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IL-17C amplifies epithelial inflammation in human psoriasis and atopic eczema

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Abbreviations:

DAMPs: Damage associated molecular patterns
FLA: Flagellin
IFN: Interferon
IHC: Immunohistochemistry
IL: Interleukin
ISD: Inflammatory skin disease

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JNK: Janus kinase

MAPK: Mitogen activated protein kinase

NF-κB: Nuclear factor kappa B

PAMPs: Pathogen associated molecular patterns

TNF: Tumour-necrosis-factor

Abstract

Background: Key pathogenic events of psoriasis and atopic eczema (AE) are misguided immune reactions of the skin. IL-17C is an epithelial derived cytokine, whose impact on skin inflammation is unclear.

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Objective: We sought to characterize the role of IL-17C in human ISD.

Methods: IL-17C gene and protein expression was assessed by immunohistochemistry and transcriptome analysis. Primary human keratinoytes were stimulated and expression of cytokines chemokines was determined by qRT-PCR and luminex assay. Neutrophil migration towards supernatant of stimulated keratinocytes was assessed. IL-17C was depleted using a new IL-17C specific antibody (MOR106) in murine models of psoriasis (IL-23 injection model) and AE (MC903 model) as well as in human skin biopsies of psoriasis and AE. Effects on cell influx (mouse models) and gene expression (human explant cultures) were determined.

Results: Expression of IL-17C mRNA and protein was elevated in various ISD. We demonstrate that IL-17C potentiates the expression of innate cytokines, antimicrobial peptides (IL-36G, S100A7, HBD2) and chemokines (CXCL8, CXCL10, CCL5, VEGF) and the autocrine induction of IL-17C in keratinocytes. Cell-free supernatant of keratinocytes stimulated with IL-17C was strongly chemotactic for neutrophils, thus demonstrating a critical role for IL-17C in immune cell recruitment. IL-17C depletion significantly reduced cell numbers of T cells, neutrophils and eosinophils in murine models of psoriasis and AE and led to a significant downregulation of inflammatory mediators in human skin biopsies of psoriasis and AE ex vivo.

Conclusion: IL-17C amplifies epithelial inflammation in Th2 and Th17 dominated skin inflammation and represents a promising target for the treatment of ISD.

Introduction

The skin immune system is a finely tuned interplay of different cell types ensuring host defence against pathogenic organisms and homeostasis under physiological conditions. Apart from classical immune cells, keratinocytes are critically involved in initiating and maintaining skin inflammation. Activated by pathogen-associated-molecular-patterns (PAMPs), keratinocytes release cytokines, chemokines and anti-microbial peptides mediating the quick recruitment of innate immune cells followed by later T cell mediated immune responses. Specific Th1, Th2 or Th17 deviated immune responses are critical for the control of viral, parasitic, bacterial or fungal

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infections of the skin. In the context of inflammatory skin diseases (ISD), however, these mechanisms can lead to exaggerated and self-amplifying skin inflammation, barrier defects and systemic inflammation (1, 2).

Interleukin-17C (IL-17C) is a member of the IL-17 cytokine family and - in contrast to IL-17A - mainly produced by epithelial cells. IL-17C binds to the receptor heterodimer IL-17RA/IL-17RE (3, 4) and is thus sharing one receptor subunit with IL-17A. Overexpression of IL-17C in keratinocytes leads to a psoriasis-like inflammation in murine models (5) and knock-down of IL-17C results in a diminished inflammation in experimental autoimmune encephalitis (6). IL-17C is required for sufficient control of intestinal infections (4), but dispensable for the control of candida infections (7). Neutralization of IL-17C reduces ear thickness and expression of inflammatory genes in mouse models of psoriasis and atopic eczema (AE) (8). The role of IL-17C in human ISD and the way how IL-17C influences skin inflammation, however, is still elusive.

Here, we report that enhanced epidermal IL-17C expression can be detected in a broad spectrum of inflammatory and autoimmune skin diseases. TNF-α potentiates the autocrine self-induction of IL-17C, thus building a pro-inflammatory circuit of IL-17C and TNF-α. IL-17C induces the release of various chemokines in keratinocytes and is critically involved in mediating neutrophil migration. Evaluation of immune cell numbers in mouse models of psoriasis and AE treated with an IL-17C neutralizing antibody (MOR106) reveals that IL-17C signalling is crucial for the recruitment of immune cells to inflamed skin. Neutralization of IL-17C in human skin biopsies of psoriasis and AE leads to downregulation of target genes comparable to the effects of the Janus-kinase (JAK) inhibitor tofacitinib. Thus, IL-17C represents an epithelial amplifier of immune reactions and is a potential therapeutic target for the treatment of ISD.

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Material and methods

Study approval

Human skin samples (skin biopsies, primary human keratinocytes) and blood cells (neutrophil granulocytes) were derived from the *Biobank Biederstein*. All patients gave their written informed consent prior to inclusion in the study. The study was approved by the ethics review committee of the Technical University of Munich (EC number 44/16S) and conducted according to ethical principles laid down in the Declaration of Helsinki.

Animal studies were performed according to ethical guidelines applied by the Animal Institutional Care and Use Committee of Galapagos controlled by French Authorities.

Antibodies and cytokines

For cell culture recombinant human TNF-α (R&D, 50 ng/ml), IL-4 (Milteny, 50 ng/ml), FLA (InvivoGen, 100 ng/ml), LPS (InvivoGen, 100 ng/ml), Poly(I-C) (InvivoGen, 20 ng/ml), HSKA (InvivoGen, 10°7/ml), IL-1β (Promokine, 50 ng/ml), IL-22 (R&D, 50 ng/ml), IL-17C (R&D, 100 ng/ml), MOR106 (50 µg/ml) and tofacitinib (Selleckchem, 50 nM) were used.

Isolation of primary human keratinocytes

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Keratinocytes were isolated by suction blister and cultured in keratinocyte growth medium (Lifeline). At 60–80% confluence keratinocytes were cultured in basal medium for 5 hours followed by stimulation for 16 hours (for RNA isolation) or 72 hours (for ELISA).

Isolation of human neutrophil granulocytes

Neutrophils were isolated from peripheral blood of healthy donors using PolymorphPrep kit (Progen) according to the manufacturer's instructions and cultured in RPMI medium containing 5 % human serum. Neutrophils were added to the top of a 5 µm pore polycarbonate membrane (ChemoTx Disposable). Migrated cells were analysed with an LSRFortessa flow cytometer (BD Biosciences) after 2 hours. Migration was performed in duplicates.

Ex-vivo skin biopsy culture

Lesional human skin biopsies of untreated patients with AE or psoriasis were divided in three parts. One part was cultured in pure RPMI medium containing 5 % human serum, one part in medium containing MOR106 (Galapagos/Morphosys, 50 µg/ml), and one in medium containing tofacitinib (Selleckchem 50 nM) for 4 hours at 37 °C and 5 % CO_2. Then, biopsies were directly transferred to RNA later stabilization solution (Thermofisher).

