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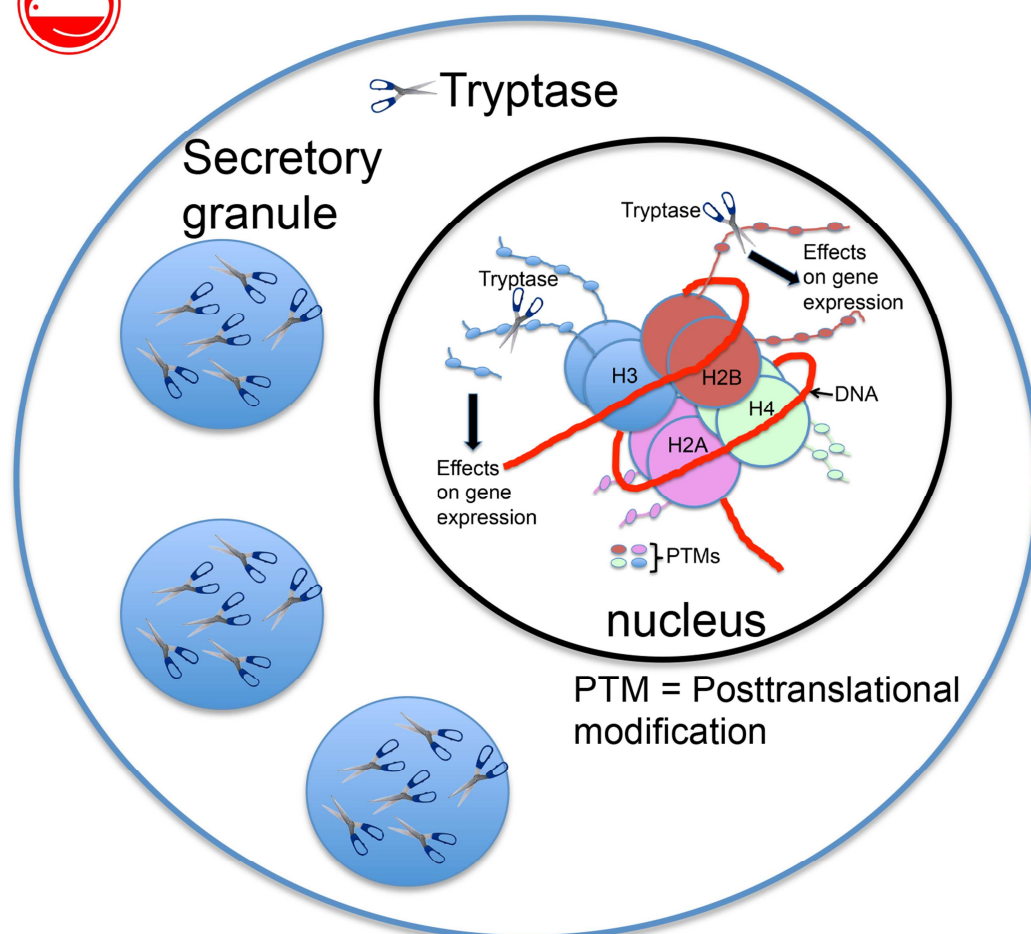
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Mast cell



Tryptase-catalyzed core histone truncation: a novel epigenetic regulatory mechanism in mast cells

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Abstract

Background: Mast cells are key effector cells in allergic reactions. When activated to degranulate, they release a plethora of bioactive compounds from their secretory granules, including mast cell-restricted proteases such as tryptase. In a previous study we showed that tryptase, in addition to its intragranular location, can be found within the nuclei of mast cells where it truncates core histones at their N-terminal ends.

Objective: Considering that the N-terminal portions of the core histones constitute sites for posttranslational modifications of major epigenetic impact, we here evaluated whether histone truncation by tryptase could have an impact on epigenetic events in mast cells.

Methods: Mast cells were cultured from wild type and tryptase null mice, followed by an assessment of their profile of epigenetic histone modifications and their phenotypic characteristics.

Results: We show that tryptase truncates nucleosomal histone 3 (H3) and H2B and that its absence results in accumulation of the epigenetic mark, lysine 5-acetylated H2B (H2BK5ac). Intriguingly, the accumulation of H2BK5ac was cell age-dependent and was associated with a profound upregulation of markers of non-mast cell lineages, loss of proliferative control, chromatin remodeling as well as extensive morphological alterations.

Conclusion: These findings introduce tryptase-catalyzed histone clipping as a novel epigenetic regulatory mechanism, which in the mast cell context may be crucial for maintaining cellular identity.

Keywords: Mast cells, epigenetics, histone acetylation, tryptase, mMCP6, serglycin, H2B, H2BK5ac

Key messages:

- Tryptase is found in the chromatin of mast cells, where it truncates N-terminal ends of core histones 3 and 2B
- The absence of tryptase leads to an age-dependent accumulation of the epigenetic mark acetylated histone 2B (H2BK5ac)
- The accumulation of H2BK5ac leads to extensive effects on the state of mast cell differentiation, including effects on gene expression, morphology and proliferative control

Capsule summary:

Tryptase is mainly implicated in events downstream of mast cell degranulation. Here we show that tryptase additionally has a role in intranuclear core histone processing, thereby affecting epigenetic regulation and cellular differentiation in mast cells.

Keywords: mast cells, epigenetics, core histones, tryptase, histone acetylation, secretory granules, serglycin proteoglycan

Abbreviations:

MC, mast cell; H, histone; CPA3, carboxypeptidase A3; mMCP, mouse mast cell protease; WT, wild type; CFSE, carboxyfluorescein diacetate succinimidyl ester; TEM, transmission electron microscopy; ChIP-seq, chromatin immunoprecipitation-sequencing

Introduction

Mast cells (MCs) are hematopoietic cells with a wide impact on various pathophysiological settings, most notably allergy but also cancer, atherosclerosis, obesity and autoimmune disease^{1, 2}. A hallmark feature of MCs is their high content of secretory granules, filled with a plethora of bioactive substances such as bioactive amines (histamine and serotonin), cytokines, growth factors, proteoglycans of serglycin type and multiple proteases including tryptases, chymases and carboxypeptidase A3 (CPA3)^{3, 4}. Although the general notion is that the default fate of these granule-contained compounds is to be delivered to the extracellular milieu where they influence various inflammatory processes, we recently showed that one of the MC granule-contained proteases, tryptase, can also be found in the nucleus⁵. Moreover, we showed that nuclear tryptase has the ability to proteolytically remove core histone N-terminal tails, thereby acting as a histone “clippase”. It is now established that the N-terminal tails of core histones constitute sites for posttranslational modification (PTM) events that have a wide epigenetic impact^{6, 7, 8}. Conceivably, tryptase-catalyzed histone clipping may thus cause changes in the pattern of epigenetic histone modifications, potentially affecting gene expression and cellular phenotype. Here we investigated this possibility and demonstrate that the absence of tryptase causes an age-dependent accumulation of one epigenetic mark – acetylated histone 2B lysine 5 (H2BK5ac). Furthermore, extensive effects on cellular phenotype accompany this change to the chromatin landscape. Thereby, tryptase-catalyzed N-terminal clipping of H2B emerges as a novel epigenetic regulatory mechanism in MCs.

Methods

Animals and cells. Wild type (WT), serglycin^{-/-}⁹ and mMCP6^{-/-}¹⁰ mice were on C57BL/6J genetic background. Bone marrow-derived MCs (BMMCs) were cultured in IL-3-containing medium as described¹¹.

Methods. Proliferation was assessed by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution. Cell morphology was assessed by Transmission Electron Microscopy (TEM)¹² or light microscopy after staining with May Grünwald/Giemsa or toluidine blue¹¹. Western blot analysis¹¹, ChIP-seq analysis¹³, oligonucleosome purification¹⁴ and quantitative real time RT-PCR (qPCR) analysis¹¹ were carried out as described. Immunohistochemical staining and confocal microscopy was performed using antibodies to H2BK5ac, mMCP6 and CPA3.

For further details, see the Methods section in this article's Online Repository.

Results

Absence of tryptase or serglycin in MCs results in age-dependent loss of proliferative control

We showed previously that MC tryptase (mouse MC protease 6 (mMCP6)) has the ability to proteolytically remove the N-terminal tails of core histones⁵. To assess whether this can affect the epigenome of the MCs such that their phenotype is altered, we developed bone marrow-derived MCs from WT and mMCP6^{-/-} mice¹⁵ and assessed their characteristics. In addition, we developed MCs from mice lacking serglycin, a proteoglycan that is crucial for the storage of mMCP6 in MCs⁹. Hence, the phenotypic characteristics of tryptase-deficient cells should be mimicked in cells lacking serglycin.

Approximately equal numbers of bone marrow cells were obtained from wild type (WT), serglycin^{-/-} and mMCP6^{-/-} mice and addition of MC-driving growth factors (IL-3-containing medium) to the bone marrow cells produced an expansion of the cultures at approximately equal rates up to ~3 months (Fig. 1A-C). However, when cultures were followed over an extended time, we noticed that cultures of mMCP6^{-/-} cells expanded at a considerably higher rate than did cultures of WT cells (Fig. 1B, D). Notably, the onset of exaggerated expansion rates differed between individual cultures, with some cultures showing an onset at ~3 months whereas a delayed onset was seen in others (Fig. 1D). Expansion of the MC niche was also seen *in vivo* when analyzing tissues from aging mMCP6^{-/-} mice (Fig. 1C and Suppl. Fig. 1). Similar to mMCP6^{-/-} cells, serglycin^{-/-} cultures exhibited age-dependent increased expansion rates (Fig. 1B, D). To evaluate whether the increased cell number expansion seen in mMCP6^{-/-} and serglycin^{-/-} cultures was a result of increased proliferation we performed a CFSE dilution experiment. Indeed, aged mMCP6^{-/-} and serglycin^{-/-} cells showed a markedly higher rate of proliferation than did corresponding WT cells (Fig. 1E-F). In contrast, similar rates of proliferation were seen in WT, mMCP6^{-/-} and serglycin^{-/-} cells taken before the observed onset of increased expansion

rates (data not shown). The numbers of apoptotic cells did not differ between WT, mMCP6^{-/-} and serglycin^{-/-} cultures (data not shown), reinforcing that the increased expansion rates seen in mMCP6^{-/-} and serglycin^{-/-} cultures is a result of increased proliferation. The increased proliferation of mMCP6^{-/-} and serglycin^{-/-} cells was not associated with reduced growth factor dependency (Fig. 1G-H). In fact, mMCP6^{-/-} and serglycin^{-/-} cells underwent cell death at a considerably higher rate than WT cells after growth factor deprivation (Fig. 1G-H).

