

Defective Collagen I Remodeling and Contraction is a Feature of Asthmatic Airway Fibroblasts

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Introduction

Asthma is a chronic inflammatory disease of the airways that is associated with airway remodeling that involves all tissues of the airway wall including the epithelial, sub-mucosal, and smooth muscle layers as well as vascular structures. In particular a well documented feature of airway remodeling in asthma, is the accumulation of fibroblasts in the epithelial-mesenchymal trophic unit (EMTU) that leads to the deposition of excess collagen in the lamina propria of the airways.

In this regard we have shown that airway epithelial cells through the production of IL-1 α regulate the phenotype of lung fibroblasts in the lung EMTU¹.

Specific Aim

The aim of this study was to assess asthmatic human airway remodeling in the context of collagen morphology and the ability of lung fibroblasts from asthmatic and non-asthmatic airways to remodel collagen I as well as the effects of IL-1 on the remodeling phenotype of lung fibroblasts using optical imaging methods

Methods & Materials

Lung tissue and airway fibroblasts were obtained from donor lungs deemed not suitable for transplant from both asthmatic (n=10) and non-asthmatic (n=10) individuals. Lung tissue was used to assess the orientation and arrangement of collagen I fibers using second harmonic generation (SHG) nonlinear microscopy with texture analysis and transmission electron microscopy. Fibroblasts were cultured until confluent in 10% FBS in DMEM with added antibiotics/antimycotics. After which they were seeded between passage 3 and 5 onto collagen I gels. To assess the effect of IL-1 on the remodeling characteristics of lung fibroblast, cell-seeded collagen I gels was also stimulated with 1ng/ml recombinant human IL-1. Gel contraction was assessed over time and then quantified as a percentage of initial gel area at 24 & 72 hours using ImageJ software (figure 3). Collagen fiber formation was also assessed using SHG nonlinear microscopy.

Second Harmonic Generation (SHG) nonlinear microscopy

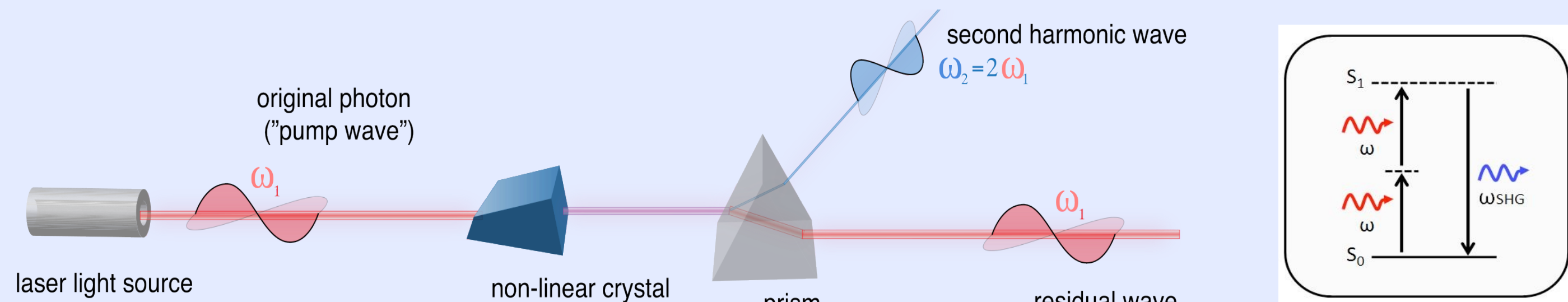


Figure 1. SHG nonlinear microscopy is an optical process where photons with the same frequency interacting with a nonlinear material are effectively "combined" to generate new photons with twice the energy, & therefore twice the frequency & half the wavelength of the initial photons. Only non-centrosymmetric structures (e.g. collagen I) are capable of emitting SHG light.

