Structural basis for the recognition and cleavage of histone H3 by cathepsin L

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H3 Peptides*	Sequence**	(%) Inhibition***
$H3_{1-41}$	ARTKQTARKSTGGKAPRKQLATKAARK SA...KPHRY	88.4 ± 0.9
$H3_{4-19}$K14ac	KQTARKSTGGK$_{ac}$APRK Q	4.3 ± 0.7
$H3_{5-29}$K14ac	QTARKSTGGK$_{ac}$APRK QLATK AARK SA	84.5 ± 0.4
$H3_{14-31}$	K APRK QLATK AARK SAPA	85.4 ± 0.3
$H3_{14-31}$L20A	K APRK QAATK AARK SAPA	13 ± 1
$H3_{14-31}$L20AK27me3	K APRK QAATK AARK$_{27}$SAPA	19 ± 2
$H3_{14-31}$Q19A	K APRK ALATK AARK SAPA	82.5 ± 0.4
$H3_{14-31}$Q19A/L20A	K APRK AAATK AARK SAPA	14.9 ± 0.6
$H3_{14-31}$K18ac	K APRK$_{ac}$QLATK AARK SAPA	68.7 ± 0.2
$H3_{14-31}$K23ac	K APRK QLATK$_{ac}$AARK SAPA	82 ± 2
$H3_{14-31}$K18acK27me2	K APRK$_{ac}$QLATK AARK$_{me2}$SAPA	72.6 ± 0.7
$H3_{14-31}$K23acK27me2	K APRK QLATK$_{ac}$AARK$_{me2}$SAPA	83.9 ± 0.3
$H3_{14-31}$K18acK23Ac	K APRK$_{ac}$QLATK$_{ac}$AARK SAPA	67.7 ± 0.1
$H3_{14-31}$K27me2	K APRK QLATK AARK$_{me2}$SAPA	85.0 ± 0.1
$H3_{14-31}$K27me3	K APRK QLATK AARK$_{me3}$SAPA	84 ± 1
$H3_{19-33}$	QLATK AARK SAPATG	32.1 ± 0.1
$H3_{19-33}$K27me1	QLATK AARK$_{me1}$SAPATG	32 ± 1
$H3_{19-33}$K27me2	QLATK AARK$_{me2}$SAPATG	44.2 ± 0.2
$H3_{19-33}$K27me3	QLATK AARK$_{me3}$SAPATG	32 ± 2
$H4_{12-27}$ K20me3	GGAKRHRK$_{me3}$VLRDNIQ	68.3 ± 0.5
$H4_{15-27}$K20me1	AKRHRK$_{me1}$VLRDN	31 ± 4
$H4_{15-27}$K20me2	AKRHRK$_{me2}$VLRDN	19.8 ± 0.6
$H4_{15-27}$K20me3	AKRHRK$_{me3}$VLRDN	44.3 ± 0.3

Supplementary Table S1. Competitive enzymatic assays for cathepsin L with histone-derived peptides.

* Peptide numbering corresponds to the sequences of the H3 and H4 histone tails.

**Sites of post-translational modification are numbered, mutations are shown in bold.

***relative to the average rate of observed inhibition obtained for the unrelated peptides PPGMR$_{me2}$GPPP and PPGMR$_{me2}$PPMGP.

H3 Peptides*	**Sequence****	**(%) Inhibition*****
$H3_{12-33}$K18ac	GGK APRK$_{ac}$QLATK AARK SAPATG	68.0 ± 0.7
$H3_{12-33}$K23ac	GGK APRK QLATK$_{ac}$AARK SAPATG	81.3 ± 0.3
$H3_{12-33}$K18acK23ac	GGK APRK$_{ac}$QLATK$_{ac}$AARK SAPATG	59 ± 4
$H3_{12-33}$K23acK27me1	GGK APRK QLATK$_{ac}$AARK$_{me1}$SAPATG	84 ± 2
$H3_{12-33}$K23acK27me2	GGK APRK QLATK$_{ac}$AARK$_{me2}$SAPATG	79.9 ± 0.5
$H3_{12-33}$K23acK27me3	GGK APRK QLATK$_{ac3}$AARK$_{me3}$SAPATG	57 ± 4
$H3_{12-33}$K18acK27me1	GGK APRK$_{ac}$QLATK AARK$_{me1}$SAPATG	61 ± 4
$H3_{12-33}$K18acK27me2	GGK APRK$_{ac}$QLATK AARK$_{me2}$SAPATG	68 ± 1
$H3_{12-33}$K18acK27me3	GGK APRK$_{ac}$QLATK AARK$_{me3}$SAPATG	61 ± 3
$H3_{14-31}$L20AK27me1	K APRK Q**A**ATK AARK$_{me1}$SAPA	nd

