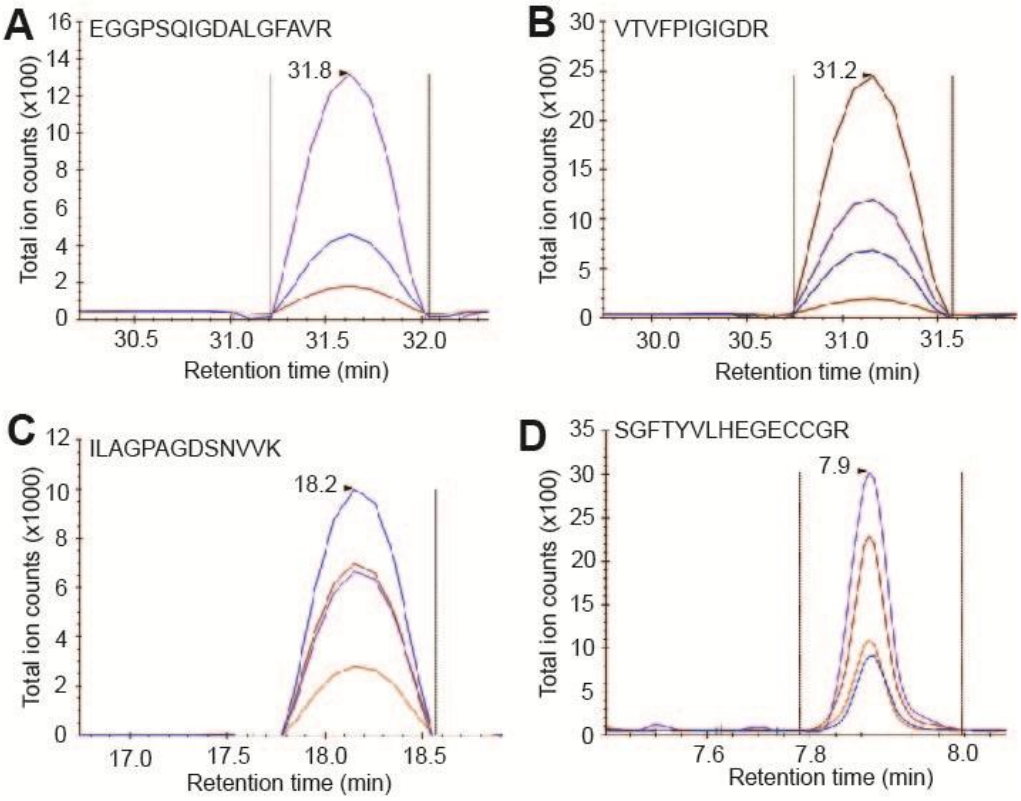
Supplemental method for mass spectrometry measurements of VWF cleavage

