Supporting Online Material C. J. Phelps 1078942,

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Materials and Methods

1. Flow cytometry analysis of pig cells with FITC-GS-IB4 lectin staining. Harvested cells were washed 3 times with phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde (Sigma, St. Louis, MO) in PBS for 30 min on ice and washed 2 times with FACS buffer (1% BSA + 0.1% sodium azide in PBS). About 5×10^5 cells in 100 µl of 1% bovine serum albumin (BSA, Sigma) in PBS were stained with 100 µg/ml of FITC-GS-IB4 lectin (EY Laboratories Inc., San Mateo, CA) at 4°C for 1 hour, washed 3 times with fluorescence-activated cell sorter (FACS) buffer, resuspended into 250 µl of 1% paraformaldehyde and analyzed by FACS.

2. Complement lysis assay. Cells in 24-well plates with 80% confluency were incubated with 250 µl of 2 µg/ml calcein AM (Molecular Probes, Eugene, OR) for 1 hour at 37°C and washed with PBS. Normal human serum (NHS, Sigma) and heat-inactivated human serum (HIA-HS) were diluted into gelatin veronal buffer (GVB, Sigma) at 20% concentration and added to the cells in triplicate wells, respectively, at 250 µl/well. The reagent control, 0.5% BSA in GVB, was added in duplicate wells. After 1 hour incubation at 37°C, all contents of wells were transferred into a 96-well plate (sample 1) and 250 µl of 0.1% Triton X-100 (Sigma) in water was added into each well to lyse remaining cells. The lysed cells were transferred into a new 96-well plate (sample 2) and the supernatants in both samples were read in a fluorimeter with excitation wavelength set at 485 and the emission filter set at 538. The percentage of calcein release due to complement lysis were calculated as % calcein release = (sample 1)/ (sample 1+ sample 2).

3. Northern blot analysis. Total RNA (20 µg) was used for Northern blot analysis. A 400-bp porcine α1,3GT cDNA fragment (exon 2 to exon 8) labeled with digoxigenin (Roche Molecular Biochemicals, Indianapolis, IN) was used as the probe.

4. Section and FITC-GS-IB4 staining of piglet tissues. OCT embedded frozen tissues were cut at 5 microns and fixed briefly in 100% methanol for 10 min. The slides were then washed with PBS (2 timesfor 5 min each) and blocked with 1% BSA in PBS for one hour at room temperature. The slides were then incubated overnight in a humid chamber at 4°C with FITC-GS-IB4 at a concentration of 40 µg/ml in BSA/PBS blocking buffer. The slides were then washed with PBS 3 times, 10 min/wash, mounted with antifade mounting medium (Vectashield H-1000, Vector Laboratories, Burlingame, CA), and photographed.

5. Isolation of islet-like cell clusters (ICC) from neonatal piglets. Neonatal pancreata from both α1,3GT DKO and wild-type piglets were processed after ~18 hours of cold ischemia time by enzymatic digestion followed by a slightly modified stationary incubation method (S1). The ICCs were cultured in CMRL medium supplemented with 10% fetal calf serum, 2 mM nicotinamide, 2 mM L-glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin, in a 5% CO_2 incubator. Viability was determined by

fluorescent staining with propidium iodide and calcein AM[BPO1]. Both DKO and wild-type (WT) pig ICCs were more than 70% viable after isolation and culture.

6. IgM antibody titration. Galactose α1,3-Galactose-BSA (Calbiochem, La Jolla, CA) was biotinylated using the FluoReporter Mini-Biotin-XX Protein Labeling Kit (Molecular Probes). The BSA-α1,3Gal antigen (250 µg) was dissolved in 200 µl of PBS, spin-column purified, and processed as directed in the kit. Lumavidin LabMap 105 beads (Luminex, Austin, Texas) in 200 µl were washed with 100 µl PBS-1% BSA and incubated at room temperature (RT) for 2 hours with 100 µl of the biotinylated antigen. The beads were then washed twice with 500 µl of PBS-1% BSA, and resuspended in PBS-1% BSA at 635 beads per µl. Control beads (LabMap 104) were prepared by substituting PBS-1% BSA for the antigen. The α1,3Gal beads and control beads were blocked for 1 hour with human IgM (20 µg/ml, Sigma) in PBS-1% BSA, pelleted, resuspended in PBS-1%B SA, and combined at a final concentration of 60 beads/µl. Assays were performed in 50 µl total volume containing 25 µl of the beads, 24.5 µl of PBS-1% BSA, and 0.5 µl serum sample. The mixtures were incubated at RT with gentle agitation for 2 hours. After centrifugation, the liquid was carefully removed and replaced with 75 µl of PE-conjugated anti-mouse IgM (BD PharMingen, San Diego, CA) (diluted 1:30 in PBS). The samples were finally analyzed in the Luminex 100 system. Results were expressed as the difference of the fluorescence of the α1,3Gal beads and the control beads averaged from duplicate tubes. The peak titer of anti-α1,3Gal IgM from the recipients of WT piglet ICCs were inhibited 80% by the addition of soluble α1,3Gal epitopes.

