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Supplemental information

Sebocytes contribute to melasma onset

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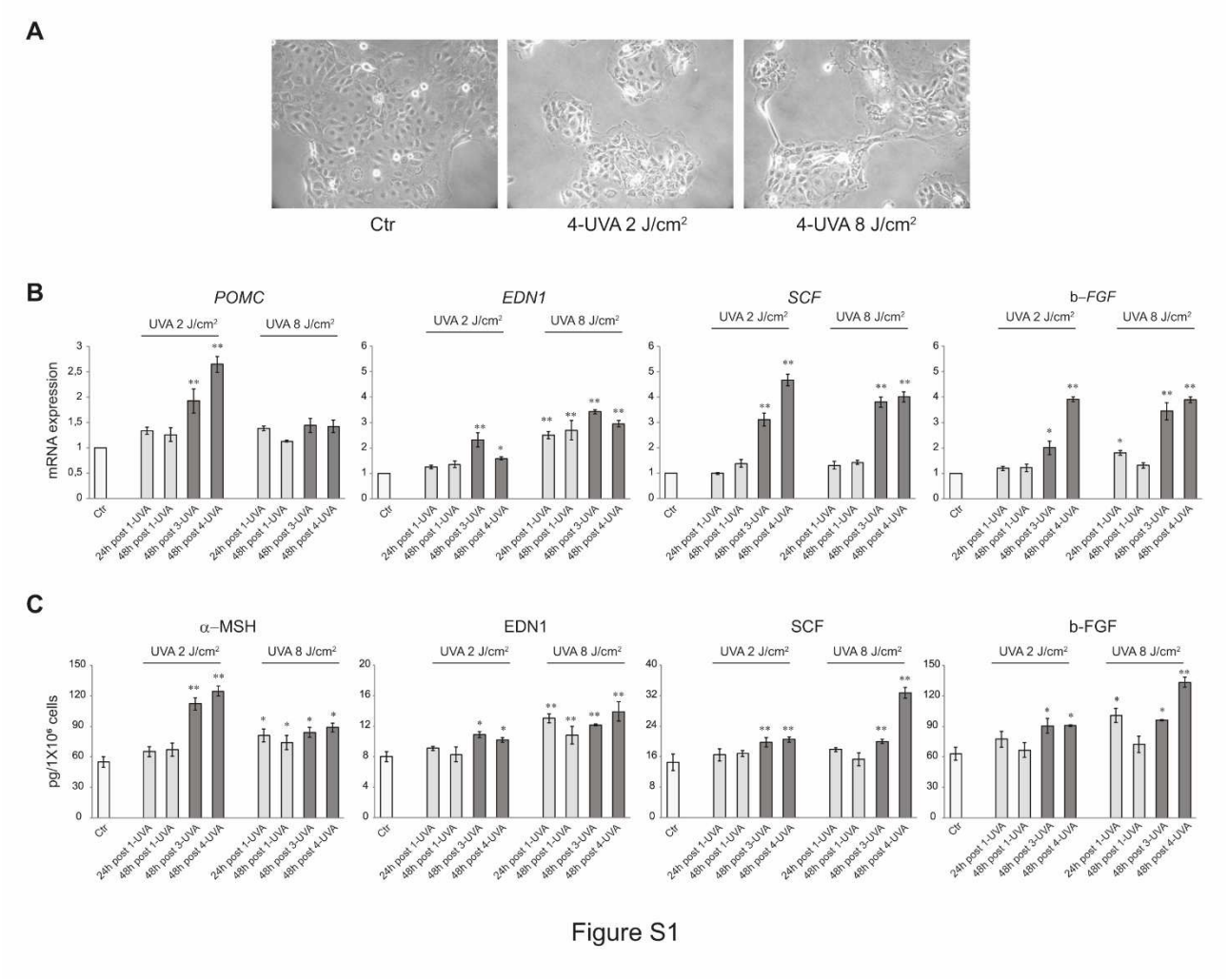

Figure S1

Figure S1. Effect of UVA 2-8 J/cm^2 irradiation on growth factors release in SZ95 sebocytes, Related to Figure 1.

