1 Mouse Single Islet β Cell Transcriptomics Reveal Sexually

2 Dimorphic Transcriptomes and Type 2 Diabetes Genes

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41 **Abstract**

42 Type 2 diabetes (T2D), characterized by malfunction of pancreatic β cells, is affected
 43 by multiple cues including sex differences. Nevertheless, mechanisms of sex
 44 differences in type 2 diabetes susceptibility and pathogenesis remain unclear. Using
 45 single-cell RNA sequencing (scRNA-seq) technology, we showed that sexual
 46 dimorphism of transcriptome exists in mouse β cells. Our analysis further revealed the
 47 existence of sex-dependent type 2 diabetes altered genes in high fat diet induced T2D
 48 model, suggesting divergences in pathological mechanisms of type 2 diabetes
 49 between sexes. Our results indicated that sex should be taken into consideration when
 50 treating diabetes, which was further validated by the sex-matched and
 51 sex-mismatched islet transplantation in mice. Compared to sex-matched transplants,
 52 sex-mismatched transplants showed downregulation of genes involved in the
 53 longevity regulating pathway in β cells and led to impaired glucose tolerance in
 54 diabetic mice. Taken together, our findings could advance current understanding of

type 2 diabetes pathogenesis with sexually dimorphic perspectives and provide new insights to the development of precision medicine.

KEYWORDS: Type 2 diabetes mellitus; Pancreatic β cell; Sex-biased gene expression; Sex-dependent T2D altered genes; Precision medicine

Introduction

The efficacy of current anti-diabetic medication varies significantly among individuals with diabetes, highlighting the importance of personalized treatment for type 2 diabetes. However, the bottleneck for precision medicine lies in the heterogeneous nature of the disease, which not only hinges on genetic predispositions that have been identified by genome wide association study (GWAS), but also other cues, including sex, diet, and aging. Among these factors, sex differences should be first considered for personalized therapy since sex is one of the most recognizable traits.

However, in most current studies on metabolism using rodents, female animals are usually neglected, because male animals have the tendency to show better disease phenotypes and are dominantly used [1]. And this experimental bias on sex hampered novel and comprehensive acknowledgement of metabolic pathological mechanisms. Thus, the National Institutes of Health (NIH) demands sex differences should be emphasized in preclinical studies [2, 3], which should be especially stressed in T2D as a global pandemic.

Glucose homeostasis is controlled by pancreatic islet, which is mainly composed of α cells, β cells, δ cells and PP cells. α cells elevate glucose level by secreting glucagon to promote hepatic glucose synthesis, and β cells release insulin to decrease glucose level by stimulating blood glucose uptake by fat, muscle, liver, and intestine cells, etc. δ cells secrete somatostatin to downregulate hormones releasing from both α cells and β cells via paracrine signaling [4]. Polypeptide from PP cells, the most infrequent islet cell type, has effects on both gastric and pancreatic secretions [5].

83 Despite the similar cell type components of islet architecture shared by both male
84 and female, there exist profound sex differences in islet physiological function and
85 metabolism, signaling pathways involved in hormone releasing, and diabetes
86 occurrence [6]. For example, female rats are more susceptible than males to
87 streptozotocin (STZ) induced diabetes, suggesting that female rat β cells are more
88 sensitive to STZ toxicity than male β cells [6]. Similarly, maternal high fat diet results
89 in insulin resistance and oxidative stress-induced β cell loss specifically in male
90 offsprings, rather than in female offsprings, indicating that female islets might have
91 self-protective machinery against oxidative stress [7]. In human, type 2 diabetes occurs
92 more frequently in men with younger age and less BMI than women [8-10]. Insulin
93 sexual dimorphism of DNA methylation was also observed in human islets by whole
94 islet genome-wide DNA methylation sequencing. It is suggested that sex differences of
95 methylome are associated with differences of islet genes expression and insulin
96 secretion level [11]. However, sexual dimorphism of pancreatic islet β cell has not
97 been investigated at the single cell level, and these studies could reveal sex differences
98 of gene expression in islet β cells between male and female and provide better
99 treatment plans for diabetic patients from both sex groups.

100 Sex differences in diabetes susceptibility, development and progression have been
101 previously reported, suggesting the existence of sex-dependent diabetes associated
102 genes. Previous studies showed that androgen receptor specifically expressed in male
103 islet β cells and plays an important role in regulating glucose-stimulated insulin
104 secretion in both mice and humans [12]. It has also been reported that *KLF14* allele
105 variants show increased female-specific T2D risk, probably via female-specific fat
106 storage and distribution [13]. Recent banding studies reported that single-cell RNA
107 sequencing technology has been applied to identify novel diabetes altered genes
108 [14-16]. Nevertheless, the diverse sex-dependent diabetes associated genes and
109 molecular pathways have not been comprehensively investigated from these studies.

Here, we systematically analyzed the single cell gene expression profiles of healthy and diabetic β cells from mice. We found a considerable number of genes had sex-biased expression in β cells. Furthermore, we identified 122 sex-dependent diabetes altered genes, suggesting that the molecular mechanisms mediating diabetes pathogenesis in males and females have important differences. Based on the recognition of the sex differences in T2D altered genes and pathways in β cells, we concluded that sex as a biological variance should be emphasized in diabetes treatment. And this conclusion was further supported by the sex-matched and sex-mismatched islet transplantation in mice. Compared to sex-matched transplants, we found that genes involved in the longevity regulating pathway tended to be down-regulated in β cells of sex-mismatched transplants, and glucose tolerance notably decreased in diabetic mice transplanted with sex-mismatched islets. Together, our results not only advanced current understanding of T2D pathogenesis, but also provided new insights and targets for developing sex-dependent precision medicine to treat diabetes.

125

126 **Results**

127 **Identification of male and female pancreatic β cell transcriptomes in mouse**

To obtain expression profiles of mouse pancreatic β cells, we employed flow cytometry to isolate single islet cells from dissociated mouse islets with live dye staining (**Figure 1A**). Totally, we collected and sequenced 5472 islet cells (3264 male; 2208 female) from both male and female mice, including 1056 islet cells (1056 male; 768 female) of 8-week-old healthy mice, 2208 islet cells (1152 male; 1056 female) of 9-month-old healthy and diabetic mice, 672 male transplanted islet cells (9 months post-transplant) in kidney capsules of both male and female recipient mice, and 768 endogenous pancreatic islet cells (384 male; 384 female) of recipient mice (detailed sample information in **Table 1**). Single-cell RNA sequencing library was constructed by a modified Smart-seq2 protocol. The average library size is 370K

reads per cell, and the average number of genes detected in these cells is about 1500 per cell. We retained 4662 cells that have at least 500 genes detected (Figure S1A). The retained cells had high total counts of unique molecular identifiers (UMIs) mapped to gene exons (Figure S1B), and low fractions of UMI counts of mitochondrial genes (Figure S1C), suggesting we obtained a set of qualified scRNA-seq profiles of mouse islet for the main purpose of this study.