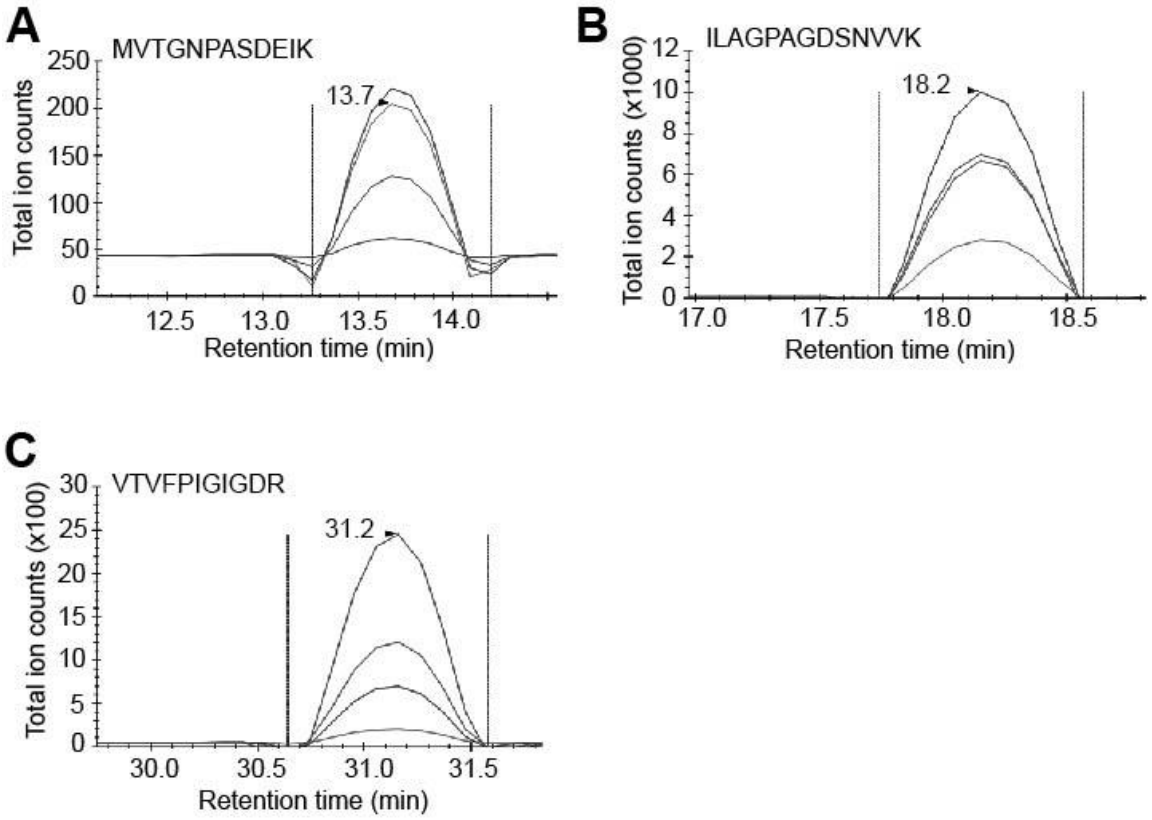
To reduce protein complexity for SRM, 14 highly abundant proteins were depleted from samples using an ÄKTA FPLC system (GE Healthcare, USA) coupled with a human IgY14 LC2 depletion column (Agilent, Santa Clara, CA). After depletion, the samples were digested with trypsin and desalted with Oasis MCX cartridges (Waters, Milford, MA).[1] SRM was performed on Agilent 6490 triple quadrupole (QQQ) mass spectrometers equipped with a nanoelectrospray ionization source (voltage: 1800V) and a HPLC ChipCube, using a large capacity Agilent HPLC chip (160 nL trap, 150 mm C18 column). Plasma samples (2 μg) were eluted over 60 min with a 0.66%/min acetonitrile slope in the presence of 0.1% formic acid. SRM data were processed using the Skyline targeted proteomics tool.[2] Internal peptide standards for all five VWF peptides were synthesized with heavy isotopic lysine ($^{13}C_6{}^{15}N_2$) or arginine ($^{13}C_6{}^{15}N_4$) at the C-termini (Thermo Fisher Scientific, Rockford, IL). The total peak area and the ratio of plasma peptide and its synthesized heavy counterpart were normalized to quantify VWF and its cleavage. VWF multimers purified from cryoprecipitate were cleaved by ADAMTS-13 [6] and tested as a control. VWF cleavage was also measured by immunoblots for data validation. VWF antigen and multimer patterns were measured by ELISA and 0.8% agarose gel electrophoresis followed by immunoblots using a VWF antibody (Dako, Carpinteria, CA), respectively.[3]

Collision energies (CE) estimated with the instrument vendor provided default formula (CE=0.036*m/z − 4.8) was optimized for charge 2 and charge 3 precursors with 4 additional CE conditions (±5V, ±10V). The best precursors and each of their best 4 transitions under optimized conditions were selected for the SRM analysis. Heavy peptides were titrated in a human plasma background to generate titration curves for the determination of the linear relationship between peak area under the curve (AUC) and the desired spike-in peptide amount of each peptide standard.

