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Electronic Supplementary Information

Hypoallergenic acid-sensitive modification preserves major mugwort allergen fold and delivers full repertoire of MHC class II-binding peptides during endolysosomal degradation

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[7] University of Belgrade, School of Medicine, Dr Subotica 8, Belgrade, Serbia
[8] University of Belgrade, Faculty of Biology, Studentski trg 16, Belgrade, Serbia
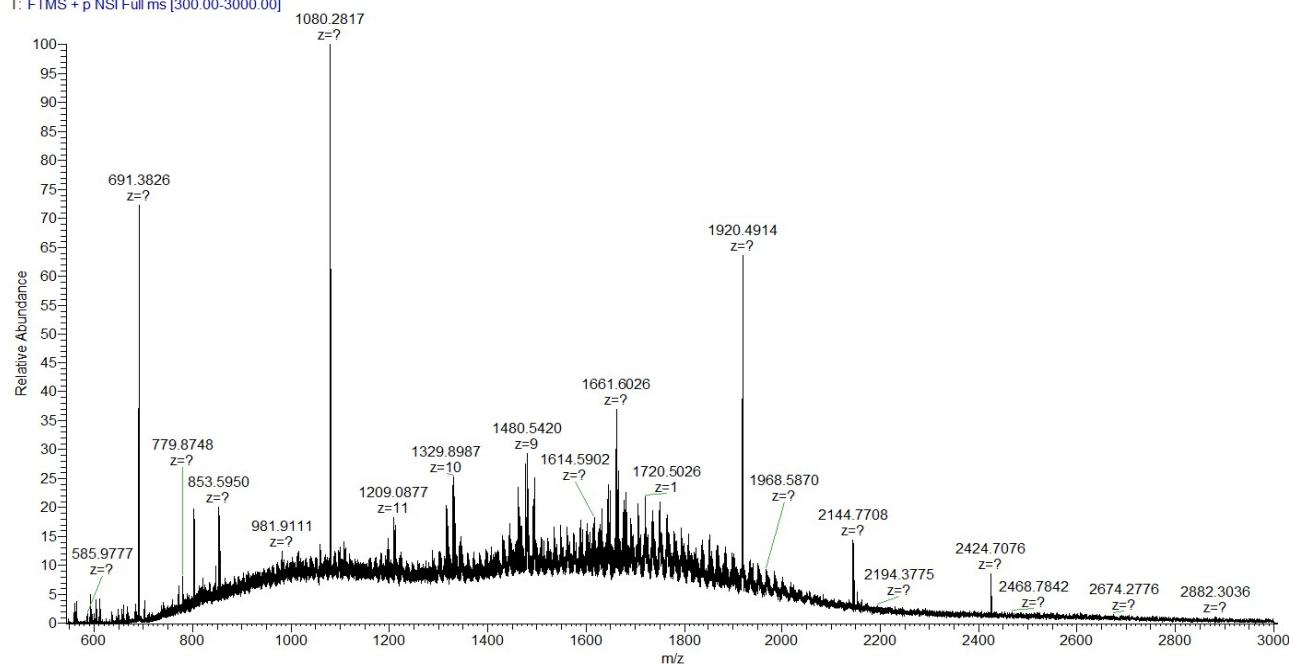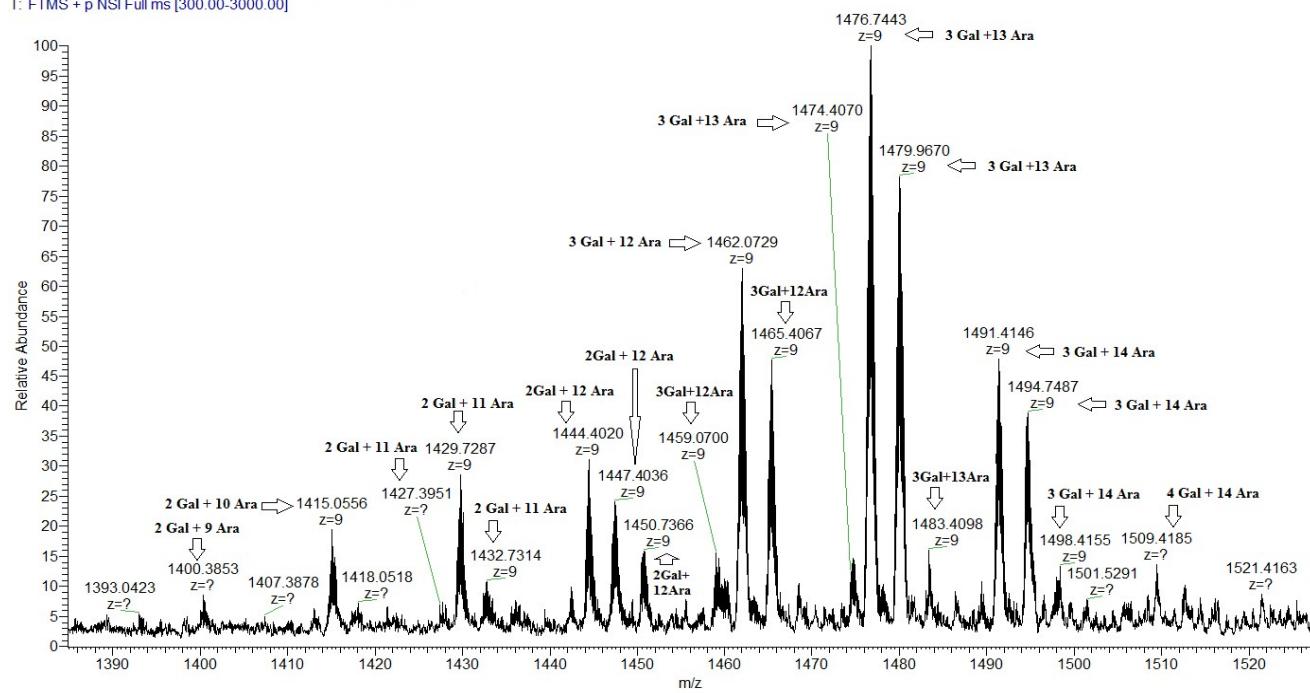
Figure S1. ESI-MS spectra of intact Art v 1. ESI-MS spectra at retention time (A) 41.83-49.88 and (B) 42.46-43-27seconds.

In mass spectra of native Art v 1 we have succeeded to resolve only few isoforms which are the most dominant individual isoforms in comparison to the rest of isoforms. Due to the extreme diversity, the rest of isoforms are represented individually in very low concentrations and therefore cannot be resolved from each other. According to the masses of resolved isoforms, ranging 12.5-13.5 kDa, they belong to Art v 1 fraction appearing as smaller set of peaks around 13 when analyzed by MALDI mass spectrometry.[2] In the study of Himly et al.[1] it was proposed that shorter set of isoforms (about 13 kDa) contain one short chain of arabinogalactan, while longer set of isoforms (14-16 kDa) contain longer one, or two, the same or different, arabinogalactan chains. From obtained mass signals with nArt v 1, they easily matched experimental masses in the range of 14-16 kDa with calculated molecular weights of Art v 1 proteins with one or two arabinogalactosyl chains, but they could not match obtained masses in the range 12.7-13.5 kDa with any of the calculated masses. However, in their further study these authors[2] proposed two models of glycan on Art v 1, the simple version having solely β-arabinose residues, which give a mass of 13 kDa, and the complex version, equipped additionally with an arabinogalactan polysaccharide resulting in a mass of 15 kDa. In contrast to their model, we have found isoforms (12.5-13.5 kDa) which contained arabinogalactan. Our isoforms contained 2-4 galactose , with far the most dominant glycans containing 3 galactose. These results are in accordance to the findings of these authors who detected dominant mass of trigalactosyl-Hyp and smaller peaks of additional di- and tetra-galactosyl series after basic and limited acid hydrlyisis of native Art v .[2] Our detected isoforms with 3 galactosyl residues contained in addition 12, 13 and 14 of arabinose residues, while for di-gaslactosyl and tetra- galactosyl series the number of arabinose was found to be 9-12 and 14, respectively. In comparison to MALDI-TOF, nLC -LTQ-Orbitrap method can much better provide resolved individual masses of such complex mixture of isoforms, not only averaged masses. For example, peaks originating from isoforms with the same glycan composition, but with small amino acid variations are resolved by nLC -LTQ-Orbitrap mass spectrometry (Fig S1). According to all these data it can be concluded that shorter Art v 1 isoforms (12.5-13.5 kDa) can bear arabinogalactan chain containing 2-4 galactose, with far dominant tri-galactosyl series. Of course, short isoforms containing solely β-arabinose are possibly present in non-resolved part of mass spectra and their existence cannot be excluded.
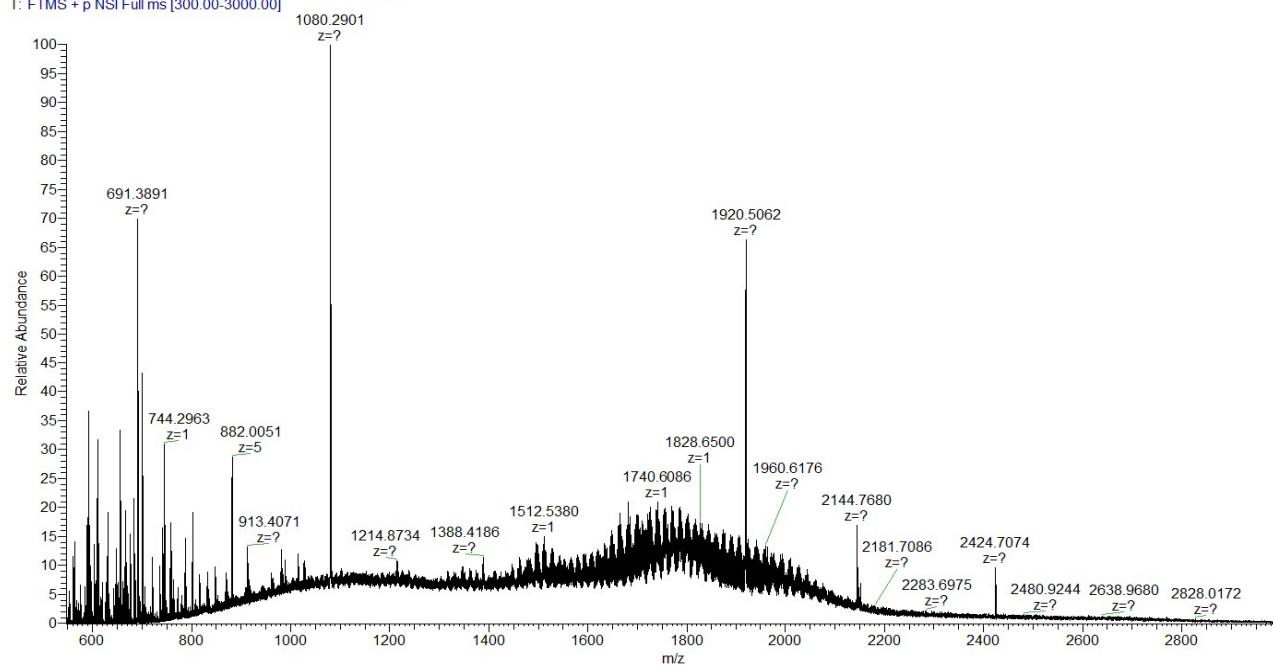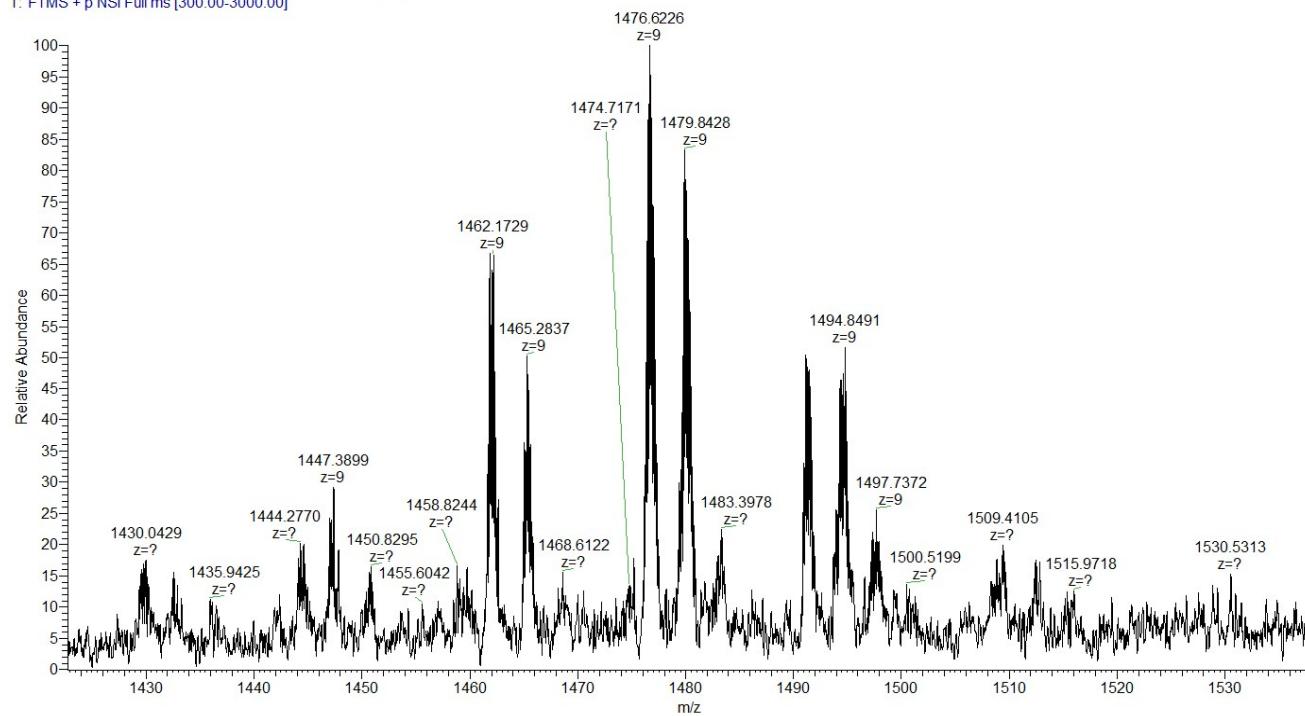
Figure S2. ESI-MS spectra of intact CAA. ESI-MS spectra at retention time (A) 39.84-52.24 and (B) 42.95-43-28 seconds. It has to be mentioned that, due to experimental conditions used during sample preparation and MS analysis, acyl groups of CAA were hydrolysed (1% formic acid) resulting in isoform profile identical to unmodified Art v 1.

A)

OB1196 #2320-3644 RT: 39.90-52.00 AV: 655 NL: 1.57E5
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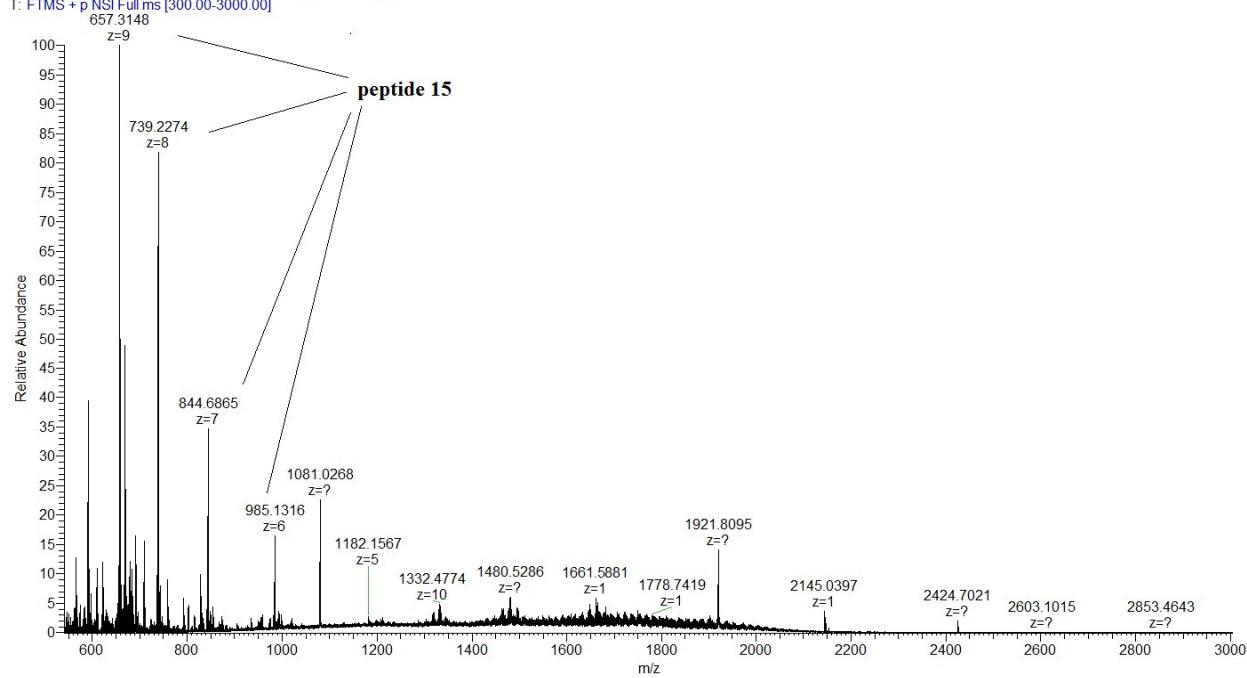
peptide 15

657.3148
z=9

739.2274
z=8

844.6865
z=7

1081.0268
z=?

985.1316
z=6

1182.1567
z=5

1332.4774
z=?

1480.5286
z=?

1661.5881
z=1

1778.7419
z=1

1921.8095
z=?

2145.0397
z=1

2424.7021
z=?

2603.1015
z=?

2853.4643
z=?

B)

OB1196 #2320-3644 RT: 39.90-52.00 AV: 655 NL: 1.57E5
T: FTMS + p NSI Full ms [300.00-3000.00]

peptide 15

657.3148
z=9

739.2274
z=8

669.1071
z=1

591.6840
z=10

597.7493
z=5

566.4296
z=2

621.3257
z=8

630.0851
z=1

691.8601
z=?

