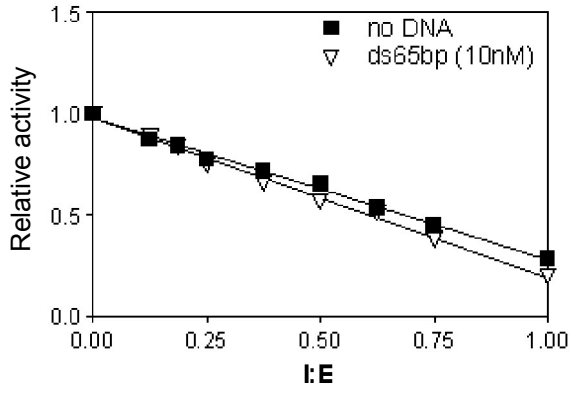
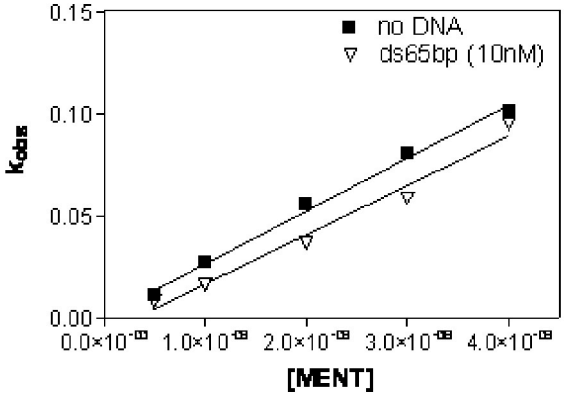
A



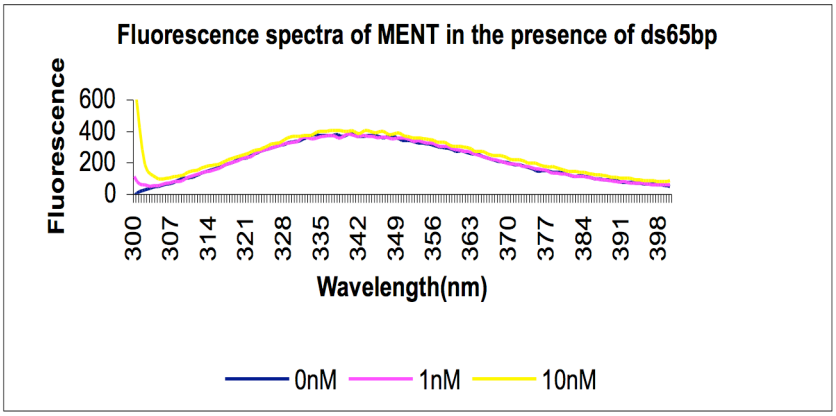
SI of MENT with cathepsin L= 1.4
SI of MENT with cathepsin L/ds65bp DNA= 1.2

B



k_{ass} of MENT with cathepsin L = 2.6 x 10^6 $M^{-1}s^{-1}$
k_{ass} of MENT with cathepsin L/ds65bp DNA = 2.4 x 10^6 $M^{-1}s^{-1}$

C



ds65bp	Peak measured at 340nM		Percentage of movement
0nM	382.27678	0	0
1nM	380.853871	-1.422909	-0.372219573
10nM	399.181674	16.90489	4.422160823

Supplementary Figure 1: Stoichiometry of inhibition (SI) (A) and rate of association (k_{ass}) (**B**) for cathepsin L inhibition by MENT in the presence and absence of ds65bp DNA. (A) Cathepsin L (10nM) with or without ds65bp (10 nM) was incubated with increasing concentrations of MENT for 20 mins at 37ºC and residual enzyme activity against the substrate N-cbz-Phe-Arg50-methylcoumarin was measured. (B) The k_{ass} values for the interaction between MENT and cathepsin L were determined as described in supplementary Materials and Methods. (**C**) The effect of ds65bp on the fluorescence emission spectra MENT. The intrinsic tryptophan fluorescence of the protein was scanned over the range of 300 to 390 nm, after excitation at 295 nm, in the absence (blue) and presence (pink and yellow) of 1 nM and 10 nM of ds65bp, respectively.

Supplementary Figure 1

Supplementary Figure 1:

Materials and Methods

Determination of kinetic parameters for interaction between MENT and cathepsin L

Human cathepsin L was expressed in *Pichia pastoris* (system kindly donated by Dr Dieter Bromme) and further purified, as described previously. All cathepsins were pre-activated by incubation in cathepsin buffer (0.1 M acetate, 1 mM EDTA, 0.1% (w/v) Brij-35, 0.02% (w/v) sodium azide, 10 mM cysteine, pH 5.5) for at least 20 min at room temperature before use. The active enzyme concentration was determined with trans-epoxysuccinyl-L-leucylamido-(4-guanidino)-butane (E-64) titration (Kirschke and Barrett, 1980). The N-Cbz-Phe-Arg-methlycoumarin enzyme substrate was purchased from Sigma. The 65bp DNA was synthesized by Gene Works.

