Data S1. Construction of the TLR4 truncation rat model.

Experimental materials

Main instrument: Microinjection system.

Experimental procedure

Program design, synthesis target oligo. Using CRISPR/Cas9 gene targeting technology, a gRNA targeting the TLR4 gene was constructed and transcribed into mRNA *in vitro*, directing the Cas9 protein to cleave the DNA duplex at a specific site.

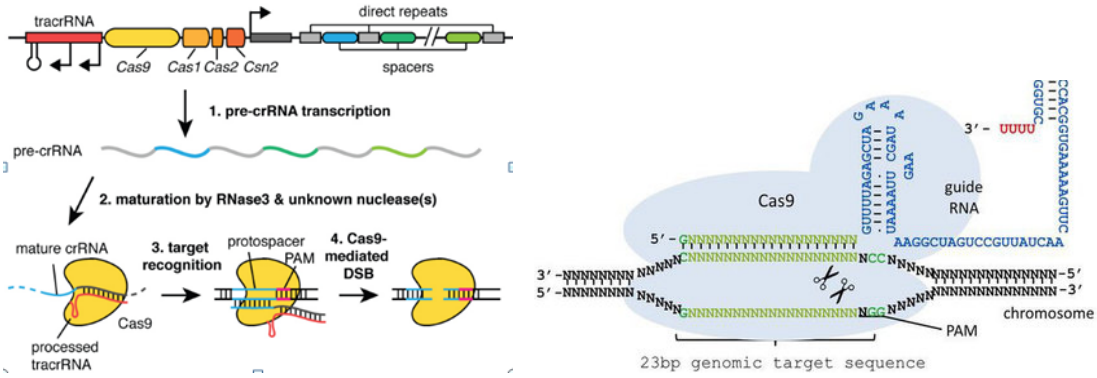


Figure 1. CRISPR/Cas9 gene targeting schematic diagram (Shanghai GenePharma Co. Ltd.).

Embryonic donor rat (SD) superovulation. The donor female rats were treated with pregnant mare serum gonadotrophin. Human chorionic gonadotrophin was injected 46 h later, the male rats were mated, and the fertilized eggs were taken for microinjection the next day.

Microinjection and embryo transfer. Microscopically, the fertilized eggs with a clear pronucleus were injected. After injection, the cultured fertilized eggs were maintained at 37°C, 5% CO_2 for 30-60 min, and transplanted into the fallopian tube of the pseudo-pregnant mother for delivery.

Founder rat identification. The embryo-transplanted rats were born ~20 days after the operation, and DNA was extracted from the tail (or toe) and identified by PCR after ~7 days.

Founder rats are mated with wild-type rats (SD) to obtain F1. Male founder rats were 10 weeks old, and female rats were 8 weeks old. They were mated with wild-type heterosexual rats, and the rats were assessed by PCR 10 days after birth. If a positive rat was born, it meant that the transgene had been integrated into the germ cell and the marker line had been successfully established.

Target information

Gene name	Gene ID	Target
TLR4	29260	Target 1: atattacctaccaatgcatgg
		Target 2: aatttctcacaacttcagtgg

Identification result

Identification conditions: 604 p; Tm value, 57˚C. Identification primers:

Sense, (5’ to 3’)　AGTGCTTGTGTGGTAGAGGCAA;

anti-sense, (5’ to 3’)　CTGCCTACCAAATAGAAACCAGG.

