Victorelli *et al*. Appendix

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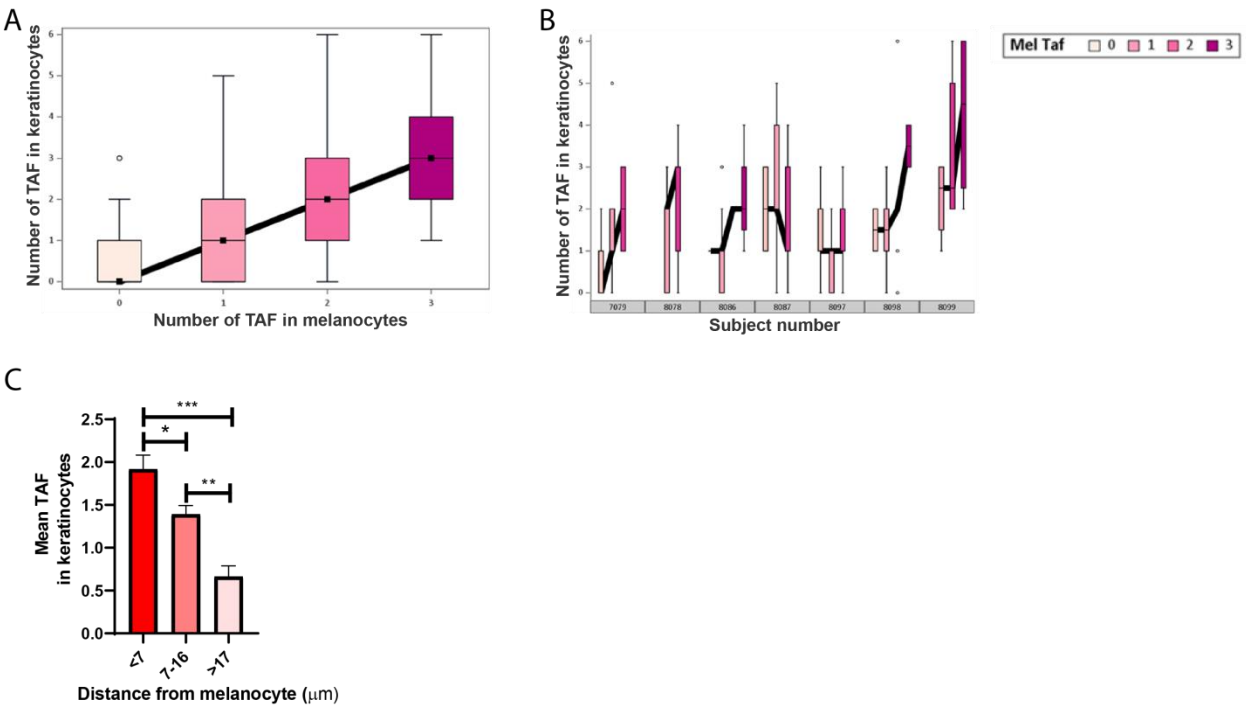
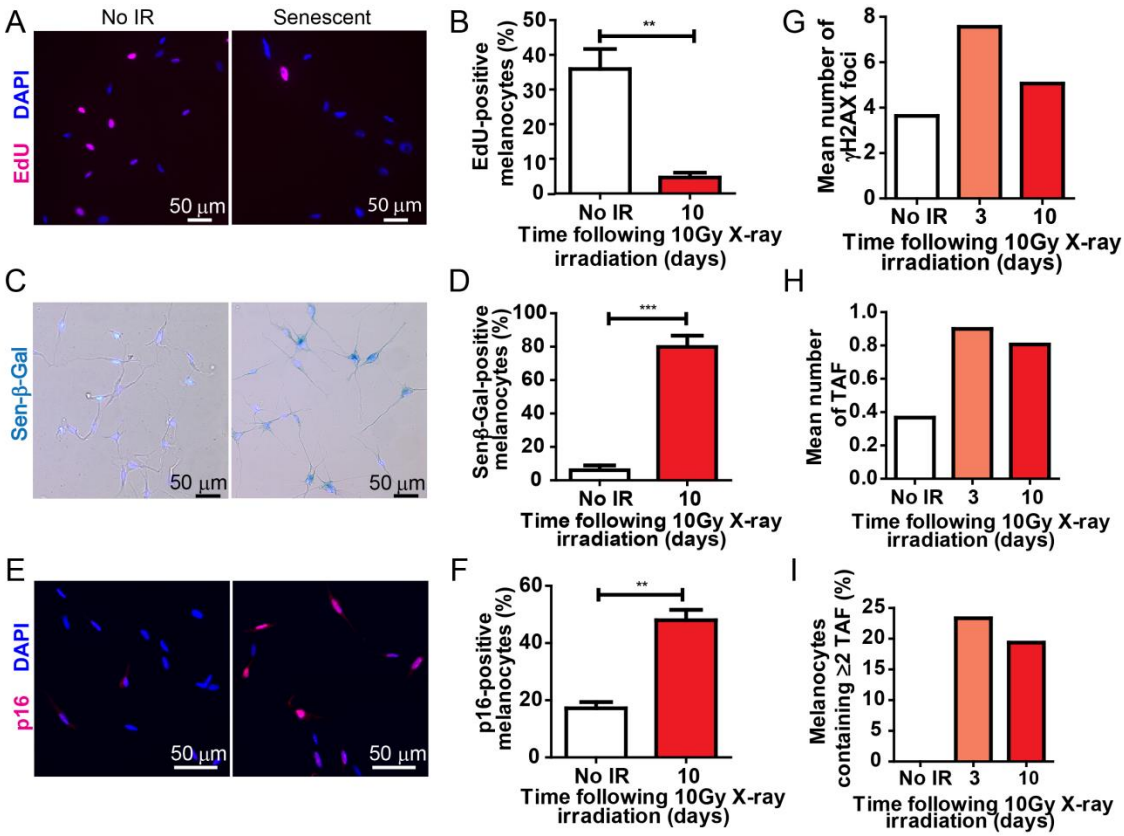


