Supplemental data for Habibovic et al.: A critical role for the NADPH oxidase DUOX1 in epithelial EGFR activation, mucous metaplasia, and airway remodeling during allergic asthma

Supplementary Figures:

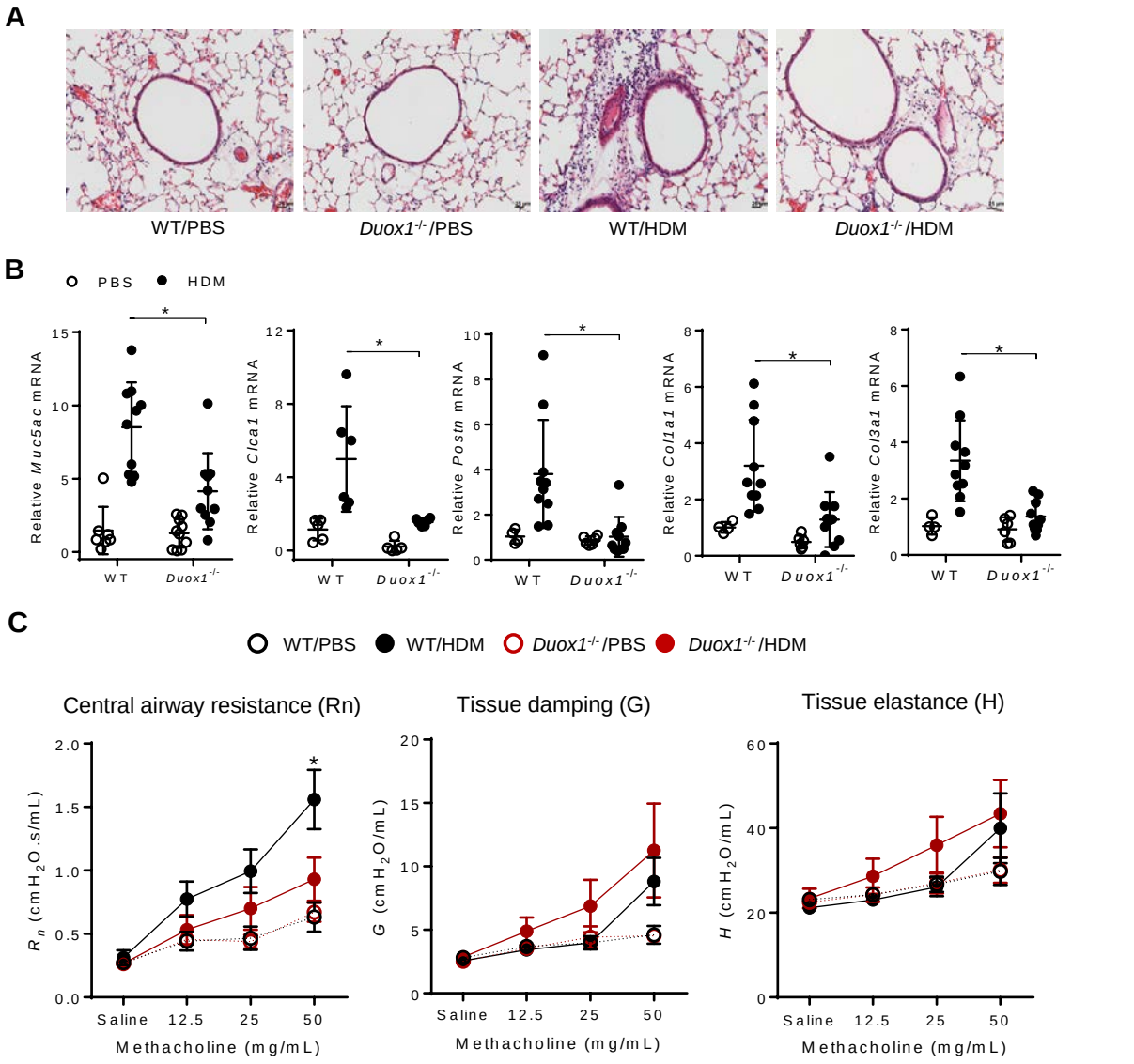

Figure S1: DUOX1 contributes to HDM-induced neutrophilia, mucous metaplasia, subepithelial fibrosis, and airways hyperresponsiveness. (**A**) Analysis of lung inflammation by H&E staining of lung tissues. (**B**) RT-PCR analysis of lung tissue markers of mucous metaplasia and fibrosis. (**C**) Analysis of airways hyperresponsiveness to methacholine, by calculation of central airway resistance (R_N), tissue damping (G) and tissue elastance (H). Histochemical images are representative of 3 separate experiments. Dot