

SUPPLEMENTARY DATA

Supplementary Table 1. PCR primer sequences used in mRNA expression analysis.

Target	5' Sequence (5' to 3')	3' Sequence (5' to 3')
<i>Insulin</i>	CCACCCAGGCTTTTGTCAA	CCCAGCTCCAGTTGTTCCAC
<i>Glucagon</i>	CATTCACCAGCGACTACAGCAA	TCATCAACCACTGCACAAAATCT
<i>Sst</i>	AACGCAAAGCTGGCTGCAAGAA	TCAGAGGTCTGGCTAGGACAACAA
<i>PP</i>	TTGCAGCCTCTCTTGTCTTCA	TAGTTTGCAAGGGAGCAGGTT
<i>Ghrelin</i>	AGCCCAGCAGAGAAAGGAATC	GGGAGCATTGAACCTGATCTC
<i>Pdx1</i>	CGGCTGAGCAAGCTAAGGTT	TGGAAGAAGCGCTCTCTTTGA
<i>FoxA2</i>	GAGCACCATTACGCCTTCAAC	AGGCCTTGAGGTCCATTTTGT
<i>Hb9</i>	AACAAGTACCTGTCTCGACCC	GCTGCGTTTCCATTTTATTTCG
<i>Ngn3</i>	TGACCCTATCCACTGCTGCTT	CCTCATCCACCCTTTGGAGTT
<i>Pax6</i>	TGGCAAACAACCTGCCTATG	TGCACGAGTATGAGGAGGTCT
<i>Isl1</i>	GCAACCCAACGACAAAATAA	CCATCATGTCTCTCCGGACT
<i>MafA</i>	CCTGTAGAGGAAGCCGAGGAA	CCTCCCCAGTCGAGTATAGC
<i>NeuroD1</i>	ACAGACGCTCTGCAAAGGTTTG	GCGGATGGTTCGTGTTTGAAG
<i>Nkx6.1</i>	CCTCTGGACCCGAACCTCTGA	GCTGCCACCGCTCGATT
<i>Arx</i>	TCCGGATACCCACTTAGCTT	GACGCCCTTTCCTTTAAGTG
<i>MafB</i>	AACGCGTCCAGCAGAAACA	AGCTGCTCCACCTGCTGAAT
<i>Kras</i>	TGAAGATGTGCCTATGGTCCTGGT	ACCCTGTCTTGTCTTTGCTGAGGT
<i>Ube4B</i>	AGCCACCAGCGAGTTCTATGACAA	TGAACTCCTCCATGAAGGTGCCAT
<i>Fzd3</i>	ACGGATCATTCCAGGCACAGTAGT	AAGCACTGGTTCCATCCTCCTCAA
<i>Rnf6</i>	AGAGAGCATAGGCAGCAAAGACCA	TGCAAATGACCCTTGACAGAAACC
<i>Glp1r</i>	TTGCCATTCTCTTTGCTATCGGC	GGCAAGCCTGCATTTGATGTCAGT
<i>G6pc2</i>	AGATGATATGGGTAGCGGTCA	TGGGCCTGTTTCGCACGTAG
<i>Kcnj11</i>	TGTGCAGAAATATCGTCGGGCTGAT	GCATGCTTGCTGAAGATGAGGGTT
<i>ATP1a1</i>	CCCAAATGCATCTGAGCCCAAACA	AAGCGTCCTCAGCTCTTCATCCA
<i>Ptprs</i>	CGCCTTTAACATGCTCAGTGGCAA	TCCGAAGTGGGACCATCACAATGA
<i>Slc2A2</i>	CAGTTCGGCTATGACATCGGT	GTTAATGGCAGCTTTCCGGTC

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<i>Gapdh</i>	AAC TTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
<i>TBP</i>	CCCCTTGTACCCTTCACCAAT	GAAGCTGCGGTACAATTCCAG
<i>Ldb1</i>	ACTCATGTGGATGCCTGTGTG	CCCCAACATTTAGCCCCTAAG
<i>Ldb2</i>	GGACGAGGACGAAAGGCTAAT	ACAGCGTAATCAGGTGCCAGT
<i>Lhx1</i>	GACCTACCCTTTGTGCCATCA	CCACCATTGACCGACAGAGAT
<i>Lhx2</i>	CAGCCCTTCACAAACGACTCT	CAGGCGAGATCCTAAAACGTG
<i>Lhx3</i>	CTTCCTGGCCACTGACAAAAC	TCTCGCATTTAGAGGCTACGC
<i>Lhx4</i>	CTTAGAGGCTTTGGCTGCTCA	CTGGTGAGAGGGATGATTTGG
<i>Lhx5</i>	GGTCGAGATTCCAAGCACATC	TTCTCTCCCCCTTCAACCTC
<i>Lhx6</i>	CTTCAGTGAGCACATGCCAAG	GTGCATCAGGACACCAAGTCA
<i>Lhx7/8</i>	TCTCGCTGCCAGGTATGTAT	ACGATCAGTCCTGCCTTTCAG
<i>Lhx9</i>	GAAGTGCTGGGACAAAACCAC	AGACCTTGGATCTCCGTGACA
<i>Lmo1</i>	CTATGAGGAGGGGCATCTCAA	AGAACAGCCACCTTCCCATC
<i>Lmo2</i>	TGGA CTCTTCCTGGGCACTAA	GAAGCCTATCAGGATGGCACA
<i>Lmo3</i>	GCCTTTGGGTAGCATGTGAAC	GGCCATCAGCTCATTAAACCAC
<i>Lmo4</i>	CACCTTTGTAGCCAGCACCAT	ATCTCCCATTAGCCCAGGTTT
<i>Lmx1a</i>	TCATAGAAGCAGAGGGGACCA	GCACCGTAATTGGAGATCAGG
<i>Lmx1b</i>	GAACGACTCCATCTTCCACGA	TGGCTCTCAGGAGGCAAAGTA

