

## SUPPLEMENTAL MATERIALS

### **Supplemental Table 1. Semi-quantitative Histopathology Severity Scoring Rubric.**

**Supplemental Figure 1. Illustrated histopathology scoring reference for alveolar inflammation.** Panels A, B, C, and D show alveolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate infiltrating macrophages in the parenchyma of the lung (panels B-D).

**Supplemental Figure 2. Illustrated histopathology scoring reference for peribronchiolar inflammation.** Panels A, B, C, and D show peribronchiolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate macrophages present in the peribronchiolar regions of the lung (panels B-D). Br = bronchiole; TB = terminal bronchiole; AD = alveolar duct.

**Supplemental Figure 3. Illustrated histopathology scoring reference for perivascular inflammation.** Panels A, B, C, and D show perivascular inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate inflammatory cells infiltrating the perivascular regions of the lung (panels B-D). BV = blood vessel.

**Supplemental Figure 4. Illustrated histopathology scoring reference for subpleural inflammation.** Panels A, B, C, and D show subpleural inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate cellular infiltration present in the subpleural area of the lung (panels B-D). P = pleural lining.

**Supplemental Figure 5. The survival curve for the complete set of 74 animals.** Rats were exposed to tobacco smoke (TS) (square markers with dashed lines; n=36) or filtered air (FA)

(round markers with solid lines; n=38) for 9 months (6 hours/day, 3 days/week, for 39 weeks), from ages 10-49 weeks, and subsequently maintained in an FA environment until death. The resulting percent survival is shown for each group over time. Survival curves were compared using a Student's T-test at a significance level of  $p < 0.05$ . The survival curve for the complete set of 74 animals is similar to that of the 22 animals (12 experimental, 10 control) randomly selected for rigorous morphometric analysis.

Supplemental Table 1. Semi-quantitative Histopathology Severity Scoring Rubric

Type of Pathology	Severity Score			
	0	1	2	3
<b>Alveolitis</b>	Normal. Thin alveolar walls, with very few free macrophages in the lumen. No inflammatory cells.	Similar to 0 score with more free macrophages in the alveolar lumen. No polymorphonuclear cells (PMNs).	Atypical cellularity in the walls and/or lumen of the alveoli with most of the alveolar spaces still clear of free cells. Over-represented cell types include macrophages, monocytes, and/or PMNs (e.g. neutrophils and eosinophils).	Thickened alveolar walls and enlarged alveolar airspace. Marked influx of mixed cells (phagocytes and/or PMNs) into the alveolar lumen forming large cellular agglomerates which occupy much of the airspace.

Supplemental Table 1 Semi-quantitative Histopathology Severity Scoring Rubric (continued)

Type of Pathology	Severity Score			
	0	1	2	3
<b>Bronchiolitis</b>	Normal respiratory epithelium, 1 cell-layer thick. Normal smooth muscle and submucosal layers.	Mild influx of macrophages and/or monocytes to the airway submucosa, but no PMNs.	Slightly thickened airway due to moderate influx of PMNs and/or phagocytes such as neutrophils, eosinophils, or macrophages, into the submucosa. PMNs encompass <15% of influxing cells.	Marked influx of inflammatory cells into the submucosal layer causing pronounced thickening of the airway. A high percentage of PMNs may be present.

Supplemental Table 1. Semi-quantitative Histopathology Severity Scoring Rubric (continued)

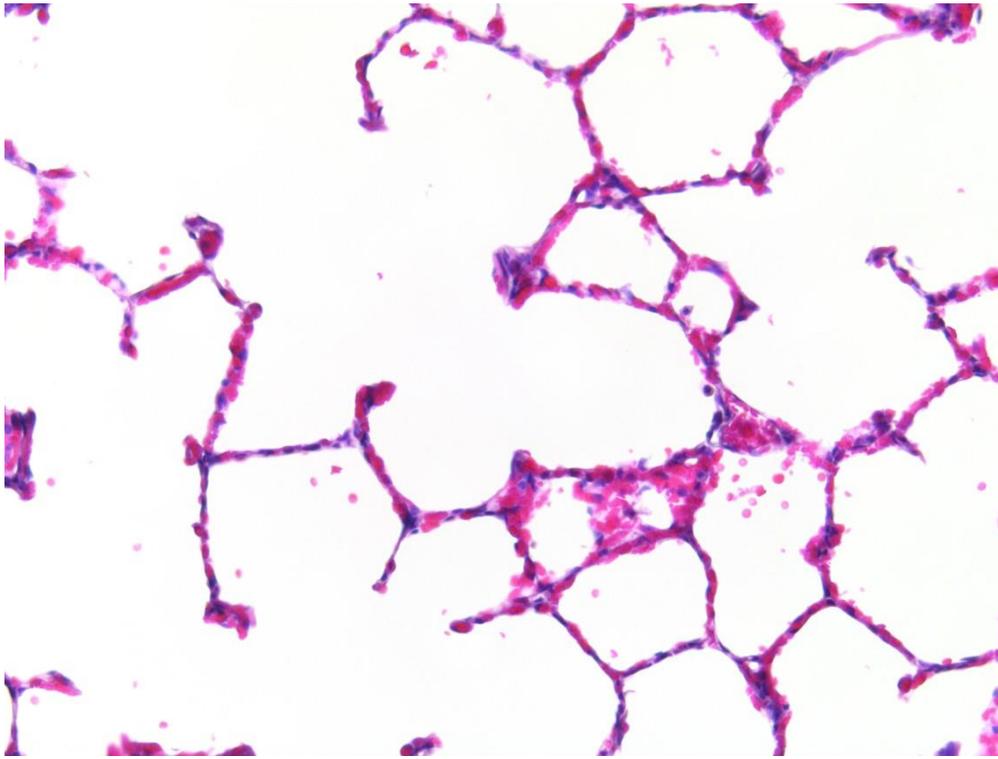
Type of Pathology	Severity Score			
	0	1	2	3
<b>Perivascular Inflammation</b>	Normal vascular endothelium.	Mild influx of a few macrophages and/or monocytes to the region, but no PMNs. Nearly all the connective tissue is still visible.	Moderate PMN and/or phagocyte infiltrates with much of the connective tissue still visible.	Marked mixed cellular infiltrates such that much of the connective tissue is obscured. A high percentage of PMNs may be present.

