Supplemental Figure 1. Expression of human factor XII (FXII) and Pro-hepatocyte growth factor activator (Pro-HGFA) with N-terminal HA tags.

To facilitate purification of recombinant Pro-HGFA, a nine amino acid hemagglutinin tag (Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala) was added to the protein’s C-terminus. Previously, we determined that adding such tags to C-termini of FXII and factor XI did not compromise protein function. As a control for the present experiment, we prepared and assessed the activity of FXII with a C-terminal HA tag. Please note that in all experiments described in this paper, the only protein with an HA tag is Pro-HGFA. Other proteins lacked HA tags.

Recombinant FXII with an HA tag underwent (A) autoactivation in the presence of polyphosphate and (B) reciprocal activation in the presence of prekallikrein, in a manner similar to untagged wild type FXII (FXII-WT).

The oligonucleotides used to introduce the HA tags into Pro-HGFA and FXII are show in the following table:

### Oligonucleotides used to put HA-tag on Factor XII

| HA-tag amino acid sequence | YPYDVPDYA |
|---------------------------|-----------|
| HA-tag DNA sequence       | 5’ TAC CCA TAC GAT GTT CCA GAT TAC GCT 3’ |
| Sense oligonucleotide     | 5’ GGG CGG GAG CAC ACC GTC'C TCC TAC CCA TAC GAT GTT CCA GAT TAC GCT TGA CGGGCCG CCT CCA GTC TAG ACC C 3’ |
| Antisense oligonucleotide | 3’G GCC TCT GTG TGG CAA AGG ATG GGT ATG CTA CAA GGT CTA ATG CGA ACT CGCCGGC GGA GCT CAG ATC TGG G 5’ |

### Oligonucleotides used to put HA-tag on Pro-HGFA

| HA-tag amino acid sequence | YPYDVPDYA |
|---------------------------|-----------|
| HA-tag DNA sequence       | 5’ TAC CCA TAC GAT GTT CCA GAT TAC GCT 3’ |
| Sense oligonucleotide     | 5’ C AGG CGG CTT GTG CTC TCC TAC CCA TAC GAT GTT CCA GAT TAC GCT TGA CCC TCC AGG GGG ACA C 3’ |
| Antisense oligonucleotide | 3’G TGT CCC GCT GGA GGG TCA ATG GGT ATG CTA CAA GGT CTA ATG CGA ACT GGA GGG AGC CAC AAG CCG CCT G 5’ |
Supplemental Figure 1. (Continued)

FXII was converted to FXIIa by incubation with dextran sulfate. Pro-HGFA was converted to the active protease HGFA by incubation with dextran sulfate and thrombin. Reactions were stopped by addition of polybrene to dissociate the proteins from the dextran sulfate and hirudin to neutralize thrombin. The proteins were repurified by anti-HA IgG affinity chromatography. Samples (2 µg) of the unactivated and activated proteins were run on 10% polyacrylamide-SDS gels under reducing conditions and stained with Coomassie Blue. The heavy chains (HC) and light chains (LC) of the activated forms are indicated at the right of each panel. Molecular mass markers on indicated at the left of each panel.

**FXII-Lys253 and FXII-ΔPRR.** (left and center panels) Samples (2 µg) of purified FXII(FXIIa) and FXII-Lys253 proteins were run on 10% polyacrylamide-SDS gels under non-reducing and reducing conditions and stained with Coomassie Blue. (right panel) Samples (2 µg) of purified FXII, the chimera FXII-PRR and FXII lacking amino acids 278 to 338 (FXII-ΔPRR) were run on 10% polyacrylamide-SDS gels under non-reducing conditions and stained with Coomassie Blue.
**Supplemental Figure 2. Factor XII/Pro-HGFA Heavy Chain/Light Chain Chimeras.** Show are amino acid sequences for human FXII and Pro-HGFA, with cysteine residues involved in disulfide bonds highlighted in black ((stderr)) and Arg^353 at the activation cleavage site highlighted in green ( hdc ). The Asp, His and Ser residues of the catalytic triad are highlighted in blue ( b , e , s ). The numbering system shown is for human FXII. Also shown are sequences for chimeric proteins containing the Pro-HGFA heavy chain and FXII light chain (HGFA^HC/FXII^LC) and the FXII heavy chain and Pro-HGFA light chain (FXII^HC/HGFA^LC). For all sequences, FXII sequence is in black and Pro-HGFA sequence in red. The arginine at position 281 (FXII numbering, highlighted in light blue) in HGFA^HC/FXII^LC, which is Arg^337 in Pro-HGFA numbering, was changed to alanine to remove a cleavage site for the protease plasma kallikrein.

