Telmisartan potentiates insulin secretion via ion channels, independent of the AT1 receptor and PPARγ

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Abstract

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28 Angiotensin II type 1 receptor blockers (ARBs), as antihypertensive drugs, have drawn

29 attention for their benefits to individuals with diabetes and prediabetes. However, the

30 effects of ARBs on insulin secretion remain unclear. Here, we investigated the

31 insulinotropic effects of ARBs (telmisartan, valsartan, and irbesartan) and the

32 underlying electrophysiological mechanism in rat islets. We found that only telmisartan

33 among the three ARBs exhibited an insulin secretagogue role. Distinct from other ARBs,

34 telmisartan exerted effects on ion channels including voltage-gated potassium (Kv)

35 channels and voltage-gated Ca^{2+} channels to promote extracellular Ca^{2+} influx, thereby

36 potentiating insulin secretion in a glucose-dependent manner. We observed that the

37 peroxisome proliferator-activated receptor γ pathway was not involved in these

38 telmisartan-induced effects. Furthermore, we identified that telmisartan at least directly

39 inhibited Kv2.1 channel through construction of a Chinese hamster ovary cell line with

40 Kv2.1 channel overexpression. Acute exposure of type 2 diabetes model (*db/db*) mice

41 to a telmisartan dose equivalent to therapeutic doses in humans resulted in lower blood

42 glucose and increased plasma insulin concentration in the oral glucose tolerance test.

43 We further observed the telmisartan-induced insulinotropic and electrophysiological

44 effects on pathological pancreatic islets isolated from *db/db* mice. Collectively, our

45 results establish an important function of telmisartan distinct from other ARBs in the

46 treatment of diabetes.

47 **Keywords:** telmisartan, insulin secretion, AT1 receptor, PPARγ，Kv channel

48

49   **Introduction**

50   Diabetes and hypertension constitute common clinical conditions that are

51   interlinked through numerous pathophysiological mechanisms *(Deedwania, 2004;*

52   *Ferrannini and Cushman, 2012)*. In particular, hypertension substantively increases the

53   risk of type 2 diabetes mellitus (T2DM), as revealed by a prospective cohort study

54   wherein subjects with hypertension were almost 2.5 times more likely to develop

55   T2DM than those with normal blood pressure *(Gress T et al., 2000)*. In turn, the

56   majority (70%–80%) of patients with T2DM also have hypertension *(Fox et al.,*

57   *2015)*.The coexistence of both conditions significantly increases the risks of developing

58   nephropathy, heart failure, and other cardiovascular disease, leading to high rates of

59   mortality and morbidity *(Deedwania, 2004; Ferrannini and Cushman, 2012)*.

60   Therefore, the identification of drugs that prevent both conditions would be of

61   considerable clinical importance.

62   Growing evidences indicated that angiotensin II type 1 (AT1) receptor blockers

63   (ARBs), an important drug class in the treatment of hypertension and heart failure,

64   provided beneficial effects for patients with diabetes and prediabetes. Several clinical

65   trials and retrospective-analyses have shown that ARBs reduce the incidence of new-

66   onset diabetes among patients with hypertension and heart failure *(NAVIGATOR Study*

67   *Group et al., 2010; Yusuf et al., 2005; Kjeldsen et al., 2006)*. Moreover, it has been

68   repeatedly demonstrated that ARBs ameliorate T2DM and its related complications

69   such as atherosclerosis and nephropathy *(Candido et al., 2004; Makino et al., 2008;*

70   *Viberti et al., 2002; Parving et al., 2001)*. In addition, ARBs have been highly

71   recommended in pharmacological therapy regimens for patients with both diabetes and

72   hypertension by the American Diabetes Association *(American Diabetes Association,*

73   *2015)*.

74   T2DM is a metabolic disorder syndrome characterized by insulin resistance and

75   deficiency. The confirmed benefits of ARBs in patients with diabetes and prediabetes

76   have been primarily attributed to blockade of the local renin–angiotensin system (RAS).

77   ARBs suppress oxidative stress and inflammatory responses resulting from overactivity

78   of this system, thereby protecting β-cells against dysfunction and improving insulin

79   sensitivity to maintain euglycemia *(van der Zijl et al., 2011; Hunyady and Catt, 2006;*

80   *Li et al., 2012; Nagel et al., 2006; Shiuchi et al., 2004)*. However, although insufficient

81   insulin secretion constitutes a fundamental process that determines the onset and

82   progression of T2DM *(Weyer et al., 1999; Levy et al., 1998)*, few studies have focused

83   on the effect of ARBs on insulin secretion or its underlying mechanism.

84   In the present study, we applied three ARBs, namely telmisartan, valsartan, and

85   irbesartan to evaluate the effects of ARBs on insulin secretion and investigate the

86   underlying electrophysiological mechanism. Notably, our data showed that unlike other

87   ARBs, telmisartan glucose-dependently elevated the intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) levels

88   of β-cells through its distinctive action on ion channels, leading to enhanced insulin

89   secretion. Our findings suggest that in addition to the typical beneficial effects of ARBs,

90   telmisartan may serve as an insulin secretagogue in the treatment of patients with both

91   diabetes and hypertension.

92 **Results**

93 *Telmisartan, but not valsartan or irbesartan, enhances glucose-stimulated insulin*

94 *secretion (GSIS)*

95   To examine the effects of ARBs on insulin secretion, firstly, isolated rat islets were

96 treated with various doses of telmisartan. As shown in Fig. 1A, telmisartan (10 and 50

97 μM) potentiated insulin secretion under 8.3 mM glucose conditions but had no effect

98 under 2.8 mM glucose. Furthermore, the data in Fig. 1 B confirmed that telmisartan-

99 induced insulin secretion was glucose-dependent. Next, the functions of other ARBs

100 were evaluated. Notably, no promotion of insulin secretion was observed following

101 treatment with valsartan and irbesartan under 8.3 and 16.7 mM glucose conditions (Fig.

102 1, C and D, and fig. S1). Considering that telmisartan, valsartan and irbesartan are

103 clinically available ARBs owing to their high specificity for AT1 receptors *(Michel et*

104 *al., 2013)*, our results suggested that telmisartan-mediated insulinotropic effect was

105 independent of AT1 receptors.

106
107 *Telmisartan, but not valsartan or irbesartan, increases ([Ca^{2+}]$_i$) concentration in β-*
108 *cells*

109   Within β-cells, eliciting an increase in [Ca^{2+}]$_i$ causes insulin granule exocytosis;

110 therefore, the elevation in [Ca^{2+}]$_i$ level is essential to induce insulin secretion *(Sabatini*

111 *et al., 2019)*. To verify whether the insulinotropic effect of telmisartan was related to

112 the change in [Ca^{2+}]$_i$, we applied the calcium-sensitive dye Fura 2-AM to detect changes

113 in fluorescence intensity. Telmisartan (10 and 50 μM) induced an acute increase in

114 fluorescence intensity dose-dependently. Moreover, the elevation only occurred under

115 high (11.1 and 16.7 mM) (Fig. 2 C, D, and E, F) but not low (2.8 mM) (Fig. 2 A and B)

116   glucose conditions. In addition, in the calcium imaging experiment, neither valsartan

117   (Fig. 2 G and H) nor irbesartan (Fig. 2 I and J) increased the $[Ca^{2+}]_i$ concentration of β-

118   cells under high glucose conditions (16.7 mM).

119

120   ***Peroxisome proliferator-activated receptor γ（PPARγ）is not involved in telmisartan-***

121   ***induced insulin secretion and elevation of $[Ca^{2+}]_i$ levels***

122   Telmisartan and irbesartan have also been reported to function as a partial agonist

123   of PPARγ *(Schupp et al., 2004, 2005)*. In view of the absence of changes in insulin

124   secretion and $[Ca^{2+}]_i$ levels with irbesartan, we speculated that PPARγ might not be

125   responsible for the effects of telmisartan on these measures. We therefore performed

126   the insulin secretion assay and calcium imaging experiment using GW9662, a selective

127   PPARγ antagonist. As shown in Fig. 3, GW9662 alone had no effect on GSIS and

128    $[Ca^{2+}]_i$ concentration, and the addition of GW9662 did not influence the effects of

129   telmisartan on insulin secretion or $[Ca^{2+}]_i$ concentration.

130

131   ***Telmisartan affects $[Ca^{2+}]_i$ concentration through extracellular Ca^{2+} influx rather***

132   ***than intracellular Ca^{2+} stores release***

133        $[Ca^{2+}]_i$ levels of β-cells are tightly maintained through the regulation of

134   extracellular Ca^{2+} influx and the movement of Ca^{2+} within intracellular stores *(Sabatini*

135   *et al., 2019)*. We thus examined the concentration of $[Ca^{2+}]_i$ in the absence of

136   extracellular Ca^{2+} to study the pathway by which telmisartan increases $[Ca^{2+}]_i$

137   concentration. Calcium imaging (Fig. 4 A and B) showed that telmisartan-induced

138   elevation of $[Ca^{2+}]_i$ levels was reversed in Ca^{2+}-free KRBH medium, although the

139   $[Ca^{2+}]_i$ level was considerably increased upon intracellular Ca^{2+} mobilization via

140   thapsigargin.

141   Moreover, telmisartan-induced effects on $[Ca^{2+}]_i$ levels were monitored following

142   the application of azelnidipine, an L-type voltage-gated Ca^{2+} channel (VGCC) blocker.

143   We observed that the increase in $[Ca^{2+}]_i$ levels with telmisartan was completely blocked

144   by azelnidipine, supporting that telmisartan enhances extracellular calcium influx

145   through L-type VGCCs. Conversely, significant elevation remained upon thapsigargin

146   addition (Fig. 4 C and D), confirming the lack of telmisartan effect on intracellular

147   calcium stores.

148

149   ***Telmisartan inhibits voltage-gated potassium (Kv) channels, and prolongs action***

150   ***potential durations (APDs) in β-cells***

151   Pancreatic β-cells are electrically excitatory. Previous studies have demonstrated

152   that Kv channels play an important role in GSIS and glucose-stimulated increase of

153   $[Ca^{2+}]_i$ *(Herrington et al., 2006; Roe et al., 1996; MacDonald and Wheeler, 2003)*;

154   therefore, we applied patch-clamp techniques to explore the effects of telmisartan on

155   the Kv channels of β-cells. Fig 5 A and B illustrate that telmisartan decreased the Kv

156   channel currents compared with that of controls.

157   Kv channels participate in the repolarization of action potentials of β-cells, so

158   that inhibition of Kv channels delays the repolarization, thus prolonging the APD,

159   namely the duration of extracellular Ca^{2+} influx *(Herrington et al., 2006; MacDonald*

160   *and Wheeler, 2003; Jacobson and Philipson, 2007)*. Therefore, we next recorded the

161   action potentials in current-clamp mode to observe the effect of telmisartan on APD. As

162   presented in Fig. 5 C, comparison of APDs with or without telmisartan indicated that

163   telmisartan extended APD.

164   ***Telmisartan directly inhibits Kv2.1 channels independent of AT1 receptor and PPARγ***

165   We evaluated the effects of valsartan and irbesartan in the voltage-clamp

166   experiment. Neither valsartan nor irbesartan exhibited similar effects on Kv channels

167   as those observed with telmisartan treatment (Fig. 6 A and B). Moreover, GW9662

168   addition did not influence the telmisartan-induced inhibition of Kv channels (Fig. 6 C

169   and D). The results indicated that telmisartan inhibited Kv channels independent of the

170   AT1 receptor and PPARγ.

171   We therefore hypothesized that telmisartan might directly inhibit Kv channels. As

172   the Kv2.1 channel constitutes the main subtype among Kv families involved in the

173   regulation of insulin release by β-cells *(MacDonald P et al., 2001; Li et al., 2013;*

174   *Jacobson et al., 2007)*, we carried out patch-clamp experiments to determine whether

175   telmisartan directly inhibited Kv2.1 channels. Chinese hamster ovary (CHO)cells,

176   which do not express any endogenous Kv channels *(Yu and Kerchner, 1998)*, were

177   utilized to establish the Kv2.1-overexpressing CHO-Kv2.1 cell line. Under whole-cell

178   voltage-clamp mode, Kv2.1 channel currents and their suppression by telmisartan were

179   both detected in CHO-Kv2.1 cells (Fig. 6 E, and F), suggesting that telmisartan exerted

180   direct inhibition on Kv2.1 channels.

