Supplementary Figures

Inhibition of the signal peptidase complex blocks cleavage of HTLV-1 ORFI encoded p12 to p8 and impairs virus transmission

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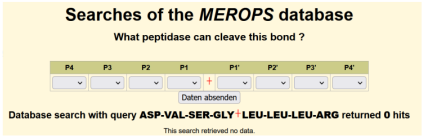
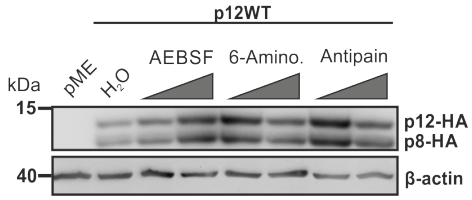
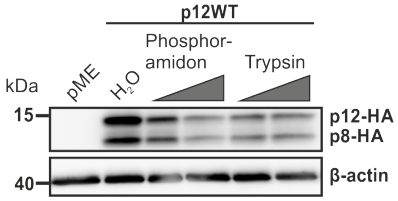
A


Searches of the MEROPS database
What peptidase can cleave this bond ?
P4 P3 P2 P1 P1' P2' P3' P4'
Daten absenden
Database search with query ASP-VAL-SER-GLY↑LEU-LEU-LEU-ARG returned 0 hits
This search retrieved no data.

