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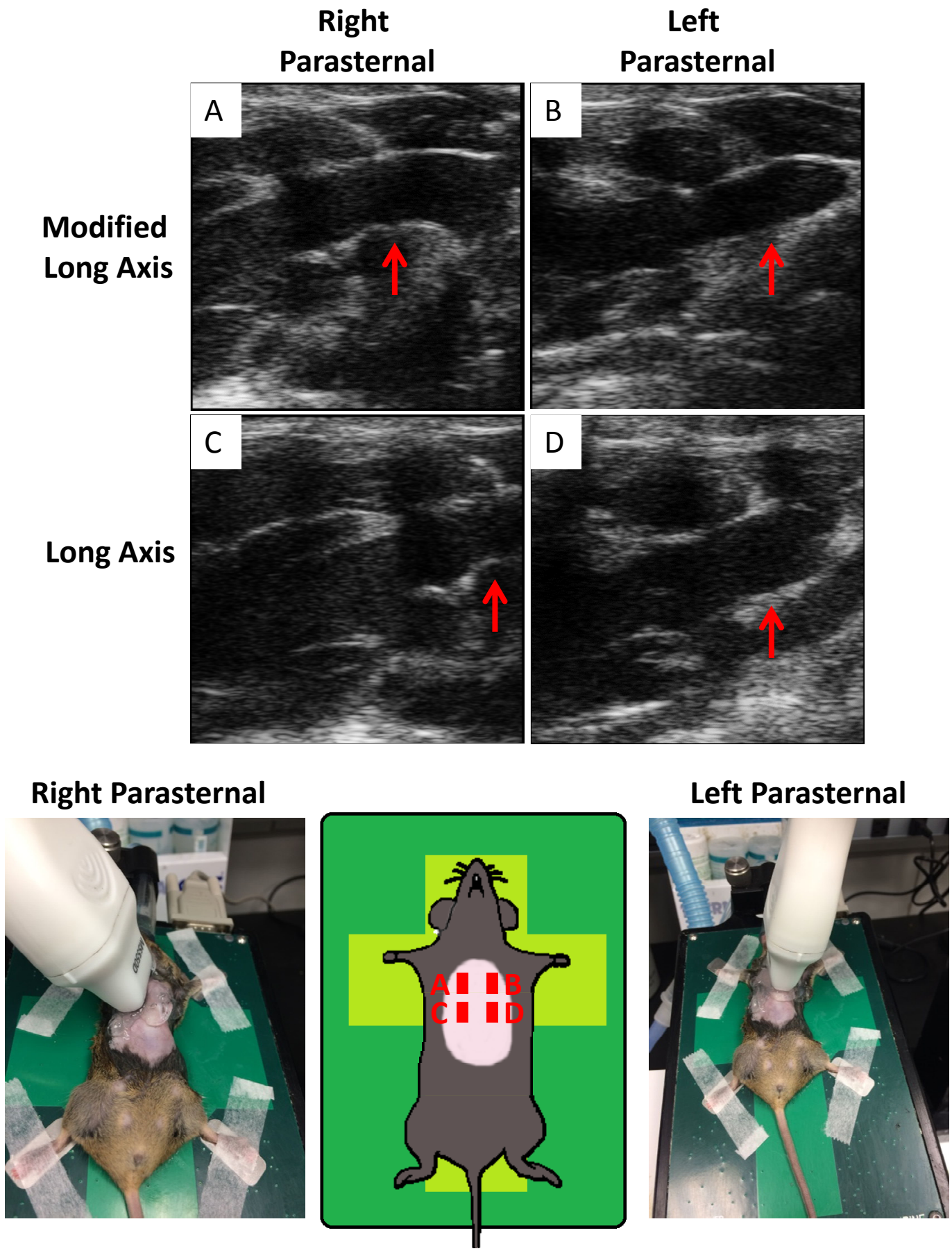
Supplementary File

