The GC box at the proximal enhancer of the *Hdc* gene is critical for *Hdc* gene transcription and histamine-mediated anaphylaxis

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Abstract

Histamine is a critical mediator of anaphylaxis, a neurotransmitter, and a regulator of gastric acid secretion. Histidine decarboxylase is a rate-limiting enzyme for histamine synthesis. However, in vivo regulation of *Hdc*, the gene that encodes histidine decarboxylase is poorly understood. We identified potential enhancers in the *Hdc* gene using H3K27a histone modification and chromatin accessibility; we assessed the enhancer activity of the potential enhancers in a reporter gene assay; and we deleted noncoding sequences that possessed enhancer activity using CRISPR sgRNA guides. Deletion of the GC box reduced *Hdc* gene transcription and histamine synthesis in the CFTL-15 mouse mast cell line and the LAD2 human mast cell line. Mast cells, basophils, brain cells, and stomach cells from GC box-deficient mice transcribed the *Hdc* gene much less than similar cells from wild-type mice and *Hdc* GC box-deficient mice failed to develop anaphylaxis. Thus, a GC box within the proximal enhancer in the mouse *Hdc* gene is essential for *Hdc* gene transcription, histamine synthesis, and histamine-mediated anaphylaxis in vivo. Our study also reveals a critical role of the GC box in human *HDC* gene transcription and histamine synthesis.

Introduction

Anaphylaxis is a life-threatening allergic reaction that rapidly affects the skin, gastrointestinal tract, respiratory system, and cardiovascular system (Simons, 2010). Anaphylaxis can be caused by allergy to foods, insect venoms, medications, and other agents (Simons, 2010). Food-induced anaphylaxis has risen dramatically in developed countries during the past several decades and continues to rise (Hogan et al., 2012; Sicherer and Sampson, 2010; Vickery et al., 2011). Understanding the molecular regulation of anaphylactic shock should promote effective prevention and treatment.

Mast cells (MCs) play a critical role in mediating anaphylaxis by releasing large amounts of histamine and other mediators. MCs express FcεRI, the high-affinity receptor for IgE, a heterotetramer composed of one IgE-binding α subunit, one membrane-tetraspanning β subunit, and a disulfide-linked γ subunit dimer. MCs can be activated by antigen cross-linking of antigen-specific IgE bound to FcεRI. Antigen cross-linking of the IgE/FcεRI complex activates MCs to release histamine that is pre-made and stored in MC granules.

Histamine belongs to a family of biogenic amines that includes neurotransmitters such as serotonin and dopamine and hormones such as adrenaline. Biogenic amines that contain one or more amine groups are formed mainly by decarboxylation of amino acids. Histamine is a monoamine synthesized from the amino acid histidine through a reaction catalyzed by the enzyme histidine decarboxylase (HDC), which removes the carboxyl group from histidine. The *Hdc* gene encodes HDC protein with a molecular mass of 74 kDa, a proenzyme with little or no enzyme activity. However, it becomes active once the proenzyme is cleaved at the site near its c-terminus, presumably by Caspase-9. HDC is the primary enzyme that catalyzes histamine synthesis.

Knowledge of how the human and mouse *Hdc* genes are transcribed is limited (Huang et al., 2018). The Mouse *Hdc* gene is located on chromosome 2 (Suzuki-Ishigaki et al., 2000). It contains 12 exons. *Hdc* mRNA is expressed in basophils, MCs, and enterochromaffin-like cells (ECL) of the stomach and is 86% homologous with the human gene. The human *HDC* gene is located in the 15q21.2 region of chromosome 15 and also contains 12 exons (Yatsunami et al., 1994). Most previous work has concentrated on analyzing the *Hdc* promoter region using reporter gene assays in transformed MC cell lines. Deletional analysis of *Hdc* promoter-driven luciferase reporter gene transcription demonstrated that the transcription factor SP1 binds to a GC box (GGGGCGGGG) found in both the human and mouse *Hdc* gene promoters (Hirasawa et al., 2006; Suzuki-Ishigaki et al., 2000). Several promoter elements have been reported to regulate *Hdc* gene transcription negatively. For example, the transcription factors YY1 and KLF4 negatively regulate the *Hdc* gene by suppressing SP1 in a gastric cancer cell line (Ai et al., 2004; Ai et al., 2006). Our group demonstrated positive regulation of *Hdc* gene expression by the transcription factor GATA binding protein 2 (GATA2), a member of the GATA family of transcription factors and by Microphthalmia-associated transcription factor (MITF) (Li et al., 2018).

Enhancers are segments of DNA located in the noncoding regions of genes. They activate gene transcription by delivering important accessory factors to the promoter (Schaffner, 2015; Schoenfelder and Fraser, 2019). Decoding enhancers has been a longstanding goal in the field of gene transcription (Vaishnav et al., 2022). *cis-regulatory* elements, such as enhancers, work from a distance in eukaryotes. Transcriptional codes hidden in gene distal regions are often required for full transcription. Locating critical enhancers can be difficult because they can be located up

to 100kb from the transcription start sites in noncoding regions that make up 99% of a genome. Remarkable progress has been made in identifying cis-regulatory elements in higher animals. The binding of TFs and co-factors to enhancers creates accessible regions within enhancers that recruit histone-modifying enzymes to add acetyl or methyl groups to histones. Monomethylation of lysine four 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. DNA regions associated with increased histone modification often coincide with chromatin accessibility, which can be measured by ATAC-seq. The combined presence of histone modifications and chromatin accessibility predicts enhancer activity (Calo and Wysocka, 2013; Creyghton et al., 2010; Heintzman et al., 2007; Rada-Iglesias et al., 2011; Spitz and Furlong, 2012).

Results

The proximal *Hdc* enhancer is essential for *Hdc* gene transcription.

Previously, we used the histone modifications H3K4me1 and H3K27ac to identify potential enhancers in the *Hdc* gene and analyzed the distal -8.8 enhancer in detail. In this study, we used H3K27ac CUT&Tag-seq and Omni-ATAC-seq to perform a higher resolution analysis of potential *Hdc* enhancers in a 40 kb region of the *Hdc* gene. In addition to the previously defined *Hdc* E-8.8, we identified proximal enhancer (PE) E+8.5, E+12.3, E+15.8, E+18.8, and E+20.8 noncoding DNA regions that showed increased H3K27ac modification and increased chromatin accessibility (Fig. 1). To determine which of the potential enhancers possess enhancer activity, we constructed a series of luciferase reporter genes in which the PE E+8.5, E+12.3, E+15.8, E+18.8, or E+20.8 was linked to the minimal *Hdc* promoter (MP) (-42 to +1 relative to the TSS). We included a DNA region that was not associated with H3K27ac modification or chromatin accessibility as a negative non-enhancer (NE) control (Fig. 1). The constructs were transfected into the CFTL-15 mast cell line, which we previously used to show *Hdc* E-8.8 enhancer activity (Li et al., 2017). We found that, in addition to *Hdc* E-8.8, the *Hdc* PE E+18.8 showed enhancer activities relative to the MP promoter (E-8.8, 40.0-fold; PE, 13.4-fold; E+18.8, 12.7-fold) (Fig. 2A). As expected, the NE region did not show enhancer activity (Fig. 2A).

