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TITLE

Interleukin-17 (IL-17)-mediated immunity controls skin infection and T helper 1 response during experimental *Microsporum canis* dermatophytosis.

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SHORT TITLE

IL-17-mediated immunity in dermatophytosis.

KEYWORDS

DERMATOPHYTES * T HELPER 17 * LANGERHANS CELLS

ABBREVIATIONS

Th17: T helper 17

Th1: T helper 1

sdLN: skin draining lymph node

HKMc**:** heat-killed *Microsporum canis* hyphae

LC: Langerhans cells

p.i.: post-infection

ABSTRACT

Despite worldwide prevalence of superficial mycoses, the immune response in dermatophytosis has scarcely been investigated. In this study, we developed a model of superficial skin infection in C57BL/6 mice with *Microsporum canis*, a highly prevalent human pathogen. This model mimics mild inflammatory human dermatophytosis, characterized by neutrophil recruitment and fungal invasion limited to the epidermis, and exhibits the establishment of a specific T helper 17 (Th17) immune response during infection. By using IL-17RA or IL-17A/F deficient mice we showed that, in the absence of a functional IL-17 pathway, *M. canis* extensively colonizes the epidermis and promotes an exaggerated skin inflammation and a shift to an IFN-γ-mediated (Th1) response. Strikingly, IL-17 signaling was not involved in neutrophil influx to skin or fungal invasion to deeper tissues. Finally, this study demonstrates that skin langerin-expressing cells contribute to the antifungal Th17 response *in vivo*.

In conclusion, these data directly demonstrate a dual function of IL-17 cytokines in dermatophytosis by controlling superficial infection and down-modulating a Th1 antifungal response.

INTRODUCTION

Microsporum canis is a zoophilic dermatophyte fungus that causes highly prevalent superficial infections of the scalp (*tinea capitis*) and body (ringworm or *tinea corporis*) with mild or severe inflammatory clinical symptoms (Chiapello et al., 2011; Hay, 2007; Spesso et al., 2013). Immunocompromised patient may experience extensive or atypical skin infection, with a high treatment failure rate (Achterman and White, 2012).

Despite worldwide prevalence of dermatophyte infections, a good understanding of immune response during dermatophytosis is still elusive. Neutrophil recruitment, enhanced epidermal proliferation and antimicrobial peptide expression have been associated with host protection in human and experimental infections (Hube et al., 2015). Furthermore, dermatophytes induce a specific adaptive immune response that promotes clinical recovery and is protective against re-infection (Hay, 2017). Host protection has been associated to delayed-type hypersensitivity response due to CD4 T lymphocytes and an IFN-γ- mediated T helper 1 (Th1) immunity. However, these studies were performed prior to the discovery of T helper 17 (Th17), when cellular immunity was known to be only dependent on Th1 (Heinen et al., 2017).

In skin, IL-17 cytokines, produced by leukocytes from both innate (ILCs, γδ T cells, iNKT) or adaptive immune systems (Th17: $CD4^+$ and $CD8^+$ T lymphocytes), play a major role in the defense against extracellular bacteria and opportunistic fungi (Kashem and Kaplan, 2016; Meller et al., 2015). The six members of IL-17 family (IL-17A to IL-17F) signal through five receptors (IL-17RA to IL-17RE) (Amatya et al., 2017), but a requisite role for IL-17RA has been demonstrated for IL-17A, C, E and F (Martin et al., 2013). In human dermatophytosis, an increased susceptibility to deep or extensive skin infections has been described in patients with inborn errors in the IL-17 pathway (Lanternier et al., 2013; Nielsen et al., 2015) In addition, C57BL/6 mice infected with dermatophytes of the genus *Arthroderma,* over-expressed TGF-β, IL-1β and IL-6 mRNA in skin which are key mediators of Th17 differentiation (Cambier et al., 2014). However, the role of IL-17 immunity has not been directly explored *in vivo* during dermatophytosis.

Dermatophyte exclusive colonization of skin surface and hair follicles suggests that Langerhans cells (LC, langerin-expressing cells from epidermis) could drive antifungal

immunity, since they are the only epidermal antigen presenting cells involved in priming T lymphocytes after migration to the lymph nodes (Merad et al., 2008). Regarding this, a previous study reported that human LC induced specific cell-mediated immunity to dermatophyte antigens *in vitro* (Braathen and Kaaman, 1983). However, dermatophyte effects on dendritic cell functions in skin are still completely unknown.

In the present study, we investigated the outcome of skin infection and cellular immune response in a dermatophytosis model with *M. canis* in wild-type (WT) and IL-17-deficient C57BL/6 mice. Furthermore, taking advantage of langerin-expressing cells depletion in *M. canis*-infected transgenic Lang-EGFPDTR mice (Kissenpfennig et al., 2005), we explored the contribution of these cells in driving the specific Th17 immunity against *M. canis.*

RESULTS AND DISCUSSION

In this study, we first established a reproducible model of dermatophytosis by epicutaneous treatment with *M. canis* hyphae along with a semi-permeable patch (Tegaderm®) on previously scarified back skin of C57BL/6 WT mice (Hay et al., 1988). All infected animals showed clinical signs of dermatophytosis and developed mild cutaneous lesions characterized by erythema, scaling and thin crusting, similar to non-inflammatory dermatophytosis in immunocompetent humans (**Figure 1a**, **Figure 2b**) (Hay, 2007). Skin lesions were only limited to the epidermis and visible from day 4 post-infection (p.i.), with a peak at day 8 p.i. and until day 18, when they spontaneously healed. Histopathological analysis revealed the presence of acanthosis and epidermal and dermal micro-abscesses, indicating neutrophil recruitment. PAS positive hyphae were localized in the *stratum corneum* and, especially, invading hair follicles (**Figure**

1b and **c**, **Figure 2b**, **Suppl. Figure S1a** and **Suppl. Table**). According to this, direct examination of hair shafts showed parasitization with arthroconidia (**Figure 1d**) resembling human *tinea capitis* (Hay, 2017). In concordance with clinical features and histopathology, *M. canis* colony forming units (CFU) were effectively isolated in Sabouraud agar plates from epidermis after 4 and 8 days p.i. (**Figure 1e**, **Figure 2d** and **e**). Afterwards, by day 18, WT mice resolved infection and cleared the fungi from skin (**Figure 1a, b** and **e**). Furthermore, flow cytometry studies showed a significant increase of neutrophils ($CD11b^{+}Gr1^{+}$ cells) in epidermis from 8-day-infected mice, compared to uninfected (sham) controls (**Figure 1f,** gating strategies displayed in **Suppl. Figure S2a**). These results resemble to the hallmarks of dermatophytosis like the sloughing of fungal mass by epidermal proliferation and the neutrophil infiltrates, which remove fungi from the skin (Calderon and Hay, 1987; Cambier et al., 2014; Heddergott et al., 2012).

