

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Flow cytometry data were collected using Life Technologies Attune software. Images were captured using Axio Observer Z1M (Zeiss), Nikon confocal microscope, ECHO Revolve (ECHO), and Olympus IX73 (Olympus) microscopes. Image adjustment was performed using ZEN software (v3.0, blue edition, Zeiss). Animal images were taken by SII Lago X imaging system. Sequencing data were generated using Illumina NovaSeq 6000 software (v1.5).
Data analysis	Single-cell RNA-seq data were processed and analyzed using Cell Ranger (v7.0.1, 10x Genomics), Seurat (v4.4.0), Scanpy (v1.9.3), scVelo (v0.3.2). Cell-cell communication was analyzed using CellChat (v1.6.1). Gene set enrichment analyses were performed with fgsea (v1.28.0). Additional analyses were performed with pandas (v2.0.3), Scissor (v2.1.0), and PHATE (v1.0.11). Spatial transcriptomic data was analyzed by Xenium Explorer (v3.2.0) and Seurat. Statistical analyses were performed using GraphPad Prism (v9.4.0, Dotmatics). No custom code was developed beyond the tools cited above.

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](#)

Xenium In Situ, scRNA-seq, and CUT&RUN data are available via the GEO database (GSE304811; log-in token for reviewers: yvzuoscbtsnbmj, GSE304907; log-in token for reviewers: elotekeelxsdvoh, GSE305003; log-in token for reviewers: ixutkeymbvujjmj). Code is available at GitHub (https://github.com/jaeilparklab/DGAC_RKP) and is archived on Zenodo under (<https://doi.org/10.5281/zenodo.21630485>).

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 recorded as a biological variable based on clinical records. No sex-based stratified analyses were performed, as the study was not designed or powered to detect sex-specific differences in gene expression.

Reporting on race, ethnicity, or other socially relevant groupings

Race and ethnicity were not analyzed in this study, as the cohort consisted of patients treated at a single institution and race/ethnicity was not considered relevant to the biological questions addressed.

Population characteristics

The study included resected tumor specimens from patients with advanced gastric cancer (pathological stage $\geq T2$). A total of 975 consecutive surgical cases were screened, and targeted DNA and RNA sequencing was conducted using the TruSight Oncology 500 628 (TSO500, Illumina) panels. The TSO500 panel comprises 523 cancer-related genes utilized for 629 calculating tumor mutation burden (TMB), 130 regions for assessing microsatellite instability 630 (MSI), and an equal number of genes as TST170 for detecting potential amplification, RNA 631 fusions, and RNA splice variants. Available clinicopathological variables, age, and Lauren classification were included. Approximately 40–50% of tumors were classified as diffuse-type gastric cancer, enabling further analyses by histological subtype.

Recruitment

Tumor specimens were retrospectively identified from consecutive surgical cases at a single institution. No active recruitment or patient contact was involved.

Ethics oversight

The study was approved by the Institutional Review Board (IRB) of the participating institution. All human tissue samples were obtained from surgical specimens, and the requirement for informed consent was waived due to the retrospective nature of the study.

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](https://www.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

For all in vitro experiments, at least three independent biological replicates were included. No formal sample-size calculation was performed for these experiments; sample sizes were selected based on established experimental practice and were considered sufficient to assess reproducibility across independent experiments. For animal studies, sample sizes were determined by power calculation based on the expected effect size and variability. The exact sample sizes are provided in the corresponding figure legends. Samples and animals were randomly allocated to experimental groups before the initiation of treatment or data collection.

Data exclusions

No data were excluded from the analyses. All collected data that passed quality control were included in the study.

Replication

All experiments presented in this study were performed using at least three biological replicates, unless otherwise stated. All presented findings were reproducible across biological replicates.

Randomization

Mice were randomly assigned to experimental groups to minimize selection bias. For in vitro assays, randomization was not relevant to the study design.

