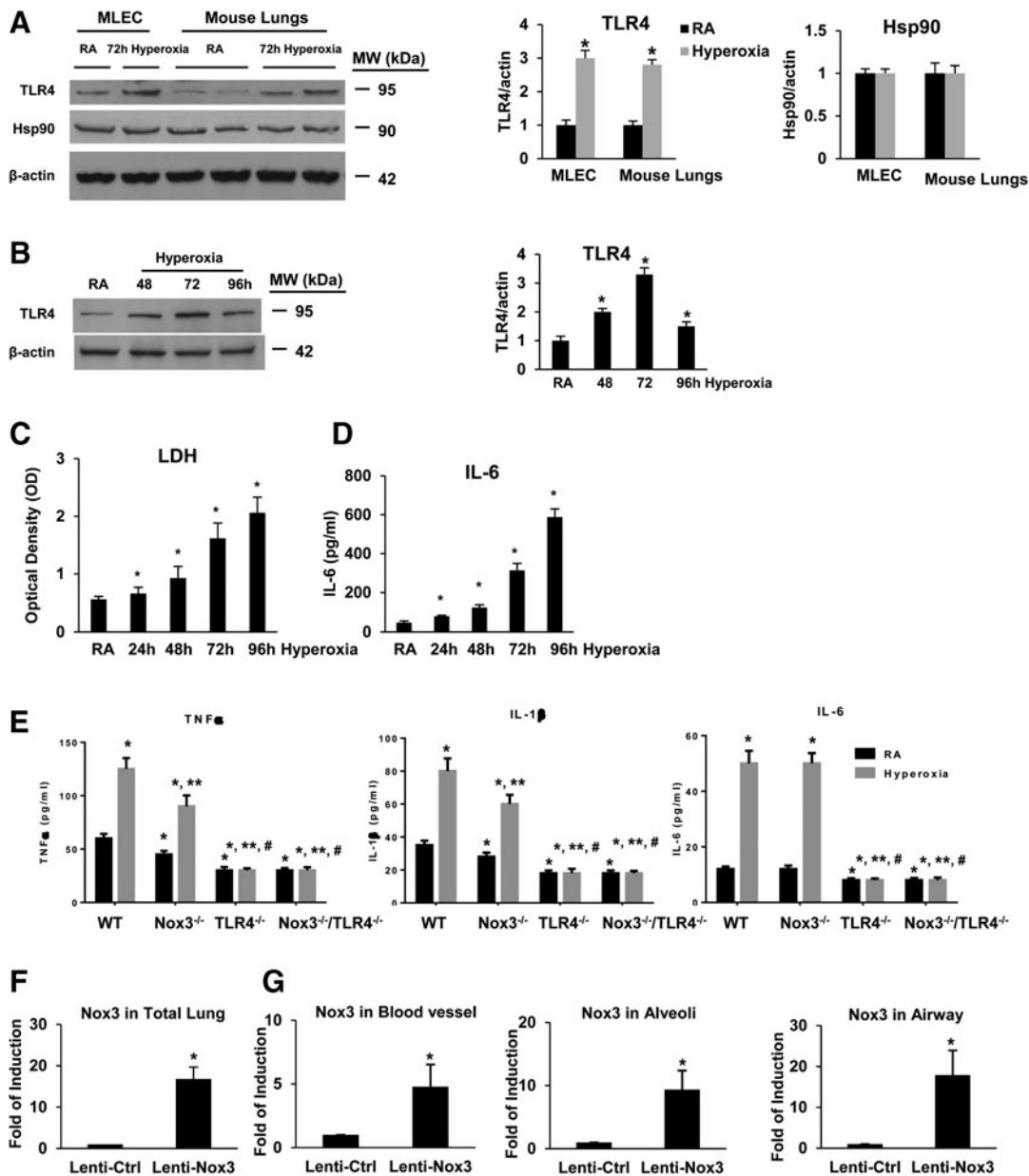


Supplementary Data



SUPPLEMENTARY FIG. S1. TLR4-Nox3 mediates hyperoxia-induced inflammation. (A) Lysates from MLECs or mouse lungs were prepared and immunoblotted against TLR4 or Hsp90 antibody. β -Actin was used as protein loading control. (B) TLR4 level in WT MLECs exposed to a course of hyperoxia. (A, B) Quantification based on densitometry for the listed proteins relative to β -actin is shown on the right panel. Experiments were performed in triplicate. The values are expressed as mean \pm SD and analyzed by Student's *t* test. * p < 0.05 versus RA. (C) LDH activity assay from MLEC supernatant exposed to a course of hyperoxia. (D) IL-6 level was detected in supernatants of WT MLECs exposed to a course of hyperoxia. (C, D) The values are expressed as mean \pm SD and analyzed by Student's *t* test. Experiments were performed in triplicate. * p < 0.05 versus RA. (E) WT, Nox3^{-/-}, TLR4^{-/-}, and Nox3^{-/-}/TLR4^{-/-} mice were exposed to RA or to hyperoxia for 72 h. Secreted TNF α , IL-1 β , and IL-6 were detected by enzyme-linked immunosorbent assay in bronchoalveolar lavage fluid obtained from the mice. The values are expressed as mean \pm SD and analyzed by Mann-Whitney test (n = 12 in each group). * p < 0.05 versus WT RA; ** p < 0.05 versus WT hyperoxia; # p < 0.05 versus Nox3^{-/-} Ad-Ctrl hyperoxia. (F, G) WT mice were administered intranasal lentivirus (lenti-Ctrl or lenti-Nox3); after 2 weeks, whole lung tissues were processed. (F) Nox3 mRNA expression was measured by real-time RT-PCR in lung tissue. (G) Real-time RT-PCR was performed on RNA extracted from laser capture microdissected tissues from blood vessels, alveolar, and large airway tissue (parenchyma) on lung sections. The values are expressed as mean \pm SD and analyzed by Student's *t* test (n = 4 for each group). * p < 0.05 versus lenti-Ctrl. lenti-Ctrl, lentiviral-control; Hsp90, heat shock protein 90; LDH, lactate dehydrogenase; MLECs, mouse lung endothelial cells; mRNA, messenger RNA; Nox, NADPH oxidase; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; TLR4, Toll-like receptor 4; TNF α , tumor necrosis factor- α ; WT, wild-type.