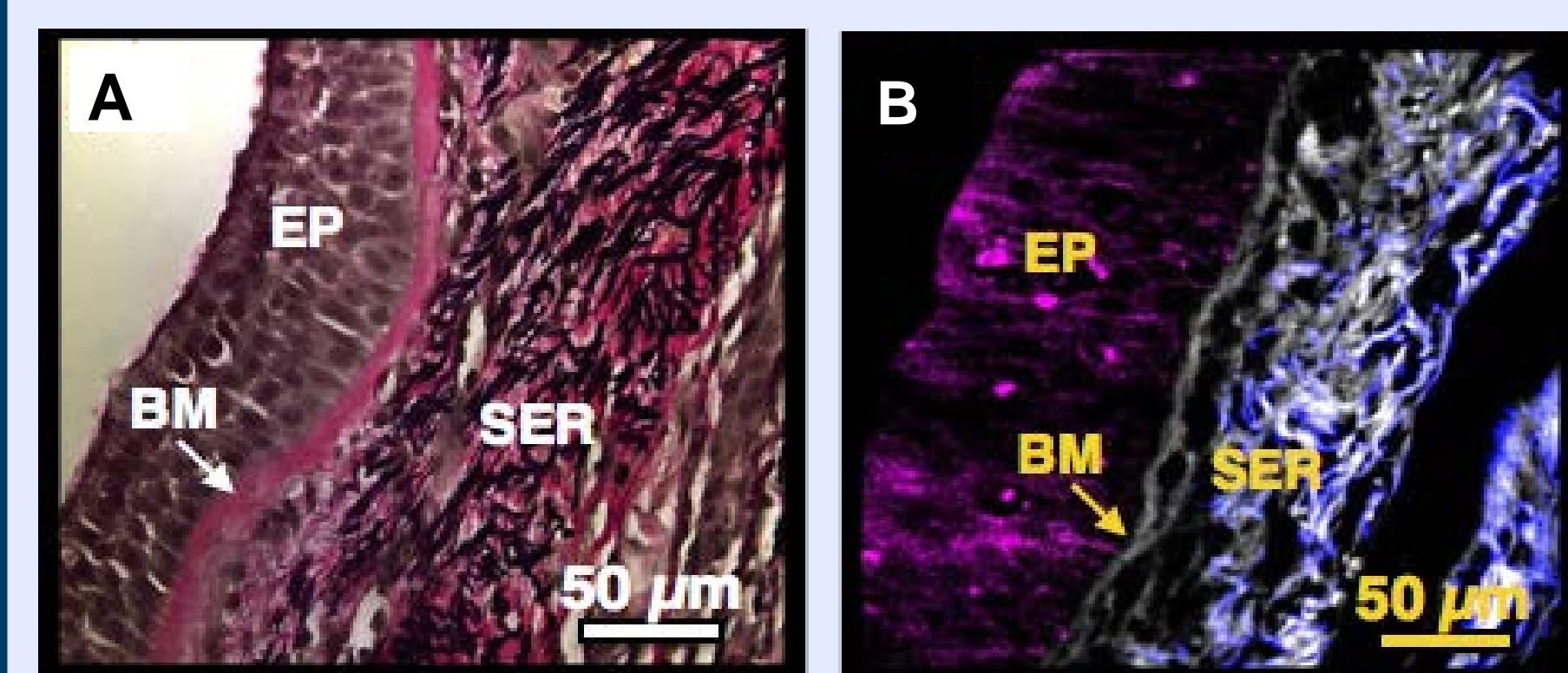


Figure 2. A) Masson trichrome stained airway compared to B) a SHG image of the same airway. In the SHG image, Epithelial cells are coloured pink (autofluorescence signal), Elastic fibers are colored white (two photon signal) & collagen I fibers coloured blue (SHG). BM: basement membrane; SER: sub-epithelial region (lamina propria); EP: epithelium;

