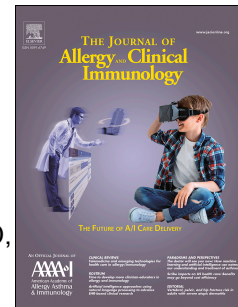


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Sirtuin 6 is a negative regulator of FcεRI signaling and anaphylactic responses

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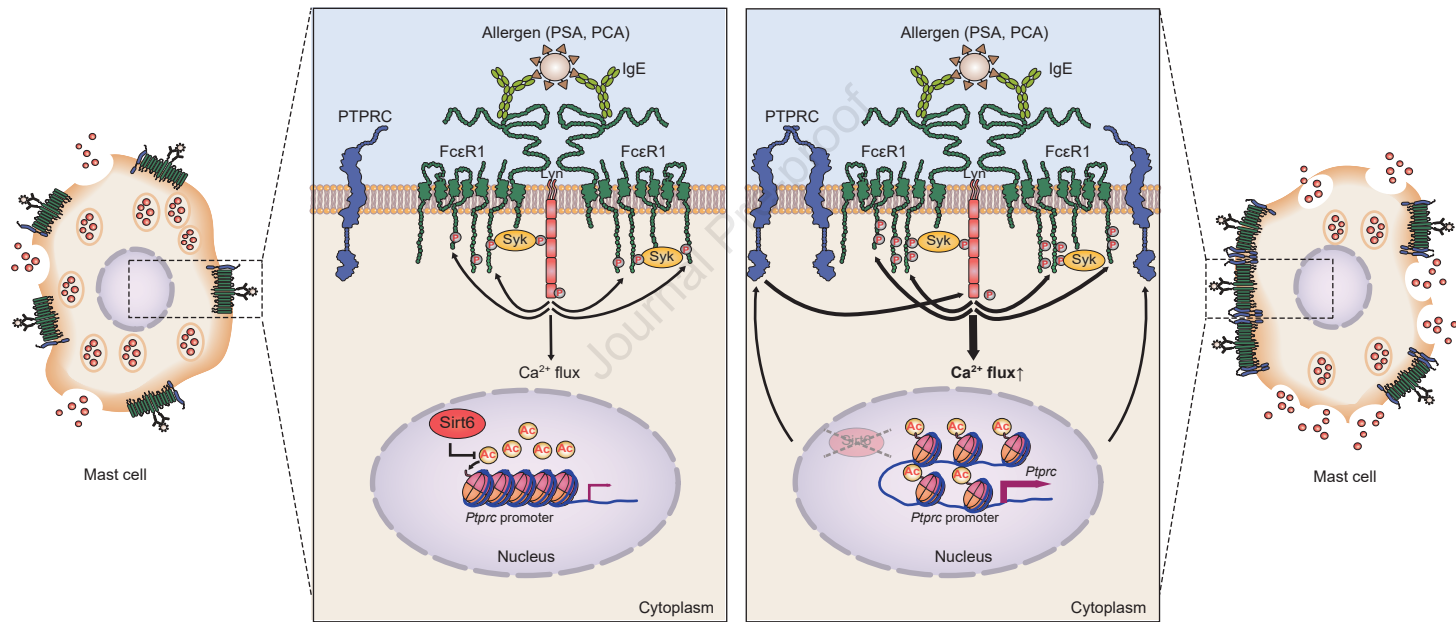
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Sirt6 deficiency enhances FcεR1 cross-linking by upregulating the expression of PTPRC



Sirt6, sirtuin 6; PTPRC, protein tyrosine phosphatase, receptor type C; PSA, passive systemic anaphylaxis; PCA, passive cutaneous anaphylaxis

Sirtuin 6 is a negative regulator of FcεRI signaling and anaphylactic responses

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Running title: Sirt6 suppresses mast cell activation.

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25

26 **Conflicts of interest**

27 The authors declare no conflicts of interest.

28

Abstract

Background: Binding IgE to a cognate allergen causes aggregation of FcεRI in mast cells, resulting in activation of receptor associated Src family tyrosine kinases (SFK), including Lyn and Syk. Protein tyrosine phosphatase, receptor type C (PTPRC), also known as CD45, has emerged as a positive regulator of FcεRI signaling by dephosphorylation of the inhibitory tyrosine of Lyn.

Objective: Sirt6, a NAD⁺-dependent deacetylase, exhibits an anti-inflammatory property. It remains to be determined, however, whether Sirt6 attenuates mast cell-associated diseases, including anaphylaxis.

Methods: FcεRI signaling and mast cell degranulation were measured after IgE crosslinking in murine bone marrow derived mast cells (BMMCs) and human cord blood derived mast cells. To investigate the function of Sirt6 in mast cell activation *in vivo*, we used mast cell dependent animal models of passive systemic anaphylaxis (PSA) and passive cutaneous anaphylaxis (PCA).

Results: Sirt6-deficient BMMCs augmented IgE-FcεRI-mediated signaling and degranulation compared to wild-type BMMCs. Reconstitution of mast cell-deficient *Kit*^{W-sh/W-sh} mice with BMMCs received from Sirt6 KO mice developed more severe PSA and PCA compared with mice engrafted with wild-type BMMCs. Similarly, genetic overexpression or pharmacologic activation of Sirt6 suppressed mast cell degranulation and blunted responses to PCA.

Mechanistically, Sirt6 deficiency increased PTPRC transcription via acetylating histone H3, leading to enhanced aggregation of FcεRI in BMMCs. Finally, we recapitulated the Sirt6 regulation of PTPRC and FcεRI signaling in human mast cells.

Conclusion: Sirt6 acts as a negative regulator of FcεRI signaling cascade in mast cells by

suppressing PTPRC transcription. Activation of Sirt6 may therefore represent a promising and novel therapeutic strategy for anaphylaxis.

Clinical implications

Activating Sirt6 could be a novel therapeutic or prophylactic for mast cell-associated diseases.

Capsule summary

Sirt6 deficiency augments IgE/Ag-mediated FcεRI cross-linking by upregulating the expression of PTPRC, resulting in mast cell hyperactivation and exacerbated anaphylaxis.

Keywords

Sirt6, mast cell, anaphylaxis, FcεRI, PTPRC

Abbreviations

FcεRI, Fcε receptor I; PLCγ, phospholipase Cγ; PTPRC, protein tyrosine phosphatase, receptor type C; PCA, passive cutaneous anaphylaxis; PSA, passive systemic anaphylactic; BMMCs, bone marrow derived mast cells; hCBMCs, human cord blood derived mast cells; DEG, differentially expressed gene; SCF, stem cell factor

Introduction

Anaphylaxis is a life-threatening reaction that affects the respiratory, cardiovascular, cutaneous, and gastrointestinal systems. This allergic reaction is typically precipitated when immunoglobulin E (IgE)-bound mast cells come into contact with allergens.¹ Mast cells constitutively express high levels of Fcε receptor I (FcεRI), a high-affinity IgE receptor, on the cell surface. FcεRI comprises a tetrameric complex consisting of IgE-binding chain FcεRIα, FcεRIβ, and FcεRIγ homodimer.² Binding FcεRI-bound IgE to the cognate antigen (Ag) induces an aggregation of FcεRI, resulting in phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) in the receptor by the Src-family tyrosine kinase Lyn, as well as subsequent recruitment and activation of Syk tyrosine kinase.³ Activated Syk triggers a signaling cascade through phospholipase Cγ (PLCγ) and calcium mobilization, which ultimately leads to the exocytosis of preformed vasoactive mediators, cytokines, and enzymes.⁴

FcεRI-mediated signaling is regulated by several transmembrane adaptor proteins.⁵ For example, protein tyrosine phosphatase, receptor type C (PTPRC, also known as CD45) positively regulates FcεRI signaling by dephosphorylating the inhibitory tyrosine residue in Lyn, thereby enhancing the recruitment of Lyn to FcεRI.⁶ Accordingly, PTPRC knockout (KO) mast cells do not respond to FcεRI-mediated degranulation and PTPRC KO mice are resistant to IgE-dependent systemic anaphylaxis.⁷ Thus, FcεRI-mediated signaling is a highly ordered and sequential molecular event. Today, antihistamines, mast cell stabilizers, and other anti-allergic drugs are used to treat IgE-mediated allergic diseases. Unfortunately, their use may also trigger adverse CNS symptoms, including drowsiness and headaches. Small molecule inhibitors that disrupt the FcεRI-mediated signaling recently exhibited marked improvement in preclinical models of anaphylaxis.^{8,9} In this regard, inhibiting mast cell

activation by targeting proximal FcεRI signaling may be an effective strategy for managing IgE-mediated diseases.¹⁰

Li et al.¹¹ recently reported that activation of NAD⁺-dependent histone deacetylase sirtuin 1 (Sirt1) attenuates FcεRI-mediated mast cell activation by inhibiting Syk phosphorylation. Sirt1-deficient mice exhibited augmented passive cutaneous anaphylaxis (PCA) in response to Ag challenge. These observations encouraged us to determine whether other sirtuin family members had similar effects on FcεRI signaling in mast cells and on anaphylactic reactions in mice. Similar to Sirt1, Sirt6 is located in the nucleus and deacetylates histone and non-histone proteins. As a histone deacetylase, Sirt6 alters chromatin structure by catalyzing the deacetylation of histone H3 lysine 9 (H3K9), H3K18, and H3K56.^{12, 13} As a non-histone protein deacetylase, Sirt6 deacetylates general control nonrepressed protein 5 (GCN5),¹⁴ pyruvate kinase M2 (PKM2),¹⁵ forkhead box protein O1 (FoxO1),¹⁶ estrogen receptor α (ERα),¹⁷ and estrogen-related receptor γ (ERRγ).¹⁸

Sirt6 is by now well-known for its ability to determine macrophage phenotypes and their associated functions,^{19, 20} suggesting that it has a regulatory role in inflammation conditions. Indeed, Sirt6 is known to have anti-inflammatory effects in a variety of inflammatory disease conditions, including allergic airway inflammation,²¹ rheumatoid arthritis,²² adipose tissue inflammation,²³ and non-alcoholic steatohepatitis.²⁴ No study, however, has been performed to determine the role of Sirt6 in the biology and function of FcεRI in bone marrow-derived mast cells (BMMCs). To address this lacuna, we investigated the role of Sirt6 in anaphylactic responses using Sirt6-deficient BMMCs and mice. Sirt6-deficient BMMCs displayed increased Ag-stimulated IgE-mediated signals and more degranulation than WT BMMCs after FcεRI stimulation. Additionally, Sirt6 deficiency led to exaggerated IgE-mediated anaphylactic responses in mouse models of PCA and passive systemic anaphylaxis (PSA).

119 Mechanistically, we found that Sirt6 specifically targeted the early phase of FcεRI signaling
120 via a transcriptional repression of PTPRC. Therefore, our results establish the physiological
121 functions of Sirt6 as a critical regulator of FcεRI-mediated signaling in mast cells.

122

Methods

Animals

Myeloid Sirt6 KO mice (*Sirt6*^{flox/flox}; *LysM-Cre*) were generated as described previously.¹⁹ Mast cell-deficient *Kit*^{W-sh/W-sh} mice of the C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). All animal experiments were performed in accordance with the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011). The study protocol was approved by the Institutional Animal Care and Use Committee of Chonbuk National University (Permit No: CBNU 2017-0124).

