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Journal

Diabetes, 68(9)

ISSN

0012-1797

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Publication Date

2019-09-01

DOI

10.2337/db19-0349

Peer reviewed

The Pdx1-Bound Swi/Snf Chromatin Remodeling Complex Regulates Pancreatic Progenitor Cell Proliferation and Mature Islet β-Cell Function

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Diabetes 2019;68:1806–1818 | https://doi.org/10.2337/db19-0349

Transcription factors positively and/or negatively impact gene expression by recruiting coregulatory factors, which interact through protein-protein binding. Here we demonstrate that mouse pancreas size and islet β-cell function are controlled by the ATP-dependent Swi/Snf chromatin remodeling coregulatory complex that physically associates with Pdx1, a diabetes-linked transcription factor essential to pancreatic morphogenesis and adult islet cell function and maintenance. Early embryonic deletion of just the Swi/Snf Brg1 ATPase subunit reduced multipotent pancreatic progenitor cell proliferation and resulted in pancreas hypoplasia. In contrast, removal of both Swi/Snf ATPase subunits, Brg1 and Brm, was necessary to compromise adult islet β-cell activity, which included whole-animal glucose intolerance, hyperglycemia, and impaired insulin secretion. Notably, lineage-tracing analysis revealed Swi/Snf-deficient β-cells lost the ability to produce the mRNAs for *Ins* and other key metabolic genes without effecting the expression of many essential islet-enriched transcription factors. Swi/Snf was necessary for Pdx1 to bind to the *Ins* gene enhancer, demonstrating the importance of this association in mediating chromatin accessibility. These results illustrate how fundamental the Pdx1:Swi/Snf coregulator complex is in the pancreas, and we discuss how disrupting their association could influence type 1 and type 2 diabetes susceptibility.

The mammalian pancreas consists of two functionally distinct compartments: the exocrine pancreas containing acinar and ductal cells essential for secreting digestive enzymes, and the endocrine pancreas containing hormone-secreting α- (glucagon), β- (insulin), δ- (somatostatin), ε- (ghrelin), and pancreatic polypeptide cells of the islets of Langerhans that are essential for regulating glucose homeostasis. All of these pancreatic cells derive from a common pool of progenitor cells at mouse embryonic day (e)8.5 that express the pancreas and duodenum homeobox 1 (Pdx1) transcription factor, a critical regulator of pancreas development, later β-cell formation, and adult islet β-cell function. In fact, pancreas agenesis occurs in mice and humans that lack PDX1 (1,2), whereas heterozygous mutations cause type 2 diabetes (T2D) because of islet β-cell dysfunction (3).

Embryonic $Pdx1^+$ pancreatic progenitor cells rapidly divide and acquire the expression of other transcription factors essential to organ expansion and lineage diversification, including Ptf1a (4) and Sox9 (5). These $Pdx1^+Ptf1a^+Sox9^+$ cells form the highly proliferative multipotent pancreatic progenitor cell (MPC) pool that differentiates into the distinct exocrine, ductal, and islet cell types (6). Notably, pancreas mass is restricted in mice by the MPC pool size (7), which is affected by early embryonic genetic removal of *Pdx1*, *Ptf1a*, or *Sox9* (2,4,5). Because of considerable variations in pancreas mass (and therefore variable β-cell number) between humans (8),

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Received 4 April 2019 and accepted 6 June 2019

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db19-0349/-/DC1.

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understanding how the transcriptional activities of these essential MPC regulators control growth rate and final organ size is of significant importance. It has been proposed that dissimilarities in pancreas mass influence diabetes susceptibility, a proposal supported by the reduced pancreas size of autoantibody-positive individuals with type 1 diabetes (9).

In the postnatal pancreas, Pdx1 is produced at much higher levels in islet β-cells than in other pancreas cell types (10). Conditional removal of Pdx1 from these cells in mice leads to a profound loss of β-cell function and identity, because these cells rapidly transdifferentiate to glucagon^{+} and insulin^{-} α-like cells (11). This remarkable control derives not only from the positive actions of Pdx1 on target gene transcription but also from its repression of key α-cell functional genes in β-cells, such as *MafB* and *Gcg*. As a result of these novel and fundamental properties, Pdx1 is viewed as one of the most critical pancreas-enriched transcription factors (6).

Transcription factors like Pdx1 predominantly control gene expression through the recruitment of coregulators, often operating as large multiprotein complexes. These coregulators affect transcriptional activity positively and/or negatively, for example, by displacing nucleosomes, epigenetically modifying histones/DNA, and affecting recruitment of the RNA polymerase II transcriptional machinery. Because of limited knowledge of the coregulators influencing Pdx1 activity, an unbiased chemical crosslinking, immunoprecipitation, and mass spectrometry analysis was used to identify interacting proteins in rodent β-cell lines. Numerous proteins with an array of cellular functions were found to associate with Pdx1, including the ATP-dependent Swi/Snf chromatin remodeling complex (12). Significantly, Pdx1 was the principal islet β-cell–enriched transcription factor binding to Swi/Snf in mouse β-cell lines (12).

The multisubunit Swi/Snf complex uses the energy of ATP hydrolysis, through the actions of the two mutually exclusive Brg1 (i.e., also referred to as *Smarca4*) and Brm (i.e., *Smarca2*) ATPase subunits (13,14) (Fig. 1*A*), to disrupt DNA-nucleosome contacts and influence DNA accessibility. We previously discovered that in vitro knockdown of Brg1 in rodent β-cell lines negatively affected Pdx1 target gene expression (e.g., *Ins*, *MafA*, and *Glut2*). Moreover, Pdx1:Swi/Snf interactions were not only acutely enhanced in islet β-cells by increased glucose concentrations but were also reduced in human T2D β-cells (12). Here we show that the Pdx1:Brg1/Swi/Snf complex is critical for mouse MPC proliferation, with embryonic conditional deletion of only the *Brg1* gene encoding ATPase resulting in a ~50% smaller pancreas. In contrast, removing both Brg1 and Brm was necessary to impact postnatal β-cells, causing severe changes in expression of *Ins* and other β-cell regulatory genes, a hallmark feature of T2D β-cells. Collectively, our results suggest that Pdx1:Swi/Snf is required for controlling the growth rate of the embryonic pancreas, and thus its final postnatal size, and for maintaining β-cell identity in adult islets.

RESEARCH DESIGN AND METHODS

Animals

Ptf1a-Cre (15) and *MIP-Cre^{ERT}* (16) mice were used to remove the *Loxp* sites surrounding exons 17 and 18 of the *Brg1* locus (*Brg1$^{f/f}$* [17]) and the *Stop* cassette in the *Rosa26-Loxp-Stop-Loxp-tdTomato* lineage reporter (*R26$^{LSL\text{-}tdTomato}$* [18]). *Brm$^{-/-}$* mice were generated using homologous recombination to insert the neomycin gene into *Brm* exon 4 (19). The following genotypes were used for the developmental studies: control, *Ptf1a-Cre;Brg1$^{f/+}$* or *Ptf1a-Cre;Brg1$^{f/+}$;Brm$^{+/-}$;* experimental, *Brg1$^{\Delta panc}$* (*Ptf1a-Cre;Brg1$^{f/f}$*), *Brm$^{-/-}$* (*Ptf1a-Cre;Brm$^{-/-}$*), and *DKO$^{\Delta panc}$* (*Ptf1a-Cre;Brg1$^{f/f}$;Brm$^{-/-}$*). Noon of the day of the vaginal plug discovery was designated day e0.5. For BrdU injections, 100 mg of BrdU (B5002; Sigma-Aldrich) per kilogram of pregnant dam body weight was injected 30 min before embryo harvest.

