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Supplemental Information

Skin Microbiome Modulates the Effect of Ultraviolet Radiation on Cellular Response and Immune Function

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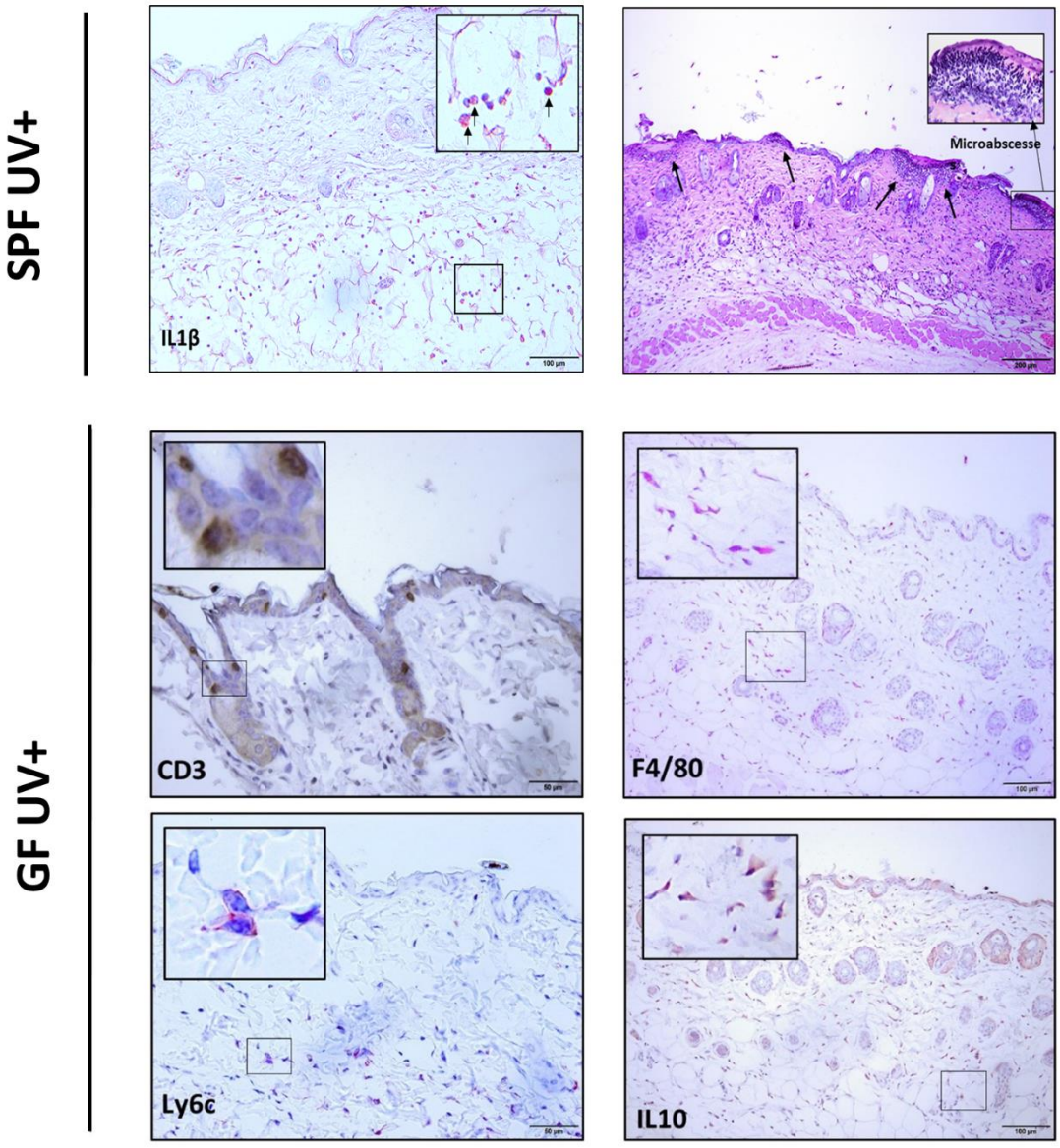

Figure S1. Representative images of quantitative analysis of neutrophilic microabscess and immunohistochemical stainings. Related to Figure 3.

