Supporting Information

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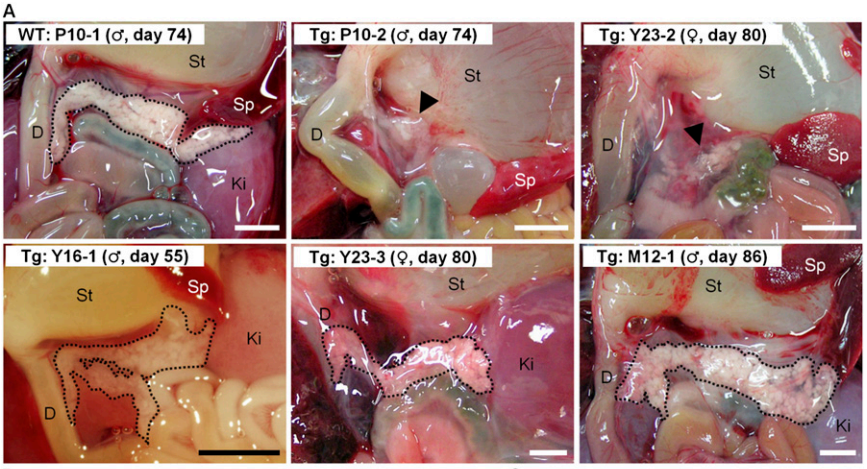
SI Materials and Methods

Fluorescence in Situ Hybridization. Fetal fibroblast cells derived from a pancreatogenesis-disabled transgenic pig were cultured in MEMα containing 15% FBS (vol/vol) for 3 d. The cells were then cultured with 30 μg/mL BrdU for 4 h, followed by incubation with 0.02 μg/mL colcemide for 2 h. After fixation with methanol-acetic acid (3:1 ratio), the cells were spread on slides and air-dried. The cells were then stained with Hoechst 33258 and treated with UV light for G-banding. *Pdx1* (pancreatic and duodenal homeobox 1)-*Hes1* (hairy enhancer split 1). DNA was labeled with digoxigenin-11-dUTP as a probe and hybridized at 37 °C overnight. After stringent washing, the bound label was detected with anti–Dig-Cy3 using Leica DRAM2 and CW4000 FISH software.



B

Transgenic fetuses carrying *Pdx1-Hes1*	Pancreatogenesis-disabled phenotype	Copy numbers of the integrated transgene	Chromosomal transgene integration site
P10-2	Distinct	15–20	7q21
Y23-2	Distinct	20–30	NA
Y16-1	Indistinguishable	1	NA
Y23-3	Indistinguishable	1	NA
M12-1	Indistinguishable	5	NA

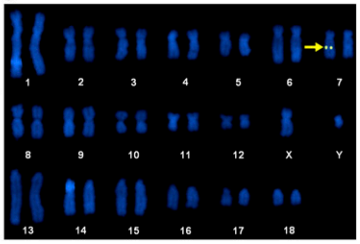


Fig. S1. Production details of pancreatogenesis-disabled *Pdx1-Hes1* transgenic pig fetuses. (*A*) Among the five transgenic fetuses obtained, a distinct pancreatogenesis-disabled phenotype was observed in one male (P10-2) and one female (Y23-2). These fetuses each had a vestigial pancreas (arrowhead) apparently less than 5% of normal size compared with that of a WT sibling fetus (P10-1). The pancreata of three other transgenic (Tg) fetuses (Y16-1, Y23-3, and M12-1) appeared indistinguishable from normal pancreata (P10-1). D, duodenum; Ki, kidney; Sp, spleen; St, stomach. (*B*) Phenotypic characteristics of the *Pdx1-Hes1* transgenic pig fetuses and integration patterns of the transgene. NA, not analyzed. (*C*) Karyogram for the pancreatogenesis-disabled *Pdx1-Hes1* transgenic fetus (P10-2) with fluorescent in situ hybridization, indicating single-site transgene integration (arrow). (Scale bars: *A*, 5 mm.)

