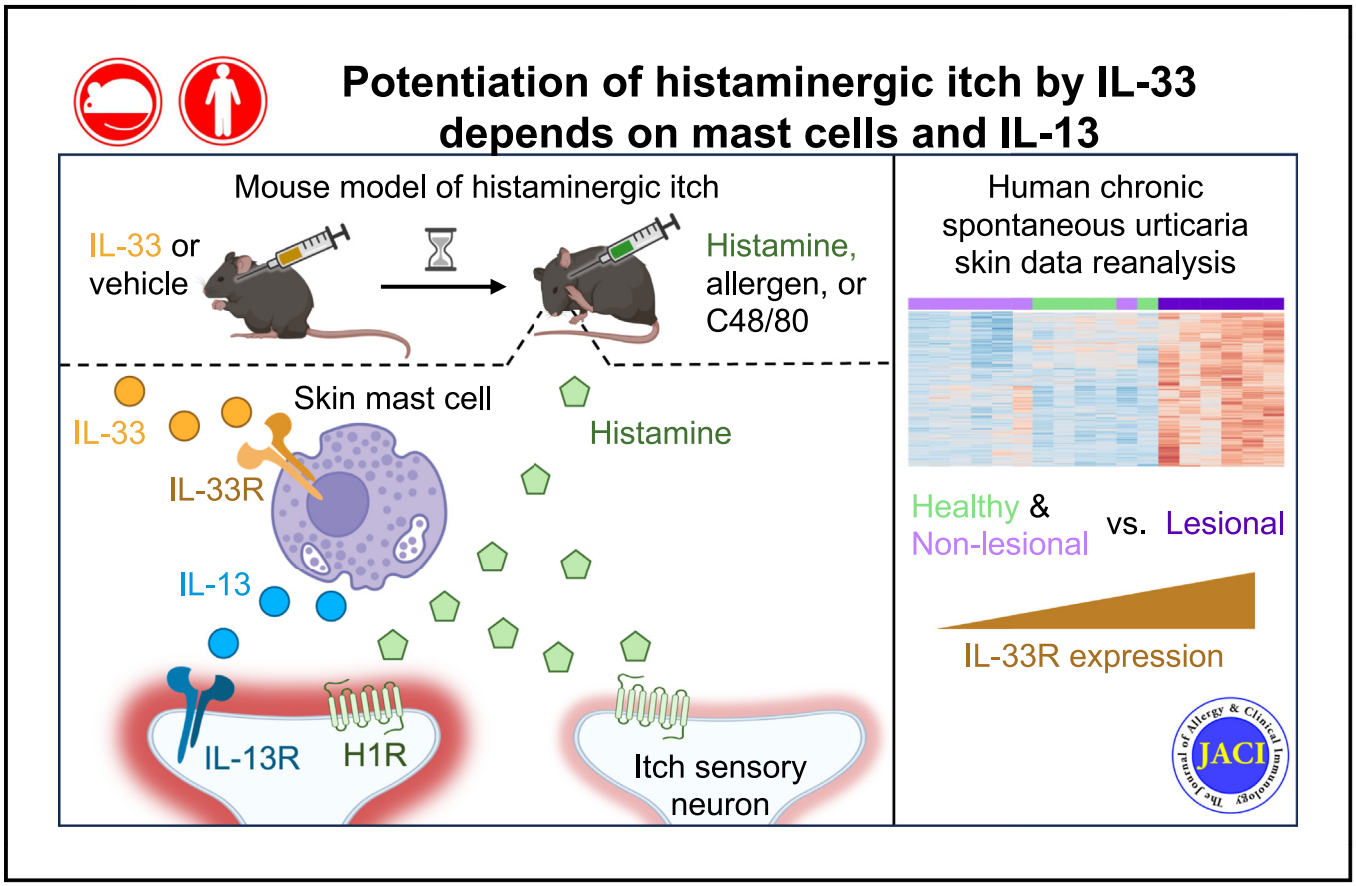
# IL-33 potentiates histaminergic itch

**Anna M. Trier, BA, Aaron M. Ver Heul, MD, PhD, Avery Fredman, BS, Victoria Le, Zhen Wang, MD, PhD, Kelsey Auyeung, BS, et al**

## GRAPHICAL ABSTRACT




**Capsule summary:** IL-33 stimulates a mast cell– and IL-13–dependent axis to exacerbate histaminergic itch. These findings provide insight into mast cell–associated itch conditions such as chronic spontaneous urticaria.

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# IL-33 potentiates histaminergic itch

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**Background: Itch is a common symptom that can greatly diminish quality of life. Histamine is a potent endogenous pruritogen, and while antihistamines are often the first-line treatment for itch, in conditions like chronic spontaneous urticaria (CSU), many patients remain symptomatic while receiving maximal doses. Mechanisms that drive resistance to antihistamines are poorly defined.**

**Objectives: Signaling of the alarmin cytokine IL-33 in sensory neurons is postulated to drive chronic itch by inducing neuronal sensitization to pruritogens. Thus, we sought to determine if IL-33 can augment histamine-induced (histaminergic) itch.**

**Methods: Itch behavior was assessed in response to histamine after IL-33 or saline administration. Various stimuli and conditional and global knockout mice were utilized to dissect cellular mechanisms. Multiple existing transcriptomic data sets were evaluated, including single-cell RNA sequencing of human and mouse skin, microarrays of isolated mouse mast cells at steady state and after stimulation with IL-33, and microarrays of skin biopsy samples from subjects with CSU and healthy controls.**

**Results: IL-33 amplifies histaminergic itch independent of IL-33 signaling in sensory neurons. Mast cells are the top expressors of the IL-33 receptor in both human and mouse skin. When stimulated by IL-33, mouse mast cells significantly increase IL-13 levels. Enhancement of histaminergic itch by IL-33 relies on a mast cell– and IL-13–dependent mechanism. IL-33 receptor expression is increased in lesional skin of subjects with CSU compared to healthy controls.**

**Conclusions: Our findings suggest that IL-33 signaling may be a key driver of histaminergic itch in mast cell–associated pruritic conditions such as CSU. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)**

***Key words:*** *Chronic spontaneous urticaria, histamine, IL-13, IL-33, itch, mast cell, neuroimmunology*

## INTRODUCTION

Histamine was one of the first molecules identified to act as a pruritogen. Antihistamines are often a first line therapy for pruritic conditions, yet many patients have minimal response or develop refractory symptoms over time.[1,2] Mechanisms underlying the limited efficacy of antihistamines in pruritic disorders remain poorly understood.

