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The mitochondrial enzyme pyruvate carboxylase restricts pancreatic β-cell senescence by blocking p53 activation

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Defective glucose-stimulated insulin secretion (GSIS) and β-cell senescence are hallmarks in diabetes. The mitochondrial enzyme pyruvate carboxylase (PC) has been shown to promote GSIS and β-cell proliferation in the clonal β-cell lines, yet its physiological relevance remains unknown. Here, we provide animal and human data showing a role of PC in protecting β-cells against senescence and maintaining GSIS under different physiological and pathological conditions. β-cell-specific deletion of PC impaired GSIS and induced β-cell senescence in the mouse models under either a standard chow diet or prolonged high-fat diet feeding. Transcriptomic analysis indicated that p53-related senescence and cell cycle arrest are activated in PC-deficient islets. Overexpression of PC inhibited hyperglycemia- and aging-induced p53-related senescence in human and mouse islets as well as INS-1E β-cells, whereas knockdown of PC provoked senescence. Mechanistically, PC interacted with MDM2 to prevent its degradation via the MDM2 binding motif, which in turn restricts the p53-dependent senescent program in β-cells. On the contrary, the regulatory effects of PC on GSIS and the tricarboxylic acid (TCA) anaplerotic flux are p53-independent. We illuminate a function of PC in controlling β-cell senescence through the MDM2–p53 axis.

diabetes | pyruvate carboxylase | cellular senescence | p53 | MDM2

Significance

β-cell senescence contributes to type 2 diabetes in aging, but the underlying mechanisms are yet to be fully understood. Apart from its widely recognized role in glucose-stimulated insulin secretion, we report that pyruvate carboxylase (PC) prevents β-cell senescence by stabilizing MDM2 protein, thereby restricting p53-mediated senescent program in both rodent and human β-cells. Therefore, targeting PC in β-cell represents a promising approach to prevent or reverse the key pathogenic events, including defective insulin secretion and senescence, in diabetes.

Pancreatic β-cell dysfunction, characterized by defective insulin secretion and reduced β-cell mass, is a central cause of type 2 diabetes (1). Glucose controls insulin secretion and maintenance of functional β-cell mass. Glucose is first metabolized to pyruvate through glycolysis, followed by the the tricarboxylic acid (TCA) cycle in mitochondria to generate coupling factors, such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), for insulin secretion. Short-term high glucose exposure induces β-cell proliferation and maintains survival, which are mediated in part by carbohydrate-responsive element-binding protein and activation of proproliferative protein kinases including protein kinase B (AKT) and extracellular signal-regulated kinase (2–4). However, chronic hyperglycemia (also known as glucotoxicity) triggers cellular senescence, mitochondrial dysfunction, oxidative stress, and dedifferentiation in β-cells, yet the underlying pathways remain poorly understood (5–8).

As nutrient sensors and metabolite generators, mitochondria play a central role in regulating β-cell functions. Mutations in mitochondrial DNA and transcription factors like *mitochondrial transcription factor-A* (*TFAM*) have been linked to diabetes (9). Several mitochondrial enzymes, including pyruvate carboxylase (PC) (10, 11), succinate dehydrogenase (SDH) (12), and pyruvate dehydrogenase (PDH) (13), along with their derived metabolites, are known to control insulin secretion and β-cell mass in rodents and humans. Glucose-derived pyruvate enters the TCA cycle via PC or PDH in pancreatic β-cells. PC mediates around half of the carbon flux from glucose into the TCA cycle, generating coupling factors ATP and NADPH for insulin secretion (14, 15). PC expression positively correlates with glucose responsiveness and is up-regulated by glucose stimulation in β-cells (16). PC-mediated anaplerotic flux to oxaloacetate (OAA) and malate from glucose is increased upon after 60% pancreatectomy (17). On the contrary, PC expression is down-regulated in pancreatic β-cells/islets under diabetic conditions in humans and rodents (18, 19). Knockdown of PC or inhibition of PC with phenylacetic acid impairs glucose-stimulated insulin secretion (GSIS) and β-cell proliferation (17, 20). Our previous work showed that PC transcription is repressed by p53 activation in pancreatic β-cells under obese conditions, resulting in defective GSIS and glucose intolerance in the mouse model (21). Beyond effects on insulin secretion and proliferation, Nika Danial's research team found that PC protects against inflammatory cytokine-induced β-cell apoptosis by activating the urea cycle to neutralize nitric oxide production (22). More recently, the same team demonstrated that PC mediates glucose-derived glutathione biosynthesis, thereby blocking inflammation and oxidative stress in human and rodent islets (23).

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While previous studies examined the role of PC in β-cell functions using cell lines or isolated islets, its physiological function in vivo has never been explored. To fill this research gap, we generated a β-cell-specific PC knockout (β-PCKO) mouse model using the Cre-LoxP system. We demonstrated that β-cell selective deletion of PC impaired GSIS and reduced β-cell mass in mice fed either a standard chow (STC) or high-fat diet (HFD). Surprisingly, we revealed an anti-senescent function of PC in β-cells through regulation of the MDM2–p53 axis, and such a beneficial effect is independent of its anaplerotic function.

Results

Conditional Deletion of *Pc* in Pancreatic β-Cells Impairs GSIS and Induces Glucose Intolerance in Mice. To determine the physiological functions of PC in pancreatic β-cells, we generated β-PCKO mice by crossing $Pc^{floxed/floxed}$ mice with transgenic mice expressing Cre recombinase under the control of rat insulin II promoter (RIP-Cre mice) (*SI Appendix*, Fig. S1*A*). The pancreas from 12-wk-old male β-PCKO mice and their WT littermates were subjected to immunofluorescent staining and islet isolation. This analysis revealed an obvious decrease of PC expression in the insulin-positive β-cells in the pancreatic sections of β-PCKO mice (Fig. 1*A*). Consistently, immunoblotting analysis showed a significant reduction of PC expression in the islets of β-PCKO mice compared to their WT littermates (Fig. 1*B*), whereas PC expressions in other tissues were comparable between the two genotypes (*SI Appendix*, Fig. S1*B*). Next, we evaluated the effect of β-cell-specific deletion of *Pc* on glucose metabolism. At the age of 8-wk-old, β-PCKO mice, WT littermates, and RIP-Cre controls displayed comparable glucose tolerance as assessed by the intraperitoneal glucose tolerance test (IPGTT) (Fig. 1*C*). However, β-PCKO mice exhibited glucose intolerance when compared to WT controls or RIP-Cre controls at 16-wk-old (Fig. 1*D*), and this impairment was mainly due to defective GSIS (Fig. 1*E*) but not insulin resistance (Fig. 1*F*). Since RIP-Cre controls and WT

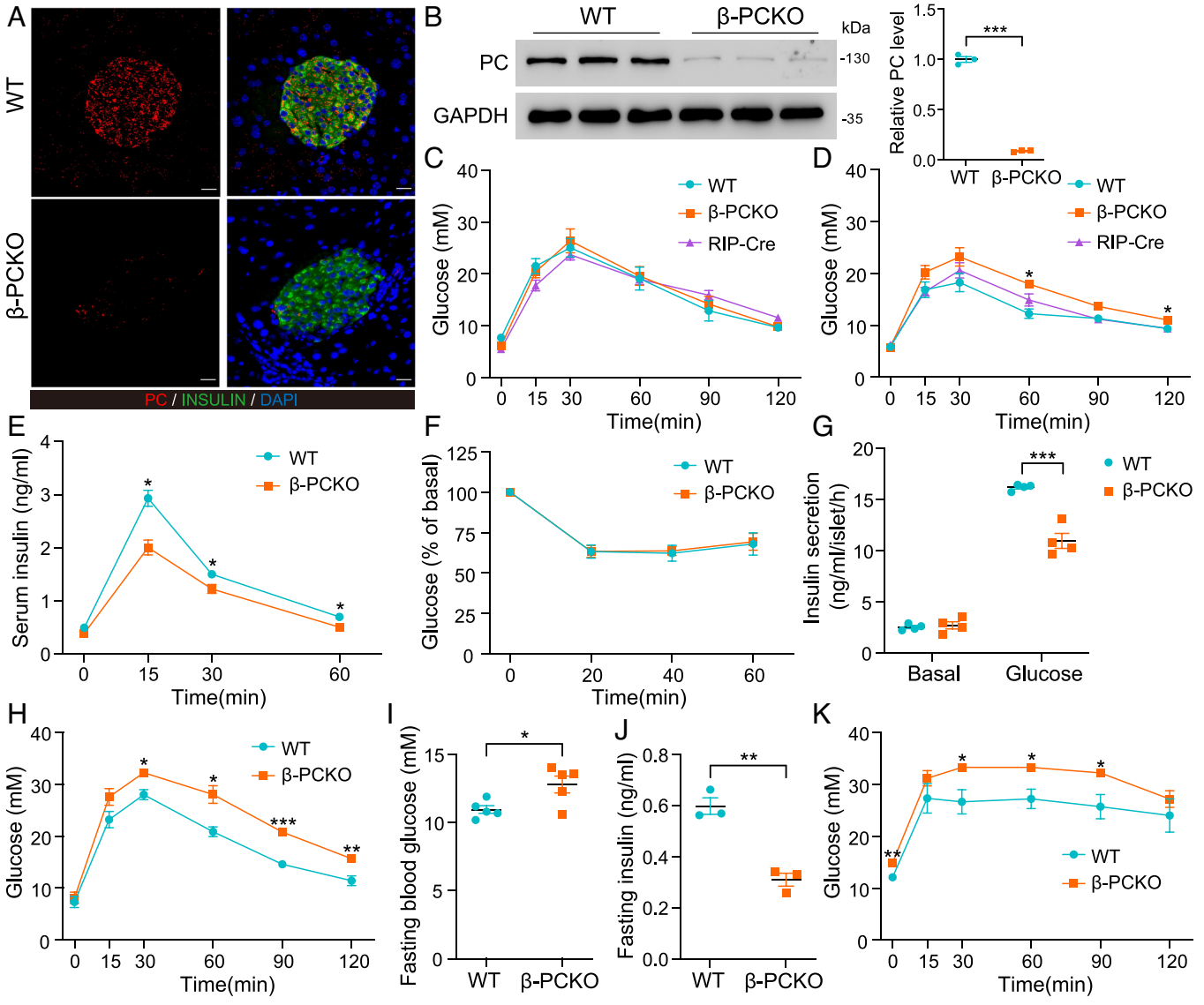

Fig. 1. β-cell-specific deletion of *Pc* induces glucose intolerance and impairs GSIS. (*A*–*G*) Male RIP-PC KO (β-PCKO) mice, their WT littermates, and RIP-Cre controls fed with standard chow (STC) were used. (*A*) Immunofluorescence (IF) staining of PC (red) and INSULIN (green) in the pancreatic sections of 12-wk-old mice. (Scale bar: 20 μm.) (*B*) Immunoblotting analysis of PC in the isolated islets and quantification. (*C*) GTT, 8-wk-old mice (n = 4). (*D*) GTT, 16-wk-old mice (n = 4). (*E*) GSIS during GTT in (*D*). (*F*) ITT, 16-wk-old mice (n = 4). (*G*) Static insulin secretion under basal (2.8 mM) and glucose (20 mM) stimulation conditions in isolated islets from 26-wk-old mice (n = 4). (*H*–*K*) 8-wk-old male β-PCKO and WT mice were fed with 60% high-fat diet (HFD) for indicated time points. (*H*) GTT in 12-wk-old mice (n = 4). (*I*) Fasting blood glucose and (*J*) fasting serum insulin level of mice (n = 3 to 5). (*K*) GTT in 40-wk-old mice (n = 4). Significance was determined using two-tailed independent Student's *t* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All values are represented as mean ± SEM.

controls displayed similar glucose metabolism, we only included WT littermates for the subsequent experiments, unless otherwise specified. Consistently, pancreatic islets from 26-wk-old β-PCKO mice also displayed a remarkable defective GSIS compared to those from the WT controls (Fig. 1*G*), along with glucose intolerance (*SI Appendix*, Fig. S1*C*).

