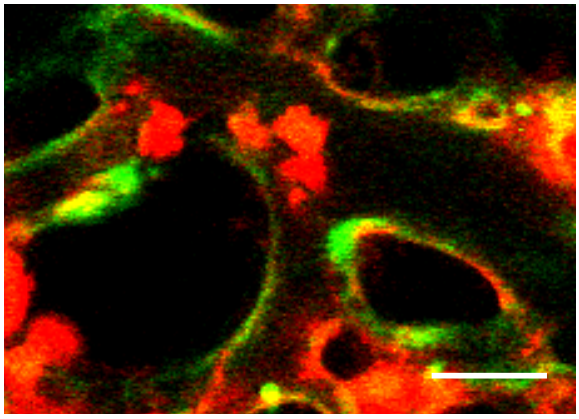
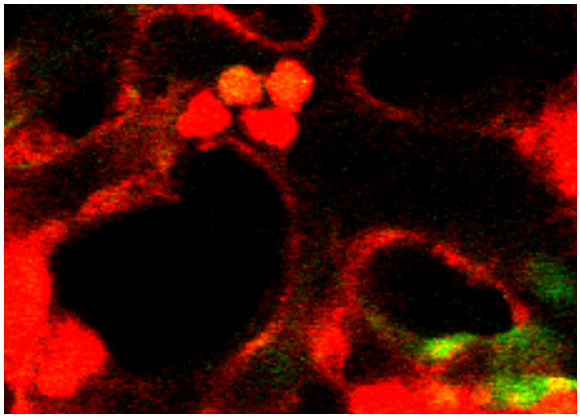
Baseline

