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Local IL-23 is required for proliferation and retention of skin-resident memory Th17 cells

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Abstract

The cytokine IL-23 is critical for development and maintenance of autoimmune inflammation in nonlymphoid tissues, however the mechanism through which IL-23 supports tissue-specific immunity remains unclear. In mice, we found that circulating memory T cells were dispensable for anamnestic protection from *C. albicans* skin infection, and tissue resident memory (T_{RM}) cell-mediated protection from *C. albicans* reinfection required IL-23. Administration of anti-IL-23R antibody to mice following resolution of primary *C. albicans* infection resulted in loss of $CD69^+$ $CD103^+$ tissue resident memory Th17 (T_{RM17}) cells from skin and clinical anti-IL-23 therapy depleted T_{RM17} from skin of psoriasis patients. IL-23 receptor blockade impaired T_{RM17} cell proliferation but did not impact apoptosis susceptibility or tissue egress. IL-23 produced by $CD301b^+$ myeloid cells was required for T_{RM17} maintenance in skin after *C. albicans* infection, and $CD301b^+$ cells were necessary for T_{RM17} expansion during the development of imiquimod dermatitis. This study demonstrates that locally produced IL-23 promotes *in situ* proliferation of cutaneous T_{RM17} to support their longevity and function and provides mechanistic insight into the durable efficacy of IL-23 blockade in the treatment of psoriasis.

One sentence summary:

IL-23 from $CD301b^+$ myeloid cells sustains the skin Th17 T_{RM} reservoir to promote antifungal immunity and psoriasis-like inflammation.

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INTRODUCTION

Barrier tissues such as skin contain memory T cells that perform local immunosurveillance critical to host defense(1–11). Tissue resident memory T cells (T_{RM}) are abundant in murine and human skin(12), where they coexist with populations of transient recirculating T cells (T_{CIRC})(3). T_{CIRC} can be defined by surface expression of lymph node homing receptor CCR7 and/or the vascular adhesion molecule L-selectin (CD62L)(3, 12, 13). Mouse and human T_{RM} express specific markers of tissue residency, most reliably CD69 and CD103, possess a unique transcriptional profile, and resist S1PR1-mediated tissue egress to persist long term in peripheral tissues(6, 8, 14–17). In skin, $CD8^+$ T_{RM} are required for local protection from secondary infection with vaccinia and herpes simplex viruses (HSV) and are implicated in the pathogenesis of organ specific autoimmune diseases including vitiligo and alopecia areata(18–22). $CD8^+$ T_{RM} localize to the epidermis, where they are maintained long-term in a manner dependent on locally produced TGF-β and IL-7/IL-15(23, 24). Considerably less is known about $CD4^+$ T_{RM}. IFNγ-producing T helper (Th) 1 T_{RM} defend the skin from reinfection with HSV and the intracellular parasite *Leishmania major*(4, 11). Th17 T_{RM} cells (T_{RM17}) arising in the skin of mice following infection with *Candida albicans* have been described (25). However, at present, knowledge of factors required for their tissue establishment and maintenance is lacking.

Th17 cells defend skin and mucosae from invading extracellular pathogens(26, 27). This effector $CD4^+$ T cell lineage is characterized by production of the cytokines IL-17A, IL-17F, IL-21, and IL-22, which elicit recruitment of inflammatory cells to promote pathogen clearance(28, 29). Th17 development variably relies on IL-6, TGF-β, IL-1β, and IL-23(26, 27, 30–32). Although discovery of the cytokine IL-23 led to the initial characterization of the Th17 lineage, and IL-23 is strongly associated with chronic inflammatory disorders including psoriasis, Crohn's disease, rheumatoid arthritis, and multiple sclerosis, its precise role in supporting autoimmunity in peripheral tissues remains unclear(29, 33–38). IL-23 is dispensable for Th17 differentiation *in vitro*, however, a multitude of reports define a requirement for IL-23 in generation of pathogenic Th17 responses *in vivo*(28, 29, 34–42). Addition of IL-23 to culture of activated or memory $CD4^+$ T cells induces their proliferation and increases the proportion of $IL\text{-}17^+$ cells(43). Maintenance of IL-17A and RORγt expression upon restimulation of differentiated effector Th17 are also dependent on IL-23(35). Thus, it has been suggested that the function of IL-23 is to stabilize the Th17 phenotype by maintaining IL-17 production from fully differentiated effector cells and/or promote their survival(27, 43, 44).

Anti-IL-23 biologic-based therapies show remarkable efficacy in reducing the organ-specific Th17 inflammation of psoriasis, inflammatory arthritis, and inflammatory bowel disease, demonstrating that IL-23 has a critical role in regulating Th17 immunity in skin and other barrier tissues(43, 45–48). Notably, anti-IL-23 therapies provide a much more durable clinical response than biologics that target IL-17A or TNF-α(49). Herein, we investigate the requirement for IL-23 in cutaneous memory Th17 cell responses using a canonical mouse model of Th17 immunity. We report selective reduction of T_{RM17} cells in murine skin after IL-23R blockade and identify $CD301b^+$ myeloid cells as a required source of IL-23 supporting skin T_{RM17} proliferation, retention, and function in host protective

and pathogenic recall responses. We further show that therapeutic responses observed in psoriasis patients after clinical IL-23 inhibition correlate with depletion of T_{RM17} from lesional skin.

RESULTS

Th17 cell accumulation in skin, but not lymph node or spleen, requires IL-23

To test the hypothesis that IL-23 is required for memory Th17 development and/or persistence in peripheral tissues, we used a well-established mouse model of Type-17 immunity in which transient *C. albicans* skin infection elicits a robust host protective Th17 response(25, 50, 51). Wildtype (WT) and *Il23a*$^{-/-}$ mice were epicutaneously infected with a strain of SC5314 *C. albicans* that has been genetically modified to express the model antigen 2W1S(50). We performed a kinetic analysis of antigen-specific CD4$^+$ T cell accumulation at various timepoints following *C. albicans* skin infection using the MHC-II tetramer I-A^b:2W1S(52). We found at 5 days following *C. albicans* skin infection, equivalent numbers and frequencies of I-A^b:2W1S$^+$ CD4$^+$ T cells were present in secondary lymphoid organs (e.g. lymph node (LN) and spleen; SLO) and skin of WT and *Il23a*$^{-/-}$ mice. At later timepoints after *C. albicans* infection, a marked reduction in I-A^b:2W1S$^+$ CD4$^+$ T cell number was observed in skin but not in secondary lymphoid organs of *Il23a*$^{-/-}$ animals (Figure 1A–D). Of note, an increased frequency of I-A^b:2W1S$^+$ CD4$^+$ T cells was observed in the SLO of *Il23a*$^{-/-}$ mice (Figure 1A–B) but total SLO CD4$^+$ T cell number was reduced, yielding no net change in antigen-specific CD4$^+$ T cell number in the SLO of *Il23a*$^{-/-}$ animals compared with WT (Figure 1D). This likely reflects the combination of a lower proliferation rate and impaired egress from lymph nodes previously observed in IL-23R-deficient CD4$^+$ T cells(53). Taken together, these data establish a requirement for IL-23 in supporting the accumulation of antigen-specific CD4$^+$ T cells in the skin during *C. albicans* infection and demonstrate that IL-23 deficiency does not alter *C. albicans*-specific CD4$^+$ T cell abundance in SLO.

Cutaneous C. albicans infection generates T_{RM17} that persist long term in skin

To better explore memory Th17 cell biology in the skin and expand on the finding that T_{RM17} accumulate in skin in response to epicutaneous *C. albicans* infection, we next used the *C. albicans* skin infection model in combination with dual *Il17a*^{Cre} *Rosa26*STOP$^{fl/fl}$tdTomato *Il17f*$^{Thy1.1/Thy1.1}$ (abbreviated as 17Tracker) reporter mice(54–56). 17Tracker mice express a red fluorescent protein variant (tdTomato) in cells that have a history of *Il17a* expression, thereby enabling us to identify cells that were activated to express *Il17a* in response to *C. albicans* infection even after they have extinguished IL-17A production. Additionally, Thy1.1 reports *Il17f* expression, allowing us to visualize cells actively synthesizing IL-17F which is expressed coordinately with IL-17A(55, 57). To validate our model, we first epicutaneously infected 17Tracker mice with *C. albicans* and quantified and phenotyped tdTomato$^+$ and Thy1.1$^+$ immune cells present in skin at various timepoints after infection. We observed that tdTomato and Thy1.1 expression were restricted to T cells. *C. albicans* infection did induce tdTomato expression by innate lymphoid cells (ILCs), natural killer (NK) cells, or NK T cells and these cells were not appreciable sources of IL-17F (Figure 2A–B). TdTomato$^+$ CD4$^+$ and CD8$^+$ T cells accumulated in skin as

early as 3 days post infection and underwent a robust expansion that was maximal at day 7. Contraction was evident on day 28, but tdTomato$^+$ αβ T cells persisted in the skin at least 120 days after primary *C. albicans* infection. TCRγδ T cells exhibited less dramatic expansion but expressed abundant tdTomato protein at all timepoints examined. Analyses of T cell populations revealed approximately 5-fold greater abundance of bulk CD4$^+$ T cells compared with bulk γδ T cells at day 28 post-infection, however, numbers of tdTomato$^+$ CD4$^+$ and γδ T cells were roughly similar. We noted enrichment of tdTomato expression at memory timepoints across all T cell types compared to naïve mice. In addition, CD4$^+$ T cells were the dominant skin source of IL-17F. In summary, 17Tracker mice enabled us to detect tdTomato$^+$ cells that expanded and expressed IL-17A/F in response to *C. albicans* infection, and these cells persisted long-term in skin.

