							0.035							0.327
Absent	53	56.99	43	47.25	38	69.09		54	56.84	48	54.55	38	66.67	
Present	40	43.01	48	52.75	17	30.91		41	43.16	40	45.45	19	33.33	
NA	2	—	1	—	2	—		1	—	2	—	1	—	
Differentiation							0.021							0.155
I–II	85	89.47	73	79.35	41	71.93		71	76.34	73	81.11	39	67.24	
III–IV	10	10.53	19	20.65	16	28.07		22	23.66	17	18.89	19	32.76	
NA	—	—	—	—	—	—		3	—	—	—	—	—	
Tumor number							0.185							0.065
Single	74	77.89	61	66.30	39	68.42		80	83.33	62	68.89	45	77.59	
Multiple	21	22.11	31	33.70	18	31.58		16	16.67	28	31.11	13	22.41	
Tumor size							0.016							0.742
≤ 5 cm	50	52.63	30	32.61	28	49.12		49	51.04	45	50.00	26	44.83	
> 5 cm	45	47.37	62	67.39	29	50.88		47	48.96	45	50.00	32	55.17	
Vascular invasion							< 0.001 ^{††}							0.015
Absent	91	95.79	81	88.04	42	73.68		90	93.75	82	91.11	46	79.31	
Present	4	4.21	11	11.96	15	26.32		6	6.25	8	8.89	12	20.69	
BCLC stage classification							0.018							0.008
0–A	68	71.58	54	58.70	28	49.12		73	76.04	58	64.44	30	51.72	
B–C	27	28.42	38	41.30	29	50.88		23	23.96	32	35.56	28	48.28	
TNM stage							0.016							0.002
I–II	74	77.89	60	65.22	32	56.14		78	81.25	70	77.78	33	56.90	
III–IV	21	22.11	32	34.78	25	43.86		18	18.75	20	22.22	25	43.10	

Supplemental Table 4 (continued). Correlation Between the Clinicopathological Characteristics and the MRS Grouping in the Primary Cohort

Characteristics	Primary training cohort						<i>P</i> *	Primary test cohort						<i>P</i> *
	MRS ^{low} (<i>n</i> = 95)		MRS ^{int} (<i>n</i> = 92)		MRS ^{high} (<i>n</i> = 57)			MRS ^{low} (<i>n</i> = 96)		MRS ^{int} (<i>n</i> = 90)		MRS ^{high} (<i>n</i> = 58)		
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
Cirrhosis							0.475							0.441
Absent	20	21.05	20	22.22	8	14.29		17	18.48	14	15.73	14	24.14	
Present	75	78.95	70	77.78	48	85.71		75	81.52	75	84.27	44	75.86	
NA	—	—	2	—	1	—		4	—	1	—	—	—	
ALBI grade							0.815							0.146
1	58	61.05	52	56.52	34	59.65		61	63.54	68	75.56	36	63.16	
2	37	38.95	40	43.48	23	40.35		35	36.46	22	24.44	21	36.84	
NA	—	—	—	—	—	—		—	—	—	—	—	—	
Child-Pugh							0.084 ^{††}							0.538 ^{††}
A	91	95.79	92	100.00	54	94.74		92	96.84	86	95.56	53	92.98	
B	4	4.21	0	0.00	3	5.26		3	3.16	4	4.44	4	7.02	
NA	—	—	—	—	—	—		1	—	—	—	1	—	
AFP							0.007							0.090
≤ 25 ng/mL	48	50.53	26	28.26	21	36.84		41	42.71	26	28.89	17	29.31	
> 25 ng/mL	47	49.47	66	71.74	36	63.16		55	57.29	64	71.11	41	70.69	
ALT (U/L)							0.316							0.239
≤ 40 U/L	58	61.05	49	53.26	28	49.12		62	64.58	50	55.56	30	51.72	
> 40 U/L	37	38.95	43	46.74	29	50.88		34	35.42	40	44.44	28	48.28	
HBsAg							0.397							0.707
Positive	84	88.42	86	93.48	50	87.72		84	87.50	82	91.11	51	87.93	
Negative	11	11.58	6	6.52	7	12.28		12	12.50	8	8.89	7	12.07	
HCVAb							1.000 ^{††}							0.040 ^{††}
Positive	1	1.05	0	0.00	0	0.00		0	0.00	1	1.11	3	5.17	
Negative	94	98.95	92	100.00	57	100.00		96	100.00	89	98.89	55	94.83	

*Calculated by Chi-Square test, unless otherwise indicated; †calculated by Kruskal-Wallis test; ††calculated by Fisher's exact test.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; NA, not applicable.

