Analysis of kallikrein-related peptidase 7 (KLK7) autolysis reveals novel protease and cytokine substrates

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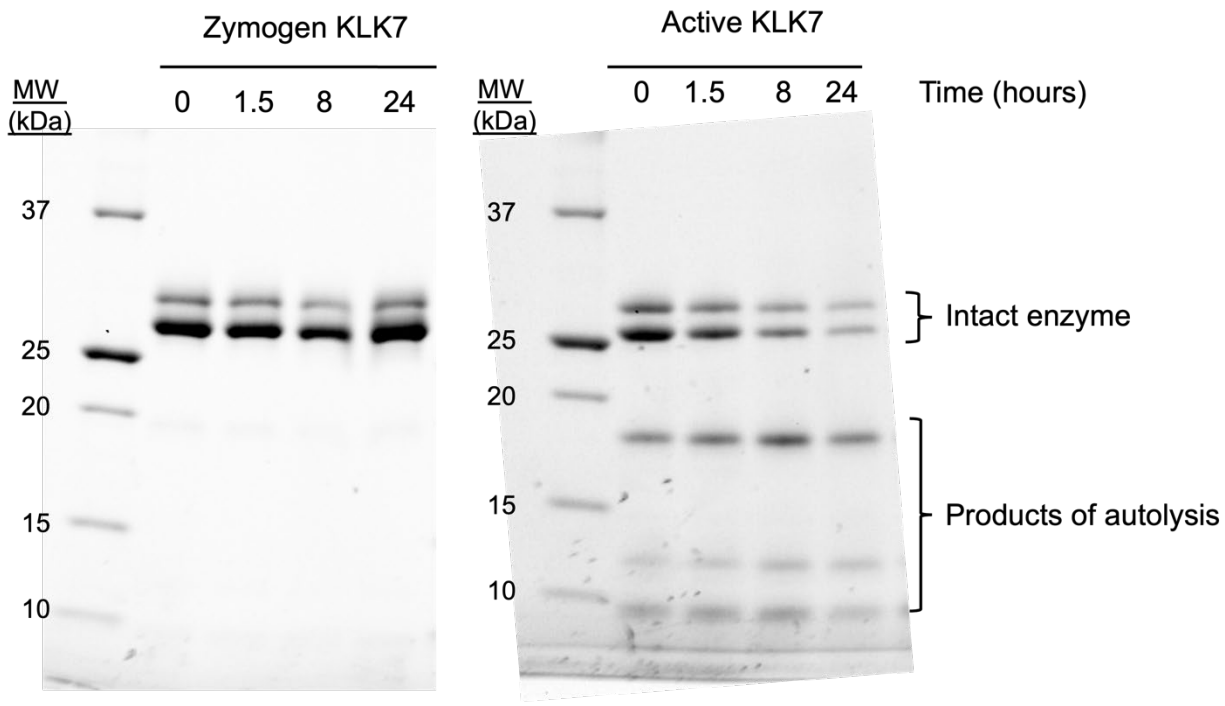
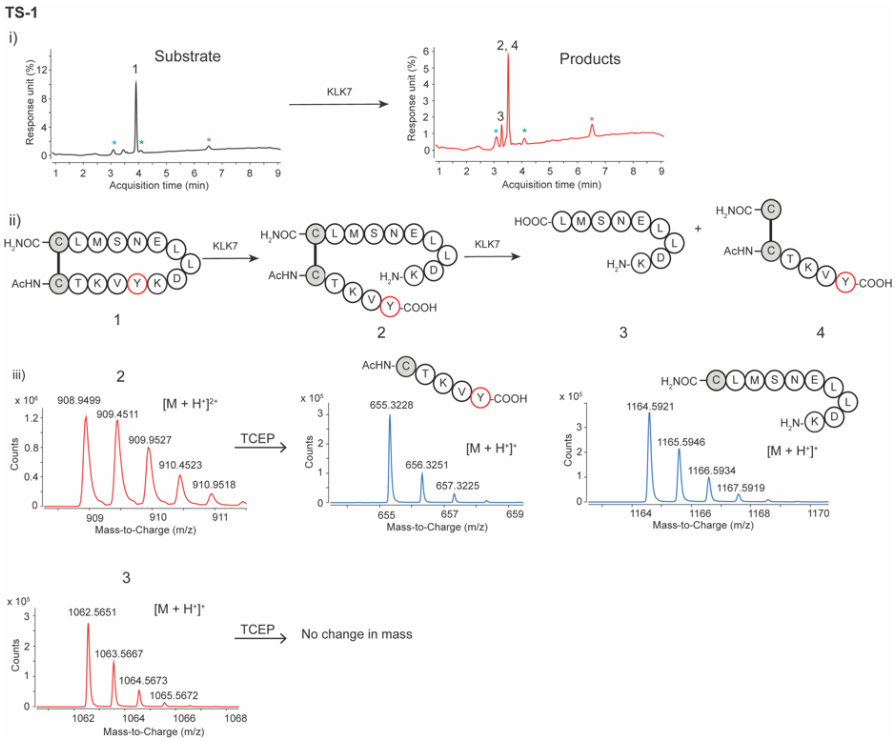
Supplementary material