Bone marrow-derived mast cells (BMMCs)

Bone marrow cells were obtained by flushing bone marrow from the femurs and tibias of mice. Cells were cultured in RPMI 1640 media supplemented with IL-3 and stem cell factor (SCF, PeproTech, Cranbury, NJ, USA). After 4–6 weeks in culture, the phenotype of BMMCs was assessed by using flow cytometry. Cells with purity > 95% were used for subsequent experiments.

Passive systemic anaphylaxis (PSA) and passive cutaneous anaphylaxis (PCA)

Myeloid Sirt6 KO mice were backcrossed for at least 8 generations to the C57BL/6 background. For PSA, BMMCs (1×10^7) from WT or KO mice were injected into 5-week C57BL/6J-*Kit*^{W-sh/W-sh} mice via tail vein. Four months later, mast cell reconstitution in these recipients was histologically confirmed by toluidine blue staining of tissues. Mice were sensitized by intravenous injection with 10 µg of anti-DNP IgE in 100 µl PBS. The following

day, mice were intravenously challenged with 100 µg of DNP-HSA in 100 µl PBS. Body temperature was measured with a rectal thermometer (eDAQ, NSW, Australia) after challenge in every ten min for 90 min.

For PCA, BMDCs (2×10^6) from WT or KO mice were intradermally injected into each ear of the *C57BL/6J-Kit^{W-sh/W-sh}* mice. Four months later, mice were sensitized by intradermal injection with 1 µg of anti-DNP-IgE in 20 µl PBS in the right ear and an equal volume of saline in the left ear. After 24 h, mice were challenged intravenously with DNP-HSA (100 µg in saline containing 1% Evans blue).

For Sirt6 activation experiments, anti-DNP IgE-sensitized C57/BL6 mice were topically administered the Sirt6 activator MDL801 (50 mg/kg), 1 h before the DNP-HSA challenge.²⁵

Generation of human cord blood derived mast cells (hCBMCs)

Umbilical cord blood was obtained from normal vaginal and cesarean deliveries. Informed consent was received prior to the collection of cord blood and the study was approved by the Institutional Review Board of Chonbuk National University Hospital (Approval No. CBNUH: 2020-07-039-004). hCBMCs were generated using a modified version of the method described by Weng et al.²⁶

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were by one-way analysis of variance followed by Turkey's post hoc analysis. P value below 0.05 was considered significant.

170 *Additional methods*

171 Additional details concerning our methods are provided in the Supplementary Methods
172 section.

173

Results

Sirt6 deficiency promotes Ag-stimulated mast cell degranulation

We first isolated bone marrow cells and cultured them in the presence of IL-3 and SCF for 4 weeks. Successful generation of the BMMCs was confirmed by measuring the release of β -hexosaminidase, histamine, LTC₄, and PGD₂, as well as by analyzing Fc ϵ RI signaling (Fig E1, A-C). Next, we measured sirtuin expression in the BMMCs after anti-DNP-IgE and subsequent DNP-HSA (Ag) stimulation. Western blotting analysis revealed selective induction of Sirt6 (Fig E1, D). qPCR analysis confirmed this observation (Fig E1, E).

To explore the physiological role of Sirt6 in anaphylactic reactions, we stimulated WT and KO BMMCs with Ag. β -hexosaminidase release in both WT and KO BMMCs followed the typical bell-shaped dose-response curve²⁷ when each was treated with increasing concentrations of Ag for 30 min (Fig 1, A). Release peaked at 30 min, followed by a mild decrease at 60 min after IgE/Ag (100 ng/ml) treatment (Fig 1, B). The degranulation response in KO BMMCs was significantly enhanced compared to that of WT BMMCs across all the doses and time-points examined. Consistently, the release of vasoactive mediators (histamine, LTC₄ and PGD₂) and proinflammatory cytokines (IL-6 and TNF- α) was higher in KO BMMCs than in WT BMMCs (Fig 1, C-G). Electron microscopy revealed a more activated appearance of the KO BMMCs after Ag stimulation compared with the WT BMMCs (Fig 1, H), supporting the degranulation data.

Because IgE-mediated degranulation is calcium dependent, we analyzed the effect of Sirt6 deficiency on calcium influx. For this, we compared the extent of phosphorylation of Lyn, Syk, LAT, and PLC γ , which are upstream signaling molecules that lead to calcium influx. Compared against WT BMMCs, Ag stimulation caused a greater phosphorylation of the aforementioned signaling molecules (Fig 2, A). Interestingly, inhibitory phosphorylation

of Lyn at Y507 was somewhat suppressed in KO BMMCs, suggesting an enhancement of Lyn signaling by Sirt6 deficiency. Tyrosine kinase Fyn is essential for PI3K/AKT activation though Gab2 and contributes to IgE-FcεRI-mediated granule translocation in mast cells.²⁸ We therefore investigated the phosphorylation of these proteins, and the results showed an increase in phosphorylation of Fyn, Gab2, and PI3K/AKT in KO BMMCs. Notably, in KO BMMCs, we observed the increased phosphorylation of MAPKs, which are required for the synthesis of lipid mediators and cytokines.²⁹ Fluo-4 fluorescence corresponding to intracellular calcium was assessed using a confocal microscope. KO BMMCs displayed sustained calcium oscillations upon Ag stimulation while WT BMMCs displayed transient calcium oscillations (Fig 2, B). The functionality of Sirt6 deficiency was confirmed by an increased acetylation of H3K9, H3K18, and H3K56 (Fig 2, C).

To ascertain whether the enhanced IgE-mediated BMMCs activation by Sirt6 was a result of altered mast cell development, BMMCs from WT or KO mice were cultured in parallel and the maturation of BMMCs was monitored. Overall, there was no distinction between genotypes in cell surface expression of c-Kit and FcεRI, mRNA levels of matured mast cell marker genes, and histamine contents (Fig E2, A-D). Together, this data indicates that Sirt6 is required for downregulating FcεRI signaling and restraining IgE-mediated degranulation, without affecting mast cell differentiation.

Genetic re-expression and pharmacological activation of Sirt6 dampen FcεRI-mediated signaling in KO BMMCs

We performed rescue experiments by re-expressing Sirt6 in KO BMMCs. As expected, reintroducing Sirt6 calmed the hyperactive degranulation machinery in Sirt6-deficient BMMCs. Specifically, the release of β-hexosaminidase, histamine, LTC₄, and PGD₂ were

significantly inhibited by Sirt6's re-expression (Fig 3, A, B). The phosphorylation of FcεRI signaling molecules was comparable with WT counterparts (Fig 3, C).

We employed MDL801, which is a recently identified Sirt6 allosteric activator.²⁵ Consistent with our Sirt6 re-expression results, MDL801 treatment suppressed Ag stimulation-mediated FcεRI signaling and mast cell degranulation in a concentration dependent manner (Fig 3, D-F), confirming Sirt6's suppression of mast cell activation.

Mast cell Sirt6 deficiency exacerbates passive systemic anaphylaxis and passive cutaneous anaphylaxis

On the basis of *in vitro* observations, we performed *in vivo* experiments using mast cell-deficient *Kit^{W-sh/W-sh}* mice. *Kit^{W-sh/W-sh}* mice were intravenously injected with BMMCs from WT or KO mice. To confirm successful BMMCs engraftment in the *Kit^{W-sh/W-sh}* mice, we counted the number of mast cells in the glandular stomach, back skin, and lung tissues, and detected toluidine blue positive mast cells in BMMCs-transferred mice (Fig E3, A). Once the BMMCs were adapted in the mice, we tested whether the presence of Sirt6 in BMMCs could affect the severity of PSA. We measured rectal temperature as an indicator of PSA. The IgE sensitized *Kit^{W-sh/W-sh}* mice that received KO BMMCs showed a progressive decrease in rectal temperature within 10 min after antigen challenge, while *Kit^{W-sh/W-sh}* mice that received WT BMMCs showed an attenuated response (Fig 4, A). Accordingly, serum levels of MCPT1 and IL-6 were higher in KO BMMCs-transferred mice than WT BMMCs-transferred mice (Fig 4, B).

Kit^{W-sh/W-sh} mice received an intradermal transfer of WT or KO BMMCs into their ear. We verified that equal numbers of WT BMMCs and KO BMMCs were present in the ears after

engraftment (Fig E3, B). We then used an established protocol of PCA. In the ears that received KO BMMCs, more ear swelling and Evans blue dye extravasation were observed than in ears that received WT BMMCs (Fig 4, C and D). Consistently, there was more pronounced mast cell degranulation in the ears of the KO BMMCs-transferred mice, as is apparent from the presence of numerous toluidine blue positive granules outside the mast cell cytoplasm (Fig 4, E).

Genetic overexpression and pharmacological activation of Sirt6 protect mice against passive cutaneous anaphylaxis

Having elucidated Sirt6's beneficial role in attenuating Ag stimulation-mediated FcεRI signaling and mast cell degranulation, we examined whether Sirt6 activation could suppress anaphylactic reactions. To do so, we performed PCA experiments using Sirt6 overexpressing BMMCs. Compared against control mice which received β-galactosidase overexpressing BMMCs (AdLacZ group), Sirt6 overexpression markedly suppressed ear swelling, microvascular leakage, and mast cell degranulation (Fig 5, A-C). Based on the results of dye extravasation and histological analysis, MDL801 treatment also effectively suppressed anaphylactic reactions (Fig 5, D-F). To reiterate, both Sirt6 adenovirus and MDL801 decreased histone H3 acetylation (Fig E4, A and B).

Sirt6 deficiency increases PTPRC expression in mast cells

In the context of gene expression, Sirt6 represses transcription by acting as a specific deacetylase for H3K9, H3K18, and H3K56.^{12, 13} We accordingly performed RNA sequencing (RNA-seq) in WT and KO BMMCs to identify any transcriptional changes caused by Sirt6

deficiency. The heatmap plot revealed that WT and KO BMMCs could be differentiated by their mRNA expression profile (Fig 6, A). The differences in biological process, molecular function, and cellular components of the differentially expressed genes (DEGs) in the WT and KO BMMCs were obtained by GO enrichment analysis (Fig 6, B). The GO categories of biological processes most highly represented were the cellular process, metabolic process and immune system process, which suggested a high degree of basic metabolic activity in the Sirt6-deficient BMMCs. The volcano plot identified 72 genes upregulated and 142 genes downregulated in the KO BMMCs (Fig 6, C). We focused on the upregulated genes, given the likelihood that downregulated genes could not be explained as direct targets of Sirt6 deficiency. Genes up-regulated 2-fold or more, including *Ptprc*, *Cadm1*, *S100a9*, and *Elmo2* were further validated using qPCR (Fig 6, D and E). Among these genes, *Ptprc* had the highest RNA transcript level in Sirt6-deficient BMMCs. We therefore hypothesized that increased expression of *Ptprc* may be responsible for the functional phenotype of Sirt6-deficient BMMCs.

Further validation of PTPRC upregulation in KO BMMCs was performed using Western blotting, flow cytometry, and immunofluorescence staining (Fig 7, A-C). ChIP was performed to test hyperacetylation on the *Ptprc* promoter using an anti-Ac-H3K9 antibody in WT and KO BMMCs with or without Sirt6 re-expression. Results showed increased acetylation of H3K9 at the *Ptprc* promoter region in KO BMMCs, which significantly decreased once Sirt6 was re-expressed (Fig 7, D-F). These findings indicate that Sirt6 controlled *Ptprc* gene transcription by deacetylating histones at the *Ptprc* promoter. Finally, immunofluorescence staining showed enhanced colocalization of FcεRI and PTPRC in KO BMMCs upon exposure to Ag (Fig 7, G). When viewed comprehensively, these results suggest that Sirt6 deficiency in mast cells promotes PTPRC expression, which causes increased colocalization with FcεRI, which in turn leads to amplified FcεRI signal

transduction.

