Supporting Information

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SI Methods

Mice. Additional genotyping of *Pdx1*-deficient mice was done with *Pdx1*-specific primers (forward, 5′-CGCCTGGTCTGACACTATCC-3′; reverse, 5′-GGCTTGTTCCTACCCTAGCC-3′) were used to distinguish wild type and heterozygotes.

Thymic Epithelial Cells. Briefly, thymi of 3- to 5-week-old mice were digested at 37°C for 30 min with collagenase, dispase (each at 0.2 mg/ml), and 25 μg/ml DNase in RPMI medium 1640 and 2% FCS. EDTA at a final concentration of 10 mM was added during the last 5 min. The single-cell suspension was separated on a discontinuous Percoll gradient, and the MEC-enriched fraction was used.

Single-Cell Sorting, Reverse Transcription, and PCR. Thymic MECs were stained with G8.8 [anti-epithelial cell adhesion molecule (EpCAM)] conjugated with fluorescein isothiocyanate (FITC), an APC-conjugated anti-CD45 antibody (eBioscience), a biotin-conjugated anti-Ly51 antibody (BD Pharmingen), and a PE-conjugated anti-A^b antibody (eBioscience). To detect the biotin-conjugated anti-Ly51, we used APC-Cy7-conjugated Streptavidin (eBioscience). Stained cells were analyzed and sorted for the phenotype $CD45^-G8.8^+Ly51^{int}MHC\text{-}II^{hi}$ on a MoFlo fluorescence-activated cell sorter (Cytomation). Cells were sorted directly into wells of 96-well PCR plates containing 10 μl of RT buffer (50 mM Tris·HCl, 75 mM KCl, 3 mM $MgCl_2$) with 2% Triton X-100, 500 μM dNTP, 10 units of RNase Inh (Promega), 30 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen), 50 ng of oligo(dT)12–18 (Invitrogen), and 500 μM gene-specific 3′ primers. Gene-specific primers were used for reverse transcription and are listed in Table S1. The plates were incubated for 90 min at 37°C, and M-MLV was inactivated at 70°C for 15 min. Fifty microliters of PCR buffer [50 mM KCl, 10 mM Tris·HCl (pH 9.3)] was added to the resulting cDNA, and cDNA was split to conduct two independent and parallel single-cell PCRs for each gene. For the first PCR round, 5 μl of the resulting cDNA was amplified by adding 45 μl of Taq buffer [50 mM KCl, 10 mM Tris·HCl (pH 9.3), 2.5 mM $MgCl_2$] containing 2.5 units of Taq polymerase, 500 μM dNTP, and 250 ng of sense and antisense primer (for *Aire* and *β-actin*, 5 min at 95°C, 30 cycles with 54°C annealing temperature; for PTAs, 40 cycles at 54°C annealing temperature; for *ins2*, 40 cycles at 58°C annealing temperature). For the second PCR round, 2.5 μl of the first amplification product was amplified under the same conditions in 50 μl of Taq buffer containing 1 unit of Taq polymerase, 500 μM dNTP, and 250 ng of sense and seminested primers (for *Aire* and *β-actin*, 30 cycles; for PTAs, 35 cycles). The PCR products were visualized on a 2% agarose gel with ethidium bromide. All primers are listed in Tables S2 and S3.

Precautions were taken against PCR contamination, with 32 control wells per 96-well PCR plate in which a mock droplet of PBS was sorted. Plates showing any trace of positive amplification in these control wells were discarded.

Single-Cell Allelic Discrimination and Sequencing. PCR products were sequenced directly by using the following primers: *S100a8* 5′-CACCCACTTTTATCACCATCG-3′, *Expi* 5′-CAGCCACAGTCTTTGTTCTGG-3′, *S100a6* 5′-CAATGGTGAGCTCCTTCTGG-3′, and *β2m* 5′-CCCCTCAAATTCAAGTATACTCACG-3′.

RT-PCR. RT-PCR using TaqMan was performed in a final volume of 25 μl of 2× TaqMan master mix (Applied Biosystems) containing AmpliTaq Gold polymerase. Nine hundred nanomolar forward and reverse primers and 200 nM of the probe were used, and duplicate reactions were cycled on an Applied Biosystems 7700 sequence detection system machine (annealing temperature of 60°C, 40 cycles). Primers and probes are listed in Table S4.

