SUPPLEMENTAL DIGITAL CONTENT (SDC)

Materials and Methods S1

Animals and study design: All animals were housed and cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Resources and published by the National Institute of Health (NIH, Publication No. 86-23, revised 1996). An autotransplantation model was developed (24) to avoid alloimmune response (Fig S1).

Anesthesia and experimental equipment: Anesthesia was induced by intramuscular injection of ketamine (20 mg/kg body weight) (Ketaminol Vet. Intervet, Boxmeer, Netherlands), xylazine 100 mg (Rompun Vet, Bayer, Solna, Sweden), and atropine (0.5 mg) (Atropine, Mylan AB, Stockholm, Sweden). Fentanyl 4 µg/kg body weight (Fentanyl B. Braun,

Melsungen, Germany) and midazolam (0.4 mg/kg body weight; Midazolam Panpharma, Panpharma S.A., Trittau, Germany) were administered intravenously through an ear vein before tracheostomy.

The animals were connected to a servo ventilator 300 (Siemens AB, Solna, Sweden). Volume-controlled and pressure-regulated ventilation was used, with a minute volume of 100–150 mL/kg body weight and a frequency of 20 breaths/min. The positive end-expiratory pressure was adjusted to 5 cm H_2O, and the inspired oxygen fraction was 0.5.

Blood grouping: Pigs had either blood group 0 or A. DiaClon Anti-A (Bio-Rad Laboratories, Solna, Sweden) was used to define blood group A. The donor erythrocytes were

crossmatched with recipient plasma and recipient erythrocytes with donor plasma. Cell suspensions were prepared in PBS buffer.

Red blood cell (RBC) washing: To prepare washed erythrocytes (RBC), a Medtronic Autolog Autotransfusion system (Minneapolis, USA) was used according to the manufacturer's instructions. Cells were collected after draining separate blood group-compatible donor pigs
(n=10) from blood via heart puncture. Anticoagulant citrate dextrose solution-A (ACD-A; Fresenius KABI, Uppsala, Sweden) was used as the washing medium.

Ex vivo perfusion device: A custom-built perfusion device was designed to allow ex vivo perfusion and reconditioning of kidneys while controlling pressure, flow, and temperature using a heat exchanger (Sorin CSC14, Sorin Group, Milan, Italy). The flow was pressurecontrolled and the resistance was automatically read from the device. The endotoxin (LPS
Adsorber, Alteco Medical AB, Lund, Sweden) and cytokine removal (CytoSorb, CytoSorbents Corp., New Jersey, USA) were integrated into the system. These were introduced during RBC washing, preparing for the second step of reconditioning, which includes the addition of RBCs to fully evaluate and oxygenate the (CX*FX05RE, Terumo, Leuven, Belgium) organs at a higher temperature.

Blood gas and creatinine analysis: A blood gas analyzer (ABL 700, Radiometer, Copenhagen, Denmark) was used to analyze the acid-base and electrolyte balance, including creatinine in plasma and urine. Creatinine in blood was measured before nephrectomy, at reperfusion,

90 minutes after reperfusion and at 10 days.

Arterial flow: Renal artery blood flow was measured using a Cardiomed CM-4000, Medistim a/s, Oslo, Norway), with Medistim® flow probes 3-5 mm.

Histology: Representative histological materials from the two groups were analyzed in this study. In all, four categories of histological material were collected as indicated below, the groups refer to Table I. Group I**:** Reconditioned uDCD kidneys from autologous transplanted animals (n=13) surviving more than 10 days after transplantation. The animals were subjected to 4.5 hours ischemia following cardiac arrest. The reconditioning protocol was subsequently applied, as described above. Kidney tissue was sampled before and after the machine perfusion procedure to analyze the effects of the reconditioning protocol. Group II: Kidneys from healthy pigs without circulatory arrest, retrieved as a live donor kidney, perfused with base solution without thrombolysis (n=8). Group III: Kidneys from uDCD pigs, perfused with base solution without thrombolysis, not transplanted followed by 4,5 hours of ischemia and then machine perfused with buffer containing no thrombolytic factors. (n=7). Group IV: uDCD followed by 4,5 hours of ischemia, followed by immediate transplantation without preceding machine perfusion protocol. (n=8)

Tissue samples were prepared by transversal incisions through the cortex and medulla for subsequent histological analysis. The slice thickness was approximately 5 mm. Tissues were fixed for 48 h in a 4% neutral-buffered formaldehyde solution. All tissue samples were processed and embedded in paraffin according to standard histological procedures. Tissue blocks were sectioned at 2 µm to allow for nephropathological evaluation. The sections were stained with hematoxylin/eosin (Sigma Aldrich: MHS16/230251), Alcian

blue-periodic acid Schiff's stain (AB-PAS) (Polysciences: 25086-1) and Trichrome according to Masson (Polysciences: 25088-1) according to the instructions of the manufacturer. The morphology was evaluated in a blinded manner by an experienced nephropathologist.

