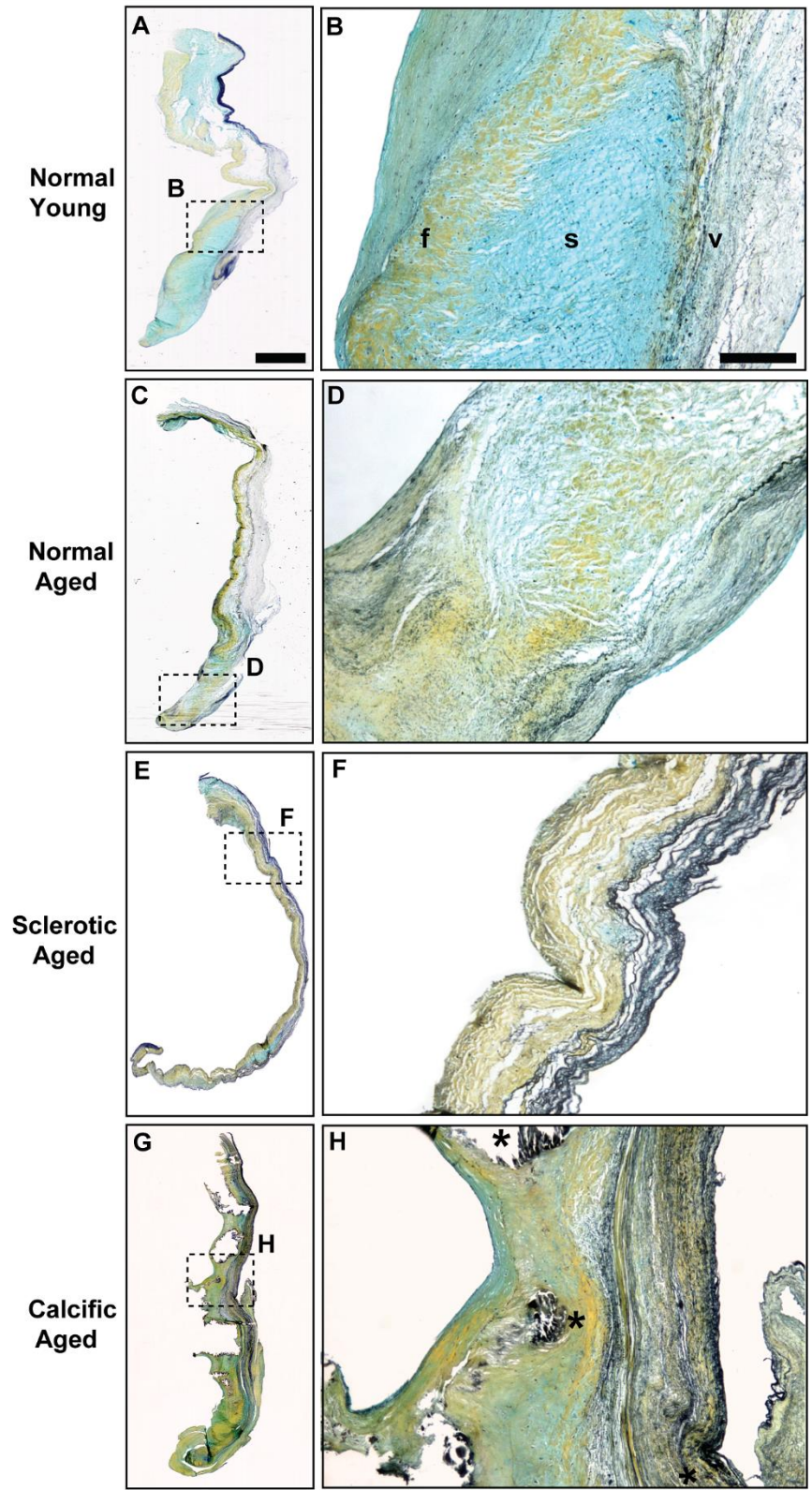
