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SUPPLEMENTARY ONLINE DATA

Allosteric antibody inhibition of human hepsin protease

Tobias KOSCHUBS*, Stefan DENGL†, Harald DÜRR†, Klaus KALUZA†, Guy GEORGES†, Christiane HARTL†, Stefan JENNEWEIN†, Martin LANZENDÖRFER†[1], Johannes AUER†, Alvin STERN‡, Kuo-Sen HUANG‡, Kathryn PACKMAN‡, Ueli GUBLER‡, Dirk KOSTREWA*, Stefan RIES†, Silke HANSEN†, Ulrich KOHNERT†, Patrick CRAMER* and Olaf MUNDIGL†[2]

*Gene Center Munich, Department of Biochemistry, Ludwig-Maximilians-Universität (LMU) München, Feodor-Lynen-Str. 25, 81377 Munich, Germany, †Roche Diagnostics GmbH, Roche Biologics Research, Nonnenwald 2, 82377 Penzberg, Germany, and ‡Roche, 340 Kingsland Street, Nutley, NJ 07110, U.S.A.

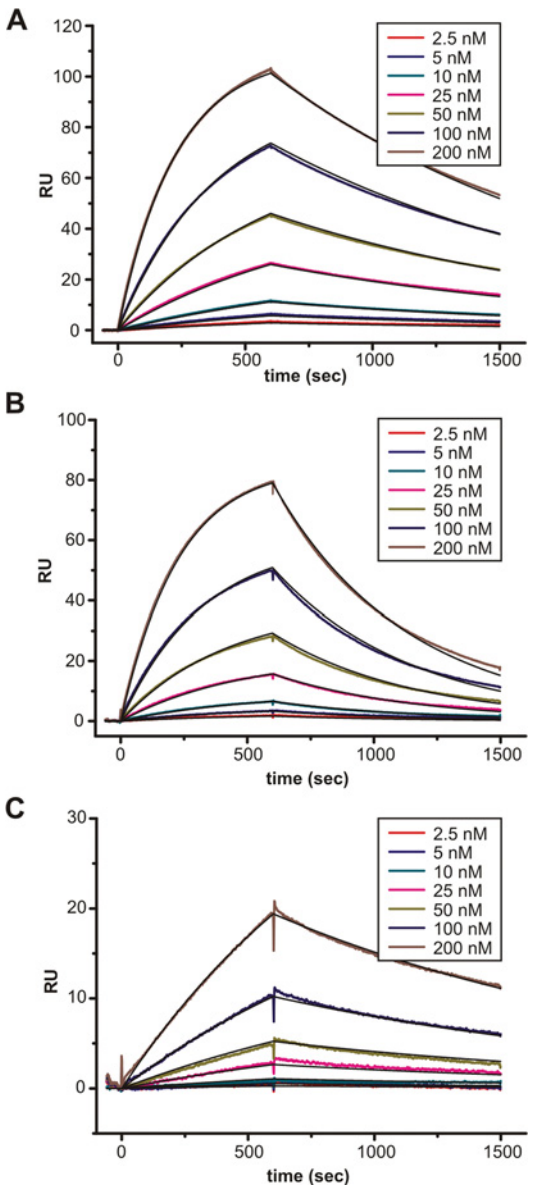

Figure S1 Adjusted SPR (Biacore) sensograms

(**A**) Analysis of antibody hH35 immobilized via Protein A on a CM5 sensor chip. Hepsin was injected at concentrations 0–200 nM. Curve fittings using a 1:1 Langmuir binding model are shown by black lines. (**B**) Analysis of antibody chH35 immobilized via Protein A. Samples were measured and analysed analogously to (**A**). (**C**) Analysis of antibody mH35 immobilized via Protein G. Samples were measured and analysed analogously to (**A**).

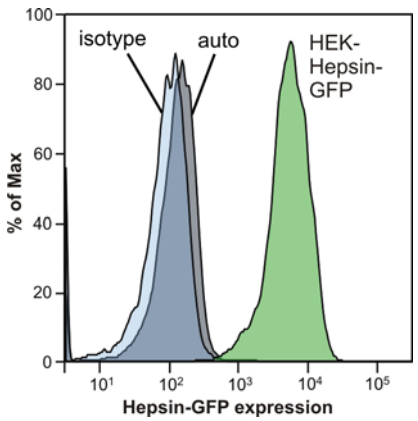

Figure S2 Characterization of the HEK-293 clone, stably overexpressing hepsin–GFP

HEK-293 cell lines that stably overexpress full-length hepsin with a C-terminal GFP-fusion tag were analysed by flow cytometry to measure the intrinsic GFP fluorescence. The diagram shows the analysis of one highly and homogeneously expressing clone that was selected for further studies.