The adult studies consisted of these genotypes: control, *MIP-Cre^{ERT};Brg1$^{f/+}$;Brm$^{+/-}$;R26$^{LSL\text{-}tdTomato/+}$*; and experimental, *Brg1$^{\Delta\beta}$;Brm$^{+/-}$* (*MIP-Cre^{ERT};Brg1$^{f/f}$;Brm$^{+/-}$;R26$^{LSLtdTomato/+}$*), *Brg1$^{\Delta\beta/+}$;Brm$^{-/-}$* (*MIP-Cre^{ERT};Brg1$^{f/+}$;Brm$^{-/-}$;R26$^{LSLtdTomato/+}$*), and *βDKO* (*MIP-Cre^{ERT};Brg1$^{f/f}$;Brm$^{-/-}$;R26$^{LSLtdTomato/+}$*). Cre^{ERT}-mediated recombination of *Brg1$^{f/f}$* and the *R26^{LSLtdTomato}* was achieved by administration of 4 mg tamoxifen (T5648; Sigma-Aldrich) by oral gavage three times over a 5-day period.

Intraperitoneal Glucose Tolerance Test and Serum Insulin Measurements

Mice (n = 5–12) were given intraperitoneal injection of D-glucose (2 mg/g body wt) after a 6-h fast. Blood glucose was measured using a FreeStyle glucometer (Abbott Diabetes Care). Serum insulin was measured by radio immunoassay at the Vanderbilt Hormone Assay and Analytical Services Core.

Glucose-Stimulated Insulin Secretion

Secreted insulin from isolated control and βDKO mice (n = 8–10) islets was performed as described previously (20). The outcome was presented as the fold change between the percentage of secreted insulin (relative to insulin content) at 16.7 mmol/L glucose and the percentage of secreted insulin (relative to insulin content) at 2.8 mmol/L glucose. Islet insulin content was calculated as the concentration of insulin per islet in each reaction (ng/mL/islet).

Tissue Preparation and Immunostaining

Whole embryos and adult pancreata were fixed in 4% (v/v) paraformaldehyde, embedded, and sectioned to 6 μm. Immunofluorescence staining was performed as previously described (21) with the antibodies listed in Supplementary Table 1. Embryos were cut on the transverse (cross-sections) plane throughout the pancreatic epithelium, and manual cell counting was performed on antibody-stained sections prepared every 60 μm (e12.5) or 90 μm (e15.5) from the superior to inferior region.

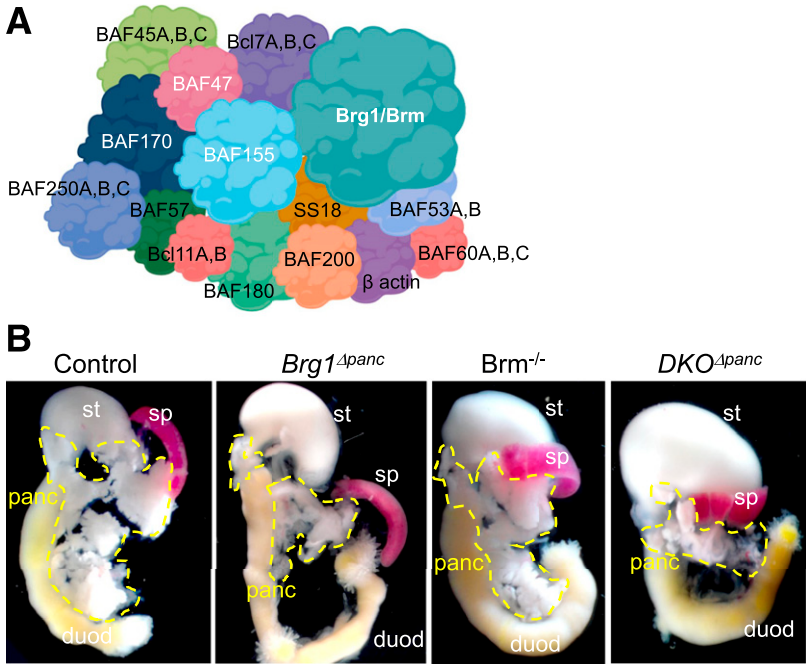

Figure 1—Pancreas size is reduced upon embryonic deletion of the mouse *Brg1* Swi/Snf ATPase subunit. *A*: Schematic illustrating the composition of the mammalian Swi/Snf complex, with the core of the coregulator containing the Brg1 or Brm ATPase and the BAF47, BAF155, and BAF170 subunits (white font). Swi/Snf complexes also contain 8–10 other BAFs (Brm- or Brg1-associated factors) and accessory subunits, which vary depending on the tissue and developmental stage (14). *B*: Representative image of the pancreas (panc), stomach (st), spleen (sp), and duodenum (duod) in P1 control, $Brg1^{\Delta panc}$, $Brm^{-/-}$, and $DKO^{\Delta panc}$ mice.

Proximity Ligation Assay

The assay was performed on e12.5 sections following the manufacturer's protocol (Sigma-Aldrich) with goat transcription factors Pdx1 (1:20,000) (AB47383; Abcam), Ptf1a (1:2,000) (from C.V.E.W.) or rabbit Sox9 (1:500) (AB5535; Millipore) in combination with rabbit ATPase Brg1 (1:400) (sc-10768; Santa Cruz Biotechnology), goat Brg1 (1:500) (AF5738; R & D Systems), mouse Brm (1:500) (sc-17828; Santa Cruz Biotechnology), or rabbit Brm (1:500) (ab1559; Abcam) antibodies. Immunofluorescence Z-Stack images were acquired on a Zeiss Axioimager M2 fluorescence scope and processed using ImageJ software.

Flow Cytometry, RNA Purification, and Quality Control of Sorted β-Cells

Isolated islets were dispersed into a single-cell suspension (Accumax; A7089; Sigma-Aldrich), stained with DAPI, and sorted by gating for $Tomato^{+}DAPI^{+}$ cells by FACS at the Vanderbilt Flow Cytometry Core. RNA was isolated from FACS-purified β-cells (control: 10,215 ± 1,589 cells [n = 3], βDKO: 16,267 ± 3,032 cells [n = 3]) using the Maxwell 16 LEV simplyRNA Tissue Kit (TM351; Promega), and then DNAse was treated and analyzed on an Agilent 2100 Bioanalyzer. Only samples with an RNA Integrity Number >8.0 were used for cDNA synthesis and library preparation.

RNA Sequencing and Analysis

cDNA libraries were constructed from RNA isolated from FACS-purified control and βDKO islet β-cells, and paired-end sequencing of three replicates was performed on an Illumina NovaSeq6000 (150 nucleotide reads). The generated FASTQ files were processed and interpreted using the Genialis visual informatics platform (https://www.genialis.com). Sequence quality checks were determined using raw and trimmed reads with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), and Trimmomatic (22) was used to trim adapters and filter out poor-quality reads. Trimmed reads were then mapped to the University of California, Santa Cruz, mm10 reference genome using the HISAT2 aligner (23). Gene expression levels were quantified with HTSeq-count (24), and differential gene expression analyses were performed with DESeq2 (25). Poorly expressed genes, which have expression count summed over all samples of <10, were filtered out from the differential expression analysis input matrix. RNA expression analysis of selected candidates was performed with the quantitative (q)PCR primers provided in Supplementary Table 2.

Chromatin Immunoprecipitation Assays

Chromatin was prepared from 1,000 to 1,400 control or βDKO islets and chromatin immunoprecipitation (ChIP) was conducted with Pdx1 (AB47383), Nkx2.2 (HPA003468; Sigma-Aldrich), or IgG antibodies as described previously (21) (n = 3). The chromatin was sheared to ~200–300 base pairs (bp). qPCR was performed with immunoprecipitated DNA over *Ins2* (i.e., −95/−35 bp) and *Nkx6.1* (−884/−720 bp). Binding enrichment is

presented as the fold enrichment of the transcription factor signal on *Ins2* or *Nkx6.1* over the control *β-actin* signal relative to IgG. Primer sequences are available in Supplementary Table 2.

Statistical Analysis

Statistical significance was determined using the two-tailed Student *t* test. Data are presented as the mean ± SEM. A threshold of $P < 0.05$ was used to declare significance.

