

## Supplementary Figure Legends

### Supplementary Figure 1. Reduced Pdx1 mRNA and protein but normal Ngn3 mRNA and

**Ngn3+ endocrine progenitor numbers in  $Pdx1^{+/-}$  mice.** (A) *Pdx1* mRNA levels in e13.5 total pancreas from  $Pdx1^{+/-}$  (gray bars) and wild-type (black bars) littermate controls (n=6, \*p<0.05), measured by real-time PCR and normalized to *HPRT* transcript levels. (B) Western Blot quantitation of total Pdx1 protein levels in e13.5 pancreata from  $Pdx1^{+/-}$  and wild-type littermate controls normalized to cyclophilin B (n=9 per group, \*p<0.05). (C) *ngn3* mRNA levels in total pancreas of e13.5 animals, measured by quantitative real-time RT-PCR. Values are normalized to *HPRT* mRNA levels and expressed relative to wild-type. N=5; \*p =0.005. (D) Number of Ngn3+ cells per unit of epithelial area (e11.5) or pancreatic area (e13.5-P1) quantified from three (e13.5-P1) or five (e11.5) tissue sections per animal. N=5 per genotype.

### Supplementary Figure 2. Decreased expression of endocrine hormones and transcription

**factors at e13.5 in  $Pdx1^{\Delta C/\Delta C}$  mice.** mRNA levels of endocrine hormones (A) and transcription factors (B) in total pancreas from  $Pdx1^{+/+}$  (black bars),  $Pdx1^{\Delta C/+}$  (gray bars) and  $Pdx1^{\Delta C/\Delta C}$  (white bars) e13.5 embryos measured by quantitative real time RT-PCR (N=7-9 per genotype, \*p<0.05). Levels were normalized to *HPRT* mRNA and expressed as fold change compared to wild-type.

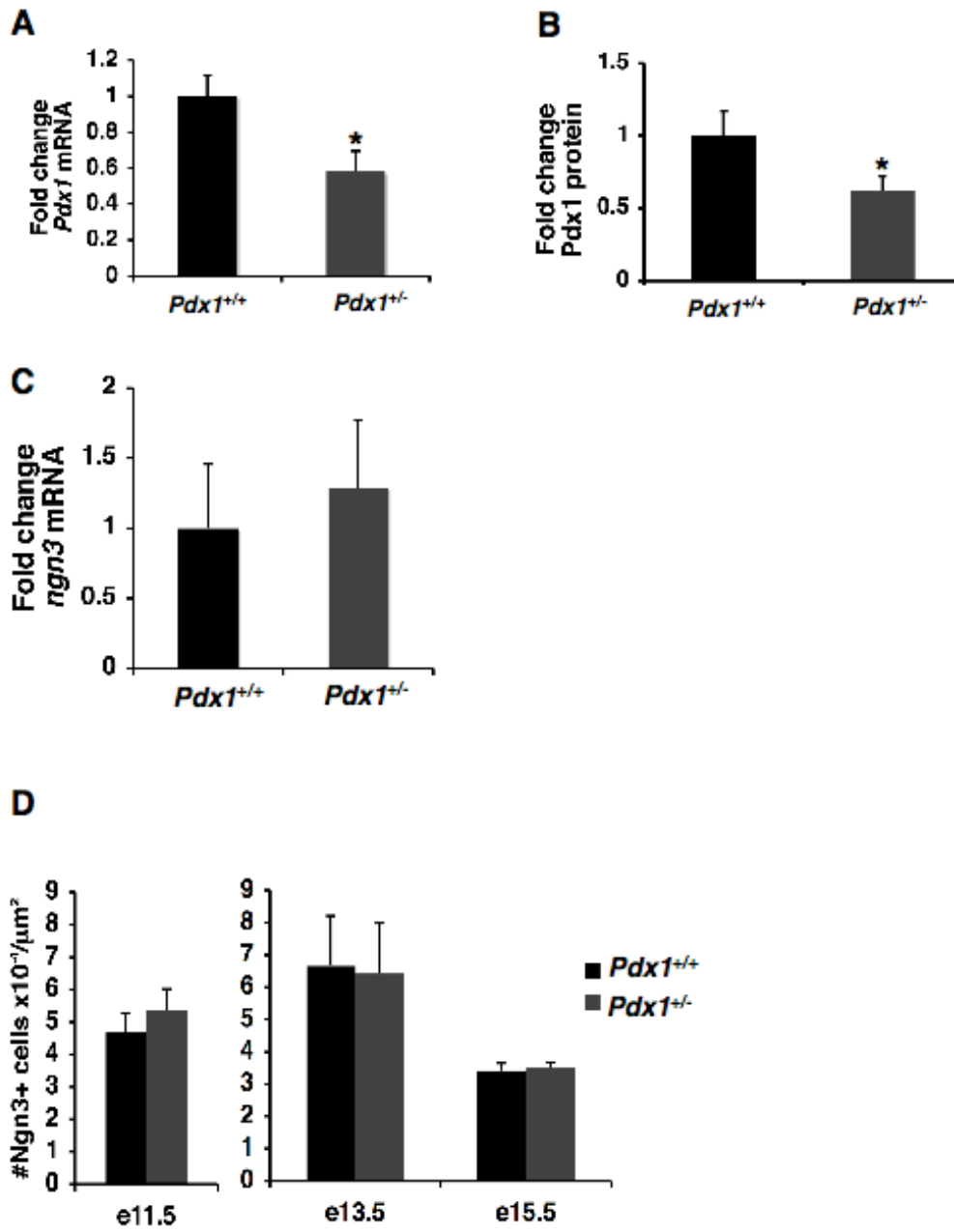
**Supplementary Figure 3. Survival of Ngn3+ endocrine progenitors in *Pdx1*<sup>ΔC/ΔC</sup> mice.**

