SUPPLEMENTAL MATERIAL:

Hyaluronan Activation of the Nlrp3 Inflammasome Contributes to the Development of Airway Hyperresponsiveness

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Running Title: Hyaluronan activation of the inflammasome and environmental airways disease

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Supplement Figures

Figure S1.

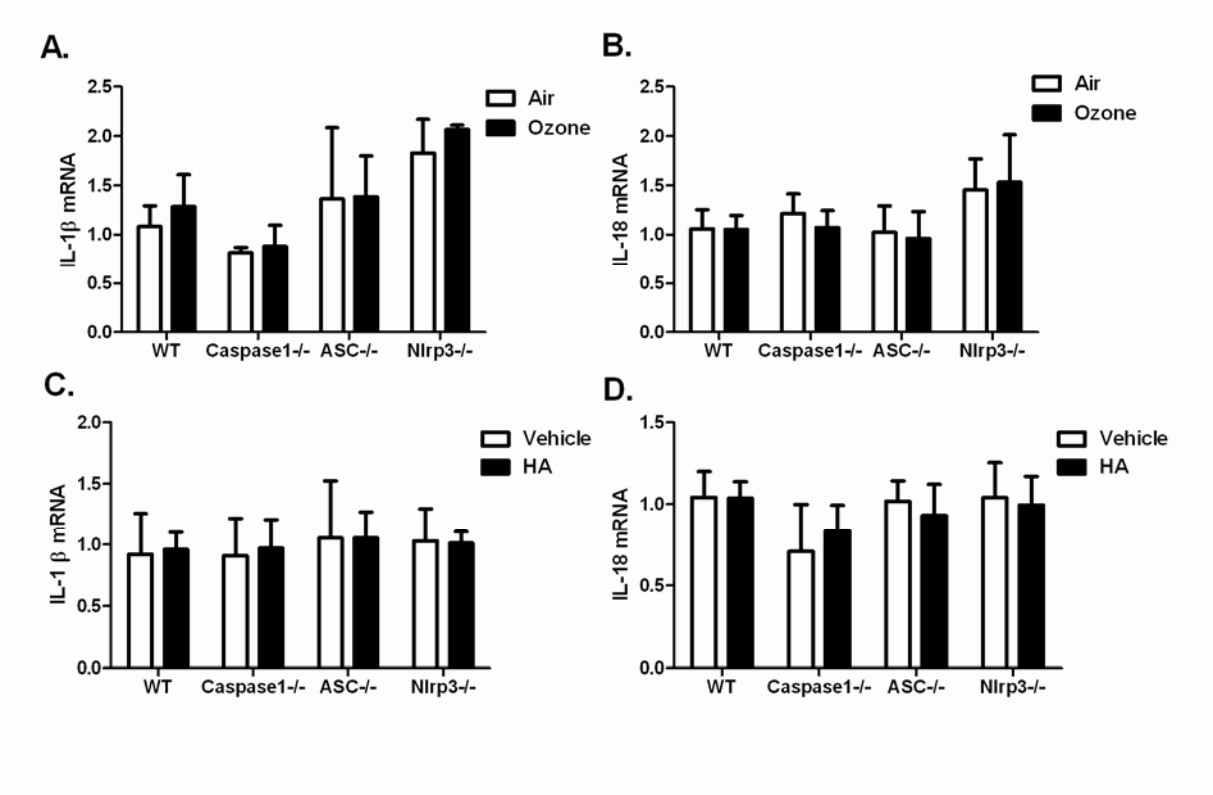

Figure S1: RNA expression of *IL-1β* and *IL-18* in whole lung macrophages. (A/B) Whole lung macrophages were isolated after exposure to ozone and evaluated for mRNA expression of *IL-1β* and *IL-18* by real-time PCR and no differences were detected. **(C/D)** Whole lung macrophages were isolated after exposure to hyaluronan fragments and evaluated for mRNA expression of *IL-1β* and *IL-18* by real-time PCR and no differences were detected. Data presented as mean ± SEM (n=5/group).

Figure S2.

