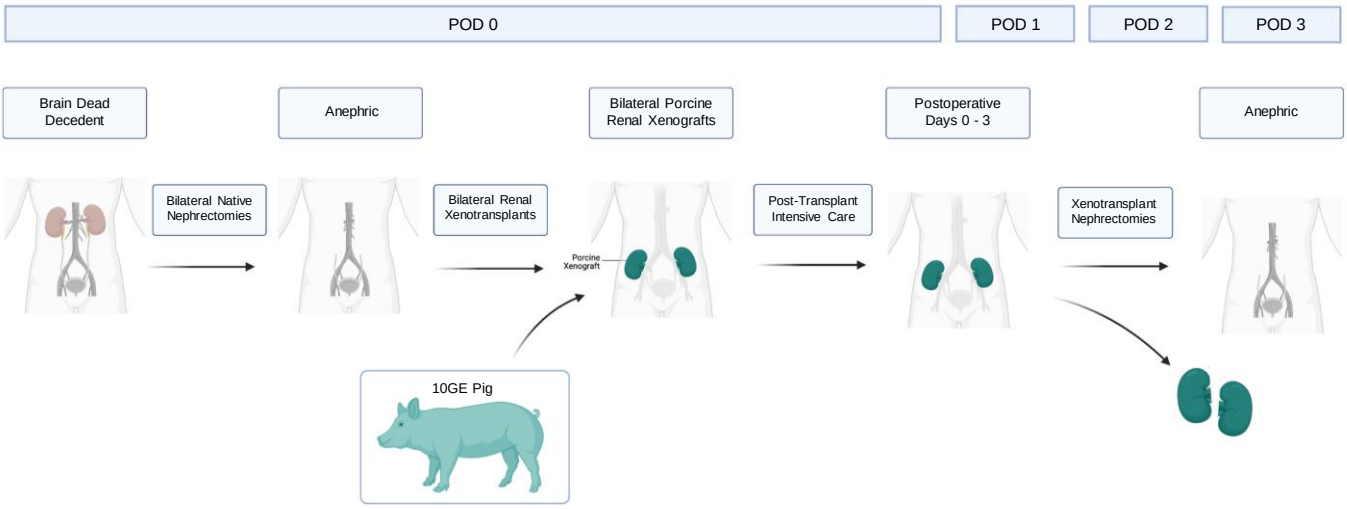
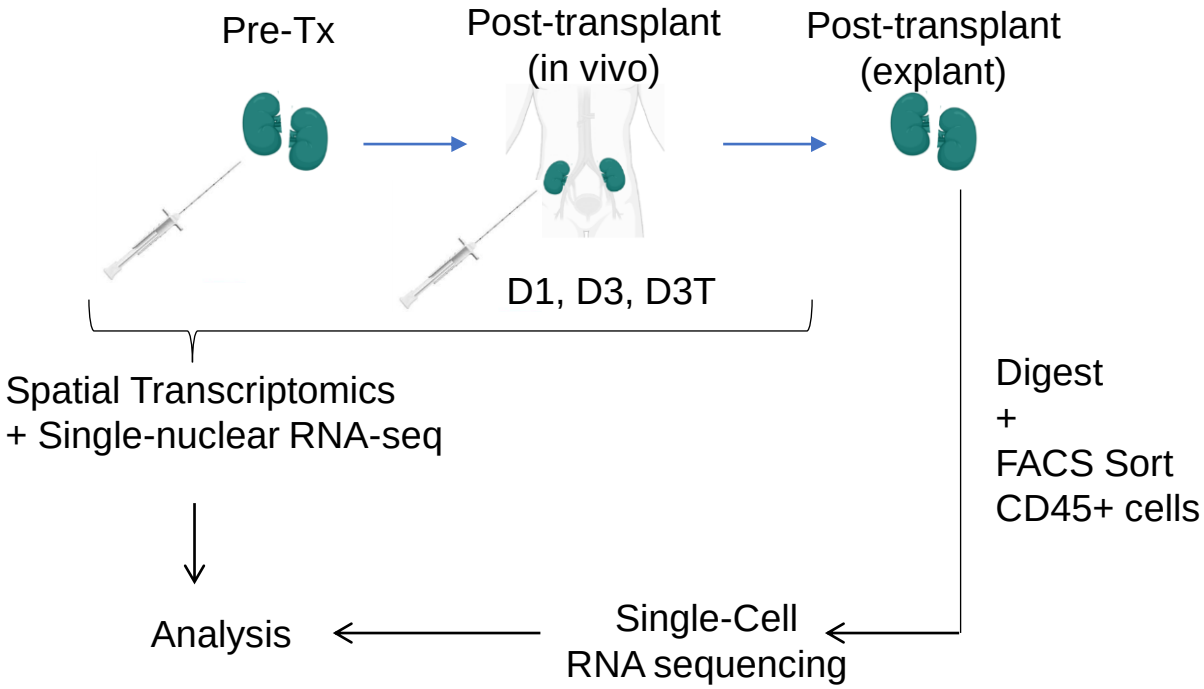
a

