MicroRNAs (miRNAs) are important regulators of gene expression programs in the pancreas; however, little is known about the role of miRNA pathways during endocrine cell specification and maturation during neonatal life. In this study, we deleted Dicer1, an essential RNase for active miRNAs biogenesis, specifically from NGN3+ endocrine progenitor cells. We found that deletion of Dicer1 in endocrine progenitors did not affect the specification of hormone-expressing endocrine cells. However, the islets in the mutant mice in the neonatal period exhibited morphological defects in organization and loss of hormone expression, and the mutant mice subsequently developed diabetes. Dicer1-deficient β-cells lost insulin expression while maintaining the expression of β-cell transcription factors such as Pdx1 and Nkx6.1 early in the postnatal period. Surprisingly, transcriptional profiling showed that the Dicer1-deficient endocrine cells expressed neuronal genes before the onset of diabetes. The derepression of neuronal genes was associated with a loss in binding of the neuronal transcriptional repressor RE-1-silencing transcription factor to its targets in Dicer1-deficient β-cells. These studies suggest that miRNAs play a critical role in suppressing neuronal genes during the maturation of endocrine cells. *Diabetes* 62:1602–1611, 2013

**A potential therapeutic approach to replenish the pancreatic β-cell mass in diabetic patients involves the transplantation of functional, glucose-responsive β-cells differentiated from human pluripotent stem cells. Several attempts have been made at differentiating β-cells in vitro from stem cells, with limited success, (1,2) because the insulin-expressing cells generated lack the characteristic hallmarks of functionally mature β-cells, such as the ability to regulate glucose-stimulated insulin-secretion. Although many transcription factors and signaling pathways underlying the stepwise cell fate acquisition during β-cell development are known (3–6), a complete understanding of the molecular basis of β-cell specification and functional maturation is lacking. Of significant interest is the role of microRNAs (miRNAs) in regulating the pancreatic developmental program. miRNAs are non-protein-coding small RNAs (~19–25 nucleotides) that negatively regulate gene expression at the post-transcriptional level (7) and have been implicated as important regulators of animal development (8). Newly transcribed miRNAs undergo a series of processing steps that require the RNase III enzymes Drosha and Dicer1 before becoming functional (9,10). Although several miRNAs have been proposed to regulate β-cell transcription factors during development (11), many of these computationally predicted miRNA–mRNA interactions have not been experimentally validated in vivo. The dysregulation of miRNAs through Dicer1 ablation in the early embryonic pancreatic progenitor cells expressing Pdx-1 resulted in severe deficiencies in the formation of all islet cell lineages (12). More recently, it has been shown that deletion of Dicer1 in β-cells leads to loss of insulin expression and to development of diabetes in adult mice (13). Although these studies reveal key functions of miRNA-dependent pathways during early pancreatic development and in adult β-cells, they preclude analysis of the role of miRNAs during the specification of endocrine cells and their functional maturation in postnatal life.

In this study, we used a mouse model where expression of Cre recombinase directed by the Ngn3 promoter conditionally deleted floxed Dicer1 alleles in endocrine progenitor cells. In addition, by crossing these mice onto the R26RYFP reporter line, we were able to trace the lineage of the Dicer1-deficient islet progenitor cells. Our data demonstrate that Dicer1-deficient endocrine progenitors differentiate into hormone-expressing endocrine cells but subsequently lose hormone expression during the neonatal period and develop diabetes. More surprisingly, we found that the Dicer1-deficient islet cells expressed neuronal genes, supporting a model in which miRNA pathways control important transcriptional networks required for suppressing neuronal fate during the maintenance and maturation of newly specified endocrine cells.

**RESEARCH DESIGN AND METHODS**

**Mice and physiology.** Mice were maintained in a 12-h light/dark cycle under standard conditions. Studies involving mice were performed in accordance with National Institutes of Health policies on the use of laboratory animals and approved by the University of California, Los Angeles (UCLA) Animal Research Committee. The mice used in this study are the conditional *Dicer1* flox allele (14), the *Ngn3-Cre* (15), and the R26R-YFP (16) lines. The control mice used throughout were heterozygous for the conditional *Dicer1* allele and the Ngn3-Cre transgene (NC-Dicer1^flflo^). All mice were maintained in the C57BL6 background.

