<u>Supplemental Table I.</u> <u>Blood Chemistry and Body Mass.</u>
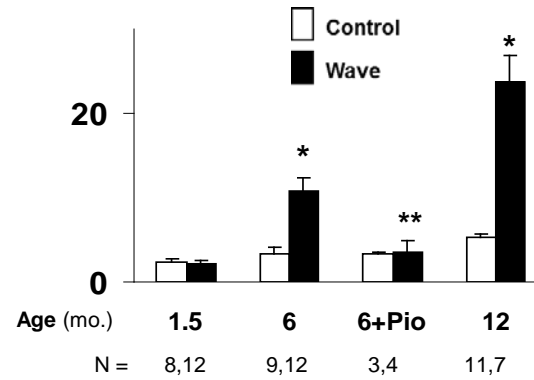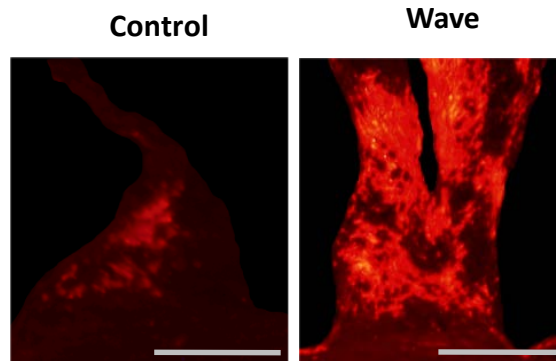
	Tchol (mg/dl)	Ca^{++} (mg/dl)	Phosphorus (mg/dl)	Body Mass(g)
1.5 month				
Control	73 ± 7	9.2 ± 0.1	7.7 ± 0.4	20 ± 1
Wave	73 ± 3	9.2 ± 0.2	7.3 ± 0.3	18 ± 1
6 month				
Control	55 ± 4	6.1 ± 0.1	9.5 ± 0.5	28 ± 1
Wave	66 ± 6	6.1 ± 0.1	9.9 ± 1.1	26 ± 1
6 month + pio				
Control	59 ± 8	6.4 ± 0.1	8.1 ± 0.2	33 ± 2
Wave	61 ±12	6.4 ± 0.1	9.1 ± 0.7	30 ± 2
