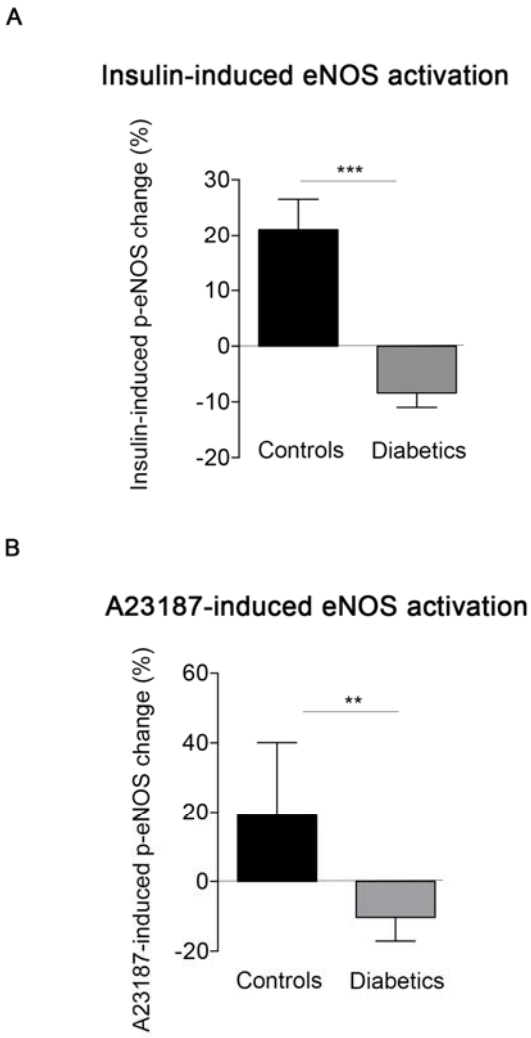

Figure SI. Diabetes is associated with altered endothelial nitric oxide activation. Venous endothelial cells from diabetic and non-diabetic patients were freshly isolated as described in the methods. Endothelial cells were identified by von Willebrand factor (vWF) staining and nuclear morphology and eNOS activation was evaluated as eNOS phosphorylation at Ser1177. A, Pooled data demonstrated that insulin (10 nM, 30 min) increased eNOS phosphorylation at Ser1177 in endothelial cells from nondiabetic controls (n=10), but not in endothelial cells from patients with diabetes (n=7, ***P<0.001). B, Pooled data demonstrated that A23187 treatment (1 μM, 5 min) increased eNOS phosphorylation at Ser1177 in endothelial cells from nondiabetic controls (n=4) but not in endothelial cells from patients with diabetes (n=5, **P=0.02).

