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Supplementary Materials for

LncRNA *PTPRE-AS1* modulates M2 macrophage activation and inflammatory diseases by epigenetic promotion of PTPRE

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This PDF file includes:

Supplementary Results

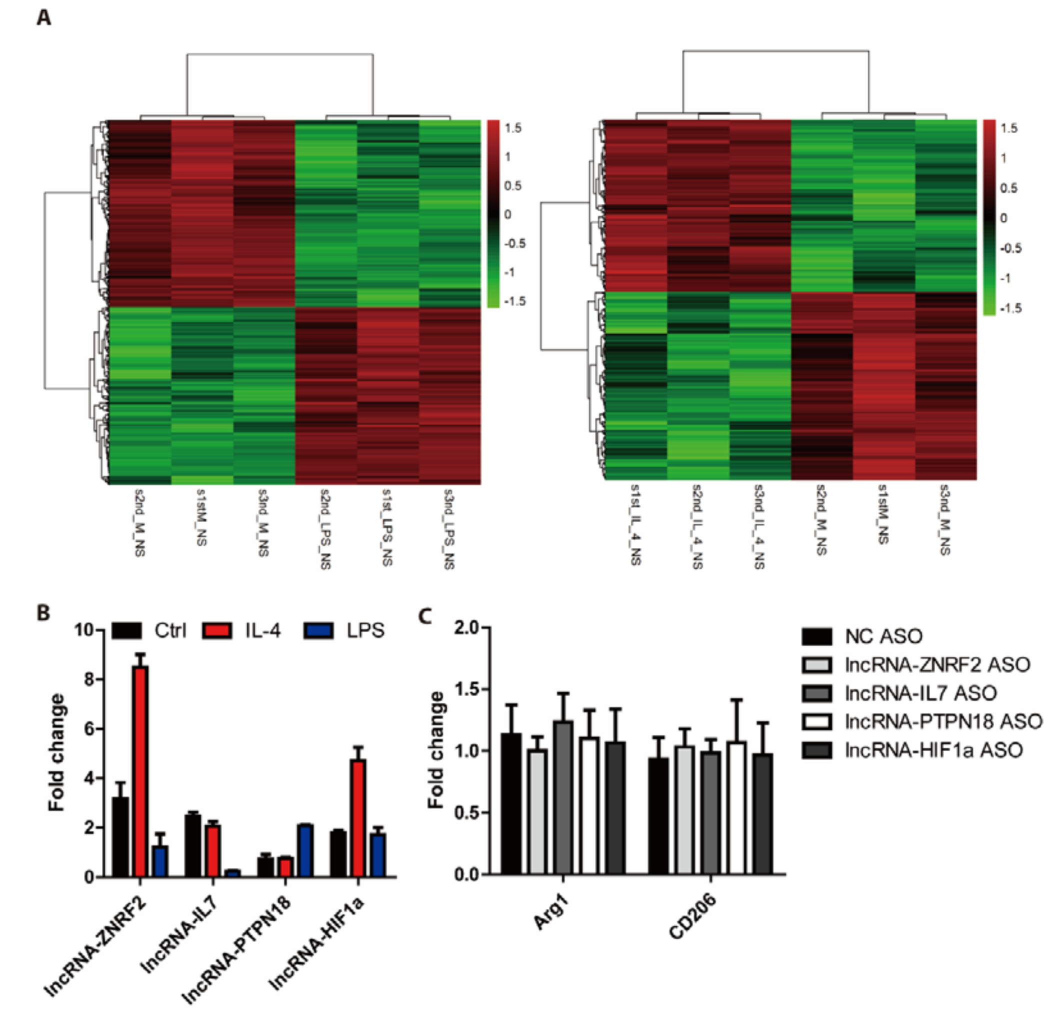


Fig. S1. Differentially expressed lncRNAs in LPS- and IL-4–stimulated BMDMs. (**A**) Heatmap of lncRNAs with significantly changed levels upon LPS (200 ng/ml, 3 h) and IL-4 (20 ng/ml, 24 h) stimulation of BMDMs. (**B**) The 5 candidate differentially expressed antisense lncRNAs in BMDMs that were identified by microarray analysis were validated by RT-qPCR. (**C**) M2-associated gene expression levels in RAW 264.7 cells transfected with lncRNAs ASO after IL-4 treatment were quantified by q-PCR.

