Supplementary Material and Methods

Labeling of free sulfhydryls using MPB. Lung tissues (n=3-4 mice/group) were lysed in Tris Buffer, pH 7.4, containing, 125mM NaCl, 1% NP-40 with 500μM biotinylated *n*-ethyl maleimide (MPB) for 1 hour at ambient temperature. Lysates were then frozen at -20℃ overnight. Thawed lysates were centrifuged at 14,000 rpm for 10 min and passed through a Micro Bio-Spin (Bio-Rad) column to separate free MPB. The eluent whole-lung lysate was immunoprecipitated using anti-Eotaxin (R&D System), Periostin (Abcam) or EGF (Santa Cruz) antibodies and sequentially probed, initially with streptavidin-horseradish peroxidase (HRP) to detect biotinylated free sulfhydryl groups, and then with anti- Eotaxin, Periostin or EGF protein specific antibodies.

Non-reducing gel electrophoresis: Lung homogenates were suspended in loading buffer without the reducing agent dithiothrietol (DTT). A separate set of samples were resuspended in loading buffer with DTT to reduce the disulfide bonds. The samples were resolved by SDS-PAGE and subjected to western blot analysis.

HDM model of allergic airways disease: For all experiments, 8 to 12 week old mice were used, as approved by the Institutional Animal Care and Use Committee. Mice (n=10/group) were anesthetized with isofluorane and exposed to 50μg of the allergen, HDM (GREER-containing 35 endotoxin units/mg) extract, suspended in PBS, via intranasal administration on day 0 and boosted again on day 7. Mice were then administered 50μg of HDM consecutively on days 14-18, and euthanized 24 hours post final exposure. The control group was given 50μl of sterile PBS alone at all-time points.

Deletion of ERp57. Confirmation of deletion of ERp57 from epithelial cells in mice containing *CCSP-rtTA/ TetOP-Cre/ERp57$^{loxP/loxP}$* following administration of doxycycline containing food for 5 days. Single cells lung suspensions were prepared and cells were sorted by flow cytometry. The EpCAM positive, CD45 negative, Sca1 low fraction of lung cells were isolated for assessment of ERp57 expression by Western Blot analysis. β-actin was used as loading control. CD45^{+} cells were selected as a non-epithelial control.

Assessment of AHR: Mice (n=8-10/group) were anesthetized with an intraperitoneal injection of pentobarbital sodium (90 mg/kg), tracheotomized using an 18 gauge cannula, then mechanically ventilated at 200 breaths/min using a FlexiVent™ computer controlled small animal ventilator (SCIREQ). While on the ventilator mice also received the paralytic, pancuronium bromide. The parameter Newtonian resistance (Rn), was calculated as previously described[49, 50]. Airway responsiveness is represented as the average of the 3 peak measurements for each animal, obtained at incremental methcholine doses.

Bronchoalveolar lavage processing: Bronchoalveolar lavage (BAL) from mice (n=8-10/group) was collected. Total and differential cell counts were performed as previously described (20). Briefly, cells were isolated by centrifugation and total cell counts were enumerated using the Advia 120 automated hematology analyzer system. Differential cell counts were obtained via cytospins using Hema3 stain reagents (Fisher Scientific). Differentials were performed on a minimum of 300 cells per animal.

Western blot analysis: Following dissection, right lung lobes were flash frozen for protein analysis. Lungs were pulverized, and lysed in buffer containing 137mM Tris·HCl (pH 8.0), 130mM NaCl, and 1% NP-40. Proteins from cell lysates were prepared in the same buffer. Insoluble proteins were pelleted via centrifugation, and following protein quantitation of the supernatant, samples were suspended in loading buffer with dithiothrietol (DTT) and resolved by SDS-PAGE. Proteins were transferred to PVDF and membranes were probed using a standard immunoblotting protocol using the following primary antibodies: GRP78, ATF6[50] and CHOP (Abcam), ERp57, GRP94 (Stressgen), and β-actin (Sigma).

