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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Total RNA was extracted from the HCC tissues using Qiagen kits. RNA sequencing (RNA-seq) was performed on Illumina Novaseq at the MIT BioMicro Center Core Facility (Cambridge, MA).

Data analysis	FastQC (V0.11.9) was performed for the quality control of RNA-seq raw data. After quality control, the low-quality bases and adaptors contamination was removed by Cutadapt (V1.18). The quality of clean yield data was examined again by FastQC software. Next, the clean data were aligned to the mouse reference genome mm39 by STAR (V2.7.9a). After data mapping, samtools (V1.3.1) and featureCounts (V2.0.1) were used to count the number of reads aligned to the gene features. DESeq2 (1.42.1) identified the differentially expressed genes. Differentially expressed genes were annotated using REACTOME databases, and the cell type enrichment analysis was performed using the xCell package (V1.1.0). For the multi-cohort RNA sequencing analysis, the data were obtained and integrated from our previous published studies; after the batch effect removal by the sva package (3.50.0), the normalized data were used for the cell type deconvolution by immunedeconv package (V2.1.0). To demonstrate the effect size of the cell type score calculated by different methods in immunedeconv, Cohen's d value was applied to measure the differences of each cell type between immunoactivator and control groups. The network plot of enriched pathways was performed by the clusterProfiler package (4.10.1). Single-sample gene set enrichment analysis (ssGSEA) was used to calculate a Breg score based on specific markers. TCGA data analyses were performed using the log-rank Mantel-Cox test using web server GEPIA2, based on TCGA liver hepatocellular carcinoma (TCGA-LIHC) data. Single-cell RNA-seq (scRNA-seq) data were processed following the Seurat package tutorial (V5.2.1). Signature scores were computed using the AddModuleScore function, and cell-cell interactions between B cells and other cell populations were examined using the CellChat package (V2.2.0) according to its standard workflow. Spatial transcriptomic data from HCC clinical samples were analyzed with Seurat. Colocalization was defined as a spot with non-zero expression of both HAVCR1 and either CD79A or CD79B. Spatial images were generated using the SpatialFeaturePlot function. The custom code used for analysis in this study is deposited in a Github repository (https://github.com/Lewisen-Liu/HCC_B_cell).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The RNA-sequencing data from this study are deposited in the Gene Expression Omnibus (GEO) under accession numbers GSE301030, GSE301138, GSE301139, GSE301140. The public scRNA-seq data of HCC patients treated with neoadjuvant anti-PD-1 therapy were obtained from GEO database with an accession number of GSE206325. The spatial transcriptomic data of HCC samples treated with neoadjuvant anti-PD-1 were obtained from Mendeley Data (DOI: 10.17632/skrx2fz79n.1). The public scRNA-seq data of murine B cells were obtained from GEO database with an accession number of GSE174739. The remaining data are available within the Article, Supplementary Information or Source Data file. Source data are provided within this manuscript.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	No human participants.
Reporting on race, ethnicity, or other socially relevant groupings	No human participants.
Population characteristics	No human participants.
Recruitment	No human participants.
Ethics oversight	No human participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculations were performed. Animal sample size was determined by the number of biological replicates necessary for ensuring statistical significance. The number of biological replicates are also reported in the relevant figure legends in the manuscript. For experiments other than animal studies, the sample size (n) of each experiment is provided in the figure captions in the manuscript and supplementary information accordingly. Sample sizes were chosen to support meaningful conclusions. In addition, we adhere to sample size

	requirements necessary for determining statistical significance.
Data exclusions	No data were excluded.
Replication	All attempts at replication were successful. Experimental repeat numbers are also reported in Figure Legends.
Randomization	All samples were randomly allocated into experimental groups.
Blinding	Investigators were blinded to group allocation during data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[ ]	[x] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

For flow cytometric analyses, harvested cells were washed with the buffer and stained with the cell surface antibodies. Anti-mouse CD16/32 antibody (clone 93, Biolegend, San Diego, California, USA) was added for FcR blockade and incubated for 5 minutes at room temperature; 7-amino-actinomycin D or zombie UV was added for live/dead staining. After another washing step, antibodies for cell phenotyping were added, and cells were incubated for 30 minutes at room temperature. The monoclonal antibodies used for flow cytometry analysis were specific for CD45 (BioLegend, 30-F11), CD3 (BioLegend, 17A2), CD19 (BioLegend, 1D3/CD19), B220 (BioLegend, RA3-6B2), CD11b (BioLegend, M1/70), CD80 (BioLegend, 16-10A1), IL-10 (BioLegend, JES5-16E3), TIM-1 (BD Biosciences, RMT1-4), PD-L1 (BioLegend, 10F.9G2), MHC II (BioLegend, M5/114.15.2), CD11c (BioLegend, N418), CD86 (BioLegend, GL-1), CD8a (BioLegend, 53-6.7), CD4 (BioLegend, GK1.5), and IFN-γ (BioLegend, XMG1.2).

Mouse anti-CD19 (clone 1D3), anti-B220 (clone RA3.3A1/6.1), anti-TIM-1 (clone 3B3), anti-PD1 (clone RMP1-14), and anti-VEGFR2 (clone DC101) were purchased from BioXCell (Lebanon, NH). Anti-CD19 and anti-B220 depleting antibodies (10 mg/kg, every 5 days for 3 weeks), anti-TIM-1 blocking antibodies antibody (10 mg/kg, every 5 days for 20 days), and anti-PD1 and anti-VEGFR2 blocking antibodies were administered by intraperitoneal (i.p.) injections (anti-PD1: 10mg/kg, anti-VEGFR2: 20mg/kg, every 3 days for 21 days). Corresponding isotypes of IgG were administered i.p. at the same frequency as the other antibodies. B-cell depletion was validated by flow cytometry analysis of peripheral blood mononuclear cells collected 5 days after the last antibody dose.

