SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods: Detailed description of materials and methods

Protein isolation and western blot analysis

For preparation of tissue protein extracts, skin biopsies (healthy, psoriatic non-lesional and psoriatic lesional) were cut into small pieces with a razor blade. 6 M guanidine hydrochloride (Sigma Aldrich, Saint Louis, Missouri, USA) solution was used as an extraction buffer for 24 h at 4°C under continuous agitation. For protein precipitation, an ethanol-based method was applied. Protein concentrations were measured using Bradford assay (Bio-Rad Laboratories, Hercules, California, USA). Protein extracts (25 µg) were separated on a 4–20% gradient SDS polyacrylamide gel under reducing or non-reducing conditions. Proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories) and blocked in 5% non-fat milk powder containing Tris-buffered saline (TBS) for 60 min at room temperature (RT). Membranes were incubated for overnight at 4°C with goat anti-human COMP primary antibody (1:2000, R&D Systems, Minneapolis, Minnesota, USA), and rabbit anti-human actin primary antibody (1:2000, Sigma Aldrich). Subsequently, membranes were incubated with horseradish peroxidase-conjugated anti-goat (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and anti-rabbit (Southern Biotech, Birmingham, USA) secondary antibodies, both diluted 1:2000, for 60 min at RT. Signal was visualized with Clarity Max Western ECL Substrate (Bio-Rad Laboratories) on a C-digit blot scanner (LI-COR Biosciences, Lincoln, Nebraska, USA).

Hematoxylin eosin staining and light microscopic analysis

To visualize the tissue structure of *ex vivo* wound model samples, hematoxylin-eosin (Leica Biosystems, Wetzlar, Germany) staining was performed according to the manufacturer's

instructions in a Leica ST5020 Multistainer device (Leica Biosystems). The stained samples were visualized with a Nikon eclipse TS100 microscope (Nikon, Minato, Tokyo, Japan).

Cell cultures and examination of cellular properties

Cell cultures

Primary dermal fibroblasts were isolated from healthy and psoriatic non-lesional skin biopsies, and normal human epidermal keratinocytes (NHEKs) were isolated from healthy skin samples. The epidermis was separated from the dermis with overnight incubation at 4°C in Dispase II (neutral protease, grade II, 2U/ml, Roche Diagnostics, Risch, Switzerland) (Szabad et al., 2007) solution. Keratinocytes were obtained from the epidermal part after trypsin digestion for 10 min at 37°C (Sigma Aldrich). NHEK cells were then grown in keratinocyte serum-free medium (KSFM, Life Technologies, Carlsbad, United States) supplemented with 1% antibiotic/antimycotic solution (AB/AM, Sigma Aldrich), brain pituitary extract (50 μg/ml, Life Technologies) and epidermal growth factor (5 ng/ml, Life Technologies).

Fibroblasts were obtained from the dermal part after digestion for 120 min at 37°C. The media (Dulbecco's Modified Eagle Medium, DMEM, supplemented with 1 g/l glucose, Lonza Group, Basel, Switzerland) also contained collagenase (from *Clostridium histolyticum*, 2.7 mg/ml, Sigma Aldrich), deoxyribonuclease I (from bovine pancreas, 0.1 mg/ml, Sigma Aldrich), hyaluronidase (from bovine testes, 1.25 mg/ml, Sigma Aldrich) and fetal bovine serum (FBS, 2.5%, EuroClone, Pero, Italy) (Gubán et al., 2016). Fibroblasts were cultured in DMEM 1 g/l glucose (Lonza Group), supplemented with 5% FBS (EuroClone), 1% antibiotic/antimycotic solution (AB/AM, Sigma Aldrich) and 1% L-glutamine (PAA Laboratories GmbH, Pasching, Austria).

The human immortalized keratinocyte cell line HPV-KER was also used for our experiments. HPV-KER is a stable cell line, generated from normal human epidermal keratinocytes transfected

with the HPV16/E6 oncogene in a pCMV vector . It was established by continuous culturing (Tax et al., 2016). It shows similar responses to NHEK cells in various immune activation protocols (Danis et al., 2018; Erdei et al., 2018). Culture conditions of HPV-KER cells are the same as NHEK cells.

Each cell type was cultured at 37°C in a humidified atmosphere with 5% v/v CO_2.

