

1 Supplementary1

(a)

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1MQLEIQVALN FIISYLYNKL PRRRVNIFGE ELERLLKKKY EGHWYPEKPY 50
                                     BoxA
51KSGGFRCIHI GEKVDPIEQ ASKESGLDID DVRGNLPQDL SVWIDPFVEVS100
                                     BoxB
101YQIGEKGPVK VLYVDDNEN GCELDKEIKN SFNPEAQVFM PISDPASSVS150
                                     BoxC
151SSPSPPFGHS AAVSPTFMPR STQPLTFTTA TFAATKFGST KMKNSGRSNK200
201VARTSPINLG LNVNDLLKQK AISSSMHSLY GLGLGSQQQPQQQQQPAQPP250
251PPPPPPQQQ QQKTSALSPN AKEFIFPNMQ GQGSSTNGMF PGDSPLNLSP300
301LQYSNAFDVF AAYGGLNEKS FVDGLNFSLN NMQYSNQQFQ PVMAN345

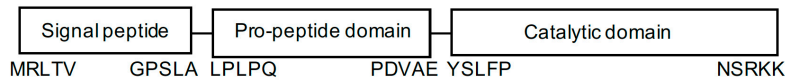
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(b)

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1 MRLTVLCAVC LLGPSLALPL PQEAGGMSEL QWEQAQDYLR FYLYDSETK 50
                                     heat
                                     APMA
                                     Trypsin 2
                                     Autoproteolysis
                                     Trypsin 1
51 NANSLEAKLK EMQKFFGLPI TGMLNSRVIE IMQKPRCGVP DVAEYSLFNP 100
                                     Pro-peptide domain
                                     Catalytic domain
101 SPKWTSKVVY YRIVSYTRDL PHITVDRLVS KALNMWVGKEI PLHFRKVVVWG150
151 TADIMIGFARG AHGDSYPFD GQGNLTAHAF APGTGLGGDA HFDEDERWTD 200
201 GSSLGINFLY AATHELGHSL GMPHSSDPNA VMYPTYGNGD PQNFKLSQDD 250
251 IKGIQKLYGK RSNSRKK 267

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4 **Supplementary data 1** Amino acid sequences of Tob1 and MMP-7

5 (a) Amino acid sequence of Tob 1

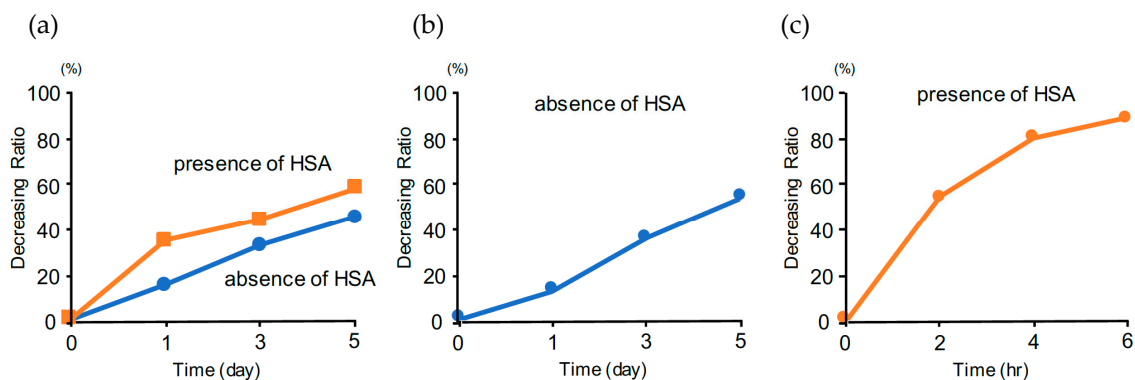
6 Three domains, BoxA, BoxB and BoxC, are highly conserved in the Tob/BTG family proteins.

7 (b) Amino acid sequence and domain structure of MMP-7

8 MMP-7 is the smallest molecule in the family and consists of a signal peptide, pro-peptide domain and
9 catalytic domain. The proteolytic activity of proMMP-7 was controlled by the cysteine-switch mechanism in
10 the non-active form. This mechanism disappeared after cleavage of the prodomain region of proMMP-7 by
11 heat, trypsin, APMA, and the active MMP-7 was produced from the cleavage by auto-catalysis.