plots indicate mean ± S.D. of 8-12 replicates from 3 independent experiments. *P<0.05 compared to corresponding controls.

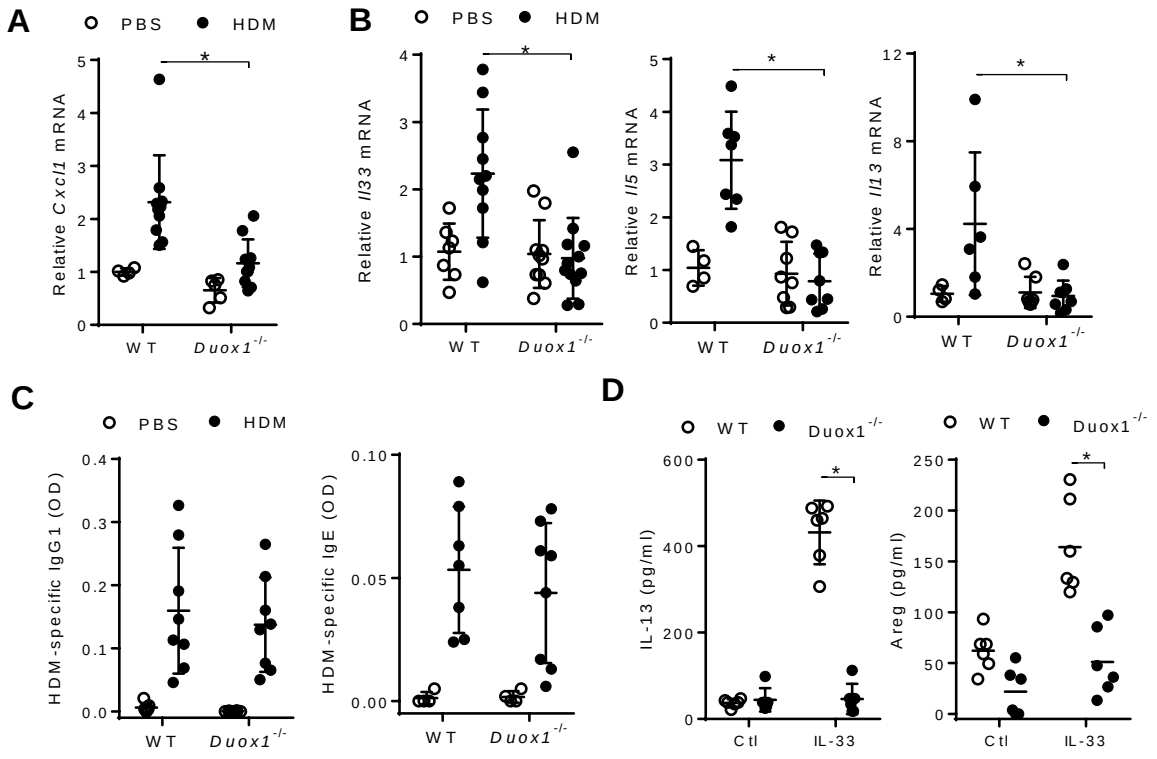

Figure S2: DUOX1-dependent type 2 cytokine responses and ILC2 activation during HDM-induced inflammation. (**A**,**B**) Lung tissue mRNA expression of Cxcl1 (**A**) and type 2 cytokines (**B**) in response to HDM-induced inflammation. (**C**) Analysis of serum levels of HDM-specific IgG1 and IgE. (**D**) MTECs from wild-type or Duox1-deficient mice were stimulated with 100 ng/ml IL-33 for 6 hrs, and production of IL-13, Areg, and Cxcl1 was analyzed in conditioned media. Dot plots indicate mean ± S.D. from 8-12 replicates from 2-3 separate experiments. **P*<0.05 compared to corresponding controls.

