ORIGINAL ARTICLE

Deletion of *Pten* in Pancreatic β-Cells Protects Against Deficient β-Cell Mass and Function in Mouse Models of Type 2 Diabetes

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OBJECTIVE—Type 2 diabetes is characterized by diminished pancreatic β-cell mass and function. Insulin signaling within the β-cells has been shown to play a critical role in maintaining the essential function of the β-cells. Under basal conditions, enhanced insulin-PI3K signaling via deletion of phosphatase with tensin homology (PTEN), a negative regulator of this pathway, leads to increased β-cell mass and function. In this study, we investigated the effects of prolonged β-cell–specific PTEN deletion in models of type 2 diabetes.

RESEARCH DESIGN AND METHODS—Two models of type 2 diabetes were employed: a high-fat diet (HFD) model and a *db/db* model that harbors a global leptin-signaling defect. A Cre-loxP system driven by the rat insulin promoter (RIP) was employed to obtain mice with β-cell–specific PTEN deletion (RIP*cre*$^+$ *Pten*$^{fl/fl}$).

RESULTS—PTEN expression in islets was upregulated in both models of type 2 diabetes. RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice were completely protected against diabetes in both models of type 2 diabetes. The islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice already exhibited increased β-cell mass under basal conditions, and there was no further increase under diabetic conditions. Their β-cell function and islet PI3K signaling remained intact, in contrast to HFD-fed wild-type and *db/db* islets that exhibited diminished β-cell function and attenuated PI3K signaling. These protective effects in β-cells occurred in the absence of compromised response to DNA-damaging stimuli.

CONCLUSIONS—PTEN exerts a critical negative effect on both β-cell mass and function. Thus PTEN inhibition in β-cells can be a novel therapeutic intervention to prevent the decline of β-cell mass and function in type 2 diabetes. ***Diabetes* 59: 3117–3126, 2010**

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The quintessential defects in type 2 diabetes are the development of peripheral insulin resistance and β-cell dysfunction (1–3). In fact, the loss of insulin secretion in β-cells in response to glucose occurs before the emergence of insulin resistance and hyperglycemia (4–6). Once insulin resistance develops, hyperglycemia, high-circulating free fatty acids, and inflammatory cytokines further abrogate glucose-stimulated insulin secretion (2,7–9). It is becoming increasingly clear that insulin/insulin-like growth factor 1 (IGF-1) signaling plays an important role in the maintenance of β-cell function under both basal and diabetic conditions. Mice with β-cell–specific deletion of IGF-1 receptor exhibit a defect in glucose-stimulated insulin secretion (10,11), whereas insulin receptor deletion in β-cells results in both attenuated insulin secretion in response to glucose and reduced β-cell mass with aging (12,13). Thus, β-cells are not only an essential source of the hormone insulin, but are also a critical target of insulin action in the maintenance of β-cell function.

Phosphoinositide 3-kinase (PI3K) signaling cascade is one of the major intracellular signaling pathways through which insulin and IGF-1 mediate their effects (14). Phosphatase with tensin homology (PTEN) is a dual-specific phosphatase and a potent negative regulator of this pathway by its ability to dephosphorylate phosphatidylinosotol-3,4,5-triphosphate (PIP3) to phosphatidylinosotol-4,5-bisphosphate (PIP2), thereby effectively removing the critical secondary messenger of this signaling cascade (15,16). Although PTEN was first discovered as a tumor suppressor, recent studies have highlighted the important physiologic role of PTEN in metabolism (16–18). Tissue-targeted deletion of PTEN in liver, fat, or muscle lead to improved insulin sensitivity in these insulin-responsive tissues and protects mice from HFD-induced diabetes (19–22). Additionally, we and others have reported that mice with PTEN deletion in pancreatic β-cells show increased β-cell mass because of both increased proliferation and reduced apoptosis without compromising β-cell function under the basal condition (23,24).

PTEN has been shown to be upregulated in models of insulin resistance, including a genetic model of combined ablation of insulin/IGF-1 signaling in β-cells (25–27). Furthermore, in vitro overexpression of PTEN in pancreatic β-cell lines showed impaired insulin secretion in response to ambient glucose (28). However, the regulation of PTEN expression in β-cells in models of type 2 diabetes in vivo was unknown. We show here that PTEN expression was increased in islets of both high-fat diet (HFD)-fed and *db/db* mice, which was accompanied by attenuation in

PI3K signaling, suggesting the potential causal role of PTEN in the pathogenesis of β-cell dysfunction in type 2 diabetes. In this report, we investigated the essential role of PTEN in β-cells in the context of type 2 diabetes models. We used the rat insulin promoter (RIP) to drive deletion of PTEN in the Cre-loxP system (RIP*cre*$^+$ *Pten*$^{fl/fl}$). RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice were protected from HFD-induced type 2 diabetes because of their increased islet mass and proliferation with intact β-cell function. β-Cells from RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice were protected against HFD-induced β-cell dysfunction both in vitro and in vivo, which can be attributed to the constitutively active PI3K signaling in their islets. Furthermore, RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice in the *db/db* background still remained euglycemic, despite being severely insulin resistant. Interestingly, their β-cell mass was not significantly different from *db/db* littermates. However, their β-cell function and islet PI3K signaling remained intact. Together, our data highlight the critical role of β-cell PTEN in the development of β-cell dysfunction in type 2 diabetes and support PTEN as a potential therapeutic target for β-cell growth and the preservation of its function.

RESEARCH DESIGN AND METHODS

Pten$^{fl/fl}$ mice (exons 4 and 5 of *Pten* flanked by *loxP* sites by homologous recombination) were mated with RIP*cre* mice (*Cre* transgene under the control of the rat insulin two promoter TgN[ins2-cre]25Mgn from Jackson Laboratories). RIP*cre*$^+$ *Pten*$^{+/fl}$ mice were intercrossed to generate RIP*cre*$^+$ *Pten*$^{+/+}$, RIP*cre*$^+$ Pten$^{+/fl}$, and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice. RIP*cre*$^+$ *Pten*$^{+/+}$ mice were used as controls. RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice were generated by breeding RIP*cre*$^+$ *Pten*$^{+/fl}$ mice with *Lepr*$^{+/db}$ mice (Jackson Laboratories) to obtain RIP*cre*$^+$ *Pten*$^{+/fl}$ *Lepr*$^{+/db}$ mice. RIP*cre*$^+$ *Pten*$^{+/fl}$ *Lepr*$^{+/db}$ mice were then intercrossed to generate RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{+/+}$ (wild-type), RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ (*db/db*), and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ (−/−; *db/db*) mice. Only male mice were used for experiments and only littermates were used as controls. Genotypes for *Cre* and *Pten* gene were determined with PCR using ear clip DNA as described previously (19,23). All mice were maintained on a mixed 129J-C57BL/6 background and housed in a pathogen-free facility on a 12-h light-dark cycle and fed ad libitum with standard irradiated rodent chow (5% fat; Harlan Tecklad, Indianapolis, IN) in accordance with the Ontario Cancer Institute Animal Care Facility protocol, with no restrictions on the animals' activities.

