An Overfeeding-Induced Obesity Mouse Model Reveals Necessity for Sin3a in Postnatal Peak β-Cell Mass Acquisition

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Diabetes 2022;71:2395–2401 | https://doi.org/10.2337/db22-0306

The increase of functional β-cell mass is paramount to maintaining glucose homeostasis in the setting of systemic insulin resistance and/or augmented metabolic load. Understanding compensatory mechanisms that allow β-cell mass adaptation may allow for the discovery of therapeutically actionable control nodes. In this study, we report the rapid and robust β-cell hyperplasic effect in a mouse model of overfeeding-induced obesity (OIO) based on direct gastric caloric infusion. By performing RNA sequencing in islets isolated from OIO mice, we identified Sin3a as a novel transcriptional regulator of β-cell mass adaptation. β-Cell–specific Sin3a knockout animals showed profound diabetes due to defective acquisition of postnatal β-cell mass. These findings reveal a novel regulatory pathway in β-cell proliferation and validate OIO as a model for discovery of other mechanistic determinants of β-cell adaptation.

Pancreatic β-cell mass has the ability to adapt to an organism's metabolic environment. Acute (i.e., pregnancy) or chronic (i.e., obesity) perturbations lead to decreased insulin sensitivity and a need for greater insulin secretion. Glucose homeostasis is maintained by meeting this increased demand through expansion of functional β-cell mass. Failure of these compensatory processes is associated with altered glucose tolerance and diabetes.

Adaptive changes in β-cell mass mostly rely on β-cell self-replication (1). While the proliferative capacity of β-cells is generally considered low, probably because of the small percentage of proliferating β-cells observed in adult pancreas sections, ablation of this replicative capacity has clear consequences on glucose homeostasis (2). Rodent models used to study the physiology and molecular mechanisms underlying β-cell proliferation include high-fat diet (HFD) feeding, pregnancy, pancreatic duct ligation, partial pancreatectomy, or pharmacologic induction of insulin resistance (3–6). These experimental models elicit mild to moderate hyperplasic responses, and in some cases, the compensatory increase of β-cell mass requires a prolonged period. As such, the rate of β-cell mass increase observed in static measurements is relatively low, and elucidation of the factors responsible for β-cell mass adaptive responses may meet with variable success.

Here, we report a novel experimental model for β-cell proliferation studies. By using gastric intubation surgery and direct gastric caloric delivery, we generated a model of rapid weight gain, termed overfeeding-induced obesity (OIO). OIO mice show a rapid expansion of β-cell mass, highlighting the intrinsic ability of β-cells to replicate in order to acclimate to alterations in the metabolic environment. To test the capability of OIO as a platform for discovery of the β-cell–autonomous mechanisms involved in these adaptive changes, we performed transcriptional profiling of islets, which revealed a cell proliferation signature. Aside from known determinants of β-cell proliferation, we identified a novel transcriptional regulator of the observed adaptive response, Sin3a. To test whether Sin3a

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Received 1 April 2022 and accepted 30 July 2022

This article contains supplementary material online at https://doi.org/10.2337/figshare.20425617.

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affects β-cell proliferation, we generated β-cell–specific Sin3a knockout (β-Sin3aKO) mice, which proved unable to increase β-cell mass after birth, resulting in overt diabetes, validating the OIO screen.

RESEARCH DESIGN AND METHODS

Animals

Six-week-old C57BL/6J male mice were obtained from The Jackson Laboratory (strain no. 000664), individually housed upon arrival, and placed on a chow diet (no. 5053; Purina Mills). After acclimation to mouse facilities, intragastric surgery to place the feeding catheter was performed as previously described (7). After a 1–2 week recovery from surgery, the overfeeding protocol was started using Vanilla Ensure original powder (Abbott Laboratories) reconstituted in deionized water to obtain a 0.92 kcal/mL solution. Mice were infused with Ensure solution or saline for 14 h (dark cycle) from 2000 h to 1000 h. The infusion of calories started at 13 kcal/day and increased daily up to 18.5 kcal/day on day 8 of the infusion protocol. Both groups had access to chow diet, and food intake was monitored as previously described (7). *Sin3a*^{tm1Rdp} was generously provided by Ronald A. DePinho (University of Texas), and *Ins1*$^{tm1.1(cre)Thor}$ was obtained from The Jackson Laboratory. Glucose tolerance tests were performed after an overnight fast by intraperitoneal injection of glucose solution (2 g/kg body weight). Insulin was detected by ELISA (Mercodia). The Columbia University Institutional Animal Care and Use Committee approved all animal procedures.