To further elucidate the functional role of PC in β-cells under metabolic stress, 8-wk-old β-PCKO mice were fed with a HFD. β-cell-specific deletion of *Pc* exacerbated HFD-induced glucose intolerance and fasting hyperglycemia, accompanied by a lower fasting insulin level (Fig. 1 *H*–*K*). Of note, body weights were similar between β-PCKO and WT littermates during the HFD feeding (*SI Appendix*, Fig. S1*D*) (24, 25), indicating that the change of glucose metabolism is unlikely due to the change of energy balance.

Deletion of *Pc* Inhibits β-Cell Proliferation. Apart from insulin secretion, PC is known to regulate proliferation and apoptosis in pancreatic β-cell lines (10, 22). We thus measured β-cell mass in β-PCKO mice. Immunohistochemical staining showed that islet mass was significantly reduced in β-PCKO mice under the STC feeding for 18 wk or HFD feeding for 32 wk (so-called long-term HFD model) (*SI Appendix*, Fig. S2*A*). The changes were associated with reduced Ki-67 positive β-cells (*SI Appendix*, Fig. S2*B*). On the other hand, β-cell apoptosis, detected by TUNEL staining, was virtually undetectable in both β-PCKO and WT littermates (*SI Appendix*, Fig. S2*C*).

To further confirm the role of PC in β-cell proliferation in vivo, we subjected the mice to HFD feeding for 7 d (26) or subjected the mice to partial pancreatectomy (PPx). The short-term HFD treatment triggers β-cell proliferation without affecting body weight and insulin resistance (26). PPx is a well-established approach to stimulate compensatory β-cell proliferation under normoglycemic status (17). Short-term HFD significantly increased number of Ki-67 positive β-cell in WT controls as previously reported (26), but such dietary effect was diminished by β-cell deletion of *Pc* (*SI Appendix*, Fig. S3*A*) (27). 60% PPx increased number of Ki-67 positive β-cells in 12-wk-old WT mice when compared to those that received sham operation (*SI Appendix*, Fig. S3 *B* and *C*). However, such induction of β-cell proliferation was partially abolished by β-cell deletion of *Pc* (*SI Appendix*, Fig. S3*C*).

PC Downregulation Enhances p53-Dependent Senescence in Pancreatic β-Cells. To gain a comprehensive understanding of PC on β-cell functions, we conducted RNA sequencing (RNAseq) in the isolated pancreatic islets of 12-wk-old β-PCKO mice and WT mice fed with STC. RNAseq analysis revealed approximately 2,000 genes were differentially expressed between the two genotypes (log2 fold ≥1 or ≤−1.0; *P* adjusted value ≤ 0.05; *SI Appendix*, Fig. S4*A*). The transcripts annotated to divergent KEGG pathways including p53 on the top, followed by adipocytokine, fatty acid metabolism, DNA replication, and TCA cycle are enriched in the islets β-PCKO mice (Fig. 2*A*). *p53* signaling pathway is known to control cell proliferation, apoptosis, and senescence, which might contribute to the reduction of β-cell mass in β-PCKO mice. The RNAseq data identified 56 genes related to p53-dependent senescence (such as *Serpine1*, *Cdkn2a*, *Cdkn1a*), apoptosis (such as *Casp9*, *Bid*, *Fas*), DNA repair (such as *Rrm2b*, *Rrm2*, *Ddb2*), and cell cycle (such as *Cdk2*, *Ccnb1*, *Chek2*) were differentially expressed between the islets of β-PCKO mice and WT littermates (Fig. 2*B* and *SI Appendix*, Fig. S4*B*). QPCR analysis confirmed the senescent gene *Cdkn1a*, and the cell cycle genes such as *Ccnb1* and *Cdk2* but not the apoptotic genes such as *Caspase9, Bid, and Fas*, were influenced by deletion of *Pc* in the pancreatic islets (Fig. 2*C*). The reason for inconsistence of the apoptotic gene expression between RNAseq and QPCR is unknown, but might be due to

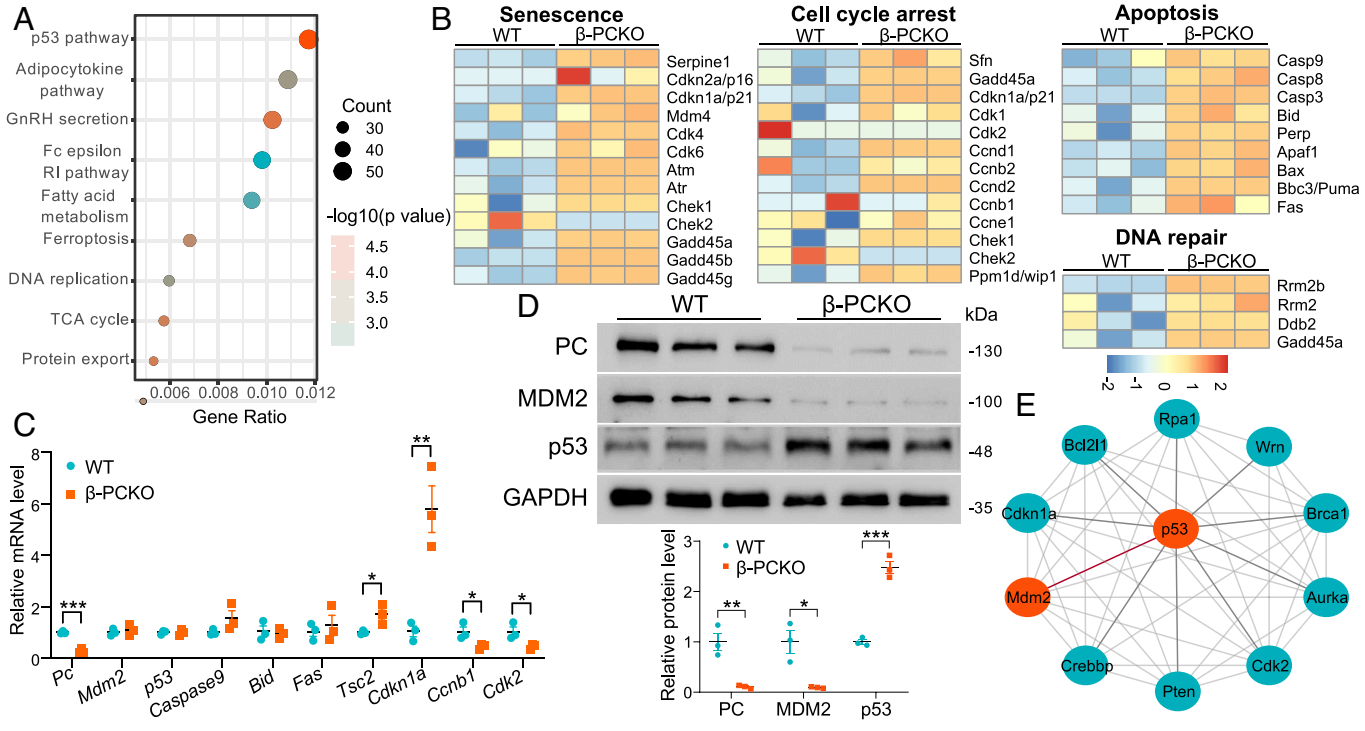

Fig. 2. *p53* pathways are activated in the islets of β-PCKO mice. Islets isolated from 12-wk-old mice β-PCKO mice and their WT littermates were subjected to RNAseq analysis (n = 3). (*A*) KEGG pathways. (*B*) Heatmap of key genes altered in the pathways controlled by p53. (*C*) QPCR analysis of genes in the *p53* signaling pathway of islets isolated from each group (n = 3). (*D*) Immunoblotting analysis of PC, p53, and MDM2 of islets isolated from each group. (*E*) Network analysis illustrating genes' significant correlation with p53 ($P < 0.05$) enriched in KEGG pathways from RNA-seq data. Significance was determined using two-tailed independent Student's *t* test for panels (*C* and *D*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All values are represented as mean ± SEM.

their low abundance (28). Indeed, the unchanged apoptotic gene is consistent with the no change in TUNEL staining of the islets between β-PCKO mice and WT controls (*SI Appendix*, Fig. S2*C*). p53 protein level was dramatically increased in the islets from β-PCKO mice, despite no change in the *p53* mRNA level (Fig. 2 *C* and *D*). Network analysis of RNAseq data identified *Mdm2* as the hub gene connecting to the p53 pathways (Fig. 2*E*). MDM2 is the major negative regulator of p53, which binds and promotes proteasomal degradation of p53 and blocks transcriptional activity of p53. Immunoblotting analysis showed that MDM2 expression was reduced in the islets of β-PCKO mice (Fig. 2*D*).