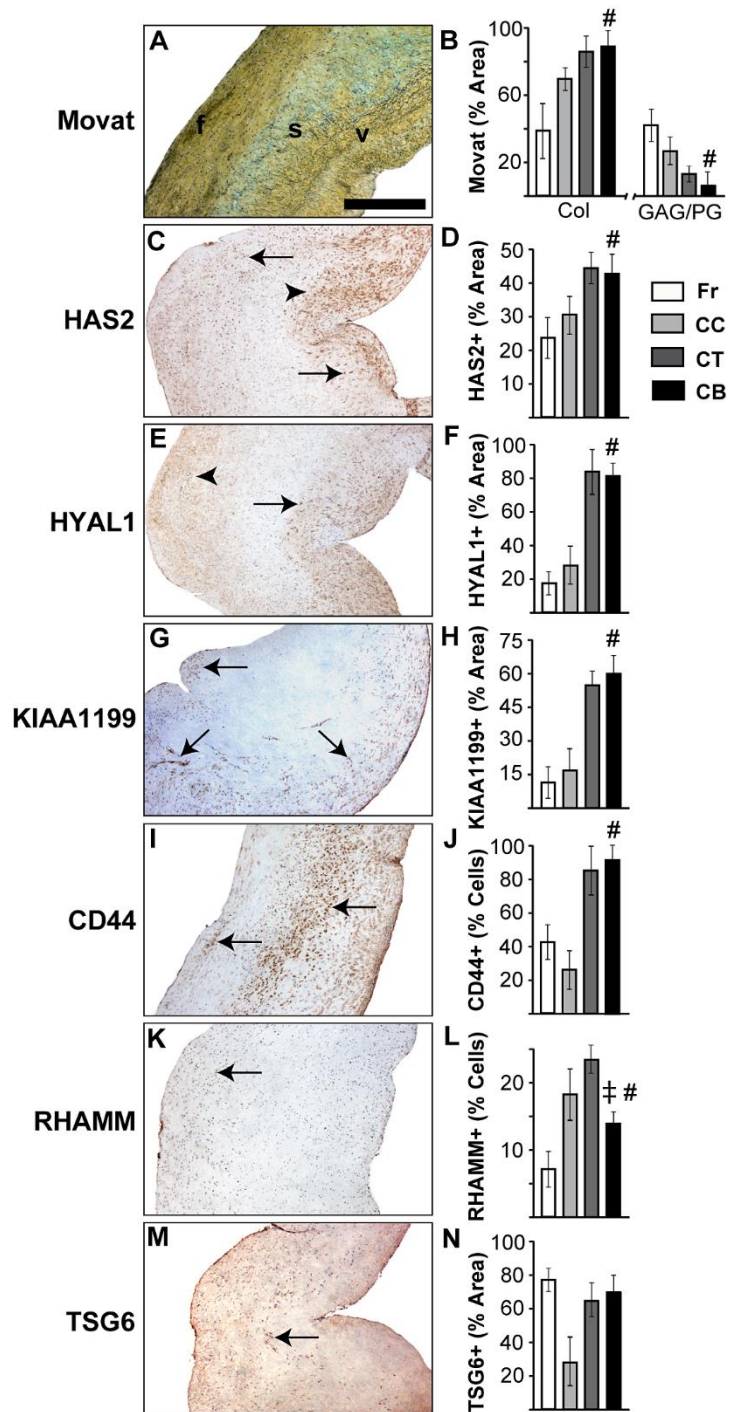


