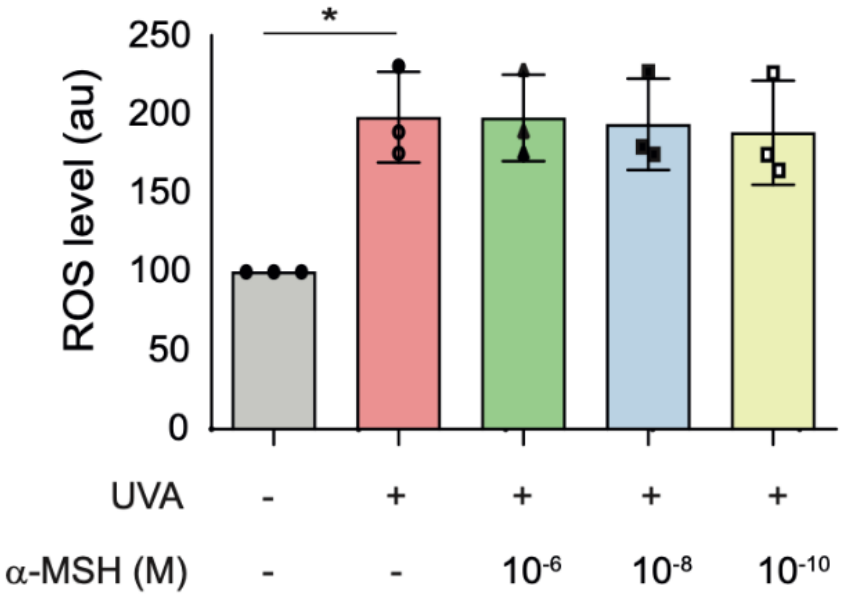
SUPPLEMENTARY DATA

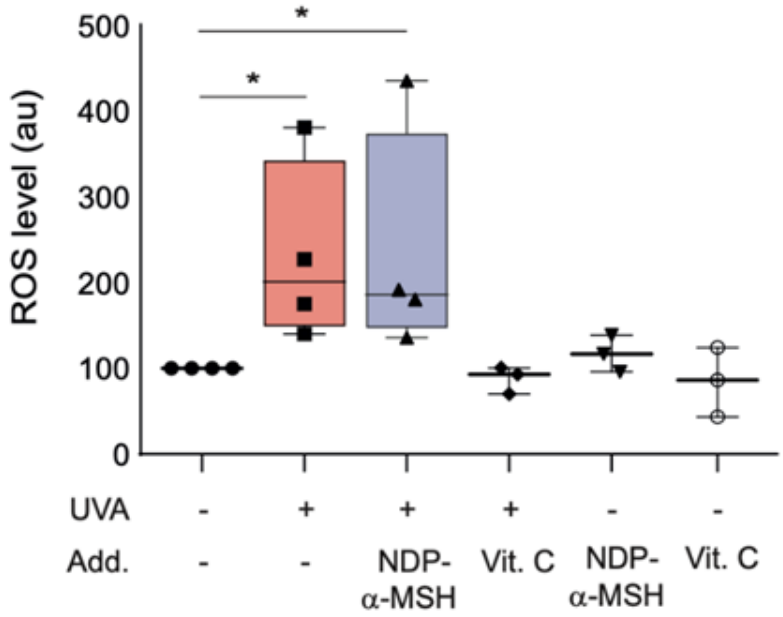
Dissecting the Impact of α-MSH-MC1R-cAMP Signaling on UVA-Induced Stress in Fibroblasts – Implications for Regulation of Cutaneous Photoaging

Markus Böhm, Agatha Stegemann, Agnieszka Wolnicka-Głubisz, Bartlomiej Olajossy, Nicole Schäfer, Stephan Niland, Johannes Eble, Verena Raker, Kerstin Steinbrink, Susanne Grässel, Lionel Larue

