

Supplementary Material

**Maturation process and characterization of a novel thermostable and halotolerant subtilisin-like protease with high collagenolytic but low gelatinolytic activity**

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Running title: A thermostable collagenolytic protease

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**TABLE S1** Predicted ion pairs in TSS and KP-43\*

|                     | TSS              |                         | KP-43                   |                         |     |
|---------------------|------------------|-------------------------|-------------------------|-------------------------|-----|
|                     | Ion pair         | Distance (Å)            | Ion pair                | Distance (Å)            |     |
| Salt bridge         |                  |                         | K9-D313                 | 3.0                     |     |
|                     |                  |                         | R37-D39                 | 3.3                     |     |
|                     |                  |                         | K151-D147               | 3.1                     |     |
|                     |                  | R217-D215               | 4.0                     |                         |     |
|                     |                  | <b><u>K219-D221</u></b> | 2.6                     | <b><u>K217-D219</u></b> | 2.7 |
|                     |                  |                         |                         | <u>K247-E44</u>         | 3.7 |
|                     |                  |                         |                         | <u>K274-E270</u>        | 2.9 |
|                     |                  | <b><u>K288-D221</u></b> | 4.0                     | <b><u>K286-D219</u></b> | 4.0 |
|                     |                  | <u>R309-D296</u>        | 3.3                     |                         |     |
|                     |                  | <u>R372-E419</u>        | 2.9                     |                         |     |
| Long-range ion pair | R3-D189          | 4.4                     |                         |                         |     |
|                     |                  |                         | R5-D11                  | 6.0                     |     |
|                     | <u>R9-D296</u>   | 4.2                     |                         |                         |     |
|                     | R39-E45          | 7.5                     |                         |                         |     |
|                     | R47-D91          | 4.7                     |                         |                         |     |
|                     | K49-E50          | 6.7                     |                         |                         |     |
|                     | R56-D59          | 4.5                     |                         |                         |     |
|                     | <u>R123-D153</u> | 5.2                     | <u>R124-D153</u>        | 5.5                     |     |
|                     |                  |                         | <u>R150-D353</u>        | 5.9                     |     |
|                     | <b>K178-D354</b> | 4.2                     | <b><u>K177-D353</u></b> | 4.5                     |     |
|                     |                  |                         | R189-D2                 | 5.4                     |     |
|                     | R190-D82         | 7.7                     |                         |                         |     |
|                     | R210-E203        | 7.6                     |                         |                         |     |
|                     |                  |                         | R215-D213               | 4.5                     |     |
|                     | R233-D239        | 4.7                     | R231-D237               | 4.9                     |     |
|                     | <u>R271-D153</u> | 4.3                     | <u>R269-D153</u>        | 4.8                     |     |
|                     |                  |                         | <u>K281-D153</u>        | 4.5                     |     |
|                     | R303-E169        | 6.0                     |                         |                         |     |
|                     | <u>R309-D202</u> | 5.3                     |                         |                         |     |
|                     | R313-D12         | 4.6                     |                         |                         |     |
| <u>R314-D296</u>    | 4.7              |                         |                         |                         |     |
| K324-D322           | 7.9              |                         |                         |                         |     |
| K328-D331           | 6.6              |                         |                         |                         |     |
|                     |                  | K332-E415               | 6.2                     |                         |     |
|                     | R363-E362        | 6.1                     |                         |                         |     |

\* The ion pairs involved in ionic networks are underlined. The ion pairs located at the interface between the catalytic and  $\beta$ JR domains are bolded.

TABLE S2 Hydrolyzed peptides of insoluble type I collagen  $\alpha 1$  chain by TSS at 60°C

