Supplemental data

Supplemental Materials and Methods

ELISA

Microtiter plates were coated overnight with histones (100 nM) in coating buffer (15.9 mM Na_2CO_3, 30 mM $NaHCO_3$, pH 9.6) and stored over night at 4°C. Plates were then washed three times in deionized water, blocked in phosphate buffered saline containing 0.05% Tween-20 (PBST) and 0.5% bovine serum albumin for 30 min at 37°C. After a washing step, plates were probed with MBP (8 nM) for 1 h at 37°C and binding was detected with a polyclonal antisera against MBP-p33 (1:5000) and a peroxidase-conjugated antibody against rabbit IgG (1:2500, 1 h, 37°C, Bio-Rad Laboratories). All incubations were followed by a washing step in PBST. The polyclonal antisera against MBP-p33 was tested to detect MBP and MBP-p33 equally in equimolar concentrations.

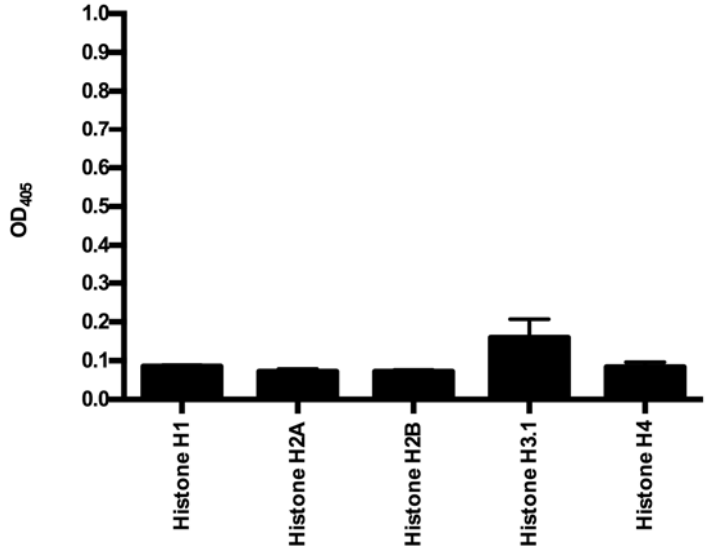
In vitro hemolysis assay

Citrated blood (1 ml) was centrifuged (2000 x g, 10 min, 4°C), plasma was removed and replaced by 1 ml PBS. The washing step was repeated two times. Histone H4 were diluted in PBS to 10 μM in presence or absence of 10 μM native p33 to a final volume of 60 μl. Tox-7 lysis buffer and PBS served as positive and negative control, respectively. Three μl washed blood cells (5% v/v) were added to each sample and the samples were incubated (60 min, 37°C on rotation) in a heat block. All samples were centrifuged (2000 x g, 10 min, RT) and the supernatants were transferred to microtiter plates. Absorbance of hemoglobin was measured at 540 nm and histone-induced hemolysis was expressed as percentage of Tox-7 lysis induced hemolysis.

In vivo imaging of MBP-p33 in mice

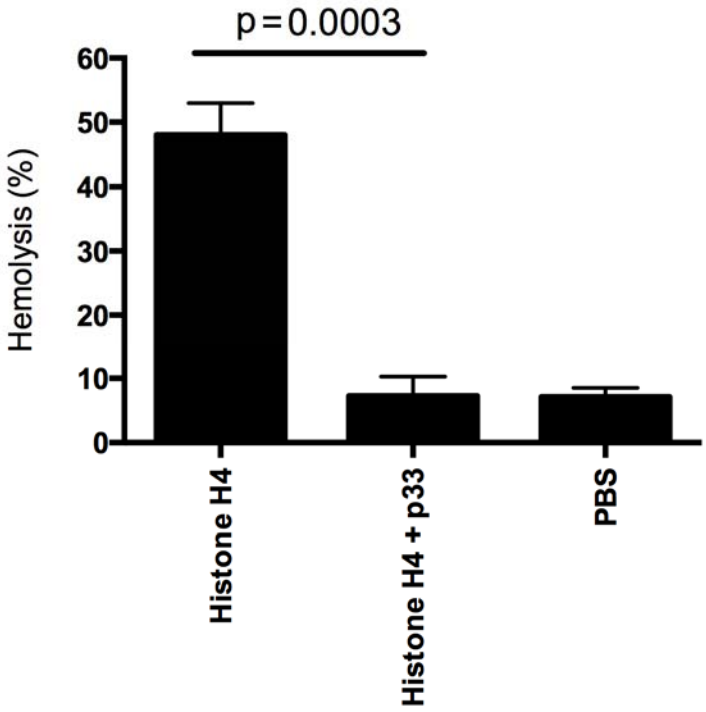
MBP-p33 was labeled with a fluorochrome (VivoTag 680XL) using Protein Labeling Kit (PerkinElmer) according to manufacturer's instructions. Mice, anesthetized with isofluorane, were injected i.v. with fluorescent MBP-p33 and its distribution was visualized every day for 7 days using the *In Vivo* Imaging System from PerkinElmer (IVIS Spectrum). At time points 1 h and 1 day, mice were sacrificed and MBP-p33 distribution in different organs was visualized. PBS injected mice served as control.

