SUPPLEMENTAL MATERIAL

Supplementary Figure Legends

Supplementary Figure 1. The effect of CORM ALF 421 and hypoxia on the proliferation of RAEC. A. BrdU incorporation was used to assess the proliferation rate of CORM ALF 421 (1-20 μM) or vehicle treated RAEC cells measured at 24 hours. Data are representative of 2 independent experiments performed in triplicate. Two-way ANOVA, $p<0.0001$. CORM vs control. Tukey post-hoc; Control vs 1μM, $p=0.505$; vs 5μM, $p=0.079$; vs 10μM, $^{**}p<0.001$; vs 20μM, $^{**}p<0.0001$ **B.** BrdU incorporation testing proliferation of RAEC cells exposed to normoxia (21%O_2) and hypoxia (1% O_2). The data are representative of 2 independent experiments in triplicate. One-way ANOVA $^{*}p<0.001$ vs normoxia.

Supplementary Figure 2. Densitometric analysis of CO-treated RAEC at 4 hr for (**A**) P-Rb; one-way ANOVA, $^{*}p=0.003$. and (**B**) P-Akt, one-way ANOVA, $^{*}p=0.04$ and 10 min for (**C**) P-eNOS, one-way ANOVA, $^{*}p=0.001$. Data represent mean ± SD of values obtained from 3 separate blots comparing CO vs Air.

Supplementary Figure 3. CO blocks the migration of macrophages in vitro and in vivo. A. Immunostaining for the macrophage marker ED-1 of carotid arteries from air and CO-treated animals 4 days after injury. Note that CO blocks inflammatory cell influx into the injured vessel. Images are representative of 6-8 fields from 4-5 animals/group. Magnification is 40x. Arrows indicate positive staining. Scale bar=50 μm. **B**. Transwell

migration of U937 monocytes treated with Air or CO for 24 hours. Data are representative of 2 independent experiments preformed in triplicate. One-way ANOVA **p<0.001. **C.** Effect of CORM ALF 421 (10-50 μM) treatment on U937 macrophage migration in Boyden chambers. Cells were treated for 24 hours and the amount of cells that migrated to the lower chambers were counted. Data represent mean ± SD from 3 independent experiments (n=3/group). Two-way ANOVA; **p<0.001 CORM vs Control (C). Tukey post-hoc; Air vs 10μM, *p=0.04; vs 20μM, **p=0.009; vs 50μM, **p<0.0001.

Supplementary Figure 4. Carbon monoxide induced sca-1$^+$ progenitor recruitment to the injured vessel in mice. Mice were treated with air or CO as described previously. Carotid artery segments were harvested 12 hrs after injury and sectioned and stained for the presence of sca1$^+$ and CD31. Vessels from naïve, untreated mice are shown as control. Note that CO increased the number of sca1$^+$ cells in the artery at 12 hr, which was not present in air-treated mice. Representative images are from 4-6 animals/group. Arrows indicate positive staining. Magnification is 20x, Scale bar=50 μm.

Supplementary Figure 5. Carbon monoxide induces recruitment of bone marrow cells to the circulation as measured by the colony outgrowth assay. **A**. Representative images of colony outgrowth (black arrows) 10 days after isolation of blood mononuclear cells from Air or CO (1h, 250ppm;) treated animals cultured in EC medium (n=4 mice/group). *Upper Panels*: Air-treated animals, *Lower Panels*: CO-treated animals. **B.** Quantitation of the outgrowth data represented as the number of colonies per field of

view (FOV) at 40x magnification. Data represent mean ± SD of 8-10 FOV from each animal/well in duplicate. One way ANOVA *p=0.007 vs air; Wilcoxon *p=0.031. Note bottom right panel showing a representative FOV showing tubule-like formation (white arrows) in a few FOV from cells harvested from animals treated with CO, which was not observed in air-treated animals.

Supplementary Materials and Methods

Immunoblotting

Cells were lysed by a freeze-thaw cycle in ice-cold lysis buffer (0.5% NP-40, 0.1% NaDOC, 0.1% SDS, 50mM Tris-HCl pH=7.5, 150mM NaCl, 1mM EDTA pH=8.0, 1mM NaF, in the presence of a protease inhibitor cocktail. Samples were centrifuged for 30 min at 14000g at 4°C and the supernatants were harvested. 20-40 μg of each protein sample were electrophoresed on NuPAGE 4-12% Bis-Tris Gel (Invitrogen, CA) followed by transfer to PVDF membrane. The membranes were then blocked with 5% non-fat dry milk, probed with appropriate primary antibodies, followed by HRP-conjugated secondary antibodies at a dilution of 1:5000. Bands were visualized using Super signal chemiluminescent substrate (Pierce, Rockford, IL) exposed to ECL Film (ISC BioExpress, Kaysville, UT).