(A) Phase-contrast analysis of SZ95 sebocytes after 4-UVA 2-8 J/cm^2 irradiations.

(B) The mRNA expression levels of POMC, EDN1, SCF and b-FGF in SZ95 sebocytes after 24 and 48h post 1-UVA and 48h post 3 or 4-UVA 2-8 J/cm^2 irradiations. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*$p < 0.05$, **$p < 0.01$).

(C) Protein quantitation by ELISA of α-MSH, EDN1, SCF and b-FGF in SZ95 sebocytes after 24 and 48h post 1-UVA and 48h post 3 or 4-UVA 2-8 J/cm^2 irradiations. Results are presented as the mean ± SD of three independent experiments and are expressed in absolute quantities (*$p < 0.05$, **$p < 0.01$ vs Ctr).

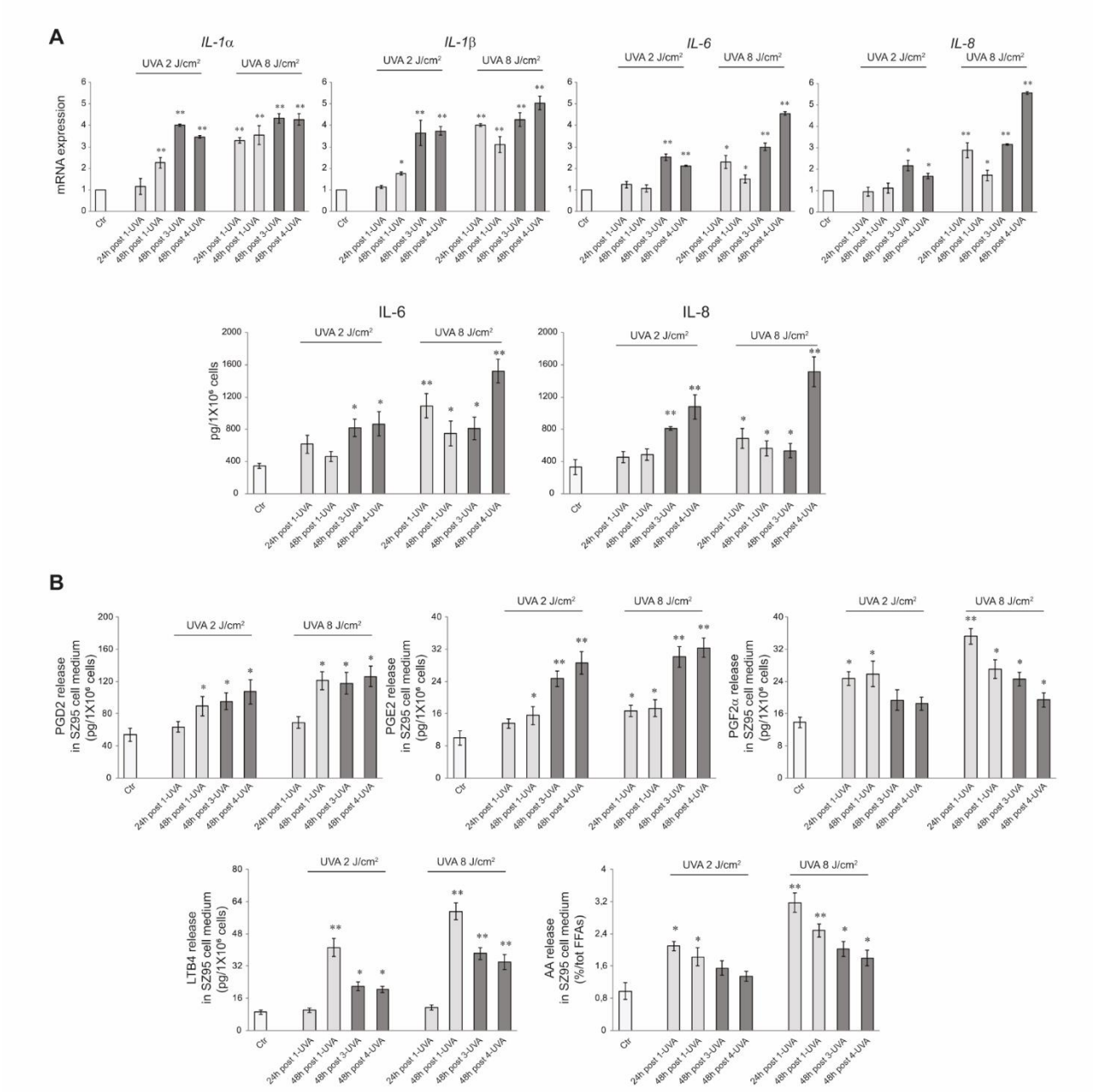

Figure S2

Figure S2. Effect of UVA 2-8 J/cm^2 irradiation on inflammatory mediators release in SZ95 sebocytes, Related to Figure 2.

(A) The mRNA expression levels of IL-1α, IL-1β, IL-6, IL-8, and protein quantitation by ELISA of IL-6 and IL-8 in SZ95 sebocytes after 24 and 48h post 1-UVA and 48h post 3 or 4-UVA 2-8 J/cm^2 irradiations. For mRNA levels, results are expressed as the fold change respect to untreated control cells (*p < 0.05, **p < 0.01). For ELISA assay, results are expressed in the absolute quantities (*p < 0.05, **p < 0.01 vs Ctr).

(B) PGD2, PGE2, PGF2α, LTB4 and AA quantitation by HPLC-MS/MS in SZ95 sebocytes after 24 and 48h post 1-UVA and 48h post 3 or 4-UVA 2-8 J/cm^2 irradiations. Results are expressed as the fold change respect to untreated control cells (*p < 0.05, **p < 0.01).

Data represent the mean ± SD of three independent experiments.

