

- Avery DT, Deenick EK, Ma CS, Suryani S, Simpson N, Chew GY, et al. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med* 2010;207:155-71.
- Fomek JL, Tygrett LT, Waldschmidt TJ, Poli V, Rickert RC, Kansas GS. Critical role for Stat3 in T-dependent terminal differentiation of IgG B cells. *Blood* 2005;107:1085-91.
- Ma CS, Avery DT, Chan A, Batten M, Bustamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. *Blood* 2012;119:3997-4008.
- Ray JP, Marshall HD, Laidlaw BJ, Staron MM, Kaech SM, Craft J. Transcription factor STAT3 and type I interferons are corepressive insulators for differentiation of follicular helper and T helper 1 cells. *Immunity* 2014;40:307-9.
- Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* 2010;32:605-15.
- Ozaki K. A critical role for IL-21 in regulating immunoglobulin production. *Science* 2002;298:1630-4.
- Lindblad EB. Aluminium compounds for use in vaccines. *Immunol Cell Biol* 2004;82:497-505.
- Phan TG, Paus D, Chan TD, Turner ML, Nutt SL, Basten A, et al. High affinity germinal center B cells are actively selected into the plasma cell compartment. *J Exp Med* 2006;203:2419-24.

Available online June 11, 2016.
<http://dx.doi.org/10.1016/j.jaci.2016.05.018>

The potential for repositioning antithyroid agents as antiasthma drugs



To the Editor:

Bronchial asthma is a common disease in which type 2 immunity is dominant.¹ Based on this immunological background, various antiasthma drugs targeting type 2 immune mediators, such as IL-4, IL-5, IL-13, and CRTH2, are now under development.² However, to develop novel drugs, particularly biologics, huge investments of time and money are required and safety risks are involved. *Drug repositioning*, which is the process of finding new therapeutic indications for existing drugs, is highly desirable as an alternative strategy.^{3,4} However, to our knowledge, there has been no instance of drug repositioning in bronchial asthma.

Airway peroxidase functions as a potent defense system against microbes by producing biocidal compounds, including hypohiocyanite (OSCN⁻), together with hydrogen peroxide generated by Duox1 and Duox2, which are members of the Nox/Duox family.^{5,6} Three heme peroxidases—myeloperoxidase (MPO), eosinophil peroxidase (EPX), and lactoperoxidase (LPO) (expressed at neutrophils, eosinophils, and epithelial cells, respectively)—are involved in this system in the lung tissues. In contrast, the deleterious effects of the airway peroxidase system in bronchial asthma remain undetermined. To define the pathological roles of peroxidase in bronchial asthma, we applied heme peroxidase inhibitors widely used as antithyroid agents to a mouse model of allergic airway inflammation. We found that these agents efficiently inhibited allergic airway inflammation. These results suggest that antithyroid agents can be repositioned as antiasthma drugs.

We first examined the expression of 3 airway peroxidase genes in a mouse model of allergic airway inflammation. The pulmonary expression of all 3 heme peroxidases (*Mpo*, *Epx*, and *Lpo*) was significantly upregulated in response to an allergen challenge (Fig 1, A). The peroxidase activities were also enhanced in the bronchoalveolar lavage fluids (BALFs) of allergen-challenged mice (Fig 1, B). We then collected bronchial biopsy

samples from 10 patients with asthma (see Table E1 in this article's Online Repository at www.jacionline.org) to determine whether the expression of the heme peroxidases was also enhanced in the bronchial tissues of patients with asthma. The peroxidase activities and the expression levels of *LPO* were not statistically enhanced. No expression of *EPX* or *MPO* was detected or was invariant between the patients and normal donors in contrast to the mouse analyses (Fig 1, C, data not shown). However, some patients showed distinctly high peroxidase activities and *LPO* expression. This may be due to the background (steps 2-4 according to the GINA2014 criteria), effects of the treatment for the patients (under good control of inhaled corticosteroids [ICSs]), and/or heterogeneity among patients with asthma regarding airway peroxidase expression. These results suggest the involvement of the airway peroxidase system at least in the mouse model of allergic airway inflammation and possibly in some patients with asthma.

We applied the heme peroxidase inhibitors to examine the role of the airway peroxidase system in allergic airway inflammation. The approach we took to understand the role of this production system during allergic inflammatory responses involved inhibiting all peroxidase-mediated events in the lung because no inhibitors specific for *LPO*, *MPO*, or *EPX* have been developed. We examined the effects of 2-mercapto-1-methylimidazole (methimazole) and 6-propyl-2-thiouracil (PTU), which are agents that inhibit all peroxidases and are widely used as antithyroid agents targeting thyroid peroxidase. For the experiments of methimazole or PTU ingestion, 0.2 mg/mL of methimazole (Wako Pure Chemical Industries, Osaka, Japan) or 0.5 mg/mL of PTU (Sigma-Aldrich, St Louis, Mo) in drinking water was administered orally every day for the indicated times. We adopted 2 protocols of methimazole administration, a long and a short administration strategy (Met-L and Met-S, respectively) (Fig 2, A). Methimazole was administered orally every day from the start of sensitization (day 0) in the long administration and from 2 days before the start of the allergen airway challenge (day 20) in the short administration.

The parameters of ovalbumin (OVA)-induced airway inflammation (ie, airway hyperresponsiveness [AHR], infiltration of inflammatory cells in BALF, and histological changes) were completely inhibited in the long administration and less so (yet significantly) in the short administration (Fig 2, B-D). The use of another peroxidase-inhibiting antithyroid agent, PTU, showed effects similar to but smaller than those of methimazole. Nonetheless, these effects showed a statistically significant improvement in AHR at the dosages used in this experiment (0.2 mg/mL of methimazole vs 0.5 mg/mL of PTU) (see Fig E1 in this article's Online Repository at www.jacionline.org). These results strongly suggest that heme peroxidase activities are critical for the setting of allergic airway inflammation in the model mice.

The Met-L decreased free T4 in serum by inhibiting thyroid peroxidase (1.32 ± 0.14 ng/dL vs 2.0 ± 0.27 ng/dL; $P < .001$) (see Fig E2 in this article's Online Repository at www.jacionline.org). We complemented the thyroid function by administering thyroxine to exclude the possibility that the inhibition of airway inflammation by methimazole was due to impaired thyroid function; however, administering thyroxine had no effect on airway responsiveness (see Fig E3 in this article's Online Repository at www.jacionline.org).

