Supplementary data:

Applying a ceramide containing formulation accelerates skin barrier repair by modulating lipid biosynthesis

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Supplemental Table S1: List of the ceramides standards used with the corresponding supplier.

Ceramide	Supplier	Ceramide	Supplier
N(24)dS(18)	Avanti*	A(24)S(18)	Avanti*
N(18)dS(18)	Avanti*	A(R)(22)S(18)	Avanti*
N(16)dS(18)	Avanti*	A(S)(22)S(18)	Avanti*
N(24)S(18)	Evonik**	A(R)(16)S(18)	Avanti*
N(22)S(18)	Avanti*	A(S)(16)S(18)	Avanti*
N(20)S(18)	Avanti*	A(R)(24)P(18)	Avanti*
N(18)S(18)	Avanti*	E(18:2)O(30)S(18)	Evonik**
N(16)S(18)	Avanti*	E(18:2)O(27)S(18)	Evonik**
N(24)P(18)	Evonik**	E(18:2)O(30)P(S18)	Evonik**
N(22)P(18)	Evonik**	E(18:2)O(27)P(18)	Evonik**
N(16)P(18)	Evonik**	N(24deu)S(18)	Evonik**

* Avanti polar lipids (Alabaster AL, USA)

** Evonik Industries (Essen, Germany)

Supplemental Table S2: Ingredients of the formulation

Super sterol esters Crodalan SWL	(Croda, UK)	49.72% w/w
Crodamol GTCC miglyol	(Croda, UK)	36.98% w/w
Squalane	(Galderma)	6.63% w/w
Cholesterol USP/NF	(Croda, UK)	3.63% w/w
Palmitic acid (FFAC16:0)	(Emery)	0.83% w/w
Stearic acid (FFA 18:0))	(Berg + Schmidt)	0.10% w/w
Ceramide NS (C40)	(Evonik, Germany)	1.18% w/w
Ceramide EOS (C66)	(Evonik, Germany)	0.83% w/w
Oxynex LM	(Merck, Germany)	0.10% w/w
[anti-oxidant; protection of Ceramide EOS] containing:		
DL-γ -Tocopherol (Vitamin E)	0.025% w/w	
Lecithin	0.025% w/w	
Ascorbyl palmitate	0.020% w/w	
Glycerin monostearate	0.020% w/w	
Glycerin monooleate	0.0025%w/w	
Citric acid	0.0025%w/w	

Supplemental Table S3: Correlations to amount of barrier disruption

The table depict the Pearson r and p-value of the correlation between the TEWL directly after barrier disruption and AUC of the barrier recovery curve. It also contains the correlations between the difference in TEWLs directly after barrier disruption and the ΔT at different recovery %.

	TEWL start vs. AUC	TEWL Dif vs. 5%	TEWL Dif vs. 10%	TEWL Dif vs. 15%	TEWL Dif vs. 20%	TEWL Dif vs. 25%	TEWL Dif vs. 30%	TEWL Dif vs. 35%	TEWL Dif vs. 40%	TEWL Dif vs. 45%
Pearson r	-0.26	0.05	0.15	0.20	0.17	-0.15	-0.26	-0.27	-0.34	-0.43
p-value	0.17	0.85	0.60	0.47	0.54	0.60	0.35	0.34	0.22	0.11
	TEWL Dif vs. 50%	TEWL Dif vs. 55%	TEWL Dif vs. 60%	TEWL Dif vs. 65%	TEWL Dif vs. 70%	TEWL Dif vs. 75%	TEWL Dif vs. 80%	TEWL Dif vs. 85%	TEWL Dif vs. 90%	TEWL Dif vs. 95%
Pearson r	-0.50	-0.40	-0.34	-0.34	-0.22	-0.09	-0.06	-0.23	-0.11	0.86
p-value	0.06	0.14	0.21	0.22	0.44	0.76	0.82	0.42	0.71	0.06

Supplemental Table 4: LLM of the formulation ceramides:

The table shows the output of the LLM of the logarithm of the amount of NS C40 (ng/SQ) and EOS C66 (ng/SQ). The effect sizes are the changes to the intercept (control site tape-strips 5-8), due to the depth, stripping, and treatment. The second part of the table shows the amount in ng/SQ. This was calculated by exponentiation of the mean log value outputted by the LLM.