From these 4662 cells, we went on to identify β cells for downstream analysis. Firstly, we applied an adjusted CPM method (adjCPM) to normalize our scRNA-seq data by excluding the union of the top 2 genes with the highest expression from all cells while calculating the normalization factors (Figure S1D and S1E; see Methods). Secondly, hierarchical clustering of scRNA-seq profiles based on the union of the top 10 highly expressed genes from all cells was used to discriminate the identity of the cells (Figure S1F). After performing principal component analysis with the identified hyper variable genes (HVGs) and subsequent visualization by t-distributed stochastic neighbor embedding (t-SNE) (Figure S1G; see Methods), the retained islet cells of healthy (8-week-old; 9-month-old) and diabetic mice were found to be aggregated into two clusters exhibiting differential expression of β cell marker gene *Ins2* (**Figure 1B and 1C**). Then, 3912 β cells (2197 male; 1715 female) from mice in different conditions were identified through high expression of *Ins2* (**Figure 1C and S4A**). The sex of mouse β cells was labeled on the t-SNE map (male in blue; female in red; **Figure 1D**), and further confirmed by X and Y chromosome genes (Figure S2B). We found male and female β cells were not completely overlapped on the t-SNE plot (Figure 1D). To verify the existence of sexually dimorphic gene expression, we applied Kolmogorov-Smirnov test (K-S test) to analyze the differential expression of *Ins2* between male and female β cells, and found its expression was significantly different between two sexes (**Figure 1D**), indicating sexual dimorphism of β cell transcriptomes exist in mouse and could be important to β cell functions.

165

166 Sex-biased gene expressions in mouse β cells under healthy and T2D conditions

167 To identify genes that display sex-biased expression pattern in mouse pancreatic β
 168 cells, we first compared the transcriptional profiles of male β cells with female β cells
 169 from 8-week-old C57BL/6J mice (**Figure 2A**). Differentially expressed genes were
 170 sorted out by MAST [17], with a FDR cutoff of 0.05 (the same cutoffs were used for all
 171 the differential analysis using MAST in our study). In total, we obtained 162
 172 differentially expressed genes (DEGs), comprising 37 genes expressed higher in male
 173 and 125 genes expressed higher in female (**Figure 2A**). Among them, only four genes
 174 were on the sex chromosomes, including X chromosome (*Xist*) and Y chromosome
 175 (*Eif2s3y*, *Ddx3y*, *Uty*) (**Figure 2B**), suggesting that considerable level of sexual
 176 dimorphism exist in healthy β cell transcriptomes.

177 To investigate whether such sexually dimorphic β cell transcriptomes also exist in
 178 diabetic conditions, β cells from high-fat-diet (HFD) induced diabetic mice were
 179 collected for scRNA-seq. Firstly, we used HFD to induce T2D model with β cell
 180 failure in C57BL/6J mice (fed with HFD from 8-week-old) as previously described
 181 [18]. Consequently, characteristic T2D phenotypes were observed in those diabetic
 182 mice of both male and female, including high body weight, increased fasting insulin
 183 level, and impaired glucose tolerance (**Figure 2C and 2D**). Notably, sex differences of
 184 [fasting serum insulin level in healthy mice](#) were also observed: the fasting serum
 185 insulin level of healthy male mice is significantly higher than healthy female mice
 186 (**Figure 2C**). In addition, the area under curve (AUC) of glucose tolerance test showed
 187 that [the sex differences of glucose tolerance exist between male and female in healthy](#)
 188 [and T2D mice](#) (**Figure 2D**). Then, to explore the genetic basis for such differences, we
 189 obtained single β cell transcriptomes from 9-month-old diabetic mice (HFD feeding
 190 up to 7 months) and age-matched healthy mice (normal diet, ND) of both sexes. The
 191 comparison of single β cell transcriptional profiles of male and female from either
 192 healthy or T2D mice was carried out by MAST with the same cutoffs as described
 193 above. As shown in the results, 394 DEGs between male and female in 9-month-old

194 healthy mice were identified, including 200 genes expressed higher in male and 194
195 genes expressed higher in female. In parallel, 81 male highly expressed genes, and 52
196 female highly expressed genes were identified in β cells from T2D mice (**Figure 2E**).

197 To further elucidate sex-biased pathways based on these comparisons, gene set
198 enrichment analysis (GSEA) was performed with the cutoff set as $FDR \leq 0.25$ (the
199 same cutoff was used for all GSEA in our study) [19]. In β cells from both 8-week-old
200 and 9-month-old healthy animals, the longevity regulating pathway was enriched in
201 males, and related genes (*Ins1*, *Ins2*, *Hspa1a*, *Hspa8*) were expressed significantly
202 higher in males (**Figure 2F**; S2C and S2D). Intriguingly, the ribosome pathway was
203 consistently enriched in females in β cells from both healthy (8-week-old;
204 9-month-old) and diabetic mice (Figure S2C; S2D and S2E). Importantly, in β cells
205 from diabetic mice, both the N-Glycan biosynthesis pathway and Notch signaling
206 pathway were enriched in males. Conversely, the JAK-STAT signaling pathway,
207 ferroptosis pathway, spliceosome pathway, carbohydrate digestion and absorption
208 pathway were enriched in female β cells from diabetic mice (Figure S2E). Expression
209 patterns of representative sex-biased DEGs involved in the GSEA results were shown
210 in the heatmap (**Figure 2F**). Above all, our results validated the existence of sex-biased
211 gene expression in β cells of both healthy and diabetic mice, indicating that the
212 pathological mechanism of T2D might differ between males and females.

213

214 **Abundant sex-dependent T2D altered genes were found in mouse β cells**

215 Given the sex-biased gene expressions detected in T2D β cells, we hypothesized T2D
216 development may differ in males and females. Previous studies compared the
217 single-cell transcriptional profiles of T2D islets with that of healthy islets to identify
218 T2D altered genes [14-16] without considering the factor of sex. To dissect that in a
219 sex-dependent manner, we compared the single-cell transcriptome of β cells from
220 T2D mice with that from age-matched healthy mice. Firstly, we performed
221 differential analysis between T2D and healthy group with scRNA-seq profiles of all

beta cells from both sexes using MAST (here both cellular detection rate (CDR) and sex were provided as covariates) and defined the DEGs identified as sex-independent T2D altered genes

Finally, we identified 98 sex-independent (40 downregulated, 58 upregulated) T2D altered genes (Table S5). Then, we compared the diabetic β cells with healthy β cells of the same sex to obtain DEGs in either male or female specific manner, and consequently compared the DEGs of each sex with the 98 sex-independent genes. The non-overlapping parts were defined as candidate genes for sex-dependent T2D altered genes, including 56 candidate genes in female and 65 candidate genes in male (Figure 3A). Based on the defined candidate genes, we introduced two additional filtering criteria to define sex-dependent T2D altered genes in a more reliable way: 1. The estimated 95% confidence interval of log2 fold change (log2FC) by MAST from the differential expression analysis in one sex does not overlap with that from the other sex. 2. The p-value in the non-significant sex group is higher than 0.2. We finally used the genes passing both of these two additional criteria as the final sex-dependent T2D altered genes. As the result, we obtained 31 female-specific (22 up and 9 down regulated in T2D compared to Healthy) and 31 male-specific (18 up and 13 down regulated in T2D compared to Healthy) T2D altered genes (Figure 3B and Table S6). Moreover, 14 selected sex-dependent T2D altered genes with basemean > 10 were validated by q-PCR experiments (Figure S3A).