Factor XII

| Position | Sequence |
|----------|----------|
| 1-100    | IPPEWAEPAKHE YKKAEEHTTV LTVTGEPEHF PFQYHRQLYH KTHKGRPQG QPVGCAATTPNF DQDQRGWYGL |
| 101-200  | IPPPEWAEPAKHE YKKAEEHTTV LTVTGEPEHF PFQYHRQLYH KTHKGRPQG QPVGCAATTPNF DQDQRGWYGL |
| 201-300  | E--PKKV-- --DH5SKSHSP QRGQTvpn MSgh--IL PQLHTQNHQ KERKFEQPLL RFFHKNEIY |
| 301-400  | RTEQAVARQ GQKGPDHADQ RLSAQARQRTN PCLHGGQRLLE VEGHRL--CH DPVGTYGAFP DVTJKASVYD |
| 401-500  | RQCHGHEVCE CEFGRGTFGT CEHTAHLSS FLNLGTQTHL IVATGTQVQA GPPFGAQGLP NIEPERPFL |
| 501-600  | GLRQCHGHEVCE CEFGRGTFGT CEHTAHLSS FLNLGTQTHL IVATGTQVQA GPPFGAQGLP NIEPERPFL |
| 601-700  | GGRKLHRGLA RTTLSGAPV PWASEATYRN VTAEQ--ARN W--GLGGHAFF RNPNDIYRPF SVFLNRDRLS |
| 701-800  | WEYDOLACQ PTQAAPPPTP VSPLHVLPLM PAQAPPKPPQ PTTRTPQPSQ TGPALPAKRE QPSLTRNGP |
| 801-900  | WEYDOLACQ PTQAAPPPTP VSPLHVLPLM PAQAPPKPPQ PTTRTPQPSQ TGPALPAKRE QPSLTRNGP |
| 901-1000 | LSQCQRLKS LSSMTYVVGQ LVALARAGHPY IAALYWGHSF CAGSLIAAPW VLTAESLQD RPAPEDLTTV |
| 1001-1100| LSQCQRLKS LSSMTYVVGQ LVALARAGHPY IAALYWGHSF CAGSLIAAPW VLTAESLQD RPAPEDLTTV |
| 1101-1200| LQERRHNSHT EFQTLAVRS YRLHEAFSPV SYQHLLLALRL LQEDADGCSA LLSPYVQPSV LPSSGARPSE |
| 1201-1300| LQERRHNSHT EFQTLAVRS YRLHEAFSPV SYQHLLLALRL LQEDADGCSA LLSPYVQPSV LPSSGARPSE |
| 1301-1400| LQHFFHNRTT DVQTGFIEQ IYPITLVSF NPSDLVLLVI RKKKKDSRA TRSQFVQPIC LPEGSTTPA |
| 1401-1500| LQHFFHNRTT DVQTGFIEQ IYPITLVSF NPSDLVLLVI RKKKKDSRA TRSQFVQPIC LPEGSTTPA |
| 1501-1600| TTQQVAGWG HQFEGAEAYA SFLQEAQVFPL SSLSGSSAPTD VHGSFSPILPM GDQGNSGDP |
| 1601-1700| TTQQVAGWG HQFEGAEAYA SFLQEAQVFPL SSLSGSSAPTD VHGSFSPILPM GDQGNSGDP |
| 1701-1800| LQDQAAER RLLTQLGIISSW GCGQRRKTP GYVTQYAVYL AWIREHTVS |
| 1801-1900| LQDQAAER RLLTQLGIISSW GCGQRRKTP GYVTQYAVYL AWIREHTVS |

*1
cDNAs for HGFA$^{\text{HC}}$/FXII$^{\text{LC}}$ and FXII$^{\text{HC}}$/HGFA$^{\text{LC}}$ were created by overlap extension PCR in three steps. Shown in this illustration is the strategy for creating HGFA$^{\text{HC}}$/FXII$^{\text{LC}}$. An similar strategy was used to create FXII$^{\text{HC}}$/HGFA$^{\text{LC}}$.

