SUPPLEMENTARY MATERIAL

Glycerol improves skin lesion development in the imiquimod mouse model of psoriasis: Experimental confirmation of anecdotal reports from patients with psoriasis

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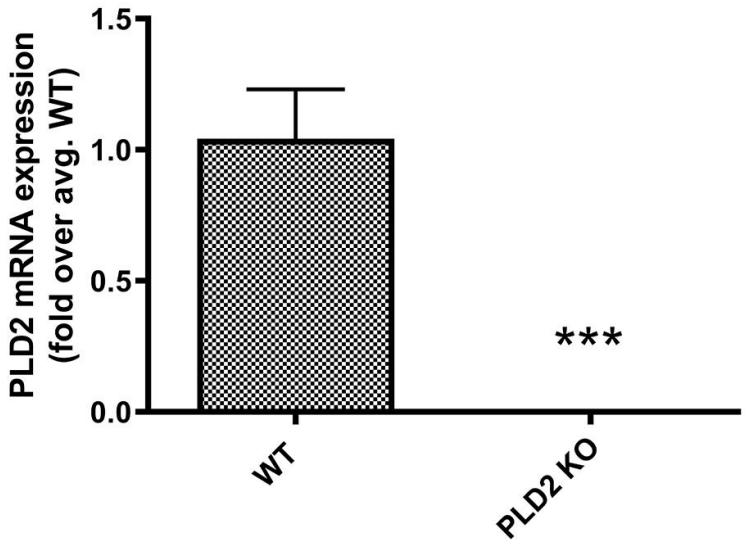

Figure S1: PLD2 mRNA expression in back skin of the PLD2 knockout mice. Mouse back skin from WT and PLD2 knockout mice was homogenized as described in Methods and RNA isolated. Quantitative RT-PCR analysis for PLD2 was performed using the delta-delta Ct method with Gapdh and Rplp0 as the endogenous control. Results represent the means ± SEM expressed as percent maximal (n= 4-6). Two tailed t-test was used to determine statistically significant differences, ***p<0.001 versus WT.

