STRUCTURAL
BIOLOGY

Volume 75 (2019)

Supporting information for article:

Crystal structures of the recombinant β-factor XIIa protease with bound Thr-Arg and Pro-Arg substrate mimetics

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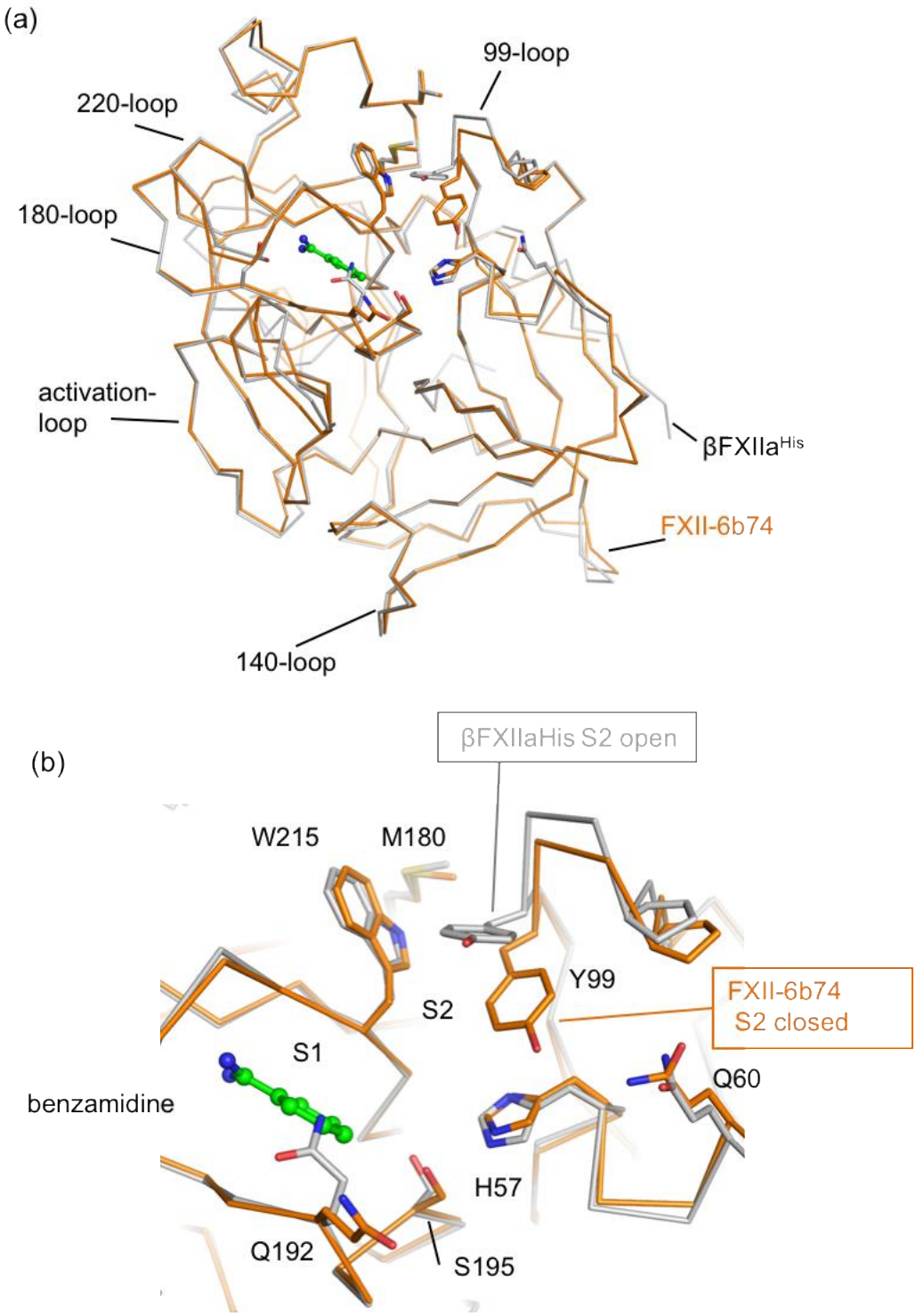

Figure S1 (a) Recombinant and plasma purified βFXIIa crystal structures compared. C-α traces are displayed for superposed recombinant protease domain βFXIIa^{His} (grey) and plasma purified βFXIIa (pdb code:6b74, orange) generated using PyMol and overall r.m.s.d. for the superposition=0.513 Å (b) Zoomed in view illustrating key differences in the region of the 99-loop and the S2 pocket with key residues highlighted as sticks.