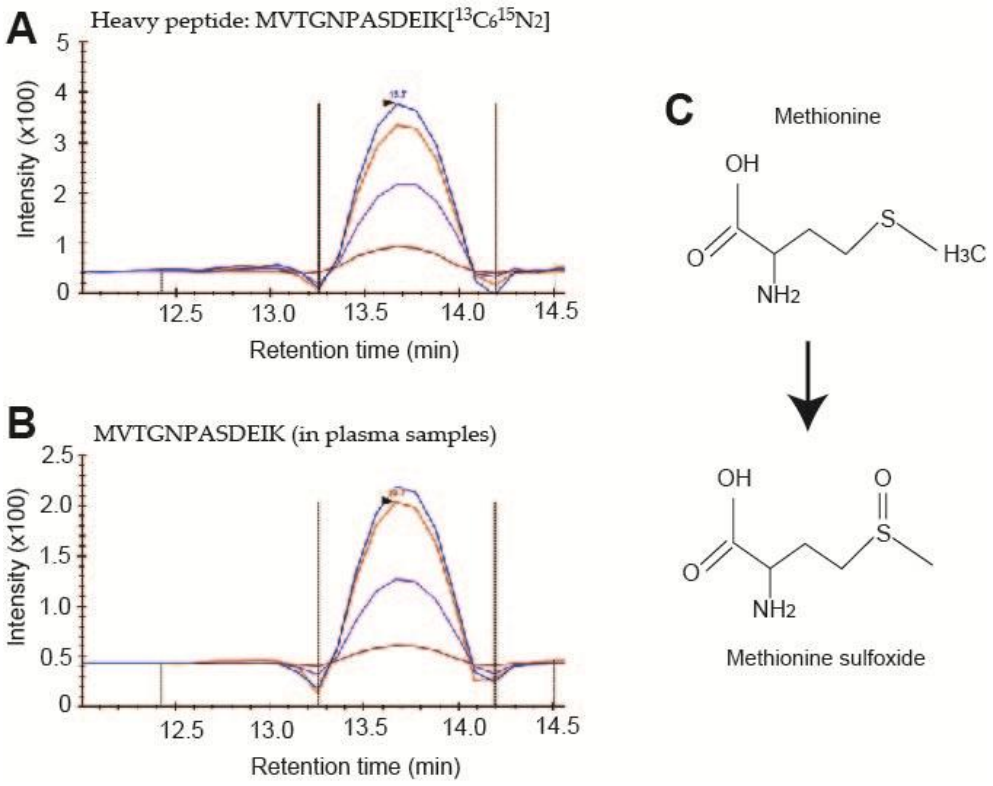
Supplemental Figures and legends



sFigure 1: Detection of VWF tryptic peptides: Mass spectrometry ionizes peptides and identify the peptide ions based on their mass to a charge ratio (m/z). In SRM analysis, we first filtered the intact peptide as precursor ion by its m/z, after fragmentation, we then measured the produced peptides as fragment ions by their m/z(s) named as transitions. Typical SRM chromatograms are shown for the four VWF peptides (**A – D**) in samples collected from a patient under baseline conditions. Curves in each chromatogram are transition peaks of corresponding peptides. Three to four transitions in each pepetide are monitored to ensure the target peptide being correctly identified and quantified.



sFigure 2: Detection of VWF tryptic peptides from recombinent A1-3 domain: A recombinant human VWF A1-3 domain polypeitde (10 μg) was digested with trypsin for two hrs at 37°C. The ADAMTS-13 cleaved A2 peptide (**A**) and two tryptic peptides from the A3 domain (**B** & **C**) were detected by SRM.



sFigure 3: the MVTGNPASDEIK peptide was oxidized in patient samples. Synthetic peptide with isotopic lysine residue (K) served as the heavy peptide (**A**) to define and quantify the light peptide from cleaved VWF by ADAMTS-13 in plasma samples from patients (**B**). **(**C**)** Methionin residue (M) was found to be oxidized to methionine sulfoxide based on the mass of the heavy peptide and light peptide.

REFERENCE

1. Qin S, Zhou Y, Lok AS, et al. SRM targeted proteomics in search for biomarkers of HCV-induced progression of fibrosis to cirrhosis in HALT-C patients. *Proteomics*. 2012;12(8):1244-1252.

2. MacLean B, Tomazela DM, Shulman N, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics*. 2010;26(7):966-968.

3. Dong JF, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100(12):4033-4039.