Deletion of protein kinase D1 in pancreatic beta cells impairs insulin secretion in high-fat fed mice

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ABSTRACT

Beta-cell adaptation to insulin resistance is necessary to maintain glucose homeostasis in obesity. Failure of this mechanism is a hallmark of type 2 diabetes (T2D). Hence, factors controlling functional beta-cell compensation are potentially important targets for the treatment of T2D. Protein kinase D1 (PKD1) integrates diverse signals in the beta cell and plays a critical role in the control of insulin secretion. However, the role of beta-cell PKD1 in glucose homeostasis in vivo is essentially unknown. Using beta-cell specific, inducible PKD1 knock-out mice (βPKD1KO), we examined the role of beta-cell PKD1 under basal conditions and during high-fat feeding. βPKD1KO mice under chow diet presented no significant difference in glucose tolerance or insulin secretion compared to mice expressing the Cre transgene alone; however, when compared to wild-type mice, both groups developed glucose intolerance. Under high-fat diet, deletion of PKD1 in beta cells worsened hyperglycemia, hyperinsulinemia and glucose intolerance. This was accompanied by impaired glucose-induced insulin secretion both in vivo in hyperglycemic clamps and ex vivo in isolated islets from high-fat fed βPKD1KO mice, without changes in islet mass. This study demonstrates an essential role for PKD1 in the beta-cell adaptive secretory response to high-fat feeding in mice.

Type 2 diabetes (T2D) is characterized by insufficient insulin secretion from the pancreatic beta cell. Functional beta-cell adaptation is a central mechanism by which the body overcomes insulin resistance to maintain glucose homeostasis in obese individuals. Over time, however, failure of this mechanism can lead to T2D (1). Insulin secretion is a tightly regulated process controlled by a number of metabolic, hormonal, and neural cues, many of which are mediated by G protein-coupled receptors (2). G protein-coupled receptors are validated targets for the treatment of T2D and among these, the fatty-acid receptor GPR40/FFAR1 has been the subject of considerable interest in recent years (3). GPR40 is predominantly expressed in beta cells and is implicated in the second phase of insulin secretion in response to fatty acids in vivo and in vitro (4-6). GPR40 preferentially couples to the G protein subunit Gαq and, as shown by our group in isolated mouse islets (7), its downstream signaling cascade involves protein kinase D1 (PKD1), a serine/threonine protein kinase of the calcium/calmodulin-dependent kinase family. PKD1 is also involved in the potentiation of insulin secretion by M3 muscarinic receptor signaling via beta-arrestins (8). Mechanistically, PKD1 promotes insulin vesicle fission at the trans-Golgi network (9; 10) and controls remodeling of the actin cytoskeleton (7). Hence, inhibition of PKD1 activity in cultured beta cells reduces insulin secretion by preventing the replenishment of secretion competent insulin granules at the plasma membrane (9; 11). Together these studies suggest that PKD1 activity regulates insulin granule formation and secretion. However, the precise role of beta-cell PKD1 in glucose homeostasis in vivo remains to be elucidated.

To this aim, we generated tamoxifen-inducible, beta-cell specific PKD1 knock-out mice and examined glucose homeostasis and beta-cell function and mass under basal conditions and in response to high-fat feeding.

RESEARCH DESIGN AND METHODS

Animals and diets

All procedures involving animals were approved by the institutional committee for the protection of animals at the Centre Hospitalier de l'Université de Montréal. Mice were housed on a 12-h light/dark cycle with free access to water and standard laboratory chow (Teklad Global 18% protein rodent diet 2918, Harlan Teklad, Madison, WI). Mice carrying LoxP sites between exon 11 and 12 and exons 14 and 15 of one *Prkd*1 allele (PKD1$^{+/fl}$) (12) and transgenic MIP-CreERT^{1Lphi} (MIP-CreERT) mice (13; 14) were backcrossed to C57Bl/6N (Charles River, Saint-Constant, QC) for 9 generations and genotyped as described (12; 13). PKD1$^{+/fl}$ female and MIP-CreERT; PKD1$^{+/fl}$ male were crossed to generate experimental groups. Tamoxifen (Sigma, Oakville, ON) was injected IP (125 mg/kg) at 48-h intervals for a total of 3 injections in 9-week-old males as described (15). All experimental animals received tamoxifen. Three weeks following tamoxifen injections (week 0), mice were either kept on standard chow or given a high-fat diet (60% fat, 15% protein and 25% carbohydrate on a caloric basis; Bioserv Diets, S3282, Frenchtown, NJ) for up to 13 weeks. Body weight composition was assessed with the EchoMRI Analyzer-700 (Echo Medical System, Houston, TX).

Oral glucose tolerance test and hyperglycemic clamps

Oral glucose tolerance tests (OGTT) were performed in 4-h fasted mice by measuring tail blood glucose and plasma insulin after oral glucose administration (2 g/kg) by gavage as described (16).

One-step hyperglycemic clamps were performed in conscious, ad libitum fed animals as described (6). Briefly, a 20% dextrose solution (Baxter, Mississauga, ON) was infused via a jugular catheter. Mice initially received a 90-second bolus (140 mg/kg/minute) and then the glucose infusion rate (GIR) was adjusted to maintain blood glucose between 18 and 21 mmol/l for 80 min. The insulin sensitivity index (M/I) was calculated as the glucose infusion rate (M) divided by the average insulinemia during the last 30 min of the clamp (I). Insulin clearance (Insulin/C-peptide) was estimated as the average insulinemia divided by the average C-peptide during the last 30 min of the clamp. Blood samples were collected from the tail to measure glucose using the hand-held glucometer Accu-Chek (Roche, Indianapolis, IN) and plasma insulin, C-peptide and proinsulin were measured with ELISA (Alpco Diagnostics, Salem, NH) at the time points indicated in the Figure legends.

Islet mass measurements

Immediately following the hyperglycemic clamp, the pancreas was removed and islet mass was measured on paraffin sections using an anti-chromogranin A antibody (Ab85554, Abcam, Toronto, ON) to label endocrine cells as described (17).

Static incubations

Islets were isolated by collagenase (Sigma, Oakville, ON) digestion as described (4) and recovered in RPMI 1640 (Gibco Life Technologies, Burlington, ON) supplemented with 10% (wt/vol) FBS (Gibco Life Technologies, Burlington, ON), 100 U/ml penicillin/streptomycin and 11 mmol/l glucose. Triplicate batches of 10 islets each were incubated in KRBH with 0.1% BSA and 2.8 mmol/l glucose twice for 20 minutes followed by a 1-h static incubation in KRBH in the presence of glucose, oleate (0.5 mmol/l; Sigma, Oakville, ON), carbachol (500 µmol/l; Millipore, Billerica, MA) or glucagon-like peptide-1 (GLP-1; 0.1 µmol/l; Bachem, Torrance, CA), as indicated in the Figure legends. Oleate was complexed for 1 h at 37°C with fatty-acid-free BSA (BAH66, Equitech-Bio, Kerrville, TX) to a final molar ratio of 1:5 prior to use as described (18). Control conditions contained the same amount of BSA and vehicle (50% (vol/vol) ethanol). Secreted insulin was measured in the supernatant and intracellular insulin content was measured after acid–alcohol extraction by radioimmunoassay using a rat insulin RIA kit (Millipore, Billerica, MA).

Western blots

Islet protein extracts were subjected to 10% SDS–PAGE, transferred to nitrocellulose membranes and immunoblotted with primary antibodies against PKD1 (1:500; Cell Signaling, New England Biolabs, Whitby, ON) or tubulin (1:5000; Abcam, Toronto, ON), then horseradish peroxidase-labeled anti-rabbit IgG secondary antibodies in 5% (wt/vol) milk, and visualized using Western Lighting Plus ECL (Perkin Elmer, Woodbridge, ON). Band intensity was quantified using Image J software (National Institutes of Health).

Statistical analyses

Data are expressed as mean ± SEM. Significance was tested using one-way ANOVA with Tukey or Dunnett post hoc test, or two-way ANOVA with post hoc adjustment for multiple comparisons, as appropriate, using GraphPad Instat (GraphPad Software, San Diego, CA). $P<0.05$ was considered significant.

