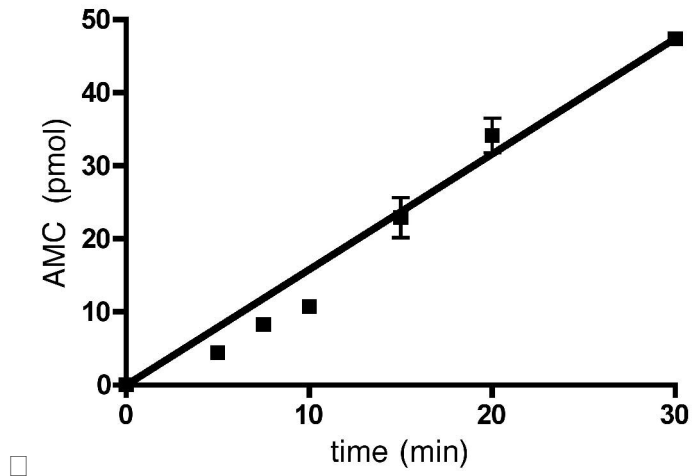


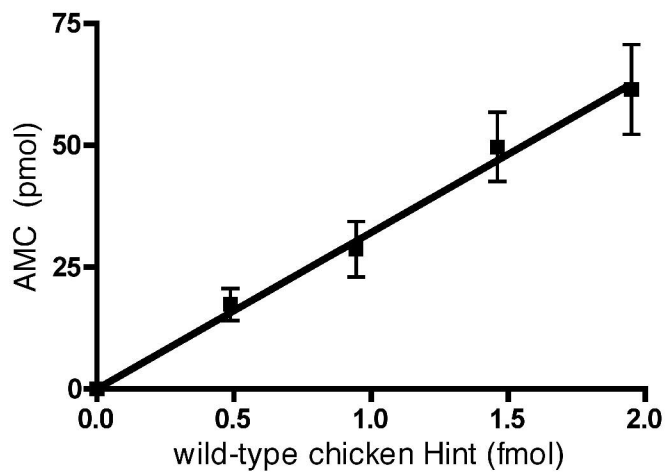
Supplementary Methods and Figures

Time and Hint-concentration dependence of tBoc-LysAMP-MCA hydrolysis were established as follows. All incubations were performed at 20 °C in black 96-well plates and quantitated using a Wallac Victor2 multilabel counter. Initially, substrate was hydrolyzed to completion with 1 - 2 µg wild-type Hint for 20 - 40 min in 25 µl 10 mM NaAcetate pH 5.5, 50 mM NaCl. It was determined that addition of 75 µl of T buffer (100 mM bis-tris propane pH 9.5, 20 mM CaCl₂, 200 mM NaCl, and 80 µg /µl trypsin (Worthington)) was sufficient to maximize production of aminomethylcoumarin (AMC) in a 10 min further incubation. The substrate concentration was corrected by calibration of Hint plus trypsin-dependent fluorescence less the small background of trypsin-dependent fluorescence against a standard curve of AMC in the 100 µl complete reaction without either enzyme. Time-dependence of the reaction required that T buffer be at a sufficiently high pH and/or a sufficiently high concentration of trypsin to arrest Hint activity. Moreover, time-dependence required that any Hint-independent fluorescence arising from potential contamination with tBoc-Lys-MCA or AMC be negligible with respect to Hint-dependent fluorescence. The time-dependence and Hint-dependence of reactions are shown in Supplemental Figs. 1 and 2. Assays to determine cation-dependence of the Hint reaction compared the 10 mM NaAcetate pH 5.5, 50 mM NaCl incubation without cations to those with cations and/or EDTA. As shown in Supplemental Fig. 3, because of the salutary effect of 1 mM EDTA on the reaction, all further reactions included 1 mM EDTA. Kinetic values (Table 1) were calculated using reactions that hydrolyzed $\leq 10\%$ of the initial substrate.

Supplemental Fig. 1. Time dependence of 15 μ M tBoc-AMPLys-MCA hydrolysis by 1.95 fmol wild-type Hint.



Supplemental Fig. 2. Hint-dependence of 15 min tBoc-AMPLys-MCA assays.



Supplemental Fig. 3. Salutary effect of 1 mM EDTA. tBoc-AMPLys-MCA assays (15 μ M) were performed with 1.95 fmol wild-type Hint for 15 min with the indicated additives.