12 month				
Control	56 ± 4	6.1 ± 0.1	NM	34 ± 2
Wave	73 ± 7	6.1 ± 0.1	NM	32 ± 1

N = 3 for 1.5 month-old, 9 or 10 for 6 month-old, 3 or 4 for Pio-treated, and 7 or 12

month-old Control and Wave mice, respectively. **Tchol** total cholesterol, **Ca^{++}** calcium,

NM not measured. p = NS for Wave vs. Control for all values.

Control **Wave** **Biglycan Immunofluorescence**

% Positive Staining

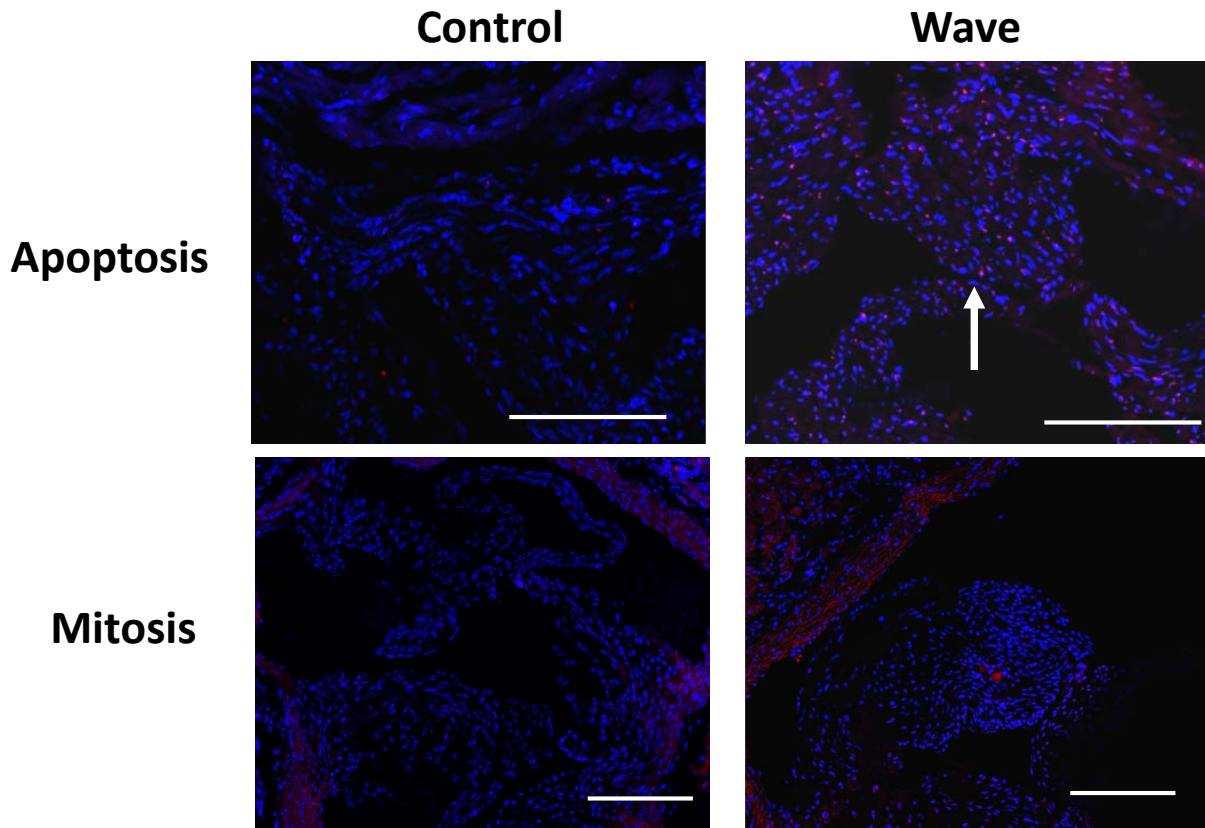|	Control	Control-Pio	Wave	Wave-Pio
N =	2	3	3	4

