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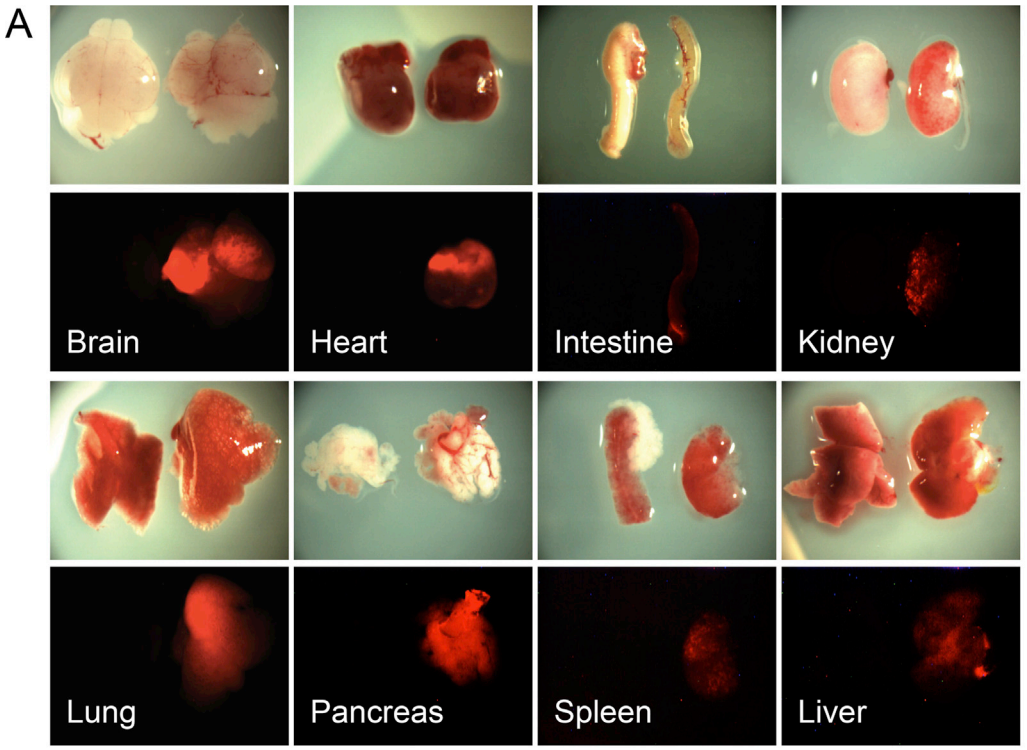

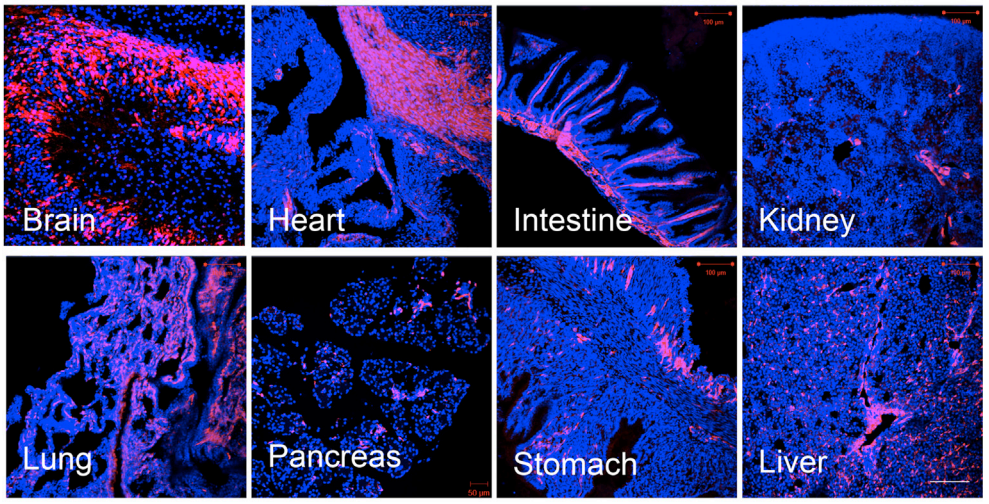

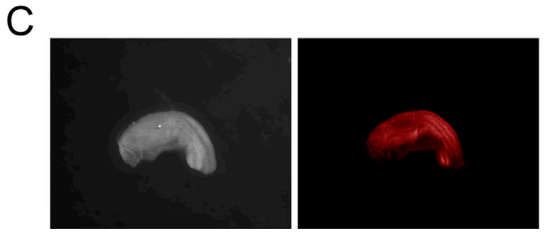

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Figure S1. Chimeric Contribution of Rat PSCs to Different Organs of Rat-Mouse Chimeras, Related to Figure 1

