COMP negatively influences keratinocyte proliferation via α5β1-integrin: Potential relevance of altered COMP expression in psoriasis

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Abbreviations: AF, Alexa Fluor; ANOVA, analysis of variance; BrdU, bromodeoxyuridine/5-bromo-2'-deoxyuridine; CI, cell index; COMP, cartilage oligomeric matrix protein/thrombospondin-5; DAPI, 4′,6-diamidino-2-phenylindole; DEJ, dermal–epidermal junction; ECM, extracellular matrix; BM, basement membrane; EDA+FN, fibronectin splice variant containing the extra domain A; FBS, fetal bovine serum; FN, fibronectin; HPV-KER, human immortalized keratinocytes; ITGA5, α5-integrin; ITGB1, β1-integrin; KRT17, Keratin 17; LAMA1, laminin alpha 1; MMP-3, matrix metalloproteinase 3; MMP-19, matrix metalloproteinase 19; MMP-12, matrix metalloproteinase 12; NGS, normal goat serum; NHEK, normal human epidermal keratinocyte; PBS, phosphate-buffered saline; rhCOMP, recombinant human cartilage oligomeric matrix protein; SEM, standard error of the mean; TBS, TRIS-buffered saline.

ABSTRACT

In psoriasis, non-lesional skin shows alterations at the dermal–epidermal junction (DEJ) compared to healthy skin. Cartilage oligomeric matrix protein (COMP) is part of the papillary dermis of healthy skin, and its expression has not yet been studied in psoriatic skin. In this study, we found that COMP localization extended deeper into the dermis and formed a more continuous layer in psoriatic non-lesional skin compared to healthy skin, while in psoriatic lesions, COMP showed a partially discontinuous deposition at the DEJ. COMP and β1-integrin showed strong co-localization in non-lesional skin, where the laminin layer within the basement membrane (BM) is discontinuous. In *in vitro* models, the presence of exogenous COMP decreased the proliferation rate of keratinocytes and this proliferation-suppressing effect was diminished by blocking α5β1-integrin. Our results suggest that COMP can interact with α5β1-integrin of basal keratinocytes through the disrupted BM, and this interaction might stabilize the epidermis in the non-lesional state by contributing to the suppression of keratinocyte proliferation. The antiproliferative effect of COMP is likely to be relevant to other skin diseases in which chronic non-healing wounds are coupled with massive COMP accumulation.

INTRODUCTION

The pathogenesis of plaque-type psoriasis (*Psoriasis vulgaris*) is only partially understood, and only symptomatic treatment is currently available. In addition to hyperproliferation, altered keratinocyte differentiation and massive immune-cell infiltration, the dermal extracellular matrix (ECM) and the basement membrane (BM) are also affected in healthy-looking non-lesional skin of patients (Bata-Csorgo et al., 1998; Gliński et al., 1993; Mondello et al., 1996; Vaccaro et al., 2002). Non-lesional epidermal keratinocytes have been shown to represent a "pre-activated" state for hyperproliferation (Chen et al., 2001): these cells are more sensitive to stress (Szabó et al., 2014) and to proliferative signals (Bata-Csorgo et al., 1995). Alterations of the ECM that are already present in non-lesional skin also affect the cell attachment modulator fibronectin (FN), which is differentially expressed in non-lesional skin. Previously, we found that fibroblasts, as well as basal keratinocytes, express high levels of the FN splice variant that contains the extra domain A (EDA+FN) in non-lesional skin (Gubán et al., 2016; Széll et al., 2004). Moreover, some integrins, including the FN-interacting α5β1-integrin, also exhibit an increased expression (Bata-Csorgo et al., 1998; Gubán et al., 2016) in keratinocytes at the dermal–epidermal junction (DEJ). The enhanced EDA+FN and α5β1-integrin production that we observed in psoriatic non-lesional skin may contribute to the induction of keratinocyte proliferation (Bata-Csorgo et al., 1995; Bata-Csorgo et al., 1998; Széll et al., 2004). Furthermore, at the DEJ in non-lesional skin, the laminin layer of the BM is discontinuous and the connection of keratinocytes to the BM is also altered (McFadden and Kimber, 2016; Mondello et al., 1996).

Cartilage oligomeric matrix protein (COMP) is a non-collagenous, glycoprotein component of the ECM. The flexible structure of the COMP homopentamer allows simultaneous interactions with multiple cellular and extracellular components (Malashkevich et al., 1996; Mörgelin et al., 1992). COMP is mainly deposited in cartilage, but it is also located in tendons,

ligaments, synovium and skin. In addition, it is expressed in vascular smooth muscle cells, cardiomyocytes and activated platelets (Müller et al., 1998; Posey et al., 2018; Tan and Lawler, 2009; Wang et al., 2010). In healthy skin, COMP is primarily produced by fibroblasts (Dodge et al., 1998) and localizes to the papillary dermis, where it is believed to take part in ECM stabilization and provide cohesion between the anchoring plaques of the upper dermis and the BM (Agarwal et al., 2012; Farina et al., 2006). Although COMP accumulation in the dermis is elevated in various fibrotic skin disorders (Agarwal et al., 2013; Inui et al., 2011), COMP has not previously been investigated in the context of psoriasis.

COMP modulates cellular behavior via direct interactions with cell-surface proteins, including the α5β1 (Chen et al., 2005), α7β1 and αvβ3 (Rock et al., 2010) members of the integrin family. α5β1-integrin modulates processes in psoriasis pathogenesis, including inflammatory responses and keratinocyte proliferation (Bata-Csorgo et al., 1998; Chen et al., 2001; Pellegrini et al., 1992).

Here we show that the COMP level is elevated in non-lesional psoriatic skin, where it co-localizes with α5β1-integrin and EDA+FN and where it has a suppressive effect on keratinocyte proliferation, which is likely mediated through α5β1-integrin. In this way, COMP can override the proliferation-promoting effect of increased EDA+FN and α5β1-integrin, which is associated with the disrupted laminin layer. (Bata-Csorgo et al., 1998; Mondello et al., 1996). These results indicate a crucial role for COMP in the pathomechanism of psoriasis.

RESULTS

COMP level is elevated in psoriatic non-lesional skin

Non-lesional skin carries several known alterations of the ECM in the papillary dermis (Bos et al., 1983; Ting et al., 2000). Since COMP has previously been reported to also be present in the papillary dermis, COMP-protein accumulation was characterized in non-lesional and lesional skin from psoriasis patients and skin from healthy individuals. COMP protein was detected with western blot analysis under reducing (Figure 1a, 1b and Figure S1) and non-reducing (Figure S2) conditions. Under reducing conditions, we detected elevated COMP monomer and fragment levels in psoriatic non-lesional protein extracts, compared to healthy skin (Figure 1a, 1b and Figure S1).

