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	Leica ASP300 (Leica Biosystems, Deer Park, IL) BD ACCURI C6 cytometer (Beckton Dickinson; Franklin Lakes, New Jersey) BioTek Cytation™ 5 reader (Winooski, Vermont) equipped with a CY5 (628/685) filter BD LSRFortessa (BD Biosciences, San Jose, CA) Roche LightCycler (Roche, Basel, Switzerland) Luminex 200 System (Millipore Sigma, USA) Nanozoomer whole slide scanner (Hamamatsu Photonics, k.k., Japan) BenchMark Ultra automatic platform (Ventana Medical Systems-Roche, Oro Valley, Arizona, USA) NanoString nCounter platform (Bruker Spatial Biology, Bothell, WA, USA) 10X GEM Chromium 5' Ver2 QuantStudio 7 Pro (Thermo)
Data analysis	FCS Express 7.18.0015 (De Novo Software, Glendale, CA) Millipex Analyst 5.1 software GraphPad Prism v9.0; v 9.5.0; and v10.0; 10.6.1 Microsoft Excel Version 16.67; v16.77.1, RStudio v.4.4.0 and Prism v9 RStudio (version 4.3.1); and immunarch R (v.4.4.0) and Seurat (v.5.0.3) NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). nSolver Analysis Software (version 4.0.70) varianceStabilizingTransformation on DESeq2 package

clusterProfiler package (v4.6.0).
ggplot2 (v3.4.0).
Sample Processing CellRanger (v.6.1.2 pipeline)

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 bulk gDNA TCRβ CDR3 sequencing data that support the findings of this study are publicly available through Adaptive Biotechnologies' ImmuneACCESS database at https://doi.org/10.21417/RM2025N.

Other data that support the findings of this study are available on request from the corresponding authors, RAM and MS. The data are not publicly available due to containing information that could compromise the privacy of research subjects. Count matrices for the generated data will be available upon publication. Due to commercial sensitivities with pig genome and peptide sequence level data, we can only make gene/protein counts available for the transcriptomic and proteomic datasets. We will endeavor to perform nucleotide or peptide resolution analyses upon reasonable analyses requests to the corresponding author, RAM, with return of composite data results.

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	Decedent sex was reported. No sex-based analyses were performed or reported due to the small sample size.
Reporting on race, ethnicity, or other socially relevant groupings	Our study did not report races, ethnicities, or other socially-relevant groupings.
Population characteristics	In our study of n=1 decedent subject, we reported decedent sex (1 male), age (57 years old), and past relevant medical history of stage IV glioblastoma.
Recruitment	The decedent model was utilized. Consent was obtained in the same manner from the authorized family decision maker for whole body donation for xenotransplantation studies. Included decedents had been ruled out for organ donation by LiveOnNY organ procurement organization.
Ethics oversight	Ethics oversight was provided by the NYU Research on Decedents Oversight Committee (RDOC) and the study was excluded from IRB review because it was deemed not human subjects research (Protocol RDOC #22-001).

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	Sample sizes were limited by decedent availability and suitability, as well as availability of limited experimental samples from the decedent or donor animal. No sample size calculations were performed for number of decedents or number of technical replicates for each assay, given that sample sizes were limited by the quantity available for experimental assays. These samples sizes were deemed sufficient given the descriptive nature of the study.
Data exclusions	No decedents or experimental data were excluded.
Replication	For decedent experiments, experiments were performed with n=1. Decedent experimental methods were replicated as documented in methods. Results were replicated as documented in the results, with any hypotheses for any dissimilar results posited in the Discussion. For non-decedent experiments, each biological replicate was run in technical replicates when possible of 2 (zoonosis PCR assays) or 3 (flow

	cytometry XM, CDCXM) with replicated results. For some non-decedent experiments (clinical labs, clinical radiography, IHC), assays were performed on n=1 due to limited experimental samples and limited personnel resources at time of radiographic study.
Randomization	No randomization was performed due to the small number of decedents (n=1). Covariates were not deemed necessary to control, given the descriptive nature of the study.
Blinding	No blinding was performed, given that it was not possible to conceal the sex/age/characteristics of the decedent from study personnel.

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	C5b-9 (monoclonal B7 mouse anti-C5b-9 neo-epitope, kind gift of Professor Paul Morgan, Cardiff Institute of Infection & Immunity, UK), anti-CD68 (clone KP1, cat# Ga60961-2, Dako-Agilent), anti-CD15 (clone Carb-3, cat#Ga06261-2, Dako-Agilent), anti-CD3 (clone SP7, cat#Ma1-90582, Thermo Scientific), anti-CD20 (clone L26, cat#M075501-2, Dako-Agilent), and anti-vWF (polyclonal rabbit anti-human vWF, cat#A008229-5, Dako-Agilent), anti-human C4d rabbit monoclonal antibody (clone SP91, cat#06732364001, Ventana Medical Systems, Roche). Violet Proliferation Dye 450 (BD Biosciences, catalog # 562158/RRID: AB_2869398), Mouse monoclonal anti-Human Vδ2 TCR (Milteny Biotec, clone 123R3, catalog # 130-121-339/RRID: AB_2784475), Mouse monoclonal anti-Human γδ TCR (Beckman Coulter, clone IMMU510, catalog # B10247/RRID: AB_3662706), Mouse monoclonal anti-Human CD45 (BD Horizon, clone HI30, catalog # 562279/RRID: AB_11154577), Mouse monoclonal anti-Human CD8 (BD Biosciences, clone SK1, catalog # 557834/RRID: AB_396892), Mouse monoclonal anti-Human CD4 (Tonbo, clone OKT4, catalog # 80-0048/RRID: AB_2621976), Mouse monoclonal anti-Human CD3 (BD Biosciences, clone SP34-2, catalog # 552852/RRID: AB_394493)
Validation	The optimal antibody dilutions and incubations were determined for each primary antibody by performing a titration experiment and serial dilutions on positive (class 4 human lupus nephritis) and negative controls (human normal peritumoral kidney tissue).

