ORIGINAL ARTICLE

Keratinocytes Present *Staphylococcus aureus* Enterotoxins and Promote Malignant and Nonmalignant T Cell Proliferation in Cutaneous T-Cell Lymphoma

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Cutaneous T-cell lymphoma is characterized by malignant T cells proliferating in a unique tumor microenvironment dominated by keratinocytes (KCs). Skin colonization and infection by *Staphylococcus aureus* are a common cause of morbidity and are suspected of fueling disease activity. In this study, we show that expression of HLA-DRs, high-affinity receptors for staphylococcal enterotoxins (SEs), by KCs correlates with IFN-γ expression in the tumor microenvironment. Importantly, IFN-γ induces HLA-DR, SE binding, and SE presentation by KCs to malignant T cells from patients with Sézary syndrome and malignant and nonmalignant T-cell lines derived from patients with Sézary syndrome and mycosis fungoides. Likewise, preincubation of KCs with supernatant from patient-derived SE-producing *S aureus* triggers proliferation in malignant T cells and cytokine release (including IL-2), when cultured with nonmalignant T cells. This is inhibited by pretreatment with engineered bacteriophage *S aureus*—specific endolysins. Furthermore, alteration in the HLA-DR–binding sites of SE type A and small interfering RNA–mediated knockdown of Jak3 and IL-2Rγ block induction of malignant T-cell proliferation. In conclusion, we show that upon exposure to patient-derived *S aureus* and SE, KCs stimulate IL-2Rγ/Jak3–dependent proliferation of malignant and nonmalignant T cells in an environment with nonmalignant T cells. These findings suggest that KCs in the tumor microenvironment play a key role in *S aureus*–mediated disease activity in cutaneous T-cell lymphoma.

Keywords: Cutaneous T-cell lymphoma, Keratinocyte, *Staphylococcus aureus*

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Abbreviations: CTCL, cutaneous T-cell lymphoma; KC, keratinocyte; MF, mycosis fungoides; NHEK, normal human epidermal keratinocyte; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome; STAT, signal transducer and activator of transcription; TME, tumor microenvironment

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INTRODUCTION

Cutaneous T-cell lymphomas (CTCLs) represent a group of lymphoproliferative neoplasms characterized by the presence of malignant T cells in the skin. Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common and aggressive leukemic forms of CTCL, respectively (Assaf et al, 2021; Willemze et al, 2019). The etiology is still largely unknown, and the pathogenesis is only partially understood (Dummer et al, 2021) partly because of a considerable disease heterogeneity (Litvinov et al, 2017). Although CTCL does not seem to be inherited (Odum et al, 2017), both genetic, epigenetic, and environmental factors have been implicated (Ghazawi et al, 2019; Han et al, 2023, 2022; Ødum and Geisler, 2024; Tensen et al, 2022; Weiner et al, 2021). Recent data suggest that certain genetic events involving fusion proteins such as Jak (Jak3–INSL3) are more prevalent than initially anticipated (Velatooru et al, 2023), indicating that genetic events may indeed play a key role in the pathogenesis (Choi et al, 2015; da Silva Almeida et al, 2015; Dobos and Assaf, 2022; Ungewickell et al, 2015).

It is well-established that infection is frequent and is a common cause of morbidity and mortality in CTCL, particularly in patients with advanced disease (Allen et al, 2020;

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Axelrod et al, 1992; Bonin et al, 2010; Dai and Duvic, 2023; Dobos and Assaf, 2022; Mirvish et al, 2011; Willerslev-Olsen et al, 2013), and is possibly associated with a deficient antimicrobial defense (Wolk et al, 2014). In a Danish twin study, we obtained evidence that susceptibility to severe bacterial infections in patients with CTCL was not genetically determined but rather a vulnerability, which developed gradually after the diagnosis had been established (Odum et al, 2017). In support, recent findings indicate that malignant T cells orchestrate changes in the tumor microenvironment (TME), leading to repression of skin barrier proteins such as FLGs and in turn a compromised skin barrier (Gluud et al, 2023). Of note, FLG elements have anti—*S aureus* properties, and skin lesions are typical ports of entry for bacteria such as *Staphylococcus aureus*, which is the most common pathogen to colonize lesional skin in patients with CTCL (Axelrod et al, 1992; Jackow et al, 1997; Nguyen et al, 2008). Importantly, the risk of skin colonization by *S aureus* increases with disease progression, and between one and two thirds of patients with advanced disease harbor *S aureus*, a large proportion of which produce toxins such as staphylococcal enterotoxins (SEs) (Willerslev-Olsen et al, 2013). In addition to the risk of serious invasive infections, colonization of skin lesions by SE-producing *S aureus* is also suspected to fuel disease activity and enhance drug resistance in malignant T cells (Kadin et al, 2020; Vadivel et al, 2024; Willerslev-Olsen et al, 2013). Of note, bacteria accelerate and aggravate disease progression in a mouse model of CTCL (Fanok et al, 2018), and antibiotic treatment often reduces skin symptoms and improves disease (Lindahl et al, 2019) at least transiently until the skin become recolonized by *S aureus* (Lindahl et al, 2022). Importantly, eradication of SE-producing *S aureus* from lesional skin by aggressive antibiotic treatment of patients with advanced disease not only reduced skin symptoms and clinical disease activity but also decreased cell proliferation and the fraction of malignant T cells in lesional skin (Lindahl et al, 2019), supporting the hypothesis that SE-producing *S aureus* can fuel disease activity and disease progression (Willerslev-Olsen et al, 2013).

As mentioned earlier, it is a characteristic feature of CTCL, particularly in MF, that predominantly the malignant but also the nonmalignant T cells tend to silently invade the epidermis without other histologically visible signs of inflammation (a phenomenon named epidermotropism) (Edelson, 1980) and, at least in the initial stage of the disease, proliferate in a TME dominated by keratinocytes (KCs) (Nickoloff and Griffiths, 1990). Recently, we discovered that malignant T cells induced the repression of FLG in KCs (Gluud et al, 2023), and single-cell sequencing of CTCL skin lesions identified distinct gene expression patterns in lesional KCs, suggesting that crosstalk takes place between malignant T cells and KCs (Calugareanu et al, 2023; Gaydosik et al, 2023; Rindler et al, 2021a, 2021b). As the most abundant cell type in the epidermis, KCs are among the first to encounter *S aureus* and its toxins upon their colonization of lesional skin. It has been known for decades that KCs in lesional skin often express HLA class II molecules (such as HLA-DR) (Auböck et al, 1986; Niedecken et al, 1988; Wood et al, 1994), which are high-affinity receptors for SE (Chintagumpala et al, 1991; Scholl et al, 1989). Accordingly, this investigation was undertaken to unravel how *S aureus* and SE impact the interplay between KCs and malignant and nonmalignant T cells. We provide the evidence that KCs can present patient-derived *S aureus* and purified SE to drive signal transducer and activator of transcription (STAT) activation and proliferation of malignant T cells in cultures with nonmalignant T cells and that the response to *S aureus* can be blocked by pretreatment with *S aureus*—specific endolysins.

RESULT

HLA-DR expression by lesional KCs correlates with IFN-γ expression in the TME

HLA class II are high-affinity receptors for SE (Johnson et al, 1991). Because KCs are the first to encounter SE and other staphylococcal toxins in the skin, we asked whether KCs express HLA-DR in lesional skin of patients with CTCL. As judged from public single-cell RNA-sequencing data, lesional KCs expressed significantly higher levels of *HLA-DRA* than KCs from healthy skin, which were largely negative (Figure 1a—d). Importantly, in all the examined cohorts of patients with CTCL, we observed a heterogeneous *HLA-DRA* expression pattern (Figure 1d), which was consistent with previous immunohistochemical studies on HLA-DR expression in CTCL (Niedecken et al, 1988; Wood et al, 1994). IFN-γ is a known inducer of HLA-DR in KCs, but the role of IFN-γ is far from understood in CTCL. Although it is well-established that disease progression is associated with an increase in T helper type 2 cytokines (IL-4, IL-5, IL-13) (Guenova et al, 2013; Papadavid et al, 2003; Vowels et al, 1992), several earlier studies reported that *IFNG* (and IP-10) are expressed in CTCL skin lesions (Daliani et al, 1998; Dong et al, 2018; Gluud et al, 2023; Nickoloff et al, 1994; Sarris et al, 1995; Vowels et al, 1994). In our analysis, we found that *IFNG* was expressed in lesional skin in a substantial fraction of patients with CTCL (Figure 1e). When compared with expression in healthy skin, a significant increase in *IFNG* expression was found in the skin of some patients at all stages of the disease (Figure 1e). Because IFN-γ is a potent inducer of HLA-DR expression in KCs, we hypothesized that heterogeneous *HLA-DRA* expression could reflect the heterogeneous IFN-γ expression pattern in the TME. In support, there was a positive correlation between *IFNG* and *HLA-DRA* expression in KCs from patients with CTCL (Figure 1f) ($P = .005$).

KCs present SEs to T cells in CTCL

To address the functionality of HLA class II—positive KCs, we established a culture system as illustrated in Figure 2a. First, we treated KCs with IFN-γ to induce HLA class II expression (Figure 2b) prior to incubation with biotinylated SE type A (SEA) and subsequent staining with APC-R700-streptavidin (Figure 2c). IFN-γ—treated KCs bound significantly higher amounts of biotinylated SEA in a concentration-dependent manner than untreated KCs (Figure 2c). Next, HaCaT cells were treated with IFN-γ and a pool of SEs prior to addition of malignant T cells (MyLa3675) and nonmalignant T cells (MyLa1850) from a patient with MF (Figure 2a and Supplementary Figure S1). As judged from Ki-67 staining, SE- and IFN-γ—treated KCs induced vigorous proliferation in malignant MF and SS T cells (Figure 2d—g), when compared with untreated KCs and KCs treated with only SE or IFN-γ

