Supporting Information

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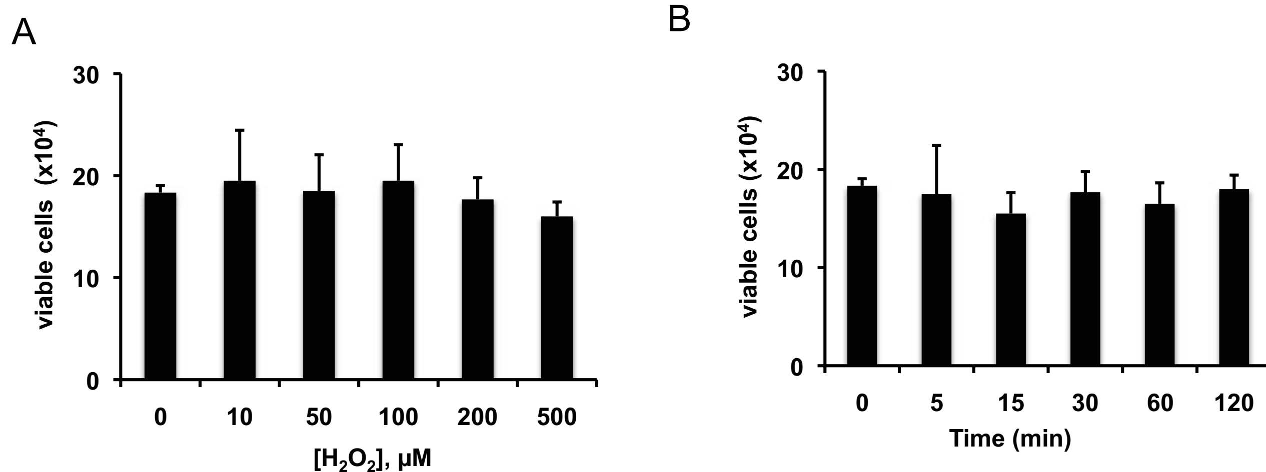

Fig. S1. Viability of H_2O_2-treated endothelial cells. Shown in this figure are the results of the viability measurements in endothelial cells treated with H_2O_2 using the trypan blue exclusion assay. Equal numbers of cells in 12-well plates were treated with H_2O_2 as indicated. Triplicate wells from each treatment were counted to determine the number of viable cells by trypan blue exclusion. (*A*) The dose-response experiment analyzed in cells stimulated with the indicated concentrations of H_2O_2 for 30 min; (*B*) shows the time-course experiment in BAECs treated with 200 μM H_2O_2 for the indicated times.