SUPPLEMENTARY DATA

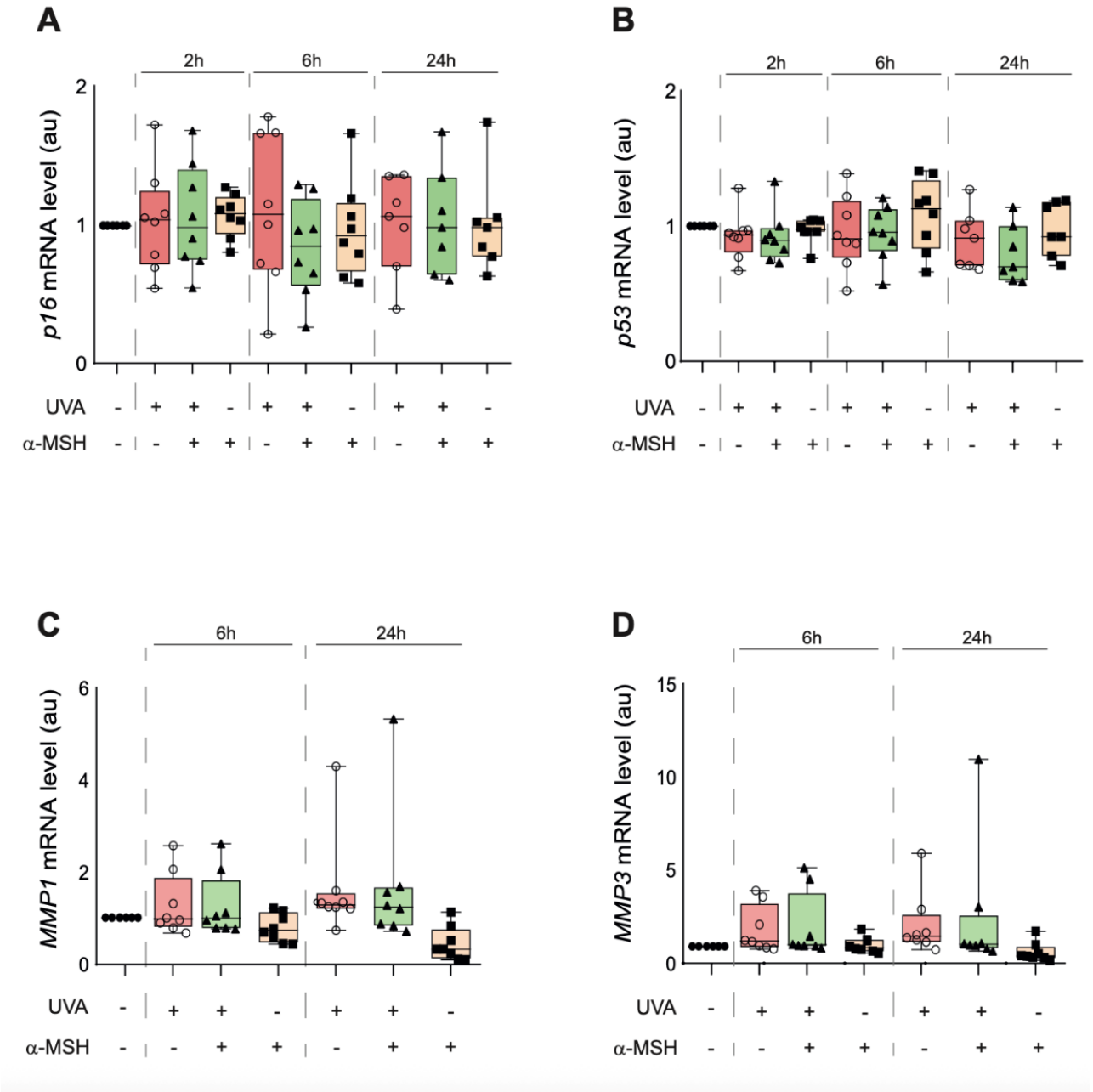


Supplementary Figure 1. Effect of different doses of α-MSH on UVA-mediated intracellular ROS generation in aHDFs. ROS was measured by FACS analysis using the redox-sensitive fluoroprobe CM-H2DCFDA. n=3 independent donors. *p<0.01, evaluated by one-way ANOVA.



