

Supplementary Information for:

Atopic dermatitis-related anti-inflammatory in vitro effects of a plant extract mixture

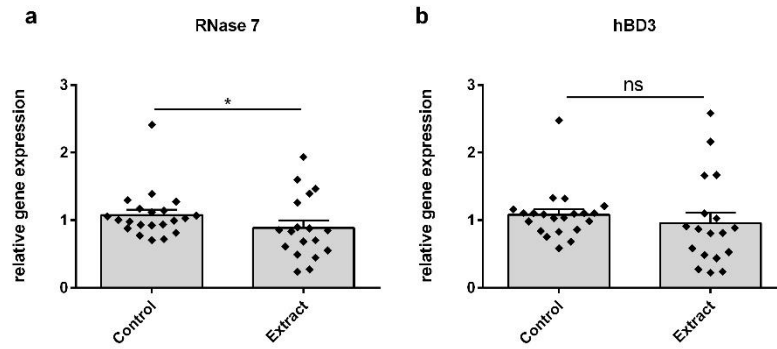
Authors: Nina Heinemann¹, Franziska Rademacher¹, Henning Vollert², Regine Gläser¹, Jürgen Harder^{1*}

¹ Department of Dermatology, Kiel University, Kiel, Germany

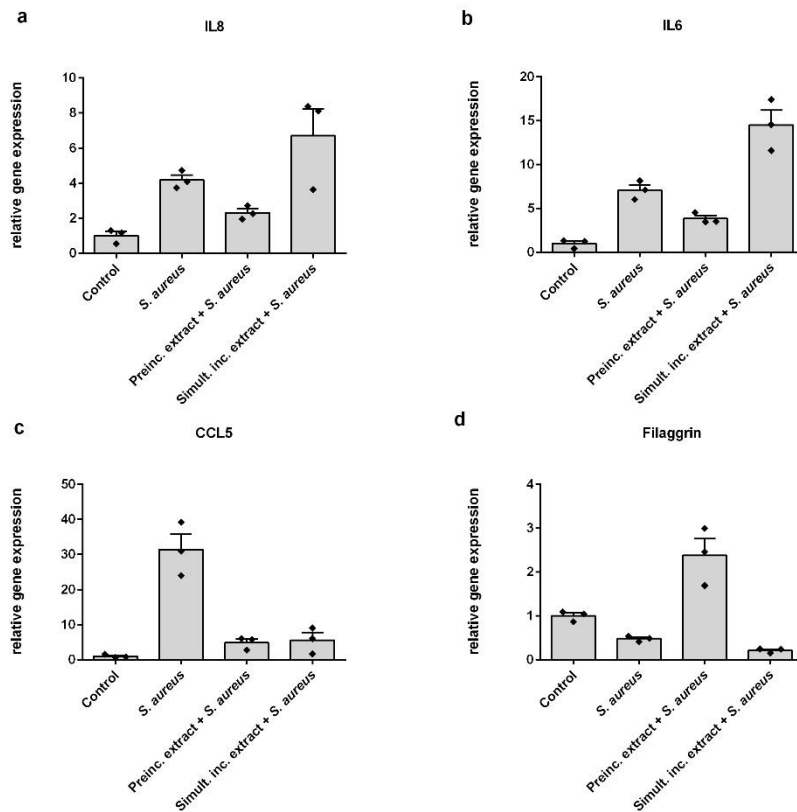
² Bioactive Food GmbH, Bad Segeberg, Germany

This file includes:

