Data file for Title:

Sex differences in impacts of early gestational and peri-adolescent ozone exposure on lung development in rats: Implications for later life disease in humans

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IUGR O3x3-JD2018 study.

Figure 1: Timeline of key life events in rats and air pollutant exposures.

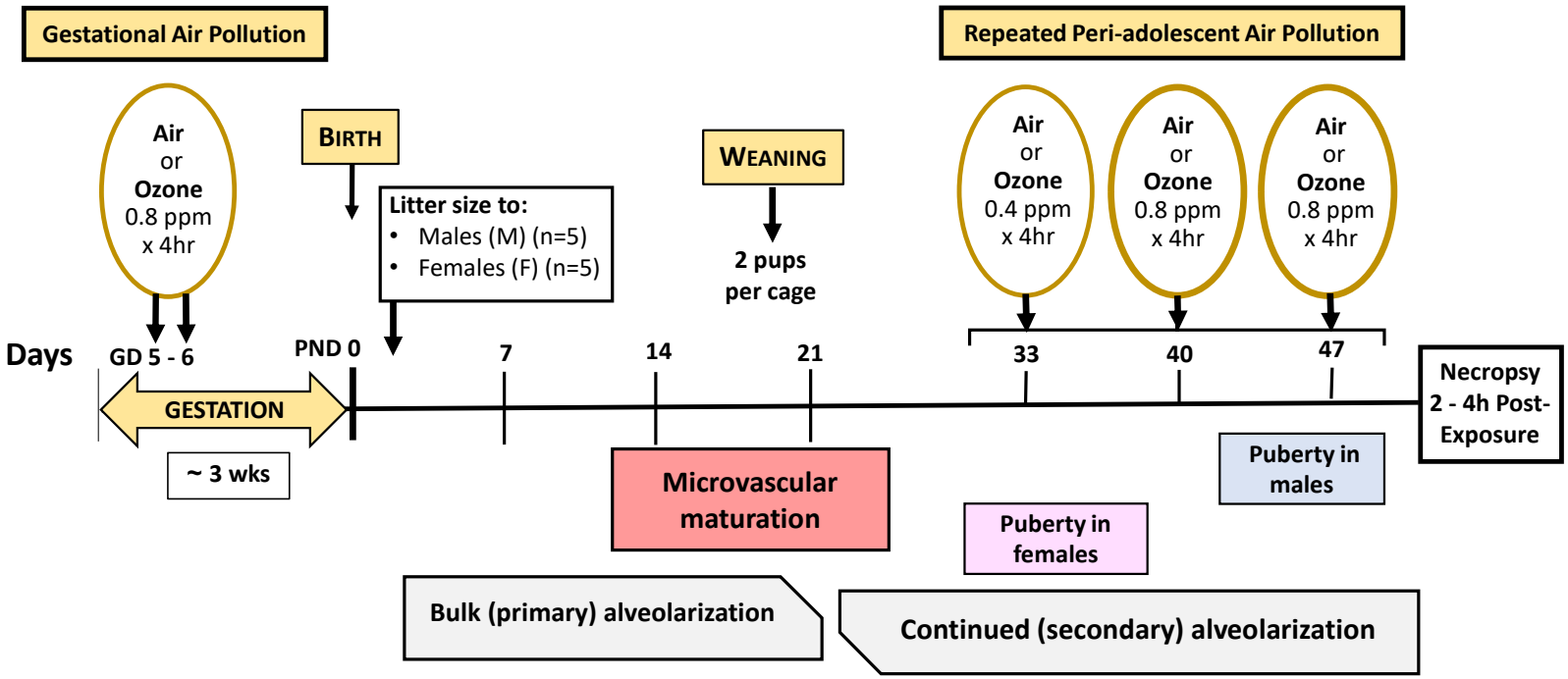


Fig. 2A-C

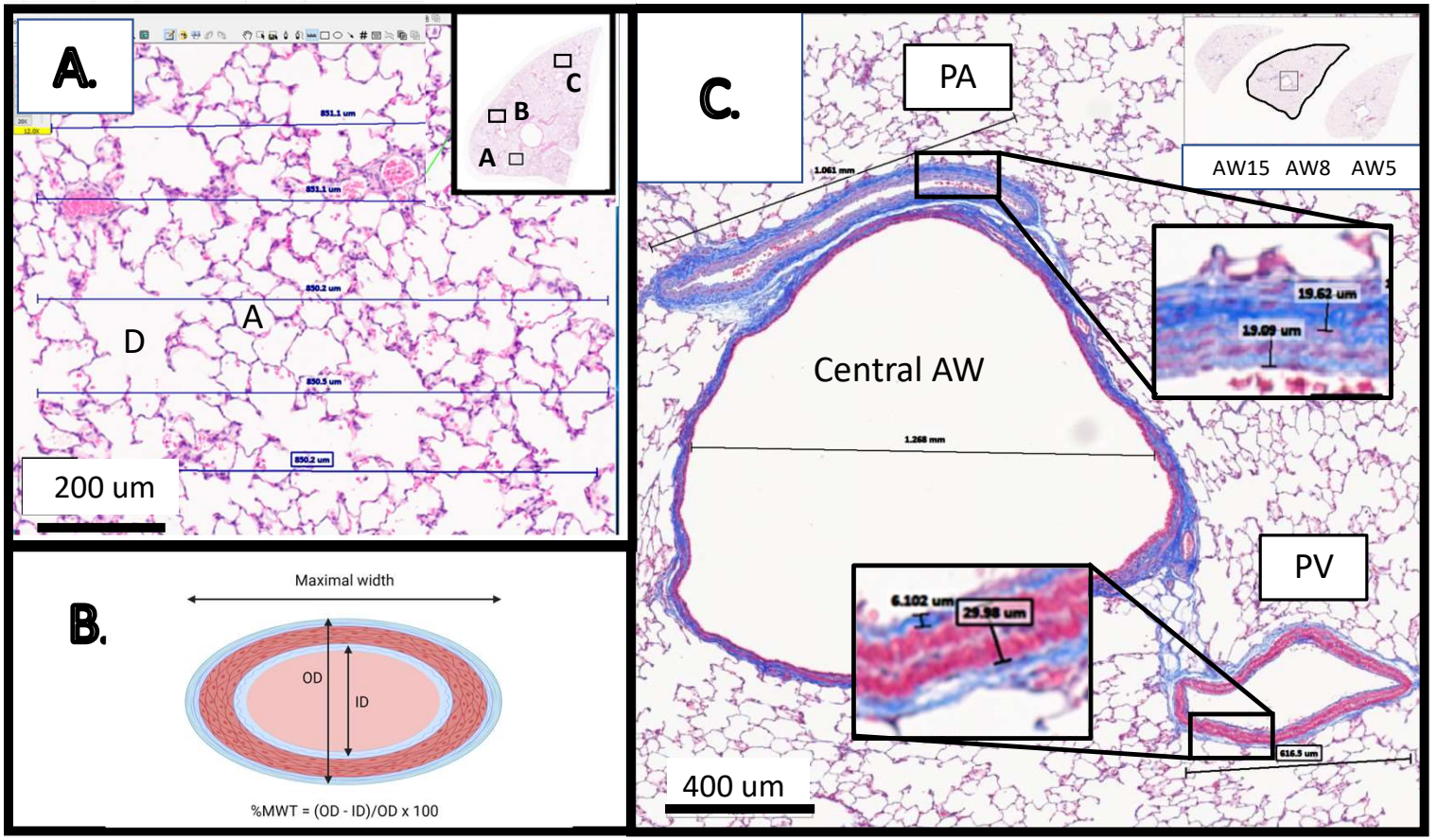


Figure 2A-C. For airspace morphometry, rectangular areas within dorsal (A), lateral (B), and ventral (C) regions were assessed. Within each rectangle, structures crossing guard lines were visually identified as alveolar (A) or ductal (D) space, and the number and chord length of the space is quantified (Fig. 2A). Schematic of the small and medium-sized vessel medial wall thickness (MWT%) based on inner diameter (ID) and outer diameter (OD) measurements (Fig. 2B). Schematic of large vessel length and wall thickness (insert) for the central airway (AW) and pulmonary artery (PA) and pulmonary vein (PV) (Fig. 2C).

Figure 3A-E. Corresponding offspring group means (± SEM) values for body weight (gm) in females (Fig. 3A, *n* = 9-12/group) and males (Fig. 3B, n = 10-12/group) prior to the 1st, 2nd, and 3rd peri-adolescent air or ozone exposure. Comparison of A:Ax3 to A:O_3x3 group-only body weights for both sexes (Fig. 3C). Comparison of final body length (cm) in males (Fig. 3D) and body mass index (kg/M^2) in females (Fig. 3E). Significant difference from the females of the same age (****$p \leq 0.0001$) and significant difference from M A:Ax3 males (λ $p \leq 0.05$).