(A) Bright field (top) and fluorescence (bottom) images showing hKO-labeled rat ESCs (DAC8) contributed to different organs of a neonatal rat-mouse chimera. Control organs from a wild-type neonatal mouse were showing on the left.

(B) Representative fluorescence images showing hKO-labeled rat iPSCs (SDFF) contributed to different tissues in a 3-week-old rat-mouse chimera. Red, hKO-labeled rat cells; blue, DAPI. Scale bar is 100 μm.

(C) Bright field (left) and fluorescence (right) images showing an isolated neonatal mouse gallbladder contributed by hKO-labeled rat iPSCs (SDFE), as shown in Figure 1F.

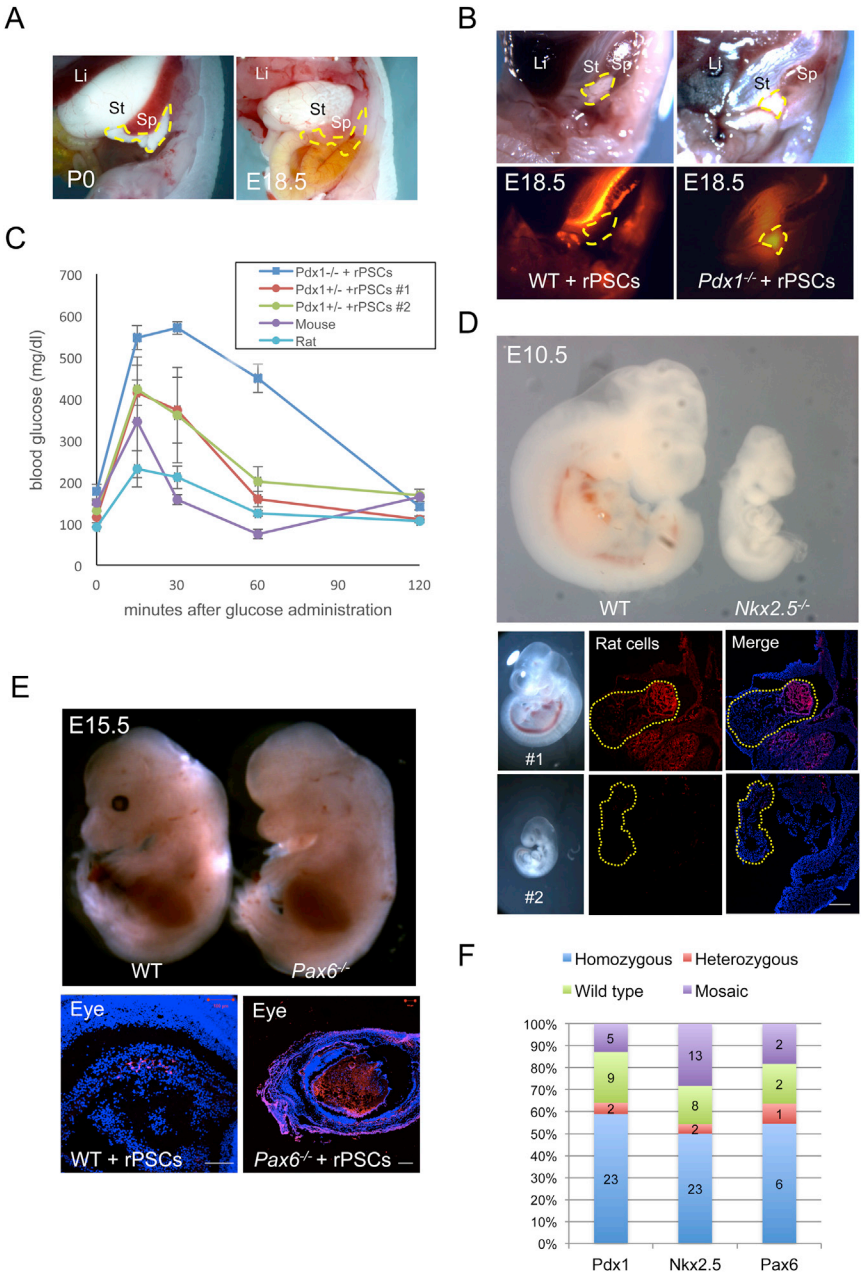

Figure S2. CRISPR-Cas9-Mediated Rat-Mouse Blastocyst Complementation, Related to Figure 2

(A) Bright field images showing a wild type (P0, left) mouse and a *Pdx1*$^{-/-}$ (E18.5, right) mouse fetus generated by zygotic co-injection of Cas9 mRNA and *Pdx1* sgRNA. Li, liver; St, stomach; Sp, spleen. Yellow-dotted line encircles the pancreas in the wild type, and indicates the lack of a pancreas in the *Pdx1*$^{-/-}$ fetus.

(B) Bright field (top) and fluorescence (bottom) images showing more chimeric contribution of rat cells in a *Pdx1*$^{-/-}$ mouse pancreas than a wild type mouse pancreas. Li, liver; St, stomach; Sp, spleen. Yellow-dotted line encircles the pancreases. Red, hKO-labeled rat cells.

