

# Different Suppressive Effects of Canthaxanthin and Fucoxanthin on Cellular Responses of Human Dermal Fibroblasts to Ultraviolet A Irradiation

Nao Wakuda<sup>1</sup>, Yuki Manabe<sup>1\*</sup>, Takuya Seko<sup>2</sup>, Tatsuya Sugawara<sup>1</sup>

1. Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University  
(Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, JAPAN)
2. Fisheries Technology Institute, Japan Fisheries Research and Education Agency  
(2-12-4 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-8648, JAPAN)

## \*Correspondence to:

Yuki Manabe, Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan  
Tel.: +81-75-753-6213

E-mail: [manabe.yuki.8c@kyoto-u.ac.jp](mailto:manabe.yuki.8c@kyoto-u.ac.jp)

## Abstract

Ultraviolet A (UVA) exposure is a major cause of skin damage and changes in the skin's appearance. UVA promotes the production of reactive oxygen species (ROS), which damages dermal fibroblasts. ROS also induce the expression of matrix metalloproteinase-1 (MMP-1), an enzyme that degrades collagen leading to wrinkle formation. We focused on carotenoids, widely distributed natural antioxidants, to find candidate cytoprotective and anti-wrinkle agents. Canthaxanthin and fucoxanthin suppressed the UVA-induced decrease in cell viability. Fucoxanthin also suppressed ROS production; hence, this may be a molecular mechanism. In contrast, canthaxanthin significantly suppressed MMP-1 mRNA expression in UVA-irradiated fibroblasts without inhibiting ROS production.

Subsequent analyses suggested that canthaxanthin could bind and inhibit p38 kinase activity by which it shows cytoprotective effect and inhibitory effect on MMP-1 mRNA expression. These two carotenoids may be potential agents for attenuating UVA-induced skin damage, and canthaxanthin may be a potential anti-wrinkle agent.

## **Keywords**

carotenoid, UVA, dermal fibroblast, p38, MMP-1

Human skin is exposed to various environmental stresses, including ultraviolet (UV) radiation. UV rays are classified into three groups: UVC (200–280 nm in wavelength), UVB (280–320 nm), and UVA (320–400 nm). UVC is the most harmful to humans; however, it is mainly absorbed in the ozone layer and the majority does not reach the Earth's surface. UVB and UVA have various biological effects on the skin. UVB induces inflammation, mainly in the epidermal layer of the skin, leading to sunburn. On the other hand, UVA reaches the dermal layer and promotes the production of reactive oxygen species (ROS) (Fujii, Soma and Matsuda 2023). ROS not only attack biomolecules, such as lipids, proteins, and nucleic acids, but also initiate signal transduction pathways, leading to alterations in gene expression levels related to skin conditions. For example, singlet oxygen, a type of ROS, activates the mitogen-activated protein kinase (MAPK) signaling pathway, leading to the expression of matrix metalloproteinases-1 (MMP-1) (Fujii, Soma and Matsuda 2023; Liu *et al.* 2022). MMP-1 is responsible for the degradation of dermal collagen fibrils that maintain the strength and elasticity of the skin. Hence, exposure to UVA causes MMP-1-mediated loss of collagen, leading to changes in skin appearance, such as deep wrinkling and laxity. As a result, natural compounds that suppress UVA-induced MMP-1 expression have attracted increasing attention.

Carotenoids are natural antioxidants with more than 1,000 chemical structures

(Yabuzaki 2017). Photosynthetic organisms produce carotenoids as photosynthetic pigments and antioxidants that protect the photosynthetic apparatus from sunlight (Sandmann 2019). Citrus red mites, *Panonychus citri*, were found to metabolize dietary  $\beta$ -carotene into astaxanthin to obtain antioxidant protection against solar UVB radiation (Atarashi *et al.* 2017). Many studies have investigated the use of carotenoids as protective agents against UV-induced skin changes. For example, crocetin was found to decrease the UVA-induced damage in human dermal fibroblasts and lipid peroxidation in mouse skin (Ohba *et al.* 2016). We have previously reported that dietary astaxanthin and fucoxanthin suppress UVA-induced changes in skin appearance (Komatsu *et al.* 2017; Liu *et al.* 2025). Moreover, a human intervention study showed orally administered mixed carotenoid supplement containing  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein, and zeaxanthin suppressed UVA-induced skin pigmentation (Baswan *et al.* 2020). However, most other carotenoids have not been investigated, even though their chemical structures are expected to exhibit antioxidant activity. In the present study, we screened carotenoids for their suppressive effects on UVA-induced damage in human dermal fibroblasts. We then evaluated the effects of the hit carotenoids, canthaxanthin and fucoxanthin, on UVA-induced biological phenomena, including ROS production, MAPK activation, and MMP-1 mRNA expression, to elucidate their mechanisms of action.

