THYMOKIDNEY AND METHODS OF CONSTRUCTING THEREOF

1. GOVERNMENT LICENSE RIGHTS

[0001] This invention was made with government support under AI045897 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

2. FIELD

[0002] The present disclosure provides porcine thymokidneys and, methods of making thereof, and methods of using thereof. The present disclosure further provides methods of treating a disease (*e.g.*, a kidney disease) in a human subject in need thereof comprising transplanting of a porcine thymokidney.

3. BACKGROUND

[0003] Xenotransplantation is a rapidly developing area of interest, with pre-clinical studies in deceased human donors already underway (*see, e.g.*, Moazami et al., *Nat Med* 29, 1989-1997 (2023); Montgomery et al., *N Engl J Med* 386, 1889-1898 (2022)). There is an ongoing and unmet need for kidney xenografts that are appropriate for patients with renal disease. Thymokidneys have been constructed to transplant life-supporting kidney grafts containing vascularized donor thymic tissue in order to induce operational transplant tolerance. Currently, thymokidneys are constructed by inserting pieces of thymus tissue under the kidney capsule using either an intra-abdominal or a lateral/flank approach. The intra-abdominal approach causes intra-abdominal adhesions and has a higher risk for complications, while the lateral/flank approach offers limited visibility and exposure of the kidney, leading to inclusion of smaller volumes of thymus tissue under the kidney capsule. As such, there is a need in the art to develop thymokidneys substantially free of adhesions comprising a high volume of thymic tissue capable of supporting thymopoiesis.

4. SUMMARY

[0004] In an aspect, the present disclosure provides an isolated porcine thymokidney comprising:

a. a porcine kidney; and

b. porcine thymic tissue,

wherein the thymic tissue covers at least 20% of the anterior surface of the kidney.

[0005] In certain embodiments, the thymic tissue covers at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney.

[0006] In certain embodiments, the thymic tissue is partially or fully covered by porcine peritoneal membrane. In certain embodiments, the thymic tissue is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane.

[0007] In certain embodiments, the kidney and/or thymic tissue and/or peritoneal membrane are from the same herd. In certain embodiments, the kidney and/or thymic tissue and/or peritoneal membrane are from a minipig. In certain embodiments, the kidney and/or thymic tissue and/or peritoneal membrane are from the same individual minipig.

[0008] In certain embodiments, the minipig is a genetically modified minipig. In certain embodiments, the genetically modified minipig has at least one genetic modification. In certain embodiments, the least one genetic modification comprises:

a. knockout of the *GGTA1* gene;

b. knockout of the *CMAH* gene;

c. knockout of the *β4GalNT* gene;

[0009] In certain embodiments, genetically modified minipig comprises at least one genetic modification that reduces or eliminates expression of a protein encoded by the *GGTA1* gene, the *CMAH* gene, and the *β4GalNT* gene. Non-limiting additional examples of the genetic modification that potential benefit for xenotransplantation include transgenic expression of human decay-accelerating factor (DAF), human CD46, human CD59, human CD39, human thrombomodulin, human endothelial protein C receptor (EPCR), human HO-1, and human A20, anti-CD2, CTLA4Ig, human CD47, PD-L1, FasL, and Class I MHC (*see e.g.* Sykes et al., *Nat Rev Nephrol.* 2022 Dec;18(12):745-761). .

[0010] In certain embodiments, the least one genetic modification comprises expression of a protein for a porcine CTLA4 fused to a hinge and CH2/CH3 regions of human IgG1 (pCTLA4-Ig). In certain embodiments, the least one genetic modification that results in the inactivation of porcine endogenous retrovirus (PERV).

[0011] In certain embodiments, the genetically modified minipig comprises a *GGTA1* genetic modification. In certain embodiments, the genetically modified minipig does not express alpha-1,3-galactosyltransferase (GalT-KO).

[0012] In certain embodiments, the thymic tissue is fully neovascularized.

[0013] In certain embodiments, the thymic tissue has a higher cell density than that of a porcine thymic tissue that is not included in the isolated porcine thymokidney. In certain embodiments, the thymic tissue has a cell density ranging from about 12000 to 15000 cells/ 1×10^{-6} cm^3 in thymic cortex tissue. In certain embodiments, the thymic tissue has a cell density ranging from about 3000 to 6000 cells/ 1×10^{-6} cm^3 in thymic medulla tissue.

[0014] In certain embodiments, the thymic tissue is capable of supporting thymopoiesis. In certain embodiments, the thymic tissue comprises $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes. In certain embodiments, the thymic tissue comprises at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes.

[0015] In certain embodiments, the thymokidney is substantially free of detectable scar tissue. In certain embodiments, the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% detectable scar tissue.

[0016] In certain embodiments, the thymokidney has at least one genetic modification. In certain embodiments, the thymokidney does not express alpha-1,3-galactosyltransferase (GalT-KO).

[0017] In certain embodiments, the thymokidney was constructed using anterior retroperitoneal surgery.

[0018] In another aspect, the present disclosure provides a method of treating a disease of the kidney in a human subject in need thereof, wherein the method comprises: transplanting the thymokidney disclosed herein into the subject.

[0019] In certain embodiments, the thymokidney is transplanted into the contralateral side in the subject compared to the donor animal.

[0020] In certain embodiments, the thymokidney is transplanted with the anterior surface of the kidney facing posteriorly. In certain embodiments, the thymokidney is transplanted such that the anterior surface of the kidney has minimal to no contact with the subject's great omentum. In

certain embodiments, less than 25%, 20%, 15%, 10%, 5%, or 1% of the anterior surface of the kidney is in contact with the subject's great omentum.

[0021] In another aspect, the present disclosure provides a method of creating the thymokidney described herein, wherein the method comprises:

a. obtaining thymic tissue from a porcine donor animal;

b. transplanting the thymic tissue obtained in step a to the anterior surface of a kidney of a recipient animal.

[0022] In certain embodiments, the thymic tissue is obtained using cervical thymectomy.

[0023] In certain embodiments, the transplanting step b is conducted using anterior retroperitoneal surgery. In certain embodiments, the transplanting step b is conducted without disrupting the peritoneal cavity. In certain embodiments, the transplanting step b further comprises inserting the thymic tissue under the kidney capsule.

[0024] In certain embodiments, at least $2cm^3$ of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal. In certain embodiments, at least 2 cm^3, 3 cm^3, 4 cm^3, 5 cm^3, or 6 cm^3 of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal.

[0025] In certain embodiments, the transplanted thymic tissue covers at least 20% of the anterior surface of the kidney of the recipient animal. In certain embodiments, the transplanted thymic tissue covers at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 75% of the anterior surface of the kidney of the recipient animal.

[0026] In certain embodiments, the method of creating the thymokidney described herein further comprises:

c. covering the anterior surface of the kidney with the peritoneum after the transplanting step b.

[0027] In certain embodiments, the thymokidney is harvested from the recipient animal at least 4 weeks after the transplanting step b. In certain embodiments, the thymokidney is harvested from the recipient animal at least 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step b.

[0028] In certain embodiments, the thymokidney is substantially free of perinephric and/or intra-abdominal adhesions for at least 6 weeks after the transplanting step b. In certain

embodiments, the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% perinephric and/or intra-abdominal adhesions.

5. BRIEF DESCRIPTION OF THE FIGURES

[0029] **FIGS. 1A-1C** depict representative images of a cervical thymectomy in a porcine donor. **FIG. 1A** shows a view of the thymic lobes before ligating the internal cervical artery and veins and recovery of the thymus. **FIG. 1B** shows that no thymic tissue was left behind in the neck of the swine. **FIG. 1C** shows the cervical thymus after removal.

[0030] **FIGS. 2A-2B** depict representative images of thymokidney (TK) construction in a porcine donor using an anterior retroperitoneal approach. **FIG. 2A** shows that a good view of the kidney was achieved without disrupting the peritoneum. **FIG. 2B** shows thymic tissue inserted underneath the kidney capsule. Arrows point to the inserted thymic tissue.

[0031] **FIGS. 3A-3D** depict representative images of a macroscopic overview of the thymokidneys (TKs) harvested 8-weeks after construction. **FIGS. 3A and 3B** represent the bilateral TKs harvested from a porcine donor with their anterior peritoneum still attached. **FIGS. 3C and 3D** represent the bilateral TKs harvested from a porcine donor after the removal of the anterior peritoneum. Good thymic engraftment can be appreciated in the images, where "a" marks thymic tissue on the TK anterior surface.

[0032] **FIGS. 4A-4E** depict representative images of histopathology of the thymokidneys. **FIG. 4A** shows a histopathological view of the posterior aspect of the TK, recovered as a negative control. Glomeruli and tubules can be appreciated. However, no thymus tissue was visible underneath the kidney capsule. **FIG. 4B** shows a histopathological view of the naïve thoracic thymus, recovered as a positive control. Thymic lobules, cortex and medulla can be appreciated. **FIG. 4C** shows a histopathological view of the TK, recovered 8-weeks after TK construction, where "a" marks the kidney capsule, "b" marks the thymic medulla, "c" marks the thymic cortex, and "d" marks the kidney parenchyma. Representative images of a 64X magnified view of the naïve thoracic thymus medulla (**FIG. 4D**) and of the TK thymus medulla (**FIG. 4E**) recovered at the time of the necropsy are shown. Arrows show Hassall's Corpuscles.

[0033] **FIGS. 5A-5C** depict flow cytometric analysis of the thymokidneys. Shown are the analyses of the kidney (**FIG. 5A**) and thoracic thymus (**FIG. 5B**) that were performed to be used as a negative and positive control, respectively. **FIG. 5C** shows the analysis performed on the thymus of TKs, showing the presence of $CD3^{-}CD4^{+}CD8^{+}$ DP cells.

6. DETAILED DESCRIPTION

[0034] Provided herein is a porcine thymokidney, methods of making thereof, and methods of using thereof.

[0035] In certain embodiments, provided herein is a porcine thymokidney (see Section 6.1). In certain embodiments, a porcine thymokidney provided herein comprises a porcine kidney and porcine thymic tissue. A porcine donor that provides tissues comprising a porcine thymokidney disclosed herein is described in Section 6.2 below.

[0036] In certain embodiments, provided herein are methods of making a porcine thymokidney (see Section 6.3). A porcine thymokidney of the present disclosure is generated, in part, from thymic tissue obtained from a porcine donor according to the methods described in Section 6.3.1 below. Also provided herein are methods of generating a porcine thymokidney using an anterior retroperitoneal surgery (see Section 6.3.2). Following anterior retroperitoneal surgery according to the methods disclosed herein, a porcine thymokidney remains in the porcine donor for further development until donor nephrectomy (see Section 6.3.3).

[0037] In certain embodiments, provided herein are methods of using a porcine thymokidney (see Section 6.4). A porcine thymokidney of the present disclosure can be transplanted into a subject in need thereof according to the methods described in Section 6.4.1 below. A subject suitable for receiving a porcine thymokidney disclosed herein is described in Section 6.4.3 below. Such a subject in need of a porcine thymokidney of the present disclosure can have, be suspected of having, or demonstrate at least one symptom of a disease described in Section 6.4.4 below.

6.1 Porcine Thymokidney

[0038] In certain embodiments, the present disclosure provides a porcine thymokidney. In certain embodiments, a thymokidney of the present disclosure comprises a kidney prepared by transplanting thymic tissue from a donor under said donor's kidney capsule. In certain embodiments, a porcine thymokidney provided herein comprises a porcine kidney and porcine thymic tissue. In certain embodiments, a porcine thymokidney disclosed herein is an isolated porcine thymokidney. In certain embodiments, an isolated porcine thymokidney provided herein comprises a porcine thymokidney removed from the porcine donor. Porcine donors and tissues derived thereof are further described in Section 6.2 and methods of making a porcine thymokidney disclosed herein are described in Section 6.3.

[0039] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is covered by porcine peritoneal membrane. The peritoneal membrane is a serous membrane that lines the abdominal cavity. The kidneys lie retroperitoneally (*i.e.*, behind the peritoneal membrane) on the posterior abdominal wall. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is fully covered by porcine peritoneal membrane. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is partially covered by porcine peritoneal membrane. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is more than about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is about 25% to about 30%, about 30% to about 35%, about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, about 95% to about 99%, about 25% to about 50%, about 25% to about 70%, about 25% to about 99%, about 50% to about 75%, about 50% to about 99%, or about 75% to about 99% covered by porcine peritoneal membrane. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane.

[0040] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers the anterior surface of the kidney. The anterior surface of the kidney is convex and positioned forward and lateralward, whereas the posterior surface of the kidney is positioned backward and medialward. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine

thymic tissue wherein the thymic tissue covers the entire anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue partially covers the anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers more than about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, about 95% to about 100%, about 10% to about 50%, about 10% to about 60%, about 10% to about 100%, about 15% to about 50%, or about 20% to about 40% of the anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers about 20% of the anterior surface of the kidney.

[0041] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue does not cover the posterior surface of the kidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue covers less than about 1%, 2%, 3%, 4%, 5%, 10% or 15% of the posterior surface of the kidney.

[0042] In certain embodiments, the transplanting of the thymic tissue is conducted using anterior retroperitoneal surgery (*see* Section 6.3.2). The anterior retroperitoneal surgery approach

for the construction of the thymokidney provides access to the entire anterior surface of the kidney and the posterior surface of the kidney. It is thus possible to construct a thymokidney wherein the thymic tissue is transplanted as two patches to one kidney or both kidneys of the recipient animal. In certain embodiments, the thymic tissue is transplanted as two patches to both kidneys of the animal recipient. In certain embodiments, the thymic tissues is transplanted as two patches to one kidney of the animal recipient but to both anterior and posterior surface of the kidney (a sandwich thymokidney). In certain embodiments, the thymic tissue obtained according to a method described herein are transplanted as two patches to one kidney of the recipient animal and to one surface (either anterior or posterior) of the kidney (a double thymokidney).

[0043] In certain embodiments, a porcine thymokidney disclosed herein is substantially free of detectable scar tissue. Without being bound by any particular theory, an injury (*e.g.*, an incision, a tear, a rupture) to the peritoneal membrane can cause scar tissue formation. In certain embodiments, a porcine thymokidney disclosed herein comprises no detectable scar tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises minimal to substantially free of detectable scar tissue. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1%, 5%, 10%, 15%, or 20% detectable scar tissue. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, or about 15% to about 20% detectable scar tissue.

[0044] In certain embodiments, a porcine thymokidney disclosed herein is substantially free of detectable adhesions. Scar tissue formation is referred herein to as "adhesions" when the scar tissue formation results in pathologic attachments between physiological structures (*e.g.*, organs, adipose tissues, abdominal wall). In certain embodiments, a porcine thymokidney disclosed herein comprises no detectable adhesions. In certain embodiments, a porcine thymokidney disclosed herein comprises minimal to substantially free of detectable adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1%, 5%, 10%, 15%, or 20% detectable adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, or about 15% to about 20% detectable adhesions.

[0045] In certain embodiments, a porcine thymokidney disclosed herein is substantially free of detectable perinephric adhesions. As used herein, "perinephric adhesions" refer to scar tissue that has formed between the renal capsule and perinephric adipose tissue. In certain

embodiments, a porcine thymokidney disclosed herein comprises no detectable perinephric adhesions. In certain embodiments, a porcine thymokidney disclosed herein comprises minimal to substantially free of detectable perinephric adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1%, 5%, 10%, 15%, or 20% detectable perinephric adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, or about 15% to about 20% detectable perinephric adhesions.