High-fat diet feeding. HFD (Dyets lard Surmwit mouse diet DYET# 182084; Fyets Inc., Bethlehem, PA) for RIPcre$^+$ Pten$^{fl/fl}$ and RIP*cre*$^+$ *Pten*$^{+/+}$ mice was started for mice at 2 months of age and continued for 7 months.

Metabolic studies. All overnight fasts were carried out between 5:00 P.M. and 10:00 A.M. All blood glucose, glucose tolerance tests, insulin tolerance tests, and glucose-stimulated insulin secretion tests were performed on overnight-fasted animals as previously described (23).

Islet perifusion assay. Detailed perifusion protocol was described in a previous publication (29). In brief, 60 islets were placed in a perifusion chamber at 37°C with a capacity of 1.3 ml, and perifused with Kreb-Ringer bicarbonate buffer at 1 ml/min. Islets were equilibrated with Kreb-Ringer bicarbonate HEPES buffer (2.8 mmol/l glucose) for 30 min. They were then stimulated with 2.8 mmol/l glucose for 10 min, followed by a 40-min incubation with 16.7 mmol/l glucose. Fractional insulin content was determined with radioimmunoassay kit (Linco Research, St. Louis, MO). At the end of each perifusion, islets were collected and lysed with acid ethanol for assessment of insulin content. First phase secretion was 10 to 25 min, and second phase secretion was 25 to 50 min. Results were presented as insulin secreted normalized to 60 islets and to total insulin content.

Immunohistochemistry and immunofluorescence. Pancreas was fixed in 4% paraformaldehyde in 0.1M PBS (pH 7.4) as previously described (25). For immunohistochemical and immunofluorescent staining antibodies against PTEN (NeoMarker, Fremont, CA), Akt (Cell Signaling Technology, Beverly, MA), p-Akt (Ser473) (Cell Signaling Technology, Beverly, MA), insulin (DAKO), glucagon (NovoCastra Laboratories), laminin (Sigma), β-catenin (BD Transduction Laboratories), GLUT2 (Chemicon, Temecula, CA), PDX-1 (Chemicon, Temecula, CA), and Ki67 (DAKO) were used. Total islet area and total pancreas area were determined on insulin immunohistochemically stained sections with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) and expressed as total islet area per total pancreas area. Proportions of different islet sizes were measured on H&E-stained pancreas sections: small (<10 cells), medium (10–200 cells), and large (>200 cells). β-Cell size was determined with insulin/DAPI costained pancreas sections. Insulin-positive area was determined with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) and divided by the number of DAPI-positive nuclei within the insulin-stained areas.

Western blotting. Islets and hypothalami were isolated and protein lysates were obtained as previously described (23). Antibodies against actin (Santa Cruz Biotechnology, Santa Cruz, CA), Akt, p-Akt, FoxO-1 (Cell Signaling Technology, Beverly, MA), p-FoxO-1 (Cell Signaling Technology, Beverly, MA), GAPDH (Cell Signaling Technology, Beverly, MA), GLUT-2, mTOR (Santa Cruz Biotechnology, Santa Cruz, CA), p-mTOR (Cell Signaling Technology, Beverly, MA), p-p53 (R&D systems), p53 (Santa Cruz Biotechnology, Santa Cruz, CA), PDX-1, and PTEN were used (antibody sources for Akt, p-Akt, GLUT-2, PDX-1, and PTEN refer to immunohistochemistry and immunofluorescence).

Gamma irradiation and quantitative PCR. Isolated islets are incubated in RPMI-1640 (10% FBS) at 37°C overnight, then irradiated with 30Gy of gamma irradiation the next day. Islets were harvested after overnight in culture. RNA was extracted with RNeasy Plus Mini Kit (Qiagen, Valencia, CA) according to protocol provided. Complimentary DNA was synthesized according to protocol published elsewhere (30). PCR was monitored in real time using the ABI Prism 7900HT Real-Time PCR system (Applied Biosysterm). Experiments were performed in triplicate for each sample. Primer sequences for Mdm2, Bax, and p21 are available upon request.

Statistics. Data are presented as mean ± SEM and were analyzed by one-sample *t* test, independent-sample *t* test, and one-way ANOVA with the post hoc Tukey least significant difference test, when appropriate. All data were analyzed using the statistical software package SPSS (version 16.0) for Macintosh.

RESULTS

Increased PTEN expression with attenuated PI3K signaling in islets of HFD-fed and *db/db* mice. PTEN transcript and protein levels were measured in islets of mice with type 2 diabetes. In both HFD-induced and *db/db* mice, PTEN transcripts in islets were significantly increased as accessed by quantitative real-time PCR (Fig. 1*A*). PTEN protein expression was also increased as demonstrated by immunohistochemistry of pancreatic sections and Western blotting of isolated islet lysates (Fig. 1*B* and *C*). The increase in islet PTEN expression in both of these type 2 diabetes models was accompanied by attenuated PI3K signaling, as demonstrated by the reduction of p-Akt, p-mTOR, and p-FoxO-1 expression (Fig. 1*C*).

RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice were protected against HFD-induced diabetes. We have previously shown that RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice exhibit an increase in β-cell mass and function under basal conditions (23). To investigate whether these positive attributes of PTEN deletion in pancreatic β-cells conferred protection against type 2 diabetes, we fed these mice a prolonged HFD for 7 months. Efficient PTEN deletion in β-cells, along with partial deletion in the hypothalamus, persisted in the RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice on prolonged HFD (Fig. 2*A* and *B*). Despite their increased weight gain upon HFD feeding, RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice remained remarkably euglycemic throughout the duration of prolonged HFD, in contrast to the gradual increase in blood glucose levels in control littermates (Fig. 3*A* and *B*). They also exhibited improved glucose tolerance (Fig. 3*C*). The attenuation of insulin secretion in response to glucose is a characteristic β-cell defect in type 2 diabetes (1–3). Indeed, this attenuation was observed in both the first and second phases of insulin secretion after in vivo glucose challenge in wild-type mice after a prolonged HFD. In contrast, insulin secretion in response to glucose was preserved in RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice (Fig. 3*D*).