## Material and methods

### Reagents

Carotenoids were obtained as previously reported (Manabe, Hirata and Sugawara 2014; Manabe *et al.* 2023). A ROS indicator, 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA), was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Anti-phosphorylated HSP27 (Ser-78 and Ser-82) rabbit monoclonal antibody (cat. no. MAB23141) was obtained from R&D Systems (Minneapolis, MN, USA). Anti-HSP27

rabbit monoclonal antibody (#95357), anti-phosphorylated p38 (Thr-180 and Tyr-182) rabbit monoclonal antibody (#9215), anti-p38 rabbit polyclonal antibody (#9212), and horseradish peroxidase (HRP)-linked anti-rabbit IgG goat polyclonal antibody (#7074) were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). All other chemicals, cell culture media, and solvents used in the experiments were commercially available and reagent grade.

## **Cell culture**

Normal human skin fibroblasts (NB1RGB) were obtained from the RIKEN Gene Bank (Tsukuba, Japan). NB1RGB cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> and passaged by trypsinization every 2–3 days.

## **Carotenoid treatment**

Carotenoids were dispersed in DMEM containing 1% FBS, using dimethyl sulfoxide (DMSO) as the vehicle. The final concentrations of the carotenoids and DMSO were 1.0 µM and 0.1%, respectively. NB1RGB cells were incubated in a carotenoid-containing medium for 24 h, followed by UVA irradiation. Un-irradiated (no UVA) cells and vehicle control cells were incubated in vehicle-containing medium for 24 h, followed by no irradiation and UVA irradiation, respectively.

## **UVA irradiation**

After carotenoid treatment, cells were exposed to 10 J/cm<sup>2</sup> UVA light (XX-15BLB; Analytik Jena GmbH+Co. KG, Jena, Germany) in Hank's Balanced Salt Solution containing calcium and magnesium without phenol red (HBSS). The dose of UVA (10

J/cm<sup>2</sup>) was approximately equal to the dose received during 30 min of midday summer sunlight (He *et al.* 2003; McAuliffe and Blank 1991). The radiative flux spectrum of the UVA lamp was previously reported (Moreira *et al.* 2010). UVA light was centered above the plate at a fixed distance of 10 cm. The no UVA cells were covered with aluminum foil to prevent exposure to UVA.

#### **Methyl thiazolyl tetrazolium (MTT) assay**

The MTT assay was performed 48 h after UVA irradiation, as previously described (Manabe *et al.* 2021).

#### **Lactate dehydrogenase (LDH) assay**

Extracellular LDH leakage was determined using an LDH-Cytotoxic Test kit (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) 24 h after UVA irradiation according to the manufacturer's instructions. Briefly, 50 µL of the supernatant and the coloring solution was mixed and incubated for 15 min at 37°C. The absorption at 560 nm was measured using a spectrophotometer. The absorption at 650 nm was also measured as a reference.

#### **Reactive oxygen species production assay**

Immediately after UVA irradiation, H<sub>2</sub>DCFDA solution containing H<sub>2</sub>DCFDA (40 µM in final concentration), probenecid (1 mM), and HEPES (20 mM) was added to each culture, followed by incubation for 30 min at 37°C. Fluorescence was measured using a microplate reader at excitation and emission wavelengths of 493 and 522 nm, respectively.

#### **Real time qRT-PCR analysis**

Total RNAs were extracted using Sepasol<sup>®</sup>-RNA I Super G reagent (Nacalai Tesque,

Kyoto, Japan) at 24 h after UVA irradiation. Aliquots of the extracted RNA were used for cDNA synthesis using ReverTra Ace<sup>®</sup> qPCR RT Master Mix (TOYOBO, Osaka, Japan). Real-time qPCR analysis was performed using the following primers: MMP1, 5'-AAAATTACACGCCAGATTTGCC-3' and 5'-GGTGTGACATTACTCCAGAGTTG-3'; GAPDH, 5'-GGCCTCCAAGGAGTAAGACC-3' and 5'-AGGGGTCTACATGGCAACTG-3'. The mRNA expression levels were normalized to the corresponding levels of GAPDH.

