Factor XII and kininogen ternary complex with gC1qR is governed by allostery

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Supplemental Data

MATERIALS AND METHODS

Expression and protein purification of FXIIFnII

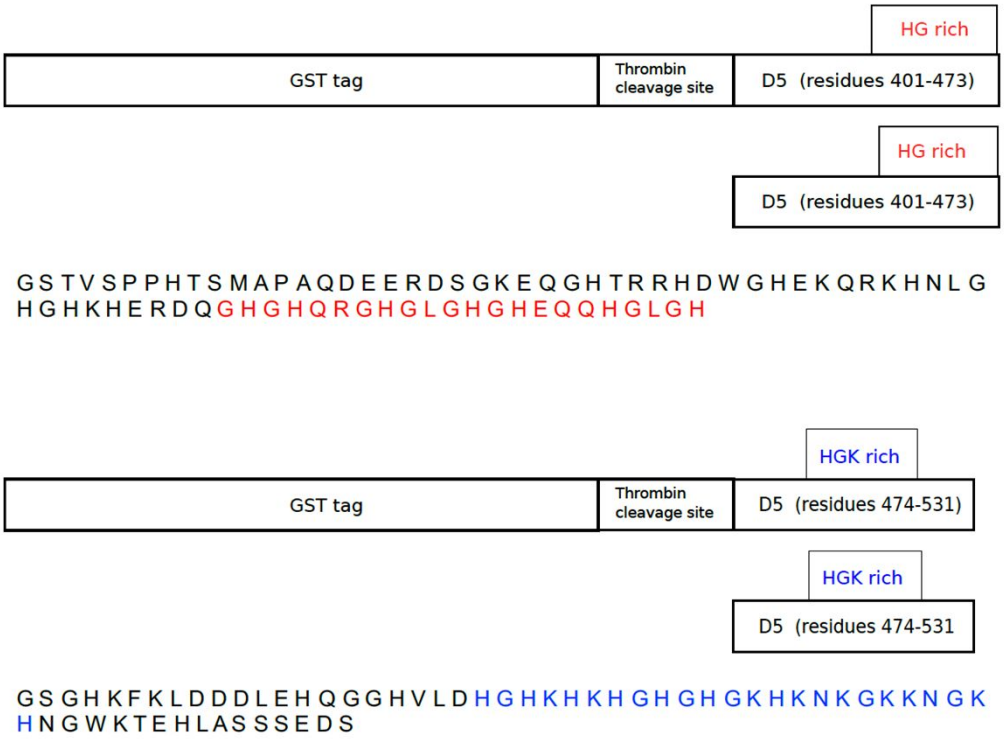
Drosophila S2 cells were grown to a cell density of 1 x 10^6 cells/ml in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum at 28°C . They were transfected with pMT-PURO-FXIIFnII DNA using calcium phosphate and grown for 24 hr prior to selection with 10 μg/ml puromycin (Sigma) to establish stable cell lines. Secreted proteins in serum-free Express Five culture medium (Invitrogen) were separated from cells and cell debris by centrifugation at 4600 rpm for 30 min. Media was collected and further centrifuged at 15 000 g for 1 hour, filtered using 0.22 μm filter and diluted with equal volume of 0.05 M MES pH 6.0. FXIIFnII was initially purified with anion-exchange chromatography using a Capto-S column (GE healthcare) with 0.05 M MES pH 6.0 and a gradient of 0-1.0 M NaCl was used for elution. Subsequently, this was applied to a HiTrap Ni^{2+} column (GE Healthcare) and eluted using an imidazole gradient concentration of 0-1.0 M and a final purification step utilized size-exclusion chromatography with a HiLoad Superdex^{TM} 75 16/60 column (GE healthcare) in 0.05 M Tris-HCl pH 8.0 and 0.1 M NaCl.

