Expanded View Figures

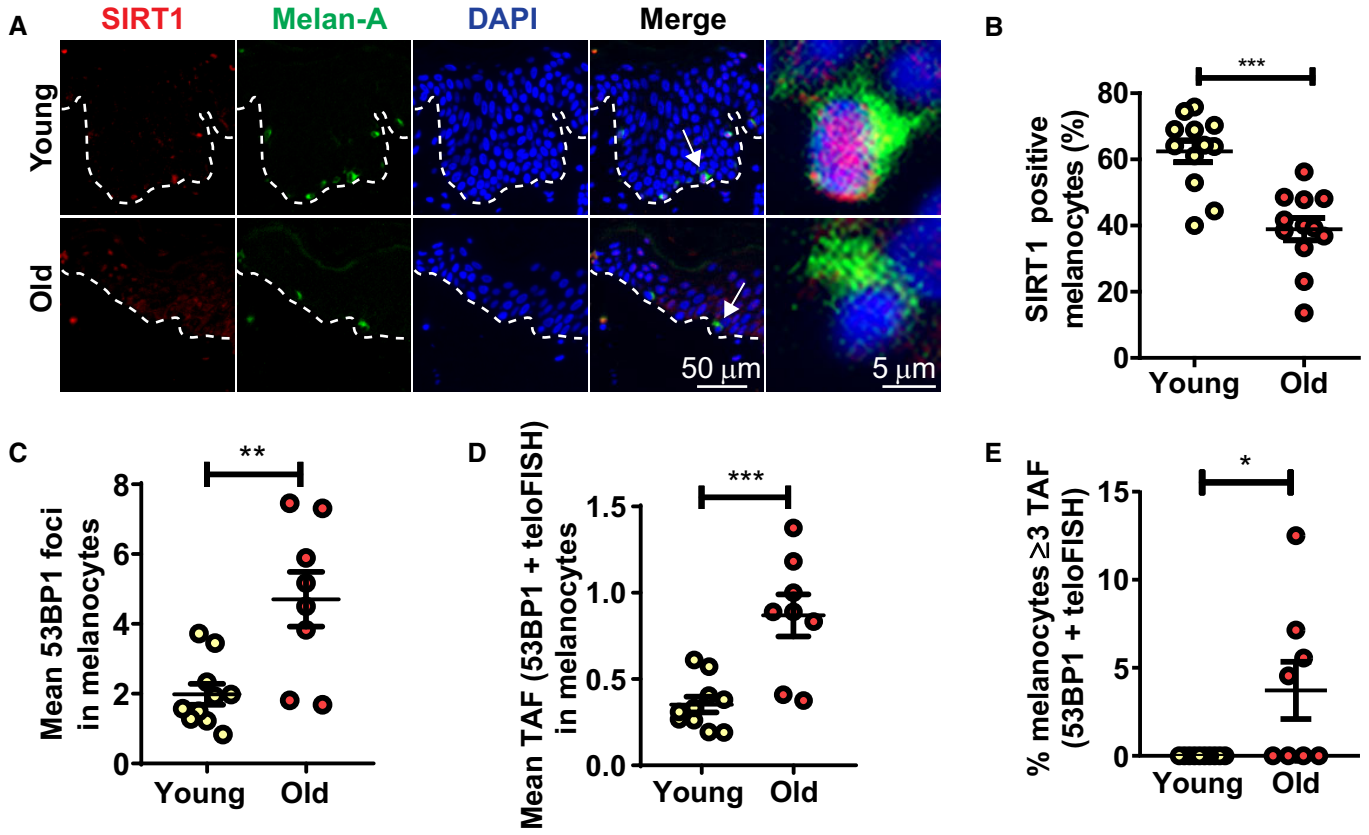

Figure EV1. Melanocytes express increased markers of senescence in human skin with age.