| No. | Peptide sequence<br>(↓: cleavage site)               | Position | Modification<br>(O*: oxidation<br>OH*: Hydroxylation;<br>P*: phosphorylation)                 | PSMs              |
|-----|------------------------------------------------------|----------|-----------------------------------------------------------------------------------------------|-------------------|
| 1   | R↓GLPGTAGLPGMKGHR↓G                                  | 92-106   | 1xO* [M11]; OH* [K/P]                                                                         | 2                 |
| 2   | L↓PGTAGLPGMKGHR↓G                                    | 94-106   | OH* [K/P]; 1xOH* [M9]                                                                         | 3                 |
| 3   | S↓GLDGAKGDAGPAGPK↓G                                  | 110-124  |                                                                                               | 1                 |
| 4   | S↓GLDGAKGDAGPAGPKGEPGSPGENGAPQMGPR↓G                 | 110-142  | 1xO* [M30]; OH* [P]<br>1xO* [M30]; 3xOH* [K/P]                                                | 2<br>7            |
| 5   | R↓GRPGAPGPAGAR↓G                                     | 149-160  | 1xOH* [P3]<br>2xOH* [P3; P6]                                                                  | 2<br>2            |
| 6   | R↓PGAPGPAGAR↓G                                       | 151-160  | 2xOH* [P1; P4]                                                                                | 1                 |
| 7   | R↓GSEGPQGVRR↓G                                       | 200-208  |                                                                                               | 1                 |
| 8   | K↓GANGAPGIAGAPGPFPGAR↓G                              | 236-253  | 3xOH* [P6; P12; P15]                                                                          | 1                 |
| 9   | T↓GAKGEPGPTGIQGPPEPAGEEGKRGAR↓G                      | 284-310  | 1xOH* [P15]                                                                                   | 1                 |
| 10  | R↓GEPGPAGLPGPPGER↓G                                  | 311-325  | 2xOH* [P9; P11]<br>3xOH* [P9; P11; P12]                                                       | 1<br>1            |
| 11  | R↓GFPAGDGVAGPKGPA↓G                                  | 332-346  | 1xOH* [P3]                                                                                    | 1                 |
| 12  | R↓GFPAGDGVAGPKGPAGER↓G                               | 332-349  | 1xOH* [P3]<br>2xOH* [P3; K12]                                                                 | 6<br>1            |
| 13  | F↓PGADGVAGPKGPAGER↓G                                 | 334-349  | 1xOH* [P1]                                                                                    | 1                 |
| 14  | D↓GVAGPKGPAGER↓G                                     | 338-349  |                                                                                               | 1                 |
| 15  | R↓GAPGPAGPK↓G                                        | 350-358  | 1xOH* [P3]                                                                                    | 1                 |
| 16  | R↓GQAGVMGFPGPKGAAGEPGKAGER↓G                         | 413-436  | 1xOH* [K/P]<br>OH* [K/P]; 1xO* [M6]<br>OH* [K/P]; 1xO* [M6]                                   | 6<br>5<br>9       |
| 17  | A↓GVMGFPGPKGAAGEPGKAGER↓G                            | 416-436  | 1xOH* [K/P]<br>1xO* [M3]; OH* [K/P]<br>1xO* [M3]; OH* [K/P]<br>1xO* [M3]; 3xOH* [P6; P8; P15] | 3<br>11<br>6<br>2 |
| 18  | V↓MGFPKGAAGEPGKAGER↓G                                | 418-436  | 1xOH* [P4]                                                                                    | 1                 |
| 19  | M↓GFPKGAAGEPGKAGER↓G                                 | 419-436  | 1xOH* [P3]<br>2xOH* [P5; P]<br>3xOH* [P3; P5; P12]                                            | 3<br>4<br>2       |
| 20  | K↓GAAGEPGKAGER↓G                                     | 425-436  |                                                                                               | 7                 |
| 21  | R↓GVQGPAGPR↓G                                        | 524-535  | 1xOH* [P6]<br>2xOH* [P5; P6]                                                                  | 2<br>1            |
| 22  | V↓QGPPGAGPR↓G                                        | 526-535  | 1xOH* [P4]                                                                                    | 1                 |
| 23  | Q↓GPPGAGPR↓G                                         | 527-535  | 1xOH* [P3]                                                                                    | 1                 |
| 24  | R↓GAAGLPGPK↓G                                        | 572-580  | 1xOH* [P6]                                                                                    | 1                 |
| 25  | R↓GAAGLPGPKGDR↓G                                     | 572-583  | 1xOH* [P6]<br>2xOH* [P6; K9]                                                                  | 2<br>1            |
| 26  | R↓GAAGLPGPKGDRGDAGPKGADGAPKDGVR↓G                    | 572-601  | 3xOH* [K/P]                                                                                   | 3                 |
| 27  | A↓GLPGPKGDRGDAGPKGADGAPKDGVR↓G                       | 575-601  | 3xOH* [P3; P5; K23]                                                                           | 1                 |
| 28  | L↓PGPKGDR↓G                                          | 577-583  | 1xOH* [P1]                                                                                    | 1                 |
| 29  | L↓PGPKGDRGDAGPKGADGAPKDGVR↓G                         | 577-601  | 3xOH* [P1; P3; K21]                                                                           | 1                 |
| 30  | K↓GDRGDAGPKGADGAPKDGVR↓G                             | 581-601  |                                                                                               | 1                 |
|     |                                                      |          | 1xOH* [K/P]<br>2xOH* [P8; P15]                                                                | 3<br>1            |
| 31  | R↓GDAGPKGADGAPKDGVR↓G                                | 584-601  |                                                                                               | 2                 |
|     |                                                      |          | 1xOH* [K/P]<br>2xOH* [K/P]                                                                    | 7<br>2            |
| 32  | R↓GDAGPKGADGAPKDGVRGLTGPVGGPAGAPGDKGEAGPSGPAGPTGAR↓G | 584-634  | 2xOH* [K14; K36]                                                                              | 1                 |
| 33  | G↓DAGPKGADGAPKDGVR↓G                                 | 585-601  | 1xOH* [P11]                                                                                   | 1                 |
| 34  | D↓AGPKGADGAPKDGVR↓G                                  | 586-601  | 1xOH* [P10]                                                                                   | 2                 |
| 35  | A↓GPKGADGAPKDGVR↓G                                   | 587-601  | 1xOH* [P9]                                                                                    | 1                 |
| 36  | G↓PKGADGAPKDGVR↓G                                    | 588-601  | 1xOH* [P8]                                                                                    | 1                 |
| 37  | K↓GADGAPKDGVR↓G                                      | 590-601  |                                                                                               | 1                 |
|     |                                                      |          | 1xOH* [P6]                                                                                    | 2                 |
| 38  | R↓GLTGPVGGPAGAPGDKGEAGPSGPAGPTGAR↓G                  | 602-634  | 1xOH* [P]<br>3xOH* [P9; P11; P15]                                                             | 4<br>2            |
| 39  | T↓GPIGPPGAGAPGDKGEAGPSGPAGPTGAR↓G                    | 605-634  | 1xOH* [P6]                                                                                    | 1                 |
| 40  | A↓GAPGDKGEAGPSGPAGPTGAR↓G                            | 614-634  | 1xOH* [K/P]                                                                                   | 2                 |
| 41  | R↓GSAGPPGATGPGAAGR↓V                                 | 704-720  | 2xOH* [P6; P12]                                                                               | 2                 |
| 42  | R↓VGPVPPSGNAGPPGPPGAGKEGSKGPR↓G                      | 721-748  | 3xOH* [K/P]                                                                                   | 3                 |
| 43  | S↓GNAGPPGPPGAGKEGSKGPR↓G                             | 728-748  | 2xOH* [P8; P]<br>3xOH* [P6; P8; P9]                                                           | 2<br>1            |
| 44  | N↓AGPPGPPGAGKEGSKGPR↓G                               | 730-748  | 2xOH* [P6; P]<br>3xOH* [P4; P6; P7]                                                           | 2<br>1            |
| 45  | R↓GVVGLPGQR↓G                                        | 797-805  | 1xOH* [P6]                                                                                    | 1                 |
| 46  | V↓VGLPGQR↓G                                          | 799-805  | 1xOH* [P4]                                                                                    | 1                 |
| 47  | R↓GETGPAGPPGAPGAPGPPVGPAGK↓S                         | 875-900  | 3xOH* [P12; P15; P18]                                                                         | 1                 |

