Melatonin and its metabolites protect human melanocytes against UVB-induced damage: Involvement of NRF2-mediated pathways

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Legend to Supplementary Figures

Supplementary Figure S1. Melatonin receptors are poorly expressed in human melanocytes. Nontreated HEMn cells were lysed in RIPA buffer and equal amounts of lysates were divided into four separate tubes and incubated with Protein A/G PLUS-Agarose beads and anti MEL1A (sc13186) (1), anti-MEL1A (sc13179) (2), anti-MEL1B (sc13177) (3), or anti-MEL1B (sc28453) (4) antibodies. Immunoprecipitates were separated by SDS–PAGE, immunoblotted with goat polyclonal antibodies against MEL1A (half of the membrane where lysates were incubated with MELA antibody) or MEL1B (the other half of the membrane where lysates were incubated with MELB antibody). The secondary antibody used was HRP anti-goat IgG.

Supplementary Figure S2. Melatonin and its metabolites induced the expression of antioxidative enzymes and NRF2 related genes. Human epidermal melanocytes were incubated with melatonin or its metabolites at the concentration of $5x10^{-5}$ M for 24 h before UV exposure. After cells were exposed to UVB, they were incubated again with fresh melatonin or its derivatives for 3 h. Cells were further harvested for RNA isolation. RT-PCR was performed using the equal amount of cDNA. B-actin served as internal control. Data were analyzed using t-test. The *p* value *, <0.05; **, <0.01; ***, <0.001, were determined *vs.* vehicle (EtOH, ethanol) control.

Supplementary Figure S3. Melanocytes used for all experiments were isolated from neonatal foreskin of African American donors. Cells in their third passage were used for the treatment with ethanol, melatonin, AFMK, 6-OHM, 5-MT or NAS 24 h before the exposure to UVB. Cells were further incubated for 3 h or 24 h in fresh media with compounds and harvested. Cell morphology did not change significantly when cells were exposed to UVB. Representative pictures of cells treated with vehicle (E) or melatonin (M) for additional 3 h or 24 h after UVB exposure (0, 25 and 50 mJ/cm^2) were taken under the light microscope magnification 10x (A). Melanin content remained similar among cells after treatment as seen as the dark color of cell pellets: UV exposure 0 mJ/cm^2 (B, D) or 50 mJ/cm^2 (C, E) of UVB, for 3 (B, C) or 24 h (D,

E). Densitometric analysis of the intensity of the cell pellets 24h after treatment was performed using Image J and presented in a graph (F).

Table 1: RT-PCR primers

Gene	Left primer	Right primer
Catalase CAT	CGTGCTGAATGAGGAACAGA	AGTCAGGGTGGACCTCAGTG
Glutathione peroxidase GPx	ACGATGTTGCCTGGAACTTT	GATGTCAGGCTCGATGTCAA
Superoxide dismutase Cu/Zn–SOD	GGCAAAGGTGGAAATGAAGA	GGGCCTCAGACTACATCCAA
Superoxide dismutase Mn–SOD	GCTCATGCTTGAGACCCAAT	CACCCGATCTCGACTGATTT
Heme oxygenase-1 (HO-1)	CCAGCGGGCCAGCAACAAAGTGC	AAGCCTTCAGTGCCCACGGTAAGG
Transcription factor NF-E2-related factor 2 (NRF2)	TTCTGTTGCTCAGGTAGCCCC	TCA GTTTGGCTTCTGGACTTGG
Glutamylcysteine synthetase (GCS)	TTGCAGGAAGGCATTGATCA	GCATCATCCAGGTGTATTTTCTCTT
Glutathione-S-transferase (GSTP1)	CCTGGTGGACATGGTGAATGA	CCTGGTGCAGATGCTCACATAGT
Glutathione reductase (GR)	GAGATGGCAGGGATCCTGTCAGC	GAGATGGCAGGGATCCTGTCAGC
NAD(P)H dehydrogenase, quinone2 (NQO2)	TTGACATCCCAGGATTCTACG	CCGTGGTTACGGAAAGGAG
Bata actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG

The source of primers is described in following articles: CAT, GPx, Cu/Zn-SOD, and Mn-SOD[1] [2]; HO-1 [3]; NRF2 [4]; GCS [5]; GSTP1 [6]; GR [7]; NQO2 [8].