ELISA and chemokine analysis

IL-17C protein in keratinocyte supernatant was determined using an IL-17C specific ELISA generated and validated by Galapagos NV. Protein amount in the supernatant of stimulated keratinocytes was determined using Bio-Plex Pro Human Cytokine and Chemokine Assay (Bio-Rad, Munich, Germany).

Mouse models

BALB/c mice (female, 8-week old) were obtained from Janvier-Labs (France).

The MC903 model of AE was performed as described before (9). In brief, 2 nmol MC903 was applied daily on the ears of BALB/c mice for five consecutive days. 50 mg/kg MOR106 or isotype control antibody (MOR03207) were administered i.p. three days before, just prior to the first MC903 application and four days after the beginning of MC903 application. Mice were sacrificed at day eight.

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The IL-23 psoriasis model was performed as described before (10). Briefly, IL-23 was injected intradermally into the left ear of BALB/c mice for four consecutive days. 10 mg/kg MOR106 or isotype control were administered i.p. three days before and at the beginning of the IL-23 injections. Mice were sacrificed at day five.

Statistics

Two-sample t test was used to test for differences in keratinocyte gene expression experiments. Results of cell influx measurements were compared using one-way Anova followed by Dunnett multiple comparisons *post hoc* test. Gene expression of cultured human skin biopsies were compared to the untreated autologous part of each biopsy. Here, one-sample t test was used to test for differences between groups.

Supplementary material and methods

Information about intracellular pathway analysis, RNA isolation, qRT-PCR, punch biopsy specimen, histology and immunohistochemistry can be found in the supplementary material and methods.

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Results

IL-17C is abundantly expressed in inflamed skin

To assess the impact of IL-17C on ISD, we determined IL-17C gene expression in transcriptome data of non-lesional skin (healthy), acute skin inflammation (acute contact dermatitis, ACD), Th2- (atopic eczema, AE) and Th17-polarized skin inflammation (psoriasis, Pso). Here, we observed an enhanced expression of IL-17C in all inflammatory conditions compared to non-lesional skin (Figure 1A). We observed that IL-17C is induced by TNF-α, IL-1β and flagellin (FLA) via the activation of MAP kinases and NFκB complex (supplementary figure 1 – 4). To further assess the expression of IL-17C related genes, we defined a gene panel consisting of IL-17C upstream signalling pathways and genes, which were described to be induced by IL-17C in keratinocytes (3). We detected a broad upregulation of IL-17C related genes in psoriasis, AE and ACD compared to non-lesional skin. IL-17RE, the specific subunit of the IL-17C receptor, however, was downregulated under inflammatory conditions (Figure 1B). Next, we performed IL-17C immunohistochemistry (IHC) of 18 inflammatory and autoimmune skin diseases. As IL-17C is produced by epithelial cells, we determined epidermal staining intensity using Image J software and colour deconvolution plugin (Figure 1C) (11, 12). Staining specificity was assured by isotype antibody control staining (supplementary figure 5). We detected a high epidermal IL-17C expression in various ISD, while its expression was low in bullous and granulomatous autoimmune diseases as well as in healthy skin (Supplementary figure 6). This observation indicates that IL-17C might be involved in the pathogenesis of ISD.

IL-17C establishes a pro-inflammatory feedback loop with TNF-α via and regulates immune cell recruitment via the induction of chemokines

IL-17C was described to stimulate epithelial cells in an autocrine manner and shares induction pathways with TNF-α (3, 13, 14). We therefore hypothesized that IL-17C might enhance skin inflammation of ISD by activating innate immune responses of keratinocytes. We observed that IL-17C potentiates the autocrine induction of IL-17C mRNA in keratinocytes in synergism with TNF-α (IL-17C: 1.6 ± 1.2 relative expression, IL-17C + TNF-α: 20.1 ± 13, p=0.013), thus building

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an amplifying circuit of IL-17C expression. Furthermore, IL-17C and TNA-α synergistically enhanced the expression of IL-36G, S100A7 and HBD2 (Figure 2A, supplementary figure 7), while it did not act synergistically with IL-6, another early pro-inflammatory cytokine (supplementary figure 8). Stimulation with IL-17C alone led to increasing release of CXCL8, CXCL10, CCL5 and VEGF by keratinocytes within 72 hours (Figure 2B). Cell-free supernatant of keratinocytes stimulated with IL-17C induced neutrophil migration *in vitro* to an extent comparable with TNF-α (IL-17C 155,7 % ± 60.8 % compared to control, IL-17C + TNF-α 160.3 % ± 35.5 % compared to control, $p>0.05$). Depletion of IL-8 via an antibody reduced the effects on neutrophil migration markedly (Figure 2C and D). Thus, IL-17C potentiates TNF-α effects and mediates immune cell recruitment by induction of chemokines in keratinocytes.

Neutralization of IL-17C reduces recruitment of immune cells in a murine model of AE

To assess the impact of IL-17C *in vivo* we tested the effect of IL-17C neutralization in a murine model of AE. Topical application of MC903 (calcipotriol) leads to the development of inflammatory skin lesions in mice, which are paralleled by increased Th2 cytokine levels in the skin and elevated systemic IgE levels (9). MOR106 is a novel, fully-human anti-IL-17C IgG1 antibody, which selectively inhibits the binding of IL-17C to the IL-17RA/IL-17RE receptor heterodimer in humans and mice. Recently, it was shown that MOR106 attenuates skin inflammation in murine models of AE and psoriasis in terms of epidermal thickness and cytokine expression in the skin (8). However, it remained elusive how IL-17C depletion affects immune cell composition in these models. As we observed that IL-17C mediates immune cell migration *in vitro*, we assessed the effect of IL-17C neutralization on the influx of inflammatory cells. MOR106 was administered i.p. three days before, at start and five days after beginning of MC903 application (Figure 3A). MC903 significantly increased the numbers of T cells, eosinophils and neutrophils as assessed by IHC (Figure 3 B – C). Neutralization of IL-17C significantly diminished the amount of all three immune cell subsets, with the most significant effects at T cells (MC903: 364.9 ± 148.5/mm², MC903 + MOR106: 204.8 ± 40.2 cells/mm², $p=0.0107$) and eosinophils (MC903: 169.4 ± 71.1 cells/mm², MC903 + MOR106: 74.7 ± 23.7 cells/mm², $p=0.0031$). Mast cell numbers were not increased by MC903 treatment and did not show significant changes after inhibition of IL-17C signalling. Thus, MOR106 reduces the number of inflammatory cells in a murine model of Th2-polarized inflammation.