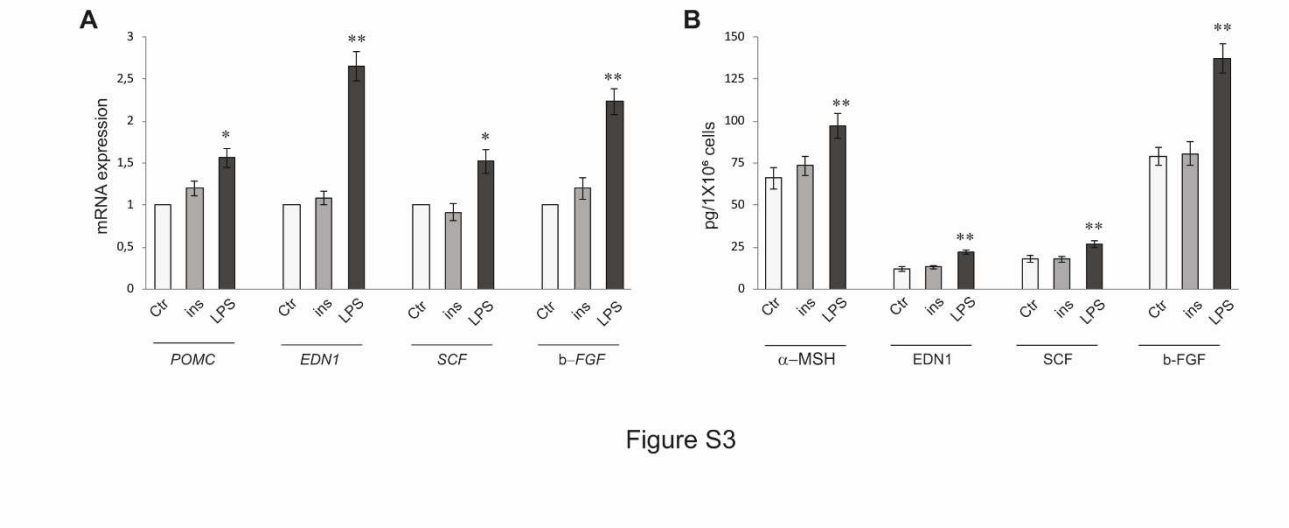

Figure S3

Figure S3. Effects of insulin and LPS on growth factors release in SZ95 sebocytes, Related to Figure 2.
(A) The mRNA expression levels of POMC, EDN1, SCF and b-FGF in SZ95 sebocytes treated with insulin 1 µM or LPS 10 µg/ml for 24h. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*p < 0.05, **p < 0.01).
(B) Protein quantitation by ELISA of α-MSH, EDN1, SCF and b-FGF in SZ95 sebocytes treated with insulin 1µM and LPS 10µg/ml for 24h. Results are presented as the mean ± SD of three independent experiments and are expressed in absolute quantities (**p < 0.01 vs Ctr).

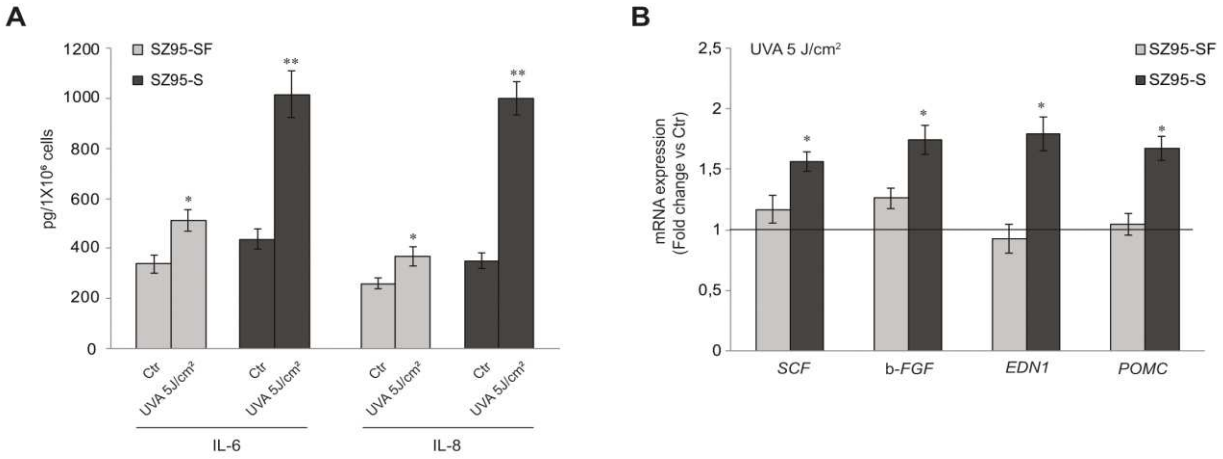

Figure S4

Figure S4. Effects of UVA irradiation on the secretion pattern of low differentiated SZ95 cells, Related to Figure 1 and Figure 2. (A) Protein quantitation by ELISA of IL-6 and IL-8 in SZ95 sebocytes maintained in serum free (SF)/serum (S) conditions. Results are presented as the mean ± SD of three independent experiments and are expressed in absolute quantities (*$p < 0.05$, **$p < 0.01$ vs Ctr).
(B) The mRNA expression levels of SCF, b-FGF, EDN1 and POMC in SZ95 sebocytes maintained in serum free (SF)/serum (S) conditions. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*$p < 0.05$ vs Ctr). The value of Ctr (=1) is represented by a black line.

