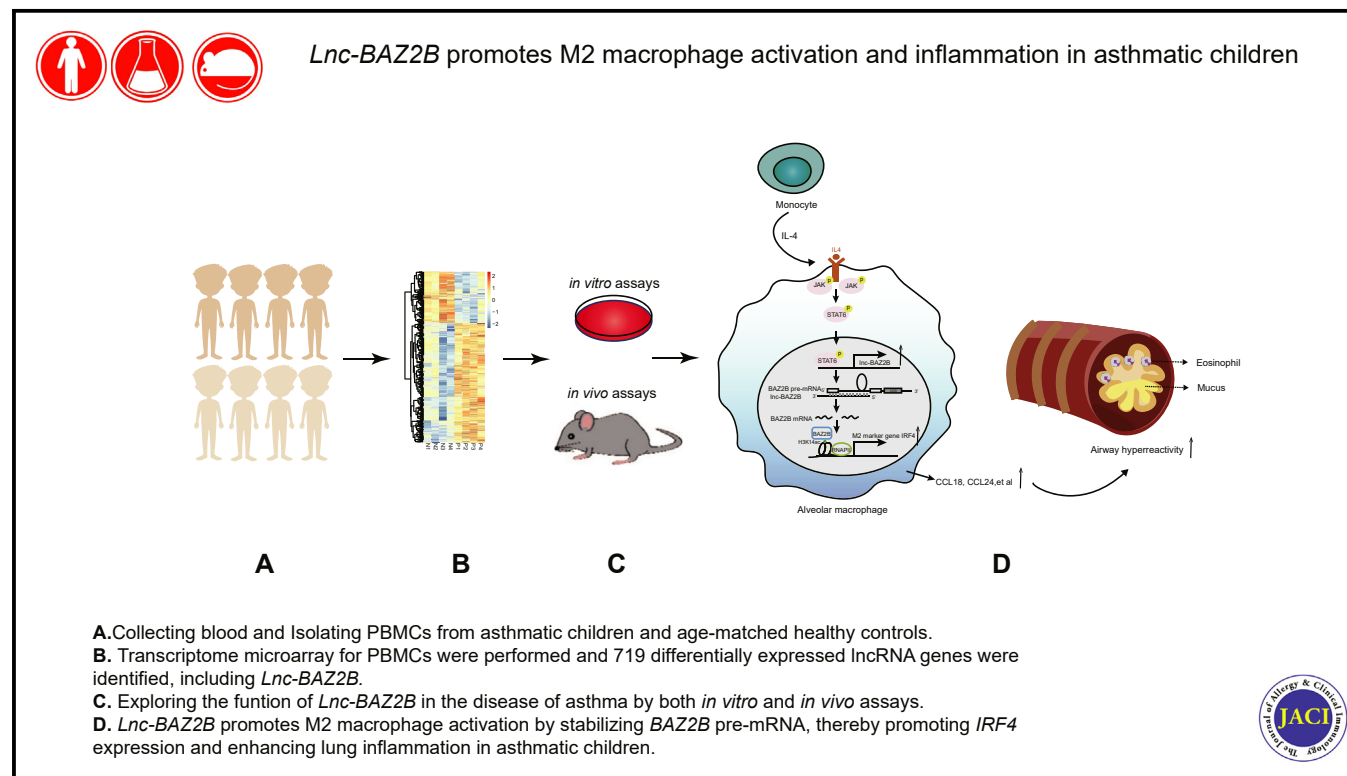


Inc-BAZ2B promotes M2 macrophage activation and inflammation in children with asthma through stabilizing *BAZ2B* pre-mRNA

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GRAPHICAL ABSTRACT



Background: Dysregulation of long noncoding RNAs (lncRNAs) is associated with a variety of human diseases; however, whether they have a role in childhood asthma is unknown.

Objective: We sought to determine the differential expression profiles of lncRNAs in PBMCs of children with asthma and the

mechanisms underlying the effects of lncRNAs on the pathogenesis of asthma.

Methods: The differential expression profiles of lncRNAs were analyzed by transcriptome microarray. The effects and mechanisms by which lncRNAs influence macrophage

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activation were detected by real-time quantitative PCR, Western blot, RNase protection assay, and chromatin immunoprecipitation assay. The roles played by lncRNAs in asthma were tested in a cockroach allergen extract (CRE)-induced mouse model.

Results: We identified 719 lncRNAs that were differentially expressed in PBMCs of children with asthma, 502 of which were upregulated and 217 were downregulated. An lncRNA of unknown function, *lnc-BAZ2B*, was dominantly expressed in monocytes and significantly upregulated in children with asthma. *lnc-BAZ2B* promotes M2 macrophage activation by enhancing *BAZ2B* expression and exacerbated lung inflammation in an M2 macrophage-associated CRE-induced asthma model. Mechanistically, *lnc-BAZ2B* promoted the expression of its *cis* target gene *BAZ2B* by stabilizing its pre-mRNA. *BAZ2B*, a reader of H3K14ac modification, enhanced the transcription of *IRF4* and promoted M2 macrophage activation. *lnc-BAZ2B* expression was correlated with that of *BAZ2B* in PBMCs from children with asthma. *Baz2b* knockdown could alleviate asthma severity in a CRE-induced asthma model.

Conclusion: *lnc-BAZ2B* promotes M2 macrophage activation and inflammation in children with asthma and may serve as a potential therapeutic and diagnostic target in children with asthma. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Asthma, lncRNA, macrophage activation, BAZ2B

Asthma is one of the most common chronic respiratory tract diseases in children and adults.¹ An important molecular mechanism of asthma is type 2 inflammation, in which T_H2 cells and T_H2-type cytokines such as IL-4 and IL-13 are thought to drive disease pathology.^{2,3} Macrophages are the most abundant immune cells in the lung, and recent studies have indicated that alternatively activated (M2) macrophages play important roles in allergic asthma dominated by type 2 responses.⁴⁻⁶ Mechanistically, IL-4 and IL-13 skew macrophages toward the M2 activation state via the activities of signal transducer and activator of transcription 6 (STAT6) and several other transcription factors, including interferon regulatory factor 4 (IRF4) and peroxisome proliferator-activated receptor gamma (PPAR γ). Subsequently, M2 macrophages secrete high levels of IL-13 and chemokines, including C-C motif chemokine 17 (CCL-17), CCL-18, CCL-22, and CCL-24, which activate T_H2 cells and promote eosinophil infiltration into the lungs.^{4,7}

The incidence of asthma has increased gradually worldwide in recent years.^{8,9} It is becoming increasingly clear that epigenetic mechanisms play an important role in the pathogenesis of allergic asthma.^{10,11} In response to environmental stimuli, 3 main epigenetic changes can occur during macrophage activation, namely, DNA methylation, histone modifications, and regulation by noncoding RNAs.^{4,12-14} Noncoding RNAs, including microRNAs (miRNAs), PIWI-interacting RNAs, endogenous small interfering RNAs (siRNAs), and long noncoding RNAs (lncRNAs), comprise a cluster of RNAs that do not encode functional proteins.^{15,16} However, increasing evidence has indicated that regulatory noncoding RNAs, especially miRNAs and lncRNAs, play important roles in gene regulation in the immune system.^{10,17} miRNAs such as miR-511-3p and miR-155 have been reported to have important roles in allergic

Abbreviations used

BAZ2B:	Bromodomain adjacent to zinc finger domain protein 2B
ChIP:	Chromatin immunoprecipitation
CRE:	Cockroach allergen extract
FC:	Fold change
IRF4:	Interferon regulatory factor 4
lncRNA:	Long noncoding RNA
miRNA:	MicroRNA
PAR γ :	Peroxisome proliferator-activated receptor gamma
RT-qPCR:	Real-time quantitative PCR
siRNA:	Small interfering RNA

asthma through the regulation of macrophage activation.¹⁸⁻²⁰ lncRNAs are defined as transcribed RNA molecules more than 200 nucleotides long, and they can interact with DNA, RNA, or proteins to mediate a variety of biologic functions.^{21,22} Significantly, several studies have revealed that lncRNAs are key in controlling the differentiation and function of mammalian cells, including innate and adaptive immune cells.²² In contrast, lncRNA dysregulation is related to a variety of human diseases, including cancer, infection, and neurologic and autoimmune diseases.²³⁻²⁶ Several studies have reported that lncRNAs such as GAS5, Mirt2, and MM2P can regulate immunologic diseases and cancer by modulating macrophage activation.^{25,27,28} However, the profiles of differentially expressed lncRNAs and the roles played by lncRNAs in regulating macrophage activation and allergic asthma are poorly understood.

In this study, we provide evidence supporting a critical role for *lnc-BAZ2B* in asthma regulation in children through the promotion of M2 macrophage activation. Mechanistically, *lnc-BAZ2B* promotes the expression of *BAZ2B*, its *cis* gene, by stabilizing its pre-mRNA. As a reader of H3K14ac modification, *BAZ2B* enhances the transcription of *IRF4*, a key transcription factor for M2 macrophage activation. In summary, we have identified a functionally unrecognized lncRNA, *lnc-BAZ2B*, which promotes M2 macrophage activation by stabilizing the pre-mRNA of *BAZ2B* in children with asthma.

METHODS

Ethical approval of the study protocol

A total of 54 children primarily diagnosed with asthma were recruited from the Children's Hospital of Fudan University. Asthma diagnosis was based on the 2016 edition of the Guidelines for the Diagnosis and Prevention of Childhood Bronchial Asthma of China.^{29,30} A total of 50 age- and sex-matched healthy children without a personal or family history of allergies were recruited as controls. The human study was approved by the research ethics board of the Children's Hospital of Fudan University. Informed consent was obtained from each of the study participants. Animal experiments complied with the relevant laws and institutional guidelines as overseen by the animal studies committee of the Children's Hospital of Fudan University.

Additional details on the methods used in this study, including regarding use of human study subjects, RNA extraction, real-time quantitative PCR (RT-qPCR), microarray analysis, cell culture, cell transfection, Western blot, RNase protection assay, subcellular fractionation and localization, isolation of human monocytes and T and B cells, chromatin immunoprecipitation (ChIP) assays, a murine model of asthma, analyses of bronchoalveolar fluid, measurement of airway hyperresponsiveness, lung pathology, and statistical analyses, are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Inc-BAZ2B is upregulated in children with asthma

To identify differentially expressed lncRNAs in children with asthma, we first analyzed the lncRNA and mRNA expression profiles in PBMCs of children with asthma ($n = 4$) and age-matched healthy controls ($n = 4$) by transcriptome microarray. We found 719 lncRNA genes (68,423 lncRNA genes in total) that were differentially expressed in children with asthma, including 502 upregulated and 217 downregulated lncRNAs ($P \leq .05$; fold change [FC] ≥ 2) (see Fig E1, A and B in this article's Online Repository at www.jacionline.org). Next, we noticed that 34 upregulated nonexonic sense lncRNAs have a Gene Symbol description ($P < .05$; FC ≥ 2 ; mean ≥ 7) (Fig 1, A). We then selected 5 antisense lncRNAs that are upregulated in children with asthma (*LOC643072*, *DAPK1-IT1*, *ASAP1-IT1*, *RARA-AS1*, and *VCAN-AS1*) for further confirmation by RT-qPCR using an increased number of PBMC samples from another 50 patients with asthma and 46 healthy controls. Detailed characteristics of the study subjects are presented in Table E1 (in this article's Online Repository at www.jacionline.org). Consistent with the microarray analysis results, the expression of these 5 lncRNAs was markedly higher in patients with asthma than in healthy controls (Fig 1, B and see Fig E1, C in this article's Online Repository at www.jacionline.org).