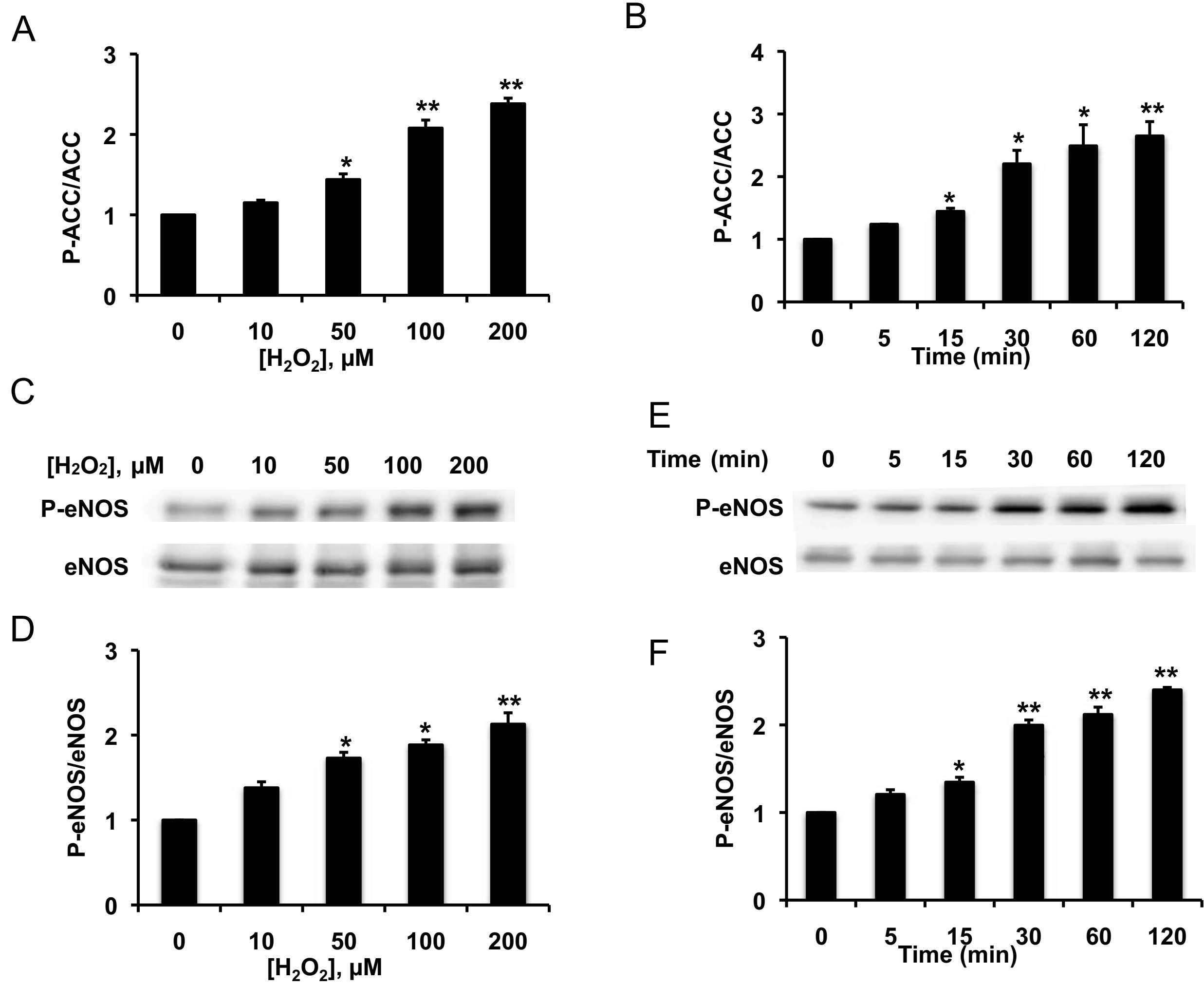

Fig. S2. H_2O_2-mediated ACC and eNOS phosphorylation in endothelial cells. Shown in this figure are the results of immunoblots analyzed in endothelial cells treated with H_2O_2. (*A* and *B*) Pooled data from five independent experiments, analyzing the intensities corresponding to phospho-ACC and total ACC by quantitative chemiluminescence. (*C*) Representative immunoblot from a dose-response experiment analyzed in cells stimulated with the indicated concentrations of H_2O_2 for 30 min and probed with antibodies as shown; (*D*) pooled data from five independent experiments, analyzing the intensities corresponding to phospho-eNOS and total eNOS by quantitative chemiluminescence. (*E*) Representative time course experiment in BAECs treated with 200 μM H_2O_2 for the indicated times and analyzed in immunoblots probed with antibodies as shown; (*D*) pooled data from five independent experiments. *, $P < 0.05$ and **, $P < 0.01$ by ANOVA.

