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 | FACS Canto (BD Biosciences, USA) was used for flow cytometry data collection. IVIS lumina (Perkin Elmer) for IVIS Spectrum bioluminescent imaging acquisition. Rotor-gene Q RT-PCR cycler (Quiagen, Germany) was used for evaluation of gene expression. CTL ImmunoSpot Analyzer (Cellular Technology, Shaker Heights, OH, USA) for IFN-γ ELISpot assay. Carl Zeiss Microscopy Gmbh (Jane, Germany) was used for immunofluorescent tissue staining. Automated analyzer (Hitachi instrument, Tokyo, Japan) was used to analyze clinical chemistry parameters for evaluating systemic toxicity. VersaMax Absorbance Microplate Reader was used to analyse cytokines. |
| Data analysis | Flowjo v10 was used for analysis of flow cytometry data. ImmunoSpot Professional Software version 5.0 for detecting IFN-γ spot-forming cells. GraphPad Prism 9.3.0 was used for statistical analysis. Fluorescent images processing: Mouse live imaging and ex vivo imaging were performed by Living ImageR 4.7.4 sortware, IVIS lumina, Perkin Elmer. Quantitative of western blot: image lab 6.0 Biorad, Excel 365 |

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 data (Figures 1-6, Supplementary Figures, Supplementary tables) that provide the finding of present study are available within the paper and its supplementary information. Additional data are available from the authors upon reasonable request.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | Not applicable |
| Population characteristics | Not applicable |
| Recruitment | Not applicable |
| Ethics oversight | Not applicable |

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 | We calculated sample size in this study to be n = 3 - 33 mice per group in vivo depending on the experiments, is sufficient to perform statistical significance with good reproducibility. The number of independent experiments was indicated in each figure legend. |
| Data exclusions | No data were excluded from the manuscript |
| Replication | All experimental replicates are reported in figure legends, the finding were reliably reproduced. |
| Randomization | All in vivo tumor model, the animals were randomly selected from different cages to house them in one new cage for tumor implantation and each mouse were used for experiment only one to make sure the starting tumor burden in different treatment groups was similar before treatment. All groups were age and sex matched. |
| Blinding | Blinded experiments were not possible. Animals were treated by blinded independent researcher. |