TABLE S2 (continued)

| No. | Peptide sequence<br>(↓: cleavage site)                | Position | Modification<br>(O*: oxidation<br>OH*: Hydroxylation;<br>P*: phosphorylation) | PSMs   |
|-----|-------------------------------------------------------|----------|-------------------------------------------------------------------------------|--------|
| 48  | R↓GETGPAGPPGAPGAPGAPGVPVGPAGKSGDR↓G                   | 875-904  | 2xOH* [P12; P15]<br>3xOH* [P9; P12; P15]                                      | 1<br>1 |
| 49  | R↓GETGPAGPPGAPGAPGAPGVPVGPAGKSGDRGETGPAGPAGPIGVPGAR↓G | 875-922  | 3xOH* [P12; P15; P]                                                           | 2      |
| 50  | A↓GKSGDRGETGPAGPAGPIGVPGAR↓G                          | 899-922  |                                                                               | 2      |
| 51  | A↓GKSGDRGETGPAGPAGPIGVPGARGPAGPQGPR↓G                 | 899-931  |                                                                               | 2      |
| 52  | K↓SGDRGETGPAGPAGPIGVPGARGPA↓G                         | 901-925  |                                                                               | 1      |
| 53  | K↓SGDRGETGPAGPAGPIGVPGARGPAGPQGPR↓G                   | 901-931  |                                                                               | 1      |
| 54  | R↓GETGPAGPAGPIGVPGAR↓G                                | 905-922  | 1xOH* [P8]                                                                    | 1      |
| 55  | R↓GETGPAGPAGPIGVPGARGPAGPQGPR↓G                       | 905-931  |                                                                               | 1      |
| 56  | R↓GETGPAGPAGPIGVPGARGPAGPQGPRGDKGETGEQDGRGK↓G         | 905-946  | 2xOH* [K30; K42]                                                              | 1      |
| 57  | T↓GPAGPAGPIGVPGAR↓G                                   | 908-922  |                                                                               | 1      |
| 58  | V↓GARGPAGPQGPR↓G                                      | 920-931  |                                                                               | 1      |
| 59  | A↓RGPAGPQGPR↓G                                        | 922-931  |                                                                               | 1      |
| 60  | R↓GPAGPQGPR↓G                                         | 923-931  |                                                                               | 1      |
| 61  | R↓GPAGPQGPRGDK↓G                                      | 923-934  |                                                                               | 2      |
| 62  | R↓GPAGPQGPRGDKGETGEQDGR↓G                             | 923-943  | 1xOH* [K/P]                                                                   | 3      |
| 63  | A↓GPQGPRGDKGETGEQDGR↓G                                | 926-943  | 1xOH* [K/P]                                                                   | 3      |
| 64  | R↓GDKGETGEQDGRGK↓G                                    | 932-946  | 1xOH* [K15]                                                                   | 1      |
|     |                                                       |          | 2xOH* [K3; K15]                                                               | 2      |
| 65  | R↓GDKGETGEQDGRGKGR↓R                                  | 932-948  | 2xOH* [K3; K15]                                                               | 2      |
| 66  | R↓GDKGETGEQDGRGKGR↓G                                  | 932-949  | 1xOH* [K15]                                                                   | 1      |
|     |                                                       |          | 2xOH* [K3; K15]                                                               | 1      |
| 67  | K↓GETGEQDGRGK↓G                                       | 935-946  |                                                                               | 1      |
|     |                                                       |          | 1xOH* [K12]                                                                   | 1      |
| 68  | K↓GETGEQDGRGKGR↓G                                     | 935-949  | 1xOH* [K12]                                                                   | 1      |
| 69  | S↓GLQGPFPSPGSPGQPSGASGPAGPR↓G                         | 953-979  | 2xOH* [P]                                                                     | 3      |

TABLE S3 Hydrolyzed peptides of insoluble type I collagen  $\alpha 2$  chain by TSS at 60°C

