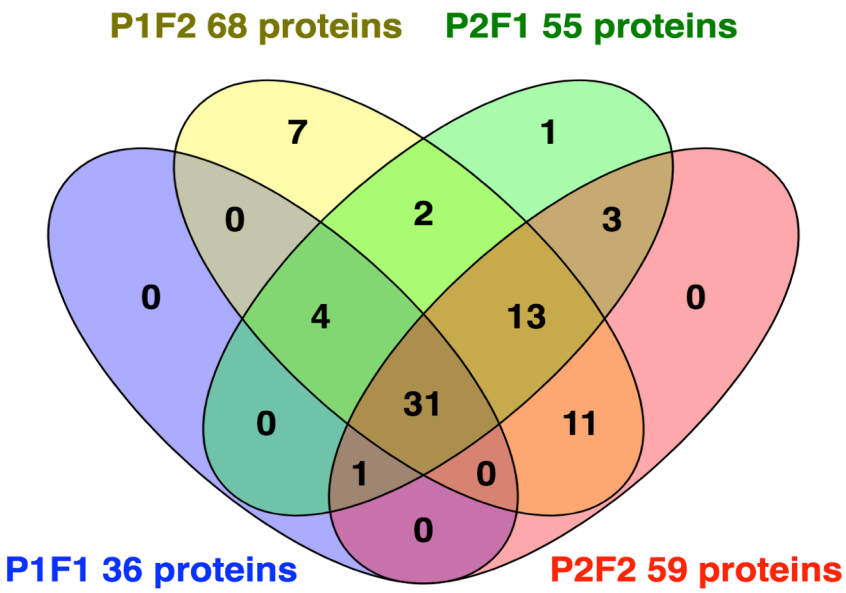
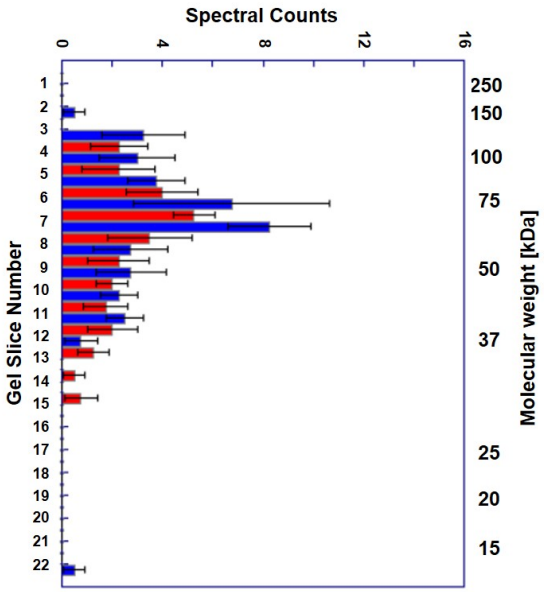
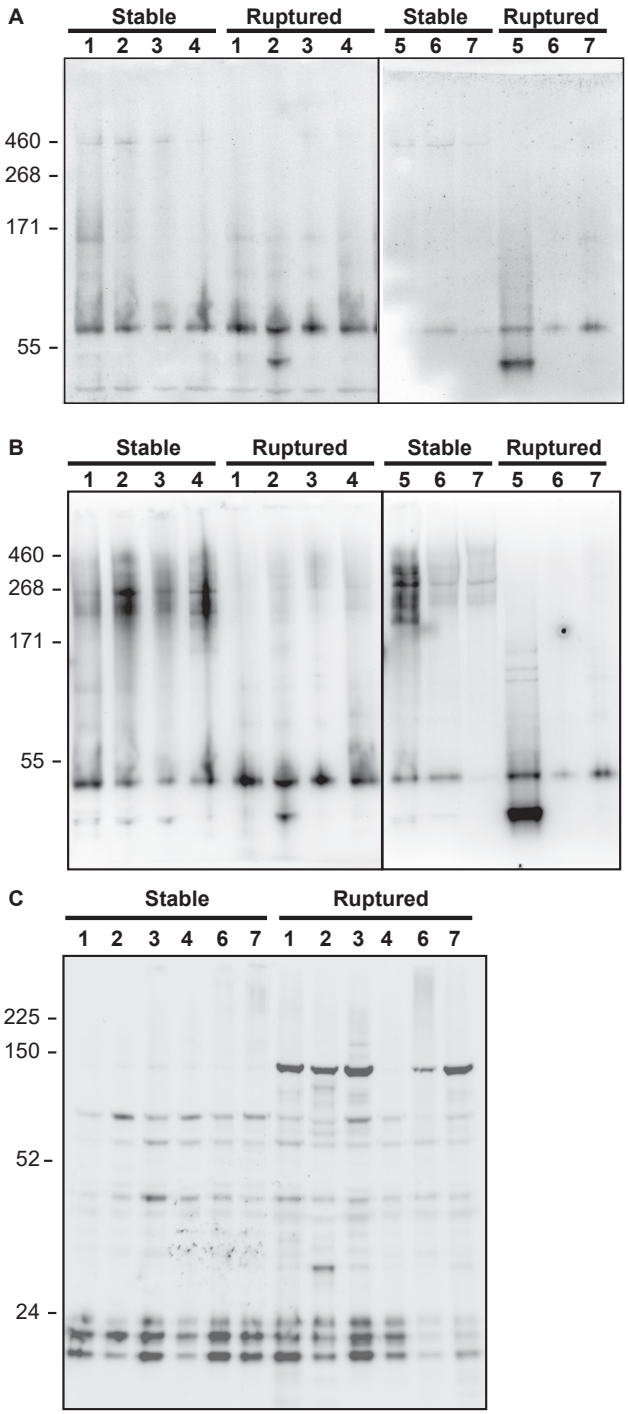
Supplemental Material (Online Figures I – VI; Online Tables I - III)



