A polysaccharide extract from the medicinal plant Maidong inhibits the IKK–NF-κB pathway and IL-1β–induced islet inflammation and increases insulin secretion

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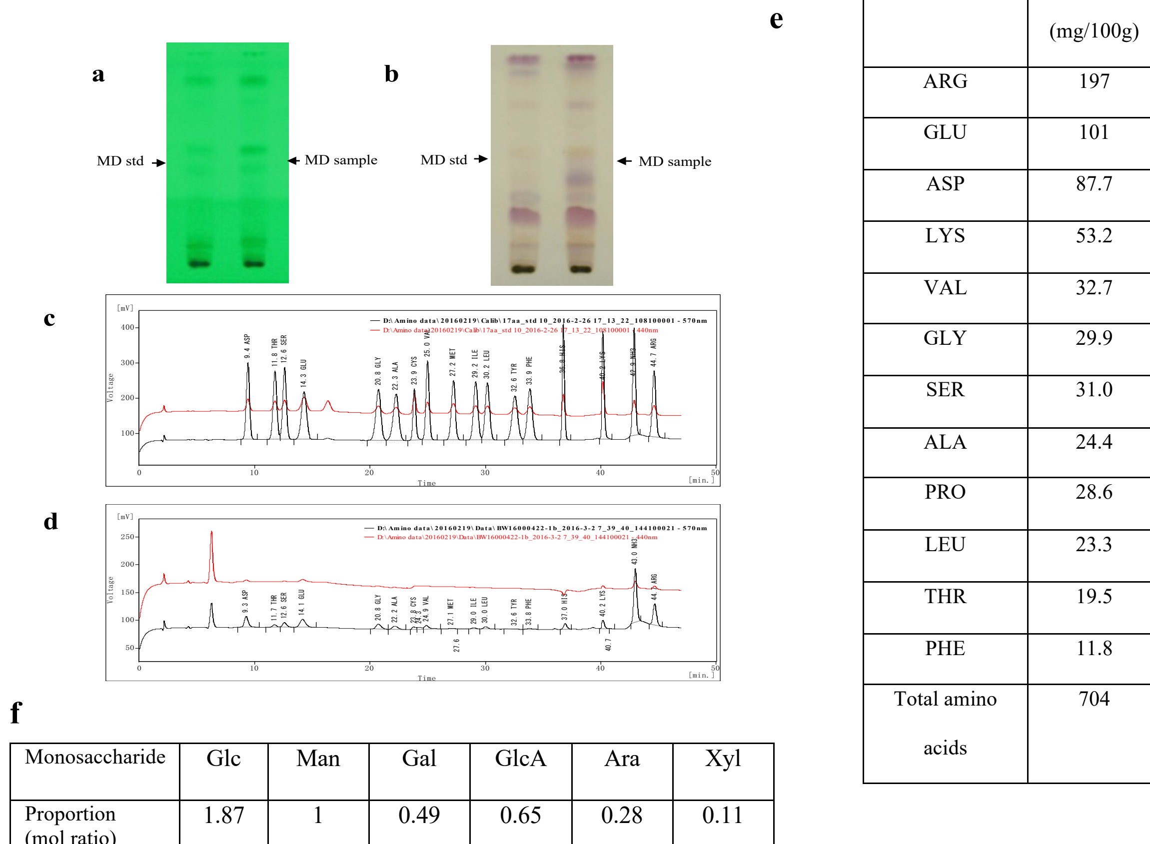
## Supplementary figures




| Amino acids | Proportion (mg/100g) |
|---|---|
| ARG | 197 |
| GLU | 101 |
| ASP | 87.7 |
| LYS | 53.2 |
| VAL | 32.7 |
| GLY | 29.9 |
| SER | 31.0 |
| ALA | 24.4 |
| PRO | 28.6 |
| LEU | 23.3 |
| THR | 19.5 |
| PHE | 11.8 |
| Total amino acids | 704 |

| Monosaccharide | Glc | Man | Gal | GlcA | Ara | Xyl |
|---|---|---|---|---|---|---|
| Proportion (mol ratio) | 1.87 | 1 | 0.49 | 0.65 | 0.28 | 0.11 |

