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Perfluoroalkyl Substances Stimulate Insulin Secretion by Islet β Cells via G Protein-Coupled Receptor 40

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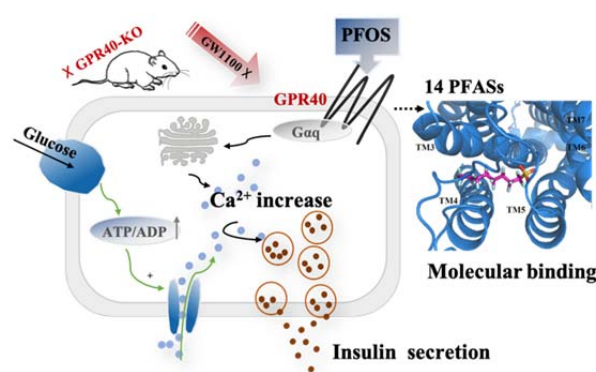
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ABSTRACT

The potential causal relationship between exposure to environmental contaminants and diabetes is troubling. Exposure of perfluoroalkyl substances (PFASs) is found to be associated with hyperinsulinemia and the enhancement of insulin secretion by islet β cells in humans, but the underlying mechanism is still unclear. Here, by combining *in vivo* studies with both wild type and gene knockout mice and *in vitro* studies with mouse islet β cells (β -TC-6), we demonstrated clearly that one-hour exposure of perfluorooctane sulfonate (PFOS) stimulated insulin secretion and intracellular calcium level by activating G protein-coupled receptor 40 (GPR40), a vital free fatty acid regulated membrane receptor on islet β cells. We further showed that the observed effects of PFASs on mouse model may also exist on human by investigating the molecular binding interaction of PFASs with human GPR40. We thus provided evidence for a novel mechanism for how insulin-secretion is disrupted by PFASs in human.

Key Words: perfluoroalkyl substances; GPR40; insulin secretion; intracellular calcium; islet β cells

42 **A graphic for the Table of Contents**



43

INTRODUCTION

Perfluoroalkyl substances (PFASs) are a class of persistent organic pollutants with great threat to environmental and human health. Perfluorooctane sulfonate (PFOS) is one of the most concerned members of PFASs. It was detected in the serum of the general public at concentrations around 0.05 $\mu\text{g/mL}$ (0.1 μM)¹, while the concentrations for occupationally exposed workers were recorded to exceed 10 $\mu\text{g/mL}$ (20 μM)². Although strict global regulation has restricted the production and usage of PFOS^{3,4}, human exposure to PFOS will persist for many years since the half-life of PFOS is around 5.4 years in the human body⁵. In addition, many other PFASs including perfluoroalkyl carboxylic acids have also been detected in various environmental media, wildlife and humans^{1,6,7}. Their levels are expected to increase over time since they are not yet listed in the Stockholm Convention.

Recently, the relationship between PFASs and diabetes has gained great concerns⁸⁻¹¹. Insulin level is a crucial indicator for diagnosis of diabetes. Many epidemiological investigations demonstrated significant positive associations between PFOS and perfluorooctanoic acid (PFOA) exposure with β cell function, insulin resistance, fasting proinsulin and insulin levels¹²⁻¹⁵. For example, Lin et al. found that, according to the data from NHANES 1999-2000 and 2003-2004, the increased serum PFOS level was accompanied with higher fasting insulin level and higher insulin secretion activity of β cells among U.S adolescents and adults¹³. More specific data indicated that an increase of serum PFOS concentration by 10 ng/mL was associated with 16% rise of serum insulin level and 12% increase of β cells insulin secretion

66 activity among overweight children in a subset of the European Youth Heart Study¹⁴.

67 Reasons for the abnormal serum insulin level may be complex, which may
68 include disruptive insulin secretion function of pancreas as well as the abnormal
69 insulin response function of hepatic or adipose tissue¹⁶. Previous toxicological studies
70 of PFASs on experimental animals mainly focused on the hepatic and adipose
71 tissues¹⁷⁻²³. However, metabolic regulations in these tissues are downstream
72 responding signal pathways of insulin. Pancreas is the only known organ to release
73 insulin²⁴, but few studies have been performed on this upstream regulation. PFOS has
74 been detected in the pancreatic tissue of human²⁵, indicating that PFOS can reach
75 human β cells. It is therefore reasonable to speculate that the insulin disorder
76 associated with PFASs exposure may be due to the dysfunction of β cells.
77 Unfortunately, up to now studies on the effects of PFASs on pancreas β cells are
78 limited to the adverse effects on pancreas organogenesis in mice and zebrafish,
79 without a clear mechanism²⁶⁻²⁸.

80 G protein-coupled receptor 40 (GPR40) - also known as the free fatty acid
81 receptor 1 (FFAR1) - is highly expressed on the membrane of islet β cells²⁹. GPR40
82 can be activated by medium- and long-chain free fatty acids (FFAs), leading to the
83 increase of intracellular calcium level and the secretion of insulin^{29,30}. Previous
84 studies demonstrated that PFASs could mimic the functions of FFAs due to their
85 structural similarity. PFASs were showed to bind with and activate peroxisome
86 proliferator-activated receptors (PPARs) and affect the cellular functions regulated by
87 these receptors^{31,32}. We therefore speculate that PFASs could also activate GPR40 in β

cells and stimulate insulin secretion. However, other studies have also demonstrated that structure-based predictions are not reliable. For example, PFASs are structurally very different from thyroid hormones (T₃, T₄) and estrogens (17 β -estradiol), and yet they have been shown to activate thyroid hormone and estrogen receptors³³⁻³⁶. Therefore, experimental verification for the predicted PFOS effects on GPR40 is vital.

In this study, we investigated and verified the role of GPR40 in the PFOS effects on insulin secretion activity of β cells by using a combined approach of *in vivo*, *in vitro* and *in silicon* experiments. We also investigated the interaction of PFOS with human GPR40 so as to provide support for our assumption that the mechanism revealed in the mouse model might also exist in humans. In addition, the binding interaction of 14 other PFASs with GPR40 was investigated to assess their potential effects on the insulin secretion activity of β cells via GPR40.

EXPERIMENTAL SECTION

Reagents. 15 PFASs (purity \geq 98%) used in this study are listed in the Table 1 and their full names and structures are shown in the Table S1. 3 PFASs (PFOS, PFHxDA and PFOcDA) were purchased from Alfa Aesar (USA). Other 12 PFASs (PFBA, PFBS, PFHxA, PFHxS, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTriDA, PFTeDA) were purchased from Sigma (USA). Lauric acid (LA, purity \geq 98%) and GW1100 (purity \geq 97%) were purchased from Sigma-Aldrich (USA). TAK-875 (purity \geq 99%) was purchased from Selleckchem (USA). All the chemicals were dissolved in DMSO and stocked in -20 °C.

Animal Treatments. C57BL/6 (6-7 weeks old) mice were purchased from Beijing Vital River Laboratory (China). GPR40 knock out (GPR40-KO) C57BL/6 mice were constructed by Beijing Biocytogen (China). After 12-hour fasting, mice were weighted by an electronic balance and administered 0, 0.5, 1, 5 and 10 mg PFOS per kilogram body weight by gavage^{21,37}. After one hour, mouse serum was collected for insulin detection by ultrasensitive insulin ELISA kit (Mercodia, Sweden). All the experimental operations were carried out according to the institutional guidelines for the care and use of laboratory animals. The details of operation process are shown in the supporting information.

Cell lines and culture condition. Mouse islet β cancer cells (β -TC-6) and human embryonic kidney 293 cells (HEK293) were purchased from Shanghai Cell Bank of Chinese Academy of Sciences. β -TC-6 cells were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 20% fetal bovine serum (FBS, HyClone, USA), 100 U/mL penicillin and 100 μ g/mL streptomycin (Invitrogen, USA). HEK293 cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 μ g/mL streptomycin. All the cells were cultured at 37°C in a humidified 5% CO₂ atmosphere. GPR40 protein is highly expressed in β -TC-6 cells, while undetectable in HEK293 cells (<https://www.proteinatlas.org>). β -TC-6 cells were used to verify the insulin-secretion disruption of PFOS on β cells and explore its mechanism underlying this biological effect. HEK293 was used to exogenously express human GPR40 protein by transient transfection, which then was applied for studying the binding effects of chemicals

with human GPR40 protein. HEK293 transfected with flag plasmid served as blank control.

