Supporting Information:

Mutations Proximal to Sites of Autoproteolysis and the α-Helix that Co-evolve under Drug Pressure Modulate the Autoprocessing and Vitality of HIV-1 Protease

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Figure S1

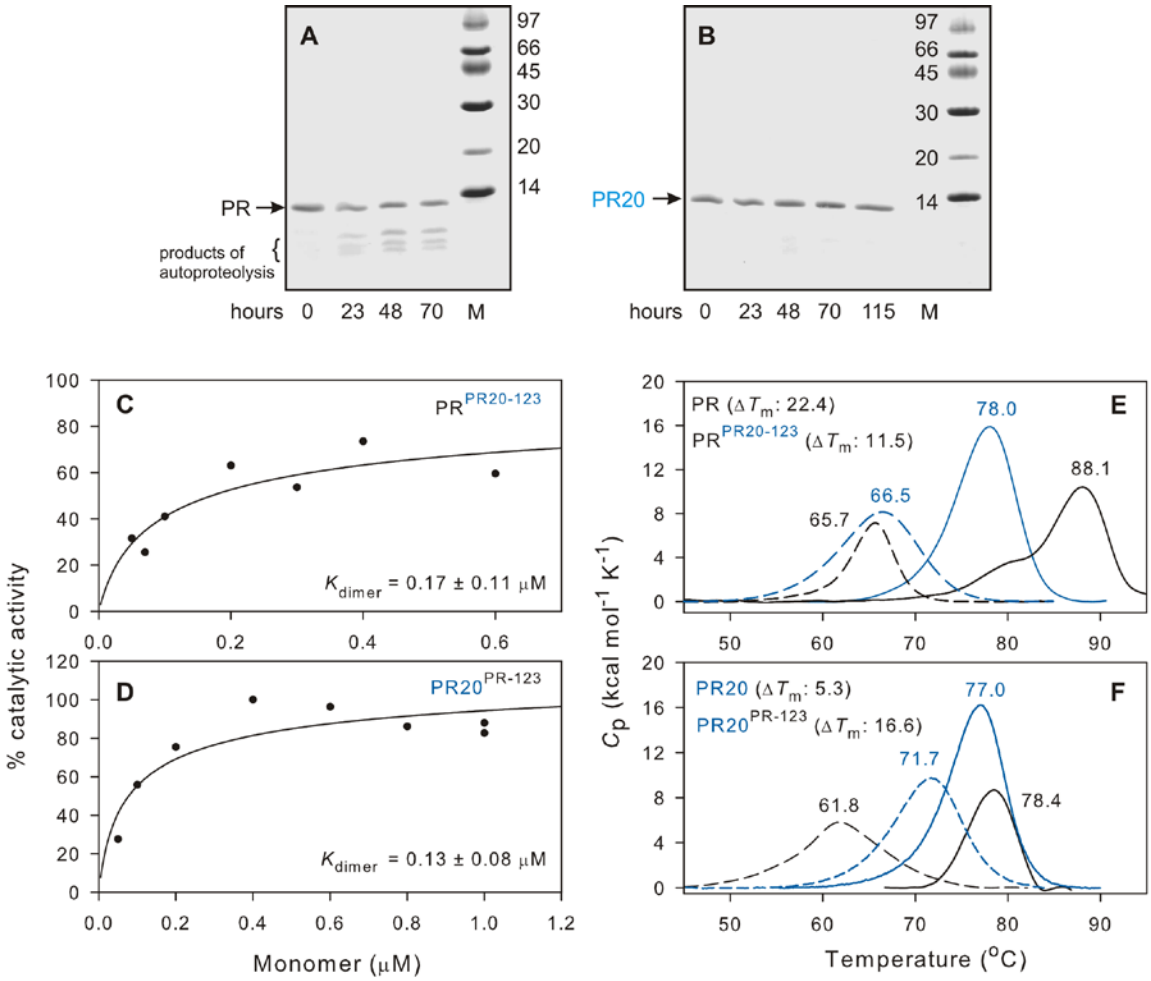

Figure S1. Evaluation of the autoproteolysis, K_{dimer} and thermal melting of mature proteases. Published results of the time course of autoproteolysis of PR (A) and PR20 (B) by SDS-PAGE are shown here solely for comparison with $PR^{PR20\text{-}123}$ and $PR20^{PR\text{-}123}$ described in the main text.[1] K_{dimer} of $PR^{PR20\text{-}123}$ (B) and $PR20^{PR\text{-}123}$ (C) in 50 mM sodium acetate, pH 5.0, containing 250 mM sodium chloride at 28 °C. K_{dimer} values were determined by fitting a previously described equation[2] (shown below) to rate data, where PR_{mono} is the concentration of PR expressed as momomers, V_x is the observed initial rate/$[PR_{mono}]$, and V_{max} is the extrapolated maximum rate/$[PR_{mono}]$ when the enzyme is fully dimeric. Data shown are scaled relative to a maximum activity of 100%.

