ROS enhance angiogenic properties via regulation of NRF2 in tumor endothelial cells

Supplementary Materials

Supplementary Figure 1: (A) Relative ROS levels in NECs (gray columns) and TECs (black columns) cultured in normoxia (20% O₂) or hypoxia (1% O₂) condition for 24 h. (B) Relative ROS levels in NECs (gray columns) and TECs (black columns) in control (medium with 5% FBS) or starved condition (medium with 0.5% FBS) for 24 h. (C) *Ho-1* mRNA in NECs and TECs were analyzed by real-time PCR. *P* < 0.01, two-sided Student’s *t*-test. Data are mean ± SD, *n* = 4 real-time RT-PCR runs.
Supplementary Figure 2: (A) Phosphorylated ERK1/2 (pERK) and total ERK1/2 (ERK) levels were analyzed in ECs stimulated with or without pyocyanin (25 µM) by western blotting. B-Actin served as loading controls. (B) Pai-1 mRNA in NECs and TECs with or without pyocyanin (25 µM) or the MEK inhibitor U0126 (1 µM) were analyzed by real-time PCR. *P < 0.01, N.S., not significant. One-way ANOVA. Data are mean ± SD, n = 4 real-time RT-PCR runs. (C) Real-time PCR confirms silenced Smad2 mRNA levels. *P < 0.01, one-way ANOVA. Data are mean ± SD, n = 4 real-time RT-PCR runs. (D) Vgf-a, Vgf2m, Cxcr7 and Ptgir mRNA expressions in TECs with or without pyocyanin treatment (25 µM) were analyzed by real-time PCR. *P < 0.01, two-sided Student’s t-test. Data are mean ± SD, n = 4 real-time RT-PCR runs.
Supplementary Figure 3: (A) Vegf-a mRNA in NECs and TECs with or without pyocyanin treatment (25 µM) were analyzed by real-time PCR. *P < 0.01. One-way ANOVA. Data are mean ± SD, n = 4 real-time RT-PCR runs. (B) Cell motilities of NECs and TECs treated with pyocyanin (25 µM) were analyzed by Boyden chamber migration assay in the presence of anti-VEGFA antibody (150ng/ml) or VEGFR2 kinase inhibitor, Ki8751 (10 nM). *P < 0.01, one-way ANOVA. Data are represented as mean ± SD, n = 4 fields. (C) Phosphorylated ERK (pERK) and total ERK (ERK) levels were analyzed in Biglycan knockdown TECs stimulated with BGN (1 µg/ml) by western blotting. B-Actin served as loading controls. (D) Phosphorylated NF-κB (pNF-κB) and total NF-κB (NF-κB) in Biglycan knockdown TECs stimulated with BGN (1 µg/ml) by western blotting. B-Actin served as loading controls.