Subsequently, the distribution of COMP in tissues was analyzed using immunofluorescence staining. In line with previous reports, COMP was detected in the papillary dermis of healthy skin (Farina et al., 2006). In psoriatic non-lesional samples, COMP deposition extended deeper into the dermis and formed a more even and continuous layer than observed in healthy samples (Figure 1c and Figure S3). In contrast, COMP deposition in lesional skin extended to the upper part of the reticular dermis and exhibited a discontinuously scattered distribution (Figure 1c and Figure S3).

Because skin fibroblasts are the major producers of COMP protein (Dodge et al., 1998), we examined the mRNA expression of COMP in primary dermal fibroblasts derived from healthy and psoriatic non-lesional skin and detected elevated COMP mRNA expression in non-lesional fibroblasts (Figure 1d).

COMP co-localization with β1-integrin of basal keratinocytes and EDA+FN is increased and with LAMA1 is decreased in non-lesional psoriatic skin

COMP is known to interact with several members of the integrin cell-surface receptor family, including α5β1-integrin (Chen et al., 2005), whose expression increases together with EDA+FN in non-lesional skin, possibly due to damaged BM (Bata-Csorgo et al., 1998; Mondello et al., 1996; Ting et al., 2000). To investigate the possible interactions of COMP with proteins in the DEJ that have been altered, confocal microscopic analysis with dual immunofluorescence staining was applied and consecutive sections of the appropriate area were analyzed.

To determine whether COMP accumulation at the DEJ allows interaction with basal epidermal keratinocytes, COMP and β1-integrin co-immunofluorescence staining was applied. In the papillary dermis, COMP staining partially co-localized with the β1-integrin from basal keratinocytes in healthy and non-lesional skin (Figure 2a and Figure S4). However, the co-localization of the two proteins was most prominent in psoriatic non-lesional skin (Figure 2d).

Laminin alpha-1 (LAMA1) is a component of the BM laminin layer, which is fragmented and occasionally completely missing in non-lesional psoriatic skin (Mondello et al., 1996; Vaccaro et al., 2002). Therefore, to examine whether the damaged BM of non-lesional skin allows the interaction of COMP and β1-integrin, LAMA1–COMP dual immunostaining was performed. In non-lesional skin, COMP–β1-integrin double-positive regions exhibited a discontinuous LAMA1 staining pattern (Figure 2b) and the co-occurrence of COMP and LAMA1 was significantly lower in non-lesional skin compared to healthy skin (Figure 2e).

In addition, fibronectin has also been reported to interact with COMP (Di Cesare et al., 2002); therefore, confocal microscopic analysis was also applied to COMP and EDA+FN. In psoriatic non-lesional skin, in which co-localization of COMP and β1-integrin was apparent, partial colocalization of COMP and EDA+FN was observed (Figure 2c), and the intensity of colocalization was significantly higher in non-lesional skin relative to healthy skin (Figure 2f).

COMP negatively influences keratinocyte proliferation via α5β1-integrin *in vitro*

To investigate whether the possible interaction between COMP and β1-integrin influences keratinocyte cellular behavior, we first performed an impedance measurement-based, real-time cellular analysis of the HPV-KER immortalized keratinocyte cell line. When the culturing plate was pre-coated with recombinant human COMP (rhCOMP), cells exhibited reduced cell index (CI) values in a manner that was dependent on COMP concentration compared to cells grown on uncoated surfaces (Figure 3a). CI is influenced by changes in cell proliferation, viability, morphology and adhesion (Dickhuth et al., 2015). To investigate whether the proliferation rate of HPV-KER cells was affected, a BrdU cell proliferation assay was performed. Pre-coating the surface with a high concentration (10 μg/ml) of rhCOMP resulted in significantly reduced proliferation rates at 24 and 72 hours, compared to cells grown on an uncoated surface. Cell proliferation of primary normal human epidermal keratinocytes (NHEK) was also reduced when the surface was coated with 10 μg/ml rhCOMP (Figure 3c).

To test whether integrins mediate the observed negative effect of COMP on cell proliferation, blocking experiments using anti-α5 and anti-β1-integrin polyclonal antibodies were performed. Blocking of either α5- or β1-integrin subunit in cells grown on a surface pre-coated with 10 μg/ml rhCOMP abolished the negative effect of COMP on HPV-KER proliferation, whereas blocking either α5- or β1-integrin alone had no negative effect on these cells (Figure 4a, 4b and Figure S5). Similarly, the negative effect of COMP on the proliferation rate of primary NHEK cells could also be abolished by blocking COMP, α5- or β1-integrin, as determined with the BrdU assay (Figure 4c, 4d) and Ki67 immunofluorescent staining (Figure 4e, 4f).

COMP has a negative effect on skin wound healing by attenuating keratinocyte proliferation and by compromising keratinocyte migration and activation in *ex vivo* wound models

To further study the effect of COMP on keratinocytes, an *ex vivo* three-dimensional skin wound-healing model was applied. Standardized wounded skin samples, with or without

rhCOMP treatment (10 μg/ml) (Figure 5a), and unwounded controls were examined at 72 hours after wounding. Immunofluorescent staining revealed COMP-localization on the dermal surface of the injured region in COMP-treated wounds, whereas COMP was not detected at the injured region of untreated wounds 72 hours after treatment (Figure 5a). By applying Ki67 immunofluorescent staining to detect proliferating cells, we found a markedly reduced number of Ki67 positive cells in COMP-treated wounds, indicating a decreased rate of proliferation (Figure 5b, 5c and Figure S6a).

Cell migration processes at the wound edge during the closure of injuries require dynamic reorganization of the actin cytoskeleton in the keratinocytes located close to wound margins. To visualize these cells, immunofluorescence staining for actin was applied. In wounds not exposed to COMP, we found that keratinocytes exhibited high levels of actin expression at wound edges, while actin expression at wound edges was markedly decreased in COMP-treated wounds, indicating that actin expression was compromised, possibly resulting in a reduction of active cell migration (Figure 5d).

Keratin-17 (KRT17) expression is known to be induced in keratinocytes at wound edges during healing (Mazzalupo et al., 2003). Therefore, we performed KRT17 immunofluorescence staining to further investigate the effect of COMP in the ex vivo wound healing model. In rhCOMP-treated wounds, KRT17 expression and re-epithelization were reduced and restricted to a smaller proportion of keratinocytes, compared to untreated control wounds (Figure 5e and Figure S6b, S6c). This suggests that the presence of COMP compromised keratinocyte activation.