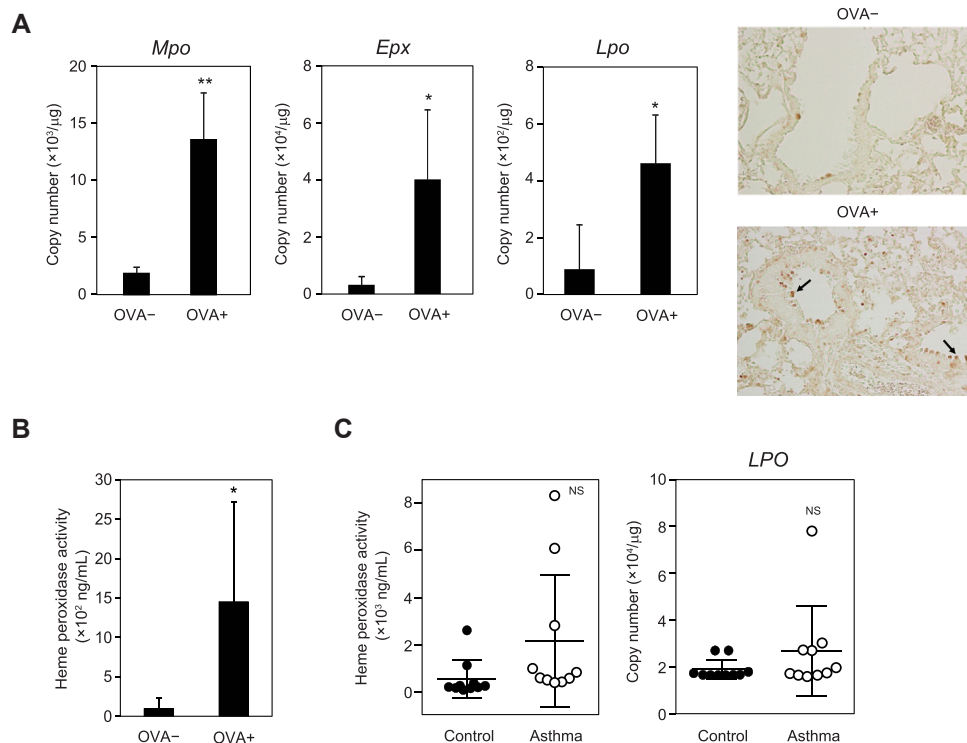


FIG 1. Enhanced expression of airway peroxidase in the lung tissues of allergen-challenged mice and patients with asthma. **A**, *Mpo*, *Epx*, and *Lpo* expression (left) and immunostaining for *Lpo* (right) in the lung tissues of mice with or without OVA inhalation. The arrows indicate *Lpo*-positive cells. **B**, Heme peroxidase activities converted to EPX concentration in BALF of mice with or without OVA inhalation. **C**, Heme peroxidase activities converted to EPX concentration in BALF of patients with asthma and *LPO* expression in the bronchial tissues of patients with asthma. The averages of control samples are set as 1-fold. NS, Not significant. * $P < .05$. ** $P < .01$.

Next, we used mice deficient in each of the 3 peroxidases (*Mpo*, *Epx*, and *Lpo*) to determine which peroxidase dominantly contributes to the setting of allergic airway inflammation. Because *Lpo*-deficient mice were not available, we generated *Lpo*-deficient animals on a BALB/c background in preparation for these studies (see Fig E4 in this article's Online Repository at www.jacionline.org). *Epx*- and *Lpo*-deficient mice showed a nominal but not statistically significant decrease in AHR compared with their control littermates (see Fig E5, A, in this article's Online Repository at www.jacionline.org). The *Mpo*-deficient mice showed no change in AHR. Furthermore, infiltration of eosinophils and T cells was decreased in the BALF of the *Lpo*-deficient mice, whereas there was no change in infiltration in the *Mpo*- or *Epx*-deficient mice (Fig E5, B). These results suggest that the contributions of the 3 peroxidases are redundant in the setting of allergic airway inflammation. However, *Lpo* appears to be dominant among the peroxidases.

Our results may help explain the findings of several reports showing the beneficial effects of the accidental administration of antithyroid agents to patients with asthma,^{7,8} although there is a conflicting report.⁹ It is of note that in most patients, bronchial asthma was exacerbated by discontinuing or tapering off of antithyroid agents.^{7,8} Currently, it remains unknown how heme peroxidase plays a role in the pathogenesis of allergic airway inflammation. We found that OSCN⁺, a product of airway

peroxidase, activates nuclear factor kappa B in airway epithelial cells (unpublished data), which partly explains the role of peroxidase in bronchial asthma. Drug repositioning is now expected as an alternative to drug discovery and development. The use of antithyroid agents could be the first example of drug repositioning in bronchial asthma.

We thank Dr Dovie R. Wylie for the critical review of this manuscript and Prof. Hidenobu Soejima and Drs Kenichi Nishioka and Hidetaka Watanabe for the instruction of Droplet Digital PCR. We also thank Maki Futamata, Tameko Takahashi, Chizuko Kondo, Kazuyo Yoshida, and Seiji Kawasaki for technical assistance.

Shoichi Suzuki, PhD^a
Masahiro Ogawa, PhD^a
Shoichiro Ohta, MD, PhD^b
Kazuhiro Arima, MD, PhD^a
Satoshi Nunomura, PhD^a
Yasuhiro Nanri, PhD^a
Yasutaka Mitamura, MD^a
Tomohito Yoshihara, MD^a
Yutaka Nakamura, MD, PhD^c
Kohei Yamauchi, MD, PhD^c
Kazuyuki Chibana, MD, PhD^d
Yoshiki Ishii, MD, PhD^d
James J. Lee, PhD^e
Yasuaki Aratani, PhD^f
Shigeru Kakuta, PhD^{g,h}
Sachiko Kubo, PhD^{h,i}