Supplemental Figure I. Biglycan in aortic valve. Histologic data were obtained at 6 months of age. Red stain = biglycan immunofluorescence; to-Pro Blue stain = nuclei. Scale bar = 100 μm. *p < 0.05 *vs*. Control.
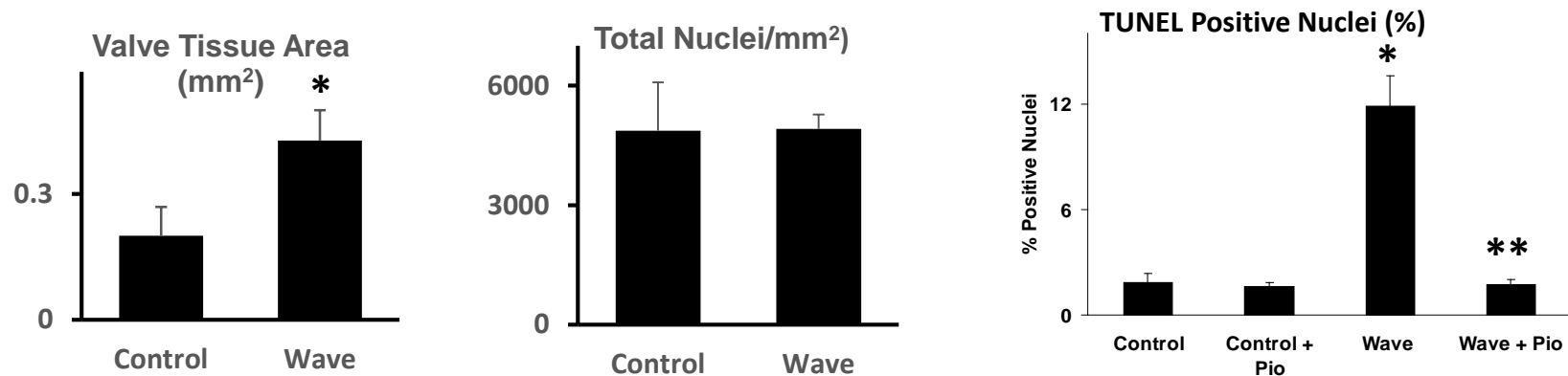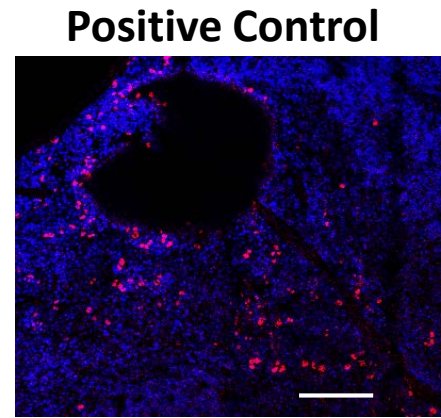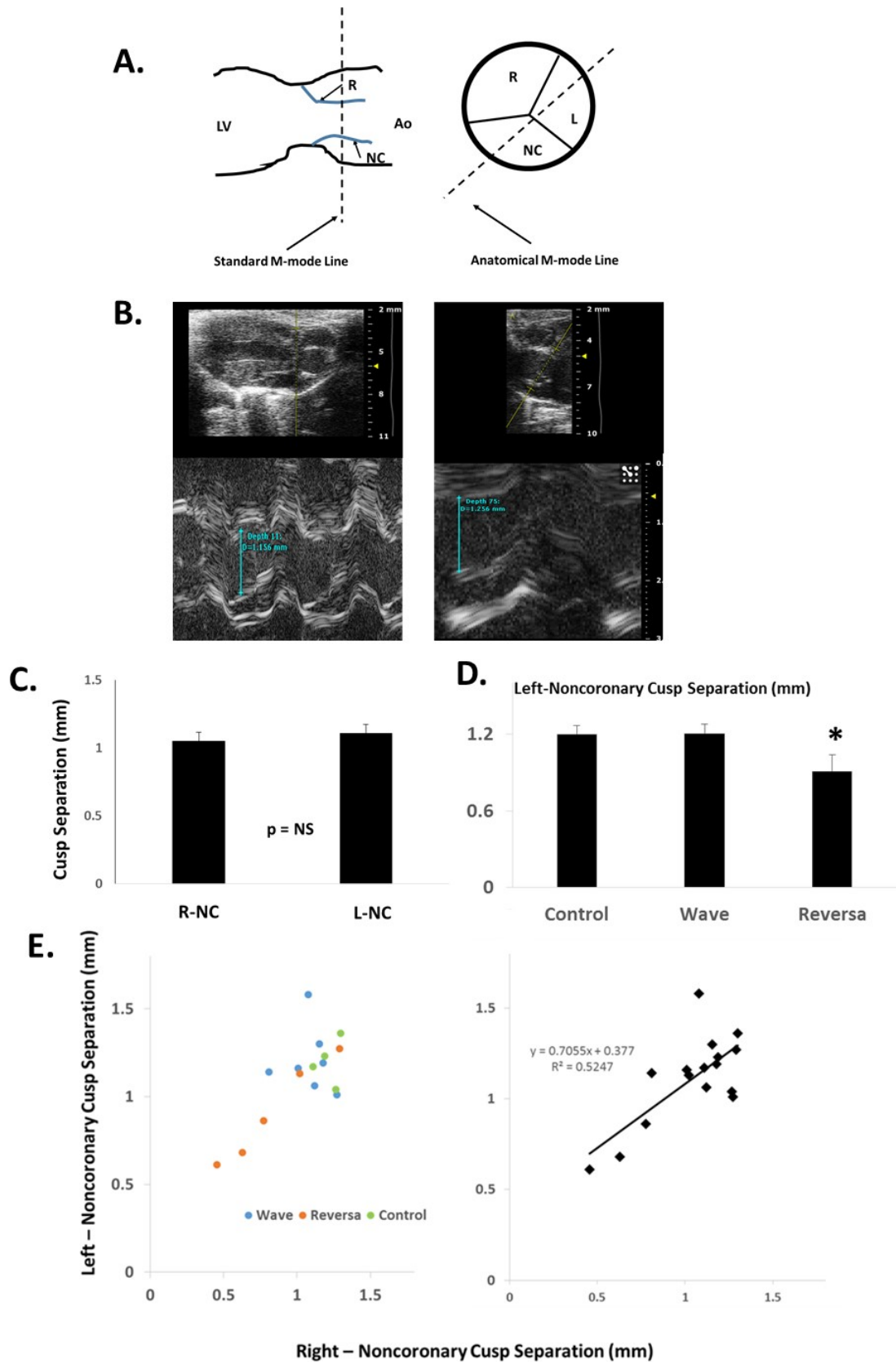
Supplemental Figure II. Immunostaining for the pro-apoptotic marker activated caspase-3 in the aortic valve. *p < 0.05 vs. age-matched Control; **p < 0.025 vs. 6 mo Wave. Scale bar = 100 µm.