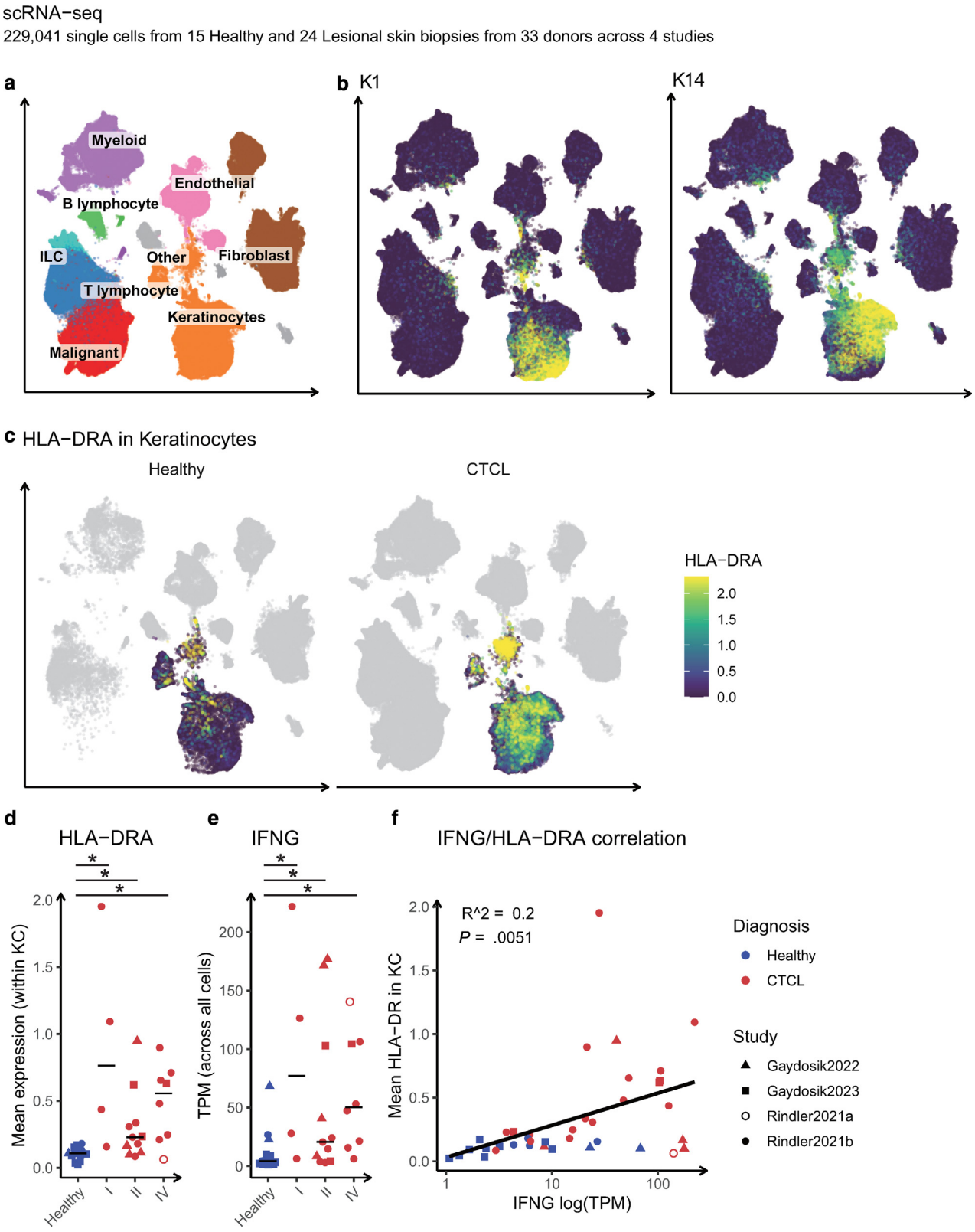


Figure 1. HLA-DR expression by lesional KCs correlates with IFN-γ in the TME. scRNA-seq data from 39 skin biopsies across 4 published studies were integrated using scANVI, visualized using Uniform Manifold Approximation and Projection, and colored by (**a**) cell types annotation, (**b**) expression of *K1* and *K14*, and (**c**) normalized KC expression of *HLA-DRA* in biopsies from healthy controls and patients with mycosis fungoides. (**d**) Mean normalized expression of *HLA-DRA* within KCs. (**e**) *IFNG* transcripts normalized to the total TPMs within each biopsy (TPM). (**f**) Correlation between mean *HLA-DRA* expression in KCs and the *IFNG* expression (as TPM) for each biopsy. CTCL, cutaneous T-cell lymphoma; ILC, innate lymphoid cell; K1, keratin 1; K14, keratin 14; KC, keratinocyte; scRNA-seq, single-cell RNA sequencing; TME, tumor microenvironment; TPM, transcript per million.

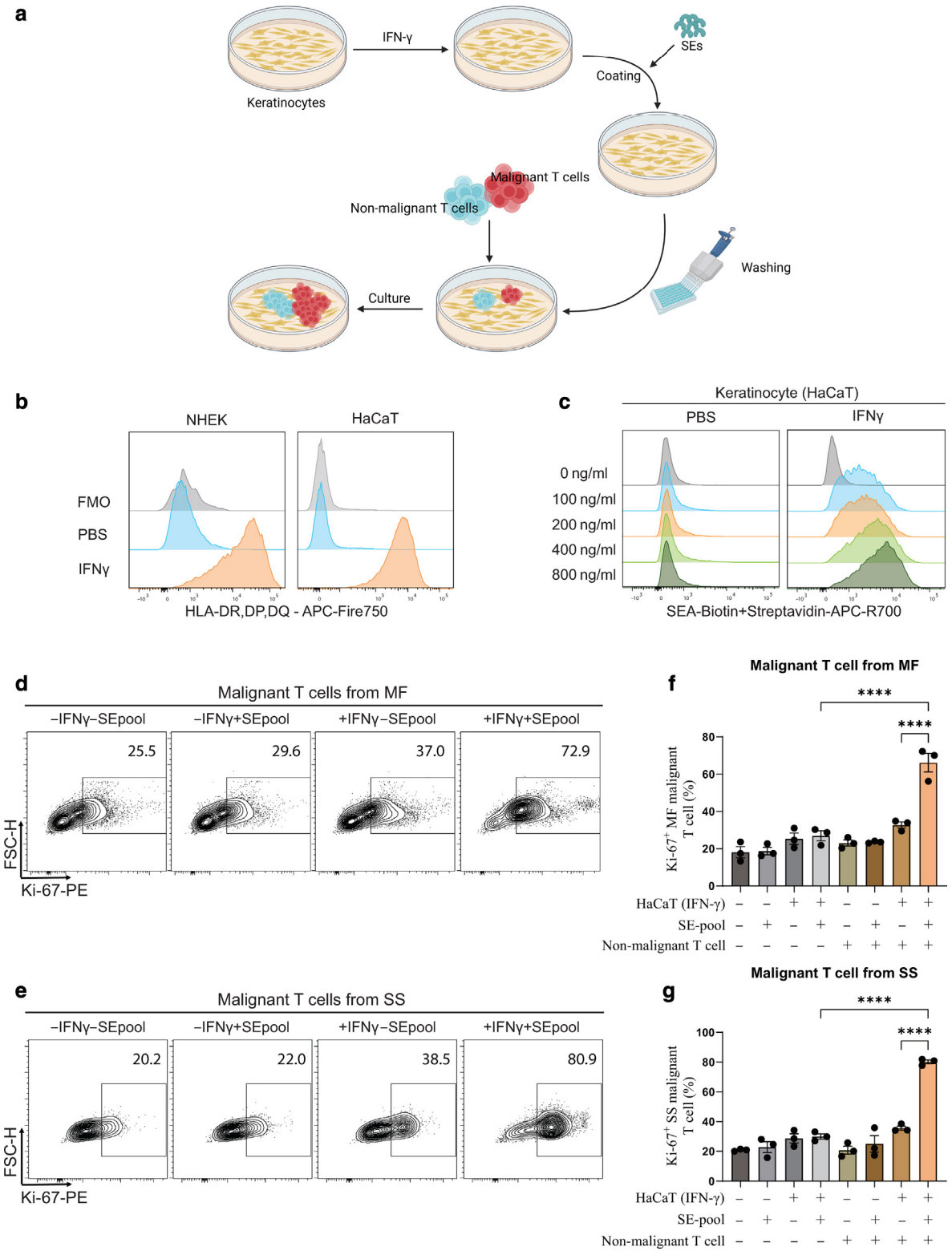


Figure 2. Keratinocytes present SEs to T cells in CTCL. (**a**) Schematic illustration of the culture setup. In short, keratinocytes were pretreated with 10 ng/ml IFN-γ prior to coating with SEs. After thorough washing, malignant and nonmalignant T cells from patients with CTCL were added and analyzed by flow cytometry. (**b**) NHEKs and HaCaT cells were treated with ±10 ng/ml IFN-γ for 24 hours and analyzed for expressions of HLA-DR, HLA-DP, and HLA-DQ using flow cytometry. (**c**) Keratinocytes (HaCaT) were treated with ±10 ng/ml IFN-γ and different concentrations of SEA-bio for 24 hours, and binding was assessed by flow cytometry. (**d**–**g**) Representative flow cytometric contour plots and quantification of Ki-67 expression in malignant T cells—(**d, e**) Myla3675 and (**f, g**) SeAx—after 24 hours culture with nonmalignant T cells (Myla1850) and SE-coated keratinocytes (HaCaT) (n = 3). (**h, i**) Flow cytometric contour plots of Ki-67 expression in malignant T cells—(**h**) Myla3675 and (**i**) SeAx—cultured 24 hours with nonmalignant T cells (Myla1850) and keratinocytes (HaCaT) pretreated with ±10 ng/ml IFN-γ and 100 ng/ml SEA^{wt} or $SEA^{F47A/D227A}$ for 24 hours. Bar plots show the mean ± SEM from 3 independent replicates. *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$. APC, allophycocyanin; CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; FSC-H, forward scatter height; NHEK, normal human epidermal

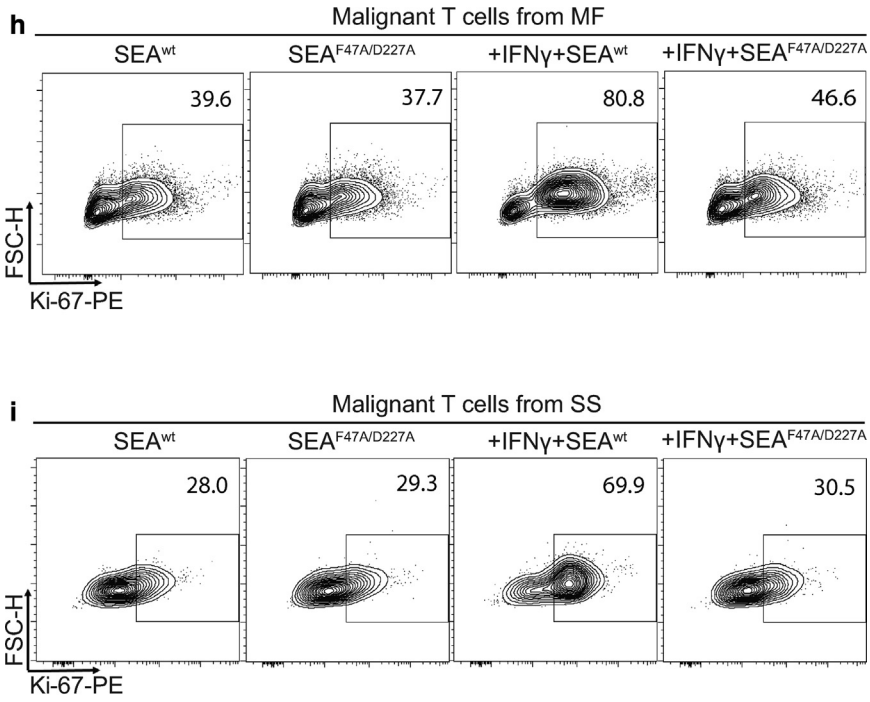


Figure 2. Continued.

(Figure 2d–g) and cultures without nonmalignant T cells (Supplementary Figure S2). Next, we compared the induction of proliferation by wild-type SEA and mutated SEA ($SEA^{F47A/D227A}$), which had lost the ability to bind HLA class II (Mehindate et al, 1995). As expected, $SEA^{wild\text{-}type}$ induced vigorous proliferation, whereas $SEA^{F47A/D227A}$ did not (Figure 2h and i), showing that HLA class II binding is a prerequisite for SEA presentation by KCs.

KCs stimulate IL-2Rγ/Jak-dependent proliferation of malignant T cells

To address how SE presentation induced malignant T-cell proliferation, cultures (as described earlier) were treated with and without the clinical grade pan-Jak inhibitor tofacitinib. As shown in Figure 3a and b, Jak inhibition blocked the SE-induced proliferation of malignant T cell lines. Likewise, small interfering RNA–mediated knockdown of *Jak3* and *IL2RG* in malignant T cells (Myla3675 and SeAx) (Supplementary Figure S3) significantly inhibited the proliferation induced by SE- and IFN-γ–treated KCs (Figure 3c–f). This indicates that KCs induce IL-2Rγ/Jak-dependent proliferation of malignant T cells. Because nonmalignant T cells in the cultures were rapidly induced to express *IL2* mRNA (Supplementary Figure S4), they are a likely source of GFs to stimulate IL-2Rγ/Jak-dependent proliferation in malignant T cells as described in other CTCL models (Woetmann et al, 2007).