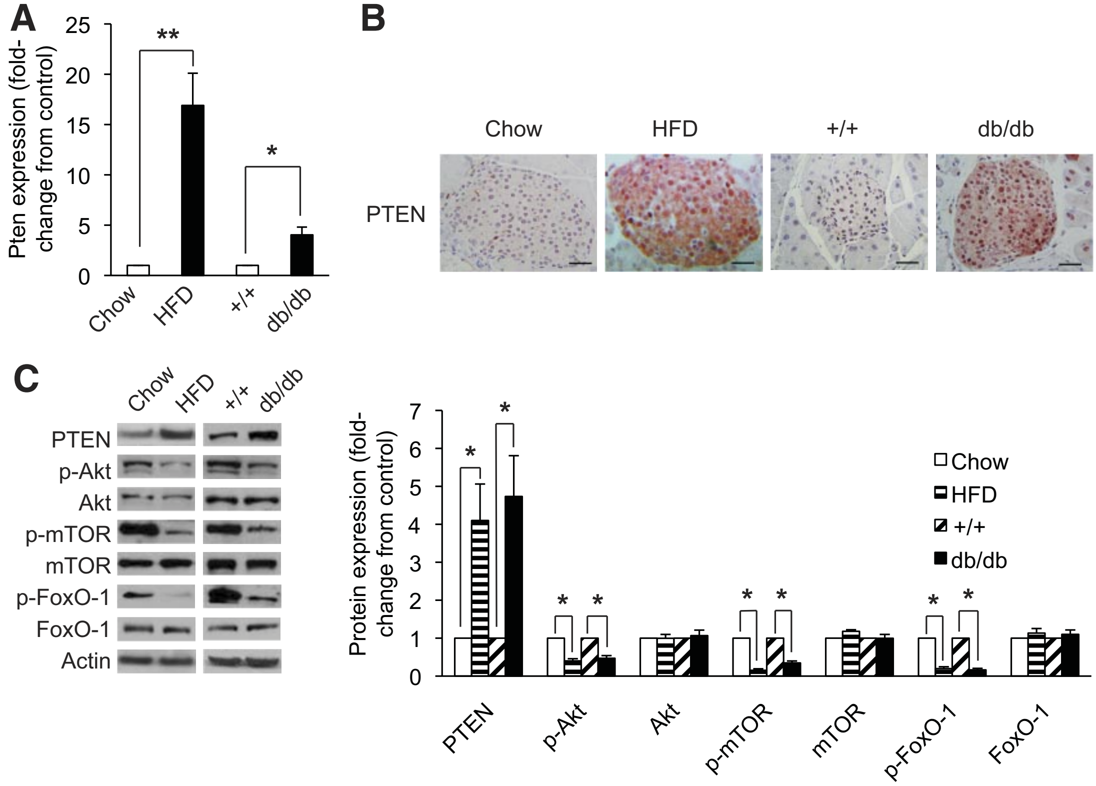

FIG. 1. Islet PTEN upregulation with concomitant attenuation of PI3K signaling in models of type 2 diabetes. *A* and *B*: PTEN transcript levels by quantitative PCR (*A*) and immunohistochemical staining (*B*) of chow- and HFD-fed (after 7 months on HFD); wild-type (+/+) and *db/db* islets (7 months of age) ($n = 3$). *C*: Western blot (left panel) and quantification (*right panel*) of PTEN, p-Akt (Ser473), total Akt, p-mTOR (Ser2448), total mTOR, p-FoxO-1 (Ser253) and total FoxO-1 in chow- and HFD-fed (7 months on HFD); wild-type and *db/db* islets (7 months of age) ($n = 3$). *$P < 0.05$; **$P < 0.005$. Scale bar, 50 μm. The results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

Increased β-cell mass and β-cell size in islets of HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice. During HFD-induced diabetes, development of peripheral insulin resistance leads to a compensatory increase in β-cell mass to meet the increasing demands for insulin. This compensatory proliferation was observed in RIP*cre*$^+$ *Pten*$^{+/+}$ islets on HFD (Fig. 4*A* and *B*). RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets showed an already increased β-cell mass under chow-fed conditions, and we observed no further increase in β-cell mass in the mice on HFD. The increased β-cell mass was due to both an increase in proliferation and β-cell size in RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice under both chow and HFD conditions, which likely reflects the direct effects of PTEN deletion in their β-cells (Fig. 4*C*, *E*, and *F*). Furthermore, age-matched chow- and HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice showed similarly increased proportion of large islets (Fig. 4*D*). Despite the increased proliferation and cellular growth in RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets, their architectures were maintained (Fig. 5*A*).

RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets were protected against HFD-induced β-cell dysfunction. In order to assess the direct effects of PTEN deletion specifically in β-cells, we measured insulin secretion during perifusion assay on isolated islets ex vivo. Under basal condition, both RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets demonstrated similar insulin release after glucose stimulation (Fig. 4*G* and *H*).

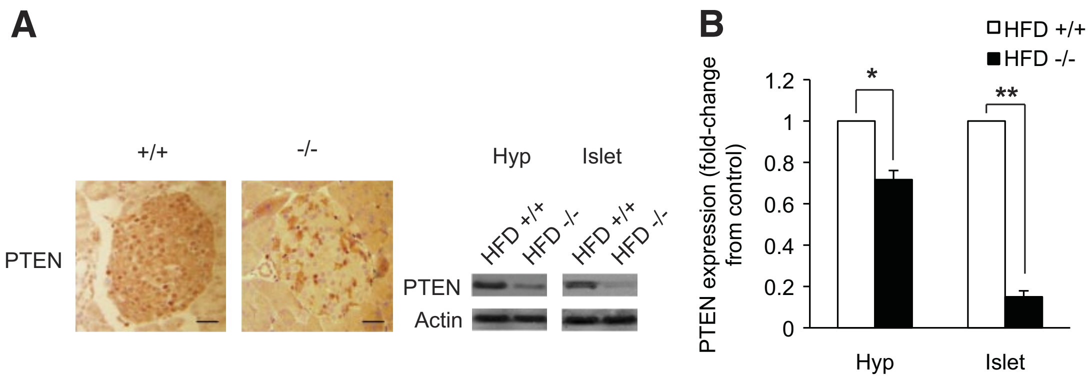

FIG. 2. PTEN deletion in RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets. *A*: Immunohistochemical staining of PTEN in RIP*cre*$^+$ *Pten*$^{+/+}$ (+/+) and RIP*cre*$^+$ *Pten*$^{fl/fl}$ (−/−) pancreas sections. *B*: Western blot (*left panel*) and quantification (*right panel*) of PTEN expression in RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets and hypothalamus (Hyp) ($n = 3$). *$P < 0.05$; **$P < 0.005$. Scale bar, 50 μm. The results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