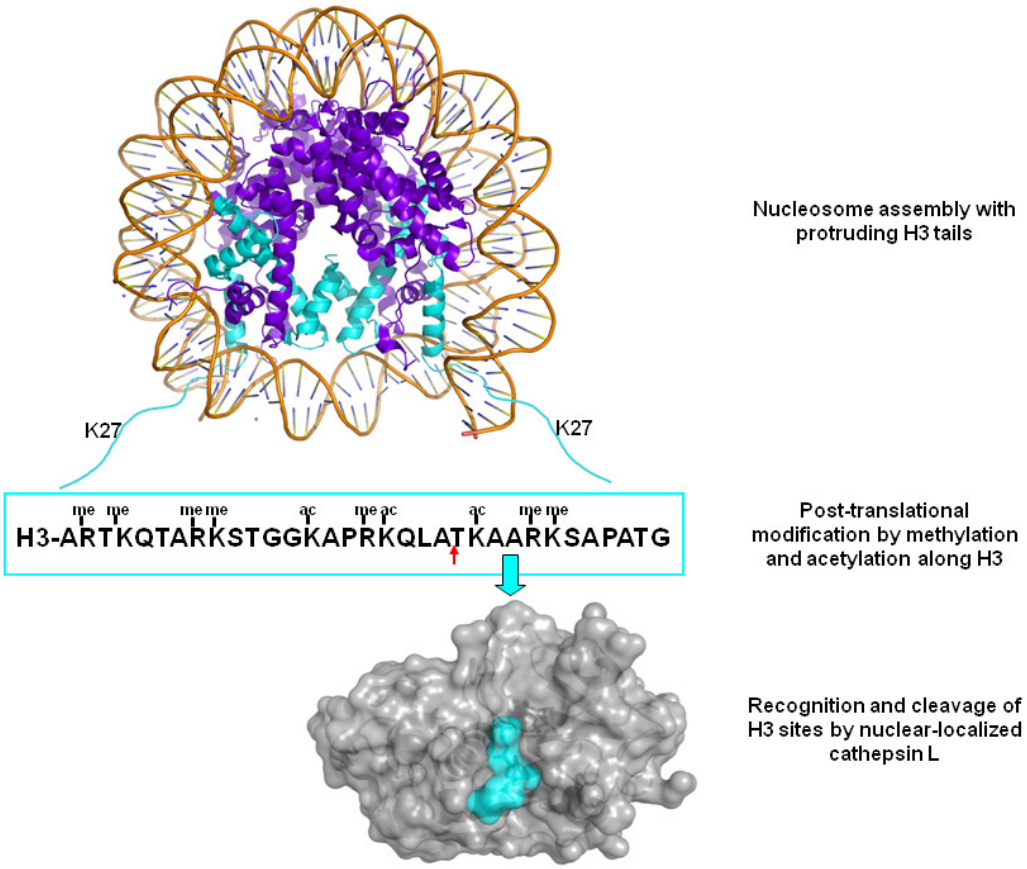
Supplementary Table S2. Competitive enzymatic assays for cathepsin L with FITC-labeled H3-derived peptides.

* Peptide numbering corresponds to the sequences of the H3 histone tails.