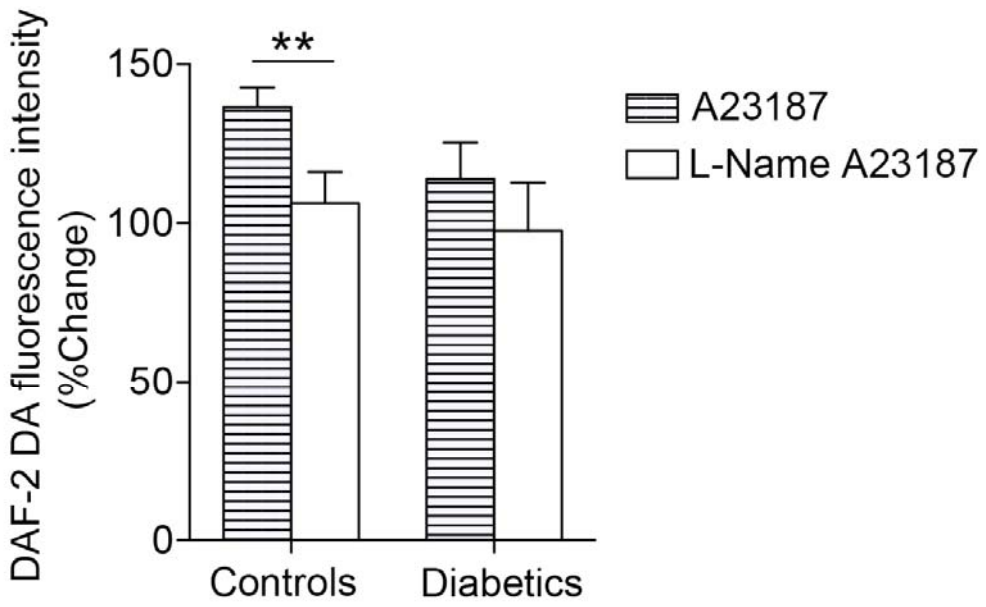

Figure SII. L-NAME blocks nitric oxide production induced by A23187. Venous endothelial cells from diabetic and non-diabetic patients were freshly isolated as described in methods and were incubated with an inhibitor of nitric oxide synthase, L-NAME (300 μM, 1h) or vehicle before loading the cells with DAF-2 DA probe. Then cells were incubated with A23187 and fluorescence intensity was quantified. Pooled data demonstrated the specificity of nitric oxide probe in the endothelial cells (n=8, **P=0.003).

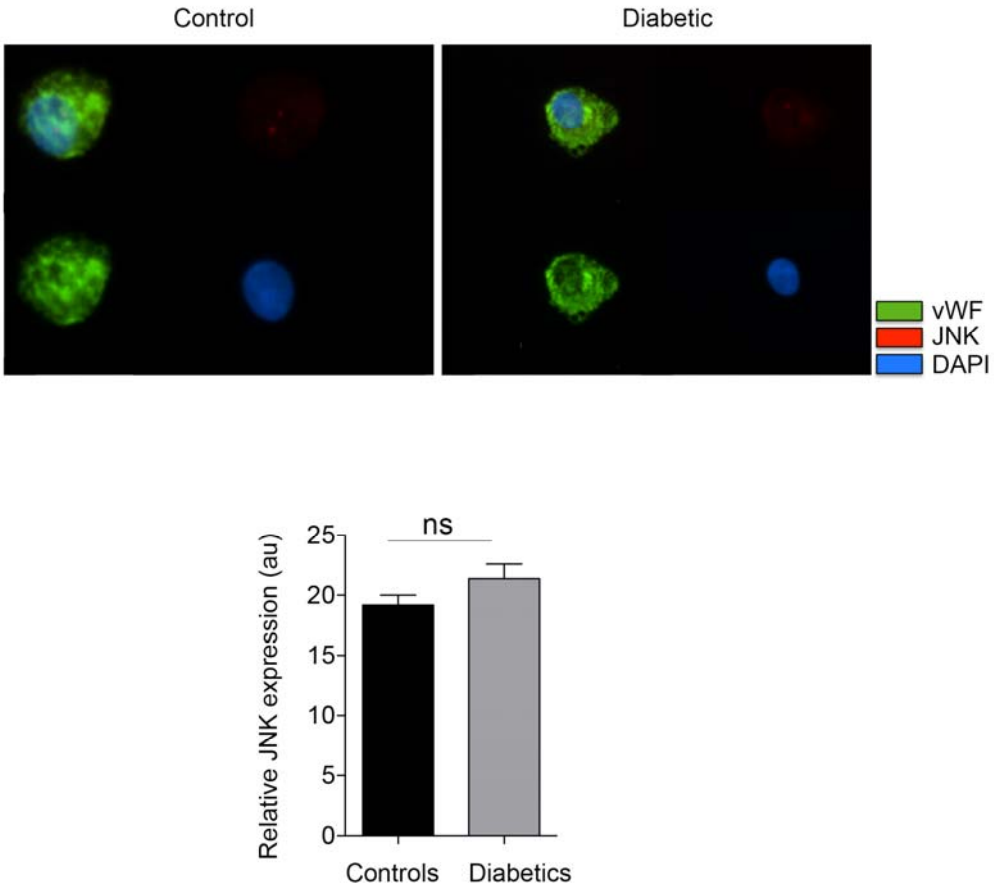

Figure SIII. Diabetes is not associated with a differential expression of JNK. Venous endothelial cells from diabetic and non-diabetic patients were freshly isolated as described in methods. Representative cell from a diabetic patient (right) shows similar JNK expression compared with a cell from a nondiabetic control (left). Pooled data show that JNK levels were comparable in the diabetic patients and diabetics (n=12) compared with the nondiabetic controls (n=12, P=0.17).

