

Supplementary material

Preservative and Safety Testing: Methods

Preservative efficacy and safety testing was performed and is described in supplementary material. Preservative efficacy was assessed in an independent contract laboratory (Silliker Australia, Regents Park, Australia) to ensure the products met the requirements listed in the United States Pharmacopeia Chapter 51 for the Efficacy of Antimicrobial Preservation test of Category - 2 Products (<http://www.usp.org/>). Repeat insult patch testing was performed by AMA laboratories (New City NY, USA) using a cohort of 50 individuals. Healthy adult individuals, free from dermatologic disorders were inducted with informed consent in accordance with US Code of Federal Regulations Title 21, Part 50. Participants were excluded from the study if they were using medications, topical or systemic, that could potentially interfere with the test results. Pregnant and lactating females were also excluded. Test patches (Parke-Davis Hypoallergenic Read Bandages) were prepared with 0.2 mL of the test material and applied to the infrascapular regions of the back. The patch was then removed after 24 hours. The procedure was repeated until 9 consecutive 24 hour exposures had been made for every Monday, Wednesday and Friday for 3 consecutive weeks. On the presentation of any adverse reactions, the area of erythema or edema was measured relative to unaffected normal skin, 24 hours post patch removal. After 10-14 days of rest following the initial 9 exposures, the participants were challenged with the test material again, applied at a previously unexposed site. Reactions were scored as above at 24 and 48 hours post application.

Preservative and Safety Testing: Results

Formulations met the preservative efficacy requirements. We tested our évolis® product base with no active compounds and évolis® with 0.095% or 0.5% MTP3 for safety. A 50 participant repeated insult patch test for the test products was performed and monitored for adverse reactions. All three products were extremely well tolerated with zero adverse reactions recorded (Supplementary Table ST1), indicating that they were non-primary irritants and non-primary sensitizers according to FDA designation.¹

Dermal Papilla Cell PCR

Dermal Papilla cells were grown as described in the methods section for the DP-ALP assay and were treated with 0.3 µg/mL FGF5 and 10nM Wnt3a (R&D Systems, USA), 10nM Wnt3a alone, or were left untreated. RNA was extracted from cells using the RNeasy mini kit (Qiagen, Germany). cDNA was prepared using the ReverTra Ace- α - kit (Toyobo, Japan). Quantitative real-time PCR was performed using appropriate forward and reverse primers and the SYBR Premix Ex TaqII (Tli RNaseH Plus, Takara Bio Inc, Japan) on a Thermal Cycler Dice® (TP-850, Takara Bio Inc, Japan). Data generated from each PCR reaction were analyzed using Thermal Cycler Dice® Real Time System Single Software ver4.02 (Takara Bio Inc, Japan). Transcript levels were normalized to β -Actin.

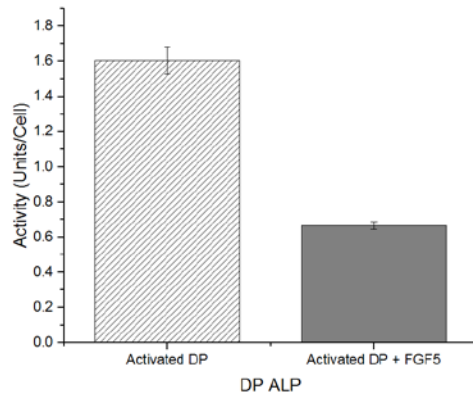
Scientifically matched photography

We performed quantitative analysis of hair volume using high resolution scientifically matched photography, carried out in a controlled environment in an independent laboratory (AMA Laboratories, New City NY, USA). Photographs were converted to a format which allowed quantitation of hair density using PhotoGrammetrix®. The procedure reduced full color pictures to a two color image, with the scalp showing black and hair showing green. Pixel density was then quantified and was reflective of hair density. The results of the analysis of 3 male and 3 female participants from the 0.095% MTP3 group are shown in Supplementary Fig. 5. All participants had an increase in the density of hair at day 56, and 5 out of 6 participants continued to show an increase in hair density up to day 112. Due to large differences in baseline, we analyzed the increase in hair density as a function of baseline (Fig.8 main text). Both groups had improvements over baseline at both time points as described above, and we observed a significant increase in hair density with respect to baseline at day 112 compared to day 56 ($p = 0.03$, Fig 8 in main body), further highlighting the continued improvement in hair density for this group of participants applying the topical formulation with 0.095% MTP3.