Expression and protein purification of HKD5, D5-1 and D5-2

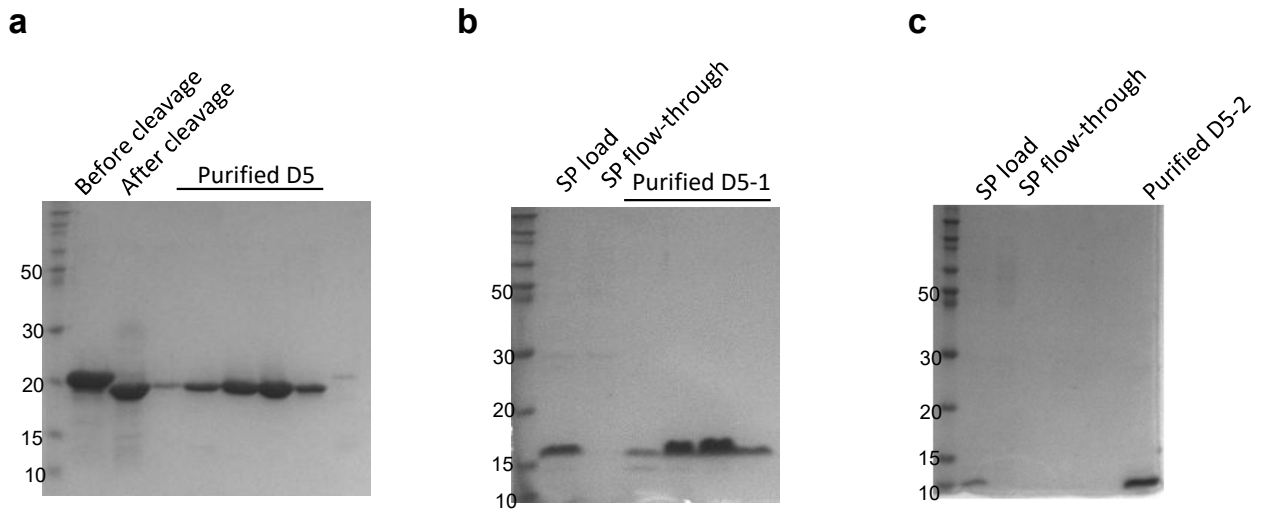
Expression was performed in 1 L of LB media with 50 μg/ml kanamycin, and induced with 1 mM IPTG for 5 hours at 30 °C. Pellets were re-suspended in 20 mM Tris HCl (pH 7.5), 150 mM NaCl and sonicated followed by centrifugation at 35,000 g for 30

mins. Cell lysates were loaded onto a HiTrap Ni^{2+} column (GE Healthcare) and eluted using an imidazole gradient concentration of 0-1.0 M imidazole. The tag was then cleaved by the addition of human alpha thrombin at a concentration of 1 U per mg of HKD5 protein. Cleavage was left for 18 hours at room temperature. The final purification step involved diluting the protein 5-fold in 10 mM potassium phosphate (pH 7) and loading onto a Hi-trap SP column (GE Healthcare). The protein was eluted using a gradient of 400-800 mM NaCl and concentrated to 5 mg/ml. The protein was dialyzed against 2 x 1 L of 20 mM potassium phosphate (pH 7.4), 100 mM NaCl.