[1] This paper is dedicated to the memory of the scientific integrity, passion and dedication of Martin Lanzendörfer, who died in September 2010, while at the peak of his career.

[2] To whom correspondence should be addressed (email olaf.mundigl@roche.com).

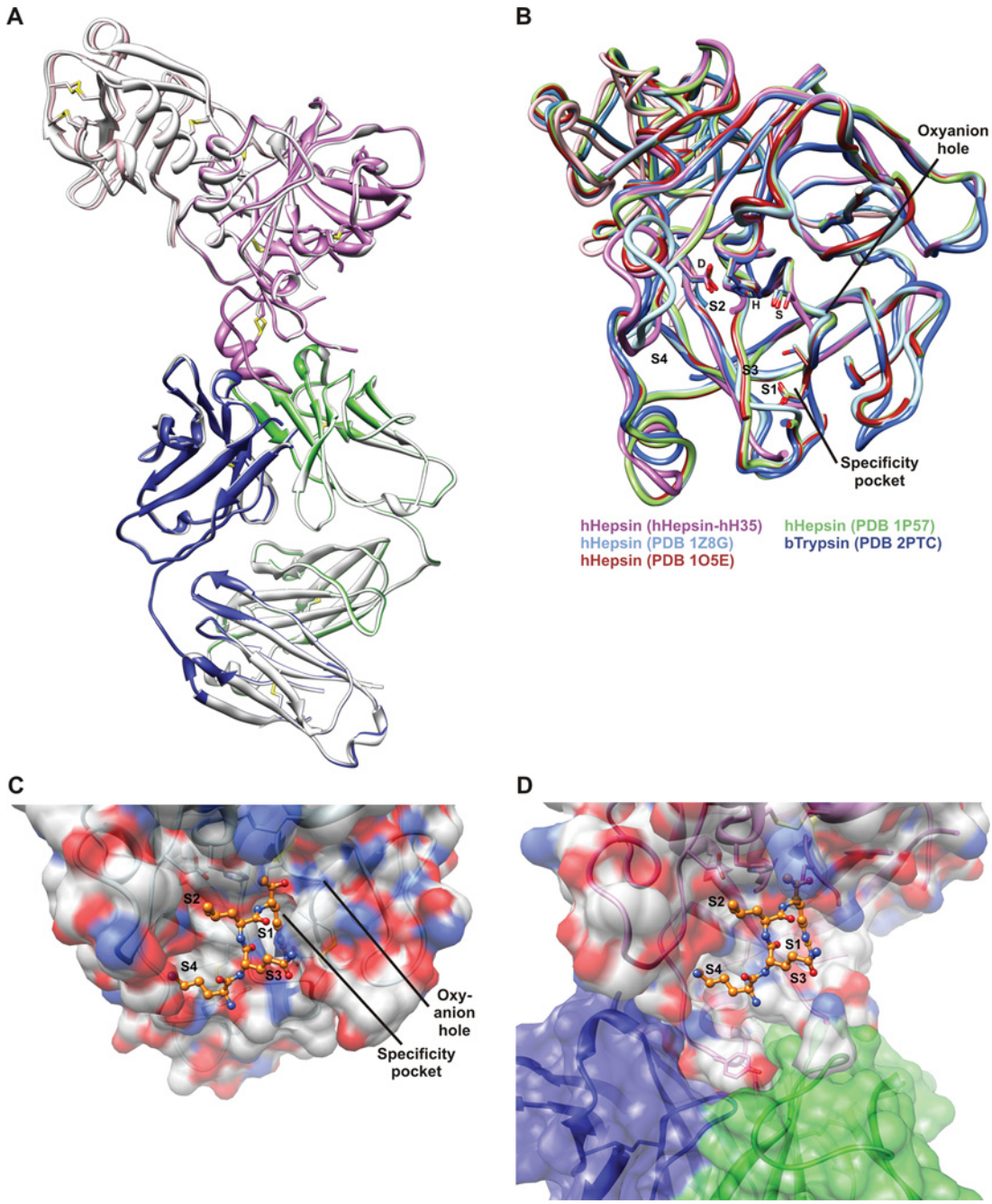

Figure S3 Analysis of the protein hHepsin–hH35 Fab crystal structure

(**A**) Ribbon-style model superimposition of the hHepsin–hH35 structure as shown in Figure 6(A) of the main text and the second NCS copy of hHepsin–hH35 present in the crystal unit cell (light grey). (**B**) Structural representation of hepsin in the hHepsin–hH35 complex superimposed on to other published hepsin structures and on bovine trypsin. Catalytic triad residues (aspartate, histidine and serine) are shown as sticks. Substrate-binding pockets are marked by S1–S4. (**C**) Semi-transparent surface view with an underlying ribbon model of human hepsin complexed with the KQLR-methylene ligand (shown in orange as a ball-and-stick representation) as found in the PDB code 1Z8G structure. Substrate-binding pockets are marked by S1–S4. (**D**) Semi-transparent surface view with an underlying ribbon model of the hHepsin–hH35 complex structure. A hypothetical model complex with the KQLR-methylene ligand (based on the superimposition on to the PDB code 1Z8G structure) is shown.