| No. | Peptide sequence<br>(↓: cleavage site)        | Position | Modification<br>(O*: oxidation<br>OH*: Hydroxylation;<br>P*: phosphorylation) | PSMs |
|-----|-----------------------------------------------|----------|-------------------------------------------------------------------------------|------|
| 1   | R↓GHNGLDGLKQPGAPGVKGEPEGAPGENGTPGQTGAR↓G      | 100-135  | 1xP* [T29]                                                                    | 1    |
| 2   | R↓GRVGPAGFPAGAR↓G                             | 142-153  | 1xOH* [P6]                                                                    | 2    |
| 3   | R↓GIPGVPVGAAGATGAR↓G                          | 247-261  | 1xOH* [P3]                                                                    | 1    |
| 4   | R↓GLPGADGRAGVMGPAGSR↓G                        | 325-342  | 1xOH* [P3]                                                                    | 1    |
| 5   | P↓GADGRAGVMGPAGSR↓G                           | 328-342  | 1xO* [M12]; OH* [P]                                                           | 1    |
| 6   | R↓AGVMGPAGSR↓G                                | 333-342  | 1xO* [M4]; 1xOH* [P6]                                                         | 2    |
| 7   | R↓GATGPAGVVR↓G                                | 343-351  | 1xO* [M4]; 1xOH* [P6]                                                         | 1    |
| 8   | R↓GATGPAGVVRGPN↓G                             | 343-354  |                                                                               | 1    |
| 9   | A↓TGPAGVVRGPN↓G                               | 345-354  | 1xOH* [P5]                                                                    | 1    |
| 10  | T↓GPAGVVRGPN↓G                                | 346-354  |                                                                               | 1    |
| 11  | N↓GDSGRPGEPGLMGPR↓G                           | 355-369  | 1xOH* [P6]                                                                    | 1    |
|     |                                               |          | 1xO* [M12]; OH* [P]                                                           | 1    |
|     |                                               |          | 1xO* [M12]; 2xOH* [P6; P9]                                                    | 2    |
| 12  | S↓GDPGKAGEKGHAGLAGAR↓G                        | 421-438  |                                                                               | 1    |
|     |                                               |          | 1xOH* [K9]                                                                    | 1    |
|     |                                               |          | 2xOH* [P3; K9]                                                                | 1    |
| 13  | K↓GHAGLAGAR↓G                                 | 430-438  |                                                                               | 1    |
| 14  | Q↓GLPGPAGTAGEACKPGER↓G                        | 475-492  | 2xOH* [K/P]                                                                   | 2    |
| 15  | I↓PGEFGLPGPAGAR↓G                             | 495-507  | 2xOH* [P1; P7]                                                                | 1    |
| 16  | I↓PGEFGLPGPAGARGER↓G                          | 495-510  | 2xOH* [P1; P7]                                                                | 1    |
| 17  | A↓GPSGSPGLPGERGAAGIPGGKGEKGETGLRGDIGSPGRDGR↓G | 553-594  | 1xP* [S35]                                                                    | 1    |
| 18  | R↓GAAGIPGGKGEKGETGLR↓G                        | 565-582  | 2xOH* [P6; K12]                                                               | 1    |
|     |                                               |          | 3xOH* [P6; K9; K12]                                                           | 2    |
| 19  | A↓AGIPGGKGEKGETGLR↓G                          | 567-582  | 2xOH* [P4; K10]                                                               | 1    |
|     |                                               |          | 3xOH* [P4; K7; K10]                                                           | 1    |
| 20  | I↓PGGKGEKGETGLR↓G                             | 570-582  | 2xOH* [P1; K7]                                                                | 3    |
|     |                                               |          | 3xOH* [P1; K4; K7]                                                            | 1    |
| 21  | R↓GDIGSPGRDGA↓R                               | 583-593  | 1xOH* [P6]                                                                    | 1    |
| 22  | R↓GAPGAIGAPGPAGANGDR↓G                        | 595-612  | 2xOH* [P3; P9]                                                                | 1    |
| 23  | R↓GAPGAIGAPGPAGANGDRGEAGPAGPAGPAGPR↓G         | 595-627  | 2xOH* [P3; P9]                                                                | 1    |
| 24  | R↓GEAGPAGPAGPAGPR↓G                           | 613-627  |                                                                               | 1    |
| 25  | R↓GEAGPAGPAGPAGPRGSPGER↓G                     | 613-633  | 1xOH* [P18]                                                                   | 1    |
| 26  | A↓GPAGPAGPAGPR↓G                              | 616-627  |                                                                               | 1    |
| 27  | R↓GSPGERGEVGPAGPENGFPAGPAGAAGQPAGKGER↓G       | 628-660  | 2xOH* [P20; P27]                                                              | 1    |
| 28  | R↓GTKGPKGENGPVGTGPVGAAGPSGPNPPGAGSR↓G         | 661-696  | 2xOH* [P17; P23]                                                              | 1    |
| 29  | R↓GDGGPPGATGFPGAAGR↓T                         | 697-713  | 2xOH* [P6; P12]                                                               | 1    |
| 30  | R↓GDQGPVGR↓S                                  | 742-749  |                                                                               | 1    |
| 31  | N↓AGPVGAAGAPGPGQPVGVPVKGH↓G                   | 873-894  | 1xOH* [P10]                                                                   | 1    |
| 32  | N↓AGPVGAAGAPGPGQPVGVPVKGHGN↓R                 | 873-896  | 1xOH* [P10]                                                                   | 1    |
| 33  | N↓AGPVGAAGAPGPGQPVGVPVKGHGNR↓G                | 873-897  | 1xOH* [P10]                                                                   | 1    |
| 34  | A↓AGAPGPGQPVGVPVKGHGNR↓G                      | 879-897  | 1xOH* [P4]                                                                    | 2    |
| 35  | A↓GAPGPGQPVGVPVKGHGNR↓G                       | 880-897  | 1xOH* [P]                                                                     | 3    |
| 36  | K↓HGNRGEPPAGAVGPAGAVGPR↓G                     | 894-915  |                                                                               | 1    |
|     |                                               |          | 1xOH* [P7]                                                                    | 1    |
| 37  | H↓GNRGEPPAGAVGPAGAVGPRGSPGQ↓G                 | 895-921  |                                                                               | 1    |
|     |                                               |          | 1xOH* [P]                                                                     | 2    |
| 38  | H↓GNRGEPPAGAVGPAGAVGPRGSPGQIR↓G               | 895-924  | 1xOH* [P6]                                                                    | 1    |
| 39  | H↓GNRGEPPGPA↓G                                | 895-903  |                                                                               | 1    |
| 40  | H↓GNRGEPPGPA↓G                                | 895-909  |                                                                               | 1    |
|     |                                               |          | 1xOH* [P6]                                                                    | 1    |
| 41  | H↓GNRGEPPGPA↓G                                | 895-915  |                                                                               | 1    |
| 42  | H↓GNRGEPPGPA↓G                                | 895-918  | 1xOH* [P]                                                                     | 2    |
| 43  | N↓RGEPPGPA↓G                                  | 897-918  | 1xOH* [P]                                                                     | 2    |
| 44  | N↓RGEPPGPA↓G                                  | 897-924  |                                                                               | 1    |
|     |                                               |          | 1xOH* [P4]                                                                    | 1    |
| 45  | R↓GEPGPA↓G                                    | 898-915  | 1xOH* [P3]                                                                    | 1    |
| 46  | A↓GAVGPAGAVGPR↓G                              | 904-915  |                                                                               | 1    |
| 47  | A↓GAVGPAGAVGPRGPS↓G                           | 904-918  |                                                                               | 1    |
| 48  | A↓GAVGPAGAVGPRGSPGQ↓G                         | 904-921  |                                                                               | 1    |
| 49  | R↓GPSGPGQIR↓G                                 | 916-924  |                                                                               | 1    |
| 50  | R↓GPSGPGQIRGDKGEPGDKGPR↓G                     | 916-936  | 1xOH* [K12]                                                                   | 1    |
|     |                                               |          | 2xOH* [K/P]                                                                   | 3    |
| 51  | A↓GHHGDQGAPGAVGPAGPR↓G                        | 955-972  |                                                                               | 1    |
|     |                                               |          | 1xOH* [P9]                                                                    | 1    |
| 52  | A↓GHHGDQGAPGAVGPAGPRGPA↓G                     | 955-975  | 1xOH* [P9]                                                                    | 2    |