3A. Body weight. Females

	F A:Ax3 Wk1	F A:Ax3 Wk2	F A:Ax3 Wk3	F O_3:Ax3 Wk1	F O_3:Ax3 Wk2	F O_3:Ax3 Wk3	F A:O_3x3 Wk1	F A:O_3x3 Wk2	F A:O_3x3 Wk3	F O_3:O_3x3 Wk1	F O_3:O_3x3 Wk2	F O_3:O_3x3 Wk3
Number of values	11	11	11	9	9	9	12	12	12	11	11	11
Mean	116.5	165.0	196.1	123.6	173.5	198.2	117.3	166.5	187.6	122.8	168.2	188.1
Std. Deviation	10.96	14.29	16.23	5.802	11.44	17.30	8.815	12.41	16.79	10.19	11.93	17.38
Std. Error of Mean	3.305	4.309	4.893	1.934	3.814	5.768	2.545	3.582	4.847	3.072	3.598	5.241

3A. Body weight. Males

	M A:Ax3 Wk1	M A:Ax3 Wk2	M A:Ax3 Wk3	M O_3:Ax3 Wk1	M O_3:Ax3 Wk2	M O_3:Ax3 Wk3	M A:O_3x3 Wk1	M A:O_3x3 Wk2	M A:O_3x3 Wk3	M O_3:O_3x3 Wk1	M O_3:O_3x3 Wk2	M O_3:O_3x3 Wk3
Number of values	12	12	12	10	10	10	12	12	12	11	11	11
Mean	133.0	207.5	260.0	129.0	216.0	275.9	131.1	199.3	253.2	138.8	213.1	265.5
Std. Deviation	11.83	15.63	21.99	13.26	14.96	27.35	10.47	17.68	20.13	13.79	18.76	23.96
Std. Error of Mean	3.415	4.512	6.349	4.194	4.730	8.648	3.024	5.103	5.812	4.157	5.656	7.225

Figure 3A-E. Corresponding offspring group means (± SEM) values for body weight (gm) in females (Fig. 3A, *n* = 9-12/group) and males (Fig. 3B, n = 10-12/group) prior to the 1st, 2nd, and 3rd peri-adolescent air or ozone exposure. Comparison of A:Ax3 to A:O_3x3 group-only body weights for both sexes (Fig. 3C). Comparison of final body length (cm) in males (Fig. 3D) and body mass index (kg/M^2) in females (Fig. 3E). Significant difference from the females of the same age (****$p \leq 0.0001$) and significant difference from M A:Ax3 males (λ $p \leq 0.05$).

3C. Body weight trend Females and Males (see 3A and 3B data)

3D. Body length. Males

	M A:Ax3 Wk3	M O_3:Ax3 Wk3	M A:O_3x3 Wk3	M O_3:O_3x3 Wk3
Number of values	12	10	12	11
Mean	15.34	16.03	15.35	15.75
Std. Deviation	0.7267	1.007	0.5962	0.6378
Std. Error of Mean	0.2098	0.3183	0.1721	0.1923

3E. BMI. Females

	F A:Ax3 Wk3	F O_3:Ax3 Wk3	F A:O_3x3 Wk3	F O_3:O_3x3 Wk3
Number of values	11	9	12	11
Mean	9.991	9.769	9.428	9.373
Std. Deviation	0.6999	0.5337	0.6310	0.9257
Std. Error of Mean	0.2110	0.1779	0.1821	0.2791

Figure 4A-C. Left lung lobe displacement volumes prorated to TLC are depicted, including volumes predicted by body weight and by body height-adjustment factors (Fig. 4A). Corresponding group means (± SEM) values of the pro-rated total lung displacement volume adjusted by height for females (Fig. 4B, *n*= 9-12) and males (Fig. 4C, *n* = 10-12). Significant difference from the corresponding control group (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

4A. Lung Volume comparison. Females

	F A:Ax3	TLC Vol	Weight	Height	F O_3:Ax3	TLC Vol	Weight	Height	F A:O3x3	TLC Vol	Weight	Height	F O3:O3x3	TLC Vol	Weight	Height
Number of values		10	10	10		9	9	9		12	12	12		11	11	11
Mean		5.625	5.514	5.620		5.028	5.550	5.683		5.271	5.258	5.637		4.955	5.265	5.659
Std. Deviation		0.5559	0.4695	0.1897		0.7336	0.4904	0.1562		0.9855	0.4786	0.2591		0.4977	0.4884	0.1529
Std. Error of Mean		0.1758	0.1485	0.0600		0.2445	0.1635	0.05207		0.2845	0.1381	0.07481		0.1501	0.1473	0.04611

4A. Lung Volume comparison. Males

	M A:Ax3	TLC Vol	Weight	Height	M O3:Ax3	TLC Vol	Weight	Height	M A:O3x3	TLC Vol	Weight	Height	M O_3:O_3x3	TLC Vol	Weight	Height
Number of values		11	11	11		10	10	10		12	12	12		11	11	11
Mean		5.955	7.358	5.932		6.025	7.725	6.193		6.042	7.110	5.931		6.273	7.435	6.080
Std. Deviation		0.9342	0.5845	0.2929		1.057	0.7660	0.3885		0.5724	0.5819	0.2309		0.7862	0.6681	0.2467
Std. Error of Mean		0.2817	0.1762	0.08831		0.3343	0.2422	0.1229		0.1652	0.1680	0.06667		0.2371	0.2014	0.07437

Figure 4A-C. Left lung lobe displacement volumes prorated to TLC are depicted, including volumes predicted by body weight and by body height-adjustment factors (Fig. 4A). Corresponding group means (± SEM) values of the pro-rated total lung displacement volume adjusted by height for females (Fig. 4B, *n*= 9-12) and males (Fig. 4C, *n* = 10-12). Significant difference from the corresponding control group (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

4B. Lung Volume / Body Height. Females

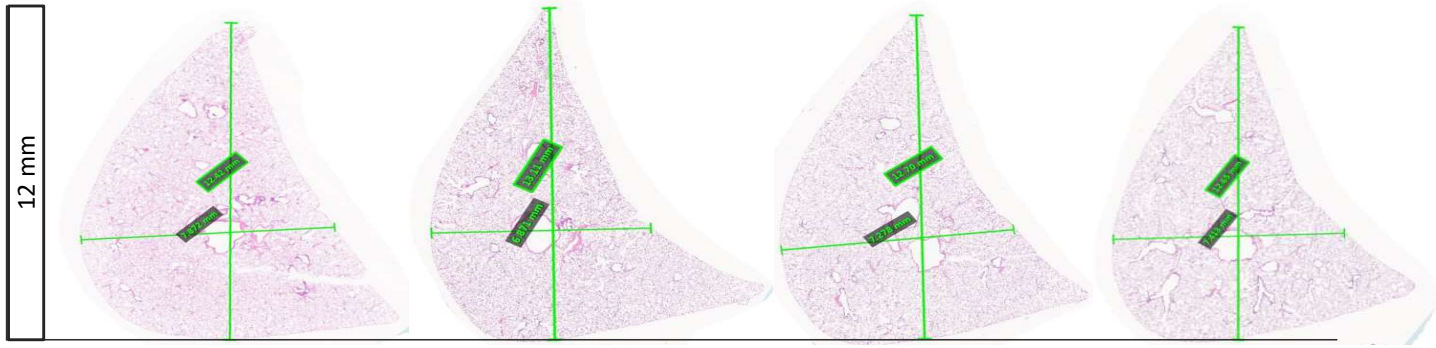
F Lung Vol/body HT	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	10	9	12	11
Mean	0.4010	0.3533	0.3742	0.3482
Std. Deviation	0.04654	0.05315	0.07103	0.03816
Std. Error of Mean	0.01472	0.01772	0.02050	0.01151

4B. Lung Volume / Body Height. Males

M Lung Vol/body HT	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	11	10	12	11
Mean	0.3873	0.3760	0.3933	0.3973
Std. Deviation	0.05985	0.07058	0.03962	0.04671
Std. Error of Mean	0.01804	0.02232	0.01144	0.01408