References

S1. G. S. Korbutt *et al*., *J. Clin. Invest.* **97**, 2119 (1996).

S2. A.D. Thall, P. Maly, J.B. Lowe, *J. Biol. Chem.* **270**, 21437 (1995).

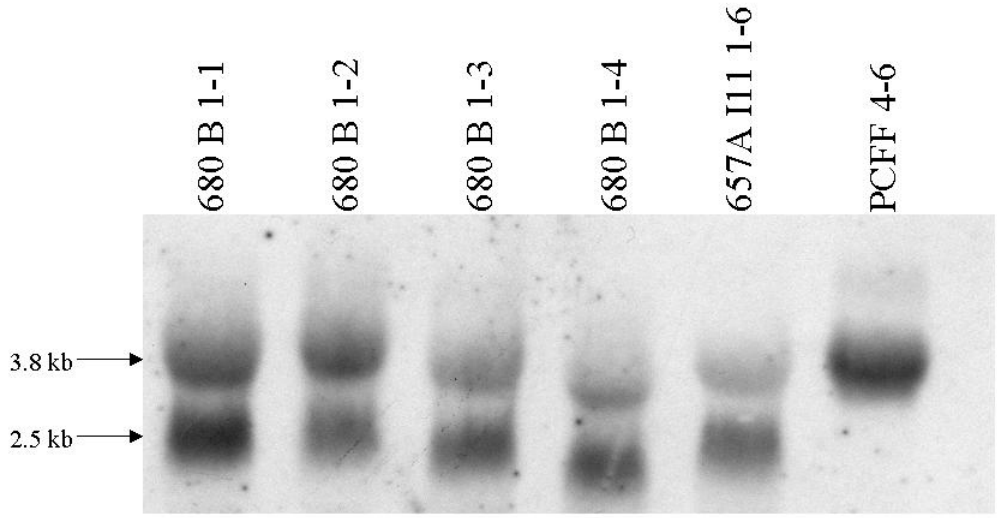

Fig. S1. Northern blot analysis of 680B1-1 to B1-4 cells. 657A-I11 1-6 cells are fetal fibroblasts with heterozygous α1,3GT knockout, and the parent cells of 680B1. PCFF4-6 cells are WT porcine fetal fibroblasts, which are the parent cells of 657A-I11. A 400-bp porcine α1,3GT cDNA fragment (exon 2 to exon 8) was used as the probe. The upper band represents the WT α1,3GT transcripts and the lower band represents the shorter transcripts from the α1,3GT allele disrupted with the insertion of an IRES-neo-polyA cassette at the 5' end of the exon 9.