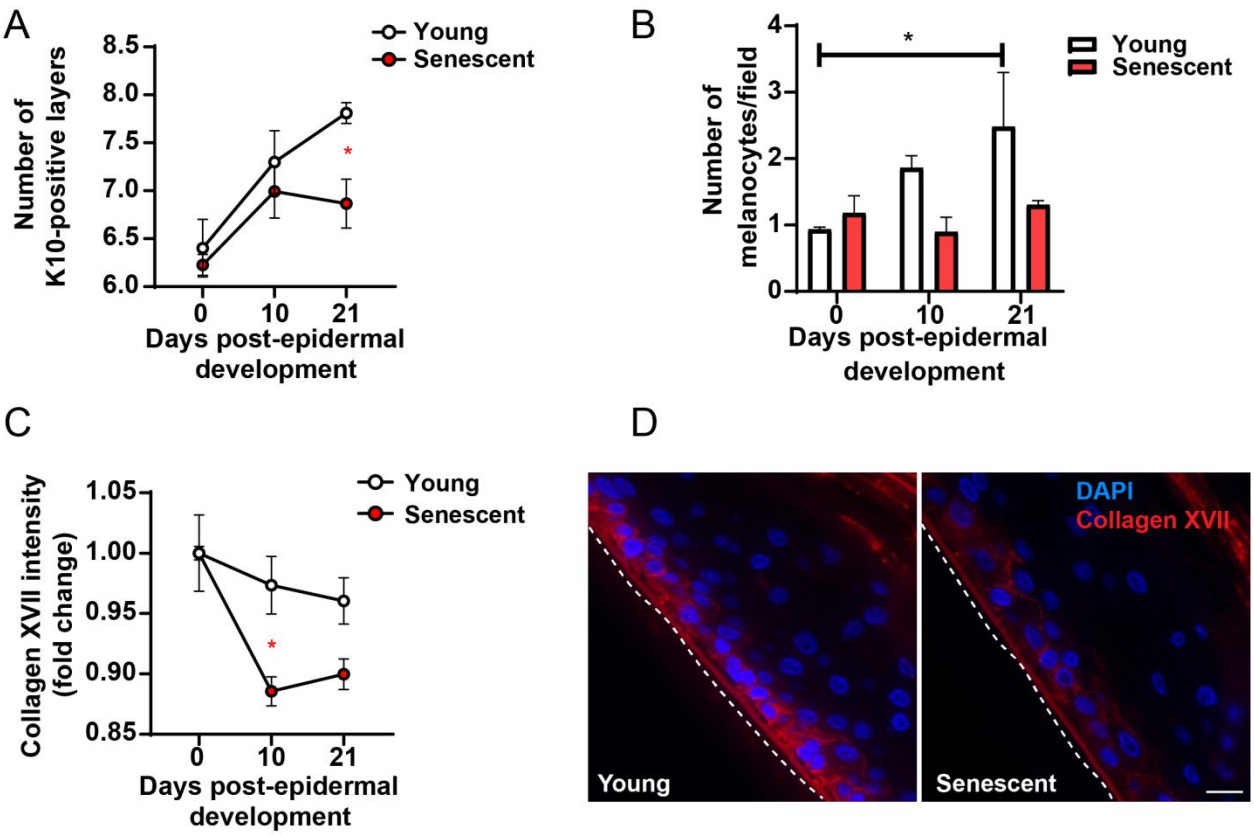
Appendix Figure S1 - Association between TAF in melanocytes and surrounding keratinocytes *in vivo*. (A) Association between number of TAF in melanocytes (X-axis) and surrounding keratinocytes (Y-axis). There were a total of 369 keratinocytes that had a TAF count in association with a melanocyte TAF count in 14 subjects. There is a positive trend in the medians of ρ=0.45 with a p-value <0.0001. **(B)** For the old group, 6/7 subjects had a positive correlation between damage in melanocytes and keratinocytes of which 3/6 are significant at a p-value<0.05. A similar pattern was not observed in young subjects (data not shown) but this was unsurprising given the lower amount of TAF damage seen (e.g. 2 or more TAF counts in melanocytes made up nearly 20% of total counts in old but <5% in young). **(C)** Mean number of TAF in keratinocytes surrounding melanocytes with 1 – 3 TAF within the distances indicated. Data are mean ± SEM (n=7 old donors). 70 – 100 keratinocytes were analyzed per donor.