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Supplementary Table 2. PCR primer sequences used for ChIP analyses.

<i>MafA</i> Region 3	CTGGAAGATCACCGCACA	ATTTACCAAGCCCCAAACG
<i>Slc2A2</i> - proximal	ATCTGGCTCCGCACTCTCATCTTG	CCCTGTGACTTTTCTGTGTCTTAGG
<i>Slc2A2</i> Re1	CATGATCTTCGCTCCCGTAT	CTCTGCAGGGCATCTTTCTC
<i>Slc2A2</i> Re2	CTTGTTCCCAAGTGACACCA	CTAACAGCAGGGAGCACACA
<i>Arx</i> Re1	CCATTTGAAGGCAAAATGCT	GTATGGGCTGCAAACACCTT
<i>Arx</i> Re2	TGAAGTGGCTGAATGAGAGC	AGTTGGAGCGCGTTTTGTAG
<i>Gpl1r</i>	TTTAACTTCCCTGCTAGCCCACCA	AATCCTGCAGAGTGAGCTGCTGAT
<i>Pepck</i>	CAACAGGCAGGGTCAAAGTTTAG	AGGCCTCAGGCCCTCTAT

SUPPLEMENTARY DATA

Supplementary Table 3. Antibodies, dilutions and conditions used for immunostaining experiments.

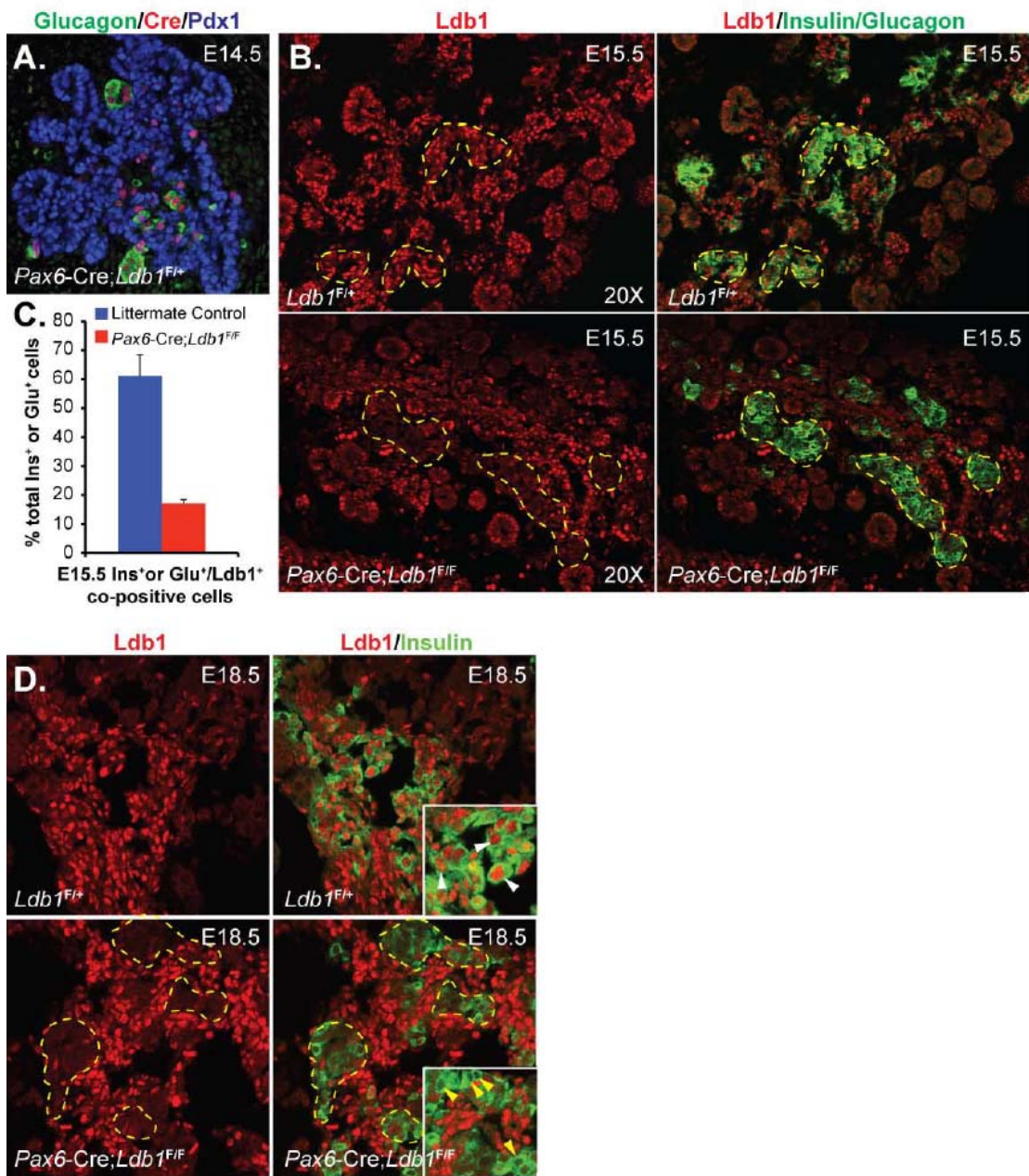
Antibody target	Dilution	Embedding Media	Antigen	
			Retrieval/Amplification	Source
Ldb1	1:3000	OCT		P. Love – NIH/NICHD
Lmo4	1:300	Paraffin	CitraPlus boil	J. Visvader
Insulin	1:1000	OCT, Paraffin		Invitrogen #180067
Glucagon	1:2000- 4000	OCT, Paraffin		Sigma G2654
Somatostatin	1:1500	OCT, Paraffin		American Research Products 13-2366
PP	1:1500	Paraffin		Linco RPP63-4
Ghrelin	1:100	OCT, Paraffin		Santa Cruz sc-10368
Pdx1	1:20000	OCT, Paraffin	TEG boil	C. Wright - Vanderbilt
Dolichos biflorus (DBA)	1:1000	Paraffin		Vector Laboratories
Cytokeratin-19	1:50	Paraffin		DSHB Troma III
Cre	1:2000	Paraffin	TEG/Citraplus boil	Novagen #69050
Amylase	1:1000	OCT, Paraffin		Sigma #A8273
Arx	1:1000	OCT		P. Collombat - INSERM
MafA	1:1000	Paraffin	TEG boil/TSA Amp.	Bethyl BL-1069