Neutralization of IL-17C reduces recruitment of immune cells in a murine model of psoriasis

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As assessed by gene expression and IHC analysis, IL-17C was expressed in various ISD (Figure 1). To test if IL-17C affects immune cell influx in different ISD, we next analysed the effects of MOR106 in a mouse model of psoriasis. Daily injection of IL-23 leads to skin inflammation accompanied by upregulation of Th1- and Th17-related genes (15). IL-23 was injected daily in one ear of the mice on four consecutive days. MOR106 was administered i.p. three days before and at start of IL-23 injections (Figure 4A). Mice treated with IL-23 alone showed significantly increased numbers of T cells, eosinophils and neutrophils in the skin, while the number of mast cells was reduced compared to untreated animals. Again, inhibition of IL-17C signalling led to a significant reduction of T cell (IL-23: 245.6 ± 85.1 cells/mm², IL-23 + MOR106: 169.3 ± 54.6 cells/mm², p=0.0281) and neutrophil (IL-23: 3.2 ± 0.9 % area/mm², IL-23 + MOR106: 1.3 ± 1.1 % area/mm², p=0.0005) influx (Figure 4B – C). Eosinophil and mast cell numbers did not show significant changes compared to isotype control. Hence, MOR106 leads to reduced cellular influx in a murine model of Th17 polarized skin inflammation.

Inhibition of IL-17C signalling reduces expression of target genes in human skin biopsies of AE and psoriasis

To further evaluate the effects of IL-17C in a human setting, we examined the effects of IL-17C neutralization in human skin biopsies of psoriasis and AE. Biopsies were divided into three parts and cultured in pure medium or medium containing MOR106 (50 nM) or the JAK inhibitor tofacitinib (50 µg/ml) for 4 hours. Gene expression was normalized to the untreated autologous part of each biopsy (Figure 5A). We observed downregulation of several marker genes of skin inflammation after IL-17C neutralization, though statistical significance was not always reached (Figure 5 D). Neutralization of IL-17C resulted in remarkable downregulation of HBD2 (MOR106: 0.43 ± 0.14 relative expression, p=0.0009 vs. untreated; tofacitinib: 0.78 ± 0.49, p>0.05 vs. untreated) and IL36G (MOR106 0.62 ± 0.12 relative expression, p=0.0026 vs. untreated; tofacitinib 0.81 ± 0.33, p>0.05e vs. untreated) in psoriasis skin biopsies (Figure 5B) and significant reduction of IL36G (MOR106: 0.69 ± 0.33 relative expression, p=0.022 vs. untreated; tofacitinib 1.07 ± 0.52, p>0.05 vs. untreated) and LCE3A (MOR106: 0.54 ± 0.33 relative expression, p=0.021 vs. untreated; tofacitinib 0.71 ± 0.21, p=0.027 vs. untreated) gene expression in AE skin biopsies (Figure 5C). The effects of IL-17C inhibition were equal and partly superior (e.g. downregulation of HBD2 in psoriasis biopsies) to the effects of tofacitinib (Figure 5 B-C).

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Discussion

In this study, we demonstrate that IL-17C establishes a self-amplifying circuit in synergy with TNF-α, which ultimately leads to secretion of pro-inflammatory cytokines by keratinocytes and recruitment of immune cells to the skin. Of note, IL-17C is not specifically related to Th2 or Th17 immunity, but amplifies keratinocyte derived pro-inflammatory signals in murine and human models of both, psoriasis and AE. Hence, IL-17C is a potential target to treat a broad variety of ISD.

On gene expression level and in IHC, we detected a strong epidermal IL-17C expression in several ISD indicating that IL-17C is not related to a specific kind of inflammation, but broadly induced in an inflammatory micro-milieu. In line with these observation, genes upstream of IL-17C and genes induced by IL-17C were upregulated under different inflammatory skin conditions (psoriasis, ACD, AE). Only IL-17RE, the IL-17C specific receptor subunit, was downregulated compared to non-lesional skin. This might be due to a compensatory negative regulation of the receptor under inflammatory conditions and emphasises the pro-inflammatory potential of IL-17C in the skin.

Barrier organs are critically involved in initiating immune responses, and cytokines produced by epithelial cells are crucial to generate inflammation (16). In this study, we identified IL-17C as an amplifier of keratinocyte mediated inflammation. As IL-17C is produced by epithelial cells of skin, airways and gut (3), the effects of IL-17C described in this study might reflect a conserved mechanism of barrier organs. Upstream inducers of IL-17C expression are FLA, IL-1β and TNF-α. Activation by FLA occurs in the context of bacterial infections. Given that IL-17C signalling was reported to be important for the control of intestinal infections (4), the natural function of IL-

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17C appears to be the control of pathogenic bacteria. However, IL-1β and TNF-α, also inducers of IL-17C, are abundantly expressed in chronic inflammatory diseases, thus explaining how IL-17C is activated in the context of misguided inflammation. We observed that IL-17C blockade significantly reduced immune cell influx in murine models of ISD, pointing towards a central role for mediating skin inflammation *in vivo*. In line with this observation, IL-17C overexpression was shown to result in a psoriasis like inflammation in mice (5, 17) and knock-down of IL-17C attenuates inflammation in a murine model of autoimmune encephalitis (6).

On a mechanistic level, we identified two modes how IL-17C mediates and amplifies skin inflammation. First, IL-17C increases the expression of antimicrobial peptides, alarmins, chemokines and the autocrine induction of IL-17C. Second, IL-17C indirectly triggers the influx of immune cells to the site of inflammation. *In vitro* stimulation of keratinocytes with IL-17C was sufficient to induce neutrophil migration via CXCL8. This observation is in concordance with a report linking IL-17C to the recruitment of neutrophils in lung cancer (18). Of note, IL-17C induced chemokines of Th1 (CXCL10), Th2 (CCL5) and Th17 immunity (CXCL8), thus pointing out that IL-17C is not exclusively related to one specific type of T cell mediated inflammation. Recently it was shown that depletion of IL-17C signalling decreases inflammation in murine models of ISD (8). Based on the mechanistic data obtained in our cell culture experiments, we could prove the pivotal role of IL-17C for immune cell recruitment in these murine models as we demonstrate that blockade of IL-17C resulted in significantly reduced T cell, neutrophil and eosinophil numbers. Though it was shown that IL-17C does not have a direct activating effect on lymphocytes (3), the disruption of the pro-inflammatory IL-17C/TNF-α circuit and the decrease of chemokines are potential mechanisms how blockade of IL-17C signalling affects recruitment of several immune cells. Besides chemokines, IL-17C also induced the release of VEGF. This might represent a potential link between epithelial inflammation and hyper-vascularization, which can be observed in several chronic inflammatory conditions.

As the incidence of ISD rises (19), novel treatment strategies are urgently needed. Using human disease models, we confirmed the high therapeutic potential of targeting IL-17C in AE and psoriasis. By culturing human skin biopsies, we achieved an *in vitro* model of inflammation close to the human *in vivo* situation. Here, we observed that genes which are related to psoriasis or AE (20, 21) were significantly downregulated after neutralization of IL-17C. MOR106 (anti-IL-17C) is currently investigated in a Phase II clinical trial for the treatment of patients with moderate or severe AE (clinicaltrials.gov identifier: NCT03568071).

