SUPPLEMENTARY INFORMATION

Supplementary Methods

Western blotting

Protein samples were heated at 95°C for 3 min and then subjected to SDS-PAGE on acrylamide gels, followed by transfer to PVDF membranes using a wet transfer unit (NOVEX; 1 h at 100 V). The membranes were then blocked for 1 h with TBS containing 0.05% (v/v) Tween 20 plus 5% (w/v) nonfat dry milk and incubated for 2 h with the appropriate primary antibodies in TBS containing 0.05% (v/v) Tween 20 plus 3% (w/v) BSA, followed by incubation for 1 h with HRP-conjugated secondary antibodies. The bands were developed using an ECL kit (Amersham Biosciences, Piscataway, NJ).

Semi-quantitative RT-PCR

Total cellular RNA was extracted using Easy Blue^{TM} (Intron Company, Sungnam, Korea), and 2 μg of the extracted RNA was reverse-transcribed using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA). IL-6, MKP-1 and GAPDH transcripts were amplified using an RT-PCR PreMix Kit (Intron Company). For the semi-quantitative analysis of the transcripts, we first determined the optimal PCR conditions for the linear amplification of GAPDH. The primers used were as follows: 5′-CCAGTACCCCCAGGAGAAGA-3′ (forward) and 5′-GCATCCATCTTTTTCAGCCA-3′ (reverse) for human IL-6; 5′-CCTCAAAGGAGGATACGAAGC-3′ (forward) and 5′-GCTCTTGTACTGGTAGTGACC-3′ (reverse) for human MKP-1; and 5′-CTGCACCACCAACTGCTTAGC-3′ (forward) and 5′-CTTCACCACCTTCTTGATGTC-3′ (reverse) for GAPDH. The PCR products were electrophoresed on an agarose gel and visualized using ethidium bromide staining.

RNA interference for MKP-1 and BLT2

The MKP-1-specific siRNA (5'-CCAAUUGUCCCAACCAUUU-3') was purchased from Dharmacon Research (Lafayette, CO), and the BLT2-specific siRNA (5′-CCACGCAGTCAACCTTCTG-3′) and control (scrambled) siRNA were purchased from Bioneer (Daejeon, Korea) (Hennig *et al.*, 2008). For the RNA interference experiments, cells were plated at a density of 4×10^5 cells per 60 mm dish. After 24 h, the cells were transfected with the above oligonucleotides using the oligofectamine reagent (Invitrogen) in accordance with the manufacturer's instructions. After 24 h, the level of each mRNA was analyzed by RT-PCR to evaluate the degree of knock-down.

Data analysis and statistics

The results are presented as the means ± SD. The analyses were performed using the Student's t test. Values of $P < 0.05$ were considered to be significant.

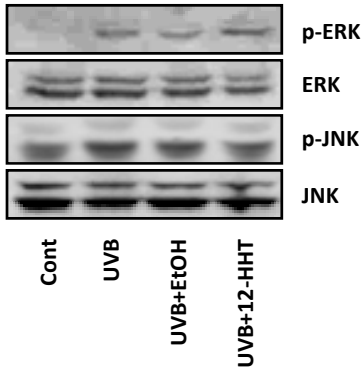
Supplementary Figure legends

Supplementary Figure 1. 12-HHT-induced MKP-1 does not affect the regulation of ERK or JNK. Upon UVB irradiation (5 mJ/cm^2), HaCaT cells were incubated with ethanol (control) or 12-HHT (150 nM) for 60 min. Cell lysates were prepared and examined by western blotting using antibodies specifically recognizing p-ERK, ERK, p-JNK and JNK.