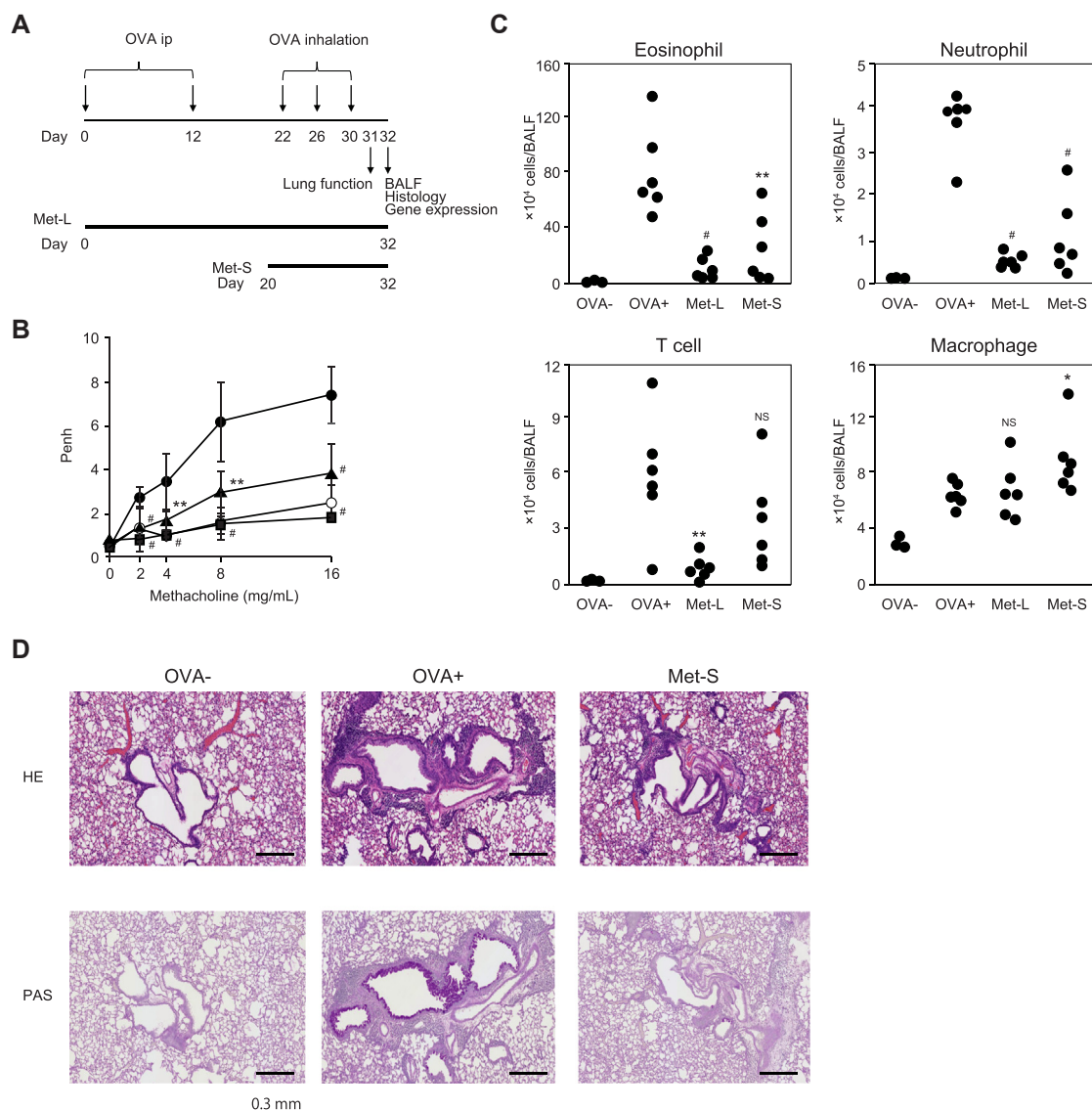


FIG 2. Inhibitors against heme peroxidases improve airway allergic inflammation in mice. **A**, The experimental protocol of the long and the short administrations of methimazole (Met-L and Met-S). **B**, Airway reactivity. Open circle: without OVA inhalation; closed circle: with OVA inhalation; closed square: Met-L; and closed triangle: Met-S. **C** and **D**, Analyses of BALF (Fig 2, C) and lung histology (Fig 2, D). HE, Hematoxylin and eosin; NS, not significant; PAS, periodic acid-Schiff. * $P < .05$, ** $P < .01$, # $P < .001$ (vs with OVA inhalation).

Yoichiro Iwakura, PhD^{h,i}
Hiroki Yoshida, MD, PhD^j
Kenji Izuhara, MD, PhD^a

From ^athe Division of Medical Biochemistry and ^bthe Department of Laboratory Medicine, Saga Medical School, Saga, Japan; ^cthe Division of Pulmonary Medicine, Allergy, and Rheumatology, Department of Internal Medicine, Iwate Medical University School of Medicine, Morioka, Japan; ^dthe Department of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University School of Medicine, Mibu, Japan; ^ethe Division of Pulmonary Medicine, Mayo Clinic Arizona, Scottsdale, Ariz; ^fthe Graduate School of Nanobioscience, Yokohama City University, Yokohama, Japan; ^gthe Department of Biomedical Science, Graduate School of Agricultural and Life Sciences, the University of Tokyo, Tokyo, Japan; ^hthe Center for Experimental Medicine and Systems Biology, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan; ⁱthe Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Japan; and ^jthe Division of Immunology, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan. E-mail: kizuhara@cc.saga-u.ac.jp.

This work was funded in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (S.S., grant no. 24591467; K.I., grant no.

25293224) and by the Grant for Joint Research Project of the Institute of Medical Science, the University of Tokyo (S. Kakuta, S. Kubo, Y.I., and H.Y., grant no. 201). Disclosure of potential conflict of interest: S. Suzuki receives research support from the Japan Society for the Promotion of Science and holds a patent prophylactic or therapeutic agent for bronchial asthma and method for screening the same. S. Ohta holds a patent prophylactic or therapeutic agent for bronchial asthma and method for screening the same. K. Arima receives payment for lectures from Chugai Pharmaceuticals and Astellas Pharmaceuticals and holds a patent prophylactic or therapeutic agent for bronchial asthma and method for screening the same. J. J. Lee receives research support from the National Institute of Health. S. Kakuta receives research support from the joint Project of the Institute of Medical Science, the University of Tokyo. S. Kubo receives research support from the joint Project of the Institute of Medical Science, the University of Tokyo. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

- Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014;133:388-94.

2. Izuhara K, Matsumoto H, Ohta S, Ono J, Arima K, Ogawa M. Recent developments regarding periostin in bronchial asthma. *Allergol Int* 2015;64:S3-10.
3. Tobinick EL. The value of drug repositioning in the current pharmaceutical market. *Drug News Perspect* 2009;22:119-25.
4. Li YY, Jones SJ. Drug repositioning for personalized medicine. *Genome Med* 2012;4:27.
5. Hawkins CL. The role of hypothiocyanous acid (HOSCN) in biological systems. *Free Radic Res* 2009;43:1147-58.
6. Barrett TJ, Hawkins CL. Hypothiocyanous acid: benign or deadly? *Chem Res Toxicol* 2012;25:263-73.
7. Settipane GA, Schoenfeld E, Hamolsky MW. Asthma and hyperthyroidism. *J Allergy Clin Immunol* 1972;49:348-55.
8. Nakazawa T, Kobayashi S. Influence of antithyroidal therapy on asthma symptoms in the patients with both bronchial asthma and hyperthyroidism. *J Asthma* 1991;28:109-16.
9. Grembiale RD, Naty S, Iorio C, Crispino N, Pelaia G, Tranfa CM. Bronchial asthma induced by an antithyroid drug. *Chest* 2001;119:1598-9.