Immunohistochemistry (IHC): Paraffin-embedded kidney biopsies (2 µm) were taken before and after machine perfusion (groups I-III) and kidney tissue at the end of evaluation from group IV. The sections were deparaffinized, rehydrated, and microwave antigen retrieval was performed in sodium citrate buffer and blocked for one hour. Sections were then stained with rabbit polyclonal anti-pig fibrinogen beta chain antibody (1:150, Abcam, ab

232793) overnight at 4°C. The MACH3 rabbit alkaline phosphatase (AP) polymer kit (Biocare Medical) was used as the detection system and immunostaining was visualized using the

Vulcan Fast Red Chromagen Kit (Biocare Medical) according to the manufacturer's protocol.

Hematoxylin was used as a counterstain.

Transmission electron microscopy (TEM): Needle biopsies were immersion-fixed in modified Karnovsky fixative (2.5% glutaraldehyde, 2% formaldehyde, 0.02% sodium azide in 0.05M Na-cacodylate buffer) for 1h at room temperature (RT) with gentle shaking followed by 8h at 4°C, and then stored for up to several weeks in fixative diluted 1:9 with 0.1M cacodylate buffer. The samples were then dissected, washed 5 times with 0.1M cacodylate buffer for a total of 1h with the third wash containing 50mM glycine, post-fixed in 1% osmium ferricyanide for 1h at 4°C, washed 5x with water for a total of 40 min, and contrasted en bloc with aq 0.5% uranyl acetate overnight at 4°C. Dehydration and

infiltration with Hard-Plus resin were performed using an automated microwave-assisted Leica EM AMW processor. Briefly, the samples were incubated in 30%, 50%, 70%, 85%, 95%, and 4 × 100% acetone for 2 min per step at 40°C. Resin without an accelerator was first applied in 25%, 50%, and 75% dilutions in acetone, 30 min per dilution, of which 10 min of microwaving at 40, 43, and 45°C, respectively. Pure resin without an accelerator was applied in at least five changes, 50 min per change, of which 10 min with microwaving at 50°C. Pure resin with accelerator was applied in at least two changes of 1h each, with 10 min of microwaving at 50°C per change. One of the 100% resin changes with or without an accelerator was applied to the samples overnight in 2 ml centrifuge tubes on a rotor at RT.

The samples were polymerized in silicon coffin molds or in flat BEEM capsules at 60°C for 48h. Sections of 80 nm thickness were collected using a Leica EM UC6 microtome, mounted on non-coated 150 hexagonal mesh copper grids, and contrasted with Raynold's lead citrate for 5 min. Images were collected on a ThermoFisher Talos L120C TEM at 120 kV using a 4 × 4 k Ceta camera.

Figure S1.

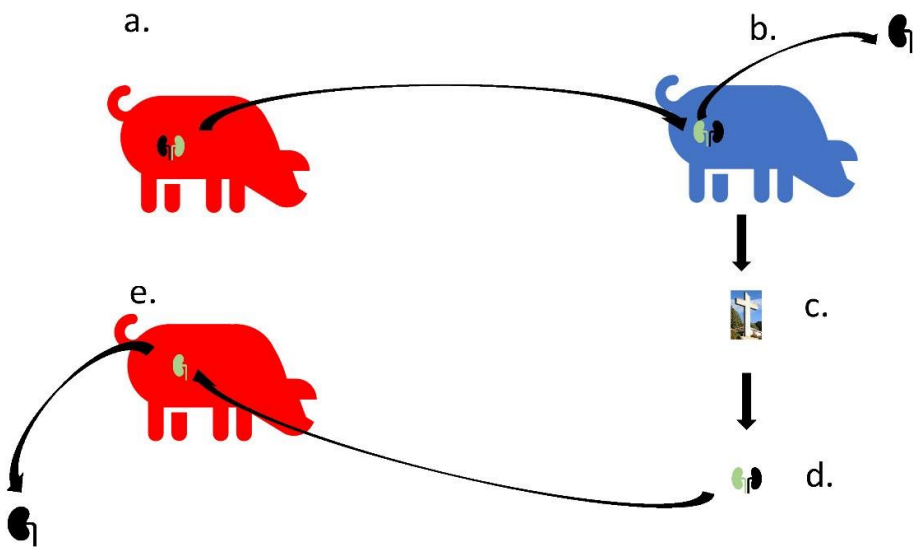

Fig S1. (a) The left kidney, in green, was transplanted from the recipient (red pig) to the donor pig (blue pig) after (b) the right kidney of the donor had been removed and discarded. (c) The donor pig was then converted to a uDCD pig. (d). After 4.5 hours, both the remaining donor kidney and the transplanted recipient kidney (in green) were removed en bloc with vena cava and aorta, followed by treatment according to Table I. (e) After completed reconditioning, the original recipient kidney, in green, was transplanted back to the recipient after the remaining right kidney of the recipient had been removed. The transplanted kidney is now an autotransplant, in a uDCD model.