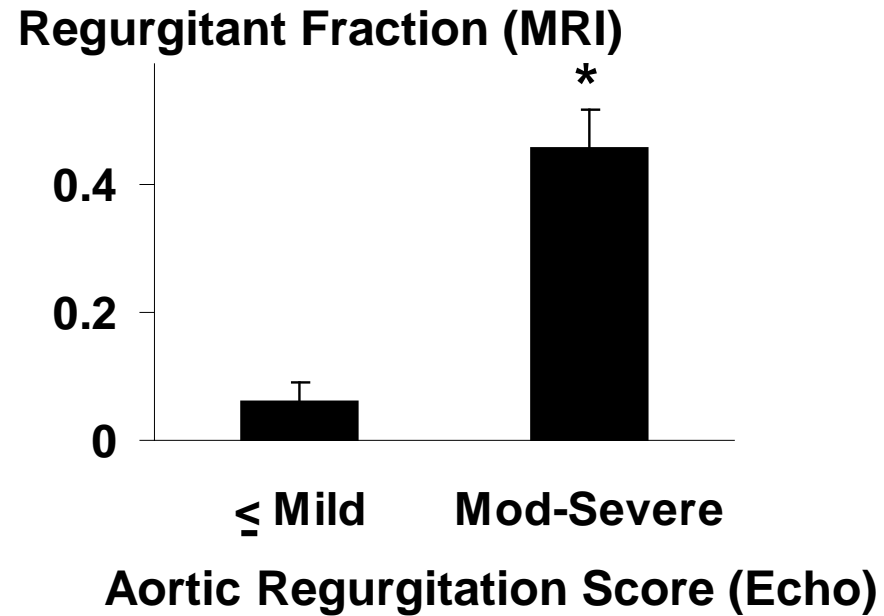
Supplemental Figure III. <u>Valve cell turnover</u>. Total valve tissue area, summed over three histologic slides from analogous anatomic locations in each of 4 mice per group, was about two-fold higher in Wave vs. Control. Cell density was not different between Wave and Control. Apoptosis (TUNEL, arrow, red staining) was significantly increased in Wave vs. Control. Mitosis (immunostaining for pHH3, red) was present in < 0.1% of valve cells in all samples analyzed (p = NS, group data not shown). Mitosis was abundant