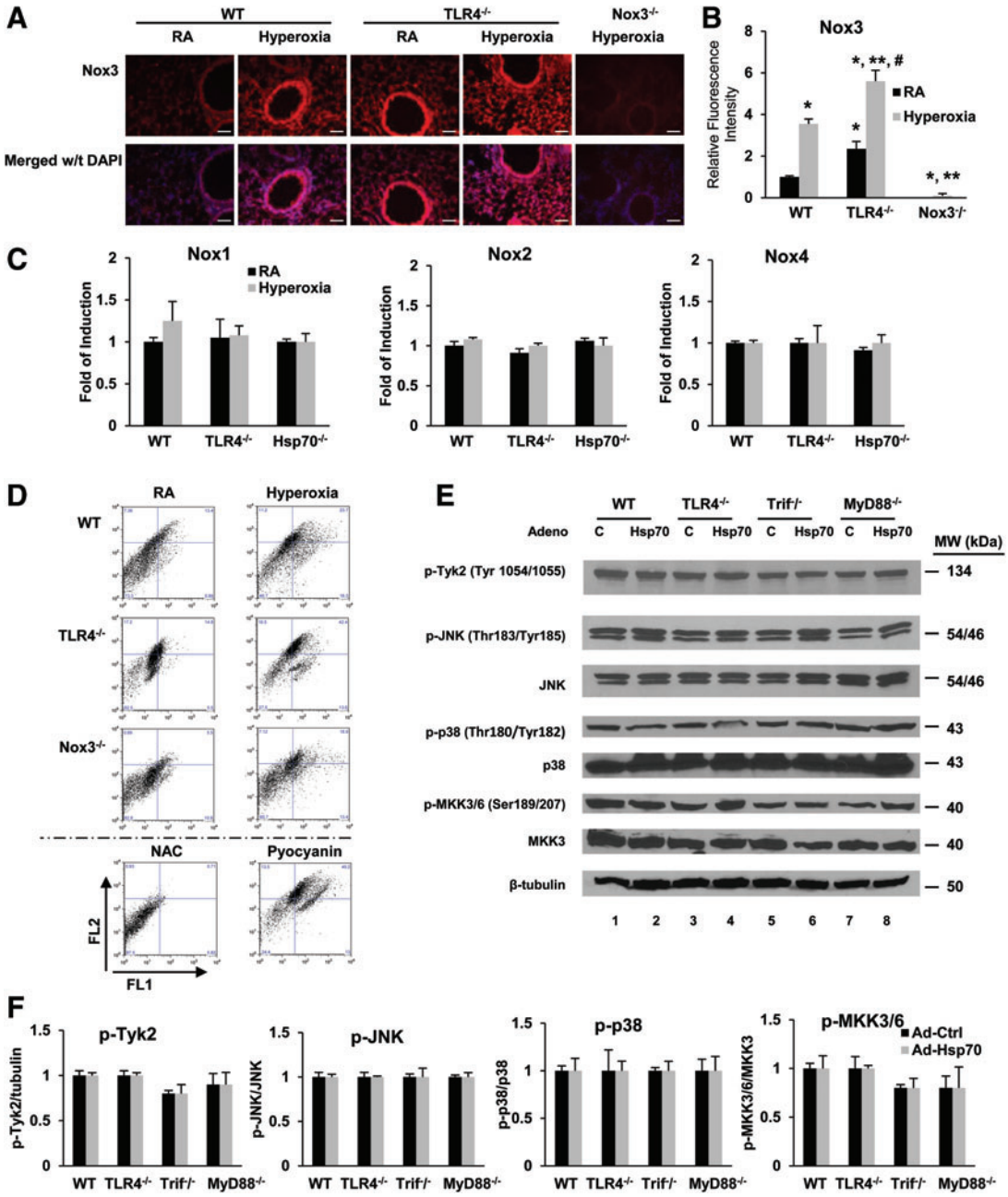

SUPPLEMENTARY FIG. S2. TLR4 deficiency increases Nox3 protein expression in mouse lungs. (A) Nox3 expression in mouse lungs with immunofluorescence staining. Nox3 protein is detected as red cytoplasmic fluorescence staining. Images are representative of five mice in each group. Scale bar = 250 μm. **(B)** The relative fluorescence intensity is expressed as mean ± SD and analyzed by Mann–Whitney test. Experiments were performed in triplicate. $*p < 0.05$ *versus* WT RA; $**p < 0.05$ *versus* WT hyperoxia; $^{\#}p < 0.05$ *versus* corresponding RA. **(C)** Nox mRNA levels in WT, *TLR4*$^{-/-}$, and *Hsp70*$^{-/-}$ MLECs exposed to 72 h of hyperoxia. The values are expressed as mean ± SD and analyzed by Mann–Whitney test. Experiments were performed in triplicate. There is no significant difference between each group. **(D)** WT, *TLR4*$^{-/-}$, and *Nox3*$^{-/-}$ MLECs were exposed to 72 h of hyperoxia. ROS-positive cells and superoxide-positive cells were measured by flow cytometry. The ROS inhibitor, N-acetyl-L-cysteine (NAC, 5 m*M*), or the ROS inducer, pyocyanin (Pyo, 200 μ*M*), was incubated with WT MLECs as the negative or positive control. Graphical quantitation of flow cytometry analysis based on log FL1 (*x*-axis) *versus* log FL2 (*y*-axis) dot plots. The representative dot plots of three experiments are shown. Cells with increased levels of oxidative stress demonstrate a *bright green* staining in the presence of the oxidative stress detection reagent and can be registered in the FL1 channel. Such cells will appear in the *upper* and *lower right* quadrants of a log FL1 (*x*-axis) *versus* a log FL2 (*y*-axis) dot plot. Cells with increased production of superoxide demonstrate *bright orange fluorescence* and will be detected using the FL2 channel. Such cells will appear in the two upper quadrants of a log FL1 (*x*-axis) *versus* a log FL2 (*y*-axis) dot plot. Graphical quantitation of flow cytometry analysis is shown in Figure 6C. **(E)** WT, *TLR4*$^{-/-}$, *Trif*$^{-/-}$, and *MyD88*$^{-/-}$ MLECs were treated with Ad-Ctrl or Ad-Hsp70. Cell lysates were immunoblotted against antibodies shown on the *left*. β-Tubulin was used as protein loading control. One representative Western blot of three independent experiments is shown. **(F)** Quantification based on densitometry for the listed proteins relative to β-tubulin. The values are expressed as mean ± SD and analyzed by Mann–Whitney test. Experiments were performed in triplicate. There is no significant difference between each group. Ad-Hsp70, adenoviral-Hsp70; ROS, reactive oxygen species.