Supplementary materials and methods

Cell culture and transfection

C2C12 (a mouse muscle-derived cell line), NIH3T3 (a preadipocyte cell line of mouse embryonic origin with fibroblast morphology), and HEK293 cells (a human embryonic kidney cell line) were cultivated in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA; Cat. no. 31600-083) with 10% fetal bovine serum (FBS: AusGeneX Industries) and 1% (v/v) penicillin-streptomycin (Sangon Biotech). NIH3T3-L1 adipoblasts were cultured in DMEM with 10% FBS, 2 mM L-glutamine, 100 units/liter penicillin, and 100 g/liter streptomycin. HL-7702 cells (a human normal hepatocyte cell line) were grown in RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were divided into plates with three replicates and cultured overnight to get a 70%-90% confluence and then transfected with plasmids using Lipofactamine3000 (Invitrogen).

Cell proliferation assays

Cells were seeded in 96-well plates in medium containing 0.1% FBS and 0.5% premium grade bovine serum albumin (free of insulin-like growth factor contaminants), and ligands were added 24 h later (concentrations: 10, 5 and 1 nM). After 48 h of incubation, 10 μL of CCK-8 reagent (China, Beyotime) was added to each well and incubated for a further 1 h. Absorbance values were read at 450 nm using a microplate analyzer.

Immunoprecipitation

The cultured cells were washed with cold PBS and treated with 1× lysis buffer containing protease inhibitors. After complete lysis, the supernatant was collected by centrifugation. This lysate was then incubated with anti-GRP78 (Abcam), normal IgG (Beyotime) working solution, and protein A+G beads (Beyotime) with gentle rocking overnight at 4 ℃. The mixture was separated by a magnetic rack, washed with lysis buffer, and then analyzed by western blotting.

Immunofluorescence staining for colocalization study

C2C12 cells grown on coverslips were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 (v/v), and then incubated with anti-insulin, anti-GRP78, and anti-PDI (Protein disulfide-isomerase) antibodies (Abcam), respectively. After incubation with goat anti-rabbit mouse universal secondary antibodies (Abmart), TSA staining solution was added and the labeled immunofluorescence signals were analyzed using an Ultra High-Resolution Laser Scanning Confocal Microscope (Carl Zeiss).

Glucose tolerance test

For the intraperitoneal glucose tolerance test (IPGTT), mice were fasted for 16 h followed by an intraperitoneal injection of D-glucose (2 g/kg body weight). The blood glucose of each mouse was measured with a glucometer (Roach) at 0, 15, 30, 60, 90, 120,150, and 180 min after glucose administration. The area under the curve (AUC) was calculated to further evaluate the IPGTT result.

Testing of the GAIS system:

Dose-dependent glucose-inducible SIA expression

The C2C12 cells expressing GAIS system were discarded the supernatant, washed with PBS for 3 times, and starved with glucose concentration 2.5 mM for 12 h. The cells were treated with medium containing different concentrations of glucose, and the supernatant was collected 4 h later for SIA expression analysis. SIA expression was measured by Elisa.

Time Kinetic Detection of SIA release

The C2C12 cells expressing GAIS system were discarded the supernatant, washed with PBS for 3 times, and starved with glucose concentration 2.5 mM for 12 h.The cells were stimulated with glucose of 15 mM, and the culture medium was collected every 30 minutes after stimulation. The culture medium was centrifuged at 1000 g for 5 min, and the supernatant was taken to detect the concentration of SIA.SIA expression was measured by Elisa.

GAIS reversibility detection

C2C12 cells expressing GAIS plasmid were discarded supernatant and washed with PBS for 3 times. After starvation with glucose concentration 2.5 mM medium for 12 h, samples were collected per 30 min in “on-off-on” group after adding 15 mM glucose concentration culture medium. After 120 min, the supernatant was discarded. After PBS washing, the cells were starved again for 12 h. The supernatant was discarded and cells were treated with fresh 2.5 mM medium, and samples were collected every 30 min until 120 min.C2C12 cells were then treated again with 15 mM glucose, and

cell supernatants were collected at intervals of 30 to 120 min. In the :“off-on-off” group, after changing the fresh medium of 2.5 mM , samples were collected once per 30 min until 120 min. Then after 15 mM sugar concentration medium treatment, the supernatant was collected once per 30 min to 120 min, PBS washing 3 times and then added glucose concentration 2.5 mM starvation for 12 h, then changed to fresh glucose concentration 2.5 mM medium, and continued sampling per 30 min until 120 min. SIA expression was measured by Elisa.