A Representative images of SIRT1 immunofluorescence in combination with the melanocyte marker Melan-A in young and older skin. Arrow indicates a melanocyte, which is amplified on the right.

B Dot plot showing percentage of SIRT1-positive melanocytes for each individual donor. The horizontal line represents the group mean (n = 12 donors for each group). Data are shown as mean ± SEM.

C–E Graphs showing (C) mean number of 53BP1 foci in melanocytes, (D) mean number of 53BP1 foci co-localising with telomeres in melanocytes and (E) percentage of melanocytes containing ≥ 3 53BP1 foci co-localising with telomeres for each individual subject. The horizontal line represents the mean for each group (n = 10 young donors; n = 8 old donors). Data are shown as mean ± SEM.

Data information: *P < 0.05, **P < 0.01, ***P < 0.001. Statistical tests: two-tailed unpaired t-test.

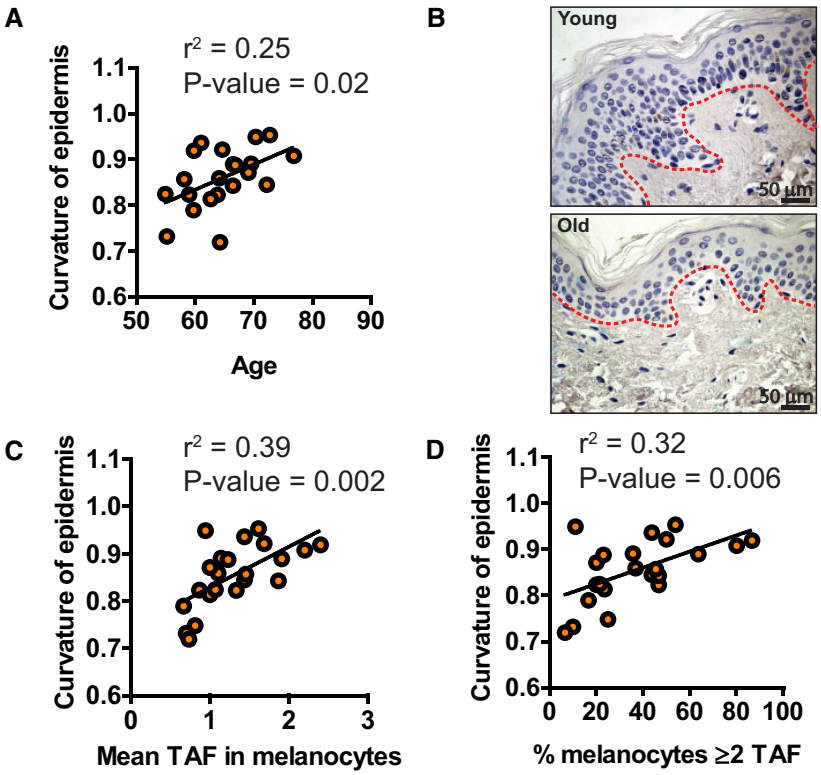

Figure EV2. Telomere dysfunction in melanocytes correlates with age-associated loss of epidermal curvature.

A Graph showing the correlation between epidermal curvature and age for each individual donor (n = 22 donors) accounting for subject age. A curvature value closer to 1 denotes a flatter epidermal–dermal junction (EDJ).

B Images illustrating the loss in epidermal curvature (red dotted lines) with age. Red dotted lines represent the EDJ.

C, D Graphs showing correlations between (C) mean number of TAF in melanocytes and (D) percentage of melanocytes containing ≥ 2 TAF and epidermal curvature for each individual donor. When accounting for subject age, the statistical significance for these correlations was (C) P = 0.007 and (D) P = 0.02.

Data information: Correlations were determined using Pearson's correlation test.