**TABLE S3 (continued)**

| No. | Peptide sequence<br>(↓: cleavage site)            | Position | Modification<br>(O*: oxidation<br>OH*: Hydroxylation;<br>P*: phosphorylation) | PSMs |
|-----|---------------------------------------------------|----------|-------------------------------------------------------------------------------|------|
| 53  | A↓GHHGDQGAPGAVGPAGPRGPAGPSGPAGKDGR↓I              | 955-986  |                                                                               | 1    |
|     |                                                   |          | 1xOH* [P9]                                                                    | 3    |
| 54  | A↓GHHGDQGAPGAVGPAGPRGPAGPSGPAGKDGRIGQPGAVGPAGIR↓G | 955-999  | 1xOH* [P9]                                                                    | 2    |
|     |                                                   |          | 2xOH* [P9; K29]                                                               | 2    |
| 55  | V↓GPAGPRGPAGPSGPAGKDGR↓I                          | 967-986  |                                                                               | 1    |
| 56  | R↓GPAGPSGPAGK↓D                                   | 973-983  |                                                                               | 1    |
| 57  | R↓GPAGPSGPAGKDGR↓I                                | 973-986  |                                                                               | 2    |
| 58  | R↓GPAGPSGPAGKDGRIGQPGAVGPA↓G                      | 973-996  | 1xOH* [P18]                                                                   | 1    |
| 59  | R↓GPAGPSGPAGKDGRIGQPGAVGPAGIR↓G                   | 973-999  |                                                                               | 2    |
|     |                                                   |          | 1xOH* [P]                                                                     | 6    |
| 60  | A↓GPSGPAGKDGRIGQPGAVGPAGIR↓G                      | 976-999  | 1xOH* [P15]                                                                   | 2    |
| 61  | A↓GKDGRIGQPGAVGPAGIR↓G                            | 982-999  |                                                                               | 1    |

**TABLE S4** Hydrolyzed peptides of solublized type I collagen  $\alpha$ 1 chain by TSS at 60°C

| No. | Peptide sequence<br>(↓: cleavage site)                | Position | Modification<br>(O*: oxidation<br>OH*: Hydroxylation;<br>P*: phosphorylation) | PSMs   |
|-----|-------------------------------------------------------|----------|-------------------------------------------------------------------------------|--------|
| 1   | K↓STGISVPGPMGPPSGPR↓G                                 | 10-25    | 1xOH* [P7]; 1xO* [M10]                                                        | 1      |
| 2   | R↓GLPGTAGLPGMK↓G                                      | 92-103   | OH* [K/P]; 1xO* [M11]                                                         | 1      |
| 3   | N↓GAPGQMGPR↓G                                         | 134-142  | 1xOH* [P3]                                                                    | 1      |
| 4   | R↓GRPGAPGPAGAR↓G                                      | 149-160  | 2xOH* [P3; P6]                                                                | 2      |
| 5   | R↓GRPGAPGPAGARG↓N                                     | 149-161  | 2xOH* [P3; P6]                                                                | 1      |
| 6   | R↓PGAPGPAGAR↓G                                        | 151-160  | 2xOH* [P1; P4]                                                                | 1      |
| 7   | R↓GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPPQGPR↓G          | 161-199  | 3xOH* [K/P]                                                                   | 3      |
| 8   | K↓GEGGPPQGPR↓G                                        | 191-199  |                                                                               | 1      |
| 9   | R↓GSEGGPPQVPR↓G                                       | 200-208  |                                                                               | 1      |
| 10  | K↓GEPGPTGIQGPPPAGEEGKR↓G                              | 287-307  | 1xOH* [P12]                                                                   | 1      |
| 11  | R↓GEPGPAGLPGPPGER↓G                                   | 311-325  | 2xOH* [P9; P11]                                                               | 1      |
| 12  | R↓GEPGPAGLPGPPGERGGPGSR↓G                             | 311-331  | 3xOH* [P9; P11; P12]                                                          | 1      |
| 13  | R↓GFPGADGVAGPKGPA↓G                                   | 332-346  | 1xOH* [P3]                                                                    | 1      |
| 14  | R↓GFPGADGVAGPKGPAGER↓G                                | 332-349  | 1xOH* [P3]                                                                    | 2      |
| 15  | F↓PGADGVAGPKGPAGER↓G                                  | 334-349  | 1xOH* [P1]                                                                    | 1      |
| 16  | R↓GAPGPAGPK↓G                                         | 350-358  | 2xOH* [P3; K9]<br>1xOH* [P3]                                                  | 1<br>1 |
| 17  | R↓GAPGPAGPKG↓S                                        | 350-359  | 1xOH* [P3]                                                                    | 1      |
| 18  | R↓GQAGVMGFPGPKGAAGEPGKAGER↓G                          | 413-436  | 1xO* [M6]; OH* [P]                                                            | 2      |
| 19  | A↓GVMGFPGPKGAAGEPGKAGER↓G                             | 416-436  | 2xOH* [P6; P8]                                                                | 1      |
| 20  | K↓GAAGEPGKAGER↓G                                      | 426-436  |                                                                               | 1      |
| 21  | R↓GVQGGPPGAGPR↓G                                      | 524-535  | 1xOH* [P6]<br>2xOH* [P5; P6]                                                  | 3<br>1 |
| 22  | R↓GAAGLPGPKGDR↓G                                      | 572-583  | 2xOH* [P6; K9]<br>1xOH* [P6]                                                  | 1<br>2 |
| 23  | K↓GDRGDAGPKGADGAPKDGVR↓G                              | 581-601  | 1xOH* [P15]                                                                   | 1      |
| 24  | R↓GDAGPKGADGAPKDGVR↓G                                 | 584-601  | 1xOH* [P12]<br>2xOH* [P5; P12]                                                | 3<br>1 |
| 25  | A↓GPKGADGAPKDGVR↓G                                    | 588-601  | 1xOH* [P9]                                                                    | 1      |
| 26  | K↓GADGAPKDGVR↓G                                       | 591-601  | 1xOH* [P6]                                                                    | 2      |
| 27  | R↓GLTGPIGPPGAPAGPKGEGAGPSGPAGPTGAR↓G                  | 602-634  | 2xOH* [K/P]<br>3xOH* [P9; P11; P15]                                           | 5<br>1 |
| 28  | A↓GPSGPAGPTGAR↓G                                      | 623-634  |                                                                               | 1      |
| 29  | S↓GPAGPTGAR↓G                                         | 626-634  |                                                                               | 1      |
| 30  | R↓GAPGDRGEPGPPGPA↓G                                   | 635-649  | 3xOH* [P3; P9; P11]                                                           | 1      |
| 31  | R↓GSAGPPGATGFFGAAGR↓V                                 | 704-720  | 2xOH* [P6; P12]                                                               | 1      |
| 32  | R↓VGPPGPPSGNAGPPGPPGAGKEGSKGPR↓G                      | 721-748  | 3xOH* [P6; P12; P13]                                                          | 1      |
| 33  | R↓GETGPAGRPGEVGGPPGPPGAGEKAGAPGADGPAGAPGTPGPQGIAGQR↓G | 749-796  | 1xP* [T3]; 2xOH* [P36; P39]<br>1xP* [T3]; 1xOH* [P36]                         | 1<br>1 |
| 34  | K↓GAPGADGPAGAPGTPGPQGIAGQR↓G                          | 773-796  | 2xOH* [P8; P12]<br>3xOH* [P8; P12; P15]                                       | 2<br>1 |
| 35  | R↓GVVGLPGQR↓G                                         | 797-805  | 1xOH* [P6]                                                                    | 1      |
| 36  | V↓VGLPGQR↓G                                           | 799-805  | 1xOH* [P4]                                                                    | 1      |
| 37  | R↓GETGPAGPPGAPGAPGPPVGPAGKSGDR↓G                      | 875-904  | 3xOH* [P9; P12; P15]                                                          | 1      |
| 38  | A↓GKSGDRGETGPAGPAGPIGPVGAR↓G                          | 899-922  |                                                                               | 2      |
| 39  | K↓SGDRGETGPAGPAGPIGPVGAR↓G                            | 901-922  |                                                                               | 1      |
| 40  | V↓GARGPAGPQGPR↓G                                      | 920-931  |                                                                               | 1      |
| 41  | A↓RGPAGPQGPR↓G                                        | 922-931  |                                                                               | 1      |
| 42  | R↓GPAGPQGPR↓G                                         | 923-931  |                                                                               | 1      |
| 43  | R↓GPAGPQGPRGDKGETGEQGDR↓G                             | 923-943  | 1xOH* [K/P]                                                                   | 4      |
| 44  | R↓GDKGETGEQDRGIK↓G                                    | 932-946  | 2xOH* [K3; K15]                                                               | 2      |
| 45  | K↓GETGEQDRGIK↓G                                       | 935-946  | 1xOH* [K12]                                                                   | 1      |
| 46  | R↓GPPGSAGSPKDGGLN↓G                                   | 980-994  | 2xOH* [P3; P9]                                                                | 1      |

