Supplementary Data:

A combined approach for the study of histone deacetylase inhibitors

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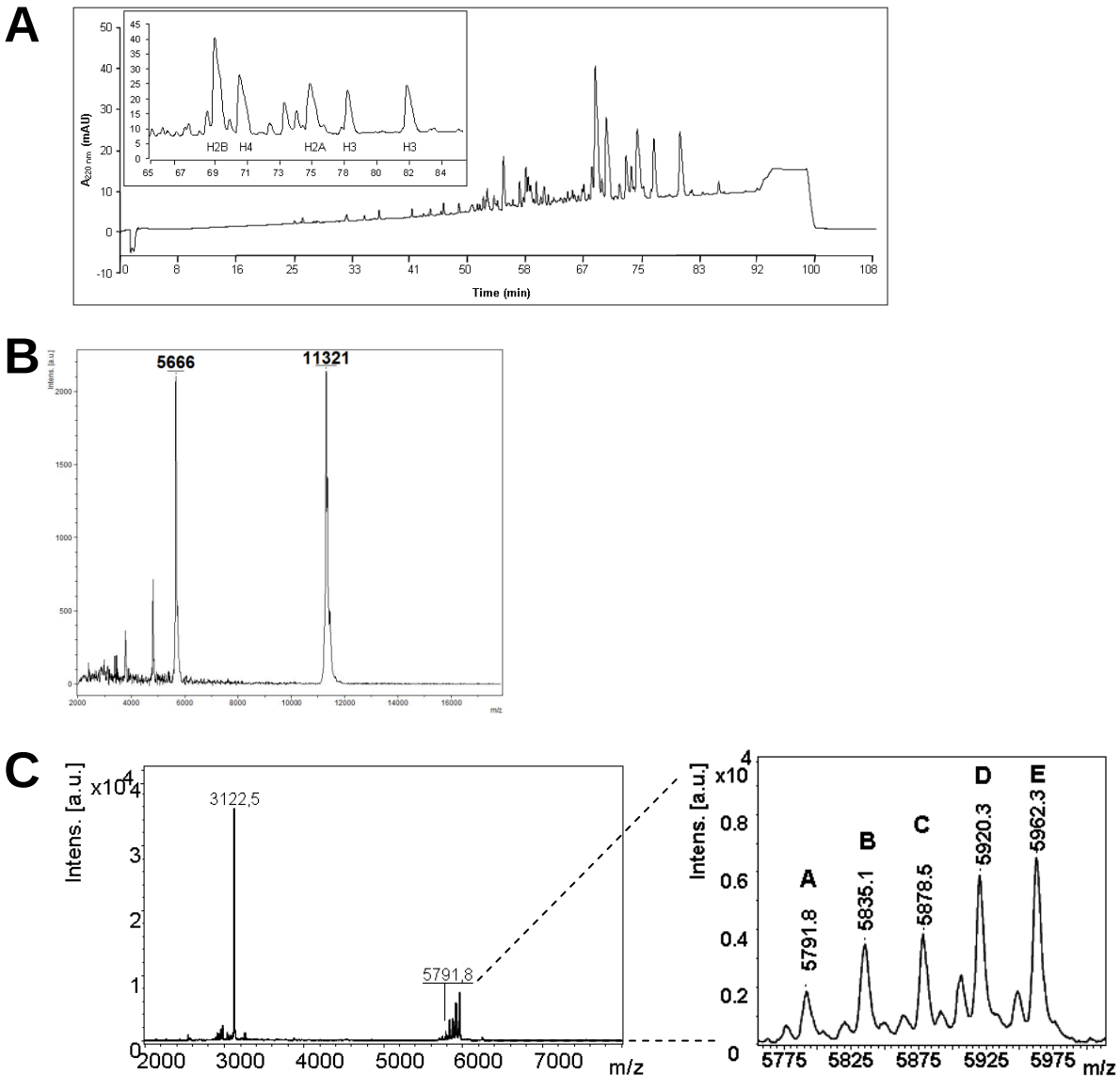
Fig. S1: MALDI-MS - TSA analysis



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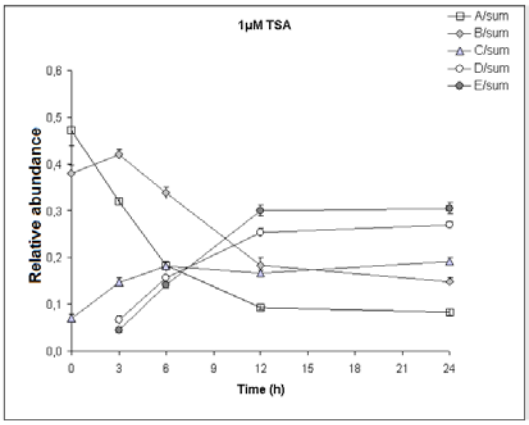

