Supplementary Material

Staphylococcus epidermidis activates aryl hydrocarbon receptor (AhR) signaling in human keratinocytes: Implications for cutaneous defense

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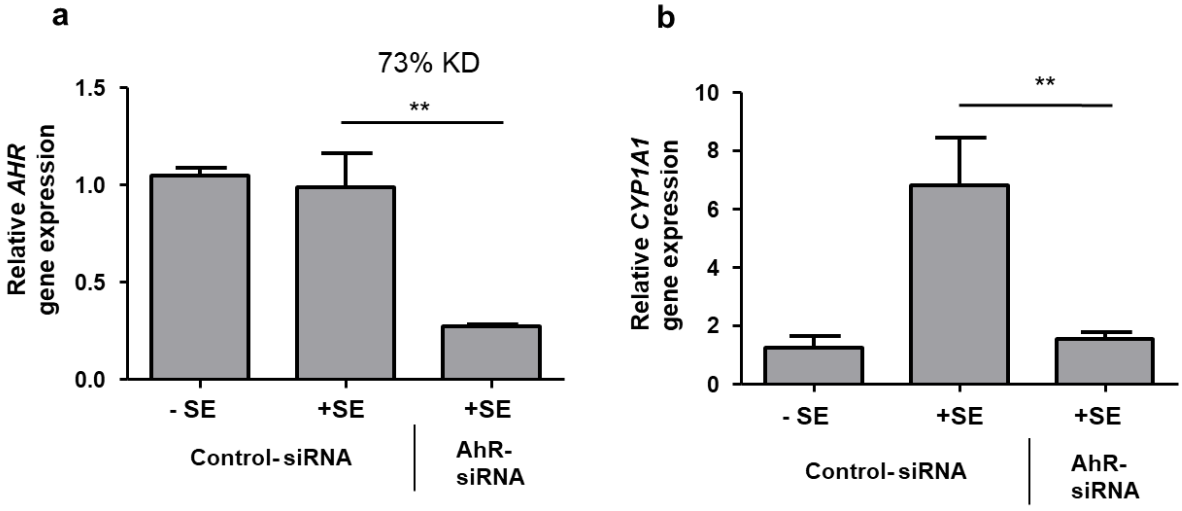
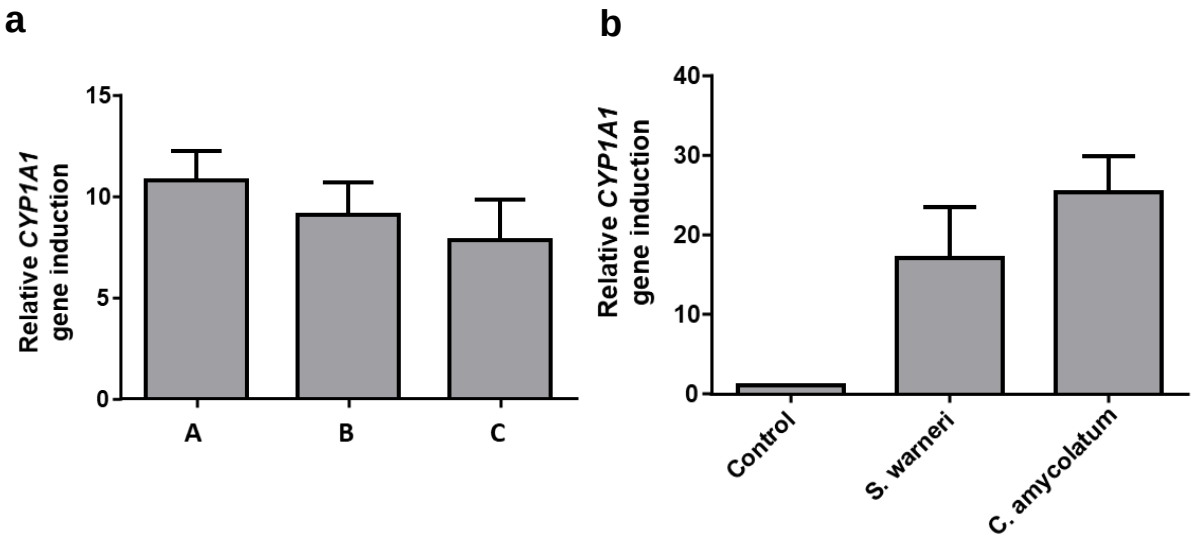
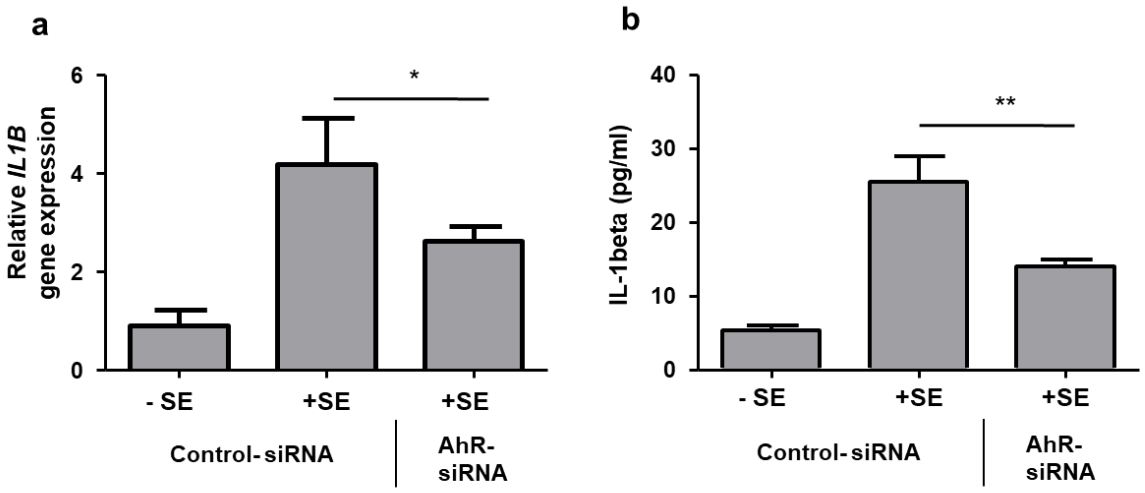