Oligonucleotides used to create the cDNAs for HGFA$^{\text{HC}}$/FXII$^{\text{LC}}$ are shown in the following table:

| PCR 1 | Template DNA | HGFA in pJv |
|-------|--------------|-------------|
| Primer A | 5’ G AAG ACA CCG GGA CCG ATC CAG 3’ |  |
| Primer B | 5’ G GAG CCG CTG CCC GCA GGC CTG GCG 3’ |  |
| Product AB | Contain HGFA HC sequence and overhang for FXII LC |  |

| PCR 2 | Template DNA | FXII in pJv |
|-------|--------------|-------------|
| Primer C | 5’ GGG GCG CCA GGC CTG CCC GCA GGC CTG GCG 3’ |  |
| Primer D | 5’ CGG GGA GCT CGG AGT CTA GA 3’ |  |
| Product CD | Contain FXII LC sequence and overhang for HGFA HC |  |

| PCR 3 | DNAs | Product AB + Product CD |
|-------|------|------------------------|
| Primer A | 5’ G AAG ACA CCG GGA CCG ATC CAG 3’ |  |
| Primer D | 5’ CGG GGA GCT CGG AGT CTA GA 3’ |  |
| Product AD | HGFA$^{\text{HC}}$/FXII$^{\text{LC}}$ DNA in pJv |  |

Oligonucleotides used to create the cDNAs for FXII$^{\text{HC}}$/HGFA$^{\text{LC}}$ are shown in the following table:

| PCR 1 | Template DNA | FXII in pJv |
|-------|--------------|-------------|
| Primer A | 5’ G AAG ACA CCG GGA CCG ATC CAG 3’ |  |
| Primer B | 5’ G GAG CCG CTG CCC GCA GGC CTG GCG 3’ |  |
| Product AB | Contain FXII HC sequence and overhang for HGFA LC |  |

| PCR 2 | Template DNA | HGFA in pJv |
|-------|--------------|-------------|
| Primer C | 5’ GG AAC GGC CCA CTG AGC TGC GGC AGG AGG 3’ |  |
| Primer D | 5’ CGG GGA GCT CGG AGT CTA GA 3’ |  |
| Product CD | Contain HGFA LC sequence and overhang for FXII HC |  |

| PCR 3 | DNAs | Product AB + Product CD |
|-------|------|------------------------|
| Primer A | 5’ G AAG ACA CCG GGA CCG ATC CAG 3’ |  |
| Primer D | 5’ CGG GGA GCT CGG AGT CTA GA 3’ |  |
| Product AD | FXII$^{\text{HC}}$/HGFA$^{\text{LC}}$ DNA in pJv |  |
Mutagenesis oligonucleotides used to change Arg337 in HGFA\textsuperscript{HC}/FXII\textsuperscript{LC} to alanine are shown in the following table:

| PCR 4 | Template DNA | HGFA\textsuperscript{HC}/FXII\textsuperscript{LC} DNA in pJ\textsuperscript{V} |
|-------|--------------|--------------------------------------------------------------------------------|
| Sense oligonucleotide | 5' GAA TCC CTC ACC \textbf{GCA} GTC CAA CTG TCA 3' | |
| Antisense oligonucleotide | 3' TGA CAG TTG GAC \textbf{TGC} GGT GAG GGA TTC 5' | |
Supplemental Figure 3. Factor XII/Pro-HGFA Heavy Chain Domain Chimeras.

Show are the amino acid sequences of the plasma forms of human FXII and Pro-HGFA, with cysteine residues involved in disulfide bonds highlighted in black (3), arginine residue 353 immediately in front of the activation cleavage site highlighted in green (4), and the Asp, His and Ser residues of the catalytic triad highlighted in blue (3). Also shown are sequences from FXII/Pro-HGFA chimeras FXII-FN2, FXII-EGF1, FXII-FN1, FXII-EGF2, FXII-KNG and FXII-PRR showing the Pro-HGFA sequence (in red) introduced into the FXII cDNA (black). The amino acid numbering system shown is for human FXII, starting with the N-terminal isoleucine of the mature plasma protein.

| Factor XII | Pro-HGFA |
|------------|----------|
| FXII-FN2   | IPPWEAPKEH KYKAEHEEVV LTVTGEPHIF PQYHRQLYH KTHKGRGPG QPEATTNPQ QPQHRQYGL |
| FXII-FN1   | E--PPPGGPA ALDPASG-P LNLGGS3NT QPQPSYHSSS PRAFTKDGQ TEE FD ETRY EYLEGGDRWA |
| FXII-EGF2  | RTEQAVARC QKGPDAH-H RLASQA3RTN PFLHGGRELE VEGHRL--SH PVGTYGAPQ DVDTKAA3ID |
| FXII-KNG   | GRTLSYGL ALDDGSA-H PWASEAYTN VTAEQ--ARN W-GLGGHAF RNPNDNIRPW RQVNLNRDLS |
| FXII-PRR   | WEY0LAQ--- CPTAAPPPT VSPRLHVPLM PAQAPPKDPQ PTTTRPPSQA TGALPAKRE QPPSLTRNGP |