To note, among these defined sex-dependent T2D altered genes of female, we found upregulated expressions of *Serp1* and *Ero1lb*, which encode endoplasmic reticulum (ER) stress associated proteins. Overexpression of *Ero1lb* in β cells results in the upregulation of unfolding protein associated genes, and leads to ER stress [20]. Interestingly, intervention of conjugated estrogens could protect postmenopausal women from diabetes by promote degradation of misfolded proteins during the process of insulin synthesis in β cells [21]. Taken together, these findings indicate sex differences of ER stress might exist in T2D pathogenesis.

250 Among male-dependent T2D altered genes, several genes have been reported to
 251 confer sex specific effect in physiological function of β cells or onset of T2D.
 252 Notably, our analysis identified *Iapp* as male dependent T2D upregulated gene.
 253 Accumulation of IAPP is toxic to pancreatic β cells. It was reported that ectopic
 254 expression of human *IAPP* in pancreatic β cells of transgenic mice resulted in more
 255 severe diabetic phenotype in males than in females, suggesting sex dependent
 256 pathogenesis [22], which is in line with our result. Moreover, insulin secretion
 257 capacity associated with mitochondrial function declines with aging which is a
 258 contributing factor to T2D. Evidence showed that elderly male Wistar rats show more
 259 significantly decreased mitochondrial function and insulin secretion than elderly
 260 females [23], consistent with our results that two mitochondrial function related
 261 genes, *Cox4i1* and *Ndufb7*, were identified as downregulated male dependent T2D
 262 altered genes.

263 For other sex-dependent T2D altered genes, there was no previous report on sex
 264 differences of them in diabetes pathogenesis, such as *Cpe*, *Scg3*, *Ttr*, *Rnase4*, *Malat1*,
 265 *Ucn3*, *Pura*, *Tmed3*. Because in most current studies on metabolism with rodent
 266 models, there is a experimental bias on dominant using of males which have the
 267 tendency to show better disease phenotypes [24], limiting understanding of T2D
 268 pathogenesis with sex specific perspective. Thus, it will be valuable to examine sex
 269 specific role of these genes in T2D pathogenesis in future study.

270 To further systematically investigate the sex differences in T2D altered pathways,
 271 GSEA was performed based on comparison of β cells from T2D and healthy mice of
 272 each sex. In females, several pathways enriched in T2D- β cells such as pancreatic
 273 secretion pathway and longevity regulating pathway (multiple species) have direct
 274 links to β cell function and onset of T2D. Conversely, oxidative phosphorylation
 275 pathway and ribosome pathway were enriched in healthy β cells. At the same time, in
 276 males, T2D- β cells had ribosome pathway enriched and healthy β cells had type 2
 277 diabetes mellitus pathway enriched (**Figure 3C** and **3D**). The expression patterns of

278 genes involved in the pathways mentioned above were shown in the heatmap (**Figure**
279 **3E**) and violin plots (Figure S3B). Altogether, both the distinctive patterns of
280 sex-dependent T2D altered genes and different T2D associated pathways suggested
281 divergence of T2D pathogenesis between sexes.

282 Although we detected that no gene set showed significant enrichment in both sexes,
283 we found many of the sex-independent DE genes (98 in total, 40 downregulated, 58
284 upregulated) (Table S5) are involved in fundamental β cell function, or T2D
285 pathogenesis. For example, in regards to regulating insulin secretion process, we
286 detected that *Slc2a2*, *Calm2*, and *c-Fos* were downregulated in sex-independent
287 manner. *Slc2a2* encodes the glucose transporter 2 (Glut-2). Impaired insulin secretion
288 is a key manifestation of T2D. Glut-2 functions as a membrane protein to transport
289 glucose into β cells to mediate glucose-stimulated insulin secretion (GSIS) which is
290 crucial for glucose homeostasis [25]. Evidence showed that mutations of *Slc2a2* result
291 in neonatal diabetes in both male and female patients [26]. It was also reported that
292 *Slc2a2* pathological variants are associated with increased risk of transition from
293 impaired glucose tolerance to T2D [27]. Moreover, *Calm2*, encodes Camodule-2 to
294 mediate calcium signaling in GSIS [28]. At the same time, overexpression of *c-Fos* in
295 β cells was reported to contribute to increased insulin secretion, whereas its
296 knock-down results in decreased insulin secretion [29].

297 Meanwhile, we also observed sex-independent upregulated genes involved in
298 critical cellular events in T2D pathogenesis, such as transdifferentiation and ER
299 stress. As reported previously, β cell to alpha cell (glucagon producing)
300 transdifferentiation occurs in T2D pathogenesis [30,31]. Consistently, *Gcg* (encoding
301 glucagon) was identified as upregulated sex-independent T2D gene in our analysis.
302 Moreover, *Hsp90aa1* was also upregulated in both male and female T2D β cells.
303 Previous study identified Hsp90 (encoded by *Hsp90aa1*) as a therapeutic target for
304 ER stress in T2D, and inhibition of Hsp90 alleviates insulin resistance and improves

glucose tolerance [32,33]. Collectively, these results suggest not only divergence of pathological mechanism but also common pathogenesis in T2D between sexes.

