Mesenchymal Stem Cell Extracellular Vesicles Reverse Sugen/Hypoxia Pulmonary Hypertension in Rats

James R. Klinger, Mandy Pereira, Michael Del Tatto, Alexander S. Brodsky, Keith Q. Wu, Mark S. Dooner, Theodore Borgovan, Sicheng Wen, Laura R. Goldberg, Jason M. Aliotta, Corey E. Ventetuolo, Peter J. Quesenberry, and Olin D. Liang

ONLINE DATA SUPPLEMENT

Online Data Supplement

Legends for Supplemental Figures

Supplemental Figure 1. Transmission electron microscope images (FEI Morgagni 268 at 80 kV) showing extracellular vesicles in resuspended pellet. (A) Magnification: 21,000 x, scale bar = 200 nm. (B) Magnification: 69,000 x, scale bar = 100 nm. Expression of typical markers of MSC-EV and FL-EV was confirmed with Western blot using specific antibodies. Albumin which is not expressed by MSC-EV or FL-EV was used as a negative control. Antibodies against human Cd9 (22-27 kDa), Cd81 (26 kDa), Cd63 (30-60 kDa) were purchased from System Biosciences (Palo Alto, CA), GAPDH (37 kDa), TSG101 (45 kDa) and Albumin (66 kDa) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX).

Supplemental Figure 2. Mesenchymal stem cell extracellular vesicle size and concentration measured by nanoparticle tracking analysis using NanoSight 5000 showing an average size of 212.5 ± 107.0 nm.

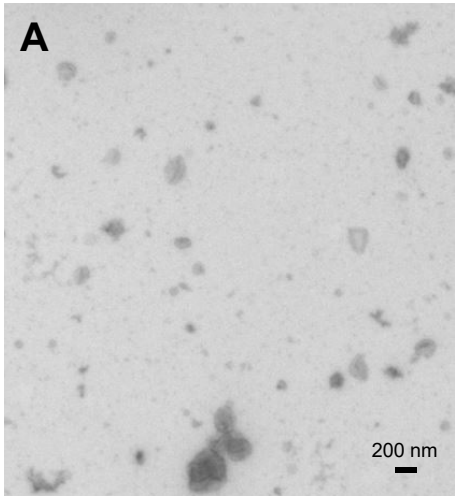
Supplemental Figure 3. Study protocol for induction of pulmonary hypertension with the Sugen/hypoxia model (A). Typical example of individual tracings of right ventricular systolic pressure (RVSP) in normoxic controls (Nx) injected with DMSO vehicle (B) and in rats injected with Sugen 5416 and kept in hypoxia (SuHx) for 3 weeks (C). RVSP (D) and right ventricle to left ventricle + septum ratio (RV/LV+S) (E) in Nx and SuHx rats measured at week 4. Photomicrographs of H&E stained lung sections showing vascular wall thickening of peripheral pulmonary vessels in SuHx (G), but not in Nx (F) rats at week 4. *** $P < 0.001$, **** $P < 0.0001$. Scale bar = 50 μm.

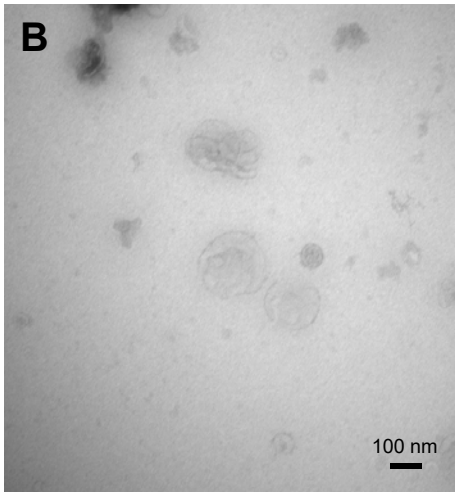
Supplemental Figure 4. Photomicrographs of lung sections stained with an antibody against α-smooth muscle actin to show muscularization of peripheral pulmonary vessels (black arrows) in normoxic control rats (Nx), rats with Sugen/hypoxia pulmonary hypertension treated with vehicle alone (SuHx) or rats with Sugen/hypoxia pulmonary hypertension that were treated with mesenchymal stem cell extracellular vesicles at the start (EV Prevention) or end (EV Reversal) of Sugen/hypoxia. Scale bar = 50 μm.