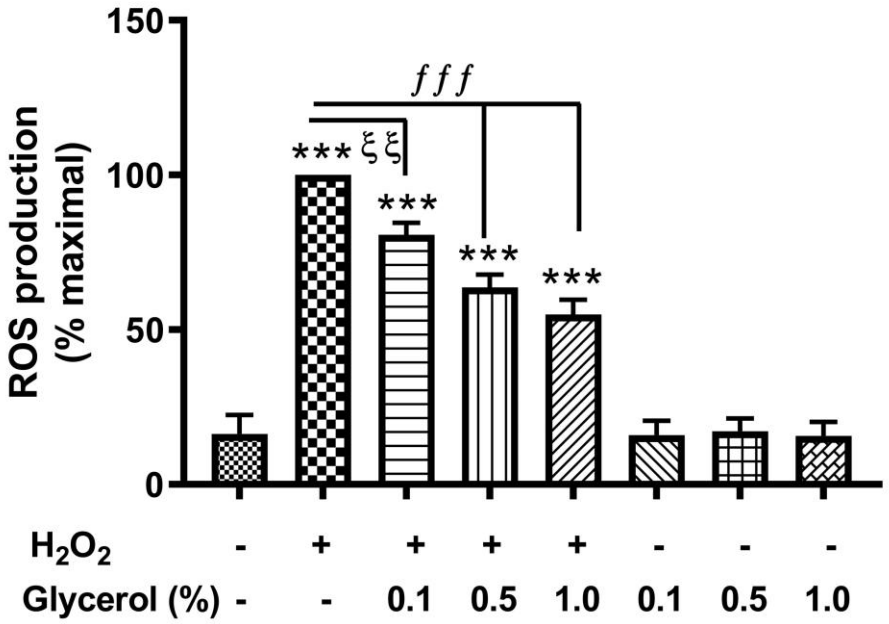

Figure S2: Glycerol inhibits hydrogen peroxide transport, as measured by intracellular ROS levels, in primary cultures of mouse keratinocytes. To measure hydrogen peroxide entry into cells, OxiSelect Intracellular ROS Assay kit (#STA-342, Cell Biolabs, Inc., San Diego, CA) was used as per the manufacturer's protocol. Briefly, primary cultures of mouse keratinocytes were plated on black cell culture fluorometric 96-well plates. The next day cells were washed twice with DPBS and 100 μL of 1x DCFH-DA/media solutions were added and incubated for 30 minutes. Cells were washed twice with DPBS and then treated with 100 μM hydrogen peroxide (H_2O_2) with or without glycerol (0.1 or 0.5 or 1.0%) as indicated for 1 hour. Fluorescence (intracellular ROS levels) was read by a fluorometric plate reader at 480 nm/530 nm from 5 min to 60 mins every 5 minutes and averaged from all time points. The ROS levels (fluorescence) are shown here as percent maximal and represent means ± SEM (n=3). One-way analysis of variance with Student-Newman-Keuls multiple comparison post-hoc tests (GraphPad Prism, La Jolla, CA) was used to determine significant differences; ***$p<0.001$ versus control; $^{\xi\xi}p<0.01$ or $^{fff}p<0.001$ as indicated.

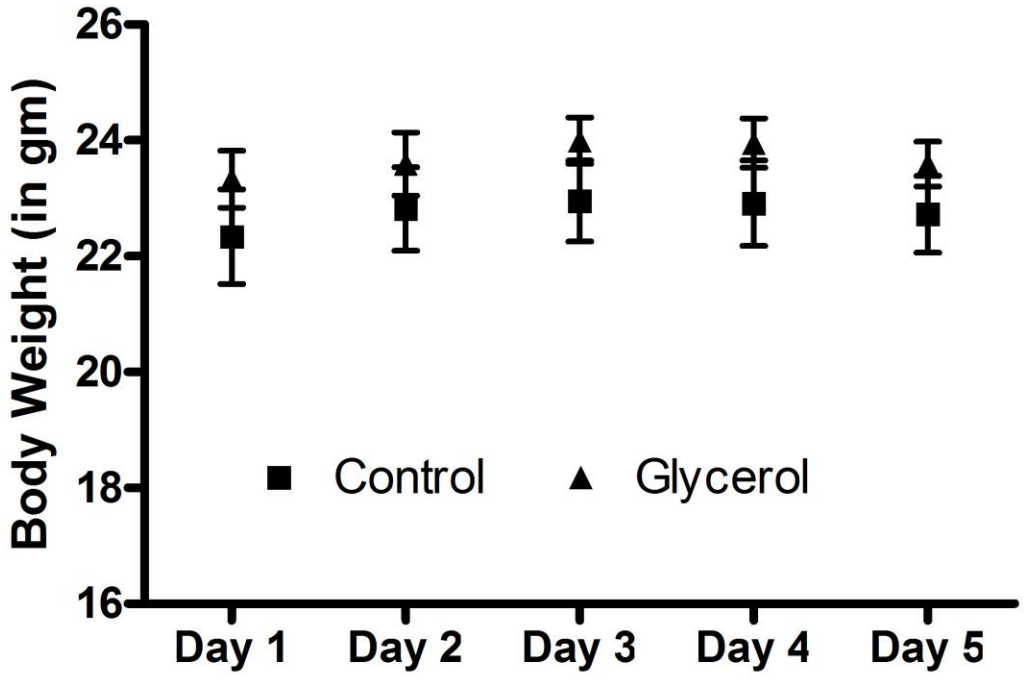

Figure S3: Oral glycerol does not affect mouse body weight. C57BL/6 mice receiving glycerol in the drinking water or not were treated with vehicle (petrolatum) or IMQ daily for 5 days. Body weights (in grams) of mice were recorded every day for 5 days. The quantitative data are presented as means ± SEM (n=4-5). One-way analysis of variance with Student-Newman-Keuls multiple comparison post-hoc tests (GraphPad Prism, La Jolla, CA) was used to determine significant differences. The body weights of glycerol-fed mice were not different from control mice on any day.