Mice with sex-matched β cell transplant exhibited better control of glucose homeostasis

Altogether, the existence of sexual dimorphism in mouse β cell transcriptome informed us that sex as an important factor in β cell function and pathogenesis of T2D should be emphasized when treating diabetes. In order to validate this critical concept, long-term islet transplantations (an experimental treatment for insulin insufficient diabetes mellitus) with sex-matched and sex-mismatched islets were performed in ICR mice. The ICR mice selected as donor and recipient in this assay for the reason that ICR mouse do not develop spontaneous insulinitis and diabetes, therefore they are more suitable for long-term assessment of the effects of islet transplantation than another inbred strain C57BL/6. Male islets were isolated from 6-8 weeks old mice and transplanted into kidney capsules of age-matched male and female mice, respectively. Transplanted islets (TX-islet) and endogenous islets (Endo-islet) from recipient mice were all collected and dissociated for scRNA-seq profiling, 9 months post-transplant (**Figure 4A**). The β cells identified by high expression of *Ins2* as previously described (**Figure 4B**). Differential expression gene analysis in β cells was performed as the following two ways. Firstly, we plotted the gene expression changes observed in comparing the transcriptomes of sex-matched and sex mismatched transplanted β cells to those of male (**Figure 4C** left panel) or female (**Figure 4C** right panel) endogenous beta cells. In both analysis, we observed a obvious correlation between the gene expression changes detected the two comparisons, which indicates that most of the differentially expressed genes showed consistent expression changes between transplanted and endogenous beta cells in male and female recipients. However, it can also be seen that the distribution of the log2 expression fold changes shown on x-axis was clearly wider than the distribution of those shown on y-axis in both plots,

suggesting that the overall sex-matched transplant- β cell transcriptomes were closer to endogenous islet β cells from both male and female recipients, than the sex-mismatched transplant- β cell transcriptomes. Secondly, transplant- β cell transcriptomes were directly compared between sex-matched and sex-mismatched groups for DEGs. Compared to sex-mismatched group, sex-matched transplant- β cells had 3 genes (*Kap*, *Sfrp5* and *Akr1c21*) significantly up-regulated and 2 genes (*Ovol2*, *Matn2*) significantly down-regulated (Figure S3C and S3D). Notably, *Sfrp5* was a conservative male-biased expression gene in 8-week-old and 9-month-old healthy β cell (Table S1 and S2), previous study reported that overexpression of *Sfrp5* (down-regulated in obesity and T2D) can ameliorate impaired glucose tolerance in mice [34]. In humans, high level of serum SFRP5 was correlated with lower risk of T2D onset [35]. Moreover, the results of GSEA showed that longevity regulating pathway-multiple species was significantly enriched in β cells of sex-matched transplant-islets and among the 16 leading-edge genes, *Ins2*, *Sod1*, *Sod2* and *Foxa2* are directly linked to β cell secretion (Figure 4D). Collectively, these results suggested that sex-matched islet transplantation could be more beneficial to the function of β cells of the transplants, thus better controlling glucose homeostasis.

To further validate the advantage of sex-matched transplantation, we transplanted islets from male and female mice into streptozotocin induced female diabetic mice, respectively (Figure 4E). Although the hyperglycemia (> 350 mg/dl) of the diabetic recipient with sex-matched or sex-mismatched transplantation restored to normal levels 1 month post-transplant (< 144 mg/dl; Figure S3E), the glucose tolerance of the diabetic mice with sex-matched transplantation was significantly better as evidenced by the oral glucose tolerance test (Figure 4F). Above all, our scRNA-seq analysis of β cells and experimental validation concluded that sex should be taken into consideration in diabetes treatment.

Discussion

361 According to previously published work, it has been well recognized that sexual
362 dimorphism exists in many organs or systems, such as heart, kidney and immune
363 responses [36-38]. What's more, a recent study in humans has shown that sexual
364 dimorphism not only exhibits in neuron cells, but also is associated with susceptibility
365 of mental diseases [39]. Herein, T2D is a complex metabolic disorder characterized
366 with islet β cell failure and impacted by sex. Sex differences in islet β cell
367 physiological function and diabetes prevalence have been recognized, but still need to
368 be better understood. To decipher the sexually dimorphic T2D pathogenesis, we used
369 scRNA-seq to comprehensively measure the transcriptomes of healthy and diabetic β
370 cells from mice of both sexes. We identified abundant genes have significantly
371 sex-biased expression in β cells of both healthy and T2D mouse.

372 Besides, we found longevity regulating pathway was male specific enriched in
373 healthy β cells. Evidence has shown that insulin and insulin like growth factor
374 signaling are involved in longevity pathway, suggesting the crucial role of metabolic
375 homeostasis of glucose for aging and life span [40-42]. Expressions of two insulin
376 encoding genes, *Ins1* and *Ins2*, were specifically upregulated in β cells of healthy
377 male mice, suggesting enhanced insulin synthesis. At the same time, we observed
378 another two longevity pathway related genes *Hsp1a* (in β cells of healthy 8-week-old
379 mice) and *Hsp1a* (in β cells of healthy 8-week-old and 9-week-old mice) exhibiting
380 healthy male specific upregulation. Both *Hsp1a* and *Hspa8* were reported to protect β
381 cells from oxidative stress, and upregulation of *Hspa1a* is associated to extended
382 longevity [43,44], which may suggest a protective mechanism against the stress
383 caused by increased insulin synthesis in healthy male mice. Furthermore, we
384 identified 62 sex-dependent T2D altered genes in mouse, suggesting important
385 differences in the molecular mechanisms of diabetes pathogenesis between males and
386 females in mouse T2D model. Collectively, these results provided innovative
387 sex-specific targets for future studies on the precision treatment of T2D.

388 In current clinical trials of islet transplantation, sex matching between donor and
 389 recipient has not been stressed. Based on the recognition of sex differences in the
 390 transcriptome of β cells, we concluded that sex as a crucial biological variance should
 391 be emphasized in the treatment of diabetes. And this conclusion was further supported
 392 by the sex-matched and sex-mismatched islet transplantation in mice. Compared with
 393 the sex-mismatched group, the β cells of transplants in the sex-matched group showed
 394 significant enrichment of the longevity regulating pathway (consistent with the results
 395 of Figure S2C and S2D) and glucose tolerance notably improved. The long-term
 396 curative effect of islet transplantation is impacted by the survival of the transplants
 397 [45]. In comparison of sex-matched transplantation and sex-mismatched
 398 transplantation, we found that the longevity regulating pathway was also enriched in β
 399 cells of sex-matched transplantation with upregulated expressions of genes related to
 400 longevity pathways. Among them, *Ins2*, *Hsp1a* and *Hspa8* were also upregulated in
 401 the sex-matched group. At same time, upregulated expressions of gene involved in
 402 extending longevity of organism were also detected, such as *Sod1*, *Sod2*, *Hdac1*
 403 [46-48]. Taken together, our results suggested that sex should be taken into
 404 consideration as an important factor for islet transplantation to achieve long-term
 405 stability and functionality of the transplants. For better curative effect, our result
 406 raised the necessity for sex-matched islet transplantation, and even for sex-matched
 407 stem cell-based cell replacement therapy for diabetes treatment.