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In summary, IL-17C is an important mediator of cutaneous inflammation as it amplifies epithelial inflammation and immune cell influx to the skin. Given the significant decrease of immune cell influx in murine models and the downregulation of target genes in human skin biopsies of psoriasis and AE after IL-17C depletion, IL-17C is a promising drug target for the treatment of ISD.

Author contributions: F.L. and M.J. contributed to design the project, performed experiments and wrote the manuscript; V.B., W.K., M.A., M.M., S.M., F.M and L.L. performed experiments, L.K. performed gene expression and statistical analysis, N.M. and F.T. supervised statistical analysis, C.A., C.S., S.E., T.B., N.V. and S.S. supervised experiments and critically reviewed the manuscript, K.E. designed and supervised the project. All authors have read and approved the final version of the manuscript.

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Figure Legends

Figure 1 IL-17C is abundantly expressed in inflamed skin

A IL-17C gene expression in non-lesional skin, acute contact dermatitis (ACD), atopic eczema (AE) and psoriasis (Pso) determined by gene expression microarray. **B** Heatmap of genes related to IL-17C induction or signalling in psoriasis (Pso), atopic eczema (AE), acute contact eczema (ACD) and non-lesional skin. † indicate different probes of IL-17RA. †† indicate different probes of IL-6. ††† indicate different probes of IL8. **C** Examples of epidermal staining intensity in IL-17C IHC of sarcoidosis (grade 0), AE (grade 1) and psoriasis (grade 2). **D** Epidermal 3,3′-Diaminobenzidine (DAB) staining intensity determined by Image J Software in IL-17C IHC of different human skin diseases (n=3/disease). Results are shown as mean and standard error of the mean. Two-sample t test was used to test for differences between groups. * $p < 0.05$; ** $p < 0.01$, ***$p<0.001$, ****$p<0.0001$.

Figure 2 IL-17C opens up a pro-inflammatory feedback loop with TNF-α and induces neutrophil migration via keratinocyte derived chemokines

A Gene expression of keratinocytes stimulated with IL-17C (100 ng/ml), TNF-α (50 ng/ml) or both for 16 hours ($n = 3$). Values are reported as 2^CT compared to untreated controls. **B** Amount of CXCL10, CCL5, CXCL and VEGF in keratinocyte supernatant after stimulation with IL-17C (100 ng/ml) for 24, 28 and 72 hours ($n = 3$). **C** Schematic representation of experimental setup. Keratinocytes were stimulated for 16 hours with IL-17C (100 ng/ml), TNF-α (50 ng/ml) or the combination of both. Then, medium was changed after 5 times washing with PBS. Supernatant was collected after additional 48 hours. Keratinocyte supernatant was used in a neutrophil migration assay compared to supernatant of untreated keratinocytes (72 h). **D** Ratio of migrated neutrophils towards supernatant of IL-17C or TNF-α stimulated keratinocytes compared to control

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supernatant. Supernatant of keratinocytes stimulated with IL-17C alone was depleted for IL-8 using a biotinylated antibody and streptavidin beads. Results are shown as mean and standard error of the mean. Two-sample t test was used to test for differences between groups. * $p < 0.05$; ** $p < 0.01$, ***p<0.001, ****p<0.0001.

Figure 3 Neutralization of IL-17C reduces cell influx in a murine model of atopic eczema
A Schematic representation of MC903 mouse model (n=8). Mice were treated with MC903 for five consecutive days. IL-17C neutralizing antibody (MOR106) or isotype control antibody were administered i.p. three days before, at start and at the end of MC903 application. **B** Representative immunohistochemistry stainings for T cells (CD3), eosinophils (EPX), neutrophils (MPO) and mast cells (MCT). Scale bars indicate 50 µm. **C** Evaluation of T cell, eosinophil and mast cell number per mm^2 and neutrophil area (%) per mm^2. Data are expressed as mean and standard error of the mean and were compared with a one-way Anova followed by Dunnett multiple comparisons *post hoc* test. Significance levels were defined as *p<0.05; **p<0.01; ***p<0.001 *versus* diseased control group.

Figure 4 Neutralization of IL-17C reduces cell influx in a murine model of psoriasis
A Schematic representation of IL-23 injection mouse model (n=10). Mice were injected IL-23 on four consecutive days. IL-17C neutralizing antibody (MOR106) or isotype control antibody were administered i.p. three days before and just prior to the first IL-23 injection. **B** Representative immunohistochemistry stainings for T cells (CD3), eosinophils (EPX), neutrophils (MPO) and mast cells (MCT). Scale bars indicate 50 µm. **C** Evaluation of T cell, eosinophil and mast cell number per mm^2 and neutrophil area (%) per mm^2. Data are expressed as mean and standard error of the mean and were compared with a one-way Anova followed by Dunnett multiple comparisons *post hoc* test. Significance levels were defined as *p<0.05; **p<0.01; ***p<0.001 *versus* diseased control group.

Figure 5 Neutralization of IL-17C downregulates selected genes in human skin biopsies of psoriasis and atopic eczema.
A Schematic representation of human skin biopsy culture. Lesional biopsies were divided into three parts and cultured for four hours with 50 µg/ml IL-17C neutralizing antibody (MOR106), 50 nM tofacitinib or pure medium. Values are reported as 2^CT compared to untreated autologous controls. **B** HBD2, IL-36G and IL-17A gene expression in cultured human skin biopsies of psoriasis (n=5). Significance level was determined versus untreated control. **C** LCE3A, IL36G and

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IL13 gene expression in cultured human skin biopsies of atopic eczema (n=6). Significance level was determined versus untreated control. **D** Heatmap of gene expression in human skin biopsies of psoriasis and atopic eczema after 4 hours culture with MOR106 compared to untreated autologous control. Genes in grey colour were not determined due to limited amounts of RNA. Results are shown as mean and standard error of the mean. One-sample t test was used to test for differences between groups. * $p < 0.05$; ** $p < 0.01$, ***$p<0.001$.