We next examined whether PC controls p53 pathways in β-cell line. To this end, we silenced *Pc* expression in INS-1E cells using siRNA. INS-1E cells transfected with siRNA against *Pc* (*siPc*) showed a significant reduction of PC at protein and mRNA levels compared with those transfected with *siScramble* (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S4*C*). This was accompanied by increased protein levels of p53 and p21 and decreased MDM2 protein levels (Fig. 3*A*). siRNA-mediated *Pc* silencing increased *Cdkn1a* expression and reduced the cell cycle genes *Cdk4* and *Ccne1* but had no obvious effect on the apoptotic gene *Puma* (*SI Appendix*, Fig. S4*C*). While PC is known to control glucose metabolism, chronic hyperglycemia has been recently shown to induce cellular senescence in β-cells (5). To further investigate the interplay between PC and glucose metabolism in the senescence response, we treated *Pc*-silencing INS-1E cells with high glucose (25 mM) for 64 h (referred to as hyperglycemia hereafter) as previously reported (5). As expected, our data demonstrated that chronic hyperglycemia up-regulated p53 protein but reduced PC and MDM2 protein levels in INS-1E cells (*SI Appendix*, Fig. S5). *P53* mRNA remained unchanged but *Mdm2* mRNA level was up-regulated in *Pc*-down-regulated INS-1E cells under normal and hyperglycemia conditions, possibly as a compensatory mechanism for the upregulation of p53-dependent senescence response and MDM2 protein reduction (Fig. 3 *C* and *D*). Chronic hyperglycemia up-regulated expression of *Cdkn2a* and *Cdkn1a* in INS-1E cells transfected with *siScramble* (Fig. 3 *E* and *F*). The senescent effect of hyperglycemia was further exacerbated by PC downregulation (Fig. 3 *E* and *F*). Apart from the general senescent hallmark genes *Cdkn2a* and *Cdkn1a* (29), we determined whether *Pc* silencing induces senescent responses in β-cells under hyperglycemia condition, by including multiple senescent hallmarks (a) senescence-associated secretory phenotype (SASP), (b) β-galactosidase (SA-β-gal) activity, (c) cell morphological changes (i.e., enlarged cell size and disrupted nuclear membrane), (d) DNA damage and (e) cell cycle arrest (30). First, we detected SASP expression levels in *Pc* knockdown INS-1E cells. QPCR analysis demonstrated that several β-cell-specific SASP (31), including *Gdf15*, *Hsp90aa1*, *Dusp3,* and *Serpine1,* were up-regulated by *Pc* silencing (*SI Appendix*, Fig. S6*A*). High-glucose up-regulated the β-cell-specific SASP markers in INS-1E cells transfected with si*Scramble*, and such high-glucose effect was further potentiated by *Pc* silencing (Fig. 3*G* and *SI Appendix*, Fig. S6 *B–D*). Next, we conducted β-gal staining in INS-1E cells transfected with *siPc* and *siScramble* under high-glucose stimulation for 60 h, followed by microscopic and flow cytometry analyses. Both analyses revealed that silencing or knockout of *Pc* significantly increased β-gal positive signals (Fig. 3*H* and *SI Appendix*, Fig. S6 *E–H*). In line with previous results, *Pc* silencing exerts no significant effect on β-cell death as assessed by propidium iodide (PI) staining (*SI Appendix*, Fig. S6*G*). The flow cytometry analysis also showed a larger cell size in the *siPc*-transfected cells (Fig. 3*I*). To assess DNA damage (double-strand breaks), we measured protein expression and foci formation of γ-H2AX phosphorylation at serine 139 by immunoblotting and immunofluorescent analyses. These analyses showed that silencing of *Pc* not only up-regulated γ-H2AX protein expression but also the number of γ-H2AX-positive nuclei in INS-1E cells (Fig. 3*A* and *SI Appendix*, Fig. S6*I*). Of note, the staining of γ-H2AX showed a typical foci pattern, confirming the DNA damage in the nucleus. In addition, loss of nuclear membrane integrity (reflected by expression of nuclear protein Lamin B1) (Fig. 3*A*) and reduced cell proliferation (reflected by BrdU incorporation assay) (Fig. 3*J*) were also observed in the INS-1E cells transfected with *siPc* compared to those transfected with *siScramble.* Our data demonstrated that β-gal positive signaling increased in the pancreatic islets of β-PCKO mice compared to WT controls under long-term HFD feeding condition (*SI Appendix*, Fig. S6*H*), which may contribute to the downregulation in proliferation of β-cells (32, 33). These data collectively indicate that PC prevents cellular senescence in β-cells.

Next, we examined whether the senescent effect of *Pc* silencing is p53 dependent. To this end, INS-1E cells were cotransfected with *siRNA* against *Pc*, *Pc, and p53* or *Scramble* as indicated in Fig. 4. The p53 upregulation induced by *Pc* silencing was counteracted by concomitant silencing of *p53* (Fig. 4*A*). In addition, *p53* silencing largely abolished the senescent responses mediated by PC downregulation, which were reflected by gene expression of *Cdkn1a* and *Cdkn2a* (Fig. 4 *B–E*), β-gal positive fluorescent signal (*SI Appendix*, Fig. S7 *A–C*), SASP signatures (Fig. 4*F* and *SI Appendix*, Fig. S7*D*), β-gal staining (Fig. 4*G*), nuclear membrane integrity (Fig.4*A*), and cell cycle arrest (*SI Appendix*, Fig. S7*E*), in INS-1E cells under normoglycemia and hyperglycemia conditions.

As expected, *Pc* silencing impaired GSIS in INS-1E cells, however, concomitant silencing of *p53* could not rescue the defective GSIS (Fig. 4*H*). Next, we investigated whether the interplay between PC and p53 is involved in glucose metabolism in INS-1E cells using the stable metabolic flux approach. After transfection with siRNA for 33 h, the INS-1E cells were incubated with $^{13}C_6$-labeled glucose for 24 h. Isotopomer distribution analysis showed that siRNA-mediated *Pc* silencing led to dramatic reduction of ^{13}C labeled TCA intermediates, including OAA, citrate, and α-ketoglutarate, which reflects a diminished PC activity (*SI Appendix*, Fig. S8). Concomitant silencing of *p53* was unable to restore anaplerotic function of PC (*SI Appendix*, Fig. S8). To mimic the metabolic changes during GSIS, we measured TCA cycle intermediates in INS-1E cells upon low (2 mM) and high (20 mM) glucose stimulation for 30 min. Targeted metabolomics analysis showed that acute high glucose up-regulated the TCA metabolites (including OAA, citrate, malate, and α-ketoglutarate) in the *siScramble* group, but this upregulation was blocked by *Pc* silencing. Concomitant silencing of *p53* was unable to restore diminished production of TCA cycle metabolites in *Pc* down-regulated INS-1E cells (*SI Appendix*, Fig. S9) (34). On the other hand, silencing of *p53* exerted no obvious effect on TCA metabolite concentration in INS-1E cells under the low or high glucose conditions (*SI Appendix*, Fig. S10). Taken together, these findings suggest the p53 activation induced by *Pc* silencing is mainly responsible for the senescence but not for the defective GSIS and glucose metabolism in the TCA cycle.

Overexpression of PC Counteracts Aging- and Diabetes-Induced Senescence in Human and Mouse Islets. Pancreatic β cell senescence is found in both aging and diabetic conditions (35). Next, we examined whether PC expression is altered in the islets/β-cells of humans in aging and diabetes. We subjected pancreatic sections from 3 young donors without diabetes (Young-ND; male, 18~45 y old), 3 young donors with diabetes (Young-DM; male, 18~45 y old), 3 aged donors without diabetes (Aged-ND; male, >60 y old) and 3 aged donors with diabetes (Aged-DM; male, >60 y old) to