Immunostaining and Antibodies

Pancreata were weighed and fixed in 4% paraformaldehyde. β-Cell mass determination and staining were performed as previously described (8). Immunostaining used antibodies against insulin (IR00261-2; Agilent-Dako), Ki67 (clone SP6, GTX16667; Genetex), Cadh1 (3195; Cell Signaling Technology [CST]), glucagon (2760; CST), phospho-H3 (Ser10) (9701; CST), phospho-H2A.X (Ser139) (2577; CST), and BrdU (ab6326; Abcam). TUNEL assay was performed with the In Situ Cell Death Detection Kit, Fluorescein (Roche Applied Sciences) according to the manufacturer's instructions, and pancreas sections were further stained with insulin and Ki67. For BrdU staining, pups were injected with 100 mg/kg BrdU (MilliporeSigma) 1 h before sacrifice, and staining was performed as previously described (8). Quantitative data from immunostaining on pups included males and females, while quantitative data on adults was gathered only in males. Images were taken with a Zeiss Axio Observer Z1 inverted microscope with LSM 710 scanning module.

RNA Sequencing, Differential Gene Expression, and Enrichment Analyses

Islets were isolated by collagenase P digestion (Roche Applied Sciences), Histopaque-1077 gradient (MilliporeSigma), and handpicking. Three samples (each with pooled islets from two mice) of control or 9-day overfed mice were used. Library construction and RNA sequencing were performed by the Columbia Genome Center. Raw counts were pseudoaligned to the mouse transcriptome (GRCm38) by kallisto and gene count tables generated. Differential gene expression analyses were performed using limma implemented in the BioJupies application (9). All data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) under accession number GSE199907. Gene set enrichment analysis (GSEA) to compare the different data sets was performed using the GSEAPreranked tool of GSEA software (https://gsea-msigdb.org); the ranked lists used and gene sets