SEs presented by primary KCs induce proliferation in primary malignant T cells

Because HaCaT cells are transformed KCs and therefore not representative for normal KCs, we also addressed whether primary KCs (normal human epidermal KCs [NHEKs]) were able to present SEs and, secondly, whether primary malignant $CD4^+$ T cells from peripheral blood of patients with SS were responsive to SE presentation by IFN-γ–treated NHEKs. IFN-γ–treated NHEKs were fully capable of presenting SEs to induce enhanced proliferation in malignant T cells (Myla3675 and SEAx) (Figure 4a) when cultured together with nonmalignant T cells (MyLa1850). Likewise, IFN-γ–treated NHEKs were able to induce SE-mediated proliferation of primary $CD4^+$ malignant T cells from all 4 investigated patients with SS (Figure 4b and c and Supplementary Figure S5).

KCs present SEs from patient-derived *S aureus* isolates to induce proliferation of malignant T cells

To examine the effect of SE-producing *S aureus* isolated from lesional skin, we expanded patient bacteria and used supernatants from SE-producing *S aureus* in our culture system as illustrated in Figure 5a. KCs treated with supernatant from *S aureus* induced high expression of pY-STAT3 and pY-STAT5 in both MF- and SS-derived malignant T cell lines (Figure 5b and c) and nonmalignant T cells (Supplementary Figure S6). Importantly, treatment of *S aureus* cultures with endolysin (XZ.700) blocked the effect (Figure 5b and c and Supplementary Figure S6). In parallel, KC presentation of patient-derived *S aureus* supernatants profoundly increased proliferation in both MF and SS cells (Figure 5d–g) and nonmalignant T cells (Supplementary Figure S7). Again, treatment of *S aureus* cultures with endolysin (XZ.700) blocked the effect (Figure 5d–g and Supplementary Figure S7). Similar results were obtained using other *S aureus*–specific recombinant endolysin (Supplementary

keratinocyte; PE, phycoerythrin; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; SEA-bio, biotinylated staphylococcal enterotoxin type A; SEA^{wt}, wild-type staphylococcal enterotoxin type A; SS, Sézary syndrome.

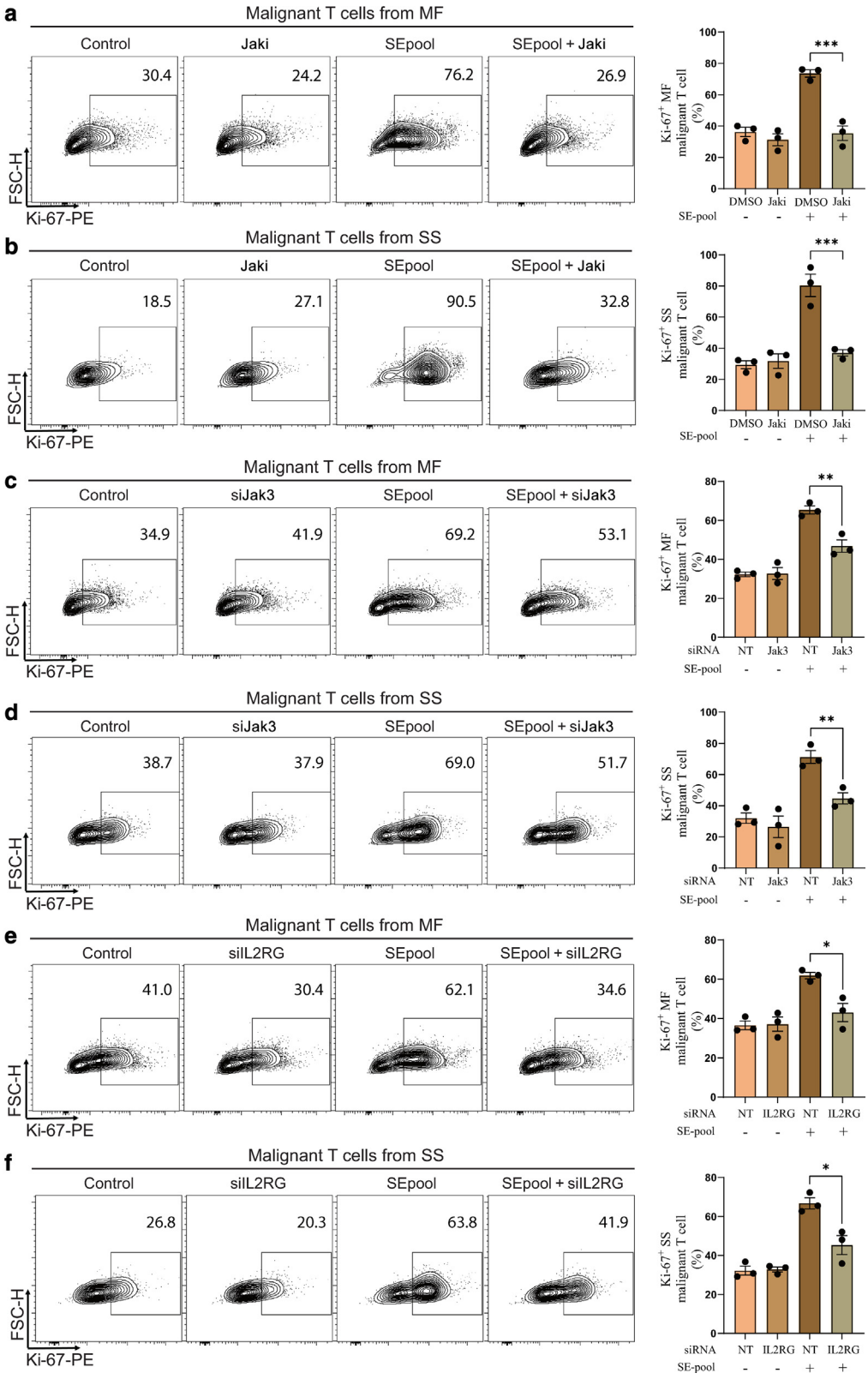


Figure 3. Keratinocytes stimulate IL-2Rγ/Jak-dependent proliferation of malignant T cells. (**a**–**f**) Representative flow cytometric contour plots and quantification of Ki-67 expression in malignant T cells. (**a**) Myla3675 and (**b**) SeAx after culture with nonmalignant T cells (Myla1850) and keratinocytes (HaCaT) treated with ±10 ng/ml IFN-γ and then coated with ±100 ng/ml SE pool in the presence of 1 μM tofacitinib (Jaki) or same amount of DMSO (n = 3). Transient transfection with siJak3 or siIL2RG or the same amount of NT siRNA control was performed in malignant T cells from (**c**–**e**) MF (Myla3675) or (**d**–**f**) SS (SeAx). After 48-hour incubation, transfected malignant T cells were further cultured for 24 hours with nonmalignant T cells (myla1850) and keratinocytes (HaCaT) treated with ±10 ng/ml IFN-γ and ±100 ng/ml SE pool (n = 3). Bar plots show the mean ± SEM from 3 independent replicates. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, and $^{****}P < .0001$. Jaki, Jak inhibitor; MF, mycosis fungoides; NT, nontargeting; PE, phycoerythrin; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; siIL2RG, IL2RG-targeted small interfering RNA; siJak3, Jak3-targeted small interfering RNA; siRNA, small interfering RNA; SS, Sézary syndrome.

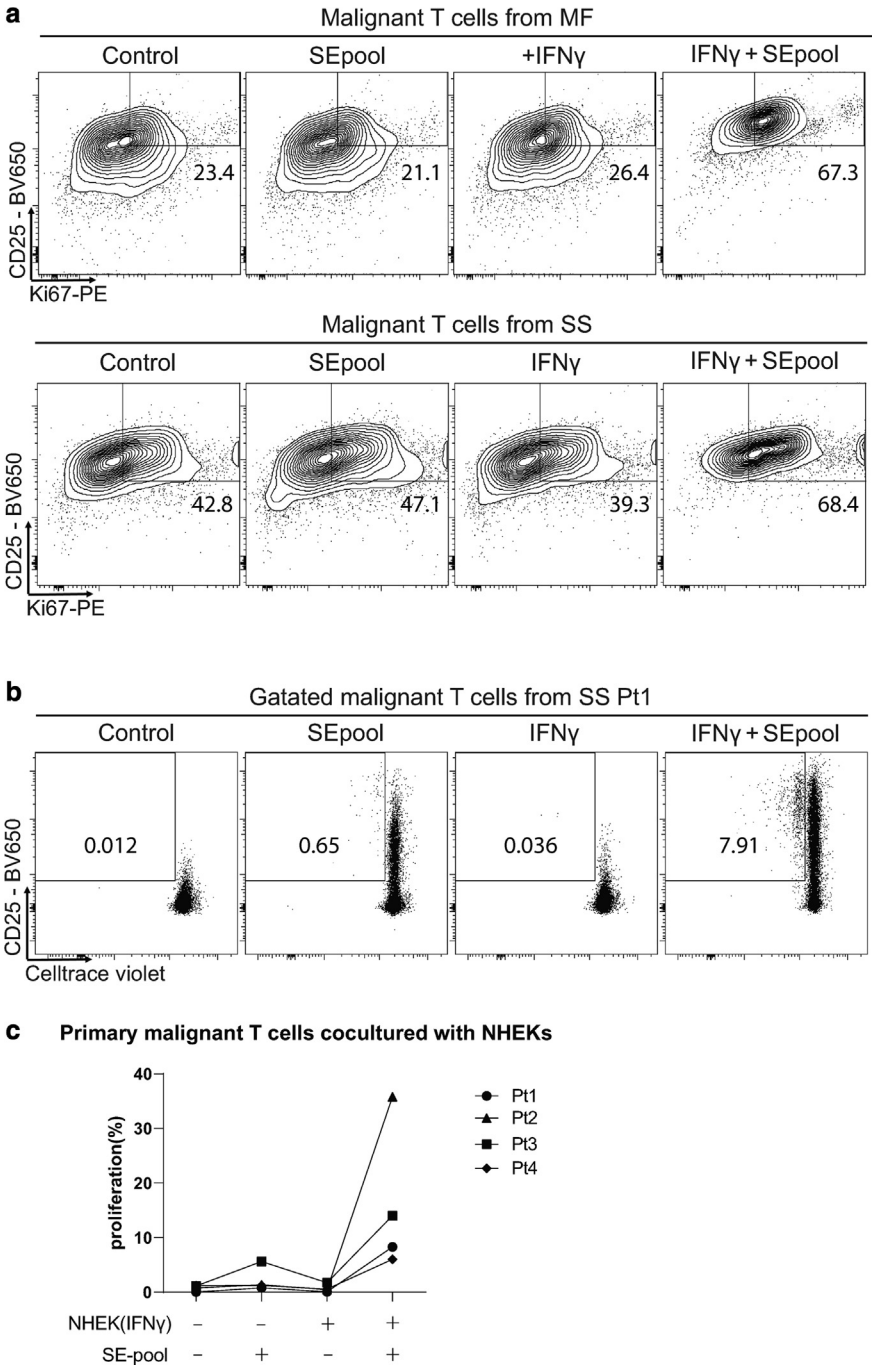


Figure 4. SEs presented by primary keratinocytes induce proliferation in primary malignant T cells. (**a**) Representative flow cytometric contour plots and quantification of Ki-67 expression in malignant T cells (Myla3675 on top, SeAx on bottom). Malignant T cells were cultured for 24 hours with nonmalignant T cells (Myla1850) and primary keratinocytes (NHEKs) treated with ±10 ng/ml IFN-γ and ±100 ng/ml SE pool. (**b**) Contour plot shows the proliferation level of malignant T cells from Pt1. Total $CD4^+$ T cells isolated from the blood of patient with SS were cultured for 5 days with NHEKs treated with ±100 ng/ml IFN-γ and ±100 ng/ml SE pool. Malignant T cells were gated as $CD3^+CD4^+TCRV\beta18^+CD26^-$. (**c**) A summary graph showing the percentage of proliferation in total of 4 primary $CD4^+$ T cells isolated from different patients with SS cultured with NHEKs treated with ±100 ng/ml IFN-γ and ±100 ng/ml SE pool. NHEK, normal human epidermal keratinocyte; PE, phycoerythrin; Pt1, patient 1; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome.