**Figure S1. Amino acids and monosaccharides components were identified in MPE.**

Maidong (MD) sample was authenticated by comparing to MD standard (std) by chromatography under UV light **(a)** or sprayed by Anisaldehyde-sulphuric acid **(b)**. The amino acids composition in Maidong polysaccharide **(d&e)** were compared to animo acid standards **(c)** by using the GB/T 14965-1994 method (Standard Method for Determination of Amino Acids in China). Monosaccharide **(f)** were also identified by using high-performance liquid chromatography (HPLC) analysis.

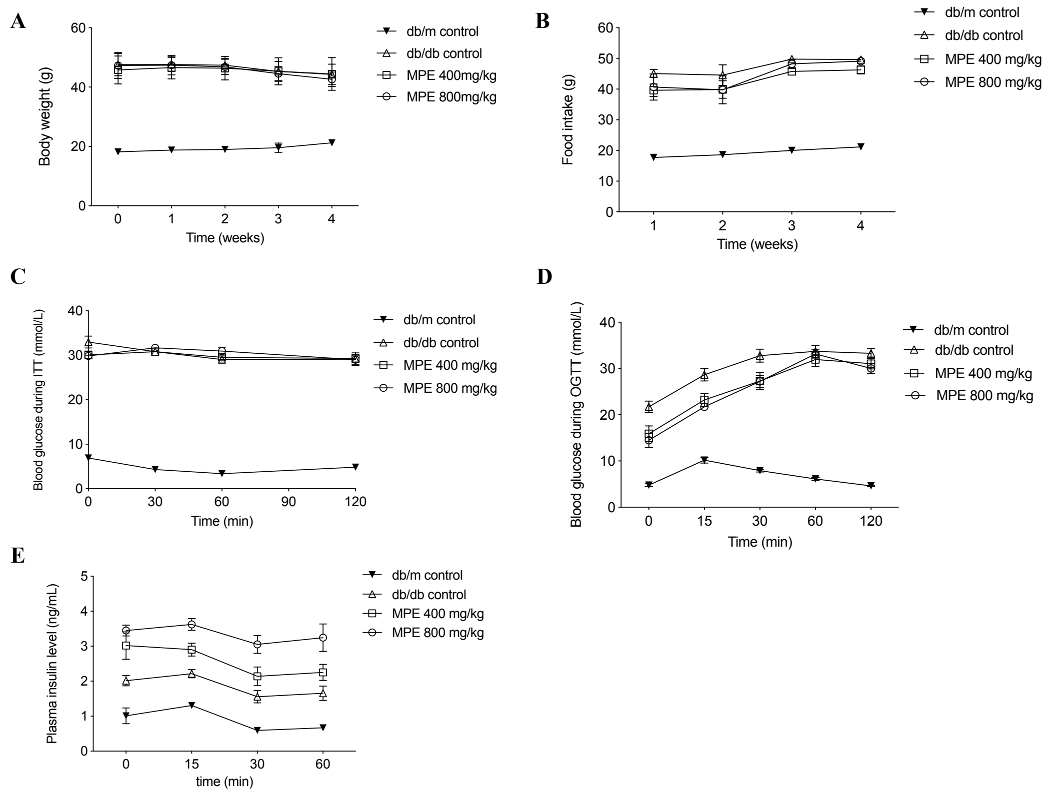



**Figure S2. MPE has no effect on body weight, food intake and insulin tolerance in db/db mice.**

Body weight **(a)** and food intake **(b)** were monitored weekly. Insulin tolerance test (ITT) **(c)** and glucose tolerance test **(d)** was performed after MP treatment for 4 weeks. Insulin secretion was measured **(e)** at OGTT.