Source of antibodies

The following antibodies were used: rabbit anti-P(Ser473)-Akt (Cell Signaling), rabbit anti-total Akt (Cell Signaling), rabbit anti-P (Ser780)-Rb (Cell Signaling), mouse anti-GAPDH (Calbiochem), rabbit anti-P-eNOS (Ser1177) and rabbit anti-total eNOS (Millipore Upstate), rabbit anti-Histone H3 (Cell Signaling), rat anti-mouse and mouse

anti-rat CD31 (BD Biosciences), anti-ICAM (BD Biosciences), mouse anti-rat mononuclear phagocyte (ED-1) (BD Pharmingen), rat anti-Ly-6A/E (Sca1) (BD Biosciences) and rabbit anti-GFP (Invitrogen, Molecular Probes).

Cell motility assay

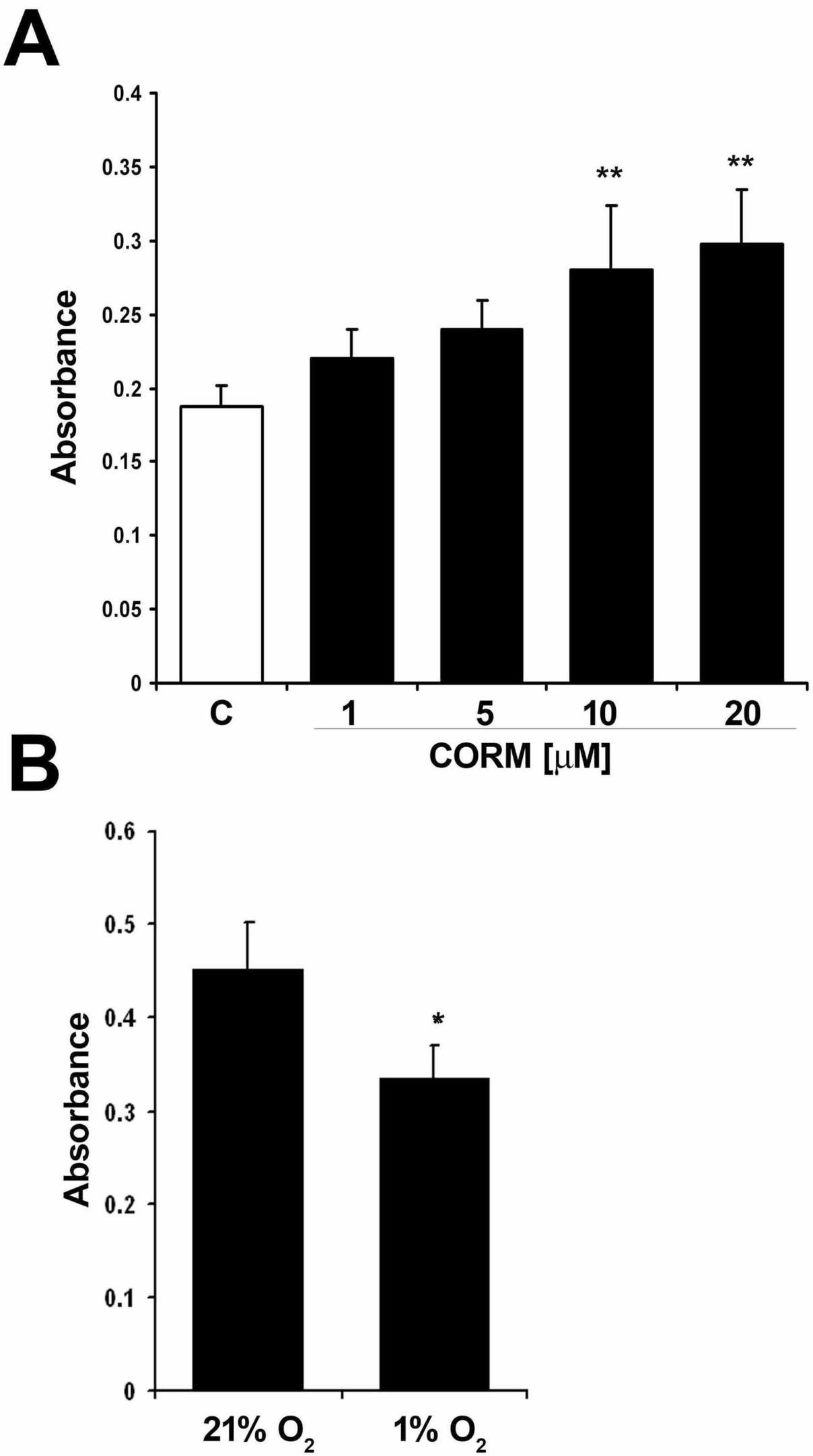
RAEC were grown to confluency on gelatin-coated coverslips. Prior to imaging, a diametric scratch was made exposing a cell-free region approximately 25 to 30 μm wide. The coverslips were loaded into 37^{o}C incubated closed chambers (FCS2®, Bioptechs, Inc, Butler, PA), aligning the scratch longitudinally with the flow of medium at 0.5 ml/hr delivered by a KDS 100 syringe pump, (KD Scientific). The coverslips were exposed to buffered medium (CO-saturated and non-saturated containing 5% CO_2/air). The medium was prepared by bubbling with 250 ppm CO continuously for 30 min and then loaded into an airtight Hamilton syringe. The system was designed and tested to be gas impermeable). Differential interference contrast (DIC) images were taken every 20 min at 12 different positions along the scratch over 24 hr with a Nikon TE300 with 20X objectives; the experiments were done simultaneously. Cellular motility was analyzed with MetaMorph 6.2 (Universal Imaging Corp.), and measured as the average speed of the cells' nuclei (n=120 cells) along their migratory pathway.

