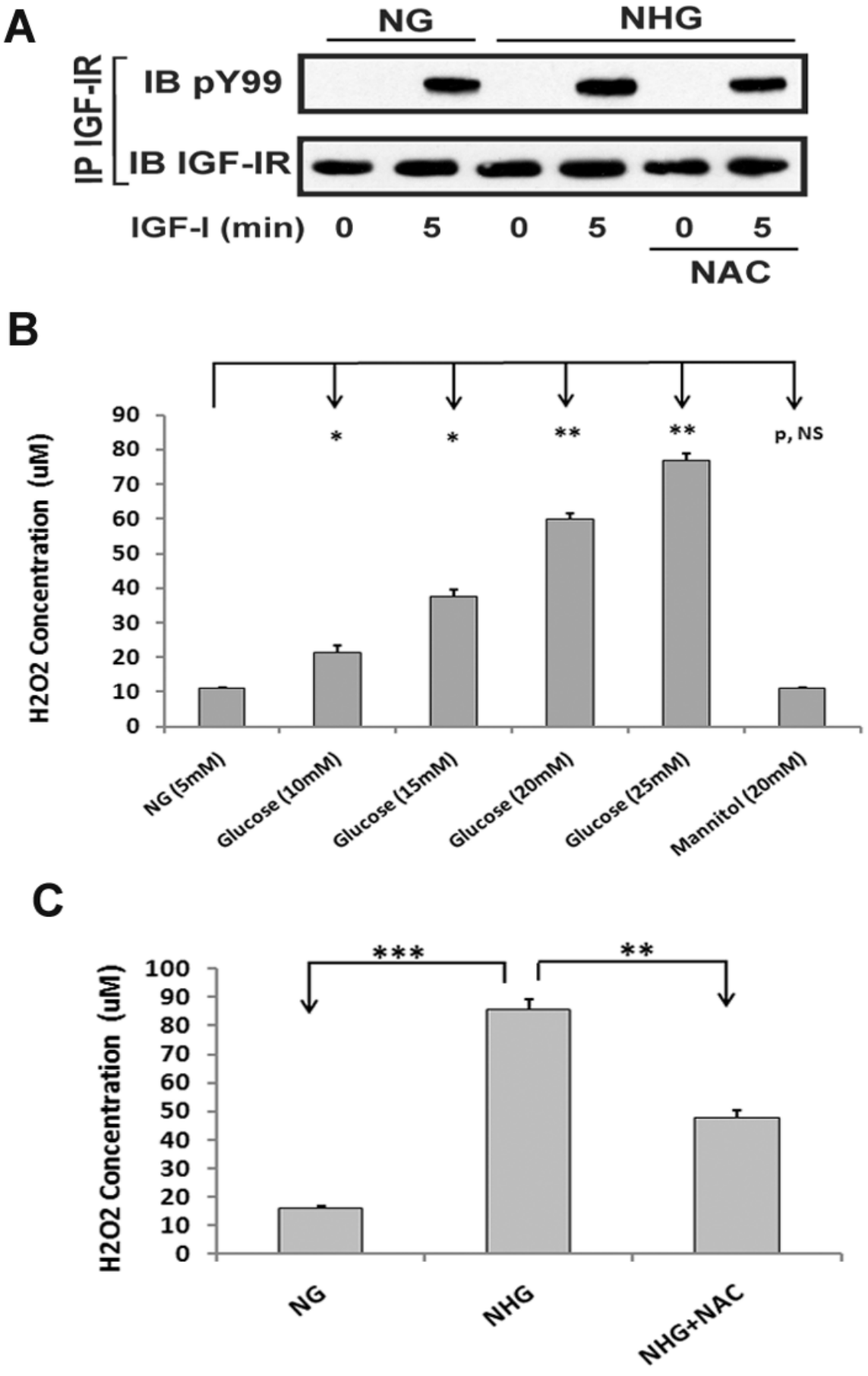
SUPPLEMENTARY DATA

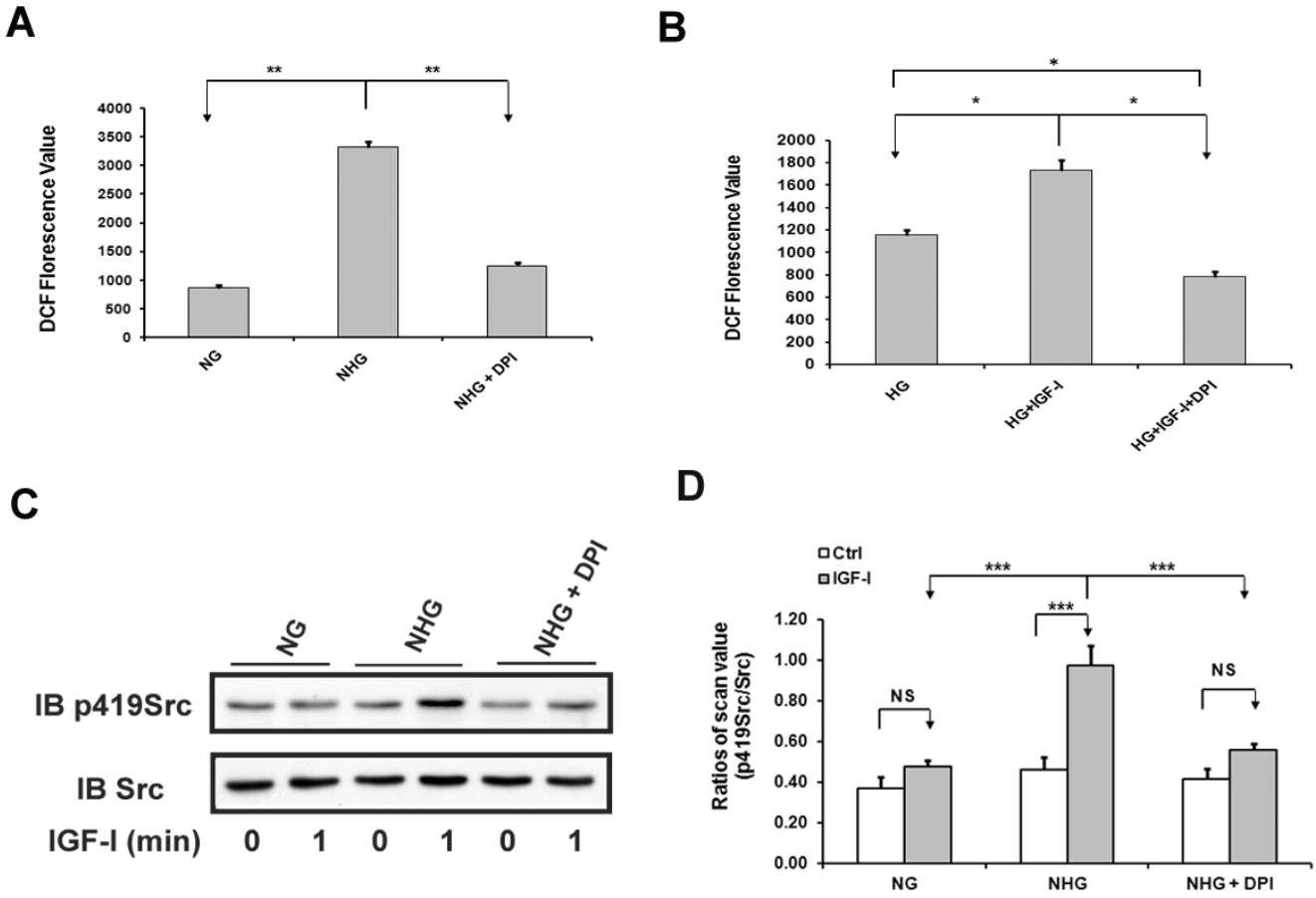
Supplementary Figure 1. (A) VSMC were cultured in DMEM containing normal glucose (5 mM, NG) plus 10% FBS and serum deprived for 16 hr before treatment with 25 mM glucose (NHG) for 24 hr or maintained in NG in the presence or absence of IGF-I (100 ng/ml). For NAC treatment, 2 mM NAC was added in serum free DMEM and incubated for 16hr before IGF-I exposure. Cell lysates were immunoprecipitated with an anti-PY99 antibody followed by immunoblotting with an anti-IGF-I receptor antibody. To control the loading, the same amount of cell lysate protein was separated and immunoblotted using an anti-IGF-I receptor antibody. (B) VSMC were cultured in DMEM containing normal glucose (5mM, NG) and serum starved for 16hr prior to the addition of the indicated glucose concentration. Mannitol (25 mM) was used as an osmotic control. (C) VSMC were cultured and treated as described in panel A.ROS generation was determined using the amplex red method described in "Research Design and Methods". * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ indicate significant differences between two treatments. NS indicates no significant difference. The figures are representative of three independent experiments.