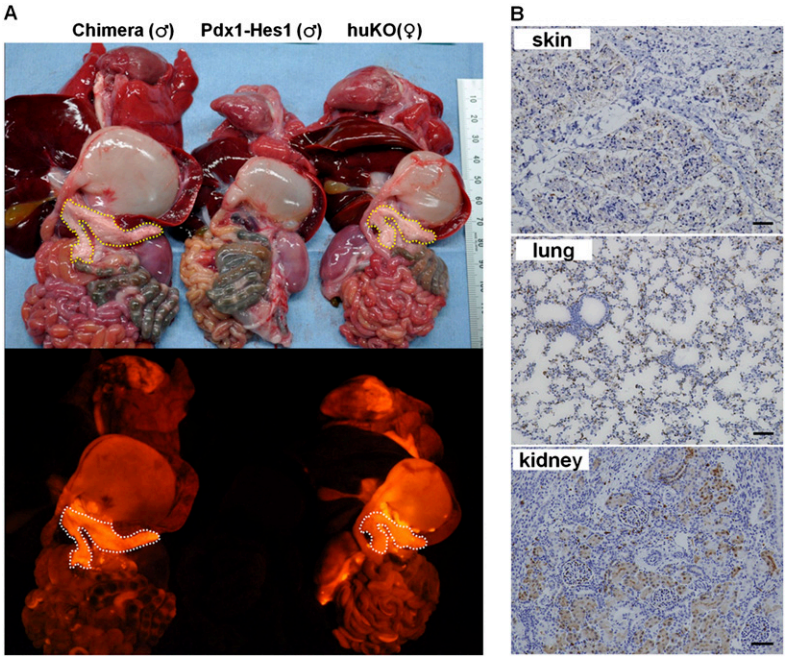


Fig. S2. Regeneration of the missing pancreata from exogenic cells in chimeric pig fetuses by complementation of *Pdx1-Hes1* clone embryos with the cloned embryos carrying the gene encoding orange fluorescent protein humanized Kusabira-Orange (*huKO*). (*A*) Orange fluorescence was observed systemically in chimeric fetuses, as seen in nonchimeric cloned fetuses derived from *huKO* cells. Orange fluorescence in the pancreata of the chimeric fetuses was prominent, indicating that these pancreata consisted primarily of donor cells expressing huKO. In contrast, a nonchimeric pancreatogenesis-disabled fetus derived from a *Pdx1-Hes1* embryo displayed no fluorescence. (*B*) Chimerism was confirmed histologically in all 10 of the organs/tissues observed, including skin, lung, and kidney, by immunostaining for huKO. (Scale bars: *B*, 50 μm.)

A

Chimeric boar	No. fetuses obtained	Fetuses displaying the pancreatogenesis-disabled phenotype* (%)	Fetuses carrying the *huKO* transgene†
W126	12	5 (41.7)	0
W127	11	7 (63.6)	0
W128	11	9 (81.8)	0
W129‡	11	6 (54.5)	-

*Confirmed by PCR to contain Pdx1-Hes1 transgene sequences.
†Confirmed by PCR.
‡Chimeric boar derived from a *Pdx1-Hes1* clone embryo and a colored-coat hybrid embryo.

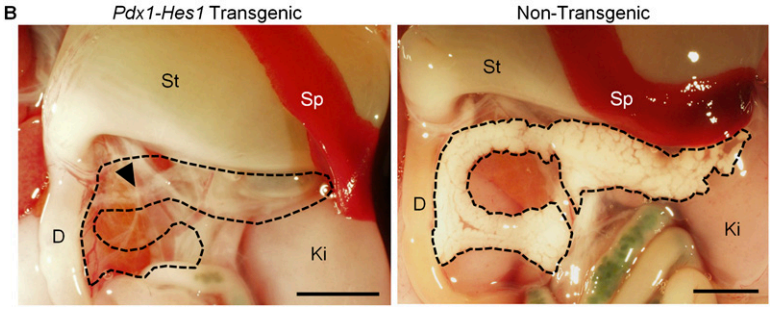


Fig. S3. Production details of chimeric pigs by blastocyst complementation and reproductive performance of the chimeric boars. (*A*) Transmission of the pancreatogenesis-disabled phenotype from chimeric boars to offspring. Each chimeric boar was mated with a WT sow, and fetuses between days 69 and 109 of gestation were examined. (*B*) Pancreatogenesis-disabled phenotype in offspring of a chimeric boar. Transgenic fetus (*Left*) and nontransgenic fetus (*Right*) littermates sired by a chimeric boar (W129) are shown at day 69 of gestation. The arrowhead (*Left*) and dotted area (*Right*) indicate a vestigial pancreas and a normally formed pancreas, respectively. D, duodenum; Ki, kidney; Sp, spleen; St, stomach. (Scale bars: *B*, 5 mm.)