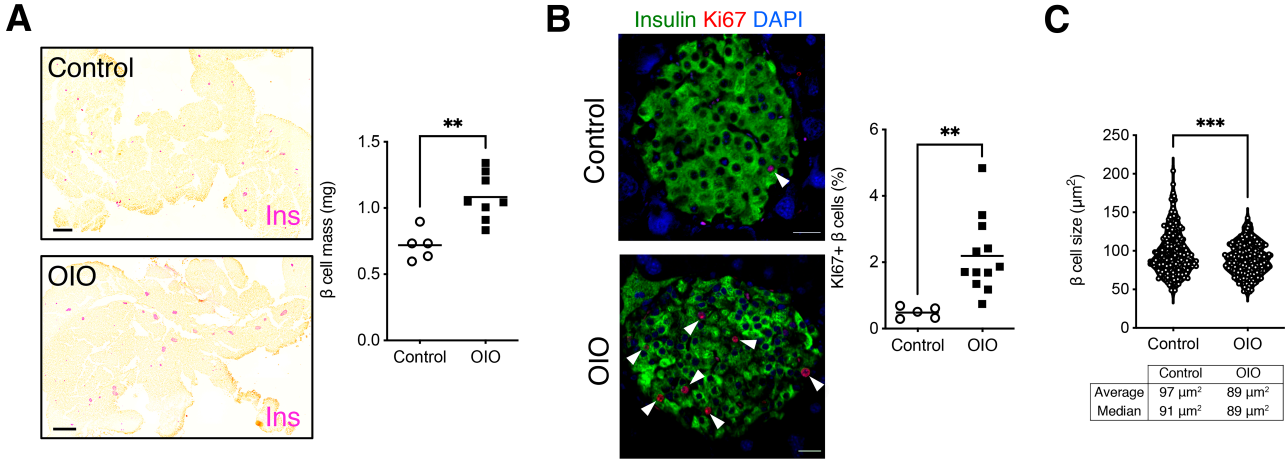

Figure 1—Overfeeding induces rapid β-cell mass acquisition. *A*: Representative insulin staining (red) in whole pancreata from saline-infused control and OIO mice and quantification of β-cell mass (n = 5–8 mice/group). Scale bars: 500 μm. *B*: Representative images from pancreatic sections of control and OIO mice stained with insulin (green), Ki67 (red), and DAPI (blue), with quantification of Ki67$^+$ β-cells (n = 5–11 mice/group). Scale bars: 20 μm. *C*: Quantification of individual β-cell size in OIO and control mice. Violin plots of all data points are shown; average and median cell size are provided in the text. Data are group means. $^{**}P < 0.01$, $^{***}P < 0.001$ by two-tailed t test.

described are listed in Supplementary Table 1. Raw data from other studies were processed using the same workflow, using the following data sets: HFD (NCBI GEO accession no. GSE78183, using data from C57BL/6J, 10 days HFD) (4), pregnancy (NCBI GEO accession no. GSE59141, pregnant females, day 9.5) (5), and S961-treated mice (NCBI GEO accession no. GSE137187, 2 day treatment) (10). Data from sorted proliferating β-cells were provided by the authors (11). Transcription factor enrichment analyses was performed using Enrichr (12); open source Cytoscape software (https://cytoscape.org/) was used to visualize the networks.

Data and Resource Availability

Transcriptomic data have been deposited in NCBI GEO (accession no. GSE199907). Other data are available upon request.

RESULTS AND DISCUSSION

To study mechanisms leading to β-cell hyperplasia, we surgically placed a gastric feeding tube for direct infusion of calories into the stomach, which enables overfeeding with the delivery of calories beyond what mice would eat if allowed to eat ad libitum, leading to rapid weight gain (7). Using this model of OIO for 2 weeks, we can recapitulate