Supplemental Figure legends

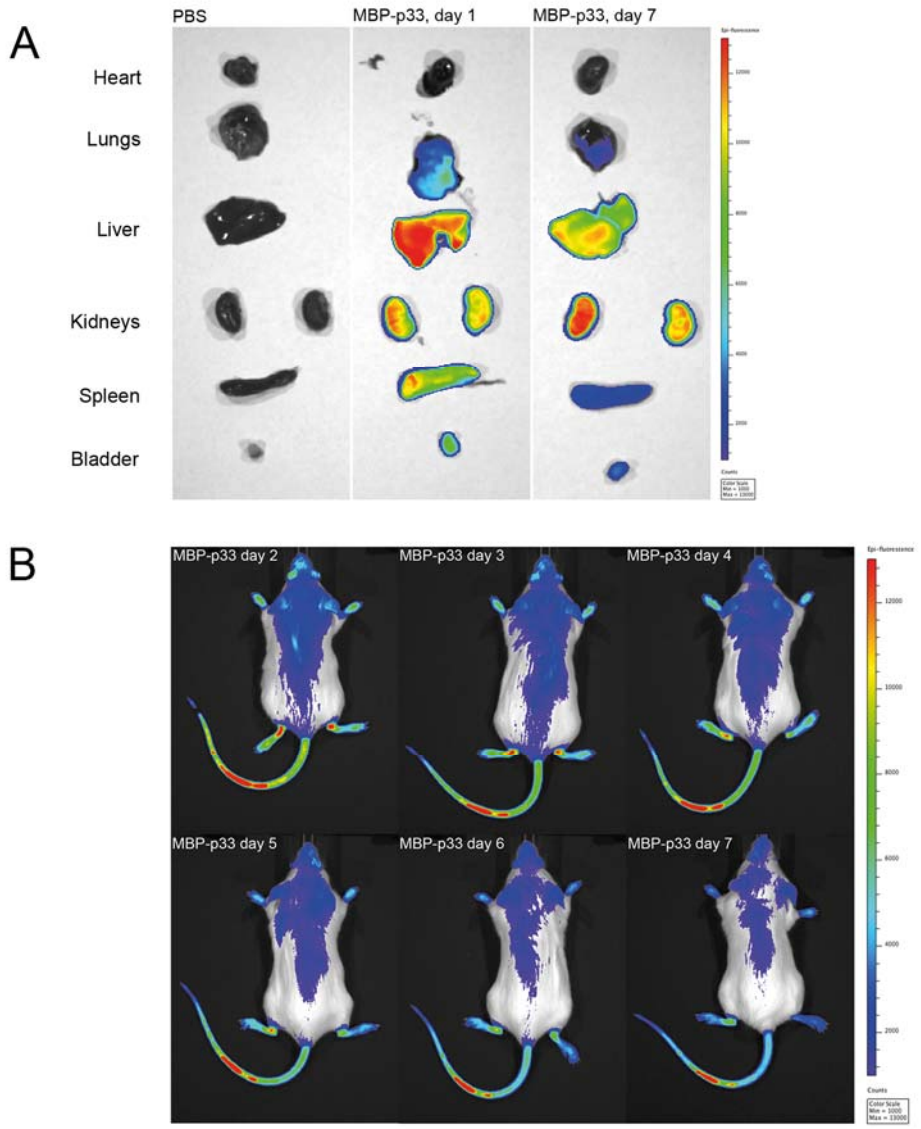


Supplemental Figure 1. **MBP does not bind to histones**

Microtiter plates were coated with different subclasses of histones (100 nM), probed with MBP (8 nM) and detected with a polyclonal antibody against MBP-p33. Mean and SE from three individual experiments are shown.



Supplemental Figure 2. **Native p33 inhibits the cytolytic activity of histone H4 against erythrocytes.** Histone H4 (10 μM) were incubated (60 min at 37^{o}C) with washed and diluted human erythrocytes (5% v/v) in the absence or presence of 10 μM p33. Samples were centrifuged and the release of hemoglobin was measured in the supernatant at 540 nm. Hemolysis was calculated as percentage of cells treated with Tox-7 Lysis buffer. Data are mean and SE values from three separate experiments.



Supplemental Figure 3. **Deposition of injected MBP-p33 in mice.**

(A) Fluorescently labeled MBP-p33 was i.v. injected into a Balb/c mouse and its distribution and deposition in animals was followed for 7 days by an *IVIS* Spectrum station. PBS was used as a control.

(B) Organs from mice injected with fluorescently labeled MBP-p33 at time points day 1 and day 7. PBS was used as a control.