712.5086
z=7

758.2258
z=8

792.0848
z=6

828.0981
z=6

844.6865
z=7

853.5864
z=5

905.6832
z=2

935.1361
z=1

958.1560
z=1

985.1316
z=6

993.5145
z=5

1041.0352
z=?
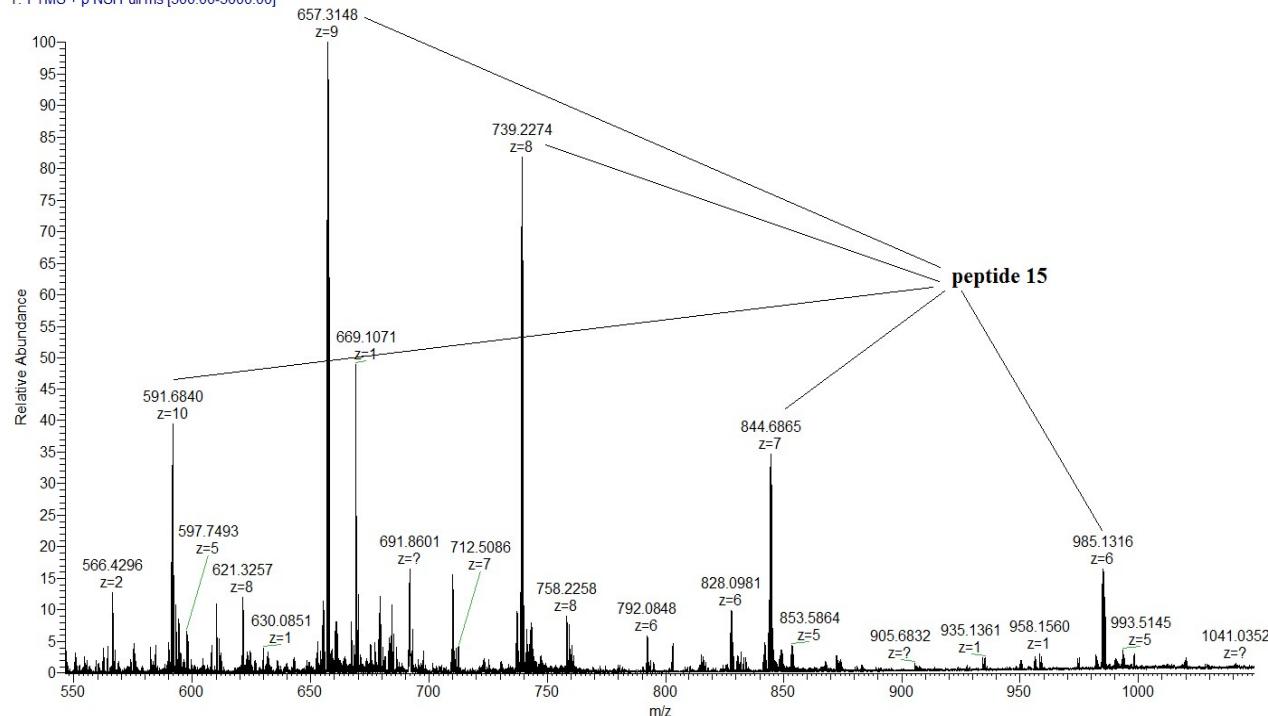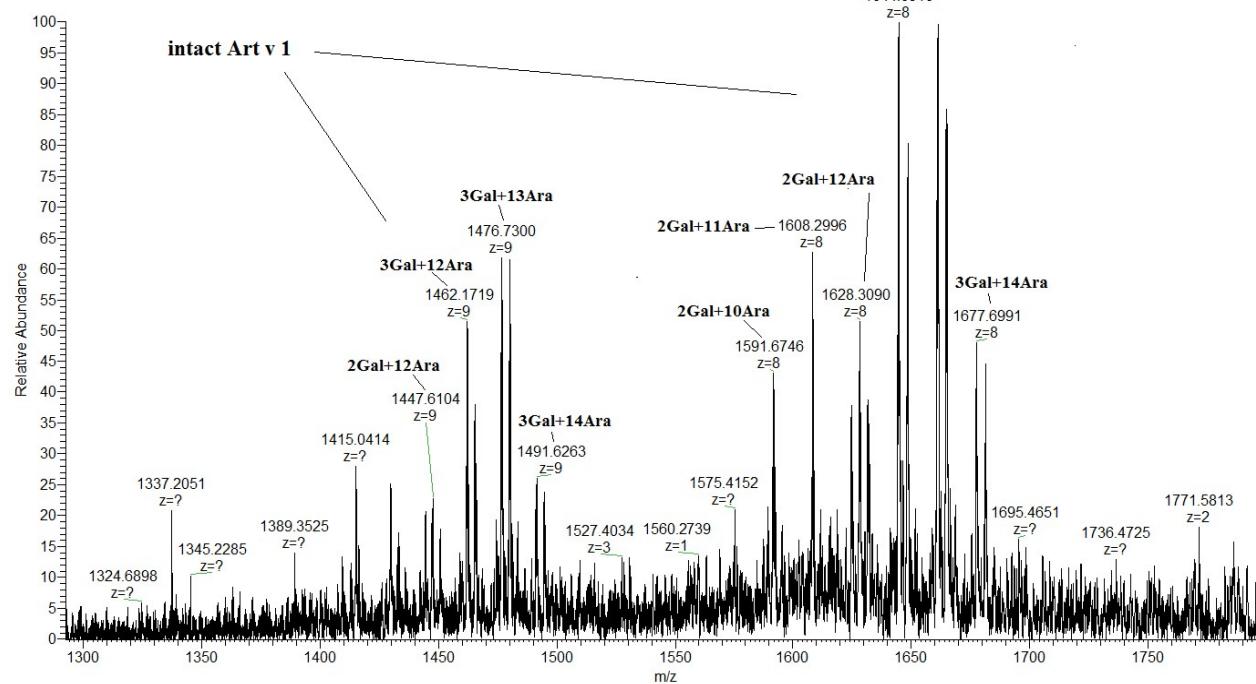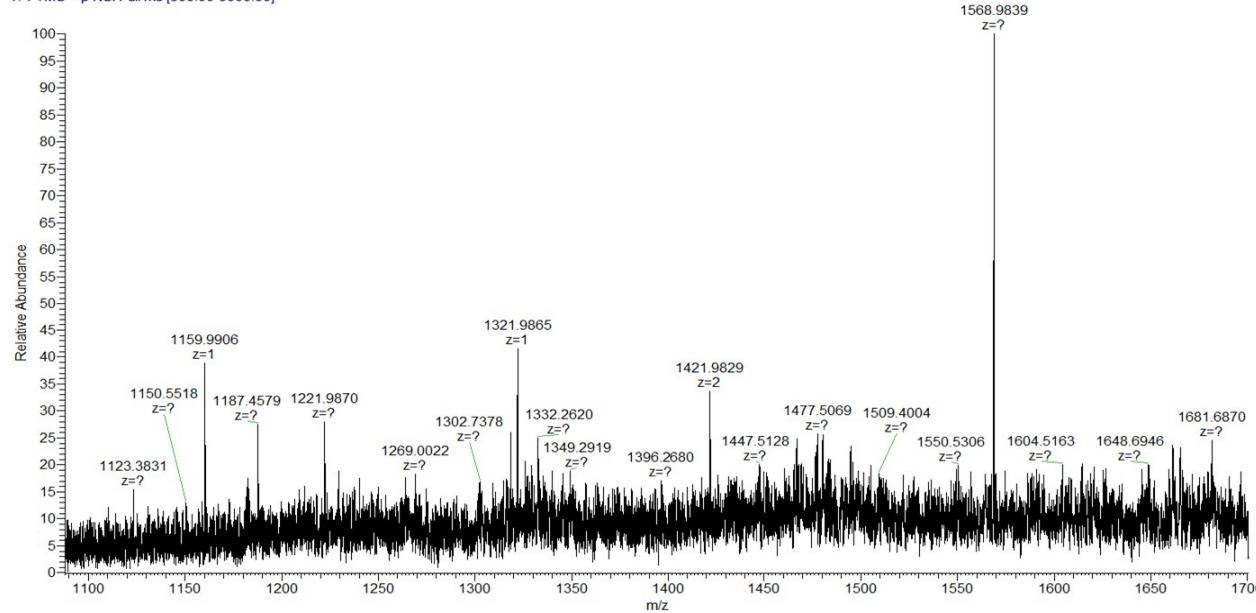
Figure S3. ESI-MS spectra of Art v 1 peptides released during 15 min of endolysosomal processing. ESI-MS spectra at retention time (A) 39.90-52.00, (B) 39.90-52.00 zoom out, (C) 41.72-42.09 and (D) 55.81-59.91 seconds. At 15 minute point, in Art v 1 sample far the most dominant peptide is peptide 15 (5909 Da). Therefore, it definitely originate from invariable part of all Art v 1 isoforms, e.g. defensin domen, and acctualy it consists of the whole defensin domen with additional starting N-terminal tripeptide. The most dominant individual isoforms of intact Art v 1 are still present.
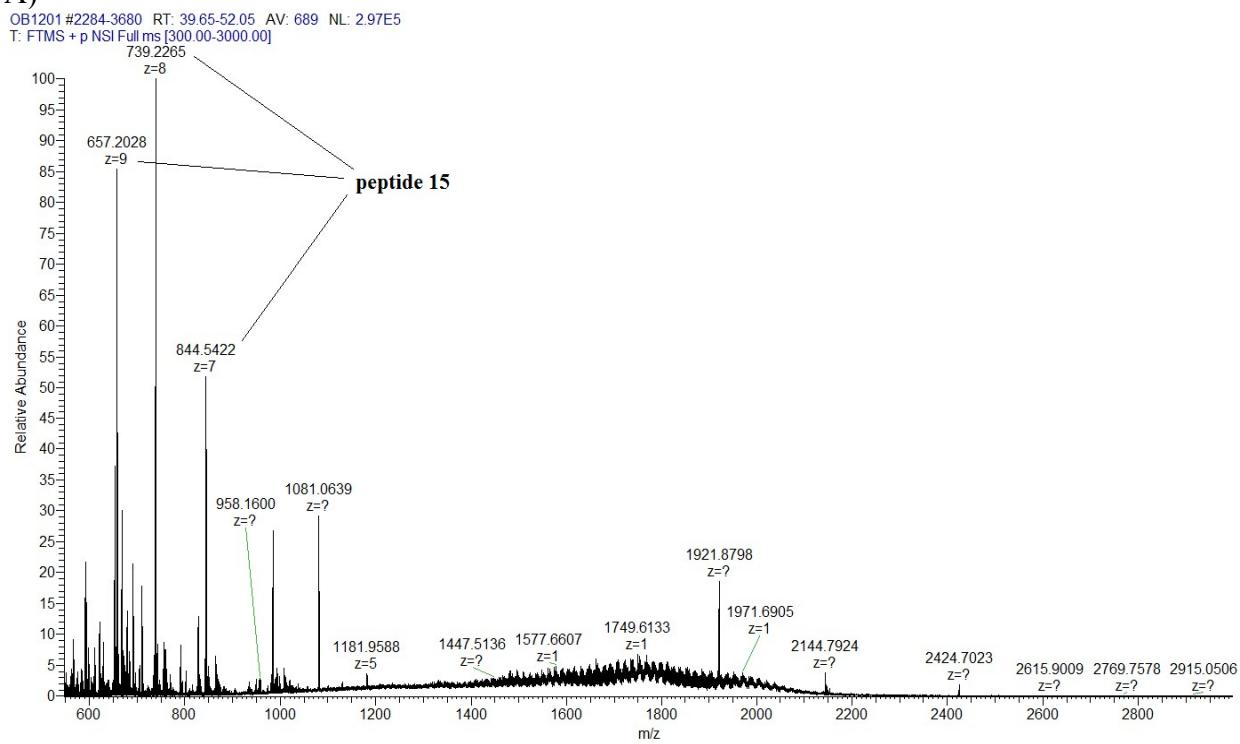
A)

B)
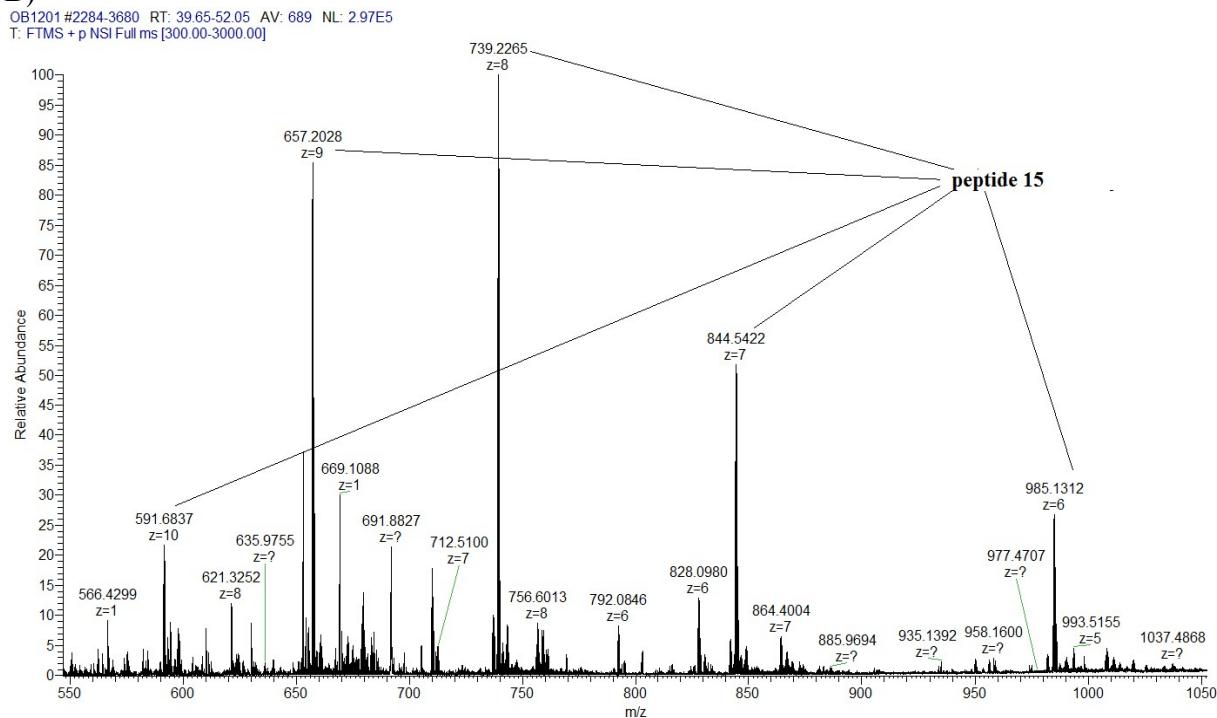
S7

C)

D)
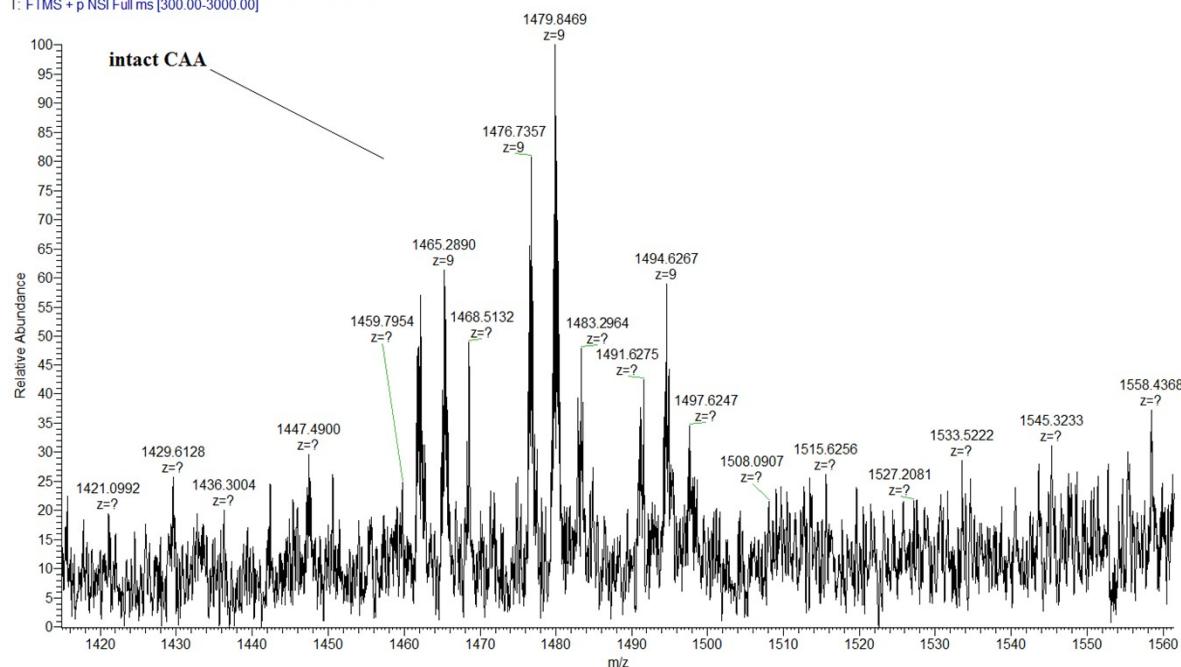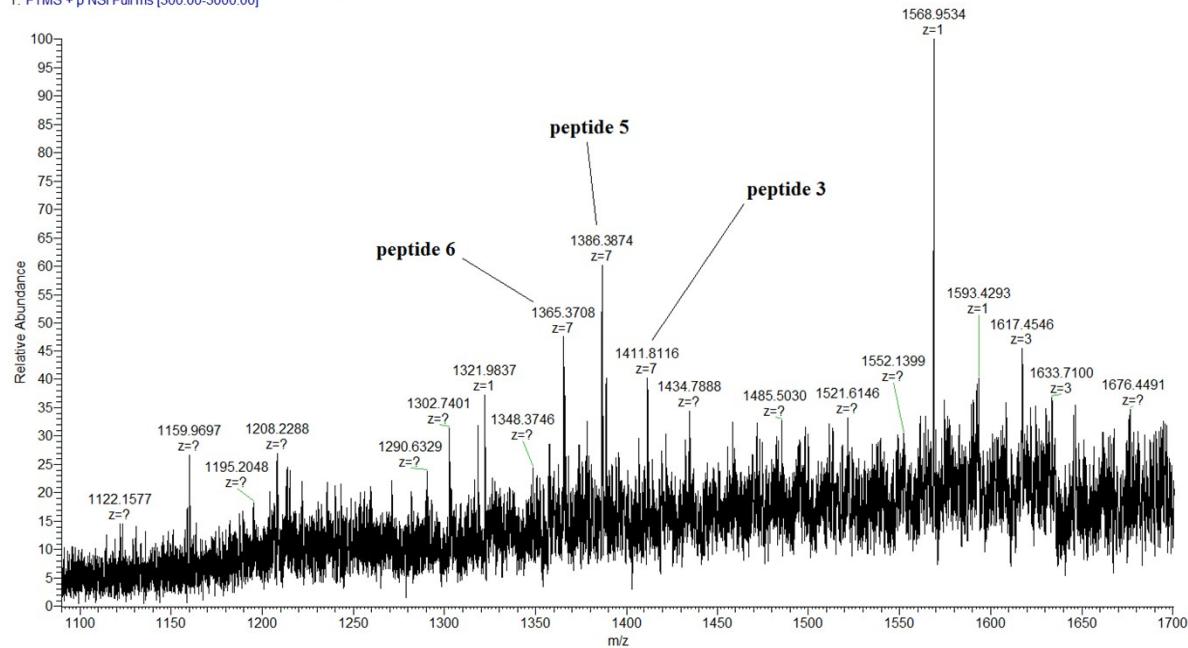
Figure S4. ESI-MS spectra of CAA peptides released during 15 min of endolysosomal processing. ESI-MS spectra at retention time (A) 39.65-52.05, (B) 39.65-52.05 zoom out, (C) 43.40-43.77 and (D) 55.99-59.93 seconds. Similary to Art v 1 sample, in CAA sample peptide 15 (5909 Da) is also far the most dominant at 15 minute point and the most dominant individual isoforms of intact CAA are still present. Also, CAA shortened by part of hydroxyproline domen (peptides 3, 5 and 6) are also present.