We also analyzed the differential mRNA expression profiles in PBMCs of patients with asthma from the same samples as those used for lncRNA analysis. We found that 382 mRNAs were differentially expressed in patients with asthma (FC ≥ 2 ; $P \leq .05$), including 311 upregulated and 71 downregulated mRNAs (see Fig E2 in this article's Online Repository at www.jacionline.org). We performed gene ontology enrichment analysis of the differentially expressed mRNA genes in PBMCs of patients with asthma and found that the categories type 2 immune response and regulation of type 2 immune response were enriched, which was consistent with previous reports indicating that the type 2 immune response plays an important role in allergic asthma (Fig 1, C).⁴⁻⁶ We also found some inflammation pathways, such as the IL-8–related pathway, were enriched. This was also consistent with previous reports that the expression level of IL-8 was significantly elevated in children with asthma.³¹⁻³⁴

Next, we assessed the expression levels of lncRNAs in T cells, B cells, and monocytes among PBMCs. We found that *LOC643072* (of unknown function) was mainly expressed in monocytes (Fig 1, D). As M2 macrophage activation is important for the type 2 immune response and allergic asthma and circulating monocytes could transform into macrophages in inflamed tissues,^{4,35} we hypothesized that *LOC643072* may regulate asthma in children by influencing the activation of M2 macrophages, and that their expression would likely be tightly controlled following macrophage activation. We therefore assessed the expression levels of the 5 selected lncRNAs after stimulation with LPS (designated operationally as M1 subsets) and IL-4 (M2 subsets). Notably, among these 5 differentially expressed antisense lncRNAs, the expression of *LOC643072* was greatly enhanced during IL-4–induced M2 macrophage activation and decreased during LPS-induced M1 macrophage activation, whereas expression of the other lncRNAs showed no clear pattern (Fig 1, E and F and see Fig E3 in this article's Online Repository at www.jacionline.org). These results suggested that *LOC643072*

may play an important role in macrophage activation. The sequence of lncRNA *LOC643072* was mapped to the reverse strand of its *cis* gene, bromodomain adjacent to zinc finger domain 2B (*BAZ2B*), and was therefore designated as *lnc-BAZ2B*.

***lnc-BAZ2B* promotes M2 macrophage activation**

To determine whether *lnc-BAZ2B* regulates macrophage activation, we evaluated the effect of *lnc-BAZ2B* loss- and gain-of-function on THP1-derived human macrophage activation following IL-4 stimulation. We found that *lnc-BAZ2B* knockdown in macrophages significantly impaired the expression of M2-specific marker genes, including C-type lectin domain containing 7A (*CLEC7A*), interleukin 1 receptor type 1 (*IL1RA*), *CCL18*, and *IRF4* following IL-4 stimulation (Fig 2, A and B). In contrast, the expression levels of M2-specific marker genes, including *CLEC7A*, *IL1RA*, mannose receptor C-type 1 (*MRC1/CD206*), and *IRF4* were markedly increased when macrophages overexpressed *lnc-BAZ2B* compared with the control (empty vector) (Fig 2, C and D). These results suggested that *lnc-BAZ2B* promotes M2 macrophage activation.

STAT6 is the predominant transcription factor in the IL-4 signaling pathway. We found that the expression level of *lnc-BAZ2B* in IL-4-stimulated M2 macrophages with short hairpin RNA–mediated STAT6 knockdown was sharply reduced compared with the level in the negative controls (Fig 2, E and F). Taken together, our results suggested a possible sequence of events whereby IL-4 induces the expression of *lnc-BAZ2B* through STAT6 activation, which then promotes M2 macrophage activation.

***lnc-BAZ2B* promotes M2 macrophages activation by enhancing *BAZ2B* expression**

As *lnc-BAZ2B* is located on the strand opposite that of the coding gene, *BAZ2B*, and lncRNAs have been reported to exert *cis*-regulatory effects on nearby genes, we investigated whether the effect of *lnc-BAZ2B* on M2 macrophage activation was due to its influence on *BAZ2B* expression. We found that *lnc-BAZ2B* knockdown did indeed result in an approximately 50% reduction in *BAZ2B* expression in THP1-derived macrophages (Fig 3, A) whereas ectopic expression of *lnc-BAZ2B* significantly increased the levels of *BAZ2B* mRNA (Fig 3, B). These findings confirmed that *lnc-BAZ2B* can positively regulate *BAZ2B* expression.

Next, we further examined whether the effect of *lnc-BAZ2B* on macrophage activation was mediated through its direct target, *BAZ2B*. We found that THP1-derived, IL-4 stimulated M2 macrophages with confirmed *BAZ2B* knockdown exhibited significantly decreased expression levels of M2-associated genes when compared with those in the negative controls (Fig 3, C and D). Similarly, the levels of M2-specific marker genes were also markedly downregulated in IL-4–stimulated RAW264.7 mouse macrophages when *Baz2b* was knocked down (Fig 3, E and F). Additionally, no difference was observed in *lnc-BAZ2B* expression when *BAZ2B* was knocked down, which is consistent with our hypothesis that *lnc-BAZ2B* is an upstream regulator of *BAZ2B* (see Fig E4, A in this article's Online Repository at www.jacionline.org).

We noted that the expression level of *IRF4*, which encodes a key transcription factor that controls M2 macrophage activation,

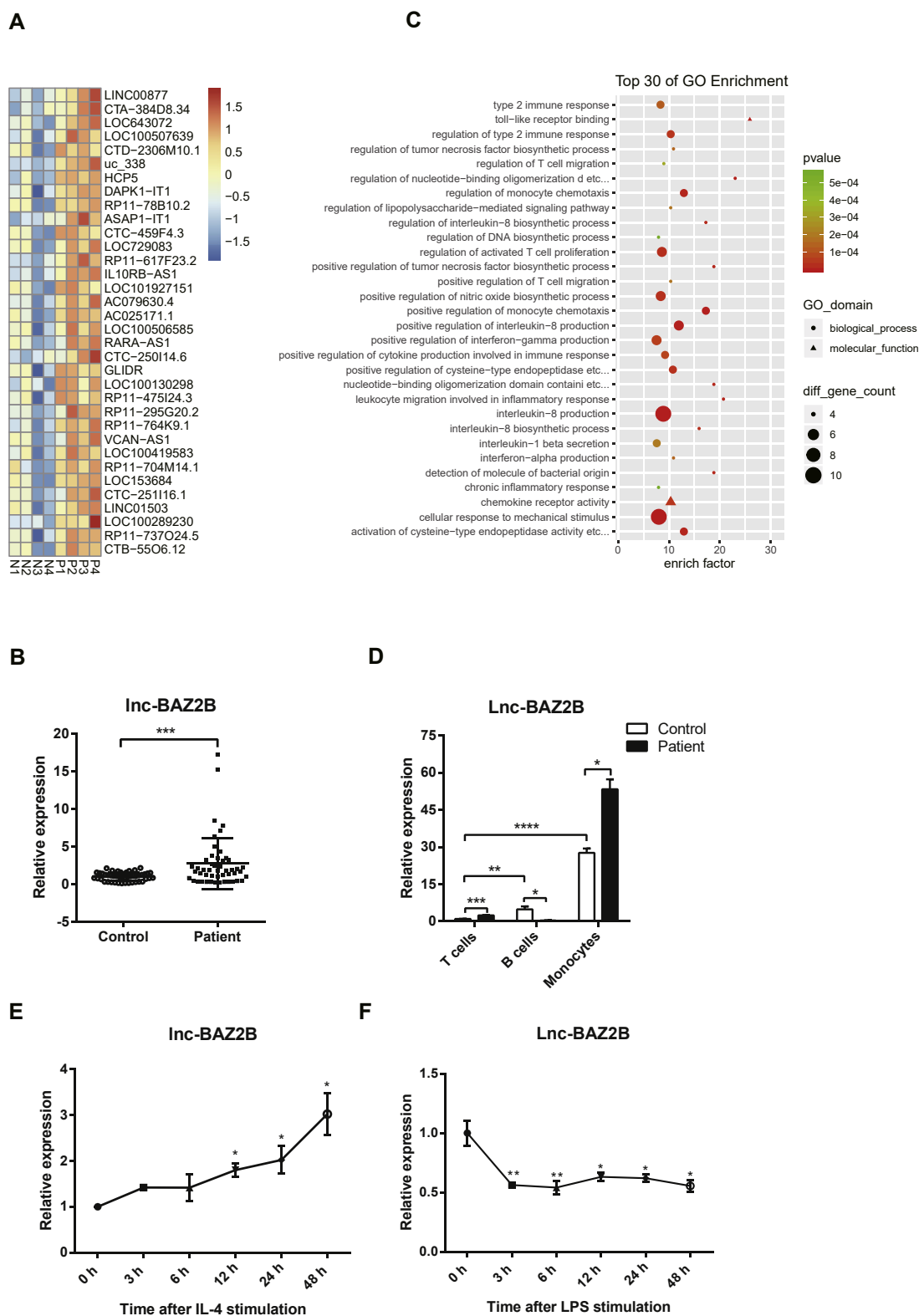


FIG 1. *lnc-BAZ2B* was upregulated in children with asthma. **A**, Nonexonic sense lncRNAs with a Gene Symbol description upregulated in children with asthma ($P < .05$; $FC \geq 2$; mean > 7). **B**, The expression level of *LOC643072* (*lnc-BAZ2B*) in children with asthma ($n = 50$) and healthy children ($n = 46$) was validated by RT-qPCR. The Mann-Whitney U test was performed for 2-group comparisons. **C**, The 30 most enriched gene ontology (GO) terms created by clusterProfiler from R/Bioconductor for mRNAs upregulated in PBMCs of children with asthma ($P < .05$; $FC \geq 2$). **D**, The expression levels of *lnc-BAZ2B* in T cells, B cells, and monocytes from the PBMCs. *RPL13A* was used as an internal control. The expression level of *LOC643072* (*lnc-BAZ2B*) in THP1-derived macrophages stimulated with IL-4 (20 ng/mL) (**E**) or LPS (100 ng/mL) (**F**) for 0 to 48 hours. Data are represented as means $\pm$ SEMs. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

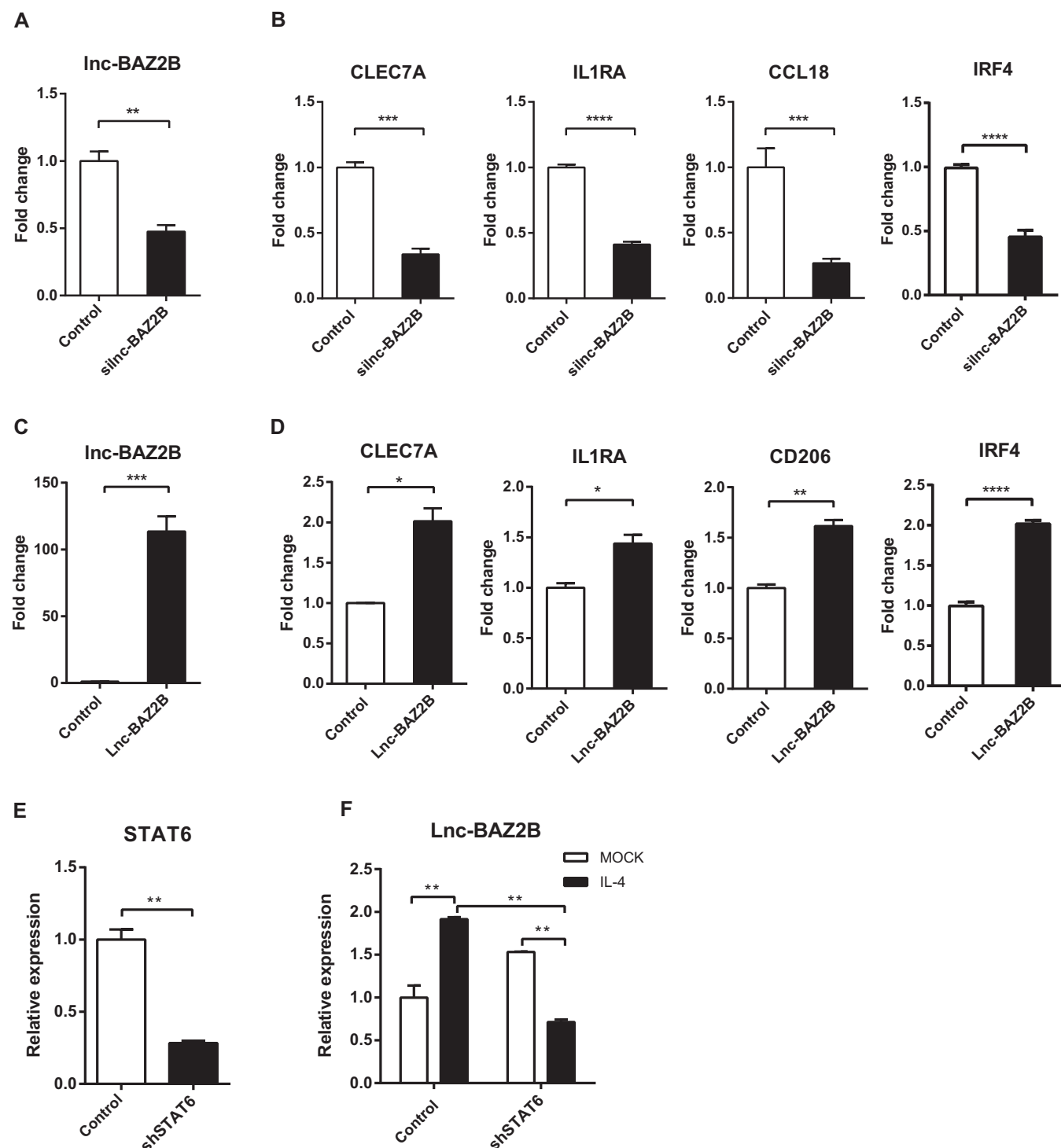
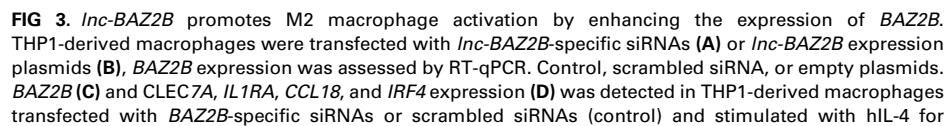