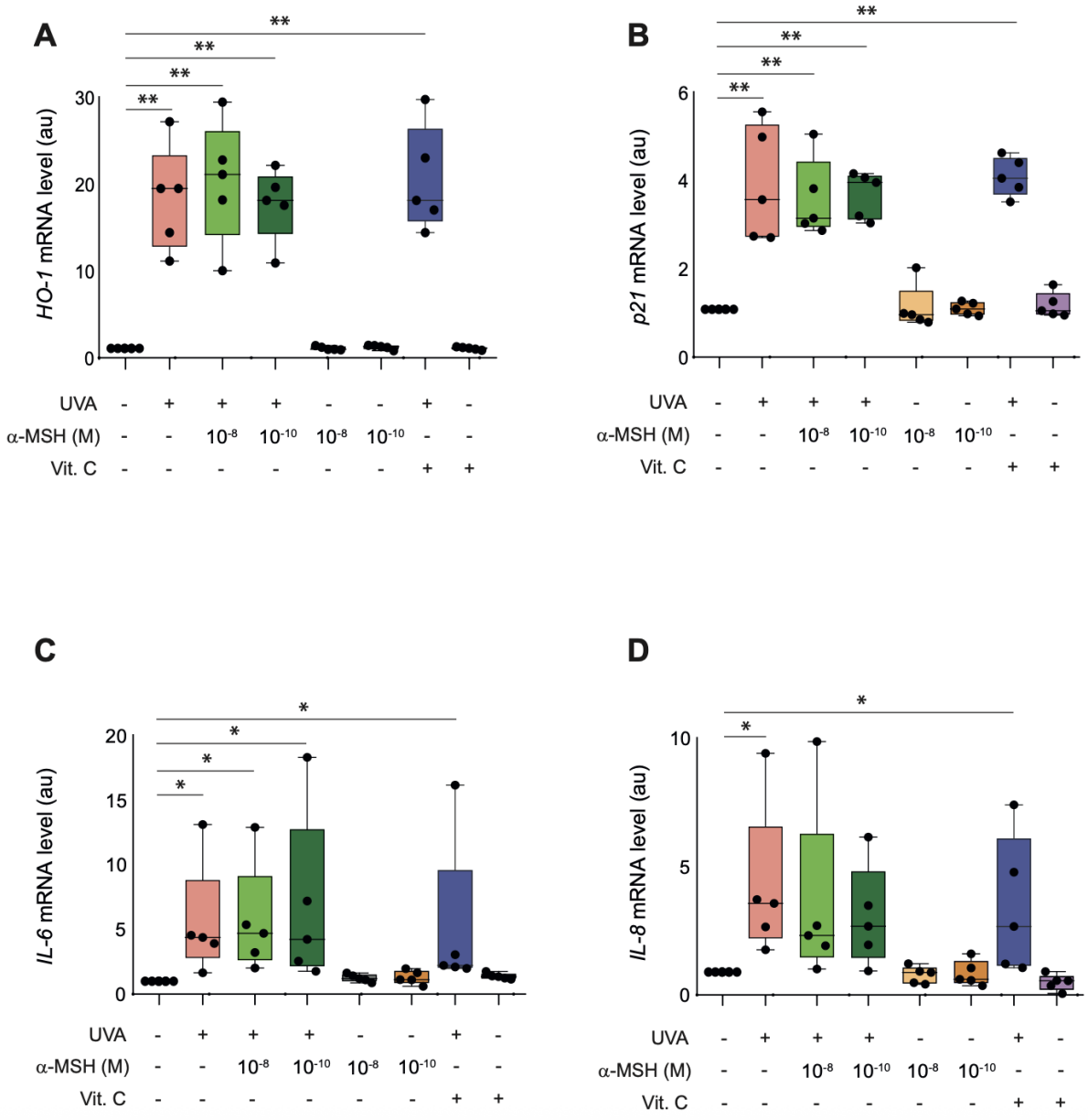
Supplementary Figure 2. Effect of the synthetic and superpotent α-MSH analogue NDP-α-MSH on UVA-induced generation of intracellular ROS in 2D cell cultures of aHDFs. Cells (n=4 independent donors) were treated with UVA (10 J/cm^2), NDP-α-MSH (10^{-6} M), or UVA plus NDP-α-MSH. Vitamin C (Vit. C) and sham-irradiated cells were used as controls (baseline); *p<0.01, evaluated by the Kolmogorov-Smirnov test.

