TITLE

Commensal Microbiota Regulates Skin Barrier Function And Repair Via Signaling Through The Aryl Hydrocarbon Receptor

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SUMMARY

The epidermis forms a barrier that defends the body from desiccation and entry of harmful substances, while sensing and integrating environmental signals. The tightly orchestrated cellular changes required for the proper formation and maintenance of this epidermal barrier occur in the context of the skin microbiome. Using germ free mice, we demonstrate the microbiota is necessary for proper differentiation and repair of the epidermal barrier. These effects were mediated by the aryl hydrocarbon receptor (AHR) in keratinocytes, a xenobiotic receptor also implicated in epidermal differentiation. Murine skin lacking keratinocyte AHR was more susceptible to barrier damage and infection, during steady state and epicutaneous sensitization. Colonization with a defined consortium of human skin isolates restored barrier competence in an AHR-dependent manner. We reveal a fundamental mechanism whereby the microbiota regulates skin barrier formation and repair, with far-reaching implications for the numerous skin disorders characterized by epidermal barrier dysfunction.

KEYWORDS

Skin; barrier; microbiome; epidermis; aryl hydrocarbon receptor; keratinocyte; microbiota; commensal

MAIN TEXT

INTRODUCTION

The skin is the primary barrier between the human body and the environment and functions to prevent desiccation and entry of foreign and/or harmful substances. The barrier properties of the skin reside in the epidermis, a semi-permeable stratified epithelium that is formed as a result of keratinocyte terminal differentiation. Though continuously exposed to xenobiotic toxins, physical insults, and pathogenic microbes, the epidermis is also associated with diverse commensal microbial communities that are critical players in regulating skin physiology [1]. These microbial communities, collectively referred to as skin microbiota, are specialized to thrive in the unique nutrient and environmental conditions of this organ. The skin microbiome is topographically diverse, temporally complex, and distinct from other organs [2, 3]. How the commensal microbiota influences development of skin's barrier function is undefined, as are the molecular mechanisms that mediate these interactions.

The barrier function of the skin may be conceptualized as four intertwined "levels" consisting of microbial, immune, chemical, and physical barriers [4]. The skin microbiome itself provides a barrier to pathogenic micro-organisms via a variety of different mechanisms e.g. production of proteases, antimicrobial peptides and antibiotics, and interference with quorum sensing [5]. This outermost microbial barrier also interacts with and mediates other functional levels of the cutaneous barrier. Skin microbiota play a fundamental role in the induction, training, and function of the skin immune barrier in part through the release of antimicrobial peptides, short-chain fatty acids, and polyamines [6]. Neonatal colonization by microbiota has long-lasting impacts on adult immune barrier as commensal skin microbes' prime immune cells to differentiate between commensal versus pathogenic bacterium [7]. Bacterial lipases can hydrolyze lipids resulting in production of free fatty acids that impact the acidic surface pH of the skin, which dictates the chemical barrier of the skin [8, 9]. While studies in gnotobiotic mice suggest that epidermal differentiation and barrier genes are microbially regulated [10],

66 mechanistic roles for the skin microbiota in development, regeneration, and function of the
67 physical barrier are not well defined.

68 The epidermal permeability barrier (EPB) comprises of the stratum corneum and a
69 complex system of tight junctions and adhesion complexes and their associated cytoskeletal
70 networks that mediate cell-cell adhesion to create a mechanical barrier between the
71 environment and underlying tissue [11]. Actively dividing keratinocytes in the stratum basale
72 commit to terminal differentiation and move progressively into suprabasal layers, i.e. stratum
73 spinosum, the stratum granulosum and eventually the stratum corneum [12]. In the stratum
74 corneum, keratinocytes become flattened and denucleated (which are then called corneocytes),
75 and plasma membranes are replaced with cornified envelopes. Lamellar bodies secrete their
76 lipid-rich contents into the intercellular space between the corneocytes and are subsequently
77 processed into barrier-providing lipid lamellae. Altogether, corneocytes, lipids and a complex
78 network of trans-membrane proteins, provide a highly hydrophobic EPB against the
79 environment. Microbial influences on this process of epidermal differentiation and EPB
80 formation are not well understood, nor are the mechanisms whereby the EPB senses and
81 responds to changes in the microbiota.

82 The sensing of xenobiotics, or compounds foreign to a living organism, is critical for
83 barrier defense and homeostasis in the skin [13]. Keratinocytes function as sentinels that sense
84 and respond to external stimuli [14]. Activation of xenobiotic receptors in keratinocytes induces
85 expression of detoxification enzymes and membrane transporters that promote elimination of
86 toxic compounds[15]. Accumulating evidence suggests that roles for xenobiotic receptors
87 extend to cellular processes beyond xenobiotic metabolism that include cellular proliferation,
88 tissue repair, and immune responses [13]. Microbes produce a plethora of small molecules and
89 secondary metabolites, which are hypothesized to mediate their interactions with the host
90 toward a mutualistic relationship [16]. The diversity of molecular signals produced by skin
91 microbes, and how keratinocytes decipher and respond to them, remain largely unexplored.

92 The aryl hydrocarbon receptor (AHR) is a xenobiotic receptor that has emerged as a
93 critical player in EPB development, function and integrity. Activation of the AHR, a ligand-
94 activated transcription factor of the basic, helix-loop-helix motif-containing Per-ARNT-Sim family
95 [17], induces a variety of epidermal differentiation and barrier genes, accelerates terminal
96 differentiation, and increases stratum corneum thickness [18-22]. The AHR can be activated by
97 halogenated and non-halogenated aromatic hydrocarbons, including dioxins such as 2,3,7,8-
98 Tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls (PCBs) and polycyclic aromatic
99 hydrocarbons (PAHs); clinically used drugs, food-derived molecules, endobiotics, and bacterial
100 metabolites [23-26]. Microbial regulation of the AHR in the context of the skin barrier remains
101 poorly understood, as well as the consequences of perturbing the commensal microbiota with
102 respect to EPB function and defense.
103 In addition to congenital barrier deficiencies, epidermal barrier dysfunction is a hallmark
104 of inflammatory skin diseases, including atopic dermatitis and psoriasis, and predisposes skin to
105 infections [27-29]. Additionally, epicutaneous sensitization, as a result of epidermal barrier
106 dysfunction, may lead to atopic and allergic disease [30].Thus, there is an urgent scientific and
107 clinical need to define the mechanistic basis by which the commensal microbiota regulate
108 homeostatic barrier function, as such mechanisms provide new targets for prevention and/or
109 intervention in skin barrier deficiencies.
110 Here, we investigated the role of commensal microbiota in regulation of permeability
111 barrier homeostasis of skin. We found that commensal microbes are necessary for normal
112 epidermal differentiation, EPB function, and repair. These effects were mediated by microbial
113 signaling through the keratinocyte AHR. Murine skin lacking keratinocyte AHR signaling
114 displayed increased barrier permeability, enhanced susceptibility to infection by *S. aureus,* and
115 increased pathology in a model of atopic dermatitis. We show that topical colonization with a
116 defined consortium of human skin commensals improves EPB function in murine germ-free skin
117 and models of barrier dysfunction. Our findings reveal a fundamental role for the commensal

118 skin microbiota in regulating the physical integrity and repair of the skin barrier, provides
119 mechanistic insights into microbial-skin crosstalk, and uncovers therapeutic targets for
120 improving skin barrier function.

121 **RESULTS**

122 **Epithelial development and differentiation programs are impaired in germ free skin**

123 To characterize microbially-mediated regulation of homeostatic epithelial gene
124 expression programs, we performed RNA-seq on epidermal sheets isolated from dorsal skin of
125 *C57BL/6* mice of 3 different colonization states (n=8 mice each. **Figure 1A**): Specific pathogen
126 free (SPF) mice that were conventionally raised in presence of microbiota, germ free (GF) mice
127 born and raised in sterile gnotobiotic isolators, and a third group of mice that were born GF and
128 then colonized (COL) with SPF microbiota for 2 weeks. Within 2 weeks, COL mice were
129 colonized with microbiota from SPF mice as validated by 16S ribosomal RNA (rRNA) gene
130 sequencing (**Figure 1B**). We identified differentially expressed genes (DEGs) between
131 colonization states by training negative-binomial linear models using DESeq2 R package and 3-
132 way comparisons. This analysis revealed 6396, 427, and 3232 DEGs for SPF vs. GF, COL vs.
133 GF and SPF vs. COL comparisons, respectively (**Figure 1C, Table S1**). We focused on the 396
134 shared DEGs of SPF and COL epidermis when compared to GF epidermis (**Figure 1D**). We
135 reasoned that this subset of DEGs meet the criteria of being induced and sustained by microbial
136 colonization, suggesting homeostatic control. The 396 DEGs were significantly enriched for
137 biological functions such as skin development, keratinocyte differentiation, and epidermis
138 development (**Figure 1E**). This result suggests the microbiota plays an important role in
139 epithelial barrier formation.

140 To further examine DEGs involved in epithelial barrier function, we manually curated
141 genes involved in different facets of epithelial barrier: keratinization, cornified envelope
142 formation, adherence and gap junction, basement membrane function, barrier development,
143 differentiation and intercellular lipid-lamellae processing (**Table S2)**. Focusing on the SPF vs.

144 GF subset of DEGs, multiple genes across each of these categories were expressed at lower
145 levels in GF mice (**Figure 1F**, **Tables S3, S4**). In particular, genes critical for cornified envelope
146 formation [e.g. *involucrin (Ivl)*, *envoplakin (Evpl)*] and its desquamation, [e.g. *Kallikrien-related*
147 *peptidases 5, 7(Klk5, 7)*] were downregulated in GF skin. We hypothesized that such significant
148 and widespread differences in gene expression would result in structural differences between
149 GF and SPF skin. However, consistent with prior reports [10, 31] we did not notice any overt
150 differences between the epithelial organization of GF vs SPF mice by traditional
151 histopathological examination (**Figure S1**). Analysis of skin ultrastructure by electron
152 microscopy showed that overall, SPF mice had a greater number of individual layers within the
153 stratum corneum than GF mice (**Figure S1**). Immunofluorescence-based analysis of molecular
154 biomarkers showed decreased expression of loricrin and cytokeratin-10 in skin of GF mice
155 (**Figure 1G**) that are implicated in barrier integrity [32-34]. Among genes that were
156 downregulated in GF skin were tight and adherens junctions family members (**Figure 1F**) such
157 as *tight junction protein 3 (Tjp3), desmogleins 1a-b (Dsg1),* and *claudin-1 (Cldn1)* that are
158 critical players involved in skin barrier formation (reviewed in [35]). Remarkably, tight junction
159 integrity appeared compromised in the suprabasal epithelium as evident by downregulated and
160 diffused expression of *Dsg1* in GF mouse epidermis (**Figure 1G)**. Disruption of *Dsg1* is
161 associated with improper formation of desmosomes in suprabasal epithelia and has been
162 associated with skin barrier impairment [36-38]. Together, these findings suggest the
163 hypothesis that skin barrier formation requires the commensal microbiota.

164 **Commensal microbiota promotes skin barrier function and repair**

165 A fully functioning stratum corneum closely controls the water concentration gradient in
166 the skin such that passive diffusion of water occurs from inner layers towards the outside.
167 Barrier disruption compromises the ability of the stratum corneum to maintain this water
168 concentration gradient and results in increased transepidermal water loss (TEWL), measured
169 using a sensor for water vapor flow density [39, 40]. Low TEWL values are indicative of intact

170 skin and increased TEWL is associated with a disrupted barrier (**Figure 2A, Panel i**). Under
171 basal conditions, GF mice had slightly increased TEWL compared to SPF mice corroborating
172 the findings from ultrastructure analysis (**Figure S2)**. Skin barrier of GF mice was perturbed
173 more readily with tape stripping than SPF skin (**Figure S2**), consistent with fewer layers of
174 stratum corneum in GF mice. After comparable insults following tape-strip injury (TEWL 15-20
175 $g/m^2/h$), SPF mice more rapidly repaired their barrier compared to GF mice when measured
176 over a period of 24 hours (**Figure 2B**). We observed similar delays in barrier recovery in GF
177 *Rag1*$^{-/-}$ mice (that lack mature T and B-cells) compared to age-matched SPF *Rag1*$^{-/-}$ mice
178 (**Figure 2C, Figure S2**) suggesting that microbially-regulated adaptive immune responses are
179 not responsible for the delayed barrier repair phenotype. Skin from GF *Rag1*$^{-/-}$ mice also
180 showed decreased expression of genes involved in terminal differentiation and formation of
181 transmembrane junctions compared to SPF *Rag1*$^{-/-}$ mice (**Figure S2)** as seen in wild-type mice.
182 Basal keratinocytes undergo a spatiotemporal and highly controlled differentiation
183 program dependent on intracellular calcium flux to establish and maintain barrier [41]. In
184 presence of high calcium, primary epidermal keratinocytes can differentiate *in vitro* to express
185 genes involved in formation of cornified envelope [42]. We derived murine primary epidermal
186 keratinocytes from GF and SPF skin, respectively, and exposed them to high calcium containing
187 medium (**Figure 2D**). Expression of genes involved in terminal differentiation [*cytokeratin-10*
188 *(Krt10)*, *involucrin (Ivl)*] and formation of transmembrane junctions [*Corneodesmosin (Cdsn),*
189 *Desmocollin-1(Dsc1), Desmoglein-1a (Dsg1a)*] were reduced in GF keratinocytes compared to
190 SPF keratinocytes (**Figure 2E, Figure S2)**. Additionally, GF keratinocytes had decreased
191 transepithelial electrical resistance (TEER) which is indicative of decreased transmembrane
192 junction strength (**Figure 2F, Figure S2**).
193 To further examine the implications of perturbing the microbiota on skin barrier function
194 we used an antibiotic depletion model (**Figure 2G**). Prior studies had shown that antibiotics
195 traditionally used to disrupt gut microbiota in mice were not sufficient to disrupt skin microbiota

196 in mice [43]. We developed a new regimen consisting of antibiotics (Metronidazole,
197 Sulfamethoxazole, Trimethoprim, Cephalexin and Enrofloxacin) that are administered orally in
198 hospitals and veterinary clinics to target skin bacteria [44-47] and were able to inhibit prominent
199 murine skin commensal *Staphylococcus xylosus* [48, 49]. Oral administration of antibiotics for
200 two weeks diminished microbial burden on skin as observed by both quantitative cultures and
201 16S rRNA gene sequencing (**Figure 2H, I**) but did not significantly affect microbial burden in the
202 gut (**Figure S3).** Antibiotic-treated mice were delayed in barrier repair compared to control mice
203 that were treated with vehicle (**Figure 2J**). Together, these data confirm a role for commensal
204 microbes in promoting skin barrier function and repair.