181   ***Telmisartan activates VGCCs independent of the AT1 receptor and PPARγ***

182   To further confirm whether Kv channels alone are involved in mediating

183   telmisartan-induced insulin secretion and increase of $[Ca^{2+}]_i$ levels,

184   tetraethylammonium chloride (TEA), a potent inhibitor of Kv channels, was employed

185   in pancreatic β-cells. Previous studies have shown that 20 mM TEA blocks the majority

186   of Kv channels and causes calcium elevation *(Roe et al., 1996; MacDonald P et al.,*

187   *2001)*. As shown in Fig. 7A, TEA stimulated insulin secretion under 11.1 mM glucose

188   conditions and telmisartan still significantly promoted insulin secretion in the presence

189   of TEA, indicating that other factors may participate in telmisartan-stimulated insulin

190   secretion. Consistent with this results, telmisartan also enhanced the $[Ca^{2+}]_i$

191   concentration in the presence of TEA under 11.1 mM glucose conditions (Fig. 7 B and

192   C).

193   As telmisartan enhances extracellular calcium influx through VGCCs, we

194   performed patch-clamp experiments to observe the effects of telmisartan on VGCCs in

195   pancreatic β-cells. As presented in Fig. 7 D and E, telmisartan increased voltage-

196   dependent inward Ca^{2+} currents densities compared with those of controls. In addition,

197   no significant difference was observed when VGCC currents were recorded following

198   treatment with valsartan or irbesartan (fig. S2, A and B); telmisartan-induced activation

199   was not inhibited by GW9662 co-administration (fig. S2, C and D). The results thus

200   demonstrated that telmisartan also activated VGCCs of β-cells; moreover, neither the

201   AT1 receptor nor the PPARγ mediated this effect.

202   ***Telmisartan ameliorates hyperglycemia by increasing insulin secretion in vivo and***

203   ***amplifies GSIS in vitro in db/db mice***

204   We applied db/db mice as T2DM model mice to determine whether telmisartan

205   induced hypoglycemic effects in vivo. Male mice were administered with telmisartan

206   (15 mg/kg) or vehicle once by gavage at the age of 8 and 11 weeks, then the oral glucose

207   tolerance test (OGTT) was performed to observe the effects of telmisartan on glucose

208   response.

209   In 8-week-old mice, blood glucose levels monitoring revealed that glucose

210   clearance at 30 min and thereafter was improved significantly in telmisartan-treated

211   mice, and noticeable difference was observed when the glycemic response was

212   measured via the area under the curve (AUC) compared with that of control (Fig. 8 A).

213   However, although the time of peak blood glucose was similarly advanced to 15 min in

214   11-week-old mice, it was not until 90 min and 120 min (approximately 1 h later than in

215   8-week-old mice) that the blood glucose values were markedly lower than those of

216   controls. Additionally, the AUC results showed no significant difference between the

217   groups (Fig. 8 B). Furthermore, the levels of plasma insulin in the telmisartan-treated

218   group were considerably higher than those in the control group at 15, 30, and 60 min

219   with the AUC differing significantly between the groups (Fig. 8 C). Therefore, the

220   glucose-lowering effect of telmisartan was accompanied by the increase in the levels of

221   plasma insulin, suggesting that the hypoglycemic effects of telmisartan were a result of

222   increased insulin secretion. We speculated that the glucose-lowering effect of

223   telmisartan was delayed and weakened in 11-week-old mice, possibly owing to the

224   progression of insulin resistance and the deterioration of β-cell function in db/db mice.

225   At the end of the experiment, islets were isolated from *db/db* mice and used for an

226   ex vivo study. The results showed that telmisartan potentiated insulin secretion under

227   16.7 and 30 mM glucose conditions (Fig. 8 D). Consistent with the results of in vivo

228   studies, telmisartan treatment similarly enhanced GSIS under the pathological

229   condition of diabetes. However, GSIS in cultured islets only occurred under 30 mM

230   glucose conditions whereas 2.8 and 16.7 mM glucose showed equivalent secretion. The

231   bluntness of GSIS might be related to impaired β-cell function caused by long-term

232   exposure to high glucose and lipids in the development of diabetes in the db/db mice

233   *(Olofsson et al., 2007)*.

234   　　In T2DM, high glucose and free fatty acids leads to adverse effects (including

235   blunted GSIS and decreased cell viability) *(Olofsson et al., 2007; Tan et al., 2013)*, with

236   the modulation of Kv and voltage-gated Ca^{2+} channels by "glucolipotoxicity" also being

237   involved *(Hoppa et al., 2011; Lee et al., 2018)*. We next performed the patch-clamp

238   experiments to ascertain whether telmisartan exerted similar electrophysiological

239   effects on pathological β-cells. We observed that both the decreased Kv channel

240   currents (fig. S3, A and B) and increased VGCC currents (fig. S3, C and D) remained

241   in telmisartan-treated β-cells of db/db mice. Therefore, under the pathological condition

242   of T2DM, telmisartan still served the function of an insulin secretagogue through its

243   action on ion channels.

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247

248 **Discussion**

249   ARBs are of critical importance to individuals with both diabetes and hypertension.

250 We therefore carried out the study to better understand the beneficial effects of ARBs

251 for diabetes. Notably, we revealed an insulin secretagogue role for telmisartan, which

252 is not present in other ARBs. In the present study, isolated islets were exposed to

253 telmisartan for only 30 minutes prior to the insulin secretion assay, and glucose-

254 lowering effects were observed in db/db mice following acute telmisartan

255 administration. The evidences both in vitro and in vivo thus demonstrated the rapid

256 insulinotropic effect of telmisartan. To the best of our knowledge, no prior reports of

257 telmisartan exist with respect to this direct effect on insulin secretion.

258   Moreover, our results showed that telmisartan, enhances insulin secretion in a

259 glucose-dependent manner. Even at high concentrations (50 μM), no insulinotropic

260 effect of telmisartan was observed under low glucose conditions (2.8 mM) (Fig. 1A).

261 This indicated that telmisartan might be applied as an insulin secretagogue without the

262 risk of hypoglycemia. Hypoglycemia is a frequent and severe adverse effect. Not only

263 can apparent hypoglycemia cause coma or the disruption of daily life, but unrecognized,

264 recurrent hypoglycemia can also lead to life-threatening cardiac complications such as

265 arrhythmias and myocardial ischemia, and cause permanent cognitive impairment that

266 may accelerate the onset of dementia*(Frier, 2014)*. In addition, emerging evidence

267 suggests that forcing the β-cells to secrete insulin constantly, termed insulin

268 hypersecretion, might have the potential to accelerate the decline in β-cell function and

269 thus may constitute a contributing factor to the progression of T2DM *(Rustenbeck et*

270 *al., 2010; Aston-Mourney et al., 2008)*. Therefore, glucose-independent insulinotropic

271 agents have exhibited poor durability in maintaining long-term glycemic control

272 *(Kahn et al., 2006)*. In comparison, our study showed that telmisartan increased insulin

273 secretion in a manner proportional to the accumulating glucose concentration, thereby

274 avoiding the risk of overstimulating the β-cells.

275 By means of its function as both an ARB and a partial agonist for PPAR-γ,

276 telmisartan provides numerous beneficial effects in ameliorating T2DM and related

277 complications*(Makino et al., 2008; Li et al., 2012; Nagel et al., 2006; Hasegawa et al.,*

278 *2009; Saitoh et al., 2009; Yamana et al., 2008; Perl et al., 2010; Goyal et al., 2008)*.

279 However, our results demonstrated that telmisartan also functioned rapidly as an insulin

280 secretagogue, consequent to its unique electrophysiological effects on ion channels,

281 which were independent of the AT1 receptor and PPARγ.

282 Glucose-induced insulin secretion and increase of $[Ca^{2+}]_i$ are tightly controlled by

283 ion channels that regulate cell membrane potential. The closure of ATP-sensitive

284 potassium (K_{ATP}) channels caused by high glucose results in membrane depolarization

285 and opening of Kv channels and VGCCs *(Sabatini et al., 2019; Kalwat and Cobb,*

286 *2017)*. Kv channels mediate repolarization of β-cells, and antagonize the Ca^{2+} influx

287 induced by VGCC activation. Blockade of Kv channels therefore prolongs action

288 potential duration, leading to an increase of insulin secretion. In support of this notion,

289 here we found that inhibition of Kv channels was linked to telmisartan-induced

290 augmentation of GSIS.

291 Moreover, we identified that telmisartan directly inhibited Kv2.1 channel. The

292 Kv2.1 channel, as a Kv family member, accounts for the majority of Kv currents on β-

293 cells, serving to not only negatively regulate GSIS but also potentiate β-cell apoptosis

294 *(Kim et al., 2012; Tingting et al., 2018)*. Previous studies attributed telmisartan-induced

295 protective effects against β-cells apoptosis and dysfunction to its action on the AT1

296 receptor and PPARγ*(Li et al., 2012; Hasegawa et al., 2009; Saitoh et al., 2009; Wang*

297 *et al., 2019)*, however, our results indicated that the inhibition of Kv2.1 might also be

298 involved. Moreover, based on its dual effects including regulation of insulin secretion

299 and β-cell apoptosis, Kv2.1 is considered as a promising therapeutic target for T2DM

300 by most researchers in the field. However, despite the occasional reports of small

301 molecule Kv2.1 inhibitor*(Tingting et al., 2018; Zhou et al., 2016)*, no specific drugs

302 have been developed for therapeutic use. Alternatively, as drug repurposing has become

303 a successful approach to accelerate novel anti-diabetic drug development*(Turner et al.,*

304 *2016)*, our favorable finding provides insight with regard to new options for anti-

305 diabetic drug discovery. Furthermore, as Kv2.1 also serves as the key channel during

306 neuronal apoptosis and its cleavage inhibits neuronal apoptosis *(Liu et al., 2018; Yao et*

307 *al., 2009)*, the potential neuroprotective role of telmisartan also warrants further

308 investigation. It should be noted here that there are many isoforms of the Kv channel

309 contributing to the regulation of GSIS in β-cells *(MacDonald P et al., 2001)*;

310 Accordingly, our data did not exclude the possibility that telmisartan also interacts with

311 other Kv channel isoforms.

312 Of note, although the potent Kv channel inhibitor TEA blocks the majority of Kv

313 channels, we found that telmisartan showed a more effective potentiation on insulin

314 secretion and $[Ca^{2+}]_i$ concentration in the presence of TEA. Indeed, our findings

315 revealed that in addition to Kv channels, VGCCs mediated the effects of telmisartan,

316 which were also independent of the AT1 receptor and PPARγ. Moreover, we concluded

317 that K_{ATP} channels were unlikely to be involved in telmisartan -regulated insulin

318 secretion for several reasons. Specifically, the insulinotropic effect of inhibition of K_{ATP}

319 channels is glucose-independent *(Dukes et al., 1994; Henquin, 2011)*, whereas

320 telmisartan did not enhance insulin secretion under low glucose (2.8 mM) conditions

321 (Fig. 1A and D). Conversely, the K_{ATP} antagonist tolbutamide increased $[Ca^{2+}]_i$

322 concentrations in β-cells under low glucose conditions (Fig. 2 A and B), suggesting that

323 telmisartan and tolbutamide act on separate targets.

324   In summary, our results showed that beyond AT1 receptor blockade or PPARγ

325 activation, telmisartan also inhibits Kv channels and activates VGCCs to promote

326 extracellular Ca^{2+} influx, thereby enhancing $[Ca^{2+}]_i$ levels and amplifying GSIS. Our

327 findings provide a new understanding of an anti-diabetes mechanism for telmisartan

328 that is distinct from that of other ARBs, and may have important implications for

329 determination of the choice of ARBs for the treatment of patients with both

330 hypertension and diabetes. In addition, our identification of telmisartan also acting as a

331 Kv2.1 inhibitor and glucose-dependent insulinotropic agent, provides a foundation for

332 the development of new anti-diabetic drugs.

