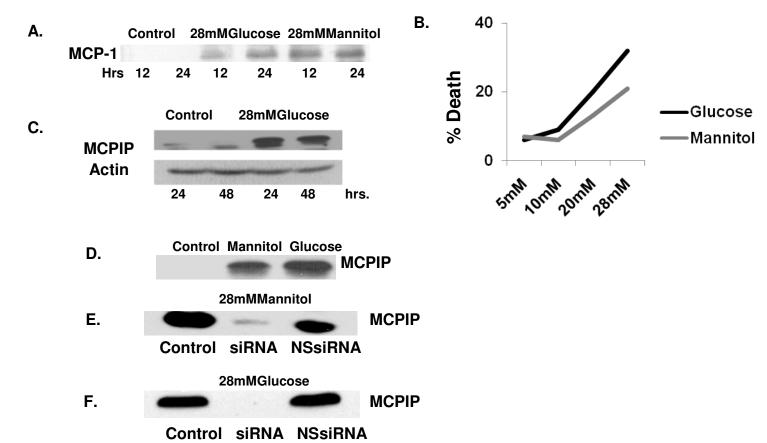
Supplemental 1:



Supplemental 1: H9c2 cardiomyoblasts were treated with/without 28mmol/L glucose. (A), At 12 and 24 hrs, cell lysate was collected and analyzed using immunoblot with MCP-1 antibody. Results were quantified against β-actin (*=p<0.03). (B), Cardiomyoblasts were treated with 5, 10, 20, and 28mmol/L glucose or mannitol and were evaluated for cell death using trypan blue. (C), Cardiomyoblasts were treated with or without 28mmol/L glucose. Cell lysate was collected at 24 and 48 hrs. and analyzed using immunoblot with MCPIP antibody. β-actin served as a control. (D), Cardiomyoblasts were treated with 28mmol/L glucose or mannitol. Cell lysate was collected at 48 hrs. and analyzed using immunoblot with MCPIP antibody. (E-F)

.Cardiomyoblasts were treated with 28mmol/L glucose or mannitol with/without siRNA specific for MCPIP or with non-specific siRNA. Cell lysate was collected and analyzed using immunoblot with MCPIP antibody at 24hrs.

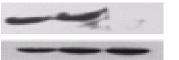
Supplemental 2:

A. B.

Control 28mM Glucose

Phox47 (Membrane)
Phox47 (Cytosol)

28mM NSsiRNA siRNA

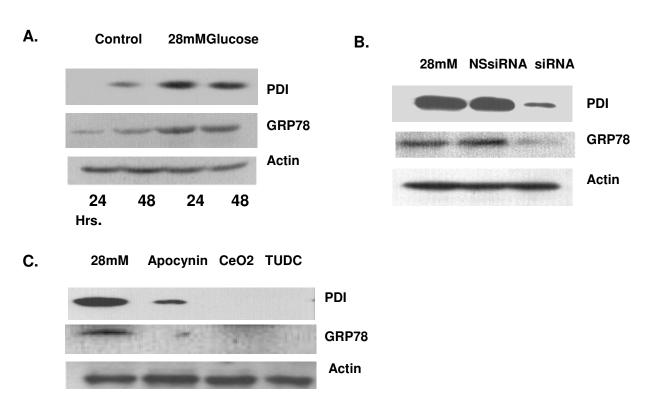


Phox47 (Membrane)
Phox47 (Cytosol)

Supplemental 2: (A), Cardiomyoblasts were treated with/without 28mmol/L glucose. Cell lysate was collected and the membrane fractions were isolated. Both cytosol and membrane fractions were evaluated using immunoblot with phox47 antibody. (B), Cardiomyoblasts were treated with 28mmol/L glucose with/without siRNA specific for MCPIP or with non-specific siRNA. Cell lysate was collected and the membrane fractions were isolated. Cytosol and membrane fractions were evaluated using immunoblot with phox47 antibody.

Supplemental 3:

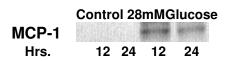


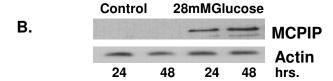


Supplemental 3: (A), H9c2 cardiomyoblasts were treated with/without 28mmol/L glucose. Cell lysate was collected and evaluated using immunoblot with antibody for PDI or GRP78. (B), Cardiomyoblasts were treated with 28mmol/L glucose with/without siRNA specific for MCPIP or with non-specific siRNA. Cell lysate was collected from the same samples and evaluated using immunoblot with antibody for PDI or GRP78. (C), Cardiomyoblasts treated with 28mmol/L glucose were treated with/without 20μM Apocynin, 300nM CeO₂ nanoparticles, or 50μM TUDC. At 24 hrs, cell lysate was collected and analyzed using immunoblot with PDI or GRP78 antibody.

Supplemental 4:

A.





Supplemental 4: Isolated cardiomyocytes were treated with/without 28mmol/L glucose. (A), At 12, and 24 hrs. cell lysate was collected and analyzed using immunoblot with MCP-1 antibody. Results normalized to β -actin (*=p<0.03). (B), Cell lysate was collected at 24 and 48 hrs. and analyzed using immunoblot with MCPIP antibody. β -actin served as control.

Cell Culture

DMEM was modified by supplementing with 10% FBS, 1% penicillin and 1% streptomycin.

Cells were treated with or without 50µmol/L 3'methyladenine; 1µmol/L LY294002; 100µmol/L tauroursodeoxycholate (TUDC); 50µmol/L 4-phenylbutiric acid (4-PBA); 50µmol/L L-NAME; 100nmol/L CeO2, or 20µmol/L apocynin prior to treatment with 28mol/L glucose.

RT-PCR

Primers designed for RT-PCR were as follows: MCPIP F-5'-TGTGCCTATCACAGACCAGCACAT-3'; R-5'-TCGGATTCATAGGCCAGCTTC-ACA-3'; GRP78 F-5'-AGCCCACCGTAACAATCAAGGTCT-3'; R-5'-CGTGT-CAATGCGCTCTTTGAGCTT-3'; PDI F-5'-CAGAATGGAAACCGCACAAACCCA-3'; R-5'-GGCCACATCACCATCA CAAACAA-3'

<u>Immunoblot Analysis</u>

Protein was extracted using cell lyses buffer (20% glycerol 0.1% TritonX 8% 0.5M EDTA and 1% 1M DTT). Polyclonal antibodies used for immunoblot analysis were as follows: anti-MCPIP (1:2000); anti-BECN1 (Santa Cruz; 1:500); anti-LC3; anti-GRP78 (1:2000); anti-PDI (1:2000) anti-phox47 (1:1000); and anti-iNOS (Upstate; 1:2000). Immunoblots were quantified as a ratio over β -actin expression.

Statistical Analysis:

The experimental data was analyzed by using SPSS statistical software (SPSS Inc.) under Windows XP. All values are presented as mean \pm SEM. Results were compared between groups by ANOVA analysis followed by t tests. Differences were considered significant at a p value of <0.05.