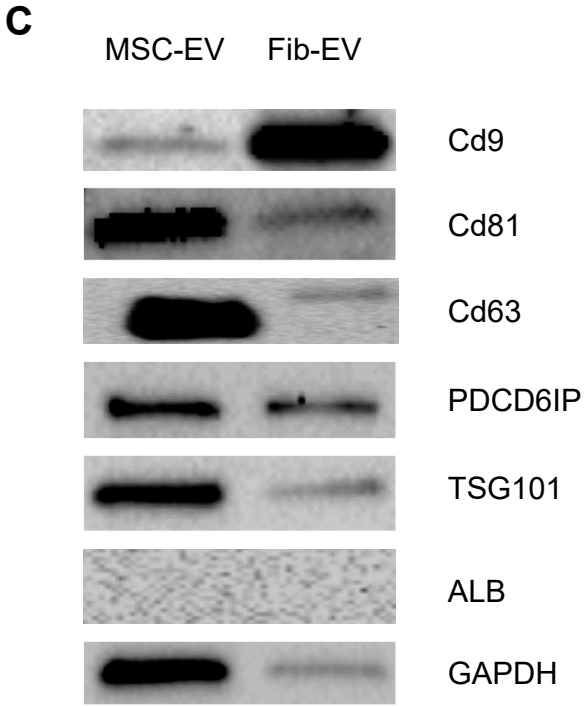
Supplemental Figure 5. Immunohistochemical staining of Arginase-1 (Arg-1)+ M2 macrophages (brown spots) in lungs of rats treated with normoxia (Nx), Sugen/hypoxia plus PBS vehicle (SuHx), Sugen/hypoxia plus mesenchymal stem cell extracellular vesicles (SuHxEV). Average number of Arg-1+ M2 macrophages per 10 x field in each group is shown in (D). ** $P < 0.01$.

Supplemental Figure 6. Inhibition of human bone marrow monocyte differentiation into M1 polarized macrophages by MSC-EV in vitro. The percentage of Cd64+Cd68+ M1 macrophages were quantified on a LSRII flow cytometer (BD).

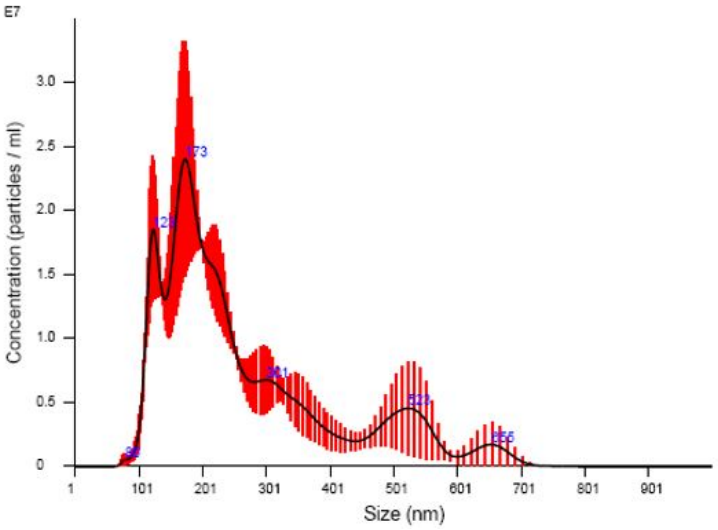
Supplemental Figure 7. Immunohistochemical staining of von Willebrand factor (vWF) + distal pulmonary vessels (black arrows) in lungs of normoxic (Nx) rats and Nx rats treated with MSC-EV (Nx EV) following Reversal Protocol I. No difference was seen in the number of vessels per 10 x field.