FIG 2. *Inc-BAZ2B* promotes M2 macrophage activation. **A**, THP1-derived macrophages were transfected with *Inc-BAZ2B*-specific siRNAs or scrambled siRNAs as a control, and the knockdown efficiency was tested by RT-qPCR. **B**, The expression levels of *CLEC7A*, *IL1RA*, *CCL18*, and *IRF4* were assessed by RT-qPCR in *Inc-BAZ2B* knockdown THP1-derived macrophages stimulated with IL-4 (20 ng/mL) for 24 hours. **C**, THP1-derived macrophages were transfected with *Inc-BAZ2B* expression plasmids or empty vectors as control, and *Inc-BAZ2B* overexpression was confirmed by RT-qPCR. **D**, The expression levels of *CLEC7A*, *IL1RA*, *MRC1*, and *IRF4* were measured in *Inc-BAZ2B* overexpressing THP1-derived macrophages stimulated with IL-4 (20 ng/mL) for 24 hours. **E**, THP1-derived macrophages were transfected with *STAT6*-specific (shSTAT6) short hairpin RNAs (shRNAs) or scrambled shRNAs (control), and *STAT6* levels were assessed by RT-qPCR. **F**, After being transfected with *STAT6*-specific shRNAs or scrambled shRNAs (control), THP1-derived macrophages were stimulated with IL-4 (20 ng/mL) for 24 hours. *MOCK*, Without IL-4 stimulation. *Inc-BAZ2B* levels were quantified by RT-qPCR. Data are represented as means $\pm$ SEMs from 3 independent experiments. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.



was significantly decreased when either *lnc-BAZ2B* or *BAZ2B/Baz2b* was downregulated (Fig 3, D and F-H); however, the expression of *PPAR γ* , another master regulator of the M2 phenotype, was virtually unchanged after *BAZ2B* knockdown (see Fig E4, B). In contrast, the levels of *IRF4* and M2 macrophage marker genes were upregulated with *lnc-BAZ2B* overexpression under stimulation by IL-4. We then knocked down *IRF4* expression in macrophages overexpressing *lnc-BAZ2B* and observed that the expression of M2 macrophage-related genes was reversed (Fig 3, I). This indicated that *lnc-BAZ2B* and *BAZ2B* promote M2 macrophage activation mainly through the upregulation of *IRF4*.

As *BAZ2B* is a H3K14ac reader,^{36,37} we hypothesized that *BAZ2B* may bind H3K14ac-modified sites present in the *IRF4* gene and promote its transcription. To assess this, we performed a ChIP assay using an anti-H3K14ac antibody and evaluated the H3K14ac levels on the *IRF4* promoter by qPCR. The results indicated that H3K14ac was indeed enriched at the *IRF4* promoter (Fig 3, J). Collectively, our results suggested that *BAZ2B*, as a H3K14ac reader, promotes M2 macrophage activation primarily by upregulating the transcription of *IRF4*.

***lnc-BAZ2B* enhances the expression of *BAZ2B* by stabilizing its pre-mRNA**

To explore how *lnc-BAZ2B* regulates *BAZ2B* expression, we first investigated *lnc-BAZ2B* cellular localization by RT-qPCR after performing subcellular fractionation. The results indicated that *lnc-BAZ2B* was expressed in both the nucleus and cytoplasm at a ratio of 35%:65% (Fig 4, A). Through bioinformatic analysis, we found that the *lnc-BAZ2B* sequence mapped to the reverse strand of *BAZ2B*, its *cis* gene. The 5' end of *lnc-BAZ2B* is complementary to that of *BAZ2B* pre-mRNA, even though the bases between nucleotides 854 and 1149 do not match (Fig 4, B). To reveal the relationship between *lnc-BAZ2B* and its *cis* gene, *BAZ2B*, we knocked down *lnc-BAZ2B* in THP1-derived macrophages by using specific siRNAs and measured the levels of *BAZ2B* pre-mRNA and mature mRNA by RT-qPCR. The results indicated that the expression levels of *BAZ2B* pre-mRNA and mature mRNA were both markedly decreased following *lnc-BAZ2B* knockdown (Fig 4, C). Next, we performed an RNase protection assay and found that the *BAZ2B* pre-mRNA could still be amplified by PCR after RNase A+T digestion when using primers located in *lnc-BAZ2B* and *BAZ2B* complementary regions (Fig 4, D). In contrast, no *BAZ2B* pre-mRNA amplicons were observed when primers targeting noncomplementary regions were used (Fig 4, D). These results suggested that *lnc-BAZ2B* and the pre-mRNA of *BAZ2B* might form a duplex that protects *BAZ2B* pre-mRNA from degradation by RNases.

To further confirm these results, full-length *lnc-BAZ2B* was exogenously expressed in THP1-derived macrophages and *lnc-BAZ2B* overexpression was confirmed by RT-qPCR (Fig 4, E). We found that the expression of both *BAZ2B* and *BAZ2B* pre-mRNA was upregulated in macrophages overexpressing *lnc-BAZ2B* (Fig 4, F and G). We then treated macrophages with actinomycin D (an inhibitor of transcription) for 6 hours and found that with overexpression of *lnc-BAZ2B*, both the pre-mRNA and mature mRNA of *BAZ2B* in macrophages were degraded more slowly than those in negative controls after actinomycin D treatment (Fig 4, F and G). These results indicated that *lnc-BAZ2B* regulates *BAZ2B* expression at the posttranscriptional level. Collectively, our findings suggested that *lnc-BAZ2B* and the pre-mRNA of *BAZ2B* could form a duplex, which could increase the stability of *BAZ2B* pre-mRNA and further increase the levels of *BAZ2B* mature mRNA.

***lnc-BAZ2B* and *Baz2b* exacerbate CRE-induced lung inflammation**

Next, we investigated the role of *lnc-BAZ2B* and its *cis* target gene *BAZ2B* in a mouse model of M2 macrophage-associated, CRE-induced pulmonary inflammation (Fig 5, A).³⁸ Asthma pathologies developed in CRE-challenged mice, as indicated by the significant recruitment of inflammatory cells to the lung, with dense peribronchial infiltrates and goblet cell hyperplasia in the histologic examination; however, this was not observed in PBS-challenged mice (Fig 5, B). Interestingly, *lnc-BAZ2B* overexpression in CRE-treated mice significantly enhanced pulmonary inflammation, including increased peribronchial infiltrates and goblet cell hyperplasia, and it increased the total number of infiltrated inflammatory cells, especially eosinophils, in bronchoalveolar lavage fluid and enhanced the expression level of Muc5ac, compared with that in mice treated with CRE only (Fig 5, B-E). Also, the airway resistance of mice with *lnc-BAZ2B* overexpression was much higher than in the green fluorescent protein-negative controls (Fig 5, F). However, *Baz2b* knockdown mice treated with CRE displayed decreased pulmonary inflammation with fewer infiltrated inflammatory cells and decreased expression level of Muc5ac when compared with the levels in CRE-treated scramble control mice (Fig 5, B, G-I).

To further verify whether *lnc-BAZ2B* and *Baz2b* exacerbated CRE-induced lung inflammation through the promotion of M2 macrophage activation *in vivo*, we compared the expression levels of mouse M2 macrophage-specific marker genes in lung tissues from different groups of mice. Compared with those in the PBS-treated group, the expression levels of M2-specific marker genes, including *Mrc1*, *Arg1*, *Clec7a*, and *Irf4*, were greatly increased in CRE-treated mice (Fig 5, J and K), which was consistent with the results of our previous study.³⁸ Moreover, *Baz2b* mRNA expression was higher in CRE-treated mice than

24 hours. Levels of *Baz2b* (E), *Clec7a*, *Mrc1*, *Arg1*, and *Irf4* (F) expression were measured in RAW264.7 macrophages transfected with *Baz2b*-specific siRNAs or scrambled siRNAs (control) stimulated with mouse IL-4 for 24 hours. G, Immunoblotting analysis of *IRF4* in THP1-derived macrophages after *lnc-BAZ2B* knockdown (G) or *BAZ2B* knockdown (H) by siRNAs or scrambled siRNAs (negative control [NC]). I, THP1-derived macrophages overexpressing *lnc-BAZ2B* were transfected with *IRF4*-specific siRNAs or scrambled siRNAs (control). The cells were stimulated with IL-4 for 24 hours and *IRF4*, *CLEC7A*, and *IL1RA* was evaluated by RT-qPCR. J, ChIP assays using an anti-H3K14ac antibody in THP1-derived macrophages. MOCK, Normal rabbit IgG control. Binding at the *IRF4* promoter region was assessed by qPCR. Data are represented as means $\pm$ SEMs from 3 independent experiments. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