Figures S8 and S9). Next, we examined the effect of patient-derived *S aureus* when presented by KCs (NHEKs) to primary malignant $CD4^+$ T cells from patients with SS. KCs pre-treated with IFN-γ and supernatant from *S aureus* induced cell division in malignant $CD4^+$ T cells, which was blocked by endolysin (XZ.700) (Figure 5h and i and Supplementary Figure S10). Similar effect was observed in nonmalignant T cells in the same culture (Supplementary Figure S11).

SEs can induce HLA class II expression in KCs through stimulation of primary SS $CD4^+$ T cells

To further decipher the SE-mediated crosstalk between KCs and malignant and nonmalignant T cells, we measured cytokines in the supernatants of primary SS patient $CD4^+$ T cells cultured together with KCs stimulated with IFN-γ and supernatant from *S aureus* or purified SE. As shown in Figure 6a, presentation of SE from patient-derived *S aureus* by KCs to primary SS $CD4^+$ T cells induced the release of several cytokines to the supernatants, including malignant T cell GFs (IL-2 and IL-13) (Geskin et al, 2015; Guenova et al, 2013) and VEGF known to play a role in angiogenesis and disease progression in CTCL (Karpova et al, 2011; Krejsgaard et al, 2006; Pileri et al, 2015; Sakamoto et al, 2018). Accordingly, these observations suggest that *S aureus*—indirectly—stimulate malignant and nonmalignant T cells in concert to release GFs that induce proliferation in both malignant and

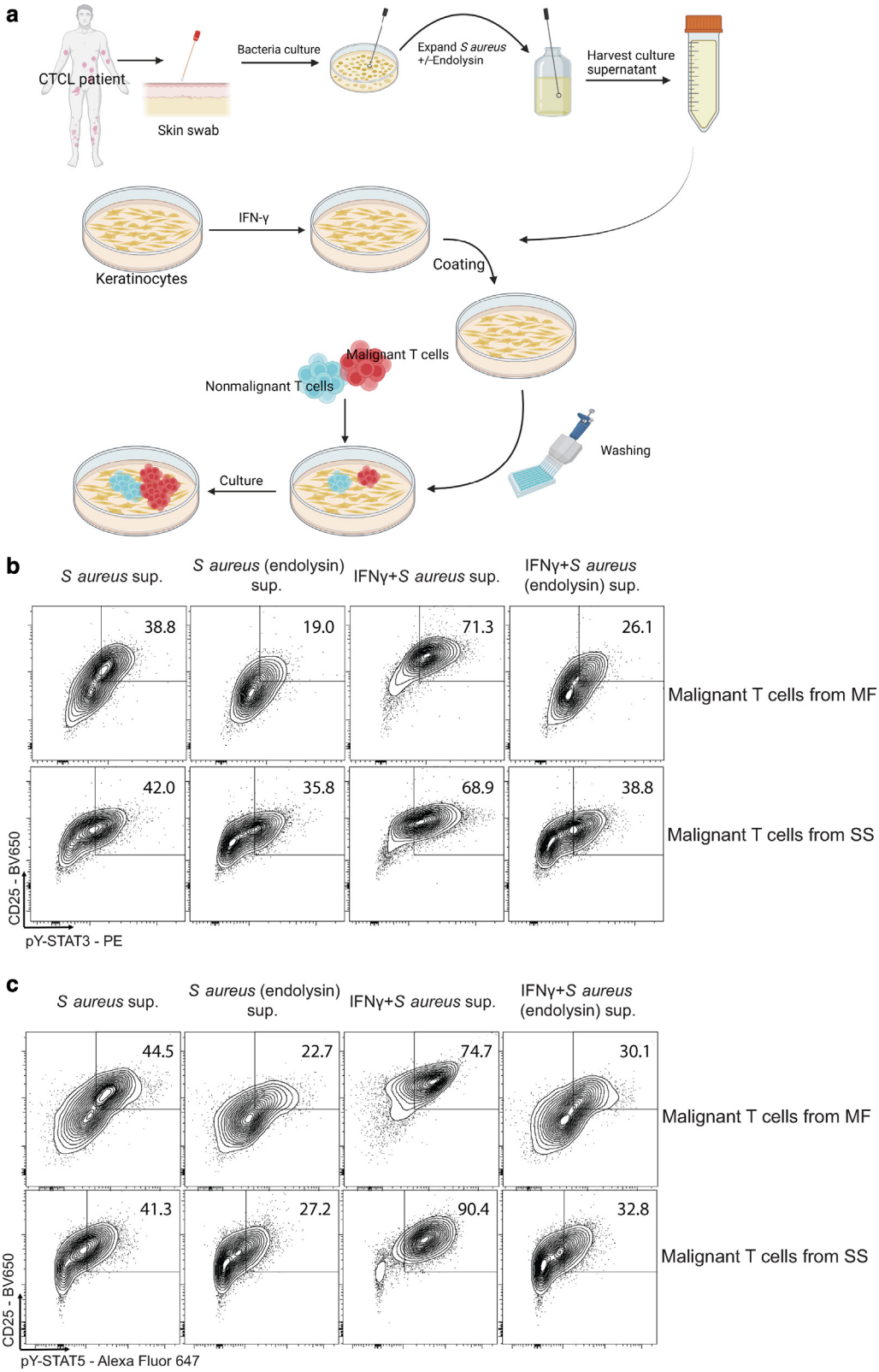
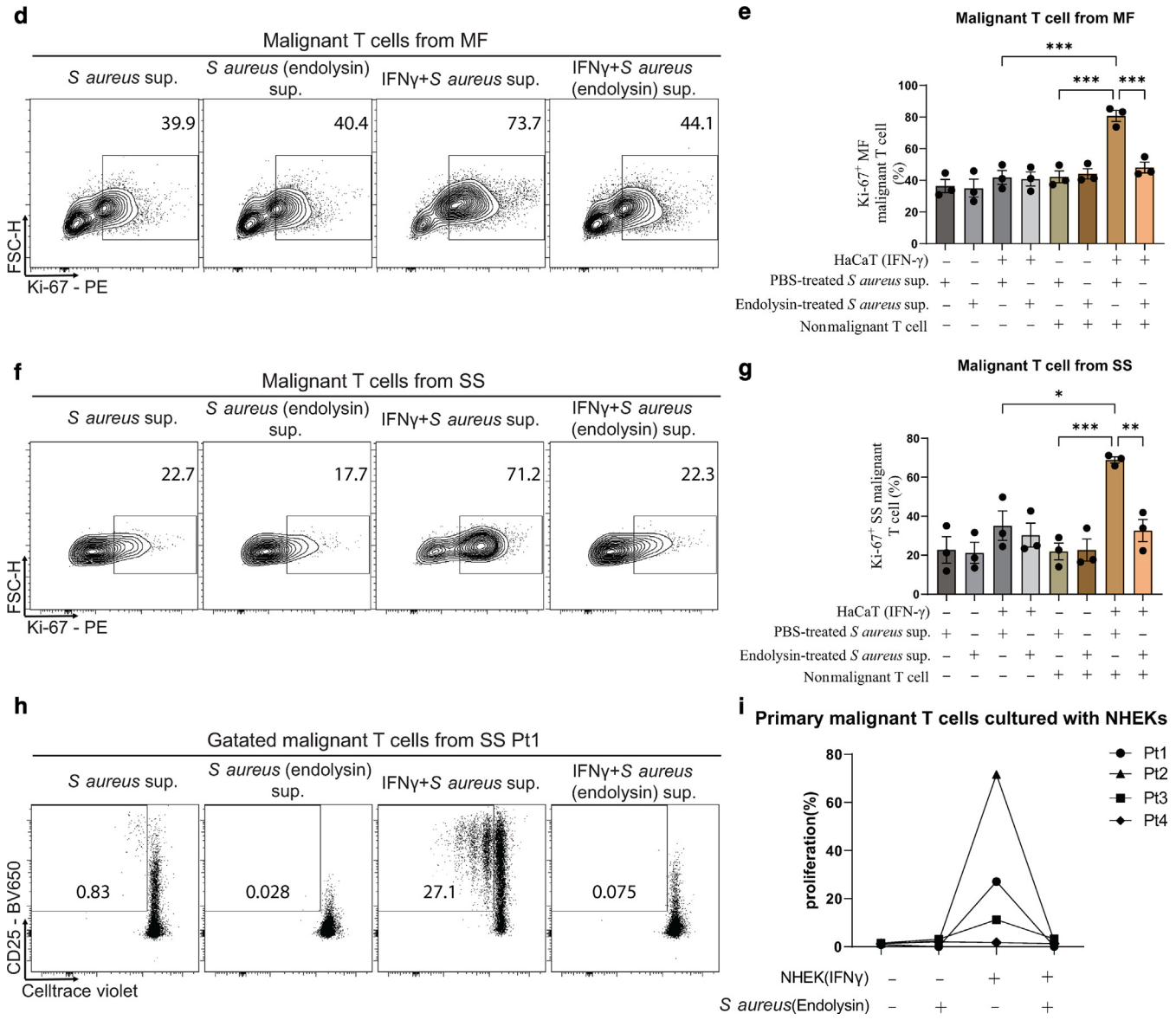


Figure 5. Keratinocytes present SEs from patient-derived *S aureus* isolates to induce proliferation of malignant T cells. (**a**) Schematic illustration of culture setup. In short, keratinocytes were pretreated with 10 ng/ml IFN-γ prior to coating with *S aureus* supernatant derived from patients with CTCL (±1 μg/ml endolysin XZ.700). After thorough washing, malignant and nonmalignant T cells from patients with CTCL were added and analyzed by flow cytometry. Flow cytometric contour plots present the expression level of (**b**) pY-STAT3 and (**c**) pY-STAT5 on malignant T cells (Myla3675, top; SeAx, bottom) after culturing with nonmalignant T cells (Myla1850) and keratinocytes (HaCaT) treated with ±100 ng/ml IFN-γ for 24 hours and coated with *S aureus* (±1 μg/ml endolysin XZ.700) supernatant for 6 hours. (**d**–**g**) Representative flow cytometric contour plots and quantification of Ki-67 expression in malignant T cells—(**d, e**) Myla3675 and (**f, g**) SeAx—after 24-hour culturing with nonmalignant T cells (Myla1850) and *S aureus* (±1 μg/ml endolysin XZ.700) supernatant-coated keratinocytes (HaCaT) (n = 3). (**h**) Primary $CD4^+$ T cells isolated from the blood of a patient woth SS (Pt1) were cultured for 5 days with NHEKs treated with ±100 ng/ml IFN-γ for 24



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nonmalignant T cells (as shown in this study and originally proposed by Woetmann et al [2007]) and VEGF to increase angiogenesis, which is associated with disease progression (Pileri et al, 2015; Sakamoto et al, 2018). Of particular interest in the present context, we observed induction of high levels of IFN-γ and the IFN-γ–inducible chemokine IP-10, which was originally associated with epidermotropism in CTCL (Sarris et al, 1995; Tensen et al, 1998) (Figure 6a). Accordingly, we examined whether supernatants from cultures stimulated with SE-producing *S aureus* and SE are sufficient to induce the expression of HLA class II on KCs. As shown in Figure 6b, supernatant from *S aureus*–stimulated cultures induced HLA class II expression by KCs, which was abrogated if the bacteria had been treated with endolysin (XZ.700). Supernatants from cultures stimulated with purified SE induced even higher levels of HLA class II expression by KCs (Figure 6b). Analysis of published microarray data (Lindahl et al, 2019) showed a decreased HLA class II expression in situ after eradication of SE-producing *S aureus* from lesional skin by aggressive antibiotic therapy in patients with CTCL with advanced disease (Supplementary Figure S12). In contrast, the expression of keratins such as keratin 14 remained essentially unchanged. These findings suggest that SE-producing *S aureus* induced HLA class II

hours and coated with *S aureus* (±1 μg/ml endolysin XZ.700) supernatant for 6 hours. Malignant T cells were gated as $CD3^+CD4^+TCRV\beta18^+CD26^-$. (**i**) Summary of the percentage of proliferation of malignant T cells isolated from 4 patients with SS cultured with NHEKs treated with different conditions. Bar plots show the mean ± SEM from 3 independent replicates. *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$. sup denotes supernatant. CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; NHEK, normal human epidermal keratinocyte; PE, phycoerythrin; Pt1, patient 1; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome; STAT, signal transducer and activator of transcription.