Supplemental Table 5. Correlation Between the Clinicopathological Characteristics and MRS Grouping in the Internal and External Validation Cohorts

Characteristics	Internal Validation Cohort						<i>P</i> *	Combined External Validation Cohort						<i>P</i> *
	MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 114)		MRS ^{high} (<i>n</i> = 72)			MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 136)		MRS ^{high} (<i>n</i> = 57)		
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
Age, years							0.180 [†]							0.822 [†]
Median	50		48		47			53		53		52		
Range	23–77		20–78		25–75			30–75		23–84		25–72		
Sex							0.823							0.607
Male	138	89.03	101	89.38	66	91.67		130	83.87	108	79.41	46	80.70	
Female	17	10.97	12	10.62	6	8.33		25	16.13	28	20.59	11	19.30	
Capsule							0.035							0.228
Absent	77	49.68	63	55.26	49	68.06		91	58.71	68	50.00	23	47.92	
Present	178	50.32	51	44.74	23	31.94		64	41.29	68	50.00	25	52.08	
Differentiation							0.064							0.001
I–II	127	83.01	89	79.46	47	69.12		129	84.31	100	74.07	34	59.65	
III–IV	26	16.99	23	20.54	21	30.88		24	15.69	35	25.93	23	40.35	
NA	2	—	2	—	4	—		2	—	1	—	—	—	
Tumor number							0.453							0.618
Single	118	76.13	79	69.30	52	72.22		134	86.45	112	82.35	41	85.42	
Multiple	37	23.87	35	30.70	20	27.78		21	13.55	24	17.65	7	14.58	
NA	—	—	—	—	—	—		—	—	—	—	9	—	
Tumor size							0.009							0.104
≤ 5 cm	67	43.23	32	28.07	19	26.39		95	61.29	69	50.74	27	48.21	
> 5 cm	88	56.77	82	71.93	53	73.61		60	38.71	67	49.26	29	50.88	
NA	—	—	—	—	—	—		—	—	—	—	1	—	
Vascular invasion							< 0.001							< 0.001
Absent	143	92.26	98	85.96	50	69.44		119	76.77	75	55.15	28	56.00	
Present	12	7.74	16	14.04	22	30.56		36	23.23	61	44.85	22	44.00	
NA	—	—	—	—	—	—		—	—	—	—	7	—	

Supplemental Table 5 (continued). Correlation Between the Clinicopathological Characteristics and MRS Grouping in the Internal and External Validation Cohorts

Characteristics	Internal Validation Cohort						<i>P</i> *	Combined External Validation Cohort						<i>P</i> *
	MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 114)		MRS ^{high} (<i>n</i> = 72)			MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 136)		MRS ^{high} (<i>n</i> = 57)		
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
BCLC stage classification							< 0.001							< 0.001
0–A	89	57.42	46	40.35	19	26.39		112	72.26	67	49.26	25	52.08	
B–C	66	42.58	68	59.65	53	73.61		43	27.74	69	50.74	23	47.92	
NA	—	—	—	—	—	—		—	—	—	—	9	—	
TNM stage							0.001							< 0.001
I–II	102	65.81	56	49.12	30	41.67		141	90.97	115	84.56	38	66.67	
III–IV	53	34.19	58	50.88	42	58.33		14	9.03	21	15.44	19	33.33	
Cirrhosis							0.854							0.288
Absent	28	18.06	23	20.18	15	20.83		19	12.26	21	15.44	11	21.15	
Present	127	81.94	91	79.82	57	79.17		136	87.74	115	84.56	41	78.85	
NA	—	—	—	—	—	—		—	—	—	—	5	—	
ALBI grade							0.892							0.011
1	104	67.53	76	66.67	45	64.29		118	76.13	82	60.29	30	62.50	
2	50	32.47	38	33.33	25	35.71		37	23.87	54	39.71	18	37.50	
NA	1	—	—	—	2	—		—	—	—	—	9	—	
Child-Pugh							0.955							0.230 ^{††}
A	142	92.21	104	91.23	64	91.43		117	96.69	82	91.11	40	93.02	
B	12	7.79	10	8.77	6	8.57		4	3.31	8	8.89	3	6.98	
NA	1	—	—	—	2	—		34	—	46	—	14	—	
AFP							0.001							0.294
≤ 25 ng/mL	59	38.06	19	16.67	22	30.56		59	38.06	40	29.41	17	35.42	
> 25 ng/mL	96	61.94	95	83.33	50	69.44		96	61.94	96	70.59	31	64.58	
NA	—	—	—	—	—	—		—	—	—	—	9	—	
ALT							0.315							0.564
≤ 40 U/L	72	46.45	62	54.39	32	44.44		75	48.39	72	52.94	27	56.25	
> 40 U/L	83	53.55	52	45.61	40	55.56		80	51.61	64	47.06	21	43.75	
NA	—	—	—	—	—	—		—	—	—	—	9	—	