Study Approval

All animal studies were reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee. Mice were housed and cared for according to the Vanderbilt Department of Animal Care and the Institutional Animal Care and Use Committee/Office of Animal Welfare Assurance standards and guidelines.

RESULTS

Embryonic Pancreas–Specific Removal of the Brg1 ATPase Results in Pancreatic Hypoplasia

To evaluate the mechanistic basis by which Swi/Snf controls pancreas mass in vivo, we crossed mice producing a Cre recombinase driven by the *Ptf1a* locus (*Ptf1a-Cre* [15]) with mice containing *Loxp* sites surrounding exons 17 and 18 of the *Brg1* gene (i.e., $Brg1^{\Delta panc}$ [17]) or constitutive *Brm*-null ($Brm^{-/-}$ [19]) alleles or in combination to create a developmental double knockout ($DKO^{\Delta panc}$). $Brm^{-/-}$ animals are viable, fertile, and have no overt physiological or morphological phenotypes (19). Brg1 removal was observed in ~50% of e12.5 pancreatic epithelial cells in $Brg1^{\Delta panc}$ and $DKO^{\Delta panc}$ mutants. Brg1 levels did not increase in $Brm^{-/-}$ embryos (Supplementary Fig. 1) and Brm did not in $Brg1^{\Delta panc}$ mutants (Supplementary Fig. 2), signifying no compensatory upregulation between these alternative ATPase Swi/Snf subunits.

At postnatal day 1 (P1), $Brg1^{\Delta panc}$ mice showed a severe reduction in pancreas size that was not observed in $Brm^{-/-}$ mice or exacerbated in $DKO^{\Delta panc}$ (Fig. 1*B*). However, the size of the spleen, liver, and kidneys was unaffected in the Swi/Snf ATPase mutants (Fig. 1*B*) (data not shown). The incomplete inactivation of *Brg1* within the MPC pool (Supplementary Fig. 1) led to the presence of nonrecombined escaper cells, with Brg1 absent from most P1 acinar cells (in which *Ptf1a* expression is enriched and thereby increasing the likelihood of inactivation of $Brg1^{f/f}$ alleles) but present in islet hormone$^+$ Ptf1a$^-$ cells (4) (Supplementary Fig. 3). The preservation of Brg1 in $DKO^{\Delta panc}$ islets presumably allows normal control of blood glucose levels postnatally, because β-cells totally deficient in all Swi/Snf activity have a significant impairment (described below). These observations suggest that the pancreatic hypoplasia reported in the 3-week-old $Brg1^{\Delta panc}$ mutants (26) results from a reduction in embryonic MPC numbers.

Pdx1 Binds to Brg1 and Brm1 in MPCs and Not Ptf1a or Sox9

The proximity ligation assay (PLA) was used to evaluate the ability of Pdx1, Ptf1a, and Sox9 to interact with Brg1 and Brm in the developing pancreas, wherein a punctate fluorescent signal is generated if the physical distance between interacting proteins is within 30–40 nm. Pdx1:Brg1 and Pdx1:Brm signals were clearly detectable in e12.5 pancreatic epithelium, but scant binding was found between Ptf1a and Sox9 with either Swi/Snf ATPase subunits (Fig. 2). These results imply that Swi/Snf actions in MPCs are mediated principally through Pdx1.

All Pancreatic Cell Lineages Are Reduced in e15.5 $Brg1^{\Delta panc}$ Mutants

To determine whether the absence of Pdx1-recruited Brg1/Swi/Snf impacted the loss of specific pancreatic cell lineages, we immunostained and quantitated serially cut control and mutant e15.5 sections for cell type markers characteristic of this developmental stage. Expression of Sox9 at e15.5 denotes a population of bipotent progenitor cells (27), and neurogenin 3 (Neurog3) marks the subset of cells destined to become islet endocrine cells (28). We found that the number of cells expressing Sox9 and Neurog3 was reduced by ~50% in $Brg1^{\Delta panc}$ pancreata, which was not decreased further in the $DKO^{\Delta panc}$ mutant (Fig. 3*A* and *B*). Moreover, the number of insulin$^+$, glucagon$^+$, and somatostatin$^+$ cells, along with carboxypeptidase 1$^+$ (Cpa1) acinar cells, was also reduced by ~50% in the $Brg1^{\Delta panc}$ and $DKO^{\Delta panc}$ mutants (Fig. 3*C* and *D*). In addition, ductal branching was less expansive in $Brg1^{\Delta panc}$ than in control embryos, which was expected due to reduced $Brg1^{\Delta panc}$ pancreatic cell numbers (Supplementary Fig. 4). Together, these results demonstrate that all pancreatic lineages are negatively influenced by the loss of the embryonic Pdx1:Brg1/Swi/Snf complex.

Brg1-Deficient MPCs Have Reduced Proliferative Capacity

We next investigated whether the reduction in pancreatic cell type formation directly resulted from depletion of the embryonic $Brg1^{\Delta panc}$ MPC pool. In agreement with this prediction, the number of coexpressing Pdx1 and Sox9 cells at e12.5 was reduced by ~40% (Fig. 4*A*). However, there was no detectable difference in TUNEL$^+$ cell numbers in the e12.5 control and $Brg1^{\Delta panc}$ E-cadherin$^+$ pancreatic epithelium (data not shown), suggesting that the decreased MPC population was not due to increased cell death. Pregnant dams were then injected with BrdU to assess the proliferation of Brg1-deficient MPCs. Control, $Brg1^{\Delta panc}$, and $DKO^{\Delta panc}$ mutants incorporated BrdU into ~30% of wild-type MPCs at e12.5, whereas incorporation in Brg1-deficient MPCs was only 14.4 ± 0.7% and 14.8 ± 1.8% in the $Brg1^{\Delta panc}$ and $DKO^{\Delta panc}$ mutants, respectively (Fig. 4*B*). These studies illustrate the novel function for Pdx1:Brg1/Swi/Snf in modulating MPC proliferation in the developing pancreas, which to our knowledge is the

first transcriptional coregulator shown to influence MPC number and pancreas mass.

Impaired Adult Islet β-Cell Function Is Only Observed Upon Removal of Both the Brg1 and Brm ATPase Subunits of Swi/Snf

To test whether Pdx1:Swi/Snf also contributed to adult β-cell function, we crossed transgenic mice containing a tamoxifen-inducible, β-cell–specific Cre (mouse *Ins1* enhancer/promoter [*MIP*]–driven Cre^{ERT} [16]) and the *Rosa26-Loxp-Stop-Loxp-tdTomato* ($R26^{LSL\text{-}tdTomato}$ [18]) lineage reporter with $Brg1^{f/f}$;$Brm^{+/-}$ (termed $Brg1^{\Delta\beta}$;$Brm^{+/-}$), $Brg1^{f/+}$;$Brm^{-/-}$ (i.e., $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$), or $Brg1^{f/f}$;$Brm^{-/-}$ (i.e., βDKO) mice. All experimental and control animals contained the $MIP\text{-}Cre^{ERT}$ transgene and received tamoxifen, because this Cre line contains the human growth hormone minigene sequence that has been reported to independently augment islet β-cell mass, insulin content, and insulin secretion (29). Brg1 removal was induced in 4-week-old mice by three tamoxifen administrations every other day over a 5-day period. At 2 weeks after the last tamoxifen treatment, ∼70% of islet β-cells expressed the fluorescent Tomato lineage reporter and >90% of these βDKO cells lacked Brg1 (Supplementary Fig. 5).