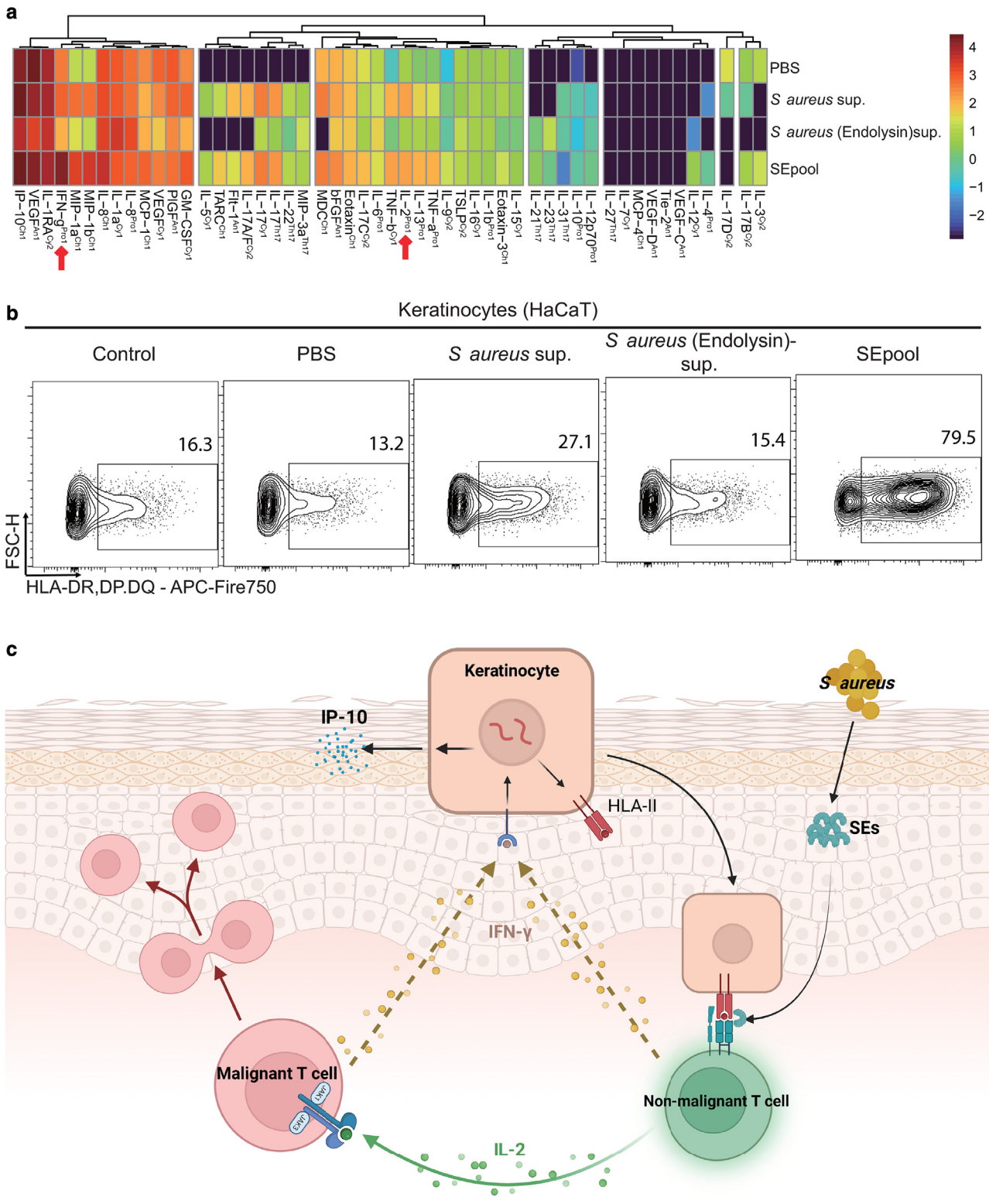

Figure 6. SEs can induce HLA class II expression in keratinocytes through stimulation of primary SS CD4$^+$ T cells. (**a**) Cytokine secretion from cell coculture supernatants with SS CD4$^+$ T cells and SE-coated (Figure 4b) or patient-derived *S aureus* supernatant–coated (Figure 5h) keratinocytes analyzed by multiplexed ELISA (Mesoscale). Superscript for each cytokines represents different meso scale panels as explained in Materials and Methods. (**b**) Supernatants from (**a**) 4 culture conditions were added to keratinocytes (HaCaT) and incubated for 24 hours. HLA class II expression levels on keratinocyte were measured by flow cytometry. (**c**) Illustration of a putative vicious circle; keratinocytes can present SEs produced by *S aureus* to nonmalignant T cells and further induce cytokine secretion such as IL-2 to promote the proliferation of malignant T cells. Activation of malignant and nonmalignant T cells could induce IFN-γ to stimulate further HLA class II expression on keratinocytes and release of IP-10, which in concert attract and activate more T cells at the site of *S aureus* colonization. sup denotes supernatant. FSC-H, forward scatter height; SE, staphylococcal enterotoxin; SS, Sézary syndrome.

expression in vivo, which is—at least partly—reversed by elimination of *S aureus* from lesional skin. As illustrated in Figure 6c, our findings suggest that KCs can present SE to malignant and nonmalignant T cells in TME. This induces enhanced proliferation of malignant T cells and release of cytokines, which in turn triggers HLA class II expression by surrounding KCs. Together, this constitutes a vicious circle fueling malignant proliferation driven by SE-producing *S aureus* (Figure 6c).

DISCUSSION

Malignant T cells in CTCL reside in a unique TME that contains KCs. Regardless, it is far from clear how KCs impact the malignant T cells and other immune cells in skin lesions of CTCL. In this study, we provide the evidence that KCs in the presence of SE-producing *S aureus* stimulated proliferation of malignant T cells. KCs displayed a heterogeneous HLA class II expression pattern in lesional skin in all disease stages, confirming previous studies showing HLA-DR expression in MF (Claesson-Welsh et al, 1986; Sarris et al, 1995; Volc-Platzer et al, 1987). Because IFN-γ is a potent inducer of HLA class II in healthy KCs (Johnson-Huang et al, 2012; Kerr et al, 1990; Nathan et al, 1986), we hypothesized that IFN-γ also induced HLA class II in KCs in CTCL. In support, *IFNG* displayed a heterogeneous expression, very similar to the HLA class II expression pattern, and HLA class II expression correlated with the expression of *IFNG*. Given the expression of T helper type 2 cytokines (IL-4, IL-5, and IL-13) by malignant T cells and the general belief that CTCL is a T helper type 2–driven disease, it may appear surprising that we observed *IFNG* expression even at late stages of the disease, which was in contrast to a previous study by Asadullah et al (1996). However, several other studies have reported on *IFNG* expression in MF—also in some patients with advanced disease (Gaydosik et al, 2023; Gluud et al, 2023; Nickoloff et al, 1990; Rindler et al, 2021a, 2021b; Saed et al, 1994; Sarris et al, 1995)—suggesting that a heterogeneous *IFNG* expression pattern is a characteristic feature of MF. Although IFN-γ is a potent and dominating driver of HLA class II expression in KCs, we cannot exclude that other cytokines in the TME could also contribute to HLA class II expression. However, cytokines expressed in MF lesions, such as T helper type 2 cytokines IL-1, IL-2, IL-10, and IL-15, do not induce HLA class II in KCs (Huang et al, 2001; Morhenn et al, 1989; Péguet-Navarro et al, 1994; Viac et al, 1994). Therefore, further studies are warranted to elucidate the complex regulation and heterogeneous expression of HLA class II in CTCL. Importantly, HLA class II–positive KCs bound SEA molecules and induced proliferation in malignant T cells in culture, an effect that was blocked if the HLA class II–binding domains of SEA were mutated. Likewise, KCs efficiently induced malignant T-cell proliferation when the KCs had been pretreated with IFN-γ and incubated with supernatant from patient-derived SE-producing *S aureus* prior to culturing with T cells. This effect was abrogated in the absence of IFN-γ and when endolysin was added to the bacterial culture. SE belong to a group of bacterial toxins—the exotoxins—that are produced and secreted by live bacteria only, which contrasts with other types of bacterial toxins that are an integral part of the bacterial wall and found in debris from dead bacteria (Cavaillon, 2018). Because endolysin inhibits the production and release of SE by killing of patient-derived *S aureus* (Pallesen et al, 2023), our findings suggest that only live, SE-producing *S aureus*—through presentation by HLA class II–positive KCs—can stimulate release of malignant T-cell GFs and proliferation of malignant and nonmalignant T cells, whereas bacterial debris from *S aureus* cannot. In support, release of cytokines and GFs (including IL-2) from cell cultures stimulated with supernatant from patient-derived *S aureus* was inhibited when the bacteria had been treated with endolysin. Taken together, these findings show that KCs—upon stimulation with IFN-γ—have the potential to present SE to induce cytokine release and proliferation of malignant and nonmalignant T cells. Thus, our findings indicate that KCs can be an active player in the TME in CTCL and that SE can promote crosstalk between KCs and malignant and nonmalignant T cells. Thus, our findings support recent single-cell sequencing studies by demonstrating crosstalk between malignant T cells and KCs (Gaydosik et al, 2023; Rindler et al, 2021a, 2021b). Other studies indicate that KCs under certain conditions produce cytokines such as IL-7, IL-15, and TSLP, which may support malignant T-cell growth (Döbbeling et al, 1998; Takahashi et al, 2016; Yamanaka et al, 2006). Thus, it is likely that KCs also engage in SE-independent cellular interactions, which support and stimulate malignant T cells during lymphomagenesis, similarly to the recently described cancer-associated KCs in head and neck cancer (Danella et al, 2021). Because it was recently reported that several subtypes of KCs are present in CTCL skin (Gaydosik et al, 2023), future studies are warranted to elucidate the role of KC subpopulations in the pathogenesis in CTCL.

S aureus has long been suspected to directly enhance disease activity in patients with CTCL (Tokura et al, 1992) (reviewed in Willerslev-Olsen et al [2013]). Skin colonization by toxin-producing *S aureus* in CTCL was first reported by Duvic et al (Jackow et al, 1997). Recently, Liu et al (2024) studied bacterial skin colonization in a larger cohort of 66 patients with CTCL and observed that one third of the patients exhibited *S aureus* colonization of skin lesions. Importantly, the frequency of lesional skin colonization by *S aureus* increased with disease progression (Liu et al, 2024). Interestingly, *S aureus* was found in cultures from both lesional and nonlesional skin in most *S aureus*–positive patients, but *S aureus*–positive patients had higher density of *S aureus* on the lesional skin than on the contralateral, nonlesional skin (Liu et al, 2024), indicating that the number of bacteria may be of importance for the clinical effect. Because more than one third of the isolated *S aureus* strains produced SE, the study was in line with the report by Duvic et al that patients with advanced disease were more prone to harbor SE-producing *S aureus* strains (Jackow et al, 1997).