DISCUSSION

In psoriasis, the non-lesional skin contains ECM alterations compared to healthy skin. COMP is localized to the papillary dermis of healthy skin (Farina et al., 2006) and, through its interactions with type XII and XIV collagens, contributes to the stabilization of the DEJ (Agarwal et al., 2012). We found that, in psoriatic non-lesional skin, COMP is localized to the papillary dermis and, in contrast to healthy skin, it forms a continuous, more compact, linear layer beneath the basal keratinocytes. Apart from this altered localization, COMP expression was also elevated in dermal fibroblasts from psoriatic samples.

Psoriatic non-lesional skin is more sensitive to stress (Sonkoly et al., 2005; Széll et al., 2016), and abnormalities at the DEJ and the BM are believed to be important in the development of the disease (Bos et al., 1983; Ting et al., 2000). Interruption of the BM (Mondello et al., 1996; Vaccaro et al., 2002) may allow ECM components, normally located directly below the BM, to come in direct contact with basal keratinocytes. COMP reportedly binds directly to the extracellular domain of β1-integrin of both cardiomyocytes (Huang et al., 2013) and cardiac fibroblasts, resulting in the stabilization of β1-integrin by preventing its degradation and, subsequently, improving cellular survival (Huang et al., 2013; Posey et al., 2018). In cartilage, COMP mediates chondrocyte attachment and stabilization partially via α5β1-integrin (Tan et al., 2009). Our confocal microscopic analysis revealed a partial co-localization of papillary dermal COMP and β1-integrin in basal keratinocytes, which indicates the possibility of a direct interaction between these two proteins *in vivo*. In non-lesional skin, the α5-integrin subunit is overexpressed in the basal layer of the epidermis, in contrast to healthy skin, where it is present at low levels or completely missing (Bata-Csorgo et al., 1998). Our findings are in line with this observation, as COMP and β1-integrin strongly co-localize in psoriatic non-lesional epidermis and expression of both are upregulated in non-lesional skin. Moreover, the BM is partially discontinuous in psoriatic non-lesional skin (Mondello et al., 1996; Vaccaro et al.,

2002), allowing direct interaction. The possibility of this interaction is supported by our finding that, in areas where COMP and β1-integrin were found to have strong co-localization in psoriatic non-lesional skin, the expression of LAMA1, a member of the BM, is reduced or completely absent.

COMP also interacts with α7β1- and αvβ3-integrins (Chen et al., 2005; Rock et al., 2010). Of these proteins, only α7β1 contains a β1-integrin subunit. There is currently no information available about α7β1-integrin expression in basal keratinocytes. Thus, we assumed that, if COMP exhibits a strong interaction with β1-integrin, its α-subunit is likely to be α5-integrin. In addition to binding to α5β1-integrin, a fibronectin receptor, COMP might also bind to the fibronectin protein itself (Di Cesare et al., 2002). Furthermore, α5β1-integrin–associated fibronectin and EDA+FN are known to play roles in psoriasis pathogenesis (Bata-Csorgo et al., 1998; Ting et al., 2000). Enriched expression of α5β1-integrin and EDA+FN in non-lesional skin are thought to be due to the incompleteness of the laminin layer (Mondello et al., 1996; Vaccaro et al., 2002). Our confocal microscopic analysis revealed partial co-localization of COMP and EDA+FN in non-lesional skin. These results suggest that, in addition to interacting with EDA+FN, COMP may also affect basal keratinocytes via interactions with both the EDA+FN and its receptor, α5β1-integrin.

Keratinocyte behavior is influenced by ECM proteins through interactions with different cell surface integrins (Hamill et al., 2012; Tjin et al., 2014), and connection of basal keratinocytes to the altered BM could enhance proliferation (Yang et al., 2016). We analyzed the biological relevance of the interaction of COMP and β1-integrin in basal keratinocytes using HPV-KER and NHEK cells *in vitro*. We found that the presence of COMP resulted in reduced keratinocyte proliferation in both cell types and that this affect was reversible by blocking COMP with a specific antibody.

We also analyzed whether the observed negative effect of COMP on keratinocyte proliferation involves interaction with α5β1-integrin. By partially blocking the function of the β1- or α5-integrin subunits with specific antibodies, the negative effect of COMP on cell proliferation was abolished, suggesting that the negative influence of COMP on keratinocyte proliferation involves α5β1-integrin.

Our *in vitro* findings were also validated in an *ex vivo* wound model: exogenous COMP treatment delayed healing of artificial wounds, and this affect was coupled with reduced keratinocyte proliferation and compromised actin expression, both important aspects of wound healing (Gurtner et al., 2008). In addition, keratinocyte KRT17 expression, considered a hallmark of normal wound healing (Mazzalupo et al., 2003), was also decreased in the presence of COMP. These results suggest that COMP has a negative influence on *ex vivo* wound healing. Interestingly, in normally healing wounds of healthy donors, COMP was hardly detectable when re-epithelialization was complete (Agarwal et al., 2013). Similarly, in psoriatic lesions, in which keratinocyte proliferation is abnormally increased, COMP was found to be discontinuous or completely absent from the papillary dermis. Although there are no data regarding keratinocyte proliferation or migration in wounds of COMP-deficient mice (Schulz et al., 2016), in human non-healing wounds, such as venous leg ulcers, the level of COMP is reported to be highly elevated (Agarwal et al., 2013). Our findings are in agreement with this observation.

In conclusion, our study shows that COMP is present at an elevated level in the papillary dermis of non-lesional psoriatic skin and that it possibly reduces keratinocyte proliferation via the α5β1-integrin. These aspects of COMP contribute to the maintenance of the non-lesional, non-hyperproliferative state of psoriatic non-lesional epidermis, despite the overexpression of EDA+FN and α5-integrin. Similar interactions may also take place in other skin diseases in which non-healing wounds are coupled with massive COMP accumulation.

MATERIALS AND METHODS

Skin samples and ethics

Skin punch biopsies (d=6 mm) were collected from healthy volunteers (n=10; age 18–70 years, Table S1), and from psoriatic patients with moderate-to-severe chronic plaque-type psoriasis from lesional (n=13) and non-lesional skin areas (n=13; minimum of 6 cm from lesional region; age 18–70 years (Table S1)). Psoriatic patients did not receive local therapy for at least 4 weeks and had not been subjected to systemic therapy for at least 8 weeks. Skin biopsies were taken from areas of skin that were not exposed to sun. Tissue collection was obtained after written informed consent, in accordance with the rules of the Helsinki Declaration. The study was confirmed by the Human Investigation Review Board of the University of Szeged (PSO-EDAFN-002, 34/2015, 3517, 23 February 2015, Szeged, Hungary; PSO-ECMPR-002 IF-562-5/2016 and; 157/2015-SZTE, 3638, 21 September 2015, Hungary).