Human Aortic Valve



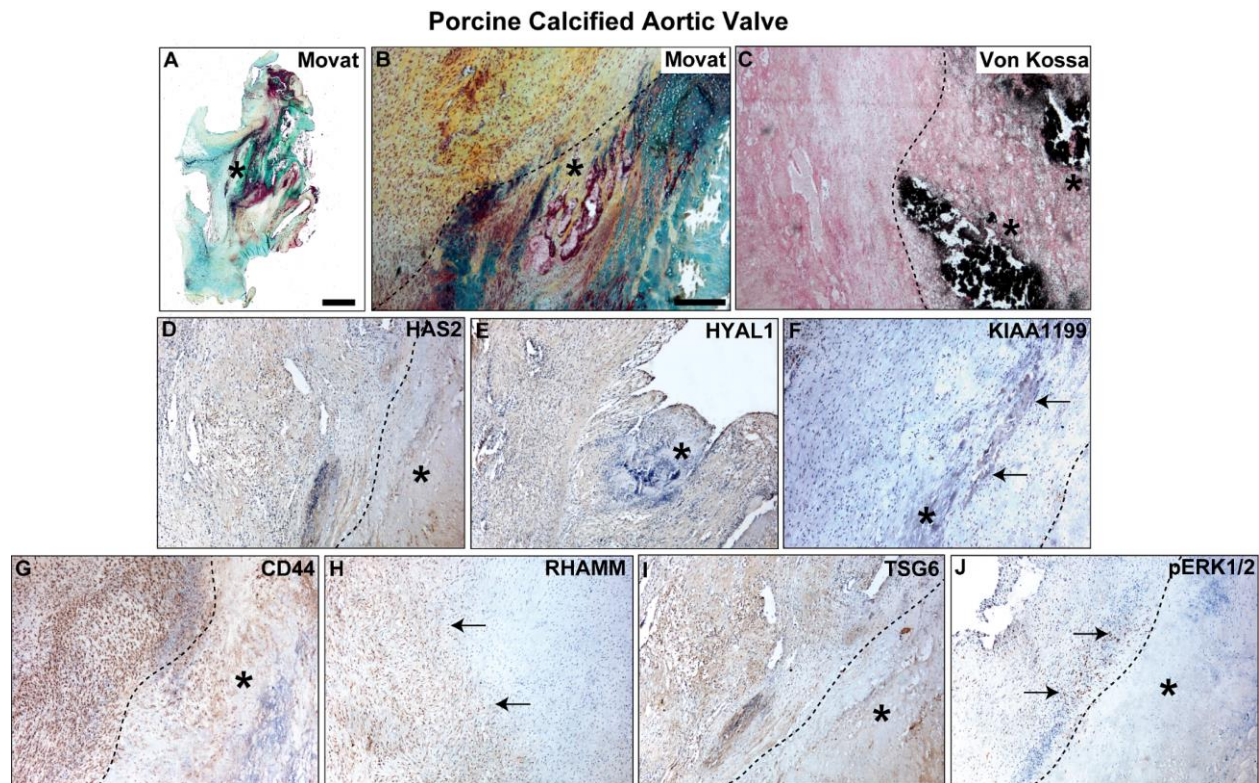
Supplemental Figure 1. Human aortic valves demonstrate fibrotic remodeling with increased collagen and reduced GAGs with age. Movat histological stain in valves classified as young (15-20 year old) or aged (>50 year old) and either normal or abnormal (sclerotic/calcific) are shown. Calcific nodules in H are indicated by asterisk. f, fibrosa; s, spongiosa; v, ventricularis. Scale bar: 0.5 mm for A, C, E, G, and 200 μ m for others.