Tryptase- or serglycin-deficiency causes age-dependent morphological alterations

No major morphological differences between WT and mMCP6^{-/-} cells were seen in young cultures (up to ~3 months; not shown). However, after extended culture times we noted that mMCP6^{-/-} cells exhibited a marked increase in cellular size, as assessed both by side/forward scatter analysis (Fig. 2A-D) and by inspection with light microscopy (Fig. 2E-F). An increase in cellular size was also observed in serglycin^{-/-} cells (Fig. 2A-E). Moreover, we noted the frequent appearance of multinucleated and giant cells in cultures of mMCP6^{-/-} MCs (Fig. 2E-F), which were absent in WT cultures (Fig. 2E). Similar morphological changes were also seen *in vivo*, as manifested in tissue MCs of aging mMCP6^{-/-} mice (Fig. 2G and Suppl. Fig. 2). It was also apparent that serglycin^{-/-} and mMCP6^{-/-} cells frequently showed dramatic changes in their nuclear architecture, with many cells displaying a multi-lobulated or kidney-shaped rather than round nucleus (Fig. 2E-F).

We also analyzed cells by TEM. As seen in Fig. 3, WT cells had a rounded nucleus with defined nucleolus, and possessed numerous cytoplasmic granules with mixed regions of dense core and electron translucent areas, in agreement with previous studies¹⁶. In contrast, the nuclei of mMCP6^{-/-} and serglycin^{-/-} cells showed signs of extensive chromatin remodeling, exhibiting marked deviation from the rounded nuclear shape of WT cells and with many cells showing a more macrophage-like or multi-lobulated morphology (Fig. 3). Together, the light microscopy

and TEM analysis indicate that the absence of mMCP6 or serglycin has a large, cell age-dependent impact on the chromatin organization of MCs.

Tryptase is localized to chromatin and causes clipping of nucleosomal H2B and H3

As chromatin remodeling is a hallmark manifestation of epigenetics, the data above support our hypothesis that the absence of the tryptase-serglycin axis may influence epigenetic regulatory mechanisms in MCs. Hence, we set out to investigate this possibility. In agreement with our previous findings⁵, the absence of mMCP6 resulted in a dramatic reduction in the levels of truncated H3 and H2B (Fig. 4A). In further agreement with this notion, incubation of WT MCs with a selective tryptase inhibitor (Nafamostat mesylate) caused a substantial reduction in the truncation of H2B and H3 (Fig. 4B). Next, we assessed whether tryptase can cleave histones that are incorporated into nucleosomes, the latter a prerequisite for having an impact on epigenetic regulation. To address this, we purified nucleosomes from HeLa cells and incubated these with recombinant tryptase. Indeed, tryptase was able to truncate histones in a nucleosomal context, with H2B and H3 being particularly susceptible (Fig. 4C). H2A was less susceptible and H4 was largely resistant to tryptase-catalyzed clipping (Fig. 4C). A dose response assay showed that tryptase was efficient at concentrations down to 0.25 ng/ml (Fig. 4C). Moreover, time course analysis revealed that tryptase-catalyzed clipping of nucleosomal histones was a very rapid event (Fig. 4D). Although we showed previously by confocal microscopy that tryptase is found in the nucleus of MCs⁵, it has not been demonstrated whether tryptase is in fact associated with chromatin. To clarify this issue, we prepared cytosolic, nuclear and chromatin fractions, and assessed the mMCP6 levels in these fractions by immunoblot analysis. As expected, the cytosolic fractions had high levels of tryptase, originating from the cytoplasmic secretory granules (Fig. 4E). It was also evident that mMCP6 could be found both in the nuclear and chromatin fractions. In contrast, another prominent granule protease, CPA3, was only detected in the cytosolic fraction. Hence, these data support that tryptase is associated with chromatin in

MCs. It was also apparent that truncated H2B was found within the chromatin (as well as in the nuclear fraction), whereas truncated H3 was seen in the nuclear but not in the chromatin fraction (Fig. 4E).

Tryptase regulates H2B lysine 5 acetylation

To evaluate whether tryptase-catalyzed histone clipping in fact influences the levels of epigenetic histone marks, we performed analysis of histone PTMs using antibodies detecting histone modifications of known major biological significance. We thereby focused on PTMs of H3 and H2B since our data above suggested that these were particularly susceptible to tryptase-catalyzed clipping (see Fig. 4). As shown in Fig. 5A and F, immunoblot signals were obtained for the H3K9me3 (H3 trimethylated at lysine 9), H3K27me3, H3K9ac (H3 acetylated at lysine 9), H3K14ac and H3K4me1 modifications of H3. However, signals were of approximately equal intensity in WT, serglycin^{-/-} and mMCP6^{-/-} cells. For H2B, signals corresponding to the H2BK5ac, H2BK9ac and H2BK12ac modifications were detected. Intriguingly, although the signals for the H2BK9ac and H2BK12ac marks were equal among the genotypes, there was an increase in H2BK5ac in both mMCP6^{-/-} and serglycin^{-/-} MCs in comparison with WT controls (Fig. 5A, B), suggesting that tryptase clipping of H2B affects this particular epigenetic modification. In agreement with this, incubation of WT MCs with tryptase inhibitor resulted in significant accumulation of the H2BK5ac mark (Fig. 5C). Further, confocal microscopy analysis revealed a markedly more intense staining for H2BK5ac in the nuclei of mMCP6^{-/-} vs. WT cells (Fig. 5D). Accumulation of H2BK5ac was only seen in cell cultures after prolonged culture times (from ~3-4 months)(Fig. 5E-G). Hence, the accumulation of H2BK5ac occurs in a cell age-dependent manner in mMCP6^{-/-} and serglycin^{-/-} cells, and there is thus a correlation between the onset of increased cellular proliferation (see Fig. 1) and accumulation of this particular PTM.

The absence of tryptase results in upregulated expression of non-MC lineage markers

Given the suggested role for H2BK5ac as a marker for active chromatin¹⁷ we next investigated how the epigenetic effects of tryptase deficiency affect gene expression. To address this issue, we first screened for genes differentially expressed in mMCP6^{-/-} cells (taken after 4 months of culture) compared to WT cells using a microarray analysis of total RNA. Indeed, the gene array analysis indicated that numerous genes were extensively upregulated in mMCP6^{-/-} cells (data not shown). Several of these represented markers of non-MC lineages, including *Emr1* (also known as F4/80 or *Adgre1*), which is a well-established macrophage marker. Also *Ctsg*, a gene expressed mainly by neutrophils, showed a strong upregulation in mMCP6^{-/-} cells. There was also a profound upregulation of *Mcpt8* (coding for mMCP8), an established marker of basophils. The upregulation of these non-MC lineage markers in tryptase-deficient cells was confirmed by qPCR analysis, whereas the expression of a control gene (*Cpa3*) was similar among the genotypes (Fig. 6A). Importantly, the upregulation of these genes was prominent in aged cell cultures, whereas approximately equal expression was seen in 6-week old WT and mMCP6^{-/-} cells (Fig. 6B). To further substantiate these findings we performed flow cytometry analysis of the expression of *Emr1* / F4/80. In agreement with the gene array and qPCR data, profoundly higher cell surface expression of F4/80 was seen in aged mMCP6^{-/-} vs. WT cells (Fig. 6C, D). Elevated F4/80 surface expression was also seen in serglycin^{-/-} cells (Fig. 6C, D). In contrast, surface expression of F4/80 was low and approximately equal among the genotypes in younger cells (7 weeks) (not shown).

Inhibition of histone acetyltransferase activity reverses the effects on gene expression seen in tryptase-deficient cells

To further address whether the aberrant gene expression seen in mMCP6^{-/-} cells was due to increased histone acetylation, we assessed the effect of anacardic acid, a histone acetyltransferase inhibitor, on the expression of non-MC lineage markers (*Emr1*, *Mcpt8*). Indeed, incubation of mMCP6^{-/-} cells with anacardic acid resulted in a significant decrease in the

expression of *Emr1* and *Mcpt8* (Fig. 6E), hence supporting a causative link between histone acetylation and increased expression of non-MC lineage markers. Notably, while anacardic acid inhibited the expression of these genes in mMCP6^{-/-} cells, no significant effect of anacardic acid was seen in WT cells (Fig. 6E). Moreover, anacardic acid had no significant effect on the expression of control genes whose expression were unaffected by the mMCP6 knockout (*Gapdh*, *Cpa3*; Fig. 6E). We also assessed the effect of anacardic acid on the total levels of RNA in WT vs. mMCP6^{-/-} cells. As seen in Fig. 6F, incubation of mMCP6^{-/-} cells with anacardic acid produced a decrease in the amount of recovered RNA while not affecting cell viability (Fig. 6G). In contrast, histone acetyltransferase inhibition did not reduce the level of total RNA recovered from WT cells (Fig. 6F). Together, these data support that the upregulated expression of non-MC lineage genes in MCs lacking tryptase is an effect of increased histone acetylation, this in turn being a consequence of an accumulation of non-clipped core histones.

ChIP-seq analysis reveals an association between H2BK5ac and non-MC lineage genes

To substantiate the link between tryptase-deficiency and accumulation of H2BK5ac we performed a genome-wide chromatin immunoprecipitation-sequencing (ChIP-seq) analysis for H2BK5ac in mMCP6^{-/-} cells and WT cells. We found on average a two-fold higher enrichment for H2BK5ac in mMCP6^{-/-} cells in enriched regions common between the two cell lines (Suppl. Fig. 3). While this could indicate a genome-wide accumulation of H2BK5ac we cannot rule out experimental variation in enrichment. Therefore, we normalized the signal in mMCP6^{-/-} cells to that in WT in order to identify differentially enriched genes. As shown for the chymase locus on chromosome 14 in Fig. 7A, ChIP using the H2BK5ac antibody revealed similar enrichment around several genes in WT and mMCP6^{-/-} cells. Strikingly however, the enrichment of *Mcpt8* was much higher in mMCP6^{-/-} than in WT cells, and the mMCP6^{-/-} cells also showed a markedly higher enrichment of *Ctsg* than did WT cells (Fig. 7B and C). Moreover, *Emr1* (*Adgre1*) revealed a highly enriched region upstream of the transcription start site (Fig. 7D) while the

control gene used in qPCR, *Cpa3*, had the same level of enrichment in both samples (Fig. 7E). Hence, these findings support a link between the accumulation of H2BK5ac and the gene expression patterns seen in the tryptase-deficient MCs. In a genome-wide comparison of the samples using read counts in larger windows (50 kb), we found that the *Mcpt8/Ctsg* region was among the ten most upregulated regions (Suppl. Table 1). Notably, a large number of genes in addition to *Mcpt8*, *Ctsg* and *Emr1/Adgre1* showed accumulation of the H2BK5ac mark as a consequence of tryptase-deficiency (Suppl. Table 1, Suppl. Fig. 3).

Reduced tryptase expression in aging wild-type cells produces similar epigenetic events as in genetically tryptase-deficient cells

To further evaluate the age-dependent effects of tryptase on histone clipping, we followed cell cultures for further extended time periods, up to 12 months. Intriguingly, when culturing MCs for such time periods, we noted a partial loss of mMCP6 expression in WT cells (Fig. 8A). In agreement with this, we also noted a profound loss of mMCP6 expression *in vivo* in MCs of aging WT mice (Fig. 2G), whereas CPA3 and chymase expression was intact (Fig. 2G and Suppl. Fig. 2). In line with the effects of tryptase deficiency seen in genetically mMCP6-ablated cells, the partial loss of tryptase expression in aging WT cells was accompanied by lesser extents of H2B and H3 clipping (Fig. 8A) and by significant accumulation of the H2BK5ac mark (Fig. 8A-B). The loss of tryptase expression in aging WT cells was also accompanied by increased rates of proliferation (Fig. 8C), increased cellular size (Fig. 8D-E) and increased F4/80 surface expression (Fig. 8F-G), all of these findings being in line with the effects seen in genetically mMCP6-deficient cells (see Figs 1, 2 and 6, respectively). An increase in cellular size was also seen *in vivo*, as manifested in MCs of aging mice in which tryptase expression is downregulated (Fig. 2G). Hence, these data provide further support for a major role for tryptase in age-dependent regulation of epigenetic mechanisms through histone clipping.