# 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 |

## Methods

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

# Antibodies

Antibodies used

For Western blotting:
Rabbit anti-ClyA (Polyclonal Ab, lab preparation),HRP-conjugated rabbit anti-mouse immunoglobulins, (polyclonal Ab, Dako/P0260), HRP-conjugated goat anti-rabbit immunoglobulins (Polyclonal Ab, Dako/P0448), Rabbit anti-FlaB (Polyclonal Ab, Chonnam National University), Rabbit anti-ALDH18A1 (Polyclonal Ab, Novus Biologicals/NBP1-83324), Rabbit anti-Mtch1 (Polyclonal Ab, Novus Biologicals/NBP1-69285), Rabbit anti-E2F8 (Polyclonal Ab, Abcam/ab109596), Rabbit anti-GluD1 (Polyclonal Ab, Thermo Fisher Scientific /PA5-28301), Anti Rabbit β-actin (Clone 13E5, cell signalling/4970L), Rabbit anti-TetR antibody (Polyclonal, Novusbio/ NB600-234)
Elisa assay:
IL-12 p70 mouse uncoated ELISA kit with plates (Thermo Fisher Scientific /88-7121-22), IL-1β Mouse Uncoated ELISA Kit with Plates (Thermo Fisher Scientific /88-7013-22), IFN-γ mouse ELISA Kit (Invitrogen/BMS606), IL-4 mouse ELISA Kit (Invitrogen/BMS606),TNF alpha mouse Elisa Kit, High Sensitivity ( Thermal Fisher, BMS607HS), Mouse IFN-γ T cell ELISpot Kit (U-CyTech/CT317-PB5)
Flow cytometry:
Anti-mouse CD16/32 (BioLegend/101320), Anti-TLR5-Alexa Fluor 488 (clone 19D579.2, IMGENEX/IMG-664AF488) , Anti-mouse CD197 (CCR7)-PE (Clone 4B12, eBioscience/12-1971-82), ant-calreticulin (clone D3E6, Cell Signaling Technology/12238S), Anti-rabbit IgG (H & L) (Cell Signaling Technology/4412S), Rabbit (DA1E) mAb IgG XP isotype control (clone DA1E, cell signalling/#55742S), Live/ dead Fixable aqua dead cell stain kit (Invitrogen/L34957), Anti-mouse CD45.2-Pacific blue (Clone 104,BioLegend/109820), , Anti-mouse CD3-FITC (Clone 17A2, eBioscience/11-0032-82), Anti-mouse CD11c-FITC (Clone N418, eBioscience/11-0114-82), Anti-mouse 11b-FITC ( Clone M1/70, BioLegend/101205), Anti-mouse CD4-APC (Clone GK1.5, BioLegend/100412), Anti-mouse CD8a-PE (Clone 53-6.7, eBioscience/12-0081-82), Anti-mouse CD274-PE (Clone 10F.9G2, BioLegend/12-0081-82), Anti-mouse F4/80-PE (Clone BM8, eBioscience/124801-82), Anti-mouse IL-1β PE (Clone CRM56, eBioscience/12-7018-82), Anti-mouse CD86-PE (Clone GL1, eBioscience/12-0862-82), Anti-mouse CD49-PE (Clone DX5, BioLegend/108908), Anti-mouse FOXP3-APC (Clone FJK-16s, eBioscience/77-5775-40), Anti-mouse CD49-APC (Clone DX5, BioLegend/108909), Anti-mouse CD4-APC (Clone GK1.5, BioLegend/100412), Anti-mouse Gr-1-APC (Clone 1A8, BioLegend/127614), Anti-mouse CD274-APC (Clone B7-H1, BioLegend/124312), Anti-mouse CD206-APC (Clone B7-H1, BioLegend/C068C2), Anti-mAnti-mouse granzyme B-PerCP-Cy5.5 (ouse CD25-PerCP-Cy5.5 (Clone PC61, BioLegend/102030), Anti-mouse CD279-PerCP-Cy5.5 (Clone RMP130, BioLegend/109120), Anti-mouse granzyme B-PerCP-Cy5.5 (Clone QA16A02, BioLegend/372212), Anti-mouse CD8-PerCP-Cy5.5 (Clone 53-6.7, BioLegend/100733), Anti-mouse CD62L-PerCP-Cy5.5 (Clone MEL-14, BioLegend/104432), Anti-mouse MHCII-PerCP-Cy5.5 (Clone M5/114.15.2, BioLegend/107625 ), Anti-mouse Ly-6G-PerCP-Cy5.5 (Clone RB6-8C5, BioLegend/127615), Anti-mouse F4/80-PerCP-Cy5.5 (Clone BM8, BioLegend/123128), Anti-mouse Ki67-PE-Cy7 (Clone SolA15, Thermo Fisher Scientific/25469882), Anti-mouse CD152-PE-Cy7 (Clone UC10-4B9, BioLegend/106313), Anti-mouse CD8 PE-Cy7 (Clone 53-6.7, eBioscience/25-0081-82), Anti-mouse CD206 PE-Cy7 (Clone C068C2, BioLegend/141719), Anti-mouse CD11c PE-Cy7 (Clone N418, Thermo Fisher Scientific/25-0114-82), Anti-mouse IFNγ-APC-Cy7 (Clone XMG1.2, BioLegend/505849), Anti-mouse PD-1-APC-Cy7 (Clone 29F.1A12, BioLegend/135224), Anti-mouse CD44-APC-Cy7 (Clone IM7, BioLegend/103028), Anti-mouse CD86-APC-Cy7 (Clone GL-1, BioLegend/105029).
Neutralizing Abs:
Anti-mouse CD4 (Clone GK 1.5, Bio X Cell/BP0003-1), Anti-mouse CD8α (Clone 2.43, Bio X Cell/BP0061), Rat IgG2b isotype control Ab (Clone LTF-1, Bio X Cell/BP0090), Anti-HMGB1, ChIP grade (Polyclonal Ab, Abcam/ab18256), Rabbit IgG, ChIP grade (Polyclonal Ab, Abcam/ab171870), InVivoMAb anti-mousr CSF1R (CD115) (Bioxcell, BE0213)

Validation

All antibodies were validated by the manufactures and used following supplied instructions. In certain cases, these antibodies also were re-validated and routinely used in our lab

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

CT26 (mouse colon carcinoma, CRL-2638), 4T1 (mouse breast cancer, CRL-2539), and HEK293T (human epithelial cell, CRL-3216) cell lines were purchased from the American Type Culture Collection (ATCC, USA). MC38 (murine colon adenocarcinoma, Kerafast ENH204-FP) and Luciferase/tdTomato dual-reporter MC38 (murine colon adenocarcinoma, Alstem LRL04) (MC38-Fluc-RFP) cell lines were purchased from Kerafast or Alstem Cell Advancements, respectively. The MDA-MB-231-FLuc-GFP (human triple-negative breast cancer, SC044, Gen Target, USA) and HepG2-FLuc (human hepatocellular carcinoma cells stably expressing firefly luciferase, HB-8065) cell lines were kindly provided by Dr. Mee Sun Yoon (Chonnam National University, Republic of Korea).