333

Methods

Animals

Adult male Wistar rats, weighing 240–260 g, were purchased from Beijing Weitong Lihua experimental animal center (Beijing, PR China). Five-week-old male diabetic *db/db* mice (BKS - Lepr^em2Cd479^/Gpt, stock number T002407) were obtained from GemPharmatech Co.,Ltd (Nanjing, China). Rats and mice were maintained in specific-pathogen-free surroundings, with a 12 h-light/dark cycles under controlled temperature ($22 \pm 2°C$) and humidity (55–60%) conditions, and with free access to water and food. All animal care and experimental procedures conformed to the ethical guidelines for animal research at Shanxi Medical University and were approved by the Animal Care and Use Committee of Shanxi Medical University (Taiyuan, China).

culture of islets and cells

The rat pancreas was isolated following injection of 1 mg/mL collagenase P (Roche, Indianapolis, IN, USA) through the common bile duct. After digestion at 37 °C for 11 min and density gradient centrifugation with Histopaque-1077 (Sigma-Aldrich, St.Louis, MO, USA) for 23 min, the expanded pancreas was dispersed, and islets remaining in the supernatant separated from the sediment. The islets were hand-collected under a dissection microscope, and single islet cells were obtained from islets using Dispase II (Roche, Indianapolis, USA) digestion. The *db/db* mouse islets were similarly obtained, although the pancreas was injected with 1 mg/mL collagenase V (Roche, Indianapolis, USA), then digested for 16 min and centrifuged twice with Hanks Balanced Salt Solution. Isolated islets and cells were cultured in RPMI 1640 (Hyclone,

356   Thermo Scientific, Waltham, MA, USA) medium containing 11.1 mM glucose,

357   supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin at 37 °C in

358   a humidified atmosphere of 5% CO2, 95% air.

359   Chinese hamster ovary (CHO) cells were obtained from the National Infrastructure

360   of Cell Line Resource (Beijing, China). Lentivirus vectors overexpressing voltage-

361   dependent potassium (Kv) 2.1 channels were constructed (Shanghai Genechem Co.,

362   Ltd., Shanghai, China) to transfect CHO cells, and the CHO-Kv2.1 cell line was

363   established. CHO-Kv2.1 cells were cultured in Dulbecco's modified Eagle's medium

364   (Hyclone, Thermo Scientific, Waltham, MA, USA) containing 4500 mg/L glucose in

365   addition to 10% fetal bovine serum, 1% penicillin and streptomycin and 0.5 μg/mL

366   puromycin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). CHO

367   cells were cultured under similar conditions except for puromycin selection.

368   *Insulin secretion assay*

369   Handpicked separated islets were cultured for 1–2 days before the experiment. A

370   total of five islets per tube were pre-incubated in Krebs Ringer bicarbonate- HEPES

371   (KRBH) buffer under 2.8 mM glucose conditions for 30 min. The KRBH buffer

372   contained 128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO$_4$, 1.2 mM MgSO$_4$, 2.5 mM

373   CaCl$_2$, 5 mM NaHCO$_3$, and 10 mM HEPES, adjusted to pH 7.4 with NaOH prior to the

374   addition of 2% bovine serum albumin. Islets were then treated with different drugs and

375   glucose conditions as indicated, and supernatant liquid was collected at the end of every

376   30 min incubation, and stored at −20 °C for insulin concentration measurement. Insulin

377   secretion was determined using an Iodine [^{125}I] Insulin Radioimmunoassay Kit (North

378   Biological Technology Research Institute of Beijing).

379   *Calcium imaging technology*

380   Calcium imaging was carried out at 28–30 °C using the calcium-sensitive dye Fura

381   2-AM (Dojindo Laboratories, Kumamoto, Japan), using an OLYMPUS IX71 inverted

382   microscope and Meta Fluor software 7.8 (Molecular Devices, Sunnyvale, CA, USA).

383   Islet cells were cultured on coverslips coated with adhesion reagent for 6–10 hour, then

384   were loaded with 2 μM Fura 2-AM in KRBH buffer with addition of 2.8 mM glucose

385   for 30 min at 37 °C. Subsequently, the loading buffer was removed, and cells were

386   washed twice with KRBH solution to remove excessive fluorescent dye. Fura-2 was

387   excited at 340 and 380 nm wavelengths in 1 s intervals with fluorescence emission

388   detected at 510–520 nm wavelengths. The ratio of fluorescence intensity (F340/F380)

389   was recorded to measure intracellular Ca^{2+} concentrations.

390   Fura 2-AM-loaded islet cells on coverslips were transferred to a glass chamber

391   containing KRBH buffer with appropriate glucose conditions. Between each test, the

392   reagent was dripped onto the coverslip and F340/F380 data points were acquired to

393   monitor the changes of intracellular Ca^{2+} level. The average value during 30 s

394   F340/F380 spikes (15 s before and after the peak of F340/F380) for each test was used

395   to compare the change of Ca^{2+} concentrations under different treatments, unless

396   otherwise stated.

397   *Electrophysiology*

398   Whole-cell recording patch-clamp technology was applied to detect voltage-

399   activated currents and record action potentials using an EPC-10 amplifier and PULSE

400   software from HEKA Electronik (Lambrecht, Germany) at room temperature. Islet cells

401   were cultured on glass coverslips coated with cell adherent reagent (Applygen

402   Technologies Inc., Beijing, China).

403   In voltage-clamp mode, to record Kv currents, patch pipettes (5–8MΩ) were

404   loaded with intracellular solution containing 10 mM NaCl, 1 mM $MgCl_2$, 0.05 mM

405   EGTA, 140 mM KCl, 0.3 mM Mg-ATP, and 10 mM HEPES, pH 7.25 adjusted with

406   KOH. Cells were transferred to a recording chamber containing extracellular solution

407   consisting of 138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2 \cdot 6H_2O$, 2.6 mM $CaCl_2$, 11.1

408   mM glucose, and 5 mM HEPES (pH 7.4 adjusted with NaOH). The β-cells were

409   identified by cell capacitance (>7 pF) *(Göpel et al., 1999)* and were clamped to a holding

410   potential of −70 mV, then test potentials were elicited by ranging from −70 mV to 80

411   mV in 10 mV steps for 400 ms.

412   For voltage-gated Ca^{2+} channel (VGCC) currents, the intracellular solution

413   contained: 120 mM CsCl, 20 mM TEA (Sigma-Aldrich), 5 mM MgATP, 1 mM $MgCl_2$,

414   0.05 mM EGTA, and 10 mM HEPES (pH 7.25 adjusted with CsOH). The extracellular

415   solution consisted of: 100 mM NaCl, 20 mM TEA, 20 mM$BaCl_2$, 4 mM CsCl, 1 mM

416     $MgCl_2$, 5 mM HEPES, and 3 mM glucose (pH 7.4 adjusted with NaOH). Ca^{2+} was

417   replaced with Ba^{2+} as the charge carrier in the extracellular solution to eliminate Ca^{2+}-

418   dependent inactivation of the VGCCs. β-cells were clamped to a holding potential of

419   −70 mV, and then elicited by test potentials of −50 mV to 30 mV in 10 mV steps for 50

420   ms.

421   In current-clamp mode, β-cells were elicited by 4 ms currents of 150 pA to record

422   action potentials. The time between the initiation and the point where membrane

423   potential returned to within 10 mV of the resting membrane potential, was considered

424   to be the measurement of action potential duration.

425   *In vivo evaluation of mice and drug administration*

426   At the age of 8 weeks, the mice were given fasting glucose teste to ensure that

427   diabetes models were successfully established. Given that a therapeutic doses of

428   telmisartan are 40–80 mg/day in humans, the conversion for mice was approximately

429   8.2–16.4 mg/kg of body weight *(Nair and Jacob, 2016)*. In our experiment, the mice

430   were administered acute oral acute oral telmisartan treatment at 15 mg/kg of body

431   weight. At 2 hours following drug intake, when the onset of action of telmisartan

432   reached a maximum, oral glucose tolerance test (OGTT) was performed *(Gohlke et al.,*

433   *2001)*.

434   *OGTT*

435   At the age of 8 weeks, following overnight fasting (14 h), the mice were

436   randomly divided into groups receiving treatment with telmisartan (in drinking water

437   containing 0.5% carboxymethyl cellulose sodium salt) or vehicle by gavage. For

438   OGTTs, groups of mice were fed with glucose at 1.5 g/kg body weight orally, then a

439   blood sample was collected from the tail vein and glucose levels were assessed using a

440   Sinocare Glucometer (Changsha, China) at baseline (0 minute) and after 15, 30, 60, 90,

441   and 120 min. At the age of 11 weeks, the mice were treated as described above and

442   additional blood samples (50 μL) were obtained in a heparinized microhematocrit tube

443   at 0, 15, 30, 60, 90, and 120 min. After centrifugation, the plasma was collected for

444   insulin concentration measurement using the Mercodia Mouse Insulin ELISA (stock

445   number 10-1247-01, Uppsala, Sweden).

446   *Statistical analysis*

447   All experimental data are presented as the means ± SEM. $P < 0.05$ was considered

448   to indicate statistical significance. Sharpiro–Wilk tests were used to analyze the

449   normality of the data. Upon normal distribution, the means of numerical variables were

450   compared using the Student's *t* test or one-way analysis of variance (ANOVA), whereas

451   data with non-normal distribution were analyzed using the Mann–Whitney Rank Sum

452   Test or Kruskal–Wallis one-way ANOVA on Ranks. If any statistically significant

453   difference was detected among three or more groups, the Student–Newman–Keuls

454   method or Tukey test was performed for post hoc comparisons, unless otherwise stated.

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467   **Author contributions**

468   Y. Z., Y. L. and T. L. conceived and designed the study; T. L. performed the ex vivo

469   experiments with assistance from L. C., H. X., X. Y., M .L., T. B., Z. L., and Q.G.; T. L.,

470   L. C., H. Y., and L. Z. carried out the in vivo experiments; T. L., M. Z., and P. H. analyzed

471   the data. T. L., Y. Z., and Y. L. wrote the manuscript.

472

473   **Competing interests**

474   All authors declare that they have no competing interests, and approve the final

475   manuscript.

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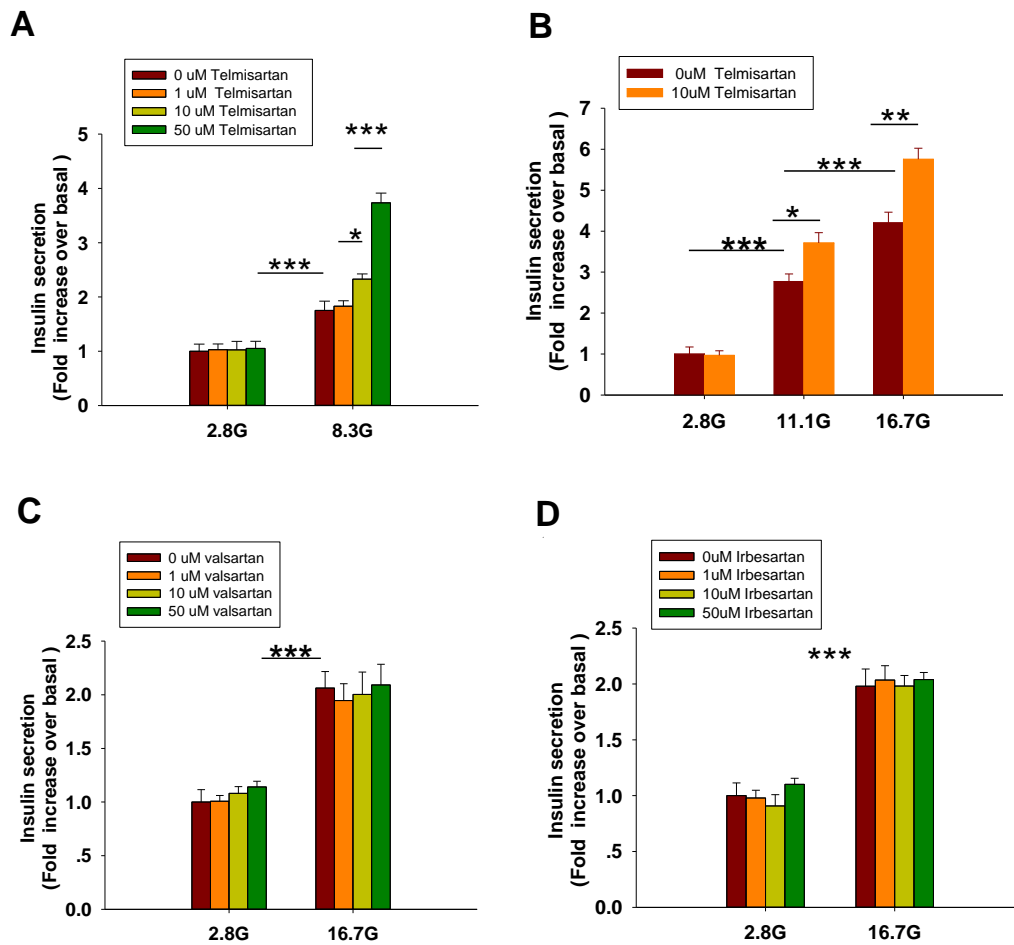
Fig. 1: Only telmisartan among the three ARBs potentiated insulin secretion in rat islets.