5′ RLM-RACE. Briefly, 10 μg of total RNA was dephosphorylated at 37°C for 1 h. RNA was then phenol/chloroform-extracted, precipitated, and resuspended in water. Four micrograms of dephosphorylated RNA was digested for 60 min at 37°C in a 10-μl reaction with tobacco acid pyrophosphate (TAP) (Ambion) and incubated for 60 min at 37°C with T4 RNA ligase (Ambion) and an RNA adaptor (5′-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3′; Ambion). Reverse transcription was conducted in the presence of random decamers and M-MLV RT (Ambion). Gene-specific PCR was conducted (see Table S5 for primers), and the products were purified on an agarose gel and sequenced. To identify TSSs, we compared sequences obtained from the 5′ RLM-RACE method with the mouse genome (Emsembl and Fantom) (1).

1. Carninci P, *et al.* (2005) The transcriptional landscape of the mammalian genome. *Science* 309:1559–1563.

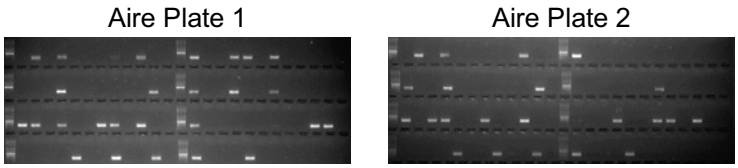

Fig. S1. Representative results from plate 1 and plate 2 of a single-cell PCR assay specific for Aire conducted in duplicate and parallel. Products were visualized on 2% agarose gels.

Relative risk to express

For a cell expressing	Aire+	s100a13	s100a8	s100a6	ins2	csnβ
	s100a13		4.3	2.15	0.58	1.18
	s100a8	2.19		1.37	1.17	1.72
	s100a6	1.79	1.46		1.67	1.83
	ins2	0.69	1.15	1.44	1.18	
	csnβ	1.15	2.02	1.87	1.26	

Fig. S2. Relative risk ratios of PTAs expressed in *Aire*-positive cells. Significant ratios are highlighted in gray. Calculation described in the text.

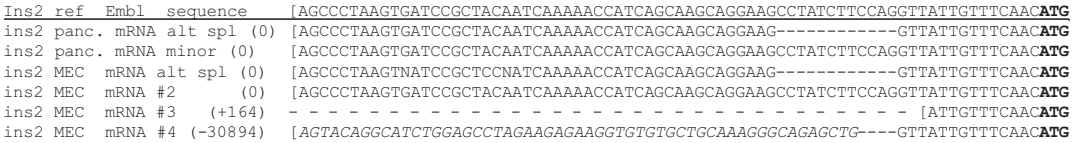

Fig. S3. Sequence alignment of *Ins2* from pancreas and MEC mRNA.

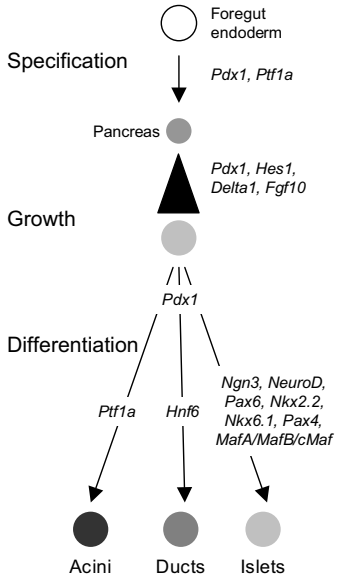

Fig. S4. Specification, growth, and differentiation of pancreatic acini, ducts, and islets.

Table S1. Gene-specific primers for reverse transcription

Gene	Primer
Aire	5′-TCATCTCTACCAGGTATAGTGAC-3′
S100a13	5′-ATCAGTCTCCAGTATTCACTGAACC-3′
S100a8	5′-TGGCTGTCTTTGTGAGATGC-3′
S100a6	5′-ACTGGATTTGACCGAGAGAGG-3′
Ins2	5′-TAGTGGTGGGTCTAGTTGCAG-3′
Csnβ	5′-TGGTGGCTTTAGCTTTAAGG-3′
β-actin	5′-ACGCTCGGTCAGGATCTTC-3′
β2m	5′-TGATGCTTGATCACATGTCTCG-3′
Expi	5′-GCACTTCATGTTGCCAGAGC-3′