However, the enhancer luciferase reporter assay does not assess enhancer activity in the context of native chromatin. To determine whether the *Hdc* PE, E-8.8, and/or E+18.8 enhancers play a role in *Hdc* gene transcription in the context of naïve chromatin, we used CRISPR/Cas9 technology to delete these enhancers. We used the NE as a negative control. The CRISPR deletion method using two sgRNA guides often resulted in deletions occurring in one chromosome, creating heterozygous deletion that does not have a phenotype. To overcome this technical challenge, we targeted one enhancer four sgRNA guides, each expressing a bicistronic gene encoding for fluorescence protein BFP, GFP, RFP, or Thy1.1 (Fig. 2B). CFTL-15 cells were transduced with lentiviruses containing the four bicistronic sgRNA guides. Cells expressing BFP, GFP, RFP, and Thy1.1 (7.8% of transfected cells were positive for four colors) were FACS-sorted, using gates shown in Fig. 2C. This method achieved 84-94% homozygous deletion of *Hdc* enhancers in bulk (Fig. 2D). Deletion of the PE enhancer resulted in the greatest reduction (73%) in *Hdc* mRNA expression (Fig 2E), while deletion of the E-8.8 enhancer resulted in a 38% reduction in *Hdc* mRNA expression (Fig. 2E). In contrast, deletion of the NE or *Hdc* E18.8 enhancer did not affect *Hdc* mRNA expression in the context of naïve chromatin (Fig. 2E). These data demonstrate that *Hdc* PE is critical in *Hdc* gene transcription, while the *Hdc* E-8.8 contributes to *Hdc* gene transcription in the context of naïve chromatin.

The GC box in the proximal *Hdc* enhancer is essential for *Hdc* gene transcription in the CFTL-15 mast cell line

To analyze transcription factor binding sites in the *Hdc* PE enhancer, we performed a deletional analysis of the -1401 bp long *Hdc* PE enhancer using a luciferase reporter gene assay. We found that most PE enhancer activity was driven by sequences located within the -48 bp fragment (Fig 3A). Transcription factor binding site analysis revealed that a GC box is located within this -48 bp fragment. Mutation in the GC box (-43 to -48) resulted in a complete loss of enhancer activity in the luciferase reporter gene assay (Fig 3B). Furthermore, CRISPR-mediated deletion of the GC box in CFTL-15 mast cells reduced *Hdc* mRNA expression by 78% (Fig 3C). These data

demonstrate that the GC box in the proximal *Hdc* enhancer is important for *Hdc* gene transcription in a mast cell line.

Mice deficient in the *Hdc* GC box have greatly reduced synthesis of histamine and IgE-mediated anaphylaxis after antigenic challenge.

To validate the findings obtained using the IL-3-dependent non-transformed mast cell line CFTL-15, we generated mice deficient in the *Hdc* GC box using a single sgRNA guide shown in Fig. 4A. A founder strain of mice with a six-nucleotide deletion of the *Hdc* GC box was verified by sequencing (Fig 4A). Mice deficient in the *Hdc* GC box ($Hdc^{GCbox-/-}$) tended to have slightly reduced numbers of peritoneal mast cells (Supplemental Fig. 1A and B). Surprisingly, FcεRIα and cKit expression on the peritoneal mast cells in the $Hdc^{GCbox-/-}$ mice tended to be elevated but this did not reach statistically significant levels compared to control $Hdc^{GCbox+/+}$ mice (Supplemental Fig. 1B). In contrast, *Hdc* mRNA expression was considerably and significantly reduced in both BMMCs (90% reduction) and basophils (67% reduction) (Fig. 4B). In addition to its activity as an allergic mediator, histamine is also a neurotransmitter and a regulator of gastric acid secretion (Huang et al., 2018). Histaminergic neurons in the basal ganglia of the brain express *Hdc* mRNA and synthesize histamine. In the stomach, enterochromaffin-like cells express *Hdc* mRNA and synthesize histamine, which stimulates parietal cells in the stomach to secrete stomach acid (Hersey and Sachs, 1995; Tanaka et al., 2002). We found that the levels of *Hdc* mRNA were significantly decreased in brain cells (83% reduction) and stomach cells (84% reduction) in the $Hdc^{GCbox-/-}$ mice (Fig. 4B). Consistent with the reduction of *Hdc* mRNA in the $Hdc^{GCbox-/-}$ mice, histamine contents in BMMCs and peritoneal mast cells derived from the $Hdc^{GCbox-/-}$ mice were dramatically decreased (91% and 97% reduction, respectively, Fig. 4C). Histamine plays a critical role in IgE/mast cell-mediated anaphylaxis. We found that the $Hdc^{GCbox-/-}$ mice developed little or no passive systemic anaphylaxis after the injection of IgE anti-TNP antibody and challenge with TNP-BSA (Fig. 4D). These mice released little histamine after challenge with IgE anti-TNP antibody and TNP-BSA (95% reduction relative to $Hdc^{GCbox+/+}$ mice, Fig. 4E). In contrast, MMCP-1 release to the blood was reduced moderately, if at all, in $Hdc^{GCbox-/-}$ mice compared to $Hdc^{GCbox+/+}$ mice (Fig. 4F), indicating that the *Hdc* GC box deletion primarily affects histamine synthesis. These data demonstrate that the GC box in the proximal *Hdc* enhancer is critical for histamine synthesis and histamine-mediated anaphylaxis *in vivo*.

The GC box plays a minor role in regulating chromatin modification and chromatin accessibility at the distal enhancers of the *Hdc* gene.

Chromatin accessibility at the enhancers plays a critical role in regulating the levels of gene transcription (Calo and Wysocka, 2013). Because the *Hdc* GC box plays an essential role in *Hdc* gene transcription, we investigated whether the *Hdc* GC box regulates chromatin structures at the distal enhancers. We performed Omni-ATAC-seq and H3K27ac ChIP-seq in WT and $Hdc^{GCbox-/-}$ BMMCs. Chromatin accessibility at the proximal enhancers near the GC box was notably reduced in $Hdc^{GCbox-/-}$ BMMCs. Reads at two out of three peaks (peak one and two) near the deleted GC Box were reduced by 65% and 43%, respectively (Fig. 5A). However, H3K27ac histone modification was not affected by the absence of the *Hdc* GC box. On the contrary, the H3K27ac peak at the -8.8 position was elevated (Fig. 5B), indicating that *the Hdc* GC box plays a minor role in chromatin accessibility at the PE enhancer and does not contribute to chromatin accessibility at the distal enhancers. These data demonstrate that the

Hdc GC box plays a minor role in regulating chromatin modification and chromatin accessibility at distal enhancers of the *Hdc* gene.