TLR4 wild-type sequence

Cagtgcttgtgtggtagaggcaattagataggaagttcaatgttatctttgactgtatagctaatttaaaagaagactgggctatctgaaag
cttgtttaaaaacaaaataaatcacgaagaatggatggataggtggatggtatattgattagcatgtatatgggagttttaccatctcattatt
catctttggagaggagtgggaacacatacagtcgaaaagaataacattttgtgtgatttttacaggtacttcctaatattacctaccaatgc
atggatcagaatctcagcaaaatccctcatgacatcccttattcaaccaagaacctagatctgagcttcaacccccctgaagatcttaaga
agctatagcttctccaatttctcacaacttcagtggctggatttatccaggtaatgaatgagttttatttgctgcagactgtgaagtagttattt
ctatatcactgcatttttggctcagaagacctagatgtttctaagtaaatttcctttactcatctattcagtagacttagtccttgctgtaaatcct
tgggacagttacttttatttacctggtttctatttggtaggcagc

4.2. F0 generation information：

10#

AGAGGAGTGGGAACACATACAGTCGAAAAGAATAACATTTTGTGTGATTT

TT.ACAGGTACTTCCTAATATTACC.....

.....AGTGGCTGGATTTATCCAGGTAATGAATG

AGTTTTAT　　10#-1 -130bp

11#

TTTT.ACAGGTACTTCCTAATATTACC.....

.....TACTACATGTGAA

GTAGTTATTTCTATATCACTGCATTTTTGGCTCAGAAGACCTAGATGTT

11#-1　　-178bp +7bp(exon -149bp)

4.3. F1 generation information：

	F1 generation from 11# founder x WT
2#♂　3#♀　5#♂ 6#♀ 8#♀　11#♀　13#♂ TTTT.ACAGGTACTTCCTAATATTACC..... .....TACTACATGTGAA GTAGTTATTTCTATATCACTGCATTTTTGGCTCAGAAGACCTAGATGTT 11#-1　　-178bp +7bp(exon -149bp)	

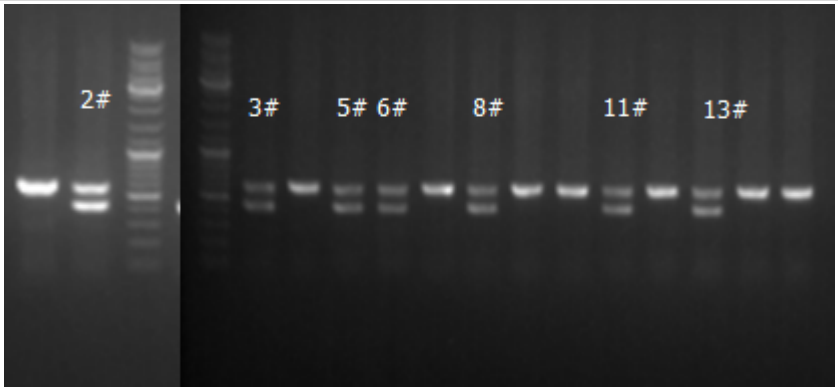


Figure 2. F1 identification result: 2#, 3#, 5#, 6#, 8#,11#,13# -178 bp +7 bp (exon -149 bp)

4.4. F2 generation information：

	F2 generation from 2# ♂ x 8# ♀ 11# ♀ F1 generation (from 11# ♀ founder x WT)

KO: 2# 8# 24# 32# 45# 49# (3 ♀ 3 ♂) -178 bp +7 bp (exon -149 bp)

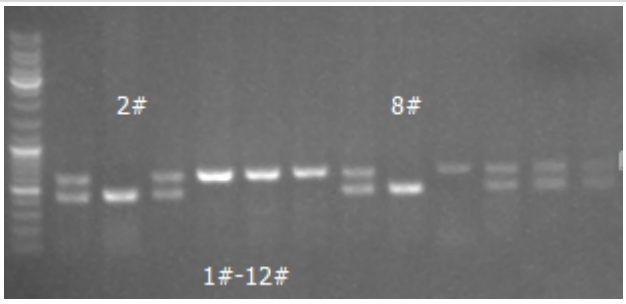
HZ: 1# 3# 7# 10# 11# 12# 13#-18#;20#-23#;25#-30#;33# 36# 37#-41#;43# 47# 48# 50# 52# 54# 55#