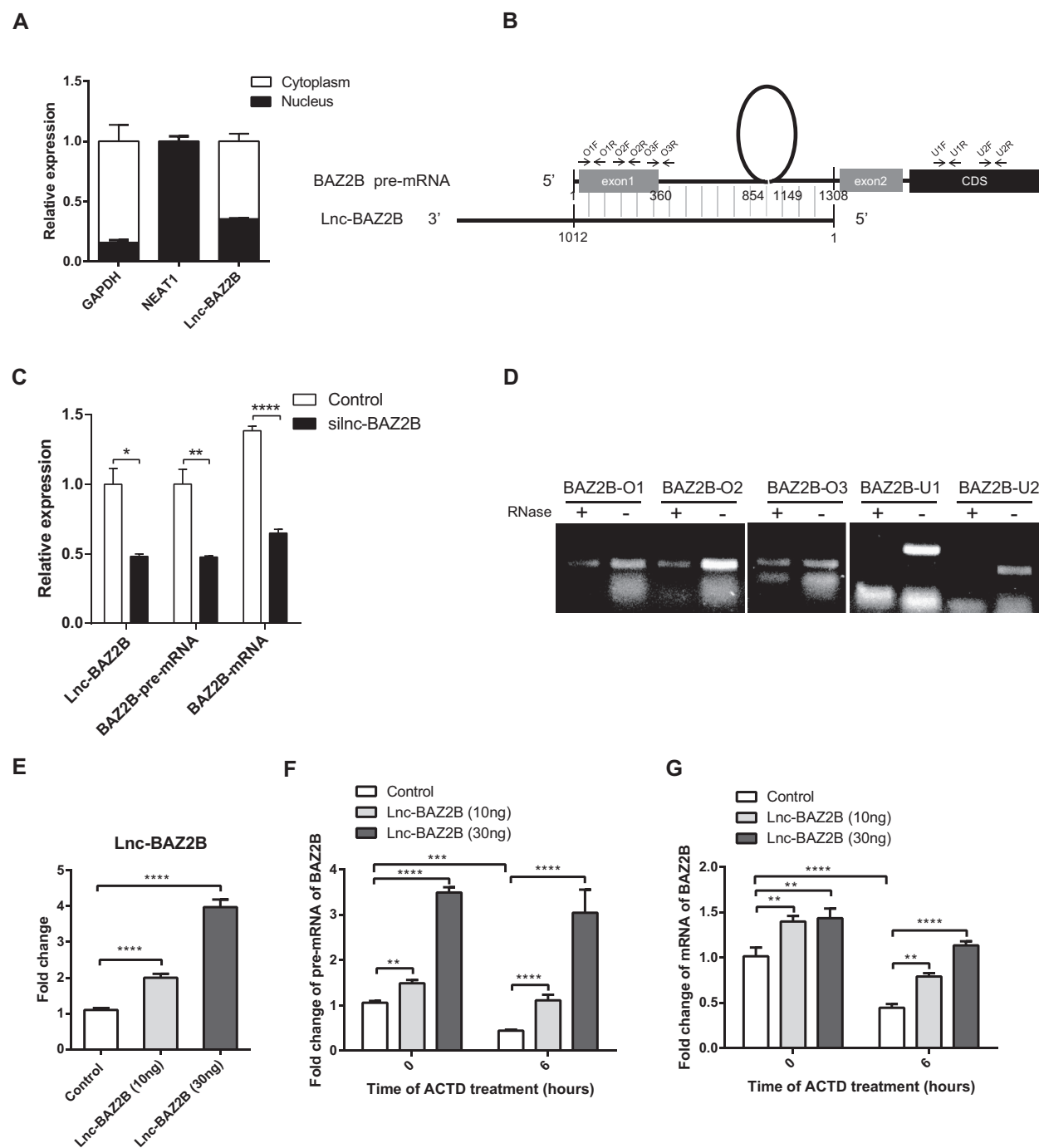


FIG 4. *Inc-BAZ2B* enhances the expression of mature *BAZ2B* mRNA by stabilizing its pre-mRNA in M2 macrophages. **A**, The localization of *Inc-BAZ2B*, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (cytoplasm control), and nuclear paraspeckle assembly transcript 1 (*NEAT1*) (nuclear control) in macrophage cytoplasmic and nuclear fractions was evaluated by RT-qPCR. **B**, A schematic representation of the reverse complementary pairing of the 5' end sequence of *Inc-BAZ2B* with that of *BAZ2B* pre-mRNA. **C**, RT-qPCR was used to measure the expression levels of *Inc-BAZ2B* and pre-mRNA and mRNA of *BAZ2B* in THP1-derived macrophages after *Inc-BAZ2B* knockdown by siRNAs or scrambled siRNAs (control). **D**, Total RNA from THP1-derived macrophages was digested by RNase A+T for 1 hour at 37°C, and RT-PCR was performed with different primer sets. Bands shown are the different PCR products (O1, O2, and O3, overlapped segments; U1 and U2, unoverlapped segments). **E**, RT-qPCR was used to measure the expression levels of *Inc-BAZ2B* in the THP1-derived macrophages transfected with *Inc-BAZ2B* plasmids (0 - 30 ng) or empty vector as a control. *BAZ2B* pre-mRNA (**F**) and *BAZ2B* mRNA (**G**) were measured by RT-qPCR in the *Inc-BAZ2B* overexpressed macrophages after actinomycin D (ACTD) treatment for 0 to 6 hours. Data are represented as means $\pm$ SEMs from 3 independent experiments. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

in PBS-challenged mice (Fig 5, L). We further found that the expression levels of M2-specific marker genes and *Baz2b* were considerably upregulated in mice treated with CRE and overexpressing *lnc-BAZ2B* than in control, green fluorescent protein-expressing mice (Fig 5, J-L). However, the expression levels of M2-specific marker genes, including *Arg1*, *Chil3*, and *Irf4*, were substantially lower in CRE-treated *Baz2b* knockdown mice than in control mice (Figure 5, M and N). Collectively, these results suggest that *lnc-BAZ2B* and *Baz2b* can exacerbate allergen-induced lung inflammation through the promotion of M2 macrophage activation.

***lnc-BAZ2B* expression is correlated with *BAZ2B* expression in PBMCs of children with asthma**

To determine the potential importance of *lnc-BAZ2B* and *BAZ2B* in allergic asthma in children, we measured the expression levels of *lnc-BAZ2B* and *BAZ2B* in PBMCs from children with allergic asthma and healthy control subjects. The expression level of *lnc-BAZ2B* in children with asthma was considerably higher than that in healthy children (Fig 1, B). Importantly, a positive correlation was detected between *lnc-BAZ2B* and *BAZ2B* in children with asthma (Fig 6, A). We did not find significant difference in *BAZ2B* expression between these 2 groups at the whole population level (see Fig E5 in this article's Online Repository at www.jacionline.org). However, the children with asthma could be divided into 2 groups, namely, those with low levels of *lnc-BAZ2B* and those with high levels based on the expression level of *lnc-BAZ2B* compared with that in the group of normal controls (Fig 6, B). In patients with asthma with higher levels of *lnc-BAZ2B*, the expression of *BAZ2B* was higher than in patients with lower *lnc-BAZ2B* expression levels (Fig 6, C). Moreover, patients with asthma with higher expression of *lnc-BAZ2B* had a greater blood eosinophil count and percentage than patients with lower *lnc-BAZ2B* expression levels (Fig 6, D and E). The ability of *lnc-BAZ2B* to differentiate patients with asthma from healthy subjects was assessed by receiver operating curve analysis, which yielded an area under the curve of 0.85 (Fig 6, F). Together, these findings imply that *lnc-BAZ2B* has potential as a biomarker in childhood asthma.

DISCUSSION

In this study, we defined the differential lncRNA expression profiles in children with asthma and found that *lnc-BAZ2B* was upregulated in these patients. And for the first time, we showed that *lnc-BAZ2B* promoted M2 macrophage activation and subsequently enhanced the severity of asthma symptoms. Furthermore, IL-4-induced expression of *lnc-BAZ2B* and STAT6 was required in this process. Knockdown of *lnc-BAZ2B* by siRNAs in macrophages impaired the expression of M2-specific marker genes. However, these effects could be reversed when *lnc-BAZ2B* was overexpressed in macrophages. Interestingly, in a mouse model of M2-associated, CRE-induced pulmonary inflammation, we showed that *lnc-BAZ2B* overexpression led to significantly enhanced pulmonary inflammation when compared with that in mice treated with CRE only. Furthermore, the expression levels of M2-specific marker genes and *Baz2b* were substantially higher

in mice overexpressing *lnc-BAZ2B* than in the controls. Collectively, these results indicated that *lnc-BAZ2B* exacerbates allergen-induced lung allergic inflammation by promoting M2 macrophage activation.

LncRNAs regulate gene expression by various mechanisms and have emerged as key modulators of both physiologic and pathologic processes.^{21,22,24,39,40} LncRNAs function as signals, decoys, guides, and scaffolds, and they modulate gene expression at the levels of transcription, translation, and mRNA stability.^{21,22} Here, we have explored the mechanisms by which *lnc-BAZ2B* regulates M2 macrophage activation in asthma. First, we found that *lnc-BAZ2B* was expressed in both the nucleus and cytoplasm at a ratio of 35%:65%. Moreover, we observed that the 5' end of *lnc-BAZ2B* is complementary to that of *BAZ2B* pre-mRNA, although the bases between nucleotides 854 and 1149 do not match. We also found that the expression level of *BAZ2B* was influenced by the expression of *lnc-BAZ2B*. These data indicate that *lnc-BAZ2B* might regulate *BAZ2B*, its *cis* gene. Furthermore, the results of the RNase protection assay and cell culture experiments suggested that *lnc-BAZ2B* and *BAZ2B* pre-mRNA can form a duplex, which enhances the stability of the *BAZ2B* pre-mRNA and further increases the expression level of *BAZ2B* mature mRNA. However, to further validate that *lnc-BAZ2B* and *BAZ2B* pre-mRNA can indeed form a duplex, fluorescence *in situ* hybridization should be performed in macrophages.^{22,41-43} In short, we newly identified that *lnc-BAZ2B* positively regulates its *cis* gene *BAZ2B* at the posttranscription level.

BAZ2B belongs to a family of bromodomain-containing proteins that can bind to acetylated lysine side chains in histones and other proteins in a substrate-specific manner.⁴⁴ However, unlike its subfamily member *BAZ2A*, the major subunit of nucleolar-remodeling complex (NoRC) transcriptional repressor complex, the biologic function of *BAZ2B* has remained uncharacterized.⁴⁵ Several studies have reported that *BAZ2B* specifically recognizes and binds H3K14ac,^{36,37} an active histone marker that is indispensable for the transcriptional activation of genes essential for embryonic development.^{46,47} Genome-wide H3K14ac and H3K9ac co-occur with the H3K4me3 and H3K27ac modifications in mouse embryonic stem cells.⁴⁸ In our study, we observed that *BAZ2B* promotes the expression of *IRF4*, but not *PPAR γ* , both of which are key transcription factors for M2 macrophage activation. Additionally, H3K14ac was enriched in the promoter region of *IRF4*, which suggests that *BAZ2B* may bind H3K14ac at the *IRF4* promoter and activate its transcription. However, further experiments, such as ChIP assay with antibodies for *BAZ2B*, are required to confirm this, although no antibodies for *BAZ2B* are currently available.

To further investigate the functions of *lnc-BAZ2B* and *BAZ2B*, we analyzed their transcriptional expression levels in another 50 PBMC samples from children with allergic asthma. The results showed that the expression level of *lnc-BAZ2B* was substantially higher in children with allergic asthma than in healthy children, whereas no difference was observed for the expression of *BAZ2B* at whole population level (see Fig E5). A possible explanation for this may be that besides being

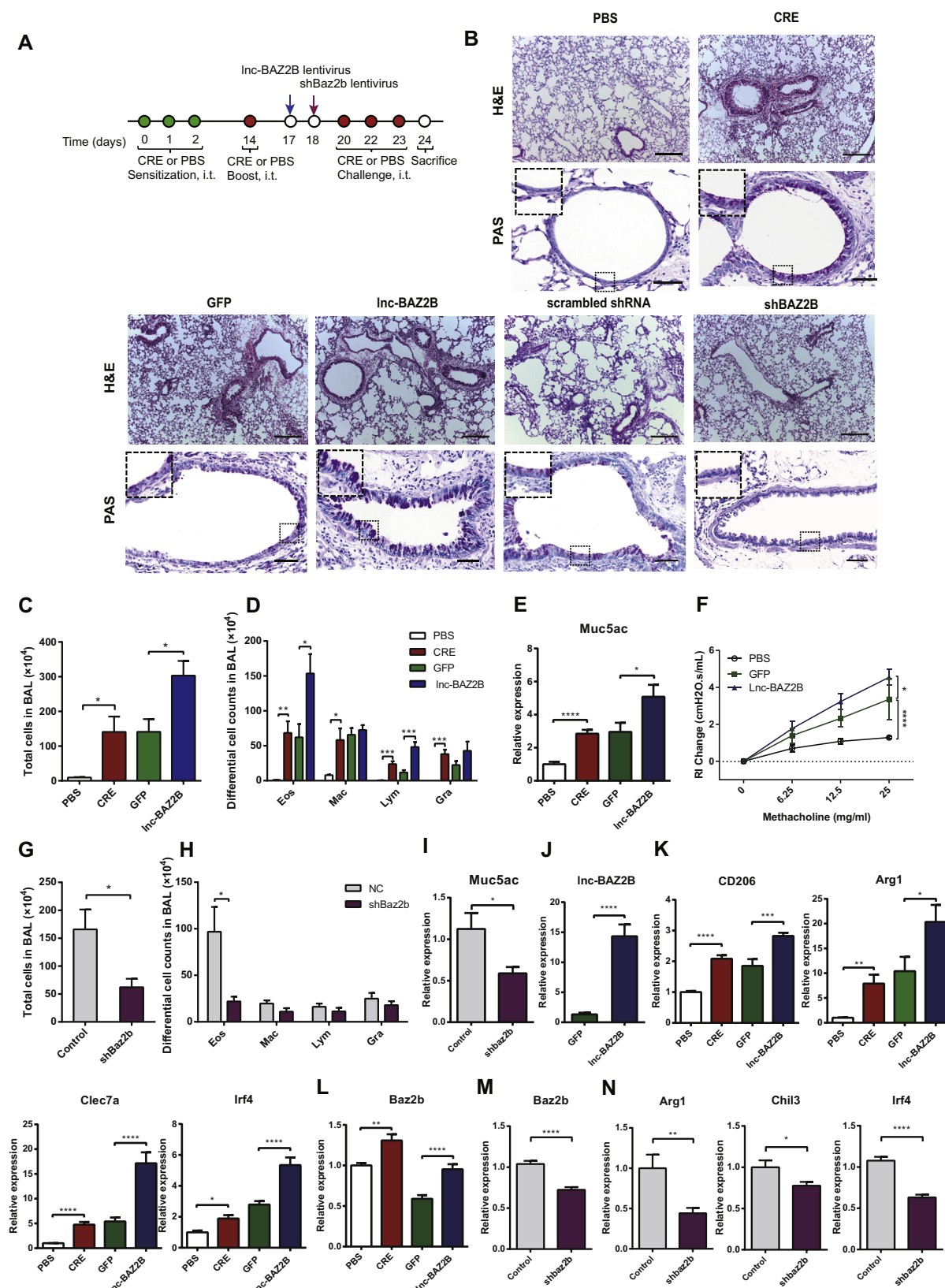


FIG 5. *Inc-BAZ2B* and *Baz2b* exacerbate CRE-induced lung inflammation in a mouse model of asthma. **A**, Schematic representation of the CRE-induced mouse model of asthma. Intratracheal (i.t.) instillation of 2×10^6 plaque-forming units of lentivirus of *Inc-BAZ2B* or the green fluorescent protein (GFP) control was performed on day 17 and sh-Baz2b lentivirus or negative control was performed on day 18. **B**, Lung sections stained by using the hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) methods.