RESULTS

Efficient beta-cell specific deletion of PKD1

MIP-CreERT;PKD1$^{fl/fl}$ (βPKD1KO) mice were born at the expected Mendelian ratio. At 9 weeks of age, male mice from all 4 experimental groups were injected with tamoxifen. Three weeks later, PKD1 protein levels were assessed by Western blotting in isolated islets. PKD1 protein levels were significantly reduced in βPKD1KO mice compared to MIP-CreERT, PKD1$^{fl/fl}$ (FL) or wild-type (WT) littermates (Suppl. Fig. 1A&B).

MIP-CreERT and βPKD1KO mice develop glucose intolerance

We first performed sequential OGTTs in a first cohort of chow-fed animals from all 4 genotypes starting 3 weeks after tamoxifen injections (week 0). As shown in Fig. 1, glucose tolerance was reduced, though not significantly, in MIP-CreERT and βPKD1KO mice compared to WT mice at week 0 (Fig. 1A). At week 8, glucose tolerance of MIP-CreERT and βPKD1KO mice became significantly different from WT mice (Fig. 1B), and it remained so for βPKD1KO mice at week 12 (Fig. 1C). Insulin levels were unaffected (Fig. 1D-F). The development of glucose intolerance in both MIP-CreERT and βPKD1KO mice suggests that this phenotype is likely due to the presence of the Cre transgene rather than PKD1 deletion.

Second, to further examine the impact of the Cre transgene on insulin secretion in vivo, animals from the 4 genotypes were subjected to hyperglycemic clamps at week 13 (Fig. 2). Mice were catheterized at the beginning of week 13 and allowed to recover for 3-5 days. During the clamp, target blood glucose levels were achieved in all 4 groups and were stable between 50 and 80 minutes (Fig. 2A). Although insulin levels during the clamp were not significantly different (Fig.

2B&C), plasma C-peptide levels were significantly reduced in MIP-CreERT and βPKD1KO mice compared to WT mice (Fig. 2D). Both MIP-CreERT and βPKD1KO mice had reduced GIR compared to WT mice (Fig. 2E), although the difference was statistically significant only for βPKD1KO mice. However, the M/I index of insulin sensitivity (Fig. 2F) and the insulin to C-peptide ratio (Suppl. Fig. 2A) were not significantly different between all 4 groups.

Third, to examine whether the lower C-peptide levels in MIP-CreERT and βPKD1KO mice during the clamp reflected an intrinsic insulin secretory defect, we performed 1-h static incubations in islets isolated at week 0 (Fig. 3). As shown in Fig. 3A, glucose-stimulated insulin secretion (GSIS) was similar in all 4 groups. Whereas the potentiation of GSIS by oleate was similar between MIP-CreERT, FL and WT islets, it was slightly but significantly diminished in βPKD1KO vs. WT islets (Fig. 3A). In contrast, the potentiation of GSIS by carbachol was diminished in MIP-CreERT, FL, and βPKD1KO vs. WT islets (Fig. 3A). Neither the potentiation of GSIS by GLP-1 nor insulin content were significantly different between the 4 genotypes (Fig. 3A&B). Overall, these data suggest that 1) expression of the MIP-CreERT transgene results in glucose intolerance, lower glucose-induced C-peptide secretion in vivo, but no obvious defect in GSIS ex vivo; and 2) beta-cell PKD1 is dispensable for normal glucose homeostasis in mice under basal conditions. Given the phenotype of the MIP-CreERT mice, this group was used as a control against which the βPKD1KO mice were compared in all subsequent experiments.

Deletion of PKD1 in beta cells exacerbates hyperglycemia, hyperinsulinemia, and glucose intolerance under high-fat diet

We administered a high-fat diet (58% calories from fat) to a second cohort of MIP-CreERT and βPKD1KO mice for 12 weeks, beginning at week 0 (3 weeks after tamoxifen injections). PKD1 protein levels assessed by Western blot of isolated islets confirmed that PKD1 remained significantly reduced in βPKD1KO islets compared to MIP-CreERT islets after 12 weeks of diet (Suppl. Fig. 1C&D). During the 12-week diet, caloric intake (Fig. 4A) and weight gain (Fig. 4B) were not different between both groups, except for a transient increase in weight gain in βPKD1KO mice between weeks 5 and 7 (Fig. 4B). Fed and fasted glucose (Fig. 4C&D) and insulin (Fig. 4E&F) levels were similar between normal-chow fed βPKD1KO and MIP-CreERT mice. In contrast, high-fat fed βPKD1KO mice became more severely hyperglycemic than high-fat fed MIP-CreERT mice after 8 weeks, especially in the fed state (Fig. 4C&D). This was accompanied by elevated levels of circulating insulin (Fig. 4E&F). Lean (Fig. 4G) and fat (Fig. 4H) mass respectively decreased and increased in response to high-fat feeding, but were not different between both genotypes after the 12-week diet. Proinsulin levels were not significantly different in βPKD1KO compared to MIP-CreERT following 8 and 12 weeks of high-fat diet (Fig. 4I).

OGTTs were performed following a 4-h fast in chow and high-fat diet fed groups at weeks 8 and 12 (Fig. 5). As expected, glucose intolerance (Fig. 5A&B) and hyperinsulinemia (Fig. 5C&D) were observed in both high-fat fed groups during the OGTT. βPKD1KO mice were more severely glucose intolerant than MIP-CreERT mice at week 8 (Fig. 5A), although this difference was no longer significant at week 12 (Fig. 5B). Insulin levels during the OGTT were not different between both genotypes (Fig. 5C&D). These data indicate that beta-cell specific PKD1 deletion exacerbates high-fat diet-induced hyperglycemia, hyperinsulinemia and glucose intolerance.

Deletion of PKD1 in beta cells is associated with defective GSIS in high-fat fed mice

A cohort of MIP-CreERT and βPKD1KO mice underwent catheterization under general anesthesia after 12 weeks of diet and were allowed to recover from surgery while on the same respective diet regimen, after which insulin secretion in vivo was assessed by hyperglycemic clamps (Fig. 6). Target blood glucose levels during the clamp were achieved in all 4 groups and were stable between 50 and 80 minutes (Fig. 6A). High-fat fed MIP-CreERT mice displayed a robust insulin response to glucose during the clamp (Fig. 6B), both at early time points (Fig. 6C) and during the steady-state period (Fig. 6D). In contrast, the glucose-stimulated insulin response of high-fat fed βPKD1KO mice was significantly reduced compared to high-fat fed MIP-CreERT mice (Fig. 6B) and indistinguishable from that of chow-fed controls (Fig. 6B-D). Similar to insulin, C-peptide levels during the steady state of the clamp were increased in high-fat fed MIP-CreERT but not βPKD1KO mice (Fig. 6E). The glucose infusion rate (Fig. 6F) during the clamp and M/I index of insulin sensitivity (Fig. 6G) trended lower in both high-fat fed groups, suggestive of insulin resistance, but were not different between βPKD1KO and MIP-CreERT mice, as was the insulin to C-peptide ratio (Suppl. Fig. 2B). Taken together, these data indicate that deletion of PKD1 in beta cells impairs insulin secretion in vivo after high-fat feeding.

To examine whether this phenotype was associated with defective GSIS ex vivo, we performed 1-h static incubations of islets isolated from high-fat fed mice. While insulin secretion in response to 2.8 and 8.3 mmol/l glucose was similar between βPKD1KO and MIP-CreERT mice (Fig. 7A), the stimulation index calculated as insulin secretion at 16.7 mmol/l glucose / insulin secretion at 2.8 mmol/l glucose was significantly reduced in βPKD1KO islets (Fig. 7B) without changes in insulin content (Fig. 7C). This was not associated with significant changes in islet area, islet mass or total islet number (Suppl. Fig. 3A-C), although high-fat fed βPKD1KO mice tended to have a greater proportion of larger islets (Suppl. Fig. 3D).