**TABLE S5** Hydrolyzed peptides of solublized type I collagen  $\alpha 2$  chain by TSS at 60°C

| No. | Peptide sequence<br>(↓: cleavage site) | Position | Modification<br>(O*: oxidation<br>OH*: hydroxylation;<br>P*: phosphorylation) | PSMs |
|-----|----------------------------------------|----------|-------------------------------------------------------------------------------|------|
| 1   | R↓GRVGAPGPAGA↓R                        | 142-152  | 1xOH* [P6]                                                                    | 1    |
| 2   | R↓GRVGAPGPAGAR↓G                       | 142-153  | 1xOH* [P6]                                                                    | 1    |
| 3   | N↓PGPAGPAGPR↓G                         | 192-201  | 1xOH* [P1]                                                                    | 1    |
| 4   | R↓GIPGPVGAAGATGAR↓G                    | 247-261  | 1xOH* [P3]                                                                    | 2    |
| 5   | I↓PGPVGAAGATGAR↓                       | 249-261  | 1xOH* [P1]                                                                    | 1    |
| 6   | R↓GSTGEIGPAGPPGPPGLR↓G                 | 301-318  | 2xOH* [P12; P14]                                                              | 1    |
| 7   | R↓GLPGADGRAGVMGPAGSR↓G                 | 325-342  | 1xOH* [P3]                                                                    | 2    |
| 8   | L↓PGADGRAGVMGPAGSR↓G                   | 327-342  | 1xOH* [P1]                                                                    | 1    |
| 9   | R↓AGVMGPAGSR↓G                         | 333-342  | 1xO* [M4]; 1xOH* [P6]                                                         | 3    |
| 10  | R↓GATGPAGVVRGPN↓G                      | 343-354  |                                                                               | 1    |
| 11  | T↓GPAGVRGPN↓G                          | 346-354  |                                                                               | 1    |
| 12  | K↓GHAGLAGAR↓G                          | 430-438  |                                                                               | 1    |
| 13  | L↓PGPAGTAGEAGKPPER↓G                   | 477-492  | 2xOH* [K/P]                                                                   | 2    |
| 14  | I↓PGGKGEKGET↓G                         | 570-579  | 1xOH* [P1]                                                                    | 1    |
| 15  | R↓GAPGAI GAPGPAGANGDRGEAGPAGPAGPAGPR↓G | 595-627  | 2xOH* [P3; P9]                                                                | 1    |
| 16  | A↓GANGDRGEAGPAGPAGPAGPR↓G              | 607-627  |                                                                               | 1    |
| 17  | N↓GDRGEAGPAGPAGPAGPR↓G                 | 610-627  |                                                                               | 1    |
| 18  | R↓GEAGPAGPAGPAGPR↓G                    | 613-627  |                                                                               | 1    |
| 19  | G↓PAGPAGPAGPR↓G                        | 617-627  |                                                                               | 1    |
| 20  | N↓GPVGPPTGFPVGAAGPSGPNPAGPAGSR↓G       | 670-696  | 1xOH* [P21]                                                                   | 1    |
| 21  | A↓GSRGDDGGPPGATGFPGAAGR↓T              | 694-713  | 2xOH* [P9; P15]                                                               | 1    |
| 22  | R↓GDGGPPGATGFPGAAGR↓T                  | 697-713  | 2xOH* [P6; P12]                                                               | 1    |
| 23  | R↓GYPGNAGFPVGAAGAPGQGPVGPVVK↓H         | 868-893  | 2xOH* [P8; P15]                                                               | 1    |
| 24  | N↓AGFPVGAAGAPGQGPVGPVVKHGN↓R           | 873-896  | 1xOH* [P10]                                                                   | 1    |
| 25  | N↓RGEPPGAVGPAGAVGPR↓G                  | 897-915  |                                                                               | 1    |
| 26  | N↓RGEPPGAVGPAGAVGPRGSPGQIR↓G           | 897-924  | 1xOH* [P4]                                                                    | 1    |
| 27  | R↓GEPGPAGAVGPAGAVGPRGSPGQ↓G            | 898-921  | 1xOH* [P]                                                                     | 2    |
| 28  | R↓GEPGPAGAVGPAGAVGPRGSPGQIR↓G          | 898-924  | 1xOH* [P5]                                                                    | 1    |
| 29  | A↓GAVGPAGAVGPRGSPGQ↓G                  | 904-921  | 1xOH* [P]                                                                     | 2    |
| 30  | G↓PAGAVGPR↓G                           | 908-915  |                                                                               | 1    |
| 31  | R↓GDKGEPGDKGPR↓G                       | 925-936  | 1xOH* [K3]                                                                    | 1    |
| 32  | A↓GHHGDQAPGAVGPAGPR↓G                  | 955-972  | 1xOH* [P9]                                                                    | 2    |
| 33  | R↓GPAGPSGPAGK↓D                        | 973-983  |                                                                               | 1    |
| 34  | R↓GPAGPSGPAGKDGR↓I                     | 973-986  |                                                                               | 1    |
| 35  | R↓GPAGPSGPAGKDGRIGQPGAVGPAGIR↓G        | 973-999  | 1xOH* [P5]                                                                    | 1    |
| 36  | R↓GPAGPSGPAGKDGRIGQPGAVGPAGIRGSO↓G     | 973-1002 | 1xOH* [P18]                                                                   | 3    |
| 37  | A↓GPSGPAGKDGRIGQPGAVGPAGIR↓G           | 976-999  | 1xOH* [P]                                                                     | 3    |
|     |                                        |          | 1xOH* [P15]                                                                   | 1    |



