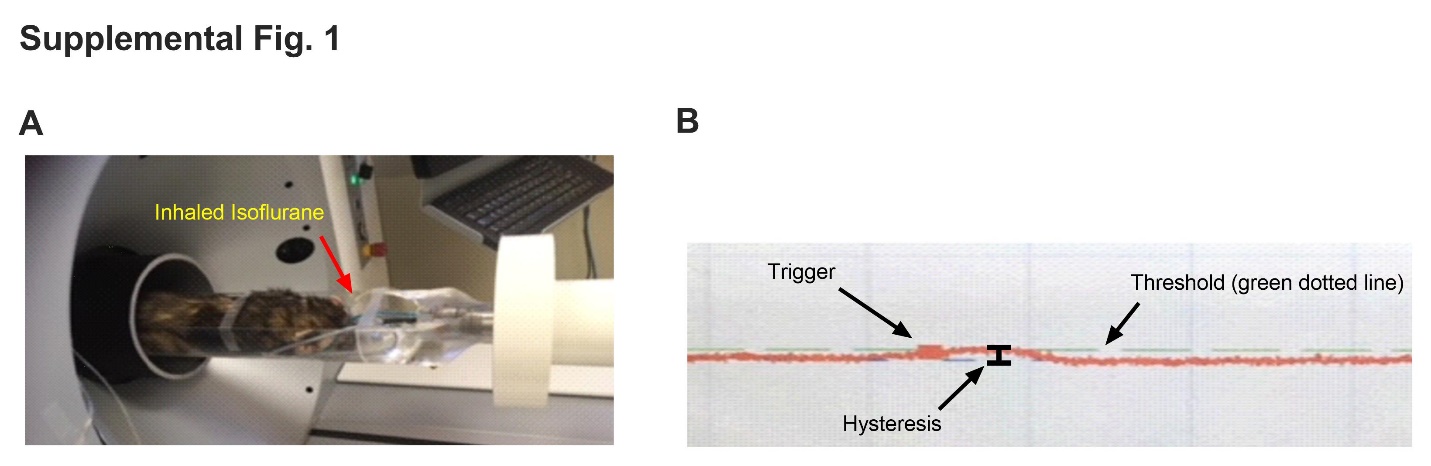
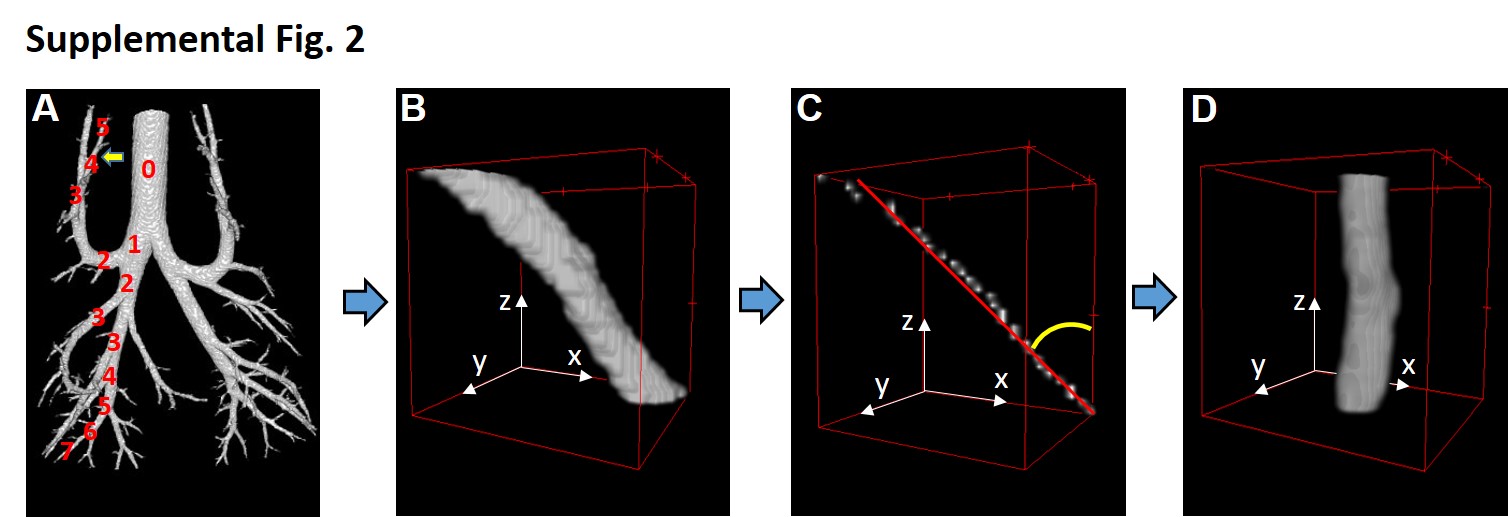
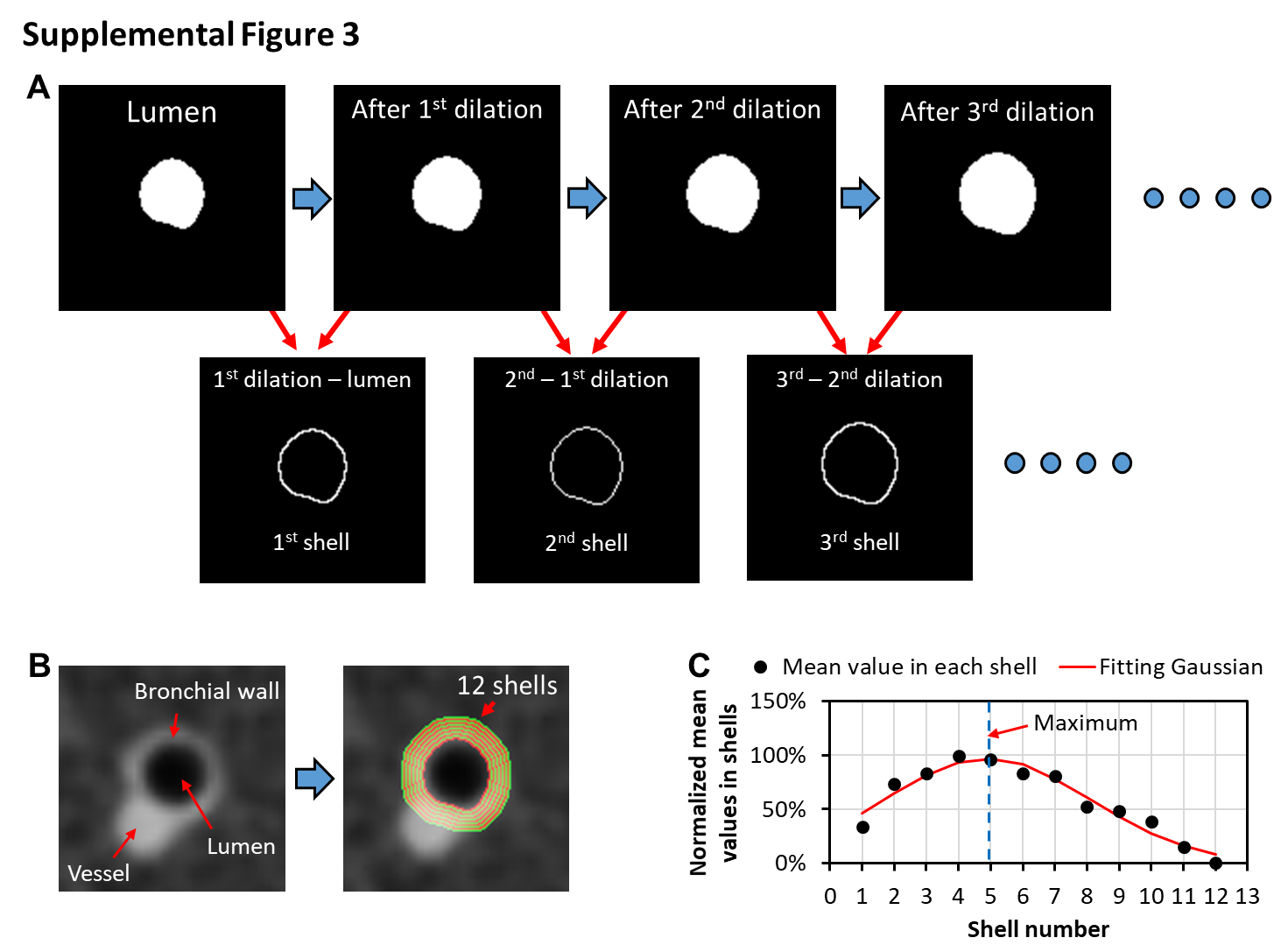
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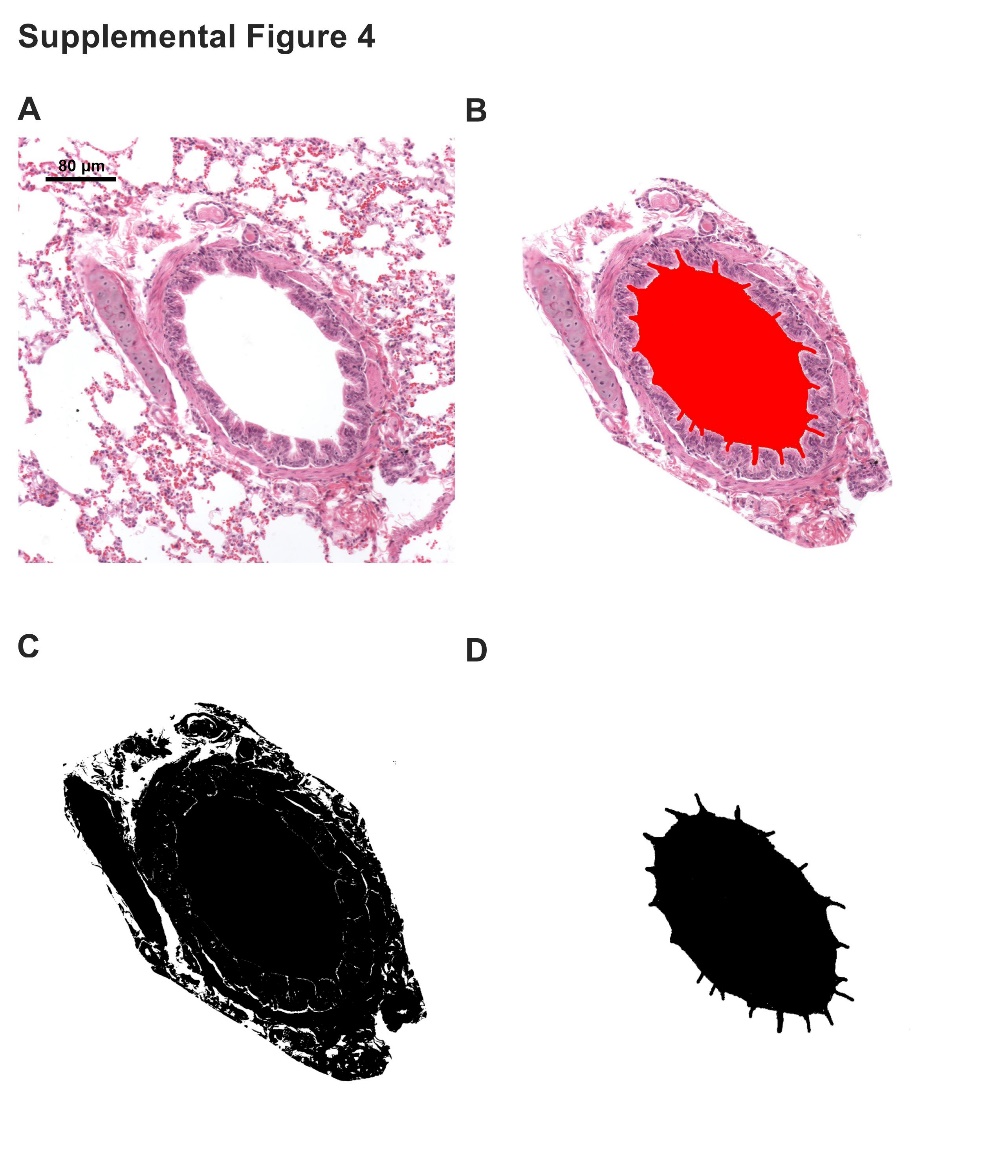
**Supplemental Figure 1. Milabs µCT Machine image with respiratory gating (A)** Representative image of ferret position on µCT bed. **(B)** Representative of respiratory gating illustrating waveform of respiration. Hysteresis is defined as the difference between baseline respiration (blue line) and peak inspiration (green line). Trigger occurs as denoted when baseline exceeds threshold value.