### **Mitogen-activated protein kinases profile**

The phosphorylation levels of 26 serine/threonine kinases, including nine MAPKs, were determined using a commercially available Proteome Profiler antibody array (Human Phospho-MAPK Array Kit, R&D Systems), according to the manufacturer's instructions. The 26 kinases and their phosphorylation sites were as follows: Akt1 (Ser-473), Akt2 (Ser-474), Akt3 (Ser-472), pan-Akt (Ser-472, Ser-473, and Ser-474), CREB (Ser-133), ERK1 (Thr-202 and Tyr-204), ERK2 (Thr-185 and Tyr-187), GSK-3 $\alpha/\beta$  (Ser-9 and Ser-21), GSK-3 $\beta$  (Ser-9), HSP27 (Ser-78 and Ser-82), JNK1 (Thr-183 and Tyr-185), JNK2 (Thr-183 and Tyr-185), JNK3 (Thr-221 and Tyr-223), pan-JNK (Thr-183, Tyr-185, Thr-221, and Tyr-223), MKK3 (Ser-218 and Thr-222), MKK6 (Ser-207 and Thr-211) MSK2 (Ser-360), p38 $\alpha$  (Thr-180 and Tyr-182), p38 $\beta$  (Thr-180 and Tyr-182), p38 $\gamma$  (Thr-183 and Tyr-185), p38 $\delta$  (Thr-180 and Tyr-182), p53 (Ser-46), p70 S6 kinase (Thr-421 and Ser-424), RSK1 (Ser-380), RSK2 (Ser-386), and TOR (Ser-2448). Briefly, cell lysates (300  $\mu$ g of protein/mL) were prepared immediately after UVA irradiation. Aliquots (400  $\mu$ L) were mixed with 1.1 mL of the buffer provided in the kit and incubated overnight with the Human Phospho-MAPK Array. The spots were visualized with HRP-conjugated streptavidin and a chemiluminescent substrate using a LAS-3000 visualizer (Fujifilm Corporation, Tokyo, Japan).

## **Western blot analysis**

Western blot analysis was performed as described previously (Manabe 2023).

## **Molecular docking simulation**

A molecular docking simulation was performed on astaxanthin, canthaxanthin, and a p38 inhibitor PH-797804 to examine their binding poses against p38. The crystal structure of human p38 with PH-797804 was obtained from the Protein Data Bank (ID: 3HLL) and was used as the target structure for the docking study. The crystal structure and structure of the inhibitor were imported into the docking simulation software Molecular Operating Environment (MOE, Chemical Computing Group, Montreal, Canada). Astaxanthin and canthaxanthin were drawn using the built-in MOE module. According to previous studies (Ishihara *et al.* 2021; Seko *et al.* 2019; Seko *et al.* 2020), 3HLL, carotenoids, and PH-797804 were charged and assigned ionization states under the AMBER 10 EHT force field in MOE. The energy minimizations of the protein and the target compounds were terminated when the root mean square gradients fell below  $41.84 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-2}$ . While the docking simulation, atoms of the protein farther than 0.8 nm from the compounds were fixed, and water molecules farther than 0.45 nm from the protein and the compounds (PH-797804, astaxanthin, or canthaxanthin) were deleted for reducing amount of calculations.

## **Statistical analysis**

All data are represented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Dunnett's test.

## **Results**

## **Canthaxanthin and fucoxanthin ameliorated UVA-induced cell damage**

We evaluated the protective effects of 11 carotenoids against UVA-induced damage in human dermal fibroblasts. UVA irradiation significantly decreased the MTT reductase activity, indicating that UVA irradiation damaged the cells (Fig. 1a). Pretreatment with canthaxanthin or fucoxanthin significantly ameliorated this decrease. We also evaluated the protective effects of these two carotenoids using an LDH assay. UVA irradiation significantly increased LDH leakage from the cells, indicating cell damage, and pretreatment with canthaxanthin or fucoxanthin significantly suppressed it (Fig. 1b).

## **Canthaxanthin and fucoxanthin exerted different effects**

We investigated the effects of canthaxanthin and fucoxanthin on UVA-induced cellular events. An increase in the ROS levels in the dermis is one of the initial events induced by UVA irradiation. ROS levels in UVA-irradiated fibroblasts were approximately eight-fold higher than those in non-irradiated cells (Fig. 2a). Pretreatment with fucoxanthin, but not canthaxanthin, significantly reduced the UVA-induced increase in ROS levels.

An increase in MMP-1 expression is a key event that leads to changes in skin appearance. UVA irradiation upregulates mRNA expression of MMP-1 in human dermal fibroblasts (Fig. 2b). Canthaxanthin pretreatment significantly suppressed MMP-1 mRNA expression, whereas fucoxanthin pretreatment significantly upregulated it.