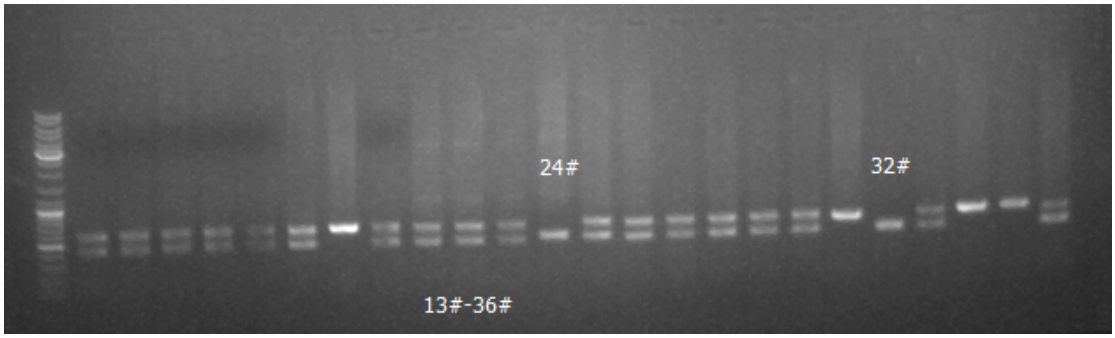
TTTT.ACAGGTACTTCCTAATATTACC.....

.....TACTACATGTGAA

GTAGTTATTTCTATATCACTGCATTTTTGGCTCAGAAGACCTAGATGTT

11#-1 -178 bp +7 bp (exon -149 bp)





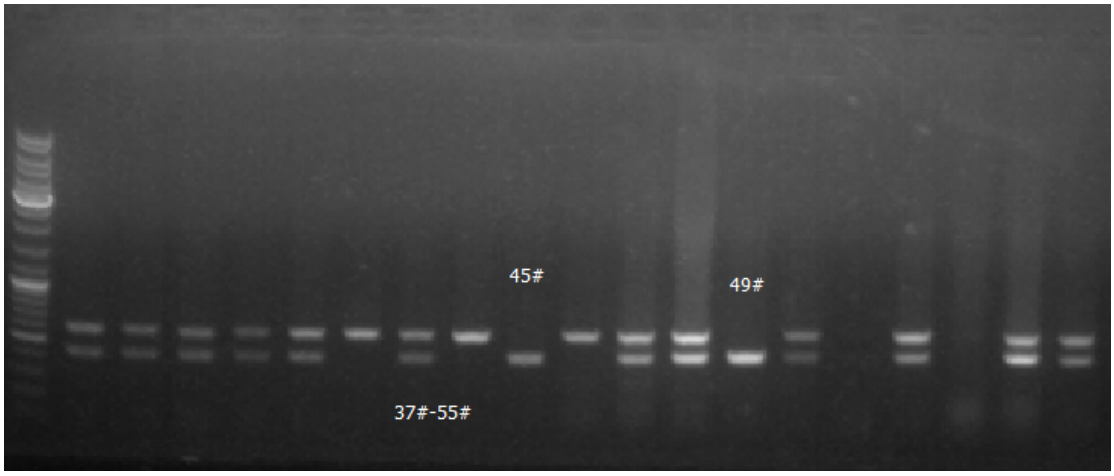
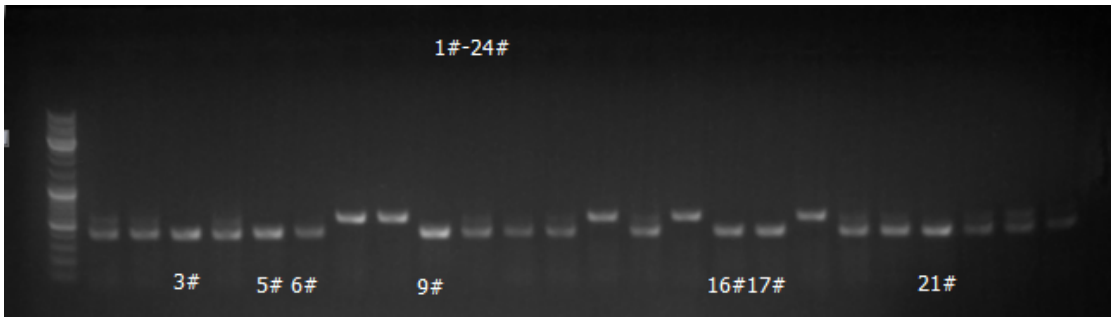