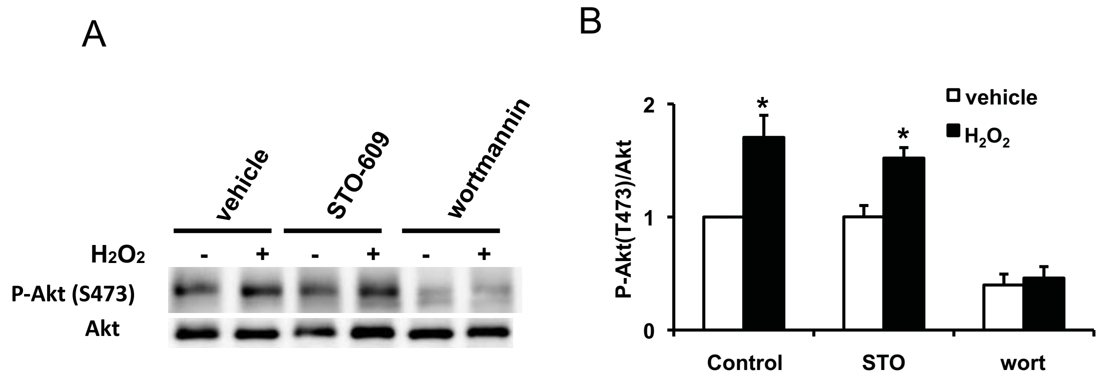

Fig. S3. Effects of protein kinase inhibitors on H_2O_2-induced Akt phosphorylation. (*A*) Representative immunoblot analyzed in endothelial cells treated with H_2O_2 (200 μM, 30 min) after being first incubated for 30 min with inhibitors as shown: STO-609 (CaMKKβ inhibitor, 10 μg/mL); wortmannin (PI3-kinase inhibitor, 10 μM). Cell lysates were subjected to immunoblotting and probed with antibodies as shown. (*B*) Quantitative analyses of pooled data from three independent experiments, measuring the intensities corresponding to phospho-Akt and total Akt by quantitative chemiluminescence. *, $P < 0.05$.