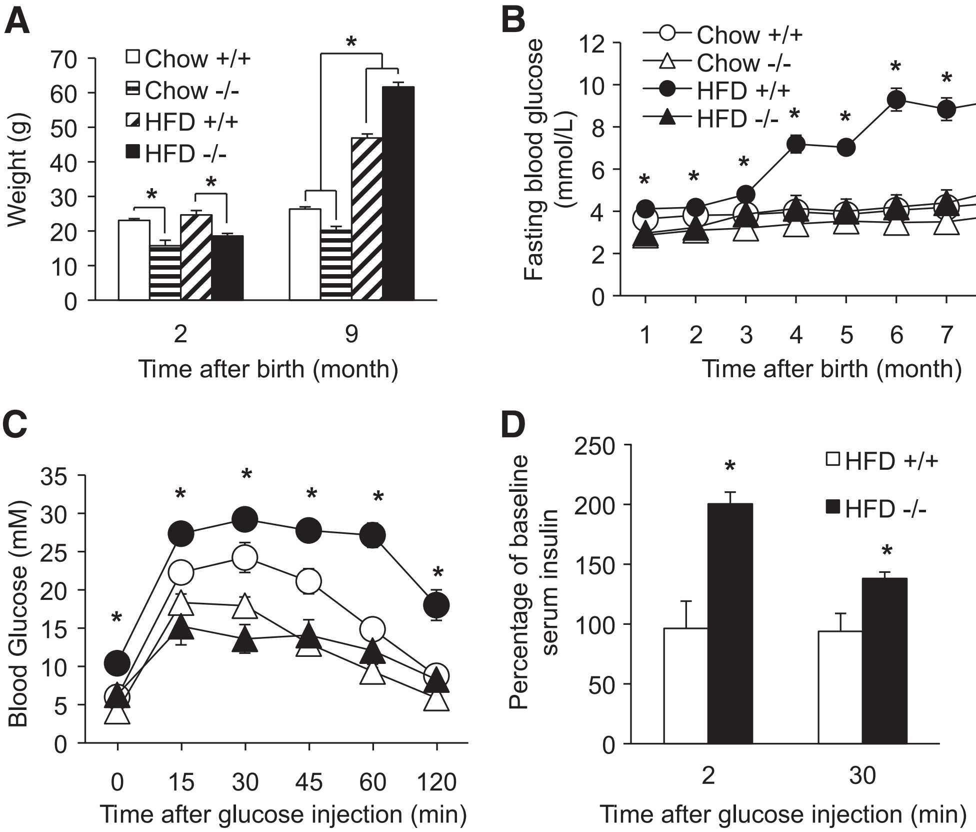

FIG. 3. RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice showed maintained glucose metabolism and in vivo glucose stimulated insulin secretion after prolonged HFD while demonstrating drastic weight gain. *A*: Weight of RIP*cre*$^+$ *Pten*$^{+/+}$ (+/+) and RIP*cre*$^+$ *Pten*$^{fl/fl}$ (−/−) mice at the start of HFD (2 months of age) and after HFD (9 months of age) with chow-fed RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice at the same time points. *B*: Fasting blood glucose of RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice fed either chow or HFD for 7 months (n >7). *C*: Glucose tolerance tests of RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice after 7 months of either chow or HFD feeding (n >7). *D*: in vivo glucose stimulated insulin secretions of RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice after 7 months of either chow or HFD feeding (n >3). **P* < 0.05. The results are presented as mean ± SE.

However, after HFD feeding, RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice maintained robust insulin secretion in response to glucose in contrast to islets of HFD-fed RIP*cre*$^+$ *Pten*$^{+/+}$ mice, which showed attenuation in both phases of insulin secretion (Fig. 4*I* and *J*). These data suggest that diabetes protection in HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice are likely due to both preserved β-cell function and mass.

Maintained high PI3K signaling in RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets after prolonged HFD. To determine the mechanisms underlying the enhanced β-cell growth and maintained function after HFD feeding in islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice, we assessed for alterations in the PI3K signaling pathway. HFD has been shown to attenuate phosphorylation of Akt in β-cells (7). However this decline was not observed in PTEN-deficient islets, which indicates persistent activation of the PI3K pathway even after prolonged exposure to HFD (Fig. 5*A* and *B*). FoxO-1 and mTOR, both downstream effectors of PI3K signaling, showed increased phosphorylation in HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets (Fig. 5*B*). The increased p-mTOR was in keeping with the increased β-cell size in RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice (Fig. 4*E* and *F*). The expression of GLUT-2, a marker of β-cell differentiation and a rate-limiting component in glucose sensing, has also been shown to decline with the progression of type 2 diabetes (31,32). GLUT2 remained high in HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets to a similar degree as the islets of chow-fed RIP*cre*$^+$ *Pten*$^{+/+}$ mice, in contrast with the decline of GLUT2 in islets of HFD-fed RIP*cre*$^+$ *Pten*$^{+/+}$ mice (Fig. 5*A* and *B*). Furthermore, PDX-1, a downstream transcriptional target of insulin signaling important in β-cell differentiation and growth (33), was also increased in RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets, which may also contribute to the preserved β-cell mass and function in HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice (Fig. 5*A* and *B*). However, RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice also exhibited increased peripheral insulin sensitivity because of partial neuronal deletion of PTEN (L.W. and M.W., unpublished data). To circumvent the potential confounding effects of enhanced insulin sensitivity, we next examined RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice in a *db/db* model of type 2 diabetes which did develop insulin resistance as described below.

RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice remained euglycemic despite severe insulin resistance. Both RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice and RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ littermates exhibited similar degrees of weight gain and insulin resistance (Fig. 6*A* and *D*). However, despite severe insulin resistance in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice, they continued to remain remarkably euglycemic and showed normal glucose tolerance (Fig. 6*B* and *C*). Furthermore, *in vivo* GSIS experiments showed robust insulin secretion in response to glucose stimulation in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice (Fig. 6*E*). Interestingly, islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice demonstrated similar degrees of hypertrophy and proliferation as those of RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ islets and did not show a further increase in β-cell mass compared with *db/db* controls (Fig. 7*B* and *C*). Islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice showed no signs of disorganized architecture (Fig. 7*A*).