Real-time, label-free cellular analysis of HPV-KER cells using the xCELLigence system

xCELLigence (ACEA Biosciences, San Diego, USA) is a real-time, impedance measurement-based cellular analysis system, where dimensionless Cell Index (CI) value is calculated (CI= (impedance at time point n – impedance in the absence of cells)/nominal impedance value). Differences in CI values could be due to altered cell proliferation rate, viability, morphology and adhesion (Dickhuth et al., 2015). This system was used to investigate the effect of the COMP protein on keratinocytes. HPV-KER cells were plated at a density of 10,000 cells/well in uncoated 96-well E-plates (ACEA Biosciences) or wells that were coated with low-concentration (1 μg/ml) or high-concentration (10 μg/ml) recombinant human COMP protein (rhCOMP, R&D Systems). Impedance measurement was performed in every 15 min for 140 h, and a dimension-free CI value was calculated for every time point. Four technical replicates were performed.

Further investigation of keratinocyte cell-proliferation

To further investigate the effect of COMP on NHEK cell's proliferation, Ki67 immunofluorescent staining (mouse anti-human Ki67 antibody, 1:100, Beckton Dickinson, Franklin Lakes, New Jersey, USA) was applied, using integrin and COMP-blocking as described in the main text. Cells were plated at a density of 20,000 cells/well in 8-well chamber slides (SPL Life Sciences, Naechon-Myeon, Pocheon-si, Korea) that were uncoated or coated with high-concentration (10 μg/ml)

rhCOMP (R&D Systems), in three biological replicates. Ki67 positive cells were counted on three randomly selected areas per group, and statistical analysis was performed.

RNA isolation and real-time RT-PCR

Total RNA was isolated from primary fibroblasts from healthy and psoriatic non-lesional skin cultured in 75 cm^2 cell culture flasks (Corning, New York, USA) and collected at the fifth passage using TRI-Reagent (Molecular Research Center; Cincinnati, United States) as described by the manufacturer. The iScript™ cDNA Synthesis kit (Bio-Rad Laboratories) was used for cDNA synthesis, and 0.5 μg total RNA was reverse transcribed. Real-time RT-PCR was performed on a C1000 Touch Thermal Cycler (Bio-Rad Laboratories) with the Universal Probe Library system (Roche Diagnostics) using qPCRBIO Probe Mix Lo-ROX (PCR Biosystem Ltd., London, UK) and the following primers: COMP FWD: CACCGACGTCAACGAGTG, COMP REV: TGGTGTTGATACAGCGGACT; 18S rRNA FWD: CGCTCCACCAACTAAGAACG, 18S rRNA REV: CTCAACACGGGAAACCTCAC. The expression of COMP was normalized to 18S rRNA expression using the ΔΔCt method.

Supplementary References

Danis, J., Janovák, L., Gubán, B., Göblös, A., Szabó, K., Kemény, L., et al., 2018. Differential Inflammatory-Response Kinetics of Human Keratinocytes upon Cytosolic RNA- and DNA-Fragment Induction. Int J Mol Sci 19.

Dickhuth, J., Koerdt, S., Kriegebaum, U., Linz, C., Müller-Richter, U.D., Ristow, O., et al., 2015. In vitro study on proliferation kinetics of oral mucosal keratinocytes. Oral Surg Oral Med Oral Pathol Oral Radiol 120, 429–435.

Erdei, L., Bolla, B.S., Bozó, R., Tax, G., Urbán, E., Kemény, L., et al., 2018. TNIP1 Regulates Cutibacterium acnes-Induced Innate Immune Functions in Epidermal Keratinocytes. Front Immunol 9, 2155.

Gubán, B., Vas, K., Balog, Z., Manczinger, M., Bebes, A., Groma, G., et al., 2016. Abnormal regulation of fibronectin production by fibroblasts in psoriasis. Br. J. Dermatol. 174, 533–541.

Szabad, G., Kormos, B., Pivarcsi, A., Széll, M., Kis, K., Kenderessy Szabó, A., et al., 2007. Human adult epidermal melanocytes cultured without chemical mitogens express the EGF receptor and respond to EGF. Arch. Dermatol. Res. 299, 191–200.

Tax, G., Urbán, E., Palotás, Z., Puskás, R., Kónya, Z., Bíró, T., et al., 2016. Propionic Acid Produced by Propionibacterium acnes Strains Contri-butes to Their Pathogenicity. Acta Derm. Venereol. 96, 43–49.