Measurement of collagen and immunohistochemistry: Collagen content was measured via the hydroxyproline assay (n=8-10/group). Briefly, lung lobes were diced and placed in 500µl of 10 mg/mL pepsin in 0.5 M acetic acid for 3h at 37^{o}C, or until lungs were completely digested. The digest was spun at 10,000g for 10min at room temperature. Fifty microliters of the supernatant was mixed vigorously with 500µL of sircol dye solution for 30min and then spun again at 10,000g for 10min. Excess dye was decanted off and the resulting pellet was dissolved in 500µL of an alkaline solution, 200µL of which was pipetted in duplicates into a 96 well plate and measured at 540 nm. To evaluate regional changes in ERp57 (Stressgen), α-smooth muscle actin (Sigma), and active caspase-3 (Cell Signaling), fixed sections were prepared for immunostaining by deparaffinizing with xylene and rehydrating through a series of ethanols. For antigen retrieval, slides were heated for 20min in 95℃ cit rate buffer (pH 6.0), then rinsed in

distilled water. Sections were then blocked for 1h in blocking serum as per manufacturer's instructions (Vectastain Alkaline Phosphatase Universal, Vector). Slides were then washed in TBS with 0.1% TWEEN-20 3x5min, followed by incubation with primary antibody for αSMA (Sigma) overnight at 4℃. Sections were washed again and incubated with a biotinylated universal secondary antibody (Vectastain Alkaline Phosphatase Universal, Vector) for 30min at room temperature. Slides were washed and incubated with the Vectastain ABC-AP reagent (prepared as per manufacturer's instructions) for 30min at room temperature. Sections were then incubated with Vector Red Alkaline Phosphatase Substrate Kit I (Vector) for 20 min at room temperature, rinsed with tap water, and counterstained with Mayer's Hemotoxylin. The ERp57 and active caspase-3 detection in Fig S3 C were performed on serial sections that are 5µm apart on the same slides.

Semi quantitative scoring: α-SMA, ERp57, periodic acid–Schiff (PAS) imaging was all performed at 20X magnification. Histochemistry staining were assessed with a blinded scoring system by three independent investigators with the following scale: 0, no reactivity; 1, minimal staining; 2, moderate staining; and 3, prominent staining. Scores were averaged according to treatment group.

Enzyme-linked immunosorbent assay: Eotaxin, G-CSF, CCL-20, IL-33 and IL-6 were detected by ELISA in lung homogenates (normalized for protein) according to the manufacturer's instructions (R&D Systems).

Measurement of Serum IgG1 and IgE: Following euthanization, blood was collected by heart puncture and immediately spun through a microtainer. Serum was collected and IgG1 and IgE content was determined using an ELISA-based method, with a 96-well plate pre-incubated with 1µg/ml of HDM.

Analysis mRNA from tissue samples: Lobes of the right lung were flash frozen, pulverized and total RNA was isolated and purified using the RNeasy kit (QIAGEN). 1 µg of RNA was reverse transcribed to cDNA for quantitative assessment of gene expression using SYBR Green (Bio-Rad) to measure periostin, transforming growth factor beta, mucin 5ac (MUC5ac), and mclca3 (Gob5). Expression values were normalized to the house keeping gene cyclophilin. The primers used in this study are as follows:

MUC5ac	F5'-CAGTGAATTCTGGAGGCCAACAAGGTAGAG-3' R5'-CTAAGCTTAGATCTGGTTGGGACAGCAGC-3'
GOB5	F5'-ACTAAGGTGGCCTACCTCCAA-3' R5'-GGAGGTGACAGTCAAGGTGAGA-3'
Periostin	F 5'-CGAAGGGGACAGTATCTCCA-3' R5'-GCTTCAGAGAGGATGCCAAG-3'
Cyclophilin	F5'-TTCCTCCTTCACAGAATTATTCCA-3' R5'-CCAGTGCCATTATGG-3'
TGFβ	F5'- GGCCTTGGAAGCATGTAGAGG -3' R5'- GGAGAACTCGTTAGAGACGACTT-3'

Supplementary Figure Legends

Figure S1. ERp57 increases are associated with increases in eosinophils and bronchodilator response. **A:** Representative images of paraffin embedded human lung tissue samples obtained from asthmatic human subjects numbers 1 to 3 were obtained from UCSF (table S1 A) and numbers 4-6 were obtained from Cleveland Clinic (table S1 B). Tissues were stained for ERp57 (red). Scale bars represents 50µm. **B:** Lung tissue samples stained using secondary antibody alone. Scale bars represents 50µm. **C**: Semi quantitative histological scores for ERp57. **D & E:** Correlations between ERp57 score and blood eosinophil counts or bronchodilator response in non-asthmatics and asthmatics.

