The Transcription Factor YY1 Is Essential for Normal DNA Repair and Cell Cycle in Human and Mouse β-Cells

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Diabetes 2022;71:1694–1705 | https://doi.org/10.2337/db21-0908

Identifying the mechanisms behind the β-cell adaptation to failure is important to develop strategies to manage type 2 diabetes (T2D). Using *db/db* mice at early stages of the disease process, we took advantage of unbiased RNA sequencing to identify genes/pathways regulated by insulin resistance in β-cells. We demonstrate herein that islets from 4-week-old nonobese and nondiabetic leptin receptor–deficient *db/db* mice exhibited downregulation of several genes involved in cell cycle regulation and DNA repair. We identified the transcription factor Yin Yang 1 (YY1) as a common gene between both pathways. The expression of YY1 and its targeted genes was decreased in the *db/db* islets. We confirmed the reduction in YY1 expression in β-cells from diabetic *db/db* mice, mice fed a high-fat diet (HFD), and individuals with T2D. Chromatin immunoprecipitation sequencing profiling in EndoC-βH1 cells, a human pancreatic β-cell line, indicated that YY1 binding regions regulate cell cycle control and DNA damage recognition and repair. We then generated mouse models with constitutive and inducible YY1 deficiency in β-cells. YY1-deficient mice developed diabetes early in life due to β-cell loss. β-Cells from these mice exhibited higher DNA damage, cell cycle arrest, and cell death as well as decreased maturation markers. Tamoxifen-induced YY1 deficiency in mature β-cells impaired β-cell function and induced DNA damage. In summary, we identified YY1 as a critical factor for β-cell DNA repair and cell cycle progression.

Pancreatic β-cells are responsible for maintaining glucose homeostasis by secreting insulin. β-Cells adapt to insulin resistance (IR) by increasing their number and hormone secretion function. When β-cells fail to adapt to IR, type 2 diabetes (T2D) occurs. Several distinct yet disconnected pathways lie at the heart of β-cell failure during the disease development; however, the specific cues and factors mediating this process remain poorly understood. For instance, the overwhelming increase in insulin secretion demand enhanced by IR affects the unfolding capacities of the endoplasmic reticulum (ER) (1). Several studies have suggested that the cellular response of pancreatic β-cells to ER stress through the activation of the unfolded protein response impairs glucose-stimulated insulin secretion (2,3). Moreover, fuel load surfeit compromises the mitochondrial oxidative phosphorylation capacity, disturbing ATP production and glucose oxidation (4) and leading to impaired insulin secretion, oxidative stress (5), and DNA damage (6).

A growing body of data supports a new model of β-cell dysfunction where DNA damage response triggers β-cell dedifferentiation and death (7–11). Patients treated with whole-body or abdominal radiation have an increased incidence of diabetes, potentially due to radiation-induced DNA damage in islets (7). Human and mouse β-cells from subjects with T2D have DNA breaks and oxidized DNA (8–10), indicating defects in DNA repair mechanisms or chronic DNA damage accumulation. Mice deficient in nonhomologous end-joining DNA repair mechanisms exhibit β-cell dysfunction, including senescence like-phenotype and T2D (11).

The zinc-finger transcription factor Yin Yang 1 (YY1) is important in many biological processes, such as development, apoptosis, metabolism, and growth (12). YY1 acts as repressor when associated with the polycomb repressor

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Received 12 October 2021 and accepted 21 April 2022

This article contains supplementary material online at https://doi.org/10.2337/figshare.19723336.

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See accompanying article, p. 1614.

complex (13) or as activator when associated with the INO80 complex (14). YY1 has also been implicated in the regulation of cell cycle, DNA damage response, and DNA repair (15–18). YY1 plays a role in several tissues, but its role in pancreatic β-cell function is not completely known (19–22). In β-cells, YY1 mutation was found to be prevalent in insulinomas (23,24), being responsible for the enhanced insulin secretion by the tumor (25). Mice with YY1 deletion in β-cells were recently described to have altered mitochondrial structure and function, impairing β-cell survival and insulin secretion (26).

We identified YY1 as a critical factor for β-cell DNA repair and cell cycle. RNA sequencing (RNA-seq) of islets from 4-week-old *db/db* mice shows a remarkable downregulation of DNA repair and cell cycle regulation gene pathways. Mechanistically, YY1 and its targeted genes are the key genes suppressed in these two pathways. In addition, YY1 is also reduced in diabetic human and mouse β-cells. Chromatin immunoprecipitation (ChIP) sequencing (ChIP-seq) profiling in EndoC-βH1 cells, a human pancreatic β-cell line, indicated that YY1 binding regions regulate cell cycle control and DNA damage recognition and repair. Conditional genetic mouse models of YY1 deficiency in β-cells confirmed that YY1 is central to β-cell mass maintenance and function.

RESEARCH DESIGN AND METHODS

Animals and Cell Line

Procedures were approved by University of Miami Institutional Animal Care and Use Committee (protocol no. 18-168-LF). Rip*β-YY1KO* and Ins1*β-YY1KO* mice were generated by crossing the *floxed-yy1* mouse with the rat insulin promoter (RIP)-cre mouse (B6.Cg-Tg(Ins2-cre)25Mgn/J; JAX stock no. 003573) and Insulin 1 (Ins1)-cre mouse (B6(Cg)-Ins1tm1.1(cre)Thor/J; JAX stock no. 026801), respectively. Males and females were used in experiments using Rip*β-YY1KO* and Ins1*β-YY1KO* mice. Male *iβ-YY1KO* mice (8 weeks old) were generated by crossing the $yy1^{f/f}$ mice with animals expressing the inducible *MIP1-*Cre^{ER} driver (B6.Cg-Tg(Ins1-Cre/ERT)1Lphi/J; JAX stock no. 024709), followed by three subcutaneous injections of tamoxifen (5 mg per animal) or corn oil (control) every other day. Male *db/db* mice (BKS.Cg-*Dock7m*$^{+/+}$ Leprdb/J; JAX stock no. 000642) were sacrificed at 4 weeks and 3 months. *Dock7m* +/*Dock7m* + mice from the same colony were used as the control. At this age, *db/db* mice exhibit elevated plasma insulin, and some animals already exhibit elevated blood glucose (between 4 and 8 weeks). Two-month-old male C57Bl/6 mice (C57BL/6J; JAX stock no. 000664) were fed a chow diet (control) or a high-fat diet (HFD) for 12 weeks. The controls for Rip*β-YY1KO*, Ins1*β-YY1KO*, and *iβ-YY1KO* mice were, respectively, Ins-cre, Rip-cre $yy1^{f/+}$ and Mip-cre + tamoxifen and Mip-cre $YY1^{f/f}$ + vehicle. The EndoC-βH1 human β-cell line was cultured in RPMI 1640 culture medium with 2 mmol/L L-glutamine supplemented with 10% FBS (v/v) and 100 units/mL penicillin and 0.1 g/L streptomycin antibiotics.