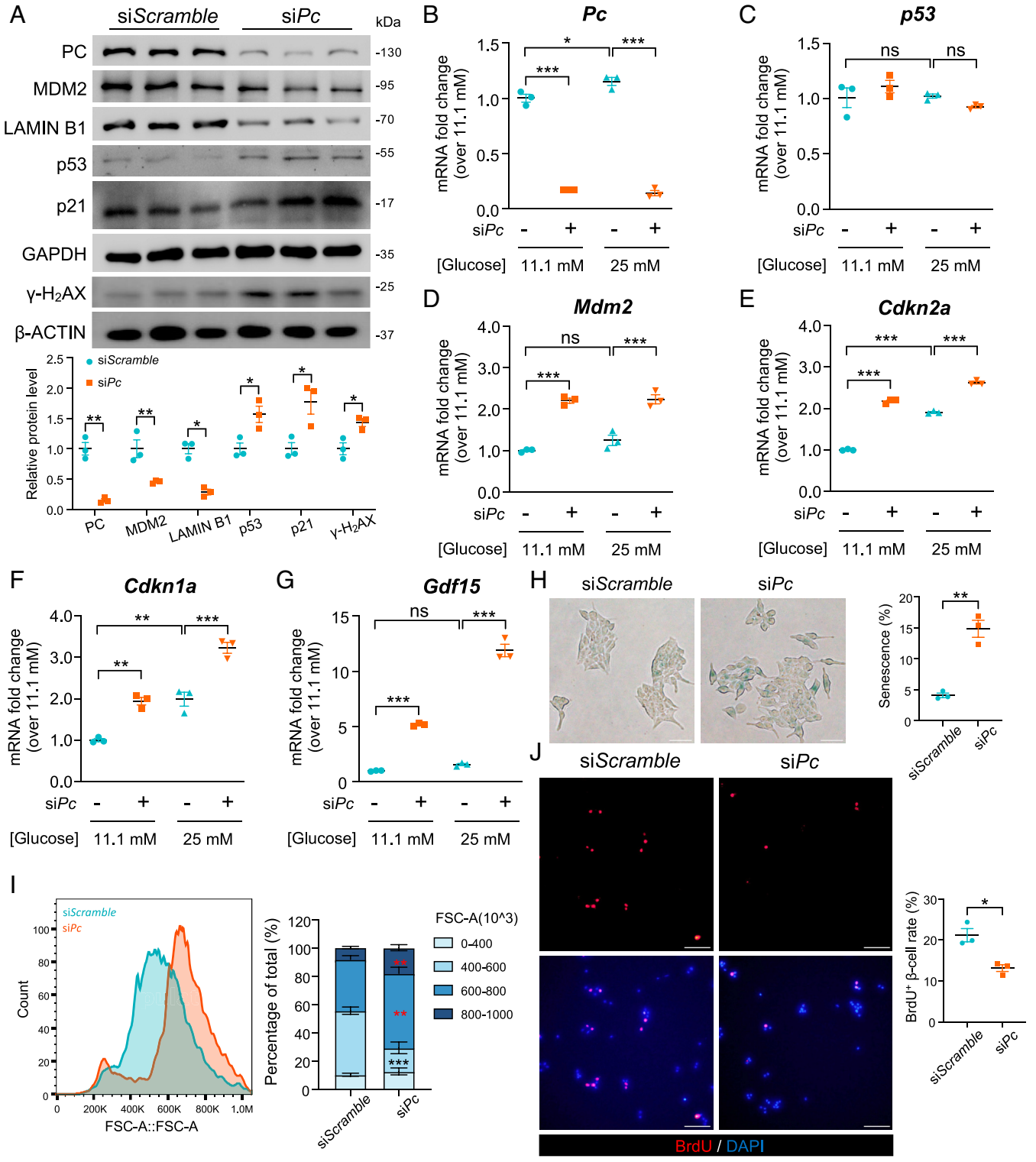

Fig. 3. Downregulation of PC aggravates β-cell senescence. (*A*) Immunoblotting analysis of PC, MDM2, LAMIN B1, p53, p21, and γ-H_2AX in INS-1E cells transfected with siRNA against *Scramble* or *Pc* in normal (11.1 mM) condition (n = 3). (*B–G*) INS-1E cells were transfected with siRNA against *Scramble* or *Pc* and cultured in normal (11.1 mM) or high-glucose (25 mM) condition. Relative expression of *Pc* (*B*), *p53* (*C*), *Mdm2* (*D*), senescence-related genes *Cdkn2a* (*E*), *Cdkn1a* (*F*), and *Gdf15* (*G*) are shown. (*H–J*) INS-1E cells were transfected with siRNA against *Scramble* or *Pc* and cultured for 12 h followed by high-glucose treatment for 60 h. (*H*) Beta-galactosidase (β-gal) staining and quantification. (Scale bar: 20 μm.) (*I*) Cell size detection by flow cytometry (n = 3). (*J*) BrdU staining (scale bar: 50 μm.) (n = 3). Significance was determined using two-tailed independent Student's *t* test for panels (*A* and *H–J*), and two-way ANOVA with Tukey correction for panels (*B–G*). *P < 0.05, **P < 0.01, ***P < 0.001. All values are represented as mean ± SEM.

immunofluorescent staining (*SI Appendix*, Table S1). The result revealed that PC was abundantly expressed in pancreatic β-cells in the Young-ND group, but sharply decreased in Aged-ND, Young-DM, and Aged-DM groups (Fig. 5 *A* and *B*). The insulin$^+$ β-cell area was comparable between young and aged groups (Fig. 5*C*). Furthermore, we analyzed *PC* mRNA expression in the islets of humans with or without T2DM, using the transcriptome dataset GSE76895 (36). This analysis showed a reduction of *PC* in the diabetic samples (*SI Appendix*, Fig. S11*A*). KEGG pathway analysis indicated that lower *PC* expression correlated with the cellular

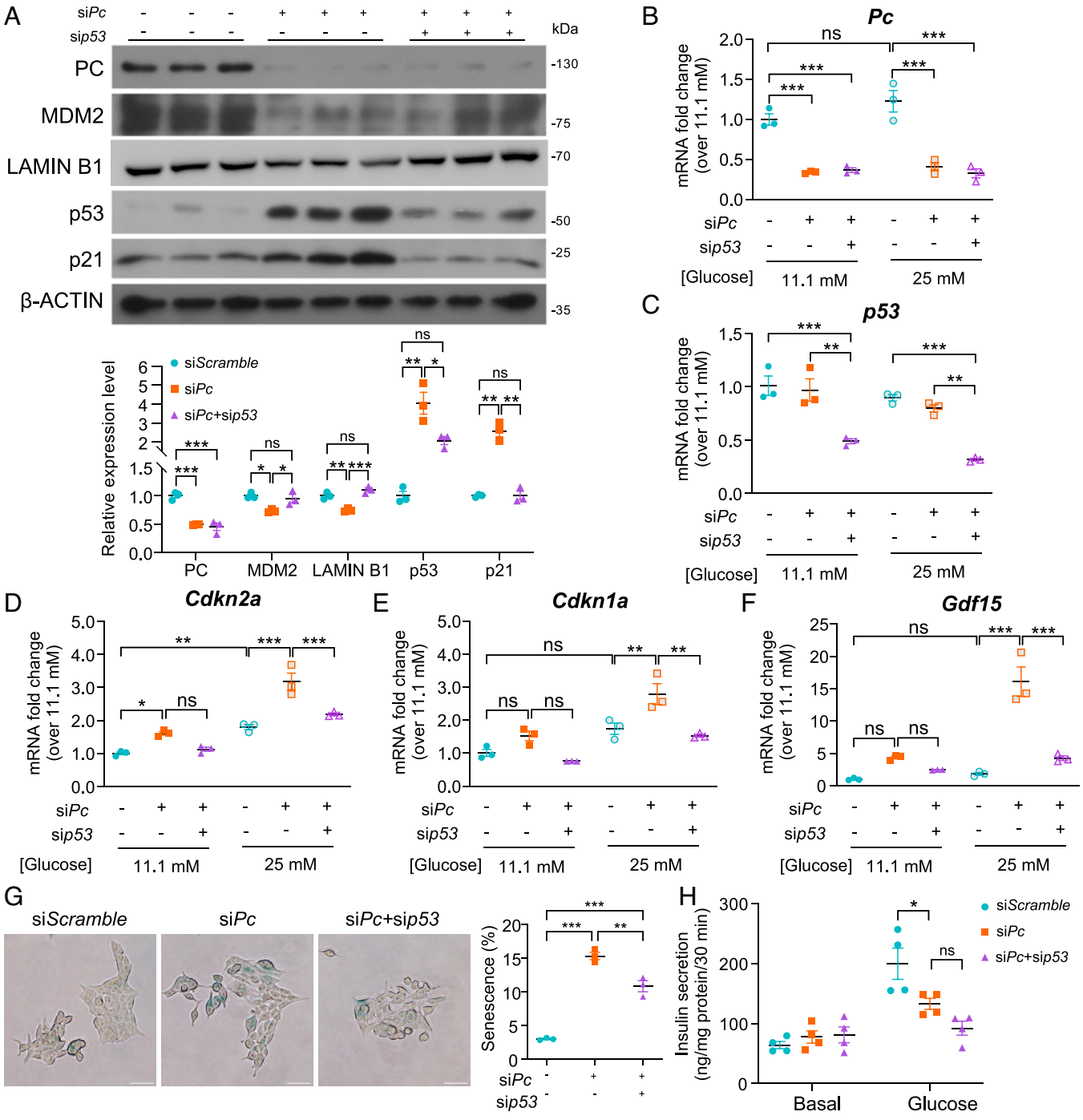

Fig. 4. *Pc* silencing induces senescence via p53 activation in pancreatic β-cells. INS-1E cells were transfected with siRNA against *Scramble*, *Pc,* or *Pc* and *p53*. (*A*) Immunoblotting analysis of PC, MDM2, LAMIN B1, p53, and p21 in the transfected cells (n = 3). (*B–F*) INS-1E cells were cultured in normal (11.1 mM) or high-glucose (25 mM) condition. Relative expression of *Pc* (*B*), *p53* (*C*), senescence-related genes *Cdkn2a* (*D*), *Cdkn1a* (*E*), and *Gdf15* (*F*) detected by QPCR. (*G*) β-gal staining in the transfected cells. (*H*) GSIS under basal (2 mM) and glucose (20 mM) stimulation conditions in the transfected cells (n = 4). Significance was determined using one-way ANOVA with Tukey correction for panels (*A* and *G*), and two-way ANOVA with Tukey correction for remaining panels. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. All values are represented as mean ± SEM.

senescence pathway (*SI Appendix*, Fig. S11*B*). A negative association was found between *PC* and *CDKN1A* mRNA expression in the human pancreatic islets (*SI Appendix*, Fig. S11*C*). To examine the *PC* expression at the single-cell level under healthy and diabetic conditions, we reexamined single-cell RNA-sequencing (RNA-seq) data of human islets conducted by Segerstolpe Å et al. (*SI Appendix*, Fig. S11*D*) (37). Consistently, *PC* significantly decreased in mature β cells of T2DM individuals compared with healthy individuals (*SI Appendix*, Fig. S11*E*). On the other hand, *MDM2* and *CDKN1A* expression but not *P53*, *CDKN2A,* or *GDF15* were up-regulated in the diabetic condition (*SI Appendix*, Fig. S11 *F–J*).

We next examined whether overexpression of PC improves aging- and diabetes-induced defective GSIS and senescence in pancreatic β-cells. We infected the pancreatic islets isolated from the diabetic patients with adenovirus expressing human PC or GFP for 48 h, followed by assessment of GSIS and senescent gene profile. Overexpression of PC significantly repressed mRNA and protein expression of P53 and P21 and up-regulated protein but not mRNA expression of *MDM2* (Fig. 5 *D* and *E*). Overexpression