The alarmin cytokine IL-33 is elevated in chronic pruritic conditions such as atopic dermatitis, chronic spontaneous urticaria (CSU), and dry skin itch.[3-5] Although the role of IL-33 in itch is only beginning to be elucidated, recent evidence suggests that IL-33 may drive chronic itch development by inducing peripheral itch sensitization. Indeed, we and others have found that signaling of the IL-33 receptor (IL-33R) in sensory neurons is required for chronic itch in certain mouse models.[4,6] We hypothesized that IL-33 may augment histaminergic itch through neuromodulatory mechanisms to limit effectiveness of antihistamines in chronic pruritic conditions. Instead, we found IL-33 can promote itch through a novel pathway involving mast cells and IL-13.

## RESULTS AND DISCUSSION

We and others have shown that IL-33 does not act like a classic pruritogen as intradermal injection of IL-33 does not induce an immediate acute itch response.[4,6] Thus, we hypothesized that IL-33 may instead promote itch by enhancing neuronal responsiveness to pruritogens like histamine, similar to IL-4 and oncostatin M (OSM).[7,8] However, in contrast to IL-4 and OSM, we observed no difference in acute scratching (itch behavioral response) between wild-type (WT) mice that received intradermal coinjection of IL-33 and histamine or histamine alone (Fig 1, *A*). Instead, IL-33 significantly increased histamine-provoked itch behavior if mice were pretreated with IL-33 6 hours before histamine injection (Fig 1, *B*). This effect was not due to IL-33 directly provoking itch, since we observed no difference in scratching 6 hours after mice were intradermally injected with IL-33 or saline control (in the absence of subsequent histamine challenge) (Fig 1, *C*). We next examined whether potentiation of histaminergic itch by IL-33 was mediated by neuronal IL-33R signaling, as we initially hypothesized. We utilized a

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The first 2 authors contributed equally to this article, and both should be considered first author.

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*Abbreviations used*
C48/80: Compound 48/80
CSU: Chronic spontaneous urticaria
His: Histamine
IL-33R: IL-33 receptor
ImmGen: Immunologic Genome Project
LM: Littermate
OSM: Oncostatin M
OVA: Ovalbumin
scRNA-Seq: Single-cell RNA sequencing
WT: Wild type

mouse strain in which IL-33R was conditionally deleted from sensory neurons (IL-33R$^{\Delta neuron}$).[4] In contrast to our hypothesis, we found no difference in itch behavior between IL-33R$^{\Delta neuron}$ and littermate (LM) control mice in the IL-33 pretreatment and histamine challenge (IL-33 + His) model (Fig 1, *D*). These findings suggest that IL-33 magnifies histaminergic itch via an intermediary cell type rather than through direct effects on sensory neurons, in contrast to mechanisms identified for other itch-promoting cytokines like IL-4 or OSM.[7,8]

As an alarmin, a canonical function of IL-33 is its ability to rapidly activate innate immune cells to mobilize a type 2 immune response.[9] However, whether this axis plays a role in itch remains unknown. Thus, to test if IL-33R signaling in immune cells is required for its ability to amplify histaminergic itch, we utilized mice that conditionally lack IL-33R expression in immune cells (IL-33R$^{\Delta immune}$).[4] Strikingly, scratching in the IL-33 + His model was significantly decreased in IL-33R$^{\Delta immune}$ mice compared to LM control mice (Fig 1, *E*).

To determine which immune cells may be responding to IL-33, we utilized the Immunological Genome Project (ImmGen, www.immgen.org) to analyze the expression level of *Il1rl1* (IL-33R) across different immune cell populations from a variety of tissues (for more information, see the Methods section in this article's Online Repository available at www.jacionline.org). In support of prior studies,[9] several mast cell populations were among the cell types with the highest expression levels of IL-33R (see Fig E1 in the Online Repository). To evaluate IL-33R expression specifically within skin tissue, we reanalyzed a previously published single-cell RNA sequencing (scRNA-Seq) data set of mouse skin containing 17 different mouse cell types (Fig 2, *A*).[10] Consistent with the ImmGen data, mast cells (cluster 12) had the highest expression of *Il1rl1* across the 17 identified cell clusters (Fig 2, *B*). However, despite the high expression of IL-33R on mast cells, how IL-33 affects mast cell function remains a highly active area of investigation.

To test the potential role of mast cells in IL-33–enhanced histaminergic itch, we first used 2 mast cell–specific reagents known to induce histaminergic itch through orthogonal pathways. A classic model of mast cell activation involves the passive transfer of anti-ovalbumin (OVA) IgE by intravenous injection, followed by intradermal OVA challenge the next day. We have previously shown that this kind of antigen-mediated itch is highly histaminergic in nature.[11,12] Alternatively, compound 48/80 (C48/80) induces degranulation by activating the mast cell–specific G protein–coupled receptor Mrgprb2.[13] When we intradermally administered IL-33 or saline control in WT OVA-sensitized mice 6 hours before intradermal OVA challenge, we observed that mice treated with IL-33 had enhanced itch behavior compared to those that received saline control (Fig 2, *C*). Similarly, we found that mice pretreated with IL-33 had enhanced itch behavior compared to those that received saline control when challenged 6 hours later with intradermal C48/80 (Fig 2, *D*). Next, we utilized *Sash*$^{-/-}$ mice, which developmentally lack mast cells as their primary phenotype.[14] In our IL-33 + His model, *Sash*$^{-/-}$ mice scratched significantly less compared to LM control mice (Fig 2, *E*). Taken together, our findings demonstrate a central role of mast cells in mediating IL-33–enhanced histaminergic itch.