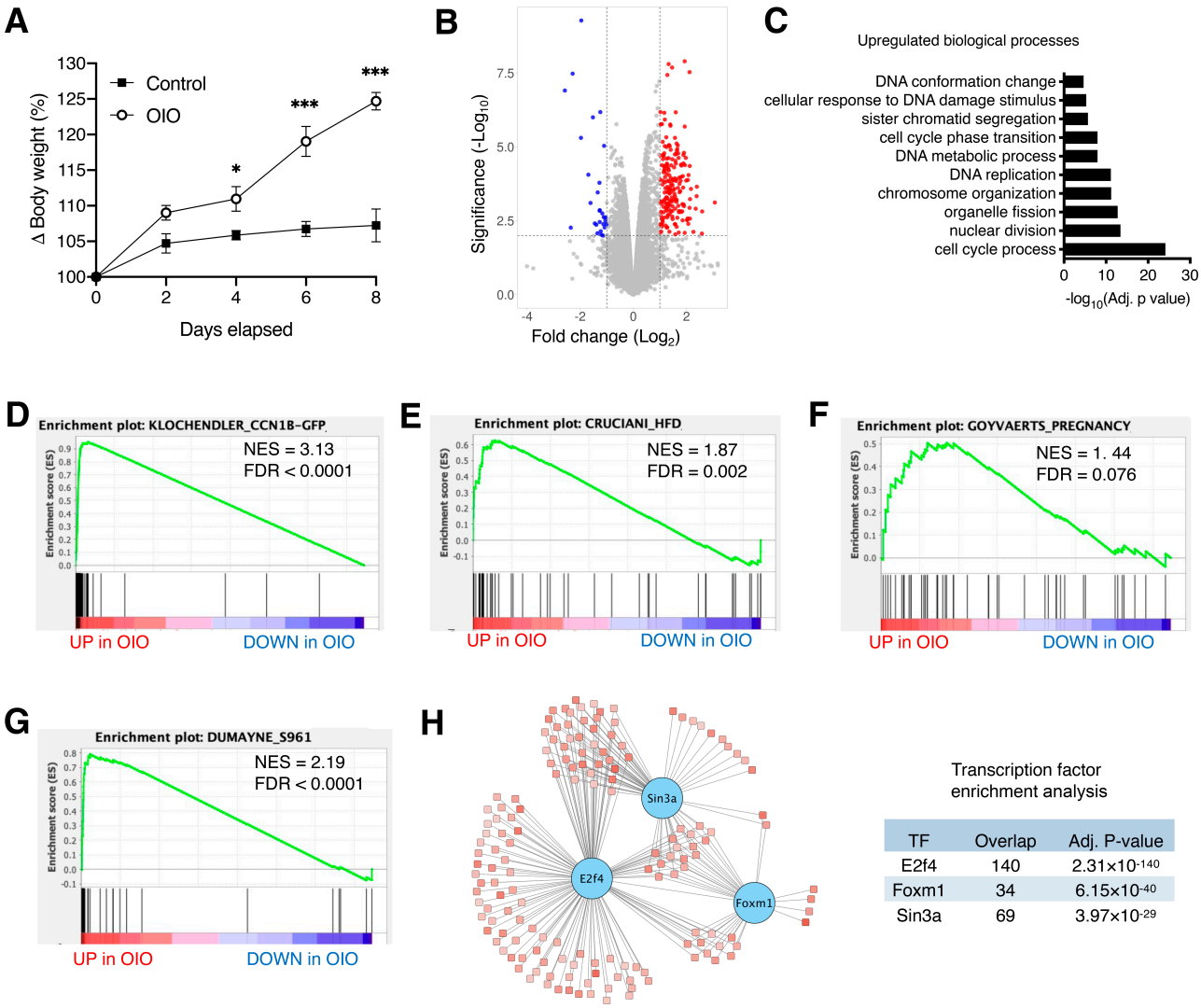

Figure 2—OIO induces profound changes in the islet transcriptome. *A*: Change in body weight from baseline in OIO and saline-infused control mice. Data are mean ± SEM. **P* < 0.05, ****P* < 0.001 by two-tailed *t* test. *B*: Volcano plot showing differentially expressed genes (DEGs) (adjusted [adj.] *P* < 0.05) in islets from 9-day OIO mice compared with saline-infused controls. *C*: Biological processes corresponding to upregulated DEGs in OIO islets, after gene ontology enrichment analysis using Enrichr. *D*–*G*: GSEA plot for upregulated DEGs in sorted proliferating β-cells (11) (*D*), isolated islets from HFD-fed mice (4) (*E*), pregnant mice (5) (*F*), or S961-treated mice (10) (*G*) compared with a ranked list of DEGs in OIO islets, with normalized enrichment score (NES) and false discovery rate (FDR) *q* value as indicated. *H*: Transcription factor (TF) enrichment of upregulated DEGs in OIO islets, showing that 158 of the top 250 upregulated DEGs are associated with E2f4, Foxm1, or Sin3a. Network shown and expanded in Supplementary Fig. 2. DOWN, downregulated; UP, upregulated.

the weight gain observed in mice fed a high-calorie diet in an additional model of ad libitum obesity (ALO) for 24 weeks (7). ALO results in increased β-cell mass, but surprisingly, we observed similar β-cell mass adaptation elicited by the 2-week OIO intervention (7). In fact, compared with saline-infused mice (controls), we found a 50% increase in β-cell mass in the OIO group (Fig. 1*A*) and a significantly increased percentage of $Ki67^+$ β-cells (Fig. 1*B*). Average β-cell size was slightly reduced in the OIO group (Fig. 1*C*), suggesting that hyperplasia and not hypertrophy accounted for increased β-cell mass.