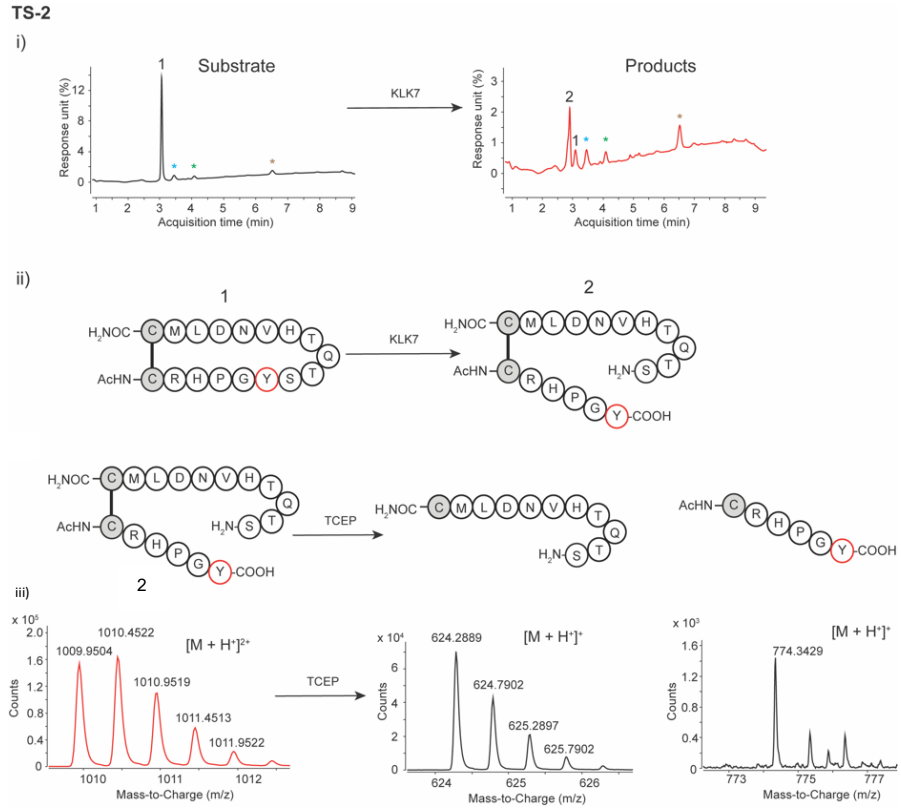


Supplementary Figure S1: KLK7 zymogen does not undergo autolysis. Inactive KLK7 zymogen form containing an N-terminal His-tag (~ 5 mg/mL), and active enzyme (~ 5 mg/mL) were separately incubated at 37 °C in 25 mM CHES pH 9.0, 100 mM NaCl. Aliquots were removed at various times and quenched by addition of Laemmli sample buffer and deactivation at 95 °C. Deactivated samples were stored at -20 °C, and were analyzed altogether using SDS-PAGE under reducing conditions. The zymogen form of KLK7 (left panel) does not change, whereas the active protease form of KLK7 (right panel) undergoes autolysis to products having lower molecular weight values of ~17 kDa, ~12 kDa, and ~8 kDa.