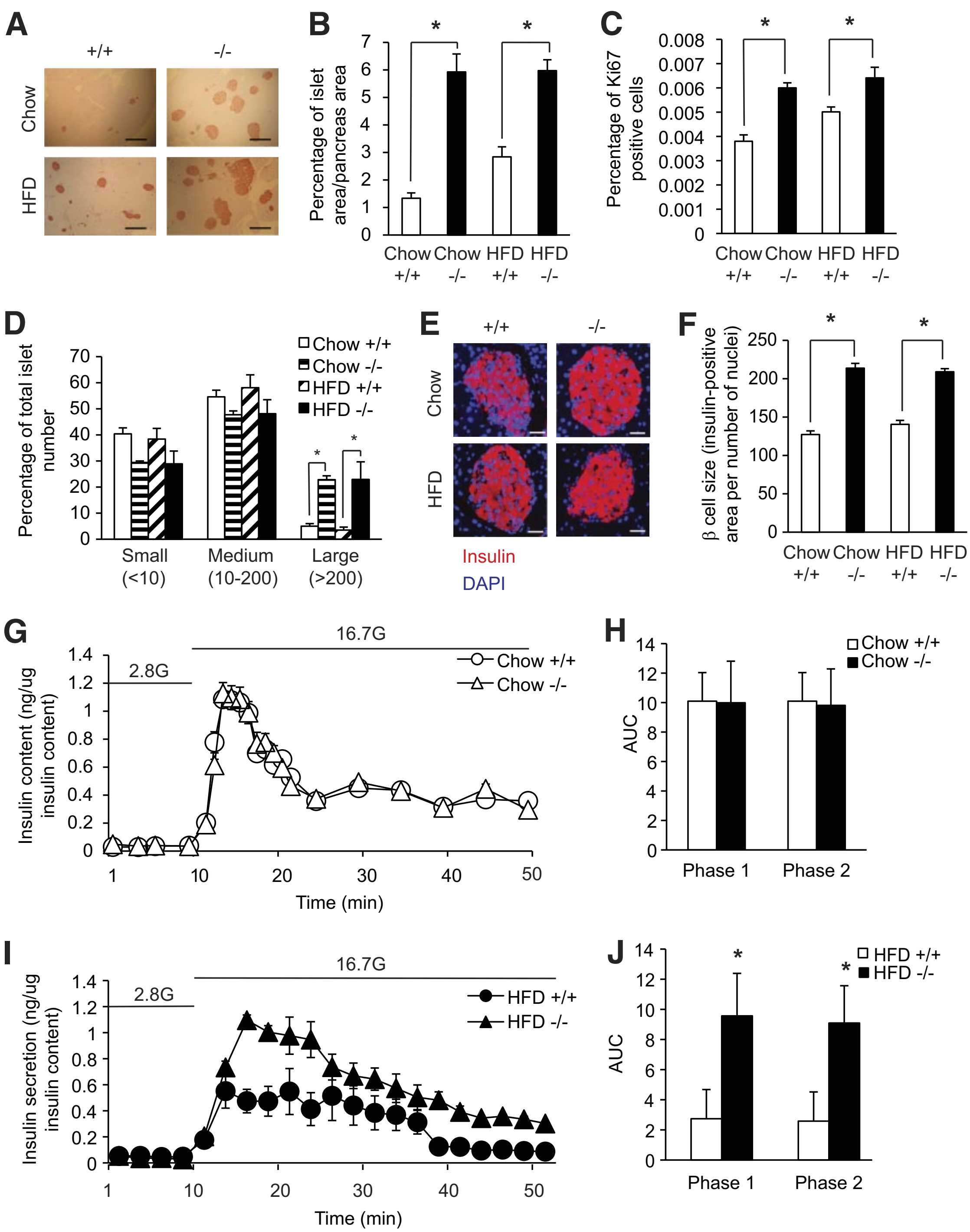

FIG. 4. RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice maintained high islet mass and β-cell size with protection against HFD-induced β-cell dysfunction. *A* and *B*: Insulin staining (*A*) and quantification (*B*) of pancreas sections of RIP*cre*$^+$ *Pten*$^{+/+}$ (+/+) and RIP*cre*$^+$ *Pten*$^{fl/fl}$ (−/−) mice fed either chow or HFD (n = 3), Scale bar, 500 μm. *C*: Percentage of Ki67 positive cells in islets from RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice fed either chow or HFD (n = 3). *D*: proportion of small (<10 cells), medium (10–200 cells) and large (>200 cells) islet in pancreas from RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice fed either chow or HFD (n = 3). *E* and *F*: Immunofluorescent staining of insulin/DAPI (*E*) and quantification of β-cell size (*F*) of pancreas from RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice fed either chow or HFD (n = 3), Scale bar, 50 μm. *G* and *H*: Insulin secretion per 60 islets during perifusion analysis (*G*) and quantification of area under the curve (AUC) (*H*) of chow-fed RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets (n = 3). *I* and *J*: Insulin secretion per 60 islets during perifusion analysis (*I*) and quantification of area under the curve (*J*) of HFD-fed RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets (n = 3). *P < 0.05. Results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets demonstrated increased β-cell function. To assess the direct effects of PTEN deletion on β-cell function in the absence of leptin signaling, we examined islet function ex vivo by perifusion. RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ islets showed diminished insulin secretion, whereas robust first phase insulin secretion was observed in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets after glucose stimulation, further confirming the preserved glucose-responsive insulin secretory function in PTEN-deficient β-cells in *db/db* mice (Fig. 6*F* and *G*). Thus, in contrast to the HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice, where the combination of increased β-cell mass and function likely

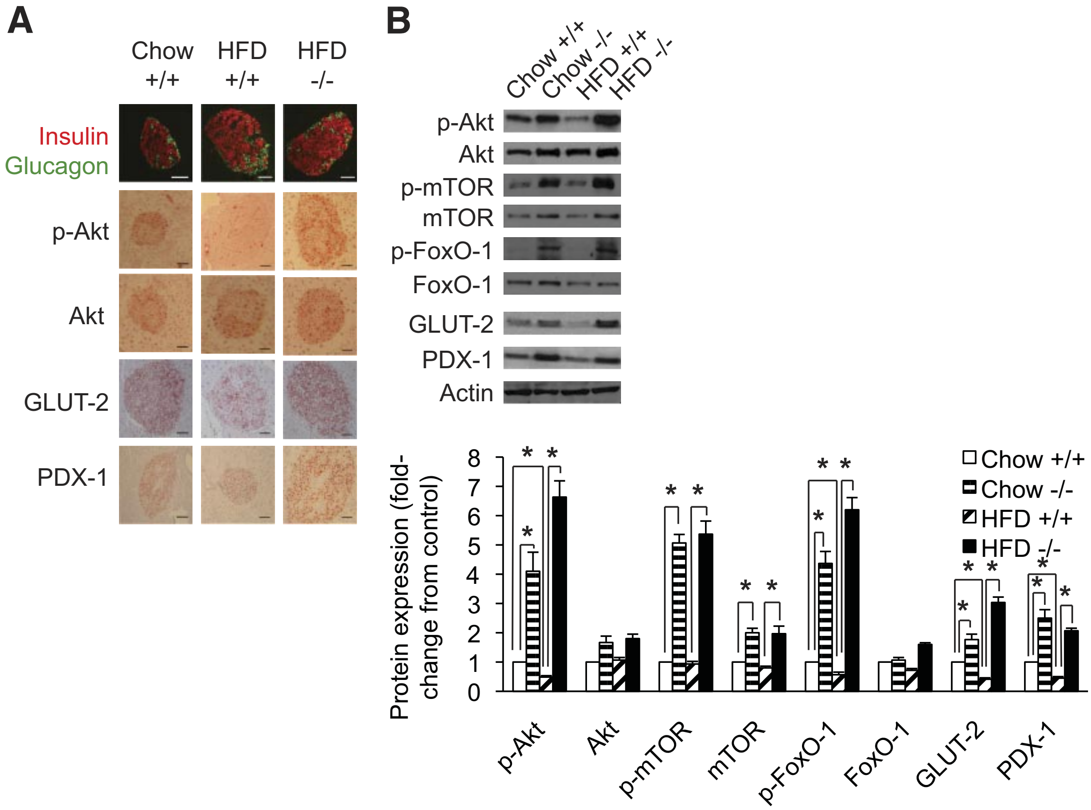

FIG. 5. RIP*cre*⁺ *Pten*$^{fl/fl}$ mice maintained islet PI3K signaling after prolonged HFD. *A*: Immunofluorescent staining of insulin/glucagon, and immunohistochemical staining of p-Akt (Ser473), total Akt, GLUT2, and PDX-1 in chow-fed, HFD-fed RIP*cre*$^+$ *Pten*$^{+/+}$ (+/+) and HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ (−/−) mice. *B*: Western blot (*left panel*) and quantification (*right panel*) of p-Akt (Ser473), total Akt, p-mTOR (Ser2448), total mTOR, p-FoxO-1 (Ser253), total FoxO-1, GLUT-2, and PDX-1 of islets from RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ mice fed either chow or HFD (n = 3). *P < 0.05. Scale bar, 50 μm. Results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

contributed to the protective effect against diabetes, increased β-cell function and not mass in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice was likely responsible for their diabetes protection.