Assuming that β-cell mass acquisition occurs linearly over time, parallel to changes in body weight (7), we hypothesized that the greater rate of β-cell mass acquisition in OIO mice may reveal key transcriptional changes below the detection threshold in experimental models that elicit a similar response but over a prolonged time. To test this potential, we assessed islet transcriptomics in a new cohort of OIO mice overfed for 9 days, a time point when mice are still accruing weight relative to saline-infused controls (Fig. 2*A*). We observed an upregulation of genes corresponding to cell proliferation in the OIO group, including DNA replication, chromosome segregation, and nuclear division, with a smaller number of downregulated genes (Fig. 2*B* and *C* and Supplementary Table 2). Next, we compared OIO islet transcriptomics with the signature of sorted proliferating β-cells (11) using GSEA, which confirmed that OIO islets contain the transcriptional profile of replicating β-cells (Fig. 2*D*). We next compared OIO islets with other published data sets of islets from models of β-cell adaptation, including HFD feeding (4), pregnancy (5), and treatment with an insulin receptor antagonist, S961 (10). Of these, OIO islets showed the greatest concordance with S961-treated mice (Fig. 2*E*–*G*). Cross comparison of these models of adaptation with the pure signature of proliferating β-cells (11) showed that OIO and S961 treatment elicited the strongest transcriptional signature of proliferation (Fig. 2*G* and Supplementary Fig. 1). Collectively, these comparative analyses suggest that OIO contains the transcriptional signature of β-cell adaptation and likely represents a far superior model to ALO to generate a fast and robust β-cell proliferative response.

We next searched the OIO islet transcriptomic profile for transcription factors associated with upregulated genes, using the chromatin immunoprecipitation sequencing data from the Encyclopedia of DNA Elements (ENCODE) project

