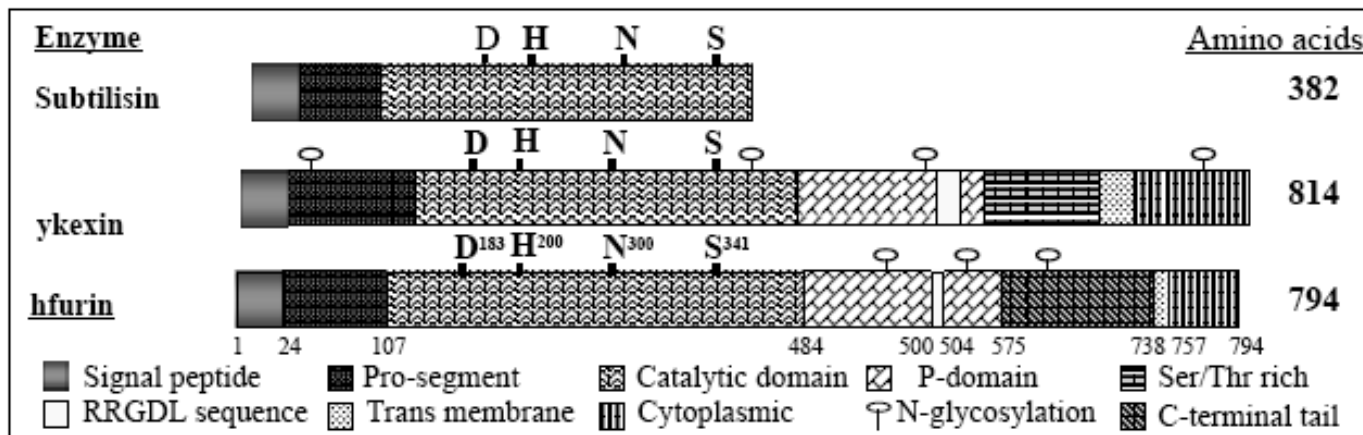
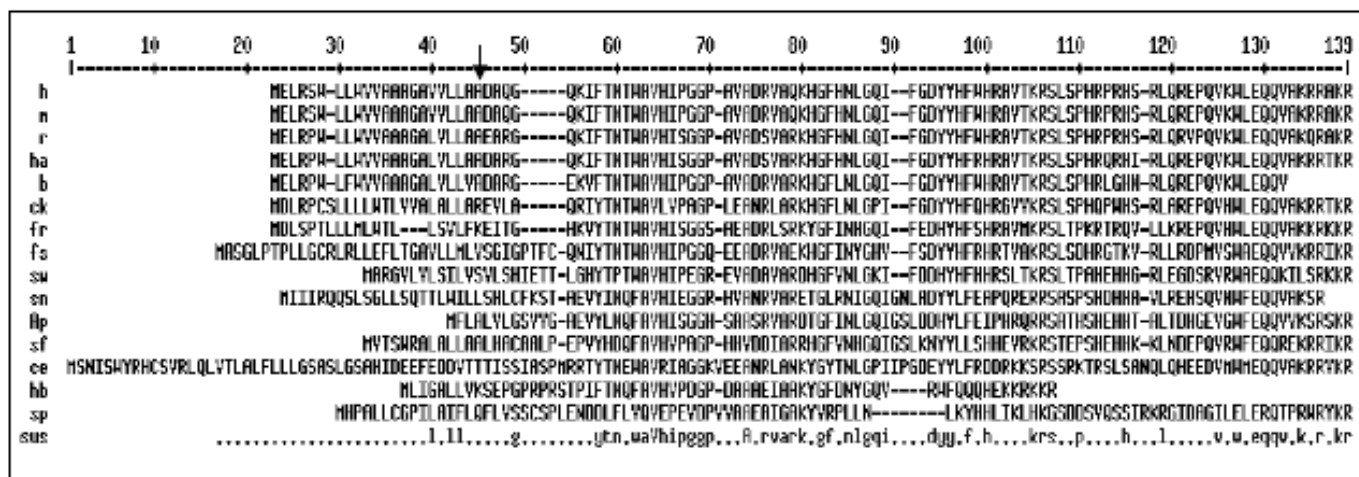


