

SUPPLEMENTARY DATA

Supplementary Data 1. The following antibodies were used in this study.

Stressgen Biotechnologies (Victoria, BC, Canada)

Anti-HSP72 antibody: SPA-810

Santa Cruz Biotechnology (Santa Cruz, CA)

Anti-insulin antibody: sc-9168

Anti-nuclear factor of activated T-cell (NFAT) c1 antibody: sc-7294

Anti-glucose transporter (GLUT) 2 antibody: sc-9117

Anti-BiP (GRP78) antibody: sc-1050

Anti-C/EBP homologous protein (CHOP) (GADD153) antibody: sc-575

8-Hydroxy-2'-deoxyguanosine (8-OHdG) antibody: sc-66036

XBP-1 antibody: sc-7160

Anti-actin antibody: sc-1615

Cell Signaling Technology Inc. (Beverly, MA)

Anti-forkhead box O1 (FOXO1) antibody: #9462

Anti-phospho-c-jun N-terminal kinase (JNK) antibody: #9251

Anti-JNK antibody: #9252

Anti-nuclear factor-kappa B (NF- κ B) p65 antibody: #4764

Anti-cleaved-caspase-3 antibody: #9661

Anti-phospho-AMPK α : #2535

Anti-AMPK α : #2532

Chemicon International Inc. (Billerica, MA)

Anti-pancreatic and duodenal homeobox (PDX)-1 antibody: AB3243

α -tubulin antibody: #05-829

Upstate Biotechnology (Lake Placid, NY)

Anti-insulin receptor substrate (IRS)-2 antibody: 06-506

Biorbyt (Riverside, UK)

Anti-Annexin V antibody: orb18007

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Supplementary Data 2. Primer sequences for quantitative real time RT-PCR.

insulin-2 FW; 5'-GCTCTCTACCTGGTGTGTGG-3',

insulin-2 RV; 5'-GTTTTATTCATTGCAGAGGG-3',

Hsp72 FW; 5'-TGGTGCTGACGAAGATGAAG-3',

Hsp72 RV; 5'-AGGTCGAAGATGAGCACGTT-3',

PDX-1 FW; 5'-GAAATCCACCAAAGCTCACG-3',

PDX-1 RV; 5'-TTCAACATCACTGCCAGCTC-3',

BiP FW; 5'-ATCGGACGCACTTGAATGAC-3',

BiP RV; 5'-TTCCCAAATACGCCTCAGCAG-3',

CHOP FW; 5'-CATACACCACCACACCTGAAAG-3',

CHOP RV; 5'-CCGTTTCCTAGTTCTTCCTTGC-3',

β -actin-FW; 5'-CGTAAAGACCTCTATGCCAA-3',

β -actin-RV: 5'-AGCCATGCCAATGTTGTCTC-3'.

To assess the specificity of the amplified PCR products, a melting curve analysis was performed after the last cycle.