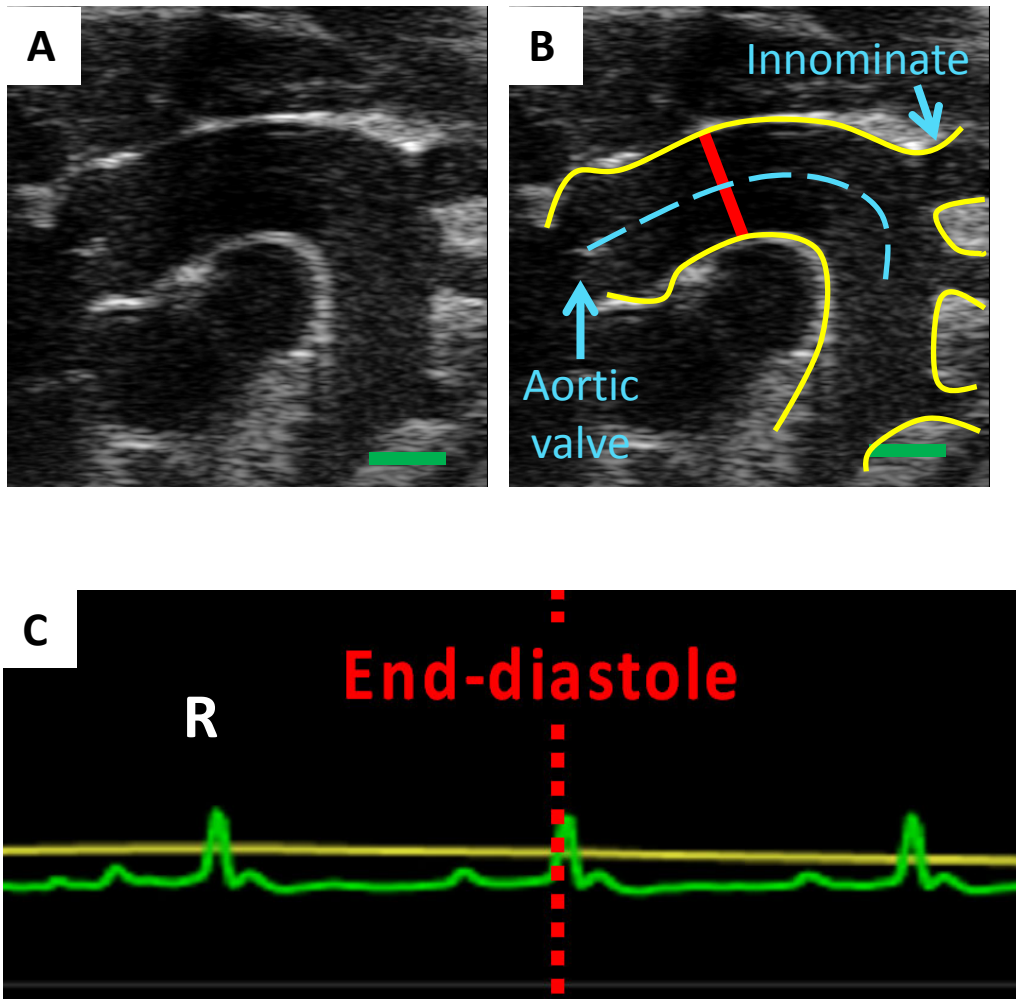
Aortic Strain Correlates With Elastin Fragmentation in Fibrillin-1 Hypomorphic Mice

Jeff Z. Chen, BSc; Hisashi Sawada, MD, PhD; Jessica J. Moorleghen, BSc; Mackenzie Weiland, BSc; Alan Daugherty, PhD; Mary B. Sheppard, MD

