

Supplementary information

Ultraviolet-irradiated endothelial cells secrete stem cell factor and induce epidermal pigmentation

Misun Kim¹, Takako Shibata², Soohyun Kwon¹, Tae Jun Park^{3,4,5}, Hee Young Kang^{1,5}

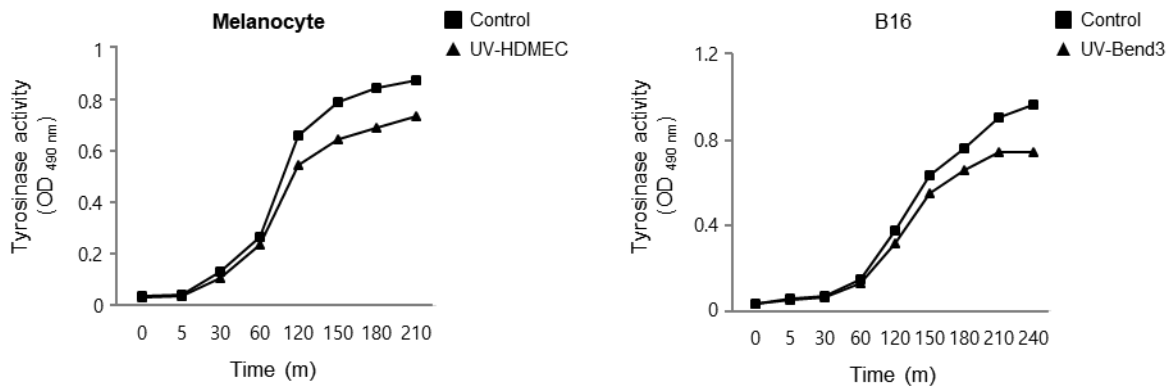
¹Department of Dermatology, Ajou University School of Medicine, Suwon, Korea

²Shiseido Global Innovation Center, Yokohama, Japan

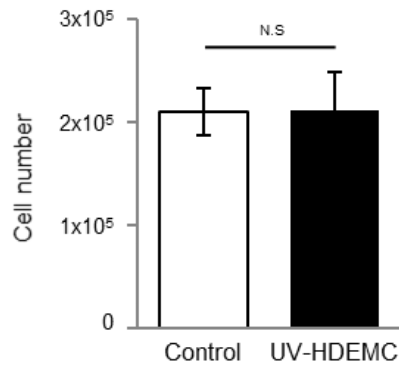
³Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon, Korea

⁴Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

⁵Department of Biomedical Science, The Graduate School, Ajou University, Suwon, Korea

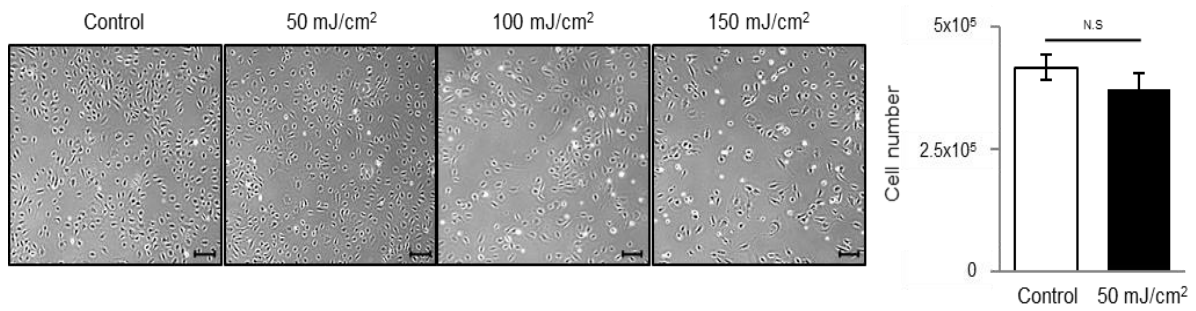


Supplementary Figure 1. The kinetic assay of tyrosinase activity.



