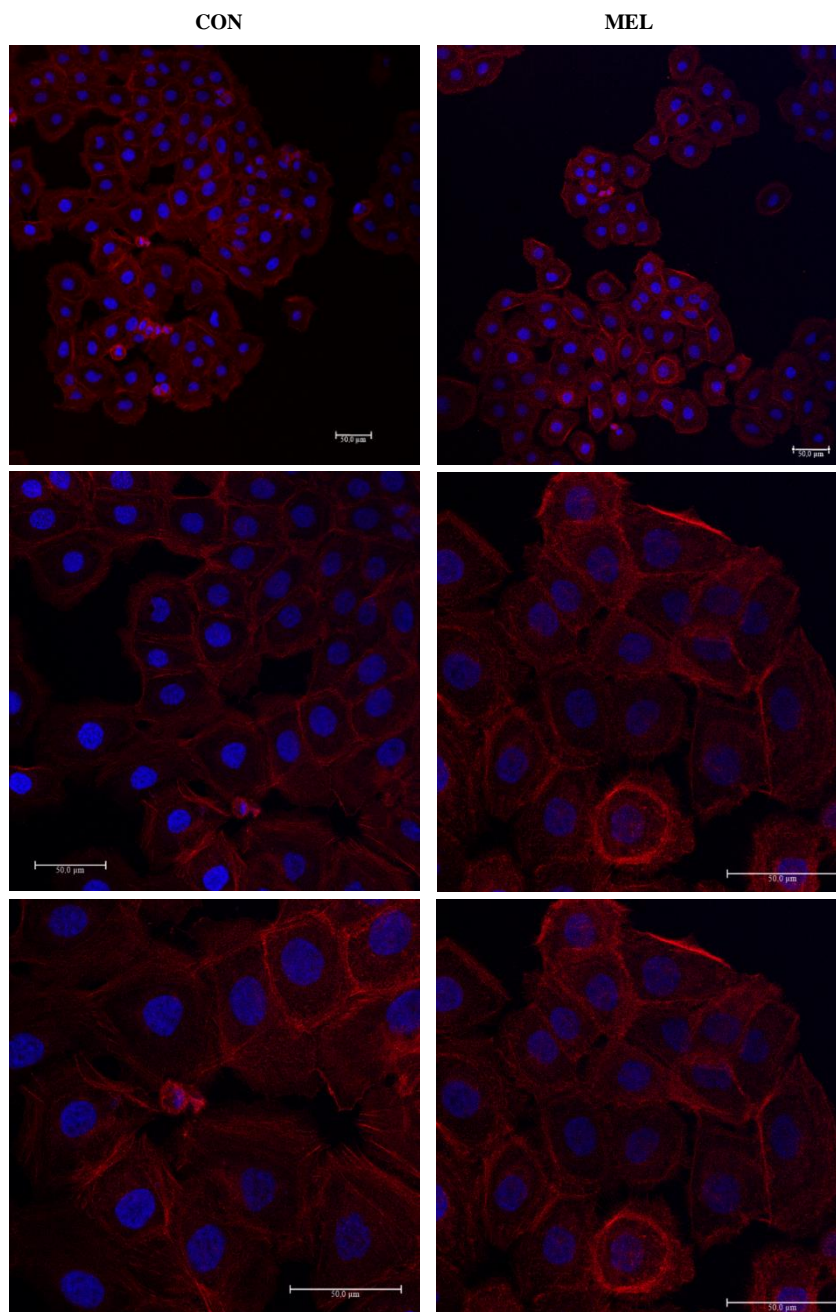


SUPPLEMENTARY MATERIALS

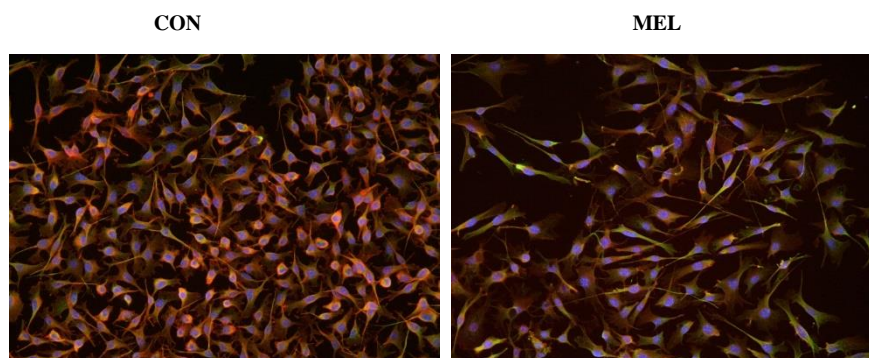
A novel study of melatonin diffusion in a 3-D cell culture model

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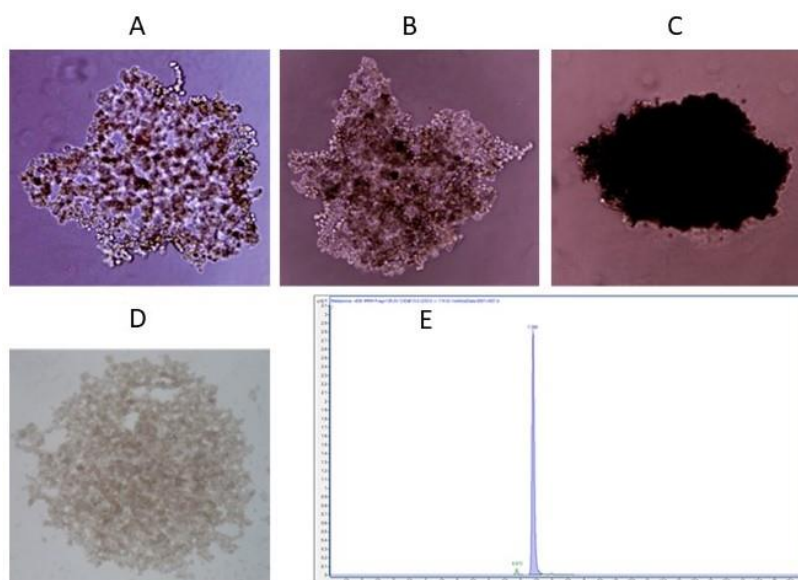


Supplementary Fig.1. Confocal microscopy images of undifferentiated HaCaT human keratinocytes.

Left panel, control cells (CON) cultured with vehicle (0.1% DMSO); right panel, 1mM melatonin (MEL) treated cells. Red-conjugated Phalloidin was used to visualize F-actin. Cells were counterstained with DAPI.

**Supplementary Fig. 2. Fluorescence microscopy images of murine melanoma B16F10 cells.**

Left, control cells (CON) cultured with vehicle (0.1% DMSO); right, 1mM melatonin (MEL) treated cells. Red-conjugated Phalloidin was used to visualize F-actin. Green-conjugated antibody was used for visualizing tubulin. Nuclei were counterstained with DAPI. Micrographs show the merge of three colors.

**Supplementary Fig. 3. Micrographs showing spheroids of B16F10 cells and HaCaT.**

Top panel, B16F10 spheroids after 24 (A), 48 (B) and 96h (C), respectively, displaying an increasing amount of melanin. Bottom panel, micrograph showing a HaCaT spheroid (D) and (E) representative HPLC chromatogram showing the elution profile of both, internal standard (5-methoxy tryptamine, 5-MT) and melatonin, with retention time of 6.8 and 7.5 min, respectively, under conditions described in the M&M section.



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