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**Tape stripping alters the microbe-host correlations in mouse skin**

To the Editor,

Allergic diseases usually begin early in life with atopic dermatitis (AD), progressing to food allergies, asthma and/or rhinitis later in life. AD is characterized by itching and scratching, dermal infiltration with T helper 2 (Th2) cells, and elevated serum IgE and eosinophilia.[1] Continuous scratching causes skin injury and induction of wound repair mechanisms from inflammatory reactions (0-48h) and tissue proliferation (2d-14d) to remodelling (<1 year).[2] Abnormal host-microbe interactions have been shown to associate with cutaneous disorders like AD, acne, and psoriasis, but the exact mechanisms underlying the microbial contributions to disease development and progression are currently unknown.[3] Our aim was to study how skin injury induced by tape-stipping changes mouse skin microbiota, genome-wide gene expression, and how these two coexisting systems interact during epithelial recovery phase up to 29 days.

To study microbiota-host interactions, standardized skin injury was induced to Balb/c mouse skin by eight consecutive tape-strippings (Figure S1). After 1d, 5d, 8d, 11d and 29d, skin swabs and biopsies were collected for 16S rRNA sequencing and genome-wide arrays, respectively. For detailed methodological information, please see online repository. Histologically, both epidermis and dermis showed enhanced thickening, peaking at 8d, and reaching the original thickness in 29d (Figure 1a and Figure S2). Neutrophils were recruited

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to the site of tape-stripping at the early time points (1d), and small number of eosinophils was present in the skin at later time points (5d – 11d) (Figure 1a).

As an injury disturbs the cutaneous homeostasis, we investigated dynamics of the skin microbiota during microbial recolonization by 16S amplicon sequencing. Microbial alpha-diversity increased until 11d, and then started to decrease but did not reach the original diversity seen in the untreated controls (Figure 1b). The operational taxonomical units (OTUs) that changed significantly in relative abundance formed four main clusters in the heatmap containing samples from time points 5d, 11d, ctrl-1d-8d, and ctrl-29d (Figure 1c). The intact skin of the control mice had high proportional abundance of Burkholderiales/Proteobacteria (Figure S3), which are shown to play an important role in skin homeostasis.[4] Alekseyenko et al. divided Proteobacteria-associated cutaneotype to healthy individuals and Actinobacteria/Firmicutes-associated cutaneotypes to psoriatic and lesional skin specimens.[5] Along with this, Firmicutes was the major phylum represented on mouse skin at 5d in our studies, represented primarily by the order Gemellales, which is also categorized into the AD-prone signature microbes in humans.[6] The distribution of bacterial OTUs and their relative abundancies did not fully recover to the original level at our last time point (29d), suggesting that skin irritation by intensive scratching and following barrier disturbance cause long-lasting effects on microbiota composition on the involved epidermal area.

In reverse to microbial changes, a month was enough for the skin transcriptome to recover. The differentially expressed genes (DEGs, Figure S4) formed two separate clusters at 1d and at 5d, and samples at time points 8d and 11d shared the third cluster, whereas gene expressions of the untreated controls and samples at d29 together formed the last cluster (Figure 1d). Gene functions related to inflammation (IL-6/-8/-17, AHR), antigen presentation, chemotaxis and acute phase responses were highly activated while helper T cell pathways (Th1/Th2) and co-stimulation signalling (ICOS, PPAR, LXR/RR) were decreased in Canonical pathway analysis (IPA) especially at the 1d time point (Figure 1e and Figure S5). Inflammation and proliferation phases were recognized in our transcriptomics analysis, and similar pathways have been reported to be activated also in previous studies.[7] Almost all stages of wound healing are affected by various matrix metalloproteinases (MMP), and also in our data, MMPs were highly upregulated especially at 1d and in lesser amount at 5d (Figure S6). Tightly regulated expression of MMPs is crucial for proper re-epithelialization

and regeneration, and their dysregulation leads to prolonged inflammation and delayed wound healing.[8] The microarray results were validated by RT-PCR (Figure S7).