The GC box is required for *HDC* gene transcription and histamine synthesis in human mast cells.

It was unclear whether human *HDC* is also regulated by the GC box in the context of naïve chromatin. To determine this, we designed four bicistronic RNA guides targeting the human *HDC* GC box in the proximal enhancers (-153 to -495, Fig 6 A). Non-transformed human mast cell line LAD2 cells were transduced with lentivirus containing the four bicistronic sgRNA guides. LAD2 cells expressing BFP, GFP, RFP, and Thy1.1 (19.3% of transfected cells were positive for 4 colors) were FACS-sorted, using gates shown in Fig. 6B. We achieved 91% homozygous deletion of the GC box (Fig. 6C). Deletion of the *HDC* GC box resulted in 77% reduction in *HDC* mRNA expression (Fig 6D). Consistent with this, histamine content in the GC box-deleted LAD2 cells was reduced by 86% (Fig 6E). These results demonstrate that the human *HDC* GC box is as critical in *HDC* gene transcription in human MCs as in mouse MCs.

Discussion

The work described in this paper contributes to the study and understanding of eukaryote gene transcription in general and *Hdc* gene transcription in particular in several ways. It: (1) moves beyond in vitro studies with luciferase reporter genes to in vivo studies that incorporate the effects of naïve chromatin; (2) reveals and validates an improved method for CRISPR-mediated homozygous deletion of a gene segment; (3) demonstrates a critical role for the proximal enhancer GC box in both mouse and human *Hdc* gene expression despite its lack of influence on the chromatin structure of *Hdc* distal enhancers; and (4) provides a novel and useful mouse model in which nearly absent *Hdc* transcription blocks histamine synthesis and IgE-mediated systemic anaphylaxis with little or no reduction in mast cell number, maturation, or degranulation.

Although chromatin structure determines the levels of gene transcription in eukaryotes, *Hdc* gene transcription has not previously been investigated in the context of naïve chromatin. Our study has demonstrated a critical role of the *Hdc* GC box in regulating both mouse and human *Hdc* genes in this context. For example, we previously demonstrated that the distal *Hdc* E-8.8 enhancer contributes to *Hdc* gene transcription as measured by a luciferase reporter gene assay in the mouse MC cell line CFTL-15. We have now extended this observation by showing that this enhancer also contributes to *Hdc* gene transcription in the context of naïve chromatin. *In addition, in vivo* validation of the GC box function in mice adds rigor to the previous conclusion which depended on studies with a single cell line that had been generated from a single mouse strain and used a mast cell line that had been cultured for a long time in vitro. Our *in vivo* validation will also allow broader generalization of our finding that the *Hdc* GC box is required for *Hdc* gene transcription, histamine synthesis, and histamine-mediated anaphylaxis.

This work that has depended on our development of a novel technique for achieving homozygous gene deletion. The CRISPR deletion method that uses two sgRNA guides often results in deletions that occur in only a single chromosome, producing heterozygous deletion that lacks a phenotype. To overcome this technical challenge, we have used four sgRNA guides, each expressing a bicistronic gene that encodes fluorescence protein BFP, GFP, RFP, or Thy1.1 molecule to target one enhancer. By coupling this with fluorescence activated cell sorting to select for cells that express all four marker genes, we achieved 84% to 94% enhancer deletion at two chromosomes without single-cell cloning. This technical improvement has allowed us to analyze mouse and human *HDC* gene transcription in the context of naïve chromatin, which has revealed a critical role of the GC box in both mouse and human *HDC* gene expression.

The GC box has a consensus sequence of GGGCGG and is often located near the promoter of eukaryotic genes. The transcription factor SP1 is known to bind to the GC box, functioning like transcription factors that bind to more distal enhancers. The GC box in the promoters can appear once, more often it appears multiple times. The GC box only appears once in the mouse and human *Hdc* proximal enhancers. Remarkably, deletion of the single GC box in the mouse or human *Hdc* proximal enhancer profoundly reduces *Hdc* gene transcription. Despite its importance in *Hdc* gene transcription, absence of the GC box in the proximal *Hdc* enhancer does not affect the chromatin structure of distal enhancers of the mouse *Hdc* gene. This is consistent with the view that other transcription factors, such as GATA2, control *Hdc* gene chromatin accessibility (Li et al., 2018). GC box thus might function as an enhancer to promote the assembly of the basic transcriptional complex.

Compared with the existing *Hdc* gene knockout mice (Ohtsu et al., 2001), the *Hdc*$^{GCbox-/-}$ mice that we have generated show only minor effects on mast cell maturation. The number of peritoneal cavity mast cells, which are considered mature mast cells, was appeared to be slightly, but not significantly reduced. Surprisingly, cKIT and FcεRIα on *Hdc*$^{GCbox-/-}$ peritoneal cavity mast cells were expressed at normal or higher levels than in WT mast cells. Consistent with the notion that the *Hdc* GC box primarily regulates *Hdc* gene transcription, mast cells protease MMCP1 levels in resting *Hdc*$^{GCbox-/-}$ mice or *Hdc*$^{GCbox-/-}$ mice that had undergone passive systemic anaphylactic treatments were reduced moderately, if at all. Any reduction might have been caused by a proportionate reduction in the number of mature mast cells in *Hdc*$^{GCbox-/-}$ mice. Because *Hdc*$^{GCbox-/-}$ mice have relatively normal mast cell differentiation, these mice are more suitable than global *Hdc*-deficient mice for studying the importance of the *Hdc* gene in mast cell/IgE-mediated anaphylaxis. Additionally, because *Hdc*$^{GCbox-/-}$ mice also have reduced *Hdc* gene transcription in stomach and brain tissues, these mice can be used to study how the *Hdc* gene is regulated in neurons and in gastric enterochromaffin-like cells.

Methods

Mice

sgRNA (TTTATGGGGGCGGGGC) was designed using the online CRISPick tool from Broad Institute (https://portals.broadinstitute.org/) and was injected into BALB/cByJby zygotes as described (Wang et al., 2013). BALB/cByJby mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The six-nucleotide deletion (GGGCGG) in *Hdc*$^{GCbox+/-}$mice was verified by sequencing. Two founder strains that have an exact GGGCGG six-nucleotide deletion were selected for further breeding. Mice deficient in the GC box was designated as *Hdc*$^{GCbox+/-}$ mice, and were backcrossed to BALB/c mice for 6 generations. Genotyping was carried out using the following primers: forward, TCAAATCAGAAAGGACGGGGA; reverse, ATTTAAGGAGTTCCCTATTCCCCTC.