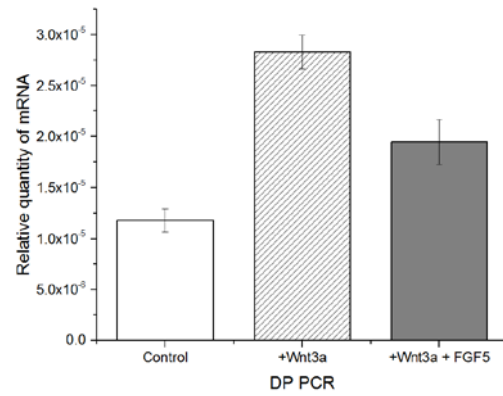
1. US FDA. Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics. 1965.

Supplementary Figure 1

A

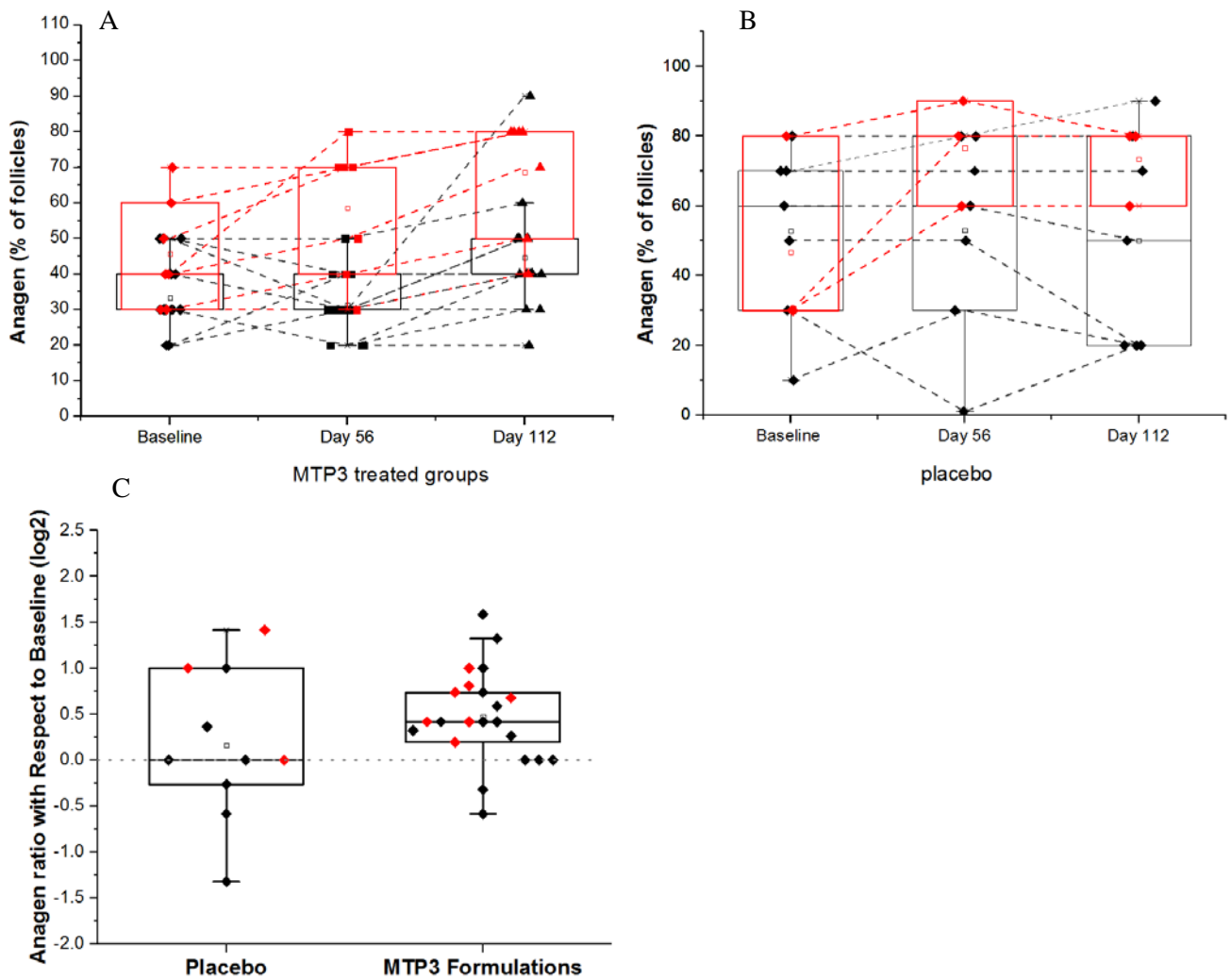


B



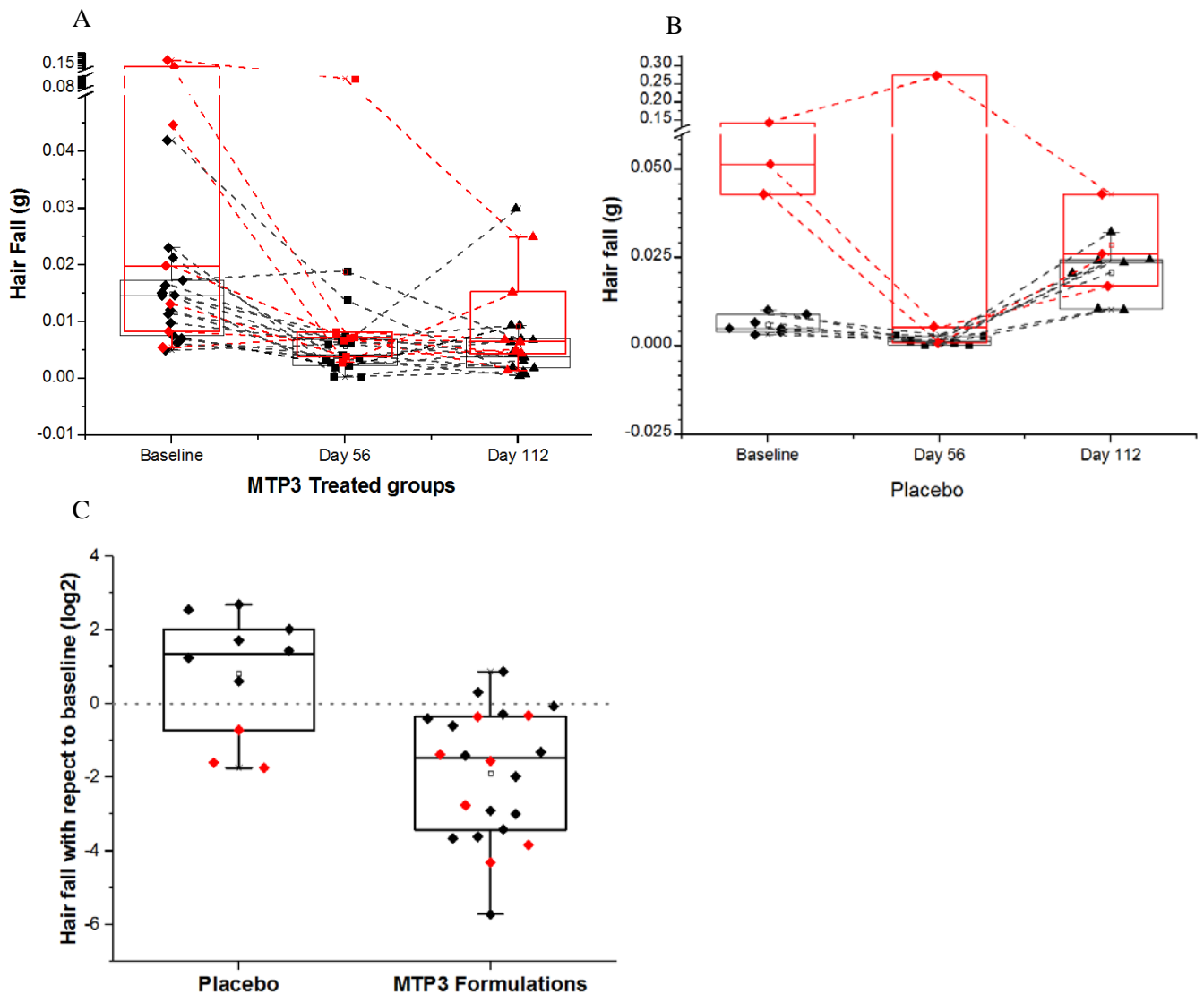
Effect of adding FGF5 to Wnt activated DP cells in culture. FGF5 added to Wnt activated DP cells reduces ALP activity (A) ($p = 0.0003$). ALP is an indicator of anagen, and a reduction of ALP is an indicator of catagen induction. Addition of FGF5 to these cells also reduces ALP transcription (B), indicating that the presence of FGF5 is having a regulatory effect. ALP mRNA levels are increased when DP cells are incubated with Wnt3a ($p = 0.0002$), subsequent treatment of Wnt3a activated cells with FGF5 results in reduced mRNA levels of ALP ($p = 0.004$)

