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The transcription factors GATA2 and MITF regulate *Hdc* gene expression in mast cells and are required for IgE/mast cell-mediated anaphylaxis

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Abstract

Background: Histamine is a critical mediator of IgE/mast cell-mediated anaphylaxis. Histamine is synthesized by decarboxylating the amino acid histidine, a reaction catalyzed by the Hdc-gene encoded enzyme histidine decarboxylase. However, regulation of the Hdc gene in mast cells is poorly understood.

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Objective: We sought to investigate the in vivo regulation of IgE/mast cell mediated anaphylaxis by transcription factors GATA2 and MITF and the mechanisms by which GATA2 and MITF regulate Hdc gene expression in mouse and human mast cells.

Methods: mice deficient in the transcription factors Gata2, Ahr, Ahrr, or Bhlhe40 were assessed for anaphylactic reactions. Chromatin immunoprecipitation sequencing analysis identified putative Hdc enhancers. Luciferase reporter transcription assay confirmed enhancer activities of putative enhancers in the Hdc gene. The shRNA knock down approach was used to determine the role of MITF in regulating mouse and human HDC gene expression.

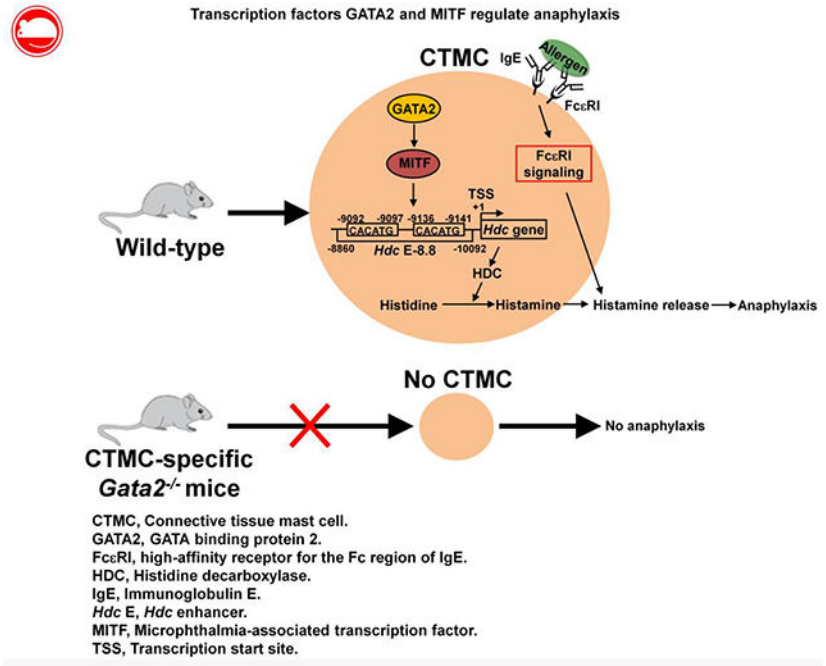
Results: Connective tissue mast cell (CTMC)-specific Gata2 deficient mice failed to develop IgE/mast cell-mediated anaphylaxis. GATA2 induced the expression of Mitf, Ahr, Ahrr and Bhlhe40 in mast cells. MITF, but not AHR, AHRR or BHLHE40, was required for anaphylaxis. MITF bound to an enhancer located 8.8 kb upstream of the transcription start site of the Hdc gene and directed enhancer activity. MITF overexpression largely restored Hdc gene expression in the Gata2 deficient-mast cells. In human mast cell line LAD2, MITF was required for the HDC gene expression and histamine synthesis.

Conclusion: The transcription factors GATA2 and MITF regulate Hdc gene expression in mast cells and are required for IgE/mast cell-mediated anaphylaxis.

Capsule summary

Our study demonstrates that the transcription factors GATA2 and MITF play an essential role in IgE/mast cell-mediated anaphylaxis mainly by regulating *Hdc* gene in mast cells.

Graphical Abstract



Keywords

mast cell; CTMC; GATA2; MITF; Hdc; enhancer; anaphylaxis; histamine synthesis

INTRODUCTION

Anaphylaxis is a serious allergic reaction that is rapid in onset and includes signs and symptoms that involve the skin, gastrointestinal track, respiratory system and cardiovascular system[1]. The most severe form of anaphylaxis is anaphylactic shock, which is characterized by hypotension and can cause death[1]. Anaphylaxis can be caused by allergies to foods, insect venoms, medications and other agents[1]. The incidence of food-induced anaphylaxis has risen at an alarming rate, especially in children, in developed countries during the past several decades and continues to rise[2–4] Understanding the molecular regulation of anaphylactic shock is an important step in developing effective prevention and treatment.

Mast cells are mononuclear protease granule-containing cells that display FcεRI, the high affinity receptor for IgE, on the cell surface. FcεRI is a heterotetramer composed of one IgE-binding α subunit, one membrane-tetraspanning β subunit and a dimer of disulfide-linked γ subunits. Mast cells can be activated by antigen (Ag) crosslinking of specific IgE that is associated with FcεRI. Mast cell activation by IgE/FcεRI crosslinking induces degranulation, with release of inflammatory mediators including histamine, and secretion of both pre-formed and newly synthesized cytokines. While cytokines induce allergic inflammation, histamine has a major role in causing IgE-mediated anaphylactic shock[5–7]. Mice genetically deficient in mast cells or depleted of mast cells by treatment with anti-stem cell factor antibody are unable to develop IgE-mediated anaphylactic shock[6, 7] Amelioration of peanut allergy by treatment with the anti-IgE monoclonal antibody omalizumab supports the importance of IgE/mast cells in mediating human anaphylactic shock[8].

Histamine is produced by the decarboxylation of the amino acid histidine, a reaction catalyzed by enzyme histidine decarboxylase[9, 10]. The *Hdc* gene encodes histidine decarboxylase, the rate-limiting enzyme that is essential for mouse and human histamine synthesis[9, 11, 12] Mice deficient in the *Hdc* gene fail to synthesize histamine and have reduced or absent IgE-mediated anaphylactic responses[13–16]. There is only limited knowledge of how *Hdc* gene expression is regulated. The transcription factor SP1 binds to a GC box found in both the human and mouse *Hdc* gene promoters[17, 18]. Several promoter elements that negatively regulate *Hdc* gene transcription have been reported. For example, YY1 and KLF4 negatively regulate the *Hdc* gene by suppressing SP1 in a gastric cancer cell line[19, 20].

GATA binding protein 2 (GATA2) is a member of the GATA family of transcription factors. GATA2 has been shown to be critical for survival and proliferation of hematopoietic stem cells[21, 22], granulocyte-monocyte progenitor differentiation[23] , and basophil and mast cell differentiation[24,25]. Recently, we demonstrated that GATA2 plays a critical role in regulating *Hdc* gene expression in mast cells[26]. However, the in vivo role of GATA2 in regulating IgE/mast cell-mediated anaphylaxis is not clear. Microphthalmia-associated transcription factor (MITF) plays a critical role in mast cell differentiation. A MITF null mutation results in loss

of cKIT expression and severely impairs mast cell differentiation in C57BL/6 mice[27, 28], although this mutation does not affect mast cell differentiation in WB mice because of overproduction of the cKIT ligand stem cell factor in this strain[29]. We reported that MITF is sufficient to induce the differentiation of common basophil/mast cell progenitor pre-BMPs into mast cells and is required to maintain mast cell identity[30]. However, it is unknown whether MITF regulates the *Hdc* gene in either mouse or human mast cells. Furthermore, the mechanisms by which GATA2 and MITF regulate the *Hdc* gene have not been investigated.

In this study, we have investigated the in vivo roles of GATA2 and MITF in IgE/mast cell-mediated anaphylaxis. We determined that GATA2 is required for CTMC differentiation and for maintaining the expression of genes required for anaphylaxis in vivo. MITF, but not other GATA2-dependent transcription factors, is critical for regulating IgE/mast cell-mediated anaphylaxis. We observed that MITF binds the −8.8 *Hdc* enhancer and is important for activities of its enhancer. Our results indicate that the transcription factors GATA2 and MITF control IgE/mast cell-mediated anaphylaxis mainly by regulating *Hdc* gene expression in mast cells.

METHODS

Mice

Mouse information is described in detail in the METHODS section of this article's Online Repository at www.jacionline.org. All animal experiments were conducted according to protocols approved by the National Jewish Health Institutional Animal Care and Use Committee (Denver, CO).

FACS analysis and sorting

Cells prepared from various tissues were stained with fluorochrome-labeled antibodies. Stained cells were acquired by CyAN (DakoCytomation, Glostrup, Denmark) and analyzed using FlowJo software (Tree Star, Ashland, OR). Cell sorting was carried out using a Moflo cytometer (DakoCytomation). A more detailed description of cell surface markers used is included in the METHODS section in this article's Online Repository at www.jacionline.org.

Quantitative RT-PCR (qPCR)

qPCR measurements were performed as described in detail under METHODS of the Online Repository. Primer sequences are listed in Table E1 of the Online Repository. qPCR analysis was carried out as described in detail in the METHODS section in this article's Online Repository at www.jacionline.org.

Passive cutaneous anaphylaxis (PCA)

Mice were sensitized with IgE anti-2, 4, 6-Trinitrophenyl (TNP) antibody and challenged with TNP-BSA (300μg /300 μl PBS) and 0.5% Evans blue dye via intravenous injection. Thirty minutes after challenge, the ears were collected and incubated in dimethylformamide for 48 hours, after which the absorbance of the dimethylformamide at 620 nm (OD620 nm)

was measured. See the METHODS in this article's Online Repository at www.jacionline.org for further details.

Passive systemic anaphylaxis (PSA)

Mice were sensitized with 10 μg of IgE anti-TNP antibody and challenged with 10 μg of TNP-BSA. Rectal temperatures of the challenged mice were measured before challenge (0 min) and at 5, 10, 15, 20, 25, 30, 40, 60, 80, 100 and 120 minutes after challenge. See the METHODS in this article's Online Repository at www.jacionline.org for further details.

Chromatin Immunoprecipitation Sequencing (ChIP-seq)

DNA fragments associated with histone H3 lysine 4 monomethylation (H3K4me1) or H3 lysine 27 acetylation (H3K27ac) were immunoprecipitated using respective specific antibodies obtained from Abcam (Cambridge, MA). The immunoprecipitated DNA fragments were repaired, ligated to adapters, and size selected to libraries, which were sequenced as reported previously[31]. Reads were aligned to the mouse reference genome and peaks were called comparing an immunoprecipated sample to its corresponding input sample. ChIP-seq data were visualized using the Integrative Genomics Viewer (IGV)[32, 33]. Data may be accessed at the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo, GSE97253). See the METHODS in this article's Online Repository at www.jacionline.org for further details.

Statistical analysis

All the error bars in this report represent standard error of mean (SEM). For ELISA, qPCR, and luciferase analyses, mean ± SEM were derived from data pooled from two or three independent experiments. Results from each independent experiment are detailed in supplemental figures in this article's Online Repository at www.jacionline.org. Differences between paired samples were analyzed using Student's t test. The changes at various time points before and after challenge in PSA analyses were analyzed using a two-way repeated measure ANOVA.

Other methods including cell culture, histology, ELISA measurements, plasmid construction, adoptive transfer of BMMCs, luciferase reporter assay, retroviral infection, ChIP, and cholera toxin treatment of BMMCs, are included in the METHODS in this article's Online Repository at www.jacionline.org.

RESULTS

GATA2 is critical for CTMC differentiation and IgE/mast cell-mediated anaphylaxis

GATA2 exerts its functions at multiple developmental stages of hematopoiesis. Previously, we reported that *Gata2* mRNA expression is upregulated in common basophil and mast cell progenitors (pre-BMPs)[26]. GATA2 is required for the differentiation of pre-BMPs into mast cells and for maintaining gene expression in mast cells in vitro[26]. However, the in vivo roles of GATA2 in mast cell differentiation and maintenance have not been investigated. To address this, we generated CTMC-specific Gata2-deficient mice by crossing *Gata2*$^{f/f}$ mice to CTMC-specific *Mcpt5*-Cre mice[34]. *Gata2*$^{f/f}$ *Mcpt5-Cre* mice exhibited a nearly complete

deficiency of mast cells in the ear skin, stomach, trachea and peritoneal cavity, while having comparable number of basophils (Fig 1, A and B) and T cells, B cells, DCs, NK cells, neutrophils and macrophages (see Fig E1 in this article's Online Repository at www.jacionline.org). Thus, we refer to *Gata2*$^{f/f}$ *Mcpt5-Cre* mice as CTMC-deficient mice.

To analyze whether connective tissue mast cell-deficient mice have compromised mast cell functions, we performed passive cutaneous anaphylaxis (PCA), an assay that measures cutaneous mast cell degranulation, as well as passive systemic anaphylaxis (PSA). We found that CTMC-deficient mice failed to develop PCA and PSA in response to antigen (Ag) and Ag-specific IgE crosslinking of FcεRI (Fig. 1, C and D). These mice failed to release histamine 5 minutes after antigen challenge (5% of controls) (Fig 1, E). MMCP-1 is a protease predominantly expressed by mucosal mast cells[35]. To determine whether CTMC-deficient mice retain the ability to produce MMCP-1, we examined MMCP-1 levels in the serum of the sensitized and challenged mice and found that CTMC-deficient mice produced 47% less MMCP-1 than WT controls (Fig 1, F), suggesting that approximately half of mucosal mast cells in *Gata2*$^{f/f}$ *Mcpt5-Cre* mice are also impaired by the absence of the *Gata2* gene. These data demonstrate that GATA2 is critical for CTMC differentiation and function in vivo and suggest that GATA2 also contributes to the differentiation and function of mucosal mast cells that express MMCP1.

To distinguish the role of GATA2 in maintaining the expression of genes that are required for mediating anaphylaxis from its role in connective tissue mast cell differentiation, we acutely deleted the *Gata2* gene in differentiated Bone Marrow-derived Mast Cells (BMMCs) and tested whether *Gata2*$^{-/-}$ BMMCs restore the ability of CTMC-deficient mice to develop anaphylactic reactions. To prepare *Gata2*$^{-/-}$ BMMCs, we treated BMMCs cultured from conditional *Gata2* knockout (*Gata2*$^{f/f}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} mice) or littermate control mice (*Gata2*$^{+/+}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} mice) with 4 hydroxytamoxifen (4HT) for 11 days to delete the *Gata2* gene as described previously[26]. YFP^{+}Gata2 $^{-/-}$ BMMCs and the control YFP^{+}*Gata2*$^{+/+}$ BMMCs were FACS-sorted and transferred into recipient CTMC-deficient mice. Our results show that *Gata2*$^{-/-}$ BMMCs failed to restore IgE-mediated anaphylaxis in CTMC-deficient mice, whereas *Gata2*$^{+/+}$ BMMCs completely restored the ability of CTMC-deficient mice to develop anaphylaxis (Fig 1, G). Our data establish that GATA2 is essential in maintaining the expression of genes that are required for anaphylaxis.

The transcription factor MITF, but not AHR, AHRR or BHLHE40, regulates *Hdc* gene expression and IgE/mast cell-mediated anaphylaxis

Our previous study showed that GATA2 is critical for *Hdc* gene expression in mast cells[26]. However, it remained unclear whether GATA2 directly regulates the *Hdc* gene or regulates it indirectly by inducing the expression of *Hdc* gene-regulating transcription factors. Mast cells express higher levels of *Hdc* transcripts[36]. Therefore, we reasoned that transcription factors that are highly expressed in mast cells, but not in basophils, are more likely to play a role in regulating the *Hdc* gene. We analyzed our published microarray data that profiled gene expression in mast cells versus basophils[30] and identified 15 transcription factor genes that are expressed to a much greater extent in mast cells than in basophils. These include *Mitf, Ahrr, Ahr, Hic1, Mecom, Bhlhe40, Batf1, Eid2, Id2, Mdfic, Meis1, Mzf1, Pgr, Tal1 and*

Zfhx3 (Fig. 2, A). In this study, we selected transcription factor genes *Mitf, Ahr, Ahrr and Bhlhe40* for further study because MITF has been shown to play a critical role in mast cell differentiation and because mice deficient in *Ahr37, Ahrr* (NIH Knockout Mice Project) and *Bhlhe40*[38] genes are available, which allowed investigation of the role of these genes in mast cell differentiation by both gene deletion and gene suppression (shRNA) approaches. Using qPCR, we verified that *Mitf, Ahr, Ahrr* and *Bhlhe40* mRNA were expressed to a much greater extent_in mast cells than in basophils [fold difference (mast cells/basophils): *Mitf* 8.8, *Ahr* 33.2, *Ahrr* 17.2 and *Bhlhe40* 19.8] (Fig. 2, B). Furthermore, expression of these transcription factor genes in mast cells is dependent on GATA2 (percentages of reduction in *Gata2*$^{-/-}$ BMMCs: *Ahr* 79.9%, *Ahrr* 94.5%, *Bhlhe40* 86.8%, and *Mitf* 60.8%) (Fig 2, C).

