



Excessive iron inhibits insulin secretion via perturbing transcriptional regulation of *SYT7* by *OGG1*

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Abstract

Although iron overload is closely related to the occurrence of type 2 diabetes mellitus (T2DM), the specific mechanism is unclear. Here, we found that excessive iron inhibited the secretion of insulin (INS) and impaired islet β cell function through downregulating *Synaptotagmin 7 (SYT7)* in iron overload model in vivo and in vitro. Our results further demonstrated that 8-oxoguanine DNA glycosylase (*OGG1*), a key protein in the DNA base excision repair, was an upstream regulator of *SYT7*. Interestingly, such regulation could be suppressed by excessive iron. *Ogg1*-null mice, iron overload mice and *db/db* mice exhibit reduced INS secretion, weakened β cell function and subsequently impaired glucose tolerance. Notably, *SYT7* overexpression could rescue these phenotypes. Our data revealed an intrinsic mechanism by which excessive iron inhibits INS secretion through perturbing the transcriptional regulation of *SYT7* by *OGG1*, which suggested that *SYT7* was a potential target in clinical therapy for T2DM.

Keywords Vesicle transport · Gene transcription · Oxidative stress · Chronic degenerative disease · Target therapy

Introduction

T2DM is a chronic metabolic disorder due to declining β cell function, and usually accompanied by a reduced sensitivity to INS [1]. This disease is fueled by interactions between multiple susceptible genetic loci and various environmental and behavioral factors [2]. Recently it has been shown

that excessive iron intake and improper use of iron supplements are closely related to the occurrence of T2DM [3]. The serum iron level of T2DM patients is higher than that of the health [4]. Until now, the causal relationship between excessive iron and β cell dysfunction and the underlying mechanism are not yet fully understood.

Oxidative stress is considered to be responsible for iron overload-induced islet β cell dysfunction as excessive iron can lead to peroxidation through the Fenton reaction [5]. In living cells, the peroxidation environment can easily cause the oxidative modification of G in genomic DNA to 7,8-dihydro-8-oxoguanine (8-oxoG) due to the low redox potential of guanine. *OGG1*, a key protein for the response to oxidative stress, binds and excises oxidized 8-oxoG in the genome to maintain the stability of the genome [6]. Mutation of *OGG1* has been implicated in the development of certain diseases, including Alzheimer's disease [7], atherosclerosis, cancer and diabetes [8, 9]. Although *Ogg1* knockout (*Ogg1*^{-/-}) mice exhibit normal development, they tend to show the characteristics of metabolic syndrome, such as impaired glucose tolerance [10]. To date, there is no clear mechanistic explanation of *OGG1* function in metabolism. Recent evidence suggests that in addition to the classic DNA repair function [6], *OGG1* is also involved in the

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transcriptional regulation of several genes, such as *TNF α* [11], *HRAS* [12], *VEGF* [13] and *c-Myc* [14]. The identification of a new downstream target of OGG1 may shed light on its role in metabolic processes.

In this study, we focused on the effect and mechanism of iron overload on islet β cell dysfunction and found that excessive iron impaired INS secretion via decreasing the expression of SYT7, a key molecule in this process. We further identified that SYT7 was a new downstream factor regulated by OGG1 and such regulation could be perturbed by excessive iron. Together, this novel finding revealed a hitherto unknown mechanism by which excessive iron inhibited INS secretion via perturbing the transcription regulation of SYT7 by OGG1, and therefore impaired islet β cell function.

Materials and methods

Mouse model

All animal experiments in this study were carried out in strict accordance with the guidance scheme formulated by the ethical review committee of experimental animal welfare of Nanjing Normal University (Approval No. IACUC-20200515). Male C57BL/6J mice, male *db/db* mice (C57BLKS/JNju background), and female and male *Ogg1*^{+/-} mice (C57BL/6J background) were purchased from GemPharmatech Co., Ltd. (N000013, T002407, T014260, Nanjing, China) and raised in the experimental animal center of Nanjing Normal University. The feeding conditions were 12 h of light/12 h of dark cycle, ambient temperature of 24°C, relative humidity of 50%, and freely available drinking water. *Ogg1*^{+/+} and *Ogg1*^{-/-} mice were obtained from *Ogg1*^{+/-} mice. According to the instructions corresponding to Article No. T014260, the mouse genotype was identified by PCR. CRISPR/Cas9 technology was used for the knock-out of this gene.

Fe²⁺ mice were obtained by feeding 4-week-old male C57BL/6J mice with FeSO₄ compound diet (according to the time for feeding like 1 month, 2 months, 3 months and 4 months, recorded as Fe²⁺-1m, Fe²⁺-2m, Fe²⁺-3m and Fe²⁺-4m respectively). The sibling mice fed on common diet (MD17121, provided by Jiangsu Mediscience Biomedical Co., Ltd.) were used as control (CW mice, CW-1m, CW-2m, CW-3m and CW-4m were recorded according to feeding for 1 month, 2 months, 3 months and 4 months respectively). The FeSO₄ compound feeding is a kind of common diet with an additional 21.5 g/kg of FeSO₄·7H₂O (Jiangsu XieTong Bioengineering Co., Ltd.).

In the rescue experiment of *Ogg1*^{-/-} mice, the initial injection time of adeno-associated virus serotype 8 vector (AAV8)-null/SYT7 was 3 weeks old. In the rescue experiment of Fe²⁺ mice, the initial injection time of AAV8-null/

SYT7 or Trulicity was 4 months after FeSO₄ compound diet intake. In the rescue experiment of *db/db* mice, the initial injection time of AAV8-null/SYT7, Trulicity or Deferoxamine (DFO) was 3 weeks old.

Trulicity (1.5 mg/0.5 ml, Lilly) was injected subcutaneously into the groin with an injection dose of 1.08 mg/kg/week. The injection method of DFO (HY-B0988, MCE) in *db/db* mice was intraperitoneal injection with an injection dose of 50 mg/kg/day.

The INS level in the serum isolated from the whole blood collected after fasting (free drinking water) for 4 h in each group of mice was defined as the basal INS level.

Intraductal pancreatic virus infusion

According to the method of Xiao et al. [15], AAV8-SYT7 or control virus (AAV8-null) (Vigene, Jinan, China) is diluted with PBS to 10¹² genome copy particles (GCPs)/ml. AAV8-SYT7 or control virus is provided in the form of ssAAV, and the vector map was shown in Fig. S5A.

Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal INS tolerance test (IPITT)

After fasting for 16 h, mice were injected with glucose (G8270, Sigma) (1.5 g/kg) IPGTT. For IPITT, INS (Novolin 50R, 300 IU/3 ml) was injected with 0.75 IU/kg on the mice after fasting for 4 h. At the set time point, we used the blood glucose meter (06870279001, Roche) to measure the blood glucose value of the tail vein, drawn the curve and calculated the area under the curve (AUC).

Hyperglycemic clamps

According to the method of Zhu et al. [16], jugular vein implantation and hyperglycemic clamp experiments were conducted on each group of mice. The time of jugular vein implantation in mice of each group is shown in Supplementary Table 3. After fasting for 12 h, mice were subjected to hyperglycemic clamp test, and the glucose infusion rate was adjusted so that the blood glucose was clamped to 17 mM (\pm 1 mM) within 30 min after infusion. Blood samples were collected at the time point of 0, 5, 10, 20, and 30 min respectively after infusion. Then the INS content was detected by enzyme-linked immunosorbent assay (ELISA). 0–10 min was defined as the first stage of INS secretion, and 10–30 min was defined as the second stage of INS release.

Cell culture and medication treatment

The mouse β cell line MIN6 was donated by Prof. Bin Xue (Shaw Hospital Affiliated to Nanjing Medical University). The Beta-TC-6 was purchased from National Collection

of Authenticated Cell Cultures. Both cell lines cultured in DMEM (glucose 1 g/L) containing 15% FBS, 1 mM sodium pyruvate and 50 μ M β -mercaptoethanol (all from Gibco). Medium needs to be replaced every 24 h. The freshly prepared complete culture medium needs to be used up within a week. TH5487 (HY-125276, MCE) dissolved in DMSO was added to fresh MIN6 complete medium to a final concentration of 5 μ M, and the control group was treated with the same amount of DMSO. The fresh culture medium was replaced every 24 h, and the supernatant and cells were collected after TH5487 treatment for 48 h.

The glucagon-like peptide-1 (GLP-1) was synthesized by Sangon Biotech (Shanghai, China). The sequence was HAE-GTFTSDVSSYLEGQAAKEFIAWLVKGRG. Final concentration was 50 nM, and the fresh medium was replaced every 24 h. The supernatant and cells were collected 48 h after the treatment of GLP-1.

The Fe^{2+} -exposed cell model was established by culturing MIN6 cells in complete medium supplemented with 100 μ M FeSO_4 . Although FeSO_4 is considered not harmful and widely used as supplement to treat iron-deficiency anemia, however, drug dosage is an issue that must be considered, as high concentrations may be toxic to cells. Therefore, in our preliminary experiment, to avoid the toxicity of high dose of FeSO_4 to β cells, a normal human serum iron concentration of 20 μ M was used as the initial reference value to evaluate the effect of different FeSO_4 concentrations on cell viability and INS release. The results showed that a FeSO_4 did not exert toxicity to β cells even with 3 mM FeSO_4 treatment, and a significant suppressive effect on INS release was observed in MIN6 cells starting at 100 μ M FeSO_4 . Therefore, 100 μ M is a safe concentration of FeSO_4 for establishing the Fe^{2+} -exposed cell model. In this study, MIN6 cells were cultured with 100 μ M FeSO_4 -containing medium for 11 days with fresh medium replaced daily.