DNA extracted from tails was used for PCR-based genotyping. Blood glucose levels were measured from tail vein blood using a FreeStyle glucometer (Abbott Diabetes Care), and pancreatic insulin content was measured using a mouse insulin ELISA kit (Mercodia) after acid ethanol (0.18 mol/L HCl in 70% ethanol) extraction according to the protocol recommended by the Animal Models of Diabetes Complications Consortium (http://www.amdcc.org/).

**Histology and immunohistochemistry.** Pancreatic tissue was processed for immunohistochemical analyses as previously described (17). Briefly, the pancreas was dissected and fixed in 4% formaldehyde for 2 h before being embedded in paraffin. Sections (5-μm thick) were deparaffinized, rehydrated,
subjected to antigen retrieval using Antibody Unmasking Buffer (Vector Laboratories), and permeabilized in 4°C Triton X-100/TBS. Tissues were subsequently blocked with 3% IgG-free BSA (Jackson ImmunoResearch Laboratories). Incubation with primary antibodies was performed overnight at 4°C in blocking solution at the following dilutions: 1:200 guinea pig anti-insulin (Dako), 1:500 rabbit anti-glucagon (Immunostar), 1:200 rabbit anti-amyloid (Sigma-Aldrich), 1:100 mouse anti-Pdx1 (DSHB), 1:200 rabbit anti-Pdx1 (Chemicon), 1:250 chicken anti-green fluorescent protein (GFP; Aves Laboratories Inc.), 1:500 rabbit anti-MafA (18), 1:100 rabbit anti-MafB (Bethyl), 1:1000 rabbit anti-Glut2 (Millipore), 1:250 rabbit anti-tyrosine hydroxylase (Th; Millipore), 1:50 mouse anti-Nkx2.2 (BCBC), 1:50 mouse anti-Nkx6.1, and 1:50 mouse anti-Ki67 (BD Pharmingen). Secondary antibodies (Jackson ImmunoResearch Laboratory) conjugated to fluorescein isothiocyanate (1:200 dilution) or biotin (1:1000 dilution) were diluted in blocking solution. Chromatin immunoprecipitation (ChIP) and ChIP-qPCR were performed using Openlab software (Perkins Elmer) and a Leica DM6000 microscope.

**RESULTS**

Dicer1-null endocrine precursor cells specify appropriately but display loss of hormone expression during neonatal phase and subsequently develop diabetes. The catalytic role of Dicer1 is central to the functional maturation of miRNAs from their precursors (9).

To investigate in vivo the role of miRNAs during islet cell specification from endocrine precursor cells expressing Neurogenin3 (Ngn3), the Dicer1 conditional allele (Dicer1<sup>fl/fl</sup>) (14) was deleted specifically from endocrine precursor cells using NC-mediated excision (15) thereby preventing the generation of active miRNAs in these cells. In addition, an R26R-YFP reporter line (16) was crossed onto the NC-Dicer1<sup>fl/fl</sup> mice to heritably label any cells undergoing recombination and deletion of Dicer1. Co-immunostaining of NC-Dicer1<sup>fl/fl</sup> pancreatic sections for insulin/YFP (Fig. 1A–F) and glucagon/YFP (data not shown) showed a high degree of overlap of these markers in the mutant islets, therefore confirming that a high percentage of specified islet cells were derived from endocrine precursor cells that had undergone Cre-mediated recombination. Real-time qPCR analysis revealed that the Dicer1 transcript was reduced by 80% in the mutant NC-Dicer1<sup>fl/fl</sup> islets at 1 week (Fig. 1G), further confirming the deletion of Dicer1 from a majority of islet cells.