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13 Supplementary2
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16 **Supplementary data 2** Auto-proteolytic activity of JAL-AK22 and JAL-TA9 in the presence or absence of HSA

17 (a) The auto-proteolytic activity of JAL-AK22 was determined by incubation at 37 °C in the presence or
18 absence of human serum albumin (HSA) in PBS. After incubation for 1, 3 or 5 days, the reaction mixture was
19 analyzed by HPLC. The amount of JAL-AK22 was plotted as the ratio between the final and original levels.

20 (b, c) The auto-proteolytic activity of JAL-TA9 was determined by incubation at 37 °C in the absence (b) or
21 presence (c) of human serum albumin (HSA) in PBS. The amount of JAL-TA9 was plotted between
22 the final and original levels.

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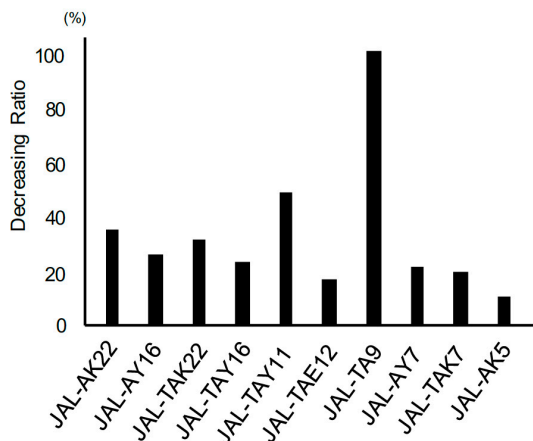
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27 Supplementary3

(a)

JAL-AK22	¹ K Y E G H W Y P E K P Y K G S G F R C I H I ²²
JAL-AY16	⁷ Y P E K P Y K G S G F R C I H I ²²
JAL-TAK22	¹ K Y E G H W Y P E K P Y K G S G F R M I H I ²²
JAL-TAY16	⁷ Y P E K P Y K G S G F R M I H I ²²
JAL-TAY11	¹² Y K G S G F R M I H I ²²
JAL-TAE12	⁹ E K P Y K G S G F R M I ²⁰
JAL-TA9	¹² Y K G S G F R M I ²⁰
JAL-AY7	¹² Y K G S G F R ¹⁸
JAL-TAK7	¹³ K G S G F R M ¹⁹
JAL-AK5	¹³ K G S G F ¹⁷

(b)



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30 **Supplementary data 3** Structure-activity relationship of JAL-TA9 derivative peptides

31 (a) Amino acid sequences of synthetic peptides derived from Tob1 BoxA domain

32 Our purpose in this study is not to investigate the unknown biological function of Tob1 protein, but to
 33 prove the proteolytic activity of synthetic peptide. Thus, we tested the auto-proteolytic activity of various
 34 peptides (1a), because we have not found any information about the substrate of the synthetic peptide.

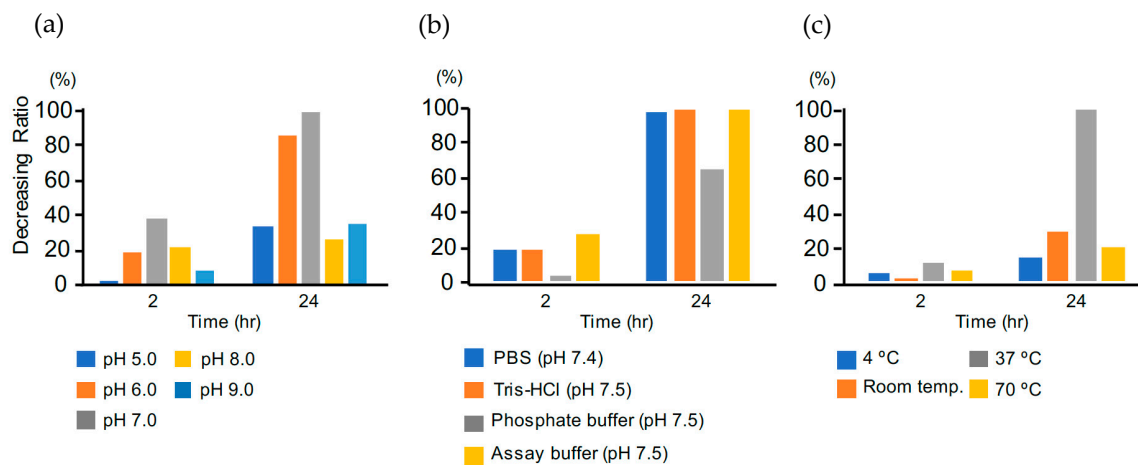
35 (b) Auto-proteolytic activity of various synthetic peptides