Mitf null mutant mice on a C57B1/6 background do not develop mature mast cells[39, 40]. To determine whether the GATA2-dependent transcription factors MITF, AHR, AHRR and BHLHE40 regulate the *Hdc* gene, we used a shRNA knockdown approach. We examined *Hdc* mRNA expression in BMMCs with knocked down *Mitf* (sh*Mitf*), *Ahr* (sh*Ahr*), *Ahrr* (sh*Ahrr*), or *Bhlhe40* mRNA expression (sh*Bhlhe40*). We found that *Hdc* mRNA expression was significantly diminished (70% reduction) in BMMCs with knocked down *Mitf* mRNA expression (Fig 3, A), but not in BMMCs with knocked down *Ahr*, *Ahrr* or *Bhlhe40* mRNA (Fig 3, B). BMMCs with knocked down *Mitf* mRNA also synthesized less histamine (68% reduction) (Fig 3, A). BMMCs with knocked down *Mitf* mRNA, but not with control shRNA targeting random sequences or with knocked down *Ahr, Ahrr*, or *Bhlhe40* mRNA, failed to restore anaphylaxis in CTMC mice (Fig 3, C).

BMMCs are considered to be immature because they contain low levels of histamine[41, 42]. To determine whether MITF is also required for *Hdc* mRNA expression and histamine synthesis in mature mast cells, we knocked down *Mitf* mRNA expression in mature peritoneal cavity mast cells (PCMCs) and found that PCMCs with knocked down *Mitf* mRNA expressed 65% less *Hdc* mRNA-and synthesized 75% less histamine than normal control mast cells (Fig 3, D). Knockdown of *Mitf* mRNA expression did not lead to mast cell death or down regulation of *Fcer1a* mRNA expression (see Fig E5 in this article's Online Repository at www.jacionline.org).

To verify the results obtained using the shRNA knockdown approach, we examined the *Hdc* mRNA in mature PCMCs of *Ahr*$^{-/-}$, *Ahrr*$^{-/-}$ or *Bhlhe40*$^{-/-}$ mice. Consistent with the results obtained using shRNAs, comparable levels of *Hdc* mRNA expression were observed in the mature PCMCs derived from *Ahr*$^{-/-}$, *Ahrr*$^{-/-}$ or *Bhlhe40*$^{-/-}$ mice and littermate control mice (see Fig E6 in this article's Online Repository at www.jacionline.org). In agreement with these findings, *Ahr*$^{-/-}$ (see Fig E7A), *Ahrr*$^{-/-}$ (see Fig E8A) and *Bhlhe40*$^{-/-}$ mice (see Fig E9A) had normal numbers of mast cells in the ear skin, stomach, trachea and peritoneal cavity. These mice also developed normal PCA (see Fig E7B, Fig E8B and Fig E9B) and PSA (see Fig E7C, Fig E8C and Fig E9C in this article's Online Repository at www.jacionline.org).

A recent study comparing mouse and human mast cell gene signatures found considerable similarity in gene expression between mast cells from these two species[43]. This observation prompted us to investigate whether MITF regulates the *Hdc* gene in human mast cells. We

knocked down human *MITF* mRNA expression in LAD2 human mast cells, which have high levels of *HDC* mRNA and histamine[44]. LAD2 cells with knocked down *Mitf* mRNA expressed 69% less *HDC* mRNA and synthesized 62% less histamine than negative control LAD2 cells (Fig 3, E). Collectively, these results demonstrate that MITF, but not any of the other GATA2-dependent transcription factors, regulates *Hdc* gene expression and histamine synthesis in both mouse and human mast cells as well as IgE/mast cell-mediated anaphylaxis in mice.

Identification of putative *Hdc* enhancers

Although our experiments established roles for MITF in regulating the *Hdc* gene and anaphylaxis, they did not provide evidence that MITF directly regulates the *Hdc* gene. To address this, we studied MITF binding of *Hdc* enhancers. Epigenomic studies demonstrate that monomethylation of lysine 4 on histone 3 (H3K4me1) marks genes that are poised to be transcribed, whereas acetylation of lysine 27 on histone 3 (H3K27ac) identifies genes that are actively being transcribed. The combined presence of H3K4me1 and H3K27ac modifications predicts enhancer activity[45–49]. To detect these marks genome-wide, we performed H3K4me1 and H3K27ac ChIP-seq analysis of BMMCs. We used BMMCs because they recapitulate the *Hdc* gene expression in peritoneal mast cells analyzed directly ex vivo. We found two putative *Hdc* enhancers (defined by both H3K4me1 and H3K27ac histone modifications) located −8.8 kb upstream and +0.3 kb downstream from the transcription start site (TSS) of the *Hdc* gene (indicated in Fig 4, A by red lines). We also note that there are two H3K4me1 peaks located around the +8.5 kb region. However, H3K27ac peaks in the locations were insignificant based on our peak calling statistical analysis (Fig 4, A). Thus, we did not consider these two H3K4me1 peaks further as putative *Hdc* enhancers.

To determine whether the putative *Hdc* enhancers function in *Hdc* gene transcription, we constructed a luciferase reporter gene in which luciferase expression is under the control of the *Hdc* minimal promoter (−44 to +1 relative to the TSS) and the −8.8 or +0.3 *Hdc* enhancer (a schematic diagram is shown in Fig 4, B *upper panel*). We found that the −8.8 *Hdc* enhancer dramatically increased the activity of the *Hdc* minimal promoter (13.4-fold increase) (Fig 4, B, *lower panel*), whereas the +0.3 *Hdc* enhancer failed to show significant enhancer activity.

MITF binds to the −8.8 *Hdc* enhancer and drives its activity

To determine whether MITF binds to the −8.8 *Hdc* enhancer, we aligned our H3K4me1 and H3K27ac ChIP-seq data from this region with the published MITF ChIP-seq data[50] and found one statistically significant MITF-binding peak in the −8.8 *Hdc* enhancer (Fig 5, A). Our ChIP-qPCR analysis of MITF binding in BMMCs revealed that among five predicted MITF-binding sites located within the −8.8 and +0.3 *Hdc* enhancers (Fig 5, A), MITF binding was only detected at two MITF-binding sites located in the −8.8 *Hdc* enhancer (e.g., MITF-binding sites 3 and 4, a 6.3-fold enrichment found in the combined binding sites) (Fig 5, B).

BMMCs and the CFTL-15 mast cells that we used to analyze *Hdc* enhancer activity are considered to be immature mast cells because they contain low amounts of histamine and express fewer receptors for IgE than mature mast cells[51–53]. To determine whether the binding of MITF to the −8.8 *Hdc* enhancer increases in mature mast cells, we measured MITF binding both in BMMCs that were induced to mature and in mature PCMCs. BMMCs were induced to mature by cholera toxin treatment as reported[54]. We found that *Hdc* mRNA expression levels and histamine content were enhanced 8.9- and 4.7-fold, respectively, by cholera toxin treatment (Fig 5, C and D). Concomitantly, the binding of MITF to the −8.8 *Hdc* enhancer also increased to levels similar to those of mature PCMCs [Fold enrichment (anti-MITF antibody/IgG): untreated BMMCs 7.4, cholera toxin-treated BMMCs 11.2 and PCMCs 11.7] (Fig 5, E).

To determine whether MITF activates −8.8 *Hdc* enhancer activity, we prepared CFTL-15 mast cells with control or knocked down *Mitf* mRNA, followed by transfection of the −8.8 *Hdc* enhancer luciferase reporter gene construct. The −8.8 *Hdc* enhancer lost ~60% of its activity in the presence of knocked down (by 80%) *Mitf* mRNA (Fig 6, A). Conversely, overexpression of MITF in a non-mast cell line greatly increased the activity of the −8.8 *Hdc* enhancer (8.2-fold increase) (Fig 6, B). Mutations in MITF-binding sites 3 and 4, but not MITF-binding site 5, abolished the MITF/−8.8 *Hdc* enhancer-driven luciferase reporter gene activity (percentages of reduction: BS3 Mut, 98%; BS4 Mut, 92% and BS345 Mut, 92%) (Fig 6, C). Taken together, our results demonstrate that MITF directly binds to the −8.8 *Hdc* enhancer in immature as well as mature mast cells and activates the enhancer.

MITF is sufficient to fully restore *Kit* and *Fcer1a* gene expression but only partially restores *Hdc* gene expression

Having established that expression of the *Mitf* gene in mast cells is GATA2-dependent and that MITF directly regulates the *Hdc* gene, we investigated whether MITF is sufficient to upregulate *Hdc* gene expression in the absence of the *Gata2* gene. To prepare $Gata2^{-/-}$ BMMCs that overexpress MITF ($Mitf^{+}$ $Gata2^{-/-}$ BMMCs), $Gata2^{f/f}$ × *ERT2-Cre* BMMCs were retrovirally transduced with recombinant retrovirus for enforced expression of MITF. Next, $Mitf^{+}$ $Gata2^{f/f}$ BMMCs were acutely depleted of the *Gata2* gene by 4HT treatment for 11 days[26]. Our results confirmed that overexpression of MITF is sufficient to drive the expression of the *Kit* and *Fcer1a* genes (Fig 7, A), but only partially restored *Hdc* gene expression ~63% relative to the wild-type control (Fig 7, B). In contrast, overexpression of GATA2 completely restored *Hdc* gene expression (146% of wild-type) (Fig 7, B). Together, these results indicate that GATA2 regulates *Hdc* expression though inducing MITF, and that additional GATA-2 dependent mechanisms are required for full expression of the *Hdc* gene in mast cells.

DISCUSSION

Our results demonstrate that a GATA2-MITF regulatory circuit controls IgE/mast cell-mediated anaphylaxis in mast cells. Previous studies established that GATA2 plays a crucial role in mast cell development. We and others have demonstrated that GATA2 is essential for the differentiation of pre-BMPs into mast cells and is required for maintaining the molecular

program that defines mast cells in vitro[26, 55]. In this study, we present evidence that GATA2 is required for the differentiation of CTMCs in vivo. Although CTMC-deficient mice failed to release histamine in response to Ag and Ag-specific IgE crosslinking of FcεRI, these mice retained the capacity to secrete mast cell protease MMCP1, albeit in reduced amounts. Our findings are consistent with evidence that CTMCs play a major role in inducing anaphylaxis[56]. Taking advantage of the CTMC-deficient mice in adoptive transfer experiments, we demonstrated that GATA2 was essential for maintaining the ability of mast cells to mediate anaphylaxis in response to Ag and Ag-specific IgE crosslinking of FcεRI.

Our study sheds light on molecular mechanisms by which transcription factors regulate IgE/mast cell-mediated anaphylaxis. We and others previously confirmed that GATA2 is the major transcription factor required for maintaining the expression of many, if not all, mast cell signature genes[26, 55]. These results indicated that GATA2 initiates a regulatory hierarchy composed of many transcription factors that synergistically control expression of mast cell-specific genes. Here, we show that GATA2 induces the expression of the transcription factor-encoding *Mitf, Ahr, Ahrr* and *Bhlhe40* genes. We also found that the expression of *Ahr, Ahrr* and *Bhlhe40* depends on MITF (Li et. al. unpublished data), indicating that MITF functions at a higher level within the hierarchy than AHR, AHRR and BHLHE40. Consistent with this model, MITF is critically involved in regulating the expression of *Hdc*, as well as additional genes in mast cells (Li et. al. unpublished data). Our data obtained with both gene deletion and shRNA approaches demonstrate that AHR, AHRR and BHLHE40 are not required for *Hdc* gene expression. However, we should interpret these findings with caution. We do not rule out the possibility that AHR, AHRR or BHLHE40 redundantly regulate the *Hdc* gene in mast cells.

Enhancers and their associated transcription factors are critical regulators of cell-specific gene expression[49, 57] We used active histone marks to localize two potential *Hdc* gene enhancers. We demonstrate that MITF directly binds and activates the −8.8 kb upstream *Hdc* enhancer. Because transfection efficiency of mature primary mast cells is inefficient, we transfected CFTL-15 mast cells with the *Mitf* and luciferase reporter genes to analyze *Hdc* enhancer activity. CFTL-15 mast cells are thought to resemble immature mast cells because they contain low amounts of histamine and express fewer receptors for IgE than mature PCMCs[58]. Our results provide evidence that MITF also regulates the *Hdc* gene expression in mature mast cells. MITF binding to the −8.8 *Hdc* enhancer concomitantly was increased when BMMCs were induced to mature to cells with higher *Hdc* gene expression. Although the increase in MITF binding was moderate compared with the increase in *Hdc* gene expression as BMMCs matured, we propose that this moderate increase in MITF binding might correlate with greater *Hdc* transcriptional output when the MITF binding is put into context with the *Hdc* promoter and other potential *Hdc* enhancers. Furthermore, we demonstrated that *Mitf* gene expression is required to maintain *Hdc* gene expression in mature mast cells. In contrast to the −8.8 enhancer, the +0.3 downstream *Hdc* enhancer lacks detectable_enhancer activity. However, the in vivo importance of this enhancer in *Hdc* gene transcription cannot be ruled out by the luciferase reporter gene transcription assay alone.

Although mouse and human mast cells share expression of similar genes programs, direct evidence that MITF plays a critical role in regulating *Hdc* gene expression and histamine

synthesis in human mast cells lays a solid foundation for future studies of how the human *HDC* gene are transcriptionally regulated. This will enable a more comprehensive understanding of how histamine synthesis and release are controlled.

Although MITF fully restored c-Kit and FcεR1αexpression, it only restored *Hdc* gene transcription to an average of 63% of wild-type amounts. This suggests that in addition to inducing MITF expression, GATA2 is directly involved in regulating the *Hdc* gene. Feed-forward loop transcription circuits regulate gene expression in many developmental systems[59]. This regulation involves three classes of factors. First, a primary transcription factor gene (gene product) regulates the expression of a second transcription factor gene. The primary and secondary transcription factors bind the regulatory region of a third (target) gene and cooperatively regulate its transcription[59]. Our data support GATA2, MITF and the *Hdc* gene form a feed-forward loop. In this mode of action, it is possible that in addition to inducing MITF expression, GATA2 maintains the accessibility of the *Hdc* promoter and/or enhancers, and that GATA2 may function by coordinating interactions between promoter and enhancers modules. It is also possible that GATA2 cooperates with MITF to optimally transcribe the *Hdc* gene. Thus, our findings that GATA2 and MITF regulate IgE/mast cell-mediated anaphylaxis mainly by regulating the *Hdc* enhancer add new knowledge how enhancers and their associated transcription factors_regulate anaphylaxis.

Genes are typically regulated by a set of transcription factors that bind together to clustered binding sites within an enhancer region[49, 60, 61]. One of the best-studied examples is the human *IFNB* enhancer, which is activated by multiple transcription factors in response to intracellular signaling pathways[62]. Our findings confirm a central role for MITF in coordinating a cluster of transcription factor-binding sites within the *Hdc* enhancer to regulate histamine synthesis. Future studies will address how *Hdc* enhancers interact with the *Hdc* promoter, as well as how other transcription factors cooperatively regulate the *Hdc* gene.

METHODS

Mice

Gata2$^{f/f}$ mice (B6.129-*Gata2*^{tm1Sac}/Mmmh)[E1] were purchased from the Mutant Mouse Regional Resource Center (the mutant mouse regional resource center at University of California-Davis, Davis, CA). Inducible *Gata2* knockout mice (*Gata2*$^{f/f}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} mice) were generated by crossing *Gata2*$^{f/f}$mice to Cre activity reporter *Rosa*$^{Yfp/Yfp}$ mice[E2] [B6.129X1-*Gt(ROSA)26Sor*$^{tm1(EYFP)Cos}$/J, the Jackson Lab, Bar Harbor, ME] and inducible Cre mice [B6. Cg-Tg(CAG-cre/Esr1*)5Amc/J][E3] (the Jackson Lab) as described [E4]. These mice were backcrossed to the C56BL/6 background for 4 generations. Connective tissue mast cell-specific *Gata2* deficient mice (*Gata2*$^{f/f}$ *Mcpt5*-Cre mice) were generated by crossing *Gata2*$^{f/f}$ mice to connective tissue mast cell-specific Cre mice[E5] [Mcpt5-Cre mice, *B6-Tg(Cma1-cre)*/ARoer, provided by Dr. Axel Roer via Dr. Jim Hagman, Denver, CO]. These mice were backcrossed to the C56BL/6 background for 5 generations. Mast cell-specific *Ahr* deficient mice (Ahr$^{f/f}$Cpa3-Cre mice) were generated by crossing *Ahr*$^{f/f}$ mice[E6] (*B6-Ahr*$^{tm3.1Bra}$*/J*, the Jackson Lab) to the mast cell-specific Cre mice[E7] [Cpa3-Cre mice, *B6-Tg(Cpa3-* cre)4Glli/J, the Jackson Lab]. Ahrr$^{-/-}$ sperm with the

FRT flanked Neo and floxed *Ahrr* gene (*Ahrr*$^{tm1a(KOMP)Wtsi}$) were purchased from the NIH knockout mouse project repository (University of California-Davis, Davis, CA). Our core facility (National Jewish Health, Denver, CO) performed in vitro fertilization and generated *Ahrr* knockout first mice (referred to as *Ahrr*$^{-/-}$ mice). *Bhlhe40*$^{-/-}$ mice (B6.129.CD1-*Bhlhe40*^{tm1Rhli}) were generated by Dr. Robert H Lipsky[E8] and provided by Dr. Mark Mattson of the National Institutes of Health (Bethesda, Maryland). These mice were on a B6.129 mixed genetic background (backcrossed to the C56BL/6 background for one generation). All animal experiments were conducted according to protocols approved by the National Jewish Health Institutional Animal Care and Use Committee.