293T was purchased from National Collection of Authenticated Cell Cultures and cultured in DMEM (4.5 g/L glucose) containing 10% FBS. The medium was replaced every 48 h. The cell incubator was set to 37°C, 5% CO_2 , and saturated humidity.

Glucose-stimulated INS secretion (GSIS), potassium-stimulated INS secretion (KSIS) and INS content determination

The isolation and culture of islets were described in Zhu et al. [17]. The methods of GSIS/KSIS of MIN6 (or isolated islets) were as described in Huang et al. [18]. In the GSIS experiment, glucose was used at concentrations of 2.8 mM and 25 mM. In the KSIS experiment, potassium chloride was used at the concentration of 50 mM. The methods for determining INS secretion or content were carried out in

accordance with the instructions of the ELISA (10-1249-01, Mercodia). INS secretion and INS content can be obtained after standardization with total protein.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed according to the method described previously [14], the probe (– 208 to – 156 bp) was designed according to the promoter region of *SYT7*. The final concentration of OGG1 (enz-253, PROSPEC) final concentration was 50 ng/ μ l in the reaction system. Fe^{2+} was added in the form of an $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ aqueous solution and dissolved before use (with final concentration 100 μ M in reaction system).

Western blot

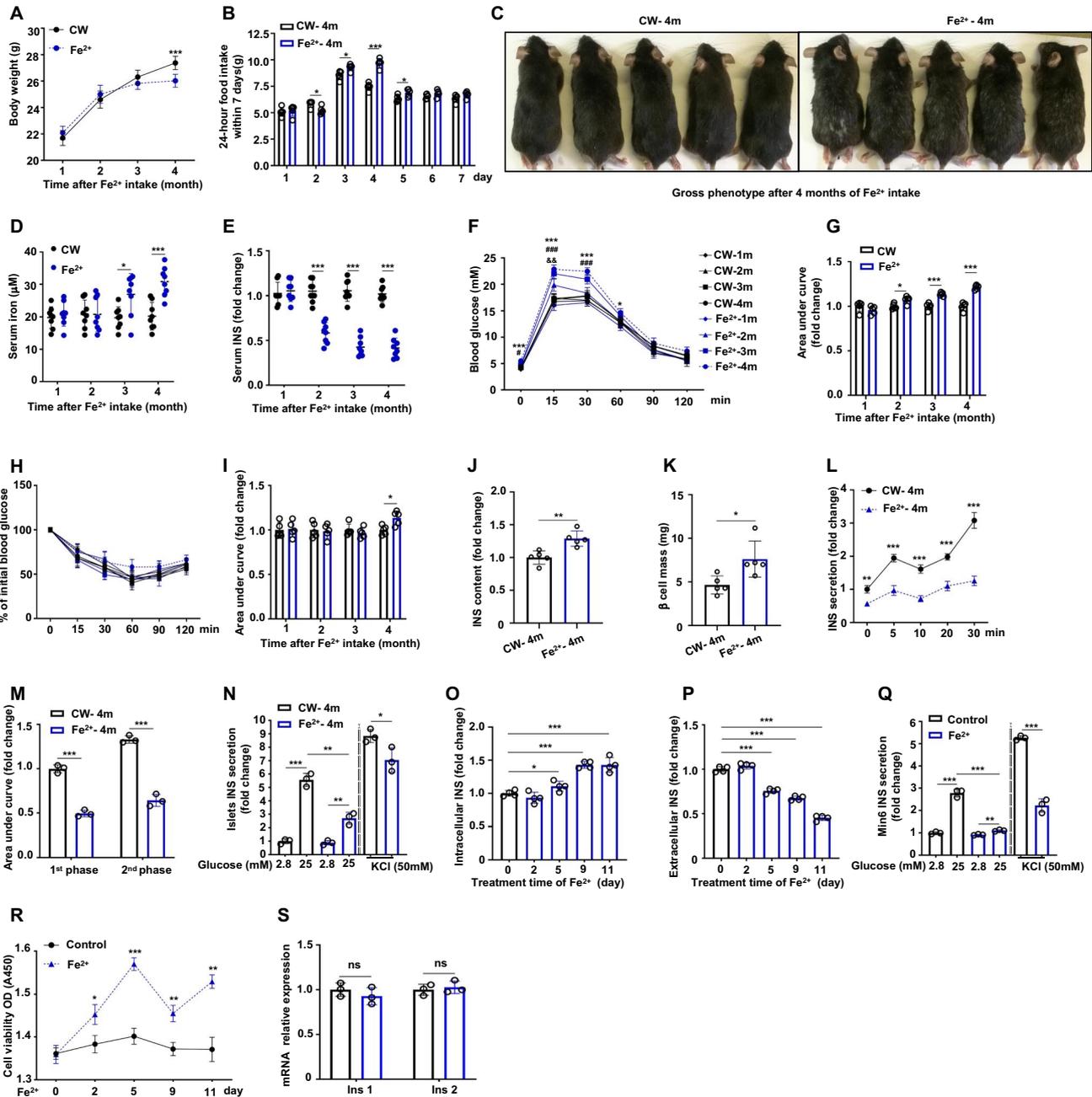
Western blot experiments were carried out according to the method of Ci et al. [19]. The primary antibodies anti-OGG1 (sc-376835) and anti-synaptotagmin VII (sc-293343) were from Santa Cruz (diluted 1:200) and anti-INS (ab181547) was from Abcam (diluted 1:1000) and Anti- β -tubulin was from Abgent (AM1031A, diluted 1:1000).

Statistical analysis

Quantitative data were obtained in triplicate from at least three independent experiments and are presented as the mean \pm standard error and inferential statistics (*P* values). Statistical significance was evaluated using paired two-tailed Student's *t* tests. *P* < 0.05 was considered statistically significant. All statistical analyses were done using GraphPad Prism 8.0.

Other assays

Cell transfection and dual luciferase reporter gene detection, intracellular Ca^{2+} content detection, serum Fe^{2+} content detection, hematoxylin and eosin (H&E) staining, immunofluorescence and oil red O staining, β cell mass, chromatin immunoprecipitation (ChIP)-qPCR, microarray analysis, transmission electron microscope (TEM) detection, cell viability assays, real-time quantitative PCR (RT-qPCR), retrospective analysis of patients with T2DM, more details about these assays are included in Supplementary Methods.



Results

Long-term high-dose FeSO₄ intake impairs INS secretion

Through analysis of mice with a long-term, high-dose FeSO₄ diet, it was found that the average weight was slightly lower than that of the CW mice when fed for 3 months, with significant weight loss at month 4 (Fig. 1A). However, food intake in the Fe²⁺ mice group did not decrease (Fig. 1B). In terms of general appearance, the Fe²⁺ mice had erect hair and lacked luster and became lighter in color (Fig. 1C). As

expected, serum iron levels gradually increased with the duration of high-dose FeSO₄ intake (Fig. 1D), while serum INS levels gradually decreased (Fig. 1E).

We performed IPGTT and IPITT to evaluate the effect of high-dose FeSO₄ intake on glucose tolerance and INS tolerance. The data showed that in the Fe²⁺ mice group, both the fasting blood glucose and blood glucose levels at 15, 30, and 60 min after glucose loading were significantly increased with the duration of FeSO₄ intake, and the AUC increased (Fig. 1F, G), while the glucose levels at all time point of IPITT were comparable to that of the CW group (Fig. 1H), and the AUC increased with FeSO₄ intake

Fig. 1 Long-term high-dose FeSO₄ intake impairs INS secretion. **A** The weight changes of the mice after a high-dose FeSO₄ supplemented diet. $n=6$ in each cohort. $***P<0.001$. **B** The amount of food consumed by the mice in Fe²⁺ mice group per 24 h for one week after ingesting high-dose FeSO₄ for 4 months. $n=5$ in each cohort. $*P<0.05$, $***P<0.001$. **C** Gross phenotype of the Fe²⁺ mice group fed a high-dose FeSO₄-supplemented diet for 4 months compared with the CW mice group. **D** Changes in serum iron content in the mice consuming high-dose FeSO₄-supplemented diets for 1–4 months. $n=8$ in each cohort. $*P<0.05$, $***P<0.001$. **E** Fold changes in serum INS content in the mice consuming high-dose FeSO₄-supplemented feed for 1–4 months compared to CW mice. $n=8$ in each cohort. $***P<0.001$. **F, G** The results of the IPGTT experiment and the corresponding AUC of the mice in the CW mice group and the mice in the Fe²⁺ mice group when they were fed a normal diet and high-dose FeSO₄ diet for 1–4 months. $n=5$ in each cohort. Mice were fed normal chow for 1 month, 2 months, 3 months and 4 months and are shown as cw-1m, cw-2m, cw-3m and cw-4m, respectively. Mice were fed high-dose FeSO₄-supplemented feed for 1 month, 2 months, 3 months and 4 months and are shown as Fe²⁺-1m, Fe²⁺-2m, Fe²⁺-3m and Fe²⁺-4m, respectively. *Indicates the Fe²⁺-4m group compared with the CW-4m group, $*P<0.05$, $***P<0.001$. #. Indicates the Fe²⁺-3m group compared with the CW-3m group, $#P<0.05$, $###P<0.001$. &Indicates the Fe²⁺-2m group compared with the CW-2m group, $&P<0.01$. In the comparison of the AUC of each group, $*P<0.05$, $***P<0.001$. **H, I** The results of the IPITT experiment and corresponding AUC of the mice in the CW group and the mice in the Fe²⁺ group when they were fed a normal diet and a high-dose FeSO₄ diet for 1–4 months. $n=5$ in each cohort. $*P<0.05$. **J** ELISA show that fold changes in INS content in isolated islets of Fe²⁺-4m and CW-4m mice. $n=5$ in each cohort. $**P<0.01$. **K** β cell mass of isolated islets of mice in Fe²⁺-4m group. $n=5$ in each cohort. $*P<0.05$. **L, M** Hyperglycemic clamp tests show INS biphasic secretion and AUC when high-dose FeSO₄ diet for 4 months. 0–10 min is defined as the first phase and 10–30 min is defined as the second phase. $n=3$ in each cohort. $**P<0.01$, $***P<0.001$. **N** GSIS/KSIS levels in isolated islets of mice in Fe²⁺-4m group. **O** ELISA results showed fold changes in intracellular INS content in MIN6 cells after FeSO₄ (100 μ M) treatment for 2–11 days compared to 0 day. **P** Fold changes in INS level in the supernatant of MIN6 culture medium after treatment with FeSO₄ (100 μ M) for 2–11 days compared to 0 day. **Q** GSIS and KSIS levels in the MIN6 cells exposed to Fe²⁺ for 11 days. **R** The viability of the MIN6 cells treated with FeSO₄ for 0–11 days. **S** The expression level of *Ins1/Ins2* mRNA in MIN6 cells treated with FeSO₄ for 11 days. **N–S**, data are presented as mean \pm s.d. of at least three separate experiments. $*P<0.05$, $**P<0.01$, $***P<0.001$, *ns* no significant difference