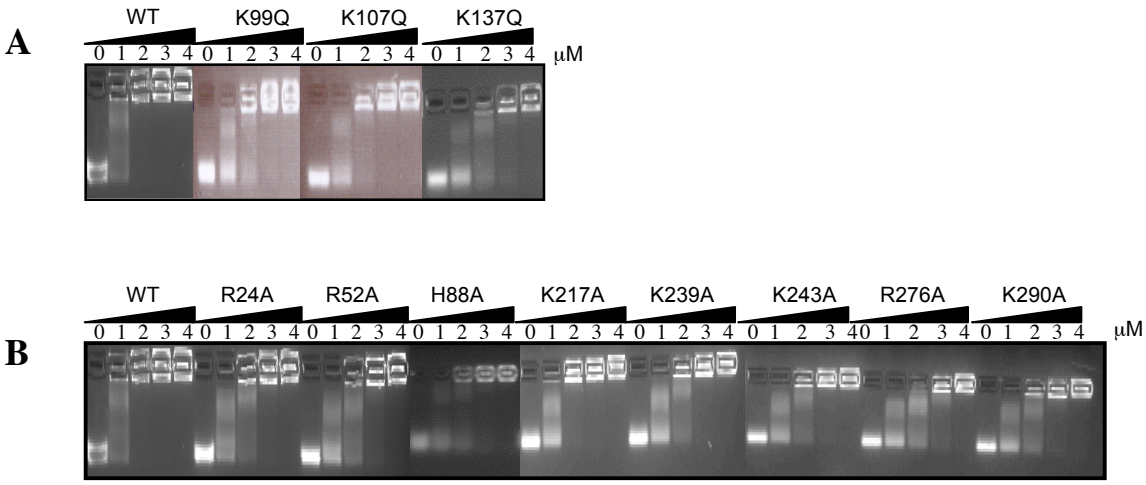
The stoichiometry of inhibition in the presence and absence of DNA was measured as dscribed previously (Irving *et al*., 2002). The discontinuous method for determination of k_{ass} values between MENT and cathepsin L was undertaken in cathepsin buffer at 30°C using the well mode in a Fluostar Galaxy plate reader (BMG Labtechnologies, Germany) with excitation/emission wavelengths of 370/460nm. For any given assay, the enzyme concentration was held constant at 0.1 nM, while MENT concentrations were at least 5-fold higher (5-40 nM). 10 nM ds65bp DNA was used to test the effects of DNA. In the assay, a final concentration of 40 mM N-Cbz-Phe-Arg-methlycoumarin substrate in a total volume of 200 μl was used to measure the residual activity of cathepsin in the absence and presence of cofactor at various time points after addition of the serpin (2-160 seconds, depending on the MENT concentration used). Initial velocities were determined by measuring every second over 2 mins, yielding linear plots of fluorescence increase versus time. Linear least squares regression of the plots using PRISM (GraphPad Software) gave estimates of the slope (initial velocity). A semi-logarithmic plot of initial activity versus time for each concentration of serpin was fitted using linear regression, producing negative linear slopes equivalent to the observed rate of inhibition for each individual serpin concentration (k_{obs}). The k_{obs} values were plotted against [I] and a linear least square regression analysis of the data gave a slope which represented the association rate constant (k_{ass} [$M^{-1}.s^{-1}$]).

Measurement of change in intrinsic tryptophan fluorescence of MENT in the presence and absence of DNA

The effect of ds65bp DNA on the fluorescence emission spectrum of MENT was examined by monitoring the intrinsic fluorescence of MENT in the presence and absence of the DNA. Each sample in an acrylic cuvette was excited at a wavelength of 295nm and the emission was scanned 5 times over the range of 300 to 390 nm at 60 nm/min, using excitation and emission slit widths of 5 nm. 200 nM MENT was incubated alone or with 1 or 10 nM ds65bp DNA in a final volume of 2ml in buffer containing 10 mM HEPES, 0.5 mM EDTA, 40 mM NaCl, 0.1% (w/v) Brij-35 at pH 7.0. A scan of buffer alone was also performed. The emission spectrum of buffer was subtracted from the emission spectra in the presence and absence of DNA.

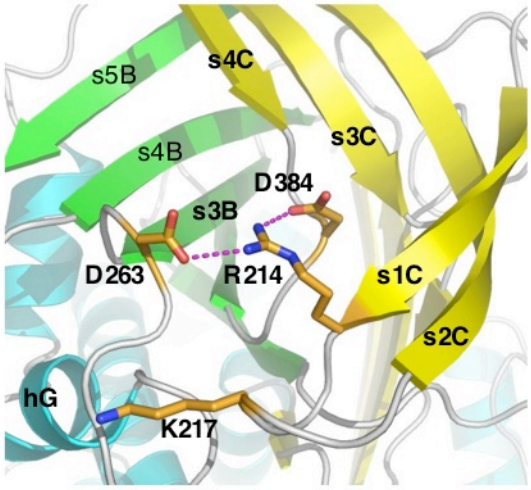
References

Barrett, AJ and Kirschke, H. (1981) Cathepsin B, Cathepsin H, and cathepsin L. *Methods Enzymol*, **80**, 535-561.



