SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Time course of TEER values of ALI-cultured Calu-3 cells.

TEER was measured at the indicated time points, and shown as the mean value ± SD of triplicate determinations.
Supplementary Figure S2. Effect of Der p 1 co-exposure on the barrier integrity of ALI-cultured Calu-3 cells on days 2 and 7, and on Ole e 1 allergen.

A) Confocal laser scanning microscopy analysis of ZO-1 (red) expression over time of exposure. Representative Z-stack projection of 12-16 individual sections are shown. White arrows indicate TJ disruptions. Scale bar = 25 μm. C, non-treated cells; O, cells exposed to Ole e 1; D, cells exposed to Der p 1 protease; OD, cells co-exposed to the combination of Ole e 1 and Der p 1.

B) MALDI-TOF-MS analysis of Ole e 1-cleavage products by Der p 1 at different time points. The mass/charge ratio (m/z) is shown: monoprotonated (16197.4 and 17518.4 m/z) and bi-protonated (8285.3 and 8612.2 m/z) species of non-glycosylated and glycosylated of Ole e 1 forms, respectively. The N-terminal sequences of full- and short-length forms of Ole e 1 protein (*) obtained by Edman degradation after protease-treatment for 0.5 h are shown. Insert, immunoblotting of Ole e 1 using a specific polyclonal antiserum over 24 h of protease-treatment. Molecular mass protein standards in kDa are indicated. Ole e 1 hydrolysed with 6N HCl was used as a negative control: HCl.