SUPPLEMENTAL DATA

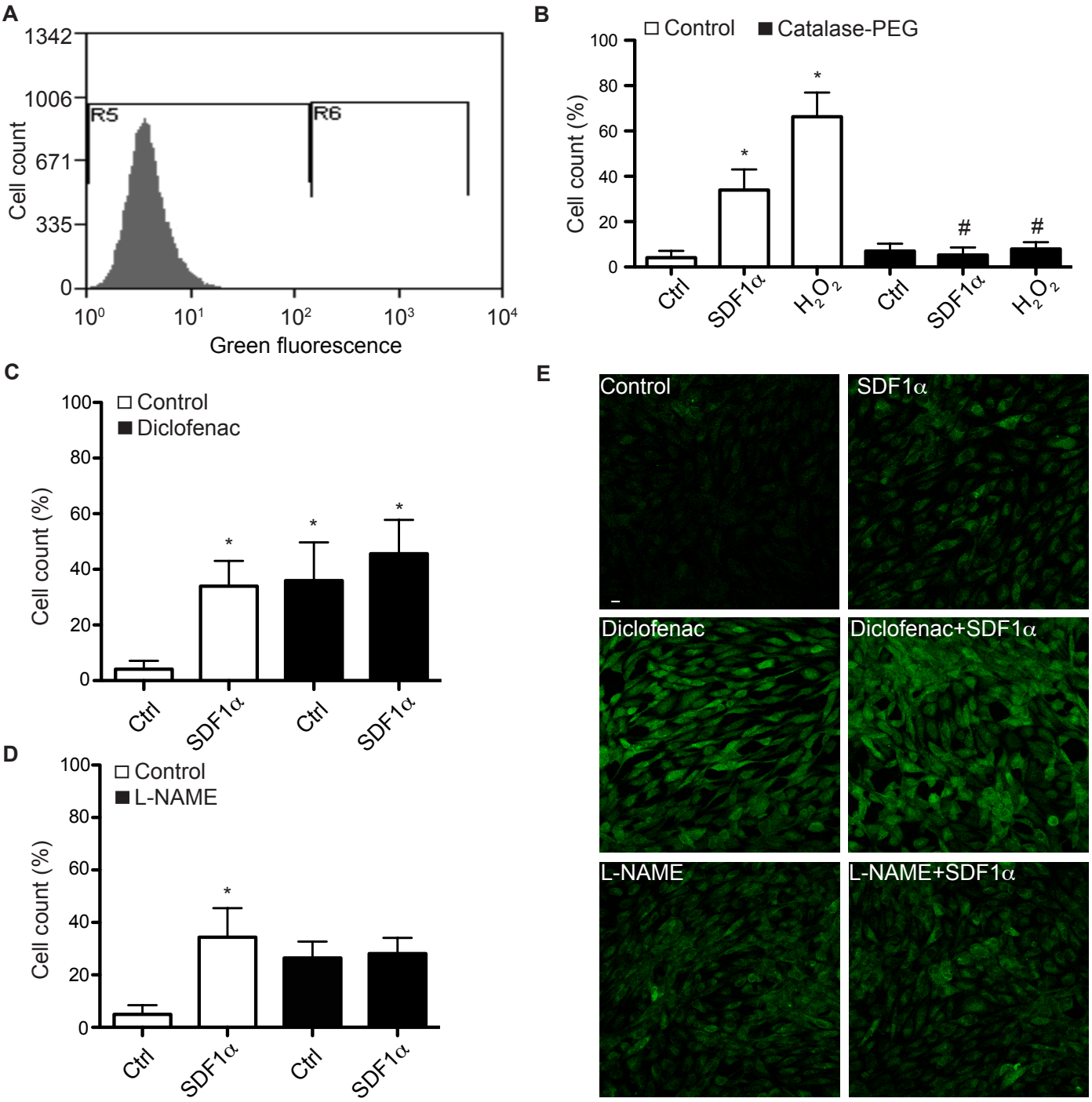

Figure SI. ROS are generated following SDF-1α treatment. **A**, Flow cytometry image of control cells without DCFDA-staining. **B-D**, BAECs were preloaded with CM-H_2DCFDA, followed by incubation with 1000 U/ml PEG-catalase (**B**), 1 mM diclofenac (**C**) or 10 mM L-NAME (**D**) for 30 minutes and SDF-1α (100 ng/ml) for 5 minutes or H_2O_2 (1 mM) for 30 minutes. The intracellular H_2O_2 production was quantified as CM-H_2DCFDA fluorescence, detected by flow cytometry analysis. *, $P<0.05$, compared to control BAECs; #, $P<0.05$; compared to control cells with SDF-1α or H_2O_2; n=3. **E**, Representative images of an endothelial cell monolayer following incubation with 1 mM diclofenac or 10 mM L-NAME for 30 minutes and SDF-1α (100 ng/ml) for 5 minutes. BAECs were fixed for CM-H_2DCFDA staining. Scale bar, 10 μm.