Supplementary Figure Legends

Figure S1: COMP monomer and fragment level is elevated in psoriatic non-lesional skin under reducing conditions.

COMP monomers and fragments were detected with western blot analysis from healthy, psoriatic non-lesional and lesional skin under reducing conditions (n=3). The band intensities of (a) COMP monomer, (b) COMP fragment separately, as well as (c) the level of monomer and fragment together were analyzed with Image Studio software (LI-COR Biosciences) and presented as fold changes normalized to actin. The graph shows mean+/-SEM (n=3), *:P<0.05 vs. healthy control, calculated by one-way analysis of variance, followed by Tukey's *post hoc* test.

Figure S2: COMP protein detection under non-reducing conditions.

COMP protein was detected with western blot analysis from healthy, psoriatic non-lesional and lesional skin under non-reducing conditions (n=3). A representative blot is shown. (H: healthy, NL: non-lesional, L: lesional).

Figure S3: Characterization of COMP deposition in healthy, non-lesional and lesional skin.

Immunofluorescence staining for COMP in (a) healthy, (b) psoriatic non-lesional and (c) psoriatic lesional skin. (n=10, DAPI: 4,6-diamidino-2-phenylindole. Zeiss Axio Imager Z1 original magnification: 20x, scale bar: 50μm).

Figure S4: Enhanced co-localization of COMP with β1-integrin the in non-lesional psoriatic skin.

Confocal microscopic immunofluorescence analysis of COMP and β1-integrin in (a) healthy and (b) psoriatic non-lesional skin using z-stack pictures. Dotted lines indicate the borders of enlarged

regions. Co-localized pixels of COMP and β1-integrin were calculated by ImageJ software (ImageJ, Wisconsin, USA; n=5, magnification: 63x; scale bar:10 μm).

Figure S5: COMP negatively influences keratinocyte cell index via α5β1 integrin.

Cell index (CI) measurement of HPV-KER cells cultured on uncoated and recombinant human COMP (rhCOMP)-coated (10 μg/ml) surfaces. CI was determined using real-time impedance measurement-based cellular analysis. The graph is representative of three independent experiments, all showing similar results. Mean CI+/-SEM of four technical replicas for each group.

Figure S6. Reduced proliferation and re-epithelization in the presence of COMP during *ex vivo* skin wound healing.

(a) Reduced number of Ki67 positive cells was detected in wound samples treated with recombinant human COMP protein (rhCOMP, 10 μg/ml), compared to untreated control wounds. The graph shows mean+/-SEM (n=3), *:P<0.05 vs. untreated control, calculated by two-tailed Student *t* test. (b) Re-epithelization of artificial untreated and rhCOMP-treated (10 μg/ml) wounds on hematoxylin-eosin stained sections (n=3; magnification: 20x; scale bar: 50μm). Representative pictures are shown. Arrowheads indicate the newly synthetized areas. (c) Re-epithelization of untreated, control- and rhCOMP-treated wounds were measured using area measurement of ImageJ software (ImageJ, Wisconsin, USA). The graph shows mean+/-SEM (n=3), *:P<0.05 vs. untreated control, calculated by two-tailed Student *t* test.

Table S1. Donor data and experiments applied to donor samples.

Figure S1.