DISCUSSION

The objective of this study was to delineate the contribution of PKD1 in beta cells to glucose homeostasis. To this aim, we analyzed the metabolic consequence of beta-cell specific PKD1 deletion in mice under basal conditions and in response to high-fat feeding. We showed that βPKD1KO mice became more hyperglycemic, hyperinsulinemic and glucose intolerant than control MIP-CreERT mice under high-fat diet. High-fat fed βPKD1KO mice had defective GSIS in hyperglycemic clamps in vivo and in isolated islets ex vivo, without significant changes in islet mass. Our findings demonstrate a key contribution of PKD1 to the beta-cell functional adaptation to high-fat feeding.

Under normal diet, both MIP-CreERT and βPKD1KO mice became glucose intolerant with age as compared to FL or WT mice (Fig. 1), suggesting that this phenotype was due to the MIP-CreERT transgene rather than deletion of PKD1. Although insulin levels were not significantly lower during the OGTT (Fig. 1), C-peptide levels during the hyperglycemic clamps were significantly reduced (Fig. 2), which likely contributes to the glucose intolerance. As all mice in our study were on the same C57Bl/6N background, the difference between Cre-expressing (MIP-CreERT and βPKD1KO) and non-Cre-expressing (FL and WT) mice is likely due to the human growth hormone minigene included in the MIP-CreERT transgene (14). In a previous study (14) we observed normal glucose tolerance in MIP-CreERT mice of similar age (equivalent to week 0 in this study) on the C57Bl/6J background, an observation subsequently confirmed by Carboneau et al. (19). We attribute this apparent discrepancy (at week 8 and 12 in this study) to the fact that both of these previous studies used MIP-CreERT mice on a C57Bl/6J background while ours were on a C57Bl/6N background. Such possibility is supported by the known phenotypic differences between the 6J and 6N sub-strains (16; 20-23). These findings further highlight the importance of including Cre-expressing mice as controls for beta-cell specific knockout mice generated with the MIP-CreERT construct (13; 14). βPKD1KO mice were very similar to MIP-CreERT mice under normal diet (Fig. 1&2), suggesting that beta-cell PKD1 is dispensable for glucose tolerance and insulin secretion under basal conditions, although we cannot entirely rule out the possibility that the glucose intolerance due to expression of the Cre transgene might have masked a subtle phenotype induced by PKD1 deletion.

As expected, oleate potentiation of GSIS was reduced in islets from βPKD1KO mice (Fig. 3), although this reduction was less pronounced than previously observed in islets from FL mice transduced with an adenovirus encoding Cre (7). In contrast to previous studies using PKD1-targeting shRNA (8), M3 muscarinic-potentiation of GSIS in βPKD1KO islets was not significantly reduced compared to MIP-CreERT islets (Fig. 3). These results suggest that sustained deletion of PKD1 for several weeks in the present study might have triggered compensatory mechanisms not observed upon acute PKD1 knockdown in isolated islets.

During the course of high-fat feeding, βPKD1KO mice exhibited more severe hyperglycemia, hyperinsulinemia, and glucose intolerance than their MIP-CreERT littermates (Fig. 4&5). The possibility that this was due to more severe insulin resistance in high-fat fed βPKD1KO mice appears unlikely since the M/I index of insulin sensitivity and the insulin to C-peptide ratio calculated from the hyperglycemic clamps at the end of the diet period were not different from that of MIP-CreERT mice (Fig. 6 and Suppl Fig. 2). The difference in glucose tolerance between high-fat fed βPKD1KO and MIP-CreERT mice was no longer significant after 12 weeks (Fig. 5). This could be due to the fact that glucose tolerance in mice is largely independent of insulin secretion dynamics, especially under elevated insulin concentrations (24). Regardless of any differences in insulin sensitivity or glucose effectiveness between βPKD1KO and MIP-CreERT mice, fasting and fed hyperglycemia (Fig. 4) and oral glucose intolerance (Fig. 5) in high-fat fed βPKD1KO mice are indicative of a defect in beta-cell compensation. This conclusion is supported by the results of the hyperglycemic clamps (Fig. 6) and static incubations of isolated islets (Fig. 7), both demonstrating a defect in GSIS after 13 weeks of high-fat diet.

The observed defect in GSIS in βPKD1KO mice is in agreement with previous findings indicating a positive correlation between PKD1 activity and insulin secretion. An increase in PKD1 activity in beta cells resulting from the loss of mitogen-activated protein kinase p38 δ (11) or peroxisome proliferator-activated receptor β/δ (25) enhances GSIS and improves glucose tolerance. Conversely, down-regulation of PKD1 in insulin-secreting cell lines reduces GSIS (9; 11) and defective PKD1 activation in phosphorylation-deficient M(3)-muscarinic receptor mutant mice (M3RKI) decreases GSIS and glucose tolerance (8). In contrast, our data indicating that islet mass was not altered in βPKD1KO mice (Suppl. Fig. 3) diverge from previous studies supporting a role of PKD1 in beta-cell proliferation and survival (11; 25). However, the present study was conducted using adult beta-cell specific knockout mice, whereas evidence supporting a role of PKD1 in beta-cell proliferation and survival are based on indirect PKD1 gain-of-function studies performed during neonatal development (25) or in a non-physiological setting of STZ-induced apoptosis (11), respectively. Hence, the contribution of PKD1 to beta-cell mass regulation may be context dependent.

Previously we demonstrated that PKD1 is necessary for GPR40-mediated potentiation of GSIS in response to long-chain fatty acids (7). High-fat fed mice deficient for GPR40 secrete significantly less insulin in response to glucose (5), a profile that resembles the phenotype of the high-fat fed βPKD1KO described in this study. This similarity is consistent with an important role of PKD1 in the GPR40 signaling cascade. PKD1 is necessary for the replenishment of insulin granules (7; 9; 10), hence it is tempting to speculate that altered vesicle formation and recruitment in βPKD1KO mice may yield a more profound phenotype under metabolic stress of high-fat feeding, when insulin demand is higher, than under basal conditions. However, the absence of a significant change in proinsulin levels in high-fat fed βPKD1KO mice (Fig. 4) suggest that insulin granule maturation is intact. Further studies will be required to elucidate the precise molecular events downstream of PKD1 controlling functional beta-cell compensation.

In conclusion, we provide the first in vivo and ex vivo analysis of a beta-cell specific PKD1 knock-out mouse and show that PKD1 in beta cells is dispensable under basal conditions but necessary for the compensatory increase in GSIS in response to high-fat feeding, furthering our understanding of the implication of this kinase in the control of beta-cell function.

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V.B. designed the experiments and acquired the data. V.B., J.G. and V.P. researched data, analyzed the results, and wrote the manuscript. K.V. acquired the data. V.P. conceived and designed the project. N.T., L.H.P. and J.F. provided key reagents. All authors revised the manuscript and approved the final version. V.P. is the guarantor of this work and, as such, takes full responsibility for the work.

The authors have no relevant conflict of interest to disclose.

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FIGURE LEGENDS

Figure 1: Age-related changes in oral glucose tolerance and insulin levels in βPKD1KO and control mice.

Blood glucose (A-C) and plasma insulin (D-F) in mice during OGTT at week 0 (A,D) and after 8 (B,E) and 12 weeks (C,F) on normal diet. Data are mean +/- SEM of 5 to 10 animals per group. * $p<0.05$ compared to WT following two-way ANOVA with Dunnett post hoc adjustment for multiple comparisons.

Figure 2: Hyperglycemic clamps in βPKD1KO and control mice.

Blood glucose (A) and plasma insulin (B) in mice during hyperglycemic clamps performed after 13 weeks on normal diet. Average plasma insulin (C), average plasma C-peptide (D), GIR (E), and M/I index (F) as assessed during the steady-state of the clamp (50 to 80 min). Data are mean +/- SEM of 7 to 11 animals per group. * $p<0.05$ and ** $p<0.01$ compared to WT following one-way ANOVA with Dunnett post hoc adjustment for multiple comparisons.

Figure 3: Insulin secretion from βPKD1KO and control mouse islets ex vivo.

Insulin secretion presented as a percentage of islet insulin content was assessed in 1-h static incubations in response to glucose in the absence or presence of oleate (OL), carbachol (Carb) and GLP-1 (A). Total islet insulin content (B). Data are mean +/- SEM of 7 to 10 animals per group. * $p<0.05$ and ** $p<0.01$ compared to WT following two-way ANOVA with Dunnett post hoc adjustment for multiple comparisons.