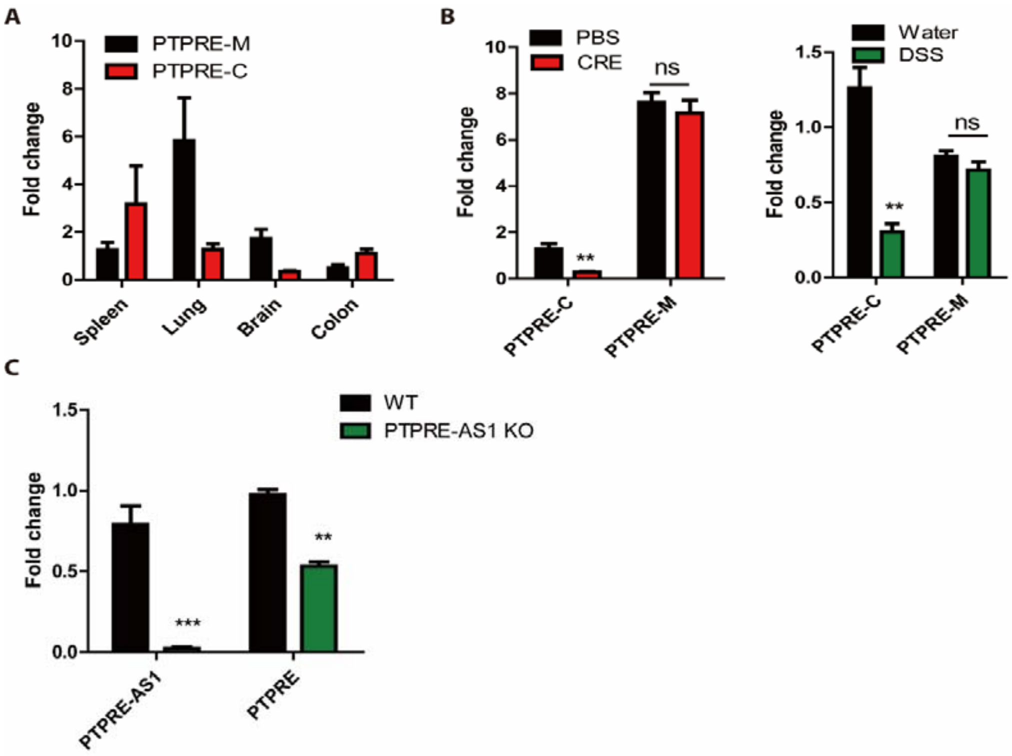


Fig. S2. *PTPRE-AS1* mediates *PTPRE* expression. (**A**) Distribution of two variant PTPRE forms, *PTPRE-M* and *PTPRE-C*, in mouse tissues. (**B**) RT-qPCR analysis of *PTPRE-M* and *PTPRE-C* levels in lung tissues from CRE-induced allergic asthma and colon tissues from DSS-induced colitis, respectively. (**C**) RT-qPCR analysis of *PTPRE-AS1* and *PTPRE* levels in BMDMs from WT and *PTPRE-AS1*-deficient mice. Data are presented as means ± SEM from three independent experiments. ***P* < 0.01; ****P* < 0.001; ns, no significance.

