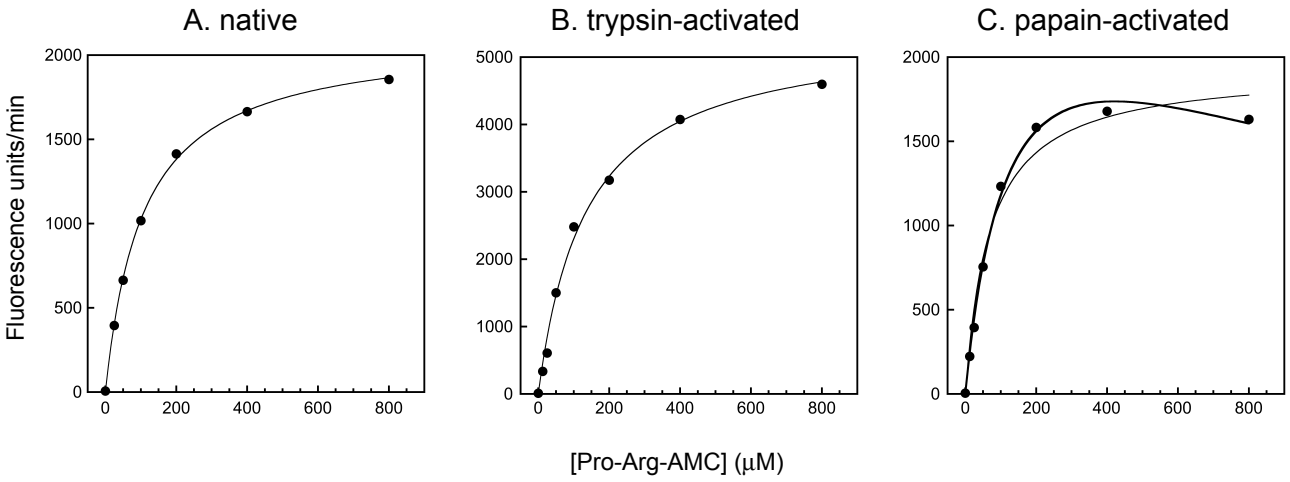

Figure S1: Rates of Pro-Arg-AMC hydrolysis for native DPAP1 (A), trypsin-activated recombinant DPAP1 (B) and papain-activated recombinant DPAP1 (C). Data points were fit to the Michaelis-Menten equation $\nu = V[s]/(K_m + [s])$ (A, B and thin line in C) or to an equation derived from a mechanism of uncompetitive substrate inhibition: $\nu = V[s]/(K_m + [s] + [s]^2/K_i)$ (C, thick line).

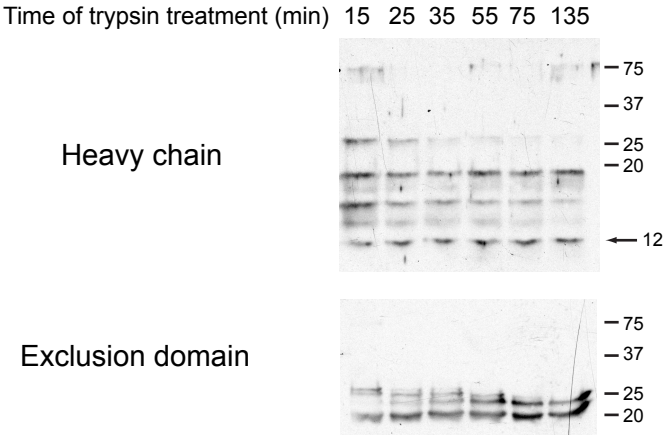

Figure S2: Time course of trypsin processing of recombinant DPAP1. At various time points after adding 1.5 ng/μL bovine trypsin to TEV protease-cleaved DPAP1, an aliquot of the reaction was taken for immunoblot analysis. DPAP1 was detected with an antibody that recognizes the heavy chain of the catalytic domains (top panel) or the exclusion domain (bottom panel). Processing of both segments of DPAP1 appears to be complete by 75 minutes. Sizes of markers are shown at right. For the heavy chain blot, markers below 20 kDa were not available. The size of a polypeptide that typically runs at ~12 kDa is indicated with an arrow.