Representative example of triple immunofluorescence for Ngn3 (green), TUNEL (red) and DAPI (blue) of e19.5 pancreas from *Pdx1*<sup>ΔC/ΔC</sup> mice. Arrow points to the same cell in all panels. Bar=10μm.

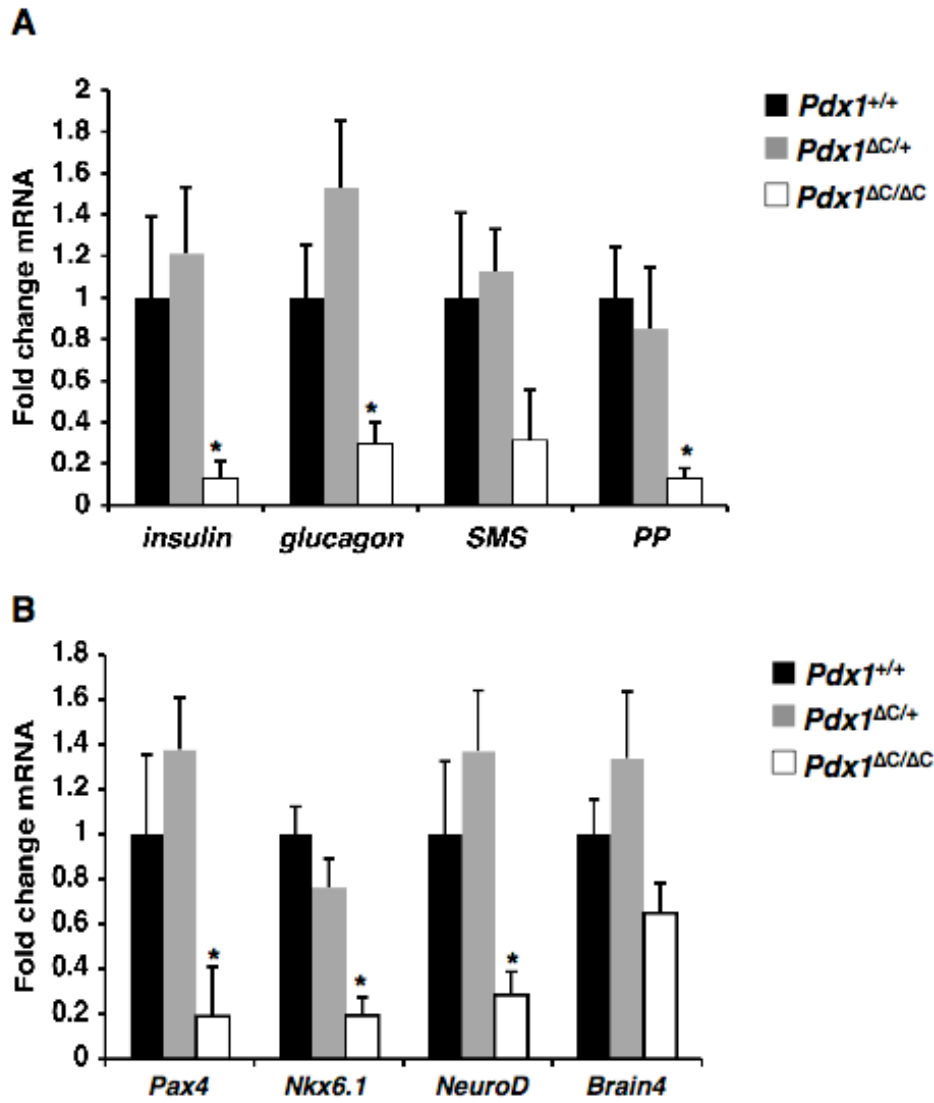
**Supplementary Figure 4. Pdx1 and Ngn3 do not co-localize at e19.5.** Double immunofluorescence for Ngn3 (green, left) and Pdx1 (red, middle) of e19.5 wild-type pancreata. Merged image is shown on the right. Bar=10μm.