Available online June 15, 2016.
<http://dx.doi.org/10.1016/j.jaci.2016.04.047>

Keratin-dependent thymic stromal lymphopoietin expression suggests a link between skin blistering and atopic disease



To the Editor:

Atopic dermatitis (AD) is a pruritic and inflammatory skin disorder characterized by barrier disruption. The cytokine thymic stromal lymphopoietin (TSLP), a common denominator of atopic diseases, promotes allergic skin inflammation and is predominantly produced by barrier epithelial cells, triggering T_H2 -mediated inflammatory responses. The mechanisms governing upregulation of TSLP in keratinocytes remain incompletely understood, although barrier disruption represents a major trigger of TSLP expression.^{1,2} Keratin (K) proteins form the intermediate filament cytoskeleton of epithelia and are crucial barrier constituents. Dominant-negative mutations in the *KRT5* or *KRT14* genes cause epidermolysis bullosa simplex (EBS), a disorder characterized by skin fragility, inflammation, and barrier defects. In addition to protecting epithelia against mechanical stress, keratins regulate immune responses by controlling alarmins, IL-18, and additional cytokines. Remarkably, gene expression and cytokine profiling of keratin-deficient mice and disorders such as AD show considerable overlap.³⁻⁵ The notion that itch of unknown cause is the most common and bothersome complication in EBS⁶ raises the question whether keratin mutations are involved in itch through upregulation of TSLP. Here, we report keratinocyte-intrinsic and keratin-dependent upregulation of TSLP independent of barrier defects in keratinocytes isolated from keratin-deficient mice and identify a link between *KRT5* and *KRT14* mutations and increased TSLP serum levels in patients with EBS.

To investigate the role of keratins in epidermal differentiation and pathogenesis, we generated mice lacking all type II (keratin type 2 gene cluster knockout [KtyII^{-/-}]) or all type I (keratin type 1 gene cluster knockout [KtyI^{-/-}]) keratin genes and corresponding keratinocyte cell lines.⁷ Both strains lack the entire epidermal keratin cytoskeleton, with highly similar phenotypes. Global gene expression and quantitative PCR analysis of prenatal KtyII^{-/-} mice revealed a strong

upregulation of *Tslp* gene expression (Fig 1, A; see Table E1, A, in this article's Online Repository at www.jacionline.org). A few cytokines and chemokines were also upregulated *in vivo* but not in cultured keratinocytes, correlating with barrier defects triggering their induction (Table E1). Tslp protein levels in sera of postnatal day 5 mosaic KtyII^{-/-} mice⁸ reached up to 3000 pg/mL whereas controls had levels below 300 pg/mL (Fig 1, A). Perinatal mortality in global KtyII^{-/-} and KtyI^{-/-} mice precluded the analysis of serum levels and the assessment of a potential scratching behavior of keratin-deficient mice due to elevated Tslp.

Tslp induction may result from barrier defects or from novel, keratinocyte-intrinsic mechanisms due to the absence of keratins. To address this, immunofluorescence of KtyI^{-/-} and wild-type (WT) mouse skin was performed. WT epidermis hardly expressed Tslp (Fig 1, B and Fig E1, A), in agreement with ELISA data (Fig 1, A), whereas the entire KtyI^{-/-} epidermis showed strong Tslp reactivity (Fig E1, A). To substantiate the inverse relationship between Tslp and keratin protein expression, the unique setting of mosaic keratin type 2 gene cluster knockout mice with keratin-deficient and positive keratinocytes in the same specimen was probed.⁸ This confirmed Tslp presence in keratin-deficient and absence in WT keratinocytes (Fig 1, B). Thus, high Tslp levels in keratinocytes and release therefrom strongly correlate with absence of keratins in mouse epidermis.

To support cell-intrinsic and barrier-independent Tslp upregulation, we analyzed its expression by quantitative PCR and ELISA in isolated KtyI^{-/-} keratinocytes. Here, very strong upregulation of Tslp RNA and protein (Fig 1, C) levels occurred, even in low Ca^{2+} medium. Most other cytokine RNAs remained unaffected by the cells' keratin status (Table E1, B). To examine keratin dependence, mouse Krt14 was reexpressed in KtyI^{-/-} keratinocytes (Kty^{-/-} keratin 14 [K14]), restoring keratin filaments together with endogenous Krt5 (Fig E1, B).⁹ This significantly reduced both Tslp RNA and protein levels (Fig 1, C), strongly supporting keratinocyte-intrinsic and keratin-dependent Tslp expression. Given that dominant KRT14 mutations cause EBS by formation of cytoplasmic keratin aggregates, murine keratinocytes stably expressing the dominant K14p.Arg₁₃₁Pro mutation (KtyI^{-/-} K14R131P) were generated, equivalent to the human mutation K14p.Arg₁₂₅Cys, which results in severe EBS with keratin aggregates in keratinocytes.¹⁰ The mutation led to an approximately 4-fold Tslp increase relative to WT controls (Fig 1, C), accompanied by extensive cytoplasmic keratin aggregation (Fig E1, B), a hallmark of severe generalized EBS.¹⁰ Collectively, keratin aggregates comprising the dominant K14 EBS mutant or lack of keratins were sufficient to trigger increased Tslp in a keratinocyte-intrinsic way. Because keratin-dependent *Tslp* regulation could be of clinical interest, we addressed molecular mechanisms controlling *Tslp* expression.

To examine the role of calcium, cells were treated for 3 hours with the intracellular calcium chelator 1,2-Bis(2-Aminophenoxy) ethane-N,N,N',N'-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM). This decreased *Tslp* RNA levels by approximately 50%, whereas chelation of residual extracellular calcium by 3 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) had negligible effects on *Tslp* (see Fig E2, A and B, in this article's Online Repository

METHODS

Induction of airway allergic inflammation in mice

Seven- to 10-week-old female mice were used. Animal studies were undertaken following the guidelines for care and use of experimental animals of the Japanese Association for Laboratory Animals Science (1987) and were approved by the Saga University Animal Care and Use Committee (Saga, Japan).