Supplemental Table 5 (continued). Correlation Between the Clinicopathological Characteristics and MRS Grouping in the Internal and External Validation Cohorts

Characteristics	Internal Validation Cohort						<i>P</i> *	Combined External Validation Cohort						<i>P</i> *
	MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 114)		MRS ^{high} (<i>n</i> = 72)			MRS ^{low} (<i>n</i> = 155)		MRS ^{int} (<i>n</i> = 136)		MRS ^{high} (<i>n</i> = 57)		
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
HBsAg							0.127							0.026
Positive	128	82.58	104	91.23	62	86.11		138	89.61	107	78.68	37	78.72	
Negative	27	17.42	10	8.77	10	13.89		16	10.39	29	21.32	10	21.28	
NA	—	—	—	—	—	—		1	—	—	—	10	—	
HCVAb							0.718 ^{††}							0.029 ^{††}
Positive	3	1.94	2	1.75	0	0.00		0	0.00	2	1.48	2	4.26	
Negative	152	98.06	112	98.25	72	100.00		152	100.00	133	98.52	45	95.74	
NA	—	—	—	—	—	—		3	—	1	—	10	—	

*Calculated by Chi-Square test, unless otherwise indicated; [†]calculated by Kruskal-Wallis test; ^{††}calculated by Fisher's exact test.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; NA, not applicable.

Supplemental Table 6. Univariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in the Primary Training Cohort

Characteristics	TTR			OS		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age, years (> 49 vs ≤ 49)	1.00	0.71–1.42	0.991	1.04	0.72–1.51	0.847
Sex (male vs female)	0.73	0.45–1.21	0.224	0.88	0.52–1.50	0.646
Tumor encapsulation (present vs absent)	1.30	0.91–1.84	0.150	0.79	0.54–1.16	0.231
Differentiation (III–IV vs I–II)	1.33	0.85–2.08	0.206	1.44	0.92–2.27	0.112
Tumor number (multiple vs single)	1.68	1.15–2.47	0.008	2.01	1.37–2.95	< 0.001
Tumor size, cm (> 5 vs ≤ 5)	1.43	1.00–2.04	0.048	2.12	1.42–3.15	< 0.001
Vascular invasion (present vs absent)	3.37	2.08–5.46	< 0.001	6.12	3.87–9.66	< 0.001
BCLC stage classification (B–C vs 0–A)	2.02	1.42–2.88	< 0.001	3.09	2.12–4.50	< 0.001
TNM stage (III–IV vs I–II)	1.73	1.20–2.51	0.004	2.94	2.02–4.28	< 0.001
T stage (T3–T4 vs T1–T2)	1.79	1.24–2.60	0.002	2.83	1.94–4.12	< 0.001
N stage (N1 vs N0)	0.05	0–1.1E25	0.923	5.40	0.74–39.25	0.096
M stage (M1 vs M0)	0.05	0–974.59	0.551	3.98	0.98–16.18	0.053
Cirrhosis (present vs absent)	2.69	1.54–4.70	< 0.001	2.97	1.55–5.70	0.001
ALBI grade (2 vs 1)	1.30	0.91–1.84	0.145	1.43	0.99–2.08	0.060
Child-Pugh (B vs A)	1.36	0.50–3.67	0.550	1.81	0.74–4.45	0.193
AFP, ng/mL (> 25 vs ≤ 25)	1.45	1.01–2.09	0.046	1.79	1.20–2.69	0.005
ALT, U/L (> 40 vs ≤ 40)	1.09	0.77–1.54	0.642	1.41	0.97–2.05	0.071
HBsAg (positive vs negative)	1.54	0.81–2.94	0.188	0.91	0.50–1.66	0.764
HCVAb (positive vs negative)	1.89	0.26–13.55	0.528	2.29	0.32–16.43	0.411
Myeloid Response Score	1.02	1.02–1.03	< 0.001	1.03	1.02–1.04	< 0.001

Univariate analysis, Cox proportional hazards regression model.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; HR, hazard ratio; NA, not applicable; OS, overall survival; TTR, time to recurrence.

