Supplementary Materials and Methods

ELISA: Levels of IL1β and TNF-α in culture supernatants of injured NHEK were measured using commercial ELISA kits (eBioscience).

Real-time PCR: Total RNA from skin wounds of TLR4 deficient, wild type mice, and in vitro wounded NHEK were extracted using TriZol, treated with DNAse I, and subjected to reverse transcription using a Retro-script kit (Invitrogen). mRNA expression of TLR2, TLR4, IL-1β, IL-6, TNF-α, and EGF was examined by a real time PCR system (StepOne Plus, Applied Biosystems, Carlsbad, CA) using SYBR Green PCR mix and gene specific primers. GAPDH was used for normalization. The primers that were used are: Mouse TLR2 primers, forward: CTGGAGCATCCGAATTGCA, Reverse: CATCCTCTGAGATTTGACGCTTT; Mouse TLR4 primers, forward: GGCAACTTGGACCTGAGGAG, Reverse: CATGGGCTCTCGGTCCATAG; Human TLR4 primers, forward: 5-TGGAAGTTGAACGAATGGAATGTG-3, reverse: 5-ACCAGAACTGCTACAACAGATACT-3. Primer sequences for human/mouse GAPDH, mouse IL-1β, IL-6 and TNF-α were as previously described (Giulietti, et al, Methods. 2001; 25(4):386-401). mRNA expression of IL-1β, IL-6, TNF-α, and TLR2 in wild type mice was used as baseline. EGF mRNA expression was analyzed by *TaqMan PCR.* Probes and primers for GAPDH and EGF were purchased from Applied Biosystems.

Laser capture microdissection (LCM): 6h wound tissues embedded in OCT from TLR4 deficient and wild type mice were sectioned and mounted on PEN membrane slides and the

sections were stained with cresyl violet. The epithelial layer within 1mm range of the wound edge was dissected using a LCM system (Leica, Wetzlar, Germany). Total RNA was extracted using a RNAqueous micro scale RNA isolation kit (Invitrogen) followed by RT-PCR to analyze IL-1β, IL-6, TNF-α, and EGF mRNA expression.

Detection of NF-kB, phosphorylated p38, and JNK MAPK. NHEK were fixed in 3.7% formaldehyde for 10 min and permeabilized by 0.15% Triton 100 with 10% FBS for 10 min. Samples were blocked with 10% goat serum for 30 min, then exposed to mouse anti-human NF-kB antibody (5μg/ml, BD Bioscience, SanDiego, CA) , rabbit anti-human phosphorylated-p38 (8.25ng/ml, Cell Signaling, Boston, MA), or mouse anti-human phosphorylated-JNK (0.575ng/ml, Cell Signaling) for 45min. Cells were then incubated with Alexa Fluor 488 goat anti-mouse IgG (Invitrogen) or Alexa fluor goat 594 anti-rabbit IgG (Invitrogen) for 45 min. DAPI was used to non-specifically stain cell nuclei. Results were observed using a fluorescence microscope, Axioskop 40, and recorded using a digital camera (AxioCam HRc) (ZEISS) with the same exposure conditions.

Detection of TLR4, CD3+ T cells, neutrophils, and proliferating cells by indirect immunofluorescence. For detection of TLR4, CD3+ T cells, neutrophils, and ki67+ proliferating cells, wounds were harvested, immediately embedded in OCT compound, and stored at -80^{o}C until sectioning. Eight μm frozen sections were prepared, air-dried, and fixed in cold acetone for 15 min. Following rehydration in PBS, sections were blocked with 10% goat serum for 30 min. Sections were then incubated with rabbit anti-human TLR4 (cross-react with mouse tissue, 10μg/ml, Abcam, Cambridge, MA) or rat anti-mouse Gr-1 (0.5μg/ml, BD Bioscicences) or rat

anti-mouse CD3 (10μg/ml, SouthernBiotech, Birmingham, AL) or rabbit anti-mouse ki67 (0.5μg/ml) for 45 min followed by Alexa fluor 488 goat anti-rat or rabbit IgG or Alexa fluor 594 goat anti-rabbit IgG (Invitrogen). Rabbit IgG and rat IgG were used as negative controls for TLR4/Ki67 and CD3/neutrophil immunohistochemical staining respectively. Results were observed using a fluorescence microscope and recorded using a digital camera (AxioCam HRc) (ZEISS) as described above. Positively stained neutrophils and CD3+T cells in the wounds and wound margins were counted and the average number per 20x field was calculated. All procedures were performed at room temperatur**e.**

Detection of macrophages: For macrophage staining, frozen sections were air-dried for 10 min, fixed in cold acetone for 5 min, and rehydrated in PBS. The sections were quenched with 0.3% H_2O_2 in PBS for 5 min and blocked with blocking buffer containing 0.5% Tween 20, 0.2% gelatin, and 3% BSA in 50mM Tris-HCl for 30 min. The sections were then incubated with rat monoclonal rat anti-mouse Moma-2 antibody (1.25 μg/ml, AbdSerotec, Raleigh, NC) followed by biotinylated rabbit anti-rat antibody (2μg/ml) for 30 min (Vector Lab, Burlingame, CA). Rat IgG was used for negative control. The sections were developed using a VECTASTAIN ABC kit (Vector). Results were observed using a fluorescence microscope and recorded using a digital camera (AxioCam HRc) (ZEISS) as described above. Positively stained cells in the wounds and wound margins were counted and the average number per 20x field was calculated.