Figure 3. F2 identification result: 2#, 8#, 24#, 32#, 45#, 49#: -178bp +7bp (exon -149bp)

Homozygous information：

	B1: Homozygous passage from F1 generation 2#♂ 5#♂ 13#♂ x 3#♀ 6#♀ 8#♀ 11#♀F1 generation (from 11# ♀ founder x WT) (-178 bp） 1#-37# B2: Homozygous passage from F2 generation HO 3♀ x F1 generation HZ 3♂ (from F0 generation 11#: -178 bp) 1#-34#
B1：3# 5# 6# 9# 16# 17# 21# 25# 26# 28#：-178 bp KO B2：1# 2# 3# 4# 5# 9# 12# 14# 15# 21# 24# 25# 26# 27# 30# 33#： -178 bp KO TTTT.ACAGGTACTTCCTAATATTACC..... .....TACTACATGTGAA GTAGTTATTTCTATATCACTGCATTTTTGGCTCAGAAGACCTAGATGTT 11#-1 -178bp +7bp(exon -149bp)	



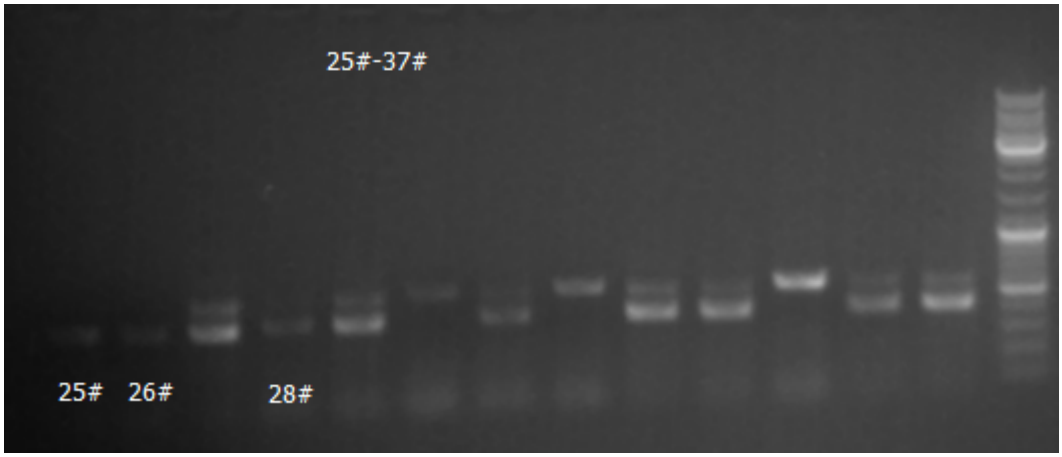
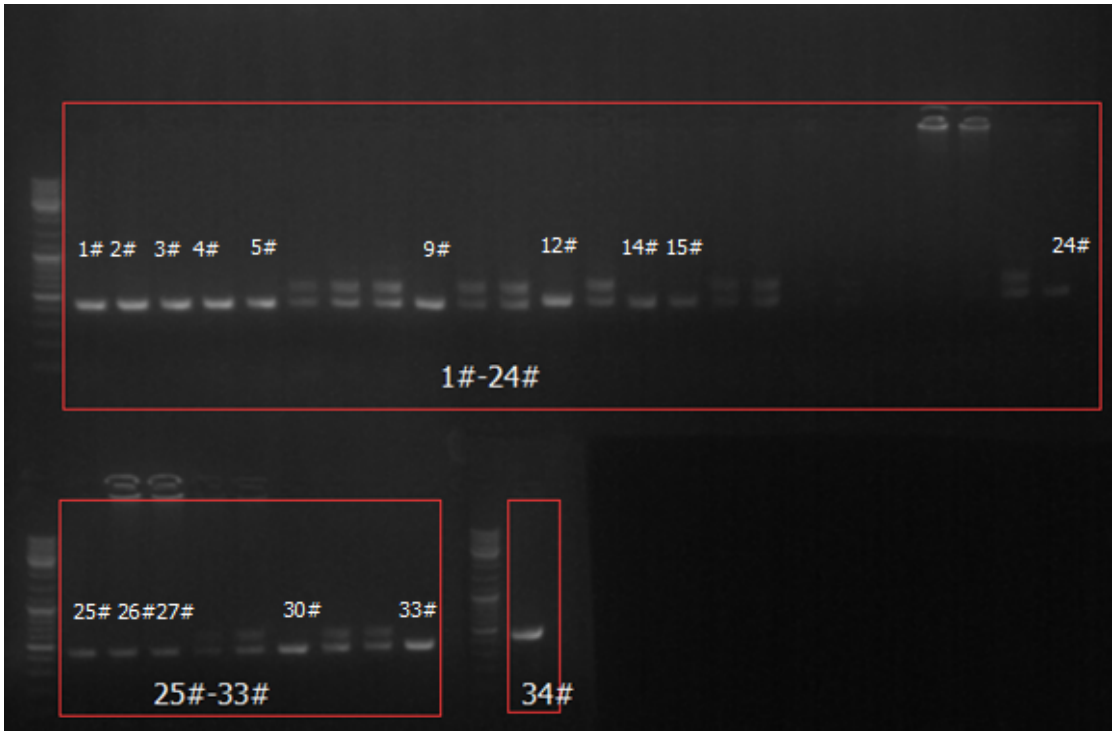


