Supplemental Figure Legends

Fig. S1. CD spectra and melts of native and cleaved α_1AT variants. *A*, CD spectra of intact control (Wt, blue), P8-P6 Asp (red), and P10 Pro, P9-P6 Asp (green) variants suggest identical, correctly folded native states. *B*, RCL cleavage for the α_1AT species (at P5 using V8 protease for control and P1 cleavage using thrombin for variants) yielded identical CD spectra that were similar to those obtained for intact α_1AT. *C*, CD melting profiles of intact α_1AT control and variants. Melting temperatures (Tm) were taken as the X-intercept of the second derivative of the smoothed profiles. *D*, Melting profiles for RCL-cleaved control and variants. No melting was observed for the cleaved control or the cleaved P8-P6 Asp variant up to the highest temperature (95°C), indicating hyperstability. The P10-P6 variant unfolded with a Tm of 81.0°C.

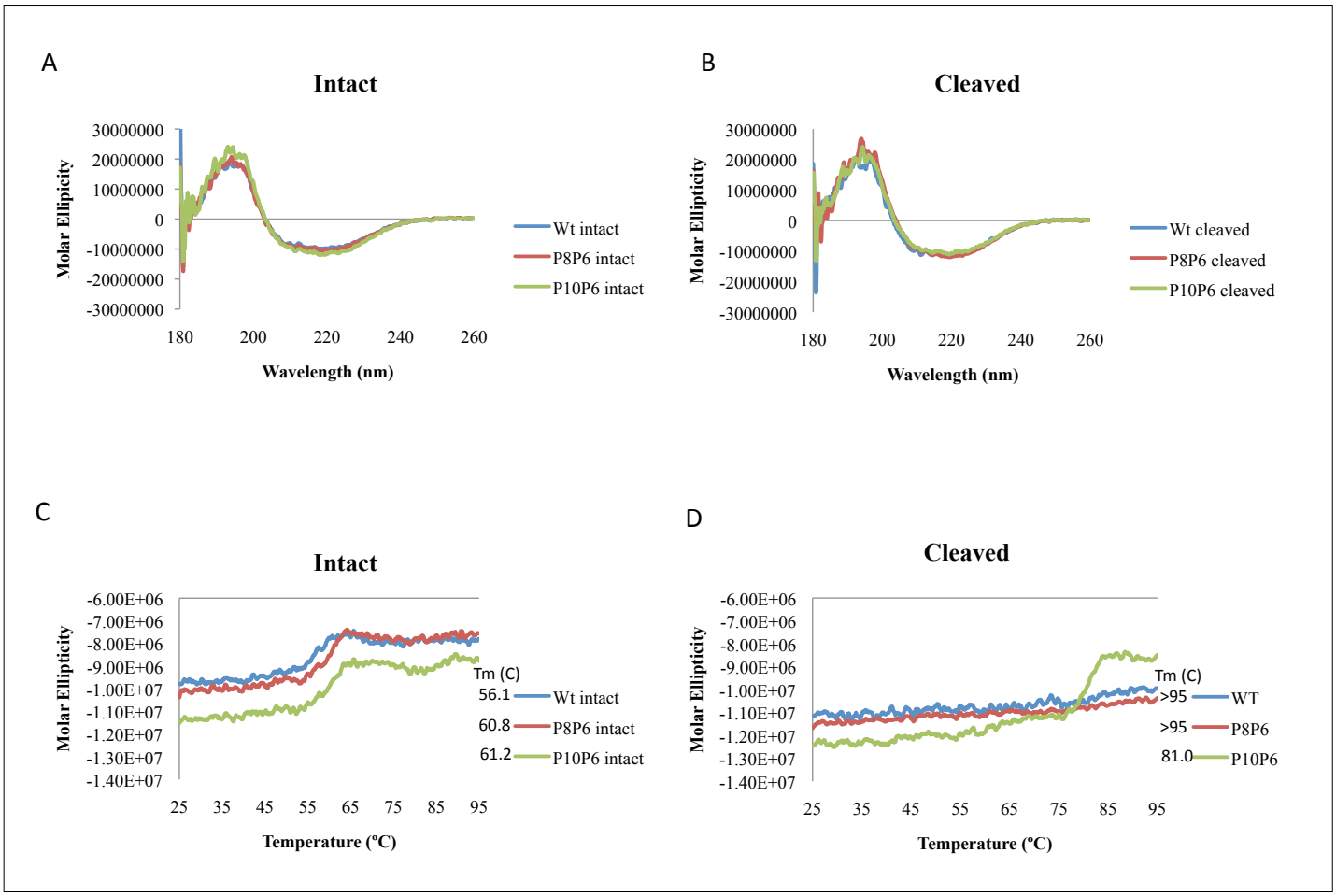
Fig. S2. SDS-PAGE of the reactions between the α_1AT control and variants with thrombin. *A*, The control (WT) is the P1 Arg, C232A variant of α_1AT, and it therefore has been converted to an inhibitor of thrombin. Flanking lanes on all gels contain size standards (as indicated). The first two lanes following the standards are thrombin (IIa) and control (WT) alone. Thrombin and α_1AT are mixed in the ratios indicated above each lane. For the control, all of the thrombin forms the higher molecular weight complex between 1:1 and 1:2. *B*, The P8-P6 Asp mutant was efficiently cleaved by thrombin (band of lower mobility than control), and formed some complex (higher molecular weight species), but even at 1:300, only about 1/3rd of the thrombin has been inhibited. Based on these numbers, an estimate of stoichiometry of inhibition of 900 is calculated, however, we cannot rule out the possibility that the complex has been destabilized by the Asp mutations. *C*, No complex is observed for the P10-P6 variant, and all native variant is efficiently cleaved by thrombin.