Collagen I gel contraction assay

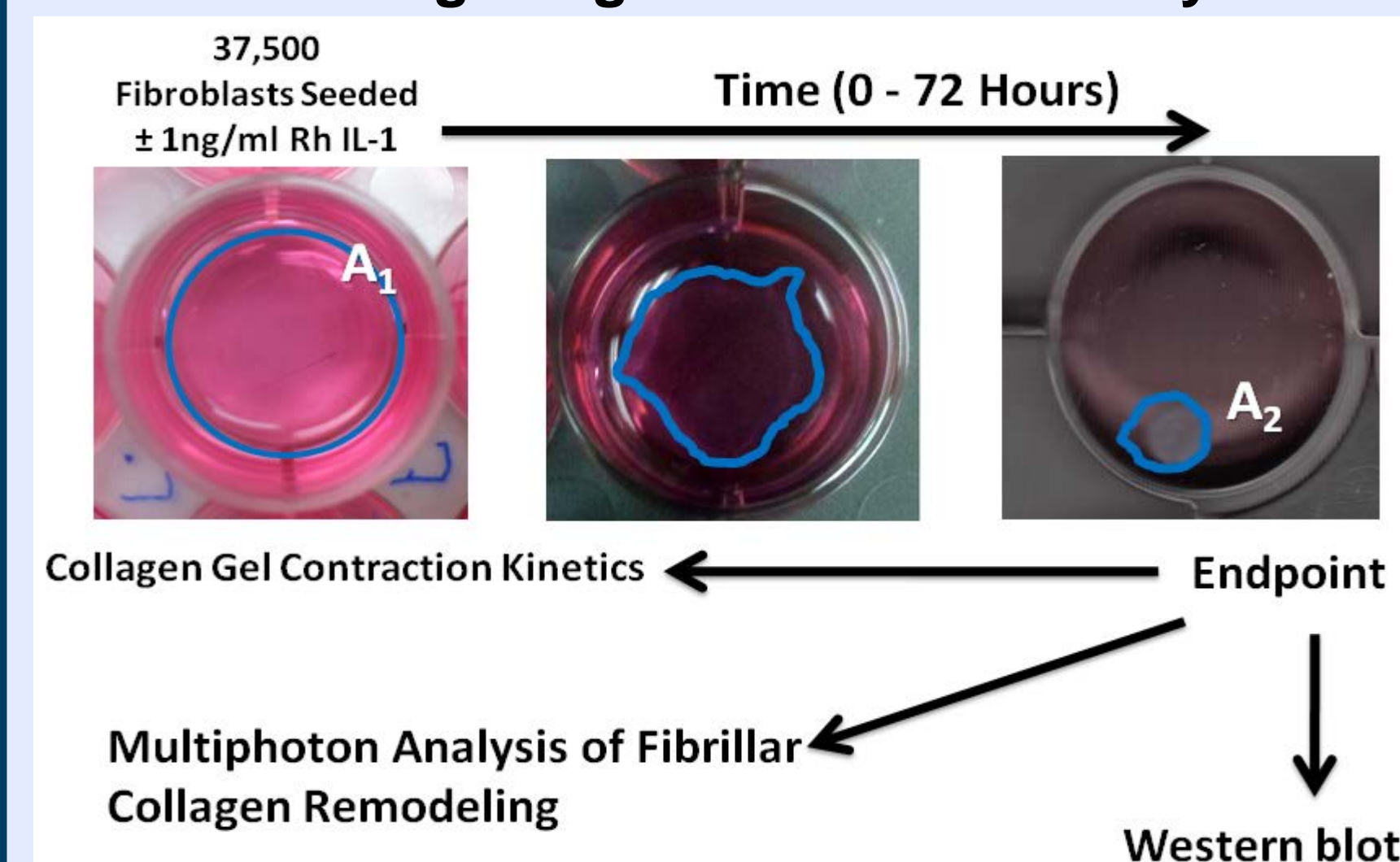


Figure 3. Collagen gel contraction assay. Airway fibroblasts are seeded in Collagen I gels with or with Rh IL-1 α & IL-1 β 1ng/ml stimulation. Gel contraction over a period of 24 or 72 hours is then quantified as a percentage of the initial gel area using image J software. The gels are then processed for SHG imaging.