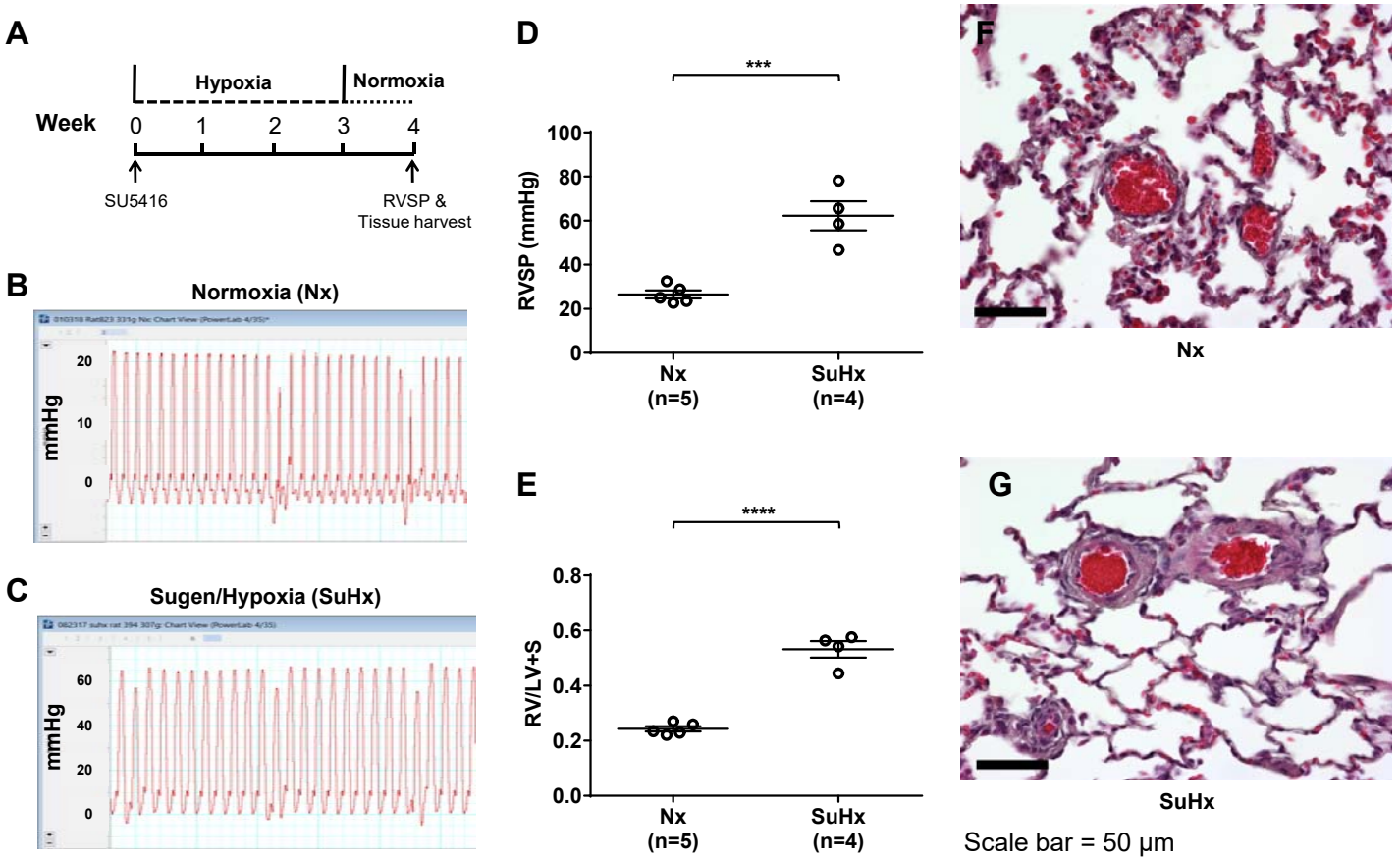




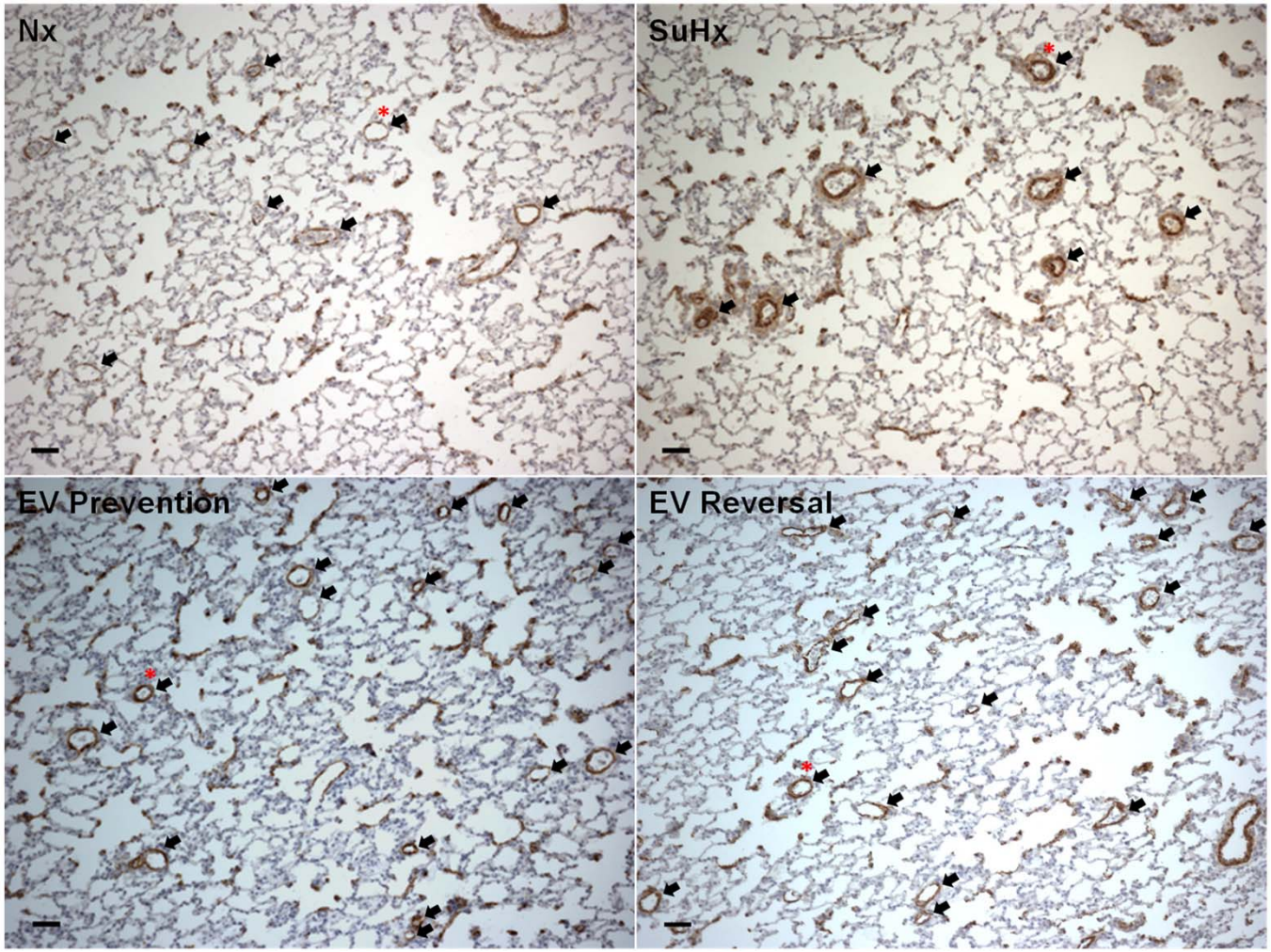