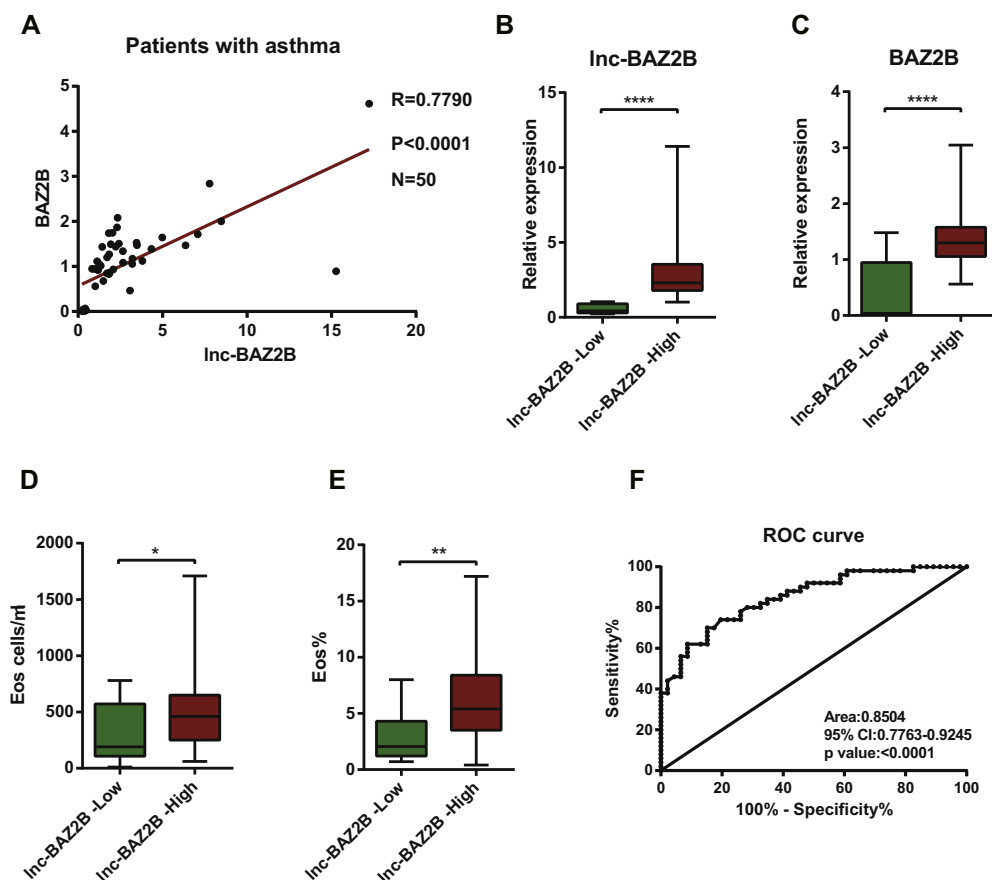


FIG 6. *Inc-BAZ2B* expression is correlated with that of *BAZ2B* in PBMCs from children with asthma. **A**, Correlation analysis of the expression levels of *Inc-BAZ2B* and *BAZ2B* in patients with allergic asthma (Spearman rank correlation). **B**, The expression level of *Inc-BAZ2B* in PBMCs from patients with asthma assessed by RT-qPCR was divided into high (value ≥ 1) or low (value < 1) expression groups. The average expression value of *Inc-BAZ2B* in healthy children was set at 1. The Mann-Whitney *U* test was performed for 2-group comparisons. **C**, The expression level of *BAZ2B* was quantified by RT-qPCR in PBMCs from patients with asthma with high or low *Inc-BAZ2B* expression levels. Blood eosinophil counts (**D**) and the percentage of eosinophils in white blood cells (Eos%) (**E**) in patients with asthma with high or low levels of *Inc-BAZ2B* expression. **F**, Receiver operating curve analysis (ROC) of the sensitivities and specificities of *Inc-BAZ2B* in the study population. The results are expressed as means $\pm$ SEMs. **P* < .05; ***P* < .01; *****P* < .0001.

upregulated by *Inc-BAZ2B* under IL-4 stimulation, *BAZ2B* expression may also be downregulated through other unknown mechanisms under LPS or IFN- γ stimulation in patients with asthma presenting with bacterial or viral infections. Intriguingly, patients with high *Inc-BAZ2B* expression levels also exhibited higher expression levels of *BAZ2B* and a higher blood eosinophil count.

In summary, we have shown for the first time that *Inc-BAZ2B* enhances the disease severity of allergic asthma by promoting

M2 macrophage activation. We also found that *Inc-BAZ2B* enhanced the stability of *BAZ2B* pre-mRNA through duplex formation. As a reader protein of the H3K14ac modification, *BAZ2B* subsequently increased the transcription of M2 macrophage marker genes, especially that of *IRF4*. Our *in vivo* results indicated that *Inc-BAZ2B* and *BAZ2B* have a crucial role in exacerbating the progression of allergic asthma and may serve as potential therapeutic and diagnostic targets in childhood asthma.

Scale bars = 200 μ m (H&E) and 50 μ m (PAS). Total cell counts (**C**) and differential cell counts (**D**) in BAL fluid. **E**, *Muc5ac* expression in the lungs of different groups of mice. **F**, Methacholine-induced airway hyperresponsiveness. Total cell counts (**G**) and differential cell counts (**H**) in BAL fluid in mice with *Baz2b* knockdown. **I**, *Muc5ac* expression in the lungs of mice with *Baz2b* knockdown. Controls, scrambled short hairpin RNAs (shRNAs). **J**, *Inc-BAZ2B* expression in the lungs of mice overexpressing *Inc-BAZ2B* via intratracheal instillation. *Mrc1*, *Arg1*, *Clec7a*, and *Irf4* (**K**) and *Baz2b* (**L**) expression in the lungs of different groups of mice. *Baz2b* (**M**) and *Arg1*, *Chil3*, and *Irf4* (**N**) expression in the lungs of mice with *Baz2b* knockdown. Data are represented as means $\pm$ SEMs from 3 independent experiments (*n* = 15-18 mice per group). **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

Key messages

- *Lnc-BAZ2B* exacerbated cockroach allergen-induced lung inflammation by promoting M2 macrophage activation.
- *Lnc-BAZ2B* promoted the expression of its *cis* target gene *BAZ2B* by stabilizing its pre-mRNA.
- *BAZ2B*, as a reader of H3K14ac modification, enhanced the transcription of *IRF4* and promoted M2 macrophage activation.

REFERENCES

- Global Initiative for Asthma. Global strategy for asthma management and prevention. 2018. Available at: www.ginasthma.org. Accessed August 27, 2018.
- Fahy JV. Type 2 inflammation in asthma—present in most, absent in many. *Nat Rev Immunol* 2015;15:57-65.
- Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. *Nat Rev Immunol* 2010;10:838-48.
- Saradna A, Do DC, Kumar S, Fu QL, Gao P. Macrophage polarization and allergic asthma. *Transl Res* 2018;191:1-14.
- Bosco MC. Macrophage polarization: Reaching across the aisle? *J Allergy Clin Immunol* 2019.
- Murray PJ. Macrophage polarization. *Annu Rev Physiol* 2017;79:541-66.
- Bouhelle MA, Derudas B, Rigamonti E, Dievart R, Brozek J, Haulon S, et al. PPAR- γ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* 2007;6:137-43.
- The Global Asthma Report 2018. Auckland, New Zealand: Global Asthma Network; 2018.
- Sha L, Shao M, Liu C, Li S, Li Z, Luo Y, et al. [The prevalence of asthma in children: a comparison between the year of 2010 and 2000 in urban China]. *Zhonghua Jie He He Hu Xi Za Zhi* 2015;38:664-8.
- Yang IV, Schwartz DA. Epigenetic mechanisms and the development of asthma. *J Allergy Clin Immunol* 2012;130:1243-55.
- Ivashkiv LB. Epigenetic regulation of macrophage polarization and function. *Trends Immunol* 2013;34:216-23.
- Yang X, Wang X, Liu D, Yu L, Xue B, Shi H. Epigenetic regulation of macrophage polarization by DNA methyltransferase 3b. *Mol Endocrinol* 2014;28:565-74.
- Ishii M, Wen H, Corsa CA, Liu T, Coelho AL, Allen RM, et al. Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* 2009;114:3244-54.
- Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 2010;11:936-44.
- Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. *Nat Rev Cancer* 2018;18:5-18.
- Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep* 2017;37:3-9.
- Turner M, Galloway A, Vigorito E. Noncoding RNA and its associated proteins as regulatory elements of the immune system. *Nat Immunol* 2014;15:484-91.
- Martinez-Nunez RT, Louafi F, Sanchez-Elsner T. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). *J Biol Chem* 2011;286:1786-94.
- Zhou Y, Do DC, Ishmael FT, Squadrito ML, Tang HM, Tang HL, et al. Mannose receptor modulates macrophage polarization and allergic inflammation through miR-511-3p. *J Allergy Clin Immunol* 2018;141:350-64.e8.
- Wang C, Zhang C, Liu L, A X, Chen B, Li Y, et al. Macrophage-derived mir-155-containing exosomes suppress fibroblast proliferation and promote fibroblast inflammation during cardiac injury. *Mol Ther* 2017;25:192-204.
- Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell* 2011;43:904-14.
- Hu W, Alvarez-Dominguez JR, Lodish HF. Regulation of mammalian cell differentiation by long non-coding RNAs. *EMBO Rep* 2012;13:971-83.
- Wapinski O, Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol* 2011;21:354-61.
- Zhang Y, Cao X. Long noncoding RNAs in innate immunity. *Cell Mol Immunol* 2016;13:138-47.
- Du M, Yuan L, Tan X, Huang D, Wang X, Zheng Z, et al. The LPS-inducible lncRNA Mirt2 is a negative regulator of inflammation. *Nat Commun* 2017;8:2049.
- Sigdel KR, Cheng A, Wang Y, Duan L, Zhang Y. The emerging functions of long noncoding RNA in immune cells: autoimmune diseases. *J Immunol Res* 2015;2015:848790.
- Chi X, Ding B, Zhang L, Zhang J, Wang J, Zhang W. lncRNA GAS5 promotes M1 macrophage polarization via miR-455-5p/SOCS3 pathway in childhood pneumonia. *J Cell Physiol* 2018.
- Cao J, Dong R, Jiang L, Gong Y, Yuan M, You J, et al. lncRNA-MM2P identified as a modulator of macrophage M2 polarization. *Cancer Immunol Res* 2018.
- Subspecialty Group of Respiratory Diseases, Society of Pediatrics, Chinese Medical Association; Editorial Board, Chinese Journal of Pediatrics. [Guideline for the diagnosis and optimal management of asthma in children (2016)]. *Zhonghua Er Ke Za Zhi* 2016;54:167-81.
- Hong J, Bao Y, Chen A, Li C, Xiang L, Liu C, et al. Chinese guidelines for childhood asthma 2016: major updates, recommendations and key regional data. *J Asthma* 2018;55:1138-46.
- Grob M, Schmid-Grendelmeier P, Joller-Jemelka HI, Ludwig E, Dubs RW, Grob PJ, et al. Altered intracellular expression of the chemokines MIP-1 α , MIP-1 β and IL-8 by peripheral blood CD4 $^{+}$ and CD8 $^{+}$ T cells in mild allergic asthma. *Allergy* 2003;58:239-45.
- Hsieh KH, Chou CC, Chiang BL. Immunotherapy suppresses the production of monocyte chemotactic and activating factor and augments the production of IL-8 in children with asthma. *J Allergy Clin Immunol* 1996;98:580-7.
- Charrad R, Kaabachi W, Raftai A, Berraies A, Hamzaoui A. IL-8 gene variants and expression in childhood asthma. *Lung* 2017;195:749-57.
- Jiang XG, Yang XD, Lv Z, Zhuang PH. Elevated serum levels of TNF- α , IL-8, and ECP can be involved in the development and progression of bronchial asthma. *J Asthma* 2018;55:111-8.
- Girodet PO, Nguyen D, Mancini JD, Hundal M, Zhou X, Israel E, et al. Alternative macrophage activation is increased in asthma. *Am J Respir Cell Mol Biol* 2016;55:467-75.
- Kostrhon S, Kontaxis G, Kaufmann T, Schirghuber E, Kubicek S, Konrat R, et al. A histone-mimicking interdomain linker in a multidomain protein modulates multivalent histone binding. *J Biol Chem* 2017;292:17643-57.
- Ferguson FM, Dias DM, Rodrigues JP, Wienk H, Boelens R, Bonvin AM, et al. Binding hotspots of BAZ2B bromodomain: histone interaction revealed by solution NMR driven docking. *Biochemistry* 2014;53:6706-16.
- Han X, Huang S, Xue P, Fu J, Liu L, Zhang C, et al. lncRNA PTPRE-AS1 modulates M2 macrophage activation and inflammatory diseases by epigenetic promotion of PTPRE. *Sci Adv* 2019;5:eaax9230.
- Qiu YY, Wu Y, Lin MJ, Bian T, Xiao YL, Qin C. lncRNA-MEG3 functions as a competing endogenous RNA to regulate Treg/Th17 balance in patients with asthma by targeting microRNA-17/ ROR γ mat. *Biomed Pharmacother* 2019;111:386-94.
- Fan M, Xu J, Xiao Q, Chen F, Han X. Long non-coding RNA TCF7 contributes to the growth and migration of airway smooth muscle cells in asthma through targeting TIMMDC1/Akt axis. *Biochem Biophys Res Commun* 2019;508:749-55.
- Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, et al. lncRNA-p21 suppresses target mRNA translation. *Mol Cell* 2012;47:648-55.
- Gong C, Maquat LE. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3' UTRs via Alu elements. *Nature* 2011;470:284-8.
- Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 2010;39:925-38.
- Filippakopoulos P, Picaut S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, et al. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* 2012;149:214-31.
- Guett C, Lienemann P, Sirri V, Grummt I, Hernandez-Verdun D, Hottiger MO, et al. The NoRC complex mediates the heterochromatin formation and stability of silent rRNA genes and centromeric repeats. *EMBO J* 2010;29:2135-46.
- Kueh AJ, Dixon MP, Voss AK, Thomas T. HBO1 is required for H3K14 acetylation and normal transcriptional activity during embryonic development. *Mol Cell Biol* 2011;31:845-60.
- Mishima Y, Miyagi S, Saraya A, Negishi M, Endo M, Endo TA, et al. The Hbo1-Brd1/Brpf2 complex is responsible for global acetylation of H3K14 and required for fetal liver erythropoiesis. *Blood* 2011;118:2443-53.
- Karmodiya K, Krebs AR, Oulad-Abdelghani M, Kimura H, Tora L. H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. *BMC Genomics* 2012;13:424.

