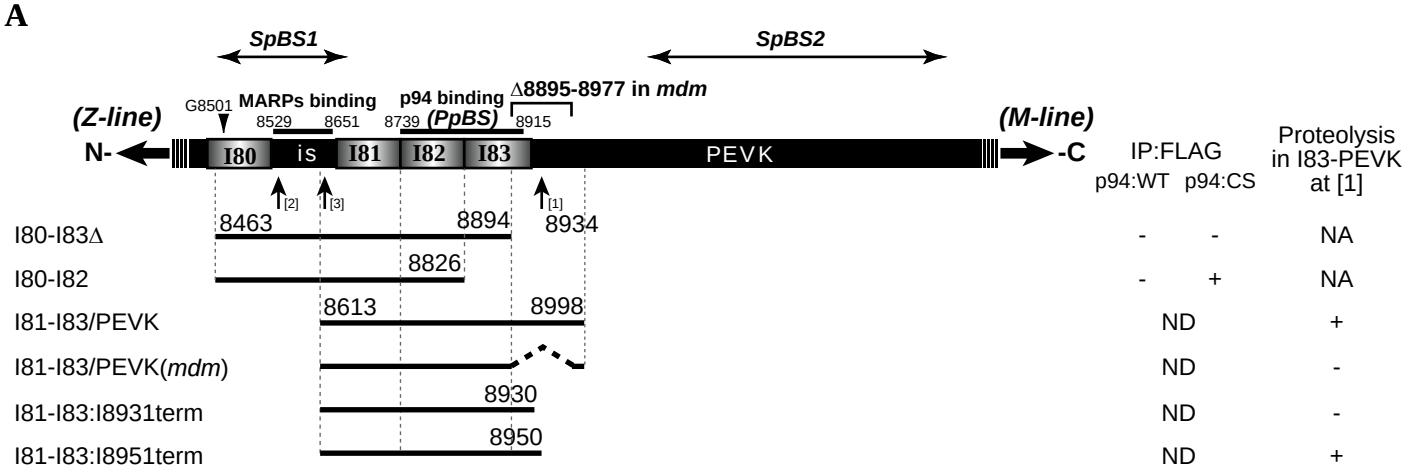
**M7:08262**

Legends for Supplementary data Hayashi *et al.*,

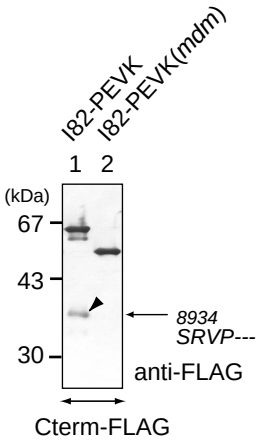
**Fig. S1. Expression and proteolysis of connectin/titin fragments.** *A*, Additional N2A-PEVK connectin/titin constructs used in this study (and not included in Fig. 1) are shown. *B*, I82-PEVK-FLAG became resistant to proteolysis by endogenous protease(s) in COS7 cells when the *mdm* deletion was introduced. The N-terminus of the C-terminal proteolyzed fragment (closed arrowhead) was determined as Ser8934 by aa sequence. *C*, A series of connectin fragments shown in *A* was expressed in COS7 cells. Generation of proteolyzed fragments were observed for I81-I83/PEVK and I81-I83:I8951term, but not for I81-I83/PEVK(*mdm*) or I81-I83:I8931term, confirming proteolysis at the N-terminus of Ser8934.

**Fig. S2. Properties of p94:DA and the effects of its protease activity on MARPs.** *A*, The lysate of Sf-9 cells expressing p94:DA or p94:CS was incubated in the presence of 1mM PMSF at 37$^{o}$C under the indicated conditions. Note that p94:DA, but not p94:CS, showed $Ca^{2+}$-dependent, leupeptin/E64c-sensitive, and calpastatin-resistant autolysis. *B*, p94:DA was expressed with (lanes 1 and 3) or without (lanes 2 and 4) His-FLAG-I80-PEVK, and immunoprecipitated with anti-FLAG. We noted that the full-length p94 (*open arrowhead*) was preferentially coprecipitated with I80-PEVK, similar to the result obtained with WT (see Fig. 1B and 5A). *C*, The lysate of Sf-9 cells, either uninfected, expressing p94:DA, or p94:CS, was incubated with recombinant His-MARP2 in the presence of 5 mM $CaCl_2$ and 1mM PMSF at 37$^{o}$C under the conditions indicated. Proteolysis of MARP2 was observed only with p94:DA, which coincided with autolysis of p94:DA and was inhibited by leupeptin/E64c/calpastatin, but not by calpastatin alone. *Open* and *closed arrowheads* indicate the full-length and autolyzed fragments, respectively.