Discussion

The concept of histone clipping as an epigenetic mechanism in mammalian cells was introduced by Duncan et al who showed that clipped H3 was present in differentiating embryonic stem cells and that silencing of cathepsin L resulted in attenuated H3 clipping¹⁸. However, it is not clear whether such cathepsin L-mediated H3 clipping in fact has a direct epigenetic effect as reflected by altered levels of H3 PTMs. Hence, although histone clipping as a concept has gained some attention^{19, 20}, there is still only limited data to support an epigenetic impact of this type of mechanism in mammalian cells. Moreover, it is notable that most previous studies on this topic have focused on the N-terminal clipping of H3 and C-terminal clipping of H2A²⁰, whereas potential epigenetic effects of clipping of other core histones have not been studied.

In this study we introduce clipping of H2B by tryptase as a novel epigenetic mechanism. The general consensus has been that tryptase has functions that are associated with its release to the extracellular milieu, while being biologically inert during intracellular storage⁴. Here we challenge this notion by showing that tryptase has a profound intracellular impact on MCs, by regulating its epigenome. Intriguingly, tryptase was found to be associated with chromatin and was shown to truncate both H3 and H2B of intact nucleosomes, thereby having the potential to abolish epigenetic modifications at these sites. Indeed, our data reveal that tryptase has an epigenetic regulatory role by preventing the accumulation of H2BK5 acetylation. Besides altering histone acetylation marks, we note that mammalian H2BK5 is adjacent to two potentially phosphorylatable residues, S4 and S6. Interestingly, phosphorylation at S14 in mammalian H2B and yeast H2B at S10 have been closely linked to apoptosis^{21, 22}, and in the case of *S. cerevisiae* deacetylation of an adjacent K11 is required to initiate this apoptotic phosphorylation²³. It remains unclear the extent to which, if at all, other PTMs besides H2BK5ac are impacted by the tryptase-mediated H2B cleavage that we have focused upon in this study.

The present study suggests that cells lacking tryptase, with the consequent accumulation of H2BK5ac, undergo cell age-dependent extensive chromatin remodeling and other morphological changes, accompanied by loss of proliferative control. Moreover, there was a dramatic upregulation in tryptase null cells of genes that serve as markers of non-MC lineages. Importantly, the locus-specific accumulation of H2BK5ac at these genes was confirmed by ChIP-seq analysis, thus providing a causal link between the absence of tryptase and upregulated expression of non-MC lineage genes. These data hence introduce the possibility that tryptase-catalyzed histone clipping may serve as a mechanism to ensure preservation of cellular identity in aging cell populations, the absence of which will lead to aberrant expression of markers of other cell lineages. This raises the intriguing possibility that regulated acetylation of H2B could represent an epigenetic mechanism that regulates cell differentiation. In line with this notion, a recent study implicated H2BK5 acetylation in the regulation of epithelial-mesenchymal transition¹⁷. It is also striking that the observed aberrant accumulation of H2BK5ac is strongly correlated with the age of the cells. It is thus possible that loss of control over H2B acetylation could be one of the epigenetic effects that may be associated with aging²⁴.

The results presented here and in a previous study⁵ indicate that tryptase has profound H3 and H2B clipping activity. However, while the epigenetic modification of H2B was affected in tryptase null cells we did not see any effect of tryptase-deficiency on the epigenetic signature of H3. Although we cannot at present with certainty explain this, it is notable that truncated H2B was found to be associated with chromatin whereas we did not see chromatin-associated truncated H3. Hence, mMCP6-mediated H3 truncation may be a non-nucleosomal event, and it is therefore plausible that mMCP6 action on H3 does not affect its epigenetic signature. In contrast to H3, little is known concerning the biological impact of epigenetic H2B modifications. In most cases, histone acetylations are regarded as activating modifications. In

agreement with such a notion, we see a dramatic increase in expression of numerous genes in cells exhibiting enhanced an H2BK5ac signal at their promoter proximal and distal regulatory elements, due to tryptase deletion. It was also evident that inhibition of histone acetylase activity has a corresponding inhibitory action on the expression of these genes, supporting a causative link between H2B acetylation and effects on gene expression.

An important question is whether tryptase-mediated H2B clipping has any biological implications beyond the MC context. Notably, although tryptase expression is largely MC-restricted, there is evidence that certain other cell types such as basophils and macrophages can express tryptase, albeit at lower levels^{25, 26, 27, 28}. Hence, it cannot be excluded that tryptase can have an epigenetic regulatory impact on non-MCs. Further, it is conceivable that trypsin-like proteases distinct from tryptase, expressed by other cell lineages, may acquire similar histone-clipping activities as seen for MC tryptase.

Our data show that the epigenetic effects of tryptase deficiency largely overlap with those seen in cells lacking the proteoglycan serglycin. Since tryptase storage is strongly dependent on serglycin⁹, these findings reinforce the impact of tryptase on the epigenome of MCs. In contrast to tryptase, serglycin expression is relatively wide-spread²⁹. Hence, serglycin might have epigenetic implications also when expressed by non-MC lineages, possibly by acting as a partner for histone-clipping proteases.

In summary, the present study introduces histone clipping by tryptase as a novel epigenetic mechanism, with implications for regulation of cell differentiation.

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Figure Legends

Figure 1. Absence of tryptase or serglycin results in age-dependent loss of proliferative

control. (A) Bone marrow cells from WT, serglycin^{-/-} or mMCP6^{-/-} mice were differentiated into MCs and were enumerated at the indicated time points. (B) MCs were cultured for extended time periods (up to 6 months) and cells were enumerated before passages (n = 3). (C) MCs were enumerated in the ear skin and tongue of young (4 months) and old (2 years) mice, using Toluidine Blue staining (n =30-34 for ear skin sections; n=12-16 for tongue sections). (D) Cell counts of individual MC cultures kept for up to 9.5 months. (E) CFSE dilution experiment performed on 5 month-old MCs. Cells were analyzed at day 0 (light gray), day 5 (intermediated gray shade) and day 10 (dark gray) after CFSE labeling. (F) Quantification of CSFE labeling data, using the gates (H-, H+) specified in (D) (n = 3). (G) 5 month-old MCs of the indicated genotypes were deprived of growth factors, followed by assessment of residual viable cells (Trypan blue staining) at the time points indicated. (H) Parallel control experiment to (G) confirming more rapid proliferation of serglycin^{-/-} and mMCP6^{-/-} cells than WT cells in the presence of IL-3.

Figure 2. Tryptase- or serglycin-deficiency causes age-dependent morphological

alterations. (A, C) Representative dot plots (A) and histogram (C) of forward/side scatter FACS analysis of 5 month-old cells showing larger size (FSC-H) of serglycin^{-/-} and mMCP6^{-/-} MCs vs. WT controls. (B) Quantification of the data from the forward/side scatter analysis (n = 3). (D) Cell size distribution as determined by cell diameter. (E) Representative May Grünwald/Giemsa-stained cytopsin slides of 4 and 10 month-old MCs. (F) Examples of dramatic changes (red arrows) in nuclear architecture of aged mMCP6^{-/-} MCs. (G) Confocal microscopy analysis of mMCP6 and CPA3 (MC granule marker) expression in tongue tissue from young (4 months) and aged (2 years) WT and mMCP6^{-/-} mice.

Figure 3. The absence of tryptase mMCP6 or serglycin in MCs causes nuclear remodeling.

(A) Transmission electron microscopy (TEM) analysis of 5 months old WT, serglycin^{-/-} and mMCP6^{-/-} MCs. Note the rounded nuclear shape of WT cells and the lobulated structure of MCs from serglycin^{-/-} and mMCP6^{-/-} MCs. (B) Quantification of nuclear characteristics.

Figure 4. Tryptase is associated with chromatin and causes clipping of nucleosome-associated H2B and H3.

(A) Western blot analysis confirming that the absence of tryptase (mMCP6) results in defective clipping of H3 and H2B. (B) WT MCs were cultured with tryptase inhibitor (Nafamostat mesylate; 1 μ M) for 48 h, followed by Western blot analysis for H2B and H3 processing. (C) Oligonucleosomes prepared from HeLa cells were subjected for 30 min to treatment with increasing concentrations of recombinant tryptase, followed by Western blot analysis for H2A, H2B, H3 and H4. (D) Time course analysis showing clipping of core histones after addition of 0.25 ng/ml tryptase to oligonucleosomes. (E) Cytosol, nuclear and chromatin fractions were extracted from MCs and were subjected to Western blot analysis for β -actin, CPA3 (MC granule marker), tryptase (mMCP6), H3 (antibody to C- and N-terminal, respectively) and H2B.

Figure 5. Tryptase regulates H2B lysine 5 acetylation.

(A) Western blot analysis showing the levels of H3 and H2B posttranslational modifications (PTMs) in WT, serglycin^{-/-} and mMCP6^{-/-} MCs (3 months old). Note the increase of H2B lysine 5 acetylation (H2BK5ac) in mMCP6^{-/-} cells. (B) Quantification of Western blot analysis for the levels of H2BK5ac relative to the levels of β -actin (n = 6). (B) WT MCs were incubated for 48 h with tryptase inhibitor (Nafamostat mesylate; 1 μ M), followed by Western blot analysis for H2BK5ac, and quantification of data (n=4). (D) Confocal microscopy analysis verifying the upregulated expression of H2BK5ac in mMCP6^{-/-} cells vs. WT controls. Nuclei were visualized using DAPI. (E) Cell numbers in WT,

serglycin^{-/-} and mMCP6^{-/-} cultures, with arrows indicating time points used for PTM analysis in (F). (F-G) Comparison of the levels of histone PTMs in 1-, 3- and 4 month-old cultures of WT, serglycin^{-/-} and mMCP6^{-/-} MCs as shown by Western blot analysis (F) and quantification of the H2K5ac bands relative to β -actin (G). Note the age-dependent increase in H2BK5ac in aging mMCP6^{-/-} and serglycin^{-/-} cells vs. WT controls.