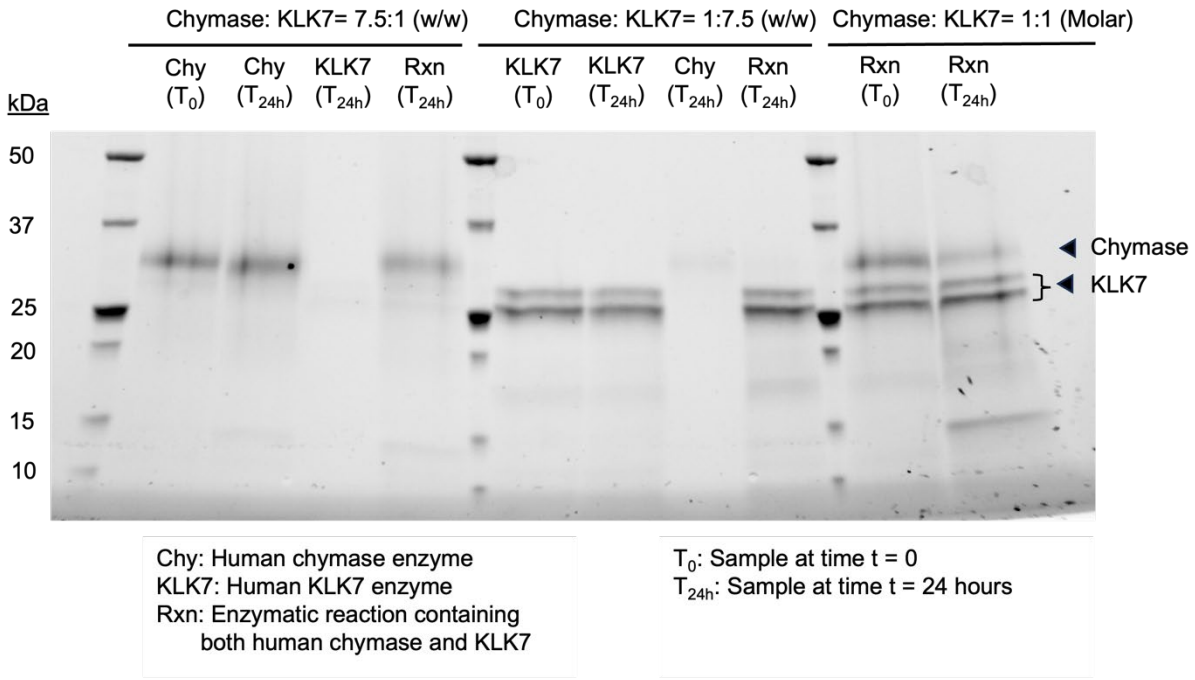
A


TS-1
i)
Substrate
Products
KLK7
Response unit (%)
Acquisition time (min)
ii)
KLK7
KLK7
1
2
3
4
iii)
2
908.9499
909.4511
909.9527
910.4523
910.9518
[M + H+]2+
TCEP
655.3228
656.3251
657.3225
[M + H+]+
1164.5921
1165.5946
1166.5934
1167.5919
[M + H+]+
Counts
Mass-to-Charge (m/z)
3
1062.5651
1063.5667
1064.5673
1065.5672
[M + H+]+
TCEP
No change in mass

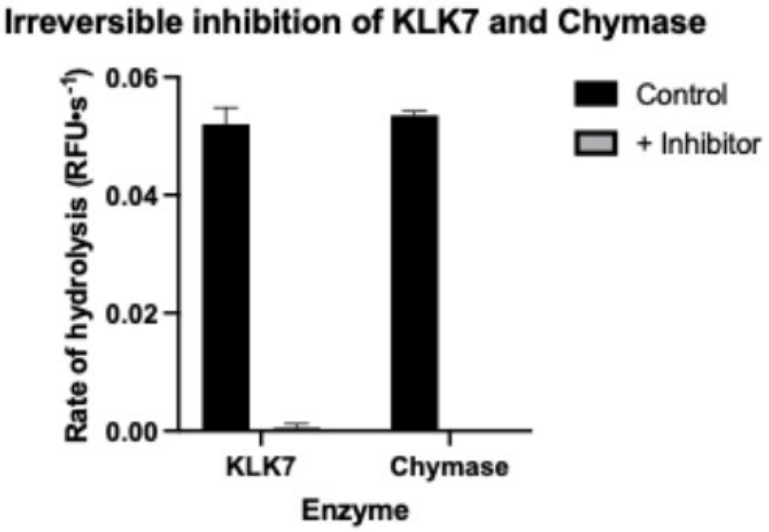
B


TS-2
i)
Substrate
Products
KLK7
Response unit (%)
Acquisition time (min)
ii)
1
2
KLK7
TCEP
2
iii)
1009.9504
1010.4522
1010.9519
1011.4513
1011.9522
[M + H+]2+
TCEP
624.2889
624.7902
625.2897
625.7902
[M + H+]+
774.3429
[M + H+]+
Counts
Mass-to-Charge (m/z)