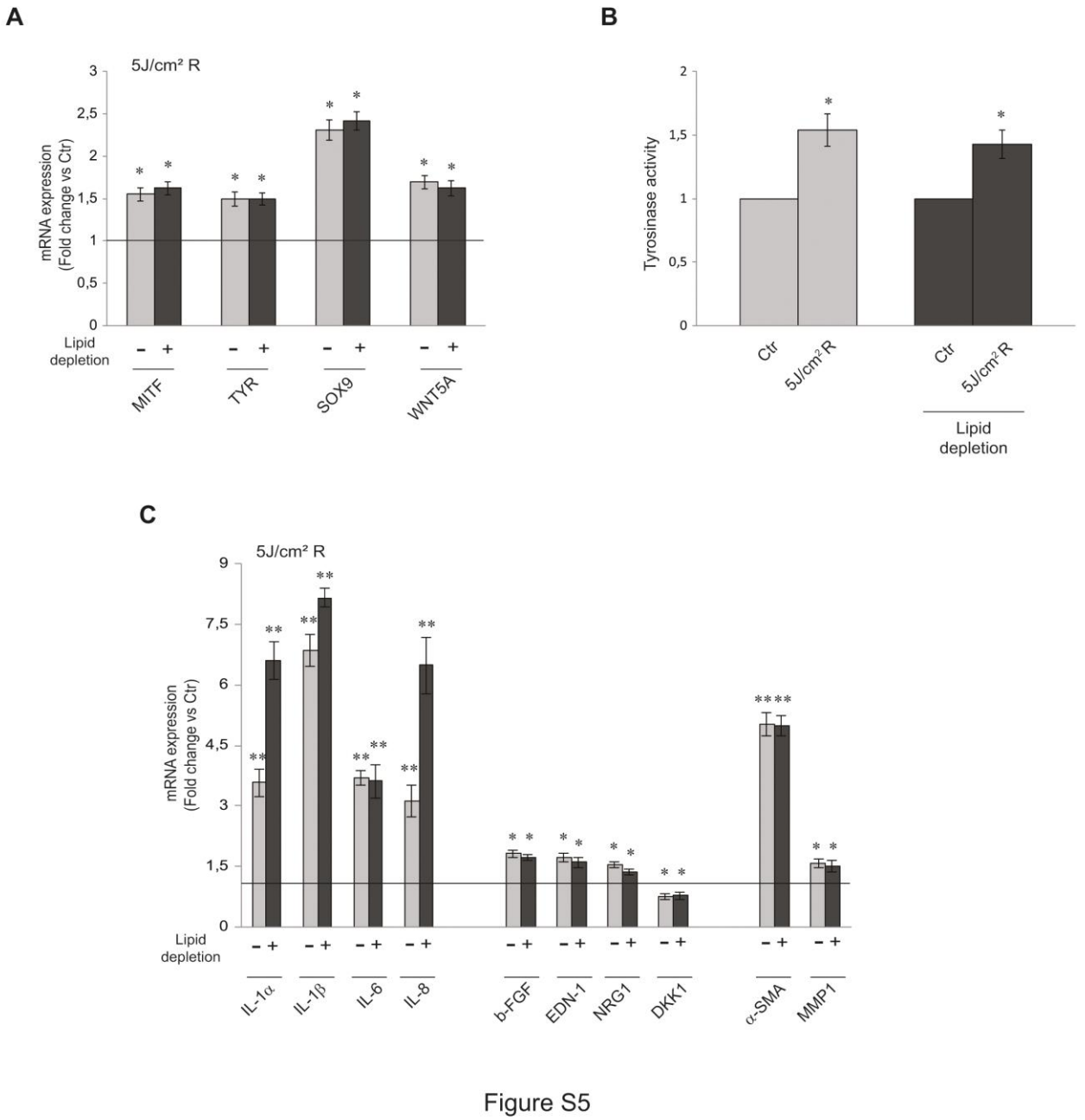

Figure S5

Figure S5. Effects of lipid-depleted SZ95 conditioned medium on NHMs and NHFs, Related to Figure 4.

(A) The mRNA expression levels of MITF, TYR, SOX9 and WNT5a in NHMs after addition with R UVA 5 J/cm^2 or lipid-depleted R. Results are expressed as the fold change respect to untreated control cells (*$p < 0.05$). The value of Ctr (=1) is represented by a black line.

(B) Analysis of tyrosinase activity on NHMs after addition with R UVA 5 J/cm^2 or lipid-depleted R for 4 days. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*$p < 0.05$).

(C) The mRNA expression levels of IL-1α, IL-1β, IL-6, IL-8, b-FGF, EDN1, NRG1, DKK1, MMP1 and αMSH in NHFs after addition with R UVA 5 J/cm^2 or lipid-depleted R for 48h. Results are expressed as the fold change respect to untreated control cells (*$p < 0.05$, **$p < 0.01$). The value of Ctr (=1) is represented by a black line. Data represent the mean ± SD of three independent experiments.