Fluorescence microscopic analysis

Biopsies were frozen in a cryogenic matrix (Thermo Fisher Scientific, Waltham, Massachusetts, USA) or were paraffin embedded and were subsequently cut into 5 μm sections. For fixation and permeabilization, 4% paraformaldehyde followed by 0.25% TritonX-100 (Thermo Fisher Scientific) or commercially available staining buffer set (eBioScience, Santa Clara, California, USA) were used. For blocking, TBS containing 1% bovine serum albumin and 1% normal goat serum (NGS, Sigma Aldrich, Saint Louis, Missouri, USA) was used, and for frozen samples, which were digested with chondroitinase ABC (5U, 1:100; Sigma Aldrich), 10% fetal bovine serum (FBS, EuroClone, Pero, Italy) and 5% NGS (Sigma Aldrich) were applied. Samples were incubated with the following primary antibodies: polyclonal rabbit anti-human COMP (1:250), a kind gift from Prof. Dr. Mats Paulsson and Dr. Frank Zaucke from the University of Cologne (Agarwal et al., 2012); mouse anti-human β1-integrin (clone: JB1B, 1:100, Abcam, Cambridge, United Kingdom), rabbit anti-human actin (1:100, Sigma Aldrich),

mouse anti-human Ki67 (1:100, Beckton Dickinson, Franklin Lakes, New Jersey, USA), mouse anti human KRT17 (ready to use, Dako, Santa Clara, California, USA), mouse anti human LAMA1 (clone: LAM-89, 1:100, R&D Systems, Minneapolis, Canada USA), mouse anti-human fibronectin (EDA+FN, clone: IST-9, 1:500, Abcam). Isotype controls were the following: rabbit polyclonal IgG (Santa Cruz Biotechnology, California, USA) and mouse IgG1 (Beckton Dickinson) antibodies. As secondary antibodies, Alexa Fluor (AF) 546 conjugated anti-rabbit IgG and AF 647 conjugated anti-mouse IgG (Life Technologies, Carlsbad, California, USA) were used. Nuclei were visualized with 4′,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich) staining. Zeiss LSM 880 or Zeiss Axio Imager Z1 microscopes (Carl Zeiss AG, Oberkochen, Germany) were used for visualization.

Pearson's correlation coefficient, R, was calculated using ImageJ/Fiji software (ImageJ, Wisconsin, USA).

Cell cultures and examination of cellular properties.

Cell-proliferation assay

To investigate the effect of COMP on keratinocyte proliferation, a bromodeoxyuridine/5-bromo-2'-deoxyuridine (BrdU) cell-proliferation colorimetric ELISA assay (Abcam) was performed. HPV-KER cells, a stable human keratinocyte cell line that has been characterized in our laboratory (Tax et al., 2016; Danis et al., 2018; Erdei et al., 2018), and NHEK cells were plated at a density of 10,000 cells/well in 96-well plates (Corning, New York, USA) that were uncoated or coated with low- (1 μg/ml) or high-concentration (10 μg/ml) rhCOMP protein (R&D Systems), in three technical replicates. For the blocking of α5-integrin and β1-integrin, the following antibodies (1 μg antibody for 10^6 cells) were used: mouse anti-human α5-integrin antibody (clone: IIA1, Beckton Dickinson), mouse anti-human β1-integrin (clone: JB1B). Goat anti-human COMP antibody (1 μg antibody for 10 μg/ml rhCOMP protein, R&D Systems) was

applied to block the COMP protein. Integrin- and COMP-blocking was applied to cells grown on uncoated plates or plates coated with 10 μg/ml rhCOMP protein. BrdU assay was performed at 24 and 72 hours after blocking, according to the manufacturer's instructions.

Ex vivo skin wound-healing assay

Healthy skin samples were collected for the *ex vivo* organotypic wound healing assay. Approximately 1 cm diameter skin pieces were cut and mildly wounded in the middle using a 4 mm punch biopsy blade (Steele Supply Company, St. Joseph, MI, USA). Wounded skin samples and unwounded control samples were incubated for 72 hours at an air–liquid interface on the upper part of transwell cell culture inserts. The dermal part was in contact with DMEM F12 culture media (Lonza Group, Basel, Switzerland) supplemented with 10% FBS (EuroClone) and 1% antibiotic/antimycotic solution (Sigma Aldrich). The middle of the wounds was treated for 72 hours with high-concentration (10 μg/ml) rhCOMP (R&D Systems) diluted in phosphate buffered saline (PBS) or PBS only as a control. Samples were fixed in formalin and embedded in paraffin for immunofluorescent staining. To determine the rate of proliferation, 50 cells on each wound edge were counted and the proportion of ki67 positive cells was determined. Re-epithelization of untreated, control (where only PBS was administered), and COMP-treated wounds were assessed by measuring the area using the ImageJ software.

Statistical analysis

For comparing only two groups, two-tailed Student *t* test was performed. One-way analysis of variance (ANOVA) with Tukey *post hoc* test was used to compare more than two groups. Differences were considered statistically significant at $^{**}P<0.01$, $^{*}P<0.05$. Data were analyzed using R-Studio software, version 3.2.2 (R-Studio, Boston, USA).

Further methods

More detailed information of the materials and methods regarding, protein isolation and western blot analysis, cell culture experiments, hematoxylin–eosin staining, RNA isolation and real time RT-PCRs are presented in the Supplementary Materials.

Data availability statement

No datasets were generated or analyzed during the current study.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: GG. Experimental design: RB and GG. Investigation: RB, ES, JD and BG. Formal analysis: RB, KS and GG. Writing - Original Draft Preparation: RB and GG. Writing - Review and Editing: KS, ZBC and GG. Supervision: LK, ZBC and GG.

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Figure Legends

Figure 1.: COMP level is elevated in psoriatic non-lesional skin.

