

# Supplemental Materials

Collagen Fiber Regulation in Human Pediatric Aortic Valve Development and Disease

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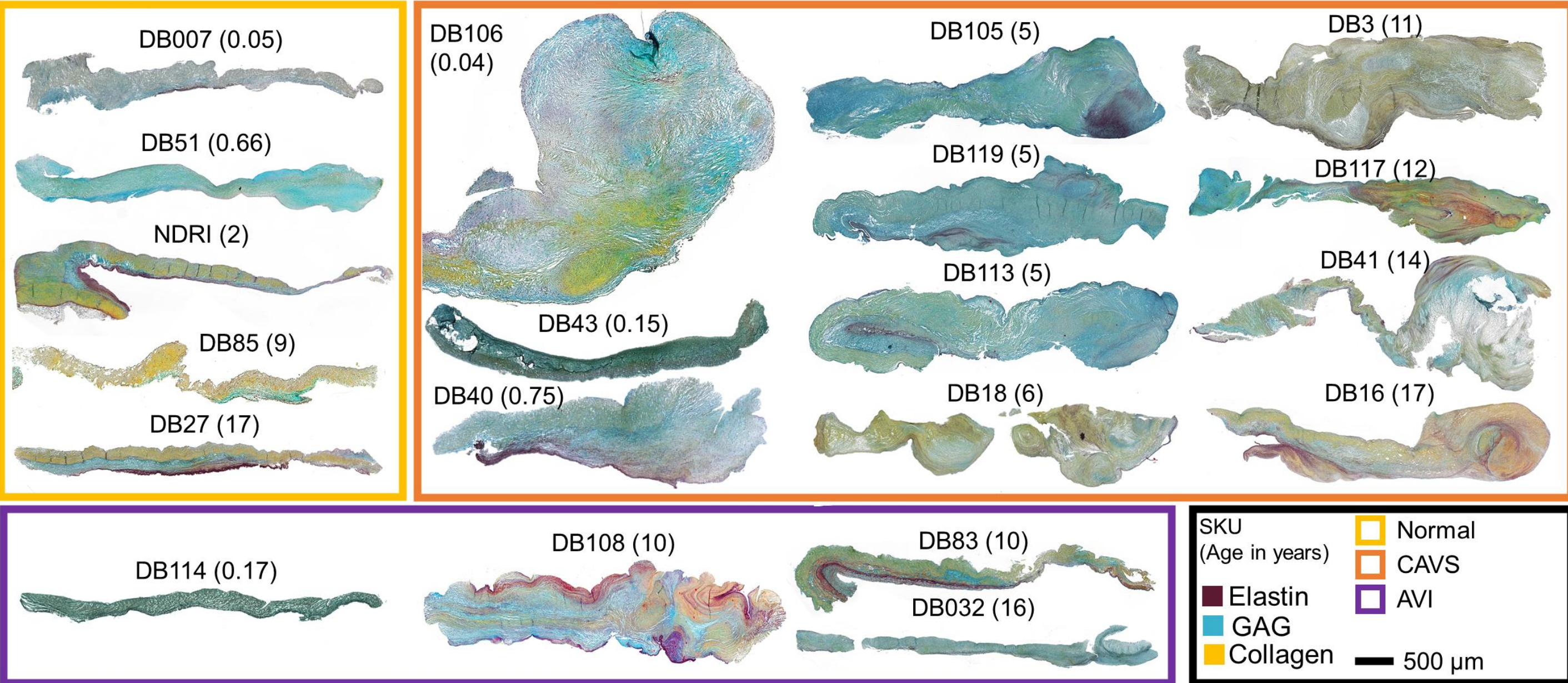
Email: [angelp@musc.edu](mailto:angelp@musc.edu) Phone: [\(843\) 792-8410](tel:(843)792-8410) Fax: [\(843\) 792-0481](tel:(843)792-0481)

**Short title:** Collagen types in human aortic valve

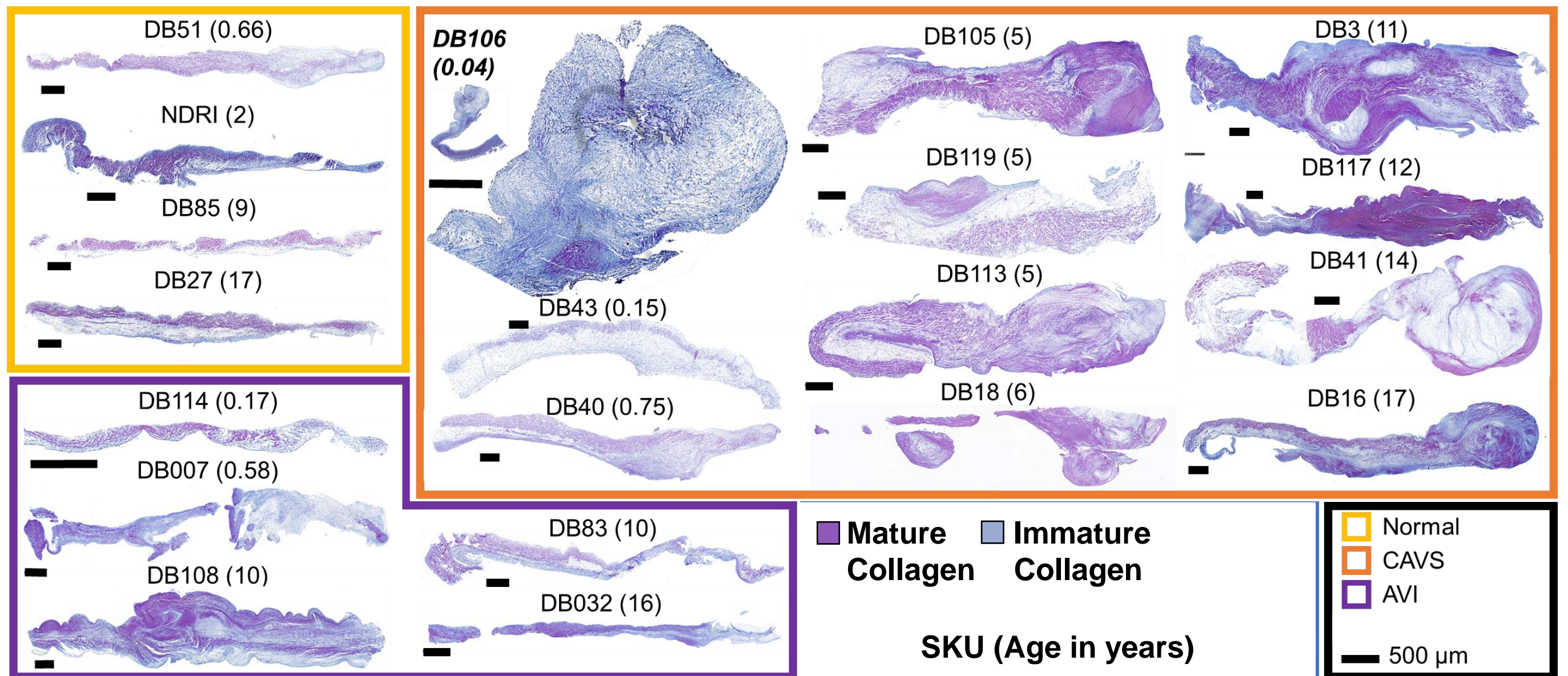
**Total word count:** 3946/4500 (not including Abstract, Methods, References, and Figure Legends)

**Key words:** aortic valve, congenital aortic valve stenosis, extracellular matrix, collagen, valve development, proteomics, collagen proline hydroxylation, collagen proteomics