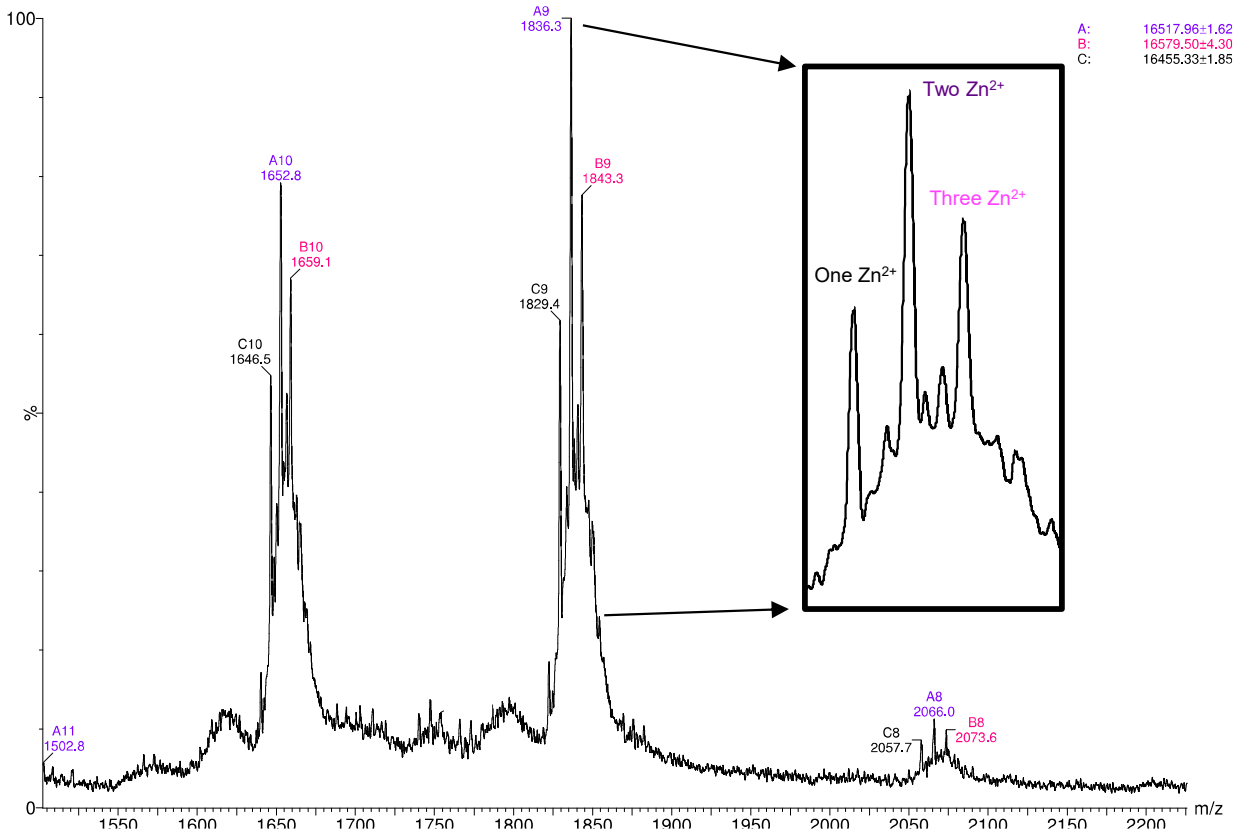
D5-1, D5-2 and HK 401-438 were expressed as GST fusion proteins in pGEX 4T-1 vector in 1 L LB media supplemented with 100 μg/ml ampicillin (Supplemental Figure 1). Lysates were purified by the addition of 1 mL of glutathione sepharose 4B (GE Healthcare). After a 1 hour incubation, beads were washed in 50 mM Tris HCl (pH 8.4), 150 mM NaCl, 25 mM calcium chloride and the GST tag was cleaved by the addition of 2.5 U human alpha thrombin. Cleavage was left for 18 hours at 4 °C. The proteins were then loaded onto a Hi-trap SP column and eluted using a gradient of 0-800 mM NaCl (Supplemental Figure 2). These proteins were concentrated to 3 mg/ml and dialysed using the same method as full length D5.