[0046] In certain embodiments, a porcine thymokidney disclosed herein is substantially free of detectable intra-abdominal adhesions. As used herein, "intra-abdominal adhesions" refer to scar tissue that has formed between two or more organs or between organs and the abdominal wall. In certain embodiments, a porcine thymokidney disclosed herein comprises no detectable intra-abdominal adhesions. In certain embodiments, a porcine thymokidney disclosed herein comprises minimal to substantially free of detectable intra-abdominal adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1%, 5%, 10%, 15%, or 20% detectable intra-abdominal adhesions. In certain embodiments, a porcine thymokidney disclosed herein has less than about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, or about 15% to about 20% detectable intra-abdominal adhesions.

[0047] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is neovascularized. As used herein, "neovascularized" or "neovascularization" refers to the formation of new blood vessels in a tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is partially neovascularized. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is fully neovascularized. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% neovascularized tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises about 50% to about 99%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about

75% to about 80%, about 90% to about 95%, or about 95% to about 99% neovascularized tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% neovascularized tissue.

[0048] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is hypercellular. As used herein, a tissue or an organ referred to as "hypercellular" has an abnormal increase in the number of cells (*i.e.*, cell density) compared to that of a tissue or organ that is not deemed hypercellular. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has a higher cell density than that of a porcine thymic tissue that is not included in the porcine thymokidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% higher cell density than that of a porcine thymic tissue that is not included in the porcine thymokidney.

[0049] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises a porcine thymic cortex tissue with a higher cell density compared to that of a porcine thymic cortex tissue that is not included in the porcine thymokidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises porcine thymic cortex tissue with at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% higher cell density compared to that of a porcine thymic cortex tissue that is not included in the porcine thymokidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has a cell density ranging from about 10000 to 17000 cells/ 1×10^{-6} cm^3, 11000 to 16000 cells/ 1×10^{-6} cm^3, or about 12000 to 15000 cells/ 1×10^{-6} cm^3 in the thymic cortex tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has a cell density of at least about or about 10000 cells/ 1×10^{-6} cm^3, 11000 cells/ 1×10^{-6} cm^3, 12000 cells/ 1×10^{-6} cm^3, 13000 cells/ 1×10^{-6} cm^3, 14000 cells/ 1×10^{-6} cm^3, 15000 cells/ 1×10^{-6} cm^3, 16000 cells/ 1×10^{-6} cm^3, 17000 cells/ 1×10^{-6} cm^3, or more than about 17000 cells/ 1×10^{-6} cm^3 in the thymic cortex tissue.

[0050] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises porcine thymic medulla tissue with a higher cell density compared to that of a porcine thymic medulla tissue that is not included in the porcine thymokidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises porcine thymic medulla tissue with at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% higher cell density compared to that of a porcine thymic medulla tissue that is not included in the porcine thymokidney. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has a cell density ranging from about 1000 to 8000 cells/ 1×10^{-6} cm^3, 2000 to 7000 cells/ 1×10^{-6} cm^3, or 3000 to 6000 cells/ 1×10^{-6} cm^3 in the thymic medulla tissue. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue has a cell density of at least about or about 1000 cells/ 1×10^{-6} cm^3, 2000 cells/ 1×10^{-6} cm^3, 3000 cells/ 1×10^{-6} cm^3, 4000 cells/ 1×10^{-6} cm^3, 5000 cells/ 1×10^{-6} cm^3, 6000 cells/ 1×10^{-6} cm^3, 7000 cells/ 1×10^{-6} cm^3, 8000 cells/ 1×10^{-6} cm^3, or more than 8000 cells/ 1×10^{-6} cm^3 in the thymic medulla tissue.

[0051] In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue is capable of supporting thymopoiesis. Thymopoiesis is the process within the thymus that transforms thymocytes into mature T cells. In order for a thymocyte to differentiate into a mature T cell, the thymocyte goes through several maturational stages including the progression and maturation to double-positive (DP) thymocytes expressing both CD4 and CD8 co-receptors. Accordingly, without wishing to be bound by any particular theory, the presence of $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes in the porcine thymic tissue disclosed herein is indicative of a tissue that is capable of supporting thymopoiesis. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the thymic tissue comprises at least about or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% $CD3^-CD4^+CD8^+$ DP thymocytes. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue

wherein the thymic tissue comprises about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, about 20% to about 40%, about 40% to about 60%, about 50% to about 70%, about 20% to about 30%, about 30% to about 40%, or about 40% to about 50% $CD3^{-}CD4^{+}CD8^{+}$ DP thymocytes. In certain embodiments, one kidney donor pig has at least two patches of thymus tissue associated with its kidney(s). The purpose of the second patch is to allow biopsy on one patch while leaving the other patch entirely intact. Such a biopsy may be useful to conduct a quality control test on the thymus tissue to ensure healthy thymus tissue before transplantation. Without being bound by theory, it is possible that a biopsy might negatively impact the health of the patch of thymus tissue. As such, having two otherwise equal patches allows for a biopsy without risking the health of the tissue that did not undergo a biopsy. The two (or more) patches can be found on the same kidney or on both kidneys. In other embodiments, the patches are arranged on the kidney as a double thymokidney or as a sandwich thymokidney or both (*see* discussion above in paragraph [0042]).

[0052] In certain embodiments, a porcine thymokidney disclosed herein comprises at least one gene modification. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the kidney comprises at least one gene modification. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the kidney comprises the gene modification(s) of the porcine donor. In certain embodiments, a porcine thymokidney disclosed herein comprises a porcine kidney and porcine thymic tissue wherein the kidney comprises the gene modification(s) of any of the porcine donors described in Section 6.2. In certain embodiments, a porcine thymokidney disclosed herein comprises at least one gene modification in the gene encoding alpha-1,3-galactosyltransferase. In certain embodiments, a porcine thymokidney disclosed herein does not express alpha-1,3-galactosyltransferase (*i.e.*, GalT-KO).

6.2 Porcine Donors

[0053] In certain embodiments, a porcine thymokidney provided herein comprises a porcine kidney and porcine thymic tissue provided from a porcine donor. The term "porcine" is used

interchangeably herein with the terms "pig" and "swine" and refers to mammals in the family Suidae. Non-limiting examples of the breeds of swine suitable for use herein includes any of the following: American Landrace, American Yorkshire, Aksai Black Pied, Angeln saddleback, Appalachian English, Arapawa Island, Auckland Island, Australian Yorkshire, Babi Kampung, Ba Xuyen, Bantu, Basque, Bazna, Beijing Black, Belarus Black Pied, Belgian Landrace, Bengali Brown Shannaj, Bentheim Black Pied, Berkshire, Bisaro, Bangur, Black Slavonian, Black Canarian, Breitovo, British Landrace, British Lop, British Saddleback, Bulgarian White, Cambrough, Cantonese, Celtic, Chato Murciano, Chester White, Chiangmai Blackpig, Choctaw Hog, Creole, Czech Improved White, Danish Landrace, Danish Protest, Dermantsi Pied, Li Yan, Duroc, Dutch Landrace, East Landrace, East Balkan, Essex, Estonian Bacon, Fengjing, Finnish Landrace, Forest Mountain, French Landrace, Gascon, German Landrace, Gloucestershire Old Spots, Gottingen minipig, Grice, Guinea Hog, Hampshire, Hante, Hereford, Hezuo, Hogan Hog, Huntington Black Hog, Iberian, Italian Landrace, Japanese Landrace, Jeju Black, Jinhua, Kakhetian, Kele, Kemerovo, Korean Native, Krskopolje, Kunekune, Lamcombe, Large Black, Large Black-White, Large White, Latvian White, Leicoma, Lithuanian Native, Lithuanian White, Lincolnshire Curly-Coated, Livny, Malhado de Alcobaca, Mangalitsa, Meishan, Middle White, Minzhu, Minokawa Buta, Mong Cai, Mora Romagnola, Moura, Mukota, Mulefoot, Murom, Myrhorod, Nero dei Nebrodi, Neijiang, New Zealand, Ningxiang, North Caucasian, North Siberian, Norwegian Landrace, Norwegian Yorkshire, Ossabaw Island, Oxford Sandy and Black, Pakchong 5, Philippine Native, Pietrain, Poland China, Red Wattle, Saddleback, Semirechensk, Siberian Black Pied, Small Black, Small White, Spots, Surabaya Babi, Swabian-Hall, Swedish Landrace, Swallow Belied Mangalitza, Taihu pig, Tamworth, Thuoc Nhieu, Tibetan, Tokyo-X, Tsivilsk, Turopolje, Ukrainian Spotted Steppe, Ukrainian White Steppe, Urzhum, Vietnamese Potbelly, Welsh, Wessex Saddleback, West French White, Windsnyer, Wuzhishanm, Yanan, Yorkshire and Yorkshire Blue and White.

[0054] In certain embodiments, the breed of a porcine donor for use in the present disclosure is a breed of minipig. Non-limiting examples of minipig breeds include Bama, Berlin, Chinese, Clawn, Czech-Republic, Gottingen, Hanford, Lee Sung, Mini-Lewe, Mini-Sib, Munich, National Institutes of Health (NIH), Ohmini, Panepinto, Sinclair, Vietnamese potbellied, Wuzhishan, WZS, and Yucatan. In certain embodiments, a porcine donor for use herein is a NIH minipig. In

certain embodiments, a porcine donor for use herein is a Yucatan minipig. In certain embodiments, a porcine donor for use herein is a Göttingen minipig.

[0055] In certain embodiments, a porcine donor of the present disclosure can be a wholly or partially inbred swine. In certain embodiments, a porcine donor of the present disclosure can be a highly inbred swine. The determination of how inbred a swine is can be determined by calculating the coefficient of inbreeding (COI). The coefficient of inbreeding (f) of an individual swine can be calculated by formula (I):

$$f_X = \sum 0.5^{n-1} \cdot (1 + f_A) \quad \text{(I)},$$

wherein n is the number of individuals in the loop, and f_A is the coefficient of inbreeding of the common ancestor of X's parents. A loop is defined as a path that runs from an individual up to the common ancestor through one parent and back down to the other parent, without going through any individual twice. In certain embodiments, a porcine donor of the present disclosure can be an inbred swine having a COI of less than about 25, less than about 20, less than about 15, or less than about 10. In certain embodiments, a porcine donor of the present disclosure can be an inbred swine having a COI of less than about 1 to about 2, about 2 to about 3, about 3 to about 4, about 4 to about 5, about 5 to about 6, about 6 to about 7, about 7 to about 8, about 8 to about 9, or about 9 to about 10. In certain embodiments, a porcine donor of the present disclosure can be an inbred swine having a COI of less than about 0.1 to about 0.2, about 0.2 to about 0.3, about 0.3 to about 0.4, about 0.4 to about 0.5, about 0.5 to about 0.6, about 0.6 to about 0.7, about 0.7 to about 0.8, about 0.8 to about 0.9, or about 0.9 to about 1. In certain embodiments, a porcine donor of the present disclosure can be a highly inbred swine having a COI of less than about 1.

[0056] In certain embodiments, a porcine donor of the present disclosure includes wholly or partially inbred swine. In certain embodiments, a porcine donor of the present disclosure is from the same herd. A "herd," as used herein, refers to a group of at least one male and one female which can breed to produce fertile male and female offspring. In certain embodiments, the herd of swine includes at least one male swine and at least one female swine capable of reproduction, *e.g.*, at least one male and one female which can produce functional gametes. In certain embodiments, a porcine donor of the present disclosure is from a herd of wholly or partially inbred swine. In certain embodiments, a porcine donor of the present disclosure is from a herd of inbred swine homozygous for a major histocompatibility complex haplotype. In certain

embodiments, a porcine donor of the present disclosure is from a herd of swine homozygous for a major histocompatibility complex haplotype and at least about 50% homozygous at all other genetic loci. In certain embodiments, a porcine donor of the present disclosure is from a herd of swine homozygous for a major histocompatibility complex haplotype and at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% homozygous at all other genetic loci. In certain embodiments, a porcine donor of the present disclosure is from a herd of swine homozygous at swine leukocyte antigens A, B, C, DR, and/or DQ. In certain embodiments, the swine leukocyte antigens A, B, C, DR, and DQ can comprise haplotype a (Aa, Ba, Ca, DRa, DQa), haplotype c (Ac, Bc, Cc, DRc, DQc) haplotype d (Ad, Bd, Cd, DRd, DQd), haplotype g (Ag, Bg, Cg, DRg, DQg), haplotype h (Ah, Bh, Ch, DRh, DQh), and/or haplotype j (Aj, Bj, Cj, DRj, DQj).

[0057] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor disclosed herein. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from the same porcine donor disclosed herein. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different porcine donors from the same breed. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different porcine donors from the same herd. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different porcine donors that are major histocompatibility complex (MHC) (i.e., swine leukocyte antigen (SLA)) matched).

[0058] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a minipig. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from the same minipig disclosed herein. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs from the same breed. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs from the same herd. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken

from two different minipigs that are major histocompatibility complex (MHC) (i.e., swine leukocyte antigen (SLA) matched).

[0059] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each homozygous for a porcine MHC haplotype. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each homozygous for a porcine MHC haplotype selected from the group consisting of SLAaa, SLAcc, and SLAdd. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each homozygous for a recombinant porcine MHC haplotype. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each homozygous for a recombinant porcine MHC haplotype selected from the group consisting of SLAff, SLAgg, SLAhh, SLAjj, and SLAkk. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each homozygous for SLAhh. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane are taken from two different minipigs that each comprises a mismatch in minor histocompatibility antigens (mHAg).

[0060] In certain embodiments, a porcine donor of the present disclosure is genetically modified. In certain embodiments, a porcine donor of the present disclosure is highly inbred and is genetically modified. In certain embodiments, a porcine donor of the present disclosure is highly inbred for a porcine MHC haplotype and is genetically modified. In certain embodiments, a porcine donor of the present disclosure is highly inbred for SLAhh and is genetically modified.

[0061] In certain embodiments, a porcine donor of the present disclosure is genetically modified. In certain embodiments, a genetically modified porcine donor (*e.g.*, a transgenic, chimeric, or mosaic swine) comprises at least one genetic modification. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is prevented (*i.e.*, knocked out). In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is prevented in at least one organ, tissue, or cell type (*e.g.*, in the kidney and/or thymus). In certain embodiments, a genetically modified porcine donor comprises

at least one genetic modification in which the expression of a porcine protein is higher compared to that of a non-genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is higher in at least one organ, tissue, or cell type (*e.g.*, in the kidney and/or thymus) compared to the at least one organ, tissue or cell type of a non-genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is lower compared to that of a non-genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is lower in at least one organ, tissue, or cell type (*e.g.*, in the kidney and/or thymus) compared to the at least one organ, tissue or cell type of a non-genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which a human protein is expressed in the genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which a human protein is expressed in at least one organ, tissue, or cell type (*e.g.*, in the kidney and/or thymus) of the genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is prevented and at least one genetic modification in which a human protein is expressed in the genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein is prevented in at least one organ, tissue or cell type (*e.g.*, in the kidney and/or thymus) and at least one genetic modification in which a human protein is expressed in at least one organ, tissue or cell type (*e.g.*, in the kidney and/or thymus) of the genetically modified porcine donor.