Cell-mediated immunity is necessary for clinical recovery and protection against infection with dermatophytes (Calderon and Hay, 1984; Zahur et al., 2014). Nevertheless, the specific response mounted against these fungi has not been previously characterized *in vivo*. Although some publications have recently suggested a role of IL-17 cytokines in host protection during dermatophytosis (Alves de Medeiros et al., 2016; Cambier et al., 2014; Lanternier et al., 2013; Nielsen et al., 2015; Sakuragi et al., 2016) this branch of cell-mediated immunity has not been directly investigated in a biologically relevant system. Here, we demonstrated that Th17 response is the adaptive immunity that predominates during *M. canis* infection, as IL-17A/F was mainly produced by heat-killed *M. canis* (HKMc)-stimulated cells from skin draining lymph nodes (sdLN) after 8 and 18 days p.i. (**Figure 1g**) without significant changes in IFN-γ, IL-10, TNF, IL-4, TGF-β or IL-6 production during infection (data not shown).

Additionally, we detected a greater frequency of IL-17A-producing antigen-specific $CD4^+$ T cells than $CD8^+$ T cells from sdLN (**Figure 1h**).

To address the role of IL-17 cytokines in the antifungal response, *M. canis* infection course was evaluated in IL-17RA (IL-17RA KO) deficient mice, which lack signaling for most members of the IL-17 family (Patel and Kuchroo, 2015). In contrast to WT (**Figures 1a**, **Figure 2a**), *M. canis*-infected KO mice developed wet and ulcerated skin lesions with greasy yellowish scales (**Figure 2a**) and abundant hyphae on the *stratum corneum* (scutulum), resembling inflammatory human dermatophytosis and experimental *favus* in susceptible mice (Hay, 2017; Hay et al., 1988). **Figures 2d and e** show that, while *M. canis* CFU counts were similar between WT and IL-17RA KO mice by day 4 p.i., a remarkable increase in fungal burden was detected in KO mice by day 8 pi (1,130±411 *vs.* 46,482±15,923 CFU/g skin, $p<0.01$, WT *vs*. KO). Accordingly, 8-day-ergosterol quantification (Arias et al., 2016; Young, 1995) of total skin homogenates also demonstrated higher fungal load in KO respect to WT mice (**Suppl. Figure S1e**). Furthermore, after 18 days p.i., and in contrast to WT, IL-17RA KO animals failed to completely heal and still produced CFU from skin cultures (**Figure 2**). However, IL-17-deficient mice eventually overcame infection and recovered hair growth by day 25 p.i. (data not shown) with no detection of fungus. Also, *M. canis* was unable to invade the dermis or disseminate to other organs in both strains of mice (data not shown). Taken together, these data demonstrate that IL-17 cytokines effectively inhibit skin dermatophyte proliferation but are not relevant in the control of fungal dissemination to deeper tissues.

In skin and mucosal defense, the IL-17 family cytokines are essential for promoting neutrophil mobilization at the site of infection, maintaining epithelia integrity and regulating antimicrobial peptide production (Conti and Gaffen, 2015). Our data show

that the increased superficial fungal burden at 8-day-infected IL-17RA KO mice correlated with an intense inflammatory response, characterized by epidermal and dermal micro-abscess mainly constituted by neutrophils (**Figure 2b** and **c**, **Figure 4d**, **Suppl. Table** and **Suppl. Figure S1b**). Infected KO mice also had a significant increase in the absolute number of sdLN cells, compared with infected WT mice (**Suppl. Figure S1d**). In addition, after 8 days p.i., IL-17RA KO mice did not seem to exhibit the epidermal thickening shown by WT (**Figure 2b** and **c**, **Figure 4d** and **Suppl. Table**), suggesting defects in epidermal proliferation that might be associated with the lack of IL-17 signaling (Pappu et al., 2012). No clinical or histological differences were observed between uninfected WT and KO mice (**Suppl. Figure S1c**).

Flow cytometry analysis from epidermis confirmed that, despite the lack of IL-17 signaling, IL-17RA KO mice experienced a significant increase in the neutrophil ($CD11b^{+}Ly6G^{+}$) percentage by day 8 p.i., compared to infected WT ($p<0.0001$) (**Figure 3a**). Moreover, neutrophils from IL-17-deficient mice efficiently surrounded *M. canis* hyphae in the tissue (**Figure 3b**) and produced levels of reactive oxygen species (ROS) similar to infected WT mice **(Figure 3d)**. In contrast, no differences in neutrophil infiltrates were detected at day 4 p.i (**data not shown** and **Suppl. Table**) while fungal burden was similar in both mice strains (**Figure 2d** and **e**). These data demonstrate that neutrophil migration and activity in epidermis are independent of IL-17 signaling during *M. canis* infection**.** In line with our data, Trautwein-Weidner K. *et al*. (Trautwein-Weidner et al., 2015) demonstrated that neutrophil recruitment to the mucosa was uncoupled from the IL-17 pathway during a murine model of oral candidiasis and observed that IL-17 mediates host protection through antimicrobial peptides production. In this regard, skin expression of these molecules has been associated with defense mechanisms in human dermatophytosis (Brasch et al., 2014; Jensen et al., 2007; Sawada

et al., 2012). Thus, current experiments are underway to examine the involvement of antimicrobial peptides in IL-17-mediated protection during *M. canis* dermatophytosis.

In the absence of IL-17 cytokines, deficiencies in epidermal barrier functions (Pappu et al., 2012) might facilitate fungal persistence in the cornified layer and, therefore, the establishment of an exacerbated inflammation. Furthermore, exaggerated skin inflammation and increased neutrophil influx have also been observed in IL-17RA KO mice with imiquimod-induced psoriasis (El Malki et al., 2013). In this sense, it has been also demonstrated that IL-17A directly inhibits IFN-γ production by suppressing the expression of Th1 'master regulator', T-bet. Thus, IL-17 deficient mice developed a severe host-*vs*-graft disease in a skin transplantation model (Vokaer et al., 2013) or an aggressive IFN-γ-mediated inflammatory disease in experimental autoimmune colitis (O'Connor et al., 2009). In agreement with these data, here we demonstrated that *M. canis*-infected IL-17RA KO mice experienced a shift to an antigen-specific Th1 response (**Figure 3d**). Although both infected mice strains produced IL-17A/F by HKMc-stimulated sdLN cells at day 8 p.i., IL-17RA KO mice also showed an increased IFN-γ production (**Figure 3d** and **f**) with higher frequency of IFN-γ- producing $CD4^+$ and $CD8^+$ T cells than WT mice (**Figure 3e**). As mentioned above, there were no differences in IFN-γ levels produced by sdLN cells from sham or infected WT mice at day 8 p.i. (**Figure 3f**) or by cells from WT and KO mice at day 4 p.i. (**Figure 3d**). These results demonstrate that *M. canis* dermatophytosis promotes an IFN-γ-mediated immune response in the absence of IL-17RA signaling.