205 **Aryl hydrocarbon receptor pathway is attenuated in germ free skin**

206 The sensing of external physiological and chemical signals is critical for barrier defense
207 and homeostasis in the skin [13]. Therefore, we hypothesized that xenobiotic receptors that act
208 as epithelial sensors and relay microbial signals would be among those genetic pathways
209 dysregulated in GF epidermis. Previous studies have identified at least 304 xenobiotic
210 processing genes (XPGs) in mice, which encode the enzymes, transporters, and transcription
211 factors required to metabolize xenobiotics [50]. We found that 52/304 XPGs were differentially
212 expressed ($P<0.05$) in skin of SPF and GF mice, and the majority were upregulated in SPF
213 mice (n=43/52; **Table S5**). The pregnane X receptor (PXR*, NR1I2*), constitutive androstane
214 receptor (CAR*, NR1I3*), peroxisome proliferator-activated receptor-alpha (PPARa) and aryl
215 hydrocarbon receptor (AHR) are key transcription factors that regulate xenobiotic processing in
216 skin [51]. Of these, only the AHR gene was differentially expressed and was upregulated in SPF
217 skin compared to GF skin. Canonically, after ligand binding, the AHR translocates to the cell
218 nucleus and binds DNA at xenobiotic responsive elements (XRE), to regulate transcription of
219 target genes [52]. Expression of key downstream target genes, i.e. cytochrome-p450 *Cyp1a1*
220 and molecular chaperones *Hsp90aa1* and *Hsp90ab1* that respond to AHR activation, were also
221 downregulated in GF murine epidermis (**Figure 3A**). Primary keratinocytes derived from GF skin

222 were also impaired in expression of these genes (**Figure S2**). Overall, microbiota-mediated
223 upregulation of *AHR* was consistent with changes in its canonical pathway, suggesting that
224 regulation of xenobiotic processing genes in the skin may be mediated through the *AHR*.
225 Since the AHR can activate multiple signaling pathways [53], we explored the impact of
226 microbial colonization on expression of genes reported to be a part of canonical and non-
227 canonical AHR signaling networks (**Figure 3A**). In this context, we saw upregulation of several
228 genes in SPF mice, including those involved in cell proliferation, differentiation, and
229 inflammation (e.g. *Hes1, Jun* and *Tgfb1).* Together these findings suggest the AHR as a
230 potential mechanism by which skin microbes modulate epithelial barrier integrity.
231 **Treatment with an AHR agonist improves barrier function and recovery in germ free mice**
232 We hypothesized that if the GF skin barrier phenotype we observed was due to
233 attenuated AHR signaling, then activation via AHR ligand would improve barrier recovery. We
234 treated adult GF mice topically with AHR ligand 6-Formylindolo[3,2-b] carbazole (FICZ), daily for
235 2 weeks at a low dose (100µg/kg) (**Figure 3B**), a regimen shown to induce expression of
236 *Cyp1a1* [54]. FICZ is a tryptophan photoproduct and is a well-characterized AHR ligand in skin
237 [55, 56]. At the end of treatment, we compared the rate of barrier recovery in tape-stripped GF
238 mice that were either treated with FICZ or vehicle (**Figure 3C**). We observed that FICZ
239 significantly accelerated barrier recovery in GF mice. In parallel, we also treated SPF mice with
240 FICZ at the same dose and observed that FICZ accelerated early stage barrier recovery
241 (**Figure3D**). FICZ treatment activated AHR signaling in the treated group as *CYP1A1*
242 expression was induced in all FICZ treated mice in comparison to the untreated group (**Figure**
243 **3E**). Additionally, FICZ treatment increased expression of genes implicated in barrier repair
244 (**Figure 3E**) in GF mice. AHR upregulation increases expression of genes involved in epidermal
245 differentiation and formation of gap junctions [57]. In primary human keratinocytes, FICZ
246 increased epithelial resistance as measured by TEER (**Figure 3F, Figure S4**), which was
247 diminished by co-treatment with an AHR inhibitor (**Figure 3G**). Overall, activation of the AHR in

248 GF skin improves epidermal barrier recovery, supporting a mechanistic role for the commensal
249 microbiota in homeostatic regulation of the AHR.

250 **Mice deficient in epithelial AHR have a defective skin barrier and are more susceptible to**
251 **infection**

252 AHR is expressed in a variety of cell types in the skin, but abrogating AHR function in
253 keratinocytes has been suggested to impact skin barrier [58]. To confirm the role of keratinocyte
254 AHR in skin barrier function, we generated mice where the *floxed Ahr* allele ($Ahr^{f/f}$) was
255 conditionally knocked out in the skin epithelia using a *Cre* driven by the keratin-14 promoter
256 ($K14^{Cre/+}Ahr^{f/f}$). While littermates that retained AHR function ($Ahr^{f/f}$) repaired their barrier within
257 24 hours following tape-strip disruption, barrier repair was significantly diminished in
258 $K14^{Cre/+}Ahr^{f/f}$ skin (**Figure 4A**). Additionally, keratinocytes derived from $K14^{Cre/+}Ahr^{f/f}$ mice
259 showed increased TEER compared to $Ahr^{f/f}$ keratinocytes (**Figure 4B**).

260 Patients with diseases of skin barrier impairment such as atopic dermatitis (AD) are
261 highly susceptible to colonization and infection by pathogens including *S. aureus* [59]. To test if
262 AHR-dependent barrier impairment leads to increased infection, we topically applied *S. aureus*
263 to tape-stripped skin [60] and quantified bacteria following 48 hours. We observed enhanced
264 infection of *S. aureus* and increased overall bacterial burden on tape stripped skin (**Figure 4C**)
265 of $K14^{Cre/+}Ahr^{f/f}$ mice compared to AHR sufficient controls (**Figure 4D**). Since barrier dysfunction
266 is a hallmark in the development of AD [61], and mice lacking AHR are impaired in barrier
267 repair, we hypothesized that these mice will be more prone to barrier damage, infection, and
268 atopic disease. We adapted a mouse model of AD (**Figure 4E**) induced by repeated
269 epicutaneous sensitization of tape-stripped skin with ovalbumin (OVA) [30]. In this model,
270 $K14^{Cre/+}Ahr^{f/f}$ skin was exacerbated in disease pathology, with enhanced TEWL (**Figure 4F**) and
271 increased susceptibility to *S. aureus* infection compared to $Ahr^{f/f}$ mice (**Figure 4G**). Together,
272 these data demonstrate that AHR function in keratinocytes is essential for barrier function, and

273 loss of AHR can lead to enhanced barrier damage in the setting of inflammatory skin disease
274 and facilitate bacterial entry.

275 **Human skin microbial consortium restores barrier repair and function via the AHR**

276 In the gastrointestinal tract, the AHR is activated by the gut microbiota [62] to enhance
277 intestinal barrier integrity by inducing tight junction proteins in intestinal epithelial cells [63].
278 However, the relation between skin commensals and cutaneous AHR-dependent barrier
279 regulation is unexplored. Given our observations that the commensal microbiota directly impacts
280 barrier repair (**Figure 1, 2**) and regulates the AHR genetic pathway (**Figure 3**) in murine skin,
281 we hypothesized that topical association of GF skin with human skin commensals would
282 activate the AHR and restore skin barrier repair. To test this hypothesis, we first curated a
283 collection of cultured skin microbes that were abundant and prevalent on healthy human skin.
284 Referred to as Flowers' Flora, the collection consists of members of Firmicutes phylum i.e.
285 *Staphylococcus epidermidis, S. warneri, S. hemolyticus* and members of Actinobacteria phylum
286 i.e. *Micrococcus luteus, Corynebacterium aurimucosum* (**Figure 5A**). These skin microbes
287 activated AHR in keratinocytes as determined by way of a reporter assay consisting of the AHR
288 reporter element conjugated with *Cyp1a1* (**Figure 5B**). Flowers' Flora colonized murine GF skin
289 as determined by bacterial culture swabs and species-specific qPCR analysis (**Figure 5C, D**).
290 After two weeks of colonizing with this defined consortium of human skin commensals (**Figure**
291 **5E**) barrier recovery function in GF skin was restored (**Figure 5F**). Skin of colonized mice
292 showed elevated expression of differentiation genes as well as *Cyp1a1* (**Figure 5G**) compared
293 to GF skin. Upon terminal differentiation, keratinocytes derived from colonized mice were
294 enhanced in TEER compared to GF controls (**Figure 5H**). Colonization of $K14^{Cre/+}Ahr^{f/f}$ mice
295 with Flowers' Flora did not improve barrier repair (**Figure 5I**), further supporting an AHR-
296 dependent mechanism. Finally, to determine if Flowers' Flora could prevent barrier damage in
297 AD-like disease, we pre-colonized wild-type SPF murine skin prior to short-term epicutaneous
298 sensitization with OVA (**Figure 5J**). Pre-colonization with Flowers' Flora significantly improved

299 barrier recovery compared to skin that was not pre-colonized (**Figure 5K**). Together, these
300 findings indicate that skin commensal microbes signal through the AHR to maintain homeostatic
301 control of epidermal barrier integrity, and suggest new targets for preventing and/or treating
302 epidermal barrier dysfunction.

303 **DISCUSSION**

304 The skin microbiome provides the first level of barrier defense to the human body. While
305 commensal skin microbes have demonstrated effects on immune and chemical barriers of the
306 skin, their regulation of the physical barrier is not well-defined. Proper epithelial differentiation
307 and cornification is essential for formation of the epithelial barrier [11, 64, 65]. By using
308 comparative transcriptomics of gnotobiotic mice, we identified genes involved in skin
309 development, differentiation and barrier function as top candidates for microbial regulation.
310 Using multiple models of microbial depletion, loss of commensal microbiota impaired the barrier
311 repair function of the skin. We mechanistically linked the xenobiotic receptor AHR in mediating
312 microbial signals to the keratinocyte to boost epithelial differentiation and adhesion, and thus
313 integrity of the EPB. The absence of such signaling resulted in exacerbated pathology and
314 increased bacterial infection in models of barrier disruption and epicutaneous sensitization.
315 Finally, we showed that topical association with a consortium of human skin commensals
316 restores skin barrier function and integrity, an effect that was dependent on the keratinocyte
317 AHR.

318 Our findings parallel those in the gastrointestinal tract, where gut commensals have
319 been demonstrated to regulate intestinal barrier formation by modulating epithelial turnover [66]
320 and controlling mucus production [67]. However, unlike the simple mucosal epithelium that
321 provides the intestinal barrier, the skin is composed of a multi-layered stratified squamous
322 epithelium that terminally differentiates. Such complexity requires tightly orchestrated signals to
323 balance differentiation with proliferation. We show here that skin commensals produce signals
324 that directly regulate epithelial stratification through the xenobiotic sensor AHR. Future studies

325 will be required to address the identity of the microbial metabolites that interact spatially within
326 the complex architecture of the skin.

327 Our studies focused on the keratinocyte AHR and its role in forming the EPB, but the
328 AHR can also be expressed by epidermal Langerhans cells, innate and adaptive immune cells,
329 and dermal cells. Previous studies suggest that repair of the EPB is not dependent on AHR
330 derived from Langerhans cells [58]. However, during epicutaneous sensitization, Langerhans
331 specific loss of AHR led to decreased Langerhans cells number and function and dysregulated
332 T cell responses [68]. Thus, AHR likely represents a sensor for Langerhans cell activation as
333 part of the immunological barrier. Further studies will need to better define the cell-type
334 specificity of AHR signaling to the different levels of barrier function, including the immune
335 barrier.

336 Through depletion models and topical association with defined microbial consortia, we
337 demonstrate the necessity and sufficiency, respectively, of commensal microbes in epithelial
338 barrier function. We note that while colonization of germ-free mice with commensal microbes for
339 two-weeks (COL) resulted in restoration of key epithelial differentiation signals, there were still
340 differences between gene expression profiles of COL mice and SPF mice raised in the
341 presence of microbes. It has been shown that skin-resident immune cells rely on imprinting by
342 early life bacterial exposures to regulate acute wound healing in adult mouse skin [69].
343 Therefore, it is possible that certain skin development genes rely on early-life exposure to the
344 microbiota for complete restoration of epithelial gene expression profiles.