To characterize the spatial location of tdTomato$^+$ CD4$^+$ T cells, we performed immunofluorescent microscopy analysis of skin sections obtained between day 0-120 post *C. albicans* infection (Figure 2C). TdTomato$^+$ CD4$^+$ T cells were present in the papillary and reticular dermis at early timepoints after *C. albicans* infection but starting at day 28 were restricted to the superficial dermis and exhibited perifollicular clustering. Using flow cytometry to phenotype skin tdTomato$^+$ CD4$^+$ at day 42 post-infection, we found that tdTomato$^+$ CD4$^+$ T cells universally expressed CD44 and the majority were CD69$^+$ CD103$^{+/-}$ (Figure 2D–E, Figure S1). We also noted a small population of CD62L$^+$ CCR7$^-$ cells that may represent a recently described population of recirculating memory T cells termed T migratory memory cells (T_{MM}) that have yet to be fully characterized(58). Our results corroborate the spatial distribution(4, 7) and surface phenotype(25, 59–61) previously reported for cutaneous CD4$^+$ T_{RM} and validate the use of tdTomato reporter expression as a surrogate for T_{RM17} present in skin after resolution of *C. albicans* infection.

IL-23 is required for T_{RM17} persistence in skin

Since *Il23a*$^{-/-}$ mice have impaired clearance of *C. albicans* following skin infection(62) and altered Th17 differentiation(53), we next devised an experimental approach to test the requirement of IL-23 on skin-resident memory CD4$^+$ T cells without these caveats. Cohorts of 17Tracker mice were epicutaneously infected with *C. albicans* and allowed to rest for 28 days which corresponds to an early memory timepoint that is well after resolution of *C. albicans* skin infection (Figure 3A)(25, 50). Mice were then treated with either isotype or a neutralizing monoclonal antibody (mAb) to the IL-23 receptor (IL-23R), 21A4(63, 64). After an additional 14 days, which is less than the estimated *in vivo* half-life of 21A4 (MJM, unpublished observations), we assessed numbers of tdTomato$^+$ CD4$^+$ T cells in skin and SLO. Total numbers of CD45$^+$ immune cells and CD4$^+$ T cells in the skin of anti-IL-23R mAb treated mice were similar to those treated with isotype antibody (Figure 3B–C). However, anti-IL-23R Ab treated mice exhibited reduced numbers of tdTomato$^+$ CD4$^+$ T cells in skin compared with isotype treated controls. CD4$^+$ tdTomato$^-$ T cell number was not impacted by IL-23R blockade. Phenotyping cutaneous CD4$^+$ tdTomato$^+$ T cells based on expression of CD69 and CD103 revealed that all tdTomato$^+$ CD4 subsets were reduced in number, but the largest reduction was evident in the CD69$^+$CD103$^+$ T_{RM} population (Figure 3D). CD4$^+$ tdTomato$^+$ T cell number in SLO was unaltered by anti-IL-23R neutralization (Figure S2A–B), suggesting that memory Th17 cell persistence in secondary lymphoid

tissues is independent of IL-23. This may relate to higher expression of *Il23R* by tdTomato$^+$ CD4$^+$ T cells within skin compared with LN and spleen (Figure S2C–D). Collectively, these data demonstrate that IL-23 is required for the persistence of cutaneous T_{RM17}, and neutralization of IL-23R is not associated with redistribution of these cells to the SLO.

IL-23 blockade diminishes T_{RM17}-mediated host defense

Previous work has shown that non-recirculating memory CD4$^+$ T cells present in skin are sufficient for protection in the *C. albicans* skin infection model(25). To confirm this result, naïve mice and memory mice that had been infected with *C. albicans* then rested for 42 days were challenged with *C. albicans* and the extent of skin infection was assessed by CFU at 24 hours (Figure 3E). As expected, memory mice showed a 1.5 log reduction in *C. albicans* CFU. To test the contribution of T_{RM} cells and their dependency on IL-23, we repeated this experiment in memory mice in the presence or absence of mAbs that neutralize TCRγδ T cells (anti-TCRγδ) and IL-23 (anti-IL23R), and compounds that prevent entry of circulating T cells into skin (FTY720, anti-CD49d, anti-VCAM-1)(10). Blockade of IL-23R resulted in impaired host defense only in mice in which the contributions of TCRγδ and recruited T cells were inhibited (Figure 3F). Diminished T_{RM}-mediated host defense was associated with a reduced number and frequency of IL-17A but not IFNγ-expressing CD4$^+$ T cells in the skin (Figure 3G–H). Notably, ablation of TCRγδ cells and inhibition of T cell recruitment did not impact host defense in IL-23-sufficient mice. Collectively, these data demonstrate that T_{RM17} generated in response to an epicutaneous *C. albicans* infection can provide host defense to a subsequent infection and this T_{RM17}-mediated protection requires IL-23.

IL-23 promotes in situ proliferation of cutaneous T_{RM17}

The established importance of IL-23 in proliferation and survival of Th17 cells in nonlymphoid tissues including intestine(26) and central nervous system(53) led us to hypothesize that IL-23 may selectively promote T_{RM17} proliferation in skin. To test this hypothesis, we compared T cell viability and proliferative capacity in isotype and anti-IL-23R treated memory mice at least 42 days post infection using the experimental approach outlined in Figure 3A. We found no significant difference in tdTomato$^+$ CD4$^+$ T cell viability between anti-IL-23R and isotype antibody treated mice, as measured by live/dead dye, Annexin-V, and TUNEL staining of both flow cytometry specimens and histological sections (Figure 4A–B). In contrast, bromodeoxyuridine (BrdU) incorporation was reduced in tdTomato$^+$ but not tdTomato$^-$ CD4 T cells in anti-IL-23R treated mice (Figure 4C–E). This was most evident in CD69$^+$ CD103$^+$ tdTomato$^+$ CD4$^+$ T cells (Figure 4F). Interestingly, proliferation of tdTomato$^+$ CD4 cells in SLO was not significantly affected by IL-23R blockade (Figure S2 E–F). These data suggest that local IL-23 is required for homeostatic proliferation of cutaneous T_{RM17} cells thereby promoting their long-term persistence in skin.

T_{RM17} persistence in skin requires IL-23 from CD301b$^+$ dermal dendritic cells and macrophages

IL-23 expressed by the CD301b$^+$ subset of conventional type 2 dendritic cells (cDC2) is required for IL-17A production by dermal γδ T cells in both *Candida*(65)

and imiquimod(66) acute inflammation models. CD301b is also expressed by tissue macrophages. To determine whether CD301b$^+$ dermal dendritic cells (dDCs) produced IL-23 is required for maintenance of cutaneous T_{RM17} cells in skin during homeostasis, we used the *Mgl2*^{DTR} mouse model(4, 5, 51). In this model, administration of diphtheria toxin (DT) induces depletion of CD301b$^+$ expressing dDCs and macrophages(4). To seed the skin of *Mgl2*^{DTR} mice with T_{RM17} cells, CD4$^+$ T cells were isolated by magnetic bead purification from the SLO of 17Tracker mice 36 days after primary *C. albicans* infection. These cells were adoptively transferred into naïve CD45.1 congenic *Mgl2*^{DTR} recipients which were then infected epicutaneously with *C. albicans* to both expand *C. albicans*-specific 17Tracker CD4$^+$ T cells and recruit them into the skin. Mice were rested for 28 days to allow transition to an early memory phase response before treatment with DT or PBS over the course of 14 days to ablate CD301b$^+$ cells (Figure 5A). On day 42, we observed a reduction in transferred 17Tracker CD4$^+$ T cell number due to the loss of tdTomato$^+$ CD4$^+$ T cells in the skin of DT treated mice (Figure 5B, D–E). TdTomato$^-$ CD4$^+$ T cell number in skin and cells numbers in SLO were unaffected by CD301b$^+$ cell depletion. Immunofluorescence microscopy visualization of skin (Figure 5C) revealed reduced numbers of tdTomato$^+$ cells (identified by arrows heads) as well as reduced numbers of GFP$^+$ CD301b$^+$ cells (identified by asterisk) which was confirmed with qPCR for *Il23a* and flow cytometry for GFP, thereby demonstrating the efficacy of DT-mediated ablation (Figure 5C, Figure S3A–B). Notably, loss of tdTomato$^+$ CD4$^+$ T cells from the skin was not accompanied by increased numbers in the SLO (Figure 5B–E), demonstrating that attrition of T_{RM17} from skin is not associated with redistribution to lymph nodes or spleen. Thus, we conclude that CD301b$^+$ cells are required to sustain T_{RM17} in skin.

Next, to confirm that maintenance of cutaneous T_{RM17} requires IL-23 from CD301b$^+$ cells, we employed the same strategy using *Il23a*$^{-/-}$ + *Mgl2*^{DTR} mixed bone marrow chimeras. A 1:1 ratio of bone marrow isolated from *Mgl2*^{DTR} and either WT or *Il23a*$^{-/-}$ mice was transferred into lethally irradiated CD45.1 WT recipients. After 12 weeks of reconstitution, these mice were used as recipients for 17Tracker memory CD4$^+$ T cells, infected with *C. albicans*, and treated with DT at an early memory timepoint (Figure 5A). DT-treated *Mgl2*^{DTR} + *Il23a*$^{-/-}$ → WT recipients in which CD301b$^+$ cells are IL-23 deficient, demonstrated reduced numbers of tdTomato CD4+ in the skin in comparison to *Mgl2*^{DTR} + WT → WT controls (Figure 5F–G). In addition, homeostatic proliferation based on BrdU incorporation was selectively reduced in tdTomato$^+$ CD4$^+$ in the absence of CD301b$^+$-derived IL-23 (Figure 5G). Taken together, these data demonstrate that cutaneous CD301b$^+$ dDCs and/or macrophages are a nonredundant source of IL-23 at steady state that is required for homeostatic proliferation and persistence of cutaneous T_{RM17} cells.

