



**HAL**  
open science

## Sensory neuron-derived TFAFA4 promotes macrophage tissue repair functions

Guillaume Hoeffel, Guillaume Debroas, Anais Roger, Rafaëlle Rossignol, Jordi Gouilly, Caroline Laprie, Lionel Chasson, Pierre Vincent Barbon, Anais Balsamo, Ana Reynders, et al.

► **To cite this version:**

Guillaume Hoeffel, Guillaume Debroas, Anais Roger, Rafaëlle Rossignol, Jordi Gouilly, et al.. Sensory neuron-derived TFAFA4 promotes macrophage tissue repair functions. *Nature*, 2021, 594 (7861), pp.94-99. 10.1038/s41586-021-03563-7 . hal-03419356

**HAL Id: hal-03419356**

**<https://amu.hal.science/hal-03419356>**

Submitted on 8 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

# **Sensory neuron-derived TFAFA4 promotes macrophage tissue repair functions**

Guillaume Hoeffel<sup>1\*#</sup>, Guillaume Debroas<sup>1\*</sup>, Anais Roger<sup>1</sup>, Rafaelle Rossignol<sup>1</sup>, Jordi Gouilly<sup>1</sup>, Caroline Laprie<sup>1</sup>, Lionel Chasson<sup>1</sup>, Pierre Vincent Barbon<sup>1</sup>, Anais Balsamo<sup>1</sup>, Ana Reynders<sup>2</sup>, Aziz Moqrich<sup>2</sup> and Sophie Ugolini<sup>1#</sup>

<sup>1</sup> Aix Marseille Univ, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, France

<sup>2</sup> Aix Marseille Univ, CNRS, IBDM, Institut de Biologie du Développement de Marseille, Marseille, France

\*Equal contributions

#Corresponding authors: hoeffel@ciml.univ-mrs.fr; ugolini@ciml.univ-mrs.fr

Keywords: Monocytes, macrophages, sensory neurons, neuropeptides, tissue repair, skin fibrosis, TFAFA4, GINIP

Running title: TFAFA4 triggers IL-10 production by dermal macrophages and promotes skin repair

25 **Summary**

26 **Inflammation is a defense response to tissue damage requiring tight regulation to**  
27 **prevent impaired healing. Tissue-resident macrophages play a key role in tissue**  
28 **repair<sup>1</sup> but the precise molecular mechanisms regulating the balance between**  
29 **inflammatory and pro-repair macrophage responses during healing remain**  
30 **poorly understood. We demonstrate here a major role of sensory neurons in**  
31 **promoting macrophage tissue repair functions. In a sunburn-like model of skin**  
32 **damage, the conditional ablation of sensory neurons expressing the Gαi-**  
33 **interacting protein (GINIP) resulted in defective tissue regeneration, and dermal**  
34 **fibrosis. Dissection of the underlying molecular mechanisms revealed a crucial**  
35 **role for the neuropeptide TAFA4, produced in the skin by C-low threshold**  
36 **mechanoreceptors (C-LTMR), a subset of GINIP<sup>+</sup> neurons. TAFA4 modulated the**  
37 **inflammatory profile of macrophages directly *in vitro*. *In vivo* analyses in *Tafa4-***  
38 **deficient mice revealed that TAFA4 promoted IL-10 production by dermal**  
39 **macrophages after UV-induced skin damage. This TAFA4/IL-10 axis also ensured**  
40 **the survival and maintenance of IL-10<sup>+</sup>Tim4<sup>+</sup> dermal macrophages, resolving skin**  
41 **inflammation and promoting tissue regeneration. These results reveal a new**  
42 **neuroimmune regulatory pathway driven by the neuropeptide TAFA4 that**  
43 **promotes macrophage anti-inflammatory functions and prevents fibrosis upon**  
44 **tissue damage, creating new therapeutic perspectives for inflammatory diseases.**

45

46

47

48

49

50 **Main text**

51 Skin overexposure to ultraviolet (UV) light causes sunburn, characterized by epidermis  
52 destruction and inflammation of the underlying dermal papilla<sup>2</sup>. Such tissue damage  
53 induces a complex inflammatory response, requiring tight regulation to prevent  
54 persistent injury. Sunburn is also characterized by a transient phase of painful  
55 hypersensitivity, mediated by the activation of specialized skin-innervating sensory  
56 neurons<sup>3,4</sup>. Studies of other pathological cutaneous conditions have shown that the  
57 peripheral nervous system regulates cutaneous inflammatory processes<sup>3,5-9</sup>. Most  
58 studies have focused on the roles of TRPV1<sup>+</sup> peptidergic primary sensory neurons and  
59 the neuropeptide CGRP<sup>3,5-7,9</sup> (calcitonin gene-related peptide). However, the potential  
60 immunoregulatory role of non-peptidergic C-fibers expressing GINIP<sup>10</sup> remains  
61 unknown.

62

63 **GINIP<sup>+</sup> neurons prevent UV-induced skin fibrosis**

64 We analyzed the role of somatosensory neurons in tissue repair in mouse ears exposed  
65 to UV-C radiation, an experimental model of sunburn (**Fig. 1a**). We assessed the  
66 expression of genes associated with tissue inflammation and repair in skin from wild-  
67 type (WT) mice, following UV overexposure. Genes encoding inflammatory  
68 molecules, such as *Il-1 $\beta$*  and *Tnf $\alpha$* , were upregulated until day 7 post-UV irradiation  
69 (pi), whereas pro-repair genes, such as *Colla1* and *Retna*, were upregulated from day  
70 14 until day 35 pi, defining the resolution/repair phase of this model (**Extended data**  
71 **Fig. 1a**).

72 We analyzed the consequences for the somatosensory nervous system of skin  
73 overexposure to UV light. Most of the neurons responsible for detecting noxious stimuli  
74 and eliciting pain perception express the sodium channel Nav1.8<sup>11-13</sup>, and have cell

75 bodies in the dorsal root ganglia (DRGs) or trigeminal ganglion. We monitored the  
76 expression of ATF3 (Activating Transcription Factor 3), a transcription factor used as  
77 a proxy for neuronal injury and activation<sup>14</sup>, in DRGs after skin exposure to UV. *Atf3*  
78 expression significantly increased in C2/C3 DRGs, which specifically innervate ear  
79 skin (**Fig. 1b, Extended data Fig. 1b-d**).

80 We determined the phenotype of ATF3<sup>+</sup> DRG neurons, by immunofluorescence  
81 staining. Very few of these neurons co-expressed CGRP pi, whereas up to 50% co-  
82 expressed GINIP (**Fig. 1c, d, Extended data Figure 1e, f**), suggesting that UV-induced  
83 skin inflammation essentially affects GINIP<sup>+</sup> somatosensory neurons.

84 We therefore investigated the role of these Nav1.8<sup>+</sup>GINIP<sup>+</sup> neurons (GINIP<sup>+</sup> neurons)  
85 in tissue repair, by conditional depletion in the GINIP-DTR model<sup>10,15</sup> (**Extended data**  
86 **Fig. 2a-c**). Between days 5 and 35 pi, ear skin was significantly thicker in GINIP-DTR  
87 mice than in control DTR (“control”) littermates (**Fig. 1e**). Macroscopic analysis also  
88 revealed greater redness, tissue shrinkage and signs of necrosis leading to ear tissue loss  
89 in GINIP-DTR mice (**Fig. 1f**). These skin lesions were not associated with an increase  
90 in scratching in GINIP-DTR mice (**Extended data Fig. 2d**). Histological analysis  
91 showed similar steady-state skin structures in GINIP-DTR and control littermates. By  
92 contrast, 35 days pi, GINIP-DTR mice displayed marked pinna thickening, with an  
93 increase in horizontally oriented collagen fiber deposition, persistent leukocytic  
94 infiltration and focal auricular cartilage obliteration, whereas control mice presented  
95 signs of skin healing (**Fig. 1g-j, Extended data Fig. 2e-j**). Thus, GINIP<sup>+</sup> neurons  
96 prevent skin over-inflammation and fibrosis after UV irradiation.

97

98 **Impaired macrophage response in GINIP-DTR mice**

99 We investigated whether the profound tissue repair defect of GINIP-DTR mice was  
100 linked to dysregulation of the cutaneous immune response. The absolute numbers of  
101 dendritic cells (DC), Langerhans cells (LC), mast cells, lymphoid cells and  
102 granulocytes were similar in GINIP-DTR and control mice, at steady state and 14 days  
103 pi, whereas GINIP-DTR mice had fewer monocytes/macrophages (Mo/M $\phi$ ) than  
104 control mice pi (**Extended data Fig. 3a-d**). None of these immune cells expressed DTR  
105 in GINIP-DTR mice, and their numbers were unaffected by DT treatment in  
106 homeostatic conditions (**Extended data Fig. 3b-d, f**).

107 Macrophages regulate all stages of tissue repair<sup>1</sup>, and dysregulation of their homeostasis  
108 can lead to chronic inflammation, excessive collagen deposition and  
109 hypertrophic/fibrotic scars<sup>1</sup>. Dermal resident M $\phi$  expressing CD206 were observed  
110 near GINIP<sup>+</sup> nerve terminals, suggesting possible functional interaction (**Fig. 2a-b**,  
111 **Extended data Fig. 3g**). We analyzed the dermal Mo/M $\phi$  response upon UV  
112 overexposure. Tissue-resident M $\phi$  emerge either during embryonic development<sup>16-18</sup> or  
113 differentiate postnatally from circulating monocytes<sup>18,19</sup>. Upon inflammation,  
114 monocyte subsets<sup>20</sup>, including “classical” Ly6C<sup>+</sup> monocytes (Mo), “patrolling” Ly6C<sup>lo</sup>  
115 monocytes (PMo) and “intermediate” Ly6C<sup>+</sup>MHC-II<sup>+</sup> monocytes (Int. Mo), can  
116 generate short-lived MHC-II<sup>+</sup> monocyte-derived DC (MoDC) and monocyte-derived  
117 CD64<sup>+</sup>CD206<sup>-</sup> inflammatory macrophages (Inflam M $\phi$ ). We identified eight Mo/M $\phi$   
118 subsets based on differential expression of CCR2, CD64, Ly6C, MHC-II, CD206 and  
119 Tim4 (**Fig. 2c**, **Extended data Fig. 4a-c**). Their steady-state distributions were similar  
120 in the skin of GINIP-DTR and control mice (**Fig. 2e, f**, **Extended data Fig. 4d-h**). By  
121 contrast, absolute numbers of Tim4<sup>+</sup> dermal M $\phi$  were very low from days 3 to 14 pi in  
122 the skin of GINIP-DTR mice (**Fig. 2c-e**), whereas Ly6C<sup>+</sup>Mo recruitment increased on  
123 day 3 pi (**Extended data Fig. 4d**). This influx of Ly6C<sup>+</sup>Mo was associated with

124 increases in Inflamm M $\phi$  numbers in GINIP-DTR mice from days 3 to 14 pi (**Fig. 2f**),  
125 consistent with the stronger skin inflammation observed in these mice (**Fig. 1e**). By  
126 contrast, the expansion of DN and MHC-II<sup>+</sup> dermal M $\phi$  subsets was little affected by  
127 the absence of GINIP<sup>+</sup> neurons (**Extended data Fig. 4e-h**). Thus, signals from GINIP<sup>+</sup>  
128 sensory neurons promote Tim4<sup>+</sup> dermal M $\phi$  maintenance and control Inflamm M $\phi$   
129 responses following UV-induced skin damage.

130

### 131 **TAFA4 promotes skin repair upon UV exposure**

132 We investigated the mechanisms underlying the immunomodulatory and pro-repair role  
133 of GINIP<sup>+</sup> neurons. GINIP is expressed by two somatosensory neuron subsets:  
134 GINIP<sup>+</sup>/IB4<sup>+</sup> neurons, which also express MRGPRD and selectively innervate the  
135 epidermis, and GINIP<sup>+</sup>/IB4<sup>-</sup> neurons, which express TAFA4, TH and VGLUT3,  
136 markers of the C-low-threshold mechanoreceptors (C-LTMRs) selectively innervating  
137 hair follicles<sup>10,21</sup> (**Extended data Fig. 5a-c**). Binding to isolectin-B4 (IB4) and TAFA4  
138 can be used to distinguish between these subsets in DRGs<sup>10,22,23</sup>. We generated anti-  
139 TAFA4 monoclonal antibodies (**Extended data Fig. 5d, g**), for C-LTMR  
140 identification. As expected, TAFA4<sup>+</sup>, TH<sup>+</sup> and IB4<sup>+</sup> neurons were ablated in GINIP-  
141 DTR mice, whereas CGRP<sup>+</sup> neurons were spared (**Extended data Fig. 2b**). DRG  
142 staining showed that 10-20% of ATF3<sup>+</sup> neurons were IB4<sup>+</sup> and 20-50% were TAFA4<sup>+</sup>  
143 (**Fig. 3a, b, Extended data Fig. 5e, f**), suggesting potential roles for these subsets of  
144 GINIP<sup>+</sup> neurons in regulating UV-induced skin inflammation.

145 C-LTMRs mediate two opposing aspects of touch sensation: pleasantness and injury-  
146 induced mechanical pain<sup>10,22</sup>. TAFA4 is a secreted protein<sup>24</sup> that can modulate injury-  
147 induced mechanical hypersensitivity and chemical pain in mice<sup>22</sup>. TAFA4 promotes

148 human macrophage chemotaxis *in vitro*<sup>25</sup>, but its impact on myeloid cell responses *in*  
149 *vivo* remains unknown.

150 We established an ELISA for TAFA4 detection in tissues. UV exposure induced  
151 increases in TAFA4 levels in WT mouse DRGs and skin (**Fig. 3c, Extended data Fig.**  
152 **5h**). We investigated the cellular source of TAFA4 in skin. Consistent with publicly  
153 available database (<http://www.immgen.org>; <http://biogps.org>), *Tafa4* (*Fam19a4*)  
154 transcription was observed in DRGs, but not in the blood, gut, lung or skin (**Extended**  
155 **data Fig. 5i**). *Tafa4* transcription has been reported in human M $\phi$  following LPS  
156 stimulation<sup>25</sup>. However, we detected no *Tafa4* transcripts in BM-derived macrophages  
157 (BMDM) activated with LPS (+/-TAFA4) or in sorted CD206<sup>+</sup> dermal M $\phi$  on days 0  
158 and 7 pi (**Extended data Fig. 5j**). *Tafa4* mRNA was undetectable in the skin, even after  
159 UV exposure, but abundant in DRGs pi (**Extended data Fig.1a, 5k**). These data  
160 strongly suggest that *Tafa4* is transcribed only in DRG neuron cell bodies, with protein  
161 release into the skin via C-LTMR nerve endings.

162 We investigated the possible role of TAFA4 in regulating tissue regeneration pi, by  
163 studying *Tafa4*-KO mice<sup>22</sup>. From days 14 to 35 pi, *Tafa4*-KO mice had thicker ear skin  
164 than control littermates, suggesting defective resolution of skin inflammation in the  
165 absence of TAFA4 (**Extended data Fig. 6a**). No histological change in the skin of  
166 *Tafa4*-KO mice was observed in homeostatic conditions. By contrast, 35 days pi,  
167 leukocyte infiltration score and epidermal thickness were higher in *Tafa4*-KO mice,  
168 which displayed more extensive, persistent dermal fibrosis (**Extended data Fig. 6b, c**).  
169 Picro Sirius Red staining revealed characteristic excessive type 1 collagen deposition  
170 in unresolved fibrotic scars in the dermis of *Tafa4*-KO mice (**Fig. 3d, e**). Thus, like  
171 GINIP-DTR mice, *Tafa4*-KO mice presented pathological fibrosis, suggesting a  
172 requirement for the neuropeptide TAFA4, produced by C-LTMRs, for skin repair pi.

173

174 **TAFA4 regulates dermal macrophage IL-10 production**

175 We investigated the mechanisms by which TAFA4 regulates skin repair. We monitored  
176 inflammatory cytokine and chemokine concentrations in skin from *Tafa4*-KO mice and  
177 control littermates from days 0 to 35 pi. TNF $\alpha$ , IL-1 $\beta$ , IL-6, CCL2, CCL4 and CXCL1  
178 (**Fig. 3f, Extended data Fig. 6d**) levels decreased between days 7 and 10 pi in control  
179 mice, but remained higher in *Tafa4*-KO mice. By contrast, expression levels of the anti-  
180 inflammatory cytokine IL-10 were lower in the skin of *Tafa4*-KO mice pi (**Fig. 3g,**  
181 **Extended data Fig. 6d**). Resolution of the inflammatory phase was therefore impaired  
182 in the absence of TAFA4.

183 IL-10 is a potent anti-inflammatory molecule. We hypothesized that impaired IL-10  
184 production might underlie the observed dysregulation of skin repair in UV-exposed  
185 *Tafa4*-KO mice. Intracellular staining and analyses in IL-10-GFP reporter mice  
186 identified resident M $\phi$  and mast cells<sup>26</sup> as the main producers of IL-10, with regulatory  
187 T cells (Tregs) producing only small amounts (**Extended data Fig. 6e-i**). Mast cell  
188 numbers and IL-10 production decreased strongly on day 3 pi (**Extended data Fig. 6j-**  
189 **m**). TAFA4 deficiency was not associated with modulation of IL-10 production by  
190 Tregs and mast cells (**Extended data Fig. 6i-m**). By contrast, *Il10* levels in sorted  
191 CD206<sup>+</sup> dermal M $\phi$  were lower in *Tafa4*-KO mice than in controls on days 7 and 10 pi  
192 (**Fig. 3h**). Intracellular staining confirmed that TAFA4 was required for the  
193 upregulation of IL-10 production in all CD206<sup>+</sup> M $\phi$  subsets after UV overexposure,  
194 with Tim4<sup>+</sup> M $\phi$  producing more IL-10 than DN and MHC-II<sup>+</sup> M $\phi$  (**Extended data Fig.**  
195 **7a-c**).

196 We investigated whether TAFA4 regulated M $\phi$  activation directly. BMDM or purified  
197 peritoneal M $\phi$  (ThioM $\phi$ ) were activated with LPS in the presence or absence of TAFA4.

198 *Tnfa*, *Il1β* and *Il6* transcripts were significantly downregulated, whereas *Il10*  
199 transcripts were upregulated by TAFA4 (**Fig. 3i, Extended data Fig.7e**). Together,  
200 these data suggest that the TAFA4-mediated regulation of IL-10 production in the skin  
201 upon UV-exposure is mediated essentially through the regulation of dermal  
202 Mφ functions.

203 Formyl peptide receptor 1 (FPR1) has been described as a TAFA4 receptor inducing  
204 the chemotaxis of human Mφ and transfected cell lines *in vitro*<sup>25</sup>. We observed no  
205 chemotactic effect of TAFA4 on mouse BMDM or isolated peritoneal Mφ *in vitro*  
206 (**Extended data Fig. 7d and data not shown**). Consistently *in vivo*, monocyte-derived  
207 cell recruitment pi was not reduced in GINIP-DTR mice (**Extended data Figure 4d**).  
208 Moreover, TAFA4 had a similar immunoregulatory effect on BMDM from FPR1-KO  
209 and WT mice showing that at least one other unknown receptor drives the functional  
210 effect of TAFA4 on Mφ (**Extended data Fig. 7f**). Thus, TAFA4 can regulate the  
211 inflammatory profile of Mφ directly, in an FPR1-independent manner. It will be  
212 important to determine whether macrophage regulation by TAFA4 differs between  
213 mice and humans.

214

### 215 **A TAFA4-IL10-Tim4<sup>+</sup> macrophage axis promotes tissue repair**

216 We then investigated whether TAFA4 affected the dynamics between dermal resident  
217 Mφ and newly recruited monocyte-derived cells during repair. We first performed a  
218 lineage-tracing analysis, tracking the fate of CX3CR1<sup>+</sup> embryonic Mφ precursors using  
219 the tamoxifen-dependent CX3CR1<sup>CreERT2</sup>:R26-YFP mouse model<sup>27</sup>. YFP expression  
220 was selectively induced in CX3CR1<sup>+</sup> cells on day 16.5 of embryonic development. In  
221 adult mice under homeostatic conditions, the MHC-II<sup>+</sup> and DN dermal Mφ subsets only  
222 weakly expressed YFP (**Extended data Fig. 8a, b**). By contrast, 20% of Tim4<sup>+</sup> Mφ

223 were YFP<sup>+</sup>, like LCs<sup>28</sup>, suggesting that Tim4<sup>+</sup> Mφ are mostly *bona fide* embryo-derived  
224 cells.