345 Our data supports the hypothesis that sensing of microbial signals by the xenobiotic
346 receptor AHR are crucial for self-renewal required of the epidermis. Topical application of coal
347 tar is one of the oldest therapies for atopic dermatitis and has been shown to activate AHR to
348 induce epithelial differentiation [70]. Recently, the natural product derived small molecule,
349 Tapinarof, was found to bind and activate the AHR to moderate inflammatory responses in
350 atopic dermatitis and psoriasis [71]. In support of the hypothesis that AHR mediates microbial

351 signals to promote barrier function, we found that treatment with the potent and selective AHR
352 ligand FICZ at a low dose was able to restore epithelial barrier repair in GF mice and induced
353 epithelial differentiation. However, the role of AHR in skin barrier regulation may be highly
354 context dependent. In murine models, exposure to pollutants can lead to hyperactivation of AHR
355 that results in skin barrier damage and inflammation, which mirrors the phenotype of mice that
356 constitutively express AHR in the keratinocyte [72, 73]. Thus, the balance in the specificity and
357 quantity of AHR ligand, from endogenous and environment sources, is likely a key factor in
358 modulating downstream signaling and impact on the skin, and requires further investigation.
359 AHR has now been recognized as an intracellular pattern recognition receptor that can
360 identify and metabolize bacterial pigmented virulence factors, and promote antibacterial defense
361 responses [74]. Our studies show that loss of AHR in skin led to increased susceptibility to the
362 skin pathogen *S. aureus.* It remains to be determined whether increased susceptibility was due
363 to impaired physical barrier, impaired antimicrobial barrier, or both. For example, the microbiota-
364 induced antimicrobial protein RELMα protects against skin infection in a Vitamin A dependent
365 manner [75]. Since there is interaction between AHR and retinoic acid signaling pathways [76,
366 77], this may represent a mechanism by which the skin microbiota mediates the antimicrobial
367 barrier. The differential roles for pathogens and commensals in regulating AHR and promoting
368 downstream effects are undefined, though are critical when considering diseases of barrier
369 impairment or wounding, which are often complicated by *S. aureus* colonization and/or infection.
370 In summary, our findings show a role for skin microbiota in regulating epithelial
371 differentiation and barrier function in stratified epithelia through AHR. These studies show that
372 skin microbiome directly impacts development of the epidermal physical barrier. Future studies
373 that address how microbial communities interact with each other to influence xenobiotic signals
374 in homeostatic versus disease states will help leverage how personalized microbiota-based
375 therapies can be used to improve the skin barrier.
376 **FIGURE LEGENDS**

377 **Figure 1. Commensal microbiota regulates epithelial barrier genes. (A)** Three
378 groups of mice were employed, specific pathogen free (SPF), germ free (GF), and germ-free
379 mice colonized (COL) with SPF microbiota for 2 weeks. **(B)** Skin microbiota composition
380 determined by 16S rRNA gene sequencing. Y-axis indicates absolute read counts of most
381 abundant phylum (by relative abundance in the dataset) for each mouse (x-axis). **(C)** RNA-seq
382 workflow. **(D)** Overlap of differentially expressed genes when comparing groups of gnotobiotic
383 mice. **(E)** Shown in white are the number of genes that were further analyzed for uniquely
384 enriched gene ontology biological process terms for aforementioned DEGs. Shown on the y-
385 axis are the uniquely enriched terms, with *p*-values indicated on the x-axis. *P*-values are based
386 on Fisher's exact test and FDR-adjusted under dependency using the "BY" method. **(F)** To
387 examine DEGs involved in epithelial barrier function, we manually curated genes involved in
388 different facets of epithelial barrier: keratinization, cornified envelope formation, adherence and
389 gap junction, basement membrane function, barrier development, differentiation and intercellular
390 lipid-lamellae processing. Shown here is a snapshot of key genes that were differentially
391 expressed in the SPF vs GF subset ($p<0.001$). Horizontal bars represent the Log2 fold-change
392 comparison (genes upregulated in SPF: log2FC> 0, downregulated in SPF: log2FC < 0). Error
393 bars represent standard error estimate for the log2 fold-change computed using the DESeq2
394 package. **(G)** Tail-skin from SPF and GF mice. Immunofluorescence-based detection of
395 differentiation markers (i) loricrin (red) and (ii) keratin-10 (purple) and adhesion marker (iii)
396 desmoglein-1(grey). Nuclei are counter-stained with Hoechst stain (blue). Images were taken at
397 constant light exposure (100ms for proteins of interest and 10ms for DAPI channel) and then
398 overlaid for representation. White dashed-line indicates boundary separating epithelial-stromal
399 compartments. Scale bar (10μm) is indicated in white (bottom-right). For quantification 10-12
400 random images were taken in a blinded fashion for each mouse tissue (n=6) at constant light
401 exposure and processed through ImageJ. For each image integrated density of signal was
402 normalized to Hoechst stain signal from the same area. Each dot corresponds to average

403 normalized signal across 10-12 images for each mouse. Asterisk indicates statistical
404 significance ($p<0.05$, T test, two-sided). See also Table S1, S2, S3, S4 and Figure S1.
405 **Figure 2. Commensal microbiota promotes skin barrier repair function. (A)** Schematic
406 depicts (i) principle of measuring transepidermal water loss (TEWL) to assess barrier repair
407 function in adult mice (6-8 weeks old). (ii) Experimental design for assessing barrier recovery.
408 Dorsal skin of mice was tape-stripped to achieve comparable insults and TEWL was measured
409 up to 24 hours post-tape stripping. Effect of colonization of microbes was assessed by
410 comparing age-matched germ-free (GF) and specific pathogen-free (SPF) mice (n=4 mice per
411 group) in **(B)** wild-type C57/BL6 mice [ANCOVA, *F (1,69) =50.649, ***P<0.001)*] and **(C)** *Rag1*$^{-/-}$
412 mice [ANCOVA*, F (1,53) =188.1, ***P<0.001)*]. **(D)** Primary mouse keratinocytes were derived
413 wild-type GF (n=4) and SPF (n=4) C57/BL6 mice and grown in 5% FBS and 1.6mM Ca^{2+} for
414 three days to induce terminal differentiation. **(E)** Expression of genes involved in differentiation
415 [Involucrin *(Ivl),* cytokeratin-10 *(Krt10)*] and adherence [Corneodesmosin *(Cdsn)*, Desmocollin-1
416 *(Dsc1)*, Desmoglein-1a *(Dsg1a)*] was assessed by qRT-PCR. Cycle thresholds were normalized
417 to housekeeping genes (*Rplp2*, *Sptbn1* and *18S rRNA*) and normalized relative to Cq values of
418 SPF condition. Each square represents average readings from keratinocytes (n= 4 technical
419 replicates) derived from an individual mouse (n=4 mice per group). **P<0.01*, ** *P<0.001* by T-
420 test adjusted by Bonferroni correction. **(F)** Primary keratinocytes were grown on transwells in
421 5% FBS and 1.6mM Ca^{2+}. Epithelial adhesion was assessed by measuring transepithelial
422 electrical resistance (TEER) at indicated time points. Data from one experiment is represented
423 for visualization (See Figure S2). One dot represents average TEER readings from technical
424 replicates (n=3) derived from one individual mouse (n=4 mice per group). ****P*<0.001 by two-
425 way ANOVA adjusted for multiple experiments. **(G)** To decrease skin microbial burden, wild-
426 type SPF mice were treated with antibiotic cocktail (n=5) or vehicle (n=6) for two weeks. **(H)** To
427 determine microbial burden, mice were swabbed 14 days after treatment and colony forming
428 units (CFU) were determined. **(I)** Genomic DNA was extracted from swabs collected at baseline

429 (Day-0) and after one week of treatment (Day 7). V1-V3 region was amplified and analyzed by
430 16S rRNA gene sequencing and overall abundance i.e. total operational taxonomic unit (OTUs)
431 counts belonging to different phyla in each sample are depicted**.** Phyla with total read count
432 <1000 are grouped into 'Other'. (See Figure S3) **(J)** At the end of two weeks the two groups of
433 mice were tape stripped to achieve comparable insults and TEWL was measured and plotted
434 against time [ANCOVA, *F (1,41) =26.315, ***P<0.001)*]. TEWL/TEER vs time readings were
435 fitted by linear modeling (in **B, C, F** and **J**) and significance was assessed by ANCOVA analysis.
436 Modeling parameters (adjusted R^2 and *F*-statistics) are indicated on top-right for each plot. Span
437 indicated by shaded area represents 95% CI. Temperature and humidity conditions during
438 TEWL measurement are indicated for each experiment. Also see Figures S2 and S3.
439 **Figure 3. Activation of aryl hydrocarbon receptor (AHR) signaling in skin rescues barrier**
440 **dysfunction in germ free mice. (A)** Differentially expressed genes (DEGs) in SPF vs GF mice,
441 as identified by RNAseq, were mapped onto the AHR pathway. Log_2FC of significant DEGs
442 (*P<0.01)* in SPF mice are represented in colored boxes according to the legend. **(B)** Schematic
443 illustrating experimental design. Age matched 6-week-old, GF and SPF (n=5 mice/group)
444 C57/BL6 mice, respectively, were treated daily with 6-formylindolo[3,2-b] carbazole (FICZ) at
445 100µg/kg or vehicle for 2 weeks. At end of treatment dorsal skin of mice was tape-stripped to
446 achieve comparable insults (~20g/m^2/h) and TEWL was measured up to 24 hours post-tape
447 stripping to assess barrier recovery. TEWL vs time readings were fitted by linear modeling and
448 covariance was assessed by ANCOVA. Barrier recovery was compared in **(C)** GF mice [*F (1,47)*
449 *=21.9, ***P<0.001)*] and **(D)** SPF mice *[F(1,57)=2.98, *P=0.0492]* that were either treated with
450 FICZ or vehicle. **(E)** Expression of genes [*Ahr, Cdsn, Cyp1a1, Cyp1b1, Dsg1a, Ivl* and Krt14]
451 was assessed by qRT-PCR in GF mouse skin treated with FICZ or vehicle (4 mice per group).
452 Cycle thresholds were normalized to housekeeping genes (*Rplp2*, *Sptbn1* and *18s rRNA*) and
453 normalized relative to Cq values of FICZ treatment. **P<0.05,* by T-test adjusted by Bonferroni
454 correction. **(F)** Primary human keratinocytes grown on transwells (in 5% FBS and 1.6mM Ca^{2+})

455 in presence of FICZ (0nM, 10nM and 100nM) for three days and transepithelial electrical
456 resistance (TEER) was measured. Cells from different donors are represented by different
457 symbol. See Figure S4. **(G)** Primary human keratinocytes (in 5% FBS and 1.6mM Ca^{2+}) were
458 treated as indicated with FICZ and/or AHR inhibitor at 100nM doses. TEER values at the end of
459 three days of treatment are reported. ***$P<0.001$ by T-test for panels F and G.

460 **Figure 4. Loss of aryl hydrocarbon receptor (AHR) in keratinocytes impairs skin barrier in**
461 **mice. (A)** *Ahr* floxed *($Ahr^{f/f}$)* allele was knocked out in mice using Cre driven by a keratin-14
462 promoter *($K14^{Cre/+}Ahr^{f/f}$)*. Dorsal skin was tape-stripped to achieve comparable insults (20-25
463 $g/m^2/h$) and TEWL recovery curves were compared between $Ahr^{f/f}$ (n=5) and $K14^{Cre/+}$ $Ahr^{f/f}$ (n=6)
464 mice [ANCOVA, $F(1,96) = 131.34$, ***$P<0.001$)]. **(B)** Primary mouse keratinocytes were derived
465 from mice and polarized in 5% FBS and 1.6mM Ca^{2+} for three days and transepithelial electrical
466 resistance (TEER) was measured (**$P<0.005$, T-test). **(C)** $Ahr^{f/f}$ (n=5) and $K14^{Cre/+}$ $Ahr^{f/f}$ (n=7)
467 mice were tape-stripped (TEWL=20 $g/m^2/h$) and 10^7 CFU *S. aureus* containing tdTomato was
468 applied to back skin. **(D)** 48 hours post-infection tissue was collected, weighed, homogenized
469 and plated. *S. aureus* (visible as red colored colonies) and total bacterial colonies were counted
470 (**$P<0.005$, T-test). **(E)** Model for atopic dermatitis induced by repeated epicutaneous
471 sensitization of tape-stripped skin with ovalbumin (OVA) or vehicle (PBS) [30] was implemented.
472 Mice were tape stripped at the beginning of the experiment (TEWL~20 $g/m^2/h$) and OVA was
473 applied daily for 7 days, 3 times with rest for 2 weeks between each treatment. At the end of
474 final treatment, mice were tape-stripped (TEWL~40 $g/m^2/h$) and 24 hours later **(F)** TEWL levels
475 were assessed and **(G)** *S. aureus* was applied to back skin, *S. aureus* CFUs were determined
476 (as described in E). Statistical significance in panels G and H were assessed using a 2-way
477 ANOVA (**$P<0.005$, ***$P<0.0005$). (See Figure S4)

478 **Figure 5. Commensal microbes curated from human skin restores skin barrier function in**
479 **germ-free mice via AHR activation. (A)** Curation of bacteria for Flowers' Flora (FF)
480 consortium. **(B)** HaCaT cells (10^6 cells/well) were co- transfected with plasmids containing a