Results

Collagen I fibers are increased and disorganized in asthmatic airways

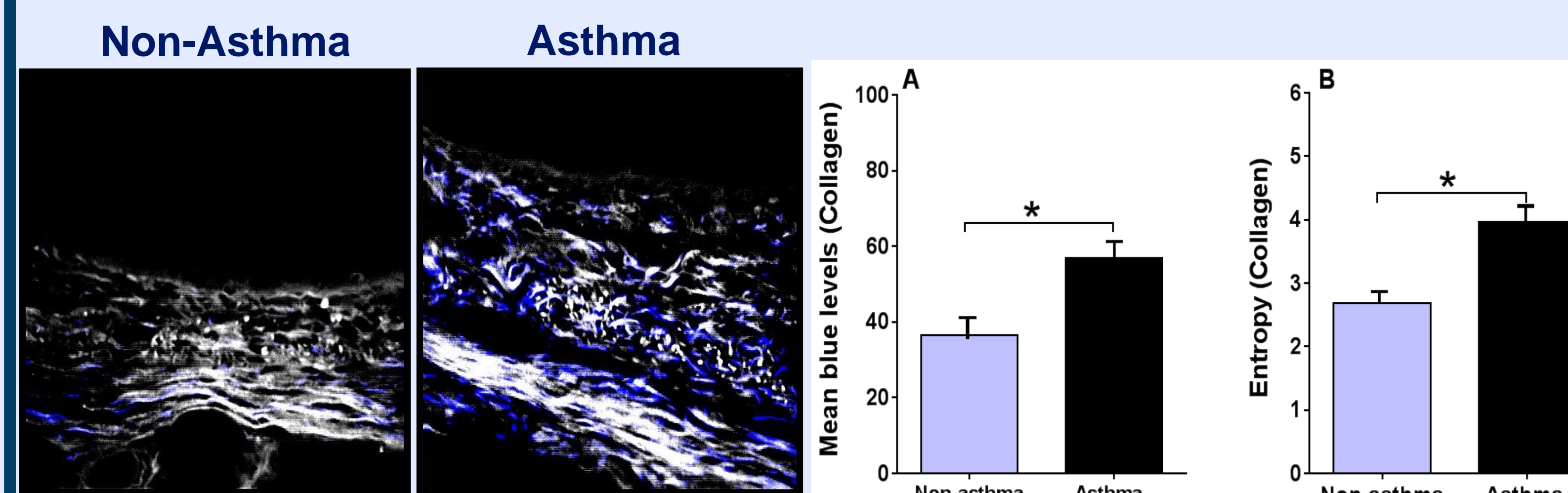


Figure 4. The accumulation and organization of collagen I fibers in airways of non-asthmatics compared to asthmatics. Representative SHG images of the sub-epithelial regions in a cross-section of the non-asthmatic and asthmatic airways and the corresponding A) levels of Collagen I and B) entropy as determined by textural analysis. Data is presented as Mean \pm SEM for an n=10 of non-asthmatics & n=10 of asthmatics. * $=p<0.05$

