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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	No code was used to collect data for this study. Please see description below of code used for data analysis.
Data analysis	All analyses of single-cell RNA-seq, single-nuclear RNA-seq, and spatial transcriptomics data sets were performed using open source packages. All packages used in the analysis have been detailed in the Methods section and appropriately referenced in the main text as well as in the supplemental references. We summarize briefly the primary resources used here: Cell Ranger (v.6.1.1), R (v.4.2.1), Seurat (v.4.2.0), SoupX (v.1.6.1), Scanpy (v.1.9), Space Ranger (v.1.3). All commands for the analysis performed using the stated packages are included within the body of the detailed methods section.

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 following data availability statement has been included in the submitted manuscript for review: "The data from this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.". The authors further specify the privacy restrictions in the cover letter to the Nature Medicine editorial team. In brief, the datasets that are not subject to privacy restrictions (i.e., human kidney biopsy data, wild-type pig kidney biopsy data) will be made publicly available and uploaded to GEO prior to publication of the accepted manuscript. This specific point will be added to the data sharing statement upon final publication. The private data sets from the gene-edited porcine kidney xenotransplants may be shared with select investigators after implementation of the appropriate and necessary MTAs and therefore may become available after contact with the corresponding and senior author (Porrett).

Human research participants

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

Reporting on sex and gender	The sex of the xenograft recipient and the donor of the human kidneys is male and is reported as such.
Population characteristics	We performed a porcine kidney xenotransplant into an adult human brain-dead man. The demographic characteristics of this recipient including age, race, cause of brain death, etc. are detailed in the methods section.
Recruitment	Recruitment and screening for the brain-dead xenograft recipient is described in the methods section of this paper and an associated paper published in the American Journal of Transplantation (reference provided). In brief, the family of the brain-dead xenograft candidate was approached by the Legacy of Hope organ procurement organization and then by the study principal investigator after exhaustion of all organ transplant wait lists.
Ethics oversight	As stated in the methods, this study was approved by the IRB of the University of Alabama at Birmingham (IRB-300004648).

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	One human brain-dead male was the recipient of a porcine kidney xenograft. This is stated throughout the manuscript.
Data exclusions	Data from cells that underwent RNA-sequencing were excluded if they did not meet quality criteria. These quality control criteria with respect to RNA content, doublets, etc. are detailed in the methods section.
Replication	Given the small sample size and the unique nature of this study (n=1 recipient), no biologic replicates were performed. However, sequential biopsies of the kidney transplants were performed over time, providing some degree of biologic replicates in this single recipient. Identification of cells from either the human recipient or the porcine recipient was reproduced by the addition of a parallel analytic pipeline that used modified genome references as described in detail in the methods.
Randomization	No randomization was performed in this study.
Blinding	No blinding was performed in this 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

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: As described in detail in the methods, fluor-conjugated antibodies were used to label and enrich immune cells from the porcine kidney explant. These included: 0.5 mg/mL anti-human CD45 FITC (Biolegend® Inc.; San Diego, CA) and 10 µL/100 µL cell suspension of mouse anti-pig CD45-Alexa Fluor® 647 conjugate antibody (Bio-Rad Laboratories, Inc).

Validation: Antibodies were obtained from trusted commercial manufacturers with established QC standards (i.e., Biolegend, Becton Dickinson, etc.) and were therefore not independently validated.

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: Kidney and blood samples were obtained from a wild-type pig and a 10 gene-edited pig as described in detail in the methods. Details regarding the sex, age, weight, and breed of the animals is provided in the methods as well.

Wild animals: No wild animals were used in this study.

Reporting on sex: See above for "laboratory animals"

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

Ethics oversight: Animal work in this study was performed as specified in the UAB IACUC protocol 22015 which provides oversight for all activities at the designated pathogen free facility and xenotransplantation campus where the pigs for this study were housed.

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: As described in detail in the methods, tissue from the explanted right kidney xenograft was digested in LiberaseTM (Millipore Sigma, Roche; Indianapolis) in RPMI 1640 (Gibco™ ThermoFisher Scientific; Grand Island, NY) prior to incubation with fluor-conjugated antibodies.

Instrument: Cells were sorted using a Becton Dickinson FACSAria in the single cell core facility at the University of Alabama at Birmingham.

Software: FACSDiva software on the BD FACSAria was used to collect the sorted CD45+ immune cells. No additional flow data was collected or analyzed for this experiment.

Cell population abundance: After sorting, tthe CD45+ cells were >99.9% pure. Subsequent single-cell RNA-sequencing of the sorted CD45+ immune cells confirmed the purity of the sorted cells.

Gating strategy

Due to our understanding of limitations on the number of extended data figures, we have not provided plots depicting the gating strategy used for the sorting of the CD45+ immune cells from the explanted porcine kidney xenograft, so we have not ticked off the boxes regarding the plots above. Nevertheless, the staining and processing of the sample as well as the antibodies used and the gating strategy for the sort are described in detail in the methods. Plots used at the time of data acquisition using FACSDiva software during the source were pseudocolor plots. The number of sorted cells that were ultimately analyzed using single-cell RNA-sequencing is provided in the methods as well as in the specific figures (Extended Data Fig. 3; n=6,513 CD45+ immune cells, and Extended Data Fig. 4 for number of cells by immune cell type and species).

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