5A. Female Rats Representative AW8 sections

5A. Female Rats Representative AW8 Lung Sections



5B. Male Rats Representative AW8 sections

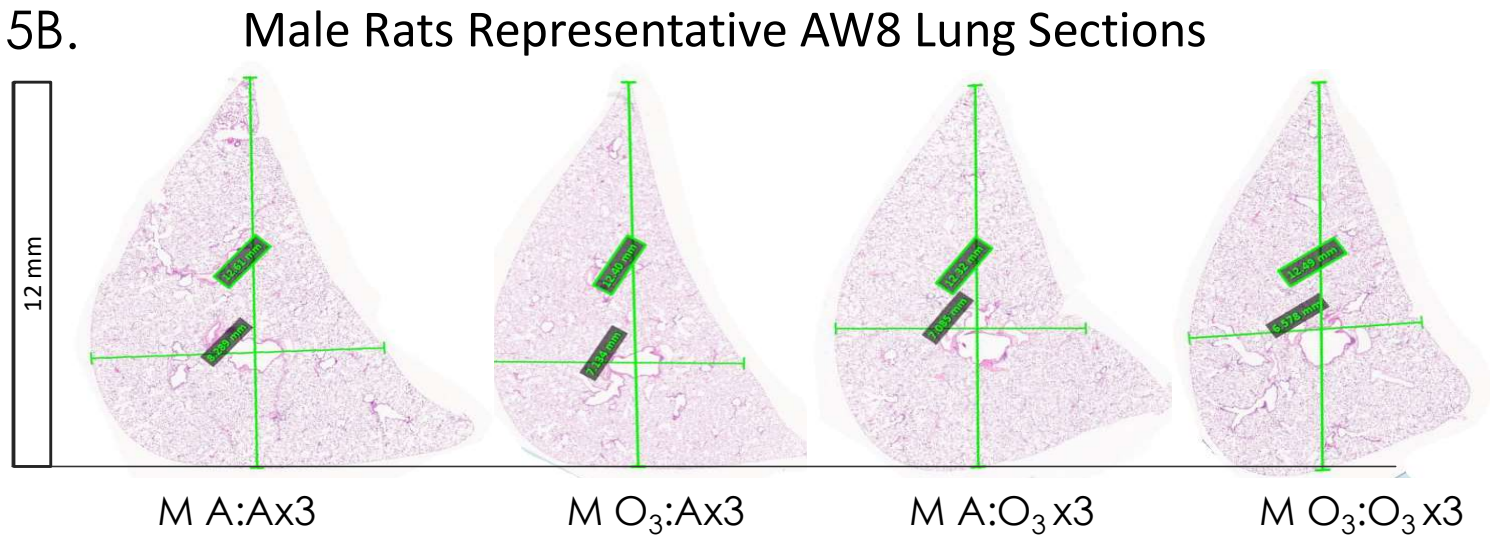


Figure 5A-C. Representative transverse AW8 lung sections are depicted for females (Fig. 5A) and males (Fig. 5B). Corresponding group mean (± SEM) values for the AW8 sectional areas (mm^2) are depicted for females (Fig. 5C, *n* = 7-8) and males (Fig. 5D, *n* = 7-9). Significant difference from the corresponding A:Ax3 group by sex (*$p \leq 0.05$).

5C. Lung (AW8) area (mm^2). Females

F Lung Area mm2	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	65.04	55.02	62.64	58.20
Std. Deviation	7.545	9.271	5.996	5.494
Std. Error of Mean	2.852	3.278	2.120	1.942

5D. Lung (AW8) area (mm^2). Males

M Lung Area mm2	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	9	7	7	8
Mean	66.27	62.66	63.95	65.47
Std. Deviation	6.759	9.018	3.212	8.329
Std. Error of Mean	2.253	3.408	1.214	2.945

Figure 6A-G. Representative photomicrographs (Fig. 6A, Black lines = 200 µm). 6B. Alveolar capillary dysplasia (ACD)-like changes are present with increased numerous of macrophages within airspaces, 6C. Mild bronchoalveolar ductal hyperplasia changes, 6D. Heterogeneous airspace size and thickened septal tips including an insert, and 6E. Cluster of thickened small vessels and thickened artery adjacent to small airway (insert). The mean (± SEM) of the modified mean linear intercept (AW8 lung section) for females (Fig. 6F, *n* = 7-8) and males (Fig. 6G, *n* = 7-9).

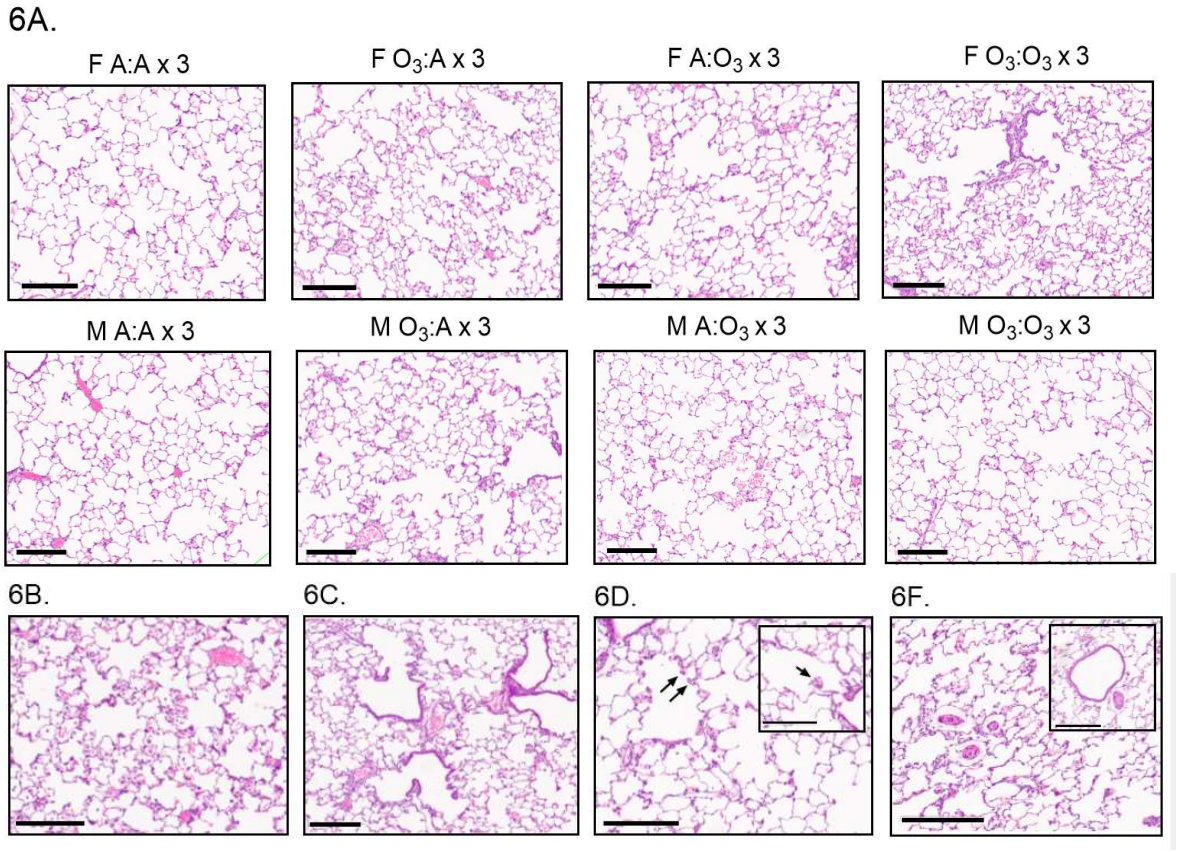


Figure 6A-G. Representative photomicrographs (Fig. 6A, Black lines = 200 µm). 6B. Alveolar capillary dysplasia (ACD)-like changes are present with increased numerous of macrophages within airspaces, 6C. Mild bronchoalveolar ductal hyperplasia changes, 6D. Heterogeneous airspace size and thickened septal tips including an insert, and 6E. Cluster of thickened small vessels and thickened artery adjacent to small airway (insert). The mean (± SEM) of the modified mean linear intercept (AW8 lung section) for females (Fig. 6F, *n* = 7-8) and males (Fig. 6G, *n* = 7-9).

6F. Modified MLI. Females

F Lung MLI-2	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	46.43	47.79	51.25	50.24
Std. Deviation	3.167	1.272	4.208	8.503
Std. Error of Mean	1.197	0.4498	1.488	3.006

6G. Modified MLI. Males

M Lung MLI-2	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	9	7	7	8
Mean	48.93	48.01	52.17	51.71
Std. Deviation	5.631	3.398	5.150	4.340
Std. Error of Mean	1.877	1.284	1.947	1.534

Figure 7A-D. Airspace morphometrics related to alveolar number estimated for the AW8 lung section in females (Fig. 7A, *n* = 7-8) and males (Fig.7B, *n* = 7-9); with corresponding alveolar area estimations in females (Fig. 7C, *n* = 7-8) and males (Fig. 7D, *n* = 7-9). Group means (± SEM) are depicted. Significant difference from the corresponding A:Ax3 group (*$p \leq 0.05$).