BMMC culture

Bone marrow-derived mast cells (BMMCs) were cultured from bone marrow cells of BALB/c mice in Iscove's DMEM (10016CV, Corning™ cellgro™, Manassas, VA) plus 10% FBS,100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM β-mercaptoethanol in the presence of 20 ng/mL IL-3 for four weeks. Over 99% of BMMCs were mast cells as determined by FACS analysis (FcεRIα^{+} c-Kit^{+}).

H3K27ac CUT&Tag-seq

The Cleavage Under Targets and Tagmentation (CUT&Tag) assay for H3K27ac modification was performed according to the published protocol (Kaya-Okur et al., 2019). Briefly, BMMCs (1×10^5) were treated with concanavalin A-coated beads (Bangs Laboratories, BP531) and incubated with rabbit anti-H3K27ac antibody (Abcam, ab4729) at 4 □ overnight. The cells were then incubated with guinea pig anti-rabbit antibody (Antibodies online, ABIN101961) at room temperature for 30 minutes. The cells were washed and incubated with pA-Tn5 adapter complex (Diagenode, C01070001) at room temperature for 1 hour on a rotator. The cell pellet was resuspended in 300 μl tagmentation buffer and incubated at 37 □ for 1 hour. The reaction was stopped by adding 10 μL 0.5M EDTA, 3 μL 10% SDS, and 2.5 μL 20 mg/mL Proteinase K, and cells were digested at 37 □ overnight. Then, DNA was extracted using phenol chloroform and diluted in 25 μl TE buffer with 1/400 RNAse A. The tagmented DNA was enriched by PCR amplification using Universal i5 primer and uniquely barcoded i7 primers under the following conditions: 72 °C for 5 min, and 13 cycles: 98 °C for 30 sec, 98 °C for 10 sec, 63 °C for 10 sec, 72°C for 1 min and hold at 8 □. The PCR products were cleaned up and size-selected using Ampure XP beads (Beckman Coulter, A63880). The quality and quantity of libraries were analyzed with the Agilent TapeStation 4200 system. Pair-ended sequencing of DNA libraries was performed on an Illumina NovaSEQ6000 platform.

Omni-ATAC-seq

Omni-ATAC-seq was performed as previously described (Corces et al., 2017; Li et al., 2021). Briefly, 50,000 BMMCs, either untreated or activated by IgE receptor crosslinking, were spun down and washed once with cold PBS. The cells were resuspended in 50 µl cold ATAC-RSB-lysis buffer (10mM Tris-HCl pH 7.4, 10mM NaCl, 3mM $MgCl_2$, 0.1% NP-40, 0.1% Tween-20 and 0.01% digitonin) and incubated for 3 minutes. The lysis buffer was immediately washed out with 1 mL ATAC-RSB buffer (10mM Tris-HCl pH 7.4, 10mM NaCl, 3mM $MgCl_2$ and 0.1% Tween-20). The cell pellet was resuspended in 50 µl transposition mix (25 µl 2X TD buffer, 2.5 µl transposase (Illumina, FC-121-1030), 16.5 µl PBS, 0.5 µl 1% digitonin, 0.5 µl 10% Tween-20, 5 µl H_2O) and incubated at 37 °C for 30 minutes. The reaction was stopped by adding 2.5 µl of 0.5M EDTA, pH 8, and transposed DNA was purified with a Qiagen MiniElute PCR purification kit (Qiagen). Purified DNA was amplified using the following conditions: 72°C for 5 min, 98 °C for 30 s, and 7 cycles: 98 °C for 10 s, 63 °C for 30 s, 72 °C for 1 min. The amplified libraries were purified, size-selected, and the quality and quantity of libraries were assessed with an Agilent Technologies 2100 Bioanalyzer. The pair-ended sequencing of DNA libraries was performed with an Illumina NovaSEQ6000 platform.

NGS data analysis

Raw sequencing reads (average 118.2 million reads, 2 biological replicates) were adaptor trimmed by Trimmomatic 0.33 and the quality of sequenced data was analyzed by FastQC. The trimmed reads were aligned to the mm10 reference genome using Bowtie2 with -very-sensitive and -x 2000 parameters. The read alignments were filtered using SAMtools (version 1.7) to remove mitochondrial genome and PCR duplicates. Peaks were identified by MACS2 with the q-value cut-off of 0.05 and the sequencing data was displayed using IGV. The representative ATAC-seq IGV tracks showed in the figures were generated from one biological sample, representing two or three biological samples with similar pattens.

Luciferase reporter constructs and 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. 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). Transcriptional activity was normalized as the ratio of luciferase activity divided by *Renilla* activity.

Deletion of GC boxes using four bicistronic sgRNA guides

The sgRNA sequences targeting *Hdc* enhancers or transcription factor exons were designed using the online CRISPick tool from Broad Institute (https://portals.broadinstitute.org/). Each of

four sgRNA sequences targeting the same enhancer was cloned into LentiCRISPRv2GFP (Addgene, Plasmid # 82416), LentiCRISPRv2-mCherry (Addgene, Plasmid #99154), LentiCRISPRv2BFP, or LentiCRISPRv2THY1.1 vectors. LentiCRISPRv2THY1.1 vector was modified by replacing the *Gfp* gene in LentiCRISPRv2GFP with the gene encoding THY1.1, using the BsmBI cloning site. CFTL-15 cells (10×10^6) were electroporated with 10 μg of each CRISPR plasmid using a Bio-Rad Gene Pulser at 450 V and 500 microfarads. Twenty-four hours after electroporation, the cells that expressed BFP, GFP, RFP, and Thy1.1 were FACS-sorted. The sorted cells were subjected to DNA analysis of GC box deletion. The intensities of DNA fragments were measured using Imagej (NIH, MD).

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 as described previously (Li et al., 2018).

Histamine and Mcpt1 measurements

Plasma was collected 5 minutes after challenge for histamine measurement, using a histamine EIA kit (Beckman Coulter, IM2015). Sera were collected one hour after challenge for determination of serum levels of mucosal mast cell protease (MMCP)1, using an MMCP1 EIA kit (Thermo Fisher Scientific).

Statistical analysis

The nonparametric Mann-Whitney U test or two-tailed student's *t* test was used to determine significant differences between two samples. The body temperature changes at various time points before and after PSA challenges were analyzed using a two-way repeated measure ANOVA.