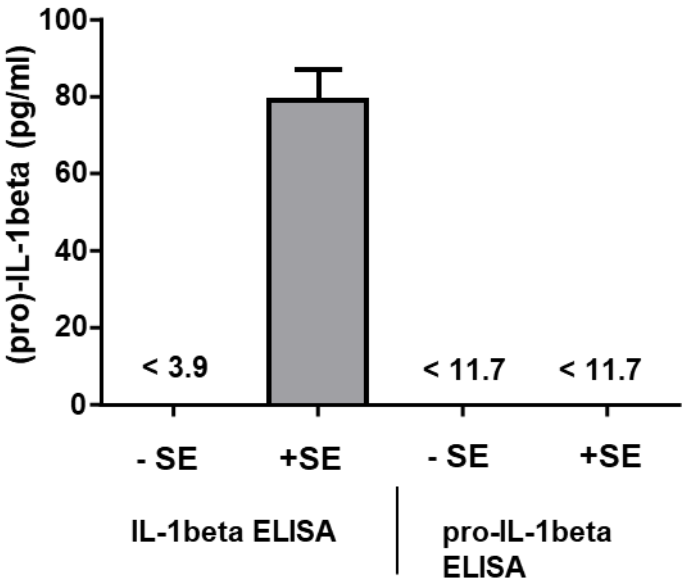
Supplementary Figure 1. Induction of the AhR-responsive gene CYP1A1 by *S. epidermidis* in human keratinocytes is mediated by the AhR. Human primary keratinocytes were transfected with a control siRNA or an AhR-specific siRNA (s1200) and stimulated with *S. epidermidis* strain ATCC 14990 (SE). Knockdown efficiency (KD) of AhR gene expression (**a**) and *CYP1A1* gene expression (**b**) were analyzed by real-time PCR. Bars are means ± SEM of 6 stimulations (**p<0.01, Student`s *t*-test).