A)

OB1197 #2419-3967 RT: 39.91-52.30 AV: 649 NL: 2.01E5
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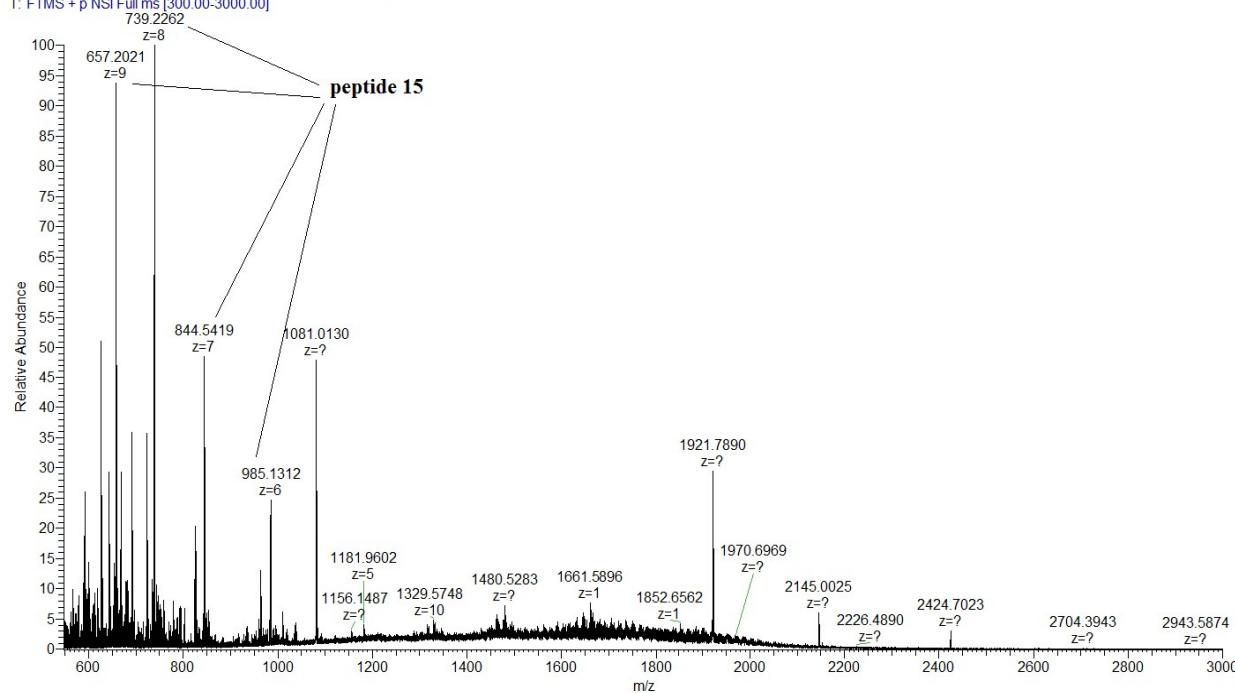
B)

OB1197 #2419-3967 RT: 39.91-52.30 AV: 649 NL: 2.01E5
T: FTMS + p NSI Full ms [300.00-3000.00]
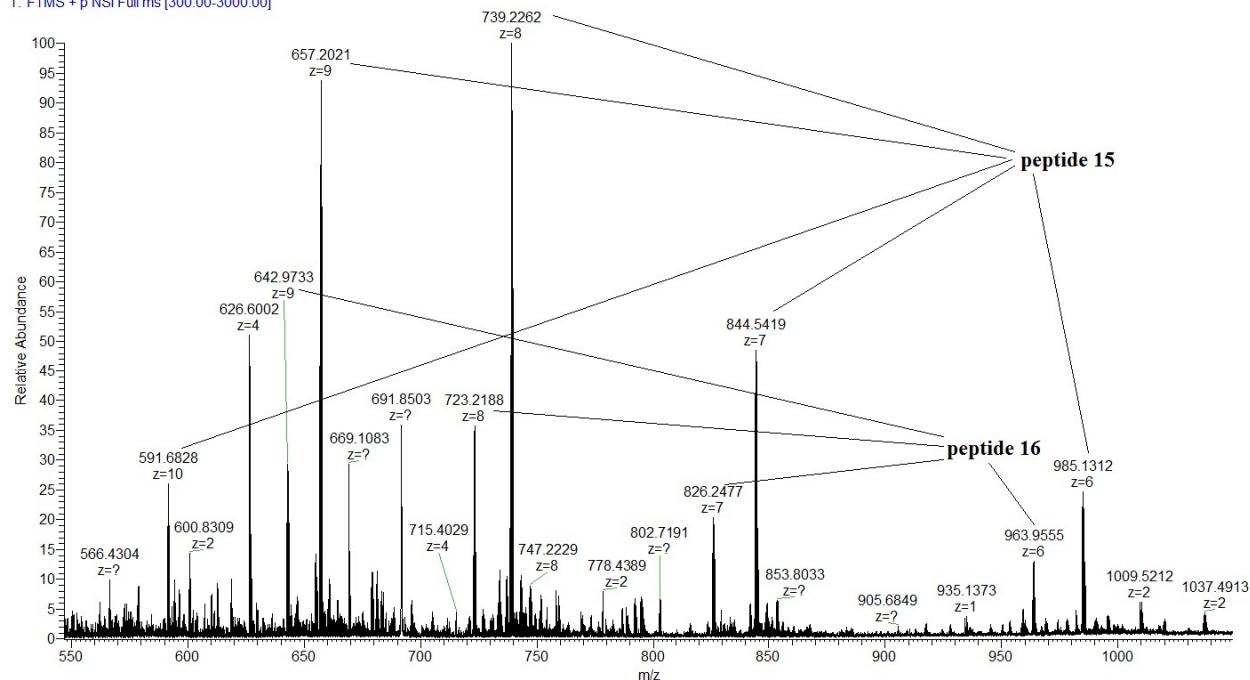
S9
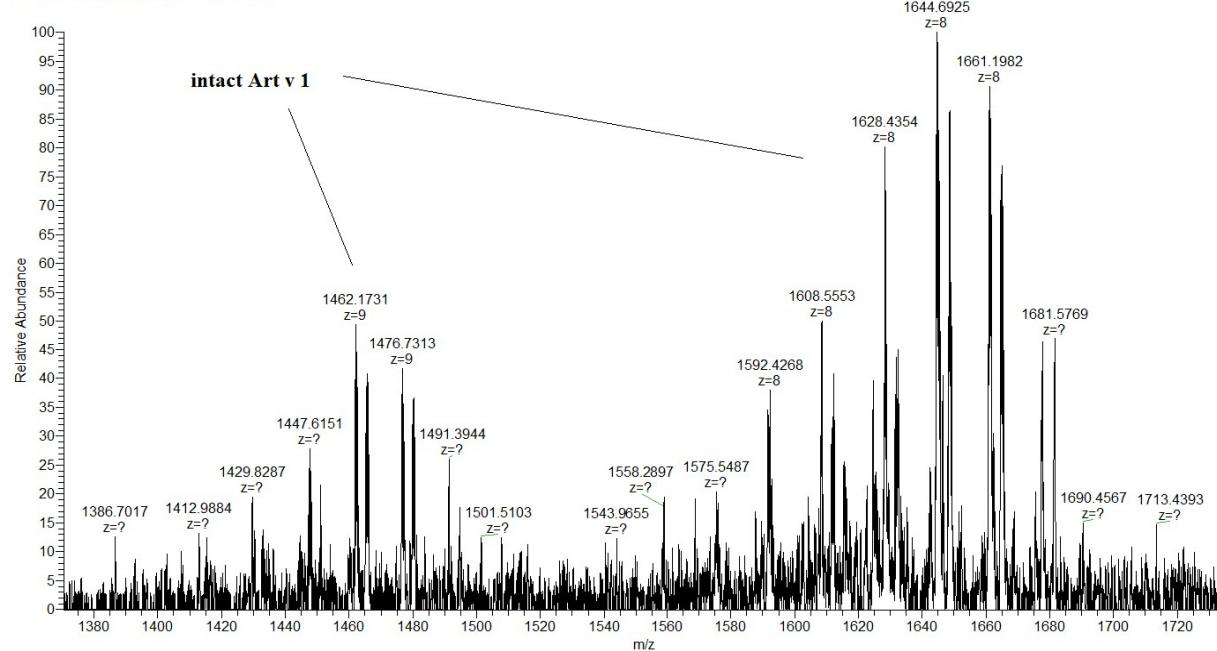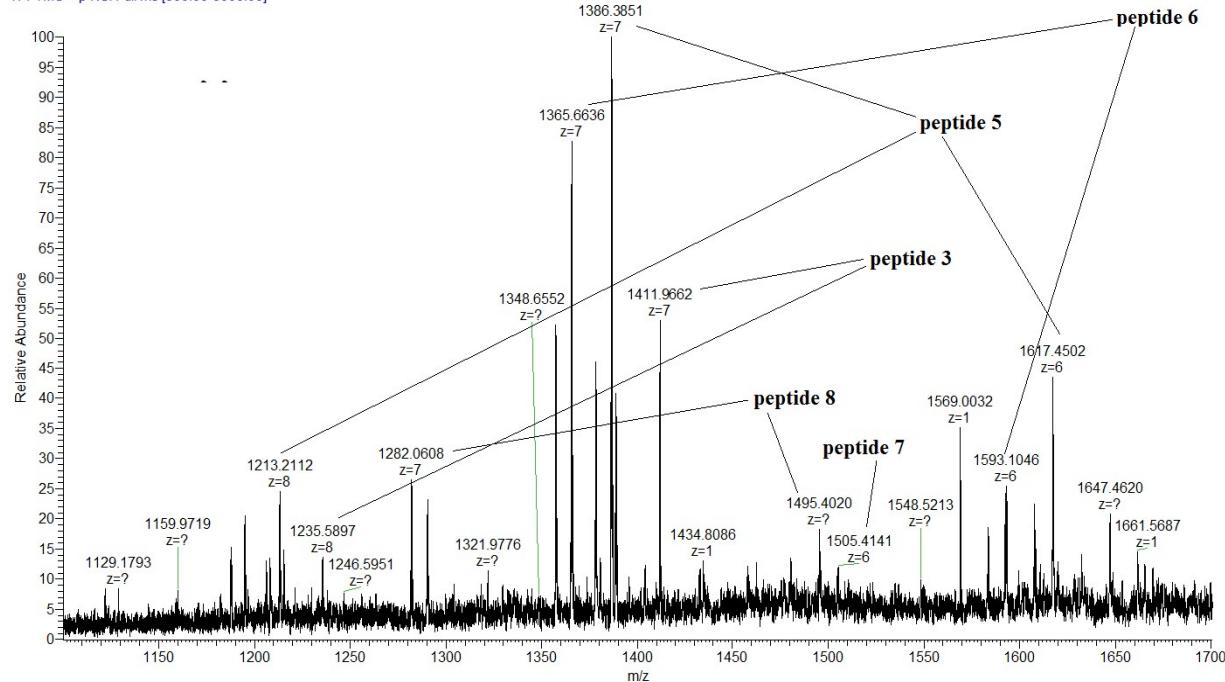
Figure S5. ESI-MS spectra of Art v 1 peptides released during 3 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.91-52.30, (B) 39.91-52.30 zoom out, (C) 41.97-42.20 and (D) 56.05-60.11 seconds. After 3h of degradation peptide 15 is still the most dominant , but appereance of aboundant peptide 16 (5781 Da) can be also observed. Peptide 16 is peptide 15 shortened by starting N-terminal dipeptide Ala-Gly. Dominant isoforms of intact Art v 1 are still present. In addition to peptides 3, 5 and 6, shorted peptides (peptides 7 and 8) appear.
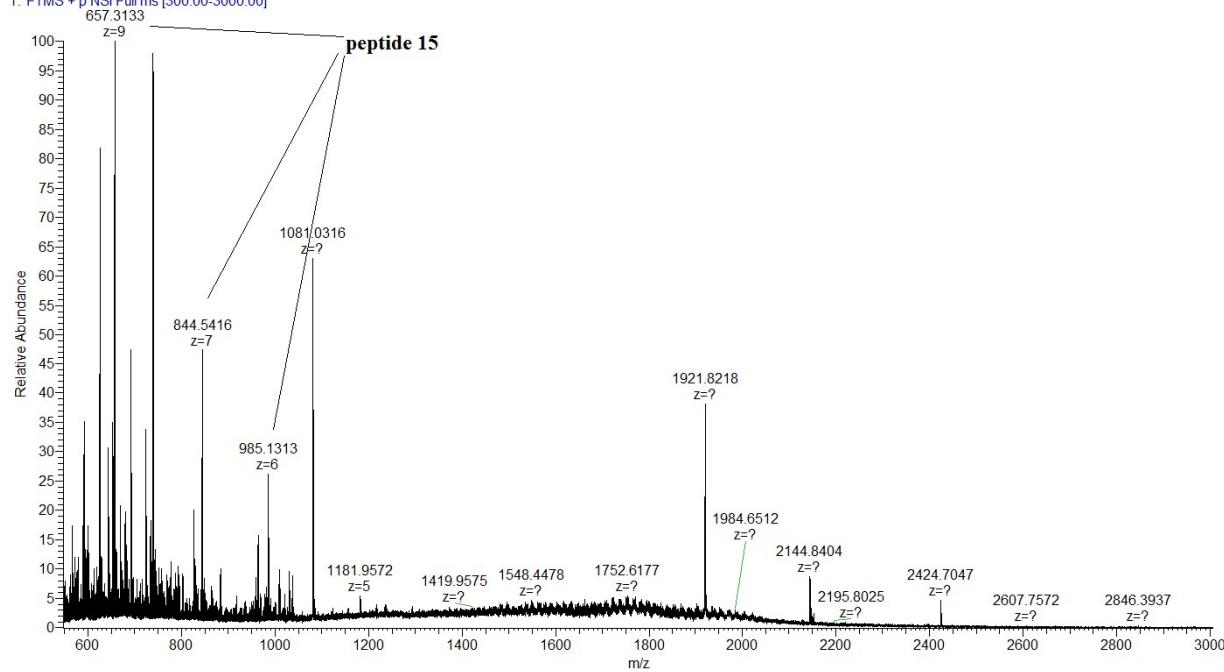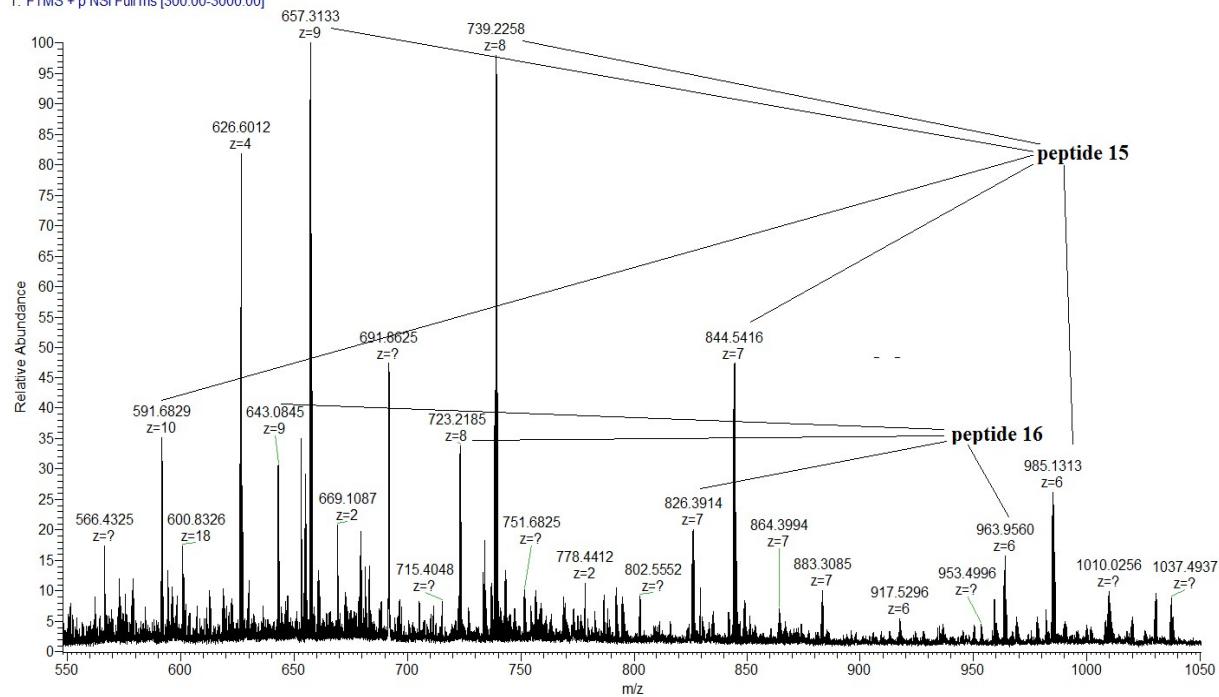
C)

OB1202 #2519-2588   RT: 41.64-42.33   AV: 34   NL: 4.03E2
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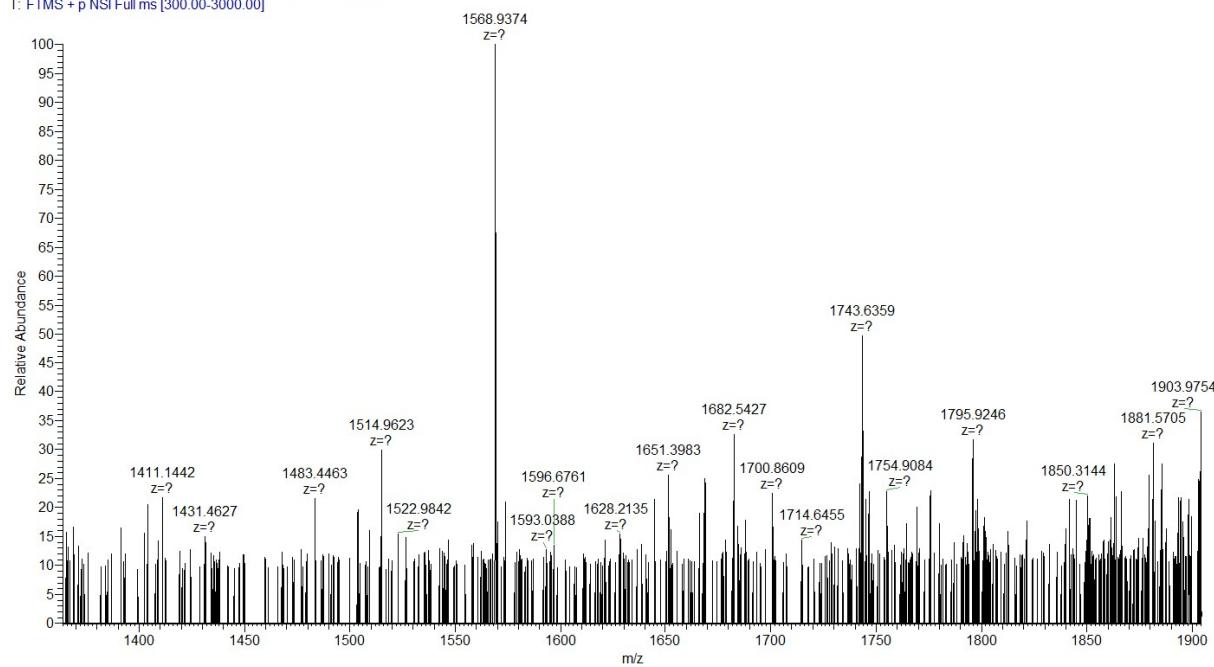
D)

OB1202 #4154-4429   RT: 56.05-60.16   AV: 265   NL: 1.55E3
T: FTMS + p NSI Full ms [300.00-3000.00]
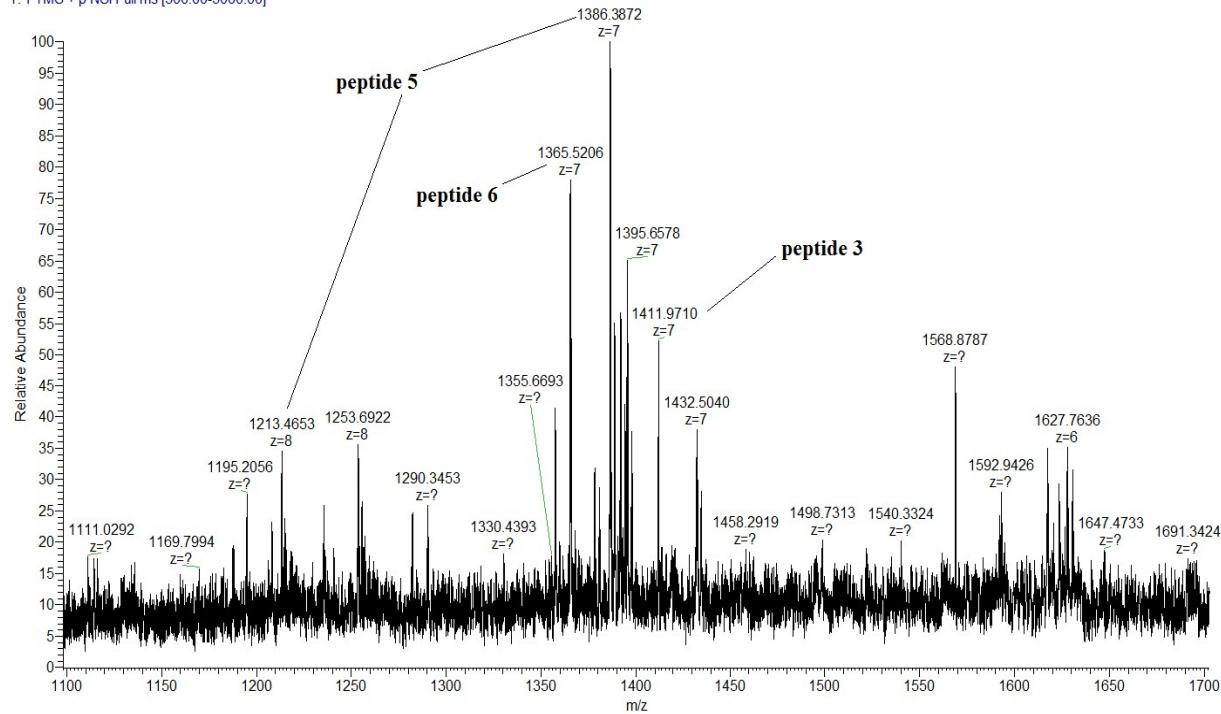
Figure S6. ESI-MS spectra of CAA peptides released during 3 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.91-50.75, (B) 39.91-50.75 zoom out, (C) 41.64-42.33 and (D) 56.05-60.16 seconds. A 3h time point in CAA sample peptide 15 is also still the most dominant, and similary to Art v 1 sample peptide 16 appears. Dominant isoforms of intact CAA are no longer seen and peptides 3,5 and 6 are still present.