$$V_x = V_{max}\left[1-\left\{(K_{dimer}/2 * [PR_{mono}])^{1/2} * [(K_{dimer}/2 * [PR_{mono}]) + 4]^{1/2} - (K_{dimer}/2 * [PR_{mono}])^{1/2}\right\}/2\right]$$

DSC thermograms of $PR^{PR20\text{-}123}$ (E) $PR20^{PR\text{-}123}$ (F) and their parent constructs PR and PR20, in the presence (solid lines) and absence (dashed lines) of a two-fold molar excess of darunavir. Data from previous work are shown for PR[3] and PR20[1] for comparison only.

Figure S2

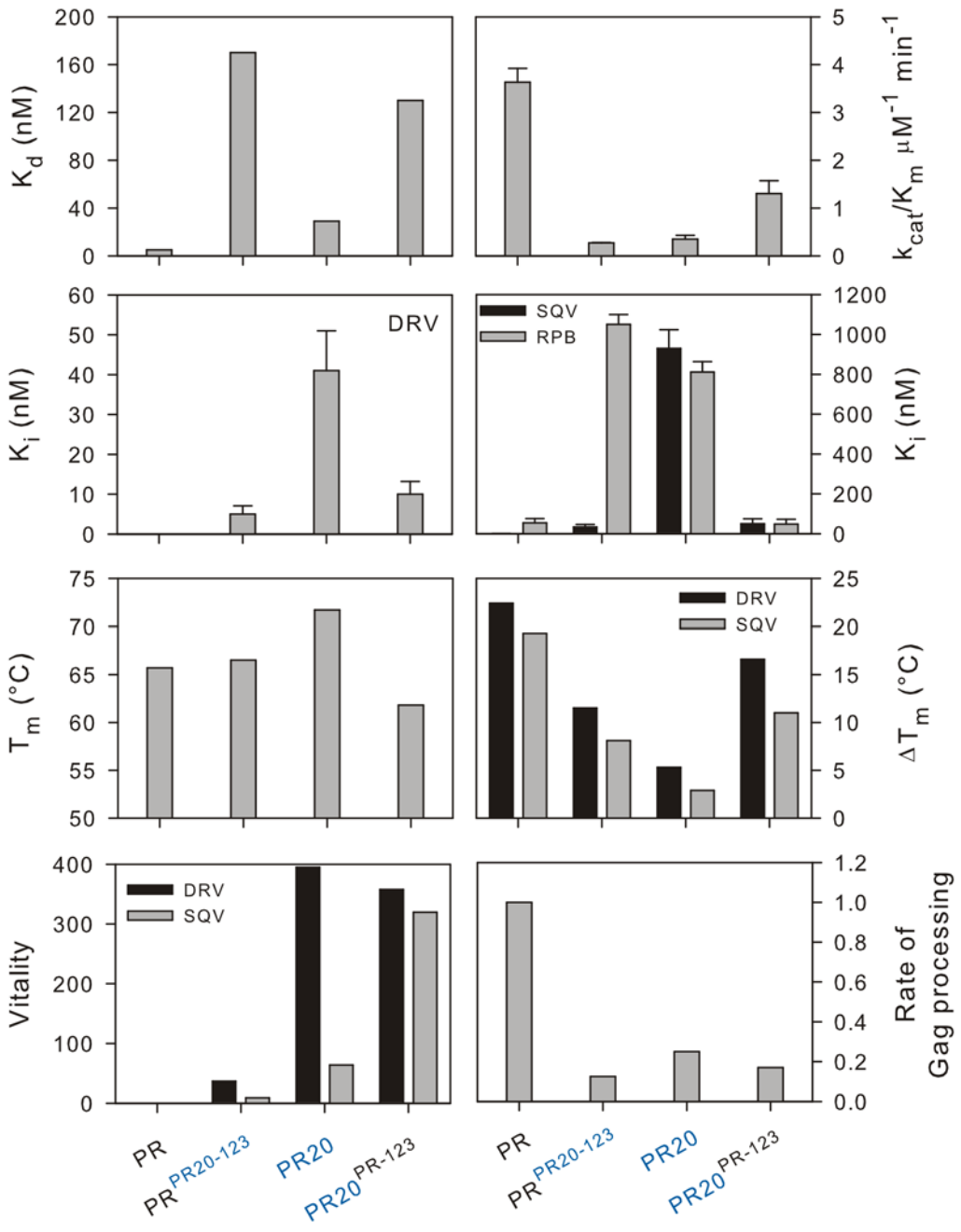

Figure S2. Graphical representation of table 1.