To determine whether loss of Dicer1 affected the specification of islet cells, pancreatic tissue isolated from control (heterozygous for the Dicer1 conditional allele NC:Dicer1<sup>fl/+</sup>) and mutant NC:Dicer1<sup>fl/fl</sup> mice were co-immunostained for insulin and glucagon at different stages of neonatal development. Immunostaining of pancreatea from e18.5 NC:Dicer1<sup>fl/fl</sup> mice for insulin and glucagon revealed that α- and β-cells specified appropriately in the pancreas, and no morphological differences in islet architecture were apparent (Fig. 1H and K). However, pancreatea from 1-week-old NC:Dicer1<sup>fl/fl</sup> mice exhibited altered islet organization, with many instances of α-cells prevalent within the core of the islet rather than at the periphery, an observation also confirmed by Pdx1 and glucagon staining at the same stage (Fig. 1F and L; Supplementary Fig. 1A and B). Pancreata from 2-week-old NC:Dicer1<sup>fl/fl</sup> mice displayed severe defects in morphology, and the expression of insulin and glucagon was severely diminished (Fig. 1J and M). Quantification at 1 day after birth revealed similar β- and α-cell mass (Fig. 1P and Q). A similar comparison showed a dramatic reduction in endocrine cell mass in the mutant NC:Dicer1<sup>fl/fl</sup> mice at 2 weeks after birth. Consistent with the reduction in β-cell mass by 2 weeks (Fig. 1P), an almost total loss of pancreatic insulin content was observed in the mutant NC: Dicer1<sup>fl/fl</sup> animals (shown at 3 weeks in Fig. 1O). However, only a very modest decrease in insulin content was noticeable in the mutant NC:Dicer1<sup>fl/fl</sup> animals at 1 week. The mutant NC:Dicer1<sup>fl/fl</sup> animals displayed an inability to metabolize glucose and developed hyperglycemia and frank diabetes within 2 weeks of birth (Fig. 1N), consistent with a nearly total loss of β-cells by that age. Taken together, our results suggest that Dicer1 in the endocrine progenitors was not required for specification of endocrine cells during embryogenesis but was required postnatally to maintain the expression of hormones and the maintenance of endocrine cell mass. These observations therefore underscore the key role miRNAs play during the neonatal period when endocrine cells became functionally mature and capable of maintaining blood glucose levels.
NEURONAL GENE EXPRESSION AND β-CELL MATURATION

1 week

A

B

C

G

Dicer1

\% YFP

\% YFP

NC:Dicer1

YFP

D

E

F

Ins Dapi

Ins YFP

Relative Dicer1 mRNA levels

0

1

1.2

Control

NC:Dicer1







Insulin Glucagon

H

I

J

Control

NC: Dicer1

\% YFP

e18.5

1 week

2 weeks

Blood glucose (mg/dl)

Non fasting

Control

NC:Dicer1







Pancreas insulin content (μg)

1 week

3 weeks

Control

NC:Dicer1







β-cell mass (μg)

Day 1

2 weeks

Control

NC:Dicer1







α-cell mass (μg)

Day 1

2 weeks

Control

NC:Dicer1








Endocrine cells from Dicer1-null mice maintain expression of characteristic transcription factors despite losing hormone expression. Next, we asked whether the loss of hormone expression in mutant NC:Dicer1fl/fl islet cells was due to any potential effect on key islet transcriptional regulators upon ablation of miRNAs. We tested this hypothesis by assessing the expression of characteristic β-cell transcription factors Pdx1, Nkx6.1, and MafA. Immunostaining of pancreata from 2-week-old control mice showed the expected coexpression of all these transcription factors with insulin (Fig. 2A–C and data not shown). In contrast, whereas the pancreata from mutant NC:Dicer1fl/fl littermates showed normal expression levels of Pdx1, Nkx6.1, and MafA in the islets, only a few of these cells were also positive for insulin, with the rest devoid of insulin expression (Fig. 2D–F). Similarly, 2-week-old mutant NC:Dicer1fl/fl pancreatic sections showed a number of cells that expressed MaB but not glucagon (Fig. 3A and D). Furthermore, real-time qPCR analyses of a set of key transcriptional regulatory genes that characterize endocrine transcriptional regulatory genes upon ablation of miRNAs.