225 We then assessed the contribution of monocytes to the various dermal Mo/Mφ subsets,  
226 by generating shield-irradiated BM chimeras (**Extended data Fig. 8c**). As expected,  
227 two months after CD45.1 BM reconstitution in CD45.2 recipients, 90% of blood  
228 monocytes were CD45.1<sup>+</sup>, whereas microglia were entirely of recipient (CD45.2<sup>+</sup>)  
229 origin. Under homeostatic conditions, dermal Ly6C<sup>+</sup>Mo, Int Mo, PMo, MoDC and  
230 Inflam Mφ were mostly CD45.1<sup>+</sup>, confirming their BM origin, whereas Tim4<sup>+</sup> Mφ  
231 were CD45.2<sup>+</sup>, demonstrating their self-maintenance over time independently of  
232 circulating monocytes (**Extended data Fig. 8d**). However, 120 days pi, the CD45.1  
233 chimerism of DN and Tim4 Mφ increased (**Extended data Fig. 8e**), showing that  
234 monocytes recruited pi acquired a resident-like Mφ phenotype with self-renewing  
235 capacity.

236 We then generated shield-irradiated BM chimeras, using WT or *Tafa4*-KO (both  
237 CD45.2) recipient mice (**Fig. 4a-f, Extended data Fig. 8f-h**). Intracellular staining on  
238 day 7 pi revealed that IL-1β expression was mostly associated with monocyte-derived  
239 cells, whereas TNFα expression was restricted essentially to Inflam Mφ (**Fig. 4c**). The  
240 number of Inflam Mφ cells in *Tafa4*-KO chimeric mice was higher than that in control  
241 chimeras, suggesting that TAFA4 reduces the number of newly derived Inflam Mφ  
242 (**Fig. 4d**). Tim4<sup>+</sup> Mφ were the predominant CD45.2<sup>+</sup> population in both chimeras (**Fig.**  
243 **4b, c**), consistent with our previous analysis. Tim4<sup>+</sup> Mφ produced large amounts of IL-  
244 10, but no IL-1β or TNFα (**Fig. 4c**), highlighting their anti-inflammatory function.  
245 TAFA4 deficiency had little effect on DN and MHC-II<sup>+</sup> Mφ homeostasis pi (**Extended**  
246 **data Fig. 8h**). By contrast, the absolute number of CD45.2<sup>+</sup> Tim4<sup>+</sup> Mφ, which was

247 similar in both chimeras under homeostatic conditions, strongly decreased in *Tafa4*-  
248 KO recipient mice pi (**Fig. 4e left**). Overall CD45.1 chimerism levels in Tim4<sup>+</sup> Mφ  
249 revealed that CD45.2<sup>+</sup> Tim4<sup>+</sup> Mφ were replaced by CD45.1<sup>+</sup> monocyte differentiation  
250 to a greater extent in *Tafa4*-KO than in WT recipients (**Fig. 4e right**). However, the  
251 overall Tim4<sup>+</sup> Mφ pool expanded to a lesser extent in the absence of TAFA4 (**Fig. 4f**),  
252 suggesting that both embryo-derived, and monocyte-derived Tim4<sup>+</sup> Mφ require TAFA4  
253 for survival pi. Consistently, the frequency of apoptotic Annexin<sup>+</sup>Tim4<sup>+</sup> cells in UV-  
254 exposed skin was higher for GINIP-DTR and TAFA4-KO mice than for control  
255 littermates, whereas DN and MHC-II<sup>+</sup> subsets were unaffected (**Extended data Fig.**  
256 **8i-l**). Thus, following exposure to UV, GINIP-DTR and TAFA4-KO mice present an  
257 imbalanced Mφ response, with impaired survival of IL-10-producing Tim4<sup>+</sup> Mφ and  
258 larger numbers of Inflamm Mφ. TAFA4 deficiency therefore affects both the  
259 maintenance of dermal Tim4<sup>+</sup> Mφ and their IL-10 production capacity.

260

261 Intradermal injections of IL-10-blocking antibodies before UV irradiation in WT mice  
262 induced a phenotype similar to that of *Tafa4*-KO mice (Extended data **Fig. 9a-c**),  
263 suggesting that IL-10 upregulation is essential for Tim4<sup>+</sup> Mφ maintenance. Similarly,  
264 IL-10-deficient mice (*Il-10<sup>gfp/gfp</sup>* mice) exposed to UV had smaller numbers of Tim4<sup>+</sup>  
265 Mφ, larger numbers of Inflamm Mφ, and greater skin damage (**Extended data Fig. 9d-**  
266 **f**). IL-10 defects are, therefore, sufficient to mimic the phenotype of GINIP-DTR and  
267 *Tafa4*-KO mice, suggesting that IL-10 acts downstream from TAFA4 signaling.

268 Consistent with the crucial role of IL-10-producing Mφ in this model, we found that  
269 resident Mφ depletion by CSF-1R-blocking antibody (AFS98)<sup>17</sup> treatment before UV  
270 exposure decreased total skin IL-10 content (**Extended data Fig. 9g-j**). Monocyte  
271 infiltration after resident Mφ depletion led to the rapid reconstitution of MHC-II<sup>+</sup> and

272 DN M $\phi$  subsets, whereas Tim4<sup>+</sup> M $\phi$  generation took up to six days (**Extended data**  
273 **Fig. 9k**). M $\phi$ -depleted mice presented increases in the number of Inflamm M $\phi$  and skin  
274 damage pi (**Extended data Fig. 9l-n**), demonstrating the requirement of a  
275 TAFA4/dermal resident M $\phi$  axis for regulating Inflamm M $\phi$  responses. Furthermore,  
276 adoptive transfer of sorted dermal Tim4<sup>+</sup> M $\phi$  in the skin of GINIP-DTR mice pi was  
277 sufficient to limit tissue damage (**Extended data Fig. 10a-d**). Thus, the early loss of  
278 dermal resident Tim4<sup>+</sup> M $\phi$  determines the tissue damage and repair defect rates in  
279 GINIP-DTR and TAFA4-KO mice.

280 Finally, the phenotype of TAFA4-KO mice was rescued by intradermal injections of  
281 recombinant TAFA4 pi (**Fig. 4g, h**). TAFA4 administration was also sufficient to  
282 upregulate Tim4<sup>+</sup> M $\phi$  IL-10 production in TAFA4-KO mice (**Extended data Fig. 10e-**  
283 **f**). These data suggest that TAFA4 production by C-LTMR nerve endings after UV  
284 exposure is necessary and sufficient to downregulate inflammatory processes by  
285 modulating dermal M $\phi$  homeostasis and IL-10 production.

286

## 287 **Discussion**

288 This study reveals a crucial immunoregulatory role of non-peptidergic GINIP<sup>+</sup>  
289 cutaneous sensory neurons in tissue repair, through TAFA4 production, promoting  
290 early and persistent IL-10 production by dermal macrophages. TAFA4 ensures the  
291 survival of embryo-derived Tim4<sup>+</sup> M $\phi$  and sustains their production of large amounts  
292 of IL-10. This neuro-immune pathway downregulates Inflamm M $\phi$  expansion, thereby  
293 reducing inflammatory cytokine levels, and promoting skin repair (**Extended data Fig.**  
294 **10g**). These data are consistent with the metabolic reprogramming capacity of IL-10<sup>29</sup>  
295 and with previous reports of IL-10 deficiency leading to excessive collagen deposition  
296 during wound healing<sup>30</sup>. The regulatory role of TAFA4 is not limited to Tim4<sup>+</sup> M $\phi$ .

297 TAFA4 can also modulate the inflammatory profile of other macrophage subsets *in*  
298 *vitro* and *in vivo*, including other CD206<sup>+</sup> dermal M $\phi$  subsets and peritoneal M $\phi$ .  
299 Recent studies of genetic or chemical ablations of sensory neuron subsets have revealed  
300 a regulatory role of the somatosensory nervous system in cutaneous immune  
301 responses<sup>3,5-9</sup>, which may be pro- or anti-inflammatory. The considerable functional  
302 and phenotypic heterogeneity of somatosensory neurons<sup>31-33</sup>, and their selective or  
303 combinatorial stimulation, may account for immunoregulatory differences between  
304 pathological contexts. Previous studies have identified an important role of TRPV1<sup>+</sup>  
305 neurons and the neuropeptide CGRP in bacterial and fungal infections and models of  
306 sterile and imiquimod-induced cutaneous inflammation<sup>3,5-7,9</sup>. Our study reveals a  
307 crucial immunomodulatory role of TAFA4 production by C-LTMRs in a sunburn-like  
308 model of skin damage. By contrast, no such effect was observed in a model of  
309 imiquimod-induced skin inflammation (**Extended data Fig. 10h**). It will therefore be  
310 important to determine whether this novel neuroimmune regulatory pathway also plays  
311 a role in the skin in other pathological contexts. The properties of TAFA4 could be  
312 exploited therapeutically to modulate inflammation in other inflammatory diseases or  
313 in patients with wound healing defects.

314

315

316

317

318

319

320

321

322 **References**

323

324 1 Wynn, T. A. & Vannella, K. M. Macrophages in Tissue Repair, Regeneration, and  
325 Fibrosis. *Immunity* **44**, 450-462, doi:10.1016/j.immuni.2016.02.015 (2016).

326 2 Merad, M. *et al.* Langerhans cells renew in the skin throughout life under steady-state  
327 conditions. *Nat Immunol* **3**, 1135-1141, doi:10.1038/ni852 (2002).

328 3 La Russa, F. *et al.* Disruption of the Sensory System Affects Sterile Cutaneous  
329 Inflammation In Vivo. *J Invest Dermatol* **139**, 1936-1945 e1933,  
330 doi:10.1016/j.jid.2019.01.037 (2019).

331 4 Lopes, D. M. & McMahon, S. B. Ultraviolet Radiation on the Skin: A Painful  
332 Experience? *CNS Neurosci Ther* **22**, 118-126, doi:10.1111/cns.12444 (2016).

333 5 Chiu, I. M. *et al.* Bacteria activate sensory neurons that modulate pain and  
334 inflammation. *Nature* **501**, 52-57, doi:10.1038/nature12479 (2013).

335 6 Riol-Blanco, L. *et al.* Nociceptive sensory neurons drive interleukin-23-mediated  
336 psoriasiform skin inflammation. *Nature* **510**, 157-161, doi:10.1038/nature13199  
337 (2014).

338 7 Kashem, S. W. *et al.* Nociceptive Sensory Fibers Drive Interleukin-23 Production from  
339 CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. *Immunity*  
340 **43**, 515-526, doi:10.1016/j.immuni.2015.08.016 (2015).

341 8 Pinho-Ribeiro, F. A. *et al.* Blocking Neuronal Signaling to Immune Cells Treats  
342 Streptococcal Invasive Infection. *Cell* **173**, 1083-1097 e1022,  
343 doi:10.1016/j.cell.2018.04.006 (2018).

344 9 Cohen, J. A. *et al.* Cutaneous TRPV1(+) Neurons Trigger Protective Innate Type 17  
345 Anticipatory Immunity. *Cell* **178**, 919-932 e914, doi:10.1016/j.cell.2019.06.022  
346 (2019).

347 10 Gaillard, S. *et al.* GINIP, a Galphai-interacting protein, functions as a key modulator  
348 of peripheral GABAB receptor-mediated analgesia. *Neuron* **84**, 123-136,  
349 doi:10.1016/j.neuron.2014.08.056 (2014).

350 11 Basbaum, A. I., Bautista, D. M., Scherrer, G. & Julius, D. Cellular and molecular  
351 mechanisms of pain. *Cell* **139**, 267-284, doi:10.1016/j.cell.2009.09.028 (2009).

352 12 Abraira, V. E. & Ginty, D. D. The sensory neurons of touch. *Neuron* **79**, 618-639,  
353 doi:10.1016/j.neuron.2013.07.051 (2013).

354 13 Abrahamsen, B. *et al.* The cell and molecular basis of mechanical, cold, and  
355 inflammatory pain. *Science* **321**, 702-705, doi:10.1126/science.1156916 (2008).

356 14 Braz, J. M. & Basbaum, A. I. Differential ATF3 expression in dorsal root ganglion  
357 neurons reveals the profile of primary afferents engaged by diverse noxious chemical  
358 stimuli. *Pain* **150**, 290-301, doi:10.1016/j.pain.2010.05.005 (2010).

359 15 Urien, L. *et al.* Genetic ablation of GINIP-expressing primary sensory neurons strongly  
360 impairs Formalin-evoked pain. *Sci Rep* **7**, 43493, doi:10.1038/srep43493 (2017).

361 16 Gomez Perdiguero, E. *et al.* Tissue-resident macrophages originate from yolk-sac-  
362 derived erythro-myeloid progenitors. *Nature* **518**, 547-551, doi:10.1038/nature13989  
363 (2015).

364 17 Hoeffel, G. *et al.* C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give  
365 rise to adult tissue-resident macrophages. *Immunity* **42**, 665-678,  
366 doi:10.1016/j.immuni.2015.03.011 (2015).

367 18 Hoeffel, G. & Ginhoux, F. Fetal monocytes and the origins of tissue-resident  
368 macrophages. *Cell Immunol* **330**, 5-15, doi:10.1016/j.cellimm.2018.01.001 (2018).

369 19 Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and  
370 tissue homeostasis. *Nat Rev Immunol* **14**, 392-404, doi:10.1038/nri3671 (2014).

371 20 Guilliams, M., Mildner, A. & Yona, S. Developmental and Functional Heterogeneity  
372 of Monocytes. *Immunity* **49**, 595-613, doi:10.1016/j.immuni.2018.10.005 (2018).

373 21 Li, L. *et al.* The functional organization of cutaneous low-threshold mechanosensory  
374 neurons. *Cell* **147**, 1615-1627, doi:10.1016/j.cell.2011.11.027 (2011).

375 22 Delfini, M. C. *et al.* TFAFA4, a chemokine-like protein, modulates injury-induced  
376 mechanical and chemical pain hypersensitivity in mice. *Cell Rep* **5**, 378-388,  
377 doi:10.1016/j.celrep.2013.09.013 (2013).

378 23 Reynders, A. *et al.* Transcriptional Profiling of Cutaneous MRGPRD Free Nerve  
379 Endings and C-LTMRs. *Cell Rep* **10**, 1007-1019, doi:10.1016/j.celrep.2015.01.022  
380 (2015).

381 24 Tom Tang, Y. *et al.* TFAFA: a novel secreted family with conserved cysteine residues  
382 and restricted expression in the brain. *Genomics* **83**, 727-734,  
383 doi:10.1016/j.ygeno.2003.10.006 (2004).

384 25 Wang, W. *et al.* FAM19A4 is a novel cytokine ligand of formyl peptide receptor 1  
385 (FPR1) and is able to promote the migration and phagocytosis of macrophages. *Cell*  
386 *Mol Immunol* **12**, 615-624, doi:10.1038/cmi.2014.61 (2015).

387 26 Grimaldeston, M. A., Nakae, S., Kalesnikoff, J., Tsai, M. & Galli, S. J. Mast cell-  
388 derived interleukin 10 limits skin pathology in contact dermatitis and chronic  
389 irradiation with ultraviolet B. *Nat Immunol* **8**, 1095-1104, doi:10.1038/ni1503 (2007).

390 27 Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue  
391 macrophages under homeostasis. *Immunity* **38**, 79-91,  
392 doi:10.1016/j.immuni.2012.12.001 (2013).

393 28 Hoeffel, G. *et al.* Adult Langerhans cells derive predominantly from embryonic fetal  
394 liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp*  
395 *Med* **209**, 1167-1181, doi:10.1084/jem.20120340 (2012).

396 29 Ip, W. K. E., Hoshi, N., Shouval, D. S., Snapper, S. & Medzhitov, R. Anti-  
397 inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages.  
398 *Science* **356**, 513-519, doi:10.1126/science.aal3535 (2017).

399 30 Eming, S. A. *et al.* Accelerated wound closure in mice deficient for interleukin-10. *Am*  
400 *J Pathol* **170**, 188-202, doi:10.2353/ajpath.2007.060370 (2007).

401 31 Cavanaugh, D. J. *et al.* Distinct subsets of unmyelinated primary sensory fibers mediate  
402 behavioral responses to noxious thermal and mechanical stimuli. *Proc Natl Acad Sci U*  
403 *S A* **106**, 9075-9080, doi:10.1073/pnas.0901507106 (2009).

404 32 Usoskin, D. *et al.* Unbiased classification of sensory neuron types by large-scale single-  
405 cell RNA sequencing. *Nat Neurosci* **18**, 145-153, doi:10.1038/nn.3881 (2015).

406 33 Sharma, N. *et al.* The emergence of transcriptional identity in somatosensory neurons.  
407 *Nature* **577**, 392-398, doi:10.1038/s41586-019-1900-1 (2020).

408  
409  
410

411 **Figure legends**

412 **Figure 1: GINIP<sup>+</sup> sensory neurons prevent fibrosis after UV exposure**

413 **a**, Experimental scheme indicating the phases of skin inflammation, resolution and  
414 repair after overexposure to UV radiation (See also **Extended data fig. 1a** for  
415 inflammatory and pro-repair gene expression pi). **b**, *Atf3* mRNA levels in C2/C3 DRGs  
416 over time in UV-irradiated (purple dots) and non-exposed mice (gray dots), (*n*=5 mice  
417 per group). **c**, Confocal images of GINIP (red), CGRP (blue) and ATF3 (green)  
418 expression in DRGs, from unexposed (No UV) and UV-exposed mice (D3 UV). (See  
419 also C2/C3 DRG characterization in **Extended data fig. 1b-f**). **d**, Percentage of ATF3<sup>+</sup>  
420 neurons from C2/C3 DRGs positive for CGRP (blue) or GINIP (red) at D3 pi (*n*=8 mice  
421 per group). **e**, Changes in ear thickness over time pi, in control DTR (blue) and GINIP-  
422 DTR mice (red), (*n*=7 mice per group). **f**, Representative control DTR (left) or GINIP-  
423 DTR mice (right) ears on D14 pi. (see also neuron depletion in GINIP-DTR skin,  
424 **Extended data fig. 2a-b**). Representative H&E staining of ear sections from control  
425 DTR (**g**), and GINIP-DTR mice (**h**), at D35 pi. Fibrosis (**i**), and cumulative  
426 histopathological score (**j**), at D35 pi (*n*=5 mice per group). (See also **Extended data**  
427 **fig. 2d-j** for detailed analysis of histopathological score, scratching behavior and back  
428 skin analysis). Data are presented as mean ± SEM and representative for at least two  
429 independent experiments. One-way ANOVA with Tukey tests, except for **b** and **e**, two-  
430 way ANOVA and **d**, Student's *t*-test.