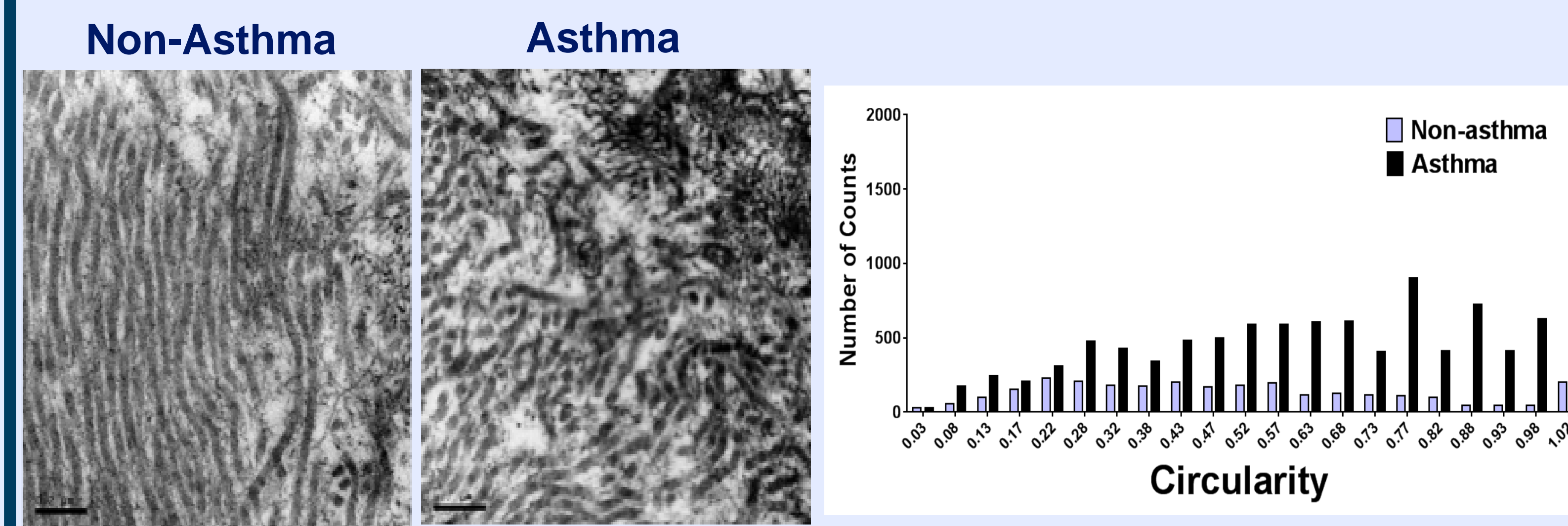


Figure 5. TEM images of a cross section of collagen fibers with their spatial position to the imaging plane and a graph of the collagen fibrils shapes score distribution. Non-asthmatics collagen I fibrils are well organized and well aligned, in contrast with Asthmatic collagen which are not well aligned within fibers. Circularity between asthmatic and non-asthmatic fibrils was significantly different $p=0.0017$.

Fibroblasts derived from asthmatic airways are defective at contracting Collagen I gels