Sirt6 suppresses the expression of PTPRC and degranulation in human cord blood derived mast cells

We used hCBMCs, as identified by flow cytometric analysis, to determine whether Sirt6 affects signaling in human mast cells similar to how it affects mouse mast cells (Fig 8, A). hCBMCs transfected with Sirt6 were stimulated with myeloma IgE and anti-IgE complex (IgE/anti-IgE). PTPRC expression and mast cell degranulation were significantly suppressed by Sirt6 overexpression when compared with control cells (Fig 8, B and C). Conversely, when these experiments were repeated with Sirt6 silencing hCBMCs, the results obtained were precisely the opposite (Fig 8, D and E). Consistent with the results obtained using Sirt6 KO BMMCs, we observed an enhanced colocalization of FcεRI and PTPRC in Sirt6 silencing hCBMCs upon exposure to IgE/anti-IgE complexes (Fig 8, F). These results suggest that Sirt6 not only inhibits IgE-mediated immune responses in murine models of anaphylaxis, but that it may also has the same effect in human patients.

Discussion

In this study, we identified a previously unknown function of Sirt6, that of a transcriptional repressor of PTPRC in the mast cell IgE/FcεRI signal transduction pathway. Loss of inhibitory Lyn phosphorylation by PTPRC proximal to FcεRI aggregation resulted in elevated and prolonged propagation of downstream pathways, including the tyrosine kinase Syk and PLCγ. This latter process is essential for calcium mobilization and subsequent mast cell degranulation. The loss of Sirt6 activity in mast cells is biologically significant, as it causes more severe IgE-mediated anaphylaxis in mice.

Our previous studies have demonstrated the anti-inflammatory role of Sirt6 in various allergic and inflammatory disease models. Sirt6 reduces Th2 cell-mediated inflammation in the lungs.²¹ Mice lacking myeloid Sirt6 develop autoimmune rheumatoid arthritis²² and severe inflammation in the affected tissues.^{19, 20} At the molecular level, Sirt6 directly modifies the process of immune cell differentiation. For example, Sirt6 suppresses macrophage polarization toward an M1 type by suppressing NF-κB-STAT3 axis¹⁹ while facilitating M2 macrophage polarization by activating the AKT pathway.²⁰ Sirt6 suppresses CD4⁺ T cell differentiation into Th2 cells by deacetylating GATA3.²¹ During mast cell development, GATA2 is essential for mast cell lineage specification, and it plays a more important role than even GATA1.^{30, 31} We therefore investigated first whether Sirt6 could affect mast cell differentiation. In contrast to the effects on macrophages and Th2 cells, expression levels of all mast cell-specific genes, including *Gata1* and *Gata2*, were not changed in BMMCs by a deficiency in Sirt6, indicating that Sirt6 is not necessary for mast cell development. FcεRI signaling, however, was markedly active in Sirt6-deficient BMMCs. For many years, IgE/FcεRI signaling has been considered a quintessential example of aggregation-driven receptor triggering of immune cells. For FcεRI to be triggered in mast

cells, receptors must be cross-linked by multivalent antigens in the optimal geometric structure that is required for phosphorylation of Src family kinases Lyn and Syk. This, in turn, activates PLC γ to transmit a signal to the degranulation mechanisms.³² Our results indicate that Sirt6 deficiency increased the phosphorylation levels of Lyn, Syk and PLC γ , suggesting an activation of the Lyn-Syk-PLC γ pathway in Sirt6-deficient BMMCs. Interestingly, it appears that Sirt6 deficiency also activated the Fyn-Gab2-PI3K pathway, an auxiliary signaling pathway that activates BMMCs. This suggests that Sirt6 deficiency augments Fc ϵ RI signaling at an upstream level of Lyn and Fyn activation.

When the IgE- Fc ϵ RI complex is aggregated by multivalent antigens, Lyn autophosphorylates itself at tyrosine (Y396) and transphosphorylates tyrosine residues in the β and γ subunits of Fc ϵ RI.³³ To identify possible signaling effector(s) for the activation of Fc ϵ RI signaling, we performed RNA seq analysis and found a higher expression of *Ptpsrc* in Sirt6-deficient BMMCs. Additionally, using confocal microscopy, we observed an enhanced colocalization of PTPRC and Fc ϵ RI in Sirt6-deficient BMMCs. Based on these findings, we hypothesize that PTPRC upregulation may contribute to the phenotype of Sirt6-deficient BMMCs. Previously, PTPRC has been reported to regulate the activity of Src family tyrosine kinases by dephosphorylating the carboxy-terminal inhibitory tyrosine in T cells, B cells, and mast cells.^{6, 34} Consistent with these reports, we observed a marked reduction of phosphorylation at Lyn Y507, which is an inhibitory tyrosine of Lyn, in Sirt6-deficient BMMCs. This would further facilitate the Fc ϵ RI-mediated signaling that is associated with mast cell degranulation. Although all of the evidence points to a direct modulation of proximal Fc ϵ RI signaling responses by Sirt6, we cannot exclude the possibility that Sirt6 may also regulate other components involved in mast cell degranulation. For example, our research, and that of others, has shown that Sirt6 interacts with microtubule-associated protein light chain 3 (LC3) to induce autophagy,^{35, 36} which plays a crucial role in mast cell

degranulation.³⁷ Collectively, these findings reinforce the conclusion that Sirt6 exhibits a negative function in FcεRI-mediated mast cell activation, specifically by downregulating the transmembrane protein PTPRC. Nevertheless, we still lack a complete understanding of the underlying mechanism.

In sum, the present study highlights a novel role for mast cell Sirt6 as a transcriptional repressor of PTPRC in response to Ag stimulation. Given the positive regulatory role of PTPRC on FcεRI signaling, Sirt6 repression of PTPRC may represent a very proximal step to limiting FcεRI activation and mast cell degranulation. Additionally, our findings of a perturbation of FcεRI signaling by the small molecule Sirt6 activator without noticeable side effects, and reproduction of this result in hCBMCs, suggests that the current study may point the way forward in the development of new treatments for human diseases in which mast cells play a pathogenic role.

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Figure legends

Figure 1. Sirt6 deficiency promotes Ag-stimulated mast cell degranulation. BMMCs (1×10^6) from WT or KO mice were sensitized with anti-DNP-IgE and stimulated with DNP-HSA (Ag). **(A, B)** Dose- and time-dependent β -hexosaminidase releases were measured (n=13). **(C-G)** Degranulation was assessed by measurement of histamine, LTC₄, and PGD₂ at 30 min, and of IL-6 and TNF- α at 6 h. **(H)** Transmission electron microscopic analysis of WT and KO BMMCs stimulated with Ag for 30 min. Values are mean $\pm$ SD. Individual values are shown as circles. ** $p < 0.01$ vs. WT-Veh; ## $p < 0.01$ vs. WT or WT-Ag. Veh, vehicle; LTC₄, leukotriene C₄, PGD₂, prostaglandin D₂

Figure 2. Sirt6 deficiency activates Fc ϵ RI-initiated signaling in mast cells. **(A, B)** After sensitization and stimulation with 1 μ g anti-DNP-IgE/100 ng DNP-HSA, Fc ϵ RI-mediated signaling was compared by Western blotting. **(B)** IgE sensitized BMMCs were labeled with Fluo-4 and then stimulated with DNP-HSA for indicated times. Representative confocal micrographs (top) and fluorescence emission spectral scans (bottom) of intracellular calcium influx. **(C)** After stimulation with Ag, lysine acetylation of histone H3 was analyzed by Western blotting.

Figure 3. Sirt6 activation restores Fc ϵ RI-mediated signaling in mast cells. **(A, B)** BMMCs from WT or KO mice were transduced with Flag or Flag-Sirt6 plasmids and then sensitized with 1 μ g anti-DNP-IgE and stimulated with 100 ng DNP-HSA for 30 min. Degranulation was measured by the release of β -hexosaminidase, histamine, LTC₄, and PGD₂. **(C)** Fc ϵ RI-mediated signaling was compared by Western blotting. **(D-F)** IgE-sensitized BMMCs were treated with various concentrations of MDL801 for 1 h and then stimulated

with DNP-HSA for 30 min. Releases of β -hexosaminidase, histamine, LTC₄, and PGD₂ and Fc ϵ RI-mediated signaling were then determined. Values are mean $\pm$ SD. Individual values are shown as circles. ** p <0.01 vs. Veh; # p <0.05 and ## p <0.01 vs. WT-Ag; \$\$ p <0.01 vs. KO-Ag; & p <0.05 and && p <0.01 vs. MDL801-0 μ M. KS, KO+Sirt6

Figure 4. Sirt6 deficiency aggravates anaphylactic response. (A, B) Mast cell-deficient *Kit*^{W-sh/W-sh} mice received PBS (*W*^{sh}/*W*^{sh} + PBS) or intravenously injected with BMMCs from WT mice (*W*^{sh}/*W*^{sh} + WT BMMCs) or KO mice (*W*^{sh}/*W*^{sh} + KO BMMCs). Four months later, BMMCs engrafted mice were sensitized with 10 μ g anti-DNP-IgE and challenged with 100 μ g DNP-HSA. Changes in body temperature and serum levels of MCPT1 and IL-6 were determined (n=7-8). Individual values are shown as circles. **(C)** BMMCs (2×10^6) from WT or KO mice were intradermally injected into *Kit*^{W-sh/W-sh} mice. Four months later, BMMCs engrafted mice were passively sensitized by intradermal injection of 1 μ g anti-DNP-IgE into the ear. Subsequently, mice were challenged by intravenous injection of 100 μ g DNP-HSA in PBS/Evans blue. Representative ear images after PCA reaction are shown. **(D)** Ear swelling calculated by ear thickness and Evans blue dye extravasation. **(E)** Representative toluidine blue staining of ear skin sections. Total and degranulated mast cells in ear skin section were counted. Black and red arrows indicate non-degranulated and degranulated mast cells, respectively. Values are mean $\pm$ SD. * p <0.05 and ** p <0.01 vs. *W*^{sh}/*W*^{sh} + PBS; ## p <0.01 vs. *W*^{sh}/*W*^{sh} + WT BMMCs. MCPT1, mast cell protease 1