for 4 months (Fig. 1I). Although INS content of islets (Fig. 1J) and β cell mass (Fig. 1K) were increased after high dose iron intake, the results of hyperglycemia clamp tests (Fig. 1L, M) and INS release tests of isolated islets (Fig. 1N) showed that long term high dose iron intake decreased the INS secretion level. To determine whether Fe²⁺ exposure directly impairs β cell function, we cultured the mouse β cell line MIN6 in complete medium containing 100 μ M FeSO₄. The intracellular INS content showed an upward trend with prolonged incubation time (Fig. 1O). However, after 5 days of incubation, the INS content in the supernatant of MIN6 culture medium with FeSO₄ was significantly reduced, and to approximately 50% of that of the control group after 11 days (Fig. 1P). The INS secretion

response to glucose and potassium chloride stimulation decreased (Fig. 1Q). Corresponding to the reduced level of INS secretion, the intracellular Ca²⁺ content was also reduced (Fig. S1A, B). Furthermore, cell vitality assays showed that 100 μ M FeSO₄ treatment slightly increased cell viability (Fig. 1R), which excluded the effects of cell growth. Such increase may be due to an adaptation of β cells to the environmental stress and compensation for the damaged secretion function. RT-qPCR results showed that this effect was not caused by INS transcription (Fig. 1S). Overall, these results suggest that high doses of FeSO₄ significantly inhibited the INS secretion of β cells before compromising cell viability.

OGG1 deficiency impaired INS secretion

Recent studies have suggested that oxidative stress is the main cause of islet β cell dysfunction induced by excessive iron [20]. Since OGG1 is constitutively expressed as a housekeeping enzyme in response to oxidative stress in living cells, OGG1 deficiency is closely related to metabolic disorders [21]. We then suspected that OGG1 may be the candidate molecule involved in excessive iron-inhibited INS secretion. To test this hypothesis, we examined the phenotype in WT (*Ogg1*^{+/+}) and *Ogg1* knockout (*Ogg1*^{-/-}) mice (Fig. S2A, B). The body weight of mice was slightly decreased with *Ogg1* knockout and this was not due to the reduced food intake (Fig. 2A, Fig. S2C). Notably, random blood glucose was slightly elevated (Fig. 2B), while serum INS levels were significantly reduced in the *Ogg1*^{-/-} mice (Fig. 2C). The IPGTT results showed that blood glucose of *Ogg1*^{-/-} mice increased significantly at 15 min and 30 min after glucose loading (Fig. 2D), and the AUC increased (Fig. 2E). IPITT data showed no significant difference between the two groups (Fig. 2F, G). *Ogg1*^{-/-} mice exhibited increased intracellular INS level in β cells (Fig. 2H, I). The hyperglycemic clamp experiment demonstrated an attenuated INS secretion level in *Ogg1*^{-/-} mice (Fig. 2J, K). Moreover, isolated islets from the *Ogg1*^{-/-} mice showed impaired GSIS (Fig. 2L) and decreased intracellular Ca²⁺ content (Fig. S3A). Next, similar results were obtained in MIN6 cells with *Ogg1* knockdown by shRNA (Fig. 2M–R, Fig. S3B, C) or with the OGG1 inhibitor TH5487 treatment (Fig. 2R–V, Fig. S3D, E). Thus, the data suggest that OGG1 plays an important role in glucose-stimulated INS secretion.

SYT7 is a new downstream target gene of OGG1

As OGG1 is involved in the regulation of gene expression [11–14], searching the appropriate downstream target gene may be the effective way to investigate OGG1 physiological function. We compared the gene expression of *Ogg1*^{+/+} and *Ogg1*^{-/-} mouse embryonic fibroblasts (MEFs) by microarray

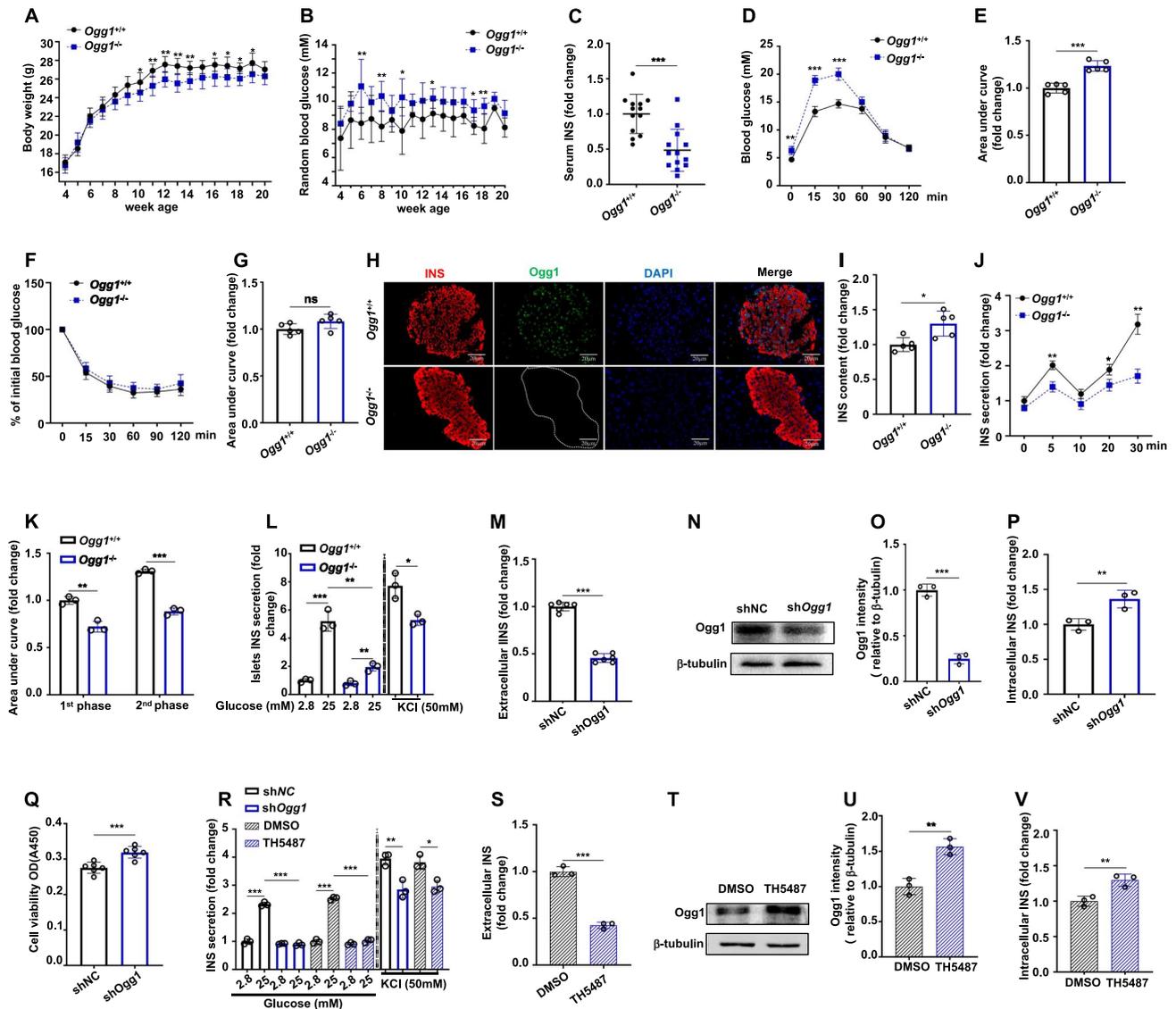


Fig. 2 OGG1 deficiency impaired INS secretion. **A** Weight changes of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 4–20 weeks. *n*=6 in each cohort. **P*<0.05, ***P*<0.01. **B** Random blood glucose of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 4–20 weeks. *n*=6 in each cohort. **P*<0.05, ***P*<0.01. **C** Serum INS content in the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks, *n*=13 in each cohort. ****P*<0.001. **D**, **E** The results of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks detected by IPGTT and the corresponding AUC. *n*=5 in each cohort. ***P*<0.01, ****P*<0.001. **F**, **G** The results of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks detected by IPITT and the corresponding AUC. *n*=5 in each cohort. ns, no significant difference. **H**, **I** The levels of Ogg1 in islets of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks were detected by immunofluorescence. **I** The levels of INS in islets of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks were detected by ELISA. *n*=5 in each cohort. **P*<0.05. Scale bar 20 μm. **J**, **K** Hyperglycemic clamp tests show INS biphasic secretion and AUC in *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks. 0–10 min is defined as the first phase and 10–30 min is defined as the second phase. *n*=3 in each cohort. **P*<0.05, ***P*<0.01, ****P*<0.001. **L**