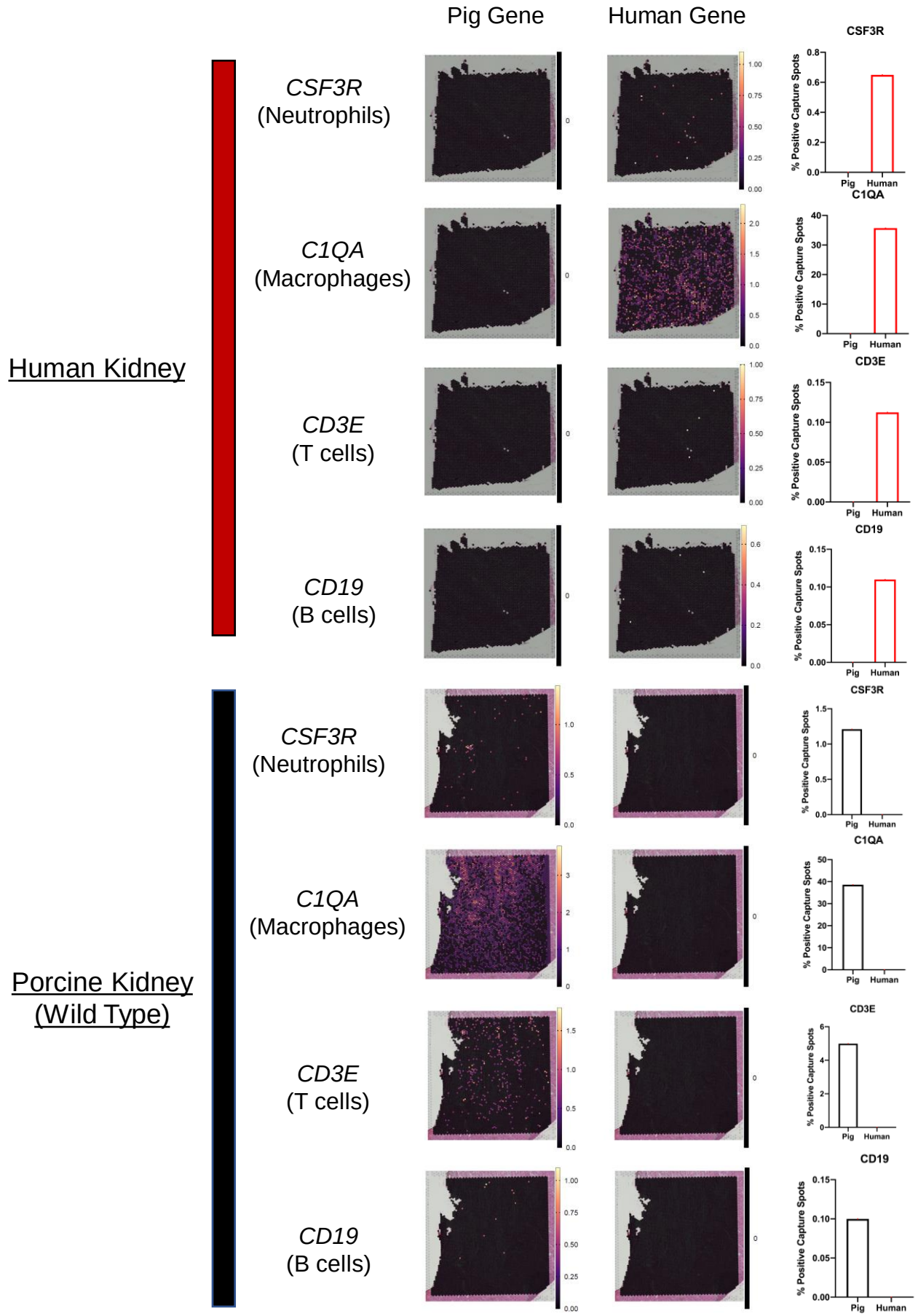


b



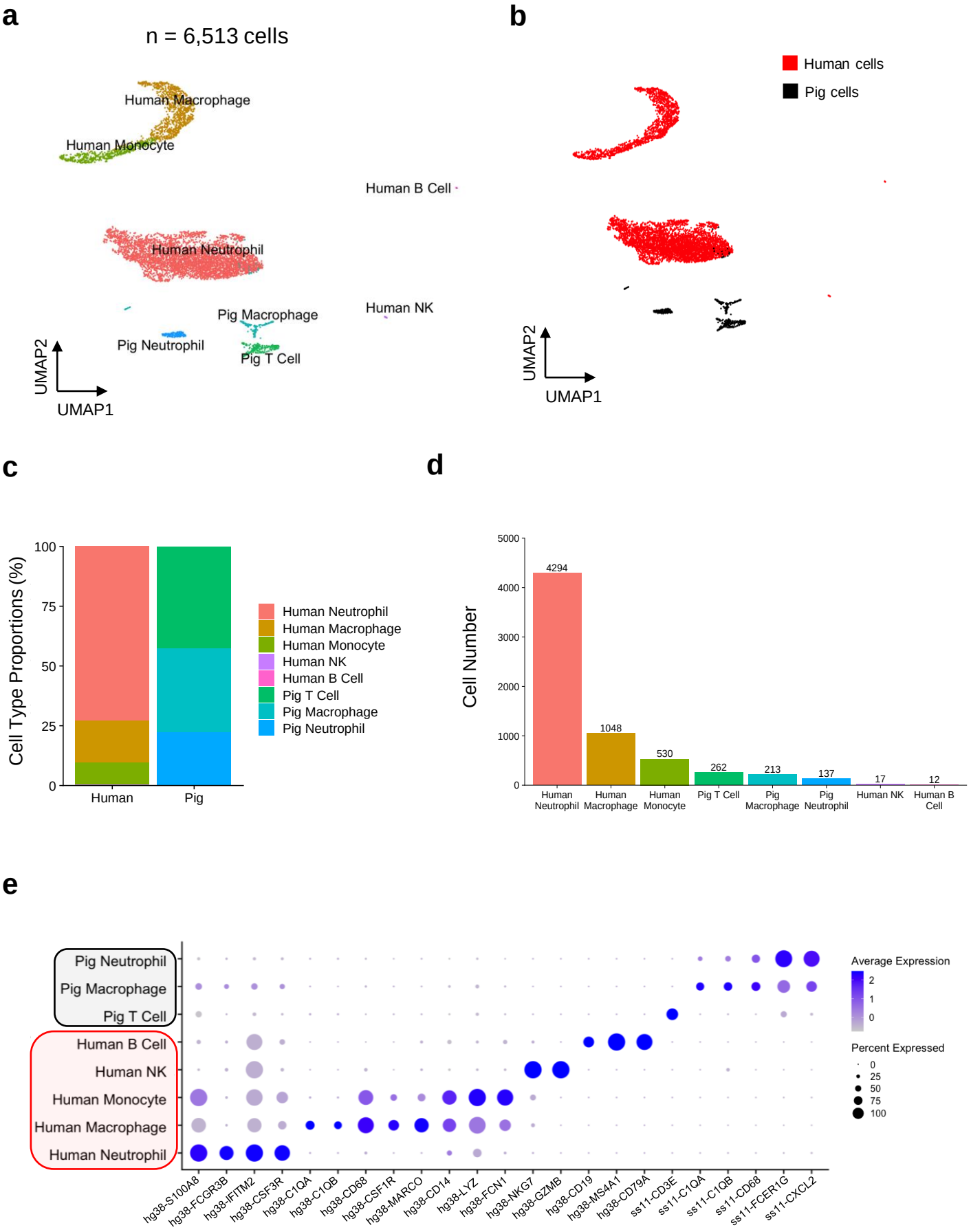
Extended Data Fig. 1

Extended Data Figure 1. Schematic of pig-to-human xenotransplant experiment and sample collection. **a**) Experimental design. Native nephrectomies were performed on a brain-dead human recipient, and bilateral porcine kidney xenografts were transplanted from a pig with 10 gene edits. Pharmacologic immunosuppression was administered from transplantation until termination approximately 74 hours later. The kidney xenografts were explanted after termination. **b)** Schematic of sample collection and wet lab workflow. Core needle biopsies were taken pre-transplant, on days 1 and 3 post-transplant, and immediately prior to termination of the experiment (i.e. day 3T). Each core biopsy was frozen in OCT and sections of the biopsy were placed on Visium slides for spatial transcriptomics. Nuclei were isolated from another section of each thawed OCT block for single-nuclear RNA-seq. A portion of the explanted kidney xenograft representing cortex through medulla was digested to a single-cell suspension, labeled with both pig-specific and human-specific CD45 antibodies, and sorted using fluorescence activated cell sorting (FACS). Single-cell RNA-sequencing was then performed on FACS-enriched CD45+ cells from the explanted xenograft.