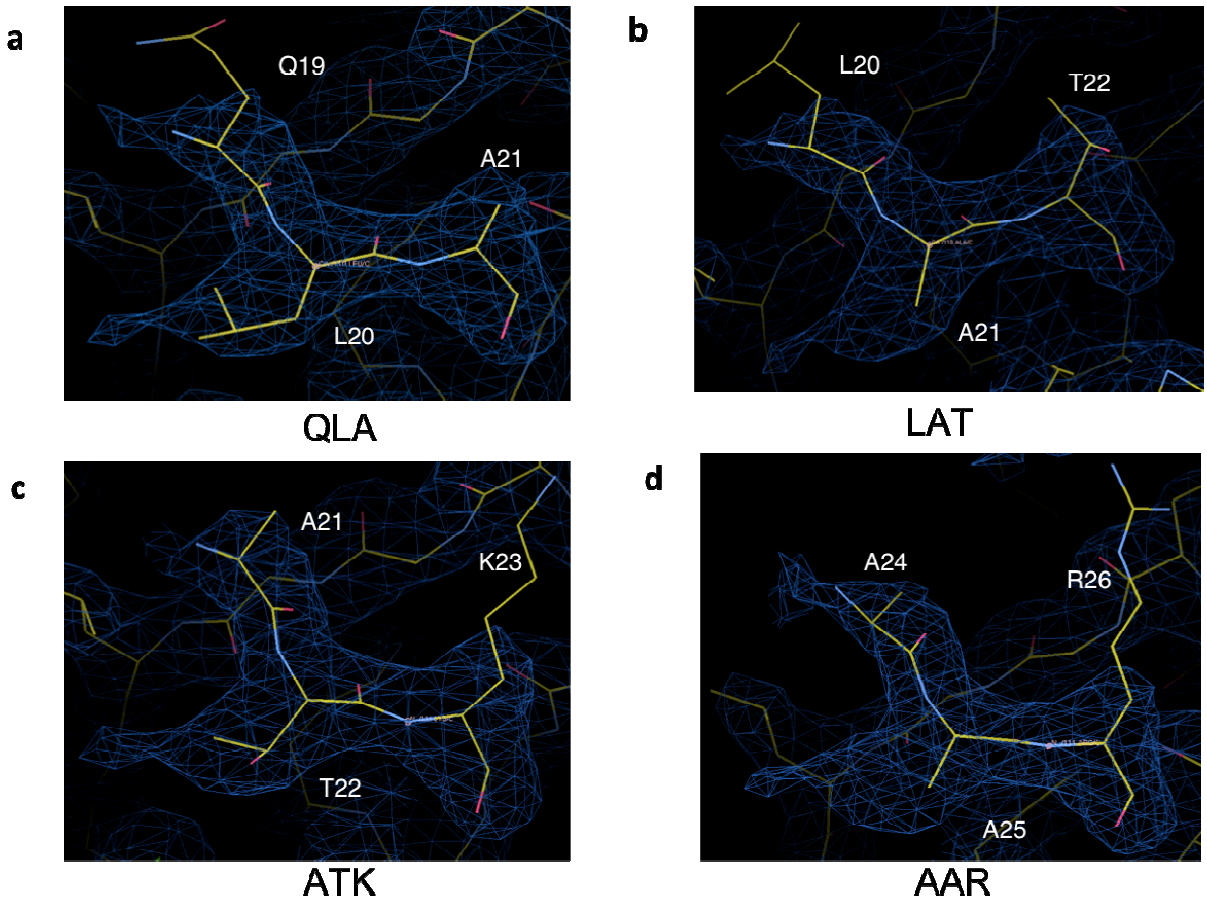
**Sites of post-translational modification are numbered, mutations are shown in bold.

***relative to the average rate of observed inhibition obtained for the unrelated, FITC-labeled peptides PPGMR$_{me2}$GPPP and PPGMR$_{me2}$PPMGP.

nd, not detected



Supplementary Figure S1. Cathepsin L cleaves accessible histone H3 tails. In this work, we have studied the molecular mechanisms of cathepsin L processing of the histone H3 tail. H3 histones are colored in cyan in the nucleosome structure (top) and a histone H3 tail is colored in cyan in the surface representation (bottom). The N-terminal sequence of histone H3 is shown in the middle panel with identified modifications. The cleavage site in marked by an red arrow.



Supplementary Figure S2. Systematic shifting of the histone H3 peptide register throughout the active site cleft placing other major cleavage sites in the S2 subsite. Only three residues from the histone H3 peptide are visible in the electron density map of the complex structure. In the previous study by Duncan *et al*[16], four major cleavage sites were identified: QLA|T|K|AAR|KSA. | denotes the scissile bond. The tripeptides tried in this test are: a: QLA; b. LAT; c, ATK; d: AAR. The electron density map is generated with the histone peptide omitted. Peptide sequence used in crystallization is QLATKAARKSAPATG