481 *Firefly* luciferase reporter conjugated to *Cyp1a*- AHR response element and a *Renilla* luciferase
482 as transfection control. Transfected cells were treated with indicated bacteria at indicated
483 multiplicity of infection (MOI) and luminescence was measured. *Firefly* luciferase activities were
484 normalized to *Renilla* luciferase levels and relative response compared to 10nM FICZ treatment
485 (positive control) was computed. **(C)** Germ-free mice were colonized with FF daily for two
486 weeks. Mice were swabbed at indicated days and CFUs were enumerated by plating on blood
487 agar plates. **(D)** To determine whether individual bacteria of FF colonized skin, genomic DNA
488 was extracted from skin swabs collected from mice at day 14 and qPCR analysis was
489 conducted using species-specific primers for each bacterium and percentage composition
490 relative to total 16S rRNA was determined. **(E)** Two-weeks post colonization mice (n=5
491 mice/group) that were either germ-free (GF) or colonized with FF were tape stripped (TEWL
492 ~20-30 g/m^2/h) and **(F)** barrier recovery was assessed by TEWL (ANCOVA, $F(1,157)=181.25$,
493 $P<0.0001$). **(G)** Expression of genes [*Ahr, Cdsn, Cyp1a1, Cyp1b1, Dsg1a, Ivl* and Krt14] was
494 assessed by qRT-PCR in mouse skin treated with FF or vehicle (GF). Cycle thresholds were
495 normalized to housekeeping genes (*Rplp2*, *Sptbn1* and *18s rRNA*) and normalized relative to
496 Cq values of FF treatment. *$P<0.05$ and **$P<0.005$ by T-test adjusted with Bonferroni
497 correction. **(H)** Primary mouse keratinocytes were derived and polarized in 5% FBS and 1.6mM
498 Ca^{2+} for three days and transepithelial electrical resistance (TEER) was measured (**$P<0.005$,
499 T-test). **(I)** To test if improved barrier recovery via FF is through AHR, $K14^{Cre/+}Ahr^{f/f}$ (n=6) were
500 pre-colonized as shown in Fig. 4F and compared to $K14^{Cre/+}Ahr^{f/f}$ (n=3) that were treated with
501 Control (Ctrl) [ANCOVA, $F(1,61)=0.1191$, $P=0.73115$]. Additionally, $Ahr^{f/f}$ mice that were
502 colonized (n=4) and untreated (n=3) were included in comparisons. **(J)** To test if pre-
503 colonization with FF could improve barrier recovery in an epicutaneous sensitization model, we
504 pre-colonized C57BL6/J mice (n=5) with FF for 2-weeks. At the end of colonization, mice were
505 tape-stripped and treated with Ovalbumin for 1-week. Mice were subjected to comparable
506 insults (TEWL~40g/m^2/h) and barrier recovery kinetics were compared in FF colonized versus

507 control (non-colonized) mice. FF colonized mice showed improved barrier recovery compared to
508 control mice [ANOVA, ***P<0.01*]. (See Figures S5).
509
510 **MATERIALS AND METHODS**
511 **RESOURCE AVAILABILITY**
512 ***Lead contact***
513 Further information and requests for resources and reagents should be directed to and will be
514 fulfilled by the corresponding author, Elizabeth Grice (egrice@pennmedicine.upenn.edu).
515 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
516 **Animal models and husbandry conditions**
517 All mouse experiments were conducted under protocols approved by the University of
518 Pennsylvania Institutional Animal Care and Use Committee (Protocol #804065). Age-matched
519 6-8 weeks old mice were used for all experiments. The following strains of mice were used in
520 these studies: C57BL6/J (JAX stock #000664), Rag1 KO (JAX stock #002216)[78], Ahr^{fx} (JAX
521 stock #006203)[79] and K14cre (JAX stock #018964)[80].
522 *Germ-free studies* were conducted in the Penn Gnotobiotic Mouse facility. Indicated
523 strains were bred and maintained as germ-free (GF) in flexible vinyl isolators at the Penn
524 Gnotobiotic Mouse facility housed in the University of Pennsylvania, School of Veterinary
525 Medicine. Mice were housed as 3-5 mice per cage, until they were euthanized for tissue
526 harvest. Aggressive mice or those that showed scratching wounds were not used in the studies.
527 SPF counterparts were purchased from Jackson Laboratories and allowed to acclimatize in the
528 facility for one week prior to beginning any experiments. One week prior to beginning of an
529 experiment, mice (GF or SPF) were transferred to hermetically-sealed cages with individually
530 filtered-positive airflow. The mice were maintained in these cages for the duration of the study.
531 This allowed similar housing conditions for both GF and SPF mice for consistent TEWL
532 readings. All mice were given autoclaved bedding, water and irradiated chow (5021

533 Autoclavable Mouse Breeder Diet, LabDiet®). Mouse handling was conducted in a laminar flow
534 cabinet through double layer protective gloves. Germ-free status of mice was confirmed by
535 weekly bacterial checks by the germ-free facility. At the end of each experiment, mice were
536 autopsied and germ-free status was confirmed by enlarged cecum. Additionally, skin swabs,
537 fecal pellet, and bedding samples were cultured by on blood-agar plates.

538 <u>*Studies in wild-type and AHR KO mice*</u> were conducted in Clinical Research Building
539 vivaria at the University of Pennsylvania. C57BL6/J mice were maintained and bred by lab
540 personnel. To generate $K14^{Cre/+}Ahr^{f/f}$ mice $Ahr^{f/f}$ mice were crossed with $K14^{Cre/+}$ mice to
541 generate $K14^{Cre/+}Ahr^{f/+}$ F1 mice, $K14^{Cre/+}Ahr^{f/+}$ F1 mice were backcrossed to $Ahr^{f/f}$ mice to obtain
542 experimental mice ($K14^{Cre/+}Ahr^{f/f}$) and litter-mate controls ($Ahr^{f/f}$) lacking *Cre*. To ensure
543 robustness of studies, mice were randomly housed. Genotyping protocol: $Ahr^{f/f}$ status was
544 determined using PCR primers oIMR6075-Reverse (5' CAG TGG GAA TAA GGC AAG AGT GA
545 3') and oIMR6076-Forward (5' GGT ACA AGT GCA CAT GCC TGC 3') and resolving on a 5%
546 polyacrylamide gel. *Cre* allele was determined using Generic Cre protocol (Protocol #22392,
547 Jax Lab, Version 1.3) and resolved on a 2.5% Agarose gel.

548 ***Primary keratinocytes from adult mouse skin***

549 Keratinocytes were derived from mouse tail or ear skin as described previously with
550 slight modifications [81]. Following euthanasia mouse ears and/or tail were excised. With the
551 help of forceps, ears were split into dorsal and ventral halves. To peel the tail skin from the
552 bone, a scalpel was used to cut along the ventral axis from base of tail to tip. The exposed tail
553 bone was peeled off using blunt-tip forceps. The resultant skin was cut into 0.75cm^2 pieces.
554 Skin obtained from ears and tails were incubated dermis side down in 6-well dishes and floated
555 in ice-cold dispase (1mg/ml) in 1X PBS overnight at 4°C. Epidermal sheets were separated by
556 lifting the epidermis using forceps. The separated epidermal sheets were cut into tiny pieces
557 using forceps and scissors and incubated in 60mm untreated culture dish containing 2ml of
558 0.25% Trypsin-EDTA at 37°C, 5% CO_2 for 15 minutes. At this point, 5ml suspension media

559 (DMEM+10% FBS+ P/S) was added to the dish and the skin pieces were pipetted vigorously
560 using a 10ml pipette to obtain a single cell suspension. Cell suspension was centrifuged at 150g
561 for 5 minutes at 4°C and supernatant was removed. The cell pellet was suspended in 10ml
562 suspension media and passed through 100µm cell strainer. Cell suspension was centrifuged at
563 150g for 15 minutes at 4°C, supernatant was removed and cells were suspended in 1ml
564 suspension media and plated in collagen-coated 60mm dishes at 0.5 mouse equivalents (i.e. 5
565 million cells/ml) in plating media (low $Ca2^{+}$ KSFM+ growth supplements+ 5% dialyzed FBS+4%
566 DMEM) containing 10µM ROCK inhibitor (abcam120129) to prevent differentiation as described
567 previously[82]. Typical cell counts were 2-5 x 10^{6} cells per mouse (cell count) and viability
568 greater than 70% (cell viability) as determined by Trypan blue exclusion assay. Dishes were
569 collagen coated by incubating 1.5ml collagen solution (50µg/ml collagen in 0.02N acetic acid)
570 for 1 hour at 37 ^{o}C, 5% CO_2 or overnight at room temperature. After incubation plates were
571 rinsed 3 times with 1X PBS. Cells were remained undisturbed for 48 hours and then passaged
572 for different experiments. Cells were maintained at 37°C in an atmosphere of 5% CO2 with
573 humidity.

574 ***Human keratinocyte cultures***

575 Primary cultures of human keratinocytes were obtained from neonatal foreskins through
576 the Penn Dermatology Skin Biology and Diseases Resource-based Center: Skin Translational
577 Research Core (STaR) Core B (visit: https://dermatology.upenn.edu/sbdrc/core-b/). Each
578 experiment was conducted with at least three donors (as indicated in text). All experiments were
579 conducted with cells at passage number less than 4. Briefly, cell suspensions were generated
580 using dispase and trypsin, and the cells were cultured in a keratinocyte growth media [50%
581 Medium 154, M154500 (Life Technologies), 50% Keratinocyte SFM, 17005042 (Life
582 Technologies), 1% HKGS supplement, S0015 (Life Technologies), 1% Antibiotic/Antimycotic,
583 15240062 (Invitrogen)]. For routine passaging, cells were split when they were less than 70%
584 confluent. Cells were washed with 1X PBS and trypsinized with 0.25% Trypsin-EDTA for 5

585 minutes, trypsin was inactivated using trypsin inhibitor (R007100, Thermo Fisher Scientific) and
586 cell suspension was centrifuged. Following removal of supernatant, cell pellet was suspended in
587 culture media and seeded as per experimental design. Cells were maintained at 37°C in an
588 atmosphere of 5% CO2 with humidity.

589 Immortalized human keratinocyte HaCaT cells [83] were used for AHR reporter assay.
590 HaCaT cells were verified for lack of mycoplasma contamination by ATCC. Experiments were
591 conducted on cells that were at passage numbers between 26-36. For routine cell culture,
592 HaCaTs were maintained in DMEM high glucose (11965092, Thermo Fisher Scientific)
593 supplemented with 1% Sodium Pyruvate, 5% FBS, 1% Antibiotic/Antimycotic, 15240062
594 (Invitrogen) and 1% Non-essential amino acids). For routine passaging, cells were split when
595 they were less than 70% confluent. Cells were washed with 1X PBS and trypsinized with 0.25%
596 Trypsin-EDTA for 5 minutes, trypsin was quenched with DMEM containing 5% FBS. Cells were
597 maintained at 37°C in an atmosphere of 5% CO2 with humidity.

598 ***Microbial strains***

599 The Flowers' Flora Consortium, consisted of *Staphylococcus epidermidis* (EGM 2-01),
600 *Staphylococcus hemolyticus* (EGM 2-08), *Staphylococcus warneri* (EGM 2-09), *Micrococcus*
601 *luteus* (EGM 2-04) and *Corynebacterium aurimucosum* (EGM 2-02), that had been isolated from
602 healthy human skin and maintained in the Grice lab culture repository (number in parenthesis
603 indicates identifier code in Grice lab culture collection). The *S. aureus* strain (AH3926) used in
604 these studies was generously provided by Dr. Alexander Horswill (University of Colorado,
605 Anschutz Medical Campus). *S. aureus* AH3926 consists of tdTomato stably integrated into *S.*
606 *aureus* LAC (AH1263) and it's construction has been described in detail previously [60].
607 Culturing conditions: All strains were cultured on solid blood agar plates at room temperature for
608 24-48 hours. For liquid cultures, all species (except *M. luteus*) were inoculated in tryptic soy
609 broth and grown by shaking at 100rpm at 37°C. *M. luteus* was inoculated in nutrient broth.

610 **METHOD DETAILS**

611 **RNA-sequencing of murine epithelia and analysis**

612 Mice were shaved, and skin was collected from dorsal region. The fat layer was scraped
613 off using a scalpel and then the skin was floated in dispase (1mg/ml) in 1X PBS overnight at
614 37°C for 1 hour in order to separate the epidermis from the dermis. The epidermis was stored in
615 RNAlater. Mouse epidermis that had been stored in RNA-Later (Thermo-Fisher) was blotted dry
616 and approximately 20 mg of tissue was placed in a Lysing Matrix A tube (MP Bio) with 600 µl
617 RLT buffer (Qiagen) containing 2-mercaptoethanol. The tissue was homogenized with three, 1
618 min bursts of bead beating in a FastPrep 24 (MP Bio). The lysate was centrifuged (14,000 x g,
619 3 min) and the supernatant was transferred to a new tube to which 1 volume of 70 % ethanol
620 was added. RNA was purified using a RNeasy Tissue Kit (Qiagen), as per manufacturer's
621 guidelines. RNA was quantified on a Qubit and RNA-integrity was assessed using BioAnalyser
622 according to manufacturer's instructions. 1µg RNA was used to construct RNA-seq libraries
623 using the stranded-TruSeq RNA Sample Prep Kit (Illumina), spiked with phiX and sequenced on
624 the Illumina NextSeq-500 Platform in 3 runs of 1x75 reads. The three runs were aggregated and
625 then analyzed and aligned against the mouse genome [Genome Reference Consortium Mouse
626 Build 38 patch release 5 (GRCm38.p5)] using AlignerBoost [84] and STAR 2.5.3[85]. Gene
627 counts were fitted into a negative binomial model where both the gnotobiotic condition (SPF, GF
628 or COL) and sex of the mouse were included using the DESeq2 [86] R package . Pairwise
629 DEGs between conditions were obtained by setting corresponding "contrasts" for each pairwise
630 comparison and filtering genes with FDP adjusted p-values less than 0.1. To identify enriched
631 Gene Ontology (GO) terms, all annotated GO terms for aforementioned DEGs were retrieved
632 using the ENSEMBL biomaRt R package[87], and significant enriched GO terms were identified
633 using the topGO R package with the FDR-adjusted *p*-values < 0.1 under dependency
634 [88].Uniquely enriched GO terms were selected by grouping similar GO terms using the online
635 GO visualization tool REVIGO[89] with default (medium) similarity settings.