36 The decreasing ratio was calculated by the comparison of peak heights on HPLC chromatogram on day 0
 37 and day 5. In the results, JAL-TA9, in which Cys residue in nature fragment peptide was substituted to Met
 38 residue, showed the most potent auto-proteolytic activity. On the other hand, JAL-AK5, which concluded SG
 39 sequence, did not show the potent auto-proteolytic activity. These data indicate that SG sequence is not
 40 enough for the auto-proteolytic activity and some secondary structure may be necessary for enzymatic activity.

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42 Supplementary4

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45 **Supplementary data 4** Determination of the optimal conditions for the auto-proteolytic activity of JAL-TA946 The auto-proteolytic activity was examined in the presence of HSA according to the same manner described in
47 Supplementary data 2.

48 (a) pH dependence

49 The optimal pH was examined in 200 mM phosphate solution, and JAL-TA9 showed the highest activity
50 at pH 7.0.

51 (b) Effects of various buffers

52 JAL-TA9 showed similar auto-proteolytic activity in PBS (pH 7.4), Tris-HCl and assay buffer, which was
53 higher than that observed in Phosphate buffer.

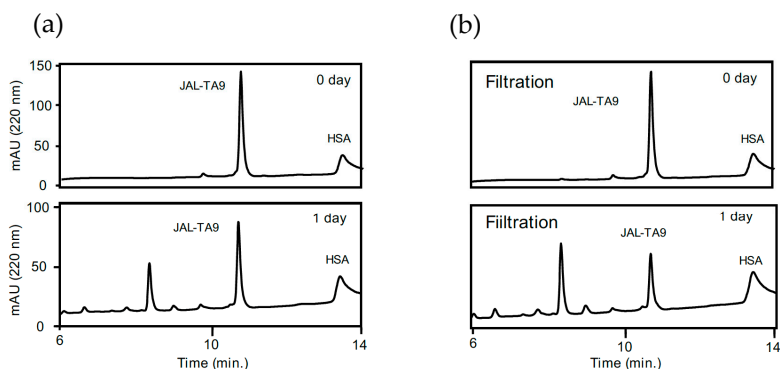
54 (c) Temperature

55 The optimal temperature was examined in PBS (pH 7.4), and JAL-TA9 showed the highest
56 auto-proteolytic activity at 37 °C.

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58 Supplementary5

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61 **Supplementary data 5** Auto-proteolytic activity of JAL-TA9 with or without filtration

62 (a) Auto-proteolytic activity of JAL-TA9 without filtration

63 The reaction mixture of JAL-TA9 was analyzed by an analytical HPLC (upper). The new peaks were
64 identified as the fragment peptides derived from JAL-TA9 after incubation for 1 day (lower).