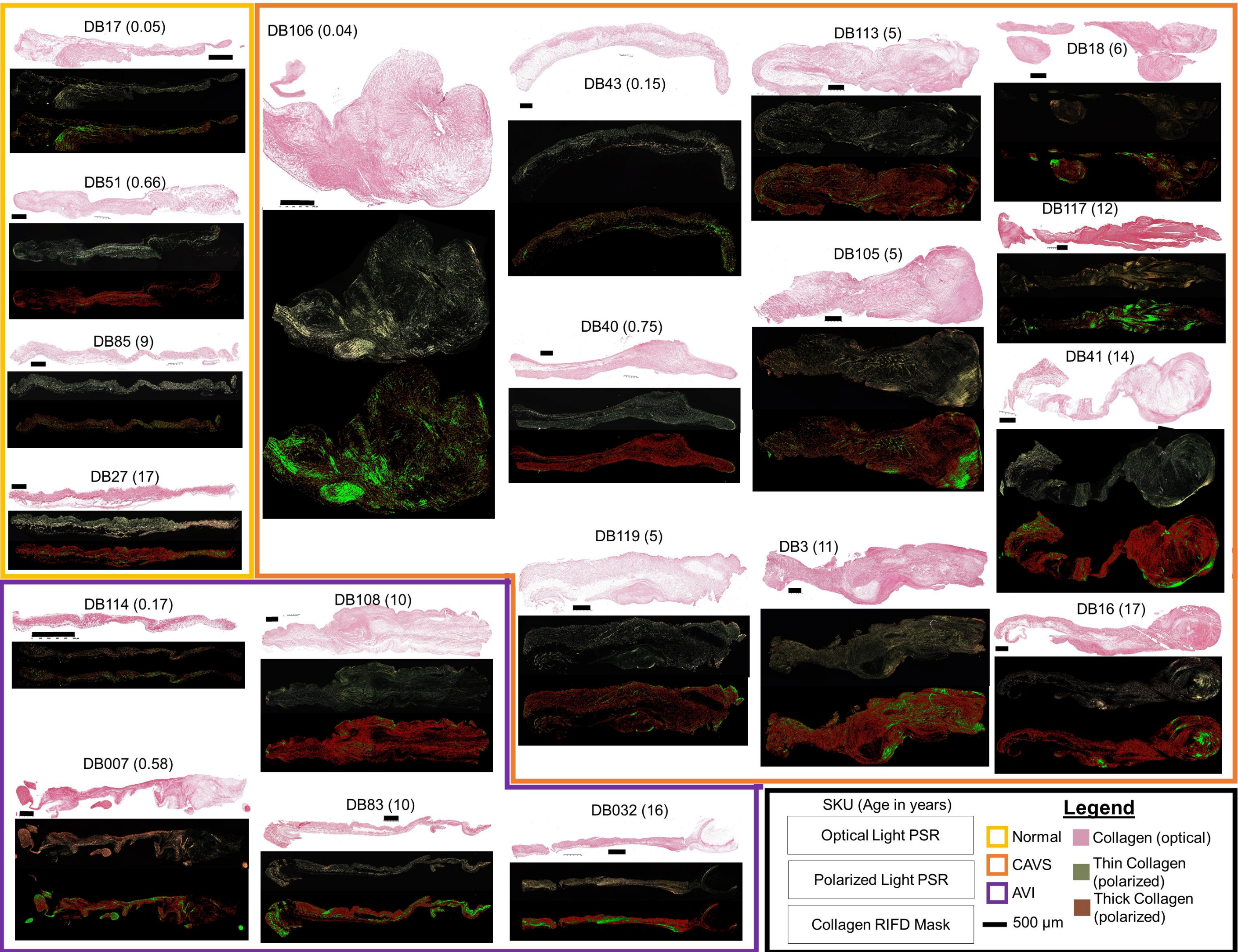
Abbreviations: AVS, Aortic Valve Stenosis; pCAVS, pediatric end-stage congenital aortic valve stenosis; AVI, aortic valve insufficiency; ECM, extracellular matrix; PTM, post-translational modification; HYP, hydroxyproline; HRAM- high resolution accurate mass; GAG, glycosaminoglycan.



**Supplemental Figure S1:** Movat's Pentachrome images of all valves studied in Figure 1. DB113 and DB32 are included as representative images only, due to delayed acquisition and manufacturer's difference in sample preparation.



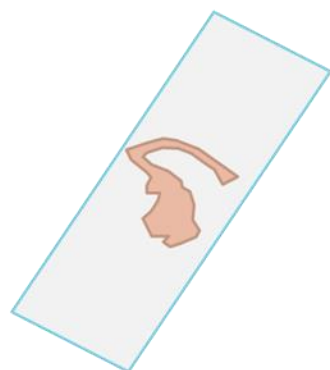
**Supplemental Figure S2:** Herovici images for all valves studied in Figure 2.



**Supplemental Figure S3:** Optical and Polarized Picosirius Red (PSR) images of valves studied in Figure 3. RIFD (Regions of Increased Fiber Density) masks are shown.

## HRAM Proteomics

### Tissue preparation



Formalin fixed paraffin embedded tissue (FFPE)

*Heat 1 Hr 60°C*

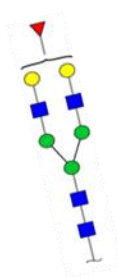
*Dewax*

Heat Induced Epitope Retrieval (HIER)