Detection of Insulin Secretion Activity of β Cells. β -TC-6 cells (3×10^5 cells/mL) were seeded in 24-well plates and allowed to adhere for 48 hours. Cells were changed into Krebs-Ringer bicarbonate HEPES buffer (KRBH, Leagene, China) with or without 0.1% bovine serum albumin (BSA) for 30 min. Then, the supernatant was removed and replaced by new assay buffer containing different concentration of glucose (Sigma, USA) and tested chemicals in KRBH buffer (with or without 0.1% BSA) for 60 min³⁸. Next, the supernatant of each well was collected into a new tube separately; cells that adhered on the bottom of each well were lysed by RIPA (Solarbio, China) for 20 min and then the cell lysate of each well was collected into another tube. Finally, the insulin level of supernatant or cell lysate was detected separately by the rat/mouse insulin ELISA kit (Milipore, USA) after dilution into the detection range; the protein content of cell lysate was quantified by a BCA protein assay kit (Beyotime, China). The detail of operation process is shown in the supporting information.

Detection of the Intracellular Calcium Level of β -TC-6 Cells. Intracellular calcium was detected by using a fluorescence indicator Fura-2AM (Invitrogen, USA)³⁹. β -TC-6 cells were harvested and incubated with 5 μ M Fura-2 AM and 0.05% pluronic acid (Invitrogen, USA) in phenol-free DMEM at 37 °C for 30 min. Cells were washed twice and incubated for another 30 min in phenol-free DMEM. Cells were re-suspended in phenol-free DMEM at a density of 1×10^7 cells/mL. Then, 10 μ L

harvested cells were mixed uniformly in 90 μ L Hank's balanced salt solution buffer (HBSS, Invitrogen, USA). Next, the intracellular calcium signal was determined by a Horiba Fluoromax-4 Spectrofluorometer (Edison, USA) before and after exposure of tested chemicals. The detail of information is shown in the supporting information.

Human GPR40 Fluorescence Competitive Binding Assay. The binding affinities of PFASs with human GPR40 (NM_005303) were measured by using a fluorescence competitive binding assay according to our previous work⁴⁰. Briefly, HEK293 cells were transfected with GPR40 plasmid by using lipofectamine 3000 transfection reagent (Life Technologies, USA). After 48 hours, cells were harvested in phenol-free DMEM (1×10^6 cells/mL) and kept on ice. Then, 50 μ L harvested cells, 100 nM F-TAK-875A (a specific GPR40 fluorescence probe) and one of tested compounds (TAK-875, LA or PFASs) were mixed in phosphate buffered saline (PBS) buffer in a total volume of 500 μ L. After incubation for 2 min at 37°C, the fluorescence signal of cells in the fluorescein isothiocyanate (FITC) channel for each sample was detected by NovoCyte flow cytometer (ACEA Biosciences, USA). At least 10,000 cells were analyzed according to the forward scatter and side scatter. Percentage values of special binding region of ligands to GPR40 were fitted with a sigmoidal model (Origin Lab, USA) to calculate the half-inhibitory concentration (IC_{50}). The relative binding affinity (RBA) compared with LA of each PFASs was obtain by dividing the IC_{50} value with that of LA.

Molecular Docking Simulation. The binding interaction between PFASs and human GRP40 was simulated by Autodock Vina v1.1.2 (San Francisco, USA). A

crystal structure of human GPR40 (4PHU) was extracted from the RCSB Protein Data Bank (<http://www.rcsb.org/pdb>). The docking process was similar to our previous work⁴⁰. Rigid docking mode was used to simulate the binding pattern of tested compounds with human GPR40 at the TAK-875 binding pocket in crystal complex. For each complex, 10 independent docking runs were conducted, and the binding mode with the lowest binding energy was chosen for comparison.

Statistical Analysis. The results of experiment were analyzed by Microsoft Excel and SPSS software. Data were represented as means \pm standard deviation ($n \geq 3$). Differences between two treatment groups were evaluated by student's *t* test. The comparisons among more than two groups were evaluated by two-way analysis of variance (ANOVA). Results were considered as statistically significant if the *p* value < 0.05 .

RESULTS AND DISCUSSION

PFOS Stimulates Insulin Secretion of Male Mice via GPR40. We aimed to investigate whether PFOS stimulates insulin secretion by β cells *in vivo*. After administrating 10 mg/kg PFOS for one hour, the fasting serum insulin level of wild type (WT) male mice (0.9 ± 0.5 ng/mL, $n = 5$) was significantly ($p < 0.05$) higher than that of solvent control group (0.2 ± 0.1 ng/mL, $n = 5$). However, no difference was observed ($p > 0.05$) between the PFOS treated group and the control group among the female mice (Figure 1A). The sex-biased effect of PFOS on mice is likely due to the protective effect of female estrogen levels, which protects pancreatic β cells

against exogenous stimulation and injuries via interacting with estrogen receptors (ERs) and G-protein estrogen receptor (GPER)⁴¹⁻⁴³. Xing *et al.* reported that the concentration of PFOS in mouse serum was around 200 µg/mL after treatment with 10 mg/kg/d PFOS for 30 days³⁷, which was about twenty folds higher than that in human. In order to reveal the toxicological mechanism of tested compounds within a short-term exposure, we used 10 mg/kg PFOS for one-time gavage in our experiment, and this exposure dose showed a stimulated insulin secretion effect on male mice.

To examine whether GPR40 is involved in the PFOS induced effect, we also employed GPR40-KO mice in our study. As shown in Figure 1B, there was no significant difference ($p > 0.05$) in the fasting serum insulin level between the 10 mg/kg PFOS treated group (0.5 ± 0.2 ng/mL, $n = 6$) and the control group (0.4 ± 0.2 ng/mL, $n = 7$) for the male mice. No obvious difference ($p > 0.05$) was observed between PFOS treated group (0.6 ± 0.1 ng/mL, $n = 4$) and control group (0.4 ± 0.2 ng/mL, $n = 4$) among the female GPR40-KO mice (Figure 1B) neither. The above results suggested that short-term administration of PFOS stimulated insulin secretion by β cells in the male mice and that GPR40 might be involved in producing this effect.

PFOS Enhances Insulin Secretion Activity and Intracellular Calcium Level of β Cells via GPR40. To further investigate the effect of PFOS on the insulin secretion activity of β cells, we detected the insulin level of supernatant on the glucose-stimulated insulin secretion (GSIS) by using mouse β -TC-6 cells *in vitro*. In previous studies, KRBH buffer with BSA was used in the GSIS experiment^{29,44-46}.

BSA is a common protein used in cell related experiments. It can increase cell adhesion and prevent mechanical damage. In addition, BSA is also a good carrier which can bind small molecules such as water, salt and hormones to strengthen cell-to-cell communication⁴⁷. Therefore, in our GSIS experiment, KRBH buffer with 0.1% BSA was also used initially to keep in line with the previous studies. Our results showed that 1.4 mM glucose had the highest activity in the GSIS of β -TC-6 cells with 1.7-fold higher than the control group (Figure S7A). This result was consistent with a previous study showing that β -TC-6 cells were more sensitive to 1.4 mM glucose³⁸.

In the presence of 1.4 mM glucose, one-hour treatment of 50 μ M or 100 μ M PFOS enhanced the secretion of insulin by 2.8 or 3.7 folds respectively by comparison with the control group (Figure 2A, 1.4 mM glucose with 0.1% BSA). Since it has been previously demonstrated that BSA can bind with PFOS⁴⁸⁻⁵⁰, this binding effect might reduce the concentration of free PFOS. KRBH buffer without BSA was used when we studied the insulin-secretion disruption effects of PFASs. Our results showed that the enhancement was further amplified by removing BSA from the KRBH buffer in the GSIS experiment. Compared with the control, 50 μ M or 100 μ M PFOS enhanced the GSIS of 1.4 mM glucose by 8.3 or 10.6 folds respectively (Figure 2A, 1.4 mM glucose, without BSA). Moreover, the lowest observed effect concentration (LOEC) was lowered from 50 μ M to 5 μ M when BSA was removed (Figure 2A). We also detected the intracellular insulin content of β -TC-6 cells after the treatment of PFOS. The result of this experiment showed that the enhancement of GSIS by PFOS induction was unassociated with a change of intracellular insulin

content (Figure S7B).