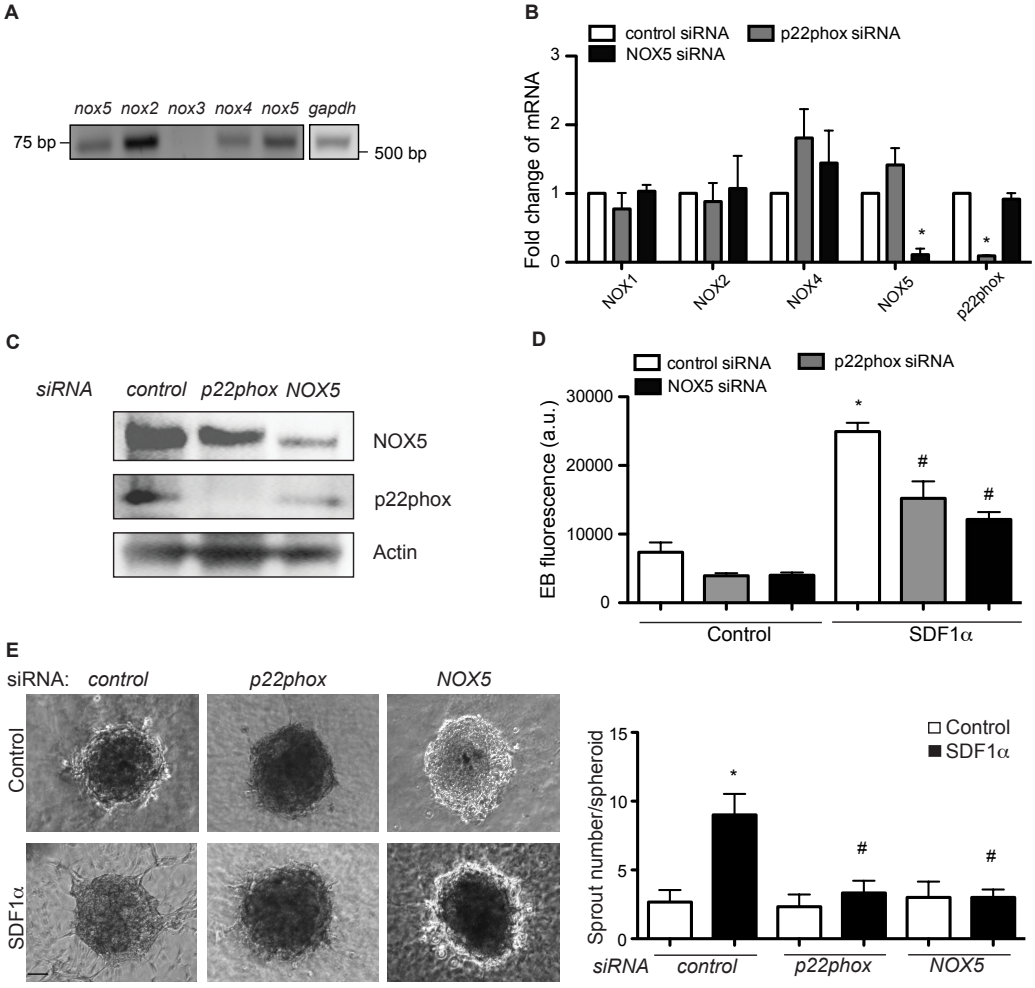

Figure SII. NOXs are required for SDF-1α-induced angiogenesis in BAECs. **A**, Total RNA of BAECs was reverse transcribed to cDNA and analyzed for the RNA levels of NOX1-5 using their specific primers. GAPDH was used as a housekeeping gene control. **B-C**, The knockdown of p22phox and NOX5 using their specific siRNAs. BAECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, the total RNA of BAECs were reverse transcribed to cDNA and analyzed for the RNA levels of NOX1, 2, 4, 5 and p22phox by quantitative PCR (**B**). In addition, the cell lysates were used for Western blotting to detect endogenous p22phox and NOX5 (**C**). **D**, BAECs were transfected with p22phox, NOX5, or control siRNAs and then treated with SDF-1α in 100 ng/ml for 5 minutes. $O_2^{\cdot-}$ production was quantified as ethidium bromide fluorescence. *, *P*<0.05, compared to control BAECs; #, *P*<0.05; compared to control cells with SDF-1α; n=3. **E**, Nox5 and p22phox were required for the sprouting angiogenesis of BAEC spheroids. The spheroid angiogenesis assays were performed with BAECs that were transfected with p22phox, NOX5 or control siRNAs. 100 ng/ml SDF-1α was used to induce sprout formation. Images of collagen-embedded BAEC spheroids demonstrate the formation of sprouts after 72 hours of treatment with SDF-1α (100 ng/ml). The number of sprouts per spheroids were counted and the quantitative data were presented. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=3. Scale bar, 100 μm.