431

432 **Figure 2: Dermal-resident Tim4<sup>+</sup> macrophage maintenance is compromised in**  
433 **UV-exposed GINIP-DTR mice**

434 **a**, Confocal analysis of ear skin from Nav1.8-RFP mice stained for GINIP<sup>+</sup> neurons  
435 (green) and CD206<sup>+</sup> dermal resident Mφ (blue). **b**, Distance (μm) between CD206<sup>+</sup> Mφ

436 and GINIP<sup>+</sup> axons (n=97 Mφ). (See also **Extended data Fig. 3g** for higher  
437 magnification). **c**, Cluster analysis and t-SNE distribution of Mo/Mφ subsets.  
438 Monocyte-related subsets (CCR2<sup>+</sup>) clustered as Ly6C<sup>+</sup> Mo (Ly6C<sup>+</sup>, orange), Int. Mo  
439 (Ly6C<sup>int</sup>, MHCII<sup>int</sup>, light green), PMo (Ly6C<sup>-</sup>, MHCII<sup>-</sup>, CD64<sup>-</sup>, dark green) and their  
440 derivatives MoDC (Ly6C<sup>-</sup>, MHCII<sup>+</sup>, blue) and Inflamm Mφ (Ly6C<sup>-</sup>, MHCII<sup>-</sup>, CD64<sup>+</sup>,  
441 CD206<sup>-</sup>, pink). Dermal resident Mφ (CD64<sup>+</sup>CD206<sup>+</sup>) subsets were identified as  
442 MHCII<sup>+</sup> Mφ (MHCII<sup>+</sup>, Tim4<sup>-</sup>, red), DN Mφ (MHCII<sup>-</sup>, Tim4<sup>-</sup>, purple) and Tim4<sup>+</sup> Mφ  
443 (MHCII<sup>-</sup>, Tim4<sup>+</sup>, cyan). (See also **Extended data Fig. 3a**, and **4a-c**; **4e-g** for detailed  
444 gating strategy and t-SNE analysis, and **Extended data Fig. 4d-f** for subset  
445 quantification). **d**, t-SNE clustering of Mo/Mφ subsets in DTR (left) or GINIP-DTR  
446 (right) mice at D14 pi. **e**, Absolute numbers of Tim4<sup>+</sup> Mφ (cyan) or **f**, Inflamm  
447 Mφ (pink), per mg ear skin (n=10-14 mice per group) from DTR (gray) or GINIP-DTR  
448 (colored) mice, between days 0 and 14 pi. Data are presented as mean ± SEM and  
449 representative for at least two independent experiments. One-way ANOVA with Tukey  
450 tests (ns: non-significant).

451

452 **Figure 3: TAFA4, a neuropeptide expressed by C-LTMRs, regulates IL-10**  
453 **production by macrophages and prevents UV-induced skin fibrosis**

454 **a**, Representative confocal images of C3 DRGs from WT mice on day 3 (D3) pi stained  
455 with IB4 (blue), anti-TAFA4 (red) and anti-ATF3 antibodies (green). **b**, Percentage  
456 ATF3<sup>+</sup> neurons positive for IB4 (blue) and TAFA4 (red) (n=8 mice per group). **c**,  
457 Quantification of TAFA4 protein (ELISA) in WT mice ear skin from D0 to D35 pi  
458 (n=6-18 mice per group). (See **Extended data Fig. 5** for C-LTMRs analysis and  
459 TAFA4 detection). **d**, Representative Picro Sirius staining of ear sections from WT  
460 (top) and *Tafa4*-KO (bottom) mice at D35 pi and **e**, collagen deposition: percentage

461 fibrosis (yellow pixels) over the total area (red + yellow pixels), ( $n=9$  mice per group).  
462 **f**, TNF $\alpha$  and **g**, IL-10 protein levels (CBA) in ear skin lysates ( $n=4-8$  mice per group).  
463 **h**, Relative *Il10* mRNA levels in sorted dermal CD206<sup>+</sup> M $\phi$  from WT (blue) and *Tafa4*-  
464 KO (red) mice between days 0 and 10 pi (pooled from 3 independent sortings, each  
465 with  $n=3$  mice per group). **i**, Relative *Tnfa*, *Il1b* and *Il10* mRNA levels (RT-qPCR) for  
466 purified peritoneal M $\phi$  (ThioM $\phi$ ) activated *in vitro* with LPS (100 ng/ml) without (blue)  
467 or with TFAFA4 (100 nM; green), ( $n=5-8$  independent samples). Data are presented as  
468 means  $\pm$  SEM and representative for at least two independent experiments. One-way  
469 ANOVA with Tukey tests, except for **b** and **f**, Student's *t*-test **c**, Kruskal-Wallis test,  
470 and **d**, two-way ANOVA (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).  
471

472 **Figure 4: The TFAFA4-IL-10 axis promotes Tim4<sup>+</sup> macrophage survival and tissue**  
473 **repair functions**

474 **a**, Experimental strategy for generating CD45.1 BM chimeras in CD45.2 WT or *Tafa4*-  
475 KO recipient mice and monitoring monocyte-derived cells pi. **b**, FACS and t-SNE  
476 analysis of skin Mo/M $\phi$  subsets 7 days pi, as described in **Fig. 2** (See also **Extended**  
477 **data fig. 8f-h** for detailed BM chimera analysis). **c**, t-SNE analysis of CD45.2<sup>+</sup> and  
478 CD45.1<sup>+</sup> clusters (yellow and black) and intracellular staining for IL-1 $\beta$ , TNF $\alpha$  and IL-  
479 10 (red). **d**, Absolute numbers of Inflamm M $\phi$  in WT control (gray) and *Tafa4*-KO BM-  
480 chimeric mice (pink). **e**, Absolute numbers of CD45.2<sup>+</sup>Tim4<sup>+</sup> M $\phi$  (left) and percentage  
481 of CD45.1 chimerism in total Tim4<sup>+</sup> M $\phi$  (right) from WT (gray) and *Tafa4*-KO BM-  
482 chimeric mice (cyan). **f**, Absolute numbers of total (CD45.1<sup>+</sup> and CD45.2<sup>+</sup>) Tim4<sup>+</sup> M $\phi$   
483 in WT (gray) and *Tafa4*-KO BM-chimeric mice (cyan), ( $n=6-10$  mice per group). **g**,  
484 Intradermal TFAFA4 protein injection in *Tafa4*-KO mice (green arrows) and changes in  
485 ear thickness over 21 days pi in saline-treated WT (blue) and *Tafa4*-KO (red) mice, or  
486 *Tafa4*-KO mice treated with TFAFA4 (green) ( $n=14$  mice per group). **h**, Absolute  
487 numbers of Inflamm M $\phi$  (left) and Tim4<sup>+</sup> M $\phi$  (right) in ear skin on day 14 pi ( $n=11$  mice  
488 per group). Data are presented as mean  $\pm$  SEM and representative for at least two  
489 independent experiments. One-way ANOVA with Tukey tests except for (**g**), two-way  
490 ANOVA (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

491

492

493 **Methods**

494 *Mice*

495 C57/Bl6J mice were bought from Janvier Labs (<https://www.janvier-labs.com>).  
496 GINIP-DTR mice were obtained by crossing GINIP<sup>flx/+</sup> line<sup>10</sup> with mice expressing  
497 the CRE recombinase from *Nav1.8* locus (*Nav1.8*<sup>Cre/Cre</sup> mice)<sup>13</sup>. One single

498 diphtheria toxin (DT; 10 $\mu$ g/kg) injection in *Nav1.8<sup>Cre/+</sup>* GINIP<sup>flx/+</sup> (hereafter called  
499 GINIP-DTR mice) allowed the specific ablation of GINIP<sup>+</sup> sensory neurons<sup>15</sup>.  
500 *Nav1.8<sup>+/+</sup>* GINIP<sup>flx/+</sup> littermates (hereafter called DTR mice) treated with DT were  
501 used as controls throughout. *Nav1.8<sup>rfp</sup>* were obtained by crossing *Nav1.8<sup>Cre/+</sup>* with  
502 flxR26-RFP mice. Homozygotes *IL-10<sup>gfp/gfp</sup>* mice, described previously<sup>34,35</sup> were  
503 used with their heterozygotes *IL-10<sup>gfp/wt</sup>* control littermates. *Tafa4*-KO mice were  
504 generated in Aziz Moqrich's lab (IBDM, AMU, France) and described previously<sup>22</sup>.  
505 CX3CR1<sup>CreERT2</sup>:R26-YFP mice were described previously<sup>27</sup>. All the mice used were  
506 bred and maintained under specific pathogen-free conditions at the Centre  
507 d'Immunophénomique (Ciphe) de Marseille and at the Centre d'Immunologie de  
508 Marseille Luminy (CIML). Mice were housed under a standard 12 h:12 h light-dark  
509 cycle with ad libitum access to food and water. Age-matched (6-12 weeks old,  
510 unless specified in figure legend) and sex-matched littermate mice were used as  
511 controls. All experiments were conducted in accordance with institutional  
512 committee recommendations (Comité d'Éthique de Marseille no. 14-APAFiS #  
513 18469-2019011417196625v2) and French and European guidelines for animal  
514 care.

515

#### 516 Bone marrow chimera generation

517 Age and sex matched WT or *Tafa4*-KO mice (CD45.2<sup>+</sup>) were anaesthetized with  
518 ketamine/xylazine (10 $\mu$ l/g, 2% Imalgene500/ 5% Rompun). Mice were then  
519 irradiated with 6,5Gy from an  $\gamma$ -ray irradiator. A lead shield was used to protect  
520 the ear's skin from  $\gamma$ -irradiation. Six hours after irradiation, 30mg/kg Busulfan  
521 (Sigma)<sup>36</sup> was injected (i.p.) to deplete remaining myeloid progenitors in recipient  
522 mice. Twelve hours later the mice were reconstituted with 5 million CD45.1<sup>+</sup> bone  
523 marrow (BM) cells. Mice were treated with Bactrim (in drinking water) for two  
524 weeks for a total recovery time of 6 weeks before analyzing the blood CD45.1  
525 chimerism and were then used for experiments.

526

#### 527 Lineage tracing study

528 CX3CR1<sup>CreERT2</sup>:R26R-EYFP<sup>+/+</sup> were used as described<sup>27</sup>. Tamoxifen (2mg Tam/mice;  
529 40mg/ml in corn oil; Sigma) was injected i.p. to pregnant mice at E16.5 days post-  
530 conception (dpc). Day of embryonic development (E) was estimated by taking the

531 day of vaginal plug observation as E0.5 dpc. Caesarian sections were carried out  
532 at term and neonates were fostered by lactating females. In this model, Tam-  
533 induced recombination leads to irreversible expression of the enhanced yellow  
534 fluorescent protein (EYFP) in CX3CR1<sup>+</sup> embryonic precursors and their progeny  
535 (i.e. fetal monocytes and differentiated M $\phi$  at the time of Tam injection; for review  
536 see<sup>37</sup>).

537

#### 538 UV-induced skin injury

539 Mice were anaesthetized with ketamine/xylazine, shaved and depilated (Veet  
540 Cream) and then left untreated or exposed to UV ( $\lambda$  254nm; voltage: 8W; source:  
541 30cm from the target) for 25 min. The protocol described previously<sup>2</sup> was adapted  
542 so that the ear skin of WT mice can recover in 35 days after UV exposure. Ear  
543 thickness was measured every other day with a caliper (under isoflurane  
544 anesthesia) and compared to the ear thickness of control untreated mice.

545

#### 546 Histology and histopathological analysis

547 At different time pi, mice were lethally anesthetized ketamine/xylazine (20 $\mu$ l/g)  
548 and perfused with 10ml of PBS. Ears were then collected and fixed with formol  
549 (for at least 1h), then cryoprotected with sucrose 30% overnight and embedded  
550 in paraffin. Skin sections of 5 $\mu$ m were stained with hematoxylin and eosin,  
551 Masson's trichrome or picosirius red. Histological scores were blindly assessed  
552 by a pathologist following the criteria described below. Leukocyte infiltration,  
553 epidermal thickness and fibrosis were evaluated and a score was assigned based  
554 on severity for each criterion. Fibrosis was also evaluated using a semi-  
555 quantitative binary score based on percentage of surface modified by fibrotic  
556 changes. A cumulative score was then obtained.

557

#### 558 Histopathological grading

##### 559 **Inflammation/leukocyte infiltration**

560 (0): No inflammation; (1): Mild dermatitis (less than 10 leukocytes per High  
561 Power Fields, HPF); (2): Moderate dermatitis (less than 50 leukocytes/HPF); (3):  
562 Marked dermatitis (less than 100 leukocytes/HPF); (4): Severe dermatitis (more  
563 than 100 leukocytes/HPF).

564 **Epidermal thickness**

565 (0): Normal thickness; (1): Mild hyperplasia (less than 4 cellular layers); (2):  
566 Moderate hyperplasia (more than 4 cellular layers); (3): Marked hyperplasia  
567 (markedly thickened, stratification and epidermal crest)

568 **Wound healing/fibrosis**

569 (0): No granulation tissue, no fibrosis; (1): Early granulation tissue and mild  
570 fibrosis; (2): Moderate fibrosis (hair follicle obliteration); (3): Marked fibrosis  
571 (persistent granulation tissue/cartilage obliteration)

572 **Fibrosis extension**

573 (1): Focal or multifocal fibrosis (less than 50% fibrosis per HPF); (2): Extensive  
574 fibrosis (more than 50% fibrosis per HPF)

575

576 *Dorsal Root Ganglia (DRG) tissue sections*

577 After lethal anesthesia and perfusion with 5 ml of PBS and 25 ml of 4%  
578 paraformaldehyde (PFA), C2/C3 DRG were carefully dissected from the spinal  
579 cord under a binocular lens, fixed for 1 hour with 4% PFA and then cryoprotected  
580 with 30% (w/v) sucrose overnight before being included in OCT (Tissue-Tek,  
581 Sakura) and stored at -80°C. DRG samples were sectioned at 10µm using a  
582 standard cryostat (Leica).

583

584 *In situ Hybridization on DRG*

585 In situ hybridization was carried out following the protocol described in<sup>15</sup>. Briefly,  
586 RNA probes were synthesized using gene-specific PCR primers and cDNA  
587 templates from mouse DRG. The following oligonucleotides were used for the  
588 nested PCRs for probe synthesis:

589 Tafa4-F1: TGCTCAGAAGTTCATAGCCAAA

590 Tafa4-R1: TAAAGGAACATTTGCAAGCTCA

591 Tafa4-F2: ATATGTGCAGTGTGG

592 Tafa4-R2 + T7: TAATACGACTCACTATAGGGCAGCCAAGTTCAAAC

593

594 DRG sections were treated with proteinase K, triethanolamine and acetic  
595 anhydride solutions. Digoxigenin labeled probes were hybridized overnight at  
596 55°C after 2h of prehybridization. The slides were treated with 0.2X SSC baths

597 then blocked at room temperature with 10% Goat serum and incubated with anti-  
598 digoxigenin antibodies (Roche). Final detection was achieved using Cyanine 3 TSA  
599 plus kit (Perkin Elmer).

600

#### 601 Immunofluorescent and confocal analysis of DRG neuron activation

602 We used the expression of ATF3 (Activating Transcription Factor 3) as a proxy for  
603 neuronal injury and activation<sup>38</sup>. DRG sections were saturated and permeabilized  
604 with a PBS 1X, 3% BSA, 0,3% Triton 100X and 10% Donkey serum (Sigma;  
605 #D9663-10ML) solution for 1 hour at room temperature (RT) before adding the  
606 primary anti-mouse TAFA4 antibody (rat clone 1D8, 1:1000), the anti-mouse  
607 GINIP (rat, 1:1000, Aziz Moqrich's lab, IBDM) or the anti-mouse GINIP (rabbit,  
608 1:1000, Invitrogen #PA5-71131), the anti-mouse ATF3 (rabbit, 1:200, Santa Cruz  
609 #SC-188 or Abcam #ab207434), the anti-TH (1:300, Merck Millipore, #AB152)  
610 and anti-mouse CGRP (goat, 1:1000, Abcam #ab36001) for 24 hours at 4°C. After  
611 several washes, donkey secondary antibodies (anti-mouse, #715-585-151; anti-  
612 rat, #712-545-153; anti-rabbit, #711-585-152; and anti-goat, #705-605-147;  
613 from Jackson ImmunoResearch, 1:500) were added for 45 minutes at room  
614 temperature. The staining with the lectin isolectin B4 (IB4) coupled to AlexaFluor  
615 647 (AF647; 1:200, Invitrogen) was performed in TNT buffer (Tris HCl 100 nM,  
616 NaCl 150 nM, Tween-20 0,1% pH 7,5) for 45 minutes at room temperature. Images  
617 were acquired using a confocal microscope LSM780 (Zeiss) and analyzed using  
618 ZEN 2.3 and ImageJ Software.

619

#### 620 Skin cell isolation

621 After lethal anesthesia and intracardiac perfusion with 5 ml of PBS, ears were  
622 collected and dorsal and ventral layer separated, finely minced with scissor in  
623 complete medium (RPMI+L-Glutamine, 10% FCS) and incubated 1h at 37°C with  
624 1mg/ml DNase (Roche) 0.2mg/ml Dispase (GIBCO) and 0.2mg/ml collagenase  
625 type IV (Sigma; #C5138-500MG). Tissues were then dissociated using 2.5 ml  
626 syringes and 18G needles and filtered on cell strainer (100 µm, BD), washed in  
627 FACS buffer (PBS-2mM EDTA, FCS 2%) to obtain a homogeneous cell suspension  
628 ready for staining.

629

630 DRG neurons isolation

631 After lethal anesthesia and intracardiac perfusion with 5 ml of HBSS 10X (Mg- Ca<sup>2+</sup>-  
632 ), HEPES 5 mM, D-Glucose 12.5 mM, and penicillin/streptomycin (P/S) 1%  
633 solution, DRGs were carefully extracted and digested twice with a 0.2 mg/ml  
634 collagenase type II (Gibco), and 0.5 mg/ml dispase (Gibco) solution for 30 minutes  
635 at 37°C. After several wash with Neurobasal complete medium (B-27 2%, L-  
636 glutamine 20 mM, P/S 1%), DRGs were mechanically dissociated using 3 needles  
637 of decreasing diameter (18G, 22G, 26G). After a filtration on a cell strainer (70 µm,  
638 Miltenyi Biotec), a Percoll gradient was used with 12.8% and 28% density in  
639 Leibovitz-15 complete medium (FCS 5% and P/S 1%) to eliminate cell debris.  
640 After centrifugation and several washes, the cell suspension was ready for  
641 staining.

642

643 Antibodies for flow cytometry

644 We adapted our FACS gating strategy from previous studies<sup>39</sup>, adding EpCAM,  
645 Langerin (CD207) or CD206 and Tim4 to decipher the Mo/Mφ compartment of the  
646 skin. Antibodies were purchased from BioLegend or BD Biosciences: CD45-BV785  
647 1:500 (30F11), CD45.1-BV605 1:200 (A20), CD11b-BV510 1:1000 (M1/70),  
648 CD64-BV711 1:300 (X54-5/7.1), Ly6C-421 1:300 (AL-21), F4/80-PECF594 1:400  
649 (T45-2342), CD11c-BUV395 1:200 (N418), EpCAM-AF594 1:500 (G8.8), CD207-  
650 APC (929F3.01; Dendritics, #DDX0362), CD206-APC 1:300 (C068C2), Tim4-  
651 PeCy7 (RMT4-54) Ly6G-APC-Cy7 1:300 (1A8), IA-IE A700 1:500 (M5/114.15.2),  
652 CD24-BUV737 1:500 (M1/69). Mast cells were characterized as previously  
653 described<sup>40,41</sup>, CD117-BV605 (c-kit; 2B8; 1:300), CD103-PerCP-Cy5.5 (2E7;  
654 1:500), anti-TNFα-PE 1:100 (MP6-XT22) anti-IL-10-PE 1:100 (JES5-16E3); CCR2-  
655 PE 1:200 (475301; R&D System), IL-1β-PE Ab 1:100 (NJTEN3; eBioscience), anti-  
656 CD4 PE-Cy7 (RM4-5; BD), anti-TCRαβ APC-Cy7 (H57-597; BD), anti-FoxP3-A488  
657 1:200 (Clone FJK-16s; eBioscience), anti-DTR 1:300 (anti-hHB-EGF; AF-259-NA;  
658 R&D System), anti-GFP (Invitrogen, #A-21311).

659

660 Flow cytometry

661 Cell suspensions were plated in 96 well U bottom plates and stained at 4°C. Cells  
662 were incubated 40min at 4°C in FACS buffer with antibodies and anti-Fc receptor

663 blocking antibody (clone: 2.4 G2). Cells were then washed and fixed (fixation  
664 buffer, from FoxP3 kit; eBioscience; #00-5521-00) until analysis. For intra-cellular  
665 cytokine staining, cell suspensions were permeabilized (Fix/perm, from FoxP3  
666 kit; eBioscience) and stained with anti-cytokine antibodies during 1h for IL-1 $\beta$  and  
667 TNF $\alpha$  or overnight for IL-10. For IL-10 expression analysis in FoxP3<sup>+</sup> Treg cells,  
668 total skin homogenates were incubated during 6h in complete medium  
669 supplemented with PMA (200ng/ml; Sigma) and ionomycin (1mg/ml; Sigma).  
670 During the last 4h, Golgi Plug (1:1000; BD Biosciences; #554724) was added. Cell  
671 suspensions were then washed extensively at 4°C, surface stained, fixed and then  
672 processed for intracellular staining. Dead cells were excluded from the analysis by  
673 counter-gating using the live/dead fixable blue Dead Cell Stain kit UV (L23105;  
674 Invitrogen). Multiparameter FACS analysis was performed using an LSR X20  
675 system (BD). Absolute numbers for each population were obtained using Quanti  
676 Beads (BD Biosciences, #556296). Data analysis was performed using Flowjo  
677 software (Tree Star, Inc.).