Monocyte migration. U937 monocytes were seeded in the upper chamber of migration Boyden chambers (8 μm, Transwell, Costar) in serum free medium. Serum containing medium was added to the lower chamber and served as the chemoattractant. Cells were treated with CO or 10-50 μM CORM ALF421 for 24 hours. The amount of cells, which migrated to the lower chambers were counted using a Neubauer hemocytometer.

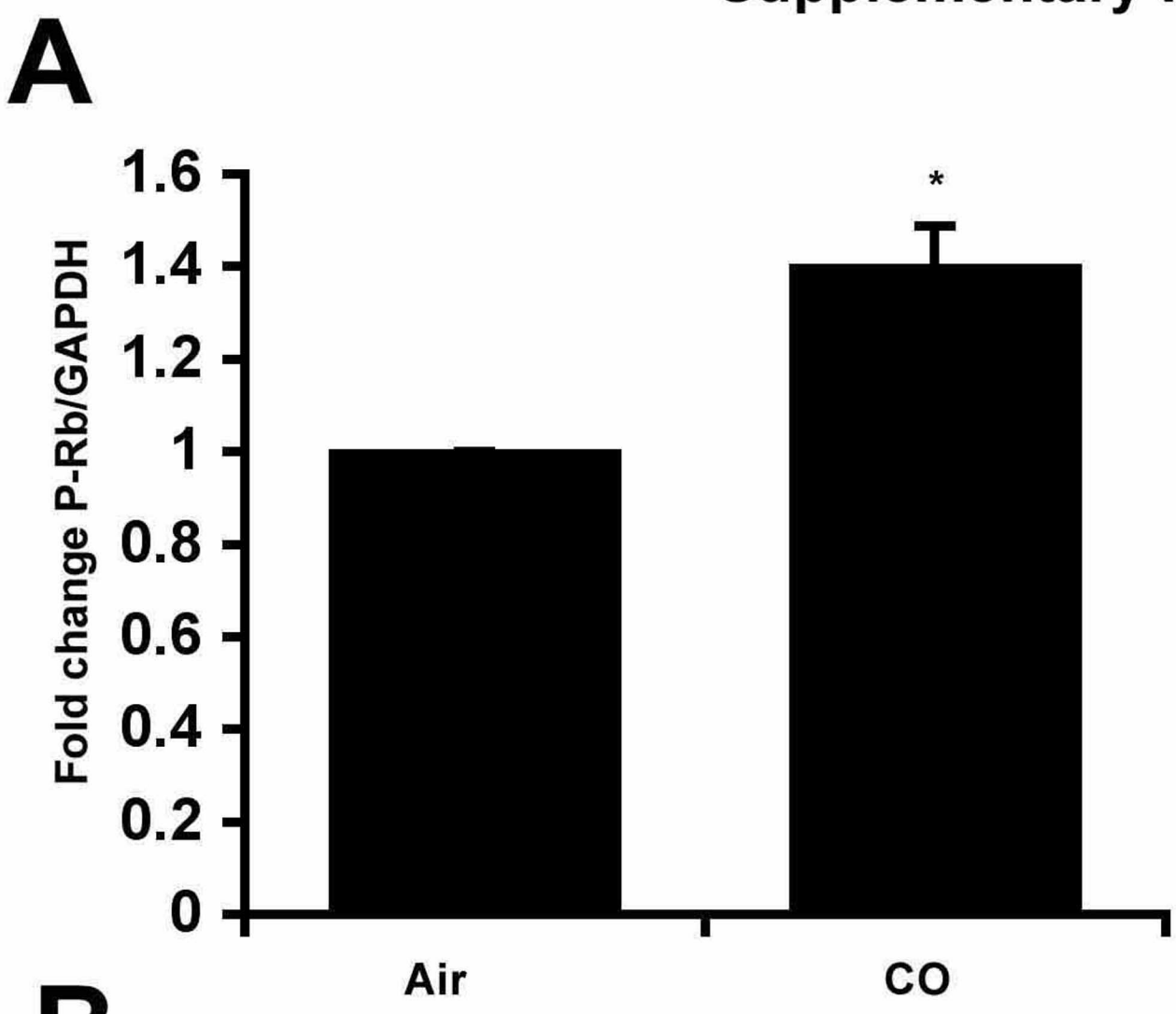
Phalloidin staining

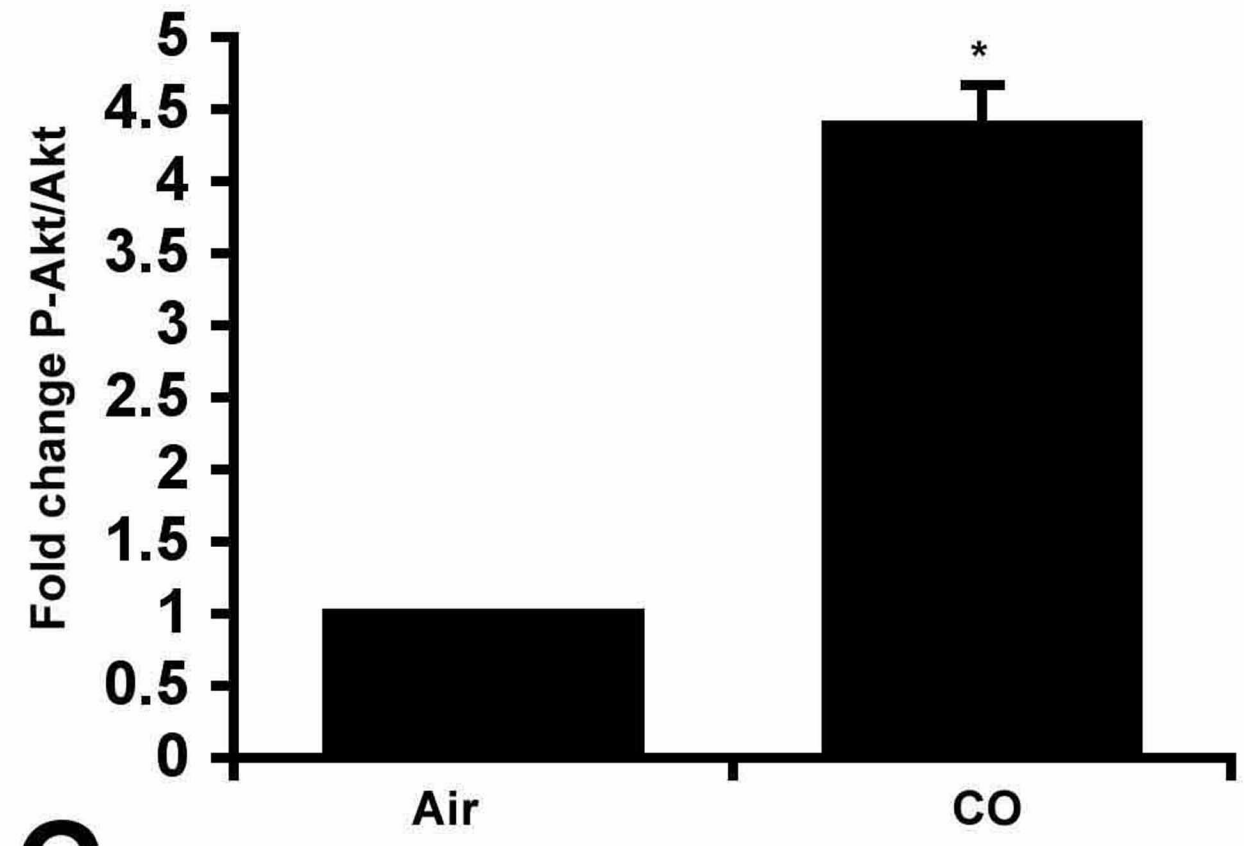
RAEC were cultured in MCD-B131 complete media (VEC Technology) on 22 mm x 22 mm coverslips in 6 well plates. 1 mm wide scratches of uniform size were then created, after which the cells were either placed in air or CO for 24 hr. Staining for actin using Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR) was performed according to manufacturer's directions. Briefly, the coverslips were fixed in 2% paraformaldehyde for 15 min at room temperature, washed with PBS and incubated with phalloidin for 15 min. Images were captured of randomized fields using a Zeiss Apotome fluorescent microscope.