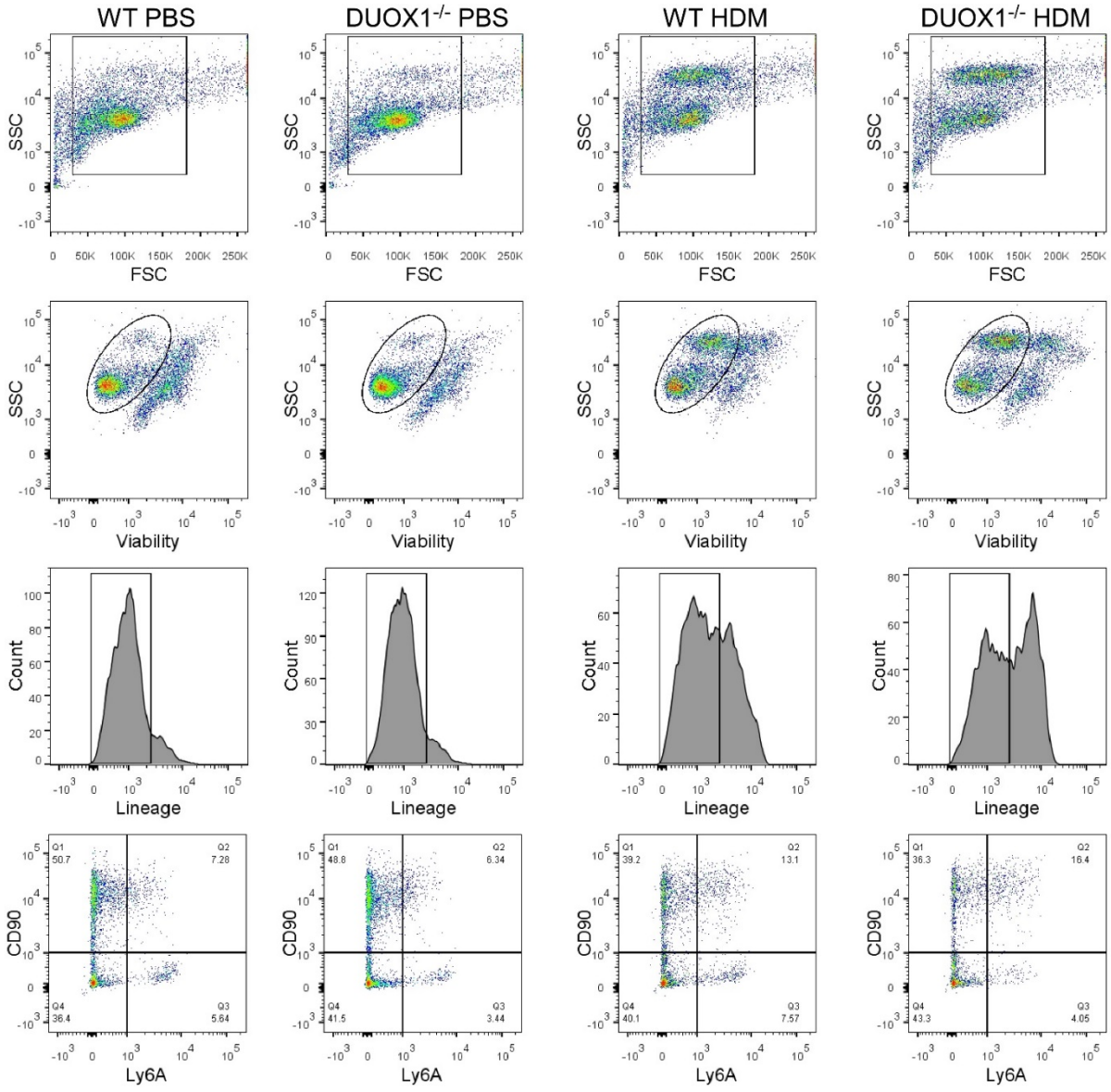

Figure S3: Gating strategy for quantitation of lung tissue ILC2 cells. Lung single-cell suspensions were prepared from various treatment groups and evaluated by flow cytometry based on forward and side scatter (top rows), viability (using UV-Blue Live/Dead stain; second row). Lineage negative cells (third rows) were evaluated for positive staining of CD90 and Ly6A to enumerate % ILC2 cells (top right quadrant in bottom rows).