S12

A)

OB1198 #2396-4032 RT: 39.78-52.06 AV: 586 NL: 1.14E5
T: FTMS + p NSI Full ms [300.00-3000.00]
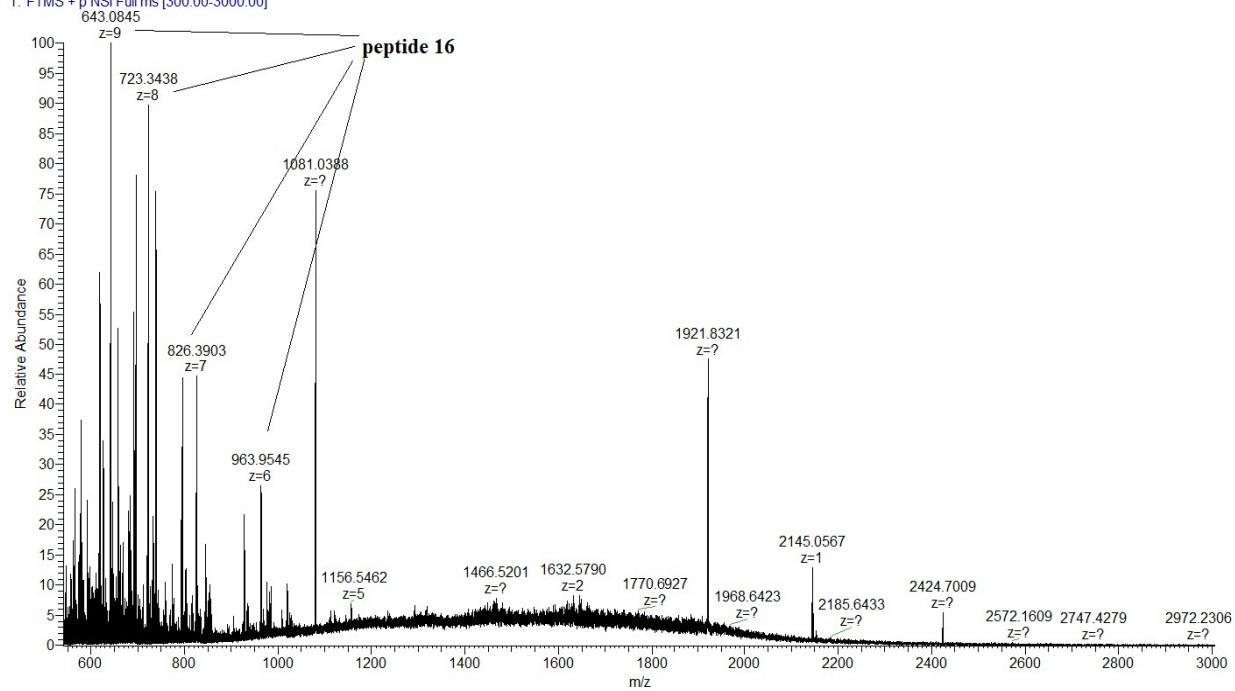
643.0845
z=9

peptide 16

723.3438
z=8

1081.0388
z=?

826.3903
z=7

963.9545
z=6

1921.8321
z=?

1156.5462
z=5

1466.5201
z=2

1632.5790
z=2

1770.6927
z=?

1968.6423
z=?

2145.0567
z=1

2185.6433
z=?

2424.7009
z=?

2572.1609
z=?

2747.4279
z=?

2972.2306
z=?

B)

OB1198 #2396-4032 RT: 39.78-52.06 AV: 586 NL: 1.14E5
T: FTMS + p NSI Full ms [300.00-3000.00]

643.0845
z=9

peptide 16

723.3438
z=8

696.4523
z=8

738.3361
z=?

peptide 15

peptide 19

619.1811
z=9

657.3130
z=9

578.9774
z=10

795.6579
z=7

826.3903
z=7

566.4298
z=?

591.7828
z=10

683.1407
z=4

758.2274
z=8

773.4058
z=6

844.6844
z=7

853.5850
z=5

866.5542
z=?

905.6841
z=?

928.0990
z=6

934.1422
z=1

963.9545
z=6

975.4456
z=2

1020.0491
z=4

S13

C)

D)
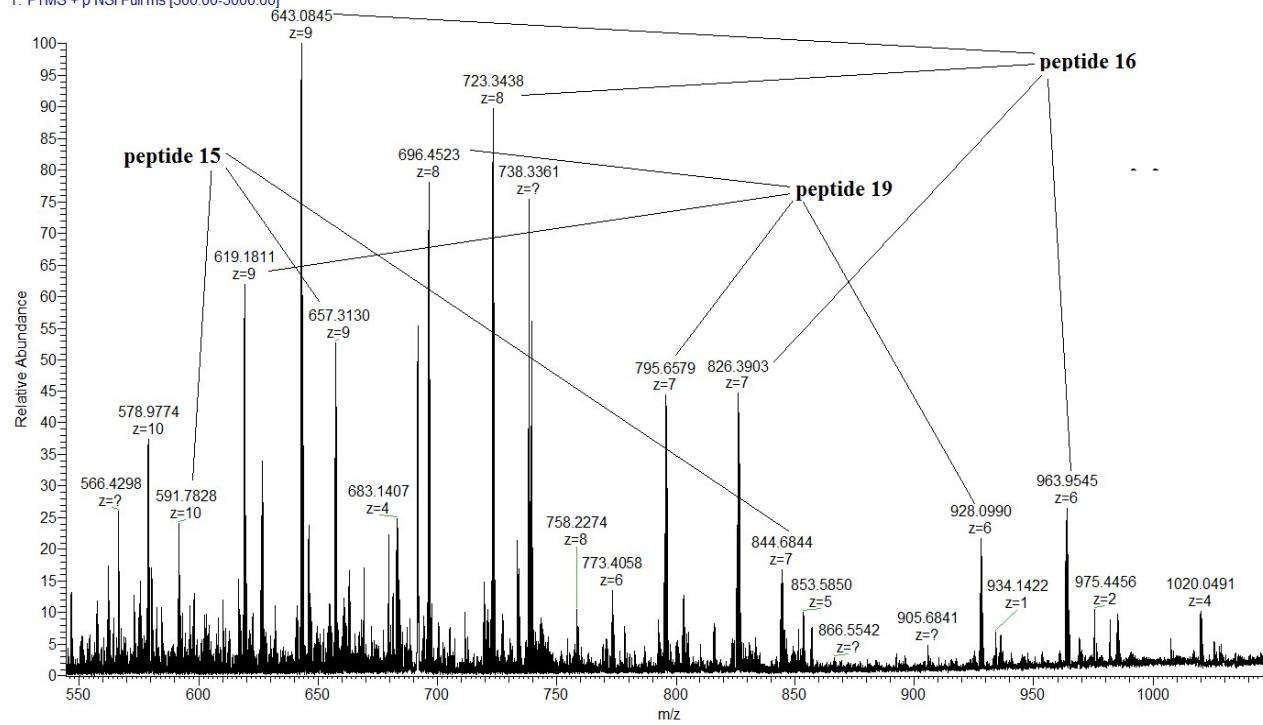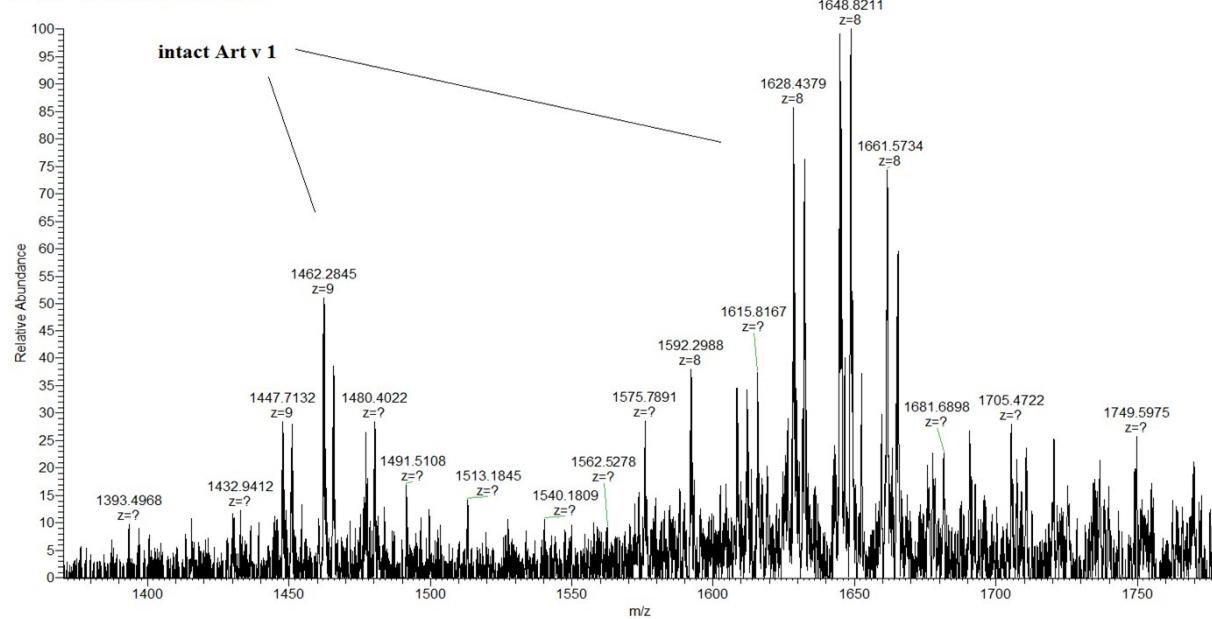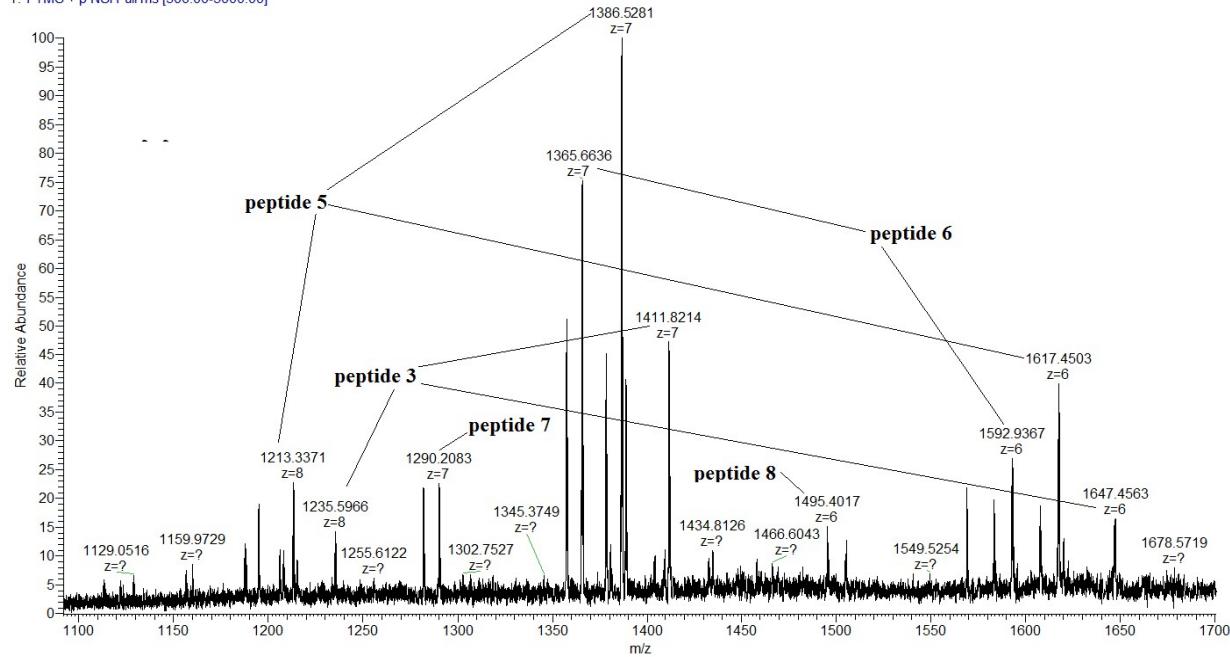
Figure S7. ESI-MS spectra of Art v 1 peptides released during 12 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.78-52.06, (B) 39.78-52.06 zoom out, (C) 42.73-43.00 and (D) 55.80-60.10 seconds. After 12h of degradation of Art v 1 peptide 15 is less aboundant, and the most dominant peptide is 16, but there is also appereence of highly aboundant peptide peptide 19 (5566 Da). Peptide 19 is peptide 16 shortened again by N-terminal dipeptide Ser-Lys, and it actually consists of whole defensin domen shortened by N-terminal Lys and C-terminal Cys. Dominant isoforms of intact Art v 1 are still present, as well as peptides 3,5,6,7 and 8.

A)

B)

S15

C)