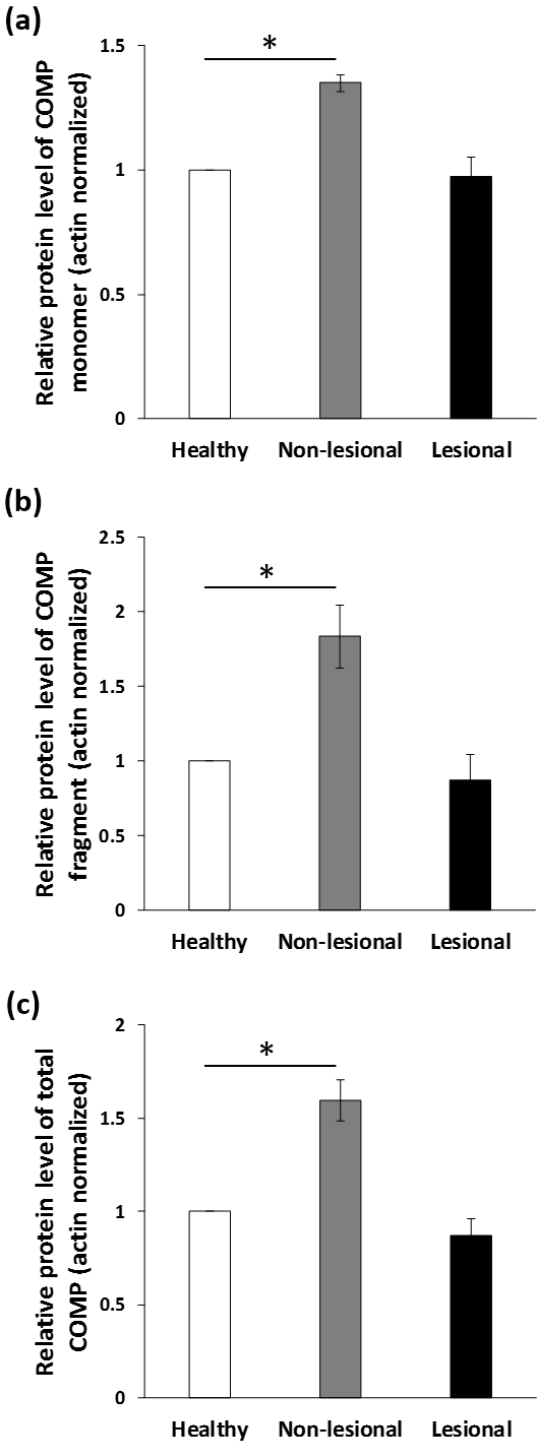

(a)
Relative protein level of COMP monomer (actin normalized)
1.5
1
0.5
0
*
Healthy
Non-lesional
Lesional
(b)
Relative protein level of COMP fragment (actin normalized)
2.5
2
1.5
1
0.5
0
*
Healthy
Non-lesional
Lesional
(c)
Relative protein level of total COMP (actin normalized)
2
1.5
1
0.5
0
*
Healthy
Non-lesional
Lesional

Figure S2.

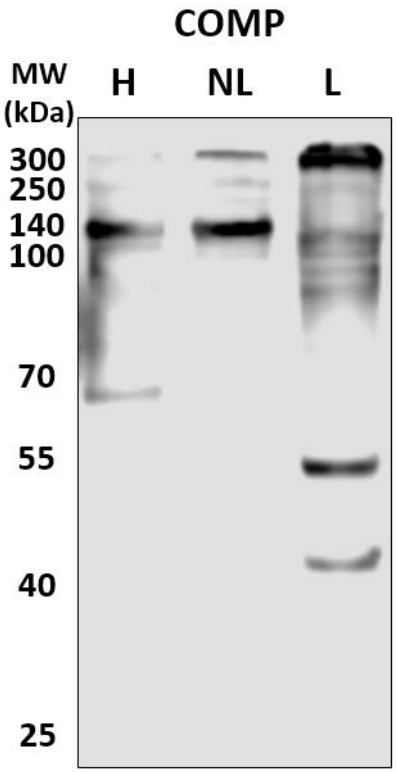


Figure S3.