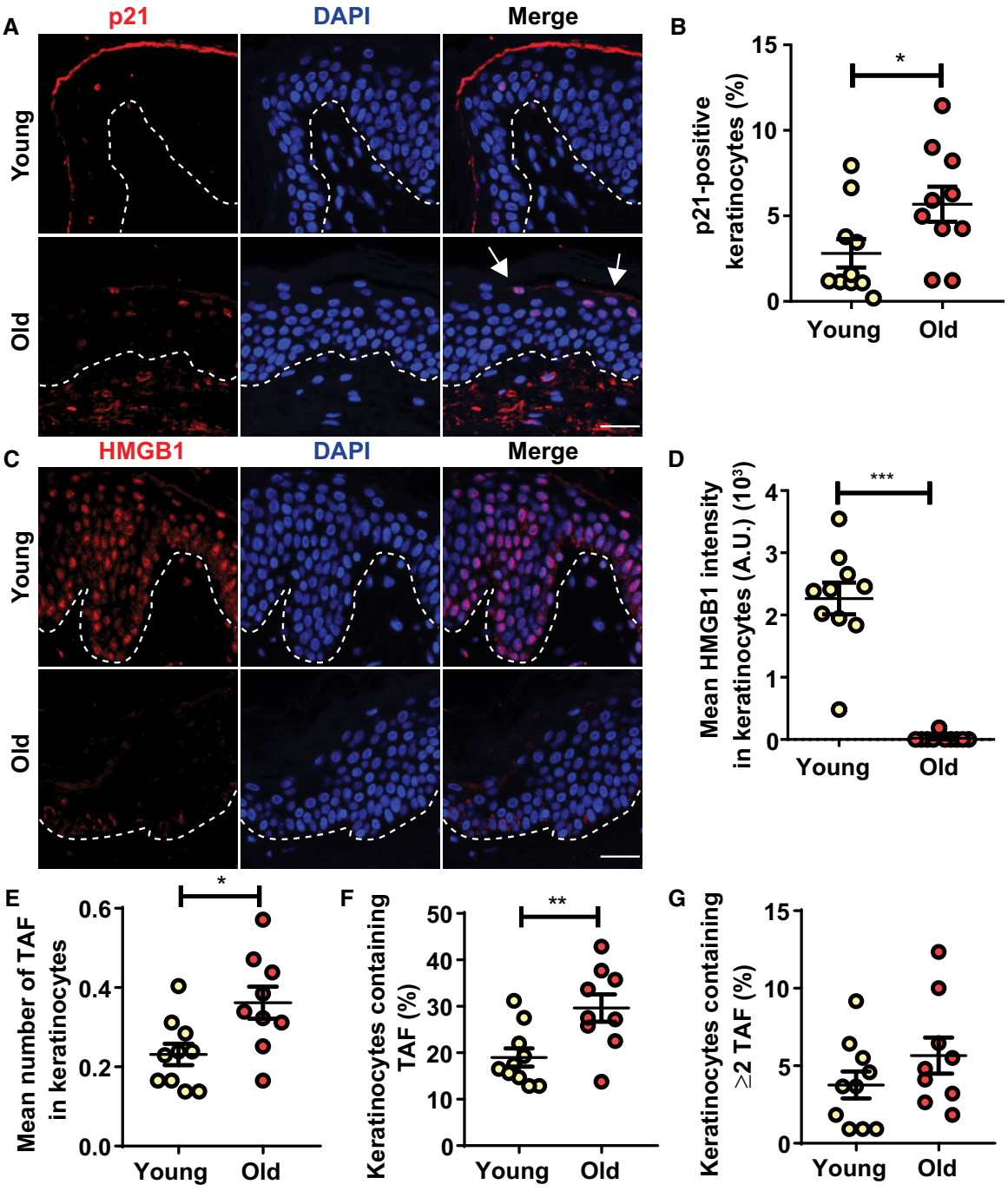

Figure EV3. Keratinocytes express senescence markers in human skin with age.

