Supplementary Data

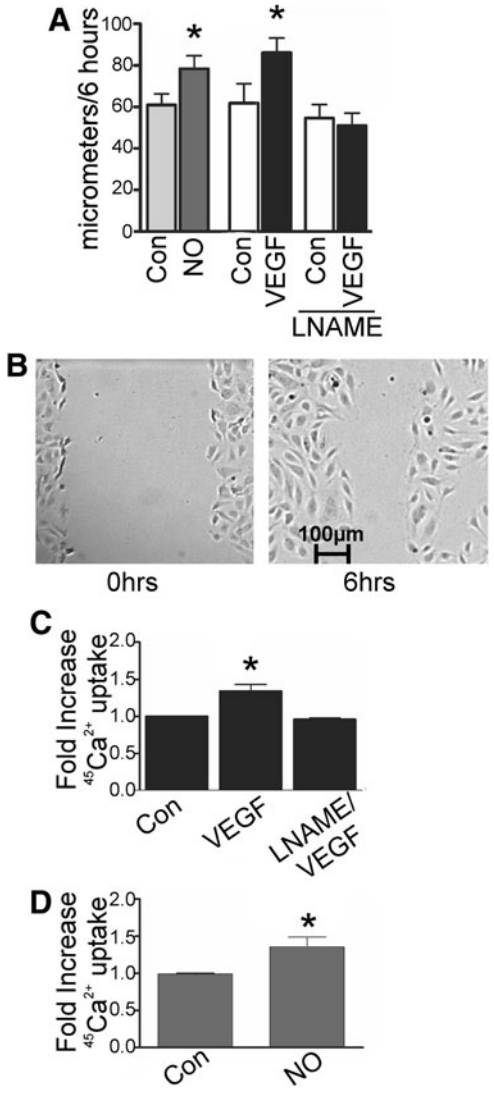
Supplementary Methods

Migration assay

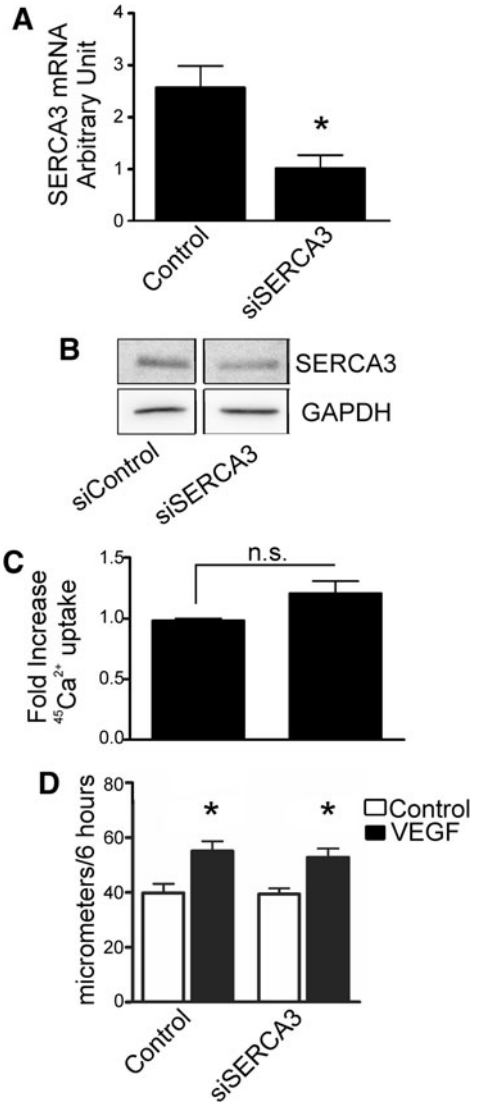
Bovine aortic endothelial cells (BAECs) or human aortic endothelial cells (HAECs) were grown to 80% confluency and quiesced overnight. Scratch wounds were applied to endothelial cell (EC) monolayers in low-serum media as previously described (11, 20). Inhibitors were given 1 h before making a scratch wound with a pipette tip and reapplied at the time of the scratch. The vascular endothelial growth factor (VEGF; 50 ng/ml) or diethylenetriamine NONOate (DETA NONOate) (30 μM, released concentration $\approx 1\ \mu M$) (32) was given at the time of the scratch in a serum-free medium. Images were taken at 0 and 6 h at three fixed locations along the scratch (Fig. S1). Migration distances were averaged from the three measurements per condition using ImageJ software, and this was considered as $n = 1$. Briefly, images were analyzed with ImageJ software (NIH) by measuring the average distance of migration for each cell bordering the scratch in a given field of view. The x and y locations of the center of each cell were taken using thresholded images and the center of the mass ImageJ function at both 0 and 6 h. Cell locations for each timepoint were averaged. The migration distance was calculated as (avg location at 6 h–avg. location at 0 h)/2.

