The NADPH oxidase NOX4 promotes the directed migration of endothelial cells by stabilizing vascular endothelial growth factor receptor 2 protein

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Running title: Stabilization of VEGFR-2 by Nox4

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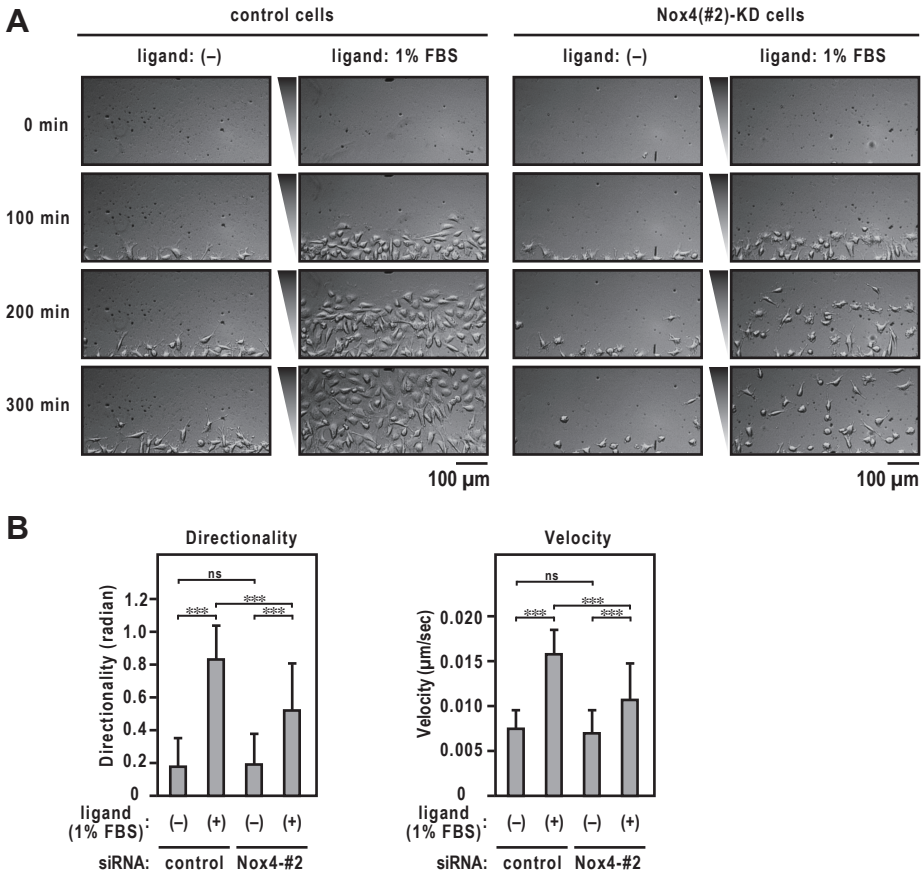
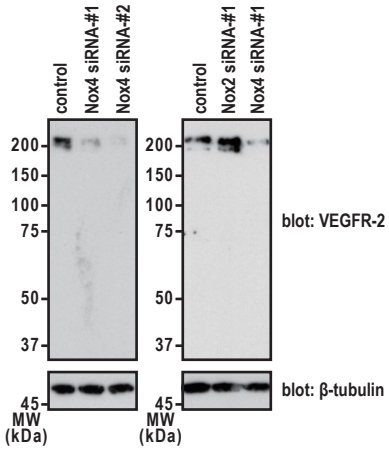
Supplemental Figure 1