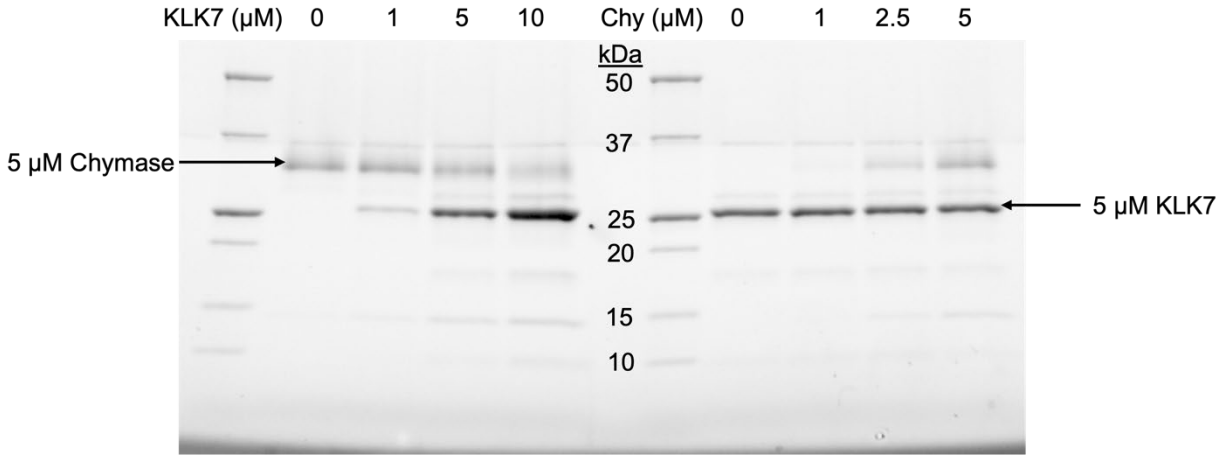
Supplementary Figure S2: LC-MS analysis of the proteolysis of TS-1, and TS-2 by KLK7. (A) Analysis of TS-1 (KLK7 170 loop mimic) proteolysis. (i) HPLC UV trace (λ = 214 nm) of the substrate and product mixtures of TS-1 respectively. The substrate (1) was fully hydrolyzed to give rise to three distinct products: 2, 3, and 4. The signals corresponding to 2 and 4 overlapped, wherein 2 was the major product and 4 was the minor product, based on extracted ion chromatograms (EICs) and the relative abundance of 2 and 3. (ii) Scheme of sequential peptide hydrolysis of 1 by KLK7 first into 2, followed by its hydrolysis to 3 and 4 respectively. (iii) Identification and confirmation of products of hydrolysis of 1 by KLK7 before and after reduction by TCEP by LC-MS. (B) Analysis of TS-2 (KLK7 99 loop mimic) proteolysis. (i) HPLC UV trace (λ = 214 nm) of the substrate and product mixtures of TS-2 respectively. The substrate (1) was partially hydrolyzed to give rise to product 2. (ii) Scheme showing enzymatic peptide hydrolysis followed by chemical reduction by TCEP. (iii) Identification and confirmation of product of hydrolysis of 1 by KLK7 before and after reduction by TCEP by LC-MS.



