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](https://nature.portfolio) contains articles on many of the points above.

**Software and code**

Policy information about [availability of computer code](https://nature.portfolio)

**Data collection**

Flow cytometry data were collected using the Invitrogen Attune NxT Acoustic Focusing Cytometer. Quantitative PCR data were obtained with the Bio-Rad CFX96 Real-Time System. Fluorescent images were obtained using the Vectra Polaris Automated Quantitative Pathology Imaging System. Immunoblotting images were obtained using the ChemiDoc Touch Imaging System (Bio-Rad) and Image Lab Touch software (Bio-Rad, version 2.3.0.07). IHC images were obtained using a fully automated digital pathology slide-system (KFBIo, KF-PRO-005).

**Data analysis**

Flow cytometry data were analysed using FlowJo (FlowJo, version 10.4). Immunofluorescent images were analysed using ImageJ (version 1.52p) or Phenochart (version 1.0.12). Statistical analysis was performed using Graphpad Prism (GraphPad software, version 8). CyTOF data were analyzed using Cytobank (https://premium.cytabank.org/cytobank/login). The correlation of expression levels of two genes was analyzed using the R corplot package and the cor function. Survival analysis was performed using the R survival package.

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](https://nature.portfolio)

All manuscripts must include a [data availability statement](https://nature.portfolio). 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](https://nature.portfolio)

GR (encoded by NR3C1) mRNA levels in paired normal pancreatic tissue and PDAC were obtained from the dataset GSE15471 in the Gene Expression Omnibus
TCGA gene expression data were obtained from The Cancer Genome Atlas data portal (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). The source data that support the findings of this study are available with no restrictions. The uncropped blots are shown in Supplementary Fig. 9. Source data are provided with this paper.

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.

- [x] 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 calculation was done either for in vitro or in vivo studies. For in vivo and in vitro studies, sample sizes were determined based on our preliminary experiments. In our experience, n = 5-7 mice per group (in vivo) and n = 3-4 samples per group (in vitro) are sufficient to detect meaningful biological differences with good reproducibility. |
| Data exclusions | No data or animals were excluded from analysis. |
| Replication | Except for the animal studies (one time), chemokine array analysis (one time), and tissue microarray analysis (one time), each experiment was repeated at least three times with similar results. |
| Randomization | Mice were randomly assigned to different treatment groups. |
| Blinding | For cell-based experiments, Western blotting, flow cytometry, and in vitro assays, blinding was not performed, because the investigator had to know the groups to load the samples or perform the assay. Blinding was not performed in mouse experiments. The investigator needed to know the treatment groups in order to perform the study. |

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

Antibodies used:

- Anti-mouse CD8α antibody (200 μg, clone 2.43, BE0061, Bio X Cell; RRID: AB_1125541)
- Anti-rat IgG2b isotype control (200 μg, LTF-2, BE0090, Bio X Cell; RRID: AB_1107780)
- Anti-mouse PD-1 antibody (100 μg, clone RMP1-14, BP0146, Bio X Cell; RRID: AB_10949053)
- Anti-rat IgG2a isotype control (100 μg, clone 2A3, BE0091; RRID: AB_1107769)
- Hamster IgG (100 μg, Bio X Cell, BE0091; RRID: AB_1107773)
- CD3 (1:200, Cell Signaling Technology, 9940S, RRID: AB_2755035)
- CDR8 (1:200, Cell Signaling Technology, 98945S, RRID: AB_2756376)
- Granzyme B (1:200, Cell Signaling Technology, 44153S, RRID: AB_2857976)
- Anti-rat IgG2a isotype control (100 μg, clone C4.3, BE0089, Bio X Cell; RRID: AB_1107769)
- Anti-GR (1:200, Sigma-Aldrich, SAB4501309, RRID: AB_10744954)
- Anti-PD-1 (1:200, Genetex, GTX01796)
- Anti-MHC-I (1:200, Santa Cruz, sc55582B, RRID: AB_831547)
CD8 (1:100, Mxb Biotechnologies, RMA-0514)