CD301b$^+$ cells expand T_{RM17} to accelerate development of psoriasiform dermatitis

CD301b$^+$ dDCs have been reported to produce high levels of IL-23 and drive imiquimod-induced psoriasis-like skin inflammation in mice(66). We used a Th17-dependent model of psoriasis recently described by Hurabielle *et al.*(67) to evaluate the capacity of *C. albicans*-specific T_{RM17} to exacerbate psoriasiform dermatitis and assess dependency of pathologic T_{RM17} responses on CD301b$^+$ cells (Figure 6A). In this model, *C. albicans* infection expands and recruits IL-17A-producing CD4$^+$ T cell into skin. During subsequent

treatment with imiquimod, these cells are reactivated to drive tissue pathology. We infected 17Tracker mice with *C. albicans* to generate cutaneous T_{RM17}, and after a rest period of 28 days, applied imiquimod topically to both infected and uninfected flank skin for 5 consecutive days. We assessed dermatitis severity daily using a modified PASI scoring system developed by van der Fits *et al*(68). As expected, skin previously infected with *C. albicans* exhibited earlier onset and more severe imiquimod-induced inflammation compared with uninfected skin (Figure 6B). After two days of imiquimod application, *C. albicans*-immune skin contained a higher percentage of Thy1.1$^+$ CD4$^+$ T cells and was enriched for tdTomato$^+$ CD4$^+$ T cells with no change in γδ T cell number or Thy1.1 expression (Figure 6C–F). TdTomato$^+$ CD4$^+$ T cells, but not γδ T cells, from *C. albicans*-sensitized mice showed enhanced imiquimod-induced proliferation (Figure 6G). These findings suggest that the accelerated development of psoriasiform dermatitis observed in *C. albicans*-immune skin is the result of T_{RM17} expansion and IL-17A/F production.

To determine if CD301b$^+$ cells are required for imiquimod-induced T_{RM17} expansion and enhanced severity of disease, we applied topical imiquimod to *C. albicans*-immune *Mgl2*^{DTR} mice 28 days post-infection, concurrent with DT or PBS treatment for 5 days (Figure 6H). DT- treated *Mgl2*^{DTR} mice exhibited reduced disease severity (Figure 6I), consistent with a previous report(69). After 3 days of imiquimod application, we observed diminished CD69$^+$CD103$^+$IL-17A$^+$ CD4$^+$ T cell (T_{RM17}) number and reduced T_{RM17} proliferation in the skin of CD301b$^+$ cell-depleted mice (Figure 6J–K), suggesting that indeed, IL-23 produced by CD301b$^+$ cells drives T_{RM17} proliferation to enhance psoriasis-like skin inflammation in mice.

T_{RM17} are depleted from lesional psoriasis skin following clinical anti-IL-23 therapy

To test the generalizability of our findings to humans, we examined the skin of psoriasis patients before and after clinical anti-IL-23 therapy. We prospectively collected punch biopsy specimens from eligible patients with moderate-severe psoriasis (n=5) immediately prior to and 1 week after start of anti-IL-23 therapy (Table S1). Immunofluorescence microscopy was used to identify CD3$^+$ T cell populations in healthy control and psoriasis skin pre- and post-treatment (Figure 7A). Previous reports suggest that both CD4$^+$ and CD8$^+$ T cells are sources of IL-17A in psoriasis skin(70, 71), and some published data suggest that T cells must enter the epidermis to drive psoriatic inflammation(72). Thus, regions of interest (ROI) within the both the epidermis and dermis were selected for NanoString GeoMX™ Digital Spatial Profiler multiplexed protein expression analysis (Figure S4). Expression of 49 human immune cell markers was quantified within spatially resolved CD3$^+$ T cell populations (Figure 7B–C). CD8$^+$ and CD4$^+$ T cells were numerous in untreated psoriasis skin but in the same plaque 1 week post anti-IL-23 therapy, reduced numbers of CD4$^+$ T cells were present without significant change in CD8$^+$ T cell number. Notably, the Ki67 proliferation index was reduced within the CD3$^+$ cell compartment post-treatment. In addition, there was increased expression of CD45RO, the survival factor Bcl-2, STING, and several inhibitory receptors in anti-IL-23 treated skin consistent with resolving inflammation (Figure 7C).

Because these analyses did not enable us to distinguish T_{RM17} from other skin T cells, we next examined relative abundance of the T_{RM} markers CD69 and CD103 in relation to CD4, IL-17A, and Ki67 in lesional skin of patients before and after anti-IL-23 therapy using immunofluorescence staining with multispectral imaging (Figure 7D–E). Nearly all $CD4^+$ T cells in psoriasis skin pre- and post-treatment co-expressed CD69 and CD103, and approximately 20% had detectable IL-17A expression. T_{RM17} represented the principal cell population diminished after anti-IL-23 treatment and exhibited reduced Ki67 expression (Figure 7E). Thus, we find that clinical anti-IL-23 therapy depletes T_{RM17} cells from lesional psoriasis skin in association with reduced proliferation, thereby demonstrating that T_{RM17} in psoriatic skin are dependent on IL-23 for proliferation and persistence as in murine skin.

DISCUSSION

Cutaneous T_{RM17} perform local immunosurveillance that is critical for host defense and are hypothesized to underlie the chronicity and high relapse rate of psoriasis(73, 74). We used a well-established experimental model of Th17 immunity to demonstrate that IL-23 is required to maintain T_{RM17} in skin following resolution of cutaneous *C. albicans* infection, identified $CD301b^+$ myeloid cells as an obligate source of this cytokine in murine skin, and determined that IL-23 neutralization impaired homeostatic proliferation of T_{RM17} without impacting migration or apoptosis susceptibility. Cutaneous T_{RM17} generated during primary *C. albicans* infection were sufficient to mount a host protective anamnestic response to subsequent *C. albicans* infection, and T_{RM17}-mediated recall responses were diminished by IL-23R blockade. We show that $CD301b^+$ cells induce T_{RM17} proliferation to accelerate onset of psoriasiform dermatitis in mice, and psoriasis patients treated with anti-IL-23 biologic agents have reduced numbers and proliferation of T_{RM17} in lesional skin. In sum, local production of IL-23 from $CD301b^+$ cells maintains murine skin-resident T_{RM17} through the promotion of homeostatic proliferation, and a similar mechanism appears to be operative in human psoriatic skin.

Previous work has suggested that IL-23 is important for Th17 survival and maintenance of the Th17 phenotype(26, 27, 43). Using the *Il23a*$^{-/-}$ mouse model, we show that *C. albicans*-specific $CD4^+$ T cell accumulation in SLO is unimpacted by IL-23 deficiency but IL-23 deficiency imparts a specific defect in Th17 cell accrual in skin. Previous work examining *Il23r*$^{-/-}$ $CD4^+$ T cells during induction of experimental autoimmune encephalomyelitis suggests that that this outcome is the result of impaired proliferation and terminal differentiation of Th17 effector cells(53). Our finding that IL-23 is required for persistence of memory Th17 cells in the skin extends this finding to include a previously unappreciated requirement for IL-23 in maintenance of Th17 immunity in skin. Our data are also significant in that they identify a role for IL-23 in supporting homeostatic proliferation of memory Th17 cells. Our results suggest that tonic IL-23 produced by $CD301b^+$ myeloid cells sustains the T_{RM17} reservoir in the skin, and this premise is further supported by clinical data demonstrating that psoriasis eventually recurs upon withdrawal of IL-23 inhibitor therapy(48). We note that although there did not appear to be any difference in frequency of Annexin V^+ or $TUNEL^+$ cells in the anti-IL-23R groups (Figure 4A–B), we cannot completely rule out an additional effect of IL-23 on survival of memory Th17 cells.

T_{RM17} that establish tissue residency have the capacity to be reactivated to provide rapid host defense from pathogens or cause autoimmune pathology. Our data examining the recall response to *C. albicans* demonstrate that T_{RM17} are dependent on IL-23 for *in situ* proliferation, and both IL-23 and recruitment of recirculating memory Th17 cells into tissue can rescue this deficit. Interestingly, recirculating memory Th17 cells (T_{CIRC}; $CD69^-$ $tdTomato^+$ d42 $CD4^+$ T cells) exhibited only partial dependency on IL-23 for skin retention (Figure 3D) and T_{CIRC} proliferation was unaffected by IL-23R blockade (Figure 4F).

We identify IL-23 as a key factor supporting skin-resident memory Th17 cell retention in skin and identify increased expression of *Il23R* as a potential basis for selective effects of IL-23 on memory Th17 cells present within skin compared with lymph node or spleen (Figure S2C–D). However, we suspect that additional signals contribute to T_{RM17} generation and long-term persistence. There are reports of T_{RM17} responding to IL-7 signals to support their retention in lung after inflammatory or infectious insult(75, 76) and a prior study identified IL-7 induction of STAT3 signaling and IL-17 production by γδ T cells(77). Thus, it is possible, as is the case with other STAT3-inducing cytokine signals (IL-1, IL-6)(78), that IL-23 synergizes with IL-7 to promote STAT3-mediated proliferation and/or survival of T_{RM17}. TGFβ, integrin receptors, FABP4/5, and IL-15 are additional factors implicated in CD8 T_{RM} skin retention(2, 3, 15, 23, 74). Further work is needed to determine if these or other local signals contribute to Th17, Th1, and Th2 T_{RM} establishment and persistence in skin and discriminate global effects on T_{RM} longevity from T helper subset or tissue specific T_{RM} retention mechanisms.

Three subsets of IL-23 producing DCs reside in skin: epidermal Langerhans cells (LCs), $CD301b^+$ dDCs, and $CD103^+$ dDCs(51). Our previous studies have demonstrated that $CD11c^+$ DCs, but not LCs or CD103+ dDCs, are required for innate IL-17 production by dermal γδ T cells following *C. albicans* infection(51, 65). We now show that memory phase depletion of $CD301b^+$ dDCs (28 to 42 days post *C. albicans* infection) results in loss of T_{RM17} from skin. An important caveat to this series of experiments is that we cannot exclude a role for $CD301b^+$ IL-23 producing macrophages in T_{RM17} maintenance *in vivo* as both $CD11b^+$ dDCs and tissue macrophages expressing the C-type lectin macrophage galactose lectin type 2 receptor are ablated in Mgl2-DTR mice. However, previous work has shown that DCs are more potent at generating and reactivating T_{RM} compared with macrophages(79) $CD11c^+$ DCs are the major myeloid population present in lesional psoriasis skin(80), and dDCs are the major $CD11b^+$ cellular source of IL-23(79). Importantly, our data rule out LCs as an obligate source of IL-23 supporting skin T_{RM17}, as LCs are radio resistant and were present in the skin of Mgl2-DTR + $Il23a^{-/-}$ bone marrow chimeric mice that exhibited reduced T_{RM17} residence.

