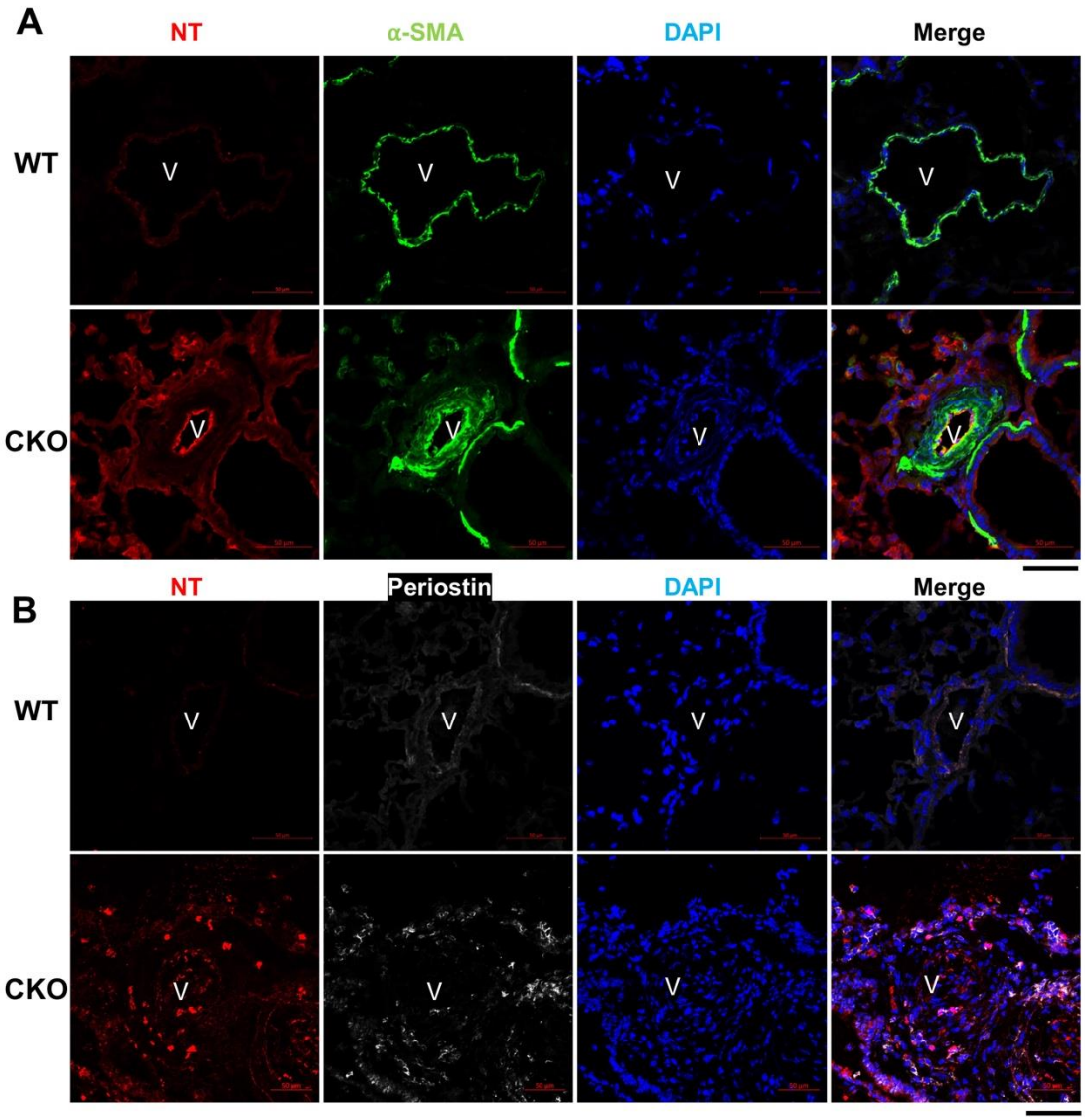
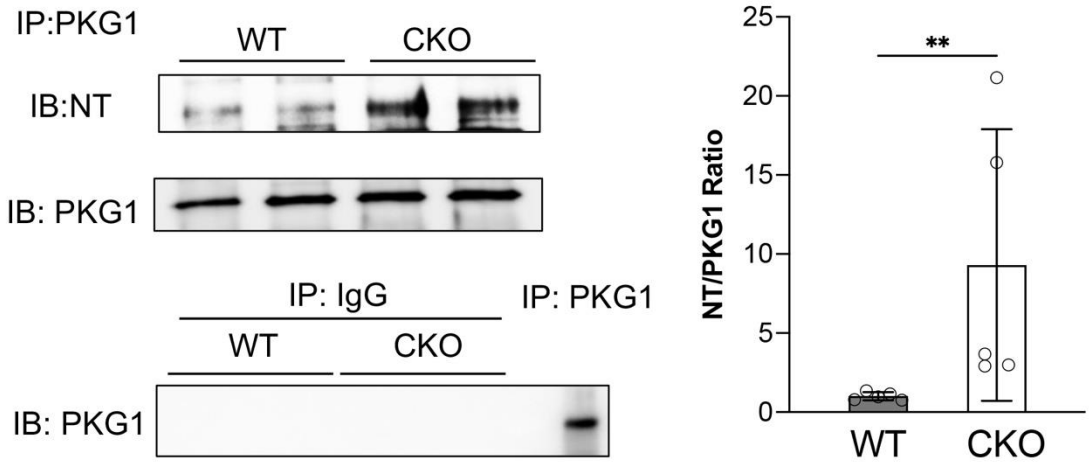
Endothelial PHD2 Deficiency Induces Nitrative Stress via Suppression of Caveolin-1 in Pulmonary Hypertension