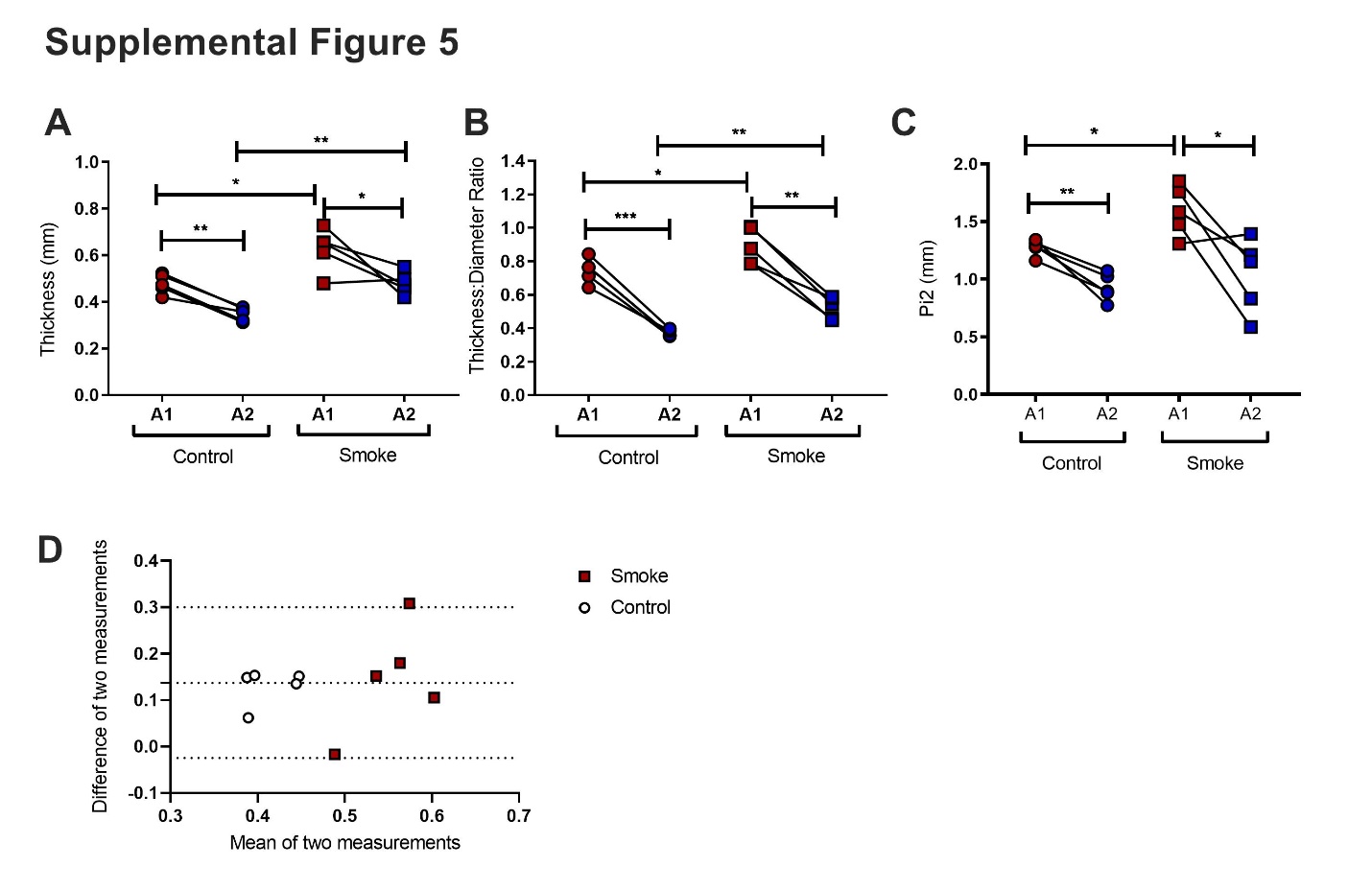
**Supplemental Figure 2. Airway selection and re-orientation. (A)** Segmented airway luminal region with generation numbers. **(B)** Close up view of the fourth-generation airway indicated with a yellow arrow in *A*. **(C)** The centroids of the lumen derived from each axial image slice (grey) plotted with the best fit line (red). The angle between the fit line and the vertical axis is indicated with a yellow arc. **(D)** The fifth-generation airway after re-orientation to bring the best fit line into plane with the vertical axis.