SUPPLEMENTARY DATA



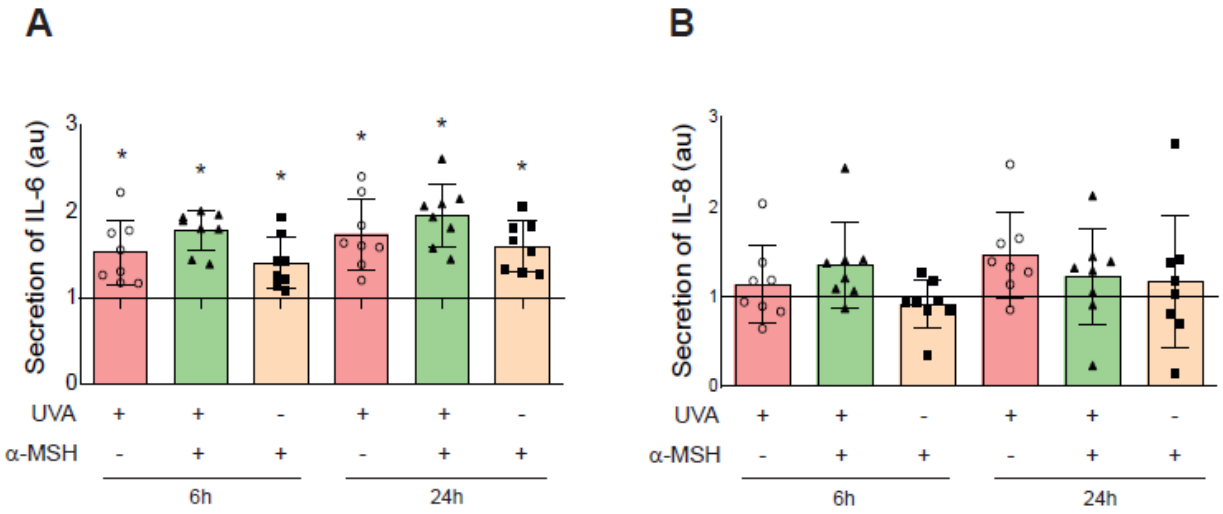
Supplementary Figure 3. Impact of UVA and α-MSH on mRNA expression of p16, p53, MMP1 and MMP3 in 2D cell cultures of aHDFs. (A-D) aHDFs (n=8 independent donors) were treated with α-MSH, UVA and a combination of both as described before (see legend of Figure 3A-D). Subsequently, the cells were harvested at indicated time points and real-time RT-PCR analysis was performed with primers for p16 **(A)**, p53 **(B)**, MMP1 **(C)** and MMP3 **(D)**.

