Supplementary Materials

Signalling pathways mediating chemokine induction in keratinocytes by cathelicidin LL-37 and flagellin

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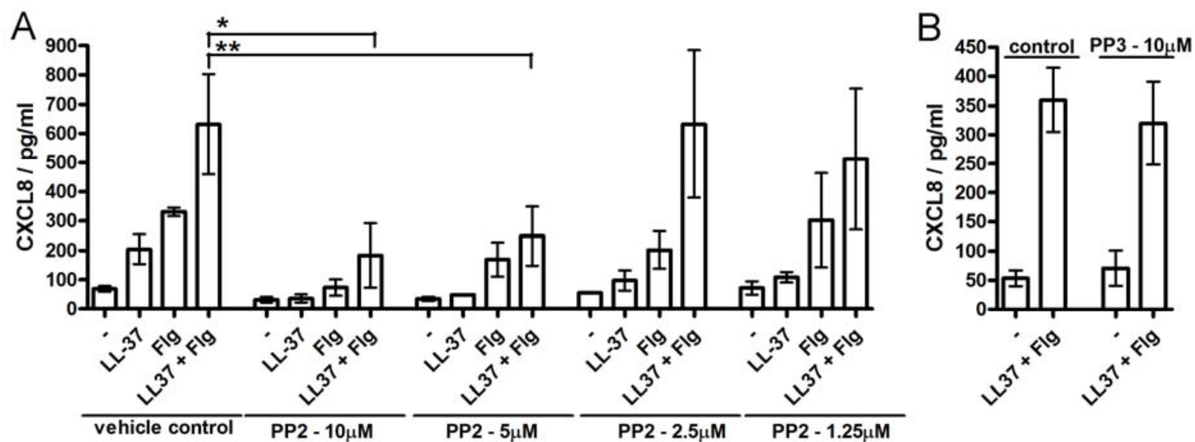
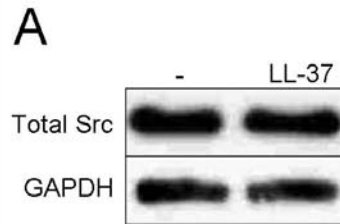
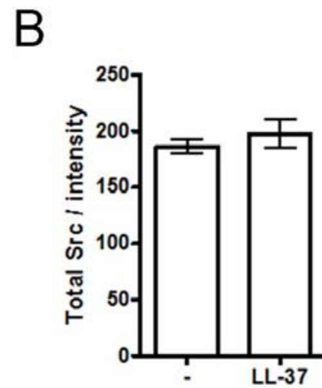
<u>* all data in this document has been generation during manuscript revision to address the reviewers' comments</u>

Supplementary Figure 1. Dose Titration of SFK Inhibitors. CXCL8 chemokine production by human primary keratinocytes stimulated with LL-37 (3 µg/ml) and flagellin (0.5 µg/ml), in the presence of (A) SFK inhibitor PP2 10-1.25µM or DMSO vehicle control (added at the same level as in the wells receiving 10 µM PP2); (B) negative control compound PP3. Bars show means ± SEM. Statistical analysis by ANOVA with Bonferroni's Multiple Comparison post-hoc test, *p<0.05, ** p<0.01.

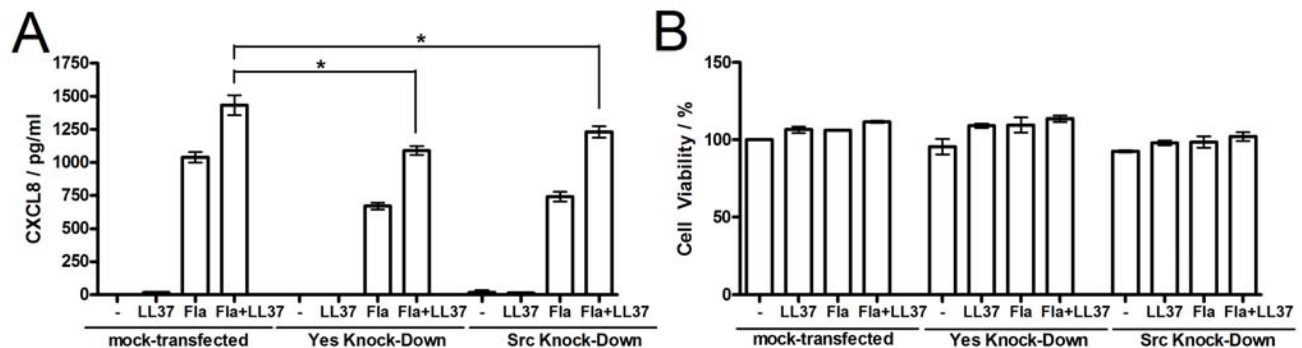


Supplementary Figure 2. Total levels of Src in primary keratinocytes were unchanged over 30 min of LL-37 stimulation. (A) Representative Western blots. (B) Blot intensities quantified using ImageJ software, and normalized against the intensity of GAPDH loading control; bars show means ± SEM.





Supplementary Figure 3. Mild reduction in CXCL8 production in response to LL-37 and flagellin stimulation in the Yes and Src knockdown keratinocytes (data from one experiment). (A) The knockdown and mock-treated control cells were stimulated with LL-37 (5 μg/ml) and flagellin (0.5 μg/ml) for 24 hours, and CXCL8 production measured by ELISA. The data is from technical replicates within one experiment, analysis by ANOVA with Bonferroni's post-hoc test, $p<0.05$. (B) Cell viability WST1 assay demonstrated no non-specific effects of the knockdown protocol on cell viability.



Methods: siRNA Gene Silencing

The cells were treated with the targeting Accell siRNAs at 1μM in Accell media (Dharmacon) for 96 hours. The cells were stimulated with LL-37 (5μg/mL), flagellin (0.5μg/mL) or the combination of the two stimuli for 24 hours. The siRNA treatments were checked for cytotoxicity using the WST-1 cell proliferation reagent (Roche). Knockdown efficiencies were confirmed by qRT-PCR, showing 76% reduction in *Yes* and 61% reduction in *Src* transcript levels in the knockdown cells. The knockdowns were also confirmed to be SFK specific, with no reduction in the *Src*-transcript levels in Yes-knockdown cells, and vice versa

Supplementary Figure 4. The effects of the LL-37, flagellin, and inhibitor treatment on the total levels of AKT and CREB proteins in keratinocytes. The cells were pretreated with SFK inhibitors PP2 or SU6656 (at 10μM) or an equivalent concentration of DMSO vehicle control for 1 hour, and stimulated with LL-37 (3 μg/ml) or/and flagellin (0.5 μg/ml) for 30 minutes. Cell-lysates were analyzed for the total levels of AKT and CREB. Blot intensities from 4 independent experiments were quantified using ImageJ 1.43u software, and standardized against the intensities of housekeeping loading control for each lane; bars show means ± SEM.