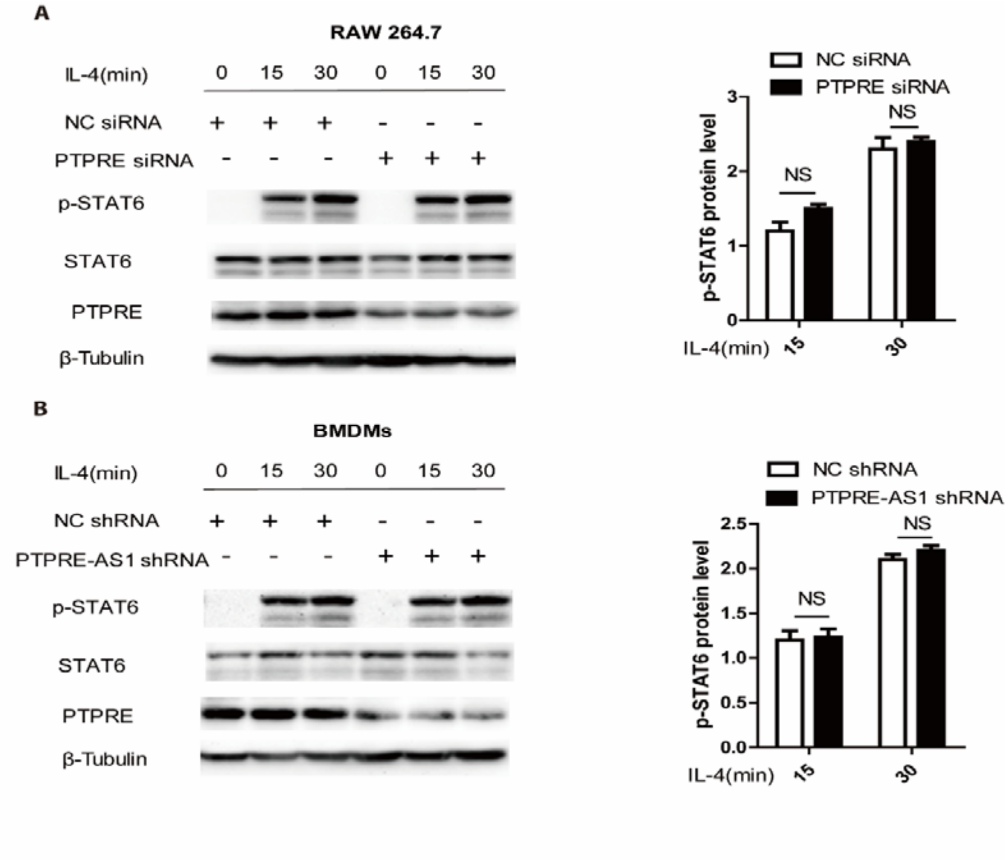
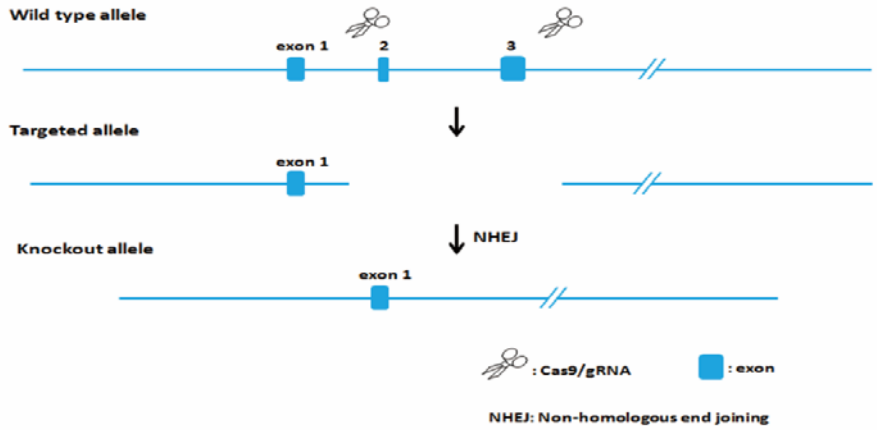