The chronic relapsing-remitting course and predilection for site specific recurrence implicate T_{RM17} in the pathogenesis of psoriasis(12, 13, 44, 58). Prior studies have examined residual T cell populations within healed psoriasis lesions following successful anti-TNFα therapy and detected psoriasis specific IL-17A-producing αβ T cell clones via high-throughput TCR sequencing(81) as well as transcriptional and histologic evidence of IL-17A competent T cell persistence, despite reversal of other features of psoriatic inflammation(82–85). Based on these studies, the prevailing hypothesis in the field is that pathogenic IL-17

producing tissue resident memory T cells (T_{RM17}) persist long term in psoriasis skin and are responsible for disease relapse after therapeutic withdrawal. We observed that in untreated psoriasis skin, >50% of immune cells were $Ki67^+$ $CD4^+$ T cells expressing both CD69 and CD103, suggesting that these cells contribute significantly to the pathogenesis of psoriasis. We found that T_{RM17} were quickly depleted after start of anti-IL-23 biologic therapy in association with rapid clinical improvement. Our results add credence to the idea that psoriasis flares represent localized sites of cutaneous T_{RM17} reactivation rather than *de novo* activation of new Th17 clones. Limitations of our study include that we have not excluded the possibility that anti-IL-23 therapy may prevent the development of newly differentiated Th17 clones and we have not established a causative link between myeloid cell produced IL-23, T_{RM17} reactivation, and disease relapse in psoriasis patients. Longitudinal studies will be needed to determine if anti-IL-23 biologics permanently reduce cutaneous T_{RM17} density and prevent relapse following drug withdrawal.

In summary, we have shown that IL-23 produced by $CD301b^+$ cells promotes *in situ* T_{RM17} proliferation required for host protective and pathologic recall responses in skin. Our finding that local IL-23-driven T_{RM17} proliferation maintains the skin T_{RM17} pool is a mechanistic insight into T_{RM} biology that may be exploited for therapeutic augmentation or diminution of T_{RM17} responses in the context of skin infections and autoimmunity, respectively. Additionally, our data suggest that the mechanism by which existing anti-IL-23 therapies resolve psoriasis inflammation in such an effective and sustained manner is by depletion of T_{RM17}.

MATERIALS AND METHODS

Study design

The objective of this study was to ascertain the role of IL-23 in promotion of tissue-specific immune memory. We designed and performed experiments using multiple techniques including cellular immunology, flow cytometry, MHC II tetramer and TUNEL assays, qPCR, immunofluorescence microscopy, NanoString™ Digital Spatial Profiling, and murine *in vivo* disease models. The sample size and number of independent experiments are indicated in each of the figure legends.

Mice

Il17f$^{Thy1.1/Thy1.1}$ mice(55) were provided by Casey Weaver (University of Alabama at Birmingham). We crossed *Il17f*$^{Thy1.1/Thy1.1}$ mice with *Il17a*-Cre mice and B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$ mice to obtain *Il17a*^{Cre} *Rosa26* $^{CAG\text{-}fl/fl\text{-}tdTomato}$*Il17f*$^{Thy1.1/Thy1.1}$ (17Tracker) reporter mice. C57BL/6 (WT), *Il17a*$^{tm1.1(icre)Stck}$/J (*Il17a*-Cre mice), B6.SJL-*PtprcaPepcb*/BoyJ (CD45.1), Mgl2$^{eGFP\text{-}DTR/wt}$ (Mgl2-DTR), and B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hz}$*/J* mice (Ai9) mice were purchased from Jackson Laboratories. Age- and sex-matched mice between 6 and 12 weeks old were used in all experiments. Mice were maintained under specific-pathogen-free conditions and all animal experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Candida albicans skin infection

C. albicans skin infection and determination of CFU were performed as described(50, 65, 86).

Dendritic cell depletion with diphtheria toxin

Mgl2-DTR mice were injected intraperitoneally (i.p.) with 1 μg of diphtheria toxin (List Biological Laboratories) 28 days after primary infection (loading dose). Maintenance dosing of 100 ng/mouse was given i.p. every 3-4 days for a total of 14 days to yield sustained depletion of $CD301b^+$ dDCs.

Inhibition of T cell migration into skin

For FTY720 treatments, mice were intraperitoneally injected with FTY720 (Cayman Chemical) 1 μg/g of body weight in normal saline containing 0.5% DMSO daily, starting 2 days prior to *C. albicans* infection. 500 μg of anti-TCRγδ (clone UC7-13D5) was administered to all animals and 500 μg of anti-CD49d (clone PS/2, BioXCell) and anti-VCAM-1 (clone M/L-2.7, BioXCell) were administered to some animals i.p. once on day −2 as previously described(10).

Adoptive T cell transfer

$CD4^+$ T cells from 17Tracker mice were purified from lymphoid tissues by magnetic bead separation (EasySep™ Mouse $CD4^+$ T Cell Isolation Kit, STEMCELL Technologies) as previously described(2). Cell purity (>95%) was determined by flow cytometry before adoptive transfer. 1-2 × 107 $CD4^+$ T cells resuspended in 100-200 μL of sterile PBS were injected intravenously into recipient mouse tail veins.

Skin digestion and flow cytometry

Preparation of single cell suspensions from tissues and staining were performed as previously described(1, 2, 24, 51). Anti-CD8α (53-6.7), CD44 (IM7), TCRβ (H57-597), CXCR3 (CXCR3-173), CD3 (17A2), Thy1.2 (30-H12), Thy1.1 (OX-7), CD69 (H1.2F3), CD103 (2E7), CD45.2 (104), CD27 (LG.3A10), Anti-KLRG1 (2F1), and CD127 (A7R34) were purchased from BioLegend. Anti-CD8α (53-6.7), CD4 (GK1.5), CD45.2 (104), CD90.1 (OX-7), CD27 (LG.3A10), and CD69 (H1.2F3) were purchased from BD Biosciences. Anti-IFNγ (XMG1.2) was purchased from Tonbo Biosciences (San Diego, CA). A BD LSR FORTESSA (BD Biosciences) and FlowJo software (TreeStar, Ashland, OR) were used for analysis. Absolute cell number was calculated using AccuCheck counting beads (Thermo Fisher), where volumetric measurement of cell sample fluid acquired during flow cytometry with a fixed concentration of beads in each sample were used to calculate cell number per unit volume by the following formula: (A/B) x (C/D) = number of cells per total volume in the sample tube (cell concentration as cells/μL). A= number of vender beads added to cell sample, B= total volume of cell sample, C= cell count form acquired data, and D= bead count from acquired data.

Immunofluorescence microscopy

Murine skin samples were fixed in 4% paraformaldehyde for 1 hour at 4°C and processed as previously described(1, 2). Sections were incubated with Alexa Fluor 647 anti-mouse CD4 antibody (BioLegend, N1UG0) for 1 hour at room temperature. If needed, sections were then incubated with secondary antibodies using a 1:1000 dilution in wash buffer. Sections were mounted with ProLong Gold Antifade with DAPI (Invitrogen). Images were taken by an Olympus fluorescent microscope (Olympus Corporation).

MHC II tetramer assay

Tetramer staining was carried out as previously described(52, 87, 88). Cell suspensions were incubated with streptavidin- and allophycocyanin-conjugated I-A^b:p2W1S MHC-II tetramers for 1 hour at room temperature. SLO samples were then enriched for bead-bound cells on magnetized columns prior to surface staining with a mixture of antibodies specific for T cell markers and viability dye. Skin samples were analyzed directly without magnetic bead enrichment of tetramer positive cells.

In situ TUNEL assay

TUNEL staining was performed using the ApopTag Fluorescein *In Situ* Apoptosis Detection Kit protocol (Millipore Sigma) according to the manufacturer's protocol. Images were taken with an Olympus fluorescent microscope.

Assessment of T cell proliferation

Mice were injected intraperitoneally with 1 mg 5-bromo-2'-deoxyuridine (BrdU; Sigma) on day 28 post-primary *C. albicans* infection, and continuously given BrdU in drinking water (0.8 mg/mL), replaced every 3 days, for 2 weeks. BrdU staining was carried out using BD Pharmingen BrdU Flow Kit (APC) according to manufacturer's instructions.

qPCR

Tissue was homogenized using the Navy RINO Lysis Kit (Net Advance, Troy, NY). RNA was isolated from whole skin using TRI Reagent (Sigma Aldrich, St. Louis, MO) according to the manufacturer's protocol. RNA to cDNA conversion was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). cDNA was analyzed using TaqMan Gene Expression assays for *Il23a, Il23r, and Il12rb1* (ThermoFisher). Ct values were normalized to *Hprt* expression and shown as relative expression ($2^{-\Delta Ct}$).

Patient specimens

Studies using human tissue were approved by the University of Pittsburgh Institutional Review Board (IRB). Normal human skin was obtained from patients undergoing elective surgery at dermatology outpatient surgery clinics. Psoriasis skin was obtained from psoriasis patients who had been off all systemic therapy for greater than 1 month prior to skin biopsy. 4 mm punch biopsies were obtained from psoriatic plaques, and in cases where specimens were obtained pre- and post-anti-IL-23 therapy, follow up biopsy was performed within

the same plaque 7-10 days after the start of drug treatment. All patients provided written informed consent prior to skin biopsy.

Immunofluorescence staining and multispectral imaging of FFPE tissue

4 mm punch biopsy specimens of human skin were formalin fixed and paraffin embedded as previously described (91). Primary antibodies used were: anti-human CD4 monoclonal antibody (Invitrogen, N1UG0), anti-human IL-17A biotinylated antibody (R&D Systems, BAF317), anti-human CD69 antibody (Novus Biologicals, 8B6), anti-human CD103 (Abcam, EPR4166-2), and recombinant anti-Ki67 (Abcam, SP6). Secondary antibodies used were: Alexa Fluor 555 rabbit anti-PE polyclonal antibody (VWR), Alexa Fluor 647 donkey anti-Rabbit IgG (H+L) antibody (Invitrogen, A21147), Alexa Fluor 555 goat anti-mouse IgG2a antibody (Invitrogen, A-21242), Alexa Fluor 633 goat anti-mouse IgG1 polyclonal antibody (Invitrogen), Alexa Fluor 488 goat anti-rabbit IgG (H+L) polyclonal antibody (Invitrogen). Slides were imaged with a Mantra Quantitative Pathology Imaging microscope (PerkinElmer) by the Human Skin Disease Resource Center at Brigham & Women's Hospital, Harvard Medical School.