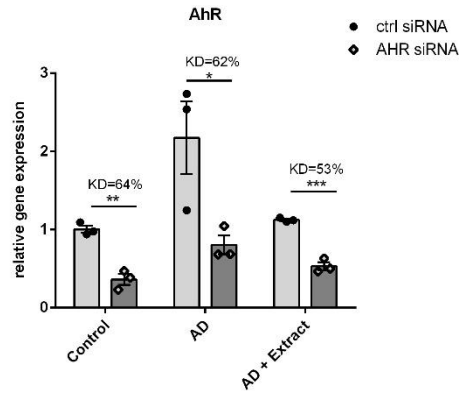
- Supplementary Figures S1-S4



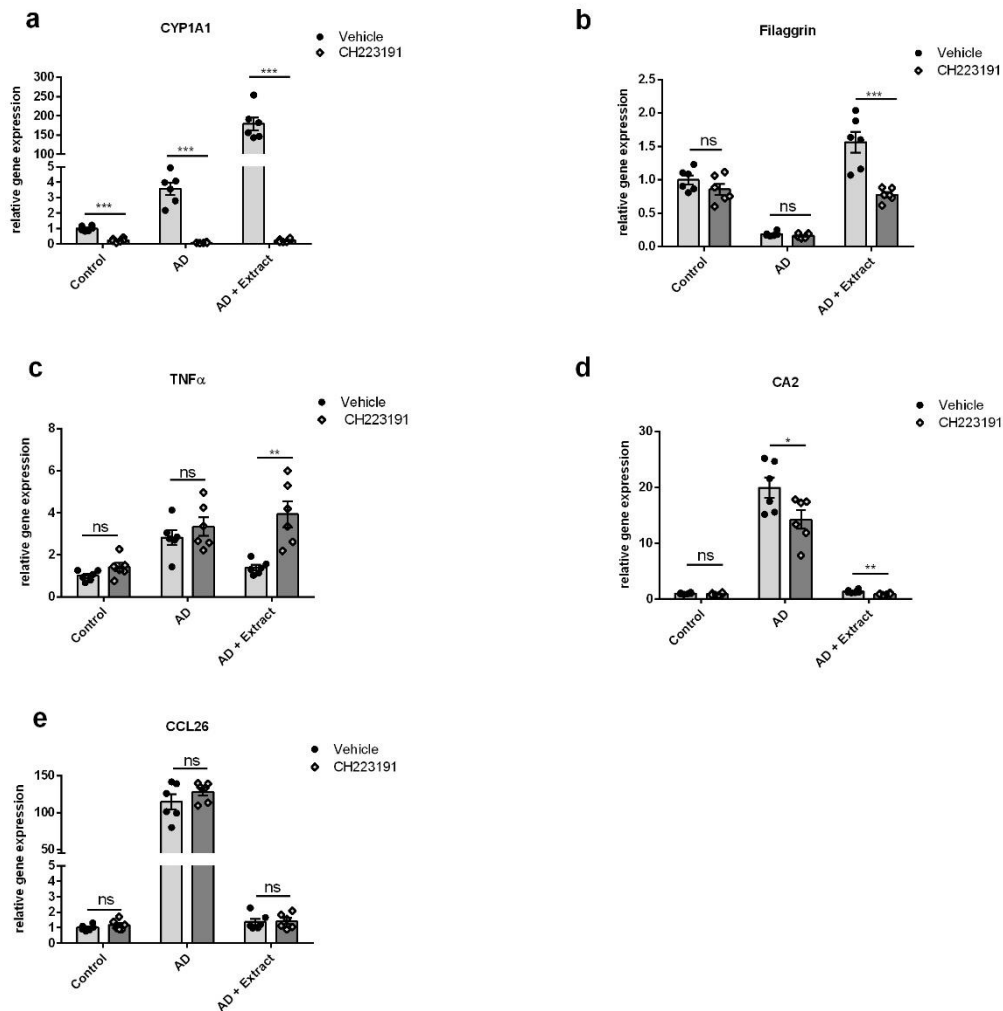
Supplementary Figure S1: Incubation with Plant Extract Mixture Did Not Induce Principal *S. aureus*-Killing Antimicrobial Peptides. CaCl₂-differentiated NHEKs were left unstimulated (Control) or stimulated with the plant extract (Extract) for 20 h. Gene expression levels of (a) RNase 7 and (b) hBD3 were determined by real-time PCR. Statistical significance was tested by Mann-Whitney test (n=18 *p < 0.05, ns = not significant). Bars indicate means + SEM.



Supplementary Figure S2: Preincubation with Plant Extract Mixture Reduced Inflammation Induced by *S. aureus* 185 P29. NHEKs were preincubated with the plant extract for 24 h and subsequently treated with the *S. aureus* strain 185 P29 for 3 h, followed by gentamycin addition and further incubation for 24 h. Gene expression levels of (a) IL8, (b) IL6, (c) CCL5 and (d) filaggrin were determined by real-time PCR. The data shown are from one experiment performed in triplicate stimulations.



Supplementary Figure S3: AhR Knockdown Efficiency. NHEKs were transfected with AhR siRNA or control siRNA. 48 h later, NHEKs were stimulated with the AD-associated cytokine mixture (IL-13, IL-4, IL-22 and TNF-alpha, each 10 ng/mL) either in the absence (AD) or in the presence of the plant extract (AD + Extract). The knockdown efficiency (KD) was indicated in percent above the corresponding bars. Gene expression levels of AhR were determined by real-time PCR. Statistical significance was tested by unpaired t-test (n=3; *p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant). Bars indicate means + SEM.



Supplementary Figure S4: Impact of AhR Inhibition on the Effects of the Plant Extract Mixture in the 2D AD Model. CaCl₂-differentiated NHEKs were preincubated with 10 μM AhR inhibitor CH223191 or vehicle for 1 h. Then, NHEKs were stimulated in the presence of 10 μM CH223191 with the AD-associated cytokine mixture (IL-13, IL-4, IL-22 and TNF-alpha, each 10 ng/mL) either in the absence (AD) or in the presence of the plant extract (AD + Extract). Gene expression levels of (a) CYP1A1 (b) filaggrin (c) TNFα (d) CA2 and (e) CCL26 were determined by real-time PCR. Statistical significance was tested by unpaired t-test (n=6; *p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant). Bars indicate means + SEM.