The insulin secretion stimulation effect of PFOS was further demonstrated by determining the upstream signal – intracellular calcium⁵¹. The increase of intracellular calcium levels promotes the fusion of insulin-containing vesicles to plasma, resulting in the secretion of insulin⁵². Therefore, the change of intracellular calcium levels in β -TC-6 cells after PFOS exposure could be used to prove that PFOS disrupts insulin secretion function of β -TC-6 cells. As shown in Figure 2B, in the presence of 1.4 mM glucose, addition of PFOS triggered an increase of intracellular calcium level in β -TC-6 cells in a concentration-dependent manner with a LOEC of 5 μ M. Combining these above results, we demonstrated that PFOS enhanced the insulin secretion activity of β -TC-6 cells by triggering the increase of intracellular calcium level.

Insulin secretion of β cells is mainly regulated by the serum glucose level through the GSIS pathway⁵³. We also investigated the effects of different concentration of glucose on the enhancement of PFOS on β -TC-6 cells. As shown in Figure 2C, 50 μ M PFOS was sufficient to stimulate the insulin secretion by β -TC-6 cells in the absence of glucose. Compared with the effect induced by 50 μ M PFOS alone, co-exposure of 1.4 mM or 5.5 mM glucose with PFOS resulted in more insulin secretion by 2.1 or 1.9 folds respectively, while 11.1 mM glucose did not (Figure 2C). The intracellular calcium level induced by PFOS in the absence or present of glucose showed a correspondence with the insulin secretion activities of β -TC-6 cells (Figure 2D). Our results showed that PFOS activates the β -TC-6 cells in a glucose-sensitive manner.

In the present study, we aimed to reveal the possible mechanism for the PFOS induced insulin secretion effect of β cells. Two GPR40 agonists (TAK-875⁵⁴ and LA⁵⁵) and one GPR40 antagonist (GW1100⁵⁶) were used to investigate whether GPR40 is involved in the effect of insulin secretion stimulation activity of PFOS on β -TC-6 cells. TAK-875 and LA also enhanced the GSIS and trigger the intracellular calcium increase of β -TC-6 cells in a concentration-dependent manner (Figure S8). When the β -TC-6 cells were pre-incubated with 10 μ M GW1100, GSIS induced by TAK-875 or LA was significantly ($p < 0.05$) inhibited by 63% or 88% respectively. Similar to these two GPR40 agonists, the enhancement of GSIS induced by 50 μ M PFOS was also inhibited by 49% (Figure 2E). With the addition of 10 μ M GW1100, the intracellular calcium levels also declined when compared with the cells exposed to TAK-875, LA and PFOS only (Figure 2F). These results suggested that the interaction of PFOS with mouse GPR40 is a possible molecular initiating event for its effects on mouse β cells. So far, our work on mice and mouse-derived cell lines has firstly identified GPR40-mediated pathway as a likely mechanism of PFOS disruption on insulin secretion.

Studying the Interaction of PFOS with Human GPR40. The demonstrated interaction of PFOS with mouse GPR40 can in principle be the molecular event that initiates the observed effects of PFOS on mouse β cells. However, due to significant species differences, this mechanism cannot be extrapolated directly to humans to provide causal linkage between PFOS exposure and health effects. We therefore studied this likely interaction of PFOS with human GPR40 by quantitatively

determining its binding affinity with human GPR40 and computationally simulating its interaction with this receptor.

HEK293 cells originate from human without GPR40 expression. After transient transfection for 48 hours, HEK293 cells exogenously expressed human GPR40 protein, which was used to study the binding effects of PFOS with human GPR40. We employed a competitive binding assay previously established in our lab⁴⁰, which used a specific fluorescent probe F-TAK-875A designed on the basis of the crystal structure of human GPR40/TAK-875 complex⁵⁷. As the concentration of LA (used as a positive control) increased, more fluorescent probes were displaced from human GPR40, indicating the binding of LA to human GPR40 (Figure 3A). Using this assay, PFOS similarly displaced the probe from human GPR40 in a concentration-dependent manner (Figure 3A), suggesting that PFOS also binds with human GPR40. By comparing the IC₅₀ value of PFOS (4.4 μ M) with that of LA (7.4 μ M), we discovered that PFOS had 1.7-fold greater binding potency than LA.

The result of the molecular docking also supported our finding that PFOS binds with human GPR40. LA was docked into the human GPR40 with its carboxylic acid substituent targeting the inner part of the receptor and forming hydrogen bonds with arginine (Arg) 183, tyrosine (Tyr) 2240, while with its carbon chain being positioned towards the entrance of the binding pocket (Figure 3C). PFOS was docked into human GPR40 with the same binding mode as LA (Figure 3B), with its sulfonic acid substituent targeting the inner part of human GPR40 and forming hydrogen bonds with Arg 183, Tyr 2240, and Arg 2258, while with its carbon chain being positioned

towards the entrance of the binding pocket (Figure 3D). This is a sensible result since they have similar chemical structures. Some previous studies based on molecular docking also showed PFOS and LA had similar binding mode in the nuclear receptors of FFAs, for example PPARs^{31,32}. Based on the above results, we demonstrated that PFOS binds with human GPR40, which might be the initiating event for the insulin-secretion disruption effect of human β cells.

Previously, several epidemiological studies showed that the serum PFOS level was positive associated with hyperinsulinemia (higher fasting serum insulin level) in human¹¹⁻¹⁴. For occupationally exposed workers, serum PFOS concentrations were reported to be in the range of 1 μ M to 20 μ M². In the present study, we found that 5 μ M PFOS was sufficient to enhance the GSIS and increase the intracellular calcium level of β -TC-6 cells (Figure 2A-B). Moreover, based on the human GPR40 competitive binding assay, 5 μ M PFOS binds with human GPR40 (Figure 3A). These results suggested a possible risk of PFOS for some occupational workers with up to micromolar serum PFOS concentrations. Thus, our study provided a possible mechanism for this observed insulin-secretion disruption effect of PFOS in human, which is by interacting with the GPR40 on the human β cells.

Predicting the insulin-secretion disruption risks of other PFASs via the GPR40 pathway. Considering the structural similarity of other PFASs with PFOS, the effects induced by PFOS are likely shared with other PFASs^{31,32,58,59}. We therefore predicted the risk of insulin-secretion disruption caused by other 14 PFASs on β cells by studying their interactions with human GPR40 using competitive binding assay

and molecular docking.

As shown in Table 1, Figure 4A-B and Figure S9, among these tested PFASs, 6 compounds (PFHxS, PFOA, PFNA, PFDA, PFUnA and PFDoA) can also bind with human GPR40 with IC_{50} values ranging from 167.7 μ M to 0.7 μ M. Compared with LA (setting the binding affinity of LA with human GPR40 as 1), PFDA [with relative binding affinity (RBA) of 1.5], PFUnA (RBA=2.6) and PFDoA (RBA=10.6) had higher binding affinity with human GPR40. However, the IC_{50} values could not be obtained for the PFASs compounds with the alkyl chain length less than 6 carbons (Table 1). As the length of alkyl chain increases from 7 carbons to 11 carbons, the RBA increased from 0.1 to 10.6 (Table 1). The RBA of the compounds with chain length greater than 11 carbons did not exceed this maximum value (Table 1). In addition, we found that PFOS (RBA = 1.7) had much higher binding affinity than PFNA (RBA = 0.3), which have the same length of fluorinated alkyl chain but with different substituents. These results demonstrated that the binding affinity of the PFASs to GPR40 is dependent on the alkyl chain length and the terminal acid group of PFASs, which is similar to the dependence observed for the binding affinity of PFASs with other proteins, such as PPARs³², ERs³⁶, thyroid hormone receptor³³, transthyretin⁶⁰, and fatty acid binding protein⁵⁹.