678

#### 679 *t-distributed Stochastic Neighbor Embedding (t-SNE)*

680 t-SNE analyses were performed with FlowJo™ version 10 software (FlowJo LLC).  
681 We used an equal number of mice for each genotype or experimental condition  
682 considered. In each group, the same number of cells was used to allow an  
683 equivalent contribution of each individual mice in the analysis. This equal cell  
684 number was selected using the DownSample plugin. Then, cells from each mouse  
685 were merged using the concatenate tool and barcoded to track and distinguish  
686 them. Finally, t-SNE analyses were performed using different markers, indicated  
687 in the figure legends. The color code representing the expression level of each  
688 marker in the heatmaps (depicted in extended data figures), is normalized on the  
689 median intensity value for a given marker. Data are represented using a four-color  
690 scale with blue, green-yellow and red indicating respectively low intermediate  
691 and high expression levels.

692

#### 693 *Immunofluorescent staining and confocal microscopy analysis of ear skin*

694 After lethal anesthesia and perfusion with 10ml PBS, ears from DT-treated GINIP-  
695 DTR and -DTR control mice were collected and ear dorsal and ventral layers were

696 separated, fixed for 1 hour with 4% PFA, washed, permeabilized and saturated  
697 with a PBS, 3% BSA, 0,2% Triton 100X and 10% donkey serum and then stained  
698 with the primary anti-mouse GINIP antibody (rat, 1:1000, Aziz Moqrich's lab,  
699 IBDM) , the anti-TH antibody (rabbit,1:300; Merck Millipore, #AB152) overnight  
700 at 4°C. After several washes, a donkey secondary anti-rat-488 and anti-rabbit-  
701 AF594 antibody (Jackson ImmunoResearch, 1:500) were added 1h at 4°C, washed,  
702 and then the anti-Beta III Tubulin-AF647 antibody (1:300 TUJI; Biolegend,  
703 #BLE801210) or the anti-CD206-APC 1:300 (rat, 1:300; clone C068C2, Biolegend,  
704 #BLE141708), were added for additional 45 minutes at room temperature. After  
705 several washes slices were then mounted on coverslips with mounting medium  
706 and image acquisition was performed on Confocal LSM780 (Zeiss) and analyzed  
707 with ZEN and ImageJ software.

708

#### 709 Bone marrow-derived macrophage (BMDM) generation

710 Femora bones were isolated from WT or FPR1-KO mice (We thank Pr Monica  
711 Lucattelli and Pr Laurence Zitvogel for providing BM cells from FPR1-KO mice),  
712 cut in half and placed in 0.5ml Eppendorf tubes pre-perforated on their bottom  
713 part. The small tubes with two ½ bone were placed within 1.5ml Eppendorf tubes.  
714 The tubes were then centrifuged 10sec at 10000rpm and BM cell pellets were  
715 resuspended in red blood cell lysis buffer for 3 minutes and washed in PBS. BM  
716 cells were then resuspended at 10<sup>6</sup>/ml in complete DMEM (DMEM, SVF 10%, P/S,  
717 1% L-Glu) supplemented with 20% conditioned L929 media during 5 days to  
718 generate bone marrow derived-macrophages (BMDM) and used as ThioMφ for *in*  
719 *vitro* experiments.

720

#### 721 Thioglycollate-elicited macrophage (ThioMφ) generation and in vitro treatment

722 Peritoneal macrophages were isolated from WT mice by peritoneal wash three  
723 days after 3% Thioglycollate (Sigma) i.p. injection. Peritoneal washes were then  
724 stained with biotin-associated lineage cocktail antibodies (CD3, CD19, NK1.1,  
725 Ly6G and Siglec F), counterstained with anti-biotin microbeads (Miltenyi Biotec)  
726 and then purified using magnetic LS columns. Peritoneal macrophage  
727 preparations were then cultured in complete DMEM (DMEM, SVF 10%, P/S, 1% L-  
728 Glu) at 10<sup>6</sup> ThioMφ/ml in a 12-well plates (BD) with or without LPS (100ng/ml,

729 Sigma) in the presence of not of 100nM TAFA4. After 16 hours, macrophages were  
730 lysed using RLT medium (Qiagen) then pass through a QIAShredder column  
731 (Qiagen) and store at -80°C.

732

#### 733 Migration assay

734 BMDM from WT or FPR1-KO mice were incubated on the top chambers of  
735 transwell plates (Costar) in complete DMEM, while the bottom chambers of the  
736 transwell plates were filled with complete DMEM for negative control, or complete  
737 DMEM containing CCL2 (MCP-1; 10nM) for positive control of migration, or  
738 increasing concentrations of TAFA4 (1 to 1000nM), during 8h at 37°C. The bottom  
739 chambers were then analyzed by FACS for macrophages content.

740

#### 741 Dermal resident macrophage depletion.

742 WT or *Tafa4*-KO mice were injected two consecutive days with 500µg of the CSF-  
743 1R blocking antibody (clone AFS98; *InVivo*MAb, BioXcell #BE0213) or the isotype  
744 control (IgG2a; clone 2A3; *InVivo*MAb, BioXcell #BE0089). One day after the last  
745 injection, mice tissues were collected and analyzed for Macrophage depletion by  
746 flow cytometry.

747

#### 748 Dermal resident macrophage isolation

749 Ears skin from WT or *Tafa4*-KO mice were dissociated and stained as previously  
750 described, and CD206<sup>+</sup> dermal resident macrophages were cell sorted on a FACS  
751 Aria III following the Figure 2 gating strategy as livedead<sup>neg</sup>, CD45<sup>+</sup> DC<sup>neg</sup>  
752 CD11b<sup>+</sup>CD24<sup>-</sup>Ly6G<sup>-</sup>CCR2<sup>-</sup>Ly6C<sup>-</sup>CD64<sup>+</sup>CD206<sup>+</sup>, then lysed using RLT medium  
753 (Qiagen) then pass through a QIAShredder column (Qiagen) and store at -80°C.  
754 Alternatively, Tim4<sup>+</sup> Mφ or Inflamm Mφ (CD64<sup>+</sup>CD206<sup>-</sup>) were sorted as presented in  
755 Extended data Figure 10b.

756

#### 757 Intradermal adoptive transfer of Inflamm and Tim4<sup>+</sup> macrophages.

758 DTR and GINIP-DTR mice were exposed to UV and then intradermally injected at  
759 D1 pi with 2000 Mφ/10µl PBS per ears.

760

#### 761 Imiquimod-induced cutaneous damage.

762 Mice (WT or *Tafa4*-KO mice) were daily treated with 5mg of 5% imiquimod cream  
763 (IMQ) applied topically to dorsal and ventral aspects of ear skin as describe<sup>6</sup>,  
764 during six days and evolution of the ear thickness ( $\mu\text{m}$ ) was followed every day  
765 with a caliper.

766

#### 767 *TAFA4 rescue and in vivo blocking of IL-10*

768 For TAFA4 rescue experiments, 20  $\mu\text{l}$  of recombinant TAFA4 (25  $\mu\text{g}/\text{ml}$ ) or saline  
769 solution only, were intradermally injected every other day following the UV-  
770 treatment until day 8 pi in each ear of *Tafa4*-KO or control mice as indicated in  
771 figures.

772 For IL-10 blocking experiments, 20  $\mu\text{l}$  of anti-IL-10 blocking antibody (0.5 mg/ml;  
773 R&D, #MAB417) or the isotype control (rat IgG1; clone JES052A5) were  
774 intradermally injected every other day following the UV-treatment until day 8 pi  
775 in each ear of *Tafa4*-KO or control mice as indicated in figures.

776

#### 777 *Gene expression analysis*

778 Total RNA was isolated from mouse ears, exposed or not to UV irradiation or from  
779 BMDM or ThioM $\phi$  activated with or without LPS and TAFA4. Ears were  
780 dilacerated by FastPrep-24 (MpBio) in lysis matrix A tubes (MpBio). RNA was  
781 isolated using a fibrous RNeasy minikit (QIAGEN). Reverse transcription was  
782 performed using Superscript RTII (Invitrogen). Preamplification was performed  
783 using specific Taqman probes (Applied Biosystems) for each targeted gene using  
784 the pre-amplification master mix (Fluidigm). Pre-amplified products (18 cycles)  
785 were diluted (1:5) in Universal PCR Master Mix then loaded in a 96.96 Dynamic  
786 Array and analyzed on a BioMark Genetic Analysis System (Fluidigm). Data were  
787 normalized ( $2^{-\Delta\text{Ct}}$ ) to the housekeeping genes, *Gapdh* and *Hprt*.

788

#### 789 *Primers used for mRNA detection in total skin samples (TaqMan references)*

790

791 *Acta2* (Mn00808218\_g1); *Areg* (Mm00437583\_m1); *Ccl1* (Mm00441236\_m1);  
792 *Ccl2* (Mm00441242\_m1); *Ccl3* (Mm00441259\_g1); *Ccl4*(Mm00443111\_m1); *Clc6*  
793 (Mm01302419\_m1); *Ccl8* (Mm01297183\_m1); *Cd36* (Mm00432403\_m1);  
794 *Cebpb/d* (Mm00786711\_s1); *Chil3* (Mm00657889\_mH); *Col1a1*  
795 (Mm00801666\_g1); *Col3a1* (Mm00802305\_g1); *Cxcl2* (Mm00436450\_m1); *Cxcl9*  
796 (Mm00434946\_m1); *Cxcl10* (Mm00445235\_m1); *Des* (Mm00802455\_m1); *Egr1*

797 (Mm00656724\_m1); *Epas* (Mm01236112\_m1); *Fam19a4* (*Tafa4*,  
798 Mm00623620\_m1); *Flt3* (Mm00439016\_m1); *Gf1r* (Mm00616224\_m1); *Ifnb1*  
799 (Mm00439552\_s1); *Il1b* (Mm00434228\_m1); *Il1r1* (Mm00434237\_m1); *Il4*  
800 (Mm00445259\_m1); *Il12a* (Mm00434169\_m1); *Il13* (Mm00434204\_m1); *Il15*  
801 (Mm00434210\_m1); *Il17a* (Mm00439618\_m1); *Il22* (Mm01226722\_g1); *Il23a*  
802 (Mm00518984\_m1); *Irf3* (Mm01203177\_m1); *Irf7* (Mm00516791\_g1); *Irf8*  
803 (Mm00492567\_m1); *Klf2* (Mm00500486\_g1); *Klf4* (Mm00516104\_m1); *Klf5*  
804 (Mm00456521\_m1); *Mmp2* (Mm00439498\_m1); *Mmp9* (Mm00442991\_m1);  
805 *Mmp10* (Mm01168399\_m1); *Mmp12* (Mm00500554\_m1); *Mpo*  
806 (Mm00447885\_m1); *Mtor* (Mm00444968\_m); *Nos2* (Mm00440502\_m1); *Nr4a1*  
807 (Mm01300401\_m1); *Nr4a3* (Mm00450071\_g1); *Pdgfra* (Mm00440701\_m1);  
808 *Pdgfrb* (Mm01262485\_m1); *Piezo1* (Mm01241544\_g1); *Piezo2*  
809 (Mm01262422\_g1); *Plod2* (Mm00478767\_m1); *Pparg* (Mm00440940\_m1);  
810 *Retna* (Mm00445109\_m1); *Ripk1* (Mm00436360\_m1); *Serpib9*  
811 (Mm00777163\_m1); *Tac1* (Mm01166994\_g1); *Tgfrb2* (Mm03024091\_m1); *Tlr3*  
812 (Mm01207402\_m1); *Tlr4* (Mm00445273\_m1); *Tlr7* (Mm04933178\_g1); *Tlr9*  
813 (Mm07299609\_m1); *Tp53* (Rn00755717\_m1); *Gapdh* (Mm99999915\_g1); *Hprt*  
814 (Mm03024075\_m1).

815

#### 816 Protein extraction from tissue

817 Mice were euthanized and their ears were snap-frozen in liquid nitrogen and then  
818 transferred into mechanical dissociation tubes (Lysis Matrix A, MPbio) containing  
819 a commercial lysis buffer (T-Per Tissue Protein Extraction Reagent,  
820 Thermofisher) with protease inhibitor cocktail (Halt Protease Inhibitor 100X,  
821 Thermofisher). Proteins extraction was performed by incubating each gram of  
822 tissue with 20 ml of lysis buffer. Tissues were dissociated using a 3 cycles program  
823 of FastPrep-24 5G (MPbio). After two centrifugations at 16 000 g for 30 minutes,  
824 the supernatants were filtered (on 70 µm cell strainers, Startedt) and stored at -  
825 80°C until use.

826

#### 827 Generation of recombinant mouse TAFA4

828 Mouse TAFA4 recombinant protein was produced in *E. coli* by Pure Biologics  
829 (<https://purebiologics.pl>). The endotoxin levels of the purified protein, measured  
830 by the LAL (Limulus Amoebocyte Lysate) method, was <0.0005 EU/µg. The  
831 recombinant protein included a Histidine (His)-Tag in the C-Terminal part of the  
832 protein allowing its detection by anti-His antibodies. The sequence of this  
833 recombinant mTAFA4 is:

834 MHLIKPGTCEVVAVHRCCNKNRIEERSQTVKCSFCFPGQVAGTTTRAQPSCVEAAIV

835 IEKWWCHMNPCLGEDCKVLPDSSGWSCSSGNKVKTTKVTRGGGSGLEHHHHHH

836

837 Generation of anti-TAFA4 monoclonal antibodies

838 Anti-TAFA4 monoclonal antibodies (mAbs) were generated by MImAbs  
839 (<https://www.mimabs.org>). Briefly, three Wistar rats were immunized with the  
840 recombinant mouse TAFA4 protein (100µg, 3 IP injections followed by a final  
841 boost). After immunization, spleen cells were harvested and fused to the myeloma  
842 cell line X63-Ag8-656. Hybridoma were seeded in methylcellulose semi-solid  
843 medium with CloneDetect agent (Molecular Devices #K8240) and secreting  
844 monoclonal IgG hybridomas were picked by ClonePix2 (Molecular Devices) to  
845 liquid medium 96 wells plates. Hybridoma clones producing anti-TAFA4  
846 antibodies were selected using an ELISA screen. The specificity of the antibodies  
847 for the native TAFA4 protein was assessed by immunofluorescent staining on  
848 mouse DRG from WT (positive control) or *Tafa4*-KO (negative control) mice.

849

850 Sandwich ELISA for TAFA4 quantification

851 To quantify TAFA4 protein, sandwich enzyme-linked immunosorbent assay  
852 (ELISA) has been developed by MImAbs (<https://www.mimabs.org>). Briefly, anti-  
853 TAFA4 antibodies (clone 33F8, 50 µl, 2µg/ml) diluted in a Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> buffer  
854 (Sigma, #C3041-100CAP, pH = 9.6) were coated on 96 well MAXISORP plates  
855 (Sigma #M5785-1CS) and incubated overnight at 4°C. Plates were then washed 3  
856 times in a washing buffer (PBS/0.1% Tween-20) and blocked with in 300 µl of  
857 StartingBlock blocking buffer (Thermofisher, #37542). Samples and serial  
858 dilutions of mTAFA4 (to establish the standard curve) were incubated 1.30 h at  
859 room temperature (RT). After 3 wash, anti-TAFA4 biotinylated antibodies (clone  
860 1D8, 50 µl, 2µg/ml) were incubated for 1.30 h at RT. After 3 wash, HRP-Conjugated  
861 Streptavidin (Thermofisher, #N100) was added, washed 3 times before adding the  
862 substrate solution TMB (Interchim, #UP664781). After 10 additional minutes, the  
863 reaction was stopped with a solution of HCl (1M) and the absorbances (at 450 nm  
864 and 620 nm) of each well were measured. The specific signal obtained for WT ear  
865 skin samples was determined by subtracting the background signal obtained with  
866 skin samples from *Tafa4*-KO mice.

867

868 Cytokine and chemokine quantification in skin samples

869 Ear skin samples were homogenized and proteins extraction was performed as  
870 described above. Cytometric Bead Array (CBA) Flex Set kit (BD Bioscience) was  
871 used to quantify chemokine ligand 1 (CXCL1), tumor necrosis factor-alpha  
872 (TNF $\alpha$ ), interleukin 6 (IL-6), interleukin 1 beta (IL-1 $\beta$ ), monocyte  
873 chemoattractant protein 1 (CCL2/MCP1) and the chemokine (C-C motif) ligands 4  
874 (CCL4). For IL-10, an enhanced sensitivity Cytometric Bead Array (CBA) Flex Set  
875 kit (BD Bioscience) was used. Samples were analyzed on a Canto II flow cytometer  
876 with FCAP Array TM Software (BD Bioscience) following manufacturer's  
877 instructions.

878

### 879 Statistical analysis

880 All the results are expressed as mean +/- SEM. Statistical analysis were performed  
881 using the GraphPad Prism for Windows software. Statistical analysis was  
882 performed using a one-way ANOVA with Tukey's multiple comparisons test  
883 throughout the study. A two-way ANOVA test was used to compare two or more  
884 than two groups in kinetic experiments after UV exposure, involving repeated  
885 measures on the same animals over time. Difference were considered significant  
886 as following: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; and \*\*\*\*p<0.0001.

887

### 888 Additional references:

889

- 890 34 Madan, R. *et al.* Nonredundant roles for B cell-derived IL-10 in immune counter-  
891 regulation. *J Immunol* **183**, 2312-2320, doi:10.4049/jimmunol.0900185 (2009).
- 892 35 Reber, L. L. *et al.* Imaging protective mast cells in living mice during severe contact  
893 hypersensitivity. *JCI Insight* **2**, doi:10.1172/jci.insight.92900 (2017).
- 894 36 Misharin, A. V. *et al.* Monocyte-derived alveolar macrophages drive lung fibrosis and  
895 persist in the lung over the life span. *J Exp Med* **214**, 2387-2404,  
896 doi:10.1084/jem.20162152 (2017).
- 897 37 Hoeffel, G. & Ginhoux, F. Ontogeny of Tissue-Resident Macrophages. *Front Immunol*  
898 **6**, 486, doi:10.3389/fimmu.2015.00486 (2015).
- 899 38 Renthal, W. *et al.* Transcriptional Reprogramming of Distinct Peripheral Sensory  
900 Neuron Subtypes after Axonal Injury. *Neuron* **108**, 128-144 e129,  
901 doi:10.1016/j.neuron.2020.07.026 (2020).
- 902 39 Tamoutounour, S. *et al.* Origins and functional specialization of macrophages and of  
903 conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* **39**, 925-  
904 938, doi:10.1016/j.immuni.2013.10.004 (2013).
- 905 40 Gentek, R. *et al.* Hemogenic Endothelial Fate Mapping Reveals Dual Developmental  
906 Origin of Mast Cells. *Immunity* **48**, 1160-1171 e1165,  
907 doi:10.1016/j.immuni.2018.04.025 (2018).

908 41 Serhan, N. *et al.* House dust mites activate nociceptor-mast cell clusters to drive type  
909 2 skin inflammation. *Nat Immunol* **20**, 1435-1443, doi:10.1038/s41590-019-0493-z  
910 (2019).

911

912

### 913 **Acknowledgments**

914 We thank Vincent Feuillet for critical reading of the manuscript and for helpful  
915 comments and discussions. We thank Justine Galluso for mouse breeding and  
916 genotyping. We thank the Centre d'Immunologie de Marseille-Luminy (CIML) mouse  
917 house and core cytometry, imaging and histology facilities. The S. U. laboratory  
918 received funding from the European Research Council (ERC) under the European  
919 Union's Horizon 2020 research and innovation program, under grant agreement No.  
920 648768; and from the *Agence Nationale de la Recherche* (ANR) (No. ANR-14-CE14-  
921 0009-01) and from the Fondation pour la Recherche Médicale (FRM, No.  
922 ECO201906009090). This work was also supported by institutional grants from  
923 INSERM, CNRS, Aix-Marseille University and Marseille-Immunopole to the CIML.