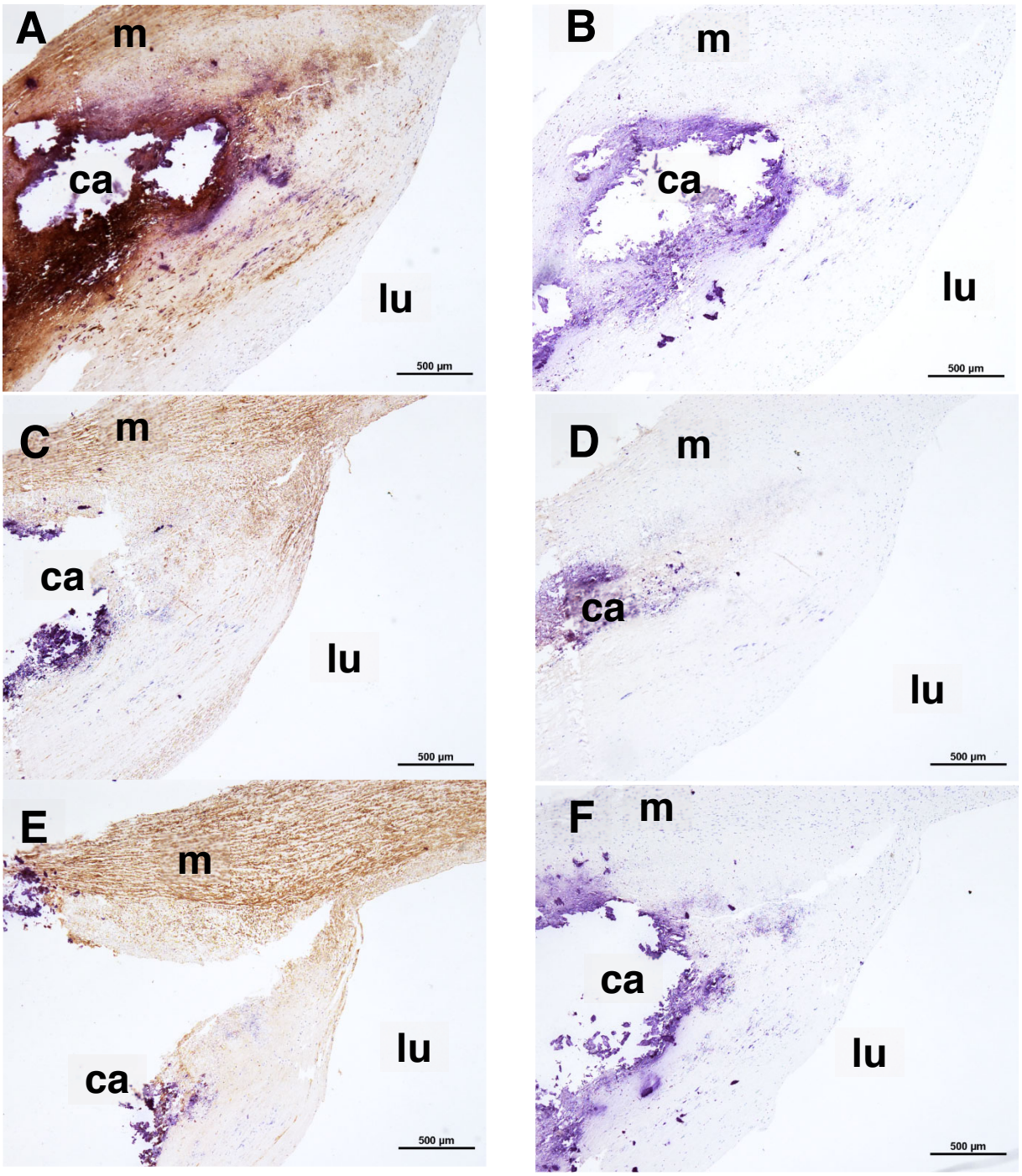
Online Figure I. Venn-diagram representation of unique extracellular matrix proteins (ECM, GO category 0031012) that were identified in each of 2 fractions of 2 separate tissue-extraction protocols applied to mouse aortas. For details of the 2 protocols (P1 and P2) and how the 2 fractions (F1 and F2 of each protocol) were generated, see Methods. The total number of unique proteins identified in each fraction is indicated next to the fraction name. The numbers of proteins identified in more than 1 fraction are indicated in cells of the diagram.