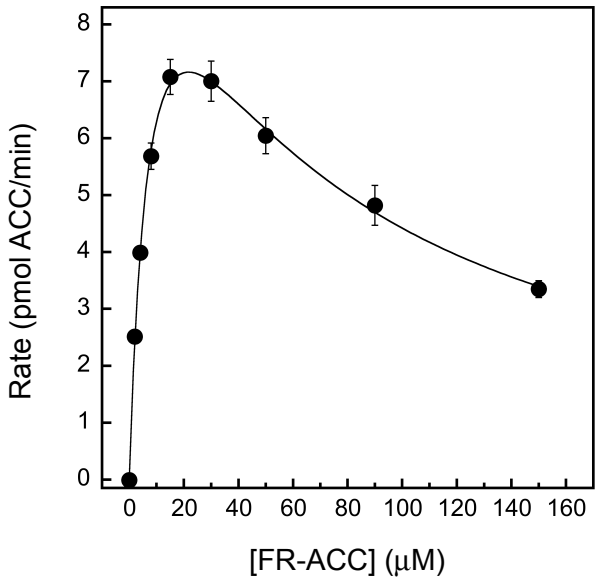

Figure S3: Substrate inhibition of cathepsin C with Phe-Arg-ACC. The data points were fit by non-linear regression to an equation for uncompetitive substrate inhibition: $\nu = Vs/(K_m + s + s^2/K_i)$. Each data point is the mean of triplicate assays with the standard deviation indicated.