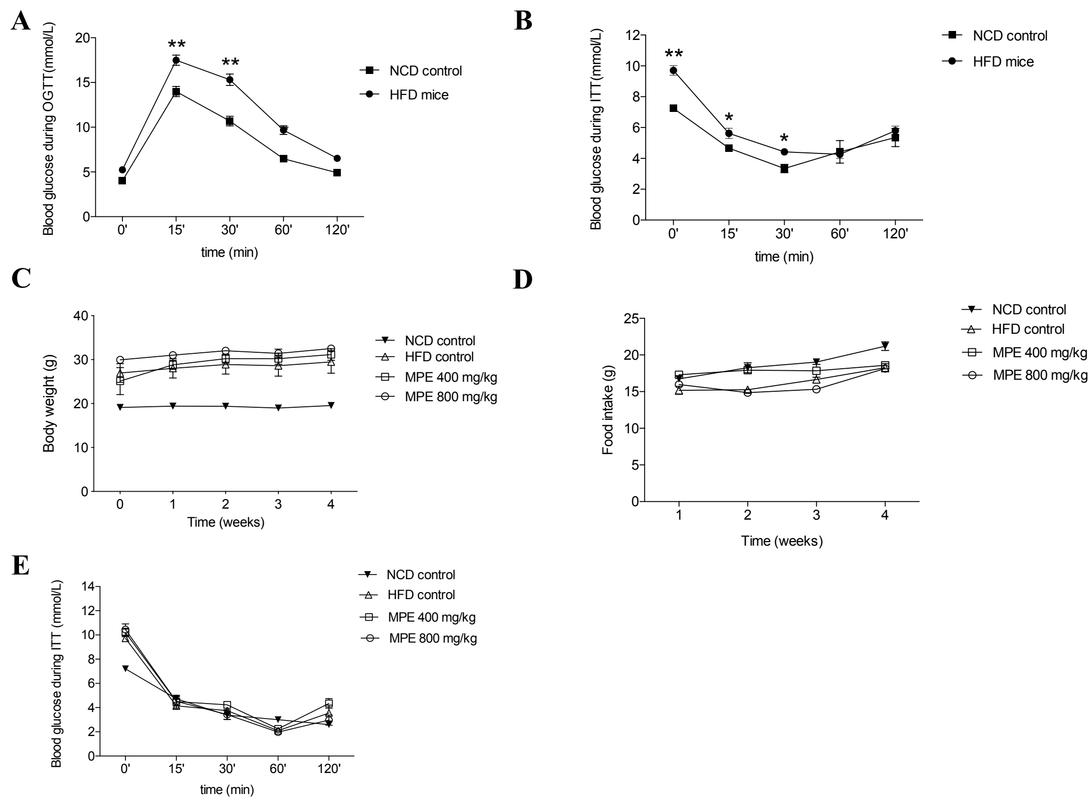



**Figure S3. 10-weeks of HFD feeding of C57 mice induce impaired glucose tolerance and insulin resistance, compared to normal chow diet fed mice. MPE has no effect on body weight, food intake and insulin tolerance in HFD-induced obese mice.**

In Maidong polysaccharide extract pretreatment, oral glucose tolerance test **(a)** and insulin tolerance test (ITT) **(b)** were performed after 10 weeks of HFD feeding. Body weight **(c)** and food intake **(d)** were monitored weekly. Insulin tolerance test (ITT) **(e)** was performed after MP treatment for 4 weeks.