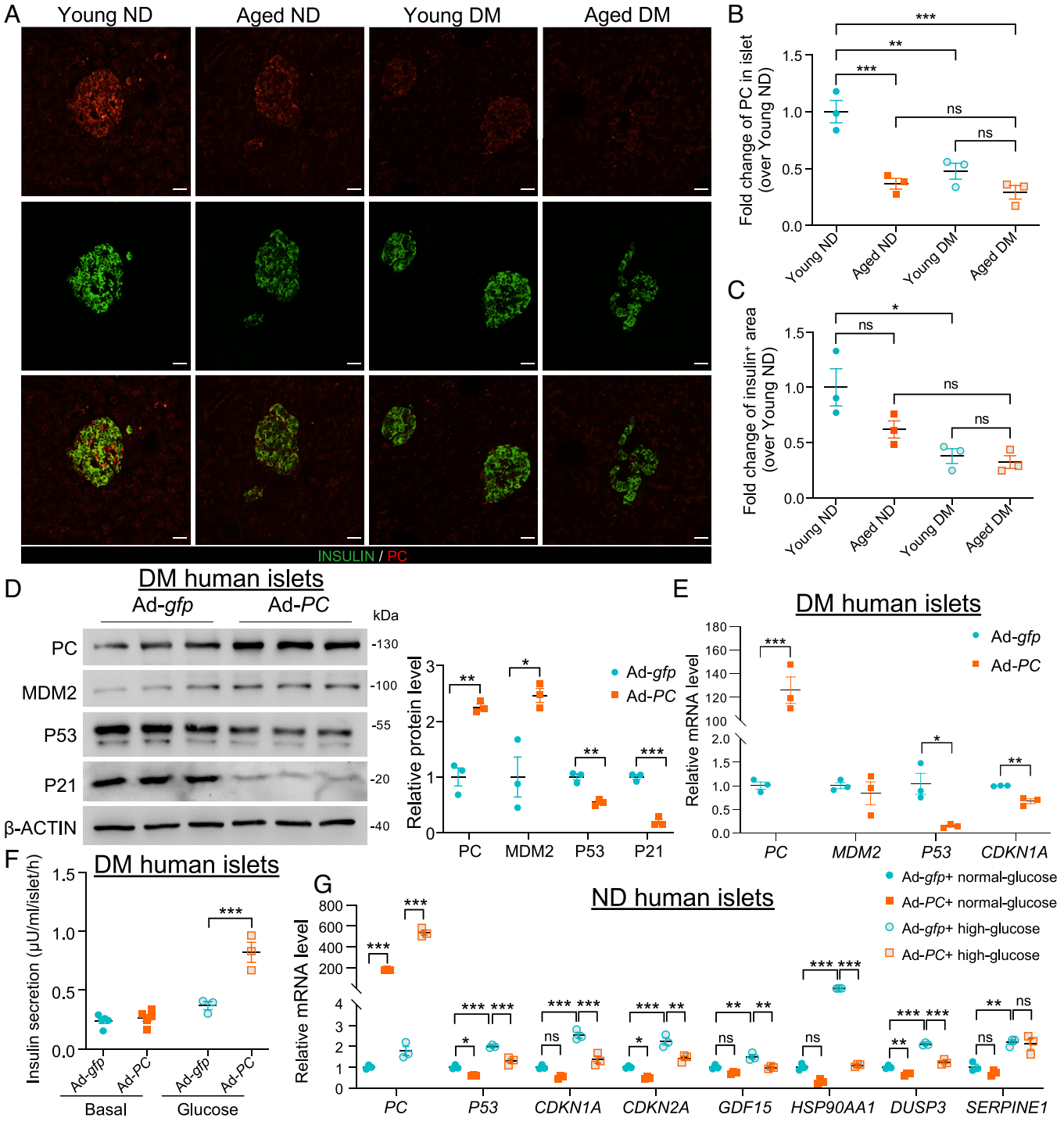

Fig. 5. Overexpression of PC restores GSIS and alleviates senescence in human pancreatic islets under diabetic conditions. (*A*) Representative IF staining images of PC (red) in paraffin pancreas sections of young nondiabetic, aged nondiabetic, young diabetic, and aged diabetic donors. Donors were divided into young (18~45 y old) and aged (>60 y old). (Scale bar: 10 μm.) (*B*) Quantification of average PC fluorescent densities colocalized with insulin in panel (*A*) (n = 3). (*C*) Quantification of average insulin-positive area in panel (*A*) (n = 3). (*D* and *E*) Diabetic human islets were infected with adenovirus-*gfp* or adenovirus-*PC* for 48 h, followed by immunoblotting analysis (*D*) and QPCR analysis (*E*) of PC, MDM2, P53, and P21. (*F*) GSIS of DM islets infected with Ad-*PC* or Ad-*gfp* for 48 h. (*G*) ND islets were infected with Ad-*PC* or Ad-*gfp* for 24 h, then cultured in 5.5 mM or 25 mM glucose for 72 h, followed by QPCR analysis. Significance was determined using two-way ANOVA with Tukey correction for panels (*B*, *C*, *F*, and *G*), and two-tailed independent Student's *t* test for panels (*D* and *E*). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. All values are represented as mean ± SEM.

of PC also restored the insulin secretory ability of diabetic islets upon glucose stimulation (Fig. 5*F*). We next tested whether PC overexpression counteracted chronic hyperglycemia-induced senescent responses in the islets from the healthy individuals. Consistent with the findings in INS-1E cells (Fig. 3 *E*–*G* and *SI Appendix*, Fig. S6 *B*–*D*), chronic hyperglycemia dramatically up-regulated the senescent genes, including *P53*, *CDKN1A*, *CDKN2A*, and SASP profiles (*GDF15*, *HSP90AA1*, *DUSP3*, and *SERPINE1*), whereas PC overexpression partially abrogated these senescent gene expressions (Fig. 5*G*). Given the nonavailability of aged human islets, we tested the effect of PC overexpression on aging-induced senescence using mouse islets. Consistent with the human data, immunofluorescent staining showed a reduction of PC in pancreatic β-cells in 22- to 25-mo-old (so-called aged;

equivalent to 70 to 80 y old in humans) C57BL6/J mice when compared to those in 3-mo-old (so-called young; equivalent to 20 to 30 y old in humans) mice (*SI Appendix*, Fig. S12*A*) (38). Given the role of aging in insulin secretion remains controversial (39–41), we only examined the effect of PC on senescence in aging. We infected the young and aging mouse islets with adenovirus expressing human PC or GFP as above. QPCR analysis showed that the senescent markers including *p53*, *Cdkn1a*, *Cdkn2a,* and *Gdf15* were up-regulated in aging mouse islets (*SI Appendix*, Fig. S12 *B–E*). The upregulation of senescent gene program was partially rectified by PC overexpression in the aging islets when compared with those aging islets infected with adenovirus expressing GFP (*SI Appendix*, Fig. S12 *B–E*). Interestingly, *Mdm2* mRNA level was increased in aging islets, which might be a compensatory response for the p53 activation and/or PC downregulation (*SI Appendix*, Fig. S12*F*). Taken together, these findings support the notion that PC downregulation contributes to p53 activation and senescence in the diabetic and aging conditions.

PC Inhibits p53 Activation by Interacting and Maintaining MDM2 Stability. Although *Mdm2* mRNA expression and its stability were up-regulated in INS-1E cells transfected with *siPc* (Figs. 3*D* and 6*A*), its protein expression was markedly reduced by *Pc* silencing (Figs. 3*A* and 4*A*). Likewise, MDM2 protein expression was lower in the islets of β-PCKO mice than that in WT controls, despite no difference in *Mdm2* mRNA levels (Fig. 2 *C* and *D*). On the contrary, overexpression of PC increased protein but not mRNA expression of MDM2 in human or mouse islets (Fig. 5 *D* and *E* and *SI Appendix*, Fig. S12*F*). The above findings suggest that PC controls MDM2 expression at the posttranslational level.

Apart from p53-mediated transcriptional upregulation and mRNA stability (42), MDM2 expression can be controlled by the proteasomal pathway. We thus examined whether PC controls MDM2 protein stability. To this end, we transfected INS-1E cells with *siPc* or *siScramble* for 48 h, followed by treatment with the protein synthesis inhibitor cycloheximide (CHX). As expected, MDM2 protein was gradually decreased in INS-1E cells transfected with *siScramble* upon CHX treatment (Fig. 6*B*). Whereas *Pc* silencing accelerated the MDM2 degradation upon inhibition of protein synthesis (Fig. 6*B*). On the other hand, treatment with the proteasome inhibitor MG132 restored MDM2 protein expression in INS-1E cells with downregulation of PC (Fig. 6*C*). On the contrary, overexpression of PC up-regulated protein expression and stability of MDM2 without affecting its mRNA expression (Fig. 6 *D–F*). Taken together, PC maintains MDM2 expression via preventing its degradation in pancreatic β-cells.

Next, we further investigated how PC maintains MDM2 protein stability and their regulatory effect on β-cell senescence. In silico analysis identified two potential MDM2 binding motifs (amino acid 1012-1016 [so-called motif 1012-1016] and amino acid 1076-1080 [so-called motif 1076-1080]) at the C terminus of PC (Fig. 7*A*). The MDM2 binding motifs can be found in the human, mouse, rat, drosophila, *Xenopus laevis*, and zebrafish. The motif 1076-1080 is identical among different species, while motif 1012-1016 is highly conserved (*SI Appendix*, Fig. S13). Coimmunoprecipitation assay confirmed that MDM2 interacted with PC in INS-1E cells, and the interaction was decreased by