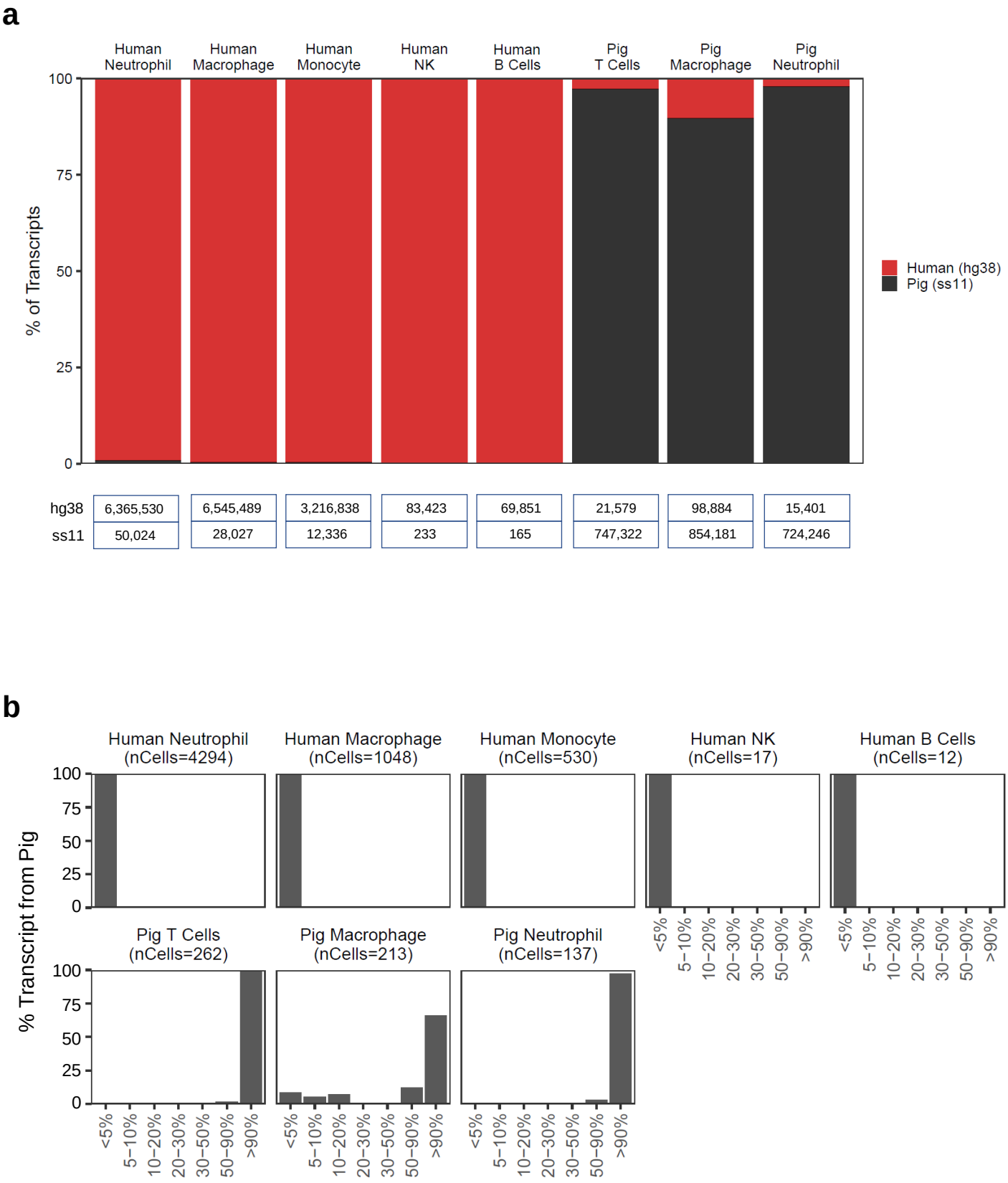
Extended Data Fig. 2

Extended Data Figure 2. Alignment of control porcine and human kidney samples to a custom porcine-human hybrid reference genome. A cortical sample of human or wild-type porcine kidney was flash frozen in optimal cutting temperature (OCT) media and a 10-µm section was placed on Visium spatial transcriptomics slides for analysis. Sequenced reads were aligned to a custom porcine-human hybrid reference genome. Read alignment of select immune cell marker genes (*CSF3R, C1QA, CD3E, and CD19*) was assessed in a species-specific fashion (i.e. reads from the porcine kidney aligned to the *ss11-CSF3R* component of the hybrid reference genome [not *hg38-CSF3R*] and vice versa). Results are quantified as a percentage of capture spots containing the gene of interest from the whole sample. Similar results to wild-type pig kidney were found for a 10-GE porcine kidney (data not shown).



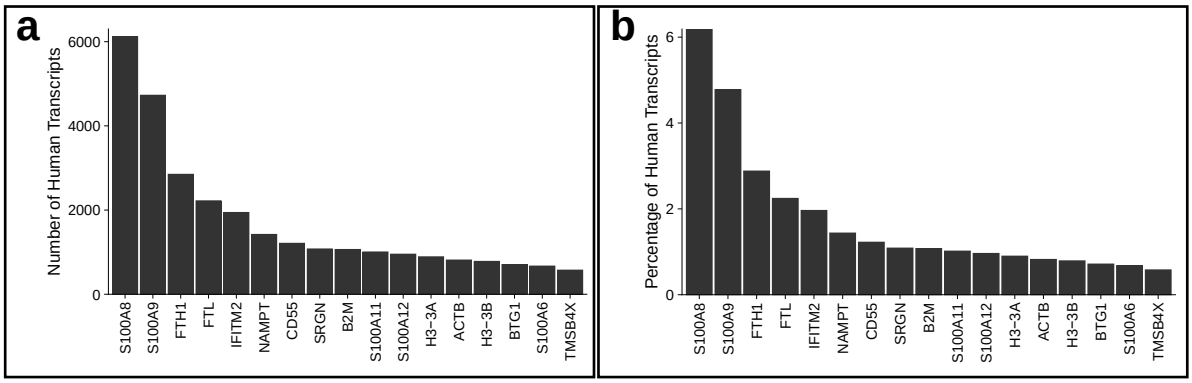
Extended Data Fig. 3

Extended Data Figure 3. Composition of CD45+ immune cells collected from the porcine xenograft explant. The 10-GE porcine xenograft was removed from the brain-dead human recipient three days after xenotransplantation, and single-cell RNA-sequencing was performed on FACS-enriched immune cells from the xenograft using pig- and human-specific CD45+ antibodies. Data were aligned to the hybrid human-porcine reference genome. **a&b)** UMAP of sorted CD45+ cells (n=6,513 cells) colored by cell type (a) and species (b). **c&d)** Enumeration of sequenced human and porcine immune cells. **e)** Expression of select marker genes in human and pig immune cell clusters.