D)
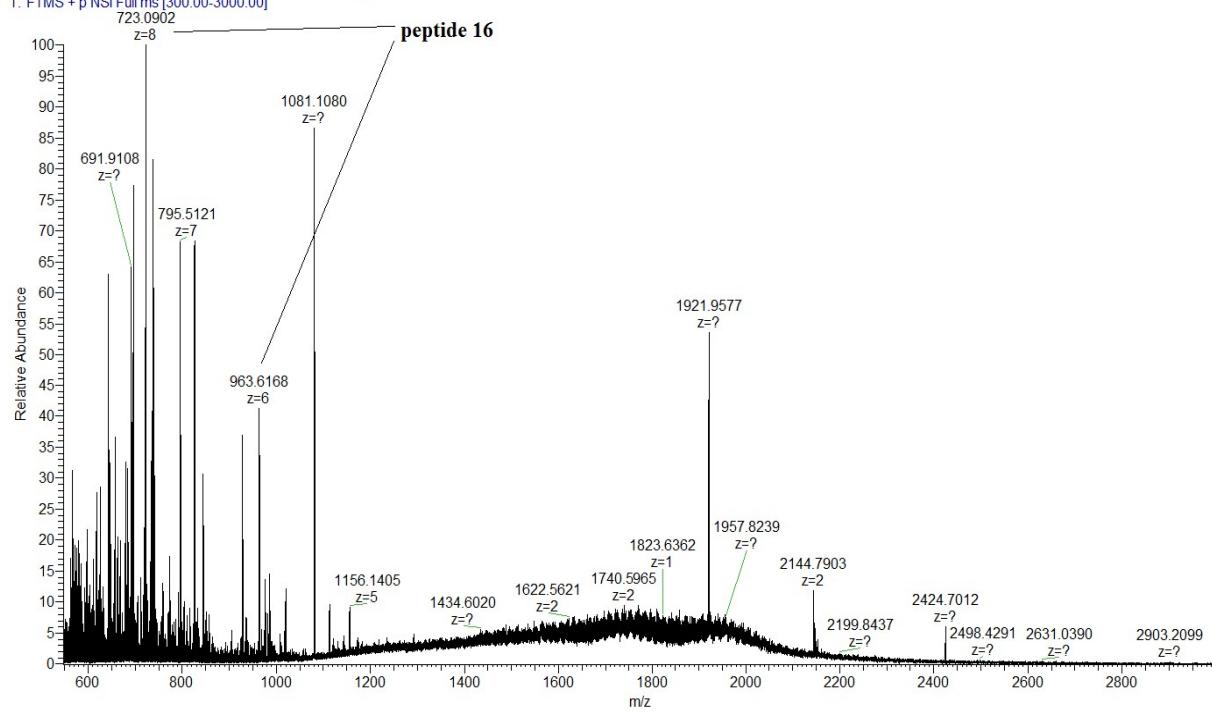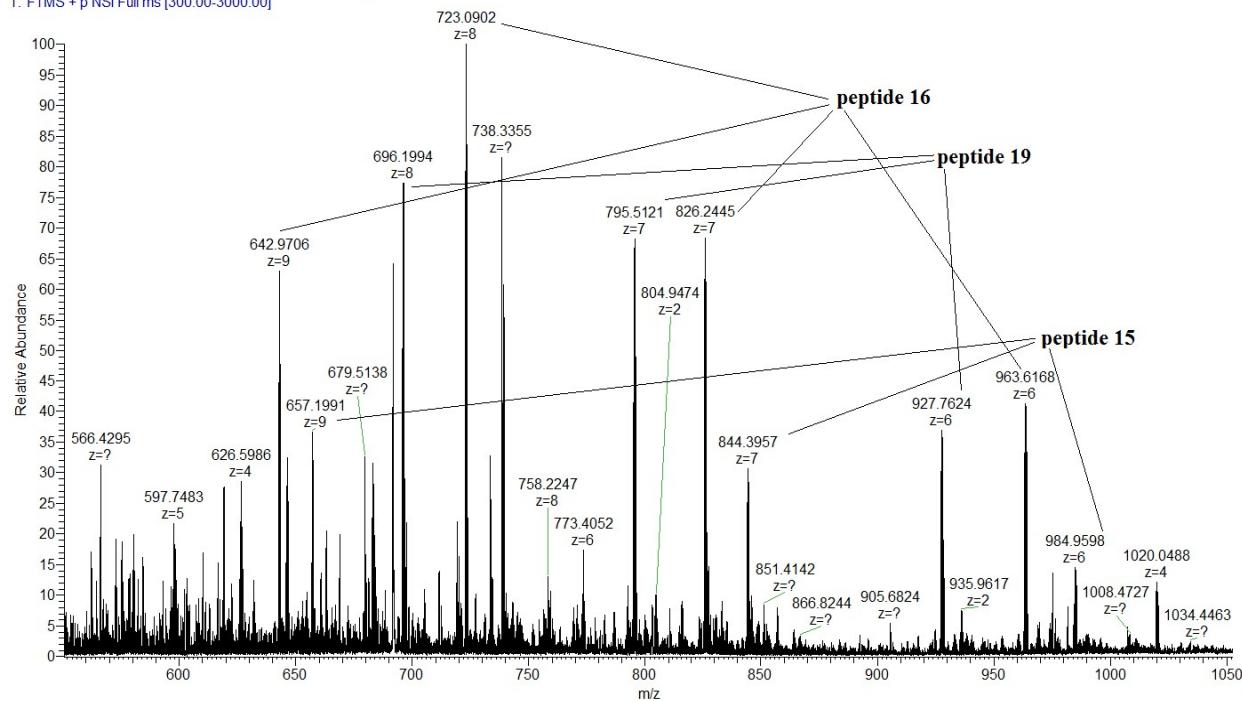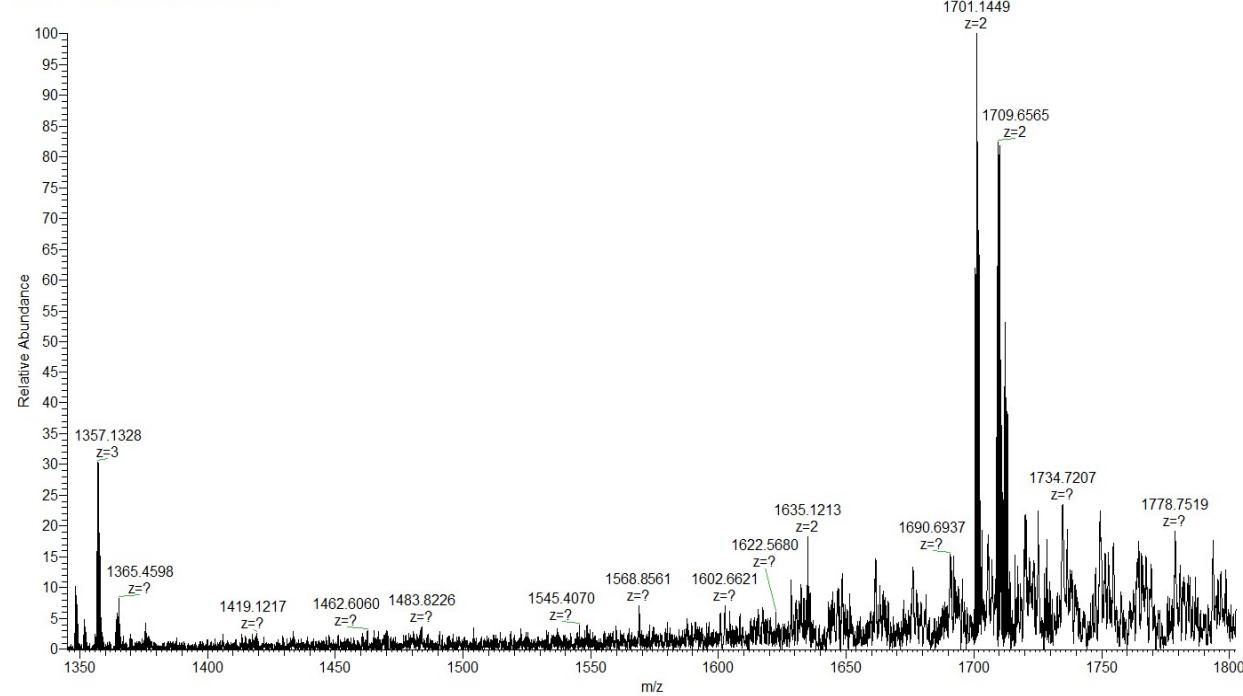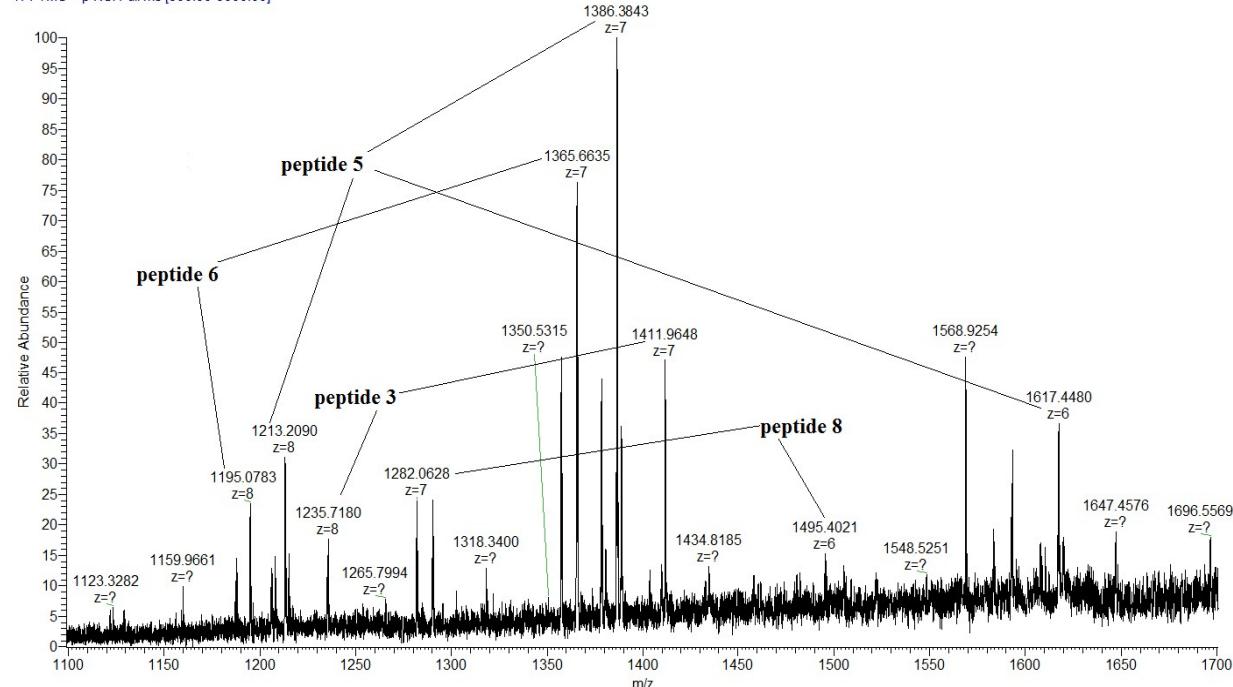
Figure S8. ESI-MS spectra of CAA peptides released during 12 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.84-52.00, (B) 39.84-52.00 zoom out, (C) 41.46-42.15 and (D) 55.81-59.97 seconds. Similary to Art v1 sample, at 12 h time point in CAA sample peptide 16 is the most dominant, with remained peptide 15 and appearance of peptide 19. In addition to peptides 3, 5 and 6, shorted peptide 8 appear.

A)

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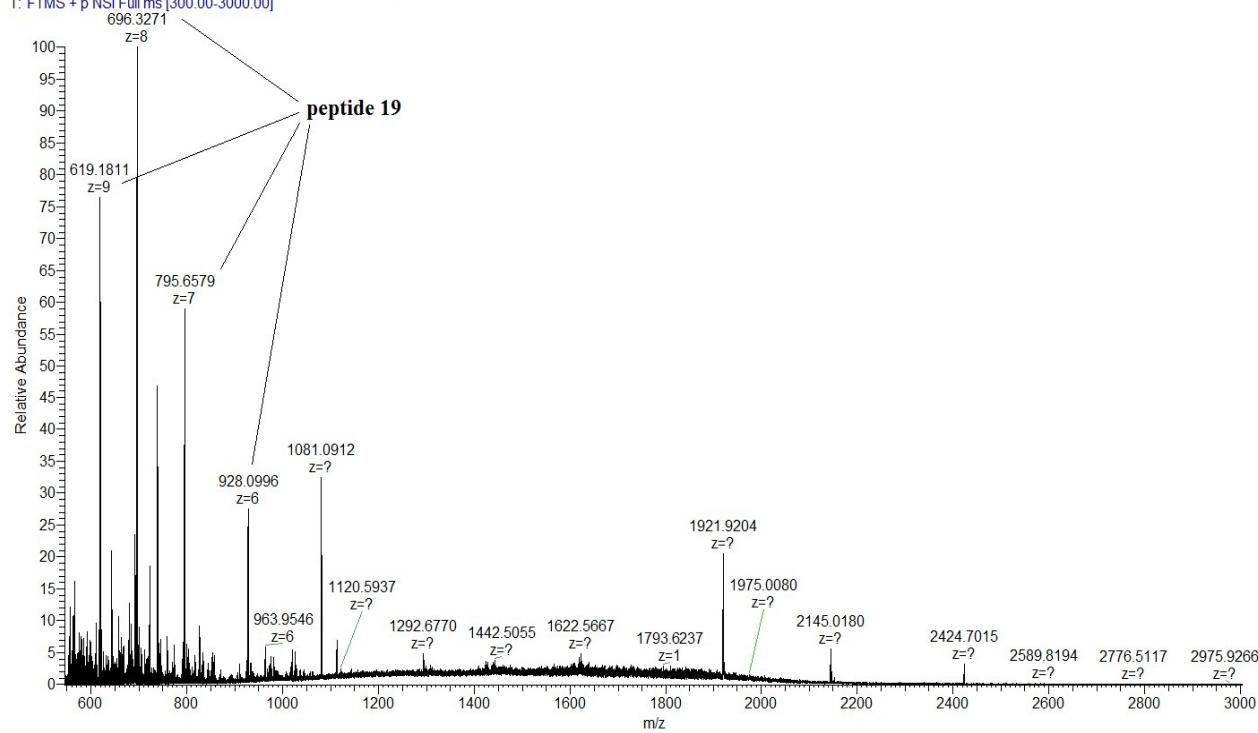
B)

OB1199 #2340-3935 RT: 39.77-52.25 AV: 591 NL: 1.59E5
T: FTMS + p NSI Full ms [300.00-3000.00]
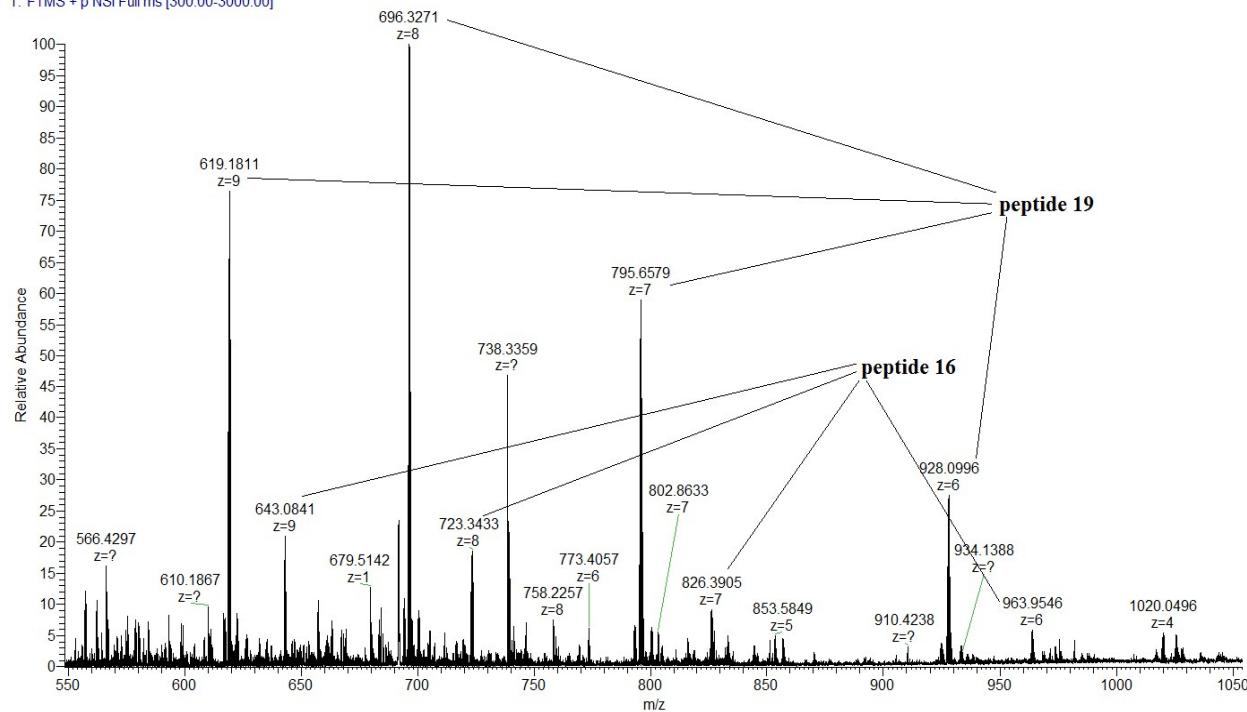
S17
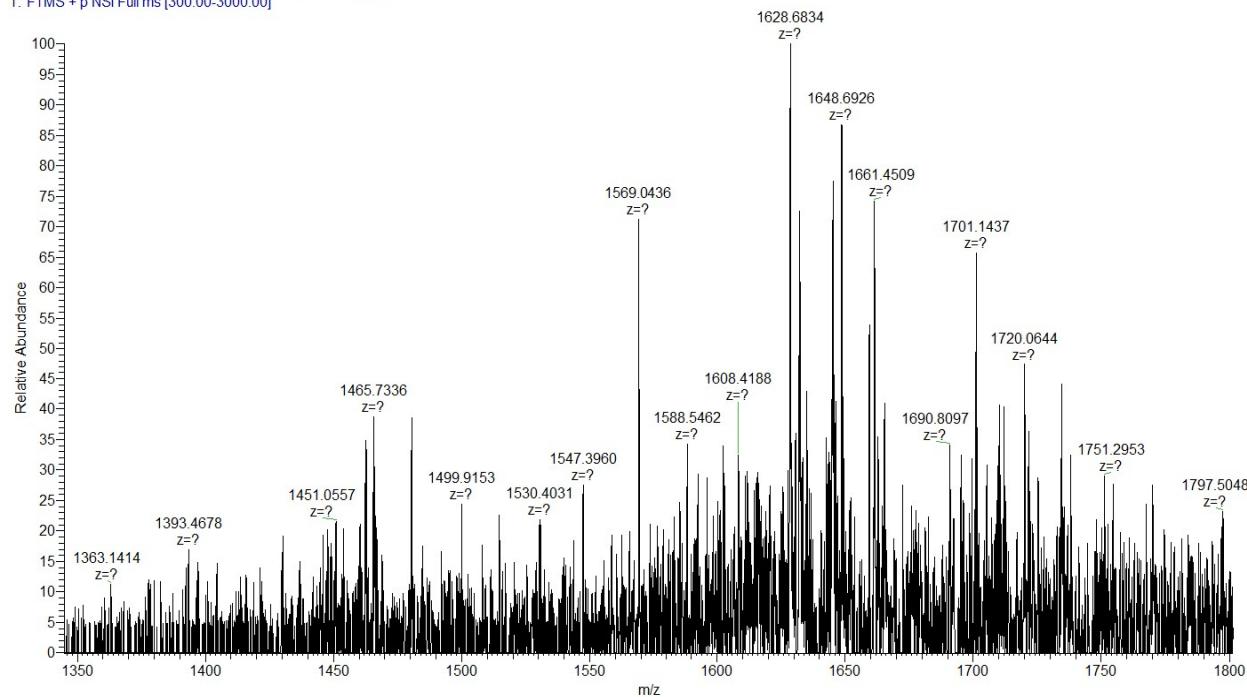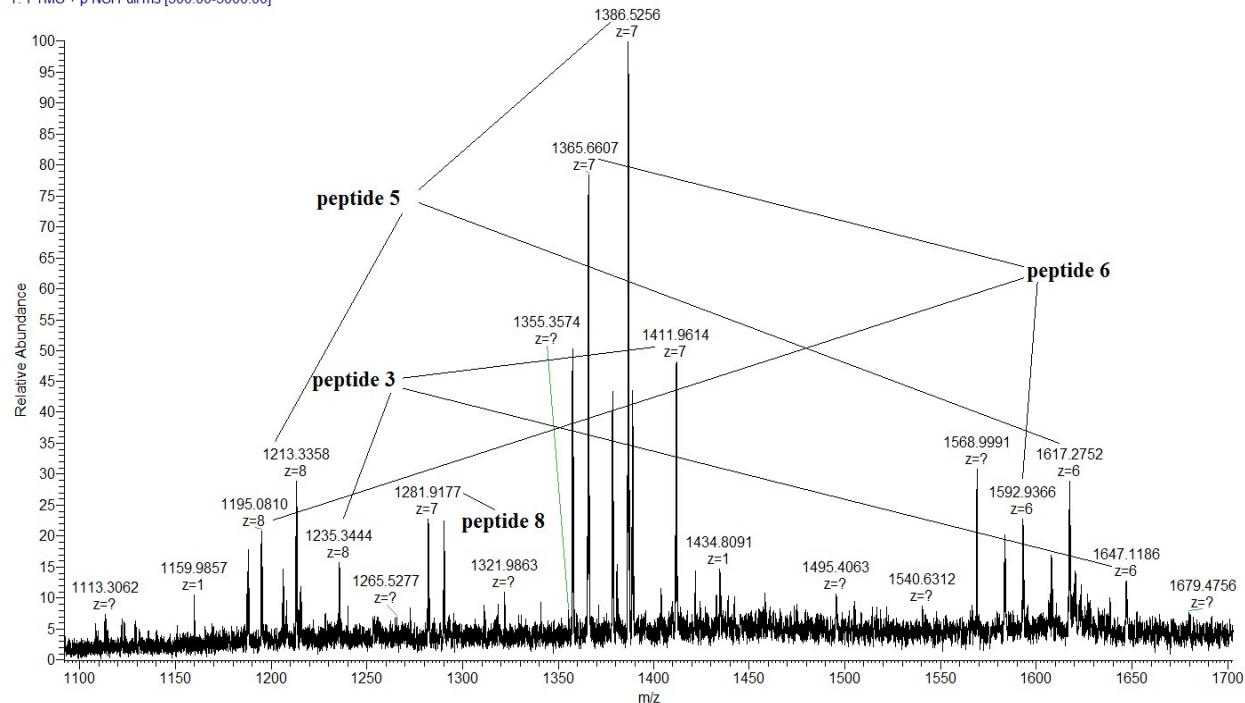
Figure S9. ESI-MS spectra of Art v 1 peptides released during 24 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.77-52.25, (B) 39.77-52.25 zoom out, (C) 42.78-43.44 and (D) 55.98-60.16 seconds. After 24 h peptide 19 predominates and peptide 15 completely disappears, while peptide 16 is still present in Art v 1 sample. Peptides 3, 5, 6 and 8 can still be detected.