To further investigate the IFN-γ contribution to skin inflammation and fungal load, *M. canis*-infected WT and IL-17RA KO mice were treated with an IFN-γ blocking antibody at days 3 and 6 p.i. **Figure 4** depicts that, in coincidence with exacerbated skin inflammation and increased neutrophil recruitment (**Figure 4a**, **Figure 2b** and **c**, **Figure**

3a), 8-day-infected IL-17RA KO mice also displayed higher cytokine production by epidermal cells than WT mice, with a significant increase in TNF, IL-6 and IL-1β production (**Figure 4b** and **e**). Differences in epidermal cytokines between WT and KO mice were not observed at day 4 p.i. (data not shown).

After treatment with anti-IFN-γ**,** 8 day-infected IL-17RA KO animals showed a significantly lower neutrophil ($CD11b^{+}Ly6G^{+}$) percentage and TNF production in epidermis in comparison to infected KO mice treated with isotype control antibody (**Figure 4a** and **b**). Strikingly, anti-IFN-γ-treated IL-17RA KO mice significantly reduced the fungal burden and, accordingly, skin histological features were similar to WT mice, after 8 days p.i. (**Figure 4c** and **d**). Additionally, these mice produced increased levels of IL-23, IL-1β, IL-6 and IL-22 by epidermal cells, all IL-17 pathway-related cytokines (**Figure 4e**). These data open the possibility that IFN-γ deregulation, in absence of IL-17 signaling during *M. canis* infection, might contribute to the superficial fungal overgrowth. Nevertheless, the role of IFN-γ in this setting remains unclear and further studies are necessary to understand a potential counter-regulation of IFN-γ- *versus* IL-17-mediated immunity in skin defense against dermatophytes.

Considering all our findings, we have demonstrated a dual function of IL-17 cytokines during *M. canis* skin infection by inhibiting superficial dermatophyte growth and suppressing Th1 antifungal response. Eventually, in the absence of IL-17RA signaling, mice were able to control dermatophytosis but at the expense of higher inflammation and tissue damage. Furthermore, we demonstrated that the biological effects observed during *M. canis* infection in IL-17RA KO mice were also reproduced in IL-17A/F double knock-out mice (Haas et al., 2012), specifically revealing the role of IL-17A/IL-17F in the anti-dermatophytic response (**Figure 5**). Our experimental data coincides with evidence in humans showing that deficiencies in IL-17 signaling predispose to a

widespread superficial infection, as seen in patients with inborn errors of IL-17 immunity, rather than deep mycosis (Nielsen et al., 2015). In contrast, deep dermatophytosis is associated with CARD9 deficiency, which controls multiple molecular pathways of fungal recognition in different cell types (Lanternier et al., 2013). According to this, we have revealed the existence of redundant host defense mechanisms that maintain the skin barrier function against dermatophyte infection.

The role of skin dendritic cells during dermatophyte infections is unknown. Langerhans cells, as well as a minor population of dermal dendritic cells, express the C- type lectin receptor langerin (CD207) (Malissen et al., 2014). Therefore, to further investigate the *in vivo* function of these cells in the Th17 response against *M. canis*, we infected transgenic Lang-EGFPDTR mice after depletion of langerin-expressing dendritic cells (LangDC) with diphtheria toxin (DT) (Bobr et al., 2010; Kissenpfennig et al., 2005; Stoecklinger et al., 2011). *M. canis*-infected mice depleted of LangDC (Lang-EGFPDTR mice + DT, **Suppl. Figure S2b** and **c**) showed a significant reduction in the frequency of IL-17-expressing $CD3^+$ and $CD3^+CD4^+$ T cells from HKMc-stimulated sdLN, respect to LangDC-competent infected animals ($p<0.05$) (**Figure 6a** and **b**)**.** No differences were observed in the frequency of IL-17-producing $CD4^+$ T cells between DT-injected WT and Lang-EGFPDTR mice or in IL-17-producing $CD8^+$ T cells percentage from the three groups studied (**Figure 6c**). However, although skin langerin-expressing dendritic cells had a significant effect on Th17 development in this model, LangDC-depleted mice did not display an increased fungal load such as that observed in IL-17 deficient mice (data not shown). These results are similar to other findings published by Igyártó *et al*. (Igyarto et al., 2011) showing that LC induced Th17 differentiation during experimental cutaneous candidiasis, but LC depletion did not promote an elevated fungal colonization. Therefore, other skin cell subsets may be

directly involved in fungal recognition and might be able to produce IL-17 cytokines (Pappu et al., 2012). Further investigations are necessary to define the cellular types involved in the IL-17-driven defense against *M. canis*.

In conclusion, in this study we have demonstrated that superficial infection with *M. canis* generates a specific IL-17-mediated adaptive response promoted by skin langerin-expressing dendritic cells and revealed a main role of IL-17 signaling in the control of dermatophytosis.

MATERIALS & METHODS

Mice

C57BL/6 wild type (WT) mice were obtained from Universidad Nacional de La Plata, Argentina. IL-17RA KO mice were kindly provided by Amgen Inc. (MTA N° 2015647778), IL-17AF double knock-out mice (Il17a/Il17f<tm1.1Impr) by Dr. Immo Prinz (Hannover Medical School, Germany) and Lang-EGFPDTR transgenic mice by Dr. Bernard Malissen (INSERM, France). Mice were housed in the Animal Facility of CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba under International Guides. All experimental protocols were approved by our Institution´s Ethics Committee (Res. Dec. N° 908/15).

Fungal strain and *M. canis* inoculum

Microsporum canis (clinical strain UNCMc01, UNC, Argentina) was grown for 15 days on Sabouraud agar. Mycelium was scraped, resuspended in pyrogen-free saline solution and filtered with stainless-steel mesh. Concentration was determined by optical density (OD) at 450 nm: OD 0.08 produced $4.28 \pm 0.02 \times 10^3$ colony forming units/ml (CFU/ml)

and OD 0.2 correlated to $10\pm1x10^3$ CFU/ml. Heat-killed *M. canis* (HKMc, OD 0.08) was obtained after 1 h-incubation at 60 ºC.

M. canis epicutaneous infection model

Eight to ten-week-old male WT or IL-17-deficient mice were anesthetized (ketamine/xylazine, 100/10 µg/kg body weight), back hair was shaved and chemically depilated and *stratum corneum* was reduced by slight abrasion with a sponge. Previous 70% ethanol-disinfection, *M. canis* suspension (200 µl, OD 0.2) or sterile saline solution was applied along with a sterile patch (Tegaderm®, 3M) (Toke et al., 2014). Infection was regularly monitored and reproducible in all mice. Mycological diagnosis was based on clinical criteria, skin direct examination (KOH 40%) and fungal isolation in Sabouraud agar at different days post-infection.