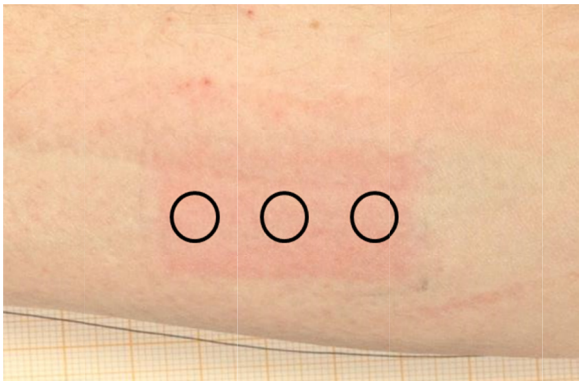
		Log NS C40		Log EOS C66	
		Estimate	p-value	Estimate	p-value
	Intercept	1.575	< 0.001	1.590	< 0.001
Effect size	Depth	-0.135	0.011	-0.135	0.011
	Stripped	+0.204	< 0.001	+0.356	< 0.001
	Treated	+0.580	< 0.001	+0.697	< 0.001
Interactions	Depth * Treated	-0.207	0.006	-0.200	0.006
	Stripped *Treated	-0.152	0.040	-0.260	0.040

Calculated Amount	NS C40		EOS C66	
ng/SQ	Depth 5-8	Depth 17-20	Depth 5-8	Depth 17-20
Control	37.61	27.57	38.93	28.54
Stripped	60.13	44.09	88.32	64.76
Treated	142.92	65.12	193.64	89.64
Stripped Treated	160.87	73.29	241.68	111.87

Supplemental Table S5: LLM of the effects on the lateral lipid packing

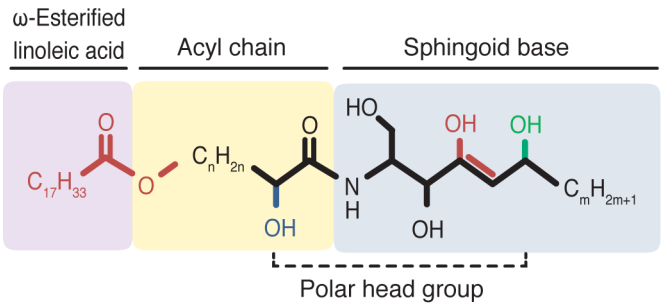
The output of linear mixed models for the FWHM of the scissoring peak. The intercept is the estimated amount at the control site at a depth of 2-10 tape-strips. Estimates are in cm^{-1}. The effect size is the amount in cm^{-1} with which the variable changes the intercept. Significance levels of all effect sizes and interactions are given.

		FWHM	
		Estimate	p-value
	Intercept	11.86	< 0.001
Effect size	Stripped	-0.37	0.19
	Treated	-0.43	0.08
	Depth 12-20	-0.59	0.02
Interactions	Stripped *Treated	0.44	0.23
	Stripped *Depth 12-20	0.67	0.07
	Treated *Depth 12-20	0.47	0.17
	Stripped *Treated *Depth 12-20	-1.32	0.01



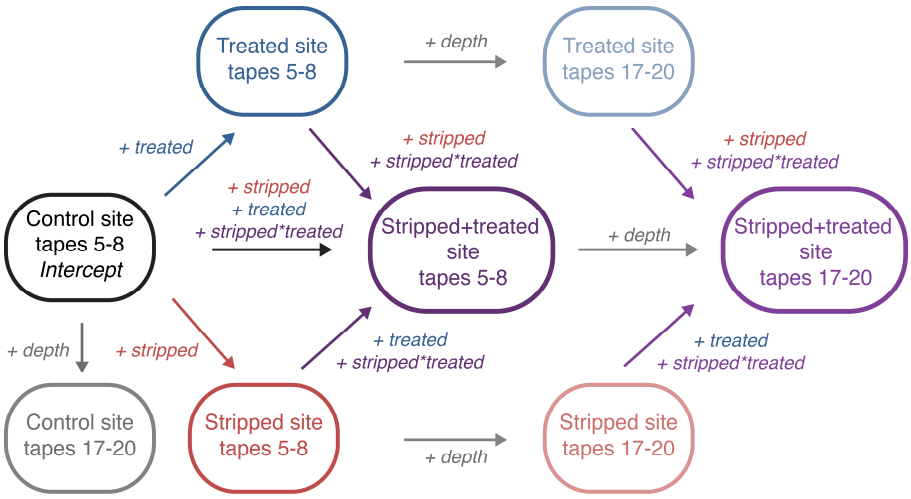
Supplemental Figure S1: Indication of TEWL measurement sites

The TEWL measurements at the sites were performed at three sections of the sites. Here the stripped site is shown which can be identified by the erythroderma. TEWL measurements were performed at the sections indicated with black circles. At every time-point TEWL was measured at the right, middle, and left section.