Supplementary Figures

S1 Triggers of IL-17C expression in keratinocytes

S2 IL-17C induction is dependent on NF-kB and MAP kinases

S3 Expression analysis of pathways potentially mediating IL-17C expression in keratinocytes

S4 Analysis of functional activation of Erk1/2, JNK, p38 and NF-κB in keratinocytes

S5 IL-17C immunohistochemistry and isotype control of psoriasis

S6 IL-17C immunohistochemistry and isotype control of healthy skin

S7 Gene expression of HBD2, IL-6 and TNF in keratinocytes

S8 Gene expression of S100A7 and IL36G in keratinocytes

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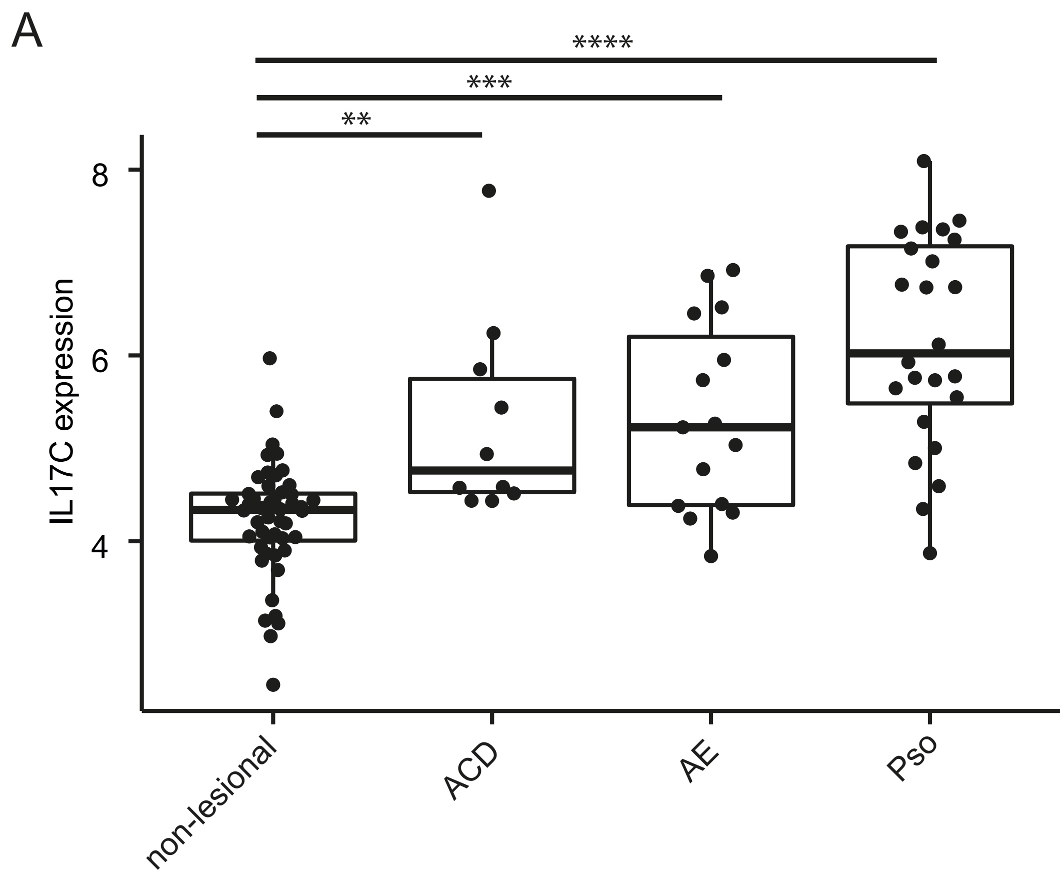
Figure 1

A



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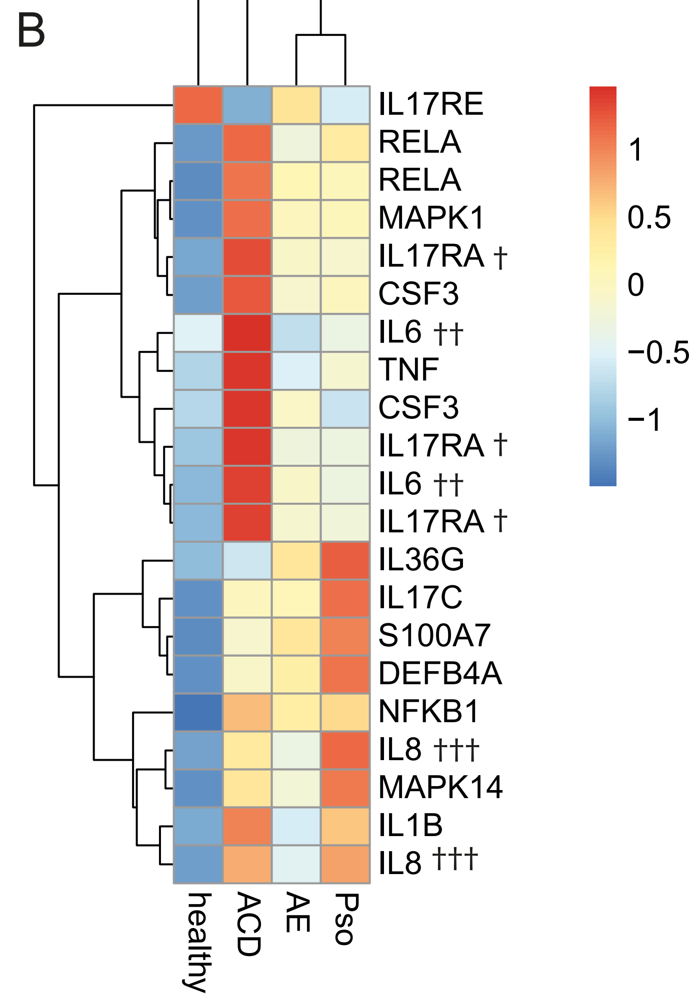

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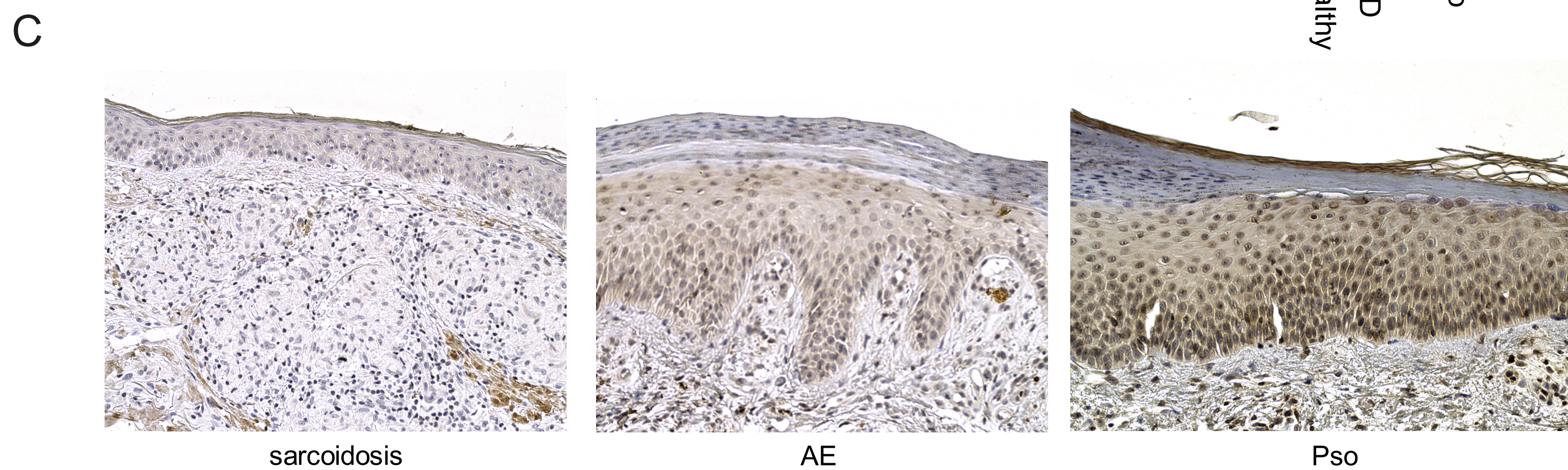