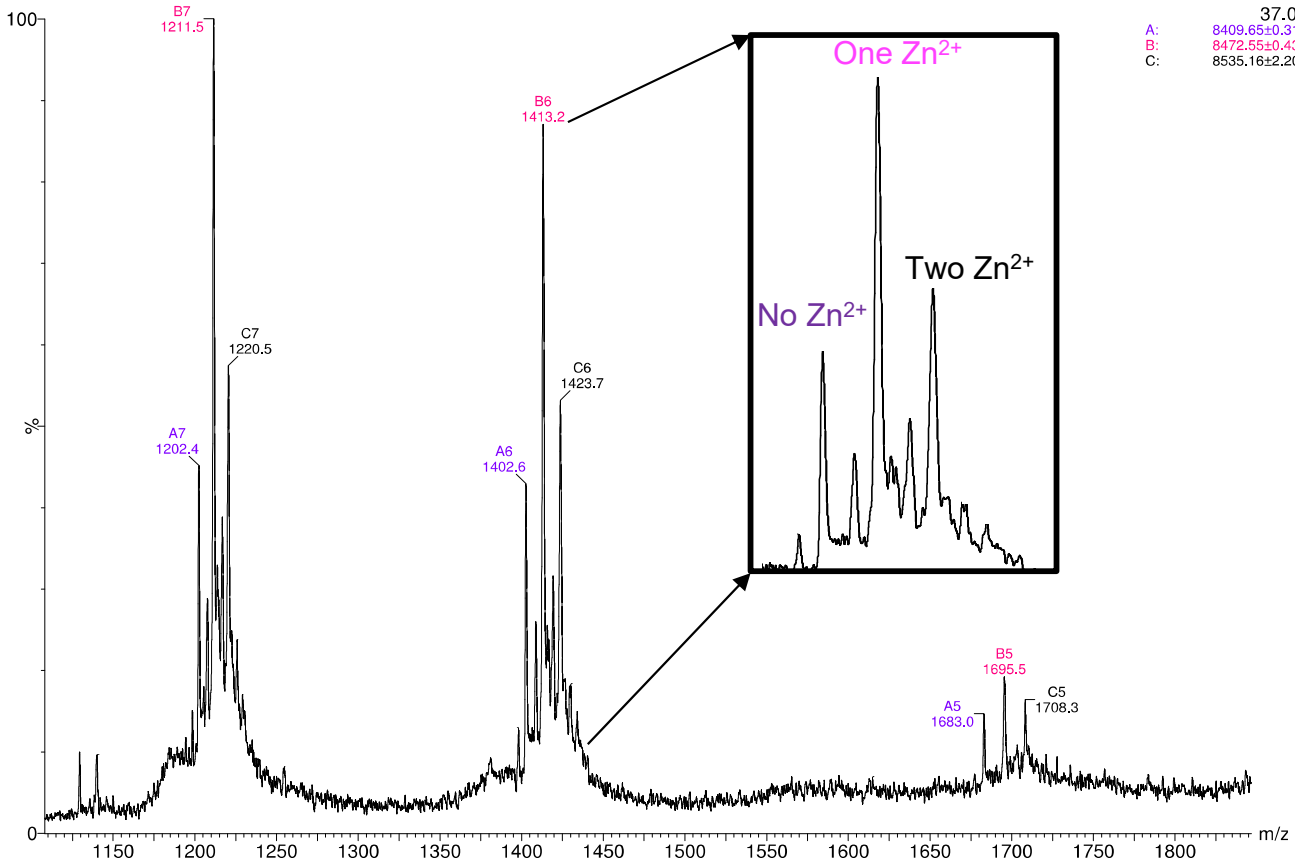
Supplemental Figure 1. Schematic representation of the D5-1 and D5-2 constructs, outlining the position of the GST tag, the thrombin cleavage site and the residues of D5. The top and bottom images represent the D5-1 and D5-2 constructs before and after thrombin cleavage respectively and the amino acid sequence for the cleaved products is shown underneath each cartoon. The His-Gly and His-Gly-Lys rich regions corresponding to HK 457-475 and HK 493-516 sequences respectively are highlighted in red or blue



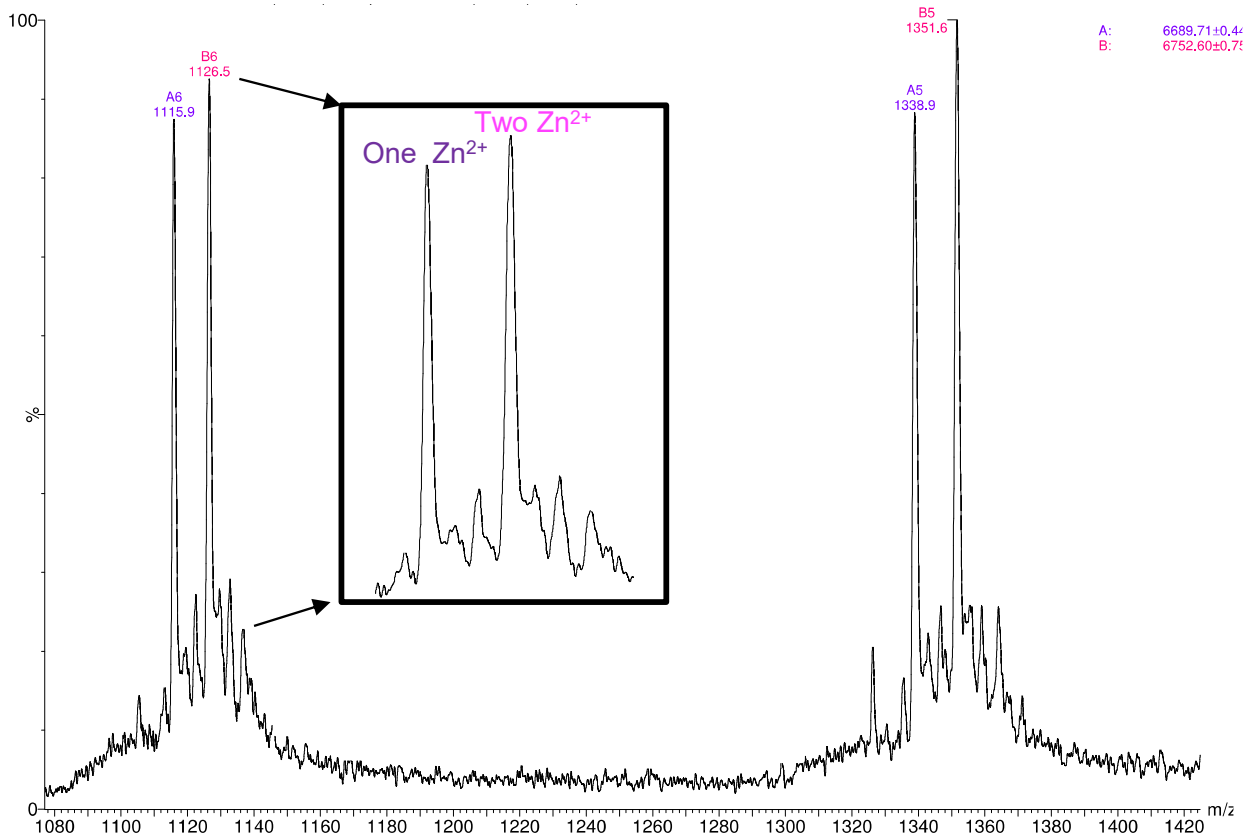
Supplemental Figure 2. Coomassie stained SDS-PAGE gels of the fractions collected for HKD5 before and after thrombin cleavage with the fractions collected from the cation exchange column also shown. The first lane of each gel shows the molecular weight protein markers with relevant sizes labelled in kDa. **b,c** show SDS-PAGE gels of D5-1 and D5-2 purifications respectively.