**Supplemental Table 1. Semi-quantitative Histopathology Severity Scoring Rubric (continued)**

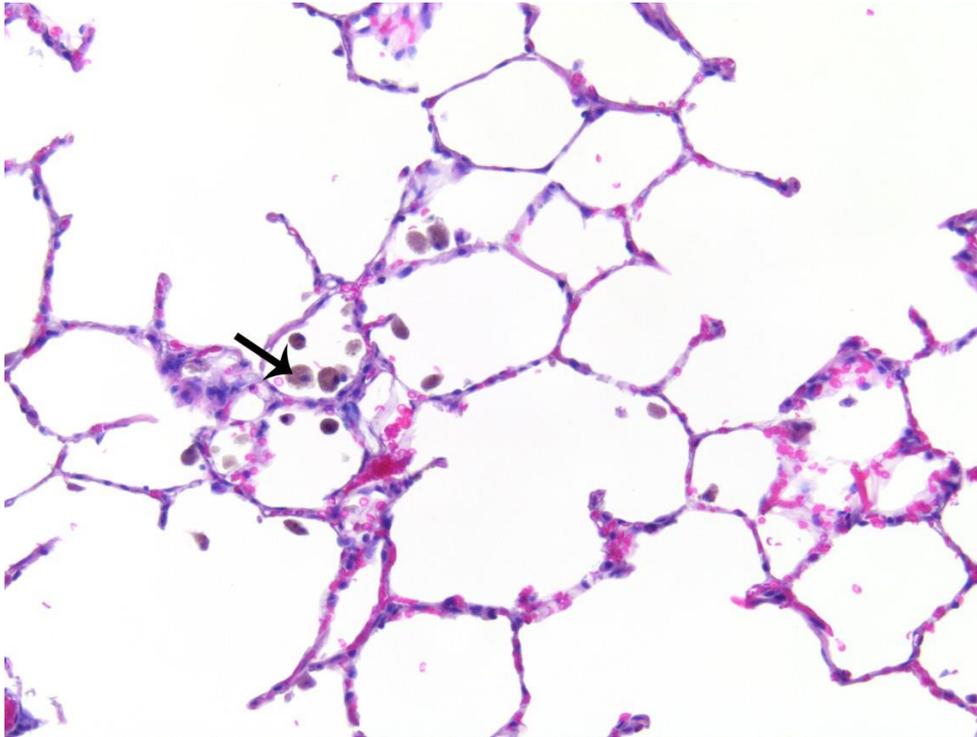
Type of Pathology	Severity Score			
	0	1	2	3
<b>Pleural Inflammation</b>	Little/no cells at the pleura.	Slightly increased cellularity at the pleura. No PMNs.	Moderately increased cellularity with PMNs and/or phagocytes.	Severe influx of cells to the pleura. A high percentage of PMNs may be present along with foamy macrophages.

*Note:* Adapted from “Pulmonary effects of silver nanoparticle size, coating, and dose over time upon intratracheal instillation,” by Silva, R.M., Anderson, D.S., Franzi, L.M., Peake, J.L., Edwards, P.C., Van Winkle, L.S. and Pinkerton, K.E. (2015), *Toxicol Sci*, **144**, 151-162, with the author’s permission to reproduce.



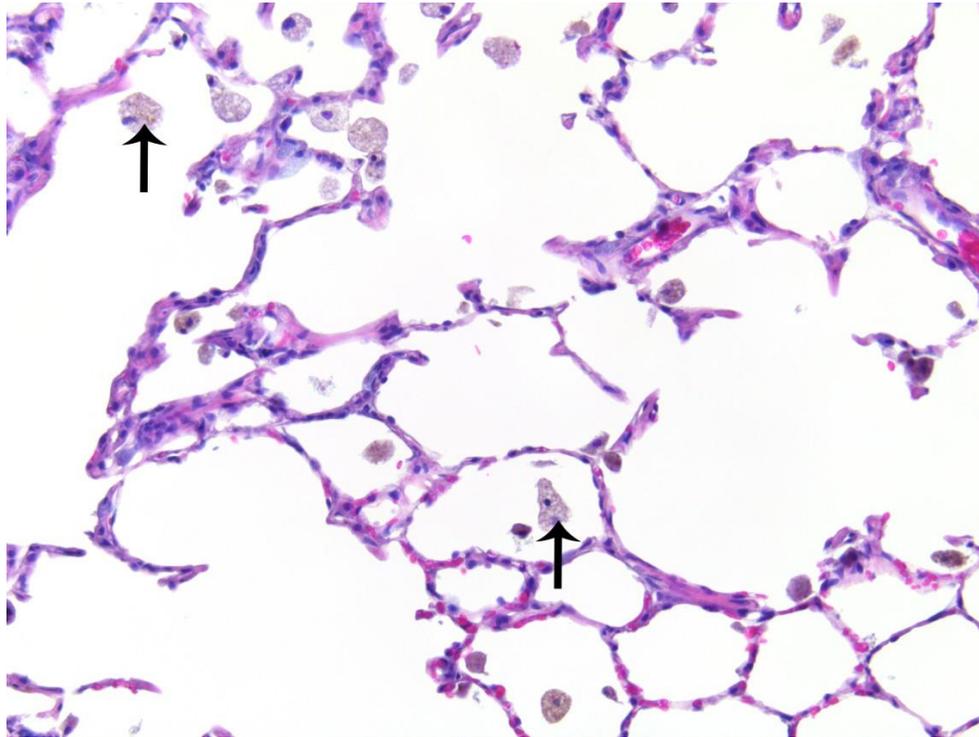


**Supplemental Figure 1A. Illustrated histopathology scoring reference for alveolar inflammation.** Panels A, B, C, and D show alveolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate infiltrating macrophages in the parenchyma of the lung (panels B-D).