Table S1. Production efficiency of pancreatogenesis-disabled pig fetuses by introduction of the *Pdx1-Hes1* transgene using intracytoplasmic sperm injection-mediated gene transfer

Recipient	No. of embryos transferred	Pregnancy	No. of fetuses, embryonic days	No. of transgenic fetuses	Pancreatogenesis-disabled phenotype
P9	106	–	—	—	—
P10	127	+	2 (day 74)	1	1*
P11	132	–	—	—	—
P12	95	+	Aborted	—	—
P20	136	–	—	—	—
P21	79	+	Aborted	—	—
P30	130	+	Aborted	—	—
P31	75	+	Aborted	—	—
Y16	62	+	1 (day 55)	1	0
Y17	124	–	—	—	—
Y23	70	+	3 (day 80)	2	1*
M11	53	+	Aborted	—	—
M12	93	+	5 (day 86)	1	0
Total	1,282	9	11 [0.9%, 11/1,282]	5 [0.4%, 5/1,282]	2 [0.2%, 2/1,282]

*A male fetus (P10-2) of recipient P10 and a female fetus (Y23-2) of recipient Y23 displayed the pancreatogenesis-disabled phenotype, whereas other fetuses had apparently normal pancreata.

Table S2. Reproduction efficiency of pancreatogenesis-disabled *Pdx1-Hes1* transgenic fetuses by somatic cell cloning

Recipient	No. of cloned embryos transferred	Pregnancy	No. of cloned fetuses obtained (%)	Age of the fetuses at laparotomy, d
P40	115	+	5 (4.3)	59
P41	114	+	4 (3.5)	110
Total	229	2	9 (3.9)	

Table S3. Production details of chimeric pig fetuses by complementation of *Pdx1-Hes1* clone embryos with *huKO* clone embryos

Type of SCNT embryos	*Pdx1-Hes1* transgenic [host embryos]* (%)	*huKO* transgenic [donor embryos]* (%)
In vitro production of chimeric blastocysts using cloned embryos		
SCNT embryos cultured	201	142
Normally cleaved embryos on day 2	154 (76.6%)	114 (80.3%)
Morula-stage embryos on day 4	146 (72.6%)†	110 (77.5%)†
Morulae used to produce chimeric embryos	97 (48.3%)	64 (45.1%)
Chimeric embryos produced	97‡	
Chimeric blastocysts developed	96 (99.0%)§	
Production efficiency of chimeric fetuses after embryo transfer		
Recipient	B53¶	B54¶
Blastocysts transferred	45	51
Fetuses obtained	5 (11.1%)‖	9 (17.6%)‖
Chimeric fetuses	1 (20.0%)**	4 (44.4%)**
Nonchimeric fetuses derived from *Pdx1-Hes1* host embryo	3 (60.0%)††	2 (22.2%)††
Nonchimeric fetuses derived from *huKO* donor embryo	1 (20.0%)‡‡	3 (33.3%)‡‡

huKO, the gene encoding orange fluorescent protein humanized Kusabira-Orange; SCNT, somatic cell nuclear transfer.

*Pdx1-Hes1 ⇔ *huKO* chimera.

†More than 70% of the cloned embryos in both the host and donor embryo groups developed to the morula stage. These morulae, selected for blastomeres of uniform size and shape, were used to produce chimeric embryos.

‡Of 97 host embryos injected with ~10 donor blastomeres, 96 (99.0%)§ developed into blastocysts after culture for 24 h. Transfer of those blastocysts on day 5 or 6 into two estrus-synchronized recipients¶ gave rise to 14 fetuses,‖ including 5 chimeric fetuses.** Nonchimeric fetuses entirely reproducing the characteristics of the host†† or donor‡‡ embryos were obtained in each litter.