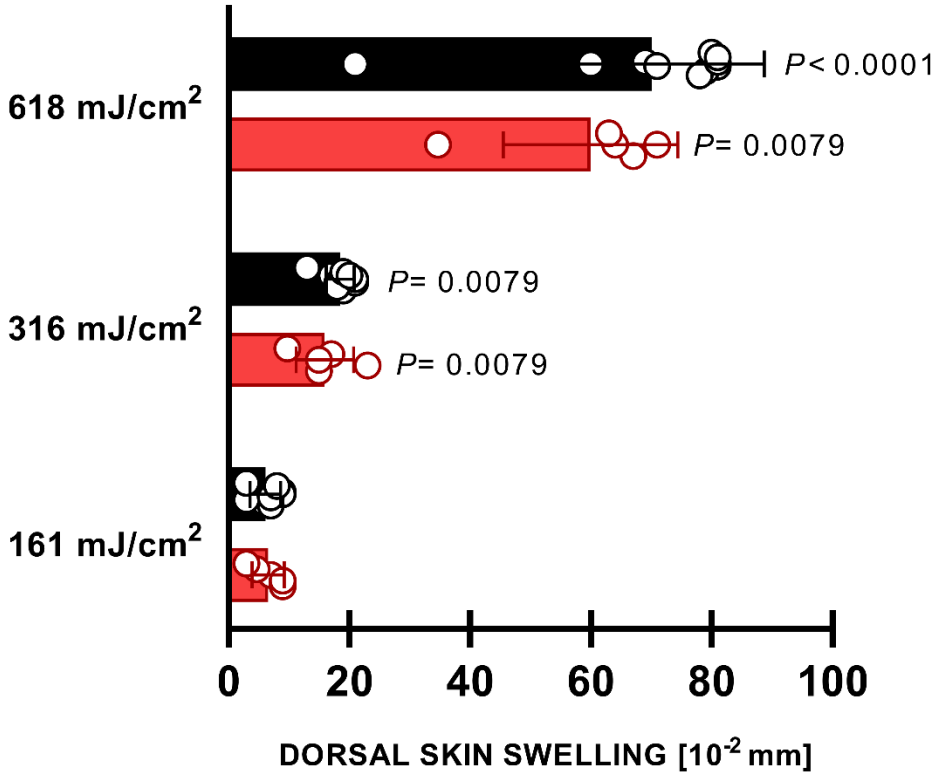

Figure S2. Minimal inflammatory dose (MID). Related to Figure 1. Data shown represent mean ± SEM. N=5 for GF and N=10 for SPF group. Stastistical analysis was performed for each dose vs 161 mJ/cm^2. The minimum inflammatory dose was 316 mJ/cm^2, as determined in a separate experiment in dose response studies (data not shown). No significance in skin swelling was observed between GF and SPF groups at any dose. *P*-value is determined by Mann-Whitney test.

REVIGO treemap of Biological Process GO terms enriched in differentially expressed genes in GF and SPF mice