A–D Representative immunofluorescence images of (A) p21 and (C) HMGB1 in skin from young and old donors. Images were taken using a 20× objective. Arrows indicate p21-positive keratinocytes in the uppermost layer of the epidermis. Scale is 30 μm. Graphs showing (B) percentage of p21-positive keratinocytes per donor and (D) HMGB1 intensity in keratinocytes. The horizontal line represents the mean for each group (n = 10 donors per group). Data are shown as mean ± SEM.

E–G (E) Mean number of TAF in keratinocytes, (F) percentage of keratinocytes containing TAF and (G) percentage of keratinocytes containing ≥ 2 TAF in skin sections from young and old donors. The mean for each group is represented by the horizontal line (n = 10 young donors; n = 9 old donors). Data are shown as ± SEM.

Data information: *P < 0.05, **P < 0.01, ***P < 0.001. Statistical tests: two-tailed unpaired t-test.

Figure EV4. Repeated UVA+B exposure induces senescence in human epidermal melanocytes *in vitro*. Melanocytes were exposed to 0.4 J/cm^2 UVA and UVB radiation once a day for five consecutive days. Cells were harvested 4 and 18 days following the last exposure.

A Representative EdU immunofluorescence images of proliferating and irradiated melanocytes (18 days post-IR) (red: EdU; blue: DAPI). Images were taken using a 20× objective.

B Graph showing the percentage of EdU-positive melanocytes at the time points indicated. Data are shown as mean ± SEM of $n = 3$ independent experiments.

C Representative images of Sen-β-Gal (blue) of proliferating and UV-irradiated melanocytes. Images were taken using a 20× objective.

D Graph showing the percentage of control and irradiated (4 days after the last exposure) Sen-β-Gal-positive melanocytes. Data are shown as mean ± SEM of $n = 3$ independent experiments.

E Representative p16 immunofluorescence images of proliferating and senescent melanocytes (18 days following the last UV exposure). Images were taken using a 20× objective.

F Graph showing the percentage of p16-positive melanocytes at the time points indicated following the last UV exposure. Data are shown as mean ± SEM of $n = 3$ independent experiments.

G Representative immuno-FISH images of proliferating (top) and senescent (bottom) melanocytes. Telomeres are shown in red, γH2AX is depicted in green, and DAPI is shown in blue. Arrows indicate co-localisation between γH2AX and telomeres, which are amplified on the right. Images are Z projections and were taken using a 63× oil objective.

H–J Graphs showing (H) mean number of γH2AX foci, (I) mean number of TAF, (J) percentage of melanocytes containing ≥ 2 TAF. Data are shown as mean ± SEM of $n = 3$ independent experiments.

Data information: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Statistical tests: two-way ANOVA (B, F), one-way ANOVA (D, H–J).