(a) COMP protein was detected with western blot analysis from healthy, psoriatic non-lesional and lesional skin. Actin was used as a loading control. A representative blot is shown under reducing condition. (b) Band intensities of COMP monomer were quantitated with Image Studio software (LI-COR Biosciences, Lincoln, Nebraska, USA) and presented as fold changes normalized to actin. The graph shows mean+/-SEM (n=6) vs. healthy control. *:$P<0.05$ calculated by one-way analysis of variance, followed by Tukey's *post hoc* test. (c) Immunofluorescence staining of COMP in healthy (left column), psoriatic non-lesional (middle column) and psoriatic lesional (right column) skin. Representative images are shown. Dotted lines indicate the border of the dermal–epidermal junction (n=10 (see also Supplementary Figure S3), magnification: 20x; scale bar: 50μm). (d) Real-time RT-PCR analysis of COMP cDNA from cultured human dermal fibroblasts of healthy and psoriatic non-lesional skin. Data were normalized to 18S rRNA using the $\Delta\Delta C_t$ method. The graph shows mean+/-SEM (n=8) vs. healthy cultured fibroblasts **:$P<0.01$, determined by two-tailed Student *t* test.

Figure 2.: COMP co-localization with basal keratinocyte β1-integrin and EDA+FN increases and with LAMA1 decreases in non-lesional psoriatic skin.

Confocal microscopic immunofluorescence analysis of (a) COMP and β1-integrin, (b) COMP and LAMA1, (c) COMP and EDA+FN co-localization in healthy (first row) and psoriatic non-lesional (second row) skin. Representative images are shown. Dotted lines indicate the enlarged regions. Co-localized pixels of the indicated proteins were calculated with ImageJ software (ImageJ, Wisconsin, USA; n=5, magnification: 63x; scale bar:10 μm). The extent of co-localization of (d) COMP–β1-integrin, (e) COMP–LAMA1, (f) COMP–EDA+FN were calculated using ImageJ/Fiji software (ImageJ, Wisconsin, USA). The graphs show mean

Pearson's correlation coefficient, R+/-SEM (n=5) vs. healthy control, *:P<0.05, **:P<0.01 determined by two-tailed Student *t* test.

Figure 3.: COMP negatively influences keratinocyte cell proliferation.

(a) Cell index (CI) measurement of HPV- KER cells cultured on surfaces that were uncoated or coated with recombinant human COMP (rhCOMP, 1 and 10 μg/ml). CI was determined using real-time impedance measurement-based cellular analysis. The graph is representative of four independent experiments, all showing similar results. The graph shows mean CI+/-SEM of four technical replicas for each group, *:P<0.05 vs. uncoated control, #:P<0.05 vs. 1 μg/ml rhCOMP coated group calculated by one-way analysis of variance, followed by Tukey's *post hoc* test. BrdU cell proliferation assay of (b) HPV-KER and (c) NHEK cells cultured on uncoated and rhCOMP protein (1 and 10 μg/ml) coated surfaces at 24 and 72 hours following seeding. The graphs show mean proliferation +/-SEM (n=3). *:P<0.05, calculated by one-way analysis of variance; followed by Tukey's *post hoc* test.

Figure 4.: COMP influences keratinocyte proliferation via α5β1 integrin.

BrdU cell proliferation assay of (a, b) HPV-KER cells cultured on surfaces that were uncoated or coated with recombinant human COMP (rhCOMP) protein (10 μg/ml) for 24 and 72 hours following seeding and treated with (a) anti-α5 or (b) anti-β1-integrin subunit antibodies. (c, d) BrdU assay, and (e, f) Ki67-positive proliferating cell number determination of NHEKs grown on surfaces that were uncoated or coated with rhCOMP protein (10 μg/ml) for 72 hours following seeding and treated with (c, e) anti-α5 and (d, f) anti-β1-integrin subunit antibody in a combination with COMP protein neutralization. The graphs show mean proliferation/mean number of Ki67-positive cells+/-SEM (n=3) vs. uncoated control, *:P<0.05, **:P<0.01 calculated by one-way analysis of variance, followed by Tukey's *post hoc* test.

Figure 5.: COMP has a negative effect on keratinocyte proliferation and is involved in keratinocyte migration and activation in *ex vivo* wound models.

(a) Representative images of the *ex vivo* skin wound healing models (n=3; magnification: 4x; scale bar: 250μm). Immunofluorescent staining for COMP at wound edges and at the middle of the wounds of untreated controls and wounds treated with recombinant human COMP (rhCOMP) (n=3; magnification: 20x; scale bar: 50μm). Immunostaining for (b) Ki67, (d) actin or (e) keratin-17 in the *ex vivo* unwounded skin (left column), and wound-healing skin models with (right column) and without (middle column) rhCOMP treatment. Representative images are shown. Dotted lines indicate the border of the dermal–epidermal junction (n=3; magnification: 20x; scale bar: 50μm). (c) Ki67 positive cells in wounds that were not treated and were treated with rhCOMP protein. The graph shows mean number of Ki67-positive cells +/-SEM (n=3) vs. uncoated, untreated control **:$P<0.01$ determined by two-tailed Student *t* test.

Figure 1.