Enhanced PI3K signaling in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets. We next determined the effects of PTEN deletion on PI3K signaling in β-cells in the *db/db* model. RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ islets showed reduced levels of p-Akt, whereas PTEN deletion completely rescued this defect (Fig. 7*A* and *D*). Phosphorylation of mTOR and FoxO-1 also remained high in RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets, consistent with constitutively active PI3K cascade (Fig. 7*D*). In keeping with its normal insulin secretion in response to glucose, GLUT2 was highly expressed in islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice in contrast to the near-complete loss of GLUT2 expression in islets of *Lepr*$^{db/db}$ mice (Fig. 7*A* and *D*). PDX-1 was also downregulated in RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ islets, whereas the RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets continued to show intense expression of PDX-1 and nuclear localization of the protein to a similar degree as the wild-type islets on chow diet (Fig. 7*A* and *D*).

Tumorigenic response to DNA-damaging stimuli is not compromised in islets of RIP*cre*$^+$ *Pten*$^{fl/fl}$ or RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice. Given PTEN's role as a tumor suppressor in various cancer-prone tissues (16–18), we assessed for changes suggestive of cellular transformation and response to DNA-damaging stimuli such as gamma irradiation. Despite the significant increase in islet mass in both HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets, intact islet integrity was observed even in the 9-month-old aged mice fed either HFD or on *db/db* background. Intact laminin staining in the basement membrane as well as localization of β-catenin to the plasma membrane both demonstrated absence of deregulated growth (Fig. 8*A*). Furthermore, induction of DNA damage with gamma irradiation showed a similar degree of DNA-repair and apoptosis response in both RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets, including phosphorylation of p53 and cleavage of MDM2 (Fig. 8*C*). The transcript levels of p53 target genes, including Bax, Mdm2, and p21 also showed similar changes in response to gamma irradiation between RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets (Fig. 8*B*). Interestingly RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets exhibited significantly reduced expression of proapoptotic gene Bax under basal condition, which is consistent with their constitutively active PI3K signaling (Fig. 8*B*). Thus, PTEN deletion in β-cells protects against β-cell dysfunction in both HFD and *db/db* models of type 2 diabetes with no finding suggestive of deregulated growth. Furthermore, PTEN-deleted β-cells still maintained intact response to DNA-damaging stimuli, which further suggests that they are not more prone to tumor formation.

DISCUSSION

In pre-diabetic individuals, insulin resistance in the classic metabolic tissues including the liver, muscle, and fat is present; however, glucose homeostasis can be maintained as long as pancreatic β-cells are able to increase insulin

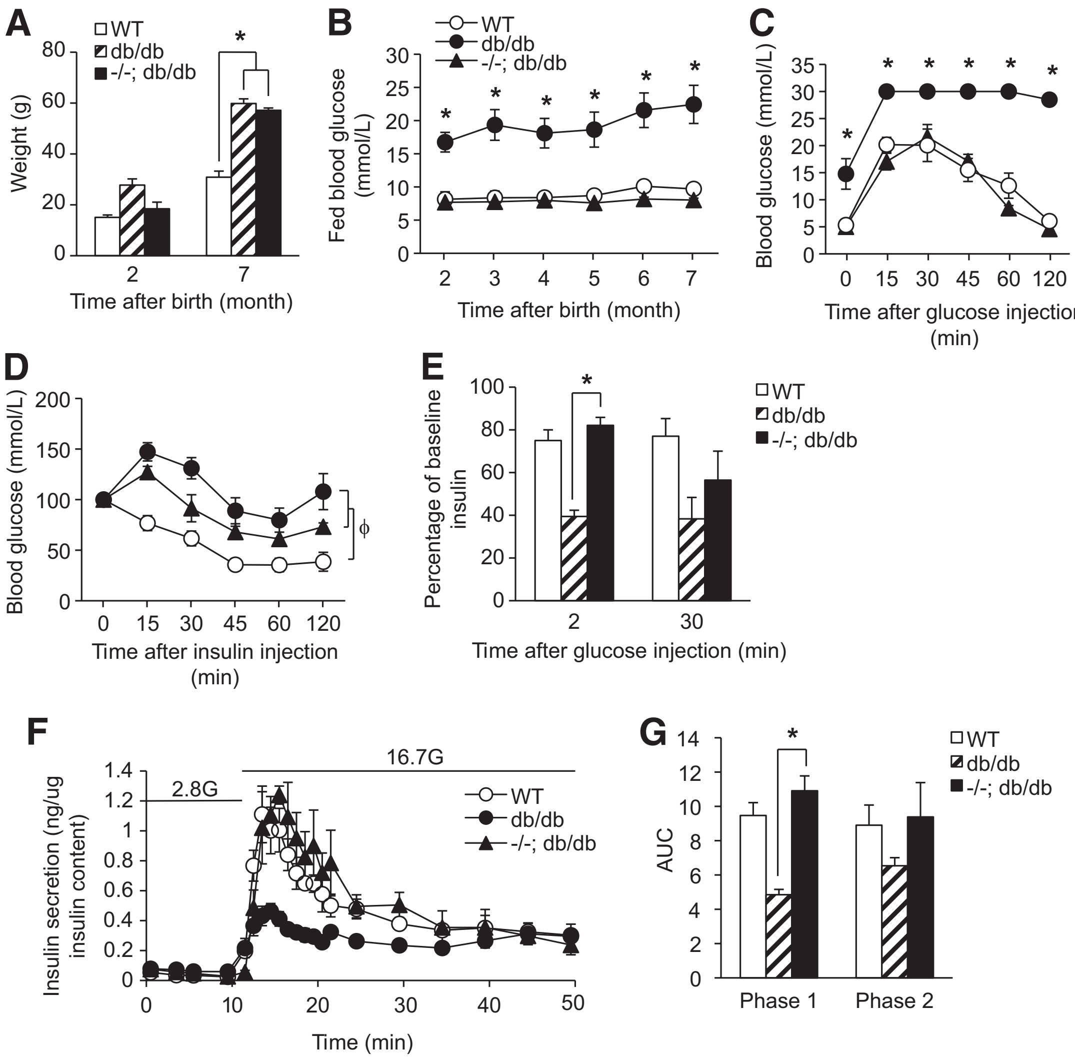

FIG. 6. RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice exhibited comparable weight gain and normal glucose tolerance despite being insulin resistant with normal β-cell function. *A*: Weight of wild-type (WT), RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ (*db/db*), and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ (−/−; *db/db*) mice at 2 and 7 months of age (n >7). *B*: Fed blood glucose of wild-type, RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice from 2 to 7 months of age (n >7). *C*: Glucose tolerance test of wild-type, RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice at 7 months of age (n >7). *D*: Insulin tolerance test of wild-type, RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice at 7 months of age (n >7). *E*: In vivo glucose-stimulated insulin secretion of wild-type, RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice at 7 months of age (n = 3). *F* and *G*: Insulin secretion per 60 islets during perifusion analysis (*F*) and quantification of area under the curve (*G*) of wild-type, RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice (n = 3). *P < 0.05 for RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice compared with RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ mice or as indicated; ϕP < 0.05 for both RIP*cre*⁺ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ and RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice compared with wild-type mice. Results are presented as mean ± SE.