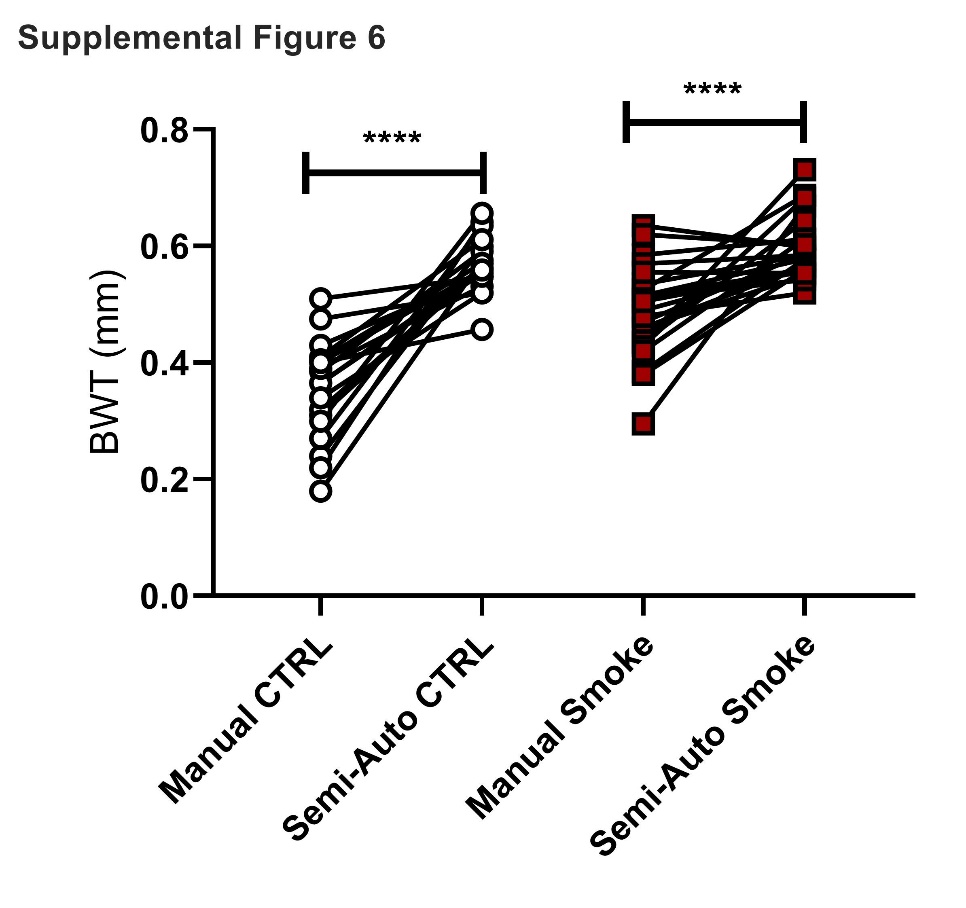
**Supplemental Figure 3. Semi-automated bronchial wall thickness measurement. (A)** Creation of iso-distance shells. The luminal region was dilated 12 times consecutively, and each shell region (40-μm thickness) was created by equation, *SRn = DLRn – DLRn-1*, where *SRn* and *DLRn* are the shell region and the dilated luminal region, respectively, at the nth iteration. **(B)** An axial CT image slice showing the lumen, bronchial wall and a vessel with/without 12 shell regions. **(C)** Mean pixel value in each shell region normalized to the maximum value with the best fitting Gaussian curve. The shell number when the fitting curve is maximal is indicated with a vertical dotted line, representing the midpoint of wall thickness. The total bronchial wall thickness (BWT) was calculated by shell number at Gaussian maximum x 2 x shell thickness (4.9 x 2 x shell thickness).