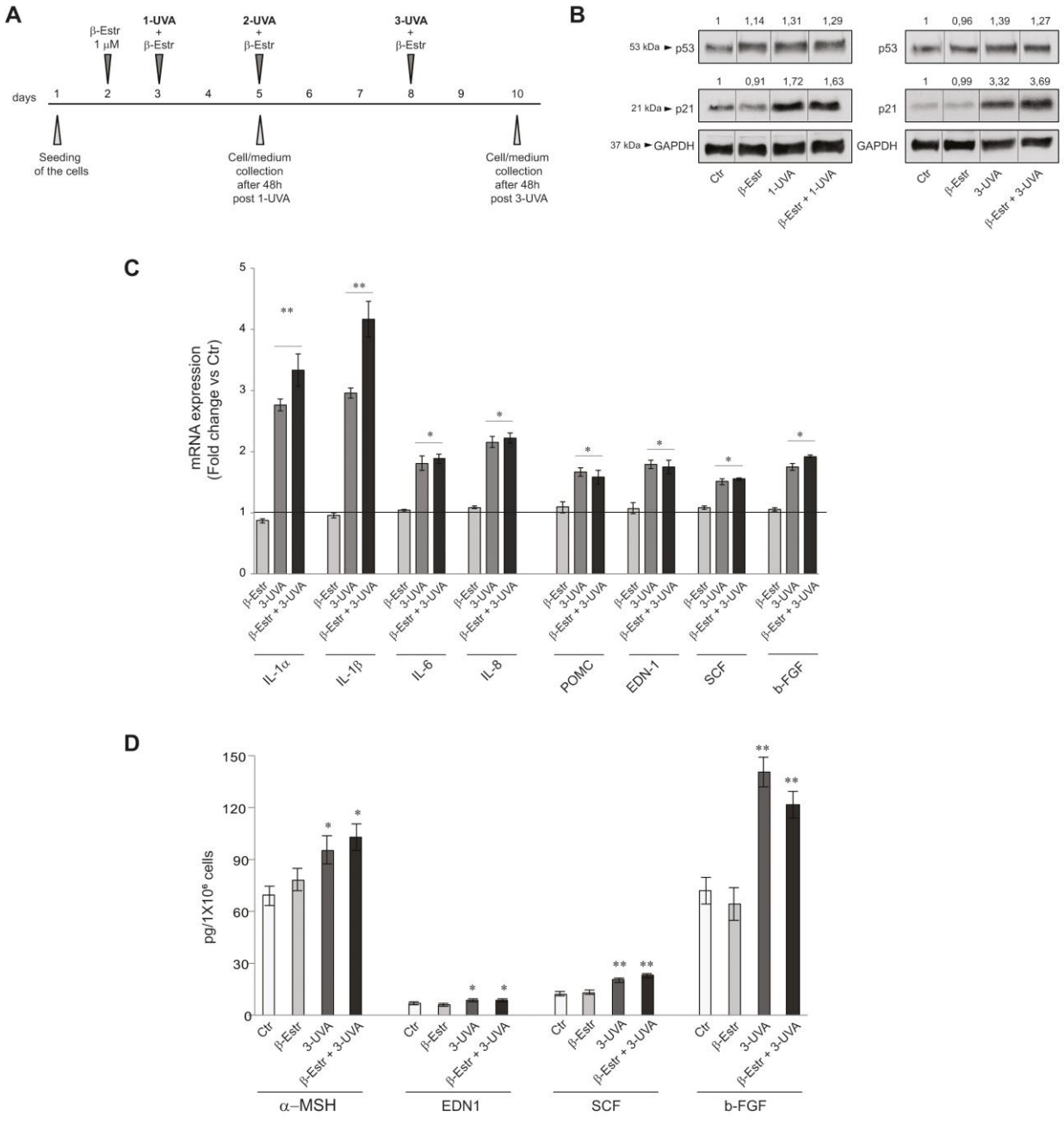

Figure S6

Figure S6. Effects of β-estradiol on SZ95 sebocytes and melanogenesis, Related to Figure 5.