We also evaluated activation of the MAPK signaling pathway using a phosphorylated MAPK-related protein antibody array. Expression of four phosphorylated proteins including cAMP response element binding protein (CREB, Ser-133), extracellular signal-regulated kinase (ERK) 1 (Thr-202 and Tyr-204), ERK2 (Thr-185 and Tyr-187), and glycogen synthase kinase (GSK)-3 $\beta$  (Ser-9) was clearly (more than 20%) decreased, and only phosphorylated heat shock protein (HSP) 27 (Ser-78 and Ser-82) was clearly increased after UVA irradiation (Fig. 2c). Pretreatment with canthaxanthin and

fucoxanthin did not revert the decrease in phosphorylation of CREB, ERK1, ERK2, and GSK-3 $\beta$ . In contrast, the increase in HSP27 phosphorylation was suppressed by canthaxanthin and further upregulated by fucoxanthin (Fig. 2d). This result was confirmed by western blotting (Fig. 2e).

### **Canthaxanthin may suppress p38 signaling in UVA-irradiated human dermal fibroblasts**

We aimed to elucidate the molecular mechanisms underlying the effects of canthaxanthin and fucoxanthin on UVA-irradiated human dermal fibroblasts. As ROS production is one of the initial events induced by UVA irradiation, the molecular mechanism of action of fucoxanthin involves the suppression of ROS production. Canthaxanthin significantly suppressed UVA-induced phosphorylation of HSP27, a well-known event downstream of p38 kinase activation (Gu, Fan and Yu 2023; Kostenko and Moens 2009). Western blot analysis indicated that the phosphorylation of p38 tended to increase upon UVA irradiation but was not clearly decreased by pretreatment with canthaxanthin (Fig. 3a). This result indicated that p38 activation itself was not suppressed by canthaxanthin and that canthaxanthin would affect between p38 activation and HSP27 phosphorylation.

By using molecular docking simulation and an animal study, Yang et al. found that astaxanthin (3,3'-dihydroxy-canthaxanthin) inhibited p38 kinase activity by occupying its ATP binding pocket similar to a previously reported p38 inhibitor, PH-797804 (Yang *et al.* 2019). Due to the similarity of its chemical structure to that of astaxanthin, we hypothesized that canthaxanthin would have a similar effect. Affinities and interactions between amino acids in the ATP-binding sites of p38 kinase, canthaxanthin, astaxanthin, and PH-797804 were speculated using an *in silico* molecular docking simulation study. The affinity of the target compounds for the binding site is presented as the docking score (kcal/mol). Lower scores indicate greater stability. The

lowest score of a p38 inhibitor, PH-797804, in the simulation was  $-9.4562$  kcal/mol. The lowest scores for astaxanthin and canthaxanthin in each simulation were  $-6.4186$  and  $-6.8470$  kcal/mol, respectively. Canthaxanthin, astaxanthin, and PH-797804 may occupy the ATP-binding pocket of the p38 kinase (Fig. 3b–d).

## Discussion

As carotenoids are well-known antioxidants, and ROS play an important role in UVA-induced cell damage, we expected that most of the tested carotenoids would ameliorate UVA-induced cell damage. However, only canthaxanthin and fucoxanthin exhibited cell protective effects. The absorption spectra of canthaxanthin and fucoxanthin are similar to those of astaxanthin and siphonaxanthin, respectively, and neither overlaps with the radiative flux spectrum of the UVA lamp. Hence, it is unlikely that the protective effects are due to the direct absorption of UVA by these carotenoids. Moreover, we did not detect any antioxidant activity of canthaxanthin under our experimental conditions, indicating that only fucoxanthin exhibited both cytoprotective and antioxidant activities. Although the data were not shown here, only fucoxanthin among the tested 11 carotenoids significantly suppressed UVA-induced ROS production. We have previously reported that fucoxanthin accumulates more readily than other carotenoids in human monocytic leukemia cells (Manabe 2021). Therefore, only fucoxanthin may accumulate in human dermal fibroblasts enough to inhibit UVA-induced ROS production. Since ROS production is one of the initial events induced by UVA irradiation, ROS suppression could be a molecular mechanism underlying the protective effect of fucoxanthin against UVA-induced cell damage.

MAPKs are well-known signal transducers involved in various cellular events, including proliferation, apoptosis, and inflammation (Wei *et al.* 2024). Activation of p38 MAPK is related to the stress responses and plays an important role in the signaling

pathway from UVA irradiation to cell death and MMP-1 expression (Wei 2024). According to a molecular docking simulation study, canthaxanthin and astaxanthin could occupy the ATP-binding pocket, indicating that these carotenoids could hinder the binding of p38 kinase to its substrate, ATP. Since various studies have indicated that astaxanthin suppresses p38 activity (Kim and Kim 2019; Yang 2019), we hypothesized that canthaxanthin would work similarly to astaxanthin. In fact, canthaxanthin significantly suppressed UVA-induced phosphorylation of HSP27, a well-known downstream event in p38 activation. Taken together, these findings indicate that the inhibition of p38 kinase would be a molecular mechanism by which canthaxanthin protects cells from death and suppresses MMP-1 mRNA expression.