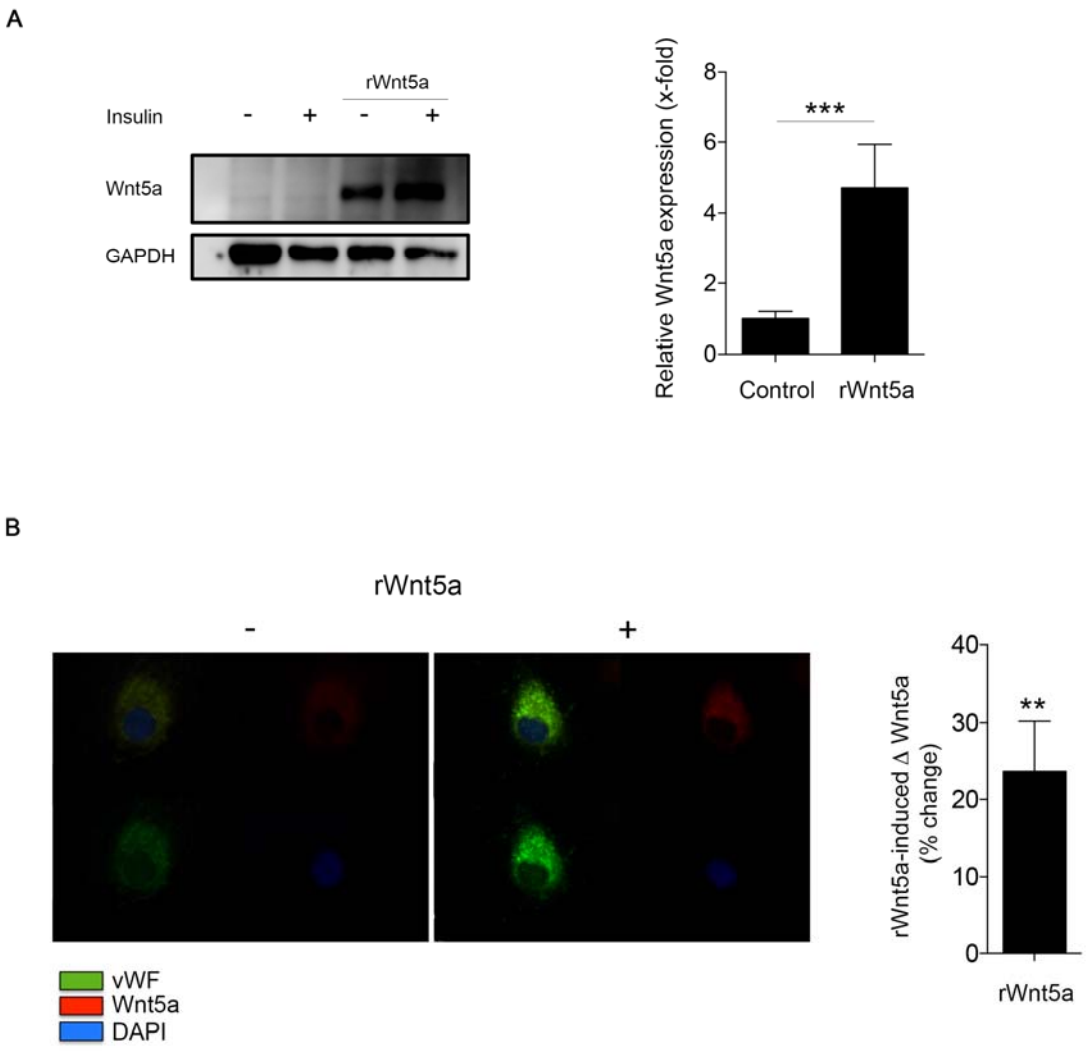

Figure SIV. Evaluation of the efficiency of recombinant Wnt5a treatment on endothelial cells. HAECs were treated with rWnt5a protein (100 ng/mL) for 1h, 37°C and treatment efficiency was evaluated by Western blot and immunofluorescence. A, rWnt5a treatment efficiency was evaluated by Western blot under non-reducing conditions. Pooled data demonstrated a 4.71-fold enhanced Wnt5a expression in endothelial cells when treated with rWnt5a. Data are shown as mean ±SEM for 6 independent experiments (***P<0.001 vs. endogenous expression). B, rWnt5a treatment efficiency was evaluated by immunofluorescence. Representative fluorescence images showing the increase upon endogenous Wnt5a