Fig. S3. Neither *PTPRE-AS1* nor PTPRE influences IL-4–induced STAT6 phosphorylation levels. (**A**) RAW 264.7 cells were transfected with *PTPRE* or control siRNA. (**B**) BMDMs were transfected with *PTPRE-AS1* or control shRNA, followed by IL-4 stimulation. Levels of STAT6 phosphorylation (p-STAT6), total STAT6, and PTPRE were examined by western blotting. p-STAT6 protein levels were normalized against those of β-Tubulin and quantified with ImageJ software. NS, no significance. Data are presented as one of three independent experiments.

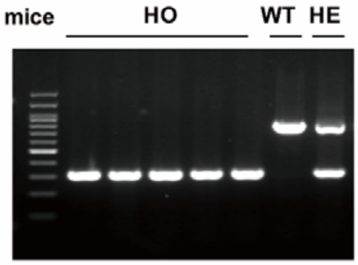
A



B

gRNA	Sequence(5'-3')
gRNA1	CTACCCTTGCACCGTGCCTT TGG
gRNA2	ACACCAAAGGCACGGTGCAA GGG

C



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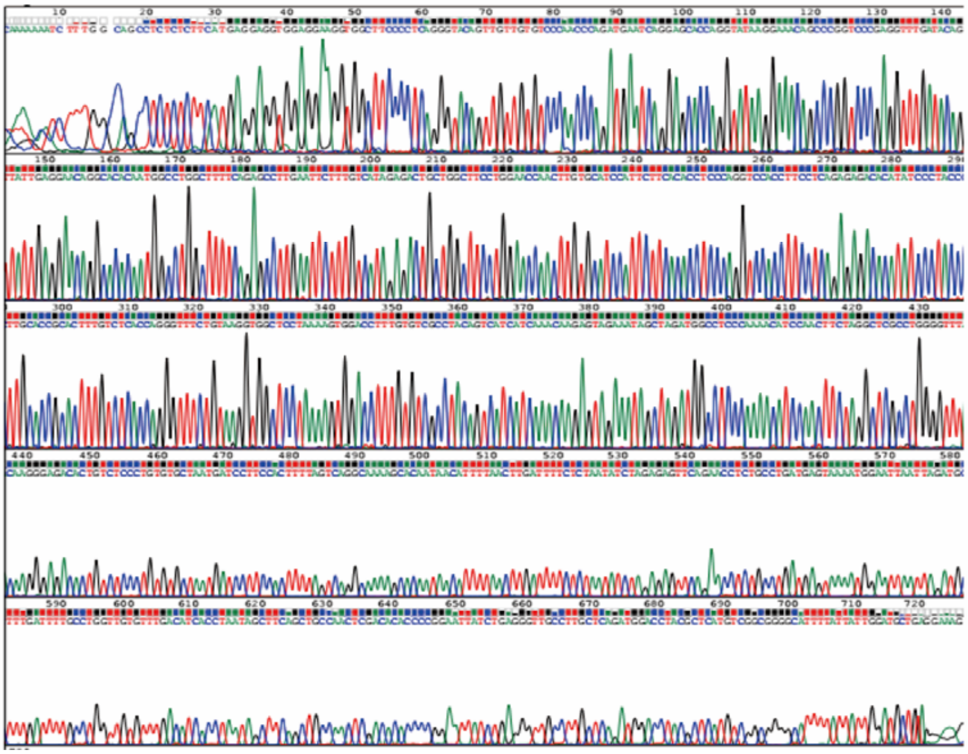

Fig. S4. Generation of *PTPRE-AS1* KO mice. (**A**) Schematic of the targeting strategy for *PTPRE-AS1* disruption using CRISPR/Cas9 technology. (**B**) The guide RNA sequences used in this project are shown. (**C**) Gel image of PCR products amplified using genotyping primers to identify homozygote (HO), WT, and heterozygote (HE) mice. (**D**) *PTPRE-AS1*-null mouse genotypes were confirmed by DNA sequencing.