924

### 925 **Author contributions**

926 G. H., G. D., A. Ro., R. R., P. V. B., A. B., designed and performed experiments and  
927 analyzed data. J.G. did the t-SNE analysis. C. L. and L.C. performed histological  
928 analysis. A.M. and A. Re. provided the GINIP-DTR and TAFA4-KO mice, reagents  
929 as well as important guidance for the characterization of sensory neurons. S. U. and G.  
930 H. designed the study, supervised experiments and co-wrote the manuscript. All authors  
931 reviewed and provided input on the manuscript.

932

### 933 **Competing interests**

934 S.U., G.H, G.D. and A.M. through Inserm transfert have filed a provisional  
935 international patent application WO2020/064907 on the clinical use of TAFA4. All  
936 other authors declare no competing interests.

937

#### 938 **Data availability**

939 All data supporting the findings of this study are found within the manuscript and its  
940 Supplementary Information. Source data are provided with this paper.

941

942

943

944

#### 945 **Extended data legends**

946 **Extended data figure 1: UV overexposure triggers a sequence of skin**  
947 **inflammation and repair over 35 days and GINIP<sup>+</sup> neurons activation**

948 **a**, Heatmap representing the expression kinetics of genes involved in skin inflammation  
949 and repair over time (days) pi. Ears from WT mice were collected before UV irradiation  
950 (D0) or at D3, D7, D14 and D35 pi and total extracted RNA were analyzed by Fluidigm.

951 Genes encoding proinflammatory cytokines and chemokines were expressed at D3 and  
952 D7 delineating the inflammatory phase (red cluster). Pro-repair genes were up-  
953 regulated at D14 and D35 (green cluster) delineating the resolution/remodeling phases.

954 The gene *Fam19a4* encoding TAFA4 is highlighted in yellow (n=4-8 mice per group).

955 **b**, Sensory neurons from C2/C3 DRGs innervate the ear skin. The fluorescent tracer

956 DiI was injected intradermally in the right ears of WT mice and left ears were injected

957 with PBS (n=3 independent DRGs per group). **c**, The trigeminal ganglia (TG) and

958 cervical DRG (C2 to C5) were collected 48h post-injection and analyzed by fluorescent

959 microscopy. **d**, Quantification of DiI<sup>+</sup> neurons per DRG fields of view; PBS-injected

960 control side (blue) or DiI-injected side (red). **e**, Additional representative confocal

961 images (related to main **figure 1c**) of C2 DRGs labeled for GINIP (red), CGRP (blue)  
962 and ATF3 (green), from unexposed (left) and UV-exposed mice at D3 pi (right). **f**,  
963 Quantification of total GINIP<sup>+</sup> and CGRP<sup>+</sup> neurons per DRGs (left) and total  
964 CGRP<sup>+</sup>ATF3<sup>+</sup> and GINIP<sup>+</sup>ATF3<sup>+</sup> (right) from unexposed (blue) and UV-exposed mice  
965 at D3 pi (red), (n=8 mice per group). All data are representative for at least two  
966 independent experiments and presented as means ± SEM. One-way ANOVA with  
967 Tukey's multiple comparisons test (ns: non-significant; \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* <  
968 0.001).  
969  
970  
971

972 **Extended data figure 2: Conditional ablation of GINIP<sup>+</sup> neurons in GINIP-DTR**  
973 **mice and skin histopathological analysis after UV exposure.**

974 **a**, Representative confocal images for DAPI (blue) and GINIP (green) staining of C3  
975 DRGs from control DTR-control (upper panels) and GINIP-DTR (lower panels) mice,  
976 10 weeks post-DT treatment. **b**, Absolute number of GINIP<sup>+</sup>, CGRP<sup>+</sup>, TAFA4<sup>+</sup>, TH<sup>+</sup>  
977 and IB4<sup>+</sup> DRG neurons were quantified in DTR-control (blue) and GINIP-DTR mice  
978 (red), (n=4-6 independent DRG per group). **c**, Representative confocal images for  
979 Beta3-tubulin (blue) and GINIP (green) staining, of mouse ear skin sections as in **a**. **d**,  
980 scratching episodes were monitored during 30 min at each time point pi indicated (n=6-  
981 10 mice per group). **e**, Representative H&E staining of ears from DTR (left) and GINIP-  
982 DTR (right) mice at D35 pi. Histopathological analysis for leukocyte infiltration (**f**),  
983 epidermal thickness (**g**), fibrosis (**h**) and fibrosis extension (**i**) (n=5 mice per group)  
984 related to the main **Fig. 1g-j**. Criteria used for the histopathological scoring are  
985 described in the **Article Methods**. **j**, Representative Masson trichrome (upper panel)  
986 and H&E staining (lower panel) of back skin from DTR-control (left) and GINIP-DTR  
987 (right) mice at D35 pi. All data are representative for at least two independent  
988 experiments and presented as mean ± SEM. One-way ANOVA with Tukey's multiple  
989 comparisons test (ns: non-significant; \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001).

990

991 **Extended data figure 3: Immune cell subsets in the skin before and after UV**  
992 **exposure.**

993 **a**, Flow cytometry gating strategy using CD11b, CD11c, CD64, MHC-II, CD103, c-  
994 kit, Ly6G and CD24 marker expression. Dendritic cells (DC; CD11c<sup>+</sup>MHCII<sup>+</sup>), mast  
995 cells (MC; CD11b<sup>-</sup>c-kit<sup>+</sup>), lymphoid cells (Lymφ; CD11b<sup>-</sup>CD103<sup>-/+</sup>), granulocytes  
996 (CD11b<sup>+</sup>CD24<sup>+</sup>, Ly6G<sup>+</sup>) and the monocyte/macrophage compartment (Mo/Mφ;  
997 CD11c<sup>-</sup>MHCII<sup>-</sup>CD11b<sup>+</sup>CD24<sup>-</sup>Ly6G<sup>-</sup>CD64<sup>int/+</sup>) are shown. **b**, Absolute numbers per mg  
998 of skin for DC, MC, Lymφ, Gr and Mo/Mφ populations at D14 pi in DTR-control (blue)  
999 or GINIP-DTR (red) mice, (n=8 mice per group). **c**, Gating strategy for skin dendritic  
1000 cell subsets (CD11c<sup>+</sup>MHCII<sup>+</sup>) and monitoring of Langerhans cells (LC;  
1001 EpCAM<sup>+</sup>Langerin<sup>+</sup>), (n=5-8 mice per group). **d**, FACS plot for LC (left) and  
1002 quantification (right) in DTR-control and GINIP-DTR mice, unexposed (No UV) or at  
1003 D14 pi (UV), (n=5-10 mice per group). **e**, Percentage of LC among DC over time post-  
1004 UV exposure in WT and Tafa4 KO mice. **f**, Representative flow cytometry analysis of  
1005 skin immune cells and DRG neurons for the expression of DTR in GINIP-DTR mice.  
1006 **g**, Confocal analysis of whole mount ear skin from Nav1.8-RFP mice stained for  
1007 GINIP<sup>+</sup> neurons and CD206<sup>+</sup> dermal resident Mφ. Hair follicles (HF) are highlighted  
1008 within white dashed squares. In the skin, Nav1.8 expression (RFP) is restricted to  
1009 sensory neuron axonal extensions. In DRG (**f**), DTR expression is restricted to neuronal  
1010 cellular bodies in DRGs. These markers were not detected in immune cells. All data  
1011 are representative for at least two independent experiments and presented as mean ±  
1012 SEM. One-way ANOVA with Tukey's multiple comparisons test (ns: non-significant;  
1013 \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001).

1014

1015

1016

1017 **Extended data figure 4: Flow cytometry and t-SNE analysis of skin**

1018 **Monocyte/Macrophage subsets in GINIP-DTR mice.**

1019 **a**, Classical flow cytometry gating strategy for Monocyte and Macrophage (Mo/M $\phi$ )

1020 subsets in the skin. **b**, t-SNE analysis including CCR2, Ly6C, MHC-II, CD64, CD206

1021 and Tim4 markers was performed to cluster Mo/M $\phi$  populations at day 14 pi in the skin

1022 of GINIP-DTR and DTR-control mice (See also **Article methods** for t-SNE analysis).

1023 **c**, Representative plots for CCR2<sup>-/+</sup>CD206<sup>-</sup> monocyte subsets on D3, D7 and D14 pi in

1024 the skin of control (DTR) or GINIP-DTR mice. **d**, Absolute numbers of Ly6C<sup>+</sup> Mo, Int.

1025 Mo, PMo and MoDC subsets, per mg of ear skin (n=9-13 mice per group) as in **c**. **e**,

1026 Representative FACS plots for CD206<sup>+</sup> dermal resident M $\phi$  as in **c**. **f**, Absolute numbers

1027 of DN and MHC II<sup>+</sup> M $\phi$  subsets, per mg of ear skin (n=9-13 mice per group) as in **c**.

1028 **g**, Representative FACS plots for CD206<sup>+</sup> dermal resident M $\phi$  subsets at D14 pi in back

1029 skin from control (DTR) or GINIP-DTR mice. **h**, Absolute numbers of M $\phi$  subsets,

1030 per mg of back skin (n=12 mice per group) from DTR-control (gray) or GINIP-DTR

1031 (colored) mice, at D14 pi. All data are representative for at least two independent

1032 experiments and presented as mean  $\pm$  SEM. One-way ANOVA with Tukey's multiple

1033 comparisons test (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

1034

1035 **Extended data figure 5: GINIP<sup>+</sup> sensory neurons innervate hair follicles and**  
1036 **release TAFA4 in the skin.**

1037 **a**, Representative confocal images of whole mount ear skin from WT mice stained with  
1038 an anti-Beta3-tubulin (blue) and anti-GINIP (green) antibodies. GINIP<sup>+</sup> lanceolate  
1039 barrel structures innervating hair follicles (HF) are highlighted within dashed squares.  
1040 **b**, Confocal analysis as in **a** with an additional anti-TH antibody staining (red) shows  
1041 GINIP<sup>+</sup>TH<sup>+</sup> C-LTMR axon terminals reaching hair follicle. **c**, Schematically, GINIP<sup>+</sup>  
1042 neurons consist of two subsets of sensory neurons projecting in the inter-follicular  
1043 regions of the epidermis as free nerve endings (IB4<sup>+</sup>) and hair follicles as C-LTMRs  
1044 (TAFA4<sup>+</sup>). **d**, Confocal images of DRG sections from control WT (left) or *Tafa4*-KO  
1045 (right) mice after immunofluorescent (IF) staining with an anti-GINIP (green) an anti-  
1046 TAFA4 antibody (1D8; red) and DAPI (blue). **e**, Additional confocal images of C3  
1047 DRGs (IF) stained with the isolectin B4 (blue), anti-TAFA4 (1D8, red), and ATF3  
1048 (green) antibodies, in WT mice (related to **main Figure 3**), unexposed (left) or at D3  
1049 pi (right). **f**, Absolute number of TAFA4<sup>+</sup> and IB4<sup>+</sup> neurons (left; n=6-12 mice per  
1050 group) and absolute number of TAFA4<sup>+</sup>ATF3<sup>+</sup> and IB4<sup>+</sup>ATF3<sup>+</sup> neurons per DRG  
1051 (right) from unexposed (blue) and exposed D3 pi (red) WT mice, (n=8 mice per group).  
1052 **g**, DAPI staining (blue) *in situ* hybridization (ISH) for *Tafa4* mRNA (red) in DRG as  
1053 in **d**. **h**, Quantification of TAFA4 levels (ELISA) in DRGs from WT mice unexposed  
1054 (D0) to D14 pi. **i**, *Tafa4* mRNA expression (RT-qPCR) in peripheral tissues and DRGs  
1055 (n=3 mice per time point; n.d: not determined). **j**, Quantification of *Tafa4* mRNA  
1056 expression in PBMC, BM, BMDM and sorted CD206<sup>+</sup> dermal Mφ compared to DRGs  
1057 (n=3 independent samples). **k**, *Tafa4* mRNA expression in C3 DRGs from D0 to D35  
1058 pi (n=3 mice per time point). All data are representative for at least two independent  
1059 experiments and presented as mean ± SEM. One-way ANOVA with Tukey's multiple

1060 comparisons test except **h**, Kruskal-Wallis (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ;  
1061 \*\*\* $p < 0.001$ ).

1062 **Extended data figure 6: Tafa4 regulates skin inflammation after UV exposure but**  
1063 **does not affect Treg and Mast cell IL-10 production.**

1064 **a**, Changes in ear skin thickness over time pi in WT control (blue) and *Tafa4*-KO (red)  
1065 mice ( $n=9$  mice per group). **b**, Representative H&E images of ears from WT (left) or  
1066 *Tafa4*-KO (right) mice at D35 pi and histopathological scoring for leukocyte  
1067 infiltration, epidermal thickness, fibrosis, fibrosis extension and then **c**, cumulative  
1068 fibrosis score and total pathological scores ( $n=12$  mice per group). See detailed scoring  
1069 in **Article methods**. **d**, IL-6, IL-1 $\beta$ , and chemokines CCL2, CXCL1 and CCL4 protein  
1070 levels (measured by CBA) and *Il10* mRNA level, in ear skin from WT (blue) or *Tafa4*-  
1071 KO (red) mice from D0 to D35 pi ( $n=3-11$  mice per group and time point). **e**, FACS  
1072 analysis and detection of IL-10<sup>+</sup> immune cells from control (WT; gray) and IL-10-GFP  
1073 (red) mice using an anti-GFP antibody and **f**, using an anti-IL-10 antibody, in skin DC  
1074 subsets, Lym $\phi$  cells, monocytes, mast cells (MC) and M $\phi$  subsets in control (IL-10<sup>gfp/gfp</sup>  
1075 mice, gray) and WT (blue) mice. **g**, Gating strategy for Tregs (CD4<sup>+</sup>TCR $\beta$ <sup>+</sup>FoxP3<sup>+</sup>)  
1076 and **h**, absolute number per mg of ear skin before (D0) or pi, in DTR-control (blue) or  
1077 GINIP-DTR (red) mice ( $n=9-18$  mice per group and per time point). **i**, Representative  
1078 FACS plots for IL-10 in Tregs from WT (left) or *Tafa4*-KO (right) mice before (blue)  
1079 or pi (red). **j**, Gating strategy (left) and absolute number (right) of mast cells (MC) per  
1080 mg of skin from DTR-control (blue) or GINIP-DTR (red) mice, showing MC depletion  
1081 (D3) and repopulation (D7) after UV exposure; ( $n=8-9$  mice per group and time point).  
1082 **k**, Representative FACS plots for IL-10 in Tim4<sup>+</sup> M $\phi$  (blue) and MC (yellow) before  
1083 (No UV, top) and at D10 pi (bottom) in WT mice. **l**, Representative FACS plot for IL-  
1084 10 in MC in WT (blue) or *Tafa4*-KO (red) mice at D10 pi and **m**, median IL-10 MFI  
1085 analysis ( $n=6-10$  mice per group). All data are representative for at least two  
1086 independent experiments and presented as mean  $\pm$  SEM. One-way ANOVA with

1087 Tukey's multiple comparisons test (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p <$   
1088 0.001).

1089

1090 **Extended data figure 7: TAFA4 promotes IL-10 production by dermal resident**  
1091 **M $\phi$  subsets *in vivo* and directly up-regulates *Il10* in M $\phi$  *in vitro*.**

1092 Representative FACS plots (upper panels) for intracellular IL-10 expression in DN M $\phi$   
1093 (a), MHC-II<sup>+</sup> M $\phi$  (b) and Tim4<sup>+</sup> M $\phi$  (c) subsets from WT (blue) and *Tafa4*-KO (red)  
1094 mice at D10 pi, and (lower panels) median IL-10 MFI quantifications in the respective  
1095 M $\phi$  subsets at D3, 7 and 10 pi (n=6-9 mice per group and time point). **d**, BMDM,  
1096 derived from WT or FPR1-KO bone marrow, were challenged in a migration assay  
1097 (transwell) using medium alone (CTL<sup>-</sup>), MCP-1 (CTL<sup>+</sup>) or increasing concentrations of  
1098 TAFA4. Absolute number of M $\phi$  in the bottom wells were analyzed by FACS (n=3  
1099 independent samples per group). **e**, *Il6* mRNA level in ThioM $\phi$  in the presence (green)  
1100 or absence (blue) of TAFA4 (See main **Fig.4i**), (n=6-8 independent sample per group).  
1101 **f**, BMDM as in (d), were activated *in vitro* by LPS alone (blue) or in the presence of  
1102 TAFA4 (green). Gene expression was analyzed by RT-qPCR for *Tnfa*, *Il6* and *Il10* and  
1103 compared to non-activated BMDM (CTL), (n=4-5 independent sample). All data are  
1104 representative for at least two independent experiments and presented as mean  $\pm$  SEM.  
1105 One-way ANOVA with Tukey's multiple comparisons test (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p <$   
1106  $< 0.001$ ).

1107

1108

1109 **Extended data figure 8: Fate-mapping and shield irradiated BM chimeras**  
1110 **revealed that TAFA4 regulates the dynamic between embryo-derived- and**  
1111 **monocyte-derived M $\phi$  after UV exposure.**

1112 **a**, Strategy for embryonic progenitor fate mapping using the CX3CR1<sup>CreERT2:R26-YFP</sup>  
1113 mice. Tamoxifen (Tam) was injected in pregnant females at E16.5 days of pregnancy  
1114 and offsprings were analyzed at 6 weeks of age. **b**, Percentages of YFP<sup>+</sup> cells in the  
1115 indicated cell types (n=5 mice). LCs: Langerhans cells. **c**, Experimental scheme for  
1116 monocyte tracing using CD45.1<sup>+</sup> BM shield-irradiated chimeras. **d**, Percentage of  
1117 CD45.1 chimerism within the indicated Mo/M $\phi$  subsets in 2 months old chimeric mice  
1118 in the steady state (n=7 mice per group), or **e**, after additional 4 months following UV  
1119 irradiation (purple) or no UV (gray), (n=5 mice per group). **f**, Gating strategy for  
1120 Mo/M $\phi$  analysis in WT CD45.1 BM Chimera before UV exposure (top panels) and  
1121 then at D7 pi in WT (middle panels) and in *Tafa4*-KO (bottom panels) recipient  
1122 chimeras. **g**, Relative expression of the markers CD45.1, Ly6C, CD64, CD206, MHCII  
1123 and Tim4 used for t-SNE analysis of skin Mo/M $\phi$  subsets from WT or *Tafa4*-KO  
1124 recipient chimeras (see also **Fig. 4a**). **h**, Absolute numbers (n=9 mice per group) per  
1125 mg of ear skin (left) and CD45.1 chimerism levels (right) (n=7 mice per group) for DN  
1126 M $\phi$  and MHC-II<sup>+</sup> M $\phi$  in WT (blue) and *Tafa4*-KO (red) BM-chimeric mice on D7 pi.  
1127 **i**, Representative Annexin-V staining of DN M $\phi$  (left), MHC-II<sup>+</sup> M $\phi$  (middle) and  
1128 Tim4<sup>+</sup> M $\phi$  (right) at D3 pi from DTR (blue) or GINIP-DTR (red) mice. **j**, median MFI  
1129 for annexin-V in M $\phi$  subsets as in (**i**), (n=4 mice per group). **k**, Representative Annexin-  
1130 V as in (**i**) at D2 pi for WT (blue) or *Tafa4*-KO (red) mice. **l**, Median fluorescence  
1131 intensity (MFI) for Annexin-V in M $\phi$  subsets as in (**j**), (n=5-6 mice per group). All data  
1132 are representative for at least two independent experiments and presented as mean  $\pm$

1133 SEM. One-way ANOVA with Tukey's multiple comparisons test (ns: non-significant;

1134 \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

1135

1136 **Extended data figure 9: IL-10 defect and dermal M $\phi$  depletion recapitulate *Tafa4*-**  
1137 **KO mice phenotype after UV exposure.**