The docking results also well explained the relationship between the binding affinity and the alkyl-chain length. 14 PFASs fitted within the ligand binding pocket of human GPR40 with similar binding geometry as LA (Figure 3B, Figure 4C-D and Figure S10). All of their sulfonic or carboxylic acid substituents were oriented

towards the inner part of GPR40 and formed hydrogen bonds with Arg 183, Tyr 2240, or Arg 2258 (Table 1). Their hydrophobic fluorinated alkyl chains were positioned towards the entrance of the binding pocket. However, their exact binding geometry was slightly different from each other. For the PFASs with chain length shorter than 11 carbons, they were enclosed within the binding core of the receptor (Figure 4C-D and Figure S10). As the chain length increased, hydrophobic interaction between PFASs and GPR40 also increased, leading to higher binding affinity. For the PFASs with chain lengths longer than 11 carbons, their molecular sizes were larger than the volume of the ligand binding pocket. The acid end group and neighboring fluorinated alkyl moiety of the long-chain PFASs occupied the binding core fully, while the remaining alkyl chain extended outside the binding pocket (Figure S10). This may destabilize the binding between PFASs and human GPR40. This dependency of binding affinity on carbon-chain length is in good accordance with our previous study on the binding affinity of 18 FFAs (C5- C23) with human GPR40⁴⁰. The above results obtained from the competitive binding assay and molecular docking demonstrated that some PFASs other than PFOS could also bind to human GPR40, suggesting the possibility of the disruption effect of these PFASs on the insulin secretion activity of β cells.

To evaluate the predictability of the above GPR40 binding results for the potential insulin-secretion disruption of PFASs, we then investigated their effects on GSIS of β cells. It is better to carry out this study by using human origin β cells, but this cell line is not available for us currently. β -TC-6 cell line that endogenously

expresses mouse GPR40 can be a good alternative. PFOA (the most commonly found PFAS in the environment) and PFDoA (having the greatest human GPR40 binding affinity according to our study) were tested. Our results show that both PFOA and PFDoA enhanced the insulin secretion by β -TC-6 cells (Figure 4E-F). In the presence of 1.4 mM glucose, the treatment of 50 μ M or 100 μ M PFOA enhanced the secretion of insulin by 1.5 or 1.6 folds respectively, and the treatment of 10 μ M or 20 μ M PFDoA enhanced the secretion of insulin by 1.4 or 1.6 folds respectively. Our results suggested that, in addition to PFOS, the potential disruption effect of other PFASs to the insulin secretion activity of β cells (possibly in humans) should not be ignored.

Notes

The authors declare no competing financial interest

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Detail information of detection of serum insulin levels of C57BL/6 mice,

396 detection of insulin secretion activity and intracellular calcium level of β -TC-6 cells.
397 Results of the active effects of glucose, PFOS, TAK-875 and lauric acid on β -TC-6
398 cells, results of competitive binding curves and molecular docking of 12 PFASs with
399 human GPR40.
400

Reference

1. Yeung, L. W., So, M. K., Jiang, G., Taniyasu, S., Yamashita, N., Song, M., Wu, Y., Li, J., Giesy, J. P., Guruge, K. S. & Lam, P. K. Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. *Environ. Sci. Technol.* **40**, 715 (2006).
2. Alexander, J., Auounsson, G. A. & Benford, D. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts scientific opinion of the panel on contaminants in the food chain. *Efsa Journal* **653**, 1-131 (2008).
3. Laura, H., Alastair, I. & Rachel, M. F. Litigating toxic risks ahead of regulation: biomonitoring science in the courtroom. *Stanf. Environ. Law. J.* **31**, 3 (2012).
4. Wang, T., Wang, Y., Liao, C., Cai, Y. & Jiang, G. Perspectives on the inclusion of perfluorooctane sulfonate into the Stockholm Convention on persistent organic pollutants. *Environ. Sci. Technol.* **15**, 5171–5175 (2009).
5. Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L. & Zobel, L. R. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **115**, 1298-1305 (2007).
6. Seo, S. H., Son, M. H., Shin, E. S., Choi, S. D. & Chang, Y. S. Matrix-specific distribution and compositional profiles of perfluoroalkyl substances (PFASs) in multimedia environments. *J. Hazard Mater.* **364**, 19-27 (2019).
7. Zafeiraki, E., Gebbink, W. A., Hoogenboom, R. L. A. P., Kotterman, M.,

- Kwadijk, C., Dassenakis, E. & van Leeuwen, S. P. J. Occurrence of perfluoroalkyl substances (PFASs) in a large number of wild and farmed aquatic animals collected in the Netherlands. *Chemosphere* **232**, 415-423 (2019).
8. MacNeil, J., Steenland, N. K., Shankar, A. & Ducatman, A. A cross-sectional analysis of type II diabetes in a community with exposure to perfluorooctanoic acid (PFOA). *Environ. Res.* **109**, 997-1003 (2009).
9. Taylor, K. W., Novak, R. F., Anderson, H. A., Birnbaum, L. S., Blystone, C., Devito, M., Jacobs, D., Köhrle, J., Lee, D. H., Rylander, L., Rignell-Hydbom, A., Tornero-Velez, R., Turyk, M. E., Boyles, A. L., Thayer, K. A. & Lind, L. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: A national toxicology program workshop review. *Environ. Health Perspect.* **121**, 774-783 (2013).
10. Lind, L., Zethelius, B., Salihovic, S., van Bavel, B. & Lind, P. M. Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. *Diabetologia.* **57**, 473-479 (2014).
11. Wang, H., Yang, J., Du, H., Xu, L., Liu, S., Yi, J., Qian, X., Chen, Y., Jiang, Q. & He, G. Perfluoroalkyl substances, glucose homeostasis, and gestational diabetes mellitus in Chinese pregnant women: A repeat measurement-based prospective study. *Environ. Int.* **114**, 12-20 (2018).
12. Jensen, R. C., Glinborg, D., Timmermann, C. A. G., Nielsen, F., Kyhl, H. B., Andersen, H. R., Grandjean, P., Jensen, T. K. & Andersen, M. Perfluoroalkyl substances and glycemic status in pregnant Danish women: the odense child

- 445 cohort. *Environ. Int.* **116**, 101-107 (2018).
- 446 13. Lin, C. Y., Chen, P. C., Lin, Y. C. & Lin, L. Y. Association Among Serum
447 Perfluoroalkyl Chemicals, Glucose Homeostasis, and Metabolic Syndrome in
448 Adolescents and Adults. *Diabetes Care* **32**, 702-707 (2008).
- 449 14. Timmermann, C. A., Rossing, L., Grøntved, A., Ried-Larsen, M., Dalgård, C.,
450 Andersen, L. B., Grandjean, P., Nielsen, F., Svendsen, K. D., Scheike, T. &
451 Jensen, T. K. Adiposity and glycemic control in children exposed to
452 perfluorinated compounds. *J. Clin. Endocrinol. Metab.* **99**, E608-614 (2014).
- 453 15. Fassler, C. S., Pinney, S. E., Xie, C., Biro, F. M. & Pinney, S. M. Complex
454 relationships between perfluorooctanoate, body mass index, insulin resistance and
455 serum lipids in young girls. *Environ. Res.* **176**, 108558 (2019).
- 456 16. Khan, S. & Kamal, M, A. Can Wogonin be used in controlling diabetic
457 cardiomyopathy. *Curr. Pharm. Des.* **25**, 2171-2177 (2019).
- 458 17. Fang, X., Gao, G., Xue, H., Zhang, X. & Wang, H. Exposure of
459 perfluorononanoic acid suppresses the hepatic insulin signal pathway and
460 increases serum glucose in rats. *Toxicology* **294**, 109-115 (2012).
- 461 18. Zheng, F., Sheng, N., Zhang, H., Yan, S., Zhang, J. & Wang, J. Perfluorooctanoic
462 acid exposure disturbs glucose metabolism in mouse liver. *Toxicol. Appl.*
463 *Pharmacol.* **335**, 41–48 (2007).
- 464 19. Hines, E. P., White, S. S., Stanko, J. P., Gibbs-Flournoy, E. A., Lau, C. & Fenton,
465 S. E. Phenotypic dichotomy following developmental exposure to
466 perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated

467 serum leptin and insulin, and overweight in mid-life☆. *Mol. and Cellular*
 468 *Endocrinol.* **304**, 97-105 (2009).