*10mM Tris pH 9*



### ECM Digest



PNGase F Digest and Deglycosylation  
*Detaches & removes N-glycans*



Collagenase III Digest  
*Detaches ECM peptides*



Incubate overnight  
*37°C, shaking*

### Acquire ECM

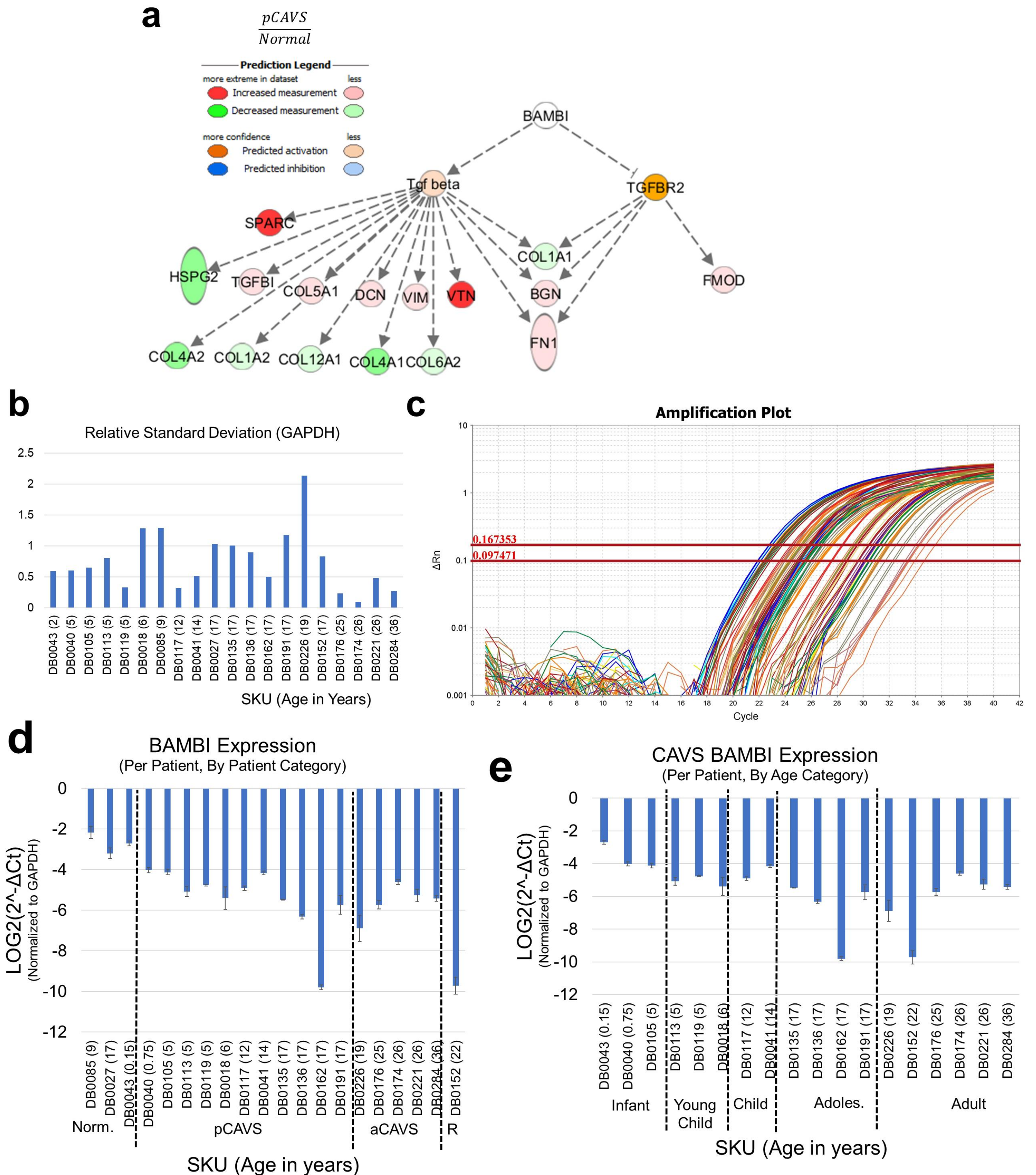


STAGE Tip Cleanup  
*Solid phase extraction of ECM peptides*

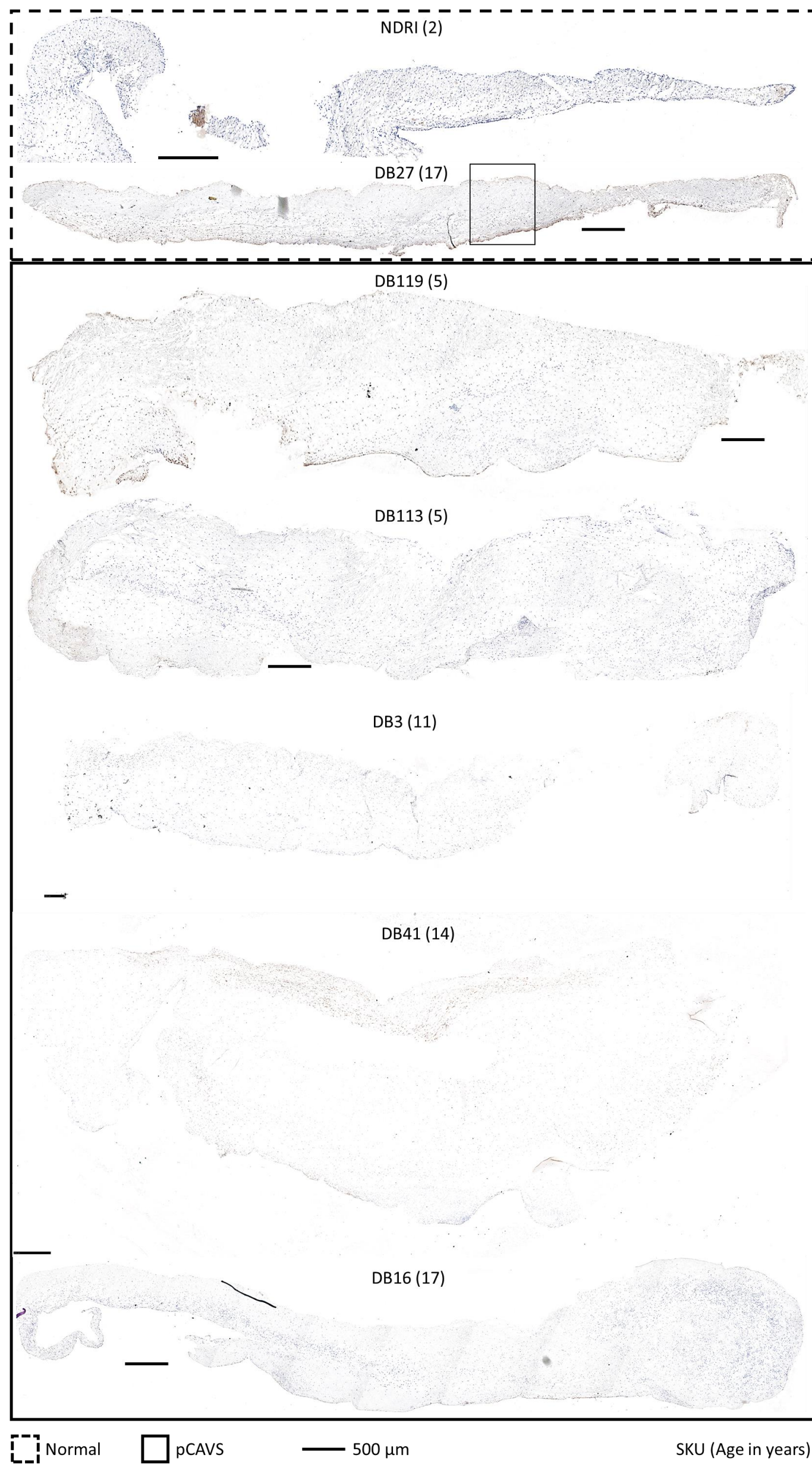


Acquire Mass Spectra  
(LC-MS/MS)

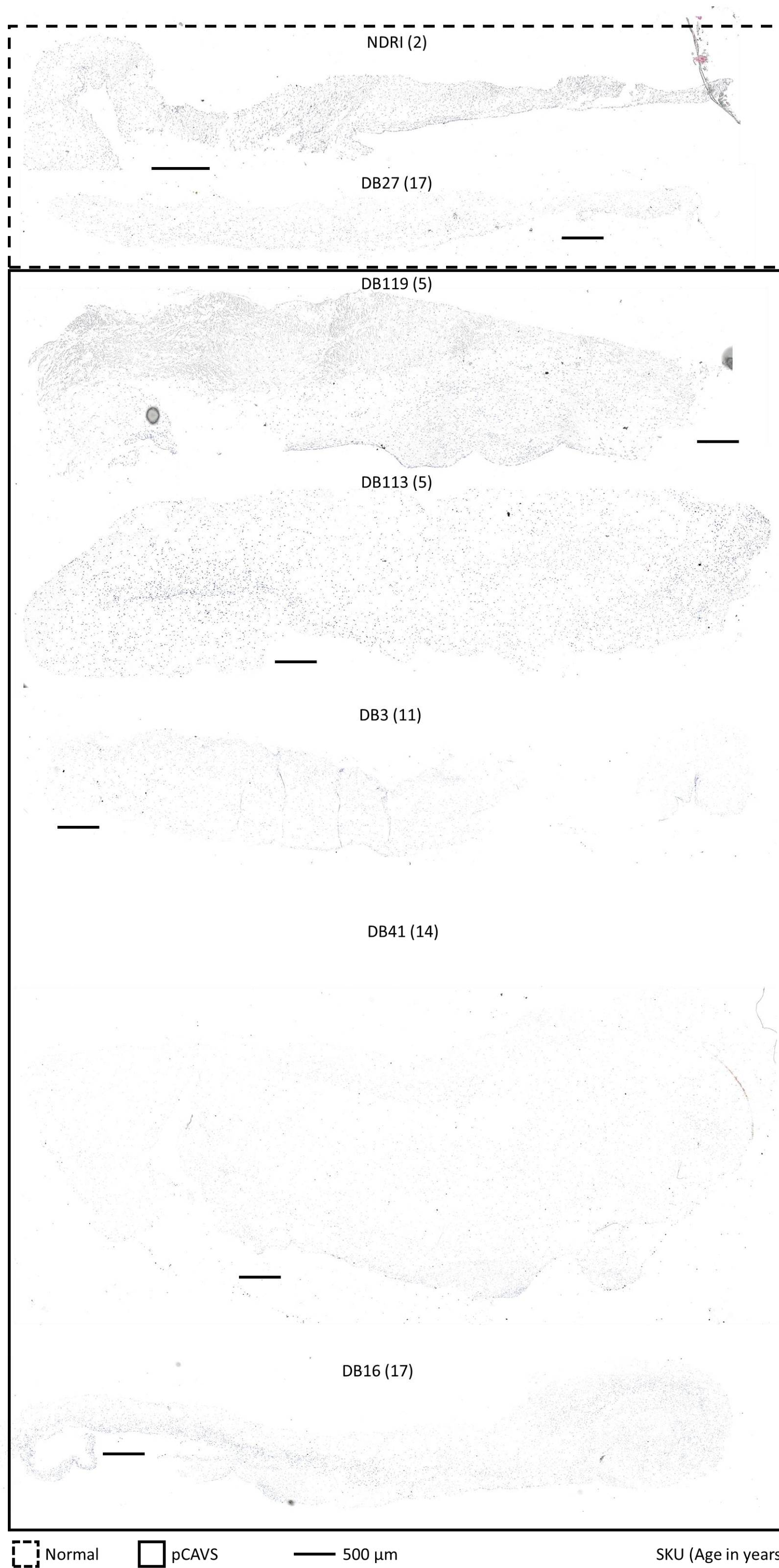
**Supplemental Figure S4:** Collagenase Type III high resolution accurate mass (HRAM) Proteomics workflow on formalin-fixed paraffin embedded tissue sections.



**Supplemental Figure S5: Causal network analysis implicating BAMBI and expression analysis. A)** Causal Network Analysis identifies a potential Master Regulator sorted by highest p-value overall and of overlap: BAMBI (BMP and activin membrane-bound inhibitor homolog, p-value 1.16E-18), based on trending expression patterns. **B)** Amplification plot of qRTPCR experiments in **Figure 6b-c; Supp Fig S5c-d**. **C)** Relative standard deviation (RSD) of GAPDH across all patient samples, sorted by age of patient. Average RSD is 0.75% ± 0.48%. **D)** Patient-by-patient expression (shown via LOG<sub>2</sub>(2<sup>-ΔCt</sup>), as normalized to GAPDH expression), organized by patient disease category. Norm: Normal; pCAVS: pediatric CAVS; aCAVS: adult CAVS; R: pulmonary valve by Ross procedure (**Fig. 6b**). **E)** Patient-by-patient expression (shown via LOG<sub>2</sub>(2<sup>-ΔCt</sup>), as normalized to GAPDH expression), of CAVS patients organized by pediatric age classification (**Fig 6c; Table 1; Supplemental Table S7**)



**Supplemental Figure S6:** Immunohistochemistry images with primary antibody BAMBI (chromogen, brown). Mayer's hematoxylin (blue) was used as a counterstain. Background subtracted samples are shown on the left, with raw images shown on the right.



**Supplemental Figure S7:** Immunohistochemistry images of negative controls corresponding to images in Supplementary Fig. S6. No primary antibody was used. Mayer's hematoxylin (blue) was used as a counterstain.