**Supplemental Figure 4. Histopathologic analysis. (A)** Representative H&E image of a ferret bronchus with submucosal gland and ciliated respiratory epithelium. **(B)** Image prepared for analysis by erasing the area external to the wall so as to leave airway adventitia, cartilage, mucus glands, and smooth muscle. Material in the lumen was erased and the lumen filled with solid red. **(C)** To measure the total area of the airway, the image was gray scaled and thresholded so as to form a representative black object. **(D)** Area of the lumen was determined by selecting the red object representing the lumen, then gray scaling, thresholding, and exporting the area in µm2 for analysis. The perimeter of the luminal object was also collected and exported.

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**Supplemental Figure 5. Between analyst differences of manual measurements of µCT-based airway metrics** Two analysts (A1 and A2, respectively) manually scored a sub-cohort of cigarette smoke exposed and control ferrets (Each point represents mean value of a single ferret derived from 6 airway measurements per ferret, n=10 total ferrets). **(A)** Bronchial wall thickness (BWT). **(B)** BWT/Luminal Diameter Ratio **(C)** Pi2. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 by Mann Whitney t-tests. **(D)** Bland Altman plot of bronchial wall thickness between two manual analysts. Mean and 95% confidence limits plotted as dotted lines.

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**Supplemental Figure 6. Manual vs. Semi-automated measurements of bronchial wall thickness.** Each point represents mean value of a single ferret derived from 6 manual airway measurements per ferret or multiple automated measurements, as in Figure 4. N=23 smoke, N=19 control. \*\*\*\*P<0.0001 by paired t-test.

**Supplemental Table 1. Ferret Demographics**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ferret Demographics** | | | |
|  | **Air Control**  **(N=14)** | **Smoke Exposed**  **(N=23)** | **P Value** |
| **Sex (Male, %)** | 5, 36% | 11, 48% | NS |
| **Weight (g)**  **mean ± SD** | Male: 1509 ± 160  Female: 748 ± 114 | Male: 1394 ± 201  Female: 651 ± 80 | NS  NS |

**Supplemental Table 2. Manual analysis of regional lung differences for bronchial wall thickness (BWT), luminal diameter (LD), and BWT:LD at 6 months.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | **Air control** | | **Smoke-exposed** | | |
|  | **Lung Region** | **Mean** | **SD** | **Mean** | **SD** | **P value** |
| **Bronchial Wall Thickness** | **Apical** | 0.354 | 0.085 | 0.487 | 0.080 | <0.001 |
| **Medial** | 0.483 | 0.083 | 0.607 | 0.148 | <0.001 |
| **Caudal** | 0.453 | 0.057 | 0.639 | 0.091 | <0.001 |
| **All** | 0.4301 | 0.093 | 0.578 | 0.127 | <0.001 |
| **Luminal Diameter** | **Apical** | 0.512 | 0.080 | 0.630 | 0.122 | <0.001 |
| **Medial** | 0.771 | 0.235 | 0.833 | 0.205 | 0.149 |
| **Caudal** | 0.611 | 0.088 | 0.728 | 0.137 | 0.006 |
| **All** | 0.631 | 0.184 | 0.730 | 0.177 | <0.001 |
| **BWT:LD Ratio** | **Apical** | 0.706 | 0.172 | 0.796 | 0.135 | 0.191 |
| **Medial** | 0.659 | 0.139 | 0.771 | 0.215 | 0.058 |
| **Caudal** | 0.765 | 0.125 | 0.909 | 0.203 | 0.015 |
| **All** | 0.710 | 0.151 | 0.825 | 0.194 | <0.001 |