nature portfolio

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Last updated by author(s): Jun 1, 2025

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	Clinical data were collected using standard hospital monitoring systems. Pathogen detection sequencing data were generated using the MGISEQ-200 platform (MGI Tech) with library preparation via the Pathogen Universal Library Prep Kit (Genskey). Tissue homogenization was performed using the GensLab Tethys Homogenizer.
Data analysis	Bioinformatics analysis was conducted using fastp (v0.23.2) for quality control, bowtie2 (v2.4.5) for alignment to the human genome (T2T-CHM13), BWA-MEM (v0.7.17) for microbial reference mapping, HISAT2 (v2.2.1) for RNA-seq alignment to GRCh38, featureCounts (v2.0.1) for expression quantification, and FlowJo (v10.8) for flow cytometry data analysis. Viral sequences were cross-referenced using the RVDB (C-RVDBv24.1). No custom or unpublished code was used.

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

This study utilized multiple publicly available reference databases for genomic alignment and pathogen detection. The human genome reference GRCh38 (accessible at https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26) was used for RNA-seq alignment, and the human T2T-CHM13 genome for host read removal is available at https://www.ncbi.nlm.nih.gov/assembly/GCA_009914755.4. The pig reference genome used was Sus scrofa (GCF_000003025.6), available at https://www.ncbi.nlm.nih.gov/assembly/GCF_000003025.6. Viral sequences were identified and validated using the Reference Viral Database (RVDB, v24.1), accessible at https://rvdb.dbi.udel.edu. Due to ethical and institutional restrictions surrounding deceased human subjects, de-identified clinical and sequencing data from the recipient are not publicly available but can be accessed upon reasonable request to the corresponding author, subject to institutional approval and a data use agreement. Requests will be reviewed within 2 weeks.

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	None.
Reporting on race, ethnicity, or other socially relevant groupings	None.
Population characteristics	The human research participant was a 39-year-old brain-dead male who had experienced intracerebral hemorrhage and brain herniation for 16 days. The decedent had no history of chronic lung disease, malignancy, or prior immunosuppressive therapy. Genetic testing was not applicable. He was declared brain-dead by four independent clinical assessments.
Recruitment	None.
Ethics oversight	The study protocol was approved by the Institutional Review Board of Guangzhou Medical University First Affiliated Hospital.

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	This study involved a single human brain-dead recipient in a first-in-human, exploratory preclinical xenotransplantation model. No formal statistical sample size calculation was performed, as the aim was to evaluate the feasibility, immunological response, and graft viability of a gene-edited pig lung in a clinically relevant human context. The sample size was determined based on ethical limitations, regulatory considerations, and institutional feasibility. This approach aligns with established practice in other published preclinical xenotransplantation studies involving deceased human subjects and is appropriate for generating proof-of-concept data.
Data exclusions	None.
Replication	This was a first-in-human, single-subject exploratory study. No replication was performed due to the ethical and logistical constraints of using brain-dead human recipients. However, key assays such as histology, immunohistochemistry, and flow cytometry were technically repeated on serial samples across multiple postoperative time points to ensure consistency and reliability.
Randomization	Randomization was not applicable because the study involved a single brain-dead human subject in a first-in-human xenotransplantation model. No experimental groups or treatment comparisons were included.
Blinding	Blinding was not performed in this study because it involved a single-subject, first-in-human xenotransplantation model without control or comparison groups. Data collection and interpretation were performed by the clinical and laboratory teams involved in the care and analysis, with predefined time points and protocols to minimize bias.

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

In this study, the following antibodies were utilized for immunohistochemistry (IHC), immunofluorescence (IF), and flow cytometry (FC) analyses to detect T cells, macrophages, antibodies, complement components, and transgene expression in lung xenografts: CD3 (DAKO, Cat# IR50361-2, Clone: POLY, Lot# 41585069), CD68 (QuanHui, Cat# NCL-L-CD68, Clone: KP-1, Lot# 20240101020), IgG (ZhongShan, Cat# ZF-0306, Lot# 24032722; Antibody System, Cat# RHJ92831), IgM (ZhongShan, Cat# ZF-0307, Lot# 24052901; Antibody System, Cat# RHJ93203), C4c (ZhongShan, Cat# ZF-0302, Lot# 24031106), C3 (ZhongShan, Cat# ZF-0301, Lot# 24032721), C3d (Abcam, Cat# ab17453), and C4d (Antibody System, Cat# PHC45301). For transgene expression analysis, CD46 (Abcam, Cat# ab108307), CD55 (Abcam, Cat# ab133684), and Thrombomodulin (TBM) (Abcam, Cat# ab109189) were used.
In flow cytometry-based serum anti-donor antibody detection, Alexa Fluor 488-conjugated Goat anti-human IgG (H+L) (Jackson ImmunoResearch, Cat# 109-545-003, 1:1000) and Alexa Fluor 647-conjugated Goat anti-human IgM (Jackson ImmunoResearch, Cat# 109-605-043, 1:1000) were utilized.

Validation

All primary antibodies used in this study were commercially available and validated by the manufacturers for use in human tissue in immunohistochemistry (IHC), immunofluorescence (IF), or flow cytometry (FC) applications. Validation information, including specificity, tissue reactivity, and assay compatibility, is available on vendor websites (e.g., Abcam, DAKO, Jackson ImmunoResearch, ZhongShan). No custom or unvalidated antibodies were used.

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

A Chinese Bama Xiang pig with a six gene-edited lung (GTKO/B4GalNT2KO/CMAHKO/CD55/CD46/TBM) was selected as the donor. The pig, a 22-month-old male weighing 70 kg, was housed under standard conditions with access to food and water ad libitum. The gene editing was performed by Clonorgan Biotechnology, Chengdu, China.

Wild animals

None.

Reporting on sex

None.

Field-collected samples

None.

Ethics oversight

This study was approved by the Laboratory Animal Ethics Committee of the Affiliated First Hospital of Guangzhou Medical University (20240419).

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

None

Study protocol

The full study protocol is available upon request. It was approved by the Institutional Review Board of Guangzhou Medical University First Affiliated Hospital (approval number ES-2024-056-02).

Data collection	Data were collected from the donor pig and brain-dead human recipient from pre-transplant to postoperative day 9. Measurements included physiological parameters, immune responses, and porcine pathogen detection through metagenomic and meta-transcriptomic analyses.
Outcomes	The primary outcome was to assess the viability and function of the xenotransplanted lung. Secondary outcomes included monitoring immune responses, hemodynamic stability, and lung function indicators, as well as the presence of porcine pathogens.

Plants

Seed stocks	None.
Novel plant genotypes	None.
Authentication	None.