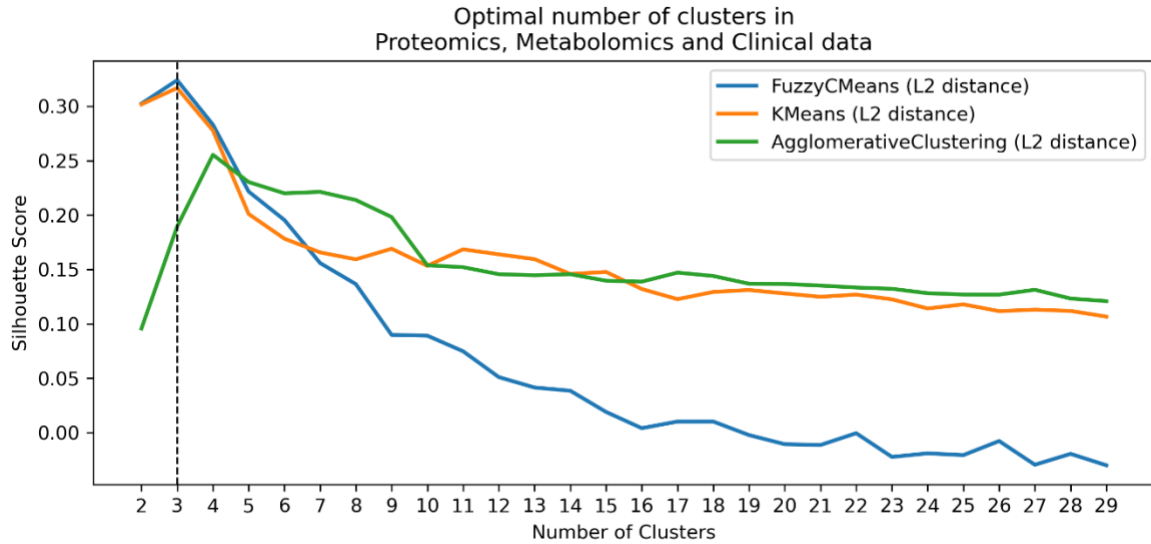
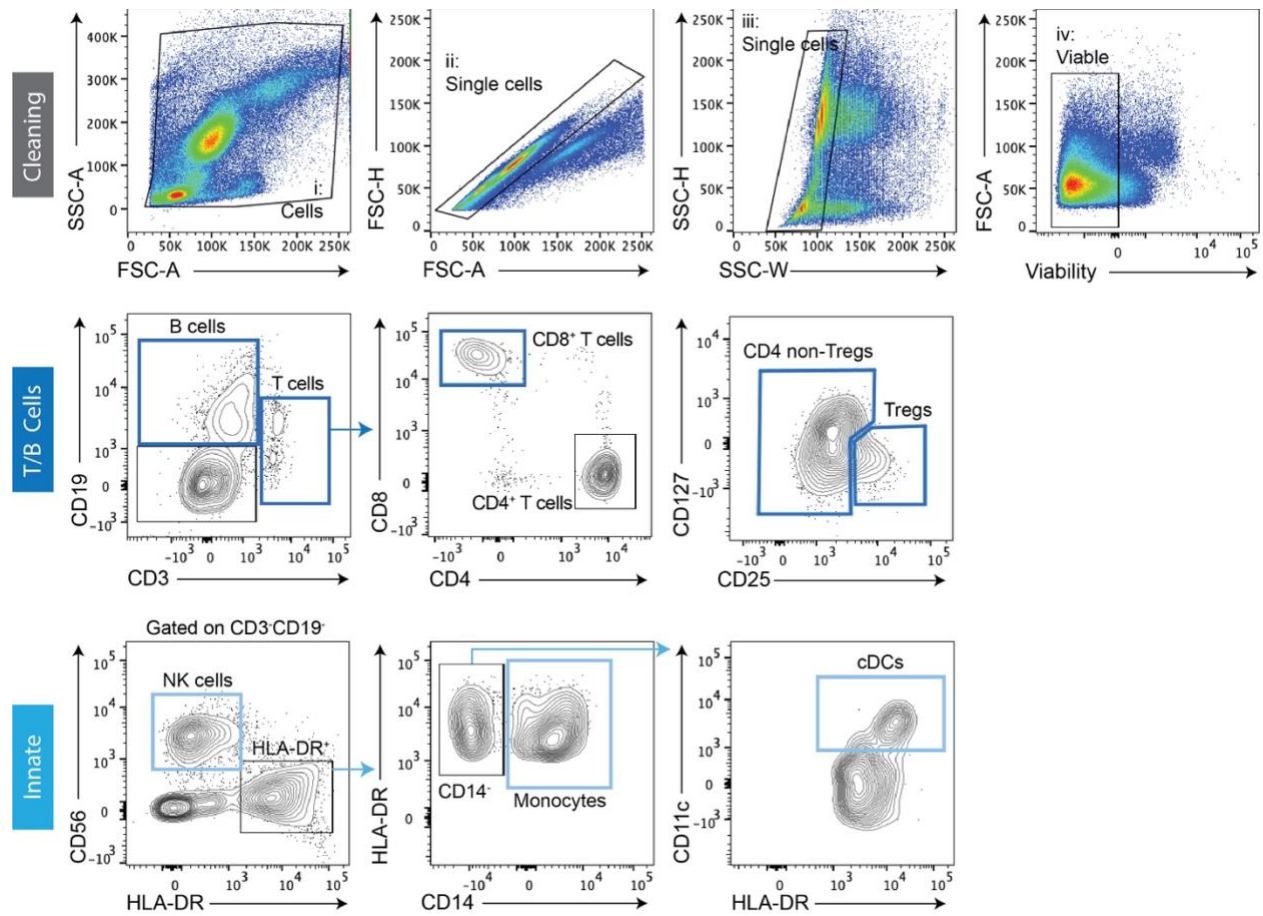