Extended Data Fig. 4

Extended Data Figure 4. Assessment of species mapping at the individual transcript and cellular levels. Single-cell RNA-sequencing was performed on FACS-enriched immune cells from the xenograft explant as in Extended Data Fig. 3. Data were aligned to the hybrid human-porcine reference genome. **a**) Species origin of all transcripts for a given immune cell cluster. Table at the bottom enumerates the transcript number for each cluster that mapped to the hg38 (human) or ss11 (pig) portion of the hybrid reference genome. Additional details of pig macrophage transcripts mapping to the hg38 component of the porcine-human hybrid reference genome are given in Extended Data Fig. 5. **b)** The frequency of cells that have low or high levels of transcripts derived from the pig.



c

S100A8

80% identical

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d

S100A9

75% identical

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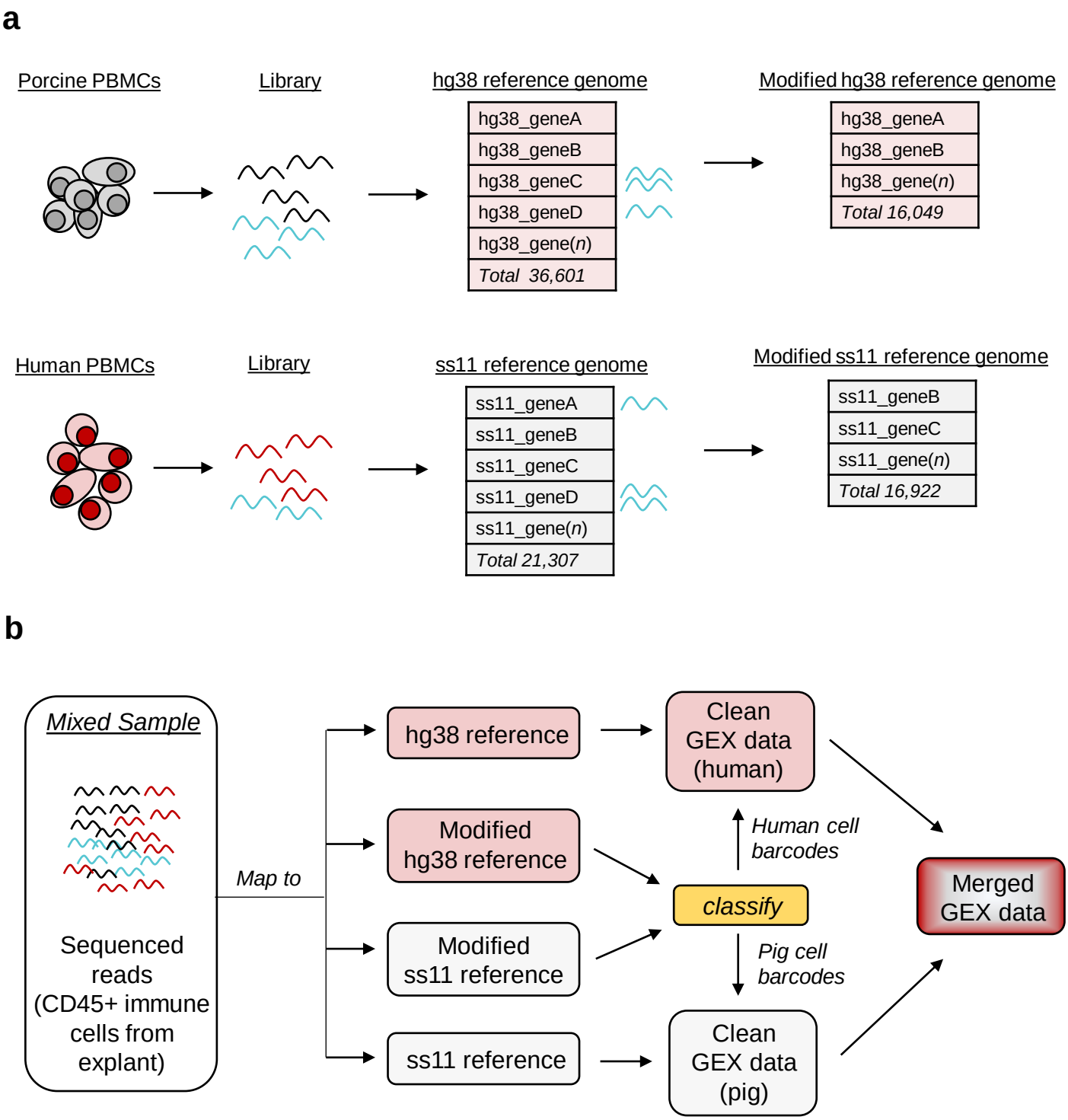
Extended Data Fig. 5