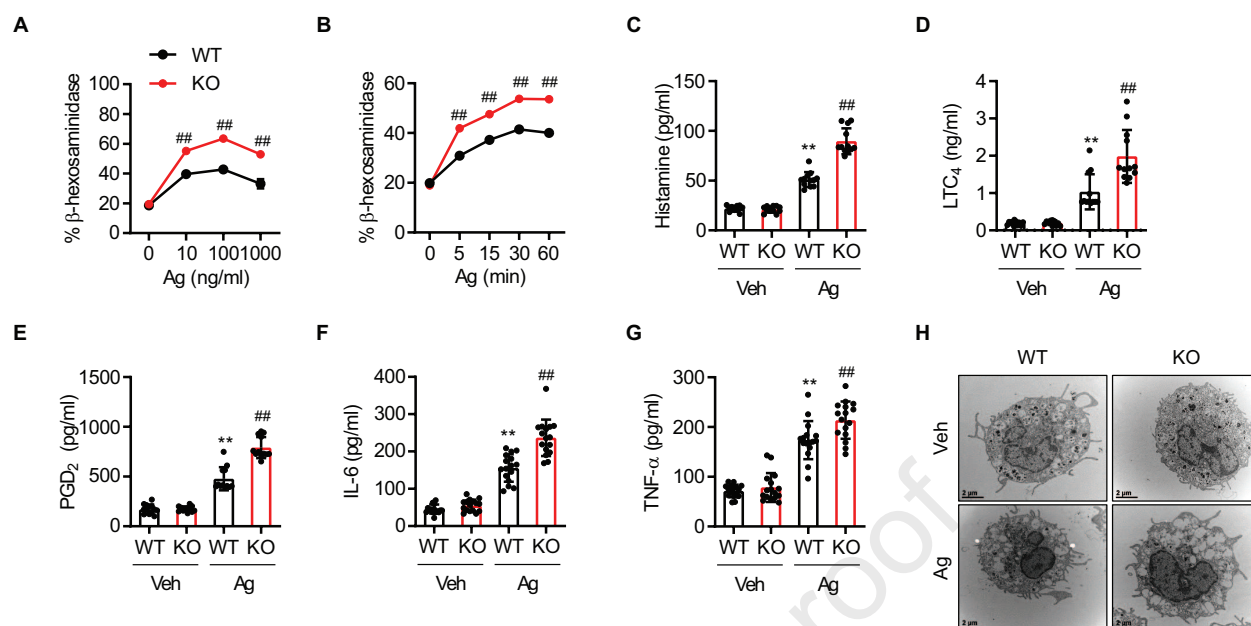
Figure 5. Sirt6 activation protects mice from anaphylactic response. (A-C) C57BL/6 mice were intradermally injected with 1×10^9 pfu of AdLacZ or AdSirt6. Three days later, PCA (1 μ g anti-DNP-IgE/100 μ g DNP-HSA) was performed. **(A)** Representative

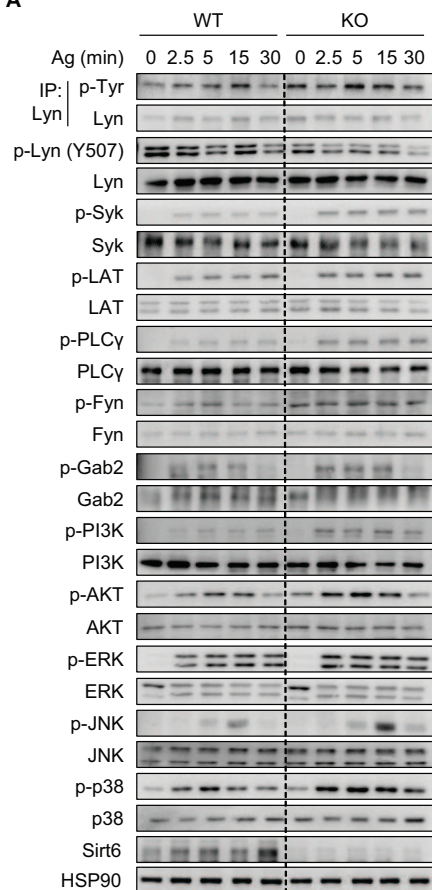
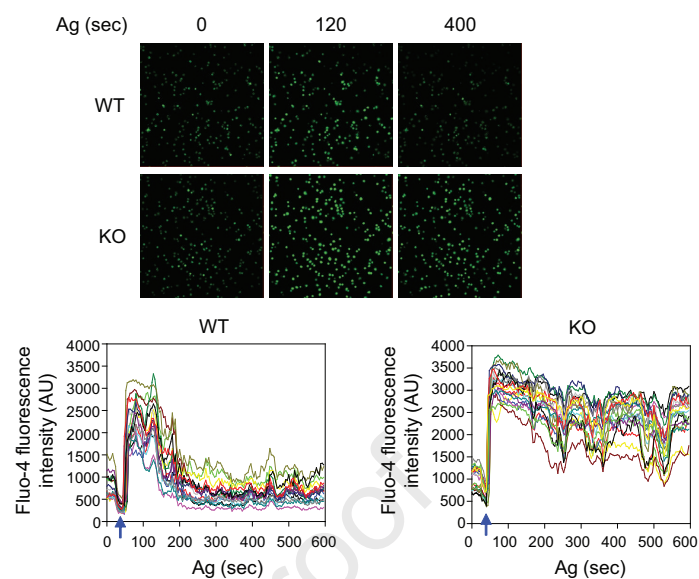
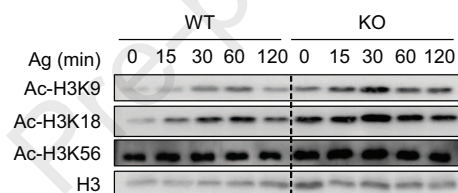
photographic images of ears. **(B)** Ear thickness and Evans blue dye extravasation. **(C)** Representative toluidine blue staining of ear skin sections. Total and degranulated mast cells in ear skin section were counted. **(D-F)** anti-DNP IgE was intradermally injected into the ears of C57BL/6 mice. After 24 h, 50 mg/kg MDL801 was topically administered 1 h prior to challenge with DNP-HSA. **(D)** Representative photographic image of ears. **(E)** Ear thickness and Evans blue dye extravasation. **(F)** Representative toluidine blue staining of ear skin sections. Total and degranulated mast cells in ear skin section were counted. Values are mean $\pm$ SD. Individual values are shown as circles. * $p < 0.05$ and ** $p < 0.01$ vs. Ctrl; ### $p < 0.01$ vs. PCA-Veh. Ctrl, control

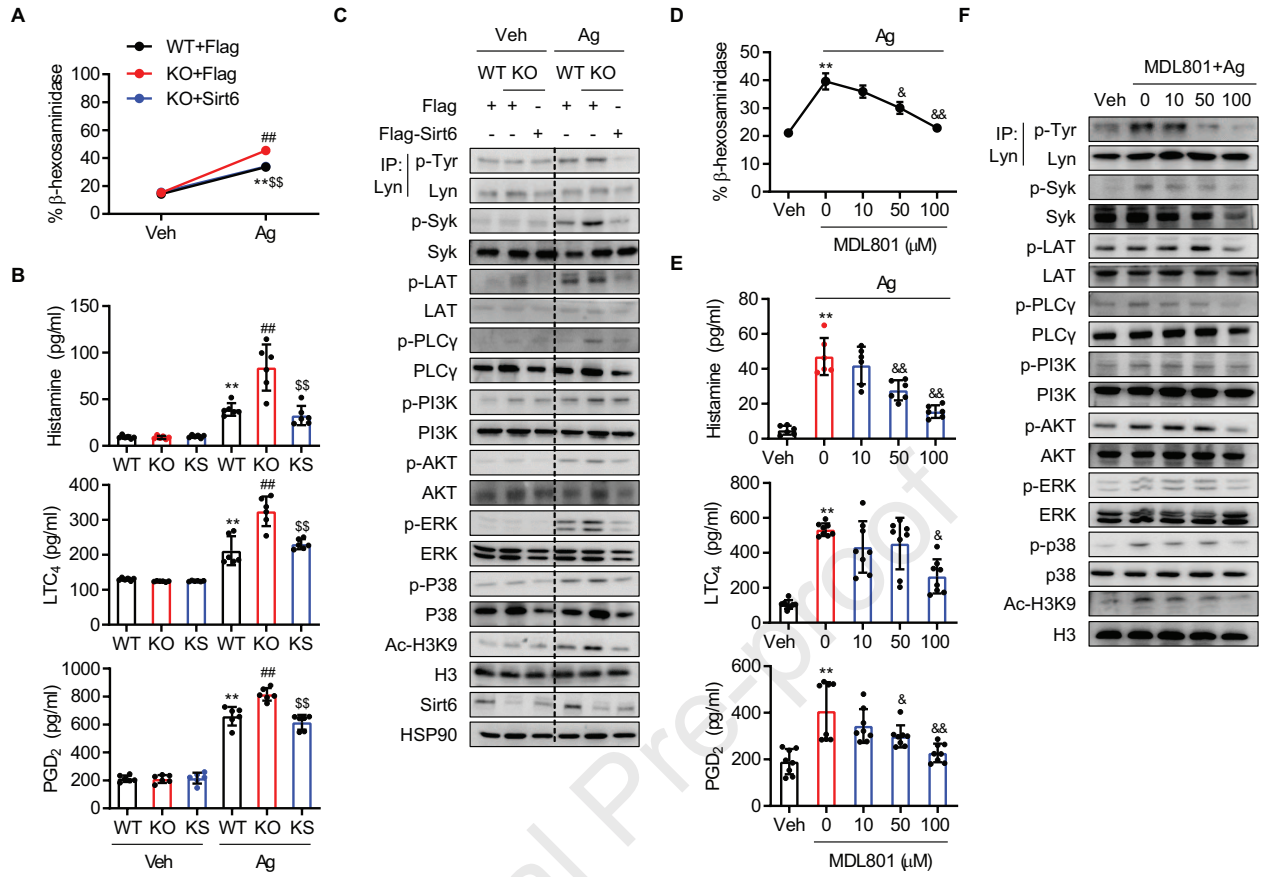
Figure 6. Sirt6 deficiency causes transcriptional reprogramming of Fc ϵ RI-mediated proximal signaling. **(A)** Total RNA from WT and KO BMMCs was used for RNA-seq analysis. DEGs are shown in a hierarchically clustered heatmap. For hierarchical clustering, distance metric between rows was calculated according to the $\log_2$ of FPKM values and then normalized by Z score. The lowest Z score values are dark blue and the highest values are bright yellow. **(B)** Enriched GO functional clustering of genes. **(C)** Volcano plot illustrating differentially regulated gene expression from RNA-seq analysis. Red and blue dots represent upregulated and downregulated DEGs with > 2 -fold change and $p < 0.05$, respectively. Each sample was analyzed in triplicate. **(D)** FPKM values are plotted for selected DEGs identified by RNA-seq. **(E)** The same genes in **(D)** were validated by qPCR. Values are mean $\pm$ SD. Individual values are shown as circles. * $p < 0.05$ and ** $p < 0.01$ vs. WT. DEG, differentially expressed genes; GO, gene ontology; FPKM, fragments per kilo base per million mapped reads