Figure 4: Glucose homeostasis in chow and high-fat diet fed βPKD1KO and MIP-CreERT mice.

Caloric intake (A), weight gain (B), fed (C&E) and 4-h fasted (D&F) blood glucose (C&D) and plasma insulin (E&F), lean (G) and fat (H) mass and proinsulin levels (I) in normal (ND) or high-fat (HF) diet fed mice at the week indicated (A-F, I) or at week 12 (G, H). Data are mean +/- SEM of 9 to 13 animals per group. & $p<0.05$ compared to respective ND control or * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ comparing βPKD1KO-HF to MIP-CreERT-HF following two-way ANOVA with Tukey post hoc adjustment for multiple comparisons. (£) values were obtained at time 0 during the OGTT.

Figure 5: Oral glucose tolerance and insulin levels in chow and high-fat diet fed βPKD1KO and MIP-CreERT mice.

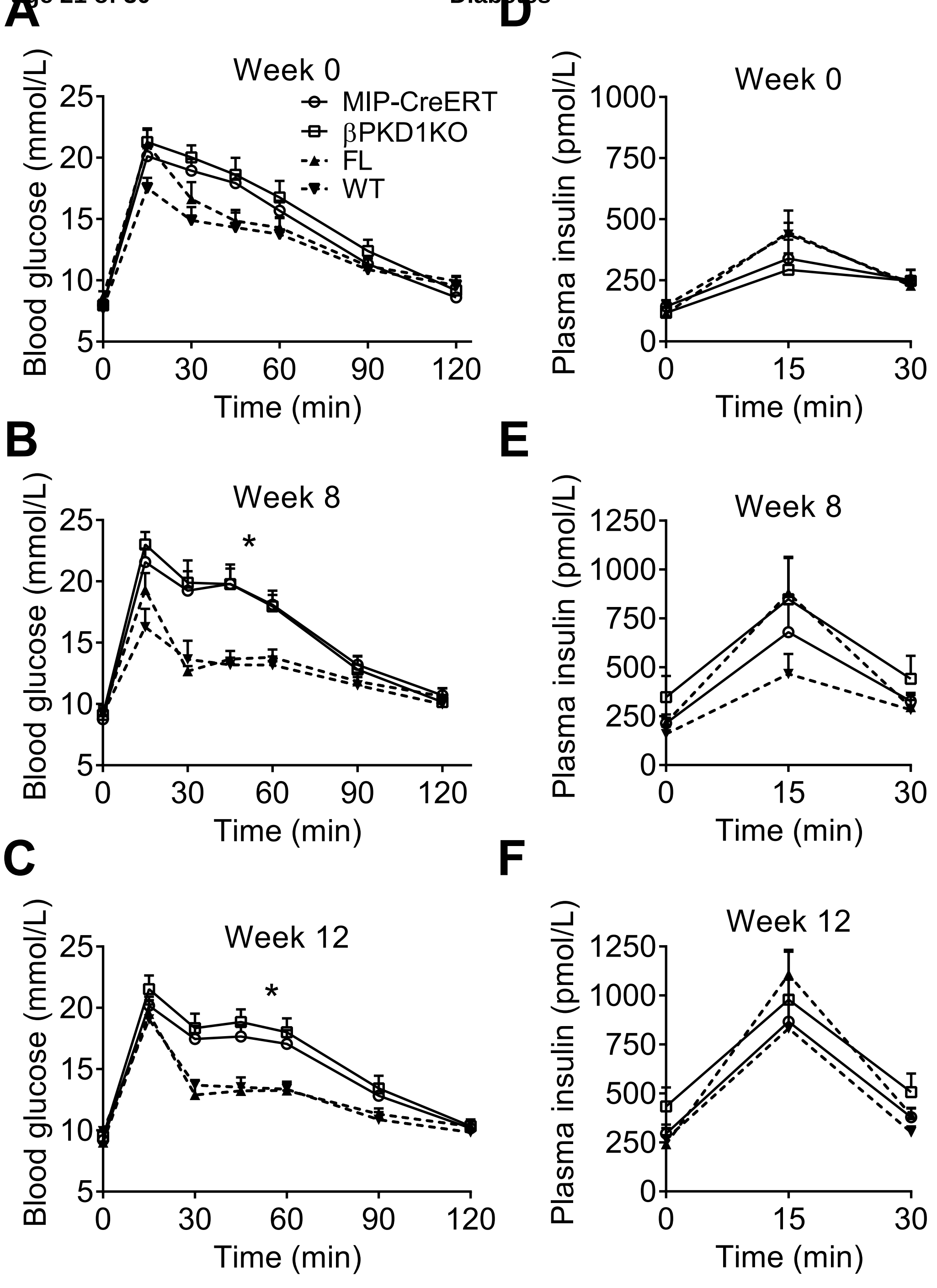
Blood glucose (A&B) and plasma insulin (C&D) in mice during OGTT following 8 (A&C) or 12 (B&D) weeks of normal chow (ND) or high-fat (HF) diet. Data are mean +/- SEM of 9 to 12 animals per group. & $p<0.05$ and && $p<0.01$ compared to respective ND control or ** $p<0.01$ compared to MIP-CreERT-HF following two-way ANOVA with Tukey post hoc adjustment for multiple comparisons.

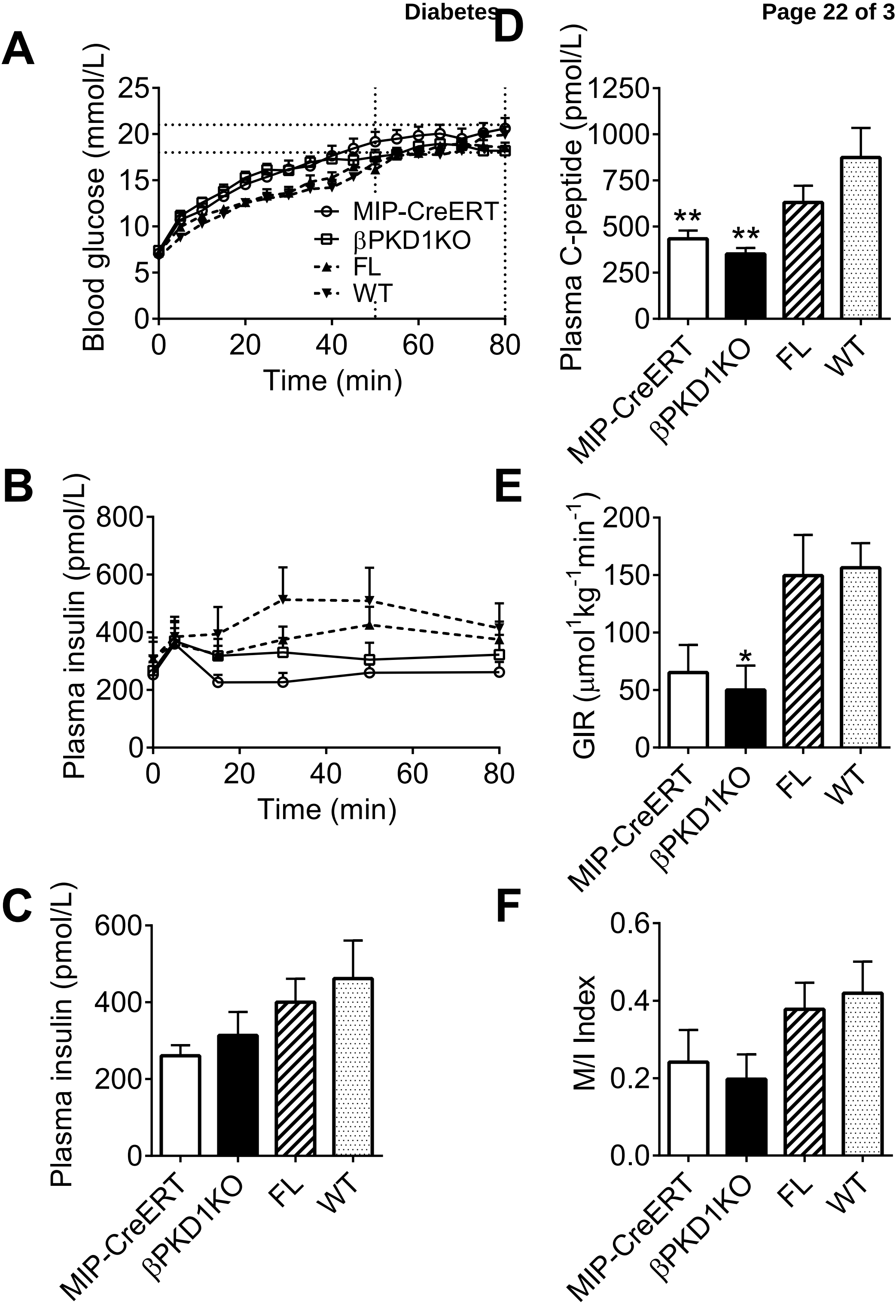
Figure 6: Hyperglycemic clamps in chow and high-fat diet fed βPKD1KO and MIP-CreERT mice.