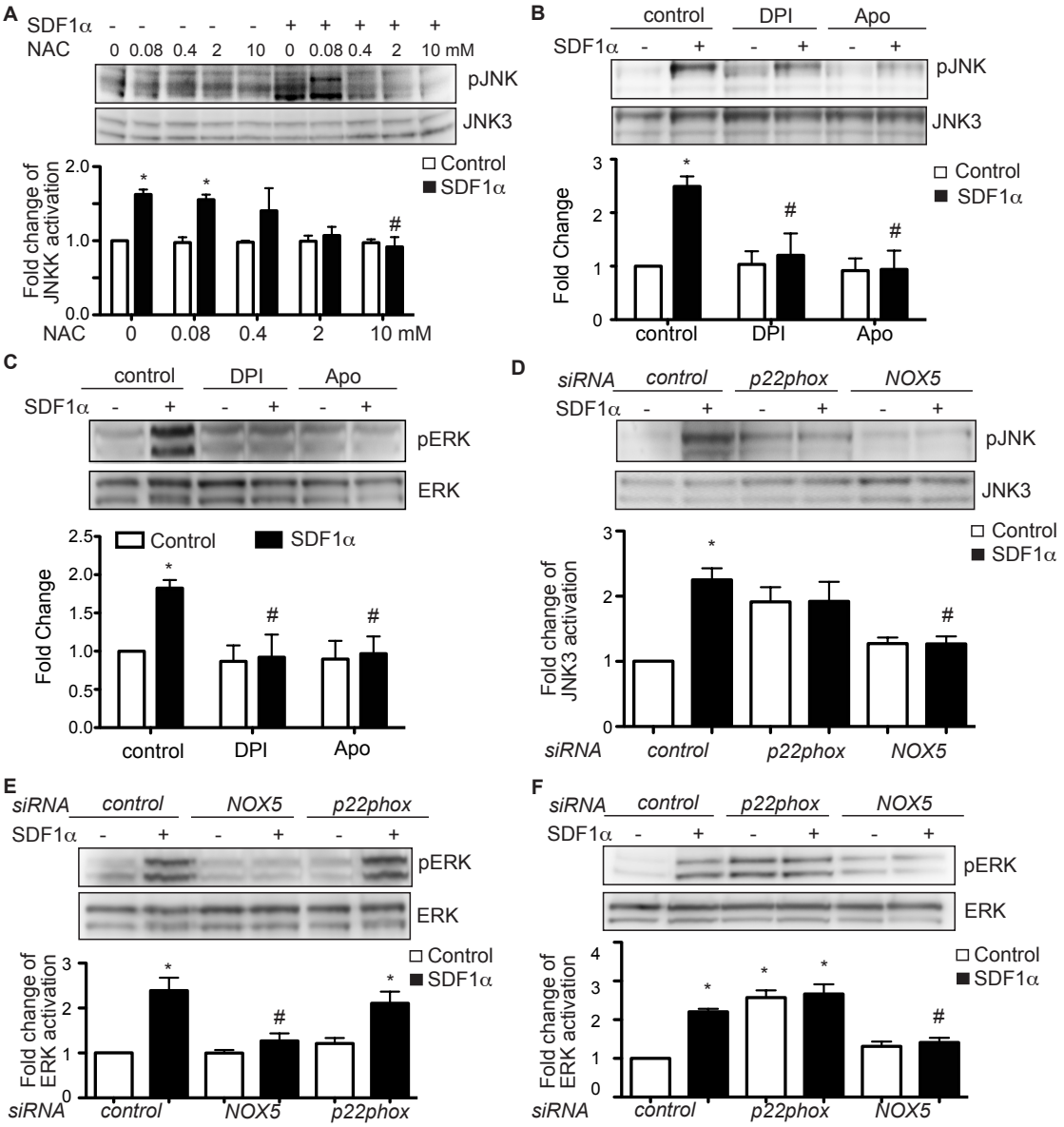

Figure SIII. NOX5 is required for SDF-1α-induced JNK3 and ERK activation in BAECs and HUVECs. **A**, Antioxidant NAC inhibited SDF-1α-induced JNK3 activation in BAECs. BAECs were incubated with NAC in different concentration for 30 minutes and then treated with SDF-1α (100 ng/ml) for 10 minutes to activate JNK3. Cell lysates were used for Western blotting to detect the activation of JNK3. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=4. **B-C**, HUVECs were incubated with NOX inhibitors DPI (10 μM) or apocynin (Apo, 100 μM) for 30 minutes and then treated with SDF-1α (100 ng/ml) for 10 minutes to activate JNK3 and ERK. Cell lysates were used for Western blotting to detect the activation of JNK3 (**B**) and ERK (**C**). *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=3. **D**, HUVECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, cells were treated with SDF-1α (100 ng/ml) for 10 minutes to activate JNK3. Cell lysates were used for Western blotting to detect the activation of JNK3. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=3. **E**, BAECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, cells were treated with SDF-1α (100 ng/ml) for 10 minutes to activate JNK3. Cell lysates were used for Western blotting to detect the activation of ERK. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells

with SDF-1α; n=4. **F**, HUVECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, cells were treated with SDF-1α (100 ng/ml) for 10 minutes to activate JNK3. Cell lysates were used for Western blotting to detect the activation of ERK. *, $P<0.05$; compared to control cells without SDF-1α. #, $P<0.05$; compared to control cells with SDF-1α; n=3.

