

**Table S1** Baseline characteristics of the included BAV patients and healthy controls

Demographics	BAV patients (n=30)	Healthy controls (n=15)	P value
Age (years)	54.1±13.1	51.1±7.0	0.403 <sup>a</sup>
Male gender	15 (50.0)	12 (80.0)	0.063 <sup>b</sup>
Hypertension	10 (33.3)	0 (0)	0.019 <sup>b</sup>
Diabetes	1 (0.3)	0 (0)	>0.999 <sup>b</sup>
Coronary artery disease	2 (0.7)	0 (0)	0.545 <sup>b</sup>
Hyperlipidemia	2 (0.7)	0 (0)	0.545 <sup>b</sup>
History of smoking	8 (26.7)	2 (13.3)	0.456 <sup>b</sup>
Ascending aortic diameter (mm)	43.9±6.9	31.8±2.2	<0.001 <sup>a</sup>
Ascending aortic diameter ≥45 mm	17 (56.7)	0 (0)	<0.001 <sup>b</sup>
Severity of aortic stenosis			<0.001 <sup>c</sup>
Mild	2 (6.7)	0 (0)	
Moderate	7 (23.3)	0 (0)	
Severe	21 (70.0)	0 (0)	

Continuous variables are expressed as means ± standard deviations, and categorical variables as numbers (percentages). Data are compared using <sup>a</sup>Student's t-test, <sup>b</sup>Fisher's exact test, and <sup>c</sup>Cochran-Mantel-Haenszel test. BAV, bicuspid aortic valve.

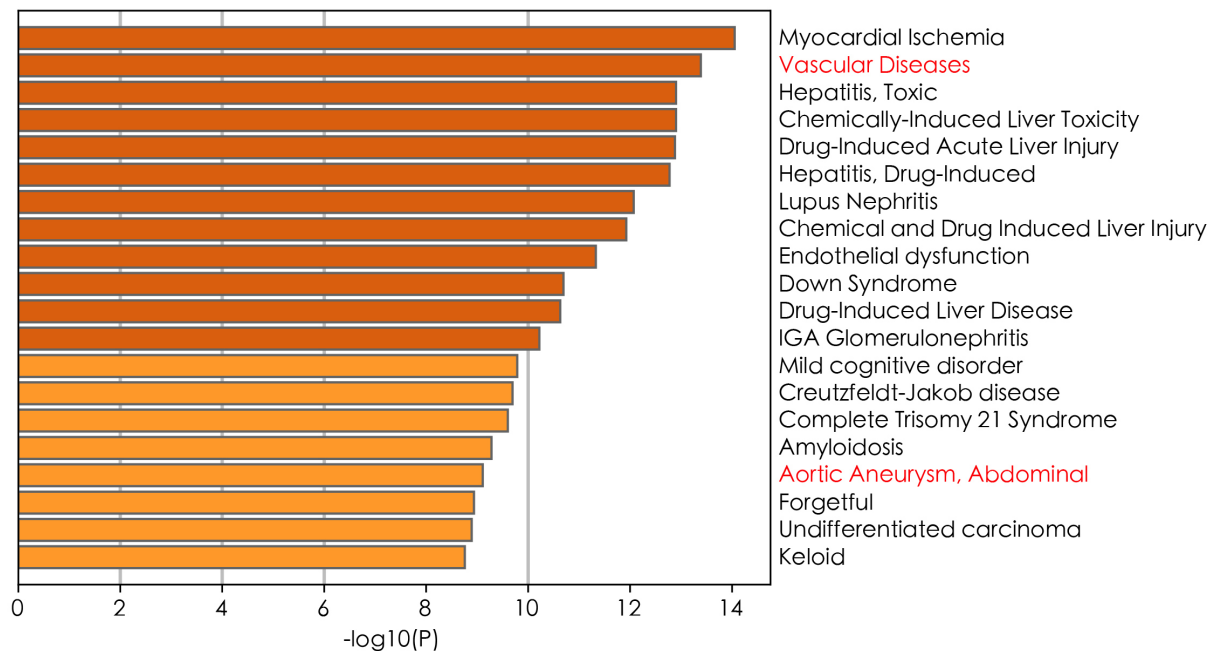
**Table S2** Baseline characteristics of the patients from whom the aortic smooth muscle cells were harvested