D

Inflammatory

Autoimmune

T-Cell mediated

B-Cell mediated

Granulomatous

Neutrophil-rich

Mean DAB intensity epidermis

150

100

50

Pso

AE

Nummular eczema

Lichen planus

Lupus erythematosus

Alopecia areata

Lichen sclerosus et atrophicus

Morphea

Pemphigus vulgaris

Bullous pemphigoid

Granuloma anulare

Sarcoidosis

Rosacea

Drug eruption

Vasculitis

Pyoderma gangrenosum

Sweet's disease

Figure 2

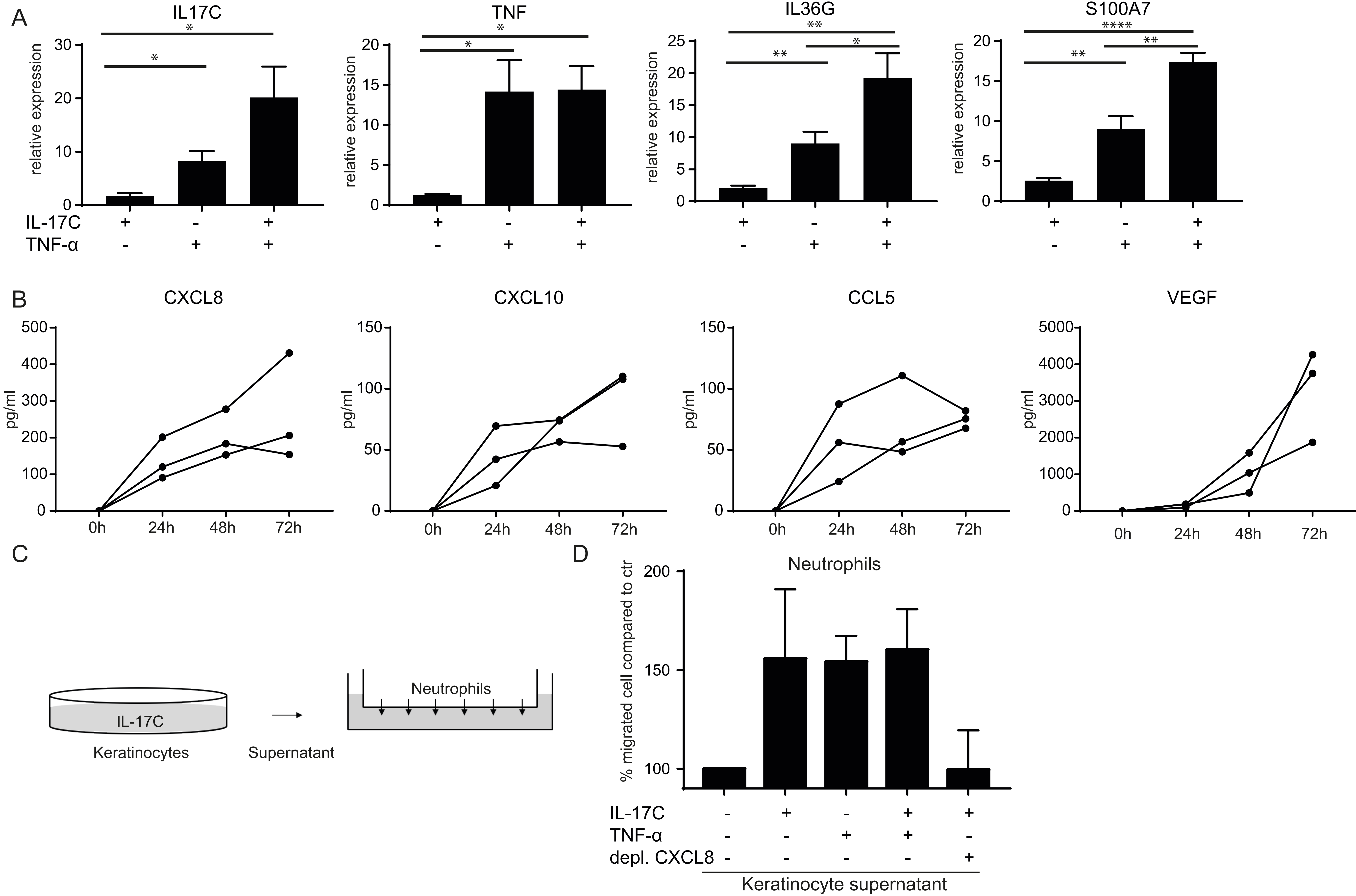

Figure 3

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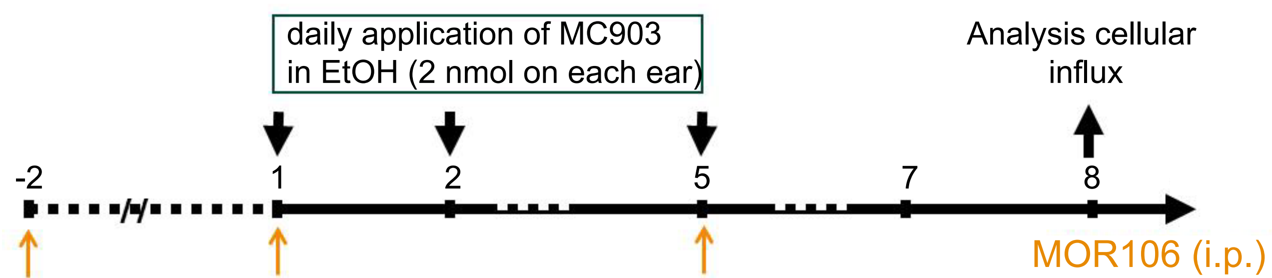