Antibodies used for immunoblotting:
- GR (1:100, Proteintech, 24050-1-AP, RRID: AB_2813890)
- Phospho-GR (Ser211) (1:1000, Cell Signaling Technology, 4161S, RRID: AB_2155797)
- GAPDH (1:2000, Proteintech, 60004-1-IG, RRID: AB_2107436)
- PD-L1 (1:100, Cell Signaling Technology, 13684S, RRID: AB_2687655)
- MHC-I (1:500, Santa Cruz Biotechnology, sc-55582, RRID: AB_831547)
- MHC-I (1:500, Santa Cruz Biotechnology, sc-32325, RRID: AB_627934)
- B2M (1:1000, Cell Signaling Technology, 2851S, RRID: AB_561284)
- PD-L1 (1:1000, Cell Signaling Technology, 149995, RRID: AB_2737027)
- PR (1:1000, Proteintech, 25871-1-AP, RRID: AB_2880277)

Antibodies used for flow cytometry:
- PD-L1: APC, 10F.9G2, 1:80, BioLegend, 124312
- CD45: PE, 30-F11, 1:100, BioLegend, 103106
- CD45: FITC, 30-F11, 1:100, BioLegend, 103108
- CD3: APC, 145-2C11, 1:40, BioLegend, 100312
- B2M: APC, A16041A, 1:40, BioLegend, 154506
- H-2Kb: PE, 29E.2A3, 1:40, BioLegend, 119703
- IFNγ: PE-Cy7, XMG1.2, 1:40, BioLegend, 135215
- PD-1: PE-Cy7, 29F.1A12, 1:80, BioLegend, 135215
- LAG-3: PE, C9B7W, 1:40, BioLegend, 135215
- TGF-β: PE-Cy7, 1F10, 1:40, BioLegend, 135215

Antibodies used for CyTOF analysis:
- For HY24409 tumors:
  - CD11b 139La M1/70 1:500 BioLegend 101249
  - CD11c 142Nd N418 1:400 BioLegend 117302
  - CD4 153Nd 145-2C11 1:500 BioLegend 100702
  - CD8a 146Nd 53-6.7 1:500 BioLegend 100702
  - F4/80 171Yb D2S9R 1:400 CST 70076BF
- For HY24160 tumors:
  - CD4 153Nd 145-2C11 1:500 BioLegend 100702
  - CD8a 146Nd 53-6.7 1:500 BioLegend 100702
  - Ly-6C 162Dy HK1.4 1:600 DVS-Fluidigm 3162014B
  - Ly-6G/Ly-6C, Gr-1 141Pr 1A8 1:400 DVS-Fluidigm 3141008B

Validation

All antibodies used are commercially available and validated by the manufacturers. Pre-validated antibodies were purchased from reputable sources. All proteins are well studied and all antibodies are widely used in the literature. The experiments included appropriate controls. We validated the GR-specific antibody by using two independent GR shRNAs.

Antibodies used for mouse experiments:
- anti-mouse CD8α antibody (BE0061, Bio X Cell; RRID: AB_1125541) https://bxcell.com/product/m-cd8a-2/
- rat IgG2b isotype control (BE0090, Bio X Cell; RRID: AB_1107780) https://bxcell.com/product/rat-igg2b-isotype-control/
anti-mouse PD-1 antibody [BP0146, Bio X Cell; RRID: AB_10049053] https://bxcell.com/product/mipinvoplus-anti-m-pd-1
anti-mouse CTLA-4 antibody [BE0032, Bio X Cell; RRID: AB_1107598] https://bxcell.com/product/m-cd152-m-ctla-4-2
rat IgG2a type control [BE0089, Bio X Cell; RRID: AB_1107769] https://bxcell.com/product/rat-igg2a-isotype-control
hamster IgG [BE0091, Bio X Cell; RRID: AB_1107773] https://bxcell.com/product/polyclonal-3