Figure S2. Deletion of ERp57 has no effect on HDM-induced mucin production. **A & B**: qRT-PCR analysis for MUC5AC and Gob5. **C:** Representative histopathological images of Periodic acid-Schiff staining (PAS) staining of WT and *ΔERp57* mice. **D:** PAS scoring on Ctr and ERp57 deleted mice lungs. *$p<0.05$ by ANOVA, denotes significant differences as compared with PBS groups. Scale bars represents 50µm.

Figure S3. Deletion of ERp57 decreases intrinsic apoptosis in HDM challenged mice lungs. **A:** non reducing SDS-PAGE showing disulfide (-S-S-) mediated of oligomerization Bak in response to HDM (T: trimer, D: dimer, M: monomer). **B:**

Caspase-3 activity as measured in whole lung lysates. *p<0.05 by ANOVA, denotes significant differences as compared with PBS groups. # p<0.05 by ANOVA, denotes significant differences compared with the HDM groups (n= 7-8 mice/group). **C:** Representative IHC images of WT and *ΔEpi-ERp57* mice challenged with HDM and stained with anti-ERp57 or anti-active caspase-3 antibody. Sequential lung sections 5µm apart were stained. Red color represents positive staining in airways epithelial cells. Scale bars represents 50µm.

Table S1 A. Baseline subject characteristics (UCSF)

	Healthy Controls	Asthmatics	p value
Sample size	6	6	
Age, years	33 ± 7	34 ± 13	0.89
BMI	26 ± 3	27 ± 4	0.73
Gender (% F)	50	33	1
Ethnicity			
White	3	3	
African-American	1	1	
Hispanic	1	1	
Asian/Pacific Islander	1	1	
FEV_1, % predicted	96 ± 7	85 ± 13	0.09
Δ FEV_1 with albuterol (% of baseline)	5.2 ± 6.1	21.1 ± 6.8	0.002
FEV_1/FVC	0.80 ± 0.065	0.69 ± 0.058	0.01
Methacholine PC_{20} (mg/ml)	> 10	0.51 (0.15-1.34)	0.0001
IgE, IU/ml	14 (4-29), (n=18)	301 (21-6556)	0.01
Blood eosinophils, $x10^9$/L	0.09 (0.05-0.30)	0.50 (0.35-0.71)	0.005
FeNO	15 (10-36)	69 (43-109), (n=5)	0.004
Skin prick positive (No.)	1 (1-2)	4 (2-11)	0.003