Supplemental Table 7. Univariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in the Primary Test Cohort

Characteristics	TTR			OS		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age, years (> 49 vs ≤ 49)	0.95	0.68–1.34	0.780	0.86	0.59–1.24	0.411
Sex (male vs female)	0.91	0.53–1.56	0.733	0.64	0.34–1.06	0.083
Tumor encapsulation (present vs absent)	0.63	0.44–0.89	0.010	0.55	0.37–0.82	0.003
Differentiation (III–IV vs I–II)	1.04	0.70–1.56	0.835	1.41	0.94–2.12	0.099
Tumor number (multiple vs single)	2.08	1.42–3.05	< 0.001	2.28	1.54–3.37	< 0.001
Tumor size, cm (> 5 vs ≤ 5)	1.41	1.00–1.98	0.047	1.40	0.97–2.03	0.072
Vascular invasion (present vs absent)	3.14	1.82–5.45	< 0.001	5.34	3.31–8.62	< 0.001
BCLC stage classification (B–C vs 0–A)	2.25	1.58–3.20	< 0.001	2.95	2.04–4.27	< 0.001
TNM stage (III–IV vs I–II)	2.56	1.77–3.70	< 0.001	3.38	2.32–4.94	< 0.001
T stage (T3–T4 vs T1–T2)	2.62	1.81–3.80	< 0.001	3.19	2.18–4.68	< 0.001
N stage (N1 vs N0)	2.35	0.86–6.41	0.096	4.13	1.67–10.20	0.002
M stage (M1 vs M0)	NA	NA	NA	241.50	15.11–3860.98	< 0.001
Cirrhosis (present vs absent)	1.38	0.88–2.15	0.158	1.44	0.85–2.45	0.176
ALBI grade (2 vs 1)	0.85	0.59–1.23	0.401	1.06	0.72–1.57	0.768
Child-Pugh (B vs A)	1.95	0.99–3.85	0.054	2.85	1.44–5.65	0.003
AFP, ng/mL (> 25 vs ≤ 25)	1.06	0.75–1.49	0.754	1.73	1.15–2.62	0.009
ALT, U/L (> 40 vs ≤ 40)	1.28	0.91–1.79	0.157	1.29	0.90–1.87	0.171
HBsAg (positive vs negative)	1.20	0.67–2.12	0.542	1.08	0.60–1.97	0.791
HCVAb (positive vs negative)	1.23	0.45–3.32	0.689	0.96	0.24–3.87	0.949
Myeloid Response Score	1.02	1.01–1.02	< 0.001	1.03	1.02–1.03	< 0.001

Univariate analysis, Cox proportional hazards regression model.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; HR, hazard ratio; NA, not applicable; OS, overall survival; TTR, time to recurrence.

Supplemental Table 8. Univariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in the Internal Validation Cohort

Characteristics	TTR			OS		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age, years (> 49 vs ≤ 49)	0.75	0.57–0.99	0.044	0.86	0.65–1.13	0.279
Sex (male vs female)	0.90	0.58–1.39	0.637	1.00	0.64–1.56	0.997
Tumor encapsulation (present vs absent)	0.54	0.40–0.71	< 0.001	0.58	0.44–0.77	< 0.001
Differentiation (III–IV vs I–II)	1.50	1.08–2.09	0.016	1.71	1.24–2.36	0.001
Tumor number (multiple vs single)	1.13	0.83–1.53	0.454	1.16	0.85–1.59	0.345
Tumor size, cm (> 5 vs ≤ 5)	1.77	1.31–2.39	< 0.001	1.65	1.22–2.23	0.001
Vascular invasion (present vs absent)	3.39	2.32–4.95	< 0.001	4.36	3.05–6.23	< 0.001
BCLC stage classification (B–C vs 0–A)	1.65	1.25–2.17	< 0.001	1.81	1.37–2.39	< 0.001
TNM stage (III–IV vs I–II)	1.45	1.15–1.99	0.003	1.49	1.13–1.96	0.004
T stage (T3–T4 vs T1–T2)	1.48	1.12–1.95	0.006	1.45	1.10–1.92	0.008
N stage (N1 vs N0)	1.09	0.68–1.75	0.682	1.38	0.91–2.10	0.130
M stage (M1 vs M0)	1.46	1.03–2.08	0.033	1.35	0.93–1.95	0.114
Cirrhosis (present vs absent)	1.17	0.82–1.67	0.387	1.34	0.92–1.95	0.122
ALBI grade (2 vs 1)	0.82	0.61–1.11	0.207	0.92	0.69–1.24	0.596
Child-Pugh (B vs A)	1.06	0.64–1.77	0.816	1.23	0.77–1.98	0.383
AFP, ng/mL (> 25 vs ≤ 25)	1.84	1.34–2.54	< 0.001	1.69	1.23–2.33	0.001
ALT, U/L (> 40 vs ≤ 40)	1.06	0.80–1.39	0.699	1.00	0.76–1.31	0.987
HBsAg (positive vs negative)	1.61	1.03–2.51	0.035	1.39	0.91–2.11	0.123
HCVAb (positive vs negative)	0.69	0.17–2.79	0.605	1.18	0.38–3.68	0.781
Myeloid Response Score	1.02	1.01–1.02	< 0.001	1.02	1.02–1.03	< 0.001

Univariate analysis, Cox proportional hazards regression model.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; HR, hazard ratio; NA, not applicable; OS, overall survival; TTR, time to recurrence.