Figure 4. B1 identification result: 3#, 5#, 6#, 9#, 16#, 17#, 21#, 25#, 26#, 28#: (Total 10) -178 bp.



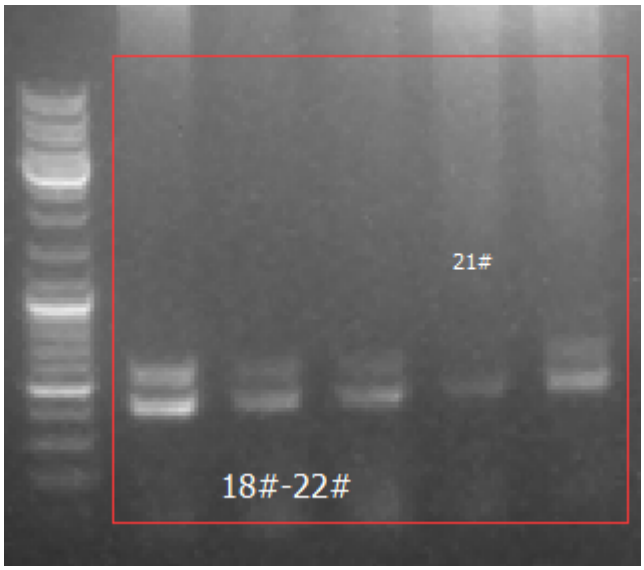


Figure 5. B2 identification result: 1#, 2#, 3#, 4#, 5#, 9#, 12# ,14#, 15# ,21#,24#, 25#, 26#, 27#, 30#, 33#: (16 in total) -178 bp.