**Supplementary Table 1. Increase in TUNEL+Ngn3+ cells in *Pdx1*<sup>ΔC/+</sup> and *Pdx1*<sup>ΔC/ΔC</sup> mice at e19.5.** Number of double positive TUNEL+/Ngn3+ cells per pancreatic section at e15.5 and e19.5. N=5-6 embryos per genotype.

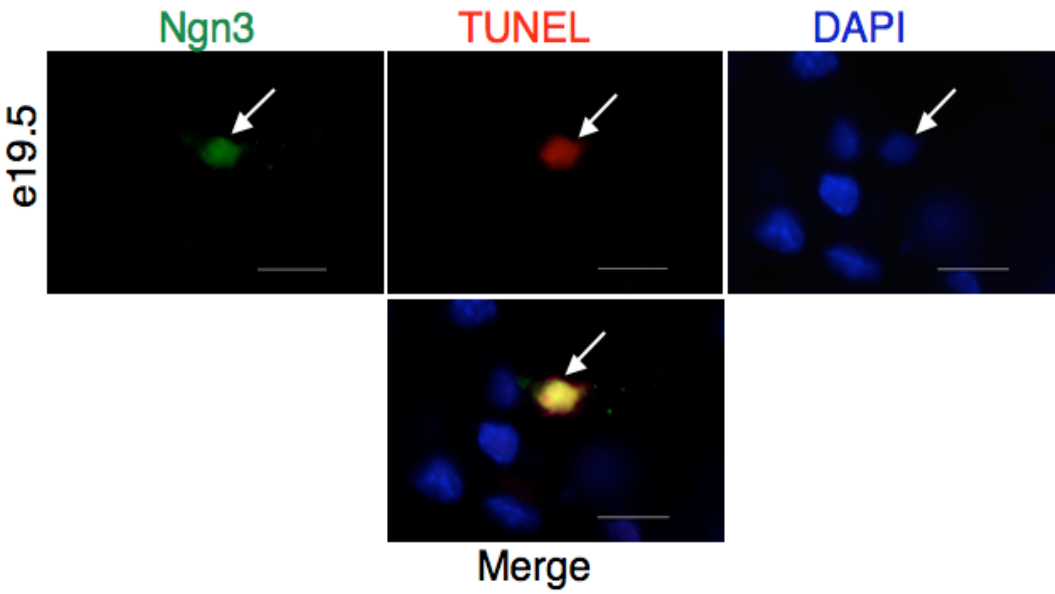
**Supplementary Table 2. Quantitative PCR primers.**