expression after recombinant treatment is shown. Pooled data demonstrated an enhanced Wnt5a expression in endothelial cells when treated with rWnt5a. Data are shown as mean ±SEM for 4 independent experiments (**P=0.007 vs. endogenous expression).

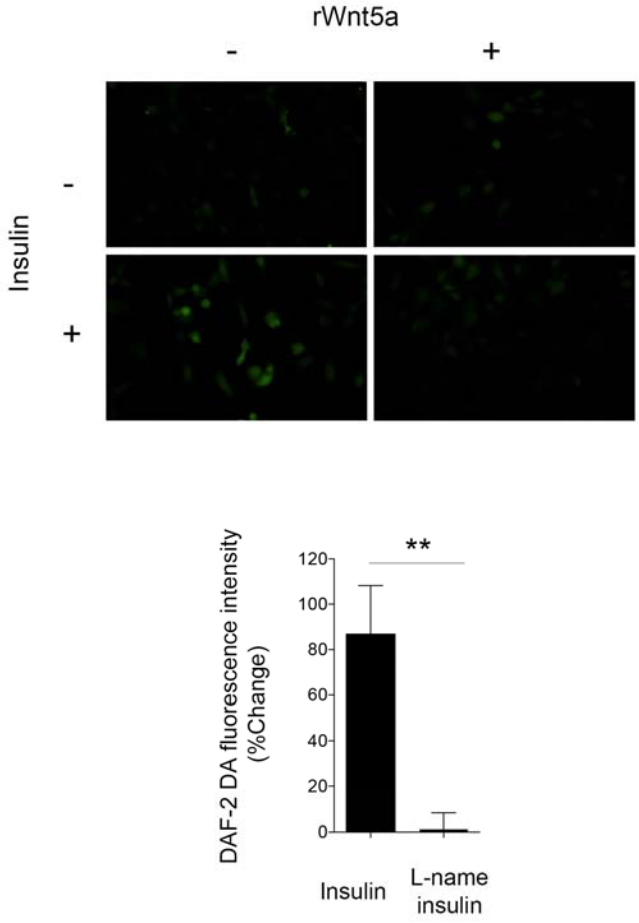

Figure SV. Nitric oxide synthase inhibitor L-NAME blocks nitric oxide production induced by insulin. HAECs were incubated with an inhibitor of nitric oxide synthase, L-NAME (300 μM, 1h) or vehicle before loading the cells with DAF-2 DA probe. Then cells were incubated with insulin and fluorescence intensity was quantified. Pooled data demonstrated the specificity of nitric oxide probe in the endothelial cells (n=8, **P=0.004).

