Phenotype and severity of asthma determines bronchial epithelial immune responses to a viral mimic

Porsbjerg C*[1,2,3], Nieto-Fontarigo JJ*[4], Cerps S[4], Ramu S[4], Menzel M[1,3,4,5], Hvidtfeldt M[1,2], Silberbrandt A[1,2], Froessing L[1,2], Klein D[1,2], Sverrild A[1,2], Uller L[4]

[1]Respiratory Research Unit, Department of Respiratory Medicine, Bispebjerg Hospital, Copenhagen, Denmark

[2]Center for Translational Research, Bispebjerg Hospital, Copenhagen, Denmark

[3]Institute for Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[4]Respiratory Immunopharmacology, University of Lund, Lund, Sweden

[5]Skin Immunology Research Center, University of Copenhagen, Copenhagen, Denmark

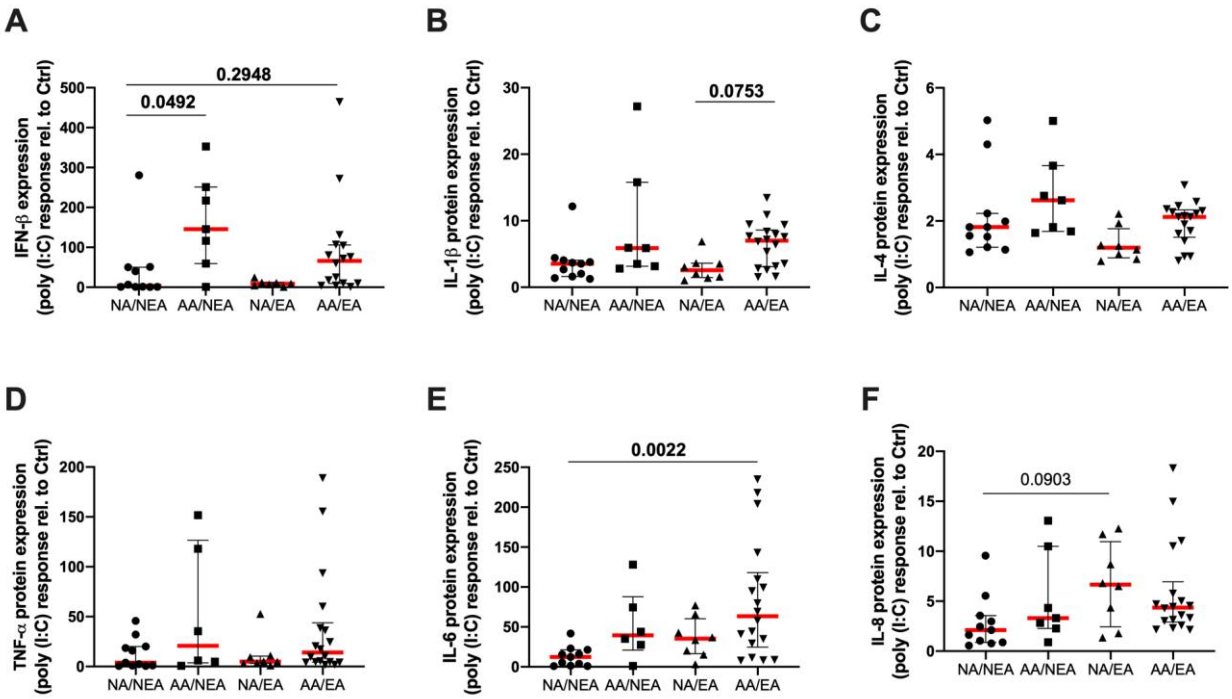
*Both authors contributed equally to the manuscript

