

SUPPORTING FIGURE LEGENDS

Figure S1. Representative SDS-PAGE analyses (A) Recombinant proteins TIMP1, COMP, MMP9, THBS2, MSLN and ENG were separated using SDS-PAGE on a pre-cast 10% Bis-Tris gel prior to staining. (B) Immunoprecipitated samples were separated using the same condition as recombinant proteins. On the basis of the migration of the molecular weight marker and the recombinant proteins, three bands from the immunoprecipitated eluent corresponding to the approximate molecular weights were excised from the gel and subjected to in-gel digestion. Each gel band corresponds to an approximate mass range: band 1(75-200 KDa) and band 2 (37-75 KDa) for multiplex five proteins, band 3 (25-37 KDa) for TIMP1. Each gel lane corresponds to one immunoprecipitation experiment.

Figure S2. MS/MS spectrum of the peptide GFQALGDAADIR of TIMP1. The assigned peaks were used to MRM analysis.

Figure S3. MS/MS spectrum of the peptide ELQETNAALQDVR of COMP. The assigned peaks were used to MRM analysis.

Figure S4. MS/MS spectrum of the peptide AVIDDAFAR of MMP9. The assigned peaks were used to MRM analysis.

Figure S5. MS/MS spectrum of the peptide ACVGDVQER of THBS2. The assigned peaks were used to MRM analysis.

Figure S6. MS/MS spectrum of the peptide TDAVLPLTVAEVQK of MSLN. The assigned peaks were used to MRM analysis.

Figure S7. MS/MS spectrum of the peptide VLPGHSAGPR of ENG. The assigned peaks were used to MRM analysis.

Figure S8. The extracted ion chromatograms (XIC) of the transitions of selected peptides from immunoprecipitation of 10 ng/mL recombinant proteins in 60 mg/mL BSA. MRM quantitation was based on stable isotope dilution. Monitored MRM transitions are depicted for the unlabeled peptide, and the 1 fmol/ μ L isotope-labeled internal standards (insets). (A) TIMP1, (B) COMP, (C) MMP9, (D) THBS2, (E) MSLN and (F) ENG.

Figure S9. Plots of the ratio of light peptide to the heavy peptide (synthetic standard) from 10 to 640 ng/mL of spiked recombinant TIMP1, COMP, MMP9, THBS2, MSLN and ENG proteins. MRM analysis was performed on the peptides shown in Table 2 from the trypsin digestion of the excised region of the gel corresponding to the molecular weight. Error bars represents standard deviation (n=3). *L*, light; *H*, heavy

Figure S10. ELISA calibration curves and plasma protein levels obtained for TIMP-1, COMP, MMP9, THBS2, MSLN and ENG proteins. All results were generated with GraphPad Prism 5, using a 5-parameter logistic data fit. Calibration curves span 0.1 to 20 ng/mL (black circles) of recombinant protein. Red squares indicate the plasma protein level with different dilutions. (A) TIMP11:100, (B) COMP 1:100, (C) MMP9 1:40, (D) THBS2 1:4, (E) MSLN 1:10, (F) ENG 1:3.

Table S1. Proteins, unique peptides and selected masses for MRM analysis

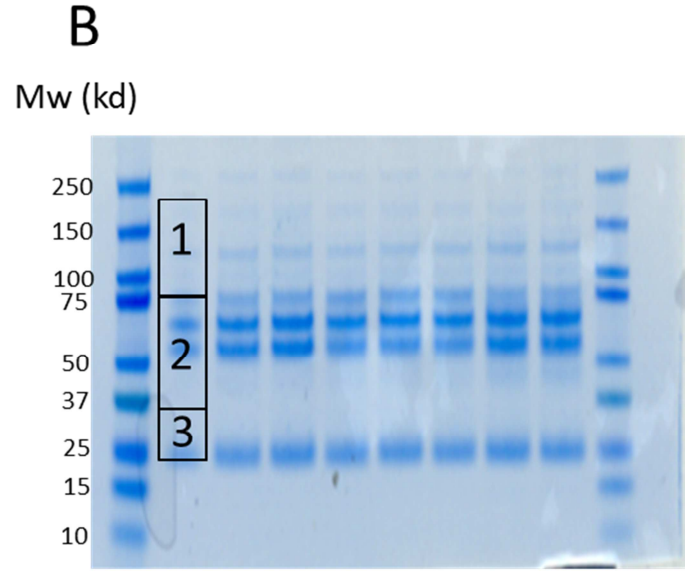
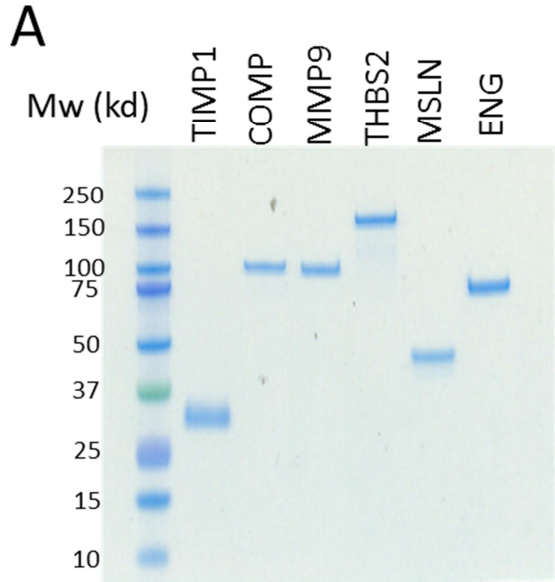