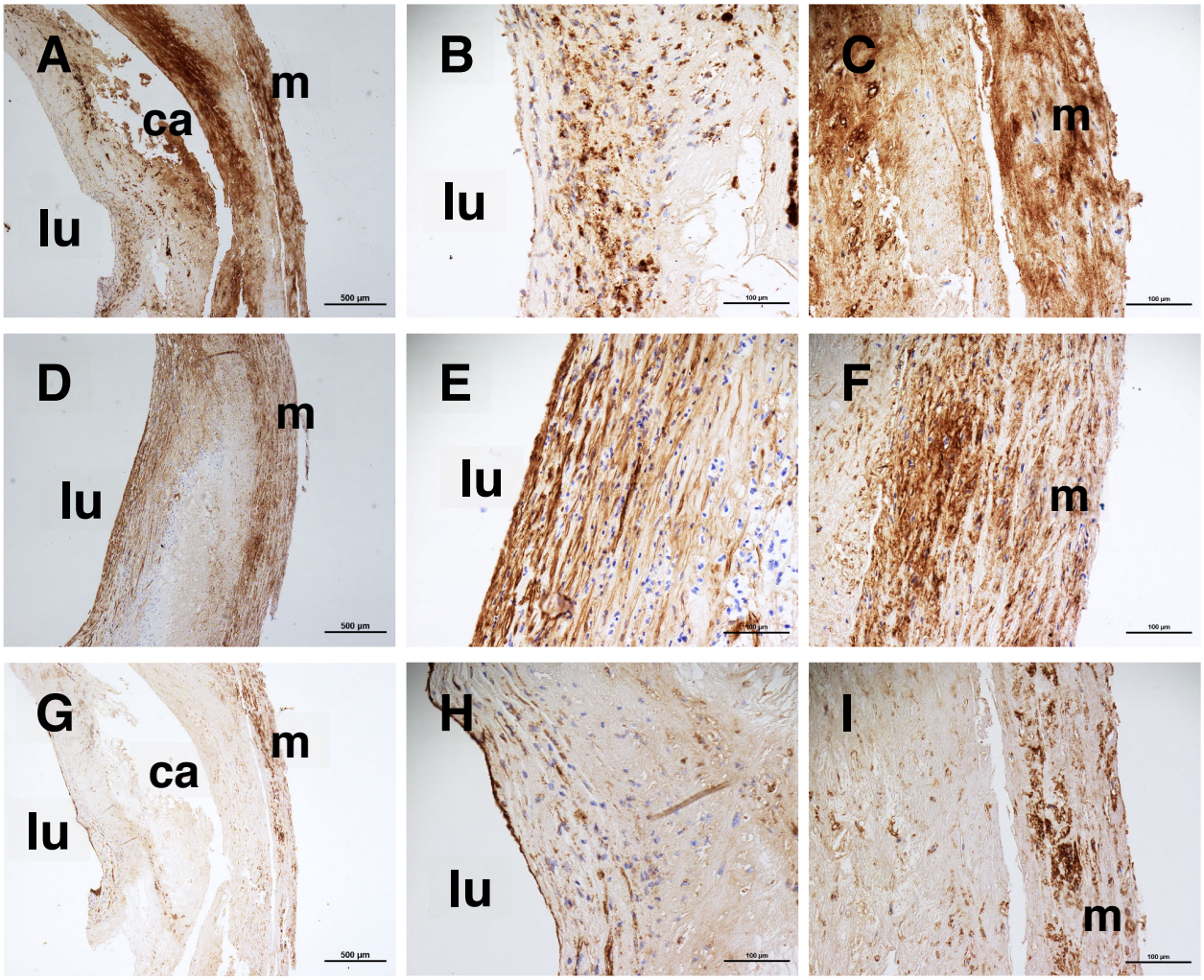
Online Figure II. **Peptograph of Nidogen2 (NID2) peptides in mouse aortic extracts.** Aortic extracts of SR-uPA$^{+/0}$ (red) and SR-uPA$^{0/0}$ (blue) mice (n = 4 samples for both, each pooled from 3 aortas) were processed and analyzed using the PROTOMAP protocol. Briefly, the extracts were subjected to SDS-PAGE and the gels were cut into 22 slices, each corresponding to a molecular-weight range. After in-gel trypsin digestion, peptides were extracted and identified by tandem mass spectrometry. Horizontal bars in each peptograph portray the number of protein-specific peptides identified in each of the 22 gel slices (mean ± SEM; n = 4). Gel-slice number is on the leftward y-axis; molecular weight of the gel slices (in kDa) is on the rightward y-axis. The x-axis indicates the total spectral counts for NID2-specific peptides in each gel slice.