[0062] In certain embodiments, a genetically modified porcine donor (*e.g.*, a transgenic, chimeric, or mosaic swine) comprises at least one genetic modification wherein the genetic modification comprises a mutation of a gene. A genetically modified porcine donor known in the art can be suitable for use in the present disclosure as such, but not limited to, those described in Denner, *J Transplant Technol Res* (2014) 4(2):133; Wei et al., *Front Cell Dev Biol* (2022) Oct 17;10:1031812; and Xi et al., *Front Cell Dev Biol* (2023) Jan 12;10:1093534, the disclosures of which are incorporated herein by reference. In certain embodiments, a genetically modified

porcine donor (*e.g.*, a minipig) comprises at least one genetic modification wherein the genetic modification comprises a mutation in the *GGTA1* gene, the *CMAH* gene, and/or the *β4GalNT* gene. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein encoded by the *GGTA1* gene, the *CMAH* gene, and/or the *β4GalNT* gene is prevented (*i.e.*, knocked out, or "KO") in the genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification in which the expression of a porcine protein encoded by the *GGTA1* gene, the *CMAH* gene, and/or the *β4GalNT* geneis prevented in at least one organ, tissue, or cell type (*e.g.*, in the kidney and/or thymus) of the genetically modified porcine donor. Non-limiting additional examples of the genetic modification that potential benefit for xenotransplantation include transgenic expression of human decay-accelerating factor (DAF), human CD46, human CD59, human CD39, human thrombomodulin, human endothelial protein C receptor (EPCR), human HO-1, and human A20, anti-CD2, CTLA4Ig, human CD47, PD-L1, FasL, and Class I MHC (see e.g. Sykes et al., Nat Rev Nephrol. 2022 Dec;18(12):745-761). In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification that results in expression of a protein for a porcine CTLA4 fused to a hinge and CH2/CH3 regions of human IgG1 (pCTLA4-Ig) in the genetically modified porcine donor. In certain embodiments, a genetically modified porcine donor comprises at least one genetic modification that results in the inactivation of porcine endogenous retrovirus (PERV) (*i.e.*, a PERV knockout). In certain embodiments, a genetically modified porcine donor (*e.g.*, a transgenic, chimeric, or mosaic swine) comprises at least one genetic modification wherein the genetic modification comprises a mutation in the *GGTA1* gene.

[0063] In certain embodiments, a genetically modified porcine donor is a genetically modified minipig. In certain embodiments, a genetically modified minipig comprises at least one genetic modification wherein the genetic modification comprises a mutation in the *GGTA1* gene. In certain embodiments, a genetically modified minipig does not express alpha-1,3-galactosyltransferase (*i.e.*, the minipig is a GalT-KO minipig). Examples of genetically modified donor minipigs that lack functional GGTA1 expression suitable for use in the present disclosure include those described in Kolber-Simonds et al., *PNAS* (2004) 101:7335-7340 and Lai et al., *Science* (2002) Feb 8;295(5557):1089-92, the disclosures of which are incorporated herein by reference.

[0064] In certain embodiments, a genetically modified porcine donor (*e.g.*, a genetically modified minipig) can be generated using any number of suitable methods known in the art for generating transgenic swine (*see, e.g.*, Lai *et al.*, *Science* (2002) Feb 8;295(5557):1089-92; Hryhorowicz et al., *Genes (Basel)* (2020) Jun 19;11(6):670). In certain embodiments, a method of generating a genetically modified porcine donor comprises micro-injection of DNA material into the male pronucleus, RNA material into the cytoplasm, or proteins into the cytoplasm or pronucleus at early embryonic stage (usually the zygote/one-cell stage). In certain embodiments, a method of generating a genetically modified porcine donor comprises sperm-mediated gene transfer (SMGT) (*see, e.g.*, Lavitrano et al., *Reprod Fertil Dev* (2006)18(1-2):19-23). In certain embodiments, a method of generating a genetically modified porcine donor comprises somatic cell nuclear transfer (SCNT). SCNT involves the transfer of the nucleus of a donor cell into an oocyte or early embryo from which the chromosomes have been removed (*see, e.g.*, Wilmut et al., *Philos Trans R Soc Lond B Biol Sci* (2015) Oct 19;370(1680):20140366). In certain embodiments, a method of generating a genetically modified porcine donor comprises a gene-targeting technique. Non-limiting examples of a gene-targeting technique suitable for use herein include homologous recombination (HR), non-homologous DNA end joining (NHEJ), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-based systems.

[0065] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor (*e.g.*, a transgenic, chimeric, or mosaic swine) disclosed herein. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from the same genetically modified porcine donor. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from are taken from two different genetically modified porcine donors from the same breed. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from are taken from two different genetically modified porcine donors from the same herd. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor wherein the genetic modification comprises a mutation of a gene. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a

genetically modified porcine donor wherein the genetic modification comprises a mutation in the *GGTA1* gene. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor that does not express alpha-1,3-galactosyltransferase. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor that does not express alpha-1,3-galactosyltransferase and is inbred for SLAhh. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor that is GalT-KO and the GalT-KO is the only genetic modification in the minipig.

[0066] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig (*e.g.*, a transgenic, chimeric, or mosaic minipig) disclosed herein. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from the same genetically modified minipig. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from are taken from two different genetically modified minipigs from the same breed. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from are taken from two different genetically modified minipigs from the same herd. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig wherein the genetic modification comprises a mutation of a gene. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig wherein the genetic modification comprises a mutation in the *GGTA1* gene. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig that does not express alpha-1,3-galactosyltransferase. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig that does not express alpha-1,3-galactosyltransferase and is inbred for SLAhh.

[0067] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a male or female porcine donor (*e.g.*, a minipig). In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue

and/or peritoneal membrane from a genetically modified male porcine donor (*e.g.*, a minipig). In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a male GalT-KO minipig. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified female porcine donor (*e.g.*, a minipig). In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a female GalT-KO minipig.

[0068] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified porcine donor, wherein the porcine donors are cytomegalovirus (CMV) negative (*see, e.g.,* Egerer S et al., *Xenotransplantation.* 2018 Jul;25(4):e12449).

[0069] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) that is at least about 6 weeks of age. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) that is at least about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, or more than about 10 weeks of age. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) that is at least about 6 weeks to 10 weeks, about 6 weeks to 9 weeks, about 6 weeks to 8 weeks, or about 7 weeks to 8 weeks. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) that is 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, or more than about 10 weeks of age.

[0070] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig that is at least about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, or more than about 10 weeks of age. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from genetically modified minipig that is at least about 6 weeks to 10 weeks, about 6 weeks to 9 weeks, about 6 weeks to 8 weeks, or about 7 weeks to 8 weeks. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from genetically modified minipig that

is 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, or more than about 10 weeks of age. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a GalT-KO minipig that is at least about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, or more than about 10 weeks of age. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a GalT-KO minipig that is at least about 6 weeks to 10 weeks, about 6 weeks to 9 weeks, about 6 weeks to 8 weeks, or about 7 weeks to 8 weeks. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a GalT-KO minipig that is about 8 weeks of age.

[0071] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) wherein the porcine donor weighs at least about 6 kg, about 7 kg, about 8 kg, about 9 kg, about 10 kg, about 11 kg, about 12 kg, or more than about 12 kg. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) wherein the weight of the porcine donor ranges from about 6 kg to 12 kg, about 7 kg to 11 kg, or about 8 kg to 10 kg. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a porcine donor (*e.g.*, a minipig) wherein the porcine donor weight is 6 kg, about 7 kg, about 8 kg, about 9 kg, about 10 kg, about 11 kg, about 12 kg, or more than about 12 kg.

[0072] In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a genetically modified minipig wherein the genetically modified minipig weighs at least about 6 kg, about 7 kg, about 8 kg, about 9 kg, about 10 kg, about 11 kg, about 12 kg, or more than about 12 kg. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a GalT-KO minipig wherein the GalT-KO minipig weighs at least about 6 kg, about 7 kg, about 8 kg, about 9 kg, about 10 kg, about 11 kg, about 12 kg, or more than about 12 kg. In certain embodiments, a thymokidney disclosed herein comprises a kidney and/or thymic tissue and/or peritoneal membrane from a GalT-KO minipig wherein the weight of the GalT-KO minipig ranges from about 8 kg to 10 kg.

6.3 Methods of Making

[0073] In certain embodiments, the present disclosure provides a method of creating a thymokidney described herein (*see* Section 6.1). In certain embodiments, a method of creating a thymokidney of the present disclosure comprises (a) obtaining thymic tissue from a porcine donor and (b) transplanting the thymic tissue obtained in step (a) to the anterior surface of a kidney of a recipient animal. Methods of obtaining thymic tissue from a porcine donor are described in Section 6.3.1 and methods of transplanting the obtained thymic tissue to the anterior surface of a kidney of a recipient animal are described in Section 6.3.2 below. In certain embodiments, the thymokidney resulting from steps (a) and (b) is incubated in the recipient animal for a period of time prior to harvesting for use. Methods of incubating a thymokidney created by methods of the present disclosure prior to harvesting are described in Section 6.3.3.

6.3.1 Obtaining Thymic Tissue from a Porcine Donor

[0074] In certain embodiments, a method of creating a thymokidney of the present disclosure comprises obtaining thymic tissue from a porcine donor (*e.g.*, a minipig) described herein (see Section 6.2). In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a thymectomy. A "thymectomy" as referred to herein is a surgical method to remove some or all of the thymus. The thymus is a soft organ with two lobes that is located anterior to the ascending aorta and posterior to the sternum. Any suitable method toward performing a thymectomy known in the art can be used to obtain thymic tissue from a porcine donor disclosed herein (*see, e.g.*, Yamada et al., *Methods Mol Biol* (2020) 2110:151-171).

[0075] In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a thymectomy to remove one or both lobes of the thymus. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial or a total (*i.e.*, 100%) thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial thymectomy wherein at least about or about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% of the thymus is removed from the porcine donor.

[0076] In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a cervical or thoracic thymectomy. Cervical and thoracic thymic tissue have similar stromal organization. Cervical thymic tissue is lying on the trachea and thyroid gland with parathyroid whereas thoracic thymic tissue is in mediastinum and anterior to pericardium. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a

thoracic thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial or a total (*i.e.*, 100%) thoracic thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial thoracic thymectomy wherein at least about or about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% of thoracic thymic tissue is removed from the porcine donor. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial thoracic thymectomy and a total cervical thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a cervical thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial or a total (*i.e.*, 100%) cervical thymectomy. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a partial cervical thymectomy wherein at least about or about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% of cervical thymic tissue is removed from the porcine donor. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a cervical thymectomy wherein one or both cervical thymus lobes are removed from the porcine donor. In certain embodiments, a method of obtaining thymic tissue from a porcine donor comprises a total cervical thymectomy.

[0077] In certain embodiments, thymic tissue obtained from a porcine donor according to methods herein is placed in a suitable physiological solution until transplanted into a recipient animal (*e.g.*, the anterior surface of a kidney of the recipient animal). Suitable physiological solutions include, but are not limited to, normal saline (about 0.9% w/v of NaCl), phosphate-buffered saline, Ringer's solution, Tris-buffered saline, and HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid)-buffered saline. In certain embodiments, thymic tissue obtained from a porcine donor according to a method disclosed herein is placed in normal saline until transplanted into a recipient animal. In certain embodiments, thymic tissue obtained from a porcine donor according to a method disclosed herein is placed in a suitable physiological solution (e.g., normal saline) until transplanted into a recipient animal wherein the physiological solution is at a temperature that is less than ambient temperature. In certain embodiments, thymic tissue obtained from a porcine donor according to a method disclosed herein is placed in a suitable physiological solution (*e.g.*, normal saline) until transplanted into a recipient animal wherein the physiological solution is at a temperature that is at least about or about 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, or more than 8°C. In certain embodiments,

thymic tissue obtained from a porcine donor according to a method disclosed herein is placed in a suitable physiological solution (*e.g.*, normal saline) until transplanted into a recipient animal wherein the physiological solution is at a temperature that is about 4°C.

[0078] In certain embodiments, thymic tissue obtained from a porcine donor according to a method disclosed herein is prepared in advance of transplanting to the anterior surface of a kidney of a recipient animal. In certain embodiments, thymic tissue obtained from a porcine donor is prepared in advance of transplanting by stripping of the thymic capsule. In certain embodiments, thymic tissue obtained from a porcine donor is prepared in advance of transplanting by cutting the thymic tissue into pieces. In certain embodiments, thymic tissue obtained from a porcine donor is prepared in advance of transplanting by cutting the thymic tissue into pieces that range in size from about 0.5 mm^3 to about 5 mm^3, about 1 mm^3 to about 4.5 mm^3, about 1.5 mm^3 to about 4 mm^3, about 2 mm^3 to about 3.5 mm^3, about 0.5 mm^3 to about 1 mm^3, about 1 mm^3 to about 1.5 mm^3, about 1.5 mm^3 to about 2 mm^3, about 2 mm^3 to about 2.5 mm^3, or about 1 mm^3 to about 2 mm^3. In certain embodiments, thymic tissue obtained from a porcine donor is prepared in advance of transplanting by cutting the thymic tissue into pieces that are at least about or about 0.5 mm^3, 1 mm^3, 1.5 mm^3, 2 mm^3, 2.5 mm^3, 3mm^3, 3.5 mm^3, 4 mm^3, 4.5 mm^3, or 5 mm^3.

6.3.2 Anterior Retroperitoneal Surgery

[0079] In certain embodiments, a method of creating a thymokidney of the present disclosure comprises (a) obtaining thymic tissue from a porcine donor according to a method described in Section 6.3.1 and (b) transplanting the thymic tissue obtained in step (a) to the anterior surface of a kidney of a recipient animal. In certain embodiments, a recipient animal comprises a porcine donor as described herein (*see* Section 6.2). In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal (*e.g.*, a porcine donor) are the same animal. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different porcine donors (*e.g.*, minipigs). In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different porcine donors (*e.g.*, minipigs) from the same breed. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different porcine donors (*e.g.*, minipigs) from the same herd. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two

different genetically modified porcine donors (*e.g.*, genetically modified minipigs). In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different genetically modified porcine donors (*e.g.*, genetically modified minipigs), wherein the genetically modified porcine donors have the same genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different genetically modified porcine donors (*e.g.*, genetically modified minipigs), wherein the genetically modified porcine donors have a different genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained is a non-genetically modified porcine donor and the recipient animal is a genetically modified porcine donor. In certain embodiments, the porcine donor from which thymic tissue is obtained is a non-genetically modified porcine donor and the recipient animal is a genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are the same breed. In certain embodiments, the porcine donor from which thymic tissue is obtained is a non-genetically modified porcine donor and the recipient animal is a genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are the same herd. In certain embodiments, the porcine donor from which thymic tissue is obtained is a non-genetically modified porcine donor and the recipient animal is a genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are major histocompatibility complex (MHC) (*i.e.*, swine leukocyte antigen (SLA)) -matched. In certain embodiments, the porcine donor from which thymic tissue is obtained is a genetically modified porcine donor and the recipient animal is a non-genetically modified porcine donor. In certain embodiments, the porcine donor from which thymic tissue is obtained is a genetically modified porcine donor and the recipient animal is a non-genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are the same breed. In certain embodiments, the porcine donor from which thymic tissue is obtained is a genetically modified porcine donor and the recipient animal is a non-genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are the same herd. In certain embodiments, the porcine donor from which thymic tissue is obtained is a genetically modified porcine donor and the recipient animal is a non-genetically modified porcine donor, wherein the non-genetically modified porcine donor and the genetically modified porcine donor are MHC

(*i.e.*, SLA) -matched. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from two different genetically modified porcine donors (*e.g.*, genetically modified minipigs), wherein the genetically modified porcine donors both comprise a *GGTA1* genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained is a non-genetically modified porcine donor and the recipient animal is a genetically modified porcine donor comprising a *GGTA1* genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained is a genetically modified porcine donor comprising a *GGTA1* genetic modification and the recipient animal is a non-genetically modified porcine donor. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from the same genetically modified porcine donor (*e.g.*, a genetically modified minipig), wherein the genetically modified porcine donor comprises a *GGTA1* genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from the same genetically modified minipig, wherein the genetically modified minipig comprises a *GGTA1* genetic modification. In certain embodiments, the porcine donor from which thymic tissue is obtained and the recipient animal are from the same genetically modified minipig, wherein the genetically modified minipig does not express alpha-1,3-galactosyltransferase (*i.e.*, a GalT-KO minipig).