Figure S1. Solvent BSA/ethanol had no effect on β-cell apoptosis, insulin secretion, oxidative stress and TLR4-NF-κB subunit P65 expression. The cells were treated with BSA/ethanol in complete medium without palmitic acid for 24 h as controls. (A) Apoptosis rate of β-cells. Magnification, 10x40. (A-a) Representative images from fluorescent microscopy in each group. (A-b) The collective analyses of all three independent experiments. (B) Insulin secretion. (C) Expression of NADPH p47 phox, NF-κB subunit P65 and TLR4. (C-a) Representative western blot images in each group. (C-b) The ratio of target protein to GAPDH. (D) The levels of ROS in β-cells. (D-a) Representative images from fluorescent microscopy in each group. (D-b) The collective analyses of all three independent experiments. NC group vs. BSA/ethanol group, P>0.05. BSA, bovine serum albumin; NC, negative control; TLR4, toll-like receptor 4; BIS, basal insulin secretion; GSIS, glucose-stimulated insulin secretion; ROS, reactive oxygen species.

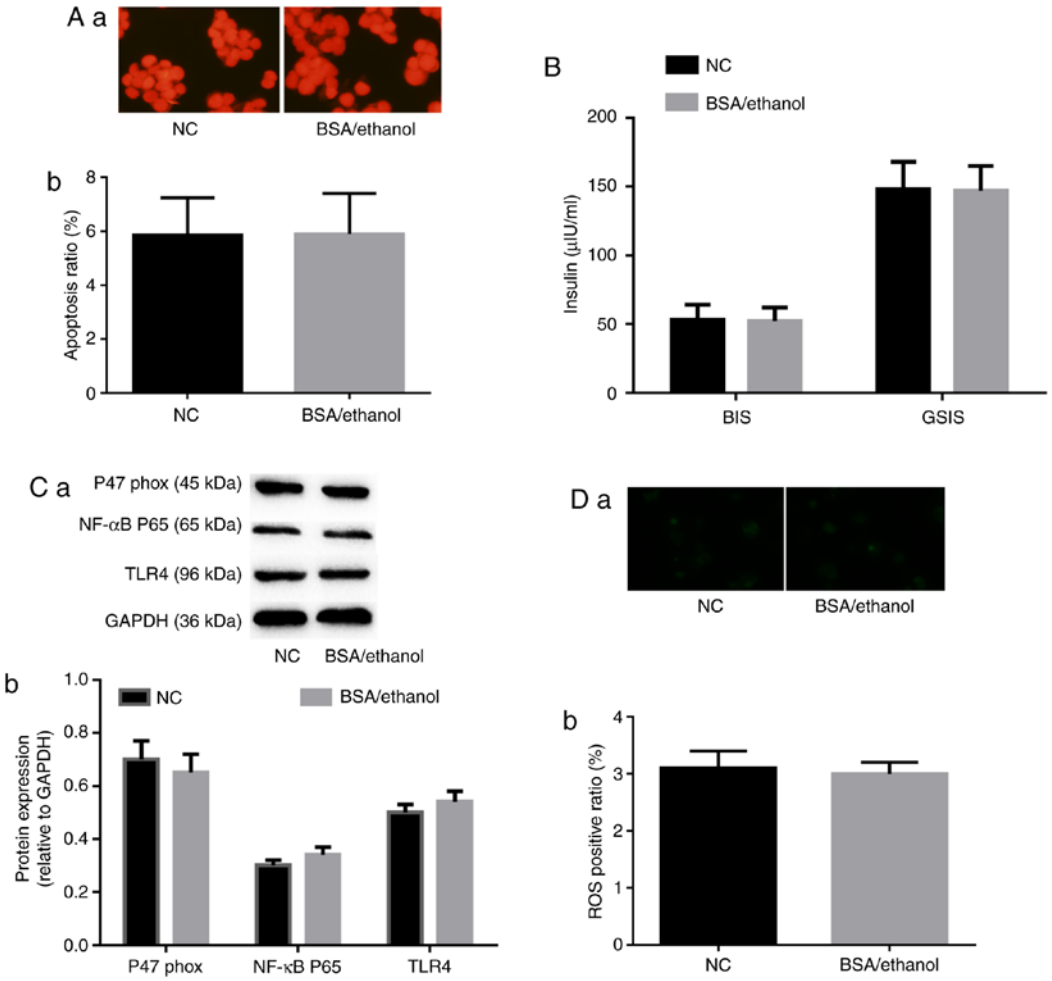


Figure S2. PCR electropherogram for the synthesis of TLR4 *in vitro*. The PCR-amplified fragment size was 2,549 bp. Marker (from top to bottom): 5 kb; 3 kb; 2 kb; 1.5 kb; 1 kb; 750 bp; 500 bp; 250 bp; 100 bp.

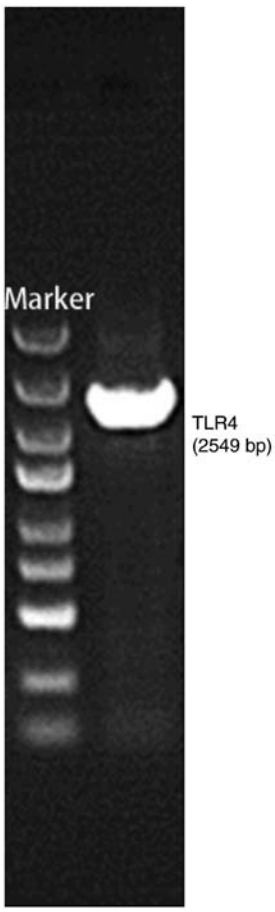


Figure S3. Effect of exenatide on the weight of HFD rats. HFD, high-fat diet; NC, negative control; EXE, exenatide.

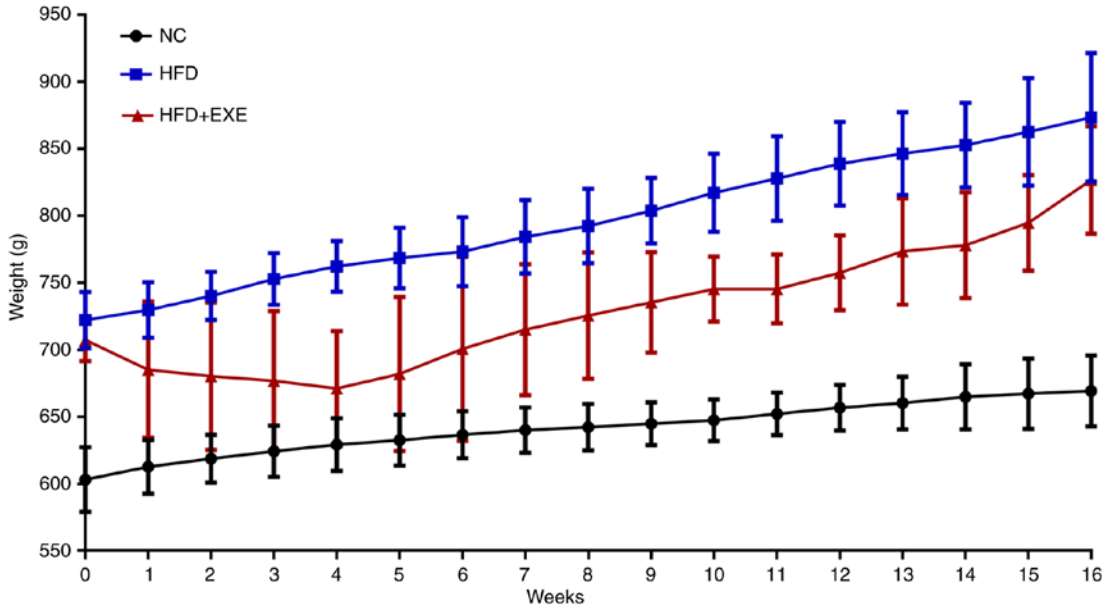


Figure S4. Expression of TLR4 mRNA in the pancreas of SD and TLR4$^{trun/trun}$ rats. The reverse transcription-semiquantitative PCR results showed that TLR4 is expressed in both SD and TLR4$^{trun/trun}$ rat pancreases. However, the molecular weight of TLR4 in the TLR4$^{trun/trun}$ group was 436 bp, while it was 600 bp in the SD group. Marker (from top to bottom): 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp. TLR4, toll-like receptor 4; trun, truncated; SD, Sprague Dawley.