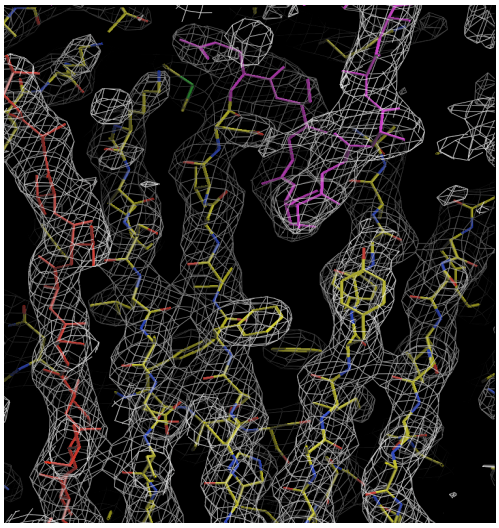
Supplementary Figure 2: EMSAs of mutants with unaltered DNA binding affinity. Representative gels of EMSA reactions using D- and E-helix (**A**) and Alanine mutant proteins (**B**). Mutant proteins are indicated at the top of each panel and final concentration (μM) of purified protein shown at the top of each lane.

Supplementary Figure 2



Supplementary Figure 3: Interactions made by R214 with D263 and D384 in native MENTWT. The "gate" region comprises strands s3C and s4C. K217 is shown in stick and labelled. Hydrogen bonds are shown as magenta broken lines.

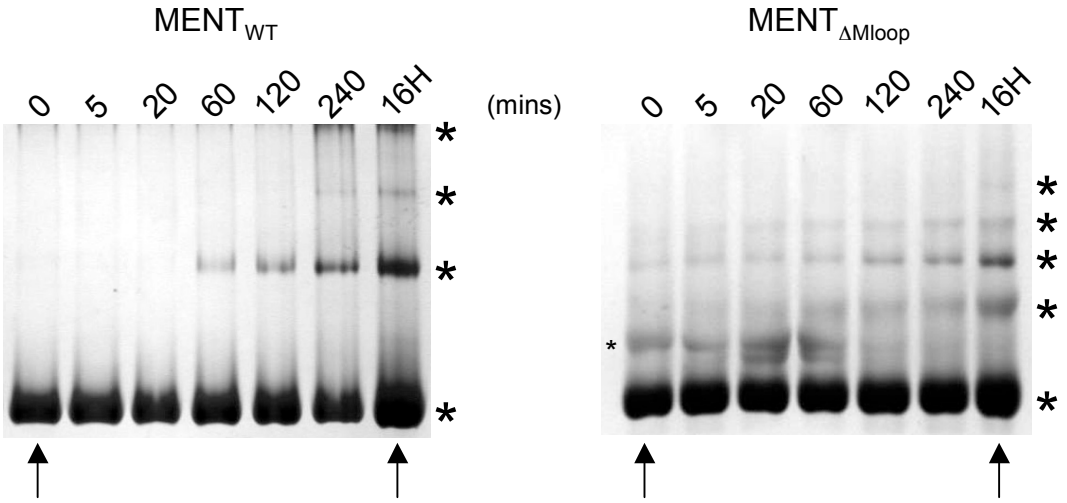
Supplementary Figure 3



Supplementary Figure 4: 2Fo-Fc electron density of the RCL of one molecule (red) with the A-sheet of a neighbouring molecule (yellow) in the asymmetric unit of native $MENT_{\Delta Mloop}$. The partially inserted RCL is in purple and the strands of the A-sheet and s7A' are labelled.

Supplementary Figure 4

A



B

	Protein	Time/44°C	SI	k_{ass} ($M^{-1}s^{-1}$ (x10^6))
→	MENT$_{WT}$	0	1.3	3.8 ± 0.1
		16H	1.4	3.8 ± 0.2
→	MENT$_{\Delta Mloop}$	0	2.2	1.5 ± 0.04
		16H	2.8	0.93 ± 0.02

Supplementary Figure 5: Heat-induced polymerization of MENT. A. Native acid PAGE (performed as described by Irving et al., 2002) of the polymerization of MENT$_{WT}$ and MENT$_{\Delta Mloop}$ incubated at 44ºC for the times indicated. Protein aliquots (10μM) were removed from heat and rapidly added to ice-cold nondenaturing acid loading buffer before electrophoresis. Protein species are indicated (*) and samples for kinetic analysis by arrows. Gels were coomassie (MENT$_{\Delta Mloop}$) and silver-stained (MENT$_{WT}$). B. Kinetic parameters (stoichiometry of inhibition, SI, and association rate constants, k_{ass}) of cathepsin L inhibition by MENT$_{WT}$ and MENT$_{\Delta Mloop}$ before and after heating (0 and 16H) at 44°C.

Supplementary Figure 5