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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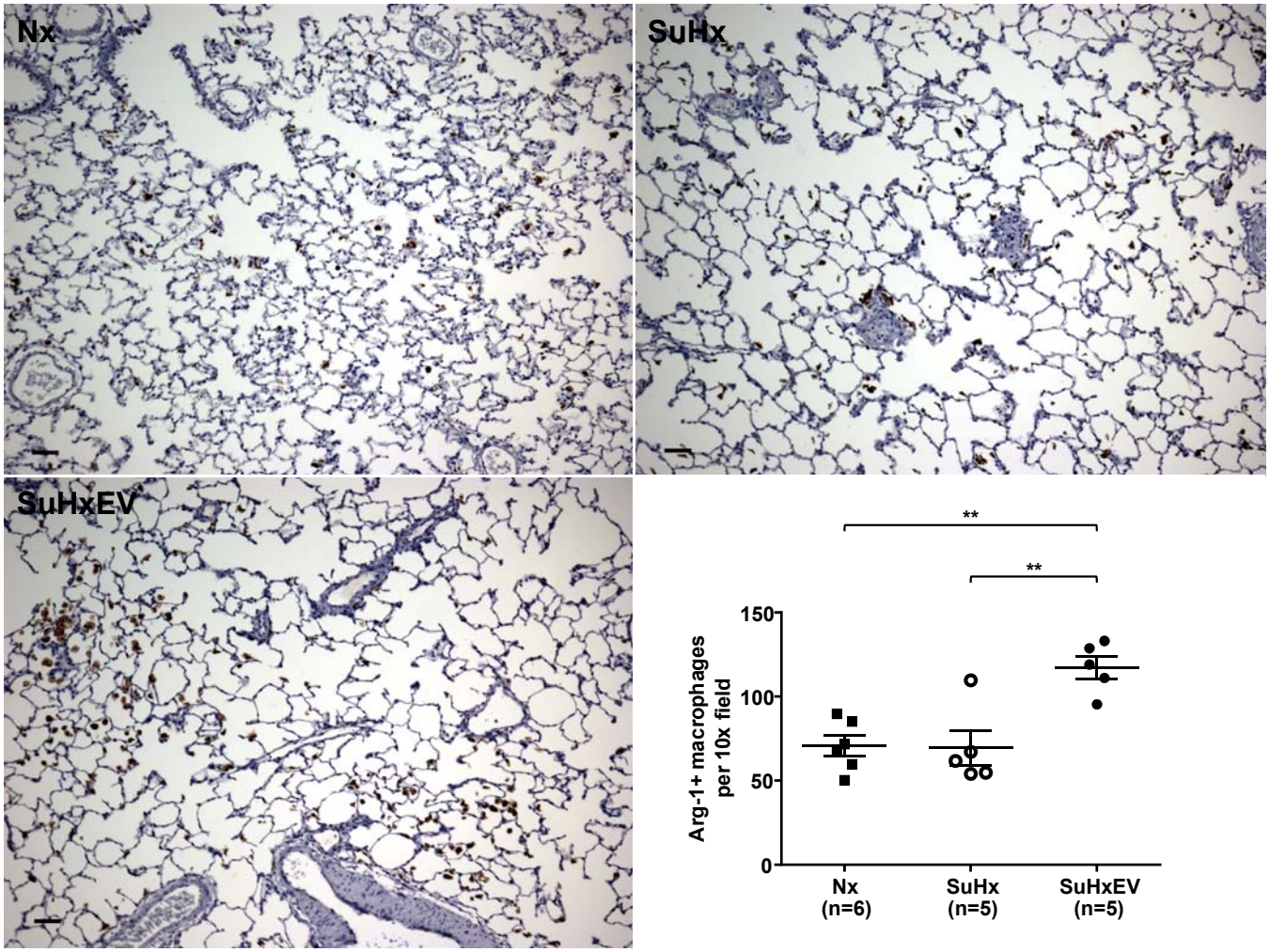
Supplemental Figure 4