Immunohistochemical & HE analysis

Intraabdominal organs (pancreas, spleen, liver, and kidney) and TA muscles were dissected from the euthanized mice, washed with PBS buffer and fixed in 4% fresh neutral paraformaldehyde solution. For immunohistochemical analysis, the samples were marked by the mouse insulin primary antibody (Abcam) followed by the goat anti-mouse secondary antibody (Abmart). The immunohistochemical images were taken by a microscope (BA400 Digital, MOTIC CHINA GROUP CO., LTD). The tissue sections were stained with hematoxylin and eosin and images were photographed by a microscope at 100 × magnifications (Leica DMI 4000B, Germany).

HbA1c and oxidative stress (MDA, SOD, GSH and T-AOC) detection

The levels of glycosylated hemoglobin (HbA1c) were detected on the 60^{th} day with a glycosylated hemoglobin assay kit (NanJing JianCheng Bioengineering Institute, China) according to the manufacturer’s instructions. The levels of SOD, T-AOC,

MDA, and GSH in serum, kidney, and heart were determined on the 60th day with commercial kits (Solarbio, China) according to the manufacturer's instructions.

Measurement of Serum IgG and IgM

The sera were collected from SIA-treated mice and healthy mice before after or one and two-week treatment. The sera were stored at -80℃ before measurement. The IgG and IgM levels were measured using ELISA kits (Solarbio; Cat#SEKM-0098 Lot No. 20210918 for IgG and Cat#SEKM-0102 Lot No. 20210918 for IgM).

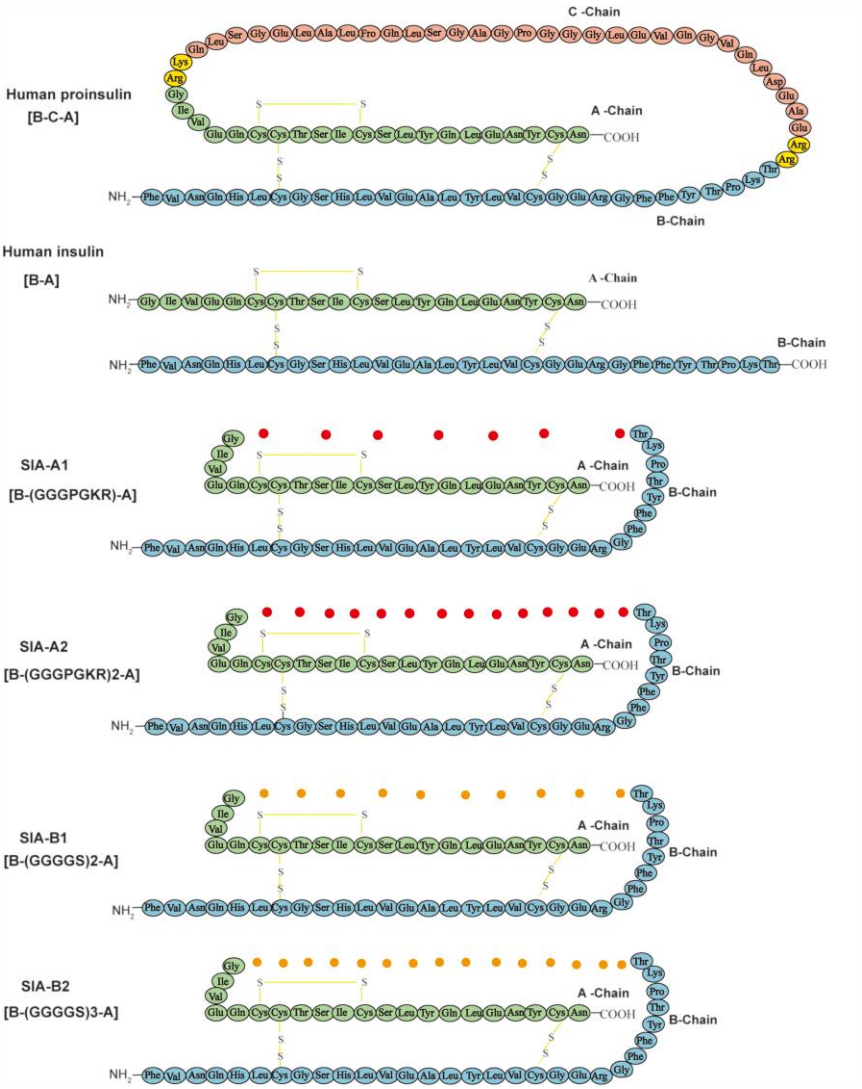
Serum inflammatory cytokines.