ChIP and Library Preparation

EndoC-βH1 cells were treated with 1% paraformaldehyde at room temperature for 10 min for chromatin cross-linking. Then, 0.125 mol/L glycine was added to quench the cross-linking reaction. Cells were then sonicated in SDS lysis buffer with protease inhibitors to shear the chromatin. Chromatin was precleared and incubated with 5 mg of anti-YY1 overnight at 4°C with rotation. After immunoprecipitation, the chromatin was harvested and the cross-links were reversed. Samples were treated with 0.2 mg/mL RNAse A, and the DNA was purified and precipitated. The resulting DNA was quantified and served as a template for library construction. Sequencing libraries were prepared from 8 ng total DNA using Accel-NGS 2S Plus DNA Library Kits according to the manufacturer's protocol. Briefly, after dephosphorylation and end repair, samples were then ligated to unique adapters and PCR amplified. Libraries were then validated using the 2100 BioAnalyzer (Agilent), normalized, and pooled for sequencing at the University of California, San Francisco Institute for Human Genetics core service.

RNA-Seq Library Preparation

The *db/db* RNA-seq library was prepared as described previously (27). Total RNA was isolated using Direct-zol RNA Microprep Plus. Sequencing libraries were prepared from 10 to 25 ng of total RNA using the SMARTer Stranded RNA-Seq Kit. Subsequently to rRNA depletion, the remaining RNA was used for cDNA synthesis and purification. Samples were then conjugated to unique adapters and amplified by PCR. Libraries were validated using a 2100 BioAnalyzer, normalized, and pooled for sequencing at the University of California, San Francisco Institute for Human Genetics core service. Gene Expression Omnibus accession number: GSE132261.

Metabolic Studies

Glycemia and insulinemia were determined from blood obtained from the tail vein using the ACCU-CHEK II glucometer (Roche) and mouse ultrasensitive ELISA (Alpco). Oral and intraperitoneal glucose tolerance tests (2 g/kg) were performed in animals fasted 5–6 h.

Pancreas Morphology

After euthanasia, pancreas was carefully removed, weighed, and fixed overnight in 4% formaldehyde solution for the experiments using the Ins-cre and Mip-cre mice, and pancreas from Rip-cre mice were fixed overnight in 10% aqueous-buffered zinc formalin solution. For β-cell mass quantification, four nonoverlapping slides separated by 200 μm per animal were used. β-Cells were stained with guinea pig anti-insulin. Fluorescent images were acquired using a microscope (Leica DM5500B) with a motorized stage using

a camera (Leica Microsystems, DFC360FX), interfaced with the OASIS-blue PCI controller, and controlled by the Surveyor software. YY1 deletion/distribution in β-cells was detected by staining pancreas slices with anti-insulin and anti-YY1 antibody. β-Cell proliferation, DNA damage, and apoptosis were determined by anti-Ki67, anti-phosphohistone H2AX (phospho-H2AX) staining, and ApopTag Red in Situ Apoptosis Detection Kit staining, respectively. β-Cell markers were detected by staining pancreas slices with anti-Glut2 and anti-Nkx6.1.

Islet Isolation and Western Blotting

Islets were isolated by the collagenase digestion method carefully described previously (28). Islets were used for experiments after overnight in RPMI 1640 supplemented with 10% FBS, 1% penicillin and streptomycin, and 5.5 mmol/L glucose. For immunoblot, 100 islets were homogenized in lysis buffer (125 mmol/L Tris [pH 6.8], 2% SDS, 1 mmol/L dithiothreitol, and phenylmethylsulfonyl fluoride) supplemented with cOmplete, Mini, EDTA-free Protease Inhibitor, and PhosSTOP. Proteins (30 μg) were separated in 10% polyacrylamide gels and transferred to polyvinylidene fluoride membranes. Membranes were blocked with Intercept Blocking Buffer for 1 h and were probed overnight with anti-YY1 and anti-tubulin antibody. LI-COR's specific second antibodies rabbit IRDye 680RD and anti-mouse IRDye 800CW (1:10,000) were incubated for 1 h at room temperature. Images were obtained using the Odyssey XF Imaging System (LI-COR) and quantified using National Institutes of Health ImageJ software (29).

RNA Isolation, RT-PCR, and Real-Time PCR

Total RNA was isolated from 80 to 100 islets using the RNeasy Plus Kit according to the manufacturer's instructions. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit. The cDNA product was used in the quantitative RT-PCR reaction with power SYBR green master mix. The real-time PCR primers sequences used are listed in Supplementary Tables 1 and 2. Real-time PCR was performed on an ABI 7000 sequence detection system.

Statistics

All data were analyzed using GraphPad Prism software and are expressed as mean ± SEM. The Student *t* test was used to compare two groups. Two-way ANOVA, followed by the Tukey post hoc test, was used to identify differences between the control and YY1-knockout groups over time. Values of $P < 0.05$ were considered statistically significant.

Data and Resource Availability

All data supporting the results are in the body of the article.

RESULTS

Islets From Insulin-Resistant *db/db* Mice Exhibit Decreased Expression of Cell Cycle and DNA Repair Genes

To identify critical factors involved in increasing the susceptibility of pancreatic β-cells to dysfunction and failure, we performed RNA-seq of islets from 4-week-old leptin receptor–deficient *db/db* mice, a model of β-cell stress (Fig. 1*A*) (30). Unbiased gene set enrichment analysis indicated that the Hallmark G2M checkpoint and Hallmark E2F targets are the two top gene pathways differentially expressed ($P < 0.001$) in *db/db* islets. Both pathways are important for cell cycle progression. While E2F transcription factors regulate the expression of genes involved in progression from the G1 phase into the S-phase, G2M checkpoint genes are involved in progression through the cell division cycle (G2 into M phase) (31). Several genes of these two pathways are remarkably downregulated in *db/db* islets, suggesting cell cycle arrest and impaired proliferation (Supplementary Fig. 1*A* and *B*). Interestingly, these two pathways are functionally associated with the Hallmark DNA repair pathway, according to the Pathway CoeXpression Network (PCxN). Indeed, genes important for DNA repair were downregulated in *db/db* islets compared with controls (Fig. 1*B*).

YY1 As a Common Factor in the Control of β-Cell Proliferation and DNA Repair: Implication in Diabetes in Humans and Mice

We used Gene Reference Into Function (GeneRIF) Biological Term Annotations to identify common genes in the G2M checkpoint and E2F target pathway in *db/db* mice. We recognized two lists of genes that are functionally related to each pathway (Supplementary Fig. 1). Among the 17 identified common genes, only two were differentially expressed in *db/db* compared with control islets in the RNA-seq analysis ($P < 0.001$): *Yy1* and its target *Ccna2* (Fig. 1*C*). Both genes were downregulated in *db/db* islets at 4 weeks of age (Fig. 1*D*). Remarkably, several YY1-sensitive genes implicated in the regulation of the G2M checkpoint, DNA repair, or E2F pathways were also less expressed in *db/db* islets (Fig. 1*D* and Supplementary Fig. 1*A* and *B*), suggesting that during the development of diabetes, the decrease in YY1 expression impairs β-cell adaptation and leads to diabetes. Therefore, we decided to analyze YY1 expression in mice fed an HFD, a mouse model of diet-induced IR. There was an ∼50% reduction in nuclei $YY1^+$ β-cells in mice fed an HFD compared with controls (Fig. 1*E*, *F*, and *H*). In diabetic *db/db* mice (12 weeks of age), the percentage of β-cells with nuclear YY1 staining was ∼70% decreased (Fig. 1*E*, *G*, and *H*), while no difference was observed in nondiabetic mice at 4 weeks (data not shown). Importantly, we observed a 40% reduction in nuclei $YY1^+$ β-cells in human subjects with T2D (Fig. 1*I–K*). The reduced nuclear YY1 staining is specific to β-cells, as nuclear YY1 staining in the surrounding acinar tissue was comparable (Fig. 1*H* and *I*).