Epidermal cell cultures

Mice back skin (2 x 3 cm) was incubated dermal side down on trypsin/EDTA solution 0.2%/0.075% (Sigma-Aldrich) in RPMI (2 h, 37°C) and filtered through stainless-steel mesh and 100 µm-cell strainer. Single cell suspensions ($1x10^6$ cells/well) were labeled with anti-CD11b, anti-Ly6G or anti-Gr-1 antibodies and neutrophil population ($CD11b^+$ $Ly6G^+$ or $CD11b^+$ $Gr\text{-}1^+$) was analyzed by flow cytometry. For reactive oxygen species (ROS) measurement, stained epidermal cells were incubated with 2′-7′-dichlorofluorescein diacetate 1mM (DCFH-DA, SIGMA-Aldrich) (Fujita et al., 2014) for 30 minutes. Cytokine production was measured in supernatant by ELISA after 5 h-culture with PMA stimulation (1 µg/ml).

Fungal burden

Skin sections were obtained, weighed and epidermal cell suspension protocol was followed as described above. For colony forming units (CFU) quantification, 100µl of epidermal cell suspension of each animal were cultured in Sabouraud agar plates. CFU values were determined at 10 days, referred to skin weight and expressed as CFU number/g skin.

Skin-draining lymph nodes cell cultures

Single cell suspensions ($3x10^5$ cells/well, 200µl) of inguinal skin draining lymph nodes (sdLN cells), recovered from each animal at day 4, 8, 18 or 45 p.i., were cultured for 48h in cRPMI alone or with HKMc (10µl/well). Cytokine production was determined in supernatants by ELISA. For intracellular cytokine detection by flow cytometry, sdLN cells were re-stimulated with PMA/Ionomycin/GolgiPlug™ in the last 5h of culture and labeled with anti-CD3, -CD4 or -CD8, anti-IL-17A, -IL-17F or anti-IFN-γ antibodies.

***In vivo* treatment with neutralizing IFN-γ antibody**

WT or IL-17RA KO mice were intraperitoneally injected with anti-IFN-γ monoclonal antibody (R4-6A2, Thermo Fisher, USA) or isotype control at day 3 and 6 p.i. (100 µg per animal). At day 8 p.i, neutrophil ($CD11b^+$ $Ly6G^+$) population and cytokine production were determined in epidermal cell suspensions ($1x10^6$ cells) by flow cytometry or ELISA**.** Epidermal cells were also used for fungal burden determination through CFU quantification. Skin histopathology was analyzed with Periodic Acid Schiff (PAS)/hematoxylin stain.

Langerin-expressing cell depletion in Lang-EGFPDTR mice

Lang-EGFPDTR transgenic mice were intraperitoneally injected with 1 μg of diphtheria toxin (DT, Merck, Germany) 24h prior to infection (Bobr et al., 2010; Kissenpfennig et al., 2005). Depletion was confirmed by flow cytometry of MHC-II$^+$ CD45$^+$ epidermal cells (LC) from back skin after 8 days p.i. (**Suppl. Figure S2a**, **b** and **c**) and fluorescence microscopy of transversal ear skin cryo-sections (**Suppl. Figure S2d**). DT-treatment did not affect the course of infection in WT mice (data not shown). At day 8 p.i., skin histopathology was performed and IL-17A secretion was determined by flow cytometry in T cell populations ($1x10^6$ cells) from unstimulated or HKMc-stimulated sdLN cells.

Statistical analysis

All experiments were performed two to three times, six mice per group were used. Statistical differences were calculated with Student T test or ANOVA. A p value <0.05 was significant and results were shown as mean ± SEM.

CONFLICTS OF INTEREST

The authors state no conflict of interest.

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FIGURE LEGENDS

Figure 1: Clinical outcome of *M. canis* dermatophytosis in C57BL/6 mice.

(a) Skin lesions after *M. canis* infection or saline treatment (sham)**,** dpi: days post-infection **(b)** Skin histopathology (PAS/H). **(c)** Higher magnification of **b** showing hyphae (arrows) and neutrophil infiltrates (asterisk) **(d)** KOH examination of hair showing arthroconidia (arrows) (8dpi). Scale bar (a-d): 50 µm **(e)** Fungal burden (CFU/g skin). Mean ± SEM (n=6) **(f)** Flow cytometry analysis of $CD11b^+Gr1^+$ epidermal cells (8 dpi, pre-gates shown in Suppl. Fig. S2a). Three independent experiments using pooled epidermal cells **(g)** IL-17A/F production (ELISA) of sdLN cells ($3x10^5$/200µl) from sham (□) or infected mice (■) in absence (saline) or with HKMc (10 µl, OD 0.08). Each symbol represents one animal **(h)** IL-17A detection in HKMc-stimulated sdLN cells gated on $CD3^+CD4^+/CD8^+$ population. Mean ± SEM (n=6), **p<0.01, ***p<0.001, *p<0.0001.

Figure 2: Comparison of clinical follow-up of *M. canis* dermatophytosis in wild-type (WT) and IL-17RA deficient mice. **(a)** Skin lesions in WT and IL-17RAKO back skin after 4, 8 and 18 d.p.i **(b)** Skin sections from *M. canis*-infected WT and IL-17RA KO mice (PAS/H) **(c)** Higher magnification of **b** showing hyphae inside hair follicles and *stratum corneum* (arrows), neutrophil infiltrates and micro-abscesses (asterisks). Scale bars (b and c): 50 µm **(d)** Fungal burden (CFU/g skin) of WT and IL-17RA KO mice during infection. Data shown are mean ± SEM (n=6). Two independent experiments. ** $p < 0.01$ **(e)** Representative images of CFU in Sabouraud agar plates.

Figure 3: *M. canis*-infected IL-17RA-deficient mice show increased neutrophil recruitment to skin and IFN-γ production by skin draining lymph node cells.

(a) $CD11b^+Ly6G^+$ percentage in epidermal cells from sham or 8-day-infected mice. Mean ± SEM (n=6), ***p<0.0001 **(b)** Skin section from IL-17RA KO mice (PAS/H, 8 dpi) revealing neutrophils (asterisks) surrounding hyphae (arrows). Scale bar: 10µm. **(c)** Histograms of ROS production by epidermal $CD11b^+Ly6G^+$ cells (8 dpi) after dichlorofluorescein diacetate (DCF-DA) labelling (flow cytometry). Triplicates of pooled epidermal cells (n=6) **(d-e)** HKMc-stimulated sdLN cells from infected WT (■) and IL-17RA KO mice (♦): **(d)** IL-17A/F and IFN-γ production (ELISA). Mean ± SEM (n=5). **p< 0.01; **(e)** Intracellular IFN-γ detection gated on $CD3^+CD4^+/CD8^+$ population (flow cytometry, 8 dpi). Mean±SEM. **p<0.01, ***p<0.0004. Triplicates of pooled sdLN cell cultures (n=5). **(f)** Cytokine production (ELISA) by HKMc-stimulated sdLN cells from sham or infected WT and IL-17RA KO mice. **p<0.005, ***p<0.0001.