1138 **a**, Experimental scheme for *in vivo* neutralization of IL-10 in WT mice. **b**, Ear thickness  
1139 at D14 pi, of WT (blue) or *Tafa4*-KO (red) mice treated with an IgG2a isotype control  
1140 (iso) and of WT mice treated with anti-IL-10 blocking antibodies (purple), (n=10 mice  
1141 per group). **c**, Absolute numbers of Inflamm M $\phi$  (left) and Tim4<sup>+</sup> M $\phi$  (right) in ear skin  
1142 of the indicated 3 groups of mice (as in **b**), (n=6-9 mice per group). **d**, Representative  
1143 FACS plots of Mo/M $\phi$  subsets at D0 and D3 pi in IL-10<sup>wt/gfp</sup> and IL-10<sup>gfp/gfp</sup> (IL-10 KO)  
1144 mice. **e**, Absolute number of Tim4<sup>+</sup> M $\phi$  (left) and Inflamm M $\phi$  (right) per mg of skin at  
1145 D7 pi in IL-10<sup>wt/gfp</sup> (gray) and IL-10<sup>gfp/gfp</sup> (colored), (n=8-10 mice per group). **f**, ear  
1146 thickness before (No UV) and at D14 pi (UV) in IL-10<sup>wt/gfp</sup> (blue) and IL-10<sup>gfp/gfp</sup>  
1147 (purple) mice, (n=9-12 mice per group). **g**, Experimental scheme for dermal resident  
1148 M $\phi$  depletion: two i.p. injections, at D-3 and D-2, of the CSF-1R blocking antibody  
1149 AFS98, are effective to deplete DN, Tim4<sup>+</sup>, MHC II<sup>+</sup> and Langerhans cells in WT mice.  
1150 **h**, Absolute number of M $\phi$  per mg of ear skin before (CTL) and after AFS98 injection  
1151 (colored), (n= 13 mice per group). **i**, IL-10 protein level detected by CBA in WT (blue)  
1152 and *Tafa4*-KO (red) after isotype control or AFS98 antibody injection (n=4-5 mice per  
1153 group). **j**, Absolute number of myeloid cell subsets per mg of ear skin after isotype  
1154 control or AFS98 antibody injection (colored), (n= 11 mice per group). **k**, (left)  
1155 Representative FACS plots of Mo/M $\phi$  subsets over the time course of M $\phi$  repopulation  
1156 (D0, D2, and D4) after AFS98 injection and complete M $\phi$  depletion (D0) in WT mice,  
1157 and, (right) Representative FACS plots at D6 after AFS98 injection in WT (blue) and  
1158 *Tafa4*-KO (red) mice showing the acquisition of Tim4 by monocytes-derived M $\phi$ , (see  
1159 bottom panels). **l**, Representative FACS plots of Mo/M $\phi$  subsets at D0 and D3 post-UV  
1160 exposure in WT and *Tafa4*-KO mice after M $\phi$  depletion with the blocking anti-CSF-

1161 1R Ab (AFS98). Absolute number of Tim4<sup>+</sup> Mφ **m** (left), and Inflamm Mφ (right), per  
1162 mg of skin at D7 pi in WT (gray) and *Tafa4*-KO (colored) treated with an IgG2a isotype  
1163 control (Iso+UV), or treated with anti-CSF-1R Ab (AFS+UV), (n=8-9 mice per group).  
1164 **n**, ear thickness in unexposed mice (No UV), and at D14 pi in WT (blue) and *Tafa4*-  
1165 KO (red) mice treated as in **m** (n=10 mice per group). All data are representative for at  
1166 least two independent experiments and presented as mean ± SEM. One-way ANOVA  
1167 with Tukey's multiple comparisons test (ns: non-significant; \**p* < 0.05; \*\**p* < 0.01;  
1168 \*\*\**p* < 0.001).  
1169

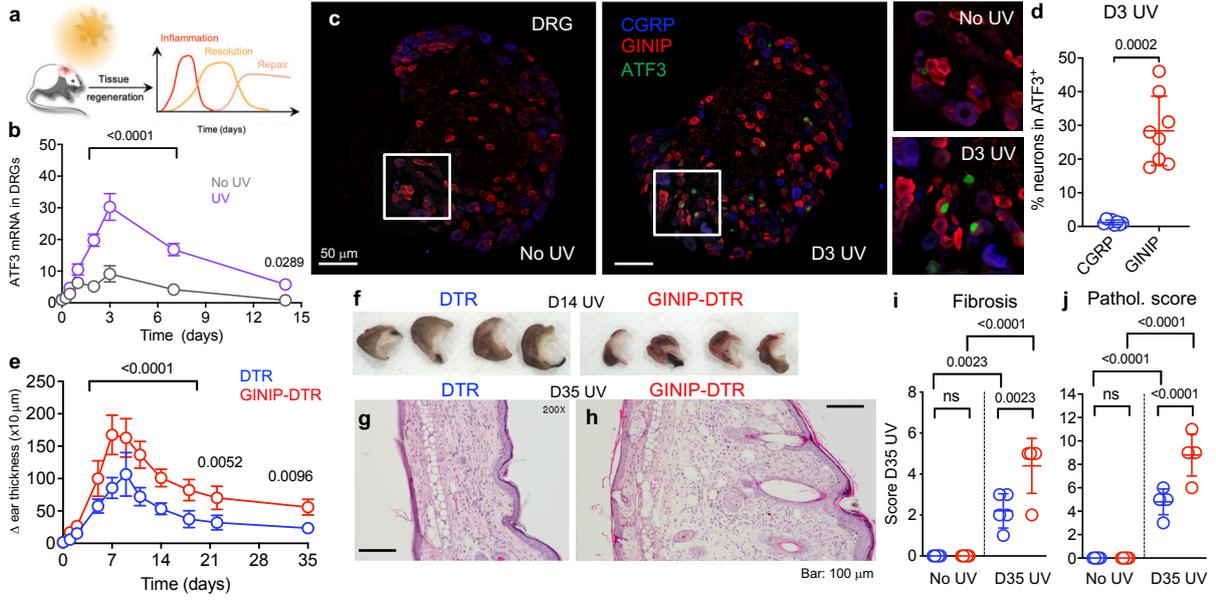
1170 **Extended data figure 10: Adoptive transfer of Tim4<sup>+</sup> macrophages is sufficient to**  
1171 **reduce tissue damage in GINIP-DTR mice.**

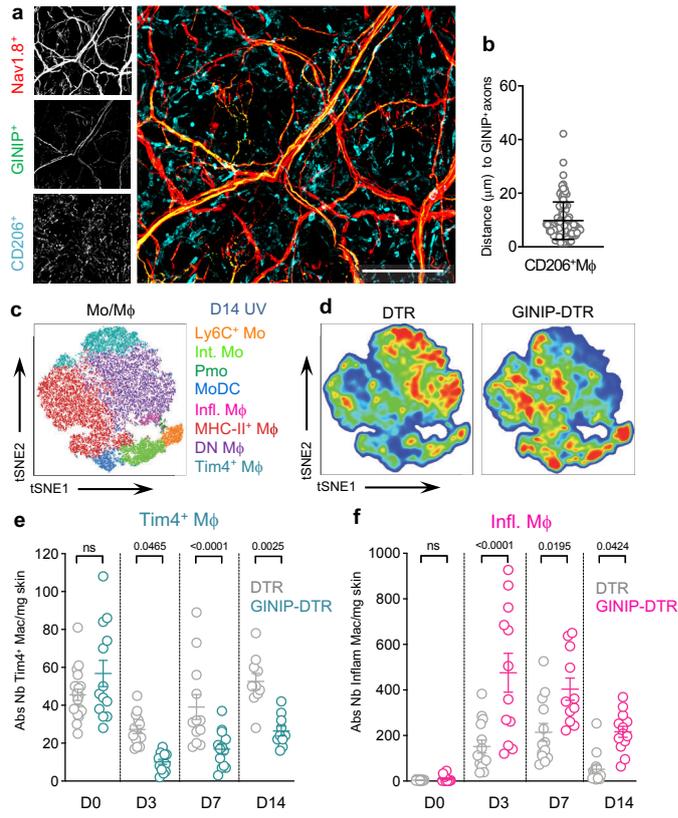
1172 **a**, Experimental scheme for intradermal adoptive transfer of CD45.1<sup>+</sup>Tim4<sup>+</sup> M $\phi$ . **b**,  
1173 Gating strategy for sorting CD45.1<sup>+</sup> Inflamm M $\phi$  (pink) and Tim4<sup>+</sup> M $\phi$  (cyan). **c**, ear  
1174 thickness for unexposed (No UV) or UV-exposed mice at D7 pi, in DTR (blue), GINIP-  
1175 DTR (red) mice and GINIP-DTR mice injected with Inflamm M $\phi$  (pink) or with Tim4<sup>+</sup>  
1176 M $\phi$  (cyan) mice, (n=7-10 mice per group). **d**, Representative images of ears at D7 pi  
1177 from each group as in (c). **e**, Experimental scheme for TAFA4 rescue in *Tafa4*-KO  
1178 mice. **f**, Representative FACS plot for intracellular staining of IL-10 in Tim4<sup>+</sup> M $\phi$  at  
1179 D14 pi, in WT mice treated with saline (blue) or *Tafa4*-KO mice treated with saline  
1180 (red) or *Tafa4*-KO mice treated with TAFA4 (green). **g**, Model for TAFA4 functions  
1181 *in vivo* after UV exposure: 1) Role of TAFA4 during the inflammatory phase. Skin  
1182 overexposure to UV induces the release of the neuropeptide TAFA4 by C-LTMRs.  
1183 TAFA4 promotes the production of IL-10 by embryonic-derived dermal resident Tim4<sup>+</sup>  
1184 M $\phi$ . The production of IL-10 is essential for their survival and protect the skin from  
1185 over-inflammation. Tissue lesions also induce the recruitment of monocytes in the skin,  
1186 where they differentiate into TNF $\alpha$ <sup>+</sup> Inflamm M $\phi$ . In the absence of TAFA4 production,  
1187 the number of Tim4<sup>+</sup> M $\phi$  and IL-10 levels are reduced, promoting the expansion of  
1188 Inflamm M $\phi$ . 2) Role of TAFA4 during the resolution phase. The TAFA4/IL-10 axis is  
1189 still active, promoting the maintenance of both embryonic- and monocyte-derived IL-  
1190 10<sup>+</sup> Tim4<sup>+</sup> M $\phi$ , which are required for tissue repair. In the absence of TAFA4  
1191 production, IL-10 production by the three subsets of CD206<sup>+</sup> dermal M $\phi$  is  
1192 compromised, leading to persistent inflammation and fibrotic scars. **h**, Repeated  
1193 measure over time of ear thickness changes ( $\mu$ m) of WT (blue) and *Tafa4*-KO (red)  
1194 mice treated daily with Imiquimod (IMQ) during six consecutive days and followed up

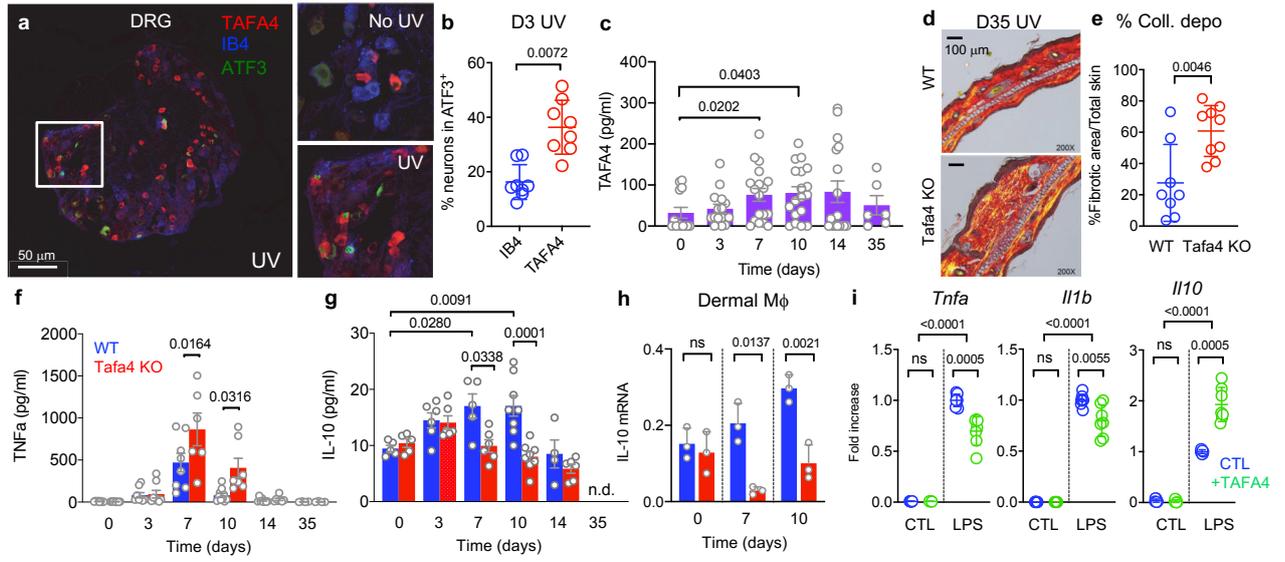
1195 to 15 days, (n=5 mice per group). All data are representative for at least two  
1196 independent experiments and presented as mean  $\pm$  SEM. One-way ANOVA with  
1197 Tukey's multiple comparisons test (ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p <$   
1198 0.001).