In every tube, five handpicked rat islets were incubated for 30 min in 500 μL Krebs

Ringer bicarbonate-HEPES (KRBH) buffer containing different drugs and glucose

concentrations, then supernatant liquid was collected for insulin measurement (n = 7

tubes per group). (A) Rat islets were treated with various doses (1, 10, and 50 μM) of

telmisartan under 2.8 and 8.3 mM glucose (denoted as 2.8 G and 8.3 G) conditions. (B)

Islets were treated with 10 μM telmisartan under different glucose concentrations (2.8,

11.1, and 16.7 mM). (C and D) Rat islets were treated with various doses (1, 10, and 50

μM) of valsartan or irbesartan under 2.8 and 16.7 mM glucose conditions. All results

are normalized to basal secretion at 2.8 G, and reported as the means ± SEM. Statistical

680   differences among three or more groups were compared using one-way analysis of

681   variance (ANOVA) and Student–Newman–Keuls method post hoc analysis. Statistical

682   differences between two groups (with or without telmisartan) under the same glucose

683   condition in (B) were determined using an unpaired two-tailed Student's t test. * P <

684   0.05, ** P < 0.01, *** P < 0.001.

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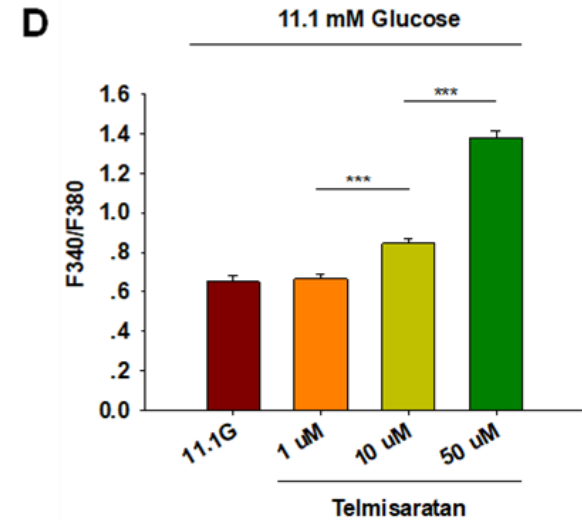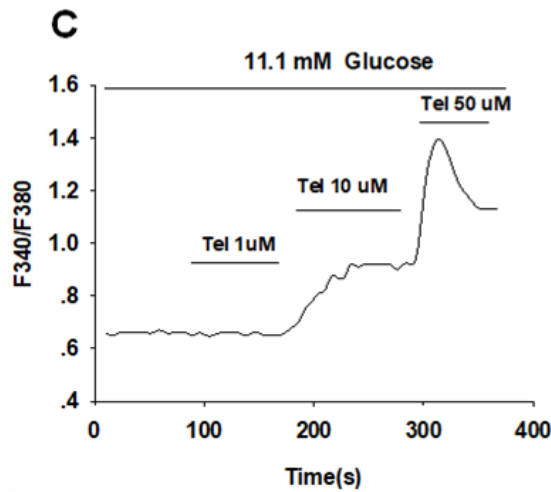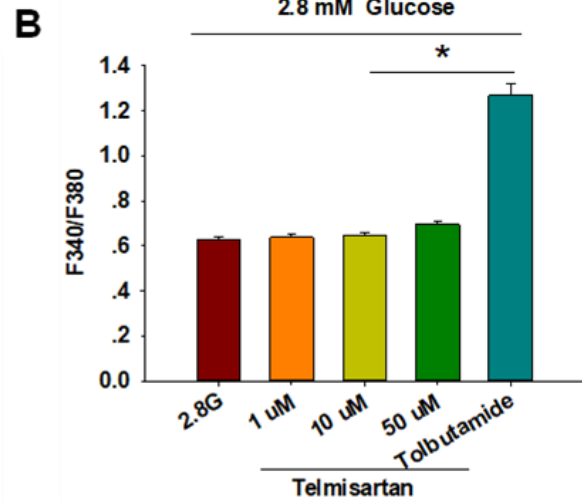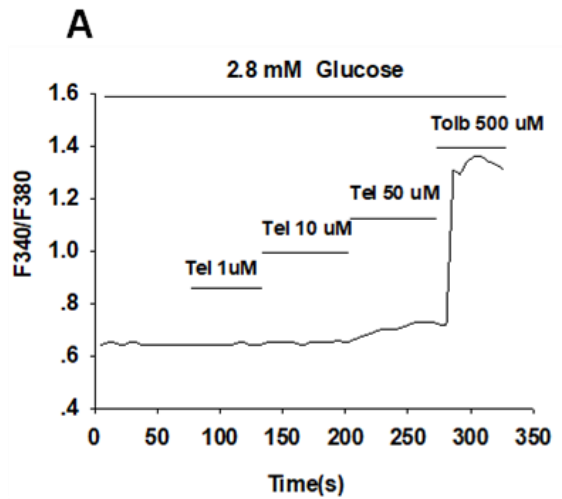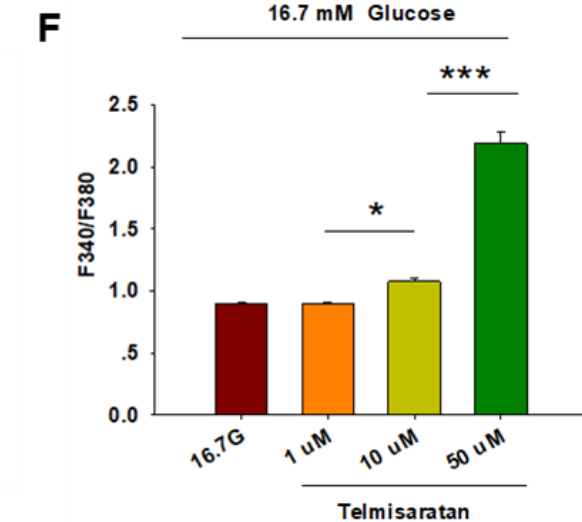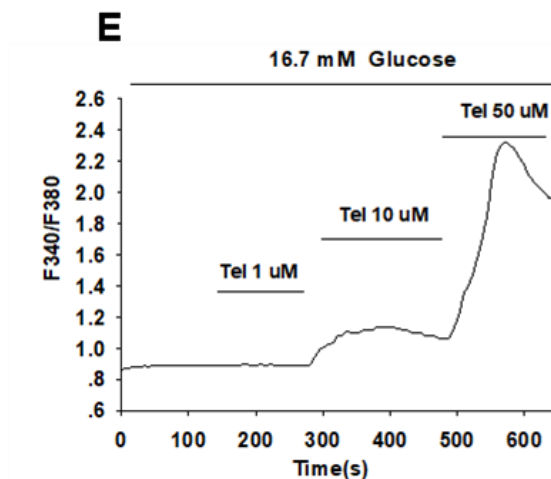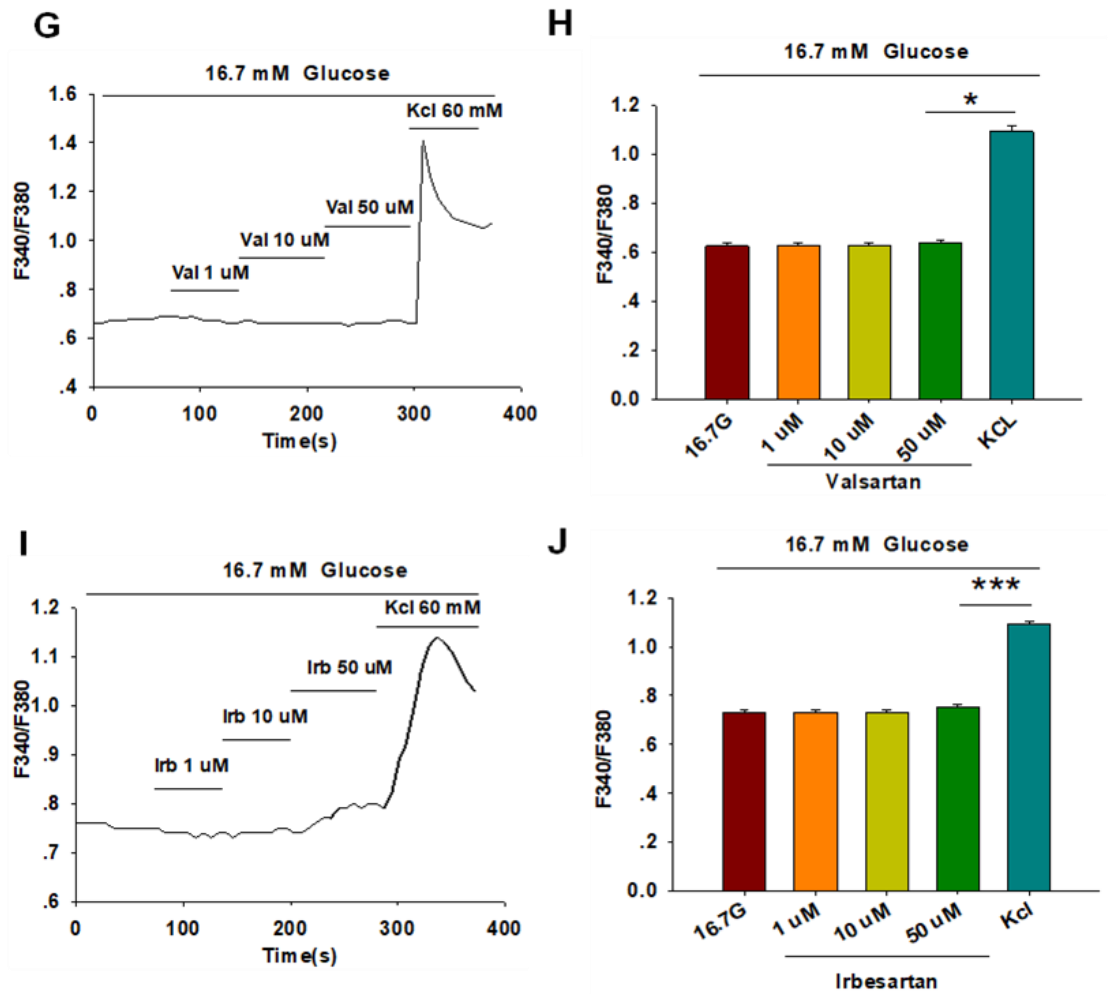
Fig. 2: Only telmisartan among the three ARBs increased intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$)

concentration in rat pancreatic β-cells. (A) The trace shows the changes of $[Ca^{2+}]_i$

concentration in β-cells treated with 1, 10, and 50 μM telmisartan (Tel) under 2.8 mM

glucose conditions; 500 μM tolbutamide (Tolb) was used as a positive control. (B) The

average value during 30 s F340/F380 spikes for each test in response to different doses

of telmisartan under 2.8 mM glucose conditions as indicated (n = 9). (C and D) The

trace shows the changes of $[Ca^{2+}]_i$ concentration in β-cells treated with different doses

of telmisartan under 11.1 mM glucose conditions, and the average value during 30s

F340/F380 spikes for each test as indicated (n = 9). (E and F) The trace shows the

changes of $[Ca^{2+}]_i$ concentration in β-cells treated with different doses of telmisartan

701 under 16.7 mM glucose conditions, and the average value during 30s F340/F380 spikes

702 for each test as indicated (n = 9). (G, H and I, J) The trace shows the changes of $[Ca^{2+}]i$

703 concentration in β-cells treated with 1, 10, and 50 μM of valsartan (Val) or irbesartan

704 (Irb) under 16.7 mM glucose conditions respectively, and the average value during 30s

705 F340/F380 spikes for each test as indicated. KCl (60 mM) was used as a positive control

706 (n = 9). All results are reported as the means ± SEM. Statistical differences among three