Supplementary Table S1: Normal peptides identified with a LOG2(Fold Change) intensity value greater than 2 compared to pCAVS. PEP: Posterior error probability.

Protein Name	Uniprot Accession Number	Uniprot Name	Gene Name	Sequence	Oxidation (P)	Oxidation (P) Probabilities	Charges	Mass	PEP	MQ Peptide		MS/MS Count	Normal Log	pCAVS Log	AVI Log
										Score	Intensity		Transform Mean	Transform Mean	Transform Mean
Basement membrane-specific heparan sulfate proteo	P98160	PGBM_HUMAN	HSPG2	LVNFTRSIEYSPQLEDAGSRE	0		3	2411.1605	0.000	122.8	2.E+07	4	2.325	-0.505	0.617
Basement membrane-specific heparan sulfate proteo	P98160	PGBM_HUMAN	HSPG2	NPSVOQGQCLPETNQAPL	1	NP(0.5)SVQGGQCLP(0.014)ETNQAP(0.486)L	2	1885.8364	0.034	81.7	3.E+06	1	1.347	-1.029	NaN
Biglycan	P21810	PGS1_HUMAN	BGN	LHSNNITKVG	0		2	1082.5720	0.088	88.8	4.E+05	1	-1.762	-4.009	-2.481
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	FLPQPPQEKAHDGGR	1	FLP(0.004)QP(0.195)P(0.801)QEKAHDGGR	2,3	1691.8380	0.025	87.7	2.E+07	4	1.623	-0.460	-0.201
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	GKDGLNGLP	0		1	870.4447	0.027	108.5	4.E+06	5	1.671	-0.993	-2.153
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	GSPGEGAPGQMGPR	1	GSP(0.5)GENGAP(0.5)GQMGPR	2	1444.5889	0.014	97.5	3.E+05	4	-1.081	-3.205	NaN
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	IAGAPGFPGAR	2	IAGAP(0.5)GFFP(0.5)GAR	2	1044.5352	0.007	111.8	6.E+06	10	1.257	-0.737	-1.330
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	POPPQEKAHDGGR	0		3	1415.6906	0.162	83.1	8.E+05	1	1.076	-5.239	NaN
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	ADGVPKDGPR	2		2	1067.5360	0.078	85.3	1.E+05	1	-1.483	-4.338	NaN
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GEPGRDGNPGSDGLPGRDGS	4	GEP(1)GRDGNP(1)GSDGLP(1)GRDGS(1)	3	2056.8682	0.000	118.7	2.E+06	7	0.687	-2.264	-4.536
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GEPGRDGNPGSDGLPGRDGS	1	GEP(0.25)GRDGNP(0.25)GSDGLP(0.25)GRDGS(0.25)	3	2056.8682	0.000	133.9	1.E+06	3	1.250	-1.104	NaN
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GPGADGVPKDGPR	1	GGP(1)GADGVPKDGPR	3	1351.6480	0.017	88.0	2.E+06	4	0.231	-3.189	-4.848
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GLKGENGLPGENGAPGPM	1	GLKGENGLP(0.5)GENGAP(0.25)GP(0.25)M	2	1743.7621	0.000	234.8	6.E+06	18	2.095	-0.397	NaN
Collagen alpha-1(IV) chain	P02462	CO4A1_HUMAN	COL4A1	QPGPKGDPGI	2	QP(1)GP(0.999)KGD(0.001)GI	1	996.4876	0.091	88.4	1.E+06	4	1.678	-1.958	-0.703
Collagen alpha-1(V) chain	P20908	CO5A1_HUMAN	COL5A1	GETGDVGQMGPP	2	GETGDVGQMGPP(0.224)P(0.776)	1	1175.4765	0.048	91.3	2.E+05	1	-1.680	-4.722	NaN
Collagen alpha-1(V) chain	P20908	CO5A1_HUMAN	COL5A1	GLQGPVGLP	1	GLQGPVGLP(1)	1	852.4705	0.118	83.6	1.E+07	3	2.498	0.273	0.355
Collagen alpha-1(VI) chain	P12109	CO6A1_HUMAN	COL6A1	FLTAGRTDPAHDVDR	0		3	1554.7903	0.122	100.2	4.E+06	2	0.523	-1.661	-1.499
Collagen alpha-1(VI) chain	P12109	CO6A1_HUMAN	COL6A1	GEAGDEGNPDPGAPGERGGPC	1	GEAGDEGNP(0.001)GP(0.005)DGAP(0.496)GERGGP(0.498)GI	3	2265.9483	0.044	89.7	1.E+05	2	-1.934	-4.896	NaN
Collagen alpha-1(VI) chain	P12109	CO6A1_HUMAN	COL6A1	LTAGRTDPAHDVDR	0		3	1407.7219	0.033	100.4	6.E+06	6	0.524	-1.646	-0.103
Collagen alpha-1(VI) chain	P12109	CO6A1_HUMAN	COL6A1	VAVVQVSGTQQRPERA	0		2,3	1844.9493	0.033	80.9	1.E+07	7	2.550	-0.394	1.859
Collagen alpha-1(XI) chain	P12107	CO8A1_HUMAN	COL11A1	MGPQEGPQQQGN	1	MGP(0.004)QGEP(0.831)GP(0.134)P(0.032)GQQQGN	2	1466.6096	0.060	76.3	6.E+06	3	1.044	-1.134	NaN
Collagen alpha-1(XVII) chain	Q07092	COG1A1_HUMAN	COL16A1	GLAGEPGP	0		1	696.3443	0.143	77.7	4.E+05	1	-0.018	-3.272	NaN
Collagen alpha-1(XVIII) chain	P39060	CO1A1_HUMAN	COL18A1	GPPGFQPFDFLQ	1	GP(0.5)P(0.5)GQFPDFLQ	2	1364.6401	0.011	119.7	1.E+06	1	0.563	-4.107	-1.940
Collagen alpha-1(XVIII) chain	P39060	CO1A1_HUMAN	COL18A1	GRFVNSDSDVPGPA	0		2	1359.6419	0.027	103.2	8.E+05	1	1.275	-2.657	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GARGSDGVSVPV	2		2	1057.5152	0.002	134.2	6.E+06	18	1.669	-1.130	-2.907
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GAVGSPGVNGAPGEA	0	GAVGSP(1)GVNGAP(1)GEA	1,2	1271.5630	0.000	166.1	1.E+07	34	2.111	-0.500	-2.597
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GAVGSPGVNGAPGEA	3	GAVGSP(1)GVNGAP(1)GEAGRDGNP(1)	2	1883.8246	0.000	126.1	8.E+05	11	-0.781	-3.090	-4.866
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GSPGVNGAPGEAGRDGNP	3	GSP(1)GVNGAP(1)GEAGRDGNP(0.012)GNDGP(0.494)P(0.494)	2	2193.9159	0.000	113.3	1.E+05	2	-2.024	-4.749	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GSPGVNGAPGEAGRDGNP	2	GSP(0.5)GVNGAP(0.5)GEAGRDGNP(0.492)GNDGP(0.254)P(0.254)	2	2209.9108	0.000	145.7	9.E+05	13	-1.047	-2.995	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	LVGEPGPA	1	LVGEP(1)GPA	1	754.3861	0.048	106.8	9.E+06	21	1.705	-0.824	-0.352
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	SPGVNGAPGEA	2	GSP(0.5)GVNGAP(0.5)GEAGRDGNP(0.492)GNDGP(0.254)P(0.254)	1	900.3825	0.036	99.1	5.E+05	4	-0.046	-2.481	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	SPGVNGAPGEA	2	SP(1)GVNGAP(1)GEA	1	987.4145	0.095	82.7	1.E+05	1	-2.541	-4.824	NaN
Collagen alpha-2(VI) chain	P12110	CO6A2_HUMAN	COL6A2	LANMTEQIR	2		2	1075.5332	0.016	88.2	3.E+06	2	-0.565	-2.723	-1.652
Collagen alpha-2(VI) chain	P12110	CO6A2_HUMAN	COL6A2	LDGSERLGEON	2		2	1216.5684	0.021	99.8	2.E+05	3	-1.145	-4.243	-5.432
Collagen alpha-2(VI) chain	P12110	CO6A2_HUMAN	COL6A2	LYRNDYAT	0		2	1015.4611	0.134	79.7	2.E+06	1	1.298	-2.462	-3.148
Collagen alpha-3(VI) chain	P12111	CO6A3_HUMAN	COL6A3	FDDEYQPEMLEKFR	0		2,3	2008.8877	0.005	112.4	6.E+07	29	3.658	1.615	1.560
Collagen alpha-3(VI) chain	P12111	CO6A3_HUMAN	COL6A3	HANTKVGLEHLR	0		3	1373.7528	0.052	89.4	4.E+05	2	-2.426	-5.142	-5.191
Collagen alpha-3(VI) chain	P12111	CO6A3_HUMAN	COL6A3	PEVPRPQAAKPA	3	P(1)EVP(1)RP(1)QAAKPA	2	1307.6834	0.065	84.2	3.E+06	2	2.204	-1.657	NaN
Collagen alpha-3(VI) chain	P12111	CO6A3_HUMAN	COL6A3	PLRLNLLLDIYE	2		2	1472.7875	0.002	120.3	7.E+05	1	0.886	-1.794	-2.687
Dentin sialophosphoprotein	Q9NZW4	DSPP_HUMAN	DSPP	RSNTNGNTDKNTQNGDV	0		2	1838.7402	0.022	88.3	2.E+06	2	0.426	-1.956	NaN
Fibromodulin	Q06828	FMOD_HUMAN	FMOD	LHLHDHNIQR	3		3	1231.6422	0.074	106.4	3.E+06	4	0.752	-1.649	-1.853
Fibromodulin	Q06828	FMOD_HUMAN	FMOD	LRELHLDHNIQR	0		3	1273.6527	0.138	90.1	2.E+06	2	-0.259	-2.861	-4.221
Fibromodulin	Q06828	FMOD_HUMAN	FMOD	LYLDHNLNTR	0		2,3	1258.6306	0.011	111.1	1.E+07	6	2.403	0.070	-0.457
Fibronectin	P02751	FN1_HUMAN	FN1	NLAPDSSSVVSG	1	NLAP(1)DSSSVVSG	2	1247.5881	0.062	81.3	4.E+06	4	1.811	-1.432	-2.549
Tenascin-X	P22105	TENX_HUMAN	TNKB	LLETTVTRTE	0		2	1189.6190	0.016	105.5	2.E+06	2	-0.110	-2.022	-1.880
Vimentin	P08670	VIME_HUMAN	VIM	KNLQEAEEWYKSK	0		3	1651.8206	0.000	161.2	8.E+06	12	1.368	-0.746	-0.918