Figure 4: IFN-γ modulates neutrophil recruitment, cytokine production and fungal growth in *M. canis*-infected IL-17RA KO mice skin.

(a) Neutrophil percentages determined as $CD11b^+Ly6G^+$ cells by flow cytometry **(b)** TNF production (ELISA) and **(c)** fungal burden (CFU/g skin) in epidermal cells from WT and IL-17RA KO mice treated with anti-IFN-γ or isotype control, at 8 days p.i. Mean ± SEM of pooled epidermal cells (n=6). Two independent experiments (*p<0.01, **p<0.001, ***p<0.0001). **(d)** Skin sections with PAS/H stain showing hyphae (arrows) and neutrophil micro-abscesses (asterisks), scale bar: 50 µm. **(e)** Cytokine production by PMA-stimulated epidermal cells (ELISA) from infected WT and infected IL-17RA KO mice treated with anti-IFN-γ or isotype control (8 d.p.i). Mean ± SEM of pooled

epidermal cells (n=6). Two independent experiments. IL-6 (*p<0.02), IL-1β (*p<0.1, **p<0.001), IL-22 (*p<0.05, **p<0.005), IL-23 (**p<0.001).

Figure 5: IL-17A/IL-17F inhibit dermatophyte superficial infection and cutaneous inflammation and induce *M. canis*-specific Th1 response. (a) Clinical features of skin lesions and **(b)** skin sections with PAS-hematoxylin stain showing hyphae (arrows) and neutrophil infiltrates (asterisks) from *M. canis*-infected WT and IL-17A/F KO mice after 8 days p.i. Scale bars: 100µm (left) and 50µm (right) **(c)** Comparison of IL-17AF and IFN-γ production (ELISA) by HKMc-stimulated sdLN cells ($3x10^5$/200µl/10µl HKMc) between 8-day infected WT, IL-17RAKO and IL-17A/F KO mice. Each symbol represents one animal. Mean ± SEM (n=5), ***p<0.0001, *p<0.01.

Figure 6. Skin langerin-expressing cells drive the Th17 immune response during *M. canis* infection.

(**a)** Representative density plots of intracellular IL-17A production by HKMc-stimulated sdLN cells gated on $CD3^+$ or **(b)** $CD3^+CD4^+$ population from 8 day-infected WT or Lang-EGFPDTR mice treated with DT (24h before infection, langerin-expressing cells depleted mice). Percentages show mean ± SEM from triplicates of pooled sdLN cell cultures (n=6), **p<0.002. **(c)** Percentage of IL-17 expression in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells from HKMc-stimulated sdLN cells from WT or Lang-EGFPDTR mice treated with DT or vehicle. Data shown are mean ± SEM (n=6). Two independent experiments. *p<0.05.

Fig.1

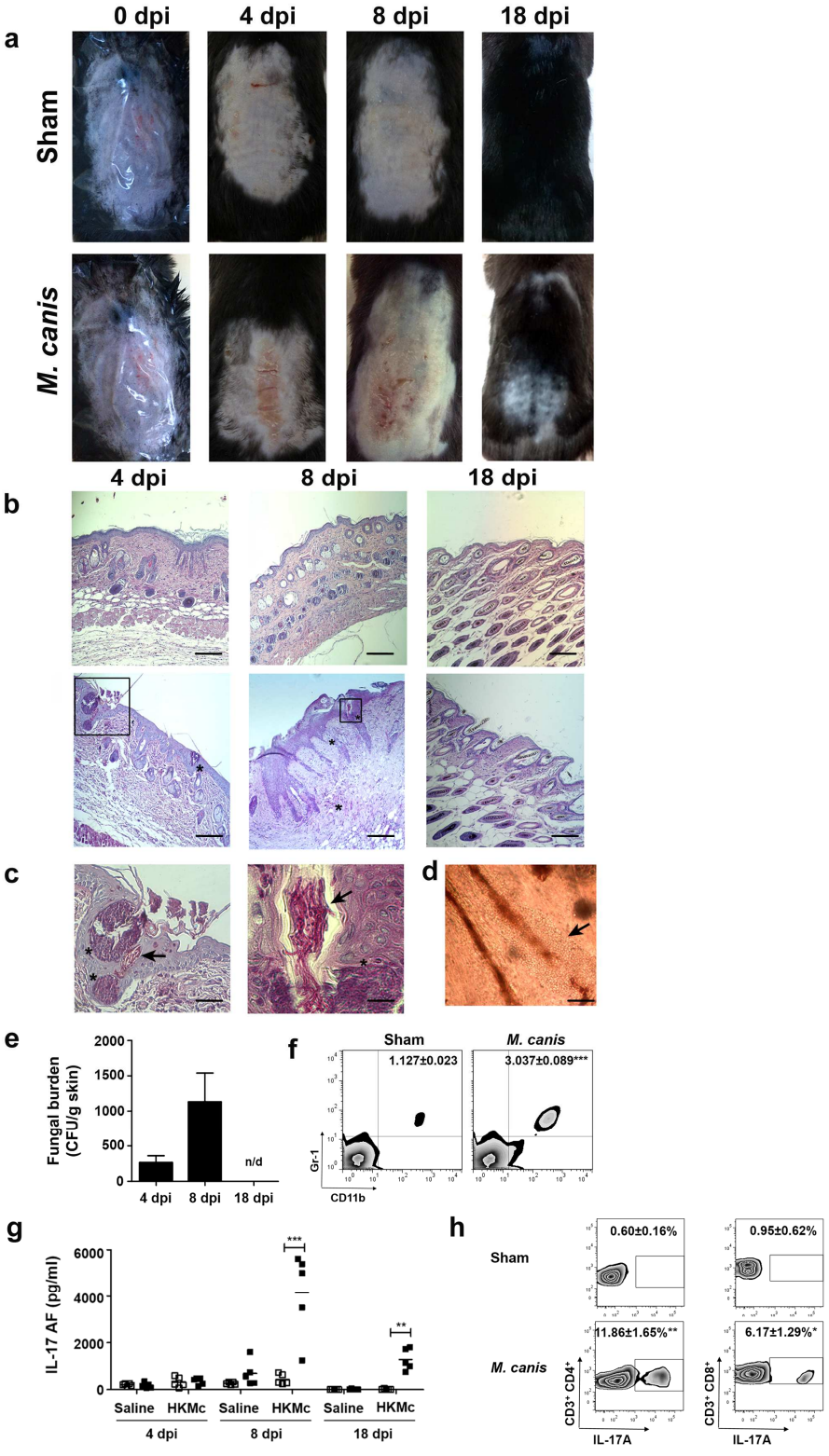

a
0 dpi
4 dpi
8 dpi
18 dpi
Sham
M. canis
b
4 dpi
8 dpi
18 dpi
c
d
e
Fungal burden (CFU/g skin)
2000
1500
1000
500
0
n/d
4 dpi
8 dpi
18 dpi
f
Sham
M. canis
1.127±0.023
3.037±0.089***
Gr-1
CD11b
g
IL-17 AF (pg/ml)
6000
4000
2000
0
***
**
Saline
HKMc
Saline
HKMc
Saline
HKMc
4 dpi
8 dpi
18 dpi
h
Sham
M. canis
0.60±0.16%
0.95±0.62%
11.86±1.65%**
6.17±1.29%*
CD3+ CD4+
CD3+ CD8+
IL-17A
IL-17A