408 Beyond the islet β cells focused in this study, sex differences of T2D susceptibility
 409 were also associated with sex steroid hormones. It has been found that endogenous
 410 estrogens are protective against T2D in females and the risk of T2D increases
 411 following menopause [49,50]. Estrogen not only improves islet β cell function and
 412 survival [51], but also stimulates the secretion of GLP-1 by both islet α cells and
 413 intestine L cells to maintain glucose homeostasis [52]. Along with that, deficiency of
 414 the male sex hormone, testosterone, increases the T2D risk in male [53,54].
 415 Furthermore, a recent report revealed that testosterone improves insulin secretion

through androgen receptor on male islet β cells in both mouse and human [12]. Taken together, sex differences in both islet β cells and sex steroid hormones need to be highlighted in the development of sex-specific precision medicine.

Materials and methods

Animals and high fat diet induced diabetic mice

We housed all the mice under the specific pathogen free grade environment of animal facility at Tongji University, Shanghai, China. Adult male and female mice (6-8 weeks old) were purchased from Shanghai Slac Laboratory Animal. To induce mouse T2D model, male and female C57BL/6J mice (n=5 per sex) were fed with high-fat diet from 8-week-old to 9-month-old as a high-fat-diet (HFD) group, and equal number of male and female mice were fed up with normal diet (ND) in an age-matched way to a control potential confounding factor, age.

Islet transplantation

Islets were isolated from 6-8 weeks old male ICR mice (n=20), and the detailed process of islet isolation was as previous description [55]. Similar size islets were handpicked after purification under a stereomicroscope, and 3 age-matched healthy male and 3 female ICR mice were selected as a recipient with about 300-400 islets were transplanted under the kidney capsule. About 9 months later, transplanted islets were dissected and scraped from the kidney capsule, and the pellet was collected and dissociated into a single cell for scRNA-seq in two batches. At the same time, the endogenous islets of recipient mice were also isolated and dissociated into a single cell for scRNA-seq. Diabetic mice and islet transplantation were both performed as previous description [55]. Adult male and female C57BL/6J mice were selected as islet

donor, and age-matched female C57BL/6J mice were chosen as recipient. C57BL/6J mice were injected with STZ at the dose of 170 mg/kg after 6 hours of fast. Mice that exhibited non-fasting hyperglycemia (> 350 mg/kg) with 3 consecutive detection were regarded as diabetic mice for islet transplantation. Each diabetic mouse was transplanted with about 350 islets, and mice that non-fasting blood glucose (< 144 mg/kg) recover normal were selected for physiological experiments 1 month post-transplant.

451

452 **Preparation of single islet cells**

453

Mouse islets were isolated from either 6-8 week old male or female C57BL/6J and ICR mice. For islets isolation, 0.5 mg/mL collagenase P ([11213873001, Roche, Basel, CH](#)) was poured into pancreas by perfusion of the common bile duct. After digestion, islets were purified through Histopaque ([11191 and 10771, Sigma Aldrich, St. Louis, MO, USA](#)) gradient centrifugation. The Histopaque buffer was made with mixing Histopaque-10771 and Histopaque-11191 together as the ratio of 5:6. Purified islets were dissociated into single cells as follows: washed the islets with cold PBS at least two times, spun at 1000 rpm for 2 minutes, then replace PBS with 1mL TrypLE Express ([12604021, Gibco, NY, USA](#)) and incubated at 37°C for 10-15 minutes with pipetting cell pellet by using P1000 pipette gently. Stop the reaction with low glucose DMEM (containing 10% FBS, 1% HEPES, 1% PenStrep), and centrifuged cells at 4°C, 1000 rpm for 2 min. Then washed the cell pellet with cold PBS for 1 time, and the resuspended cells in PBS (containing 0.5%BSA) was filtered with a 40-micrometer strainer to get single cell suspension. To obtain live single cell, Calcein Blue, AM ([C1429, Invitrogen, Carlsbad, CA, USA](#)) was used to measure the viability of cell and high viability single cell was sorted by using BD FACS Aria II flow cytometry ([Pleasanton CA, USA](#)). Single islet cell was sorted into 96-well plates containing lysis buffer.

472

473 **Library construction and NGS sequencing**

474

475 Single-cell RNA-seq libraries were constructed according to Smart-seq2 protocol
 476 except that oligod (T) primers comprising 16bp cell barcode sequence and 9bp
 477 molecular barcode sequence were used to allow sample pooling and molecular
 478 counting. In addition, part of Truseq read2 sequence was used to replace ISPCR
 479 sequence in the oligod (T) primer thus to be compatible with Illumina sequence
 480 platform. Cells in lysis buffer were denatured, reverse transcribed in the presence of
 481 template switching oligos and pre-amplified by adding both ISPCR and ISPCR-read2
 482 primers with 24 PCR cycles. cDNA of cells with different barcodes were then pooled
 483 together and were purified using 0.8x AMPure XP beads. Homemade Tn5 enzyme was
 484 used to tagment cDNA. Final amplification was processed using P7-index primers
 485 (Truseq) and P5-index primers (Nextera). Sequencing libraries were purified twice
 486 using 0.6x AMPure XP beads and once with 1x AMPure XP beads, and were
 487 sequenced on Illumina HiSeq X10 platform with default parameters. Primer and
 488 adaptor sequences were all listed in supplementary [Table S8](#) (information of barcode).

489

490 **Quantitative real-time PCR**

491

492 The cDNA for quantitative real-time PCR synthesized following the protocol that we
 493 described in previous part (library construction). The β cell expressing *Ins2* from
 494 healthy and T2D mice of both sexes were identified by single cell qPCR. Quantitative
 495 real-time PCR was performed with SuperReal PreMix Plus (SYBR Green) (EP205,
 496 TIANGEN, Beijing, China) by using *Sdha* gene as an internal control. The relative
 497 expression levels of selected genes was analyzed based on the formula of $2^{-\Delta\Delta Ct}$. The
 498 primers used for qPCR are listed in [Table S7](#).

499

500 **Physiology experiment**

501

502 For intraperitoneal injection glucose tolerance test, normal diet and high-fat diet
503 feeding mice fasted for 16 hours. 1 g/Kg body weight of glucose was intraperitoneally
504 injected, and blood glucose was measured at time point of 0 min, 15 min, 30 min, 60
505 min, 120 min after glucose injection using glucometer ([ACCU-CHEK, Roche](#)). Blood
506 samples were collected from the mice that fasted for 6 hours, to measure the insulin
507 level by using Mouse Ultrasensitive Insulin ELISA Kit ([80-INSMSU-E01, ALPOC,](#)
508 [Salem, NH, USA](#)). For oral glucose tolerance test, mice were fasted for 6 hours, and
509 glucose was gavaged at a dose of 2 g/Kg. Tail blood was collected for blood glucose
510 detection, at time point of 0 min, 15 min, 30 min, 60 min, 120 min, using glucometer
511 (ACCU-CHEK).