MMP-1 is a collagenase-degrading fibrillar type I collagen and is one of the key enzymes responsible for UVA-induced changes in skin appearance, such as wrinkle formation. Expression of the active form of MMP-1 is regulated by several steps: transcription to mRNA, translation to protein, secretion into the extracellular space, and conversion into the active form by cleavage at a specific site. Human dermal fibroblasts cultured in a monolayer fail to cleave pro-MMP-1 into MMP-1, whereas those cultured in 3D collagen lattices do (Sonoki, Okano and Yoshitake 2018). Therefore, the next important step in the development of canthaxanthin as a new anti-wrinkle agent is to evaluate its efficacy in a 3D culture model.

According to our antibody array analysis, UVA irradiation clearly decreased the phosphorylation levels of ERK1/2, GSK-3 $\beta$ , and CREB. Changes in the phosphorylation levels of ERK1/2 upon UVA irradiation are controversial. Some studies have reported that UVA induces the phosphorylation of ERK1/2 (Wei 2024), while others have reported no change (Terazawa *et al.* 2021). Furthermore, in confluent quiescent and senescent human dermal fibroblasts, UVA irradiation decreases the phosphorylation of ERK1/2 (Le Panse, Dubertret and Coulomb 2003; Park and Park 2019). In this study, fibroblasts

reached confluence prior to irradiation, which explains why UVA irradiation decreased the phosphorylation of ERK1/2. ERK1/2 have been reported to be involved in the phosphorylation of GSK-3 $\beta$  at Ser-9 (Ding *et al.* 2005; Pal *et al.* 2017), and hence, a decrease in the GSK-3 $\beta$  phosphorylation levels could be caused by a decrease in ERK1/2 phosphorylation. In addition, the antibody array used in this study only detected phosphorylated proteins and not the corresponding unphosphorylated proteins. Changes in the phosphorylated protein levels may be caused by changes in the corresponding unphosphorylated proteins. Fu *et al.* reported that ROS decreased the protein expression level of CREB (Fu *et al.* 2019); therefore, a UVA-induced decrease in phosphorylated CREB could be caused by a decrease in unphosphorylated CREB. With regards to the UVA-induced decrease in the phosphorylation levels of ERK1/2, GSK-3 $\beta$ , and CREB, only that of CREB showed slight recovery (about 11%) by fucoxanthin. Overexpression of CREB and an increase in its phosphorylated form attenuates cellular oxidative stress and protects cells from cytotoxicity (Fu 2019). Moreover, phosphorylated CREB induces MMP-1 mRNA expression by binding to its promoter region (Nakayama 2013). Although further studies are required, CREB phosphorylation could be a molecular mechanism by which fucoxanthin prevents cell damage, suppresses ROS, and increases MMP-1 mRNA expression.

Fucoxanthin significantly increased MMP-1 mRNA expression and HSP27 phosphorylation in UVA-irradiated fibroblasts. In some cell culture models, fucoxanthin activates AMP-activated protein kinase (AMPK), which indirectly promotes phosphorylation of HSP27 (Angé *et al.* 2020; Chang *et al.* 2018; Dragoni *et al.* 2021; Kang *et al.* 2012; Ou *et al.* 2019). AMPK also promotes phosphorylation of CREB at Ser-133 (Thornton *et al.* 2016). Taken together, fucoxanthin may activate AMPK, leading to the phosphorylation of HSP27 and CREB in UVA-irradiated fibroblasts.

In this study, we set the concentration of the test carotenoids at 1.0  $\mu$ M. Notably,

this value is lower than those reported in previous studies. Phillips *et al.* showed that 15  $\mu$ M of lutein significantly protected human dermal fibroblasts from cytotoxicity of UVA (Phillips *et al.* 2007). Camera *et al.* reported that astaxanthin at 10  $\mu$ M slightly but not significantly suppressed UVA-induced cytotoxicity in human dermal fibroblasts (Camera *et al.* 2009). The low carotenoid concentration could be a reason why well-known antioxidant carotenoids, including lutein and astaxanthin, did not show cytoprotective effects in this study. In contrast, siphonaxanthin markedly decreased MTT reductase activity in UVA-irradiated fibroblasts probably due to its strong cytotoxicity (Ganesan *et al.* 2011). We have previously reported on the stronger biological activities of siphonaxanthin compared with those of other carotenoids (Li *et al.* 2015; Manabe, Hirata and Sugawara 2019; Manabe, Takii and Sugawara 2020; Manabe 2021; Sugawara *et al.* 2014; Zheng *et al.* 2018); therefore, evaluation at lower carotenoid concentrations would be of interest in future studies.