(A) Experimental scheme of 1μM β-estradiol treatment and UVA 5 J/cm^2 irradiation of SZ95 sebocytes.

(B) Western blot analysis of p53 and p21 protein expression in SZ95 sebocytes after treatment with 1μM β-estradiol and 1 or 3-UVA 5 J/cm^2 irradiations. GAPDH was used as an equal loading control. Representative blots are shown. Densitometric scanning of band intensities was performed to quantify the change of protein expression (control value taken as one fold in each case).

(C) The mRNA expression levels of IL-1α, IL-1β, IL-6, IL-8, POMC, EDN1, SCF, b-FGF in SZ95 sebocytes after treatment with 1μM β-estradiol and 3-UVA 5 J/cm^2 irradiations. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*$p < 0.05$, **$p < 0.01$). The value of Ctr (=1) is represented by a black line.

(D) Protein quantitation by ELISA of α-MSH, EDN1, SCF and b-FGF in SZ95 sebocytes after treatment with 1μM β-estradiol and 3-UVA 5 J/cm^2 irradiations. Results are presented as the mean ± SD of three independent experiments and are expressed in absolute quantities (*$p < 0.05$, **$p < 0.01$ vs Ctr).

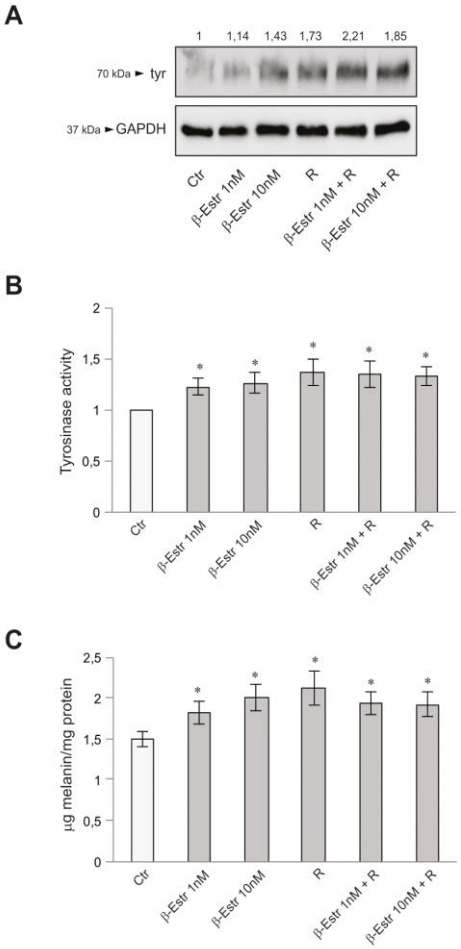

Figure S7

Figure S7. Synergistic effect of UVA irradiation, 1 and 10nM 17β-estradiol treatment and exposure to SZ95 conditioned medium on NHMs melanogenesis, Related to Figure 5.

(A) Western blot analysis of tyrosinase protein expression in NHMs after treatments with 1 and 10nM β-estradiol and R UVA 5 J/cm^2 for 48h. GAPDH was used as an equal loading control. Representative blots are shown. Densitometric scanning of band intensities was performed to quantify the change of protein expression (control value taken as one fold in each case).

(B) Analysis of tyrosinase activity on NHMs after treatments with 1 and 10nM β-estradiol and R UVA 5 J/cm^2 for 4 days. Results are presented as the mean ± SD of three independent experiments and are expressed as the fold change respect to untreated control cells (*$p < 0.05$).

(C) Melanin content evaluation in NHMs after treatments with 1 and 10nM β-estradiol and R UVA 5 J/cm^2 for 5 days. Results are presented as the mean ± SD of three independent experiments and are expressed in absolute values as ratio µg melanin/mg protein (*$p < 0.05$ vs Ctr).