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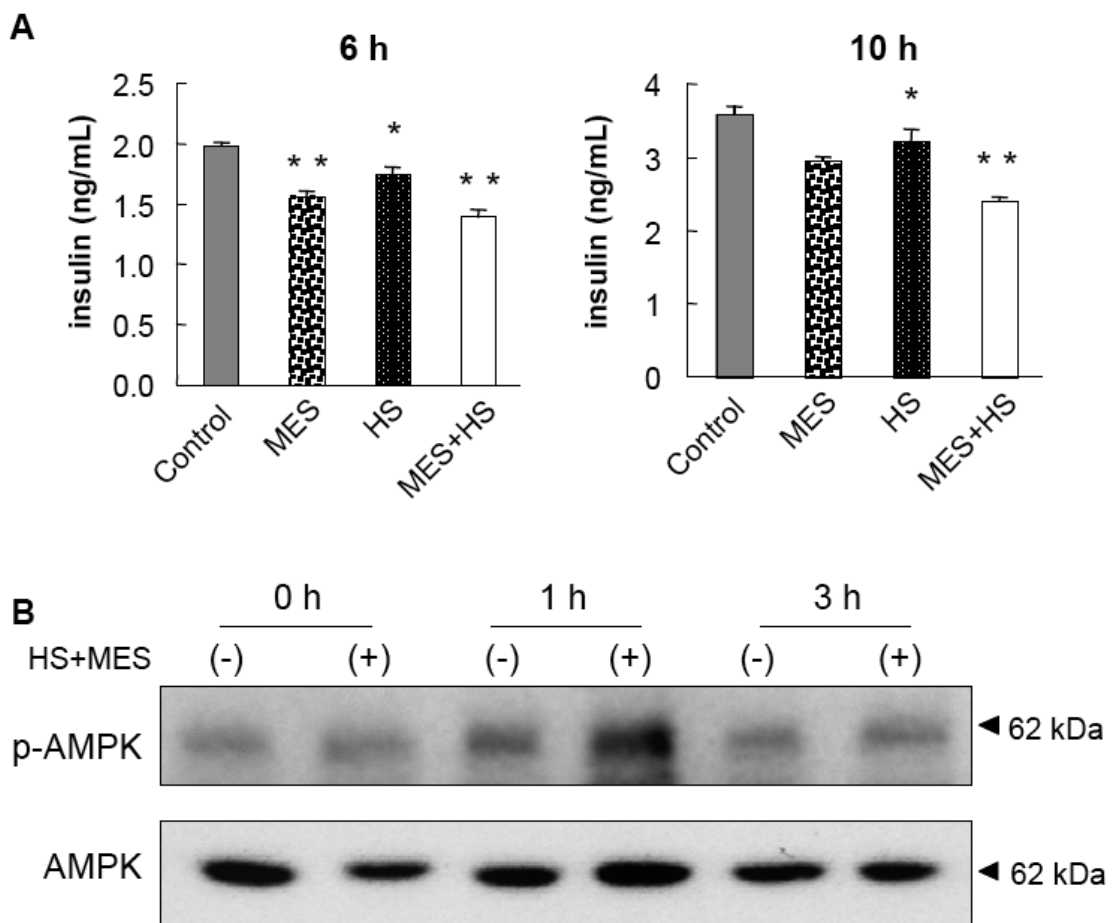
Supplementary Figure 1. Insulin secretion capacity in MIN6 cells.

A: MIN 6 cells were incubated with 400 μ M palmitate for 24hr, then sham, MES, HS or HS+MES treatment were performed for 10min. After 6 and 10 hrs of these treatments, insulin concentrations in culture medium were measured by ELISA.

B: MIN 6 cells were incubated with 400 μ M palmitate for 24hr, then sham or HS+MES treatment were performed for 10min. After 0, 1 and 3 hrs of these treatments, cell lysates were isolated, and AMPK and phospho-AMPK levels were determined by Western blot.

* p <0.05, ** p <0.01 vs. sham-treated control.

Supplementary Figure 1.