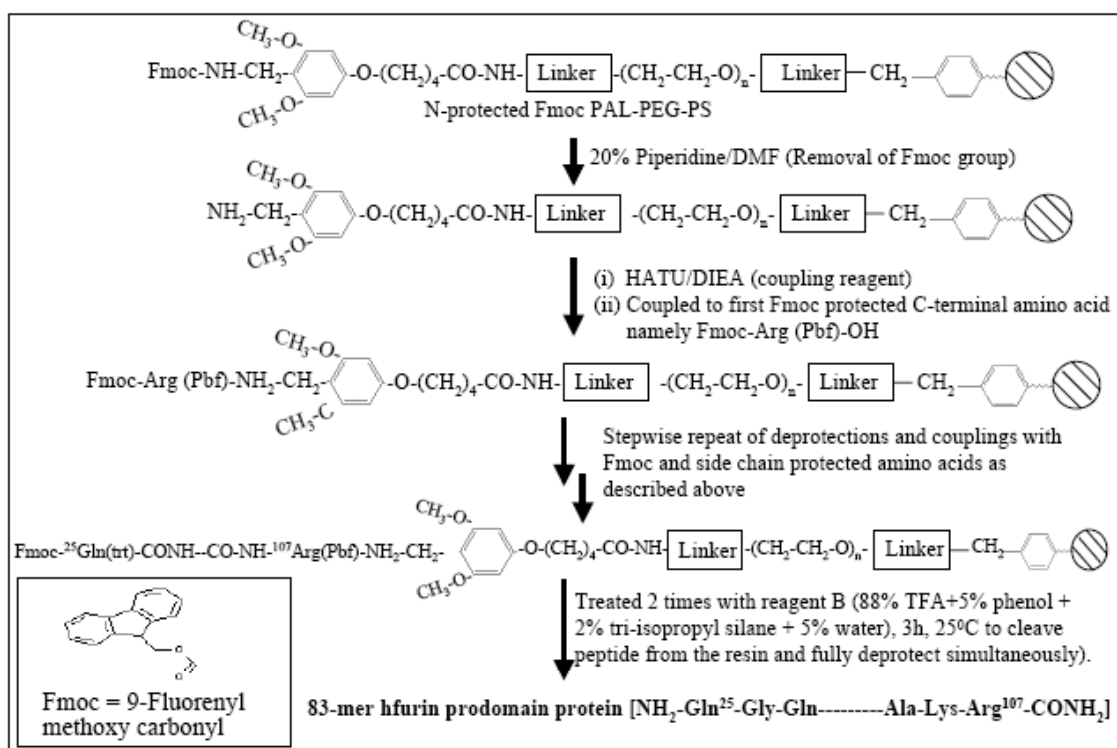
SUPPLEMENTARY MATERIAL



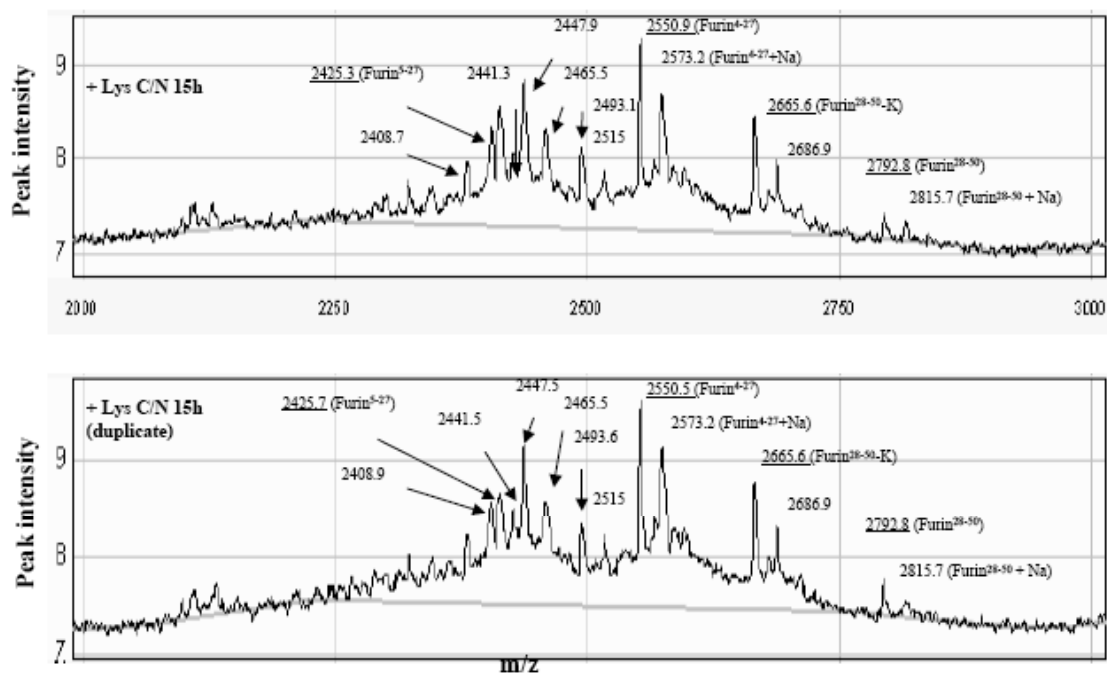
Supplementary Fig. (1A). Schematic presentation of molecular structure of hfurin compared to bacterial subtilisin and yeast (y) kexin. Various characteristic domains are shown in different patterns as indicated in comparison to bacterial subtilisin and yeast kexin homologs.



