

INTRODUCTION

Endothelial cells line the internal vasculature to form a semi-permeable barrier to maintain vascular integrity. General anesthetics comprise a heterogeneous group of drugs with multiple functions and mechanisms of action, which are not yet fully elucidated. It is suggested that general anesthetics threaten endothelium stability by causing changes in vascular tones. Sevoflurane has been shown to promote growth and proliferation of human endothelial progenitor cells and thus may support tumor metastasis by enhancing angiogenesis and tumor cell survival in hypoxic environments. Different from inhaled sevoflurane, intravenous propofol could the protection against oxidative stress in endothelial cells due to its phenol-based structure as a free radical scavenger. However, for most general anesthetics, its functional role on endothelium remains obscure. Here we characterized the effects of commonly used general anesthetic agents on mouse pulmonary endothelial cells (MPECs) in vitro and in vivo and explored the potential targets.

METHODS

- RT-PCR and Western blot:** RNA and proteins samples were extracted from HUVEC/MPECs treated with different anesthetics or lung lysate
- ELISA analysis of VEGF** from HUVEC/MPEC cells, mouse serum, and lung lysate
- Immunofluorescent staining (IF):** HIF 1 α were stained in HUVEC/MPECs
- In vitro permeability assay:** HUVEC/MPEC monolayers formed on PET membranes were treated with sevoflurane or propofol. After treatments, monolayer staining and FITC-Dextran permeability testing were performed. Disruption of monolayer integrity by sevoflurane was observable both visually and by quantification of fluorescence.
- Trans-endothelial electric resistance (TEER):** HUVEC/MPEC monolayers formed on PET membranes were treated with sevoflurane (1.3 MAC) or propofol (4 μ g/mL) for three hours. After treatments, TEER was measured to quantify the endothelium integrity.
- Rhodamine-dextran in vivo vascular permeability assay:** In Balb/c mice, one day after exposure to general anesthetics, rhodamine-dextran were i.v. injected at 100 mg/kg. Three hours later, each mouse was anesthetized and perfused with saline and followed by 5 mL of 4% formaldehyde. Lung were cryosectioned and stained with Lycopersicon Esculentum lectin. Vascular leakage was examined by fluorescence microscope.
- AngioSense® 750 fluorescent in vivo vascular permeability assay:** In Balb/c mice, one day before exposure to general anesthetics, AngioSense® 750 were i.v. injected. On the second day, mice were exposure to sevoflurane or propofol for three hours and afterwards mouse lung was harvested for Biofluorescent imaging using PerkinElmer IVIS machine.