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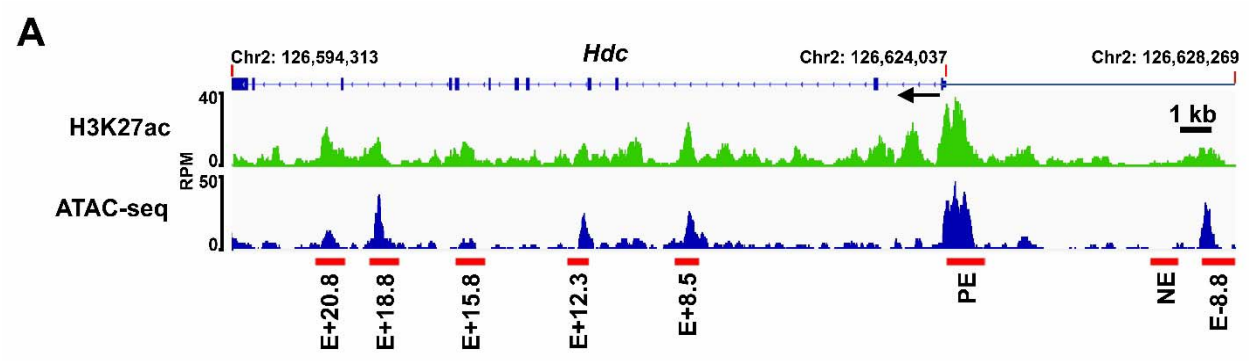


Figure 1. Identification of the putative *Hdc* enhancers. Representative Integrated Genome Viewer (IGV) tracks from H3K27ac CUT&Tag-seq and Omni-ATAC-seq. BMMCs were used for H3K27ac CUT&Tag-seq and Omni-ATAC-seq. Red bars indicate putative *Hdc* enhancers that show increased H3K27ac modifications and chromatin accessibility. RPM: reads per million mapped reads; E: enhancer; PE: proximal enhancer. The numbers following E indicate the distance (kb) of enhancer to the TSS of the *Hdc* gene; + means the enhancers are located downstream of the TSS, and – means the enhancers are located at the upstream of the TSS. One IGV track was from one biological sample, representing two (Omni-ATAC-seq) or three (CUT&Tag-seq) biological samples with similar patterns.

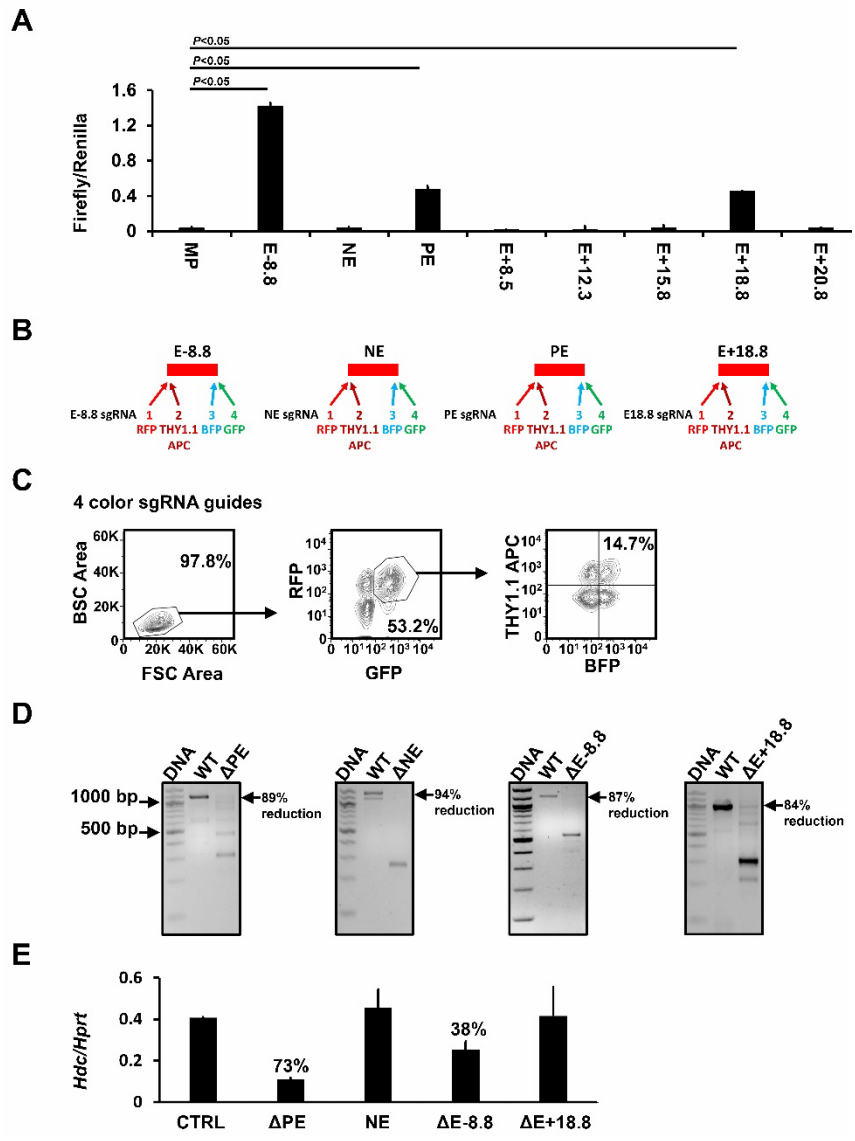


Figure 2. *Hdc* E-8.8, PE, E+2.5, E+4.6, and E+19.9 possess enhancer activity. A. Luciferase reporter analysis of the *Hdc* enhancer activities in CFTL-15 mast cells. Error bars represent mean ± SEM, n=4 transfectants from 2 independent experiments. **B.** sgRNA guide constructs that co-expressed cistronic BFP, GFP, RFP, and Thy1.1 genes. **C**. FACS sorting gates. CFTL15 cells were electroporated with BFP, GFP, RFP, and Thy1.1 sgRNA guides. BFP^+, GFP^+, RFP^+, and $Thy1.1APC^+$ transfectant cells were FACS-sorted using sorting gates indicated. **D**. DNA deletion efficiency analysis. WT and deleted DNA fragments in FACS-sorted BFP^+, GFP^+, RFP^+, and $Thy1.1APC^+$ cells were analyzed with PCR. **E**. *Hdc* mRNA expression in the FACS-sorted cells in bulk was measured by qPCR (mean ± SEM, n=3 transfectants from 3 independent experiments). The percentages indicate the percent reduction in *Hdc* mRNA expression relative to CTRLs. *P* values were calculated using the Mann-Whitney U test.