$Brg1^{\Delta\beta}$;$Brm^{+/-}$ and $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$ mice were physiologically normal 2 weeks after the last tamoxifen

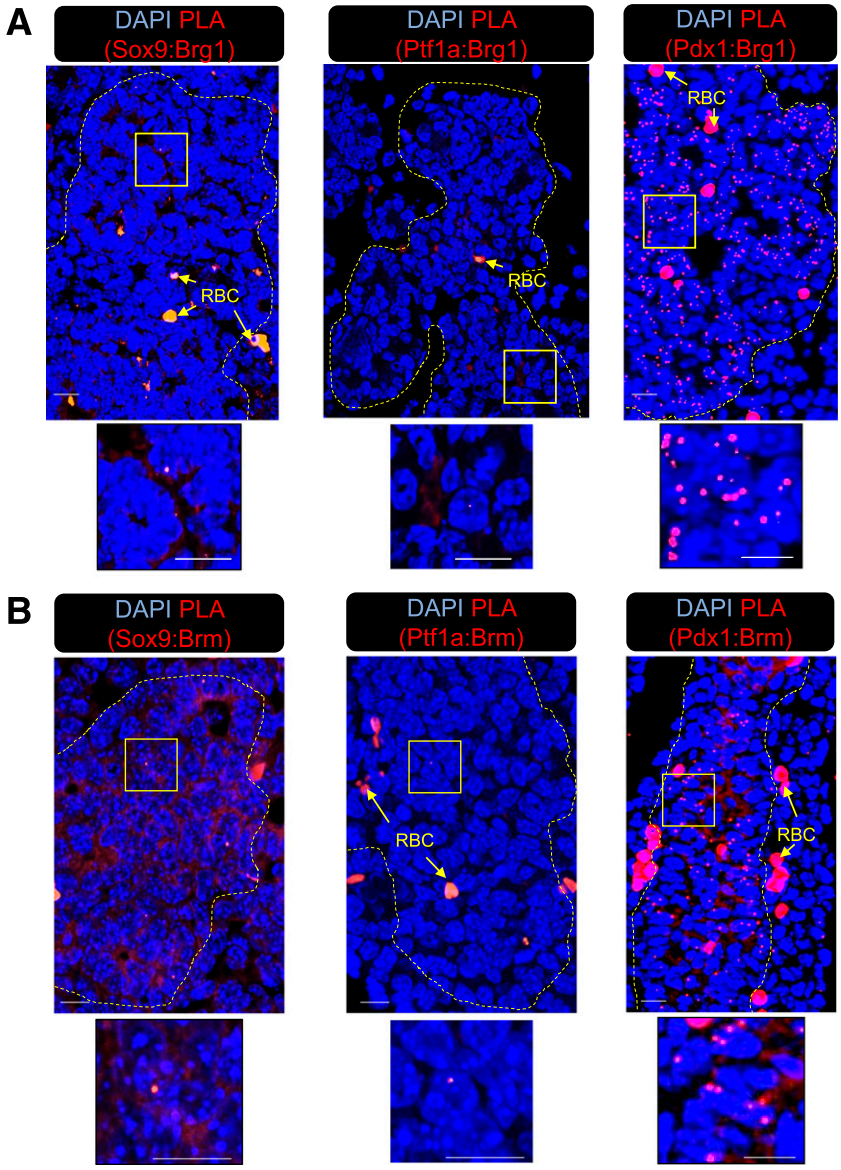

Figure 2—Pdx1, but not Sox9 or Ptf1a, interacts with Brg1 and Brm in wild-type e12.5 pancreatic epithelium. PLA was performed with antibodies specific for Brg1 (*A*) or Brm (*B*) and Sox9, Ptf1a, or Pdx1. Distinct fluorescence PLA signals were easily visible in the Pdx1:Brg1 and Pdx1:Brm experiments, but were nearly absent in the Brg1:Sox9, Brg1:Ptf1a, Brm:Sox9, and Brm:Ptf1a assays. The yellow square demarks the magnified area displayed below, and the dashed yellow marks outline the pancreatic epithelium. RBC, red blood cell. Scale bar = 10 μm.

administration, whereas βDKO animals suffered from fasting hyperglycemia, glucose intolerance, and reduced serum insulin levels (Fig. 5*A* and *B*). Glucose intolerance did not worsen in older mutant animals, and males and females both exhibited similar phenotypes (Supplementary Fig. 6). Glucose-stimulated insulin secretion (GSIS) was also compromised in size-matched islets isolated from βDKO animals, whereas $Brg1^{\Delta\beta}$;$Brm^{+/-}$ and $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$ were unaffected (Fig. 5*C*). In addition, we observed a severe and specific reduction in βDKO islet insulin content (Fig. 5*D*), suggesting that their secretion deficiency results, at least in part, from limited hormone content.

Loss of Swi/Snf Activity in βDKO Islet β-Cells Severely Reduces Insulin Production Despite Retention of Many Important Enriched Transcription Factors

To gain insight into the cause of the significant decrease in insulin content in βDKO islets, we first took a candidate approach to monitor for the presence of various β-cell markers in control and βDKO pancreata. Most strikingly, nearly every Tomato-labeled cell in βDKO islets had little to no insulin immunoreactivity (Fig. 6*A*). There was also no overt change in islet cell mass or apoptosis in mutant islets, contrasting with the reduced pancreas mass of $Brg1^{\Delta panc}$ mice caused by the developmental loss of this ATPase. As expected, Tomato-labeled $Brg1^{\Delta\beta}$;$Brm^{+/-}$ and $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$ β-cells had no obvious defects in insulin production (Supplementary Fig. 7).

Severe insulin deficiency is commonly observed upon β-cell ablation of key lineage-determining transcription factors such as Pdx1 (11), Nkx6.1 (30), Nkx2.2 (31), Pax6 (32), and Mnx1 (33), whereas removal of MafA impacts islet architecture and GSIS but has little effect on insulin content (34). Interestingly, Pdx1, Nkx6.1, Nkx2.2, Pax6, and Mnx1 levels were unaffected in Swi/Snf-deficient Tomato$^+$ cells by immunofluorescence analysis, although

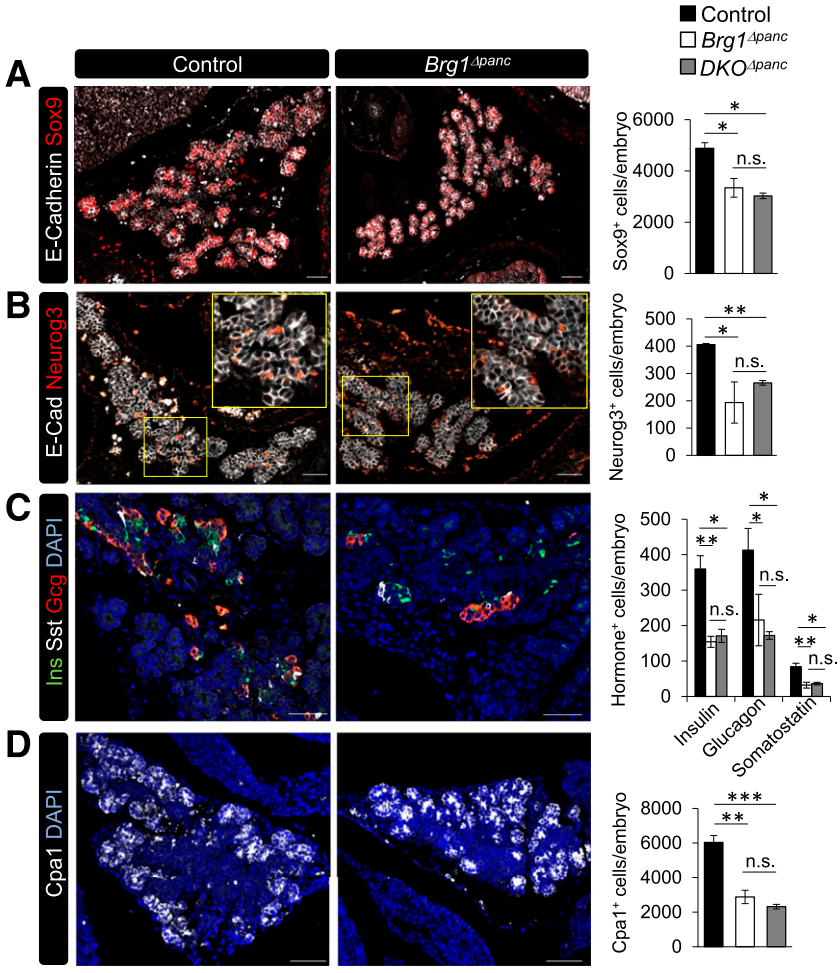

Figure 3—All pancreatic cell lineages are reduced in e15.5 $Brg1^{\Delta panc}$ and $DKO^{\Delta panc}$ epithelium. Control, $Brg1^{\Delta panc}$, and $DKO^{\Delta panc}$ mutant embryos were stained with antibodies specific for E-cadherin and Sox9 (*A*), E-cadherin and Neurog3 (*B*), insulin (Ins), somatostatin (Sst), and glucagon (Gcg) (*C*), or Cpa1 (*D*). The yellow square in *B* marks the magnified area in the panel. DAPI nuclear staining is also provided in *C* and *D*. Cell type counting was performed on sections 90-μm apart that spanned the entire pancreatic region ($n = 3$). *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. n.s., not significant. Scale bar = 50 μm.

