Supplemental Material

MATERIALS AND METHODS

Keratinocyte cell culture and treatments.

Primary human keratinocytes (Cascade Biologics; Grand Island, NY) were grown in serum-free keratinocyte growth medium (EpiLife; Cascade Biologics), with 1% human keratinocyte growth supplement (Cascade Biologics), 0.06 mM $CaCl_2$, and antibiotics. Keratinocytes were plated at 100,000 per well in a 24 well plate, coated with collagen matrix (Cascade Biologics) and were allowed to adhere overnight before treatment. When indicated, cells were treated with media control, *E. coli* derived LPS, *S. aureus* derived PG, or LTA (Sigma; St. Louis, MO), or with *S. aureus* toxins TSST or SEB (Toxin Technology; Sarasota, FL). The MyD88 peptide inhibitor or control peptide (the control peptide is identical to the inhibitor, but lacks the homodimerization sequence: RDVLPGT) (Imgenex, San Diego, CA). Peptides were used at 100 μM, and cells were pre-treated for 6 hours prior to addition of LTA. When indicated, cells were treated with IL-4 and IL-13 cytokines (50 ng/ml each) or TNF-α (20 ng/ml) from R&D systems (Minneapolis, MN) for 24 hours. In some cases (where indicated) cytokine treatment was followed by further treatment with media or LTA for an additional 24 hours.

Quantitative real-time PCR (RT-PCR).

Total RNA was isolated by RNeasy Mini Kits (Qiagen, Inc.; Valencia, CA) according to the manufacturer's protocol. One microgram of RNA was reverse-transcribed using the Qiagen Quantiscript kit according to manufacturers protocol. RT-PCR was performed and analyzed by the dual-labeled fluorogenic probe method by using an ABI Prism 7300 sequence detector (Applied Biosystems; Foster City, CA). Primers and probes for human MMP-1, 9, and 10, STAT6, Keratin 14, TIMP-1, 2, and 3 and beta actin were purchased from Applied Biosystems.

Amplification reactions were performed in MicroAmp optical plates (Applied Biosystems) in a 25-μL volume as previously described (Howell *et al.*, 2007). All reactions were normalized to beta actin.

siRNA transfection.

Second-passage keratinocytes of 50-60% confluence were transfected according to the manufacturer's instructions using Lipofectamine 2000 (Invitrogen; Grand Island, NY) with 20 μM non-targeting (microarray tested for minimal targeting of human genes) or STAT6 Smartpool siRNA (Dharmacon-Fisher Scientific; Pittsburgh PA) in antibiotic free media.

Wound scratch assay

The migration of human primary keratinocytes in monolayers was analyzed using an *in vitro* wound scratch assay. Keratinocytes were seeded into Collagen I coated, 24-well plates and treated as indicated. Cell monolayers were then scratched using a 20 μl pipette tip creating a cell free area as previously described (Eyerich *et al.*, 2009). "Wounded" monolayers were then washed three times with PBS to remove detached cells, and media was replaced. Wells were then photographed (t = 0) with a 3i Marianas microscope (Intelligent Imaging Innovations; Denver, CO) with a 5x objective lens. The residual gap area between the migrating cell front at 24 hours (t = 24) was measured and expressed relative to the initial scratch area. Quantitation was performed using Slidebook software (Denver, CO).

MMP-1, 9, 10 ELISAs

MMP levels in the culture supernatant were measured with a commercial ELISA assay (R&D Systems, Minneapolis, MN). Keratinocytes grown on a 24- well

plate were stimulated as indicated. Cell-free supernatants were tested for MMP levels according to the manufacturer's instructions.

Statistical analyses.

All statistical analysis was conducted using Graph Pad Prism. Comparisons of expression levels were performed using analysis of variance (ANOVA) techniques and Student's *t* tests as appropriate.