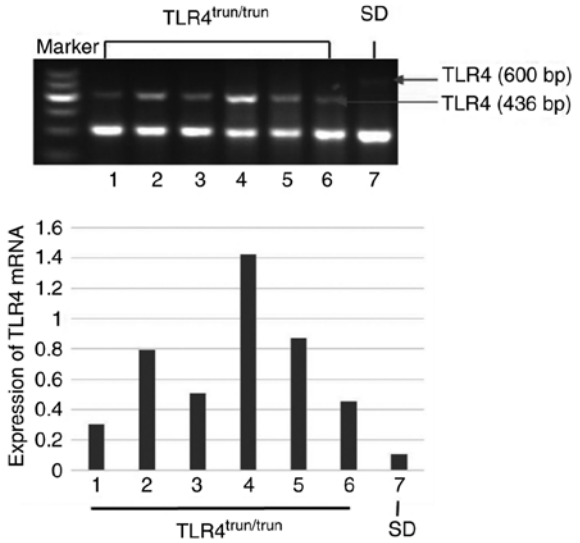


Table SI. Ingredients in the high-fat diet.

Ingredient	Quantity, g	kcal
Casein, 80 Mesh	200	800
L-Cystine	3	12
Corn starch	0	0
Maltodextrin 10	125	500
Sucrose	68.8	275.2
Cellulose, BW200	50	0
Soybean oil	25	225
Lard[a]	245	2,205
Mineral mix, S10026	10	0
Di-calcium phosphate	13	0
Calcium carbonate	5.5	0
Potassium citrate, 1 H_2O	16.5	0
Vitamin mix, V10001	10	40
Choline bitartrate	2	0
FD&C blue dye #1	0.05	0
Total	773.85	4,057

Formulated by E. A. Ulman, Ph.D., Research Diets, Inc., 26th August 1998 and 3rd November 1999. [a]Typical analysis of cholesterol in lard=0.95 mg/g. Cholesterol (mg)/4,057 kcal=232.8. Cholesterol (mg)/kg=300.8.

Table SII. EXE inhibits HFD-induced oxidative stress in β-cells independent of blood glucose fluctuations.

Parameter	NC	HFD	HFD+EXE	$TLR4^{trun/trun}$	$TLR4^{trun/trun}$+HFD	$TLR4^{trun/trun}$+HFD+EXE	P-value
NADPH p47 (phox)	0.45±0.06	1.2±0.05	0.5±0.03	0.35±0.04	1.10±0.03	0.4±0.02	<0.05
Apoptosis ratio, %	0.76±.021	1.69±0.04	0.95±0.03	0.76±0.20	1.12±0.24	0.24±0.21	<0.05
ROS positive ratio, %	2.2±0.2	10.0±0.8	5.0±0.4	2.0±0.2	3.8±0.3	3.0±0.2	<0.05
α-cell/β-cell area ratio, %	0.07±0.02	0.37±0.04	0.21±0.05	0.12±0.02	0.30±0.05	0.12±0.03	<0.05

After controlling the blood glucose levels, the differences in the expression of NADPH p47 (phox) protein, cell apoptosis, level of ROS, and the ratio of α- to β-cells in the pancreas between the groups were observed ($P<0.05$). HFD, high-fat diet; TLR4, toll-like receptor 4; trun, truncated; EXE, exenatide; ROS, reactive oxygen species; NC, negative control.