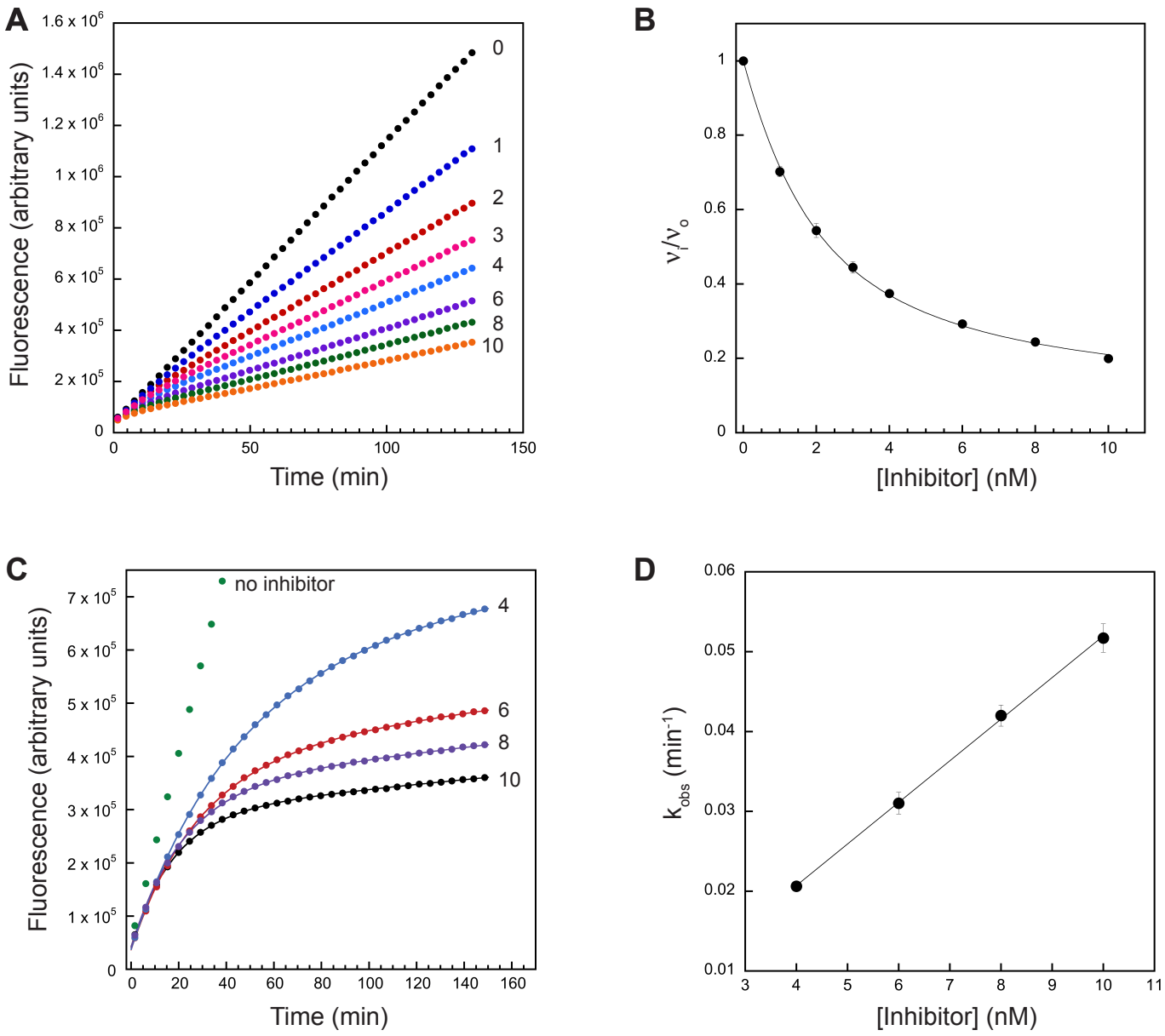

Figure S4: Inhibition of recombinant DPAP1 and cathepsin C by the semicarbazide inhibitor **1** in Fig. 4. (A) Progress curves for DPAP1 in the presence of 0 - 10 nM inhibitor (concentration shown at the right of the curves). For clarity, every second data point is shown. (B) Morrison plot for the determination of the K_i value for DPAP1. Each data point is the mean of triplicate assays with error bars showing the standard deviation. The line is a fit to the Morrison equation for tight binding inhibition (see Supplementary Methods). (C) Cathepsin C progress curves at the indicated inhibitor concentrations. For clarity, every third data point is shown. Lines are fits to an equation for slow, tight binding inhibition (see Supplementary Methods). (D) The apparent first order rate constant (k_{obs}) from the fits in (C) increases linearly with inhibitor concentration. Error bars show the standard deviation of the mean of triplicate determinations.

SUPPLEMENTARY METHODS

Determination of K_i values for compounds **1** and **2** in Figure 4

DPAP1 inhibition assays were conducted as described in the first paragraph of section 2.11, with 100 μM Pro-Arg-AMC as the substrate. Initial surveys suggested that the K_i would be close to the enzyme concentration, which indicated that these needed to be treated as tight-binding inhibitors. For both inhibitors, enzyme rates were measured for uninhibited and inhibited rates over a range of inhibitor concentrations (0 – 10 nM for **1**, 0 – 100 nM for **2**). For both native and recombinant DPAP1, a steady state was not immediately reached with inhibitor **1** but was obtained by around 30 minutes (Supplementary Figure S4A). ν_i/ν_o was plotted as a function of [I] (where ν_i is the inhibited steady-state rate and ν_o is the uninhibited rate) and the data were fit to the Morrison equation with [E] and K_i^{app} as fitted parameters [1]:

$$\frac{v_i}{v_o} = 1 - \frac{([E] + [I] + K_i^{app}) - \sqrt{([E] + [I] + K_i^{app})^2 \; - \; 4[E][I]}}{2[E]}$$

The fitted data for inhibitor **1** and recombinant DPAP1 are shown in Fig. S4B. K_i was determined from K_i^{app} according to the relationship $K_i^{app} = K_i\,(1 + [S]/K_m)$ using the K_m values in Table 1.

The K_i value for inhibition of cathepsin C by compound **2** was determined as described above for DPAP1, except that a concentration range of 0 – 200 nM was used. Inhibition of cathepsin C by compound **1** was time-dependent to a much greater extent than was observed for DPAP1 (Fig. S4C); presumably the slow step involves a conformational change of an initial low affinity enzyme-inhibitor complex (EI) to form a tight complex (EI*; Scheme 1) [2]. Therefore, this inhibitor was analyzed as a slow, tight-binding inhibitor with concentrations ranging from 0 – 10 nM. Progress curves (0-150 minutes) were fit to the following equation [2]:

$$[P] = \nu_s t + \frac{(\nu_i - \nu_s)(1-\gamma)}{k_{obs}\gamma} \ln\left\{\frac{1 - \gamma e^{(-k_{obs}t)}}{1-\gamma}\right\} + C$$

where $\gamma = \frac{[E_t]}{[I_t]}\left(1 - \frac{\nu_s}{\nu_i}\right)^2$, and [P] is product concentration, ν_i is the initial rate, ν_s is the steady state rate, k_{obs} is the apparent first order rate constant for appearance of the inhibited enzyme and C is a constant that accounts for the increase in fluorescence during the dead time of the reaction

(Fig. S4C). Because v_o did not vary with inhibitor concentration, there was no evidence for an accumulation of the EI species. Thus, the equation $k_{obs} = k_6 + \left(\frac{k_6}{K_i^{*app}}\right)[I]$ is operative [2] and k_{obs} was plotted against [I] and fit to a straight line (Fig. S4D). k_6 was determined from the relationship $k_6 = k_{obs}\frac{v_s}{v_i}$ [2] and was used to calculate K_i^{*app} from the slope (k_6/K_i^{*app}) of the linear fit. K_i^* was determined from K_i^{*app} as described above for K_i.

Scheme 1

$$E + I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EI^* \qquad K_i = \frac{k_4}{k_3},\ K_i^* = \frac{K_i k_6}{k_5 + k_6}$$

References

[1] Morrison JF. Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. Biochim Biophys Acta 1969;185:269-86.

[2] Morrison JF, Walsh CT. The behavior and significance of slow-binding enzyme inhibitors. Adv Enzymol Relat Areas Mol Biol 1988;61:201-301.