GSIS and KSIS levels in islets of the *Ogg1*^{-/-} and *Ogg1*^{+/+} mice aged 6 weeks. Data are presented as mean ± s.d. of three separate experiments. **P*<0.05, ***P*<0.01, ****P*<0.001. **M** The INS level in the supernatant of MIN6 cells after knockdown of *Ogg1* were detected by ELISA. Data are presented as mean ± s.d. of six separate experiments. ****P*<0.001. **N–P** *Ogg1* knockdown in MIN6 cell line and OGG1 content. **O**, the gray analysis statistical value of three independent experiments. **P** detection of intracellular INS level after shOgg1 by ELISA. **Q** Cell viability of MIN6 cells with *Ogg1* knockdown was detected by CCK8 assay. **R** The effect of knocking down *Ogg1* and using OGG1 inhibitor TH5487 on GSIS and KSIS levels in MIN6 cells. **S** The INS secretion level of MIN6 with OGG1 inhibitor TH5487 treatment were detected by ELISA. **T–V** The OGG1 content of MIN6 cells with or without Th5487 treatment. **U** The OGG1 intensity (relative to β-tubulin) of three independent experiments. **V** ELISA results show the fold changes in intracellular INS levels after Th5487 pretreatment compared to DMSO. **N–V** Data are presented as mean ± s.d. of three separate experiments. **P*<0.05, ***P*<0.01, ****P*<0.001

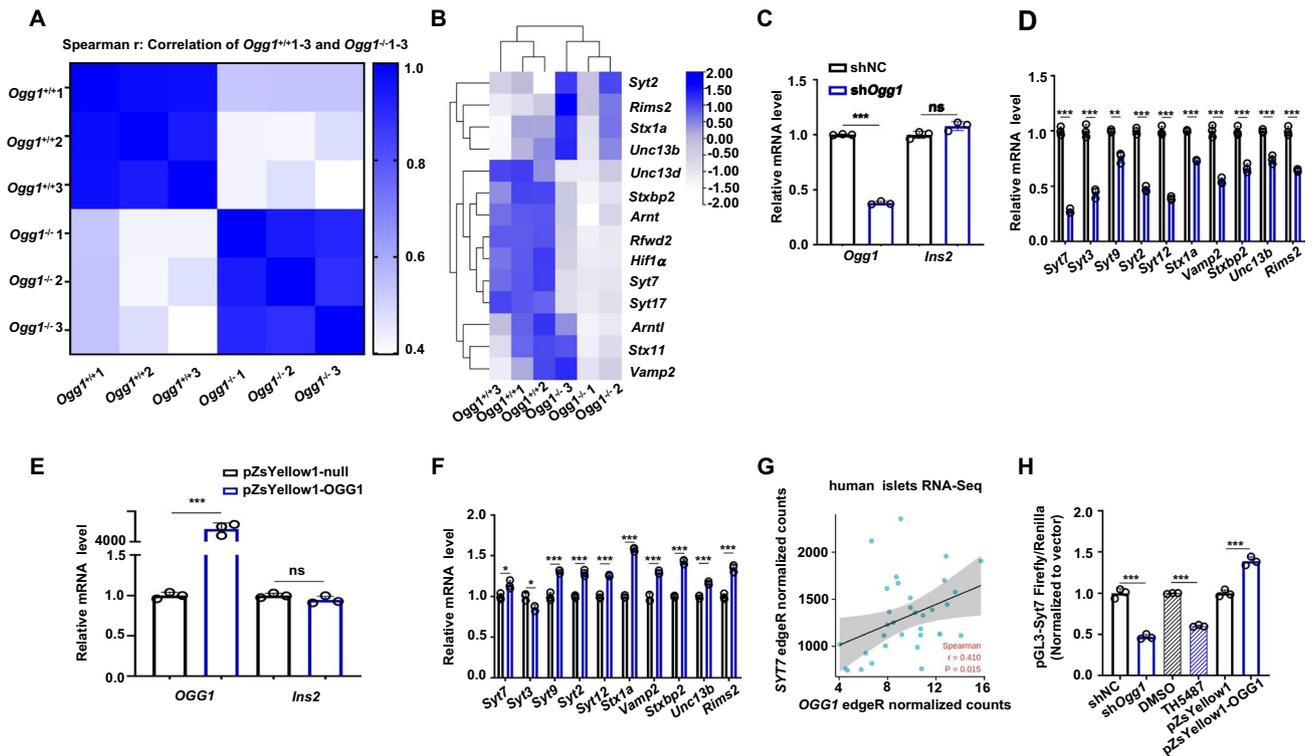


Fig. 3 *SYT7* is a new downstream target gene of *OGG1*. **A, B** The *Ogg1*^{+/+} and *Ogg1*^{-/-} MEF gene microarray test results. $n=3$ in each cohort. **C, D** Gene expression in MIN6 cells after knockdown of *Ogg1*. Data are presented as mean \pm s.d. of three separate experiments. ** $P < 0.01$, *** $P < 0.001$. *ns* no significant difference. **E, F** Gene expression in MIN6 cells after overexpression of *OGG1*. Data are presented as mean \pm s.d. of three separate experiments. * $P < 0.05$, *** $P < 0.001$, *ns* no significant difference. **G** RNA-Seq data in

the GEO database show the correlation between *OGG1* and *SYT7* expression in human β cells (data from GEO: GSE165121). Spearman related analysis. $P=0.015$. **H** Double luciferase reporter system was used to detect the effect of *Ogg1* knockdown, TH5497 and *OGG1* overexpression on the fluorescence signal intensity of pGL3-Syt7. Data are presented as mean \pm s.d. of three separate experiments. *** $P < 0.001$

from our previous study. The results showed that 14 of the genes affected by *Ogg1* knockout involved in the INS secretion process [22–29], of which 10 genes were significantly downregulated (Fig. 3A, B). Since MEFs are characterized by their ability to secrete cytokines [30], such downregulation may also be present in INS-secreting cells. To confirm the microarray results in β cells, we performed RT-qPCR in MIN6 cells with *Ogg1* knockdown or overexpression. The results show that there was no significant difference in the expression level of the *Ins2* gene (Fig. 3C, E); however, the expression of 10 genes, such as *Syt7*, was significantly changed (Fig. 3D, F). SYTs family members play crucial roles in the regulatory exocytosis of the nervous and endocrine systems [31]. To investigate whether there is a relationship between *OGG1* and *SYT7*, we used public RNA-seq data from the Gene Expression Omnibus (GEO) and analyzed the expression of *OGG1* and *SYT7* in islets from healthy people (GEO: GSE165121). The data showed a positive correlation between the two genes (Fig. 3G). It has been established that under oxidative stress, one important role

of *OGG1* is binding to oxidized guanine and participating in gene transcriptional regulation [32]. To explore whether *OGG1* is involved in *SYT7* transcription, we performed a classic dual-luciferase reporter assay. The results show that both *Ogg1* knockdown and inhibitor treatment noticeably decreased the luciferin signal, while *OGG1* overexpression increased the signal, which suggested that *SYT7* is a new transcriptional target of *OGG1* (Fig. 3H).

OGG1/SYT7 is indispensable for INS secretion

To determine whether *SYT7* is the exact downstream protein mediating *OGG1* effect on INS secretion, we overexpressed *SYT7* in *Ogg1*-knockdown MIN6 cells. *SYT7* overexpression significantly reversed the increase of intracellular INS levels and the decrease of extracellular INS levels induced by *Ogg1* deficiency (Fig. 4A–D). Correspondingly, the reduced intracellular Ca^{2+} content also partially rescued (Fig. S4A). To explore the important role of *OGG1/SYT7* in INS secretion, we first chose GLP-1 (an incretin hormone increasing