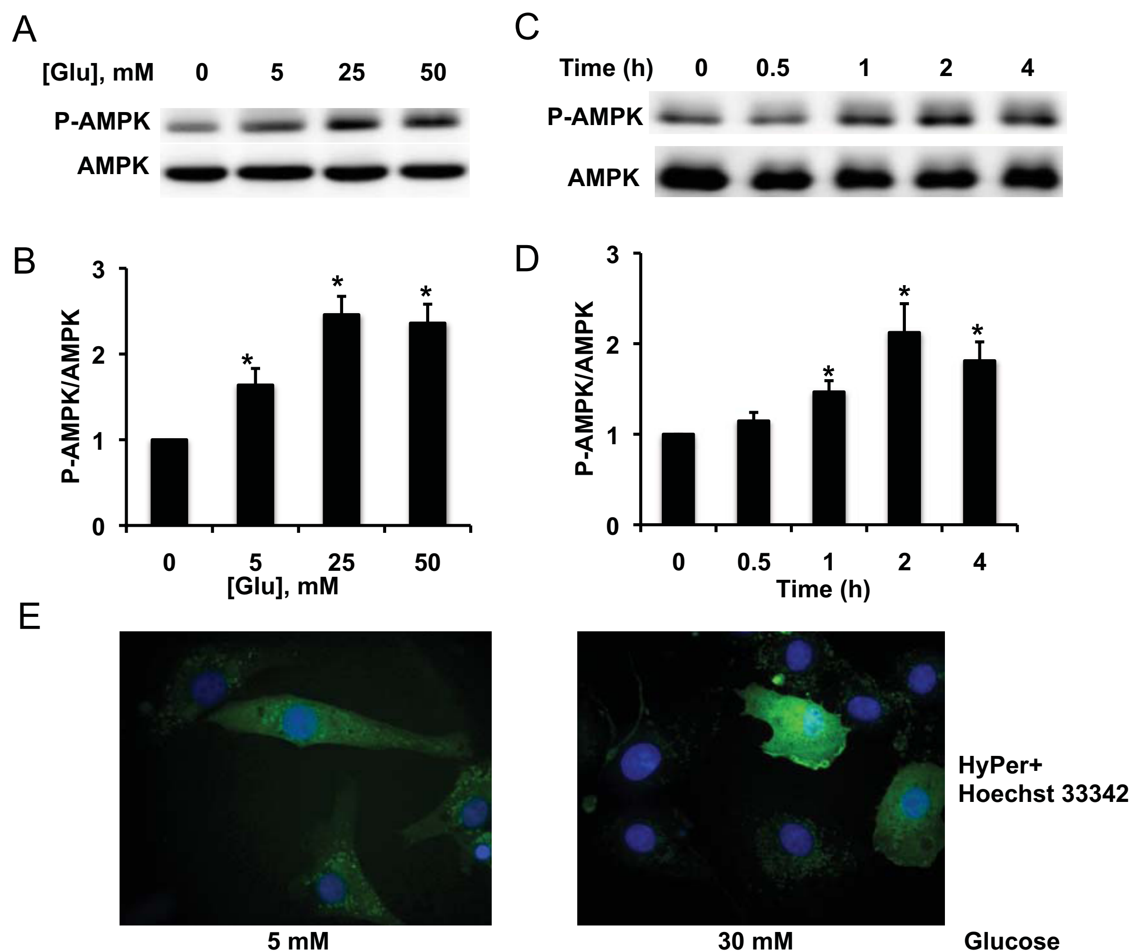

Fig. S4. High glucose induces AMPK phosphorylation and HyPer responses in endothelial cells. Shown in this figure are the results of immunoblots and single cell imaging analyzed in endothelial cells treated with high glucose. (*A*) Representative immunoblot from a dose-response experiment analyzed in cells stimulated with the indicated concentrations of high glucose for 1 h and probed with antibodies as shown; (*B*) pooled data from three independent experiments, analyzing the intensities corresponding to phospho-AMPK and total AMPK by quantitative chemiluminescence. (*C*) Representative time-course experiment in BAECs treated with 30 mM high glucose for the indicated times and analyzed in immunoblots probed with antibodies as shown; (*D*) pooled data from three independent experiments. (*E*) Representative single cell images from one experiment; the cells were treated with glucose (5 mM or 30 mM for 1 h, respectively). Then the cells were fixed and stained with Hoechst 33342, and analyzed as described in *Materials and Methods*. *, $P < 0.05$.

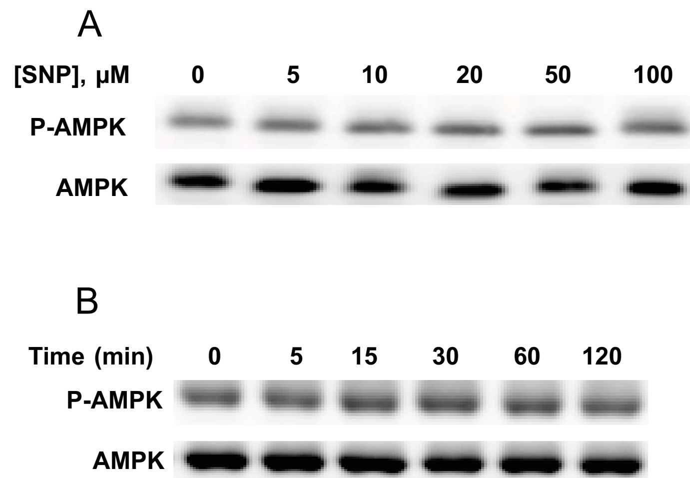

Fig. S5. Effect of SNP on AMPK phosphorylation in endothelial cells. Shown in this figure are the results of immunoblots analyzed in endothelial cells treated with NO donor, SNP and NOC-18. (*A*) Representative immunoblot from a dose-response experiment analyzed in cells stimulated with the indicated concentrations of SNP for 1 h and probed with antibodies as shown; (*B*) representative time course experiment in BAECs treated with 50 μM SNP for the indicated times and analyzed in immunoblots probed with antibodies as shown.