MafA levels were significantly reduced (Fig. 6*B* and Supplementary Fig. 8). Moreover, βDKO Tomato$^+$ cells did not produce the α-cell–specific glucagon hormone, an effect seen upon removal of Pdx1 from adult β-cells (11). In addition, somatostatin expression was not induced in this cell population (Supplementary Fig. 8), which occurs upon deletion of, for example, Mnx1 from β-cells (33). Overall, there was also no difference in the islet α- and δ-cell mass between control and βDKO islets (Supplementary Fig. 8).

Expression of Pdx1-Regulated Genes Involved in Cell Maturation, Insulin Production, and Insulin Secretion Is Affected in βDKO β-Cells

To more comprehensively define the molecular influence Swi/Snf has on β-cells, RNA sequencing was performed on FACS Tomato$^+$ cells from control and βDKO islets. Using a ± twofold cutoff and false discovery rate of <0.05, there were 1,789 downregulated and 1,273 upregulated genes in βDKO β-cells (Fig. 7*A*). Gene ontology analysis (35,36) of these differentially expressed βDKO genes using Database for Annotation, Visualization and Integrated Discovery (DAVID) led to the identification of a very diverse array of biological pathways associated with Swi/Snf control, including cell adhesion, ion transport, cell differentiation, cell migration, cell proliferation, and carbohydrate metabolism (Supplementary Table 3). Expression of some of the most upregulated genes, including *Creg1*, *Scg3*, *Gucy1a3*, and *Gucy1b3*, was confirmed by qPCR analysis (Fig. 7*B*); however, the impact of these genes on β-cells is unclear.

Greater insight into how Pdx1:Swi/Snf regulated gene expression was obtained upon comparing genes bound by Pdx1 in ChIP-sequencing analysis of mouse islets (37) to those upregulated or downregulated in βDKO β-cells. Genes upregulated and bound by Pdx1 (507/1,273) resided in biological pathways linked to cell adhesion and neuromuscular functions, whereas downregulated βDKO genes bound by Pdx1 (917/1,789) defined functional networks linked to insulin secretion and glucose homeostasis (Fig.

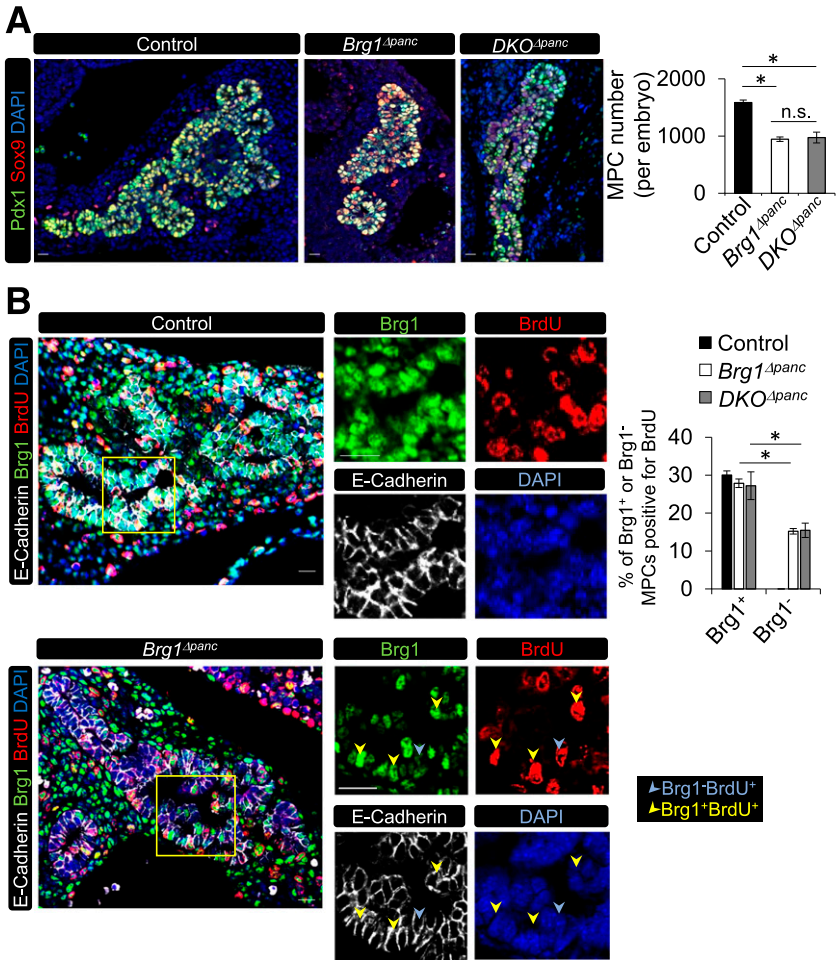

Figure 4—MPC proliferation is dependent on the Brg1 ATPase subunit of Swi/Snf. *A*: A representative immunostaining image of Sox9$^+$ and Pdx1$^+$ cells in control, *Brg1*$^{\Delta panc}$, and *DKO*$^{\Delta panc}$ pancreata at e12.5. Sox9$^+$Pdx1$^+$ MPC numbers were determined in sections obtained every 60 μm of the entire pancreatic region. *B*: E-cadherin, Brg1, and BrdU staining of pregnant dams injected with BrdU 30 min before the e12.5 embryos were harvested. Also provided is the percentage of Brg1$^+$ or Brg1$^-$ cells that incorporated BrdU ($n = 3$). The yellow square illustrates the magnified area displayed in the panels on the right. *$P < 0.05$. Scale bar = 20 μm. DAPI nuclear staining is shown in *A* and *B*.

8*A* and Supplementary Table 4). Consequently, we concluded that Pdx1:Swi/Snf represents an essential positive regulator of β-cell function. Supportive evidence of these changes came from immunofluorescence and qPCR analysis of independently derived flow-sorted βDKO β-cells. These genes included those strongly linked to mature cell identity, such as *Ins1*, *Ins2*, *Slc2a2* (Glut2), *MafA*, *Slc30A8*, and *Ucn3* (Fig. 8*B* and Supplementary Fig. 9). However, their production was unaffected in $Brg1^{\Delta\beta}$;$Brm^{+/-}$ and $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$ islets (Supplementary Figs. 7 and 10). Collectively, our analyses revealed that Swi/Snf-deficient β-cells have reduced expression of numerous Pdx1-regulated genes that are essential for sustaining β-cell identity.

Pdx1 Binding to the *Ins* Enhancer Is Compromised in βDKO β-Cells

Rodent *Ins1* and *Ins2* gene expression is largely mediated by transcription factors that bind within *cis*-acting enhancer sequences found roughly between −340 and −90 bp upstream of the transcription start site (38), a region well conserved in the human *INS* gene (39). In addition to Pdx1 binding at the A3/A4 (−201 to −195 bp in *Ins2*) and proximal A1 (−63 to −59 bp) elements (40), enhancer activity is mediated by several islet-enriched transcription factors with vital roles in developing and adult β-cells, including Pax6 (−317 to −311 bp) (41), Nkx2.2 (−128 to −122 bp) (42), and MafA (−126 to −101 bp) (43).