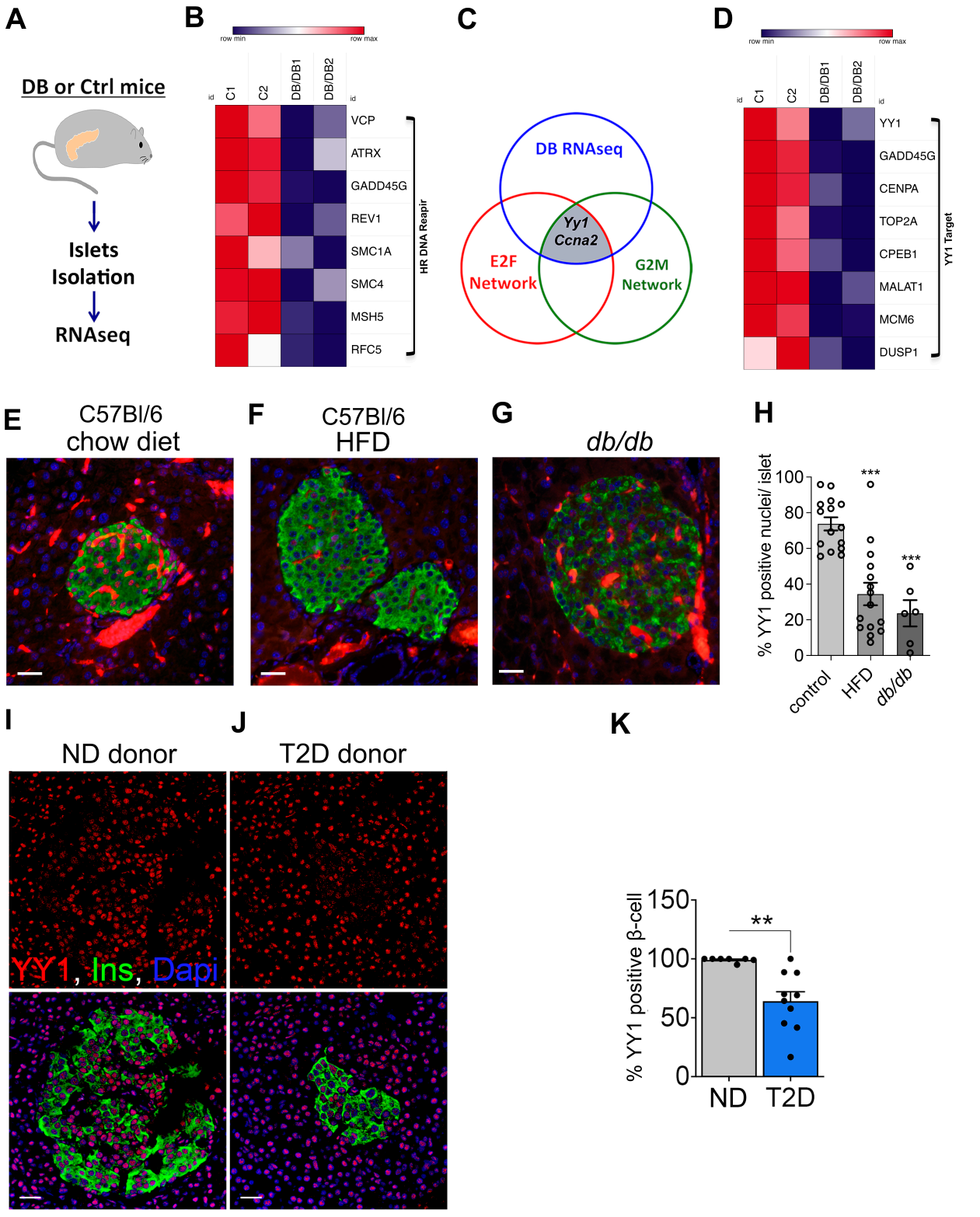

Figure 1—YY1 is a common factor in the control of β-cell cycle and DNA repair and is implicated in diabetes in humans and mice. *A*: Diagram of the experimental profile used for RNA-seq studies performed in 4-week-old mice. DB, diabetic. *B*: RNA-seq heat map showing gene expression of DNA repair genes. *C*: Venn diagram summarizing the overlap between differentially expressed genes from RNA-seq. This shows that Yy1 and Ccna2 genes as the only two common genes (*db/db* RNA-seq, EF2 Network and G2M Network) that were differentially expressed in *db/db* islets compared with control. *D*: Heat map analysis of RNA-seq gene expression of YY1 target genes. Representative images of YY1 staining in control mice fed the chow diet (C57BL/6J) (*E*), HFD (12 weeks on HFD) (*F*), and 3-month-old *db/db* (*G*) mice and in nondiabetic (ND) ($n = 3$) (*I*) and in T2D human donors ($n = 3$) (*J*). Percentage of YY1 staining in the nucleus of β-cells in mice (*H*) and human (*K*) pancreata. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with control assessed by one-way ANOVA, followed by the Tukey post hoc test (*H*) or *t* test (*K*).

YY1 Binds to Genes of the DNA Repair Pathway and Cell Cycle Checkpoints in Human β-Cells

To reveal the pathways controlled by YY1 in human β-cells, we performed ChIP-seq profiling in EndoC-βH1 cells, a human pancreatic β-cell line (33,34). EndoC-βH1 cells highly express YY1 in the nuclei (Fig. 2*A*). Using an anti-YY1 antibody, we conducted ChIP on EndoC-βH1 cells. De novo prediction of highly enriched DNA sequences revealed the YY1 motif AAnATGGC as a top motif, with 80% of binding regions containing this specific motif (Fig. 2*B*). Pathway enrichment analysis for YY1 binding regions indicates that the top pathways (five- to sixfold enriched) are involved in the establishment of sister chromatid cohesion, cohesion loading onto chromatin, mitochondria translation (initiation, elongation, and termination), mRNA processing, and mitotic telophase/cytokinesis (Fig. 2*C*). Interestingly, the cohesin complex is essential to allow DNA repair by homologous recombination during the cell cycle (35). It is also required for the DNA damage-induced G2M checkpoint (36). Outside the regulation of cell cycle controls and checkpoints, the cohesin complex is important to maintain the integrity and structure of DNA in postmitotic cells (35). The cohesin complex is required for the recruitment of the DNA repair machinery, and the loss of the cohesin complex leads to the accumulation of DNA damage (35). Additional pathways (two- to threefold enriched) have been identified as a direct target of YY1, including DNA damage recognition in global genome nucleotide excision repair, ATM-mediated double-stranded breaks repair, and the G1/S DNA damage checkpoint (Fig. 2*D*). To further increase the significance of our findings, we assessed expression for a few of the YY1 target genes identified in the EndoC-βH1 ChIP-seq studies. We observed that genes from both the G2M checkpoint and the E2F pathways from the ChIP-seq data were differentially regulated in the RNA-seq from *db/db* islets (Supplementary Fig. 1*C*).