Authentication

Cell lines purchased from ATCC, Karafast, Alstem were frozen at early passage, thus did not require additional authentication.

Mycoplasma contamination

Negative mycoplasma testing in all cell lines were carry out in this study

| | |
|---|---|
| Commonly misidentified lines (See ICLAC register) | No cell line used in this study is listed in ICLAC database |

## 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 | Female (6-week-old) BALB/c, C57BL/6, and BALB/c athymic nu−/nu− mice were purchased from Orient (Seongnam, Korea) and maintain in specific pathogen-free animal facility for one week prior to performing any experiments. The mice were grouped and housed 5 mice per plexiglass cage (22.3 x 26.8 x 12.0 cm), maintained 12h/12h at ligh/dark cycle in a temperature-controlled room (22°C) and 40-60% humidity, with free access to water and food (PMI Nutrition International, LLC 4001 Lexington Avenue North, Arden Hills, MN 55126). Cages were arranged with full autoclave wood fiber (Northeastern Product Corp., Warrensburg, NY, 12885) and changed by clean cages 2-3 times/week. No enrichment material was used inside the cage. At the moment of the experiments, mice weighed approximately 19-20 grams and aged 7 weeks. The animals were randomly selected from different cages to house them in one new cage for tumor implantation, and each mouse was used for experiment only once. |
| Wild animals | This study did not involve wild animals. |
| Reporting on sex | All the mice were conducted on female mice. |
| Field-collected samples | This study did not involve samples collected from the filed. |
| Ethics oversight | All in vitro and animal care and experimental procedures were implemented in accordance with the guidelines of the Animal Experimental and Ethics Committe at Chonnam National University (Ethics Approval no. CNU IACUC-H-20222-16) and the National Centre of the Replacement, Refinement, and Reduction on Animals in Research |

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

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|---|---|
| Sample preparation | Solid tumors, tumor-draining lymph nodes (TdLNs), spleens and well perfused metastatic livers were extracted from mice 9 or 12 days after bacterial treatment and soaked in 2 ml of isolation buffer (RPMI 1640 medium containing 5% FBS, 1% L-glutamine, 1% penicillin-streptomycin, and 10 mM HEPES). After mechanical homogenization, the cell preparations were treated with 1 mg/ml collagenase type IV (Roche, Switzerland) and 50 µg/ml DNase I (Roche, Switzerland) and then incubated for 45 min at 37°C. Next, 2 ml of each sample were mixed with an equal volume of 1× lysis buffer (BioLegend, USA) and incubated for 4 min at 37°C to remove red blood cells. The cells were then filtered through 40-µm cell strainers (BD Falcon, USA). Finally, the cells were washed sequentially with isolation buffer and FACS buffer (2% FBS in DPBS). Cells were then incubated for 10 min at room temperature with FcR blocking buffer (BioLegend) to prevent non-specific binding. The samples were then stained for 30 min on ice with specific fluorescent-dye-conjugated antibodies. Dead cells were excluded by staining with Live/Dead cell viability reagent. To detect tumor-antigen-specific cytokine production by T cells, tumor-infiltrated lymphocytes (TILs) were stimulated for 6 h at 37°C in a CO2 incubator with PMA/ionomycin cell activation cocktail in the presence of Golgi Plug (containing brefeldin A) (BD Biosciences). Subsequent surface staining and intracellular staining were performed using FOXP3 transcription factor staining Kit (eBioscience). |
| Instrument | FACS Canto flow cytometer (BD Biosciences, USA) |
| Software | FlowJo software V10.8.1 (Becton, Dickinson & Company, USA). |
| Cell population abundance | N/A |

Gating strategy

All samples are gated on live cells from population of total cells (using SSC-A and FSC-A) and sing cells (using FSC-H/FSC-A). CD45+ cells were gated for analysis of either T cells and the other immune cells. Endogenous T cells were gated on CD45 +CD3+CD4+/CD8+ and analyzed for phenotype and cytokine production. Neutrophils, M1-like macrophages, M2-like macrophages, activated dendritic cells, Treg cells, memory CD4+ T cells, Memory CD8+ T cells were gated on CD45+CD11b +,Gr-1+, CD45+F4/80+CD86+, CD45+F4/80+CD206+, CD45+CD11c+CD86+MHCIIHi, CD45+CD3+CD4+FOXP3+CD25+, CD45 +CD3+CD4+CD44+CD62L+, CD45+CD3+CD8+CD44+CD62L+, respectively. Proportion of positive gates is evaluated using a biologic control or an isotype or FMO control.

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