We compared Pdx1 binding to the *Ins2* and *Nkx6.1* enhancers in ChIP experiments performed on control and βDKO islets, noting that *Nkx6.1* was viewed as an internal control because expression was unaffected in βDKO islets (Fig. 8*B*). Nkx2.2 binding to both enhancers was also evaluated, with the Nkx2.2 and Pdx1 elements found roughly −807 to −796 bp upstream of the *Nkx6.1* transcription start site (44)). Pdx1 binding was selectively reduced on the *Ins2* gene in βDKO islets, whereas Nkx2.2 binding was unchanged, as was Pdx1 and Nkx2.2 to the *Nkx6.1* enhancer (Fig. 8*C*). These data reveal that the presence of the Brg1/Brm-associated Swi/Snf complex was necessary for Pdx1 binding to the *Ins* gene enhancer.

DISCUSSION

The binding of transcription factors to *cis*-acting DNA control elements is by itself not sufficient to regulate target gene expression (45). Accordingly, their associated coregulators serve as an essential layer of control by, for example, influencing chromatin structure, enabling interactions among transcription factors, and recruitment of other effectors of RNA polymerase II. Among the hundreds

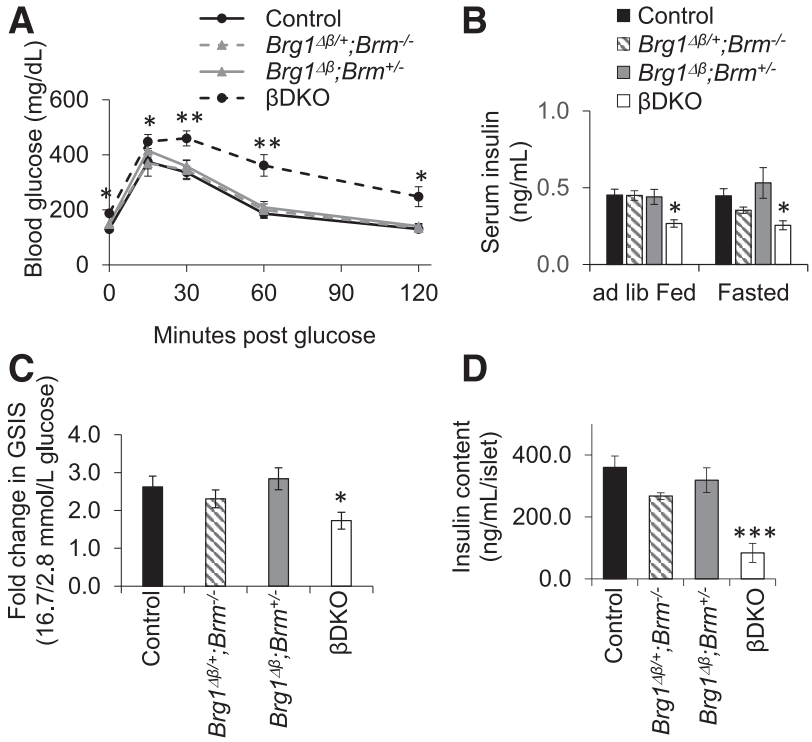

Figure 5—Severe defects in adult islet β-cell function are only produced in βDKO mice. Control, $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$, $Brg1^{\Delta\beta}$;$Brm^{+/-}$, and βDKO mice were subjected to testing 2 weeks after the last tamoxifen treatment. All statistical comparisons are to controls. *A*: Fasting blood glucose and glucose tolerance were specifically compromised in βDKO mice during the intraperitoneal glucose tolerance test. *B*: Serum insulin levels were also only reduced in ad libitum (ad lib) fed or fasted βDKO animals. n = 5–9. *$P < 0.05$; **$P < 0.01$. *C*: GSIS was reduced in βDKO islets and not in $Brg1^{\Delta\beta/+}$;$Brm^{-/-}$ or $Brg1^{\Delta\beta}$;$Brm^{+/-}$. n = 3–6. *D*: Islet insulin content was only decreased in the βDKO mutant (n = 4–8).

of positive- and negative-acting transcriptional coregulators, very few have been directly linked to β-cell–enriched transcription factor activity, despite strong evidence that Pdx1 (1,2,11), Nkx2.2 (31), Mnx1 (33), and Nkx6.1 (30) are essential to core programs of pancreas formation and β-cell activity. Here we evaluated the significance of Pdx1 recruitment of the Swi/Snf chromatin remodeling complex to pancreas formation developmentally and in islet β-cells. Conditional and constitutive mutants of the core ATPase subunits in mice were used to modulate Swi/Snf activity in vivo. Our results revealed that the Brg1 ATPase subunit regulates pancreas size by stimulating Pdx1:Swi/Snf-mediated MPC proliferation, while both Brg1 and Brm regulate expression of Pdx1-driven genes required for islet β-cell identity, including *Ins*.

Adult pancreas mass is limited by the size of the embryonic MPC pool (7). Here our data demonstrated that the inability of Pdx1 to recruit Brg1/Swi/Snf in $Brg1^{\Delta panc}$ mice reduced acinar, ductal, and islet cell numbers and pancreas mass by ∼50%, without affecting formation of other organs. Moreover, we showed that this resulted from reduced proliferation of the MPC pool. Notably, only Pdx1 was found in the PLA to bind to Brg1 and Brm in MPCs, whereas no interactions were observed with the MPC-enriched and functionally important Ptf1a (4) or Sox9 (5) transcription factors. This evidence indicated that Swi/Snf regulation of MPC expansion was principally through Pdx1.

Interestingly, pancreas mass was only affected in $Brg1^{\Delta panc}$ mice and not further in the double-ATPase $DKO^{\Delta panc}$ mutant. This may simply mean that the Brm ATPase, which has a much less impactful global regulatory phenotype in relation to Brg1 (19,46), has no influence on pancreatogenesis. This possibility is supported by a recent report that found *BRM* transcript levels increased (∼17-fold) during directed differentiation of human embryonic stem cells from stage 5 MPC-like cells to stage 6 β-like cells in vitro (Supplementary Table 5, data mined from