Mice With Targeted YY1 Disruption in β-Cells Develop Diabetes Early in Life due to Severe β-Cell Loss

To study the role of YY1 in β-cells, we crossed the *floxed-yy1* mouse with the RIP-cre mouse. As expected, Rip*β-YY1KO* islets exhibit 80% reduction in β-cells with nuclear YY1 (Fig. 3*A–C*). At 3 weeks of age, Rip*β-YY1KO* mice had similar weight but were severely hyperglycemic (Fig. 3*D* and *E*). In addition, 6 h fasting insulin levels were lower, and they fail to secrete insulin in response to glucose (Fig. 3*F*). This is explained by a 50% reduction in β-cell mass at 3 weeks of age (Fig. 3*G–J*). Remarkably, β-cells are almost gone in 3-month-old mice (Fig. 3*I*). We did not observe differences in β-cell mass of newborn mice, suggesting that YY1 deletion did not impair β-cell embryonic development (Fig. 3*G* and *J*). Owing to ectopic cre-recombinase expression outside the pancreas in the *RIP-cre* mice (37), we decided to confirm the phenotype using the *Ins1-Cre* mouse (38) (Ins1*β-YY1KO* mice). The Ins1*β-YY1KO* mice have few nuclei YY1$^+$ β-cells, and isolated islets express 50% less YY1 at 2 weeks of age (Fig. 4*A–C*). The YY1 target cyclin A2 was also reduced, suggesting that some DNA damage associated candidates identified in the *db/db* RNA-seq were also decreased in Ins1*β-YY1KO* mice

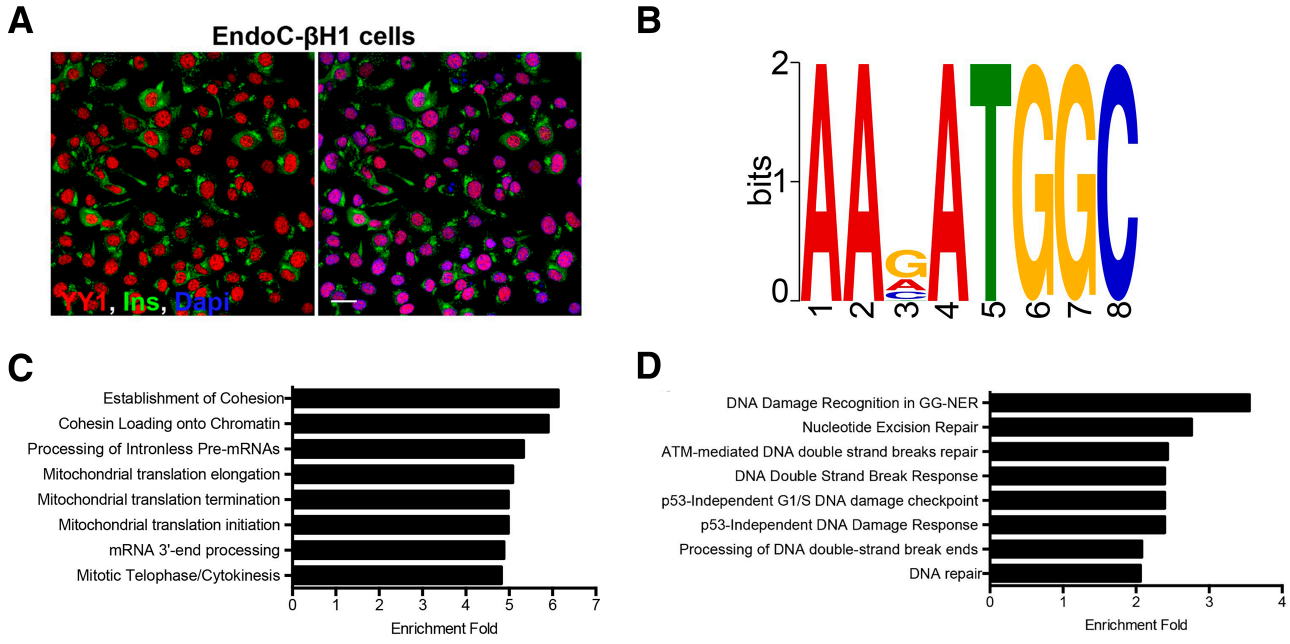

Figure 2—YY1 binds to genes of the DNA repair pathway and cell cycle checkpoints in human β-cells. *A*: Representative images of immunostaining for YY1 and insulin in EndoC-βH1 cells. *B*: Diagram showing a putative YY1 binding motif. *C* and *D*: Pathway enrichment analysis for YY1 binding regions.