FACS analysis and sorting

For fluorescence activated cell sorting (FACS) analysis of basophils, mast cells, cells obtained from the bone marrow and peritoneal cavity were stained with the following antibodies: basophils and mast cells were stained with APC-CY7-conjugated anti-c-Kit (2B8) and PE-CY7-conjugated anti-FcεRIα (MAR-1) antibodies; peritoneal mast cells were identified as CD3ε^{-}B220^{-} FcεR1α^{+}c-Kit^{+}; T cells and B cells were stained with PE-CY5-conjugated anti-CD3ε (145–2C11) and PE-CY7-conjugated anti-CD19 (1D3) antibodies, respectively; dendritic cells (DCs) were stained with APC-conjugated anti-MHC Class II (MHC II) (M5/114.15.2) and PE-conjugated anti-CD11c (N418) antibodies; neutrophils and macrophages were stained with PE-conjugated anti-Gr-1 (RB6–8C5) and APC-conjugated anti-CD11b (M1/70) antibodies; NK cells were stained with APC-conjugated anti mouse pan-NK cells (CD49b) (DX5) and PE-conjugated anti-NK1.1 (PK136) antibodies. Dead cells were identified by staining with propidium iodide (PI) and excluded from all FACS plots. Stained cells were acquired by using CyAN (DakoCytomation, Glostrup, Denmark) and analyzed using FlowJo software (Tree Star, Ashland, OR). The total number of positive cells was calculated by multiplying the total number of cells harvested from the bone marrow or peritoneal cavities by the percentage of positive cells. The YFP^{+} or YFP^{+}GFP^{+} BMMCs, and basophils (FcεR1α^{+}c-Kit^{-} CD49b^{+}CD11b^{+}) were FACS-sorted using a MoFlo cytometer (DakoCytomation, Glostrup, Denmark). All Abs used for FACS analysis and sorting were purchased from BD Pharmingen (San Diego, CA) or eBioscience (San Diego, CA).

Cell culture

Bone marrow-derived mast cells (BMMCs) were prepared by culturing bone marrow cells from mice in the presence of IL-3 for 4weeks as described[E4]. Greater than 99% of BMMCs were mast cells (FcεRIα^{+} c-Kit^{+}) as determined by FACS analysis. Peritoneal Cavity Mast Cells (PCMCs) were cultured from cells collected from the peritoneal cavity of mice in Iscove's DMEM (10016CV, Corning™ cellgro™, Manassas, VA) plus 10% FBS,100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM beta-mercaptoethanol in the presence of 20 ng/mL IL-3 and 20 ng/mL recombinant murine SCF (250–03, Peprotech, Rocky Hill, NJ). After 4 weeks of culture, over 99% of cells were mast cells (FcεRIα^{+} c-Kit^{+}). Basophils were prepared as described[E4]. Briefly, IL-3 (10 μg) and anti-IL-3 antibody (5 μg; MP2-8F8; BD PharMingen) complex (IL-3C) was i.p. injected into mice 3 days before bone marrow harvest. HEK293 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM plus 10% FBS, 100 units/ml penicillin, and

100 μg/ml streptomycin at 5% CO_2 and 37 °C. CFTL-15, a murine IL-3-dependent mast cell line[E9], was provided by Dr. Melissa Brown (Northwestern University, Chicago, IL). CFTL-15 cells were cultured in complete RPMI 1640 containing 10% FBS in the presence of 20 ng/ml IL-3[E10].

The human mast cell line LAD2 was provided by Dr. Dean Metcalfe (National Institutes of Health, Bethesda, MD)[E11] and cultured in StemPro™-34 SFM (10639011, Thermo Fisher Scientific, Waltham, MA) plus 2.6% StemPro-34 Nutrient Supplement (GIBCO), 2 mM L-Glutamine (25005CI, Corning™ cellgro™), 100 units/ml penicillin, and 100 μg/ml streptomycin in the presence of 100 ng/ml recombinant human SCF (300-07, Peprotech)[E12].

Histology

Tissues from *Gata2*$^{f/f}$*Mcpt5*-Cre mice or littermate control *Gata2*$^{+/+}$*Mcpt5*-Cre mice, *Ahr*$^{f/f}$ *Cpa3*-Cre mice or littermate control *Ahr*$^{+/+}$*Cpa3*-Cre mice, *Ahr*$^{-/-}$ mice or littermate control *Ahr*$^{+/+}$ mice, *Bhlhe40*$^{-/-}$ mice or littermate control *Bhlhe40*$^{+/+}$ mice, were fixed in 4% paraformaldehyde, cut into 5 μm sections and stained with toluidine blue as described previously[E4]. Histological images were captured on a Nikon E800 microscope (Nikon, Melville, NY).

Passive cutaneous anaphylaxis (PCA)

Gata2$^{f/f}$*Mcpt5*-Cre mice or littermate control *Gata2*$^{+/+}$*Mcpt5*-Cre mice, *Ahr*$^{f/f}$ *Cpa3*-Cre mice or littermate control *Ahr*$^{+/+}$*Cpa3*-Cre mice, *Ahr*$^{-/-}$ mice or littermate control *Ahr*$^{+/+}$ mice, *Bhlhe40*$^{-/-}$ mice or littermate control *Bhlhe40*$^{+/+}$ mice, were sensitized by IgE anti-2, 4, 6-Trinitrophenyl (TNP) antibody (100 ng/10 μl) (IGEL 2a, ATCC, Manassas, VA)[E13] or PBS (10 μl) via intradermal injection. Twenty-four hours later, the sensitized mice were challenged with TNP-BSA (300 μg/300 μl PBS) (T-5050–100, LGC Biosearch Technologies, Novato, CA) with 0.5% of Evans blue dye via intravenous injection. Thirty minutes after challenge, ears were collected and incubated in dimethylformamide for 48 hours, which was then measured for absorbance at 620 nm (OD620 nm)[E14].

Passive systemic anaphylaxis (PSA)

Mice were intraperitoneally sensitized with 10 μg of IgE anti-TNP antibody (IGEL 2a, ATCC)[E13] in 100 μl PBS. Twenty-four hours later, the sensitized mice were intraperitoneally challenged with 10 μg of TNP-BSA in 100 μl PBS. Rectal temperature of the challenged mice was measured before challenge (0 min) and at 5, 10, 15, 20, 25, 30, 40, 60, 80, 100 and 120 minutes after challenge as described[E15].

Histamine measurement

Plasma was collected 5 minutes after antigen challenge for histamine release measurement._For histamine content measurement, BMMCs, PCMCs, and human mast cell line LAD2 cells were frozen and thawed three times. Histamine in the plasma or cell lysates was measured by using an enzyme immunoassay kit (Beckman Coulter, Fullerton, CA) according to the manufacturer's instruction.

Measurement of Mouse Mast Cell Protease 1 (MMCP-1)

Serum was collected 4 hours after antigen challenge. Mouse Mast Cell Protease 1 (MMCP-1) concentrations in the serum were measured with an ELISA kit (Penicuik, Scotland) according to the manufacturer's instruction.

Lentiviral short hairpin (sh) RNA

shRNA targeting genes of interest (sh*Mitf*, TRCN0000362536, sh*Ahr* TRCN0000226005, sh*Ahrr* TRCN0000445538, and sh*Bhlhe40* TRCN0000226078) were designed and tested by The RNAi Consortium (TRC) at the Broad Institute and distributed as Mission® shRNA library by Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). ShRNA targeting random sequences was used as a control (shRNA CTRL, SHC216-pLKO.5-puro Nontarget shRNA control). Lentiviral transduction particles were prepared by transfecting HEK293 cells with shRNA plasmid (2 μg) and packaging plasmids (1.5 μg of *pΔ8.9* and 0.5 μg of *pCMV-VSV-G*, Sigma-Aldrich). Lentiviral supernatant was collected and filtered 48 hours after transfection. Lentivirus was added to BMMCs (1×10^6 cells/ml) or CFTL-15 cells (1×10^6 cells/ml) in a 12-well plate in the presence of polybrene (8 μg/ml; Millipore, Billerica, MA) and centrifuged at 1,350 *g* for 90 minutes at room temperature. Transduced cells were then cultured in complete IDMEM (BMMC) or RPMI160 (CFTL-15) in the presence of 20 ng/ml of IL-3 and 2 μg/ml (BMMC) or 4 μg/ml (CFTL-15) puromycin (Sigma-Aldrich) for three days. Ten days after puromycin selection, cells were used for experiments.

Adoptive transfer of BMMCs

Gata2$^{-/-}$ and control *Gata2*$^{+/+}$ BMMCs were prepared as described[E4]. Briefly, *Gata2*$^{-/-}$ BMMCs were cultured from bone marrow of *Gata2*$^{f/f}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} and Gata2$^{+/+}$BMMCs were cultured from control *Gata2*$^{+/+}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} mice for 4 weeks. The cultured BMMCs were treated with 25 nM 4-hydroxytamoxifen (4HT) for 11 days. YFP$^+$ BMMCs were FACS-sorted and used as donor BMMCs. Deletion of the floxed *Gata2* gene in the sorted YFP$^+$*Gata2*$^{f/f}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi} BMMCs was determined to be near 100%. Donor BMMCs with knocked down *Mitf* mRNA, *Ahr* mRNA, *Ahrr* mRNA, *Bhlhe40* mRNA, or with a shRNA control were prepared by using the shRNA approach. BMMCs from bone marrow of *Gata2*$^{+/+}$*Mcpt5*-Cre mice were transduced with sh*Mitf* lentivirus, sh*Ahr* lentivirus, shAhrr lentivirus, sh*Bhlhe40* lentivirus, or shRNA control virus. Donor cells (1×10^6 in 200 μl PBS) were intraperitoneally injected into *Gata2*$^{f/f}$*Mcpt5*-Cre mice. Three days later, the reconstituted mice were analyzed for PSA.

Quantitative PCR (qPCR)

Total RNA from FACS-sorted basophils, cultured BMMCs, PCMCs, or human mast cell line LAD2 cells was isolated using TRIzol® (Ambion Inc, Austin, TX). cDNA was synthesized by reverse transcription. Quantitative PCR (qPCR) was performed in an ABI PRISM 7700 Sequence Detection System. Primer sequences are listed in Table E1. Relative mRNA amounts were calculated as follows: Relative mRNA amount = $2^{[Ct(Sample)-Ct(Hprt)]}$.

Chromatin immunoprecipitation assay

ChIP analysis of MITF recruitment to the *Hdc* enhancers was performed as described[E4]. Anti-MITF antibody (C5) was purchased from Thermo Scientific (Fremont, CA). IgG control was purchased from Santa Cruz Biotech. Fold enrichment was calculated using the following equation: Fold Enrichment= $2^{[\Delta Ct(IgG)-\Delta Ct(IP)]}$, where $\Delta Ct(IgG)=Ct_{(IgG)}-[Ct_{(input)} - Log_2(\text{Input Dilution Factor})]$, $\Delta Ct(IP)= Ct_{(IP)}-[Ct_{(input)}-Log_2(\text{Input Dilution Factor})]$[E16].

Plasmid construction

The *Hdc* minimal promoter (−44 to −1 relative to the transcription start site (TSS) of the *Hdc* gene) was amplified and cloned into a pGL3-basic luciferase vector (Madison, WI) via the Bg1II and HindIII restriction sites. The −8.8 enhancer (−8860 to −10092 relative to the TSS of the *Hdc* gene) and the +0.3 enhancer (+273 to +1762) were generated by PCR amplification and cloned into the *Hdc* minimal promoter luciferase vector via the KpnI and MluI restriction sites. The MITF binding site mutations were generated by overlapping PCR. The primers used for plasmid constructions are listed in Table E1. All constructed plasmids were verified by sequencing.

Luciferase reporter assay

CFTL-15 cells (5×10^6) were electroporated with 10 μg of luciferase plasmid and 0.5 μg of *Renilla* vector at 450 V and 500 microfarads using a Bio-Rad Gene Pulser. HEK293 cells were transfected with 1 μg of luciferase plasmid and 0.1 μg of *Renilla* vector plus MITF expression plasmid or control plasmid using calcium phosphate. Twenty hours after electroporation or transfection, cells were collected, and luciferase activities were measured by using an InfiniteMlOOO® microplate reader (Tecan Systems, Inc., San Jose, CA) and the Dual-Luciferase reporter assay system (E1960, Promega). The transcription activity was normalized as the ratio of luciferase activity divided by *Renilla* activity.

Retroviral infection

Full-lengths *Gata2* and *Mitf* cDNAs were amplified from mouse BMMC mRNA and cloned into the retroviral expression vector MSCV2.2-Ires-*Gfp* through BglII and NotI restriction sites. The retroviral particles preparation and infection were conducted as described previously [E4]. In brief, bone marrow cells from the control (*Gata2*$^{+/+}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi}, *Gata2*$^{+/+}$) mice and inducible *Gata2* knockout (*Gata2*$^{f/f}$*Rosa*$^{Yfp/Yfp}$*TgCreErt2*^{hemi}, *Gata2*$^{f/f}$) mice were spin infected with 1 mL of recombinant retrovirus containing the *Gata2, Mitf*, or *Gfp* (CTRL) gene. Infected cells were cultured in complete IDMEM in the presence of 20 ng/ml of IL-3 for two days before they were treated with 25 nM 4HT for 11 days to delete the *Gata2* gene as described[E4]. The treated cells were analyzed by FACS analysis or subjected to FACS sorting. YFP^+GFP^+ cells were used for qPCR analysis.

ChIP-seq

DNA fragments associated with H3K4me1 or H3K27ac histone marks were immunoprecipitated with anti-H3K4me1 antibody (ab8895, Abcam, Cambridge, MA) or anti-H3K27ac antibody (ab4729, Abcam), respectively. The immunoprecipitated DNA

fragments were repaired, ligated to adapters, and size selected with the Ion Plus Fragment Library Kit (#4471252, Thermo Fisher Scientific) according to the Ion ChIP-Seq Library Preparation protocol. Libraries were analyzed for size and quantity using the High Sensitivity Bioanalyzer Kit (#5067–4626, Agilent Technologies, Santa Clara, CA). The ChIP library was sequenced on an Ion Torrent Proton Semiconductor Sequencer. Reads were aligned to the mouse mm9 reference genome using Torrent Suite v4.4.6 for the H3K4me1 samples and v5.0.4 for the H3K27ac samples using default parameters. Peaks were identified by comparison with matched input DNA using MACS version 2.0.10.20130306 (tag: beta) for H3K4me1 and v2.1.1.20160309 for H3K27ac with a q-value threshold of 0.001 (macs2 callpeak -t .. -c .. -f BAM -g mm -n … -q 0.001 -B -- SPMR)[E14]. A bedGraph coverage file to display the fold enrichment (FE) of each immunoprecipated sample with respect to its matched input DNA was created using MACS (macs2 bdgcmp -c …_control_lambda.bdg -t …_treat_pileup.bdg - o …_FE.bedgraph -m FE). ChIP-seq data were displayed using Integrative Genomics Viewer (IGV)[E17, 18]. The ChIP-seq data have been deposited in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo, GSE97253). These data will be released to the public upon acceptance of this manuscript.

Cholera toxin treatment of BMMCs

BMMCs were not treated or treated with 0.08 μg/mL cholera toxin (#100B, List Biological Laboratories Inc., Campbell, California) for 48 hours according to the published report[E19]. Then, the cells were used for qPCR analysis of *Hdc* mRNA expression and ELISA analysis of histamine content.