RESULTS

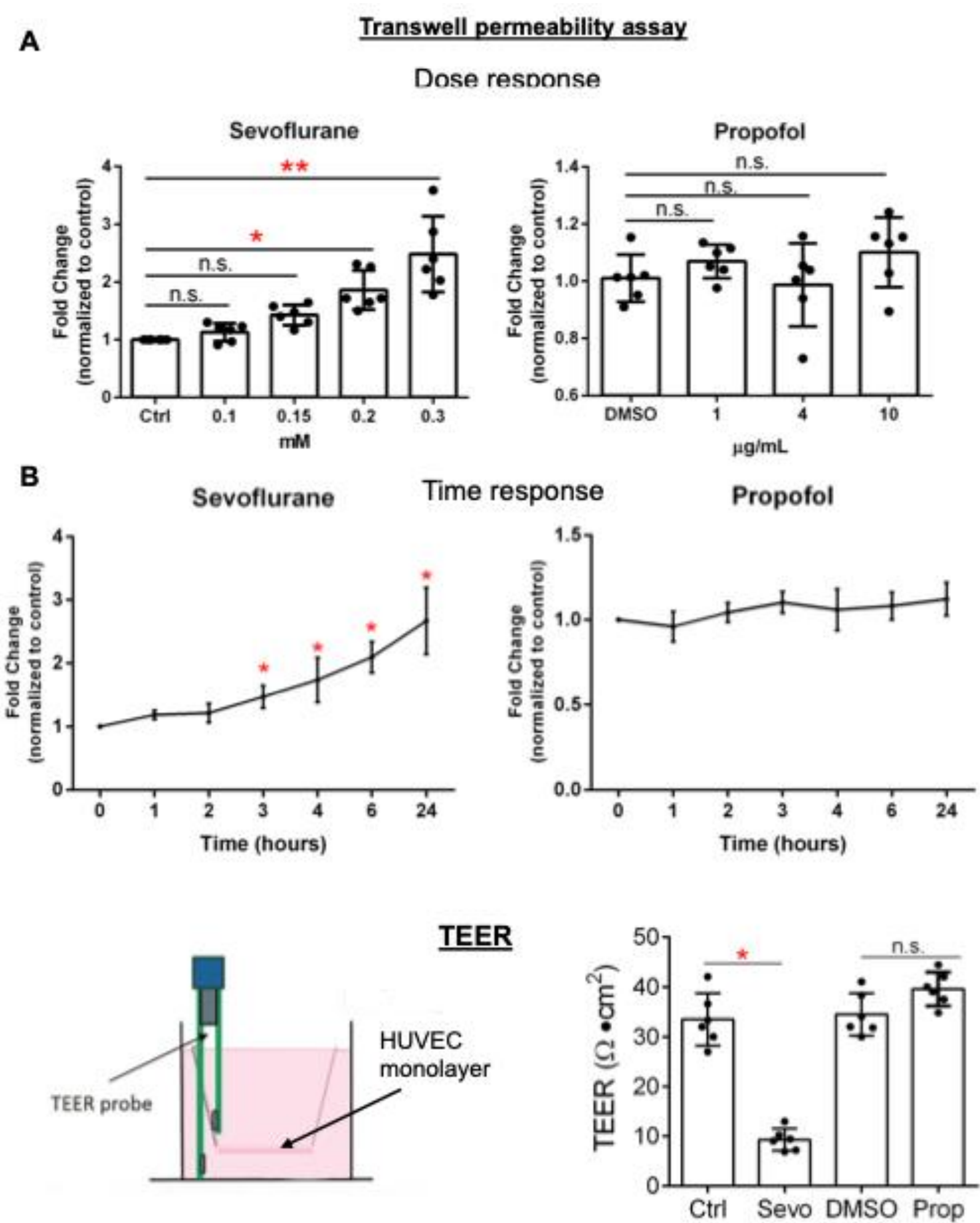


Figure 1. Sevoflurane but not propofol disrupts the integrity of HUVECs in vitro. (A) Dose responses of trans-HUVEC permeability to sevoflurane or propofol. (n = 6, for sevoflurane: control vs 0.2 mM p = 0.04, control vs 0.3 mM p = 0.001). (B) Time responses of trans-HUVEC permeability to sevoflurane or propofol. (n = 6, for sevoflurane control vs 3-hour p = 0.046, control vs 4-hour p = 0.03, control vs 6-hour p = 0.005, control vs 24-hour p < 0.0001). (C) TEER of HUVECs. (DMSO as the control to propofol) (n = 6, control vs sevoflurane p = 0.02)

