*Appendix S1*

## MATERIALS AND METHODS

### P. acnes *strains and culture conditions*

*P. acnes* bacterial strains were cultured and stored as described previously in detail (10). Briefly, *P. acnes* clinical isolates *889* (type IA), *6609* (type IB) and *ATCC 11828* (type II) strains were cultured on pre-reduced Columbia agar base supplemented with 5% (v/v) bovine blood, vitamin K1 and haemin (Oxoid, UK). Bacteria were grown under anaerobic conditions (anaerobic chamber; Bactron Sheldon Man, Oregon, USA) at 37°C. A single colony forming unit (CFU) was inoculated in brain heart infusion (BHI, pH 7.4; Oxoid) broth cultures, and the samples were incubated at 37°C for 48 h. Bacterial suspensions were then pelleted by centrifugation at 2,600×g for 10 min, and the supernatant was discarded. After 3 washes in phosphate-buffered saline-ethylene diaminetetraacetic acid (PBS-EDTA) for 10 min each, the cells were harvested in 5 ml PBS-EDTA, and the cell number was estimated by measuring the optical density (OD) at 600 nm of the suspension with a spectrophotometer. According to our previous observations, optical density $OD_{600}$=2 was equivalent to $1.5 \times 10^9$ cfu/ml. The number of bacterial cells was adjusted with PBS-EDTA to $1 \times 10^9$ cfu/ml, aliquoted and stored at −80°C until further use.

### *Human cells and ethics*

Washed human erythrocytes were obtained from peripheral blood samples with the subjects' written informed consent and the approval of the Scientific and Research Ethics Committee of the Medical Research Council, Hungary. The studies were performed in accordance with the principles of the Declaration of Helsinki and its later revision.

### *Immortalized human keratinocyte culture and treatment*

For the generation of an immortalized keratinocyte cell line, normal human adult keratinocytes (NHEK) were obtained from a healthy individual undergoing routine plastic surgery at our department and transfected with the pCMV vector containing the HPV16/E6 oncogene. A stable cell line was established by continuous culturing over 70 passages before the start of our experiments (Polyánka & Szabó, in preparation).

The HPV-KER immortalized human keratinocyte cell line was cultured using a keratinocyte serum-free medium (KC-SFM, Invitrogen) supplemented with 1% of an antibiotic/antimycotic (AB/AM) solution (Sigma-Aldrich, St Louis, USA) for most of the experiments. The cells were kept under standard conditions (37°C in a humidified atmosphere containing 5% (v/v) $CO_2$) at all times.

For the bacterial treatments, HPV-KER cells were plated in AB/AM-free KC-SFM culture medium and incubated for 24 h, before co-culturing with live *P. acnes* cultures using different human cell:bacterium ratios (multiplicity of infection, MOI, refers to the number of bacteria that were added to the cell cultures during infection).

For the visualization of *P. acnes*-induced pH shifts, the colour changes of a culturing media supplemented with phenol-red dye was used. The colour of this pH indicator molecule exhibits a gradual transition from yellow to red over the pH range 6.8–8.2. Thus, the HPV-KER cells were plated in serum-free Dulbecco's Modified Eagle Medium containing 4.5 g/l glucose (DMEM-HG, LONZA, Basel, Switzerland) and supplemented with prequalified human recombinant epidermal growth factor 1-53 (EGF 1-53) and bovine pituitary extract (BPE). Bacterial treatments were performed as described above.

### *Real-time PCR (RT-PCR) analysis*

NHEK, HPV-KER and HaCaT cells were cultured in 6-well plates at a starting density of 300,000 cells/well. After 48 h, the cells were treated with the *P. acnes* 889 strain (MOI=100) in triplicate. RNA samples were collected at 3, 6, 12 and 24 h post-treatment and isolated with phenol-chloroform extraction using TRI Reagent (Molecular Research Center, Inc., Cincinnati, USA). cDNA was synthesized from 1 µg total RNA using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, USA) and the RT-PCR experiments were performed using the Universal Probe Library system (Roche, Basel, Switzerland). The mRNA expression levels were normalized to the expression of 18S rRNA gene.

### *Real-time, label-free analysis of the interaction between HPV-KER or NHEK cells and* P. acnes

Cellular properties of the control and bacterium-treated HPV-KER and NHEK cells were followed in real-time using the xCELLigence system (ACEA Biosciences, San Diego, USA), allowing the real-time, label-free monitoring of various cellular events using impedance-based measurement. HPV-KER or NHEK cells were plated at a density of 10,000 cells/well in fibronectin coated 96-well E-plates. Twenty-four hours after plating, the *P. acnes* strains were diluted in cell culture medium and the cells were treated with various amounts of the 3 bacterium strains in order to achieve MOIs of 25, 50, 100, 200 and 300. HPV-KER treatments were performed in 5, and NHEK treatments were performed in 3, technical replicates. Impedance values were measured every 60 min for 90 or 94 h, from which a dimension free cell index (Ci) was calculated. Ci tracings (mean of the technical replicates) were normalized to values recorded at the addition of the bacterium to the cultures, and the resulting nCi values were plotted. Each data-point represents mean ± standard error of the mean (SEM).