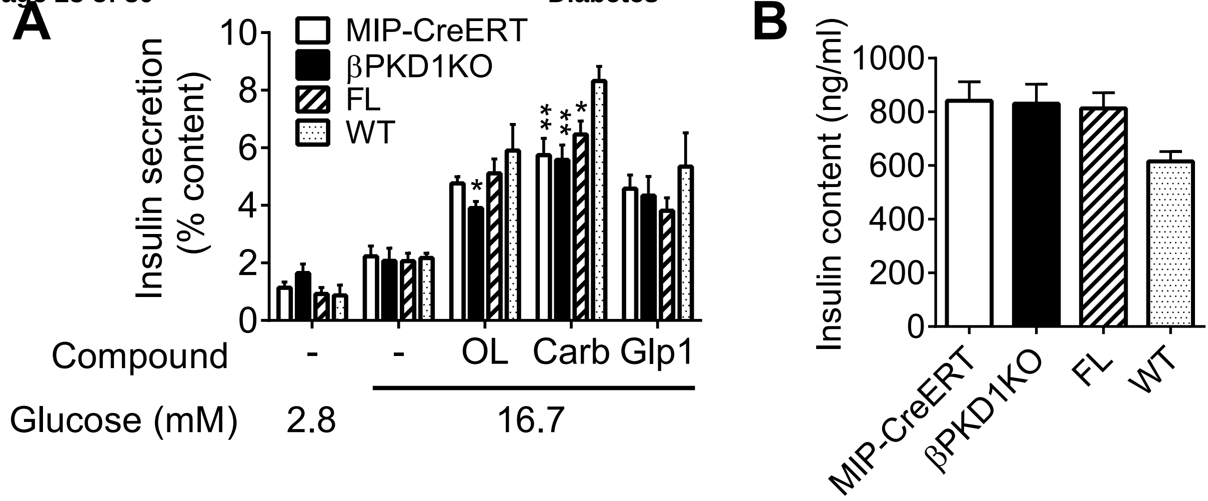
Blood glucose (A) and plasma insulin (B) in mice during hyperglycemic clamps after 13 weeks on normal chow (ND) or high-fat (HF) diet. Area under the curve (AUC) for plasma insulin during the first 15 min (C) and average insulin (D), average C-peptide (E), GIR (F) and M/I index (G) as assessed during the clamp (50 to 80 min). Data are mean +/- SEM of 7 to 12 animals per group. & $p<0.05$ compared to respective ND control or * $p<0.05$ comparing βPKD1KO-HF to MIP-CreERT-HF following two-way ANOVA with Tukey post hoc adjustment for multiple comparisons.

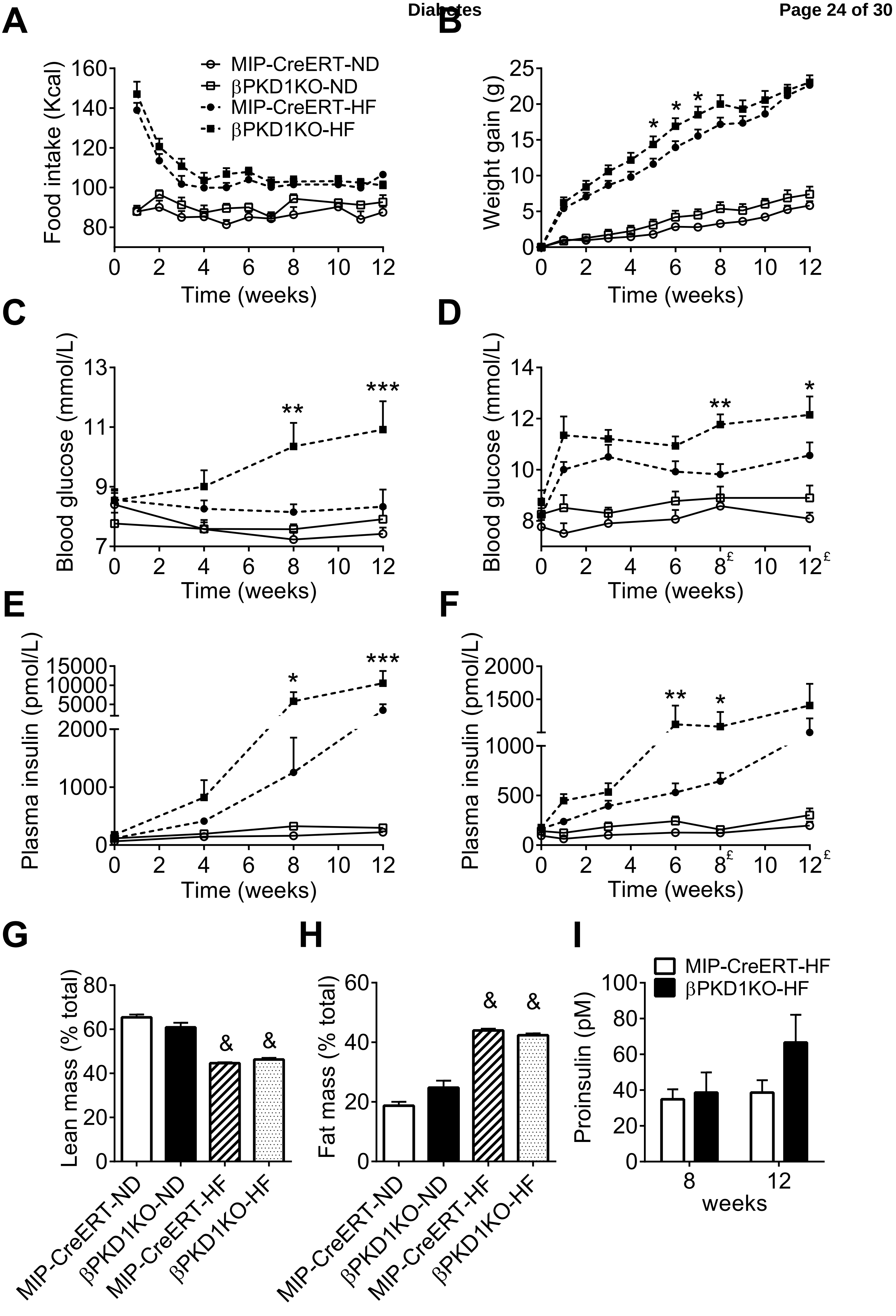
Figure 7: Insulin secretion from high-fat fed βPKD1KO and MIP-CreERT mouse islets ex vivo.