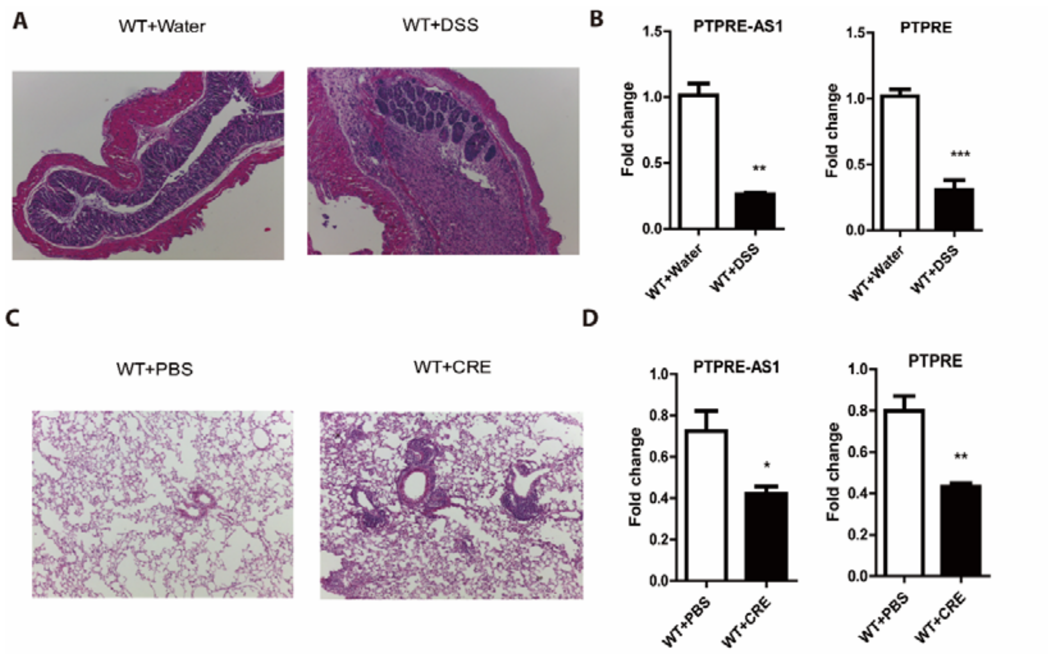

Fig. S5. *PTPRE-AS1* and *PTPRE* are reduced in both DSS-induced colitis and CRE-induced allergic asthma. (**A**) In DSS-induced colitis models, representative images of the histopathological changes in colon tissue examined by H&E staining (magnification, ×100). (**B**) Levels of *PTPRE-AS1* and *PTPRE* in colon tissues were determined by RT-qPCR. (**C**) In CRE-induced allergic asthma, representative images of H&E staining of lung tissues from PBS- and CRE-challenged WT mice (magnification, ×100). (**D**) Levels of *PTPRE-AS1* and *PTPRE* in lung tissue samples were determined by RT-qPCR. The data shown are from one of two independent experiments. Data are presented as means ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