Extended Data

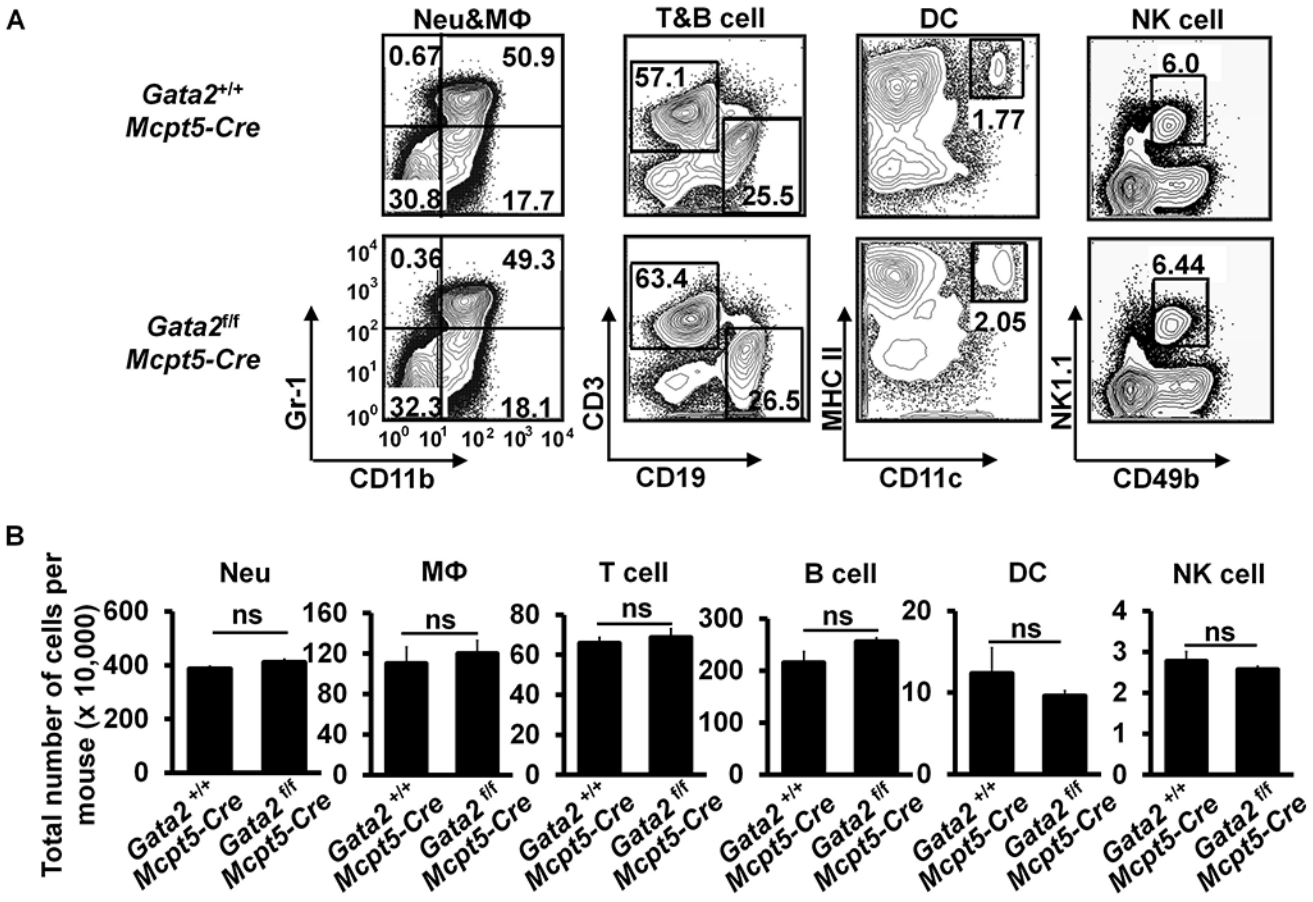


FIG E1.

Neutrophils (Neu), Macrophages (MΦ), T cells, B cells, Dendritic cells (DCs), and NK cells are not affected in *Gata2*$^{f/f}$ *Mcpt5*-Cre mice. A. Flow analysis of the indicated cell lineages in the bone marrow (Neutrophils and macrophages) and spleen (T cells, B cells, Dendritic cells and NK cells). B. Total number of cells (mean ± SEM, n=3 mice). FIG E1 related to FIG 1.

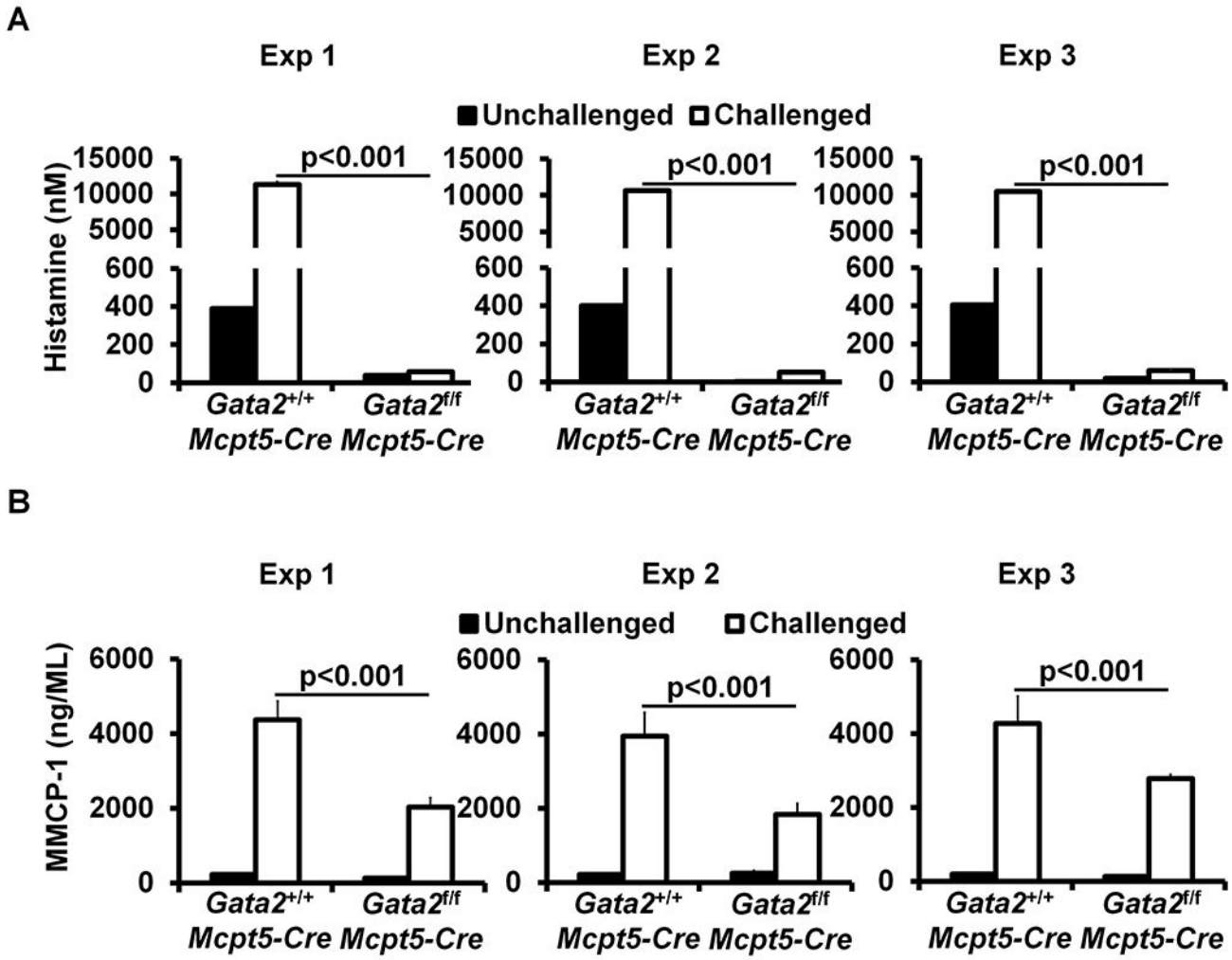


FIG E2.

Plasma histamine and serum MMCP-1 levels are decreased in *Gata2*$^{f/f}$ *Mcpt5*-Cre mice. **A.** ELISA analysis of plasma histamine level (mean ± SEM, triplicates). The left, middle, and right panels show three independent experiments. **B.** ELISA analysis of serum MMCP-1 level (mean ± SEM, triplicates). The left, middle, and right panels show three independent experiments. FIG E2A related to FIG 1E; FIG E2B related to FIG 1F.

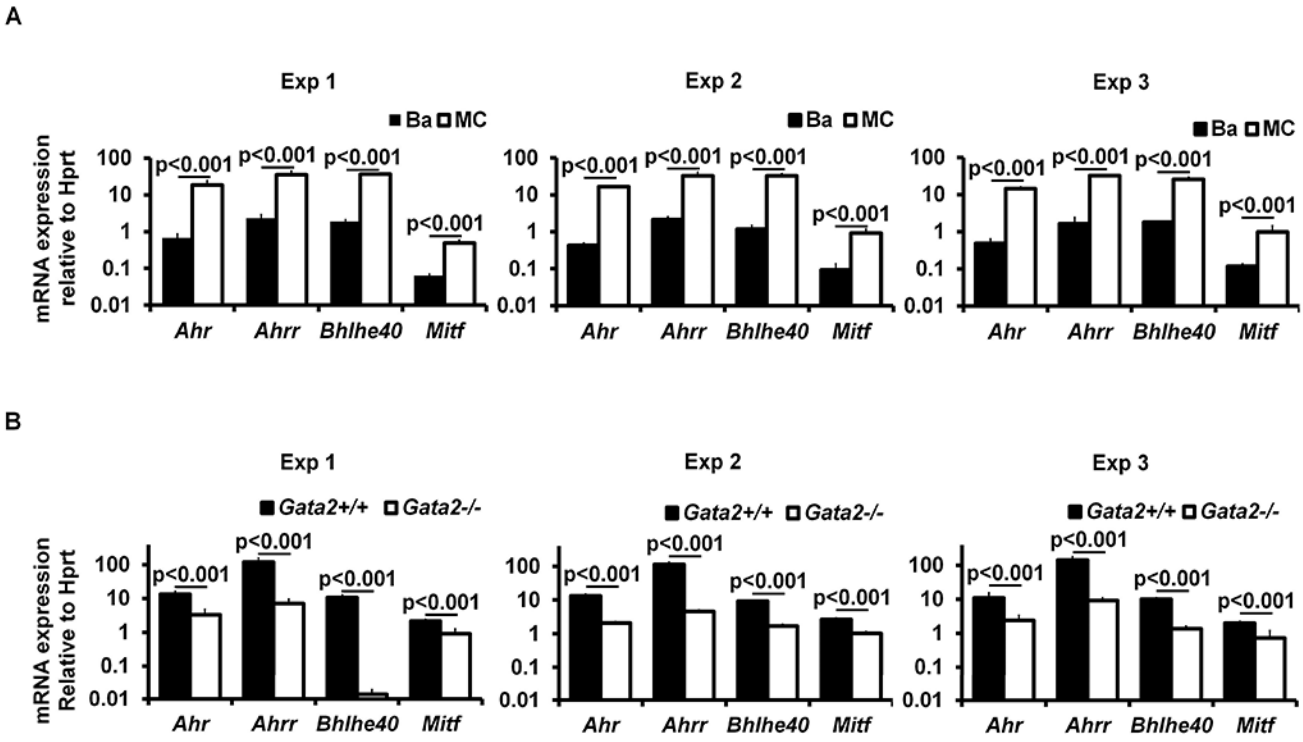


FIG E3.

Transcription factors AHR, AHRR, BHLHE40 and MITF depend on GATA2 for their expression in mast cells. qPCR analysis of the mRNA expression in BMMCs (MC) versus basophils (Ba) **(A)**, and *Gata2*$^{+/+}$ BMMCs versus *Gata2*$^{-/-}$ BMMCs **(B)** (mean ± SEM, triplicates). The left, middle, and right panels each show three independent experiments. FIG E3A related to FIG 2B; FIG E3B related to FIG 2C.

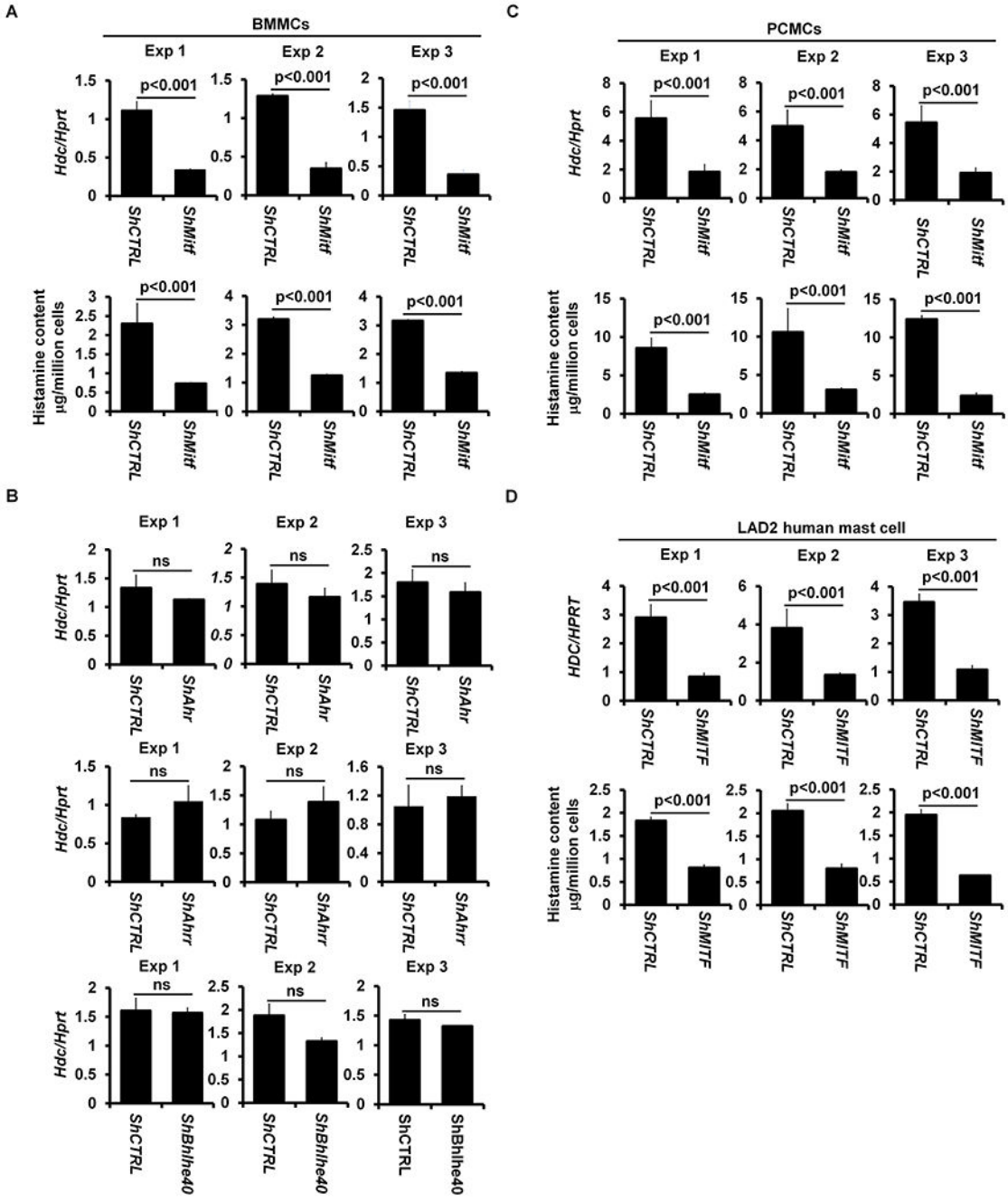


FIG E4.

The transfection factor MITF, but not AHR, AHRR or BHLHE40 regulates the *Hdc* gene expression. qPCR analysis of the *Hdc* mRNA expression and ELISA analysis of histamine content in BMMCs **(A)**, PCMCs **(C)**, and human mast cell line LAD2 **(D)** with knocked down *Mitf* mRNA (Sh*Mitf*) or with shRNA control (ShCTRL) (mean ± SEM, triplicates); **B.** qPCR analysis of the *Hdc* mRNA expression in BMMCs with knocked down *Ahr* mRNA (Sh*Ahr*), *Ahrr* mRNA (Sh*Ahrr*), and *Bhlhe40* mRNA (Sh*Bhlhe40*) or with shRNA control (ShCTRL) (mean ± SEM, triplicates). The left, middle, and right panels each show three

independent experiments. FIG E4A related to FIG 3A; FIG E4B related to FIG 3B; FIG E4C related to FIG 3D; FIG E4D related to FIG 3E.