This study provides, to our knowledge, a previously unreported mechanism whereby KCs—among the first cells to encounter *S aureus*—can stimulate malignant T cells and nonmalignant T cells in the TME. Of note, KCs often display some degree of HLA class II expression in lesional skin, and therefore they are presumably ready to present SE upon skin colonization and epithelial invasion by SE-producing *S aureus* in CTCL. Because supernatants from SE-stimulated

cultures contained IFN-γ and could induce and amplify HLA class II expression in KCs, we propose a vicious cycle where the initial stimulation by SE-binding KCs induces malignant T-cell proliferation and IFN-γ release, which in turn elicit further HLA class II expression and SE presentation by KCs, as illustrated in Figure 6c. Our analysis of patients with CTCL being treated by antibiotics showed a link—in vivo—between enterotoxin-producing *S aureus* and enhanced HLA class II expression. Thus, aggressive antibiotic treatment that eliminated SE-producing *S aureus* from colonized skin lesions was associated with a decreased HLA class II expression in situ. Because these findings suggest that eradication of *S aureus* inhibited HLA class II expression in vivo, they provide hope that killing of *S aureus* by more selective drugs may also reverse *S aureus*–induced HLA class II expression in patients.

In recent years, it has become clear that multiple signaling pathways are dysregulated in CTCL and that this aberrant and complex signaling network converge in driving proliferation and survival in malignant T cells—and reversely—that combinatorial treatment with inhibitors targeting different key regulators in this network offers new possibilities for a more efficient therapies (Ardigò et al, 2024; Cao et al, 2023; Ninosu et al, 2023; Shih et al, 2024; Sorger et al, 2022). Of note, recent data show that *S aureus* and its toxins can induce drug resistance through the upregulation of several players in the deregulated signaling network in malignant T cells from patients with SS (Vadivel et al, 2024). For instance, SE induced enhanced STAT3, protein kinase C, NF-κB, and TCR signaling in malignant T cells, whereas blockage of the corresponding pathways inhibited the induction of drug resistance to histone deacetylase inhibitors (romidepsin, vorinostat, and resminostat) and chemotherapeutic drugs (doxorubicin and etoposide) used in treatment of CTCL (Vadivel et al, 2024). Thus, it appears that *S aureus* through the release of SE can enhance signaling pathways already overly activated and dysregulated in malignant T cells, or in other words, SE seem to speed up an already ongoing pathogenic process. Therefore, elimination of *S aureus* could be considered as an adjuvant therapeutic approach to bring back the baseline cancer activity and drug sensitivity seen prior to *S aureus* colonization. In support, clinical findings suggest that antibiotic treatment can have a beneficial effect on disease activity in patients with CTCL colonized by *S aureus* (Lewis et al, 2018; Lindahl et al, 2019; Talpur et al, 2008), suggesting that antibiotics may break the vicious circle proposed earlier. However, in most cases, skin lesions quickly become recolonized by *S aureus* after termination of the antibiotic treatment (Lindahl et al, 2022). Aggressive antibiotic treatment is relevant in clinically severe and life-threatening *S aureus* infections, whereas long-term antibiotic prevention of skin colonization by *S aureus* is potentially dangerous owing to the risk of severe side effects and antibiotic resistance. In fact, skin colonization by methicillin-resistant *S aureus* is frequent in some cohorts of patients with CTCL (Emge et al, 2020). Therefore, there is a need for novel nonantibiotic treatments that selectively target *S aureus* to control and prevent its disease-promoting effects (Guenova and Ødum, 2024; Kadin et al, 2020). Bacteriophage-derived, engineered endolysin can effectively eliminate patient-derived *S aureus* from colonizing lesional patient skin ex vivo (Pallesen et al, 2023), and as shown in this study, endolysins efficiently block induction of malignant T-cell proliferation in response to presentation of patient-derived *S aureus* supernatant by KCs. Encouragingly, a recent study in a pig model of wound healing showed in vivo efficacy of endolysin XZ.700 in inducing efficient killing of *S aureus* without safety issues and side effects in vivo (Wilkinson et al, 2024). Although an effect in patients with CTCL ultimately must be determined in clinical trials, these findings suggest that topical drugs targeting *S aureus* may contribute to better treatment of CTCL without the downsides of antibiotics.

In conclusion, we provide evidence for a role of KCs in *S aureus*–mediated pathogenesis in CTCL and outline a putative mechanism for SE/KC-driven proliferation of malignant and nonmalignant T cells. Moreover, we show that nonantibiotic anti–*S aureus* endolysins potentially can block *S aureus*–induced disease aggravation.

MATERIALS AND METHODS

Cell lines, cell culture, and patient materials

NHEKs (number C12006, PromoCell) were cultured in KC growth medium 2 (number C-201110, PromoCell). Immortalized human KC cell line HaCaT (Boukamp et al, 1988) was cultured in RPMI-1640 (number R2405, Sigma-Aldrich) containing 10% fetal bovine serum (number 04-007-1A, Biological Industries) and 1% penicillin/streptomycin (number P7539, Sigma-Aldrich). The malignant T-cell lines Myla3675 and SeAx and the nonmalignant T-cell line Myla1850 were established from patients with CTCL (Woetmann et al, 2007). Malignant and nonmalignant T cells were cultured in RPMI-1640 (number R2405, Sigma-Aldrich) containing 10% human serum (Bloodbank, Copenhagen University Hospital, Copenhagen, Denmark), 1% penicillin/streptomycin, and 10^3 U/ml IL-2. A total of 500 U/ml IL-4 was applied for Myla1850 and Myla3675. Before the experimental setup, CTCL cell lines were washed twice with RPMI-1640 and starved in RPMI-1640 with 10% human serum and 1% penicillin/streptomycin without cytokines. Malignant T-cell lines were stained with CellTrace Blue Cell Proliferation Kit (number C34568, Thermo Fisher Scientific) to be identified. PBMCs were isolated from blood obtained from patients with SS by density gradient centrifugation, following the manufacturer's instruction (number 86460, StemCell Technologies). PBMCs used for this study were all cryopreserved. Patient PBMCs were thawed 1 day before the experiment and cultivated in XVIVO-15 (number BE02-053Q, Lonza). The SEs (SEA, SE type B, SE type C1, SE type D, and biotinylated SEA) were purchased from Toxin Technology (Sarasota, FL). Mutant SEA ($SEA^{F47A/D227A}$) was provided by Active Biotech (Lund, Sweden). A total of 1 μM of the clinical grade pan-Jak inhibitor tofacitinib (CP6905590, InvivoGene) was used in the cell culture experiments. Four patients with SS were included in this study. Malignant T cells were identified on the basis of their monoclonal TCR Vβ as well as low expression of CD26, as previously shown (Buus et al, 2018; Willemze et al, 2019).

S aureus supernatants preparation and *S aureus* enterotoxin determination

S aureus (SA4.1.1.1) was isolated, and the presence of SEs was verified as previously shown (Lindahl et al, 2019). Bacterial culture supernatants were harvested from patients with CTCL derived *S aureus,* which was grown in tryptic soy broth, starting in an optical desnity$_{600nm}$ = 0.01 and then cultured for 4 hours at 37 °C under

180 r.p.m. shaking as previously described (Pallesen et al, 2023). A total of 1 μg/ml recombinant endolysin XZ.700, MEndoB, or MEndoC (Micreos Pharma B.V.) (Eichenseher et al, 2022) was used to kill *S aureus*.

KC/T-cell culture system

For HaCaT/T-cell cultures, HaCaT cells were cultured until 100% confluence and pretreated with ±10 ng/ml IFN-γ for 24 hours. Cells were further coated with 50% patient-derived *S aureus* supernatants for 6 hours or coated with 100 ng/ml SEA or 100 ng/ml SE pool (containing SEA, SE type B, SE type C1, SE type D) for 2 hours. After coating, HaCaT cells were washed multiple times with PBS. Myla3675/Myla1850 or SeAx/Myla1850 were cultured with HaCaT in the medium composed of 75% RPMI fetal bovine serum (RPMI-1640 containing 10% fetal bovine serum) and 25% RPMI human serum (RPMI-1640 containing 10% human serum) medium for 24 hours. For the T-cell lines cultured with NHEKs, malignant/nonmalignant T cells were starved 1 day before the setup. NHEKs were first treated with ±10 ng/ml IFN-γ for 24 hours and then coated with 100 ng/ml SE pool (SEA, SE type B, SE type C1, SE type D) for 2 hours. After coating, NHEKs were washed multiple times with PBS. T-cell lines were cultured with NHEKs with different treatment in XVIVO-15 for 24 hours. For primary T cells/NHEKs culture, NHEKs were first cultured until 100% confluence, then treated with ±10 ng/ml IFN-γ for 24 hours, coated with 100 ng/ml SE pool (SEA, SE type B, SE type C1, SE type D) or 50% patient-derived *S aureus* (±1 μg/ml endolysin) supernatants for 6 hours, and washed several times with PBS. EasySep Human $CD4^+$ T Cell Isolation Kit (number 17952, STEMCELL) was used for $CD4^+$ T-cell isolation from CTCL PBMCs. Primary $CD4^+$ T cells were cultured with NHEKs for 5 days in XVIVO-15.

Flow cytometry

Fluorochrome-conjugated antibodies against CD3, CD4, CD7, CD8, CD25, CD26, CD69, Ki-67, pY-STAT3, pY-STAT5, TCRCβ1, TCRVβ1, and TCRVβ18 and respective fluorochrome-conjugated isotype control antibodies used for flow cytometry were purchased from BD Bioscience (San Jose, CA) and BioLegend (San Diego, CA). Cells were stained with Fixable Viability Stain Dye eFluor780 (number 65-0865-14, Thermo Fisher Scientific) prior to surface staining. Fixation/Permeabilization Kit (BD Bioscience) was used to fix and permeabilize cells prior to anti-human Ki-67 antibody staining. For phospho-specific antibodies staining, cells were fixed with fixation buffer (number 554655, BD Bioscience), permeabilized by Perm Buffer III (number 558050, BD Bioscience), and finally stained anti−pY-STAT3, anti−pY-STAT5 antibodies. Flow cytometric data were acquired from 5-laser BD LSR-Fortessa flow cytometer (BD Biosciences) and analyzed with the FlowJo software (Tree Star, Ashland, OR).

Reverse transcription quantitative PCR (RT-qPCR)

RNA from cell lines was purified (number 74034, Qiagen) and transcribed into cDNA (number 4368814, Thermo Fisher Scientific), and Light-Cycler480 II (Roche) was used for quantitative (q)PCR to detect gene expression. *GAPDH* was used as reference gene for normalization. All the TaqMan assays are from Thermo Fisher Scientific. Data were calculated according to the $2^{\Delta\Delta CT}$ method.

Transient transfections

Malignant T-cell lines (2×10^6 cells per sample) were transfected with 500 pmol small interfering RNA against *IL2RG* (number L-007985-00-0005) or 250 pmol small interfering RNA against *Jak3* (number L-003147-00-0005) or equal amount of nontargeting control (number D-001810-01-05, Dharmacon, ON-TARGETplus) in 100 μl of Ingenio Solution (number MIR-50111, Mirus Bio). After 48 hours of incubation, the effect of the knockdown was validated by qPCR, and cells were cultured with nonmalignant T cells and HaCaT cells.

Cytokine secretion profile analysis

Culture supernatants were harvested and stored at −80 °C. Mesoscale technology was performed using Mesoscale human V-plex assay kits (Meso Scale Discovery), following the manufacturer's protocol (Ch1 = V-PLEX Chemokine Panel 1 Human Kit, Cy1 = V-PLEX Cytokine Panel 1 Human Kit, Cy2 = V-PLEX Cytokine Panel 2 Human Kit, An1 = V-PLEX Angiogenesis Panel 1 Human Kit, Pro1 = V-PLEX Proinflammatory Panel 1 Human Kit, and Th17 = V-PLEX Th17 Panel 1 Human Kit).