NanoString GeoMx™ Digital Spatial Profiler

NanoString GeoMx™ Digital Spatial Profiler (DSP) technology was used as previously described(89) to perform spatially resolved assessment of protein biomarkers in 5 psoriasis and 5 healthy control FFPE skin samples. Target protein expression values for $CD3^+$ T cells were normalized to both area and housekeeping gene expression.

Quantification and statistical analysis

Groups were compared with Prism software (GraphPad) using the two-tailed unpaired Student's t test for comparison of two groups, ordinary one-way ANOVA for comparison of 3 or more groups, and or two-way ANOVA with Tukey's post-t test for human analyses focused on a single individual before and after a treatment intervention. Data are presented as individual data points and mean ± SEM. $p < 0.05$ was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing interests:

D.J.C. is employed by Janssen Research and Development, Spring House, PA, which developed the anti-IL-23 antibody guselkumab. D.H.K serves on the Janssen Global Medical Affairs IL-23 Pathway Advisory Board. L.K.F. has consulted or engaged in company-sponsored research for Arcutis, Dermavant, Boehringer Ingelheim, Bristol Myers Squibb, Amgen, Pfizer, AbbVie, Janssen, Novartis, Eli Lilly, UCB and Acelyrin. The other authors declare no competing interests.

Data and Materials Availability:

All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials.

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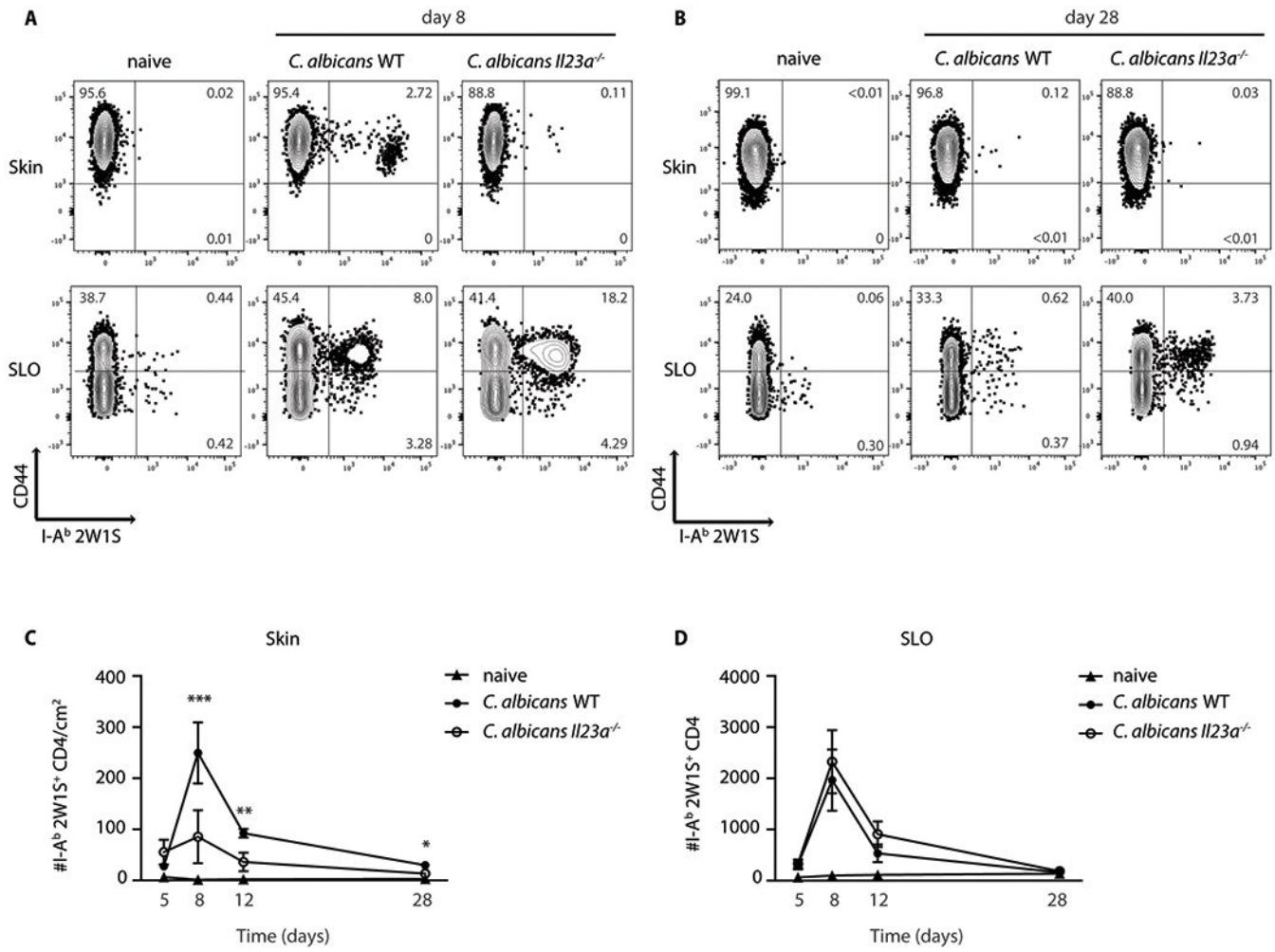

Figure 1: Accumulation of *C. albicans*-specific CD4$^+$ T cells in skin but not in secondary lymphoid organs requires IL-23.

C57BL/6 or *Il23a*$^{-/-}$ mice were infected on their shaved dorsum with 2x10^8 CFU recombinant *C. albicans* expressing 2W1S peptide. (A) Representative flow plots displaying *C. albicans*-specific 2W1S$^+$CD4 T cells on day 8 or (B) day 28 post infection *C. albicans*. Plots are gated on live TCRβ$^+$ CD4$^+$ T cells from skin and secondary lymphoid tissue (SLO) as indicated. Absolute number of *C. albicans*-specific I-A^{b}2W1S$^+$ CD4$^+$ T cells in skin (C) and SLO (D) are shown at the indicated day post infection. Data are expressed per unit area of skin (C) or per mouse (D). Results are representative of at least 2 independent experiments with 2-4 mice per cohort and are represented as mean ± SEM. Significance was calculated using unpaired Student's t-test, *p<0.05, ****p*<0.001.

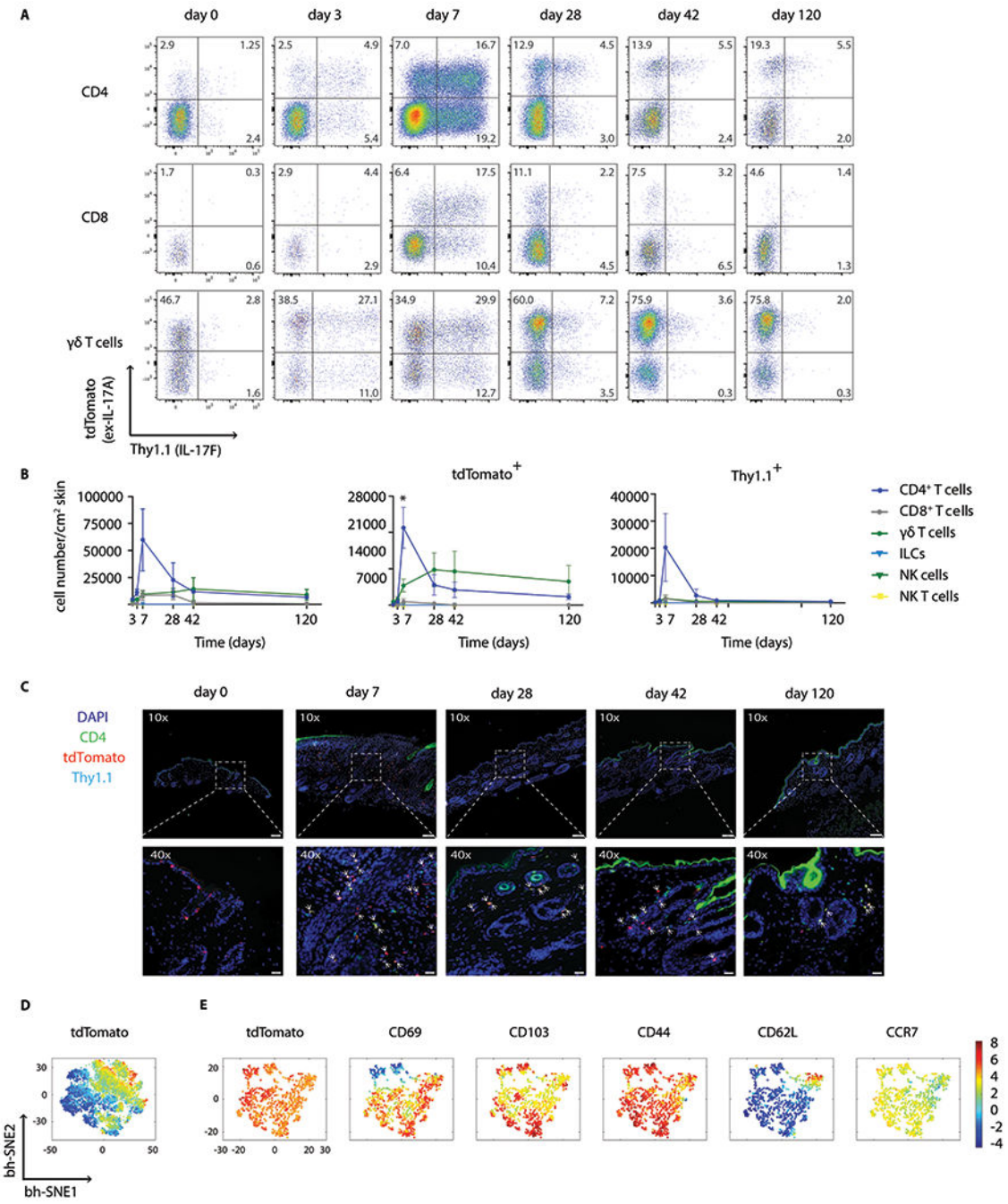

Figure 2: *C. albicans* skin infection generates long-lived cutaneous T_{RM17}.

