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 *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	Immunoblot data was collected using a ChemiDoc MP Imaging Station (Bio-Rad) and associated ImageLab software . RTqPCR data was collected using a QuantStudio-5 (ThermoFisher Scientific). ELISA data was collected using a FLUOstar microplate reader (BMG Labtech) and associated Omega software. Real-time impedance-based cytolysis data was acquired using the xCELLigence® RTCA SP instrument software (Agilent Technologies). BD FacsDiva software was used for flow cytometry raw data acquisition. In vivo imaging data was collected using IVIS Living Image Software (PerkinElmer).
Data analysis	TCGA data was acquired and analyzed using using Bioconductor v3.18, "RTCGA.rnaseq" RStudio package. Flow cytometry data analysis was performed using FlowJo Software v10.9 (FlowJo LLC). GraphPad Prism was used for all otherd ata analysis and data visualization.

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 that support the findings of this study are available from the corresponding author upon reasonable request. Data availability statement included in 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	De-identified healthy human donors were acquired at random from vendor (StemCell Technologies), and thus no relevant reports on sex and gender were collected.
Reporting on race, ethnicity, or other socially relevant groupings	De-identified healthy human donors were acquired at random from vendor (StemCell Technologies), and thus no relevant reports on race, ethnicity, or other socially relevant groupings were collected.
Population characteristics	De-identified healthy human donors were acquired at random from vendor (StemCell Technologies), and thus no relevant population characteristics were collected.
Recruitment	No patient recruitment. Human PBMCs for study were acquired at random from vendor (StemCell Technologies).
Ethics oversight	Human PBMCs for study were acquired at random from vendor (StemCell Technologies). IRB and ethics oversight available upon request from vendor.

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	For all in vivo efficacy/safety studies, >5 mice per treatment group was used per standard experimental design principles.
Data exclusions	None
Replication	All in vitro studies (where applicable) were performed with three separate healthy human T cell donors to ensure reproducibility. All in vivo efficacy studies employed a representative donor.
Randomization	For all efficacy studies, mice were assigned to treatment groups based on equitable average tumor burden.
Blinding	Blinding to treatment groups was only necessary for caliper measurements of DLD-1 subcutaneous colorectal challenge (Fig 3A) and histopathology evaluation (Fig 5G). All other efficacy studies were evaluated and analyzed by objective measurements.

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

αDSG2 primary antibody; Abcam, #ab150372
αGAPDH primary antibody; Cell Signaling Technology, #2118
anti-rabbit horseradish peroxidase-conjugated secondary antibody; Jackson ImmunoResearch Labs, #111-035-144
αDSG2-PE flow antibody, Clone: CSTEM28; ThermoFisher Scientific, #12-9159-42
IgG2bκ-PE isotype control flow antibody, Clone: eBMG2b; ThermoFisher, #12-4732-81
αCD4-BV421 flow antibody, Clone: SK1; BioLegend, #344706
αCD8-PE flow antibody, Clone: OKT4; BioLegend, #317434
αIFNγ-APC flow antibody, Clone: XMG1.2; BioLegend, #505810
αTNFα-PE/Cγ7 flow antibod, Clone MP6-XT22; BioLegend, #506324

Validation

αDSG2 primary antibody; Abcam, #ab150372 - @1:1000
αGAPDH primary antibody; Cell Signaling Technology, #2118 - @1:1000
anti-rabbit horseradish peroxidase-conjugated secondary antibody; Jackson ImmunoResearch Labs, #111-035-144 - @1:15000
αDSG2-PE flow antibody, Clone: CSTEM28; ThermoFisher Scientific, #12-9159-42 - @1:200
IgG2bκ-PE isotype control flow antibody, Clone: eBMG2b; ThermoFisher, #12-4732-81 - @1:200
αCD4-BV421 flow antibody, Clone: SK1; BioLegend, #344706 - @1:200
αCD8-PE flow antibody, Clone: OKT4; BioLegend, #317434 - @1:200
αIFNγ-APC flow antibody, Clone: XMG1.2; BioLegend, #505810 - @1:200
αTNFα-PE/Cγ7 flow antibod, Clone MP6-XT22; BioLegend, #506324 - @1:200

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

All human cell lines used in this study are described in Supplementary Table 1. No primary human cell lines were generated for this study. All primary human T cells were isolated from peripheral blood leukopaks (STEMCELL Technologies, #200-0470) with male/female representation.