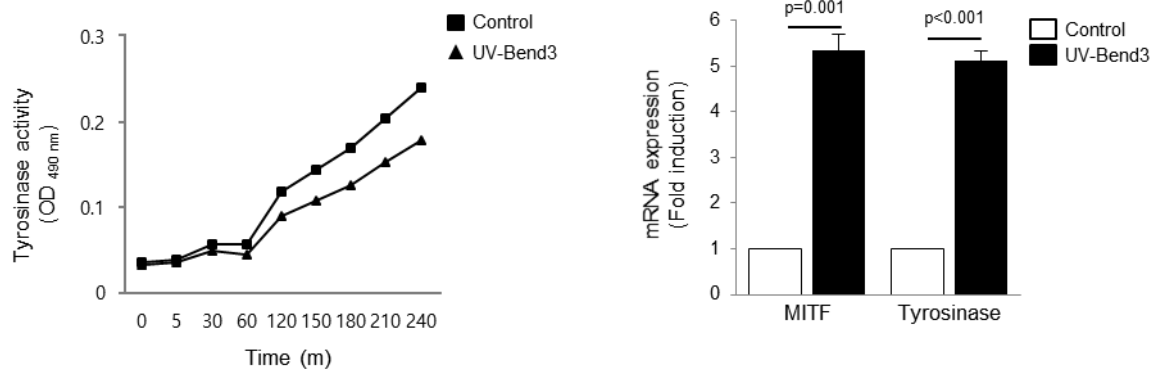
Supplementary Figure 2. UV-irradiated endothelial cells did not affect melanocyte proliferation.

Normal human melanocytes were treated with the conditioned medium (CM) obtained from UV-irradiated HDMECs (UV-HDMECs) and cell proliferation was measured using direct counting. N.S represents no significance.



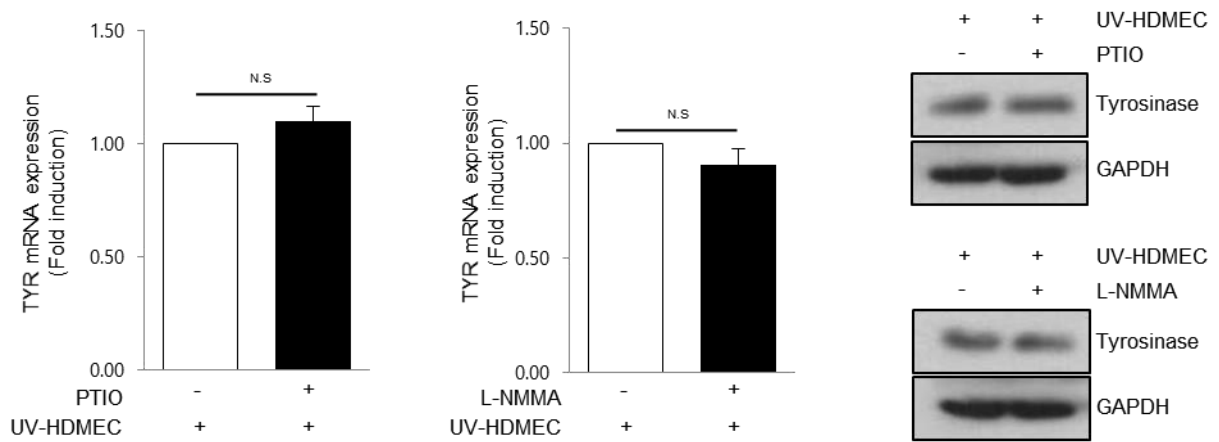
Supplementary Figure 3. The non-cytotoxic level of UVB.

Human endothelial cells (HDMECs) were irradiated with 50-150 J/cm² UVB. After 24 h, the cell morphology was examined and cytotoxicity was analyzed by means of direct counting.