(A) Expression of tdTomato and Thy1.1 (IL-17F) by live T cells ($LIN^-CD45^+CD90.2^+$ β or γδ TCR^+), innate lymphoid cells (ILCs; $LIN^-CD45.2^+$ $CD90.2^+$ $TCR\beta^-TCR\gamma\delta^-$), natural killer (NK) cells ($LIN^-CD45.2^+CD3^-NK1.1^+$), and NK T cells ($LIN^-CD45.2^+CD3^+NK1.1^+$) harvested from skin of 17Tracker reporter mice at the indicated day after epicutaneous infection with $2x10^8$ CFU *C. albicans*. (B) Total cell number (left), total number of $tdTomato^+$ cells (middle) and total number of $Thy1.1^+$ cells (right) per cm^2 of skin as in (A). (C) Immunofluorescent microscopic visualization

of tdTomato (red), CD4 (green), Thy1.1 (cyan), and DAPI nuclear label in dorsal skin of 17Tracker reporter mice at the indicated times after infection. Lower panels represent magnified views of the dashed square show in the top panel. Cells co-expressing DAPI, tdTomato, and CD4 are denoted by arrows. (D-E) viSNE analysis was performed on day 42 TCRβ^+ cells in skin as in (A), manually gated on $CD4^+$ T cells (D) or tdTomato$^+$ $CD4^+$ T cells (E). Default computational parameters were used as per Amir *et al*(90) with heatmap overlays. Results represent mean ± SEM from 4-6 independent experiments with 1-2 mice/group. Scale bars represent 100 μm (C, upper panels) and 20 μm (C, lower panels).

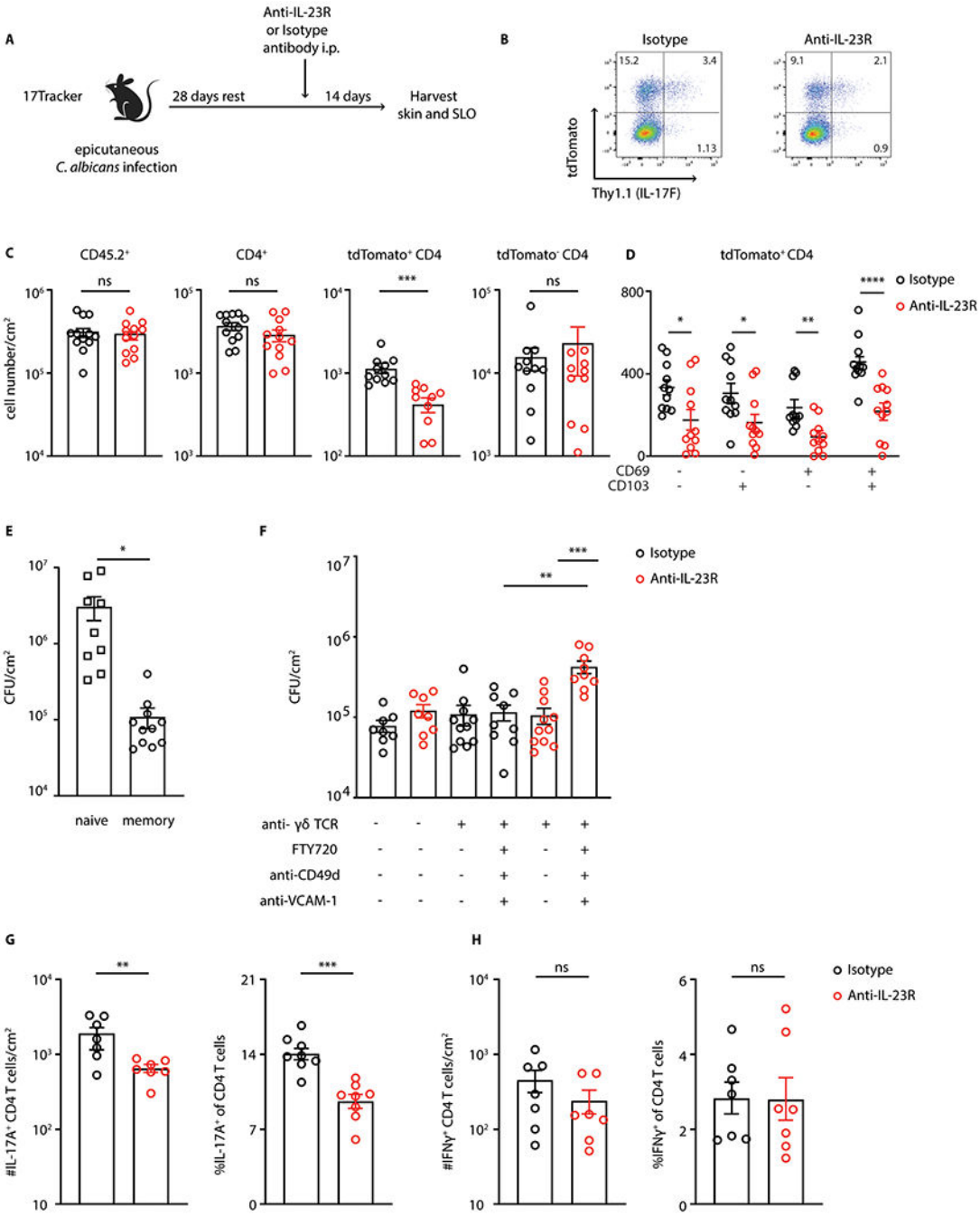

Figure 3: IL-23 is required for retention of skin-resident memory Th17 cells and T_{RM17} mediated protection from *C. albicans*.

(A) Experimental scheme for assessment of memory T cell response to *C. albicans*. 17Tracker mice were epicutaneously infected with *C. albicans* and 28 days later, isotype control or anti-IL-23R neutralizing antibody (700 μg) was administered via intraperitoneal injection. 2 weeks later immune cells were isolated from whole skin and secondary lymphoid organs (SLO) and immune cell frequency, number, and phenotype were assessed. (B) Representative flow plots gated on live $CD4^+$ T cells. (C, D) Total numbers of the indicated cell type per cm^2 skin after isotype antibody (black circles) or anti-IL-23R

treatment (red circles), assessed by flow cytometry at day 42 post-infection. (E) Cohorts of C57/BL6 mice were either uninfected (naïve) or infected on their shaved dorsum with $2x10^8$ CFU *C. albicans* (memory), and 42 days later rechallenged epicutaneously with *C. albicans*. CFU per area from homogenized skin 24 hours after infection is shown. (F) *C. albicans* CFUs isolated from memory mice as in (A) 24 hours after reinfection. Previously infected memory mice (as in E) were injected intraperitoneally with a single dose of isotype (black circles) or anti-IL-23R antibody (red circles) on day 28 post-infection. In addition, mice were given 500μg of TCRγδ T cell depleting antibodies, anti-VCAM-1 and CD49d (500μg), and FTY720 as indicated. CFU/cm^2 of skin 24 hours after a second *C. albicans* infection is shown. (G) Absolute number (left) and frequency (right) of IL-17A-expressing and (H) IFNγ-expressing live PMA/Ionomycin stimulated $TCR\beta^+CD4^+$ T cells isolated from whole skin of memory mice treated with FTY720, VCAM-1, CD49d, and anti-IL-23R antibody in (F) are shown. All data points represent individual animals. Data are representative of 3 independent experiments with cohorts of 3-5 mice. Significance was calculated using unpaired Student's t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

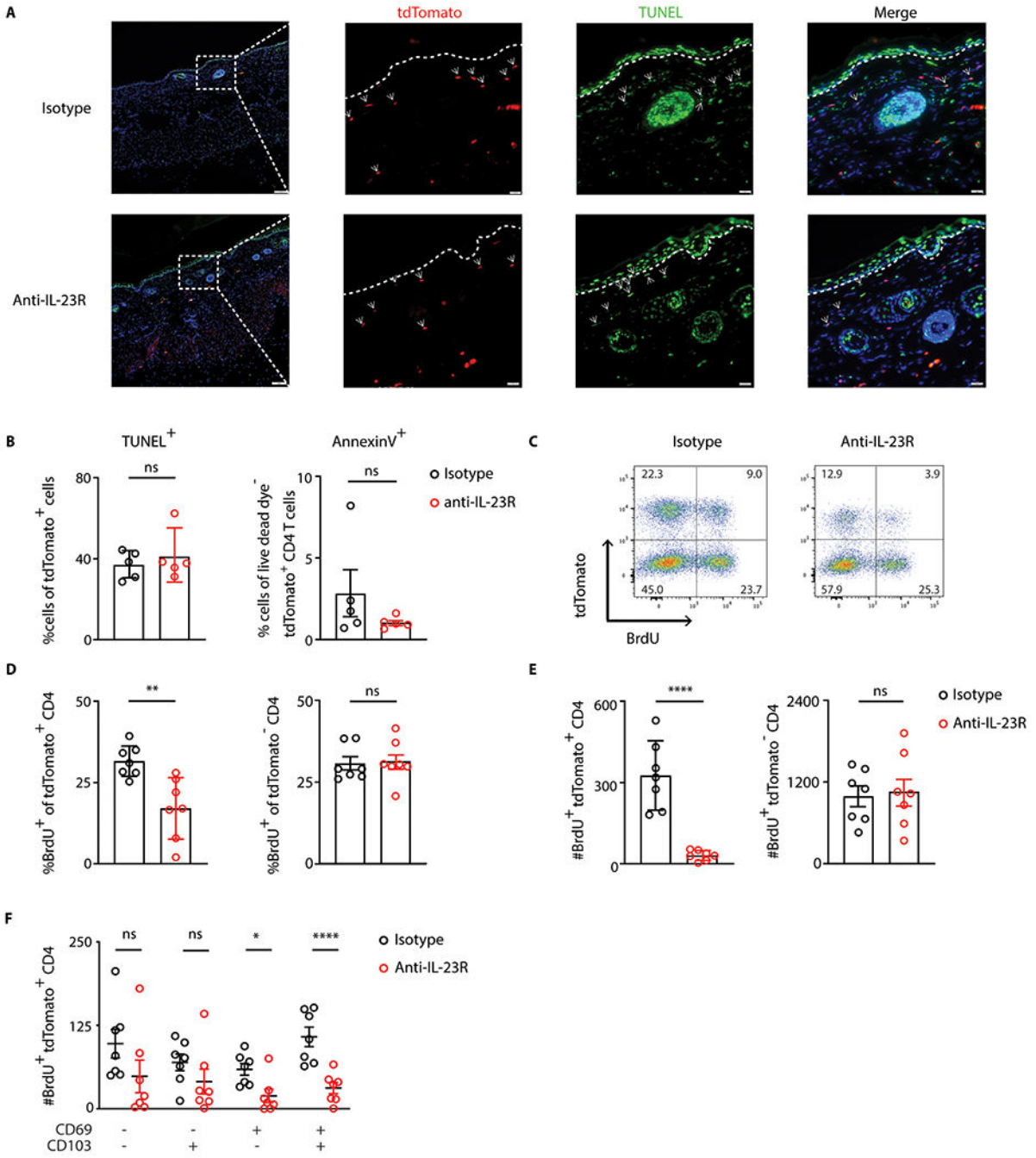

Figure 4: IL-23 promotes *in situ* proliferation of T_{RM17} in skin.