*p<0.05, **p<0.01, ***p<0.001



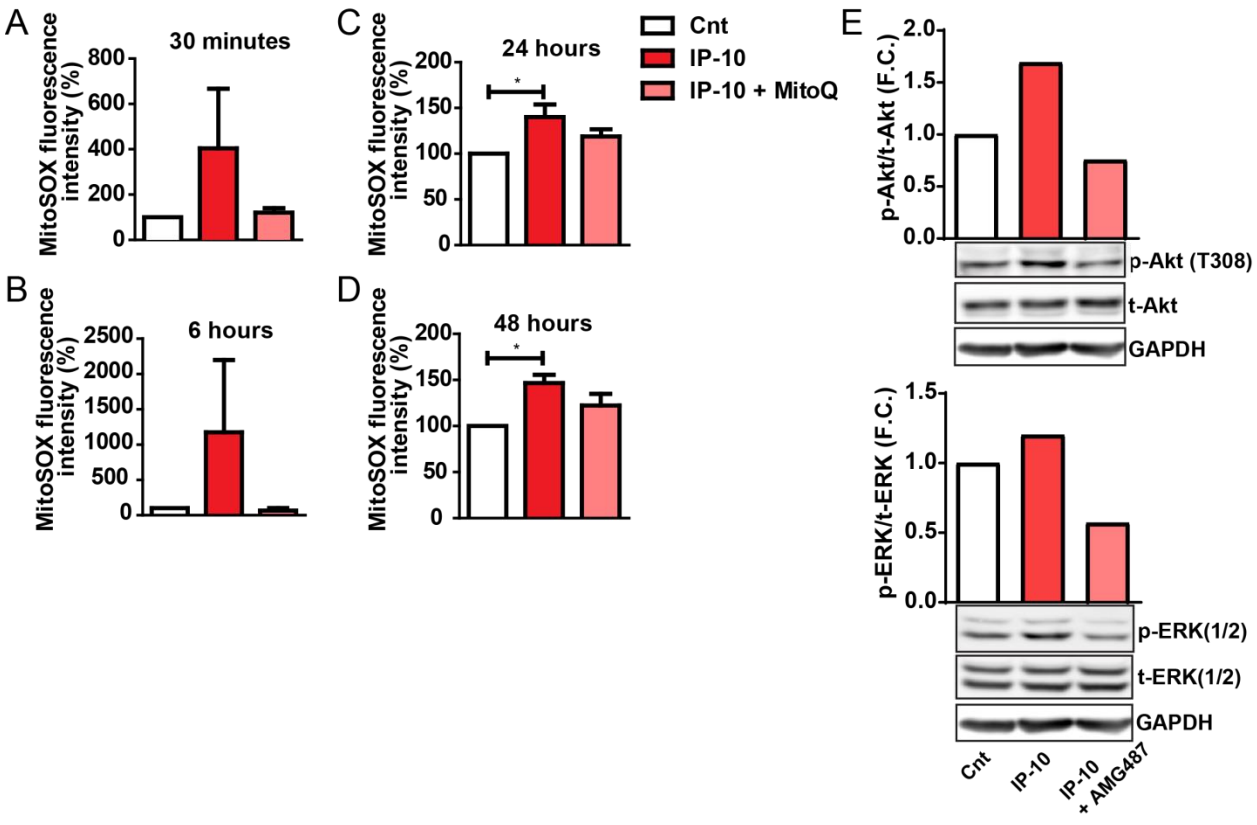
Appendix Figure S2 - X-ray irradiation induces senescence in human epidermal melanocytes *in vitro*. **(A)** Representative immunofluorescence images of EdU staining of non-irradiated melanocytes and cells 10 days after X-ray irradiation (red: EdU; blue: DAPI). Images were taken using a 20x objective. **(B)** Percentage of EdU-positive melanocytes at control, proliferating conditions (No IR) and 10 days after 10 Gy X-ray irradiation. Data are mean ± S.E.M. of N=3 independent experiments. **(C)** Representative Sen-β-Gal (blue) images of non-irradiated melanocytes and cells 10 days following X-ray irradiation. Images were taken using a 20x objective. **(D)** Percentage of Sen-β-Gal-positive untreated and irradiated melanocytes. Data are mean ± S.E.M. of N=3 independent experiments. **(E)** Representative p16 immunofluorescence images of non-irradiated and senescent (10d 10Gy) melanocytes (Red: p16; blue: DAPI). Images were taken using a 40x objective. **(F)** Percentage of p16-positive untreated and irradiated melanocytes 10 days after the initial exposure. Data are mean ± S.E.M. of N=3 independent experiments. Graphs showing **(G)** mean number of γH2AX foci, **(H)** mean number of TAF, and **(I)** percentage of melanocytes containing ≥2 TAF. Data are mean of 30 cells obtained from a minimum of 10 random planes (n=1).