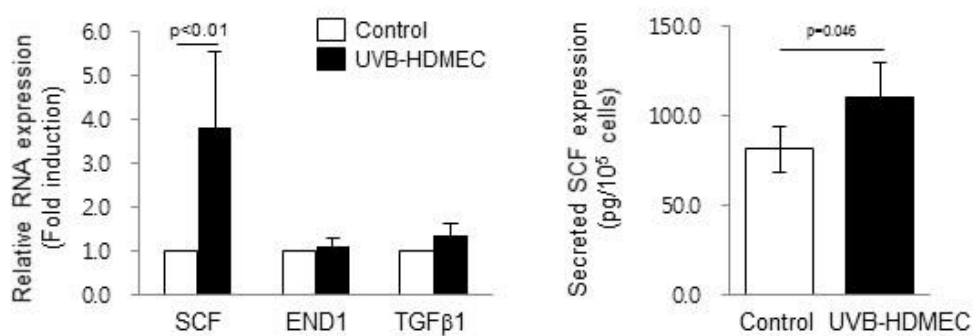


Supplementary Figure 4. Effects of UV-irradiated endothelial cells on the pigmentation of B16 melanoma cells cultured in RPMI medium.

A stimulatory effect of the Bend3-derived conditioned medium on melanogenesis was consistently reproduced in RPMI medium.

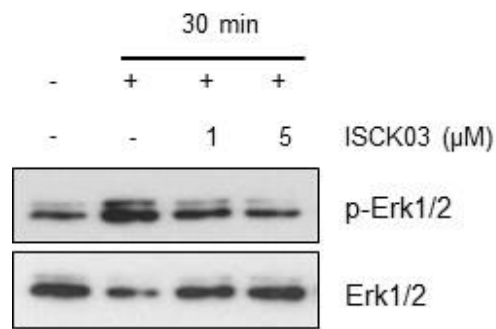


Supplementary Figure 5. Inhibition of NO activation did not affect the pigmentation induced by UV-HDMECs.



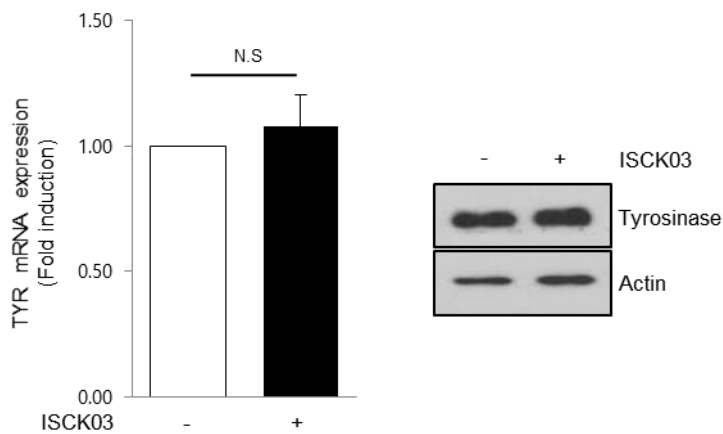
Supplementary Figure 6. Endothelial cells secrete SCF upon UVB irradiation.

Expression levels of the mRNA of SCF, EDN1, and TGFβ1 analyzed by real-time PCR (left panel). Levels of secreted SCF in a cultured medium measured by ELISA (right panel).