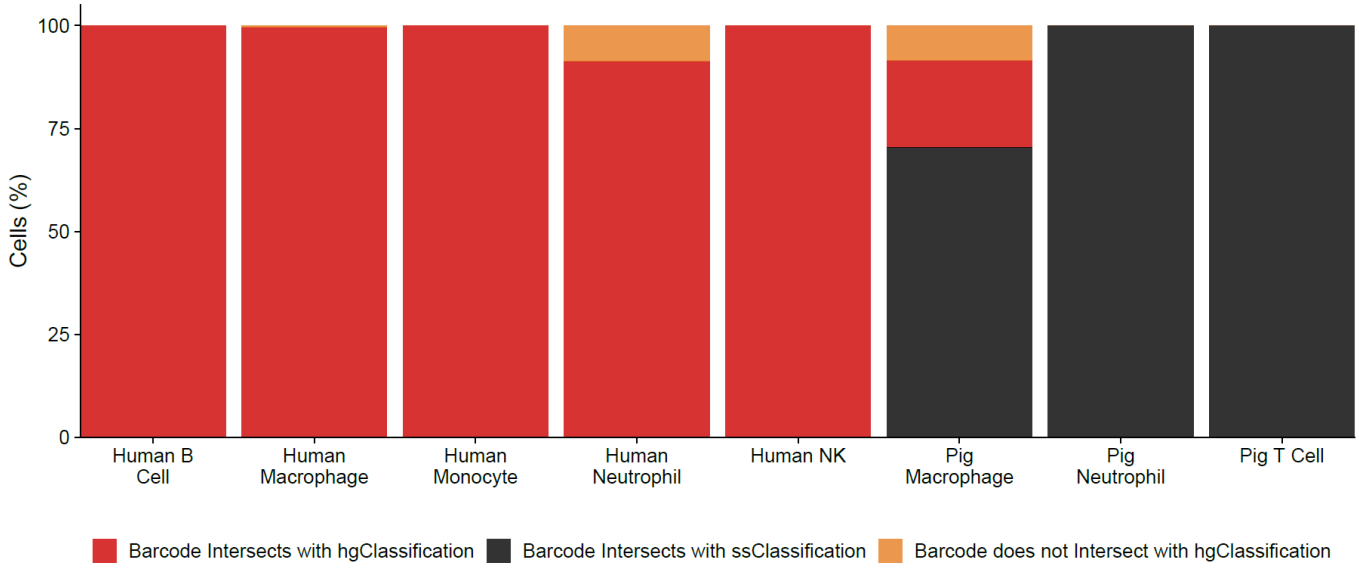
Extended Data Figure 5. Sequence homology between human and porcine genes in macrophage populations. Pig macrophages were selected from Extended Fig. 3 and the 98,884 transcripts aligning to the hg38 component of the human-porcine hybrid reference were identified (Extended Fig. 4). **a&b**) Porcine macrophage transcripts for *S100A8* and *S100A9* were the most common transcripts which aligned to human genes. Top 11 human genes to which transcripts from porcine macrophages aligned are shown. **c&d**) BLAST analysis reveals 75-80% sequence homology between *S100A8* and *S100A9*. For *S100A8* (**c**), "query" is human transcript NM_001319198.2, and "sbjct" is porcine transcript NM_001160271.3. For *S100A9* (**d**), "query" is human transcript NM_002965.4, and "sbjct" is porcine transcript NM_001177906.1.



Extended Data Fig. 6

Extended Data Figure 6. Alternative mapping strategy using species-specific modified reference genomes. **a)** Conceptual schematic of construction of modified species-specific genome references. PBMCs from the 10-GE donor pig and the human recipient were collected prior to donation or transplantation, respectively. Libraries were prepared from single cells and scRNA-seq was performed. To identify homologous genes between the species which resulted in ambiguous read mapping, reads from each PBMC sample were aligned to the reference genome of the other species. Genes that mapped >3 reads were subsequently removed from the reference genome. All remaining genes from the reference genome were included in the modified reference genome for each species. Red: Human transcripts and genes. Black/grey: Pig transcripts and genes. Light blue: Transcripts which map to homologous genes in the opposite species. **b)** Analysis workflow for validation of species assignment by the hybrid reference approach using modified references. FASTQ files from sequenced reads of CD45+ immune cells sorted from the pig kidney xenograft explant were mapped to four different references during processing with Cell Ranger. Outputs from mapping to each of the four references are successively merged to generate an expression matrix for the mixed sample. Species-specific cellular barcodes in this expression matrix have been identified based on a subset of non-homologous genes in the modified references. ss11 = *sus scrofa* genome, assembly 11.1. hg38 = human genome assembly 38.

Cluster	nCells	Barcode Intersects with human classification	Barcodes Not Intersecting with hgClassification	Barcode Intersects with ssClassification
Human Neutrophil	4294	3921	373	0
Human Macrophage	1048	1044	4	0
Human Monocyte	530	530	0	0
Pig T Cell	262	0	262	262
Pig Macrophage	213	45	168	150
Pig Neutrophil	137	0	137	137
Human NK	17	17	0	0
Human B Cell	12	12	0	0

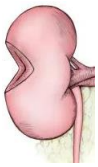


Extended Data Fig. 7

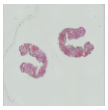
Extended Data Figure 7. Validation of immune cell origin in the porcine kidney xenograft explants using the alternative mapping approach. scRNA-seq was performed on CD45+ immune cells FACS-enriched from the explanted porcine kidney after termination (see Extended Figs. 1&3). Cells were initially identified as porcine or human in origin based on alignment against the custom human-porcine reference genome (see table column “nCells” and Extended Fig. 3). Data were then independently processed against species-specific modified reference genomes (see Extended Fig. 6). Concordance between the two methods was assessed and is portrayed in the table and bar plot. Barcodes that do not intersect with either the hg38 or ss11 classifications (orange) were excluded from the modified reference alignment due to differences in data quality and filtration steps between the workflows.