Table S4. Comparison of features of chimeric and nonchimeric cloned fetuses (days 110 and 111)

Type of fetuses	Fetus	Body length, cm	Body weight, g	Sex	Weight of pancreas or vestigial pancreas, g	Pancreas weight per body weight, %
Chimera	B53-1	30	1,392	♂	1.416	0.102
	B54-1	25	802	♂	0.448	0.056
	B54-5	22.5	452	♂	0.344	0.076
	B54-8	19	288	♂	0.262	0.091
	B54-9	18	216	♂	0.183	0.085
Average		22.9 ± 2.2	630 ± 215.7		0.531 ± 0.4513	0.082 ± 0.016
huKO clone*	B53-2	27	842	♀	0.678	0.081
	B54-3	23	702	♀	0.521	0.074
	B54-4	24.5	688	♀	0.588	0.085
	B54-7	20	378	♀	0.383	0.101
Average		23.6 ± 1.5	652.5 ± 97.9		0.543 ± 0.1076	0.085 ± 0.010
Pdx1-Hes1 clone†	B53-3	23.5	680	♂	0.008	0.001
	B53-4	23	650	♂	0.041	0.006
	B53-5	21	458	♂	0‡	0
	B54-2	23	596	♂	0‡	0
	B54-6	22.5	490	♂	0.014	0.003
Average		22.6 ± 0.4	574.8 ± 43.6		0.013 ± 0.0076	0.002 ± 0.002

huKO, the gene encoding orange fluorescent protein humanized Kusabira-Orange.
*Nonchimeric cloned fetuses derived from donor embryos.
†Nonchimeric cloned fetuses derived from host embryos.
‡No vestigial pancreatic tissue was collectable.

Table S5. Production efficiency of chimeric embryos and offspring using somatic cell nuclear transfer embryos: *Pdx1-Hes1* ⇔ *huKO* chimera

Type of SCNT embryos	*Pdx1-Hes1* (host embryos)	*huKO* (donor embryos)
In vitro production of chimeric blastocysts using SCNT embryos		
SCNT embryos cultured	199	148
Normally cleaved embryos on day 2	174 (87.4%)	106 (71.6%)
Morula-stage embryos on day 4	145 (72.9%)	92 (62.2%)
Morulae used to produce chimeric embryos	113 (56.8%)	76 (71.6%)
Chimeric embryos produced	113	
Chimeric blastocysts developed	109 (96.5%)	
Production efficiency of chimeric pigs after embryo transfer		
Recipient	B77	B78
Blastocysts transferred	58 (day 6)	51 (day 5)
Piglets obtained	6 (10.3%)	7 (13.7%)
Chimeric piglets	3 (50.0%)	1 (14.3%)
Nonchimeric piglets derived from *Pdx1-Hes1* host embryo	3 (50.0%)	5 (71.4%)
Nonchimeric piglets derived from *huKO* donor embryo	0 (0%)	1 (14.3%)

huKO, the gene encoding orange fluorescent protein humanized Kusabira-Orange; SCNT, somatic cell nuclear transfer.

Table S6. Production efficiency of chimeric embryos and offspring using somatic cell nuclear transfer embryos: *Pdx1-Hes1* ⇔ WT chimera

Type of SCNT embryos	*Pdx1-Hes1* (host embryos)	WT* (donor embryos)
In vitro production of chimeric blastocysts using SCNT embryos		
SCNT embryos cultured	103	74
Normally cleaved embryos on day 2	81 (78.6%)	56 (75.6%)
Morula-stage embryos on day 4	66 (64.1%)	56 (75.6%)
Morulae used to produce chimeric embryos	92 (96.1%)	70 (94.6%)
Chimeric embryos produced	92	
Chimeric blastocysts developed	80 (87.0%)	
Production efficiency of chimeric pigs after embryo transfer		
Recipient	B83	
Blastocysts transferred	80 (day 6)	
Piglets obtained	6 (7.5%)	
Chimeric piglets	1 (16.7%)	
Nonchimeric piglets derived from *Pdx1-Hes1* host embryo	2 (33.3%)	
Nonchimeric piglets derived from WT donor embryo	3 (50.0%)	

SCNT, somatic cell nuclear transfer.
*Cloned embryos derived from a colored-coat WT sow (Duroc × Berkshire hybrid) were used as donor embryos.