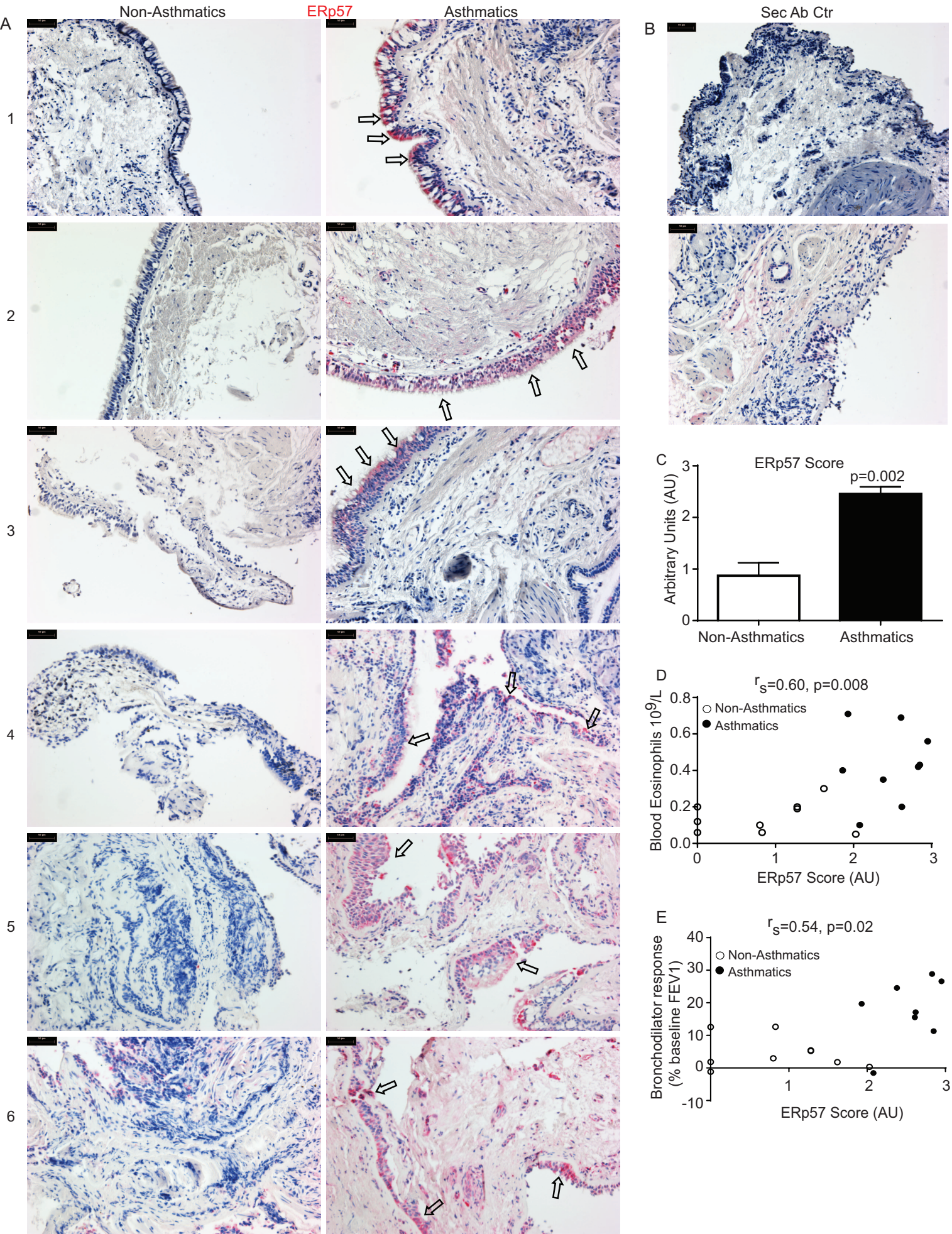
Values reported as mean ± standard deviation, or median (range). All data are from the baseline visit except spirometry, which are reported from visit 2 (immediately before initiation of ICS). P-values are based on Welch's t-test (where mean/standard deviation reported), chi-square test for proportions (gender), Wilcoxon rank sum test (where median/range reported), or poisson regression (skin prick tests). All subjects were non-smokers defined as never smoker or former smoker with no smoking for at least 1 year prior to enrollment and total pack-years ≤15.

Table S1 B. Baseline subject characteristics (Cleveland Clinic)

	Healthy Controls	Asthmatics	p value
Sample size	3	3	
Age, years	33 ± 7	34 ± 13	0.192
BMI	26 ± 5	34 ± 2	0.013*
Gender (% F)	33	33	1
Ethnicity			
White	2	1	
African-American	1	2	
FEV_1, % predicted	95 ± 4	68 ± 4	0.007*
Δ FEV_1 with albuterol (% of baseline)	6.1 ± 2.0	12.9 ± 3.5	0.161
FEV_1/FVC	0.76 ± 0.033	0.69 ± 0.033	0.227
Methacholine PC_{20} (mg/ml)	NA	1.75 (0.969-2.30)	
IgE, IU/ml	39 (16-63)	224 (19-334)	0.275
Blood eosinophils, $x10^9$/L	0.17 (0.1-0.2)	0.23 (0.1-0.4)	0.637
FeNO	21.5 (16-26.5)	33.3 (24-43), (n=2)	0.248
Skin prick positive (No.)	3 (0-6)	4 (3-5)	0.512

Values reported as mean ± standard deviation, or median (range). All data are from the baseline visit except spirometry, which are reported from visit 2 (immediately before initiation of ICS). p-values are based on Welch's t-test (where mean/standard deviation reported), chi-square test for proportions (gender), Wilcoxon rank sum test (where median/range reported), or poisson regression (skin prick tests). All subjects were non-smokers defined as never smoker or former smoker with no smoking for at least 1 year prior to enrollment and total pack-years ≤15.