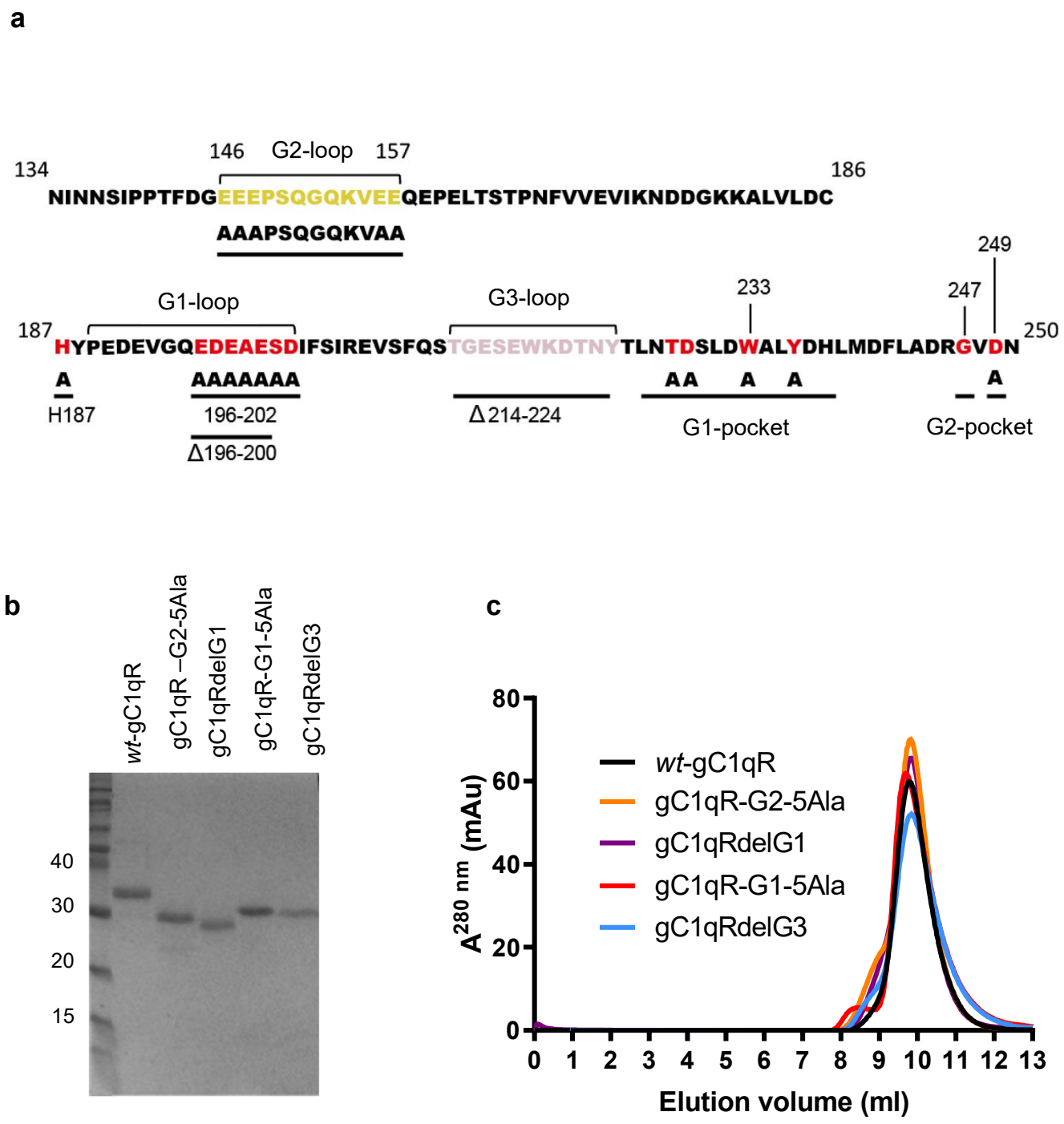
Supplemental Figure 3. Native ESI-FT mass spectrum of HKD5 (20 μM) in the presence of 100 μM zinc chloride. Three different species can be seen in this spectrum corresponding to HKD5 bound to one (species C), two (species A) and three (species B) zinc ions. The charge states and M/Z ratios are indicated above each peak, and the calculated mass for each species is shown on the right. A zoomed in image showing one set of peaks corresponding to the three species is also shown.