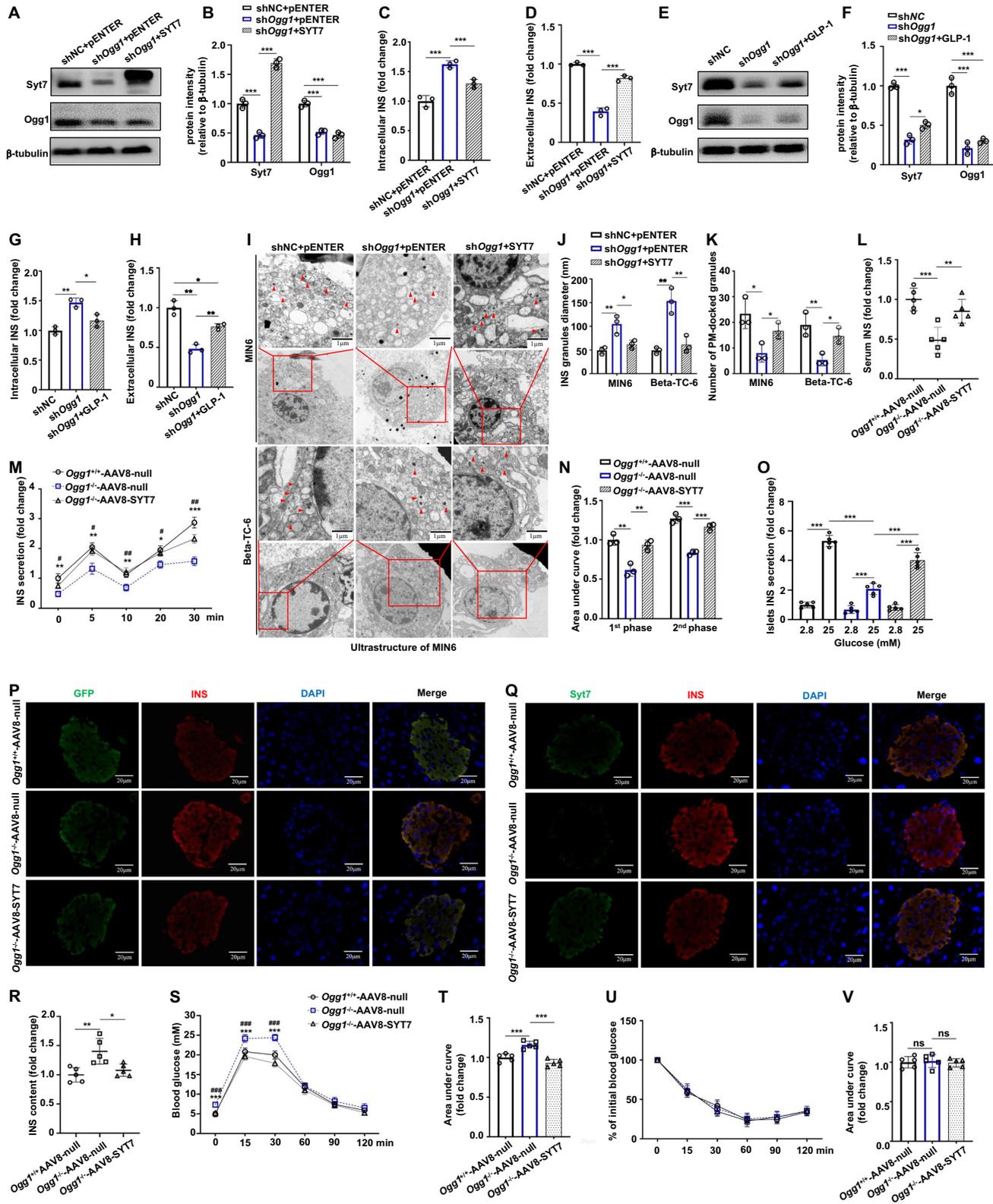


Fig. 4 OGG1/SYT7 is indispensable for INS secretion. **A–C** **A** The SYT7 level in *Ogg1*-knockdown MIN6 cells with SYT7 overexpression. **B** The gray analysis statistical value of three independent experiments. **C** Intracellular INS level in *Ogg1*-knockdown MIN6 cells with SYT7 overexpression. Data are presented as mean \pm s.d. of three separate experiments. *** $P < 0.001$. **D** The content of INS in cell culture supernatant from *Ogg1*-knockdown MIN6 cells with SYT7 overexpression. Data are presented as mean \pm s.d. of three separate experiments. *** $P < 0.001$. **E–G** **E** Syt7 level of *Ogg1*-knockdown MIN6 cells treated with GLP-1 were detected by Western blot. **F** The gray analysis statistical value of three independent experiments. **G** Intracellular INS level of *Ogg1*-knockdown MIN6 cells treated with GLP-1 were detected by ELISA. Data are presented as mean \pm s.d. of three separate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **H** The INS level in the supernatant of the cell culture medium of *Ogg1*-knockdown MIN6 cells treated with GLP-1 was detected by ELISA. Data are presented as mean \pm s.d. of three separate experiments. * $P < 0.05$, ** $P < 0.01$. **I–K** **I** The intracellular ultrastructure of MIN6 and Beta-TC-6 cells. Scale bar 1 μ m. **J** The average INS granules diameter in cells of each group. **K** Number of PM docked granules in each group. Data are presented as mean \pm s.d. of three separate experiments. * $P < 0.05$, ** $P < 0.01$. **L** Serum basal INS levels in *Ogg1*^{-/-} mice 3 weeks after AAV8-SYT7 injection. $n = 5$ in each cohort. ** $P < 0.01$, *** $P < 0.001$. **M, N** Hyperglycemic clamp tests show INS biphasic secretion and AUC in *Ogg1*^{-/-} mice 3 weeks after AAV8-SYT7 injection. 0–10 min is defined as the first phase and 10–30 min is defined as the second phase. $n = 3$ in each cohort. *Indicates the *Ogg1*^{-/-}-AAV8-null group compared with the *Ogg1*^{+/+}-AAV8-null group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. #Indicates the *Ogg1*^{-/-}-AAV8-SYT7 group compared with the *Ogg1*^{-/-}-AAV8-null group, # $P < 0.05$, ## $P < 0.01$. In the comparison of the AUC of each group, ** $P < 0.01$, *** $P < 0.001$. **O** GSIS levels of isolated islets from *Ogg1*^{-/-} mice 3 weeks after AAV8-SYT7 injection. $n = 5$ in each cohort. ** $P < 0.01$, *** $P < 0.001$. **P, Q** The expression of the AAV8 vector tag GFP and Syt7 in the islets of the *Ogg1*^{+/+}-AAV8-null, *Ogg1*^{-/-}-AAV8-null and *Ogg1*^{-/-}-AAV8-SYT7 mice 3 weeks after AAV8-SYT7 injection were detected by immunofluorescence. Scale bar 20 μ m. **R** INS content in isolated islets from *Ogg1*^{-/-} mice 3 weeks after AAV8-SYT7 injection. $n = 5$ in each cohort. * $P < 0.05$, ** $P < 0.01$. **S, T** *Ogg1*^{-/-}-AAV8-SYT7 mice 3 weeks after AAV8-SYT7 injection, the results of the IPGTT experiment and the corresponding AUC. $n = 5$ in each cohort. For **S**, *Indicates the *Ogg1*^{-/-}-AAV8-null group compared with the *Ogg1*^{+/+}-AAV8-null group, *** $P < 0.001$. #Indicates the *Ogg1*^{-/-}-AAV8-SYT7 group compared with the *Ogg1*^{-/-}-AAV8-null group, ### $P < 0.001$. In the comparison of the AUC of each group, *** $P < 0.001$. **U, V** *Ogg1*^{-/-}-AAV8-SYT7 mice 3 weeks after AAV8-SYT7 injection, the results of the IPITT experiment and the corresponding AUC. $n = 5$ in each cohort. ns, no significant difference

INS secretion in glucose-dependent manner) to evaluate whether OGG1 deficiency-induced INS secretion dysfunction could be rescued by this peptide. Results showed that although the extracellular INS level and Ca²⁺ content in the *Ogg1*-knockdown group were significantly increased by GLP-1, it was still lower than those in the control group (Fig. 4E, H, Fig. S4B), which suggests that OGG1/SYT7 was very important for INS secretion. The TEM images showed that *Ogg1* knockdown increased the volume of INS granules and reduced the number of INS granules docked on the plasma membrane (PM). Importantly, these phenotypes were restored after SYT7 overexpression (Fig. 4I–K).

Next, we injected AAV8-SYT7 into the *Ogg1*^{-/-} mice in situ through the bile duct to examine the recovery effect of SYT7. Our data showed that serum INS level, INS biphasic secretion and extracorporeal GSIS of islets were increased (Fig. 4L–O), and no damage to major organs was detected with AAV8-SYT7 injection (Fig. S5B). The immunofluorescence and ELISA results show that the intracellular INS level was decreased with the successful expression of SYT7 in islets (Fig. 4P–R). IPGTT results demonstrated that AAV8-SYT7 injection significantly decreased the blood glucose level of *Ogg1*^{-/-} mice (Fig. 4S, T). No significant change was observed by IPITT (Fig. 4U, V). Taken together, the data suggest that OGG1/SYT7 is indispensable for INS secretion.

Excessive iron inhibited OGG1 binding to the SYT7 promoter

Our data have shown that high-dose iron increased *Ogg1* protein levels in islets. However, contrary to expectation, *Syt7* expression was gradually decreased (Fig. 5A, B), which is contradictory to the positive correlation between the two genes. Since OGG1 binding to the substrate could be inhibited by heavy metal [33], we then speculate whether excessive iron has the similar effect. To test this hypothesis, we first detected the expression level of *Syt7* in MIN6 cells cultured with 100 μ M FeSO₄ in different time periods. The RT-qPCR results showed that the mRNA level of *Syt7* gradually decreased with culture time and was approximately 0.6-fold of the untreated group on the 11th day (Fig. 5C). Interestingly, by screening the RNA-seq data of the GEO database (GSE128945), we also found that *Ogg1* expression was upregulated and *Syt7* expression was downregulated in β cells of β -DKO mice whose islet function was destroyed (conditional knockout of *Swi/Snf* genes, which encode SWI/SNF chromatin-remodeling complex controlling the induction of iron transport genes), showing a negative correlation (Fig. 5D, E). To verify whether FeSO₄ directly impairs the binding of OGG1 to the SYT7 promoter, we synthesized the SYT7 promoter sequence and carried out an EMSA with purified recombinant OGG1 protein. The results showed that OGG1 specifically bound with 8-oxoG and the binding was abolished by FeSO₄ (Fig. 5F). ChIP-qPCR result was consistent with that of EMSA (Fig. 5G). Notably, the dual luciferase reporter assay showed that even in the case of overexpression of OGG1, the luciferin signal was still strongly suppressed by FeSO₄, which stressed the inhibitory effect of iron on the transcriptional regulatory function of OGG1 (Fig. 5H). These results also suggest that SYT7 may be an effector of FeSO₄ and OGG1 deficiency, leading to insufficient INS secretion. Next, to determine whether SYT7 can improve the impaired INS secretion caused by long-term high-dose FeSO₄ intake. We performed IPGTT and IPITT assay in iron overload mice with AAV-SYT7 injection. At