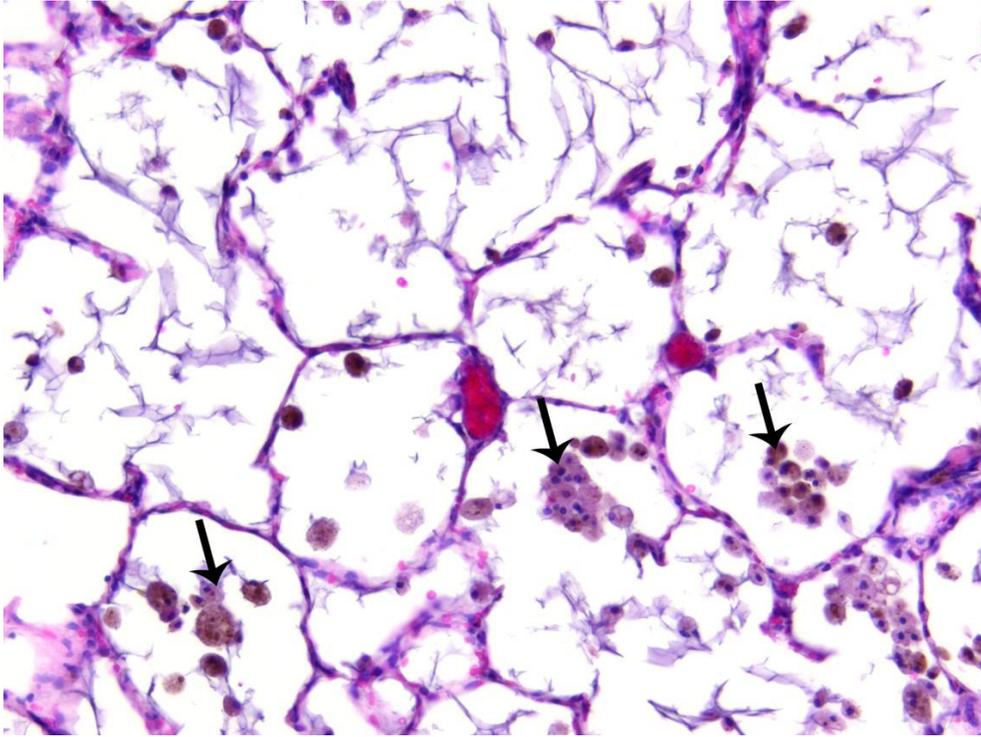


**Supplemental Figure 1B. Illustrated histopathology scoring reference for alveolar inflammation.**

Panels A, B, C, and D show alveolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate infiltrating macrophages in the parenchyma of the lung (panels B-D).

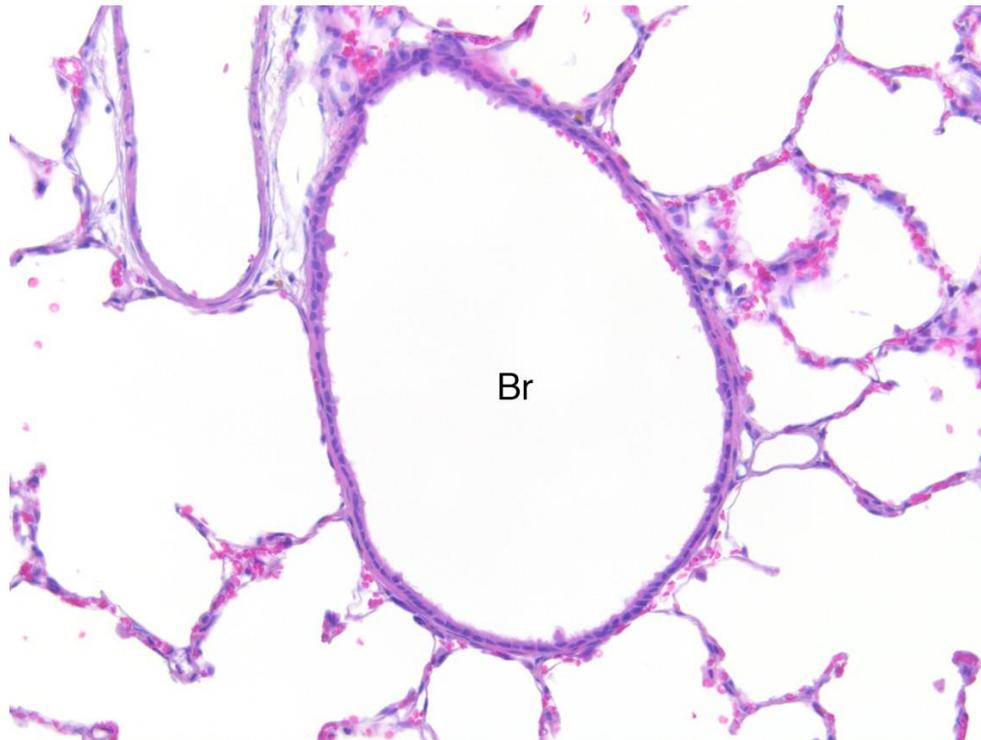


**Supplemental Figure 1C. Illustrated histopathology scoring reference for alveolar inflammation.** Panels A, B, C, and D show alveolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate infiltrating macrophages in the parenchyma of the lung (panels B-D).