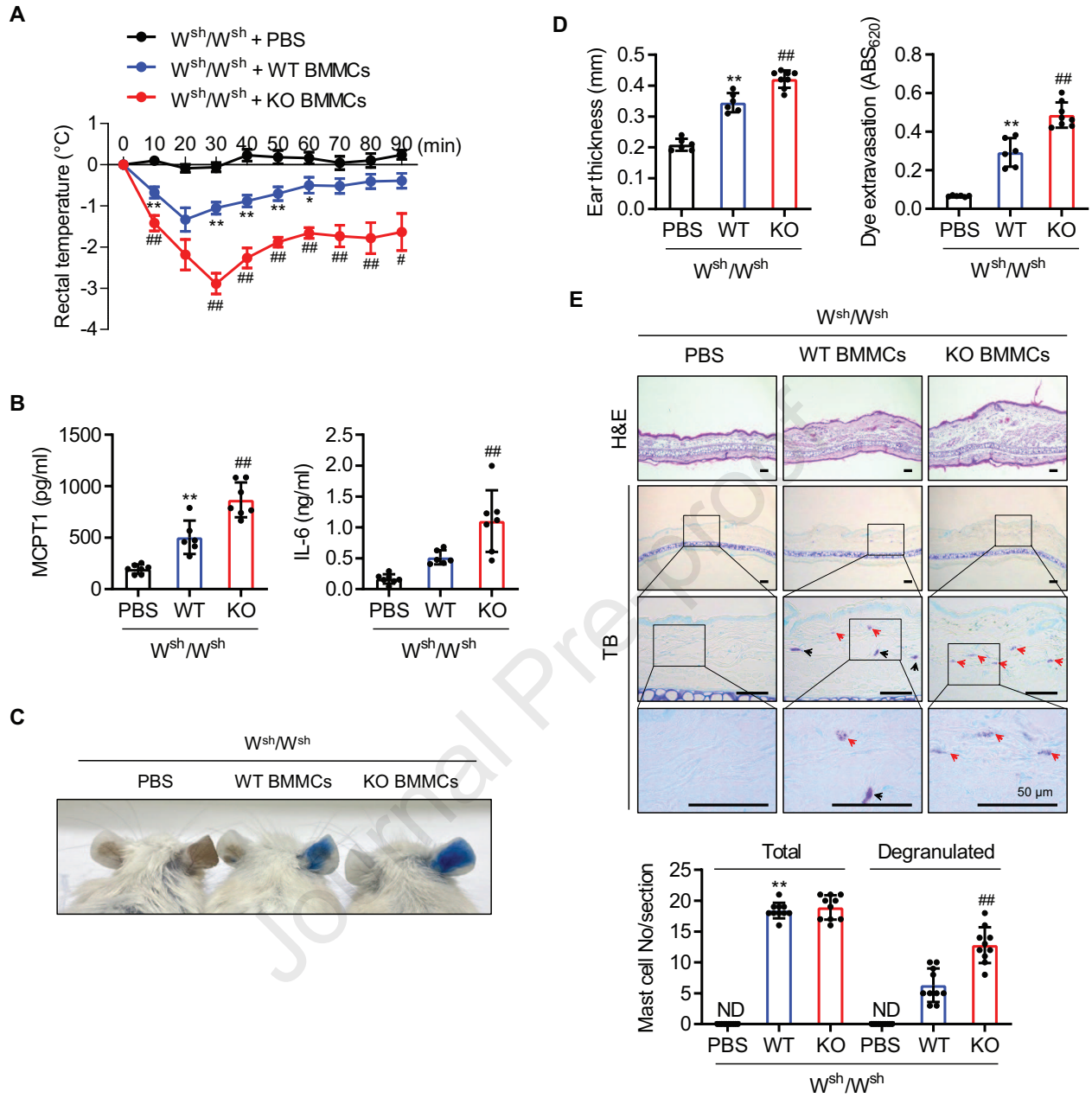
Figure 7. Sirt6 deficiency increases PTPRC expression in BMMCs. (A-C) Protein levels of PTPRC in BMMCs were compared by Western blotting, flow cytometry and immunofluorescence staining. (D) Enrichment of Ac-H3K9 on the *Ptprc* promoter region was evaluated by ChIP assay. (E, F) BMMCs from KO mice were transduced with Sirt6. Protein levels of PTPRC in BMMCs and acetylation of H3K9 at *Ptprc* promoter region were determined by Western blotting and ChIP assay, respectively. (G) Double immunofluorescence images of PTPRC and FcεRI in BMMCs. Quantification of colocalization was carried out by calculating Pearson's correlation coefficient and by measuring colocalization area. Values are mean ± SD. Individual values are shown as circles. * $p < 0.05$ and ** $p < 0.01$ vs. WT-IgG or WT-Veh; ### $p < 0.01$ vs. WT, WT-Ac-H3K9 or WT-Ag; \$\$ $p < 0.01$ vs. KO-Ac-H3K9.

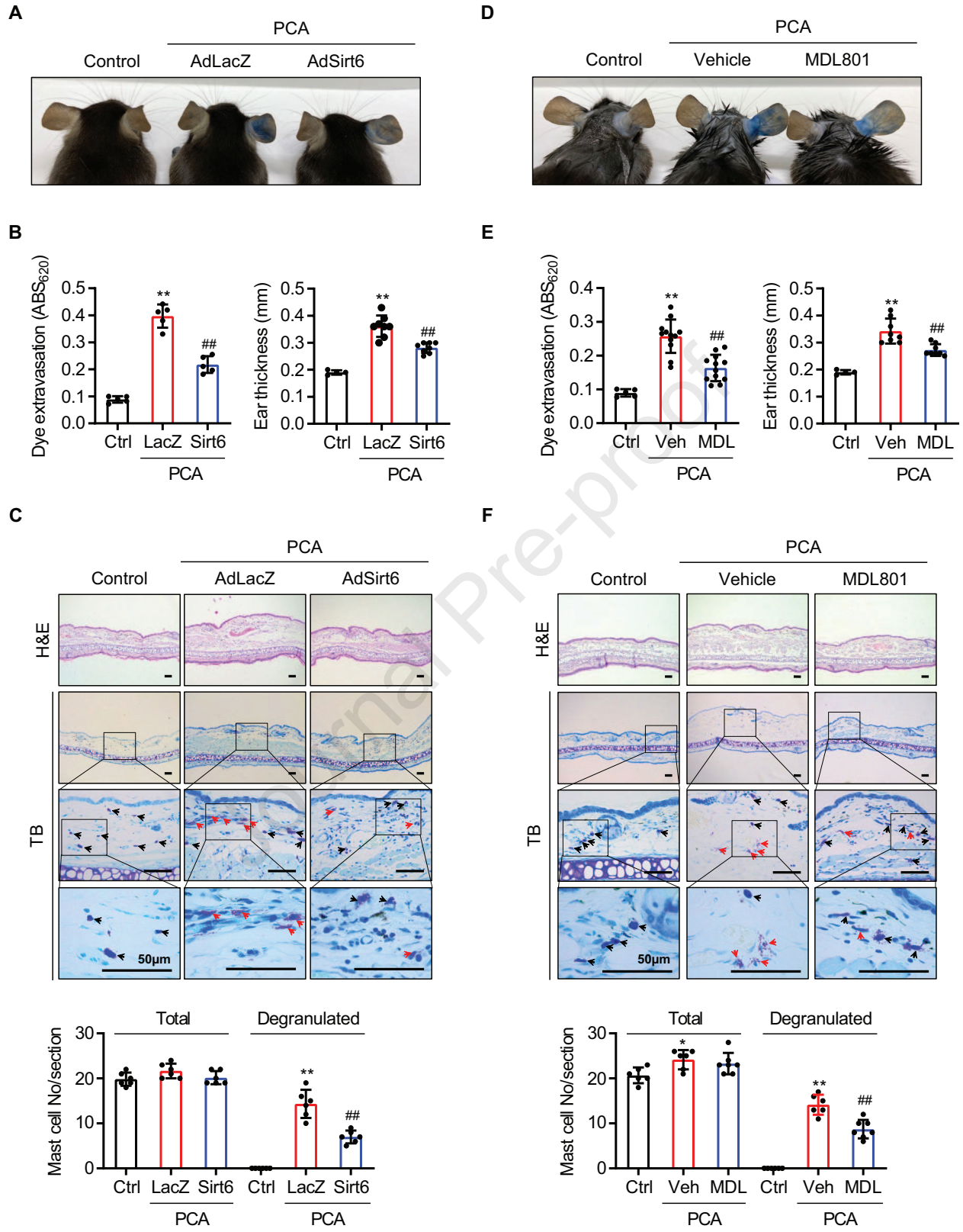
Figure 8. Sirt6 overexpression suppresses anti-IgE-mediated mast cell degranulation in hCBMCs. (A) Immunofluorescence staining and flow cytometric analysis of tryptase in human cord blood derived mast cells (hCBMCs). (B) hCBMCs were transduced with Sirt6 and PTPRC protein levels were determined by Western blotting. (C) Degranulation was determined at 30 min after anti-IgE stimulation by analysis of β-hexosaminidase releases and flow cytometric analysis of LAMP1. (D, E) hCBMCs were transduced with shSirt6. Protein levels of PTPRC in hCBMCs and degranulation were analyzed. (G) Double immunofluorescence images of PTPRC and FcεRI in hCBMCs. Colocalization was quantified using Pearson's correlation coefficient and colocalization area. Values are mean ± SD. Individual values are shown as circles. ** $p < 0.01$ vs. Veh; # $p < 0.05$ and ### $p < 0.01$ vs. anti-IgE-GFP. NTC, non-targeting control