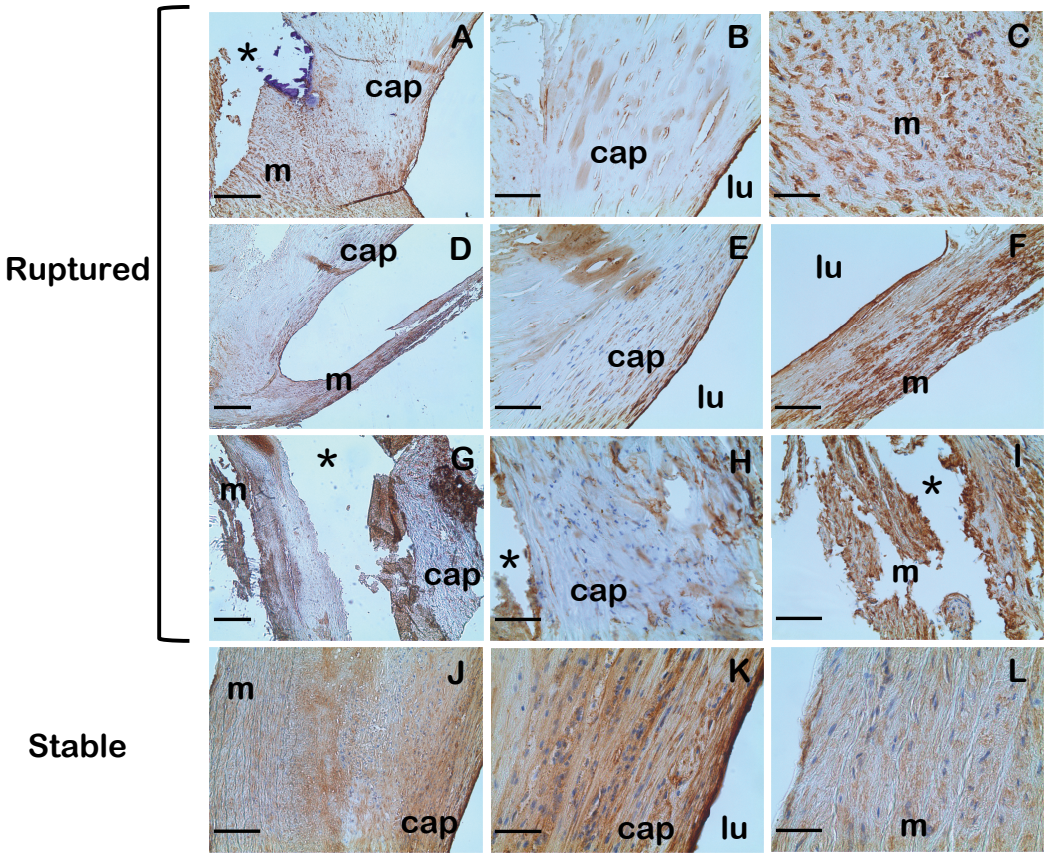
Online Figure III. Western blots of human carotid plaque extracts. Paired protein extracts of 7 human carotid plaques were separated by SDS-PAGE and probed with antibodies to 3 basement-membrane proteins: (**A**) LAMA5; (**B**) HSPG2; (**C**) COL18A1. Each pair of samples includes extracts from a stable and a ruptured area of the same plaque. The volume of the extracts of sample 5 was insufficient for the COL18A1 analysis. (**A**, **B**) Two gels were run, each with paired samples. Size markers are in kDa. Molecular weights of intact proteins are: LAMA 5: 400 kDa; HSPG2: 468 kDa; COL18A1: 178 kDa.