Immune profiling in a living human recipient of a gene-edited pig kidney

In the format provided by the
authors and unedited

Figure or Table #	Figure/Table Title	Figure/Table Legend
Supplementary Fig. 1	Optimal number of clusters in proteomics, metabolomics, and clinical data.	The optimal number of clusters was defined by calculating the silhouette score, varying the number of clusters from 2 to 29. The peak for FuzzyCMeans and KMeans using the L2 distance is on 3 clusters. FuzzyCMeans and KMeans showed similar optimal silhouette scores, 0.32 and 0.31, respectively. Since FuzzyCMeans is robust for noisy data, we chose this instead of KMeans. Agglomerative Clustering on the Linkage Average model showed a lower optimal silhouette score of 0.25 for 4 clusters.
Supplementary Fig. 2	Gating strategy for circulating immune cells.	Related to Fig. 2d .
Supplementary Table 1	Flow cytometry antibodies.	
Supplementary Table 2	Details of the Orion primary antibody and ArgoFlour panel used for the xenotransplant analysis.	



Supplementary Fig. 1 | Optimal number of clusters in proteomics, metabolomics, and clinical data. The optimal number of clusters was defined by calculating the silhouette score, varying the number of clusters from 2 to 29. The peak for FuzzyCMeans and KMeans using the L2 distance is on 3 clusters. FuzzyCMeans and KMeans showed similar optimal silhouette scores, 0.32 and 0.31, respectively. Since FuzzyCMeans is robust for noisy data, we chose this instead of KMeans. Agglomerative Clustering on the Linkage Average model showed a lower optimal silhouette score of 0.25 for 4 clusters.



Supplementary Fig. 2 | Gating strategy for circulating immune cells. Related to Fig. 2d.

Supplementary Table 1. Flow cytometry antibodies.

Target	Color	Concentration	Clone	Brand	Cat no
CD8	BUV737	1:20	SK1	BD Biosciences	612754
CD4	BUV395	1:20	SK3	BD Biosciences	563550
CD3	AF700	1:50	UCHT1	BioLegend	300424
CD19	PE-Cy5	1:20	SJ25C1	BioLegend	363042
CD127	PerCP-Cy5.5	1:20	A019D5	BioLegend	351322
CD25	PE-Cy7	1:20	BC96	BioLegend	302612
CCR7	PE-Dazzle 594	1:20	G043H7	BioLegend	353236
CD45RA	APC	1:20	HI100	BioLegend	304112
CD56	BUV737	1:20	NCAM16.2	BD Biosciences	612766
HLA-DR	BV650	1:20	L243	BioLegend	307650
CD14	BV510	1:50	M5E2	BioLegend	301842
CD11c	AF700	1:20	3.9	BioLegend	301648
CD3	PerCP-Cy5.5	1:50	UCHT1	BioLegend	300430
CD19	PerCP-Cy5.5	1:50	HIB19	BioLegend	302230

Supplementary Table 2. Details of the Orion primary antibody and ArgoFlour panel used for the xenotransplant analysis.