Figure S1

dl_20110923_TIMP1_P1 #2844-2862 RT: 49.23-49.49 AV: 4 NL: 1.03E5
F: + c NSI Full ms2 617.315 [200.000-1250.000]

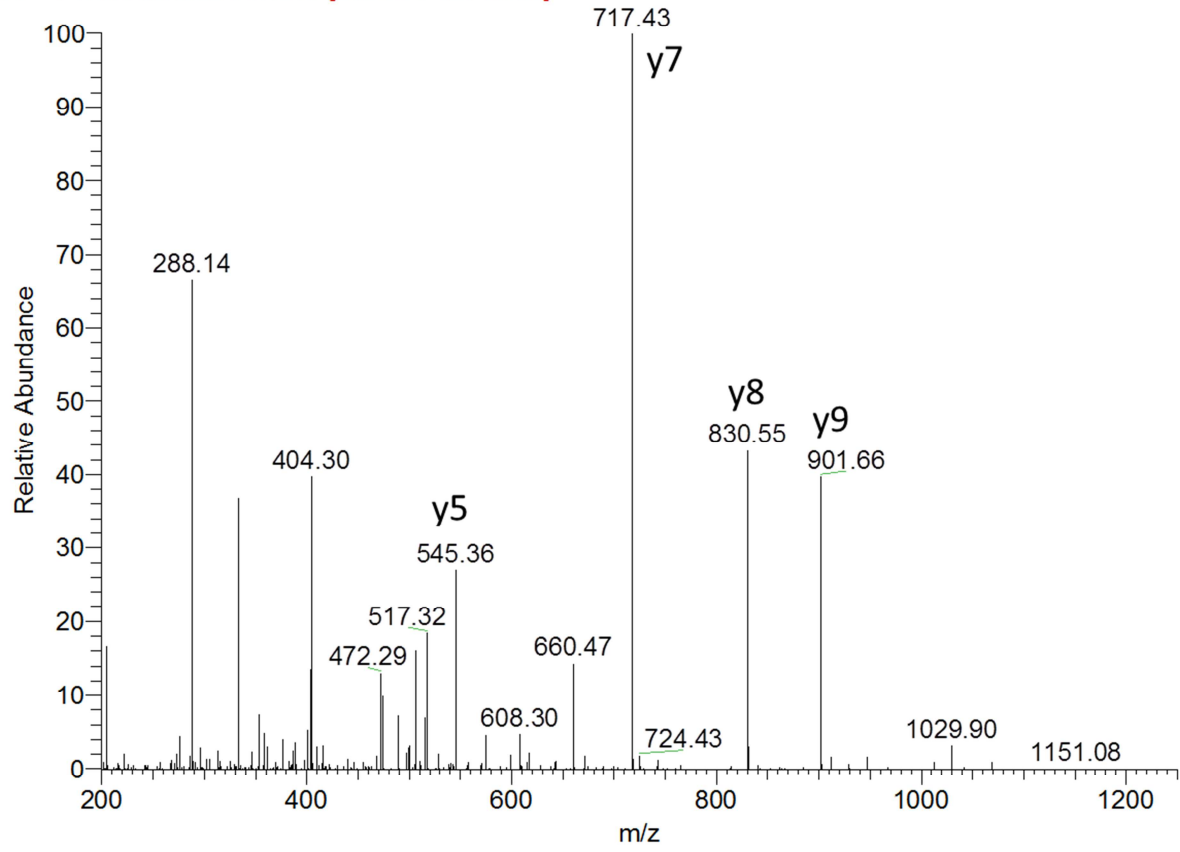


Figure S2

dl_20111003_COMP_p2 #3594-3617 RT: 25.86-26.01 AV: 5 NL: 2.22E4