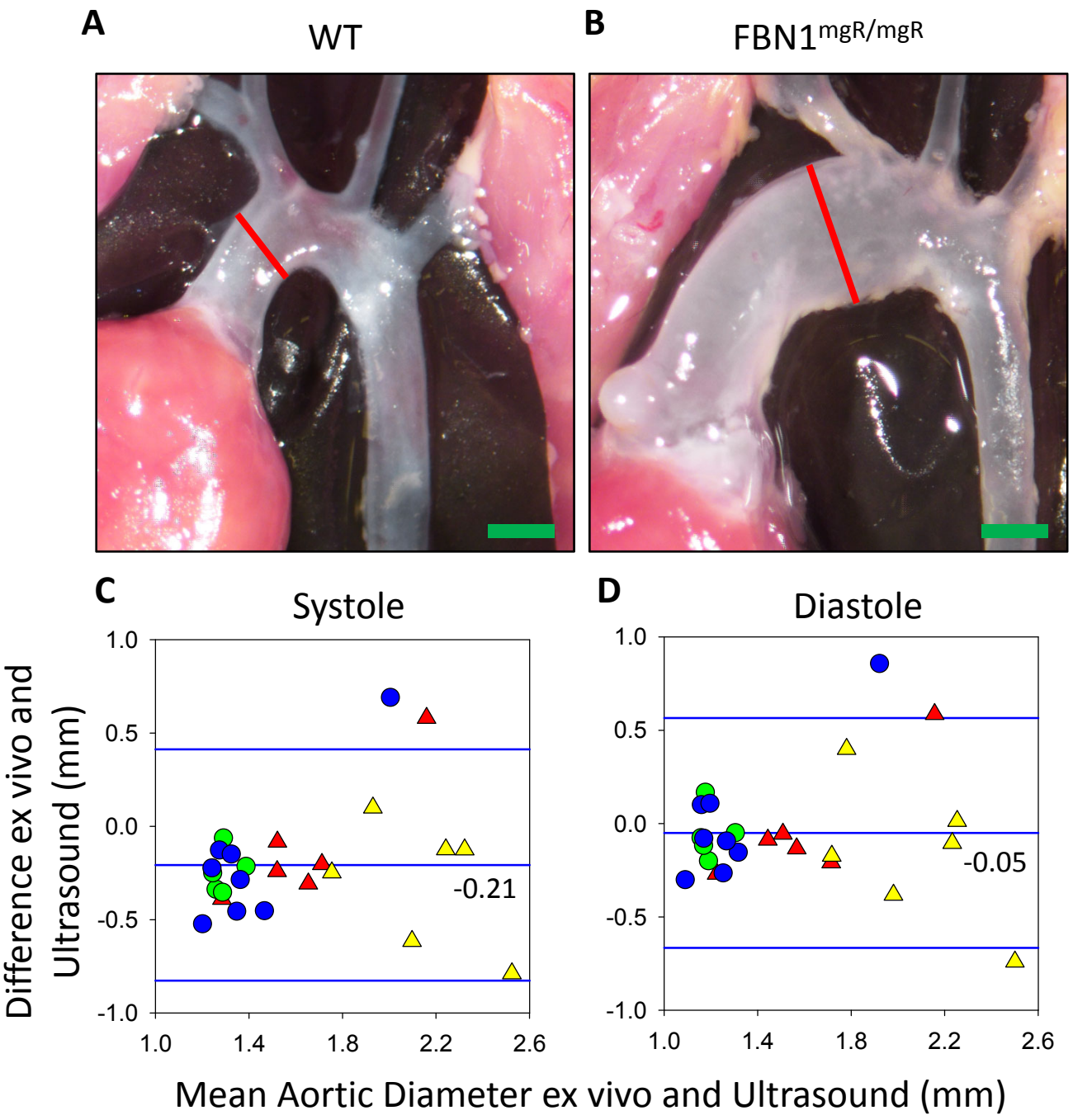
Supplementary Figure 1



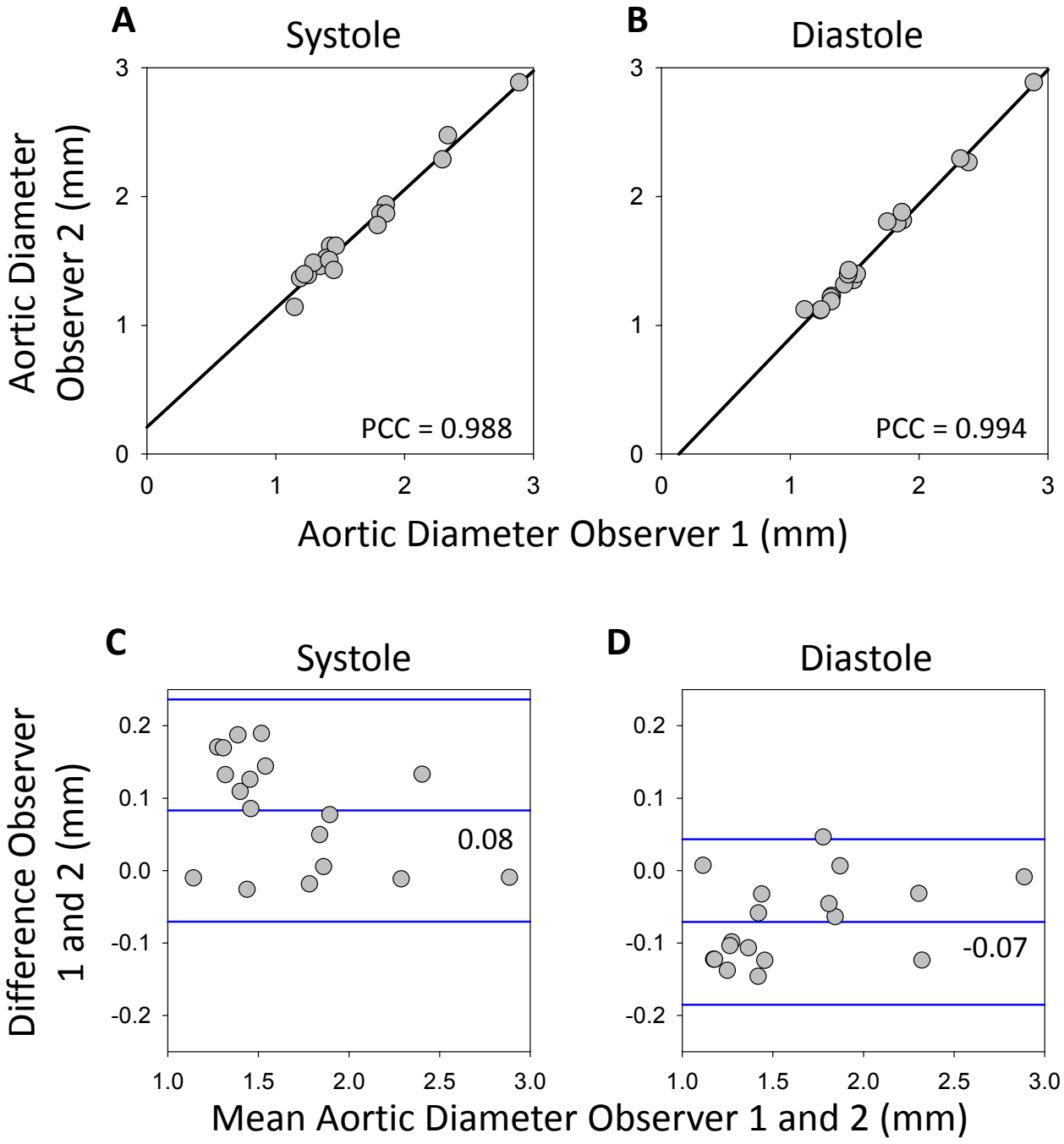
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 1. Probe positions used to measure aortic diameter. Aortas from a 12-week-old wild-type (WT) mouse was imaged in (**A**,**C**) right parasternal long axis and (**B**,**D**) left parasternal long axis views. Modified long axis views were taken from a location that was 1–2 ribs above the long axis view to optimize visualization of the ascending aorta. Red arrows, ascending aorta.