[0080] In certain embodiments, the transplanting of the thymic tissue obtained according to a method described herein (*see* Section 6.3.1) to the anterior surface of a kidney of a recipient animal (*e.g.*, porcine donor) is conducted using anterior retroperitoneal surgery. In certain embodiments, anterior retroperitoneal surgery comprises accessing the kidney by dissection into the anterior retroperitoneal space of the porcine donor. In certain embodiments, anterior retroperitoneal surgery comprises accessing the kidney by dissection into the anterior retroperitoneal space of the porcine donor without disrupting the peritoneal cavity.

[0081] In certain embodiments, the transplanting of the thymic tissue obtained according to a method described herein to the anterior surface of a kidney of a recipient animal (*e.g.*, porcine donor) comprises inserting the thymic tissue underneath the kidney capsule of the recipient animal. In certain embodiments, thymic tissue is inserted underneath the kidney capsule of the recipient animal on the anterior surface of the kidney. In certain embodiments, thymic tissue is inserted underneath the kidney capsule of the recipient animal through at least about or about 1 incision, 2 incisions, 3 incisions, 4 incisions, or more than 4 incisions. In certain embodiments,

thymic tissue is inserted underneath the kidney capsule of the recipient animal through at least one incision wherein the incision ranges in length from about 0.5 cm to about 2 cm, about 0.5 cm to about 1.5 cm, or about 0.5 cm to about 1 cm.

[0082] In certain embodiments, thymic tissue obtained according to a method described herein is inserted underneath the kidney capsule of the recipient animal on the anterior surface of the kidney wherein the total about of thymic tissue transplanted on the anterior surface of a kidney is at least about or about 2 cm^3, 2.5 cm^3, 3 cm^3, 3.5 cm^3, 4 cm^3, 4.5 cm^3, 5 cm^3, 5.5 cm^3, or 6 cm^3. In certain embodiments, thymic tissue is inserted underneath the kidney capsule of the recipient animal on the anterior surface of the kidney wherein the total about of thymic tissue transplanted on the anterior surface of a kidney is between about 2 cm^3 to about 2.5 cm^3, about 2.5 cm^3 to about 3 cm^3, about 3 cm^3 to about 3.5 cm^3, about 3.5 cm^3 to about 4 cm^3, about 4 cm^3 to about 4.5 cm^3, about 4.5 cm^3 to about 5 cm^3, about 5 cm^3 to about 5.5 cm^3, about 5.5 cm^3 to about 6 cm^3, about 2 cm^3 to about 6 cm^3, about 2.5 cm^3 to about 5 cm^3, or about 3 cm^3 to about 4 cm^3. In certain embodiments, thymic tissue is inserted underneath the kidney capsule of the recipient animal on the anterior surface of the kidney wherein the total about of thymic tissue transplanted on the anterior surface of a kidney is at least about or about 1/3, about 1/2, or about 2/3 of one cervical thymus lobe. In certain embodiments, thymic tissue is inserted underneath the kidney capsule of the recipient animal on the anterior surface of the kidney wherein the total about of thymic tissue transplanted on the anterior surface of a kidney is from about 1/3 to about 2/3 or from about 1/3 to about 1/2 of one cervical thymus lobe.

[0083] In certain embodiments, thymic tissue obtained according to a method described herein is inserted underneath the kidney capsule of the recipient animal wherein the total amount of transplanted thymic tissue covers at least about or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 75% of the anterior surface of the kidney of the recipient animal. In certain embodiments, thymic tissue obtained according to a method described herein is inserted underneath the kidney capsule of the recipient animal wherein the total amount of transplanted thymic tissue covers about 20% to about 75%, about 25% to about 70%, about 30% to about 65%, about 35% to about 60%, about 40% to about 55%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%,

about 65% to about 70%, about 70% to about 75%, or about 40% to about 60% of the anterior surface of the kidney of the recipient animal.

[0084] In certain embodiments, a method of creating a thymokidney of the present disclosure can further comprise covering the anterior surface of the kidney with the peritoneum after the transplanting step (*i.e.*, after transplanting thymus tissue under the kidney capsule). Without wishing to be bound by any particular theory, covering the anterior surface of the kidney with the peritoneum after the transplanting the thymus tissue underneath the kidney capsule on the anterior surface of the kidney prevents displacement of the thymic fragments without the need to surgically close the incisions to the capsule. In certain embodiments, a method of creating a thymokidney of the present disclosure can further comprise covering the anterior surface of the kidney with the peritoneum after the transplanting step wherein the incisions made to the kidney capsule remain open.

6.3.3 Post Anterior Retroperitoneal Surgery

[0085] In certain embodiments, the thymokidney resulting from a method described in Sections 6.3.1 and 6.3.2 is incubated in the recipient animal for a period of time prior to harvesting for use.

[0086] In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney for at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney for about 4 weeks to 12 weeks, about 4 weeks to 11 weeks, about 4 weeks to 10 weeks, about 4 weeks to 9 weeks, about 4 weeks to 8 weeks, about 4 weeks to 7 weeks, about 4 weeks to 6 weeks, about 4 weeks to 5 weeks, about 5 weeks to 12 weeks, about 5 weeks to 11 weeks, about 5 weeks to 10 weeks, about 5 weeks to 9 weeks, about 5 weeks to 8 weeks, about 5 weeks to 7 weeks, about 5 weeks to 6 weeks, about 6 weeks to 12 weeks, about 6 weeks to 11 weeks, about 6 weeks to about 10 weeks, about 6 weeks to about 9 weeks, about 6 weeks to 8 weeks, about 6 weeks to 7 weeks, about 7 weeks to 12 weeks, about 7 weeks to 11 weeks, about 7 weeks about 10 weeks, about 7 weeks to 9 weeks, about 7 weeks to 8 weeks, about 8 weeks to 12 weeks, about 8 weeks to 11 weeks, about 8 weeks to 10 weeks, about 8 weeks to 9 weeks, about 9 weeks to 12 weeks, about 9 weeks to 11 weeks, about 9 weeks to 10 weeks, about 10 weeks to 12 weeks, about 10 weeks to 11 weeks, or about 11 weeks to about 12 weeks after

the transplanting step. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney for about 3 months, about 3.5 months, about 4 months, about 4.5 months, about 5 months, about 5.5 months, about 6 months, about 6.5 months, about 7 months, about 7.5 months, about 8 months, about 8.5 months, about 9 months, about 9.5 months, about 10 months, about 10.5 months, about 11 months, about 11.5 months, or about 12 months after the transplanting step In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney for about 3 months to 3.5 months, about 3.5 months to 4 months, about 4 months to 4.5 months, about 4.5 months to 5 months, about 5 months to 5.5 months, about 5.5 months to 6 months, about 6 months to 6.5 months, about 6.5 months to 7 months, about 7 months to 7.5 months, about 7.5 months to 8 months, about 8 months to 8.5 months, about 8.5 months to 9 months, about 9 months to 9.5 months, about 9.5 months to 10 months, about 10 months to 10.5 months, about 10.5 months to 11 months, about 11 months to 11.5 months, or about 11.5 months to 12 months after the transplanting step.

[0087] In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney after the transplanting step until the thymus tissue covering the anterior surface of the kidney is fully neovascularized. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney after the transplanting step until the thymus tissue covering the anterior surface of the kidney is partially neovascularized. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney after the transplanting step until the thymus tissue covering the anterior surface of the kidney comprises at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% neovascularized thymus tissue. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney after the transplanting step until the thymus tissue covering the anterior surface of the kidney comprises 50% to about 99%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 90% to about 95%, or about 95% to about 99% neovascularized thymus tissue. In certain embodiments, a method of incubating the thymokidney in the recipient animal comprises incubating the kidney after the transplanting step until the thymus tissue

covering the anterior surface of the kidney wherein the thymus tissue is non-vascularized. In certain embodiments, without being bound by theory, the thymokidney continues to neovascularize the thymic tissue following transplantation into the human recipient. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney comprised of fully neovascularized thymic tissue at least about or about 4 weeks, about 6 weeks, 7 weeks, 8 weeks, 9 weeks, about 10 week, about 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney comprised of at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% neovascularized thymus tissue at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step.

[0088] In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that is substantially free of detectable scar tissue for at least about or about 4 week, 5 week, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that has less than about 1%, 5%, 10%, 15%, or 20% detectable scar tissue for at least about or about 6 weeks, 7 weeks, 8 weeks, 9 weeks, or 10 weeks after the transplanting step.

[0089] In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that is substantially free of detectable adhesions for at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that has less than about 1%, 5%, 10%, 15%, or 20% detectable adhesions for at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step.

[0090] In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that is substantially free of detectable perinephric adhesions for at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that has less than about 1%, 5%, 10%, 15%, or 20% detectable perinephric adhesions for at least about or about 4 weeks, 5 weeks,

6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step.

[0091] In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that is substantially free of detectable intra-abdominal adhesions for at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step. In certain embodiments, a method of creating a thymokidney of the present disclosure results in a thymokidney that has less than about 1%, 5%, 10%, 15%, or 20% detectable intra-abdominal adhesions for at least about or about 6 weeks, 7 weeks, 8 weeks, 9 weeks, or 10 weeks after the transplanting step.

6.4 Methods of Use

[0092] In certain embodiments, the present disclosure provides a method of using a thymokidney described herein (*see* Section 6.1). In certain embodiments, a method of using a thymokidney of the present disclosure comprises (a) obtaining the thymokidney from a porcine donor (*see* Section 6.4.1(a)) and (b) transplanting the thymokidney obtained in step (a) into a subject (*i.e.*, a recipient) in need thereof (*see* Section 6.4.1(b)). Subjects suitable for receiving a thymokidney according to the methods disclosed herein are described in Section 6.4.3. In certain embodiments, one or more additional treatments can be administered to the thymokidney recipient as described in Section 6.4.2.

[0093] In certain embodiments, the present disclosure provides methods of treating a disease in a subject in need thereof, wherein the method comprises transplanting a thymokidney described herein (*see* Section 6.1). Methods of evaluating the effectiveness of the treatment of the disease in the thymokidney recipient is described in Section 6.4.4.

6.4.1 Method of Transplantation of Thymokidney

(a) Donor Nephrectomy

[0094] In certain embodiments, the present disclosure provides methods of obtaining a thymokidney prepared according to the methods described in Section 6.3. In certain embodiments, a porcine thymokidney is obtained from a porcine donor by donor nephrectomy. A "donor nephrectomy" is a surgical procedure to remove a donor kidney (*e.g.*, a thymokidney) from the donor for transplant into a patient in need thereof (*e.g.*, the "recipient"). In certain embodiments, a thymokidney prepared according to the methods described herein is obtained

from the donor using either an open donor nephrectomy, a laparoscopic donor nephrectomy, or a robotic-assisted donor nephrectomy.

[0095] In certain embodiments, a donor nephrectomy is performed at least about or about 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after creating a thymokidney according to the methods described in Section 6.3. In certain embodiments, a donor nephrectomy is performed about 4 weeks to about 5 weeks, about 5 weeks to about 6 weeks, about 6 weeks to about 7 weeks, about 7 weeks to about 8 weeks, about 8 weeks to about 9 weeks, about 9 weeks to about 10 weeks, about 10 weeks to about 11 weeks, about 11 weeks to about 12 weeks, or about 6 weeks to about 8 weeks after creating a thymokidney according to the methods described in Section 6.3. In certain embodiments, a donor nephrectomy is performed about 3 months, about 3.5 months, about 4 months, about 4.5 months, about 5 months, about 5.5 months, about 6 months, about 6.5 months, about 7 months, about 7.5 months, about 8 months, about 8.5 months, about 9 months, about 9.5 months, about 10 months, about 10.5 months, about 11 months, about 11.5 months, or about 12 months after creating a thymokidney according to the methods described in Section 6.3.

[0096] In certain embodiments, a donor nephrectomy removes a thymokidney generated according to the methods described herein en-bloc. As used herein, "en-bloc" means that all of the tissues associated with the thymokidney are removed during the nephrectomy. In certain embodiments, a thymokidney is removed from a porcine donor en-bloc with the anterior peritoneum.

[0097] In certain embodiments, during the transition period after the thymokidney is removed from the donor animal but before being transplanted in the recipient, the thymokidney is stored in certain container to maintain the viability of the thymokidney. In certain embodiments, the container is capable of perfusion of the thymokidney at warm and/or cold temperatures to extend the useful life of the thymokidney. In certain embodiments, the thymokidney is stored in the container with proper fluid samples. In certain embodiments, the thymokidney is stored in the container with ample supply of oxygen. An example of such a container is provided in U.S. Pat. No. 6,673,594, which is hereby incorporated by reference in its entirety.

(b) Transplantation of Thymokidney

[0098] In certain embodiments, the present disclosure provides a method of transplanting a thymokidney prepared according to the methods described in Section 6.3 into a subject in need thereof. A subject suitable for receiving a thymokidney disclosed herein is described in Section 6.4.3 below. Methods of transplanting a porcine donor kidney into a donor animal are generally known in the art. *See, e.g.,* Kozlowski et al., *Transplantation* (1999) Jan 15;67(1):18-30; Bühler et al., *Transplantation* (2001) Dec 15;72(11):1743-52; Griesemer et al., *Am J Transplant* (2009) Dec;9(12):2669-78; and Yamada et al., *Methods Mol Biol* (2020) 2110:151-171.

[0099] In certain embodiments, a thymokidney disclosed herein is transplanted into the contralateral side in the subject (*e.g.*, thymokidney recipient) compared to the donor animal. In certain embodiments, a left thymokidney of the porcine donor is transplanted into the right flank side of the subject. In certain embodiments, a thymokidney disclosed herein is transplanted into a subject such that the anterior surface of the thymokidney is facing posteriorly. In certain embodiments, a thymokidney disclosed herein is transplanted into a subject such that the surface of the thymokidney that comprises thymic tissue is facing posteriorly.

[00100] In certain embodiments, a thymokidney disclosed herein is transplanted into the subject (*e.g.*, thymokidney recipient) such that the anterior surface of the kidney has minimal to no contact with the subject's great omentum. The greater omentum is a large fold of visceral peritoneum that extends from the greater curvature of the stomach to the transverse colon and hangs over the intestines. In certain embodiments, a thymokidney disclosed herein is transplanted into the subject such that less than about 25%, 20%, 15%, 10%, 5%, or 1% of the anterior surface of the thymokidney is in contact with the subject's great omentum. In certain embodiments, a thymokidney disclosed herein is transplanted into the subject such that about 25% to about 20%, about 20% to about 15%, about 15% to about 10%, about 10% to about 5%, about 5% to about 1%, or less than about 1% of the anterior surface of the thymokidney is in contact with the subject's great omentum.

6.4.2 Additional Treatments

[00101] In certain embodiments, a recipient treated in accordance with the methods described herein (*e.g.*, transplantation of a thymokidney) undergoes additional treatment. A recipient may undergo additional treatment by one or more different methods. Additional treatment may occur prior to, concurrently with, or subsequent to the method of treatment provided herein (*e.g.*, transplantation of a thymokidney).