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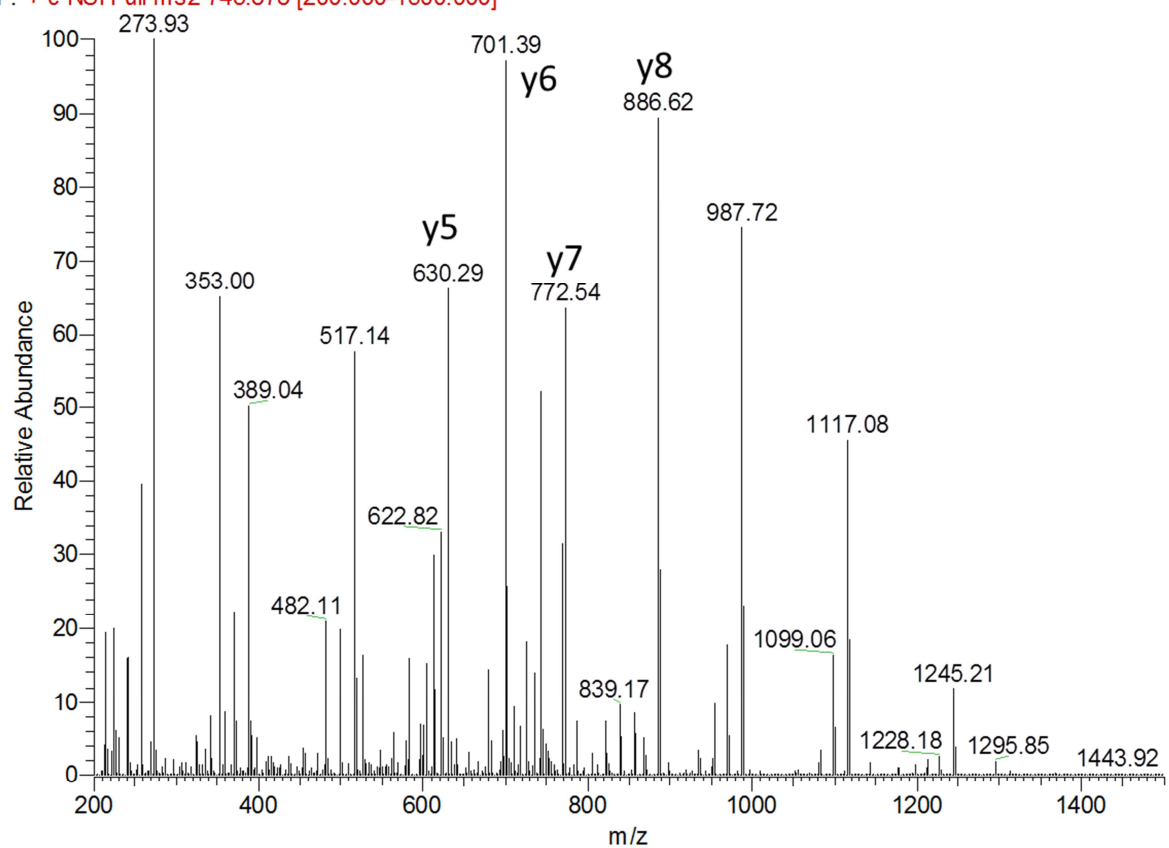


Figure S3

dl_20110923_MMP9_P1 #4692-4734 RT: 43.77-44.05 AV: 4 NL: 1.29E5
F: + c NSI Full ms2 489.256 [200.000-960.000]