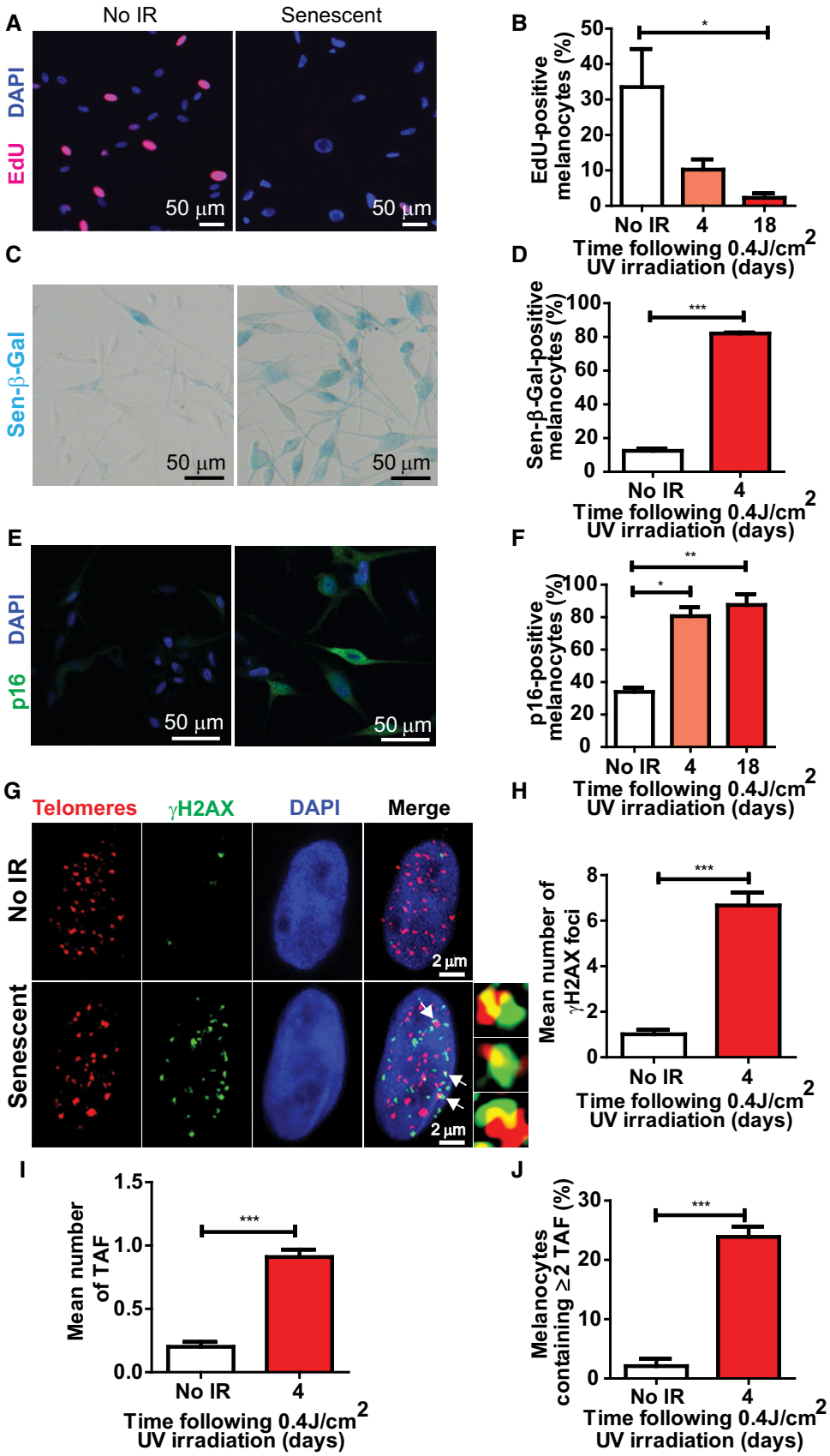

Figure EV4.