Cultured in β GP

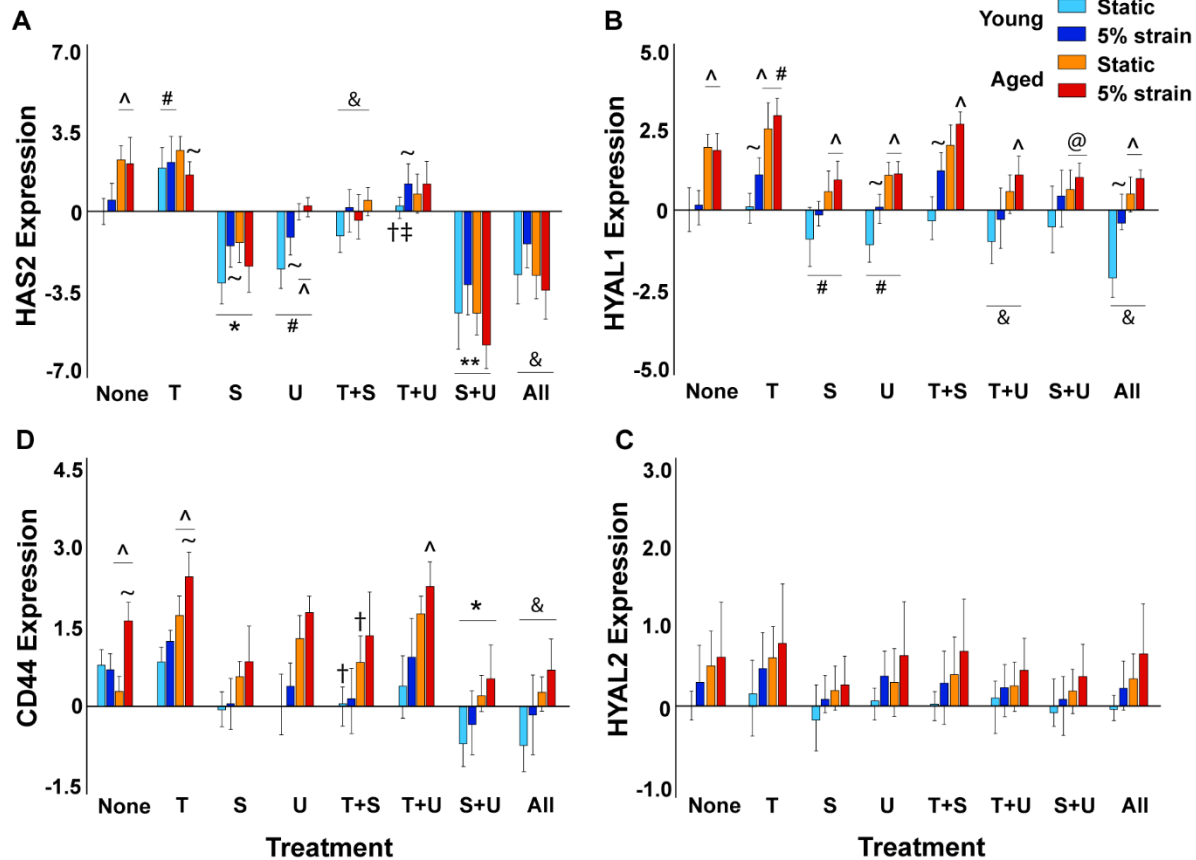


Supplemental Figure 2. β GP causes aberrant regulation of HA homeostasis in porcine aortic valve tissue similar to TGF β 1. Movat histochemical staining (A, B) and expression patterns of HAS2 (C, D), HYAL1 (E, F), KIAA1199 (G, H), CD44 (I, J), RHAMM (K, L) and TSG6 (M, N) in