Figure S1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ERp57
Asthmatics
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2
3
4
5
6
B
Sec Ab Ctr
C
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Arbitrary Units (AU)
0
1
2
3
Non-Asthmatics
Asthmatics
D
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Non-Asthmatics
Asthmatics
Blood Eosinophils 10^9/L
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0.2
0.4
0.6
0.8
0
1
2
3
ERp57 Score (AU)
E
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Non-Asthmatics
Asthmatics
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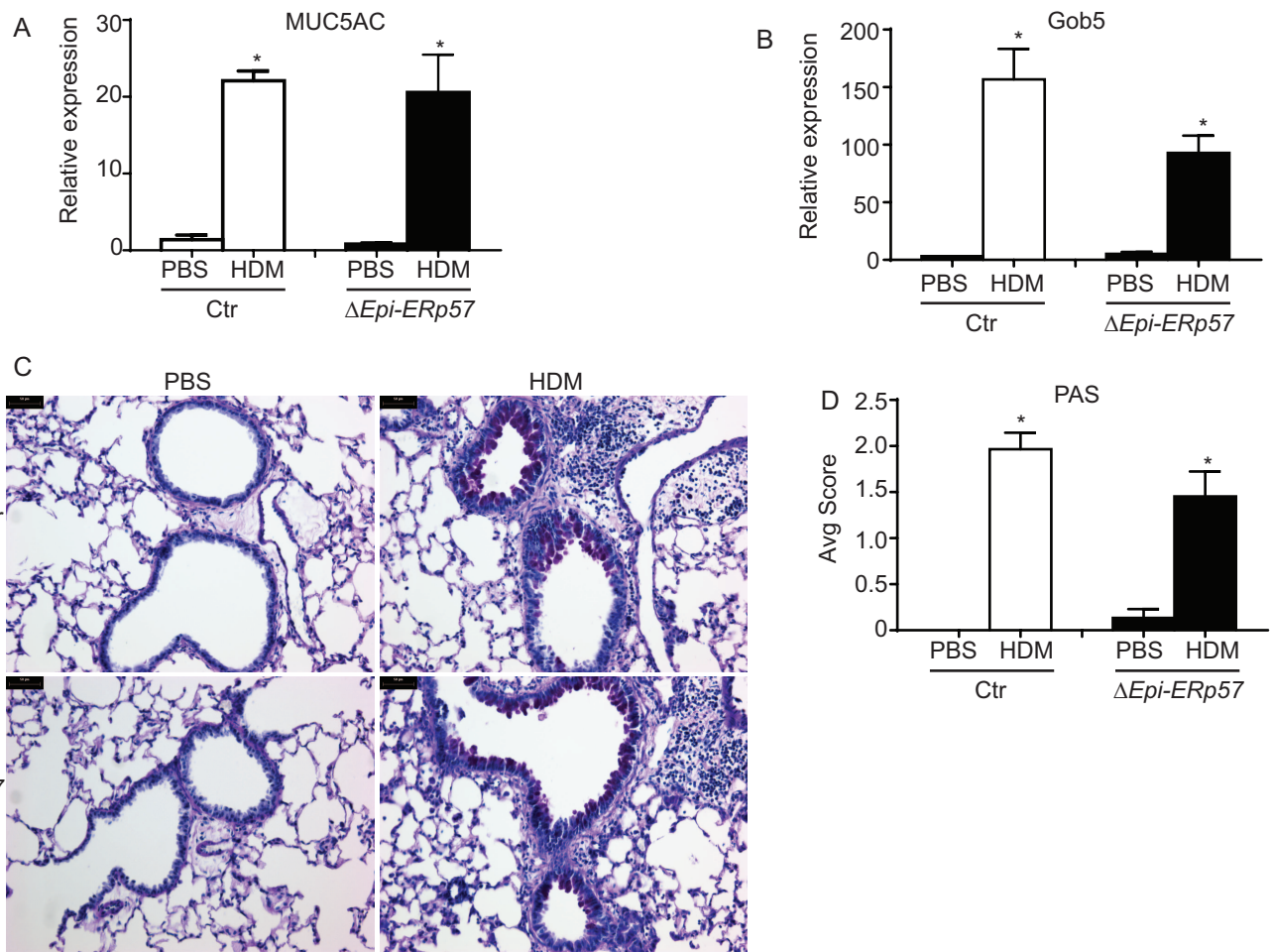
0
10
20
30
40
1
2
3
ERp57 Score (AU)