We next sought to investigate potential effector molecules that may promote IL-33 enhancement of histaminergic itch. Prior studies, largely *in vitro,* have shown that IL-33 can modulate mast cell mediator release in response to activating stimuli.[15-17] To examine whether IL-33 stimulation modulates expression of pruritogenic pathways, we reanalyzed 2 previously published microarrays of mast cells to determine differentially expressed genes after acute or chronic treatment with IL-33 (Fig 3, *A* and *B*).[18,19] We found distinct, but overlapping, transcriptional profiles of upregulated genes depending on duration of IL-33 treatment (Fig 3, *C*). Interestingly, only acute IL-33 stimulation induced expression of its own receptor, *Il1rl1* (Fig 3, *D*), while chronic stimulation led to increased expression of the histamine receptor *Hrh1* (Fig 3, *E*), suggesting mast cell responses to IL-33 are dynamic. Among 320 genes upregulated by both acute and chronic IL-33 stimulation was tryptase (*Tpsab1*) (Fig 3, *F*), which can potentially cleave IL-33 into more potent forms to further drive activation of this circuit.[20] Critically, of known pruritogenic cytokines, only IL-13 (*Il13*) was significantly upregulated by both acute and chronic IL-33 treatment (Fig 3, *A, B,* and *F*), in agreement with prior studies.[15-17] Indeed, IL-13 can directly activate sensory neurons and induce neuronal sensitization to various pruritogens.[7,21] Thus, we hypothesized that IL-13 is a key mediator of IL-33–enhanced histaminergic itch. To test this, we utilized IL-13 knockout mice and observed significantly less scratching in *Il13*$^{-/-}$ mice compared to WT mice in the IL-33 + His model (Fig 3, *G*). While additional studies are required to further dissect this axis, and other immune cells and/or pruritogenic cytokines like IL-31 may also be involved, our findings indicate that mast cells and IL-13 are critical mediators of IL-33–enhanced histaminergic itch.

Increasing evidence suggests that IL-33 can potentiate mast cell–mediated inflammation.[15-17] Yet the significance of this axis in itch has been largely unexplored. Our findings suggest that IL-33 augments histaminergic itch through mast cell activation and IL-13 production. While IL-33 signaling on sensory neurons modulates nonhistaminergic dry skin itch,[4] we found it was dispensable in our model of histaminergic itch. This discrepancy may arise from an indirect immune axis overriding the direct neuronal axis or distinct downstream effects of IL-33R activation on modulating activity of pruritogen receptors.

Our observations have important implications for clinical use of antihistamines that warrant further investigation. For example, mast cells are recognized as critical pathogenic mediators of CSU, a disorder characterized by the development of itchy hives that last longer than 6 weeks.[22] The only treatments approved by the US Food and Drug Administration available for CSU are high-dose antihistamines (first line) and the anti-IgE monoclonal antibody omalizumab (second line).[22] Yet despite the central role