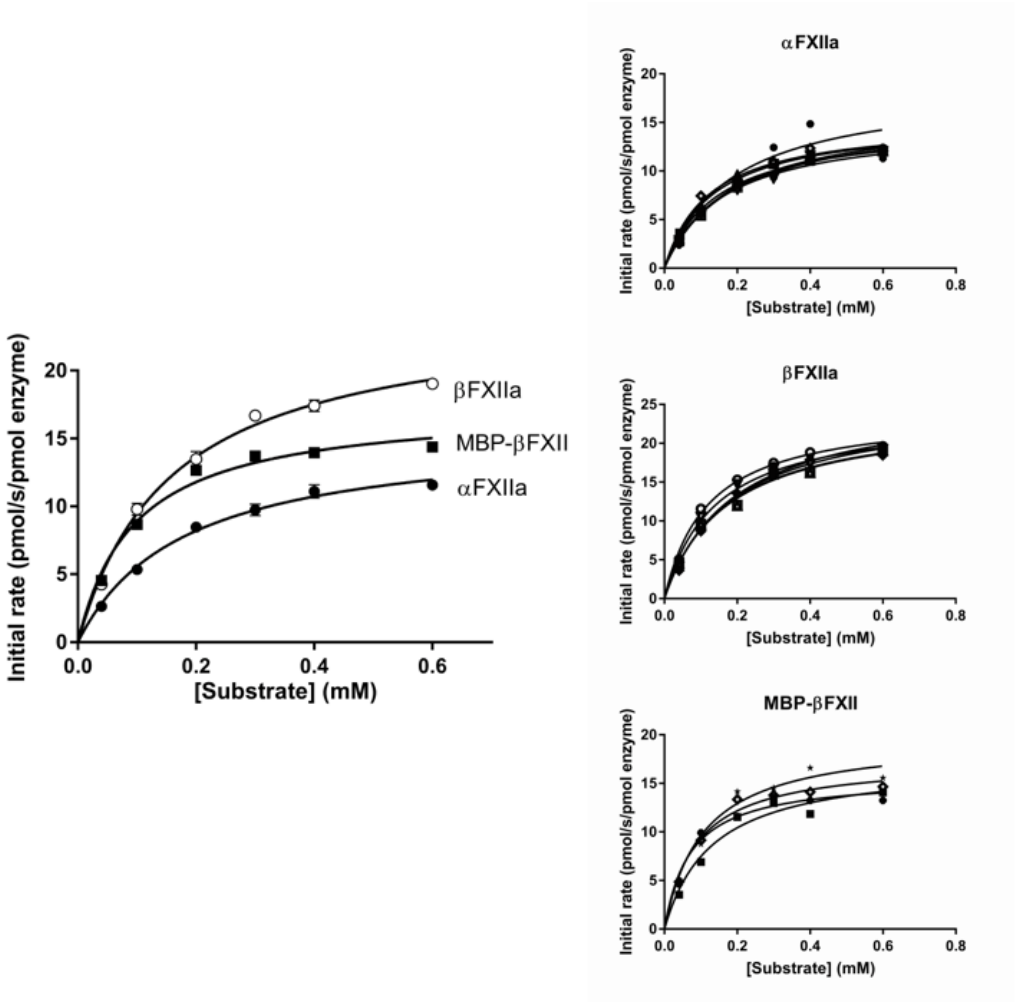

Figure S2 Plot of enzyme substrate S2302 hydrolysis as function of substrate concentration for recombinant MBP-βFXIIa^{His}, commercial βFXIIa and commercial αFXIIa. OD at 405 nm (as a function of time (min)) for different concentrations of S2302 added to βFXIIa was used to calculate initial rate (pmols^{-1}) of S2302 hydrolysis (left panel). An average of four experiments were used to determine the enzyme kinetics with independent plots superposed shown on the right panels.