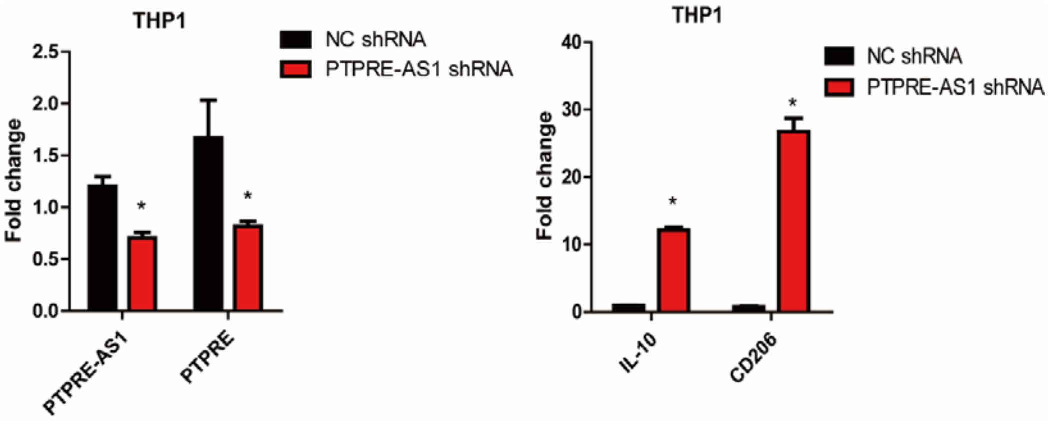

Fig. S6. *PTPRE-AS1* inhibits the expression of M2 macrophage–associated genes in THP1 macrophages. Macrophages were induced using THP1 cells treated with PMA (500 ng/ml). Knockdown of *PTPRE-AS1* in THP1 macrophages using *PTPRE-AS1* or control shRNA. After transfection (72 h), levels of *PTPRE-AS1*, *PTPRE* and M2-associated genes in IL-4-stimulated (20 ng/ml, 24 h) THP1 macrophages were quantified by RT-qPCR analysis. Experiments showing identical results were performed at least twice. $^{*}P < 0.05$.