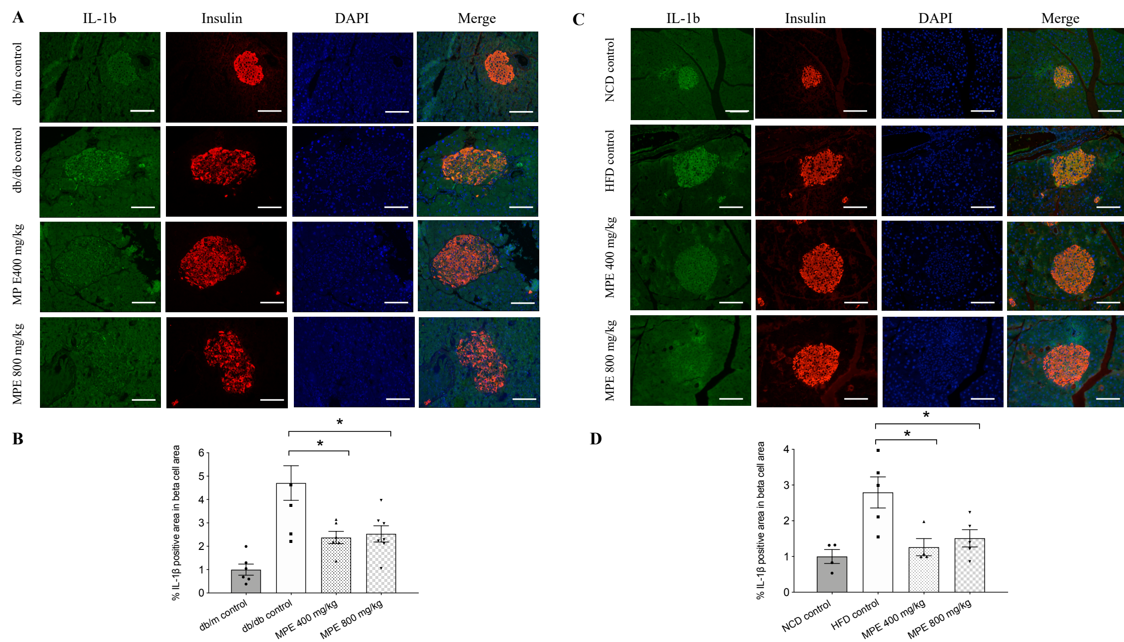



**Figure S4. MPE decreases IL-1β expression in pancreatic islets of *db/db* mice and HFD-induced obese mice.**

**(a)** Immunofluorescence staining of IL-1β and insulin in the pancreas of *db/db* mice (green: IL-1β, red: insulin, blue: DAPI, magnification: ×200, white scalebar represent 100 μm). **(b)** Relative quantification of % of β-cell area stained positive for IL-1β signal in *db/db* mice **(c)** Immunofluorescence staining of IL-1β and insulin in the pancreas of HFD-induced obese mice. **(d)** Relative quantification of % of β-cell area stained positive for IL-1β in HFD-induced obese mice by Image J software (n=5).