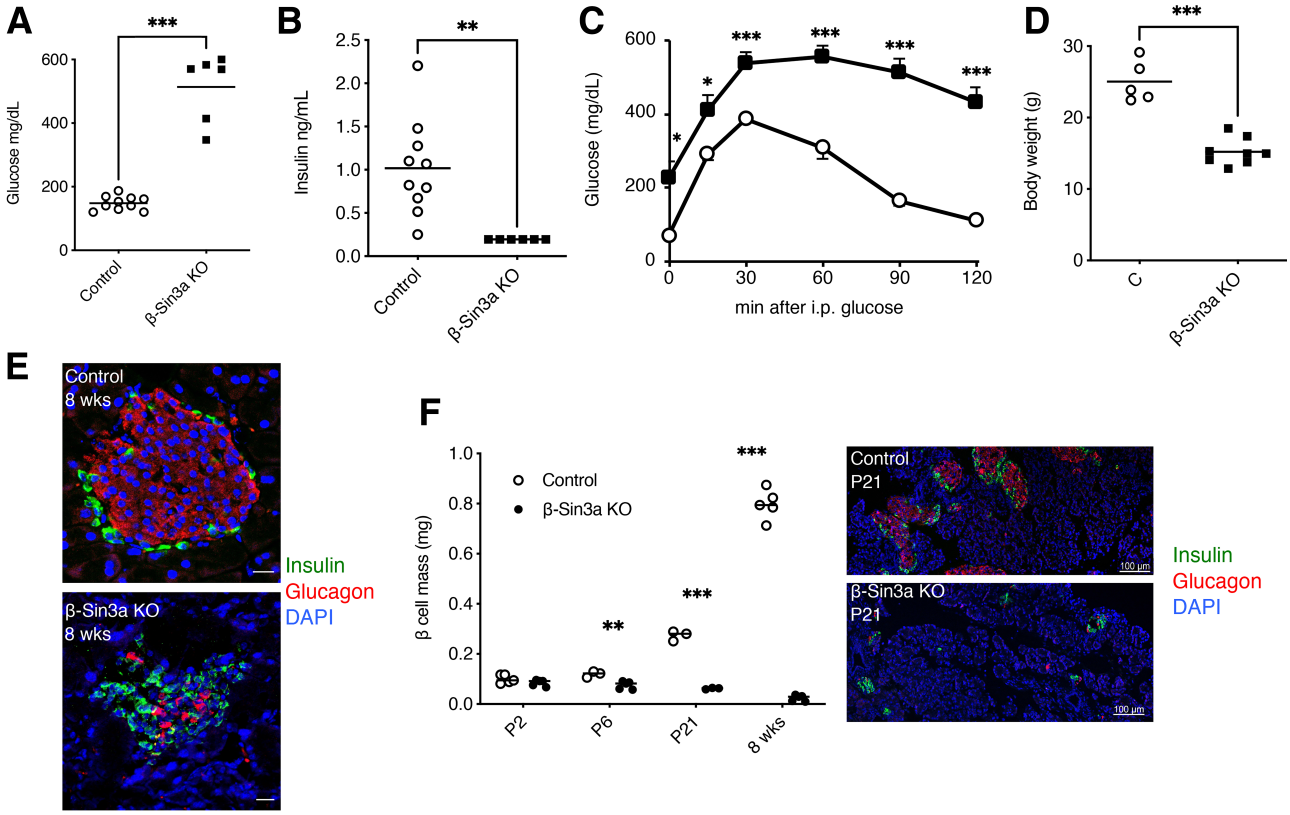

Figure 3—β-Sin3aKO mice develop diabetes and impaired β-cell mass expansion. *A* and *B*: Glucose (*A*) and insulin (*B*) levels in 8-week-old male β-Sin3aKO and control mice after 4 h of fasting. *C*: Glucose tolerance test in 8-week-old male β-Sin3aKO (■) and control mice (○). *D*: Body weight of 8-week-old male β-Sin3aKO and control mice. *E*: Representative images from pancreatic sections of 8-week-old male β-Sin3aKO and control mice stained with insulin (green), glucagon (red), and DAPI (blue). Scale bars: 20 μm. *F*: Quantification of β-cell mass at different postnatal stages in β-Sin3aKO and control mice (n = 3–5 mice/group). Data are group mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by two-tailed t test.

(13). The three most significantly enriched transcription factors were E2f4, Foxm1, and Sin3a, which were collectively associated with approximately two-thirds of the most differentially expressed upregulated genes (Fig. 2*H* and Supplementary Fig. 2). Transcriptional factor enrichment analysis with upregulated genes in sorted proliferating β-cells (11), or human β-cells treated with a proliferation-inducing drug cocktail (14), revealed these same three transcriptional factors (Supplementary Fig. 3). Foxm1 and E2f family genes have well-characterized roles in β-cell proliferation. Foxm1 determines expression of genes that regulate cell cycle progression and the mitotic machinery (15), and pancreas-specific deletion showed the importance of Foxm1 in postnatal β-cell mass expansion (16) and pregnancy (2). E2f4 is part of the E2f transcriptional factor family associated with transcriptional repression of cell cycle genes (17) and is one of three (with E2f1 and E2f6) expressed in β-cells (18). Whole-body KO of the transcriptional activator E2f1 results in impaired β-cell mass and function (19). The role of Sin3a is less well understood, although it is believed to act as a molecular scaffold that coordinates the binding of transcriptional factors and chromatin remodeling enzymes. Sin3a can act as a transcriptional activator or repressor and controls a wide array of biological processes, including cell cycle regulation and differentiation (20).

On the basis of these data, we hypothesized that Sin3a may have similar roles as Foxm1 and E2f family proteins