SUPPLEMENTARY DATA



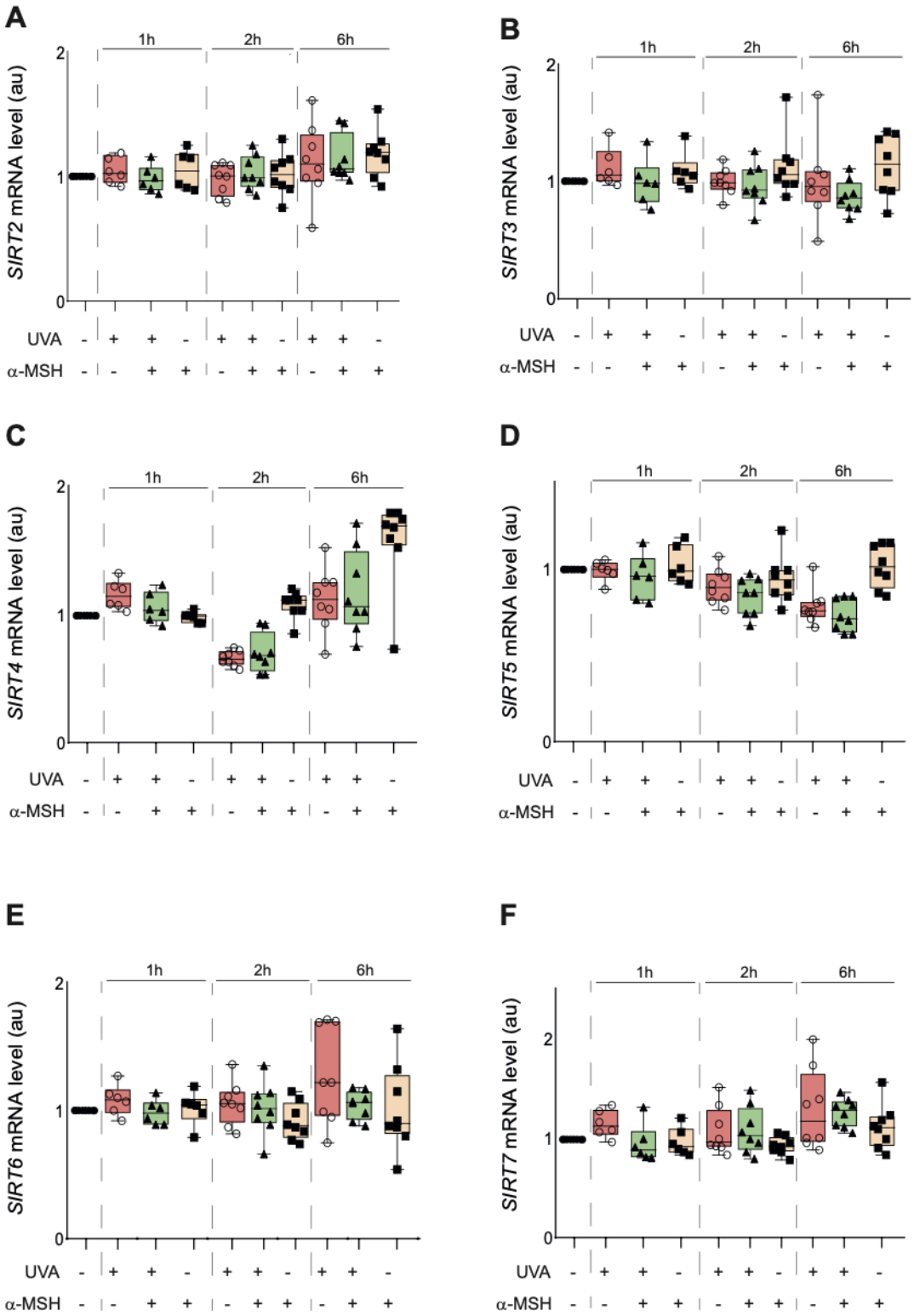
Supplementary Figure 4. Effects of different α-MSH doses on UVA-induced gene expression in 2D cell cultures of aHDFs. (A-D) Cells (n=5 independent donors) were treated with UVA (10 J/cm^2), α-MSH at 10^{-8} M or 10^{-10} M alone or in combination with UVA. Sham-irradiated cells and cells treated with vitamin C (Vit. C, 10 µM) served as controls. At indicated time points, cells were harvested and mRNA levels of HO-1 **(A)**, p21 **(B),** IL-6 **(C)** and IL-8 **(D)** were determined by real-time RT-PCR; *p<0.05; **p<0.01, evaluated by the Kolmogorov-Smirnov test.

