**Double targeting inflammasome activation and accelerated cellular senescence in alveolar macrophages exposed to cigarette smoke and COPD**

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**Supplementary Data**

**Text S1. Previous publications on the NLRP3 and cleaved IL-1β antibodies**

The NLRP3 antibody H53 (SC66846) and cleaved IL-1β antibody h117 (SC23459) have been used in multiple publications by our and other groups

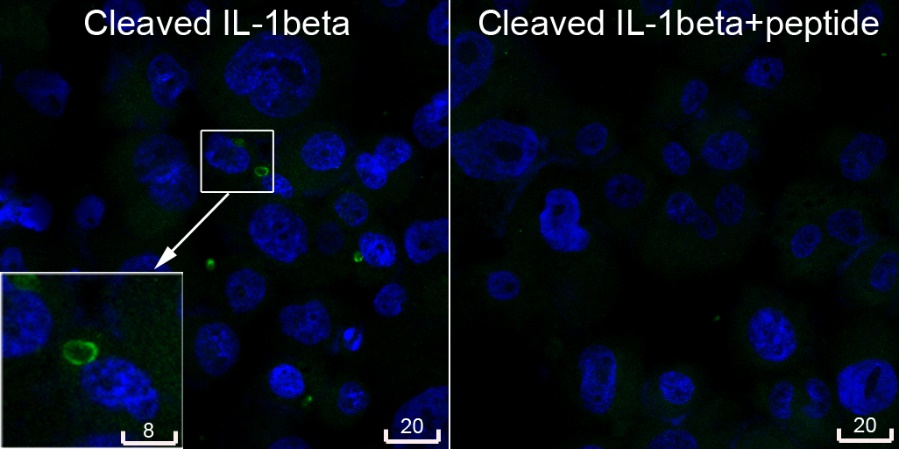
Tran et al. Immunolocalization of NLRP3 Inflammasome in Normal Murine Airway Epithelium and Changes following Induction of Ovalbumin-Induced Airway Inflammation. J Allergy (Cairo). 2012; 2012:819176. J Allergy 2012.

Chen et al. Multiple inflammasomes may regulate the interleukin-1-driven inflammation in protracted bacterial bronchitis. ERJ Open Res. 2018; 4(1):00130-2017.

Yang et al. Small heterodimer partner interacts with NLRP3 and negatively regulates activation of the NLRP3 inflammasome. Nat Commun. 2015;6:6115).