Single-cell RNA sequencing in skin biopsies

Count matrices from 4 single-cell RNA-sequencing studies were obtained or generated from the Gene Expression Omnibus (accession numbers GSE165623 [Rindler et al, 2021a], GSE173205 [Rindler et al, 2021b], GSE182861 [Gaydosik et al, 2022], and GSE206123 [Gaydosik et al, 2023]). Only skin biopsies from healthy controls or lesional CTCL skin samples containing an identifiable malignant T-cell population were included in the analysis. Low-quality cells were removed when having <300 expressed genes or >50% mitochondrial transcripts or if part of a cluster with uniformly low gene detection and high mitochondrial transcripts. Malignant cells were identified on the basis of expression of a major expanded TCR clonotype (from single-cell TCR-sequencing data) and validated by exhibiting high expression of malignant-associated genes (such as *KIR3DL2* and *TOX*). Samples were batch corrected and integrated using scVI (Lopez et al, 2018) using expression of the top 3000 variable genes. Subsequent semisupervised integration providing only malignant cell label was done using scANVI (Xu et al, 2021). Additional malignant cells were then inferred on the basis of coclustering with TCR-defined malignant cells and expression of T cell− and malignant-associated genes in the absence of any single-cell TCR-sequencing clonotype (ie, owing to drop out). Cell types were manually annotated on the basis of expression of signature genes. Total biopsy *IFNG* expression was quantified as transcripts per million by dividing the unique molecular identifier count with the total sample unique molecular identifier count divided by 10^6.

Statistics

All the bar graphs were created using GraphPad Prism 10.1.1 (GraphPad Software). One-way ANOVA with Tukey's multiple comparison test was used for comparing the significance of differences. Data are represented as the mean ± SEM from 3 independent replicates. Statistical significance was considered for $P < .05$ (*$P <$ .05, **$P <$ .01, ***$P <$ .001, and ****$P <$ .0001).

ETHICS STATEMENT

Blood samples were collected from 4 patients with SS after obtaining written, informed consent from all patients as well as institutional approvals from Denmark (Committee on Health Research Ethics, H-16025331). The patient characteristics are provided in Supplementary Table S1.

DATA AVAILABILITY STATEMENT

Single-cell RNA-sequencing data used from previously published studies are available from Gene Expression Omnibus under accessions GSE165623, GSE173205, GSE182861, and GSE206123. Code used for analysis of single-cell RNA-sequencing data is available at https://github.com/Terkild/CTCL_KC_SA_presentation. All other data are available from the corresponding authors upon reasonable request.

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CONFLICT OF INTEREST
NØ has received consulting honoraria from Mindera, Micreos Human Health, PS Consulting, LEO Pharma, and Almirall. SBK laboratory has previously received funding from Micreos, Dracen Pharmaceuticals, Kymera Therapeutics, and Bristol Myers Squibb. SBK is supported by National Institutes of Health R01 R01CA271245. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: ZZ, NØ; Investigation: ZZ, TBB; Methodology: ZZ, TBB, NØ; Resources: MRK; Funding Acquisition: NØ; Writing - Original Draft Preparation: ZZ, NØ; Writing - Review and Editing: ZZ, CKV, MG, MRJN, LY, SA, MBH, JC, TM, SBK, CMB, AW, CG, EG, MRK, TL, L-MRG, TBB, NØ

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2024.04.018.

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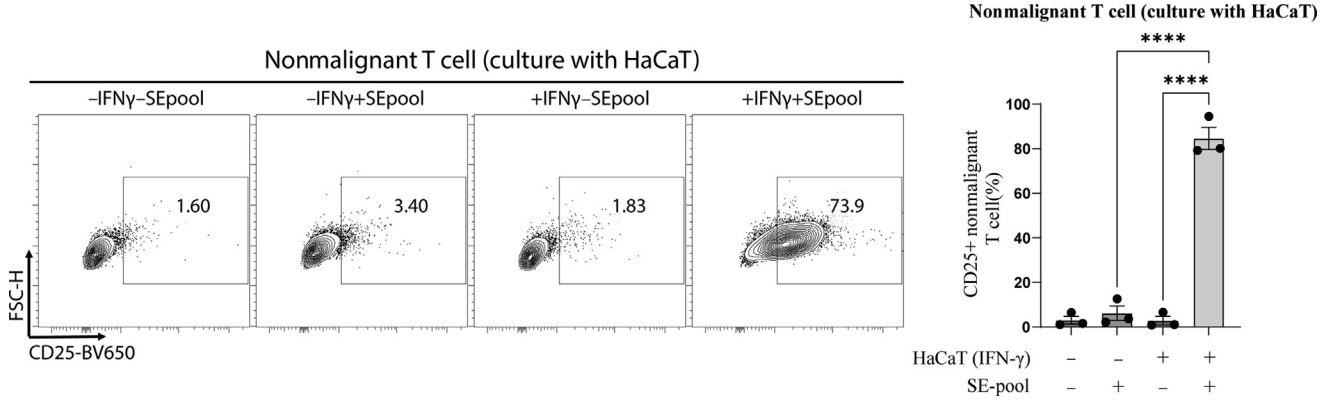
SUPPLEMENTARY MATERIALS

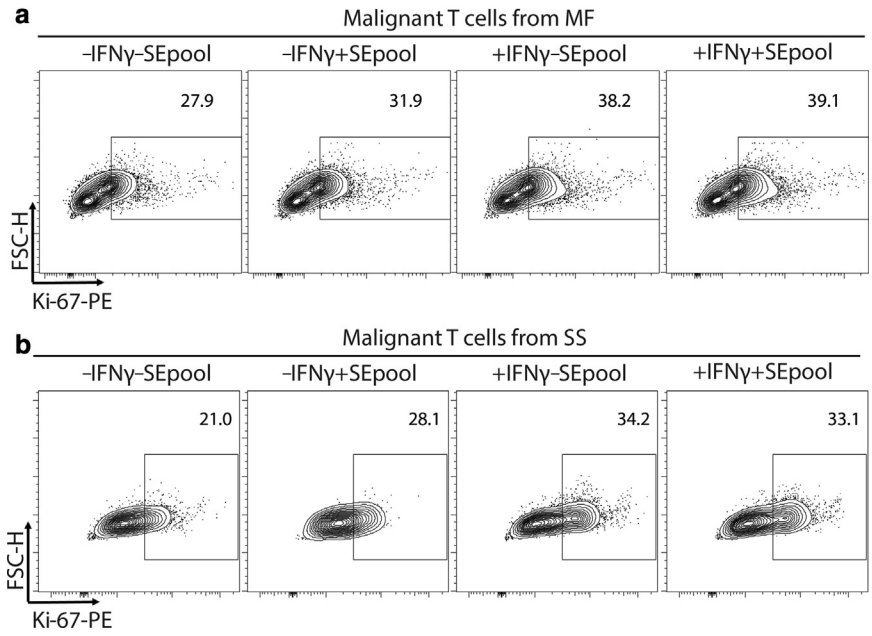
SUPPLEMENTARY REFERENCE

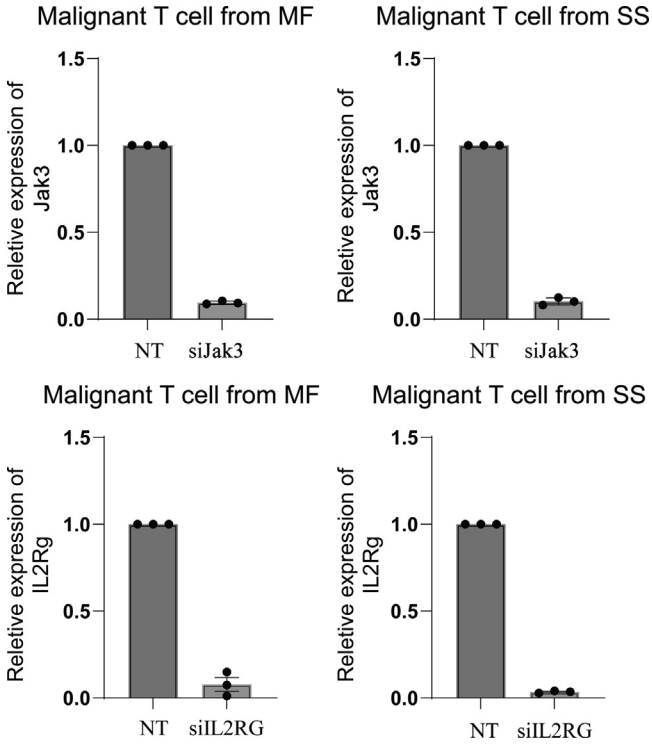
Lindahl LM, Willerslev-Olsen A, Gjerdrum LMR, Nielsen PR, Blümel E, Rittig AH, et al. Antibiotics inhibit tumor and disease activity in cutaneous T-cell lymphoma. Blood 2019;134:1072–83.



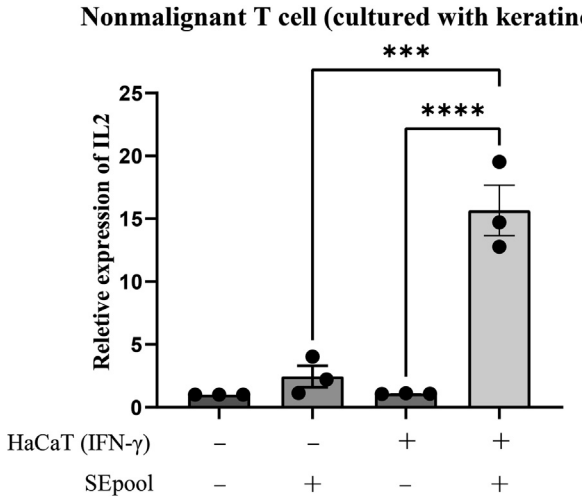
Supplementary Figure S1. Activation of nonmalignant T cells cultured with keratinocytes treated with IFN-γ and SEs. Left: contour plots shows the CD25 expression level after nonmalignant T cells (Myla1850) cultured with keratinocytes (HaCaT) treated with ±10 ng/ml IFN-γ for 24 hours and coated with ±100 ng/ml SE pool for 2 hours. Flow cytometry was run after 24 hours of culturing. Right: representative quantification of CD25 expression level of nonmalignant T cell (Myla1850) after culturing (n = 3). Data are represented as the mean ± SD from 3 independent replicates. *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$. FSC-H, forward scatter height; SE, staphylococcal enterotoxin.

Supplementary Figure S2. Malignant T cells cultured with keratinocytes in the absence of nonmalignant T cells do not enhance their proliferation. Flow cytometric contour plots shows the Ki-67 expression level on malignant T cells from (**a**) MF (Myla3675) and (**b**) SS (SeAx) cultured with nonmalignant T cells (Myla1850) and keratinocytes (HaCaT) treated with ±10 ng/ml IFN-γ for 24 hours and coated with ±100 ng/ml SE pool for 2 hours. Flow cytometry was run after 24 hours of culturing. FSC-H, forward scatter height; MF, mycosis fungoides; PE, phycoerythrin; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome.