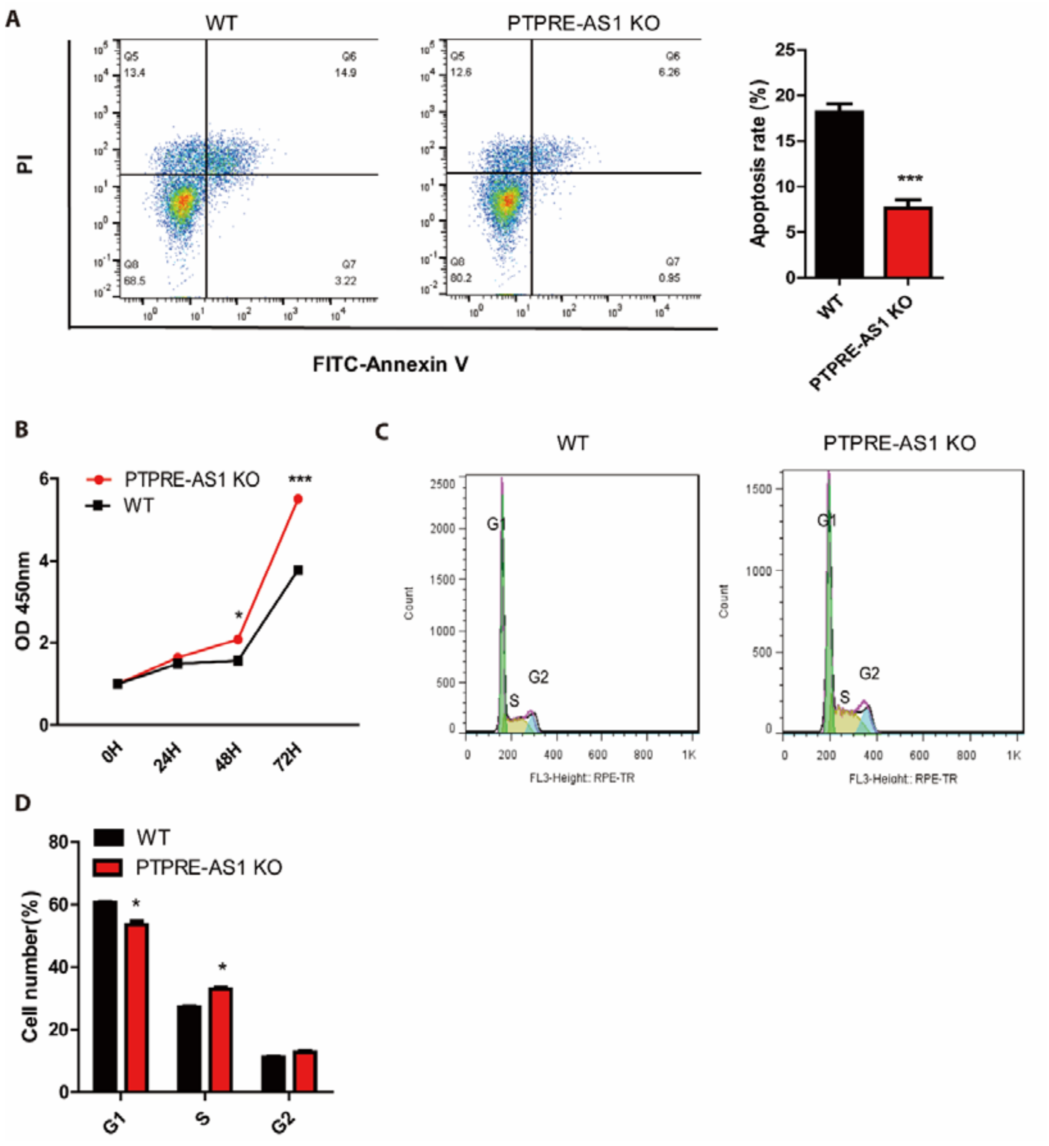

Fig. S7. *PTPRE-AS1* deficiency promotes the proliferation and expansion of lung resident macrophages stimulated by IL-4. (**A**) Flow cytometry was used to measure the apoptosis rate of lung macrophages from WT and *PTPRE-AS1* deficient mice after IL-4 stimulation for 24 h. Quantification of apoptotic cells were analyzed. (**B**) CCK-8 assays were performed to determine the proliferation of lung macrophages from WT and *PTPRE-AS1* deficient mice after IL-4 stimulation. (**C**) (**D**) The cell cycle status of WT and *PTPRE-AS* KO lung macrophages after IL-4 treatment was measured by PI analysis. The percentages of total cells in each gate (G1, S, G2) are displayed. Data are presented as mean ± SEM. *$P < 0.05$; ***$P < 0.001$.

Table S1. Demographic and respiratory health characteristics of patients with allergic asthmatic and healthy controls.

Characteristic	Normal (N=42)	Asthma(N=40)
Male/Female, no.	34/8	32/8
Age, years	9.3 ± 3.8	8.5 ± 3.9
Asthma duration, month	N/A	6.12 ± 6.6
Blood eos[1] (cells/uL)	Not done	368.5 ± 237.03
Total IgE (ku/L)	Not done	165.3 ± 114.3
ACQ score [2]	Not done	0.95 ± 0.6

Values are presented as mean ±SEM. [1]. eos, eosinophil. [2]. ACQ, Asthma Control Questionnaire

Table S2. The primer sequences.

Identification of PTPRE-AS1 KO mice

genes	Forward primer(5’-3’)	Reverse primer(5’-3’)
PTPRE-AS1 KO	ACCCCCAGCCTCCAGCACACT	GGGGCCTCCTACAGCATCCAATAA

Mouse qPCR analysis

genes	Forward primer(5’-3’)	Reverse primer(5’-3’)
GAPDH	CAGAACATCATCCCTGCATC	GCAGAGCCCTTTTTGATAATGT
LncRNA-PTPRE variant1	TTGAGAGGGGTGAAAGTTGAAC	CCCAGTGAACGAACATCACCAT
PTPRE-AS1	CAGTGAATGAGTGTGGCTCCTG	ACATGTAGAGTGTCCCTCGTTG
LncRNA-PTPRE variant3	CTGGGGACACTCCTACCTGAAG	ACAGGAGCCACACTCATTCACT
PTPRE-M	ATGGAGCCCTTGTGTCCACTCCT	TGAAGTGAGCTGTGAGACC
PTPRE-C	GTTCCTGAAGAAAGTGAAGAC	TCCCCATAGAGGTAGTATTCC
WDR5	CTCCTTGTGTCTGCCTCTGATG	CCTGAGACGATGAGGTTGGACT
IL-10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
Arg-1	CATTGGCTTGCGAGACGTAGAC	GCTGAAGGTCTCTTCCATCACC
CD206	GTTCACCTGGAGTGATGGTTCTC	AGGACATGCCAGGGTCACCTTT