**p<0.01, ***p<0.001



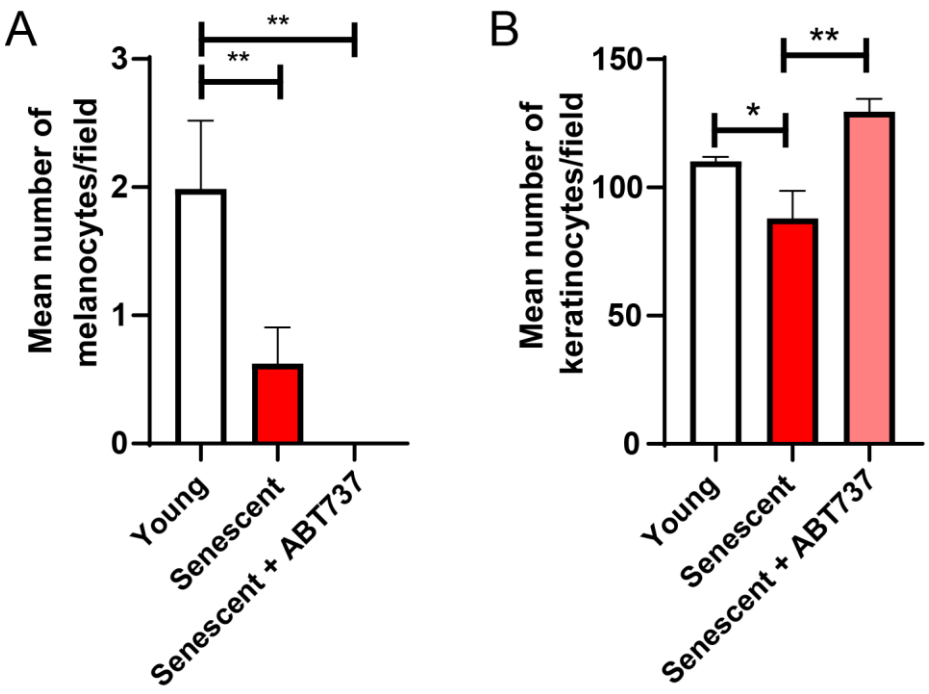
Appendix Figure S3 - Senescent melanocytes impair keratinocyte differentiation in 3D epidermal equivalents. (A) Number of cell layers positive for keratin 10 in melanoderms containing either young or senescent melanocytes at the time points indicated. Data are mean ± SEM of n=3 melanoderms per group. **(B)** Number of young or senescent melanocytes in melanoderms at the time points indicated. Data are mean ± SEM of n=3 melanoderms for each condition. **(C)** Collagen XVII intensity in keratinocytes in melanoderms containing either young or senescent melanocytes. Values are fold change normalised to day 0 for each group. Data are mean ± SEM of n=3 melanoderms per group. **(D)** Representative immunofluorescence images of collagen VXII in melanoderms containing young or senescent melanocytes. Images were taken using a 20x objective. Scale is 30µm.

*P<0.05



Appendix Figure S4 - IP-10 triggers increased mitochondrial ROS production. Graphs showing MitoSOX fluorescence intensity of fibroblasts treated with IP-10 with or without MitoQ for **(A)** 30 minutes, **(B)** 6 hours, **(C)** 24 hours and **(D)** 48 hours. Values are a percentage fold change normalised to controls (untreated). Data are mean ± S.E.M. of N=3 independent experiments. *p<0.05. **(E)** Representative Western blot showing expression of p-Akt (T308), t-Akt, p-ERK(1/2), t-ERK(1/2), and GAPDH as a loading control in dermal fibroblasts treated with IP-10 with or without AMG487 for 1 hour. Graphs show quantification of the respective Western blots.

*P<0.05



Appendix Figure S5 - Clearance of senescent melanocytes rescues the number of keratinocytes in 3D epidermal equivalents. (A) Mean number of melanocytes in melanoderms in the conditions indicated. Data are mean ± SEM of n=3 melanoderms per group. **(B)** Mean number of keratinocytes in melanoderms in the conditions indicated. Data are mean ± SEM of n=3 melanoderms per group.

*P<0.05, **P<0.01