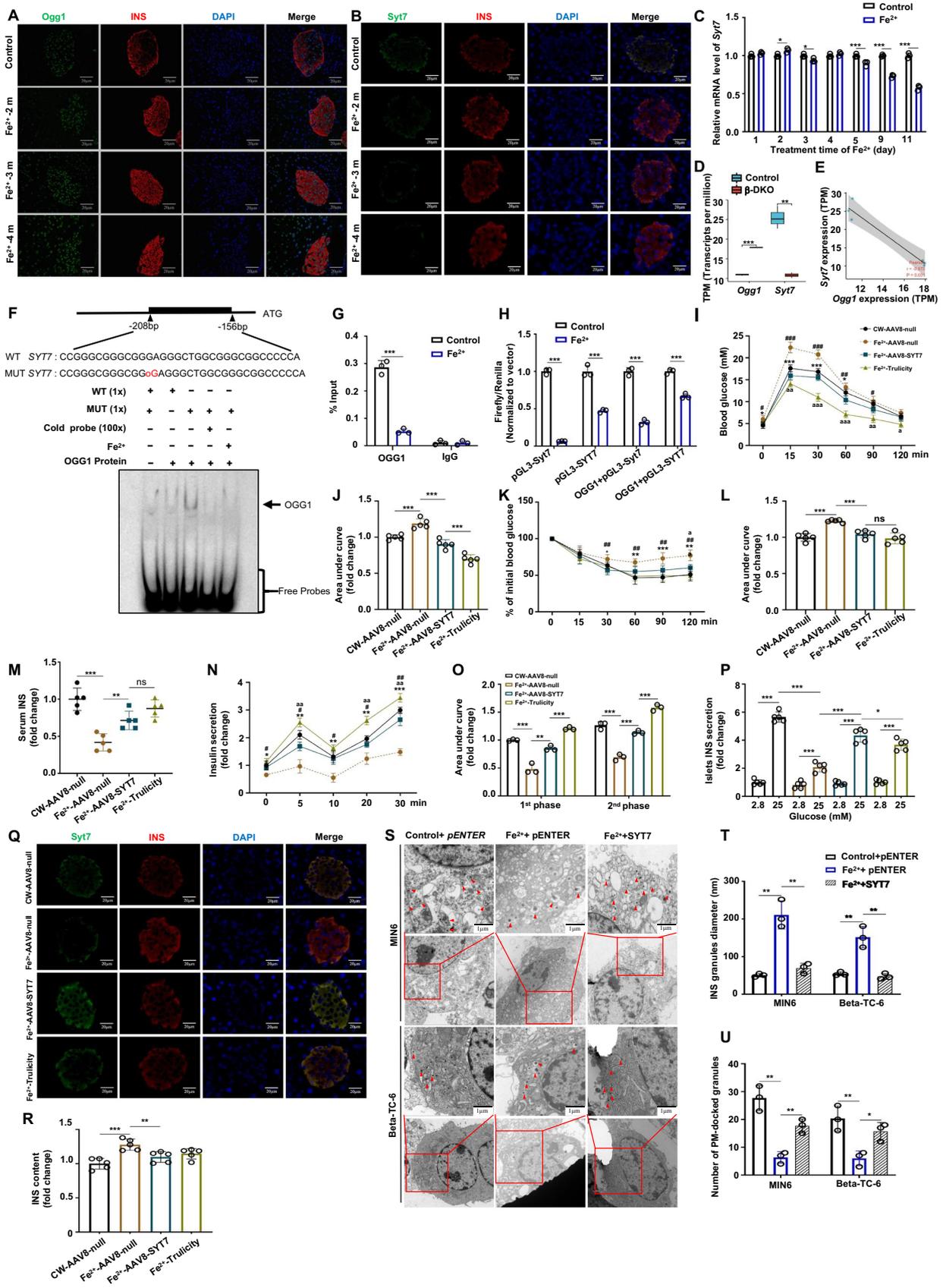


Fig. 5 Excessive iron inhibited OGG1 binding to the *SYT7* promoter. **A, B** Immunofluorescence staining of Ogg1, INS and *Syt7* proteins in mouse β cells with FeSO_4 intake for 2–4 months. Scale bar 20 μm . **C** The relative expression of the *Syt7* gene in the MIN6 cells treated with FeSO_4 for 1, 2, 3, 4, 5, 9, and 11 days. Data are presented as mean \pm s.d. of three separate experiments. $*P < 0.05$, $***P < 0.001$. **D, E** The expression of *Ogg1* and *Syt7* in mouse β cells were detected by RNA-seq. (data from GEO: GSE128945). $n = 3$ in each cohort. $***P < 0.01$, $****P < 0.001$. **E** Person related analysis. $P = 0.001$. **F** EMSA shows the binding of OGG1 to the *SYT7* promoter after FeSO_4 (100 μM) treatment. **G** ChIP-qPCR analysis of OGG1 binding to the *Syt7* promoter in MIN6 cells treated with FeSO_4 . Data are presented as mean \pm s.d. of three separate experiments. $***P < 0.001$. **H** Dual luciferase reporter analysis of the effect of FeSO_4 treatment on *Syt7* promoter activity in 293 T cells with OGG1 overexpression. Firefly luciferase activity was measured 24 h post transfection and normalized to Renilla luciferase activity. Data are presented as mean \pm s.d. of three separate experiments. $***P < 0.001$. **I, J** Fe^{2+} -4m mice 3 weeks after AAV8-SYT7 or Trulicity injection, the results of the IPGTT experiment and the corresponding AUC. $n = 5$ in each cohort. *Indicates the Fe^{2+} -AAV8-null group compared with the CW-AAV8-null group, $*P < 0.05$, $***P < 0.001$. #Indicates that the Fe^{2+} -AAV8-SYT7 group was compared with the Fe^{2+} -AAV8-null group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$. a Indicates that the Fe^{2+} -Trulicity group was compared with the Fe^{2+} -AAV8-SYT7 group, $^aP < 0.05$, $^{aa}P < 0.01$, $^{aaa}P < 0.001$. In the comparison of the AUC of each group, $***P < 0.001$. **K, L** Fe^{2+} -4m mice 3 weeks after AAV8-SYT7 or Trulicity injection, the results of IPITT experiment and corresponding AUC. $n = 5$ in each cohort. *Indicates the Fe^{2+} -AAV8-null group compared with the CW-AAV8-null group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. #Indicates that the Fe^{2+} -AAV8-SYT7 group was compared with the Fe^{2+} -AAV8-null group. $\#P < 0.01$. a Indicates that the Fe^{2+} -Trulicity group was compared with the Fe^{2+} -AAV8-SYT7 group, $^aP < 0.05$. In the comparison of the AUC of each group, $***P < 0.001$, ns no significant difference. **M** Serum basal INS content of Fe^{2+} -4m mice 3 weeks after AAV8-SYT7 or Trulicity injection. $n = 5$ in each cohort. $**P < 0.01$, $***P < 0.001$. ns , no significant difference. **N, O** Hyperglycemic clamp tests show INS biphasic secretion and AUC of Fe^{2+} -4m mice 3 weeks after AAV8-SYT7 or Trulicity injection. 0–10 min is defined as the first phase and 10–30 min is defined as the second phase. $n = 3$ in each cohort. **N** *Indicates the Fe^{2+} -AAV8-null group compared with the CW-AAV8-null group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. #Indicates the Fe^{2+} -AAV8-SYT7 group compared with the Fe^{2+} -AAV8-null group, $\#P < 0.05$, $\#\#P < 0.01$. a Indicates that the Fe^{2+} -Trulicity group was compared with the Fe^{2+} -AAV8-SYT7 group, $^{aa}P < 0.01$. In the comparison of the AUC of each group, $**P < 0.01$, $***P < 0.001$. **P** Effect of AAV8-SYT7 injection on GSIS of isolated islets in each group. $n = 5$ in each cohort. $*P < 0.05$, $***P < 0.001$. **Q** After 3 weeks of Fe^{2+} -4m mice 3 weeks injected with AAV8-SYT7 or Trulicity, immunofluorescence show the staining of *Syt7* in the β cells of each group. Scale bar 20 μm . **R** The effect of AAV8-SYT7 on the INS content in islets in each group was detected by ELISA. $n = 5$ in each cohort. $**P < 0.01$, $***P < 0.001$. **S–U** **S** The intracellular ultrastructure of MIN6 and Beta-TC-6 Fe^{2+} model after SYT7 overexpression. Scale bar 1 μm . **T** The average INS granules diameter in cells of each group. **U** Number of PM docked granules in each group. Data are presented as mean \pm s.d. of three separate experiments. $*P < 0.05$, $**P < 0.01$

the same time, Trulicity, a long-acting GLP-1 RA preparation promoting INS secretion, was also used as a positive control. The result showed that AAV8-SYT7 injection reversed the effect of FeSO_4 and improved glucose tolerance and INS sensitivity (Fig. 5I–L). The decreased serum INS level, the

impaired biphasic INS secretion and GSIS levels of isolated islets induced by high-dose iron feeding were all restored by AAV8-SYT7 (Fig. 5M–P). Immunofluorescence and isolated islets ELISA results further confirmed that AAV8-SYT7 decreased the INS content accumulated in β cells (Fig. 5Q, R). In addition, the ultrastructure showed that SYT7 overexpression partially restored the volume of INS granules and the number of INS granules docked on PM (Fig. 5S–U). In conclusion, these results suggest that iron exposure inhibits the binding of OGG1 to the *SYT7* promoter and suppresses *SYT7* transcription. Overexpression of SYT7 can improve the decrease in INS secretion levels, the reduction in secretory vesicles numbers and the increase in blood glucose levels caused by iron overload.