References:

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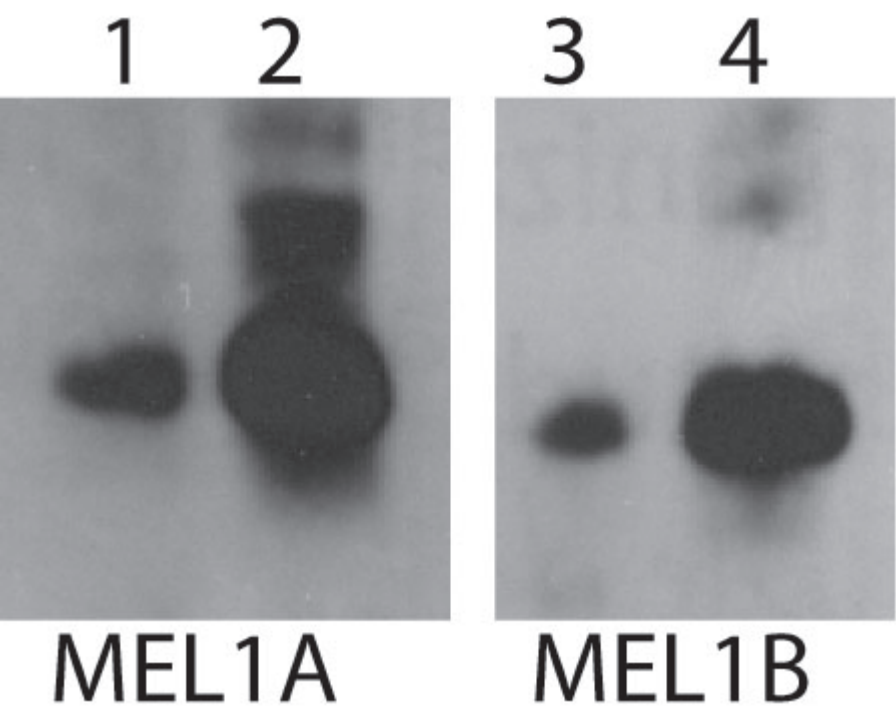
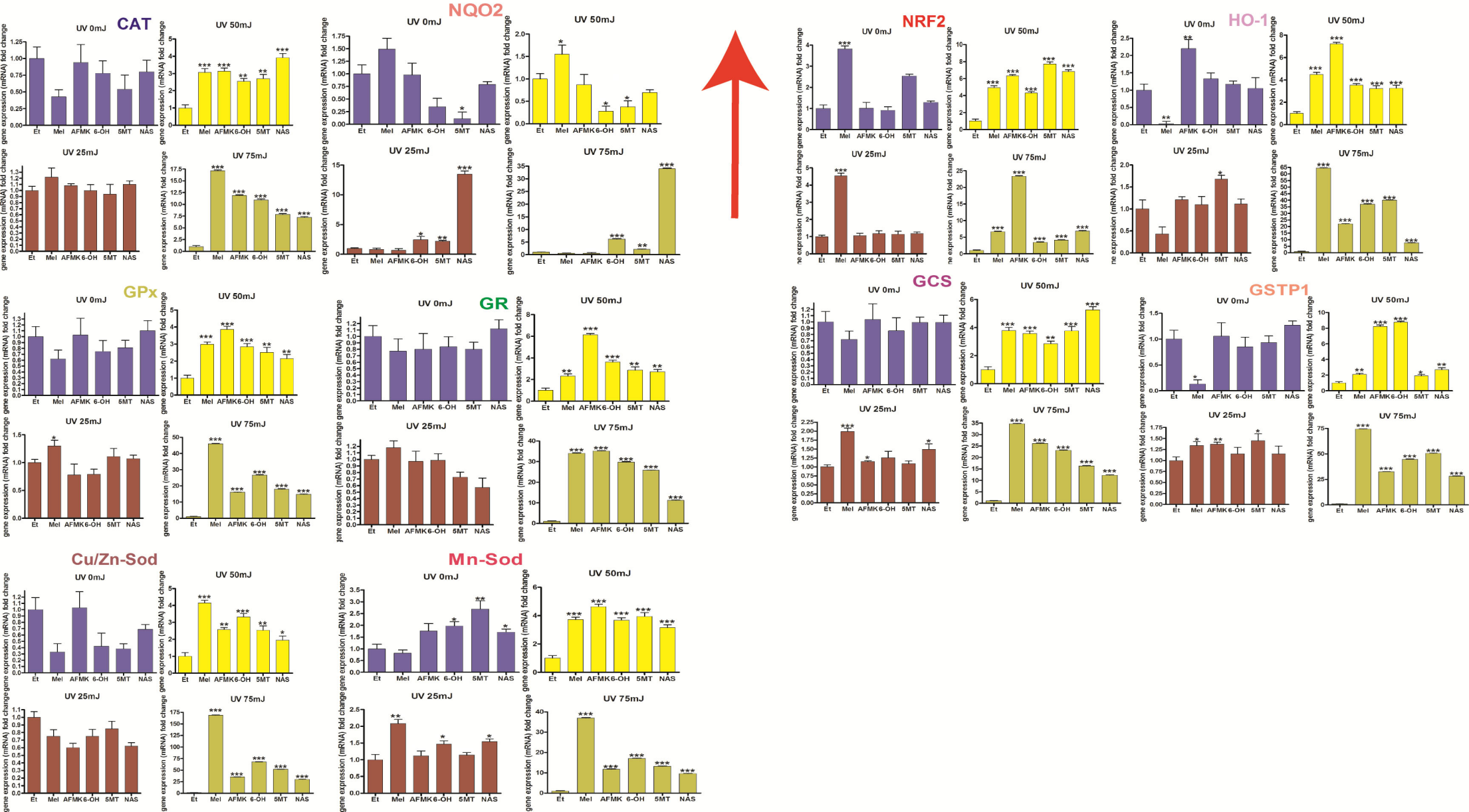
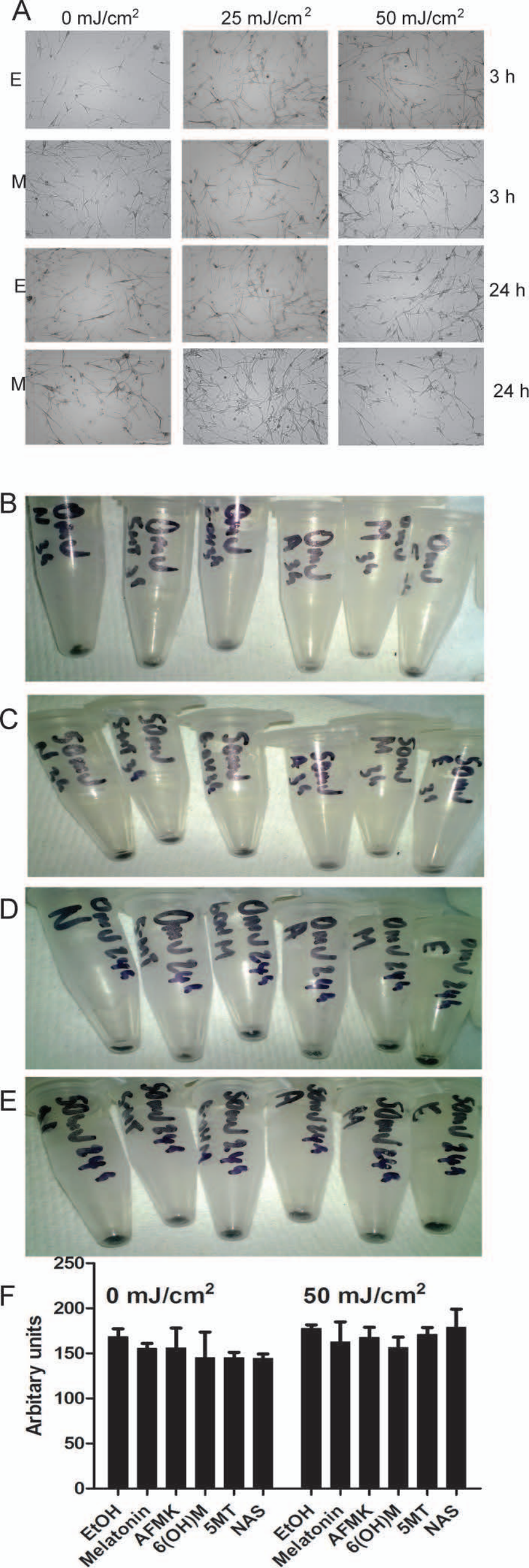

1
2
3
4
MEL1A
MEL1B

ANTIOXIDANT ENZYMES AND NRF2- DEPENDENT GENES




A
0 mJ/cm2
25 mJ/cm2
50 mJ/cm2
E
3 h
M
3 h
E
24 h
M
24 h
B
C
D
E
F
0 mJ/cm2
50 mJ/cm2
Arbitary units
250
200
150
100
50
0
EtOH
Melatonin
AFMK
6(OH)M
5MT
NAS
EtOH
Melatonin
AFMK
6(OH)M
5MT
NAS