## **Conclusion**

Our results indicate that canthaxanthin and fucoxanthin exert protective effects against UVA-induced damage in human dermal fibroblasts. Their mechanisms of action are different; canthaxanthin exerts cytoprotective effects, probably due to the inhibition of p38 kinase activity, whereas fucoxanthin suppresses ROS production. Thus, both compounds are potential agents for attenuating UVA-induced dermal fibroblast damage. In addition, canthaxanthin suppressed UVA-induced MMP-1 mRNA expression, indicating its potential as an anti-wrinkle agent.

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## **Data availability**

The data are available from the corresponding author upon reasonable request.

## **Disclosure statement**

The authors declare that they have no conflicts of interest with the contents of this article.

## **Author contributions**

Y.M. Conceptualization, Funding acquisition, Resources; N.W., Y.M., and T.S. Data curation, Formal analysis, and Writing – original draft; N.W. and T.S. Investigation; Y.M. and T.S. Methodology and Visualization; Y.M. and T.S. Project administration and Supervision; Y.M., T.S., and T.S. Writing – review and editing.

All authors reviewed and approved the final version of the manuscript.

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### Graphical abstract caption

Fucoxanthin protects dermal fibroblasts from UVA-induced damage via antioxidant activity, while canthaxanthin exerts an inhibitory effect on p38 MAPK.

## Figure captions

### Figure 1. Effects of carotenoids on UVA induced damage of NB1RGB cells

NB1RGB cells were incubated in 1% FBS-containing medium with or without 1  $\mu$ M of each carotenoid for 24 h, followed by UVA irradiation. After irradiation, the cells were further incubated in 10% FBS-containing medium for 48 h (a) or 24 h (b), followed by the measurement of MTT reductase activity in the cells (a) and LDH activity in the supernatants (b). Values represent the mean  $\pm$  SD ( $n = 4$ ). Asterisks \* and \*\* indicate significant differences from the vehicle control groups ( $p < 0.05$  and  $p < 0.01$ , respectively; Dunnett's test). No UVA, DMSO-treated and UVA un-irradiated cells; Vehicle, DMSO-treated and UVA-irradiated cells; Can, canthaxanthin; Fxn, fucoxanthin; Vlx, Violaxanthin; Lut, lutein; Zea, zeaxanthin; Ast, astaxanthin; Cry,  $\beta$ -cryptoxanthin; Car,  $\beta$ -carotene; hEch, 3-hydroxy-echinenone; Spx, siphonaxanthin; Spn, siphonein.

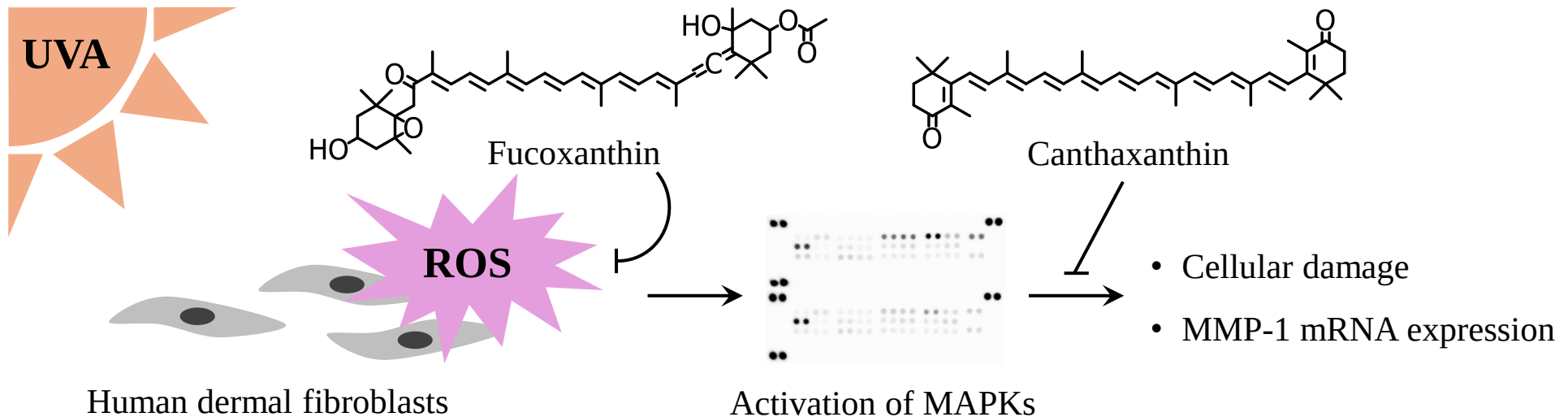
### Figure 2. Effects of canthaxanthin and fucoxanthin on the cellular responses of NB1RGB cells to UVA irradiation

