

## Supplementary Information

# Biological Impact of the Ratio of E-Cigarette Liquid Base Constituents, Propylene Glycol and Vegetable Glycerin, on Primary Human Melanocytes

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### Supplementary Methods

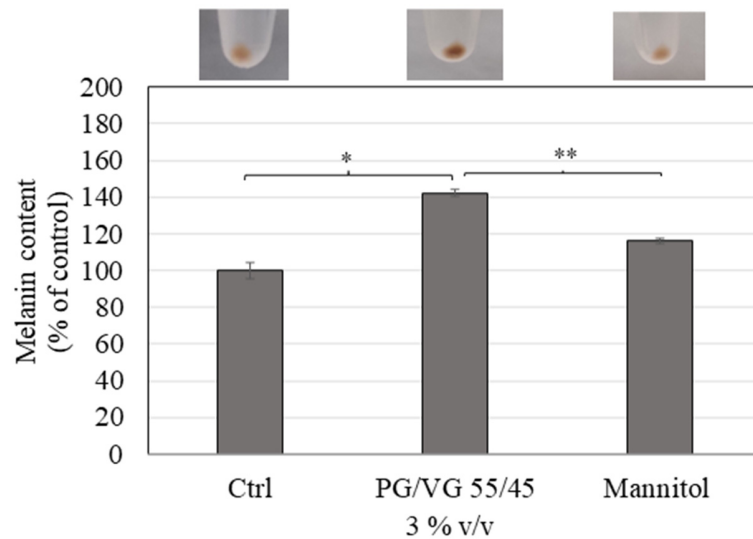
#### *Melanin Content Assay With Osmotic Control*

HEMn-LP cells ( $1.3 \times 10^5$  cells/well) were cultured in 12-well plates for 72 h followed by the replacement of culture medium with 3 % PG/VG (55/45) or 408 mM mannitol (Sigma-Aldrich) and cultures maintained for another 48 h, after which the cells were processed for the determination of intracellular melanin contents similar to the methods outlined in the main text.

#### *IL-6 Cytokine Assay*

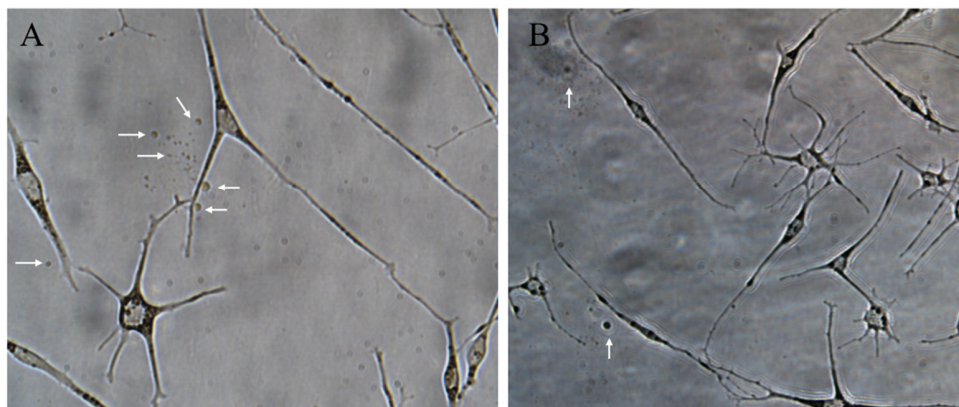
HEMn-LP cells ( $2 \times 10^4$  cells/well in 0.2 mL medium) were cultured in 48-well plates for 72 h followed by the replacement of medium with 10  $\mu$ g/mL LPS (O128: B12, Sigma, Cat# L2755) without or with PG/VG groups (0/100, 20/80, 55/45, 80/20, and 100/0) at 2 % v/v and cultures maintained at 37 °C in a CO<sub>2</sub> incubator for 48 h. After this step, the culture supernatants were collected, centrifuged, and stored at -80 °C until use. The concentration and serotype of LPS used in this experiment has been previously shown to elicit significant IL-6 production in HEMn-LP cells in our previous study [102]. An ELISA kit for human IL-6 (Raybiotech™, Norcross, GA) was used to determine IL-6 levels in supernatants according to the manufacturers' instructions. The levels of IL-6 were calculated in pg/mL and expressed as fold-change over negative control group.

## Supplementary Figures

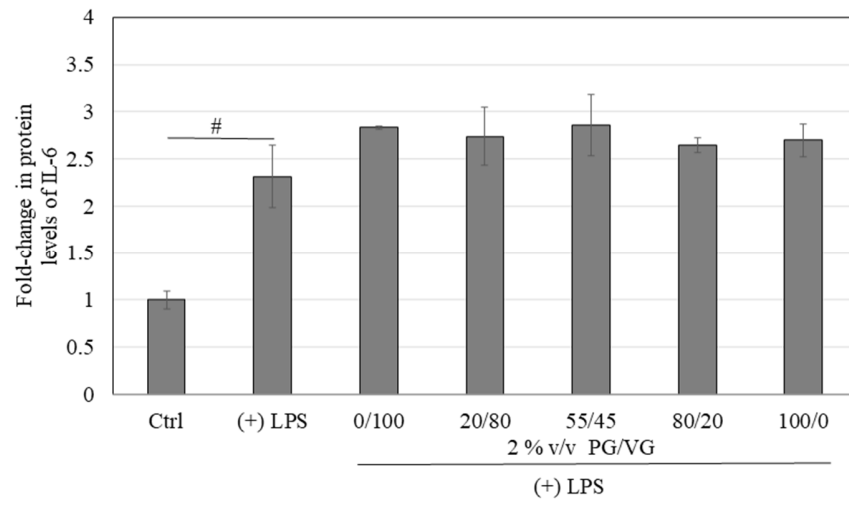


**Figure S1.** Intracellular melanin levels of HEMn-LP cells treated with 3 % PG/VG 55/45 or mannitol (408 mM) for a duration of 48 h with the corresponding photos of cell pellets shown above the bars; data is mean  $\pm$  SD of duplicate values; \* $p$  < 0.05 vs. Ctrl; \*\* $p$  < 0.01 vs. PG/VG 55/45;  $p$  > 0.05 for Ctrl vs. mannitol; pairwise unpaired t-test with Welch's correction.

## 2 % PG/VG 80/20



**Figure S2.** Two representative photomicrographs **A)** and **B)** taken from different regions at 40 $\times$  magnification in culture well of HEMn-LP cells treated with 2 % PG/VG 80/20 for a duration of 48 h; white arrows indicate the released pigment globules; dark melanin pigment aggregates in dendrite body and tips are visible in both images.



**Figure S3.** IL-6 levels measured in culture supernatants of cells treated with 10  $\mu\text{g}/\text{mL}$  LPS alone or co-treated with LPS and PG/VG at 0/100, 20/80, 55/45, 80/20, and 100/0, all at concentration of 2 % v/v for a duration of 48 h; cytokine data is expressed as fold-change and was normalized to negative control (Ctrl). Data is mean  $\pm$  SD of triplicate determinations. (# $p < 0.0001$  vs. Ctrl; one-way ANOVA with Tukey's test).