Figure S2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Ctr
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B
Gob5
Relative expression
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150
100
50
0
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Ctr
ΔEpi-ERp57
C
PBS
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PAS
Avg Score
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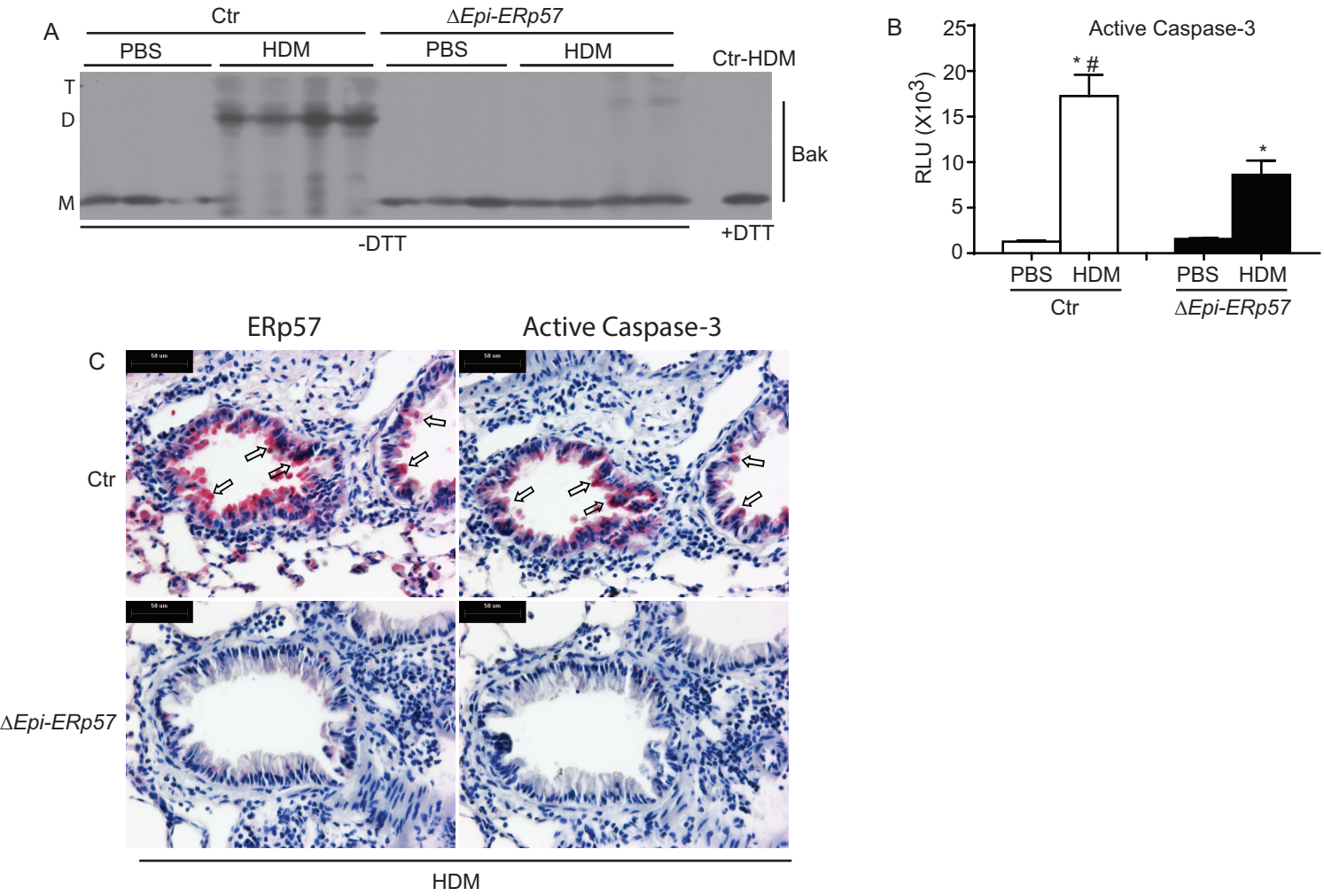
Figure S3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Ctr
ΔEpi-ERp57
C
ERp57
Active Caspase-3
Ctr
ΔEpi-ERp57
HDM