production to compensate for increased insulin demands (7,34,35). Accumulating evidence shows that a defect in insulin-PI3K signaling in pancreatic β-cells may contribute to the development of β-cell dysfunction, leading to the onset of type 2 diabetes. Indeed, mice with genetic ablation of insulin/IGF-1 receptors specifically in the β-cells demonstrate reduced β-cell function (10,12,13). Therefore, a new paradigm of type 2 diabetes pathogenesis suggests that diminished insulin responsiveness specifically in the pancreatic β-cells plays a central role in disease development.

Interestingly, PTEN, a critical negative regulator of the PI3K signaling pathway, has been shown to be upregulated in β-cells that have complete absence of insulin/IGF-1 signaling (25), leading to a hypothesis that PTEN may play a causal role in the pathogenesis of β-cell dysfunction. Here we have shown that when type 2 diabetes was induced, either by HFD or global leptin signaling deficiency, increased PTEN expression was observed in islets along with diminished PI3K signaling, suggesting that β-cell PTEN may indeed play a critical role in the development of type 2 diabetes. We and others have already shown that tissue-specific increase in PI3K signaling in pancreatic β-cells resulting from deletion of PTEN leads to increased β-cell mass and function under basal conditions (23,24). However, the biologic outcome from continued deletion of PTEN in β-cells under metabolically stressed conditions was elusive. In this report, we show that after prolonged HFD, RIP*cre*⁺ *Pten*$^{fl/fl}$ mice continued to exhibit increased islet mass and were protected against the loss of glucose-stimulation insulin secretion. The enhanced β-cell function was demonstrated not only in vivo, but also in vitro, which supports the direct effect of PTEN in β-cells. However, RIP*cre*⁺ *Pten*$^{fl/fl}$ mice exhibited enhanced insulin sensitivity, which may have masked the full potential effects of PTEN deletion in pancreatic β-cells against metabolic stress in the HFD model.

In the *db/db* model, RIP*cre*⁺ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice

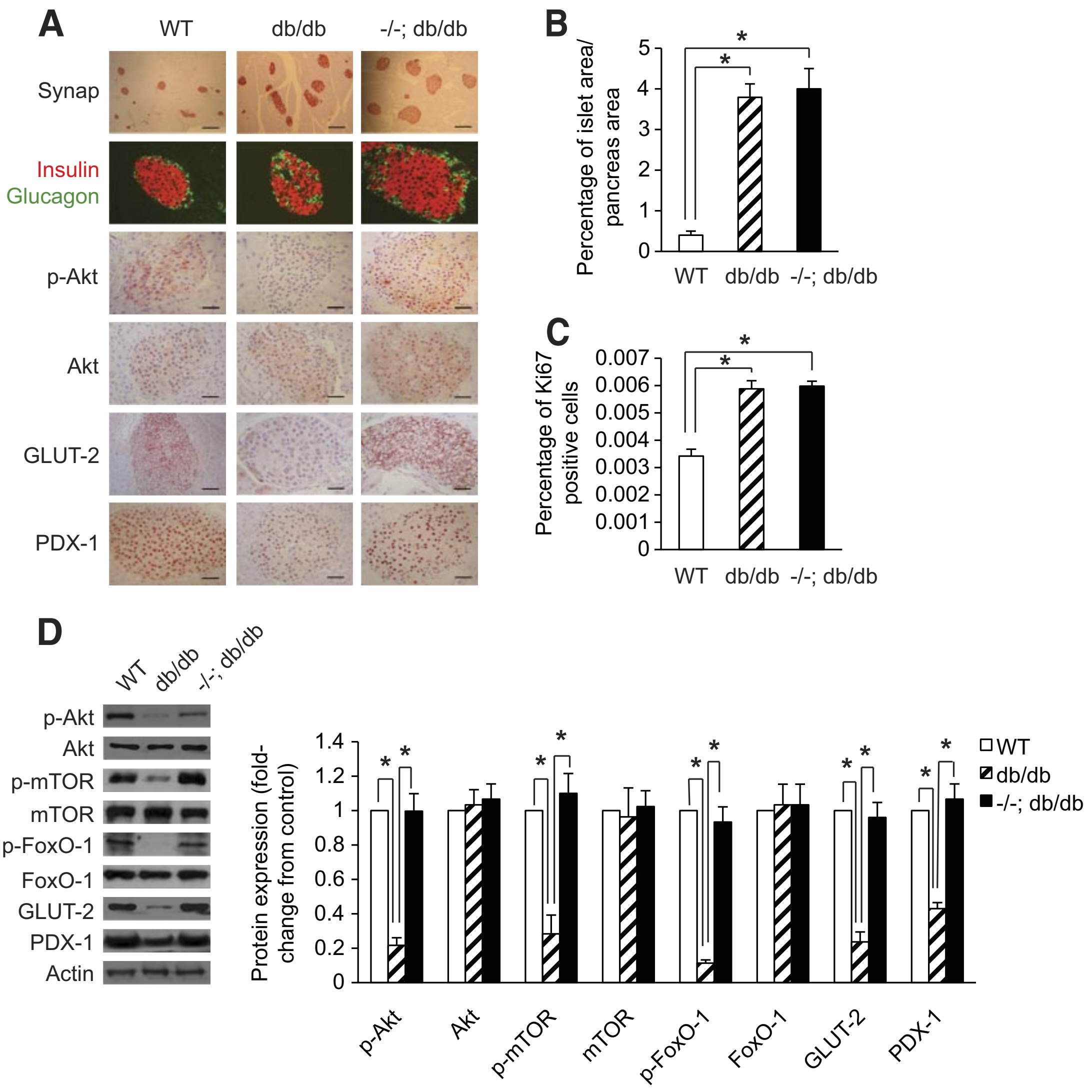

FIG. 7. RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice exhibit islet hypertrophy with normal islet architecture and enhanced PI3K signaling. *A*: Immunofluorescent staining of insulin/glucagon, and immunohistochemical staining of synaptophysin, p-Akt (Ser473), total Akt, GLUT2, and PDX-1 in wild-type (WT), RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ (*db/db*), and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ (−/−; *db/db*) mice at 7 months of age. *B*: Quantification of islet area in wild type, RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ at 7 months of age ($n = 4$). *C*: Percentage of Ki67 positive cells in wild-type, RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice at 7 months of age ($n = 4$). *D*: Western blot (*left panel*) and quantification (*right panel*) of p-Akt (Ser473), total Akt, p-mTOR (Ser2448), total mTOR, p-FoxO-1 (Ser253), total FoxO-1, GLUT-2, and PDX-1 of islets from wild-type, RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$, and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice at 7 months of age ($n = 3$). *$P < 0.05$. Scale bar, 500 μm (*A*, *top panel*); 50 μm (*A*, *rows 2 to 6*). Results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