The mouse model of airway allergic inflammation was generated as previously described^{E1} with minor modifications. Mice were sensitized by intraperitoneal injections of 50 μ g OVA (Sigma-Aldrich) and 1 mg alum (LSL, Tokyo, Japan) in 500 μ L saline on days 0 and 12. Mice inhaled 1% OVA in saline on days 22, 26, and 30 or on days 22, 23, and 24, followed by assessment of airway responsiveness on the following day and of BALF, lung histology, and gene expression 2 days later. In some experiments, 0.05 μ g/mouse thyroxine (Sigma-Aldrich) was administered intraperitoneally every day for the indicated times.

Assessment of AHR

Airway responsiveness to methacholine (Sigma-Aldrich) was assessed with a 4-chamber whole-body plethysmograph from Buxco Electronics (Wilmington, NC) or flexiVent from SCIREQ (Montreal, Canada).

Digital PCR

Droplet Digital PCR was performed on QX200 Droplet Digital PCR system (Bio-Rad, Hercules, Calif) using ddPCR supermix for probes or ddPCR EvaGreen supermix. The sequences of primers are as follows: *Mpo*, 5'-CCCTTCCTAAACTGAACCTGAC-3' and 5'-ATGGCCTCCGTCCTTC TC-3'; *Epx*, 5'-CTCCTGACTAACCCTCTGC-3' and 5'-TCACTTGACCG AGTGTCACC-3'. Pre-designed TaqMan Gene Expression Assays (Life Technologies, Carlsbad, Calif) for *Lpo* (Mm00475466_m1) and *LPO* (Hs00976400_m1) were used.

Analysis of BALF

After mice were anesthetized, lungs were lavaged with 500 μ L of BAL liquid (0.1% BSA/50 μ mol/L EDTA/PBS), and BALF was collected. This procedure was repeated 3 times. BALF cell counts were determined with a particle counter (CDA500, Sysmex, Japan). Eosinophils, neutrophils, T cells, and macrophages were defined as siglec-F⁺ cells (BD Bioscience, San Diego, Calif), Gr-1^{high} cells (eBioscience, San Diego, Calif), CD3⁺ cells (eBioscience), and strongly autofluorescent/bigger-size cells,^{E2} respectively.

Lung histology

Paraffin-embedded lung sections were prepared and stained with hematoxylin and eosin or periodic acid-Schiff. For immunostaining, sections were incubated with anti-Lpo antibodies (Thermo Fisher Scientific, Waltham, Mass) followed by visualization according to a standard procedure using 3,3'-diaminobenzidine.

Heme peroxidase activity

One hundred microliter of TMB solution (1-step Ultra TMB-ELISA, Thermo Fisher Scientific) was added to 100 μ L of BALF in the presence or

absence of 1 mmol/L sodium azide (Wako Pure Chemical Industries). The reaction was stopped by adding 50 μ L of 2 mol/L sulfuric acid and the enzyme activity was evaluated by the absorbance at 450 nm. The peroxidase activity was converted to peroxidase concentrations using a standard curve generated with commercial human EPX (Lee BioSolutions, Maryland Heights, Mo). Heme peroxidase activity was calculated by subtracting azide-insensitive peroxidase activity from total peroxidase activity.

Free T4

Free T4 was measured by Elecsys FT4II (Roche Diagnostics, Basel, Switzerland).

Patients with asthma

Ten patients with asthma and 10 healthy donors were recruited from the Iwate Medical University Hospital. The backgrounds of the patients with asthma are described in Table E1. The patients with asthma had no other medical disorders and were not current smokers; the 10 control subjects were nonallergic and nonasthmatic patients. All the patients with asthma were at step 2 to 4 according to the GINA2014 criteria and were well controlled with inhaled corticosteroids. Biopsy samples were taken from the bifurcations of the subsegmental bronchi under bronchoscopy. This study was approved by the Iwate Medical University Hospital Ethics Committee. Healthy donors had both normal spirometry and required a lung resection for treatment of lung carcinoma. They were not current smokers and had no history of asthma and allergy or systemic diseases.

Peroxidase-deficient mice

Mpo- or *Epx*-deficient mice were prepared as previously described (Fig E6).^{E3,E4} *Lpo*^{fllox/+} chimeric mice were obtained by injecting *Lpo*^{tm1a(EUCOMM)Wtsi} embryonic stem cells (JM8.N4, C57BL/6N background, European Mouse Mutant Cell Repository, Neuherberg, Germany) into blastocysts from ICR mice (Japan SLC, Hamamatsu, Japan). *Lpo*^{fllox/+} mice were selected by mating *Lpo*^{fllox/+} chimeric mice and C57BL/6 mice (Kyudo, Tosu, Japan). Then, *Lpo*-deficient mice were generated by mating *Lpo*^{fllox/+} mice and Cre transgenic mice (BALB/c-Tg(CMV-cre)1Cgn/J #3465, The Jackson Laboratory, Bar Harbor, Maine) and were backcrossed onto BALB/c mice (Japan SLC) for 8 generations.

REFERENCES

- E1. Matsushita H, Ohta S, Shiraishi H, Suzuki S, Arima K, Toda S, et al. Endotoxin tolerance attenuates airway allergic inflammation in model mice by suppression of the T-cell stimulatory effect of dendritic cells. *Int Immunol* 2010;22:739-47.
- E2. van Rijt LS, Kuipers H, Vos N, Hijdra D, Hoogsteden HC, Lambrecht BN. A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J Immunol Methods* 2004;288:111-21.
- E3. Aratani Y, Koyama H, Nyui S, Suzuki K, Kura F, Maeda N. Severe impairment in early host defense against *Candida albicans* in mice deficient in myeloperoxidase. *Infect Immun* 1999;67:1828-36.
- E4. Denzler KL, Borchers MT, Crosby JR, Cieslewicz G, Hines EM, Justice JP, et al. Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. *J Immunol* 2001;167:1672-82.