(A) Representative *in situ* terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay of dorsal skin obtained from isotype- and anti-IL-23R antibody-treated 17Tracker mice on day 42 post infection showing incorporation of TUNEL reagent (green), tdTomato (red) and DAPI nuclear stain (blue). Arrows denote cells positive for tdTomato, TUNEL, or merged image as indicated. (B) Quantification of $TUNEL^+$ (left) $tdTomato^+$ cells show in (A), and Annexin V^+ (right) viability dye^- cell quantification of $tdTomato^+$ $CD4^+$ by flow cytometry on day 42 post infection. (C) Analysis of BrdU incorporation by

tdTomato$^+$ and tdTomato$^-$ CD4$^+$ T cells after *in vivo* administration of BrdU days 28-42 post-infection with *C. albicans*. Representative flow plots gated on CD4$^+$ TCRβ$^+$ T cells are shown. (D) Quantification showing the percentage of BrdU$^+$ tdTomato$^+$ (left) and tdTomato$^-$ (right) cells are shown. (E) As in (D) showing total numbers of cells. (F) As in (E) showing number of BrdU$^+$ tdTomato$^+$ cells based on expression of CD69 and CD103. All data points represent individual animals. Data are representative of 3 independent experiments with cohorts of 3-5 mice. Significance was calculated using unpaired Student's t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

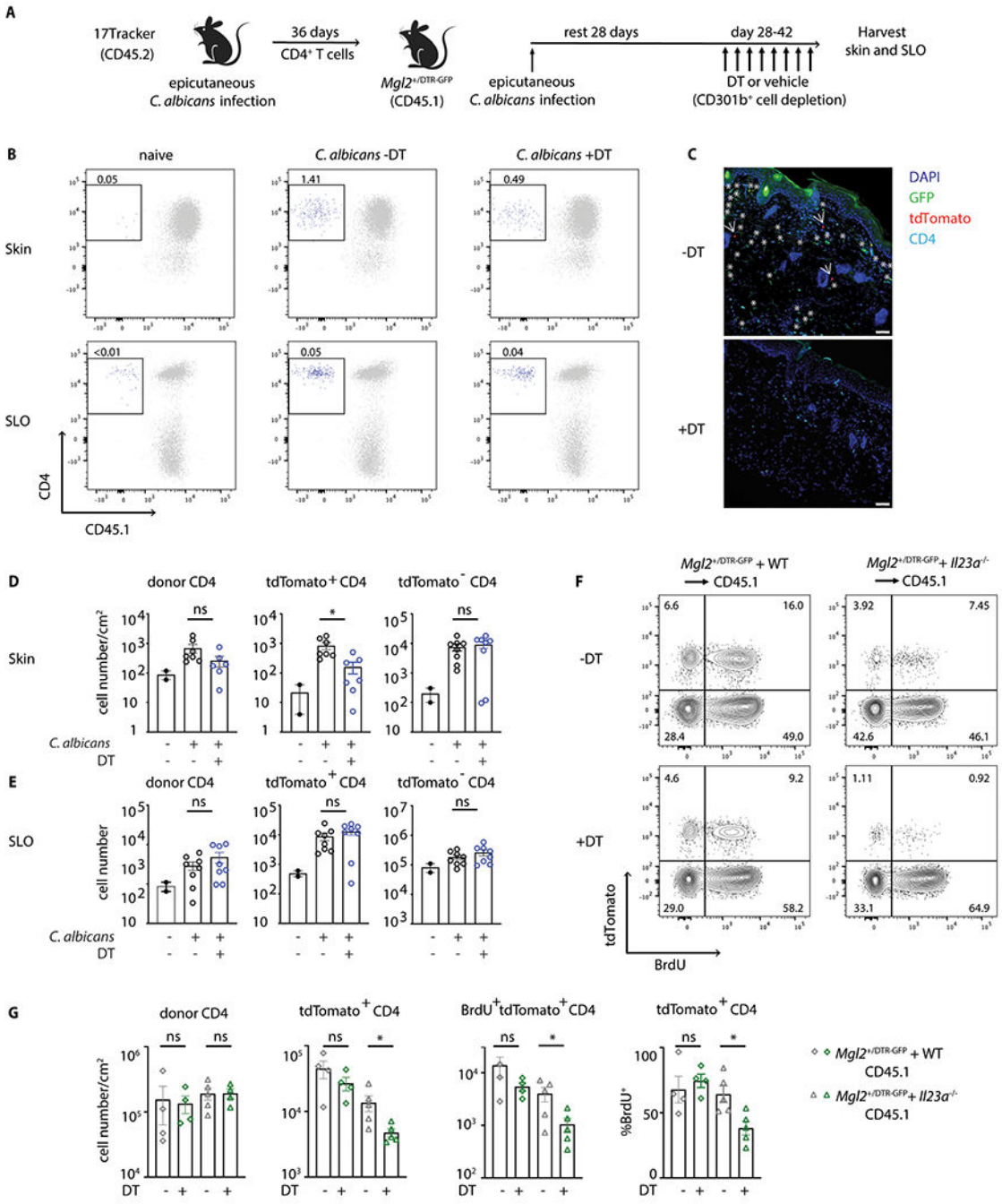

Figure 5: IL-23 from CD301b$^+$ myeloid cells is required for maintenance of cutaneous T_{RM17}.

(A) CD45.2$^+$ 17Tracker mice were epicutaneously infected with *C. albicans* and 36 days later, CD4$^+$ T cells were isolated from pooled spleen and lymph nodes. 1-2 x10^7 CD4$^+$ T cells were adoptively transferred into naïve congenic (CD45.1$^+$) *Mgl2*$^{+/DTR-GFP}$ recipients, and epicutaneously infected 1 day later with *C. albicans*. *Mgl2*$^{+/DTR-GFP}$ recipients were allowed to rest for 28 days, then 1 μg (loading dose) followed by 100 ng (maintenance dose) of diphtheria toxin (DT) was administered every 3-4 days via intraperitoneal injection to deplete CD301b$^+$ cells. On day 42 immune cells were isolated from whole skin and

SLO and T cell frequency, number, and phenotype were assessed. (B) Representative flow cytometry plots gated on live CD90.2^{+}TCRβ^{+} T cells from skin (top) and SLO (bottom) of *Mgl2*$^{+/DTR\text{-}GFP}$ mice that were sham infected (naïve, left) or infected with *C. albicans* in the absence (middle, -DT) or presence (right, +DT) of DT treatment are shown. CD45.2^{+} adoptively transferred 17Tracker cells are gated. (C) Immunofluorescent visualization of GFP (CD301b), CD4, and tdTomato (IL-17A) reporter expression in skin of DT and vehicle-treated mice at day 42 post-infection are shown. Arrows denote tdTomato^{+} cells and stars denote GFP^{+} CD301b-expressing cells. (D) Summary data from (B) of total numbers of the indicated cell type in skin per cm^{2} of skin as assessed by flow cytometry is shown. (E) As in (D) showing total cell numbers from the SLO. (F,G) Lethally irradiated B6.SJL-Ly5.2 (CD45.1) mice were reconstituted with a 1:1 ratio of bone marrow from *Mgl2*$^{+/DTR\text{-}GFP}$ mice and either WT or *Il23a*$^{-/-}$ mice. After 12 weeks, mice were subject to *C. albicans* infection and adoptive transfer of CD45.2^{+} 17Tracker CD4^{+} T cells as in (A). BrdU and DT or vehicle (PBS) were administered days 28-42 post-infection as in (A). (F) Representative flow plots gated on live CD4^{+} T cells isolated from skin 42d post-infection. (G) Total numbers of the indicated cell type per cm^{2} skin, assessed by flow cytometry at day 42 post-infection. All data points represent individual animals. Data are representative of at least 2 independent experiments with cohorts of 2-5 mice. Significance was calculated using unpaired Student's t-test, *$p<0.05$, **$p<0.01$.