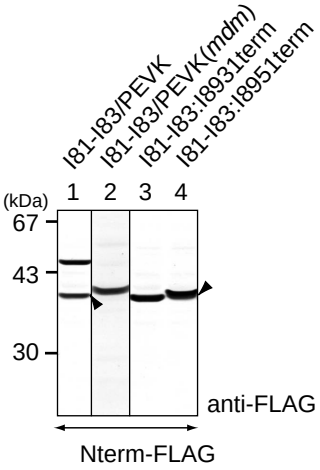
**A**


SpBS1
SpBS2
(Z-line)
G8501
MARPs binding
8529
8651
p94 binding
8739
(PpBS)
Δ8895-8977 in mdm
8915
(M-line)
N-
I80
is
I81
I82
I83
PEVK
-C
[2]
[3]
[1]
8463
8894
8934
8826
8613
8998
8930
8950
I80-I83Δ
I80-I82
I81-I83/PEVK
I81-I83/PEVK(mdm)
I81-I83:I8931term
I81-I83:I8951term
IP:FLAG
p94:WT
p94:CS
Proteolysis in I83-PEVK at [1]
- - NA
- + NA
ND +
ND -
ND -
ND +

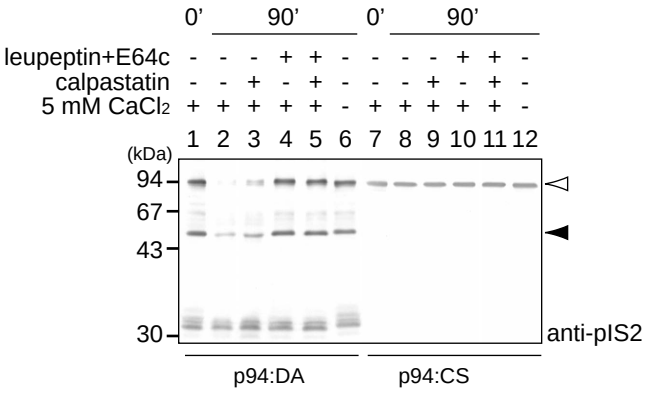
**B**


I82-PEVK
I82-PEVK(mdm)
1 2
(kDa)
67
43
30
8934
SRVP---
anti-FLAG
Cterm-FLAG

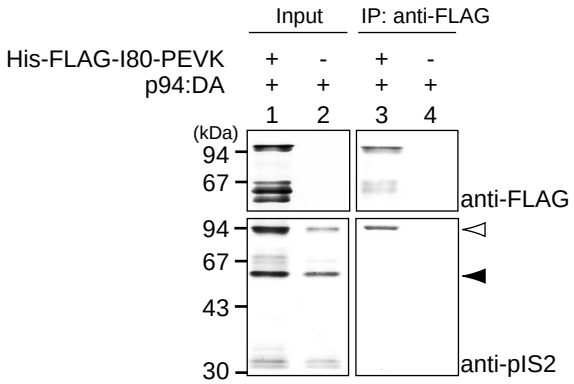
**C**


I81-I83/PEVK
I81-I83/PEVK(mdm)
I81-I83:I8931term
I81-I83:I8951term
1 2 3 4
(kDa)
67
43
30
anti-FLAG
Nterm-FLAG

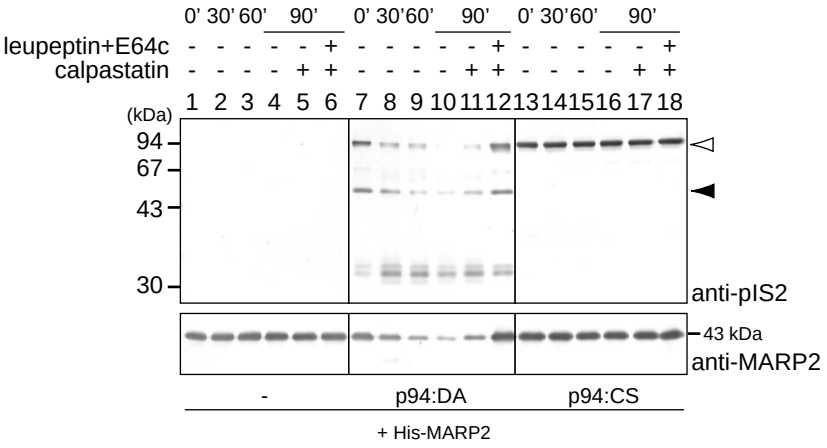
**A**


0’ 90’ 0’ 90’
leupeptin+E64c - - - + + - - - - + + -
calpastatin - - + - + - - - + - + -
5 mM CaCl2 + + + + + - + + + + + -
1 2 3 4 5 6 7 8 9 10 11 12
(kDa)
94
67
43
30
anti-pIS2
p94:DA
p94:CS

**B**


Input
IP: anti-FLAG
His-FLAG-I80-PEVK + - + -
p94:DA + + + +
1 2 3 4
(kDa)
94
67
anti-FLAG
94
67
43
30
anti-pIS2

**C**


0’ 30’ 60’ 90’ 0’ 30’ 60’ 90’ 0’ 30’ 60’ 90’
leupeptin+E64c - - - - - + - - - - - + - - - - - +
calpastatin - - - - + + - - - - + + - - - - + +
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
(kDa)
94
67
43
30
anti-pIS2
43 kDa
anti-MARP2
-
p94:DA
p94:CS
+ His-MARP2