The enrichment of genes involved in nervous system development, synaptic transmission, transcription of nerve impulse, and neurogenesis (Fig. 3D). The upregulated genes included pan-neuronal markers such as Seq10, Stmn3, and the neurofilament markers Nefl and Nefm, as well as molecular markers of noradrenergic neurons, such as the neurotransmitter-synthesizing enzymes Th and dopamine β-hydroxylase (Ddbh), the vesicular transport molecule–vesicle monoamine transporter 2 (Vmat2), and the plasma membrane transporter–norepinephrine transporter (Net). In fact, many components of the noradrenergic program were upregulated. A number of these neuronal genes are expressed to some extent early during endocrine pancreas development, but their expression declines significantly by 3 to 4 weeks of age (24). For example, real-time qPCR analysis indicated that the expression of neuropeptide Y (NPY), which is normally downregulated upon maturation, was expressed at high levels in the mutant NC:Dicer1fl/fl pancreata.

To test whether the increase in transcript levels of the neuronal genes was associated with a corresponding increase in protein levels, we immunostained 1-week control and NC:Dicer1fl/fl pancreatic sections for insulin and Th. Very few Th and insulin double-positive cells were observed in control sections (Fig. 4A–C). In contrast, the mutant NC:Dicer1fl/fl sections displayed a larger percentage of cells that coexpressed insulin and Th (Fig. 4D–F). Thus, the upregulation of mRNA of neuronal genes due to the absence of Dicer1 also results in a concomitant increase at the protein level. To assess whether the upregulation of Th was limited to β-cells, we costained Th with Pdx1. All Th-positive cells in the NC:Dicer1fl/fl islets also coexpressed Pdx1 (Fig. 4H–M), suggesting that β-cells upregulated the neuronal marker Th in the absence of Dicer1. To verify the endocrine lineage of these Th-positive cells, we performed immunostaining for the lineage trace marker YFP and Th in the mutant NC:Dicer1fl/fl pancreata. All of the Th-positive cells were also positive for YFP staining (Fig. 4G and J), therefore confirming that the Th-positive cells were derived from endocrine precursor cells that had undergone recombination and lost Dicer1 expression.

We also examined Th expression in the mutant NC:Dicer1fl/fl islets at 2 weeks. Consistent with observations in islets from 1-week-old control mice, costaining of Th with insulin (Fig. 4N–S) revealed that control islets from 2-week-old mice also typically expressed only a few Th-positive cells that also stained for insulin (Fig. 4P). In contrast, mutant NC:Dicer1fl/fl islets displayed a massive upregulation of Th staining (Fig. 4Q–S). A few insulin and Th-copositive islet cells were evident, but most of the Th-positive cells did not show any insulin staining, suggesting that neuronal gene upregulation persists in mutant islet cells that have already downregulated endocrine hormones. Taken together, our results suggest that miRNAs likely play an important role in suppressing a neuronal gene program during the maturation phase of endocrine islet cells.