636 **16S rRNA Gene Sequencing**

637 *Sample collection*

638 Mice were swabbed prior to shaving with sterile foam-tipped applicators (Puritan) as
639 described previously [49]. The swabs were snap frozen and stored at −80°C immediately
640 following collection. Bacterial DNA was extracted from swabs as described [90]. In brief, swabs
641 were incubated for one hour at 37°C with shaking in 300μL yeast cell lysis solution (from
642 Epicentre MasterPure Yeast DNA Purification kit) and 10,000 units of ReadyLyse Lysozyme
643 solution (Epicentre). Samples were subjected to bead beating for ten minutes at maximum
644 speed on a vortex mixer with 0.5 mm glass beads (MoBio), followed by a 30-minute incubation
645 at 65°C with shaking. Protein precipitation reagent (Epicentre) was added and samples were
646 spun at maximum speed. The supernatant was removed, mixed with isopropanol and applied to
647 a column from the PureLink Genomic DNA Mini Kit (Invitrogen). Instructions for the Invitrogen
648 PureLink kit were followed exactly, and DNA was eluted in 50 mL elution buffer (Invitrogen). At
649 each sampling event, swab control samples that never came into contact with the skin were
650 collected, prepared and sequenced exactly as the experimental samples. No significant
651 background contamination from either reagent and/or collection procedures was recovered.

652 *Sequencing and analysis*

653 Amplification of the 16S rRNA gene V1–V3 region was performed as described
654 previously [90]. Sequencing was performed at the PennCHOP microbiome core on the Illumina
655 MiSeq using 300 bp paired-end chemistry. The mock community control (MCC; obtained from
656 BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from
657 Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S rRNA Gene
658 Sequencing, HM-782D) was sequenced in parallel. Sequencing of the V1-V3 region was
659 performed using 300 bp paired-end chemistry. Sequences were preprocessed and quality
660 filtered prior to analysis, including size filtering to 460-600 nucleotides. HmmUFOtu was used
661 for sequence alignment and phylogeny-based OTU clustering as described previously [91].

662 Statistical analysis and visualization was performed using the phyloseq package [92] in the R
663 statistical computing environment.

664 **Barrier recovery**

665 Barrier was assessed as described [93, 94] by using noninvasive probe
666 (Courage+Khazaka, Cologne, Germany) to measure transepidermal water loss (TEWL) by
667 diffusion (Tewameter ®, TM300) according to the manufacturer's instructions. The dorsal flanks
668 of mice were shaved 24 hours prior to beginning of barrier analyses. Basal epidermal
669 permeability barrier function was assessed 24 hours after shaving. Barrier was disrupted by
670 tape stripping (3M Scotch High Performance Packaging Tape, 2"X800") to achieve comparable
671 insults between experimental and control groups as indicated for each experiment. Mice were
672 anesthetized using isoflurane during TEWL measurements. TEWL measurements were
673 averaged at 1-second intervals for a 30 second period. Indoor ambient temperature and mean
674 relative humidity were recorded for each experiment. For barrier recovery assessment, TEWL
675 was measured by placing probe at the same location on the dorsal flank of the mouse each
676 time. For consistency, the same person made all TEWL measurements.

677 **Antibiotic treatment of mice**

678 An antibiotic cocktail consisting of Metronidazole (1g/L), Sulfamethoxazole (0.8g/L),
679 Trimethoprim (0.16g/L), Cephalexin (4g/L) and Baytril (0.025g/L) dissolved in drinking water
680 containing Splenda (1 packet/250ml) as sweetener was provided to the mice for two weeks. To
681 ensure decreased microbial burden, cages were changed 3 times a week for antibiotic treated
682 mice as described previously [49]. Control cages were changed once a week, as per
683 conventional policies to ensure microbial biodiversity.

684 **Differentiation assay**

685 Confluent cells were trypsinized (0.25% Trypsin EDTA) and plated at 10^4 cells/well in 12
686 well collagen coated dishes without ROCK inhibitor. Cells were allowed to grow to confluency
687 (typically 2-3 days) and then media was switched to cornification medium i.e. traditional E-media

688 without EGF (3 parts DMEM+ 1 part DMEM/F12 + 5% FBS + cholera toxin + insulin + adenine +
689 hydrocortizone + antibiotics) for three days, to induce polarization and transmembrane junction
690 formation in keratinocytes [95]. At the end of three days cells were scraped off and processed
691 for qPCR analyses.

692 **Transepithelial electrical resistance (TEER) measurements**

693 Keratinocytes were trypsinized (0.25% Trypsin EDTA) and plated at 10^4 cells/well on
694 collagen coated 12mm transwells with 0.4μm pore (Sigma-Aldrich; CLS3460). Twenty-four
695 hours post-plating cells were 100% confluent and media was switched to KSFM containing 5%
696 FBS and 1.6mM Ca^{2+}. Every 24 hours, transepithelial electrical resistance (TEER) was
697 measured using an epithelial volt/ohm meter (EVOM) using a STX2 manual electrode. To
698 measure TEER, three readings were taken per chamber to cover three different areas of the
699 transwell membrane longitudinally over three days. The electrode was cleaned with 0.5%
700 bleach followed by 70% ethanol between each transwell. Readings are reported in ohms-cm^2.

701 *FICZ and AHR inhibitor treatments:* For experiments described in Figure 4, primary human
702 keratinocytes were seeded on transwells and grown in presence of 100nM each of AHR ligand
703 6-formylindolo[3,2-b] carbazole (FICZ) (Sigma-Aldrich, #SML1489) and/or AHR inhibitor (Sigma
704 Aldrich, #CH-223191) throughout the course of the experiments.

705 **Tissue preparation and immunofluorescence analysis**

706 Murine skin tissue was collected and fixed in 4% paraformaldehyde and embedded in
707 paraffin and sectioned at 6μm, as described previously[10]. Immunofluorescence protocols are
708 described in detail at dx.doi.org/10.17504/protocols.io.k95cz86 [96]. Briefly, tissue sections were
709 deparaffinized with xylenes and rehydrated with graded ethanol. Heat-induced antigen retrieval
710 was performed in 0.01M citrate buffer, pH 6.0 and blocked in 10% normal goat serum.
711 Antibodies against the following proteins were used at indicated dilution: Cytokeratin-10 1:1000
712 (Biolegend, #905401), Desmoglein 1a 1:200 (Abcam, #ab124798), Loricrin 1:500 (Biolegend,
713 #905101). Alexafluor 594 conjugated goat-anti rabbit 1:1000 (ThermoFisher Scientific,

714 #A32740) was used as secondary antibodies. Tissue was counterstained with Hoechst stain.
715 Wide-field fluorescent images were acquired using by means of a 20X lens objective on a Leica
716 DM6000 Widefield Fluorescence Microscope at the University of Pennsylvania, School of
717 Veterinary Medicine Imaging core. For purpose of quantification 10-12 random images were
718 taken in a blinded fashion at constant light exposure of 100 miliseconds for the Alexafluor 594
719 channel and 10 seconds for the Hoechst Stain. Images were processed using ImageJ software
720 version 10.2 (NIH, Bethesda, MD). Krt10, Dsg1a, and Loricrin levels of each image were
721 calculated by the integrated density of the signal, normalized to the Hoechst stain signal from
722 the same area. For statistical analysis, each stain was calculated by taking average levels in
723 each corresponding to 10-12 images per mouse and a two-sided T-test was used to determine
724 the significance of signal differences between groups.

725 **Electron Microscopy**

726 The ultrastructural analysis by EM was performed as described previously [97] at the VA
727 Medical Center and Department of Dermatology at the University of California, San Francisco,
728 United States. Skin samples were fixed in 2% glutaraldehyde and 2% paraformaldehyde and
729 post-fixed in reduced ruthenium tetroxide before epoxy embedding. The samples were cut on a
730 Leica Ultracut E microtome (Leica microsystems, Wetzlar, Germany) and imaged on a JEOL
731 100CX transmission electron microscope (JEOL, Tokyo, Japan) using a Gatan digital camera.
732 For quantification, the thickness of the cornified envelope was measured in at least 25 randomly
733 selected positions in 5 random high-powered electron micrographs of the mid stratum corneum
734 from three mice of each colonization state. The observer recording these measurements was
735 blinded to the groups.

736 **AHR reporter assay**

737 Immortalized human keratinocyte HaCaT cells [83] were seeded in 96 well plates at 10^4
738 cells/well in 100µl KSFM media supplemented with supplements. Twenty-four hours later, when
739 cells were 80-90% confluent, each well was co-transfected with 20ng of *Renilla* luciferase DNA

740 pGL4.74 (Promega) and 180ng of *Firefly* luciferase reporter plasmids: pGL4.23 which has
741 xenobiotic response element (XRE) corresponding to *Cyp1A1* activity. Construction of plasmid
742 is described previously[20]. Transient transfections of HaCaT cells was performed with
743 Fugene® HD (Promega, #E2311) at 3:1 Fugene transfection reagent: DNA ratio according to
744 manufacturer instructions. Twelve hours post-transfection, media containing transfection
745 complexes was removed and replaced with media containing either indicated bacterial strains to
746 represent MOI=0.1, 1 and 10, peptidoglycan (10ng) or FICZ (10nM). Twenty-four hours post-
747 infection, dual luciferase readings (*Renilla* and *Firefly*) were read using Dual-Luciferase®
748 reporter assay system (Promega, #E1910) on the BioTek Synergy HT fluorescence plate
749 reader. Background correction was performed by subtracting readings of empty wells from
750 observed readings and *Firefly* luciferase activity was normalized to *Renilla* luciferase. Relative
751 response ratio to compare *CYP1A1* induction by each bacterial species to treatment with known
752 AHR ligand FICZ (10nM) was computed as follows: RRR= [(experimental sample ratio)-
753 (negative control ratio)]/ [(positive control ratio)-(negative control ratio)][98]. Experimental
754 sample refers to cells treated with indicated bacteria or peptidoglycan; negative control refers to
755 unstimulated cells and positive control refers to cells stimulated with 10nM FICZ.

756 **cDNA synthesis for qPCR analyses**

757 Mouse skin or epidermis that had been stored in RNA-Later (Thermo-Fisher) was blotted
758 dry and approximately 20 mg of tissue was placed in a Lysing Matrix A tube (MP Bio) with 600
759 µl RLT buffer (Qiagen) containing 2-mercaptoethanol. The tissue was homogenized with three,
760 1 min bursts of bead beating in a FastPrep 24 (MP Bio). The lysate was centrifuged (14,000 x
761 g, 3 min) and the supernatant was transferred to a new tube to which 1 volume of 70 % ethanol
762 was added. RNA was purified using a RNeasy Tissue Kit (Qiagen). Traces of DNA were
763 removed with DNase-1 and the RNA was stored at -80°C.RNA was quantified (Qubit) and 10 ng
764 RNA was used as a template for Superscript III (Invitrogen) reverse transcription with random
765 hexamer primers. Following treatment with RNase H, the cDNA was stored at -20°C.

766 **Colonizing mice with human skin commensals**

767 For liquid cultures of human skin commensals, *S. epidermidis, S. warneri, S.*
768 *hemolyticus* and *C. aurimucosum* were inoculated in tryptic soy broth (TSB) and *M. luteus* was
769 inoculated in nutrient broth media, respectively and grown by shaking at 200rpm for 16 hours at
770 37°C. Cultures were centrifuged, media was removed and bacterial pellets were suspended in
771 PBS to obtain 10^9 CFU/ml. Equal amount of each bacteria (10^9 CFU) was combined in 5ml PBS
772 and inoculated in bedding of mouse cages daily for 2 weeks. For each inoculum, a fresh batch
773 culture was grown overnight.

774 **Mouse treatment with AHR ligand FICZ**

775 Mice were treated with a low dose (100µg/kg) of AHR ligand 6-Formylindolo[3,2-b]
776 carbazole (FICZ) (Sigma-Aldrich, #SML1489), that has been shown to be sufficient to induce
777 expression of *Cyp1a1*[54]. A stock solution (4.5mg/ml) of FICZ was made in DMSO and diluted
778 in 50% Acetone. Mice were shaved 24 hours prior to treatment and FICZ was applied topically
779 by directly pipetting onto shaved skin, daily for 2 weeks (**Figure 3B)**.

780 ***S. aureus* skin infection protocol**

781 *Epicutaneous infection:* Infection protocol for epicutaneous infection with *S. aureus* has
782 been described previously [99] and was implemented with slight modifications. Briefly, mice
783 were anesthetized with isoflurane, tape stripped (TEWL=20 g/m^2/h) and 24 hours later, 10^7 CFU
784 *S. aureus* in 100µl was applied to back skin and spread using a swab. The inoculum was
785 allowed to dry for 10 minutes and mice were returned to their cages.

786 *CFU enumeration:* Forty-eight hours post-infection, mice were euthanized and
787 approximately 1cm^2 infected skin area was collected and weighed and transferred to tubes
788 containing 300µl 1X PBS. Tissue was homogenized by bead beating for twenty minutes at
789 maximum speed on a vortex mixer with 0.5 mm ceramic beads and CFUs were enumerated by
790 serial dilution on blood agar plates after overnight incubation at 37°C. Both *S. aureus* and total
791 bacterial counts were determined and normalized to weight of tissues. *S. aureus* colonies were

792 visible as red colonies due to stable expression of tDTomato and could be distinguished from
793 total bacteria.