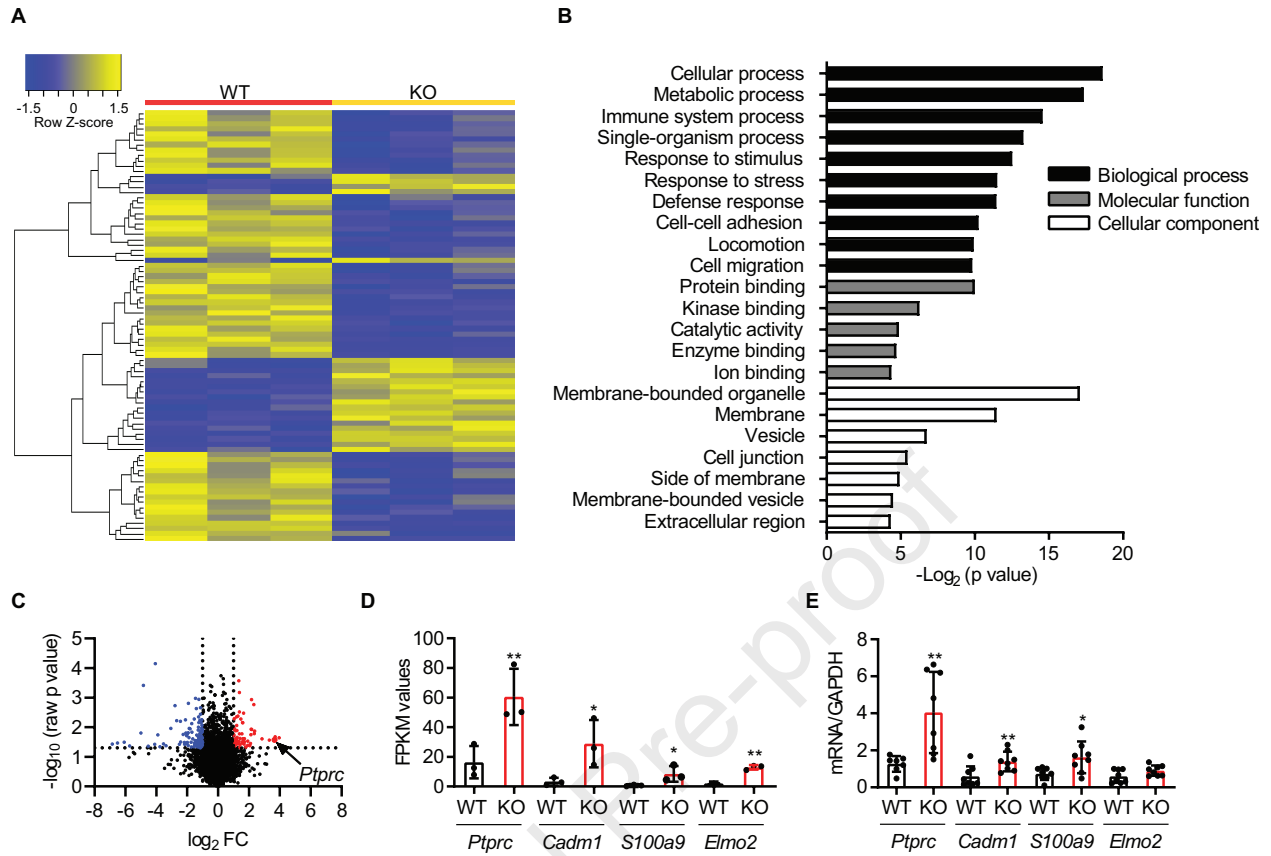


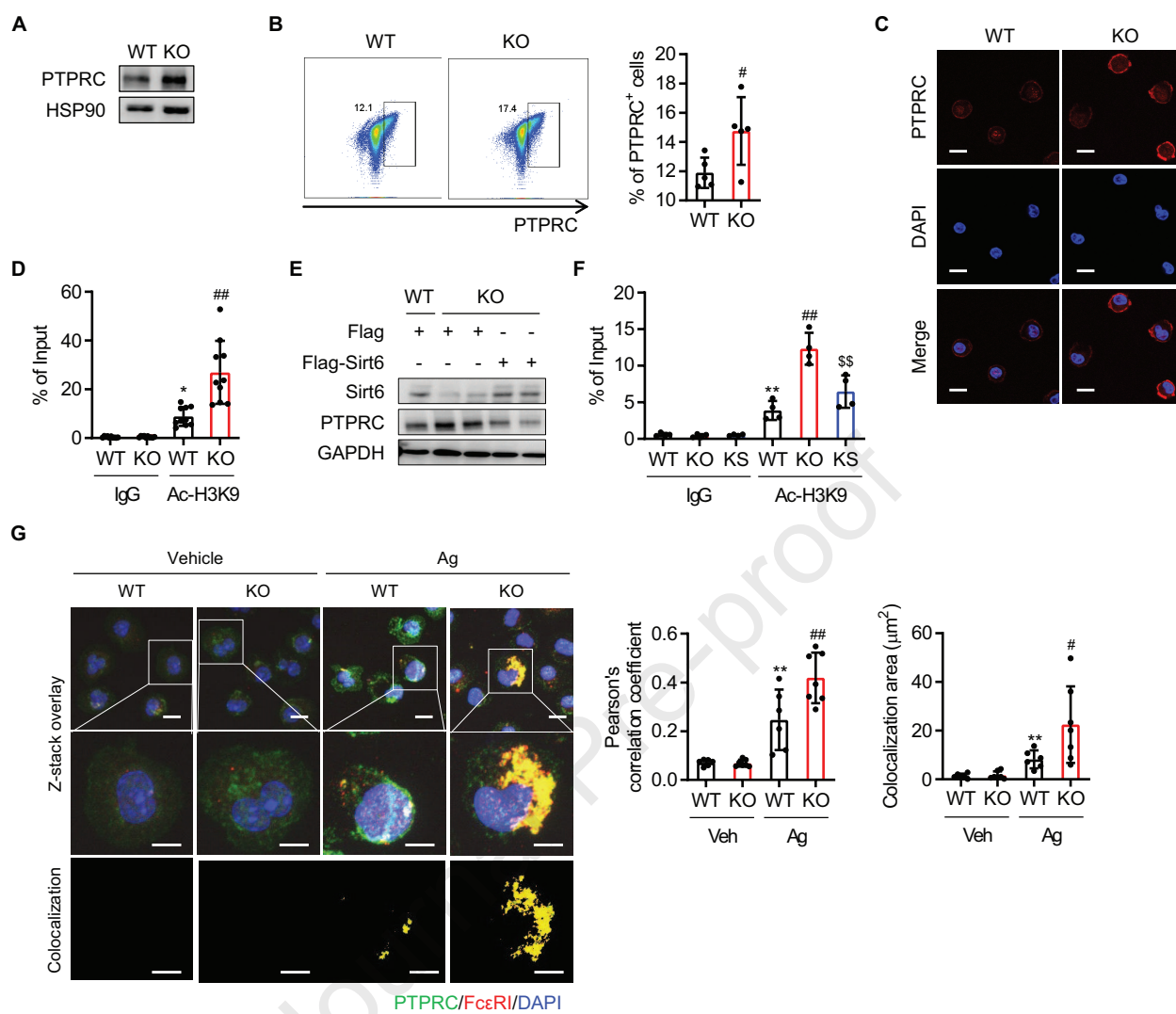
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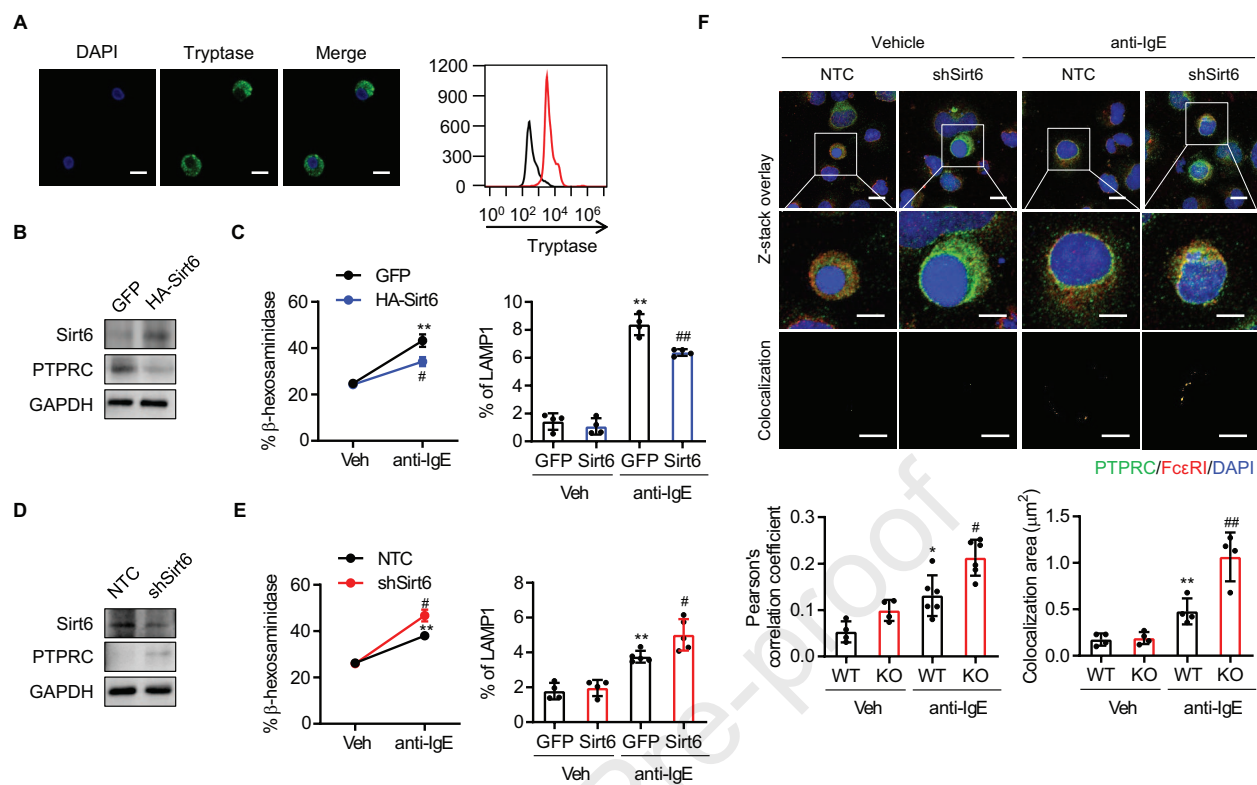


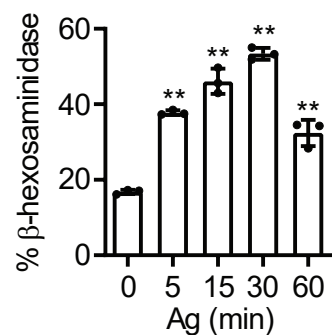
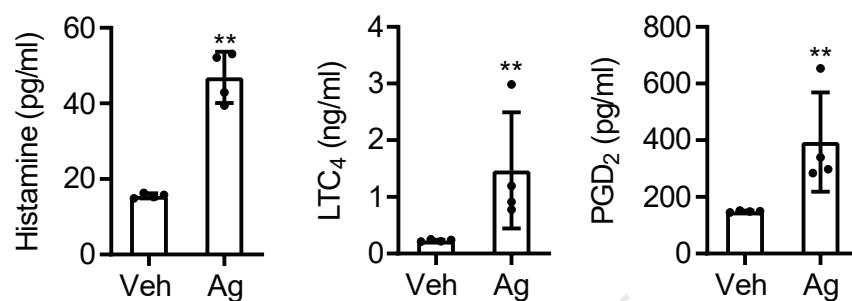
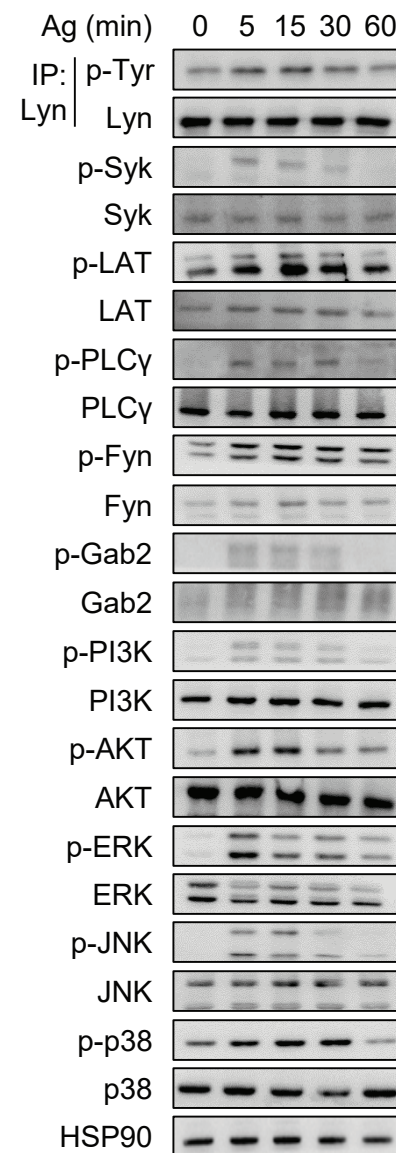
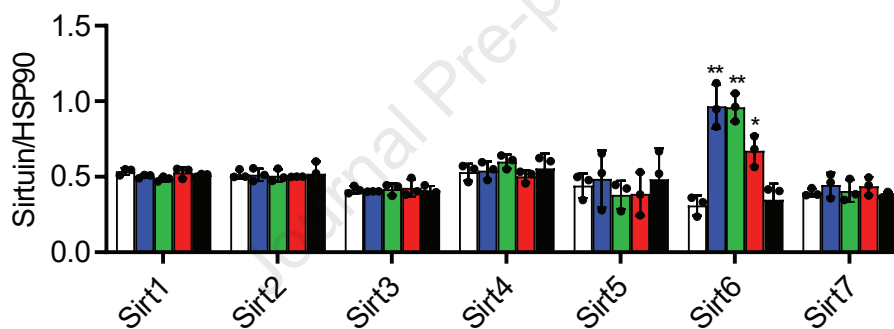
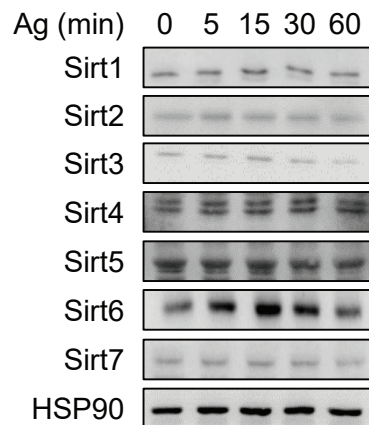
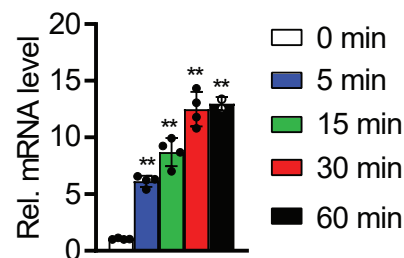