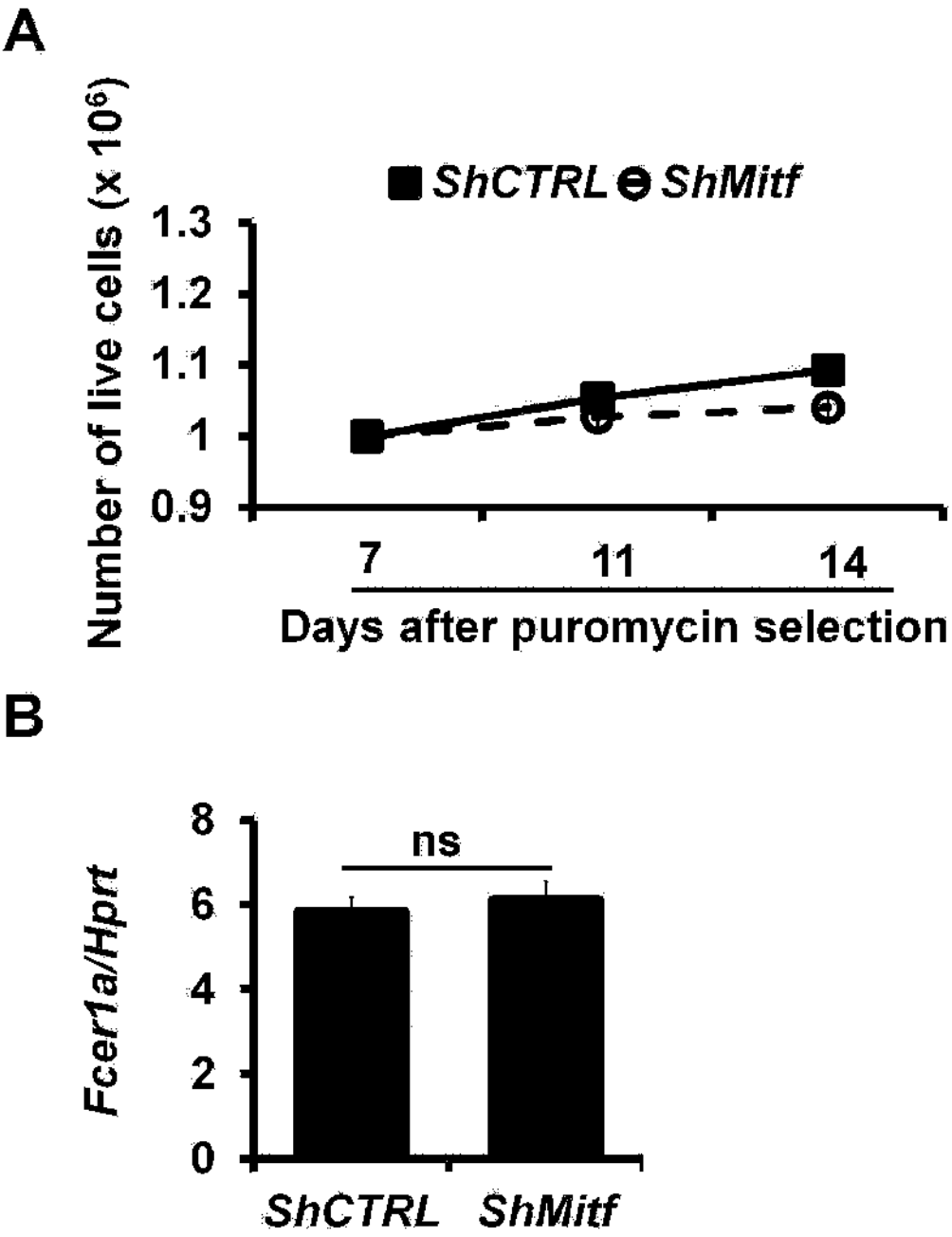


FIG E5.

Knockdown of *Mitf* mRNA expression does not lead to mast cell death or down regulation of *FceR1a* expression. **A.** Number of live *ShCTRL* or *ShMitf* PCMCs at different days after puromycin selection (mean ± SEM, n=3 samples). **B.** qPCR analysis of the *Fcer1a* mRNA

expression in *ShCTRL* or *ShMitf* PCMCs (mean ± SEM, n=3 samples). FIG E5 related to FIG 3.

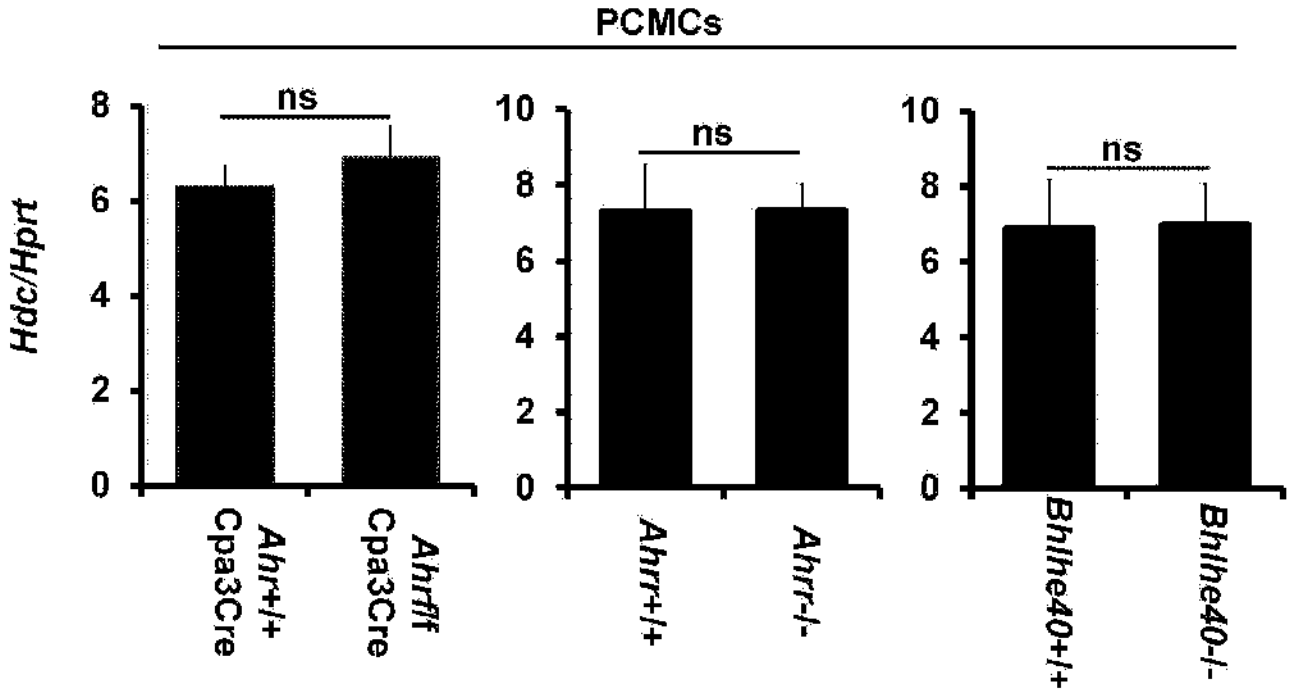


FIG E6.

The transfection factors AHR, AHRR and BHLHE40 are not required for the *Hdc* gene expression in PCMCs. qPCR analysis of the *Hdc* mRNA expression in PCMCs of the indicated mice (mean ± SEM, triplicates). FIG E6 related to FIG 3.

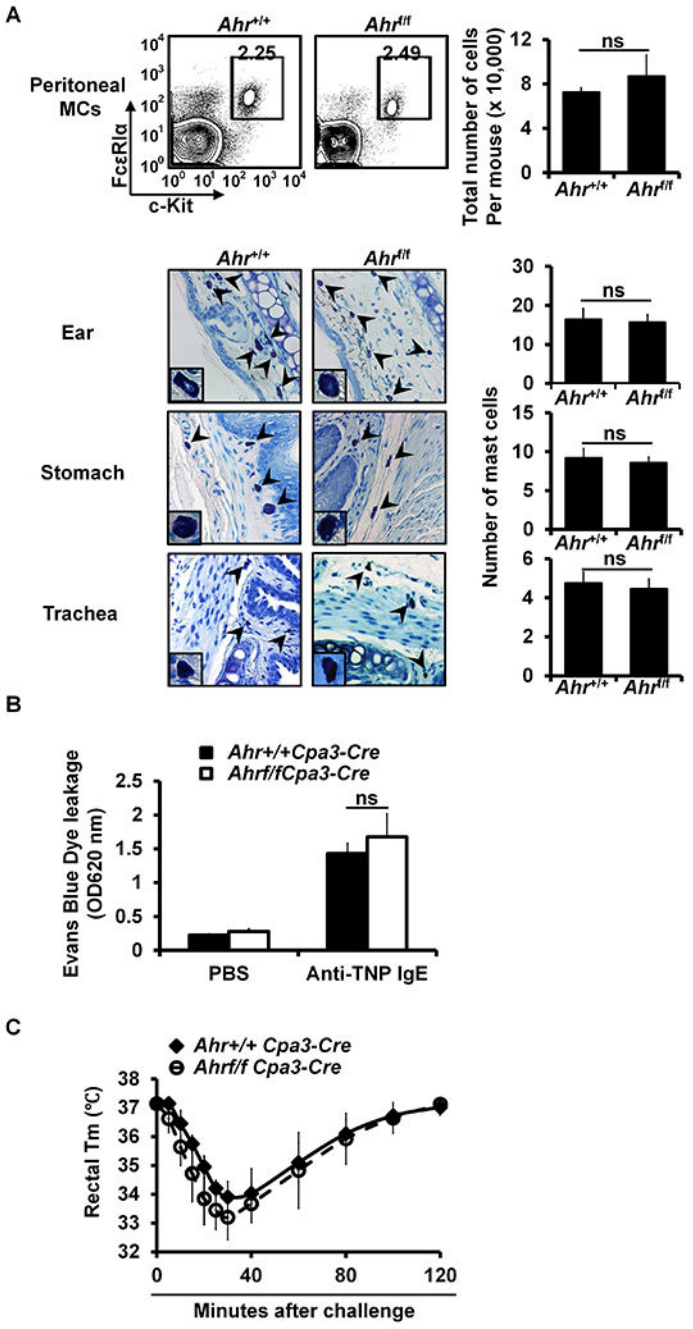


FIG E7.

Ahr$^{f/f}$*Cpa3*-Cre mice have normal numbers of mast cells and develop passive cutaneous anaphylaxis and passive systemic anaphylaxis normally. **A.** Flow analysis of peritoneal cavity mast cells and total number of peritoneal cavity mast cells (mean ± SEM, n=3 mice) (*left panel*). Toluidine blue staining of mast cells (magnification ×40; insert, ×100). Mast cells are indicated by arrows. Average number of mast cells in ten randomly selected fields (×40) (mean ± SEM, n=3 mice) (*right panels*). **B.** PCA analysis, mean ± SEM, n=3 mice. **C.** PSA analysis, mean ± SEM, n=3 mice. FIG E7 related to FIG 3.

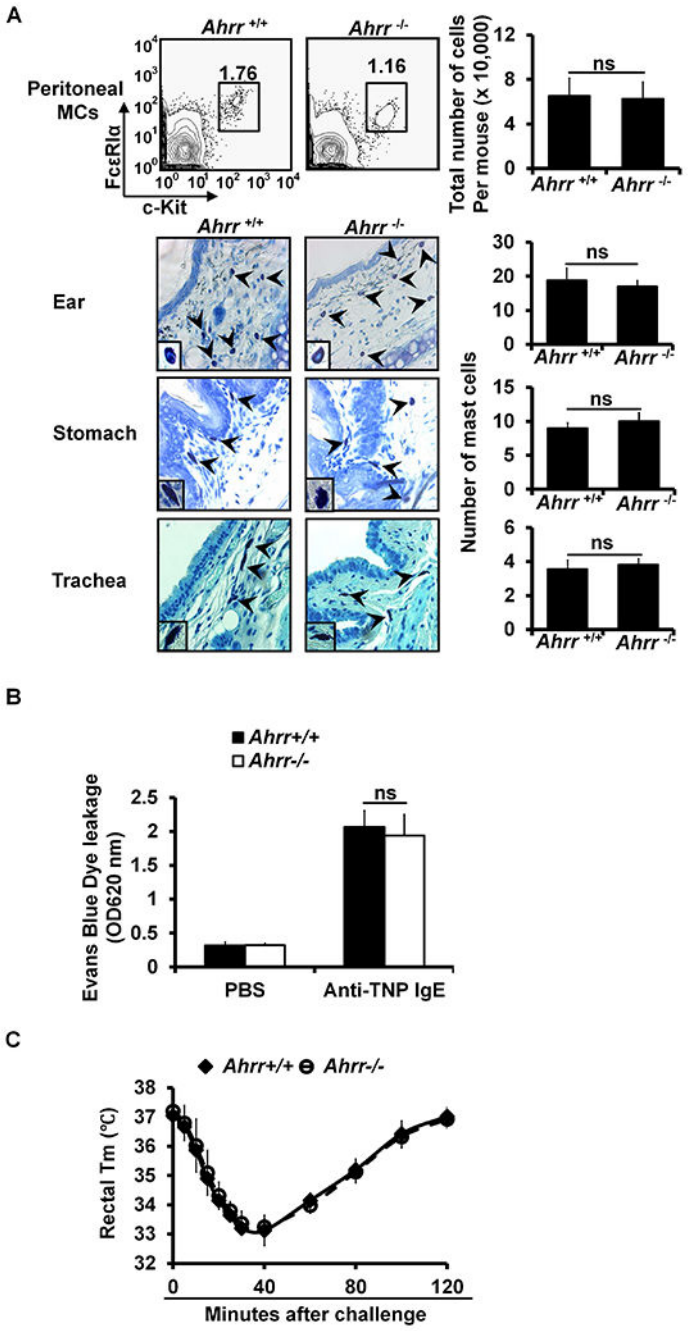


FIG E8.

Ahrr$^{-/-}$ mice have normal numbers of mast cells and develop passive cutaneous anaphylaxis and passive systemic anaphylaxis normally. **A.** Flow analysis of peritoneal cavity mast cells and total number of peritoneal cavity mast cells (mean ± SEM, n=3 mice) (*left panel*). Toluidine blue staining of mast cells (magnification ×40; insert, ×100). Mast cells are indicated by arrows. Average number of mast cells in ten randomly selected fields (×40) (mean ± SEM, n = 3 mice) (*right panels*). **B.** PCA analysis, mean ± SEM, n = 3 mice. **C.** PSA analysis, mean ± SEM, n=3 mice. FIG E8 related to FIG 3.

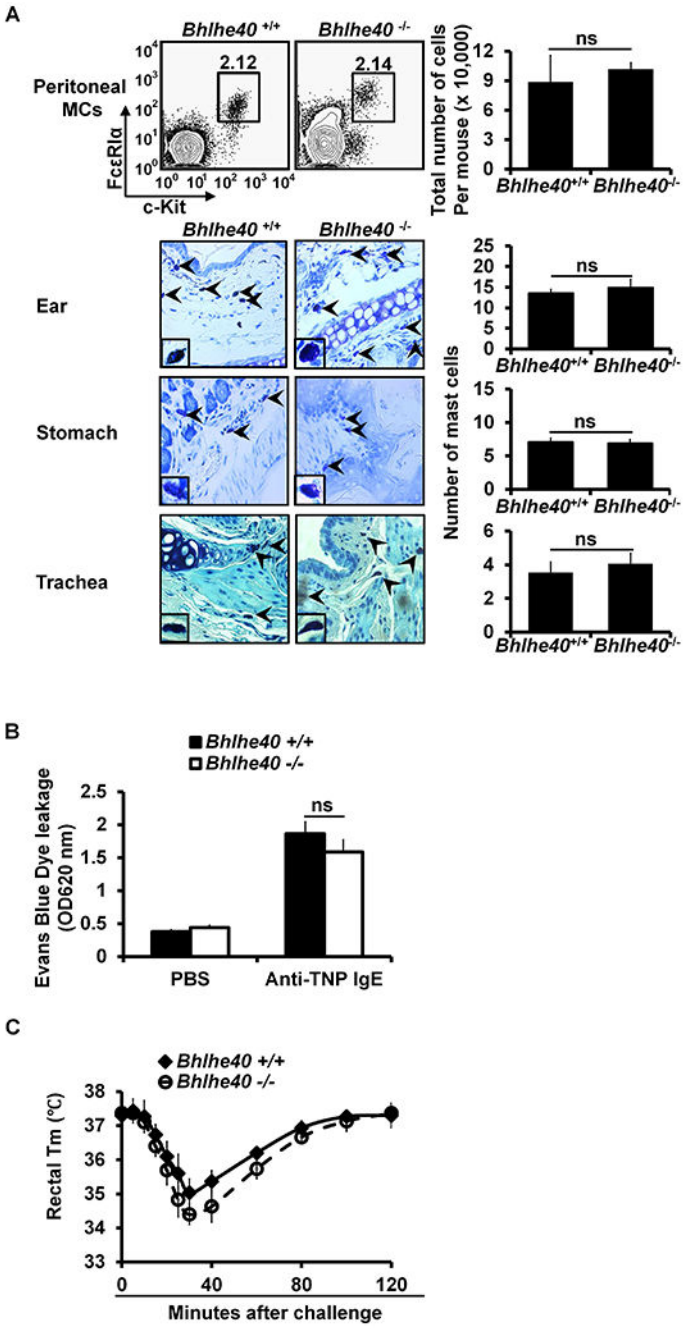


FIG E9.