NB1RGB cells were incubated with each carotenoid, followed by UVA irradiation. (a, b) Intracellular ROS levels (a) and MMP-1 mRNA expression (b) were evaluated 24 h after irradiation. (c–e) Expression of phosphorylated MAPK-related kinases (c, d) and phosphorylated and total HSP27 (e) was evaluated immediately after irradiation using an antibody array (c, d) and western blot analysis (e). Values represent the mean  $\pm$  SD ( $n = 4$  for a, b;  $n = 1$  for c, d;  $n = 3$  for e). Asterisks \* and \*\* indicate significant differences from the vehicle control groups ( $p < 0.05$  and  $p < 0.01$ , respectively, Dunnett's test). No UVA, DMSO-treated and UVA un-irradiated cells; Vehicle, DMSO-treated and UVA-irradiated cells; Can, canthaxanthin; Fxn, fucoxanthin.

**Figure 3. Effects of canthaxanthin on p38 kinase**

(a) NB1RGB cells were incubated with each carotenoid, followed by UVA irradiation. Phosphorylated and total p38 were evaluated immediately after the irradiation by using western blot analysis. Values represent the mean  $\pm$  SD ( $n = 3$ ). Statistical analysis was performed by one-way ANOVA, followed by Dunnett's test. No UVA, DMSO-treated and UVA un-irradiated cells; Vehicle, DMSO-treated and UVA-irradiated cells; Can, canthaxanthin; Fxn, fucoxanthin. (b–d) The most stable positions and structures of PH-797804 (b), astaxanthin (c), and canthaxanthin (d) at the ATP-binding pocket of human p38 kinase (ID: 3HLL) in the docking simulations.

*Different Suppressive Effects of Canthaxanthin and Fucoxanthin  
on Cellular Responses of Human Dermal Fibroblasts to Ultraviolet A Irradiation*