SUPPLEMENTARY DATA



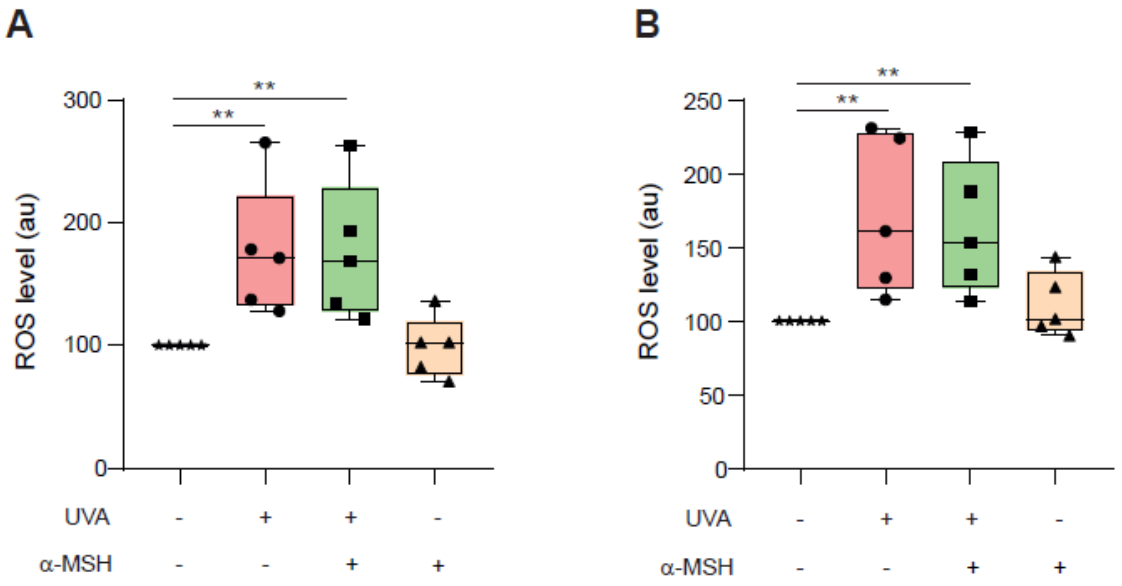
Supplementary Figure 5. Effects of UVA and α-MSH on IL-6 and IL-8 protein secretion in aHDFs. Cells (n=8 independent donors) were treated with UVA (10 J/cm^2), α-MSH (10^{-6} M) or UVA plus α-MSH. Sham-irradiated cells served as controls and were set as 1. Secreted protein levels of IL-6 **(A)** and IL-8 **(B)** at different time points after treatment were analysed by Luminex ELISA. *p<0.05, evaluated by one-way ANOVA.

SUPPLEMENTARY DATA



Supplementary Figure 6. Effects of α-MSH and UVA irradiation on mRNA levels of SIRT2-7 in 2D cell cultures of aHDFs. (A-F) Expression analysis of SIRT2-7 in aHDFs (n=6 or n=8 independent donors). Cells were treated with α-MSH and UVA as described before, harvested at the indicated time points and analysed for expression of SIRT2 **(A)**, SIRT3 **(B)**, SIRT4 **(C)**, SIRT5 **(D)**, SIRT6 **(E)** and SIRT7 **(F)** by real-time RT-PCR analysis.

SUPPLEMENTARY DATA



Supplementary Figure 7. Effects of UVA and a-MSH on intracellular ROS generation in aHDFs with LOF MC1R. Effect of UVA irradiation (10 J/cm^2) alone, in combination with α-MSH (10^{-6} M), and α-MSH (10^{-6} M) alone in aHDFs with R151C homozygosity **(A)** and R160W homozygosity **(B)** was measured by FACS analysis using the redox-sensitive fluoroprobe CM-H_2DCFDA. n=5 biological replicates. **p<0.01, evaluated by one-way ANOVA.