in the positive control sample from spleen. Data are shown for mice at 6 months of age. *p < 0.05 vs. Control. **p < 0.025 vs. Wave. Scale bar = 100 μm.
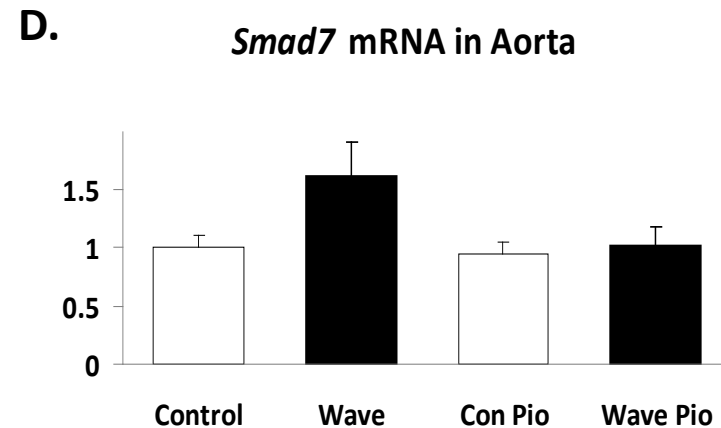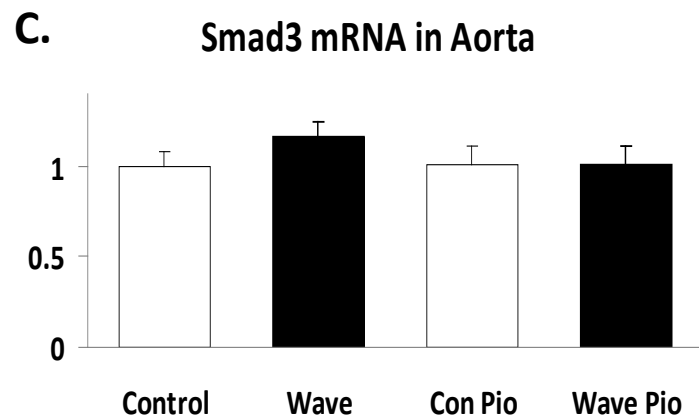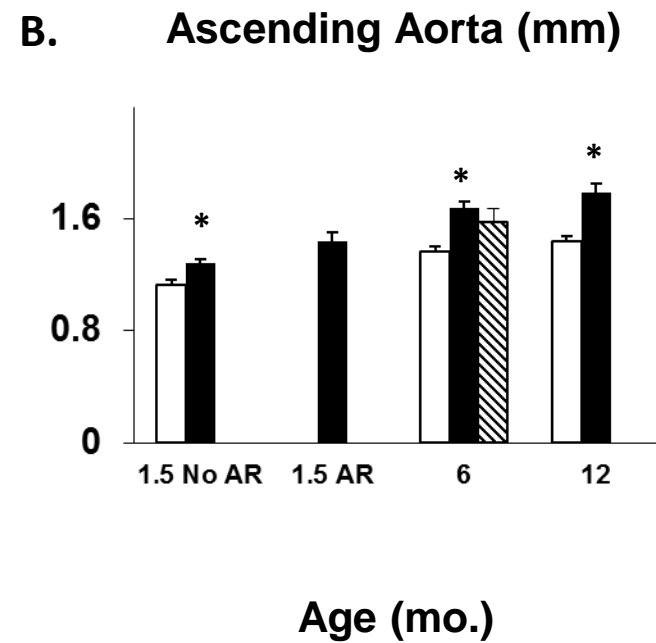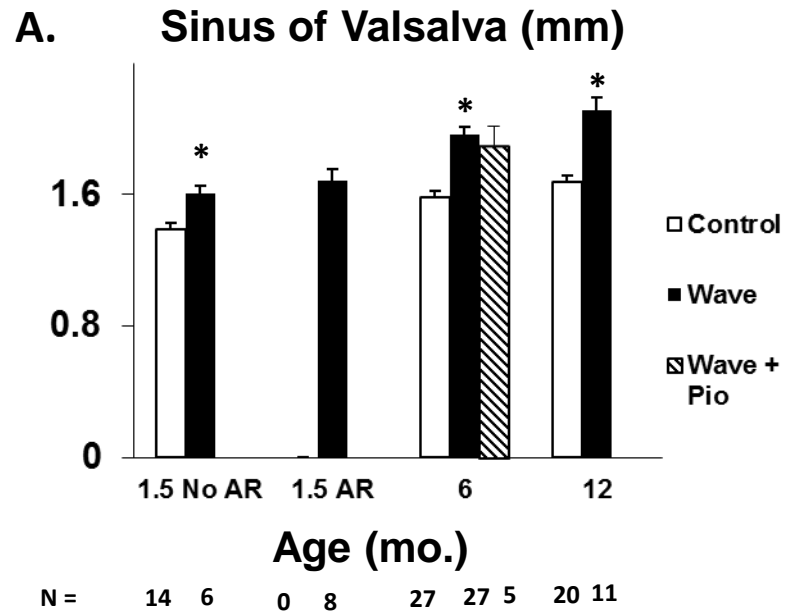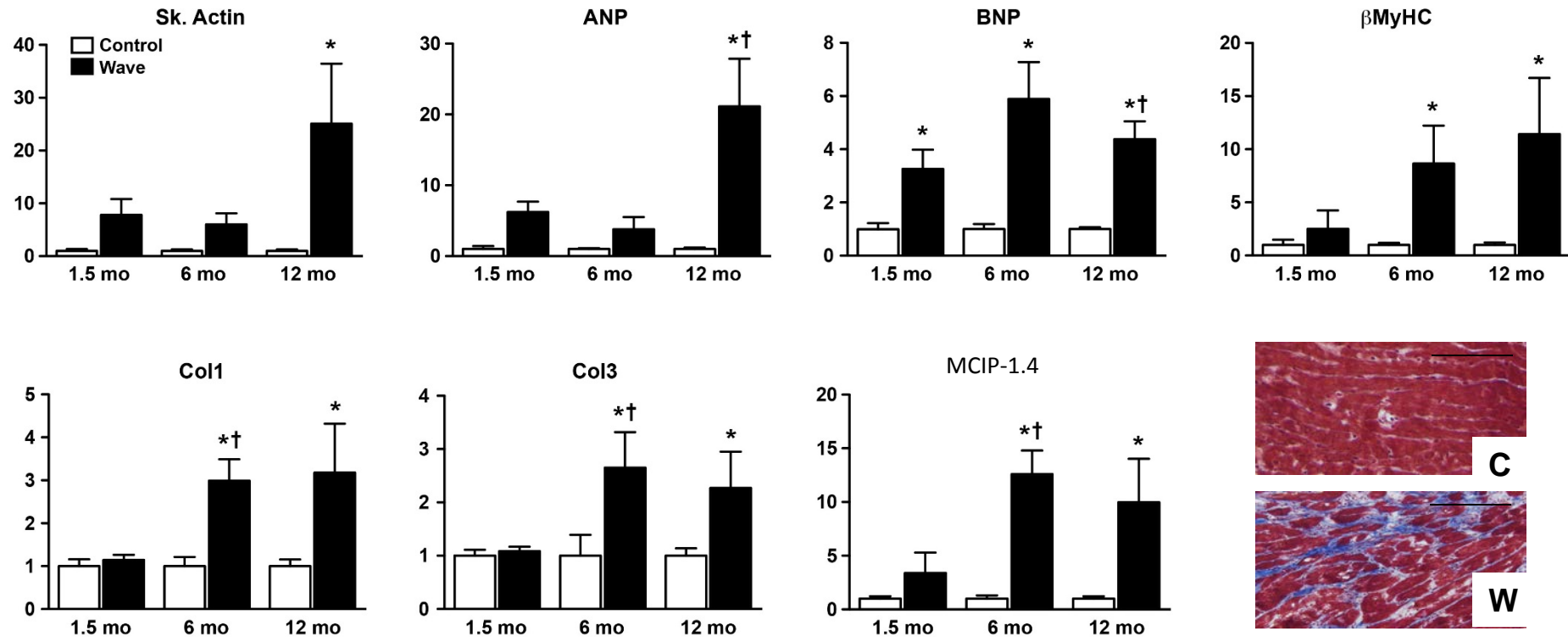
Supplemental Figure IV. Symmetry of systolic aortic valve cusp separation. **A**: schematic of standard M-mode and Anatomical M-mode imaging of the aortic valve. **B**: representative images from a single