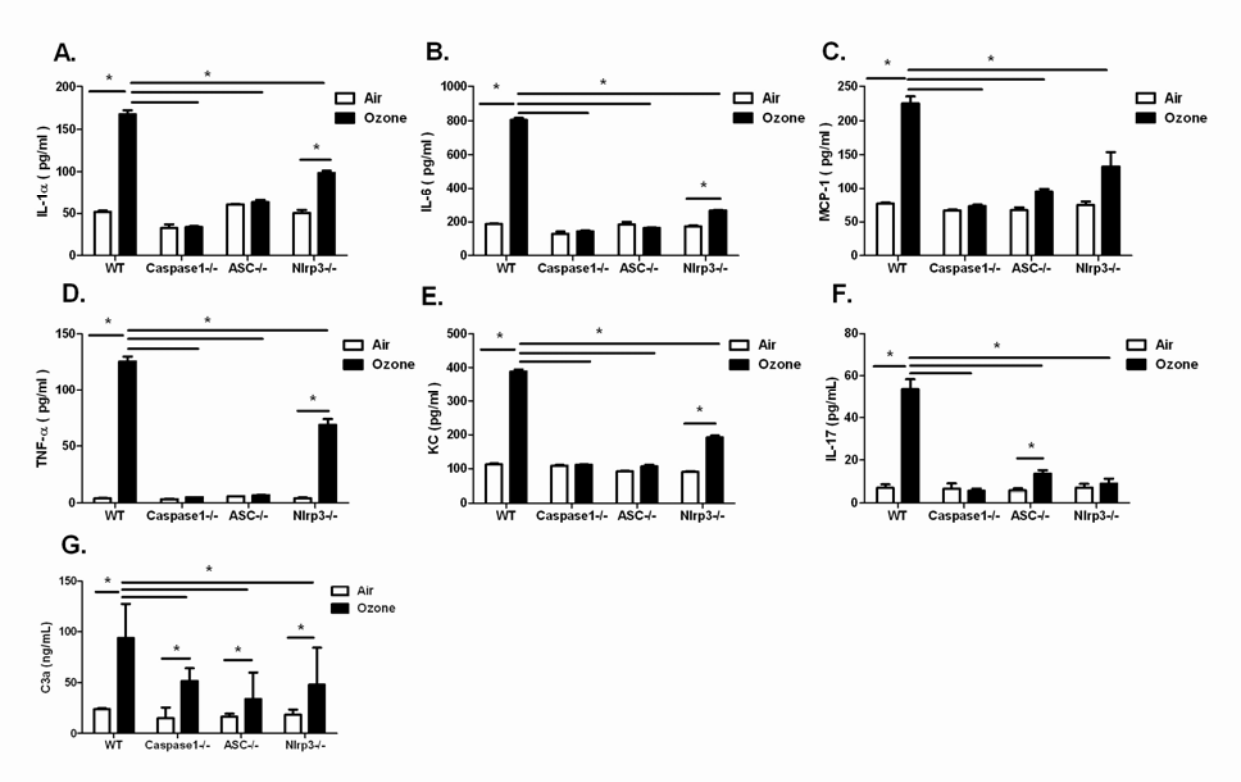

Figure S2. Level of non-inflammasome pro-inflammatory factors in BAL at 24 hours after ozone exposure. BAL IL-1α, IL-6, monocyte chemoattractant protein-1(MCP-1), tumor necrosis factor-α (TNF-α), keratinocyte-derived chemokine (KC), IL-17, and C3a were increased with ozone exposure, and MCP-1 was totally dependent on the presence of Nlrp3-inflammasome, including caspase1, ASC and Nlrp3; IL-1α, IL-6, TNF-α and KC were totally dependent on caspase1 and ASC and partially dependent on Nlrp3; IL-17 was partially dependent on ASC; C3a was partially dependent on Nlrp3-inflammasomes after ozone exposure. Data presented as mean ± SEM and are representatives from two similar experiments(n=5, $*p < 0.05$).

Figure S3.

