

Material and methods

CCK-8 assay

Cell survival rates were estimated by the CCK-8 assay (MedChemExpress, HY-K0301). Approximately 5×10^4 cells were seeded in 96-well plates with 100 μ L medium each well. After 24 h cultivation, different doses of glucose and/or MET were added respectively and incubated for 72 h. Each well was incubated with 10 μ L CCK-8 solution for 2 h away from light before measuring the absorbance at 450 nm by Microplate Reader (Molecular Devices, Flexstation® 3).

Figure legends

Fig. S1 Effects of different concentrations of sugar and metformin on cell proliferation and migration. (A, B, C, D) A scratch wound assay was performed in the presence of 5.5, 15, 35 or 55 μ M glucose (A) and 35 mM glucose with/without 25, 50, 100 or 200 μ M MET (C) treatment for 72 h. Cell monolayers were imaged at 0, 24, and 48 after wounding. White vertical lines indicate the wound area borders. Space bar=100 μ m. The images shown here were captured at 0 and 24 h. The cell migration distance was measured and shown in (B) and (D) respectively. (E, F) CCK8 assay was used to detect the chemo-sensitivity of 5.5, 15, 35 or 55 μ M glucose (E) and 35 mM glucose with/without 25, 50, 100 or 200 μ M MET (F) treatment. The survival rate = (mean absorbance of experimental group/mean absorbance of control group) \times 100%. All the values displayed are mean \pm SEM of 5 independent experiments. *P < 0.05, ***P < 0.001 vs. 5.5 mM D-Glucose group; #P < 0.05, ###P < 0.01, ####P < 0.001 vs. 35 mM D-Glucose group.

Fig. S2 The effect of MET on oxidative stress under high glucose treatment in HUVECs. (A, B) Western blot analysis of the HO-1 level in HUVECs treated with 5.5 or 35 mM D-Glucose with or without MET (100 μ M) or Bay11-7082 (0.5 μ M) for 72 h. The HO-1 level was quantified and shown in (B). (C, D) ELISA assay was performed to evaluate the level of protein carbonyl (C) and 8-isoprostane (D) in HUVECs treated with 35 mM D-Glucose with or without MET (100 μ M). All the values displayed are mean \pm SEM of 5 independent experiments. ***P < 0.001 vs. 5.5 mM D-Glucose group;

#P < 0.05, ##P < 0.01, ###P < 0.001 vs. 35 mM D-Glucose group.