**Table S1. Patients’ characteristics**

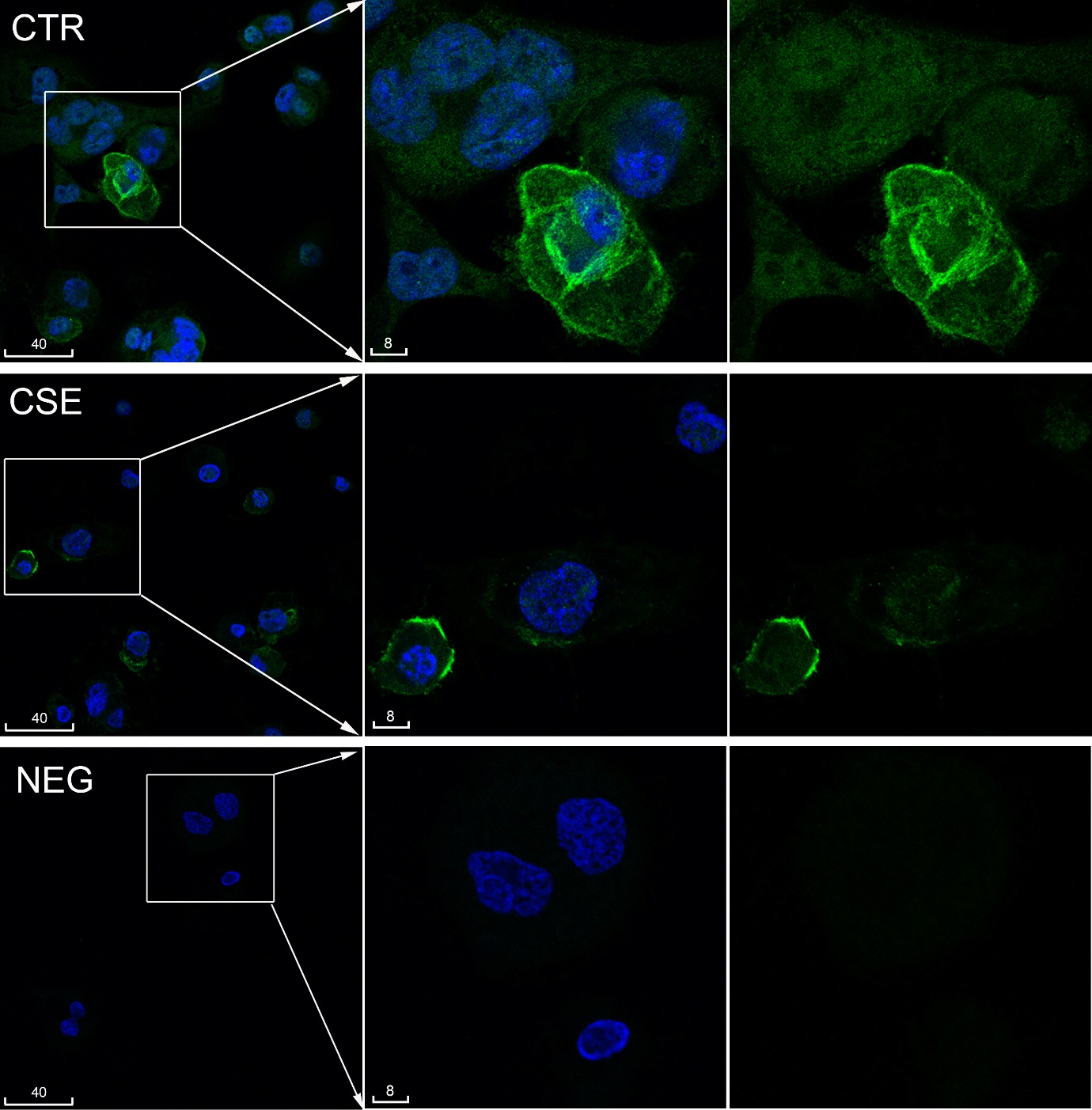




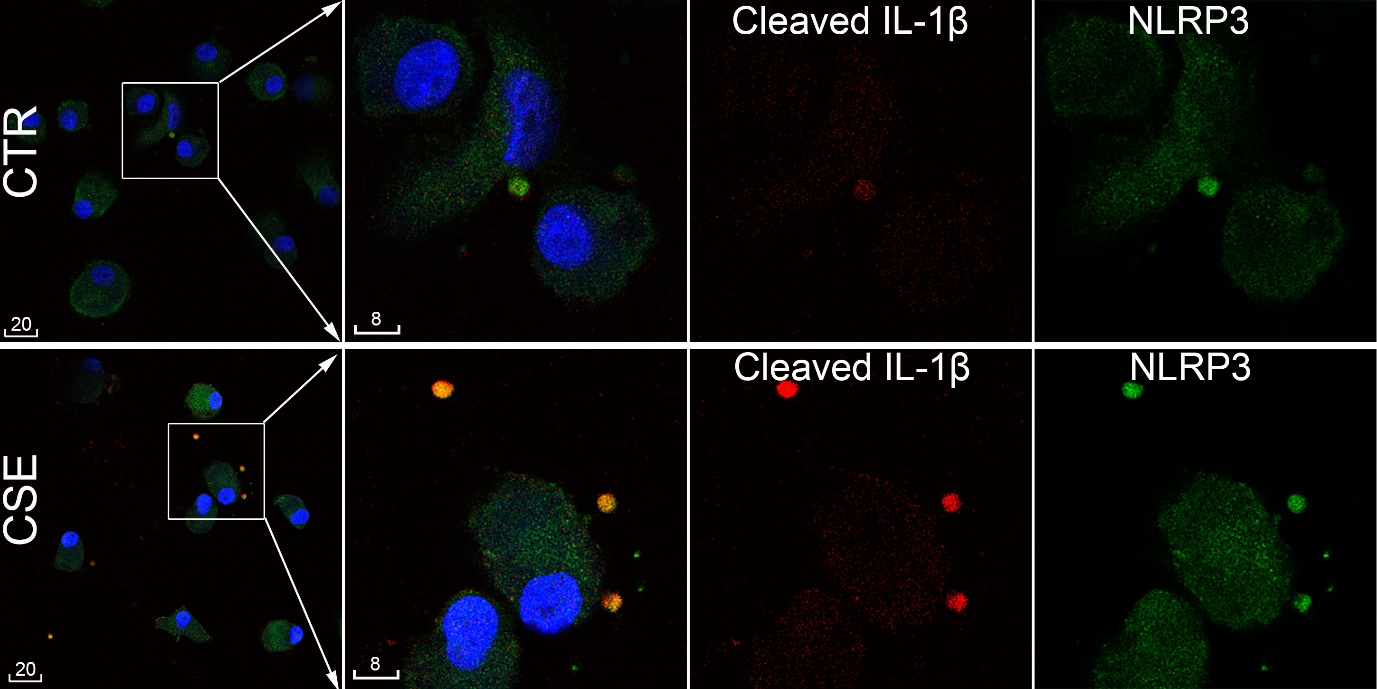
**Figure S1. Complete blocking of the cleaved IL-1β antibody h117.** Confocal images of THP-1 macrophages stained with the antibody h117 (green), incubated with (right) or without (left) specific blocking peptide. Blue is DAPI. Scale bars are in micrometers.

A graph of different colored bars

Description automatically generated with medium confidence**Figure S2. Manders’ split coefficients analysis of colocalization.** M1 (red): the fraction of NLRP3 overlapping with cleaved IL-1β immunofluorescence; M2 (green): the fraction of cleaved IL-1β overlapping with NLRP3 immunofluorescence. Error bars are standard errors from 5 measurements. Shown data for CSE-stimulated BAL-derived macrophages from 4 donors, and for CSE-stimulated THP-1-derived macrophages.



**Figure S3. Low level expression of CD9 (green) in THP-1 macrophages.** In basal state THP-1 macrophages showed surface expression of CD9 in only a few cells. Blue is DAPI. Scale bars are in micrometers. NEG, conjugate alone negative control.



**Figure S4**. Cigarette smoke extract induced extracellular cleaved IL-1β (red) colocalized with NLPR3 (green, yellow is colocalization with cleaved IL-1β) in blood monocyte-derived macrophages. Blue is DAPI. Scale bars are in micrometers.



**Movie S1.** Subcellular localization of CD81 and lamin B1 in blood monocyte-derived macrophages by confocal Z-analysis. While CD81 revealed bright cell surface staining, lamin B1 showed marginalized nuclear localization, consisting with its association with nuclear envelope and marginalized chromatin. Exposure to cigarette smoke extract induced reduction of both CD81 and lamin B1.