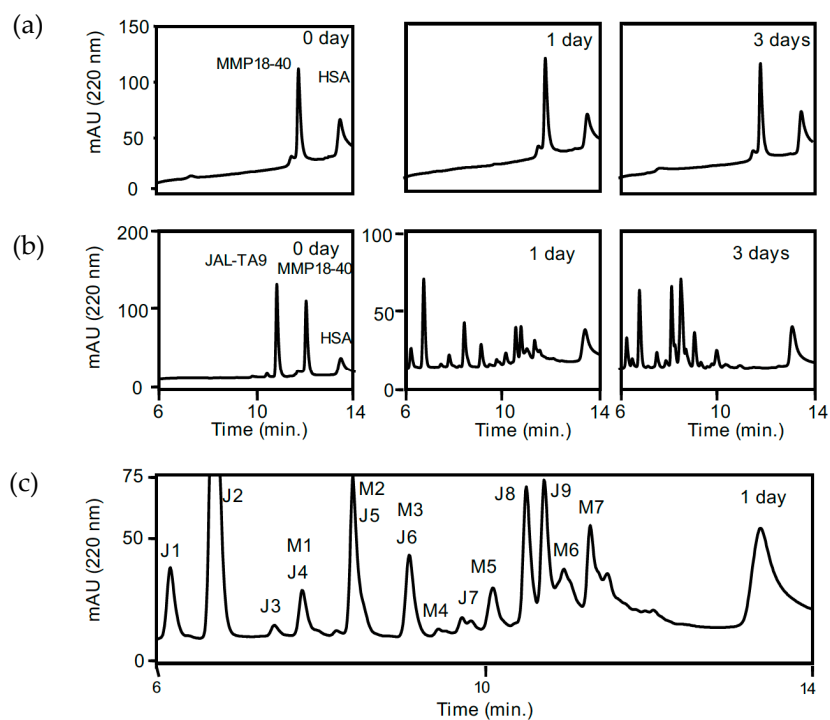
65 (b) Auto-proteolytic activity of JAL-TA9 with filtration

66 The reaction mixture was filtered using MILLEX-GV (Millipore, 0.22um Filter Unit) and TERUMO
67 Syringe (TERUMO, 26G, 1 mL) to remove contaminants and then analyzed by the same manner to the reaction
68 mixture without filtration (upper). The chromatograms obtained after incubation for 1 day were the same to
69 those obtained without filtration (lower). These data proved that the auto-proteolytic activity of JAL-TA9 was
70 not due to contamination of some bacteria.

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73 Supplementary6



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75 **Supplementary data 6** Cleavage reaction of MMP-7 fragment peptides by JAL-TA9

76 (a) Time dependent analysis of MMP18-40

77 (b) Time dependent analysis of the reaction mixture of JAL-TA9 and MMP18-40

78 (c) Peak collection of the reaction mixture of JAL-TA9 and MMP18-40

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80 **Supplementary data 7** Identification of cleavage sites

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Peak	Fragment	Theoretical MS	Experimental MS
M1	LPQ	356.21	356.2464
M2	AQDY	495.20	494.3074
M3	EQAQDYLK	993.48	933.6076
M4	QAQDYLK	864.43	864.5859
M5	LPLPQ	566.34	566.4081
M5	WEQAQDYLK	1179.56	1179.6898
M6	LPLPQEAG	710.36	709.4516
M6	LPLPQEA	766.42	766.4808
M7	EAGGMSELQWEQAQDYLK	2081.94	2082.1584