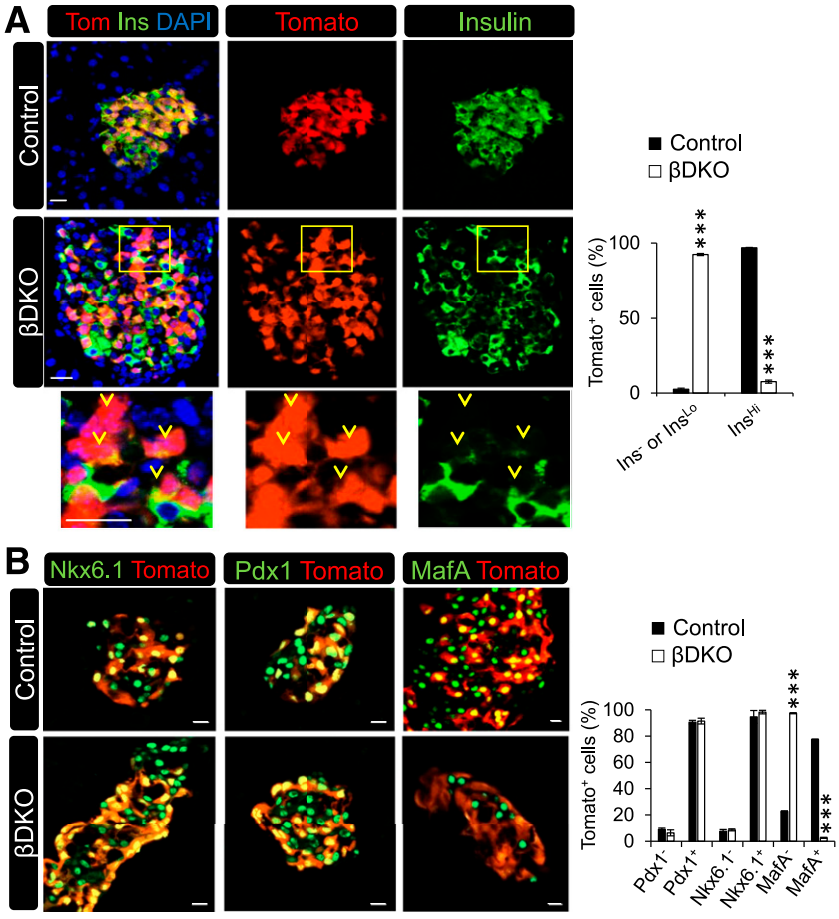

Figure 6—βDKO β-cells have very reduced insulin protein levels yet retain Nkx6.1 and Pdx1. *A*: Representative confocal images of lineage-labeled control and βDKO Tomato$^+$- and insulin$^+$-stained islet cells. Most Tomato$^+$ βDKO cells are insulin^{Lo} or insulin$^-$, as illustrated in the lower panels by the yellow arrowheads of the magnified squared field. The graph depicts the percentage of Tomato$^+$ insulin$^-$ or insulin$^+$ cells to the total number of Tomato$^+$ cells. *B*: Islet β-cell–enriched Pdx1 and Nkx6.1 are present in control and βDKO Tomato$^+$ cells, whereas MafA is absent. Scale bar = 20 μm. The graph illustrates the percentage of Tomato$^+$ transcription factor$^-$ or transcription factor$^+$ cells to the total number of Tomato$^+$ cells. ***$P < 0.001$.

reference [47]). Further, this increase in *BRM* provides a plausible explanation about why eliminating both Brg1 and Brm in adult islet βDKO β-cells had such a penetrant effect. Alternatively, Pdx1 may be recruiting a distinct Swi/Snf-related complex, termed PBAF (polybromo-associated BAF), that is only regulated by Brg1 (48). Although many subunits are shared between PBAF and the Brg1- and Brm-regulated BAF (Brg1-/Brm-associated factor) complex, each were shown to possess unique regulatory properties in controlling vitamin D receptor–driven anti-inflammatory and prosurvival responses in islet β-cells (49).

More broadly, our results suggest that coregulators of Pdx1, Ptf1a, and Sox9 influence pancreas size, a global physical determinant linked to type 1 diabetes and T2D susceptibility (9,50). Future efforts should involve not only identifying coregulators affecting transcription factor activity but also the processes and factors that influence their recruitment to target loci. These would be expected to include posttranslational modification mechanisms that positively or negatively affect transcription factor:coregulator interactions. For example, phosphorylation of the p53 transcription factor increases CBP/p300 histone acetyltransferase association, amplifying transcriptional activity (51).

T2D is ultimately caused by the inability of islet β-cells to produce sufficient amounts of insulin to cause transport of blood glucose into insulin-resistant tissues to maintain normoglycemia. A hallmark of this disease is an increase in the number of "empty" β-cells, defined by their lack of insulin immunoreactivity (52). This was also a novel characteristic of βDKO β-cells (Fig. 6) and associated with a lack of Pdx1 binding to the endogenous *Ins2* gene enhancer. These results are consistent with a recent report showing that DNA binding by the REST transcription factor relies on the remodeling activity of SWI/SNF in embryonic stem cells (53). In contrast to Pdx1, there was no apparent change in Nkx2.2 transcription factor binding to the *Ins2* gene or in Pdx1, Nkx2.2, and Nkx6.1 nuclear protein levels. In fact, MafA was the only other core *Ins* regulator apparently absent in βDKO β-cells (Fig. 7). Because loss of MafA alone does not abolish *Ins* production in $MafA^{\Delta panc}$ (34) or $MafA^{\Delta\beta}$ (54) mice, insulin deficiencies in βDKO "empty" β-cells likely reflect the combined loss of the MafA protein and inability of Pdx1 to bind to the *Ins* enhancer. This proposal is supported by the ability of Pdx1 + MafA to reprogram human islet α-cells to β-like cells (55), or in transgenic mice, when combined with the embryonic islet cell determination factor, Neurog3, to produce β-like cells in the intestine of mice (56).

Circularized Chromosome Conformation Capture (i.e., 4C) and PDX1-binding enhancer elements were used as anchor bait sites in the human EndoC-βH1 pancreatic β-cell line (57,58), and the human *INS* locus was found to physically contact many distinct genes affecting β-cell secretory processes. Knockdown of *INS* mRNA levels in EndoC-βH1 cells demonstrated that expression of 259 genes was affected, with 45 residing in 4C contact regions and ~40% associated with metabolic pathways. Moreover, these investigators proposed that the chromosomal interactions with the *INS* locus were conjoined with the transcriptional machinery at the various loci. If

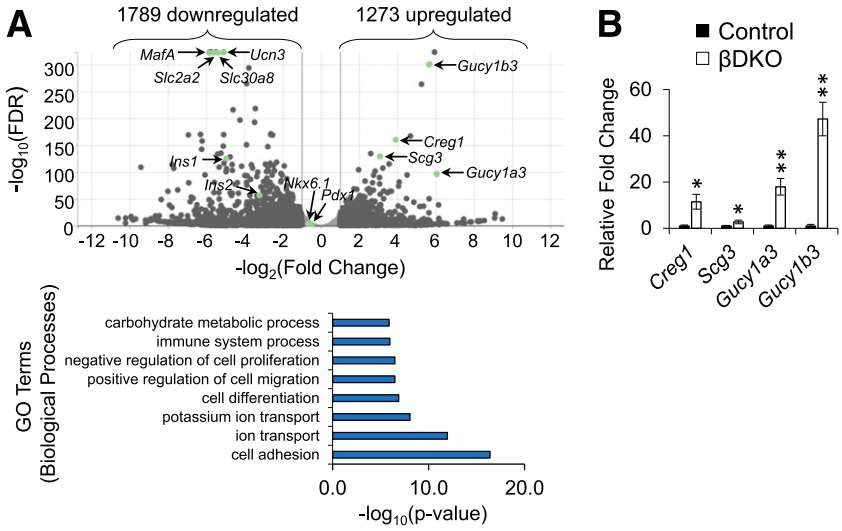

Figure 7—Swi/Snf affects a broad range of metabolic processes in islet β-cells. *A*: Top, volcano plot shows the most differentially expressed genes in βDKO β-cells. Bottom: The most significant biological pathways identified by gene ontology (GO) analysis. Swi/Snf contributed to very divergent regulatory processes, varying from cell adhesion (most significant) to ion transport, cell migration, and carbohydrate metabolism (least significant). Expression of Swi/Snf subunits BAF250A (*Arid1a*), BAF53B (*Actl6b*), and BAF60C (*Smarcd3*) was increased in βDKO β-cells by 2.11-, 2.08-, and 10.3-fold, respectively. FDR, false discovery rate. *B*: qPCR analysis from FACS-purified Tomato$^+$ β-cells of various βDKO genes upregulated in the RNA sequencing. $n = 3$. $^*P < 0.05$; $^{**}P < 0.01$.

PDX1:Swi/Snf contributed to such control, we predicted some overlap between the βDKO-regulated genes and those identified in the human 4C *INS* knockdown analysis. However, not one of their 45 genes was differentially expressed in βDKO β-cells. Possible explanations for this difference could involve the unique variable number of tandem repeats regions upstream of the human *INS* enhancer (39) and/or simply the experimental context (i.e., human EndoC-βH1 cells vs. the mouse βDKO model). Alternatively, our ability to detect Nkx2.2 binding to the *Ins2* enhancer in βDKO islets raises the possibility that 4C-detected interactions persist because they are regulated by transcription factors binding independently of Pdx1.