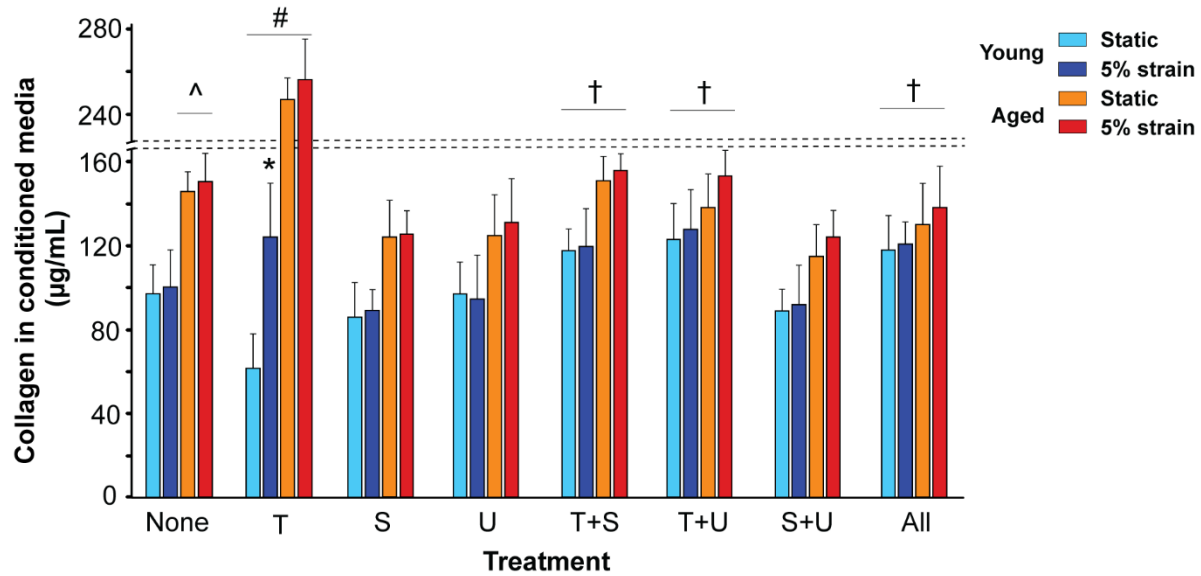
valves cultured in β GP are shown. Arrows and arrowheads denote cell and ECM expression respectively. f, fibrosa; s, spongiosa; v, ventricularis. #: vs fresh $p < 0.05$. Scale bars: 200 μ m.



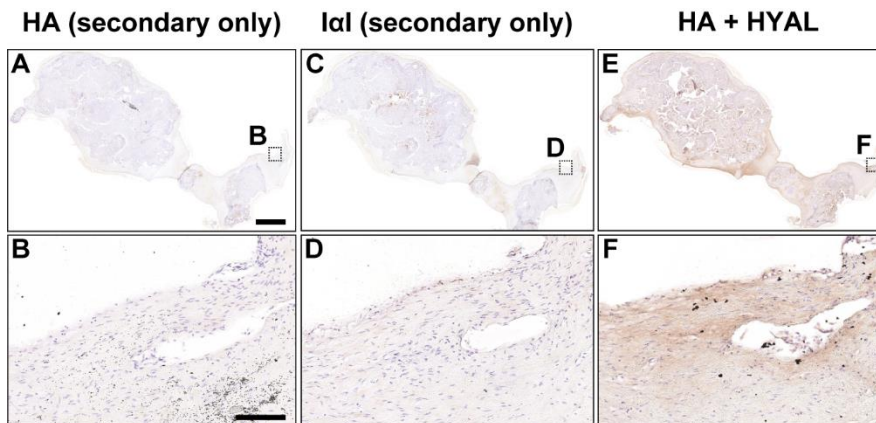
Supplemental Figure 3. Calcified porcine aortic valve demonstrates misexpression of HA homeostatic (D-I) and TGF β pathway (J) markers. Dotted lines denote the boundary between calcific and non-calcific regions of the valve tissue. Calcific nodules are indicated by asterisk. Arrows denote the region of expression. Scale bars for A: 400 μ m, and 200 μ m for others.



Supplemental Figure 4. Regulation of HA homeostatic marker gene expression by exogenous TGF β 1, its inhibitors, biomechanical strain and aging in cultured porcine aortic VICs as measured by qRT-PCR. This is the same Flexcell data as Fig 4, but with more elaborate statistics. Gene expression (on y-axis) is presented as relative log₂ expression, and all data for each gene is normalized to young, static with no treatment. “None”, no treatment; “T+S”, TGF β 1 + SB431542; “T+U”, TGF β 1 + U0126; “S+U”, SB431542 + U0126; “All”, TGF β 1 + SB431542 + U0126. #, vs. none, $p < 0.05$; *, vs. none, $p < 0.001$; **, vs. S and U, $p < 0.0001$; &, vs. T, $p < 0.001$; †, vs. T age-matched static, $p < 0.001$; ‡, vs. T+S young static, $p < 0.001$; ^, vs. young, $p < 0.0001$; ~, vs. age-matched static, $p < 0.05$; @, vs. aged none, $p < 0.05$.