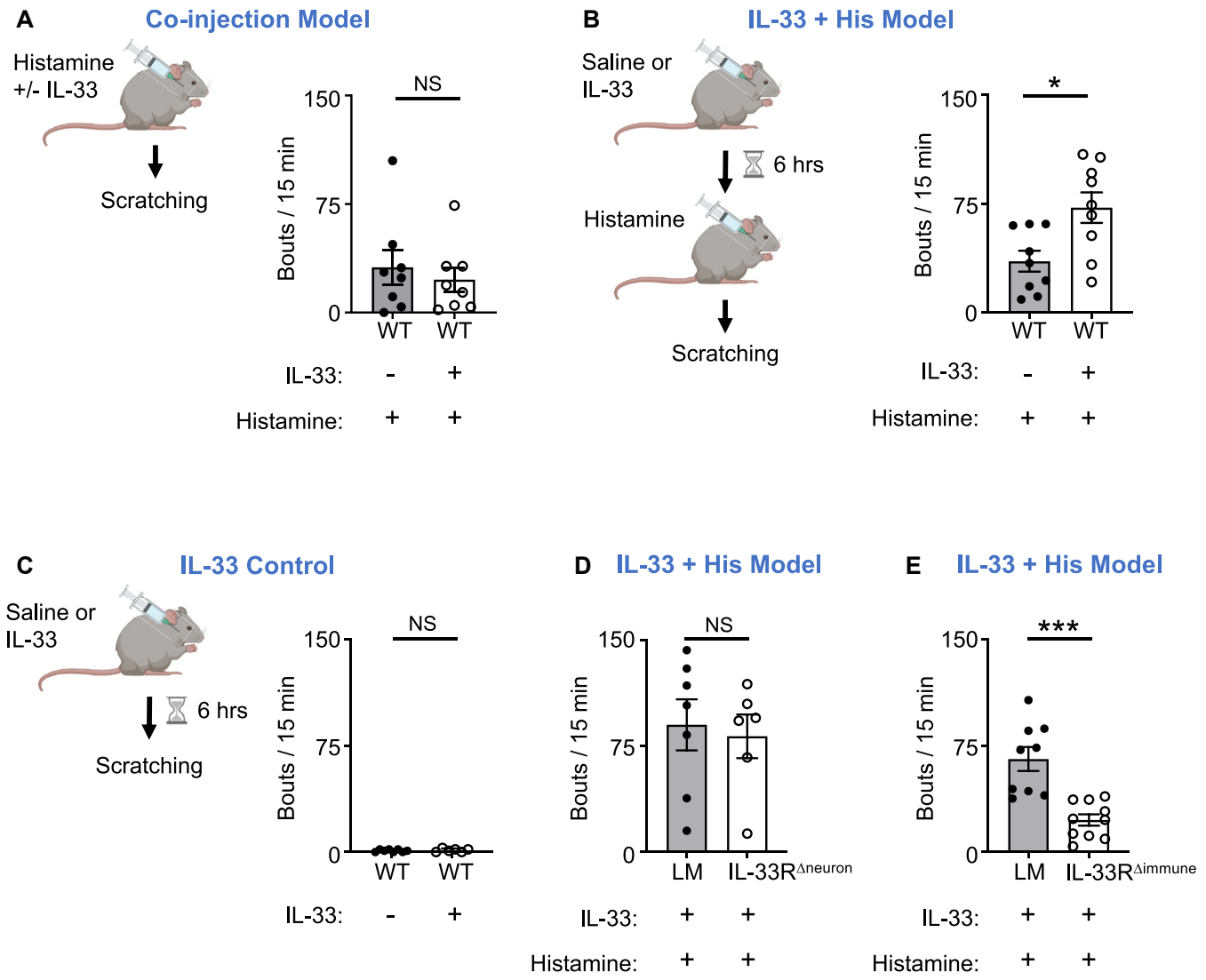


**FIG 1.** IL-33 enhances histaminergic itch. **(A)** Number of scratching bouts after intradermal (i.d.) coinjection of histamine and IL-33 (20 μg histamine + 300 ng IL-33 in 20 μL) or histamine alone (20 μg in 20 μL) in WT mice. **(B)** Number of scratching bouts after i.d. injection of IL-33 (300 ng in 20 μL) or saline control and i.d. injection of histamine (20 μg in 20 μL) 6 hours later in WT mice. **(C)** Number of scratching bouts 6 hours after i.d. injection of IL-33 (300 ng in 20 μL) or saline control. Number of scratching bouts after IL-33 pretreatment and subsequent histamine challenge in **(D)** IL-33R$^{\Delta neuron}$ ($Na_V1.8^{Cre}$ × IL-33$^{fl/fl}$) and LM mice or **(E)** IL-33R$^{\Delta immune}$ ($Vav^{Cre}$ × IL-33$^{fl/fl}$) and LM mice. *(A-E)* Six to 10 mice per group with 2 combined independent experiments per figure. *NS,* Not significant, *$P < .05$, ***$P < .001$ by unpaired 2-tailed *t* test. Data are represented as means ± SDs.

mast cell–derived histamine plays in the pathogenesis of urticaria, up to 50% of CSU patients continue to have symptoms even with maximal on-label therapy.[1,22]

Given our observations suggesting that IL-33 can expand the pruritogenic capacity of histamine in mice, we wondered if similar mechanisms may contribute to CSU pathogenesis in humans. To test this, we first analyzed a published scRNA-Seq data set of healthy human skin consisting of 40 previously identified cellular subtypes (Fig 4, *A*).[23] Consistent with our findings in mice, mast cells (cluster 19, identified by high expression of *CPA3* and *CMA1*) most highly expressed *IL1RL1* (IL-33R) in human skin (Fig 4, *B*). Next, we examined differentially expressed genes in 2 published microarray data sets of skin biopsy samples from healthy controls and subjects with CSU.[24,25] Notwithstanding the limitations of bulk transcriptional analysis to assign differences in gene expression to specific cell types, we found *IL1RL1* and *HRH1* (histamine receptor H1) were significantly upregulated in lesional CSU skin compared to healthy controls in both data sets (Fig 4, *C* and *D*).

These findings suggest skin in CSU is primed to respond to IL-33 and histamine, and provide a mechanism by which IL-33–enhanced histaminergic itch may contribute to symptoms in CSU. Furthermore, the ability of IL-33 to expand the pruritogenic capacity of histamine may explain the poor responses to