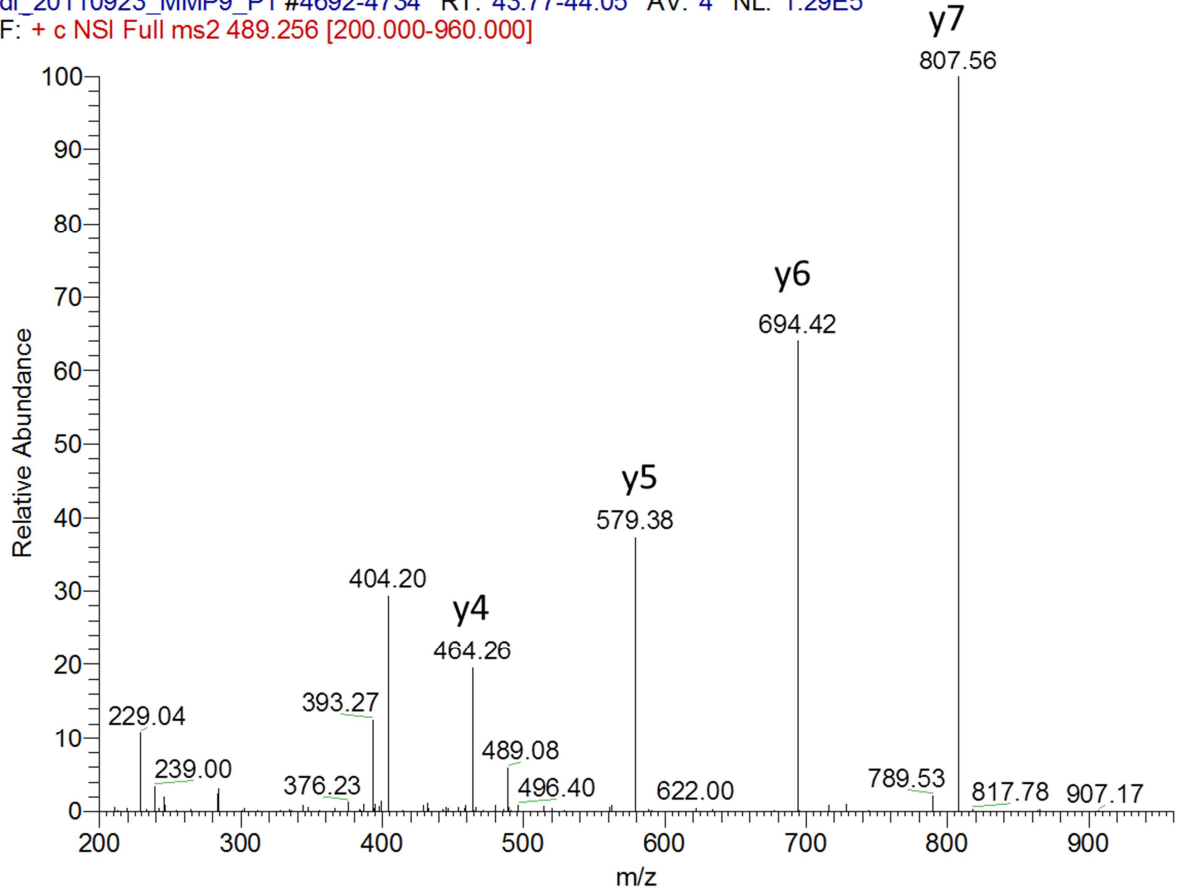


Figure S4

dl_20110927_THBS2_p1 #3058-3085 RT: 31.15-31.33 AV: 3 NL: 1.40E5
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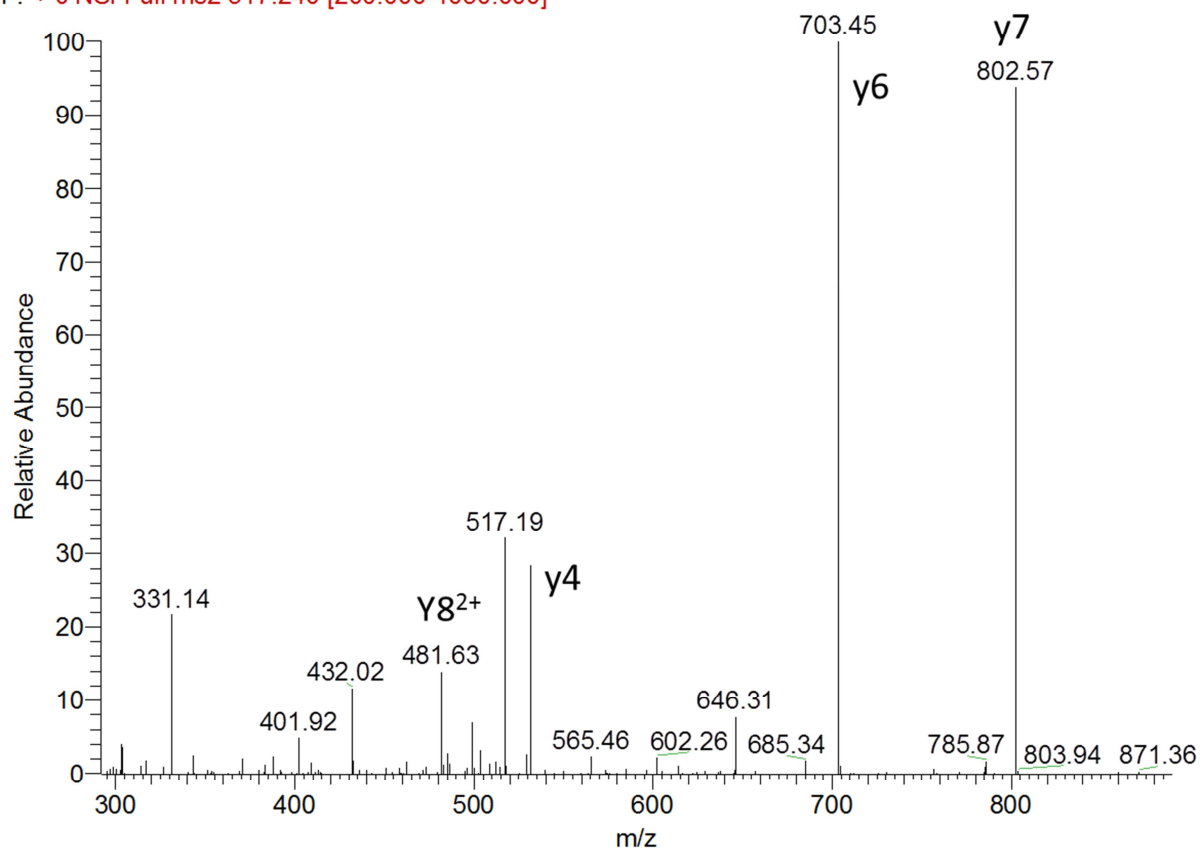