Supplementary Figure 2



We separated the data into male (BLACK) and female (RED), and performed statistical analysis on the combined treated groups. For the AT ratio, there was a significant difference from baseline in both male ($p = 0.007$) and female ($p = 0.003$) (A), with the effect most apparent at day 112 ($p = 0.02$ and $p = 0.016$ for female and male respectively). No differences were evident in the placebo group (B). Separation into the different treatment arms also identified significant differences in female and male 0.5%MTP3 arms ($p = 0.046$ and $p = 0.049$ respectively), and trends in the female and male 0.095%MTP3 arms ($p = 0.075$ and $p = 0.054$ respectively). Effect comparisons with respect to baseline between groups (treated vs placebo) (C) suffered from low sample numbers and high variance in both the male and the female placebo group, but in the male subgroup we saw a borderline significant difference compare to placebo ($p = 0.08$), that was not seen in the whole cohort analysis. All females improved in AT ratio compared to baseline.

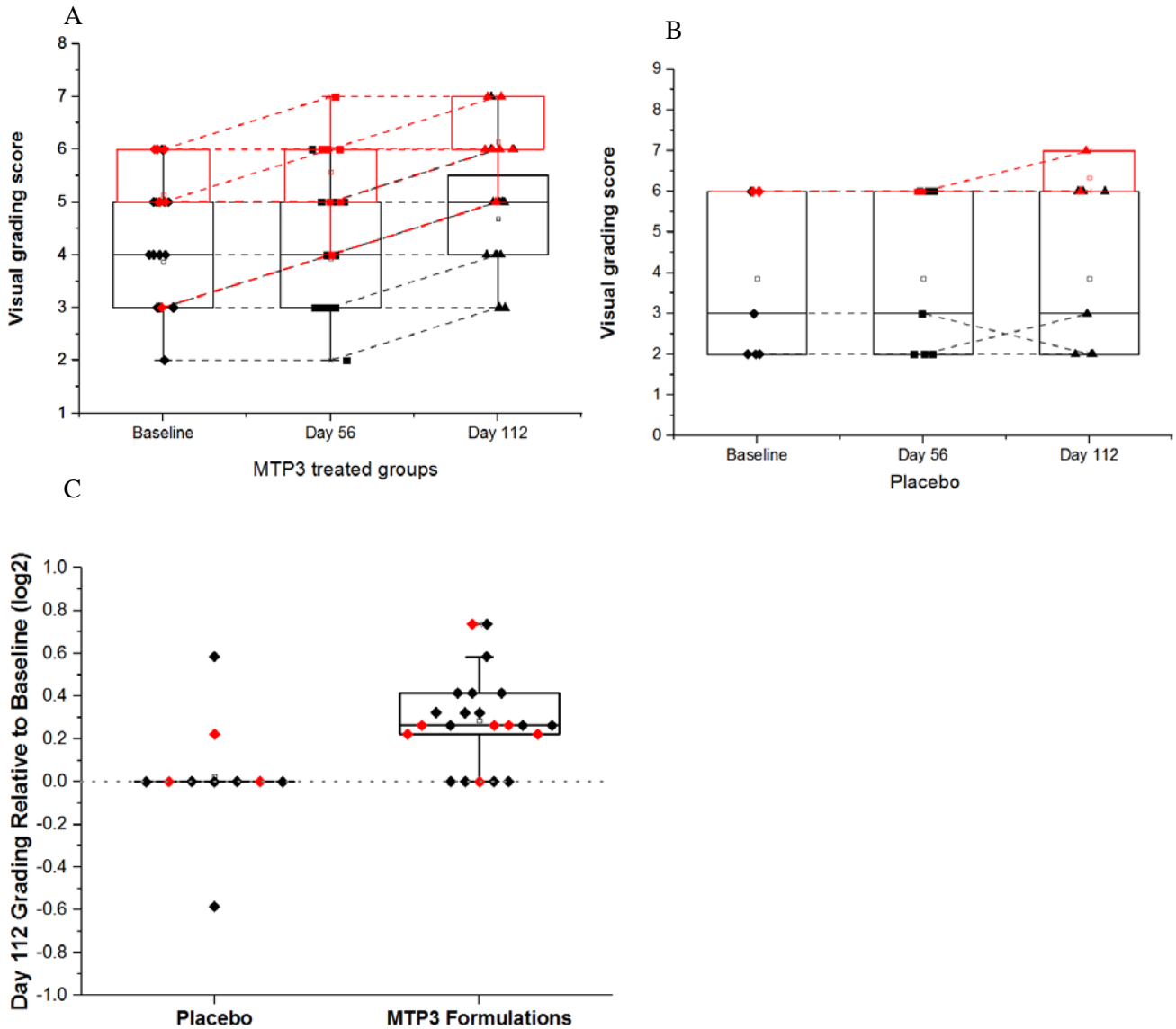
Supplementary Figure 3



We separated the data into male (BLACK) and female (RED), and performed statistical analysis on the combined treated groups (i.e. 0.095 plus 0.5% MTP3). For hair fall, a significant difference from baseline in both male ($p = 0.002$) and female ($p = 0.012$) groups was evident (A), with the effect seen at both time points but most apparent at