469 20. Lv, Z., Li, G., Li, Y., Ying, C., Chen, J., Chen, T., Wei, J., Lin, Y., Jiang, Y., Wang,
 470 Y., Shu, B., Xu, B. & Xu, S. Glucose and lipid homeostasis in adult rat is
 471 impaired by early-life exposure to perfluorooctane sulfonate. *Environ. Toxicol.*
 472 **28**, 532-542 (2013).

473 21. Yan, S., Zhang, H., Zheng, F., Sheng, N., Guo, X.. & Dai, J. Perfluorooctanoic
 474 acid exposure for 28 days affects glucose homeostasis and induces insulin
 475 hypersensitivity in mice. *Sci. Rep.* **5**, 11029 (2015).

476 22. Qiu, T., Chen, M., Sun, X., Cao, J., Feng, C., Li, D., Wu, W., Jiang, L. & Yao, X.
 477 Perfluorooctane sulfonate-induced insulin resistance is mediated by protein
 478 kinase B pathway. *Biochem. Biophys. Res. Commun.* **477**, 781-785 (2016).

479 23. Xu, J., Shimpi, P., Armstrong, L., Salter, D. & Slitt, A. L. PFOS induces
 480 adipogenesis and glucose uptake in association with activation of Nrf2 signaling
 481 pathway. *Toxicol. Appl. Pharmacol.* **290**, 21-30 (2016).

482 24. Tunaru, S., Bonnavion, R., Brandenburger, I., Preussner, J., Thomas, D.,
 483 Scholich, K. & Offermanns, S. 20-HETE promotes glucose-stimulated insulin
 484 secretion in an autocrine manner through FFAR1. *Nat. Commun.* **9**, 177 (2018).

485 25. Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Fabris, F., Danesino, P. &
 486 Imbriani, M. Determination of perfluorooctanoic acid and
 487 perfluorooctanesulfonate in human tissues by liquid chromatography/single
 488 quadrupole mass spectrometry. *Rapid. Commun. Mass Spectrom.* **20**, 2728–2734

- 489 (2006)
- 490 26. Bodin, J., Groeng, E. C., Andreassen, M., Dirven, H. & Nygaard, U. C. Exposure
491 to perfluoroundecanoic acid (PFUnDA) accelerates insulinitis development in a
492 mouse model of type 1 diabetes. *Toxicol. Rep.* **3**, 664-672 (2016).
- 493 27. Sant, K. E., Jacobs, H. M., Borofski, K. A., Moss, J. B. & Timme-Laragy, A. R.
494 Embryonic exposures to perfluorooctanesulfonic acid (PFOS) disrupt pancreatic
495 organogenesis in the zebrafish, *Danio rerio*. *Environ. Pollut.* **220**, 807-817 (2017).
- 496 28. Sant, K. E., Venezia, O. L., Sinno, P. P. & Timme-Laragy, A. R.
497 Perfluorobutanesulfonic acid disrupts pancreatic organogenesis and regulation of
498 lipid metabolism in the zebrafish, *Danio rerio*. *Toxicol. Sci.* **167**, 258-268 (2019).
- 499 29. Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi,
500 K., Hosoya, M., Tanaka, Y., Uejima, H., Tanaka, H., Maruyama, M., Satoh, R.,
501 Okubo, S., Kizawa, H., Komatsu, H., Matsumura, F., Noguchi, Y., Shinohara, T.,
502 Hinuma, S., Fujisawa, Y. & Fujino, M. Free fatty acids regulate insulin secretion
503 from pancreatic β cells through GPR40. *Nature* **422**, 173-6 (2003).
- 504 30. Rorsman, P. & Braun, M. Regulation of insulin secretion in human pancreatic
505 islets. *Annu. Rev. Physiol.* **75**, 155-179 (2013).
- 506 31. Li, C. H., Ren, X. M., Cao, L. Y., Qin, W. P. & Guo, L. H. Investigation of
507 binding and activity of perfluoroalkyl substances to the human peroxisome
508 proliferator-activated receptor beta/delta. *Environ. Sci. Process. Impacts.*
509 **21**, 1908-1914 (2019).
- 510 32. Zhang, L. Y., Ren, X. M., Wan, B. & Guo, L. H. Structure-dependent binding and

- 511 activation of perfluorinated compounds on human peroxisome
512 proliferator-activated receptor gamma. *Toxicol. Appl. Pharmacol.* **279**, 275-283
513 (2014).
- 514 33. Ren, X. M., Zhang, Y. F., Guo, L. H., Qin, Z. F., Lv, Q. Y. & Zhang, L. Y.
515 Structure–activity relations in binding of perfluoroalkyl compounds to human
516 thyroid hormone T3 receptor. *Arch. Toxicol.* **89**, 233-42 (2014).
- 517 34. Ren, X. M., Qin, W. P., Cao, L. Y., Zhang, J., Yang, Y., Wan, B. & Guo, L.
518 H.Binding interactions of perfluoroalkyl substances with thyroid hormone
519 transport proteins and potential toxicological implications. *Toxicology* **366-367**,
520 32-42 (2016).
- 521 35. Gao, Y., Li, X. & Guo, L. H. Assessment of Estrogenic Activity of Perfluoroalkyl
522 Acids Based on Ligand-induced Conformation State of Human Estrogen
523 Receptor. *Environ. Sci. & Technol.* **47**, 634-641 (2013).
- 524 36. Benninghoff, A. D., Bisson, W. H., Koch, D. C., Ehresman, D. J., Kolluri, S. K. &
525 Williams, D. E. Estrogen-like activity of perfluoroalkyl acids in vivo and
526 interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol. Sci.*
527 **120**, 42-58 (2011).
- 528 37. Xing, J. L., Wang, G., Zhao, J. C., Wang, E. Y., Yin, B. X. Fang, D. S., Zhao, J.
529 X., Zhang, H., Chen, Y. Q. & Chen, W. Toxicity assessment of perfluorooctane
530 sulfonate using acute and subchronic male C57BL/6J mouse models. *Environ.*
531 *Pollution* **210**, 388e396 (2016).
- 532 38. Niu, B., Su, H., Xia, X. S., He, Q., Xue, Y. M. & Yan, X. M. The role of

interleukin-1 β and extracellular signal-regulated kinase 1/2 in
glucose-stimulated insulin secretion. *Kaohsiung J. Med. Sci.* **33**, 224-228, (2017).

39. Cao, L. Y., Ren, X. M., Li, C. H., Zhang, J., Qin, W. P., Yang, Y., Wan, B. & Guo,
L. H. Bisphenol AF and Bisphenol B Exert Higher Estrogenic Effects than
Bisphenol A via G Protein-Coupled Estrogen Receptor Pathway. *Environ. Sci.*
Technol. **51**, 11423-11430 (2017).

40. Ren, X. M., Cao, L. Y., Zhang, J., Qin, W. P., Yang, Y., Wan, B. & Guo, L. H.
Investigation of the binding interaction of fatty acids with human G
protein-coupled receptor 40 using a site-specific fluorescence probe by flow
cytometry. *Biochemistry* **55**, 1989-1996 (2016).

41. Le-May, C., Chu, K., Hu, M., Ortega, C. S., Simpson, E. R., Korach, K. S., Tsai,
M. J. & Mauvais-Jarvis, F. Estrogens protect pancreatic beta-cells from apoptosis
and prevent insulin-deficient diabetes mellitus in mice. *Proc. Natl. Acad. Sci.*
USA **103**, 9232-9237 (2006).