**Supplemental Table S2:** pCAVS peptides identified with a LOG2(Fold Change) intensity value greater than 2 compared to Normal. PEP: Posterior error probability.

Protein Name	Uniprot Accession Number	Uniprot Name	Gene Name	Sequence	Oxidation (P)	Oxidation (P) Probabilities	Charges	Mass	PEP	MQ Peptide		MS/MS Count	Normal Log Transform Mean	pCAVS Log Transform Mean	AVI Log Transform Mean
										Score	Intensity				
Asporin	Q9BXN1	ASP_N_HUMAN	ASP_N	SEIPLNLPKS	0		1	1096.6128	0.007	120.3	4.E+06	7	-2.1048	0.1160	NaN
Basement membrane-specific heparan sulfate proteoglycan core protein															
Biglycan	P21810	PGS1_HUMAN	BCN	LAPTNEAFKIPSETLNR	0		2,3	2029.0480	0.040	79.8	7.E+07	6	0.1579	2.4777	1.1328
Biglycan	P21810	PGS1_HUMAN	BCN	ILEDLRLYSKLYR	0		3	1809.9889	0.092	73.8	1.E+07	2	-0.9563	1.4436	-0.9051
Biglycan	P21810	PGS1_HUMAN	BCN	LARVPSGLPDLK	0		2,3	1264.7503	0.003	136.3	7.E+07	38	-0.5734	1.6439	1.6777
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	GLPGTAGLP	2	GLP(1)GTAGLP(1)	1,2	813.4232	0.009	126.2	2.E+08	21	0.8426	4.3823	-0.9657
Collagen alpha-1(I) chain	P02452	CO1A1_HUMAN	COL1A1	PPOEKADGGRRY	0		2	1516.7059	0.049	85.8	8.E+07	5	-0.2527	2.1825	1.0390
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	FAPYYG	0		1	716.3170	0.158	77.5	6.E+07	2	-1.6375	2.8232	1.1229
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GAPGLKGENGLP	1	GAP(0.5)GLKGENGLP(0.5)	2	1125.5666	0.029	96.1	8.E+07	11	-3.4981	3.7819	2.7979
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GLAGTAGEP(1)GRDGNP(1)GSDGLP(1)	3		2	1942.8504	0.000	123.1	2.E+06	1	-2.2755	-0.1059	NaN
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GLKGENGLP	1	GLKGENGLP(1)	2	900.4553	0.029	107.3	1.E+07	9	-3.1905	1.1839	0.2164
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GQPGVMGFP	2	GQP(1)GVMGFP(1)	1,2	936.4011	0.017	118.0	8.E+07	19	1.0131	3.3746	-0.4589
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GQPGVMGFP	1	GQPGVMGFP(1)	1,2	936.4011	0.020	113.1	1.E+07	10	-1.1158	2.2727	-0.0280
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	GRPGRGPERGLPGPPGKIGKPA	1	GRP(0.061)GRP(0.935)GERGLP(0.003)GP(0.001)PGIKGPA	2	2038.1184	0.056	78.5	9.E+06	1	-1.7151	0.3776	0.0850
Collagen alpha-1(III) chain	P02461	CO3A1_HUMAN	COL3A1	LAGTAGEP(0.5)GRDGNP(0.5)GSDGLP(0.5)	1		2	1868.8500	0.000	132.2	2.E+07	32	-1.5434	2.5790	0.1992
Collagen alpha-1(IV) chain	P02461	CO3A1_HUMAN	COL3A1	VAVGGLAGYPPG	1	VAVGGLAGYPP(0.5)GP(0.5)	1	1088.5502	0.148	71.7	2.E+07	1	-2.1409	-0.0839	2.2532
Collagen alpha-1(IV) chain	P02462	CO4A1_HUMAN	COL4A1	DGLPGDMGPPG	0		1	1011.4331	0.158	73.7	2.E+07	1	-0.8143	1.6616	-1.7121
Collagen alpha-1(V) chain	P20908	CO5A1_HUMAN	COL5A1	GAPGKPGDGL	1	GAP(0.5)GKPG(0.452)GP(0.048)DGL	1	980.4927	0.058	90.7	1.E+07	12	-1.3273	1.2474	0.0534
Collagen alpha-1(V) chain	P20908; P12107	CO5A1_HUMAN; COBA1_HUMAN	COL5A1; COL11A1	GPPGTMLMLP	1	GP(0.5)P(0.5)GTMMLP	1,2	1044.4984	0.000	138.4	5.E+07	40	-1.5024	1.2157	0.5055
Collagen alpha-1(V) chain	P20908	CO5A1_HUMAN	COL5A1	GSIGFPFG	2	GSIGFP(1)GFP(1)	1	909.4232	0.030	106.8	6.E+06	7	0.5141	2.8417	-0.7466
Collagen alpha-1(V) chain	P12109	CO6A1_HUMAN	COL6A1	IDTIVDMKNNVEQV	0		2	1729.8920	0.000	178.7	3.E+06	6	-2.8423	-0.5475	-2.4121
Collagen alpha-1(V) chain	P12109	CO6A1_HUMAN	COL6A1	LVTIDGRSDTQRDTPLNV	0		2,3	2100.0811	0.037	59.2	4.E+07	2	-1.0554	1.2432	1.4888
Collagen alpha-1(V) chain	P12109	CO6A1_HUMAN	COL6A1	QYSHSQMGEHS	0		2	1459.6150	0.048	52.7	1.E+07	8	-2.6176	-0.0316	-0.5003
Collagen alpha-1(XIV) chain	Q05707	COEA1_HUMAN	COL14A1	VFTTEEVPAQQVLEIDVTTDSFR	0		2,3	2816.3392	0.000	166.7	6.E+06	11	-2.4274	-0.3436	-0.8350
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GARGLPERGRVGA	1	GARGLP(1)GERGRVGA	2	1618.8652	0.113	69.3	1.E+08	1	0.7930	2.8586	0.4042
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GSPGSPGLPER	1	GSPGSPGLP(1)GER	2	1125.5410	0.028	97.8	4.E+05	1	-2.7069	-0.1752	-4.5097
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GSPGNIGPAGKGPVGLPGIDGRP	3	GSP(1)GNIGPAGKGP(0.002)VGLP(0.998)GIDGRP(1)	3	2246.1291	0.064	86.0	5.E+06	1	-3.0070	-0.1115	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	GVRGPGDAGRPGEPLM	2	GPAGVRGPGDAGRP(1)GEP(1)GLM	2,3	1794.8112	0.019	91.1	5.E+06	3	-2.9732	-0.3157	-1.1215
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	SGGVDFYVGDYFRA	0		2	1745.6958	0.000	145.2	1.E+07	14	-1.6850	0.7243	NaN
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	COL1A2	VGLGPGPMG	0		1	799.3898	0.152	77.9	2.E+06	2	-2.5026	-0.2940	NaN
Collagen alpha-2(V) chain	P05997	CO5A2_HUMAN	COL5A2	GPVSPGLP	3	GP(1)VGSP(1)GLP(1)	1	827.4025	0.091	89.7	6.E+07	6	3.2119	5.2425	NaN