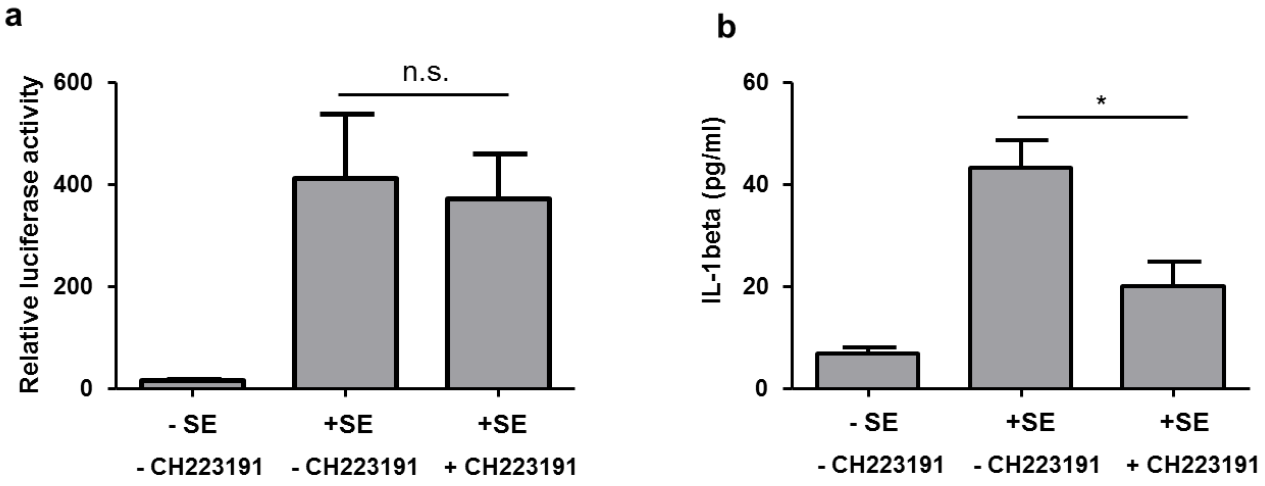
Supplementary Figure 2. Different bacterial isolates induce CYP1A1 gene expression. Human primary keratinocytes were stimulated with supernatants of three skin-derived isolates of *S. epidermidis* (A-C) derived from different individuals (**a**) or with supernatants of *Staphylococcus warneri* and *Corynebacterium amycolatum* (clinical isolates). Relative *CYP1A1* gene induction was determined by real-time PCR. Data are presented as means ± SEM of two (**a**) or three (**b**) stimulations.