Overexpression of SYT7 restored the impaired INS secretion in *db/db* mice

Islet β cell dysfunction is the key determinant of T2DM [34]. At present, the treatment of T2DM is mainly focused on oral hypoglycemic drugs and INS injections. However, what cannot be avoided is that β cell dysfunction continues to deteriorate with the passage of time [35]. Our retrospective analysis of 130 T2DM cases showed the ability of endogenous INS in response to the glucose load of β cells gradually decreased with the extension of medical history (Fig. S6), which is consistent with the discovery of Fonseca et al. [35]. Because *db/db* mice are animal models of INS resistance similar to human T2DM, and they also exhibit iron overload [36], we then used these mice to continuously detect the serum basal INS level from 3 weeks of age. The results show that *db/db* mice aged 3–6 weeks exhibited lower serum INS level than the WT group (Fig. 6A), indicating that iron overload-induced dysfunction of INS secretion in β cells may play a role in the progression of T2DM, and if so, improving pancreatic islet β cell function in pre-diabetic stage may be an effective strategy to prevent or treat T2DM. Therefore, we overexpressed SYT7 in *db/db* mice to verify whether it has the effect of alleviating T2DM symptoms. Meanwhile, Trulicity was used as the positive control, and DFO, an iron chelating agent, was used to verify whether reduced iron levels can improve the symptoms of T2DM. SYT7 was successfully overexpressed in β cells of *db/db* mice by in situ injection of AAV8-null or AAV8-SYT7 into the bile duct (Fig. S7A). There was no significant difference in weight between the *db/db* groups after injection, except for a slight increase in AAV8-SYT7 group from 6 weeks (Fig. 6B). All *db/db* mice showed an increasing serum INS level, which was gradually decreased with AAV8-SYT7/Trulicity/DFO treatment after 5 weeks. Noticeably, SYT7 significantly increased serum INS level 1 week after injection and had a better effect in maintaining

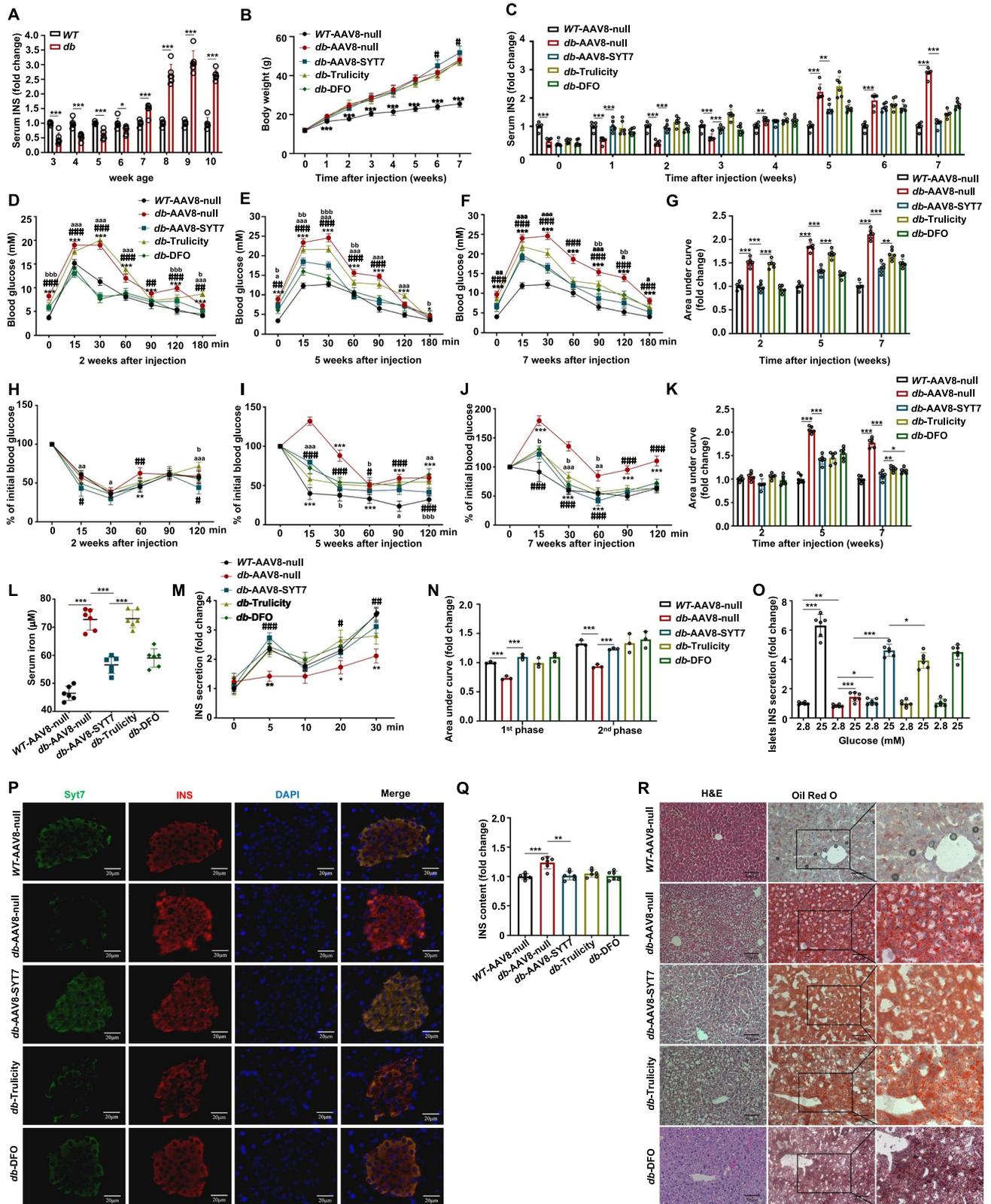


Fig. 6 Overexpression of SYT7 restored the impaired INS secretion in *db/db* mice. **A** Serum basal INS levels of the WT and *db/db* mice at 3–10 weeks of age. $n=6$ in each cohort. $*P<0.05$, $***P<0.001$. **B** Body weight changes of *db/db* mice after 0–7 weeks of AAV8-SYT7/Trulicity/DFO injection. The injection started at the age of 3 weeks, which was recorded as the 0 week after injection. $n=6$ in each cohort. *Indicates the *db*-AAV8-null group compared with the WT-AAV8-null group, $***P<0.001$. #Indicates that the *db*-AAV8-SYT7 group was compared with the *db*-AAV8-null group. $^{\#}P<0.05$. **C** The serum basal INS content of *db/db* mice after AAV8-SYT7/Trulicity/DFO injection for 0–7 weeks. $n=6$ in each cohort. $**P<0.01$, $***P<0.001$. **D–G** After AAV8-SYT7/Trulicity/DFO was injected for 2 weeks, 5 weeks and 7 weeks, the results of IPGTT experiment and corresponding AUC. $n=6$ in each cohort. *Indicates the *db*-AAV8-null group compared with the WT-AAV8-null group, $*P<0.05$, $***P<0.001$. #Indicates that the *db*-AAV8-SYT7 group was compared with the *db*-AAV8-null group. $^{\#}P<0.01$, $^{\#\#}P<0.001$. ^aIndicates that the *db*-Trulicity group was compared with the *db*-AAV8-SYT7 group, $^aP<0.05$, $^{aa}P<0.01$, $^{aaa}P<0.001$. ^bIndicates that the *db*-DFO group was compared with the *db*-AAV8-SYT7 group, $^bP<0.05$, $^{bb}P<0.01$, $^{bbb}P<0.001$. In the comparison of the AUC of each group, $**P<0.01$, $***P<0.001$. **H–K** After AAV8-SYT7/Trulicity/DFO injection for 2 weeks, 5 weeks and 7 weeks, the results of IPITT experiment and corresponding AUC. $n=6$ in each cohort. *Indicates that the *db*-AAV8-null group compared with the WT-AAV8-null group, $*P<0.05$, $**P<0.01$, $***P<0.001$. #Indicates that the *db*-AAV8-SYT7 group was compared with the *db*-AAV8-null group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. ^aIndicates that the *db*-Trulicity group was compared with the *db*-AAV8-SYT7 group, $^aP<0.05$, $^{aa}P<0.01$, $^{aaa}P<0.001$. ^bIndicates that the *db*-DFO group was compared with the *db*-AAV8-SYT7 group, $^bP<0.05$, $^{bb}P<0.001$. In the comparison of the AUC of each group, $*P<0.05$, $**P<0.01$, $***P<0.001$. **L** Serum iron level of *db/db* mice in each group 3 weeks after AAV8-SYT7/Trulicity/DFO injection. $n=6$ in each cohort. $***P<0.001$. **M, N** 3 weeks after AAV8-SYT7/Trulicity/DFO injection, the INS biphasic secretion level of *db/db* mice was detected by hyperglycemic clamp test and the AUC was calculated. 0–10 min is defined as the first phase and 10–30 min is defined as the second phase. $n=3$ in each cohort. *Indicates the *db*-AAV8-null group compared with the WT-AAV8-null group, $*P<0.05$, $**P<0.01$. #Indicates the *db*-AAV8-SYT7 group compared with the *db*-AAV8-null group, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. In the comparison of the AUC of each group, $***P<0.001$. **O** The effect of AAV8-SYT7 on GSIS of isolated islets of *db/db* mice was detected by ELISA. $n=5$ in each cohort. $*P<0.05$, $**P<0.01$, $***P<0.001$. **P** 3 weeks after AAV8-SYT7/Trulicity/DFO was injected into *db/db* mice, INS and Syt7 protein levels in β cells were detected by immunofluorescence. Scale bar 20 μm . **Q** The effect of AAV8-SYT7 on the content of INS in islets of *db/db* mice was detected by ELISA. $n=5$ in each cohort. $**P<0.01$, $***P<0.001$. **R** 7 weeks after *db/db* mice were injected with AAV8-SYT7/Trulicity/DFO, the liver tissues were observed by H&E and oil red O staining. Scale bar 50 μm

the INS stationarity (Fig. 6C). Importantly, the IPGTT results showed that blood glucose levels in the AAV8-SYT7 group were lower than that in the Trulicity group at 2 weeks, 5 weeks and 7 weeks after injection (Fig. 6D–G). The IPITT results showed that AAV8-SYT7 could also improve INS sensitivity to a certain extent accordingly (Fig. 6H–K). Interestingly, SYT7 overexpression or DFO treatment significantly downregulated the serum excessive iron levels, by contrast, the Trulicity group had no such effect (Fig. 6L). In vivo and ex vivo experimental