Collagen alpha-2(V) chain	P12110	CO6A2_HUMAN	CO6A2	FSHVAG	0		1	673.3184	0.036	104.9	1.E+06	3	-3.8602	-1.1458	NaN
Collagen alpha-2(V) chain	P12110	CO6A2_HUMAN	CO6A2	LGSDVDMVLT	0		1,2	1280.5806	0.013	111.0	8.E+07	40	0.2976	2.6329	1.2369
Collagen alpha-2(V) chain	A6NMZ7	CO6A6_HUMAN	CO6A6	LLNANLDS	0		1	971.5288	0.031	106.4	1.E+05	2	-3.1398	-0.8015	NaN
Collagen alpha-2(V) chain	P12110	CO6A2_HUMAN	CO6A2	VTMQSPDILL	0		1,2	1216.6373	0.013	104.0	3.E+07	10	-2.1749	0.9699	-0.0771
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	AAEGIPKLLV	0		2	1122.7012	0.033	95.5	9.E+06	5	-2.9678	0.2197	0.8449
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	FMYDRPLRLNLDLDE	1	FMYDRP(1)LRNLDLDE	3	2201.0827	0.023	87.5	3.E+06	1	-2.1313	0.3091	NaN
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	FRELPSLEQL	2,3		2,3	1358.7558	0.000	147.7	4.E+08	61	1.1809	3.2767	4.2496
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	KSVLLDKIKNLQVA	0		2,3	1567.9661	0.100	73.1	4.E+07	2	-0.6385	2.7378	1.6081
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LGPTPTNGAALFVFLRN	1		2	1625.9686	0.000	193.5	1.E+07	10	-1.6325	0.5093	0.4463
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LOPVLQPLSPGVGKRDVWF	0		2	2202.2525	0.000	117.1	6.E+06	7	-3.0584	-0.7657	-0.2520
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LRNLDLDE	0		2	1376.7187	0.000	136.0	3.E+05	2	-4.3541	0.9138	-3.1668
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LTEDTLKIVLYNK	-2,3		2,3	1435.7922	0.026	102.5	2.E+07	3	-2.0618	0.1143	-0.3290
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LTLGLGPTNTGAALFVFLRN	0		2	2153.1845	0.000	191.6	4.E+06	7	-2.9610	-0.6990	-0.4187
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	LVLPAGRDLTDFLENV	2		2	1770.9516	0.001	122.3	2.E+07	30	-1.8448	0.5836	-1.6825
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	PGEDGYRGYPGDEGGPGER	1	GIPGEDGYRGYP(0.967)GDEGGP(0.033)GER	2,3	1979.8246	0.046	79.3	6.E+06	5	-1.7773	0.2732	NaN
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	PGVGGKRDVVF	0		1	1129.6244	0.131	78.1	4.E+06	1	-3.7122	-0.5140	-1.4834
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	VAQKPFESKVDAILNR	0		2,3	1773.9737	0.000	139.5	2.E+08	15	-1.8115	2.7017	3.0137
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	VLOPLPSVGGV	0		1,2	1119.6288	0.000	157.3	4.E+07	32	-0.8885	1.4184	-0.2886
Collagen alpha-3(V) chain	P12111	CO6A3_HUMAN	COL6A3	VREFRELPIIEERIMNS	2,3		2,3	2131.0844	0.000	113.1	1.E+08	10	1.6446	3.9760	2.2189
Decorin	P07585	PGS2_HUMAN	DCN	LHLDNNKLTFRVPGG	3		3	1532.8423	0.007	125.5	2.E+07	5	-1.1238	1.1035	-0.3927
Decorin	P07585	PGS2_HUMAN	DCN	LILVNKISKVSPGA	0		2,3	1551.9348	0.036	82.7	3.E+07	5	-1.3148	1.1352	-0.3433
Fibronectin	P35555	FBN1_HUMAN	FBN1	LGPIPPVLVPPGFPP	2		2	1592.9330	0.002	109.3	2.E+07	17	-2.7646	-0.6033	-1.0630
Fibronectin	P35555	FBN1_HUMAN	FBN1	SPNISACIDINEGEL	0		2	1620.7011	0.090	72.3	6.E+06	2	-2.0677	0.5099	NaN
Lumican	P51884	LUM_HUMAN	LUM	IARLPSGLP	2		2	922.5600	0.114	84.6	2.E+07	1	-1.9802	0.7323	0.3523
Lumican	P51884	LUM_HUMAN	LUM	IARLPSGLPVSL	0		2	1221.7445	0.012	119.5	8.E+07	20	-0.8069	1.2787	1.5403
Lumican	P51884	LUM_HUMAN	LUM	KNIPVTNENLENY	0		2	1710.8101	0.022	110.1	2.E+07	5	-2.1286	0.1970	-1.0631
Lumican	P51884	LUM_HUMAN	LUM	LSFNQIARLPSGLPVSL	2		2	1811.0305	0.036	79.9	2.E+06	1	-4.5068	-1.2079	-3.8748
Microfibril-associated glycoprotein 4	P55083	MFAP4_HUMAN	MFAP4	LIYPSGSPVVP	0		1,2	1224.6754	0.002	145.6	9.E+07	29	0.0929	2.3167	1.2361
Serpin H1	P50454	SERPH_HUMAN	SERPHIN	RSALQSINAWA	2		2	1275.6095	0.030	96.0	6.E+07	4	-4.1829	5.4669	4.4257
Tenascin	P24821	TENA_HUMAN	TNC	IATVDSYVISYTGKVPETIR	3		3	2340.2213	0.173	69.1	9.E+06	1	-2.4106	1.4159	-0.9055
Tenascin	P24821	TENA_HUMAN	TNC	IKDVPGRDRTTDLTDEDNQYS	2,3		2,3	2408.1343	0.000	90.9	2.E+07	5	-1.4715	0.6487	0.7481
Tenascin	P24821	TENA_HUMAN	TNC	NKKSIPVSARVATY	2		2	1533.8515	0.121	71.0	9.E+07	1	-0.2405	1.9431	1.6421
Tenascin-X	P22105	TENX_HUMAN	TNXB	IQFIPTTEGASPPFTARVPS	2		2	2202.1321	0.000	163.9	1.E+07	11	-1.5615	0.6085	-0.7754
Tenascin-X	P22105	TENX_HUMAN	TNXB	LLVDGQSKILISGLEPSTPYRF	0		3	2547.3585	0.002	101.6	3.E+06	2	-1.7289	0.7801	NaN
Tenascin-X	P22105	TENX_HUMAN	TNXB	MRVPEGPGAHFEVLPGDVRQA	3		3	2243.1117	0.004	109.1	1.E+07	4	-1.8527	0.7799	0.7046
Tenascin-X	P22105	TENX_HUMAN	TNXB	WTVPEGQDFH	1,2		1,2	1361.6041	0.000	160.8	2.E+08	43	0.4036	2.9801	2.2208
Vimentin	P08670	VIME_HUMAN	VIM	AKNLQEAEEWYKSK	-2,3		2,3	1722.8577	0.000	156.0	2.E+07	11	-2.1688	0.7391	-0.6449
Viment															