Antibodies used for immunofluorescent staining:
CD3 (Cell Signaling Technology, 999405, RRID: AB_2063055) https://www.cellsignal.com/products/primary-antibodies/cd3e-d4v8l-rabbit-mab-99940
CD8 (Cell Signaling Technology, 989415, RRID: AB_2756376) https://www.cellsignal.com/products/primary-antibodies/cd8a-d4v22-xp-rabbit-mab-mouse-specific-98941
Granzyme B (Cell Signaling Technology, 441535, RRID: AB_2857975) https://www.cellsignal.com/products/primary-antibodies/granzyme-b-e5v2l-rabbit-mab-mouse-specific-44153

Antibodies used for immunoblotting:
GR (Proteintech, 24050-1-AP, RRID: AB_2813890) https://www.ptglab.com/products/NR3C1-Antibody-24050-1-AP.htm
Phospho-GR (Ser211) (Cell Signaling Technology, 4161S, RRID: AB_2155797) https://www.cellsignal.com/products/primary-antibodies/phospho-glucocorticoid-receptor-ser211-antibody-4161
GAPDH (Proteintech, 60004-1-IG, RRID: AB_2155797) https://www.cellsignal.com/products/GAPDH-Antibody-60004-1-Ig.htm
MHC-I (Santa Cruz, sc-55582, RRID: AB_831547) https://www.scbt.com/p/mhc-class-i-antibody-f-3
GAPDH (Proteintech, 60004-1-IG, RRID: AB_2155797) https://www.cellsignal.com/products/GAPDH-Antibody-60004-1-Ig.htm
MHC-I (Santa Cruz, sc-55582, RRID: AB_831547) https://www.scbt.com/p/mhc-class-i-antibody-f-3
PD-L1 (GeneTex, GTX01796) https://www.genetex.com/Product/Detail/PD-L1-antibody-ZR3/GTX01796
B2M: BioLegend, 316304 https://www.biolegend.com/en-us/products/fitc-anti-human-beta2-microglobulin-antibody-3079
B2M: BioLegend, 154506 https://www.biolegend.com/en-us/products/apc-anti-mouse-beta2-microglobulin-antibody-15126

Antibodies used for IHC:
GR (Sigma-Aldrich, SAB4501309, RRID: AB_10744954)  https://www.sigmaaldrich.com/US/en/product/sigma/sab4501309
Foxp3: DVS-Fluidigm 3158003A https://store.fluidigm.com/Cytometry/ConsumablesandReagentsCytometry/MaxparAntibodies/Anti-Mouse%20CD4%20-30-F11-89Y%20%5B40100%5D?tests?cclcl=en_US
CD45: BioLegend, 103108 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd45-antibody-99
CD4: BioLegend, 100714 https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd8a-antibody-2269
CD8: BioLegend, 100406 https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd8a-antibody-248
H-2Kb: BioLegend, 116520 https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-h-2kb-antibody-15162
H-2Kb: BioLegend, 116520 https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-h-2kb-antibody-15162
CD11c: BioLegend, 117302 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd11c-antibody-117302
CD19: BioLegend, 115502 https://www.biolegend.com/en-us/products/apc-anti-human-cd19-antibody-15132
CD25: BioLegend, 101902 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd25-antibody-954
TNFα: BioLegend, 506305 https://www.biolegend.com/en-us/products/pe-anti-mouse-tnf-alpha-antibody-978
IL-12: BioLegend, 506305 https://www.biolegend.com/en-us/products/pe-anti-mouse-tnf-alpha-antibody-978
LAG-3: BioLegend, 125207 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd223-lag-3-antibody-4486

Antibodies used for flow cytometry:
CD5: BioLegend, 124312 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd5-antibody-66557
CD4: BioLegend, 103106 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd4-antibody-100
CD8: BioLegend, 100714 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd8a-antibody-2269
CD4: BioLegend, 100406 https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd4-antibody-248
B2M: BioLegend, 154504 https://www.biolegend.com/en-us/products/apc-anti-mouse-beta2-microglobulin-antibody-15126