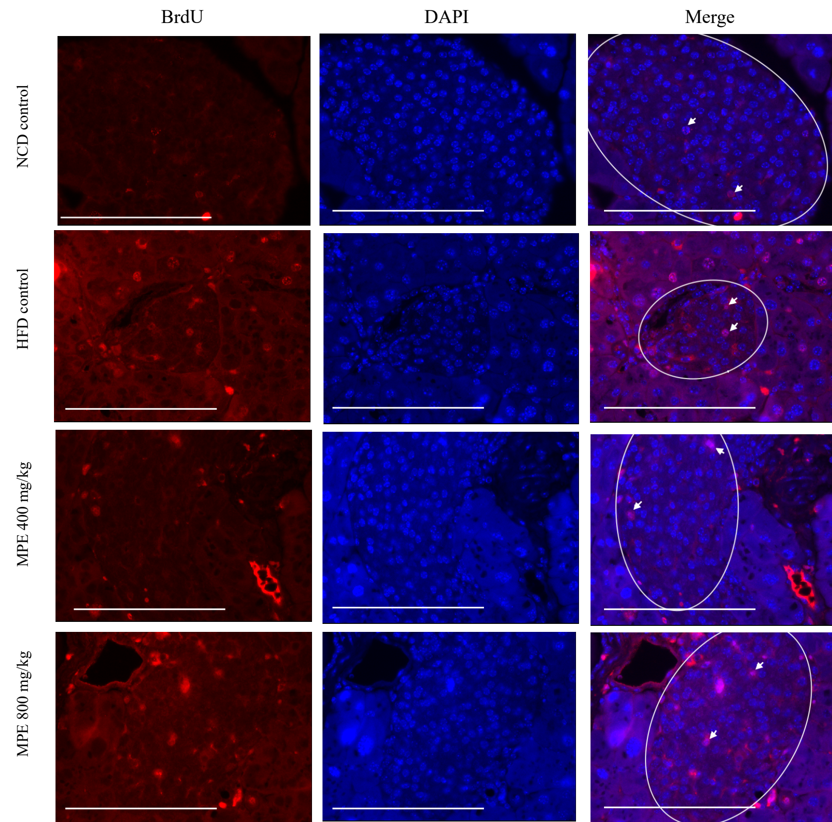



**Figure S5. MPE has no effect on cell proliferation in HFD-induced obese mice.**

Immunofluorescence staining of BrdU and DAPI in the pancreas of HFD-induced obese mice (red: BrdU, blue: DAPI, magnification: ×400, white scalebar represent 100 µm). White arrow represents positive signal in the islets.

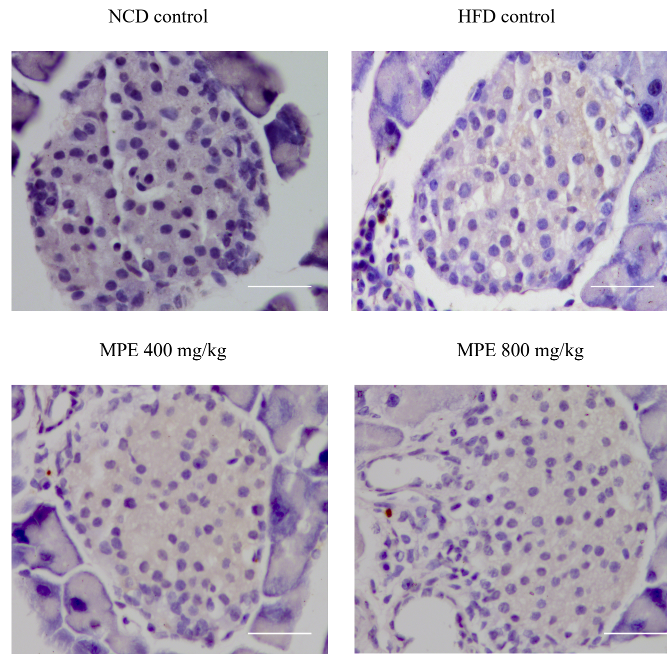



**Figure S6. The apoptosis-positive signal was not identifiable in HFD-induced obese mice with no change after MPE treatment**
TUNEL assay of apoptosis in the pancreas of HFD-induced obese mice (magnification: ×400, white scalebar represents 50 μm).