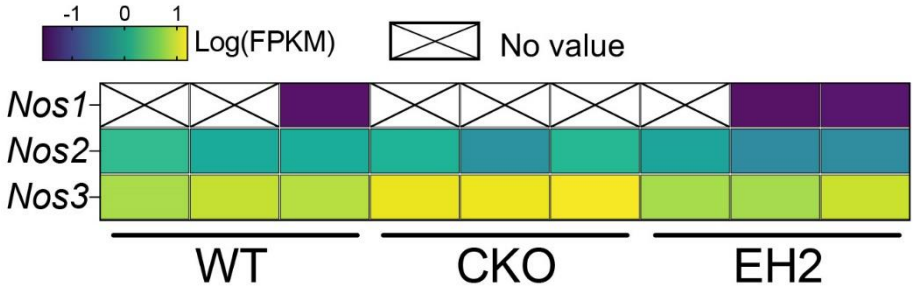
Supplemental Figures and Figure Legends



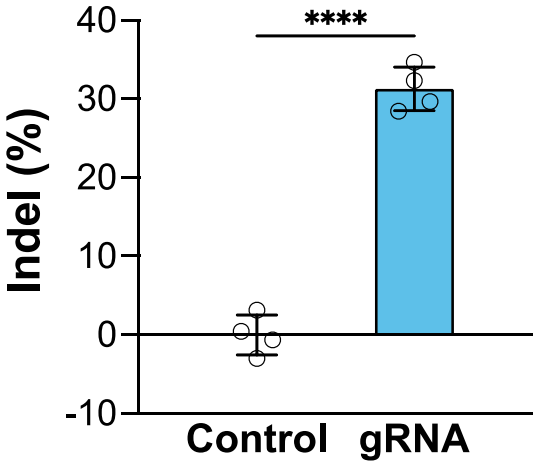
Supplementary Figure S1. Nitrative stress in SMCs and fibroblasts in the pulmonary vascular lesions of *Egln1*^{Tie2Cre} mice. (A, B) Representative micrographs of immunostaining showing relatively low levels of NT expression in SMCs and fibroblasts in pulmonary vascular lesions of CKO mice (3.5 months old). Lung sections were immunostained with anti-NT antibody (red) for detection of nitrative stress. Anti-α-SMA antibody was used to label SMCs (green, **A**). Anti-Periostin antibody was used to label fibroblasts (white, **B**). Nuclei were counterstained with DAPI (blue). The strong positive NT signal in (**A**) was from pulmonary vascular ECs not SMCs.



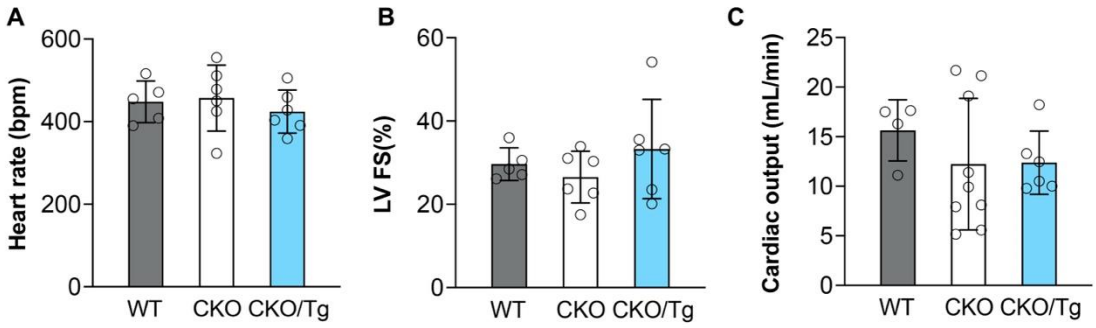
Supplementary Figure S2. Immunoprecipitation assay demonstrating that PKG nitrotyrosine modification was markedly increased in *Egln1*^{Tie2Cre} mice. Lung tissues were collected from 3.5 months old mice for homogenization. 450 µg of lysates/mouse were then immunoprecipitated with anti-PKG antibody and blotted with anti-NT antibody. The same membrane was also blotted with anti-PKG antibody as a loading control. IgG was also used as a control for precipitation.



Supplementary Figure S3. *Nos3* was upregulated in *Egln1*^{Tie2Cre} mice. RNA-sequencing analysis demonstrated the upregulation of *Nos3* in CKO mice and restoration in *Egln1*^{Tie2Cre}/*Hif2a*^{Tie2Cre} (EH2) lungs. RNA-sequencing analysis was performed with lung tissues of 3.5 months old WT, CKO and EH2 mice.



Supplementary Figure S4. Genomic editing efficiency of Nos 3 in lung ECs after nanoparticle delivery of CRISPR-Cas9 plasmid DNA. Mixture of nanoparticles:Nos3 CRISPR plasmid DNA was administered to CKO mice at age of 7, 8, and 9 weeks (total 3 injections) and lung tissuses were collected at 14 weeks of age for EC isolation. Genomic DNA isolated from ECs was used for quantitative PCR analysis with a primer specific for the predicted indel site. The genomic editing efficiency in the lung ECs was approximately 30%. ****, $P < 0.0001$, Student's *t* test.



Supplementary Figure S5. Echocardiography analysis showing similar left heart function and cardiac output in CKO/Tg mice and CKO mice. At age of 3.5 months, the mice were subject to echocardiography to assess heart rate (**A**), left ventricular fraction shortening (LV FS), indicative of contractility (**B**), and cardiac output (**C**). bpm= beats per minute. One-way ANOVA with Tukey post-hoc analysis (**A**-**C**).