Supplemental Table 9. Univariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in the Combined External Validation Cohort

Characteristics	TTR			OS		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age, years (> 49 vs ≤ 49)	1.2	0.92–1.56	0.187	1.03	0.78–1.36	0.839
Sex (male vs female)	1.22	0.87–1.71	0.250	1.24	0.87–1.80	0.238
Tumor encapsulation (present vs absent)	1.13	0.87–1.47	0.355	1.30	0.98–1.71	0.065
Differentiation (III–IV vs I–II)	1.44	1.08–1.92	0.014	1.44	1.06–1.97	0.020
Tumor number (multiple vs single)	1.76	1.26–2.45	0.001	1.72	1.21–2.45	0.002
Tumor size, cm (> 5 vs ≤ 5)	1.56	1.21–2.02	0.001	1.92	1.46–2.53	< 0.001
Vascular invasion (present vs absent)	2.14	1.64–2.79	< 0.001	2.56	1.94–3.39	< 0.001
BCLC stage classification (B–C vs 0–A)	2.22	1.71–2.88	< 0.001	2.82	2.13–3.73	< 0.001
TNM stage (III–IV vs I–II)	3.41	2.49–4.68	< 0.001	4.73	3.41–6.55	< 0.001
T stage (T3–T4 vs T1–T2)	3.45	2.49–4.78	< 0.001	4.77	3.40–6.67	< 0.001
N stage (N1 vs N0)	2.24	0.83–6.03	0.11	2.9	1.08–7.79	0.035
M stage (M1 vs M0)	NA	NA	NA	NA	NA	NA
Cirrhosis (present vs absent)	1.66	1.10–2.50	0.015	1.55	1.01–2.40	0.047
ALBI grade (2 vs 1)	1.31	1.00–1.72	0.049	1.22	0.91–1.63	0.182
Child-Pugh (B vs A)	1.31	0.73–2.36	0.361	1.62	0.88–2.99	0.125
AFP, ng/mL (> 25 vs ≤ 25)	1.41	1.06–1.86	0.017	1.53	1.13–2.08	0.007
ALT, U/L (> 40 vs ≤ 40)	1.16	0.90–1.50	0.259	1.17	0.88–1.54	0.279
HBsAg (positive vs negative)	1.06	0.74–1.51	0.768	0.91	0.50–1.66	0.764
HCVAb (positive vs negative)	1.48	0.55–3.98	0.438	1.47	0.47–4.62	0.506
Myeloid Response Score	1.02	1.01–1.02	< 0.001	1.02	1.02–1.03	< 0.001

Univariate analysis, Cox proportional hazards regression model.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; HR, hazard ratio; NA, not applicable; OS, overall survival; TTR, time to recurrence.

Supplemental Table 10. Multivariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in Each Cohort

Characteristics	Primary-training (<i>n</i> = 244)			Primary-test (<i>n</i> = 244)			Internal validation (<i>n</i> = 341)			Combined External validation (<i>n</i> = 348)		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
TTR												
Age, years (> 49 vs ≤ 49)	—	—	—	—	—	—	—	—	—	—	—	—
Sex (male vs female)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor encapsulation (present vs absent)	—	—	—	—	—	—	0.67	0.50–0.91	0.011	—	—	—
Differentiation (III–IV vs I–II)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor number (multiple vs single)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor size, cm (> 5 vs ≤ 5)	1.58	1.09–2.30	0.017	1.44	1.01–2.07	0.108	1.46	1.06–2.00	0.021	—	—	—
Vascular invasion (present vs absent)	3.17	1.90–5.31	< 0.001	2.21	1.21–4.03	0.010	2.31	1.52–3.51	< 0.001	1.56	1.11–2.21	0.011
BCLC stage classification (B–C vs 0–A)	—	—	—	—	—	—	—	—	—	—	—	—
TNM stage (III–IV vs I–II)	—	—	—	2.24	1.49–3.36	< 0.001	—	—	—	2.32	1.55–4.44	0.002
Cirrhosis (present vs absent)	2.78	1.59–4.89	< 0.001	1.77	1.11–2.83	0.017	—	—	—	2.11	1.31–3.46	< 0.001
ALBI grade (2 vs 1)	—	—	—	—	—	—	—	—	—	—	—	—
Child-Pugh (B vs A)	—	—	—	—	—	—	—	—	—	—	—	—
AFP, ng/mL (> 25 vs ≤ 25)	—	—	—	—	—	—	1.50	1.07–2.10	0.019	—	—	—
ALT, U/L (> 40 vs ≤ 40)	—	—	—	—	—	—	—	—	—	—	—	—
HBsAg (positive vs negative)	—	—	—	—	—	—	—	—	—	—	—	—
HCVAb (positive vs negative)	—	—	—	—	—	—	—	—	—	—	—	—
Myeloid Response Score	1.03	1.02–1.03	< 0.001	1.02	1.01–1.03	< 0.001	1.01	1.01–1.02	< 0.001	1.02	1.01–1.03	< 0.001