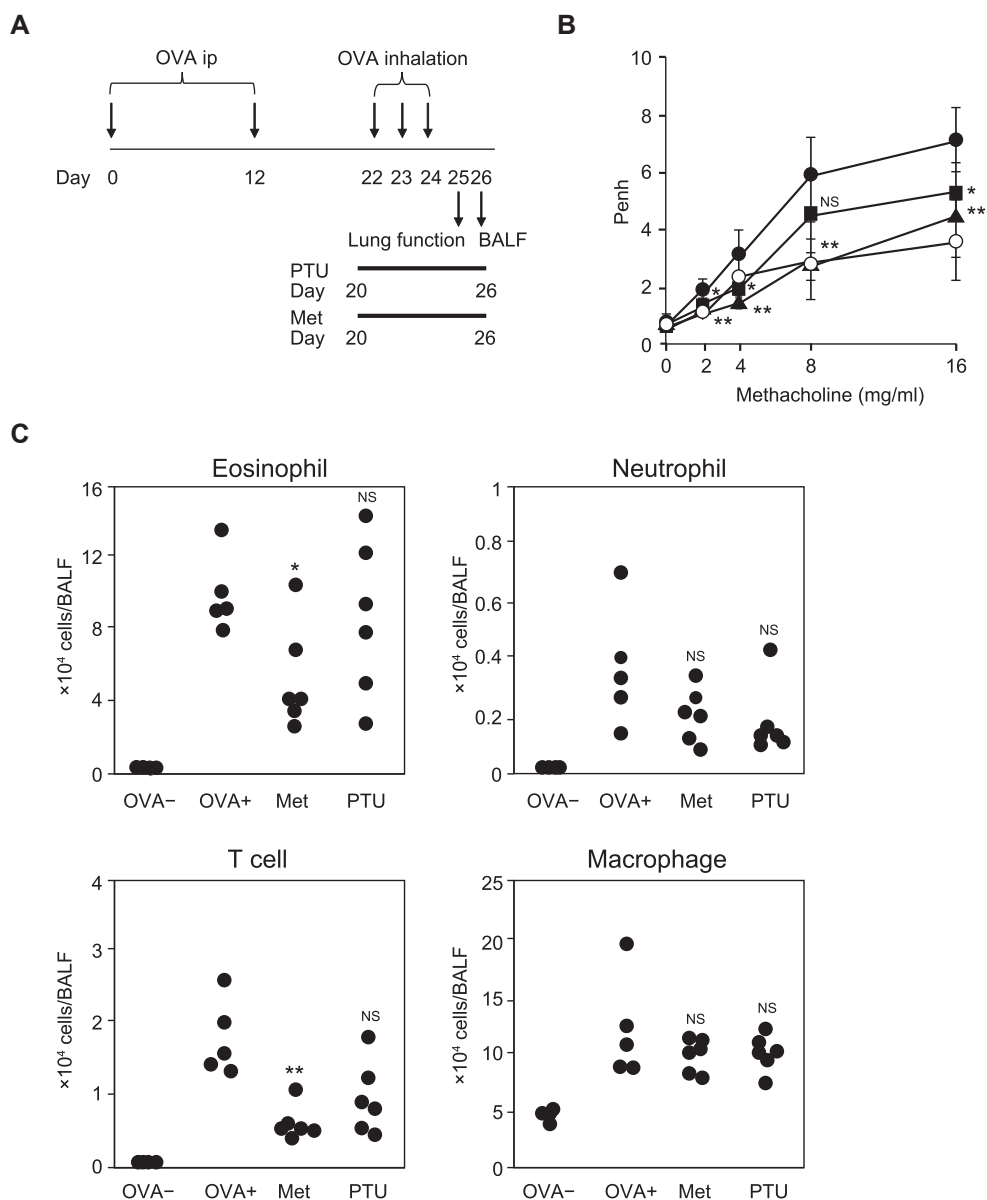


FIG E1. Effects of PTU on allergic airway inflammation in mice. **A**, The experimental protocol. Oral administration of 0.5 mg/mL PTU or 0.2 mg/mL methimazole (Met) in water every day from day 20 to day 26. **B**, Airway reactivity assessed by a whole-body plethysmograph. *Open circle*, without OVA inhalation; *closed circle*, with OVA inhalation; *closed triangle*, methimazole; and *closed square*, PTU. **C**, Analyses of BALF. *ip*, Intraperitoneal. * $P < .05$, ** $P < .01$, and NS: not significant (vs with OVA inhalation).

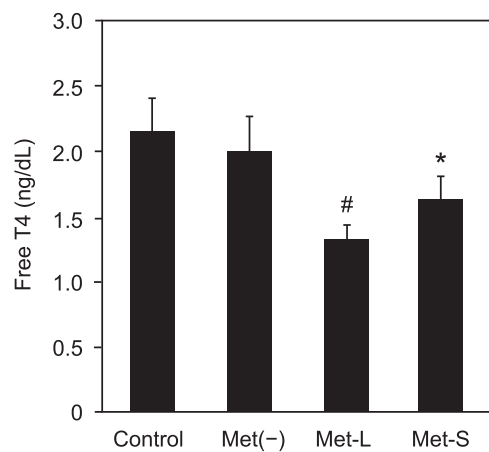


FIG E2. Effects of methimazole on free T4 in mice. Serum-free T4 in methimazole-free (Met(-)), Met-L, and Met-S mice. Serum was taken on day 31. * $P < .05$, # $P < .001$ (vs Met(-) mice).