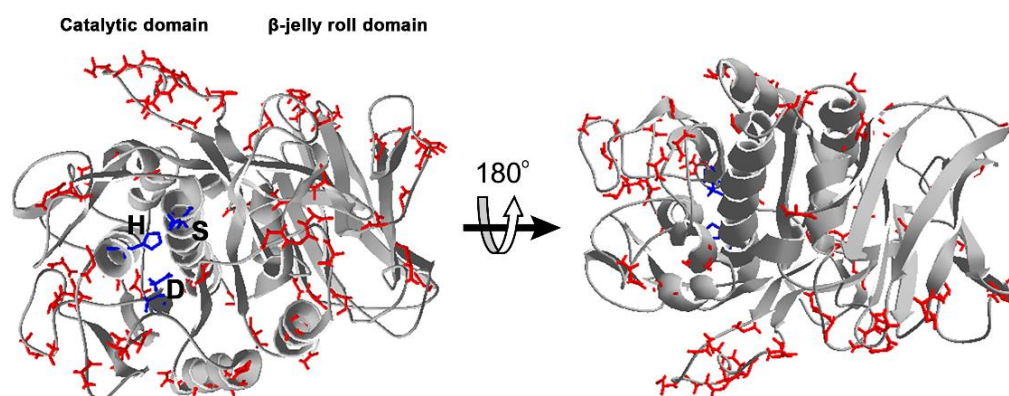
**TABLE S6** Primers used in this study

| Primer  | Oligonucleotide sequence (5' to 3')*             |
|---------|--------------------------------------------------|
| TSS-f   | GGAATTC <u>CCATATGAAGCGGGAAACAGGG</u>            |
| TSS-r   | CCGCTCGAGACGGGAGCGGGA                            |
| S257A-f | CTATCTCAGCGGGACA <u>GC</u> CATGGCGACG            |
| S257A-r | G <u>GC</u> TGTCCCCTGAGATAGGCGTAGAAGG            |
| S257C-f | CTATCTCAGCGGGACAT <u>GC</u> ATGGCGACG            |
| S257C-r | <u>GC</u> ATGTCCCCTGAGATAGGCGTAGAAGG             |
| ΔP-r    | GGCTCGAGCCTGTTTTTTCGTGAC                         |
| ΔP'-r   | GGCTCGAGGCCGTTGGC                                |
| ΔβP-r   | CCGCTCGAGCGTCTCGATCGCACG                         |
| ΔN-f    | CGCATATGGACAGGGCGGCGGCGATCATC                    |
| N-f     | GGCATATGCAACCACCACCACCACAAGCGGGAAACAGGGCGCAAG    |
| N-r     | GGCTCGAGTCAGCGCTGTTTTTCGTTCCGGAAC                |
| βJR-f   | GGCCATATGGGCTTTGTGGATGAG                         |
| PPC-f   | CCGCATATGGAAGTGGTGTCCGAC                         |
| E187A-f | GCCTCCG <u>C</u> AAACGACCGGCCGGAGC               |
| E187A-r | GTCGTTT <u>G</u> CGGAGGCGCCACGGCG                |
| N368D-f | CTGGTCAAC <u>G</u> ACCTGAACCTGCGAG               |
| N368D-r | CAGGT <u>C</u> GTTGACCAGGGTGCGCTCG               |
| N370D-f | CAACAACCTG <u>G</u> ACCTGCGAGTGGTC               |
| N370D-r | CAGGT <u>C</u> CAGGTTGTTGACCAGGGTG               |
| D385A-f | CAACG <u>C</u> CTTTTTCCACTACCCCTACG              |
| D385A-r | GTGGAAAAAG <u>G</u> CGTTGCCGTTCAAGG              |
| D393A-f | CTACGACG <u>C</u> CGAAGTGGACAACCTCAAC            |
| D393A-r | CACTTCG <u>G</u> CGTCGTAGGGGTAGTGG               |
| D396A-f | CGAAGTGG <u>C</u> CAACCTCAACAACGTG               |
| D396A-r | GAGGTTG <u>G</u> CCACTTCGTCGTCGTAG               |
| E402A-f | CAACGTGG <u>C</u> GCAGGTCTGGATCAAG               |
| E402A-r | GACCTGC <u>G</u> CCACGTTGTTGAGGTTG               |
| GFP-f   | CTTTAAGAAGGAGATATACATATGAGTAAAGGAGAAGAAGTCTTCACT |
| GFP-r   | CCCAAGCTTTTTGTATAGTTCATCCATGC                    |
| GFP'-r  | GCTTCCTCCTCCTCCGCTTCTTTGTATAGTTCATCCATGC         |
| βJR'-f  | GGAAGCGGAGGAGGAGGAAGCGGCTTTGTGGATGAGAAAAA        |
| βJR'-r  | GTGGTGGTGGTGGTGTCTCGAGGCCGTTGGCCGCCAGGGCGT       |
| PPC'-f  | GGAAGCGGAGGAGGAGGAAGCGAAGTGGTGTCCGACAATTG        |
| PPC'-r  | GTGGTGGTGGTGGTGTCTCGAGACGGGAGCGGGACGGGTAGG       |