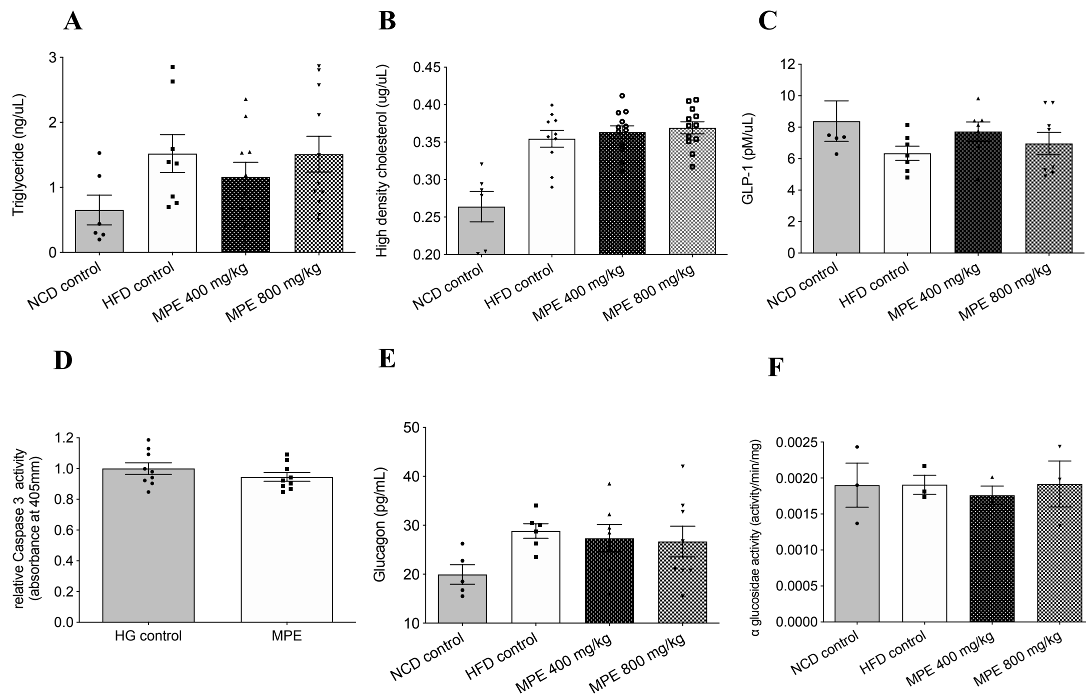



**Figure S7. MPE has no effect on lipid profile, glucagon like peptide (GLP-1), glucagon production and α glucosidase activity in HFD-induced obese mice and caspase 3 activity in MIN6 cells.**

Serum triglyceride **(a)**, high density lipoprotein cholesterol (HDL-C) **(b)**, GLP-1 **(c)**, glucagon **(e)** and α glucosidase activity **(f)** were measured after MP treatment for 4 weeks. Caspase 3 activity **(d)** was measured in the supernantant of MIN6 cells after treatment with MP for 72h.