Fig. S1 Analysis of H4 N-terminus after TSA treatment. MEC-1 cells were treated with 1 µM TSA, histone extracts were fractionated by RP-HPLC, histone H4 was digested by Glu-C and analysed by MALDI-TOF MS. (A) Representative RP-HPLC chromatogram of the core histones separation. Gradient elution was performed using solvents A [water:ACN:TFA (100:2:0.1, v/v/v)] and B [water:ACN:TFA (100:2:0.1, v/v/v)]: from A/B 100/0 (v/v) to A/B 20/80 (v/v) over 110 min, at a flow rate of 9 µL/min. (B) The purity of each fraction corresponding to histone H4 was check by MALDI-TOF MS; representative MALDI-TOF MS spectrum of intact histone H4. (C) Representative MALDI-TOF MS spectra of histone H4 after Glu-C digestion; signals corresponding to non-, mono-, di-, tri-, and tetraacetylated variants (peaks A–E) of H4 N-terminus are indicated (peak 3122.5 corresponds to another peptide of histone H4). (D) Dependence of relative abundance of H4 N-terminal variants on incubation time with TSA (means and 95 % confidence limits, n = 15). The relative abundance of the ion signal was determined as the ratio of the single ion signal intensity to the sum of the intensities of all H4 isoforms within the spectrum. The histones were chromatographed for 110 min at 70 °C, with a constant flow of 9 µL/min with a multi-step acetonitrile gradient.

Fig. S2: Image analysis AUT1D

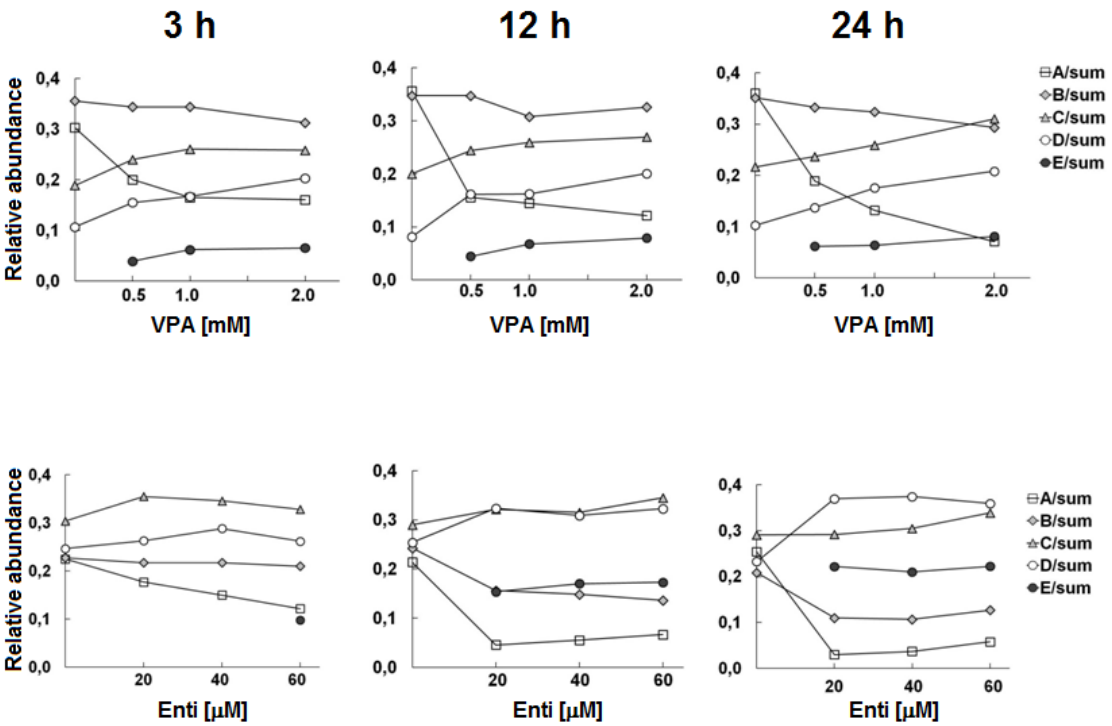

Fig. S2 Dependence of relative abundance of H4 isoforms on incubation time with VPA and Enti. 25 µg of each protein extract was separated by AUT PAGE (12 % AUT PAGE, 5 M urea), proteins were visualized with Bio-Safe coomassie stain (Bio-Rad). Image analysis of histone H4 variants was done with Quantity One 4.6.1 (Bio-Rad). The relative abundance of the band density was determined as the ratio of the single band density to the sum of the densities of all H4 variants within the gel.