707 or more groups were compared using one-way ANOVA, and followed by Student–

708 Newman–Keuls Method post hoc analysis in (D), (F), and (J), or Tukey post hoc

709 analysis in (B) and (H). * P < 0.05, *** P < 0.001

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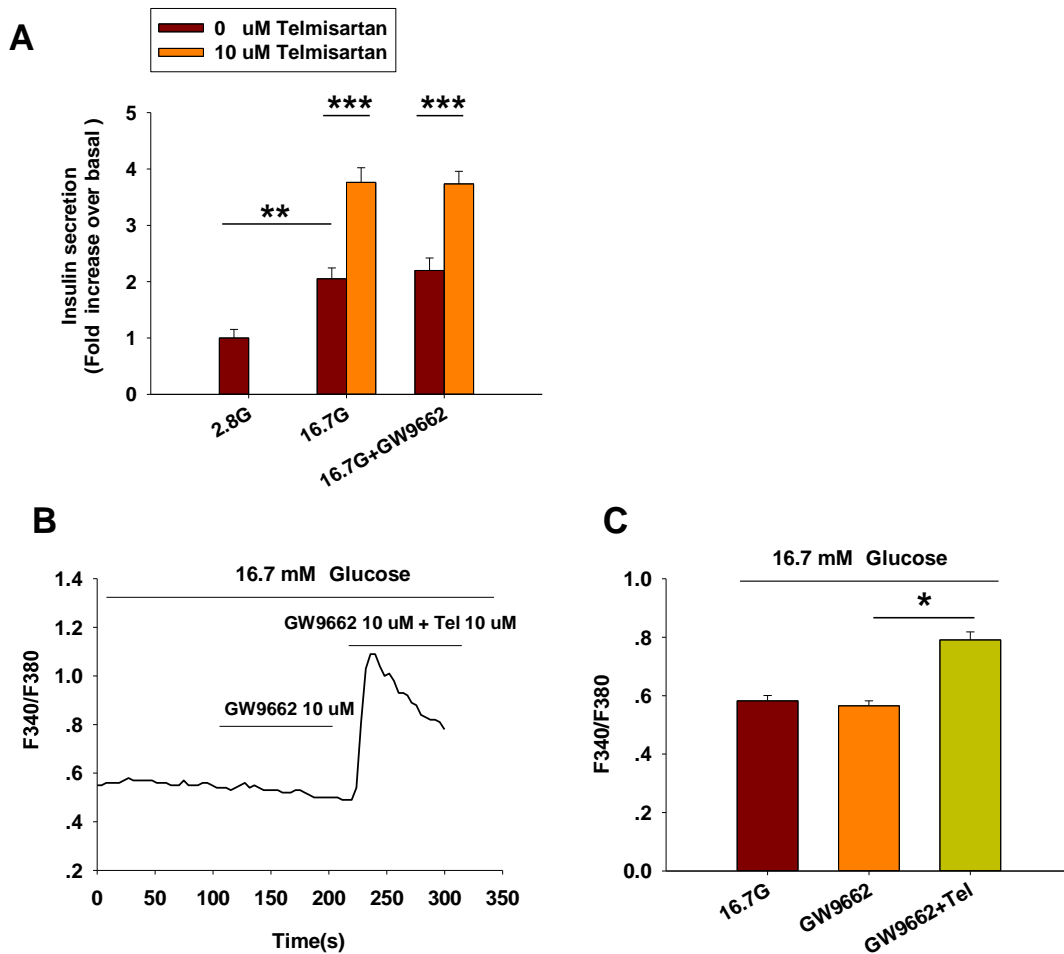
Fig. 3: PPARγ does not participate in the pathway of telmisartan-induced insulin secretion and elevation of $[Ca^{2+}]_i$ levels (A) Telmisartan (10 μM) potentiated glucose-stimulated insulin secretion in the presence or absence of the PPARγ inhibitor GW9662 (10 μM) (n = 7). All insulin secretion results are normalized to basal secretion at 2.8 Mm glucose concentration. (B) The trace shows the changes of $[Ca^{2+}]_i$ concentration in β-cells treated with GW9662 (10 μM) alone or in combination with telmisartan (Tel 10 μM) under 16.7 mM glucose conditions. (C) The average value during 30s F340/F380 spikes for each test in response to GW9662 alone or in combination with telmisartan under 16.7 mM glucose conditions as indicated (n = 9). All results are reported as the means ± SEM. In (A), statistical differences between two

722   groups (with or without telmisartan) were compared using an unpaired two-tailed

723   Student's *t* test, and difference among three groups without telmisartan were compared

724   using one-way ANOVA and Student-Newman-Keuls method post hoc analysis. In (C),

725   difference among three groups was determined by one-way ANOVA and Tukey Test

726   post hoc analysis. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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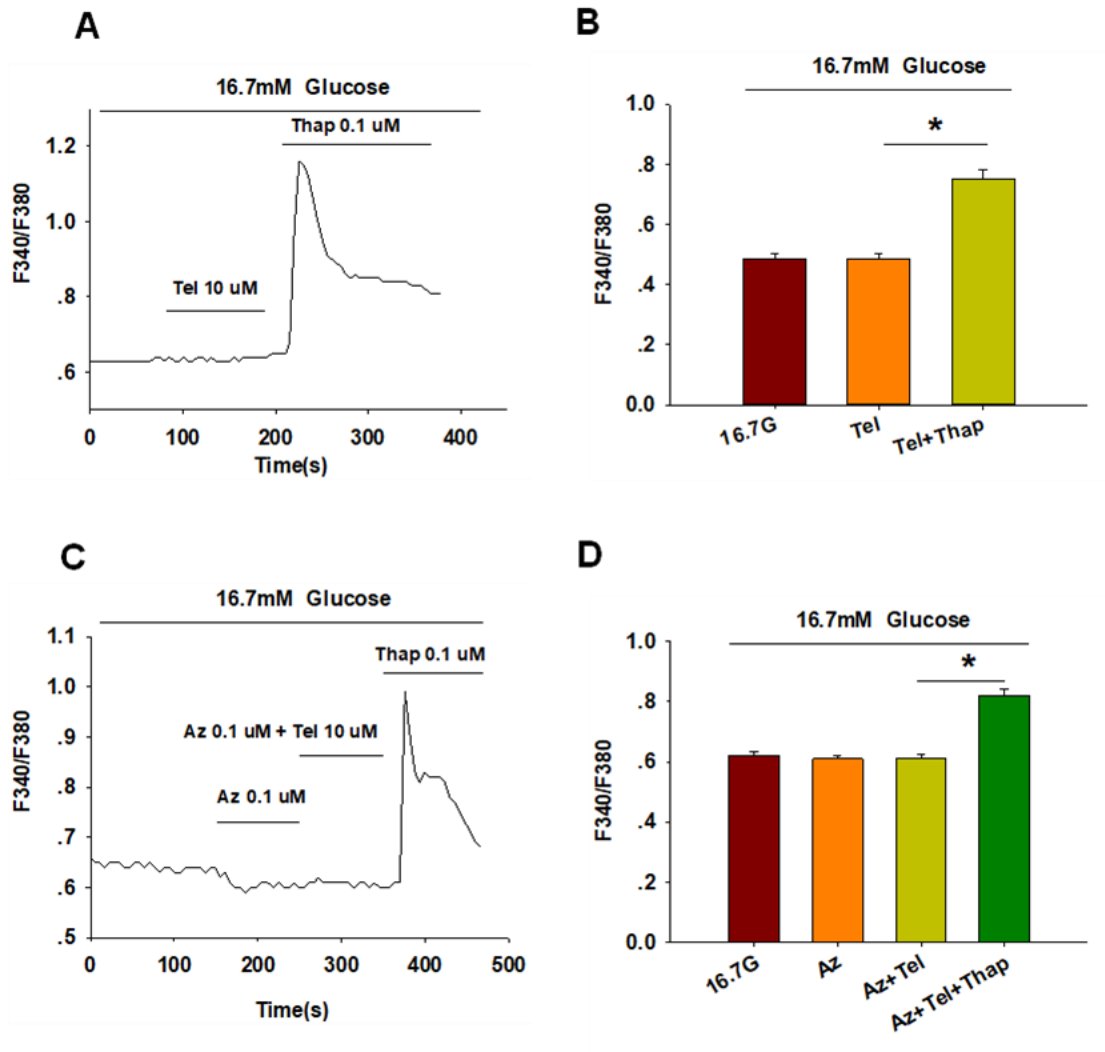
Fig. 4: Telmisartan enhances $[Ca^{2+}]_i$ levels through extracellular Ca^{2+} influx, rather than

intracellular Ca^{2+} stores release. (A) The trace shows the changes of $[Ca^{2+}]_i$

concentration in β-cells treated with telmisartan (Tel, 10 μM) under 16.7 mM glucose

conditions in Ca^{2+}-free KRBH medium. (B) The average value of F340/F380 during

each test in response to telmisartan (Tel, 10 μM) in Ca^{2+}-free KRB medium (n = 23).

(C) The trace shows the changes of $[Ca^{2+}]_i$ concentration in β-cells treated with

telmisartan (Tel 10 μM) under 16.7 mM glucose conditions with addition of the L-type

VGCC blocker azelnidipine (Az, 0.1 μM). (D)The mean value of F340/F380 during

each test in response to telmisartan (10 μM) with added azelnidipine (0.1 μM).

35

738   Thapsigargin (Thap,0.1 μM) was used as a positive control (n = 12). All results are

739   reported as the means ± SEM. Statistical differences among three or more groups were

740   determined by one-way ANOVA, followed by Tukey Test post hoc analysis. * $P < 0.05$.

741

A

Control

Telmisartan

500 pA

100 ms

B

742

C

743

744
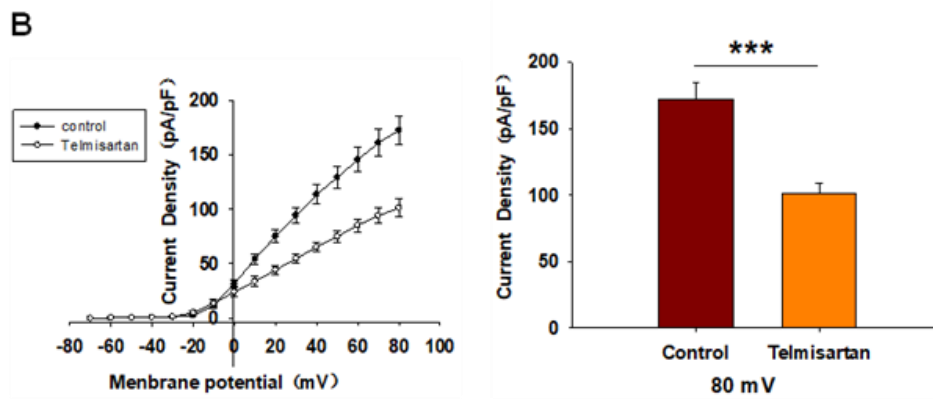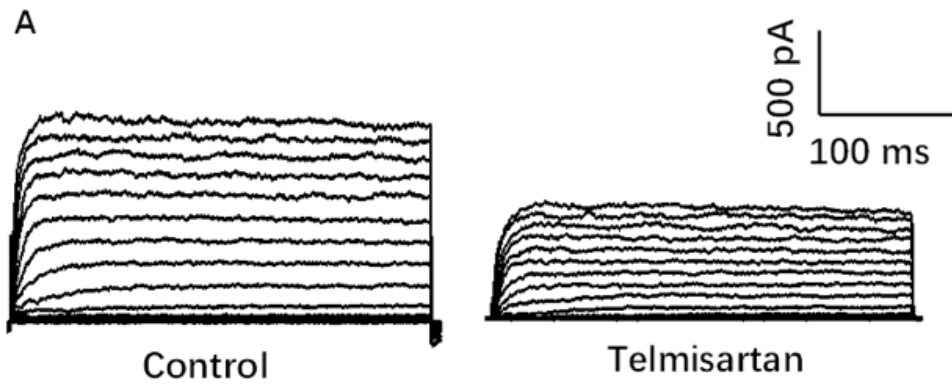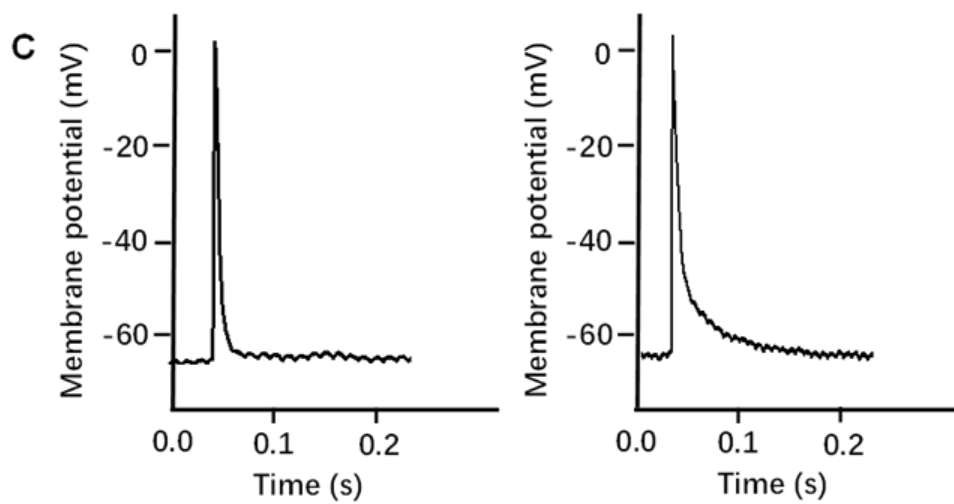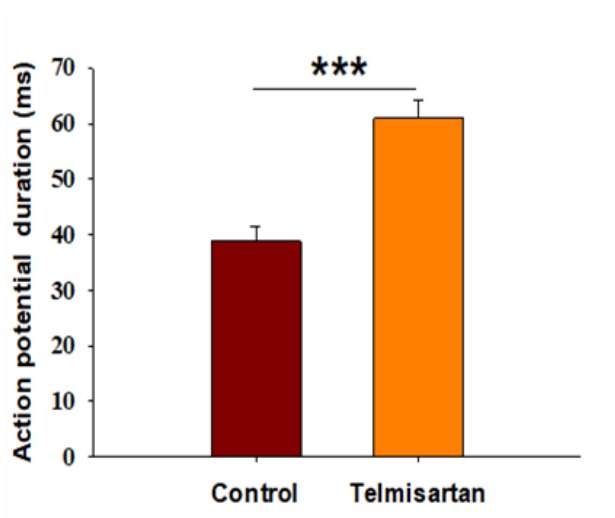
Fig. 5: Pancreatic β-cells treated with telmisartan exhibit reduced Kv currents and

extended APD. (A) Kv currents were recorded in voltage-clamp mode with holding

potential from −70 to +80 mV in 10 mV increments. Representative current traces

recorded in control and treated with telmisartan-treated (10 μM) β-cells. (B) Current-

voltage relationship curves of Kv channels and summary of the mean current density

of Kv channels recorded at 80 mV depolarization (control $n = 9$, telmisartan $n = 7$). (C)