Fig. 2

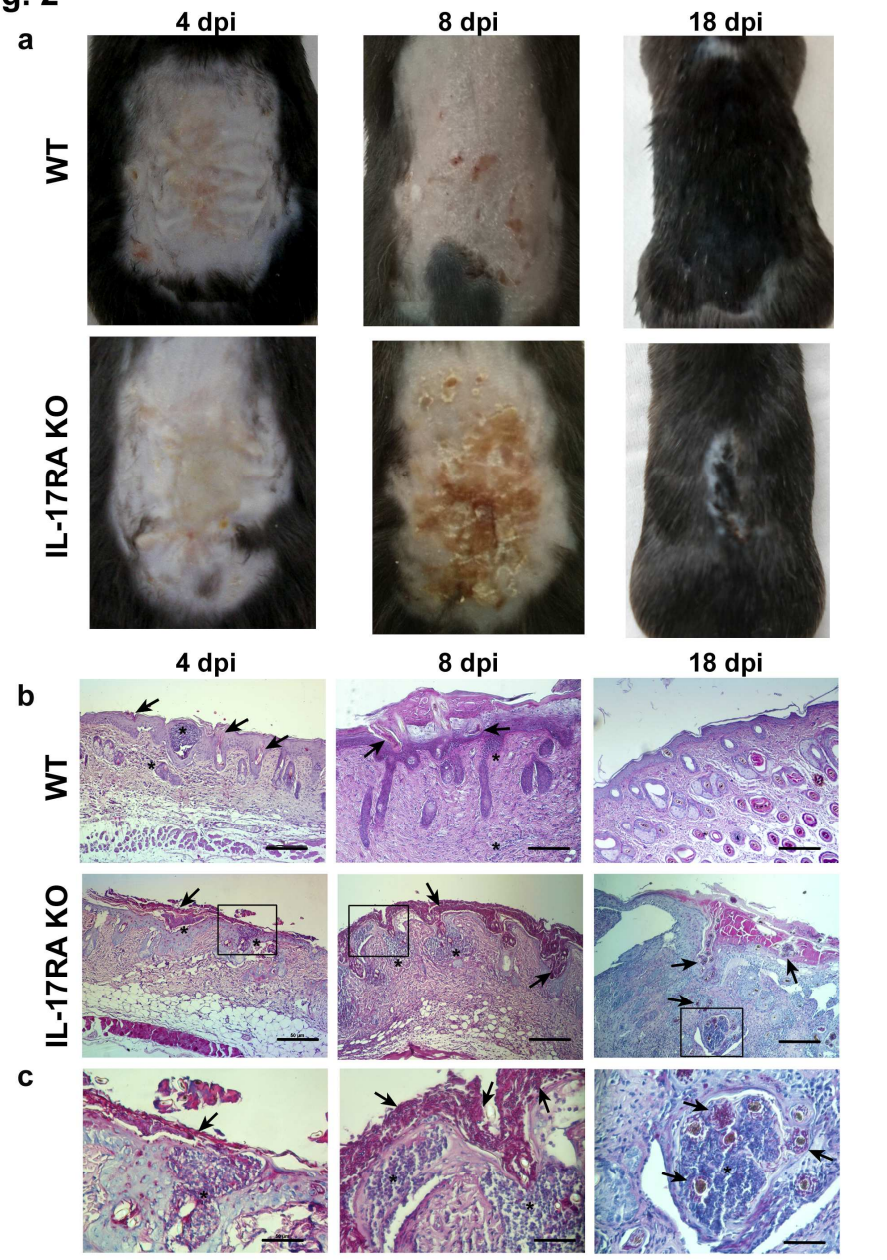

a
4 dpi
8 dpi
18 dpi
WT
IL-17RA KO
b
4 dpi
8 dpi
18 dpi
WT
IL-17RA KO
c
d
Fungal load (CFU/g skin)
8x10⁴
6x10⁴
4x10⁴
4x10³
2x10³
0
**
WT
IL-17RA KO
4 dpi
8 dpi
18 dpi
e
4 dpi
8 dpi
18 dpi
WT
IL-17RA KO