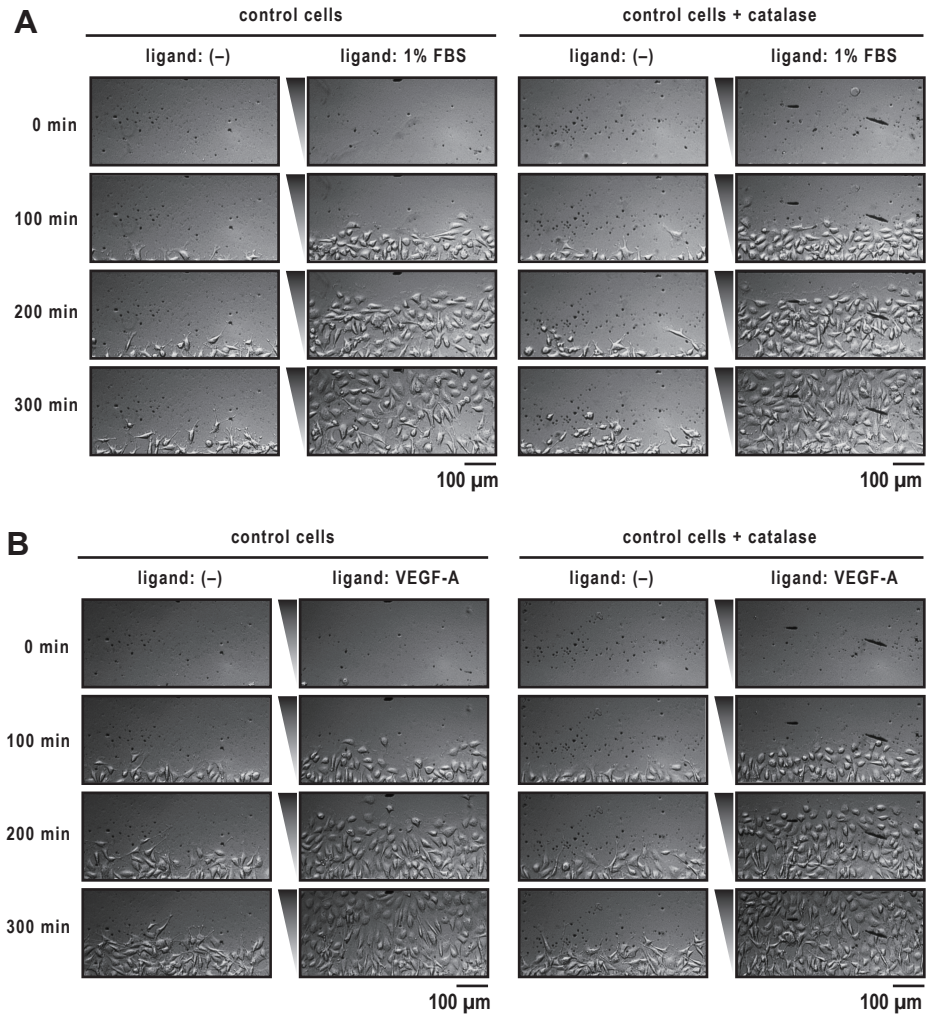
Supplemental Figure 1. Reduced directed migration of Nox4-knocked down ECs.
(A) Images of ECs migrating toward FBS in TAXIScan channels. The concentration of injected FBS was 1%. The distance between the start line (bottom) and finish line (top) of each channel was 260 μm. Scale bar: 100 μm.
(B) Statistical analysis, VD plots. Each graph represents the mean ± SD of pooled data of migrating cells ($n \geq 30$) from three independent experiments. ***$p < 0.001$. Statistical analysis used the Tukey-Kramer test.
The data are representative of results from three independent experiments.

Supplemental Figure 2



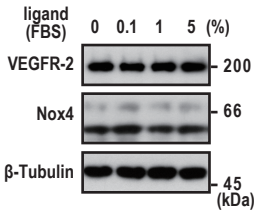
Supplemental Figure 2. Maintenance of VEGFR-2 protein levels by Nox4.
The protein expression levels of Nox4 or Nox2 siRNA-transfected ECs were analyzed by immunoblotting using the indicated antibodies. The positions of the marker proteins are indicated in kDa.
The data are representative of results from three independent experiments.

Supplemental Figure 3



Supplemental Figure 3. Directed migration of catalase-treated ECs.
(A and B) Images of ECs migrating toward FBS in TAXIScan channels. The concentration of injected FBS was 1% (A). The concentration of injected VEGF-A was 500 pg/mL (B). The distance between the start line (bottom) and finish line (top) of each channel was 260 µm. Scale bar: 100 µm.
The data are representative of results from three independent experiments.

Supplemental Figure 4



Supplemental Figure 4. Nox4 and VEGFR-2 protein levels with VEGF-A as ligand for migration assay.

EA.hy926 cells were exposed to each concentration of FBS for 6 h. Protein expression levels were analyzed by immunoblotting using the indicated antibodies. The positions of the marker proteins are indicated in kDa.

The data are representative of results from three independent experiments.