86 Supplementary8

87 **Supplementary data 8** Identification of the complex with JAL-TA9 and AEBSF

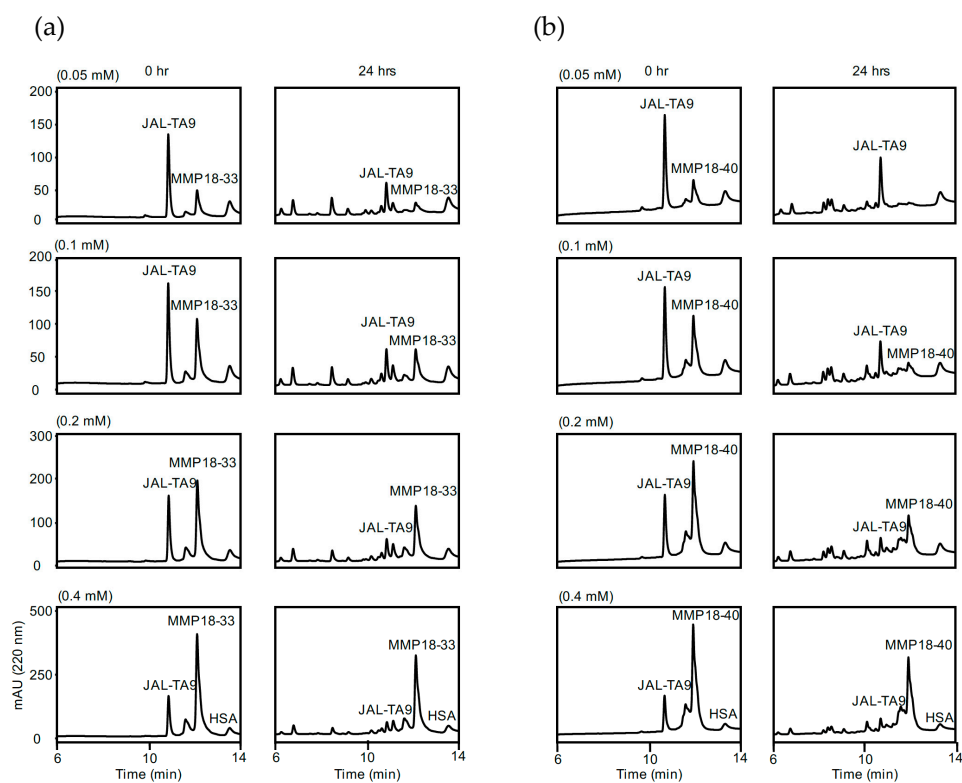
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Peak	Fragment	Theoretical MS	Experimental MS
C1	YKGS ₂ (AEBSF) GFRMI	1240.76	1240.7322
C2	YKGS ₂ (AEBSF) GFRMI	1240.76	1240.7325
C3	MMP 18-40 (AEBSF)	2813.49	2813.6094

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91 Supplementary9



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96 **Supplementary data 9** Kinetic parameters

97 (a) Chromatogram of the reaction mixture of JAL-TA9 and MMP18-33

98 (b) Chromatogram of the reaction mixture of JAL-TA9 and MMP18-40

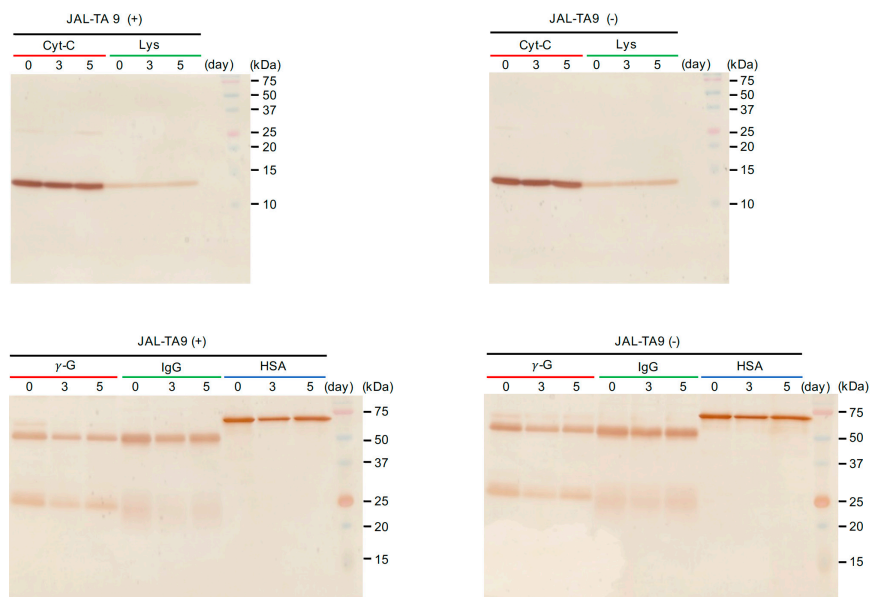
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101 Supplementary10

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105 **Supplementary data 10** Proteolytic activity of JAL-TA9 against native proteins

106 First, 0.2 mM JAL-TA9 was incubated with 0.01 % of various proteins at 37 °C in PBS. Next, 10 mL of the
107 reaction mixture was mixed with 2x sample buffer (10 μ L) and then boiled for ten min. SDS-PAGE was carried
108 out using 12.5 or 15 % polyacrylamide gel in a Tris-glycine buffer system, followed by silver staining. JAL-TA9
109 did not cleave g-globin (g-G), rabbit immunoglobulin G (IgG), cytochrome C (Cyt-C), lysozyme (Lys) or
110 human serum albumin (HAS).

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