Bhlhe40$^{-/-}$ mice have normal numbers of mast cells and develop passive cutaneous anaphylaxis and passive systemic anaphylaxis normally. **A.** Flow analysis of peritoneal cavity mast cells and total number of peritoneal cavity mast cells (mean ± SEM, n=3 mice) (*left panel*). Toluidine blue staining of mast cells (281 magnification ×40; insert, ×100). Mast cells are indicated by arrows. Average number of mast cells in ten randomly selected fields (×40) (mean ± SEM, n=3 mice) (*right panels*). **B.** PCA analysis, mean ± SEM, n=3 mice. **C.** PSA analysis, mean ± SEM, n=3 mice. FIG E9 related to FIG 3.

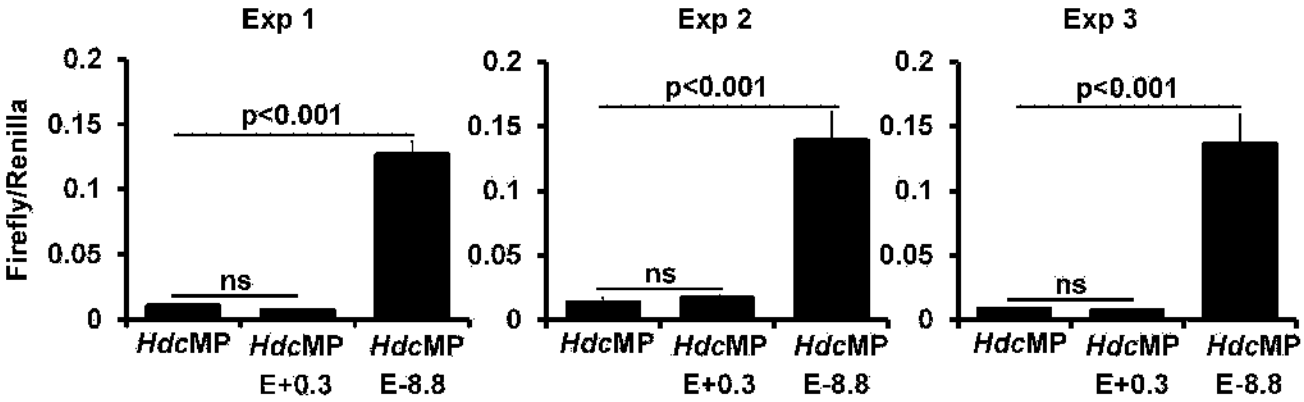


FIG E10.

Identification of putative *Hdc* enhancers. Luciferase reporter analysis of the *Hdc* enhancer activities in the CFTL-15 mast cell line. Error bars represent mean ± SEM, triplicates. The left, middle, and right panels each show three independent experiments. *Hdc*MP: *Hdc* minimal promoter. FIG E10 related to FIG 4B.

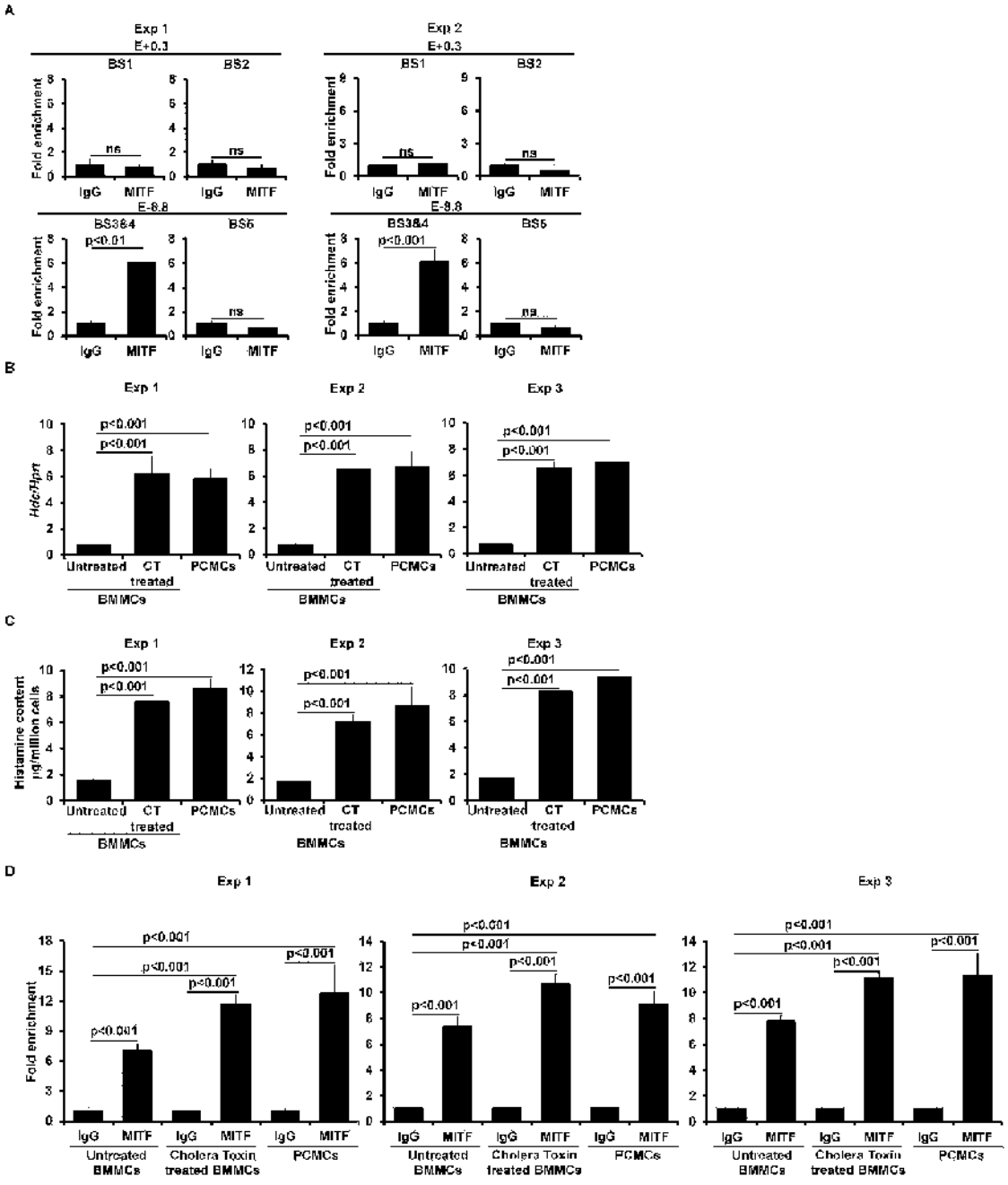


FIG E11.

MITF binds to the -8.8 enhancer. **A.** ChIP-qPCR analysis of MITF binding (mean ± SEM, triplicates). **B**, qPCR analysis of the *Hdc* gene expression in untreated or cholera toxin (CT) treated BMMCs, and PCMCs (mean ± SEM, triplicates). **C.** ELISA analysis of histamine content in untreated or cholera toxin (CT) treated BMMCs and PCMCs (mean ± SEM, triplicates). **D.** ChIP-qPCR analysis of MITF binding in untreated or cholera toxin (CT) treated BMMCs and PCMCs (mean ± SEM, triplicates). The left and right panels (**A**) or the left, middle, and right panel (**B, C,** and **D**) each show two or three independent experiments,

respectively. FIG E11A related to FIG 5B; FIG E11B related to FIG 5C; FIG E11C related to FIG 5D; FIG E11D related to FIG 5E.

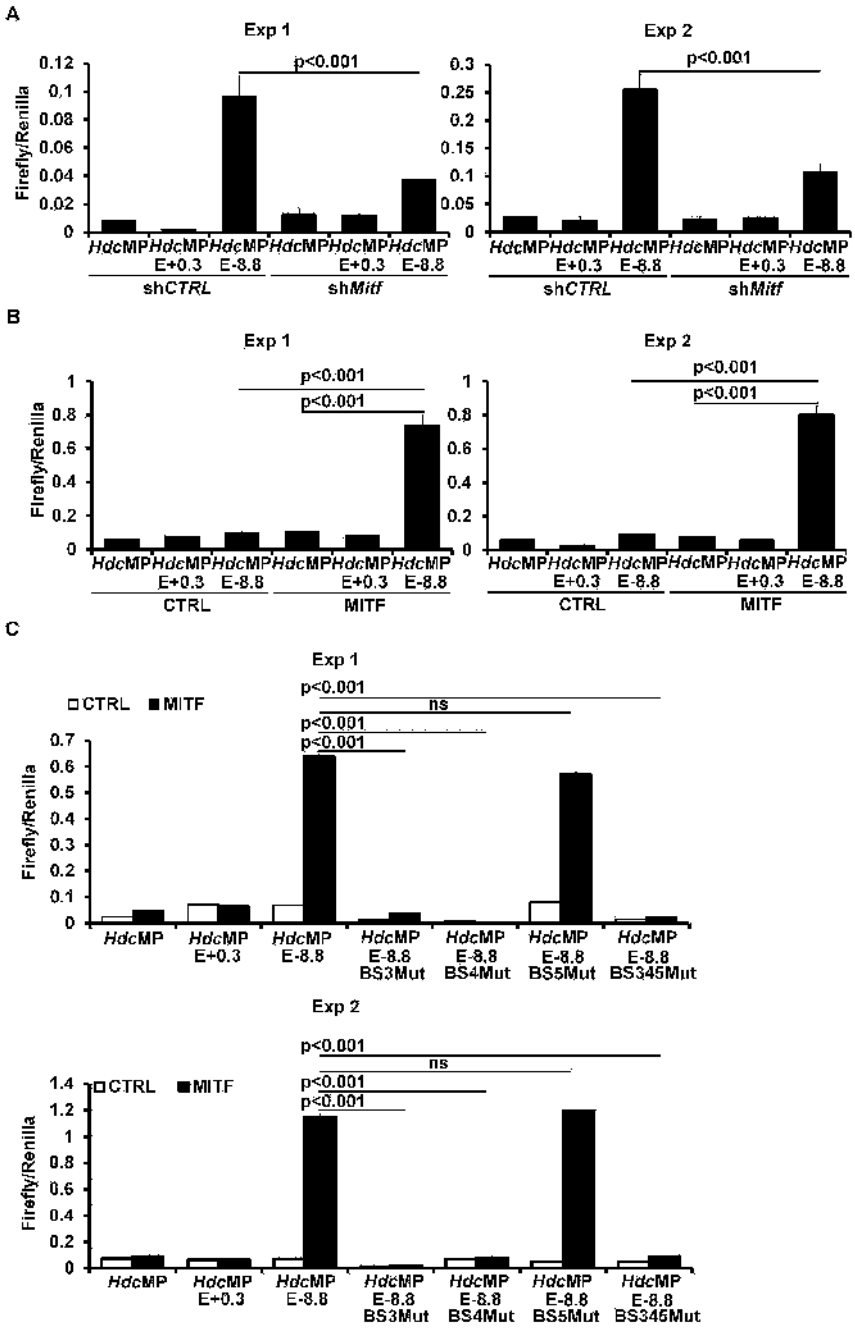


FIG E12.

MITF drives the −8.8 enhancer activity. **A**, Luciferase reporter gene analysis of *Hdc* enhancer activities in CFTL-15 cells with knocked down *Mitf* mRNA or shRNA control (mean ± SEM, triplicates). **B.** Luciferase reporter gene analysis of the *Hdc* enhancer activities in HEK293 cells co-transfected with vector control or the MITF expression

construct (mean ± SEM, triplicates). **C.** Luciferase reporter gene analysis of the activity of *Hdc* enhancers that have a mutated MITF binding site(s) (mean ± SEM, triplicates). The left and right panels (**A, B,** and **C**) or the upper and lower panel (**D**) each show two independent experiments. FIG E12A related to FIG 6A; FIG E12B related to FIG 6B; FIG E12C related to FIG 6C.

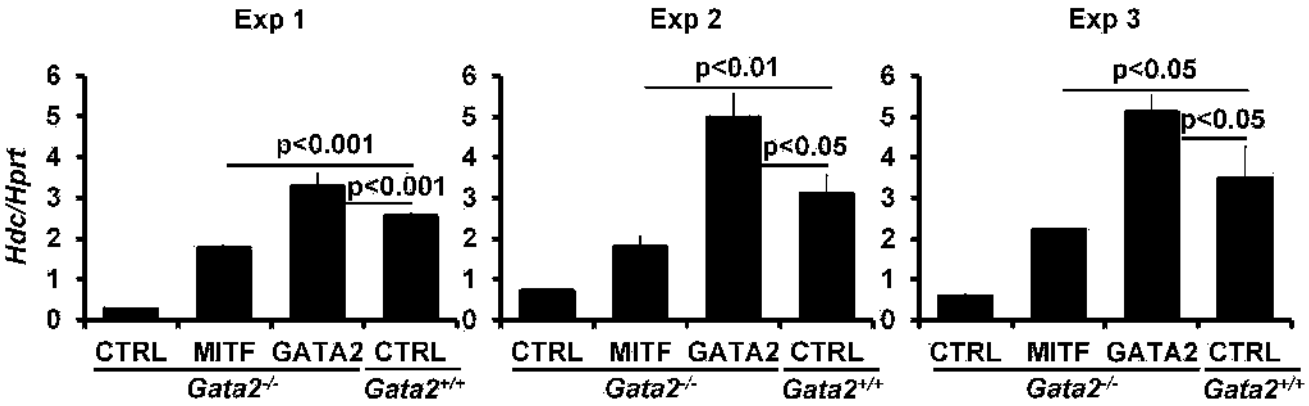


FIG E13.

MITF partially restores *Hdc* gene expression in *Gata2* deficient mast cells. qPCR analysis of the *Hdc* mRNA expression in *Gata2*$^{-/-}$ BMMCs overexpressing MITF, GATA2 or a control vector (CTRL) (mean ± SEM, triplicates). The left, middle, and right panels each show three independent experiments. FIG E13 related to FIG 7B.

Table E1

Primer sequences:

Primers for qPCR and ChIP-qPCR		
Gene	**5′ primer**	**3′ primer**
Mitf	AACCGACAGAAGAAGCTGGA	TGATGATCCGATTCACCAGA
Ahr	AGCAGCTGTGTCAGATGGTG	CTGAGCAGTCCCCTGTAAGC
Ahrr	AGAGGAGGACCAGAAGCACA	TCTGTGCTCCAGTTCAGGTG
Bhlhe40	CATGAGAACACTCGGGACCT	TCTCTTTCAAGTCCACGGCT
Hdc	TTCCAGCCTCCTCTGTCTGT	GGTATCCAGGCTGCACATTT
Hprt	CTCATGGACTGATTATGGACAGGAC	GCAGGTCAGCAAACTTATAGCC
Human HDC	TTCTGCCTGTGGATGACAAC	TAAGCAGCATCGATGTGGAG
Human HPRT	CTCATGGACTAATTATGGACAGGAC	GCAGGTCAGCAAAGAATTTATAGCC
*Hdc*E+0.3BS1 ChIP	GGGGCCACAGTGTATGAAAC	AGGAAAATGTGTCCCACTGC
*Hdc*E+0.3BS2 ChIP	GAAGGGTTTCTGGTTGCTCA	CTTGTCACACGGCTTCCAC
*Hdc*E-8.8BS3&4 ChIP	GCAAAGCCTCAAGGTGGTAA	GAAGCTGCCCGAATACTTGT
*Hdc*E-8.8BS5 ChIP	CTGCAAGCTACATTTTCAGCA	TGTGAGAACAAACTGCACTGG
Primers for constructions		
Constructs	5′ primer	3′ primer
pGL3-*Hdc*MP	AATAAGATCTGGCTAAAGGAGGGGAATAGG	AATAAAGCTTGCATCTCTTATTTAAGGAGTTCCC
pGL3-*Hdc*MPE+0.3	AATAGCTAGCGTAGATCAGAGTTTTAACAGGTCCCT	AATAAGA TCTCTTCTTCTTCTTCTTCTTTTTTTTTTT

Primers for qPCR and ChIP-qPCR		
Gene	**5′ primer**	**3′ primer**
pGL3-*Hdc*MPE-8.8	AATAGGTACCAACCAATTAGACTCCTCATGCCTT	AATAACGCGTAAGGAACTGATAAACGAGGGGTG
BS3 Mut	GTTCTGCTGGAGACAATTAAATACAG	ATTACATCCTGTATTTAATTGTCTCCAG
BS4 Mut	CAGCTGCAGGCGAATTAATGT	GTGAAAGCAACATTAATTCGCCT
BS5 Mut	AAGCCGTTGGAGAATTAATTGCT	ACACAAACCCAAGCAATTAATTCTC

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Abbreviations used:

4HT	4 hydroxytamoxifen
AHR	Aryl hydrocarbon receptor
AHRR	Aryl-hydrocarbon receptor repressor
BHLHE40	Basic helix-loop-helix family member E40
BMMCs	Bone marrow-derived mast cells
ChIP-seq	Chromatin immunoprecipitation sequencing
CTMC	connective tissue mast cell
FACS	Fluorescence activated cell sorting
GATA2	GATA binding protein 2
H3K4me1	Monomethylation of lysine residue 4 on histone 3
H3K27Ac	Acetylation of lysine residue 27 on histone 3
HDC	Histidine decarboxylase
MMCP1	Mouse mast cell protease 1
MITF	Microphthalmia-associated transcription factor
PCA	Passive cutaneous anaphylaxis
PCMCs	Peritoneal Cavity Mast Cells

pre-BMPs	Pre-basophil and mast cell progenitors
PSA	Passive systemic anaphylaxis
TNP	2, 4, 6-Trinitrophenyl
TSS	Transcription start site
YFP	Yellow fluorescent protein.