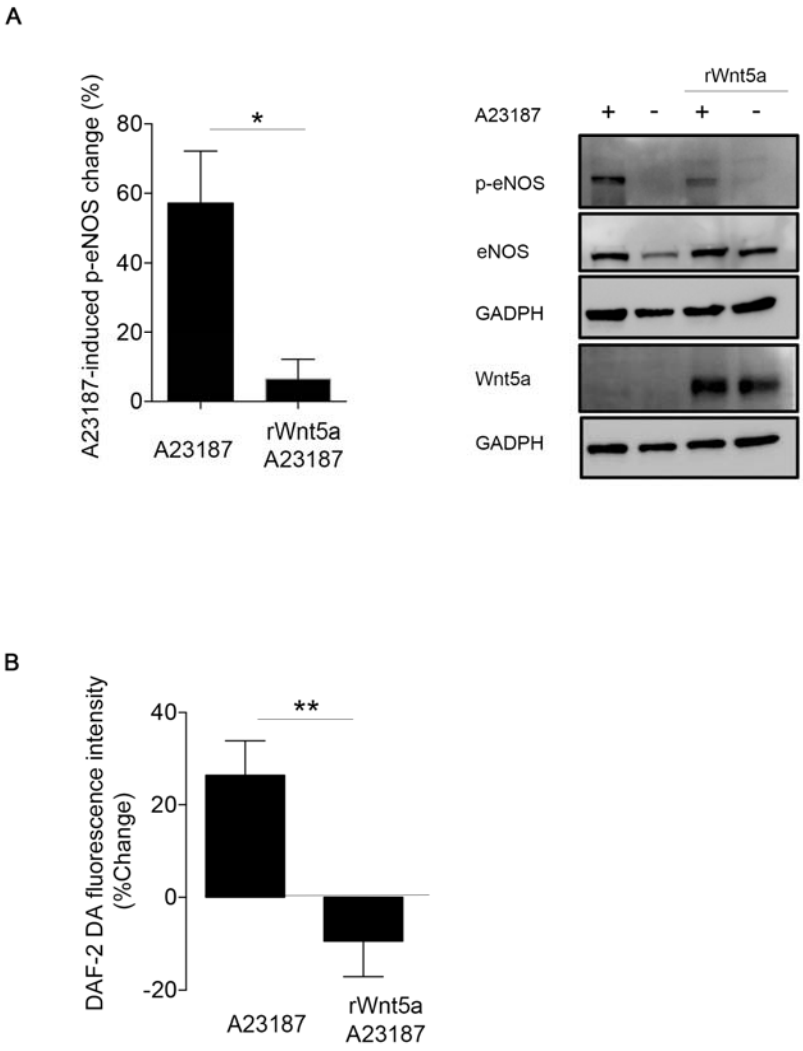

Figure SVI. Wnt5a promotes endothelial dysfunction. HAECs were treated with rWnt5a protein (100 ng/mL) for 1h, 37°C and treatment A23187 (1 μM, 5 min)-mediated eNOS activation and NO production were evaluated. A. A23187-mediated eNOS activation was evaluated as eNOS phosphorylation at Ser1177. Pooled data demonstrate that insulin increased eNOS phosphorylation at Ser1177, but treatment with rWnt5 impaired A23187-induced eNOS phosphorylation at Ser1177 (n=7, *P=0.01) as observed after immunofluorescence analysis (left panel). A representative immunoblot probed with antibodies as shown yielded equivalent results (right panel). B. HAECs were loaded DAF-2 DA and then treated with 0 μmol/L or 1 μmol/L A23187 for 5 minutes in the absence or the presence of rWnt5a. The bar graph represents the mean values ±SEM independent experiments (n=7, **P=0.004).

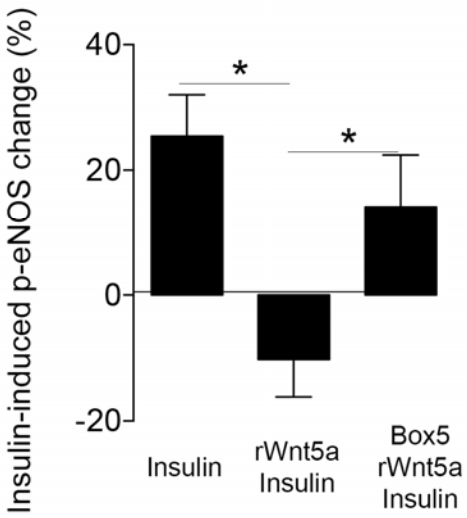

Figure SVII. Wnt5a inhibition restores endothelial function in HAECs. Insulin mediated eNOS activation in HAECs was evaluated as eNOS phosphorylation at Ser1177. Pooled data demonstrate that insulin increased eNOS phosphorylation at Ser1177. Treatment with rWnt5 impaired insulin-mediated changed in eNOS phosphorylation at Ser1177 (n=5, *P=0.01). Pre-treatment with Wnt5a competitive antagonist Box5 (100 μM, 1h) restored insulin-mediated eNOS phosphorylation at Ser1177 in HAECs treated with rWnt5a (n=4, *P=0.05).