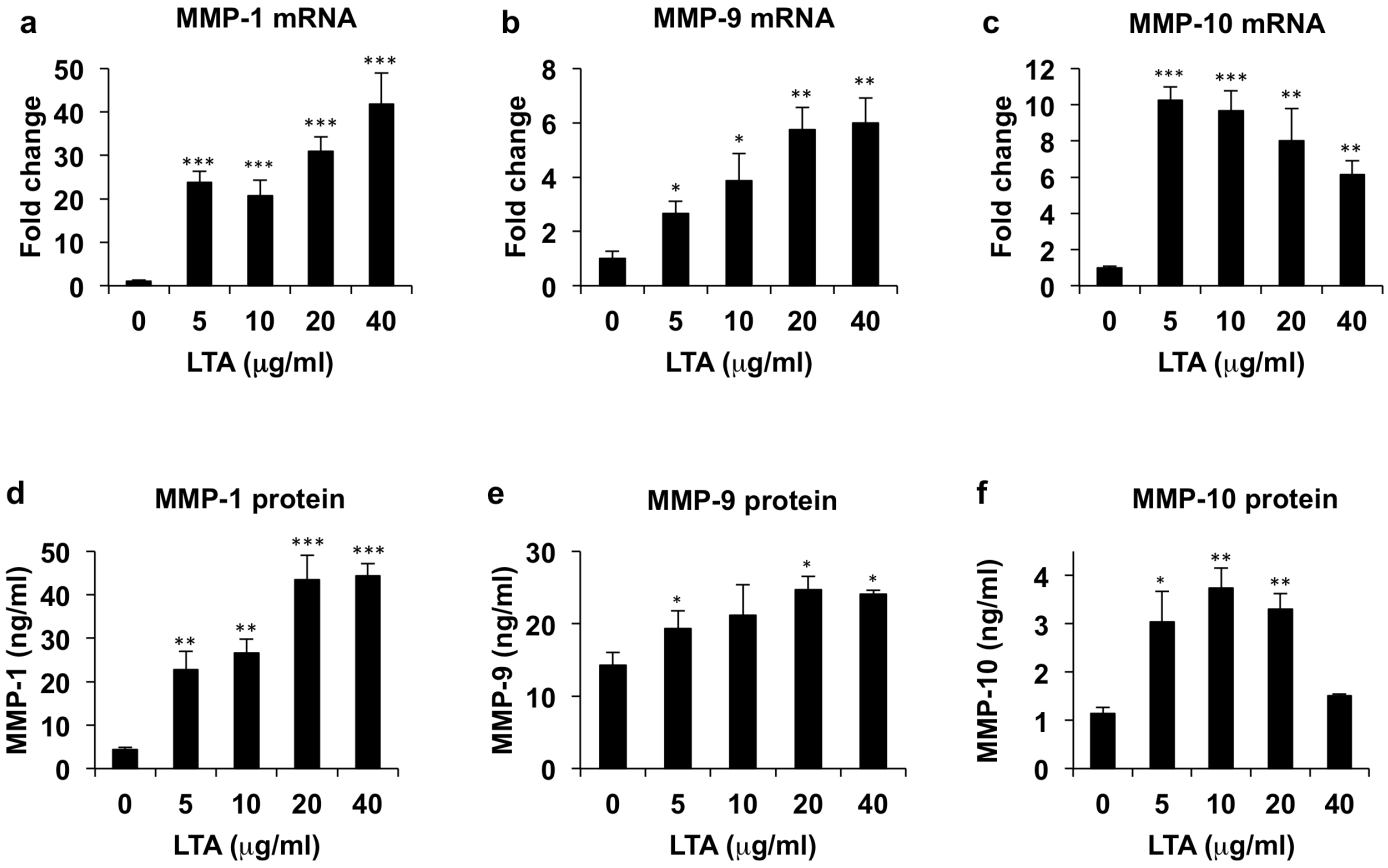

Figure S1. ***S. aureus*** **LTA induces MMP-1, 9, and 10 mRNA and protein expression in a dose dependent manner**. Primary keratinocytes were cultured in the presence of the indicated concentration of *S. aureus* LTA for 24 hours. Expression of mRNA was measured by real-time PCR for (a) MMP-1, (b) MMP-9, and (c) MMP-10, and normalized to beta actin. Fold changes in MMP expression were calculated relative to medium control. Levels of protein were measured by ELISA for (d) MMP-1, (e) MMP-9, and (f) MMP-10. Data are mean ± SEM, n = 3. *P<0.05; **P<0.01; ***P<0.001 (as compared to control medium).