[00102] Additional treatments are generally intended to improve the tolerance of the thymokidney in recipients, but other treatments are contemplated. In certain embodiments, a method of transplantation of a thymokidney in accordance with the methods described herein can thus include administering one or more additional treatments, *e.g.*, a treatment that inhibits T cells, blocks complement, remove or degrade antibodies, or otherwise down regulates the recipient's immune response to the thymokidney. In certain embodiments, a recipient is thymectomized and/or splenectomized.

[00103] In certain embodiments, a recipient receives radiation, for example, total body irradiation. In certain embodiments, a recipient receives 5-10 Gy or 10-15 Gy irradiation. In certain embodiments, thymic irradiation can be used. In certain embodiments, the recipient is administered low dose radiation (*e.g.*, a sub lethal dose of between 100 rads and 400 rads whole body radiation). In certain embodiments, local thymic radiation is administered to the recipient.

[00104] The blood of a subject undergoing transplantation by a method described herein may contain preexisting, xenoreactive antibodies that target the thymokidney. Without being bound by theory, these pre-existing antibodies can be major effectors of rejection. Any method known to the skilled artisan can be used to eliminate or reduce the titer of these pre-existing antibodies. Such methods include for example immunoadsorption by donor-species organ hemoperfusion, immunoaffinity columns of synthetic oligosaccharides and treatment with anti-Ig antibodies. In certain embodiments, the thymokidney may be genetically modified such that it is not recognized by antibodies present in the host (*e.g.*, the donor thymic tissue and/or donor the kidney is a-1,3-galactosyltransferase deficient) per Section 6.2.

(a) Immunosuppressive Therapy

[00105] In certain embodiments, a patient receiving a thymokidney in accordance with the methods described herein can further receive immunosuppressive therapy. The immunosuppressive therapy may be any FDA-approved treatment indicated to reduce transplant rejection and/or ameliorate the outcome of xenotransplantation. Non-limiting examples of immunosuppressive therapy include calcineurin inhibitors (*e.g.*, tacrolimus or cyclosporine), antiproliferative agents (*e.g.*, anti-metabolites such a mycophenolate, 6-mercaptopurine or its prodrug azathioprine), inhibitors of mammalian target of rapamycin (mTOR) (*e.g.*, sirolimus, rapamycin), steroids (*e.g.*, prednisone), cell cycle inhibitors (azathioprine or mycophenolate mofetil), lymphocyte-depleting agents (*e.g.*, anti-thymocyte globulin or antibodies such as

alemtuzumab, siplizumab or basiliximab) and co-stimulation blockers (*e.g.*, belatacept). *See, e.g.*, Chung et al., *Ann Transl Med* (2020) Mar; 8(6): 409; van der Mark et al., *Eur Respir Rev* (2020) 29: 190132; and Benvenuto et al., *J Thorac Dis* (2018) 10:3141-3155.

[00106] In certain embodiments, immunosuppressive therapy can be administered as induction therapy (perioperative, or immediately after surgery), a maintenance dose, or for an acute rejection. Induction therapy commonly includes basiliximab, anti-thymocyte globulin or alemtuzumab. Immunosuppressive therapy can also be administered as maintenance therapy, which is often required to continue for the life of the recipient. Maintenance immunosuppressive therapy commonly includes a calcineurin inhibitor (tacrolimus or cyclosporine), an antiproliferative agent (mycophenolate or azathioprine), and corticosteroids. Immunosuppressive therapy for acute rejections commonly includes thymoglobulin or steroids. *See, e.g.*, Chung et al., *Ann Transl Med* (2020) Mar; 8: 409 and Benvenuto et al., *J Thorac Dis* (2018)10:3141-3155.

[00107] Non-limiting examples of immunosuppressants include, (1) antimetabolites, such as purine synthesis inhibitors (such as inosine monophosphate dehydrogenase (IMPDH) inhibitors, *e.g.*, azathioprine, mycophenolate, and mycophenolate mofetil), pyrimidine synthesis inhibitors (*e.g.*, leflunomide and teriflunomide), and antifolates (*e.g.*, methotrexate); (2) calcineurin inhibitors, such as tacrolimus, cyclosporine A, pimecrolimus, and voclosporin; (3) TNF-alpha inhibitors, such as thalidomide and lenalidomide; (4) IL-1 receptor antagonists, such as anakinra; (5) mammalian target of rapamycin (mTOR) inhibitors, such as rapamycin (sirolimus), deforolimus, everolimus, temsirolimus, zotarolimus, and biolimus A9; (6) corticosteroids, such as prednisone; and (7) antibodies to any one of a number of cellular or serum targets (including anti-lymphocyte globulin and anti-thymocyte globulin).

[00108] Non-limiting exemplary cellular targets and their respective inhibitor compounds include, but are not limited to, complement component 5 (*e.g.*, eculizumab); tumor necrosis factors (TNFs) (*e.g.*, infliximab, adalimumab, certolizumab pegol, afelimomab and golimumab); IL-5 (*e.g.*, mepolizumab); IgE (*e.g.*, omalizumab); BAYX (*e.g.*, nerelimomab); interferon (*e.g.*, faralimomab); IL-6 (*e.g.*, elsilimomab); IL-12 and IL-13 (*e.g.*, lebrikizumab and ustekinumab); CD3 (*e.g.*, muromonab-CD3, otelixizumab, teplizumab, visilizumab); CD4 (*e.g.*, clenoliximab, keliximab and zanolimumab); CDI la (*e.g.*, efalizumab); CD18 (*e.g.*, erlizumab); CD20 (*e.g.*, afutuzumab, ocrelizumab, pascolizumab); CD23 (*e.g.*, lumiliximab); CD40 (*e.g.*, teneliximab,

toralizumab); CD62L/L-selectin (*e.g.*, aselizumab); CD80 (*e.g.*, galiximab); CD147/basigin (*e.g.*, gavilimomab); CD154 (*e.g.*, ruplizumab); BlyS (*e.g.*, belimumab); CTLA-4 (*e.g.*, ipilimumab, tremelimumab); CAT (*e.g.*, bertilimumab, lerdelimumab, metelimumab); integrin (*e.g.*, natalizumab); IL-6 receptor (*e.g.*, tocilizumab); LFA-1 (*e.g.*, odulimomab); and IL-2 receptor/CD25 (*e.g.*, basiliximab, daclizumab, inolimomab).

[00109] In certain embodiments, a method provided herein can comprise steps to induce tolerance in the recipient, *e.g.*, by inducing mixed chimerism. "Mixed chimerism" is commonly understood to describe a state in which the lymphohematopoietic system of the recipient of allogeneic hematopoietic stem cells comprises a mixture of host and donor cells. This state is usually attained through either bone marrow or mobilized peripheral blood stem cell transplantation. Mixed chimerism can be transient or stable. *See, e.g.*, Sachs et al., *Cold Spring Harb Perspect Med* (2014) 4:a015529; U.S. Patent No. 6,296,846; and U.S. Patent No. 6,306,651.

(b) Hematopoietic Stem Cell Transplant

[00110] In certain embodiments, a patient receiving a thymokidney in accordance with the methods described herein can further receive a hematopoietic stem cell transplant. Stem cell engraftment and hematopoiesis across disparate species barriers can be enhanced by providing a hematopoietic stromal environment from the donor species. The stromal matrix supplies species-specific factors that are required for interactions between hematopoietic stem cells and their stromal environment, such as hematopoietic growth factors, adhesion molecules, and their ligands.

[00111] As liver is the major site of hematopoiesis in the fetus, fetal liver can also serve as an alternative to bone marrow as a source of hematopoietic stem cells. As an alternative or an adjunct to implantation, fetal liver cells can be administered in fluid suspension. The thymus is the major site of T cell maturation. Each organ includes an organ specific stromal matrix that can support differentiation of the respective undifferentiated stem cells implanted into the host. Thymic stromal tissue can be irradiated prior to transplantation.

[00112] Porcine hematopoietic chimeras can lead to donor-specific nonresponsiveness in the mixed lymphocyte reaction, lack of anti-donor IgG antibody production, and acceptance of donor grafts. Accordingly, mixed chimerism is capable of inducing tolerance in a highly disparate xenogeneic combination and can have the clinical potential to prevent xenograft rejection. *See,*

e.g., Griesemer et al., *Immunol. Rev* (2014) 258(1): 241-258; Sachs et al., *Cold Spring Harb Perspect Med* (2014) 4:a015529. Bone marrow cells (BMC), or another source of hematopoietic stem cells, *e.g.*, a fetal liver suspension, of the donor can be injected into the recipient in order to induce mixed chimerism. The hematopoietic stem cells may be taken from any source, for example from the bone marrow or peripheral blood stem cells. *See, e.g.*, Sachs et al., *Cold Spring Harb Perspect Med* (2014) 4:a015529. Donor BMC home to appropriate sites of the recipient and grow contiguously with remaining host cells and proliferate, forming a chimeric lymphohematopoietic population. By this process, newly forming B cells (and the antibodies they produce) are exposed to donor antigens, so that the transplant will be recognized as self. Tolerance to the donor is also observed at the T cell level in animals in which hematopoietic stem cell, *e.g.*, bone marrow cell, engraftment has been achieved. For bone marrow transplant, the recipient can be administered low dose radiation. In certain embodiments, the recipient can be treated with an agent that depletes complement, such as cobra venom factor (*e.g.*, at day -1).

(c) Thymus-derived Regulatory T-cells (thyTreg) co-transplantation with thymokidney

[00113] In certain embodiments, a patient receiving a thymokidney (e.g., a porcine thymokidney) in accordance with the methods described herein can further receive a co-transplantation of donor-specific thymus-derived Tregs (thyTreg) to promote dominant regulatory tolerance. *See, e.g.*, Bernaldo-de-Quirós E et al., *J Exp Med.* 2023 Dec 4;220(12):e20231045. In certain embodiments, a patient treated in accordance with the method described herein is administered about $2x10^7$ to about $3x10^7$ cells/kg thyTregs, wherein the thyTregs are isolated from the thoracic and cervical thymus harvested from the donor of the thymokidney. In certain embodiments, a patient treated in accordance with the method described herein is administered about $2x10^7$ to about $3x10^7$ cells/kg thyTregs, wherein the thyTregs are isolated from the thymus harvested from the donor. In certain embodiments, the thyTregs described herein can be isolated by sorting $CD3^+CD4^+CD8^-CD25^+CD127^-$ thymocytes through a combination of FACS (Fluorescence-activated cell sorting) and MASC (Magnetic activated cell sorting). In certain embodiments, the isolated thyTregs described herein can be administered intravenously immediately following the thymokidney transplantation. In certain embodiments, the isolated thyTregs described herein can be administered intravenously a few days (e.g., about 10 to 14 days) after the thymokidney transplantation.

6.4.3 Thymokidney Recipients

[00114] In certain embodiments, the present disclosure provides a method of treating a patient in need thereof with a thymokidney described herein (*see* Section 6.1). In certain embodiments, a patient treated with a thymokidney described herein can be a thymokidney recipient. In certain embodiments, a patient treated in accordance with the methods described herein (*e.g.*, the recipient of a thymokidney) is a human patient. As used herein, the terms "subject" and "patient" are used interchangeably and include any human or non-human mammal. Non-limiting examples include members of the human, equine, porcine, bovine, rattus, murine, canine and feline species. In certain embodiments, the subject is a non-human primate. In certain embodiments, the subject is human. In certain embodiments, the subject is a human adult. In certain embodiments, the subject is a human child. In certain embodiments, the subject is human and receives one or more donor grafts from a porcine donor. In certain embodiments, the subject is a non-human primate (*e.g.*, a baboon, a cynomolgus monkey or a rhesus macaque) and receives one or more grafts from a porcine donor. Methods of evaluating the effectiveness of treatment of a disease in the thymokidney recipient is described in Section 6.4.4. In certain embodiments, the subject is a human subject having a disease of the kidney.

[00115] In certain embodiments, a patient treated in accordance with the methods described herein is in need of a kidney transplant. A patient may be in need of a kidney transplant due to renal failure or the rejection of a donor kidney. Renal failure can have a number of causes, including but not limited to high blood pressure (hypertension), physical injury, diabetes, kidney disease (polycystic kidney disease, glomerular disease) and autoimmune disorders such as lupus. Renal failure may be acute or chronic. Kidney failure can also be diagnosed by laboratory tests such as glomerular filtration rate, blood urea nitrogen, and serum creatinine, by imaging test (ultrasound, computer tomography) or a kidney biopsy.

[00116] In certain embodiments, a patient treated in accordance with a method described herein has Stage 1 kidney disease. In certain embodiments, a patient treated in accordance with a method described herein has Stage 2 kidney disease. In certain embodiments, a patient treated in accordance with a method described herein has Stage 3 kidney disease. In certain embodiments, a patient treated in accordance with a method described herein has Stage 4 kidney disease. In certain embodiments, a patient treated in accordance with a method described herein has Stage 5 kidney disease.

[00117] In certain embodiments, a patient treated in accordance with a method described herein has a glomerular filtration rate (GFR) of about 90 or higher. In certain embodiments, a patient treated in accordance with a method described herein has a GFR of about 60-90. In certain embodiments, a patient treated in accordance with a method described herein has a GFR of about 30-60. In certain embodiments, a patient treated in accordance with a method described herein has a GFR of about 15-30. In certain embodiments, a patient treated in accordance with a method described herein has a GFR of about 15 or less.

6.4.4 Methods of Evaluating Transplantation

[00118] In certain embodiments, use of a thymokidney produced according to the present disclosure results in a decreased occurrence of transplant rejection compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in a decreased occurrence of acute transplant rejection compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in a decreased occurrence of short-term transplant rejection compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in an about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% decreased occurrence of transplant rejection (*e.g.*, acute, short-term, long-term) compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in an at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% decreased occurrence of transplant rejection (*e.g.*, acute, short-term, long-term) compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure.

[00119] In certain embodiments, use of a thymokidney produced according to the present disclosure results in a more viable transplant compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in a transplant comprising about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% more viability compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in a transplant comprising about at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% more viability compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, the viability of the thymokidney produced according to the present disclosure is prolonged by about 1 to about 5, about 5 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, or more than about 30 more years compared to that of current standard of care (*i.e.*, a kidney transplant) or to the viability of a thymokidney produced according to methods other than those of the present disclosure.

[00120] In certain embodiments, use of a thymokidney produced according to the present disclosure results in a more functional transplant compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. Examples of methods for assessing kidney transplant function include laboratory tests such as glomerular filtration rate, blood urea nitrogen, and serum creatinine, imaging tests (*e.g.*, ultrasound, computer tomography), and/or kidney biopsy. In certain embodiments, use of a thymokidney produced according to the present disclosure results in a transplant comprising more than about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% function compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In

certain embodiments, use of a thymokidney produced according to the present disclosure results in a transplant comprising more than about at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% function compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure.

[00121] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in normal and/or improved function of at least one untargeted organ system within the thymokidney recipient as compared to the function of the other untargeted organ system before transplantation of the thymokidney to the patient. In certain embodiments, use of a thymokidney produced according to the present disclosure results in more than about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% improved function of at least one untargeted organ system compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% improved function of at least one untargeted organ system compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure.