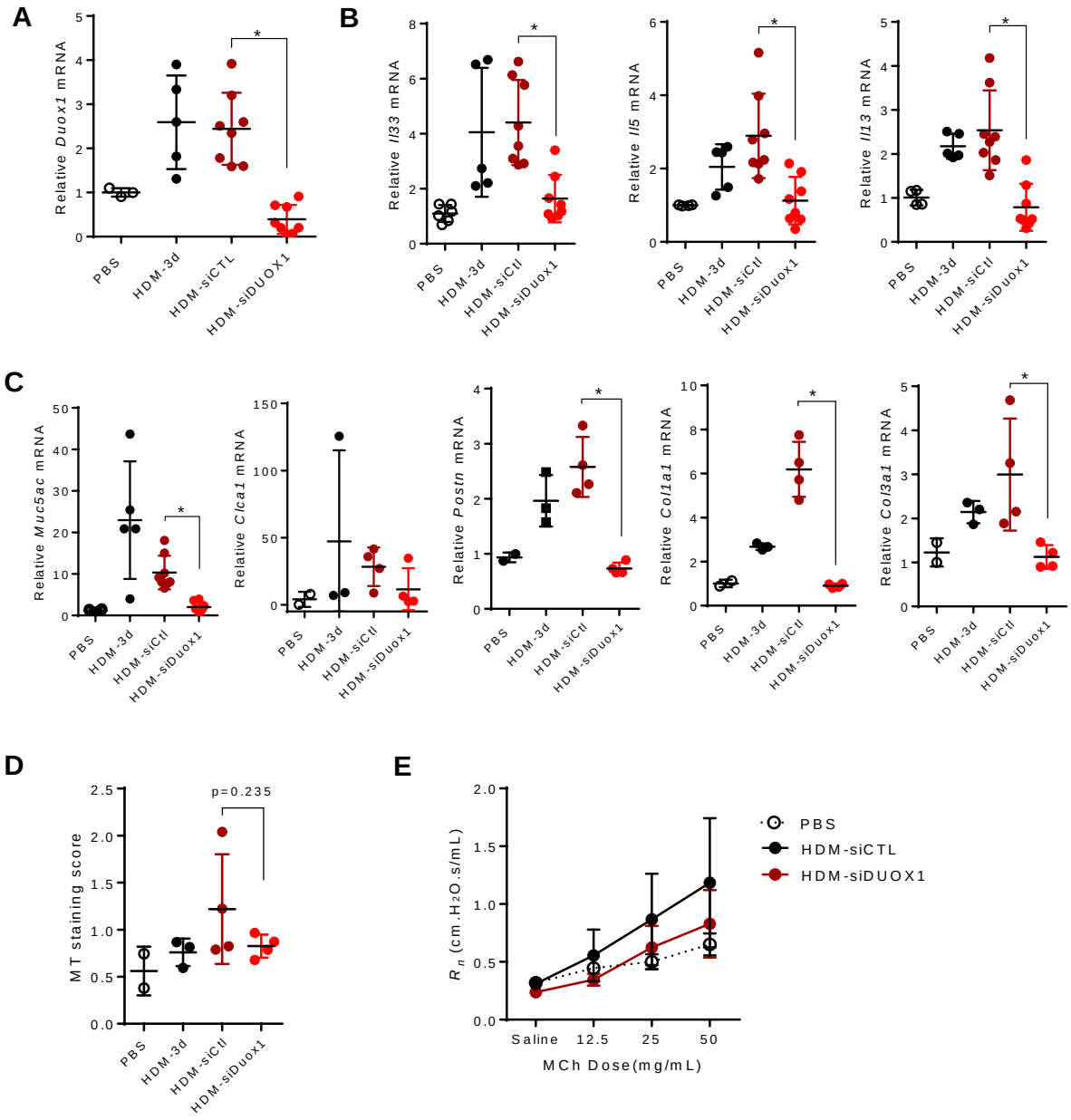

Figure S4: DUOX1 siRNA targeting reverses HDM-induced type 2 immune responses, airway remodeling, and airways hyperresponsiveness. (**A**) Mice were subjected to HDM challenge and DUOX1 siRNA as in Fig. 5A, and efficacy against DUOX1 mRNA expression was evaluated in lung tissues. (**B**) RT-PCR analysis of lung tissue mRNA levels of type 2 cytokines. (**C**) RT-PCR analysis of lung tissue mRNA levels mucus metaplasia markers and collagen genes. (**D**) Quantification lung tissue MTA staining. (**E**) Analysis of central airway resistance (R_N) in response to increasing doses of methacholine. Dot plots indicate mean ± S.D. from 6-10 replicates from 2 separate experiments. **P*<0.05 compared to corresponding controls.

