SUPPLEMENTARY INFORMATION

Comparative Study of Curcumin and its Hydrogenated Metabolites, Tetrahydrocurcumin, Hexahydrocurcumin and Octahydrocurcumin on Melanogenesis in B16F10 and MNT-1 Cells

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Supplementary Methods:

1. *Calculation of Cell viability from Alamar Blue assay.*

The data from Alamar Blue assay was used for calculation of cellular viability based on manufacturer's instructions where the % of Alamar Blue reduction between sample and control group was calculated as: (S/C) x 100, where
$S = [(\varepsilon_{OX})\lambda_2) \times (A_{\lambda 1}) - ((\varepsilon_{OX})\lambda 1) \times (A_{\lambda 2}]$ of sample, and $C = [(\varepsilon_{OX})\lambda 2) \times (A'_{\lambda 1}) - (\varepsilon_{OX})\lambda 1) \times (A'_{\lambda 2})\ ]$ of control.
In the above equation, $\lambda 1$ is wavelength at 570 nm, $\lambda 2$ is wavelength at 600 nm, $(\varepsilon_{OX})\lambda 1$ is the molar extinction coefficient of oxidized Alamar Blue at 570nm (80,586), $(\varepsilon_{OX})\lambda 2$ is the molar extinction coefficient of oxidized Alamar Blue at 600 nm (117,216), A is the absorbance of sample and A' is the absorbance of control.

2. *Cytotoxicity of PC to MNT-1 Cells.*

MNT-1 cells were cultured in a 96-well plate at a seeding density of 1×10^4 cells/well and test compound PC at concentration range (5- 40 μM) was added the next day, and cells were cultured for a period of 5 d, with the compound replenished on third day of culture. At the end of treatments, the viability was measured by Alamar Blue assay and calculations were based on the aforementioned equation.

3. *Cytotoxicity of PC after L-Cysteine co-treatment in B16F10 Cells.*

B16F10 cells were cultured in a 96-well plate at a seeding density of 0.5×10^4 cells/well and the next day, PC (10 μM) in the absence or presence of L-Cysteine solution (diluted from 0.3 M solution in DPBS) was added and cells were cultured for 3 d. At the end of treatments, the viability was measured by Alamar Blue assay.

Supplementary Figures:

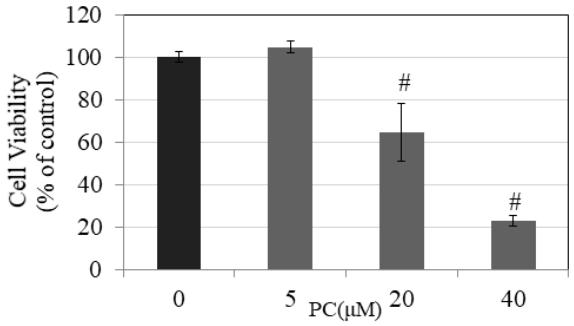

Figure S1: Viability of MNT-1 human melanoma cells treated with PC at various concentrations (5- 40 μM) for duration of 5 d tested by Alamar Blue assay; # $p < 0.001$ vs. control; One-way ANOVA with Dunnett's test; Data is mean ± SD of triplicate determinations.

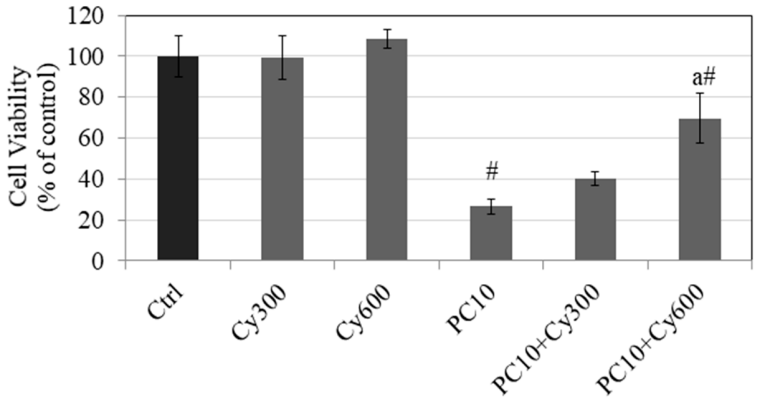

Figure S2: Viability of B16F10 mouse melanoma cells treated with PC (10 μM) in presence or absence of L-Cysteine (Cy) for duration of 3 d tested by Alamar Blue assay. Co-addition of L-cysteine (Cy) restores cytotoxicity of PC indicating a role of the Michael reaction acceptor on the β-diketone bond; PC10 indicates PC at 10 μM; L-Cysteine (Cy)at 300 μM and 600 μM was added; (#- $p < 0.001$ vs. Ctrl; letter a- $p < 0.001$ vs. PC10; One-way ANOVA with Tukey's test); Data is mean ± SD of triplicate determinations.

Table S1: Summary of the effects of THC, HHC and OHC on melanogenesis in both melanoma cells

Cell Model	THC	HHC	OHC
B16F10 mouse melanoma cells	↑ Melanin synthesis	✖ Melanin synthesis	↑ Melanin synthesis
	✖ Cellular tyrosinase activity and protein levels	✖ Cellular tyrosinase activity and protein levels	✖ Cellular tyrosinase activity and protein levels
MNT-1 human melanoma cells	↑ Melanin synthesis	↑ Melanin synthesis	✖ Melanin synthesis
	✖ Cellular tyrosinase activity and protein levels	✖ Cellular tyrosinase activity and protein levels	✖ Cellular tyrosinase activity and protein levels

The cross symbol denotes no effect, the arrow denotes a higher increase in melanin synthesis and smaller arrow shows moderate increase in melanin synthesis