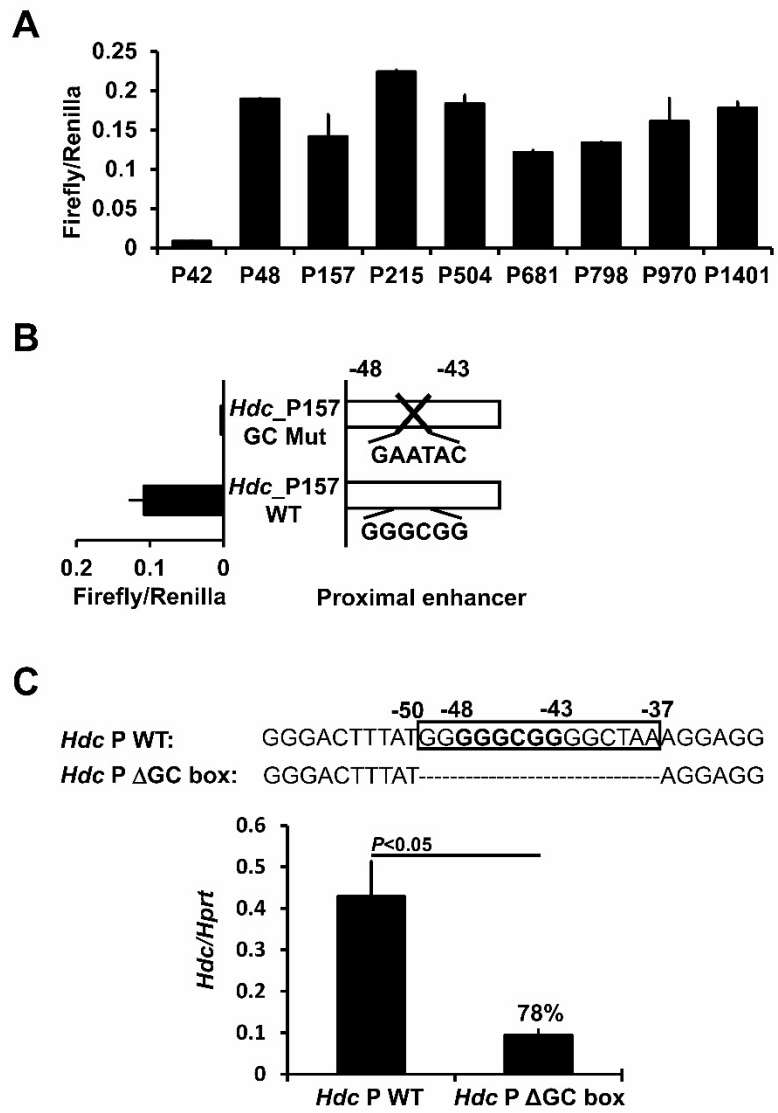


Figure 3. The GC box in the *Hdc* PE is required for *Hdc* gene transcription. A. Deletional analysis of the *Hdc* PE in CFTL-15 mast cells. Luciferase reporter genes were transfected into CFTL-15 mast cells. Error bars represent mean ± SEM, n=4 transfectants from 2 independent experiments. **B.** Mutational analysis of the GC box in the -157 *Hdc* PE using luciferase reporter gene. Luciferase reporter gene containing the GC box mutation was transfected into CFTL-15 mast cells. Mean ± SEMs were calculated from 3 transfectants from 3 independent experiments. **C.** qPCR analysis of *Hdc* mRNA expression in GC box deleted CFTL15 mast cell clones (mean ± SEM, n=3 colonies). The percentages indicate the percentages of reduction in *Hdc* mRNA expression. *P* values were calculated using the Mann-Whitney U test.

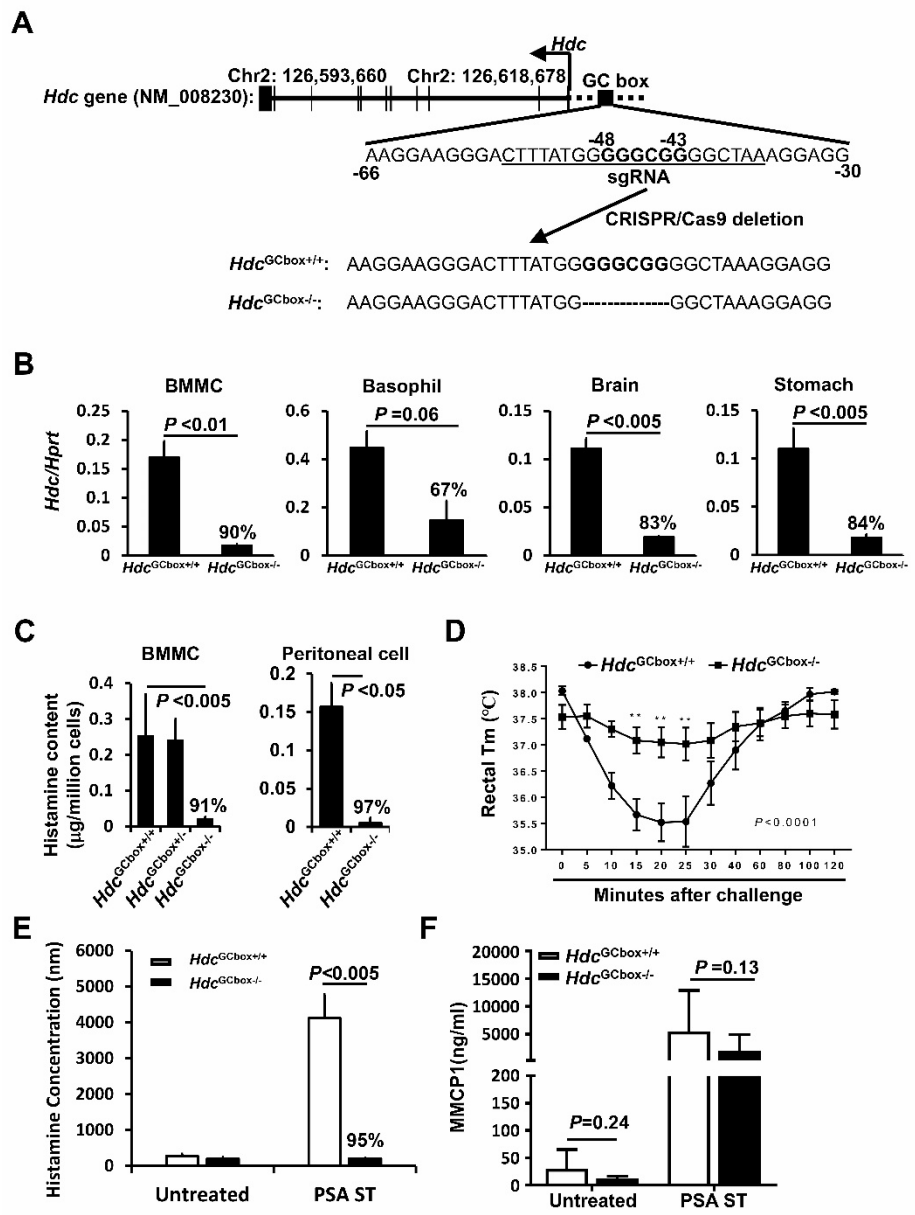
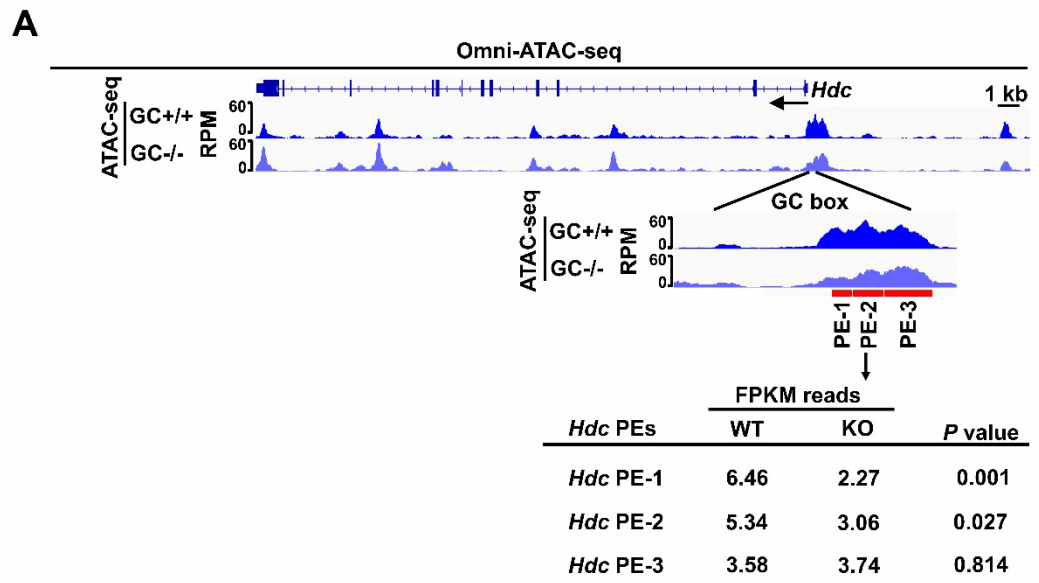
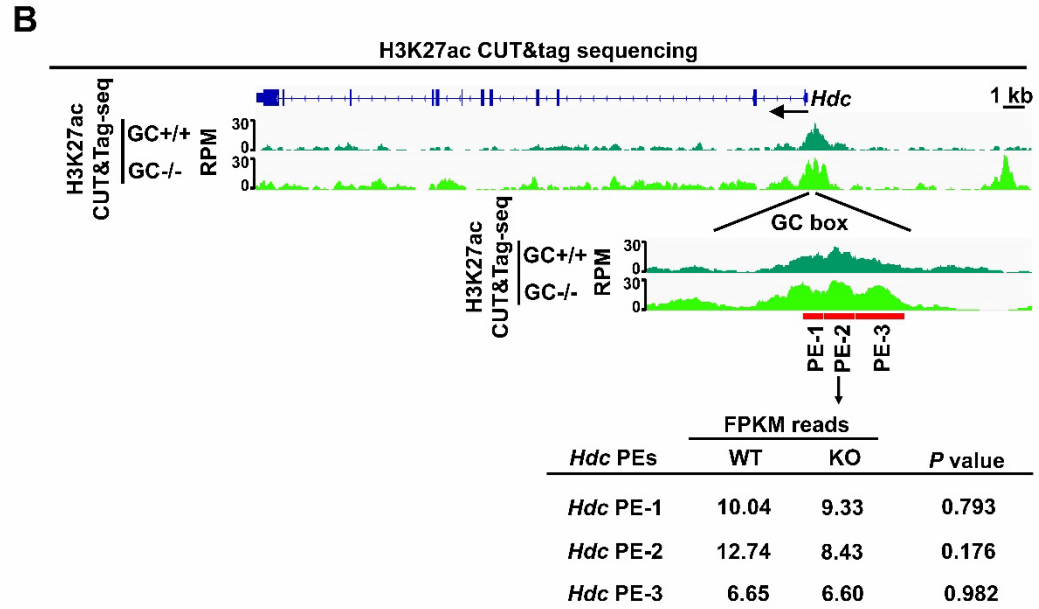