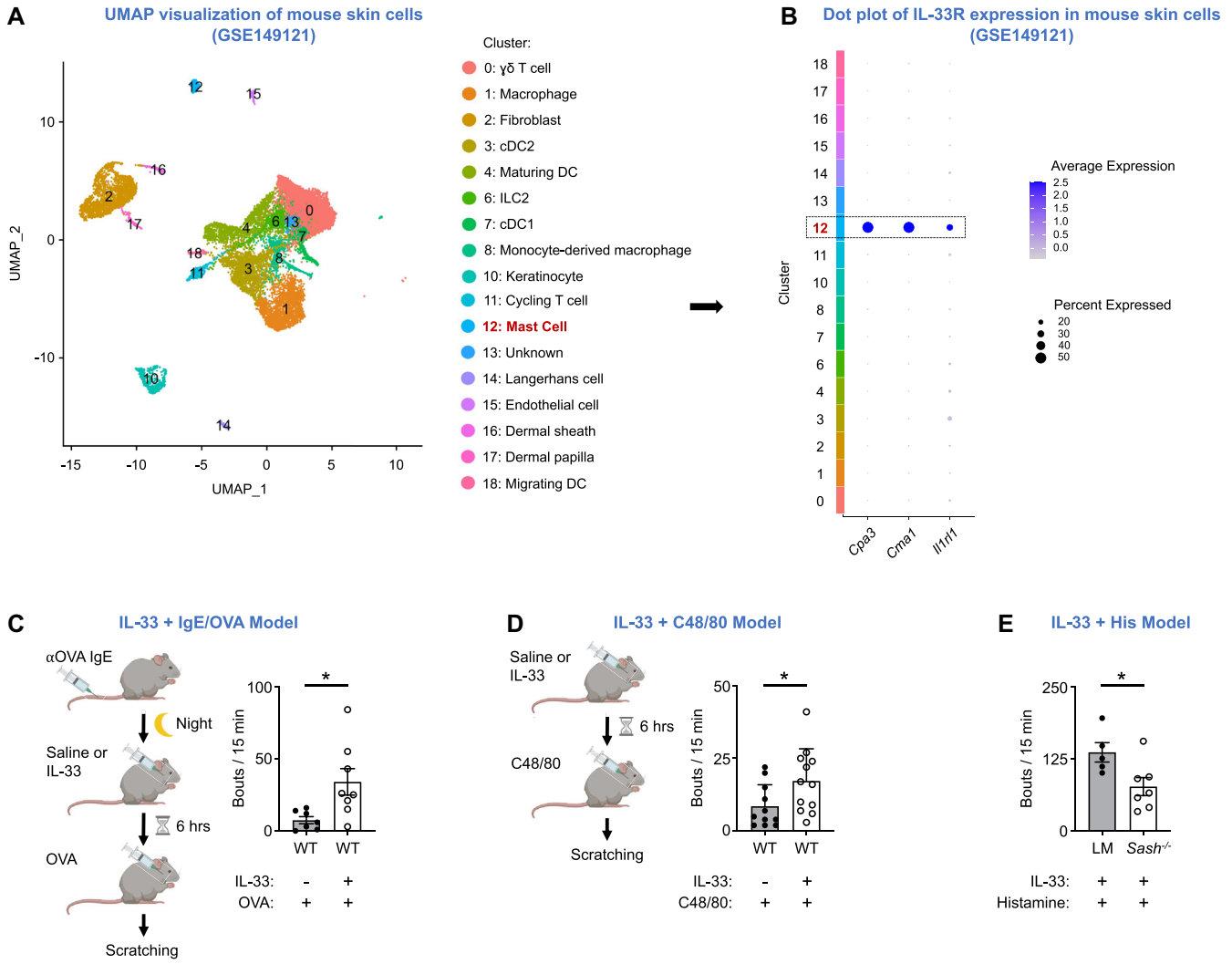


**FIG 2.** Mast cells are top expressors of IL-33R and mediate IL-33–enhanced histaminergic itch. **(A)** UMAP visualization shows 17 cell clusters in mouse skin (GSE149121, n = 3, clusters 5 and 9 omitted). **(B)** Dot plot of *Cpa3, Cma1,* and *Il1rl1* gene expression across clusters in *(A).* **(C)** Number of scratching bouts after IL-33 or saline control pretreatment and subsequent OVA challenge (intradermal [i.d.], 50 μg) in WT mice. Mice were sensitized 1 day before with anti-OVA IgE (αOVA IgE; 2 μg in 100 μL provided intravenously [i.v.]). **(D)** Number of scratching bouts after saline vehicle control or IL-33 pretreatment and subsequent challenge with C48/80 in WT mice. **(E)** Number of scratching bouts after IL-33 pretreatment and subsequent histamine challenge in *Sash*$^{-/-}$ and LM mice. *(C-E)* Five to 10 mice per group with 2 combined independent experiments per figure. *$P < .05$ by unpaired 2-tailed *t* test. Data are represented as means ± SDs.

antihistamines not only in CSU but also in other pruritic conditions traditionally considered nonhistaminergic, such as atopic dermatitis, drug rash, and allergic contact dermatitis.

## DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: B. S. Kim is founder of Klirna Biotech; he has served as a consultant for 23andMe, ABRAX Japan, AbbVie, Almirall, Amagma Therapeutics, Amgen, Arcutis Biotherapeutics, Arena Pharmaceuticals, argenx, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Cara Therapeutics, Clexio Biosciences, Eli Lilly and Company, Escient Pharmaceuticals, Evommune, Galderma, Genentech,