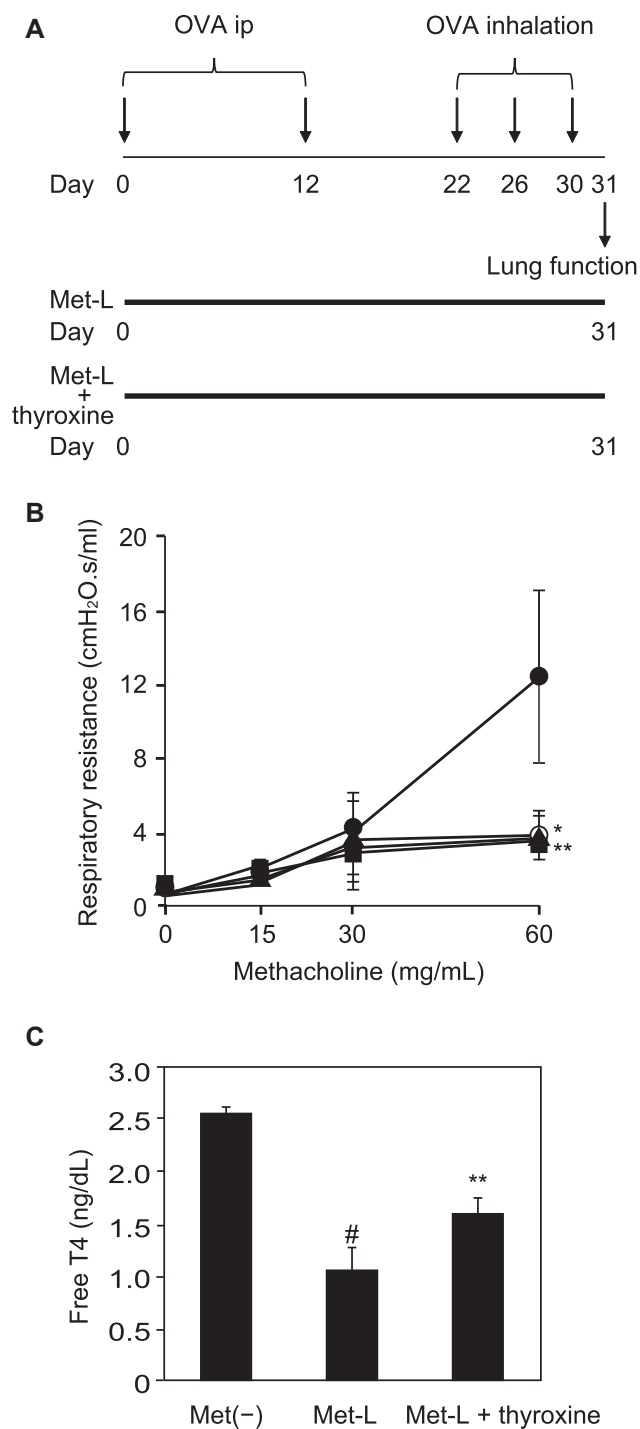


FIG E3. Effects of thyroxine on the improvement in allergic airway inflammation by methimazole in mice. **A**, The experimental protocol. Oral administration of 0.2 mg/mL methimazole (Met) in drinking water alone or with intraperitoneal (ip) administration of 0.05 μ g thyroxine per mouse every day from day 0 to day 31. **B**, Airway reactivity assessed by flexiVent (SCIREQ, Montreal, Canada). *Open circle*, without OVA inhalation; *closed circle*, with OVA inhalation; *closed triangle*, methimazole alone; and *closed square*, methimazole and thyroxine. * $P < .05$, ** $P < .01$, (vs with OVA inhalation). **C**, Serum-free T4 in Met(-), Met-L, and Met-L supplemented with thyroxine mice. Serum was taken on day 31. ** $P < .01$, # $P < .001$ (vs Met(-) mice).

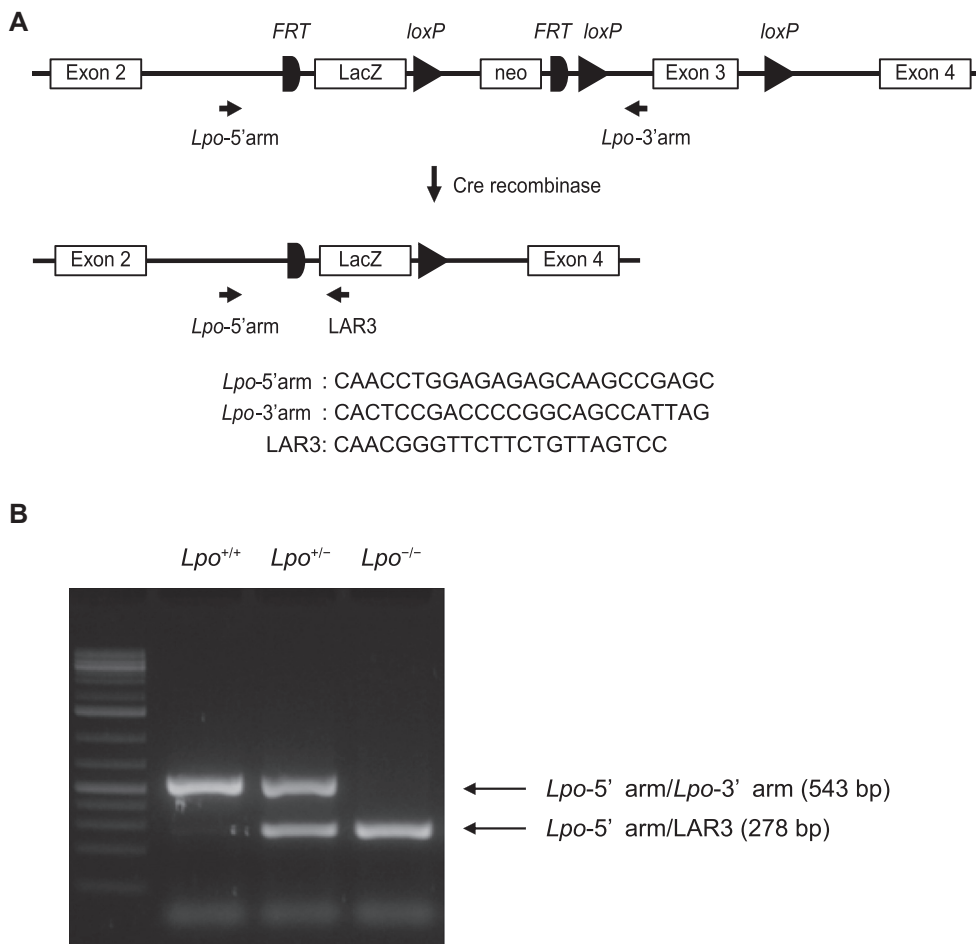


FIG E4. Genotyping of *Lpo*-deficient mice. **A**, Genomic structures of the *Lpo*-floxed allele and the *Lpo*-deficient allele generated by cleavage with Cre recombinase. The positions and the directions of 3 primers (*Lpo*-5'arm, *Lpo*-3'arm, and LAR3) are depicted. **B**, Genotyping of *Lpo*^{+/+}, *Lpo*^{+/-}, and *Lpo*^{-/-} mice. The *Lpo*-5'arm/*Lpo*-3'arm band (543 bp) and the *Lpo*-5'arm/LAR3 (278 bp) correspond to the wild-type and knockout alleles, respectively.