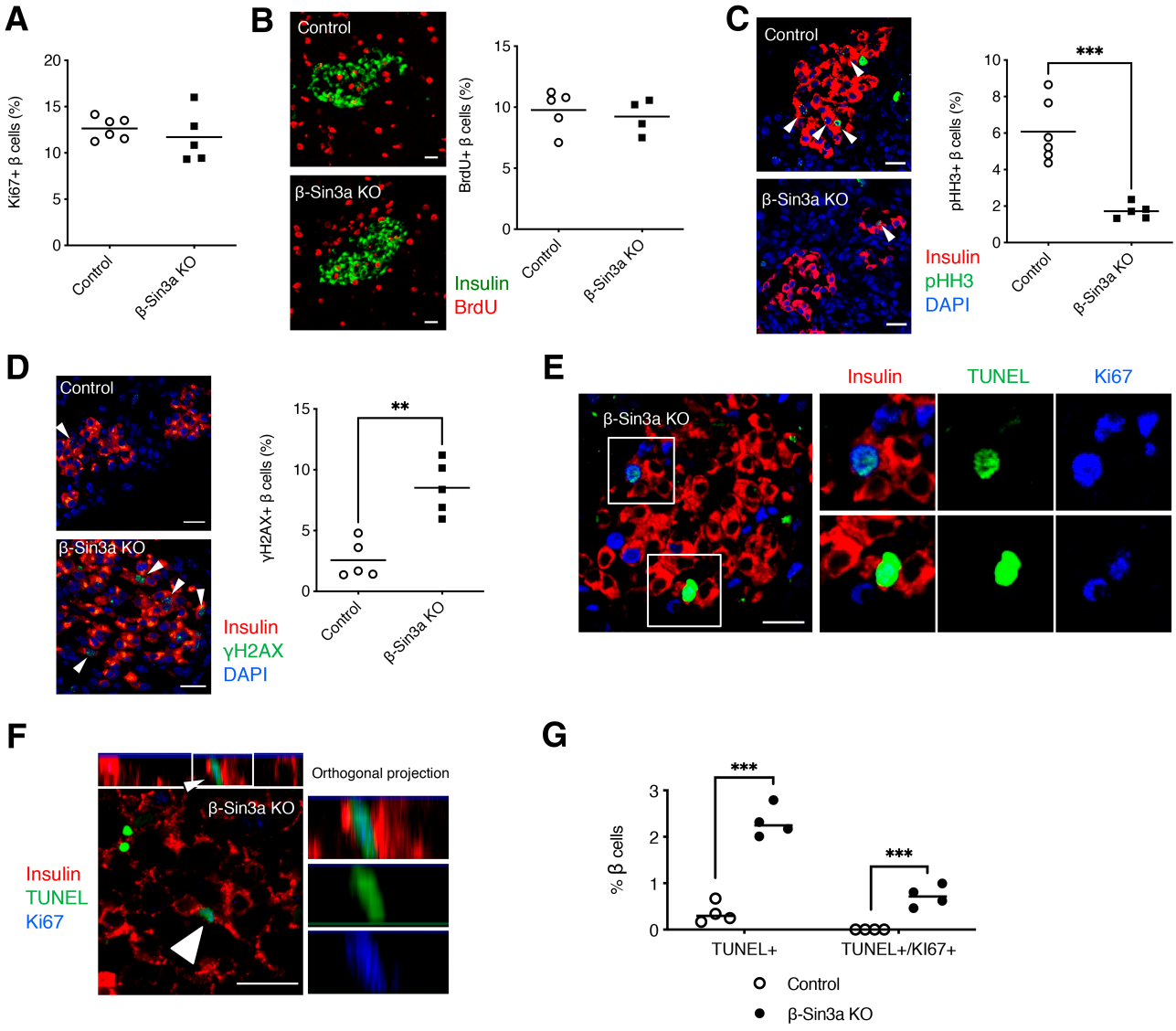

Figure 4—Impaired cell cycle progression and β-cell death in β-Sin3aKO mice. *A*: Quantification of Ki67$^+$ β-cells in pancreatic sections in P2 β-Sin3aKO and control mice (n = 5–6 mice/group). *B–D*: Representative images and quantification of BrdU$^+$ (*B*), phospho-histone H3$^+$ (pHH3) (*C*), and phospho-histone H2A.X$^+$ (γH2AX) (*D*) β-cells in pancreatic sections in P2 β-Sin3aKO and control mice (n = 4–5 mice/group). *E* and *F*: Representative images of TUNEL and Ki67 staining in pancreatic sections from β-Sin3aKO, with orthogonal projection shown in *F*. *G*: Quantification of TUNEL$^+$ and TUNEL$^+$/Ki67$^+$ β-cells in pancreatic sections from P2 β-Sin3aKO and control mice (n = 4 mice/group). Scale bars: 20 μm. Data are group means. $^{**}P < 0.01$, $^{***}P < 0.001$ by two-tailed t test.

in postnatal β-cell mass expansion. Deletion of Sin3a in pancreatic endocrine progenitors (*Ngn3-Cre*, $Sin3a^{\Delta endo}$) leads to diabetes development early in life, with β-cell functional defects and decreased survival, but it has shown no defects on differentiation or cell proliferation as assessed by Ki67 staining (21). To determine the postnatal role of Sin3a, we intercrossed Sin3a floxed and *Ins1-Cre* mice to generate mice lacking Sin3a in developed β-cells (β-Sin3aKO). Interestingly, 8-week-old β-Sin3aKO male mice showed marked glucose intolerance and, in fact, overt diabetes, with virtually no serum insulin detected (Fig. 3*A*–*C*). Hyperglycemia was associated with a significant reduction in body weight (Fig. 3*D*). Upon sacrifice, we observed very few β-cells in β-Sin3aKO islets (Fig. 3*E*). Because we surmised that this was the proximate cause of hyperglycemia, we next determined β-cell mass at different developmental stages. β-Cell mass was unchanged at postnatal day 2 (P2), but normal rapid postnatal β-cell mass acquisition was completely blocked in β-Sin3aKO mice (Fig. 3*F*). Female β-Sin3aKO mice showed similar β-cell deficits and glucose intolerance, albeit with more heterogeneity (Supplementary Fig. 4*A*–*C*).