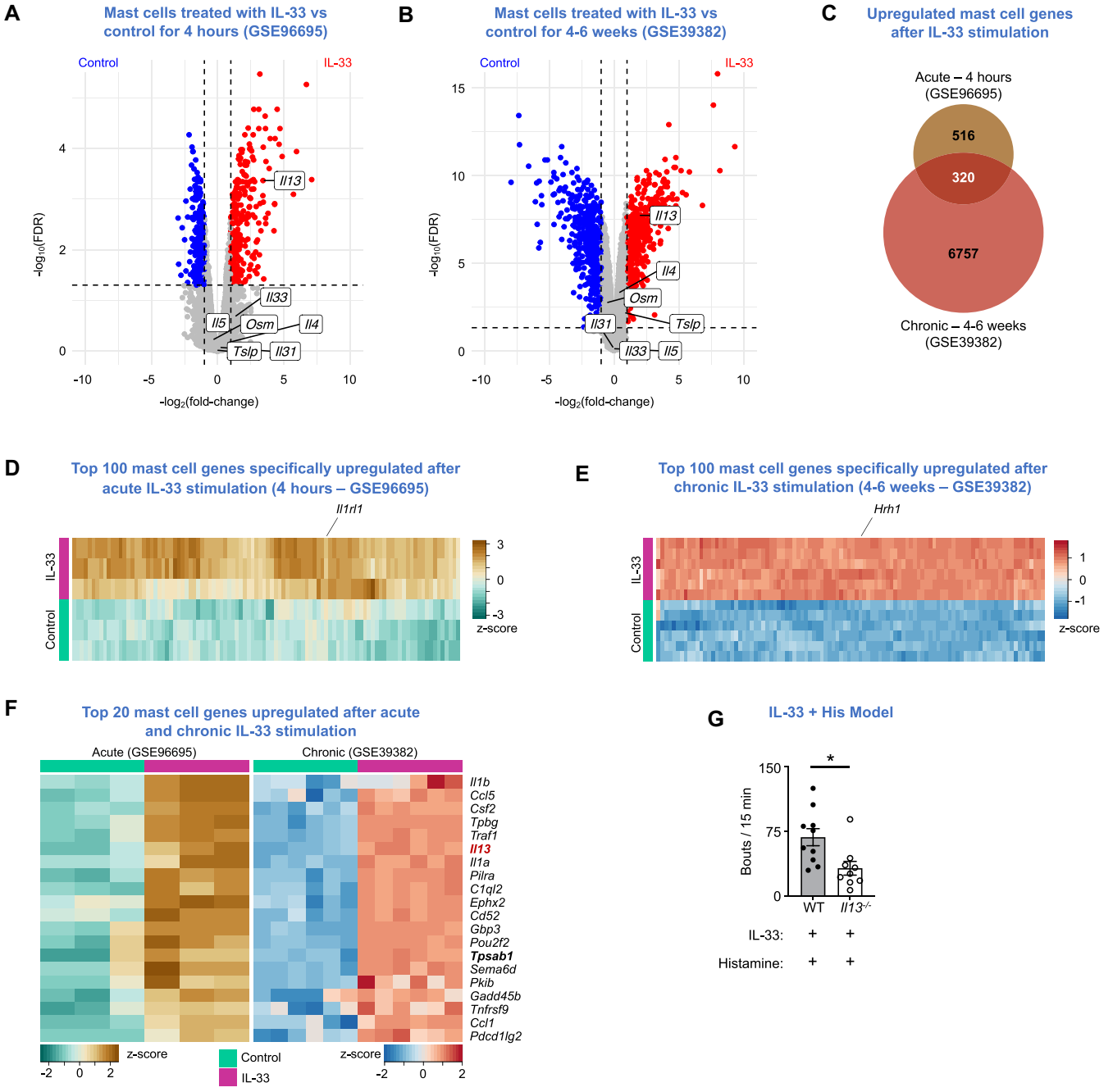


**FIG 3.** IL-13 is induced by IL-33 in mast cells and mediates IL-33–enhanced histaminergic itch. Volcano plots of differential gene expression from microarrays of bone marrow-derived mast cells (BMMCs) after **(A)** acute—4 hours (GSE96695) and **(B)** chronic—4-6 weeks (GSE39382) treatment with IL-33 versus control. Key pruritogenic cytokines are indicated. Bonferroni adjusted *P* value (false discovery rate [FDR]) cutoff 0.05, $\log_2$-fold change cutoffs <−1 and >1. **(C)** Overlap of significantly upregulated (FDR < 0.05) genes between treatment and control for acute (4 hours) and chronic (4-6 weeks) IL-33 treatment of BMMCs. Gene expression heat maps of top 100 genes upregulated compared to control only in BMMCs treated with IL-33 for **(D)** 4 hours or **(E)** 4-6 weeks. **(F)** Gene expression heat maps of top 20 genes upregulated by both acute and chronic IL-33 treatment of BMMCs. **(G)** Number of scratching bouts after IL-33 or saline control pretreatment and subsequent histamine challenge in *Il13*$^{-/-}$ and WT mice. *(G)* Five to 10 mice per group with 2 combined independent experiments per figure. **P* < .05 by unpaired 2-tailed *t* test. Data are represented as means ± SDs.

