nature portfolio

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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<br>*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<br>*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 | Leka scanner, Odessey scanner, Confocal microscopy(Cell Observer, ZEISS), FACS Celesta (BD Biosciences) equipped with FACS Diva software (BD Biosciences), BGISEQ-T7, Illumina NovaSeq platform, Cell Ranger (v.6.1.1) |
| Data analysis | R(v.4.2.0), IOBR(v.0.99.0), GSVA(v.1.52.3), Seurat (v.5.2.0), DoubletFinder (v2.0.3), NMF(v0.28), Monocle (v.2.26.0), pySCENIC (v.0.12.1), SCENIC (v.1.3.1), CellChat (v.1.6.1), BANKSY(v1.0.0), Python(3.7-3.10), Cell2location(v0.1.5), SpaGCN(v1.2.7), OpenCV-python(v4.9.0.80), Hallo software(v4.2.6399.280) |

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 raw sequence data generated in this study have been deposited the Genome Sequence Archive67 in the National Genomics Data Center68, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences , under accession number HRA012807 [https://ngdc.cncb.ac.cn/gsa-human/

browse/HRA012807] (raw sequencing data). These data are available under controlled access for ethical restrictions on human genetic resources, in compliance with the Regulation on the Management of Human Genetic Resources of China. Access requests can be submitted through the GSA-Human platform (https://ngdc.cncb.ac.cn/gsa-human/policy/policy.jsp) and are subject to review by the GSA-Human data access committee, with approved access typically granted within one month. The publicly available data used in this study are available in the GSA-Human database under accession numbers HRA005478 [https://ngdc.cncb.ac.cn/gsa-human/browse/HRA005478], HRA002336 [https://ngdc.cncb.ac.cn/gsa-human/browse/HRA002336], and HRA000051 [https://ngdc.cncb.ac.cn/gsa-human/browse/HRA000051]. The remaining data are available within the Article, Supplementary Information or Source Data file.

## 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 | Sex was considered as a standard baseline demographic variable in our study design. The sex of participants (defined as biological attribute) was determined based on administrative medical records assigned at admission. While sex data were collected, specific sex-based mechanistic or survival analyses were not performed a priori, as the primary focus of this study is the fundamental interaction between neutrophil aging, EBV reactivation, and TLS formation within the tumor microenvironment, which are immunological and virological processes not currently known to be predominantly driven by sex differences. |
| Reporting on race, ethnicity, or other socially relevant groupings | The study cohort consists of patients of Asian descent (predominantly Han Chinese), accurately reflecting the local demographic admitted to Peking University Cancer Hospital & Institute. Information regarding patient ethnicity was obtained from administrative electronic medical records collected upon admission. Because our cohort was ethnically homogeneous and geographically specific, race or ethnicity was not included as an active categorization variable in our statistical analyses, nor was it used as a proxy for socioeconomic status or other confounding variables. |
| Population characteristics | The study included human participants diagnosed with locally advanced gastric cancer. Covariate-relevant characteristics assessed include age, sex, treatment, EBV status , clinical TNM stage, Tumor Grade, Tumor Location, Lauren classification, lymphovascular invasion, perineural invasion, and treatment response categorized by Tumor Regression Grade (TRG) . Detailed population characteristics and clinico-pathological features are comprehensively listed in Supplementary Table 1/2/3. |
| Recruitment | Participants were retrospectively recruited from the clinical databases and pathology archives of Peking University Cancer Hospital & Institute between 2004 and 2023. Inclusion strictly required a confirmed diagnosis of EBVaGC, completion of neoadjuvant chemotherapy, and availability of sufficient formalin-fixed paraffin-embedded (FFPE) tissue samples for spatial analysis. Given the retrospective design, potential self-selection and survivorship biases may exist (e.g., patients with inoperable advanced disease or those who abandoned chemotherapy were excluded). To mitigate bias, we consecutively included all eligible patients who met the criteria during the specified timeframe. We believe these inherent limitations of retrospective sampling do not impact the fundamental biological and prognostic findings regarding TLS formation and neutrophil dynamics reported in this study. |
| Ethics oversight | This study has been approved by the Ethics Committee of Peking University Cancer Hospital (No. 2021KT15). |