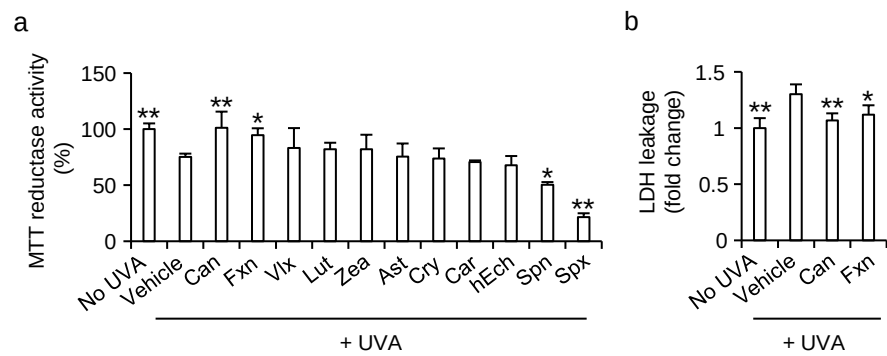


Figure 1

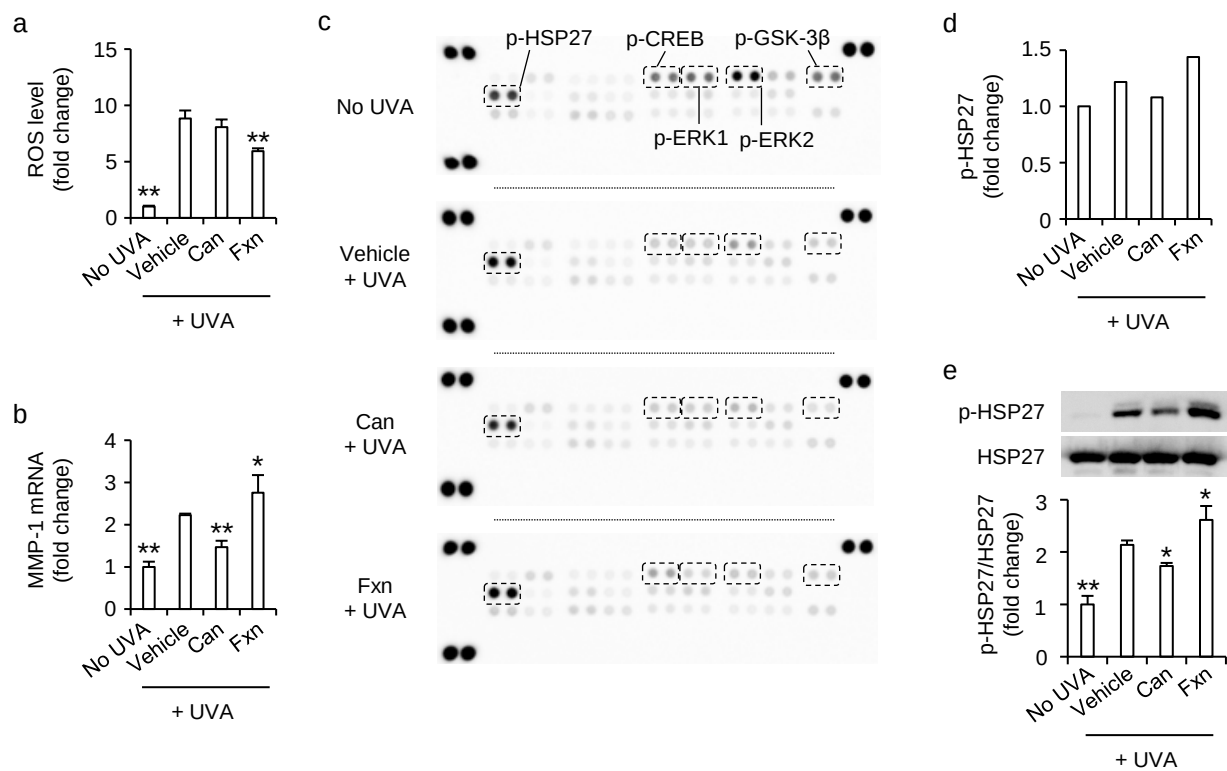


Figure 2

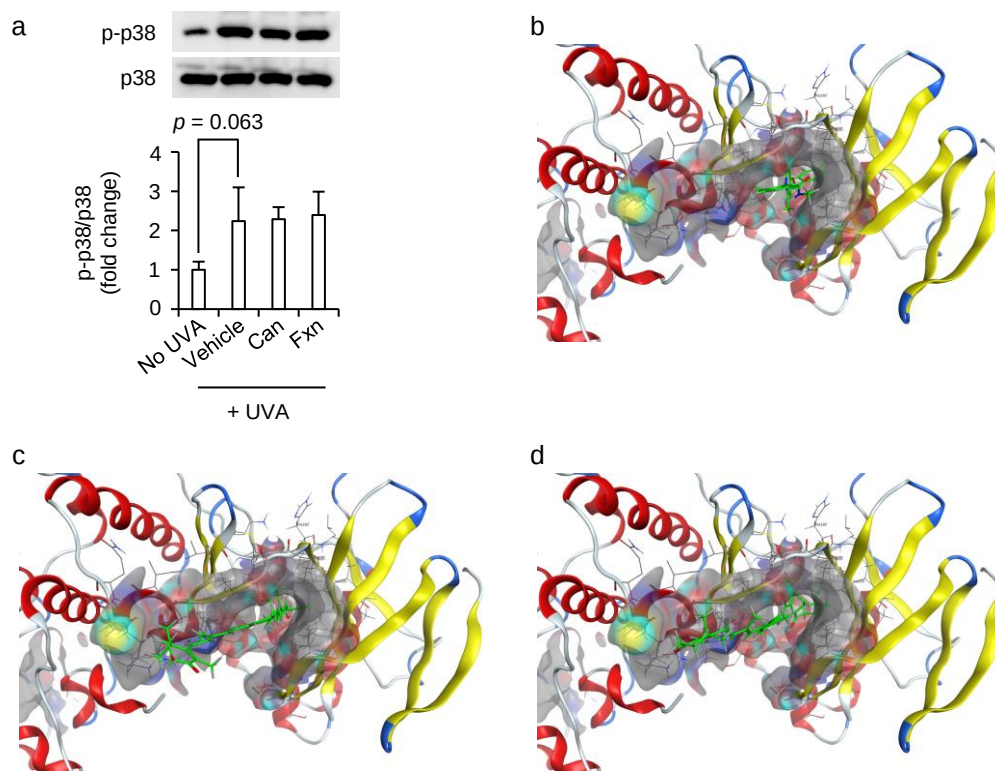


Figure 3