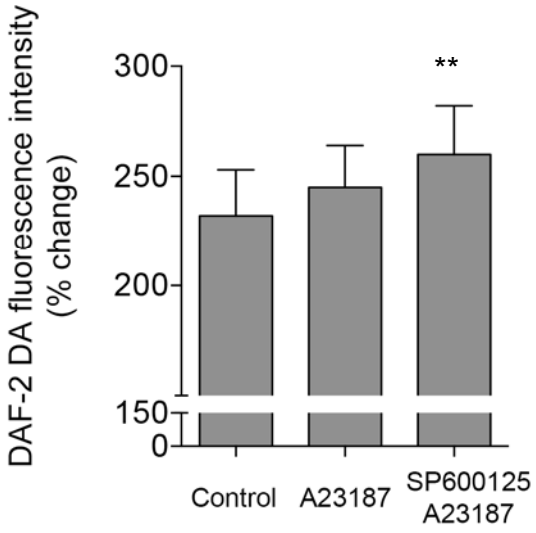

Figure SVIII. JNK inhibition restores endothelial function in endothelial cells from diabetic patients. Venous endothelial cells from diabetic patients were freshly isolated and loaded with nitric oxide specific probe DAF-2 DA fluorescence probe before treatments. Pooled data demonstrate that JNK inhibition restored A23187-induced nitric oxide production in endothelial cells from patients with diabetes (**P=0.002 vs. control).

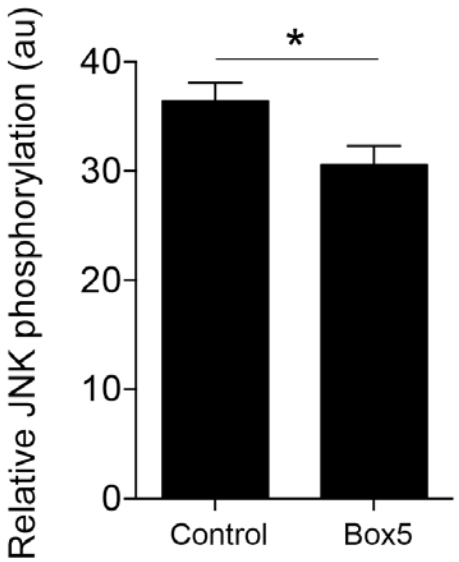

Figure SIX. Wnt5a inhibition reduces JNK activation in endothelial cells from diabetic patients. Venous endothelial cells from diabetic patients were freshly isolated as described in methods and treated with Box5 (100 uM) for 1 hour. Pooled data show that JNK phosphorylation was reduced after Box5 treatment (n=9, *P=0.017).