FIG. 1. Mutant NC:Dicer1fl/fl islet cells specify normally but lose hormone expression and develop hyperglycemia during neonatal development. Immunostaining of 1-week-old Cre negative (A–C) and mutant NC:Dicer1fl/fl (D–F) pancreatic sections (n = 3) for insulin (red) (B and F), YFP that marks recombined cells (green) (A and D), and DAPI to visualize the nuclei (blue) (C and F). Most of the insulin-β-cells colocalize with YFP in the mutant islets but not in the control islets. G: Transcript levels of Dicer1 determined by real-time qPCR using RNA isolated from islets of control and mutant NC:Dicer1fl/fl mice (n = 3) at P7. Expression levels in control were set as one arbitrary unit. Representative pancreatic sections (n = 3 for each age and genotype) from c18.5 and 1- and 2-week-old control (H–J) and mutant NC:Dicer1fl/fl (K–M) littersmates were immunostained for insulin (green) and glucagon (red). N: Nonfasting blood glucose levels in neonatal control and mutant NC:Dicer1fl/fl mice at 1, 2, and 4 weeks (n = 5 for each age group). NC:Dicer1fl/fl mutant mice are hyperglycemic by 2 weeks. 0: Total pancreatic insulin content in control and NC:Dicer1fl/fl mice at 1 and 3 weeks reveals minimal pancreatic insulin content remaining in NC:Dicer1fl/fl mice at 3 weeks (n ≥ 3). P–Q: β- and α-cell mass in control and mutant NC:Dicer1fl/fl mice at P1 and 2 weeks was assessed as described in research design and methods (n = 4). The error bars represent the SEM. *P < 0.05, **P < 0.01, ***P < 0.005. In all cases, at least two to three pancreas sections were used for each animal.
genes showed that REST was bound to the RE1 sites of the four genes examined in FACS-purified control β-cells at P7. In contrast, REST binding to its target genes was significantly reduced in the mutant NC:Dicer1fl/fl β-cells (Fig. 5C), and a concomitant increase in mRNA expression of these genes was observed in the mutant NC:Dicer1fl/fl pancreata (Fig. 3E). This suggests that miRNAs play an important role in regulating the recruitment of the REST repressive complex, which in turn represses the transcription of its target neuronal genes. In addition to REST repression of the neuronal genes, we also identified other potential candidate transcription factors using the Interactome analysis, including the neuronal homeobox transcription factors Phox2a and Phox2b, which are important regulators of the noradrenergic phenotype in vertebrates (29–31). Real-time qPCR analysis indicated that both transcripts were significantly upregulated in the mutant NC:Dicer1fl/fl pancreata (Fig. 5D). Taken together, our results reveal an important role of miRNAs in regulating key transcriptional programs required for maintaining islet cell identity during neonatal development.

**Dicer1-null islet cells die by early adulthood.** To determine the ultimate fate of the Dicer1-null islet cells that had acquired an altered transcriptome, we immunostained the islets of control and NC:Dicer1fl/fl pancreatic sections for the islet hormone markers at 6 weeks. By 6 weeks, most insulin expression (Fig. 6A and B, Supplementary Fig. 3A and B) and other hormone expression in the islet was lost (data not shown). To determine whether the hormone-negative Dicer1-null islet cells survived into adulthood, immunostaining of control and NC:Dicer1fl/fl pancreatic sections with the lineage tracer marker YFP revealed that by 6 weeks, very few YFP-positive cells remained in the mutant NC:Dicer1fl/fl pancreatic sections compared with the littermates (Fig. 6C and D). Because the genetic ablation of Dicer1 is associated with apoptosis during development (14,32,33), we assessed the apoptosis rate in control and mutant NC:Dicer1fl/fl islets using TUNEL immunostaining. A significant increase (11.7-fold) in apoptosis was observed in mutant NC:Dicer1fl/fl β-cells at P12 (Fig. 6E–G) as well as other islet cells (data not shown), therefore suggesting that Dicer1 was ultimately responsible for the survival of mature islet cells.

**DISCUSSION**

Our study illustrates an essential role of Dicer1-dependent miRNA pathways in the suppression of a subset of neuronal markers and the maintenance of endocrine hormone expression during the maturation of newly specified islet cells in neonatal development. Surprisingly, we found that deletion of the miRNA-processing enzyme Dicer1 in islet progenitors cells (NC:Dicer1fl/fl mice) did not affect the specification of endocrine cells. However, these newly specified endocrine cells acquired an altered transcriptome in which neuronal genes were upregulated and endocrine hormone expression was lost, resulting in failure to regulate blood glucose and onset of diabetes by age 2 weeks. One possible explanation for this is that the loss of miRNAs early in endocrine development may alter later developmental pathways involved in the maturation of endocrine cells.