Action potentials were elicited by 4 ms, 150 pA current. Representative action potential

waveforms for β-cells treated without or with telmisartan (10 μM) and summary of the

mean APDs ($n = 7$). Statistical differences between two groups were determined using

an unpaired two-tailed Student's *t* test. *** $P < 0.001$.

755

A

Control   Valsartan   Irbesartan

B

756

C

Control   GW9662

Telmisartan   GW9662+Telmisartan

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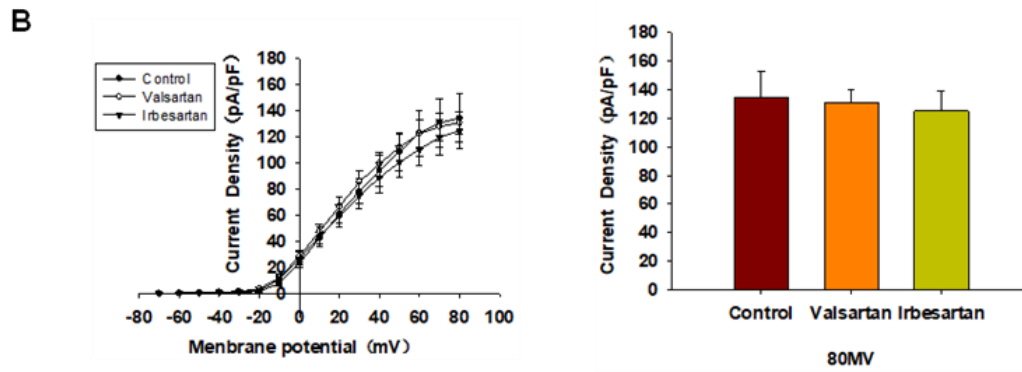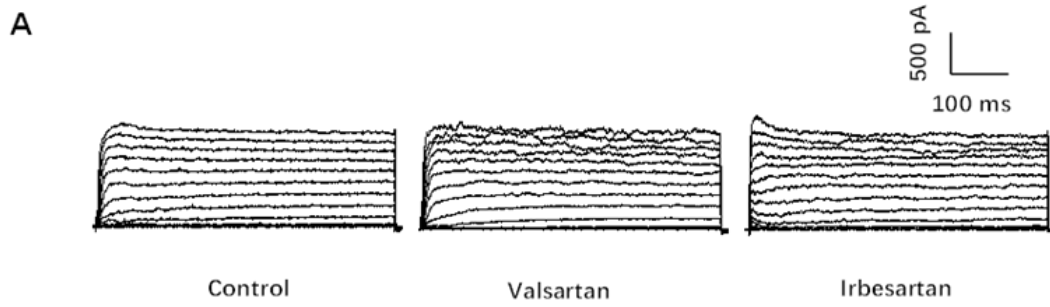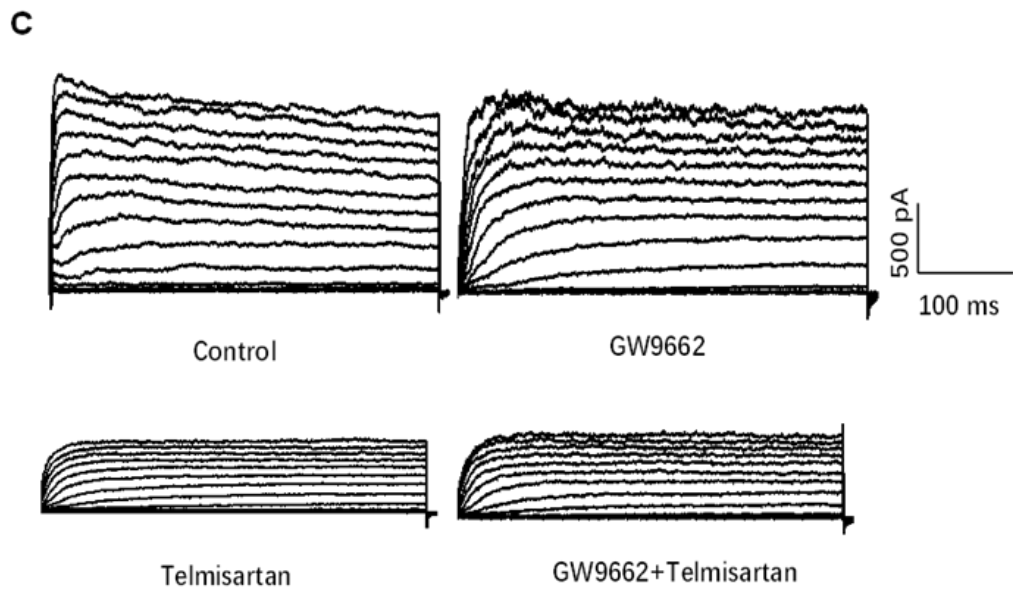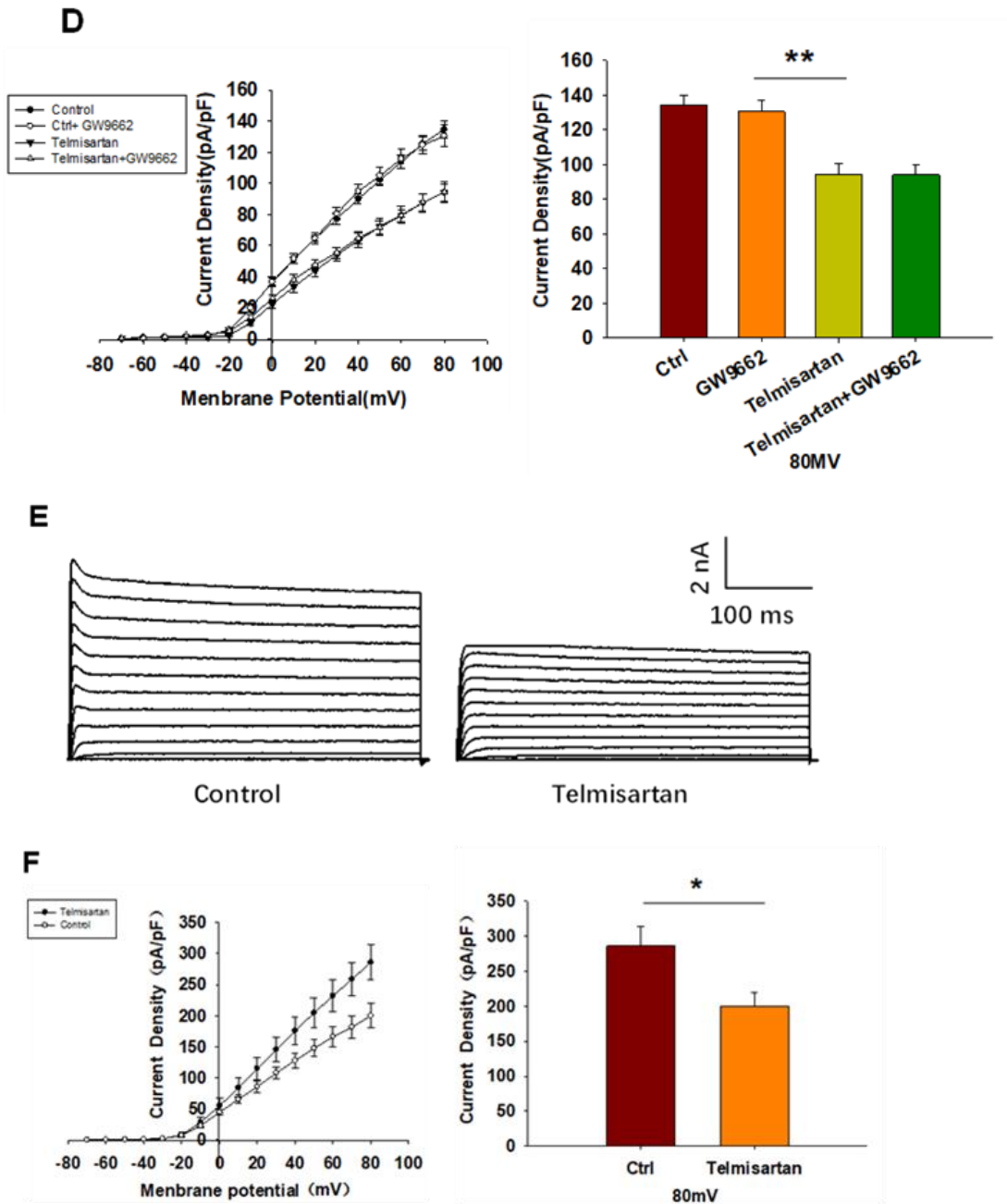
758

759

760 **Fig. 6:** The AT-1 receptor and PPARγ are not involved in the telmisartan-induced

761 inhibition of Kv channels, whereas telmisartan exerts a direct effect on Kv2.1 channels.

762 (A) Representative current traces recorded upon treatment with valsartan (10 μM) and

763 irbesartan (10 μM) in β-cells. (B) Current-voltage relationship curves and the summary

764 of the mean current density of Kv channels recorded at 80 mV depolarization (control

765 n = 7, valsartan n = 8, irbesartan n = 6). (C) Representative current traces recorded

766 under treatment of telmisartan (10 μM) alone or in combination with GW9662 (10 μM)

767 in β-cells. (D) Current-voltage relationship curves and the summary of the mean current

768   density of Kv channels recorded at 80 mV depolarization (control n = 8，GW9662 n =

769   12, telmisartan n = 7, telmisartan+GW9662 n = 10). (E) The CHO-Kv2.1 cell line was

770   constructed using a lentivirus vector overexpressing Kv2.1 channels. Representative

771   current traces recorded without or with telmisartan (10 μM) in CHO-Kv2.1 cells. (F)

772   Current-voltage relationship curves and the summary of the mean current density of Kv

773   channels recorded at 80 mV depolarization (control n = 10, telmisartan n = 8). All

774   results are reported as the means ± SEM. Statistical differences between two groups

775   were determined using an unpaired two-tailed Student's t test. Statistical differences

776   among three or more groups were compared using one-way ANOVA. For comparing

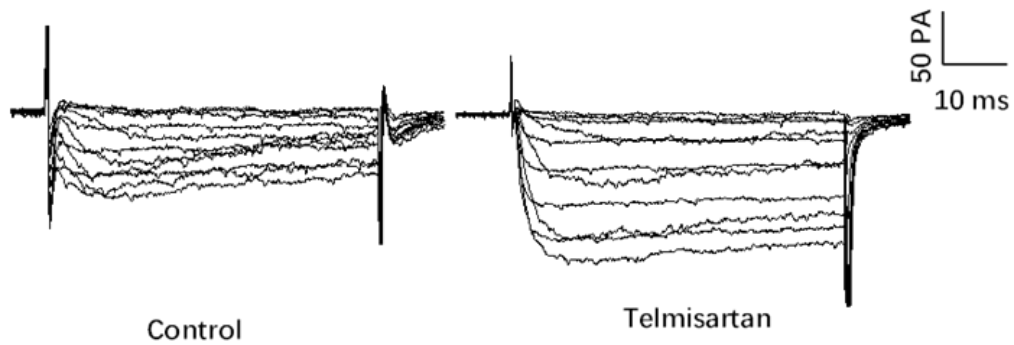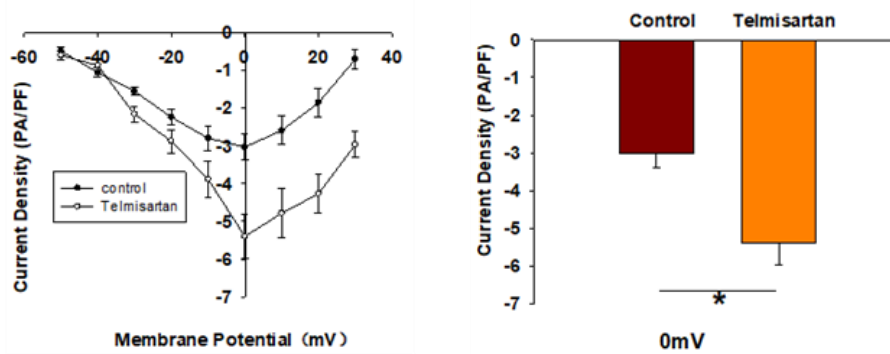777   the effects of GW9662 groups, Tukey Test post hoc analysis was applied. * P < 0.05,

778   ** P < 0.01.