Factor XII/Pro-HGFA HGFA chimeras.
Supplemental Figure 3. Factor XII/Pro-HGFA Heavy Chain Domain Chimeras (Continued).

cDNAs for FXII/HGFA heavy chain domain chimeras were prepared using the following strategy. The illustrations below uses creation of the chimera FXII-EGF1 as an example:

**Step 1:** *EcoR1* cleavage sites were inserted into the FXII cDNA in sequence encoding amino acids in linker regions between domains.

**Step 2.** Sequence for the FXII domain to be replaced is removed by digestion with *EcoR1*.

**Step 3.** Sequence for the Pro-HGFA domain to be inserted into FXII is copied from the Pro-HGFA cDNA using oligonucleotides that introduce *EcoR1* cleavage sites at the ends of the PCR product.

**Step 4.** The Pro-HGFA sequence is ligated into the *EcoR1* site introduced into the FXII cDNA.

**Step 5.** The sequences encoding the *EcoR1* sites are replaced with sequence encoding FXII and Pro-HGFA.
Supplemental Figure 3. Factor XII/Pro-HGFA Heavy Chain Domain Chimeras (Continued).

The Oligonucleotides used to copy parts of Pro-HGFA to create the FXII-HGFA heavy chain domain chimeras are listed in the following table. Sequence representing the EcoRI cleavage sites are shown in red.

| Pro-HGFA Domain | Oligonucleotide Sequence       |
|-----------------|--------------------------------|
| EGF1            | Sense 5'gatcgaattcaccggcctcgagggggggccccag3' |
|                 | Anti-Sense 5'gatcgaattcgccggaagttcctgtccggtgag3' |
| FN1             | Sense 5'gatcgaattcataacagctgtgtgtgatgagaccg3' |
|                 | Anti-Sense 5'gatcgaattgcgcttgccaccgaggtctgccag3' |
| EGF2            | Sense 5'gatcgaattcataacagctgtgtgtgatgagaccg3' |
|                 | Anti-Sense 5'gatcgaattgcgcttgccaccgaggtctgccag3' |
| KNG             | Sense 5'gatcgaattcataacagctgtgtgtgatgagaccg3' |
|                 | Anti-Sense 5'gatcgaattgcgcttgccaccgaggtctgccag3' |
| PRR             | Sense 5'gatcgaattcataacagctgtgtgtgatgagaccg3' |
|                 | Anti-Sense 5'gatcgaattgcgcttgccaccgaggtctgccag3' |

A different strategy was used to create FXII-FN2 as shown in the following schematic diagrams:

**Step 1.** NdeI and PpuMI restriction endonucleases were used to remove the original FN2 domain sequence.

**Step 2.** Sequence encoding the HGFA FN2 domain connected to the EGF1 and FN1 domains of FXII was synthesized.

**Step 3.** The sequence was digested with NdeI and PpuMI and ligated into the FXII from Step 1.
Supplemental Figure 3. Factor XII/Pro-HGFA Heavy Chain Domain Chimeras (Continued).

Schematic diagrams showing the relationships of domains in FXII, Pro-HGFA and FXII-HGFA domain chimeras. The disulfide bond that connects the heavy and light chains after activation are indicated. The activation cleavage sites (Arg353 for factor XII and Arg 372 for Pro-HGFA) are indicated by black arrows, while the active site serine residues (Ser544 for factor XII and Ser563 for Pro-HGFA) are indicated by white arrows and black bars.
Supplemental Figure 3. Factor XII/Pro-HGFA Heavy Chain Domain Chimeras (Continued).

Schematic Diagram of the human FXII heavy chain showing the location of cysteine residues involved in disulfide bonds. The individual domains of the heavy chain are indicated, as are the N-terminal isoleucine (1) and the C-terminal cysteine (340). Removal of the sequence shown in red is reported to disrupted FXII-surface binding and surface-dependent activity (Manuscript Reference 13).