developed obesity with significantly diminished insulin sensitivity to a similar degree as the RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ littermate controls (Fig. 6*A* and *D*). Yet, RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice remained completely glucose tolerant and euglycemic throughout the 7-month period. Islets from RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice demonstrated an increase in islet area to a similar degree as those of RIP*cre*$^+$ *Pten*$^{+/+}$ *Lepr*$^{db/db}$ mice. However RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ islets remained responsive to glucose-stimulated insulin secretion. This is likely attributed by the PI3K signaling which remained activated in these islets. As such GLUT-2 and PDX-1, which are important in glucose sensing and cell differentiation, are maintained. These data illustrate the importance of the negative regulation that PTEN exerts on β-cells, whereupon PTEN deletion leads to enhanced PI3K signaling and protection against β-cell dysfunction that occurs in type 2 diabetes.

Given the well known tumorigenic effects of PTEN deletion in some tissues, we assessed for evidence of deregulated growth (16–18). PTEN deficient islets showed intact architecture and demonstrated intact DNA repair response to gamma irradiation as wild-type counterparts. Furthermore, previously we have shown that PTEN deletion in combination with the activation of the cMyc oncogene is still not able to lead to tumor formation (36). However, more extensive analysis and longitudinal observation are required to conclusively demonstrate the full impact of PTEN deletion in pancreatic β-cells on tumorigenesis. It is worth noting that PTEN-deleted β-cells behave differently than β-cells that express constitutively active Akt. Although the RIP-Akt transgenic mice exhibit increased islet mass, these mice have compromised β-cell function (37,38). PTEN-deficient β-cells, in contrast, do not show loss of function even after a prolonged exposure to HFD, despite the continued proliferation and PI3K signaling within these cells. This important distinction between PTEN-deleted and Akt-overexpressing β-cells illustrates the fundamental difference between removing a

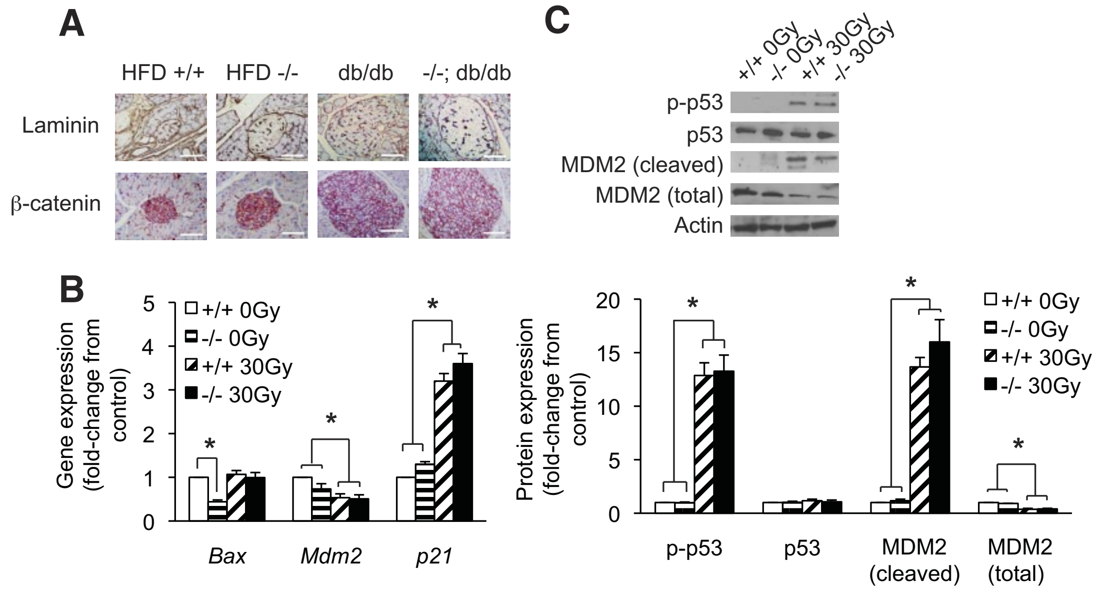

FIG. 8. HFD-fed RIP*cre*$^+$ *Pten*$^{fl/fl}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ mice showed intact islet integrity and uncompromised response to gamma irradiation. *A*: Laminin and β-catenin staining demonstrated intact basement membrane and cell-to-cell adhesion in HFD-fed (9 months of age) RIP*cre*$^+$ *Pten*$^{fl/fl}$ (−/−) and RIP*cre*$^+$ *Pten*$^{fl/fl}$ *Lepr*$^{db/db}$ (−/−; *db/db*) (9 months of age) mice. *B*: Transcripts levels of p53-related gene including *Bax*, *Mdm2*, and *p21* during quantitative PCR of RIP*cre*$^+$ *Pten*$^{+/+}$ (+/+) and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets with or without 30Gy gamma irradiation ($n = 2$). *C*: Western blotting (*left panel*) and quantification (*right panel*) of p-p53 (Ser392), total p53, cleaved MDM2, and total MDM2 in RIP*cre*$^+$ *Pten*$^{+/+}$ and RIP*cre*$^+$ *Pten*$^{fl/fl}$ islets with or without 30Gy gamma irradiation ($n = 2$). *$P < 0.05$. Scale bar, 50 μm. Results are presented as mean ± SE. (A high-quality digital representation of this figure is available in the online issue.)

physiologic brake, which would allow for PI3K signaling to return to physiologic levels, whereas forced expression of Akt may lead to supraphysiologic levels of PI3K signaling that can lead to cell proliferation and dedifferentiation. Further investigations to delineate other important genes or pathways that distinguish between these two modes of enhancing PI3K signaling may unveil clinically safe therapeutic targets for β-cells.

In summary, our findings show that PTEN plays a critical role in the pathogenesis of β-cell dysfunction in type 2 diabetes and highlight the tissue-specific role of PTEN in attenuating PI3K signaling in β-cells. Our results show that PTEN deletion in β-cells leads to an increase in β-cell mass and function as a result of restoring PI3K signaling to a physiologic level in diabetic condition. Indeed, PTEN deletion leads to complete protection against β-cell defects even in the face of severe insulin resistance, providing genetic evidence of the critical negative regulation that PTEN exerts in impeding β-cell function and growth that occurs in type 2 diabetes. Our results highlight opportunities for therapeutic targeting of PTEN in β-cells for treatment or prevention of type 2 diabetes.

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L.W. researched data, contributed to discussion, wrote the manuscript, and reviewed/edited the manuscript. Y.L., S.Y.L., K.-T.T.N., S.A.S., and A.S. researched data. T.W.M. and H.G. contributed to discussion. M.W. contributed to discussion and reviewed/edited the manuscript.

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