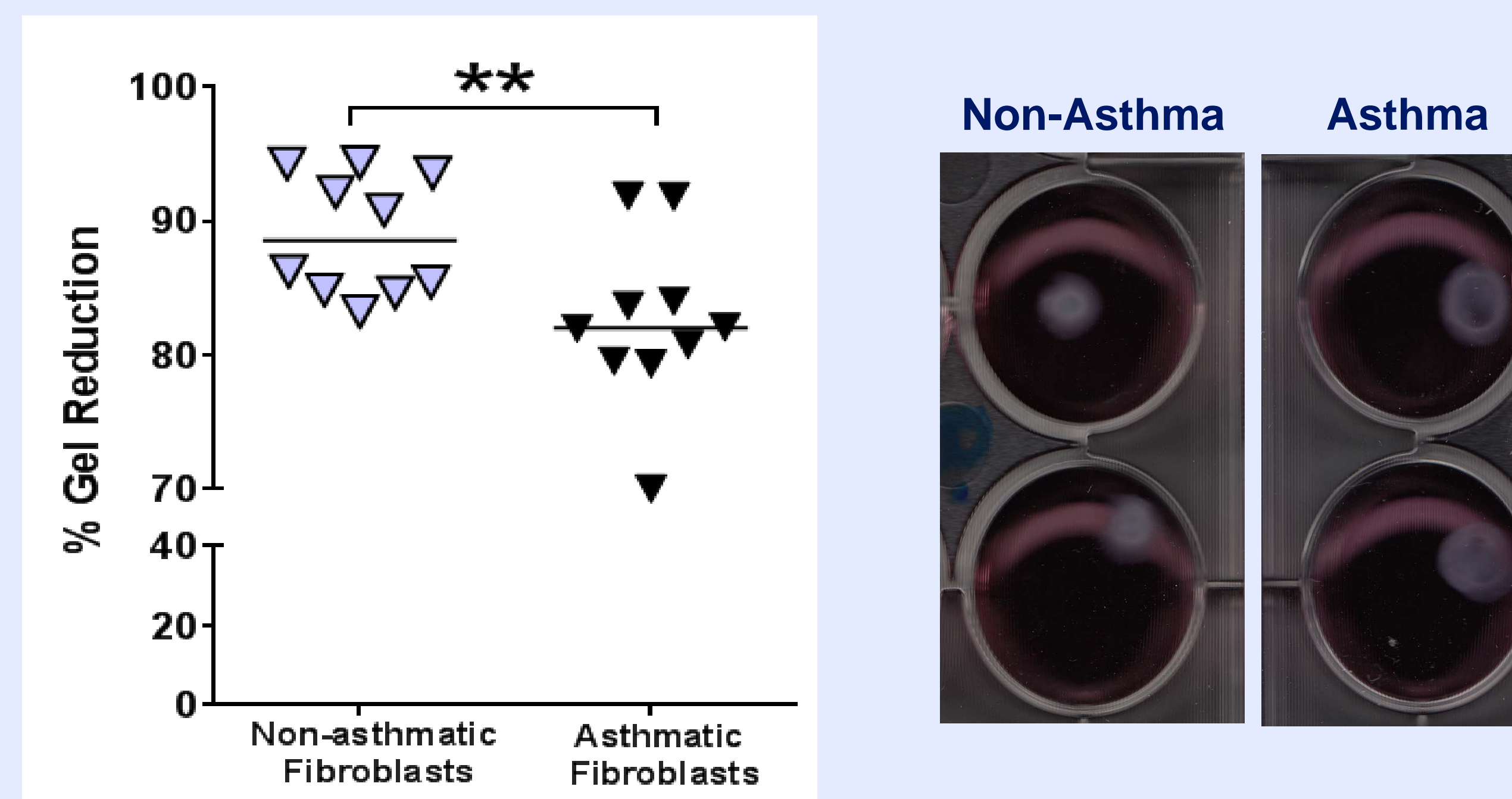


Figure 6. Percentage reduction of collagen I gels seeded with primary fibroblasts from airways. Fibroblasts derived from non-asthmatic & asthmatic airways were grown to confluence and seeded in Collagen I gels. Gel contraction over time was then observed. Data is presented as a percentage of initial gel area (with median) after 72 hours. ** $=p<0.01$