(C) Glucose tolerance test results of adult (>7 months) *Pdx1*$^{-/-}$ and *Pdx1*$^{+/-}$ mice complemented with rat PSCs. Age-matched wild type mice and rats were included as controls. Error bars indicate s.d.

(D) Top, a representative bright field image showing a wild type (left) and *Nkx2.5*$^{-/-}$ (right) E10.5 mouse embryos Middle, rat ESC-derivatives rescued retarded growth of E10.5 *Nkx2.5*$^{-/-}$ mouse embryo and were enriched in the heart. H, heart. Bottom, an E10.5 *Nkx2.5*$^{-/-}$ embryo showing retarded growth with little to no rat cells contribution to the heart. Yellow-dotted line encircles the heart. Scale bar is 100μm.

(E) Top, a representative bright field image showing a wild type (left) and a *Pax6*$^{-/-}$ (right) E15.5 mouse fetuses. Bottom, fluorescence images showing more chimeric contribution of rat cells in the eye of *Pax6*$^{-/-}$ (right) than wild type (left) chimeras. WT+rPSCs, control rat-mouse chimera without Cas9/sgRNA injection. Red, hKO-labeled rat cells. Blue, DAPI. Scale bar is 100μm.

(F) Frequencies of wild type, homozygous, heterozygous mutants and mosaic mutant mice generated by zygotic co-injection of Cas9 mRNA and sgRNAs for *Pdx1, Nkx2.5*, or *Pax6*.