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Key messages:

- GATA2 is required for connective tissue mast cell differentiation
- GATA2 and MITF regulate IgE-mediated anaphylaxis in vivo
- MITF regulates *Hdc* gene expression by directly activating an *Hdc* enhancer

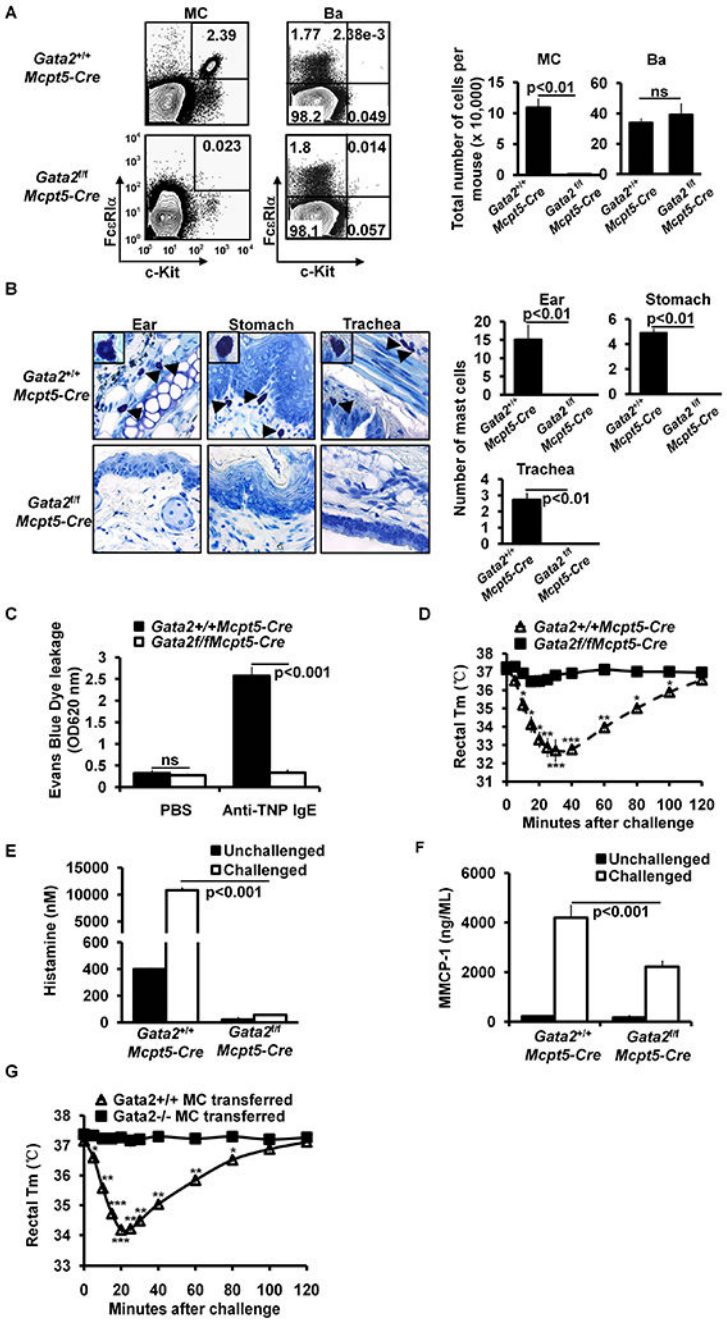

FIG 1.

GATA2 is critical for connective tissue mast cell differentiation and IgE/mast cell-mediated anaphylaxis. **A,** Flow analysis of peritoneal cavity mast cells (MC) and bone marrow basophils (Ba). Total number of cells (mean ± SEM, n=3 mice) (*right panel*). **B,** Toluidine blue staining of mast cells (magnification 3×40; insert, 3×100). Mast cells are indicated by arrows. Average number of mast cells in ten randomly selected fields (mean ± SEM, n=3 mice). **C,** PCA analysis, mean ± SEM, n = 3 mice; **D,** PSA analysis, mean ± SEM, n = 6 mice. **E,** ELISA analysis of histamine, mean ± SEM, n=3 samples from 3 individual mice. **F,**

ELISA analysis of MMCP-1 (mean ± SEM, n=3 samples from 3 individual mice). **G,** PSA analysis of the *Gata2*$^{f/f}$*Mcpt5-Cre* mice reconstituted BMMCs (mean ± SEM, n=5 mice). Statistical differences were analyzed by student's *t* test (**A**, **B**, **C**, **E**, **F**) or by ANOVA (**D**, **G**). *: p<0.05; **: p<0.01; ***: p<0.001.

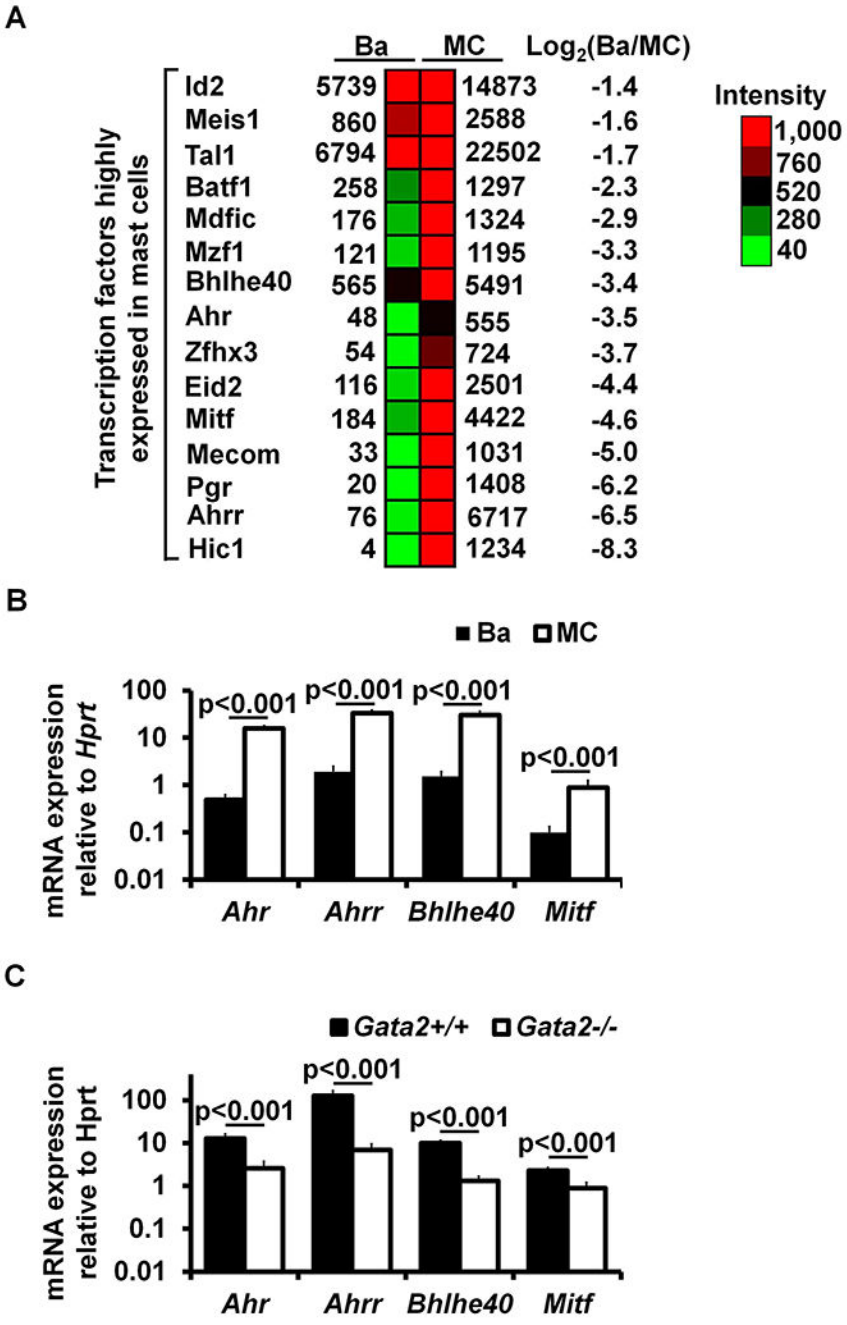

FIG 2.

Transcription factors AHR, AHRR, BHLHE40, and MITF depend on GATA2 for their expression in mast cells. **A,** Microarray analysis of transcription factor expression profiles in BMMCs versus basophils. **B,** qPCR analysis of mRNA expression in BMMCs versus basophils. The numbers indicate the fold of difference in mRNA expression between basophils and BMMCs (mean ± SEM, n=3 samples from 3 individual mice). **C,** qPCR analysis of mRNA expression in *Gata2*$^{+/+}$ or *Gata2*$^{-/-}$ BMMCs (mean ± SEM, n=3 samples from 3 individual mice).

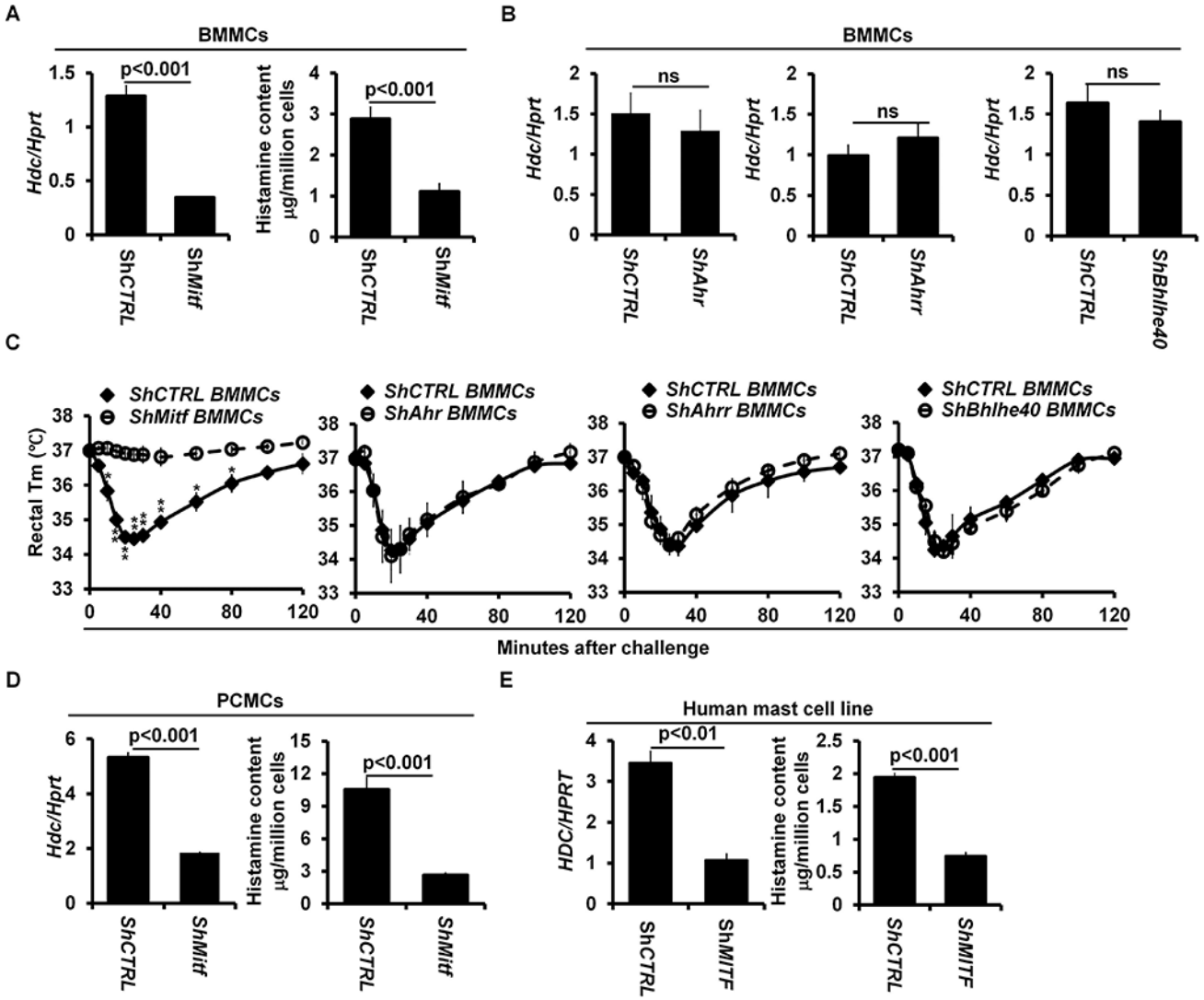

FIG 3.

Transcription factors MITF, but not AHR, AHRR or BHLHE40, regulate *Hdc* gene expression and IgE/mast cell-mediated anaphylaxis. **A,** qPCR analysis of *Hdc* mRNA expression in BMMCs with knocked down *Mitf* mRNA (sh*Mitf*) or with shRNA control (shCTRL) (mean ± SEM, n=3 samples from 3 individual mice). ELISA analysis of the histamine contents in BMMCs with knocked down *Mitf* mRNA (sh*Mitf*) or with shRNA control (mean ± SEM, n=3 samples from 3 individual mice) (*right panel*). **B,** qPCR analysis of *Hdc* mRNA expression in BMMCs with knocked down *Ahr* mRNA (sh*Ahr*), *Ahrr* mRNA (sh*Ahrr*) and *Bhlhe40* mRNA (sh*Bhlhe40*) or with shRNA control (shCTRL) (mean ± SEM, n=3 samples from 3 individual mice). **C,** PSA analysis of *Gata2*$^{f/f}$ *Mcpt5*-Cre mice reconstituted with BMMCs with knocked down *Mitf* mRNA (n=4 mice), *Ahr* mRNA (n=3 mice), *Ahrr* mRNA (n=3 mice), *Bhlhe40* mRNA (n=3 mice) or with shRNA control (n=4 mice). Statistical differences in PSA analysis were analyzed by ANOVA. *: p<0.05; **: p<0.01. **D,** qPCR analysis of *Hdc* gene expression (*left panel*) and ELISA analysis of

histamine content (*right panel*) in PCMCs with knocked down *Mitf* mRNA (sh*Mitf*) or with shRNA control (sh*CTRL*) (mean ± SEM, n=3 samples from 3 individual mice). **E,** qPCR analysis *HDC* gene expression (*left panel*) and ELISA analysis of the histamine contents (*right panel*) in the human mast cell line LAD2 with knocked down *Mitf* mRNA (sh*Mitf*) or with shRNA control (sh*CTRL*) (mean ± SEM, n=3 transduced samples).