Fig. S3. Crystal structures of P1-P1' cleaved α_1AT variants reveal a normal RCL-inserted conformation. *A*, Cartoon representation of the structure of the thrombin-cleaved P8-P6 Asp variant, with β-sheet A colored red and the RCL in yellow. The P17-P1 region is shown as rods with surrounding electron density (2Fo-Fc, contoured at 1σ). A close-up of the P9-P4 region is shown on the right, to illustrated how a water molecule and the likely protonation of P5 Glu contribute to stabilizing the RCL-inserted state. *B*, A high-resolution structure of the P10-P6 variant, with a close up of the mutated region on the right.

Table S1. Crystals, data processing, refinement and models

Crystals	P8-P6	P10-P6
Space Group	$P2_1$	$P2_1$
Cell dimensions (Å)	a=37.45 b=83.27 c=61.09 β=99.66˚	a=37.23 b=121.88 c=43.22 β=113.88˚
Solvent content (%)	47.3	38.16
Data Processing Statistics		
Wavelength (Å)	1.542	0.9795
Resolution (Å)	48.80-2.70 (2.85-2.70)	34.04-1.50 (1.58-1.50)
Total reflections	19494	195961
Unique reflections	9871	55516
$\langle I/\sigma(I)\rangle$	14.6 (6.1)	10.4 (2.4)
Completeness (%)	96.9 (99.4)	98.9 (98.8)
Multiplicity	2.0 (1.9)	3.5 (3.5)
R_{merge}	0.042 (0.129)	0.068 (0.496)
Model		
Number of protein/water atoms	2828/22	2979/216
Average B-factor ($Å^2$)	21.1	21.8
Refinement statistics		
Reflections in working/free set	8867/989	52634/2822
R-factor/R-free	21.09/26.79	15.98/21.12
r.m.s. deviation of bonds(Å)/angles (°) from ideality	0.003/0.62	0.026/2.08
Ramachandran plot; residues in		
Most favored region (%)	95.1	98.6
Allowed region (%)	4.6	1.4
Outlier region (%)	0.3 (Gln393)	0