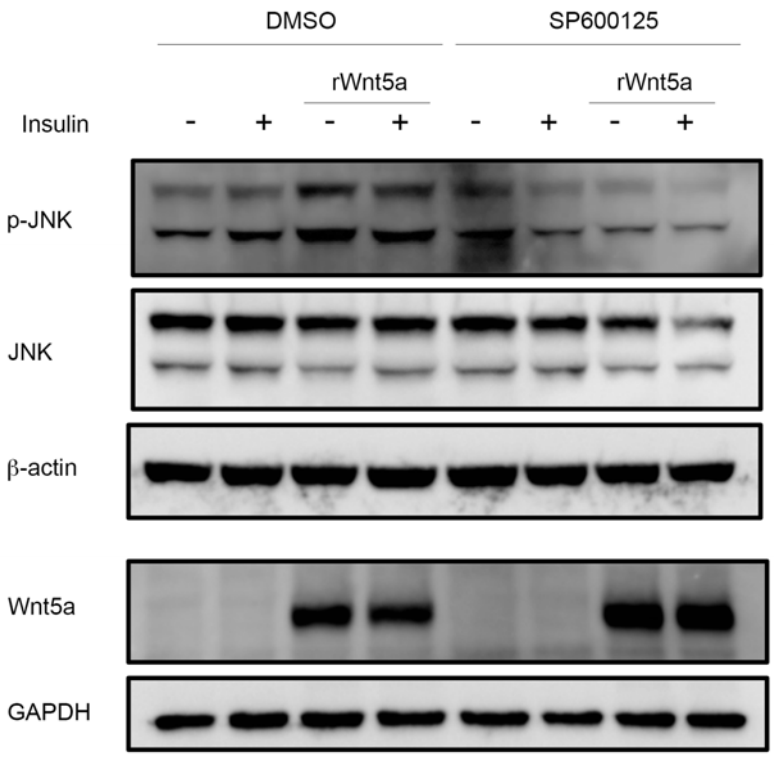

Figure SX. SP600125 treatment prevents Wnt5a-mediated JNK activation. JNK activation was studied by Western blot by evaluating JNK phosphorylation at Thr183/Tyr185 after rWnt5a treatment in HAECs. rWnt5a induced JNK phosphorylation at Thr183/Tyr185, but JNK activation mediated by rWnt5a was blocked in the presence of JNK chemical inhibitor SP600125. The blots shown are representative of at least three independent experiments that yielded equivalent results.

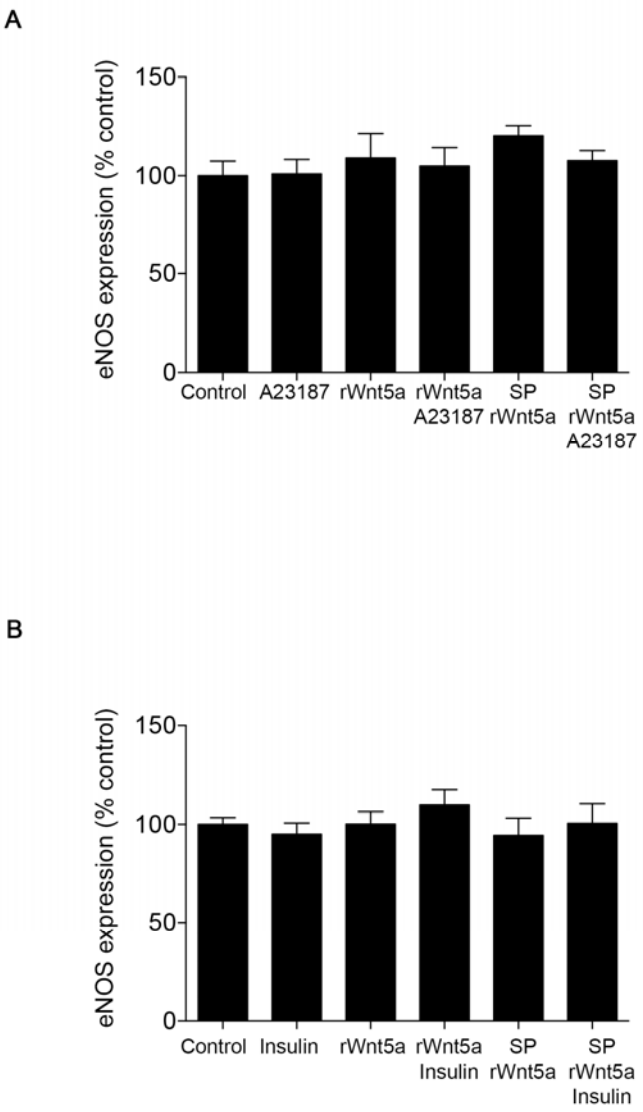

Figure SXI. Wnt5a treament does not change eNOS expression. HAECs were treated with rWnt5a protein (0 to 100 ng/mL, 1h) in the presence of absence of JNK chemical inhibitor SP600125 (10 μM, 30 min), and A23187- (1 μM, 5 min) and insulin- (10 nM, 30 min) mediated eNOS expression was evaluated by quantitative inmunofluorescence. A, Pooled data demonstrate that neither A23187, rWnt5a, nor SP600125 treatments changed eNOS expression The bar graph represents the mean values ±SEM independent experiments (n=4-8). B, Pooled data demonstrate that neither insulin, rWnt5a, nor SP600125 short treatments changed eNOS expression The bar graph represents the mean values ±SEM independent experiments (n=7).