Intracellular Ca^{2+} imaging

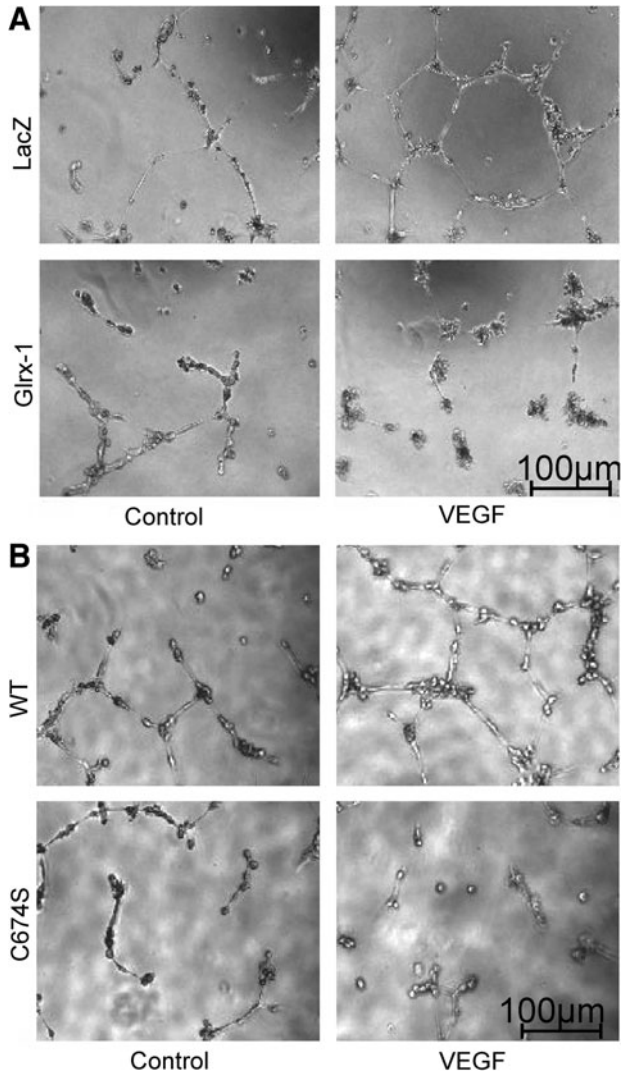
HAECs plated on gelatin-coated glass coverslips were loaded with 2 μM Fura2-AM (Invitrogen) in the presence of 0.02% pluronic F127 (Invitrogen) in serum-free endothelial growth media, and right before the experiment were transferred to nominally Ca^{2+}-free physiological saline solution (PSS) supplemented with 2.5 mM probenecid (Alfa Aesar, Ward Hill, MA). Changes in intracellular Ca^{2+} (F_{340}/F_{380}) were monitored as previously described (4, 19). Briefly, cells were allowed to equilibrate in nominally Ca^{2+}-free PSS for 1 min before addition of VEGF, $^{\bullet}$NO, or thapsigargin (TG). After 2.5 min, Ca^{2+} (2 mM) was added to the PSS. Ca^{2+} influx was recorded for 2 min before addition of ionomycin (2 μM) to permeablize the membrane and manganese (8 mM) to quench the Fura2. A dual-excitation fluorescence imaging system (Intracellular Imaging, Cincinnati, OH) was used for studies of individual cells (see Supplementary Methods for expanded details). The system was calibrated using Ca^{2+} solutions of known concentration ($R_{max} = 7.595$ at 1.35 μM Ca^{2+} and $R_{min} = 0.193$ at 0 nM Ca^{2+}), and background was subtracted from each defined region of interest. The changes in intracellular Ca^{2+} were recorded in each individual cell, and expressed as ΔRatio, which was calculated for each cell as the difference between the maximal F_{340}/F_{380} ratio after extracellular Ca^{2+} was added, and its level right before Ca^{2+} addition. Representative traces show average $\pm$ standard error of the mean (SEM) of Ca^{2+} responses from 15 to 50 cells on the same coverslip recorded simultaneously. Summary data represent the mean $\pm$ SEM of ΔRatio from at least three cell preparations, with three to five coverslips studied for each condition.