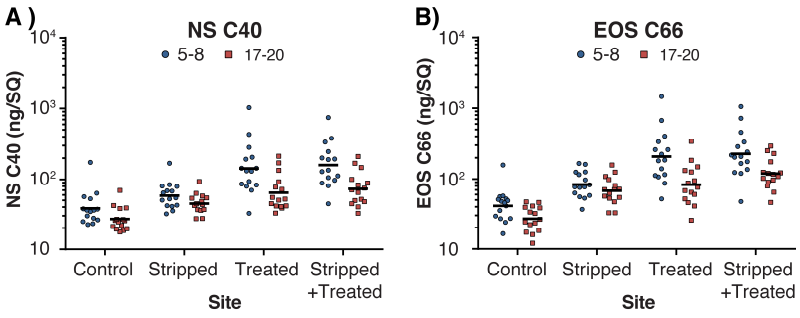
Supplemental Figure S2: General structure of a ceramide

A ceramide can be composed of three parts which determine the subclass of the ceramide. The polar head group consists of a sphingoid base and an acyl chain. There are 4 different sphingoid bases in human SC: the sphingoid (S), a dihydro-sphingoid (dS), a 4-hydroxy or phyto-sphingoid (P), and a 6-hydroxy-sphinogoid (H). The acyl chain of human SC occurs in 3 different forms. A non-hydroxyl acyl chain (N), an α-hydroxy-acyl, and a Ω-hydroxy-acyl chain (O). In SC a specific type of ceramide occurs where the Ω-hydroxyl group becomes esterified with a linoleic acid called the EO subclass of ceramides. Together these form 16 different subclasses. Both the acyl and sphingoid chain can vary in carbon chain length.



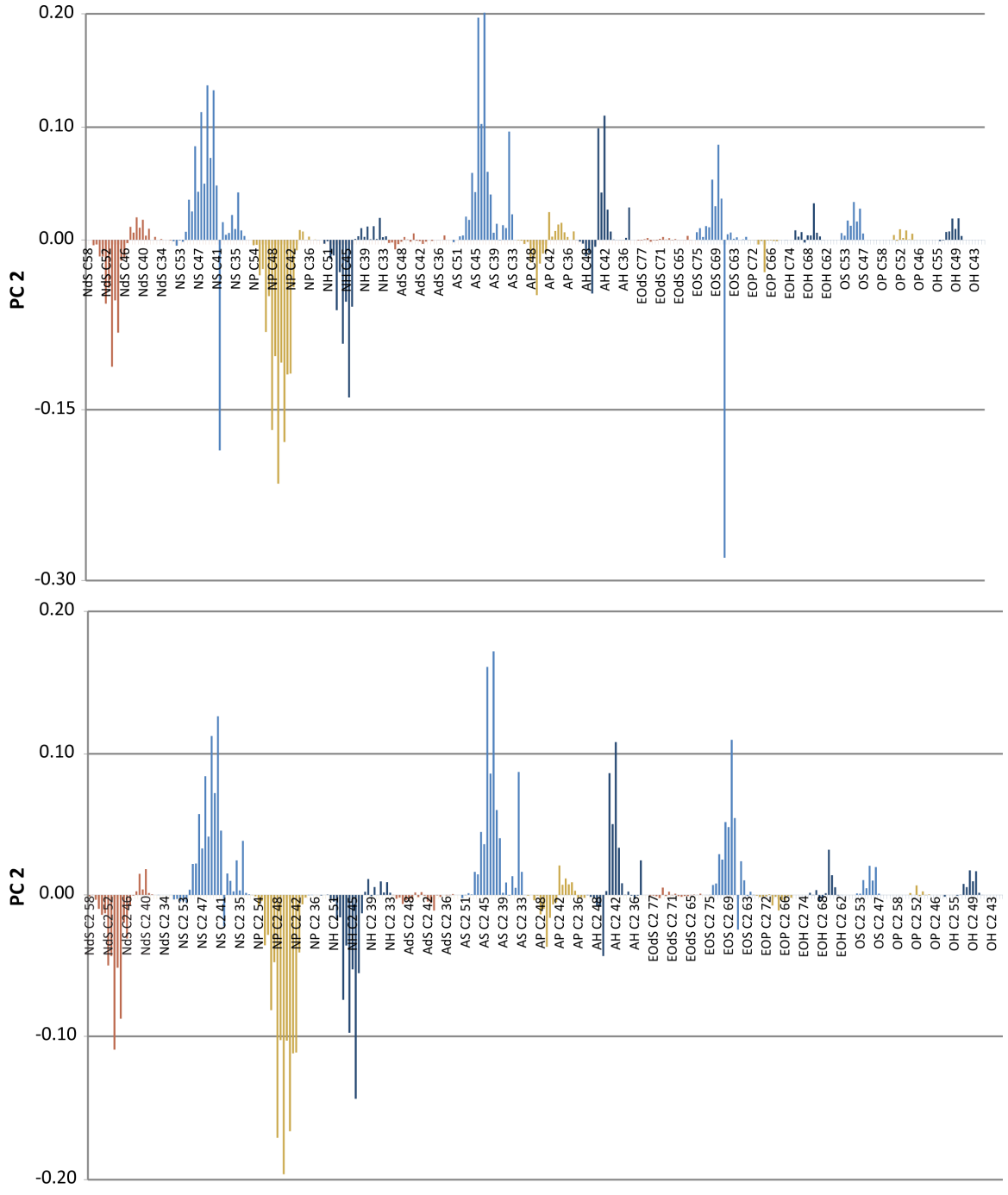
Supplemental Figure S3: Linear mixed modelling