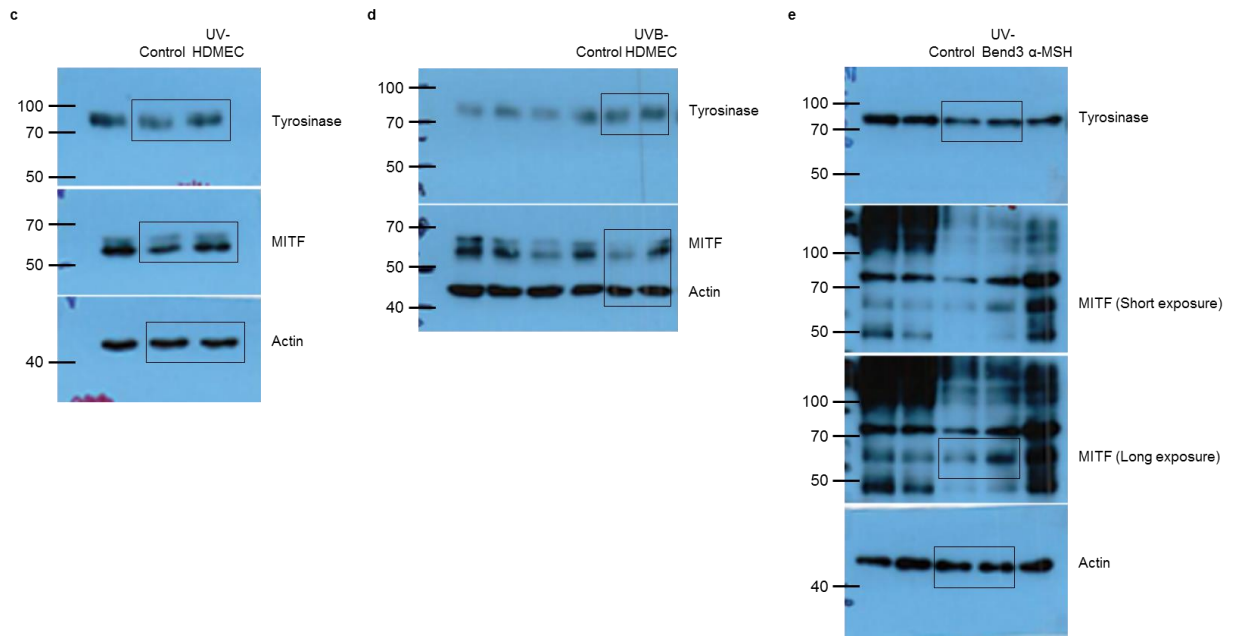


Supplementary Figure 7. The effective dose of ISCK03 in melanocytes.

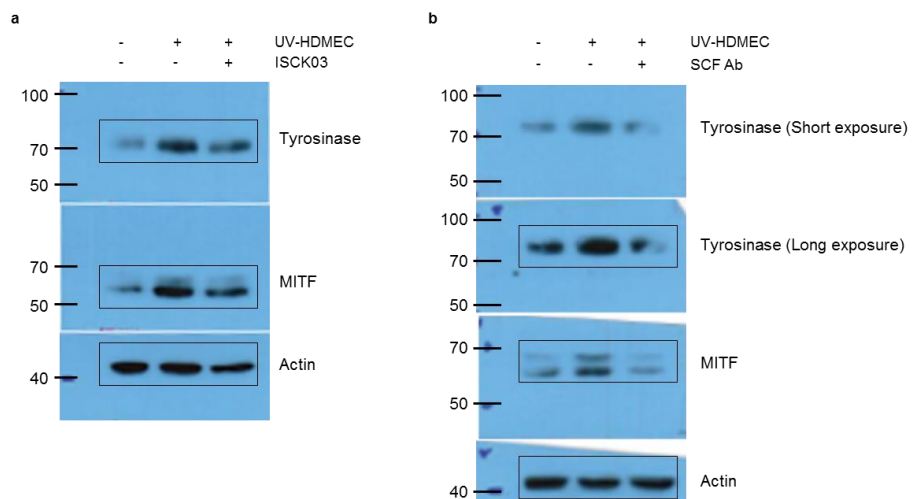
Melanocytes were treated with a KIT-specific inhibitor (ISCK03) in conjunction with CM from UV-HDMECs. Phosphorylation of extracellular-signal-regulated kinase (Erk)1/2 was analyzed by western blotting. 5 μg/mL ISCK03 was the appropriate concentration required to inhibit Erk1/2 phosphorylation, a signaling molecule acting downstream of SCF/c-KIT in melanocytes.



Supplementary Figure 8. Blocking c-kit receptors with ISCK03 treatment did not affect melanogenesis in melanocytes.



Supplementary Figure 9. Full-length western blots for Figures 1c, 1d, and 1e.



Supplementary Figure 10. Full-length western blots for Figures 5a and 5b.