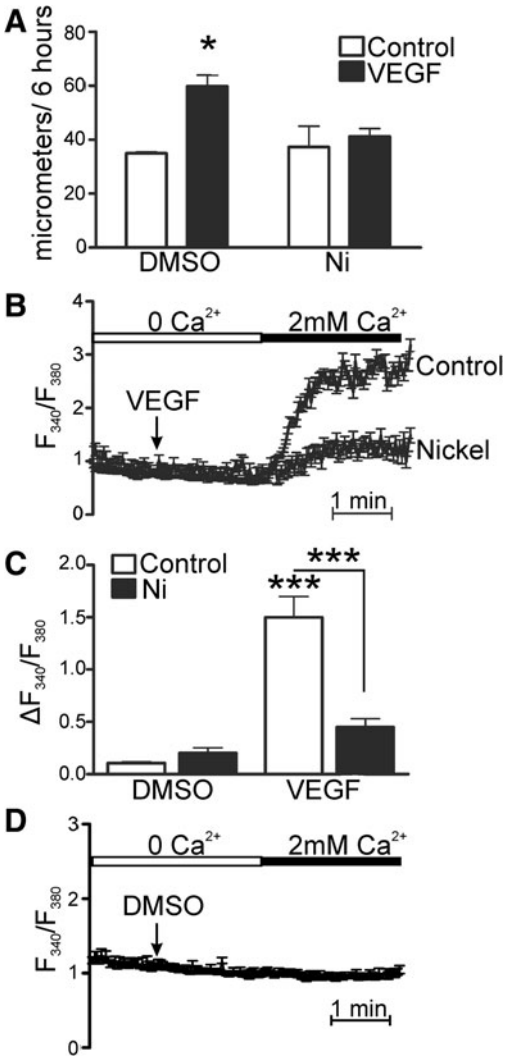
SUPPLEMENTARY FIG. S1. VEGF and •NO increase endothelial cell migration and SERCA activity in BAEC. **(A)** BAEC monolayers were treated with VEGF (50 ng/ml) or DETA NONOate (30 μM), and then migration into a scratch wound was measured over 6 h. The •NO synthase inhibitor, LNAME (30 μM), was added just before the scratch ($n=3$). **(B)** Representative migration images at 0 and 6 h. **(C, D)** SERCA activity was assessed by TG-sensitive $^{45}Ca^{2+}$ uptake in saponin-permeablized HAECs treated with VEGF or VEGF with LNAME **(C)**, or DETA NONOate [**(D)**, $n=4$]. *$p<0.05$. BAEC, bovine aortic endothelial cell; DETA NONOate, diethylenetriamine NONOate; HAEC, human aortic endothelial cell; LNAME, N (G)-nitro-L-arginine methyl ester •NO, nitric oxide; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; TG, thapsigargin; VEGF, vascular endothelial growth factor.



SUPPLEMENTARY FIG. S2. Knockdown of SERCA3 does not inhibit VEGF-induced EC migration. Knockdown of low levels of SERCA3 mRNA was confirmed by qRT-PCR **(A)** and immunoblot [**(B)**, $n=4$]. **(C)** $^{45}Ca^{2+}$ uptake into saponin-permeablized HAECs ($n=3$). **(D)**. Migration over 6 h was assessed after treatment with SERCA3 siRNA ($n=6$, *$p<0.05$). EC, endothelial cell.



SUPPLEMENTARY FIG. S3. Representative images of HAEC tube formation. **(A)** Tube formation in response to VEGF treatment in HAECs overexpressing either LacZ or Glrx-1. **(B)** VEGF-induced tube formation in HAECs overexpressing either WT SERCA2b or SERCA2b C674S. WT, wild type.



SUPPLEMENTARY FIG. S4. Nickel inhibits both EC migration and Ca^{2+} influx. **(A)** Migration of HAECs treated with or without nickel (100 μM) over 6 h ($n = 3$). **(B, C)** VEGF-induced EC Ca^{2+} influx in the presence or absence of nickel [**(B)**, representative trace] and [**(C)**, $n = 3$]. $^{*}p < 0.05$ and $^{***}p < 0.001$. **(D)** Representative trace of HAECs treated with DMSO vehicle for VEGF before addition of Ca^{2+}. DMSO, dimethyl sulfoxide.