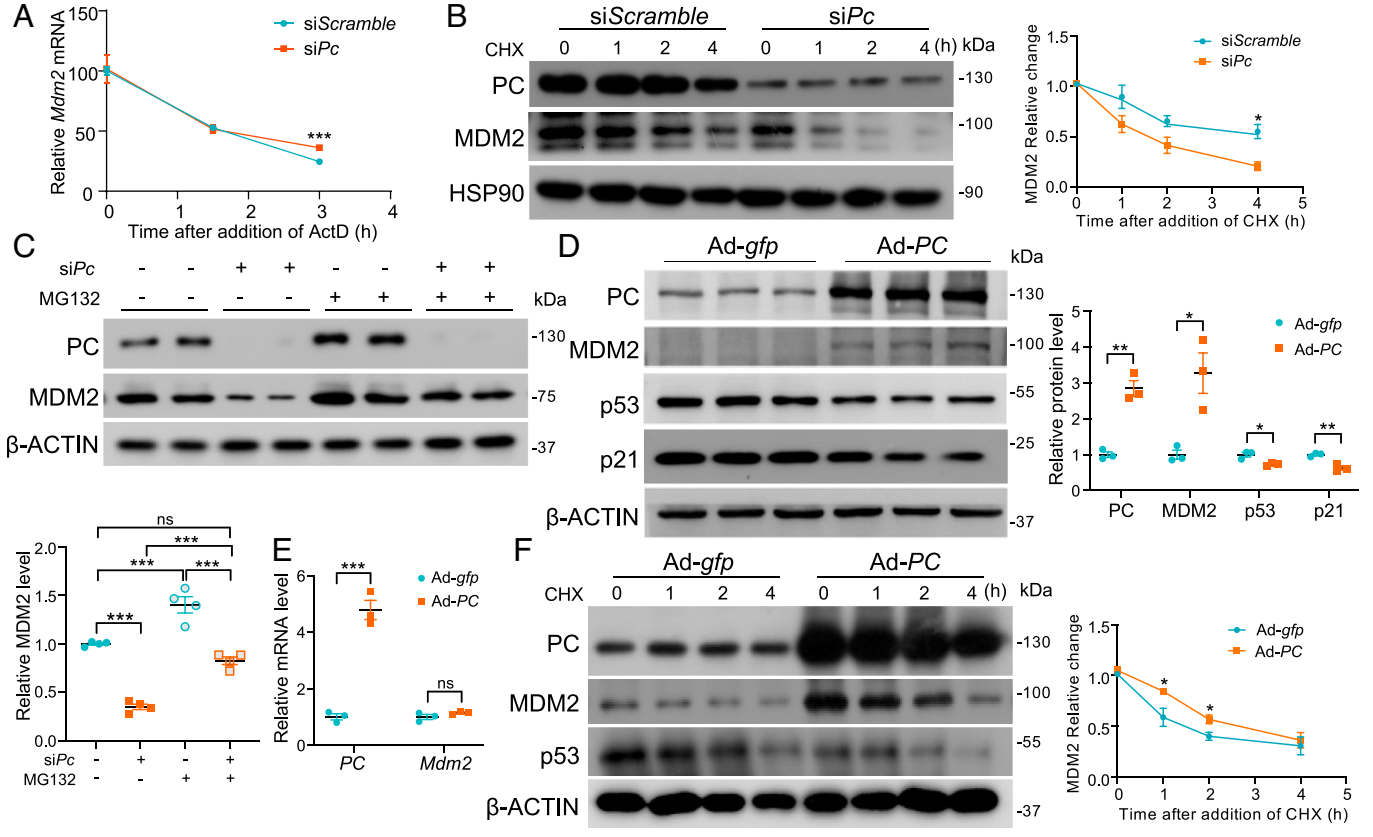

Fig. 6. PC maintains MDM2 expression and restricts p53 activation by preventing MDM2 degradation. (*A–C*) INS-1E cells were transfected with siRNA against *Scramble* or *Pc* for 48 h. (*A*) *Mdm2* mRNA stability was detected by QPCR in each group. (*B*) MDM2 protein degradation rate was detected. (*C*) MDM2 protein level was detected when treated with vehicle or MG132. (*D–F*) INS-1E cells were infected with adenovirus-*gfp* or adenovirus-*PC* for 48 h, followed by immunoblotting analysis of PC, MDM2, p53, and p21 (*D*) and QPCR analysis of *PC* and *Mdm2* (*E*) (n = 3). (*F*) MDM2 protein degradation rate in each group. Significance was determined using one-way ANOVA with Tukey correction for panel (*C*), and two-tailed independent Student's *t* test for remaining panels. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. All values are represented as mean ± SEM.

short-term high-glucose treatment (Fig. 7*B*). In addition, p53 was also coprecipitated with PC, but such interaction was not affected by high glucose (Fig. 7*B*). To further investigate whether and which MDM2 binding motif(s) in PC mediates its interaction with MDM2, we created three human PC mutants. The first mutant changed M1012, Y1013, and V1016 in motif 1012-1016 to alanine residues (so-called PC-MT1), the second mutant changed V1076, F1077, and L1080 of motif 1076-1080 to alanine residues (so-called PC-MT2), and the third mutant is the combination of the first and second mutants (i.e., M1012A, Y1013A, V1016A, V1076A, F1077A, and L1080A) (so-called PC-MT3). Coimmunoprecipitation showed that MDM2 only interacted with wild-type (WT) PC and the first mutant (M1012A/Y1013A/V1016A-PC) but not the second (V1076A/F1077A/L1080A-PC) or the third (M1012A/Y1013A/V1016A /V1076A/F1077A/L1080A-PC) mutant (Fig. 7*C*). The inhibitory effects of PC on MDM2 degradation and senescence were lost when the MDM2 binding motifs were mutated to the alanine in the V1076A/F1077A/L1080A PC mutant in INS-1E cells (Fig. 7 *D*–*F*). These findings suggest that PC binds with MDM2 and prevents its degradation via the MDM2 binding motif, which in turn inhibits p53-dependent senescent program in pancreatic β-cells (Fig. 7*G*).

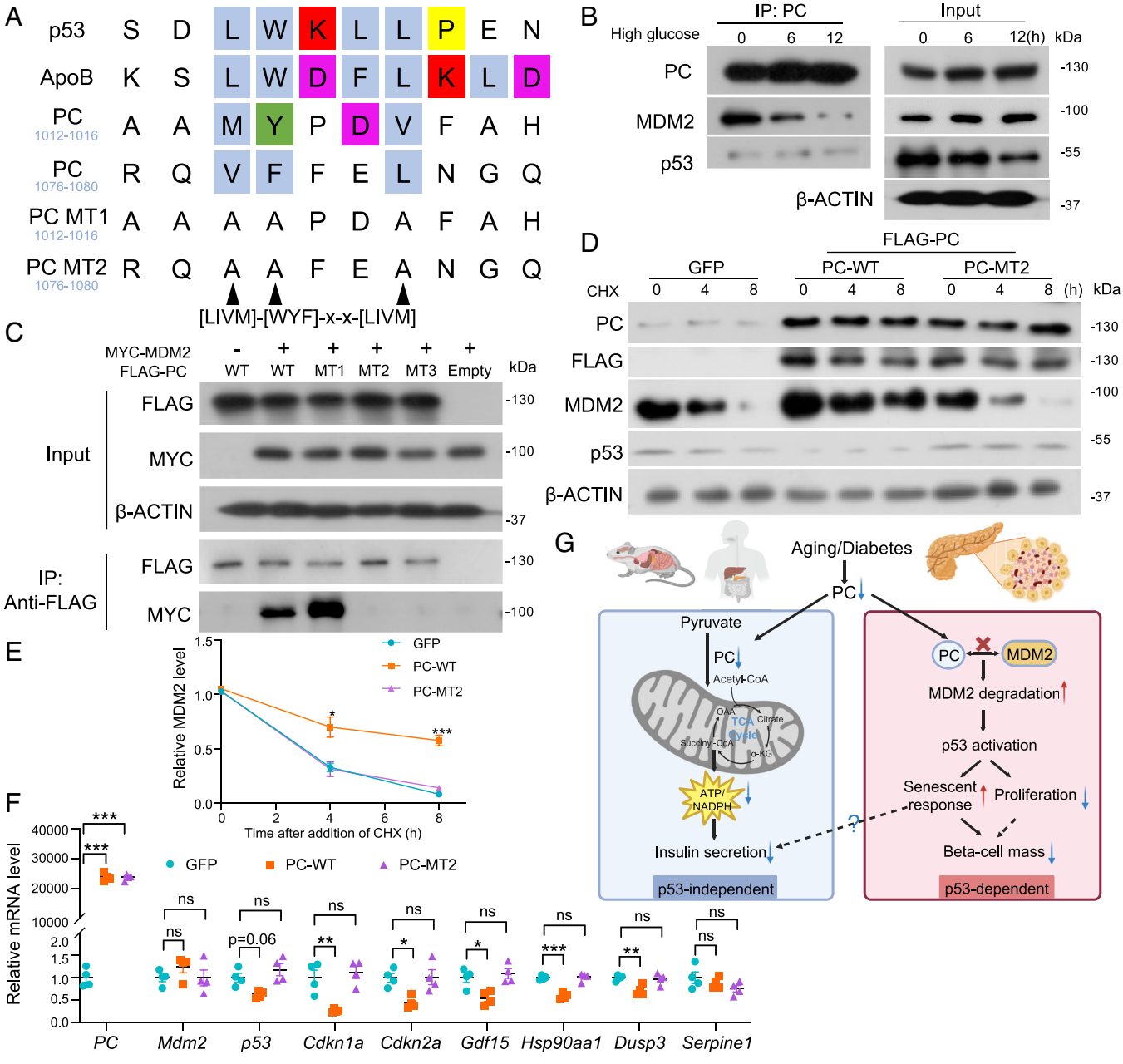

Fig. 7. PC controls MDM2 expression and restricts senescence via the protein–protein interaction in β-cells. (*A*) In silico analysis of putative MDM2 binding motifs in PC. L: leucine, I: isoleucine; V: valine; M: methionine; W: tryptophan; Y: tyrosine; F: phenylalanine; X: any amino acid. Potential MDM2 binding motifs in PC and mutant sites of PC were presented using the Clustal coloring method. (*B*) INS-1E cells after treatment with high-glucose were subjected to immunoprecipitation (IP) using an antibody against PC, followed by immunoblotting as indicated. (*C*) HEK 293 cells were transfected with plasmids encoding MYC-tagged MDM2 and FLAG-tagged PC and its mutants for 48 h. Total cell lysates were subjected to IP using anti-MYC or anti-FLAG antibodies, followed by immunoblotting analysis as indicated. (*D*–*F*) INS-1E cells were transfected with plasmids encoding FLAG-tagged PC and its mutant for 16 h, followed by high-glucose treatment for 64 h. (*D*) Immunoblotting analysis of PC, FLAG, MDM2, and p53. (*E*) Quantification of MDM2 protein levels in panel (*D*). (*F*) QPCR analysis of *PC*, *Mdm2*, *p53*, *Cdkn1a*, *Cdkn2a,* and β-cell-specific SASP genes. (*G*) Graphical abstract. Significance was determined using one-way ANOVA with Tukey correction. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. All values are represented as mean ± SEM.

Discussion

Aberrant glucose metabolism and mitochondrial dysfunction contribute to β-cell dysfunction in T2DM (43). In this study, we demonstrated the critical role of the mitochondrial enzyme PC in regulating GSIS and β-cell senescence in humans and rodents through distinct mechanisms. On the one hand, PC restricts p53 activation by interacting with and preventing MDM2 degradation, thereby inhibiting β-cell senescence under diabetic and aging condition. On the other hand, PC mediates glucose flux into the TCA cycle via production of OAA, which produces the coupling factors for insulin secretion. Notably, most of our studies were performed in a rat cell line (INS-1E), and key findings were validated in human and mouse islets.