[00122] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in better rate of survival of the thymokidney recipient as compared to the rate of survival before transplantation of the thymokidney to the patient. In certain embodiments, transplantation of a thymokidney produced according to the present disclosure improves the rate of survival of the thymokidney recipient by about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% compared to the rate of survival before transplantation of the thymokidney to the patient. In certain embodiments, transplantation of a thymokidney produced according to the present disclosure improves the rate of survival of the thymokidney recipient by at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% compared to the rate of

survival before transplantation of the thymokidney to the patient. In certain embodiments, transplantation of a thymokidney produced according to the present disclosure improves the rate of survival of the thymokidney recipient by about 3 months to about 6 months, about 6 months to about 1 year, about 1 year to about 3 years, about 3 years to about 5 years, about 5 years to about 10 years, about 10 years to about 15 years, about 15 years to about 20 years, about 20 years to about 25 years, about 25 years to about 30 years, about 30 years to about 40 years, about 40 years to about 50 years, or about more than 50 years compared to the rate of survival before transplantation of the thymokidney to the patient. In certain embodiments, transplantation of a thymokidney produced according to the present disclosure improves the rate of survival of the thymokidney recipient by at least about or about 3 months, 6 months, 1 year, 3 years, 5 years, 10 years, 15 years, 20 years, 25 years, 30 years, 40 years, 50 years, or more than 50 years compared to the rate of survival before transplantation of the thymokidney to the patient.

[00123] In certain embodiments, a method of treating a patient (*i.e.*, a recipient) in need thereof with a thymokidney produced according to the present disclosure can result in reduced administration of immunosuppressive therapy to the recipient when compared to current standard of care after the transplantation of the donor organs (*i.e.*, a kidney transplant). In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in a reduced amount of immunosuppressive agents administered to the recipient when compared to the amount of immunosuppressive agents generally administered to a comparable transplant recipient (*e.g.*, a person of the same sex and of comparable age, height, and/or weight). In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 10% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 10% to about 20% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 20% to about 30% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 30% to about 40% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 40% to about 50% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 50% to about 60% administered to the recipient. In certain embodiments, the

method provided herein results in a reduced amount of immunosuppressive agents by about 60% to about 70% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 70% to about 80% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by about 80% to about 90% administered to the recipient. In certain embodiments, the method provided herein results in a reduced amount of immunosuppressive agents by more than about 90% administered to the recipient.

[00124] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in a reduced administration frequency of immunosuppressive therapy to the recipient when compared to the one which is typically administered to a comparable recipient (*e.g.*, a person of the same sex and of comparable age, height, and/or weight). In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 10% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 10% to about 20% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 20% to about 30% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 30% to about 40% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 40% to about 50% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 50% to about 60% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 60% to about 70% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 70% to about 80% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by about 80% to about 90% administered to the recipient. In certain embodiments, the method provided herein results in a reduced frequency of immunosuppressive agents by more than about 90% administered to the recipient.

[00125] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in a shortened treatment duration of immunosuppressive therapy administered to the recipient when compared to the one which is typically administered to a comparable recipient (e.g., a person of the same sex and of comparable age, height, and/or weight). In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 10%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 10% to about 20%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 20% to about 30%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 30% to about 40%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 40% to about 50%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 50% to about 60%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 60% to about 70%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 70% to about 80%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by about 80% to about 90%. In certain embodiments, the method provided herein results in a shortened treatment duration of immunosuppressive therapy by more than about 90%.

[00126] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in an improvement of the corresponding disease that the transplantation aims to intervene within the thymokidney recipient as compared to the disease before transplantation of the thymokidney to the patient. In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in a greater improvement of the corresponding disease that the transplantation aims to intervene within the thymokidney recipient compared to that of a patient transplanted according to the current standard of care (*i.e.*, a kidney transplant) or with a thymokidney produced according to methods other than those of the present disclosure. One of skill in the art can appreciate that an improvement of the corresponding disease following transplantation of a thymokidney produced according to the

present disclosure is assessed according to the disease itself, severity of the disease, the health of the patient before and after transplant, and a myriad of other factors than can be disease and/or patient specific. In certain embodiments, an improvement of the corresponding disease can include, but is not limited to, amelioration and/or ablation of at least one symptom associated with the disease, slowing progression of the disease, preventing further progression of the disease, reversing disease progression, and/or returning the thymokidney recipient to a healthy status (*e.g.*, comparable to a healthy, disease-free patient of comparable age, gender, and background). In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in an improvement of the corresponding disease that the transplantation aims to intervene within the thymokidney recipient as indicated by at least one biomarker corresponding to the disease. In certain embodiments, use of a thymokidney produced according to the present disclosure results in about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% improvement of the corresponding disease compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% improvement of the corresponding disease compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure.

[00127] In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in an improvement of kidney disease and/or at least one symptom associated with kidney disease within the thymokidney recipient as compared to before transplantation of the thymokidney to the patient. Non-limiting examples of symptoms associated with kidney disease include abnormal GFR or eGFR (*e.g.*, less than about 90 ml/min/1.73 m^2 in a middle-aged adult), proteinuria (*e.g.*, over about 150 mg protein/day), abnormal serum creatinine levels (*e.g.*, over about 1.4 mg/dl), and abnormal blood urea nitrogen (BUN) levels (*e.g.*, over about 60 mg/dl). In certain embodiments, transplantation of a thymokidney produced according to the present disclosure results in a greater improvement of kidney disease and/or at least one symptom associated with kidney disease within the

thymokidney recipient compared to that of a patient transplanted according to the current standard of care (*i.e.*, a kidney transplant) or with a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or more than about 90% improvement of kidney disease and/or at least one symptom associated with kidney disease compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure. In certain embodiments, use of a thymokidney produced according to the present disclosure results in at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% improvement of kidney disease and/or at least one symptom associated with kidney disease compared to that of current standard of care (*i.e.*, a kidney transplant) or to the use of a thymokidney produced according to methods other than those of the present disclosure.

[00128] In certain embodiments, the severity of proteinuria is reduced in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. Proteinuria is characterized by increased levels of protein in the urine and can be a symptom of decreased kidney function and potentially renal failure. Clinically, proteinuria is defined as the excretion of over 150 mg protein per day in the urine. In certain embodiments, the severity of proteinuria is reduced by at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or over 95% in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. In certain embodiments, a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure does not have measurable proteinuria. In certain embodiments, a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure can experience transient proteinuria that resolves about 1, 2, 3, 3-7, 7-10, 10-14 days, or about 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8 weeks, or about 1, 2, 3, 4, 5, 6 months after the transplantation. In certain embodiments, a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure can experience urinary excretion of less than about 60 mg protein per day, less

than about 80 mg protein per day, less than about 100 mg protein per day, less than about 120 mg protein per day, less than about 140 mg protein per day, less than about 160 mg protein per day, less than about 200 mg protein per day, less than about 220 mg protein per day, less than about 240 mg, per day, less than about 260 mg protein per day, less than about 280 mg protein per day, less than about 300 mg protein per day, less than about 320 mg protein per day, less than about 340 mg protein per day, less than about 360 mg protein per day, less than about 380 mg protein per day, or less than about 400 mg protein per day.

[00129] In certain embodiments, creatinine clearance is improved in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. Creatinine clearance (often expressed as ml/min) can be determined by comparing the level of creatinine in urine with the creatinine level in blood, usually based on assessments of a 24-hour urine sample and a blood sample drawn at the end of the 24-hour period, and is used to estimate GFR (eGFR). Estimated glomerular filtration rate (eGFR) can be calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2021 equation (*See*, Inker et al., *N Engl J Med* (2021) 385(19): p. 1737-1749)). In certain embodiments, GFR or eGFR is improved in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. Normal GFR or eGFR can vary according to age, gender, and body weight. In young adults, GFR is approximately 120-130 ml/min/1.73 m^2. In certain embodiments, GFR or eGFR is improved by at least about or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or over 95% in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. In certain embodiments, a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure can experience a GFR or eGFR of at least about or about 15 ml/min/1.73 m^2, 20 ml/min/1.73 m^2, 25 ml/min/1.73 m^2, 30 ml/min/1.73 m^2, 35 ml/min/1.73 m^2, 40 ml/min/1.73 m^2, 45 ml/min/1.73 m^2, 50 ml/min/1.73 m^2, 55 ml/min/1.73 m^2, 60 ml/min/1.73 m^2, 65 ml/min/1.73 m^2, 70 ml/min/1.73 m^2, 75 ml/min/1.73 m^2, 80 ml/min/1.73 m^2, 85 ml/min/1.73 m^2, 90 ml/min/1.73 m^2, 95 ml/min/1.73 m^2, 100 ml/min/1.73 m^2, 105 ml/min/1.73 m^2, 110 ml/min/1.73 m^2, 115 ml/min/1.73 m^2, 120 ml/min/1.73 m^2, 125 ml/min/1.73 m^2, 130 ml/min/1.73 m^2, or more than 130 ml/min/1.73 m^2. In certain

embodiments, a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure can experience a GFR or eGFR ranging from about 15 ml/min/1.73 m^2 to about 20 ml/min/1.73 m^2, about 20 ml/min/1.73 m^2 to about 25 ml/min/1.73 m^2, about 25 ml/min/1.73 m^2 to about 30 ml/min/1.73 m^2, about 30 ml/min/1.73 m^2 to about 35 ml/min/1.73 m^2, about 35 ml/min/1.73 m^2 to about 40 ml/min/1.73 m^2, about 40 ml/min/1.73 m^2 to about 45 ml/min/1.73 m^2, about 45 ml/min/1.73 m^2 to about 50 ml/min/1.73 m^2, about 50 ml/min/1.73 m^2 to about 55 ml/min/1.73 m^2, about 55 ml/min/1.73 m^2 to about 60 ml/min/1.73 m^2, about 60 ml/min/1.73 m^2 to about 65 ml/min/1.73 m^2, about 65 ml/min/1.73 m^2 to about 70 ml/min/1.73 m^2, about 70 ml/min/1.73 m^2 to about 75 ml/min/1.73 m^2, about 75 ml/min/1.73 m^2 to about 80 ml/min/1.73 m^2, about 80 ml/min/1.73 m^2 to about 85 ml/min/1.73 m^2, about 85 ml/min/1.73 m^2 to about 90 ml/min/1.73 m^2, about 90 ml/min/1.73 m^2 to about 95 ml/min/1.73 m^2, about 95 ml/min/1.73 m^2 to about 100 ml/min/1.73 m^2, about 100 ml/min/1.73 m^2 to about 105 ml/min/1.73 m^2, about 105 ml/min/1.73 m^2 to about 110 ml/min/1.73 m^2, about 110 ml/min/1.73 m^2 to about 115 ml/min/1.73 m^2, about 115 ml/min/1.73 m^2 to about 120 ml/min/1.73 m^2, about 120 ml/min/1.73 m^2 to about 125 ml/min/1.73 m^2, about 125 ml/min/1.73 m^2 to about 130 ml/min/1.73 m^2, or about 130 ml/min/1.73 m^2 to more than about 130 ml/min/1.73 m^2.

[00130] In certain embodiments, the stage of chronic kidney disease is improved in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. The various stages of chronic kidney disease are associated with GFR or eGFR levels as follows: stage 1 (kidney damage with normal or increased GFR), GFR or eGFR greater than or equal to 90 ml/min/1.73 m^2; stage 2 (kidney damage with mild decrease in GFR), GFR or eGFR of 60-89 ml/min/1.73 m^2; stage 3 (kidney damage with moderate decrease in GFR), GFR or eGFR of 30-59 ml/min/1.73 m^2; stage 4 (kidney damage with severe decrease in GFR), GFR or eGFR of 15-29 ml/min/1.73 m2; and stage 5 (kidney failure), GFR or eGFR less than 15 ml/min/1.73 m^2. In certain embodiments, the stage of chronic kidney disease does not change in a thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. In certain embodiments, the stage of chronic kidney disease is decreased in a thymokidney recipient who was transplanted with a thymokidney produced according to the

present disclosure as compared to that before the transplantation of the thymokidney to the patient. In certain embodiments, the stage of chronic kidney disease is decreased by at least one stage in thymokidney recipient who was transplanted with a thymokidney produced according to the present disclosure as compared to that before the transplantation of the thymokidney to the patient. In certain embodiments, the stage of chronic kidney disease in a thymokidney recipient is decreased from a stage 5, as diagnosed before thymokidney transplantation, to stage 4, 3, 2, or 1. In certain embodiments, the stage of chronic kidney disease in a thymokidney recipient is decreased from a stage 4, as diagnosed before thymokidney transplantation, to stage 3, 2, or 1. In certain embodiments, the stage of chronic kidney disease in a thymokidney recipient is decreased from a stage 3, as diagnosed before thymokidney transplantation, to stage 2 or 1. In certain embodiments, the stage of chronic kidney disease in a thymokidney recipient is decreased from a stage 2, as diagnosed before thymokidney transplantation, to stage 1.

7. EXAMPLES

[00131] The following is a description of various methods and materials used in the studies. They are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent that the experiments below were performed and are all of the experiments that may be performed. It is to be understood that exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate the data and the like associated with the teachings of the present invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, percentages, etc.), but some experimental errors and deviations should be accounted for.

7.1 Example 1

[00132] Novel immunosuppression therapies and the generation of genetically modified swine have been able to circumvent hyperacute, acute, and subacute rejection in kidney xenotransplantation (KXTx), but chronic rejection still remains a challenge (Sykes & Sachs, *Nat Rev Nephrol* 18, 745-761 (2022); Sykes & Sachs, *Sci Immunol* 4 (2019)). One way in which chronic rejection may be overcome is through the development of xenotransplantation tolerance, via thymus transplantation with appropriate immunosuppressive conditioning (Duggan & Griesemer, *Curr Opin Organ Transplant* 25, 457-463 (2020); Griesemer et al., *Am J Transplant*

9, 2669-2678 (2009); Yamamoto et al., *Transplantation* 80, 1783-1790 (2005)). In KXTx, vascularized thymic lobe (VTL) transplantation was utilized to achieve such an effect (Yamamoto et al., *Transplantation* 80, 1783-1790 (2005)). However, VTL transplantation requires knowledge of and training in microsurgery and carries its own complications. To overcome these challenges, thymokidney (TK) organs have been prepared as an alternative approach, allowing the simultaneous transplantation of the life-supporting kidney graft along with vascularized donor thymic tissue.

[00133] Historically, TKs were constructed 6-8 weeks before transplantation to allow for neo-vascularization of the thymus tissue under the kidney capsule (Yamada et al., *Transplantation* 68, 1684-1692 (1999)). A cervical thymectomy was performed, and autologous thymic pieces were prepared and inserted underneath the kidney capsule using either an intra-abdominal or lateral/flank approach (Yamada et al., *Transplantation* 68, 1684-1692 (1999); Yamada et al., *J Immunol* 164, 3079-3086 (2000); Yamada et al., *Transplantation* 76, 530-536 (2003); Yamada et al., *Methods Mol Biol* 2110, 151-171 (2020)). Of these procedures, the intra-abdominal approach allowed for the insertion of bigger volumes and multiple pieces of thymus tissue but led to intra-abdominal adhesions that presented a challenge during donor nephrectomy. The flank approach allowed an intra-abdominal, adhesion-free, donor nephrectomy operation but did not allow for the insertion of multiple thymic fragments. In addition, the presence of perinephric adhesions still maintained a challenge in keeping the transplanted thymus intact during kidney harvesting.

[00134] To allow for a less challenging and adhesion-free donor nephrectomy and to maximize the thymus volume in TKs which, without wishing to be bound by any particular theory, positively correlates with the rate of thymopoiesis (*see* Kolte et al., *J Infect Dis* 185, 1578-1585 (2002)), the present disclsore provides novel TK construction methods. Described in the exemplary study herein is an anterior retroperitoneal approach for creating TKs in swine to be used in experimental models of operational transplant tolerance and potentially in future clinical trials.