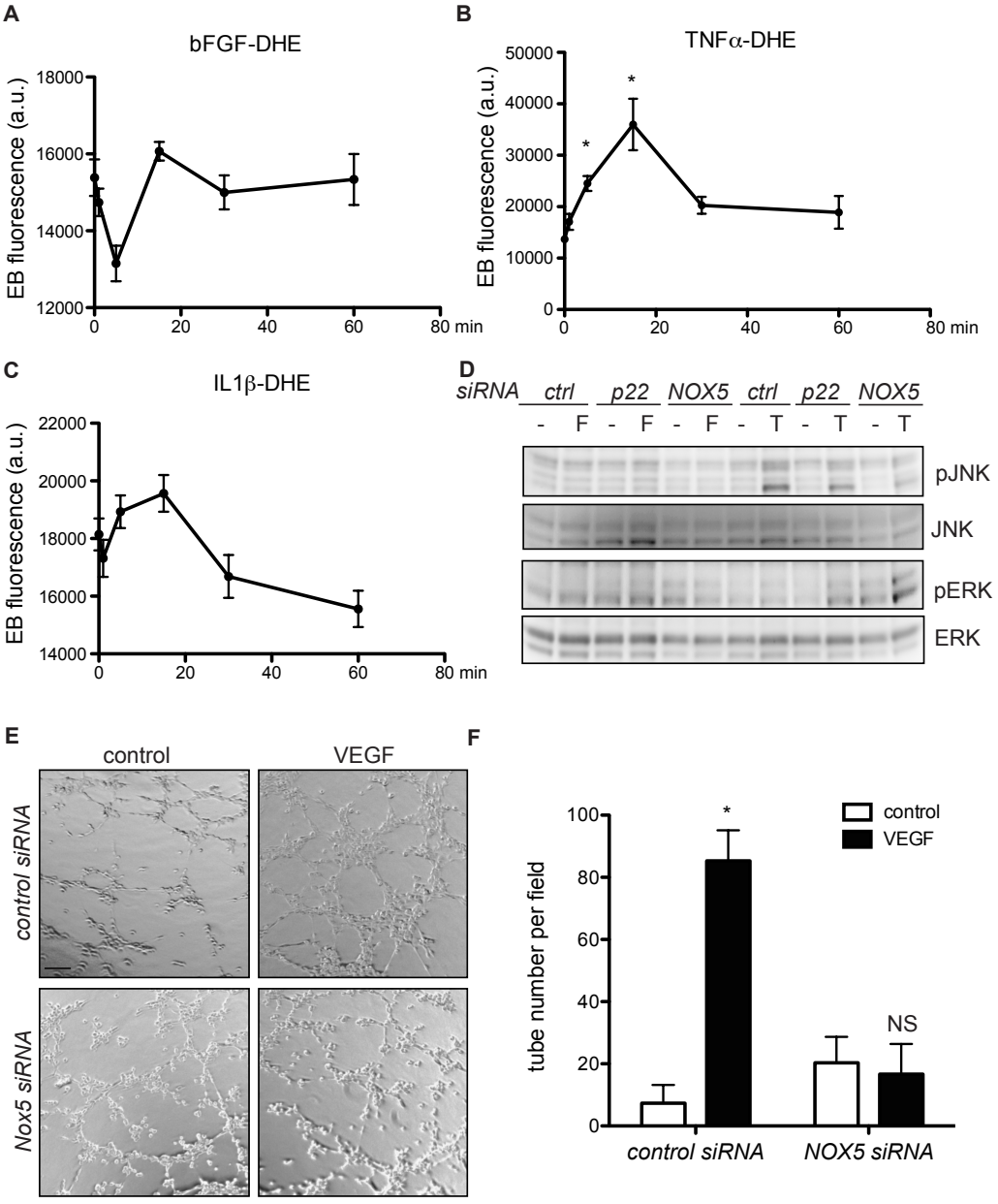

Figure SIV. Multiple cytokines induce ROS generation, TNFα-induced JNK3 activation and VEGF-induced tube formation are mediated by NOX5. **A-C**, BAECs were treated with 100 ng/ml b-FGF (A), 50 ng/ml TNFα (B) and 50 ng/ml IL1β (C) for different time periods. $O_2^{\cdot -}$ production was quantified as ethidium bromide fluorescence. *, *P*<0.05, compared to control BAECs. n=4. **D**, BAECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, cells were treated with 100 ng/ml b-FGF (F) or 50 ng/ml TNFα (T) for 10 minute. Cell lysates were used for Western blotting to detect the activation of JNK3 and ERK. **E-F**, In vitro Matrigel angiogenesis assays were performed with HUVECs that were transfected with NOX5 or control siRNAs. 