METHODS

Human study subjects

A total of 54 children primarily diagnosed with asthma were recruited from the Children's Hospital of Fudan University. Asthma diagnosis was based on the 2016 edition of the Guidelines for the Diagnosis and Prevention of Childhood Bronchial Asthma of China.^{E1,E2} A total of 50 age- and sex-matched healthy children without a personal or family history of allergies were recruited as controls. Patients' information is listed in the Table E1. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation solution (General Electric Company, New York, NY). Briefly, peripheral blood was mixed with PBS and overlaid onto Ficoll (density = 1.077 g/mL). Following centrifugation at 400 g for 30 min, the PBMCs were aspirated from the Ficoll-plasma interface. The cell pellet was washed twice with PBS and resuspended in TRIzol reagent (Thermo Fisher Scientific, Carlsbad, Calif). The study was approved by the research ethics board of the Children's Hospital of Fudan University. Informed consent was obtained from each of the study participants.

RNA extraction and RT-qPCR

Total cellular RNA was extracted by using TRIzol reagent, followed by reverse transcription of mRNAs and lncRNAs by using the PrimeScript II 1st Strand cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's instructions. RT-qPCR was performed to measure the expression levels of mRNAs and lncRNAs by using SYBR Premix Ex Taq II (Takara) in a LightCycler 480 real-time PCR system (Roche, Basel, Switzerland). β -Actin was used as an internal control. Differences in expression levels (FCs) were calculated by using the $2^{-\Delta\Delta CT}$ method. The sequences of primers used for qPCR are listed in Table E2 (in this article's Online Repository at www.jacionline.org).

Microarray analysis

Microarray analysis was performed by Shanghai Biotechnology Corporation (Shanghai, China). Specifically, total RNA was extracted and purified by using the miRNeasy Mini Kit (catalog no. 217004, QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. RNA integrity was assessed with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, Calif). Then, total RNA was amplified and labeled by using a Low Input Quick Amp Labeling Kit, One-Color (catalog no. 5190-2305, Agilent Technologies) following the manufacturer's instructions. Labeled RNA was purified by using a RNeasy Mini Kit (catalog no. 74106, QIAGEN). Subsequently, each slide was hybridized with 1.65 μ g of Cy3-labeled cRNA by using a Gene Expression Hybridization Kit (catalog no. 5188-5242, Agilent Technologies) in a hybridization oven (catalog no. G2545A, Agilent Technologies) according to the manufacturer's instructions. After 17 hours of hybridization, the slides were washed in staining dishes (catalog no. 121, Thermo Shandon, Waltham, Mass) by using a Gene Expression Wash Buffer Kit (catalog no. 5188-5327, Agilent Technologies) following the manufacturer's instructions. Finally, slides were scanned on an Agilent Microarray Scanner (catalog no. G2565CA, Agilent Technologies) with default settings (dye channel, green; scan resolution, 3 μ m; PMT, 100%; 20 bits). Data were extracted with Feature Extraction software, version 10.7 (Agilent Technologies). Raw data were normalized by using the Quantile algorithm of the R package limma. The microarray data have been deposited in the Gene Expression Omnibus catalog under accession number GSE143192.

Cell culture

The THP1, RAW264.7, and HEK293T cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, Va). THP1 cells were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10% inactivated FBS and 1% penicillin/streptomycin (both from Gibco). RAW264.7 cells were cultured in Dulbecco modified Eagle medium with high glucose (4.5 g/L) supplemented with 10% FBS and 1% penicillin/streptomycin. (Gibco, Thermo Fisher Scientific). HEK293T cells were maintained in Dulbecco modified Eagle medium supplemented with 10%

FBS and 1% penicillin/streptomycin. To differentiate human THP1 monocytes to macrophages, 50 ng/mL of phorbol-12-myristate-13-acetate was added to the cells, followed by incubation for 48 hours.

siRNAs, short hairpin RNAs, and plasmids

siRNAs and short hairpin RNAs (shRNAs) for *lncRNA-BAZ2B*, *BAZ2B*, and *STAT6* were designed by GenePharma (Shanghai, China). Specific primers for siRNAs and short hairpin RNAs are listed in Table E2. To generate plasmids containing full-length *lnc-BAZ2B*, the full-length sequence of *lnc-BAZ2B* was amplified by using the primers 5'-CCGCTCGAGGGGG TAGCGACAAGAAGAT-3' (forward) and 5'-CGAGCTCTAATAAT TAAAAACATATCAGCACAC-3' (reverse) and cloned into the pENTR4 no ccdB vector (686-1; Addgene). Then, the *lnc-BAZ2B* sequence was subcloned into the pLenti CMV Hygro DEST vector (w117-1; Addgene) by using Gateway LR Clonase II Enzyme mix (Thermo Fisher Scientific). Lentiviruses for *lnc-BAZ2B* overexpression or *BAZ2b* knockdown (sh-Baz2b) were packaged by Zorin, Shanghai, China.

Cell transfection

Transfection of siRNAs or transient transfection of the ectopic *lnc-BAZ2B* expression vector was performed by using Lipofectamine RNAiMAX Transfection Reagent or Lipofectamine 2000 Reagent (Thermo Fisher Scientific) following the manufacturer's instructions.

Western blot

Total protein was extracted with radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors (Thermo Fisher Scientific) and quantified by using the Protein BCA Assay Kit (Bio-Rad, Hercules, Calif). Antibodies against STAT6, phospho-STAT6 (Tyr641), IRF4, and PPAR γ were purchased from Cell Signaling Technology (Boston, Mass). Antibodies against β -tubulin were purchased from Abcam (Cambridge, UK).

RNase protection assay

Total RNA from THP1-derived macrophages was extracted and incubated with reaction buffer for 1 hour at 37°C. The reaction buffer contained 10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 5 mM EDTA (pH 7.5), and double-distilled H₂O. Then, RNA samples were incubated with an RNase Cocktail Enzyme Mix (Thermo Fisher Scientific) for 30 minutes at 37 °C. The quantity of RNA in each sample was assessed by RT-qPCR.

Subcellular fractionation and localization

Nuclear and cytosolic fractions were separated by using a Nuclear and Cytoplasmic Extraction Kit (Cwbio, Shanghai, China). A total of 1×10^7 THP1-derived macrophages cells were harvested, resuspended in 1 mL of Nc buffer A and 55 μ L of Nc Buffer B, and incubated for 20 minutes on ice. Cells were then centrifuged for 15 minutes at 12,000 g, and the resulting supernatants containing the cytoplasmic component and pellets containing the nuclear component were used for RNA extraction.

Isolation of human monocytes and T and B cells

CD14⁺, CD3⁺, and CD19⁺ cells were sorted from PBMCs by using a magnetic cell sorting system according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Sorted cells were subjected to RNA extraction and RT-qPCR.

ChIP assays

THP1-derived macrophages were stimulated by IL-4 (20 ng/mL) for 24 hours, after which ChIP was performed by using a SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology) according to the manufacturer's instructions. Anti-H3K14ac antibodies were purchased from

Cell Signaling Technology. Normal rabbit IgG was used as a negative control. Primer sequences for ChIP-qPCR are listed in Table E2.

Murine model of asthma

Female C57BL/6 mice, 6 to 8 weeks old, were purchased from Shanghai Slack Laboratory Animal Company (Shanghai, China) and housed in pathogen-free facilities. All of the animal experiments complied with all relevant ethical regulations for animal testing and research and were performed in accordance with protocols approved by the Committee on Animal Research and Ethics of Fudan University. The asthma model was created according to our previously published method.^{E3,E4} In brief, mice were sensitized by intratracheal instillation of 20 µg of CRE (Greer Laboratories, Lenoir, NC) per mouse on days 0, 1, 2; boosted on day 14; and challenged on days 20 and 22 with the same amount of CRE. Control mice received PBS during the sensitization and challenge phases. In several groups of mice, intratracheal instillation of 2×10^6 plaque-forming units of lentivirus for overexpression of *lnc-BAZ2B* or green fluorescent protein (control) was performed on day 17. Similarly, intratracheal instillation of 2×10^6 plaque-forming units of sh-Baz2b lentivirus or negative control was carried out on mice on day 18 (see Fig 5, A in the print text).

Collection and analyses of cells from BALF

On day 23, all of the mice were humanely killed, and bronchoalveolar lavage fluid (BALF) was harvested by 2 consecutive flushes with 0.8 mL of ice-cold PBS. After centrifugation, the supernatant was kept at -20°C and the cell pellets were resuspended in an appropriate amount of PBS. The total cell numbers were counted by using a Countess II Automated Cell Counter. Differential cell counts in BALF were obtained by flow cytometry. The following antibodies (Thermo Fisher Scientific) and their respective isotype controls were used to stain the cells of the BALF: phycoerythrin-conjugated anti-Siglec F, fluorescein isothiocyanate-conjugated anti- α -MAC3, eFluor 780-conjugated anti-Gr-1, and phycoerythrin-cyanine-5.5-conjugated anti-CD3e/CD19. Eosinophils were defined as SSC^{high} SiglecF⁺ MAC3⁺ cells, alveolar macrophages were identified as SSC^{high} SiglecF⁺ MAC3⁺ cells, granulocytes were identified as SSC^{high} Gr-1⁺ cells, and lymphocytes were identified as $\text{FSC}^{\text{low}}/\text{SSC}^{\text{low}}$ cells that also expressed CD3 or CD19.^{E3,E4}

Lung pathology

After euthanasia, mouse lungs were perfused with 10 mL of ice-cold PBS injected into the right ventricle followed by excision and fixation in 10% neutral buffered formalin. Sections (5 µm) were prepared from paraffin-embedded lungs and stained by using the hematoxylin and eosin and periodic acid-Schiff methods, respectively, according to the manufacturer's protocols (Wuhan Servicebio Technology, China).