Table S7. Serum biochemical profile in *Pdx1-Hes1* cloned piglets

	Pdx1-Hes1 cloned offspring		Control† (n = 5)
	No. 1	No. 2	
Parameters	3-d-old	4-d-old	4- to 5-d-old*
TP, g/dL	6.7	5.4	5.0 ± 0.39
BUN, mg/dL	130.6	47.9	9.16 ± 2.76
CRE, mg/dL	1.8	0.9	0.36 ± 0.05
Na, mEq/L	157	130	136.4 ± 1.50
K, mEq/L	7.8	4.8	4.62 ± 0.65
Cl, mEq/L	121	97	94.8 ± 2.86
Ca, mg/dL	6.82	6.22	6.04 ± 0.43
IP, mg/dL	>4.84‡	4.38	2.57 ± 0.79
AST, U/L	220	27	78.6 ± 61.93
ALT, U/L	95	108	44.2 ± 17.46
TCHO, mg/dL	143	121	139.2 ± 66.17
TG, mg/dL	109	33	106.6 ± 17.23
GLU, mg/dL	>600‡	>600‡	140.6 ± 36.64
Insulin, ng/mL	ND	ND	2.003 ± 0.99

ALT, alanine aminotransaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium; Cl, chloride; CRE, creatinine; GLU, glucose; IP, inorganic phosphate; K, potassium; Na, sodium; ND, nondetectable; TCHO, total cholesterol; TG, triglyceride; TP, total protein.
*Mean ± SD values are provided.
†WT piglets of the same strain and age were used as controls.
‡Above the highest detectable level.

Table S8. Serum biochemical profile in chimeric pigs

	Chimera		Control	
Parameters	6-mo-old (n = 3)	12-mo-old (n = 4)	6-mo-old (n = 5)	12-mo-old (n = 4)
TP, g/dL	7.6 ± 0.33	8.1 ± 0.19	7.58 ± 0.40	7.0 ± 0.72
ALB, g/dL	3.6 ± 0.26	3.8 ± 0.54	3.28 ± 0.35	3.7 ± 0.26
A/G	0.9 ± 0.26	1.0 ± 0.26	0.8 ± 0.20	1.2 ± 0.08
BUN, mg/dL	9.9 ± 0.96	11.7 ± 2.32	13.22 ± 2.80	10.4 ± 2.14
CRE, mg/dL	1.02 ± 0.08	1.63 ± 0.15	1.294 ± 0.13	1.74 ± 0.19
Na, mEq/L	144 ± 2.05	141 ± 2.28	141.8 ± 1.47	140.5 ± 1.50
K, mEq/L	4.4 ± 0.12	4.8 ± 0.11	5.22 ± 0.65	4.5 ± 0.33
Cl, mEq/L	98 ± 4.78	99 ± 2.86	99.2 ± 3.19	101.0 ± 3.74
Ca, mg/dL	10.0 ± 0.08	9.8 ± 0.19	10.2 ± 0.36	9.4 ± 0.55
IP, mg/dL	7.2 ± 0.17	7.0 ± 0.58	7.7 ± 1.03	5.5 ± 0.53
AST, IU/L	59 ± 32.22	35 ± 3.90	32.6 ± 7.42	27 ± 5.24
ALT, IU/L	44 ± 7.41	39 ± 15.35	29.4 ± 3.26	38 ± 4.50
ALP, IU/L	335 ± 24.57	206 ± 61.64	504 ± 210.34	256 ± 75.72
γ-GT, IU/L	34 ± 16.58	53 ± 30.41	24.8 ± 11.05	48 ± 9.41
LIP, IU/L	10 ± 1.25	6.5 ± 1.50	10.6 ± 1.74	6.8 ± 1.30
TCHO, mg/dL	68 ± 7.12	60 ± 9.01	85.4 ± 8.28	58 ± 10.62
TG, mg/dL	30 ± 5.66	39 ± 10.71	44 ± 13.84	29 ± 16.32
HDL-C, mg/dL	28 ± 0.47	25 ± 5.36	39.6 ± 5.61	25 ± 2.92
TBIL, mg/dL	0.07 ± 0.03	0.06 ± 0.04	0.04 ± 0.01	0.07 ± 0.03
GLU, mg/dL	105 ± 14.73	88 ± 25.16	110 ± 37.22	86 ± 9.94
GA, %	4.5 ± 1.14	5.2 ± 1.12	3.96 ± 0.84	6.0 ± 0.31
Insulin, ng/mL	1.851 ± 0.88	1.360 ± 0.803	3.220 ± 0.85	1.907 ± 2.44

Mean ± SD values are provided. A/G, albumin-globulin ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium; Cl, chloride; CRE, creatinine; GA, glycoalbumin; GLU, glucose; γ-GT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphate; K, potassium; LIP, lipase; Na, sodium; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglyceride; TP, total protein.