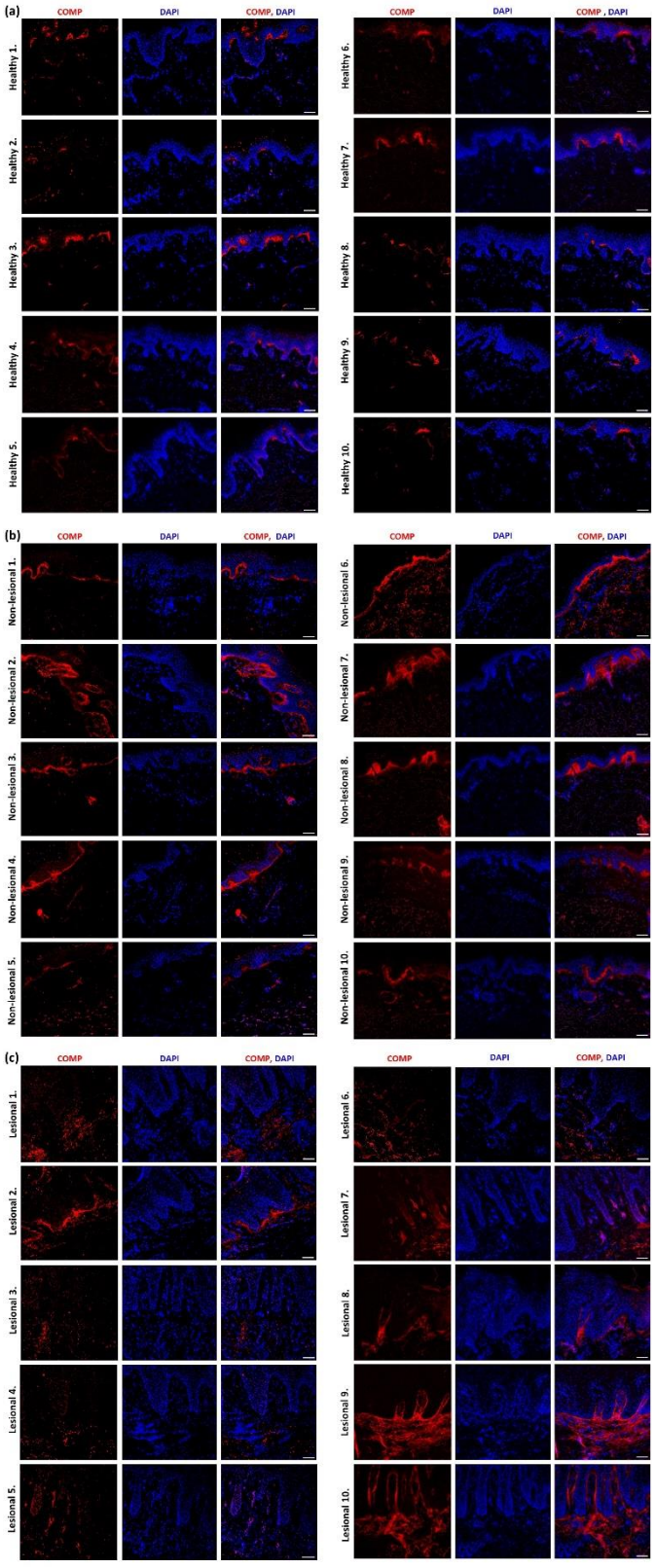

(a)
COMP
DAPI
COMP, DAPI
Healthy 1.
Healthy 2.
Healthy 3.
Healthy 4.
Healthy 5.
Healthy 6.
Healthy 7.
Healthy 8.
Healthy 9.
Healthy 10.
(b)
Non-lesional 1.
Non-lesional 2.
Non-lesional 3.
Non-lesional 4.
Non-lesional 5.
Non-lesional 6.
Non-lesional 7.
Non-lesional 8.
Non-lesional 9.
Non-lesional 10.
(c)
Lesional 1.
Lesional 2.
Lesional 3.
Lesional 4.
Lesional 5.
Lesional 6.
Lesional 7.
Lesional 8.
Lesional 9.
Lesional 10.

Figure S4.