Wave mouse. **C**: R-NC vs. L-NC cusp separation in a group of Wave mice (N = 7). **D**: L-NC cusp separation is normal in Wave mice, but is reduced in aortic stenosis-prone Reversa mice[16] (N = 5). **E**: agreement between L-NC separation and R-NC separation in individual mice. **LV** left ventricle, **Ao** aorta, **R** right cusp, **L** left cusp, **NC** noncoronary cusp, *p < 0.05 *vs*. Control.

Regurgitant Fraction (MRI)
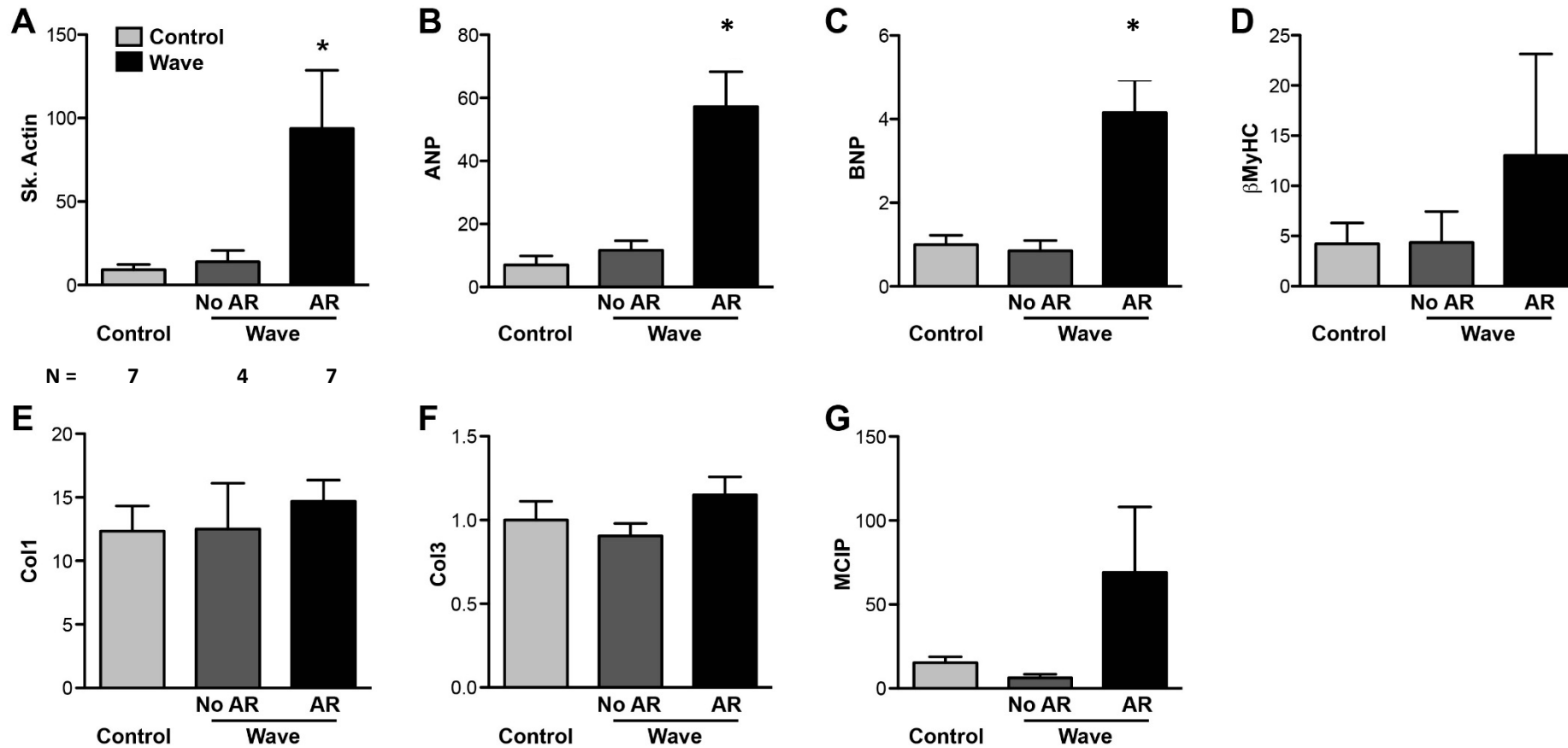
Aortic Regurgitation Score (Echo)