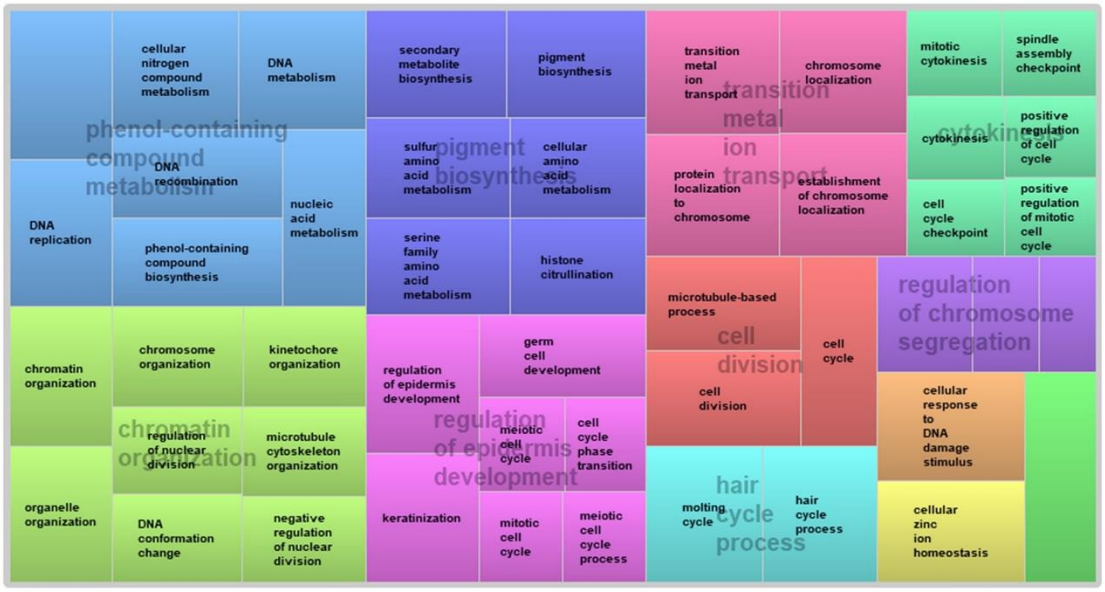

Figure S3. Biological process of differentially expressed genes in unexposed GF vs SPF skin. Related to Figure 4.

Table S1. Differential expression of AMPs and other innate immune response genes in GF and SPF skin after UV-B irradiation. Related to Figure 6.

Gene	GF UV+ / GF UV-		SPF UV+ / SPF UV-	
	p-value	Fold change	p-value	Fold change
Antimicrobial Peptides				
S100a7a	3.199E-06	**-13.00**	0.0192862	**1.93**
S100a3	1.288E-06	**-31.34**	0.0426101	**1.91**
S100a4	1.202E-07	**4.12**	0.0019311	**1.44**
S100a8	0.303135	**1.39**	8.363E-05	**9.01**
S100a9	0.0046878	**2.65**	0.0001055	**5.90**
Defb8	0.459292	**-1.40**	0.0011003	**-8.45**
Defb3	0.0713878	**1.31**	0.0005708	**2.05**
Defb14	2.649E-05	**4.81**	7.909E-06	**6.36**
Defb6	0.0007831	**2.14**	0.464353	**-1.12**
Defb14	2.649E-05	**4.81**	7.909E-06	**6.36**
Rnasel	0.0002004	**2.20**	0.0862219	**1.27**
Rnaset2b	1.404E-07	**-2.42**	0.535204	**-1.03**
Rnaset2a	1.103E-07	**-2.42**	0.419315	**-1.04**
Rnase1	0.0003325	**-2.55**	0.0010736	**-2.19**
Toll-like receptors				
Tlr13	0.0282753	**2.77**	0.0018269	**5.72**
Tlr1	0.0646121	**1.70**	0.0123469	**2.22**
Serotonin signalling genes				
Htr2a	0.0006754	**2.05**	0.0725447	**1.32**
SLC6A4	0.0028866	**1.29**	2.125E-06	**-2.05**

Table S2. Canonical pathway analysis of UV-B-exposed GF skin using ingenuity pathway analysis. Related to Figure 4.