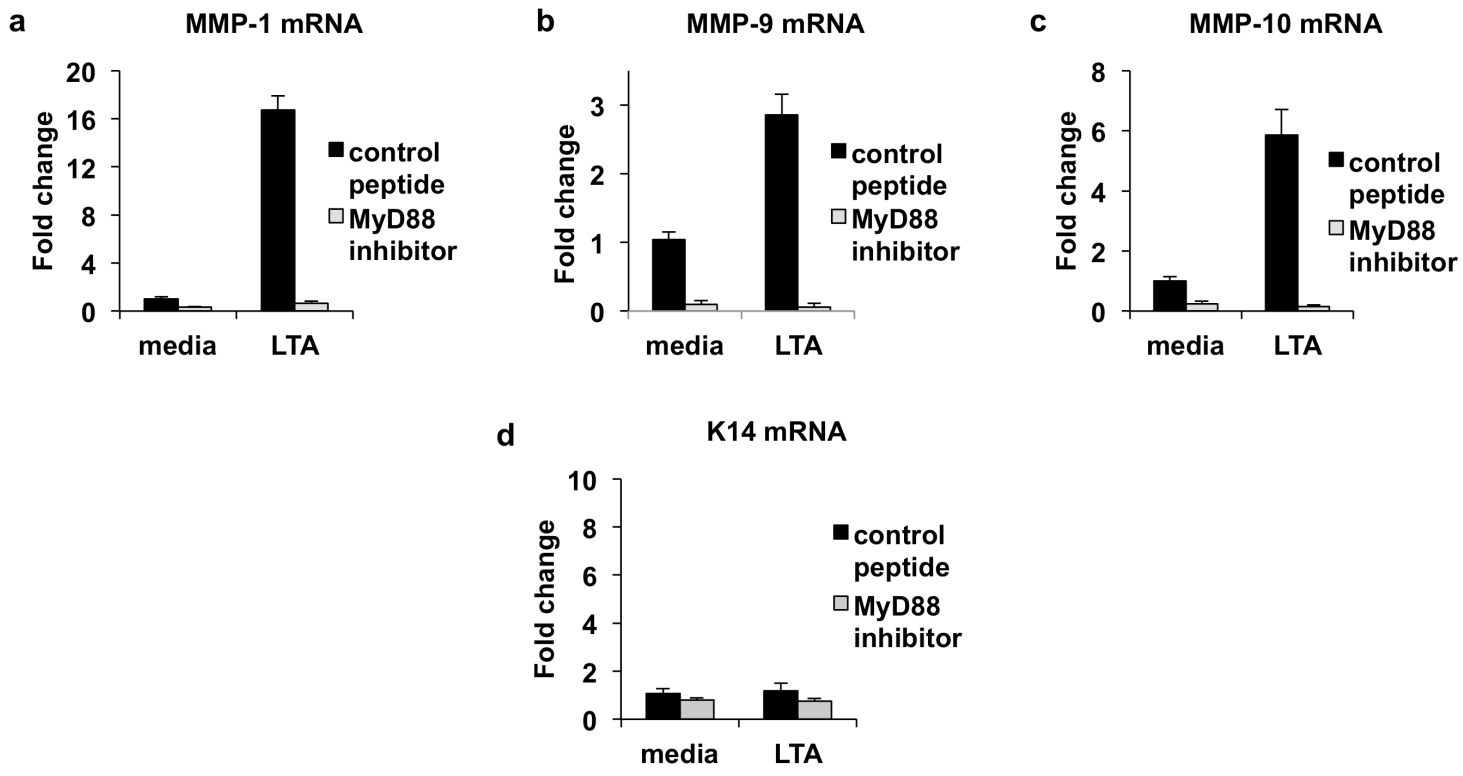

Figure S2. LTA induced MMP production is dependent on MyD88 signaling. Keratinocytes were pretreated with a peptide inhibitor of MyD88, or control peptide as indicated. LTA was then added for 24 hours. Gene expression was analyzed by real-time PCR for (a) MMP-1, (b) MMP-9, (c) MMP-10, and (d) K14 (control) and normalized to beta actin. Fold change in MMP expression was measured relative to medium control. Data are mean ± SEM, n = 3.