Figure S3

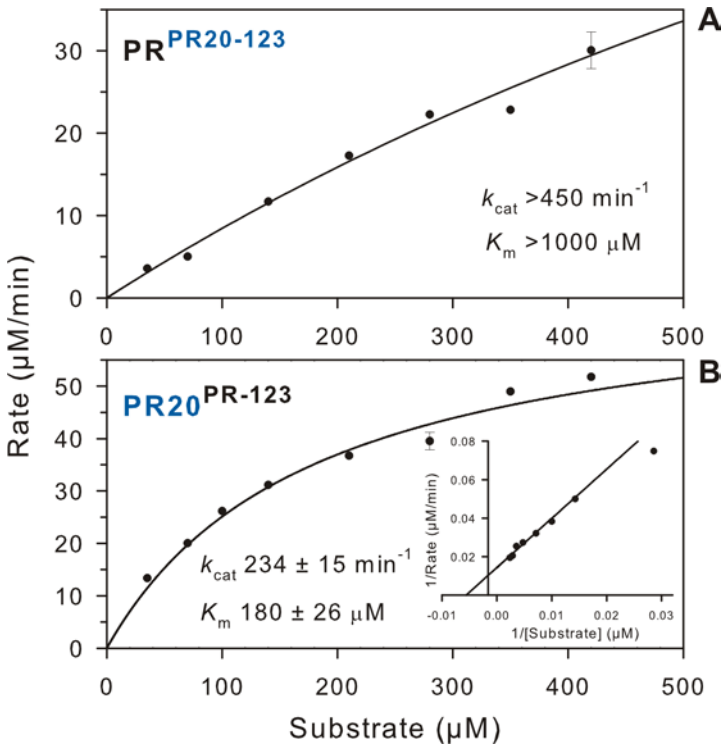

Figure S3. Michaelis-Menten plots for hydrolysis of chromogenic substrate (measured at 310 nm) by 0.3 µM $PR^{PR20\text{-}123}$ (A) and $PR20^{PR\text{-}123}$ (B) in 50 mM sodium acetate, pH 5.0, containing 250 mM sodium chloride at 28 °C. As higher substrate concentrations (> 450 µM) lead to weak inhibition by one of the cleavage products, the highest substrate concentration that could be used is below K_m for $PR^{PR20\text{-}123}$ (A). Thus, only the estimated lower limits for these kinetic parameters are given. For $PR20^{PR\text{-}123}$ (B) both Michaelis-Menten and Lineweaver-Burk plots are shown.