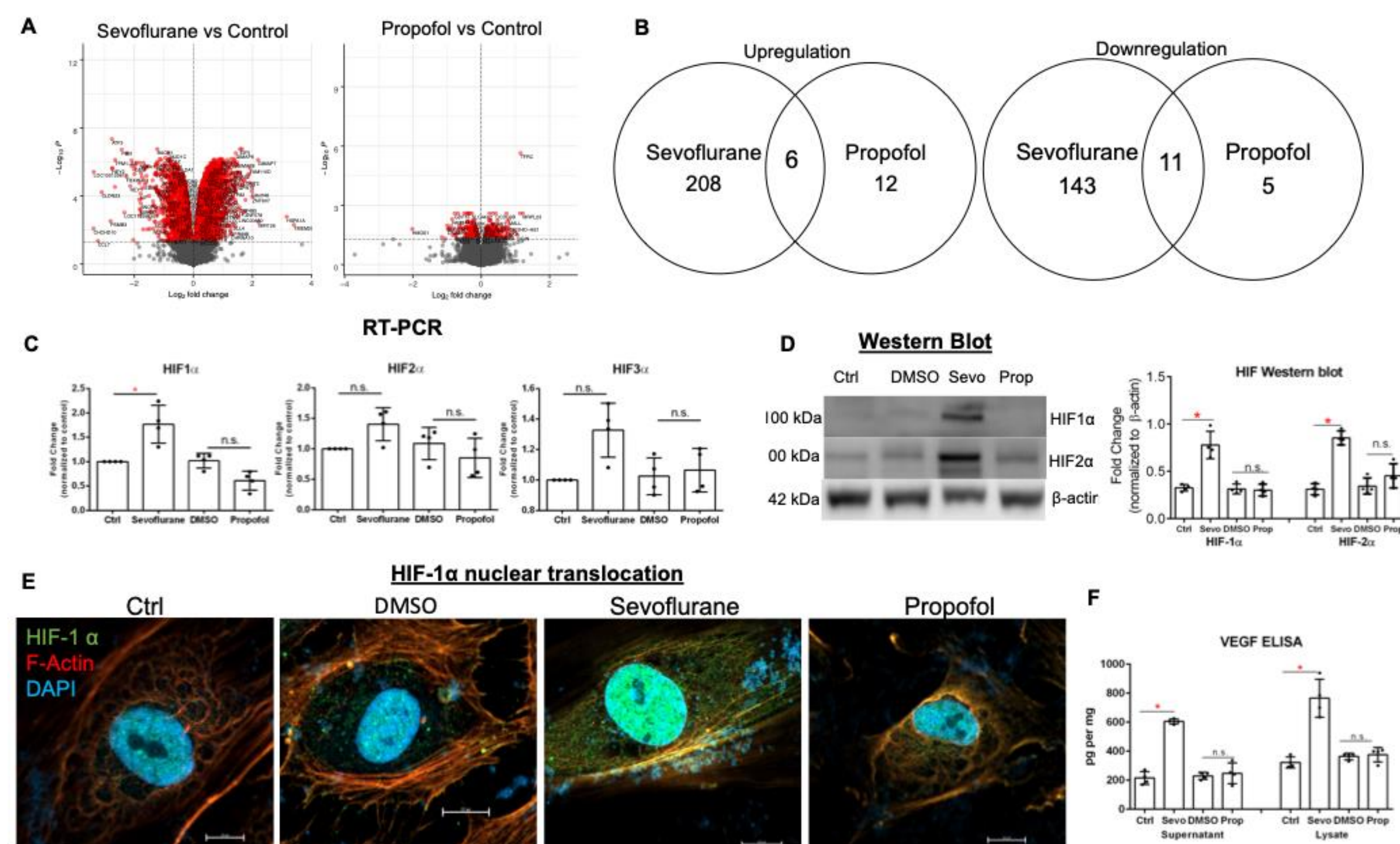


Figure 3. Sevoflurane mediates activation of HIF/VEGF signaling and disrupts the integrity of MPEC. MPECs were isolated from 6 weeks old Balb/c mice and subject to treatment with sevoflurane (0.2 mM) or propofol (4 μ g/mL) for four hours. (A) RT-PCR and (B) Western blot analysis of HIF- α isoforms in MPEC after sevoflurane and propofol exposures. (RT-PCR: n = 5, control vs sevoflurane p = 0.03; western blot: n = 4, control vs sevoflurane p = 0.015). (C) Immunofluorescence of HIF-1 α subcellular localization in MPECs. (D) The knockdown of HIF-1 α by siRNA prevented the increased expression of HIF-1 α induced by sevoflurane as evidenced by Western blot (n = 4, control vs sevoflurane p = 0.01). (E) Sevoflurane increased VEGF levels in the supernatant and lysate of MPECs, which were abolished by HIF- α siRNA (n = 5; supernatant: control vs sevoflurane p = 0.002, cell lysate: control vs sevoflurane p = 0.01). (F) In vitro transwell model with MPEC. (n = 6, control vs sevoflurane p = 0.02), and (G) increased FITC-Dextran migration as quantified by fluorescence (n = 5, control vs sevoflurane p = 0.02).