Figure 6. Upregulation of non-MC lineage markers in aging tryptase-deficient MCs. (A, B)

Total RNA was extracted from aging (5 month-old; A) or 6 week-old (B) WT and mMCP6^{-/-} MCs and was analyzed by qPCR for the expression of non-MC lineage genes (*Emr1*, *Mcpt8*, *Ctsg*). Results are shown as expression relative to the house-keeping gene (*Hprt*). (C) Flow cytometry analysis verifying upregulated cell surface expression of *Emr1*/F4/80 in 5 month-old mMCP6^{-/-} MCs vs. WT controls. Light gray-shaded area, isotype control. (D) % of *Emr1*/F4/80^{high} WT, serglycin^{-/-} and mMCP6^{-/-} cells in 5 month-old cell cultures (n = 3). (E) WT and mMCP6^{-/-} MCs were treated with histone acetyltransferase inhibitor (anacardic acid; 60 μ M) for 4h or left untreated, followed by qPCR analysis for the expression of *Gapdh*, *Emr1*, *Mcpt8* and *Cpa3* as indicated. Results are shown as % of control (untreated samples) relative to *Hprt* expression. (F) Quantification of the amount of total RNA extracted from WT and mMCP6^{-/-} MCs (equal numbers of cells from each genotype) after 4h treatment with different concentrations of anacardic acid. (G) Cell viability of WT and mMCP6^{-/-} MCs treated for 4h with 60 μ M anacardic acid.

Figure 7. ChIP-seq enrichment for H2BK5ac. (A) H2BK5ac signals in a 400 kb window of the chymase locus on chromosome 14. MACS wiggle tracks were normalized to reads per million. (B-D) Signals around *Mcpt8*, *Ctsg* and *Emr1* (*Adgre1*) in WT (gray) and mMCP6^{-/-} (black) cells.

Figure 8. Reduced tryptase expression in aging WT MCs produces similar epigenetic events as in genetically tryptase-deficient cells. (A) Western blot analysis of control (7 weeks old) and aged (12 months) WT MCs taken from 3 independent cell cultures. Cells were analyzed for the levels of tryptase (mMCP6), H2B, H2BK5ac and H3 as indicated. (B) Quantification of H2BK5ac relative to H3 (n = 3). (C) Quantification of cell numbers in the 3 independent WT MC cultures. The arrow indicates the time point for the Western blot analysis shown in (A). (D) Flow cytometry analysis showing density side/forward scatter plots of control- and aged WT MCs. (E) Quantification of the data in (D) (n = 3). (F) Flow cytometry analysis of the expression of Emr1 / F4/80 in control and aged WT MCs. Gray-shaded area, isotype control. (G) % of Emr1 / F4/80^{low} and Emr1 / F4/80^{high} cells in control (7 weeks) and in 3 independent cultures (WT1, WT2 and WT3) of aged (12 months old) WT MCs (n = 3).

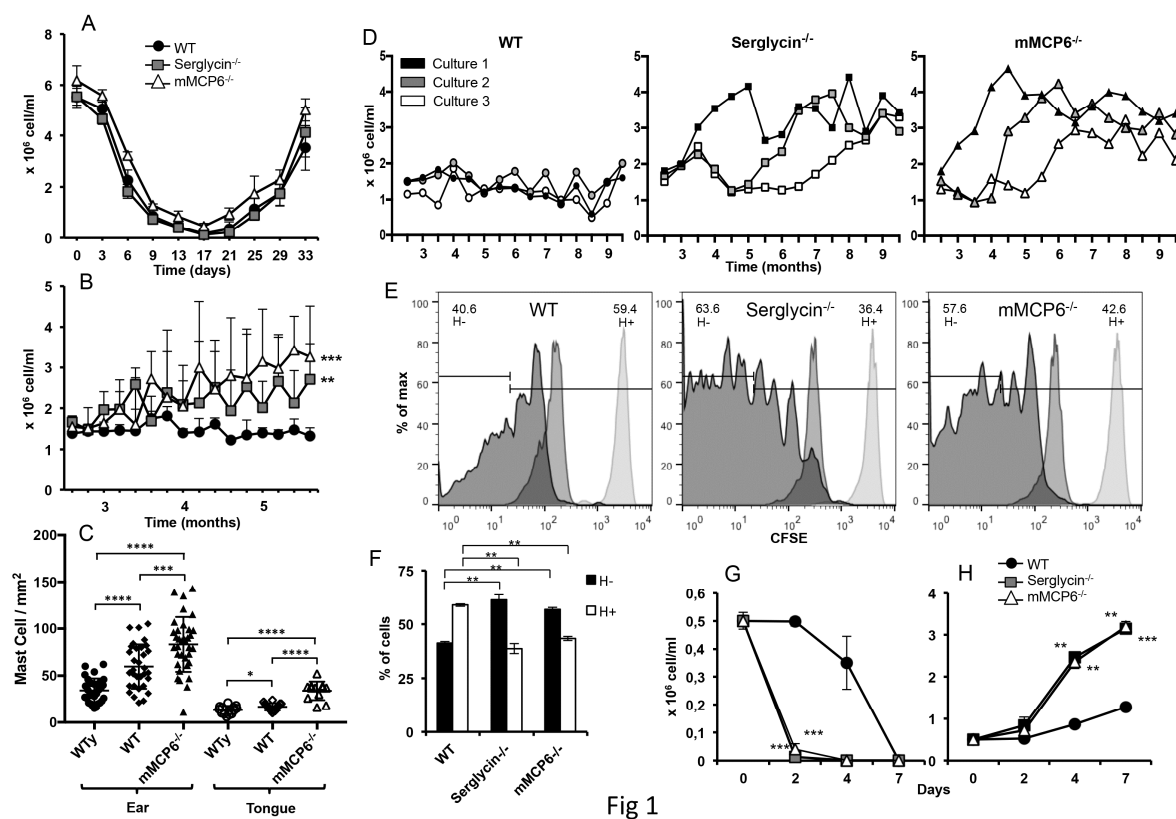
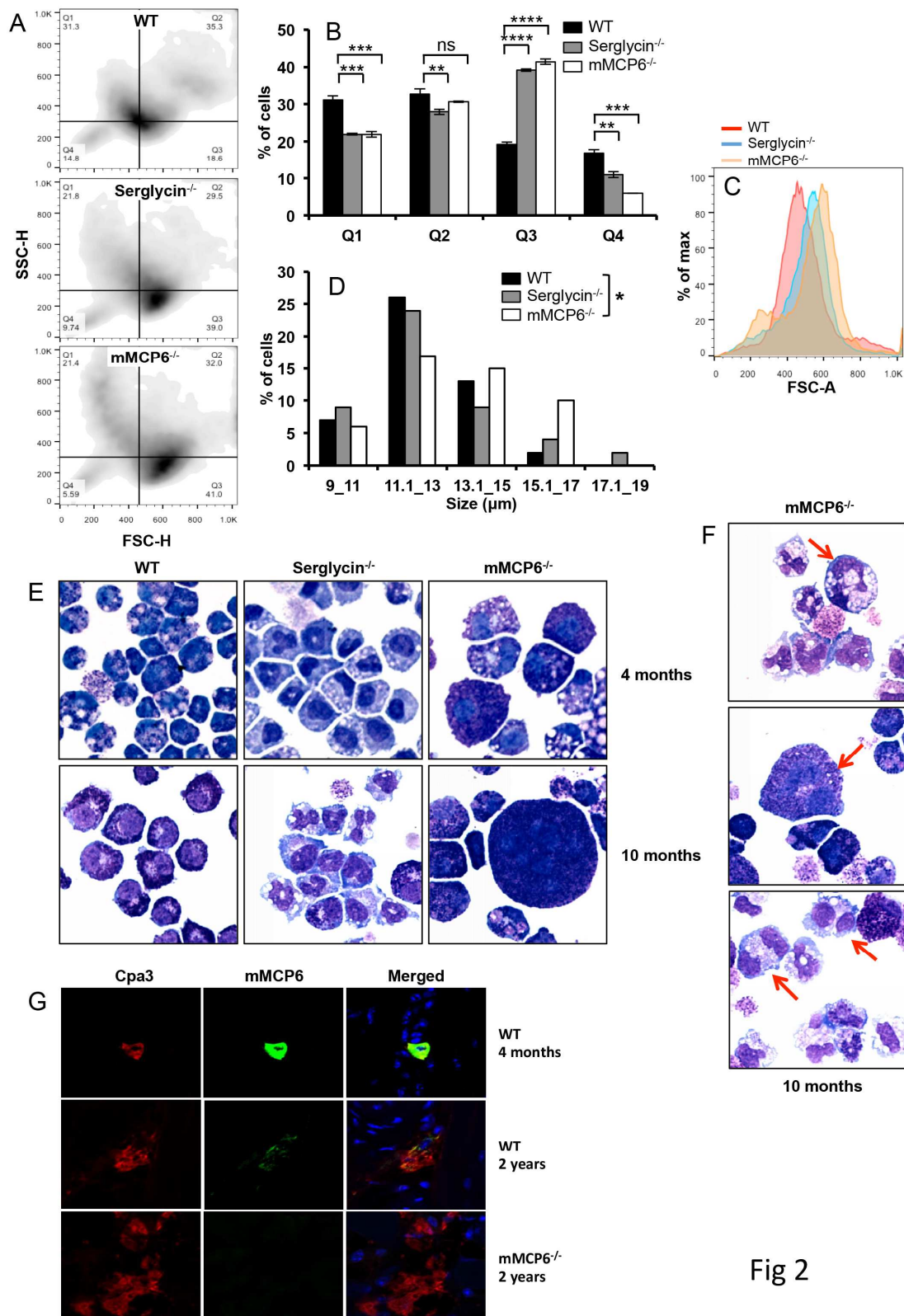
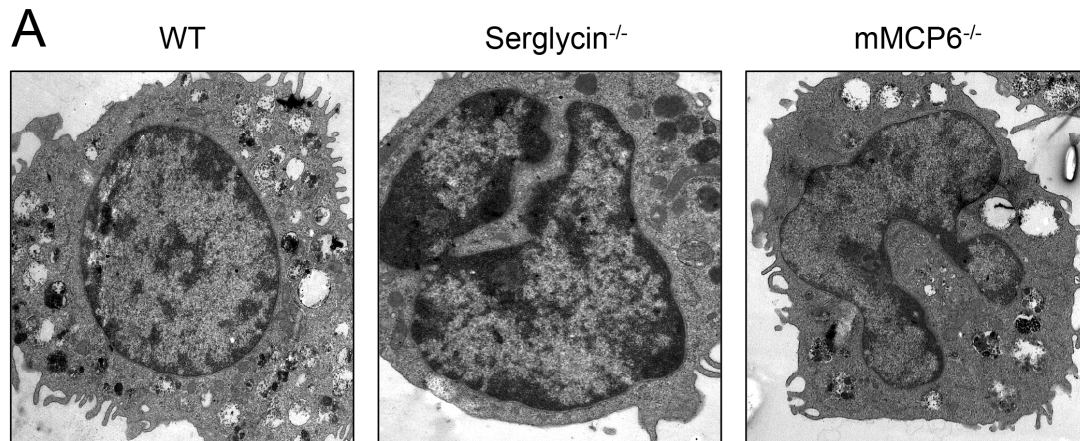


Fig 1





B

	WT	Serglycin ^{-/-}	mMCP6 ^{-/-}
Round nuclei #	1.0 ± 0.06 (18)	0.32 ± 0.11 (19) p < 0.0001 vs WT	0.06 ± 0.06 (18) p < 0.0001 vs WT
Nuclear lobes #	0.020 ± 0.02 (18)	0.47 ± 0.25 (19) ns* vs WT	0.22 ± 0.15 (18) ns vs WT
Small contraction #	0.56 ± 0.56 (18)	0.54 ± 0.16 (19) ns vs WT	0.92 ± 0.17 (18) ns vs WT
Deep contraction #	0.028 ± 0.028 (18)	0.26 ± 0.1 (19) p < 0.05 vs WT	0.44 ± 0.20 (18) p < 0.05 vs WT

Values are the mean/cell section of the indicated number of cell sections () ± SEM.

p is calculated according to Student's t test, unpaired and two tailed.