B

EtOH + isotype
MC903 + isotype
MC903 + MOR106

CD3

EPX

MPO

MCT

C

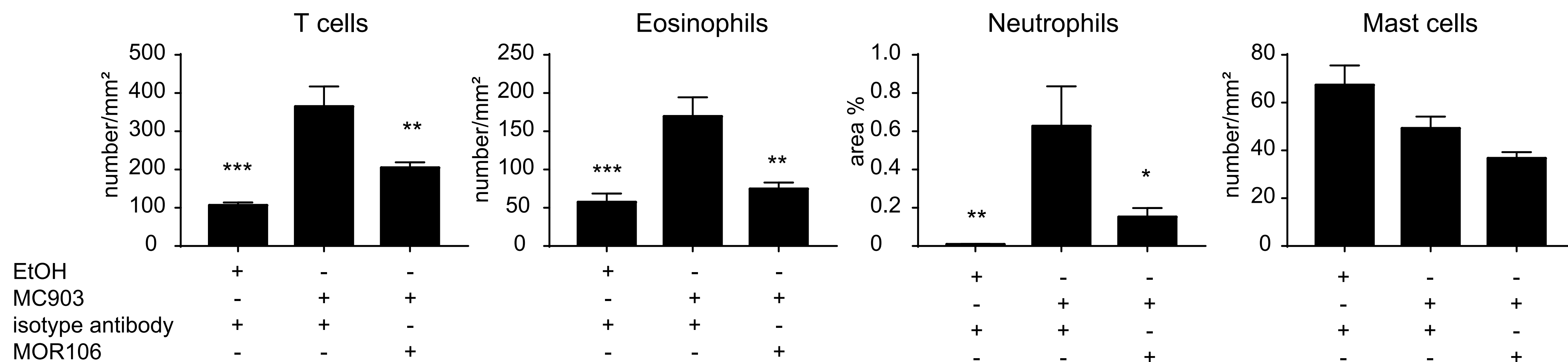
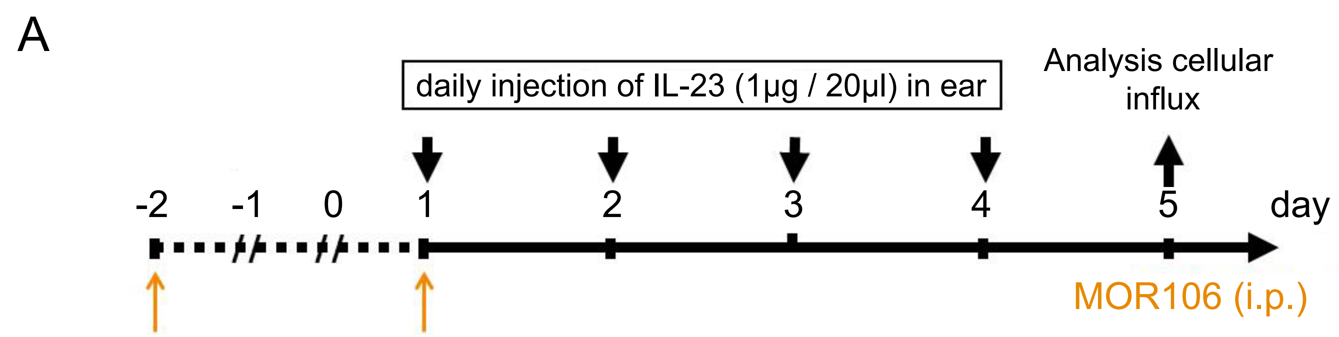


Figure 4

A



B

PBS/BSA + isotype

IL-23 + isotype

IL-23 + MOR106

CD3

EPX

MPO

MCT

C

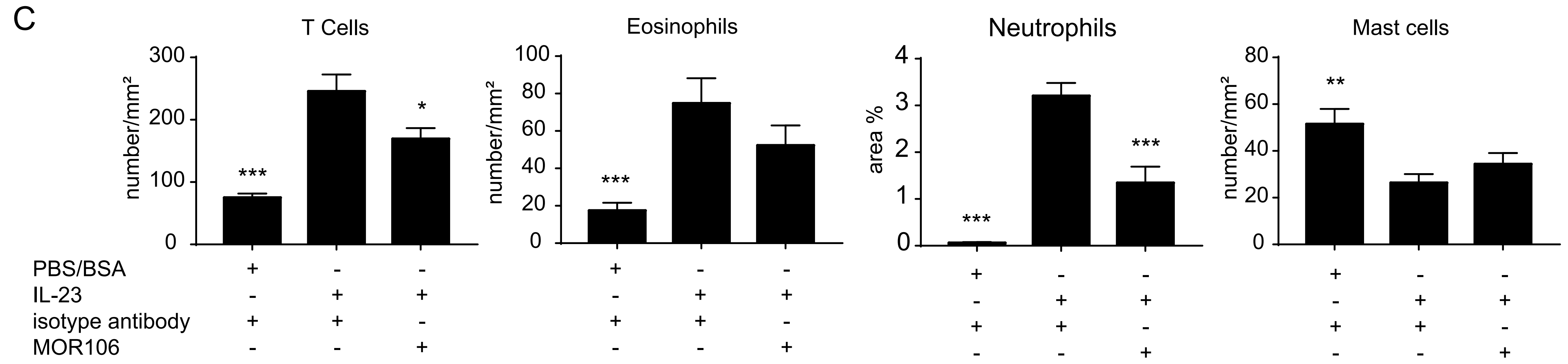
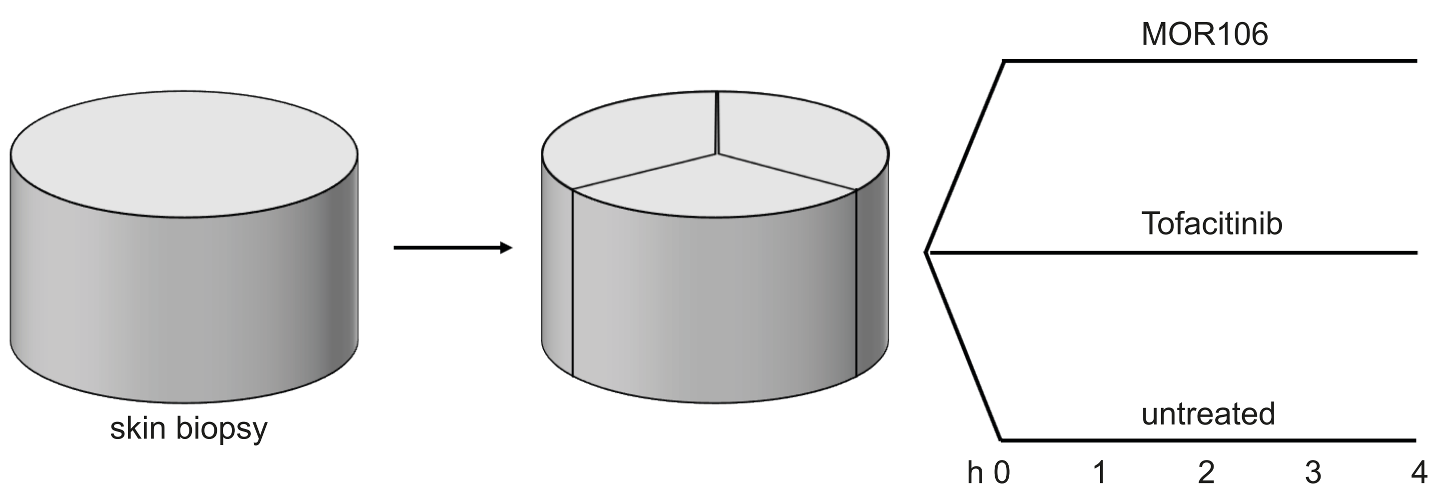
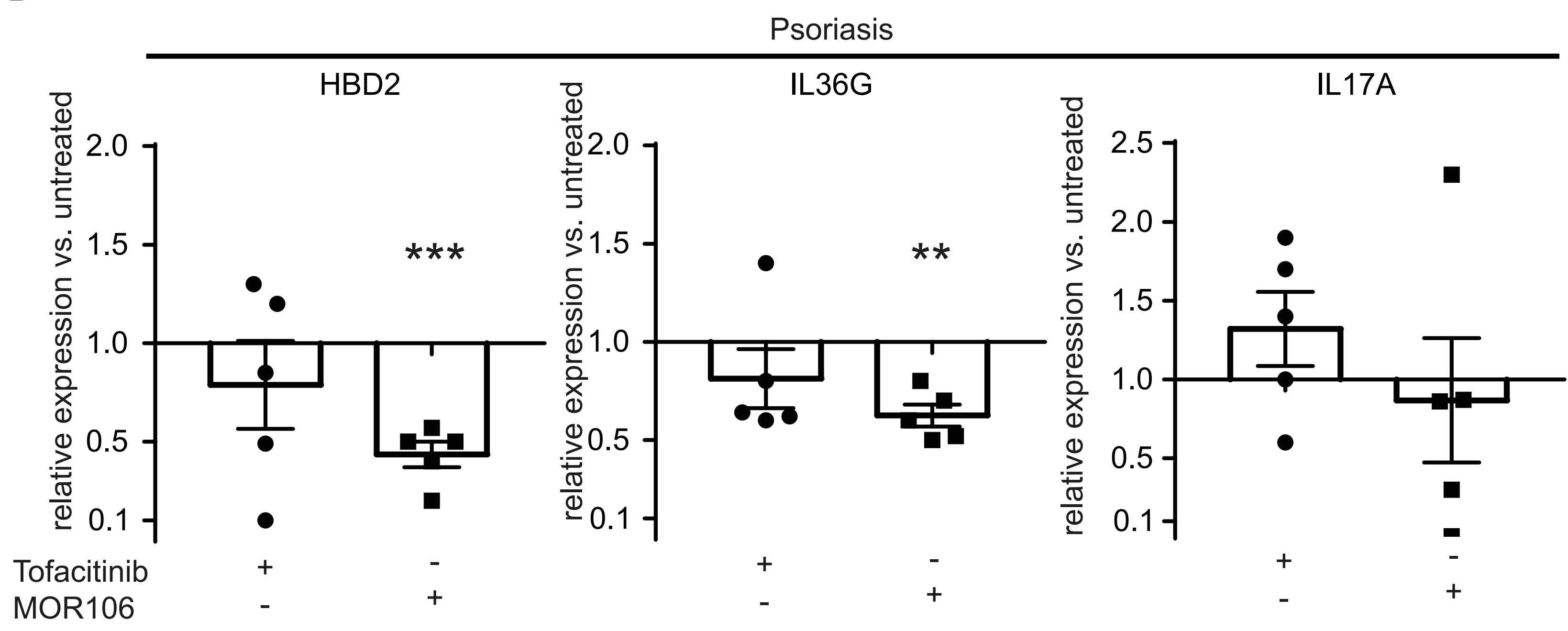