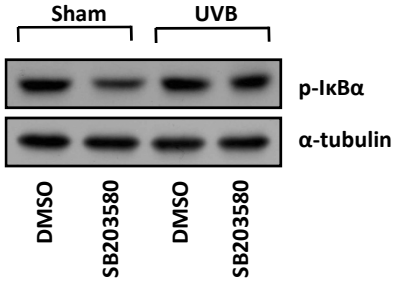
Supplementary Figure 2. NF-κB acts downstream of p38 MAPK in UVB-irradiated signaling to IL-6 synthesis in HaCaT cells. Starved HaCaT cells were pretreated with SB203580 (20 μM) for 60 min and then irradiated with UVB (5 mJ/cm^2) for 60 min. Cell lysates were prepared and examined by western blotting using antibodies specifically recognizing p-IκBα. α-Tubulin was used as the loading control.

Supplementary Figure 3. Knock-down of MKP-1 in HaCaT cells. HaCaT cells were transfected with control or MKP-1 siRNA at various doses, and the level of MKP-1 transcript was analyzed by semi-quantitative RT-PCR.

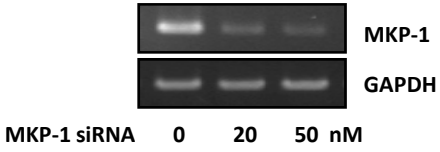
Supplementary Figure 4. 12-HHT increases MKP-1 expression in UVB-irradiated HaCaT cells in a BLT2-dependent manner. (A) Starved HaCaT cells were pre-incubated with DMSO or LY255283 (10 μM) for 60 min, followed by UVB irradiation (5 mJ/cm^2). The cells were then treated with 12-HHT (150 nM) for 60 min. Total RNA was isolated, and the level of MKP-1 transcript was determined by semi-quantitative RT-PCR. (B) HaCaT cells were transfected with control or BLT2 siRNA (20 nM) for 24 h. These transfected cells were starved with serum-free DMEM for 6 h, and then treated with ethanol (control) or 12-HHT (150 nM).



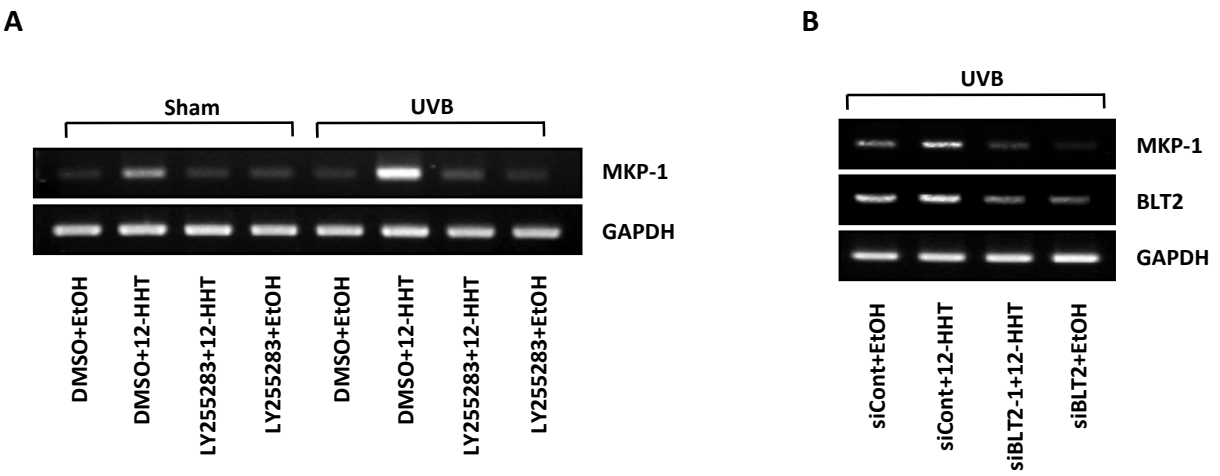
12-HHT as anti-inflammatory mediator in keratinocytes Supplementary Figure 1



12-HHT as anti-inflammatory mediator in keratinocytes Supplementary Figure 2



12-HHT as anti-inflammatory mediator in keratinocytes Supplementary Figure 3



12-HHT as anti-inflammatory mediator in keratinocytes Supplementary Figure 4