7.1.1 Methods and Materials

(a) Animals

[00135] Eight (8)-week-old female GalT-KO Miniature Swine (8-10 kg) (n=2) were used for autologous TK (n=4) construction (*see, e.g.*, Lai et al., Science 295, 1089-1092 (2002); Kolber-

Simonds et al., *PNAS* 101, 7335-7340 (2004)). The properties of these animals have been previously described (Sachs et al., *Transplantation* 22, 559-567 (1976); Pennington et al., *Transplantation* 31, 66-71 (1981)). Animals were housed in single cages following Columbia University Institute of Comparative Medicine (ICM) guidelines.

(b) TK Construction using Anterior Retroperitoneal Approach

[00136] The animals were sedated, intubated, and draped. To recover the cervical thymus for TK construction, a midline incision on the neck was made between the sternum and mandibular angles. On both sides of the neck, the sternohyoid and sternocephalic muscles were retracted, and the cervical thymus lobes were visualized (**FIG. 1A**). On the medial sides, the cervical thymus lobes were dissected from the trachea, thyroid, and carotid sheaths. On the lateral sides, the thymus lobes were dissected from the muscles and external jugular veins (**FIG. 1B**). Dissection was continued posteriorly, and the thymus lobes were dissected from the prevertebral attachments. The internal cervical artery and vein of each thymus lobe were ligated using 2/0 silk. The thymus was removed and placed in a cold saline solution (**FIG. 1C**).

[00137] On the same animal, a midline abdominal incision was made and dissected until the peritoneum was identified. The peritoneum was carefully dissected laterally from the transverse abdominal muscle and its fascia using a pair of cotton-tipped applicators until the kidneys were identified. An incision was made on the lateral aspect of Gerota's fascia. The anterior wall of the kidneys was dissected from the peritoneum and Gerota's fascia, allowing direct visualization of the kidneys without disrupting the peritoneal cavity (**FIG. 2A**). Care was taken to minimize compression of the peritoneal cavity and the diaphragm so as not to disrupt ventilation during the surgery. On the back table, the cervical thymus was stripped of its capsule and cut into 1-2 mm^3-sized pieces. Two to three 1 cm incisions were made on the kidney capsule with the tip of an 18 gauge needle. A right-angle clamp was used to sweep the kidney capsule from the parenchyma in a circular motion, to create space for thymic fragments, which were inserted underneath the kidney capsule using a right-angle clamp and fine forceps under magnification (Loupes 2.5x magnification) (**FIG. 2B**). The peritoneum was carefully laid on the kidney, covering the insertion sites to prevent displacement of the thymic fragments. The abdominal fascia and skin were approximated with 2/0 PDS (polydioxanone) suture and staples, respectively. The neck incision was closed with 2/0 braided, absorbable suture and staples. One dose each of cefazolin and sustained release (SR) buprenorphine were administered for perioperative antibiotic

prophylaxis and postoperative pain control, respectively. The animals were observed for 8 weeks before TK harvest.

(c) Necropsy and Tissue Collection

[00138] The animals were euthanized 8 weeks after TK construction using 100 mg/kg intravenous Potassium Chloride (KCl) solution. Usage of Euthasol (Virbag, Westlake, TX) was avoided as it negatively impacts tissue processing and histopathological and flow cytometric results. The thoracic and abdominal cavities were opened. The renal arteries and veins and the ureters were ligated. The TKs were recovered en-bloc with the anterior peritoneal layer on the kidney. On the back table, the anterior peritoneum was carefully dissected from the TK and sent for histopathology. Macroscopically, the thymus portion of the TKs under the kidney capsule was identified in all 4 constructed TKs. The TK samples were recovered as 1 cm^3 pieces for histopathology. The thymus portion of the TKs was further stripped from the kidney for subsequent processing and flow cytometric analysis. A piece of the posterior surface of the kidneys and the native thoracic thymus were collected for histopathology and flow cytometric analysis, to be used as negative and positive controls, respectively.

(d) Tissue Processing

[00139] Tissues were collected in RPMI-1640 medium (Cytiva, Marlborough, MA) and stored at 4°C before processing into a single-cell suspension for flow cytometry analysis. Tissue samples collected from four TK, kidney, and native thoracic thymus were processed using the same technique.

[00140] On a tissue culture dish (Thermo Fischer, Waltham, MA), 10 ml of cold RPMI-1640 medium was added. A 70 µm strainer (Alkali Scientific, Fort Lauderdale, FL) was placed in the dish. A piece from a tissue sample was cut into smaller pieces and placed inside the 70 µm strainer. The end of a 20 ml syringe (McKesson, Irving, TX) was used to crush the tissue through the strainer. The cells were collected from the dish into a 50 ml conical and washed with Hanks' Balanced Salt Solution (HBSS) medium (Cytiva, Marlborough, MA, USA). Red blood cells were lysed using ammonium-chloride-potassium (ACK) lysing buffer (Thermo Fischer, Waltham, MA, USA). The cells were washed, resuspended in HBSS medium, and stored at 4°C for further analyses.

(e) Flow Cytometry

[00141] The single cell suspensions from the tissues were Fc-blocked with normal porcine serum and stained with mouse-anti-pig CD3 (Clone: BB23-8E6-8C8), CD4 (Clone: 74-12-4), and CD8α (Clone: 76-2-11) (BD, Franklin Lakes, NJ) antibodies for 30 minutes at 4°C. The tubes were washed twice with Fluorescent Activated Cell Sorting (FACS) Buffer (BD, Franklin Lakes, NJ). DAPI (4',6-diamidino-2-phenylindole; Abcam, Waltham, MA) was added before acquisition to distinguish dead cells. The data was acquired on a 5 Laser Cytek Aurora Flow Cytometer (Cytek, Fremont, CA) and analyzed using FCS Express (De Novo Software, Pasadena, CA). Live and singlet cells were selected and plotted using CD4 and CD8 to select $CD4^+CD8^+$ double-positive (DP) thymocytes. $CD4^+CD8^+$ DP thymocytes were further gated using CD3 to indicate the presence of $CD3^-CD4^+CD8^+$ DP thymocytes, which is a specific cell phenotype found only in the thymus.

(f) Histopathology

[00142] Tissues were collected and fixed in 10% buffered formalin (McKesson, Irving, TX) for 24-48 hours for histopathology studies. Samples were processed and stained with H&E (hematoxylin and eosin) according to standard methods known in the art. Slides were analyzed using Aperio ImageScope (Leica Camera, Teaneck, NJ) and HALO, a computational pathology software for image analysis (Indica Labs, Albuquerque, New Mexico).

(g) Xenotransplantation

[00143] Eight weeks after TK construction according to the methods of the exemplary study, the TK was harvested from the donor animals and transplanted into a recipient animal. After native right-side nephrectomy of the recipient animal, the kidney graft (e.g., TK) was transplanted. The renal artery and renal vein were anastomosed end-to-side to the recipient's infrarenal aorta and vena cava, retrospectively. Both vascular anastomoses were done by 7-0 Prolene running suture. The ureter was anastomosed to the urinary bladder in a standard way with 7-0 Maxon running suture and no stent was used. Intraoperative urine production, by the transplanted graft (e.g., TK), was observed.

7.1.2 Results

(a) Construction of Thymokidneys using the Anterior Retroperitoneal Approach

[00144] Bilateral TKs (n=4) were constructed in 2-month-old GalT-KO Miniature Swine (n=2) using the exemplary anterior retroperitoneal approach described in Section 7.1.1. Both

animals underwent total cervical thymectomy and subsequent autologous TK construction without any intra and postoperative complications (**FIGS. 1A-1C and 2A-2B**). The thymus was allowed to neovascularize and engraft for a period of 8 weeks. Thereafter, the animals were euthanized, and tissues were collected for histopathologic and flow cytometric analysis. The exemplary anterior retroperitoneal approach yielded successful engraftment of the thymus tissue, which covered approximately 30-50% of the anterior surface of the kidneys, as confirmed macroscopically (**FIGS. 3A-3D**) and by histopathology (**FIGS. 4A-4E**) and flow cytometric (**FIGS. 5A-5C**) analysis.

(b) The Anterior Retroperitoneal Approach Allowed Greater Visualization of the Kidneys and Thymic Tissue Implantation

[00145] During the TK construction, the exemplary anterior retroperitoneal approach allowed for greater visualization of the entire kidney compared to the flank approach (**FIGS. 2A-2B**). This level of kidney visualization was previously available only through the intra-abdominal approach. The exemplary anterior retroperitoneal approach provided access to the entire anterior surface of the kidney for subsequent thymic tissue implantation. During thymic tissue insertion under the kidney capsule, the inserted thymus pieces covered around 30-50% of the anterior surface of one kidney, which amounted to approximately 3-4 cm^3 (1/2 to 2/3 of one cervical thymus lobe) (**FIGS. 1A-1C and 2A-2B**). The exemplary anterior retroperitoneal approach resulted in an increase of approximately 4-6 fold more thymic tissue implanted, compared to the flank approach.

(c) Recovering the Thymokidneys Without Adhesions

[00146] The TKs were procured en-bloc with the anterior peritoneum to avoid disruption of the thymic tissue during procurement (**FIGS. 3A-3B**). No adhesions were encountered either intra-abdominal or around the kidney, as the peritoneum gently covered the implants. At the back table, a gentle dissection of the anterior peritoneum was carried out and a piece was subjected to histopathology to confirm whether any thymic tissue was left behind. After removing the anterior peritoneum, macroscopically, all 4 TKs had very successful engraftment of the thymic tissue, covering 40-60% of the anterior surface of the kidneys (**FIGS. 3C-3D**). Tissues were collected for flow cytometry and histopathology as described below.

(d) Histopathological and Flow Cytometry Analysis

[00147] At the time of necropsy, the thoracic thymus and a biopsy from the posterior surface of the kidneys was collected and used as positive and negative controls, respectively. One (1) cm^3 pieces were also cut from each TK for histopathology and the rest of the thymic tissue was stripped for flow cytometric analysis. The thymus on the TKs was hypercellular, with 13000-14000 cells/1×10-6 cm^3 in the cortex and 4000-5000 cells/1×10-6 cm^3 in the medulla. The cell count on the thymus of TK was almost identical to that of the thoracic thymus (**FIGS. 5A-5C**). Additionally, Hassall's corpuscles were noted in the medulla of the TK, indicating the presence of a healthy and functioning thymus tissue (**FIGS. 4D-4E**). In single-cell phenotype analysis using flow cytometry, the presence of thymus tissue and thymopoiesis was detected by measuring $CD3^-CD4^+CD8^+$ DP cells, which are unique to thymus and thymopoiesis in pigs. All four thymus tissues recovered from TKs, as well as the native thoracic thymus, demonstrated 60-65% $CD3^-CD4^+CD8^+$ DP cells, indicating the presence and function of the thymus tissue in the TKs (**FIGS. 5A-5C**).

7.1.3 Discussion

[00148] Xenotransplantation is a rapidly developing research area, with pre-clinical studies in deceased human donors already underway (*see, e.g.*, Moazami et al., *Nat Med* 29, 1989-1997 (2023); Montgomery et al., *N Engl J Med* 386, 1889-1898 (2022)). Even though TK construction has been performed for over 20 years, the development of operational xenotransplantation tolerance is currently lacking in the field and would undoubtedly boost xenotransplantation efforts in clinical trials. To achieve tolerance, the present disclosure provides simultaneous vascularized donor thymus transplantation together with the target organ. The TK construction exemplified herein is optimized and standardized to maximize its benefits across xenotransplantation barriers and allow other persons of skill in the art to reproduce the TK xenotransplantation model described herein readily and robustly.

[00149] Historically, the first reported TK in swine was constructed using the intra-abdominal approach, which allowed for greater visualization of the kidneys and thymic tissue implantation (Yamada et al., *Transplantation* 68, 1684-1692 (1999)). However, this technique was abandoned due to intra-abdominal adhesions that proved to be challenging during donor nephrectomy, and the lateral/flank approach was adopted to prevent such adhesions (Yamada et al., *Methods Mol Biol* 2110, 151-171 (2020)). However, the lateral/flank approach offers limited visibility and exposure of the kidney, leading to inclusion of smaller volumes of thymus tissue

under the kidney capsule. On the other hand, it has been shown that the volume of transplanted thymus corresponds to posttransplant thymopoiesis in TK (*see* Kolte et al., *J Infect Dis* 185, 1578-1585 (2002)). As such, there is a need in the art to overcome these challenges and standardize TK construction. The novel anterior retroperitoneal approach presented herein provides for excellent kidney visualization and for a large volume of thymic tissue implantation without the presence of intra-abdominal adhesions as an easily achievable result.

[00150] TK construction was divided into three sections: 1) autologous thymus procurement, 2) autologous TK construction and recovery 3) surgical and postoperative complexity. For autologous thymus procurement, total cervical thymectomy was performed herein for multiple reasons including, but not limited to the following: 1) 80% of the pig thymus resides in the cervical and 20% in the thoracic regions (data unshown). Cervical thymectomy is a relatively safe surgical procedure whereas, in contrast, harvesting the thoracic thymus requires a thoracotomy and meticulous arch dissection, as well as requiring more intensive postoperative care; 2) The volume of bilateral cervical lobes provides enough tissue to construct two autologous TK in the same donor; 3) A subtotal or a total thymectomy prior to TK construction enhances the thymus development in the TK, as newly produced thymocytes migrate into the main thymus tissue now localized under the kidney capsule and stimulating its growth. By performing only a cervical (subtotal, 80%) thymectomy, additional risk associated with resection of the thoracic thymus was avoided, and as shown previously, the thoracic thymus is not necessary for construction of TK grafts (Yamada et al., *Transplantation* 68, 1684-1692 (1999)). As demonstrated by the exemplary results herein, constructing TKs with a cervical subtotal thymectomy gave excellent and reproducible results with minimal surgical complexity and no postoperative complications (**FIGS. 1A-1C and 3A-3D**).

[00151] After anterior retroperitoneal implantation of the tissue under the kidney capsule, the peritoneum was gently laid on the kidney to firmly cover the insertion areas by the positive pressure created by the peritoneal cavity. This mechanism prevented the displacement of inserted thymic tissue which was previously accomplished using a suture placed on the insertion sites. Therefore, suturing of insertion holes in the capsule was not needed in the anterior retroperitoneal approach, and the use of the anterior retroperitoneal technique helped to ensure good results despite possible implantation imperfection while establishing the TK construction technique from the beginning.

[00152] In addition to allowing large thymus volume implantation, the anterior retroperitoneal technique also prevented the formation of perinephric or intra-abdominal adhesions. During the TK retrieval, no intra-abdominal nor perinephric adhesions were observed as the thymus was gently covered by the peritoneum that was procured en-bloc with the TK. This lack of adhesions left the abdominal cavity virgin, making any subsequent organ donation with *in situ* cold organ perfusion or intra-abdominal surgery after the construction of TK, such as thymic implantation of the islet to construct thymo-islet-kidney, relatively easy to perform.

[00153] Procurement of the TK en-bloc with the peritoneum also prevented any potential injury to the thymic tissue. Such damage was especially encountered in the intra-abdominal approach, where meticulous dissection was being performed due to adhesions, as well as in the flank approach, where the thymus would adhere to the perinephric fat, requiring further dissection with significant risk for implanted thymus tissue damage. Despite the significantly larger volume of thymus implantation, the operation time required for the anterior retroperitoneal approach exemplified herein was similar to the bilateral flank approach, in which the animal had to be repositioned and re-draped, and to the intra-abdominal approach, in which the abdominal cavity had to be opened, and the kidneys needed to be dissected. The recovery and postoperative care after the anterior retroperitoneal approach exemplified herein were the same as after the intra-abdominal or flank approach.

