

## **SUPPLEMENTAL DATA**

**FIGURE S1. MS/MS and C-terminal amino acid sequence analysis.** The prominent fragment of hFibrinogen's  $\alpha$  chain that formed after this plasma protein was incubated with a recombinant hTryptase- $\beta$ •heparin complex for 1 min (75:1 enzyme:substrate ratio) was purified by SDS-PAGE, and then subjected to exhaustive digestion, LC-MS/MS, and C-terminal amino acid sequence determination. Shown are the susceptible peptides that were identified in the analyses. Although the entire fragment was mapped, only the region C terminal to residue 481 is shown for clarity. Twenty-two spectra were acquired whose C-terminal sequences terminate at Lys<sup>575</sup>, an amino acid that agrees with the specificity of hTryptase- $\beta$  but orthogonal to the specificity of the enzymes used in the mapping assay. In addition, MS/MS spectral counts diminish significantly C terminal to Lys<sup>575</sup>. We expect these to be due to co-isolated minor degradation products or incomplete cleavage of hFibrinogen by the hTryptase- $\beta$ •heparin complex. The vertical red line highlights Lys<sup>575</sup>, which is the preferred site of cleavage by the hTryptase- $\beta$ •heparin complex used in the study.

**FIGURE S2. MS/MS analysis of a 7-kDa peptide in the digest of hFibrinogen.** hFibrinogen was incubated with a recombinant hTryptase- $\beta$ •heparin complex. The resulting peptides in the digest were subjected to spin-column filtration, trichloroacetic acid precipitation, and SDS-PAGE. The obtained 7-kDa peptide was exhaustively digested with trypsin, and the resulting peptides subjected to MS/MS analysis. Ten peptides were identified, seven of which had different sequences. The identification of these peptides are shown, as well as their location in native  $\alpha$  chain of hFibrinogen (GenBank accession # NP\_068657). Nine of the ten peptides reside in the 69-mer C terminus of the protein's  $\alpha$  chain as expected.

Figure S1



Figure S2

**Amino acid sequence of hFibrinogen**

mfsmrivclvlsvvgtaawtadsggegdf laegggvrgprvverhqsackdsdwpfc sdedwnykc  
psgcrmkglidevnqdftrinklknslfeyqknnkdshsltt nimeilrgdfssannrdntyn  
rvsedlrsrievlkrkviekvqhiqlqknvraqldmkrlevdidikirscrgscsralarev  
dlkdyedqqkqleqviakdllpsrdrqhlplikmkpvpdlvpgnfksqlqkvppewkaldmpq  
(1)\*  
mrmelerpggneitrggstsygtgsetesprnpssagswnsgsgpgstgnrnp gssgtggtat  
wkpgsspggstgswns gssgtgsgtgnqnp gsprpgstgtwnpgssergsaghwtsessvsgstg  
qwhsesgsfrpdspgsgnarpnpdwgtfeevsgnvspgtrreyhteklvt skgdkelrtgkek  
vtsgstttrrrscsktvtktvigpdghkev tkevvt sedgsdcpeamdlgtlsgigtldgfrhr  
hpdeaaffdtastgktfpgffspmlgef vsetesrgsesgiftntkessshhpgiaefpsrgKs  
(2)\* (1)\*\*(3) (1)\* \*(2)  
ssyskqftsstsynrgdstfesksykmadeagseadhegthstkrghaksrpvr gihtsplgkp  
slsp

**Peptides identified by MS/MS analysis of the 7-kDa fragment of hFibrinogen formed when this protein encounters hTryptase-β**

- (r) gdstfes k (2)
- (r) gdstfesksy k (1)
- (r) ggstsygtgsetespr (1)
- (k) m\*adeagseadhegthstk (2)
- (k) qftsstsynrgdstfes k (2)
- (n) rgdstfes k (1)
- (k) sykm\*adeagseadhegthstk (1)