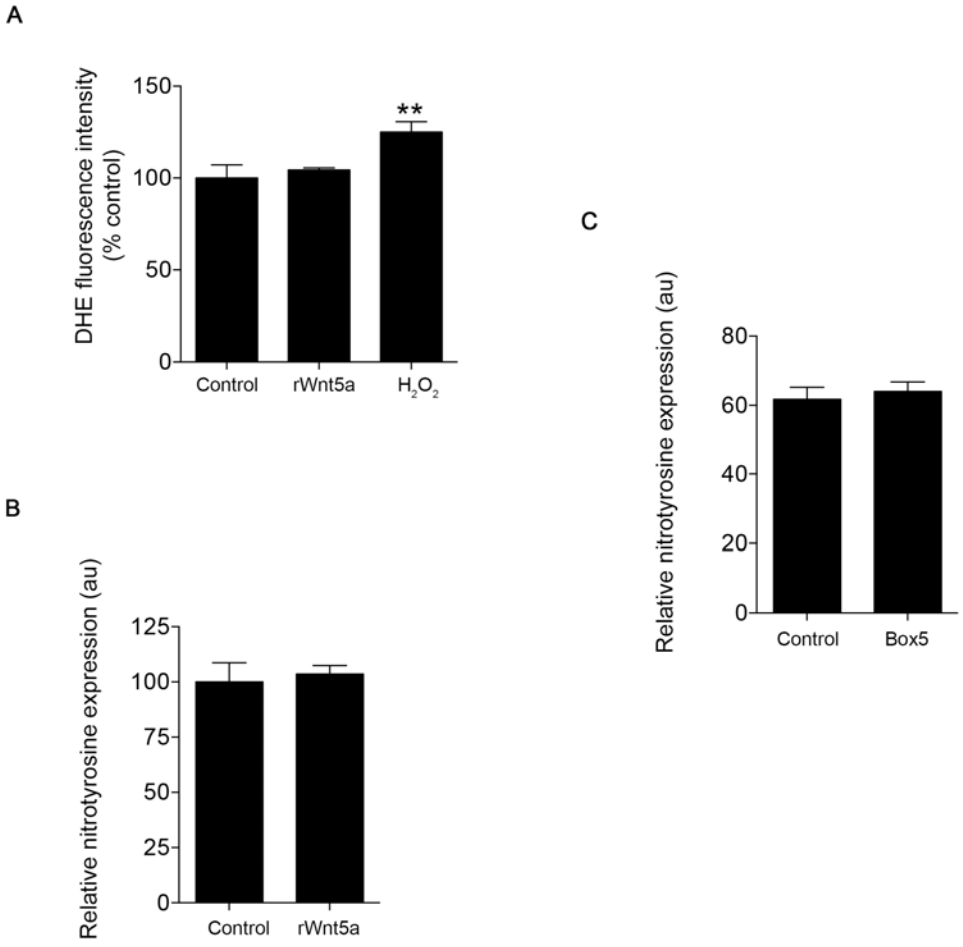

Figure SXII. Wnt5a does not change ROS production. A, HAECs were treated with rWnt5a (100 ng/ml) or H_2O_2 (500 μM) for 1 hour and ROS production was determined by DHE (n=5, P= 0.167 rWnt5a vs control; **P=0.01 H_2O_2 vs control). B, Pooled data from 7 independent experiments show that Wnt5a does not induce an increase in nitrotyrosine staining as a marker of oxidative stress (P=0.6922). C, Freshly isolated endothelial cells from patients with diabetes were isolated and treated with 0 or 100 μmol/L Box5, Wnt5a competitive antagonist, for 1 hour, JNK phosphorylation at Thr183/Tyr185 was quantified by evaluating >20 cells per patient in each condition. Pooled data demonstrate that treatment with Box5 does not change nitrotysorine staining. (n=5, P= 0.57).