Online Figure IV. Immunohistochemical detection of basement-membrane proteins in stable human plaque segments. Sections of a stable segment of a human carotid plaque were stained with antibodies directed at: (**B**) COL18A1 (Santa Cruz Biotechnology sc-3270, generated against human endostatin, a COL18A1 fragment); (**D**) HSPG2 (Abcam ab2501); (**F**) LAMA5 (rabbit anti-mouse LAMA5 serum from Dr. Lydia Sorokin). (**A**, **C**, **E**) Sections from same plaque as in (**B**, **D**, and **F**) are stained with corresponding control antibodies (see Methods). (**A**-**F**) hematoxylin counterstain. ca = calcified nodule; lu = lumen; m = media. Scale bars = 500 μm.



Online Figure V. Basement-membrane proteins detected in media, fibrous cap, and luminal endothelial layer of stable human plaque segments. Sections of stable segments of human carotid plaques were stained with antibodies directed at: (**A**-**C**) COL18A1; (**D**-**F**) HSPG2; (**G**-**I**), LAMA5. The same antibodies were used as in Figure VI. (**A**-**I**) hematoxylin counterstain.
ca = calcified nodule; lu = lumen; m = media. Scale bars: (**A**, **D**, **G**) = 500 μm; (**B**, **C**, **E**, **F**, **H**, **I**) = 100 μm.