Fig. 3

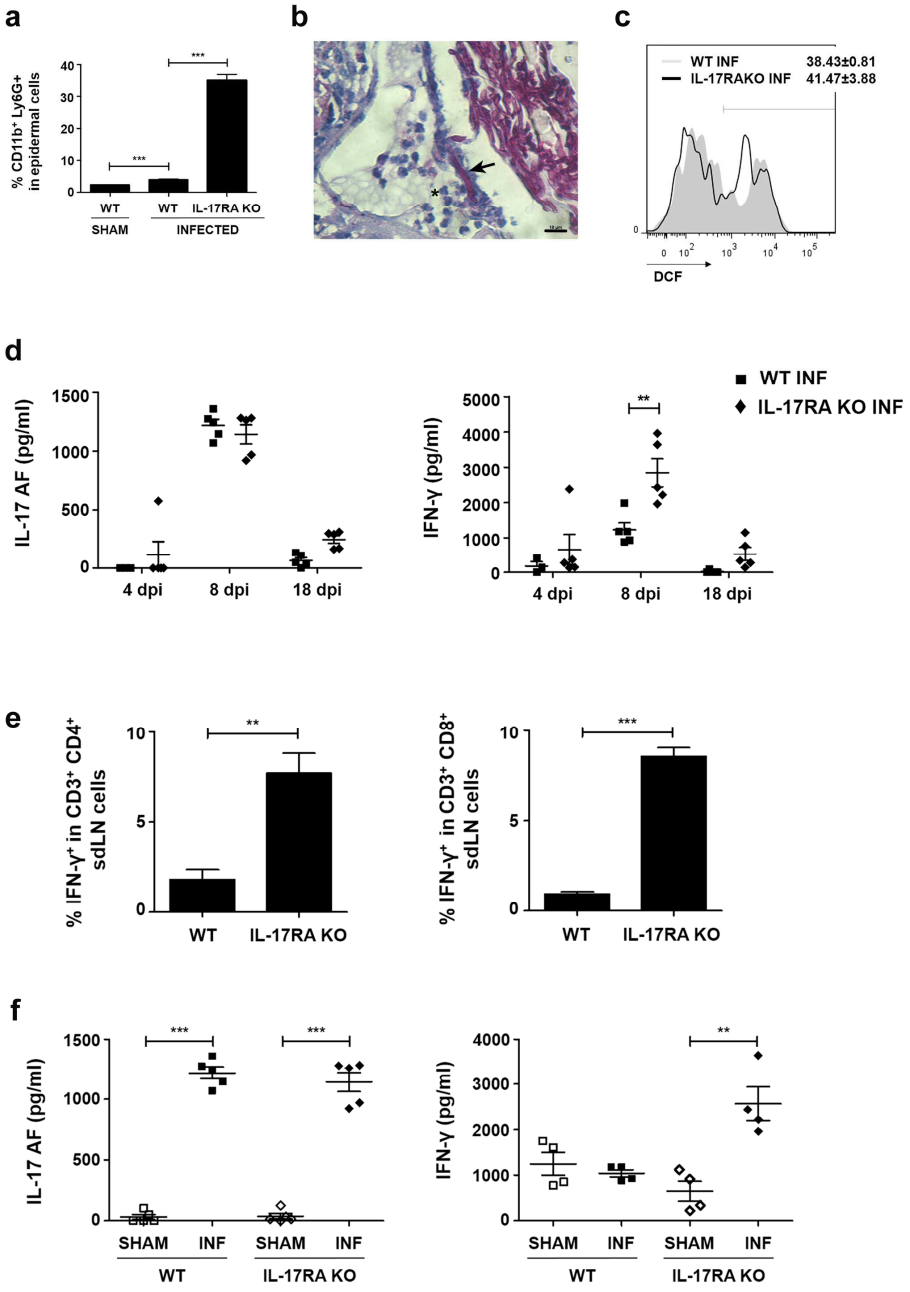

a
% CD11b+ Ly6G+ in epidermal cells
WT
WT
IL-17RA KO
SHAM
INFECTED
b
c
WT INF 38.43±0.81
IL-17RAKO INF 41.47±3.88
DCF
d
IL-17 AF (pg/ml)
4 dpi
8 dpi
18 dpi
IFN-γ (pg/ml)
WT INF
IL-17RA KO INF
e
% IFN-γ+ in CD3+ CD4+ sdLN cells
% IFN-γ+ in CD3+ CD8+ sdLN cells
WT
IL-17RA KO
f
IL-17 AF (pg/ml)
IFN-γ (pg/ml)
SHAM
INF
WT
IL-17RA KO

Fig. 4

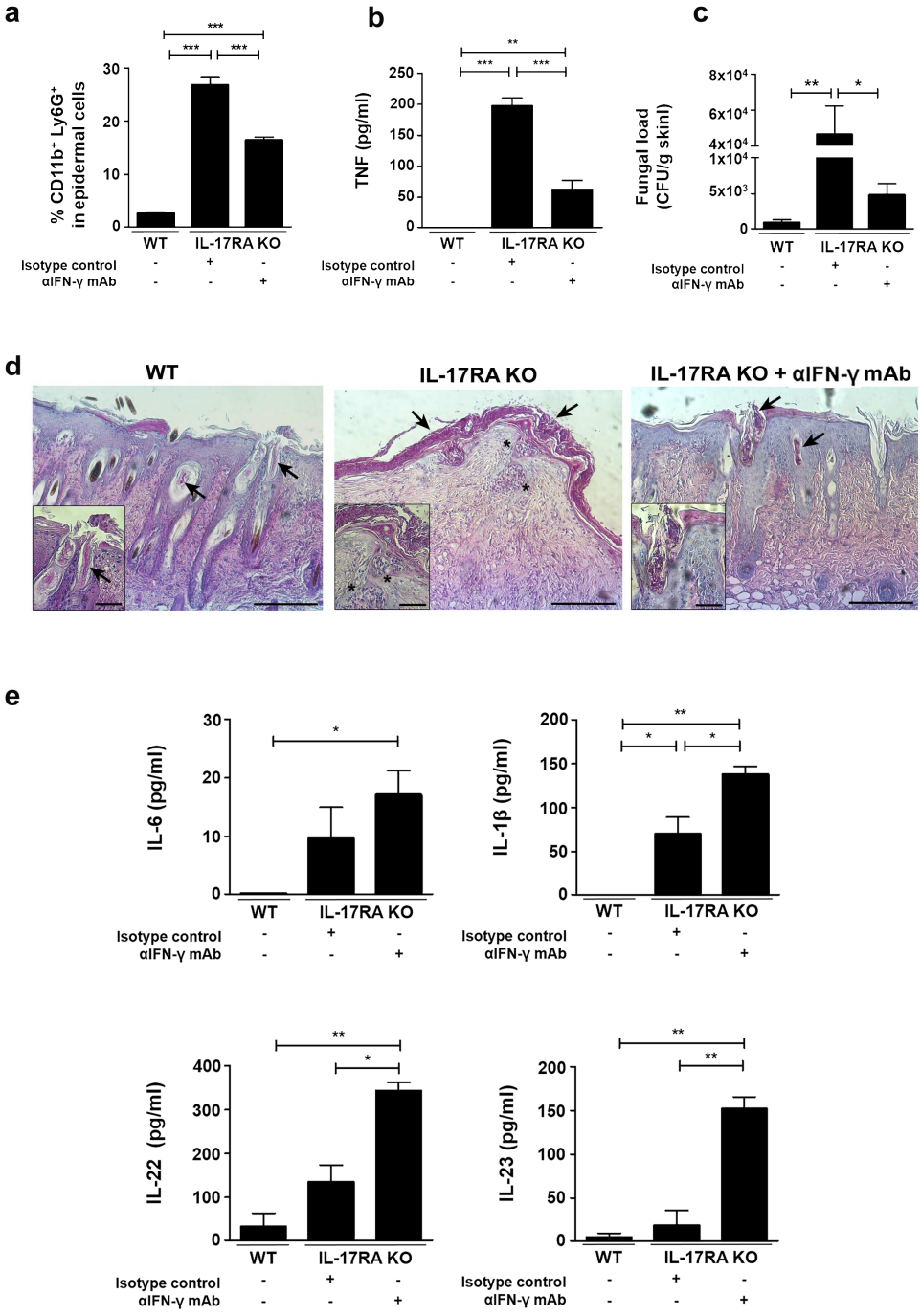

a
% CD11b+ Ly6G+ in epidermal cells
30
20
10
0
***
***
***
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
b
TNF (pg/ml)
250
200
150
100
50
0
**
***
***
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
c
Fungal load (CFU/g skin)
8x10^4
6x10^4
4x10^4
1x10^4
5x10^3
0
**
*
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
d
WT
IL-17RA KO
IL-17RA KO + αIFN-γ mAb
e
IL-6 (pg/ml)
30
20
10
0
*
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
IL-1β (pg/ml)
200
150
100
50
0
**
*
*
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
IL-22 (pg/ml)
400
300
200
100
0
**
*
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +
IL-23 (pg/ml)
200
150
100
50
0
**
**
WT
IL-17RA KO
Isotype control - + -
αIFN-γ mAb - - +