Supplemental Figure 4: Mass spectrum of recombinant D5-1. Native ESI-FT mass spectrum of D5-1 (20 μM) in the presence of 100 μM zinc chloride. Three different species can be seen in this spectrum corresponding to unbound D5-1 (species A), D5-1 bound to one (species B) and two zinc ions (species C). The charge state and M/Z ratios are indicated above the peak, and the calculated mass for each species is shown on the right. A zoomed in image showing one set of peaks corresponding to the three species is also shown boxed.

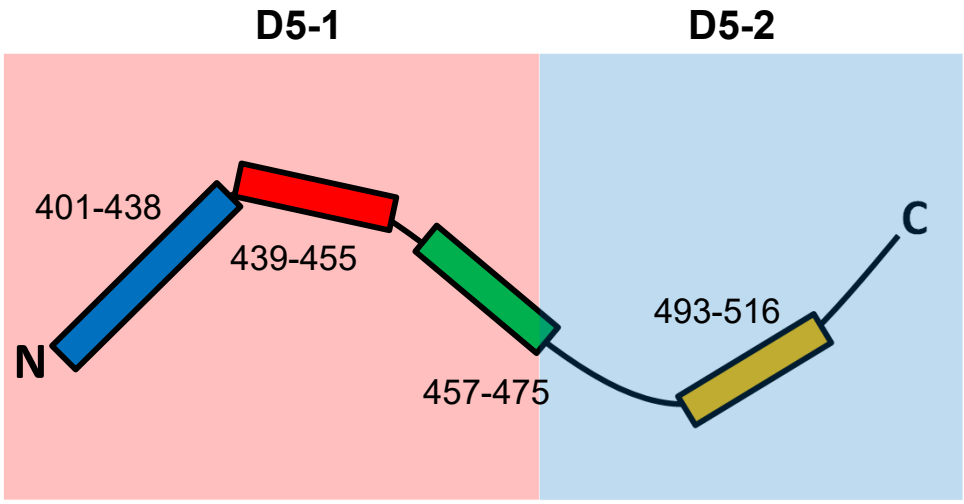


Supplemental Figure 5: Mass spectrum of recombinant D5-2. Native ESI-FT mass spectrum of D5-2 (20 μM) in the presence of 100 μM zinc chloride. Two different species can be seen in this spectrum corresponding to D5-2 bound to one (species A) and two (species B) Zn^{2+} ions. The charge state and M/Z ratios are indicated above the peak and the calculated mass for each species is shown on the right. A zoomed in image showing one set of peaks corresponding to the two species is also shown boxed.