SUPPLEMENTARY DATA



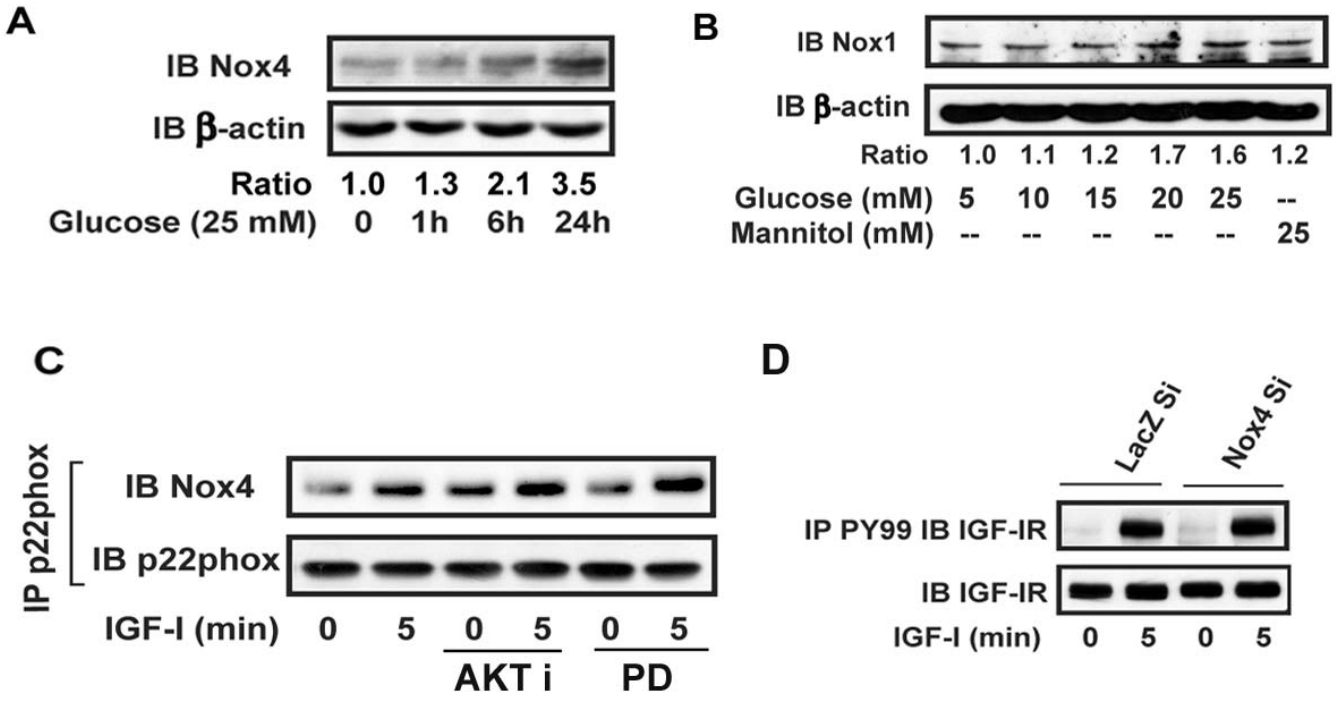
SUPPLEMENTARY DATA

Supplementary Figure 2. (A) VSMC were cultured in DMEM containing normal glucose (5 mM, NG) plus 10% FBS and serum deprived for 16 hr before treatment with 25 mM glucose (NHG) or maintained in NG. DPI (10 nM) was added 1 hr before glucose change and ROS measurement. (B) VSMC were cultured in DMEM containing high glucose (25 mM, HG) plus 10% FBS and serum deprived for 16 hr before any treatment. DPI (10 nM) was added 1 hr before IGF-I treatment (100 ng/ml) followed by ROS measurement. ROS generation was determined using the procedure described in "Research Design and Methods". (C) Cell lysates were immunoblotted with an anti-p419Src antibody and probed with an anti-Src antibody as a loading control. (D) The value of each bar is the ratio of the scan value of the p419Src band divided by the value of Src band. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ indicate significant differences between two treatments. NS indicates no significant difference. The figures are representative of three independent experiments.