* ns = not significant.

Fig 3

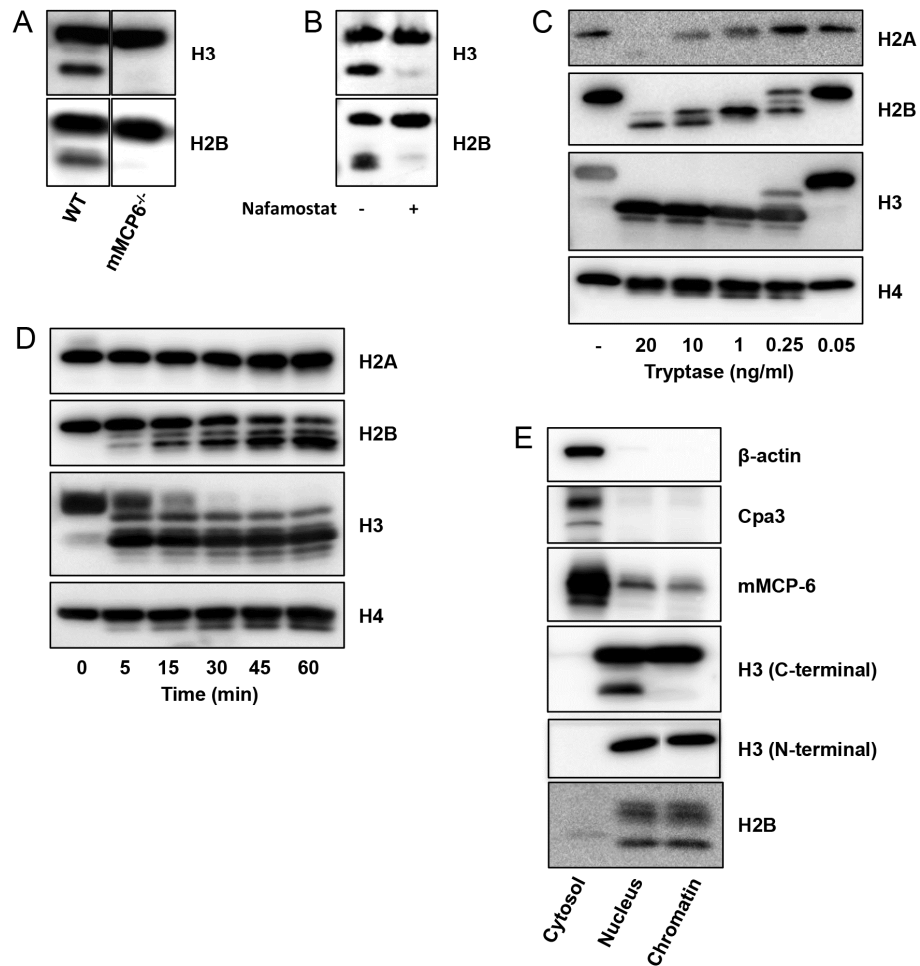


Fig 4

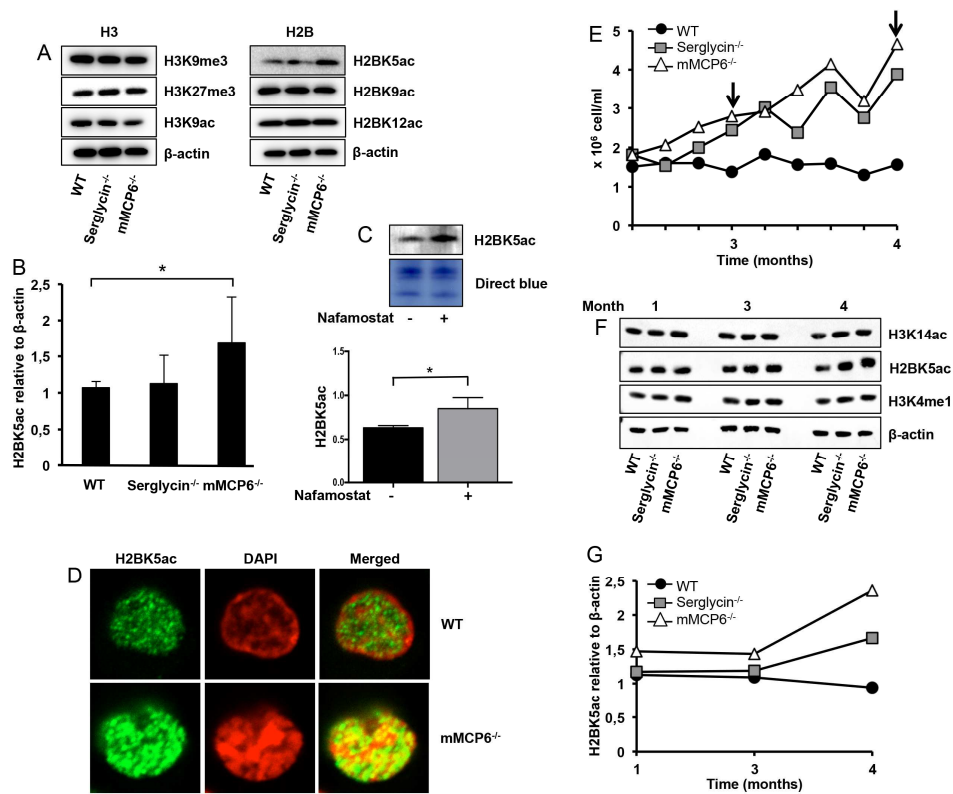


Fig 5

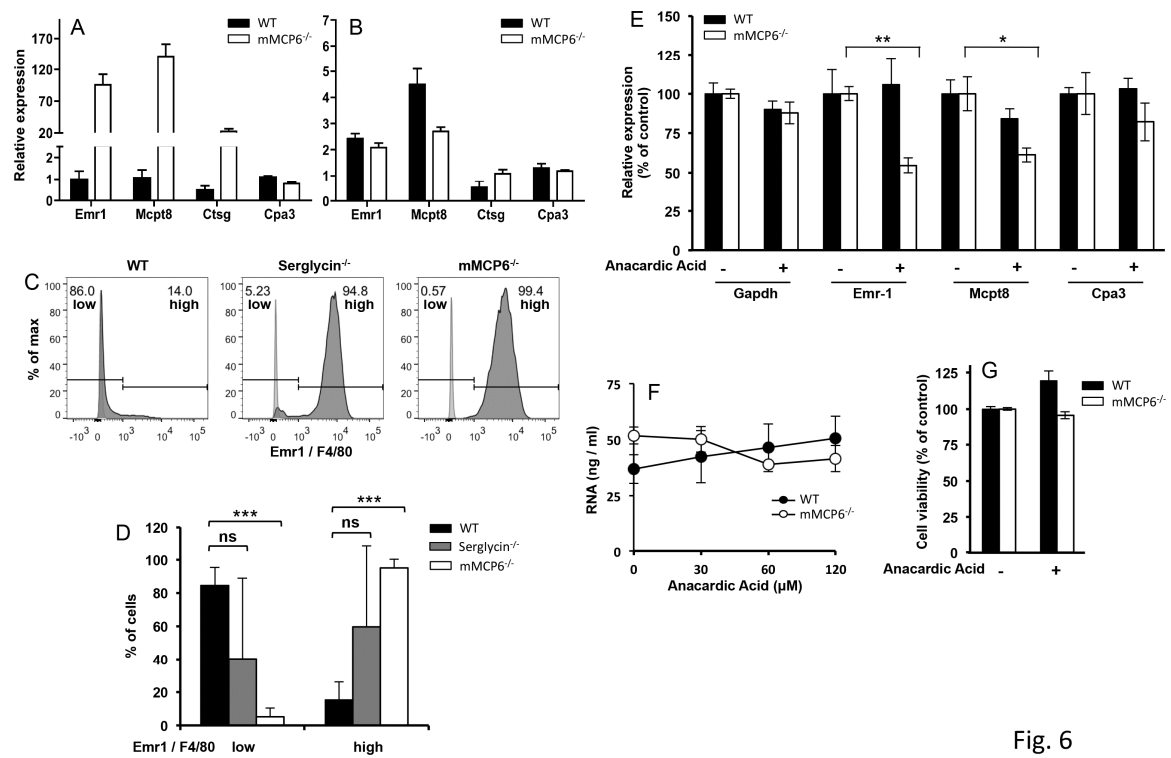


Fig. 6

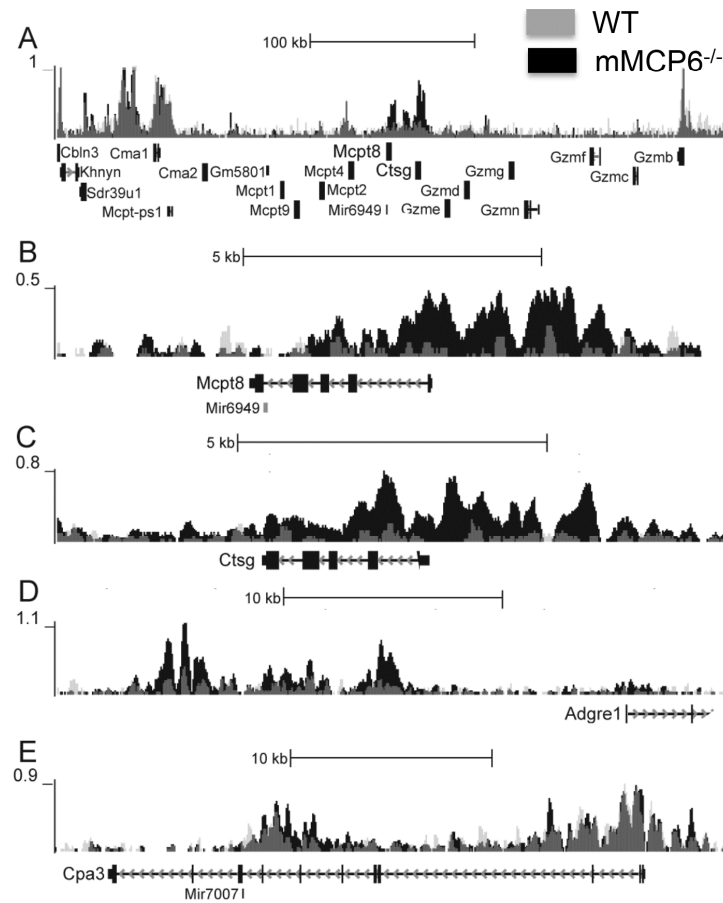


Fig 7

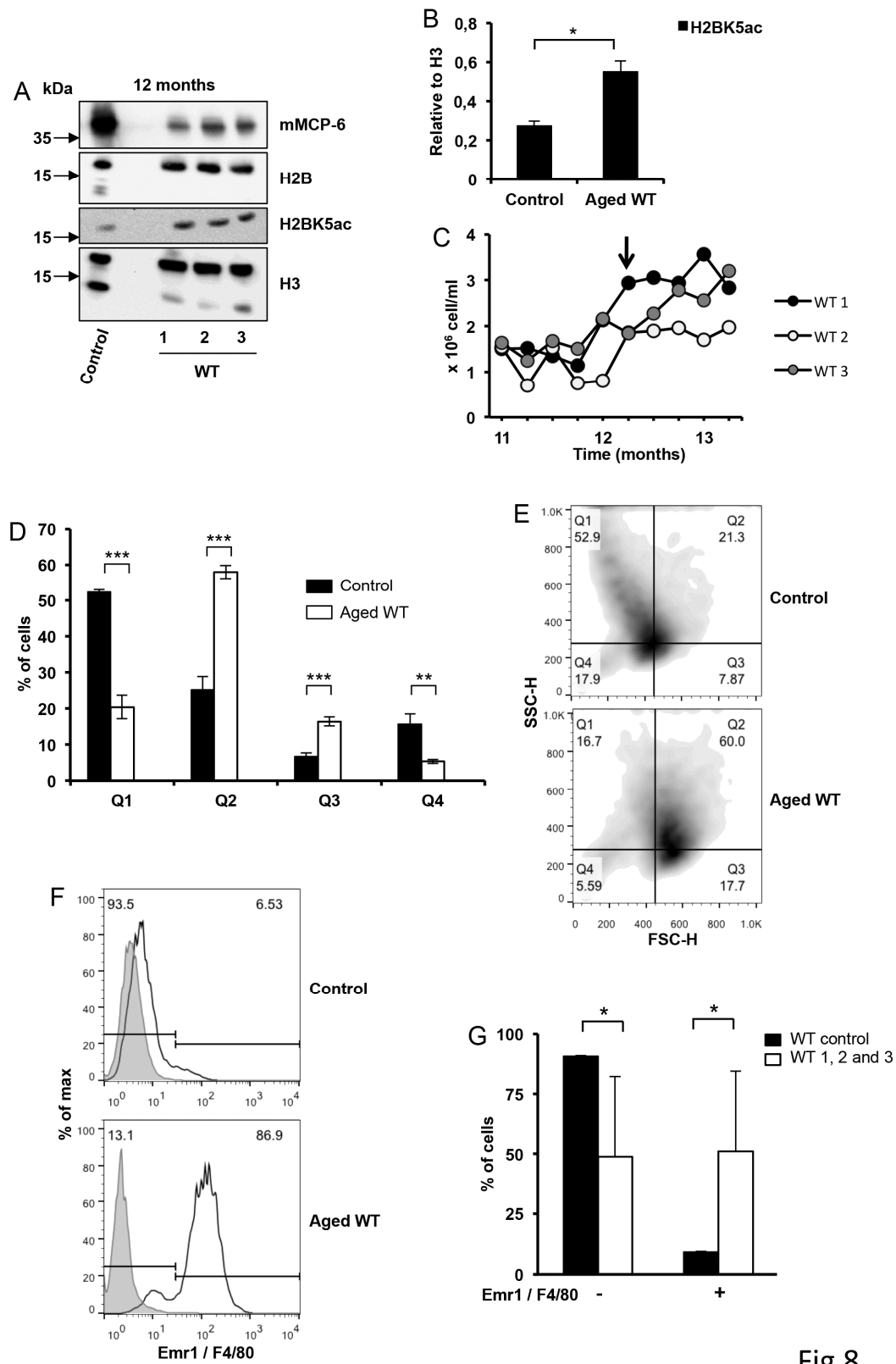


Fig 8

Methods

Reagents. Anacardic acid was from Sigma-Aldrich (Steinheim, Germany). Rabbit anti-histone H2A, H2B, H3 and H4 polyclonal antibodies, mouse anti-H3K27me3 monoclonal and rat anti-F4/80-FITC polyclonal antibodies were from Abcam (Cambridge, UK). Rabbit anti-H3K9me3 was from Active Motif (Carlsbad, CA). Rabbit anti-H2BK12ac polyclonal was from UBI (Hauppauge, NY). Rabbit anti-H2BK12ac and H3K9ac polyclonals were from EMD-Millipore (Darmstadt, Germany). Rabbit anti-H3K4me1, H2BK5ac and H3K14ac were from Cell Signaling Technology (Danvers, MA). Goat polyclonal to β -actin was from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant human skin β -tryptase was from Promega (Madison, WI). CellTrace™ CFSE cell proliferation kit was from Life Technologies (Carlsbad, CA). Nafamostat mesylate was from Sigma-Aldrich (Steinheim, Germany).

Animals. Mice (8-18 weeks old) deficient in serglycin (serglycin^{-/-})^{E1}, mMCP-6^{-/-}^{E2} as well as wild-type (WT) controls were all on C57BL/6J genetic background. All animal experiments were approved by the local ethical committee.

Bone marrow-derived MCs. Femurs and tibiae from mice of the same gender and age were recovered and MCs were obtained by culturing bone marrow cells in Dulbecco's modified Eagle's medium (SVA, Uppsala, Sweden), supplemented with 30% WEHI-3B conditioned medium, 10% heat-inactivated fetal bovine serum (Invitrogen), 50 μ g/ml streptomycin sulfate, 60 μ g/ml penicillin G, 2 mM L-glutamine (SVA) and 10 ng/ml mouse recombinant IL-3^{E3}. The cells were kept at 0.5×10^6 cells/ml, at 37°C in 5% CO₂, and the medium was changed once a week.

CFSE labeling. Cells (10^6 cells/ml) were incubated with 10 μ M CFSE at 37°C for 15 min. Five volumes of ice-cold culture medium was added to quench the staining, followed by incubation on ice for 5 min. The supernatant was removed by centrifugation (0.3 x g, 8 min) and samples were washed 3 times in fresh medium. The cells were kept at 0.5×10^6 cells/ml, at 37°C in 5% CO₂, and FACS analysis were performed after 5, 10 and 15 days (FACScan®, BD Biosciences). Data from 10,000 events/sample were collected and analyzed by the FlowJo software (Ashland, OR).

Growth factor deprivation. Cells (0.5×10^6 cells/ml) were either cultured in complete MC medium or in medium deprived of growth factors (Dulbecco's modified Eagle's medium, supplemented with 10% heat-inactivated fetal bovine serum, 50 μ g/ml streptomycin sulfate, 60 μ g/ml penicillin G and 2 mM L-glutamine). Cells were kept at 37°C in 5% CO₂, and cell counting was performed at day 2, 4 and 7 using an automated cell counter (Countess™ II FL; Life Technologies, Bothell, WA).

Morphology of cells. Transmission electron microscopy (TEM) was performed as previously described^{E4}, and for light microscopy assays cells were collected on to cytospin slides and stained with May Grünwald/Giemsa. Cell size analyses were performed by flow cytometry using 10,000 events/sample and analyzed by FlowJo software. Cell size distribution was based on cell diameter from micrographs taken using an inverted microscope (Nikon Eclipse Ti) and analyzed by Nikon's imaging software NIS-Elements (Melville, NY). Quantitative ultrastructural analysis was carried out on pictures taken at the same magnification. The percentage of cells showing a round nucleus and the presence of separated nuclear lobes (relative to 100

cell sections), were evaluated. Since a deep nuclear invagination can result in the appearance of separated lobes when observed in two-dimensions, we also evaluated the number of deep clefts (relative to 100 cell sections).

Western blot analysis. Samples of $0.5\text{--}2 \times 10^6$ MCs were collected by centrifugation and immediately solubilized in SDS-PAGE sample buffer. Samples corresponding to equal numbers of cells were subjected to Western blot analysis as previously described^{E3}. Membranes were developed with ECL (GE Healthcare) using a Gel DocTM Imager (Bio-Rad).