Supplemental Table 10 (continued). Multivariate Analyses of Clinicopathological Characteristics Associated with the TTR and OS in Each Cohort

Characteristics	Primary-training (<i>n</i> = 244)			Primary-test (<i>n</i> = 244)			Internal validation (<i>n</i> = 341)			Combined External validation (<i>n</i> = 348)		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
OS												
Age, years (> 49 vs ≤ 49)	—	—	—	—	—	—	—	—	—	—	—	—
Sex (male vs female)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor encapsulation (present vs absent)	—	—	—	—	—	—	0.74	0.55–1.01	0.054	—	—	—
Differentiation (III–IV vs I–II)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor number (multiple vs single)	—	—	—	—	—	—	—	—	—	—	—	—
Tumor size, cm (> 5 vs ≤ 5)	2.38	1.56–3.63	< 0.001	—	—	—	1.47	1.06–2.02	0.021	1.51	1.05–2.17	0.027
Vascular invasion (present vs absent)	4.18	2.58–6.77	< 0.001	3.64	2.13–6.21	< 0.001	3.41	2.28–5.09	< 0.001	1.56	1.11–2.21	0.011
BCLC stage classification (B–C vs 0–A)	—	—	—	—	—	—	—	—	—	1.75	1.16–2.63	0.007
TNM stage (III–IV vs I–II)	—	—	—	3.00	1.96–4.59	< 0.001	—	—	—	2.61	1.60–4.25	< 0.001
Cirrhosis (present vs absent)	2.72	1.41–5.24	0.003	1.95	1.11–3.45	0.021	1.62	1.11–2.38	0.013	2.63	1.52–4.55	0.001
ALBI grade (2 vs 1)	—	—	—	—	—	—	—	—	—	—	—	—
Child-Pugh (B vs A)	—	—	—	—	—	—	—	—	—	2.05	1.10–3.84	0.025
AFP, ng/mL (> 25 vs ≤ 25)	—	—	—	2.02	1.30–3.14	0.002	1.44	1.03–2.03	0.033	—	—	—
ALT, U/L (> 40 vs ≤ 40)	—	—	—	—	—	—	—	—	—	—	—	—
HBsAg (positive vs negative)	—	—	—	—	—	—	—	—	—	—	—	—
HCVAb (positive vs negative)	—	—	—	—	—	—	—	—	—	—	—	—
Myeloid Response Score	1.03	1.02–1.04	< 0.001	1.02	1.02–1.03	< 0.001	1.02	1.02–1.03	< 0.001	1.02	1.01–1.03	< 0.001

The clinicopathological characteristics were put into a Cox proportional hazards regression model, the final equation was determined using the forward selection based on likelihood ratio test. HR, 95% CI and *P* values of the selected parameters in the final regression model are shown. “—” indicates that this parameter was not included in the final regression models.

Abbreviations: AFP, alpha-fetoprotein; BCLC stage, Barcelona Clinic Liver Cancer stage; CI, confidence interval; OS, overall survival; TTR, time to recurrence.

Supplemental Table 13. Characteristics of the Patients before Sorafenib Treatment

Characteristics	MRS ^{low} (n = 25)		MRS ^{int} (n = 13)		MRS ^{high} (n = 13)		P*
	No.	%	No.	%	No.	%	
Age, years							0.290 [†]
Median		53		46		53	
Range		31–70		29–63		23–64	
Sex							0.464
Male	24	96.00	11	84.62	12	92.31	
Female	1	4.00	2	15.38	1	7.69	
Intrahepatic recurrence							0.837
Positive	21	84.00	10	76.92	11	84.62	
Negative	4	16.00	3	23.08	2	15.38	
Extrahepatic spread							0.720
Positive	15	60.00	7	53.85	9	69.23	
Negative	10	40.00	6	46.15	4	30.77	
Macrovascular invasion							0.501
Absent	21	84.00	9	69.23	11	84.62	
Present	4	16.00	4	30.77	2	15.38	
Cirrhosis							0.455
Absent	6	24.00	3	23.08	1	7.69	
Present	19	76.00	10	76.92	12	92.31	
AFP							0.189
≤ 25 ng/mL	13	56.52	3	27.27	8	61.54	
> 25 ng/mL	10	43.48	8	72.73	5	38.46	
NA	2	—	2	—	—	—	
ALT							0.309
≤ 40 U/L	7	31.82	7	53.85	7	53.85	
> 40 U/L	15	68.18	6	46.15	6	46.15	
NA	3	—	—	—	—	—	
HBsAg							0.629
Positive	22	95.65	12	100.00	12	92.31	
Negative	1	4.35	0	0.00	1	7.69	
NA	2	—	1	—	—	—	
HCVAb							NA
Positive	0	0.00	0	0.00	0	0.00	
Negative	23	100.00	12	100.00	13	100.00	
NA	2	—	1	—	—	—	

*Calculated by Chi-Square test, unless otherwise indicated; [†]calculated by Kruskal-Wallis test.