Table S2. Primers for first round of single-cell PCR

Gene	Sense	Antisense
Aire	5′-CTCTTGGAAACGGAATTCAGAC-3′	5′-TCATCTCTACCAGGTATAGTGAC-3′
S100a13	5′-CTTCTTTCCCGGTCAATCTG-3′	5′-TCCAGTATTCACTGAACCTC-3′
S100a8	5′-GAGAAGGCCTTGAGCAACC-3′	5′-TCTTTGTGAGATGCCACACC-3′
S100a6	5′-ACCGTGCGCTTCTTCTAGC-3′	5′-ATCAGCCTTGCAATTTCAGC-3′
Ins2	5′-GCCTATCTTCCAGGTTATTGTTTCA-3′	5′-AGGTTTTATTCATTGCAGAGGGGTA-3′
Csnβ	5′-TCCTCTGAGACTGATAGTATTTCC-3′	5′-TGGTGGCTTTAGCTTTAAGG-3′
β -actin	5′-GTGAAAAGATGACCCAGATCATGT-3′	5′-ACGCTCGGTCAGGATCTTC-3′
β 2m	5′-CCCCTCAAATTCAAGTATACTCACG-3′	5′-TGATGCTTGATCACATGTCTCG-3′
Expi	5′-CAGCCACAGTCTTTGTTCTGG-3′	5′-CAGAGCACGATCCATCTCC-3′

Table S3. Primers for second round of single-cell PCR

Gene	Sense	Antisense
Aire	5′-CTCTTGGAAACGGAATTCAGAC-3′	5′-GCCTTGTTCTTCAAATTGCC-3′
S100a13	5′-CTTCTTTCCCGGTCAATCTG-3′	5′-CTGAGTCCTGATTCACATCC-3′
S100a8	5′-GAGAAGGCCTTGAGCAACC-3′	5′-CACCCACTTTTATCACCATCG-3′
S100a6	5′-ACCGTGCGCTTCTTCTAGC-3′	5′-CAATGGTGAGCTCCTTCTGG-3′
Ins2	5′-GCCTATCTTCCAGGTTATTGTTTCA-3′	5′-GTGGGTCTAGTTGCAGTAGTTCTCC-3′
Csnβ	5′-TCCTCTGAGACTGATAGTATTTCC-3′	5′-AGCTTTAAGGAAGGATTCCAG-3′
β -actin	5′-GTGAAAAGATGACCCAGATCATGT-3′	5′-GGAGAGCATAGCCCTCGTAG-3′
β 2m	5′-CCCCTCAAATTCAAGTATACTCACG-3′	5′-GCTTGATCACATGTCTCGATCC-3′
Expi	5′-CAGCCACAGTCTTTGTTCTGG-3′	5′-CACGATCCATCTCCTGTGC-3′

Table S4. Primers used for RT-PCR

Gene	Sense	Antisense
Aire	5′-GTACAGCCGCCTGCATAGC-3′	5′-CCCTTTCCGGGACTGGTT-3′
Hprt	5′-GACCGGTCCCGTCATGC-3′	5′-CAGTCCATGAGGAATAAACACTTTTTC-3′
Ins2	5′-GACCCACAAGTGGCACAA-3′	5′-ATCTACAATGCCACGCTTCTG-3′
	Probe	
Aire	5′-FAM-CTGGACGGCTTCCCAAAAGATGT-TAMRA-3′	
Hprt	5′-VIC-CCGCAGTCCCAGCGTCGTGATT-TAMRA-3′	
Ins2	5′-FAM-AGGCCCGGGAGCAGGTGACCTT-TAMRA-3′	

Table S5. Primers used for PCR of RLM-RACE products

Gene	Outer	Inner
S100a9	5′-TAAAGGTTGCCAACTGTGCTTC-3′	5′-CCATTTGTCTGAATTCCTTCTTGC-3′
S100a6	5′-TTGCTCAGGGTGTGCTTGTC-3′	5′-CACCTTCCTTGCCAGAGTACTTGT-3′
S100a13	5′-CGCCCCTGCTGTCACTAGC-3′	5′-CATCCCAACACAGCGCCA-3′
Ins2	5′-AAGCCACGCTCCCCACAC-3′	5′-GGTGCTGCTTGACAAAAGCCT-3′
Cyp1a2	5′-TTCCCCACAGTCAGCATGTG-3′	5′-CTTTGGGAACCTGGGTCCTT-3′
Csnγ	5′-AACACGCTATTGTCCAGCTTAGACT-3′	5′-GATGCTGGCAGATGATTCCTC-3′
Csnβ	5′-CCTTCTGAAGTTTCTGCTCATTGATAT-3′	5′-GTTCAACAGATTCCTCACTGGAAA-3′
Spt1	5′-TCTGCCTGAGTTTCAGAGCCA-3′	5′-TGGCAAGAGACTAGGATAGTGGAAA-3′
Muc6	5′-TTGGAAATTATACTCATGGCCGT-3′	5′-GGCCGTCAAAGGTGGAAAAGT-3′