50 ng/ml VEGF was used to induce tube formation. Images of formed tubes were taken (**E**) and tube numbers were counted (**F**). *, *P*<0.001; compared to control cells without VEGF. NS, not significant. Scale bar, 50 μm.

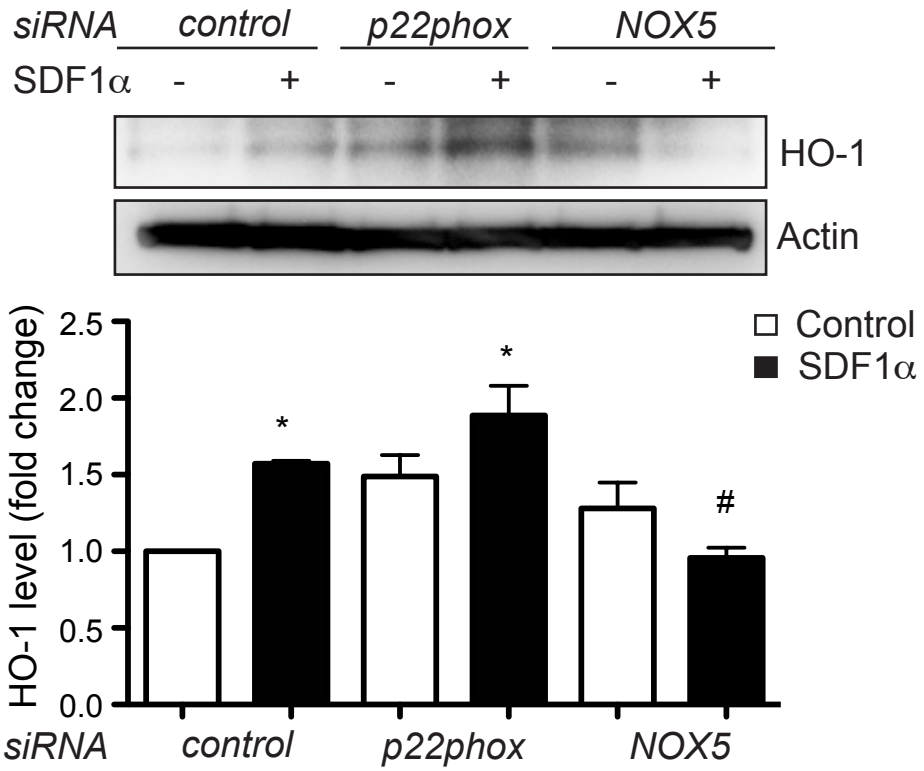

Figure SV. HO-1 induction in response to SDF-1α stimulation is mediated by NOX5. BAECs were transfected with p22phox siRNA, NOX5 siRNA or control siRNA. Three days later, cells were treated with SDF-1α (100 ng/ml) for 8 hours. Cell lysates were used for Western blotting to detect the HO-1 protein level. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=5.