Figure 5

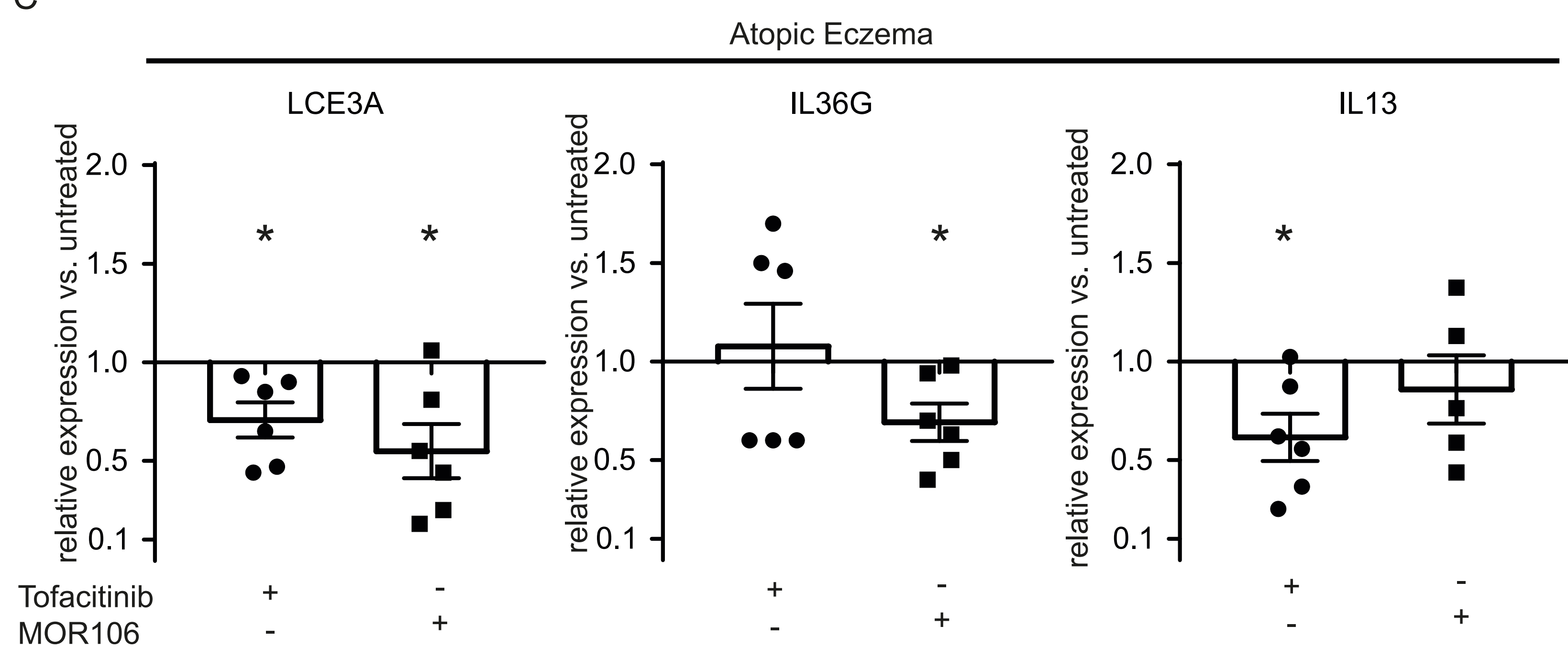
A



B



C



D