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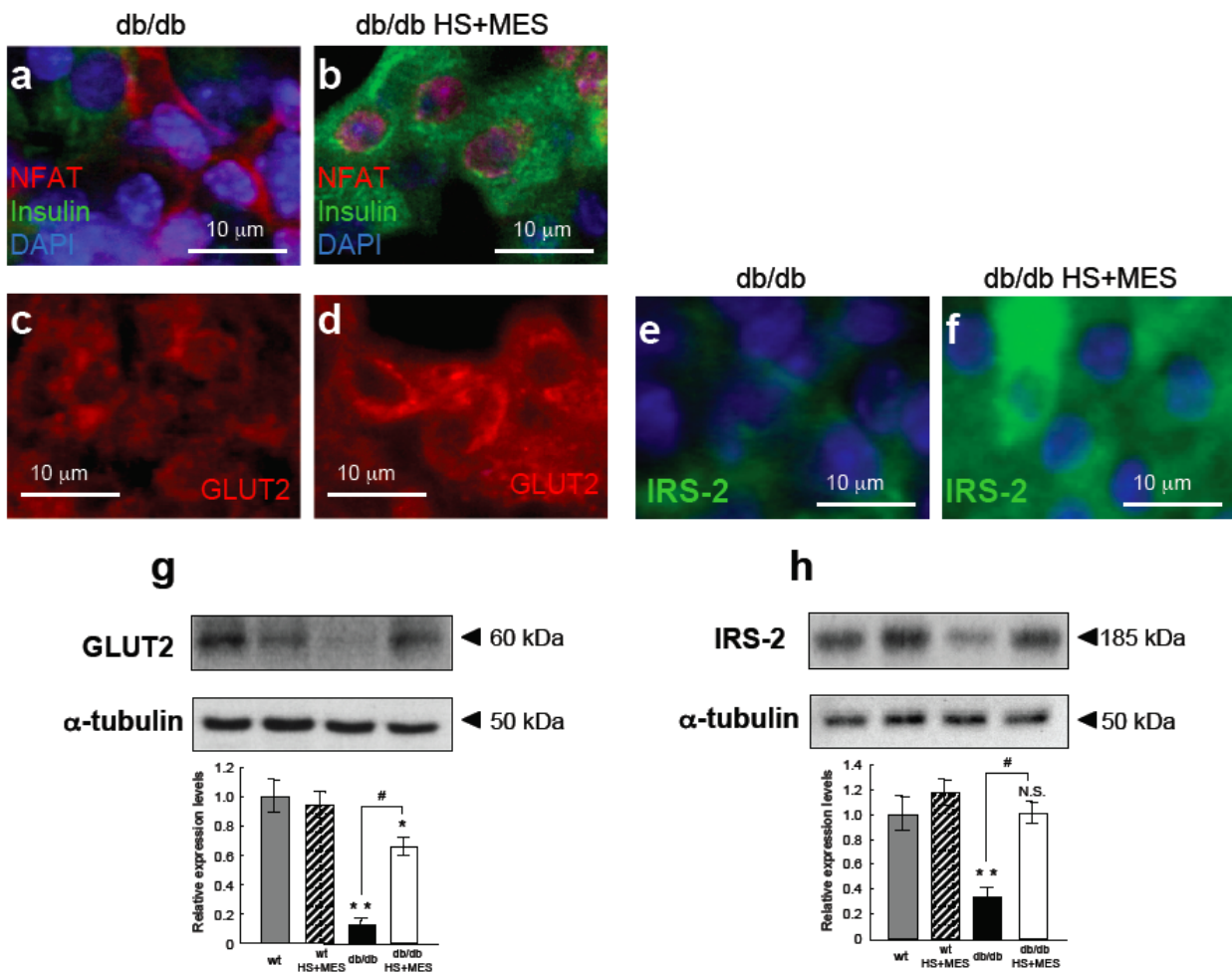
Supplementary Figure 2. Molecular markers of pancreatic β -cell integrity and function.

Immunohistochemical analysis of molecular markers associated with pancreatic β -cell integrity and function (a and b; NFAT/insulin, c and d; GLUT2, e and f; IRS-2).

GLUT2 and IRS-2 protein were determined by Western blotting, and the expression levels were corrected using internal control, α -tubulin.

* $p < 0.05$, ** $p < 0.01$, N.S. vs. sham-treated wt control. # $p < 0.05$ vs. indicated group.

Supplementary Figure 2.



SUPPLEMENTARY DATA

Supplementary Figure 3. Molecular markers of apoptotic signal, inflammation and oxidative stress in pancreatic β -cells.

Immunohistochemical analysis of molecular markers associated with apoptosis (A: a and b; Cleaved caspase-3, c and d; Annexin-V), inflammation (B: a and b; NF- κ B) and oxidative stress (B: c and d; 8-OHdG) in sham-treated db/db and HS+MES treated db/db islets.

Cleaved caspase-3 protein were determined by Western blotting, and the expression levels were corrected using internal control, α -tubulin.

** $p < 0.01$, N.S. vs. sham-treated wt control. # $p < 0.05$ vs. indicated group.

Supplementary Figure 3.