794 **Epicutaneous sensitization with ovalbumin**

795 Procedures to induce barrier defects that mimic atopic dermatitis by repeated
796 epicutaneous sensitization by ovalbumin (OVA) [30, 100] followed by infection with *S. aureus*
797 [101] have been described previously. The dorsal skin of mice was shaved and TEWL was
798 assessed to give a baseline reading. To measure and compare barrier repair between wild-type
799 and AhR null mice, we standardized the amount of initial barrier disruption to give identical
800 TEWL values. To achieve this, mice were tape stripped to give a reading of TEWL ~20g/m^2/h.
801 Twenty-four hours post tape-stripping, the mice were treated daily for 7 days with 100 µg OVA
802 (Sigma Aldrich, # A5503) suspended in 100 µl PBS was applied onto backs of mice and spread
803 using a skin swab, and allowed to dry for 2 minutes. For inducing atopic dermatitis-like
804 condition, the 7-day OVA treatment regime was repeated twice more, with 2 weeks rest
805 between subsequent treatments. At the end of treatment mice were mice were tape stripped to
806 give a reading of TEWL ~40g/m^2/h and *S. aureus* was applied as described earlier or barrier
807 recovery was assessed.

808 **QUANTIFICATION AND STATISTICAL ANALYSIS**

809 **Data visualization and statistics.**

810 All statistical analysis was performed using functions built into the R statistical environment
811 (RStudio Version 1.3.1056). Data was visualized using ggplot2 [102] package and GraphPad
812 Prism version 8.0.0 for Mac OS X, GraphPad Software, San Diego, California USA,
813 www.graphpad.com. TEWL/TEER vs time readings were fitted by linear modeling function in R
814 statistical package and visualized using ggplot2 package. Significance was assessed by
815 ANCOVA analysis. Fit parameters (adjusted R^2 and F-statistics) are indicated for each plot.
816 Span indicated by shaded area represents 95% CI. Gene expression analysis from qPCR was
817 conducted as per guidelines described previously[103]. Cycle thresholds were normalized to

818 housekeeping genes (Rplp2, Sptbn1 and 18s rRNA) and normalized relative to quantitative
819 cycle (Cq) values of control. Heatmaps for qPCR analysis were made using Morpheus heat map
820 viewer from Broad Institute (https://software.broadinstitute.org/morpheus). Each square
821 represents average normalized readings (n= 3 technical replicates). Bonferrroni correction was
822 used to adjust for multiple comparisons. The AHR pathway was originally downloaded from
823 wikipathways (https://www.wikipathways.org/index.php/Pathway:WP2873), then modified by
824 highlighting DEGs identified in our analysis using a customized Perl script. The log2 fold-change
825 values between SPF and GF mice were used to determine the color hue (red/blue: up/down in
826 SPF vs. GF) and saturation of the highlighted DEGs.
827
828
829 **KEY RESOURCES TABLE**
830 **SUPPLEMENTARY MATERIALS**
831 **Table S1. Related to Figure 1.**
832 Results from differential expression analysis of RNA-seq data for genes depicted in Fig. 1F. As
833 a result, we identified 6,396, 427, and 3,232 DEGs for SPF vs. GF, COL vs. GF and SPF vs.
834 COL comparisons, respectively. DEGs defined as those with FDR adjusted p-values < 0.1. In
835 Sheet SPF vs GF (upregulatedin SPF: log2FC> 0, downregulated in SPF: log2FC < 0); in sheet
836 COLvsGF (upregulated in COL: log2FC> 0, downregulated in COL: log2FC< 0); in sheet
837 SPFvsCOL (upregulated in SPF: log2FC > 0, downregulated in SPF: log2FC < 0).
838 **Table S2. Related to Figure 1.** Contains list of genes manually curated based on literature
839 analyses that have been implicated in barrier function. Genes are listed under the following
840 categories based on their functions: adherence junction formation, lipid-lamellae formation,
841 keratin network, differentiation, skin barrier development, formation of cornified envelope and
842 basement membrane.

843 **Table S3. Related to Figure 1.** Contains the key genes involved in skin barrier function that
844 were differentially expressed between SPF and GF murine skin and were used to generate
845 graph depicted in Figure 1F.
846 **Table S4. Related to Figure 1.** Contains list of differentially expressed barrier genes involved in
847 skin barrier function that were differentially expressed between SPF and GF murine skin by
848 using genes described in Table S2 as reference.
849 **Figure S1. Related to Figure 1.** Structural analysis of murine dorsal skin from SPF and GF
850 mice. **(i)** Histopathological analysis of Hematoxylin and Eosin stained tissue did not demonstrate
851 overt differences between age-matched GF and SPF mice. **(ii)** Electron Microscopy (EM) was
852 performed on skin and number of layers of cornified envelope were counted. *** indicates
853 *P<0.01,* T-test.
854 **Figure S2. Related to Figure 2.** Assessment of **(A)** TEWL and **(B)** pH at baseline in age-
855 matched age-matched germ-free (GF) and specific pathogen-free (SPF) C57BL6/J mice (n=4
856 mice per group). **(C)** Shown is indicated TEWL corresponding to number of tape strips in GF vs
857 SPF mice. **(D)** GF and SPF *Rag1-/-* mice were swabbed colony forming units (CFU) were
858 determined and **(E)** TEWL was measured at baseline. **(F)** Dorsal skin from GF and SPF *Rag1-/-*
859 mice was collected and gene expression was analyzed by qRT-PCR. Cycle thresholds were
860 normalized to housekeeping genes (*Rplp2*, *Sptbn1* and *18s rRNA*) and normalized relative to
861 Cq values of SPF condition. Each square represents average readings from three technical
862 replicates from each mouse. **P<0.01*, ** *P<0.001* by T-test adjusted by Bonferrroni correction.
863 **(G)** Primary keratinocytes were derived from germ free mice and grown on transwells in basal
864 medium before switching to cornification media containing 5% FBS and 1.6mM Ca^{2+}. Shown
865 here is expression of genes between GF and SPF mice before and after calcium switch.
866 Expression of genes involved in differentiation [Involucrin *(Ivl),* cytokeratin-10 *(Krt10)*] and
867 adherence [Corneodesmosin *(Cdsn)*, Desmocollin-1 *(Dsc1)*, Desmoglein-1a *(Dsg1a)*] and AHR
868 pathway (*Cyp1a1, Cyp1b1, Hsp90ab1*) was assessed by qRT-PCR. Cycle thresholds were

869 normalized to housekeeping genes (*Rplp2*, *Sptbn1* and *18s rRNA*) and normalized relative to
870 Cq values of SPF condition. Each square represents average readings from technical replicates
871 (n= 4 technical replicates) derived from an individual mouse (n=4 mice per group). *$P<0.01$, **
872 $P<0.001$ by T-test adjusted by Bonferrroni correction. **(H)** Primary keratinocytes grown on
873 transwells were grown in 5% FBS and 1.6mM Ca^{2+} epithelial adhesion was assessed by
874 measuring transepithelial electrical resistance (TEER) at indicated time points. Data from three
875 individual experiments are shown. One dot represents average TEER readings from technical
876 replicates (n=3) derived from one individual mouse (n=4 mice per group).
877 **Figure S3. Related to Figure 2.** To decrease skin microbial burden, wild-type SPF mice were
878 treated with antibiotic cocktail (n=5) or vehicle (n=6) for two weeks. Genomic DNA was
879 extracted from skin swabs and fecal samples collected at baseline (Day 0 i.e. D0) and after one
880 week of treatment (Day 7 i.e. D7). V1-V3 region was amplified and analyzed by 16S rRNA gene
881 sequencing. **(A)** Microbiota composition of each sample shown as the relative abundance of the
882 top 15 most abundant genus of the entire dataset**. (B)** Fecal pellets collected at end of treatment
883 (i.e. 14 days later) were weighed, homogenized and plated on blood agar plates and CFUs were
884 enumerated. **(C)** Within-sample alpha diversity for samples from groups shown as the Shannon
885 index. The within sample Shannon index was calculated using the Phyloseq R package. **(D)**
886 Principle Coordinates Analysis (PCoA) plot of between microbiota sample beta-diversity for
887 different sample types (skin or fecal) and antibiotic treatment (control or antibiotic). Ctrl: control
888 treatment, Abx: Antibiotic treatment. Treatment timepoint was color labeled. The Bray-Curtis
889 dissimilarity was used for the between-sample beta-diversity metric.
890 **Figure S4. Related to Figure 4.** TEWL dynamics during the course of ovalbumin sensitization
891 treatment regime. **(A)** Schematic depicts sequence of manipulations to mice during OVA
892 sensitization. Mice were tape stripped at the beginning of the experiment (TEWL~20 $g/m^2/h$)
893 and OVA was applied daily for 7 days, 3 times with rest for 2 weeks between each treatment. At
894 the end of final treatment, mice were tape-stripped (TEWL~40 $g/m^2/h$) and *S. aureus* was

895 applied to back skin. **(B)** TEWL readings were taken at indicated time points in **(i)** *K14*$^{Cre/+}$*Ahr*$^{f/f}$
896 and **(ii)** *Ahr*$^{f/f}$ mice treated with OVA or vehicle (PBS). Endpoint readings are reported in Figure
897 4. **(C)** Total bacterial counts per gram of tissue are reported for *K14*$^{Cre/+}$*Ahr*$^{f/f}$ (KO) and *Ahr*$^{f/f}$
898 (WT) mice for indicated comparisons.
899 **Figure S5. Related to Figure 5.** TEWL dynamics during the course of short-term ovalbumin
900 sensitization treatment regime after Flowers' Flora colonization **(A)** Schematic depicts sequence
901 of manipulations to C57BL6/J mice. Mice were pre-colonized with Flowers' Flora daily for 2
902 weeks. At the end of pre-colonization, mice were tape stripped at the beginning of the
903 experiment (TEWL~20 g/m^2/h) and OVA was applied daily for 7 days. At the end of final
904 treatment, mice were tape-stripped (TEWL~40 g/m^2/h) and barrier recovery was assessed. **(B)**
905 TEWL readings were taken at indicated time points in **(i)** mice pre-colonized with Flowers' Flora
906 and **(ii)** those treated with vehicle (PBS).
907