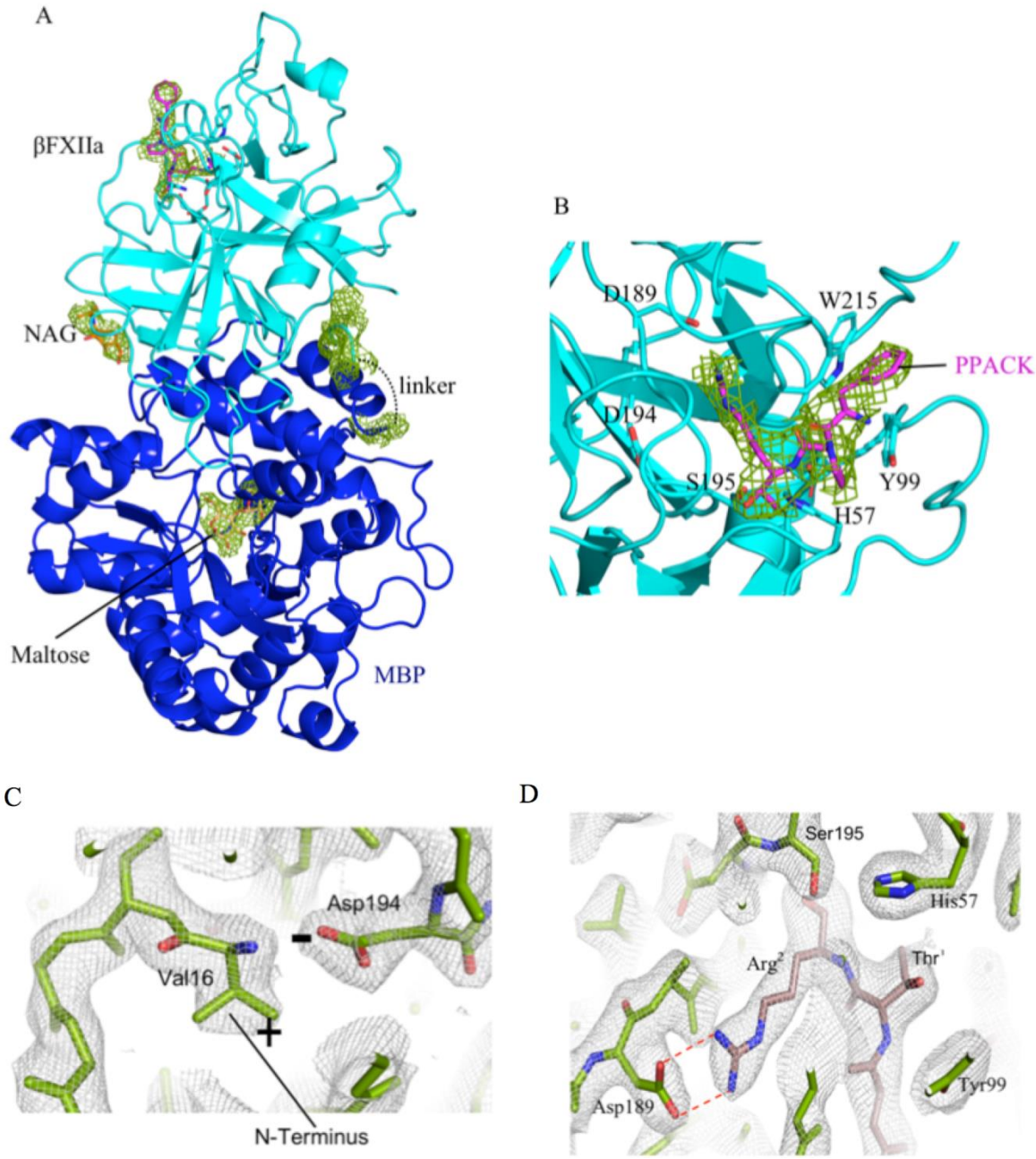

Figure S3 Cartoon diagram of the MBP-βFXIIa^{His} structure. MBP and βFXIIa^{His} are shown in blue and cyan respectively. *A,* Electron density 2Fo-Fc maps at 4 Å resolution are contoured at 1.0 σ are shown (green) for the bound maltose and N-linked glycan. *B.* Electron density Fo–Fc difference map showing the position of PPACK in the βFXIIa^{His} active site cleft. Side chains interacting with PPACK and the catalytic triad are drawn as sticks. *C,* Electron density 2Fo-Fc maps at 2.54 Å resolution are shown contoured at 1.0 σ (grey) for βFXIIa^{His} in the region of the N-terminus and *D* shows the S1 pocket with crystal symmetric residue Arg^{2} (brown) forming electrostatic interactions (red dotted lines).

Table S1 Summary of the docked EcTI and βFXIIa interfacial interactions and bond distances.

	βFXIIa [atom]	**Dist. [Å]**	**EcTI [atom]**
Hydrogen Bonds			
1	GLN 192[NE2]	2.91	ASN13[O]
2	TYR 99[OH]	2.79	THR 61[OG1]
3	GLY 215[N]	3.18	PRO62[O]
4	GLY 193[N]	2.71	ARG64[O]
7	SER 177[OG]	2.77	HIS114[O]
11	SER 214[O]	3.24	ARG64[N]
17	GLN 60[OE1]	2.83	THR69[OG1]
Salt Bridges			
1	GLU 149[OE2]	3.45	ARG12[NH1]
2	GLU 149[OE1]	2.74	ARG12[NH1]
3	GLU 149[OE2]	2.81	ARG12[NH2]
4	GLU 149[OE1]	2.73	ARG12[NH2]
5	ASP 189[OD1]	2.73	ARG64[NH1]
6	ASP 189[OD2]	3.69	ARG64[NH1]
7	ASP 189[OD1]	3.18	ARG64[NH2]
8	ASP 189[OD2]	2.73	ARG64[NH2]
9	ASP 222[OD2]	3.95	ARG92[NE]
10	ASP 222[OD2]	2.76	ARG92[NH1]
11	GLU 146[OE1]	3.11	ARG92[NH1]
12	GLU 146[OE1]	3.8	ARG92[NH2]
13	GLU 149[OE2]	3.42	ARG174[NH1]