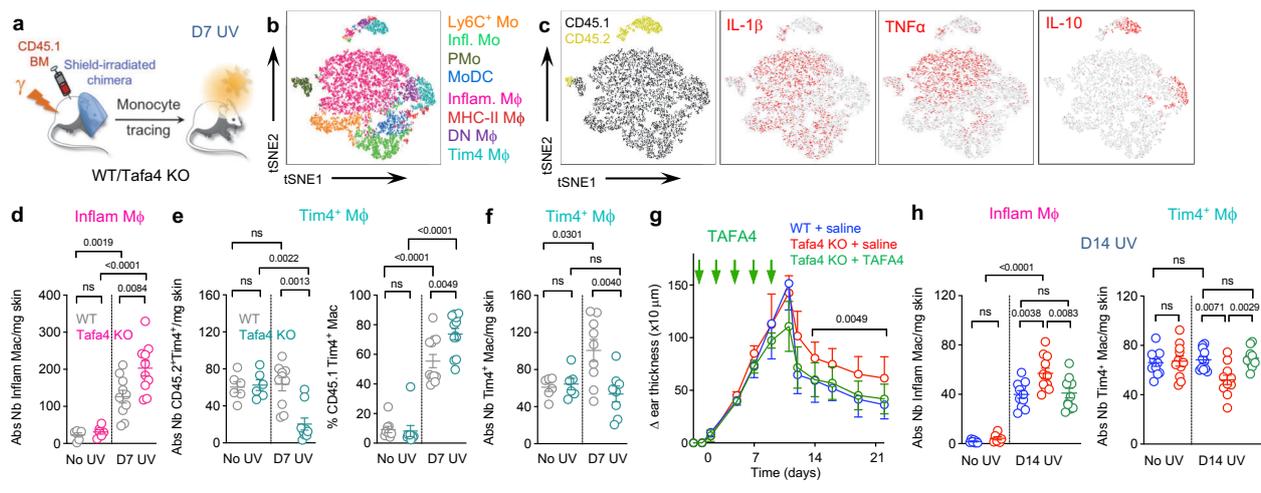
1199

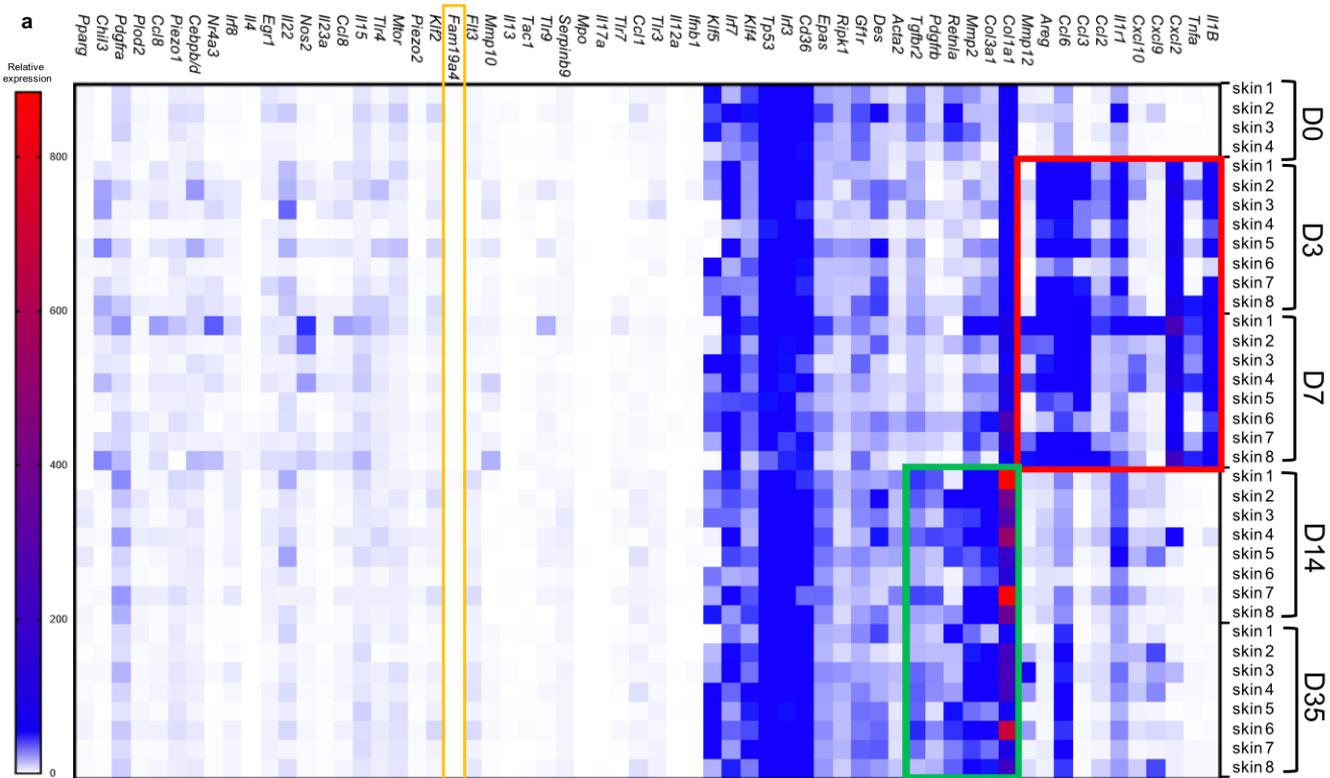
1200



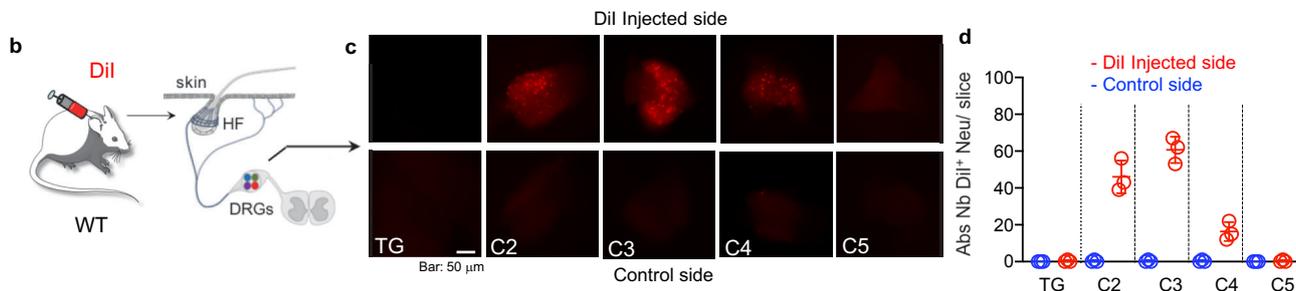




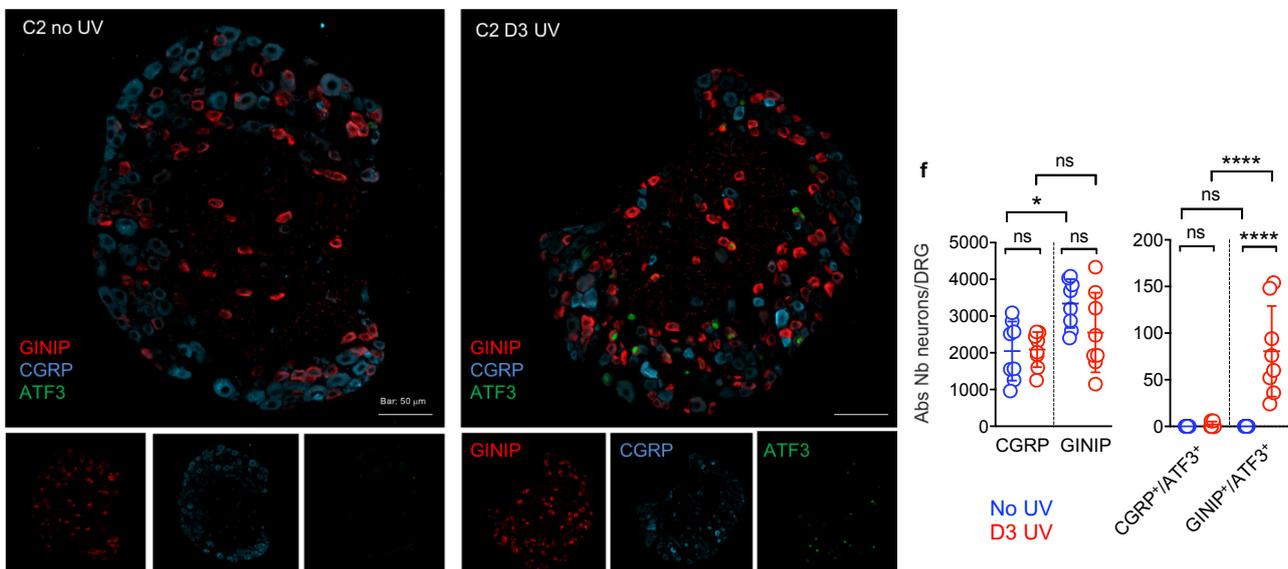


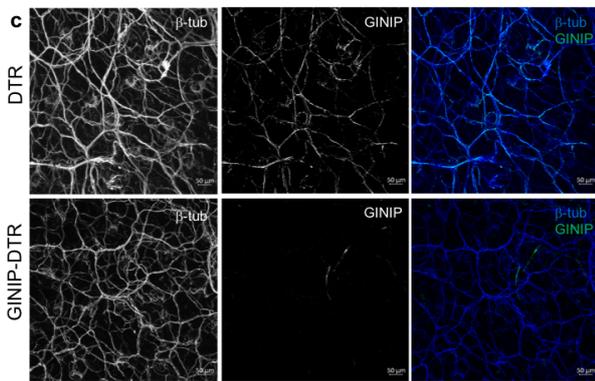
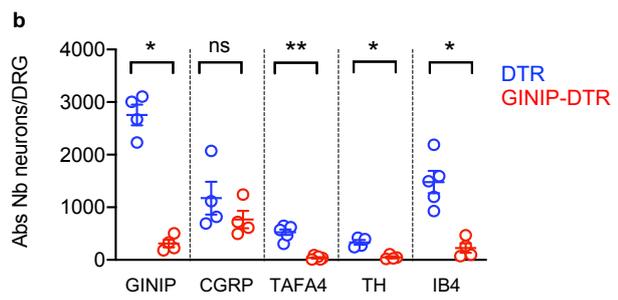
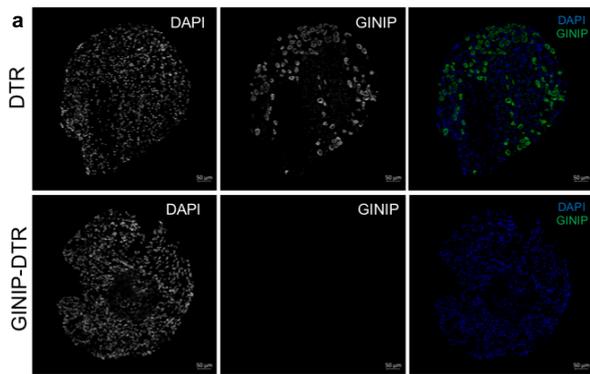


Identification of DRGs innervating the ear skin by Dil retrolabeling

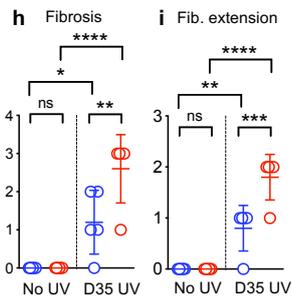
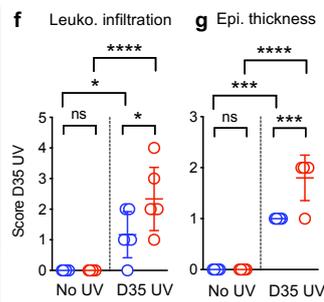
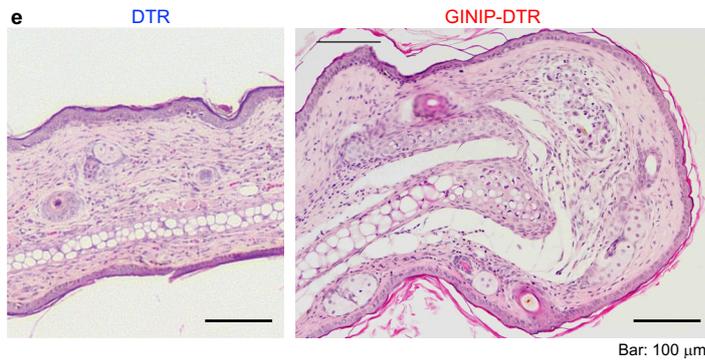
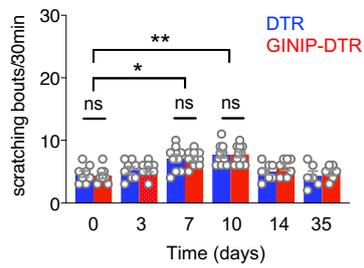


**e** UV exposure selectively activate GINIP<sup>+</sup> neurons in C2/C3 DRGs

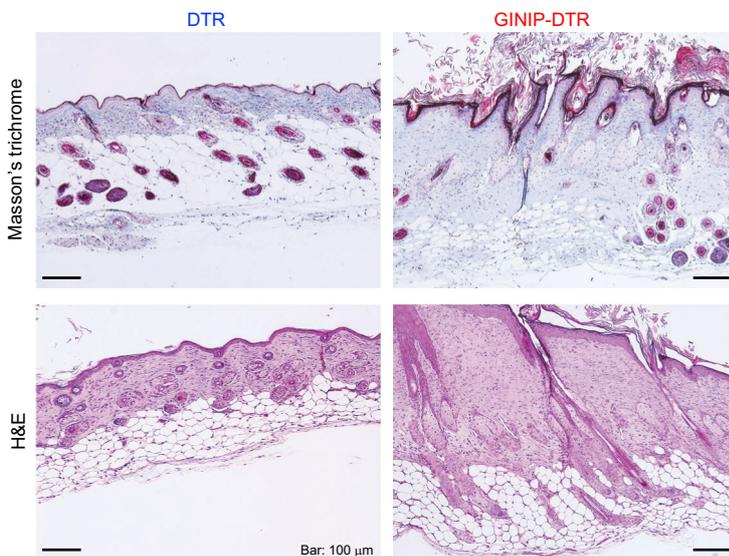




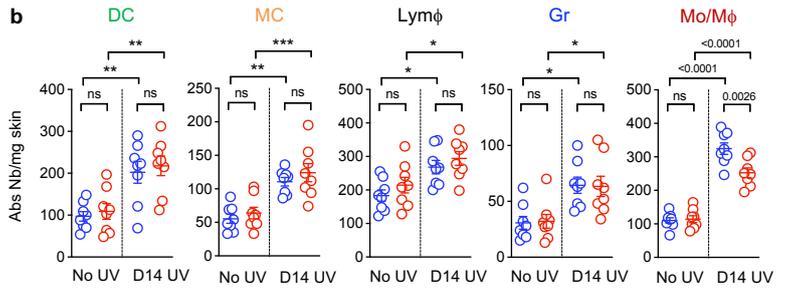
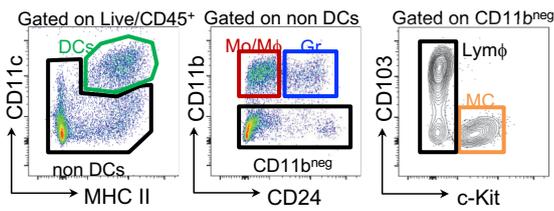
**d** Scratching bouts monitoring



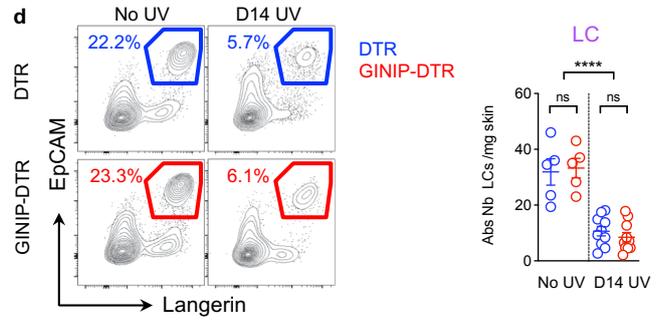
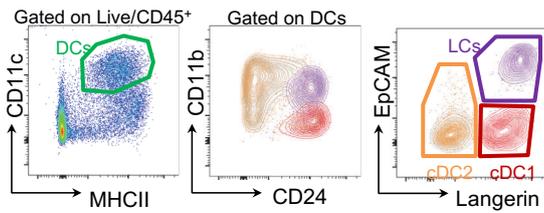
**j** Representative image of back skin at D35 UV:



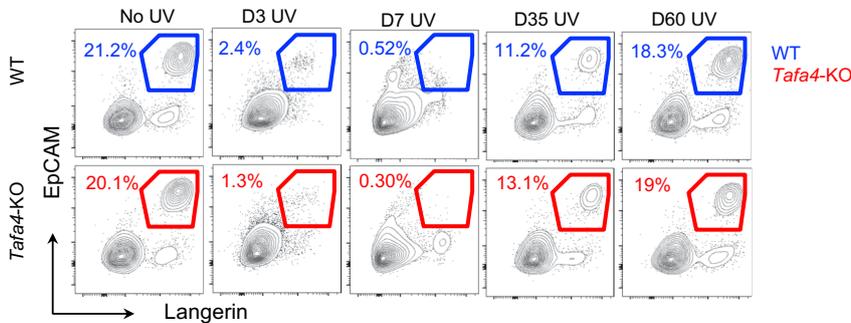
**a** Gating strategy for skin immune cell subsets:



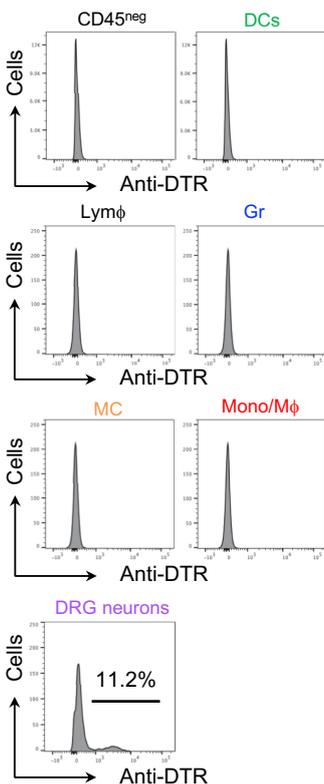
**c** Gating strategy for Langerhans cells (LCs):



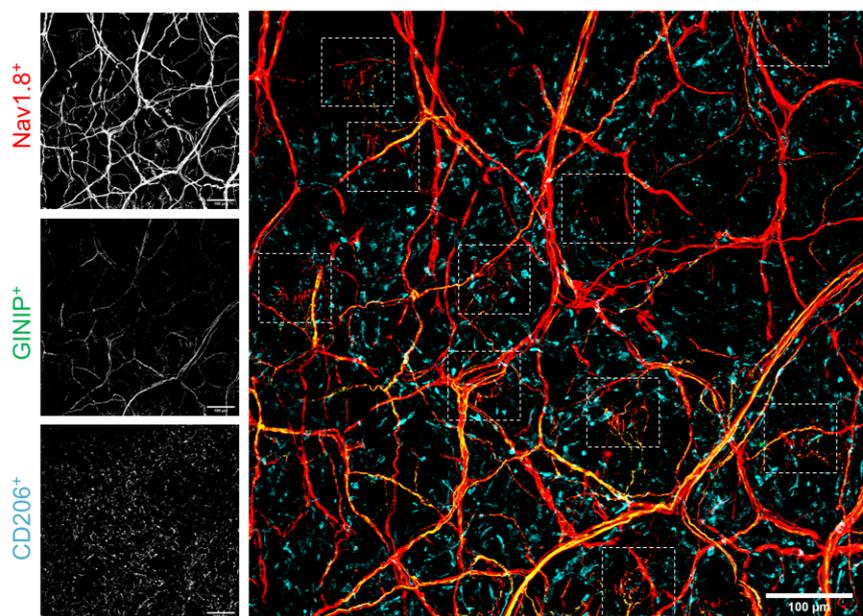
**e** Langerhans cells are depleted by UV irradiation:

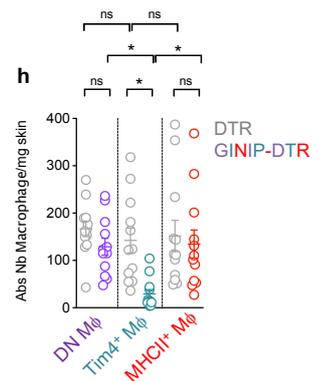
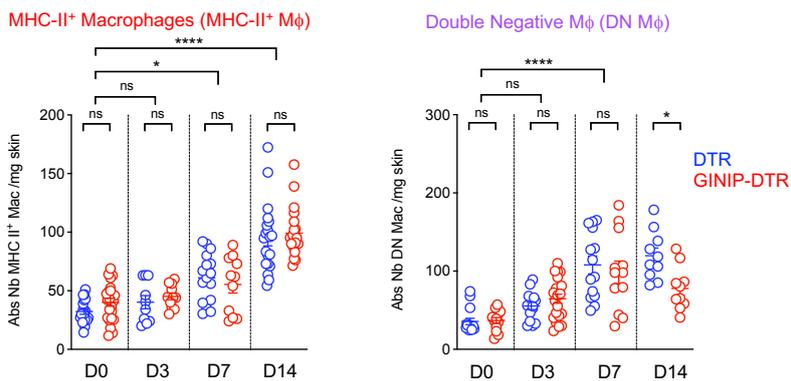
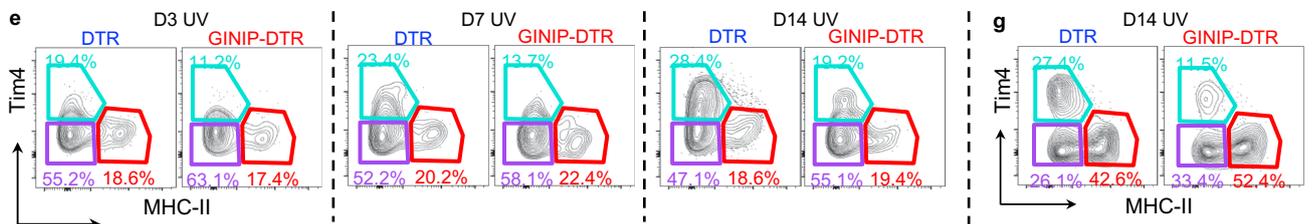
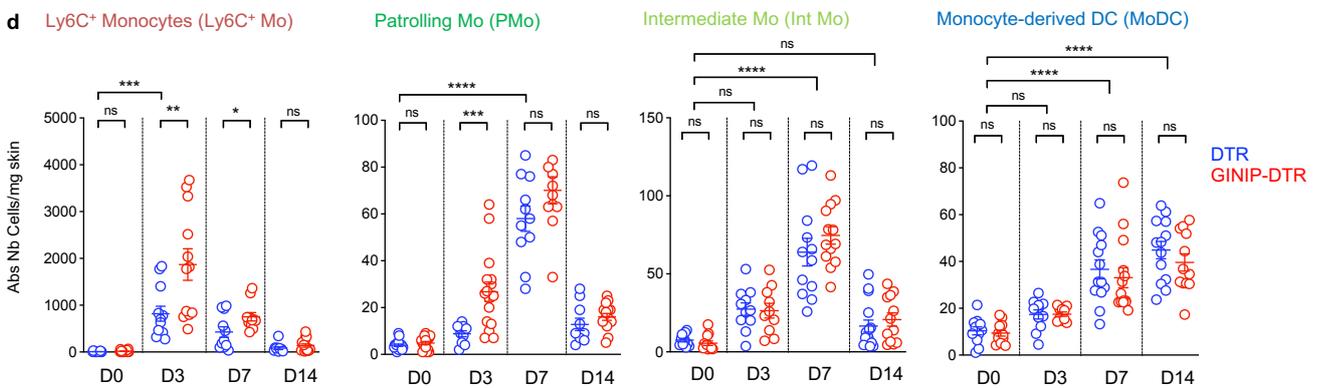
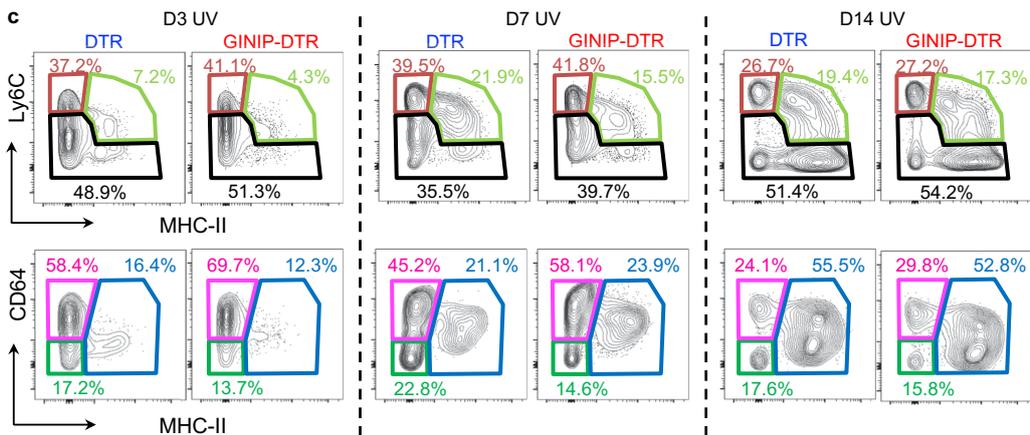
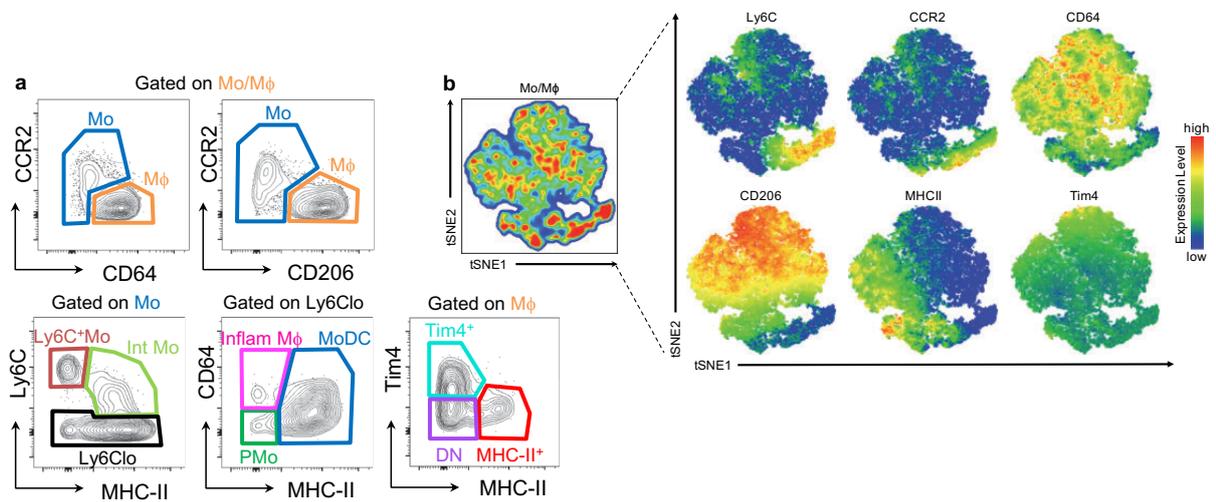