Antibodies used for CyTOF analysis
CD11b BioLegend 101249 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd11b-30-F11-89Y%20%5B40100%5D?tests?cclcl=en_US
CD8a BioLegend 100702 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd8a-antibody-9159
CD11c BioLegend 117302 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd11c-antibody-117302
CD19 BioLegend 115502 https://www.biolegend.com/en-us/products/pe-anti-human-cd19-antibody-15132
CD25 BioLegend 101902 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd25-antibody-954
CD3, CD8, CD104, CD11c BioLegend 100105 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd3-antibody-100105
CD45:BioLegend 100315 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd45-antibody-99

Request summary
March 2021

Nature Portfolio
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  The BXPC-3 (ATCC), Mlapaca-2(ATCC), Capan-1(ATCC), ASPC-1(ATCC), Colo357/FG, L3.6PL, Panc28, Capan-2(ATCC), CF-Pac-1(ATCC), Panc3, Panc1(ATCC), Panc48, HPAC(ATCC), HS766T(ATCC), SU86.86(ATCC), and SW1990 (ATCC) cell lines were from Dr. Mien-Chie Hung's lab stock. HY24409, HY19636, and HY24160 cell lines were from Dr. Haoqiang Ying. MCF-7 and HEK293T were purchased from ATCC.

Authentication  Short tandem repeat (STR) profiling was done by ATCC and MD Anderson's Characterized Cell Line Core Facility.

Mycoplasma contamination  All cell lines were confirmed to be mycoplasma free with a mycoplasma detection kit and treated with Plasmocin for the Prevention of mycoplasma contamination.

Commonly misidentified lines  No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals  Male and female C57BL/6 mice were from MD Anderson’s internal supply or the Jackson Laboratory, and we performed the surgery when the mice were 6-8 weeks old. Male NSG (non-obese diabetic; severe combined immunodeficiency; interleukin-2 receptor gamma chain null) mice were from MD Anderson’s internal supply, and six-week-old NSG mice received subcutaneous injection of tumor cells.

Mice were housed at 70F-74F (set point: 72F) with 40%-55% humidity (set point: 45%). The light cycle of animal rooms is 12 h of light and 12 h of dark.

Wild animals  This study did not involve wild animals.

Field-collected samples  This study did not involve samples collected in the field.

Ethics oversight  All animal studies were performed in accordance with a protocol (PI: Li Ma) approved by the Institutional Animal Care and Use Committee of MD Anderson Cancer Center.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics  Our research did not involve human subjects, but used human samples from the Cancer Hospital of the University of Chinese Academy of Sciences. All PDAC specimens were obtained with informed consent from patients who underwent surgical resection of primary tumors. They were de-identified specimens.

Recruitment  We did not recruit patients.

Ethics oversight  The collection and use of human samples were approved by the Ethics Committee of Cancer Hospital of the University of Chinese Academy of Sciences, following the Declaration of Helsinki ethical guidelines.

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

For cultured cell lines, cells were incubated with the Accutase Cell Detachment Solution (BioLegend, 423201) and were washed twice with PBS. For the immunophenotyping of tumors, tumor samples were dissociated on the gentleMACS Dissociator (Miltenyi Biotec) using the Mouse Tumor Dissociation Kit (Miltenyi Biotec, 130-096-730) and were depleted of red blood cells using RBC Lysis Buffer (BioLegend, 420301). 1 × 10^6 cells per sample were used for staining.

**Instrument**

Cells were analyzed on an Invitrogen Attune NxT Acoustic Focusing Cytometer.

**Software**

Cells were analyzed on an Invitrogen Attune NxT Acoustic Focusing Cytometer and analyzed by FlowJo software (FlowJo, LLC, version 10.4).

**Cell population abundance**

At least 10,000 cells were analyzed for each sample.

**Gating strategy**

Gating strategies are described in the Methods section (Flow cytometry) and Supplementary Table 2.

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