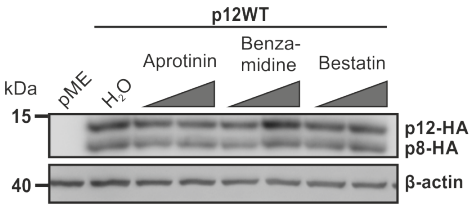
B


p12WT
AEBSF
6-Amino.
Antipain
kDa
pME
H2O
15
40
p12-HA
p8-HA
β-actin

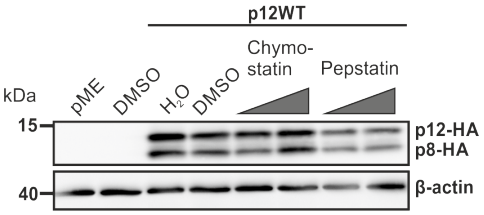
C


p12WT
Phosphor-
amidon
Trypsin
kDa
pME
H2O
15
40
p12-HA
p8-HA
β-actin

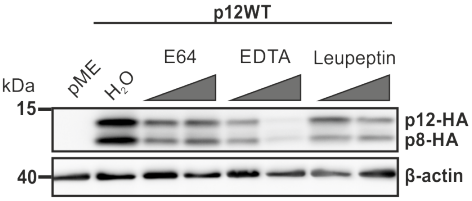
D


p12WT
Aprotinin
Benza-
midine
Bestatin
kDa
pME
H2O
15
40
p12-HA
p8-HA
β-actin

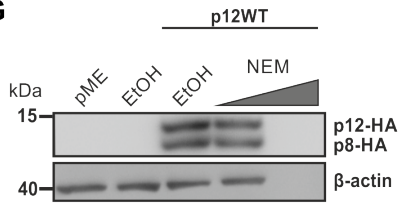
E


p12WT
Chymo-
statin
Pepstatin
kDa
pME
DMSO
H2O
DMSO
15
40
p12-HA
p8-HA
β-actin

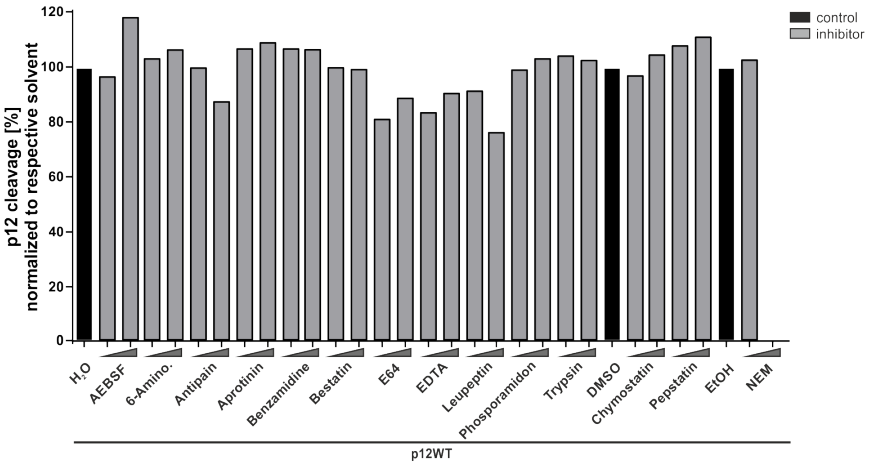
F


p12WT
E64
EDTA
Leupeptin
kDa
pME
H2O
15
40
p12-HA
p8-HA
β-actin

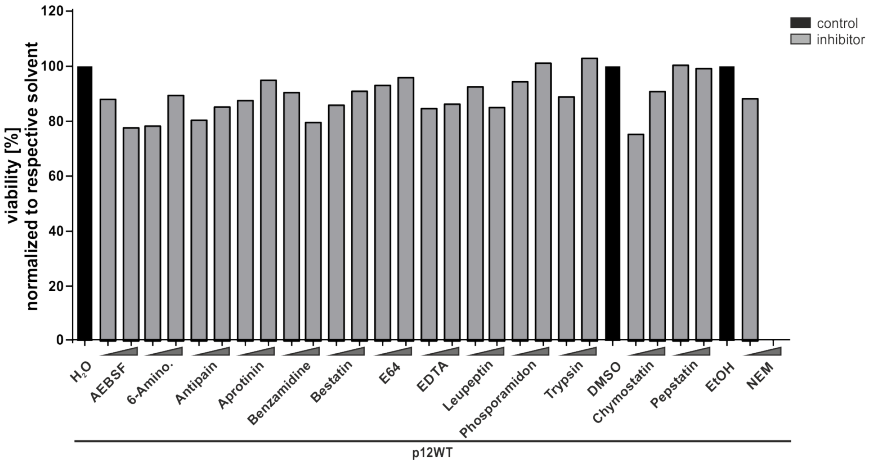
G


p12WT
NEM
kDa
pME
EtOH
EtOH
15
40
p12-HA
p8-HA
β-actin

H

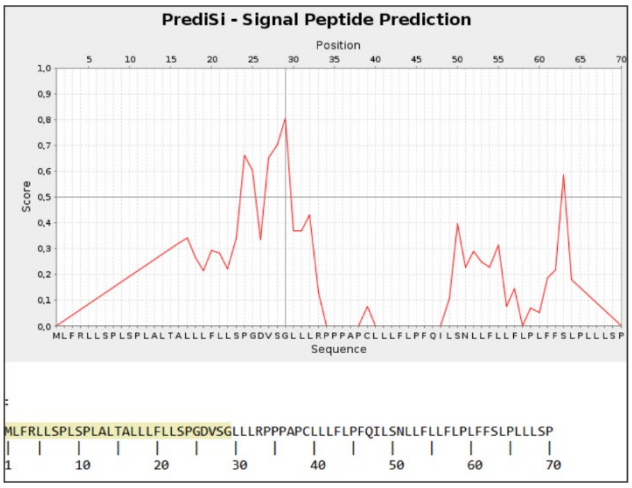

control
inhibitor
p12 cleavage [%]
normalized to respective solvent
0
20
40
60
80
100
120
H2O
AEBSF
6-Amino.
Antipain
Aprotinin
Benzamidine
Bestatin
E64
EDTA
Leupeptin
Phosporamidon
Trypsin
DMSO
Chymostatin
Pepstatin
EtOH
NEM
p12WT

I


control
inhibitor
viability [%]
normalized to respective solvent
0
20
40
60
80
100
120
H2O
AEBSF
6-Amino.
Antipain
Aprotinin
Benzamidine
Bestatin
E64
EDTA
Leupeptin
Phosporamidon
Trypsin
DMSO
Chymostatin
Pepstatin
EtOH
NEM
p12WT

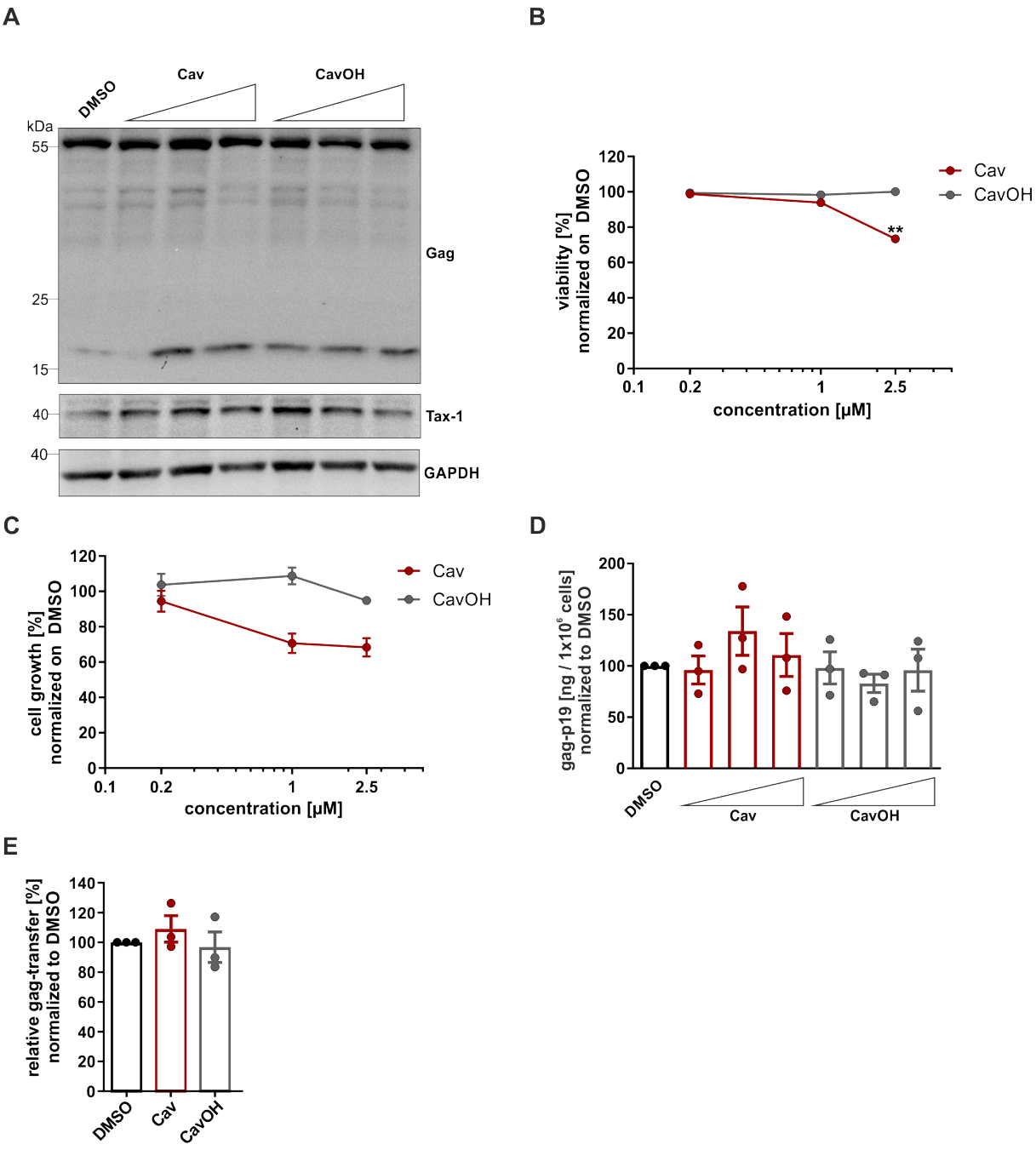
Supplementary Figure 1: Commercial protease inhibitors do not interfere with p12 cleavage in Jurkat.