7A. Number Alveoli in AW8 lung section. Females

	F Lung # alv AW8	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values		7	8	8	8
Mean		7829	6287	6658	6468
Std. Deviation		1203	970.3	948.8	1326
Std. Error of Mean		454.8	343.0	335.4	469.0

7B. Number Alveoli in AW8 lung section. Males

	M Lung # alv AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values		9	7	7	8
Mean		7537	7284	6731	6792
Std. Deviation		1163	834.1	465.1	980.7
Std. Error of Mean		387.8	315.3	175.8	346.7

Figure 7A-D. Airspace morphometrics related to alveolar number estimated for the AW8 lung section in females (Fig. 7A, *n* = 7-8) and males (Fig.7B, *n* = 7-9); with corresponding alveolar area estimations in females (Fig. 7C, *n* = 7-8) and males (Fig. 7D, *n* = 7-9). Group means (± SEM) are depicted. Significant difference from the corresponding A:Ax3 group (*$p \leq 0.05$).

7C. Number Total alveolar area (μm^2) in AW8 lung section. Females

F Lung Tot alv area AW8	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	7.986e+006	6.324e+006	7.749e+006	6.759e+006
Std. Deviation	1.308e+006	1.725e+006	1.460e+006	1.112e+006
Std. Error of Mean	494344	609865	516273	393294

7D. Number Total alveolar area (μm^2) in AW8 lung section. Males

Number of values	M Lung Tot alv area AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
		9	7	7	8
Mean		8.707e+006	8.026e+006	7.803e+006	7.430e+006
Std. Deviation		1.933e+006	1.689e+006	1.067e+006	922512
Std. Error of Mean		644360	638569	403448	326157

Figure 8A-F. Airspace morphometrics related to ductal number in the AW8 lung section in females (Fig. 8A, *n* = 7-8) and males (Fig. 8B, *n* = 7-9); corresponding ductal areas in females (Fig. 8C, *n* = 7-8) and males (Fig. 8D, males, *n* = 7-9); and ratio of the ductal area to alveolar area in females (Fig. 8E, *n* = 7-8) and males (Fig. 8F, *n* = 7-9). Group mean (± SEM) values are depicted. Significant difference from the corresponding A:Ax3 group (**p* ≤ 0.05; ***p* ≤ 0.01).

8A. Number Ducts in AW8 lung section. Females

F Lung Duct # AW8	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	958.6	857.6	1012	953.3
Std. Deviation	247.1	208.1	85.51	194.0
Std. Error of Mean	93.40	73.59	30.23	68.58

8B. Number Ducts in AW8 lung section. Males

M Lung Duct # AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	9	7	7	8
Mean	1024	903.9	1017	1168
Std. Deviation	228.3	251.2	85.55	258.3
Std. Error of Mean	76.10	94.95	32.33	91.33

Figure 8A-F. Airspace morphometrics related to ductal number in the AW8 lung section in females (Fig. 8A, *n* = 7-8) and males (Fig. 8B, *n* = 7-9); corresponding ductal areas in females (Fig. 8C, *n* = 7-8) and males (Fig. 8D, males, *n* = 7-9); and ratio of the ductal area to alveolar area in females (Fig. 8E, *n* = 7-8) and males (Fig. 8F, *n* = 7-9). Group mean (± SEM) values are depicted. Significant difference from the corresponding A:Ax3 group (**p* ≤ 0.05; ***p* ≤ 0.01).

8C. Total ductal area (µm^2) in AW8 lung section. Females

F Lung Total Duct area AW8	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	7.956e+006	8.430e+006	1.063e+007	9.266e+006
Std. Deviation	2.519e+006	1.727e+006	2.191e+006	2.868e+006
Std. Error of Mean	952048	610445	774580	1.014e+006

8D. Total ductal area (µm^2) in AW8 lung section. Males

M Lung Total Duct area AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	9	7	7	8
Mean	9.117e+006	9.439e+006	1.135e+007	1.297e+007
Std. Deviation	3.276e+006	2.357e+006	2.588e+006	3.335e+006
Std. Error of Mean	1.092e+006	890926	978154	1.179e+006

Figure 8A-F. Airspace morphometrics related to ductal number in the AW8 lung section in females (Fig. 8A, *n* = 7-8) and males (Fig. 8B, *n* = 7-9); corresponding ductal areas in females (Fig. 8C, *n* = 7-8) and males (Fig. 8D, males, *n* = 7-9); and ratio of the ductal area to alveolar area in females (Fig. 8E, *n* = 7-8) and males (Fig. 8F, *n* = 7-9). Group mean (± SEM) values are depicted. Significant difference from the corresponding A:Ax3 group (**p* ≤ 0.05; ***p* ≤ 0.01).

8E. Ratio of the ductal area to alveolar area in AW8 lung section. Females

F Ratio Duct-to-Alv area AW8	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	7	8	8	8
Mean	1.021	1.410	1.426	1.383
Std. Deviation	0.3711	0.4013	0.4225	0.4110
Std. Error of Mean	0.1403	0.1419	0.1494	0.1453

8F. Ratio of the ductal area to alveolar area in AW8 lung section. Males

M Ratio Duct-to-Alv area AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	9	7	7	8
Mean	1.081	1.180	1.477	1.753
Std. Deviation	0.4166	0.2076	0.3859	0.4456
Std. Error of Mean	0.1389	0.07847	0.1459	0.1576

Figure 9A-D. Medial wall thickness (MWT%) of small-sized (<125 µm) vessels in females (Fig. 9A, *n* = 6/group) and males (Fig. 9B, *n* = 6/group); and medium-sized (>125 µm) vessels in females (Fig. 9C, n=6/group) and males (Fig. 9D, *n* = 6/group) from H&E-stained sections are depicted. Data are expressed as means ± SEM. Significant difference from the corresponding A:Ax3 group (*$p \leq 0.05$; **$p \leq 0.01$). Representative small- and medium-sized vessel H&E-stained images are provided for the F A:Ax3 and F O_3:Ax3 groups (Fig. 9E) and M A:Ax3 and M O_3:O_3x3 groups (Fig. 9F). Black lines = 200 µm.

9A. MWT% Small Pulmonary arteries. Females

F small Pul Art % MWT	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	6	6	6	6
Mean	32.08	49.18	33.38	42.25
Std. Deviation	3.556	10.93	5.567	7.335
Std. Error of Mean	1.452	4.463	2.273	2.995

9B. MWT% Small Pulmonary arteries. Males

M small Pul Art % MWT	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	6	6	6	6
Mean	39.12	41.73	39.52	51.00
Std. Deviation	4.428	7.532	8.250	6.829
Std. Error of Mean	1.808	3.075	3.368	2.788

Figure 9A-D. Medial wall thickness (MWT%) of small-sized (<125 µm) vessels in females (Fig. 9A, *n* = 6/group) and males (Fig. 9B, *n* = 6/group); and medium-sized (>125 µm) vessels in females (Fig. 9C, n=6/group) and males (Fig. 9D, *n* = 6/group) from H&E-stained sections are depicted. Data are expressed as means ± SEM. Significant difference from the corresponding A:Ax3 group (*$p \leq 0.05$; **$p \leq 0.01$). Representative small- and medium-sized vessel H&E-stained images are provided for the F A:Ax3 and F O_3:Ax3 groups (Fig. 9E) and M A:Ax3 and M O_3:O_3x3 groups (Fig. 9F). Black lines = 200 µm.

9C. MWT% Medium Pulmonary arteries. Females

F Medium Pul Art % MWT	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3
Number of values	6	6	6	6
Mean	31.60	45.72	32.15	40.65
Std. Deviation	4.858	11.47	5.912	6.934
Std. Error of Mean	1.983	4.683	2.414	2.831
Sum	189.6	274.3	192.9	243.9

9D. MWT% Medium Pulmonary arteries. Males

M Medium Pul Art % MWT	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	6	6	6	6
Mean	39.52	39.83	38.58	47.87
Std. Deviation	5.588	5.986	5.803	9.248
Std. Error of Mean	2.281	2.444	2.369	3.775
	237.1	239.0	231.5	287.2

Fig. 9E-F. Small and medium vessel MWT%.