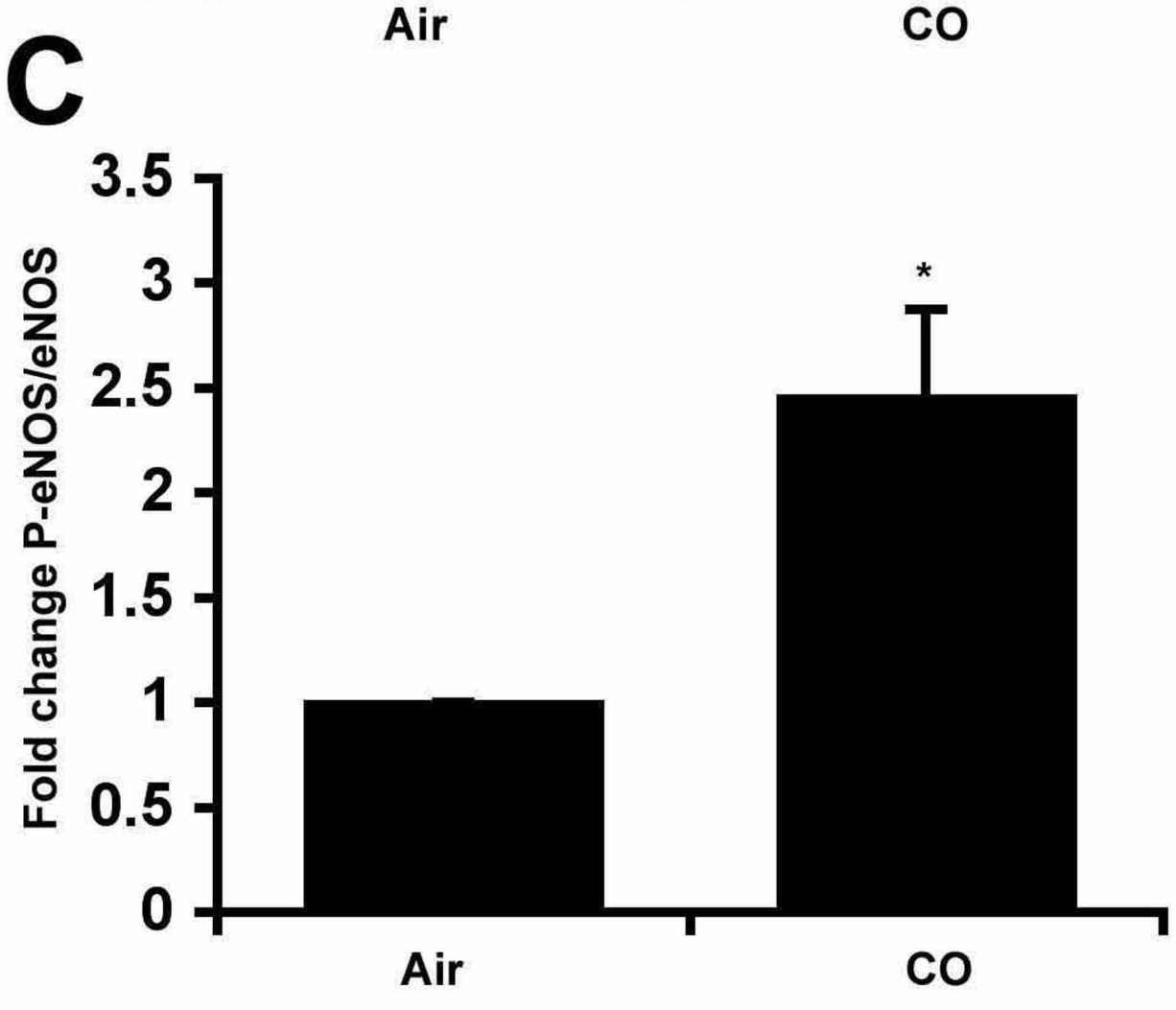
Supplementary Figure 1