Abbreviations: AFP, alpha-fetoprotein; ALBI grade, albumin–bilirubin grade; ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C virus antibody; NA, not applicable.

Supplemental Table 14. The Correlation Between the MRS and the Immune Class in 21 HCC Samples with Gene Expression Profiles

No. (% of row)	Rest	Immune class	Total	<i>P</i> (χ^2 test)
MRS low	8 (80%)	2 (20%)	10	0.0157
MRS high	3 (27%)	8 (73%)	11	
Total	11	10	21	

Supplemental Table 15. The Correlation Between the Myeloid Response State and the Immune Class in HCC Samples from the TCGA-LIHC Dataset

No. (% of row)	Rest	Immune class	Total	<i>P</i>
Myeloid response state 1	87 (97%)	3 (3%)	90	< 0.0001 (χ^2 test)
Myeloid response state 2	34 (69%)	15 (31%)	49	
Myeloid response state 3	51 (70%)	22 (30%)	73	< 0.0001 (χ^2 test for trend)
Myeloid response state 4	36 (67%)	18 (33%)	54	
Myeloid response state 5	22 (21%)	83 (79%)	105	
Total	230	141	371	

Supplemental Table 16. Antibody Sources and Staining Conditions for Immunohistochemical and Immunofluorescence Assays

Markers	Antibody Source	Clone	Species	Dilution	Pretreatment	Cellular localization	Application
CD11b	Abcam, UK	EPR1344	Rabbit monoclonal	1:2000	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC, TSA
CD14	Sino Biological, China	001	Rabbit monoclonal	1:2000	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC, TSA
CD15	ZSBio, China	MMA+BY87	Mouse monoclonal	1:200	Citrate buffer (pH 6.0) steam 10 min	Membranous and cytoplasmic	IHC, TSA
CD163	ZSBio, China	10D6	Mouse monoclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC, TSA
CD169	R&D Systems, USA	NS0	Sheep polyclonal	1:200	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC, TSA
CD204	Transgenic, Japan	SRA-C6	Mouse monoclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC
CD206	R&D Systems, USA	685645	Mouse monoclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC
CD33	Leica, Germany	PW44	Mouse monoclonal	1:50	EDTA antigen retrieval solution (pH 9.0) steam 10 min	Membranous	IHC
CD68	Dako, USA	PG-M1	Mouse monoclonal	1:200	Citrate buffer (pH 6.0) steam 10 min	Cytoplasmic	IHC, TSA
CD8	ZSBio, China	EP334	Rabbit monoclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC
PD-L1	Cell Signaling Technology, USA	E1L3N	Rabbit monoclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Membranous	IHC, TSA
S100	ZSBio, China	/	Rabbit polyclonal	1:100	Citrate buffer (pH 6.0) steam 10 min	Cytoplasmic	IHC

Supplemental Table 17. Antibody Information for Flow Cytometry Assays

Markers	Antibody Source	Clone	Conjugation
CD11b	Beckman Coulter, USA	Bear1	Phycoerythrin/Cy7
CD14	BD	M5E2	Alexa Fluor 700
CD15	eBioscience, USA	MMA	eFluor 450
CD169	Biolegend, USA	7-239	Allophycocyanin
CD3	eBioscience, USA	OKT3	Alexa Fluor 700
CD4	Biolegend, USA	OKT4	Brilliant Violet 421
CD45	Beckman Coulter, USA	J.33	Krome Orange
CD8	Beckman Coulter, USA	B9.11	Fluorescein isothiocyanate
CD86	Beckman Coulter, USA	HA5.2B7	Phycoerythrin
DCFDA	Sigma-Aldrich		
HLA-DR	BD	G46-6	Brilliant Violet 421
PD-1	eBioscience, USA	J105	Allophycocyanin

