Data Supplement

DNA and Factor VII-activating protease protect against the cytotoxicity of histones

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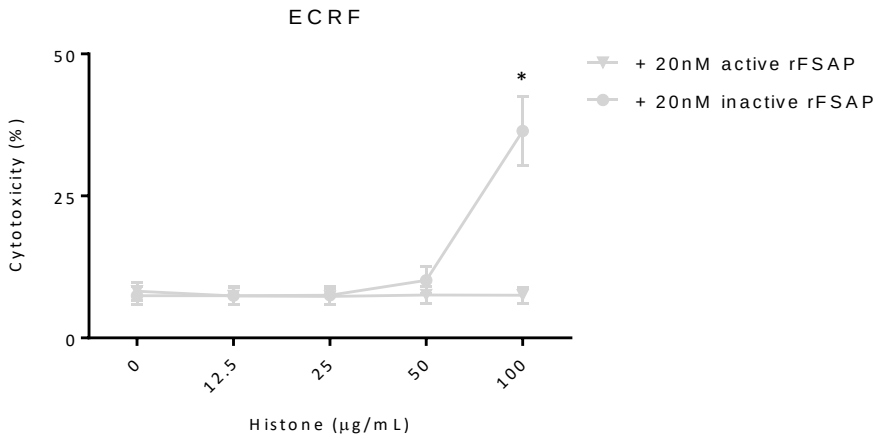
A



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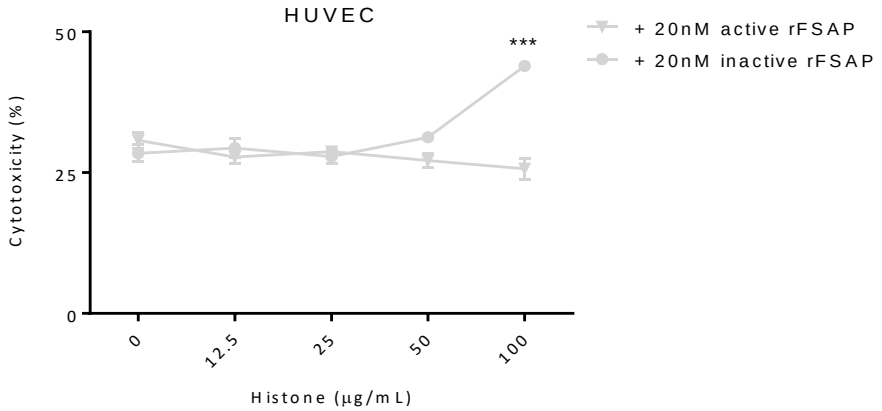
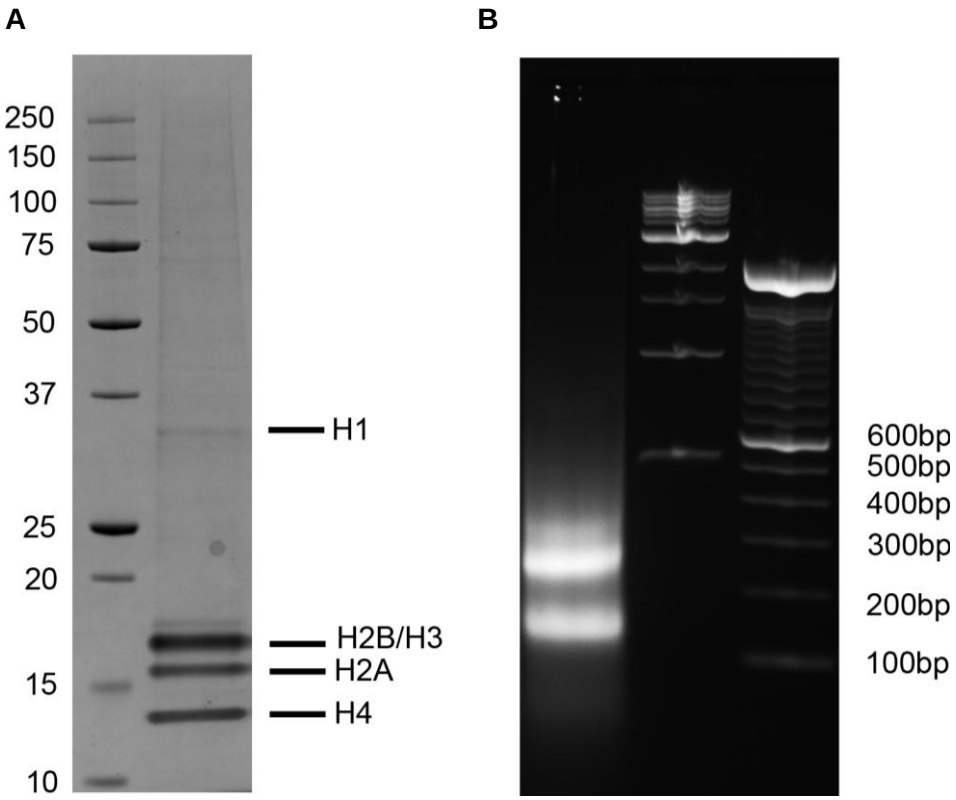