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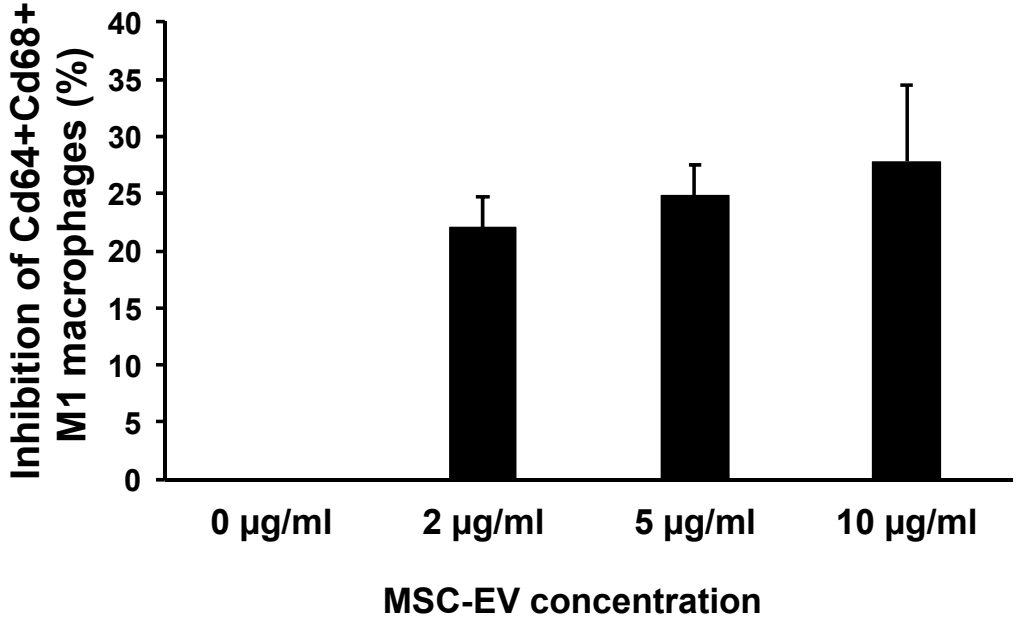
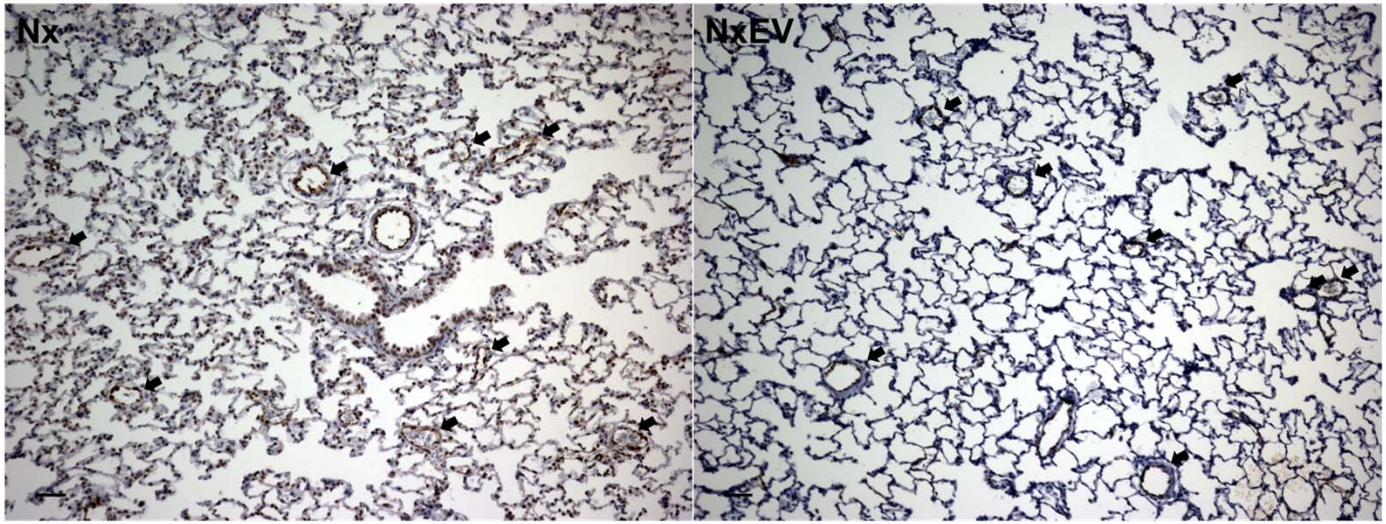
Supplemental Figure 5



Supplemental Figure 6



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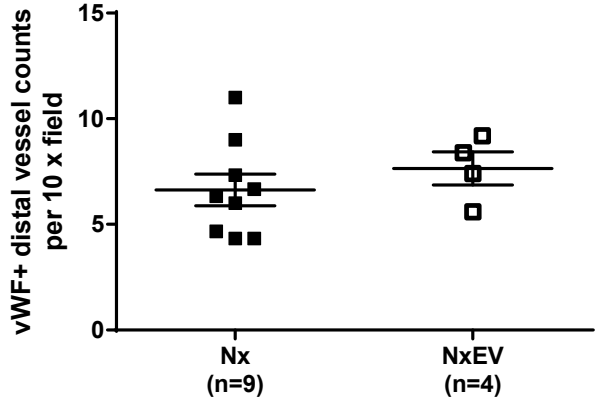

Supplemental Figure 7