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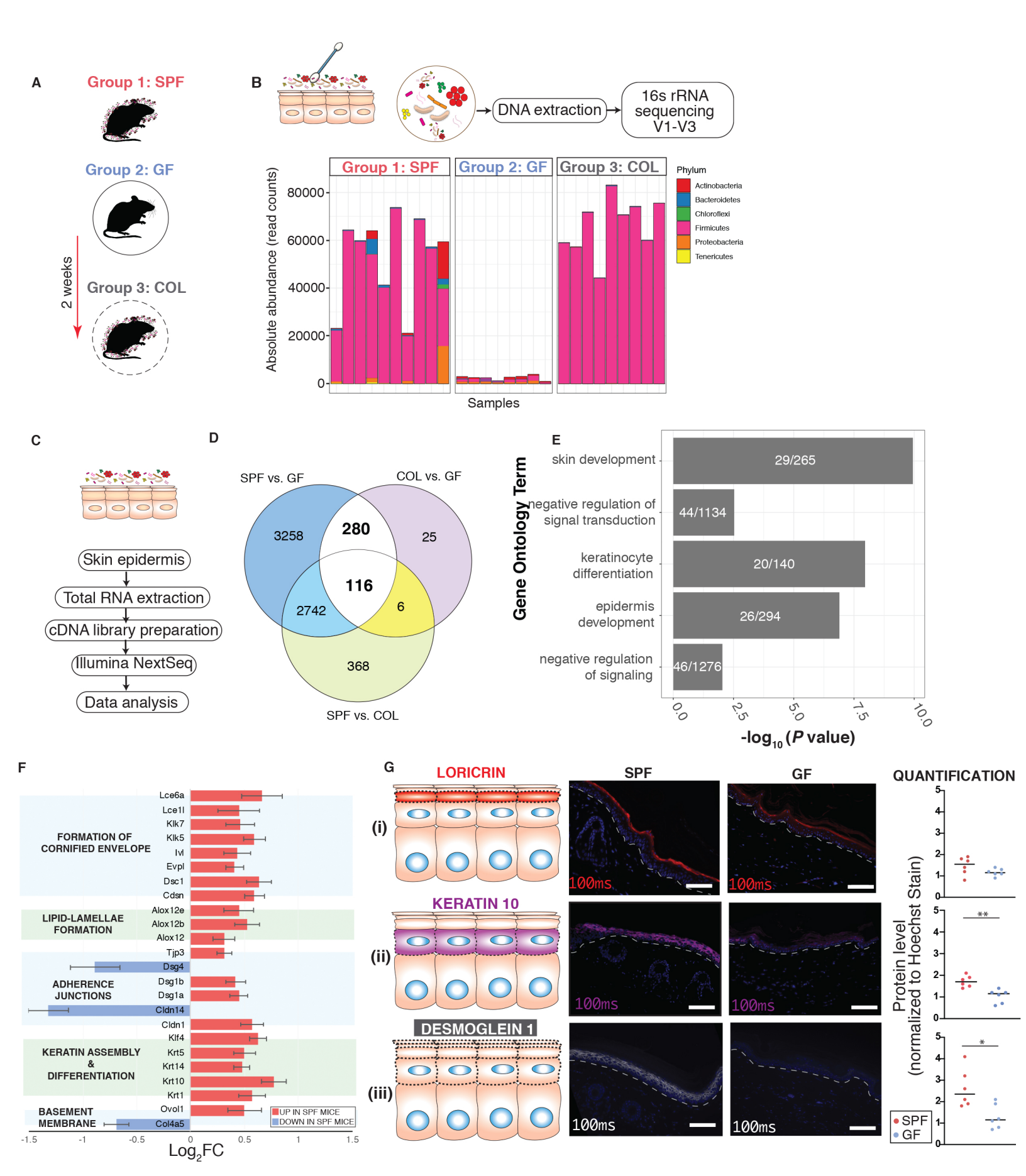
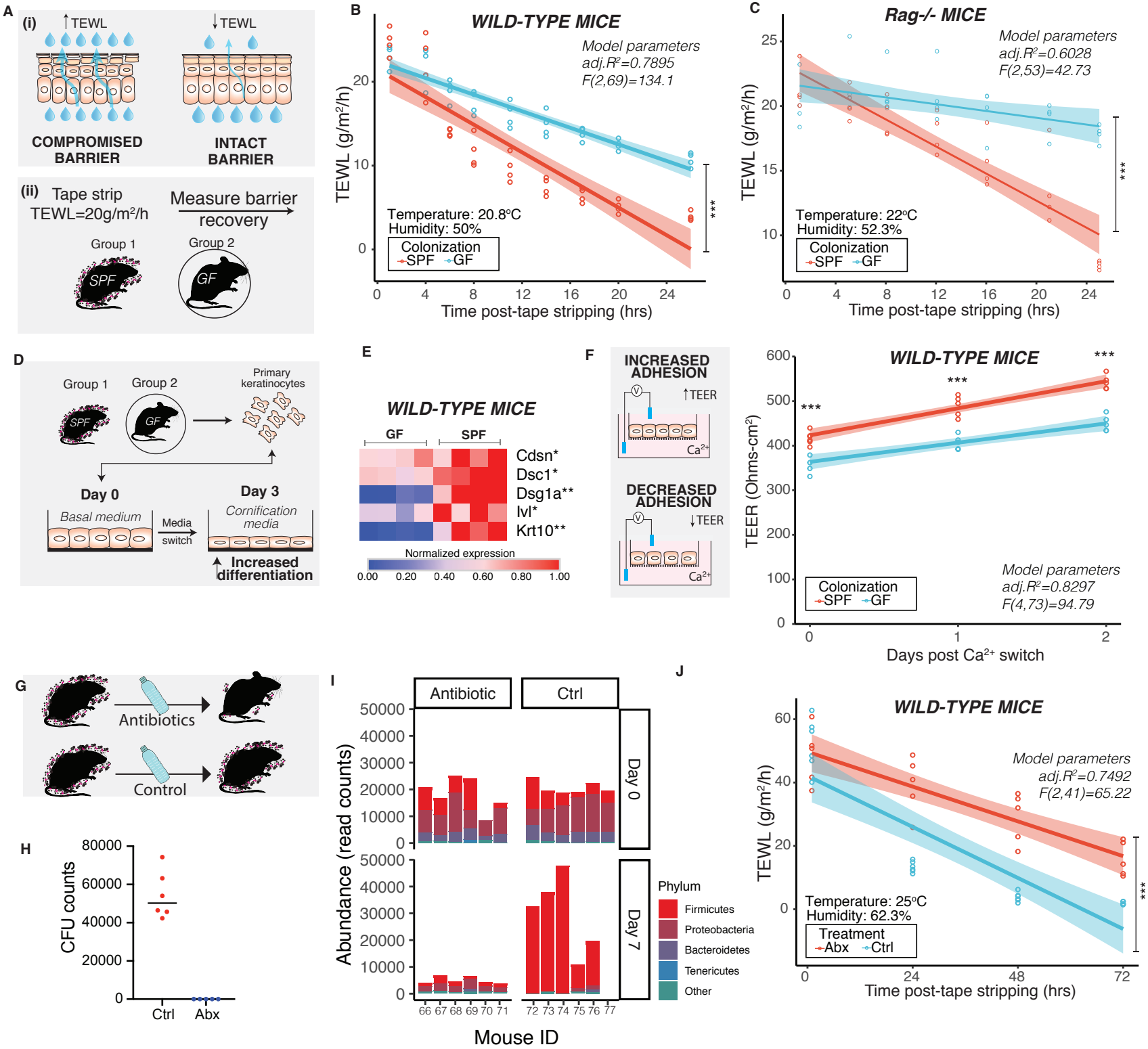
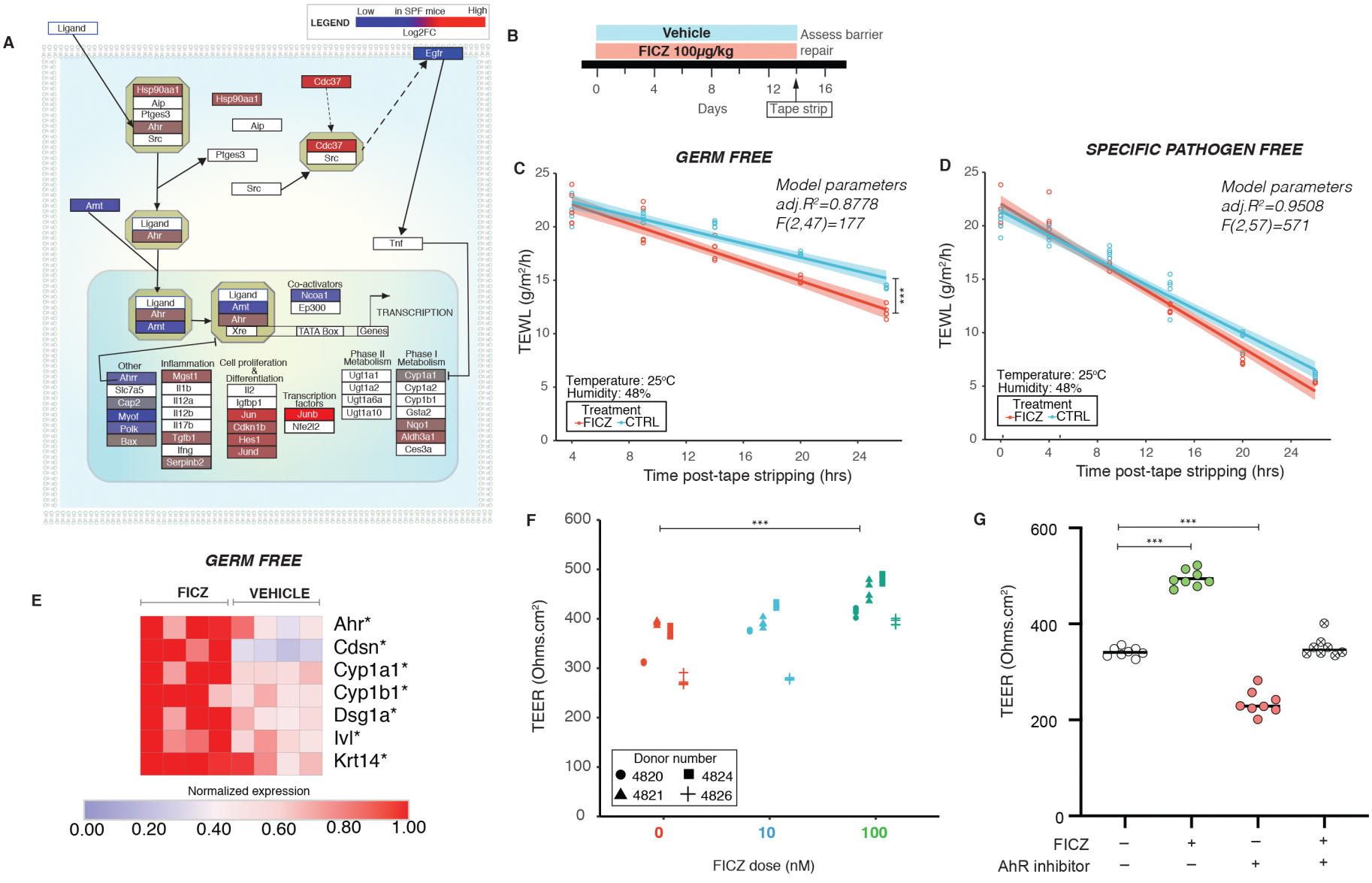
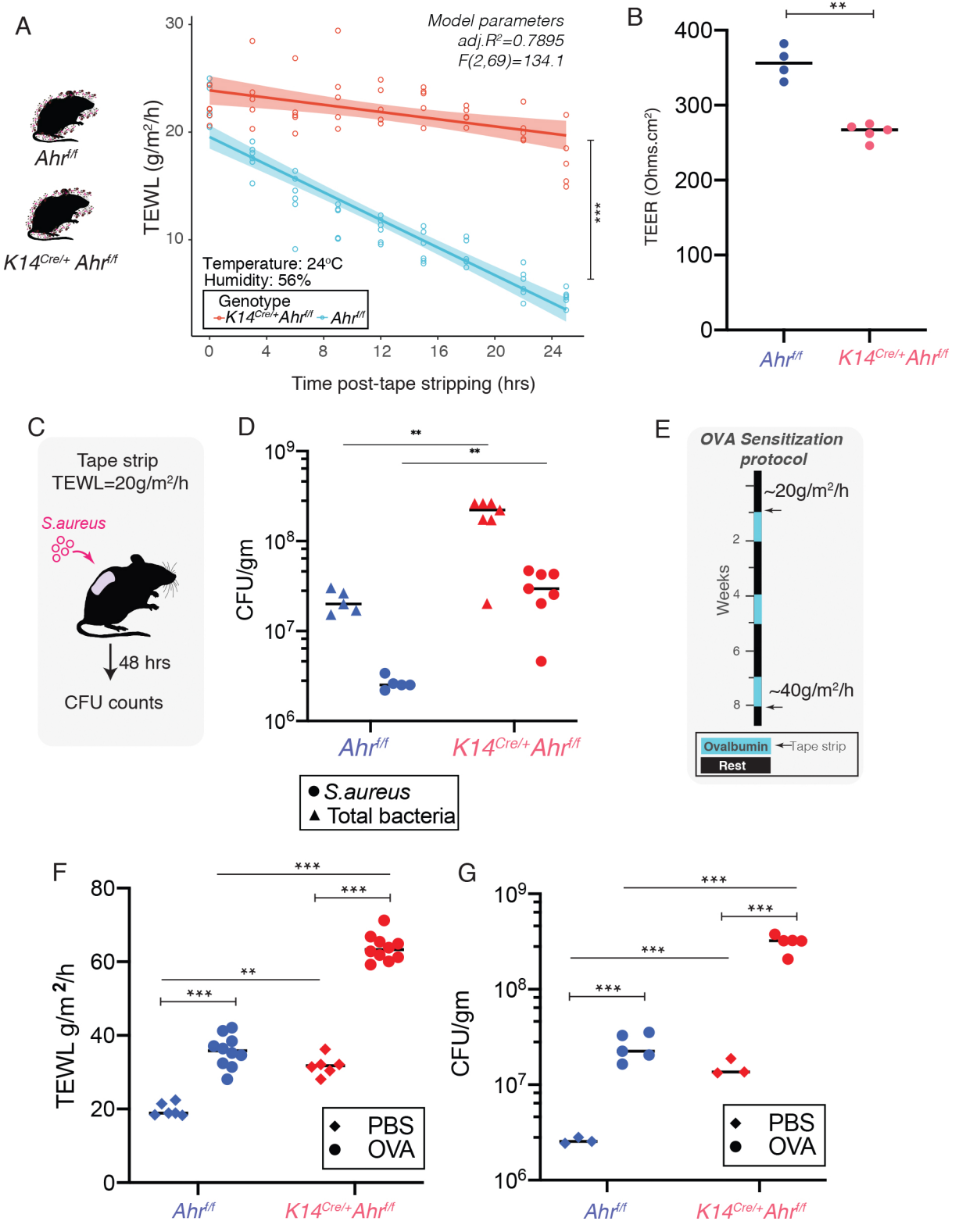
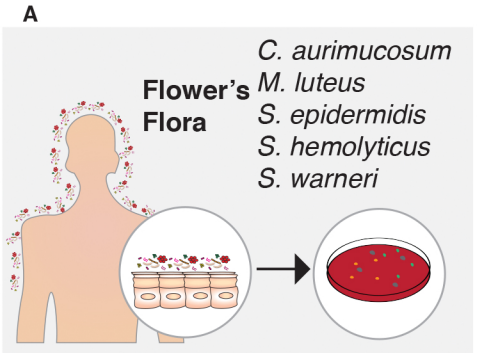
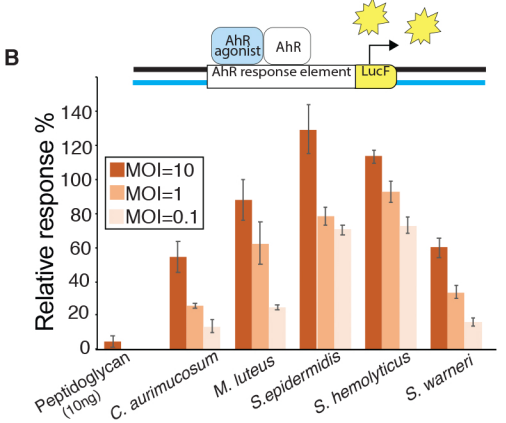