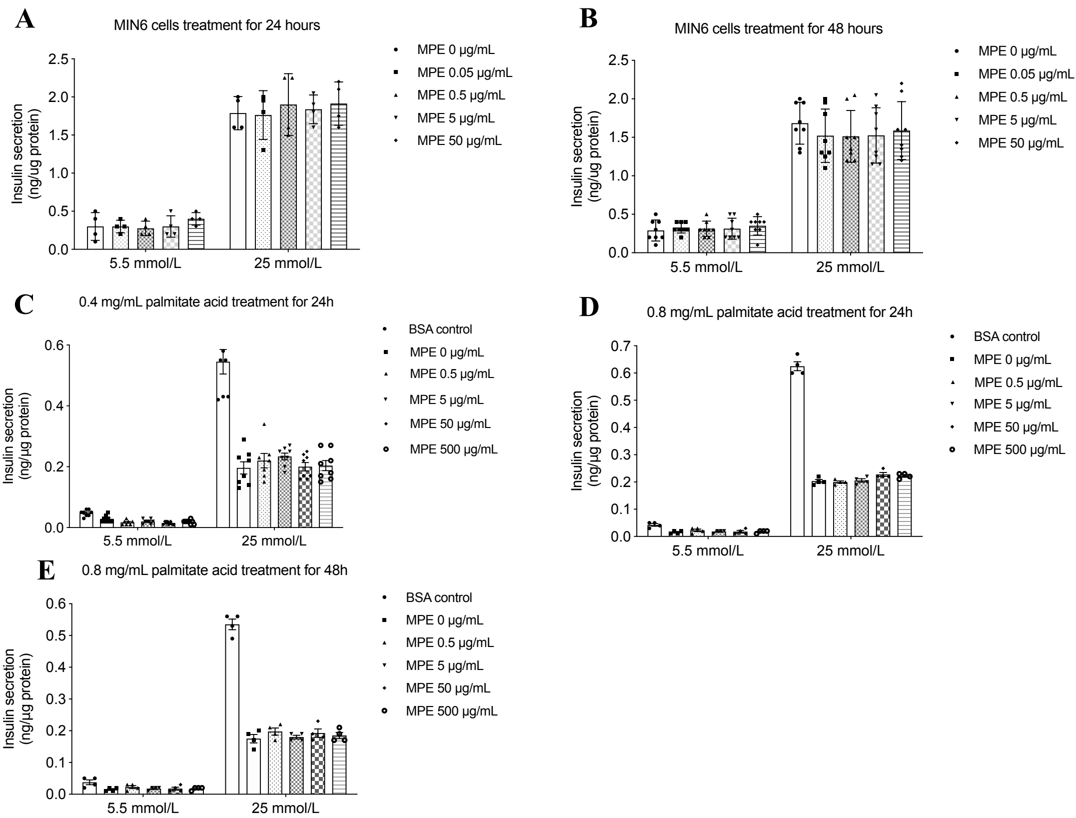



**Figure S8. MPE has no effect on GSIS in MIN6 cells treated with or without palmitate acid in time and dose dependent manner.**

MIN6 cells were treated with MPE at concentration of 0, 0.5, 5, 50 and 500 µg/mL for 24 **(a)**, or 48h **(b)**. After 48h-MPE-treatment, MIN6 cells were also treated with palmitate acid at concentration of 0.4 **(c)** or 0.8 **(d)** mg/mL for 24h. In figure **e**, after 24h-MP-treatment, MIN6 cells were also treated with palmitate acid at 0.8 mg/mL for 48h. GSIS was performed thereafter. BSA control represents vehicle group treated with bovine serum albumin for comparison with palmitate acid, which was dissolved in BSA.

**A. Primers are used for RT-qPCR**

| Gene name | Primers |
|---|---|
| *il1b* | Forward: GAAATGCCACCTTTTGACAGTG<br>Reverse: TGGATGCTCTCATCAGGACAG |
| *gapdh* | Forward: AGGTCGGTGTGAACGGATTTG<br>Reverse: TGTAGACCATGTAGTTGAGGTCA |
| *csf-2* | Forward: GGCCTTGGAAGCATGTAGAGG<br>Reverse: GGAGAACTCGTTAGAGACGACTT |
| *pik3cd* | Forward: GTAAACGACTTCCGCACTAAGA<br>Reverse: GCTGACACGCAATAAGCCG |
| *pik3ca* | Forward: CCACGACCATCTTCGGGTG<br>Reverse: ACGGAGGCATTCTAAAGTCACTA |
| *nfκb1* | Forward: ATGGCAGACGATGATCCCTAC<br>Reverse: TGTTGACAGTGGTATTTCTGGTG |

**B. Primary antibody were used for IF or WB.**

| Primary antibody | Company | Catalogue No. | Dilution | Application |
|---|---|---|---|---|
| Insulin | DAKO, USA | A056401 | 1:700 | IF |
| Glucagon | Abcam, UK | 92518 | 1:300 | IF |
| IL-1β | Novus Biologicals, US | NBP1-19775 | 1:300 | IF |
| BrdU | Abcam, UK | 8152 | 1:200 | IHC |
| phospho-P65 | Abcam, UK | 86299 | 1:500 | WB |
| P65 | Abcam, UK | 7970-1 | 1:1000 | WB |
| phospho-IκB | Abcam, UK | 133462 | 1:500 | WB |
| IκB | Abcam, UK | 32518 | 1:1000 | WB |
| GAPDH | Cell Signaling Technology, USA | 2118 | 1:5000 | WB |

**C. Second antibody were used for IF or WB**

| Second antibody | Company | Catalogue No. | Dilution | Application |
|---|---|---|---|---|
| goat anti-guinea pig 488 | Invitrogen, USA | A11073 | 1:1400 | IF |
| goat anti-rabbit 568 | Invitrogen, USA | A-11011 | 1:400 | IF |
| HRP-linked anti-rabbit IgG | Cell Signaling Technology, USA | 7074S | 1:2000/ 1:400 | WB/ IHC |
| HRP-linked anti-mouse IgG | Cell Signaling Technology, USA | 7076S | 1:2000 | WB |
| goat anti-mouse 568 | Invitrogen, USA | A-11004 | 1:400 | IF |

**Table S1. Primers are used for RT-qPCR. Sequences are printed in the 5' to3'direction (A). Primary (B) and second antibody (C) were used for IF or WB.**