Schematic Diagram representing the human FXII heavy chain in which individual domains have been replaced by sequence from Pro-HGFA. In the case of the fibronectin type II domain, the amino acids that are deleted from the factor XII sequence are shown. The colors used for each domain are also used in graphs in the manuscript figures.
Supplemental Figure 4. Chromogenic substrate Cleavage by FXIIa and HGFA. FXIIa (5 nM) or HGFA (30 nM) was incubated with varying concentrations of S-2302 or S-2366, respectively at room temperature in Reaction Buffer in the wells of a microtiter plate. Generation of p-nitroaniline (pNA) was determined by following changes in OD at 405 nm. Rates of pNA generation were determined with an extinction coefficient of 9920 M⁻¹.cm⁻¹ (405 nm). \(K_m\) and \(k_{cat}\) were determined by non-linear least squares fitting performed with Scientist Software (Micromath, St. Louis, MO, USA).

![Graphs showing cleavage by FXIIa and HGFA](image)

Supplemental Figure 5. Autoactivation of FXII, Pro-HGFA, HGFA\(^{HC}/FXII\(^{LC}\)) and FXII\(^{HC}/HGFA\(^{LC}\) by dextran sulfate and ellagic acid. FXII (blue), Pro-HGFA (violet), HGFA\(^{HC}/FXII\(^{LC}\) (orange) and FXII\(^{HC}/HGFA\(^{LC}\) (light blue), each 200 nM, were incubated with S-2302/S-2366 (200μM) and (A) dextran sulfate (10 μg/mL) or (B) ellagic acid (20 μM). Changes in OD 405 nm were continuously monitored.

![Graphs showing autoactivation of FXII, Pro-HGFA, HGFA\(^{HC}/FXII\(^{LC}\)) and FXII\(^{HC}/HGFA\(^{LC}\)](image)
Supplemental Figure 6. Autoactivation of FXII/HGFA heavy chain domain chimeras in the presence of ellagic acid or dextran sulfate. FXII (blue), FXII-FN2 (violet), FXII-EGF1 (brown), FXII-FN1 (pink), FXIII-EGF2 (grey), FXII-KNG (light green), or FXII-PRR (dark green), each 200 nM, were incubated with S-2302 (200μM) and (A) ellagic acid (20 μM) or (B) dextran sulfate (10 μg/ml). (C) FXII (blue), FXII-ΔPRR (green), and FXII-Ser^{340,467} (magenta), each 200 nM, were incubated with ellagic acid (20 μM) or dextran sulfate (10 μg/mL) and S-2302 (200μM). For all panels, dashed lines indicate proteins incubated in the absence of ellagic acid or dextran sulfate. Changes in OD 405 nm were continuously monitored.
Supplemental Figure 7. Estimations of domain charge for domains in the FXII and Pro-HGFA heavy chain, and binding of FXII/HGFA domain chimeras to heparin. The table shows predicted net charges for each of the domains in the heavy chains of FXII and Pro-HGFA. The column on the right indicates the NaCl concentration (mM) required to elute wild type proteins or chimeras off of a heparin-sepharose column.

| Protein | Calculated charge | Heparin Elution |
|---------|-------------------|-----------------|
|         | FXII | pro-HGFA | [NaCl] mM |
| FXII    | -    | -        | 350       |
| Pro-HGFA| -    | -        | 150       |
| FN2     | +0.225 | +1.043 | 330       |
| EGF1    | +1.041 | -2.057 | 150       |
| FN1     | +0.996 | -4.042 | 270       |
| EGF2    | -0.959 | +0.579 | 350       |
| KNG     | +0.186 | -4.775 | 270       |
| PRR     | +3.215 | -1.616 | 330       |

Supplemental Figure 8. Initial rates for protease activation. Shown are average initial rates of activation of PK (60 nM) by FXIIa, ΔFXIIa and chimeric proteases (60 pM) in the absence or presence of 70 μM poly-P. Rates derived from curves in figures 3A through 3D, represent nM product generated per minute over the first 5 minutes of reactions. Data represent means ± one standard deviation for at least three separate runs.