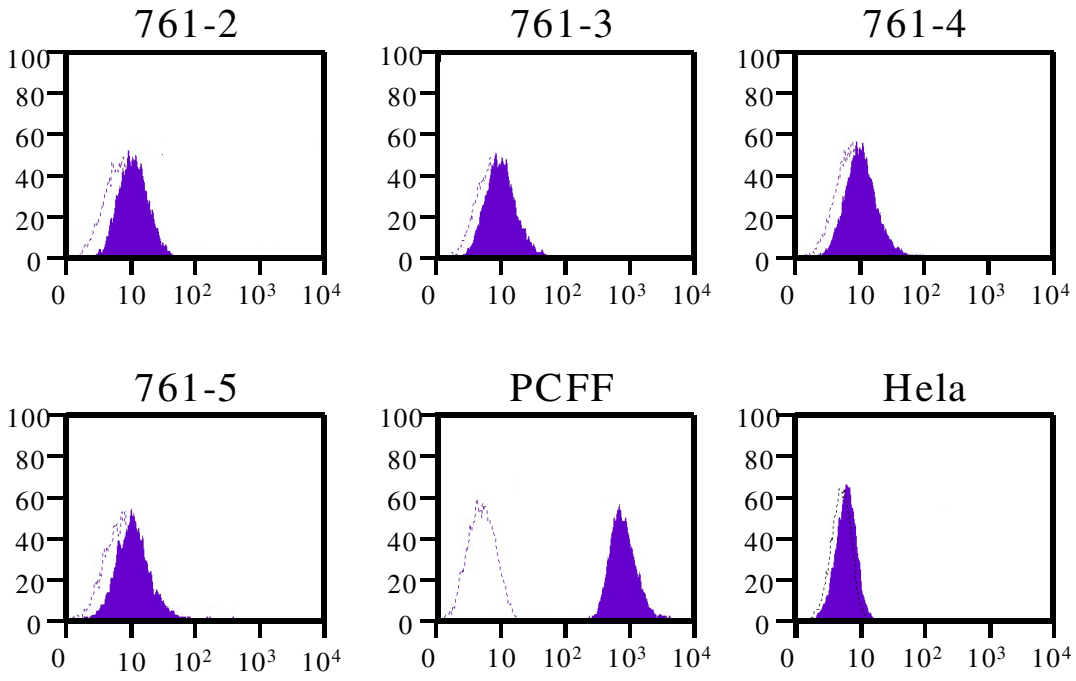

Fig. S2. Flow cytometry analysis of neonatal tail fibroblasts from four healthy α1,3GT DKO piglets with FITC-GS-IB4 lectin staining. Neonatal fibroblasts were isolated from tails of four healthy α1,3GT DKO piglets (761-2 to 762-5) and stained with 100 μg/ml of FITC-GS-IB4 lectin. HeLa cells and PCFF4-6 cells were used as negative and positive controls, respectively. The dotted line represents unstained cells, and the shadow area represents cells stained with FITC-GS-IB4 lectin. The slight differences in the FACS results between α1,3GT DKO piglet cells and HeLa cells may be due to different backgrounds between pig and human cells.