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 statistical methods were used to predetermine the sample size for the retrospective clinical cohort. The sample size was determined by the availability of eligible patient tissue samples matching our inclusion criteria (diagnosed with EBVaGC and received neoadjuvant chemotherapy) at our institution between 2004 and 2023. This sample size (n=144) is comparable to or larger than those reported in similar published studies and was sufficient to reach statistical significance. For in vitro and in vivo experiments, sample sizes were determined based on standard practices in the field and prior laboratory experience, with a minimum of 3 independent biological replicates. |
| Data exclusions | Yes, data exclusions were pre-established. For the clinical cohort, patients were excluded if they met any of the following criteria: (1) incomplete clinical or follow-up records; (2) received prior anti-tumor treatments before neoadjuvant chemotherapy; (3) insufficient tumor tissue quality for multiplex immunofluorescence or histological evaluation. No data were excluded from the in vitro experimental analyses. |
| Replication | All experimental findings were successfully replicated. For in vitro studies, all experiments were independently repeated at least three times with similar results, unless otherwise stated in the figure legends. For the clinical tissue analyses (e.g., IHC and multiplex immunofluorescence), scorings of Tertiary Lymphoid Structures (TLS) and neutrophils were performed independently by two experienced pathologists to ensure reproducibility, and concordant results were achieved. |
| Randomization | For the retrospective clinical cohort, randomization is not relevant and was not applied, as patient allocation into groups (e.g., Responders vs. |

Non-responders) was determined objectively by clinical outcomes (e.g., Tumor Regression Grade) rather than investigator assignment. Covariates in clinical analyses were controlled through multivariable regression models.

Blinding

Yes, blinding was implemented. Investigators who performed the histological scoring, quantitative analysis of TLS, and multiplex immunofluorescence evaluations of clinical tissues were strictly blinded to the patients' clinical metadata, treatment responses, and survival outcomes.

# 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

mIHC or IHC: PanCK (1:2000, Clone PDH09-10, Cat# HA601138, HUABIO), BZLF1 (1:400, Clone BZ1, Cat# sc-53904, SANTA CRUZ), CD20 (1:2000, Clone #06, Cat# 11007-MM06, Sino Biological), CD23 (1:500, Clone PD00-03, Cat# HA721139, HUABIO), CD66b (1:20000, Clone EPR25354-2, Cat# ab300122, Abcam), MPO(1:200, Polyclonal, Cat# AF3667, R&D), CitH3(1:200, Polyclonal, Cat# ab5103, Abcam), CXCR4 (1:500, Clone UMB2, Cat# ab124824, Abcam), CXCL12 (1:100, Polyclonal, Cat# 17402-1-AP, proteintech), αSMA (1:8000, Polyclonal, Cat# 14395-1-AP, proteintech), NE (1:500, Clone EPR7479, Cat#ab131260, Abcam), CD8 (1:100, Clone C8/144B, Cat#ab17147, Abcam), CD68 (1:1000, Polyclonal, Cat#ab125212 Abcam), PD-L1 (1:500, Clone EPR19759, Cat#ab213524, Abcam )

FACS: Fluorophore-conjugated antibodies against human CD45-PerCP/Cyanine5.5 (0.5 µg per 106 cells in 100 µl volume, Clone HI30, Cat#304027, BioLegend), CD16-PE (0.5 µg per 106 cells in 100 µl volume, Clone 3G8, Cat#302007, BioLegend), CD10-PE/Cyanine7 (5 µL per 106 cells in 100 µl volume, Clone HI10a, Cat#312213, BioLegend), and CD184(CXCR4)-BV421 (5 µL per 106 cells in 100 µl volume, Clone 12G5, Cat#306517, BioLegend) , isotype control BV421 Mouse IgG2a, κ Isotype Ctrl (5 µL per 106 cells in 100 µl volume, Clone MOPC-173, Cat#400259, BioLegend)

ICC: goat anti-MPO(1:40, Polyclonal, Cat#AF3667, R&D) and rabbit anti- CitH3(1:200, Polyclonal, Cat#ab5103, Abcam), donkey anti-goat IgG H&L (Alexa Fluor® 647, ab150135), donkey anti-rabbit IgG H&L (Alexa Fluor® 488, ab150061)