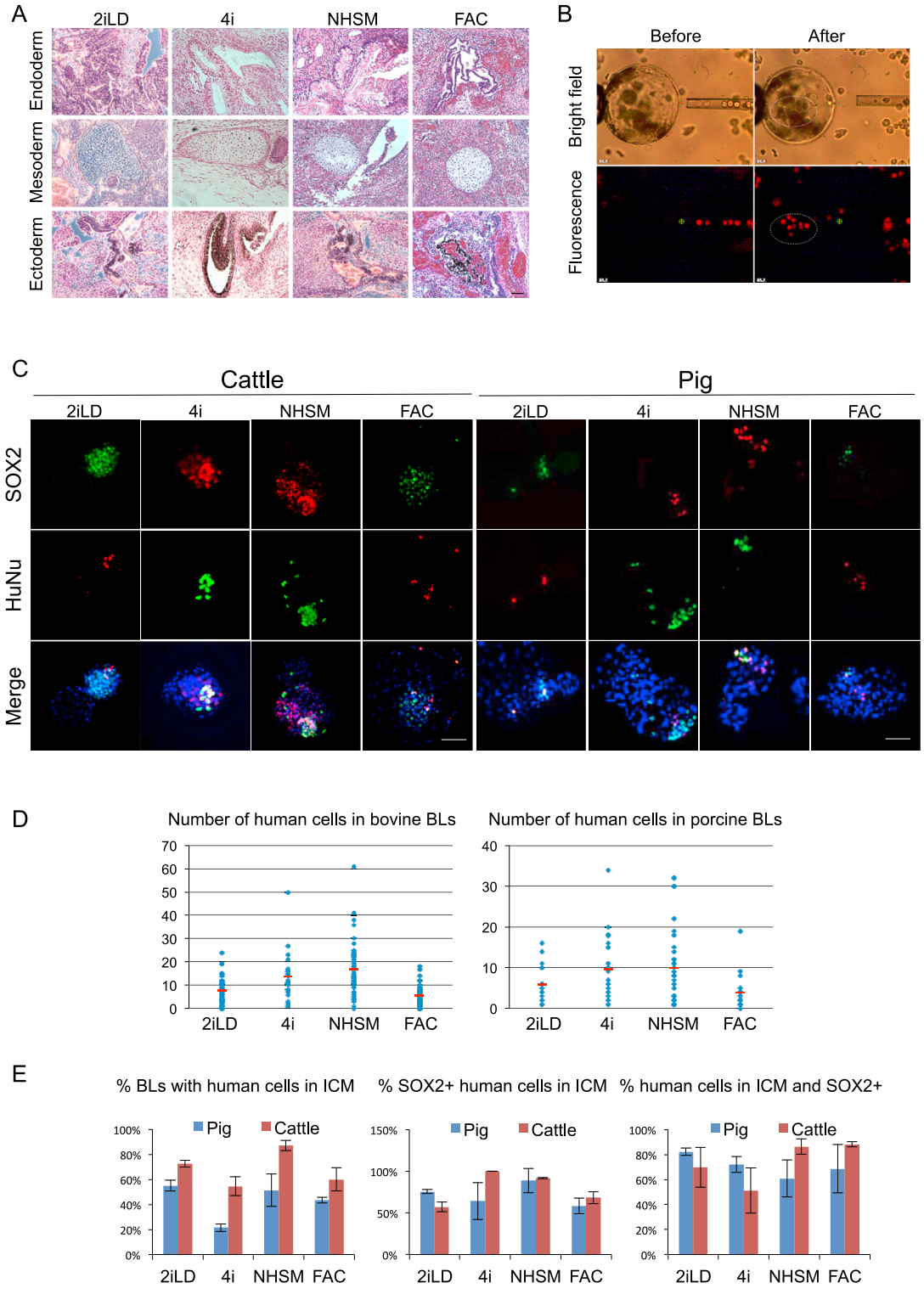

Figure S3. Teratoma Assay and Interspecies ICM Incorporation of Different Types of hiPSCs to Cattle and Pig Blastocysts, Related to Figure 4

(A) Representative images showing hematoxylin and eosin staining of histological sections derived from teratomas generated by 2iLD-hiPSCs, 4i-hiPSCs, NHSM-hiPSCs and FAC-hiPSCs. hiPSC-derived teratomas contained tissues from all three germlineages: endoderm (top), mesoderm (middle) and ectoderm (bottom). Scale bar is 100 μm.

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(B) Laser-assisted microinjection of hiPSCs into a bovine blastocyst. Left, before injection laser beam was used to perforate the zona pellucida. Right, a blunt end pipette was used to transfer hiPSCs to the blastocoel. Red color indicates hKO-labeled hiPSCs (bottom).
(C) Representative immunofluorescence images showing ICM incorporation of naive and intermediate hiPSCs. Top, SOX2; Middle, HuNu, Bottom, merged images. Blue, DAPI. Scale bar is 100 μm.
(D) Number of hiPSCs remained in the cattle (left) and pig (right) blastocysts after injection of 10 hiPSCs followed by 2 days in vitro culture. Red line, average number of cells. Blue dot, number of ICM-incorporated hiPSCs in each blastocyst. BL, blastocyst.
(E) Comparison of several parameters of ICM incorporation for each type of hiPSCs between pig and cattle. Error bars indicate s.d.