Ingenuity Canonical Pathways	Total genes	p-value	z-score
TREM1 Signaling	18/69	3.16E-06	**4.24**
Role of NFAT in Regulation of the Immune Response	22/170	1.15E-02	**3.57**
PI3K Signaling in B Lymphocytes	16/124	2.88E-02	**3.5**
Dendritic Cell Maturation	27/159	7.94E-05	**3.4**
Leukocyte Extravasation Signaling	23/202	3.80E-02	**3.27**
IL-6 Signaling	24/124	2.24E-05	**3.12**
FcÎ³ Receptor-mediated Phagocytosis in Macrophages and Monocytes	13/90	2.00E-02	**3.05**
NF-ÎºB Signaling	25/168	1.10E-03	**3.00**
CD28 Signaling in T Helper Cells	18/118	3.98E-03	**3.00**
Toll-like Receptor Signaling	13/70	2.40E-03	**2.71**
iCOS-iCOSL Signaling in T Helper Cells	18/110	1.82E-03	**2.66**
PKCÎ¸ Signaling in T Lymphocytes	17/120	1.05E-02	**2.66**
Retinoic acid Mediated Apoptosis Signaling	8/43	1.55E-02	**2.64**
Acute Phase Response Signaling	25/157	3.98E-04	**2.55**
Actin Cytoskeleton Signaling	24/217	4.68E-02	**2.50**
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	20/127	1.66E-03	**2.49**
UVA-Induced MAPK Signaling	14/99	1.95E-02	**2.49**
Interferon Signaling	6/30	2.45E-02	**2.44**
Oncostatin M Signaling	6/33	3.80E-02	**2.44**
NF-ÎºB Activation by Viruses	13/86	1.41E-02	**2.30**
Death Receptor Signaling	17/88	3.47E-04	**2.18**
Role of IL-17F in Allergic Inflammatory Airway Diseases	9/37	1.62E-03	**2.12**
PCP pathway	8/62	0.04677	**-2.12**
Basal Cell Carcinoma Signaling	12/69	0.00603	**-2.64**
PPAR Signaling	18/89	0.00013	**-3.30**
LXR/RXR Activation	19/108	0.00055	**-3.63**

Table S3. Canonical pathway analysis of UV-B-exposed SPF skin using ingenuity pathway analysis. Related to Figure 4.

Ingenuity Canonical Pathways	Total genes	p-value	z-score
Acute Phase Response Signaling	21/157	1.6218E-07	**3.15**
TREM1 Signaling	12/69	4.7863E-06	**2.88**
LPS/IL-1 Mediated Inhibition of RXR Function	21/197	6.9183E-06	**2.33**
Toll-like Receptor Signaling	12/70	5.6234E-06	**2.12**
Cholecystokinin/Gastrin-mediated Signaling	8/97	0.02137962	**2.12**
Inflammasome pathway	4/19	0.00389045	**2.00**
Oncostatin M Signaling	4/33	0.02818383	**2.00**
MIF Regulation of Innate Immunity	4/38	0.04466836	**2.00**
LXR/RXR Activation	19/108	6.9183E-09	**-2.66**
PPAR Signaling	10/89	0.0011749	**-3.16**

Table S4. Primer pairs used in the study. Related to Figure 6.

Gene	Sequence (5'->3')
IL1F8	ACAAAAAGCCTTTCTGTTCTATCAT CCATGTTGGATTTACTTCTCAGACT
IL1F9	AGAGTAACCCCAGTCAGCGTG AGGGTGGTGGTACAAATCCAA
IL1β	CAG GCA GGC AGT ATC ACT CA AGG TGC TCA TGT CCT CAT CC
IL4RA	TGACCTACAAGGAACCCAGGC CTCGGCGCACTGACCCATCT
IL6	TGAACAACGATGATGCACTTGCAGA TCTGTATCTCTCTGAAGGACTCTGGCT
IL10	CCA AGC CTT ATC GGA AAT GA CCA AGC CTT ATC GGA AAT GA
IL10RA	CCCATTCCTCGTCACGATCT TTTCCAGTGGAGGATGTGCT
IL16	AACCGAGGACAGGAACCACT CTTGAGAGATTTGCCATTGA
IL18RAP	TGCAATGAAGCGGCATCTGT CCGGTGATTCTGTTCAGGCT
IL20RA	AAGTCGAGAAGAACGTGGTC GGGTGTTTTTCCTTGCCAAC
IL20RB	AATGCTCACCGACCAAAAGT AGGACAGTTGCATTTCGGTT
IL31RA	TTCAAGACATTGTCAATCAGTGTG GTCACTGTTTTGATGCTAAGTAGAAGA
IL33	GAT GGG AAG AAG CTG ATG GTG TTG TGA AGG ACG AAG AAG GC-3
IL34	ACTCAGAGTGGCCAACATCACAAG ATTGAGACTCACCAAGACCCACAG
CXCL2	AGGGCGGTCAAAAAGTTTGC CGAGGCACATCAGGTACGAT
CXCL16	AAA CAT TTG CCT CAA GCC AGT GTT TCT CAT TTG CCT CAG CCT
CCL2	TTAAAAACCTGGATCGGAACCAA GCATTAGCTTCAGATTTACGGGT
CCL3	ACTGCCTGCTGCTTCTCCTACA