The LLMs used here compare the effects of treatment, stripping, and depth to the control site of the subject. Using subject as a random factor, gives each subjects control site (starting point) a different value, so it can start at any random point. It then determines the effect size of the fixed factors (treatment, stripping, and depth). 'Fixed' means that the model will say that all these effects have the same size for each subject and for each time this effect occurs, so also the effect at treatment at the stripped site (difference between stripped and stripped+treated). Because a stripped site can respond differently to treatment, an interaction term is added to the model, in this case stripped*treated. In this model the interaction is the effect size of treatment additional to the effects of stripping and treatment (the fixed factors). For most data we were interested in the effect of treatment on the stripped sites. This is the sum of the effect sizes of treatment and stripped*treated (illustrated above as the arrow from stripped site to Stripped+treated site). In the schematic above it is shown which steps are required to calculate the predicted values of the mixed model. The effect sizes are indicated in italic. Here, we were not interested in the mean predicted by the model but in the effect sizes of the different factors, as these calculate how the experimental conditions changed and if these were significant.



Supplemental Figure S4: LLM of the formulation ceramides

Figure A,B : Plots of the amounts of NS C40 and EOS C66 (log (ng/SQ)) in all quantified samples. Lines are geometric means. Statistical outputs of the LMMs are reported in supplementary Table S4.

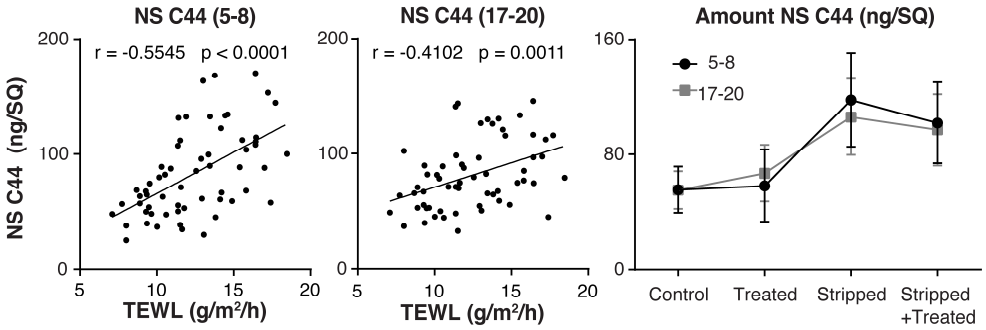


Supplemental Figure S5: PC2 plot

The two graphs depict the scores/correlation of all ceramides to PC2. The first graph shows all ceramides detected in samples 5-8 and the second graph all ceramides detected in samples 17-20. Subclasses were given different colors. High positive scores indicate a correlation to the stripped sites and a high negative score to the control sites.

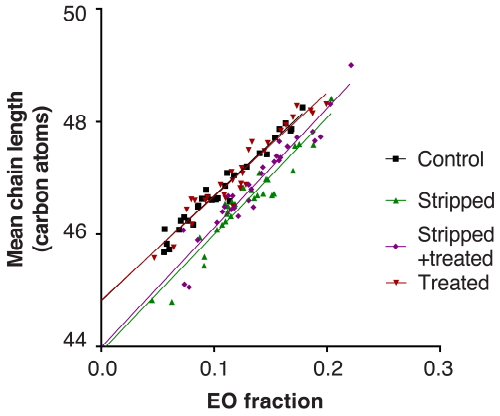
Supplemental Figure S6: TEWL at day 16

The TEWL ($g/m^2/h$) of all 4 sites after 16 days of treatment and/or barrier recovery. The red line is the mean value and the significance levels that are indicated were tested with a 1-way ANOVA with Bonferroni post-test.



Supplemental Figure S7: The amount of NS C44 and the correlation to the TEWL

Pearson r and the corresponding p-value are indicated above the data. The amount in ng/SQ of NS C44 at the 4 site and at both depths (5-8 and 17-20 tape-strips).



Supplemental Figure 8: correlation between mean chain length and EO fraction

The correlations between the mean chain length and the fraction of EO ceramides are depicted per site. Each site showed significant Pearson correlations coefficients: control (0.9817), stripped (0.9565), treated (0.9600), and stripped+treated (0.9568).