Figure S4

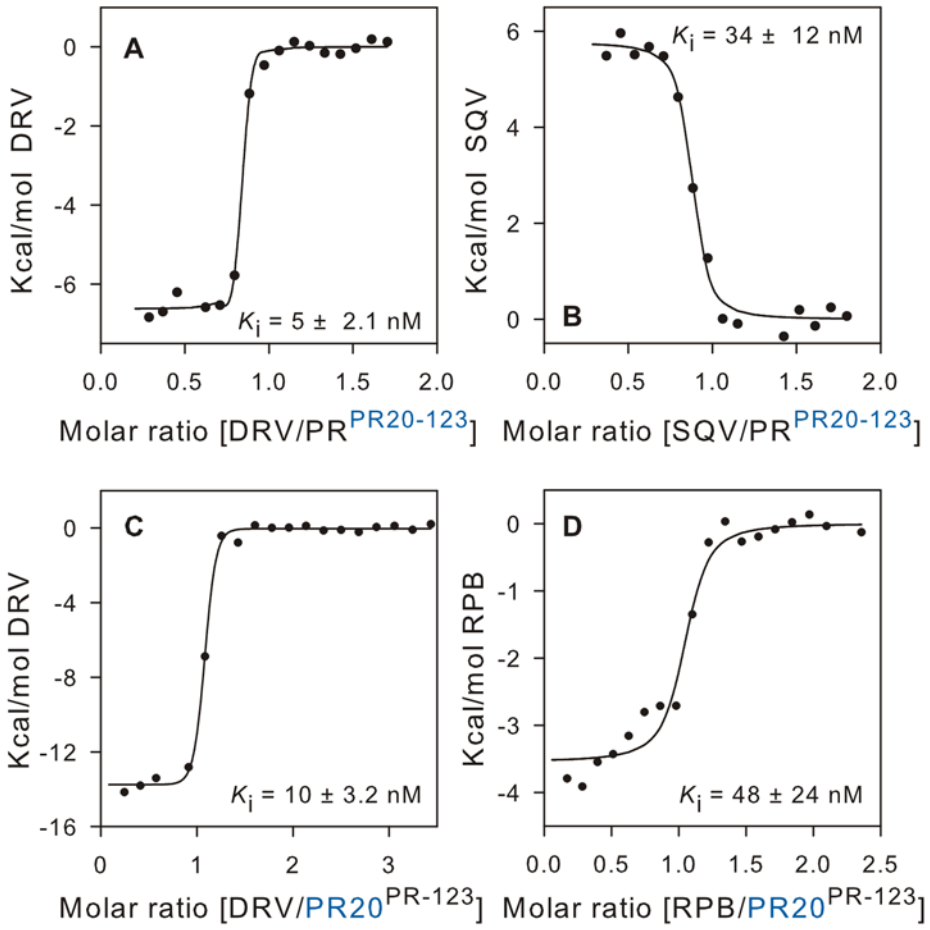

Figure S4. Determination of K_i ($1/K_{association}$) for binding of inhibitors to PR$^{PR20-123}$ and PR20^{PR-123} by ITC in 50 mM sodium acetate, pH 5.0, at 28 °C. For competitive inhibitors, $1/K_{association}$ for inhibitor binding by ITC is the same as determined kinetically.[4] The apparent stoichiometry (N-value, indicated by the midpoint of the binding isotherm) for both titrations of PR20^{PR-123}, was lower than expected for the 1:1 ratio of the enzyme-inhibitor complex, likely due to autoproteolysis (see Figure 2). Therefore, the concentration was scaled in panels (C) and (D) to give an N-value of 1. No concentration correction was applied for PR$^{PR20-123}$ [panels (A) and (B)] as expected. DRV, SQV and RPB denote darunavir, saquinavir and reduced peptide bond inhibitor, respectively.

Figure S5

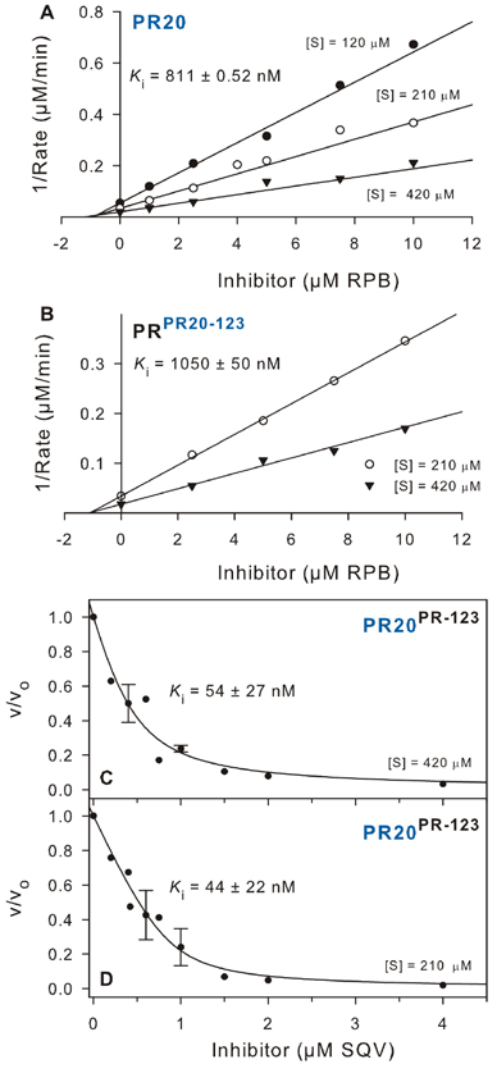

Figure S5. K_i determination for reduced peptide bond inhibitor (RPB) binding to (A) 0.5 μM PR20 and (B) 0.6 μM $PR^{PR20\text{-}123}$ by use of Dixon plots for hydrolysis of chromogenic substrate. Each complete data set comprising 2 or 3 substrate concentrations was processed together by use of the enzyme kinetics module of Sigmaplot 10. (C and D) Kinetic determinations of IC_{50} and K_i for saquinavir (SQV) mediated inhibition of 0.54 μM $PR20^{PR\text{-}123}$ at two substrate concentrations. For duplicate measurements average values with error bars are shown. The solid lines are curve fits of the Morrison equation[5] (shown below) to the data with parameters IC_{50} and E, where V_o and V_{obs} are initial rates in the absence and presence of inhibitor, respectively, and I and E are total concentrations of inhibitor and active sites respectively.

$$V_{obs}/V_o = 1-\left\{\left[I+E+IC_{50} - [(I+E+IC_{50})^2 - 4*I*E]^{1/2}\right]/2*E\right\}$$

K_i values shown were calculated from IC_{50} by use of the equation $K_i = IC_{50}/(1+ [\text{Substrate}]/K_m)$.