Supplemental Figure V. Aortic regurgitation assessed by MRI and echocardiography. < Mild, N = 18; Moderate or Severe, N = 8. Data were obtained at 6 and 12 mo. of age. *p < 0.05 vs. < Mild.
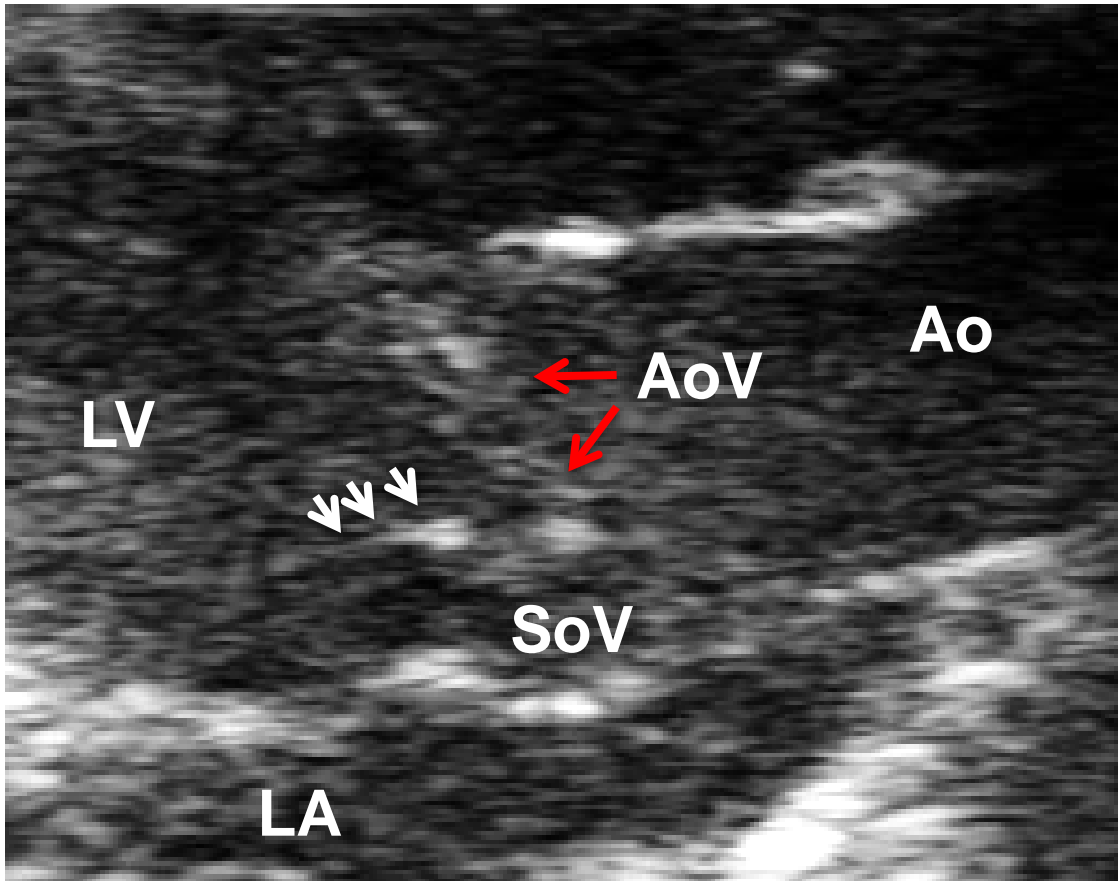
Supplemental Figure VI. <u>Aorta</u>. **A,B:** aortic dimensions, assessed by echocardiography. N = 14, 27, 7, 11 for 1.5 mo., 6 mo., Wave + Pio, and 12 mo., respectively. **C,D:** *Smad3* or *Smad7* expression as a fraction of Control (**Con**), in aortas from mice at 6 months of age (N = 6 each). **Pio** pioglitazone-treated; *p < 0.05 *vs.* age-matched and treatment-matched Control.