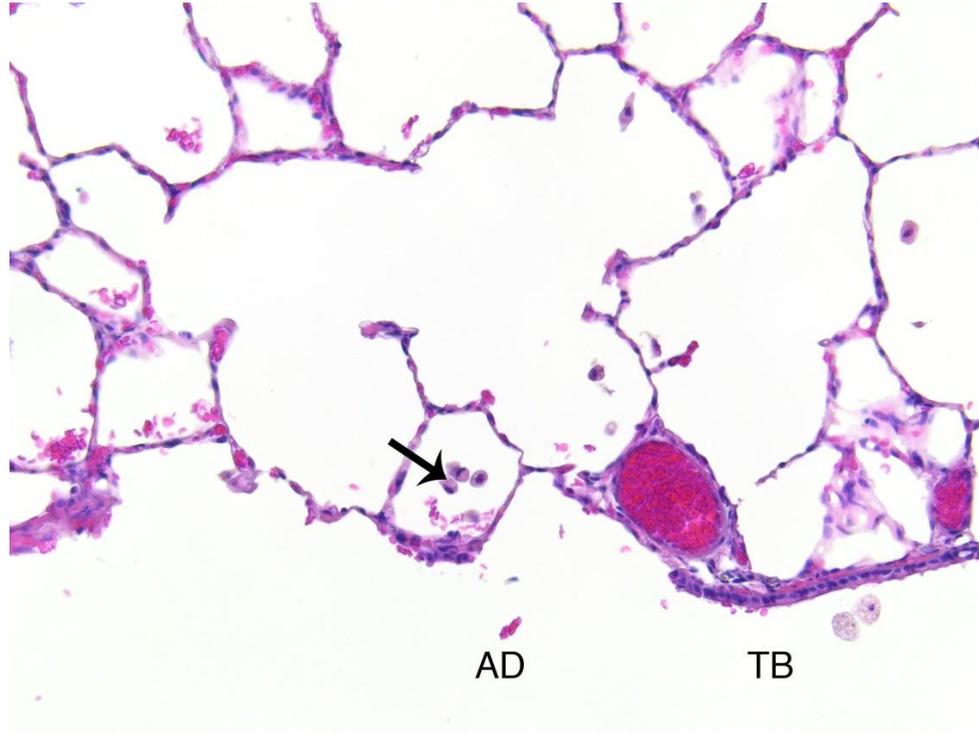


**Supplemental Figure 1D. Illustrated histopathology scoring reference for alveolar inflammation.**

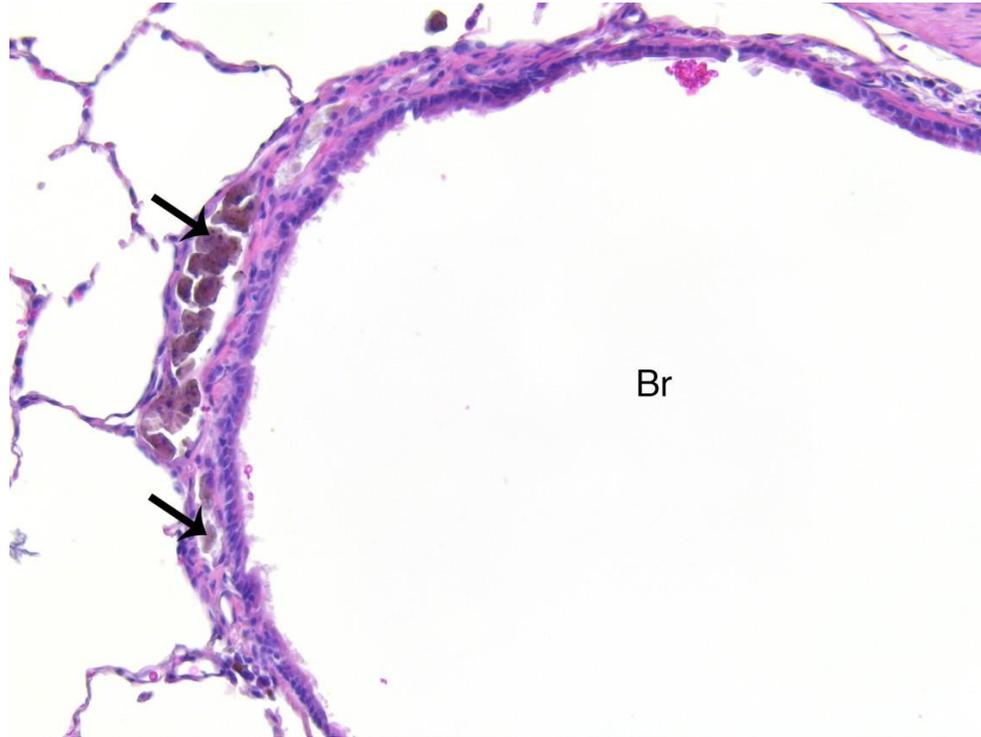
Panels A, B, C, and D show alveolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate infiltrating macrophages in the parenchyma of the lung (panels B-D).



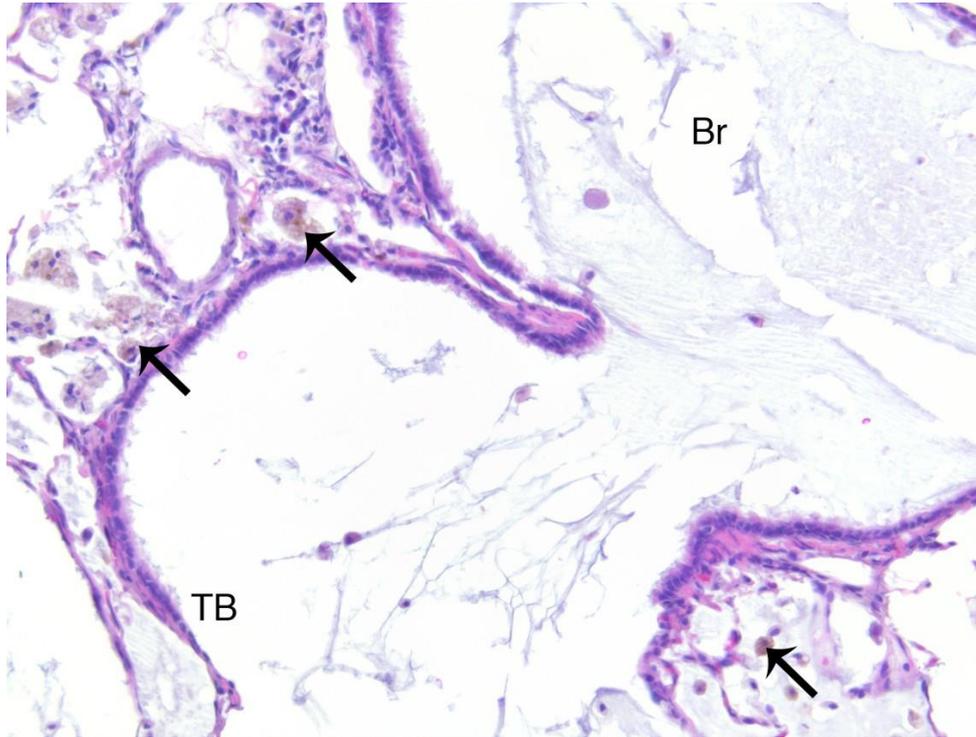
**Supplemental Figure 2A. Illustrated histopathology scoring reference for peribronchiolar inflammation.** Panels A, B, C, and D show peribronchiolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate macrophages present in the peribronchiolar regions of the lung (panels B-D). Br = bronchiole; TB = terminal bronchiole; AD = alveolar duct.