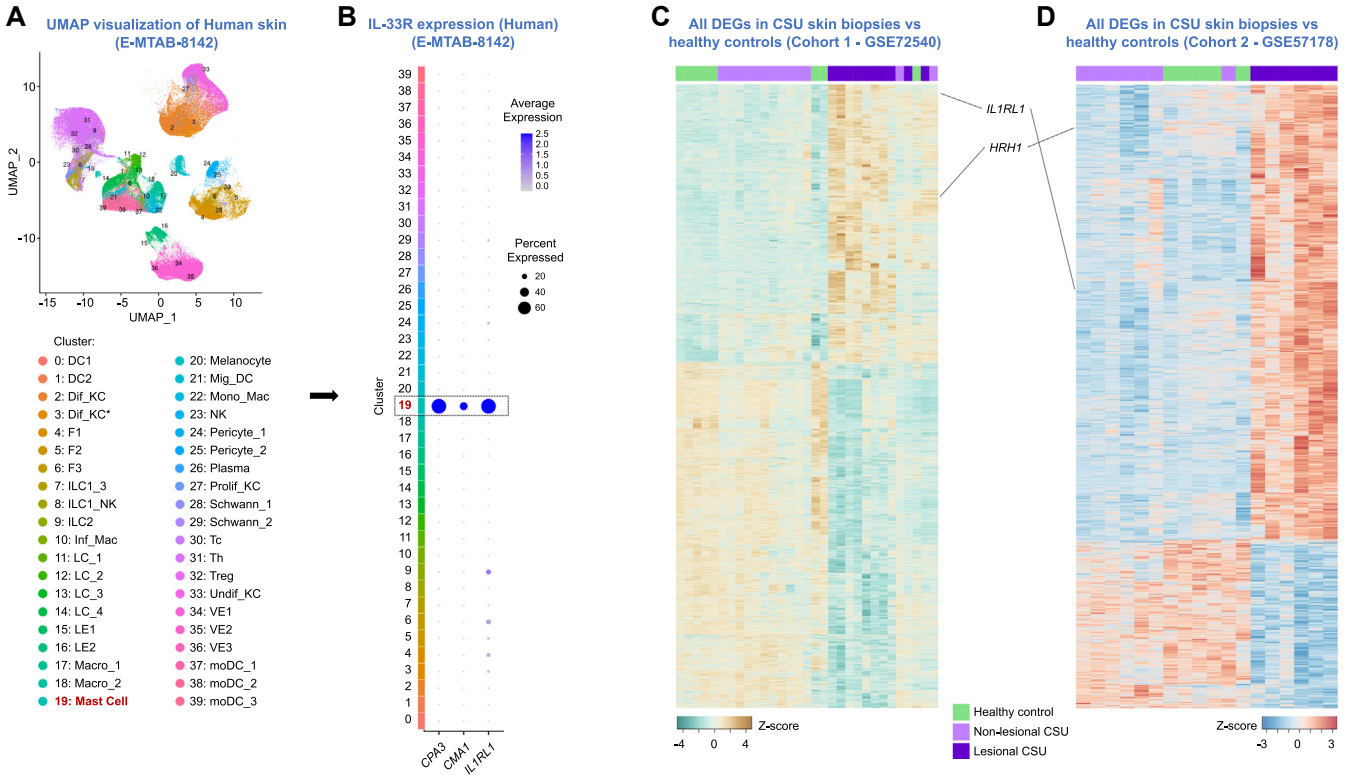


**FIG 4.** IL-33R is highly expressed in human skin mast cells and increased in skin from subjects with CSU. **(A)** UMAP visualization shows 40 cell clusters in human skin (E-MTAB-8142, n = 5). **(B)** Dot plot of *CPA3, CMA1,* and *IL1RL1* gene expression across clusters. Gene expression heat maps of all differentially expressed genes (Bonferroni-adjusted *P* value cutoff <.05) in microarrays of skin biopsy samples from lesional and nonlesional skin from subjects with CSU and from healthy controls in 2 different study cohorts for **(C)** Cohort 1 (GSE72450) and **(D)** Cohort 2 (GSE57178).

GlaxoSmithKline, Granular Therapeutics, Incyte Corporation, Innovaderm Research, Janssen, Kiniksa, LEO Pharma A/S, Maruho, Novartis, Pfizer, Recens Medical, Regeneron Pharmaceuticals, Sanofi, Septerna, Vial, WebMD; he has stock in ABRAX Japan, KliRNA Biotech, Locus Biosciences, and Recens Medical; he holds a patent for the use of JAK1 inhibitors for chronic pruritus; and he has a patent pending for the use of JAK inhibitors for interstitial cystitis. M. J. Holtzman is founder of NuPeak Therapeutics and a scientific advisor for Lonza Bend. A. M. Ver Heul has served as a consultant for Galderma and has received clinical research funds from Amgen. P. Lovato is shareholder and employee of LEO Pharma A/S, which sponsored part of this study. The rest of the authors declare that they have no relevant conflicts of interest.

Figures and graphical abstract were created in part with Biorender.com.

**Key messages**

- **Potentiation of histaminergic itch by IL-33 depends on mast cells and IL-13 but not its activity on sensory neurons.**
- **IL-33R expression is increased in lesional skin of patients with CSU.**

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# METHODS

## Research animals

All animal protocols were approved by the Washington University Institutional Animal Care and Use Committee. Standard husbandry conditions were maintained (social housing with 12:12 hour light–dark cycle and free access to food and water). WT C57BL/6J (#000664), *Sash*$^{-/-}$(C57BL/6-*Kit*$^{\text{W-sh/W-sh}}$, #030764), and Vav-Cre (#008610) animals were purchased from The Jackson Laboratory. *Il13*$^{-/-}$ mice were generated and maintained as described previously.[E1,E2] IL-33R$^{\Delta\text{immune}}$ mice and IL-33R$^{\Delta\text{neuron}}$ mice were generated by crossing IL-33R$^{\text{flox}}$ mice (generated by Cyagen Biosciences) with Vav-Cre and $Na_V1.8$-Cre (from Rohini Kuner, Heidelberg University), respectively.[E3] Independent cohorts of male and female mice (8-20 weeks old) were used. No phenotypic differences based on sex were observed.

## Mouse models

All experiments were performed on age- and sex-matched adult mouse cohorts. At least 2 days before any treatments, the right cheeks of mice were shaved, as this was where all intradermal (i.d.) injections were administered. Acute itch behavior assessment measured scratching over an interval of 15 minutes, given the short duration of histamine-induced acute itch. To test if IL-33 alone can induce acute itch, itch behavior was assessed 6 hours after WT mice received i.d. injection of recombinant mouse (rm) IL-33 (300 ng in 20 μL; R&D Systems) or saline control (20 μL). To test if IL-33 alters histamine-induced itch, several models were utilized. In the first model, mice received either i.d. coinjection of IL-33 and histamine (300 ng IL-33 + 20 μg histamine in 20 μL) or histamine alone (20 μg in 20 μL; Sigma-Aldrich) and immediate itch behavior assessment. In the second model (IL-33 + His model), mice received i.d. injection of IL-33 (300 ng in 20 μL) or saline control (20 μL, if indicated) 6 hours before i.d. injection of histamine (20 μg in 20 μL) and immediate itch behavior assessment. For the third model, (IL-33 + C48/80 model), mice received i.d. injection of IL-33 (300 ng in 20 μL) or saline control (20 μL, if indicated) 6 hours before i.d. injection of C48/80 (80 ng in 20 μL; Sigma-Aldrich) and immediate itch behavior assessment. For the fourth model (IL-33 + IgE/OVA model), mice were passively sensitized intravenously with anti-OVA IgE (2 μg in 100 μL; Bio-Rad). The next morning, the sensitized mice received i.d. injection of either IL-33 (300 ng in 20 μL) or saline control (20 μL) 6 hours before i.d. OVA challenge (50 μg in 20 μL; Sigma-Aldrich) and immediate itch behavior assessment.

## Itch behavior assessment

Mice were acclimated to individual test chambers 2 days before experiment initiation. Video recordings were manually scored to count the number of scratching bouts directed to the right side within the 15-minute interval immediately after intradermal injection. A scratching bout was defined as continuous hind paw scratching that ended with movement of the hind paw to the mouse's mouth or the chamber floor.

## Data analysis

We reanalyzed scRNA-Seq data sets of mouse and human skin, microarray data sets of bone marrow–derived mast cells treated with IL-33, and skin biopsy samples from subjects with CSU or healthy controls (Figs 2, 3, and 4).

A processed mouse skin scRNA-Seq data set was downloaded from the National Institutes of Health's Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) as accession GSE149121.[E4] A processed human skin scRNA-Seq data set was obtained from Zenodo (zenodo.org/record/4569496#.Y4NhX7LMIl8).[E5] Normalized microarray data sets for bone marrow–derived mast cells treated with IL-33 (GSE96695 and GSE39382)[E6,E7] and skin biopsy samples from subjects with CSU or healthy controls (GSE72450 and GSE57178)[E8,E9] were downloaded from GEO.