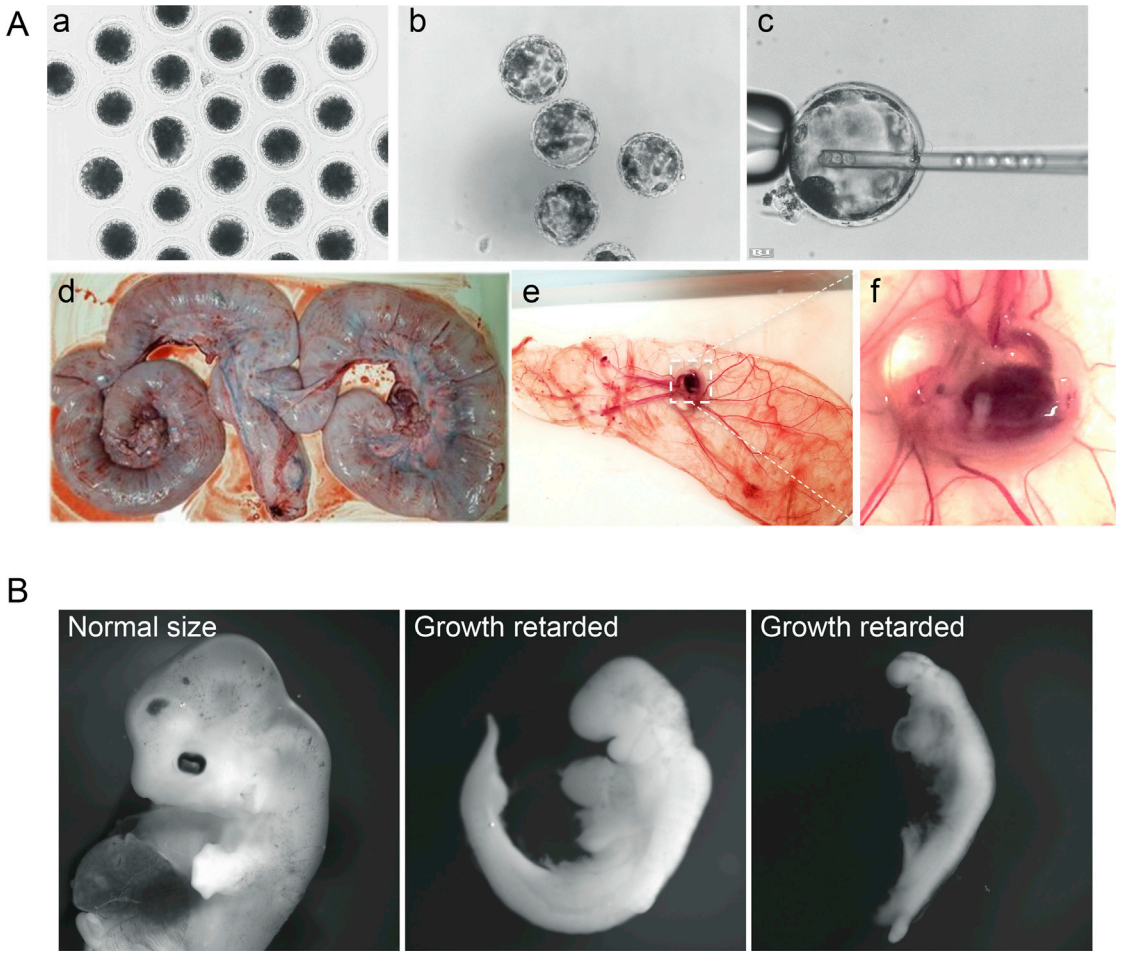

Figure S4. Generation of Post-implantation Pig Embryos Derived from Blastocyst Injection of hiPSCs, Related to Figure 5

(A) Representative bright field images showing: a) freshly collected pig zygotes; b) in vitro derived pig blastocysts; c) hiPSCs are being injected into a pig blastocyst; d) Pig female reproductive tract at day-28 of pregnancy; e) a day 28 pig conceptus displaying the embryo proper and the amniotic and allantoic membranes; f) a magnified image of the day-28 pig embryo showing in e.

(B) Representative bright field images showing a normal size day-28 pig embryo (left) and growth retarded day-28 embryos (middle and right).