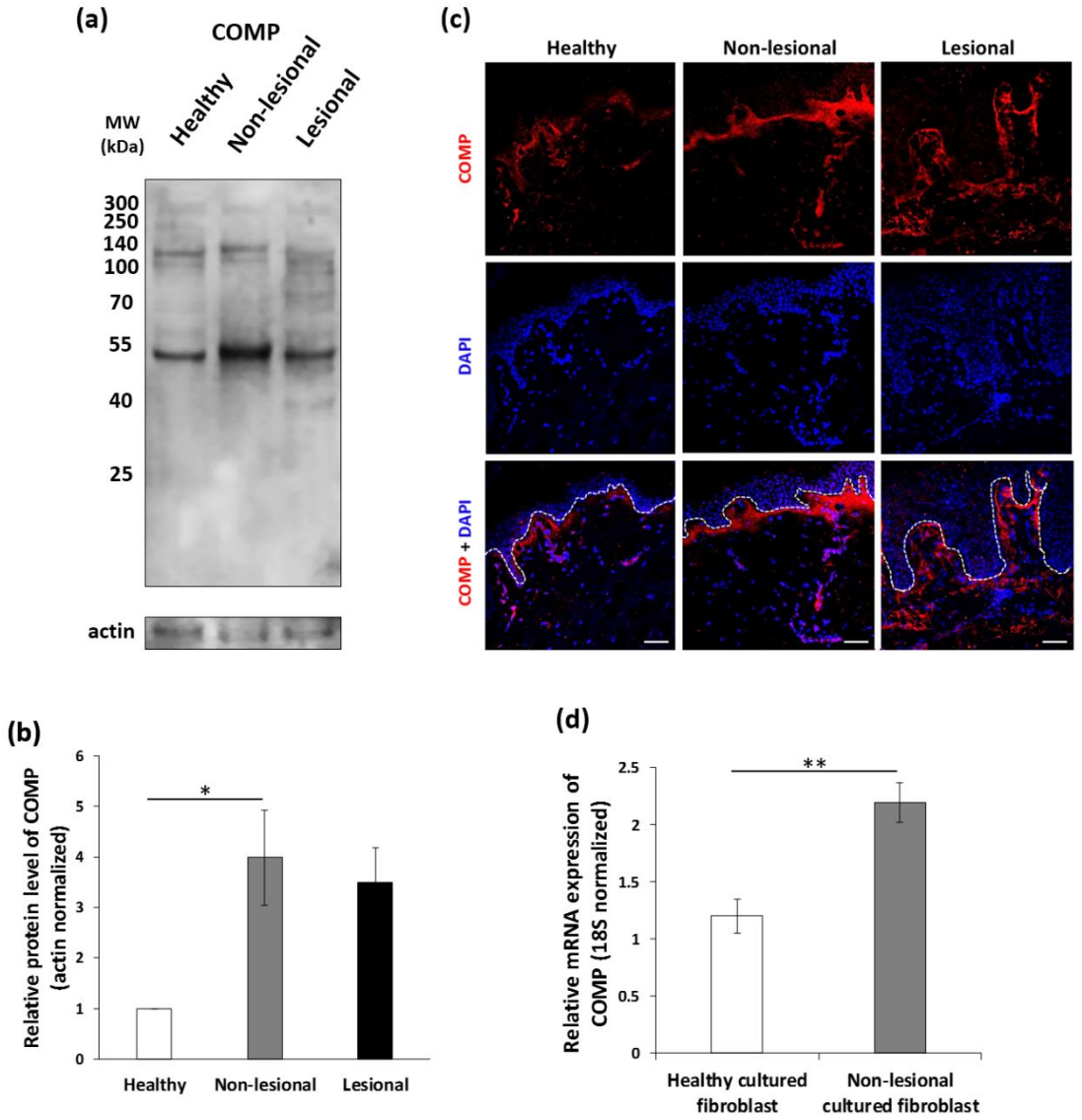

(a)
COMP
Healthy
Non-lesional
Lesional
MW (kDa)
300
250
140
100
70
55
40
25
actin
(c)
Healthy
Non-lesional
Lesional
COMP
DAPI
COMP + DAPI
(b)
Relative protein level of COMP (actin normalized)
*
Healthy
Non-lesional
Lesional
(d)
Relative mRNA expression of COMP (18S normalized)
**
Healthy cultured fibroblast
Non-lesional cultured fibroblast

Figure 2.

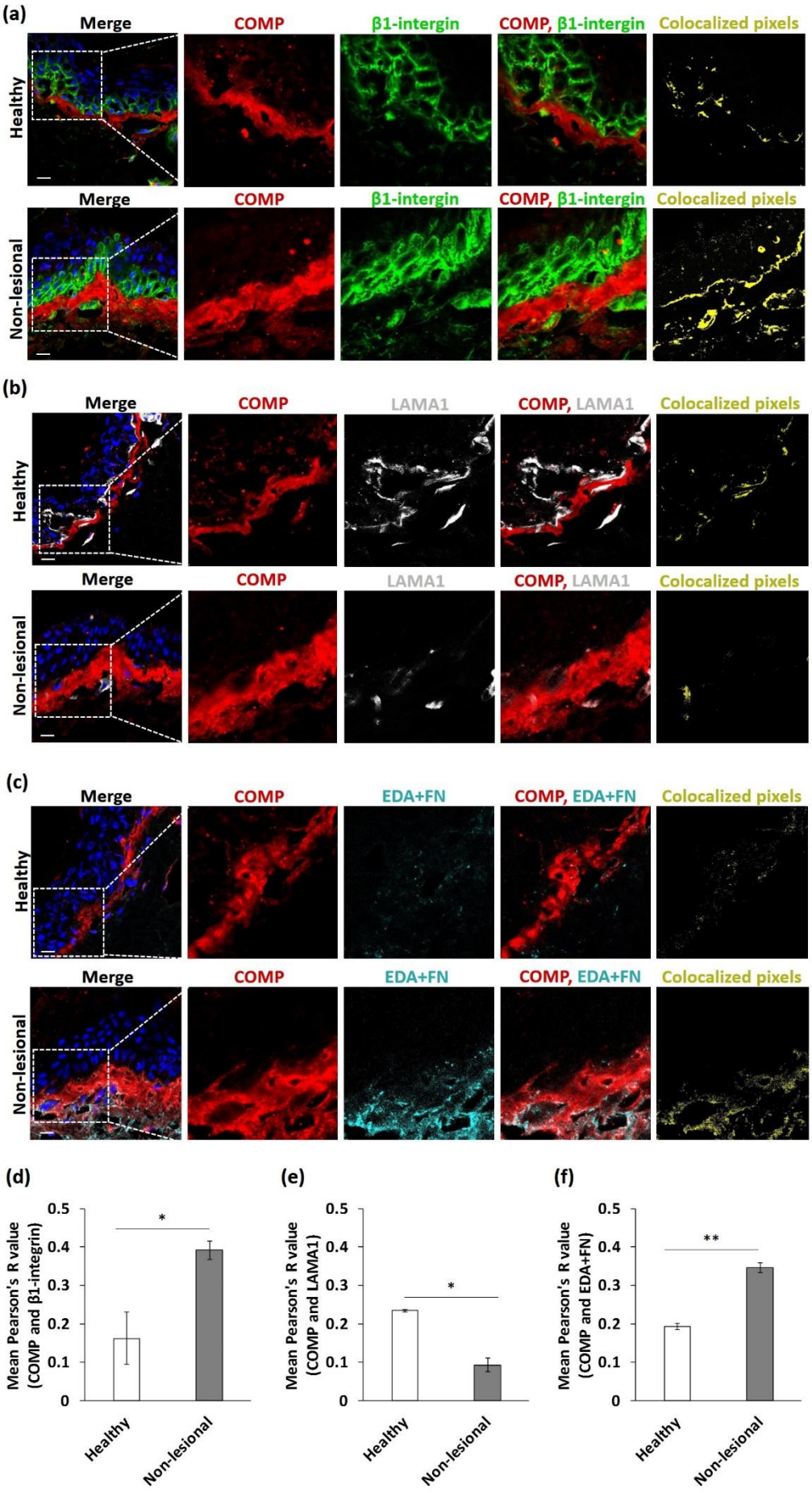

(a)
Merge
COMP
β1-intergin
COMP, β1-intergin
Colocalized pixels
Healthy
Non-lesional
(b)
LAMA1
COMP, LAMA1
(c)
EDA+FN
COMP, EDA+FN
(d)
Mean Pearson's R value (COMP and β1-integrin)
(e)
Mean Pearson's R value (COMP and LAMA1)
(f)
Mean Pearson's R value (COMP and EDA+FN)
0
0.1
0.2
0.3
0.4
0.5
*
**
Healthy
Non-lesional

Figure 3.