A)

peptide 19

B)

peptide 19

S19
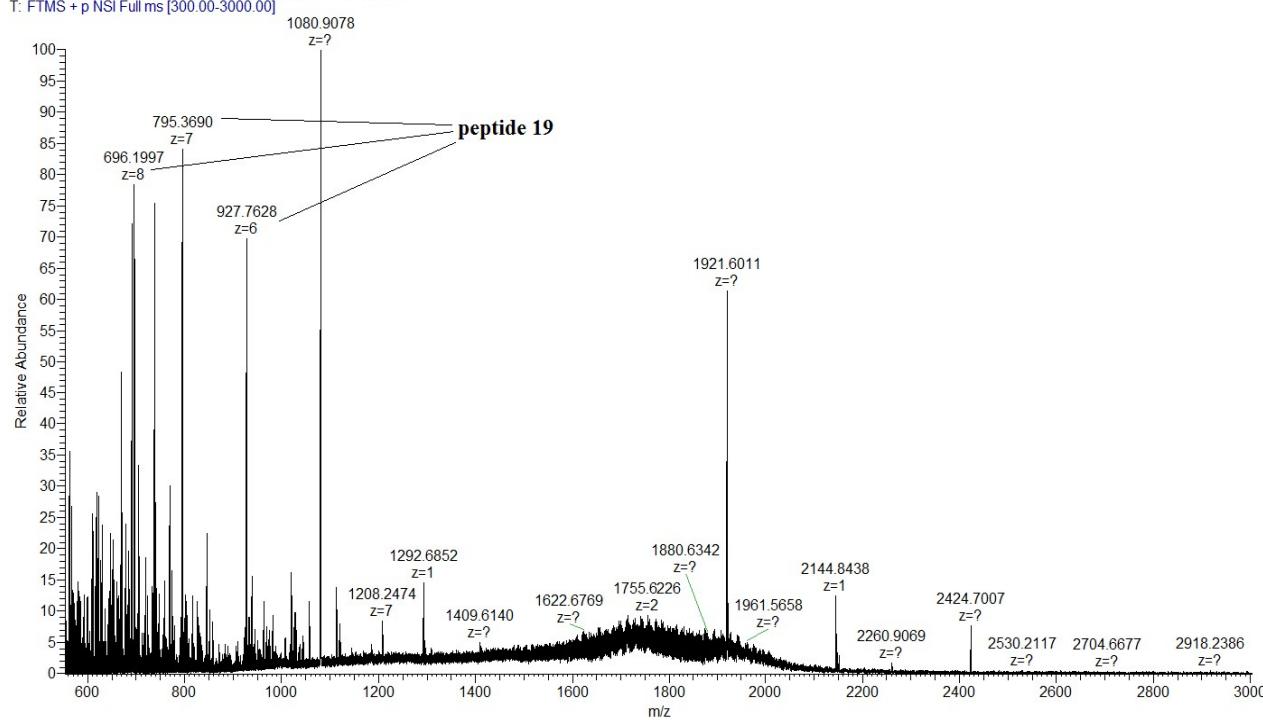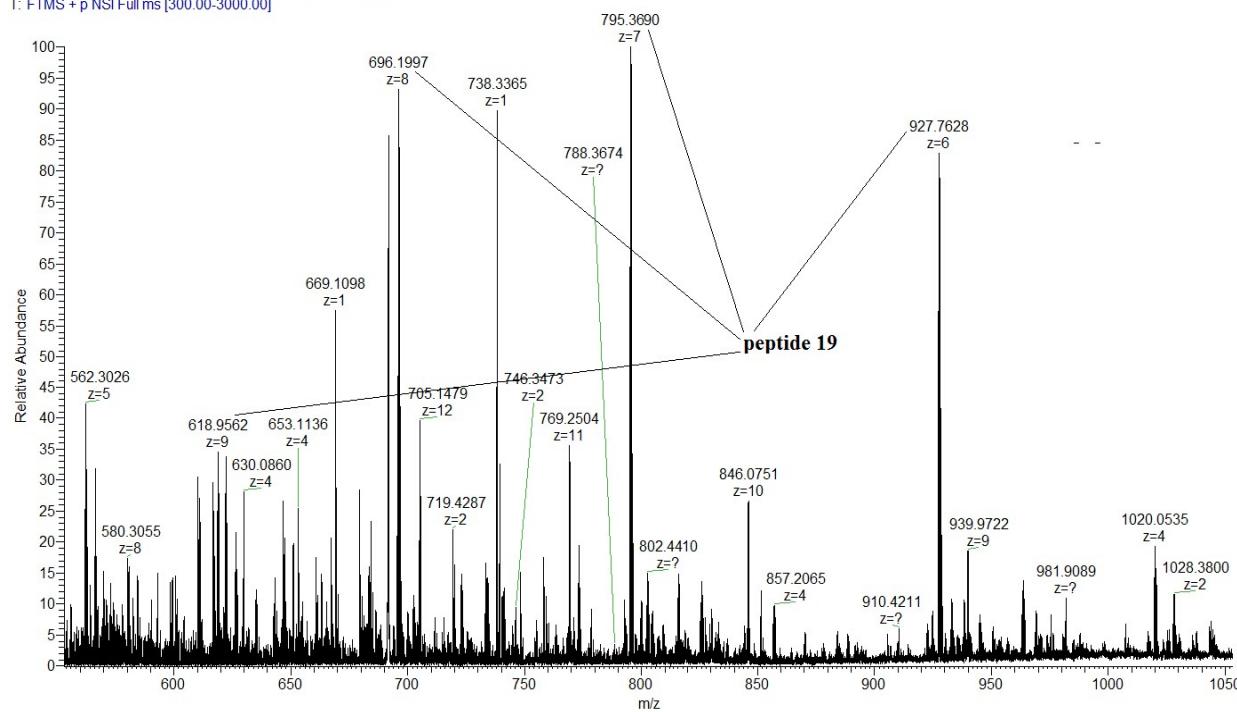
C)

OB1208 #2770-2884 RT: 41.58-42.52 AV: 41 NL: 8.49E2
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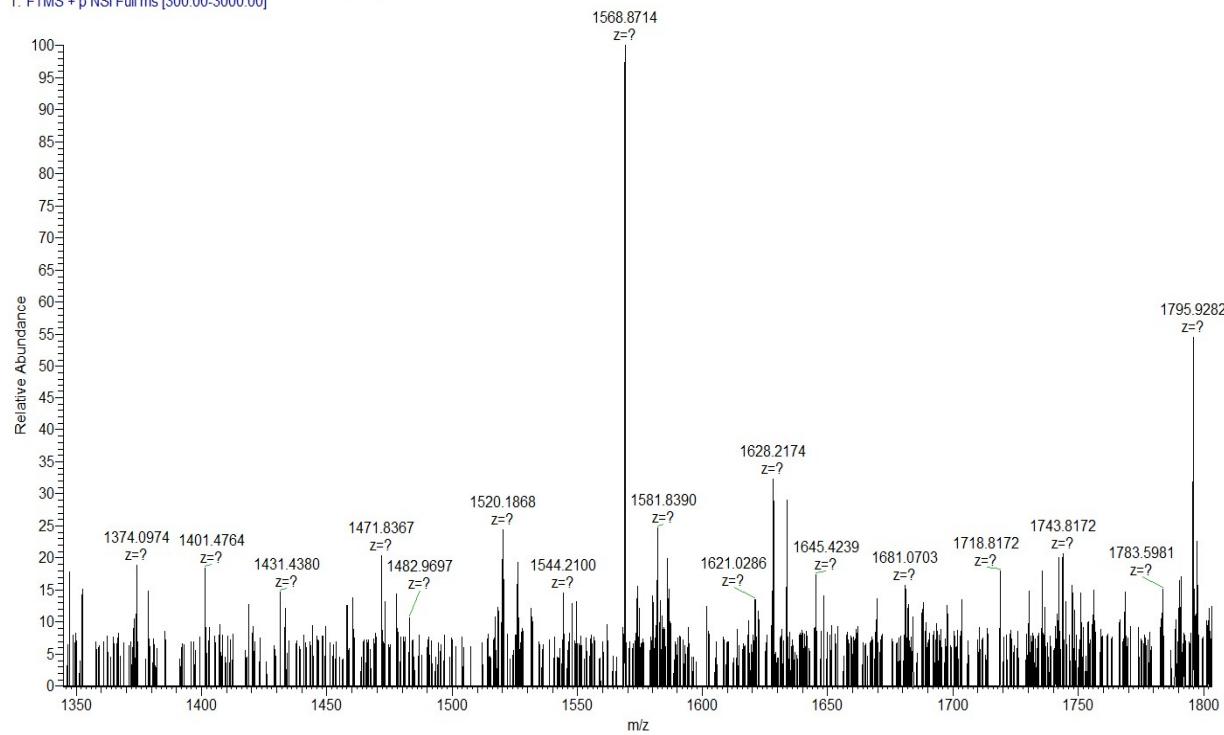
D)

OB1208 #4570-4846 RT: 55.93-60.16 AV: 272 NL: 9.37E2
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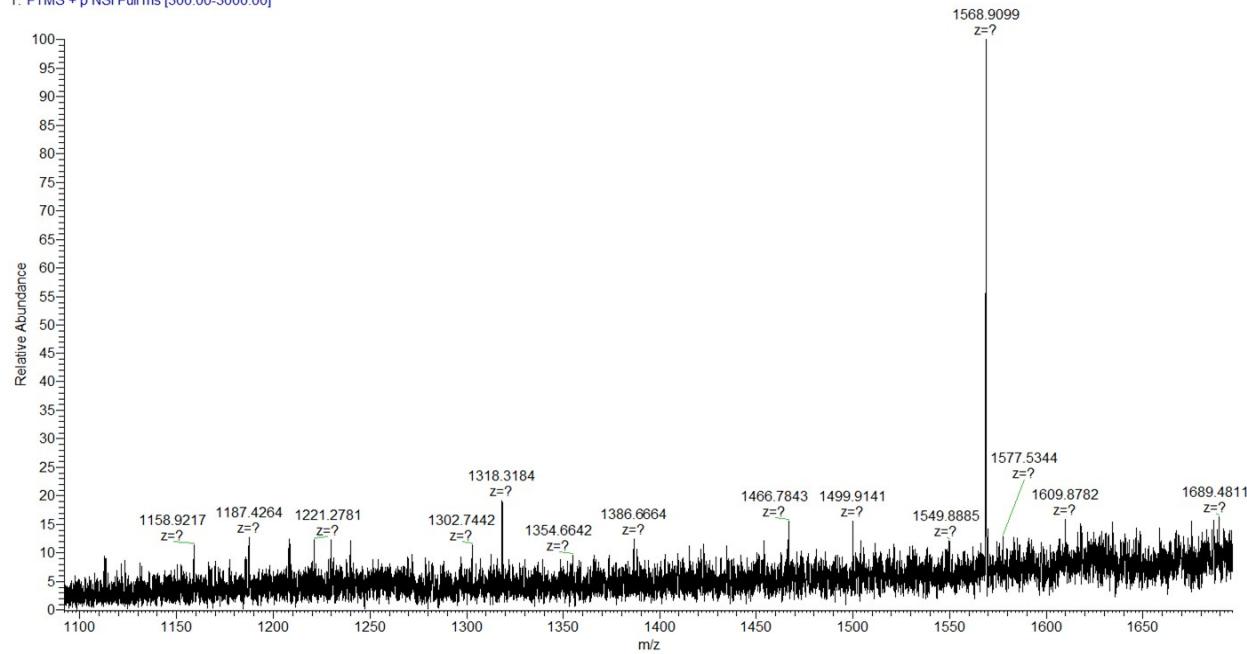
Figure S10. ESI-MS spectra of CAA peptides released during 24 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.78-52.00, (B) 39.78-52.00 zoom out, (C) 41.58-42.52 and (D) 55.93-60.16 seconds. In CAA sample after 24 h peptide 19 also predominates and peptide 15 completely disappear. Peptides 3, 5, 6 and 8 disappear.

A)

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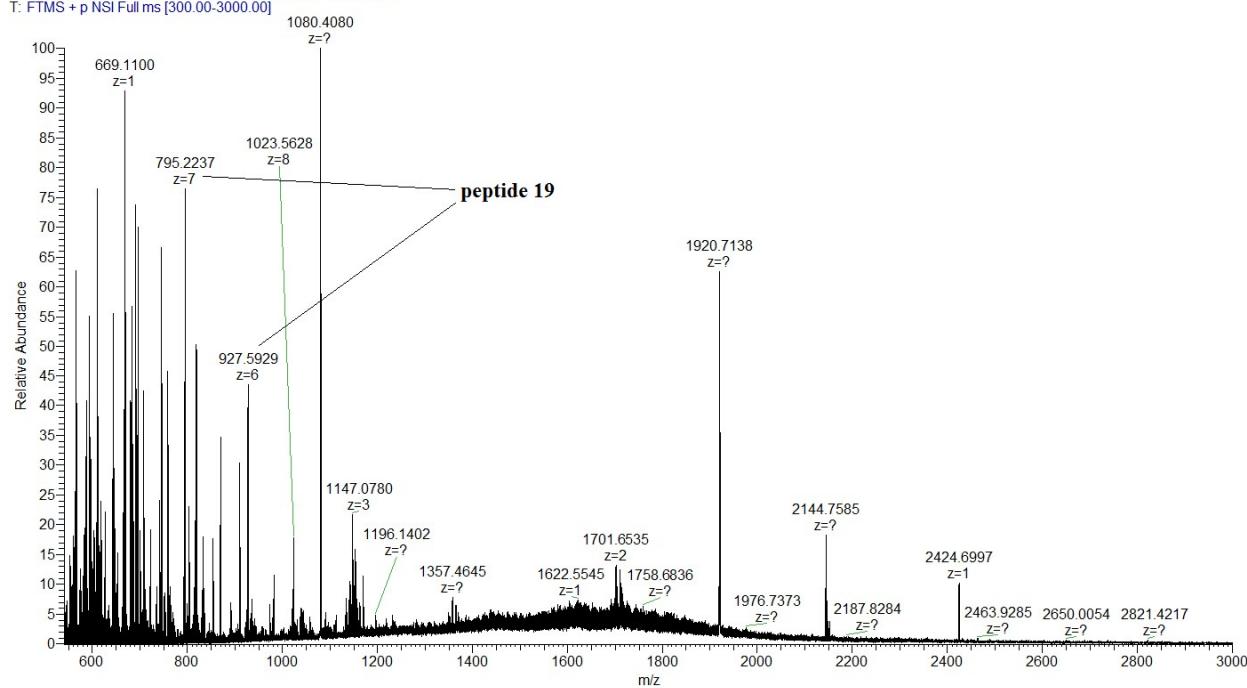
B)

OB1221 #2510-3755   RT: 39.84-51.87   AV: 645   NL: 2.41E4
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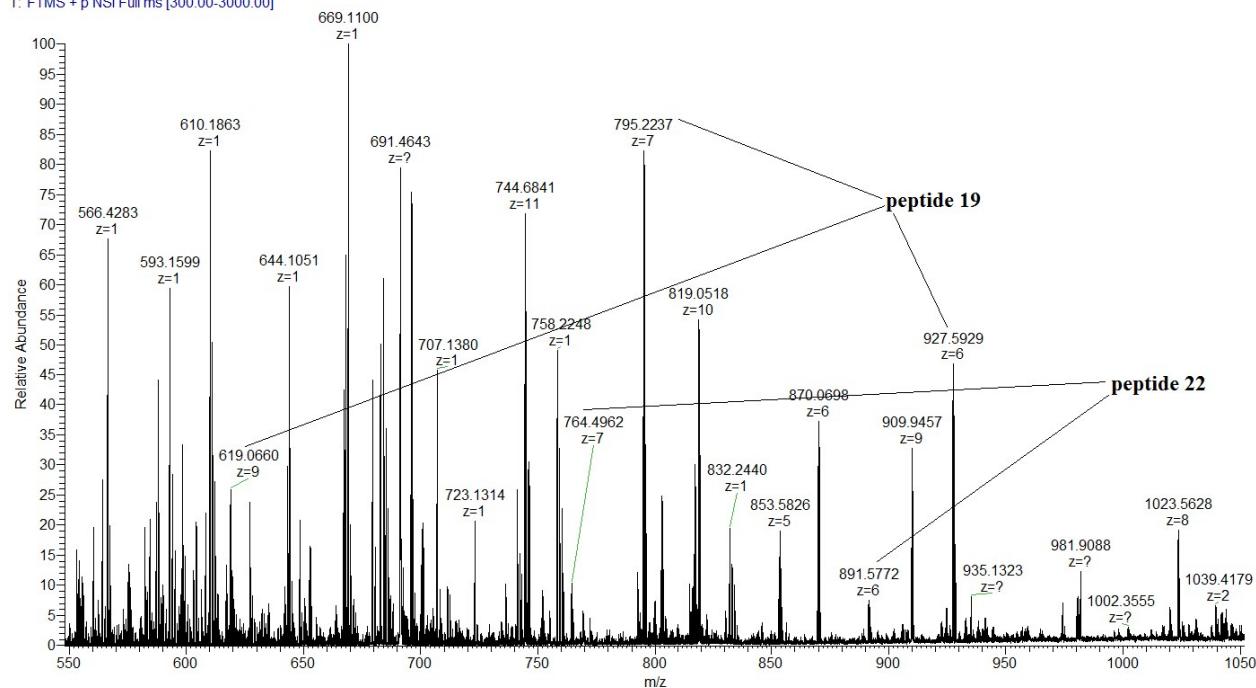
S21

C)

D)
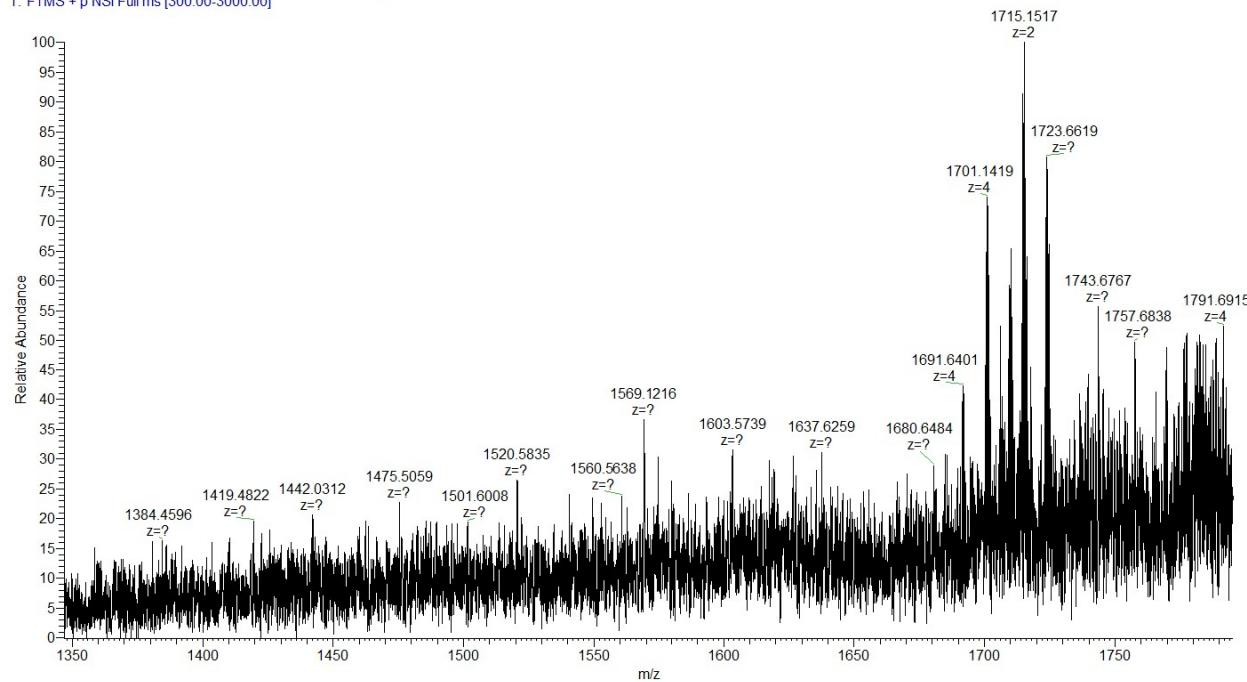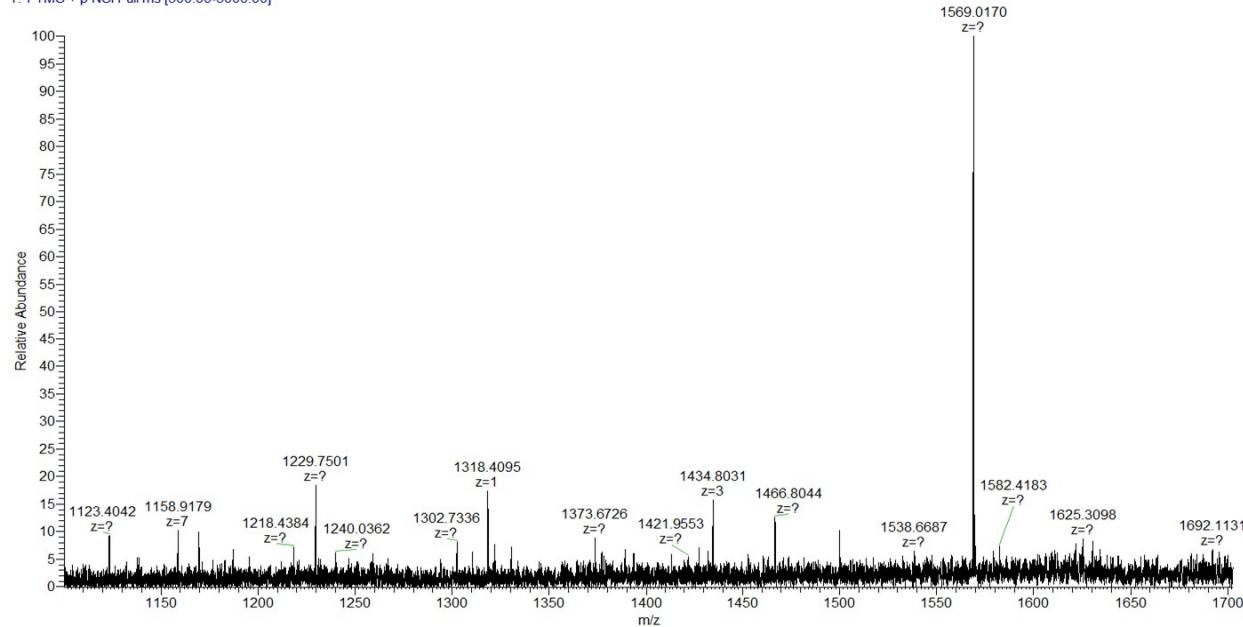
Figure S11. ESI-MS spectra of Art v 1 peptides released during 48 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.84-51.87, (B) 39.84-51.87 zoom out, (C) 41.90-42.70 and (D) 55.99-60.11 seconds. After 48 h peptide 19 is still the most abundant peptide, and peptide 22 appears (5344 Da). Peptide 22 is peptide 19 shortened by N-terminal dipeptide Leu-Cys. This suggests that even after 48 h of digestion defensin domen is still resistant to degradation due to its compact structure. Peptides 3, 5, 6 and 8 disappear.