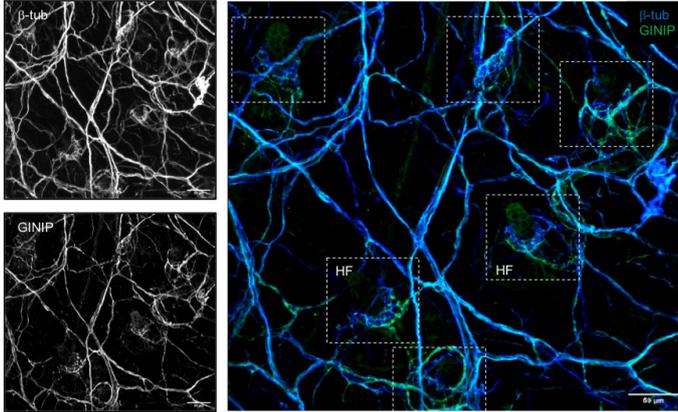
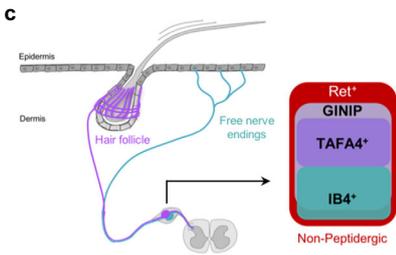
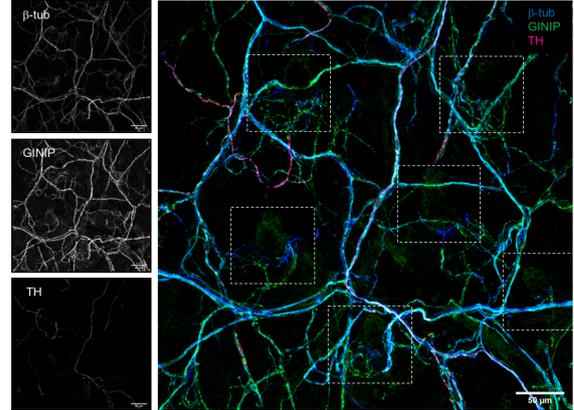
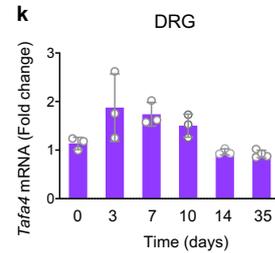
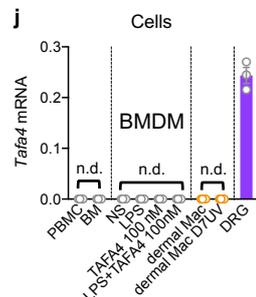
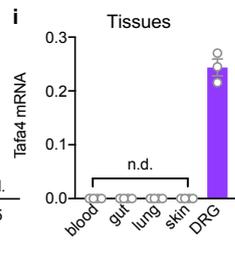
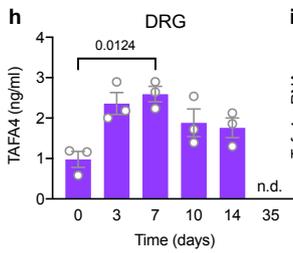
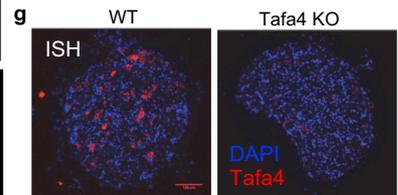
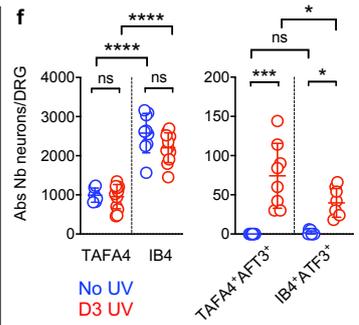
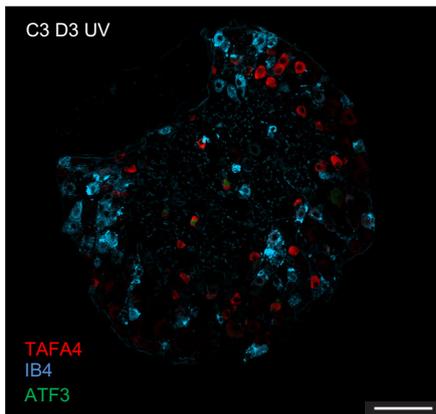
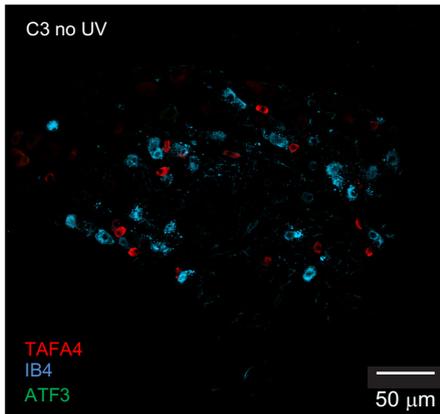
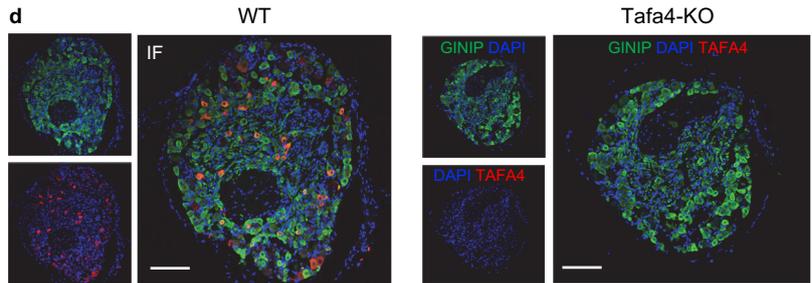
**f** DTR expression in GINIP-DTR mice:

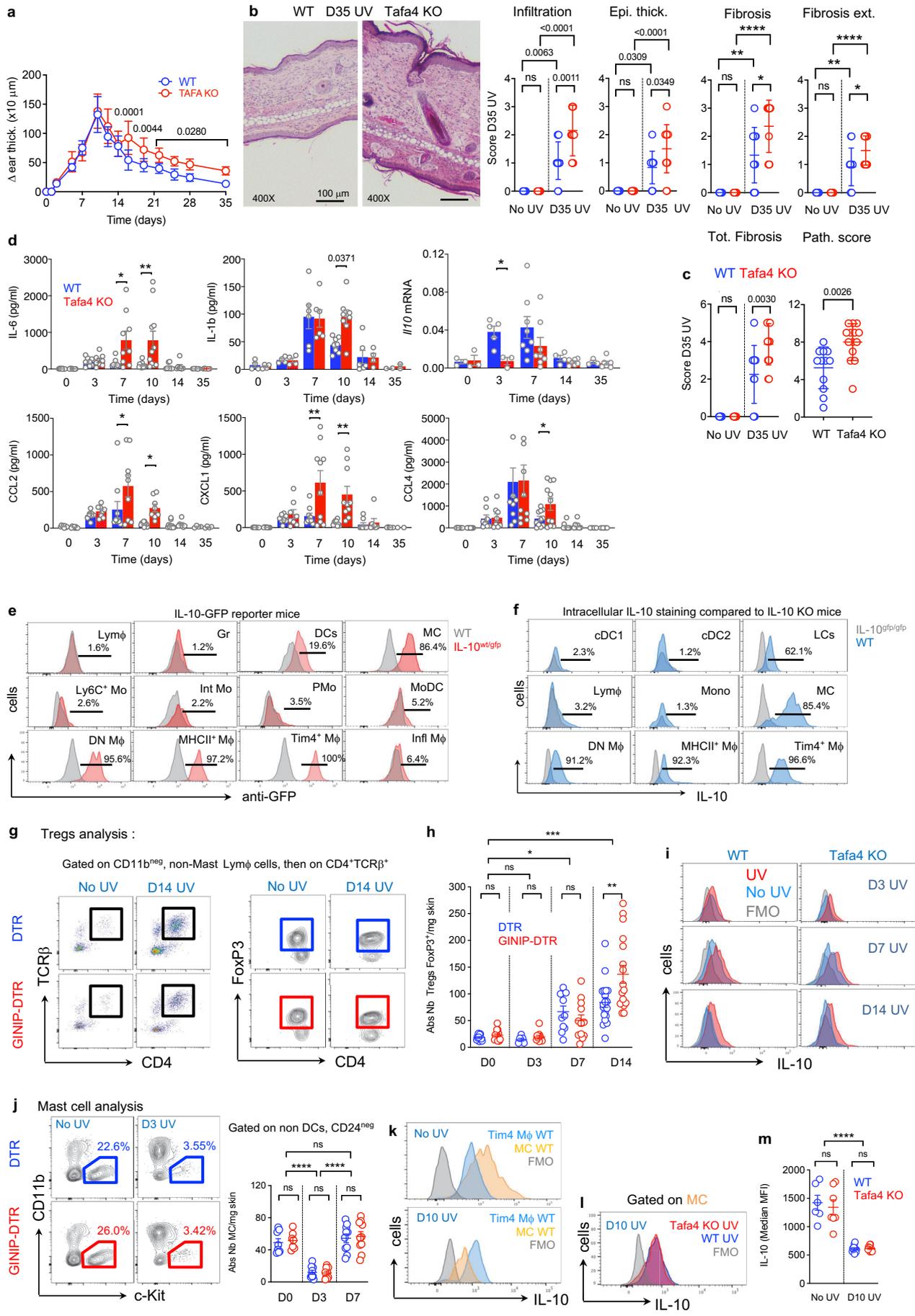


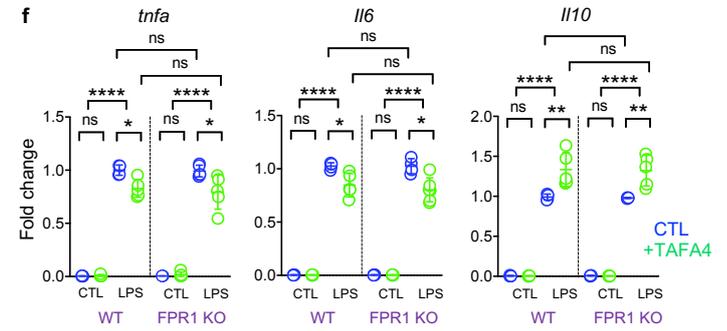
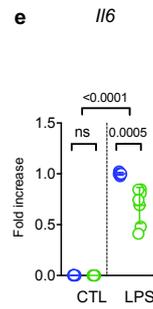
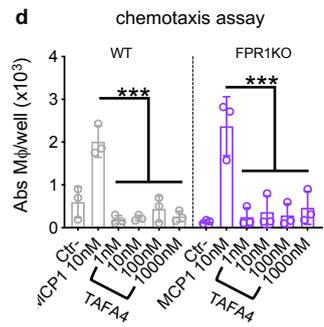
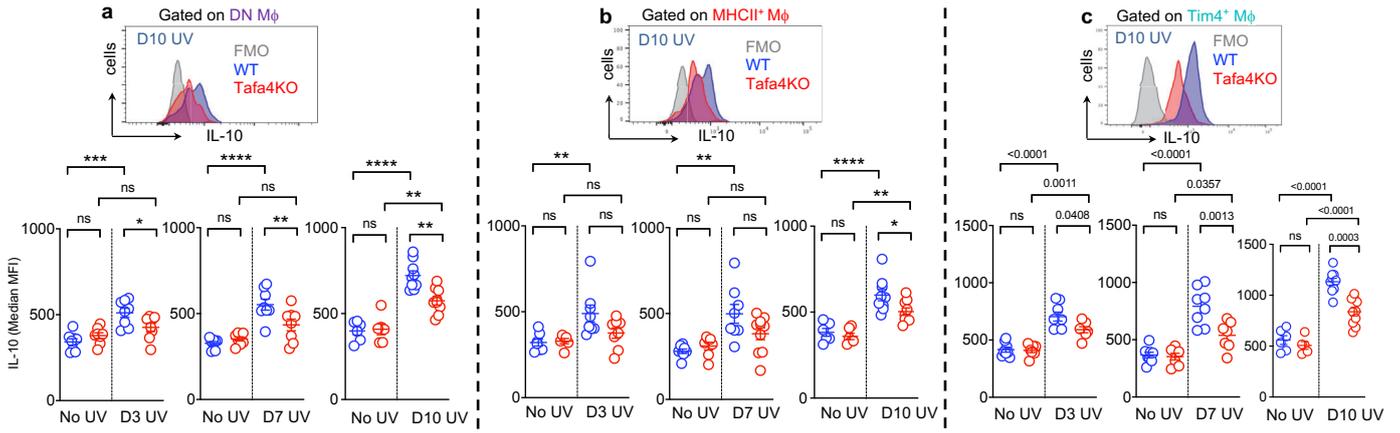
**g** CD206<sup>+</sup> dermal macrophages are near GINIP<sup>+</sup> sensory neuron



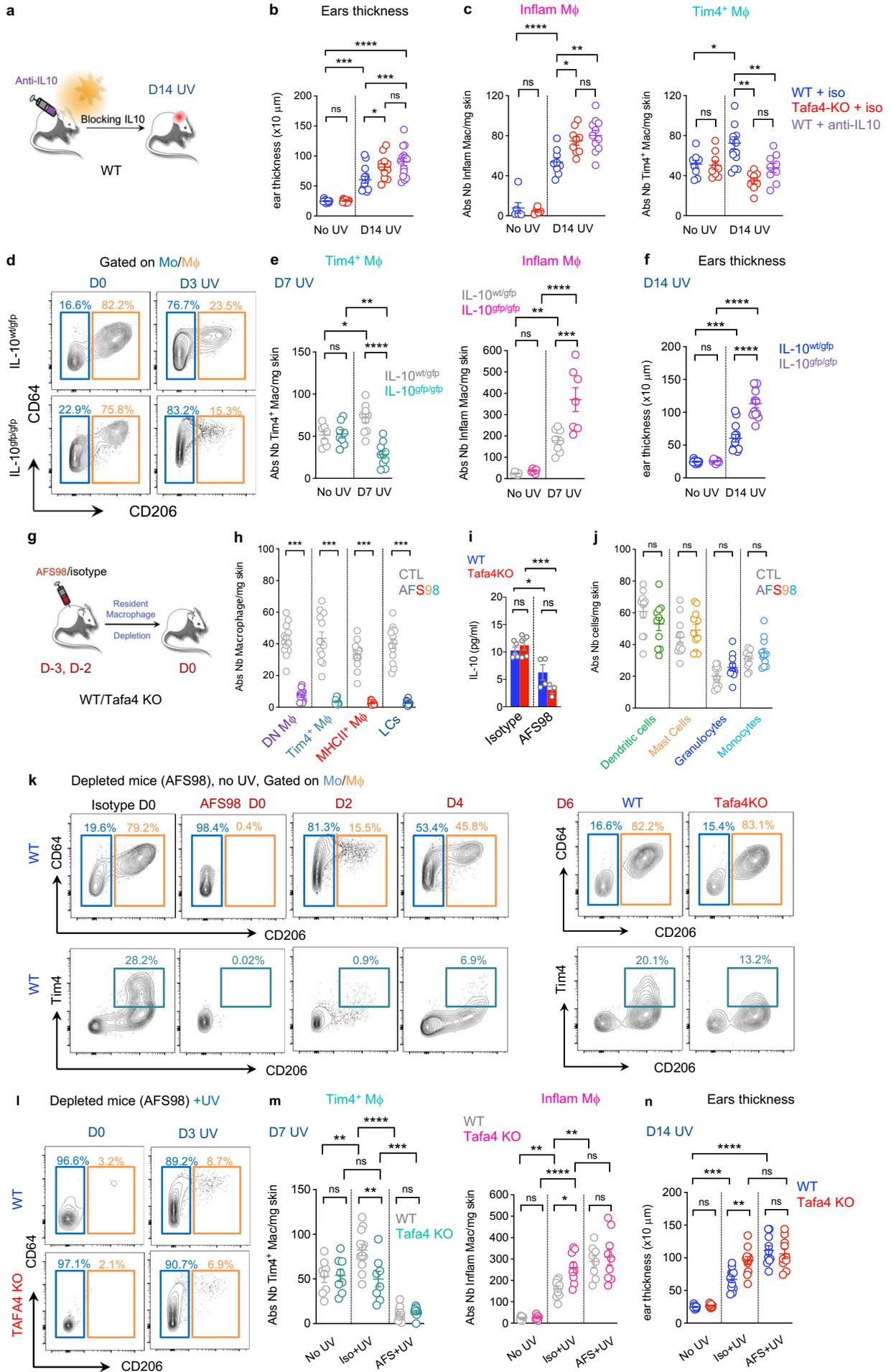


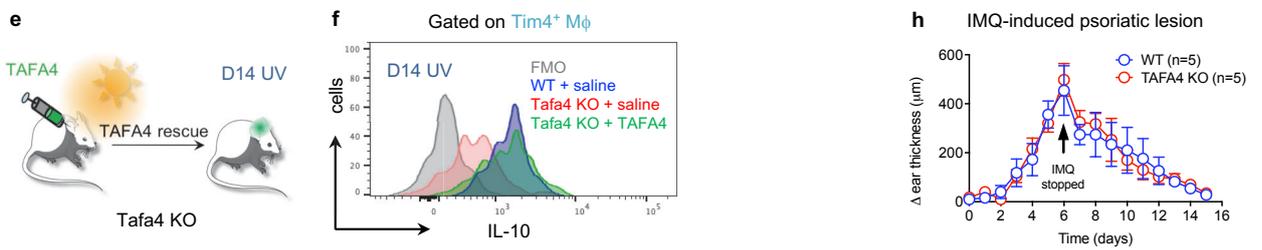
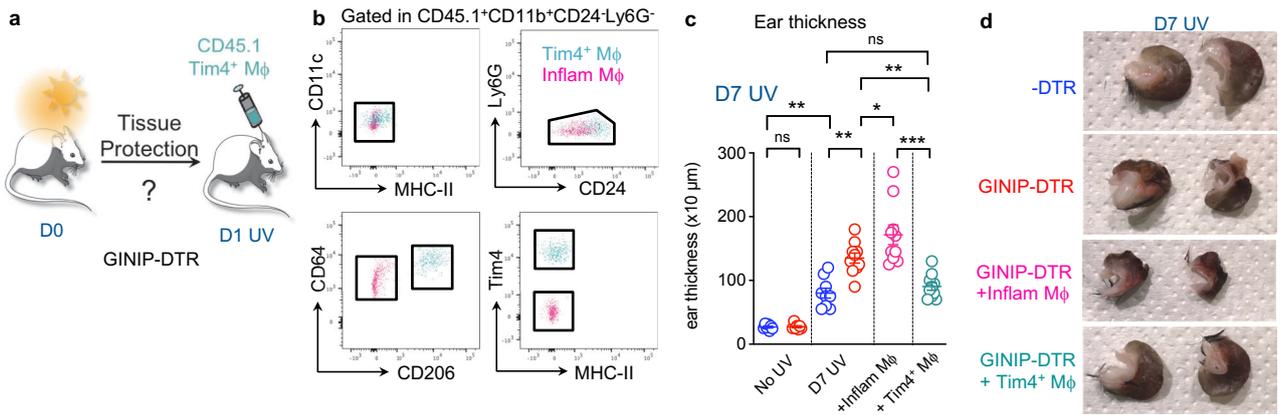
**a** GINIP<sup>+</sup> neurons innervate hair follicles**b** GINIP<sup>+</sup> neurons include TH<sup>+</sup> C-LTMR**e**











**g** Model

1) Role of Tafa4 during the Inflammatory phase

2) Role of Tafa4 during the Resolution phase