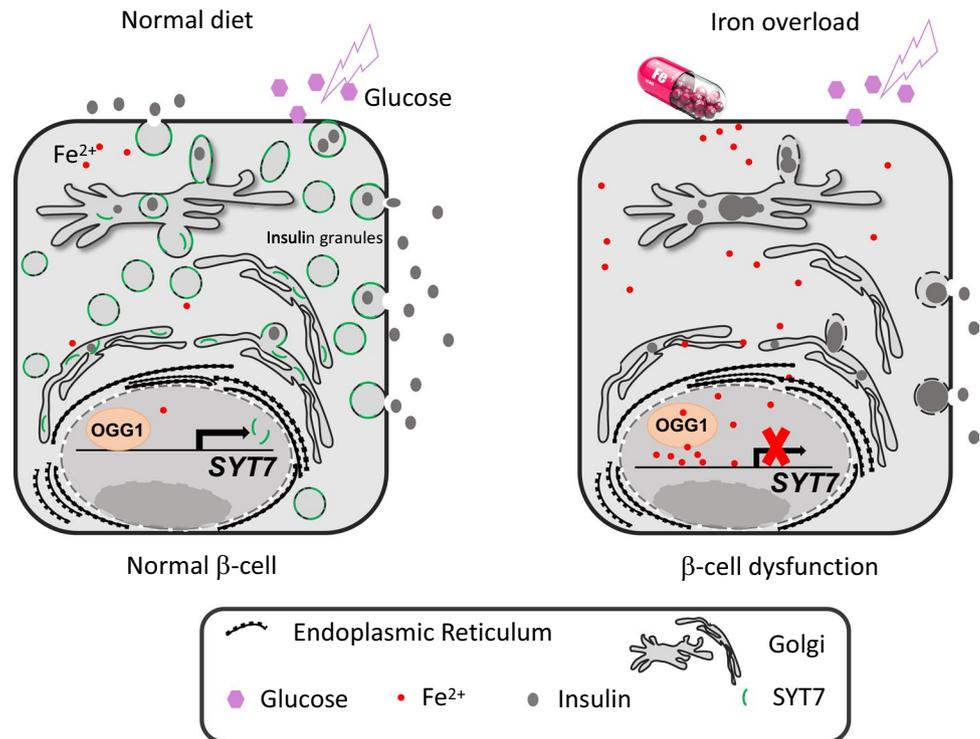
results showed that overexpression of SYT7 improved INS secretion after glucose loading (Fig. 6M–O). Immunofluorescence and ELISA results showed increased INS content in islet β cells of *db/db* mice and such increase was restored by the treatment of AAV8-SYT7, Trulicity or DFO (Fig. 6P, Q, Fig. S7B). In addition, since T2DM is usually complicated by fatty liver, the regression of intrahepatic fat can partially indicate an effective control of T2DM [37]. To further confirm the effect of AAV8-SYT7, Trulicity or DFO on *db/db* mice, 7 weeks after injection, mouse liver sections were stained with H&E and oil red O. AAV8-SYT7, Trulicity and DFO treatment all improved the swelling, disordered arrangement and lipid deposition of hepatocytes in *db/db* mice, and AAV8-SYT7 had the best effect (Fig. 6R). In conclusion, our results indicate that overexpression of SYT7 could effectively restore the impaired INS secretion in *db/db* mice and partially improve diabetes symptoms.

Discussion

This study reveals a novel mechanism for the effect of iron overload on islet β cell dysfunction. We find that excessive iron inhibits INS secretion through suppressing the transcriptional regulation of SYT7 by OGG1 (Fig. 7). SYT7, a major component of the exocytotic machinery in neurons, is also the major Syt in islet β cells shown to mediate glucose-stimulated INS secretion [38]. Previous studies have clarified that SYT7 functions not only in exocytotic fusion but also in the replenishment of releasable secretory granule (SG) pools in human β cells [38, 39]. However, SYT7's precise expression regulation is still not fully understood. Velde et al. report that CREB is critical for β cell gene expression including SYT7 via enhancer activation [40]. Human SYT7 is also discovered to be regulated by TWIST1 in non-small cell lung cancer [41]. In our work, we identify SYT7 as a new downstream target gene of OGG1 and demonstrate that OGG1/SYT7 axis is essential for INS secretion.

Indeed, OGG1 has long been considered to be an important factor in the metabolic process. *Ogg1*^{-/-} mice exhibit liver and skeletal muscle lipid metabolic disorders and impaired glucose tolerance [10]. OGG1 is of great importance responsible for oxidative DNA damage in rat model of diabetes [42]. Overexpression of OGG1 could protect β cell from free fatty acid-induced apoptosis [43]. However, the exact mechanism of OGG1 in β cells is not clear. In this study, we find that *Ogg1* deletion down-regulated not only *Syt7*, but also the other genes in the SYTs family, which suggest that OGG1 may be involved in a wider range of vesicle transport. Further investigation will be necessary to understand how OGG1 regulates SYTs expression. Given that the

Fig. 7 Schematic diagram demonstrating INS secretion upon glucose stimulation. Left panel: β cell responds to glucose signal when the Fe^{2+} level is maintained in a constant range. OGG1 binds to *SYT7* promoter and upregulates *SYT7* expression, the latter is required for transport vesicles formation and INS secretion. Right panel: excessive iron disrupts the transcriptional regulation of *SYT7* by OGG1, reduces the number of INS transport vesicles, agglomerates INS granules and inhibits INS secretion



neurotransmitter release is the classical function of SYTs family, OGG1/*SYT7* axis may also provide insight into a new pathway for neurotransmitter release under oxidative stress.

Interestingly, knockdown of *Ogg1* down-regulated many secretion-related genes, most of which were also down-regulated with FeSO_4 treatment (Fig. S8). Our data show that OGG1 positively regulates *SYT7* transcription, however, *db/db* mice and β -DKO (*Swi/Snf* deficiency leads to dysfunction) mice exhibit negative correlation between *Ogg1* and *Syt7*. Excessive iron may be appropriate to explain this contradictory phenomenon. It has been established that ROS levels are increased in diabetic mice [44], as the key factor responding to oxidative stress, OGG1 is increased accordingly, but *SYT7* could not be effectively up-regulated by OGG1 even if there are a large amount of OGG1 in cells, because excessive iron perturbs this regulation. Similarly, in our iron overload model, excessive iron increases intracellular ROS and *Ogg1* levels, while inhibits *Syt7* expression.

Such mechanism of action of iron also applies to HFD model. We isolated the serum of long-term (more than 6 months) HFD mice and found that the serum Fe^{2+} level was significantly increased (Fig. S9). Cheng et al. show that the use of iron chelation deferoxamine (DFO) improved the expression of genes related to glucose transport and glycolysis in HFD mouse and human islets, and also improve INS secretion capacity [29]. Together, iron plays a key role in occurrence and development of diabetes. However, the application of iron chelation therapy in the treatment of

diabetes is controversial [45]. Therefore, finding the exact downstream pathway of iron is important for the treatment of diabetes.

After confirming that *SYT7* is the downstream effector mediating excessive iron-induced β cell dysfunction, we thus investigated the efficacy of AAV8-*SYT7* in the treatment of T2DM. As expected, AAV8-*SYT7* effectively improves impaired glucose tolerance, alleviated IR, and improves liver lipid deposition. Importantly, blood INS level fluctuates less compared with GLP-1RA (Fig. 6C), which indicates that *SYT7* overexpression has an advantage in controlling the stability of blood glucose. H&E staining shows that there is no damage to major organs by AAV8-*SYT7* (Fig. S5B). Importantly, our data showed that the use of iron chelating agent or AAV8-*SYT7* effectively improved IR, which suggests that iron clearance or *SYT7* overexpression increases the sensitivity of target tissues to INS. However, whether this increase is an indirect feedback caused by reduced glucose toxicity or a direct effect of increased INS utilization due to improved INS release still requires further exploration. In conclusion, our study suggests that AAV8-*SYT7* is a valuable treatment modality for T2DM. Notwithstanding, there are still many issues that need to be considered. First, AAV8-*SYT7* may be effective in patients diagnosed at early stage, because those with a long history of T2DM are usually associated with progressive β cell loss. Second, our data show that *SYT7* overexpression in *db/db* mice increases weight to a certain extent, which may be due to the increased availability of INS. Therefore, for those obese

diabetic patients, SYT7 overexpression combined with weight loss strategies may be necessary. Considering the effects of off-target, immunogenicity and other issues of AAV vectors [46–48], exploring safer ways to overexpress or phosphorylate SYT7 may be a new idea for the treatment of T2DM [49].

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00018-023-04802-y>.

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Author contributions XZ and FP designed the study. XZ and YM obtained the data. MS, JX, SC and ZH contributed to and supervised the in vivo experimentation. MH and LH helped with the imaging analysis. MC and TJ provided support for the collection of outpatient cases. ZG provided funding support and support for communication with the administrative department. XZ drafted the manuscript. FP provided support for cooperation with the technology platform and manuscript revision. All authors reviewed and approved the manuscript. FP and ZG are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials All data associated with this study are present in the paper or Supplementary Materials.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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