	AGGAAAATGACACCTGGCTGG
CCR1	TTAGCTTCCATGCCTGCCTTATA TCCACTGCTTCAGGCTCTTGT
CXCR2	CAC CGA TGT CTA CCT GCT GA CAC AGG GTT GAG CCA AAA GT
CCL12	GTCCTCAGGTATTGGCTGGA GGGTCAGCACAGATCTCCTT
S100A3	GTGAGTTCCGGGAGTGTGAC TGGCAGTAGAGACAGAGGCT
S100A4	GCTGCCCAGATAAGGAACCC TGCGAAGAAGCCAGAGTAAGG
S100A8	AAATCACCATGCCCTCTACAAG CCCACTTTTATCACCATCGCAA
S100A9	GTTGATCTTTGCCTGTCATGAG AGCCATTCCCTTTAGACTTGG
S100A10	CGCCCTCTGTACCCGCC CAGCCAGAGGGTCCTTTT GA
DEFB6	TGGTGATGCTGTCTCCACTT CATGAACGCTGGCATGAG
DEFB14	GTA TTC CTC ATC TTG TTC TTG G AAG TAC AGC ACA CCG GCC AC
NE	CTTTGAGAACGGCTTTGA CC CACATTGAGCTCTTGGAG CA
House keeping genes	
18s	CCTGCGGCTTAATTTGACTC AACTAAGAACGGCCATGCAC
Ywhaz	AACAGCTTTCGATGAAGCCAT TGGGTATCCGATGTCCACA

Transparent Methods

Animals

Cesarean-born, axenic 4-8 weeks old female C57BL/6 mice were derived at the Core Facility for Germfree Research (CFGR) at Karolinska Institutet, Stockholm; housed and bred in a sterile environment, and regularly monitored to ensure their germ-free status. All short-term experiments with axenic mice were conducted in ISOcage positive cages from Tecniplast, using the appropriate standard operating procedures (SOPs) at CFGR, Karolinska Institutet. Protocols involving the use of germ-free animals were approved by the Regional Animal Research Ethical Board, Stockholm, Sweden (Stockholms norra djurförsöksetiska nämnd), following proceedings described in EU legislation (Council Directive 86/609/EEC). Animal husbandry was done in accordance with Karolinska Institutet guidelines and approved by the above-mentioned board (Ref: N190/15). All animal care and treatment protocols were also approved by the Austrian Federal Ministry for Science and Research, through protocol number BMWFW-66.010/0137-WF/V/3b/2014. Animal experiments adhered to 3R (replacement, reduction, and refinement) policy to ensure the use of minimum numbers of animals to maximize data mining.

UV-B source and irradiation

The backs of the mice were shaved using an electric clipper 24 hours before UV-B exposure. UV radiation was performed using a Waldmann 236 light source (Waldmann Medizintechnik, Villingen-Schwenningen, Germany), equipped with two Waldmann UV6 fluorescent tubes (emission range 280–360 nm; peak, 320 nm) and positioned upside down on top of the cage. The mean UVB irradiance of the lamp was 1.9 mW/cm^2, as measured by a Waldmann UV photometer with a UV6 detector head appropriate to the radiation device. Each mouse in the CHS experiments was administered a UV-B radiation dose of 618 mJ/cm^2, being equal to 2 (minimal inflammatory doses) MID (Fig. S2) (average time of exposure 5 min, 42 sec). During the UV exposure, ears of all the mice in CHS experiments were shielded by covering with black electric tape. All the procedures were performed under sterile conditions in a laminar air flow unit.