To study the interactions between microbiota and host gene expression, we performed Regularized Canonical Correlation Analysis, which showed a significant correlation between 3 OTUs and 270 DEGs throughout the time points (Figure 2a). OTUs 7 (*Gemella*/Firmicutes) and 8 (*Ralstonia*/Proteobacteria) made up 78.9-87.4% of the skin microbiota composition at all time points. Enhanced abundance of the pathobiont *Gemella* was previously associated with AD[6], whereas abundance of *Ralstonia* was shown to be decreased in the skin of allergic dogs.[9] Especially at 5d, *Gemella* positively correlated with the upper cluster of 118 genes, and anticorrelated with the bottom cluster of 152 genes, whereas *Ralstonia* showed correlations in the opposite manner (Figure 2b). According to the Panther Classification System, the upper cluster genes play roles either in cell division and chromosome organization (Bub1), or in formation of cornified envelope (Lce genes) during keratinocyte differentiation (Figure 2c). In reverse, the lower cluster of genes was involved in muscle cell development and muscle construction, and many of these genes were related to titin (Ttn), troponin (Tnnt3, Tnni2) or actin/myosin (Acta1, Mylk2, Mybpc1, Myl1) filaments (Figure 2d). These genes act mainly during the proliferation phase, when activated fibroblasts and keratinocytes divide and migrate into the injury site to establish a viable barrier, and lesioned tissue area is diminished by wound contraction. In addition to the impact on wound healing and allergic sensitization, better understanding of these interactions might offer instruments for plastic surgery and subcutaneous drug delivery systems.

In conclusion, our results show that tape-stripping disturbs the skin barrier, reversibly changes gene expression in the host, and has long-lasting effects on the skin microbiota. When the microbial OTUs and DEGs were integrated, we found a correlation of proliferation and muscle contraction gene functions with certain Firmicutes and Proteobacteria genera. Although deeper sequencing and identification of the interacting bacterial species is needed, we believe that *Gemella* and *Ralstonia* are examples of bacteria, which play a role in wound healing and (dys)regulation of skin homeostasis. Our results highlight the importance of skin homeostasis, and relevance to further investigate the interactions between microbiota and host gene expression as prolonged dysbiosis may lead to the establishment of skin diseases.

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**Author contribution**

Piia Karisola, Alina Suomalainen and Nanna Fyhrquist involved in the concept and design, in vivo experiments, acquisition of data, analysis, and interpretation of data, drafted the article, and finally approved the version to be published. Vittorio Fortino, Noora Ottman, Johanna Vendelin, Henrik Wolff, Lasse Ruokolainen and Dario Greco involved in acquisition of data, analysis and interpretation of data, critically revised the article, and finally approved the version to be published. Harri Alenius involved in study concept and design, analysis, and interpretation of data, critically revised the article, and gave final approval of the version to be published.

**Conflicts of interest**

The authors state no conflict of interest.