WB: mouse anti-EBV ZEBRA (BZLF1) (1:100, Clone BZ1, Cat#sc-53904, SANTA CRUZ) , rabbit anti-NE (1:500, Clone EPR7479, Cat#ab131260, Abcam), rabbit anti-PR3 (1:2000, Polyclonal, Cat#A17522, Abclonal), rabbit anti-PD-L1 (1:1000, Clone EPR19759, Cat#ab213524, Abcam), rabbit anti-LOX1 (1:1000, Clone EPR20750, Cat#ab214427, Abcam), rabbit anti-MMP9 (1:1000, Clone D6O3H, Cat#13667T, CST) , rabbit anti-VEGFA (1:1000, Polyclonal, Cat#A12303, Abclonal) , and rabbit anti-α/βTubulin (1:1000, Polyclonal, Cat#2148, CST)

Validation

All primary antibodies used in this study are commercially available and have been extensively validated by their respective manufacturers (including Abcam, Cell Signaling Technology, Santa Cruz Biotechnology, R&D Systems, Proteintech, and alphaxbio) for the specific species (Human/Mouse) and applications (WB, IHC, mIHC, ICC, FACS) utilized in this study. Detailed validation statements, expected molecular weights for Western Blot, and standard cellular localization patterns can be found on the manufacturers' official websites.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

AGS (human gastric adenocarcinoma, EBV-negative) was obtained from ATCC (Cat# CRL-1739); SNU-719 (human gastric carcinoma, EBV-positive) was purchased from Nanjing Kebai Biotechnology (Cat# CBP60511); C666-1 (human nasopharyngeal carcinoma, EBV-positive) was purchased from Shangon Biotech (Cat# SNL-516).

Authentication

All cell lines (SNU719, AGS, and C666-1) were authenticated by Short Tandem Repeat (STR) DNA profiling.

Mycoplasma contamination

All cell lines were confirmed to be negative prior to all in vitro and in vivo experiments.

Commonly misidentified lines
(See ICLAC register)

None of the cell lines used in this study are listed in the ICLAC database of commonly misidentified cell lines.

## 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.

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|---|---|
| Clinical trial registration | Not Applicable |
| Study protocol | Not Applicable |
| Data collection | Clinical data and archived formalin-fixed paraffin-embedded (FFPE) tumor tissue samples were retrospectively collected from patients diagnosed with EBV-associated gastric cancer at Peking University Cancer Hospital & Institute, Beijing, China. The data collection covered a cohort of patients who were diagnosed and received neoadjuvant chemotherapy between 2004 and 2023. |
| Outcomes | The primary clinical outcome measure was the pathological response to neoadjuvant chemotherapy, which was assessed using the Tumor Regression Grade (TRG) system (or pathological Complete Response, pCR) evaluated from surgical resection specimens. Secondary outcomes included Overall Survival (OS) and Disease-Free Survival (DFS), calculated from the date of initial diagnosis to the date of death, disease recurrence, or last follow-up. |

## Plants

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| Seed stocks | Not Applicable |
| Novel plant genotypes | Not Applicable |
| Authentication | Not Applicable |

## 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

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| Sample preparation | Neutrophils were isolated and resuspended in PBS buffer. Prior to antibody staining, Fc receptors were blocked using Human TruStain FcX™ (BioLegend) to prevent non-specific binding. True-Stain™ Multi-Fluor Buffer (BioLegend) was added to mitigate spectral spillover. The cell suspension was then incubated with a multicolor antibody cocktail on ice for 20 minutes. |
| Instrument | Data collection was performed using a BD FACSCelesta™ flow cytometer (BD Biosciences). |
| Software | Data acquisition was performed using BD FACSDiva™ software (BD Biosciences). Post-acquisition data analysis was conducted using FlowJo software (Tree Star). |
| Cell population abundance | Not Applicable |
| Gating strategy | Preliminary gating utilized FSC and SSC to identify the main cell population and exclude debris/doublets. Dead cells were subsequently excluded using eBioscience™ Fixable Viability Dye eFluor™ 506. From the live cell gate, neutrophils were precisely defined and gated as CD45+ CD16+ CD10+ SSChi. To evaluate the senescent/aged neutrophil subpopulation (CXCR4 +), the boundary between "positive" and "negative" CXCR4 expression was strictly determined by using an appropriate isotype control (BV421 Mouse IgG2a, κ Isotype Ctrl) to establish accurate background fluorescence thresholds. |

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