Minimal inflammatory dose (MID)

To determine the effects of the minimal inflammatory dose (Schweintzger et al., 2015; Wolf et al., 1993) and mulitples of it, 5 GF and 10 SPF mice were irradiated on the shaved dorsal skin and macroscopic skin thickness was measured immediately before and 24 hrs after UV exposure by spring-loaded engineer's micrometer and skin swelling was calculated.

Contact hypersensitivity assay

Groups of mice were sensitized (3 days after UV exposure) by applying 50 µl of freshly prepared 0.5% 1-fluoro-2,4-dinitrobenzene (DNFB; Sigma #D1529) in acetone to the shaved abdomen (Fig 1A). Five days later, the ears were challenged with 20 µl of freshly prepared 0.25% DNFB in acetone. Ear thickness before and 24 hours after the challenge was measured using a spring-loaded engineer's micrometer (Mitutoyo) to calculate ear swelling, and the following formula was used for calculating percent suppression of CHS:

$$\boldsymbol{CHS = \left[1 - \left(\frac{Ear\ swelling\ of\ UV+\ senstized+mice}{Ear\ swelling\ of\ UV-\ sensitized+mice}\right) * 100\right].}$$

Skin sample collection

Mice were sacrificed 24 hours after UV exposure (Fig 2A) and skin samples were then collected, fixed in 4% formaldehyde, and paraffin-embedded for histological and immunohistochemical analysis. Parts of the skin samples were snap frozen in liquid nitrogen and stored at -80º C until RNA isolation.

RNA extraction, microarray, and data analysis

Total RNA was extracted from the whole skin on the same location on the dorsal skin across all the mice. RNA was extracted using the miRNeasy Kit (Qiagen, Hilden, Germany; Cat. No. 217004) including DNase treatment steps on the column according to the protocol. We obtained RNA quality of a RIN between 5 and 6 (checked on the BioAnalyzer BA2100 (Agilent; Foster City, CA; Cat.No. 5065-4476)). GeneChip® Mouse Gene 2.0 ST Arrays (Affymetrix; Santa Clara, CA; Cat No. 902118) were used for the whole transcript with 500 ng of the total RNA as input . The protocol was followed according to the manual. The amplified cDNA was analyzed using the BioAnalyzer BA2100 (Agilent, Foster City,

CA) and RNA 6000 Nano LabChip (Agilent; Foster City, CA; Cat.No. 5065-4476). The given fragment size of < 2000 nt over all samples was satisfactory for ss-cDNA synthesis, fragmentation, and labeling according to the manual. We hybridized 18 hours at 45°C as suggested in the manual while rotating in the hybridization oven. Washing and staining (GeneChip® HT hybridization, Wash, and Stain Kit; Affymetrix, Santa Clara, CA; Cat No. 900720) were done with the Affymetrix Genechip® Fluidics Station 450 according to the manual (protocol on Fluidics Station: FS450_0002). Arrays were then scanned with the Affymetrix GeneChip scanner GCS3000. For evaluation of the hybridization controls and pre-analysis, Affymetrix Expression Console software version 1.3.1. was used. Raw data are available at the Gene Expression Omnibus (GEO; accession number GSE117359). Heat maps were constructed using heat mapper (http://www.heatmapper.ca/) (Babicki et al., 2016). We used the online tool GeneMANIA (https://genemania.org/) (Warde-Farley et al., 2010) to predict the interactions and functional association of the upstream regulator genes. Statistical analysis was done using Partek Software v.6.6 (Partek Inc, St Louis, MO). CEL files with the probe intensity data are imported using the robust multi-chip average (RMA) algorithm. This included background correction, quantile normalization across all arrays, and median polished summarization based on log-transformed expression values.