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	We used glycoprotein alpha-galactosyltransferase 1 (GGTA1)-knockout Landrace pigs (Sus scrofa domesticus) that were 199 days old.
Wild animals	Study did not include wild animals.
Reporting on sex	No sex-based analysis could be performed given that only one animal was used. There were no exclusions of donor animals based on sex.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Ethics oversight provided by both Revivicor, Inc. and NYU Grossman School of Medicine IACUCs.

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 flow cytometry crossmatch assays: Peripheral blood mononuclear cells (PBMCs) were isolated from whole heparinized pig blood using Lymphocyte Separation Medium (Corning, Corning, NY) according to the manufacturer's protocol. Red blood cells were removed with Pharm Lyse solution (BD Biosciences, San Jose, California). Decedent sera was serially diluted in phosphate buffered saline (PBS) as follows: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. PBMC (5x105/tube) were incubated with serially diluted sera from both decedents for 1 hour at 4°C and washed three times in cold PBS. Non-specific binding sites were blocked by incubating PBMCs with 10% heat-inactivated goat serum for 20 minutes at 4°C. After two washes, PBMCs were incubated for 1 hour at 4°C with goat anti-human IgG (Jackson Lab; product no 109-546-088) or goat anti-human IgM (Jackson Lab; product no 709-546-073). After two washes, cells were resuspended in fixation buffer (BioLegend, San Diego, CA) and analyzed with the BD ACCURI C6 cytometer (Beckton Dickinson; Franklin Lakes, New Jersey). The positive control serum was from a veterinarian at the pig farm known to have high levels of non-alpha-Gal xenoantibody.

For peripheral blood flow cytometry studies: Whole blood was incubated with antibodies specific for pig or human cells. Flow cytometry of cells stained with antibodies to human and pig antigens. Pig-specific antibody clones were: Pan-Pig (030H-1-19); Pig CD3 (BB23-8E6-8C8, BD biosciences, San Diego CA); Pig CD4 (74-12-4, BD biosciences); Pig CD8 (76-2-11, BD biosciences); pig CD45 (K252.1E4, Bio-Rad Antibodies, Hercules, California). Human-specific antibody clones were: Human HLA-A,B,C (W6/32, Biolegend, San Diego, CA or G46-2.6, BD biosciences), Human CD3 (SP34-2, BD biosciences). Whole blood flow cytometry was performed by incubating whole blood from the xenograft recipients, normal pig control and normal human control with monoclonal antibodies for 30 minutes. Red blood cell (RBC) lysis was then performed with lysis buffer. Cells were centrifuged, washed and resuspended in FACS buffer. They were then acquired on a BD LSRFortessa.

Instrument

BD Accuri C6 and BD LRSFortessa

Software

BD Accuri C6 and FCS Express 7

Cell population abundance

No cell sorting performed prior to flow analysis. For FCXM: 5x10^5 PBMCs were incubated with serially diluted sera from decedents, non-specific binding blocked by incubating w/ 10% goat serum. After incubating cells with goat anti-human IgG or goat anti-human IgM, samples were analyzed by flow and the median channel shift was determined by calculating the change from median fluorescence of negative control.

For peripheral blood FC, lymphocytes (CD3+) comprised ~40% of cell population. To ensure analysis of live, single cells, doublets and DAPI+ cells were excluded from analysis. For macrochimerism FC, porcine CD45+/human MHC class I+ cells made up 0.1% of live single cells.

Gating strategy

For flow cytometetry crossmatch: The Accuri C6 analyzer was setup to acquire 10,000 events in the "lymphocytes" gate. The lymphocyte population was gated by back-gating on anti-CD3-positive cells (FITC, Jackson Labs) vs forward scatter (FSC). Briefly, on a CD3/FSC plot we first gated the CD3-positive cells. This gate was then used to identify the lymphocyte population on the side scatter (SSC)/FSC plot. After back-gating the lymphocytes, the total IgG and IgM (PerCP, Jackson Labs) was detected on the events collected on the "lymphocyte" gate. Sample were analyzed based on their Median Channel Shift (MCS) change from the median fluorescence of the negative control (all antibody reagents/no serum = auto fluorescence + non-specific binding).

For peripheral blood flow cytometry: During analysis, primary gating was performed on FSC-SSC plots to select populations of

lymphocytes, monocytes and granulocytes and to gate out cell fragments and remaining RBC. Doublets were then gated out, and dead cells gated out using DAPI. Cells were then analyzed using Human Pan-HLA class I antibody and either Pan-pig or Pig CD45. Human and pig controls were used to confirm appropriate antibody binding and specificity and to determine the gating for the experimental sample. The presence of circulating pig cells was determined by the detection of either Pan-pig+/ Human class I- cells or Pig CD45+/Human class I- cells. Further characterization of pig cells would have been performed using T-cell specific pig markers in pig cells were detected.

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