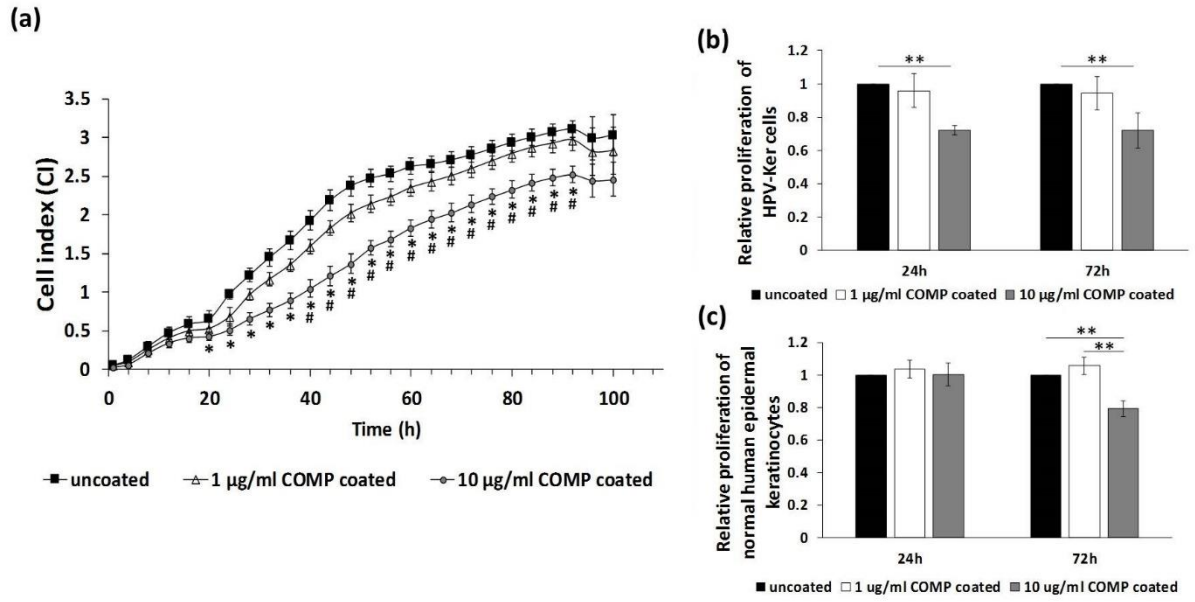

(a)
Cell index (CI)
Time (h)
uncoated
1 µg/ml COMP coated
10 µg/ml COMP coated
(b)
Relative proliferation of HPV-Ker cells
24h
72h
uncoated
1 µg/ml COMP coated
10 µg/ml COMP coated
(c)
Relative proliferation of normal human epidermal keratinocytes
24h
72h
uncoated
1 ug/ml COMP coated
10 ug/ml COMP coated

Figure 4.