A
Group 1: SPF
Group 2: GF
Group 3: COL
2 weeks
B
DNA extraction
16s rRNA sequencing V1-V3
Group 1: SPF
Group 2: GF
Group 3: COL
Absolute abundance (read counts)
80000
60000
40000
20000
0
Samples
Phylum
Actinobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Proteobacteria
Tenericutes
C
Skin epidermis
Total RNA extraction
cDNA library preparation
Illumina NextSeq
Data analysis
D
SPF vs. GF
COL vs. GF
SPF vs. COL
3258
280
25
116
2742
6
368
E
Gene Ontology Term
skin development
negative regulation of signal transduction
keratinocyte differentiation
epidermis development
negative regulation of signaling
29/265
44/1134
20/140
26/294
46/1276
0.0
2.5
5.0
7.5
10.0
-log10 (P value)
F
FORMATION OF CORNIFIED ENVELOPE
Lce6a
Lce1l
Klk7
Klk5
Ivl
Evpl
Dsc1
Cdsn
LIPID-LAMELLAE FORMATION
Alox12e
Alox12b
Alox12
Tjp3
ADHERENCE JUNCTIONS
Dsg4
Dsg1b
Dsg1a
Cldn14
KERATIN ASSEMBLY & DIFFERENTIATION
Cldn1
Klf4
Krt5
Krt14
Krt10
Krt1
BASEMENT MEMBRANE
Ovol1
Col4a5
UP IN SPF MICE
DOWN IN SPF MICE
-1.5
-1
-0.5
0
0.5
1
1.5
Log2FC
G
LORICRIN
KERATIN 10
DESMOGLEIN 1
(i)
(ii)
(iii)
SPF
GF
100ms
QUANTIFICATION
Protein level (normalized to Hoechst Stain)
**
*
SPF
GF

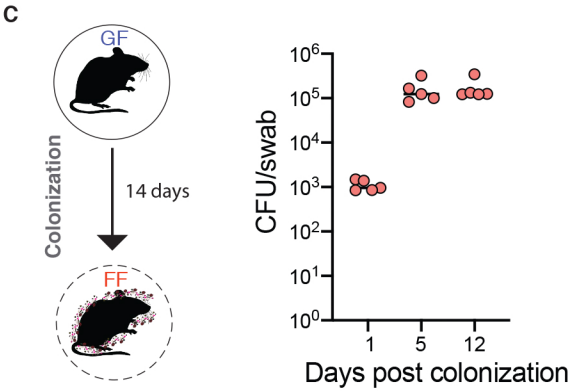

A
(i)
TEWL
TEWL
COMPROMISED BARRIER
INTACT BARRIER
(ii)
Tape strip
TEWL=20g/m²/h
Measure barrier recovery
Group 1
Group 2
SPF
GF
B
WILD-TYPE MICE
Model parameters
adj.R²=0.7895
F(2,69)=134.1
TEWL (g/m²/h)
Temperature: 20.8°C
Humidity: 50%
Colonization
SPF
GF
***
Time post-tape stripping (hrs)
C
Rag-/- MICE
Model parameters
adj.R²=0.6028
F(2,53)=42.73
TEWL (g/m²/h)
Temperature: 22°C
Humidity: 52.3%
Colonization
SPF
GF
***
Time post-tape stripping (hrs)
D
Group 1
Group 2
Primary keratinocytes
SPF
GF
Day 0
Day 3
Basal medium
Media switch
Cornification media
Increased differentiation
E
WILD-TYPE MICE
GF
SPF
Cdsn*
Dsc1*
Dsg1a**
Ivl*
Krt10**
Normalized expression
0.00
0.20
0.40
0.60
0.80
1.00
F
INCREASED ADHESION
TEER
Ca2+
DECREASED ADHESION
TEER
Ca2+
WILD-TYPE MICE
***
***
***
TEER (Ohms-cm²)
Colonization
SPF
GF
Model parameters
adj.R²=0.8297
F(4,73)=94.79
Days post Ca2+ switch
G
Antibiotics
Control
H
CFU counts
Ctrl
Abx
I
Antibiotic
Ctrl
Day 0
Day 7
Abundance (read counts)
Phylum
Firmicutes
Proteobacteria
Bacteroidetes
Tenericutes
Other
Mouse ID
J
WILD-TYPE MICE
Model parameters
adj.R²=0.7492
F(2,41)=65.22
TEWL (g/m²/h)
Temperature: 25°C
Humidity: 62.3%
Treatment
Abx
Ctrl
***
Time post-tape stripping (hrs)

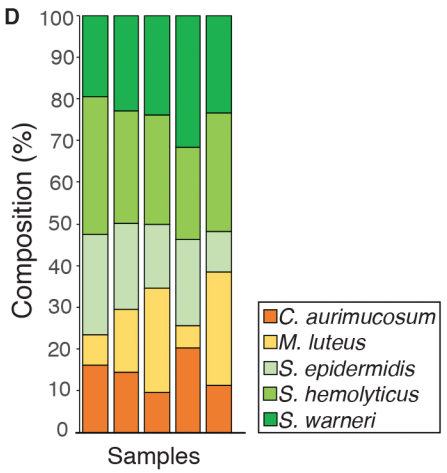

A
LEGEND
Low
in SPF mice
High
Log2FC
Ligand
Egfr
Hsp90aa1
Aip
Ptges3
Ahr
Src
Cdc37
Cdc37
Src
Ptges3
Arnt
Ligand
Ahr
Tnf
Co-activators
Ncoa1
Ep300
TRANSCRIPTION
Xre
TATA Box
Genes
Other
Ahrr
Slc7a5
Cap2
Myof
Polk
Bax
Inflammation
Mgst1
Il1b
Il12a
Il12b
Il17b
Tgfb1
Ifng
Serpinb2
Cell proliferation & Differentiation
Il2
Igfbp1
Jun
Cdkn1b
Hes1
Jund
Transcription factors
Junb
Nfe2l2
Phase II Metabolism
Ugt1a1
Ugt1a2
Ugt1a6a
Ugt1a10
Phase I Metabolism
Cyp1a1
Cyp1a2
Cyp1b1
Gsta2
Nqo1
Aldh3a1
Ces3a
B
Vehicle
FICZ 100μg/kg
Assess barrier repair
0
4
8
12
16
Days
Tape strip
C
GERM FREE
Model parameters
adj.R²=0.8778
F(2,47)=177
TEWL (g/m²/h)
Temperature: 25ºC
Humidity: 48%
Treatment
FICZ
CTRL
***
Time post-tape stripping (hrs)
D
SPECIFIC PATHOGEN FREE
Model parameters
adj.R²=0.9508
F(2,57)=571
TEWL (g/m²/h)
Temperature: 25ºC
Humidity: 48%
Treatment
FICZ
CTRL
Time post-tape stripping (hrs)
E
GERM FREE
FICZ
VEHICLE
Ahr*
Cdsn*
Cyp1a1*
Cyp1b1*
Dsg1a*
Ivl*
Krt14*
Normalized expression
0.00
0.20
0.40
0.60
0.80
1.00
F
TEER (Ohms.cm²)
***
Donor number
4820
4824
4821
4826
0
10
100
FICZ dose (nM)
G
TEER (Ohms.cm²)
***
***
FICZ
AhR inhibitor


A
Ahr f/f
K14 Cre/+ Ahr f/f
Model parameters
adj.R²=0.7895
F(2,69)=134.1
TEWL (g/m²/h)
Temperature: 24°C
Humidity: 56%
Genotype
K14 Cre/+ Ahr f/f
Ahr f/f
***
Time post-tape stripping (hrs)
B
**
TEER (Ohms.cm²)
Ahr f/f
K14 Cre/+ Ahr f/f
C
Tape strip
TEWL=20g/m²/h
S.aureus
48 hrs
CFU counts
D
**
**
CFU/gm
Ahr f/f
K14 Cre/+ Ahr f/f
S.aureus
Total bacteria
E
OVA Sensitization protocol
~20g/m²/h
Weeks
~40g/m²/h
Ovalbumin
Tape strip
Rest
F
***
***
**
***
TEWL g/m²/h
PBS
OVA
Ahr f/f
K14 Cre/+ Ahr f/f
G
***
***
***
***
CFU/gm
PBS
OVA
Ahr f/f
K14 Cre/+ Ahr f/f

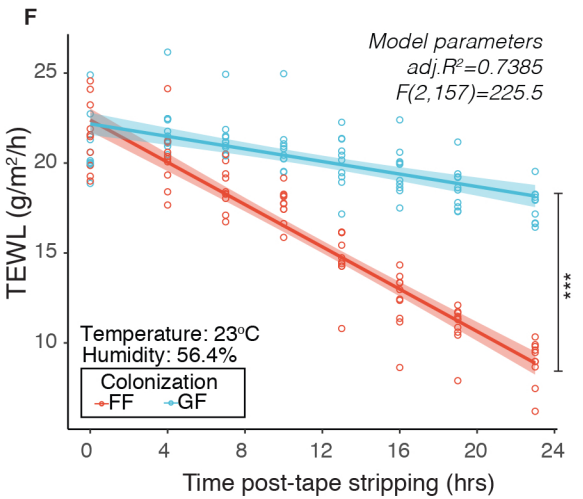

A
Flower's Flora
C. aurimucosum
M. luteus
S. epidermidis
S. hemolyticus
S. warneri

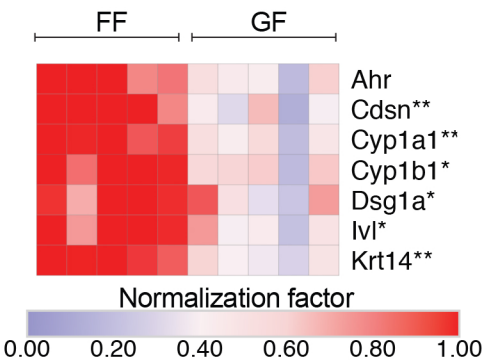

B
AhR agonist
AhR
AhR response element
LucF
Relative response %
MOI=10
MOI=1
MOI=0.1
Peptidoglycan (10ng)
C. aurimucosum
M. luteus
S.epidermidis
S. hemolyticus
S. warneri

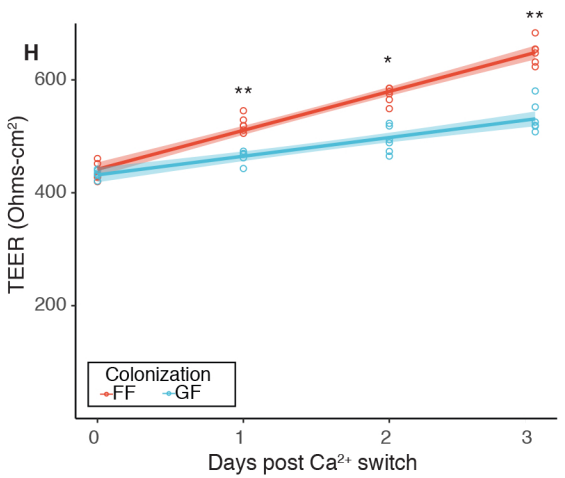

C
GF
Colonization
14 days
FF
CFU/swab
Days post colonization

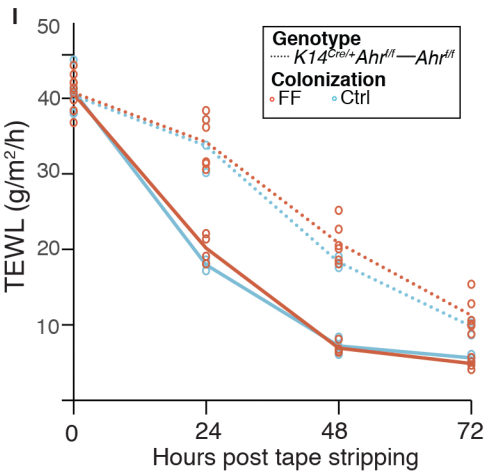

D
Composition (%)
Samples
C. aurimucosum
M. luteus
S. epidermidis
S. hemolyticus
S. warneri

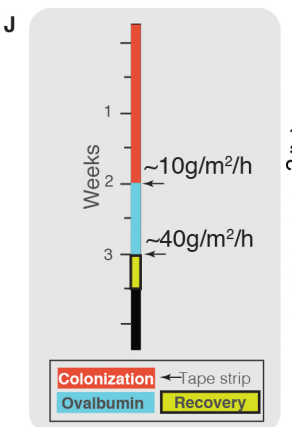
E
Colonization
2 weeks
Tape strip
TEWL=20g/m²/h
24 hours
Measure barrier recovery

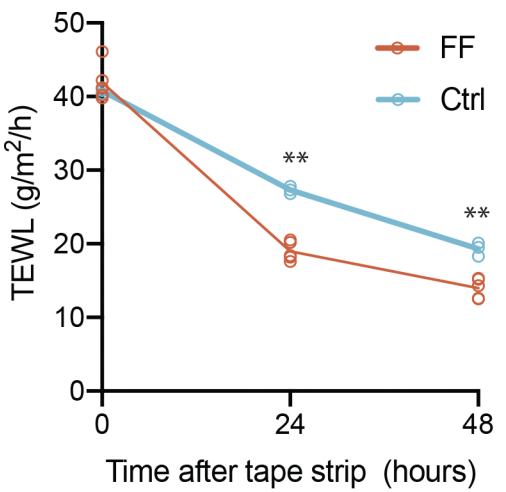

F
Model parameters
adj.R²=0.7385
F(2,157)=225.5
TEWL (g/m²/h)
Temperature: 23°C
Humidity: 56.4%
Colonization
FF
GF
***
Time post-tape stripping (hrs)


FF
GF
Ahr
Cdsn**
Cyp1a1**
Cyp1b1*
Dsg1a*
Ivl*
Krt14**
Normalization factor
0.00
0.20
0.40
0.60
0.80
1.00


H
TEER (Ohms-cm²)
Colonization
FF
GF
Days post Ca²⁺ switch


I
Genotype
K14Cre/+Ahrf/f—Ahrf/f
Colonization
FF
Ctrl
TEWL (g/m²/h)
Hours post tape stripping


J
Weeks
~10g/m²/h
~40g/m²/h
Colonization
Tape strip
Ovalbumin
Recovery


FF
Ctrl
TEWL (g/m²/h)
Time after tape strip (hours)