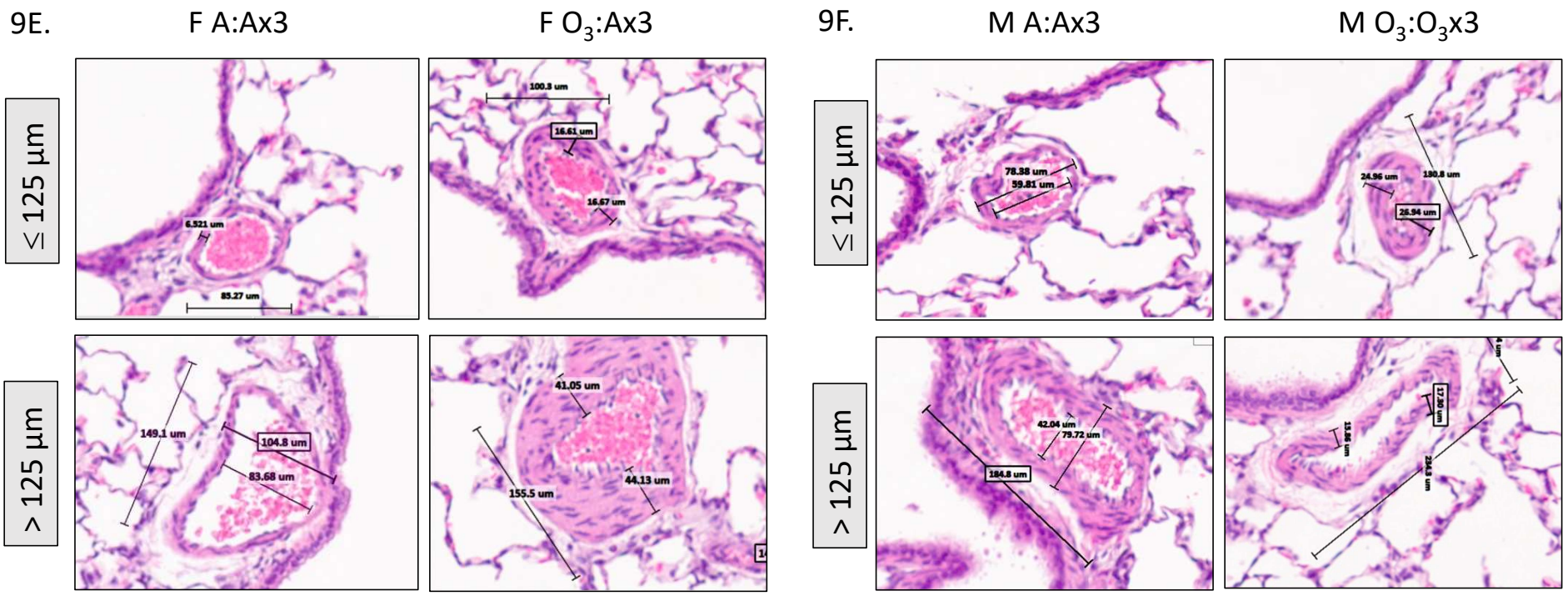


Figure 10A-H. Correlations for F A:Ax3 (white circle) vs. F O_3:Ax3 (pink circle) groups, with and without the F A:O_3x3 subject with visibly enlarged heart (larger pink checkered symbol) (Fig. 10A, 10C, 10E; n = 6/group). The Pearson r correlation (r) and correlation significance are provided within graphs. Significance of correlations inclusive of pup with an enlarged heart are provided within brackets. Correlations for M A:Ax3 (white square) vs. M O_3:O_3x3 (blue hatched square) groups are depicted (Fig. 10B, 10D, 10F; n = 6/group). For the significant correlations, lines are provided only for ease of visualizing the direction of change. Histologic images of the F O_3:Ax3 offspring with an enlarged heart revealed greatly enlarged large airspaces (Fig. 10G) and extensive medial hypertrophy of the pulmonary artery (Fig. 10H). Arrow shows poor tethering of a small airway (AW). Black line = 200 µm.

10A. Females # alv vs Duct size in AW8.

	Ductal Size um (AW8 slice) vs. # Alv in (AW8 slice)
Pearson r	
r	-0.6327
95% confidence interval	-0.8647 to -0.1782
R squared	0.4003
P value	
P (one-tailed)	0.0057
P value summary	**
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	15

10C. Females %MWT vs # alv in AW8.

	# Alv in (AW8 slice) vs. Small (<125) MWT%
Pearson r	
r	-0.6945
95% confidence interval	-0.9137 to -0.1623
R squared	0.4824
P value	
P (one-tailed)	0.0089
P value summary	**
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	11

10E. Females PA I+M vs Duct size in AW8.

	Ductal Size um (AW8 slice) vs. AW8 PA Medial Thickness
Pearson r	
r	0.5066
95% confidence interval	-0.09484 to 0.8371
R squared	0.2567
P value	
P (one-tailed)	0.0464
P value summary	*
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	12

Figure 10A-H. Correlations for F A:Ax3 (white circle) vs. F O_3:Ax3 (pink circle) groups, with and without the F A:O_3x3 subject with visibly enlarged heart (larger pink checkered symbol) (Fig. 10A, 10C, 10E; n = 6/group). The Pearson r correlation (r) and correlation significance are provided within graphs. Significance of correlations inclusive of pup with an enlarged heart are provided within brackets. Correlations for M A:Ax3 (white square) vs. M O_3:O_3x3 (blue hatched square) groups are depicted (Fig. 10B, 10D, 10F; n = 6/group). For the significant correlations, lines are provided only for ease of visualizing the direction of change. Histologic images of the F O_3:Ax3 offspring with an enlarged heart revealed greatly enlarged large airspaces (Fig. 10G) and extensive medial hypertrophy of the pulmonary artery (Fig. 10H). Arrow shows poor tethering of a small airway (AW). Black line = 200 µm.

10B. Males # alv vs Duct size in AW8.

	Ductal Size um (AW8 slice) vs. # Alv in (AW8 slice)
Pearson r	
r	-0.3892
95% confidence interval	-0.7327 to 0.1125
R squared	0.1514
P value	
P (one-tailed)	0.0613
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	17

10D. Males %MWT vs # alv in AW8.

	# Alv in (AW8 slice) vs. Small (<125) MWT%
Pearson r	
r	-0.3068
95% confidence interval	-0.7488 to 0.3242
R squared	0.09411
P value	
P (one-tailed)	0.1661
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	12

10F. Males PA I+M vs Duct size in AW8.

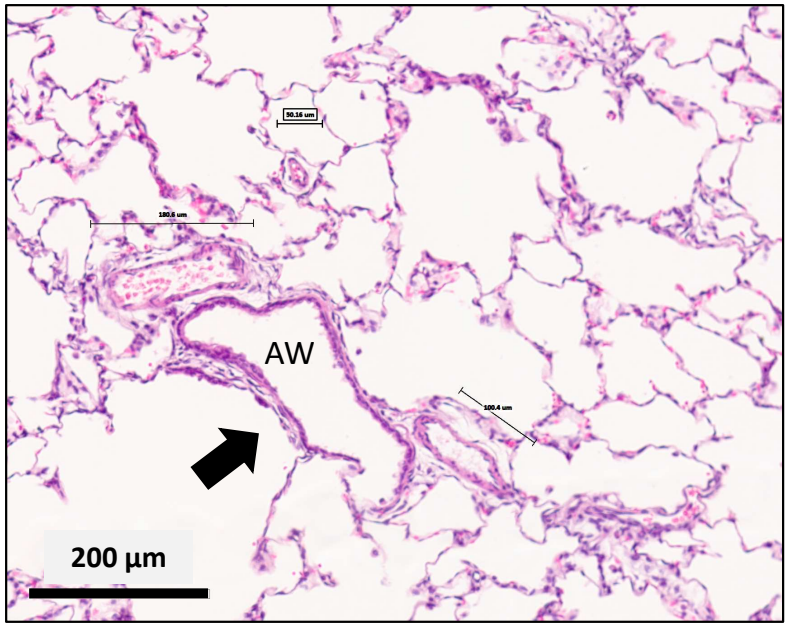
	Ductal Size um (AW8 slice) vs. AW8 PA Medial Thickness
Pearson r	
r	-0.3816
95% confidence interval	-0.7839 to 0.2462
R squared	0.1457
P value	
P (one-tailed)	0.1104
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	12

Final Fig. 10G

Fig. 10H.

Histologic images of the F O_3:Ax3 offspring with an enlarged heart revealed greatly enlarged large air-spaces (Fig. 10G) and extensive medial hypertrophy of the pulmonary artery (Fig. 10H). Arrow shows poor tethering of a small airway (AW). Black line = 200 µm.

G.



H.