a

Sorted CD45+ cells
from xenograft explant



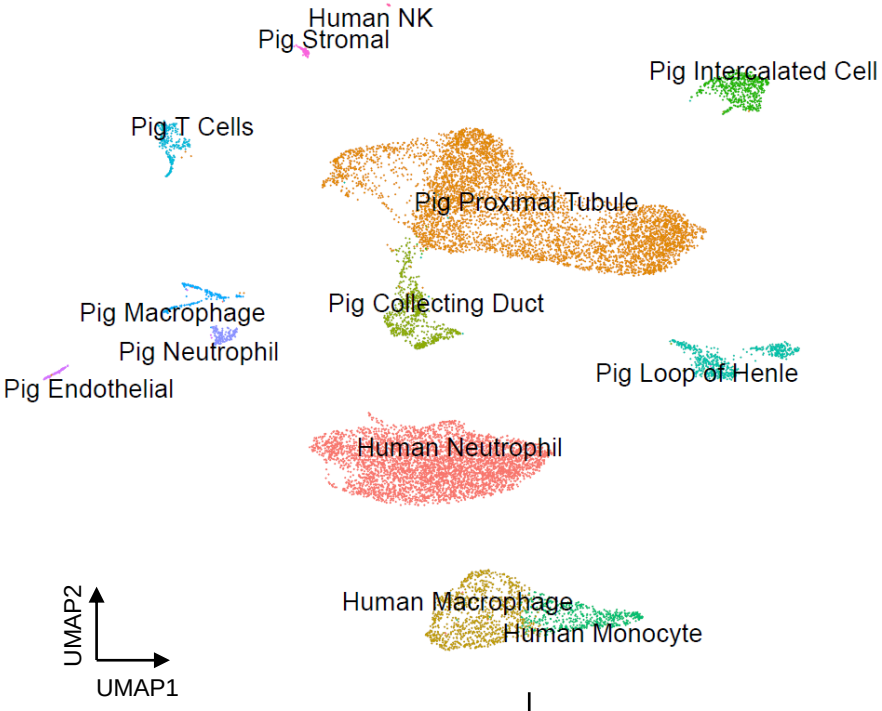
scRNA-seq

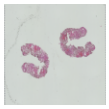


snRNA-seq

Single nuclei
(Biopsy)

Reference transcriptomes



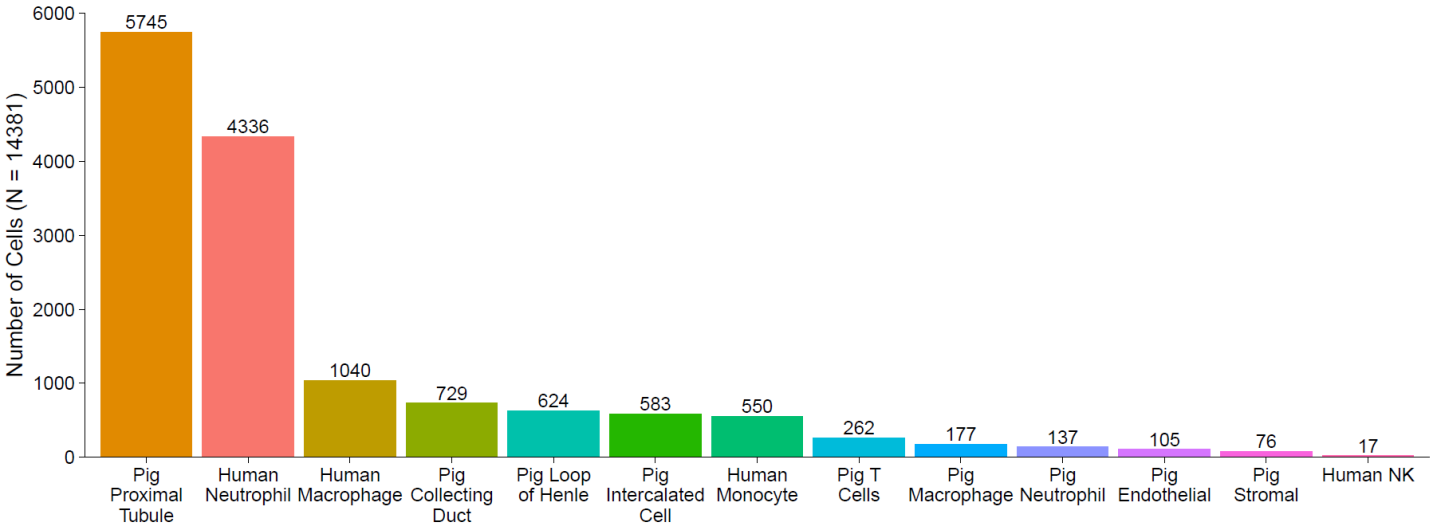


(Biopsy)