Physiological concentration and acute stimulation of glucose promote insulin secretion and β-cell proliferation, but chronic hyperglycemia induces mitochondrial dysfunction and apoptosis in β-cells (5, 44, 45). Chronic hyperglycemia has recently been shown to induce senescence in β-cells via p53 activation (5, 46). We and others demonstrated that β-cells exhibit modest senescence upon high glucose treatment for 60 to 72 h (5). However, this hyperglycemia treatment is relatively short in duration, for example, when compared to replicative senescence. Further investigation is warranted to determine whether the PC–MDM2–p53 axis is also involved in senescence induced by other insults such as DNA damage, cytokines, toxic lipids, etc. Treatment with palmitic acid hydroxy steric acid (PAHSAs), a lipid mainly derived from adipose tissue with anti-inflammatory and insulin- and GLP-1 secretory effects (47), up-regulates *Mdm2* mRNA expression, which in turn down-regulates p53 activation in β-cells under the metabolic stress conditions (5). High glucose has been shown to reduce *Mdm2* mRNA and protein level, which is accompanied by reduced p53 ubiquitination and increased nuclear p53 expression in RINm5F rat insulinoma cells (48). Our study identified a mechanism by which PC controls senescence by targeting MDM2–p53 balance in β-cells. Unlike the previous study (48), we showed that high glucose treatment does not affect *MDM2* mRNA levels but acutely decreases MDM2 protein expression in INS-1E cells and human islets, suggesting the glucose effect on MDM2 expression is at a posttranscriptional event. Short-term high glucose decreases the MDM2–PC interaction, which accelerates MDM2 proteasomal degradation and induces subsequent p53 activation and senescence in INS-1E cells. In the aging condition, we showed that *Mdm2* and *p53* mRNA were induced in the islets isolated from the 22- to 25-mo-old C57BL6/J mice when compared to those isolated from 3-mo-old counterparts. Unfortunately, expression data of MDM2 and p53 proteins are currently not available, due to limited sample availability. We speculate that the increase of *Mdm2* mRNA is a compensatory response for the MDM2 protein reduction mediated by the PC pathway and/or chronic p53 activation in the aged pancreatic islets. Since we observed the obvious PC protein reduction and *p53* upregulation in aged islets by immunofluorescent staining and QPCR analysis, respectively. In addition, *Mdm2* is a downstream target gene of p53, it is possible that the induction of p53 leads to the increased *Mdm2* mRNA expression in the aging condition. Apart from senescence, p53 activation has been shown to induce β-cell apoptosis (46, 49–51), but such p53 action is not observed in our animal and cell models with PC downregulation. Indeed, β-cell-specific p53 activation induced by MDM2 deletion does not alter β-cell mass and apoptosis, as shown in our previous study (21). We speculate that PC deficiency only induces senescence but not apoptosis, perhaps due to p53 activity not reaching the threshold of apoptosis (52). Indeed, there is no difference in several p53-downstream apoptotic genes between β-PCKO mice and their WT controls. However, it is currently unknown how the PC–MDM2 interaction maintains MDM2 protein expression. Previous studies showed that the E3 ligases including MDM2 itself and FKBP25 induce MDM2 polyubiquitination and subsequent degradation, whereas TCTP as the interaction partner blocks MDM2 autoubiquitination (53–56). Interestingly, TCTP is a glucose-regulated protein and its genetic deletion also leads to p53 activation, reduces β-cell mass, and induces hyperglycemia (57, 58), the phenotype similar to that in β-PCKO mice. Whether these MDM2 targeting E3 ligases and interaction partner involve in the glucose-regulatory effects on PC–MDM2 interaction and the subsequent MDM2 degradation, p53 activation, and senescence warrant further investigation.

Both defective GSIS and β-cell senescence are the key features of type 1 and type 2 diabetes. Removal or inhibition of senescent cells using genetic or pharmacological approaches improves β-cell functions including insulin secretory ability in chemically induced insulin resistance, HFD-fed, and aging mouse models (43, 59). On the contrary, p16 ^{Ink4a}-mediated senescence has been shown to enhance GSIS in aging (25) and stress-induced p21-dependent senescence recruits M2 macrophages to store islet function in T1D (60). It appears that the detrimental or beneficial effects of senescence on β-cells depend on sources of stress and stimuli, types, and stages of diabetes (60, 61). We showed that the anaplerotic function of PC is crucial for GSIS, while the binding ability of PC to MDM2 is to restrict p53-induced senescence response. PC is known to control GSIS via the anaplerotic reaction in the TCA cycle in pancreatic β-cells. Apart from this, our study demonstrates that silencing of *Pc* triggers senescence in β-cells under hyperglycemia condition. However, it is currently unknown whether the *Pc* silencing-mediated senescence further exacerbates defective GSIS. On the other hand, knockdown of *p53* largely attenuated *Pc* silencing-induced senescence but not defective GSIS and the anaplerotic reaction. Thus, we believe that the regulatory role of PC on GSIS is independent of its role in senescence, but whether *Pc* silencing-mediated senescence per se exacerbates other β-cell dysfunction such as defective GSIS or reduced β-cell mass requires further investigation. It is interesting to further investigate the individual effect of PC on insulin secretion and senescence in the animal model, by replenishing WT-PC, PC mutant without MDM2 binding ability in β-PCKO mice in the future study. This will indicate whether PC-deficiency-induced senescence contributes to reduced β-cell mass and defective GSIS in vivo.

PC expression is reduced in the diabetic and aging conditions as shown in our current study and previous studies (10, 11, 22, 23). We previously demonstrated short-term glucose treatment increases promoter activity of PC in MIN6 β-cells, and such increase is counteracted by p53 activation. At the molecular level, p53 directly binds to the p53 response element in the promoter region of PC and inhibits its transcription. Given that p53 is activated in β-cells under the diabetic and aging conditions (21, 46, 49–51), we therefore propose that p53 activation inhibits PC expression, leading to MDM2 degradation, which further increases p53 expression. Not only reduced PC expression, the inhibitory effect of high-glucose on PC–MDM2 interaction also leads MDM2 degradation. Thus, we proposed that reduced PC level under diabetic and aging conditions triggers a positive feedback loop involving PC-MDM2-p53, which impairs GSIS, exacerbates senescence and reduces β-cell proliferation. Our study showed that replenishing PC expression is sufficient to block this feedback loop, resulting in better β-cell functions in human and rodent islets with diabetes and aging. Therefore, β-cell-specific restoration of PC expression might represent a feasible approach for diabetes treatment.

Mitochondrial dysfunction, such as increased mitochondrial ROS and dysregulated nutrient sensing pathways is known to cause senescence (62), but the role of mitochondrial enzymes in senescence is less explored. Consistent with our findings in β-cells, a recent study conducted by Igelmann et al. showed that the cytosolic form of PC reduces senescence in clonal human fibroblast via forming a hydride transfer complex together with the TCA cycle enzyme malate dehydrogenase 1 (MDH1) and malic enzyme 1 (ME1). This complex catalyzes NAD^+ regeneration and NADPH production to reduce oxidative stress and bypass cellular senescence (63). This study mainly focused on the enzymatic function of HTC complex, but the nonenzymatic function of this complex remains to be determined. Our study identified the anti-senescence function of PC is independent of its anaplerotic function. These findings supported PC as a potential target for anti-senescence treatment. Interestingly, knockdown of ME1 leads to reduced MDM2 expression and hence activates p53-dependent senescence, but not apoptosis, in IMR-90 fibroblast (64). Taken together, these findings suggest that the interplay between mitochondrial enzymes such as PC and ME1 and MDM2–p53 axis might be crucial for senescence in multiple cell types, and further reinforce the role of mitochondrial dysfunction in senescence.

In conclusion, we provided in vivo evidence demonstrating the protective role of PC in pancreatic β-cell functions under various pathological conditions. In addition to its well-established role in promoting β-cell proliferation and GSIS, we also uncovered PC as the negative regulator of β-cell senescence. Combined with our previous study showing p53 activation by MDM2 deletion represses PC expression and impairs GSIS (21), we here propose two critical axes: 1) the MDM2–p53–PC axis in the control of GSIS, and 2) the PC–MDM2–p53 axis in the control of cellular senescence. These pathways are essential for maintaining healthy β-cell functions in diabetic and aging conditions.

Materials and Methods

Human Pancreas Studies. Human pancreatic tissues were procured from the Department of Liver Surgery and Transplantation (Department of Hepatology) of Zhongshan Hospital Affiliated to Fudan University from 2022 to 2024 with approval from the Ethics Committee of Zhongshan Hospital Affiliated to Fudan University (Ethics approval number: B2021-853R). Written informed consent for the scientific and clinical research was obtained by the participants. The sample collection and process are described in *SI Appendix, SI Materials and Methods*.

Animal Studies. β-PCKO mice were generated by crossing $Pc^{floxed/floxed}$ mice with RIP-Cre mice, and other animal experiments are described in *SI Appendix*, SI Materials and Methods.

To explore the role of PC in β-cell senescence, we manipulated PC expression in mice islets, clonal β-cell line (INS-1E), and human islets, followed by mRNA-seq, QPCR, western blot, histological studies, insulin secretion assay, flow cytometry, and LC-MS/MS. For mechanism studies, we constructed PC mutant plasmids and conducted CHX chase assay, mRNA stability analysis, and coimmunoprecipitation assay. Detail procedures, reagents, primer sequences are included in *SI Appendix, SI Materials and Methods*.

Data, Materials, and Software Availability. Next-generation RNA sequence data supporting the findings in this study have been deposited in the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE278865) (accession number GSE278865) (65). All other data are included in the article and/or *SI Appendix*.