Supplementary Fig. (1B). Alignment of amino acid sequence of prodomains of furin of various mammalian species as indicated in the figure. Note the high degree of homology near the primary (KR¹³⁹↓) and secondary activation (KR¹⁰⁶↓) sites. **Note:** h: human, m: mouse, r: rat, ha: hamster (Chinese), b: bovine, ck: chicken (gallus gallus), fr: frog (african clawed) (xenopus laevis), fs: fish (Japanese rice) (Oryzias latipes, Japanese medaka), sw: silk worm, sn: snail (great pond) (lymnaea stagnalis), Ap: aplysia (Aplysia California), sf: Spodoptera frugiperda (fall armyworm), ce: Caenorhabditis elegans, hb:honey bee (Apis mellifera), sp: Schizosaccharomyces pombe, sus: consensus, ↓= Signal peptide cleavage site.

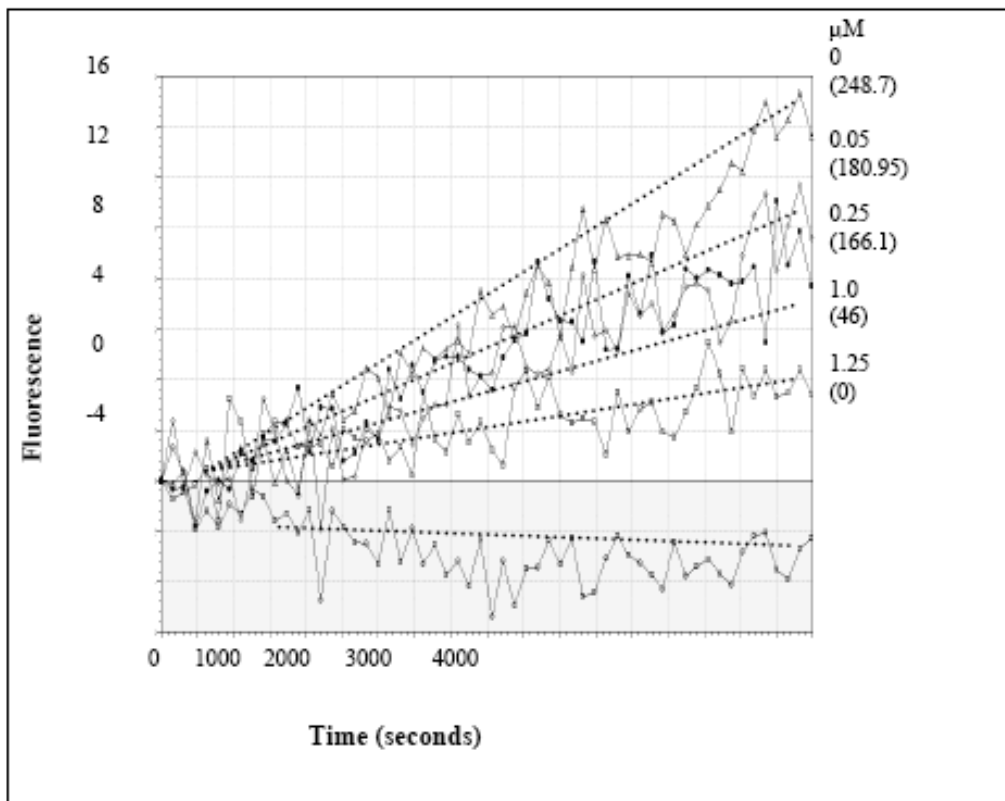


Supplementary Fig. (2). Scheme showing various steps involved in the solid phase synthesis of 83-mer hFurin prodomain protein. For each step, Fmoc protecting group was removed by treatment with 20% piperidine in DMF (dimethyl formamide) whereas the peptide coupling reaction was accomplished by the activating agent HATU/DIEA (see *Materials and Methods*).



Supplementary Fig. (3). SELDI-tof mass spectra of crude Lys-C/N digests (duplicate samples) of hprofurin²⁵⁻¹⁰⁷. SELDI-tof mass spectra of crude Lys-C/N digests (in duplicates) of 83-mer profurin protein. The digestion was performed for 15.5 h at 25°C in buffer at pH 7.4 (see *Materials and Methods* for details). The mass spectra were performed on normal phase chips using CHCA matrix and ciphergen mass spectrometer. Various fragments corresponding to most cleavages N and C-terminus to Lys residue as shown by vertical arrow (shown below) were detected in the mass spectrum. The two sets of data obtained duplicate experiments were in good agreement to each other.

¹QGG↓K⁴↓VFTNTWAVRIPG GPAVANSVAR ↓K²⁷↓HGFLNLGQI FGDYYHFWHR GVT↓K⁵⁰↓RSLSPH RPHSRLQRE PQVQWLEQQV A↓K⁷⁸↓RRT↓K⁸²↓R⁸³



Supplementary Fig. (4). Progress curves showing inhibition of furin by hprofurin²⁵⁻¹⁰⁷ protein at various concentrations. Furin activity was measured against Boc-RVRR-MCA (50 μM) as substrate. In the figure the numbers in parentheses show the measured slope of each curve as indicated by dotted lines.