Airway hyperresponsiveness

Invasive measurements of airway responsiveness were made on a FinePointe Resistance and Compliance System (Buxco Research Systems, Wilmington, NC), as described previously.^{E5,E6} Briefly, mice were anesthetized with phenobarbital sodium (0.5 µg/g body weight). After induction of anesthesia, the mouse tracheas were cannulated with a steel 18-gauge cannula. The mice were mechanically ventilated at a rate of 160 breaths per minute and tidal volume of 0.15 mL in a closed chamber. After stabilization for 3 minutes, PBS or increasing concentrations of aerosolized methacholine were nebulized into the mouse airway. Finally, airway resistance was calculated by using FinePointe software (Buxco Research Systems). Briefly, the normalization function of the software was used and data were represented as deviations from 0 mg/mL. Regular 2-way ANOVA was performed.

Statistical analysis

Results from 3 independently repeated experiments were analyzed and calculated by using GraphPad Prism software, version 6.0 (GraphPad Software, La Jolla, Calif) and data were presented as means plus or minus SEMs for each group. A 2-tailed Student *t* test or Mann-Whitney *U* test was performed for 2-group comparisons, and regular 2-way ANOVA was performed for comparisons between more than 2 groups. The relationship between *lnc-BAZ2B* and *BAZ2B* was tested by using Spearman rank correlation. *P* values of .05 or less were deemed significant.

REFERENCES

- E1. Subspecialty Group of Respiratory Diseases, Society of Pediatrics, Chinese Medical Association; Editorial Board, Chinese Journal of Pediatrics. [Guideline for the diagnosis and optimal management of asthma in children (2016)]. *Zhonghua Er Ke Za Zhi* 2016;54:167-81.
- E2. Hong J, Bao Y, Chen A, Li C, Xiang L, Liu C, et al. Chinese guidelines for childhood asthma 2016: major updates, recommendations and key regional data. *J Asthma* 2018;55:1138-46.
- E3. Han X, Huang S, Xue P, Fu J, Liu L, Zhang C, et al. *lncRNA PTPRE-AS1* modulates M2 macrophage activation and inflammatory diseases by epigenetic promotion of PTPRE. *Sci Adv* 2019;5:eaax9230.
- E4. Zhou Y, Do DC, Ishmael FT, Squadrito ML, Tang HM, Tang HL, et al. Mannose receptor modulates macrophage polarization and allergic inflammation through miR-511-3p. *J Allergy Clin Immunol* 2018;141:350-64.e8.
- E5. Poole JA, Wyatt TA, Oldenburg PJ, Elliott MK, West WW, Sisson JH, et al. Intranasal organic dust exposure-induced airway adaptation response marked by persistent lung inflammation and pathology in mice. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L1085-95.
- E6. Sun L, Fu J, Lin SH, Sun JL, Xia L, Lin CH, et al. Particulate matter of 2.5 µm or less in diameter disturbs the balance of T_H17/regulatory T cells by targeting glutamate oxaloacetate transaminase 1 and hypoxia-inducible factor 1alpha in an asthma model. *J Allergy Clin Immunol* 2020;145:402-14.

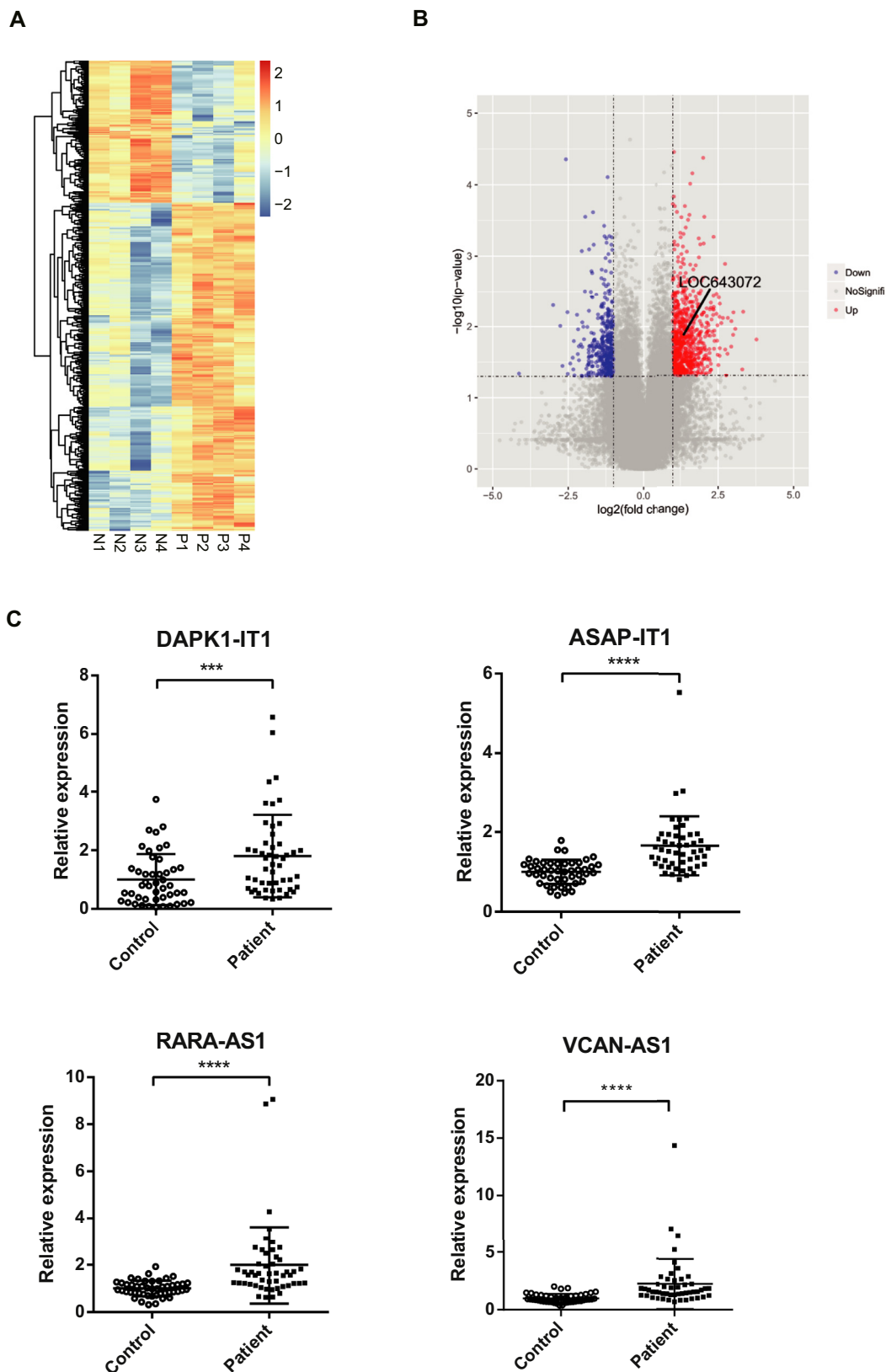


FIG E1. The profile of differential lncRNA expression in children with asthma. **A**, A hierarchical clustering heatmap of dysregulated lncRNA expression in PBMCs between children with asthma (4 patient samples: P1, P2, P3, and P4) and healthy children (4 control samples: N1, N2, N3, and N4) ($P < .05$; $\text{FC} \geq 2$). **B**, The differentially expressed lncRNAs are represented by a volcano map. **C**, Validation of the expression levels of *DAPK1-IT1*, *ASAP1-IT1*, *RARA-AS1*, and *VCAN-AS1* in PBMCs from children with asthma (n = 50) or healthy children (n = 46) by RT-qPCR. The Mann-Whitney U test was performed for 2-group comparisons. The results are expressed as means $\pm$ SEMs. *** $P < .001$; **** $P < .0001$.

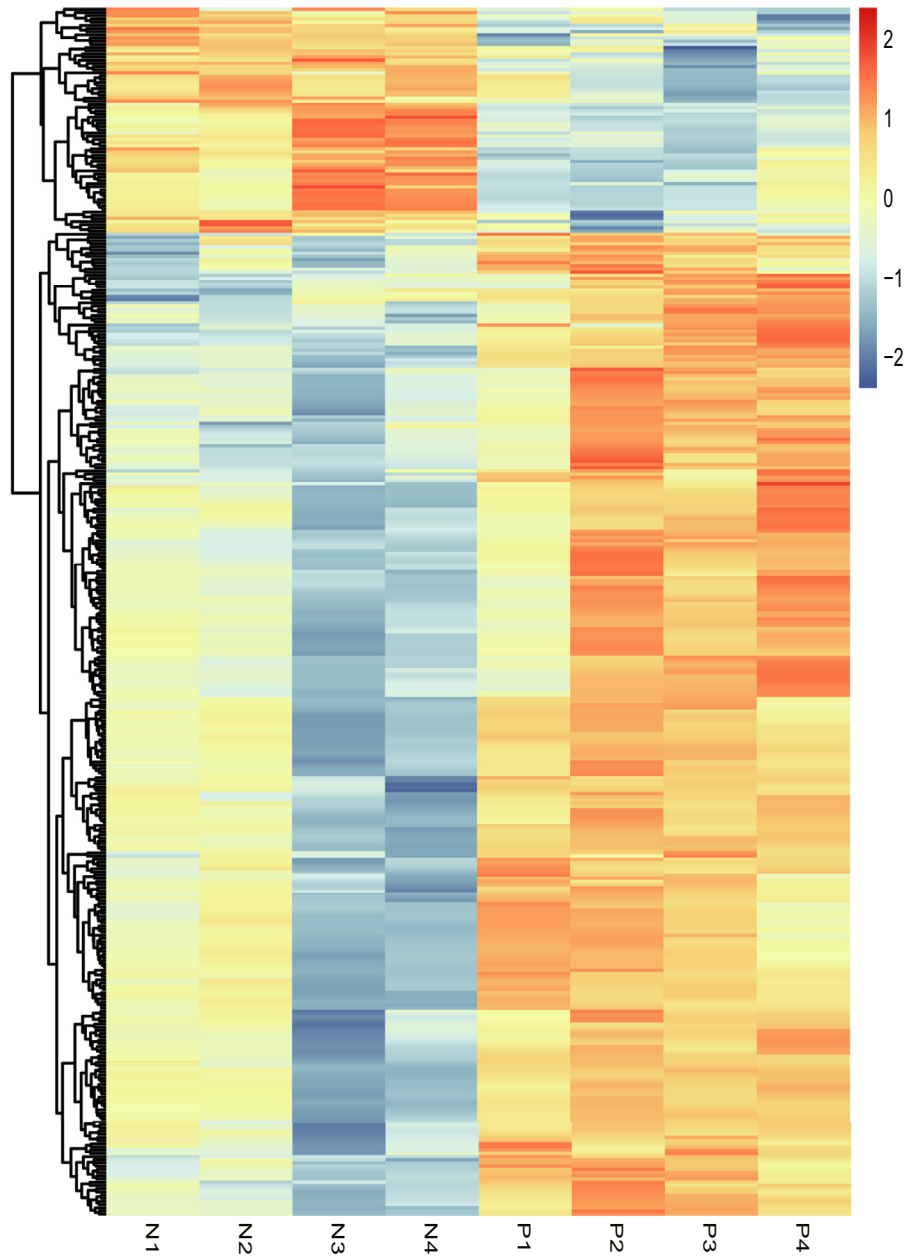


FIG E2. A hierarchical clustering heatmap of dysregulated mRNA expression in PBMCs between children with asthma. (4 patients: P1, P2, P3, and P4) and healthy children (4 controls: C1, C2, C3, and C4) ($P < .05$; $FC \geq 2$).