For tumor growth experiments, drug administration and tumor measurements were performed independently by investigators blinded to group allocation. Investigators were also blinded to group allocation during data 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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies Source Cat #
Rat anti-MKI67 Thermo Fisher Scientific Cat# 14-5698-82, 1:200
RRID: AB_10854564
Rabbit anti-cleaved CASPASE3 Cell Signaling Technology Cat# 9664S, 1:200
RRID:AB_2070042
Rat anti-CD45-CoraLite Plus 488 Proteintech Cat#: CL488-65139, 1:100
RRID: AB_2883199
Rabbit anti-EPCAM Cell Signaling Technology Cat#: 93790, 1:200
RRID: AB_2800214
Rabbit anti-MUC1 Proteintech Cat#: 23614-1-AP, 1:200
RRID: AB_2879304
Mouse anti-MUC4 Thermo Fisher Scientific Cat#: 35-4900, 1:200
RRID: AB_2533206
Rabbit anti-YAP1 Cell Signaling Technology Cat#: 14074,1:200
RRID: AB_2650491
Rabbit anti-Amphiregulin Proteintech Cat#: 16036-1-AP, 1:200
RRID: AB_2227602
Rabbit anti-CD3 Cell Signaling Technology Cat# 99940, 1:200
RRID:AB_2755035
Rabbit anti-CTLA4 Cell Signaling Technology Cat#: 53560, 1:200
RRID: AB_3107072
Rabbit anti-PDCD1 Cell Signaling Technology Cat# 84651, 1:200
RRID:AB_2800041
InVivoPlus anti-mouse CD3e Bio X Cell Cat# BE0001-1
RRID: AB_1107634
InVivoMab anti-mouse CD28 Bio X Cell Cat# BE0015-1
RRID: AB_1107624
Goat anti-Amphiregulin R&D Systems Cat# AF989
RRID: AB_2060663
Pacific Blue™ anti-mouse TNF-α Biolegend 506318, 1:50
PE/Cyanine7 anti-mouse IFN-γ Antibody Biolegend 505825, 1:50
PE anti-mouse CD45 Biolegend 103106, 1:200
FITC anti-mouse CD3 Biolegend 100306, 1:50
APC anti-mouse CD8a Biolegend 100712, 1:50
Brilliant Violet 421™ anti-mouse CD279 (PD-1) Biolegend 135217, 1:50
PE anti-mouse CD152 Biolegend 106305, 1:50
Alexa Fluor 647 goat anti-rabbit IgG Invitrogen A-21245, 1:200
Alexa Fluor 555 goat anti-rabbit IgG Invitrogen A-21434, 1:200
Alexa Fluor® 488 goat anti-rabbit IgG (H+L) Invitrogen A-11008, 1:200

Validation

All antibodies are well-recognized clones in the field and validated by the manufacturers.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Mouse GC cell lines (EKP and RKP; Mus musculus, male and female) were established from genetically engineered mouse models (GEMMs) as described in the Methods section.
Authentication	Cell lines were confirmed by genotyping and morphological verification.
Mycoplasma contamination	Cell lines have been routinely tested and confirmed negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines listed in the ICLAC register were used in this study.

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	C57BL/6 mice were purchased from The Jackson Laboratory. For in vivo tumor studies, subcutaneous and orthotopic gastric injections were performed, followed by pharmacological treatment to assess therapeutic efficacy. Mice were housed under specific pathogen-free conditions with a 12-hour light/12-hour dark cycle at an ambient temperature and humidity of 40-70%. Animals had ad libitum access to food and water.
Wild animals	Not applicable.
Reporting on sex	Both male and female mice were used.
Field-collected samples	This study did not involve any field-collected samples.
Ethics oversight	All mice were maintained in compliance with the guidelines of the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center. All animal procedures were performed based on the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care and institutional (MD Anderson) approved protocols. This study was compliant with all relevant ethical regulations regarding animal research.

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	Not applicable. This study did not involve a prospective clinical trial.
Study protocol	This study was a retrospective, observational analysis of archived surgical specimens and clinicopathological data, conducted under institutional IRB approval (IRB No. 4-2014-0638).
Data collection	Data collection included clinicopathological variables such as TNM stage, WHO and Lauren classifications. Covariate-relevant characteristics of the human research participants included age, sex, and available clinical information. These characteristics are summarized in Supplementary Table S1.
Outcomes	Targeted sequencing results on CDH1 and RHOA1 genes were assessed, and their associations were analyzed.

Plants

Seed stocks	Not applicable. This study did not involve plants or plant-derived materials.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.

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	Details are descried in the method section.
Instrument	Flow cytometry data were acquired using an Attune NxT Flow Cytometer (Thermo Fisher Scientific).
Software	Data were collected using Attune NxT software (Thermo Fisher Scientific) and analyzed using FlowJo software (version 10, BD Biosciences).
Cell population abundance	Cell populations were gated based on CD45+ expression.
Gating strategy	Sequential gating was applied using FSC/SSC to exclude debris and doublets, followed by CD45 ⁺ selection for immune populations. CD3 ⁺ T cells were subsequently separated into CD4 ⁺ and CD8 ⁺ subsets. R
☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	