Panel 1							
Channel	Fluorophore	Target	Purpose	Clone	Brand	Cat no	Notes
1	Hoechst 33342	DNA	Nuclear localization	N/A			
2	N/A	Autofluorescence1	Tissue architecture	N/A			
3	AlexaFluor 520	CD45	Leukocyte	D9M8I	RareCyte	62-1001-501	
4	ArgoFluor 548	NKG2A	NK cell	EPR2373 7-127	Abcam	ab273516	in-house conjugation to ArgoFluor 548 (RareCyte Cat no: 62-1026-503)
5	N/A	Autofluorescence2	Tissue architecture	N/A			
6	ArgoFluor 602	CD163	Macrophage	EDHu-1	Novus	NB110-40686	in-house conjugation to ArgoFluor 602 (RareCyte Cat no: 62-1031-603)
7	ArgoFluor 624	CD8a	T Cytotoxic	AMC908	RareCyte	62-1007-601	
8	ArgoFluor 706	CD4	T Helper	EPR6855	RareCyte	62-1098-701	
9	ArgoFluor 760	CD68	Macrophage	D4B9C	RareCyte	62-1013-701	
10	ArgoFluor 784	Podocin	Glomerulis	Polyclonal	Sigma-Aldrich	P0372	in-house conjugation to ArgoFluor 784 (RareCyte Cat no: 52-1039-703)
11	ArgoFluor 845	β -catenin	Tubule Epithelium	D10A8	Cell Signaling	84441SF	in-house conjugation to ArgoFluor 845 (RareCyte Cat no: 62-1040-803)
Panel 2							
Channel	Fluorophore	Target	Purpose	Clone	Brand	Cat no	Notes
1	Hoechst 33342	DNA	Nuclear localization	N/A			
2	N/A	Autofluorescence1	Tissue architecture	N/A			
3	FITC	HLA-ABC	Human HLA class I	W6/32	Thermo Fisher	11-9983-41	
4	N/A	Autofluorescence2	Tissue architecture	N/A			
5	ArgoFluor 698	CD31	Endothelium	Polyclonal	Novus	NB100-2284	Primary: In-house conjugated to NWS epitope using oYo-Link® technology (from AlphaThera)

							Secondary: ArgoFluor 698 anti-NWS epitope (RareCyte Cat no: 62- 1196-602)
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Kidney Biopsy Report (day 34)

FINAL PATHOLOGIC DIAGNOSIS

- NO EVIDENCE OF ACUTE CELLULAR REJECTION
- NO EVIDENCE OF ANTIBODY MEDIATED REJECTION
- FOCAL INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY ~30%
- FOCAL HEMORRHAGE ~10%
- FOCAL ARTERIAL INTIMAL HYPERPLASIA WITH REACTIVE ENDOTHELIA
- FOCAL TUBULAR INJURY
- NO EVIDENCE OF INTRARENAL THROMBOTIC MICROANGIOPATHY

INTERPRETATION

There is insufficient inflammation, and tubulitis, and no endothelialitis, so that an acute cellular rejection cannot be diagnosed.

The prior rejection on day 8 has resolved.

The dense interstitial fibrosis in one-third of the specimen is attributed to the resolved prior rejection.

The two arteries with mild intimal hyperplasia and reactive endothelia are adjacent to the dense fibrosis and interpreted as associated with the resolving vascular rejection and not deemed currently as pathological.

An antibody-mediated rejection is not favored as there is no microvascular inflammation, the C4d is negative both by immunofluorescence and immunohistochemistry, and, by report, no donor-specific antibody is detected.

In addition, there is insufficient evidence to diagnose an intrarenal thrombotic microangiopathy. A zone of dense fibrosis is present ~30% of the cortex, which is interpreted as resolved prior acute cellular rejection.

There is some fine diffuse fibrosis between some peritubular capillaries, which currently is attributed to the resolved acute cellular rejection.

As there is no classification of porcine xenograft rejection in humans, this report is based on extrapolations from human allografts.

BANFF CRITERIA

i0, t0-1, v0, g0, ci1-2, ct1-2, cv0-1, cg0, mm0, ah0, ptc0, C4d0, ti0, iIFTA0

IMMUNOFLUORESCENCE

- Number of Glomeruli: 5
- Globally Sclerotic: None
- Segmental Sclerotic: None
- Linear GBM: 1+ Albumin, IgG, C1q
- Granular Mesangium: 1+ IgA, Kappa/Lambda, 1-2+ IgM, trace C3; all segmental
- Granular GBM / Segmental / Tubules: None
- Reabsorption Droplets: None
- C4d: Negative
- Fibrin Thrombi: None

ELECTRON MICROSCOPY

- GBM Structure: Normal
- Duplication: None
- GBM Endothelia: Reactive; some leukocytes
- GBM Deposits: None
- Mesangial Deposits: None
- Podocyte Effacement: Moderate
- Peritubular Capillary Laminations: None
- Tubular Epithelia: Some injury; vacuolization
- Peritubular Epithelia: Reactive
- Peritubular Capillary Interstitium: Focal fibrosis