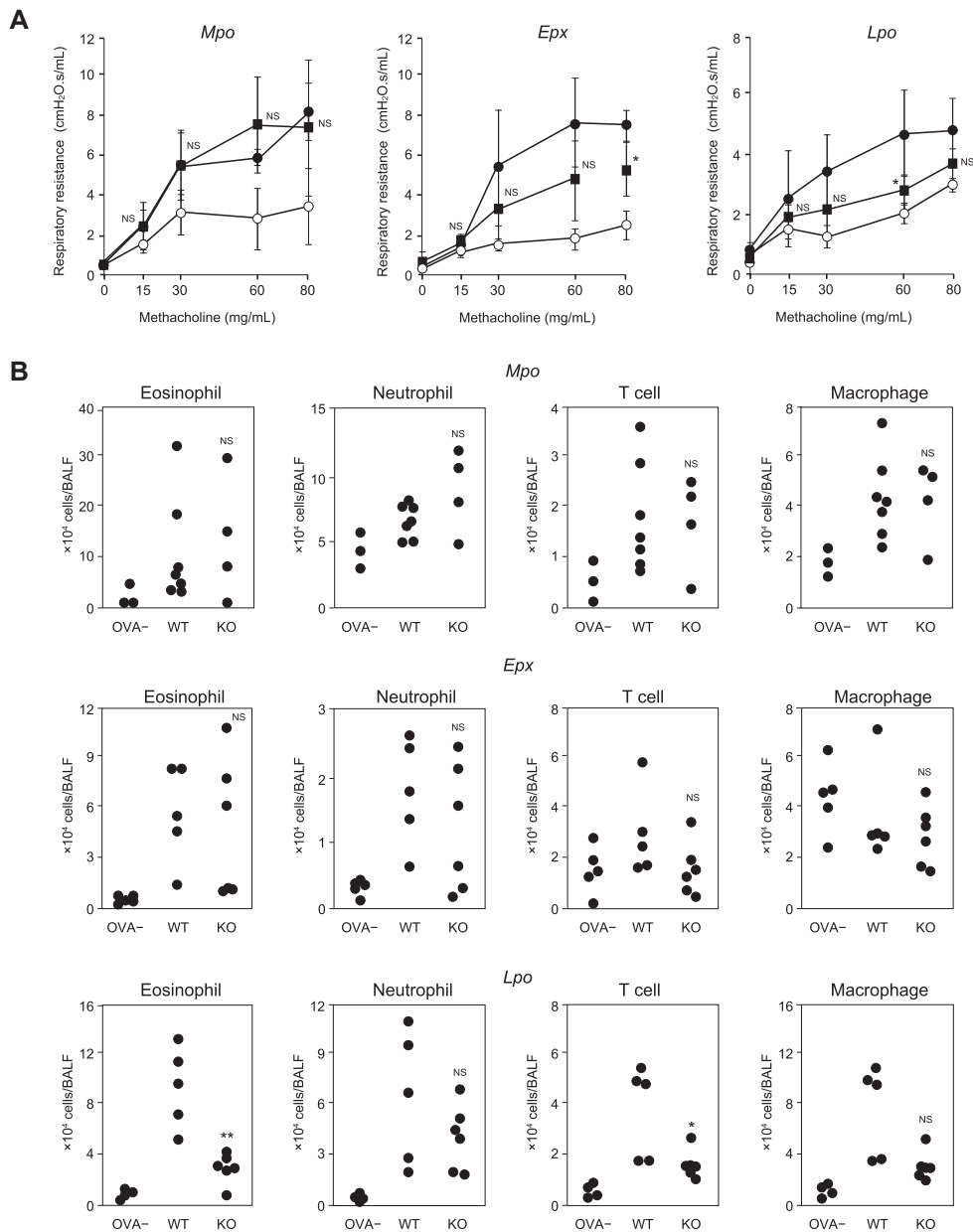


FIG E5. Redundancy of contribution to airway allergic inflammation in 3 peroxidases. **A**, Airway reactivity as assessed by the flexiVent. *Open circle*, WT littermates without OVA inhalation; *closed circle*, WT littermates with OVA inhalation; and *closed square*, knocked-out mice with OVA inhalation. **B**, Analyses of BALF. OVA, WT littermates without OVA inhalation; WT, wild-type littermates with OVA inhalation; KO, knocked-out mice with OVA inhalation. * $P < .05$, ** $P < .01$, and NS: not significant (vs WT littermates with OVA inhalation).

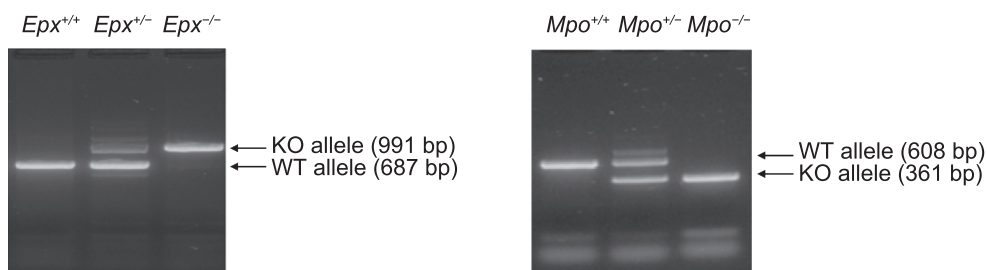


FIG E6. Genotyping of *Epx*- and *Mpo*-deficient mice. Genotyping of *Epx*^{+/+}, *Epx*^{+/-}, and *Epx*^{-/-} mice (*left panel*) and *Mpo*^{+/+}, *Mpo*^{+/-}, and *Mpo*^{-/-} mice (*right panel*). The 687 bp and the 991 bp bands for *Epx* and the 608 bp and the 361 bp bands for *Mpo* correspond to the wild-type (WT) and knockout (KO) alleles, respectively.

TABLE E1. Clinical backgrounds, *LPO* expression, and heme peroxidase activity in patients with asthma

S. no.	Age (y)	Sex	FENO (ppb)	Treatment	IgE (IU/mL)	FEV ₁ (%)	Eosinophil (/μL)	<i>LPO</i> mRNA (copy number/μg)	Heme peroxidase activity (ng/mL)
1	61	M	44	FP 200 μg/d	528	98.0	33	11,600	809
2	35	M	134	FP 500 μg/d	82	53.3	286	4,100	2,792
3	36	M	52	FP 500 μg/d	2657	96.8	659	130	367
4	38	M	34	FP 400 μg/d	1286	63.0	296	640	8,317
5	40	M	34	FP 200 μg/d	293	96.3	280	16,400	567
6	36	F	30	BUD 200 μg/d	572	82.8	341	76,500	483
7	65	M	39	FP 200 μg/d	909	48.1	252	12,300	392
8	48	M	25	FP 400 μg/d	88	65.9	391	ND	550
9	39	M	62	FP 100 μg/d	134	104.8	293	ND	6,083
10	40	F	46	BUD 400 μg/d	190	59.1	179	960	967

BUD, Budesonide; *F*, female; *FENO*, fractional exhaled nitric oxide; *FP*, fluticasone propionate; *M*, male; *ND*, not determined.