Spatially barcoded
library

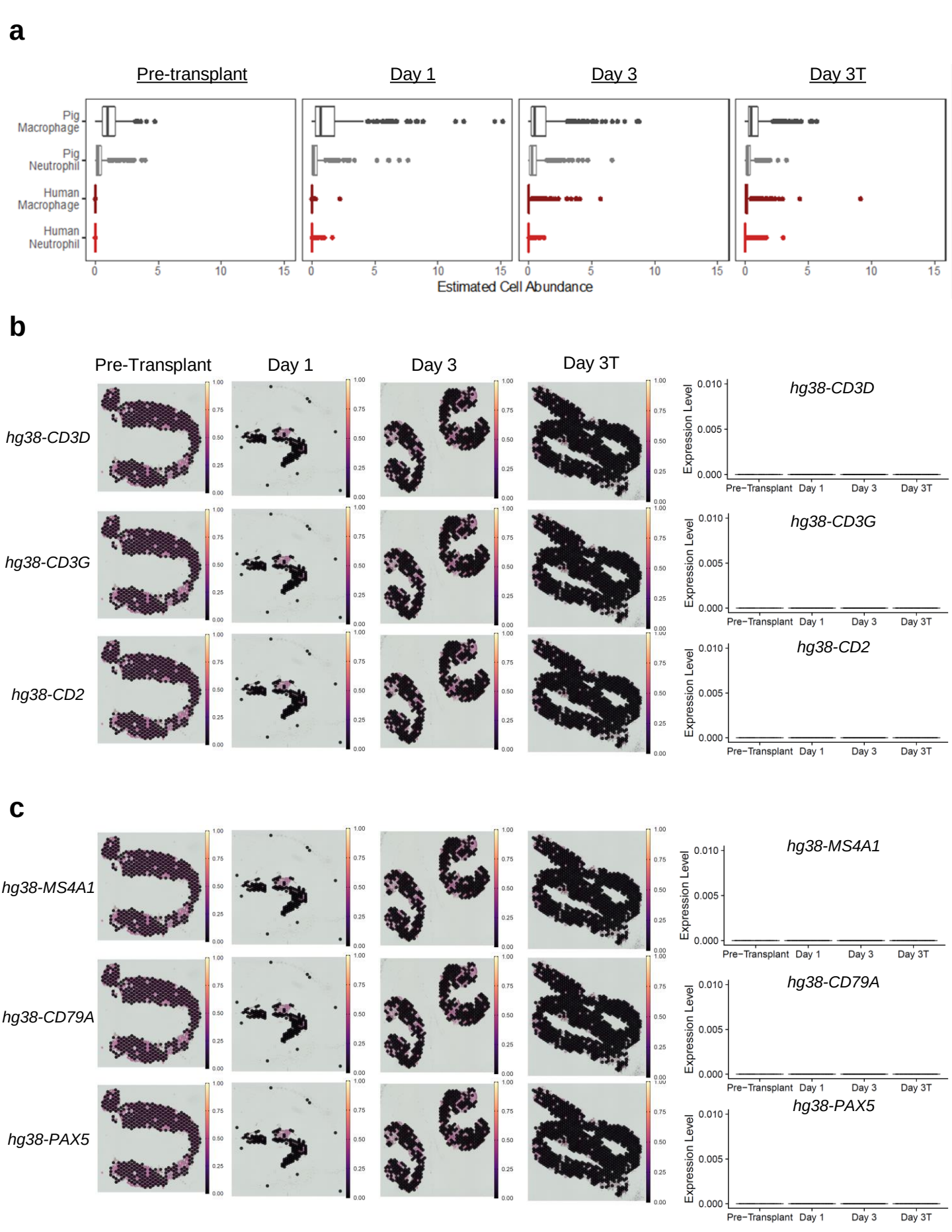
Cell2location

b



Extended Data Fig. 8

Extended Data Figure 8. Cell2location workflow for estimation of cell type abundance in porcine xenograft biopsies. **a**) Schema of Cell2location workflow, which includes input of spatially barcoded biopsy data and reference transcriptomes. Reference transcriptomes that were input into Cell2location were created from sequencing of 1) single nuclei from porcine kidney xenograft biopsies and 2) single CD45+ cells enriched from the porcine kidney xenograft after removal from the recipient (explant) (see Extended Figs. 1&3). A biopsy of the porcine kidney was taken pre-transplant and sequentially after transplant (Extended Fig. 1). Each biopsy was sectioned and placed on a spatial gene expression slide. The remainder of each biopsy was thawed and nuclei from the block were isolated for snRNA-seq. For generation of reference transcriptomes, all four libraries generated from the biopsy nuclei were aggregated together with the CD45+ immune cells. UMAP of reference transcriptomes represents 14,381 cells. **b**) Enumeration of reference transcriptomes represented in UMAP in (**a**).



Extended Data Fig. 9

Extended Data Figure 9. Distribution of species-specific immune cells in the porcine kidney xenograft. As in Fig. 1, spatial transcriptomics was performed on serial needle core biopsies of 10-GE porcine kidneys before and after transplantation into a brain-dead human recipient. Cell type signatures were identified from reference transcriptomes using Cell2location (**a**) or expression of individual marker genes (**b&c**) for cell types missing from the Cell2location reference transcriptomes (see Extended Data Fig. 8). **a**) Distribution of human and pig myeloid cells over time in the porcine kidney xenograft. **b&c**) Negligible expression of additional human T cell (**b**) and B cell (**c**) genes in the porcine kidney xenograft. These additional T and B cell genes were selected for evaluation in order to avert potential alignment errors due to sequence homology.