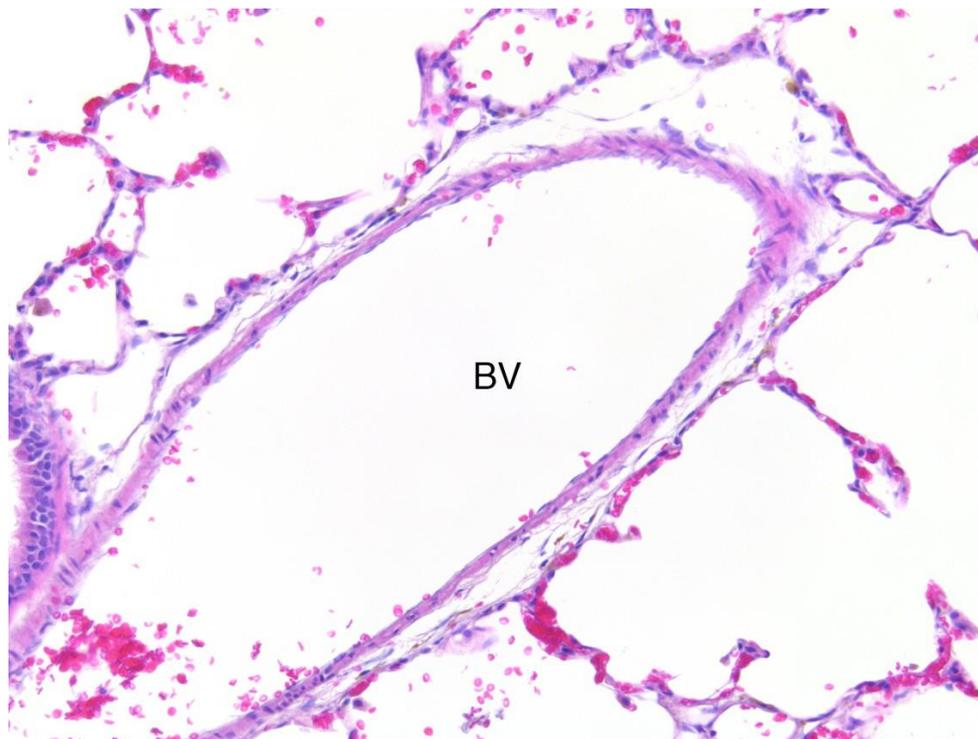
**Supplemental Figure 2B. Illustrated histopathology scoring reference for peribronchiolar inflammation.** Panels A, B, C, and D show peribronchiolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate macrophages present in the peribronchiolar regions of the lung (panels B-D). Br = bronchiole; TB = terminal bronchiole; AD = alveolar duct.



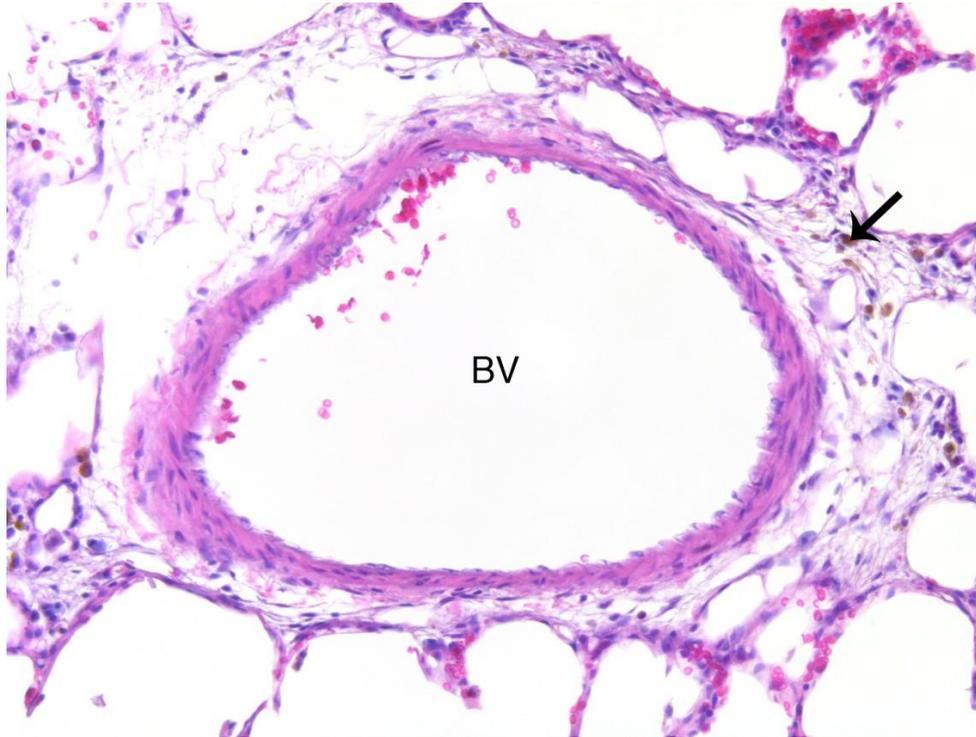
**Supplemental Figure 2C. Illustrated histopathology scoring reference for peribronchiolar inflammation.** Panels A, B, C, and D show peribronchiolar inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate macrophages present in the peribronchiolar regions of the lung (panels B-D). Br = bronchiole; TB = terminal bronchiole; AD = alveolar duct.