779

A

B

**C**

780

D

Control
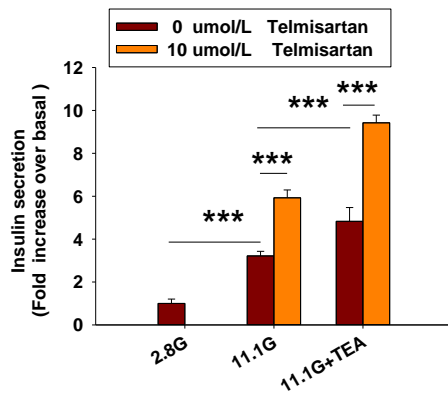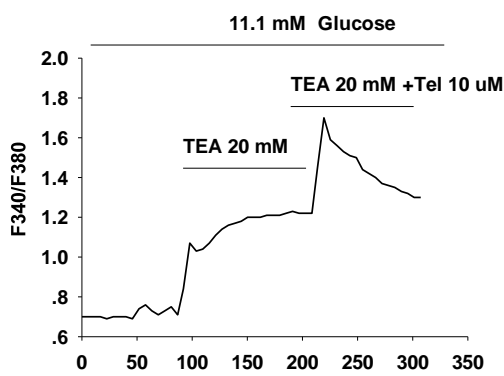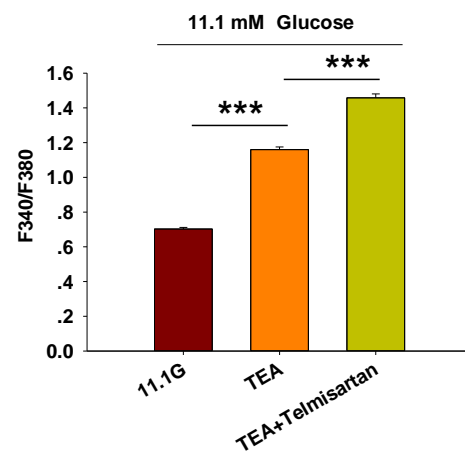
Telmisartan

E

781

782

783   **Fig. 7:** Kv channels partly mediate telmisartan-induced insulin secretion and increase

784   of $[Ca^{2+}]_i$ levels. (A) Rat islets were treated with telmisartan (10 μM) in the presence

785   or absence of TEA (20 mM) under 2.8 and 11.1 mM glucose conditions and insulin

786   secretion was measured (n = 7). All insulin secretion results are normalized to basal

787   secretion at 2.8 Mm glucose condition. (B) The trace shows the changes of ($[Ca^{2+}]_i$)

788   concentration in β-cells treated with 20 mM TEA and in combination with 10 μM

789   telmisartan (Tel) under 11.1 mM glucose conditions. (C) The average value during

790   30 s F340/F380 spikes for each test (n = 9). (D) VGCCs were recorded in voltage-clamp

791   mode with test potentials from −50 to 30 mV in 10 mV increments. Representative

792   current traces recorded in control and telmisartan-treated (10 μM) β-cells. (E) Current-

793   voltage relationship curves of VGCCs and summary of the mean Ca^{2+} current density

794   recorded at 0 mV depolarization (control, n = 7; telmisartan, n = 8). All results are

795   reported as the means ± SEM. Statistical differences between two groups were

796   determined using an unpaired two-tailed Student's *t* test. Statistical differences among

797   three groups were compared using one-way ANOVA and Student–Newman–Keuls

798   method post hoc analysis. Effects on VGCCs between telmisartan and control were

799   compared using the Mann–Whitney Rank Sum Test. * P < 0.05, and *** P < 0.001.

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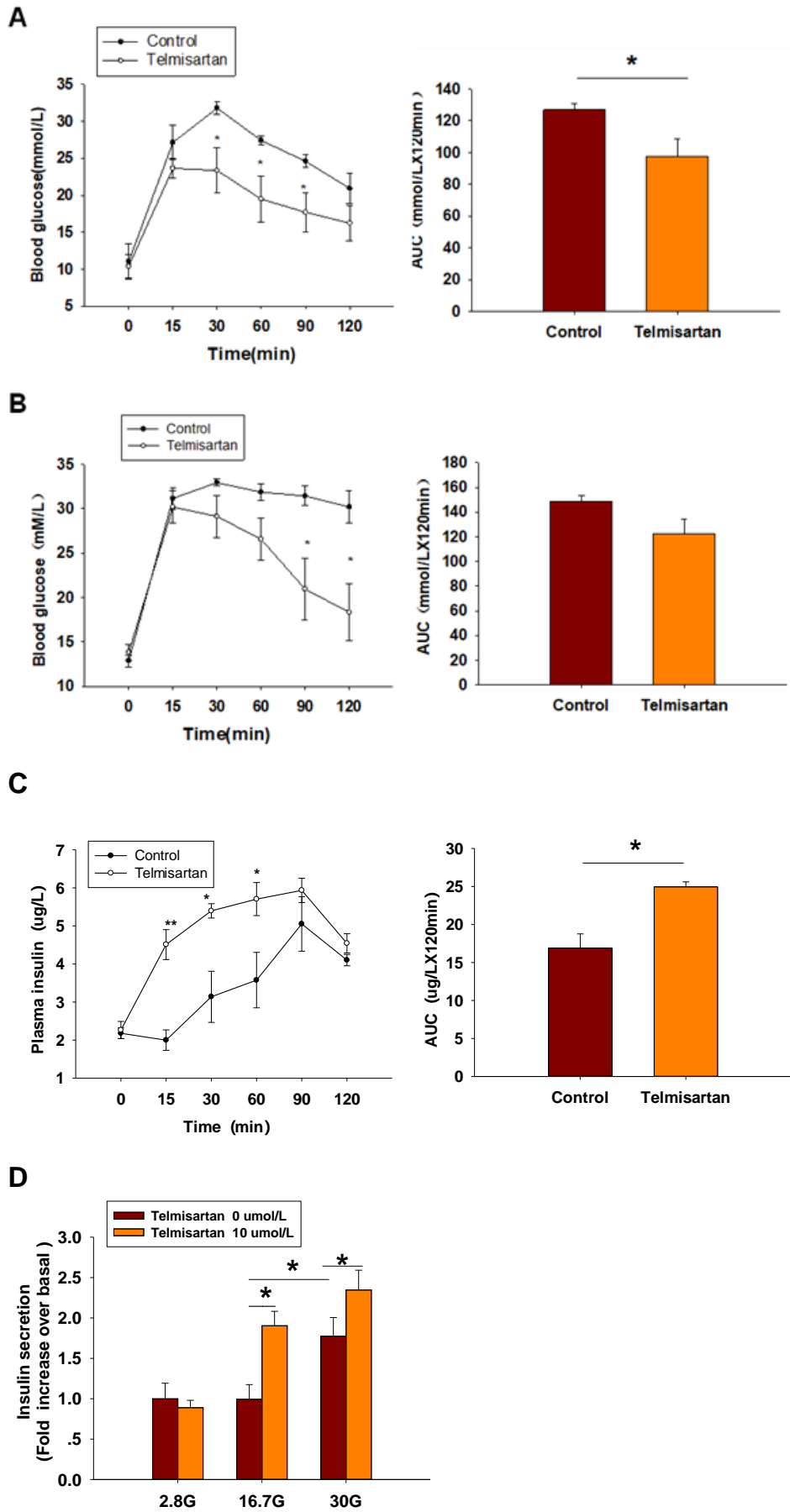
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803 **Fig. 8:** Telmisartan improves glucose tolerance in db/db mice, and elevates GSIS levels

804 in isolated islets of db/db mice. After 14 h fasting, db/db mice were administered with

805 telmisartan (15 mg/kg) or vehicle (0.5% carboxymethyl cellulose sodium) (n = 4 mice

806 per group). After 2 h, mice were fed with glucose (1.5 g/kg), and glucose level and

807 plasma insulin concentration in tail blood were determined. Finally, islets were isolated

808 from the db/db mice to perform insulin secretion assays. (A) OGTT was performed and

809 AUCs for OGTT were calculated from the data in 8-week-old mice. (B) OGTT and

810 AUC for OGTT in 11-week-old mice. (C) Serum insulin levels at corresponding times

811 and AUC in 11-week-old mice. (D) Db/db mice islets were treated with or without

812 telmisartan (10 μM) under different glucose concentrations (2.8, 16.7, and 30 mM) (n

813 = 6). All insulin secretion results are normalized to basal secretion at 2.8 Mm glucose

814 concentration. All results are reported as the means $\pm$ SEM. Statistical differences

815 between two groups were determined using the unpaired two-tailed Student's t test

816 unless otherwise stated. Glucose levels in 11-week-old mice at indicated time points

817 were compared using the Mann–Whitney Rank Sum Test except at 0 min. AUCs

818 calculated from the data of glucose levels or plasma insulin levels in 11-week-old mice

819 were compared using the Mann–Whitney Rank Sum Test. As for the insulin assay in

820 (D), statistical differences among three groups (without telmisartan) were compared

821 using one-way ANOVA and followed by Student–Newman–Keuls method post hoc

822 analysis, and differences between two groups under the same glucose conditions were