Patients	Age (year)	Gender	Hypertension	Ascending aortic diameter (mm)	Aortic stenosis
Control-1	38	Female	No	N/A	No
Control-2	22	Male	No	N/A	No
BAV-1	50	Female	No	43	Severe
BAV-2	46	Male	Yes	53	Severe
BAV-3	74	Male	Yes	53	Severe

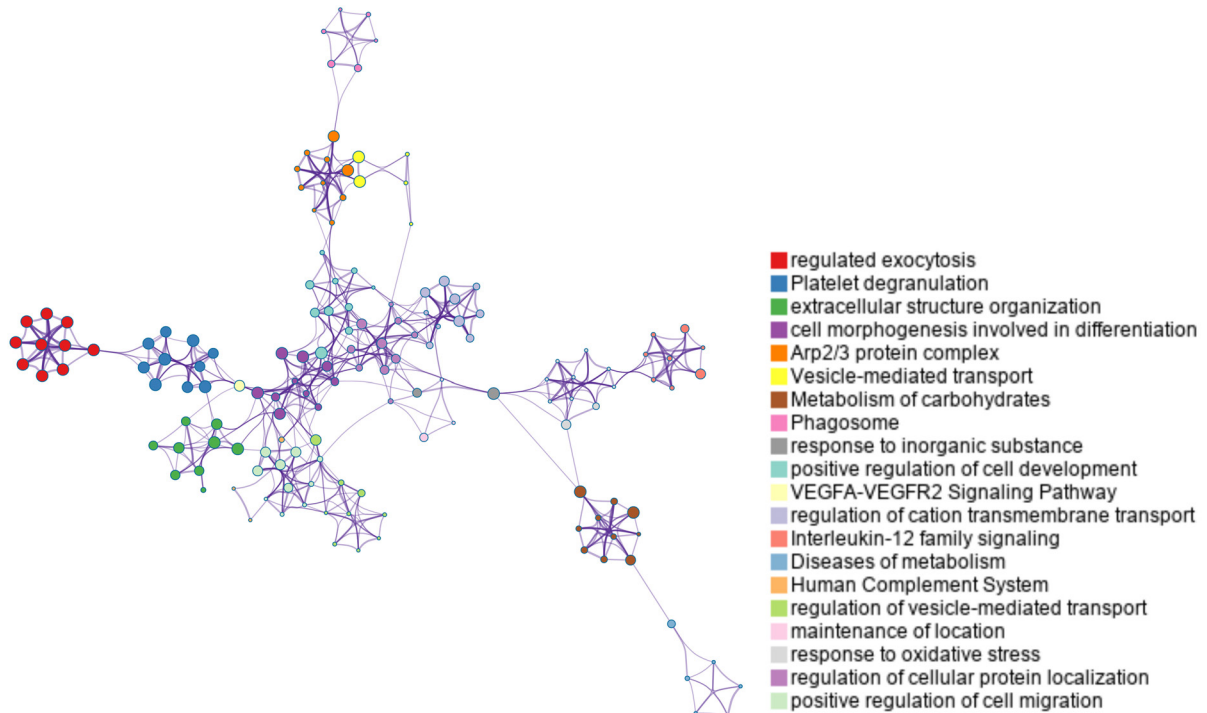
BAV, bicuspid aortic valve.