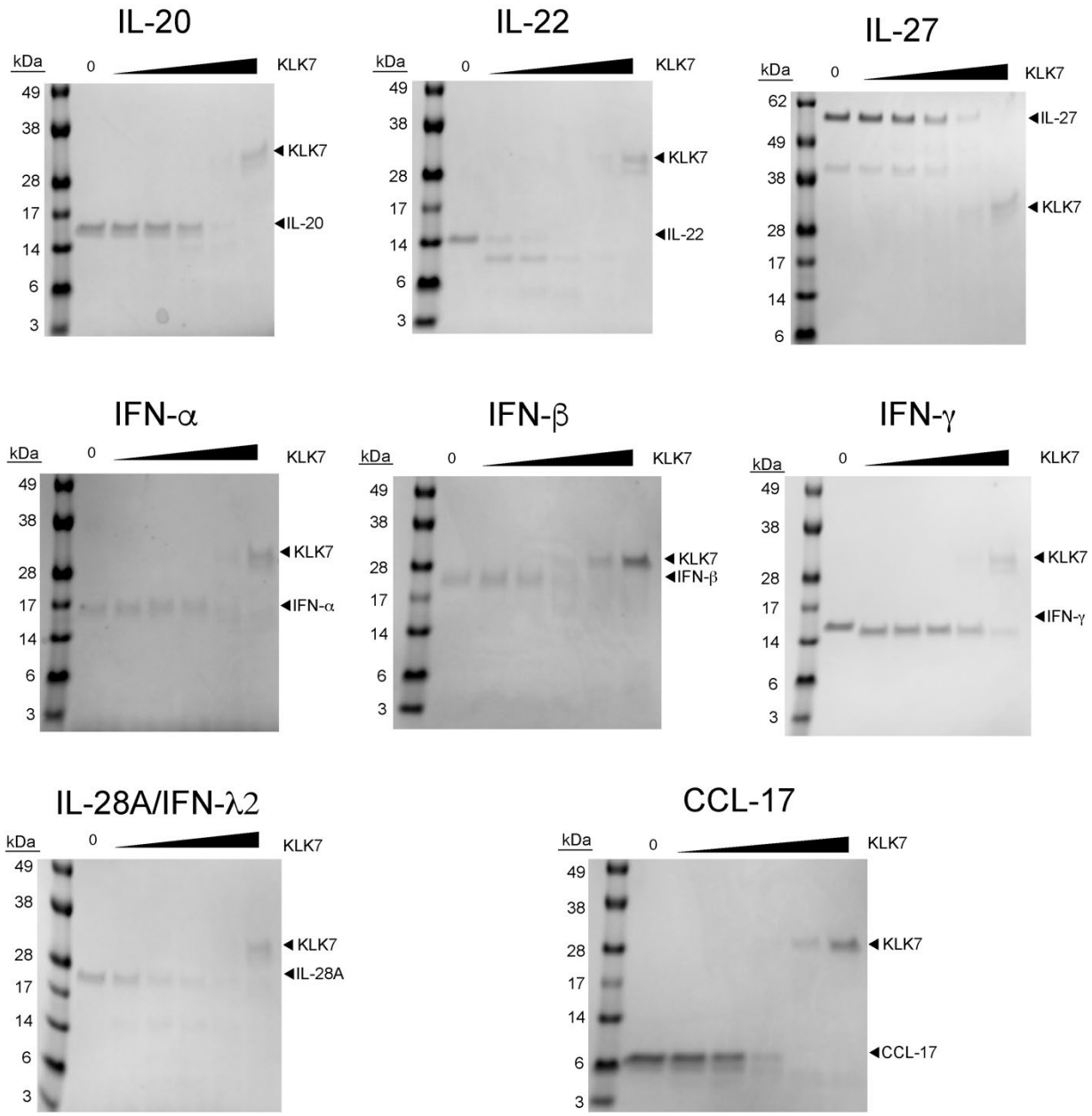
Supplementary Figure S3: Test of interaction between KLK7 and Chymase. Chymase and KLK7 were incubated with one another at various relative concentrations in 50 mM Tris pH 7.5 at 37 °C overnight. Neither Chymase nor KLK7 displayed any significant autolysis under these conditions. However, when incubated at comparable concentrations, KLK7 was able to partially hydrolyze Chymase. Samples were analyzed using SDS-PAGE under reducing conditions and stained with InstantBlue.



Supplementary Figure S4: Activity test of enzyme inhibitor complexes of KLK7 and Chymase. Serine protease irreversible inhibitors AEBSF and TPCK were able to inhibit KLK7 and Chymase activities effectively. Fluorogenic substrates ES002 (R&D Biosystems) and Suc-LLVY-AMC (Boston Biochem) were used to determine the activities of KLK7 and Chymase, respectively. Note that the inactive enzyme-inhibitor complexes AEBSF-KLK7 (In.KLK7) and TPCK-Chymase (In.Chy) were used as control reagents in Figure 5. Values are the means ± SDs of duplicate independent experiments.



Supplementary Figure S5: Titration of KLK7 and Chymase against one another. (Left) A fixed concentration of Chymase (5 μM) was titrated against increasing concentrations of KLK7, and the reaction was incubated at room temperature for 4 h in 50 mM Tris pH 7.5, 150 mM NaCl. (Right) A fixed concentration of KLK7 (5 μM) was titrated against increasing concentrations of Chymase, and the reaction was incubated under identical conditions. The amount of Chymase left unproteolyzed decreases as the concentration of KLK7 increases (left), but the amount of intact KLK7 remains unchanged when higher concentration of Chymase is added (right). Samples were analyzed using SDS-PAGE under reducing conditions and stained using InstantBlue® stain.



Supplementary Figure S6: Validation of new cytokine and chemokine substrates of KLK7. KLK7 was titrated against a fixed concentration of each cytokine independently. Each sample contained ~1 μM cytokine or chemokine in PBS buffer and varying concentrations (0, 0.0625, 0.125, 0.25, 0.5, and 1 μM respectively) of KLK7 and was incubated at 37 °C for four hours. Enzymatic reactions were quenched and analyzed using SDS-PAGE under reducing conditions as described in section 4.12 in the main manuscript.