Neuronal and β-cells both share the expression of a large number of proteins, and many similarities exist in the transcription factors involved in their differentiation...
FIG. 3. Unchanged endocrine gene expression but increased neuronal gene expression in mutant NC:Dicer1^{fl/fl} islet cells at the presymptomatic stage (P7). A: Representative scatter plot of global gene expression obtained from microarray analysis of control and mutant NC:Dicer1^{fl/fl} pancreas at P7. A high similarity (R^2) in gene expression is observed between control and mutant NC:Dicer1^{fl/fl} samples. Red lines indicate a twofold difference in expression. B: The expression levels of many neuronal markers as identified by the Gene Ontology database are upregulated in the mutant NC:Dicer1^{fl/fl} samples. Key upregulated neuronal genes (red dots) are circled. The expression levels of most endocrine cell markers are unchanged (black dots). C: List of key neuronal genes (circled in B) upregulated in microarray ChIP analyses of mutant NC:Dicer1^{fl/fl} vs. control samples. D: Gene ontology analysis displaying biological processes associated with gene function of the differentially expressed genes with fold-change greater than two. The x-axis values are in logarithmic scale and correspond to raw binomial P values. E: Real-time qPCR validation of representative upregulated neuronal gene transcripts Nefm, Stmn2, Stmn3, Syn1, Npy, Th, and Dbh using RNA isolated from pancreata of control and mutant NC:Dicer1^{fl/fl} mice. Expression levels in control pancreata were set as one arbitrary unit. All data points represent means ± SEM of at least three biologically independent experiments. *P < 0.05, **P < 0.01, ***P < 0.005. (A high-quality color representation of this figure is available in the online issue.)
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FIG. 4. Expression of Th in mutant NC:Dicer1fl/fl pancreas. Pancreatic sections from 1-week-old control and mutant NC:Dicer1fl/fl littermates showing immunostaining for insulin (Ins; green) (A and D), Th (red) (B and E), and merged view (C and F) reveals sharp upregulation of Th expression in the mutant β-cells (n = 3). Immunostaining of 1-week-old control (G and H) and NC:Dicer1fl/fl mutant (J and K) pancreatic sections for Th (red)/YFP (green) and Th (red)/Pdx1 (green; n = 3). Most Th expression is limited to Pdx1-positive cells. I and L: Magnified inset of an area in H and K is shown. M: Quantification of Th-positive cells expressed as percentage of insulin-positive and Pdx1-positive cells in 1-week-old mice. Data points represent means ± SEM of at least three independent experiments. **P < 0.005. Immunostaining of 2-week-old control and mutant NC:Dicer1fl/fl littermates for insulin (Ins; green) (N and Q), Th (O and R), and merged view (P and S) indicates the presence of many insulin-negative β-cells that express Th (n = 3). In all cases, at least two to three pancreas sections were used for each animal.

program (34). The neuronal genes could be direct targets of miRNAs, such that Dicer1 ablation would result in their derepression in the mutant NC:Dicer1fl/fl islet cells. Alternatively, it is a more likely scenario where transcriptional regulators of the neuronal markers that are direct or indirect targets of a combination of miRNAs could be derepressed in the NC:Dicer1fl/fl islet cells, resulting in the derepression of neuronal transcripts. The decreased binding of the neuronal transcriptional repressor REST to target neuronal genes, and increased levels of the neuronal transcriptional activators Phox2a and Phox2b in mutant NC:Dicer1fl/fl β-cells, suggests that these miRNA-controlled transcriptional regulators are likely part of a broader transcriptional program that actively suppresses neuronal differentiation genes to maintain islet cell identity.