day 112 for females ($p = 0.02$) and day 56 for males ($p = 0.001$). No differences were seen in the female placebo group, and in the male placebo group there was a significant increase in hair fall at day 112 ($p = 0.02$) (B). Separation into the different treatment arms also identified significant differences in male 0.095 %MTP3 arm ($p = 0.0076$), and trends in the female and male 0.5%MTP3 arms ($p = 0.1$ and $p = 0.075$ respectively), and in the female 0.095% MTP3 arm ($p = 0.074$). Effect comparisons with respect to baseline between groups (treated vs placebo) (C) suffered from low sample numbers which resulted in poor statistical power, but in the male subgroup we saw a significant difference in both treatment arms compared to placebo: 0.095% $p = 0.001$ and

0.5% $p = 0.006$). All treated females improved in hair fall compared to baseline and to a larger magnitude on average than placebo.

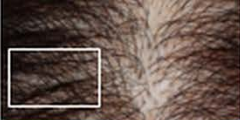


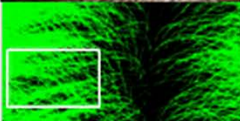
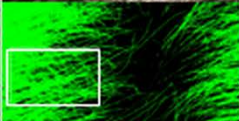
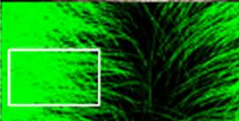
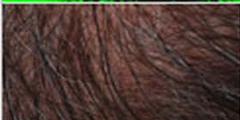
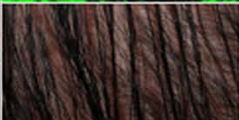

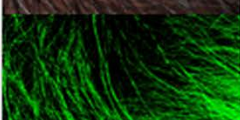
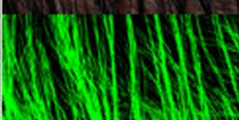
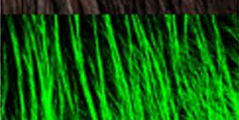
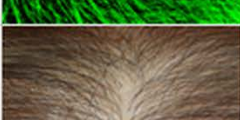
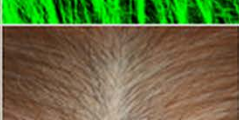
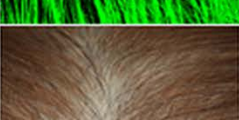


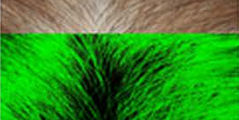
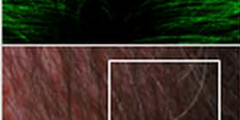