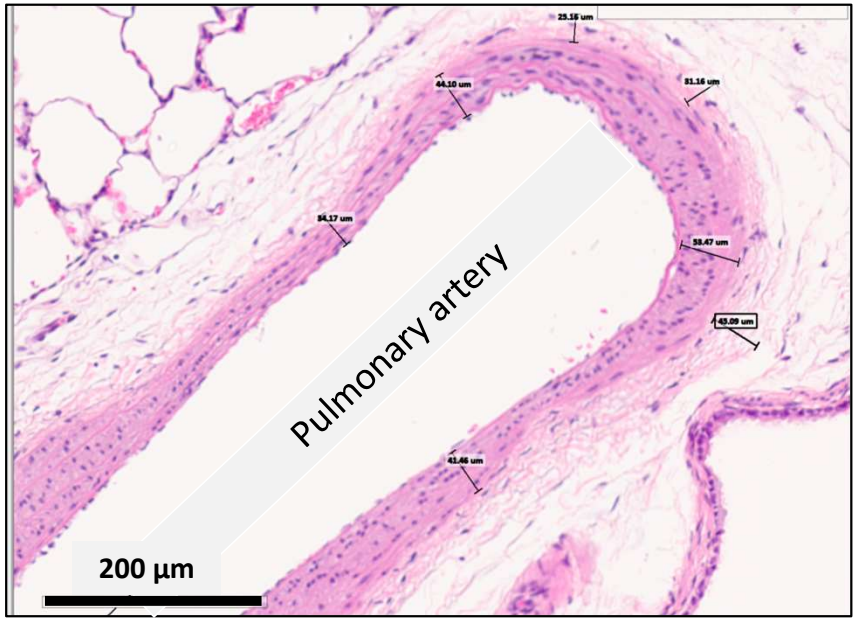


Figure 11A-F. Summary of the % space occupied by alveoli, as estimated by volume within the AW8 lung section (Fig. 11A, *n* = 7-8 females and *n* = 7-9 males). Lung protein content (normalized to lung mass) (Fig. 11B *n* = 9-12 females and *n* = 10-12 males). Data are expressed as means ± SEM. Simple plots of group mean values for % alveolar volume to lung protein content were assessed for females (Fig. 11C) and males (Fig. 11D). Correlations of % alveolar volume to lung protein content for F A:Ax3 vs. F O_3:Ax3 groups (Fig. 11E, *n* = 7-8) and M A:Ax3 vs. M O_3:O_3x3 groups (Fig. 11F, *n* = 7-9). The Pearson r correlation (r) and correlation significance are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

11A. % Alveolar space by Volume in AW8 section. Females and males

F % Alv by Vol AW*	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3	M % Alv by Vol AW8	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	7	8	8	8		9	7	7	8
Mean	27.16	19.48	20.23	20.53		27.49	21.96	18.99	16.00
Std. Deviation	6.992	6.447	6.240	5.956		9.397	4.607	5.503	4.070
Std. Error of Mean	2.643	2.279	2.206	2.106		3.132	1.741	2.080	1.439

11B. Lung protein content (µg/mg tissue). Females and males

F Lung TP	F A:Ax3	F O_3:Ax3	F A:O_3x3	F O_3:O_3x3	M Lung TP	M A:Ax3	M O_3:Ax3	M A:O_3x3	M O_3:O_3x3
Number of values	11	9	12	11		12	10	12	11
Mean	51.95	45.97	45.50	44.26		42.51	40.93	40.59	38.24
Std. Deviation	8.388	15.15	13.56	13.13		12.74	9.780	9.569	6.768
Std. Error of Mean	2.529	5.048	3.914	3.960		3.678	3.093	2.762	2.041

Figure 11A-F. Summary of the % space occupied by alveoli, as estimated by volume within the AW8 lung section (Fig. 11A, *n* = 7-8 females and *n* = 7-9 males). Lung protein content (normalized to lung mass) (Fig. 11B *n* = 9-12 females and *n* = 10-12 males). Data are expressed as means ± SEM. Simple plots of group mean values for % alveolar volume to lung protein content were assessed for females (Fig. 11C) and males (Fig. 11D). Correlations of % alveolar volume to lung protein content for F A:Ax3 vs. F O_3:Ax3 groups (Fig. 11E, *n* = 7-8) and M A:Ax3 vs. M O_3:O_3x3 groups (Fig. 11F, *n* = 7-9). The Pearson r correlation (r) and correlation significance are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

	11C. Female all groups – Lung Protein vs % Alv by Vol (AW8).	11D. Male all groups – Lung Protein vs % Alv by Vol (AW8).
	Group Mean % Alv by Vol vs. F Lung TP	Group Mean % Alv by Vol vs. M Lung TP
Pearson r		
r	0.9568	0.9083
95% confidence interval	-0.05283 to 0.9991	-0.4156 to 0.9981
R squared	0.9155	0.8250
P value		
P (one-tailed)	0.0216	0.0459
P value summary	*	*
Significant? (alpha = 0.05)	Yes	Yes
Number of XY Pairs	4	4

Figure 11A-F. Summary of the % space occupied by alveoli, as estimated by volume within the AW8 lung section (Fig. 11A, *n* = 7-8 females and *n* = 7-9 males). Lung protein content (normalized to lung mass) (Fig. 11B *n* = 9-12 females and *n* = 10-12 males). Data are expressed as means ± SEM. Simple plots of group mean values for % alveolar volume to lung protein content were assessed for females (Fig. 11C) and males (Fig. 11D). Correlations of % alveolar volume to lung protein content for F A:Ax3 vs. F O_3:Ax3 groups (Fig. 11E, *n* = 7-8) and M A:Ax3 vs. M O_3:O_3x3 groups (Fig. 11F, *n* = 7-9). The Pearson r correlation (r) and correlation significance are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

11E. Female 2 groups – Lung Protein vs % Alv by Vol (AW8).

	F Lung Protein (ug/mg tissue) vs. F % Alv by Vol in (AW8 slice)
Pearson r	
r	0.2112
95% confidence interval	-0.3376 to 0.6528
R squared	0.04460
P value	
P (one-tailed)	0.2249
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	15

11E. Male 2 groups – Lung Protein vs % Alv by Vol (AW8).

	M Lung Protein (ug/mg tissue) vs. M % Alv by Vol in (AW8 slice)
Pearson r	
r	0.6784
95% confidence interval	0.2934 to 0.8740
R squared	0.4602
P value	
P (one-tailed)	0.0014
P value summary	**
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	17

Figure. 12A-B. Using qRT-PCR, lung mRNA expression was assessed for female offspring (*n* = 7–11/ exposure group; Fig. 12A) from dams exposed to air (white bars) or ozone (pink bars) during gestation, and for male offspring (*n* = 8–11/group; Fig. 12B) from dams exposed to air (white bars) or ozone (blue bars) during gestation. Additional peri-adolescent ozone exposures are indicated by hatching of the bars. Genes included hypoxia inducible factor-1 alpha *(Hif-1α),* vascular endothelial growth factor *(Vegfa),* Vegf trans-membrane receptor 2 (*Vegfr2)*, Angiopoietin1 (*Angpt1),* Nitric oxide synthase 3 (*Nos3*), Platelet endothelial cell adhesion molecule-1 (*Pecam-1),* Dual-specificity phosphatase 1 (*Dusp1)* and Endothelin-1 *(Et-1).* Data are expressed as means ± SEM. Significant difference from the corresponding A:Ax3 groups (*$p \leq 0.05$; **$p \leq 0.01$, and ***$p \leq 0.001$).

12A. Lung gene expression. Females.

F Hif-1⍰	FAA	FOA	FAO	FOO	F Vegfa	FAA	FOA	FAO	FOO	F Flt (R1)	FAA	FOA	FAO	FOO	F Flk (R2)	FAA	FOA	FAO	FOO
Number of values	8	7	11	10		9	7	11	9		8	8	11	10		9	7	10	9
Mean	1.013	0.8561	1.103	0.6235		1.146	1.718	1.254	1.308		1.057	1.124	1.347	0.9872		1.110	1.528	1.251	1.392
Std. Deviation	0.1729	0.1051	0.3445	0.3998		0.5857	0.4807	0.4160	0.2751		0.3968	0.2476	0.4568	0.3649		0.4629	0.5119	0.2985	0.4361
Std. Error of Mean	0.06111	0.03973	0.1039	0.1264		0.1952	0.1817	0.1254	0.09171		0.1403	0.08753	0.1377	0.1154		0.1543	0.1935	0.09440	0.1454