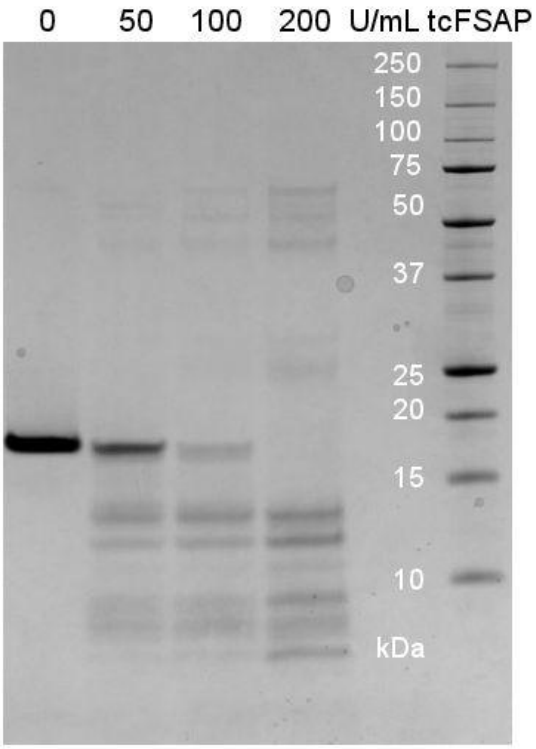
Supplemental Figure 1. Active FSAP provides protection against histone-induced cytotoxicity of ECRF cells and HUVEC

Histones (100 µg/mL) were incubated with 20 nM active recombinant FSAP or inactive recombinant FSAP for 30 minutes prior to overnight incubation with ECRF (A) or HUVEC (B) cells. To determine cytotoxicity, LDH levels were determined in the supernatant. Cytotoxicity was expressed as a percentage of the maximal cytotoxicity induced using 0.1% saponin. Data are expressed as mean ±SEM obtained from two independent experiments performed in triplicate. * $p<0.05$ *** $p<0.001$ were calculated using an unpaired, two-tailed Student's *t*-test.



Supplemental Figure 2 Characterization of purified nucleosomes

Purified nucleosomes were separated on SDS-PAGE and proteins were visualized by Instant Blue staining (A) or nucleosomes were separated on 1.4% agarose gel and DNA was visualized by dsRED (B). The different histone subtypes present in the purified nucleosomes (A) and the mono- and di-nucleosome sized DNA (B) are clearly visible.



Supplemental Figure 3. N-terminal sequencing of the 13 kDa histone H3 cleavage product upon FSAP-mediated proteolysis

Recombinant histone H3.1 (100 µg/mL, New England Biolabs) was cleaved with 0-200 U/mL tcFSAP for 1h at 37°C and the cleavage products separated on 10% SDS-PAGE followed by Instant Blue staining (Expedeon). The 13 kDa band that was visible using 200 U/mL FSAP was excised and sent for N-terminal sequencing by Alta Biosciences. The N-terminus of the 13 kDa fragment consisted of the amino acids SAPATGG, indicating that FSAP had cleaved histone H3 between K27 and S28. A slightly smaller band of 12 kDa was also sent for N-terminal sequencing and revealed the same N-terminal amino acids, suggesting that some C-terminal clipping to give rise to the 12 kDa band had occurred.