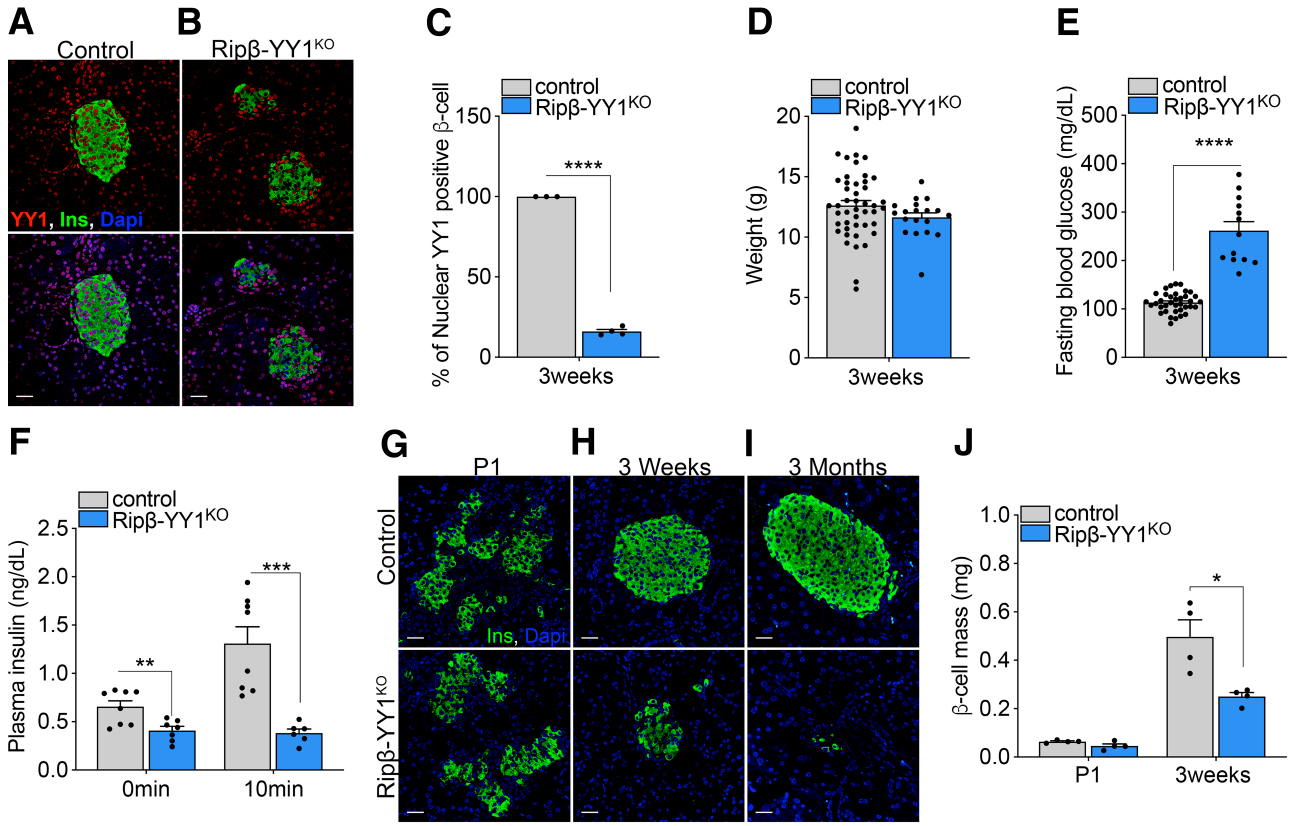

Figure 3—Rip*β-YY1KO* mice with targeted YY1 disruption in β-cells develop diabetes early in life due to severe β-cell loss. Representative images of YY1 and insulin staining in control (*A*) and Rip*β-YY1KO* pancreata (*B*). Quantification of positive YY1 nuclei in β-cells by immunostaining (*C*), body weight (*D*), fasting blood glucose (*E*), and glucose-induced insulin secretion (*F*) in 3-week-old mice. Representative β-cell staining (insulin) at postnatal day 1 (P1) (*G*), 3 weeks (*H*), and 3 months (*I*). *J*: β-Cell mass quantification in 3-week-old mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ compared with control assessed by *t* test. Comparisons in panel *F* were made using two-way ANOVA, followed by the Šidák posttest.

(Fig. 4*D* and *E*). Ins1*β-YY1KO* mice exhibit hyperglycemia at 2 weeks of age that progresses to diabetes by 3 weeks (Fig. 4*F*), with no difference in body weight (Fig. 4*G*). Nonfasting plasma insulin levels are reduced at 2 and 3 weeks of age (Fig. 4*H*). β-Cell area was reduced in Ins1*β-YY1KO* by 50% at 2 weeks of age and further decreased by 75% at 3 weeks of age (Fig. 4*I* and *J*). Similar to the Rip*β-YY1KO* mice, newborn Ins1*β-YY1KO* mice exhibit normal glucose, insulin levels, β-cell area (Fig. 4*F*, *H* and *I*), and cell death assessed by TUNEL staining (data not shown).

Impaired β-Cell Differentiation and Increased Cell Death Explain the β-Cell Loss in the Mice Lacking YY1

To understand the mechanism behind β-cell loss in mouse models of β-cell YY1 deficiency, we assessed β-cell differentiation, proliferation, and death (Fig. 5). The mRNA expression of canonical pancreatic β-cell markers, such as *Glut2*, *Nkx6.1*, *Ins1*, *Ins2*, and *Mafa*, was reduced in islets from 3-week-old Rip*β-YY1KO* mice (Fig. 5*A*). In contrast, α-cell markers *Gcg* and *Arx* expression was not different (Fig. 5*A*). The number of positive β-cells for GLUT2 and NKX6.1 was similar in newborn mice (postnatal day 1) but 45% and 70% reduced in the Rip*β-YY1KO* mice, respectively (Fig. 5*B–E*). The number of apoptotic β-cells assessed by TUNEL was higher in 3-week-old Rip*β-YY1KO* islets compared with littermate controls (Fig. 5*F* and *G*). β-Cell proliferation was similar in newborn and 3-week-old Rip*β-YY1KO* islets (Fig. 5*H*). Similar to Rip*β-YY1KO*, Ins1*β-YY1KO* mice also exhibit low levels of *Glut2*, *Ins2*, and *Pdx1* mRNA expression (Fig. 5*I*). β-Cell proliferation was comparable, while β-cell death was increased in Ins1*β-YY1KO*, consistent with the findings from Rip*β-YY1KO* (Fig. 5*J* and *K*). Additionally, we observed a decrease in phosphohistone H3, a marker of mitosis, in Ins1*β-YY1KO*, suggesting cell cycle arrest (Fig. 5*L*).

Disruption of YY1 in Adult Mature β-Cells Impairs Glucose Metabolism

We crossed the $yy1^{f/f}$ mice with animals expressing the inducible *MIP1-Cre*^{ER} driver in order to understand the role of YY1 in mature β-cells (*iβ-YY1KO* mice). *iβ-YY1KO* mice developed mild hyperglycemia (~150 mg/dL) 2 weeks after tamoxifen injection, and blood glucose levels remained