F Angpt1	FAA	FOA	FAO	FOO	F Nos3	FAA	FOA	FAO	FOO	F Pecam1	FAA	FOA	FAO	FOO	F Dusp1	FAA	FOA	FAO	FOO	F Et-1	FAA	FOA	FAO	FOO
	8	8	11	10		7	8	11	10		8	8	11	10		8	8	11	10		9	7	11	10
	1.043	0.6175	0.7715	0.5319		0.9339	0.4800	0.7931	0.6435		1.025	0.6022	0.6830	0.4535		1.066	0.7490	0.8012	0.6871		1.122	1.166	1.244	1.071
	0.3306	0.2979	0.2028	0.3764		0.3398	0.4089	0.4437	0.4029		0.2511	0.2514	0.2156	0.3356		0.4025	0.1738	0.2043	0.1700		0.4643	0.3268	0.4932	0.2710
	0.1169	0.1053	0.06116	0.1190		0.1284	0.1446	0.1338	0.1274		0.08876	0.08887	0.06500	0.1061		0.1423	0.06145	0.06159	0.05377		0.1548	0.1235	0.1487	0.08569

Figure. 12A-B. Using qRT-PCR, lung mRNA expression was assessed for female offspring (*n* = 7–11/ exposure group; Fig. 12A) from dams exposed to air (white bars) or ozone (pink bars) during gestation, and for male offspring (*n* = 8–11/group; Fig. 12B) from dams exposed to air (white bars) or ozone (blue bars) during gestation. Additional peri-adolescent ozone exposures are indicated by hatching of the bars. Genes included hypoxia inducible factor-1 alpha *(Hif-1α),* vascular endothelial growth factor *(Vegfa),* Vegf trans-membrane receptor 2 (*Vegfr2)*, Angiopoietin1 (*Angpt1),* Nitric oxide synthase 3 (*Nos3*), Platelet endothelial cell adhesion molecule-1 (*Pecam-1),* Dual-specificity phosphatase 1 (*Dusp1)* and Endothelin-1 *(Et-1).* Data are expressed as means ± SEM. Significant difference from the corresponding A:Ax3 groups (*$p \leq 0.05$; **$p \leq 0.01$, and ***$p \leq 0.001$).

12B. Lung gene expression. Males.

	M Hif-1⍰	MAA	MOA	MAO	MOO	M Vegfa	MAA	MOA	MAO	MOO	M Flt (R1)	MAA	MOA	MAO	MOO	M Flk (R2)	MAA	MOA	MAO	MOO
Number of values		10	9	11	10		11	9	11	10		10	9	11	10		11	9	11	10
Mean		1.051	0.8024	0.9564	0.9063		1.026	1.204	0.7543	1.199		1.081	0.7758	0.8836	0.8567		1.029	1.259	0.7772	1.387
Std. Deviation		0.3304	0.4122	0.3709	0.3937		0.2467	0.4469	0.2681	0.4076		0.4316	0.2747	0.4216	0.2351		0.2550	0.3761	0.3199	0.4383
Std. Error of Mean		0.1045	0.1374	0.1118	0.1245		0.07437	0.1490	0.08084	0.1289		0.1365	0.09158	0.1271	0.07435		0.07689	0.1254	0.09646	0.1386

M Angpt1	MAA	MOA	MAO	MOO	M Nos3	MAA	MOA	MAO	MOO	M Pecam1	MAA	MOA	MAO	MOO	M Dusp1	MAA	MOA	MAO	MOO	M Endothelin1	MAA	MOA	MAO	MOO
	10	8	11	10		8	8	11	10		10	9	11	10		10	9	11	10		11	9	11	10
	1.057	0.6573	0.8169	0.8963		0.7031	0.3275	0.2488	0.3254		1.059	0.6408	0.6724	0.6595		1.017	0.9707	0.8074	0.9234		1.069	1.230	0.7416	1.017
	0.3659	0.2426	0.2638	0.2792		0.5362	0.2494	0.1067	0.1317		0.3973	0.4454	0.1729	0.2376		0.2010	0.2743	0.1465	0.2861		0.4031	0.4538	0.3100	0.3201
	0.1157	0.08575	0.07955	0.08830		0.1896	0.08819	0.03217	0.04165		0.1256	0.1485	0.05215	0.07514		0.06355	0.09144	0.04417	0.09047		0.1215	0.1513	0.09345	0.1012

Figure 13. Schematic of gene expression changes for significant dam or pup exposure effects with predicted health impacts in endothelial cells (EC) and vascular smooth muscle cells (VSMC). Arrows indicate direction of change.

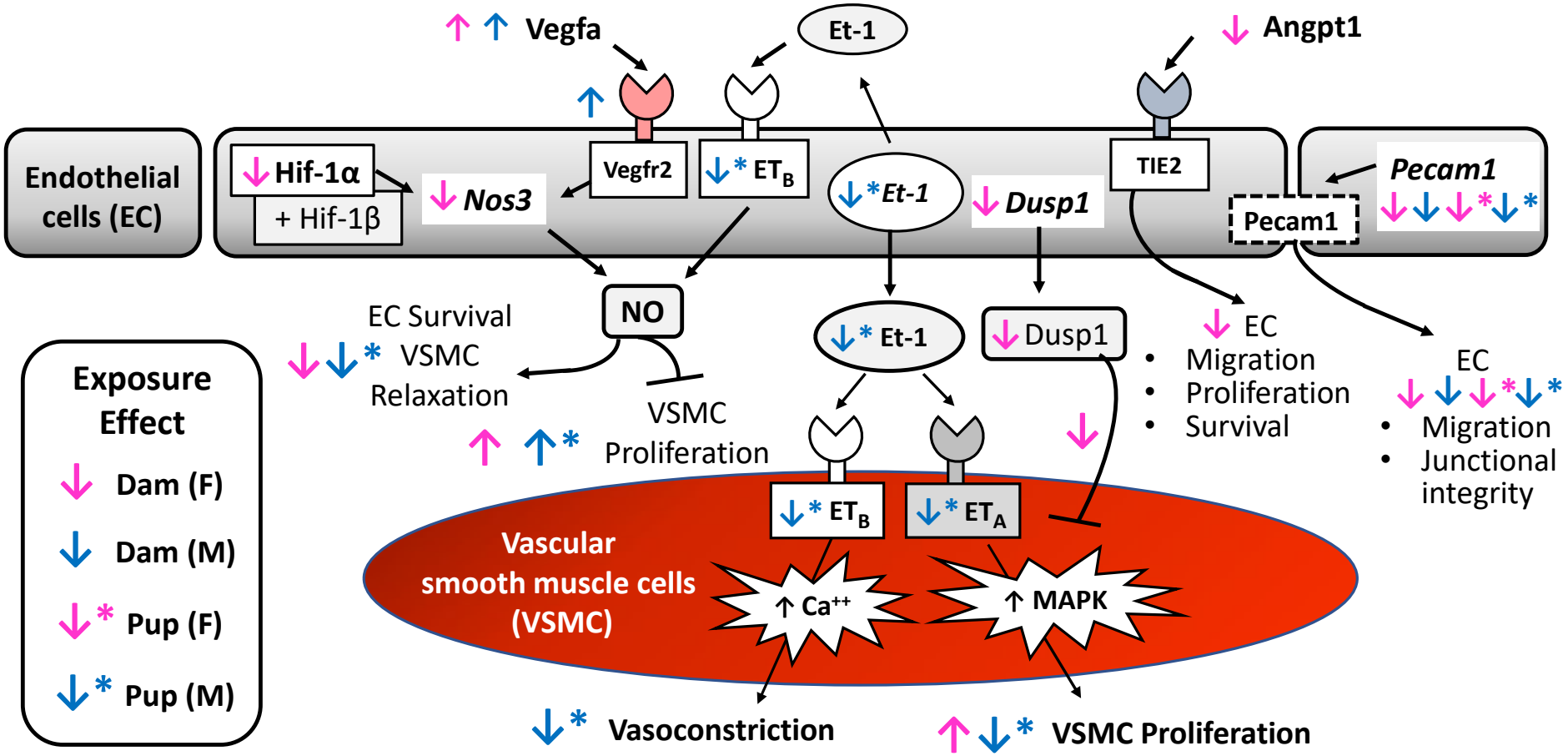


Table 1. qRT-PCR primer sequences designed for target genes.