[00154] Lastly, in the pig-to-baboon xenotransplantation studies exemplified herein, the left donor kidney was transplanted to the right recipient flank to, among other things, minimize surgical complexity and maximize success. Compared to the lateral/flank approach, the anterior retroperitoneal approach allowed for creation of the thymus on the anterior surface of the left kidney, which, when transplanted in the right flank, had the thymus portion facing posteriorly. Positioning of the thymus posteriorly prevents or minimizes its interaction with the recipient's greater omentum, which was observed to be a pro-inflammatory organ that adheres to the thymus portion of the TK grafts after transplantation (data not shown). When the TK is transplanted en-bloc with the donor peritoneum, the donor peritoneum can protect the xenothymus from the greater omentum.

[00155] In sum, described herein is the successful development of a novel anterior retroperitoneal approach to construct TKs in swine to be used in experimental and clinical trials. The anterior retroperitoneal approach disclosed herein is reproduceable, gives excellent kidney

exposure, allows for more thymus tissue implantation under the kidney capsule than in the currently practiced flank approach, and does not cause intra-abdominal adhesions, making TK procurement easier and safer than the currently practiced intra-abdominal approach.

7.1.4 Conclusion

[00156] Using an exemplary anterior retroperitoneal approach in porcine donors as described above, thymokidneys with functional thymus tissue under the kidney capsule were successfully constructed as verified by histopathology and flow cytometry. No surgical complications were observed, and no adhesions were observed intra-abdominally nor around the kidney since the peritoneum covered the implanted tissue.

[00157] The anterior retroperitoneal approach to constructing the thymokidneys was easy to perform, offered excellent kidney exposure, allowed a large volume of thymus tissue to be implanted, and did not cause intra-abdominal adhesions. The anterior retroperitoneal procedure exemplified herein simplified subsequent harvesting of the thymokidney for transplantation, with minimal risk of tissue injury of the vascularized thymus.

8. ILLUSTRATIVE EMBODIMENTS

[00158] The present disclosure provides the following non-limiting embodiments:

1. An isolated porcine thymokidney comprising:
    a. a porcine kidney; and
    b. porcine thymic tissue,

wherein the thymic tissue covers at least 20% of the anterior surface of the kidney.

2. The thymokidney of embodiment 1, wherein the thymic tissue covers at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney.

3. The thymokidney of embodiment 1 or 2, wherein the thymic tissue is partially or fully covered by porcine peritoneal membrane.

4. The thymokidney of embodiment 3, wherein the thymic tissue is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane.

5. The thymokidney of any one of embodiments 1-4, wherein kidney and/or thymic tissue and/or peritoneal membrane are from the same herd.

6. The thymokidney of any one of embodiments 1-5, wherein kidney and/or thymic tissue and/or peritoneal membrane are from a minipig.

7. The thymokidney of embodiment 6, wherein kidney and/or thymic tissue and/or peritoneal membrane are from the same individual minipig.

8. The thymokidney of embodiment 6 or 7, wherein the minipig is a genetically modified minipig.

9. The thymokidney of embodiment 8, wherein the genetically modified minipig has at least one genetic modification.

10. The thymokidney of embodiment 9, wherein the least one genetic modification comprises:

a. knockout of the *GGTA1* gene;

b. knockout of the *CMAH* gene;

c. knockout of the *β4GalNT* gene.

11. The thymokidney of embodiment 10, wherein the least one genetic modification comprises no expression of a protein encoded by the *GGTA1* gene, the *CMAH* gene, and/or the *β4GalNT* gene.

12. The thymokidney of embodiment 10, wherein non-limiting additional examples of the genetic modification that potential benefit for xenotransplantation include transgenic expression of human decay-accelerating factor (DAF), human CD46, human CD59, human CD39, human thrombomodulin, human endothelial protein C receptor (EPCR), human HO-1, and human A20, anti-CD2, CTLA4Ig, human CD47, PD-L1, FasL, and Class I MHC (see e.g. Sykes et al., Nat Rev Nephrol. 2022 Dec;18(12):745-761).

13. The thymokidney of embodiment 9, wherein the least one genetic modification comprises expression of a protein for a porcine CTLA4 fused to a hinge and CH2/CH3 regions of human IgG1 (pCTLA4-Ig).

14. The thymokidney of embodiment 9, wherein the least one genetic modification results in the inactivation of porcine endogenous retrovirus (PERV).

15. The thymokidney any one of embodiments 8-14, wherein the genetically modified minipig comprises a *GGTA1* genetic modification.

16. The thymokidney any one of embodiments 8-15, wherein the genetically modified minipig does not express alpha-1,3-galactosyltransferase (GalT-KO).

17. The thymokidney of any one of embodiments 1-16, wherein the thymic tissue is fully neovascularized.

18. The thymokidney of any one of embodiments 1-17, wherein the thymic tissue has a higher cell density than that of a porcine thymic tissue that is not included in the isolated porcine thymokidney.

19. The thymokidney of embodiment 18, wherein the thymic tissue has a cell density ranging from about 12000 to 15000 cells/ 1×10^{-6} cm^3 in thymic cortex tissue.

20. The thymokidney of embodiment 18 or embodiment 19, wherein the thymic tissue has a cell density ranging from about 3000 to 6000 cells/ 1×10^{-6} cm^3 in thymic medulla tissue.

21. The thymokidney of any one of embodiments 1-19, wherein the thymic tissue is capable of supporting thymopoiesis.

22. The thymokidney of embodiment 21, wherein the thymic tissue comprises $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes.

23. The thymokidney of embodiment 22, wherein the thymic tissue comprises at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% $CD3^-CD4^+CD8^+$ double-positive (DP) thymocytes.

24. The thymokidney of any one of embodiments 1-23, wherein the thymokidney is substantially free of detectable scar tissue.

25. The thymokidney of embodiment 24, wherein the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% detectable scar tissue.

26. The thymokidney of any one of embodiments 1-25, wherein the thymokidney has at least one genetic modification.

27. The thymokidney of any one of embodiments 1-26, wherein the thymokidney does not express alpha-1,3-galactosyltransferase (GalT-KO).

28. The thymokidney of any one of the preceding embodiments, wherein the thymokidney was constructed using anterior retroperitoneal surgery.

29. A method of treating a disease of the kidney in a human subject in need thereof, wherein the method comprises: transplanting the thymokidney of any one of the preceding embodiments into the subject.

30. The method of embodiment 29, wherein the thymokidney is transplanted into the contralateral side in the subject compared to the donor animal.

31. The method of embodiment 29 or 30, wherein the thymokidney is transplanted with the anterior surface of the kidney facing posteriorly.

32. The method of any one of embodiments 29-31, wherein the thymokidney is transplanted such that the anterior surface of the kidney has minimal to no contact with the subject's great omentum.

33. The method of embodiment 32, wherein less than 25%, 20%, 15%, 10%, 5%, or 1% of the anterior surface of the kidney is in contact with the subject's great omentum.

34. A method of creating the thymokidney of any one of embodiments 1 to 28, wherein the method comprises:

a. obtaining thymic tissue from a porcine donor animal;

b. transplanting the thymic tissue obtained in step a to the anterior surface of a kidney of a recipient animal.

35. The method of embodiment 34, wherein the thymic tissue is obtained using cervical thymectomy.

36. The method of embodiment 34, wherein the transplanting step 34.b is conducted using anterior retroperitoneal surgery.

37. The method of any one of embodiments 34-36, wherein the transplanting step 34.b is conducted without disrupting the peritoneal cavity.

38. The method of any one of embodiments 34-37, wherein the transplanting step 34.b further comprises inserting the thymic tissue under the kidney capsule.

39. The method of any one of embodiments 34-38, wherein at least $2cm^3$ of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal.

40. The method of embodiment 39, wherein at least 2 cm^3, 3 cm^3, 4 cm^3, 5 cm^3, or 6 cm^3 of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal.

41. The method of any one of embodiments 34-40, wherein the transplanted thymic tissue covers at least 20% of the anterior surface of the kidney of the recipient animal.

42. The method of embodiment 41, wherein the transplanted thymic tissue covers at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 75% of the anterior surface of the kidney of the recipient animal.

43. The method of any one of embodiments 34-42, the method further comprising:

c. covering the anterior surface of the kidney with the peritoneum after the transplanting step 34.b.

44. The method of any one of embodiments 34-43, wherein the thymokidney is harvested from the recipient animal at least 6 weeks after the transplanting step 34.b.

45. The method of embodiment 44, wherein the thymokidney is harvested from the recipient animal at least 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks after the transplanting step 34.b.

46. The method of any one of embodiments 34-45, wherein the thymokidney is substantially free of perinephric and/or intra-abdominal adhesions for at least 4 weeks after the transplanting step 34.b.

47. The thymokidney of embodiment 46, wherein the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% perinephric and/or intra-abdominal adhesions.

9. EQUIVALENTS

[00159] Although the invention is described in detail with reference to specific embodiments thereof, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00160] All patents and publications mentioned in this specification are incorporated herein by reference in their entireties. From the foregoing description, it will be apparent that variations and modifications can be made to the invention described herein to adopt it to various uses and conditions. Such embodiments are also within the scope of the following claims.

WHAT IS CLAIMED:

1. An isolated porcine thymokidney comprising:
   a. a porcine kidney; and
   b. porcine thymic tissue,

wherein the thymic tissue covers at least 20% of the anterior surface of the kidney.

2. The thymokidney of claim 1, wherein the thymic tissue covers at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the anterior surface of the kidney.

3. The thymokidney of claim 1 or 2, wherein the thymic tissue is partially or fully covered by porcine peritoneal membrane.

4. The thymokidney of claim 3, wherein the thymic tissue is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% covered by porcine peritoneal membrane.

5. The thymokidney of any one of claims 1-4, wherein kidney and/or thymic tissue and/or peritoneal membrane are from the same herd.

6. The thymokidney of any one of claims 1-5, wherein kidney and/or thymic tissue and/or peritoneal membrane are from a minipig.

7. The thymokidney of claim 6, wherein kidney and/or thymic tissue and/or peritoneal membrane are from the same individual minipig.

8. The thymokidney of claim 6 or 7, wherein the minipig is a genetically modified minipig.

9. The thymokidney of claim 8, wherein the genetically modified minipig has at least one genetic modification.

10. The thymokidney of claim 9, wherein the least one genetic modification comprises:

a. knockout of the *GGTA1* gene;

b. knockout of the *CMAH* gene;

c. knockout of the *β4GalNT* gene.

11. The thymokidney of claim 10, wherein the least one genetic modification comprises no expression of a protein encoded by the *GGTA1* gene, the *CMAH* gene, and/or the *β4GalNT* gene.

12. The thymokidney of claim 9, wherein the least one genetic modification comprises expression of a protein for a porcine CTLA4 fused to a hinge and CH2/CH3 regions of human IgG1 (pCTLA4-Ig).

13. The thymokidney of claim 9, wherein the least one genetic modification results in the inactivation of porcine endogenous retrovirus (PERV).

14. The thymokidney any one of claims 8-13, wherein the genetically modified minipig comprises a *GGTA1* genetic modification.

15. The thymokidney any one of claims 8-14, wherein the genetically modified minipig does not express alpha-1,3-galactosyltransferase (GalT-KO).

16. The thymokidney of any one of claims 1-15, wherein the thymic tissue is fully neovascularized.

17. The thymokidney of any one of claims 1-16, wherein the thymic tissue has a higher cell density than that of a porcine thymic tissue that is not included in the isolated porcine thymokidney.

18. The thymokidney of claim 17, wherein the thymic tissue has a cell density ranging from about 12000 to 15000 cells/ 1×10^{-6} cm^3 in thymic cortex tissue.

19. The thymokidney of claim 17 or claim 18, wherein the thymic tissue has a cell density ranging from about 3000 to 6000 cells/ 1×10^{-6} cm^3 in thymic medulla tissue.

20. The thymokidney of any one of claims 1-18, wherein the thymic tissue is capable of supporting thymopoiesis.

21. The thymokidney of claim 20, wherein the thymic tissue comprises $CD3^-$ $CD4^+CD8^+$ double-positive (DP) thymocytes.

22. The thymokidney of claim 21, wherein the thymic tissue comprises at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% $CD3^-$ $CD4^+CD8^+$ double-positive (DP) thymocytes.

23. The thymokidney of any one of claims 1-22, wherein the thymokidney is substantially free of detectable scar tissue.

24. The thymokidney of claim 23, wherein the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% detectable scar tissue.

25. The thymokidney of any one of claims 1-24, wherein the thymokidney has at least one genetic modification.

26. The thymokidney of any one of claims 1-25, wherein the thymokidney does not express alpha-1,3-galactosyltransferase (GalT-KO).

27. The thymokidney of any one of the preceding claims, wherein the thymokidney was constructed using anterior retroperitoneal surgery.

28. A method of treating a disease of the kidney in a human subject in need thereof, wherein the method comprises: transplanting the thymokidney of any one of the preceding claims into the subject.

29. The method of claim 28, wherein the thymokidney is transplanted into the contralateral side in the subject compared to the donor animal.

30. The method of claim 28 or 29, wherein the thymokidney is transplanted with the anterior surface of the kidney facing posteriorly.

31. The method of any one of claims 28-30, wherein the thymokidney is transplanted such that the anterior surface of the kidney has minimal to no contact with the subject's great omentum.

32. The method of claim 31, wherein less than 25%, 20%, 15%, 10%, 5%, or 1% of the anterior surface of the kidney is in contact with the subject's great omentum.

33. A method of creating the thymokidney of any one of claims 1 to 27, wherein the method comprises:

a. obtaining thymic tissue from a porcine donor animal;

b. transplanting the thymic tissue obtained in step a to the anterior surface of a kidney of a recipient animal.

34. The method of claim 33, wherein the thymic tissue is obtained using cervical thymectomy.

35. The method of claim 33, wherein the transplanting step 33.b is conducted using anterior retroperitoneal surgery.

36. The method of any one of claims 33-35, wherein the transplanting step 33.b is conducted without disrupting the peritoneal cavity.

37. The method of any one of claims 33-36, wherein the transplanting step 33.b further comprises inserting the thymic tissue under the kidney capsule.

38. The method of any one of claims 33-37, wherein at least 2cm^3 of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal.

39. The method of claim 38, wherein at least 2 cm^3, 3 cm^3, 4 cm^3, 5 cm^3, or 6 cm^3 of thymic tissue is transplanted on the anterior surface of a kidney of the recipient animal.

40. The method of any one of claims 33-39, wherein the transplanted thymic tissue covers at least 20% of the anterior surface of the kidney of the recipient animal.

41. The method of claim 40, wherein the transplanted thymic tissue covers at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 75% of the anterior surface of the kidney of the recipient animal.

42. The method of any one of claims 33-41, the method further comprising:

c. covering the anterior surface of the kidney with the peritoneum after the transplanting step 33.b.

43. The method of any one of claims 33-42, wherein the thymokidney is harvested from the recipient animal at least 6 weeks after the transplanting step 33.b.

44. The method of claim 43, wherein the thymokidney is harvested from the recipient animal at least 6 weeks, 7 weeks, 8 weeks, 9 weeks, or 10 weeks after the transplanting step 33.b.

45. The method of any one of claims 33-44, wherein the thymokidney is substantially free of perinephric and/or intra-abdominal adhesions for at least 6 weeks after the transplanting step 33.b.

46. The thymokidney of claim 45, wherein the thymokidney has less than about 1%, 5%, 10%, 15%, or 20% perinephric and/or intra-abdominal adhesions.