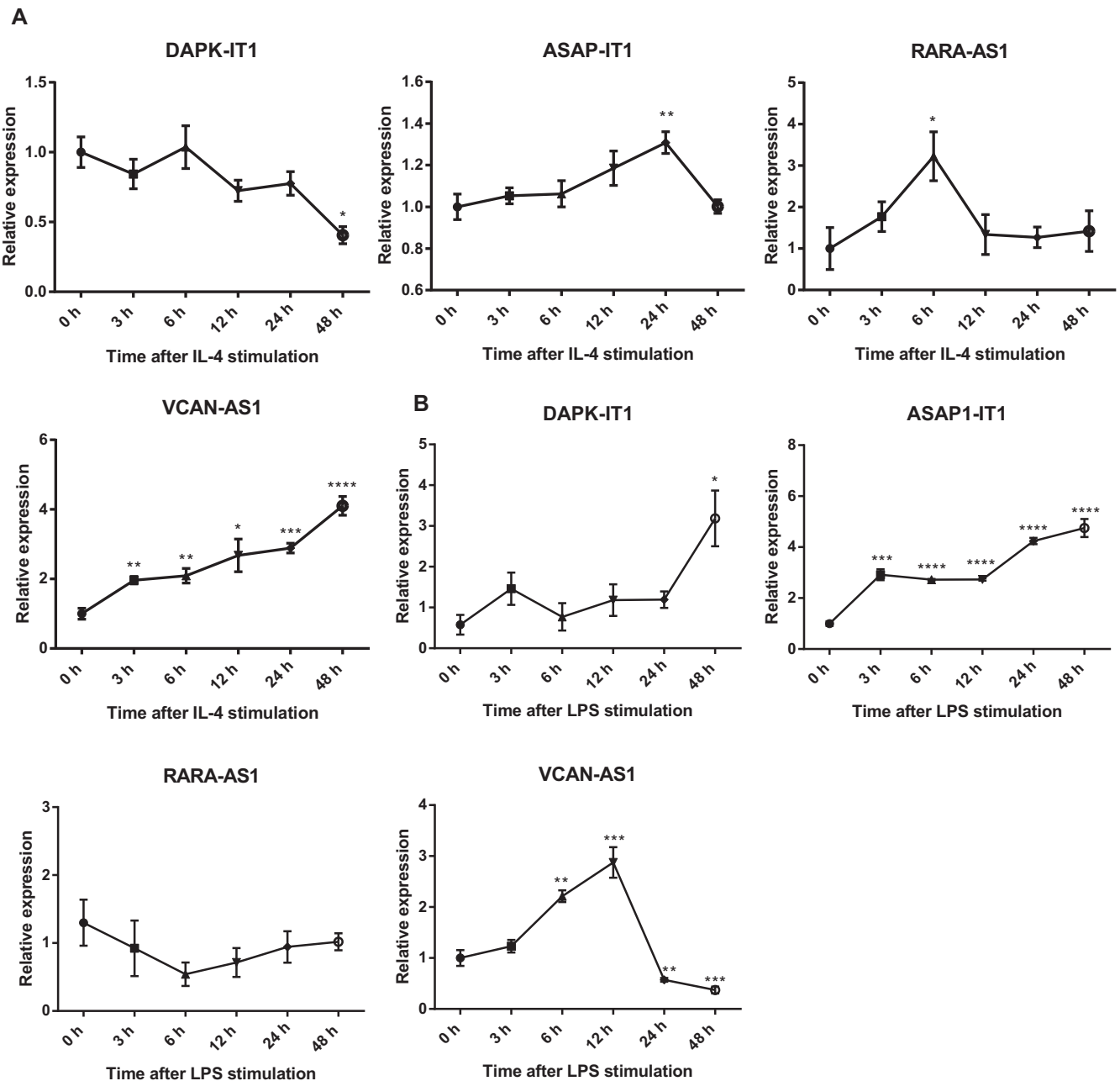


FIG E3. Dynamic expression of lncRNAs during macrophage activation. RT-qPCR was used to measure the expression levels of *DAPK1-IT1*, *ASAP1-IT1*, *RARA-AS1*, and *VCAN-AS1* in THP1-derived macrophages stimulated with IL-4 (20 ng/mL) (A) or LPS (100 ng/mL) (B) for 0 to 48 hours. The results are expressed as means $\pm$ SEMs. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

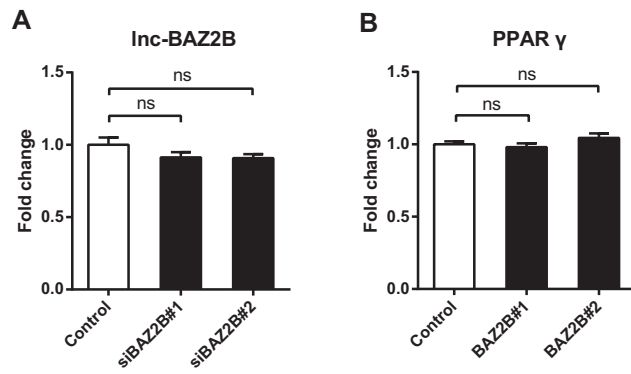


FIG E4. Knockdown of *BAZ2B* in macrophages did not affect the expression of *PPAR γ* . RT-qPCR was used to measure the expression levels of *Inc-BAZ2B* (**A**) and *PPAR γ* (**B**) in THP1-derived macrophages following *BAZ2B* knockdown by 2 siRNAs. The results are expressed as means $\pm$ SEM. *ns*, Not significant by the Student *t* test.

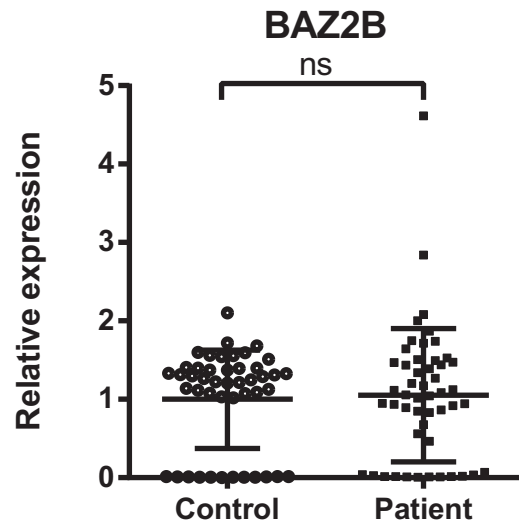


FIG E5. There were no differences in the expression level of *BAZ2B* between children with asthma and healthy children at the whole population level. Validation of the expression level of *BAZ2B* in PBMCs from children with asthma ($n = 50$) or healthy children ($n = 46$) by RT-qPCR. The results are expressed as means $\pm$ SEMs. The Mann-Whitney U test was performed for 2-group comparisons. *ns*, Not significant.

TABLE E1. Demographic and respiratory health characteristics of patients with allergic asthma and healthy controls

Characteristic	Normal (n = 46)	Asthma (n = 50)
Male/female, no.	37/9	37/13
Age (y), mean $\pm$ SD	8.98 $\pm$ 3.84	8.02 $\pm$ 1.92
Asthma duration, mo	Not available	10.92 $\pm$ 14.39
Blood eosinophils (cells/uL)	Not done	569.00 $\pm$ 416.71
Total IgE (kU/L)	Not done	156.29 $\pm$ 91.26
C-ACT score	Not done	17.4 $\pm$ 3.9

C-ACT, Childhood Asthma Control Test.

Values are presented as means $\pm$ SDs.

TABLE E2. The sequences of primers

Primers for q-PCR		
Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>CCL18</i>	GTTGACTATTCTGAAACCAGCCC	GTCGCTGATGTATTTCTGGACCC
<i>IRF4</i>	GAACGAGGAGAAGAGCATCTTCC	CGATGCCTTCTCGGAACCTTCC
<i>PPARG</i>	AGCCTGCGAAAGCCTTTTGGTG	GGCTTCACATTACGAAACCTGG
<i>CLEC7A</i>	ACAATGCTGGCAACTGGGCTCT	AGAGCCATTGGTACCTCAGTCTG
<i>IL1RA</i>	GTGCTTTGGTACAGGGATTCTTG	CACAGTCAGAGGTAGACCCCTTC
<i>CD206/MRC1</i>	AGCCAACACCAGCTCCTCAAGA	CAAAACGCTCGCGCATTGTCCA
<i>STAT6</i>	CCTTGAGAACAGCATTCCTGG	GCACTTCTCCTCTGTGACAGAC
<i>GAPDH</i>	CGGAGTCAACGGATTGGTGC	TCTCGCTCCTGGAAGATGGTGAT
<i>LOC643072</i>	GCTGACATAGACCTTCCAGAAAGCAAA	GGACTTCGCTGGAGTAGGGC
<i>RARA-AS1</i>	GAAACGCAGGGAACCGAGAG	GGCGAGTTCAGCGAGAGTTC
<i>DAPK1-IT1</i>	GATGGAGTCTCGGTCTGT	GGGTTCCTTTTGTGT
<i>ASAP1-IT1</i>	GCAGCTGACAAGTGTGACCA	CACCCAGAATGCCACCCAAT
<i>BAZ2B</i>	GCGTAAACTAGAGCAGCGAAGAT	CCTGGAATACGAGGCAACTCTG
<i>MSR1</i>	TGCACAAGGCAGCTCACTTTGG	GTGCAAGTGACTCCAGCATCTTC
<i>MMP9</i>	GCCACTACTGTGCCTTTGAGTC	CCCTCAGAGAATCGCCAGTACT
<i>CCL24</i>	TGAGAACCGAGTGGTCAGCTAC	TTCTGCTTGGCGTCCAGGTTCT
<i>BAZ2B pre-mRNA</i>	TAAGGAGGTGGTGGGGTTTC	CAGTTTCTCCGCTGCTGTTT
<i>NEAT1</i>	GCTGGACCTTTCATGTAACGGG	TGAACCTCTGCCGGTACAGGGAA
<i>Baz2b</i>	TAGCAGAAGCTCGCAGTG	AGCCGTCGCAGAGTAGA
<i>CD206/Mrc1</i>	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
<i>Clec7a</i>	CCAGCTAGGTGCTCATCTACTG	CCTTCACTCTGATTGCGGGAAAG
<i>Arg1</i>	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA
<i>Irf4</i>	GAACGAGGAGAAGAGCGTCTTC	GTAGGAGGATCTGGCTTGTCGA
<i>Gapdh</i>	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTACG
<i>Pparg</i>	GTA CTGTCGGTTTCAAGAAGTGCC	ATCTCCGCCAACAGCTTCTCCT
Sequences of siRNAs		
Gene	Sense (5'-3')	Antisense (5'-3')
<i>LOC643072-homo-2127</i>	GGAGGAUCUUAUUAUAUTT	AUACUAAUGAAGAUCUCCTT
<i>BAZ2B-homo-2956</i>	CCAGAUAGACAACGAGCAATT	UUGCUCGUUGUCUAUCUGGTT
<i>BAZ2B-homo-6854</i>	GCCACAAUAUGAGGAAGUATT	UACUCCUCAUAUUGUGGCTT
<i>IRF4-homo-409</i>	GCGCUUUGAACAAGAGCAATT	UUGCUCUUGUCAAAGCGCTT
<i>IRF4-homo-1445</i>	CCGCCAUUCCUCUAUUAATT	UUGAAUAGAGGAAUGGCGGTT
<i>Baz2b-mus-1</i>	GCAAGAGAAACGUGAUGAGTT	CUCAUCACGUUUCUCUUGCTT
<i>Baz2b-mus-2</i>	AUCACUUGUGUAGUAAAUTT	AUUUAACUACACAAGUGAUTT
Sequences of short hairpin RNAs		
Genes	Sense (5'-3')	Antisense (5'-3')
<i>STAT6</i>	GTCGCAGTTCAACAAGGAGAT	ATCTCCTTGTTGAACTGCGAC
Primers for ChIP-qPCR		
Name	Forward primer (5'-3')	Reverse primer (5'-3')
-1893	ATGACTAAACCCGGTGCAGA	AGAGGAGTTTGCTGAGGACA
-1431	TGGTTAGGAGACATTGACCCA	TTTTAGACCACCACCACCGT
-1231	GGTGAAAGCTCAGGGGGTTA	AAGTGGACCAGGGATTTTACAT
-850	TAACTGCTTTCTCCAGGTTGA	CTCCCTAAGGATCCAAGTGC
-648	ATGTCAGACACGCAGAGACAG	CTCCGCGGTGTTTAGAGAAC
-485	CAACCTCCACCTCCAGTTCT	GCGTGTAGTAGCGGGAATCT
-360	TGATTGACTGGCTCCTGAG	CTGTCACTGGGGCCGTTT
-13	ACCTCGCACTCTCAGTTTCA	GTATCAGCCTCACACCCCTC
Primers for RNase protection assay		
Names	Forward primer (5'-3')	Reverse primer (5'-3')
O1	CCTCTACCAACACTGTGGCG	GCAAGGCAATGGGAGGGAA
O2	GCCTGCCTTTGAAACAGGGT	CAACAGGCCACCACAACACA
U1	TCTGCTGCTGGATAGGA	GTTGCGGTAGATGGGAT
U2	ATCTCCTGCTTCAATCTGC	TTTTGCCTTTCCCCA