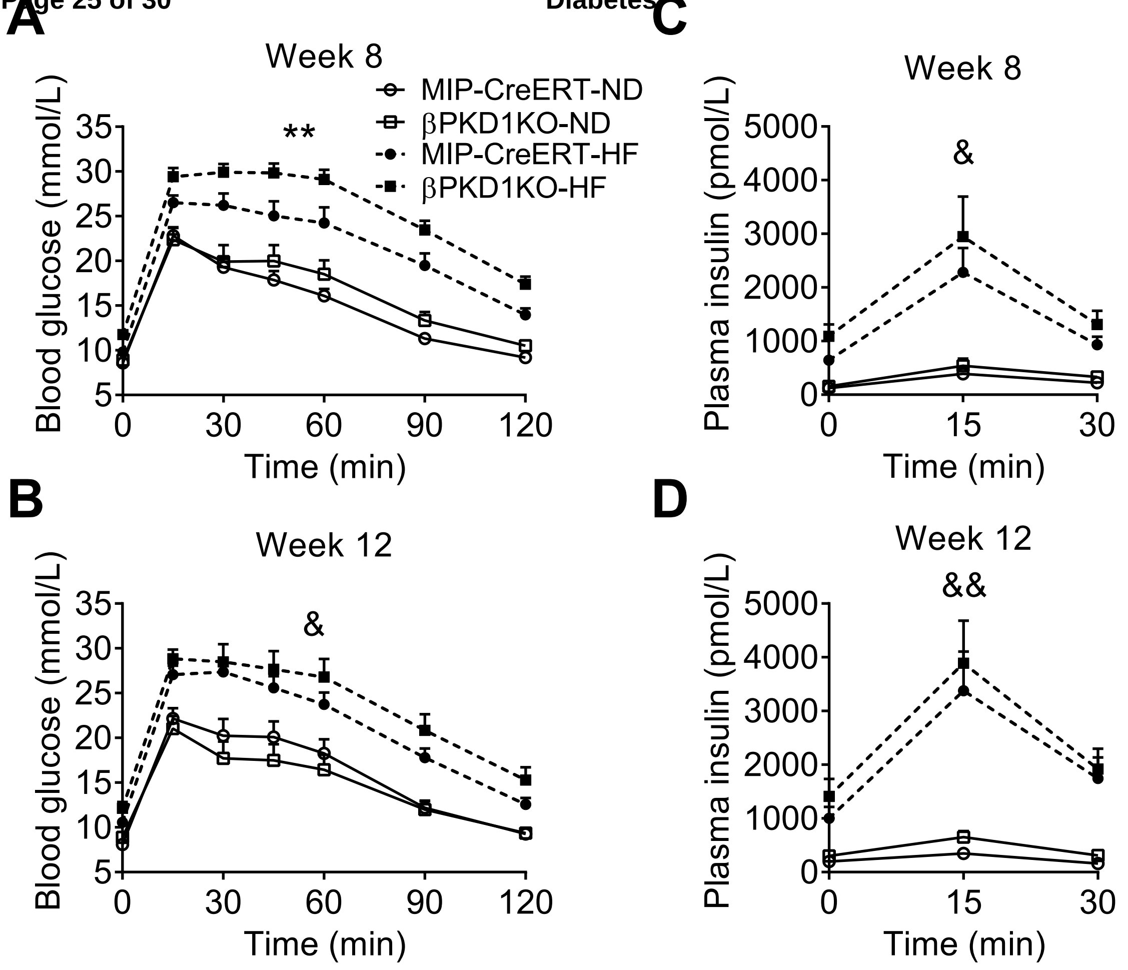
Insulin secretion presented as a percentage of islet insulin content was assessed in 1-h static incubations in response to glucose (A). Stimulation index calculated by the ratio of insulin secretion at 16.7 mmol/l / insulin secretion at 2.8 mmol/l glucose (B) and total islet insulin content (C). Data are mean +/- SEM of 6 to 7 replicate experiments. * $p<0.05$ compared to MIP-CreERT following one-tailed Student's *t*-test.

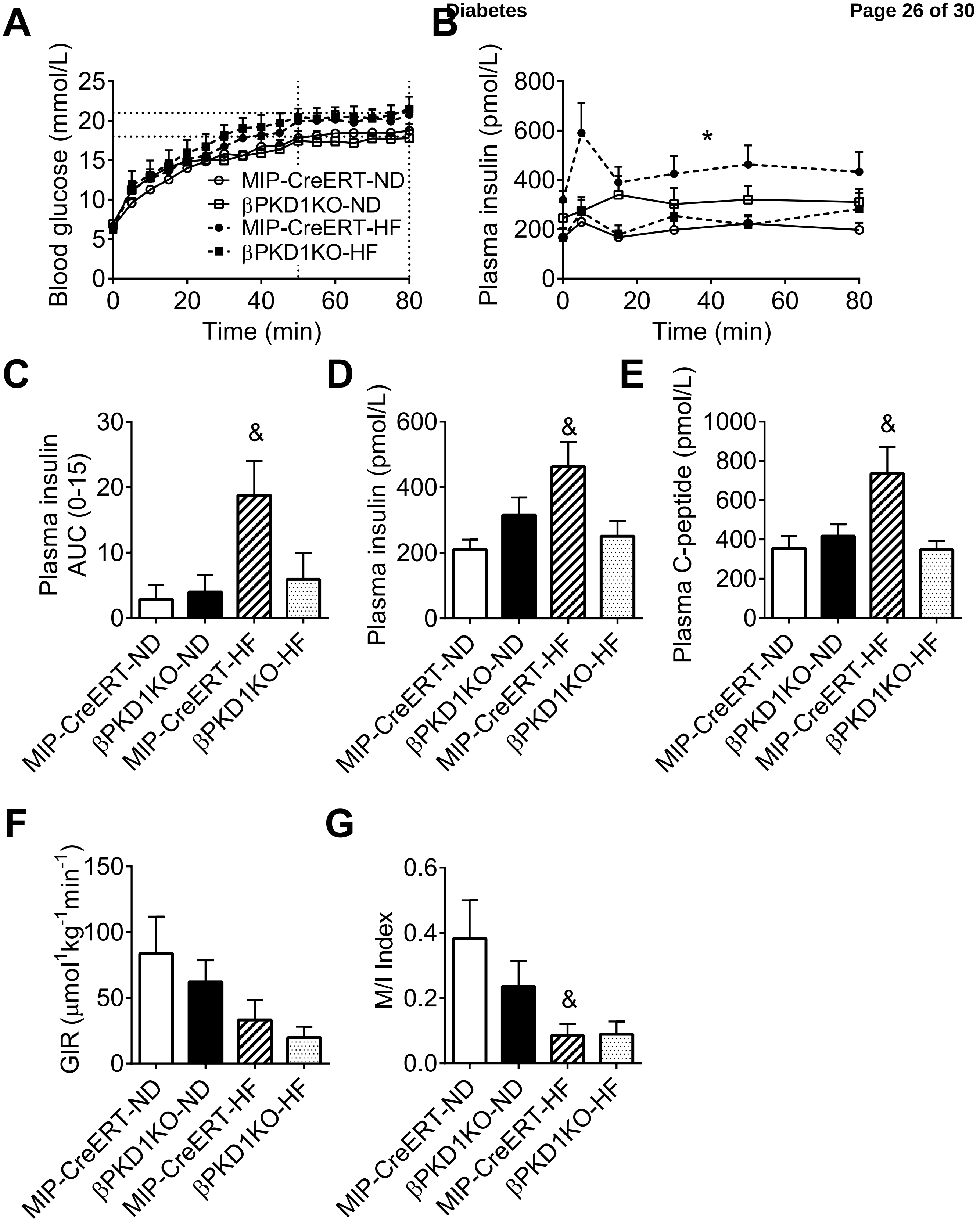

A
Week 0
Blood glucose (mmol/L)
25
20
15
10
5
0
30
60
90
120
Time (min)
MIP-CreERT
βPKD1KO
FL
WT
D
Week 0
Plasma insulin (pmol/L)
1000
750
500
250
0
15
30
Time (min)
B
Week 8
Blood glucose (mmol/L)
*
Time (min)
E
Week 8
Plasma insulin (pmol/L)
1250
1000
750
500
250
0
Time (min)
C
Week 12
Blood glucose (mmol/L)
*
Time (min)
F
Week 12
Plasma insulin (pmol/L)
Time (min)