512

513 **Quantification and statistical analysis**

514

515 *Preprocessing before normalization*

516 Reads were stored in paired-end fastq format. Reads of one end of fragment contain cell
517 barcode and UMI information which was subsequently extracted and added to the name
518 of corresponding reads of the other end in fastq file containing molecular sequence.
519 That barcode/UMI information was in fastq-R2 files. Next, the generated single-end
520 fastq files were cleaned by Trim Galore!
521 http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/ with the parameter
522 --length 30.

523 Then, the FastQC <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> was
524 applied to check reads quality before subsequent alignment on mm9 genome using
525 STAR [\[56\]](#). The parameters of STAR software were --AlignEndsType EndToEnd --out
526 FileterMismatchNoverReadLmax 0.04 --outSAMAttrIHstart 0 --outSAMmultNmax 1
527 --outFilterMultimapNmax 1. GTF file of mm9 reference genome was derived from the

RefSeq gene annotation [57] file downloaded from UCSC genome browser database ,
<http://genome.ucsc.edu/cgi-bin/hgGateway?db=mm9>. After alignment, SAM files
underwent demultiplexing by Catadapt <https://github.com/marcelm/cutadapt/releases>
with --overlap 16 --no-indels --match-read-wildcards parameters; reads were removed
if they were assigned to more than one cells. Then, we removed PCR duplicates by
UMI-tools [58]. Next, featureCounts [59] was utilized to quantify gene expression
levels according to the above mentioned GTF file. Once the expression profiles were
generated, cells with fewer than 500 expressed genes (UMI count > 1) were considered
low-quality and were removed (Figure S1). The fraction of transcripts from
mitochondrial genes in each single cell was investigated as it was used as an indicator
for general quality control. Part of our ICR cells had high transcripts fraction of
mitochondrial genes, which can be accounted for that kidney cells intrinsically express
high-level mitochondrial genes [60]. So, we kept those cells for analysis because our
transplantation cells were under renal capsule and either kidney cells or affected
transplant cells may well be introduced in our data. Next, only genes expressed (UMI
count > 1) in 5 or more cells were used for further analysis. Eventually, there are 14,152
genes and 4662 cells retained in mouse scRNA-seq data. The sample information of the
Illumina high-throughput sequencing data is listed in Table 1.

546

547 *Normalization*

Here we used an adjusted count per million (CPM) normalization method, named
adjCPM. The CPM method assumes that the total molecule reads are equal among
cells. Similarly, our adjCPM method has an assumption except excluding a few genes
in calculating the total number of molecular reads of each cell. It is based on the
observation that sometimes a few top expressed genes can possess more than 50% total
UMI count (Figure S1). These genes were selected as union set of the top 2 expressed
genes of single cells in which the sum of the corresponding top 2 genes' UMI counts is
beyond 50% of the total count of the cell. Specifically, we obtained 9 genes (*Gcg*, *Ins1*,

Ins2, Malat1, Ppy, Pyy, Rn45s, Ttr, mt-Rnr2) and they were excluded when calculating the total count of each cell. It is well known that the scRNA-seq experiments are affected by drop-out events especially for UMI method. Here, we also observed that many genes have zero expression value because of insufficient detection power or intrinsically under-expressed. To address this issue, SAVER [61] was applied to recover gene expression in the entire matrix.

Cell type identification

Since cell type identification is critical for the rest analysis, two steps were carried out to robustly define the cell type. Firstly, we selected the top 10 expressed genes of each cell, resulting in a total of 118 genes to perform the subsequent hierarchical clustering analysis. Accordingly, we primarily assigned 4 clusters of single cells after removing 111 cells which had ulterior distance in clustering dendrogram and annotated each cluster based on marker gene expression (Figure S1).

Secondly, by a lowess regression between the Log2 mean and Log2 coefficient of variance of each gene's normalized UMI count across all single cells, we selected 3000 genes according to the residue of lowess regression as hypervariable genes (HVGs). Next, principal component analysis (PCA) was applied to the Log2 transformed normalized expression of these HVGs using *sklearn* [62] after centering and scaling, and top 25 PCs were selected based on the statistical significance of the fraction of total variance explained by each of them, which was estimated using jackstraw [63]. We used the top 25 PCs as input for subsequent t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis. To have a stable t-SNE visualization, we tried a lot of combinations of parameters (perplexing [10, 15, 20, 25, 30], early exaggeration [12, 15, 20], learning rate [200, 300, 400, 500, 600, 800] and 50 random seeds) using *sklearn* [62], and found they generally gave very similar results. We finally chose perplexing:15, early exaggeration:12, learning rate:500 to generate the t-SNE plots shown in the figures. Next, Density-based spatial clustering algorithm, DBSCAN

[64] with parameters $\text{eps}=5$ and $\text{MinPts}=5$ was applied on the resulting two-dimensional t-SNE map to group the single cells into cliques (Figure S1G). After removing singletons, we examined each single cell clique in order of clique size with following criteria: 1. only cells with consistent cluster/clique labels generated by hierarchical clustering using the top expressed genes and by DBSCAN were retained; 2. differential expression analysis of single cells in clique 3 (C3) compared the single cells in other cliques demonstrated that the single cells in C3 should be excluded because they highly expressed cell cycle related genes (Figure S1I). Finally, we kept 4467 high-quality mouse single cells for downstream analysis, and the cell type labels were inferred from the marker genes highly expressed in each single cell clique (Figure S1H).

Differential expression analysis and gene set enrichment analysis

MAST [17] was used to perform differential expression analysis between male and female or between T2D and healthy scRNA-seq profiles of β cells. Cellular detection rate (CDR, which means the number of genes detected in each single cell) was controlled as a covariate while estimating treatment effects. Significantly regulated genes were identified by using false discovery rate ≤ 0.05 as cutoff. For identification of functional pathways enriched in the detected differentially expressed genes between different sexes or between T2D and healthy conditions in our scRNA-seq data, we performed gene set enrichment analysis (GSEA) [19] on genes ranked based on their z-statistics, which were derived by mapping their MAST p-values to the standard normal distribution and using the sign of the $\text{Log}_2(\text{fold change})$ of their expression values to represent the direction of regulation. Technically, the z-statistic of each gene was calculated as:

$$\text{qnorm}(p) \times \text{sign}(\text{lfc})$$

where p and lfc are p-value and $\text{Log}_2(\text{fold change})$ of this gene obtained from MAST output, qnorm is the standard normal quantile function and sign is the signum function.