(A) The p12 cleavage site was analysed with the MEROPS data base**. (B-I)** At 4 h prior electroporation, Jurkat T-cells were treated with two concentrations of one of the protease inhibitors (AEBSF: 0.01 mM, 0.1 mM; 6-Aminohexanoic acid: 0.025 mg/ml, 0.25 mg/ml; Antipain dihydrochloride: 1 µM, 100 µM; Aprotinin: 10 nM, 800 nM; Benzamidine: 0.5 mM, 4 mM; Bestatin: 4 µM, 40 µM; Chymostatin: 10 µM, 100 µM; E64: 1 µM, 10 µM; EDTA: 0.1 mM, 1 mM; NEM: 0.01 mM, 0.1 mM; Leupeptin: 10 µM, 100 µM; Pepstatin: 0.5 µg/ml, 1 µg/ml; Phosphoramidon: 1 µM, 10 µM; Trypsin: 10 µg/ml, 100 µg/ml). Next, cells were electroporated with 50 µg HA-tagged pME-p12WT or pME and treated with the respective protease inhibitor for 24 h. Cells were lysed with the respective protease inhibitor added to corresponding lysis buffer and analysed via **(B-G)** immunoblotting with anti-HA and anti-β-actin antibodies. **(H)** Densitometric analysis of p12 and p8 specific bands was performed. p12 cleavage was calculated as p8/(p12+p8) and normalized to respective solvent control. The mean of two independent experiments is depicted. **(I)** Cells were stained with propidium iodide (PI). Cell viability was analysed via flow cytometry and normalized to respective solvent control. The mean of two independent experiments is depicted.



Truncation:	70 residues
Cleavage position:	29
Score:	0.8058

Supplementary Figure 2: p12 is predicted to carry a signal peptide using PrediSi. The p12 wildtype (WT) sequence was analysed with the signal peptide prediction tool PrediSi.



Supplementary Figure 3: Inhibition of p12 cleavage does not affect HTLV-1 cell-to-cell transmission from C91-PL to Jurkat.

(A-D) Chronically HTLV-1 infected C91-PL cells were treated with DMSO or increasing doses (0.2 µM, 1 µM or 2.5 µM) of either Cav or CavOH. After 48 h of treatment, cells were analysed via **(A)** immunoblotting with anti-Gag, anti-Tax-1 and anti-GAPDH antibodies. One representative of three independent experiments is shown. **(B)** Cell viability was analysed via flow cytometry. **(C)** Cell growth was analysed via automated

cell counting. **(D)** Viral release was analysed via Gag-p19 ELISA. The means of three independent experiments ± SEM are depicted. **(E)** C91-PL were treated with DMSO, 1 µM Cav or CavOH for 24 h before co-culture with Jurkat-IRES-zsGreen cells. After 1 h, cells were fixed, stained intracellularly against Gag, and analysed via flow cytometry. Quantitative analysis of gag-p19-APC-positive cells normalized to DMSO control was conducted. The means of three independent experiments ± SEM are depicted. Data were analysed by Kruskal-Wallis test followed by Dunn's multiple comparisons correction. *$p<0.05$, **$p<0.01$.