Figure S5

dl_20111010_MSLN_20x_p2 #3228-3247 RT: 56.05-56.25 AV: 3 NL: 3.06E4
F: + c NSI Full ms2 742.422 [300.000-1400.000]

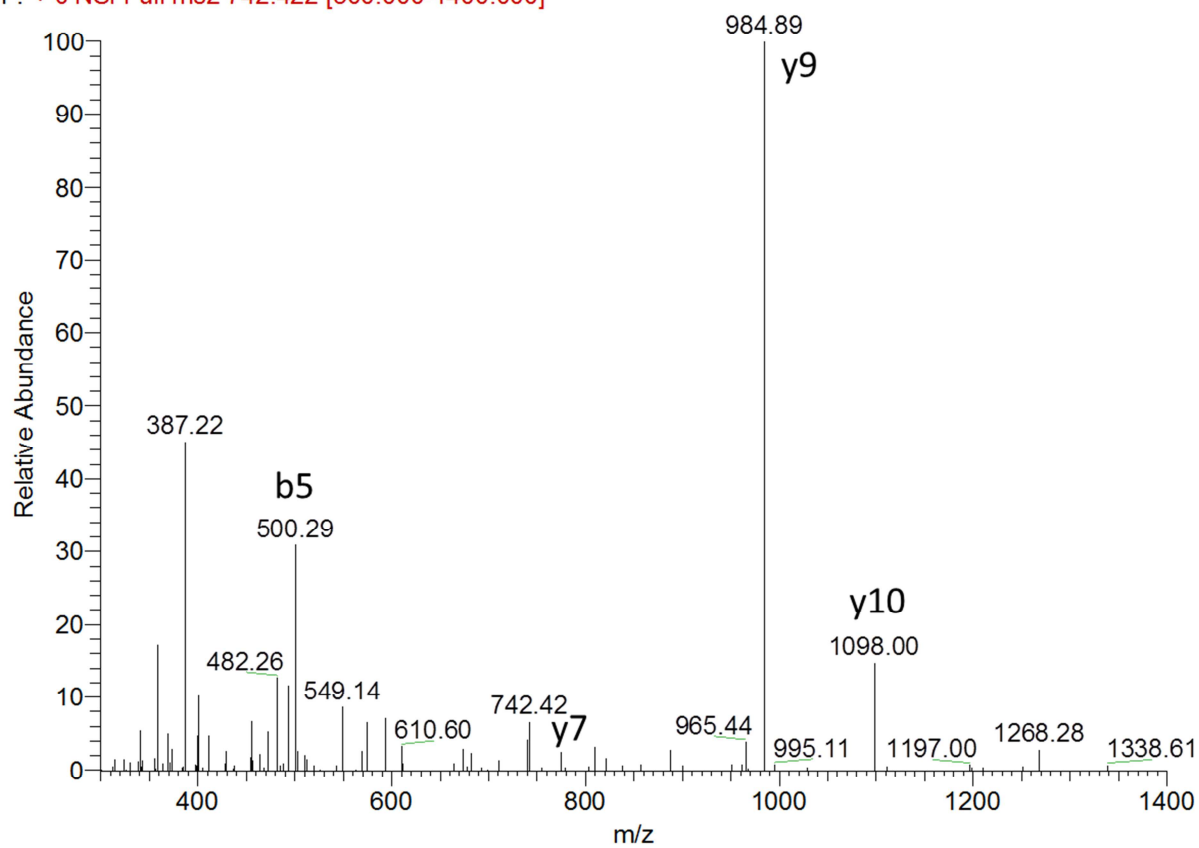


Figure S6

dl_20110923_ENG_P1 #2990-3028 RT: 28.23-28.49 AV: 5 NL: 3.06E5
F: + c NSI Full ms2 495.778 [200.000-1000.000]

