Supplementary material to article by X. Gu et al. "Oxidation Reduction is a Key Process for Successful Treatment of Psoriasis by Narrowband UVB Phototherapy"

Appendix S1

#### NB-UVB phototherapy

NB-UVB irradiation was administered to the whole body 2–3 times a week using a cabinet (PCL 8000, Puva Combi Light – ARKADE, Heverlee, Belgium) equipped with fluorescent lamps (UVB TL100W/01, Philips, Eindhoven, The Netherlands). Treatment comprising approximately 24 sessions was given during 2–3 months.

## Sample processing and microarray hybridisation

Samples were cut into 10 µm thick sections and placed on membrane-covered glass slides (PALM Membrane Slides, PALM Microlaser Technologies, Bernried, Germany). Laser Capture Microdissection (LCM) was then carried out using a PALM® microlaser system (PALM GmbH, Bernried, Germany). Epithelium only was used in further analysis, and RNA extracted using RNAqueous-Micro Kit from Ambion. RNA quality was confirmed by Agilent RNA 6000 Nano kit (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA). RNA with RIN value more than 6 was considered acceptable. Fifty ng of total RNA was used for cRNA production by using the TargetAmp-Nano Labeling Kit for Illumina Expression BeadChip (Epicenter Biotechnologies, Madison, WI, USA). Following the instructions for Illumina Expression BeadChip, a total of 750 ng cRNA was hybridised to Illumina HumanHT-12 v4 Expression BeadChip (Illumina Inc., San Diego, CA, USA). iScan Reader was used to image BeadChips. Images were visualised and intensity data extraction was conducted using GenomeStudio Data Analysis Software. Raw data were deposited in the Gene Expression Omnibus database and are available under the accession number GSE53431.

## Microarray data validation

Gene expression data were confirmed by quantitative real-time PCR (qRT-PCR). Two hundred ng of total RNA was used to synthesise cDNA using First-Strand cDNA Synthesis Kit (USB, Cleveland, OH, USA). Real time RT-PCR was performed using an IQ5 multicolor real-time PCR detection system with IQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Each sample was measured in duplicate and results normalised in relation to the housekeeping gene *RPL13A*. Primer information is summarised in Table SII<sup>1</sup>. Primers for TYR, TYRP1, SOX10, MITF and MC1R were from Real Time Primers (Real Time Primers, LLC, Philadelphia, PA, USA).

In order to study gene expression in non-involved normal skin from psoriasis patients, another set of cDNA samples from heal-

thy controls, non-involved psoriatic skin and lesional psoratic skin was used, and prepared from whole biopsies as described previously (13). *T*-test was conducted to compare qRT-PCR data and two-tailed *p*-value less than 0.05 was considered statistically significant. For immunohistochemistry, formalin-fixed and paraffin-embedded tissues prepared from the above-mentioned healthy controls, non-involved psoriatic skin and lesional psoratic skin were used. Immunohistochemical staining was performed using monoclonal antibodies directed against TYR, TYRP1 and MITF (Abcam, Cambridge, UK). Antibodies were used at concentrations 1:50 (TYR), 1:800 (TYRP1) and 1:10 (MITF), respectively. Staining was performed using a Ventana staining machine (Ventana Medical Systems, Tucson, AZ, USA) and reagents according to the supplier's recommendation.

#### Cell culture and stimuli

Human epidermal keratinocytes adult (HEKa, Life technologies, Carlsbad, USA) were maintained in medium 154 supplemented with human keratinocyte growth supplement (HKGS) (Life technologies) and cultured at 37°C with 5% CO<sub>2</sub>. Cells (between passage 3 to 6) were stimulated with Poly(dA:dT)/Lyovec or Poly(I:C) (1 μg/ml, Invivogen, San Diego, CA, USA) in 6-well plates. Experiments were performed in duplicate and repeated in 3 independent experiments. Cells were collected after 24 h and Trizol reagent (Life technologies) was used to isolate RNA. RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) was used for cDNA synthesis. Real time RT-PCR was performed using an IO5 multicolor real-time PCR detection system with IQ SYBR Green Supermix (Bio-Rad Laboratories). Primers used are shown in Table SII<sup>1</sup>. Statistical analysis for the effect of poly(dA:dT) or poly(I:C) stimulation was assessed by a paired-sample t-test, 2-tailed p-value < 0.05 was regarded as statistically significant.

# Western blotting

Protein extracted from cultured HEKa cells were separated on 10% ClearPAGE SDS gels (C.B.S. Scientific, San Diego, USA) and transferred to PVDF membranes using the Trans-Blot Turbo Blotting System (Bio-Rad, Hercules, USA). Blots were incubated with 1:400 diluted polyclonal anti-SPATA18 antibody (Atlas antibodies, Stockholm, Sweden). Mouse anti-actin monoclonal antibody from Millipore (Billerica, MA, USA) was used as a loading control at a dilution of 1:20,000. Polyclonal goat antirabbit immunoglobulins/HRP or polyclonal rabbit anti-mouse immunoglobulins/HRP (Dako UK Ltd, Cambridgeshire, UK) were used as secondary antibodies at a dilution of 1:50,000 and signals were detected with Amersham ECL select western blotting detection reagent (GE Healthcare, Pittsburgh, PA, USA).