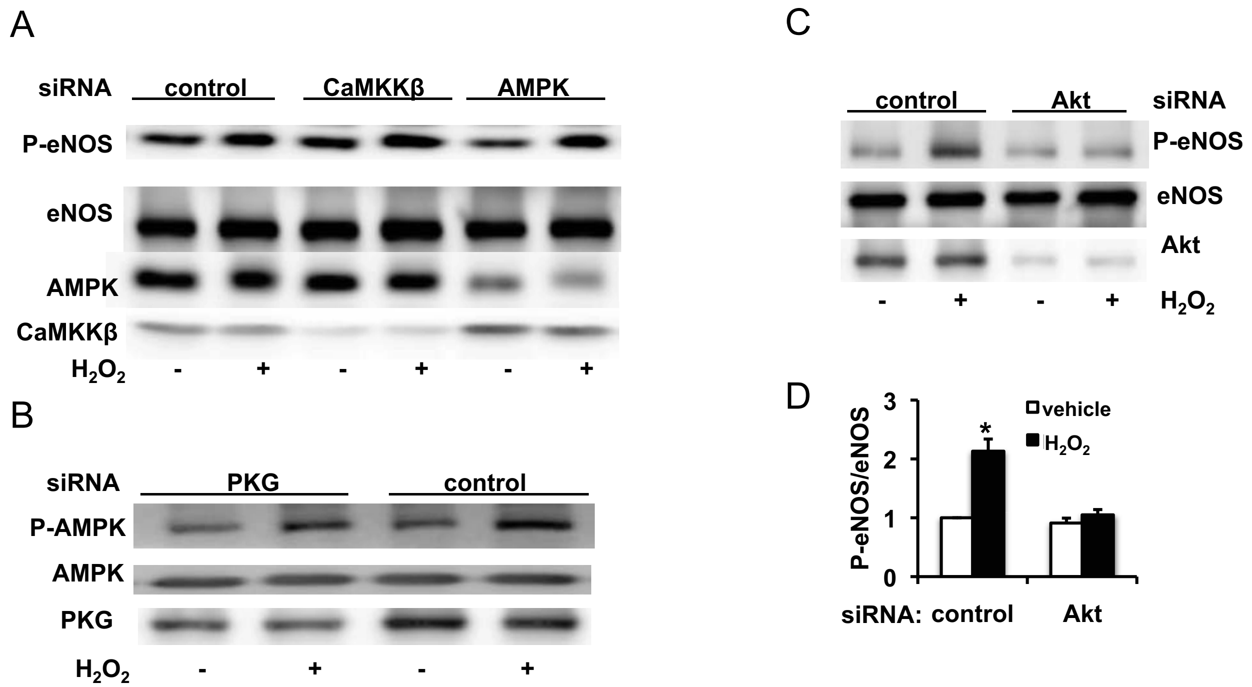

Fig. S6. Effects of siRNA-mediated knockdown of CaMKKβ, AMPK, PKG, or Akt on H_2O_2-mediated phosphorylation responses. In the experiment shown in (*A*), endothelial cells were transfected with control, CaMKKβ, or AMPK siRNA; 48 h after transfection, cells were treated with indicated concentrations of H_2O_2 for 30 min. The blot shown is a representative of five similarly designed experiments that yielded equivalent results. (*B*) Endothelial cells were transfected with control siRNA or with siRNA targeting PKG; 48 h after transfection, cells were incubated with H_2O_2 (200 μM for 30 min) or vehicle as indicated. The blot shown is a representative of three similar experiments. (*C*) Endothelial cells were transfected with control siRNA or with siRNA targeting Akt; 48 h after transfection, cells were incubated with H_2O_2 (200 μM for 30 min) or vehicle as indicated. The blot shown is a representative of three similar experiments. (*D*) Pooled data from three experiments quantitated by chemiluminescence analysis. *, $P < 0.05$.