For mouse skin data, the 3 processed vehicle-treated control skin available as 10X Genomics cell ranger outputs (labeled OXA-C replicate 1, OXA-C replicate 2, and OXA-C replicate 3) were merged and then integrated by Seurat (v3.0.0 and v4.0.3).[E10] Briefly, samples were isolated, and cells with >10% mitochondrial genes and ≤200 genes detected were filtered from downstream analyses. Each of the 3 samples was normalized and processed with the NormalizeData and FindVariableFeatures functions with default parameters. Integration anchors were determined with these 3 objects using the first 30 dimensions and integrated using the IntegrateData function on the first 30 dimensions. Using this integrated object, dimensionality reduction was carried out after ScaleData with principal component analysis (PCA) using the RunPCA function. On UMAP visualization, we did not find any notable batch effects. Clustering was performed at resolution 0.4 after FindNeighbors was run on the first 30 dimensions. At this clustering resolution, differential expression gene lists were generated using FindAllMarkers, and clusters were annotated by inferred cell type based on the highest differentially expressed markers. Dot plots were generated by the Seurat DotPlot function for visualization of gene expression by cell type. Clusters 5 and 9 were spiked in human cells for initial control purposes and were gated out. Mast cells were identified by high expression of *Cpa3* and *Cma1*.

For human skin, processed and annotated data were converted to a Seurat object in R using Seurat. Cells with >10% mitochondrial genes and ≤200 genes detected were filtered from downstream analyses. We isolated healthy skin samples for further expression analysis (n = 5). Dot plots were created using the Seurat DotPlot function and argument group.by="full_clustering" to visualize gene expression by previously annotated cell types.[E5] Annotated mast cells were confirmed to have high and unique expression of *CPA3* and *CMA1*.

Microarray data (robust multiarray average [RMA]-normalized values) and RNA-Seq data (expression values normalized by DESeq2) of IL-33 receptor expression (*Il1rl1*) in different mouse immune cell types (Fig E1) were obtained from ImmGen (www.immgen.org).[E11] Differential gene expression analysis of microarray data from GSE96695, GSE39382, GSE72450, and GSE57178 was performed using standard workflows in 'limma.'[E12]

Graphical results and statistical testing were conducted for the remaining studies by GraphPad Prism v8 (GraphPad Software). All information is presented as mean ± standard deviation (SD). Statistical significance was determined by 2-tailed unpaired Student *t* test, given normal distribution. Outliers were identified by the ROUT method ($Q = 1\%$). Significance is labeled as follows: *NS,* not significant, $*P < .05$, $***P < .001$.

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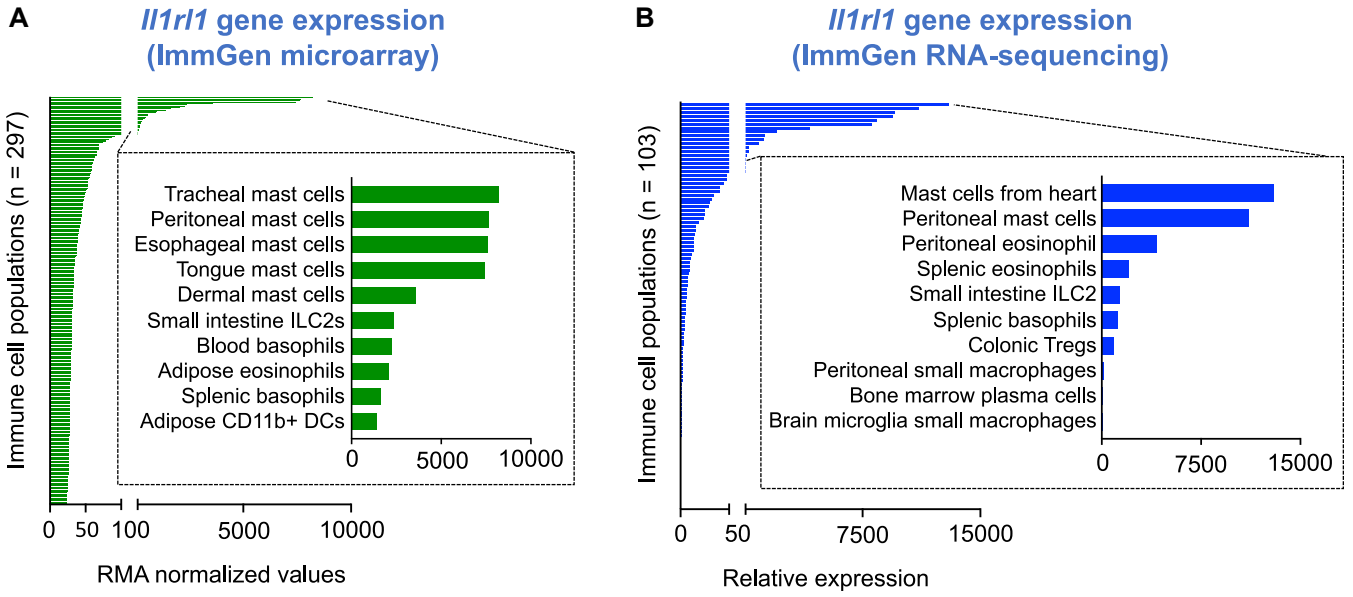


**FIG E1.** Mast cells are among the top expressors of IL-33R across tissue compartments. Expression of IL-33R *(Il1rl1)* across a diversity of tissues in **(A)** 298 mouse immune cell types as determined by microarray and **(B)** 103 immune cell types as determined by RNA-Seq. Top 10 cell populations with highest expression are shown as expanded views in *insets.* Raw data were obtained from ImmGen (www.immgen.org).