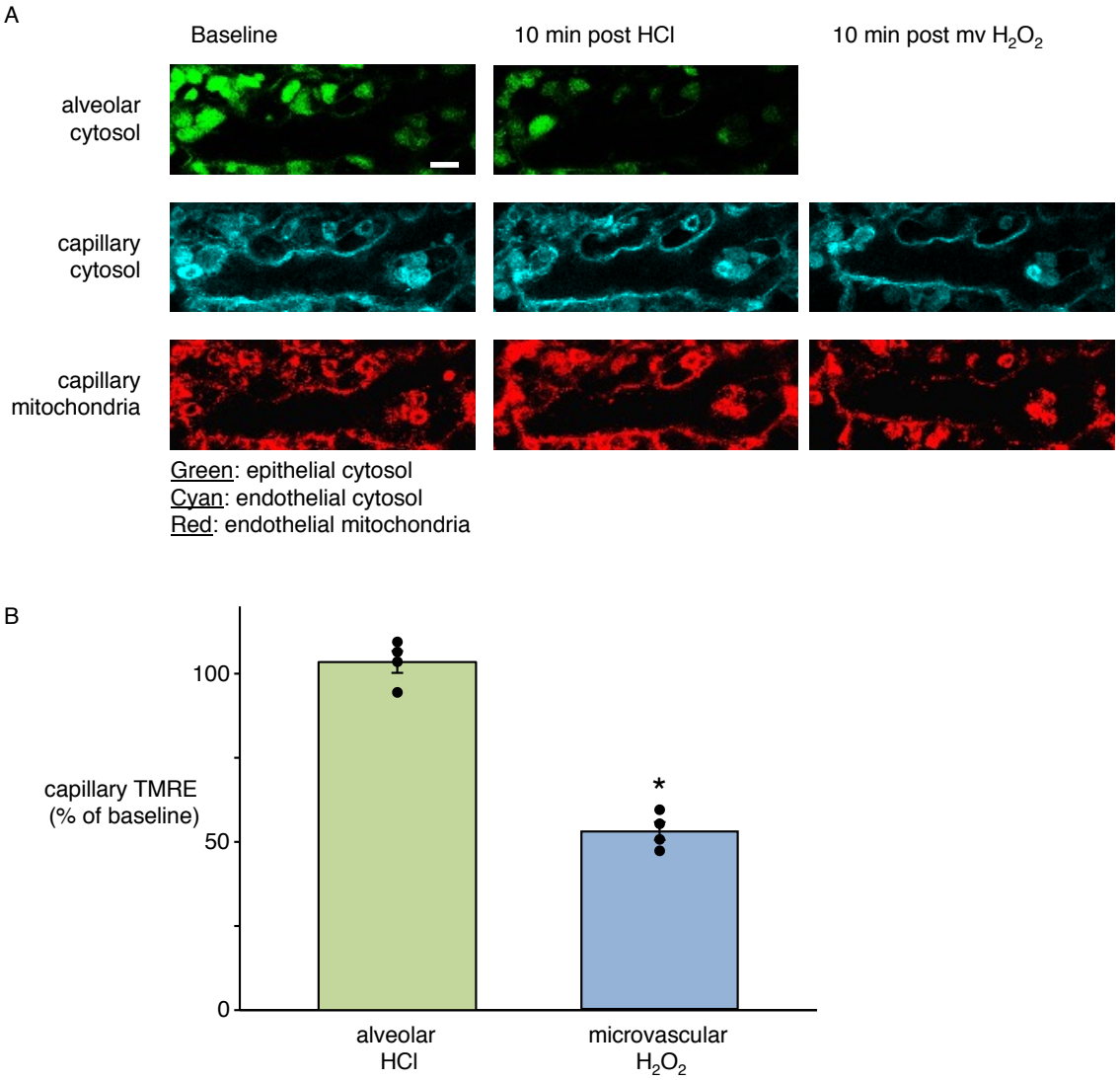


10 min post microvascular triton

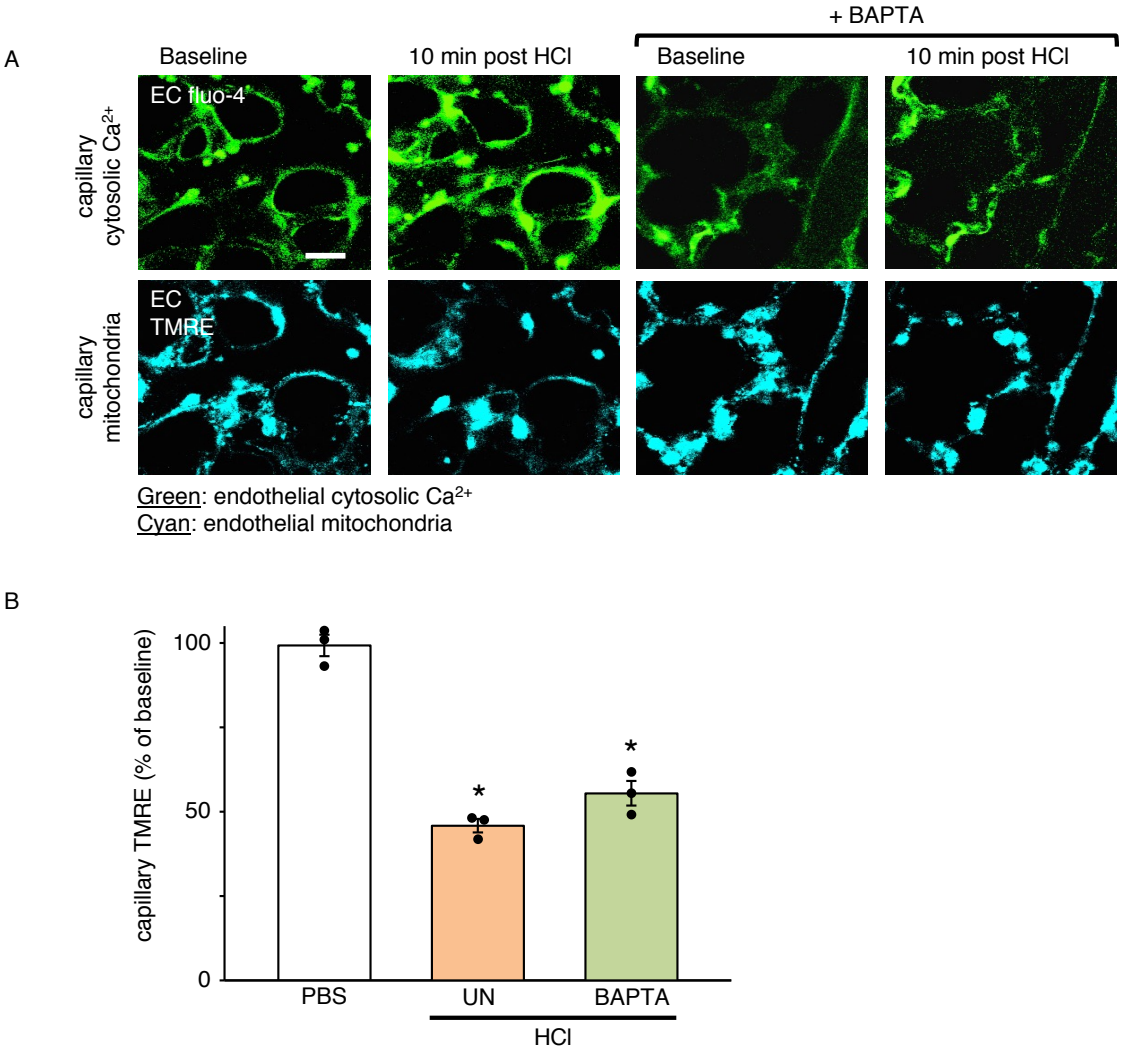


Green: endothelial cytosol
Red: endothelial mitochondria

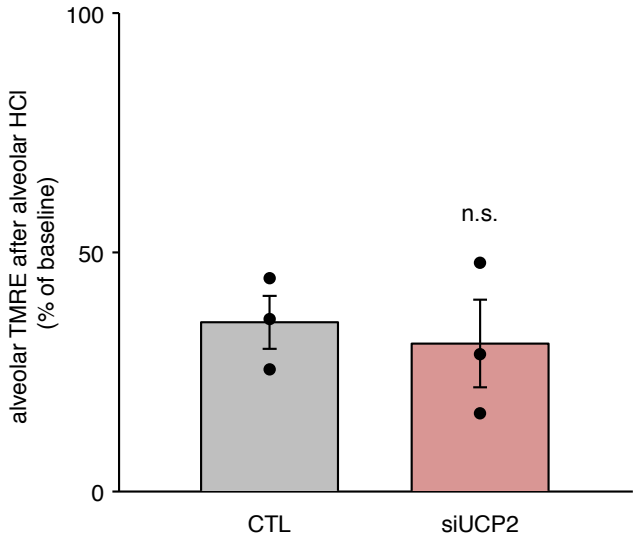
Supplemental figure 1. TMRE is sequestered in endothelial mitochondria. Effect of microvascular injection of triton detergent on EC dyes. Vessels were infused with CG (2.5 μM, 10 min) to mark EC, and TMRE to mark EC mitochondria. 0.1% Triton X-100 (5 min) was infused in microvessels. $n = 2$. Scale bar: 20 μm.



Supplemental figure 2. Epithelial transfection of catalase does not prevent microvascular H_2O_2-induced endothelial mitochondrial depolarization. Fluorescence intensity after intranasal transfection with catalase plasmid. Alveoli were microinjected with CG to mark AEC cytosol. Vessels were infused with celltracker deep red (1.5 μM, 10 min) to mark EC, and TMRE to mark EC mitochondria. Images were obtained 10 min after alveolar injection of HCl, then 10 min after microvascular infusion of H_2O_2. $n = 3$. Scale bar: 20 μm. *$p<0.05$ versus alveolar HCl.



Supplemental figure 3. Alveolar HCl-induced endothelial cytosolic calcium does not depolarize mitochondria. (A) Representative confocal images showing cytosolic Ca^{2+} and mitochondrial response to alveolar injection of HCl. Vessels were infused with fluo-4, AM (10 μM, 45 min) and TMRE. Alveoli were injected with HCl. To chelate Ca^{2+}, BAPTA-AM (80 μM, 30 min) was infused in vessels prior to HCl injection. Scale bar: 20 μm. (B) Fluorescence intensity of EC TMRE following alveolar injection of HCl. $n = 3$. *$p<0.05$ versus PBS control.



Supplemental figure 4. UCP2 knockdown by vascular injection does not prevent alveolar HCl-induced depolarization of alveolar mitochondria. Fluorescence intensity of epithelial TMRE following alveolar HCl microinjection. Alveoli were microinjected with TMRE (2 μM, 5 min) to mark alveolar mitochondria. *CTL*, no siRNA; *siUCP2*, i.v. UCP2 siRNA.