**Supplementary Table S3: Proteins identified via Collagenase Type III LC-MS/MS Proteomics. MWU: Mann Whitney U-Test**

Protein Name	Protein Accession Numbers (Uniprot)	Gene Symbol	Normal Peptide counts (all)	pCAVS Peptide counts (all)	AVI Peptide counts (all)	Normal Peptide Counts (unique)	pCAVS Peptide Counts (unique)	AVI Peptide Counts (unique)	Normal Sequence Coverage [%]	pCAVS Sequence Coverage [%]	AVI Sequence Coverage [%]	Normal LOG2 Transform Intensity	pCAVS LOG2 Transform Intensity	AVI LOG2 Transform Intensity	Log2(FC) (pCAVS/N)	Log2(FC) (AVI/N)	Log2(FC) (CAVS/AVI)	Norm-pCAVS MWU p value	Normal-AVI MWU p value	pCAVS-AVI MWU p value
Collagen alpha-6(VI) chain	sp A6NMZ7 CO6A6	COL6A6	57	170	56	57	170	56	7.0	7.9	5.5	19.87	19.06	19.51	-0.81	-0.36	-0.45	0.226	0.730	0.510
Collagen alpha-1(I) chain	sp P02452 CO1A1	COL1A1	307	1049	266	303	1035	264	23.0	29.6	19.4	26.36	26.34	25.11	-0.02	-1.25	1.23	0.949	0.016	0.001
Collagen alpha-1(III) chain	sp P02461 CO3A1	COL3A1	196	687	167	130	437	99	21.5	26.8	18.3	23.74	23.37	22.58	-0.37	-1.16	0.79	0.661	0.063	0.180
Collagen alpha-1(IV) chain	sp P02462 CO4A1	COL4A1	90	229	73	90	227	71	13.4	14.8	9.5	21.80	21.00	21.16	-0.80	-0.64	-0.16	0.343	0.413	1.000
Prelamin-A/C	sp P02545 LMNA	LMNA	11	48	20	11	48	20	6.6	9.7	8.7	17.01	17.10	17.59	0.09	0.58	-0.49	1.000	1.000	1.165
Complement C1q subcomponent subunit A	sp P02745 C1QA	C1QA	16	51	8	16	51	8	12.3	13.1	5.1	17.17	17.37	18.03	0.19	0.86	-0.66	0.851	1.000	0.513
Fibronectin	sp P02751 FN1	FN1	50	334	95	50	334	95	6.4	14.5	9.7	19.77	21.35	21.92	1.59	2.15	-0.57	0.040	0.032	0.320
Vitronectin	sp P04004 VTNC	VTNC	9	45	5	2	17	1	4.4	8.3	1.9	0.00	17.25	0.00	17.25	0.00	17.25	UTC	UTC	UTC
Collagen alpha-2(V) chain	sp P05997 CO5A2	COL5A2	81	323	61	81	322	61	11.9	15.9	7.7	22.56	22.51	21.59	-0.05	-0.97	0.92	0.949	0.032	0.005
Decorin	sp P07595 PGS2	DCN	112	392	89	112	390	89	43.0	52.4	41.4	21.72	22.17	21.53	0.45	-0.19	0.64	0.226	0.413	0.180
Collagen alpha-2(I) chain	sp P08123 CO1A2	COL1A2	487	1538	380	485	1533	379	33.9	37.7	28.2	26.23	26.09	25.28	-0.14	-0.95	0.81	1.000	0.016	0.001
Collagen alpha-2(IV) chain	sp P08572 CO4A2	COL4A2	68	136	55	68	134	54	6.9	6.1	5.4	20.66	19.91	19.96	-0.75	-0.70	-0.05	0.489	0.556	0.827
Vimentin	sp P08670 VIME	VIM	300	1224	456	294	1196	446	72.6	83.2	83.9	23.95	24.37	24.68	0.42	0.73	-0.31	0.489	0.190	0.827
SPARC	sp P09486 SPRC	SPARC	4	18	2	4	18	2	5.8	9.2	1.7	0.00	16.67	0.00	16.67	0.00	16.67	UTC	UTC	UTC
Collagen alpha-1(XI) chain	sp P12107 COB1	COL11A1	57	202	45	18	85	19	6.7	9.2	5.2	20.31	19.58	18.84	-0.72	-1.47	0.74	0.190	0.667	0.333
Collagen alpha-1(VI) chain	sp P12109 CO6A1	COL6A1	256	944	262	254	936	261	37.5	45.1	31.2	24.37	24.20	24.20	-0.18	-0.17	0.00	0.226	0.286	0.913
Collagen alpha-2(VI) chain	sp P12110 CO6A2	COL6A2	243	848	238	241	840	235	30.9	34.6	27.1	23.79	23.78	23.43	-0.02	-0.36	0.35	0.949	0.190	0.221
Collagen alpha-3(VI) chain	sp P12111 CO6A3	COL6A3	724	2839	842	724	2834	842	32.0	38.9	31.8	25.23	25.62	25.86	0.39	0.63	-0.24	0.138	0.063	0.441
Collagen alpha-1(V) chain	sp P20908 CO5A1	COL5A1	147	503	118	108	385	91	12.1	14.7	9.5	22.94	23.03	22.95	0.09	-0.59	0.68	0.753	0.063	0.027
Biglycan	sp P21810 PGS1	BGN	207	722	235	207	720	235	44.0	50.9	43.5	23.78	23.88	24.29	0.10	0.50	-0.41	1.000	0.413	0.583
Tenascin-X	sp P22105 TNX	TNXB	114	660	134	115	660	134	11.5	20.9	11.0	20.95	22.75	21.87	1.80	0.93	0.87	0.056	0.730	0.320
Fibulin-1	sp P223142 FBLN1	FBLN1	5	20	9	5	20	6	3.0	4.2	2.9	16.80	17.88	0.00	1.08	-16.80	17.88	0.056	UTC	UTC
Tenascin	sp P24821 TENA	TNC	18	163	43	18	163	43	2.7	8.9	5.0	18.95	19.77	19.65	0.81	0.69	0.12	0.225	0.143	0.827
Fibrillin-1	sp P35555 FBN1	FBN1	25	113	32	25	113	32	2.1	2.5	1.5	19.85	20.89	21.03	1.03	1.18	-0.14	0.280	0.190	0.827
Myosin-10	sp P35580 MYH10	MYH10	15	73	26	15	73	26	3.6	3.0	3.0	17.70	18.27	19.28	0.57	1.57	-1.01	1.000	0.533	0.412
Collagen alpha-1(XV) chain	sp P39059 COFA1	COL15A1	46	139	41	46	139	41	8.0	8.4	5.1	20.28	19.85	19.79	-0.43	-0.49	0.06	0.753	0.413	0.827
Collagen alpha-1(XVIII) chain	sp P39060 CO1A1	COL18A1	35	39	15	35	39	15	6.9	2.9	2.4	19.97	18.37	18.96	-1.60	-1.01	-0.59	0.374	1.000	0.545
Cartilage oligomeric matrix protein	sp P49747 COMP	COMP	16	69	15	12	56	14	5.5	9.0	5.3	18.48	18.85	18.82	0.37	0.34	0.03	0.769	1.000	0.949
Serpin H1	sp P50454 SERPH	SERPINH	3	18	12	3	18	12	3.0	5.3	7.9	0.00	0.00	18.44	0.00	18.44	-18.44	UTC	UTC	UTC
Lumican	sp P51884 LUM	LUM	103	418	130	60	271	86	28.0	42.0	35.8	22.24	22.40	22.40	0.17	0.00	0.489	0.556	0.827	0.827
Microfibril-associated glycoprotein 4	sp P55083 MFAP4	MFAP4	62	171	43	62	171	43	31.0	34.3	21.3	20.59	20.93	20.05	0.34	-0.54	0.88	0.571	0.486	0.177
Basement membrane-specific heparan sulfate	sp P98160 PGBM	HSPG2	159	422	161	159	422	161	8.7	8.7	7.3	21.68	20.90	21.80	-0.78	0.12	-0.90	0.280	0.905	0.377
Collagen alpha-1(XIV) chain	sp Q05707 COE1	COL14A1	52	159	90	52	159	90	8.7	9.1	11.2	19.82	19.09	21.39	-0.73	1.57	-2.30	0.280	0.286	0.090
Fibromodulin	sp Q06828 FMOD	FMOD	121	443	104	117	430	103	44.3	50.7	34.3	21.64	21.65	21.11	0.00	-0.53	0.54	1.000	0.190	0.267
Collagen alpha-1(XVI) chain	sp Q07092 COG1	COL16A1	30	123	32	28	111	29	5.0	8.2	4.9	20.28	20.03	19.01	-0.25	-1.27	1.02	0.753	0.114	0.138
Dermatopontin	sp Q07507 DERM1	DPT	19	73	21	19	73	21	17.2	22.0	18.4	18.74	18.74	18.31	0.00	-0.43	0.42	0.641	0.133	0.280
Nidogen-2	sp O14112 NID2	NID2	11	50	17	11	50	17	2.1	3.5	2.4	17.35	17.87	18.03	0.52	0.67	-0.15	0.571	1.000	1.000
Transforming growth factor-beta-induced prc	sp Q15582 BGH3	BGH3	47	277	70	47	277	70	15.7	29.9	19.2	19.44	21.28	21.08	1.84	1.64	0.21	0.018	0.190	0.441
Collagen alpha-1(XXI) chain	sp Q96P44 COL1A1	COL21A1	18	55	14	18	50	14	6.3	7.5	4.3	19.00	19.24	18.58	-0.23	-0.42	0.65	0.489	0.057	0.126
Collagen alpha-1(XII) chain	sp Q99715 COCA1	COL12A1	56	197	64	56	197	64	5.6	7.0	5.4	19.61	19.35	19.29	-0.25	-0.32	0.06	0.753	0.905	1.000
Asporin	sp Q9BXN1 ASPN	ASPN	42	115	38	42	115	38	4.2	22.9	17.3	23.02	22.74	23.00	-0.28	-0.02	0.00	0.571	1.000	0.743
Dentin sialophosphoprotein	sp Q9NZW4 DPPP	DPPP	2	11	0	2	11	0	0.7	1.3	0.0	0.00	0.00	0.00	0.00	0.00	0.00	UTC	UTC	UTC
EMILIN-1	sp Q9Y6C2 EMIL1	EMILIN1	24	109	32	24	109	32	7.5	10.9	7.2	18.64	19.04	19.21	0.40	0.57	-0.17	0.280	0.286	0.661