Fig. 5

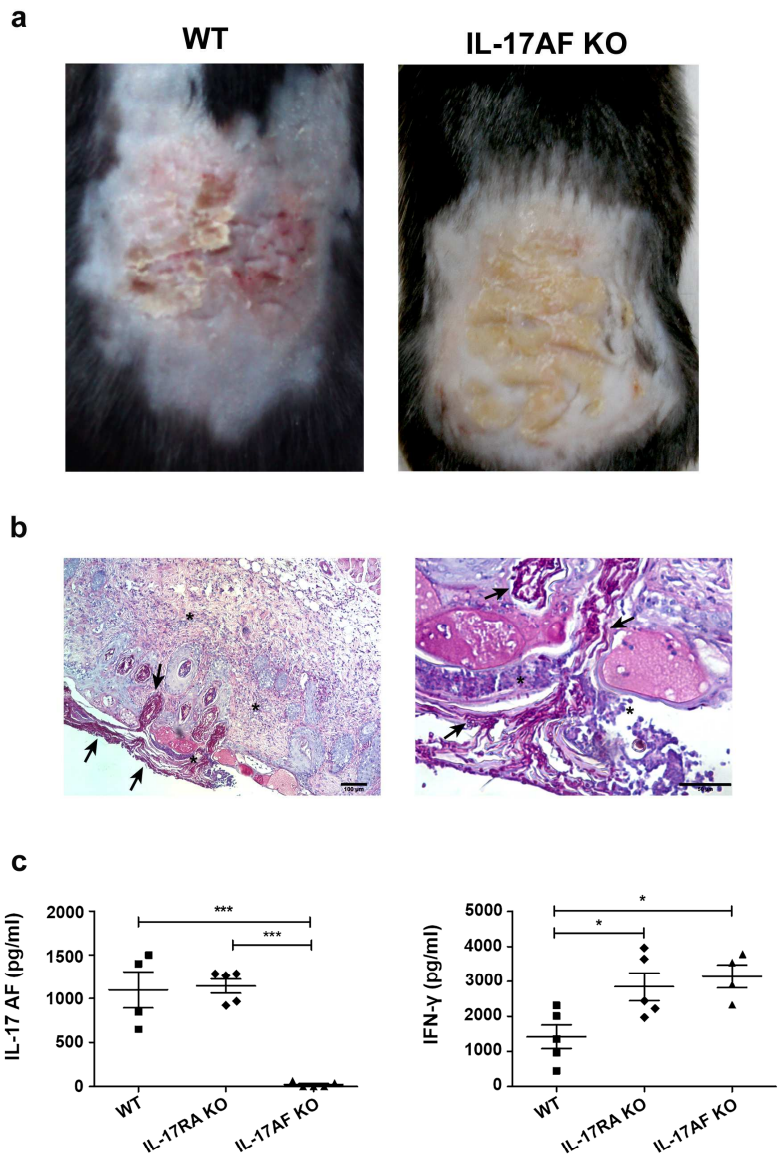
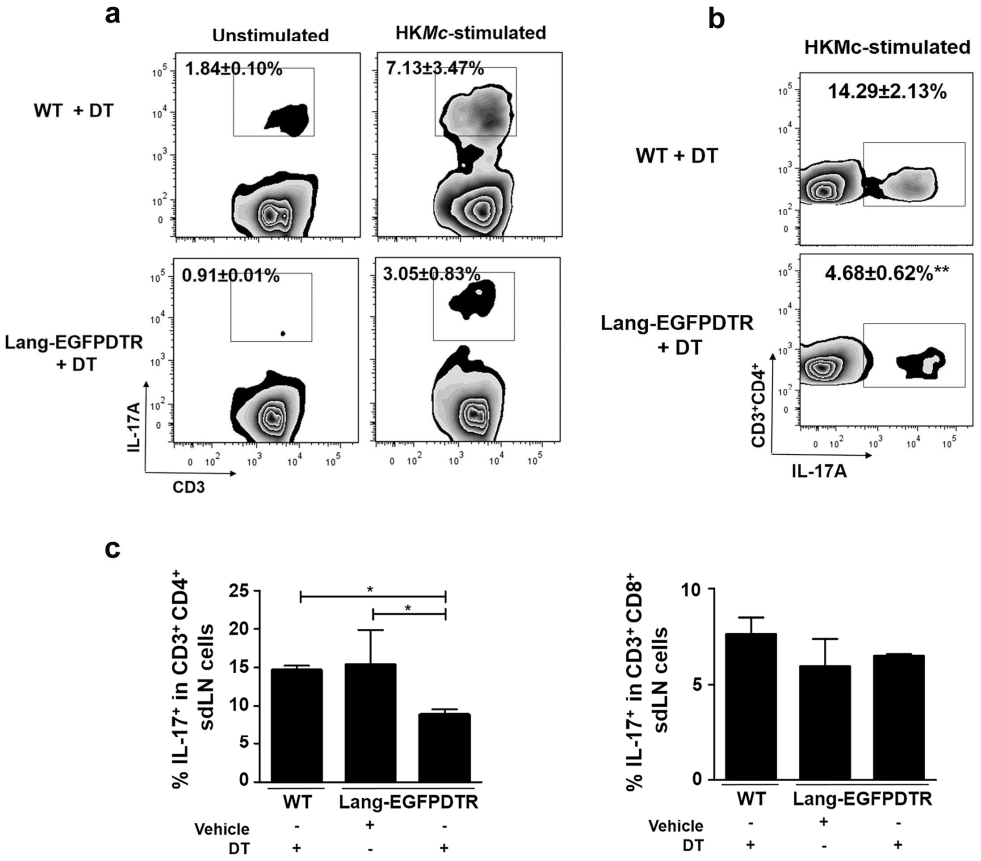

a
WT
IL-17AF KO
b
c
IL-17 AF (pg/ml)
2000
1500
1000
500
0
***
***
WT
IL-17RA KO
IL-17AF KO
IFN-γ (pg/ml)
5000
4000
3000
2000
1000
0
*
*
WT
IL-17RA KO
IL-17AF KO

Fig. 6


a
Unstimulated
HK*Mc*-stimulated
WT + DT
1.84±0.10%
7.13±3.47%
Lang-EGFPDTR + DT
0.91±0.01%
3.05±0.83%
IL-17A
CD3
b
HKMc-stimulated
WT + DT
14.29±2.13%
Lang-EGFPDTR + DT
4.68±0.62%**
CD3+CD4+
IL-17A
c
% IL-17+ in CD3+ CD4+ sdLN cells
25
20
15
10
5
0
*
*
WT
Lang-EGFPDTR
Vehicle - + -
DT + - +
% IL-17+ in CD3+ CD8+ sdLN cells
10
5
0
WT
Lang-EGFPDTR
Vehicle - + -
DT + - +