Mouse serum levels of interferon (IFN)-γ and IL-6 were quantified with mouse Ultrasensitive IFN-γ ELISA (Solarbio; catalog no. SEKM-0031) and IL-6 ELISA (Solarbio; catalog no. SEKM-0007) (all from Solarbio).

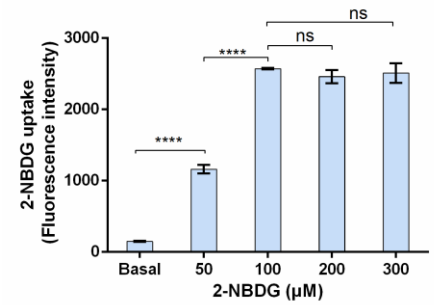
Homeostatic model assessment (HOMA-IR) in mice

HOMA-IR analysis was used to assess insulin resistance. After overnight fasting, HOMA-IR were calculated by using the following formula: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (mU/L)]/22.5.

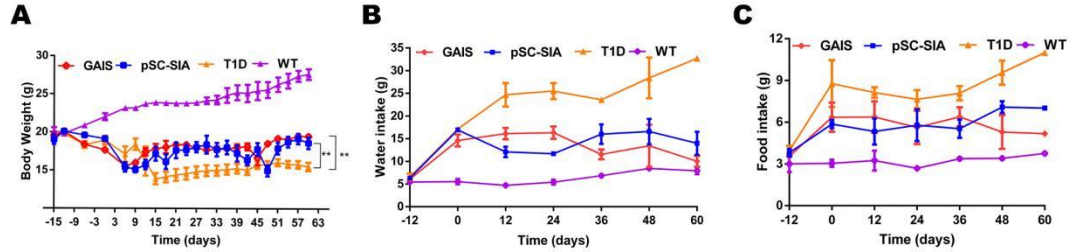
Supplementary figures



Supplementary Figure S1. Primary sequences of human proinsulin, human insulin and SIAs

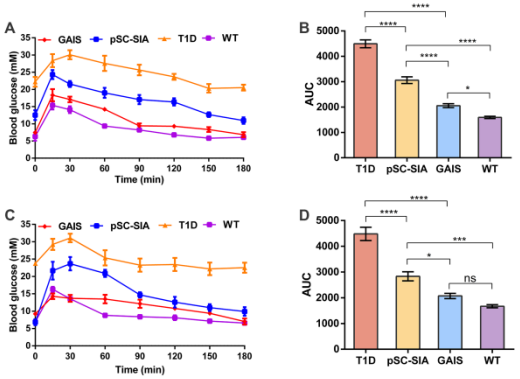


Supplementary Figure S2. 2-NBDG uptake in 3T3-L1 adipocyte cells



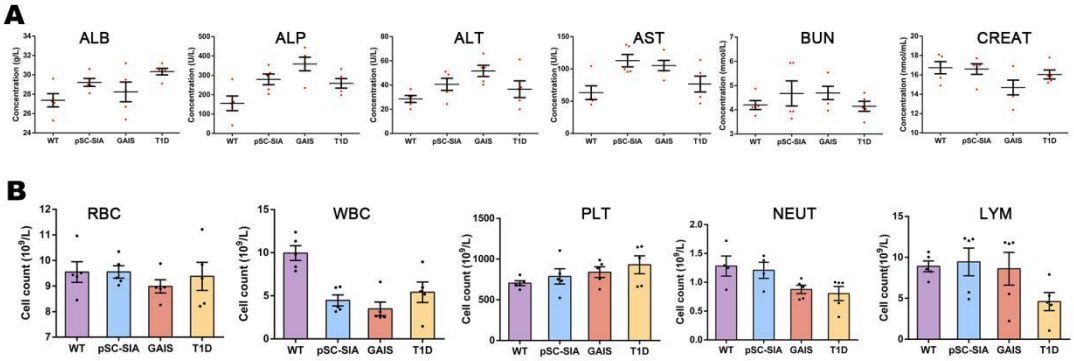
Supplementary Figure S3. Metabolism amelioration by GAIS in T1D model mice

(A) Body weight. (B) Water intake. (C) Food intake. Data were presented as the mean ± SEM; one-way ANOVA, **p < 0.01. n = 6.