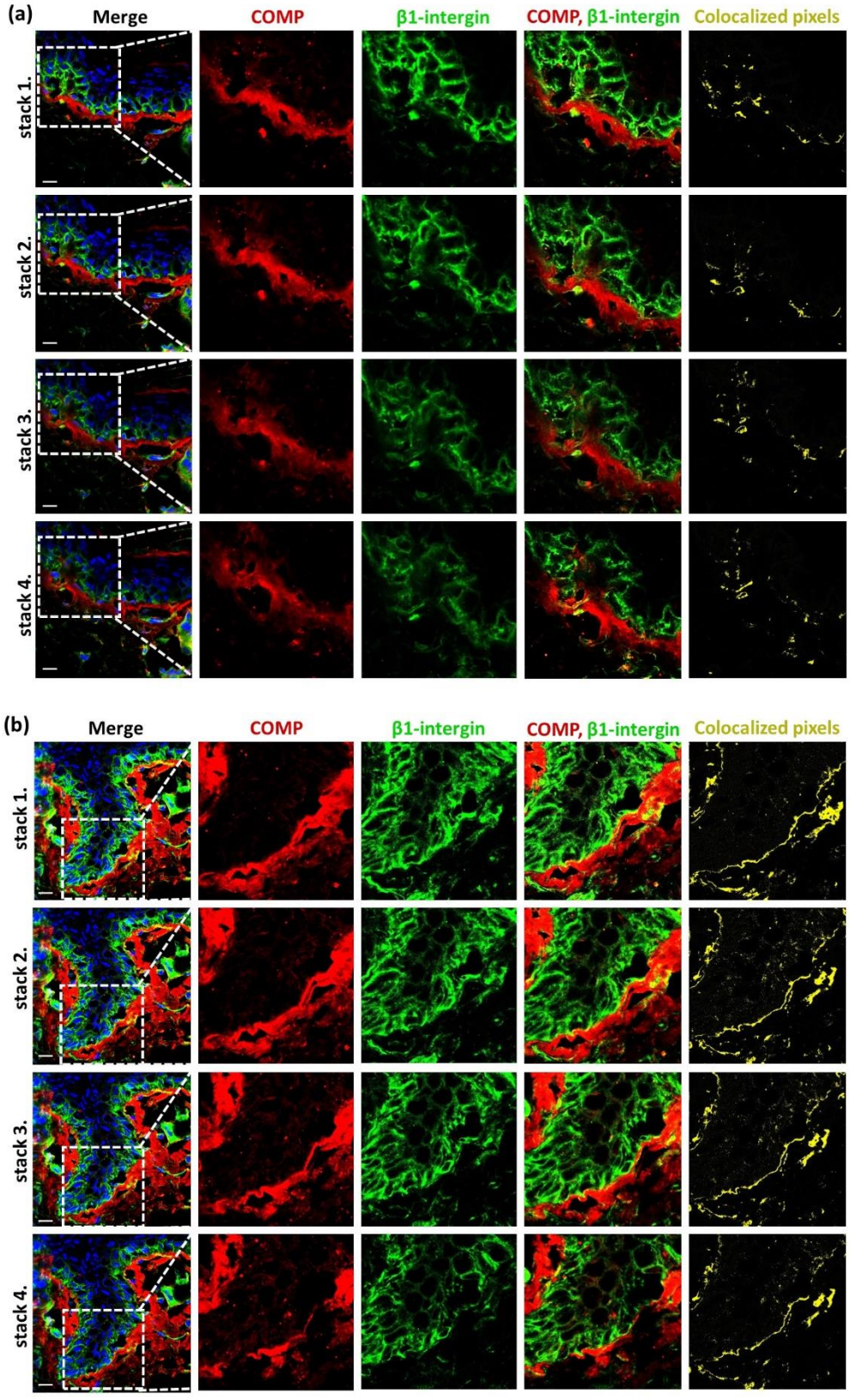

(a)
Merge
COMP
β1-intergin
COMP, β1-intergin
Colocalized pixels
stack 1.
stack 2.
stack 3.
stack 4.
(b)
Merge
COMP
β1-intergin
COMP, β1-intergin
Colocalized pixels
stack 1.
stack 2.
stack 3.
stack 4.

Figure S5.