Cell fractionation. MCs (2×10^6 cells) were collected by centrifugation, washed with PBS and resuspended in 200 μl of 10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl_2 , 0.34 M sucrose, 1 mM DTT, 1:100 protease inhibitor cocktail (Sigma-Aldrich) and 0.1% Triton X-100. Samples were incubated for 8 min on ice, followed by centrifugation (1,300 $\times$ g, 5 min, 4°C). The supernatant (cytosolic fraction) was discharged and pellets (nuclear fractions) were washed 3 times using the same buffer. The pellets were then lysed for 30 min with 100 μl of 3 mM EDTA, 0.2 mM EGTA, 1 mM DTT and 1:100 protease inhibitor cocktail, followed by centrifugation (1,700 $\times$ g, 5 min, 4°C). The supernatant was discharged and pellets (chromatin-enriched sample) were washed 3 times using the same buffer. All pellets were resuspended in 100 μl of SDS-sample buffer and analyzed by Western blot.

Oligonucleosomes from HeLa cells. Oligonucleosomes were purified from HeLa cells by MNase digestion of nuclei followed by resolution over a linear 5–30% sucrose gradient, as previously described^{E5}. Sucrose gradient fractions corresponding to greater than tetra-nucleosomes were pooled and used.

Effect of tryptase on oligonucleosomes. Oligonucleosomes extracted from HeLa cells (15 ng in Tris-buffered saline (TBS)) were incubated with different concentrations of recombinant human skin β -tryptase for 30 min at 37°C. The reaction was stopped by adding SDS-PAGE sample buffer, followed by heating at 100°C for 10 min. The time course assay was performed using 0.25 ng/ml of recombinant human skin β -tryptase and 15 ng oligonucleosomes diluted in 30 μl TBS. At different time points (5, 15, 30, 45 and 60 min) the reaction was stopped by adding 10 μl of 4x concentrated SDS-PAGE sample buffer, followed by heating at 100°C for 10 min. Samples were analyzed by Western blot using antibodies against histones H2A, H2B, H3 and H4 C-terminals.

Confocal microscopy. Aliquots of 100 μl from 0.5×10^6 cells/ml suspension were dropped into round areas on microscopic glasses marked with liquid-repellent slide marker pen. The suspension was allowed to settle for 15 min after which liquid was removed carefully with filter paper. Cells were left to dry for 15 min and then fixed with 4% paraformaldehyde in PBS for 30 min, followed by a 15 min drying step. 100 μl of a 50 $\mu\text{g/ml}$ digitonin solution in PBS was added to each slide and incubated for 10 min at room temperature. Next, 50 μl anti-H2BK5ac antibody (1:1000) in TBS/1% BSA and/or isotype control at the same concentration were added for 1 h, followed by washing 3 times with TBS-T. Fifty μl of Alexa-488 conjugated antibody against rabbit Ig in TBS/1% BSA was added. For staining of tissue sections, paraffin-embedded tissues were sectioned into 6 μm sections. Samples were deparaffinized and stained with hematoxylin and eosin, toluidine blue (0.1% toluidine blue in 0.17

mM NaCl, pH 2) for 15 min, or chloroacetate esterase for 40 min prior to mounting. All photos were taken using a Nikon Ni-U bright field microscope. Immunohistochemistry was performed in deparaffinized slides using anti-mouse mMCP6 immune serum from rabbit (1:500) incubated for 3 h, followed by incubation with anti-rabbit Alexa546 secondary antibody for 1 h, both at room temperature. Slides were then incubated overnight at 4°C with anti-mouse Cpa3 immune serum from rabbit (1:500), followed by incubation with anti-rabbit Alexa633 secondary antibody for 1 h at room temperature. The slides were kept in the dark and were washed 3 times with TBS-T between every step. Finally, 50 µl of 1 µg/ml DAPI in TBS/1% BSA was added for 2 min, followed by 3 times washing with TBS-T. The slides were mounted with SlowFade® gold antifade mounting medium (Life Technologies, Eugene, OR). Samples were analyzed using a laser-scanning microscope equipped with ZEN 2009 software (LSM 710; Carl Zeiss, Berlin, Germany).