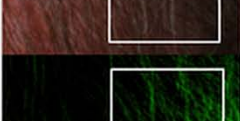
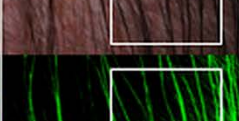
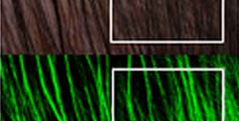
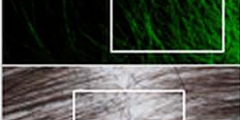
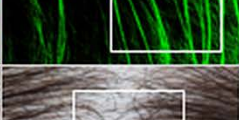
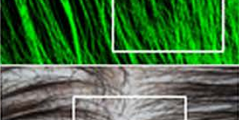
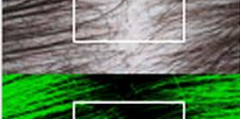
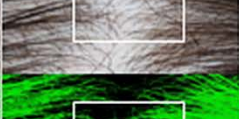
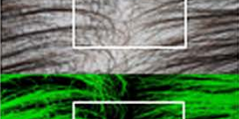
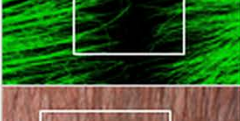
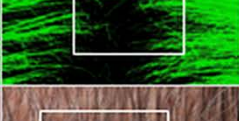




Supplementary Figure 4



We separated the data into male (BLACK) and female (RED), and performed statistical analysis on the combined treated groups. For visual grading, there was a significant difference from baseline in both male ($p = 0.002$) and female groups ($p = 0.038$), with the effect most apparent at day 112 for females ($p = 0.026$) and males ($p = 0.005$) (A). No differences were seen in the placebo groups (B). Separation into the different treatment arms also identified significant differences in female 0.5 %MTP3 arm ($p = 0.01$), and the male 0.095% MTP3 arm ($p = 0.000005$), and a borderline result for the male 0.5% MTP3 arm ($p = 0.14$). Effect comparisons with respect to baseline between groups (treated vs placebo)(C) suffered from low sample numbers which resulted in poor statistical power, but in the male subgroup

we saw significant differences in compared to placebo ($p = 0.03$) All but one treated female improved in grading compared to baseline group in which only one of three placebo females did, and we saw a trend for improvement in females compared to placebo ($p = 0.075$).

Supplementary Figure 5

			Female 1		
			Baseline	Day 56	Day 112
					
			54218 PX	55488 PX	66567 PX
			100%	102.3%	122.7%
					
			Male 1		
			Baseline	Day 56	Day 112
					
			351738 PX	659570 PX	656159 PX
			100%	187.5%	186.5%
					
			Female 2		
			Baseline	Day 56	Day 112
					
			188264 PX	372079 PX	521528 PX
			100%	197.6%	277.0%
					
			Male 2		
			Baseline	Day 56	Day 112
					
			5829 PX	63009 PX	140574 PX
			100%	1081%	2411.6%
					
			Female 3		
			Baseline	Day 56	Day 112
					
			19526 PX	27583 PX	36949 PX
			100%	141.3%	189.2%
					
			Male 3		
			Baseline	Day 56	Day 112
					
			11219 PX	84552 PX	112717 PX
			100%	753.65%	1004.7%
					

Supplementary Figure 5. Scientifically matched photography of participants from the 0.095% MTP3 treatment arm. There was an increase in pixels (PX) from baseline to day 112, and a continued increase

day 0 - day 56 - day 112 for 5 out of 6 participants (illustrated in row 4). One participant, male 1, had a very slight decrease day 56-112 after a large increase between days 0-56. Those images with boxes indicate the area from which the pixel counts were taken. Images without those gates indicate entire image was used for assessment. All Males were Hamilton-Norwood scale 3. Females 2 and 3 were Ludwig scale I-3, Female 1 was Ludwig scale I-2

Safety: Repeated insult patch testing results	
Response	Explanation
0	No evidence of any effect
?	Barely perceptible, minimal faint, light pink, uniform or spotty erythema
1	Mild pink uniform erythema covering most of contact site
2	Moderate pink/red erythema visibly uniform in entire contact area
3	Marked bright red erythema with accompanying edema, petechiae or papules
4	Severe deep red erythema with vesiculation or weeping with or without edema
D	Patch eliminated due to reaction
Dc	Discontinued due to absence of subject on application date
M	Patch applied on adjacent site after strong test reaction
N/A	Score not calculated for subjects discontinued before challenge
S	Skin stained from pigment in product
T	Tan