Fizz1	CAAGGAACTTCTTGCCAATCCAG	CCAAGATCCACAGGCAAAGCCA
Ym1	TACTCACTTCCACAGGAGCAGG	CTCCAGTGTAGCCATCCTTAGG
MALAT1	CATGGCGGAATTGCTGGTA	CGTGCCAACAGCATAGCAGTA
NEAT1	GCTGGACCTTTCATGTAACGGG	TGAACTCTGCCGGTACAGGGAA
U1	GGGAGATACCATGATCACGAAGGT	CCACAAATTATGCAGTCGAGTTTCCC

Human qPCR analysis

genes	**Forward primer(5’-3’)**	**Reverse primer(5’-3’)**
ACTIN	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
PTPRE-AS1	TCTAACCGCACGTACACCAG	GGAGCTGACACTTGTGTGTTG
PTPRE	TGATTGACCTCATCGCAGCCGT	CTCGCTCCAAAATGTTGCTGAGG
WDR5	AGTGCCTCAAGACTTTGCCAGC	CGATGAGCGTCTTCAGGCACTG
IL-10	TCTCCGAGATGCCTTCAGCAGA	TCAGACAAGGCTTGGCAACCCA
Arg-1	TCATCTGGGTGGATGCTCACAC	GAGAATCCTGGCACATCGGGAA
CD206	AGCCAACACCAGCTCCTCAAGA	CAAAACGCTCGCGCATTGTCCA

CHIP-qPCR analysis

genes	Forward primer(5’-3’)	Reverse primer(5’-3’)
PTPRE promoter1	CATGCTTATCTGTGGCCAAAGGTG	CTCCTCGAGGCTGGAGAATGTATT
PTPRE promoter2	CCTTAAGTGGAAAGTCACTGGGGT	CTGTGCTATGCTGACTTACTCTGG
PTPRE promoter3	GATAGGAGGGCACCCTAATAGGTT	TCCACCTTCCTCAGAGAGACACAT
PTPRE promoter4	GAGAGAGTGTGTGAGAGAGAGAGC	TTCAGTTCTTTCCAGGGGACAGAG

Table S3. The sequences of PTPRE-AS1 ASO, shRNA, PTPRE siRNA, and WDR5 siRNA.

GENES	Sense (5’-3’)	Antisense(5’-3’)
NC ASO	TCTACTCGTCGCTACGTACC	
PTPRE-AS1 ASO1	TGTCTGTTGCAGATTGGTGC	
PTPRE-AS1 ASO2	CCTTTTCTCTTCCTCTTCCT	
NC shRNA	TTCTCCGAACGTGTCACGT	
PTPRE-AS1 shRNA1	GGCACACACAAGATGTGTTAG	
PTPRE-AS1 shRNA2	GCACCAATCTGCAACAGACAG	
NC siRNA	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

PTPRE siRNA1	GGAUGCUCAAGUUCCUGAATT	UUCAGGAACUUGAGCAUCCTT
PTPRE siRNA2	CCAGACGGAUGUUCAGUAUTT	AUACUGAACAUCCGUCUGGTT
WDR5 siRNA1	CCUGGUGUAUAUCUGGAAUTT	AUUCCAGAUAUACACCAGGTT
WDR5 siRNA2	CACCAGUUAAGCCAAACUATT	UAGUUUGGCUUAACUGGUGTT