**Supplementary Table S4:** Gene Ontology analysis results of non-collagen type proteins identified via Collagenase Type III LC-MSMS Proteomics. P-value is Fisher's exact p-value. FDR: false discovery rate

Fold Enrichment	Function	GO	Proteins	Protein Count	p-value	FDR	-LOG10(FDR)
88.65	Basement Membrane	GO:0005604	TNC, LOXL1, HSPG2, BGH3, VTN, NID2, SPARC, FN1, FBN1, FBLN1	10	1.66E-17	1.11E-14	13.95
44.61	Collagen-containing ECM	GO:0062023	TNC, LOXL1, C1QA, TNXB, ASPN, MFAP4, DPT, BGN, FMOD, HSPG2, DCN, LUM, EMILIN1, COMP, BGH3, VTN, NID2, SPARC, FN1, FBN1, FBLN1	21	4.58E-33	9.2E-30	29.04
96.53	Collagen fibril organization	GO:0030199	TNXB, DPT, FMOD, LUM, COMP	5	2.64E-09	5.26E-06	5.28
100	Collagen binding	GO:0005518	ASP, DCN, LUM, COMP, BGH3, VTN, NID2, SPARC, FN1	10	1.24E-16	1.96E-13	12.71
62.06	Collagen metabolism	GO:0010712	MFAP4, VIM, EMILIN-1	3	1.84E-05	0.00666	2.18

**Supplemental Table S5:** Table outlining which valves were used for each experiment in the manuscript. #: Representative valves in **Supplemental Figure S1**. NI-Not included. Movat's Pentachrome and Herovici stain inclusion were based on sections with sufficient valve structure reporting. Proteomic inclusion was based on system suitability performance. IHC-BAMBI inclusion was done to verify presence of BAMBI protein in representative valve tissue sections. FFPE: Formalin-Fixed Paraffin Embedded. For qPCR studies, see **Supplemental Table S7**.

	SKU	Age (years)	Movat's Pentachrome	Herovici	Picrosirius Red	Proteomics	IHC-BAMBI
<b>Tissue Type</b>	-	-	FFPE	FFPE	FFPE	FFPE	FFPE
<b>Normal</b>	DB17	0.05	x	NI	x	NI	NI
	DB051	0.66	x	x	x	x	NI
	NDRI	2	x	x		x	x
	DB85	9	x	x	x	x	NI
	DB27	17	x	x	x	x	x
<b>pCAVS</b>	DB106	0.04	x	x	x	x	NI
	DB43	0.15	x	x	x	x	NI
	DB40	0.75	x	x	x	x	NI
	DB105	5	x	x	x	x	
	DB119	5	x	x	x	x	x
	DB113	5	#	x	x	x	x
	DB18	6	x	x	x	x	NI
	DB3	11	x	x	x	x	x
	DB117	12	x	x	x	x	NI
	DB41	14	x	x	x	x	x
	DB16	17	x	x	x	x	x
<b>AVI</b>	DB114	0.17	x	x	x	x	
	DB007	0.58		x	x	x	
	DB108	10	x	x	x	x	
	DB83	10	x	x	x	x	
	DB032	16	#	x	x	x	

**Supplemental Table S6.** Primary literature and reviews associated with aortic valve development & disease related to the BAMBI interactive network. Data were acquired by searching Pubmed and Pubmed Central with the gene names AND “aortic valve” or “aortic valve development” of “bicuspid”. The majority of the literature on aortic valve disease covers adult calcific aortic valve stenosis and very little has been done on pediatric end-stage aortic valve stenosis.

Gene	Gene Symbol	Role in Aortic Valve	References
Activin receptor-like kinase 2	ACVR1	Depletion in cushion mesenchyme leads to bicuspid aortic valve Epithelial-mesenchymal transformation in valve development	1,2
Bone Morphogenetic Protein Receptor Type 1A	BMPR1A	Activation required for aortic valve calcification	3
B-catenin	CTNNB1	Endocardial cushion development Pressure overload induced fibrosis	4,5
Dishevelled Segment Polarity Protein 2	DVL2	Upregulated in adult stenotic aortic valves	6
Estrogen Receptor Beta	ESR2	Increases in patients with aortic valve stenosis Sex specific disease features in adult aortic valve disease	7 ;Review <sup>8</sup>
Histone Acetylation and Deacetylation	HDAC1	Epigenomic alterations in aortic valve stenosis	9
LDL Receptor Related Protein 6	LRP6	Essential for outflow tract development Increased receptor expression in adult calcific aortic valve Promotes calcification in aortic valve	10,11 ; Review <sup>12</sup>
Lamin A/C	LMNA	Genetic mutation with congenital aortic valve malformation Activation of osteogenic differentiation in aortic valve interstitial cells Decreased in bicuspid aortic valve	13-15
Nuclear Factor kappa B	NFkB	Valvular endocardial cushion development Cell type differentiation during calcific aortic valve disease Inflammatory regulation in early calcific aortic valve disease	16-19
SMAD Family Member 6	SMAD6	Epithelial-mesenchymal transformation in valve development Alteration of ECM in aortic development Variants in congenital aortic valve disease	2,20-22
SMAD Family Member 7	SMAD7	Outflow tract malformations Increases valvular calcification with the downregulation of miR-195 by SMAD7 targeting	23,24
SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4	SMARCA4	Mutational effects in bicuspid aortic valve	25
Transforming Growth Factor Beta Receptor 2	TGFBR2	Epithelial-mesenchymal transformation in valve development & disease Ligand regulation of AV cushion endocardial cells Mutations in bicuspid aortic valve	26-28

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**Supplemental Table S7:** Descriptive statistics of patient characteristics for aortic valve samples used for rt-qPCR studies (**Fig. 6**). Normal aortic valves are from cardiac transplant patients where normal valve function is defined via 2D echocardiogram. 2D Echocardiogram mean gradients define pre-operative valve function where a normal gradient is defined as <5 mmHg, mild stenosis as 5-25 mmHg, moderate stenosis as 25-50 mmHg, and severe stenosis as >50 mmHg. BSA: body surface area; C: Caucasian. DB43 was included in pCAVS due to stenosis, but main diagnosis was Hypertrophic Cardiomyopathy

	SKU	Age (years)	Pediatric Age Group	Gender	Race/Ethnicity	BSA (m <sup>2</sup> )	Leaflet Morphology	Aortic Valve Function
Normal	DB85	9	Child	F	C	0.95	Trileaflet	Normal
	DB27	17	Adolescent	M	C	2.2	Trileaflet	Normal
	<b>Normal Averages</b>	<b>7.17 ± 7.5</b>		<b>75%M 25%F</b>	<b>75%C 25%L</b>	<b>1.03</b>		
pCAVS	DB43	0.15	Infant	M	C	0.24	Trileaflet	Moderate stenosis with AVI*
	DB40	0.75	Infant	M	C	0.37	Bicuspid	Moderate Stenosis
	DB105	5	Young Child	M	C	0.72	Bicuspid	Moderate Stenosis with AVI
	DB119	5	Young Child	F	-	-	Bicuspid	Moderate Stenosis with AVI
	DB113	5	Young Child	-	-	-	-	Trivial Stenosis; Severe AVI
	DB18	6	Child	F	C	0.7	Bicuspid	Moderate Stenosis with AVI
	DB117	12	Adolescent	M	-	-	Bicuspid	Moderate Stenosis with AVI
	DB41	14	Adolescent	M	C	1.6	Bicuspid	Moderate Stenosis with AVI
	DB135	17	Adolescent	M	-	-	-	Moderate Stenosis with AVI
	DB136	17	Adolescent	M	-	-	Bicuspid	Moderate Stenosis with AVI
	DB162	17	Adolescent	F	-	-	Bicuspid	Moderate Stenosis with AVI
	DB191	17	Adolescent	M	-	-	Bicuspid	Moderate Stenosis with AVI
<b>pCAVS Averages</b>	<b>6.90 ± 5.8</b>		<b>73%M 27%F</b>	<b>100%C</b>	<b>0.93</b>			
aCAVS	DB226	19	Adult	F	-	-	Trileaflet	Moderate Stenosis; no calcification
	DB176	25	Adult	F	-	-	Trileaflet	Moderate Stenosis with AVI; calcification
	DB174	26	Adult	M	-	-	Bicuspid	Moderate Stenosis; no calcification
	DB221	26	Adult	M	-	-	Bicuspid	Moderate Stenosis with AVI; no calcification
	DB284	36	Adult	F	-	-	Bicuspid	Severe Stenosis; no calcification
<b>aCAVS Averages</b>	<b>26.4 ± 6.1</b>		<b>40%M 60%F</b>	<b>100%C</b>	<b>-</b>			
Ross	DB152	22	Adult	M	-	-		Pulmonary-AV Autograft with AVI