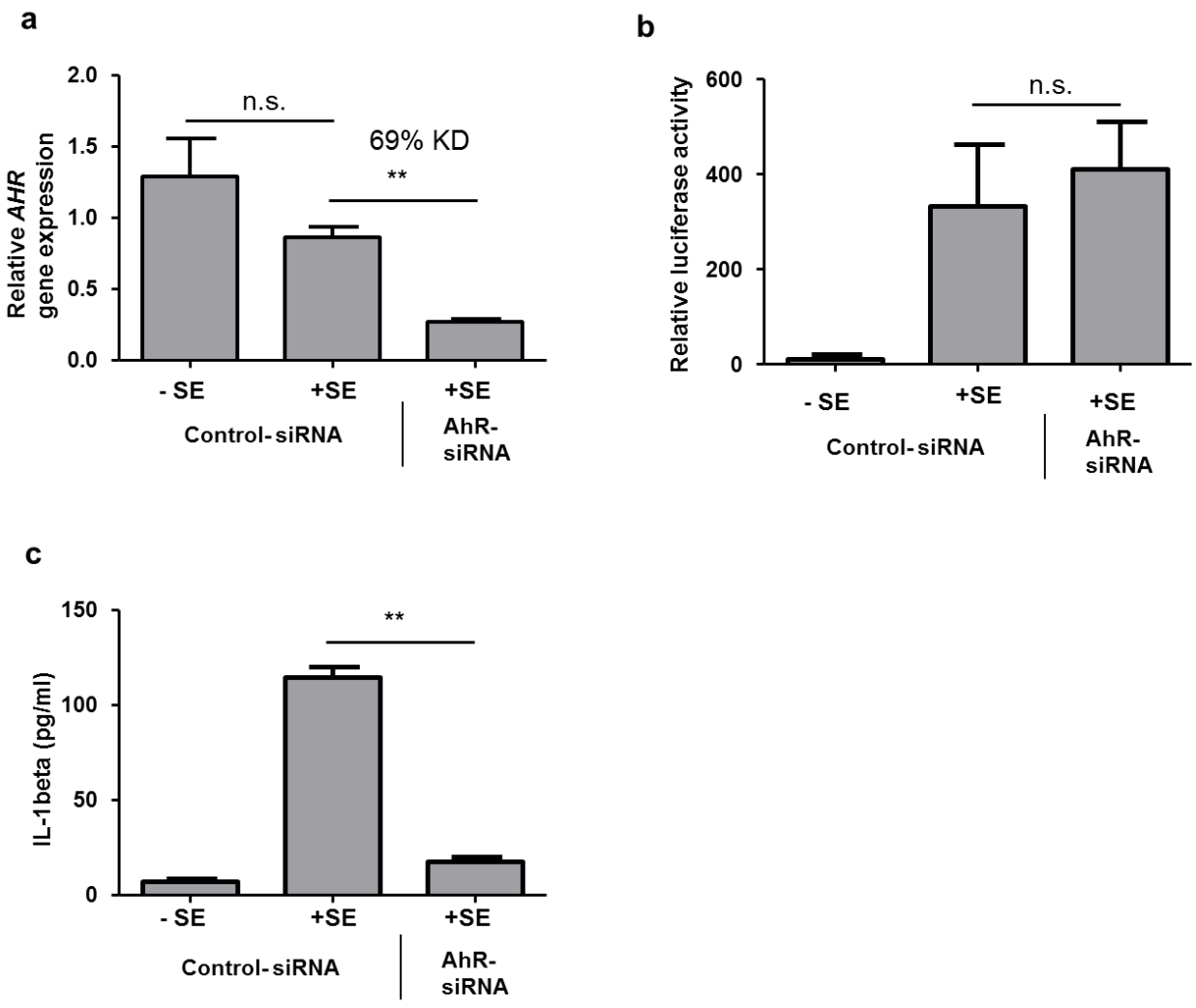
Supplementary Figure 3. Induction of IL-1beta by *S. epidermidis* in human keratinocytes is mediated by the AhR. Human primary keratinocytes were transfected with a control siRNA or an AhR-specific siRNA (s1200; 73% gene expression knockdown efficiency (see Fig. S1a)) and stimulated with S. epidermidis strain ATCC 14990 (SE). IL-1beta gene expression and protein secretion was determined by real-time PCR (**a**) and ELISA (**b**), respectively. Bars are means ± SEM of 6 stimulations (**p<0.01, Student`s *t*-test).