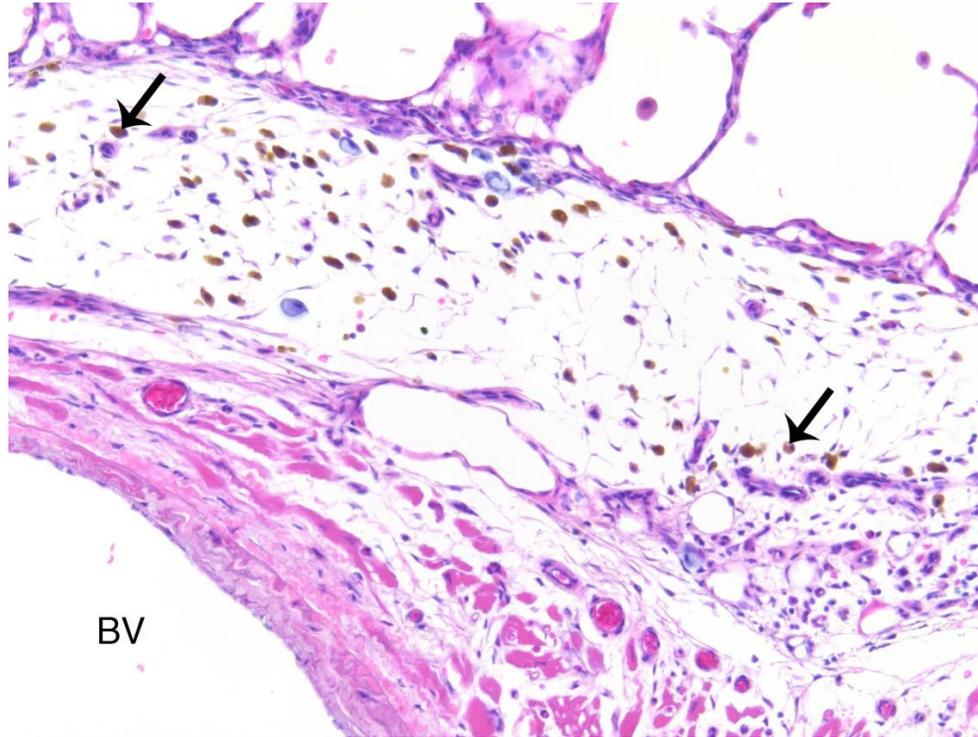
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**Supplemental Figure 3A. Illustrated histopathology scoring reference for perivascular inflammation.** Panels A, B, C, and D show perivascular inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate inflammatory cells infiltrating the perivascular regions of the lung (panels B- D). BV = blood vessel.



**Supplemental Figure 3B. Illustrated histopathology scoring reference for perivascular inflammation.** Panels A, B, C, and D show perivascular inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate inflammatory cells infiltrating the perivascular regions of the lung (panels B- D). BV = blood vessel.



**Supplemental Figure 3C. Illustrated histopathology scoring reference for**

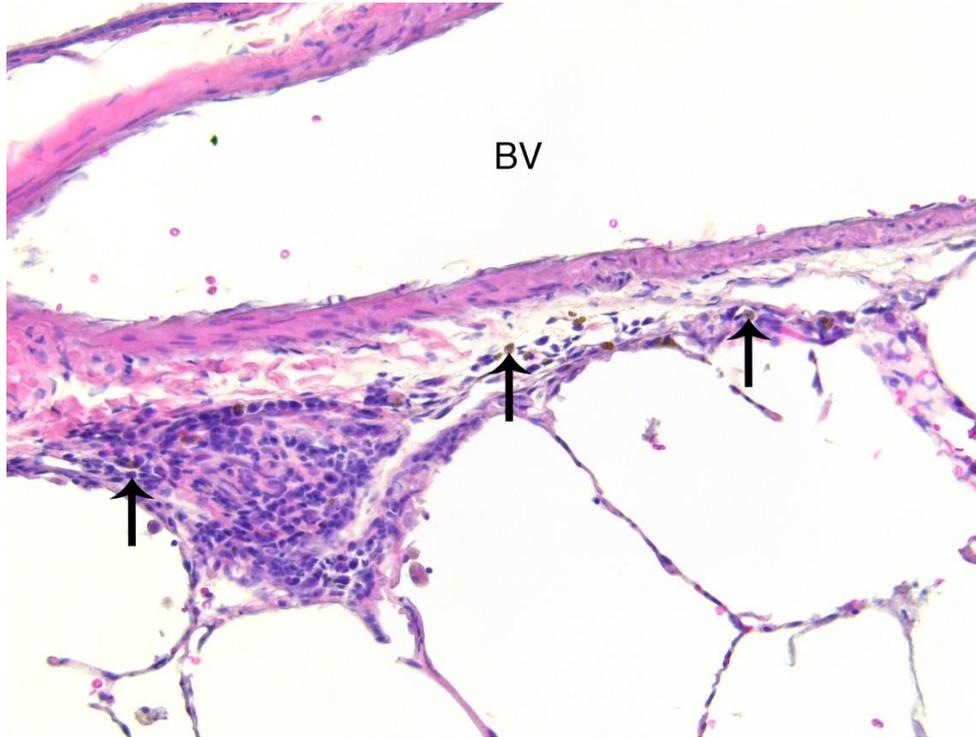
**perivascular inflammation.** Panels A, B, C, and D show perivascular