Supplemental Figure 5. Regulation of collagen secretion in the conditioned media of cultured young and aged porcine VICs by exogenous TGF β 1, its inhibitors, biomechanical strain and aging. “None”, no treatment; “T+S”, TGF β 1 + SB431542; “T+U”, TGF β 1 + U0126; “S+U”, SB431542 + U0126; “All”, TGF β 1 + SB431542 + U0126. ^, vs. young, $p < 0.001$; *, vs. static T, $p < 0.0001$; #, vs. none, $p < 0.001$; †, vs. T, $p < 0.05$.



Supplemental Figure 6. Secondary only controls for hyaluronan-binding protein and Inter alpha inhibitor, and hyaluronan-binding protein with hyaluronidase in calcified human aortic valves. Scale bar: 0.5 mm.

Supplemental Table 1. Patient information for the human aortic valves.

Valves	Age/Race/Sex	Height/Weight, Cause of Death	Medical History
Control (Young)	<ul style="list-style-type: none"> • Patient 1, 19/C/M • Patient 2, 20/B/M • Patient 3, 16/C/F 	<ul style="list-style-type: none"> • 65" / 165 lbs, MVA • 69" / 180 lbs, MVA • 60" / 129 lbs, MVA 	<ul style="list-style-type: none"> • No HTN/DM, no T, rare D, yes A • No HTN/DM, rare T, rare D, yes A • No HTN/DM, no T, no D, no A
Control (Aged)	<ul style="list-style-type: none"> • Patient 1, 67/C/M • Patient 2, 55/C/M • Patient 3, 51/C/F • Patient 4, 85/C/F 	<ul style="list-style-type: none"> • 70" / 200 lbs, MVA • 68" / 180 lbs, Respiratory • 64" / 200 lbs, Cardiac arrest • 68" / 95 lbs, Head trauma 	<ul style="list-style-type: none"> • No HTN/DM, no T, no D, rare A • No HTN/DM, rare T, no D, no A • No HTN/DM, no T, no D, no A • No HTN/DM, rare T, no D, yes A
Sclerotic (Aged)	<ul style="list-style-type: none"> • Patient 1, 64/C/M • Patient 2, 79/C/F • Patient 3, 86/C/M 	<ul style="list-style-type: none"> • 72" / 180 lbs, P. Embolism • 65" / 192 lbs, CHF • 72" / 129 lbs, Cardiac arrest 	<ul style="list-style-type: none"> • Yes HTN, no DM, rare T, yes D, yes A • Yes HTN, yes DM, yes HL, yes CAD, yes Stroke, rare T, no D, yes A • Yes HTN, no DM, yes HL, yes CAD s/p CABG, yes CHF, yes CRD, no T, no D, rare A
Calcific (Aged)	<ul style="list-style-type: none"> • Patient 1, 70/U/F • Patient 2, 59/B/F • Patient 3, 78/A/M • Patient 4, 85/U/F 	<ul style="list-style-type: none"> • All leaflets from aortic valve replacement surgeries 	<ul style="list-style-type: none"> • Unknown

Race: C - Caucasian, B - African American, A - Asian, U - Unknown. Cause of death: MVA - motor vehicle accident, P. Embolism - pulmonary embolism, CHF - congestive heart failure. Medical history: HTN - hypertension, DM - diabetes mellitus, HL - hyperlipidemia, CAD s/p CABG -

coronary artery disease status post coronary artery bypass graft, CRD - chronic renal disease, T
- tobacco use, D - drug use, A - alcohol use.