ONLINE SUPPLEMENTARY TABLES

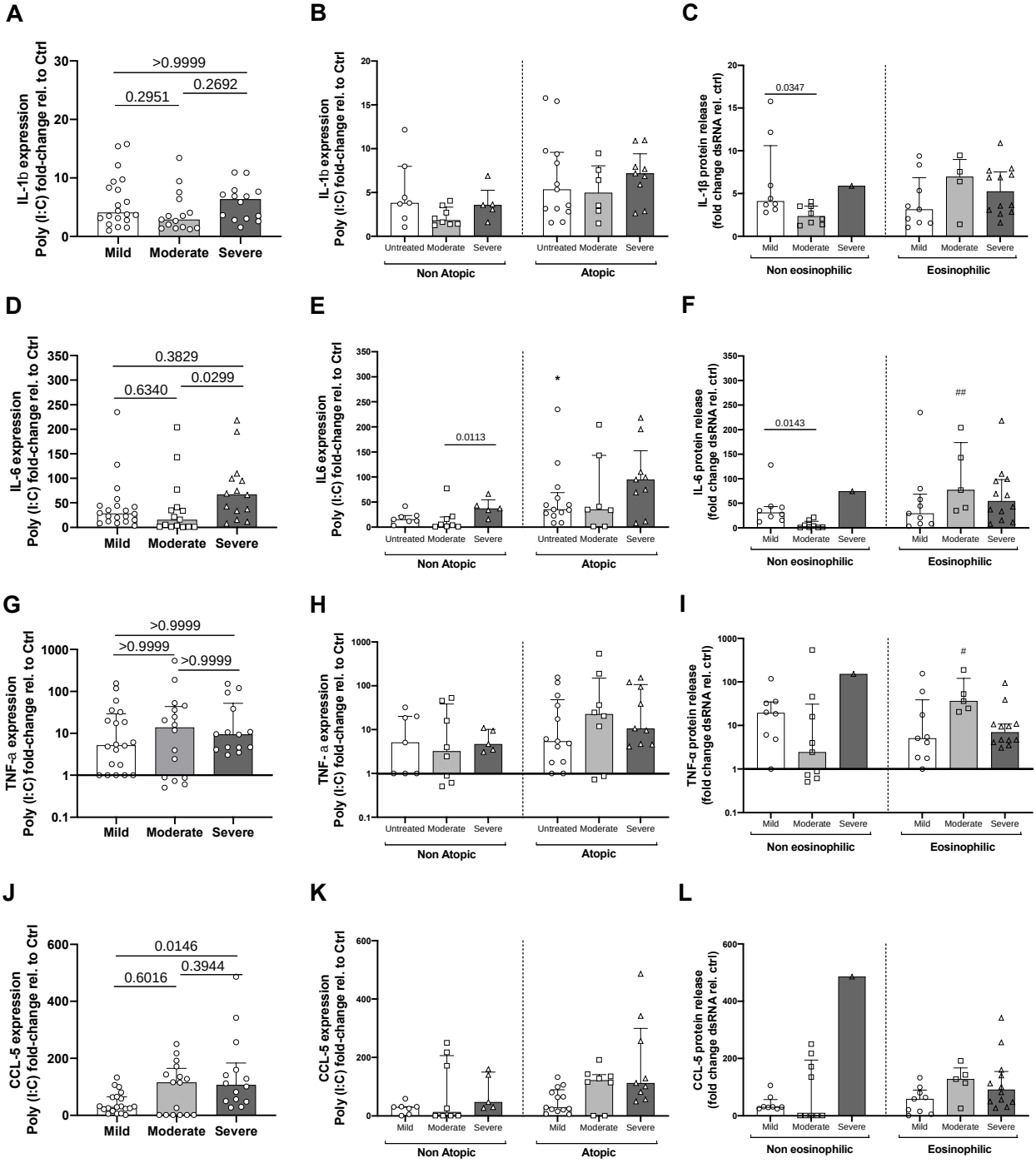
Supplementary Table S1. List of primers used in qPCR analyses

Gene	Primer	Sequence
IFN-β	Sense	-TTACTTCATTAACAGACTTACAGGT-
	Antisense	-TACATTAGCCATCAGTCACTTAAAC-
IL-8	Sense	-CAGAGACAGCAGAGCACAC-
	Antisense	-AGCTTGGAAGTCATGTTTACAC-

ONLINE SUPPLEMENTARY FIGURES



Supplementary Figure S1. Predominant effect of atopy in the immunoreactivity responses of the bronchial epithelium to poly I:C stimulation. IFN-β (A), IL-1β (B), IL-4 (C), TNF-α (D), IL-6 (E), and IL-8 (F) protein release in cell culture supernatants of human bronchial epithelial cells (HBECs) from asthmatic patients with different asthma phenotypes: non-atopic/non-eosinophilic (NA/NEA; N = 11), atopic/non-eosinophilic (AA/NEA; N = 7), non-atopic/eosinophilic (NA/EA; N = 8), and atopic/eosinophilic (AA/EA; N = 18). All data is expressed as log2 fold-change expression of poly (I:C) stimulated HBECs relative to unstimulated cells. Kruskal Wallis with Dunn´s multiple comparison test. Outliers have been removed using the ROUT test with Q set at 0.1%. Numbers on the graph represent p-values.



Supplementary Figure S2. Impact of severity on proinflammatory response of bronchial epithelium to the viral stimulation. IL-1β (A-C), IL-6 (D-F), TNF-α (G-I), and CCL-5 (J-L) release in cell culture supernatants of human bronchial epithelial cells (HBECs) from asthmatic patients with different severity degrees (Mild, N = 20; Moderate, N = 16; Severe, N = 14) in response to 24h poly (I:C)-stimulation in the overall study population, or within the different asthma phenotypes: Atopic (Mild, N = 13; Moderate, N = 8; Severe, N = 9) vs non atopic (Mild, N = 7; Moderate, N = 8; Severe, N = 5) (B, E, H, K), and eosinophilic (Mild, N = 9; Moderate, N = 5; Severe, N = 12) vs. non-

eosinophilic (Mild, N = 8; Moderate, N = 9; Severe, N = 1) (C, F, I, L). All data is expressed as log2 fold-change expression of poly (I:C) stimulated HBECs relative to unstimulated cells. Kruskal Wallis with Dunn´s multiple comparison test. Outliers have been removed using the ROUT test with Q set at 0.1%. Numbers on the graph represent p-values. *$p<0.05$ mild atopic vs mild non-atopic; #$p<0.05$ moderate eosinophilic vs moderate non-eosinophilic; Mann-Whitney U test.