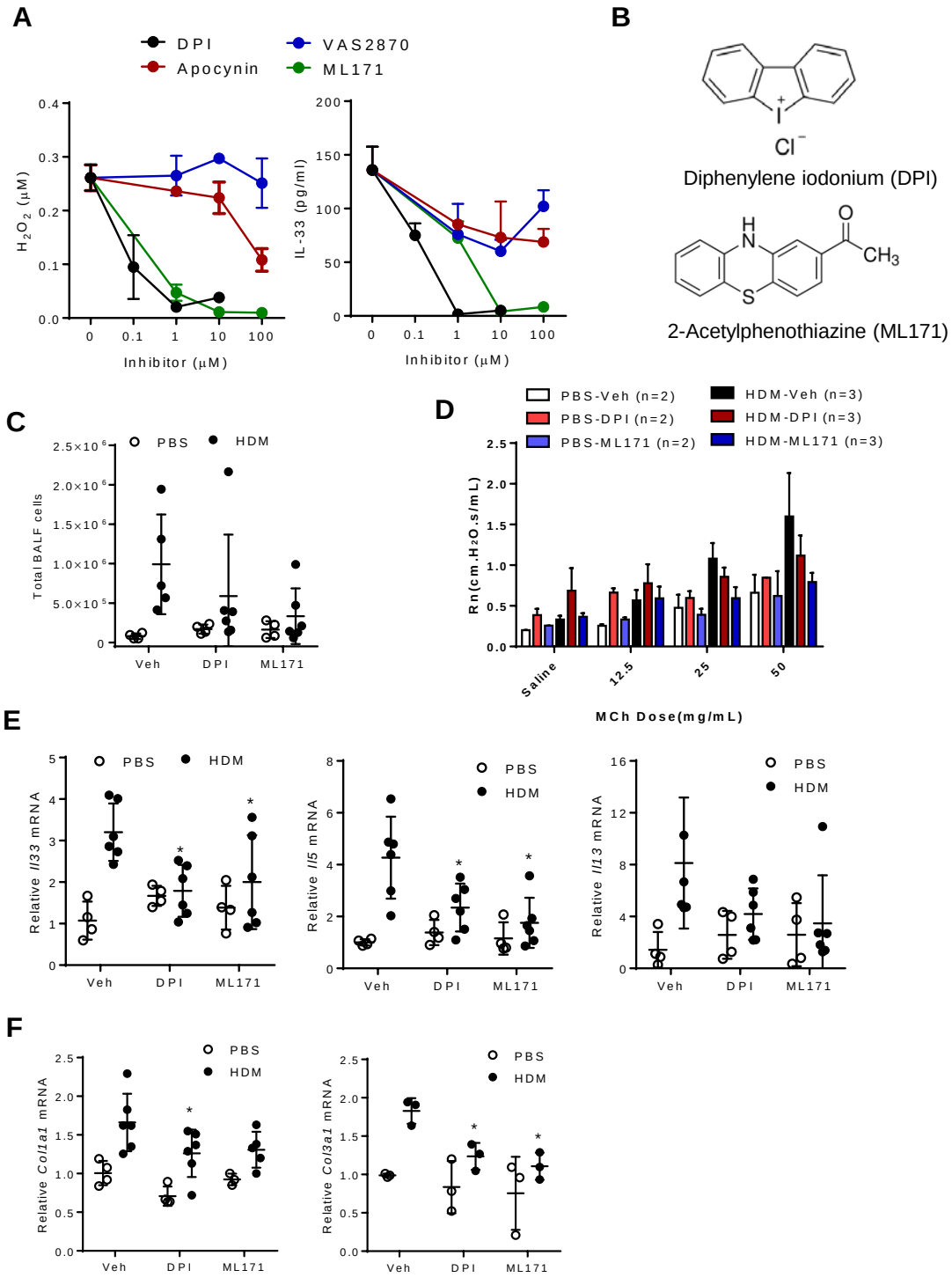

Figure S5: Pharmacological NADPH oxidase inhibitors inhibit epithelial EGFR activation and HDM-induced inflammation and remodeling (**A**) Analysis of ATP (100 μM; 15 min) mediated activation of extracellular H_2O_2 production (after 15 min), measured as in (*23*), or IL-33 secretion (after 2 hrs) in the absence or presence of various NADPH oxidase inhibitors (15-min preincubation). Data are expressed relative to ATP-induced responses in the absence of

inhibitors, and reflect mean ± S.E. of 4 measurements. (**B**) Molecular structures of DPI and ML171. (**C**) Effect of in vivo administration of DPI or ML171 on HDM-induced inflammation, measured on day 25 (see Fig. 5A). (**D)** Effects of DPI or ML171 on HDM-induced central airway resistance (Rn) in response to methacholine (MCh). **(E,F**) Effects of DPI and ML171 on HDM-induced type 2 cytokine mRNA (**E**) and collagen mRNA (**F**) expression in lung tissues.