inflammation, in the lungs of spontaneously hypertensive rats, representing

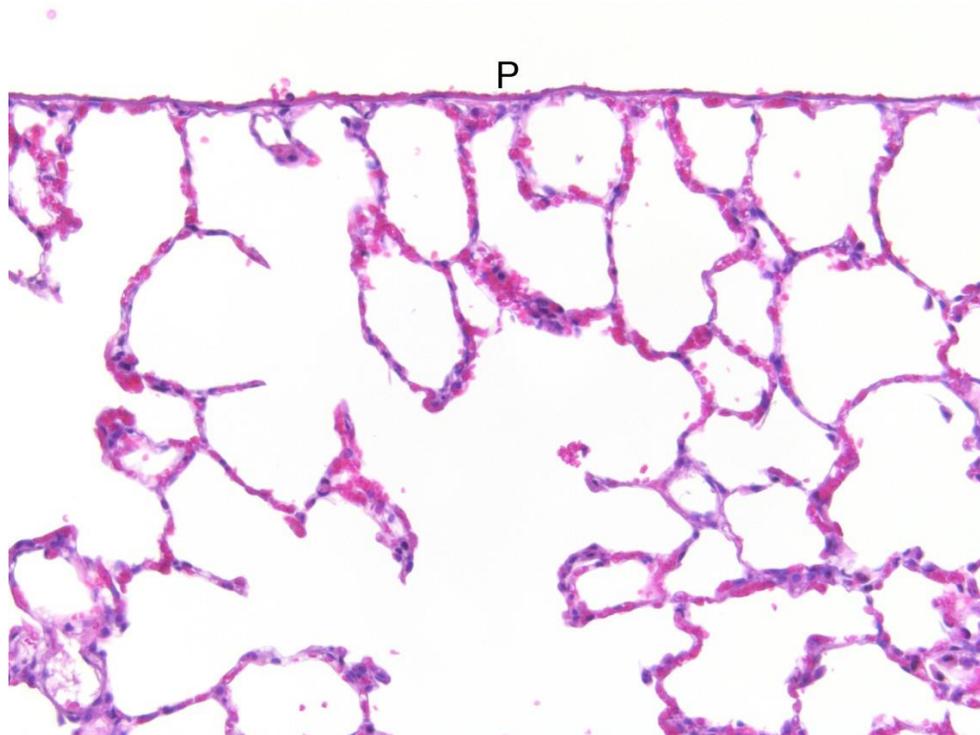
severity scores 0, 1, 2, and 3, respectively. Arrows indicate inflammatory cells

infiltrating the perivascular regions of the lung (panels B- D). BV = blood

vessel.



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**Supplemental Figure 4A. Illustrated histopathology scoring reference**

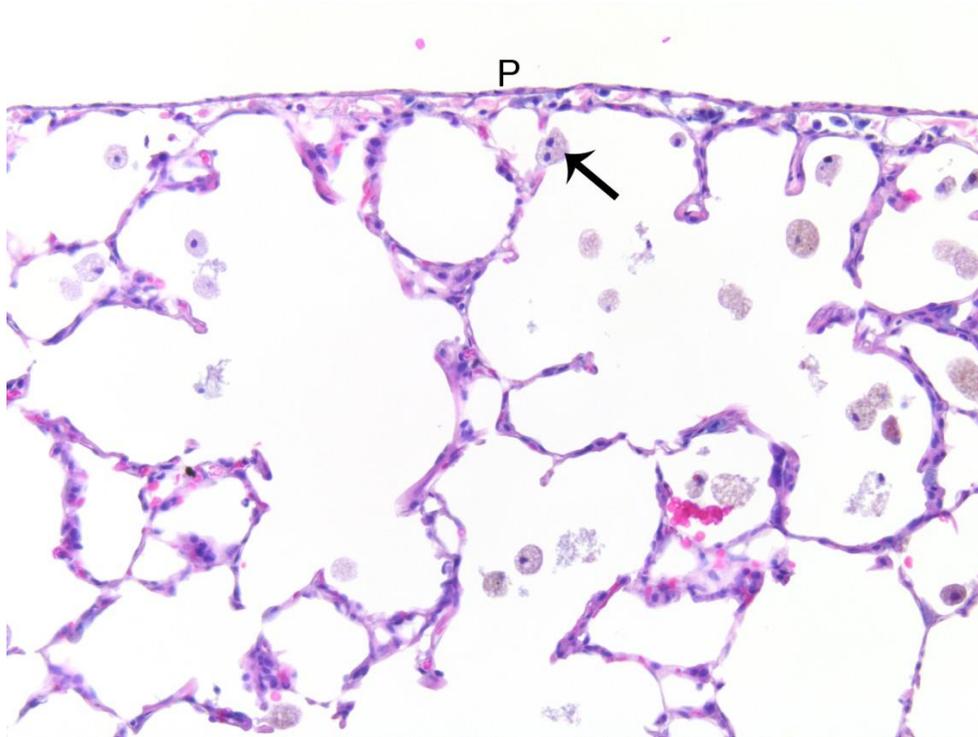
**for subpleural inflammation.** Panels A, B, C, and D show subpleural

inflammation, in the lungs of spontaneously hypertensive rats,

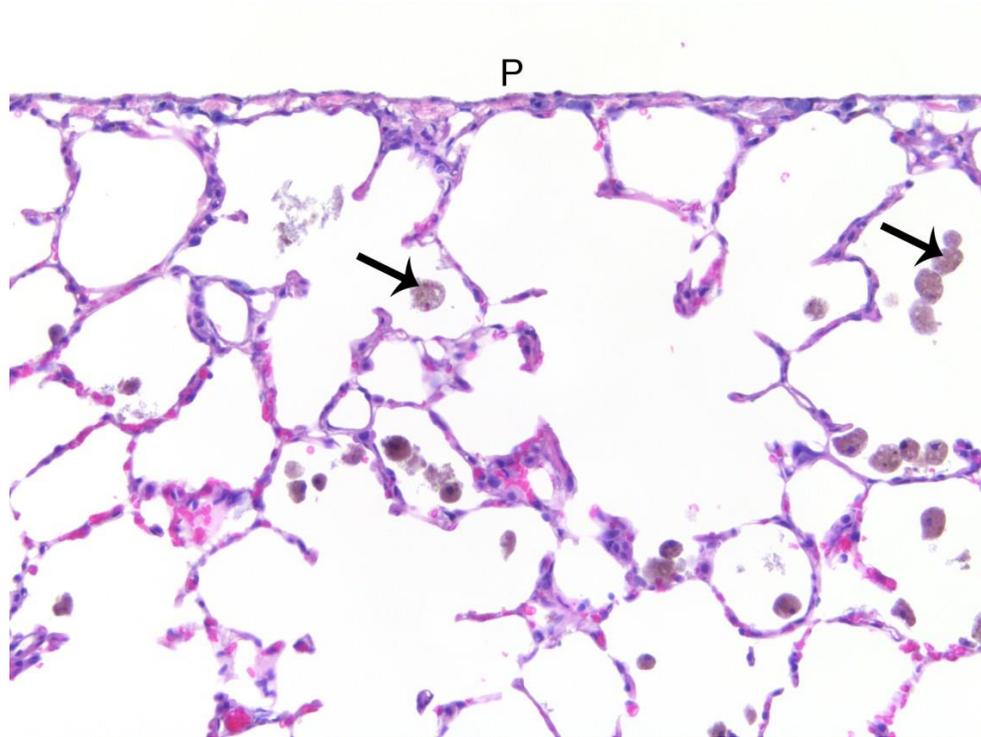
representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate

cellular infiltration present in the subpleural area of the lung (panels B-

D). P = pleural lining.



**Supplemental Figure 4B. Illustrated histopathology scoring reference for subpleural inflammation.** Panels A, B, C, and D show subpleural inflammation, in the lungs of spontaneously hypertensive rats, representing severity scores 0, 1, 2, and 3, respectively. Arrows indicate cellular infiltration present in the subpleural area of the lung (panels B-D). P = pleural lining.



**Supplemental Figure 4C. Illustrated histopathology scoring reference**

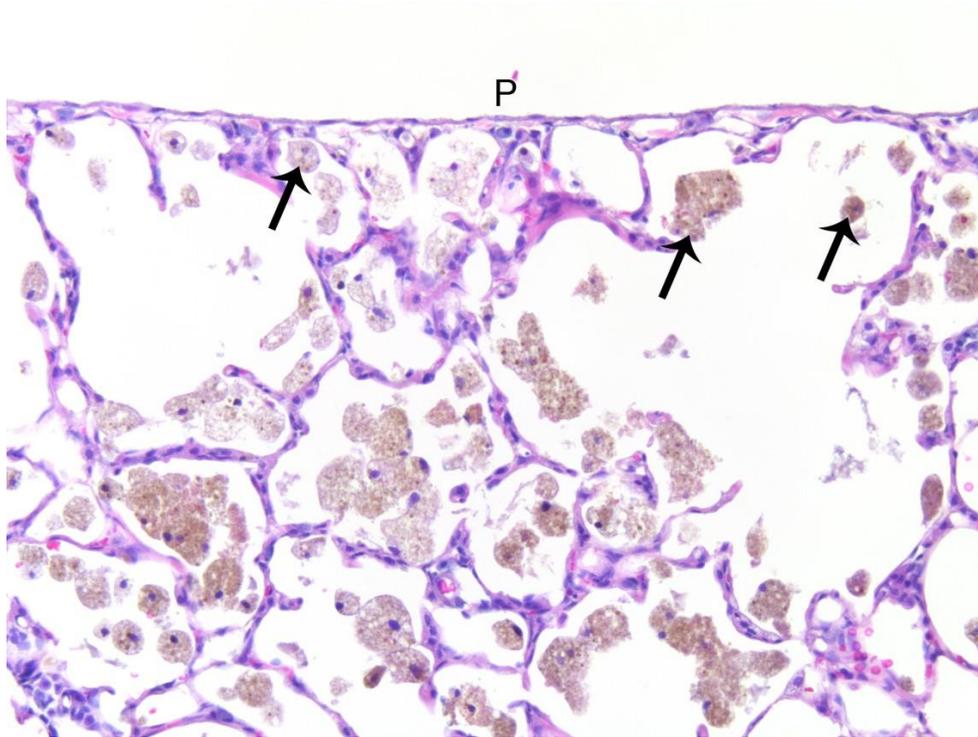
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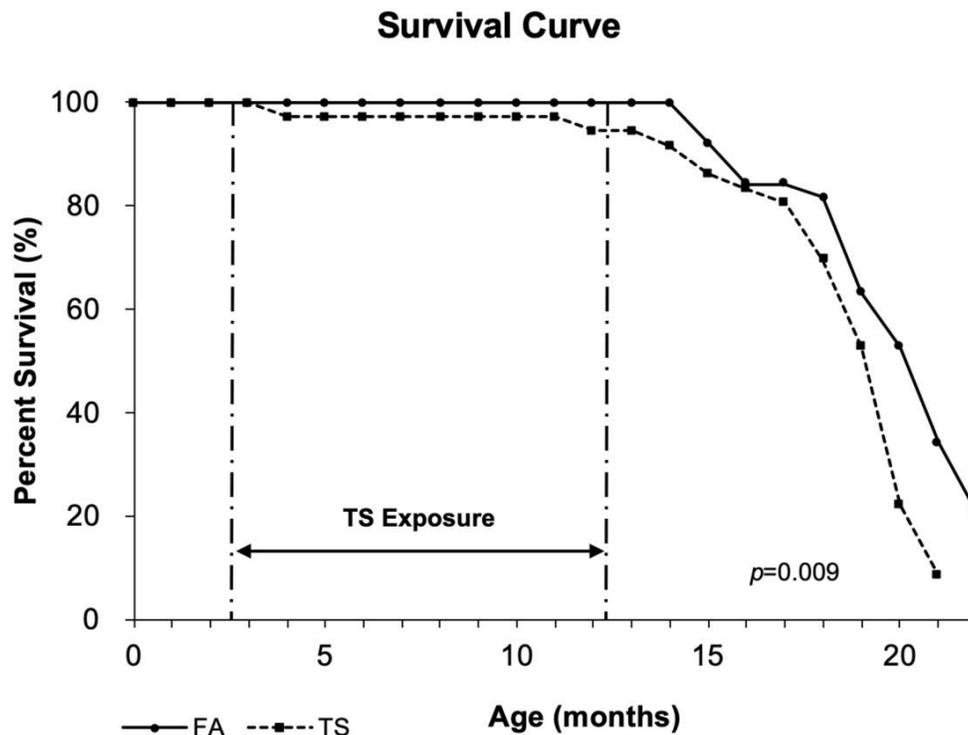
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