42. Liu, S., Le-May, C., Wong, W. P., Ward, R. D., Clegg, D. J., Marcelli, M.,
Korach, K. S. & Mauvais-Jarvis, F. Importance of extranuclear estrogen
receptor- α and membrane G protein-coupled estrogen receptor in pancreatic
islet survival. *Diabetes* **58**, 2292-2302 (2009).

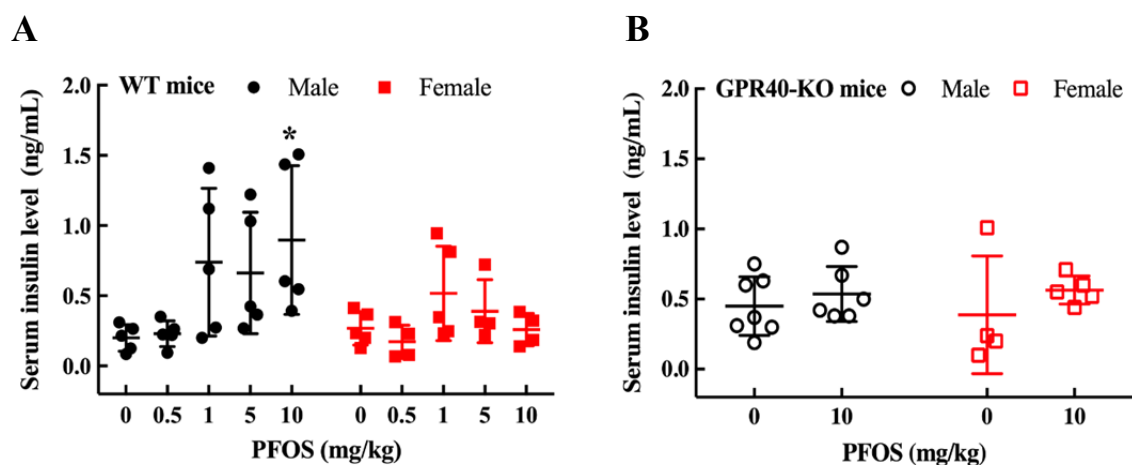
43. Balhuizen, A., Kumar, R., Amisten, S., Lundquist, I. & Salehi, A. Activation of G
protein-coupled receptor 30 modulates hormone secretion and counteracts
cytokine-induced apoptosis in pancreatic islets of female mice. *Mol. Cell.*
Endocrinol. **320**, 16-24 (2010).

- 555 44. Cho, J. H., Lee, K. M., Lee, Y. I., Nam, H. G. & Jeon, W. B. Glutamate
556 decarboxylase 67 contributes to compensatory insulin secretion in aged
557 pancreatic islets. *Islets* **11**, 33-43 (2019).
- 558 45. Minami, K., Yano, H., Miki, T., Nagashima, K., Wang, C. Z., Tanaka, H.,
559 Miyazaki, J. I. & Seino, S. Insulin secretion and differential gene expression in
560 glucose-responsive and -unresponsive MIN6 sublines. *Am. J. Physiol.*
561 *Endocrinol. Metab.* **279**, E773–E781 (2000).
- 562 46. Ishihara, H., Asano, T., Tsukuda, K., Katagiri, H., Inukai, K., Anai, M., Kikuchi,
563 M., Yazaki, Y., Miyazaki, J. I. & Oka, Y. Pancreatic beta cell line MIN6 exhibits
564 characteristics of glucose metabolism and glucose-stimulated insulin secretion
565 similar to those of normal islets. *Diabetologia* **36**, 1139-1145 (1993).
- 566 47. Klimek, K., Benko, A., Pałka, K., Ludwiczuk, A. & Ginalska, G. Ion-exchanging
567 dialysis as an effective method for protein entrapment in curdlan hydrogel. *Mater.*
568 *Sci. Eng. C Mater. Biol. Appl.* **105**, 110025 (2019).
- 569 48. Li, L., Song, G. W. & Xu, Z. S. Study on the interaction between bovine serum
570 albumin and potassium perfluorooctane sulfonate. *J. Dispers. Sci. Technol.* **31**,
571 1547-1551 (2010).
- 572 49. Chen, H., He, P., Rao, H., Wang, F., Liu, H. & Yao, J. Systematic investigation of
573 the toxic mechanism of PFOA and PFOS on bovine serum albumin by
574 spectroscopic and molecular modeling. *Chemosphere* **129**, 217-224 (2015).
- 575 50. Wang, Y., Zhang, H., Kang, Y. & Cao, J. Effects of perfluorooctane sulfonate on
576 the conformation and activity of bovine serum albumin. *J. Photochem. Photobiol.*

- 577 *B.* **159**, 66-73 (2016).
- 578 51. Ashcroft, F. M., Proks, P., Smith, P. A., Ammala, C., Bokvist, K. & Rorsman, P.
579 Stimulus-secretion coupling in pancreatic beta cells. *J. Cell. Biochem.* **55**, 54-65
580 (1994).
- 581 52. Ghislain, J. & Poitout, V. The Role and Future of FFA1 as a Therapeutic Target.
582 *Handb. Exp. Pharmacol.* **236**, 159-180 (2016).
- 583 53. Fujimoto, S., Mizuno, R., Saito, Y. & Nakamura, S. Clinical application of wave
584 intensity for the treatment of essential hypertension. *Heart Vessels.* **19**, 19-22
585 (2004).
- 586 54. Tsujihata, Y., Ito, R., Suzuki, M., Harada, A., Negoro, N., Yasuma, T., Momose,
587 Y. & Takeuchi, K. TAK-875, an orally available G protein-coupled receptor
588 40/free fatty acid receptor 1 agonist, enhances glucose-dependent insulin
589 secretion and improves both postprandial and fasting hyperglycemia in type 2
590 diabetic rats. *J. Pharmacol. Exp. Ther.* **339**, 228-237 (2011).
- 591 55. Hu, H., He, L. Y., Gong, Z., Li, N., Lu, Y. N., Zhai, Q. W., Liu, H., Jiang, H. L.,
592 Zhu, W. L. & Wang, H. Y. A novel class of antagonists for the FFAs receptor
593 GPR40. *Biochem. Biophys. Res. Commun.* **390**, 557–563 (2009).
- 594 56. Briscoe, C. P., Peat, A. J., McKeown, S. C., Corbett, D. F., Goetz, A. S., Littleton,
595 T. R., McCoy, D. C., Kenakin, T. P., Andrews, J. L., Ammala, C., Fornwald, J. A.,
596 Ignar, D. M & Jenkinson, S. Pharmacological regulation of insulin secretion in
597 MIN6 cells through the fatty acid receptor GPR40: identification of agonist and
598 antagonist small molecules. *Br. J. Pharmacol.* **148**, 619-628 (2006).

57. Srivastava, A., Yano, J., Hirozane, Y., Kefala, G., Gruswitz, F., Snell, G., Lane, W., Ivetac, A., Aertgeerts, K., Nguyen, J., Jennings, A. & Okada, K. High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. *Nature* **513**, 124-127 (2014).
58. Zhang, L. Y., Zhu, X. J. & Xu, J. X. Binding and activation of G protein-coupled receptor 40 by perfluorodecanoic acid. *J. Environ. Appl. Biores.* **05**, 16-19 (2017).
59. Zhang, L. Y., Ren, X. M. & Guo, L. H. Structure-based investigation on the interaction of perfluorinated compounds with human liver fatty acid binding protein. *Environ. Sci. Technol.* **47**, 11293-11301 (2013).
60. Weiss, J. M., Andersson, P. L., Lamoree, M. H., Leonards, P. E., van Leeuwen, S. P. & Hamers, T. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicol. Sci.* **109**, 206-216 (2009).

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616 **Figure 1** Effects of PFOS on the fasting insulin level of wild type (WT) and GPR40
 617 knock out (GPR40-KO) mice. Fasting serum levels of WT mice (**A**) and GPR40-KO
 618 mice (**B**) after administration of PFOS for one hour. * means $p < 0.05$, compared with
 619 solvent control of each group (0 mg/kg, 0.5% methyl cellulose and 0.5% dimethyl
 620 sulfoxide).