**Table S3** DEPs between BAV patients and healthy controls using cutoff values of P<0.05 and fold-change ratio >1.5

logFC	AveExpr	t	PValue	adj.P.Val	B	UNIPROT	Gene name
2.767056	23.70452	3.008633	0.004247	0.022369	-2.51371	P20742	PZP
2.628587	23.89302	5.256278	3.70E-06	9.55E-05	4.167766	Q9P2P1	NYNRIN
2.620848	19.26652	7.382316	2.44E-09	3.04E-07	11.26183	P07602	PSAP
2.326093	24.50175	6.706861	2.51E-08	1.56E-06	9.00065	P59666	DEFA3
1.821323	18.34192	6.975828	9.90E-09	1.06E-06	9.902823	Q6UY14	ADAMTSL4
1.781201	17.69439	7.788113	6.08E-10	1.14E-07	12.60953	Q8WZ42	TTN
1.740143	20.04425	6.785227	1.91E-08	1.30E-06	9.263643	P0DMV9	HSPA1B
1.668907	17.62123	5.757642	6.67E-07	2.38E-05	5.822482	Q07954	LRP1
1.625418	22.24402	5.873392	4.48E-07	1.86E-05	6.207936	Q8NBJ4	GOLM1
1.577001	20.68018	5.577753	1.24E-06	4.21E-05	5.225652	P07237	P4HB
1.531367	21.56249	4.959956	1.01E-05	0.000228	3.205701	P63241	EIF5A
1.525534	21.61898	8.305536	1.05E-10	3.01E-08	14.30949	P40925	MDH1
1.504118	20.60417	8.264965	1.21E-10	3.01E-08	14.17707	P04179	SOD2
1.446448	21.18032	6.859462	1.48E-08	1.20E-06	9.512687	O15511	ARPC5
1.434917	20.59583	5.983239	3.07E-07	1.43E-05	6.574571	P14314	PRKCSH
1.413736	21.89214	4.452534	5.37E-05	0.000855	1.598457	O15143	ARPC1B
1.383253	19.35201	4.002136	0.000226	0.002731	0.229096	P16109	SELP
1.355334	18.20865	3.483163	0.001098	0.009255	-1.25935	P02452	COL1A1
1.343631	20.96687	3.208893	0.002429	0.015141	-1.9983	O15144	ARPC2
1.335867	21.95904	6.11232	1.96E-07	1.05E-05	7.00626	P80188	LCN2
1.32445	17.94713	2.89298	0.005813	0.028606	-2.80131	P11216	PYGB
1.306756	19.73355	2.736812	0.008788	0.037139	-3.17724	P62873	GNB1
1.276435	18.91974	3.180461	0.002632	0.015811	-2.07277	Q9NZK5	ADA2
1.273504	21.82659	3.24801	0.002173	0.014014	-1.89517	O15037	KHNYN
1.244134	20.18369	4.280279	9.37E-05	0.001323	1.06744	Q86U17	SERPINA11
1.230921	19.33215	4.24268	0.000106	0.001464	0.952675	P24821	TNC
1.213669	23.9209	5.105618	6.16E-06	0.000154	3.676816	O95810	CAVIN2
1.202976	18.60707	4.912257	1.18E-05	0.000259	3.052263	P48637	GSS
1.197772	19.5404	3.947472	0.000268	0.002997	0.067391	Q9Y5C1	ANGPTL3
1.1814	21.86502	5.90132	4.07E-07	1.79E-05	6.301078	P18669	PGAM1
1.158584	19.44166	2.867433	0.006225	0.029284	-2.8638	P36871	PGM1
1.152961	18.34589	4.311577	8.48E-05	0.001219	1.163292	Q16706	MAN2A1
1.136316	18.14065	2.964793	0.004787	0.024525	-2.62363	P22897	MRC1
1.119627	17.90809	3.114158	0.003171	0.017834	-2.2448	P29350	PTPN6
1.114146	21.15054	4.466956	5.13E-05	0.000834	1.643291	P52209	PGD
1.100415	21.5133	5.29247	3.27E-06	8.97E-05	4.286204	P50395	GDI2
1.095313	18.11763	4.350417	7.48E-05	0.001119	1.282638	P08758	ANXA5
1.09237	18.88366	4.103652	0.000164	0.002085	0.532145	Q9H4A9	DPEP2
1.072839	19.86737	3.402571	0.001391	0.01062	-1.48023	P51149	RAB7A