Supplementary Table 1. Primer sequences for end-point RT-PCR.

Gene	Forward primer 5'->3'	Reverse primer 5'->3'
SIRT1	ACGCTGGAACAGGTTGCGGG	AGCGGTTCATCAGCTGGGCAC
Sirt2	TCACACTGCGTCAGCGCCAG	GGGCTGCACCTGCAAGGAGG
Sirt3	AAGTGTTGTTGGAAGTGGAG	TGTGAAAGAAGAATGGGAGT
Sirt4	AGACTCCTTGTGATGACTGG	AGTACAGCTTTCCGAGTTTC
Sirt5	AGCTATATTGTGGCCTGAAG	CACTTTCTGCACTAACACCA
Sirt6	AGTTCGACACCACCTTTGAG	CGTACTGCGTCTTACACTTG
Sirt7	CGTCCGGAACGCCAAATAC	GACGCTGCCGTGCTGATT

Supplementary Table 2. Primer sequences for quantitative real-time RT-PCR.

Gene	Forward primer 5'->3'	Reverse primer 5'->3'
Sirt1	ACGCTGGAACAGGTTGCGGG	AGCGGTTCATCAGCTGGGCAC
Sirt2	TCACACTGCGTCAGCGCCAG	GGGCTGCACCTGCAAGGAGG
Sirt3	AAGTGTTGTTGGAAGTGGAG	TGTGAAAGAAGAATGGGAGT
Sirt4	AGACTCCTTGTGATGACTGG	AGTACAGCTTTCCGAGTTTC
Sirt5	AGCTATATTGTGGCCTGAAG	CACTTTCTGCACTAACACCA
Sirt6	AGTTCGACACCACCTTTGAG	CGTACTGCGTCTTACACTTG
Sirt7	CGTCCGGAACGCCAAATAC	GACGCTGCCGTGCTGATT
p16	CCAACGCACCGAATAGTTACG	GCGCTGCCCATCATCATG

SUPPLEMENTARY DATA

CDKN1A	TGGAGACTCTCAGGGTCGAAA	GGCGTTTGGAGTGGTAGAAATC
p53	GAGCTGAATGAGGCCTTGGA	CTGAGTCAGGCCCTTCTGTCTT
MMP1	GGGAATAAGTACTGGGCTGTTCAG	CCTCAGAAAGAGCAGCATCGATATG
MMP3	CACTCACAGACCTGACTCGGTTC	CCAAGGACAAAGCAGGATCACAG
IL-6	AGCCACTCACCTCTTCAGAACG	GGTTCAGGTTGTTTTCTGCCAG
IL-8	CTTGGCAGCCTTCCTGATTTC	TTCTGTGTTGGCGCAGTGTG
HO-1	CCAGCGGGCCAGCAACAAAGTGC	AAGCCTTCAGTGCCCACGGTAAGG
RPL23	TCCAGCAGTGGTCATTCGAC	GCAGAACCTTTCATCTCGCC

Supplementary Table 3. Detailed information on used antibodies.

Antibody	**Company**	**Catalogue number**	**dilution**
Pp38	Cell Signalling (Denvers, MA, USA)	#4511	1:2000
p38	Cell Signalling	#9212	1:2000
p21	Cell Signalling	#2947	1:2000
p53	Santa Cruz (Dallas, TX, USA)	#2527	1:1000
HO-1	Invitrogen (Darmstadt, Germany)	#70081	1:1000
MC1R	Bioss (Freiburg, Germany)	#bs-23517R	1:1000
SIRT1	Cell Signalling	#9475	1:1000
SIRT1	ThermoFisher (Darmstadt, Germany)	#OTI5B2	1:1000
α-tubulin	Calbiochem (Taufkirchen, Germany)	#CP06	1:1000
β-actin	Santa Cruz	#3700	1:1000
GAPDH	Cell Signalling	#5174	1:2000