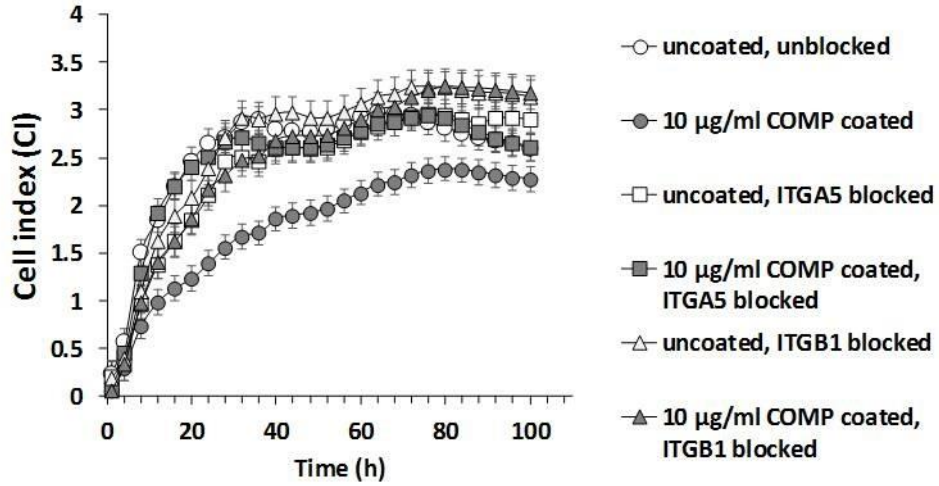
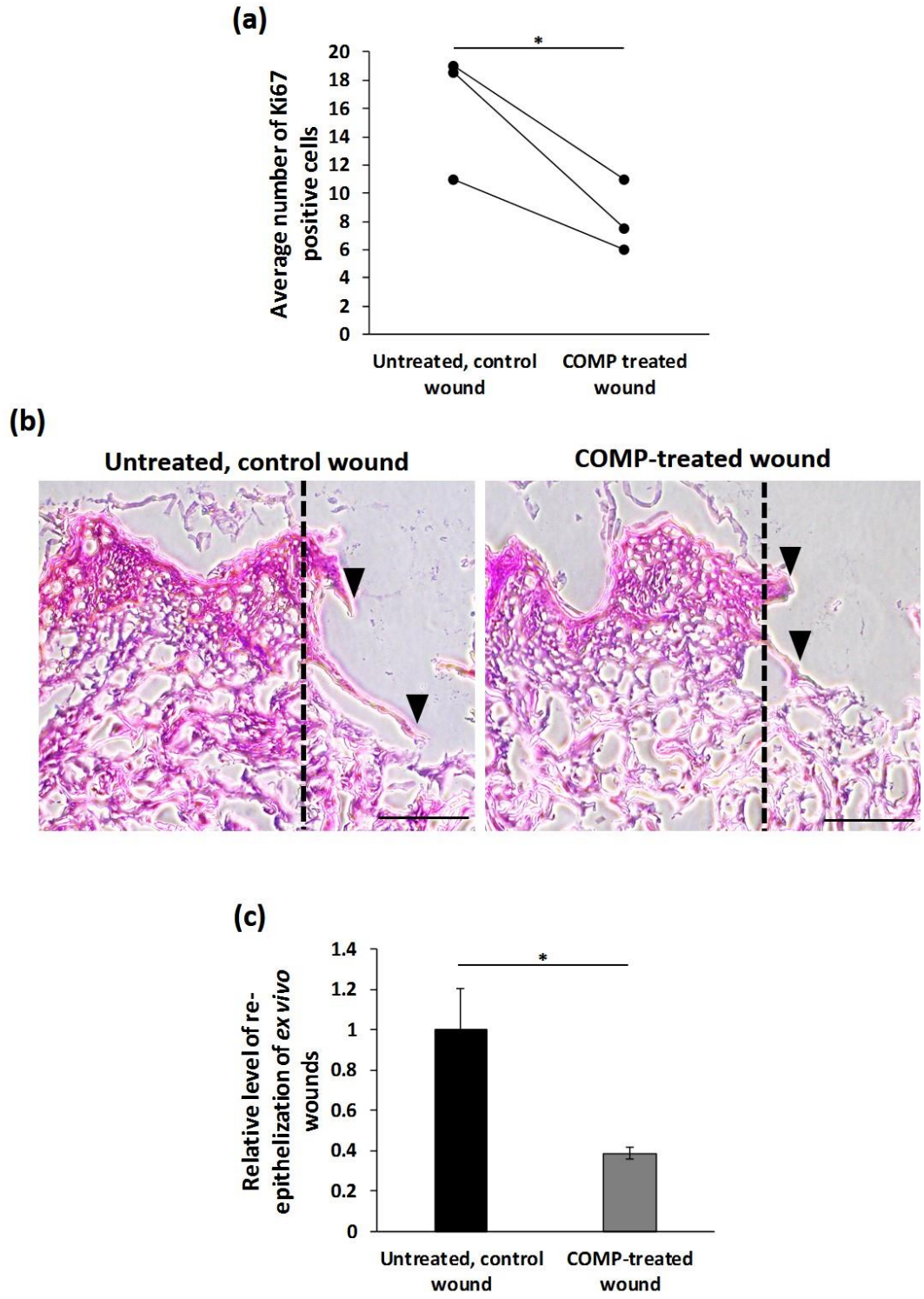

Cell index (CI)
Time (h)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
20
40
60
80
100
uncoated, unblocked
10 µg/ml COMP coated
uncoated, ITGA5 blocked
10 µg/ml COMP coated, ITGA5 blocked
uncoated, ITGB1 blocked
10 µg/ml COMP coated, ITGB1 blocked

Figure S6.


(a)
*
Average number of Ki67 positive cells
20
18
16
14
12
10
8
6
4
2
0
Untreated, control wound
COMP treated wound
(b)
Untreated, control wound
COMP-treated wound
(c)
*
Relative level of re-epithelization of ex vivo wounds
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Untreated, control wound
COMP-treated wound