1.065709	17.47763	3.974212	0.000247	0.002842	0.146358	P07942	LAMB1
1.003138	21.14033	4.127838	0.000152	0.001999	0.604856	Q04917	YWHAH
0.998304	19.06763	2.30747	0.02558	0.080393	-4.1308	P05067	APP
0.972807	21.38062	3.332812	0.001704	0.01219	-1.66895	O15145	ARPC3
0.968451	23.04386	5.070737	6.93E-06	0.000167	3.563667	P31146	CORO1A
0.967585	17.72127	3.126464	0.003064	0.017361	-2.21304	P22314	UBA1
0.967195	23.10594	5.988343	3.01E-07	1.43E-05	6.591626	P26038	MSN
0.964596	20.40342	2.783331	0.00778	0.033836	-3.0668	P55072	VCP
0.942494	18.14881	2.885752	0.005927	0.028751	-2.81903	Q07960	ARHGAP1
0.937264	18.75228	3.478534	0.001114	0.009255	-1.27212	P40189	IL6ST
0.921912	26.92135	4.343473	7.65E-05	0.001122	1.261272	P02671	FGA
0.918051	20.36725	2.899247	0.005716	0.028314	-2.78592	Q14847	LASP1
0.916045	19.98295	4.769317	1.90E-05	0.000384	2.595146	Q9BY67	CADM1
0.91168	25.42008	3.464393	0.001161	0.009543	-1.31106	Q9BXR6	CFHR5
0.911158	19.97956	2.879318	0.00603	0.028751	-2.83478	P31150	GDI1
0.905598	20.76018	3.51109	0.001011	0.008763	-1.18212	P29401	TKT
0.879173	22.29589	2.867647	0.006221	0.029284	-2.86328	Q9ULV4	CORO1C
0.871934	22.81204	6.166436	1.63E-07	9.37E-06	7.187463	Q99497	PARK7
0.869974	23.75752	4.722391	2.22E-05	0.000425	2.446018	P08637	FCGR3A
0.857112	20.5012	3.108323	0.003223	0.017916	-2.25982	P07384	CAPN1
0.848499	23.88176	2.39104	0.020948	0.069028	-3.95491	P62979	RPS27A
0.831939	19.43033	2.458389	0.017781	0.060731	-3.80964	Q9Y696	CLIC4
0.831847	19.13566	2.591887	0.01275	0.049673	-3.51268	P07307	ASGR2
0.831545	18.10959	2.197487	0.033059	0.097984	-4.35471	P07911	UMOD
0.827684	21.20568	2.343397	0.023487	0.075724	-4.05578	Q9Y2K3	MYH15
0.819518	18.53301	2.403387	0.020332	0.067593	-3.92851	O43396	TXN1
0.816386	23.23592	2.81119	0.007229	0.032575	-3.00002	Q92686	NRGN
0.809804	19.98158	2.188888	0.033718	0.099258	-4.37184	Q7Z7G0	ABI3BP
0.808821	18.19872	3.1974	0.001769	0.012368	-1.70405	P10619	CTSA
0.806301	23.3074	3.179156	0.002642	0.015811	-2.07618	P60709	ACTB
0.794206	20.13511	3.283499	0.001964	0.012998	-1.80093	P09493	TPM1
0.785136	19.71834	3.35652	0.001591	0.011782	-1.60507	P06576	ATP5F1B
0.765696	21.31632	2.91681	0.005452	0.027368	-2.74268	P68036	UBE2L3
0.743636	22.49809	3.535293	0.000941	0.008508	-1.1149	Q15942	ZYX
0.742917	21.5304	2.419202	0.019567	0.065339	-3.89455	Q92496	CFHR4
0.737743	26.19028	3.594723	0.000789	0.007402	-0.94875	P37802	TALN2
0.735802	26.77309	4.560714	3.77E-05	0.000642	1.936072	P19652	ORM2
0.726218	22.70613	4.182818	0.000128	0.00174	0.770853	P22392	NME2
0.722578	19.42471	2.355916	0.022794	0.07448	-4.02943	P54289	CACNA2D1
0.717511	22.65345	3.209774	0.002423	0.015141	-1.99599	Q3ZCW2	LGALS1
0.706609	18.52895	2.747383	0.008549	0.036542	-3.15226	P16152	CBR1
0.700815	26.00444	2.670776	0.010427	0.042388	-3.33173	P02675	FGF