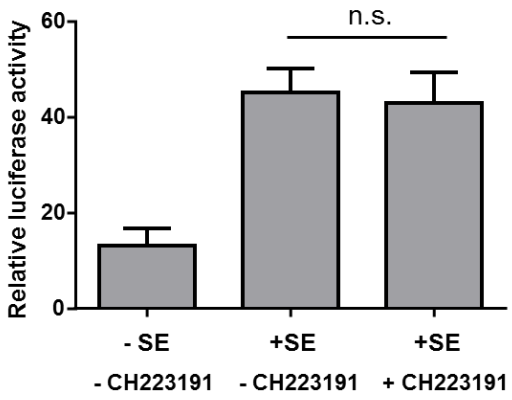
Supplementary Figure 4. *S. epidermidis* induces release of mature IL-1beta by human keratinocytes. Human primary keratinocytes were stimulated for 6 h with *S. epidermidis* strain ATCC 14990 (SE). Supernatants were analyzed by an IL-1beta and pro-IL-1beta ELISA. Bars are means ± SEM of three stimulations.



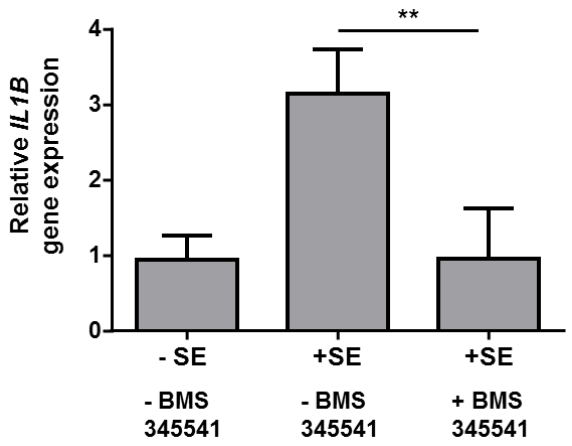
Supplementary Figure 5. AhR has no influence on proteolytic processing of pro-IL-1beta. Human primary keratinocytes were transfected with iGLuc, a luciferase reporter that releases luciferase activity in the supernatant upon cleavage of pro-IL-1beta. The cells were stimulated with a clinical isolate of *S. epidermidis* (SE) in the presence or absence of the specific AhR inhibitor CH223191. (**a**) Luciferase activity was determined to analyze proteolytic processing of pro-IL-1beta. (**b**) Release of IL-1beta was determined by ELISA. Data are presented as means ± SEM of three stimulations (*$p<0.05$, n.s. = not significant, Student`s *t*-test).



Supplementary Figure 6. AhR has no influence on proteolytic processing of pro-IL-1beta. Human primary keratinocytes were transfected with an AhR-specific siRNA (s1199). 1 day before stimulation cells were additionally transfected with iGLuc, a luciferase reporter that releases luciferase activity in the supernatant upon cleavage of pro-IL-1beta. Cells were stimulated with a clinical isolate of *S. epidermidis* (SE). (**a**) Knockdown efficiency (KD) of AhR gene expression was determined by real-time PCR. (**b**) Luciferase activity was determined to analyze proteolytic processing of pro-IL-1beta. (**c**) Release of IL-1beta was determined by ELISA. Data are presented as means ± SEM of three stimulations (**p<0.01, n.s. = not significant, Student`s *t*-test).



Supplementary Figure 7. AhR has no influence on NF-kappaB. Human primary keratinocytes were transfected with a NF-kappaB *firefly* luciferase reporter plasmid and a *renilla* luciferase control plasmid. The cells were stimulated with a clinical isolate of *S. epidermidis* (SE) in the presence or absence of the specific AhR inhibitor CH223191. NF-kappaB activation was determined by analyzing luciferase activity which was determined as the ratio between *firefly* and *renilla* luciferase activities in each sample. Data are presented as means ± SEM of three stimulations. (n.s. = not significant, Student`s *t*-test).



Supplementary Figure 8. The *S. epidermidis*-induced IL-1beta gene expression requires NF-κB. Human primary keratinocytes were stimulated with a clinical isolate of S. epidermidis (+SE) in the presence or absence of the specific NF-κB inhibitor BMS 345541. IL-1beta gene expression was determined by real-time PCR. Bars are means ± SEM of six stimulations (**P <0.01, Student`s *t*-test).