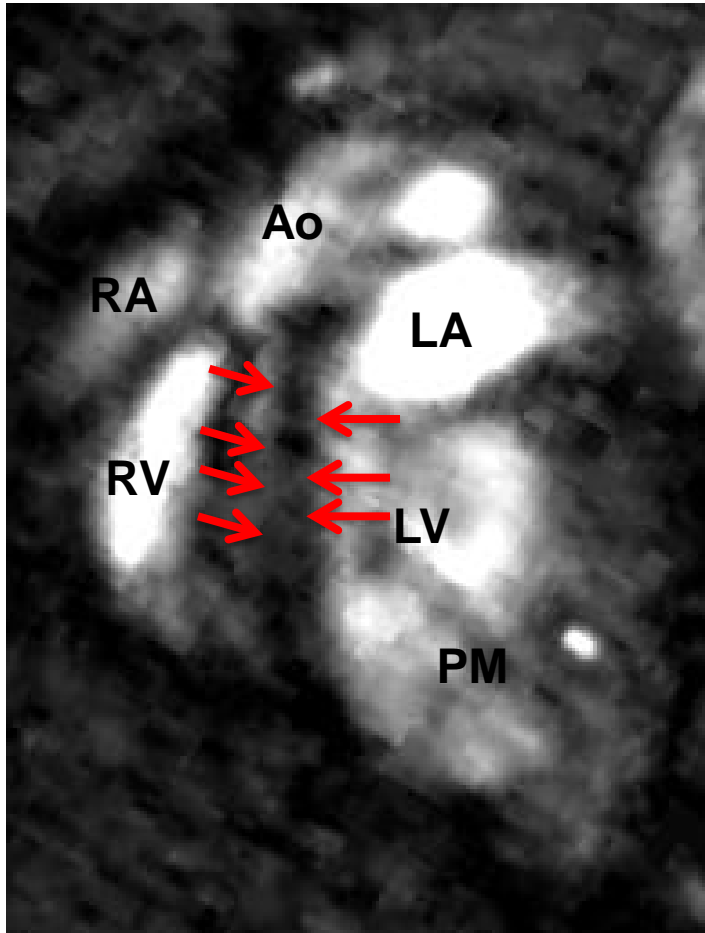
Supplemental Figure VII. Gene expression and collagen in myocardium. **Sk** skeletal muscle isoform, **ANP** atrial natriuretic peptide, **BNP** brain-type natriuretic peptide, **β-MyHC** beta-myosin heavy chain, **Col** collagen, **MCIP** myocyte-enriched calcineurin-interacting protein-1.4. Expression levels are normalized to values for Control mice at each age; N = 7 – 11 for each group. **C** Masson's Trichrome staining for collagen in Control myocardium at age 12 mo., **W** Masson's Trichrome staining for collagen in Wave myocardium at age 12 mo. Calibration bar = 100μm. *p < 0.05 vs. Control. † p < 0.05 vs. Wave at 1.5 mo.

Supplemental Figure VIII. Effects of genotype and aortic regurgitation (AR) upon myocardial gene expression at 1.5 months of age. *p < 0.05 vs. Control.

Key to Supplemental Video SV1. Parasternal long-axis 2D echocardiogram in mid-diastole from a Wave mouse with a myxomatous aortic valve (AoV, red arrows) and cusp prolapse (white arrows). The movie also shows restricted systolic motion of the aortic valve. **LV** left ventricle, **LA** left atrium, **SoV** sinus of Valsalva, **Ao** proximal ascending aorta.

Key to Supplemental Video SV2. Mid-diastole cine-MRI frame from a Wave mouse, showing severe aortic regurgitation (arrows). **Ao** aorta, **LV** left ventricle, **RV** right ventricle, **LA** left atrium, **RA** right atrium, **PM** papillary muscle.