0.700047	26.09667	2.700721	0.009652	0.040109	-3.26202	P02679	FGG
0.699753	19.04766	2.124067	0.039072	0.108646	-4.49926	P49721	PSMB2
0.69882	23.53883	3.620495	0.00073	0.007	-0.87622	P78417	GSTO1
0.695439	18.9113	2.496297	0.016195	0.059078	-3.72652	Q9NY97	BGN2
0.691181	18.62286	2.059213	0.04516	0.120216	-4.6236	Q16851	UGP2
0.68586	21.72962	2.423178	0.019378	0.065	-3.88598	Q15555	MAPRE2
0.683538	19.73187	2.132891	0.038302	0.108468	-4.4821	P17174	GOT1
0.681045	21.99428	3.534363	0.000944	0.008508	-1.11749	P61158	ACTR3
0.674793	18.95101	2.156041	0.036344	0.104159	-4.4368	Q9UEW3	MARCO
0.662929	22.62578	3.095966	0.003336	0.018214	-2.29159	P21926	CD9
0.660237	17.99514	2.63897	0.011312	0.044771	-3.40517	Q9UQ80	PA2G4
0.659068	21.20019	2.694965	0.009797	0.040485	-3.27546	P37837	TALDO1
0.640482	21.61244	2.119224	0.0395	0.10943	-4.50866	P61160	ACTR2
0.633471	20.83872	2.84901	0.006539	0.030191	-2.90863	Q8NBP7	CKSR9
0.627065	23.93667	3.340123	0.001668	0.012114	-1.64928	P27797	PICALR
0.619891	24.00249	2.508425	0.015716	0.058194	-3.69972	Q02985	CFHR3
0.619094	23.17463	3.579187	0.000826	0.007628	-0.99233	P62258	YWHAE
0.612037	21.75361	3.155757	0.002822	0.016588	-2.13714	P99999	CYCS
0.59931	18.83744	2.092803	0.041911	0.114832	-4.5596	O75874	IDH1
0.595465	21.32069	2.506177	0.015803	0.058232	-3.7047	P06744	GPI
0.59379	22.67773	2.18733	0.033839	0.099258	-4.37494	P10124	SRGN
-0.58716	21.87824	-2.87903	0.006035	0.028751	-2.83548	P08493	MGP
-0.58849	18.70042	-2.30208	0.025908	0.080745	-4.14198	O00194	RAB27B
-0.58932	27.74555	-3.10717	0.003233	0.017916	-2.26278	O75636	FCN3
-0.59167	21.25995	-2.90088	0.005691	0.028314	-2.78191	O75144	ICONS1
-0.59196	18.7551	-2.29254	0.026497	0.08224	-4.1617	P35590	TIE1
-0.59801	20.68082	-2.51436	0.015486	0.057628	-3.68658	AAO075B6H9	IGLV4-69
-0.59855	27.78072	-4.60247	3.29E-05	0.000597	2.067192	P29622	SERPINA4
-0.6164	18.06488	-2.7871	0.007704	0.033698	-3.05779	P30085	CMFPK1
-0.61804	19.69274	-3.44969	0.001212	0.009856	-1.35145	Q9ULI3	HEG1
-0.62678	19.32313	-2.31836	0.024928	0.079685	-4.10816	Q96HR3	MED30
-0.62733	19.11292	-2.17555	0.034763	0.101179	-4.39831	P24666	ACP1
-0.62896	18.24661	-2.15658	0.0363	0.104159	-4.43575	P35237	SERPINB6
-0.63872	30.22683	-3.25388	0.002137	0.013901	-1.87963	P02656	APOC3
-0.6389	18.76625	-2.30393	0.025795	0.08073	-4.13814	Q15185	PTGES3
-0.64104	32.99921	-5.53509	1.43E-06	4.66E-05	5.084573	P02766	TTR
-0.645	20.84826	-2.55222	0.014089	0.053769	-3.60214	Q9UJJ9	GNPTG
-0.6547	21.39109	-3.80408	0.000418	0.004405	-0.35156	Q14515	SPARCL1
-0.665	18.11266	-2.10879	0.040437	0.111612	-4.52883	Q9BS26	ERP44
-0.66778	21.61836	-2.09333	0.041861	0.114832	-4.55858	P48059	LIMS1
-0.6922	20.12897	-2.35388	0.022906	0.07448	-4.03373	O14672	ADAM10
-0.69248	20.61653	-2.3131	0.025241	0.080304	-4.11911	O7536	