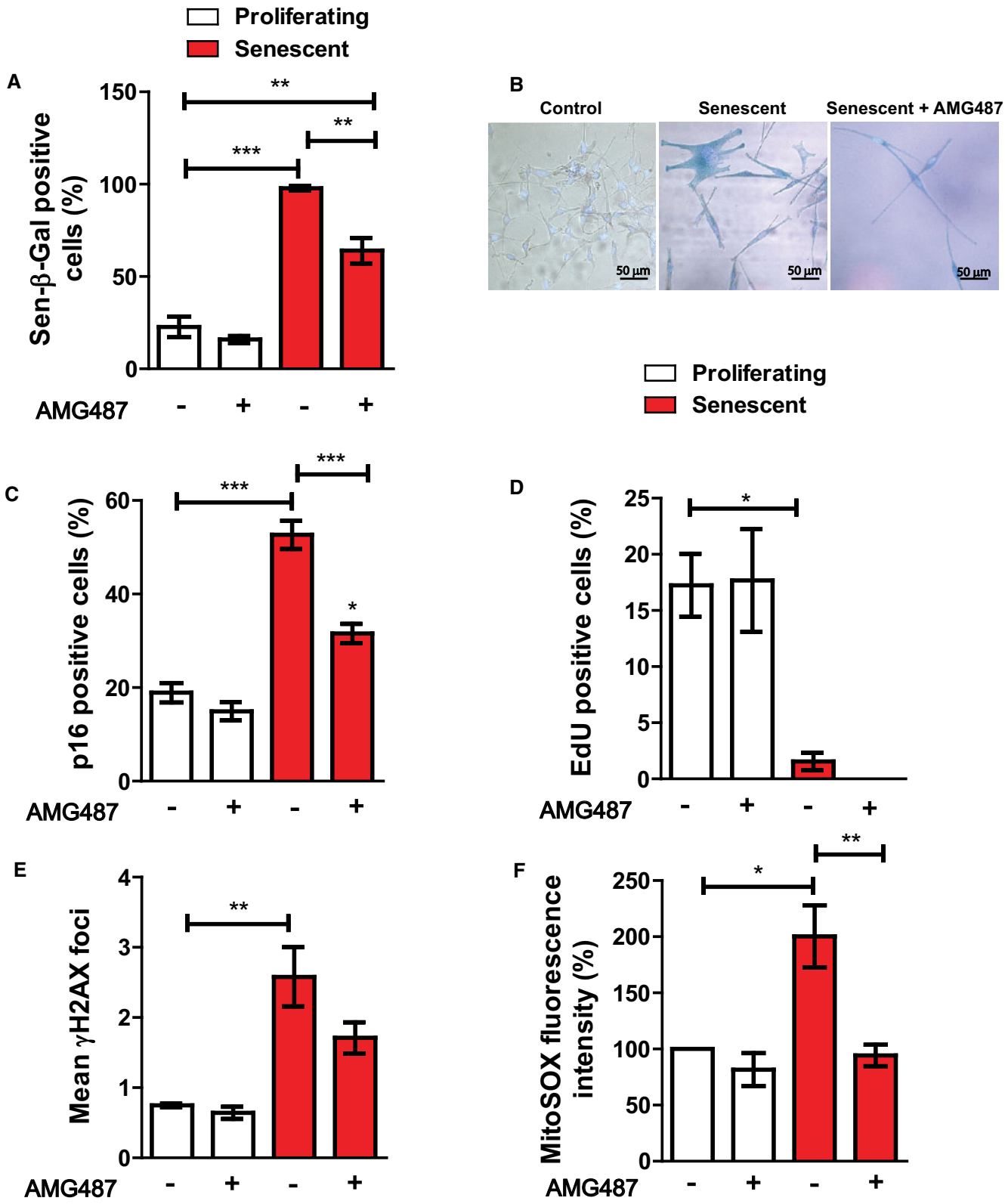

Figure EV5.

Figure EV5. CXCR3 signalling is involved in the initiation of melanocyte senescence. Senescence was induced in melanocytes by 10 Gy X-ray irradiation, and cells were treated with either AMG487 or vehicle DMSO (control) for 10 days.

A Graph showing the percentage of Sen-β-Gal-positive melanocytes at the conditions indicated (white: proliferating melanocytes; red: senescent melanocytes). Data are shown as ± SEM (n = 3 independent experiments).

B Representative Sen-β-Gal images of proliferating and senescent melanocytes with or without AMG487 treatment. Images were taken using a 20× objective.

C–F Graphs showing the percentage of (C) p16-positive and (D) EdU-positive melanocytes at the conditions indicated. (E) Mean number of γH2AX foci in proliferating and senescent melanocytes with or without AMG487 treatment. (F) MitoSOX fluorescence intensity of melanocytes at the conditions indicated. Values are a percentage fold change normalised to proliferating controls (untreated). ROS measurements were performed at 3 days following irradiation. Data are shown as mean ± SEM of N = 3 independent experiments.

Data information: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Statistical tests: one-way ANOVA.