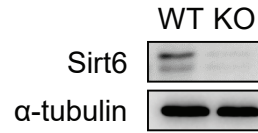
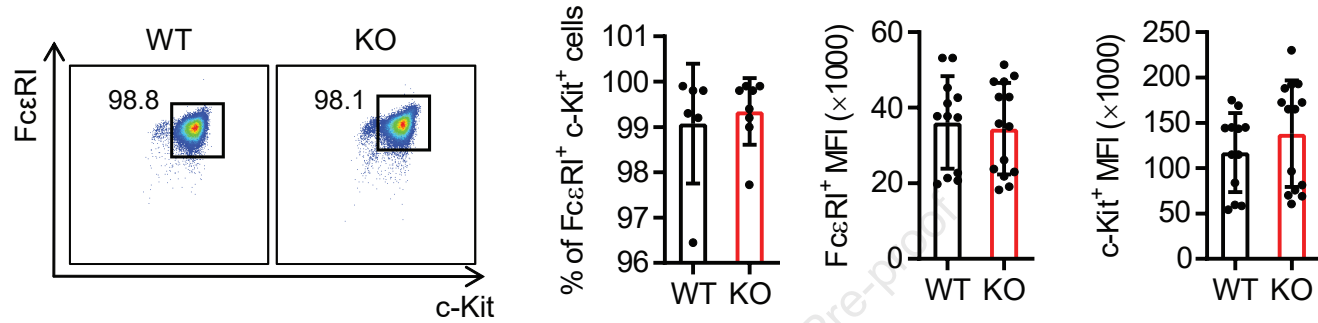
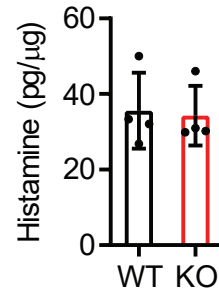
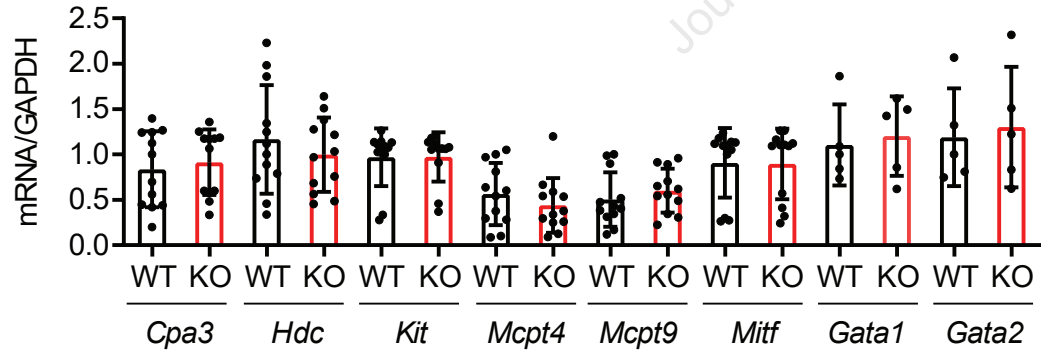


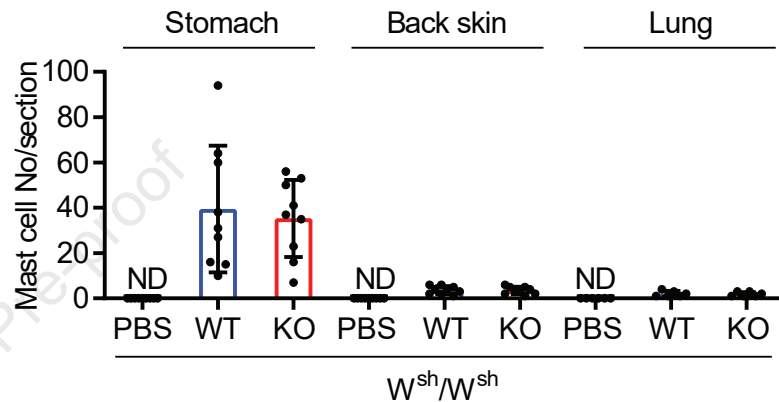
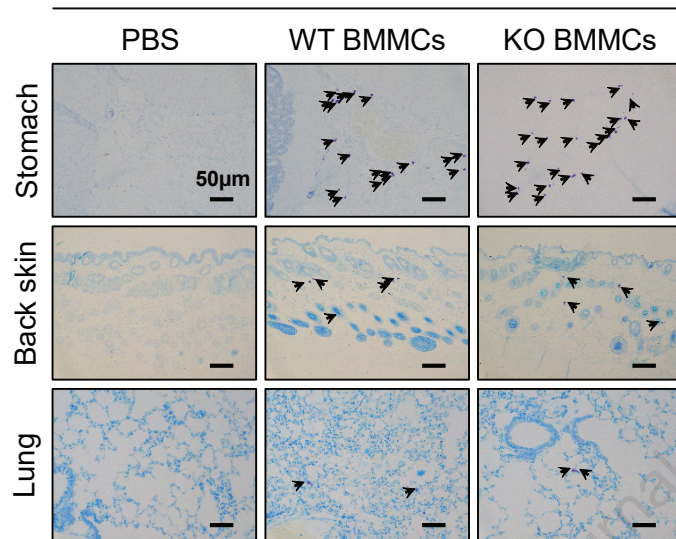
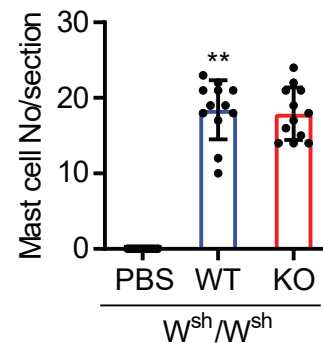
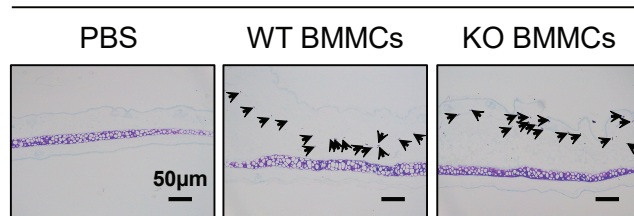


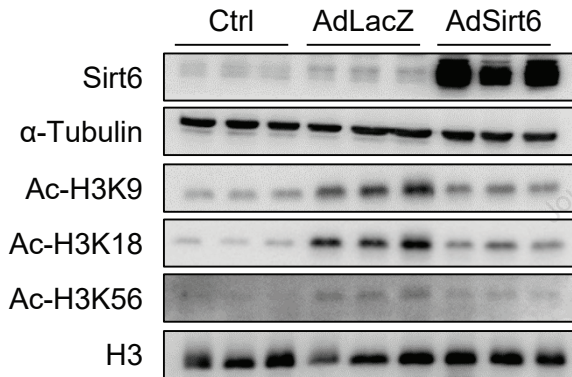
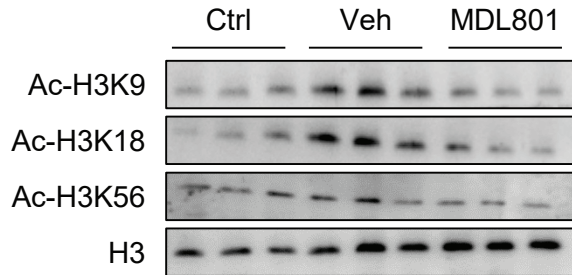




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Sirtuin 6 is a negative regulator of FcεRI signaling and anaphylactic responses

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1. Supplementary methods

Bone marrow–derived mast cells (BMMCs)

Bone marrow cells were obtained by flushing bone marrow from the femurs and tibias of mice. Cells were cultured in BMMC media (RPMI 1640 media contained 2 mM L-glutamine, 10% FBS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.05 mM 2-mercaptoethanol, 25 mM HEPES, 10 ng/ml IL-3, and 10 ng/ml stem cell factor (SCF, PeproTech, Cranbury, NJ, USA). After 4–6 weeks in culture, BMMCs were stained to confirm the surface expression of FITC–anti-mFcεRI (MAR-1; eBioscience, San Diego, CA, USA) and PE-anti-mCD117 (2B8; BD Biosciences, San Jose, CA, USA). Cells with purity > 95% were used for subsequent experiments.

Generation of human cord blood derived mast cells (hCBMCs)

Mononuclear cells were isolated by layering heparin-treated cord blood onto a Ficoll-Paque solution (GE healthcare, Chicago, IL, USA). CD34⁺ progenitor cells were isolated by magnetic cell sorting kit (CD34 Microbead kit; Miltenyi Biotech, Auburn, CA, USA). For the first week, CD34⁺ progenitor cells were cultured in Iscove modified Dulbecco medium supplemented with 1% insulin-transferrin-selenium, 0.1% β-mercaptoethanol, 50 ng/ml rhIL-3, 50 ng/ml rhIL-6, and 50 ng/ml rhSCF (all recombinant cytokines from PeproTech). After 6 weeks, the cells were cultured in 50 ng/ml rhIL-6 and 50 ng/ml rhSCF. hCBMCs cultured for at least 15 weeks were used for experiments, and cell purity was greater than 98%. hCBMCs were identified by immunofluorescence staining for tryptase (G3; Santa Cruz Biotechnology, Dallas, TX, USA) or flow cytometric analysis.

Degranulation assay

BMMCs were incubated overnight at 37°C with 1 µg/ml anti-DNP-IgE (SPE-7, Sigma-Aldrich, St. Louis, MO, USA) and then stimulated for 30 min at 37°C with 100 ng/ml DNP-HSA antigen in 100 µl Tyrode's buffer. Culture supernatants were collected and β-hexosaminidase activity was measured by the addition of p-nitrophenyl-N-acetyl-β-D-glucosaminide. For hCBMCs degranulation assay, cells were incubated overnight with 100 ng/ml human myeloma IgE (Millipore, Temecula, CA, USA) and stimulated for 30 min with 100 ng/ml mouse-anti-human IgE (Invitrogen, Carlsbad, CA, USA). Cells were then stained to confirm the surface expression of FITC-anti-hLAMP1 (H4A3) and PE-anti-hCD117 (104D2; Biolegend, San Diego, CA, USA) by flow cytometric analysis.

Enzyme linked immunosorbent assay (ELISA)

The levels of MCPT1, IL-6 (eBioscience), LTC₄, PGD₂ (Cayman, Ann Arbor MI, USA), and histamine (Beckman Coulter, Brea, CA, USA) in culture supernatants and mice serum were measured using commercial ELISA kits.