At the same time, we also tried other methods to perform differential expression analysis, such as edgeR. performing differential analysis using edgeR combined with

zingeR to incorporate an estimate of the dropout rate per cell (edgeR-zingeR), which is a DE-tool for UMI-based scRNA-seq data in the paper [65]. Using the same cutoff of MAST, abundant DEGs were also identified by edgeR-zingeR. In general, most significant DE genes from two separate methods are similar, most of which detected by MAST could also be covered by edgeR-zingeR (Figure S4), suggesting that the results of MAST are stable and could be repeated by another differential tool. The DEGs detected by edgeR are listed in Table S9 for reference. KEGG pathways [66] were used as input gene sets for GSEA, and *fdr* cutoff of 0.25 was used to select statistically significant pathways.

Ethical statements

All experiments were performed in accordance with the University of Health Guide for the Care and Use of Laboratory Animals and were approved by the Biological Research Ethics Committee of Tongji University.

Data availability

Raw data (Fastq files) for single pancreatic β cell RNA-seq in this study have been submitted to GSA at the National Genomics Data Center with accession number CRA003921 and can be accessed at <https://bigd.big.ac.cn/gsa/s/FP8dq927>.

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Competing interests

The authors have declared no competing interests.

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856

857 **Figure legends**

858 **Figure 1 Single β cell transcriptomes reveal sexually dimorphic genes in** 859 **healthy and T2D mice**

860 **A.** Schematic diagram for mouse single β cell RNA-seq data preparation and analysis.
861 Mouse single living islet cells are labelled by Calcein Blue, AM, and collected
862 through Fluorescence activated cell sorting (FACS). β cells are sorted out and divided
863 into different groups according to donor conditions. 8W, 8-week-old; 9M,
864 9-month-old. **B.** t-SNE map of cells from mouse in different conditions. The cells
865 from healthy and T2D mice are colored with different red-colored items. **C.** Mouse β
866 cells are identified by highly expressed marker gene *Ins2*. **D.** Mouse β cells are
867 colored by sex information. And the expression of β cell marker *Ins2* is significantly
868 different between cells of males and females (K-S test) as shown in the box plot.

869

870 **Figure 2 Single β cell transcriptomes reveal sex-biased genes in healthy and** 871 **T2D mice**

872 **A.** Comparison between β cells of male and female 8W (8-week-old) C57 mice. In the
873 MA plot, the sex-biased genes are highlighted by blue (male-biased) and red
874 (female-biased) dots. See also Table S1. **B.** Nested pie chart depicting genomic
875 location of sex-biased genes. **C.** Diabetes associated physiological phenotypes. Body
876 weight, fasting insulin serum level are detected. The blue line represents the sample of
877 male healthy (n=5 mice) and male T2D (n=4 mice); red line represents the sample of
878 female healthy (n=4 mice) and female T2D (n=5 mice). Results are presented as mean
879 $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$, two sample t-test. **D.** Intraperitoneal injection glucose
880 tolerance test with area under curve (AUC). Results are presented as mean $\pm$ SEM;* p
881 ≤ 0.05 , ** $p \leq 0.01$; *** $p \leq 0.001$. Red *, **, *** represent female T2D versus
882 female healthy mice; blue *, **, *** represent male T2D versus male healthy mice,
883 two sample t-test. **E.** Comparisons between male and female β cells of 9M
884 (9-month-old) healthy and T2D mice. In the MA plots, sex-biased genes are

highlighted by blue (male-biased) and red (female-biased) dots. See also Table S2 and S3. F. Heatmap of genes selected from sex-biased genes that overlapped with the leading-edge genes of GSEA. See also Table S4.

Figure 3 Single β cell transcriptomes reveal sex-dependent T2D altered genes and pathways in β cells

A. Venn diagram depicting the definition of candidate genes for sex-dependent T2D altered genes. B. Venn diagram show the definition of sex-dependent T2D altered genes. The genes in the overlapped part of venn diagram are sex-dependent T2D altered genes. “Raw” represents the candidate genes defined in Figure 3A. “CI” represents the genes that confidence interval of log2FC from analysis of one sex does not overlap with that from the other sex. “ $p > 0.2$ ” represents the p-values in the non-significant sex group are high. See also Table S6. C. Results of GSEA showing pathways significantly enriched in the healthy group and the T2D group in male (labeled in blue) and female (labeled in red). Pathways with NES > 0 are enriched in T2D β cells and pathways with NES < 0 are enriched in healthy β cells. D. GSEA plots of pathways involved in the onset of diabetes or the function of β cell. E. Heatmap of leading-edge genes of the selected pathways.

Figure 4 Sex-matched islet transplantation confers better glucose tolerance than sex-mismatched transplantation

A. Schematic diagram of sex-matched and sex-mismatched islet transplantation for scRNA-seq. Single islet cells are collected for scRNA-seq 9 months post-transplant. M.Endo-islet, endogenous islets of male recipients; F.Endo-islet, endogenous islets of female recipients; Match.TX-islet, transplanted islets in male recipients; Mismatch.TX-islet, transplanted islets in female recipients. B. t-SNE map of endogenous and transplanted islet cells in both sex-matched and sex-mismatched transplantation, and β cells are identified by highly expressed *Ins2*. Details see also

Figure S2A. **C.** Scatterplot depicting correlation of Log2(fold change) generated by comparing β cells of TX- β (sex-matched or sex-mismatched) and endo- β . M.Endo- β , endogenous β cells of male recipients; F.Endo- β , endogenous β cells of female recipients; Match.TX- β , sex-matched transplant- β cells in male recipients; Mismatch.TX- β , sex-mismatched transplant- β cells in female recipients. **D.** Pathways enriched in sex-matched transplant- β cells. Leading-edge genes of longevity regulating pathway are zoomed in with heat-map. M.Endo- β , endogenous β cells of male recipients; F.Endo- β , endogenous β cells of female recipients; Match.TX- β , sex-matched transplant- β cells in male recipients; Mismatch.TX- β , sex-mismatched transplant- β cells in female recipients. **E.** Schematic diagram of sex-matched and sex-mismatched islet transplantation for β cell functional test in STZ induced diabetic mouse. **F.** Oral glucose tolerance test (OGTT) for mice after sex-matched (n=3 mice) and sex-mismatched (n=4 mice) islet transplantation and results are summarized by the area under curve (AUC). 2 g/Kg glucose is gavaged after 6 hours fasting, and blood glucose level is detected at 0, 15, 30, 60, 120 minutes after glucose gavage. Results are presented as mean $\pm$ SEM; * $p \leq 0.05$, two sample t-test. TX, transplantation.