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1. J. E. Campbell, C. B. Newgard, Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nat. Rev. Mol. Cell Biol.* **22**, 142–158 (2021).
2. M. R. Metukuri *et al.*, ChREBP mediates glucose-stimulated pancreatic beta-cell proliferation. *Diabetes* **61**, 2004–2015 (2012).
3. S. Srinivasan, E. Bernal-Mizrachi, M. Ohsugi, M. A. Permutt, Glucose promotes pancreatic islet beta-cell survival through a PI 3-kinase/Akt-signaling pathway. *Am. J. Physiol. Endocrinol. Metab.* **283**, E784–E793 (2002).
4. A. F. Stewart *et al.*, Human beta-cell proliferation and intracellular signaling: Part 3. *Diabetes* **64**, 1872–1885 (2015).
5. M. F. Rubin de Celis *et al.*, PAHSAs reduce cellular senescence and protect pancreatic beta cells from metabolic stress through regulation of Mdm2/p53. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2206923119 (2022).
6. V. Poitout, R. P. Robertson, Glucolipotoxicity: Fuel excess and beta-cell dysfunction. *Endocr. Rev.* **29**, 351–366 (2008).
7. M. F. Brereton *et al.*, Hyperglycaemia induces metabolic dysfunction and glycogen accumulation in pancreatic beta-cells. *Nat. Commun.* **7**, 13496 (2016).
8. E. Haythorne *et al.*, Diabetes causes marked inhibition of mitochondrial metabolism in pancreatic beta-cells. *Nat. Commun.* **10**, 2474 (2019).
9. G. A. Rutter, V. Sidarala, B. A. Kaufman, S. A. Soleimanpour, Mitochondrial metabolism and dynamics in pancreatic beta cell glucose sensing. *Biochem. J.* **480**, 773–789 (2023).
10. J. Xu, J. Han, Y. S. Long, P. N. Epstein, Y. Q. Liu, The role of pyruvate carboxylase in insulin secretion and proliferation in rat pancreatic beta cells. *Diabetologia* **51**, 2022–2030 (2008).
11. N. M. Hasan *et al.*, Impaired anaplerosis and insulin secretion in insulinoma cells caused by small interfering RNA-mediated suppression of pyruvate carboxylase. *J. Biol. Chem.* **283**, 28048–28059 (2008).
12. S. Lee *et al.*, Beta-cell succinate dehydrogenase deficiency triggers metabolic dysfunction and insulinopenic diabetes. *Diabetes* **71**, 1439–1453 (2022).
13. M. Srinivasan *et al.*, ss-Cell-specific pyruvate dehydrogenase deficiency impairs glucose-stimulated insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **299**, E910–917 (2010).
14. A. Khan, Z. C. Ling, B. R. Landau, Quantifying the carboxylation of pyruvate in pancreatic islets. *J. Biol. Chem.* **271**, 2539–2542 (1996).
15. S. Farfari, V. Schulz, B. Corkey, M. Prentki, Glucose-regulated anaplerosis and cataplerosis in pancreatic beta-cells: Possible implication of a pyruvate/citrate shuttle in insulin secretion. *Diabetes* **49**, 718–726 (2000).
16. M. J. MacDonald, J. H. Kaysen, S. M. Moran, C. E. Pomije, Pyruvate dehydrogenase and pyruvate carboxylase. Sites of pretranslational regulation by glucose of glucose-induced insulin release in pancreatic islets. *J. Biol. Chem.* **266**, 22392–22397 (1991).
17. Y. Q. Liu, J. Han, P. N. Epstein, Y. S. Long, Enhanced rat beta-cell proliferation in 60% pancreatectomized islets by increased glucose metabolic flux through pyruvate carboxylase pathway. *Am. J. Physiol. Endocrinol. Metab.* **288**, E471–478 (2005).
18. M. J. MacDonald *et al.*, Decreased levels of metabolic enzymes in pancreatic islets of patients with type 2 diabetes. *Diabetologia* **52**, 1087–1091 (2009).
19. J. Han, Y. Q. Liu, Reduction of islet pyruvate carboxylase activity might be related to the development of type 2 diabetes mellitus in Agouti-K mice. *J. Endocrinol.* **204**, 143–152 (2010).
20. D. Lu *et al.*, 13C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2708–2713 (2002).
21. X. Li *et al.*, The MDM2-p53-pyruvate carboxylase signalling axis couples mitochondrial metabolism to glucose-stimulated insulin secretion in pancreatic beta-cells. *Nat. Commun.* **7**, 11740 (2016).
22. A. Fu *et al.*, Glucose-dependent partitioning of arginine to the urea cycle protects β-cells from inflammation. *Nat. Metab.* **2**, 432–446 (2020).
23. A. Fu *et al.*, Glucose metabolism and pyruvate carboxylase enhance glutathione synthesis and restrict oxidative stress in pancreatic islets. *Cell Rep.* **37**, 110037 (2021).
24. T. Gregg *et al.*, Pancreatic β-cells from mice offset age-associated mitochondrial deficiency with reduced KATP channel activity. *Diabetes* **65**, 2700–2710 (2016).
25. A. Helman *et al.*, p16(Ink4a)-induced senescence of pancreatic beta cells enhances insulin secretion. *Nat. Med.* **22**, 412–420 (2016).
26. N. Kitao *et al.*, The role of glucokinase and insulin receptor substrate-2 in the proliferation of pancreatic beta cells induced by short-term high-fat diet feeding in mice. *Metabolism* **85**, 48–58 (2018).
27. R. Kehm *et al.*, Age-related oxidative changes in pancreatic islets are predominantly located in the vascular system. *Redox Biol.* **15**, 387–393 (2018).
28. C. Everaert *et al.*, Benchmarking of RNA-sequencing analysis workflows using whole-transcriptome RT-qPCR expression data. *Sci. Rep.* **7**, 1559 (2017).
29. A. Hernandez-Segura, J. Nehme, M. Demaria, Hallmarks of cellular senescence. *Trends Cell Biol.* **28**, 436–453 (2018).
30. E. González-Gualda, A. G. Baker, L. Fruk, D. Muñoz-Espín, A guide to assessing cellular senescence in vitro and in vivo. *FEBS J.* **288**, 56–80 (2021).
31. A. Midha *et al.*, Unique human and mouse β-cell senescence-associated secretory phenotype (SASP) reveal conserved signaling pathways and heterogeneous factors. *Diabetes* **70**, 1098–1116 (2021).
32. A. Helman *et al.*, Effects of ageing and senescence on pancreatic β-cell function. *Diabetes Obes. Metab.* **18**, 58–62 (2016).
33. Y. Tian, Y. Zhang, X. Fu, β cell senescence as a common contributor to type 1 and type 2 diabetes. *Trends Mol. Med.* **25**, 735–737 (2019).

34. F. Kruiswijk, C. F. Labuschagne, K. H. Vousden, p53 in survival, death and metabolic health: A lifeguard with a licence to kill. *Nat. Rev. Mol. Cell Biol.* **16**, 393–405 (2015).
35. J. Cha, C. Aguayo-Mazzucato, P. J. Thompson, Pancreatic β-cell senescence in diabetes: Mechanisms, markers and therapies. *Front. Endocrinol. (Lausanne)* **14**, 1212716 (2023).
36. M. Solimena *et al.*, Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. *Diabetologia* **61**, 641–657 (2018).
37. Å. Segerstolpe *et al.*, Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. *Cell Metab.* **24**, 593–607 (2016).
38. S. Dutta, P. Sengupta, Men and mice: Relating their ages. *Life Sci.* **152**, 244–248 (2016).
39. G. Santulli *et al.*, Age-related impairment in insulin release: The essential role of β(2)-adrenergic receptor. *Diabetes* **61**, 692–701 (2012).
40. L. Li *et al.*, Defects in β-cell Ca2+ dynamics in age-induced diabetes. *Diabetes* **63**, 4100–4114 (2014).
41. C. Aguayo-Mazzucato *et al.*, β cell aging markers have heterogeneous distribution and are induced by insulin resistance. *Cell Metab.* **25**, 898–910.e5 (2017).
42. A. M. Klein, R. M. de Queiroz, D. Venkatesh, C. Prives, The roles and regulation of MDM2 and MDMX: It is not just about p53. *Genes Dev.* **35**, 575–601 (2021).
43. C. Aguayo-Mazzucato *et al.*, Acceleration of β cell aging determines diabetes and senolysis improves disease outcomes. *Cell Metab.* **30**, 129–142.e4 (2019).
44. Y. Li *et al.*, TXNIP exacerbates the senescence and aging-related dysfunction of β cells by inducing cell cycle arrest through p38–p16/p21-CDK-Rb Pathway. *Antioxid. Redox Signal.* **38**, 480–495 (2023).
45. G. D. Kim, S. Park, Effects of Cudrania tricuspidata on anti-senescence in high glucose-treated endothelial cells via the Akt/p53/p21 pathway. *Food Sci. Nutr.* **8**, 5999–6006 (2020).
46. V. Sidarala, A. Kowluru, Exposure to chronic hyperglycemic conditions results in Ras-related C3 botulinum toxin substrate 1 (Rac1)-mediated activation of p53 and ATM kinase in pancreatic β-cells. *Apoptosis* **22**, 597–607 (2017).
47. M. M. Yore *et al.*, Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. *Cell* **159**, 318–332 (2014).
48. R. Barzalobre-Gerónimo *et al.*, Hyperglycemia promotes p53-Mdm2 interaction but reduces p53 ubiquitination in RINm5F cells. *Mol. Cell Biochem.* **405**, 257–264 (2015).
49. A. Hoshino *et al.*, Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic β-cell function in diabetes. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 3116–3121 (2014).
50. S. Tornovsky-Babeay *et al.*, Type 2 diabetes and congenital hyperinsulinism cause DNA double-strand breaks and p53 activity in β cells. *Cell Metab.* **19**, 109–121 (2014).
51. C. Uhlemeyer *et al.*, Selective ablation of P53 in pancreatic beta cells fails to ameliorate glucose metabolism in genetic, dietary and pharmacological models of diabetes mellitus. *Mol. Metab.* **67**, 101650 (2023).
52. M. Kracikova, G. Akiri, A. George, R. Sachidanandam, S. A. Aaronson, A threshold mechanism mediates p53 cell fate decision between growth arrest and apoptosis. *Cell Death Differ.* **20**, 576–588 (2013).
53. R. Amson *et al.*, Reciprocal repression between P53 and TCTP. *Nat. Med.* **18**, 91–99 (2011).
54. H. Inuzuka *et al.*, Phosphorylation by casein kinase I promotes the turnover of the Mdm2 oncoprotein via the SCF(beta-TRCP) ubiquitin ligase. *Cancer Cell* **18**, 147–159 (2010).
55. J. He, B. Baoyinna, S. J. Taleb, J. Zhao, Y. Zhao, USP13 regulates cell senescence through mediating MDM2 stability. *Life Sci.* **331**, 122044 (2023).
56. A. M. Ochocka *et al.*, FKBP25, a novel regulator of the p53 pathway, induces the degradation of MDM2 and activation of p53. *FEBS Lett.* **583**, 621–626 (2009).
57. F. Diraison *et al.*, Translationally controlled tumour protein (TCTP) is a novel glucose-regulated protein that is important for survival of pancreatic beta cells. *Diabetologia* **54**, 368–379 (2011).
58. M. J. Tsai *et al.*, TCTP is essential for β-cell proliferation and mass expansion during development and β-cell adaptation in response to insulin resistance. *Endocrinology* **155**, 392–404 (2014).
59. P. J. Thompson *et al.*, Targeted elimination of senescent beta cells prevents type 1 diabetes. *Cell Metab.* **29**, 1045–1060.e10 (2019).
60. H. Lee *et al.*, Stress-induced β cell early senescence confers protection against type 1 diabetes. *Cell Metab.* **35**, 2200–2215.e9 (2023).
61. S. S. Varghese, S. Dhawan, Senescence: A double-edged sword in beta-cell health and failure? *Front. Endocrinol. (Lausanne)* **14**, 1196460 (2023).
62. S. Miwa, S. Kashyap, E. Chini, T. von Zglinicki, Mitochondrial dysfunction in cell senescence and aging. *J. Clin. Invest.* **132**, e158447 (2022).
63. S. Igelmann *et al.*, A hydride transfer complex reprograms NAD metabolism and bypasses senescence. *Mol. Cell* **81**, 3848–3865.e19 (2021).
64. P. Jiang, W. Du, A. Mancuso, K. E. Wellen, X. Yang, Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. *Nature* **493**, 689–693 (2013).
65. Y. M. Yang *et al.*, The mitochondrial enzyme pyruvate carboxylase restricts pancreatic β-cell senescence by blocking p53 activation. Gene Expression Omnibus database. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE278865. Deposited 5 October 2024.