Calcium (Ca²⁺) measurement

BMMCs (1×10^6) were suspended in 1 ml culture medium and sensitized with 1 µg/ml anti-DNP-IgE overnight. Cells were washed twice with PBS and then loaded with 4 µM Fluo-4 AM (Molecular Probes, Eugene, OR, USA) for 30 min. Cells were then washed again and further incubated in PBS for 30 min at room temperature. DNP-HSA (100 ng/ml) was then used to induce calcium flux, which was measured using a confocal microscope (Nikon, Tokyo, Japan) to monitor the fluorescent emission.

Transient transfection of BMMCs.

Exogenous proteins were expressed by transfecting BMMCs with 1 µg of plasmid DNA using mouse cell line Kit V (Lonza, Basel, Switzerland). After transfection, cells were allowed to recover for 24 h in medium. Cells were then sensitized with anti-DNP-IgE overnight and stimulated with DNP-HSA.

Antibodies for western blots

Antibodies were against: Sirt1, Sirt5, LAT, Fyn, Ac-H3K18 (Abcam, Cambridge, UK), Sirt2, FcεRI, p-LAT (Santa Cruz Biotechnology), Sirt4, Sirt7 (Biovision, Milpitas, CA, USA), Sirt3, Sirt6, p-Syk (C87C1), Syk, p-Lyn, Lyn, p-Fyn, p-PLCγ, p-PI3K, PI3K, p-Gab2, Gab2, p-AKT, AKT, p-ERK, ERK, p-JNK, JNK, p-p38, p38, Ac-H3K9, H3 (Cell Signaling Technology, Beverly, MA, USA), GAPDH, α-Tubulin (Bioworld Technology, St. Louis Park, MN, USA), Ac-H3K56 (Active motif, Carlsbad, CA, USA), p-Tyr (4G10) (Millipore), and HSP90 (Enzo Life Sciences, Farmingdale, NY, USA).

RNA isolation and real-time quantitative RT-PCR (qPCR)

Total RNA was extracted from frozen tissue or cultured cells using an RNA Iso kit (TaKaRa, Tokyo, Japan). First-strand cDNA was generated using the random hexamer primer provided in the first-strand cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). Specific primers for each gene (Table E1) were designed using qPrimerDepot (<http://mouseprimerdepot.nci.nih.gov>). qPCR reactions were performed in a final volume of 10 µl containing 10 ng of reverse-transcribed total RNA, 200 nM of forward and reverse

primers, and PCR master mix. qPCR was performed in 384-well plates using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems).

Confocal microscopy

Double-color immunofluorescence analysis was performed to determine the colocalization in BMMCs. Cells were incubated overnight with a combination of anti-FcεRI (Santa Cruz Biochemicals) and anti-PTPRC (Abcam) at 4°C. After incubation with the corresponding fluorochrome-conjugated secondary antibodies, cells were mounted and visualized using a LSM880 confocal laser scanning microscope (Carl Zeiss).

Transmission electron microscopy

The BMMCs were fixed using the conventional method (2% glutaraldehyde and 2% formaldehyde in 0.05 M sodium cacodylate buffer, pH 7.2). Fixed samples were embedded in Spurr's resin (Sigma-Aldrich), and thin sections (80 nm) were cut and stained with uranyl acetate and lead citrate for observation under a transmission electron microscope (H7650, accelerating voltage 100 kV, Hitachi) at the Center for University-wide Research Facilities (Chonbuk National University, Jeonju, Korea).

RNA-Seq

Total RNA (1 µg) was used for mRNA-seq library preparation using the TruSeq RNA Sample Prep kit (Illumina, San Diego, CA, USA). Sequencing was performed on an Illumina HiSeq4000 with standard protocols. After quality filtering according to the Illumina pipeline, 101-bp paired-end reads were aligned to the reference genome sequence of *Mus musculus* (mm10) using HISAT2 version 2.1.0. After alignment, StringTie v2.1.3b was used to

assemble aligned reads into transcripts and to estimate their abundance. Expression abundances of transcripts and genes were calculated as FPKM value (Fragments Per Kilobase of exon per Million fragments mapped) per sample and used to analyze differentially expressed gene (DEG) profiles. Genes with one FPKM value over zero in the samples were excluded. To facilitate $\log_2$ transformation, 1 was added to each FPKM value of filtered genes. Filtered data were $\log_2$ -transformed and subjected to quantile normalization. Statistical significance of the differential expression data was determined using fold change. For DEG set, hierarchical clustering analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Gene-enrichment analysis for differentially expressed genes (DEGs) were also performed based on Gene Ontology (<http://geneontology.org/>) database.

Chromatin Immunoprecipitation (ChIP) assay

The BMMCs were cross-linked by incubating cells in 1% formaldehyde for 10 min at room temperature. Cross-linking was stopped by 5 min of incubation with 125 mM glycine. Cells were lysed with cytosolic lysis buffer. After centrifugation, DNA preparation was performed with Simple Chip Enzymatic Chromatin IP Kits (Cell Signaling Technology, Danvers, MA, USA). Each sample was immunoprecipitated with 2 μ g of anti-Ac-H3K9 or non-specific IgG (Cell Signaling Technology). The obtained DNA was analyzed by quantitative PCR (qPCR) with *Ptpcr* promoter primer (forward: 5'-GCAATCCCACCATCTCC- 3'; reverse: 5'- GACTCATGTGAAAGGGTTGTTC- 3').

Preparation of recombinant adenovirus

Adenoviruses expressing Sirt6 (AdSirt6) or β -galactosidase (AdLacZ) were prepared as described previously (Lee et al., 2013). Viruses (1×10^9 pfu) were intradermally administrated to mice before anaphylaxis induction.

Histology

Tissues were fixed with 10% formalin and embedded in paraffin. Fixed tissues were cut into 5 μ m sections and deparaffinized. Sections were stained with hematoxylin-eosin (H&E) or toluidine blue for light microscopic examinations or mast cell infiltration assays, respectively. Degranulated cells were analyzed using iSolution DT36 software (Carl Zeiss, Oberkochen, Germany).

2. Supplementary table

Table E1. Sequences and accession numbers for primers (forward, FOR; reverse, REV) used in real-time RT-PCR and PCR analyses

Gene	Sequences for primers	Accession No.
<i>Sirt6</i>	FOR: GGTACCCAGGGTGACAGACA	NM_181586
	REV: ACACCATTCTGGACTGGGAG	
<i>Cpa3</i>	FOR: ACCAAAACCTCCACCTGCATTGGCAC	NM_007753
	REV: GGTCCTGGTGGTTAGGAGGCAGTTT	
<i>Hdc</i>	FOR: CCCCATCTACCTCCGACATGCCAA	NM_177680
	REV: CCCGAAGGACCGAATCACAAACCA	
<i>Kit</i>	FOR: CGATGTGGGCAAGAGTTCCGCCTT	NM_001122733
	REV: ACAGAGTGTGGGCCTGGATTTGCT	
<i>Mcpt4</i>	FOR: CTTCTCTTGCCTTCTGGGGCTGGA	NM_010779
	REV: GGCCATGTAAAGGGCGAGAATGTGGT	
<i>Mcpt9</i>	FOR: GGGCAGTTCCACAAAGGTGGCATCA	NM_010782
	REV: GGGCTTTGCATTTCCGCGTCCA	
<i>Mitf</i>	FOR: GGGGAGGAGTTTCACGAAGAACCCA	NM_001113198
	REV: GCTGGCGTAGCAAGATGCGTGA	
<i>Gata1</i>	FOR: CAGAACCGGCCTCTCATCC	NM_008089
	REV: TAGTGCATTGGGTGCCTGC	
<i>Gata2</i>	FOR: GCAGAGAAGCAAGGCTCGC	NM_008090
	REV: CAGTTGACACACTCCCGGC	
<i>Ptprc</i>	FOR: GAACATGCTGCCAATGGTTCT	NM_001111316
	REV: TGTCCCACATGACTCCTTTCC	
<i>Cadm1</i>	FOR: TGCCTGTTGGGGTTCAGTAG	NM_207675
	REV: CACAGGTGATGATGGGCAGAATC	
<i>S100a9</i>	FOR: AGCCTTGAAGAGCAAGAAGAT	NM_009114
	REV: TCAGGGTGTCTTCCTTCCT	
<i>Elmo2</i>	FOR: GATACTTCCCCTTGCCCTCAG	NM_080287
	REV: GCTTCCTGAGACCTACAATGG	
<i>Gapdh</i>	FOR: TTGATGGCAACAATCTCCAC	NM_008085
	REV: CGTCCCGTAGACAAAATGGT	

3. Supplementary Figure Legends

Figure E1. Sirtuin expression in Ag-stimulated mast cells. BMMCs (1×10^6) from C57BL/6 mice were sensitized with 1 μ g anti-DNP-IgE overnight and stimulated with 100 ng DNP-HSA as indicated. **(A, B)** Degranulation was assessed by measurement of β -hexosaminidase, histamine, LTC₄ and PGD₂. Individual values are shown as circles. **(C)** Fc ϵ RI-mediated proximal signaling was analyzed by Western blotting. **(D)** Protein levels of sirtuin family members were analyzed by Western blotting and their protein density was quantified. **(E)** mRNA levels of *Sirt6* were analyzed by qPCR. Values are mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$ vs. Veh or 0 min. Veh, vehicle

Figure E2. Sirt6 deficiency does not affect BMMC differentiation. Bone marrow cells from WT and KO mice were cultured for 4 weeks in the presence of SCF and IL-3 as described in the Materials and Methods section. **(A)** Expression of FcεRI and c-Kit receptor in bone marrow-derived mast cells (BMMCs) was analyzed by flow cytometry. Individual values are shown as circles. **(B, C)** Protein levels of Sirt6 and mRNA levels of mast cell differentiation related genes were analyzed by Western blotting and qPCR, respectively. **(D)** Histamine content in BMMCs was assessed by EIA. Values are mean ± SD. MFI, mean fluorescence intensity

Figure E3. Adoptive transfer of BMMCs into *Kit^{w-sh/w-sh}* mice. (A) All experimental procedures for PSA were the same as those described in Figure 4's legend. Tissue mast cells in the stomach, back skin and lung from the indicated chimeric mice were assessed by toluidine blue staining. Total number of mast cells in tissue sections were counted. Arrowheads indicate individual mast cells. Individual values are shown as circles. (B) All experimental procedures for PCA were the same as those described in Figure 4's legend. Toluidine blue staining and quantification of mast cells per section of the ears. Arrowheads indicate individual mast cells.

Figure E4. Genetic overexpression and pharmacological activation of Sirt6. (A) C57BL/6 mice were intradermally injected with 1×10^9 pfu of AdLacZ or AdSirt6. Three days later, protein levels of Ac-H3K9, Ac-H3K18, Ac-H3K56, and total histone H3 were determined by Western blotting in ear. (B) After treatment of C57BL/6 mice with 50 mg/kg MDL801, protein levels of Ac-H3K9, Ac-H3K18, Ac-H3K56, and total histone H3 were determined by Western blotting in ear.

4. Supplementary reference

Lee HS, Ka SO, Lee SM, Lee SI, Park JW, Park BH Overexpression of sirtuin 6 suppresses inflammatory responses and bone destruction in mice with collagen-induced arthritis. *Arthritis Rheumatol* 2013; 65:1776-85.