Figure 2. Sevoflurane activates HIF-1 α and induces VEGF expression in HUVEC cells. (A) mRNA-sequencing analysis of HUVECs treated with sevoflurane or propofol. Volcano plots show differential expression of genes in HUVECs treated with sevoflurane (0.2 mM) or propofol (4 μ g/mL) for four hours. (B) Venn diagrams show upregulated and downregulated genes identified by comparing expressions after sevoflurane or propofol treatment. (C) Validation of HIF-1 α mRNA levels in HUVECs treated with general anesthetics. Sevoflurane led to significantly increase of HIF-1 α mRNA level compared to propofol (n = 4, control vs sevoflurane p = 0.03). (D) Western blot analysis of HIF- α isoforms in HUVEC treated with general anesthetics. Sevoflurane increased HIF-1 α and HIF-2 α protein level compared to propofol or control (n = 4, HIF-1 α control vs sevoflurane p = 0.001, HIF-2 α control vs sevoflurane p = 0.001). (E) Subcellular localization of HIF-1 α in HUVEC cells treated with general anesthetics. After treatment, HUVEC cells were fixed and co-stained with HIF-1 α antibody, Texas Red Phalloidin for F-actin, and DAPI for the location of nucleus. Sevoflurane promoted the translocation of HIF-1 α from cytoplasm to nucleus. (F) Sevoflurane significantly increased VEGF levels in HUVEC cells compared to propofol or control (n = 4, supernatant control vs sevoflurane p = 0.028; cell lysate control vs sevoflurane p = 0.007).