Figure 4. The GC box in the *Hdc* PE is critical for histamine synthesis and IgE/mast cell-mediated anaphylaxis. A. mice deficient in GC box (GGGCGG) were generated by a single sgRNA (underlined) targeting the GC box located from-48 to -43 in the proximal promoter of the *Hdc* gene. **B**. qPCR analysis of *Hdc* mRNA expression in cells isolated from WT and $Hdc^{GC-/-}$ mice (n=3, pooled from two experiments). **C**. histamine contents in BMMCs derived from WT and $Hdc^{GC-/-}$ mice (n=6, two experiments). **D**. PSA of WT and $Hdc^{GC-/-}$ mice (n=6, two experiments). **E**. histamine release in the plasma of WT and $Hdc^{GC-/-}$ mice that were treated with IgE anti-TNP antibody and BSA-TNP for five minutes (n=6, two experiments). **F**. MMCP1 concentrations in the sera of WT and $Hdc^{GC-/-}$ mice that were treated with IgE anti-TNP antibody and BSA-TNP for one hour (n=6, two experiments). *P* values were calculated using the Mann-Whitney U test.



Hdc PEs	FPKM reads WT	FPKM reads KO	*P* value
Hdc PE-1	6.46	2.27	0.001
Hdc PE-2	5.34	3.06	0.027
Hdc PE-3	3.58	3.74	0.814



Hdc PEs	FPKM reads WT	FPKM reads KO	*P* value
Hdc PE-1	10.04	9.33	0.793
Hdc PE-2	12.74	8.43	0.176
Hdc PE-3	6.65	6.60	0.982

Figure 5. GC box only promotes chromatin accessibility, but not H3K27ac modification, at the *Hdc* PE. A. Representative Integrated Genome Viewer (IGV) tracks from Omni-ATAC-seq (n=3, 1 experiment). **B.** Representative Integrated Genome Viewer (IGV) tracks from H3K27ac CUT&Tag-seq representing three biological samples (n=3). Statistical significance was analyzed with a two-tailed student's *t* test.