Supplemental References

1. Xu J, et al. An in situ molecular signature to predict early recurrence in hepatitis B virus-related hepatocellular carcinoma. *J Hepatol.* 2012;57(2):313-21.
2. Liu CQ, et al. Expression patterns of programmed death ligand 1 correlate with different microenvironments and patient prognosis in hepatocellular carcinoma. *Br J Cancer.* 2018;119(1):80-8.
3. Li XF, et al. Increased autophagy sustains the survival and pro-tumourigenic effects of neutrophils in human hepatocellular carcinoma. *J Hepatol.* 2015;62(1):131-9.
4. Meng YM, et al. Monocytes/Macrophages promote vascular CXCR4 expression via the ERK pathway in hepatocellular carcinoma. *Oncoimmunology.* 2018;7(3):e1408745.
5. Wu C, et al. Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis. *J Clin Invest.* 2018;128(8):3425-38.
6. Ritchie ME, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 2015;43(7):e47.
7. Leek JT, et al. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics.* 2012;28(6):882-3.
8. Yu G, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS.* 2012;16(5):284-7.
9. Colaprico A, et al. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res.* 2016;44(8):e71.
10. Trapnell C, et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol.* 2014;32(4):381-6.
11. Heagerty PJ, Lumley T, and Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics.* 2000;56(2):337-44.
12. Vickers AJ, and Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Medical decision making : an international journal of the Society for Medical Decision Making.* 2006;26(6):565-74.
13. Camp RL, Dolled-Filhart M, and Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res.* 2004;10(21):7252-9.
14. Li B, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol.* 2016;17(1):174.
15. Newman AM, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods.* 2015;12(5):453-7.
16. Becht E, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol.* 2016;17(1):218.
17. Bindea G, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* 2013;39(4):782-95.
18. Gabilovich DI, and Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* 2009;9(3):162-74.
19. Bronte V, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* 2016;7:12150.

20. Okita Y, et al. Role of tumor-infiltrating CD11b+ antigen-presenting cells in the progression of gastric cancer. *J Surg Res.* 2014;186(1):192-200.
21. Cui TX, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity.* 2013;39(3):611-21.
22. Holness CL, and Simmons DL. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood.* 1993;81(6):1607-13.
23. Ding T, et al. High tumor-infiltrating macrophage density predicts poor prognosis in patients with primary hepatocellular carcinoma after resection. *Human Pathology.* 2009;40(3):381-9.
24. Kuang DM, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. *Hepatology.* 2010;51(1):154-64.
25. Fan QM, et al. Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancer Lett.* 2014;352(2):160-8.
26. Kong LQ, et al. The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma. *PLoS One.* 2013;8(3):e59771.
27. Zhang Y, et al. CD169 identifies an anti-tumour macrophage subpopulation in human hepatocellular carcinoma. *J Pathol.* 2016;239(2):231-41.
28. Li JQ, et al. Distinct patterns and prognostic values of tumor-infiltrating macrophages in hepatocellular carcinoma and gastric cancer. *J Transl Med.* 2017;15(1):37.
29. Jensen TO, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. *J Clin Oncol.* 2009;27(20):3330-7.
30. Yeung OW, et al. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. *J Hepatol.* 2015;62(3):607-16.
31. Wang B, et al. High CD204(+) tumor-infiltrating macrophage density predicts a poor prognosis in patients with urothelial cell carcinoma of the bladder. *Oncotarget.* 2015;6(24):20204-14.
32. Hou YC, et al. Coexpression of CD44-positive/CD133-positive cancer stem cells and CD204-positive tumor-associated macrophages is a predictor of survival in pancreatic ductal adenocarcinoma. *Cancer.* 2014;120(17):2766-77.
33. Shu QH, et al. Prognostic value of polarized macrophages in patients with hepatocellular carcinoma after curative resection. *J Cell Mol Med.* 2016;20(6):1024-35.
34. Dong P, et al. CD86(+)/CD206(+), Diametrically Polarized Tumor-Associated Macrophages, Predict Hepatocellular Carcinoma Patient Prognosis. *Int J Mol Sci.* 2016;17(3):320.
35. Tan-Garcia A, et al. Intrahepatic CD206(+) macrophages contribute to inflammation in advanced viral-related liver disease. *J Hepatol.* 2017;67(3):490-500.
36. Kuang DM, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol.* 2011;54(5):948-55.

37. Li YW, et al. Intratumoral neutrophils: a poor prognostic factor for hepatocellular carcinoma following resection. *J Hepatol.* 2011;54(3):497-505.
38. Zhou SL, et al. Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. *Hepatology.* 2012;56(6):2242-54.
39. He M, et al. Peritumoral stromal neutrophils are essential for c-Met-elicited metastasis in human hepatocellular carcinoma. *Oncoimmunology.* 2016;5(10):e1219828.
40. Ouyang FZ, et al. Dendritic cell-elicited B-cell activation fosters immune privilege via IL-10 signals in hepatocellular carcinoma. *Nat Commun.* 2016;7:13453.