	H-Bonds NCS copy 1			H-Bonds NCS copy 2		
CDR	Fab hH35	Hepsin	Distance (Å)	Fab hH35	Hepsin	Distance (Å)
CDR-L1	THR 29 [OG1]	GLN 385 [NE2]	3.9	THR 29 [OG1]	GLN 385 [NE2]	3.6
				TYR 32 [OH]	TYR 341 [N]	3.9
CDR-L2	ASP 50 [O]	GLN 385 [N]	3.4	ASP 50 [O]	GLN 385 [N]	3.2
	ASN 52 [OD1]	GLN 385 [N]	3.3	ASN 52 [OD1]	GLN 385 [N]	3.4
	ASN 53 [ND2]	LEU 383 [O]	2.8	ASN 53 [ND2]	TYR 328 [OH]	3.9
	ARG 54 [O]	TYR 328 [OH]	3.3	ARG 54 [O]	TYR 328 [OH]	3.2
CDR-H1	ASP 31 [O]	GLN 331 [N]	3.7	ASP 31 [O]	GLN 331 [N]	3.5
	ASP 31 [O]	LYS 333 [NZ]	3.6			
	SER 33 [N]	GLY 329 [O]	2.6	SER 33 [N]	GLY 329 [O]	2.8
	ARG 35 [NH2]	ASP 326 [O]	2.4	ARG 35 [NH2]	ASP 326 [O]	2.4
CDR-H2	THR 52A [N]	GLN 331 [OE1]	2.9	THR 52A [N]	GLN 331 [OE1]	2.9
CDR-H3	PHE 96 [N]	PHE 327 [O]	2.6	PHE 96 [N]	PHE 327 [O]	2.7
	ALA 101 [N]	PHE 327 [O]	3.0	ALA 101 [N]	PHE 327 [O]	3.1
Other-H	THR 30 [O]	GLN 331 [NE2]	3.0	THR 30 [O]	GLN 331 [NE2]	2.8
	ARG 94 [NH2]	TYR 328 [O]	3.6	ARG 94 [NH1]	TYR 328 [O]	3.6

	Salt bridges NCS copy 2		
CDR	Fab hH35	Hepsin	Distance (Å)
CDR-H1	ASP 31 [OD1]	LYS 333 [NZ]	3.8

	Hydrophobic interactions NCS copy 1		Hydrophobic interactions NCS copy 2	
CDR	Fab hH35	Hepsin	Fab hH35	Hepsin
CDR-L1	TYR 32	ILE 317	TYR 32	ILE 317
		GLY 324		
		ALA 325		ALA 325
		GLY 340		GLY 340
		TYR 341		TYR 341
		PRO 387		PRO 387
CDR-L2	ALA 55	PHE 327	ALA 55	PHE 327
		TYR 328		TYR 328
	PRO 56	TYR 328	PRO 56	TYR 328
CDR-L3	TRP 91	VAL 321	TRP 91	VAL 321
		GLY 324		GLY 324
		ALA 325		ALA 325
	PHE 96	GLY 324	PHE 96	GLY 324
		ALA 325		ALA 325
Other-L	TRP 35	PHE 327	TRP 35	PHE 327
	GLY 46	PHE 327	GLY 46	PHE 327
	LEU 47	PHE 327	LEU 47	PHE 327
	ILE 48	PHE 327	ILE 48	PHE 327
	GLY 49	PHE 327	GLY 49	PHE 327
		ALA 384		
CDR-H1	TYR 32	GLY 324	TYR 32	
		PHE 327		
		TYR 328		TYR 328
		GLY 329		GLY 329
CDR-H2	TRP 50	ILE 317	TRP 50	
		GLY 324		GLY 324
CDR-H3	GLY 95	ALA 325	GLY 95	
		PHE 327		PHE 327
		TYR 328		TYR 328
		GLY 329		GLY 329
	PHE 96	ALA 325	PHE 96	
		PHE 327		PHE 327
		TYR 328		TYR 328
	ALA 101	ALA 325	ALA 101	
		PHE 327		PHE 327
		TYR 328		TYR 328
	TYR 102	TYR 328	TYR 102	TYR 328
Other-H	TRP 47	GLY 324	TRP 47	GLY 324