Histone acetyltransferase inhibition. 2 ml of MCs (0.5×10^6 cells/ml) were incubated with 60 µM anacardic acid for 4 h at 37°C. Cells were harvested by centrifugation ($0.3 \times g$, 8 min) and pellets were used for total RNA extraction (NucleoSpin® RNA, Machery-Nagel, Germany). The cytotoxicity of anacardic acid was examined using Cell Titer-Blue® reagent cell viability assay (Promega-Invitrogen, Carlsbad, CA).

ChIP-seq. Cross-linking ChIP was performed with anti-H2BK5ac (Abcam 175046) as previously described^{E6}, using 15×10^6 cells per immuno-precipitation. 20-30 ng ChIP and input DNA was end repaired and A-tailed (Fast end repair and Klenow exo⁻, Fermentas) before ligation (Enzymatics ligase and BIOO Scientific adapters) and amplification (KAPA HiFi, 15 cycles) before sequencing on a NextSeq500 sequencer. Approximately 25×10^6 50 bp reads were generated per sample and aligned to the mm10 reference genome using BWA aln^{E7}. MACS^{E8} v1.42 was used for peak calling. For visualization, the counts of overlapping fragments in the mMCP6^{-/-} sample were divided by a factor of two to account for the overall higher enrichment level. A list of read counts over genes (Suppl. Table 1) was made using 50 kb windows centered on the transcription start sites for all refSeq genes.

Quantitative real time RT-PCR. Total RNA preparation and quantitative real-time PCR (qPCR) were performed as previously described^{E3}. The following primers were used:

Hprt: Forward 5'-GATTAGCGATGATGAACCAGGTTA-3'; Reverse 5'-GACATCTCGAGCAAGTCTTTCAGTC-3'; Gapdh: forward 5'-CTCCCACTCTTCCAC CTTCG-3'; reverse 5'-CCACCACCCTGTTGCTGTAG-3'; Emr1, forward 5'-AGGGTATCATGAGTTGATGGCA-3'; reverse 5'-TGGAGCTTCATAGTTGTAAGGCA-3'; Mcpt8, forward 5'-TCAGTCTATCGCTGTGGTGG-3'; reverse 5'-ACAGGAATGAGCTGGGTGTTT-3'; Ctsg, forward 5'-AGGGTTTCTGGTGGGAGAAG -3'; reverse 5'-GTTCTGCGGATTGTAATCAGGAT -3'; Cpa3, forward 5'-TGACAGGGAGAAGGTATTCCG-3'; reverse 5'-CCAAGTTGACTGGATGGTCT-3'.

Statistical analysis. Data were analyzed by either Student's t-test or by the Mann-Whitney test, and are presented as mean values $\pm$ SEM; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

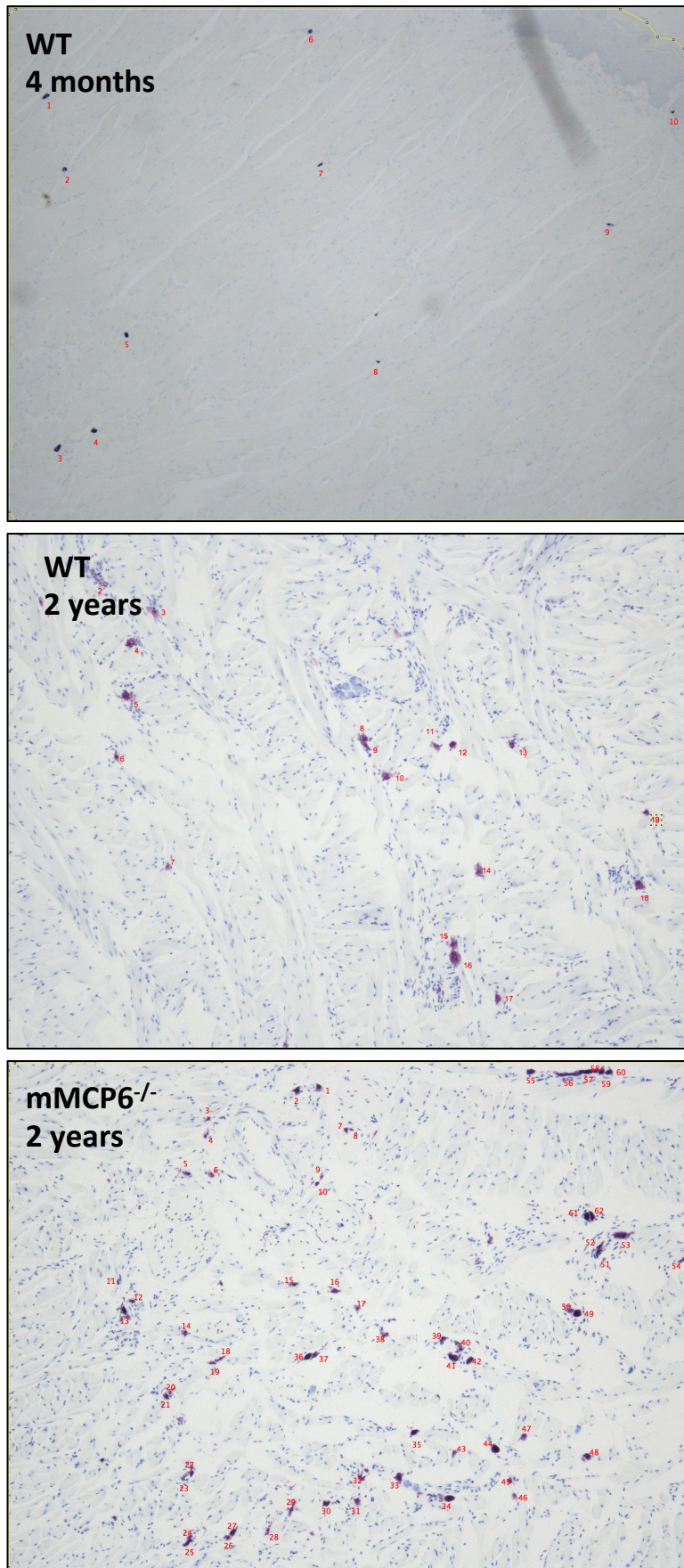
- E1. Åbrink, M., Grujic, M. & Pejler, G. Serglycin is essential for maturation of mast cell secretory granule. *J Biol Chem* **279**, 40897-40905 (2004).
- E2. Shin, K. *et al.* Mouse mast cell tryptase mMCP-6 is a critical link between adaptive and innate immunity in the chronic phase of *Trichinella spiralis* infection. *J Immunol* **180**, 4885-4891 (2008).
- E3. Ronnberg, E. & Pejler, G. Serglycin: the master of the mast cell. *Meth Mol Biol* **836**, 201-217 (2012).
- E4. Braga, T. *et al.* Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells. *Biochem J* **403**, 49-57 (2007).
- E5. Fang, J., Wang, H. & Zhang, Y. Purification of histone methyltransferases from HeLa cells. *Methods in enzymology* **377**, 213-226 (2004).
- E6. Sabari, B.R. *et al.* Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Mol Cell* **58**, 203-215 (2015).
- E7. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)* **25**, 1754-1760 (2009).
- E8. Zhang, Y. *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**, R137 (2008).

Raw counts in windows +/-25kb centered on transcription start sites. Genes with high input counts (>1000) were removed, as were genes where abs(KO -WT) were less than 200 after input sub