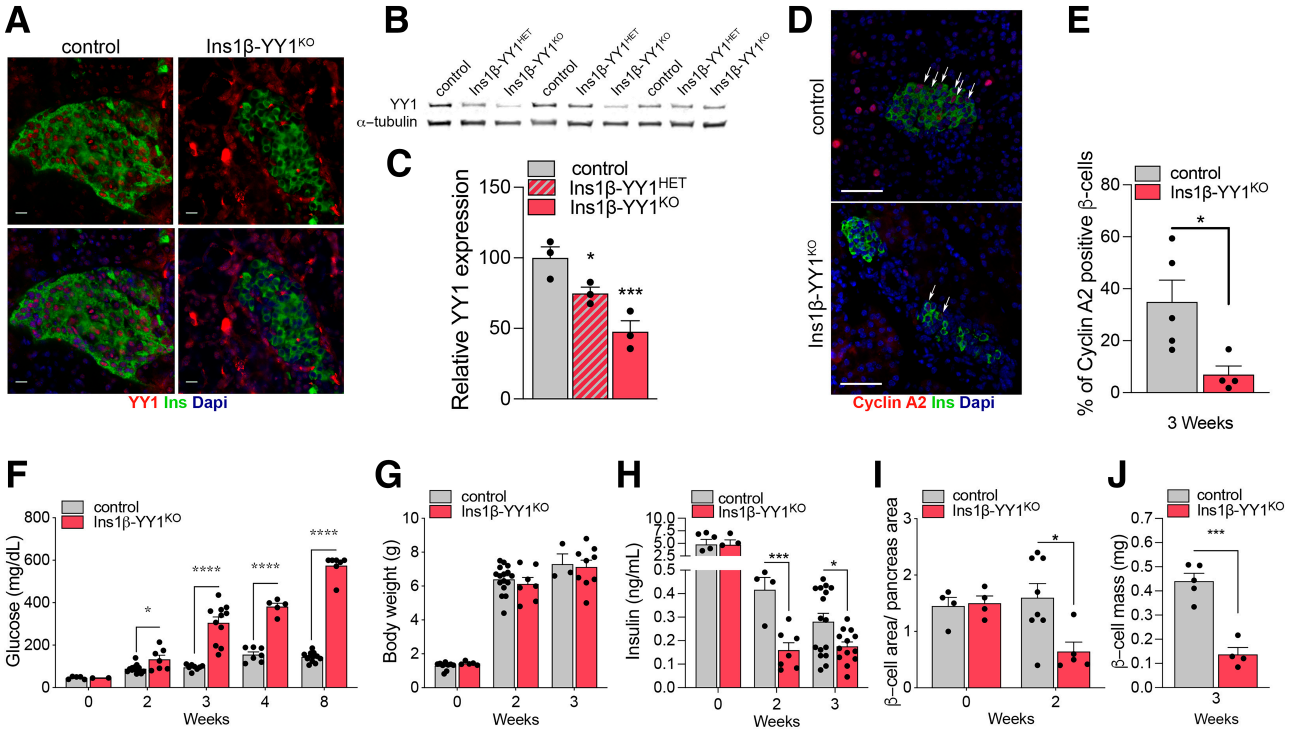

Figure 4—YY1 deletion in Ins1*β-YY1KO* mice also induces diabetes early in life due to severe β-cell loss. *A*: Representative images of YY1 and insulin staining in control and Ins1*β-YY1KO* in 2-week-old mice. Representative image (*B*) and quantification (*C*) of Western blotting for YY1 in isolated islets from Ins1*β-YY1KO* and controls. Representative images (*D*) and quantification (*E*) of cyclin A2 and insulin staining in control and Ins1*β-YY1KO* in 3-week-old mice. Blood glucose levels from birth to 2-months old (*F*), body weight (*G*), random insulin level (*H*), β-cell–to–pancreas area ratio (*I*) and β-cell mass (*J*) in Ins1*β-YY1KO* and control mice at the indicated times. *$P < 0.05$, ***$P < 0.001$ compared with control assessed by *t* test.

elevated up to 14 weeks (Fig. 6*A* and *B*). Insulinemia was normal in *iβ-YY1KO* (Fig. 6*C*), but these mice were glucose intolerant at 8 and 14 weeks after tamoxifen (Fig. 6*D* and *E*). Remarkably, glucose-stimulated insulin secretion was blunted in *iβ-YY1KO* mice (Fig. 6*F*), and there was a tendency to increase in β-cell death (Fig. 6*G*); however, β-cell mass was preserved (Fig. 6*H*).

DNA Damage Is Increased in YY1-Deficient β-Cells

We next tested whether the lack of YY1 induces DNA damage. We assessed the accumulation of DNA damage by phospho-H2AX staining. The number of phospho-H2AX/insulin double-positive cells is significantly increased in Rip*β-YY1KO* islets compared with littermate controls (Fig. 7*A* and *B*). Phospho-H2AX staining is also increased in Ins1*β-YY1KO* (Fig. 7*C* and *D*) and in *iβ-YY1KO* β-cells compared with controls (Fig. 7*E* and *F*).

DISCUSSION

Our current studies place the YY1 transcription factor as a key player in the diabetes progression. Unbiased transcriptome assessment revealed that downregulation of *Yy1* and its target *Ccna2* are the common genes explaining the suppression of DNA repair and cell cycle pathways in islets from IR *db/db* mice before they develop severe hyperglycemia. Remarkably, YY1 protein expression is suppressed in β-cells of diabetic *db/db* mice, mice fed an HFD, and human donors with T2D. We validated these findings in vivo by genetic disruption of *Yy1* specifically in β-cells. The lack of YY1 since embryonic stages led to diabetes very early in life (~2–3 weeks of postnatal life) due to a severe β-cell loss, impaired β-cell differentiation, and increased cell death and DNA damage. *Yy1* deletion in adult mature β-cells impaired glucose metabolism and also induced DNA damage. Taken together, these studies show that YY1 is an important regulator of β-cell maintenance and function.

The RNA-seq results in *db/db* mice islets showing decreased expression of DNA repair genes are in line with previous work showing accumulation of DNA damage and double-stranded breaks in islets from *db/db* mice and human T2D samples (8–10). RNA-seq and gene set enrichment analysis also detected a downregulation of cell cycle progression pathways, including GM2 checkpoint and E2F targets (Fig. 1*B–D*). The decrease in G1/S and G2/M in 4-week-old *db/db* mice is not sufficient to alter cell cycle progression and β-cell expansion at this stage, but a more sustained and prolonged DNA damage accumulates with age, and DNA repair defects can cause cell cycle arrest senescence and apoptosis at later stages (39). The E2F