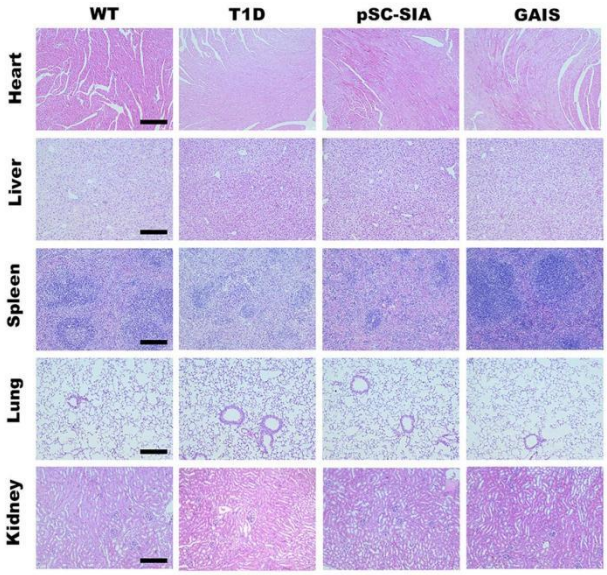
Supplementary Figure S4. IPGTT of type-1 diabetic mice treated with GAIS.

(A) IPGTT on the day 39. n = 5. (B) Area under the curve (AUC) analysis of the IPGTT on the day 39. n = 5. (C) IPGTT on the day 48. n = 5. (D) AUC analysis of the IPGTT on the day 48. n = 5.



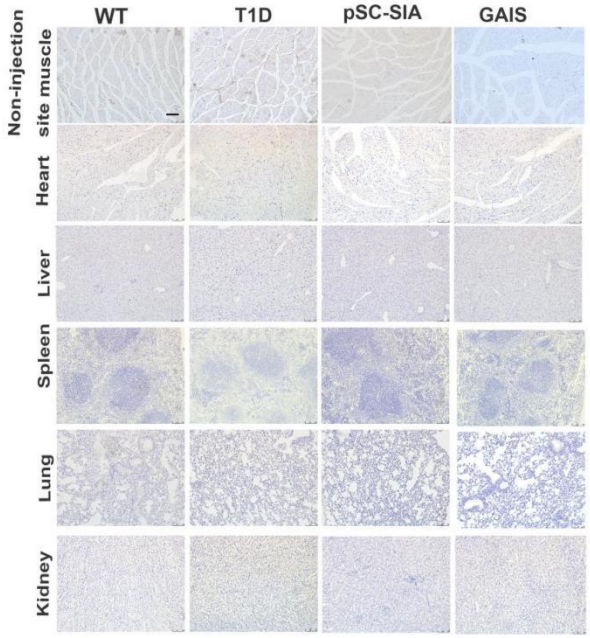
Supplementary Figure S5. Tests of liver function, kidney function and blood routine of T1D mice

(A) Values of serum albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (CREAT). (B) Values of red blood cell (RBC), white blood cell (WBC), platelet (PLT), neutrophil (NEUT), and lymphocyte (LYM). Time point: the day 18 posttreatment. Data were presented as the mean ± SEM; one-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. n = 5.



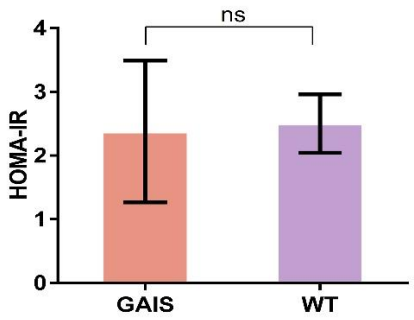
Supplementary Figure S6. Pathological analysis of different organs by HE staining

Time point: the day 60 posttreatment. Scale bar: 200 μm. n = 3.



Supplementary Figure S7. SIA immunostaining of tissue sections from various organs 18 days after gene delivery

Gene therapy vector types are given above the panels. Intraabdominal tissues used in the analysis are listed on the left side of each panel. Immunohistochemistry analysis was performed on paraffin-embedded tissues using an anti-SIA antibody. Scale bar: 100 μm. n = 3.



Supplementary Figure S8. Homeostasis model assessment of insulin resistance (HOMA-IR) was analyzed on day 24 after GAIS system treatment.n=5.