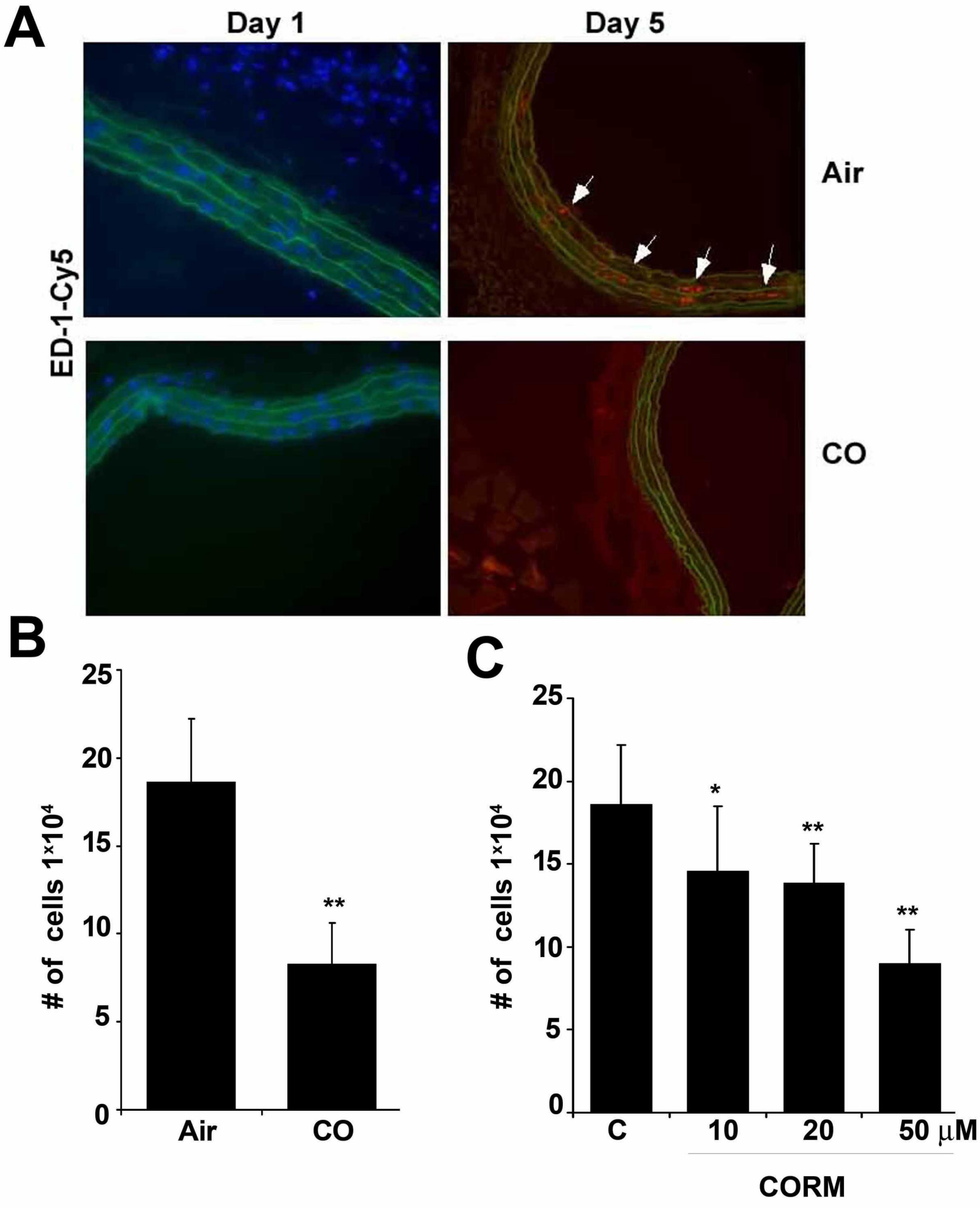
Supplementary Figure 2



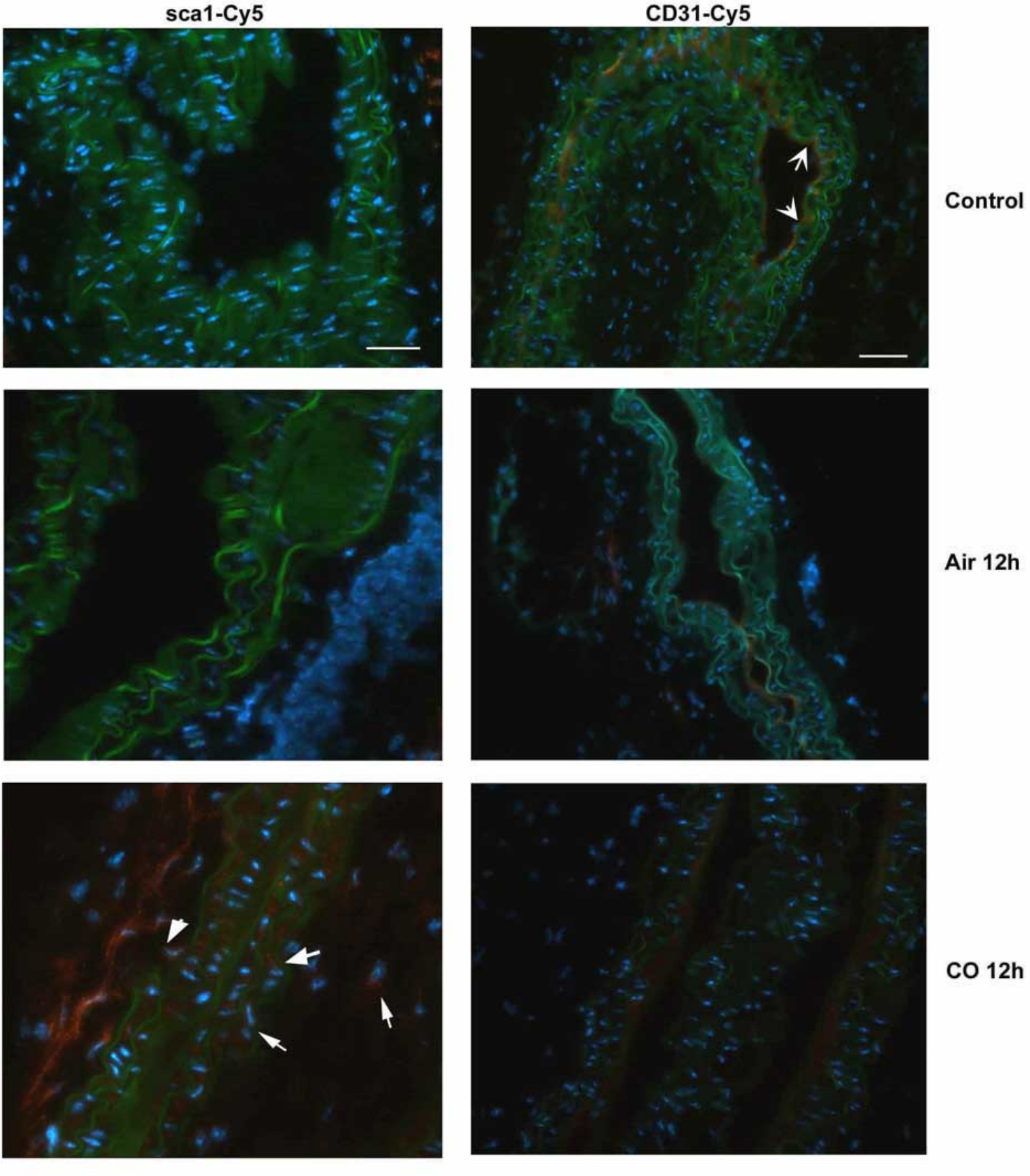




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5