Defective collagen remodeling by asthmatic airway fibroblasts

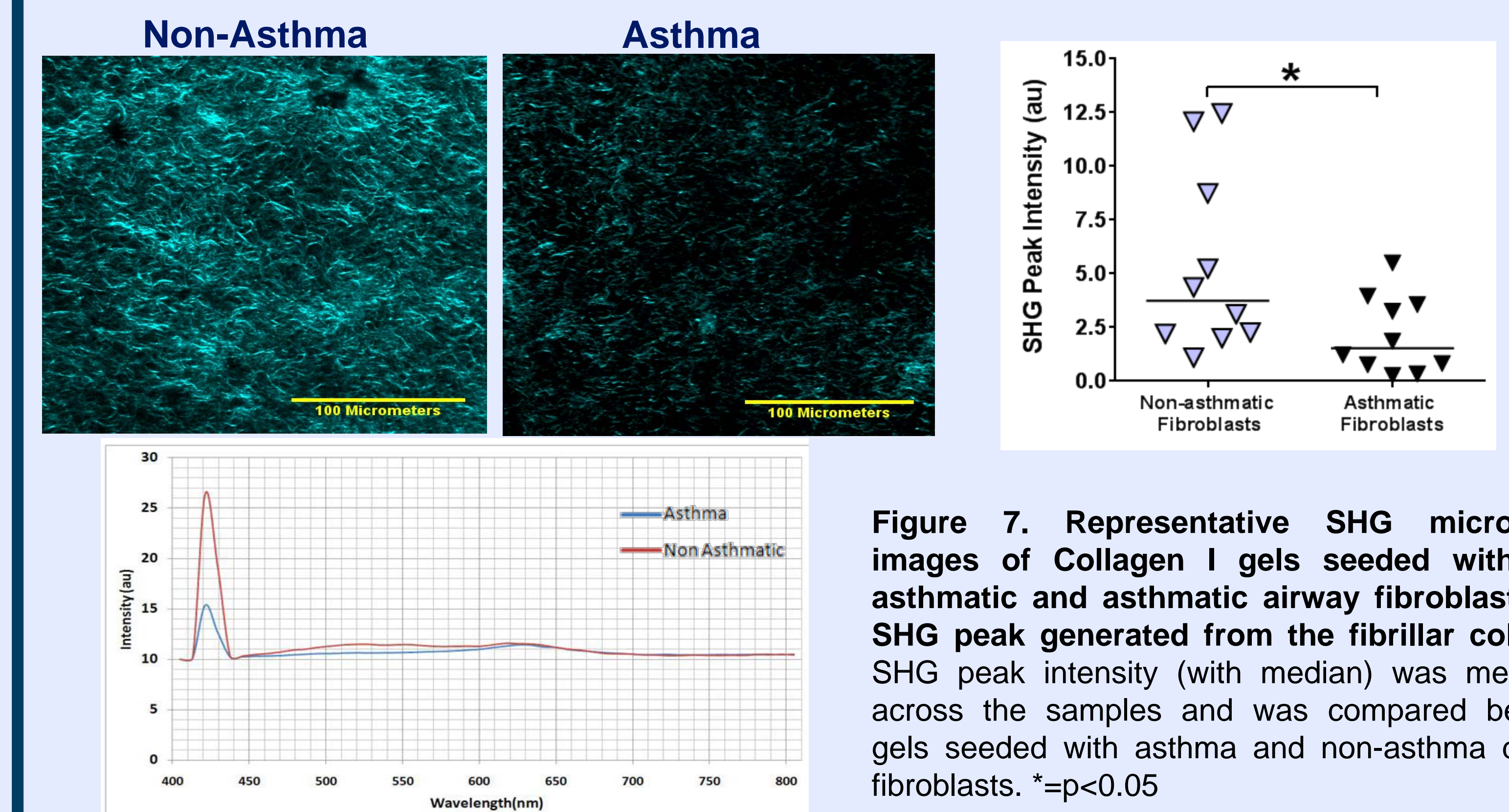


Figure 7. Representative SHG microscopy images of Collagen I gels seeded with non-asthmatic and asthmatic airway fibroblasts and SHG peak generated from the fibrillar collagen. SHG peak intensity (with median) was measured across the samples and was compared between gels seeded with asthma and non-asthma derived fibroblasts. * $=p<0.05$

Increased IL-1 expression in asthmatic airway epithelium may affect fibroblast remodelling phenotype in the lung EMTU