**Figure S1** Disease enrichment analysis of the DEPs between BAV patients and controls. Cardiovascular diseases such as vascular diseases and abdominal aortic aneurysm (labeled red) are highly enriched. DEPs, differentially expressed proteins; BAV, bicuspid aortic valve.



**Figure S2** The top 20 clusters of the enriched biological processes of the DEPs between BAV patients and control. DEPs, differentially expressed proteins; BAV, bicuspid aortic valve.

**Table S4** DEPs between BAV patients with and without progressive aortic dilation using cutoff values of P<0.05 and fold-change ratio >1.5

P value	logFC	Adjust P value	UNIPROT	Gene name
0.023843	-0.78052	0.375216	A0A075B6K4	<i>IGLV3-10</i>
0.007486	-1.77323	0.294705	A0A0J9YXX1	<i>IGHV5-10-1</i>
0.015418	0.391122	0.365986	O00533	<i>CHL1</i>
0.017985	-1.40035	0.365986	O14672	<i>ADAM10</i>
0.005385	-2.06706	0.260209	O43488	<i>AKR7A2</i>
0.002163	1.22667	0.257634	O43493	<i>TGOLN2</i>
0.010086	1.664424	0.328	O43852	<i>CALU</i>
0.018944	1.0609	0.366444	O76074	<i>PDE5A</i>
0.03157	-0.57577	0.390214	P00441	<i>SOD1</i>
0.020188	-0.54302	0.366444	P00533	<i>EGFR</i>
0.049681	-0.32422	0.502177	P00568	<i>AK1</i>
0.005015	-0.58994	0.260209	P00740	<i>F9</i>
0.00932	-0.19366	0.316878	P00742	<i>F10</i>
0.000971	0.304493	0.242217	P01008	<i>SERPINC1</i>
0.021269	-0.37487	0.366444	P01031	<i>C5</i>
0.029746	-1.04776	0.390214	P01876	<i>IGHA1</i>
0.03172	-0.8792	0.390214	P02655	<i>APOC2</i>
0.002227	-2.45432	0.257634	P02741	<i>CRP</i>
0.035287	-0.42657	0.410234	P02753	<i>RBP4</i>
0.000256	0.508344	0.191618	P05090	<i>APOD</i>
0.028416	-2.59689	0.390214	P05121	<i>SERPINE1</i>
0.005419	-0.36726	0.260209	P05156	<i>CFI</i>
0.017291	-0.48839	0.365986	P07357	<i>C8A</i>
0.005568	1.556691	0.260209	P07911	<i>UMOD</i>
0.026802	0.436539	0.384033	P08185	<i>SERPINA6</i>
0.000635	-3.24178	0.237391	P09972	<i>ALDOC</i>
0.026296	-0.56556	0.384033	P0C0L4	<i>C4A</i>
0.017998	1.462011	0.365986	P10321	<i>HLA-C</i>
0.02168	-1.16282	0.366444	P10644	<i>PRKAR1A</i>
0.0031	0.686498	0.257634	P10646	<i>TFPI</i>
0.036714	-2.05085	0.410234	P13224	<i>GP1BB</i>