Fig. S1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-30000000
180
200
220
240
260
Wavelength (nm)
Wt intact
P8P6 intact
P10P6 intact
B
Cleaved
Molar Ellipticity
30000000
20000000
10000000
0
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-20000000
-30000000
180
200
220
240
260
Wavelength (nm)
Wt cleaved
P8P6 cleaved
P10P6 cleaved
C
Intact
Molar Ellipticity
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-7.00E+06
-8.00E+06
-9.00E+06
-1.00E+07
-1.10E+07
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35
45
55
65
75
85
95
Temperature (°C)
Tm (C)
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Wt intact
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P8P6 intact
61.2
P10P6 intact
D
Cleaved
Molar Ellipticity
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-8.00E+06
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-1.10E+07
-1.20E+07
-1.30E+07
-1.40E+07
25
35
45
55
65
75
85
95
Temperature (°C)
Tm (C)
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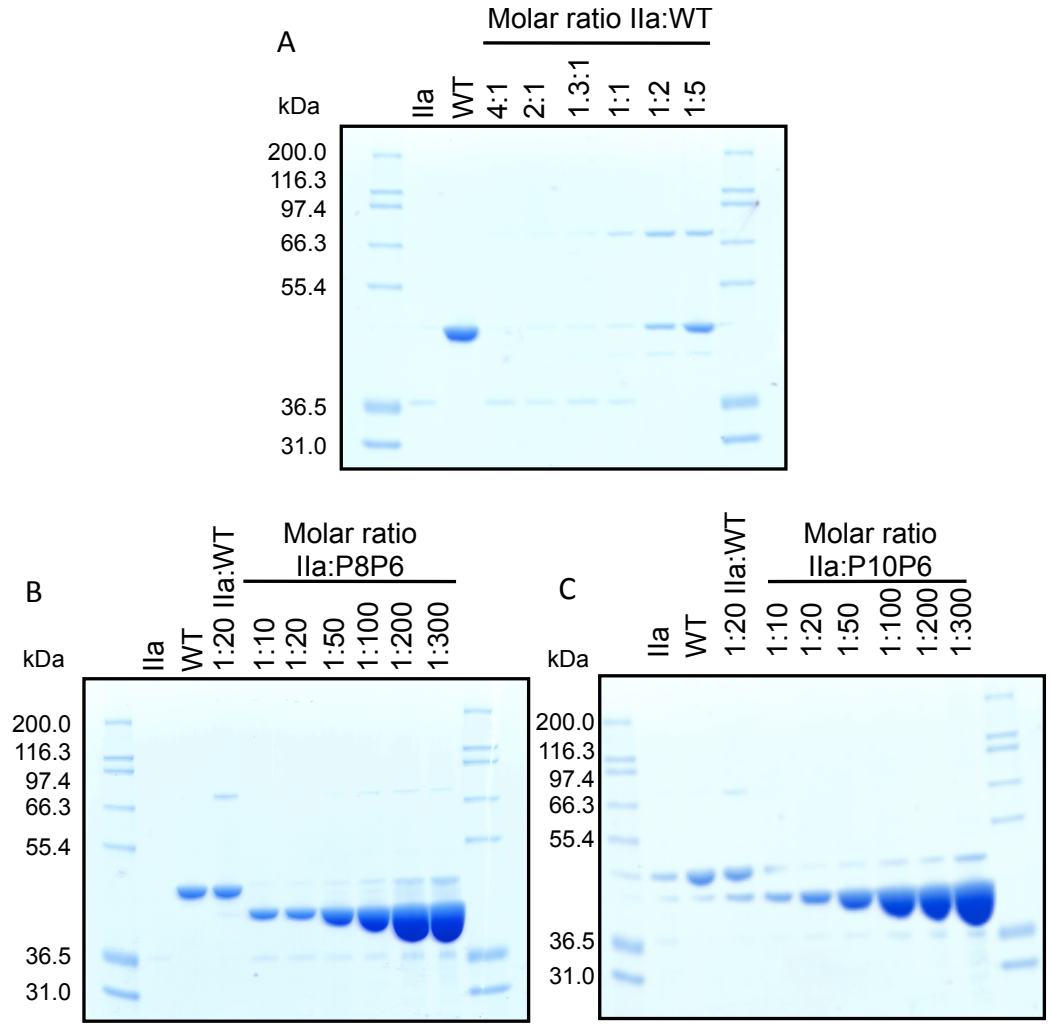
WT
>95
P8P6
81.0
P10P6

Fig. S2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WT
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2:1
1.3:1
1:1
1:2
1:5
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116.3
97.4
66.3
55.4
36.5
31.0
B
Molar ratio IIa:P8P6
kDa
IIa
WT
1:20 IIa:WT
1:10
1:20
1:50
1:100
1:200
1:300
200.0
116.3
97.4
66.3
55.4
36.5
31.0
C
Molar ratio IIa:P10P6
kDa
IIa
WT
1:20 IIa:WT
1:10
1:20
1:50
1:100
1:200
1:300
200.0
116.3
97.4
66.3
55.4
36.5
31.0

Fig. S3