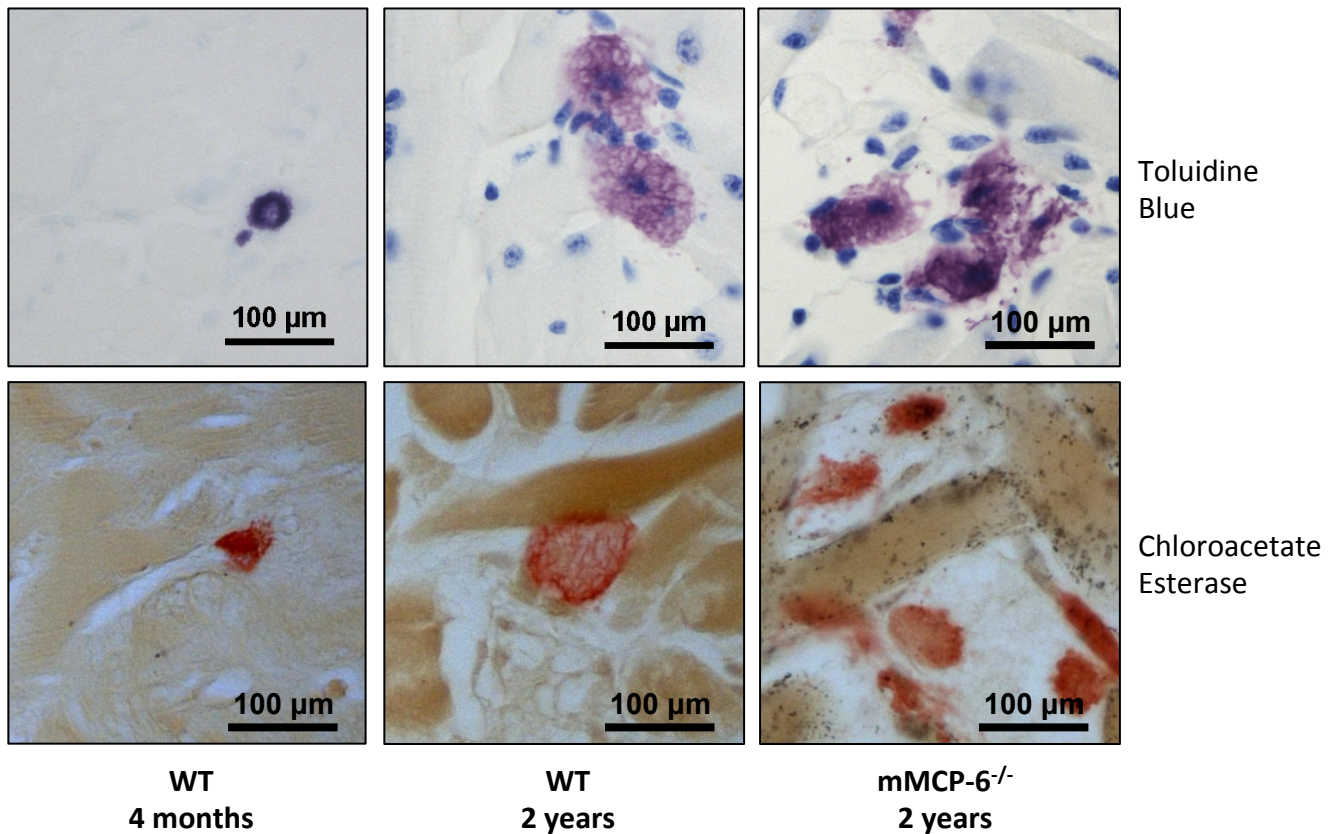
gene	KO input	KO ChIP	WT ChIP	WT input	KO-input	WT-inp	KO/WT	KO-inp/WT-inp	normKO	normWT	normKO/normWT		
Tas2r121	471	1534	477	462	1063	15	3.21593291	70.8666667	1048	3.25690021	1.03246753	3.15448197	
Plec	366	2426	792	363	2060	429	3.06313131	4.8018648	1631	6.6284153	2.18181818	3.03802368	
Mir1942	381	2537	851	376	2156	475	2.98119859	4.53894737	1681	6.65879265	2.26329787	2.94207525	same region as
Ubash3b	358	2697	1008	388	2339	620	2.67559524	3.77258065	1719	7.53351955	2.59793814	2.89980713	
Pigl	437	3086	1156	473	2649	683	2.66955017	3.87847731	1966	7.0617849	2.44397463	2.88946735	
Serpinb1a	450	3347	1263	490	2897	773	2.65003959	3.74773609	2124	7.43777778	2.57755102	2.88559866	
Grp	444	1462	469	410	1018	59	3.11727079	17.2542373	959	3.29279279	1.14390244	2.87856086	
Ncor1	436	3079	1146	465	2643	681	2.68673647	3.88105727	1962	7.06192661	2.46451613	2.86544142	same region as
Ppa1	423	2362	936	474	1939	462	2.52350427	4.1969697	1477	5.58392435	1.97468354	2.82775656	
Gm10637	560	1562	457	434	1002	23	3.41794311	43.5652174	979	2.78928571	1.05299539	2.64890591	
Tas2r122	474	1282	501	490	808	11	2.55888224	73.4545455	797	2.70464135	1.02244898	2.64525801	same region as
Ctsg	448	2029	753	439	1581	314	2.69455511	5.03503185	1267	4.52901786	1.71526196	2.64042343	
Mcpt8	453	2040	779	444	1587	335	2.61874198	4.73731343	1252	4.50331126	1.7545045	2.56671399	Same region as
Bet1l	388	2363	998	415	1975	583	2.36773547	3.38765009	1392	6.09020619	2.40481928	2.53250057	
Olfr1258	498	2157	950	555	1659	395	2.27052632	4.2	1264	4.3313253	1.71171171	2.53040583	
Ric8	386	2508	1070	413	2122	657	2.34392523	3.22983257	1465	6.49740933	2.59079903	2.50787855	
Hdc	400	1714	746	434	1314	312	2.29758713	4.21153846	1002	4.285	1.71889401	2.49288204	
Mir6949	463	2101	815	446	1638	369	2.57791411	4.43902439	1269	4.53779698	1.82735426	2.48326068	
Actr3b	409	1394	560	408	985	152	2.48928571	6.48026316	833	3.40831296	1.37254902	2.48319944	
Wee1	433	1634	717	471	1201	246	2.27894003	4.88211382	955	3.77367206	1.52229299	2.47893938	
Gm4884	451	1456	637	489	1005	148	2.28571429	6.79054054	857	3.22838137	1.30265849	2.47830219	
Fgd3	375	2399	1072	415	2024	657	2.23787313	3.08066971	1367	6.39733333	2.58313253	2.4765796	
Lmna	396	2827	1196	414	2431	782	2.36371237	3.10869565	1649	7.13888889	2.88888889	2.47115385	
Trerf1	358	2409	1124	409	2051	715	2.14323843	2.86853147	1336	6.72905028	2.74816626	2.44856011	
Mpp4	482	2658	991	440	2176	551	2.68213925	3.9491833	1625	5.51452282	2.25227273	2.44842587	
Ptk2b	398	2856	1176	399	2458	777	2.42857143	3.16344916	1681	7.1758794	2.94736842	2.43467337	
Ccl1	445	2738	1168	459	2293	709	2.34417808	3.23413258	1584	6.15280899	2.54466231	2.41792751	
Dock8	442	2702	1173	463	2260	710	2.30349531	3.18309859	1550	6.11312217	2.53347732	2.4129374	
Cadm3	417	2191	987	451	1774	536	2.21985816	3.30970149	1238	5.25419664	2.18847007	2.40085379	
Nav1	307	1012	505	367	705	138	2.0039604	5.10869565	567	3.29641694	1.3760218	2.39561389	
Slc6a9	363	1966	882	390	1603	492	2.22902494	3.25813008	1111	5.41597796	2.26153846	2.39482019	
Iqgap1	438	2115	941	463	1677	478	2.24760893	3.5083682	1199	4.82876712	2.03239741	2.37589711	
Ndst3	448	1813	839	488	1365	351	2.16090584	3.88888889	1014	4.046875	1.7192623	2.35384386	
Mob2	395	2233	948	389	1838	559	2.35548523	3.28801431	1279	5.65316456	2.43701799	2.31970571	
Rara	354	2910	1424	400	2556	1024	2.04353933	2.49609375	1532	8.22033898	3.56	2.30908398	

Toluidine Blue

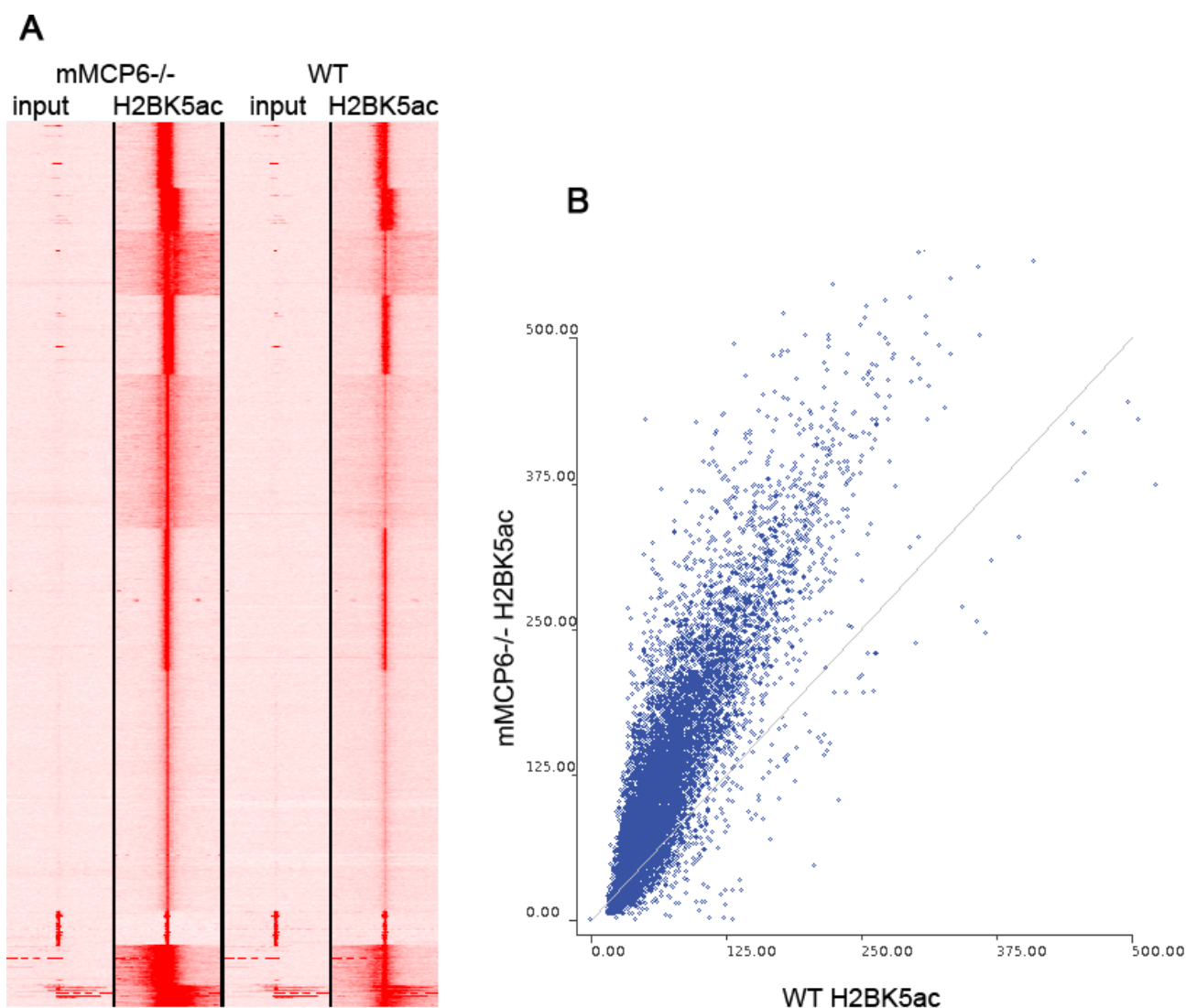
Tongue



Supplemental Figure 1. Toluidine blue staining of tongues of either 4-month- or 2-years old wild type (WT) and tryptase-deficient (mMCP6^{-/-}) mice. Note the expansion of the mast cell population (indicated by numbers) in mMCP6^{-/-} vs. WT tissue of 2-year old mice. Note also the frequent appearance of large-sized mast cells in aging animals (both in WT and mMCP6^{-/-} mice).



Supplemental Figure 2. Upper panels: Enlarged images of toluidine blue-stained tongue sections from 4-month- or 2-year-old wild type (WT) and tryptase-deficient (mMCP6^{-/-}) mice. Lower panels: Chloroacetate esterase staining of tongue sections from 4-month- or 2-year-old wild type (WT) and tryptase-deficient (mMCP6^{-/-}) mice. Chloroacetate staining detects chymase activity. Note the increase in size of mast cells from aging (both WT and mMCP6^{-/-}) mice. Note also that the chloroacetate staining is similar in young and aged mice, as well as in WT vs. mMCP6^{-/-} mast cells, indicating that neither the aging nor the absence of tryptase affects chymase levels in mast cells.



Supplemental Figure 3. More extensive H2BK5 acetylation in mMCP6^{-/-} vs. WT mast cells. ChIP-seq enrichment for H2BK5ac in mMCP6^{-/-} and WT mast cells. A) Clustering of input and ChIP read counts plotted in +/- 5 kb of peaks identified in the mMCP6^{-/-} sample. B) Read counts in 1 kb windows centered on peaks called in the WT sample. For these plots, not read count normalization was done. We obtained 26.4 M aligned reads for the H2BK5ac sample in the mMCP6^{-/-} cells and 30.9 M for the WT sample, and similar numbers for the input samples (24.7 M and 26.1 M aligned reads).