cDNA synthesis and qPCR

Total RNA (1 µg) extracted for microarray analysis was used to prepare cDNA using the Script cDNA synthesis kit (#1708890; Bio-Rad Laboratories). Reverse transcription (RT) was then performed on the 1 µl RNA sample by adding iScript reagents including 4 µl 5x iScript reaction mix, 1 µl iScript reverse transcriptase, and nuclease-free water to a reaction volume of 20 µl. The reaction was incubated for priming at 25°C for 5 min, RT at 42°C for 30 min, and RT inactivation at 85°C for 5 min. cDNA diluted 1:5 was used for qPCR. PCR was performed in 384-well Hard-Shell® PCR plates (#HSP3805, Bio-Rad Laboratories) using 1 µl of cDNA, 5 µl of iTaq ™ universal SYBR® Green Supermix (#1725121, Bio-Rad Laboratories), 1 µl 10 pM forward primer, 1 µl 10 pM reverse primer, and sufficient nuclease-free water to reach a total reaction volume of 10 µl. mRNA was then quantified using quantitative PCR on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). Primer pairs used are listed in Table S4. Expression of mRNA was analyzed using the change-in-cycle-threshold (ΔΔCT) method.

Histological analysis

Paraffin-embedded skin samples were sectioned (3.5 µm) and stained with hematoxylin and eosin (H&E). Cellular infiltrate in the dermis was quantified using an ocular grid with area coverage of 0.25 mm^2 at 5 randomly selected sites per sample. Epidermal thickness and epidermal layers were determined at 5 randomly selected locations per H&E sample under a microscope at 40× magnification. Sunburn cells (as defined as cells having pyknotic nucleus and eosinophilic cytoplasm) were counted in interfollicular epidermis in at least 10 random fields at a magnification of 10x. All measurements were performed in blinded fashion. Finally, the results were averaged per mouse and per treatment group for the statistical analysis. Images of stainings were acquired with a DP71 digital camera (Olympus, Vienna, Austria), attached to an Olympus BX51 microscope.

Immunohistochemistry

FFPE tissue sections (3.5 µm) were deparaffinized and rehydrated for immunohistochemical staining. Slides with tissues sections were incubated for heat-induced antigen retrieval in Dako Target Retrieval Solution Citrate pH 6.0 (Dako S2369) or Dako Target Retrieval Solution pH 9.0 (Dako S2367) for 30 min in a steamer. The staining was then performed manually at 4°C by antibody incubation using the Dako REAL™ Detection System, Peroxidase/AEC, and antibodies directed against anti-CD3 (1:200; #ab16669, Abcam, Cambridge), anti-Ly6c (1:500, ab15627, Abcam, Cambridge), anti-IL1 beta (1:200, ab9722, Abcam, Cambridge) and anti-IL10 (1:400, ab189392, Abcam, Cambridge). The ImmPRESS™ HRP Anti-Rat IgG (Peroxidase) Polymer Detection Kit (MP-7444, Vector Laboratories, Burlingame, California, USA) was used as secondary antibody for anti-Ly6c and anti-IL10 antibodies. F4/80 (1:200, MA5-16630, Thermo Scientific) staining was performed using EXPOSE mouse- and rabbit-specific HRP detection IHC kits (ab80436, Abcam, Cambridge) according to the manufacturer's instructions. Images of stainings were acquired with a DP71 digital camera (Olympus, Vienna, Austria), attached to an Olympus BX51 microscope.

Statistical analysis

The Mann-Whitney test was used to determine statistical significance between groups in CHS experiments and histological analyses. For microarray analyses, 1-way ANOVA with FDR < 5% was used for filtering genes. Differentially expressed genes were filtered when $P < 0.05$ and fold change was ±2. An unpaired T-test was used to determine statistical significance for qPCR analysis. 2-Way ANOVA was used to determine statistical significance for immunohistochemical quantitative analysis. The statistical analysis was performed using GraphPad Prism version 8 (GraphPad). Statistical significance was set at $P < 0.05$.

Supplemental References:

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