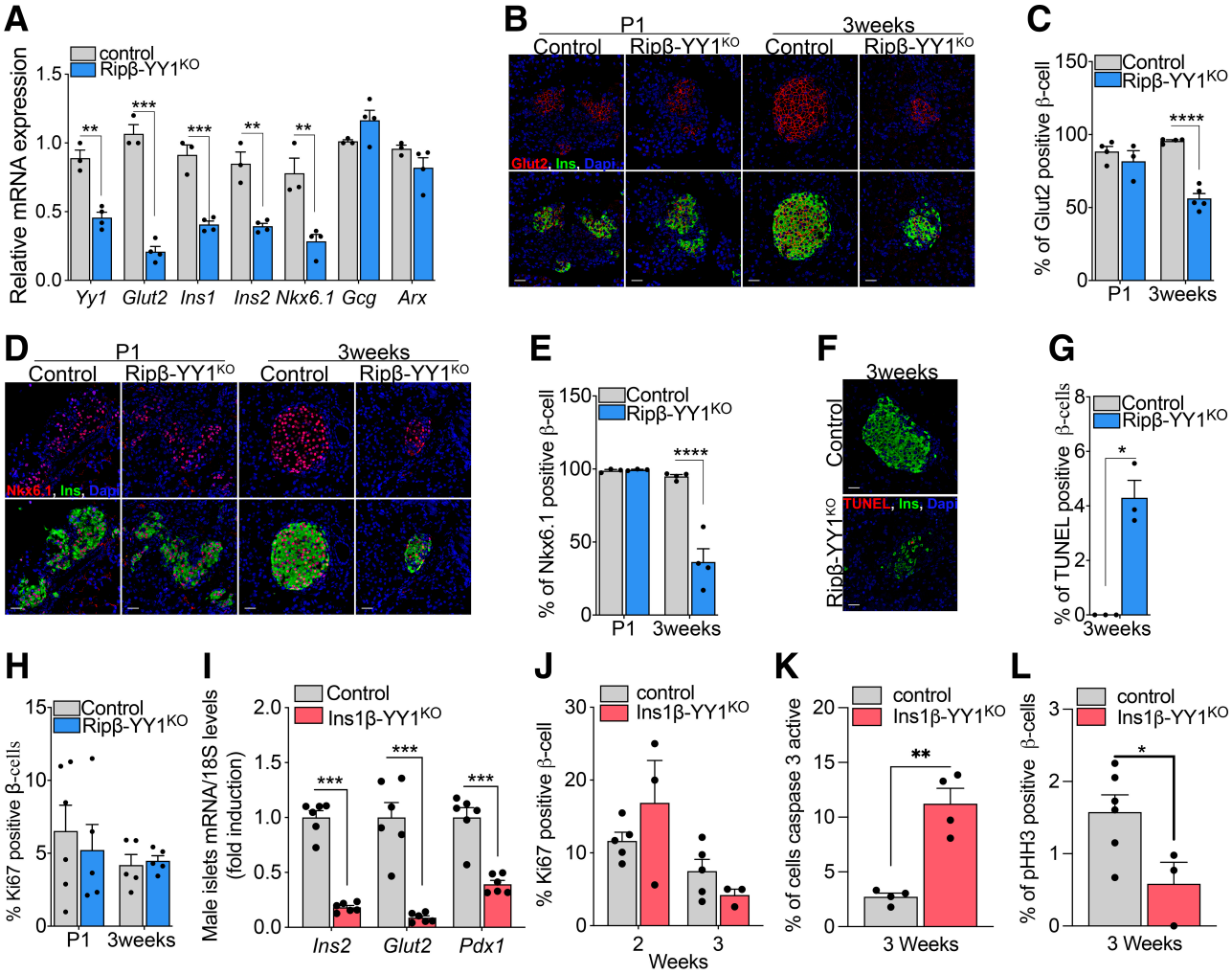

Figure 5—Impaired β-cell differentiation and increased cell death explain the β-cell loss in the mice lacking YY1. *A*: mRNA expression of key β-cell genes in isolated islets from control and Rip*β-YY1KO*. *B*: Representative images of GLUT2 staining (*B*) and quantification of GLUT2-positive β-cells (*C*). P1, postnatal day 1. Representative images of Nkx6.1 staining (*D*) and quantification in neonates and 3-week-old mice (*E*). Representative images of pancreas TUNEL staining (*F*) and quantification in β-cells from control and Rip*β-YY1KO* at the indicated ages (*G*). *H*: β-Cell proliferation in Rip*β-YY1KO* and controls determined by Ki67 immunostaining. *I*: mRNA expression for *Insulin2, Glut2,* and *Pdx1* in isolated cells islets from control and Ins*β-YY1KO*. *J*: β-Cell proliferation. *K*: Cleaved caspase 3 quantification in Ins1*β-YY1KO* mice. *L*: Phosphohistone H3 (pHH3) quantification in β-cells from control and Ins*β-YY1KO*. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 compared with control assessed by *t* test.

family regulates cell cycle progression in β-cells (40), and global E2F1-knockout mice have impaired pancreas growth, β-cell mass, and β-cell proliferation and function (41). In addition, β-cell–specific E2F1 deletion specifically in mouse β-cells resulted in impaired glucose tolerance, defective insulin secretion, and loss of β-cell identity compared with controls (42). Therefore, DNA integrity and cell cycle progression are compromised in *db/db* islets, suggesting that β-cell failure occurs when pancreatic β-cells lose the ability to activate the machineries of DNA damage and checkpoint response. We then identified *Yy1* and its target *Ccna2* as common genes to both pathways (Fig. 1*C*). Importantly, the regulation of the specificity and function of E2F by interacting with YY1 (43) and the peak of YY1 activity during G2M transition suggesting a crucial role of this transcription factor in regulating G2M transition (16). Moreover, YY1 also controls cell proliferation indirectly by inactivating the negative regulator of E2F1 (44). Taken together, these studies support a model in which suppression of YY1 expression in β-cells during the progression of the diabetes leads to impaired DNA repair capacity, ultimately resulting in DNA damage, cell death, and β-cell loss.

The results in *db/db* mice prompted us to generate mouse models with inactivation of YY1 in β-cells. YY1 disruption resulted in diabetes early in life due to β-cell loss. During the course of our study, Song et al. (26) also generated a *βYY1KO* mice. The aim was to extend their

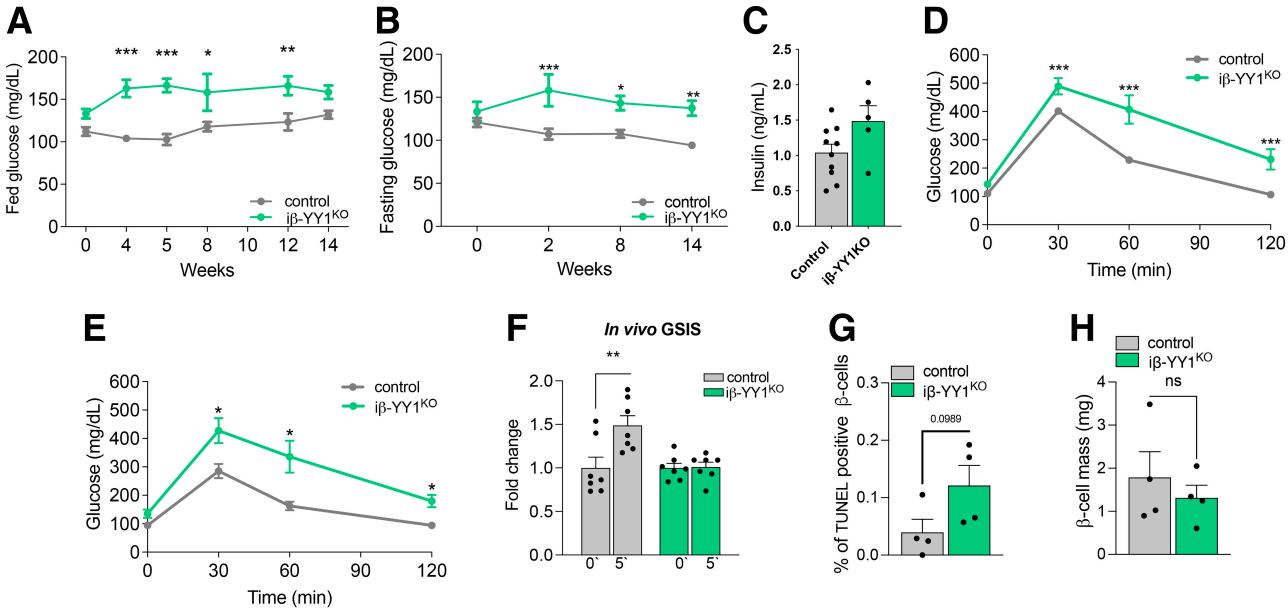

Figure 6—Disruption of YY1 in adult mature β-cells impairs glucose metabolism. Fed blood glucose (*A*), fasting blood glucose (*B*), and nonfasting plasma insulin (*C*) in mice with inducible deletion of YY1 in β-cells (*iβ-YY1KO*) and controls. Glucose tolerance test at 8 weeks (*D*) and 14 weeks (*E*) after tamoxifen treatment. *F*: Glucose-stimulated insulin secretion (GSIS) in isolated islets. *G*: TUNEL quantification in β-cells from control and *iβ-YY1KO* 16 weeks after tamoxifen treatment. *H*: β-Cell mass 16 weeks after tamoxifen treatment. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with control assessed by multiple *t* test (two-stage step-up [Benjamini, Krieger, and Yekutieli]). Comparisons in panel *F* were made using two-way ANOVA, followed by the Šidák posttest.

own previous studies, where gain-of-function mutations determined tumor growth and function (23). They reported similar reduction in β-cell mass, and this was accompanied by diabetes (26). Impaired proliferation, reduced activity of mitochondrial oxidative phosphorylation, and mitochondrial dysfunction were implicated as mechanisms for β-cell loss in *βYY1KO* mice. In contrast, we failed to show differences in β-cell proliferation. This is likely explained by the age of the animals used for analysis of β-cell proliferation (6 weeks old vs. 2 weeks old in our studies). Our data validate the phenotype and the islet morphologic changes but extend our current knowledge from this previous publication by: *1*) Reduction in β-cell YY1 expression in genetic models of IR and HFD. *2*) Reduction of YY1 expression in islets from human donors with T2D and identification of YY1 targets using ChIP-seq in human β-cell lines. Candidate YY1 target genes from both the G2M checkpoint and the E2F pathways identified by ChIP-seq in data were differentially expressed in the RNA-seq from *db/db* islets, suggesting that these putative target genes were regulated in a model of T2D. *3*) Identifying the role of YY1 in regulation of β-cell cycle progression and a previously unknown role of this transcription factor in DNA damage in β-cells. *4*) The higher DNA damage with similar β-cell survival in the *iβ-YY1KO* mice implies that adult/mature β-cells are less vulnerable to the YY1 loss and DNA damage/repair. *5*) The increase vulnerability and β-cells loss early in *βYY1KO* mice suggest that YY1 plays a key role during the maturation of β-cells. Interestingly, Song et al. (26) documented greater reactive oxygen species production, supporting the fact that DNA damage is increased in all of our in vivo knock-out mouse models. Therefore, the two studies are complementary regarding the mechanisms to explain the β-cell loss and dysfunction. In addition, we show that lack of YY1 decreased the expression of important β-cell identity markers such as Ins1, Glut2, Nkx6.1, and PDX1.