Supplementary Figure 2. Modified right parasternal long axis view and measurement protocol of the ascending aorta. (**A**) B-mode imaging was standardized to include visualization of 2 distinct anatomical landmarks in the same field: the aortic valves and the innominate artery. (**B**) Center line was defined as the midpoint between aortic walls (blue line). Measurements were taken inner-edge to inner-edge at the largest diameter (red line) perpendicular to the center line between the aortic root and innominate artery. Yellow lines depicting the aortic vessel wall were included for clarity in this diagram, but were not used for measurement purposes. Green bar, 1 mm. (**C**) Concurrent electrocardiogram monitoring allowed standardization of end-diastole (dashed red line 1 frame within the QRS complex) to facilitate imaging at a specific interval of the cardiac phase. Images at systole were taken at physiologic systole.

Supplementary Figure 3. Ultrasonographic measurements of aortic diameter in diastole closely reflected ex vivo aortic diameter measurements. (**A**,**B**) Representative imaging of in situ aortas from (**A**) wild-type (WT) and (**B**) *fibrillin-1* (*FBN1*)$^{mgR/mgR}$ mice in which measurements were recorded at the largest ascending aortic diameter. Red line, region measured; green line, 1 mm. (**C**,**D**) Correlation of ex vivo diameters more closely to ultrasonographic measurements in diastole than systole on Bland-Altman analysis. (Bias in systole, −0.21 mm; in diastole, −0.05 mm.) Measurements were less reliable at larger aortic diameters as shown by the divergence from bias in the Bland-Altman plot in ascending aortic diameters >1.8 mm. Center

blue line, bias; outer blue lines, 95% limits of agreement. WT: male, blue circles; female, green circles; *FBN1*$^{mgR/mgR}$: male, yellow triangles; female, red triangles.

Supplementary Figure 4. (**A**,**B**) Interobserver correlation in aortic diameter measurement on Bland-Altman analysis between 2 independent observers blinded to the experimental design in (**A**) systole (R^2=0.976) and (**B**) diastole (R^2=0.988; P<0.001). (**C**,**D**) Interobserver difference in ascending aorta B-mode imaging measurements by 2 independent, observers, blinded to the image identification, in (**C**) systole and (**D**) diastole on Bland-Altman analysis. Bias: systole, 0.08±0.08 mm; diastole, −0.07±0.06 mm. Limit of agreement: systole, 95%CI: −0.70 to 0.26 mm; diastole, 95%CI: −0.19 to 0.04 mm. n=aggregate measurements from 28 mice. Center blue line, bias; outer blue lines, 95% limits of agreement. PCC, pearson correlation coefficient.