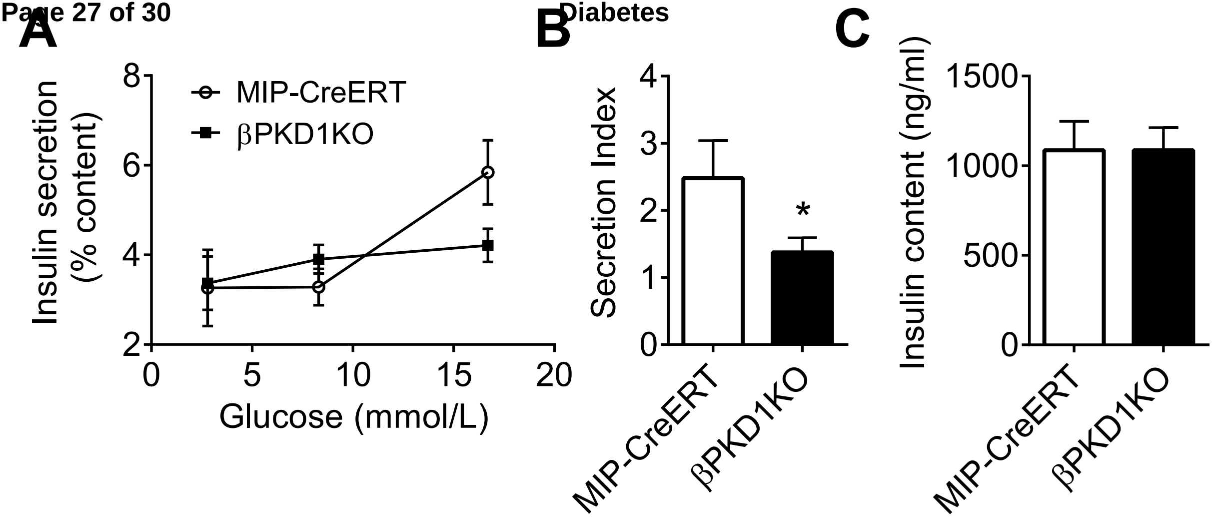

A
Blood glucose (mmol/L)
0
5
10
15
20
25
0
20
40
60
80
Time (min)
MIP-CreERT
βPKD1KO
FL
WT
B
Plasma insulin (pmol/L)
0
200
400
600
800
0
20
40
60
80
Time (min)
C
Plasma insulin (pmol/L)
0
200
400
600
MIP-CreERT
βPKD1KO
FL
WT
D
Plasma C-peptide (pmol/L)
0
250
500
750
1000
1250
**
**
MIP-CreERT
βPKD1KO
FL
WT
E
GIR (μmol^{1}kg^{-1}min^{-1})
0
50
100
150
200
*
MIP-CreERT
βPKD1KO
FL
WT
F
M/I Index
0.0
0.2
0.4
0.6
MIP-CreERT
βPKD1KO
FL
WT


A
Insulin secretion (% content)
0
2
4
6
8
10
MIP-CreERT
βPKD1KO
FL
WT
*
**
**
*
Compound
-
-
OL
Carb
Glp1
Glucose (mM)
2.8
16.7
B
Insulin content (ng/ml)
0
200
400
600
800
1000
MIP-CreERT
βPKD1KO
FL
WT


A
Food intake (Kcal)
160
140
120
100
80
Time (weeks)
0
2
4
6
8
10
12
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
B
Weight gain (g)
25
20
15
10
5
0
Time (weeks)
*
*
*
*
C
Blood glucose (mmol/L)
13
11
9
7
Time (weeks)
**
***
D
Blood glucose (mmol/L)
14
12
10
8
Time (weeks)
8£
12£
**
*
E
Plasma insulin (pmol/L)
15000
10000
5000
2000
1000
0
Time (weeks)
*
***
F
Plasma insulin (pmol/L)
2000
1500
1000
500
0
Time (weeks)
8£
12£
**
*
G
Lean mass (% total)
80
60
40
20
0
&
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
H
Fat mass (% total)
60
40
20
0
&
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
I
Proinsulin (pM)
100
80
60
40
20
0
MIP-CreERT-HF
βPKD1KO-HF
8
12
weeks


A
Week 8
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
**
Blood glucose (mmol/L)
35
30
25
20
15
10
5
0
30
60
90
120
Time (min)
B
Week 12
&
Blood glucose (mmol/L)
Time (min)
C
Week 8
&
Plasma insulin (pmol/L)
5000
4000
3000
2000
1000
0
15
30
Time (min)
D
Week 12
&&
Plasma insulin (pmol/L)
Time (min)


A
Blood glucose (mmol/L)
25
20
15
10
5
0
0
20
40
60
80
Time (min)
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
B
Plasma insulin (pmol/L)
800
600
400
200
0
*
0
20
40
60
80
Time (min)
C
Plasma insulin AUC (0-15)
30
20
10
0
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
D
Plasma insulin (pmol/L)
600
400
200
0
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
E
Plasma C-peptide (pmol/L)
1000
800
600
400
200
0
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
F
GIR (μmol^{1}kg^{-1}min^{-1})
150
100
50
0
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF
G
M/I Index
0.6
0.4
0.2
0.0
&
MIP-CreERT-ND
βPKD1KO-ND
MIP-CreERT-HF
βPKD1KO-HF


A
B
C
Insulin secretion (% content)
8
6
4
2
0
5
10
15
20
Glucose (mmol/L)
MIP-CreERT
βPKD1KO
Secretion Index
4
3
2
1
0
*
MIP-CreERT
βPKD1KO
Insulin content (ng/ml)
1500
1000
500
0
MIP-CreERT
βPKD1KO

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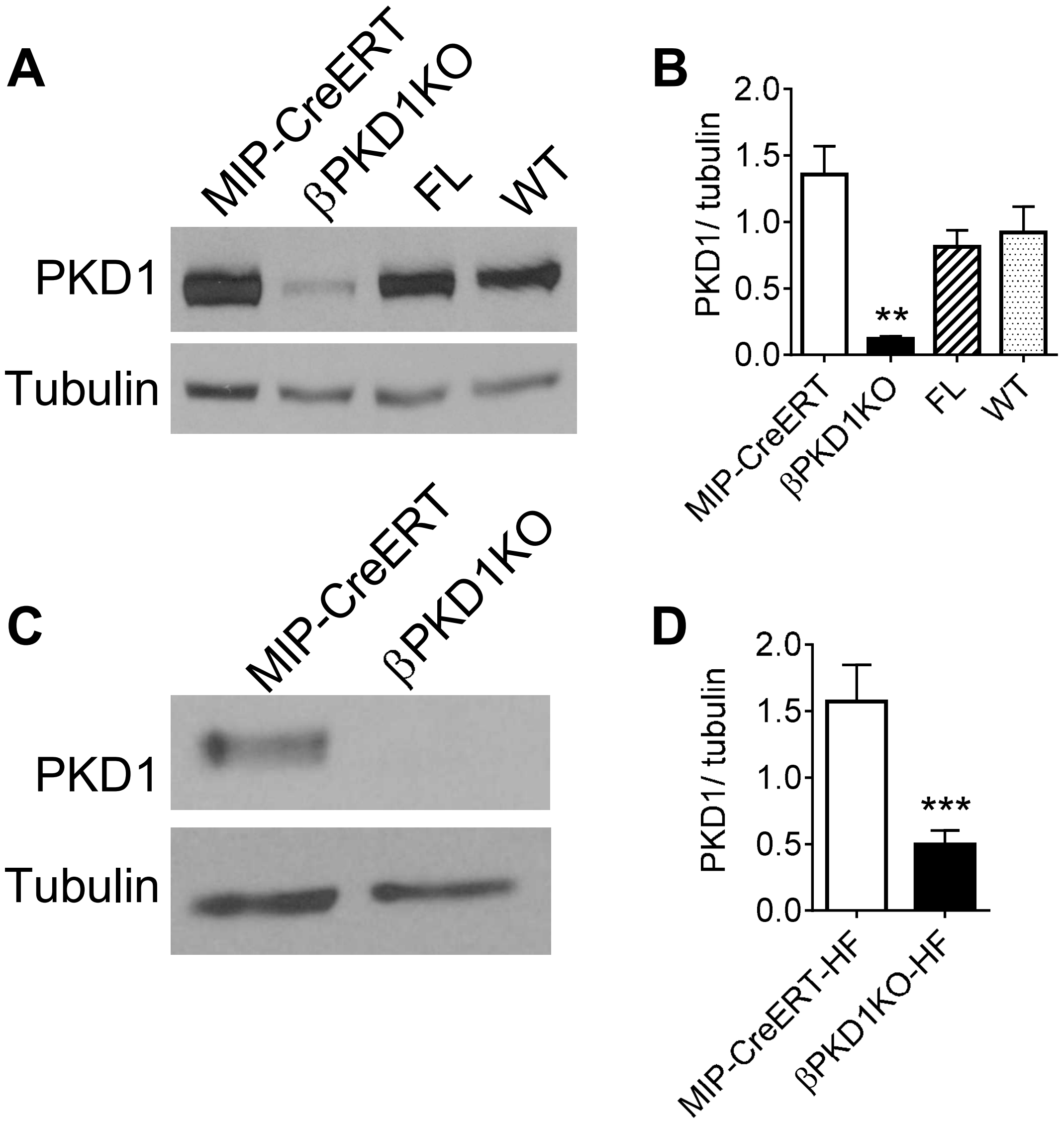

Supplementary Figure 1: PKD1 protein levels in βPKD1KO and control mice.