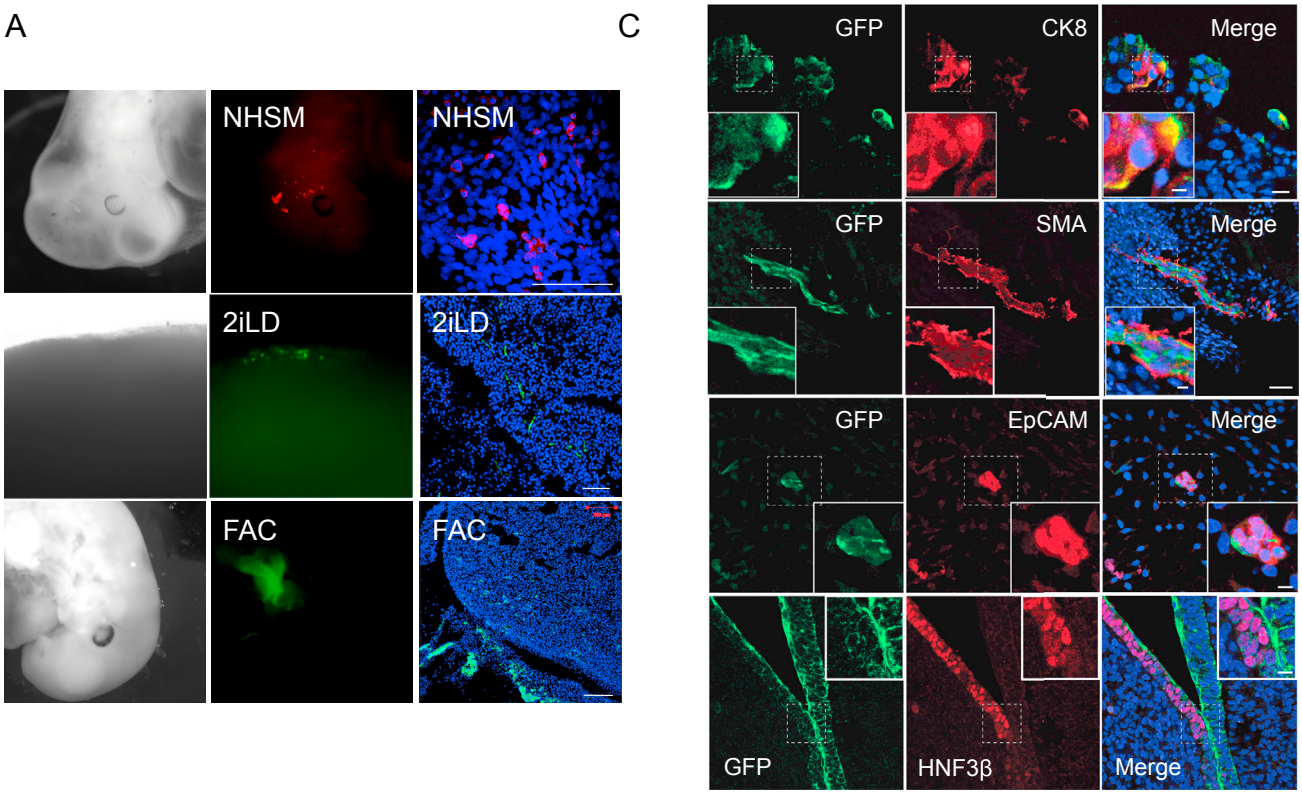

Figure S5. Chimeric Contribution of hiPSCs to Post-implantation Pig Embryos, Related to Figure 6

(A) Representative bright field (left), fluorescence (middle) and immunofluorescence (right) images showing the contribution of hKO-labeled NHSM-hiSPCs, GFP-labeled 2iLD-hiPSCs and FAC-hiPSCs to normal size day 28 pig embryos. Scale bar is 200 μm.

(B) Representative immunofluorescence images showing the contribution of FAC-hiPSCs to six additional normal size day 21-28 pig embryos (FAC #2-#7). Scale bar is 200 μm.

(C) Representative immunofluorescence images showing the chimeric contribution and differentiation of FAC-hiPSCs within a normal size, day 28 pig embryo (FAC #3). Embryo sections were stained with antibodies against GFP (green, left), EpiCAM, HNF3β, CK8 and SMA (red, middle). Right, merged images. Insets are higher magnification images of boxed regions. Scale bar is 100 μm.