Supplemental Figure 6. **a**, gC1qR amino acid sequence illustrating residues affected by the preparation of gC1qR variants. **b**, SDS-PAGE gel of the four purified gC1qR mutants compared with wild type gC1q-R (WT). The first lane is the protein marker, with sizes in kDa indicated. **c**, Analytical gel filtration traces of *wt*-gC1qR and gC1qR mutants (10 μM each), highlighting that all the mutants are able to form a trimeric structure indicated by the single elution peak.

a



b

HK 401-438 – T V S P P H T S M A P A Q D E E R D S G K E Q G H T R R H D W G H E K Q R K

HK 439-455 – H N L G H G H K H E R D Q G H G H

HK 457-475 – R G H G L G H G H E Q Q H G L G H G H

HK 493-516 – H G H K H K H G H G H G K H K N K G K K N G K H

c

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HKD5 493-516    HGHKHKHGHGHGKHKNKGKKNGKH
LyP-1                        CGNKRTRGC
Histone H4             SGRGKGGKGLGKGGAKRHRK
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Supplemental Figure 7. a, Schematic diagram showing the boundaries (above) and residues numbers. **b,** Amino acid sequence of the synthetic HKD5 derived peptides tested for binding to gC1qR. The sequence numbering includes the 18 amino acid signal sequence derived from UniProt - P01042 (KNG1_HUMAN). Thus HK 493-516 peptide corresponds to the peptide named HKH20 previously described spanning residues 479-498 numbered without the signal sequence. **c,** Sequence alignment of HKD5 493-516 peptide with other gC1qR binding peptide sequences derived from high diversity peptide screening (LyP-1) and from the histone H4 tail. The are aligned based on two glycines (red) separated by 5 amino acids which are basic in nature (blue).

See uploaded file - Video1.mov

Video1: Rocking movie showing the gC1qR-FXII complex. Cartoon diagram of the crystal structure showing the FXIIFnII domain (cyan) in complex with gC1qR trimers (wheat, purple, green) with Zn^{2+} ions in blue and the anionic G1-loop in red and key interacting side chains as sticks. Electrostatic interactions are shown as purple dotted lines.

See uploaded file - Video2.mov

Video2: Movie showing the negatively charged gC1qR pockets interacting with FXII. Cartoon diagram of the gC1qR-FXIIFnII crystal structure showing the FXIIFnII domain (cyan) in complex with gC1qR shown as a charged molecular surface (red=negative, blue=positive). The bound Zn^{2+} ion is a blue sphere on the left. The anionic G1-loop and G1 pocket are in the middle with Arg36 from FXII shown as sticks. Electrostatic interactions are shown as purple dotted lines. On the right FXII Arg65 is shown interacting with the gC1qR G2 pocket.

See uploaded file – Video3.mov

Video3: Movie showing the conformational change of key gC1qR residues between Zn^{2+} ion bound and unbound structures. Cartoon diagram of the crystal structure showing the gC1qR (green) in the region of the Zn^{2+} (blue sphere) binding site with coordinating residues His187 and Asp185 shown as sticks. Arg207 is illustrated as sticks forming electrostatic interactions (purple dotted lines) to residues Glu190 and Asp229. The conformational change was calculated as a morph in pymol and shows Arg207 switches between a position interacting with Asp185 in the absence of Zn^{2+} to form interactions with the G1 loop which may allosterically influence FXII binding.

See uploaded file – Video4.mov

Video4: Rocking movie showing the molecular surface of the gC1qR monomer and the relative disposition of binding sitess. Cartoon diagram of the gC1qR-FXIIFnII crystal structure showing the FXIIFnII domain (cyan) in complex with one gC1qR subunit (wheat) with the anionic G1-loop in red. The G1 pocket surface is purple and key side chain Trp233 is shown as sticks. The FXIIFnII domain is shown as a cartoon with residue Arg36 as sticks. The Zn^{2+} ion is depicted as a sphere (blue) with co-ordinating residues Asp185, His187 as sticks. The G3 loop implicated in HKD5 binding is in blue and the G3 pocket is defined by this loop and the gC1qR β-sheet. Overall the anatomy of the gC1qR monomer is such that it resembles a hand with the FXII binding site formed between the ring finger (G1-loop, red) and the little finger (Trp233, purple) and the palm of the hand contains the Zn^{2+} binding site adjacent to which is the thumb (G3-loop,blue) defining the principal HK binding site.