Figure S4 Continued

	Crystal contacts NCS copy 1			Crystal contacts NCS copy 2		
CDR	Fab hH35	Hepsin	Distance (Å)	Fab hH35	Hepsin	Distance (Å)
Other-L	GLN 1 [N]	SER 213 [O]	3.2	GLN 1 [NE2]	ARG 214 [O]	3.0
	THR 18 [OG1]	ARG 124 [NH1]	3.6			
	VAL 159 [O]	ARG 208 [NH1]	3.6	VAL 159 [O]	ARG 208 [NH1]	3.0
				GLY 199 [O]	THR 128 [N]	3.8
				THR 201 [OG1]	ARG 124 [NE]	3.6
CDR-H1				TYR 32 [OH]	GLU 252 [OE2]	3.2
CDR-H2				ASP 61 [O]	SER 411 [OG]	3.6
	ASP 62 [OD1]	SER 213 [N]	3.5	ASP 62 [OD1]	LEU 212 [N]	3.8
	ASP 62 [OD1]	SER 213 [OG]	2.8	ASP 62 [OD1]	SER 213 [N]	3.3
	ASP 62 [OD2]	SER 213 [OG]	3.0	ASP 62 [OD2]	SER 213 [OG]	2.6
Other-H	GLN 43 [OE1]	ARG 214 [NH2]	3.9			
	LYS 83 [NZ]	MET 413 [O]	3.7	LYS 83 [NZ]	MET 413 [O]	3.5
	GLU 85 [OE1]	ARG 210 [NH2]	2.8	GLU 85 [OE1]	ARG 210 [NH2]	2.9
Other-L	GLU 160 [OE2]	ARG 208 [NH1]	3.7	GLU 160 [OE1]	ARG 208 [NH1]	3.4
Other-H	GLU 85 [OE1]	ARG 210 [NE]	3.9	GLU 85 [OE1]	ARG 210 [NE]	4.0
	GLU 85 [OE1]	ARG 210 [NH2]	2.8	GLU 85 [OE1]	ARG 210 [NH2]	2.9

Figure S4 Residue contact analysis between hH35 Fab fragment and human hepsin

Residue contacts between the hH35 Fab fragment and human hepsin were analysed using CCP4 CONTACT [4] and PISA [5] software.

Table S1 K_m values of serine proteases using the acetyl-KQLR-AMC peptide as a substrate

Values are means ± S.D., from at least three independent tests performed in triplicate. The K_m in column two refers to KQLR as the substrate, whereas the K_m in column four refers to the referenced 'ideal' substrate.

Enzyme	Apparent K_m (μM)*	Ideal peptide substrate†	Apparent K_m (μM)	Reference
Hepsin	10.1 ± 0.8			
Trypsin	34.0 ± 2.4	N^{alpha}-p-Tos-Gly-Pro-Lys-AMC	14	[1]
Trypsin	34.0 ± 2.4	Boc-Phe-Ser-Arg-4-MCA	16.5 ± 0.9	[2]
Bovine enteropeptidase	22.2 ± 1.2	Trypsinogen	5.6 ± 0.9	[3]
HAT	123.6 ± 16.3	ABZ-Arg-Gln-Asp-Arg-ANB-NH_2	25.4 ± 2.1	[2]
Matriptase	26.5 ± 2.7	ABZ-Arg-Gln-Asp-Arg-ANB-NH_2	68.5 ± 4.2	[2]
Matriptase	26.5 ± 2.7	Boc-Phe-Ser-Arg-4-MCA	12.1 ± 1.9	[2]

*Hydrolysis rates of at least six different peptide concentrations were monitored for determination of the apparent K_m values. Data were fitted to the Michaelis–Menten equation.
†As reported in the stated references.

Table S2 Initial (v_0) and steady-state (v_s) velocities of Figure 4(C) (in the main text) measurements

Measurement	v_0 (nM · s^{-1})	v_s (nM · s^{-1})
No hH35	12.8	11.8
18 nM hH35	12.1	10.4
55 nM hH35	11.8	7.8
166 nM hH35	9.2	4.0
500 nM hH35	6.8	1.2

Table S3 Comparison of different inhibition models

The calculated results are ranked according to the R^2 equation. AICc, Akaike information criterion with a correction for finite sample size; Sy.x, S.D. of the residuals.

Rank by runs	Equation*	R^2	AICc	Sy.x	Test	Convergence
1	Mixed Tight	0.99553	−933.779	1.13E−02	Pass	Yes
2	Non-competitive Tight	0.99549	−935.124	1.13E−02	Pass	Yes
3	Competitive Tight	0.99273	−885.02	1.43E−02	Pass	Yes
4	Uncompetitive Tight	0.99063	−858.376	1.63E−02	Pass	Yes

*Study type: tight-binding inhibition with three replicates, fitted in SigmaPlot®.

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