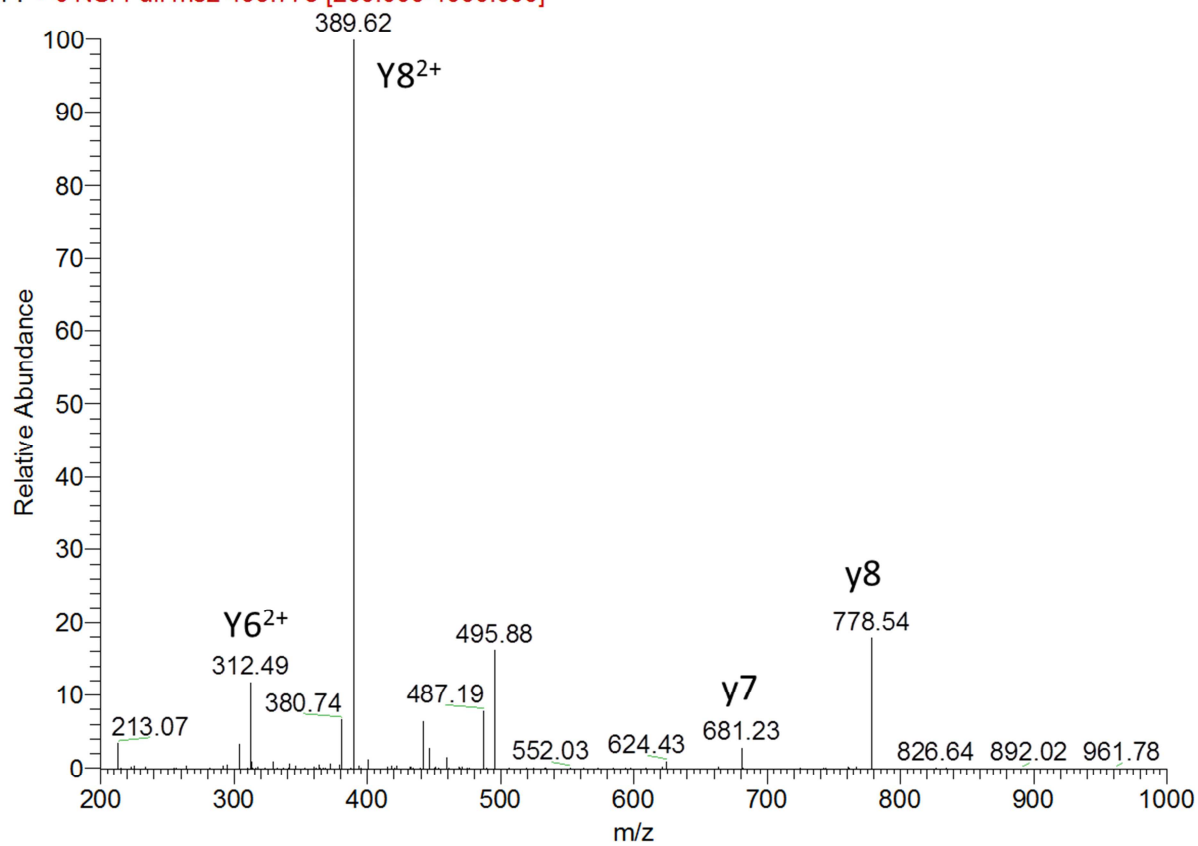


Figure S7

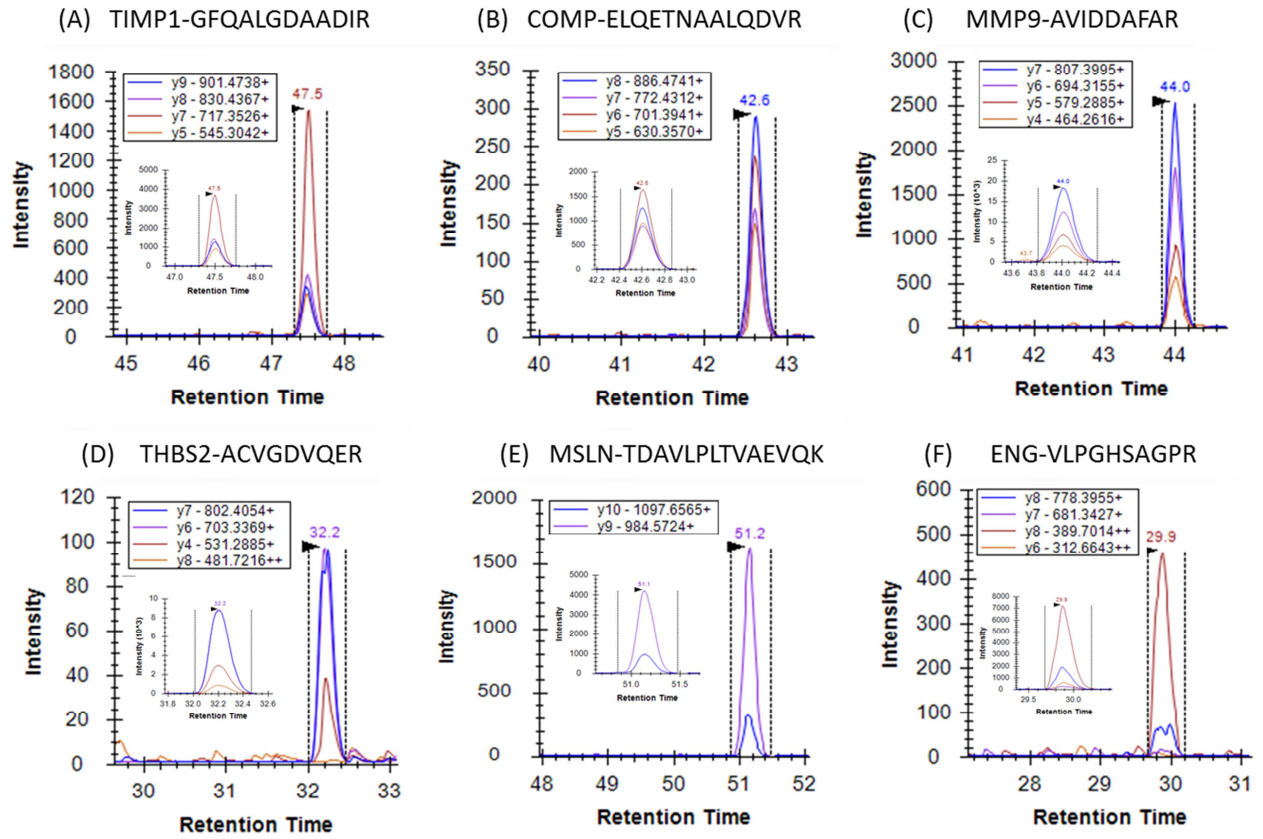


Figure S8

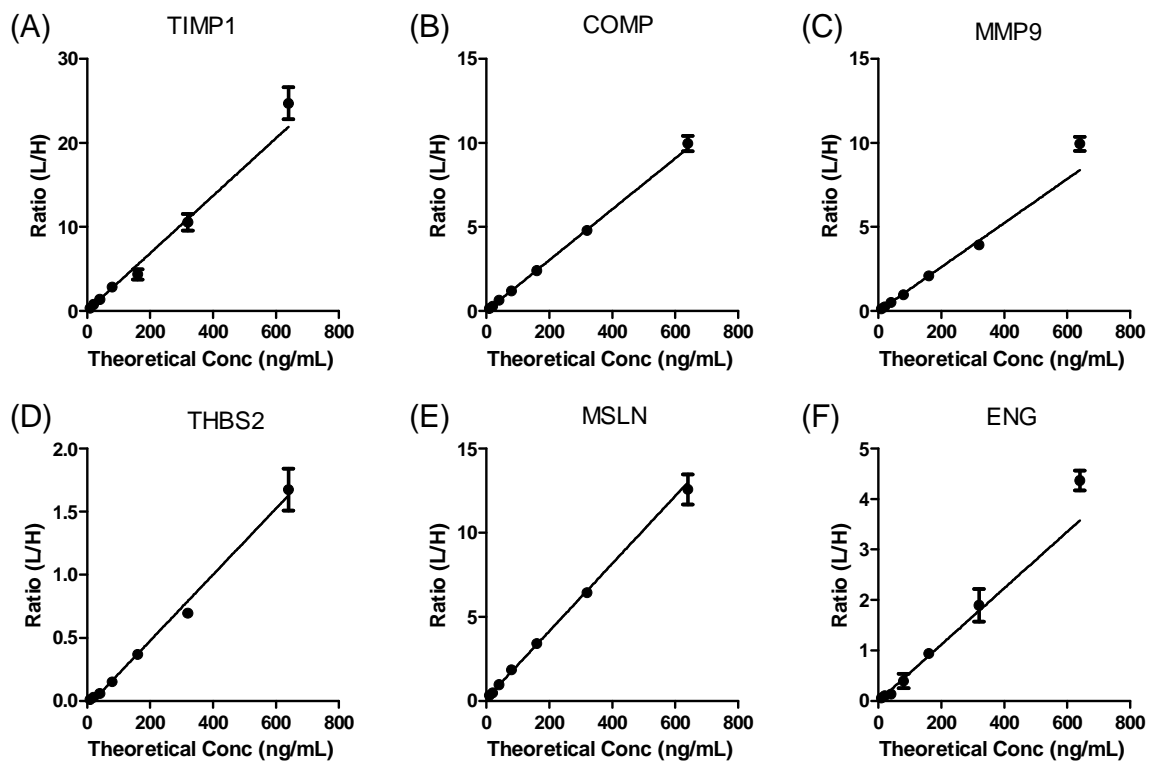


Figure S9

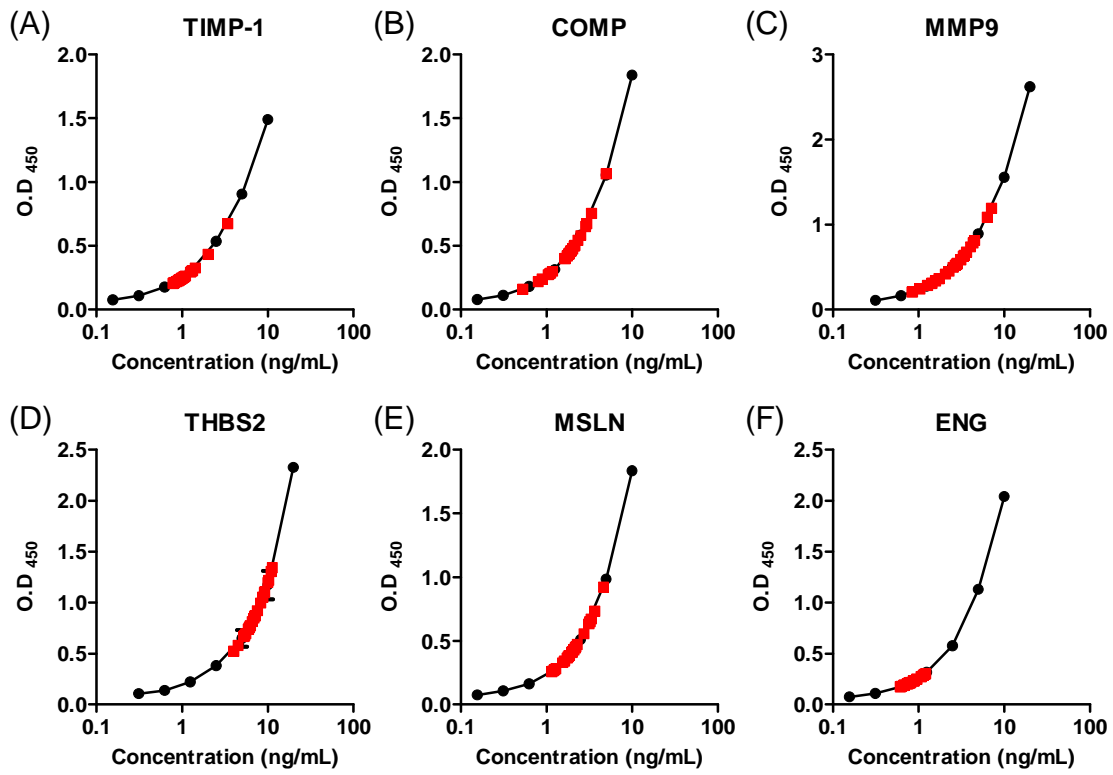


Figure S10

Table S1. Proteins, unique peptides and selected masses for MRM analysis

Gene name	Protein Mw	Gel Fraction	Peptide sequence	Q1 ^{\$}	Q3 [@]			
					Tr1	Tr2	Tr3	Tr4