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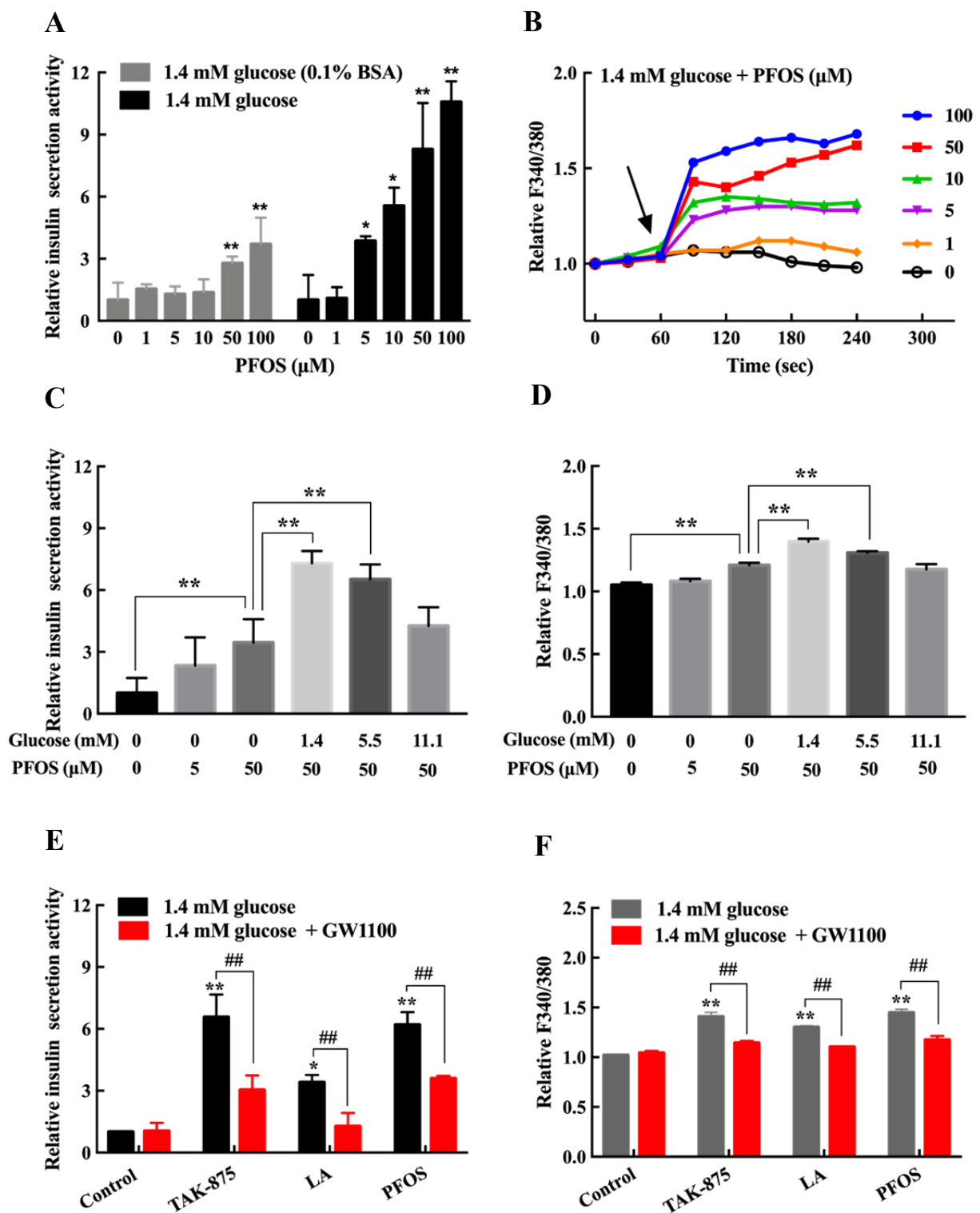
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631 **Figure 2** Effects of PFOS on the insulin secretion activity (A, C, E) and intracellular
 632 calcium level (B, D, F) of β -TC-6 cells and the inhibitory effects of GW1100. (A)
 633 Effects of different concentration of PFOS on the insulin secretion activity of β -TC-6

634 cells in presence of 1.4 mM glucose, with or without 0.1% BSA in the KRBH buffer.

635 **(B)** Effects of different concentration of PFOS on intracellular calcium level of

636 β -TC-6 cells in the presence of 1.4 mM glucose. **(C, D)** Effects of 5 μ M and 50 μ M

637 PFOS on the insulin secretion activity and intracellular calcium level of β -TC-6 cells

638 in the absence of glucose, and the effects of 50 μ M PFOS on the insulin secretion

639 activity and intracellular calcium level of β -TC-6 cells in the presence of different

640 concentration of glucose. **(E, F)** Effects of 10 μ M TAK-875, 250 μ M lauric acid (LA)

641 and 50 μ M PFOS on the insulin secretion activity and intracellular calcium level of

642 β -TC-6 cells in the presence of 1.4 mM glucose, and the inhibitory effects of 10 μ M

643 GW1100 on them. * means $p < 0.05$ and ** means $p < 0.01$, compared with the

644 corresponding control group, ## means $p < 0.01$ compared with the groups not

645 pre-treated with GW1100.