A)

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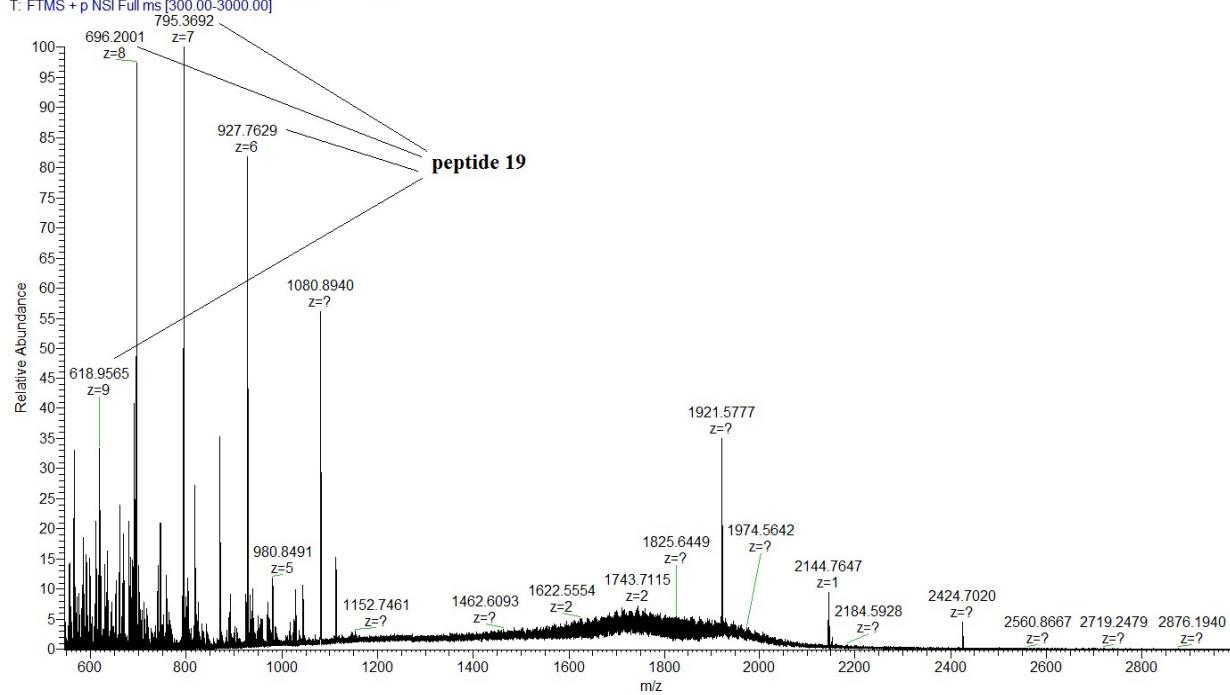
B)

OB1209 #2571-4196 RT: 39.84-52.00 AV: 579 NL: 1.03E5
T: FTMS + p NSI Full ms [300.00-3000.00]
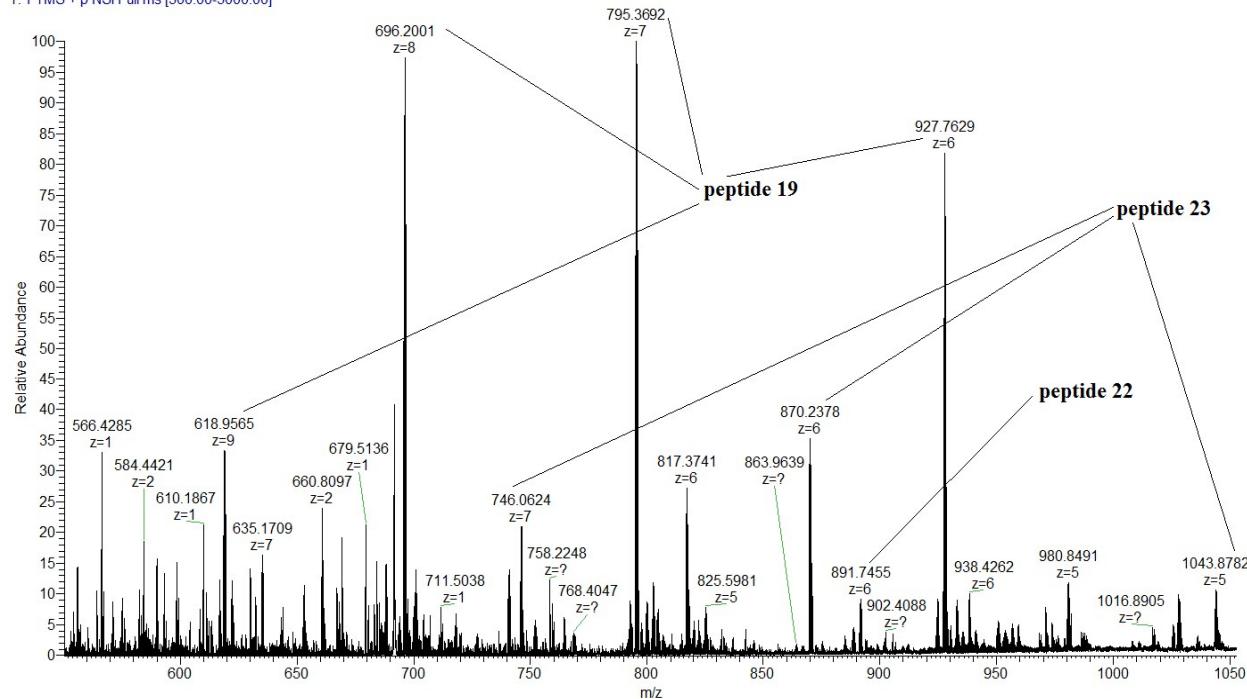
C)

D)
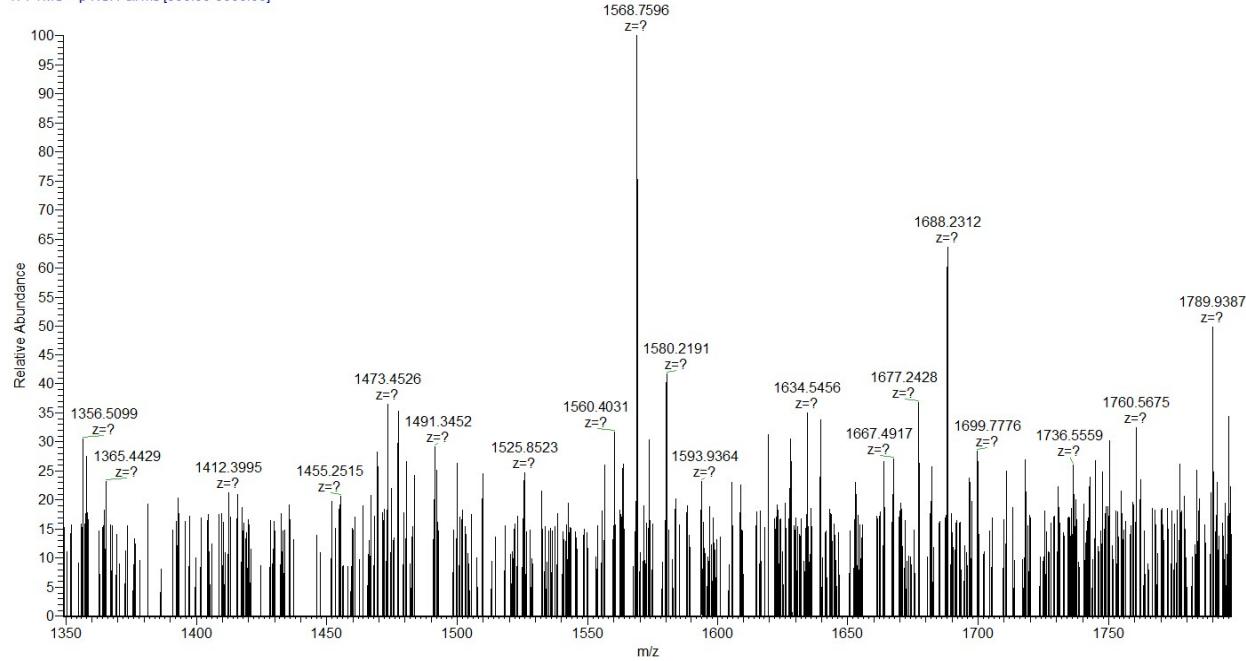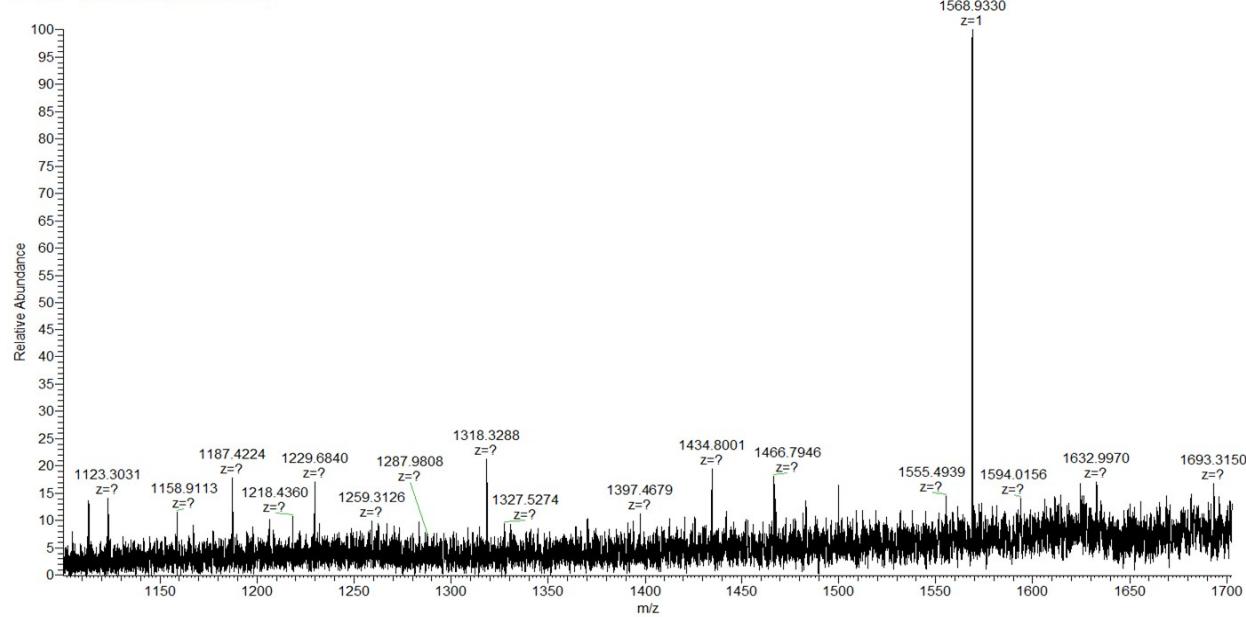
Figure S12. ESI-MS spectra of CAA peptides released during 48 h of endolysosomal processing. ESI-MS spectra at retention time (A) 39.84-52.00, (B) 39.84-52.00 zoom out, (C) 41.85-42.51 and (D) 55.99-59.98 seconds. Similarly to Art v 1 sample, in CAA sample after 48 h peptide 19 is also the most abundant peptide, and peptide 22 appears (5344 Da). In this sample presence of peptide 23 (5215 Da), which is peptide 22 shortened by N-terminal E, can be observed. This suggests that after 48 h defensin domen of CAA is also still resistant to degradation as those of Art v 1.

Table S1. Masses of intact Art v 1 and CAA isoforms. Glycan type – the type of arabinogalactosyl proposed by Himly et al.,[1] HP – proline hydroxylation, Gal – galactose mass, Ara – arabinose mass, SH- disulfide bond, n.d. – not defined.

Glycan type	Sequence mass	Modification					Calculated mass	m/z	z	Experimental mass	Δ mass
		HP	Gal	Ara	SH	Sequence conflict					
Intact Art v 1											
n.d.	10800.07	+256	+648	+1848	-8	+93	13637.07	1516.087	9	13635.79	1.2843
n.d.	10800.07	+256	+648	+1848	-8	+32	13576.07	1509.419	9	13575.77	0.3035
17	10800.07	+256	+486	+1848	-8	+32	13414.07	1491.415	9	13413.73	0.3395
17	10800.07	+256	+486	+1848	-8	+61	13443.07	1494.638	9	13442.74	0.328
17	10800.07	+256	+486	+1848	-8	+93	13475.07	1498.416	9	13476.74	-1.6722
16	10800.07	+256	+486	+1716	-8	+14	13264.07	1474.742	9	13263.68	0.3929
16	10800.07	+256	+486	+1716	-8	+32	13282.07	1476.633	9	13280.7	1.3703
16	10800.07	+256	+486	+1716	-8	+61	13311.07	1479.967	9	13310.7	0.3697
16	10800.07	+256	+486	+1716	-8	+93	13343.07	1483.41	9	13341.69	1.3809
15	10800.07	+256	+486	+1584	-8	+14	13132.07	1459.071	9	13122.64	9.4346
15	10800.07	+256	+486	+1584	-8	+32	13150.07	1462.073	9	13149.66	0.4139
15	10800.07	+256	+486	+1584	-8	+61	13179.07	1465.407	9	13179.66	-0.5903
13	10800.07	+256	+324	+1584	-8	+32	12988.07	1444.402	9	12990.61	-2.5435
13	10800.07	+256	+324	+1584	-8	+61	13017.07	1447.403	9	13017.63	-0.5606
13	10800.07	+256	+324	+1584	-8	+93	13049.07	1450.737	9	13047.63	1.4397
13	10800.07	+256	+324	+1584	-8	+14	12970.07	1442.168	9	12970.51	-0.4447
11	10800.07	+256	+324	+1452	-8	+14	12838.07	1427.725	9	12840.53	-2.4586
11	10800.07	+256	+324	+1452	-8	+32	12856.07	1429.729	9	12858.56	-2.4874
11	10800.07	+256	+324	+1452	-8	+61	12885.07	1432.732	9	12885.59	-0.5216
9	10800.07	+256	+324	+1320	-8	+32	12724.07	1415.056	9	12726.51	-2.4358
9	10800.07	+256	+324	+1320	-8	+61	12753.07	1418.053	9	12753.47	-0.4034
7	10800.07	+256	+324	+1188	-8	+32	12592.07	1400.385	9	12594.47	-2.3977
Intact CAA											
n.d.	10800.07	+256	+648	+1848	-8	+61	13605.07	1512.406	9	13602.65	2.4196
n.d.	10800.07	+256	+648	+1848	-8	+93	13637.07	1515.972	9	13634.75	2.3238
17	10800.07	+256	+486	+1848	-8	+61	13443.07	1494.849	9	13444.64	-1.5719
17	10800.07	+256	+486	+1848	-8	+93	13475.07	1497.737	9	13470.63	4.4352
16	10800.07	+256	+486	+1716	-8	+14	13264.07	1474.717	9	13263.45	0.6161
16	10800.07	+256	+486	+1716	-8	+32	13282.07	1476.623	9	13280.6	1.4666
16	10800.07	+256	+486	+1716	-8	+61	13311.07	1479.843	9	13309.59	1.4848
16	10800.07	+256	+486	+1716	-8	+93	13343.07	1483.398	9	13341.58	1.4898

15	10800.07	+256	+486	+1584	-8	+32	13150.07	1462.173	9	13150.56	-0.4861
15	10800.07	+256	+486	+1584	-8	+61	13179.07	1465.284	9	13178.55	0.5167
15	10801.07	+256	+486	+1584	-8	+93	13212.07	1468.612	9	13208.51	3.5602
13	10800.07	+256	+324	+1584	-8	+32	12988.07	1444.277	9	12989.49	-1.423
13	10800.07	+256	+324	+1584	-8	+61	13017.07	1447.39	9	13017.51	-0.4391
13	10800.07	+256	+324	+1584	-8	+93	13049.07	1450.83	9	13048.47	0.6045
11	10800.07	+256	+324	+1452	-8	+14	12838.07	1427.725	9	12840.53	-2.4586
11	10800.07	+256	+324	+1452	-8	+32	12856.07	1429.729	9	12858.56	-2.4874
11	10800.07	+256	+324	+1452	-8	+61	12885.07	1432.732	9	12885.59	-0.5216
9	10800.07	+256	+324	+1320	-8	+32	12724.07	1414.935	9	12725.41	-1.3441

Table S2. Masses of identified peptides released during 15 min, 3, 12, 24 and 48 h of endolysosomal processing of Art v 1 and CAA. Peptide name – number assigned to identified peptide, HP – proline hydroxylation, Gal – galactose mass, Ara – arabinose mass, SH- disulfide bond, H2O – internal peptide bond hydrolysis.