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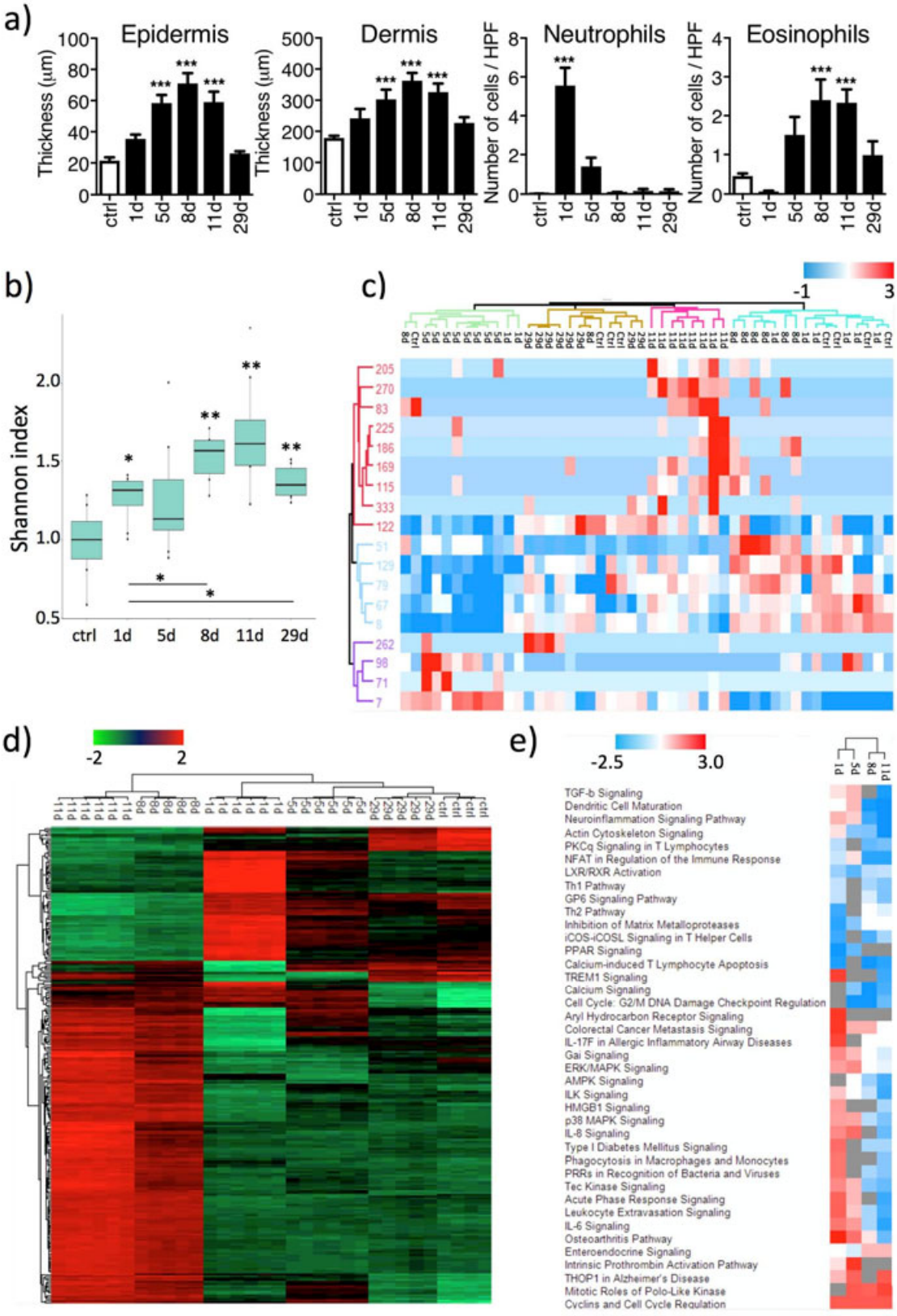
**Figure legends**

**Figure 1.** Tape-stripping thickens skin layers, changes microbiome and modifies gene expressions. (a) Skin thicknesses were measured, and number of infiltrating cells were counted from the H&E stained sections. (b) Shannon diversity index. (a-b) For comparisons nonparametric MannWhitney U-test was used, and results are expressed as mean ±SEM. *P=0.05; **P=0.01; ***P=0.001. (c) The most significantly changed 18 microbial operational taxonomic units (OTUs) were clustered based on their normalized (Z-scored) abundancies according to time with Pearson distance method. (d) Differentially expressed genes (DEGs) were hierarchically clustered by similarity based on Euclidean correlation. (e) Ingenuity pathway analysis (IPA) of DEGs on Canonical pathways.

**Figure 2.** Data integration shows correlation between microbiota and skin transcriptome. (a) Heatmap and hierarchical clustering of interacting 270 differentially expressed genes (DEGs) correlating with 3 microbial operational taxonomic units, OTUs 7, 8 and 67. (b) Relative expression of top and bottom clusters of the DEGs in (a) with relative abundancies of OTUs 7 and 8. Fifteen of the most significant GO biological processes and their interrelating genes in the top (c) and bottom (d) clusters according to the Panther Classification System.