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Glut2	1:100	Paraffin	TEG boil	Alpha Diagnostic #GT21-A
Glp1r	1:300	Paraffin		Abcam AB39072
Pax6	1:300	Paraffin	TEG boil	Covance PRB- 278P

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Supplementary Figure 1. *Ldb1* is removed from pancreatic endocrine cells of *Pax6-Cre;Ldb1^{F/F}* mice by E15.5. A) Immunofluorescence staining of E14 *Pax6-Cre;Ldb1^{F/+}* tissue demonstrating nuclear Cre expression (red) marking both *Pdx1⁺* (blue) and glucagon⁺ (green) cells. B) *Ldb1* (red) localizes to the nuclei of insulin⁺ and glucagon⁺ cells (green) of control E15.5 *Ldb1^{F/+}* pancreata (top), with levels greatly reduced or absent in the *Pax6-Cre;Ldb1^{F/F}* mutant (bottom). The yellow dashed lines encircle hormone⁺ clusters. Notably, *Ldb1* is not removed from non-hormone⁺ cells, as expected from the endocrine cell specific expression pattern of the *Pax6-Cre* transgene (37, data not shown. C) Insulin, glucagon, and *Ldb1* co-positive cells were quantified in littermate control (*Ldb1^{F/F}* and *Ldb1^{F/+}*, blue bar) and *Ldb1* mutant E15.5 pancreata (red bar). D) *Ldb1* (red) is essentially absent from all *Pax6-Cre;Ldb1^{F/F}* (bottom) insulin⁺ (green) cells at E18.5, compared with *Ldb1^{F/+}* littermate control pancreata (top). The yellow dashed lines encircle insulin⁺ clusters in *Ldb1* mutant pancreata, with the arrows depicting the presence (white) or absence (yellow) of *Ldb1* in the control and *Ldb1* mutant, respectively.



SUPPLEMENTARY DATA

Supplementary Figure 2. Ldb2 loss does not impact pancreatic islet development. Analysis of insulin (green), glucagon (red) and amylase (blue) expression at E18.5 in *Ldb1*^{F/+} control (left), *Ldb2* mutant (*Ldb1*^{F/F};*Ldb2*^{-/-}, center) and *Ldb1*/*Ldb2* double mutant (*Pax6-Cre*;*Ldb1*^{F/F};*Ldb2*^{-/-}, right) pancreata. Islet endocrine cells were lost upon deletion of *Ldb1* (right) and not *Ldb2* (center).