Online Figure VI. Immunohistochemical detection of HSPG2 in media and fibrous caps of stable and ruptured plaques. (**A**-**I**) Three ruptured plaques show faint staining in the fibrous cap tissue (**A** and **B**, **D** and **E**, **G** and **H**) with more intense staining in the media (**A** and **C**, **D** and **F**, **G** and **I**). (**J**-**L**) A stable plaque segment has strong staining in the cap (**K**), with fainter staining in the media (**L**). Antibody was from Boster Biological Technology; PB9277. cap= fibrous cap; lu = lumen; m = media. (**A**, **G**, **H**, and **I**) Spaces (*) are sectioning artifacts. Scale bars: (**A**, **J**) = 200 μm; (**B**, **C**, **E**, **F**, **H**, **I**, **K**, **L**) = 50 μm, (**D**, **G**) = 400 μm.

Online Table I. Demographics and Clinical Features of Donors of Experimental Carotid Plaques

Cohort	1 (n = 6)	2 (n = 6)	3 (n = 5)
Age	68±7	63±7	63±8
Male sex (%)	67	100	100
Caucasian (%)	100	100	100
Hypertension (%)	100	100	100
Hyperlipidemia (%)	100	100	100
Diabetes (%)	67	50	40
Active smoker (%)	0	50	40
Aspirin (%)	60	83	80
Statin (%)	100	100	100
ACE inhibitor (%)	17	67	80
ARB (%)	17	17	20
Metformin (%)	33	0	0
Insulin (%)	33	0	0
Warfarin (%)	50	0	20
Clopidogrel (%)	17	17	20

Cohort 1 = donors of first set of plaques, extracts used for the initial shotgun proteomics study. Cohort 2 = donors of second set of plaques; extracts used for the second (“validation”) shotgun proteomics study. Cohort 3 = Donors of plaques from which adequate PROTOMAP data were obtained (includes 4 individuals from cohort 2 and 1 individual from cohort 1).

Online Table II. Proteins detected by PROTOSORT tool with peptographs showing altered fragmentation between ruptured and stable plaque segments.

Proteins with increased fragmentation in ruptured plaque segments	Proteins with increased fragmentation in stable plaque segments
Ceruloplasmin	Laminin subunit beta 2
Plasminogen	Supervillin
Integrin alpha-X	
Angiotensinogen	
Inter-alpha-trypsin inhibitor heavy chain H4	
Lipopolysaccharide-binding protein	
Phosphatidylinositol-glycan specific phospholipase D	
Coagulation factor V	
Pigment epithelium-derived factor	
6-phosphogluconate dehydrogenase	
Heparin cofactor 2	
Phospholipase D3	
Carbonic anhydrase 1	
Ribosomal protein L8	
Probable ATP-dependent RNA helicase DDX5	
ATP binding cassette subfamily F, member 1	

Online Table III. PROTOMAP quantification in stable and ruptured human plaque segments (from patient cohort 2) of proteins that were significantly decreased in ruptured plaque samples from patient cohort 1 (as measured by shotgun mass spectrometry) and are in GO category extracellular matrix.