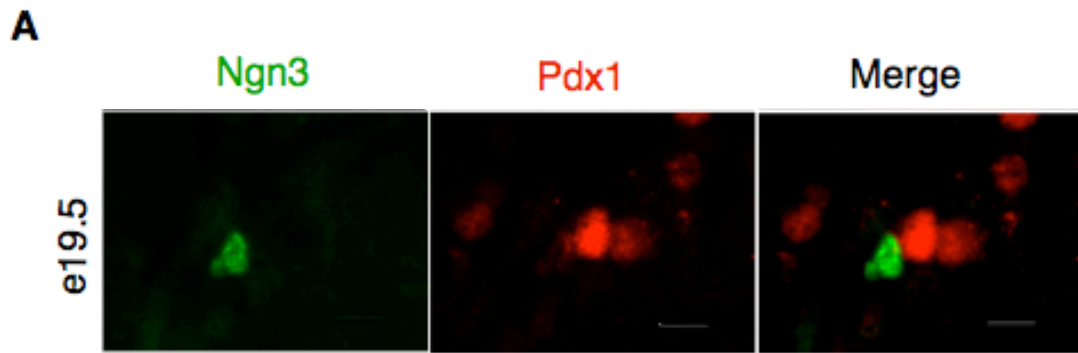
Supplementary Figure 1



Supplementary Figure 2



**Supplementary Figure 3**



**Supplementary Figure 4**

**Supplementary Table 1.**

| Genotype   | TUNEL+Ngn3+/Total Ngn3+ (%) |             |
|--|-----------------------------|-------------|
|  | e15.5                       | e19.5       |
| <i>Pdx1</i> <sup>+/+</sup>                                       | 0/105 (0)                   | 0/53 (0)    |
|  | 0/123 (0)                   | 1/35 (2.9)  |
|  | 0/116 (0)                   | 0/58 (0)    |
|  | 0/129 (0)                   | 0/39 (0)    |
|  | 0/89 (0)                    | 0/60 (0)    |
| <i>Pdx1</i> <sup>+/<math>\Delta</math>C</sup>                    | 1/62 (1.6)                  | 0/47 (0)    |
|  | 1/110 (0.9)                 | 2/66 (3.0)  |
|  | 0/167 (0)                   | 0/61 (0)    |
|  | 0/56 (0)                    | 2/18 (11.1) |
|  | 0/64 (0)                    | 1/30 (3.3)  |
| <i>Pdx1</i> <sup><math>\Delta</math>C/<math>\Delta</math>C</sup> | 0/62 (0)                    | 1/39 (2.6)  |
|  | 0/72 (0)                    | 1/21 (4.8)  |
|  | 2/44 (4.5)                  | 2/27 (7.4)  |
|  | 0/41 (0)                    | 0/13 (0)    |
|  | 0/56 (0)                    | 0/5 (0)     |
|  | 0/37 (0)                    | 0/19 (0)    |
|  | 0/66 (0)                    | 1/31 (3.2)  |

## Supplementary Table 2.

| Target              | Forward Primer (5'-3')      | Reverse Primer (5'-3') |
|---------------------|-----------------------------|------------------------|
| cDNA                |                             |                        |
| <i>Hnf4a</i>        | atgacacgtccccatctgaag       | ctcgaggctccgtagtgttg   |
| <i>Hnf1a</i>        | accactgcatccctcctatca       | acctcaggcttgaggctgtat  |
| <i>Ptf1a</i>        | tctgcagggcactctctttc        | tcctctggggtccacactta   |
| <i>Brain4</i>       | ggctgattcatccacaggaag       | ttccagtacgcccttgacact  |
| <i>Pax4</i>         | tcctgtggcttctcctcata        | gaggcctcttatggccagttt  |
| <i>Nkx6.1</i>       | aacacaccagaccacgttct        | atccccagagaataggccaag  |
| <i>NeuroD</i>       | gccagcttaatgccatctt         | caaaagggctgccttctgtaa  |
| <i>ins</i>          | tggtctctctacacaccaag        | acaatgccacgcttctgcc    |
| <i>glu</i>          | gcacattcaccagcgactaca       | cggttctcttggttcatc     |
| <i>SMS</i>          | ctgaggcaaggaagatgctgt       | gcagaaactgacggagtctgg  |
| <i>PP</i>           | ttgcagcctctctgtcttca        | tagtttgcaaggagcagggt   |
| <i>Pdx1</i>         | gaacccgaggaaaacaagagg       | gttcaacatcactgccagctc  |
| <i>Ngn3</i>         | tctcaagcatctcgctcttc        | acagcaagggtagcagatgaga |
| <i>Hnf6</i>         | caaatcaccatctcccagcag       | cagactcctcctctggcatt   |
| <i>Sox9</i>         | cttctgtgggagcgacaactt       | agggagggaaaacagagaacg  |
| <i>Foxa2</i>        | gagcaccattacgcctcaac        | aggccttgagggtccattttgt |
| <i>Hnf1b</i>        | catctgcaatggtggtcacag       | ggcttgagtggtgactggtt   |
| <i>Hes1</i>         | agaggctccaaggtttttg         | tcccactgttgctggtgtaga  |
| <i>GDF11</i>        | cagccctctctgctgtcattt       | tcccagttaggggtttagt    |
| <i>HPRT</i>         | ggccagactttgtggatttg        | tgcgctcatcttaggctttgt  |
| Promoter            |                             |                        |
| <i>Ngn3</i> (-4464) | ggcccaggattatgtcagga        | gcgacatatgttaggcagctc  |
| <i>Ngn3</i> (-4199) | actttgtttcctggctctgc        | gtcctcgaacaaagggagaag  |
| <i>Ngn3</i> (-3872) | catgagcagctcgcttatct        | ggctaccaaccccatattc    |
| <i>Ngn3</i> (-3670) | gccttgacagccaacctgtat       | ctgacacatggatttggcactg |
| <i>Ngn3</i> (-3527) | ccggatttatcacggcaaag        | tgtaagcctccccacaact    |
| <i>Ngn3</i> (-3332) | agactcccgccagagatggtt       | gaaaagaaagccccacctgtc  |
| <i>Foxa2</i> (+1)   | gtgagcggctggtgatatag        | tgtgagctgattattcaaatgg |
| <i>Foxa2</i> (+550) | aagcaggtgacagccaattt        | gccagcgagttaaaggtgtg   |
| <i>Hnf1β</i>        | ctgagccatcttctcctg          | acagggctgaagctggagt    |
| <i>Sox9</i>         | atctctccatattttgtattaagggta | ggccttagggcattttgaa    |
| <i>Hnf6</i>         | caagctggcttgaagtaggg        | ctctgctgggtctcaacatt   |