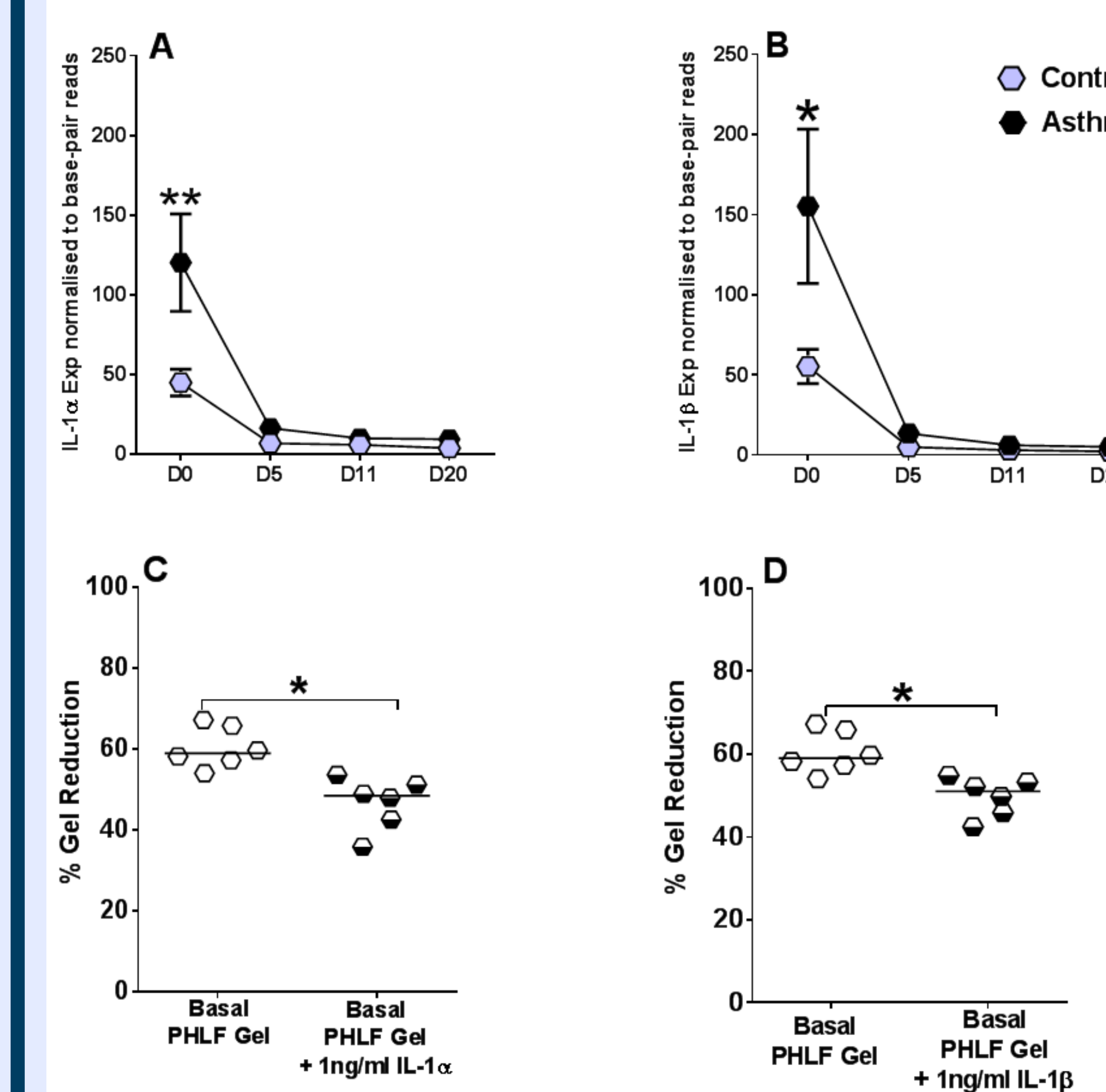


Figure 8. IL-1 expression in asthma-derived epithelium and effect on lung fibroblast collagen contraction. Primary airway epithelial cells from non-asthmatic and asthmatics were cultured at an air-liquid interface and RNA collected at Days 0, 5, 11 and 20. A) Basal IL-1 α & B) Basal IL-1 β expression from epithelial cells expressed as normalized base pair reads and presented as Mean \pm SEM for n=5 non-asthmatics & n=10 Asthmatics. Lung fibroblasts were grown to confluence and seeded in Collagen I gels in the presence or absence of 1ng/ml C) IL-1 α or D) IL-1 β . Data is presented as a percentage of initial gel area (with median) after 24 hours. * $=P<0.05$ & ** $=P<0.01$

Conclusions

- Collagen I fibers and fibrils are disorganized in the lamina propria of the airways in asthmatic patients.
- Fibroblasts derived from the airways of asthmatic patients are deficient in their ability to repair and remodel collagen I compared to non-asthmatic –derived airway fibroblasts.
- IL-1 expression is increased in airway epithelium from asthmatic patients and may influence the remodeling phenotype of lung fibroblasts.
- This helps further our understanding of collagen deposition and remodeling in asthma and create potential opportunities for therapeutic intervention

REFERENCE

1. Osei ET, Brandsma C-A, Noordhoek JA, Timens W, Postma D, Heijink I. Crosstalk between epithelium and fibroblasts; implications for COPD. *European Respiratory Journal* 2014; 44(Suppl 58)