Figure S6

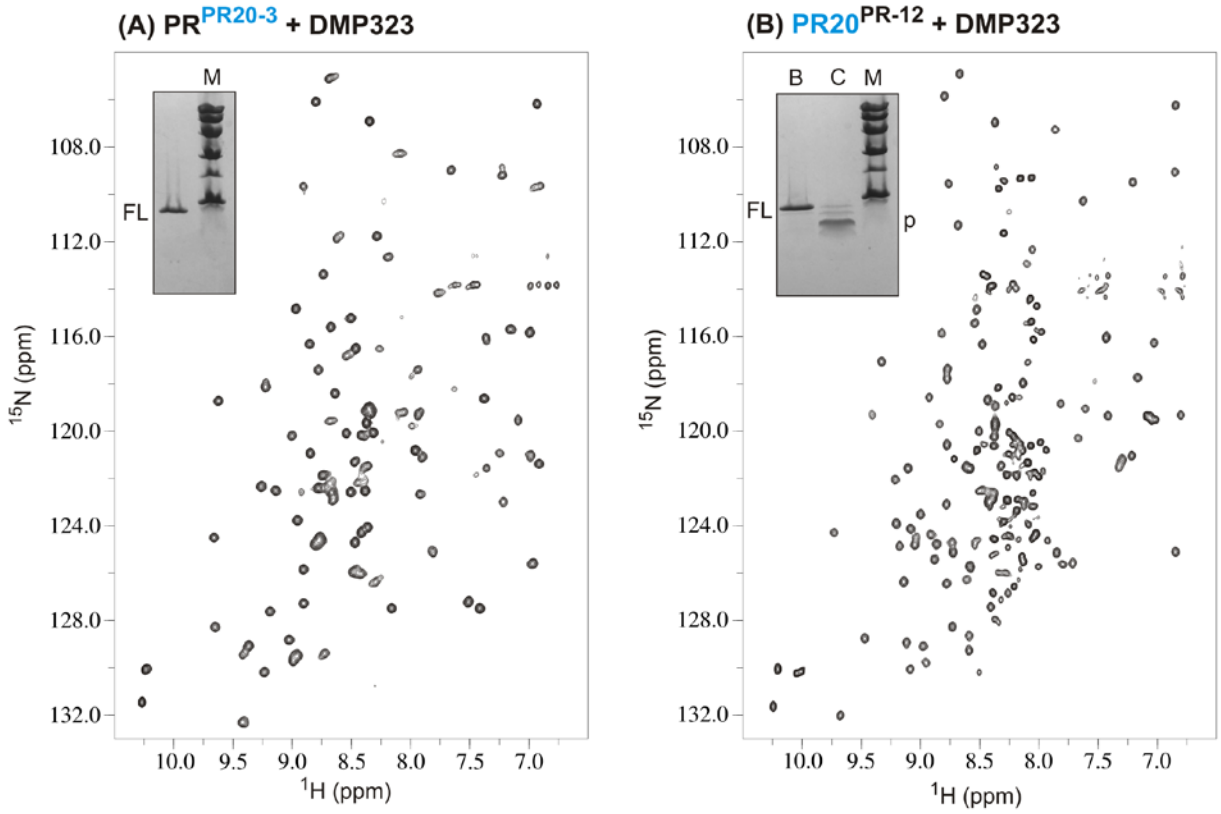

Figure S6. 600 MHz ^{1}H-^{15}N TROSY correlation spectra of freshly prepared ^{15}N or ^{15}N/^{13}C labeled proteins in 20 mM sodium phosphate buffer, pH 5.7, 20 °C. Spectra were acquired in the presence of the symmetric inhibitor DMP323.[6] After acquiring the NMR spectra, samples were subjected to SDS-PAGE on homogeneous 20% Phastgels and stained with Phastgel blue R to visualize the bands. Inset lanes B and C denote PR20^{PR-12} + DMP323 and PR20^{PR-12}, respectively. PR20^{PR-12} undergoes rapid autoproteolysis in the absence of DMP323. M, FL and p denote molecular weight standards in kDa, full-length mature protease and products of autoproteolysis, respectively.

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