0.033175	0.678009	0.400237	P13716	<i>ALAD</i>
0.020199	0.772203	0.366444	P16112	<i>ACAN</i>
0.036097	-1.69914	0.410234	P20023	<i>CR2</i>
0.04191	0.774869	0.454324	P41240	<i>CSK</i>
0.029134	-1.58522	0.390214	P48426	<i>PIP4K2A</i>
0.027211	-0.56463	0.384033	P48637	<i>GSS</i>
0.02432	-1.90319	0.375216	P49407	<i>ARRB1</i>
0.011427	-0.47361	0.356128	P51149	<i>RAB7A</i>
0.014439	0.561058	0.365986	P51884	<i>LUM</i>
0.008061	0.913406	0.301471	P54802	<i>NAGLU</i>
0.008881	-1.92216	0.316316	P54819	<i>AK2</i>
0.013363	-1.41434	0.365986	P55056	<i>APOC4</i>
0.018104	-1.13536	0.365986	P61088	<i>UBE2N</i>
0.044376	-0.638	0.474189	P61916	<i>NPC2</i>
0.046453	-0.86744	0.482101	P62942	<i>FKBP1A</i>
0.006589	-0.30224	0.273802	P62979	<i>RPS27A</i>
0.013144	0.63441	0.365986	Q12860	<i>CNTN1</i>
0.005392	-1.72557	0.260209	Q14141	<i>SEPTIN6</i>
0.025081	-1.45493	0.375216	Q15019	<i>SEPTIN2</i>
0.020735	0.698228	0.366444	Q15166	<i>PON3</i>
0.037294	0.884489	0.410234	Q16706	<i>MAN2A1</i>
0.037136	-2.90432	0.410234	Q4LDE5	<i>SVEP1</i>
0.02485	-1.22303	0.375216	Q6EEV6	<i>SUMO4</i>
0.005914	1.008333	0.260209	Q76LX8	<i>ADAMTS13</i>
0.030199	0.852641	0.390214	Q7L591	<i>DOK3</i>
0.04705	0.741053	0.482101	Q86TH1	<i>ADAMTSL2</i>
0.046495	-0.89383	0.482101	Q8NBJ4	<i>GOLM1</i>
0.02245	0.743088	0.366444	Q92820	<i>GGH</i>
0.014649	-0.94753	0.365986	Q96HR3	<i>MED30</i>
0.017085	1.489395	0.365986	Q96NZ9	<i>PRAP1</i>
0.015545	-3.026	0.365986	Q99439	<i>CNN2</i>
0.030678	-2.25868	0.390214	Q99685	<i>MGLL</i>
0.003553	-1.2312	0.257634	Q9BR76	<i>CORO1B</i>
0.013768	-1.65934	0.365986	Q9BS26	<i>ERP44</i>
0.003369	-0.38306	0.257634	Q9BXR6	<i>CFHR5</i>
0.016244	-2.33835	0.365986	Q9HBB8	<i>CDHR5</i>
0.022535	-1.10941	0.366444	Q9HDC9	<i>APMAP</i>
0.034891	-1.40687	0.410234	Q9NZ08	<i>ERAP1</i>
0.020586	0.870296	0.366444	Q9UBQ6	<i>EXTL2</i>
0.001567	-1.17653	0.257634	Q9UJC5	<i>SH3BGRL2</i>
0.002935	-2.53814	0.257634	Q9UJU6	<i>DBNL</i>
0.031822	-1.11919	0.390214	Q9UQ80	<i>PA2G4</i>
0.003789	-2.23932	0.257634	Q9Y696	<i>CLIC4</i>

DEPs, differentially expressed proteins; BAV, bicuspid aortic valve.