### *Trypan blue exclusion assay*

HPV-KER cells were cultured in 12-well plates at a starting density of 125,000 cells/well. After 48 h, the *P. acnes* strains were diluted in cell culture medium and the HPV-KER cells were treated with various amounts of the 3 bacterium strains (MOI 100 and 300) in triplicate. Samples were collected by trypsinization at 0, 6, 12, 24, 36 and 48 h post-treatment, washed with PBS and stained with Trypan blue dye (Sigma-Aldrich, St Louis, USA). Viable cells were counted using a haemocytometer.

### *Fluorescence microscopic analysis of the P. acnes and PA-treated HPV-KER cultures*

The HPV-KER cells were cultured in 6-well plates onto the surface of glass coverslips (18 × 18 mm) at a density of 300,000 cells/well. Forty-eight hours later they were treated with the different *P. acnes* strains using high bacterial dose (MOI 300) or with 2mM PA.

Samples were collected at 48 h post-treatment, by washing the cells with PBS, and then fixed in 2% paraformaldehyde (PFA) solution for 10 min at room temperature. After washing twice in PBS, the cells membranes were permeabilized by 0.1% Triton X-100 detergent (3–5 min at RT). The slides were washed again, and the filamentous actin (F-actin) was stained by Alexa Fluor 488® phalloidin (Life Technologies, Carlsbad, USA) in a 1:100 dilution prepared in PBS, also containing 1% BSA. The slides were incubated for 20–30 min at RT. After the final PBS washes, the coverslips were mounted in Fluoromount-G mounting media (SouthernBiotech, Birmingham, USA).

*Spectrophotometric haemoglobin and lactate dehydrogenase assays*

Erythrocytes were isolated from 5 ml peripheral blood samples from healthy individuals using a Ficoll gradient separation method and subsequently resuspended in 10 ml RPMI 1640 media lacking phenol red dye (LONZA, Basel, Switzerland). Erythrocyte suspensions (500 µl) were treated with *P. acnes* strains (MOI: ~300). The supernatants were collected at 72 h post-treatment, and the quantity of free haemoglobin was estimated by spectrophotometric analysis of the collected supernatant samples (optical density at 540 nm).

The cytolytic effect of the *P. acnes* strains on HPV-KER cells was also measured by the colorimetric lactate dehydrogenase (LDH) assay using the Cytotoxicity Detection Kit PLUS (Roche, Basel, Switzerland), according to the manufacturer's instructions. Briefly, HPV-KER cells were plated in a 96-well plate at a density of 10,000 cells/well. After 24 h incubation, the cells were treated with the *P. acnes* strains from the stock suspensions (MOI 100 and 300). Released LDH was quantitated after 72 h incubation by spectrophotometric analysis (optical density at 492 nm). The measured values were corrected with the background values of the culture medium, and the *P. acnes*-treated samples were also normalized to the values measured in corresponding *P. acnes*-treated cell-free controls.

*Mass spectrophotometry*

All measurements were conducted on a Shimadzu GCMS-QP2010 SE device with a ZB-WaxPlus column of 30 m length. The column was heated at a constant rate (20°C/min) during all measurements starting at 50°C up until 230°C. The head pressure was 117.6 kPa and a 2 ml/min column flow was chosen. The mass spectrometer was operated in scan mode, where all m/z values between 10 and 500 were acquired after the 3.5 min (solvent elution) to protect the filament. Serial dilutions of acetic (AA) and propionic acids (PA) were used for calibration.

*Growth curve analysis of the different* P. acnes *strains*

Bacteria were grown in brain heart infusion (BHI, pH 7.4; Oxoid) broth cultures, and the samples were incubated at 37°C for 48 h under anaerobic conditions (anaerobic chamber; Bactron Sheldon Man, Oregon, USA). The cell numbers of the bacterial suspensions were estimated by measuring the optical density at 600 nm with a spectrophotometer. The $OD_{600}$ values were adjusted with BHI to 0.02. Bacterial suspensions were incubated (A) at 37°C under anaerobic conditions or (B) under standard cell culturing conditions (at 37°C in a humidified atmosphere containing 5% (v/v) $CO_2$) and the $OD_{600}$ values of the samples were measured at 24, 36, 48, 60 and 72 h.

*Statistical analysis*

Unless otherwise noted, all the data were presented as mean ± SEM for 3 experiments, where each treatment was performed at least in triplicate. Data were compared using one-way analysis of variance (ANOVA) followed by Dunnett or Tukey's *post hoc* test to determine statistical differences after multiple comparisons (SPSS, SPSS Inc., Chicago, IL). A probability value of less than 0.05 was considered significant.