Supplementary Methods

1. **Complement-dependent cytotoxicity (CDC) assay**

   CDC was measured by detecting lysed cells with fluorescent viability stain 7-actinoaminomycin D (7-AAD, Sigma) using flow cytometry (FACS Canto II, BD Biosciences).

2. **Anti-non-Gal IgM and IgG bindings (AbFCM)**

   Serum antibody binding to cells was detected by flow cytometry (FACS Canto II, BD Biosciences) using FITC-conjugated anti-human IgM (clone SA-DA4, eBioscience #11-9998) or IgG antibody (clone EM-07, Thermo Fisher Scientific #MA1-10379). Data were analyzed using FCS Express (De Novo Software).

3. **Surgical procedure**

   Thymectomy and central venous line placement for recipient was performed one week prior to transplantation as described[1,2]. Central venous lines were used for drug administration and blood sampling. Simultaneous vascularized thymic lobe (VTL) and kidney pig-to-baboon XTx were performed in all cases. Bilateral native nephrectomies, splenectomy and bilateral oophorectomy (female baboons) were performed at the time of Tx. All surgical procedures were performed by the senior author of this article, who has more than 500 cases of experiences in pig-to-NHP kidney transplantation. In all cases in Group1 and Group2, cold ischemia time of the grafts was less than five minutes. In Group3, transportation of the kidney grafts from centralized pig facility was required, and thus cold ischemia time was up to 5 hours. Vascular anastomosis time was less than 25 minutes in all cases.

4. **Immunosuppression**

   All baboons received anti-CD40L or anti-CD40 mab-based regimen[3]. Rabbit anti-thymocyte globulin (Thymoglobulin) and anti-CD20 monoclonal antibodies (Rituximab) were administered to deplete T cells and B cells prior to transplantation. Mycophenolate mofetil (MMF) was started at Day -6. Maintenance therapy included anti-CD40 or anti-CD40L monoclonal antibodies twice a week from POD2, and cytotoxic T lymphocyte-associated protein 4 immunoglobulin (CTLA4-Ig) once a week from POD2. Baboons that received kidneys with hCD47-Tg did not receive CTLA4-Ig[3].

5. **Posttransplant outcomes**

   Kidney graft function was assessed by urine volume from the graft (every six hours), serum creatinine (Cre) (mg/dL) on postoperative day (POD) 1 and 3. We defined graft dysfunction based on macroscopic and/or microscopic findings of the kidney grafts, urine volume (< 0.5ml/kg/hr), serum Cre level (> 4.0mg/dL), or severe proteinuria (>3+).

References

1. Yamada K, Ariyoshi Y, Pomposelli T, Sekijima M. Co-transplantation of vascularized thymic graft with kidney in pig-to-nonhuman primates for the induction of tolerance across xenogeneic barriers. *Methods in Molecular Biology*. 2020;2110:151-171. doi:10.1007/978-1-0716-0255-3_11/FIGURES/2

2. Yamada K, Scalea J. Thymic transplantation in pig-to-nonhuman primates for the induction of tolerance across xenogeneic barriers. *Methods in Molecular Biology*. 2012;885:191-212. doi:10.1007/978-1-61779-845-0_12/FIGURES/4

3. Takeuchi K, Ariyoshi Y, Shimizu A, et al. Expression of human CD47 in pig glomeruli prevents proteinuria and prolongs graft survival following pig-to-baboon xenotransplantation. *Xenotransplantation*. 2021;doi:10.1111/xen.12708