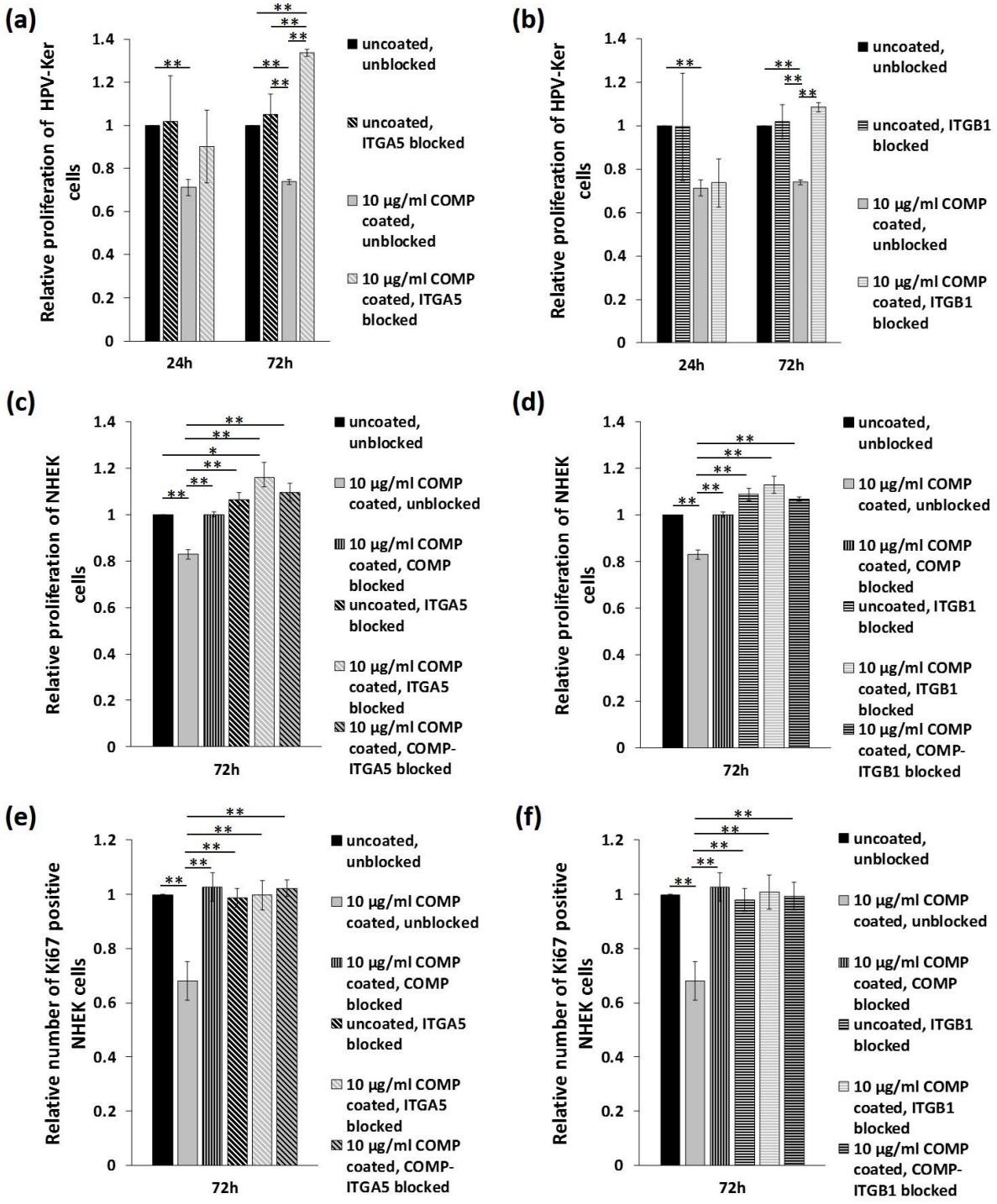
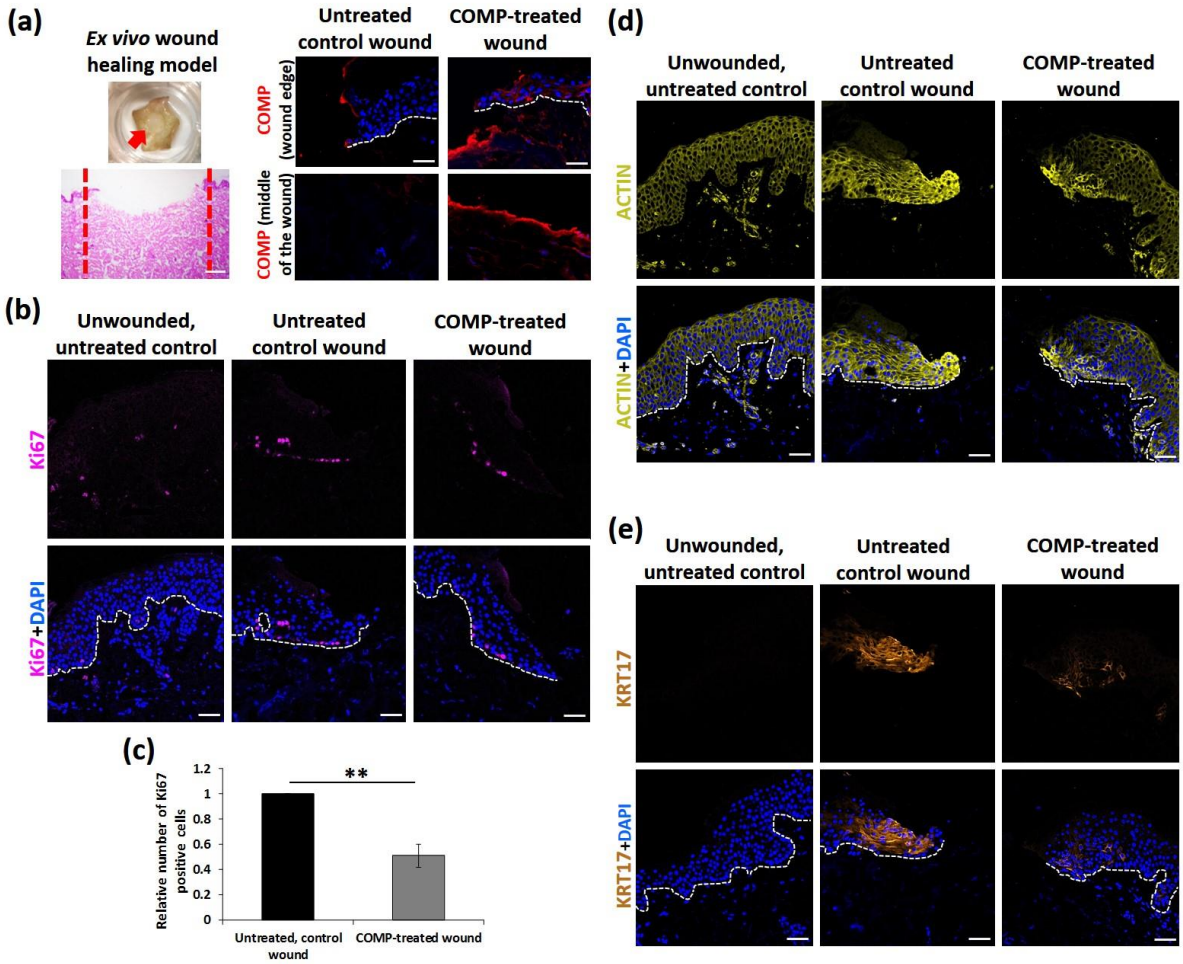

(a)
Relative proliferation of HPV-Ker cells
1.4
1.2
1
0.8
0.6
0.4
0.2
0
24h
72h
uncoated, unblocked
uncoated, ITGA5 blocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, ITGA5 blocked
(b)
Relative proliferation of HPV-Ker cells
uncoated, unblocked
uncoated, ITGB1 blocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, ITGB1 blocked
(c)
Relative proliferation of NHEK cells
72h
uncoated, unblocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, COMP blocked
uncoated, ITGA5 blocked
10 µg/ml COMP coated, ITGA5 blocked
10 µg/ml COMP coated, COMP-ITGA5 blocked
(d)
Relative proliferation of NHEK cells
72h
uncoated, unblocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, COMP blocked
uncoated, ITGB1 blocked
10 µg/ml COMP coated, ITGB1 blocked
10 µg/ml COMP coated, COMP-ITGB1 blocked
(e)
Relative number of Ki67 positive NHEK cells
1.2
1
0.8
0.6
0.4
0.2
0
72h
uncoated, unblocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, COMP blocked
uncoated, ITGA5 blocked
10 µg/ml COMP coated, ITGA5 blocked
10 µg/ml COMP coated, COMP-ITGA5 blocked
(f)
Relative number of Ki67 positive NHEK cells
72h
uncoated, unblocked
10 µg/ml COMP coated, unblocked
10 µg/ml COMP coated, COMP blocked
uncoated, ITGB1 blocked
10 µg/ml COMP coated, ITGB1 blocked
10 µg/ml COMP coated, COMP-ITGB1 blocked

Figure 5.


(a)
Ex vivo wound healing model
Untreated control wound
COMP-treated wound
COMP (wound edge)
COMP (middle of the wound)
(b)
Unwounded, untreated control
Untreated control wound
COMP-treated wound
Ki67
Ki67+DAPI
(c)
Relative number of Ki67 positive cells
1.2
1
0.8
0.6
0.4
0.2
0
**
Untreated, control wound
COMP-treated wound
(d)
Unwounded, untreated control
Untreated control wound
COMP-treated wound
ACTIN
ACTIN+DAPI
(e)
Unwounded, untreated control
Untreated control wound
COMP-treated wound
KRT17
KRT17+DAPI