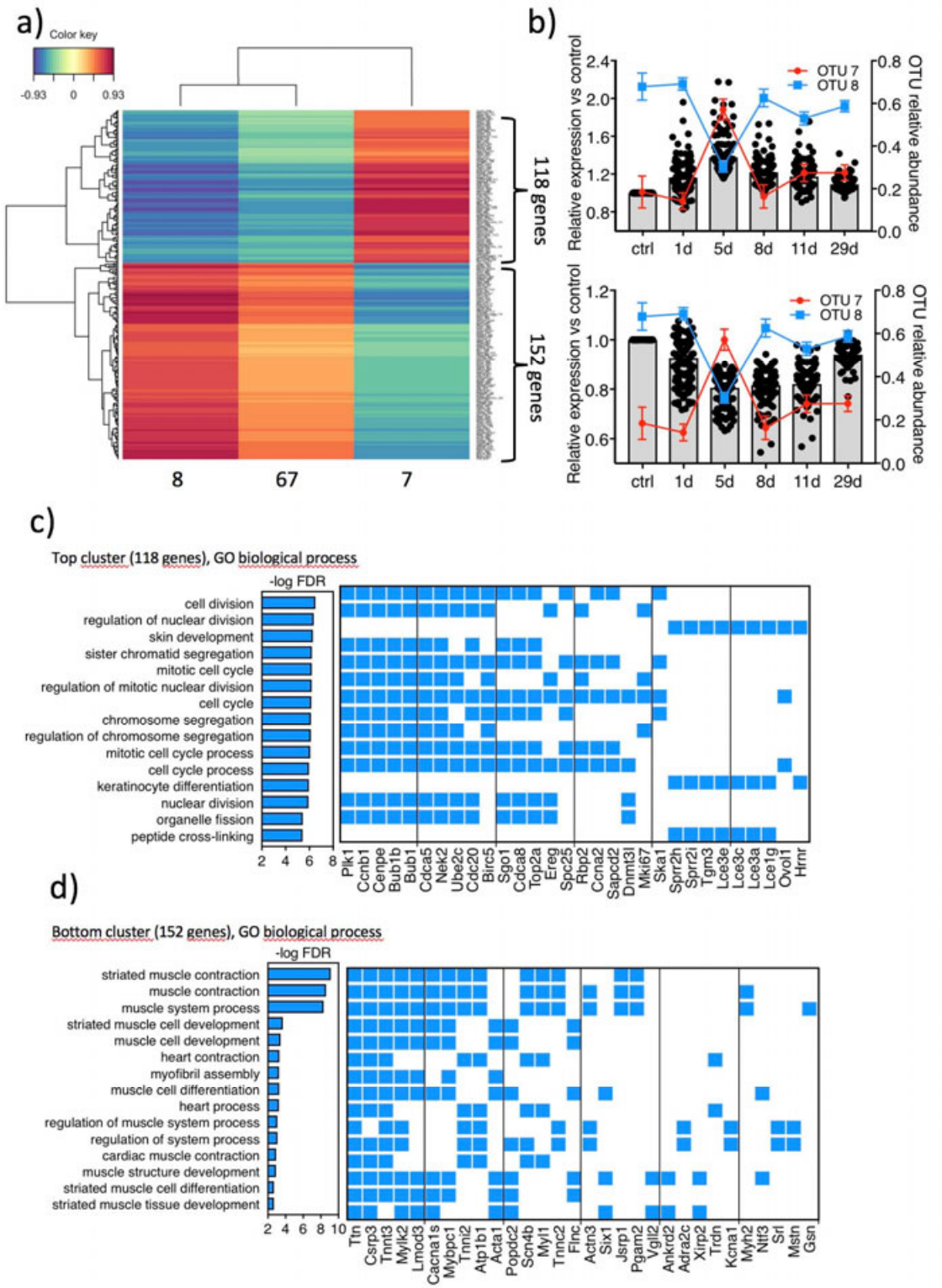



Figure 2