930

931 **Table 1 The number of collected cells from mice in different conditions**

932

933 **Supplementary material**

934 **Figure S1 Data processing identifies a set of qualified single-cell RNA**
935 **sequencing profiles of mouse islet**

936 **A.** Distribution of detected gene numbers (Log2 transformed). 810 out of 5472 cells are
937 removed from further analysis due to number of genes with more than 1 UMI count less
938 than 500. **B.** Distribution of total exon counts (Log2 transformed). The retained cells
939 (shown in orange) have relatively higher exon counts. **C.** Distribution of UMI fractions
940 of mitochondrial genes (Log2 transformed). The retained cells (shown in orange) have

relatively lower UMI fractions of mitochondrial genes. **D.** Boxplot of fraction of UMI count of top k most expressed genes. The fraction is beyond 0.5 for most cells when k = 2. Each gray line represents a cell. **E.** Distribution of UMI count fraction of top 1 (shown in red) and top 2 (shown in blue) most expressed genes. In 86.7% of the mouse islet cells, the UMI count fraction of the top 2 most expressed genes are more than 0.5. The union of the top 2 most expressed genes from all these cells includes 9 genes (*Gcg*, *Ins1*, *Ins2*, *Malat1*, *Ppy*, *Ppy*, *Rn45s*, *Ttr*, *mt-Rnr2*), which are excluded while calculating total count for normalization using adjCPM method. **F.** Hierarchical clustering for 4,662 mouse islet single cells. Using union of the top 10 expressed genes in all cells (total 141 genes) with “euclidean” metric and “complete” linkage and 110 cells are excluded. 8W, 8-week-old; 9M, 9-month-old; 11M, 11-month-old; Endo-islet, endogenous islets of recipients; TX-islet, transplanted islets in recipients. **G.** t-SNE map of retained cells. Cells are colored by the clustering result of the previous hierarchical clustering (up panel), conditions (middle panel) and clique labels from DBSCAN clustering (down panel), respectively. **H.** Violin plots of marker genes for C0, C1, C2, C4, C5 after filtering cells with inconsistent cluster labels given by previous hierarchical clustering. C1: *Ins2* (a known marker gene of β cells) highly expressing cells from mice of C57 strain (labeled as β cells); C2: *Ins2* highly expressing cells from 11-month-old mice of ICR strain (labeled as β cells); C0: *Gcg* (a known marker gene of α cells) highly expressing cells (labeled as α cells); C4 and C5: two groups of cells with high expression of *Slc34a1* and *Pvalb* (two known marker genes of kidney proximal tubule and distal convoluted tubule cells), respectively, which were labeled as kidney-like cells and were discarded. **I.** GO enrichment analysis for up- and down-regulated genes in C3. Key metabolic processes such as ATP biosynthetic process associated genes are down-regulated and cell cycle related genes are up-regulated in C3, so this clique is removed as cells in cell cycle.

Figure S2 Single-cell RNA sequencing reveals sexually dimorphic gene expression and gene set enrichment in β cells

A. Barplot showing the cell type composition of male and female mice under different conditions. 8W, 8-week-old; 9M, 9-month-old; 11M, 11-month-old; Endo-islet, endogenous islets of recipients; TX-islet, transplanted islets in recipients. **B.** t-SNE map of retained mouse cells colored by expression of sex chromosome linked genes. The reds for X-chromosome linked gene *Xist*, and the blues for Y-chromosome linked genes *Eif2s3y*, *Ddx3y*, *Uty*. **C.** GSEA for sex comparisons of β cells from 8-week-old C57 mice. The gene sets with NES > 0 are enriched in males and NES < 0 in females. **D.** GSEA for sex comparisons of β cells from healthy 9-month-old C57 mice. The gene sets with NES > 0 are enriched in males and NES < 0 in females. **E.** GSEA for sex comparisons of β cells from T2D 9-month-old C57 mice. The gene sets with NES > 0 are enriched in males and NES < 0 in females.

Figure S3 Single-cell RNA sequencing reveals sexually dimorphic genes in β cells from T2D model and transplanted islet

A. The qPCR analysis of sex-dependent T2D altered genes. Sex-dependent T2D altered genes with basemean > 10 are selected for validation. n=3 biological replicates. For each replicate, cDNA of 5 *Ins2*⁺ cells that randomly selected from scRNA-seq cDNA library was mixed with equal volume. The cDNA for qPCR is synthesized following the protocol of sequencing library construction. The relative expression (fold change) is calculated relative to the gene expression level of healthy condition within the same sex. Results are presented as mean $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, n.s., not significant, two sample t-test. **B.** Violin plots of sex-dependent T2D altered genes involved in ribosome pathway. The left 3 down-regulated genes in female T2D β cells are female-dependent T2D altered genes; the right 11 up-regulated genes in T2D β cells are male-dependent T2D altered genes. **C.** MA plot showing the comparison between

sex matched and mismatched transplant- β cells. Up-regulated and down-regulated genes in sex matched transplant- β cells are highlighted in blue and red, respectively. See also in [Table S5](#). TX- β , transplant- β cells. **D.** Violin plot of expression of *Sfrp5* in endogenous and transplanted β cells. M.Endo- β , endogenous β cells from male recipient; F.Endo- β , endogenous β cells from female recipient; Match.TX- β , sex matched transplant- β cells; Mismatch.TX- β , sex mismatched transplant- β cells. **E.** Blood glucose level before and after islet transplantation in diabetic mice. The blood glucose level of diabetic mice with sex-matched (blue) and sex-mismatched (red) transplanted islet are all recovered normal (green baseline) from hyperglycemia (orange baseline) about 3 weeks after transplantation. TX, transplantation.

Figure S4 Differential expression genes called by MAST and edgeR have overlap Scatter plots showing that p-value derived z-statistics of genes obtained by MAST and edgeR-zingeR are similar in different analysis. And venn diagrams showing the overlap of DEGs detected by MAST and edgeR-zingeR. The number in brackets represent the number of cells that were used for analysis in each condition.

Table S1 Sex-biased expression genes in β cell of 8-week-old healthy mice

Table S2 Sex-biased expression genes in β cell of 9-mounth-old healthy mice

Table S3 Sex-biased expression genes in β cell of 9-mounth-old T2D mice

Table S4 Sex biased genes included in the leading edge gene of GSEA

Table S5 Sex-independent differential expression genes

Table S6 Sex-dependent T2D altered genes

Table S7 Primers for qPCR

Table S8 Primers for single-cell RNA sequencing library construction

Table S9 Differential expression genes detected by edgeR

1025

1026

1027

A

B

C

D

A

B

C

Body weight

Insulin level

D

Glucose tolerance test

Area under curve

E

DEGs in healthy (9M) β cells DEGs in T2D (9M) β cells

F

A

Sex-matched and sex-mismatched islet transplantation

Preparation of single islet cell 9 months post-transplant

Single cell RNA sequencing

B

C

Correlation to male endogenous β cells

Correlation to female endogenous β cells

E

D

F

A

B

C

D

E

A

B

C

D

E

A

Male(534).vs.Female(460)
from T2D

Male(541).vs.Female(399)
from healthy

T2D(460).vs.Healthy(399)
from female

T2D(534).vs.Healthy(541)
from male