Primer	Forward Sequence	Reverse Sequence
Rps15α	AGGTTGAACAAGTGTGGAGTTA	GAAACCAAACTGCCGTGATG
Angpt1	GACACCTTGAAGGAGGAGAAAG	GTTGTTGGTAGCTCTGCTAAGT
Dusp1	TGGTCTGCCCTCACAAATG	GCCTGCTCTGGGTCTATTTAC
Et-1	GAACATCTGTCCGGCTTCTAC	GGAACACCTCAACCTCTCTTG
Hif-1α	GAAGTTAGAGTCAAGCCCAGAG	CTCAGGTGAGCTTTGTCTAGTG
Nos3	TCCCAGCTGTGTCCAATATG	CCCTCATGCCAATCTCTGAA
Pecam-1	CCCAGTGACATTCACAGACA	ACCTTGACCCTCAGGATCTC
Vegfa	GCTCCTTCACTCCCTCAAATTA	GGTCTCTCTCTCTCTCTCTTC
Vegfr1	TACGTCACAGATGTGCCAAAC	GCAGTGCTCACCTCTAACGA
Vegfr2	GACGACCCATTGAGTCCAATTA	GTGAGGATGACCGTGTAGTTTC

Table 2. Morphometrics (in µm) of the large central airway (AW) and associated pulmonary artery (PA) and pulmonary vein (PV). Data are expressed as means ± SEM. * Indicates different than A:Ax3 group; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Groups	A:Ax3	O_3:Ax3	A:O_3x3	O_3:O_3x3	ANOVA
Females	**n = 6**	**n = 6**	**n = 6**	**n = 6**	**Exposure Effect**
AW5					
Central AW diameter	1535 ± 110	1840 ± 114	1700 ± 102	1670 ± 81	n.s.
PA length	1280 ± 100	1200 ± 60	1230 ± 81	1060 ± 62	n.s.
Intimal + Medial layer	24.1 ± 1.4	33.6 ± 1.1***	26.2 ± 1.4	28.3 ± 1.4	***Dam effect *Interaction
Adventitial layer	24.8 ± 1.9	25.9 ± 2.2	24.7 ± 1.3	21.2 ± 1.3	n.s.
PV length	1270 ± 120	1300 ± 114	1300 ± 115	1160 ± 146	n.s.
Muscular layer	50.4 ± 3.9	45.9 ± 4.9	42.5 ± 4.0	37.5 ± 2.4	*Pup Effect
AW8					
Central AW diameter	1180 ± 69	1310 ± 98	1160 ± 85	1220 ± 77	n.s.
PA length	812 ± 45	821 ± 110	950 ± 83	801 ± 58	n.s.
Intimal + Medial layer	23.5 ± 1.2	30.5 ± 0.21*	28.9 ± 2.6	27.1 ± 1.4	*Interaction
Adventitial layer	20.7 ± 2.1	25.5 ± 1.9	25.9 ± 0.95	22.2 ± 1.2	*Interaction
PV length	642 ± 120	773 ± 120	734 ± 96	650 ± 57	n.s.
Muscular layer	24.4 ± 5.6	26.0 ± 3.5	23.9 ± 4.1	15.0 ± 2.2	n.s.

Groups	A:Ax3	O_3:Ax3	A:O_3x3	O_3:O_3x3	ANOVA
Males	**n = 6**	**n = 6**	**n = 6**	**n = 6**	**Exposure Effect**
AW5					
Central AW diameter	1520 ± 100	1620 ± 47	1760 ± 110	1450 ± 84	n.s.
PA length	1220 ± 100	1280 ± 75	1270 ± 140	1280 ± 51	n.s.
Intimal + Medial layer	34.1 ± 1.9	30.8 ± 1.2	31.7 ± 1.5	31.8 ±	n.s.
Adventitial layer	28.6 ± 3.2	27.4 ± 1.8	28.6 ± 2.3	27.8 ± 0.98	n.s.
PV length	1260 ± 120	1380 ± 75	1560 ± 110	1240 ± 82	*Interaction
Muscular layer	57.5 ± 4.1	58.2 ± 4.0	60.5 ± 3.0	49.4 ± 2.8	n.s.
AW8					
Central AW diameter	1180 ± 75	1180 ± 120	1270 ± 56	1240 ± 84	n.s.
PA length	946 ± 51	920 ± 60	963 ± 130	1040 ± 110	n.s.
Intimal + Medial layer	32.8 ± 2.1	29.6 ± 0.74	30.5 ± 1.5	32.4 ± 0.89	n.s.
Adventitial layer	26.8 ± 1.1	25.7 ± 1.9	23.6 ± 1.3	27.7 ± 0.58	n.s.
PV length	726 ± 71	758 ± 70	957 ± 80	809 ± 25	*Pup Effect
Muscular layer	28.0 ± 4.1	26.2 ± 4.5	26.4 ± 3.3	20.7 ± 4.5	*Interaction

Supplemental figures.

Figure S1 A-H. Summary of correlations for vessel morphometric changes with vascular genes showing increased expression relative to air controls. Group mean values for females (circles) (Fig. S1 A, C, E, G) and males (boxes) (Fig. S1 B, D, F, H). The Pearson r correlation (r) and correlation significance (*p*) are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

	S1A. Female all groups %MWT vs *Vegfa*	S1B. Male all groups %MWT vs *Vegfa*	S1C. Female all groups %MWT vs *Vegfr2*	S1D. Male all groups %MWT vs *Vegfr2*
	Group Mean MWT% vs. F Vegfa	Group Mean MWT% vs. M Vegfa	Group Mean MWT% vs. F Vegfr2	Group Mean MWT% vs. M Vegfr2
Pearson r				
r	0.9263	0.6662	0.9636	0.8265
95% confidence interval	-0.3172 to 0.9985	-0.8197 to 0.9921	0.03383 to 0.9993	-0.6543 to 0.9962
R squared	0.8580	0.4439	0.9285	0.6832
P value				
P (one-tailed)	0.0369	0.1669	0.0182	0.0867
P value summary	*	ns	*	ns
Significant? (alpha = 0.05)	Yes	No	Yes	No
Number of XY Pairs	4	4	4	4

Supplemental figures.

Figure S1 A-H. Summary of correlations for vessel morphometric changes with vascular genes showing increased expression relative to air controls. Group mean values for females (circles) (Fig. S1 A, C, E, G) and males (boxes) (Fig. S1 B, D, F, H). The Pearson r correlation (r) and correlation significance (*p*) are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

	S1E. Female all groups PA Medial thick AW5 vs *Vegfa*	S1F. Male all groups PA Medial thick AW5 vs *Vegfa*	S1G. Female all groups PA Medial thick AW5 vs *Vegfr2*	S1H. Male all groups PA Medial thick AW5 vs *Vegfr2*
	Group Mean AW5 PA I+M Thickness vs. F Vegfa	Group Mean AW5 PA I+M Thickness vs. M Vegfa	Group Mean AW5 PA I+M Thickness vs. F Vegfr2	Group Mean AW5 PA I+M Thickness vs. M Vegfr2
Pearson r				
r	0.9829	-0.2070	0.9716	-0.2743
95% confidence interval	0.3929 to 0.9997	-0.9743 to 0.9414	0.1580 to 0.9994	-0.9777 to 0.9327
R squared	0.9660	0.04285	0.9439	0.07526
P value				
P (one-tailed)	0.0086	0.3965	0.0142	0.3628
P value summary	**	ns	*	ns
Significant? (alpha = 0.05)	Yes	No	Yes	No
Number of XY Pairs	4	4	4	4

Supplemental figures.

Figure S2 A-F. Summary of correlations for vessel morphometric changes with vascular genes showing decreased expression relative to air controls. Group mean values for females (circles, inclusive of the pup with an enlarged heart) (Fig. S2 A, C, E) and males (boxes) (Fig. S2 B, D, F). The Pearson r correlation (r) and correlation significance (*p*) are provided within graphs. Lines are provided for ease of visualizing the direction of significant correlation.

	S2A. Female all PA Medial thick AW5 vs *Angpt1*	S2B. Male all PA Medial thick AW5 vs *Angpt1*	S2C. Female all PA Medial thick AW5 vs *Pecam1*	S2D. Male all PA Medial thick AW5 vs *Pecam1*	S2E. Female all PA Medial thick AW5 vs *Dusp1*	S2F. Male all PA Medial thick AW5 vs *Dusp1*
Pearson r r	Group Mean AW5 PA I+M Thckness vs. F Angpt-1	Group Mean AW5 PA I+M Thickness vs. M Angpt-1	Group Mean AW5 PA I+M Thickness vs. F Pecam1	Group Mean AW5 PA I+M Thickness vs. M Pecam1	Group Mean AW5 PA I+M Thickness vs. F Dusp1	Group Mean AW5 PA I+M Thckness vs. M Dusp1
95% confidence interval R squared	-0.8947 -0.9931 to -0.05922 0.8006	0.9483 -0.1445 to 0.9989 0.8992	-0.8099 -0.9869 to 0.2536 0.6559	0.9600 -0.01382 to 0.9992 0.9216	-0.8186 -0.9876 to 0.2291 0.6702	0.4617 -0.8977 to 0.9855 0.2132
P value P (one-tailed)	0.0202	0.0259	0.0483	0.0200	0.0451	0.2691
P value summary Significant? (alpha = 0.05)	* Yes	* Yes	* Yes	* Yes	* Yes	ns No
Number of XY Pairs	5	4	5	4	5	4