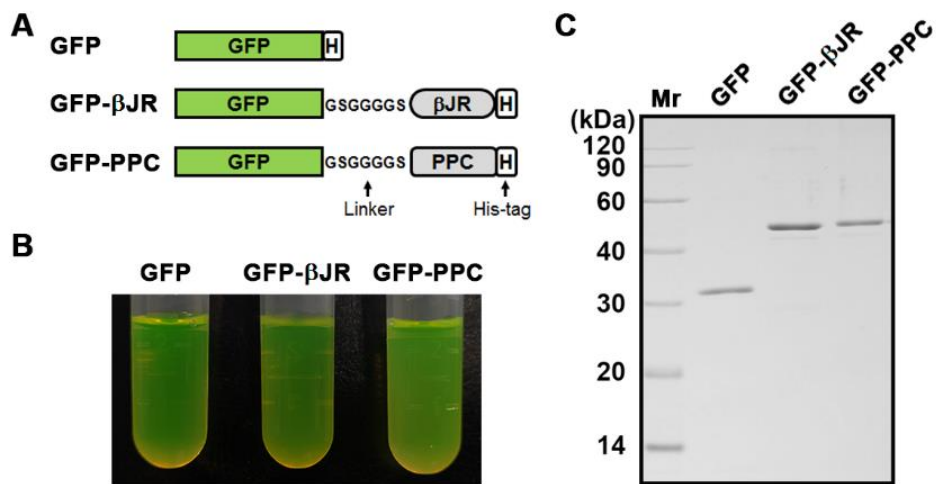
\* Underlined sequences indicate restriction enzyme sites. The mutated nucleotides are boxed. The italicized section indicates the His6 tag-encoding DNA sequence.

**TABLE S7** Primer pairs, templates, and restriction enzyme sites used in plasmid construction

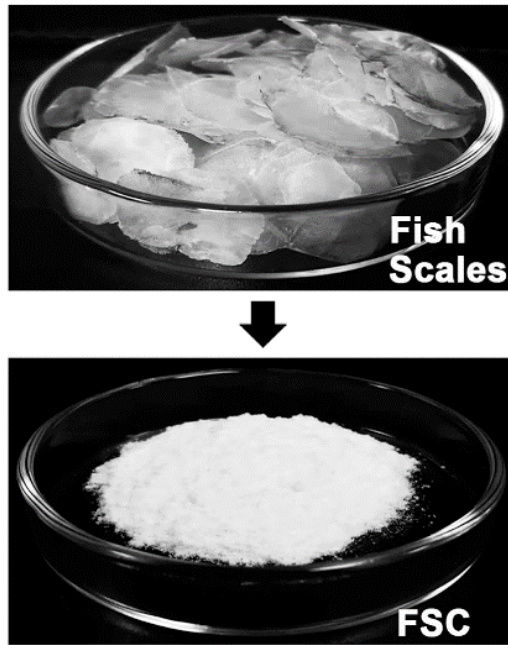
| Plasmid                  | Primer pair                    | Template                    | Restriction site              |
|--------------------------|--------------------------------|-----------------------------|-------------------------------|
| pET26b- <i>pro-TSS</i>   | TSS-f/TSS-r                    | Genomic DNA of strain WF146 | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- $\Delta P$       | TSS-f/ $\Delta P$ -r           | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- $\Delta P'$      | TSS-f/ $\Delta P'$ -r          | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- $\Delta \beta P$ | TSS-f/ $\Delta \beta P$ -r     | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- $\Delta N$       | $\Delta N$ -f/TSS-r            | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- <i>N</i>         | <i>N</i> -f/ <i>N</i> -r       | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- $\beta JR$       | $\beta JR$ -f/ $\Delta P'$ -r  | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- <i>PPC</i>       | <i>PPC</i> -f/TSS-r            | pET26b- <i>pro-TSS</i>      | <i>Nde</i> I- <i>Xho</i> I    |
| pET26b- <i>pro-S257A</i> | S257A-f/S257A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-S257C</i> | S257C-f/S257C-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-E187A</i> | E187A-f/E187A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-N368D</i> | N368D-f/N368D-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-N370D</i> | N370D-f/N370D-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-D385A</i> | D385A-f/D385A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-D393A</i> | D393A-f/D393A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-D396A</i> | D396A-f/D396A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>pro-E402A</i> | E402A-f/E402A-r                | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- $\Delta N/S257A$ | S257A-f/S257A-r                | pET26b- $\Delta N$          |                               |
| pET26b- <i>GFP</i>       | <i>GFP</i> -f/ <i>GFP</i> -r   | pAD123                      | <i>Nde</i> I- <i>Hind</i> III |
| pET26b- <i>GFP-βJR</i>   | <i>GFP</i> -f/ <i>GFP'</i> -r  | pAD123                      | Seamless cloning              |
|                          | $\beta JR'$ -f/ $\beta JR'$ -r | pET26b- <i>pro-TSS</i>      |                               |
| pET26b- <i>GFP-PPC</i>   | <i>GFP</i> -f/ <i>GFP'</i> -r  | pAD123                      | Seamless cloning              |
|                          | <i>PPC'</i> -f/ <i>PPC'</i> -r | pET26b- <i>pro-TSS</i>      |                               |



**FIG S1** Distribution of acidic amino acid residues of protease TSS. The ribbon model of TSS was generated using SWISS-MODEL (<http://swissmodel.expasy.org>) with KP-43 (PDB ID: 1WMF) as the template. The side chains of Asp and Glu (red), as well as those of the catalytic triad D-H-S (blue) are indicated.



**FIG S2** Schematic representation of primary structure (A), photographic record (B) and SDS-PAGE analysis (C) of purified recombinant GFP, GFP-βJR, and GFP-PPC.



**FIG S3** Prepared FSC powder from fish scales.