All mouse models of YY1 deficiency used in our studies exhibited higher levels of DNA damage (Fig. 7). Cell cycle analysis showing comparable levels of Ki67 (marker for all cell cycle phases) and lower phosphohistone H3 (G2M) suggest that β-cells deficient in YY1 enter cell cycle normally but fail to progress beyond the G2M phase by defects in DNA damage and repair and that these abnormalities ultimately result in cell death. These findings are also consistent with the peak of YY1 activity during the G2M transition suggesting a crucial role of this transcription factor in regulating G2M transition (16). This is consistent with the data obtained in *db/db* mice and human donors with T2D who also exhibit markers of DNA damage and p53 activity (45). Hyperglycemia triggers DNA damage by inhibiting the nucleotide excision repair pathway and changing the levels of DNA repair genes and proteins (46). Mice deficient in DNA repair display reduced β-cell area, increased apoptosis, and impaired insulin secretion (6). These published data, together with our results, suggest that DNA damage can be a critical factor in regulating

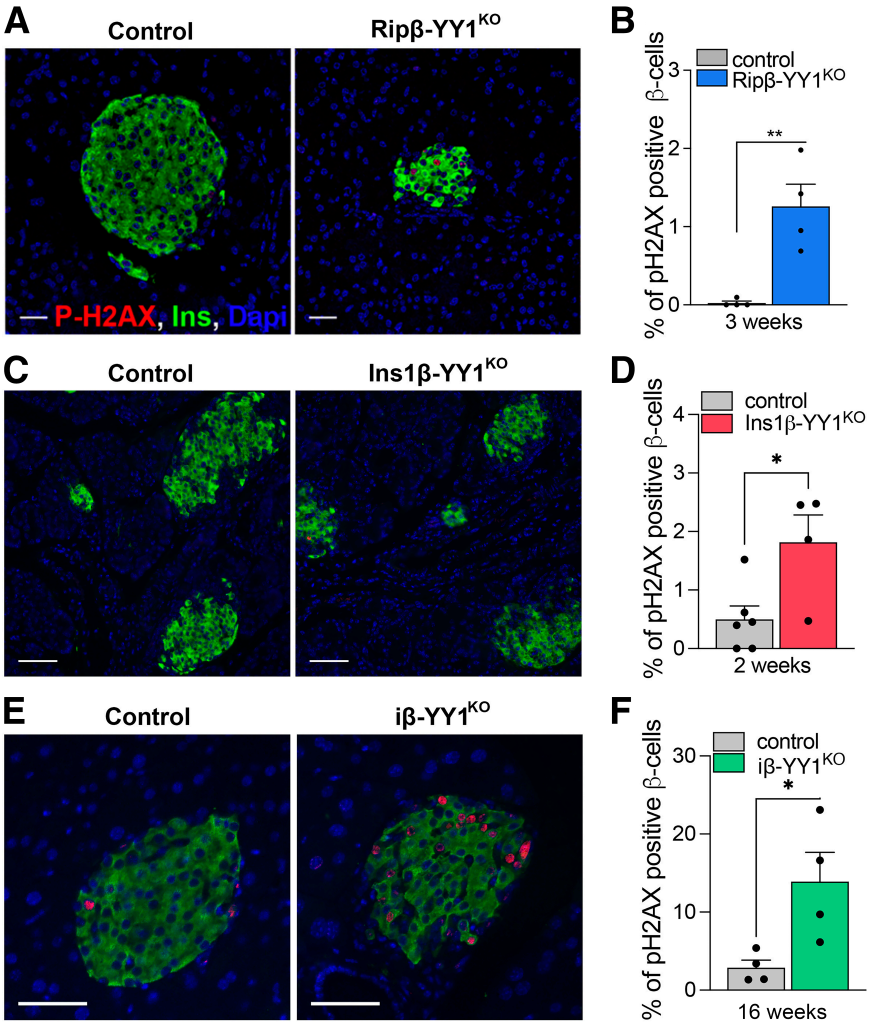

Figure 7—DNA damage is increased in YY1-deficient β-cells. Staining for phospho-H2AX (pH2AX) in 3-week-old Ripβ-*YY1KO* mice (*A*), 2-week-old Ins1β-*YY1KO* mice (*C*), and *i*β-*YY1KO* mice at 16 weeks (*E*) and quantification (*B*, *D*, and *F,* respectively) after tamoxifen treatment. *$P < 0.05$, **$P < 0.01$ compared with control assessed by *t* test.

β-cell loss in diabetes and that YY1 is implicated in this process. The mechanisms for induction of DNA damage in YY1-deficient β-cells is not completely clear, but our ChIP analysis of the human β-cell line identified DNA repair pathways, including ATM-mediated double-stranded breaks repair, as a direct target of YY1. ATM and p53 are key mediators of the DNA damage response, and both play a role in β-cell death induced by streptozotocin (47). The previously identified interaction of YY1 with p53 induces its ubiquitination, and the increase in YY1 levels inhibits the accumulation of active p53 as well as the expression of p53 target genes after DNA damage (48,49). In support for a YY1/p53 axis in regulation of survival, loss of YY1 has been shown to cause an increase in p53 levels and apoptosis (49). We also found a decrease in the expression of DNA repair genes in nonobese *db/db* mice, including GADD45 (Fig. 1*B*). Published data showed that YY1 can inhibit p53-dependent transcription of target genes such as GADD45 and $p21^{Waf1}$ (50). Finally, YY1 can also induce DNA damage by regulating mitochondrial function and reactive oxygen species production (26,51,52). In summary, our data, together with published studies, suggest that YY1 can regulate DNA damage in β-cells by different pathways.

In summary, our data show that YY1 is downregulated in β-cells from different models of mouse and human diabetes and that this transcriptional regulator orchestrates a gene expression profile that regulates key biological processes, including cell identity and DNA repair mechanisms that are critical for survival of the β-cell. These studies demonstrate that YY1 emerges as an important regulator of β-cell maintenance and function.

Funding. This work was supported by the National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK073716 and U.S. Department of Veterans Affairs Merit Review Award no. IBX002728A to E.B.-M. and NIH R01DK118099 grant to A.B.

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

Author Contributions. F.L.M.P. and R.J. contributed to design, data collection and analysis, and writing the manuscript. J.P.W.-d-C. contributed to data collection and analysis and writing the manuscript. C.-C.A. contributed to data collection and analysis. A.B. contributed to design, funding, and data discussion. E.B.-M. contributed to design, funding, data discussion, and writing the manuscript. E.B.-M. 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 accuracy of the data analysis.

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