Supplementary Table S1: qPCR primer sequences used in this study

Gene	Forward primer	Reverse primer
Human:		
EGF:	CAGGGAAGATGACCACCACT	TTCCCACCACCTCAGGTCTC
AREG:	GTGTGGGGAAAAGTCCATGA	CTGGAAAGAGGACCGACTCA
TGFA:	AGTGGTCTGAAGAGCCCAGA	ATCTCTGGCAGTGCTGTC CT
GAPDH:	GAAGGCTGGGGCTCATTTG	AGGCTGTTGTCATACTTCTCATGG
Mouse:		
Duox1:	ATCTGGGTGACAAGGACTCA	CAGACTCCTGTTCAGCACCT
Duox2:	GGCCTAAAGAAGAGGTTTGGCAAA	GCCTCCGTGTACAGCCGGG
Areg:	AACGGTGTGGAGAAAAATCC	TTGTCCTCAGCTAGGCAATG
Cxcl1:	TCGTCTTTCATATTGTATGGTCAAC	CGAGACGAGACCAGGAGAAAC
Il33:	GATGGGAAGAAGGTGATGGGTG	TTGTGAAGGACGAAGAAGGC
Il5:	ATGGAGATTCCCATGAGCAC	CCCACGGACAGTTTGATTCT
Il13:	CCACGGCCCCTTCTAATGA	GCCTCTCCCCAGCAAAGTCT
Muc5ac:	AGTCTCTCTCCGCTCCTCTCAAT	CAGCCGAGAGGAGGGTTTGATCT
Postn:	CACGGCATGGTTATTCCTTCA	TCAGGACACGGTCAATGACAT
Col1a1:	CACCCTCAAGAGCCTGAGTC	AGACGGCTGAGTAGGGAACA
Col3a1:	CCGAACTCAAGAGTGGAGAA	GGCCATAGCTGAACTGAAAA
Clca1:	CCCTCATCCAACTGAACAAC	TCAGCGTTTTTGAAGGTTTC
Gapdh:	CTGGAGAAACCTGCCAAGTA	TGTTGCTGTAGCCGTATTCA