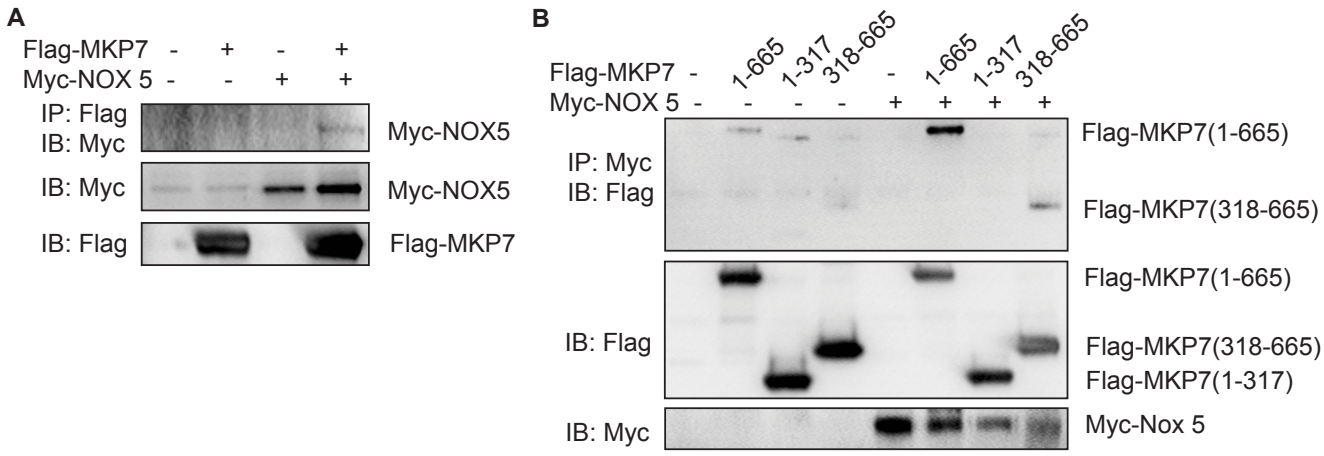

Figure SVI. NOX5 is associated with MKP7. **A**, NOX5 is associated with MKP7. HEK293 cells were transfected with Flag-MKP7 and Myc-NOX5 constructs. One day later, total cell lysates were used for immunoprecipitation for Flag-MKP7 and immunoblotting for Myc-NOX5. **B**, HEK293 cells were transfected with different Flag-MKP7 mutants and Myc-NOX5 constructs. One day later, total cell lysates were used for immunoprecipitation for Myc-NOX5 and immunoblotting for Flag-MKP7.

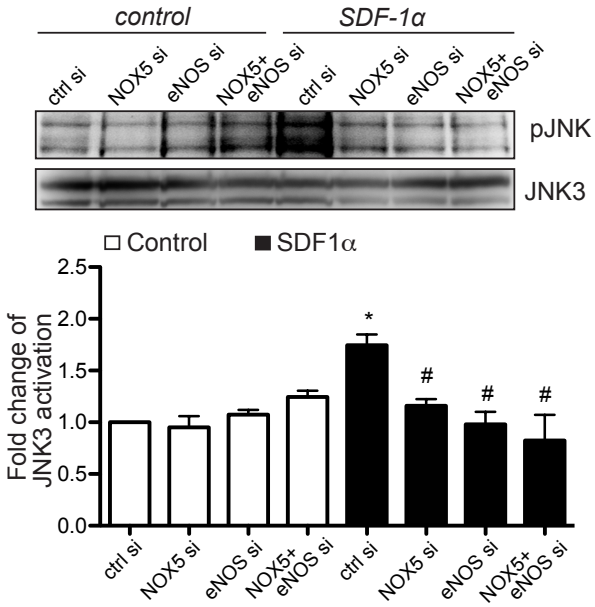

Figure SVII. JNK3 activation in response to SDF-1α stimulation is mediated by both NOX5 and eNOS. **B**, BAECs were transfected with NOX5 siRNA, eNOS siRNA or control siRNA. Three days later, cells were treated with SDF-1α (100 ng/ml) for 10 minutes. Cell lysates were used for Western blotting to detect the activation of JNK3. *, *P*<0.05; compared to control cells without SDF-1α. #, *P*<0.05; compared to control cells with SDF-1α; n=3.