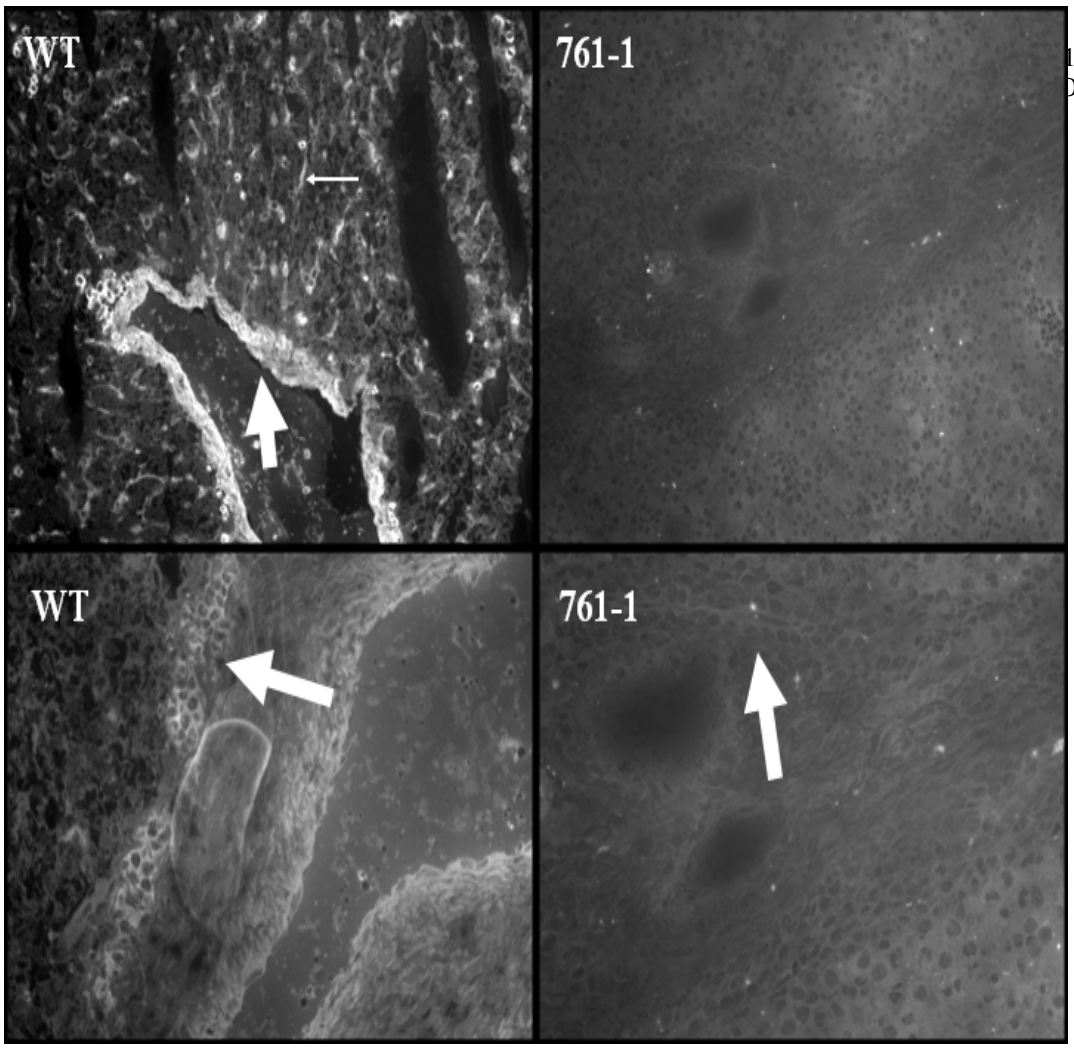

Fig. S3. Images of FITC-GS-IB4 lectin staining of the liver section from the newborn WT piglet and the α1,3GT DKO piglet 761-1. The image shows completely negative staining in the α1,3GT DKO piglet 761-1 (right panels), but diffuse sinusoidal (small arrow upper left), venous endothelial (large arrow upper left), and biliary epithelial cell (large arrow bottom left) in the WT piglet. The higher magnification of piglet 761-1 liver section (lower right) shows, in greater detail, the absence of biliary epithelial cell or venous endothelial cell staining in piglet 761-1.

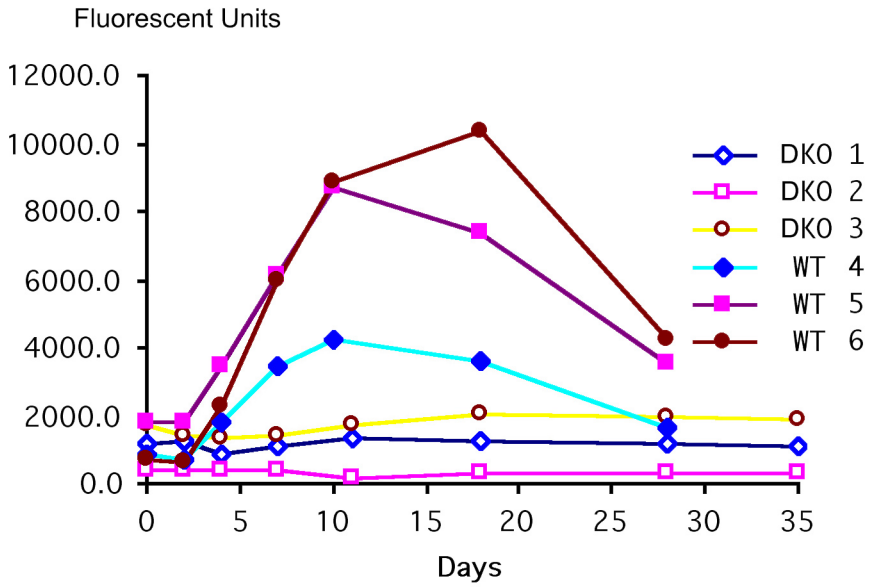

Fig. S4. Anti-α1,3Gal IgM levels before and after injections of piglet ICCs in α1,3GT KO mice (*S2*). Each mouse received three serial ICC injections via i.p. (200-500 ICC per injection) over 4 days. All three recipients of wild-type (WT) piglet ICCs showed a significant elevation of anti-α1,3Gal IgM titer and subsequent return to baseline 4 weeks after ICC implants. Sera from all three mice injected with α1,3GT DKO piglet ICCs maintained low baseline values of anti-α1,3Gal IgM titer during the observation time of 35 days.

[BPO1]calcein AM- Does AM need to be explained? Probably not to people who read Methods, but you will know.