Increases in β-cell mass are mostly due to proliferation (1), which in rodents, peaks during the first week of life and declines thereafter (22). At P2, we could not find differences in the number of $Ki67^+$ or $BrdU^+$ cells between control and β-Sin3aKO mice (Fig. 4*A* and *B*), consistent with previous reports on $Sin3a^{\Delta endo}$ (21). In these same sections, however, we observed aborted cell cycle progression from the G2 to M phase, as indicated by suppressed histone H3 phosphorylation in β-cells from β-Sin3aKO mice (Fig. 4*C*). H3 phosphorylation was not assessed in $Sin3a^{\Delta endo}$ mice, but these findings are in line with previous studies showing that Sin3a is essential for cell cycle progression of pluripotent stem cells or epithelial progenitors (23,24). Similar to these findings, incomplete cell cycle progression in β-cells was previously reported on human islets overexpressing HNF4A (25). β-Sin3aKO islets also showed increased β-cell γH2AX, a marker of DNA damage (Fig. 4*D*); $TUNEL^+$; and $TUNEL^+/Ki67^+$ double-labeled β-cells (Fig. 4*E*–*G*), a rare sight in tissues with a low proliferative rate and, to our knowledge, never previously described for β-cells in vivo, including in $Sin3a^{\Delta endo}$ mice, which showed similar β-cell defects despite unaltered expression of important factors for β-cell identity and proliferation, such as Pdx1 or Nkx6.1 (21). Consistent with this finding, we confirmed normal Pdx1 in β-Sin3aKO mice (Supplementary Fig. 4*D*).

We conclude that loss of Sin3a impairs cycle progression and effective replication in postnatal β-cells, culminating in severe defects in β-cell mass and overt diabetes. Gene ontology analysis of the Sin3a-associated genes from the OIO data set yields a picture completely focused on cell cycle processes (Supplementary Fig. 5), consistent with data from β-Sin3aKO mice showing that β-cells are insufficiently equipped to progress through the cell cycle. Our discovery of Sin3a stems from unbiased transcriptomics from an overfeeding-induced acute β-cell mass adaptation model. S961 treatment to induce acute hyperglycemia, a well-reported driver of β-cell expansion (26), has been proposed as a platform for profiling systemic factors that affect β-cell proliferation (27,28) but without success to date. We hypothesize that OIO, which leads to a similar β-cell expansion, drives a systemic response that is more reflective of physiological challenges, including the expansion of fat mass (7), a potential source of factors that may alter β-cell behavior. One of the limitations of the current study is that only male mice were used for the OIO paradigm, and sex-mediated differences remain to be determined. Sin3a is just one example of β-cell–autonomous factors, but we postulate that the OIO model may help to decipher other regulators of physiology of β-cell compensatory mechanisms.

Acknowledgments. The authors thank Ana Flete, Thomas Kolar, and Jiani Liang (Columbia University Irving Medical Center) for excellent technical support as well as members of the Pajvani and Ferrante laboratories for insightful discussion.

Funding. This work was supported by National Institutes of Health grants R01DK103818 and R01DK132661 (U.B.P.), a Russell Berrie Foundation award (A.B.), and American Diabetes Association grant 1-17-PMF-025 (A.B.).

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

Author Contributions. A.B. conducted the experiments and contributed to the experimental design, discussion, and writing of the manuscript. Y.R. and J.Y. conducted experiments and contributed to the review and editing of the manuscript. A.W.F. and U.B.P. oversaw the research and contributed to the experimental design, discussion, and writing of the manuscript. All authors reviewed and approved the final manuscript. U.B.P. 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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