SUPPLEMENTARY DATA

Supplementary Figure 3. (A) VSMC were cultured in DMEM containing normal glucose (5 mM, NG) plus 10% FBS and serum deprived for 16 hr before adding 25 mM glucose for indicated time points. Cell lysates were immunoblotted with an anti-Nox4 antibody and reprobed with an anti- β-actin antibody as a loading control. (B) VSMC were cultured in DMEM containing NG plus 10% FBS and serum deprived for 16 hr before adding different concentration of glucose as indicated for 24 hr. Mannitol was used as an osmotic control. Cell lysates were immunoblotted with an anti-Nox1 antibody. The blots were striped and reprobed with an anti-β-actin antibody. (C) Cells were cultured in DMEM containing high glucose (25 mM, HG) plus 10% FBS and serum deprived for 16 hr before IGF-I treatment (100ng/ml) for the indicated times. Prior to IGF-I exposure, cells were preincubated with a MAP kinase inhibitor PD98059 (PD, 50 uM) or an AKT inhibitor (AKT i, 1 uM) for 1 hr. Cell lysates were immunoprecipitated with an anti-p22phox antibody and immunoblotted with an anti-Nox4 antibody. To control the loading, the blots were striped and reprobed with an anti-p22phox antibody. (D) Cells were cultured in DMEM containing high glucose (25 mM, HG) plus 10% FBS and serum deprived for 16 hr before IGF-I treatment for the indicated times. Cell lysates were immunoprecipitated with an anti-PY99 antibody and immunoblotted with an anti-IGF-I receptor (IGF-IR) antibody. To control the protein input, the same amount of lysates was immunoblotted with an anti-IGF-IR antibody. The figures are representative of three independent experiments.



SUPPLEMENTARY DATA

Supplementary Figure 4. (A) VSMC expressing the shRNA targeting LacZ (LacZ Si) and PKCζ (PKCζ Si) were maintained in DMEM containing high (25 mM) or normal (5 mM) glucose. ROS measurement was determined following the procedure described in "Research Design and Methods". (B) VSMC expressing the shRNA targeting LacZ (LacZ Si) and PKCζ (PKCzeta Si) were maintained in DMEM containing high (25 mM) glucose and serum deprived for 16 hr before IGF-I treatment (100 ng/ml) for the indicated times. Cell lysates were immunoblotted with anti-pErk1/2 or pAKT (S473) antibodies. The blots were striped and reprobed with anti-Erk1/2 or anti-AKT antibodies, respectively. (C) VSMC were cultured in DMEM containing normal glucose (5 mM, NG) plus 10% FBS and serum deprived for 16 hr before adding 25 mM glucose (NHG) or maintained in normal glucose (NG) for 24 hrs. Cell lysates were immunoprecipitated with an anti-PY99 antibody and immunoblotted with an anti-SHPS-1 antibody. To control the protein input, the same amount of lysates was immunoblotted with an anti-SHPS-1antibody. (D) The aortic extracts from the normal or diabetic mice that were treated as described in "Research Design and Methods" were immunoblotted with an anti-Nox1 antibody. To control the loading, the blot was striped and reprobed with an anti-β-actin antibody. (E-G) VSMC expressing the shRNA targeting LacZ (LacZ Si) and Nox1 (Nox1 Si) were maintained in DMEM containing high (25 mM) and serum deprived for 16 hr before IGF-I treatment (1 min) or no treatment. Cell lysates were immunoblotted with anti-Nox1 and β-actin antibodies (E). Src was immunoprecipitated with an anti-Src antibody. Oxidized Src levels were measured following the procedure described in "Research Design and Methods" (F). Cell lysates were immunoblotted with anti-pSrc(Y419) and Src antibodies (G). (H) VSMC were cultured in DMEM containing normal glucose (5 mM) and serum deprived for 16hr prior to the addition of high glucose (25 mM) for indicated time periods. Cell lysates were immunoblotted with anti-pSrc (Y419) and Src antibodies. The figures are representative of three independent experiments.

SUPPLEMENTARY DATA