Representative Western blots (A&C) and quantification (B&D) of PKD1 and tubulin (as a loading control) protein expression in islet extracts from mice 3 weeks following tamoxifen injection (A&B) or after 12 weeks of high-fat diet (C&D). Data are mean +/- SEM of 4 to 9 animals in each group. ** p<0.01 compared to WT following one-way ANOVA with Dunnett post hoc test (B) or *** p<0.001 compared to MIP-CreERT following two-tailed Student's t-test (D).

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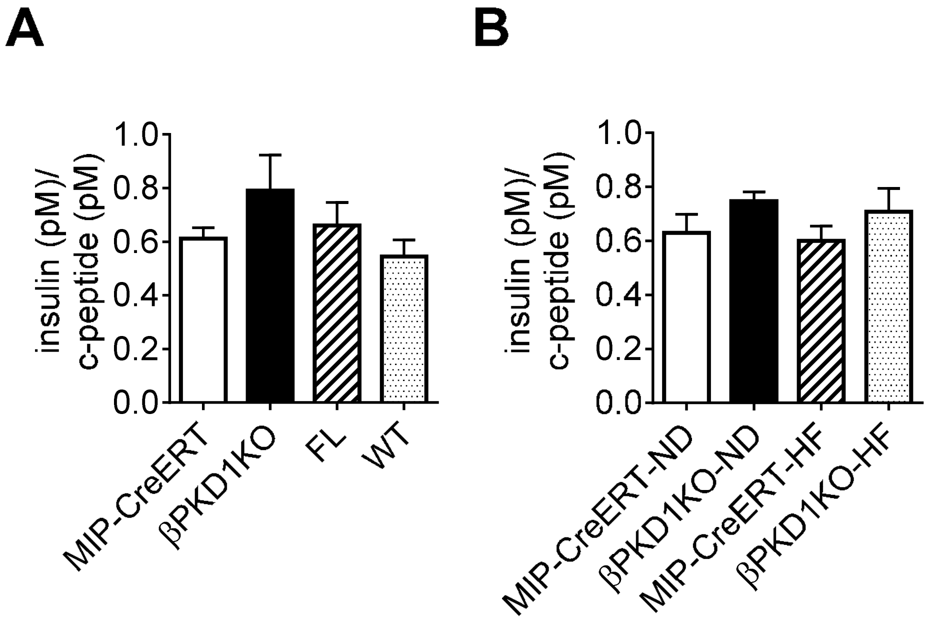
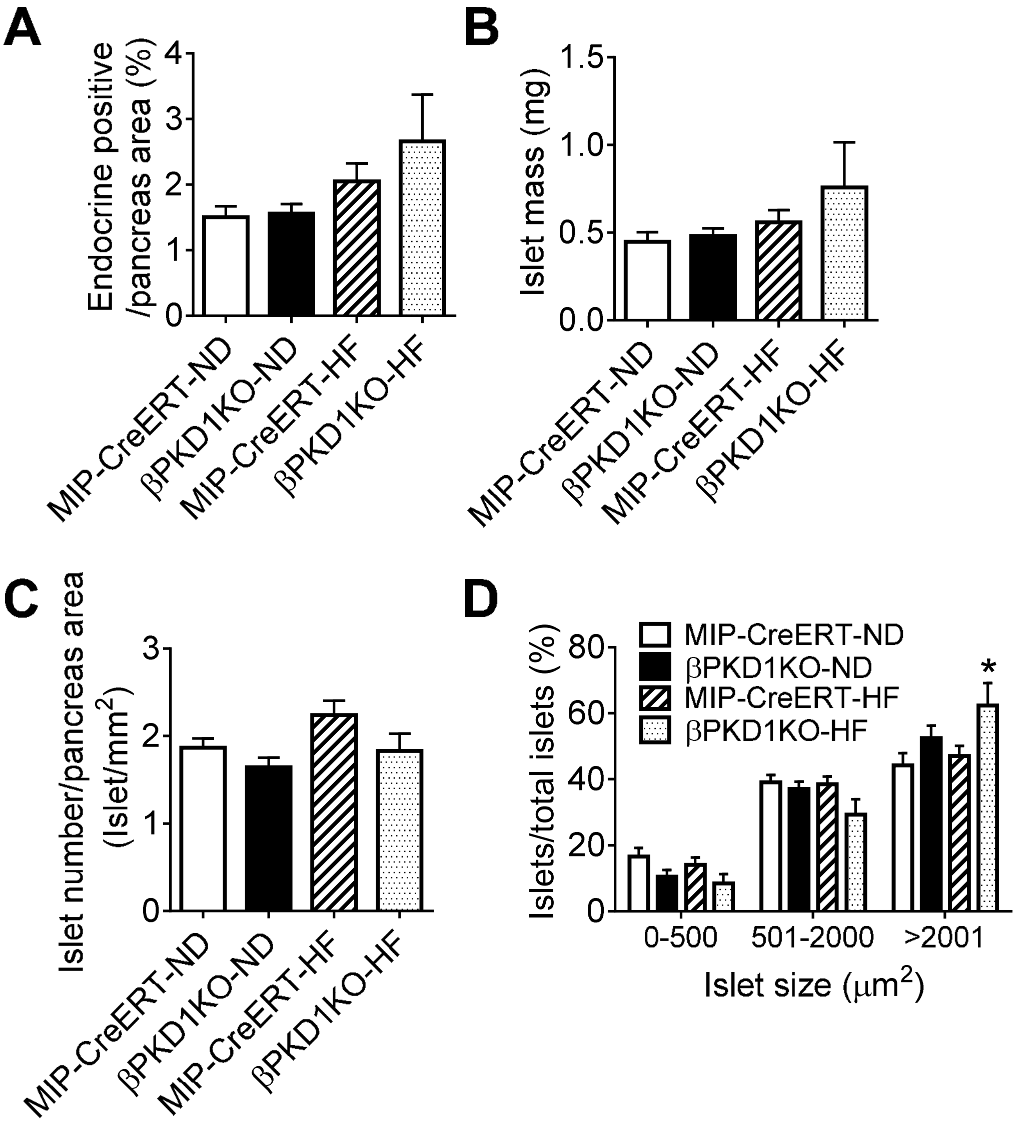


Supplementary Figure 2: Insulin clearance of chow and high-fat fed βPKD1KO and control mice.

Ratios of insulin on c-peptide levels measured during the steady state of the hyperglycemic clamp for chow fed animals 13 weeks following tamoxifen injections (A) or after 13 weeks on normal (ND) or high-fat (HF) diet (B). Data are mean +/- SEM of 7 to 12 animals in each group.



Supplementary Figure 3: Islet morphometry in chow and high-fat diet fed βPKD1KO and MIP-CreERT mice.

Beta-cell area (A), islet mass (B), islet number per pancreas section (C) and islet size distribution (D) after 13 weeks on normal (ND) or high-fat (HF). Data are mean +/- SEM of 7 to 12 animals in each group. * $p<0.05$ compared to MIP-CreERT-HF following two-way ANOVA with Tukey post hoc adjustment for multiple comparisons.