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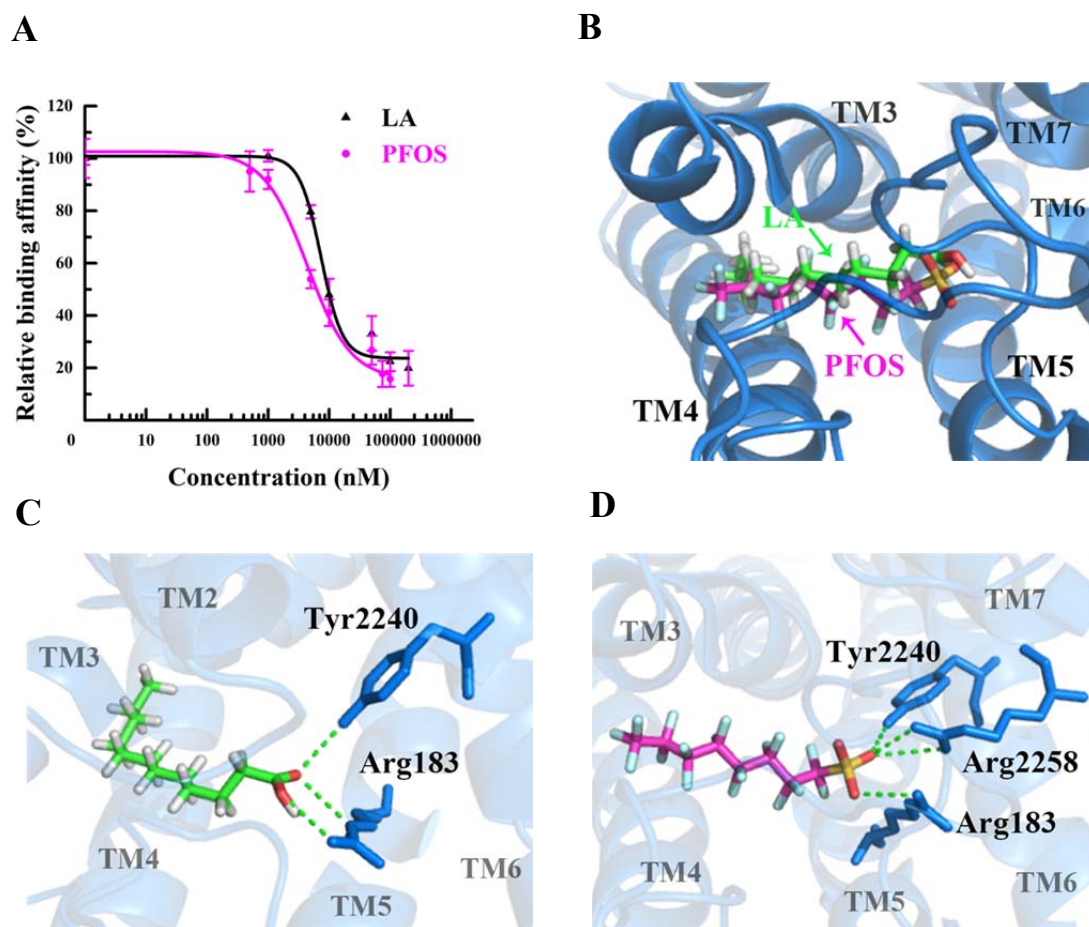
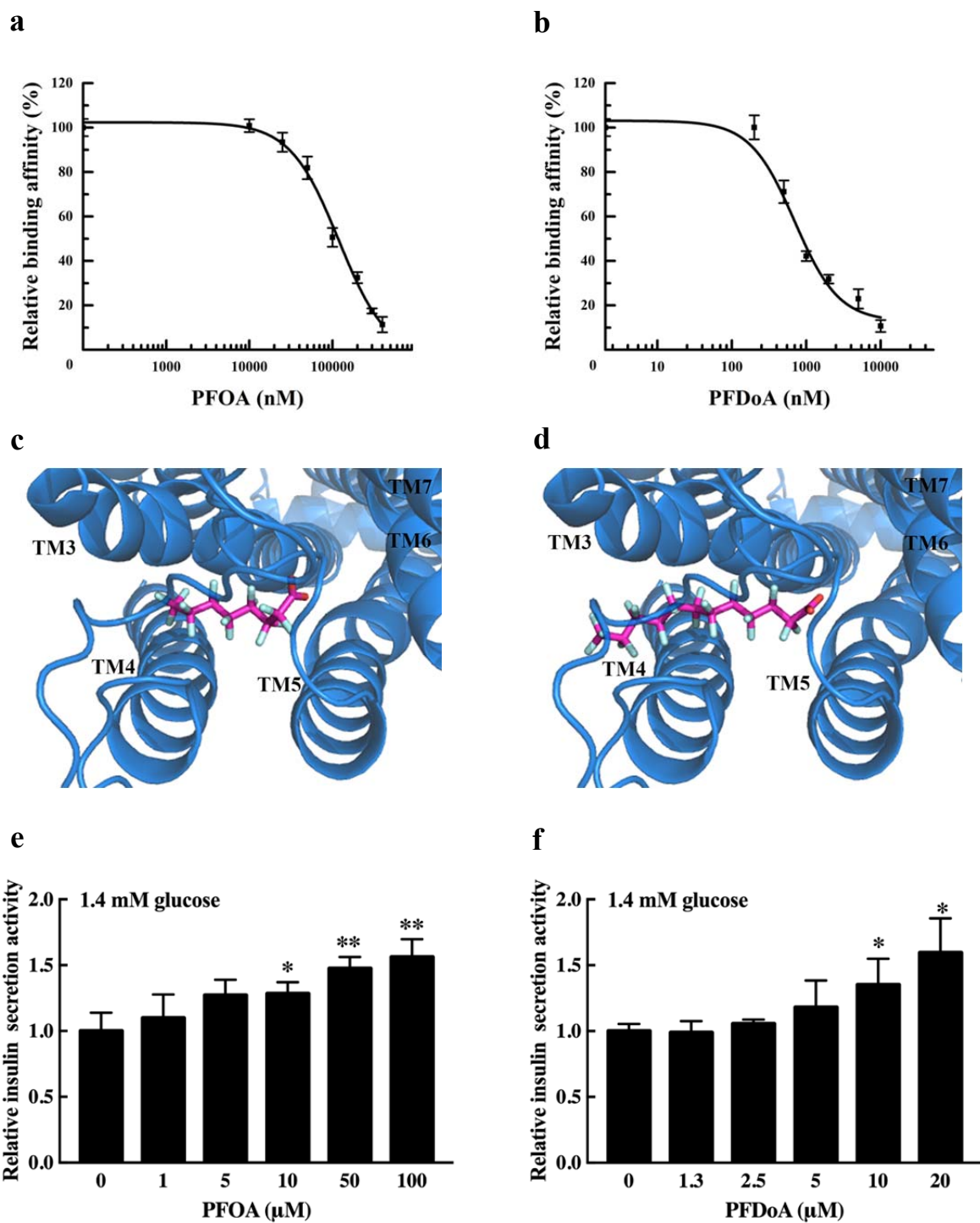


Figure 3 Studies of the interactions of lauric acid (LA) and PFOS with human GPR40 by competitive binding assay and molecular docking. **(A)** Competitive binding curves of LA and PFOS with human GPR40. **(B)** Molecular docking simulation of LA and PFOS into the binding pocket of human GPR40. **(C, D)** Interactions of LA **(C)** and PFOS **(D)** with amino acid residues of GPR40. LA and PFOS are shown as stick **(C:** magenta or green, F: cyan, S: yellow, O: red, H: white); GPR40 is shown as marine-blue helix; amino acid residues of GPR40 are shown as marine-blue stick and hydrogen bonds are indicated by green dotted lines.



656 **Figure 4** Predictions of the insulin-secretion disruption risks of PFOA and PFDoA via
657 GPR40 pathway. (A, B) Competitive binding curves of PFOA and PFDoA with
658 human GPR40. (C, D) Molecular docking analysis of PFOA and PFDoA to human
659 GPR40. (E, F) Effects of PFOA and PFDoA on the insulin secretion activity of

660 β -TC-6 cells in the present of 1.4 mM glucose. * means $p < 0.05$, ** means $p < 0.01$,

661 compared with the control group (0.1% DMSO).

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664 **Table 1** Abbreviation (Abbre.), highest tested concentration (HTC), half inhibitory
665 concentration (IC₅₀), relative binding affinity (RBA) values, and docking results of
666 lauric acid (LA), TAK-875 and 15 PFASs with human GPR40.

Compound	Abbre.	HTC (μ M)	IC ₅₀ (μ M)	RBA	Hydrogen bond
C ₁₂ H ₂₄ O ₂	LA	200	7.4 \pm 0.6	1	Arg183, Tyr2240
C ₂₉ H ₃₂ O ₇ S	TAK-875	2	< 0.1	616.7	Arg183, Arg2258
C ₄ HF ₇ O ₂	PFBA	400	ND	ND	Arg183, Arg2258
C ₄ HF ₉ O ₃ S	PFBS	400	ND	ND	Tyr 2240
C ₆ HF ₁₁ O ₂	PFHxA	400	ND	ND	Tyr 2240, Arg2258
C ₆ HF ₁₃ O ₃ S	PFHxS	400	167.7 \pm 9.8	< 0.1	Arg183, Arg2258
C ₇ HF ₁₃ O ₂	PFHpA	400	ND	ND	Tyr 2240, Arg2258
C ₈ HF ₁₅ O ₂	PFOA	400	119.3 \pm 19.3	0.1	Arg183, Tyr2240, Arg2258
C ₈ HF ₁₇ O ₃ S	PFOS	100	4.4 \pm 0.7	1.7	Arg183, Tyr2240, Arg2258
C ₉ HF ₁₇ O ₂	PFNA	100	24.3 \pm 18.5	0.3	Arg183, Tyr2240, Arg2258
C ₁₀ HF ₁₉ O ₂	PFDA	50	5.0 \pm 1.0	1.5	Tyr2240, Arg2258
C ₁₁ HF ₂₁ O ₂	PFUnA	10	2.9 \pm 0.6	2.6	Tyr2240, Arg2258
C ₁₂ HF ₂₃ O ₂	PFDoA	10	0.7 \pm 0.1	10.6	Tyr2240, Arg2258
C ₁₃ HF ₂₅ O ₂	PFTriDA	10	ND	ND	Tyr2240, Arg2258
C ₁₄ HF ₂₇ O ₂	PFTeDA	10	ND	ND	Arg183, Tyr2240
C ₁₆ HF ₃₁ O ₂	PFHxDA	10	ND	ND	Arg183, Tyr2240, Arg2258
C ₁₈ HF ₃₅ O ₂	PFOcDA	10	ND	ND	Tyr2240

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