In vivo genome-wide mapping of REST binding sites by ChIPSeq analysis has previously identified the critical islet-cell development transcription factors neurogenic differentiation 1 (NEUROD1), hepatocyte nuclear factor (HNF) 4α, HNF6, Hes1, and Ngn3 as targets of REST (35). Interestingly, genes encoding these endocrine cell regulators were bound relatively poorly by REST compared with terminally differentiated neuronal genes such as Smmn2, Smmn3, and the neurofilament genes, which bound with greater affinity. This suggests that REST might not play a major role during islet cell differentiation during embryogenesis, but may instead control terminally differentiated neuronal genes that might be involved in maturation of endocrine cells. Dicer1 deletion results in deregulation of REST recruitment, resulting in the derepression of its target neuronal genes. The eventual loss of islet cells is not surprising given that the loss of Dicer1 expression is associated with massive cell death in a range of different tissues during development (14). Our results are supported by a recent study on the deletion of Dicer1 in β-cells using the transgenic RIP-Cre line, which showed that the neonatal pancreas contained ultrastucturally normal β-cells, although these mice developed progressive hyperglycemia and full-blown diabetes in adulthood (36). The derepression of neuronal genes in β-cells, however, was not explored.

Given the similarities in the neuronal and islet cell differentiation programs, our studies demonstrate a novel role of miRNA pathways in maintaining islet cell identity through the repression of neuronal markers while maintaining the expression of endocrine hormones. Identifying the individual miRNAs involved in the maturation and maintenance of newly specified islet cells, particularly β-cells, will aid in the development of therapeutic strategies to generate functionally mature replacement β-cells. These results also have important implications for engineering β-cells from embryonic stem cells.
FIG. 5. Loss of REST binding to neuronal targets in mutant NC: Dicer1^fl/fl β-cells. A: MetaCore Interactome analysis (GeneGo, Inc.) revealed that 16% of the dysregulated genes (more than twofold change) are targets of the neuronal transcriptional repressor REST. B: Real-time qPCR analysis of Ins1, Ngn3, and REST mRNA transcript levels in adult lung tissue and brain tissue isolated at e14.5, FACS-purified Ngn3-positive pancreatic endocrine progenitor cells at e14.5, and MIP-GFP β-cells at 2 weeks. C: ChIP analysis of FACS-purified control and mutant NC: Dicer1^fl/fl mice β-cells at P7. REST binding to the RE1 regions of Stmn2, Stmn3, Th, and Syn1 locus was reduced in mutant β-cells. ChIP data are reported as fold-change of REST binding at the RE1 locus of the respective genes relative to a negative control region and represent means ± SEM of at least three biologically independent experiments. D: Real-time qPCR analysis of REST, Pdx1, and Th mRNA transcript levels in isolated islets from pancreata of control and mutant NC: Dicer1^fl/fl mice at P7. E: Transcript levels of Phox2a and Phox2b as determined by real-time qPCR using RNA isolated from pancreata of control and mutant NC: Dicer1^fl/fl mice at P7. Expression levels in the control were set as one arbitrary unit. All data points represent means ± SEM of at least three biologically independent experiments. *P < 0.05, **P < 0.01, ***P < 0.005. (A high-quality color representation of this figure is available in the online issue.)
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M.K. designed and performed research, analyzed data, and wrote the manuscript. M.G.M. designed research and reviewed and edited the manuscript. A.B. designed research, analyzed data, and wrote the manuscript. A.B. 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.

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FIG. 6. Dicer1-null islet cells undergo apoptosis during neonatal period. A–D: Representative pancreatic sections (n=3) from 6-week-old control and mutant NC:Dicer1fl/fl mice co-immunostained for insulin (green)/amyrase (red) (A and B) and YFP (green)/DAPI (blue) (C and D). E and F: Immunostaining of pancreatic sections (n=3) from 12-day-old control and mutant NC:Dicer1fl/fl mice for insulin and TUNEL reveals an increase in TUNEL-positive apoptotic cells in the mutant mice. G: Quantification of TUNEL-positive cells expressed as percentage of β-cells in 12-day-old mice. Data points represent means ± SEM of at least three independent experiments. ***P<0.005. In all cases, at least two to three pancreas sections were used for each animal.
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