Authentication

Cell lines directly acquired from ATCC are authenticated at point of sale. For all human cell lines acquired elsewhere, ATCC STR profiling (ATCC, #135-XV) was used to confirm reference cell identity with American Type Culture Collection (ATCC).

Mycoplasma contamination

All cell lines tested using ATCC, #30-1012K. All negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

None

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

Immunocompromised NSG-MHC I/II DKO mice age 8-10 weeks were used for all human tumor efficacy studies. Immune-competent human DSG2 transgenic (hDSG2tg) mice age 8-10 weeks were used for all toxicity/safety studies. All mice were housed and cared for in the Thomas Jefferson University animal facilities in a pathogen-free environment under a strict 12hr light/dark cycle in sterile IVC cages.

Wild animals

None

Reporting on sex

All animal experiments used sex-matched mice in both experimental/controls groups unless otherwise specified. The MDA-MB-231 breast cancer xenograft model used exclusively female mice, while the DU145 prostate cancer xenograft model used exclusively male mice.

Field-collected samples

None

Ethics oversight	All animal protocols and experiments were conducted in compliance with Thomas Jefferson University Institutional Animal Care and Use Committee (IACUC)–approved protocol #01529.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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 DSG2 Cell Surface Quantification: DSG2 protein cell surface quantification was enumerated via flow cytometry with BD QuantiBRITE-PE beads (BD Biosciences, #340495) for reference. Cell lines were thawed, cultured in appropriate media (Supplementary Table 1) until ~90% confluency was achieved, at which point cells were trypsinized and then quenched with ice-cold media. Cell suspensions were transferred to polystyrene FACS tubes, washed twice with cold FACS buffer, and incubated for 15 min with FC Block (BD Bioscience, #564219) on ice to minimize non-specific staining. Cells were then washed, incubated with DSG2-PE antibody (Clone: CSTEM28; ThermoFisher Scientific, #12-9159-42) for 45 min at 4°C in the dark, washed again, and finally spiked with SYTOX-Red LIVE/DEAD (ThermoFisher Scientific, #S34859) viability stain just prior to analysis. For CAR-T Transduction Efficiency Determination: Primary CD4+/CD8+ CAR-T cells were collected at day=14 in culture, allocated to polystyrene FACS tubes, washed 2x with cold PBS, incubated for 15 min with FC Block (BD Bioscience, #564219) on ice, and then stained with αCD4 (BioLegend, #344706) and αCD8-PE (BioLegend, #317434) flow antibodies for 45mins @ 4°C in FACS buffer. SYTOX-Red LIVE/DEAD (ThermoFisher Scientific, #S34859) viability stain was added just prior to analysis. For Intracellular Cytokine Staining: 48-well polystyrene plates were pre-coated 24 hours in advance with 10 µg/mL of recombinant DSG2 protein (R&D Systems; #947-DM-100, #7699-DM-050) or positive control αHIS antibody (ThermoFisher Scientific, MA1-21315) at 4°C. 1x106 αDSG2 CAR-T cells were then added to wells along with protein transport inhibitor (ThermoFisher Scientific, #00-4980-03) and incubated for 6 hours alongside PMA/Ionomycin-treated (ThermoFisher Scientific, #00-4970-03) positive control T cells. T cells alone without antigen served as a negative control. Cells were then collected, washed, surface stained (BioLegend, #100708), permeabilized (BD Biosciences, #554723), stained intracellularly with anti-IFNγ (BioLegend, #505810) and anti-TNFα (BioLegend, #506324) antibodies, and fixed (BD Biosciences, #554714) prior to analysis.
Instrument	BD LSR II, BD FACSymphony™ A5, BD FACSCelesta™
Software	Analysis via FlowJo v10.9
Cell population abundance	Population abundance/quantification provided in representative gate and annotation in Supplementary Figure S5
Gating strategy	Gating strategy outlined in Supplementary Figure S5

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