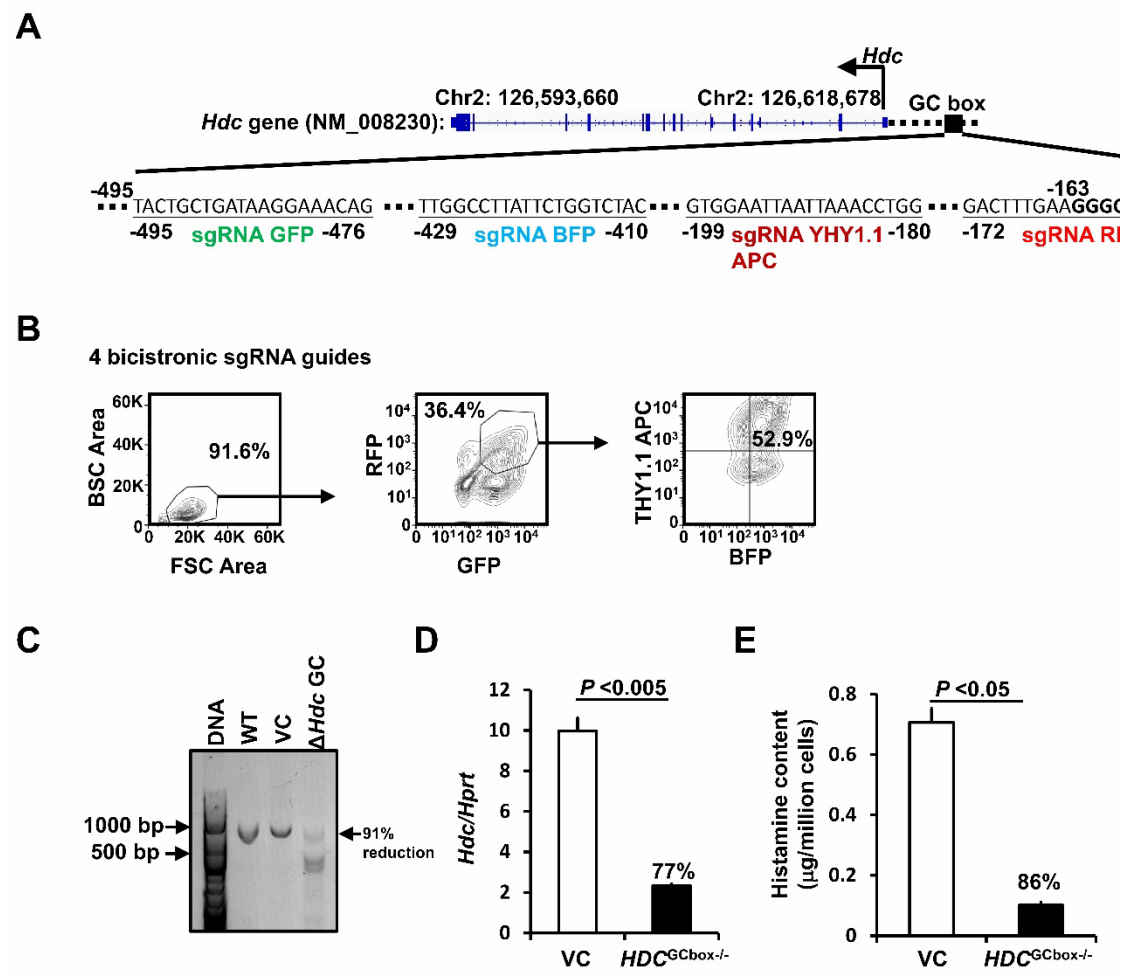
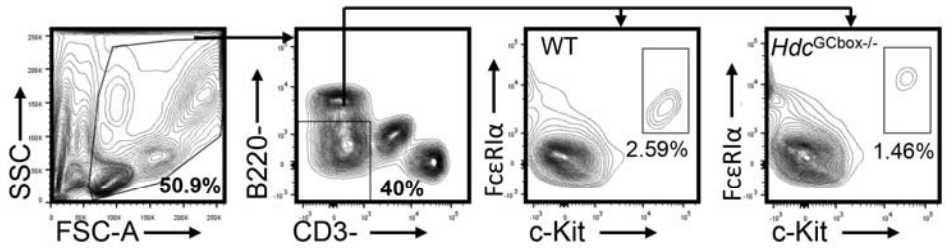


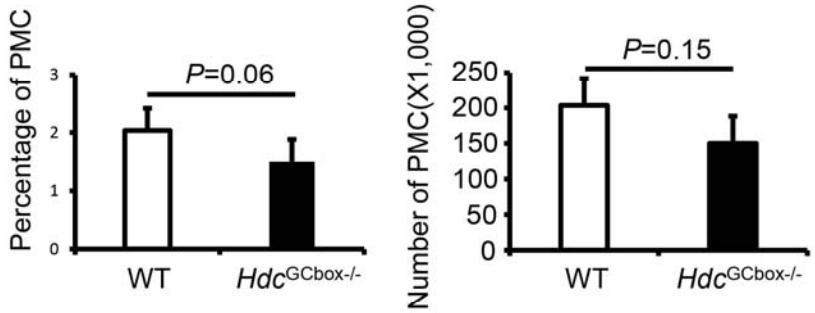
Figure. 6 The *HDC* GC box in the human *HDC* gene is critically important for *HDC* gene transcription and histamine synthesis.

A. Bicistronic BFP, GFP, RFP, and Thy1.1 sgRNA guides targeting the human *HDC* GC box located in -153 to -495. **B**. FACS sorting gates. LAD2 cells were transduced with lentivirus containing four Bicistronic sgRNA guides. BFP$^+$, GFP$^+$, RFP$^+$, and Thy1.1 APC$^+$ transfectant cells were FACS-sorted using the sorting gates indicated. As expected, the NE region did not show enhancer activity (Fig. 2A). **C.** DNA deletion efficiency analysis. GC box fragments from untransduced WT, or FACS-sorted BFP$^+$, GFP$^+$, RFP$^+$, and Thy1.1APC$^+$ cells transduced with lenti vector without sgRNA guide vector controls (VCs) and with four sgRNA guides targeting the human *HDC* GC box were analyzed with PCR. Percentage of reduction was calculated by reduction in density of the WT band. D. *Hdc* mRNA expression in the FACS-sorted BFP$^+$, GFP$^+$, RFP$^+$, and Thy1.1APC$^+$ cells was measured by qPCR (mean ± SEM, n=4 from 2 independent experiments). E. Histamine content in the FACS-sorted BFP$^+$, GFP$^+$, RFP$^+$, and Thy1.1APC$^+$ cells was measured by ELISA (mean ± SEM, n=4 from 2 independent experiments). The percentages indicate the percent reduction in *Hdc* mRNA expression and histamine contents relative to VCs. *P* values were calculated using the Mann-Whitney U test.

A



B



C

	WT	*Hdc*$^{GCbox-/-}$	*P*-value
MFI of c-Kit	35493±6504	37489±4438	>0.99
MFI of FcεR1α	7118±2322	9620.5±2467	0.34

Supplement figure 1. Mice deficient in the *Hdc* GC box had slightly reduced numbers and expressed higher levels of FceRIa and cKit expression levels in the peritoneal mast cells

A. Peritoneal cells were collected from WT and *Hdc* GC box (*Hdc*$^{GCbox-/-}$) mice and analyzed by FACS, DAPI^{-} CD3ε^{-} B220^{-} FcεRIα $^{+}$ c-Kit^{+} peritoneal mast cells were shown in the gates. **B**. Percentage and total numbers of peritoneal mast cells in the WT and *Hdc* GC box (*Hdc*$^{GCbox-/-}$) mice (mean ± SEM, n=4 from 2 independent experiments). **C**. MFI of FcεRIα and cKit of the peritoneal mast cells in the WT and *Hdc* GC box (*Hdc*$^{GCbox-/-}$) mice (mean ± SEM, n=4 from 2 independent experiments). *P* values were calculated using the Mann-Whitney U test.

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