823 compared using the paired t test. *$P < 0.05$, **$P < 0.01$.
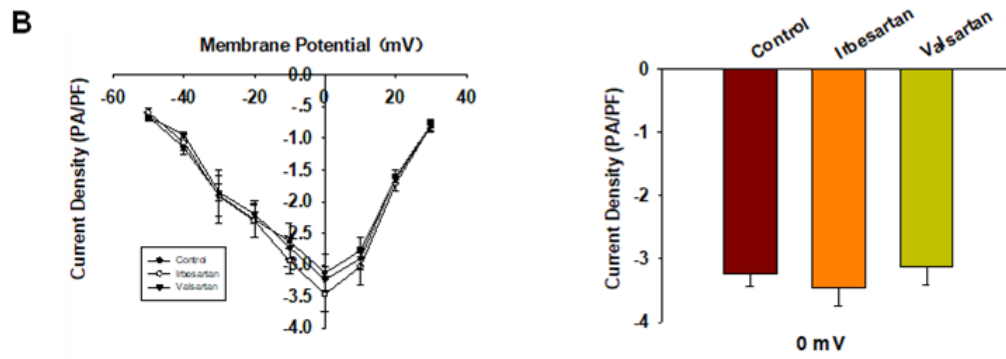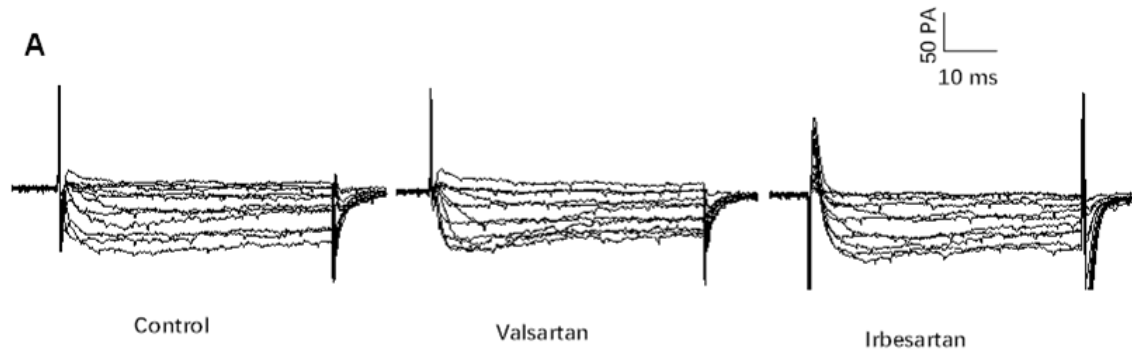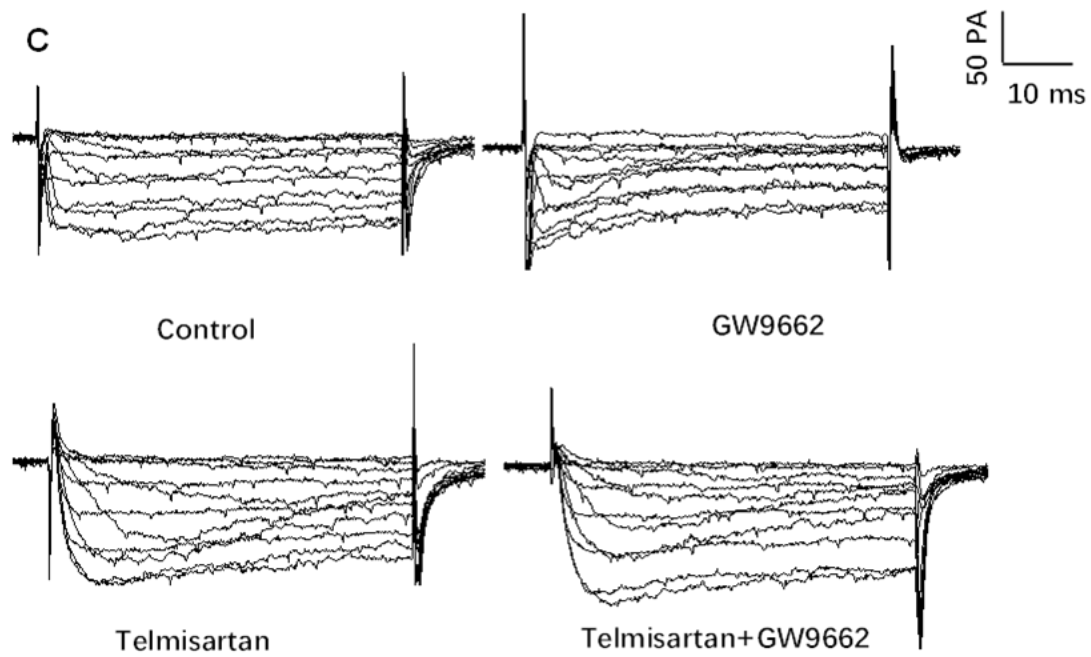
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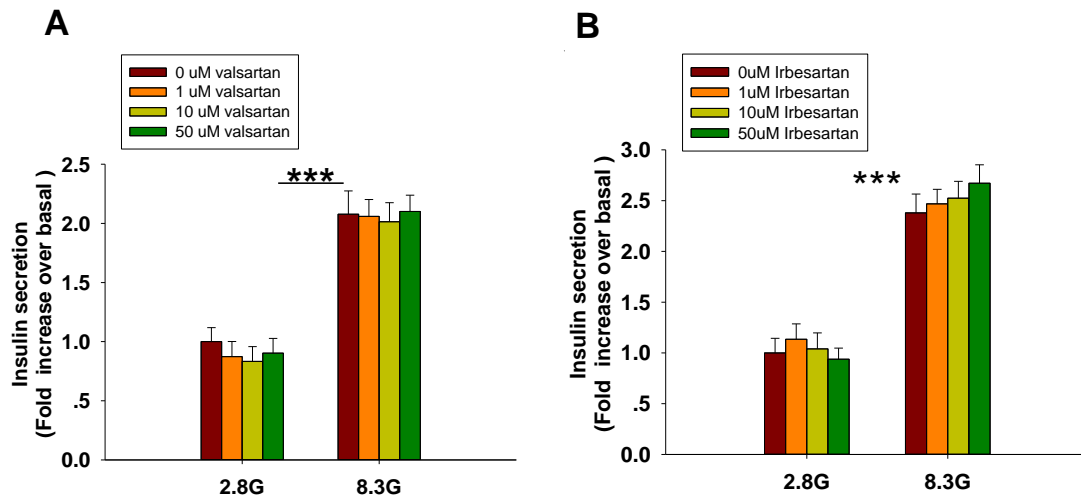
826

827 **Fig. S1:** Neither valsartan nor irbesartan potentiated insulin secretion in rat islets (n = 7 tubes

828 per group). (A) Rat islets were treated with various doses (1, 10, and 50 μM) of valsartan under

829 2.8 mM and 8.3 mM glucose (denoted as 2.8 G and 8.3 G) conditions. (B) Rat islets were

830 treated with various doses (1, 10, and 50 μM) of irbesartan under 2.8 mM and 8.3 mM glucose

831 conditions. All results are normalized to basal secretion at 2.8G, and reported as the means ±

832 SEM. Statistical differences among groups were compared using one-way analysis of variance

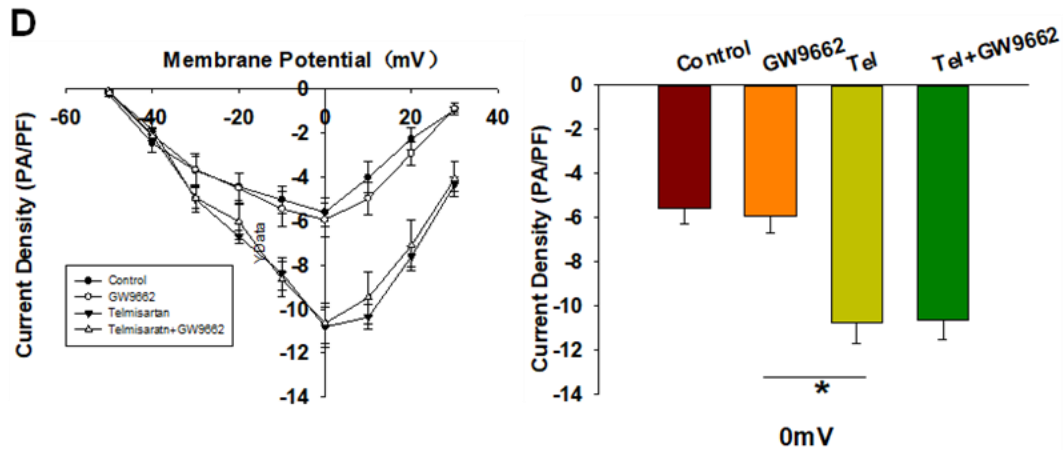833 (ANOVA) and Student–Newman–Keuls method post hoc analysis. *** $P < 0.001$.

834

A

50 PA

10 ms

Control   Valsartan   Irbesartan

B

835

C

50 PA

10 ms

Control   GW9662

Telmisartan   Telmisartan+GW9662

836

47

837

Fig. S2: The AT-1 receptor and PPARγ are not involved in telmisartan-induced activation of VGCCs. (A) Representative current traces recorded with treatment of valsartan (10 μM) and irbesartan (10 μM) in β-cells. (B) Current-voltage relationship curves and the summary of the mean Ca^{2+} current density recorded at 0 mV depolarization (n = 7). (C) Representative current trances recorded under treatment of telmisartan (10 μM) alone or in combination with GW9662 (10 μM) in β-cells. (D) Current-voltage relationship curves and the summary of the mean Ca^{2+} current density recorded at 0 mV depolarization (control, n = 10; GW9662, n = 6; telmisartan, n = 8; telmisartan+GW9662, n = 6). All results are reported as the means ± SEM. Statistical differences among three or more groups were compared using one-way ANOVA. For comparing the effects of GW9662 groups, Dunn's method post hoc analysis was applied.

*$P < 0.05$
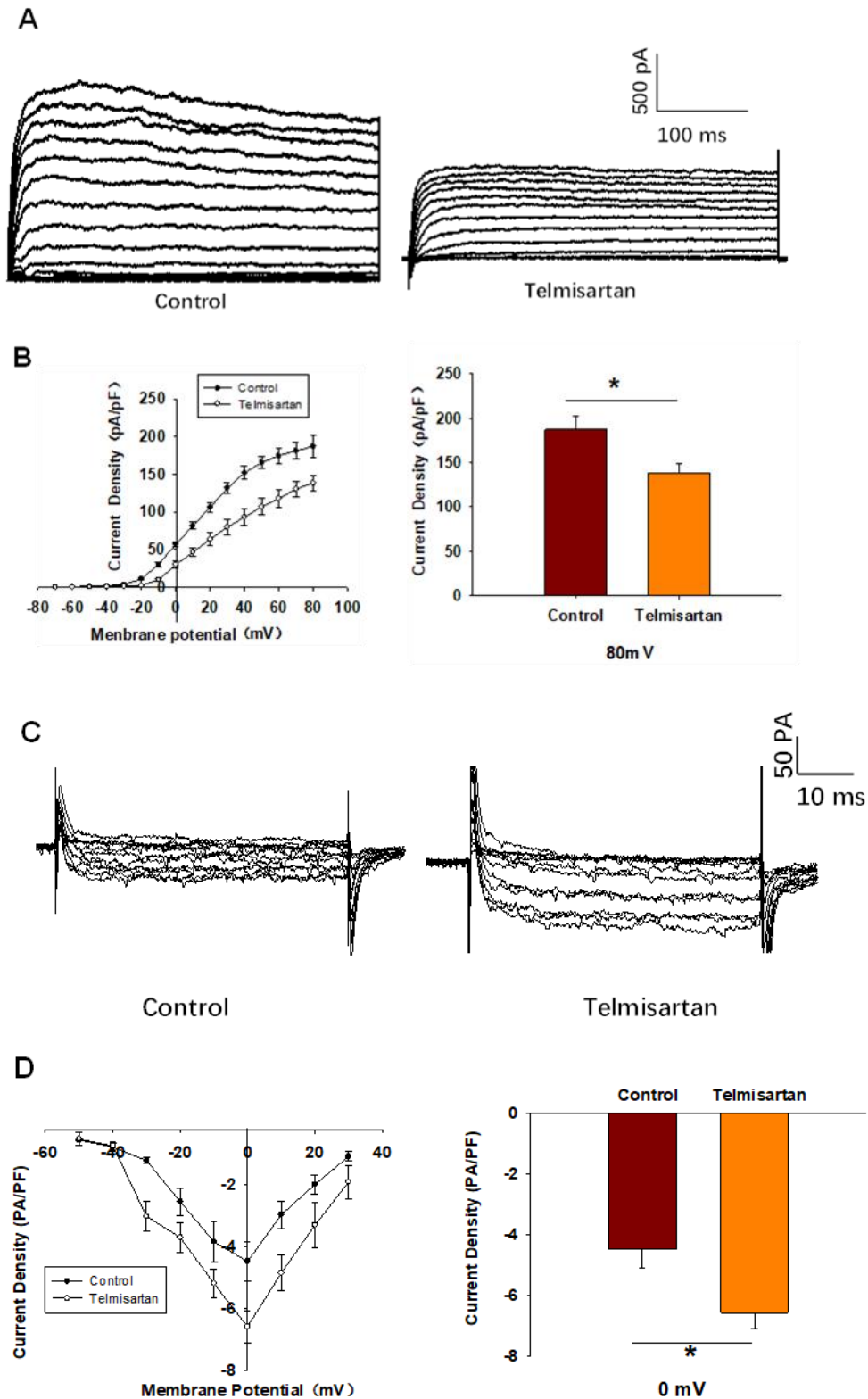
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852

853 **Fig. S3:** Telmisartan exerts similar electrophysiological effects on β-cells of db/db mice.

854 (A) Representative Kv channels current trances recorded with treatment of telmisartan

855 (10 μM) in β-cells. (B) Current-voltage relationship curves and the summary of the

49

856   mean current density of Kv channels recorded at 80 mV depolarization (n = 6). (C)

857   Representative Ca^{2+} current trances recorded with treatment of telmisartan (10 μM) in

858   β-cells. (D) Current-voltage relationship curves and the summary of the mean Ca^{2+}

859   current density recorded at 0 mV depolarization (control, n = 6; telmisartan, n = 7). All

860   results are reported as the means ± SEM. Statistical differences between two groups

861   were determined using an unpaired two-tailed Student's *t* test. *P< 0.05.

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