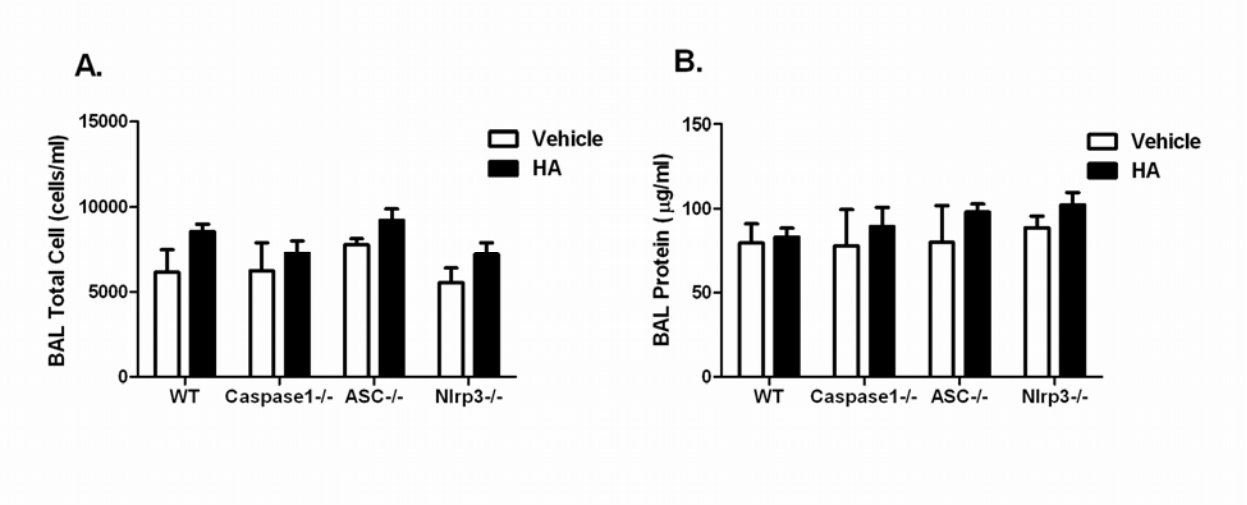

Figure S3. Biological response to hyaluronan and genes of the Nlrp3-inflammasome. (A) The level of total cells in bronchoalveolar lavage (BALF) was independent of challenge to hyaluronan. **(B)** The level of BALF protein was independent of hyaluronan exposure. Data presented as mean ± SEM (n=5/group).

Figure S4.

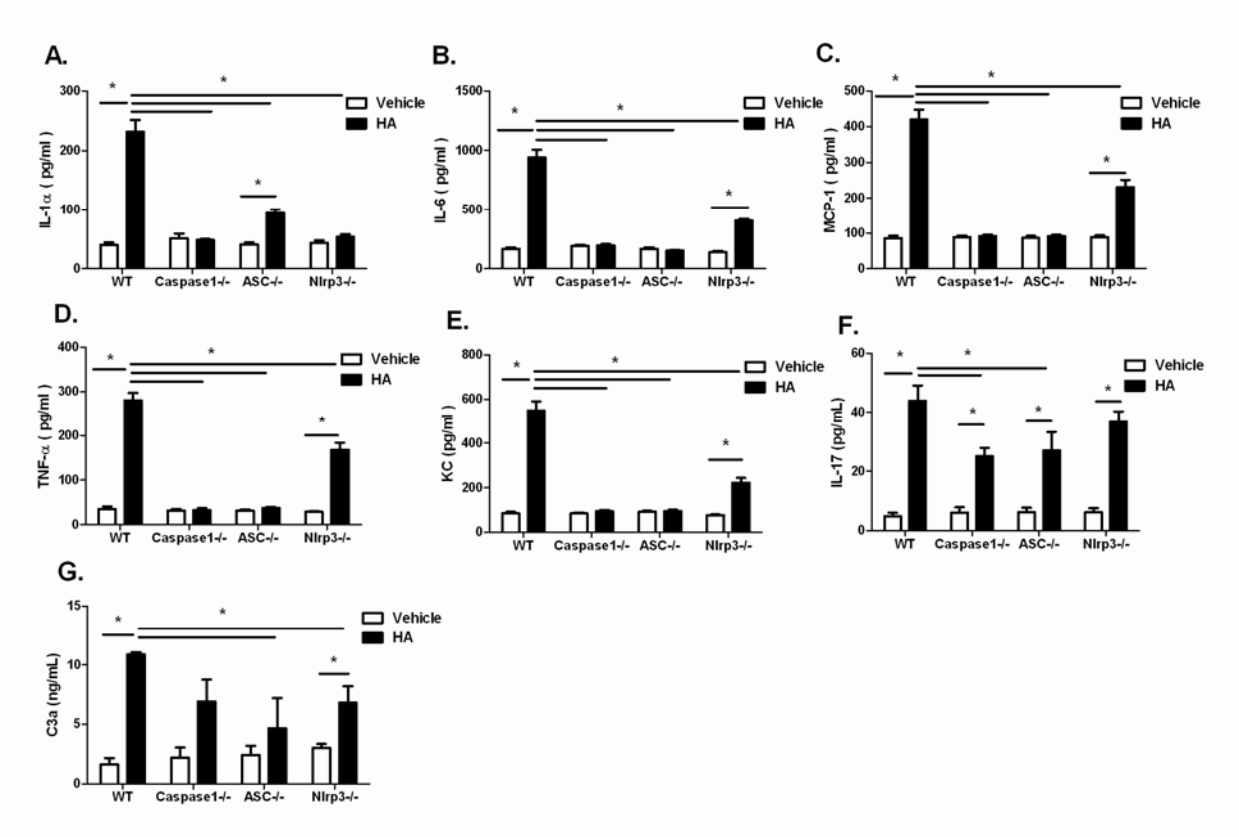

Figure S4. Level of non-inflammasome pro-inflammatory factors in the BAL after HA exposure. The release of BAL IL-6, MCP-1, TNF-α and KC were totally dependent on ASC and caspase1 and partially dependent on Nlrp3; IL-1α was partially dependent on ASC. The level of IL-17 was partially dependent on caspase1 and ASC, but not dependent on Nlrp3; C3a was totally dependent on ASC and caspase1, and partially dependent on Nlrp3 after hyaluronan exposure. Data presented as mean ± SEM and are representatives from two similar experiments (n=5, *: $p < 0.05$).