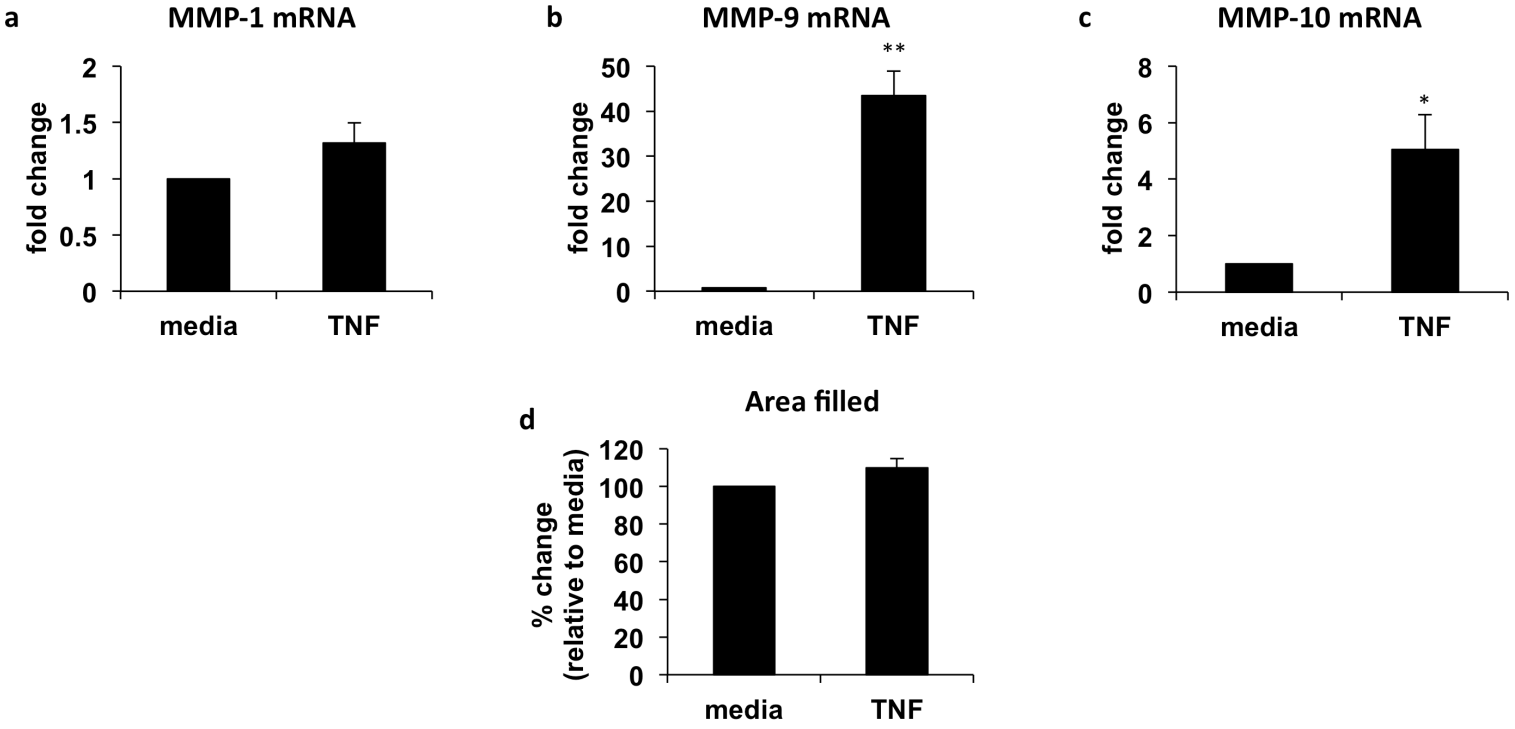

Figure S3. MMP expression and keratinocyte migration are not inhibited by TNF-α. Primary human keratinocytes were pre-treated with medium or TNF-α for 24 hours. Gene expression was analyzed by real-time PCR, for (a) MMP-1, (b) MMP-9, and (c) MMP-10, and normalized to beta actin. Fold change in MMP expression was measured relative to media control. Data are mean ± SEM, n = 3. (d) Primary human keratinocytes were cultured with keratinocyte medium alone, or in the presence of TNF-α for 24 hours. The cells were then scratched with a pipet tip. The defined area of the wound was photographed under phase-contrast microscopy at time 0 h and at 24 h. The closure of the wounded area at 24 h was quantitated. The change in closure with TNF-α is expressed relative to media control. Data are mean ± SEM, n = 3.

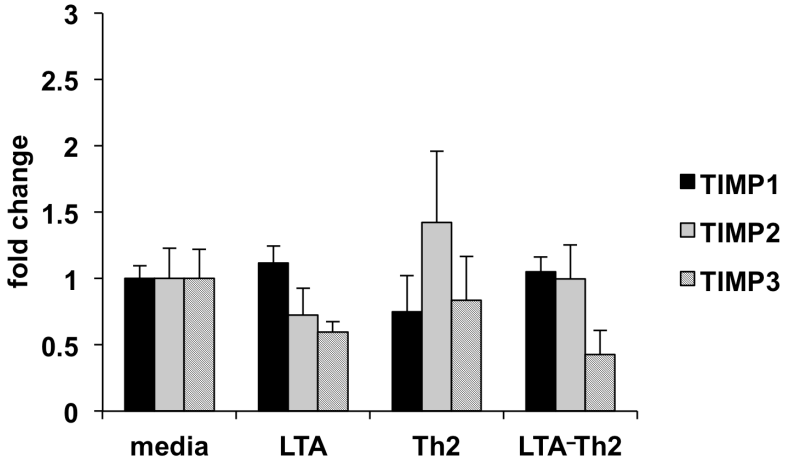

Figure S4. TIMP induction is not inhibited by Th2 cytokines. Primary human keratinocytes were pre-treated with medium, or IL-4/IL-13 for 24 hours. Following pre-treatment, cells were then cultured in the presence or absence of LTA for an additional 24 hours. Gene expression was analyzed by real-time PCR, for TIMP-1, -2, and -3, and normalized to beta actin. Fold change in TIMP expression was measured relative to medium control. Data are mean ± SEM, n = 3