Genome-wide association studies have found numerous genomic loci linked to T2D (59), but the Swi/Snf subunits have not been associated directly to diabetes pathogenesis. Notably, our results suggest that only genetic variants leading to a complete loss of Swi/Snf activity would yield a βDKO diabetic phenotype. However, a recent study found that genomic deletions and rearrangements in Swi/Snf subunits exist in approximately one-third of pancreatic cancers containing alterations in known tumor-associated genes (e.g., *MYC*, *KRAS*, *CDKN2A*, *TGFBR2*, *MAP2K4*, and *SMAD4*) (60). Specifically in regards to the ATPase subunits, heterozygous and homozygous mutations in the *BRG1* or *BRM* genes occur in 9.6% and 2.6%, respectively, of human pancreatic cancer samples. Moreover, combined mutations in BRG1 and BRM were found in several pancreatic cancer cell lines, but double mutations were absent from primary tumor samples, although the latter involved a limited sample number (60). Notably, ~80% of individuals with pancreatic cancer often present with new-onset T2D or impaired glucose tolerance at diagnosis (61). Although these studies do not directly link the prevalence of Swi/Snf subunit mutations to pancreatic cancer–associated diabetes, loss-of-function mutations in BRG1 and BRM provide a potential intersection for pancreatic cancer and T2D, especially given the influence Swi/Snf has on mature β-cell function.

Functional heterogeneity within the β-cell population was first described more than two decades ago (62,63), with recent observations identifying distinct normal and T2D β-cell populations that differ in their molecular composition and glucose-stimulated insulin secretion properties (64). Interestingly, Pdx1, MafA, and Nkx6.1

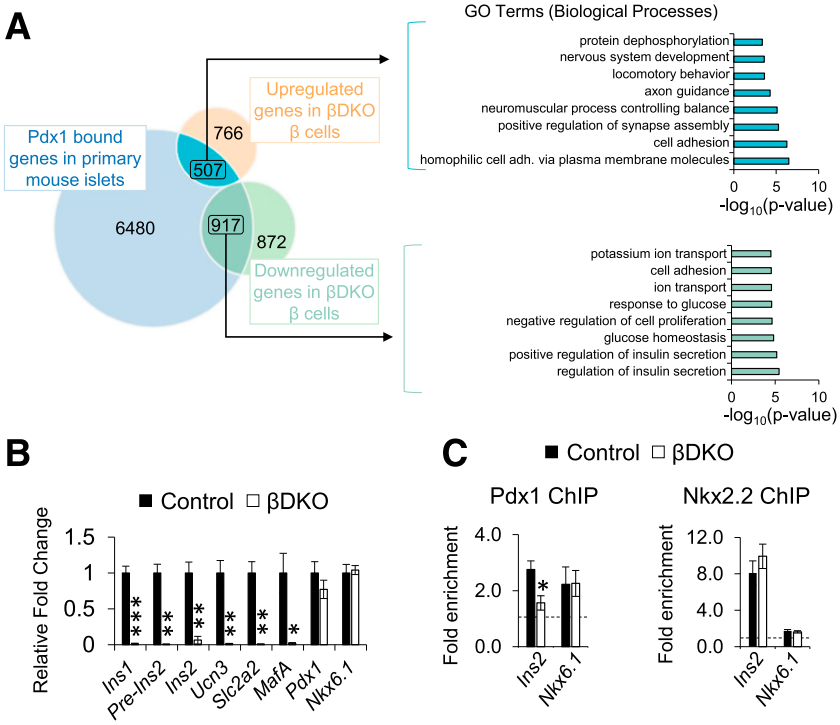

Figure 8—Pdx1:Swi/Snf controls genes essential to islet β-cell function. *A*: Left, Venn diagram of upregulated and downregulated βDKO genes overlaid with Pdx1-bound genes obtained from ChIP experimentation of primary mouse islets (37). Right: Most of the pathways identified by gene ontology (GO) analysis of the 507 genes that were Pdx1 bound and upregulated in βDKO β-cells were associated with cell adhesion (adh.) and neuromuscular functions, whereas the 917 genes that were Pdx1 bound and downregulated in βDKO β-cells were associated with β-cell function, such as glucose homeostasis and insulin secretion. *B*: Expression of a key subset of islet β-cell regulators identified in βDKO RNA sequencing and Pdx1 ChIP studies was confirmed upon qPCR of FACS-purified Tomato$^+$ β-cells. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. *C*: Pdx1 ChIP binding to the *Ins2* enhancer was decreased in βDKO compared with control islets, whereas there was no impact on binding to the *Nkx6.1* enhancer. Nkx2.2 binding to the *Ins2* and *Nkx6.1* enhancer was unchanged between βDKO and control islets. The dashed line represents IgG enrichment on the target gene after normalization to *β-actin*. $n = 3$. *$P < 0.05$.

levels appear similar between these β-cell subtypes, raising an intriguing possibility that variations in transcription factor:coregulator interactions could be affecting activity. This possibility is supported by our previous findings that Pdx1:Swi/Snf interactions were not observed in all healthy human islet β-cells in the PLA (12), which adds another level of heterogeneity to a subpopulation of human β-cells. Such observations emphasize the importance of investigating how coregulator recruitment by endocrine cell–enriched transcription factors contribute to human β-cell functional heterogeneity under normal physiological conditions.

Collectively, our study provides fundamental insight into the role of Pdx1:Swi/Snf complexes in pancreas organogenesis and in maintaining principal features of the mature β-cell state. The translational significance of our findings is underscored by the knowledge that in humans, a subset of T2D β-cells lose their ability to produce insulin and that overall pancreas size, with otherwise normal cell-type proportional allocations, is a risk factor in the development of diabetes. Furthermore, our findings that Swi/Snf activity is crucial for driving *Ins* expression and that Pdx1:Swi/Snf interactions are negatively affected in human T2D (12) indicate that therapies that enhance such interactions are an attractive target in T2D. Upon broader consideration, we propose that transcriptional coregulator recruitment is essential to the formation and function of the other islet cell types (α, β, δ, ε, and pancreatic polypeptide). In this context, our studies are currently focused on additional Pdx1-interacting proteins, such as the multisubunit nucleosome remodeler and deacetylase (NuRD) complex, the Myst2 histone acetyltransferase, and the Tif1β corepressor.

Acknowledgments. The authors thank Dr. Xin Tong and Dr. Emily Walker (both from Vanderbilt University) for critically reading the manuscript.

Funding. This work was supported by grants from the American Diabetes Association (1-16-PDF-109 to J.M.S.), National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (K01-DK-115633 to J.M.S. and R01-DK-050203 to R.S.), and National Cancer Institute (R01-CA-172045 to M.H.). The authors thank the Vanderbilt Flow Cytometry Shared Resource (National Cancer Institute P30-CA-68485), Vanderbilt Technologies for Advanced Genomics core (VANTAGE), supported in part by Clinical and Translational Science Awards (5UL1-RR-024975-03), the Vanderbilt Ingram Cancer Center (National Cancer Institute P30-CA-68485), the Vanderbilt Vision Center (National Eye Institute P30-EY-08126), and National Institutes of Health/National Center for Research Resources (G20-RR-030956), and the Vanderbilt Hormone Assay and Analytical Services Core (National Institute of Diabetes and Digestive and Kidney Diseases DK-020593) for core laboratory and technical assistance.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. J.M.S., J.-H.L., D.P., M.G., A.B.O., F.M., and N.R. designed, executed, and analyzed experiments. J.M.S. and R.S. wrote the manuscript. A.B., M.A.M., M.H., C.V.E.W., and R.S. designed and analyzed experiments. All authors reviewed the manuscript. R.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data and Resource Availability. Raw and analyzed RNA sequencing data sets have been deposited in GEO (accession number GSE128945). All noncommercially available resources generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Prior Presentation. Parts of this study were presented in abstract form at the 76th Scientific Sessions of the American Diabetes Association, New Orleans, LA, 10–14 June 2016, and at the 77th Scientific Sessions of the American Diabetes Association, San Diego, CA, 9–13 June 2017.

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