Immunofluorescence: We used an anti-CD31 antibody (Millipore, clone 2G8) to identify endothelial cells, an anti-α-SMA antibody (Sigma, clone 1A4) to identify perivascular cells, anti-CD3 (Abcam #ab135372), anti-CD8 (CST, #98941) and anti-CD4 (Abcam, #ab288724) antibodies for T cells staining, and anti-CD19 (Abcam #ab245235, Proteintech #65290-1-Ig) and anti-B220 (R&D #MAB1217) antibodies for B cells staining. Anti-TIM-1 (Abcam #ab316854), anti-IL-10 (Abcam, #ab313401), anti-CD21 (Abcam, #ab318999), and anti-PNAd (BioLegend, #120805) were used for co-staining with anti-B220. All secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

Validation

All primary antibodies were purchased from commercial vendors (BD, Biolegend, eBioscience, Abcam and Cell Signaling Technology) and used per manufacturers' recommendations. All the antibodies were validated by the suppliers. Validations can be found in the manufacturer's website. In addition, many have literature references and species specifications on their websites. CD4, CD8, CD11c, CD80, CD86 et al. are routinely used antibodies. Dilution factors were pre-determined in the lab. Flow data suggests clear isolation of the cell populations.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Cell line sources were provided under "Method: Cells and culture condition" section. HCA-1 from C3H mice, established in our laboratory, and RIL-175 (a p53/Hras mutant HCC cell line from C57Bl/6 mice, a kind gift from Dr. Tim Greten, NIH).
Authentication	The cell lines were certified by the manufacturers (surface markers, morphology).
Mycoplasma contamination	Mycoplasma contamination was routinely performed before in vivo studies for all cell lines using MycoAlert Mycoplasma Detection Kit (Lonza #LT07-318).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Laboratory animals were provided under "Method: Animal studies" and "Orthotopic HCC mouse models" sections. Animal experiments were performed in the animal facility of Massachusetts General Hospital under specific pathogen-free conditions. All animal experiments were performed under the Institutional Animal Care and Use Committee (IACUC) at Massachusetts General Hospital-approved protocol (2020N000023). Studies complied with all guidelines outlined regarding animal research in the IACUC Policies and Guidance of MGH Research Institute. In the therapeutic studies, orthotopic HCCs were induced by intrahepatic injection of HCA-1 cells in syngeneic male C3H mice, while RIL-175 cells were implanted in syngeneic male C57Bl/6 mice. The mice were purchased from the MGH Center for Comparative Medicine. Six-to-8-week-old male mice were used for experiments. Carbon tetrachloride (CCl4) is administered for 12 weeks after tail-vein injection of Cre-expressing adenovirus (adeno-Cre) in Stk4−/−Stk3F/− (also known as Mst1−/−Mst2F/−) mice to induce HCC concomitantly with liver fibrosis.
Wild animals	No wild animals were used in this study.
Reporting on sex	Male mice were used for experiments.
Field-collected samples	The samples did not involve samples collected from the field.
Ethics oversight	All animal experiments were performed after approval by the Institutional Animal Care and Use Committee of the Massachusetts General Hospital.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	No clinical data in this study.
Study protocol	No clinical data in this study.
Data collection	No clinical data in this study.
Outcomes	No clinical data in this study.

Plants

Seed stocks	No plants in this study.
Novel plant genotypes	No plants in this study.
Authentication	No plants in this study.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Harvested cells were washed with the buffer and stained with the cell surface antibodies. Anti-mouse CD16/32 antibody (clone 93, Biolegend, San Diego, California, USA) was added for FcR blockade and incubated for 5 minutes at room

	temperature; 7-amino-actinomycin D or zombie UV was added for live/dead staining. After another washing step, antibodies for cell phenotyping were added, and cells were incubated for 30 minutes at room temperature. The monoclonal antibodies used for flow cytometry analysis were specific for CD45 (BioLegend, 30-F11), CD3 (BioLegend, 17A2), CD19 (BioLegend, 1D3/CD19), B220 (BioLegend, RA3-6B2), CD11b (BioLegend, M1/70), CD80 (BioLegend, 16-10A1), IL-10 (BioLegend, JES5-16E3), TIM-1 (BD Biosciences, RMT1-4), PD-L1 (BioLegend, 10F.9G2), MHC II (BioLegend, M5/114.15.2), CD11c (BioLegend, N418), CD86 (BioLegend, GL-1), CD8a (BioLegend, 53-6.7), CD4 (BioLegend, GK1.5), and IFN-γ (BioLegend, XMG1.2).
Instrument	The stained cells were measured on flow cytometers (BD LSR II and Cytek Aurora)
Software	The stained cells were analyzed by FlowJo software (Flowjo V10.8.1)
Cell population abundance	The numbers presented in the flow cytometry analysis images are percentage based
Gating strategy	Based on the pattern of FSC-A/SSC-A, cells were used for analysis of tumor immune microenvironmet. Singlets were gated according to the pattern of FSC-H vs FSC-A. Positive populations were determined by the specific antibodies, which were distinct form negative populations. Gating was first based on FSC/SSC together with viability dyes and singlet populations. The cell populations within the gate were further analysized based on expression of markers.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.