Protein	Total spectral counts	Spectral counts in stable samples (mean ± SD; n = 5)	Spectral counts in ruptured samples (mean ± SD; n = 5)	P-value
ABI3BP	1317	205 ± 112	59 ± 35	0.04
ACAN	944	157 ± 75	32 ± 41	0.02
ACTN1	7330	898 ± 364	569 ± 354	0.23
AGRN	71	3.4 ± 1.7	11 ± 9	0.15
APP	104	14 ± 5.2	7.2 ± 8.7	0.24
CAPN2	81	11 ± 8.1	5 ± 5.3	0.24
CAST	795	108 ± 73	51 ± 53	0.25
COL14A1	3929	291 ± 119	474 ± 185	0.14
COL18A1	1758	1107 ± 221	651 ± 130	0.20
COL5A1	202	19 ± 9.6	22 ± 15	0.73
CPXM2	338	39 ± 21	28 ± 17	0.43
CRIP2	185	22 ± 11	15 ± 10	0.32
CTGF	48	4.6 ± 2.1	5 ± 3.8	0.86
DAG1	163	26 ± 14	6.2 ± 8.7	0.04
DMD	345	48 ± 22	21 ± 19	0.09
ELN	368	51 ± 20	22 ± 24	0.10
FBLN2	1401	201 ± 199	70 ± 52	0.21
FBLN5	798	113 ± 43	47 ± 24	0.29
FLNA	20,460	2336 ± 872	1756 ± 885	0.38
FMOD	1,404	215 ± 23	66 ± 50	0.0007
HAPLN1	807	139 ± 83	22 ± 25	0.03
HAPLN3	132	22 ± 7	4.4 ± 6.0	0.004
HMCN1	177	25 ± 15	30 ± 28	0.76
HSPB1	868	119 ± 26	55 ± 28	0.01
HSPG2	8792	1135 ± 467	623 ± 366	0.12
ILK	442	60 ± 36	29 ± 23	0.18
ITGA1	234	29 ± 16	18 ± 15	0.37
ITGA3	73	10 ± 3.6	5.0 ± 5.5	0.2
ITGA7	82	15 ± 6.1	1.2 ± 0.7	0.11
ITGA8	187	29 ± 15	8.2 ± 8.9	0.04
ITGAV	362	46 ± 22	26 ± 14	0.17
LAMA2	344	50 ± 40	19 ± 24	0.21
LAMA4	1004	135 ± 118	65 ± 51	0.31
LAMA5	3519	561 ± 256	142 ± 145	0.02
LAMB1	1167	150 ± 108	84 ± 57	0.31

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Protein	Total spectral counts	Spectral counts in stable samples (mean ± SD; n = 5)	Spectral counts in ruptured samples (mean ± SD; n = 5)	P-value
LAMB2	2424	382 ± 152	102 ± 82	0.01
LAMC1	3356	443 ± 210	228 ± 171	0.15
LGALS1	734	114 ± 35	32 ± 18	0.003
LMCD1	271	39 ± 39	15 ± 9.6	0.26
LMNA	3754	223 ± 74	528 ± 265	0.06
LOX	9	1.8 ± 1.1	0 ± 0	0.01
LOXL4	6	0.6 ± 0.8	0.6 ± 1.2	1.0
LTBP1	1582	248 ± 140	68 ± 63	0.047
LTBP2	3474	498 ± 326	196 ± 142	0.13
LTBP4	1620	262 ± 191	62 ± 50	0.08
MATN2	96	14 ± 12	5.6 ± 4.4	0.25
MFAP4	957	156 ± 35	36 ± 24	0.0004
MFGE8	2633	348 ± 77	178 ± 107	0.03
MYL6	1266	195 ± 71	58 ± 58	0.02
NID1	1373	179 ± 105	96 ± 77	0.23
NID2	1023	129± 105	76 ± 60	0.40
NOV	378	64 ± 47	12 ± 7.1	0.06
NPNT	36	5.4 ± 4.4	1.8 ± 3.6	0.24
OGN	2359	298 ± 56	174 ± 96	0.06
OMD	147	24 ± 17	5.6 ± 4.8	0.07
PFKP	186	21 ± 5.7	16 ± 14	0.53
PODN	179	21 ± 10	15 ± 13	0.53
PRELP	6815	758 ± 266	605 ± 253	0.43
RARRES2	15	2.6 ± 2.4	0.4 ± 0.4	0.11
RPL27	21	2.0 ± 1.6	2.2 ± 2.5	0.90
RPS13	34	1.6 ± 2.0	5.2 ± 6.1	0.30
SBSPON	182	32 ± 7.6	4.6 ± 5.2	0.0004
SOD3	650	90 ± 9.2	40 ± 14	0.0004
SPARC	48	9.2 ± 5.1	0.4 ± 0.8	0.009
SPARCL1	307	52 ± 36	9.4 ± 13.6	0.06
TGFB1I1	432	60 ± 19	26 ± 19	0.04
TINAGL1	1098	146 ± 51	74 ± 30	0.04
VCAN	4794	672 ± 155	287 ± 184	0.01
VIM	8055	693 ± 281	918 ± 361	0.36