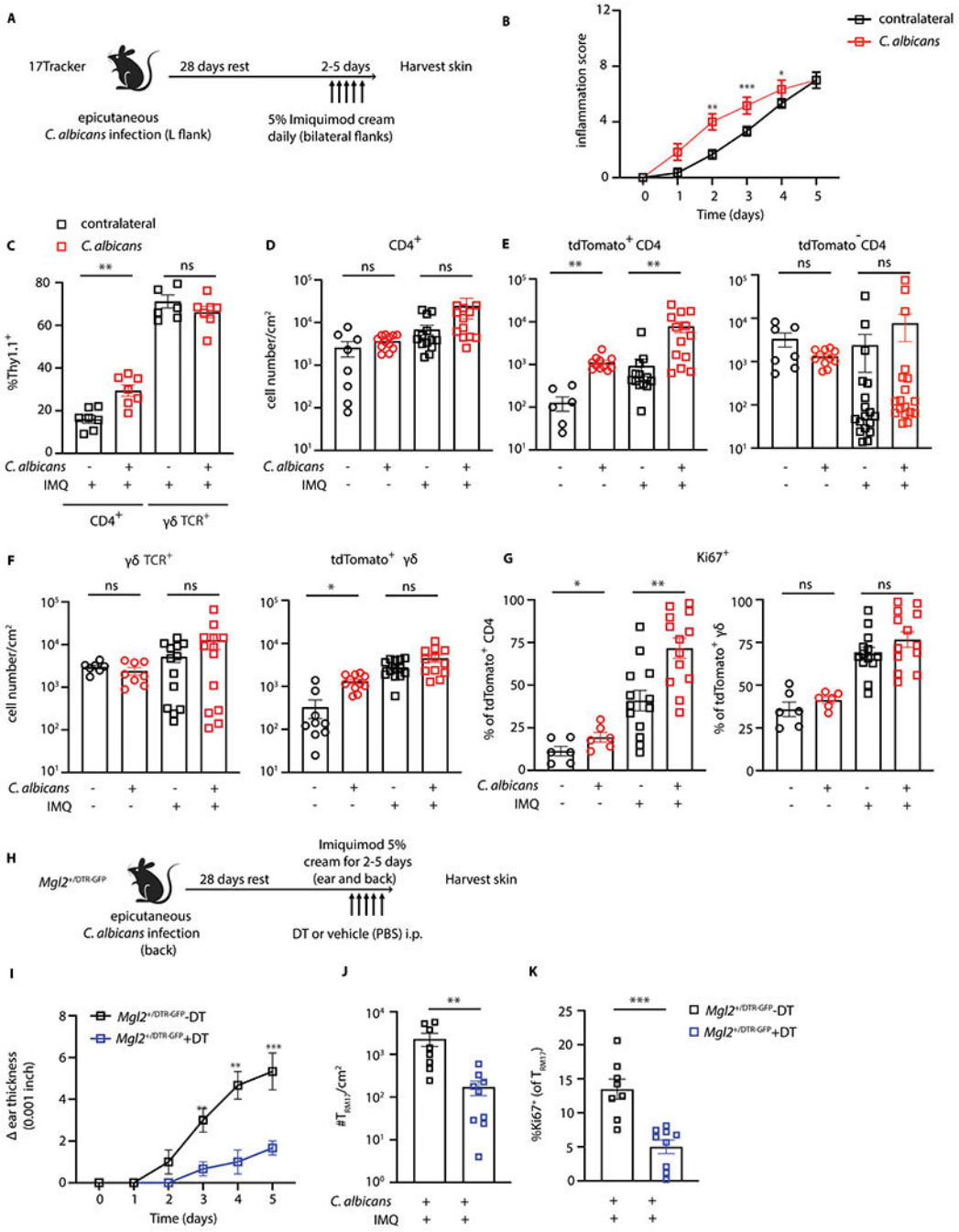

Figure 6: CD301b$^+$ myeloid cells induce T_{RM17} proliferation to accelerate development of psoriasiform dermatitis.

(A) Experimental scheme for assessment of psoriasiform dermatitis. 17Tracker mice were epicutaneously infected with *C. albicans* on the right flank and starting 28 days later, 5% imiquimod cream was applied to shaved skin on the right and left flanks daily for 5 days. Erythema (0-4), scaling (0-4), and thickness (0-4) of the back skin were assessed daily using a modified PASI scoring system as previously described(68). (B) Cumulative inflammation score of 0-12 is depicted for previously infected (red) or naïve (black) mice. (C) Frequency of Thy1.1 (IL-17F)$^+$ CD4$^+$ T cells (left) and dermal TCRγδ cells (right) isolated from

back skin previously (red) or naïve (black) mice as determined by flow cytometry. (D) As in (C) showing total numbers of $CD4^+$ T cells, $tdTomato^+$ $CD4^+$ T cells (E, left) and $tdTomato^-$ $CD4^+$ T cells (E, right) in mice treated with imiquimod or previously infected with *C. albicans* as indicated. (F) As in C, showing total numbers of dermal $TCR\gamma\delta^+$ T cells and tdTomato $TCR\gamma\delta^+$ T cells. (G) Frequency of proliferating $Ki67^+$ $tdTomato^+$ $CD4^+$ T cells (left) and $TCR\gamma\delta^+$ T cells. (H) *Mgl2*$^{+/DTR\text{-}GFP}$ mice were infected with *C. albicans* and starting 28 days later, 5% imiquimod cream was applied daily to ear and shaved back skin for 5 consecutive days. On days −1 and +3, diphtheria toxin (DT) was administered to deplete $CD301b^+$ cells. (I) Ear thickness at the indicated time points during imiquimod treatment is shown. (J) Absolute number of $CD69^+CD103^+IL\text{-}17A^+$ $CD4^+$ T cells and (K) $Ki67^+$ expression in $tdTomato^+$ $CD4^+$ T cells present in skin of DT (+DT) or vehicle-treated (−DT) mice on day 3 of imiquimod treatment is shown. All data points represent individual animals. All data are representative of 3 independent experiments with cohorts of 2-6 mice. Significance was calculated using paired (B) or unpaired (C-K) Student's t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

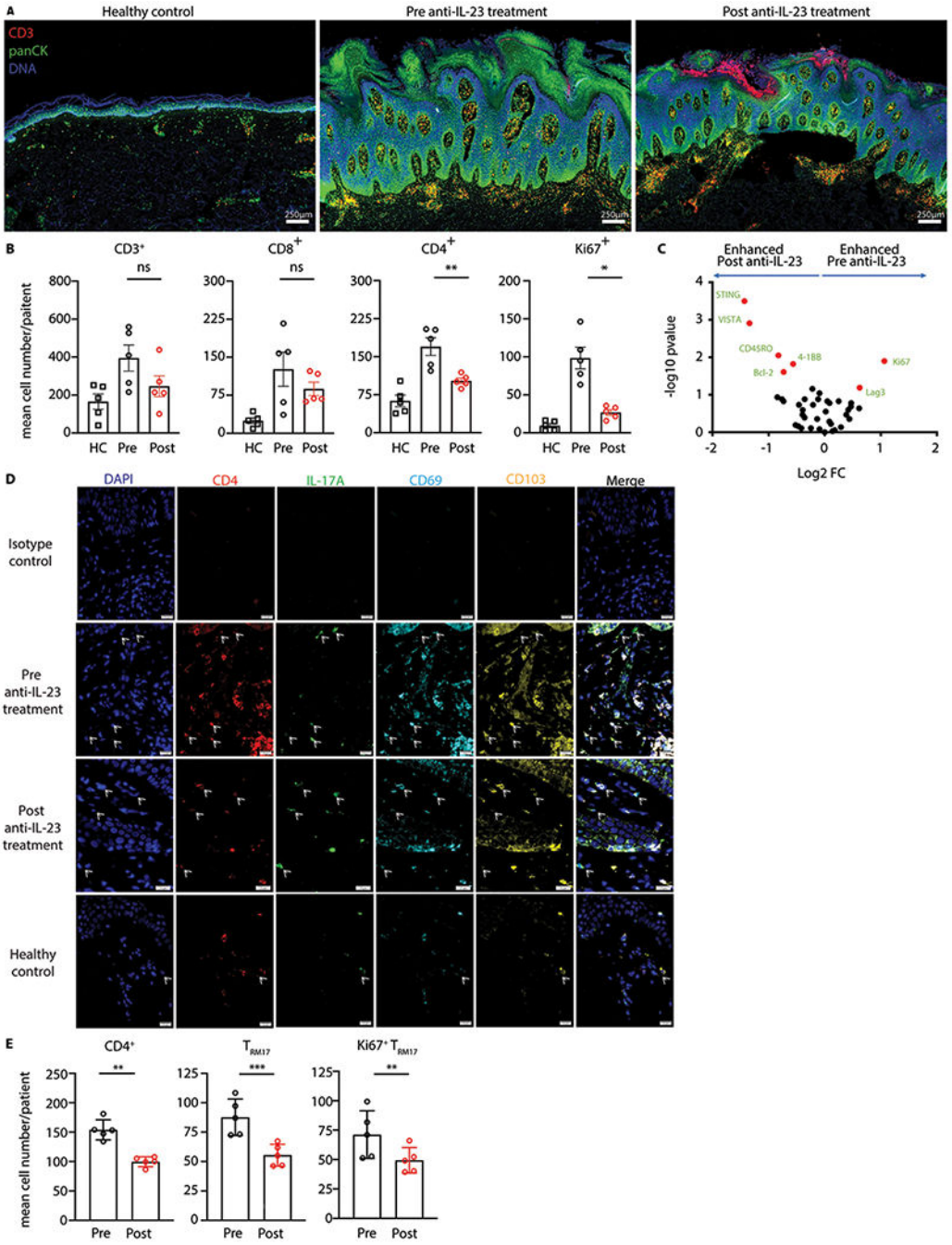

Figure 7: Clinical anti-IL-23 therapy depletes T_{RM17} from psoriatic skin.

(A) Representative immunofluorescent staining from healthy control (left), lesional skin from a single psoriatic plaque before (middle) and 1 week after (right) initiation of systemic treatment with an anti-IL-23 monoclonal antibody (150 mg risankizumab or 100 mg guselkumab). Pan-cytokeratin (green), CD3 (red), and nuclear stain (blue). Scale bar 250 μm. (B) Absolute numbers of the indicated cell type or protein assessed by NanoString GeoMX™ Digital Spatial Profiler (DSP) protein quantification of healthy control (HC), psoriasis pre-anti-IL-23 (Pre), or post anti-IL-23 (Post) treatment skin. (C) Volcano plot

displaying proteins expressed by $CD3^+$ cells satisfying threshold criteria of >0.5 fold change (x axis) and −log10 p-value >1 (y-axis). (D) Representative immunofluorescent microscopic images of samples as in (A) showing expression of CD4 (red), IL-17A (green), CD69 (cyan), CD103 (yellow) and DAPI nuclear stain (blue). Scale bar 20 μm. Arrows denote $CD4^+IL\text{-}17A^+CD69^+CD103^+DAPI^+$ cells. (E) Quantification of numbers of the indicated cells as in (D). Data are representative of 3 independent experiments and include specimens from 5 individual psoriasis patients analyzed pre and post anti-IL-23 treatment. Each data point (B,E) represents an individual patient, with an average of four high powered fields quantified per patient. Significance was calculated using two-way ANOVA with Tukey's post-t test, *$p<0.05$, **$p<0.001$, ***$p<0.001$.