Peptide name	Sequence mass	Modification						Calculated mass	m/z	z	Experimental mass	Δ mass
		HP	Gal	Ara	SH	Sequence conflict	H_2O					
Art v 1 15 min												
1	10800.07	+256	+324	+1584	-6	+61	0	13019.07	1447.61	9	13019.49	-0.4236
1	10800.07	+256	+486	+1584	-8	+32	0	13150.07	1462.172	9	13150.55	-0.4771
1	10800.07	+256	+486	+1716	-8	+32	0	13282.07	1476.73	9	13281.57	0.5
1	10800.07	+256	+486	+1848	-6	+32	0	13416.07	1491.626	9	13415.64	0.4333
1	10800.07	+256	+324	+1320	-6	+32	0	12726.07	1591.675	8	12725.4	0.6732
1	10800.07	+256	+324	+1452	-6	+32	0	12858.07	1608.3	8	12858.4	-0.3268
1	10800.07	+256	+324	+1584	-6	+61	0	13019.07	1628.309	8	13018.47	0.598
1	10800.07	+256	+486	+1584	-8	+32	0	13150.07	1644.692	8	13149.53	0.5372
1	10800.07	+256	+486	+1848	-8	+32	0	13414.07	1677.699	8	13413.59	0.4772
15	5909.78	0	0	0	6	0	0	5903.78	985.1316	6	5904.79	-1.0096
15	5909.78	0	0	0	4	0	0	5905.78	844.6865	7	5905.806	-0.0255
15	5909.78	0	0	0	4	0	0	5905.78	739.2274	8	5905.819	-0.0392
15	5909.78	0	0	0	4	0	0	5905.78	657.3148	9	5906.833	-1.0532
15	5909.78	0	0	0	4	0	0	5905.78	591.684	10	5906.84	-1.06
24	4966.67	0	0	0	4	0	0	4962.67	828.0981	6	4962.589	0.0814
24	4966.67	0	0	0	4	0	0	4962.67	709.9423	7	4962.596	0.0739
24	4966.67	0	0	0	4	0	0	4962.67	621.3257	8	4962.606	0.0644
24	4966.67	0	0	0	4	0	0	4962.67	993.5145	5	4962.573	0.0975
24	4966.67	0	0	0	4	0	+18	4980.67	712.5086	7	4980.56	0.1098
12	6068.99	0	0	0	8	0	0	6060.99	758.2259	8	6057.807	3.1828
26	4743.48	0	0	0	0	0	0	4743.48	792.0848	6	4746.509	-3.0288
32	2988.45	0	0	0	4	0	0	2984.45	597.7493	5	2983.747	0.7035
29	4249.9	0	0	0	6	0	+18	4261.9	853.5864	5	4262.932	-1.032
CAA 15 min												
1	10800.07	+256	+486	+1584	-8	+61	0	13179.07	1465.289	9	13178.6	0.469
1	10800.07	+256	+486	+1716	-8	+32	0	13282.07	1476.736	9	13281.62	0.4487
1	10800.07	+256	+486	+1716	-8	+61	0	13311.07	1479.847	9	13309.62	1.4479
1	10800.07	+256	+486	+1848	-8	+61	0	13443.07	1494.627	9	13442.64	0.4297
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1365.371	7	9550.596	1.9044
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.387	7	9697.712	0.9482
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1411.812	7	9875.681	0.9288
15	5909.78	0	0	0	-6	0	0	5903.78	985.1312	6	5904.787	-1.0072

15	5909.78	0	0	0	-6	0	0	5903.78	844.5422	7	5904.795	-1.0154
15	5909.78	0	0	0	-4	0	0	5905.78	739.2265	8	5905.812	-0.032
15	5909.78	0	0	0	-4	0	0	5905.78	657.2028	9	5905.825	-0.0452
15	5909.78	0	0	0	-4	0	0	5905.78	591.6837	10	5906.837	-1.057
24	4966.67	0	0	0	-4	0	0	4962.67	709.942	7	4962.594	0.076
24	4966.67	0	0	0	-4	0	0	4962.67	621.3252	8	4962.602	0.0684
24	4966.67	0	0	0	-4	0	0	4962.67	828.0979	6	4962.587	0.0826
24	4966.67	0	0	0	-4	0	0	4962.67	993.5155	5	4962.578	0.0925
13	6028.92	0	0	0	-4	0	+18	6042.92	864.4004	7	6043.803	-0.8828
13	6028.92	0	0	0	-4	0	+18	6042.92	756.6013	8	6044.81	-1.8904
26	4743.48	0	0	0	0	0	0	4743.48	792.0846	6	4746.508	-3.0276
24	4966.67	0	0	0	-4	0	+18	4980.67	712.5086	7	4980.56	0.1098
Art v 1 3 h												
1	10800.07	+256	+486	+1584	-8	+32	0	13150.07	1462.173	9	13150.56	-0.4879
1	10800.07	+256	+486	+1716	-8	+32	0	13282.07	1476.731	9	13281.58	0.4883
1	10800.07	+256	+324	+1320	0	+32	0	12732.07	1592.427	8	12731.41	0.6556
1	10800.07	+256	+324	+1452	-4	+32	0	12860.07	1608.555	8	12860.44	-0.3724
1	10800.07	+256	+324	+1584	-6	+61	0	13019.07	1628.435	8	13019.48	-0.4132
1	10800.07	+256	+486	+1584	-8	+32	0	13150.07	1644.693	8	13149.54	0.53
1	10800.07	+256	+486	+1716	-8	+32	0	13282.07	1661.198	8	13281.59	0.4844
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1235.59	8	9876.718	-0.1076
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1411.966	7	9876.763	-0.1534
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1213.211	8	9697.69	0.9704
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.385	7	9697.696	0.9643
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1617.45	6	9698.701	-0.0412
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1365.664	7	9552.645	-0.1452
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1593.105	6	9552.628	-0.1276
7	6906.92	+32	+486	+1584	-2	0	+18	9024.92	1505.414	6	9026.485	-1.5646
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1282.061	7	8967.426	-0.7056
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1495.402	6	8966.412	0.308
15	5909.78	0	0	0	-4	0	0	5905.78	985.1311	6	5904.787	0.9934
15	5909.78	0	0	0	-4	0	0	5905.78	844.5419	7	5904.793	0.9867
15	5909.78	0	0	0	-4	0	0	5905.78	739.2262	8	5905.81	-0.0296
15	5909.78	0	0	0	-4	0	0	5905.78	657.2021	9	5905.819	-0.0389
15	5909.78	0	0	0	-2	0	0	5907.78	591.6827	10	5906.827	0.953
16	5781.65	0	0	0	-4	0	0	5777.65	963.9555	6	5777.733	-0.083
16	5781.65	0	0	0	-4	0	0	5777.65	826.2477	7	5776.734	0.9161
16	5781.65	0	0	0	-4	0	0	5777.65	723.2188	8	5777.75	-0.1004
16	5781.65	0	0	0	-4	0	0	5777.65	642.9733	9	5777.76	-0.1097
33	2859.33	0	0	0	-2	0	0	2857.33	715.4029	4	2857.612	-0.2816
37	2500.82	0	0	0	0	0	0	2500.82	626.6002	4	2502.401	-1.5808

CAA 3 h												
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1213.465	8	9699.722	-1.0624
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.387	7	9697.71	0.9496
2	7863.97	+96	+486	+1584	-8	0	0	10021.97	1253.692	8	10021.54	0.4324
2	7863.97	+96	+486	+1584	-8	0	0	10021.97	1432.504	7	10020.53	1.442
4	7605.74	+96	+486	+1584	-8	0	0	9763.74	1395.658	7	9762.605	1.1354
4	7605.74	+96	+486	+1584	-8	0	0	9763.74	1627.764	6	9760.582	3.1584
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1411.971	7	9876.797	-0.187
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1365.521	7	9551.644	0.8558
15	5909.78	0	0	0	-4	0	0	5905.78	985.1315	6	5904.789	0.991
15	5909.78	0	0	0	-4	0	0	5905.78	844.5416	7	5904.791	0.9888
15	5909.78	0	0	0	-4	0	0	5905.78	739.2258	8	5905.806	-0.0264
15	5909.78	0	0	0	-4	0	0	5905.78	657.3133	9	5906.82	-1.0397
15	5909.78	0	0	0	-4	0	0	5905.78	591.6828	10	5906.828	-1.048
16	5781.65	0	0	0	-4	0	0	5777.65	963.9561	6	5777.737	-0.0866
16	5781.65	0	0	0	-4	0	0	5777.65	826.3914	7	5777.74	-0.0898
16	5781.65	0	0	0	-4	0	0	5777.65	723.3437	8	5778.75	-1.0996
16	5781.65	0	0	0	-4	0	0	5777.65	643.0845	9	5778.761	-1.1105
20	5498.27	0	0	0	0	0	0	5498.27	917.5296	6	5499.178	0.0976
10	6187.12	0	0	0	-8	0	0	6179.12	883.3083	7	6176.158	2.9619
13	6028.92	0	0	0	-4	0	+18	6042.92	864.3995	7	6043.797	-0.8765
37	2500.82	0	0	0	0	0	0	2500.82	626.6011	4	2502.404	-1.5844
Art v 1 12h												
1	10800.07	+256	+486	+1716	-6	+32	0	13284.07	1661.574	8	13284.59	-0.5236
1	10800.07	+256	+486	+1584	-4	+61	0	13183.07	1648.821	8	13182.57	0.4996
1	10800.07	+256	+324	+1584	-6	+61	0	13019.07	1628.438	8	13019.5	-0.434
1	10800.07	+256	+324	+1320	0	+32	0	12732.07	1592.422	8	12731.38	0.6932
1	10800.07	+256	+486	+1584	-6	+32	0	13152.07	1462.285	9	13151.56	0.5095
1	10800.07	+256	+324	+1584	-4	+61	0	13021.07	1447.713	9	13020.42	0.6512
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1213.337	8	9698.697	-0.0368
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.528	7	9698.697	-0.0367
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1617.45	6	9698.702	-0.0418
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1411.821	7	9875.75	0.8602
3	7664.61	+144	+486	+1584	0	0	0	9878.61	1647.456	6	9878.738	-0.1278
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1235.597	8	9876.773	-0.1628
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1592.937	6	9551.62	0.8798
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1365.664	7	9552.645	-0.1452
7	6906.92	+32	+486	+1584	-2	0	+18	9024.92	1290.208	7	9024.458	0.4619
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1495.402	6	8966.41	0.3098
15	5909.78	0	0	0	-4	0	0	5905.78	844.6843	7	5905.79	-0.0101
15	5909.78	0	0	0	-8	0	0	5901.78	738.3361	8	5898.689	3.0912

15	5909.78	0	0	0	-4	0	0	5905.78	657.3129	9	5906.816	-1.0361
15	5909.78	0	0	0	-2	0	0	5907.78	591.7828	10	5907.828	-0.048
16	5781.65	0	0	0	-4	0	0	5777.65	963.9545	6	5777.727	-0.077
16	5781.65	0	0	0	-4	0	0	5777.65	826.3903	7	5777.732	-0.0821
16	5781.65	0	0	0	-4	0	0	5777.65	723.3437	8	5778.75	-1.0996
16	5781.65	0	0	0	-4	0	0	5777.65	643.0845	9	5778.761	-1.1105
16	5781.65	0	0	0	-2	0	0	5779.65	578.9774	10	5779.774	-0.124
19	5566.39	0	0	0	-4	0	0	5562.39	928.0989	6	5562.593	-0.2034
19	5566.39	0	0	0	-4	0	0	5562.39	795.6579	7	5562.605	-0.2153
19	5566.39	0	0	0	-4	0	0	5562.39	696.4522	8	5563.618	-1.2276
19	5566.39	0	0	0	-4	0	0	5562.39	619.1811	9	5563.63	-1.2399
12	6068.99	0	0	0	-8	0	0	6060.99	758.2274	8	6057.819	3.1708
27	4642.35	0	0	0	-8	0	0	4634.35	773.4057	6	4634.434	-0.0842
29	4249.9	0	0	0	-6	0	+18	4261.9	853.5849	5	4262.925	-1.0245
31	4072.71	0	0	0	0	0	0	4072.71	1020.049	4	4076.196	-3.4864

CAA 12h

6	7376.5	+112	+486	+1584	-6	0	0	9552.5	1195.078	8	9552.626	-0.1264
6	7376.5	+112	+486	+1584	-6	0	0	9552.5	1365.664	7	9552.645	-0.1445
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.384	7	9697.69	0.9699
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1213.209	8	9697.672	0.988
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1617.448	6	9698.688	-0.028
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1411.965	7	9876.754	-0.1436
3	7664.61	+144	+486	+1584	-2	0	0	9876.61	1235.718	8	9877.744	-1.134
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1495.402	6	8966.413	0.3074
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1282.063	7	8967.44	-0.7196
16	5781.65	0	0	0	-6	0	0	5775.65	963.6167	6	5775.7	-0.0502
16	5781.65	0	0	0	-4	0	0	5777.65	826.2444	7	5776.711	0.9392
16	5781.65	0	0	0	-4	0	0	5777.65	723.0901	8	5776.721	0.9292
16	5781.65	0	0	0	-4	0	0	5777.65	642.9705	9	5777.735	-0.0845
15	5909.78	0	0	0	-6	0	0	5903.78	984.9598	6	5903.759	0.0212
15	5909.78	0	0	0	-6	0	0	5903.78	844.3957	7	5903.77	0.0101
15	5909.78	0	0	0	-8	0	0	5901.78	738.3355	8	5898.684	3.096
15	5909.78	0	0	0	-4	0	0	5905.78	657.1991	9	5905.792	-0.0119
19	5566.39	0	0	0	-6	0	0	5560.39	927.7624	6	5560.574	-0.1844
19	5566.39	0	0	0	-6	0	0	5560.39	795.512	7	5561.584	-1.194
19	5566.39	0	0	0	-6	0	0	5560.39	696.1993	8	5561.594	-1.2044
12	6068.99	0	0	0	-8	0	0	6060.99	758.2245	8	6057.796	3.194
27	4642.35	0	0	0	-8	0	0	4634.35	773.4052	6	4634.431	-0.0812
31	4072.71	0	0	0	0	0	0	4072.71	1020.049	4	4076.195	-3.4852
32	2988.45	0	0	0	-4	0	0	2984.45	597.7483	5	2983.742	0.7085
37	2500.82	0	0	0	0	0	0	2500.82	626.5986	4	2502.394	-1.5744

6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1195.081	8	9552.648	-0.148
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1365.661	7	9552.625	-0.1249
6	7376.5	+96	+486	+1584	-8	0	+18	9552.5	1592.937	6	9551.62	0.8804
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1213.336	8	9698.686	-0.0264
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1386.526	7	9698.679	-0.0192
5	7534.66	+96	+486	+1584	-2	0	0	9698.66	1617.275	6	9697.651	1.0088
3	7664.61	+144	+486	+1584	-4	0	0	9874.61	1235.344	8	9874.755	-0.1452
3	7664.61	+144	+486	+1584	-4	0	0	9874.61	1411.961	7	9876.73	-2.1198
3	7664.61	+144	+486	+1584	-4	0	0	9874.61	1647.119	6	9876.712	-2.1016
8	6738.72	+16	+486	+1716	-8	0	+18	8966.72	1281.918	7	8966.424	0.2961
19	5566.39	0	0	0	-4	0	0	5562.39	928.0995	6	5562.597	-0.207
19	5566.39	0	0	0	-4	0	0	5562.39	795.6578	7	5562.605	-0.2146
19	5566.39	0	0	0	-4	0	0	5562.39	696.327	8	5562.616	-0.226
19	5566.39	0	0	0	-4	0	0	5562.39	619.1811	9	5563.63	-1.2399
16	5781.65	0	0	0	-4	0	0	5777.65	963.9545	6	5777.727	-0.077
16	5781.65	0	0	0	-4	0	0	5777.65	826.3905	7	5777.734	-0.0835
16	5781.65	0	0	0	-4	0	0	5777.65	723.2192	8	5777.754	-0.1036
16	5781.65	0	0	0	-4	0	0	5777.65	643.084	9	5778.756	-1.106
12	6068.99	0	0	0	-8	0	0	6060.99	758.2253	8	6057.802	3.1876
21	5411.2	0	0	0	-4	0	0	5407.2	773.4057	7	5406.84	0.3601
18	5595.39	0	0	0	-2	0	+18	5611.39	802.8633	7	5613.043	-1.6531
29	4249.9	0	0	0	-6	0	+18	4261.9	853.5849	5	4262.925	-1.0245
31	4072.71	0	0	0	0	0	0	4072.71	1020.05	4	4076.198	-3.488

9	6218.11	+32	+486	+1716	-2	0	0	8450.11	939.9722	9	8450.75	-0.6398
9	6218.11	+32	+486	+1716	-2	0	0	8450.11	846.075	10	8450.75	-0.64
9	6218.11	+32	+486	+1716	-2	0	0	8450.11	705.1479	12	8449.775	0.3352
9	6218.11	+32	+486	+1716	-2	0	0	8450.11	769.2504	11	8450.754	-0.6444
19	5566.39	0	0	0	-6	0	0	5560.39	927.7628	6	5560.577	-0.1868
19	5566.39	0	0	0	-6	0	0	5560.39	795.369	7	5560.583	-0.193
19	5566.39	0	0	0	-6	0	0	5560.39	696.1997	8	5561.598	-1.2076
19	5566.39	0	0	0	-6	0	0	5560.39	618.9562	9	5561.606	-1.2158
27	4642.35	0	0	0	-6	0	0	4636.35	580.3055	8	4634.444	1.906
34	2811.18	0	0	0	-2	0	0	2809.18	562.3026	5	2806.513	2.667
36	2515.84	0	0	0	0	0	0	2515.84	630.086	4	2516.344	-0.504
35	2606.99	0	0	0	0	0	0	2606.99	653.1136	4	2608.454	-1.4644
31	4072.71	0	0	0	0	0	0	4072.71	1020.054	4	4076.214	-3.504

14	6006.85	+16	+486	+1584	-6	0	+18	8104.85	1158.918	7	8105.425	-0.5753
11	6077.23	+16	+486	+1584	-2	0	+18	8179.23	1023.563	8	8180.502	-1.2724

11	6077.23	+16	+486	+1584	-2	0	+18	8179.23	909.9457	9	8180.511	-1.2813
11	6077.23	+16	+486	+1584	-2	0	+18	8179.23	819.0518	10	8180.518	-1.288
11	6077.23	+16	+486	+1584	-2	0	+18	8179.23	744.6841	11	8180.525	-1.2951
19	5566.39	0	0	0	-6	0	0	5560.39	927.5929	6	5559.557	0.8326
19	5566.39	0	0	0	-6	0	0	5560.39	795.2237	7	5559.566	0.8241
19	5566.39	0	0	0	-6	0	0	5560.39	619.066	9	5562.594	-2.204
22	5350.09	0	0	0	-6	0	0	5344.09	891.5772	6	5343.463	0.6268
22	5350.09	0	0	0	-6	0	0	5344.09	764.4962	7	5344.473	-0.3834
29	4249.9	0	0	0	-6	0	+18	4261.9	853.5825	5	4262.913	-1.0125

CAA 48h

17	5626.45	0	0	0	-2	0	0	5624.45	938.4261	6	5624.557	-0.1066
19	5566.39	0	0	0	-6	0	0	5560.39	927.7628	6	5560.577	-0.1868
19	5566.39	0	0	0	-6	0	0	5560.39	795.3692	7	5560.584	-0.1944
19	5566.39	0	0	0	-6	0	0	5560.39	696.2	8	5561.6	-1.21
19	5566.39	0	0	0	-6	0	0	5560.39	618.9585	9	5561.627	-1.2365
22	5350.09	0	0	0	-6	0	0	5344.09	891.7455	6	5344.473	-0.383
23	5220.97	0	0	0	-6	0	0	5214.97	870.2377	6	5215.426	-0.4562
23	5220.97	0	0	0	-6	0	0	5214.97	746.0624	7	5215.437	-0.4668
23	5220.97	0	0	0	-6	0	0	5214.97	1043.878	5	5214.391	0.579
25	4904.62	0	0	0	-6	0	0	4898.62	817.3741	6	4898.245	0.3754
25	4904.62	0	0	0	-4	0	0	4900.62	980.849	5	4899.245	1.375
28	4442.06	0	0	0	-2	0	0	4440.06	635.1709	7	4439.196	0.8637
30	4125.71	0	0	0	-4	0	0	4121.71	825.5979	5	4122.99	-1.2795

Table S3. Identified peptides released during 15 min, 3, 12, 24 and 48 h of endolysosomal processing of Art v 1(A) and CAA (C).

	A15`	C15`	A3h	C3h	A12h	C12h	A24h	C24h	A48h	C48h
1	1	1	1		1					
2				2						
3		3	3	3	3	3	3			
4				4						
5		5	5	5	5	5	5			
6		6	6	6	6	6	6			
7			7		7					
8			8		8	8	8			
9								9		
10				10						
11									11	
12	12				12	12	12			
13		13		13						
14									14	
15	15	15	15	15	15	15				
16			16	16	16	16	16			
17										17
18							18			
19					19	19	19	19	19	19
20				20						
21							21			
22								22		22
23										23
24	24	24								
25										25
26	26	26								
27					27	27		27		
28										28
29	29				29		29		29	
30										30
31					31	31	31	31		
32	32					32				
33			33							
34								34		
35								35		
36								36		
37			37	37		37				

References

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