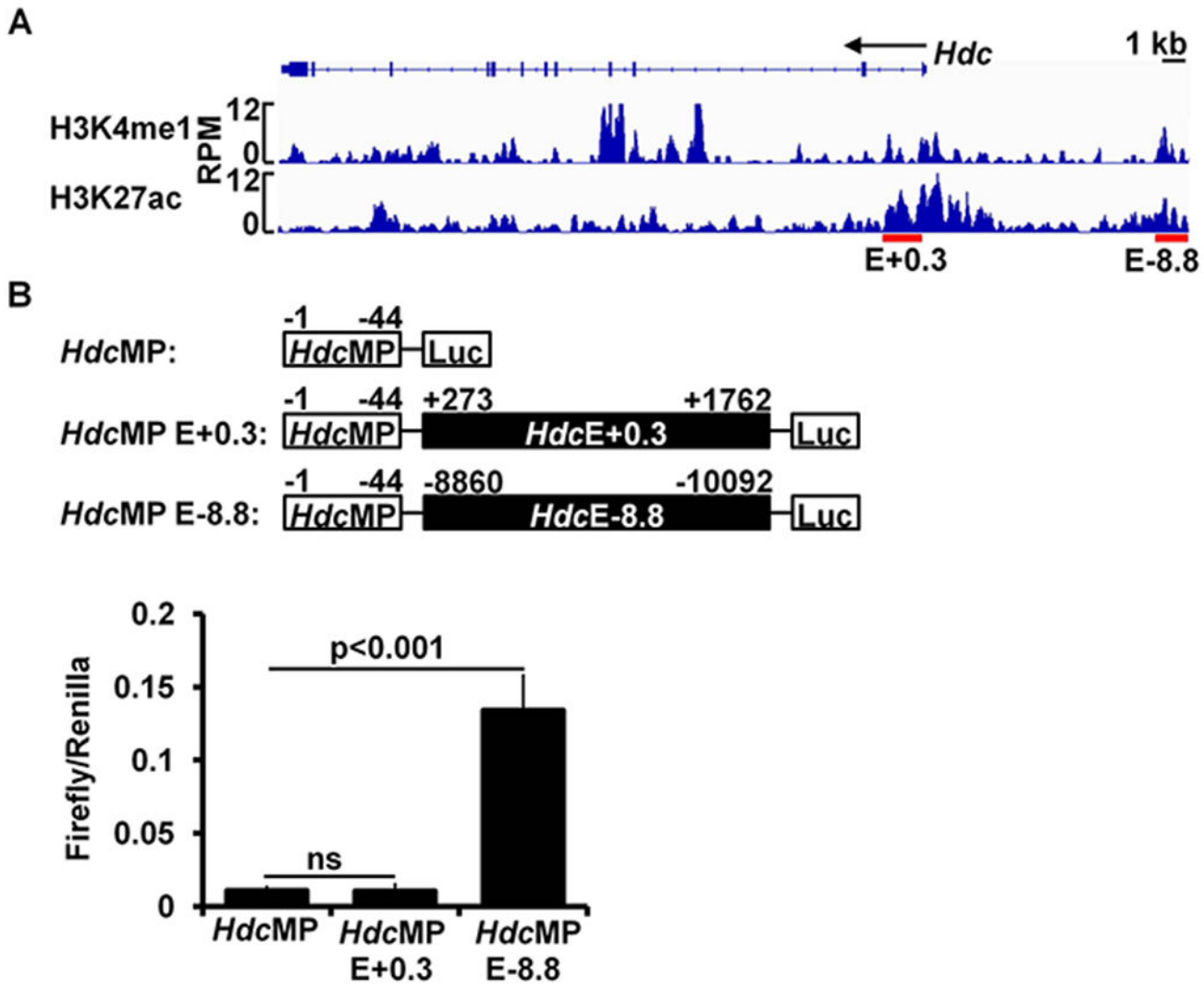

FIG 4.

Identification of putative *Hdc* enhancers. **A,** BMMCs were used for ChIP-seq (duplicate samples were used for H3K4me1 ChIP-seq and triplicate samples were used for H3K27ac ChIP-seq). H3K4me1 and H3K27ac ChIP-seq data were analyzed with the Integrative Genomics Viewer. Red bars indicate putative *Hdc* enhancers that show significant H3K4me1 and H3K27ac modifications. RPM: reads per million nucleotides; E: enhancer, +0.3 indicates the distance from the beginning of the downstream *Hdc* enhancer to the TSS of the *Hdc* gene, and −8.8 indicates the distance from the beginning of the upstream *Hdc* enhancer to the TSS of the *Hdc* gene. **B,** Scheme of the luciferase constructions (upper panel) and luciferase reporter analysis of the *Hdc* enhancer activities in the CFTL-15 mast cell line_(lower panel). Error bars represent mean ± SEM, n=3 transfectants. *Hdc*MP: *Hdc* minimal promoter.

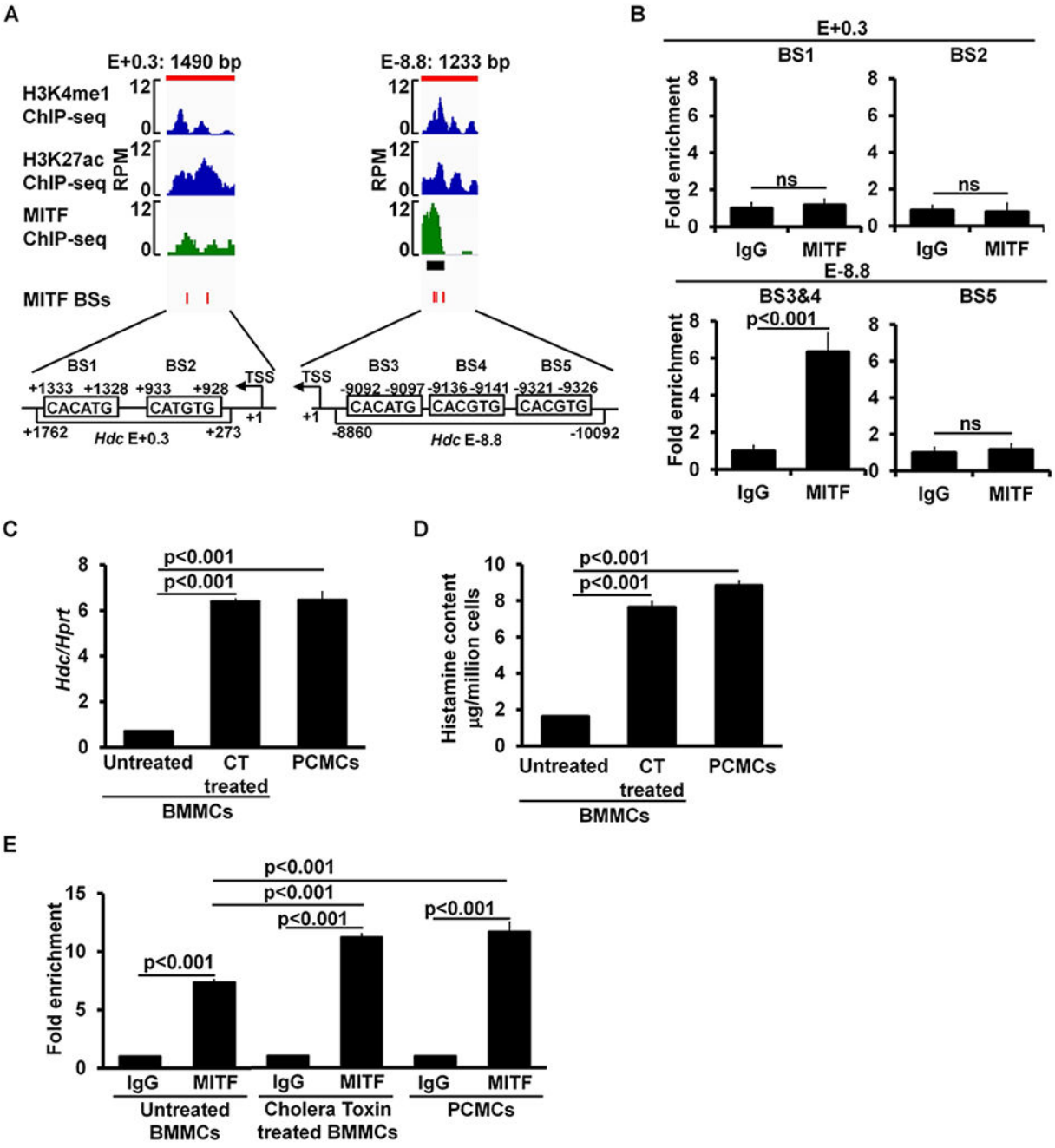

FIG 5.

MITF binds to the −8.8 enhancer in immature and mature mast cells. **A,** Enlarged ChIP-seq peak plots of the *Hdc* E-8.8 region and *Hdc* E+0.3. The published MITF ChIP-seq data were aligned with our H3K4me1 and H3K27ac ChIP data using the Integrative Genomics Viewer. The black bar beneath the MITF ChIP-seq peak plot indicates the MITF-binding peak that is statistically significant. The red bars indicate the positions of consensus MITF-binding sites (BSs). **B,** ChIP-qPCR analysis of MITF binding (mean ± SEM, n=2 samples from 2 independent experiments). BMMCs, anti-MITF antibody or control IgG was used for

immunoprecipitation. **C,** qPCR analysis of the *Hdc* mRNA expression (mean ± SEM, n=3 samples from 3 individual mice); **D,** ELISA analysis of histamine content (mean ± SEM, n=3 samples from 3 individual mice); and **E,** ChIP-qPCR analysis of MITF binding in untreated BMMCs, cholera toxin treated BMMCs, and PCMCs (mean ± SEM, n=3 samples from 3 independent experiments). CT: Cholera Toxin.

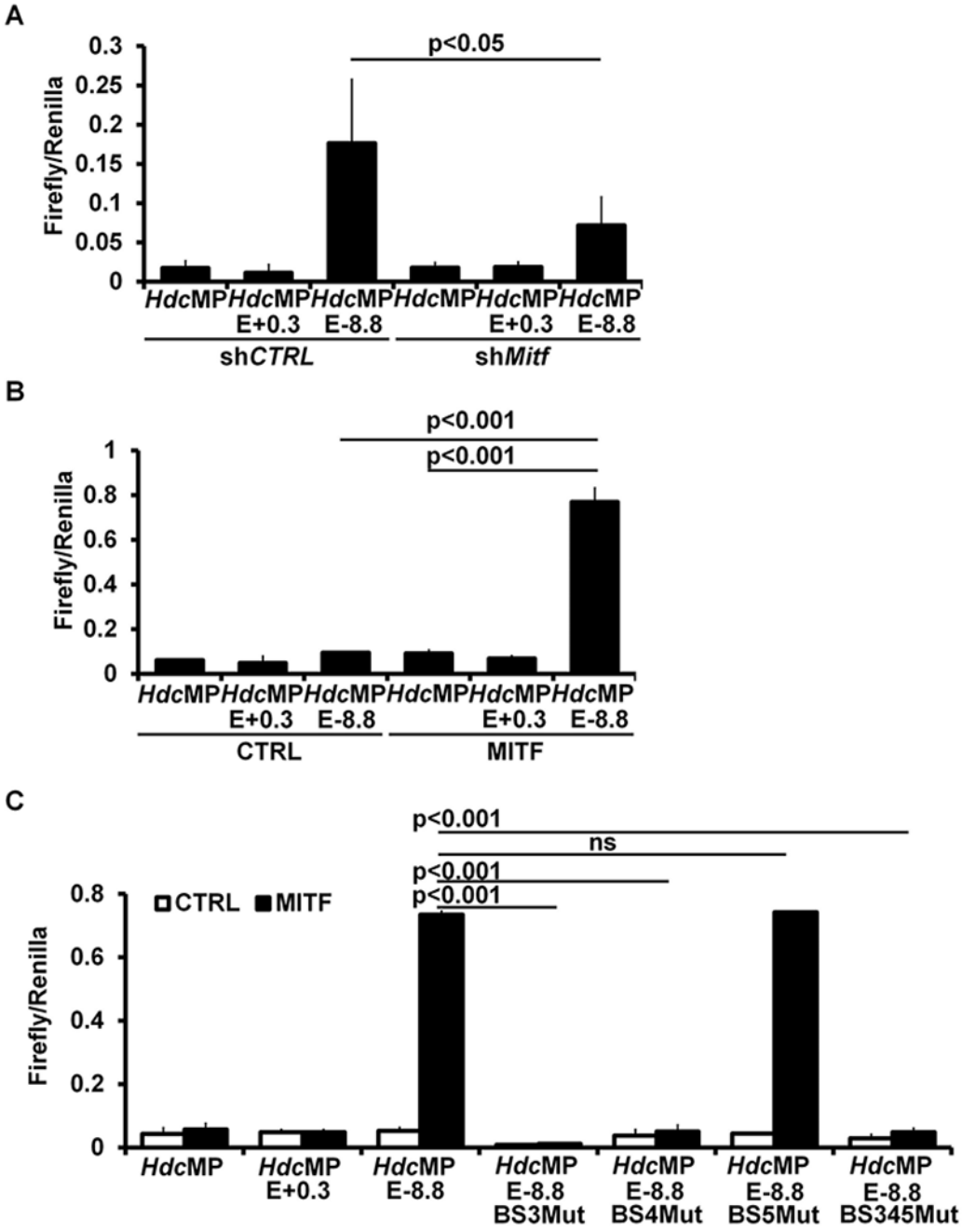

FIG 6.

MITF drives the −8.8 *Hdc* enhancer activity. **A**, Luciferase reporter gene analysis of *Hdc* enhancer activities in CFTL-15 cells with knocked down *Mitf* mRNA or shRNA control (mean ± SEM, n=2 transfectants). **B.** Luciferase reporter gene analysis of the *Hdc* enhancer activities in HEK293 cells co-transfected with vector control or the MITF expression construct (mean ± SEM, n=2 transfectants). **C.** Luciferase reporter gene analysis of the activity of *Hdc* enhancers that have a mutated MITF binding site(s) (mean ± SEM, n=2 transfectants).

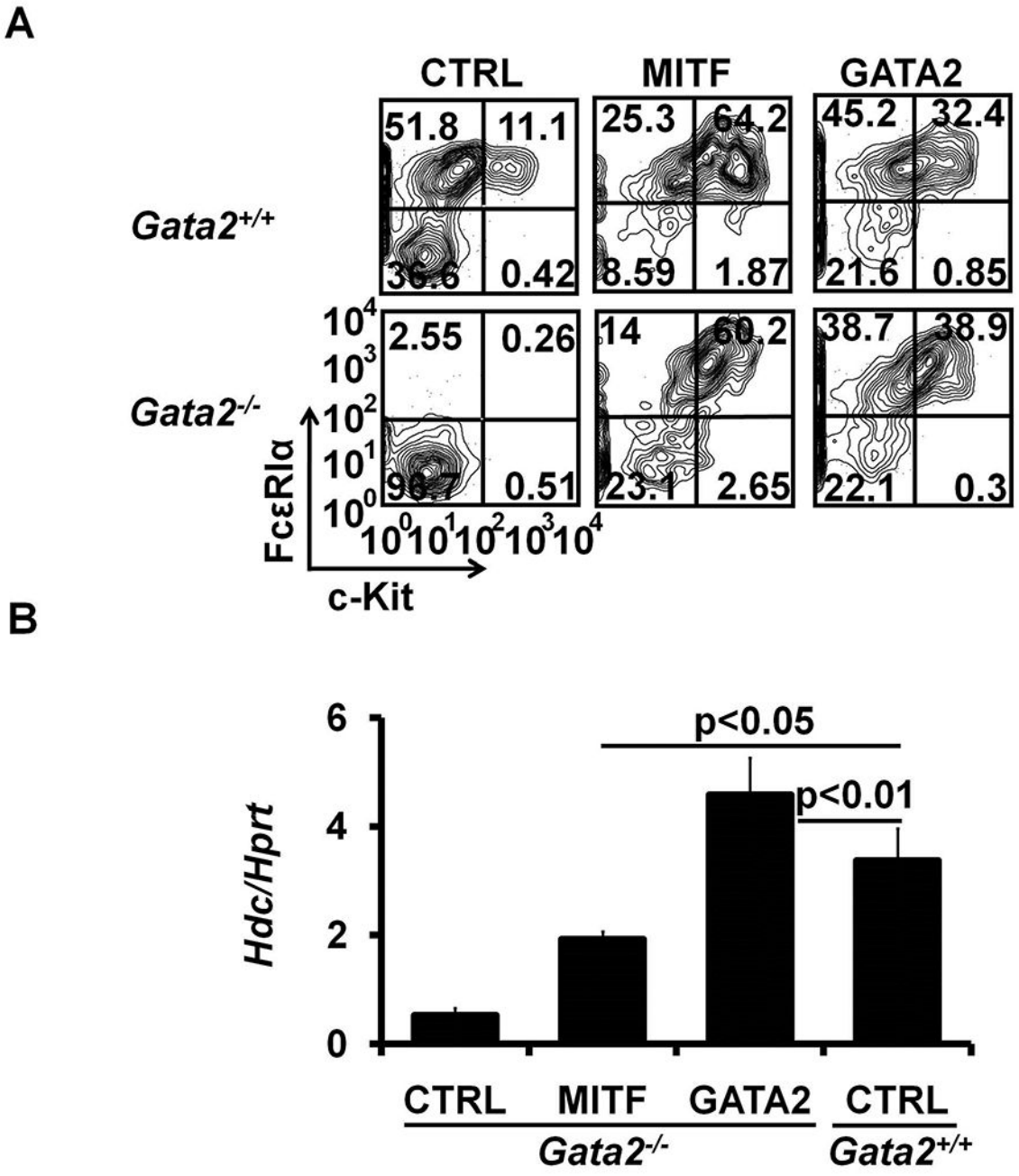

FIG 7.

MITF is sufficient to fully restore cKIT and FcεRI expression but can only partially restore *Hdc* gene expression. **A,** Flow analysis of cKIT and FcεRI expression in *Gata2*$^{-/-}$ BMMCs overexpressing MITF, GATA2 or control vector (CTRL). YFP^{+} GFP^{+} cell populations are shown. Data represent three independent experiments with similar results. **B,** qPCR analysis of the *Hdc* mRNA expression in *Gata2*$^{-/-}$ BMMCs overexpressing MITF, GATA2 or a

control vector (CTRL). YFP^+ GFP^+ BMMCs were FACS-sorted for qPCR analysis. Error bars represent mean ± SEM, n=3 samples from 3 independent experiments.