RESULTS

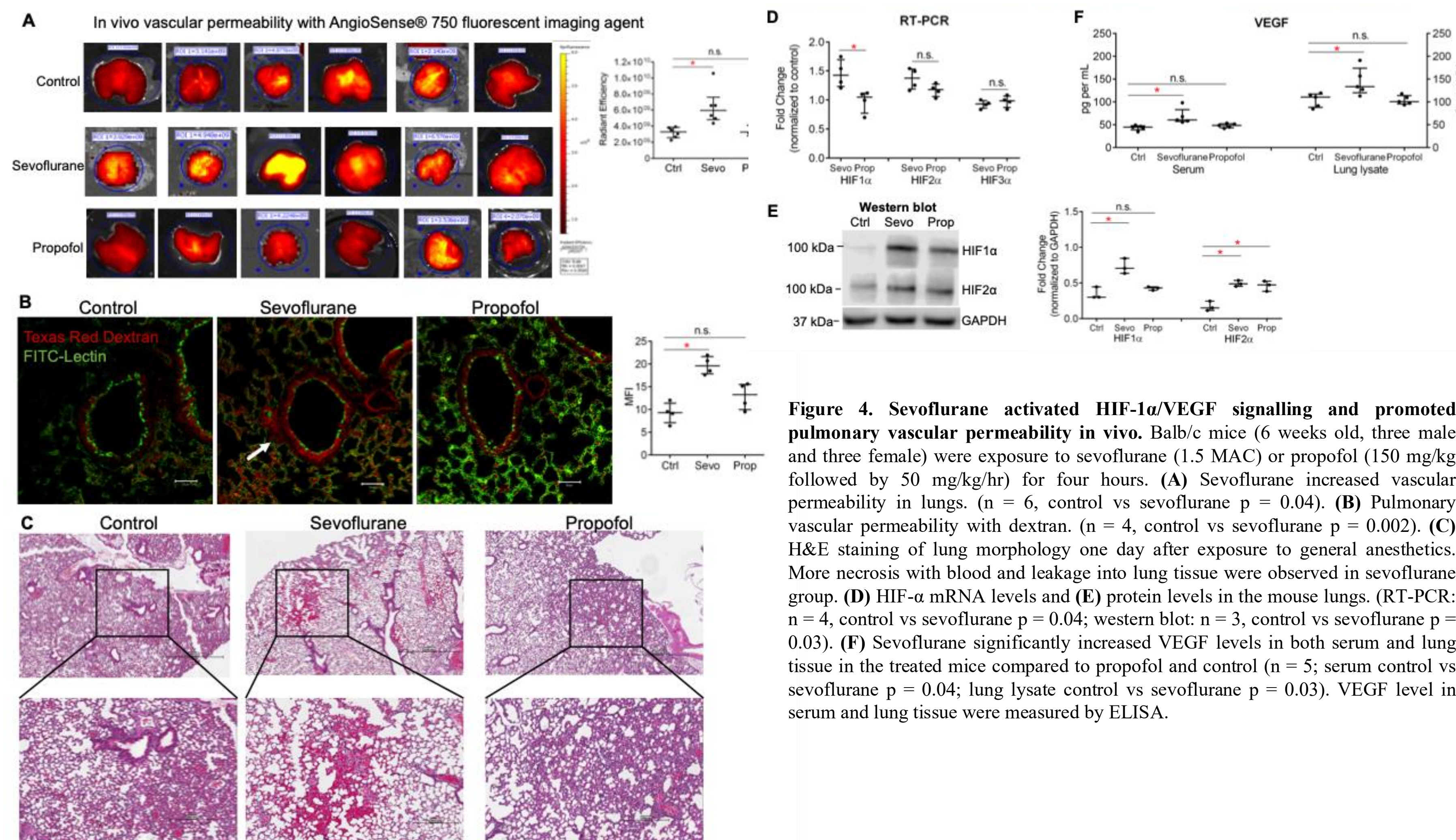


Figure 4. Sevoflurane activated HIF-1 α /VEGF signalling and promoted pulmonary vascular permeability in vivo. Balb/c mice (6 weeks old, three male and three female) were exposure to sevoflurane (1.5 MAC) or propofol (150 mg/kg followed by 50 mg/kg/hr) for four hours. (A) Sevoflurane increased vascular permeability in lungs. (n = 6, control vs sevoflurane p = 0.04). (B) Pulmonary vascular permeability with dextran. (n = 4, control vs sevoflurane p = 0.002). (C) H&E staining of lung morphology one day after exposure to general anesthetics. More necrosis with blood and leakage into lung tissue were observed in sevoflurane group. (D) HIF- α mRNA levels and (E) protein levels in the mouse lungs. (RT-PCR: n = 4, control vs sevoflurane p = 0.04; western blot: n = 3, control vs sevoflurane p = 0.03). (F) Sevoflurane significantly increased VEGF levels in both serum and lung tissue in the treated mice compared to propofol and control (n = 5; serum control vs sevoflurane p = 0.04; lung lysate control vs sevoflurane p = 0.03). VEGF level in serum and lung tissue were measured by ELISA.

CONCLUSIONS

This study demonstrates that **sevoflurane**, but not propofol, promotes endothelial and pulmonary vascular leakage through activation of the **HIF-1 α /VEGF signaling pathway**, highlighting a mechanistic distinction between commonly used anesthetics. These findings have important clinical implications. The ability of surgical interventions—particularly exposure to general anesthetics and mechanical ventilation—to disrupt pulmonary endothelial integrity underscores the critical need to better understand how perioperative factors influence vascular function and postoperative recovery.

Despite the central role of the endothelium in maintaining barrier stability and regulating inflammatory responses, our knowledge of how anesthetic agents modulate these processes at the cellular and molecular levels remains incomplete. The identification of HIF-1 α /VEGF-mediated dysfunction as a driver of sevoflurane-induced leakage provides a foundation for exploring targeted strategies to mitigate perioperative endothelial injury. Further investigation into these mechanisms will be essential for developing safer anesthetic approaches, improving perioperative management, and ultimately reducing pulmonary complications in surgical patients.