| Reaction | PK activation by FXIIa | PK activation by FXIIa with Poly-P | Protease         |
|----------|------------------------|-----------------------------------|------------------|
|          | FXIIa | βFXIIa | HGFA<sup>LC</sup> | FXIIa-FN2 | FXIIa-EGF1 | FXIIa-FN1 | FXIIa-EFG2 | FXIIa-KNG | FXIIa-PRR |
| PK activation by FXIIa Fig.3A & 3B | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.1 |
| PK activation by FXIIa with Poly-P Fig.3C & 3D | 5.5 ± 0.5 | 0.8 ± 0.2 | 0.9 ± 0.2 | 4.8 ± 0.8 | 0.8 ± 0.2 | 3.5 ± 0.3 | 5.4 ± 0.4 | 5.4 ± 0.5 | 5.0 ± 0.7 |
**Supplemental Figure 9. Reciprocal activation of PK and FXII or Pro-HGFA.** Western blots (reduced) of time courses of 200nM FXII (left) or Pro-HGFA (right) with 200 nM Prekallikrein (PK, both images in right hand column) at 37°C. Detection of FXII/FXIIa and PK/PKa were with polyclonal antibodies to the respective proteins. Pro-HGFA/HGFA was detected with an antibody to the C-terminal HA tag. Positions of standards for zymogen (Z) FXII, PK and Pro-HGFA and the heavy chains (HC) and light chains (LC) of FXIIa, and PKa are indicated at the right of the image.

**Supplemental Figure 10. Activation of PK by proteins with FXIIa catalytic domains in the absence of a surface.** PK (60nM) was incubated with 60 pM FXIIa (blue), ΔFXIIa (khaki), HGFA^{HC/FXIIa^{LC}} (orange), or β-FXIIa (black) in the absence of a surface. At the indicated times, aliquots were removed and tested for PKa generation by chromogenic assay.

**Supplemental Figure 11. ΔPro-HGFA.** Time course of ΔPro-HGFA formation (SDS-PAGE stained with GelCode Blue). Pro-HGFA (260 nM) with incubated with PKa (6 nM) in PBS at 37°C. Positions of standards for Pro-HGFA, the heavy chain of cleaved Pro-HGFA (HGFA-ΔHC) and ΔPro-HGFA, are indicated at the right of the image, and positions of molecular mass standards in kilodaltons are shown to the left.
**Supplemental Figure 12. ΔPro-HGFA.** Shown are average initial rates of activation FXII, ΔFXII and chimeric zymogens (100 nM) by PKa (12.5 nM) in the absence or presence of 70 μM poly-P. Rates derived from curves in figures 3A through 3D, represent nM product generated per minute over the first 5 minutes of reactions. Data represent means ± one standard deviation for at least three separate runs.

| Reaction                              | FXII | ΔFXII | HGFA<sub>HCG</sub>F<sub>LC</sub>-FXII | FXIII-FN2 | FXII-EGF1 | FXII-FN1 | FXII-EFG2 | FXII-KNG | FXII-PRR | FXII-Lys<sub>253</sub> |
|---------------------------------------|------|-------|-------------------------------------|-----------|-----------|----------|-----------|----------|---------|-------------------------|
| FXII activation by PKa Fig.5H & 5I    | 0.5±0.1 | 6.3±0.4 | 6.4±0.6                         | 5.7±0.5  | 1.9±0.2    | 2.2±0.2 | 2.2±0.2    | 6.3±0.5  | 0.9±0.1 | 6.9±0.8                 |

**Supplemental Figure 13. Kringle domain lysine binding sequences.** Shown is sequence from the FXII kringle domain between residues 244 and 262 indicating the position of a consensus binding site for lysine, and corresponding kringle domain sequences from Pro-HGFA, human plasminogen (Plg) and human tissue plasminogen activator (tPA). Note that the third kringle domain from plasminogen appears to lack a consensus lysine binding site, and this domain does not bind lysine.

| Substrate | Sequence                        |
|-----------|---------------------------------|
| FXII-KNG  | HAFCRNPOND<sub>IR</sub>-PWCFVLN |
| Pro-HGFA-KNG | HAYCRNPOND<sub>ER</sub>-PWCVVK   |
| Plg-KNG1  | ENYC<sub>N</sub>RNPNDPQGPW<sub>C</sub>YTTD |
| Plg-KNG2  | KNYCRNP<sub>D</sub>RELR-PWCF<sub>T</sub>T |
| Plg-KNG3  | ENYC<sub>R</sub>NPD<sub>G</sub>KRA-PWCHTTN |
| Plg-KNG4  | MNYC<sub>R</sub>NPDAD<sub>K</sub>G-PW<sub>C</sub>F<sub>T</sub>T |
| Plg-KNG5  | KNYC<sub>R</sub>NPDGDVGGPW<sub>C</sub>YTTN |
| tPA-KNG1  | HNYCRNP<sub>D</sub>RSK-PWCV<sub>V</sub>FK |
| tPA-KNG2  | HNYCRNP<sub>D</sub>GDAK-PWCHVLK |