SUPPLEMENTARY INFORMATION

Inhibitory Effects of the Bioactive Thermorubin Isolated from the Fungus *Thermoactinomyces Antibioticus* on Melanogenesis

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MITF protein expression in B16F10 cells.

4.5 × 10^3 B16F10 cells/well were inoculated in a 96-well plate and grown for 48 h after which TR was added in the presence or absence of α-MSH and treatment was continued for 72 h. At the end of treatments, the cells were processed using a cell-based MITF ELISA kit (LSBio, Seattle, USA) according the manufacturer instructions. Relative levels of MITF protein of compound-treated and untreated control were normalized by crystal violet stain absorbances to account for cell density variations in each well and results were expressed as % of control.

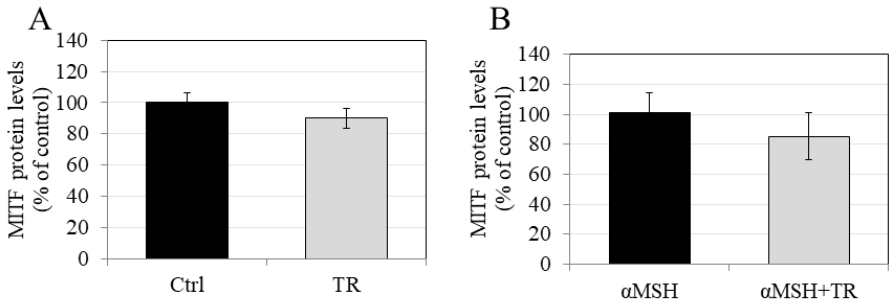

Figure S1. MITF protein levels in B16F10 cells treated with TR (25 μM) under (**A**) Basal and (**B**) α-MSH stimulated conditions; Data are mean ± SD of triplicates. No significance was found by students unpaired t-test.

MTS Cytotoxicity Assay in Human Keratinocytes.

HaCaT cells were procured from AddexBio (San Diego, CA, USA) and grown using DMEM supplemented with 10% HI-FBS and 1% antibiotics. For testing cytotoxicity of TR to HaCaT cells, we inoculated 5 × 10^3 cells/well in a 96-well tissue culture plate and after 24 h, the medium was replaced by fresh medium containing TR at various concentrations and cultures maintained for another 72 h. At the end of treatments, wells were aspirated and 100 μL of fresh culture medium with 20 μL of MTS reagent was added and the plate was incubated for a period of 30 min at 37 °C. The absorbance was read at 490 nm using a microplate reader and cell viability was reported as % normalized to control group.

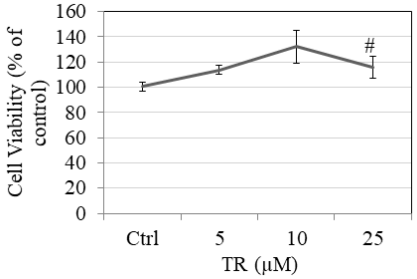

Figure S2. Viability of HaCaT cells treated with TR (5–25 μM) for 72 h. One-way ANOVA with Dunnett's test, # $p < 0.01$ vs. Ctrl; Data is mean ± SD of triplicate determinations.