TIMP1	23171	3	GFQALGDAADIR GFQALGDAADIR*	617.3 622.3	901.5 911.5	830.4 840.4	717.4 727.4	545.3 555.3
ENG	70578	2	VLPGHSAGPR VLPGHSAGPR*	495.8 500.8	778.4 788.4	681.3 691.3	389.7 394.7	312.7 317.7
MSLN	68986	2	TDAVLPLTVAEVQK TDAVLPLTVAEVQK*	742.4 746.4	1097.7 1105.7	984.6 992.6	774.4^ 782.4^	574.3^ 582.3^
THBS2	129991	1	AC#VGDVQER AC#VGDVQER*	517.2 522.2	802.4 812.4	703.3 713.3	531.3 541.3	481.7 486.7
MMP9	78458	1	AVIDDAFAR AVIDDAFAR*	489.3 494.3	807.4 817.4	694.3 704.3	579.3 589.3	464.3 474.3
COMP	82860	1	ELQETNAALQDVR ELQETNAALQDVR*	743.9 748.9	886.5 896.5	772.4 782.4	701.4 711.4	630.4 640.4

*: Stable isotope peptide (13C and 15N); #: Carbamidomethylated cysteine (Cys-CAM);

\$: Precursor [M+2H]²⁺; @: Product [M+H]⁺;

^: Transitions were not used for quantitation due to interference by signals from background peptide.