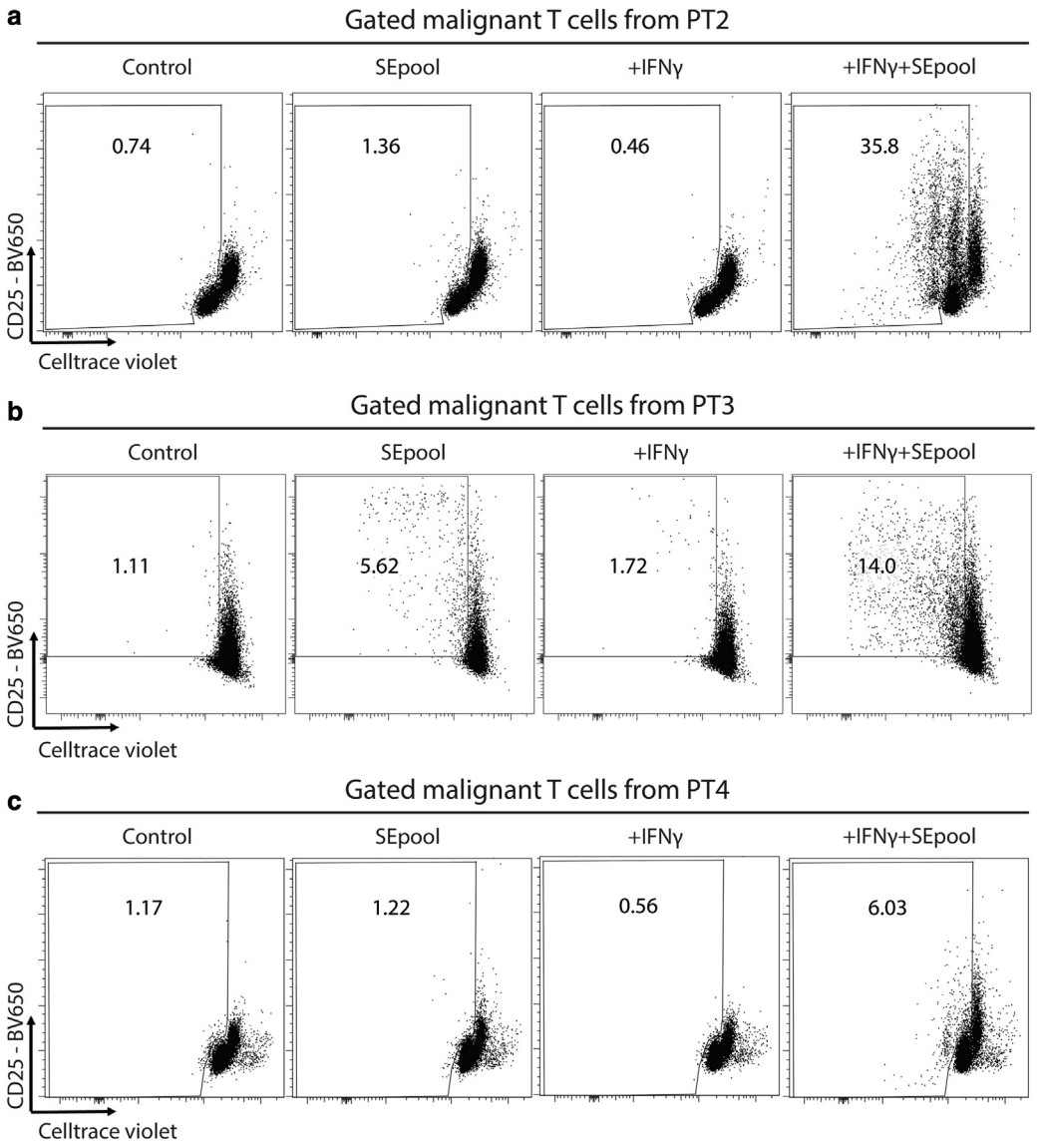




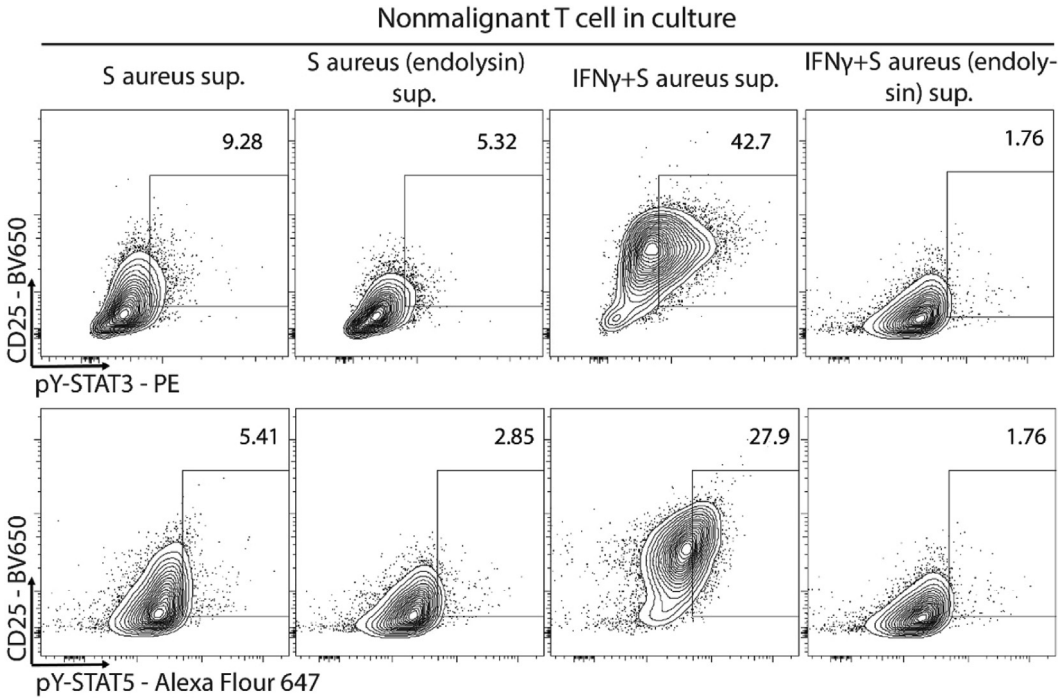
Supplementary Figure S3. qPCR analyzed the expression of IL-2RG and Jak3 expression on malignant T cells after siRNA transfection. qPCR analyzed the Jak3 expression on malignant T cells (Myla3675, top left; SEAx, top right) after 48 hours of Jak3—siRNA transfection. qPCR analyzed the IL-2RG expression on malignant T cells (Myla3675, bottom left; SEAx, bottom right) after 48 hours of IL-2RG—siRNA transfection. NT, nontargeting; SEA, staphylococcal enterotoxin type A; siIL2RG, IL2RG-targeted small interfering RNA; siRNA, small interfering RNA.



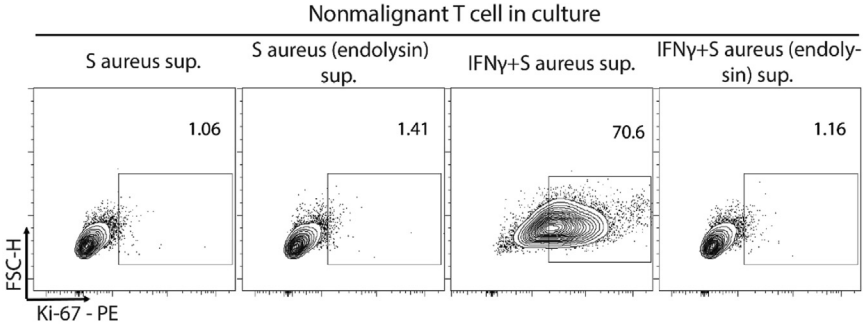
Supplementary Figure S4. IL-2 expression on nonmalignant T cells after culturing with keratinocytes treated with IFN-γ and SE pool. qPCR analyzed the IL-2 expression change on nonmalignant T cells (Myla1850) after 3-hour culturing with keratinocytes (HaCaT) treated with ±10 ng/ml IFN-γ and ±100 ng/ml SE pool. Data are represented as the mean ± SD from 3 independent replicates. SE, staphylococcal enterotoxin.



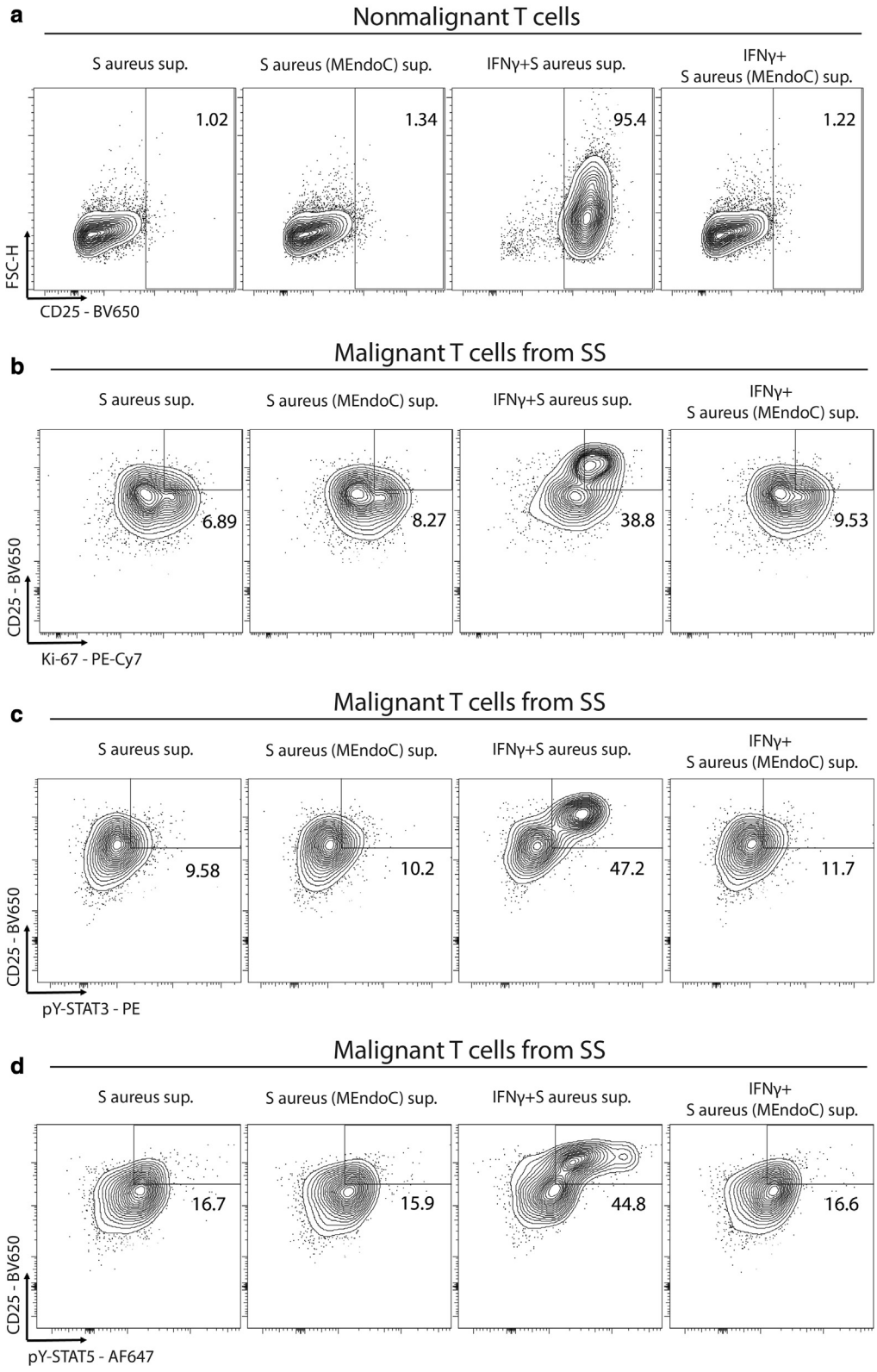
Supplementary Figure S5. Proliferation of primary CD4$^+$ malignant T cells isolated from the blood of patients with SS cultured with primary keratinocytes treated with IFN-γ and SE pool. (**a**–**c**) Flow cytometric contour plots were shown on gated malignant T cells from 3 patients with SS. Proliferation was analyzed using Celltrace violate proliferation dye stained on primary T cells isolated from 3 patients with SS after 5-day culturing with NHEKs treated with ±10 ng/ml IFN-γ and ±100 ng/ml SE pool. (**a**) Malignant T cells were gated as CD3$^+$CD4$^+$TCRCβ$^-$CD26$^-$. (**b**) Malignant T cells were gated as CD3$^+$CD8$^-$TCRCβ$^-$CD26$^-$. (**c**) Malignant T cells were gated as CD3$^+$CD4$^+$TCRVβ1$^+$CD26$^-$. PT2, PT3, and PT4, denote patients 2, 3, and 4, respectively. NHEK, normal human epidermal keratinocyte; SE, staphylococcal enterotoxin; SS, Sézary syndrome.



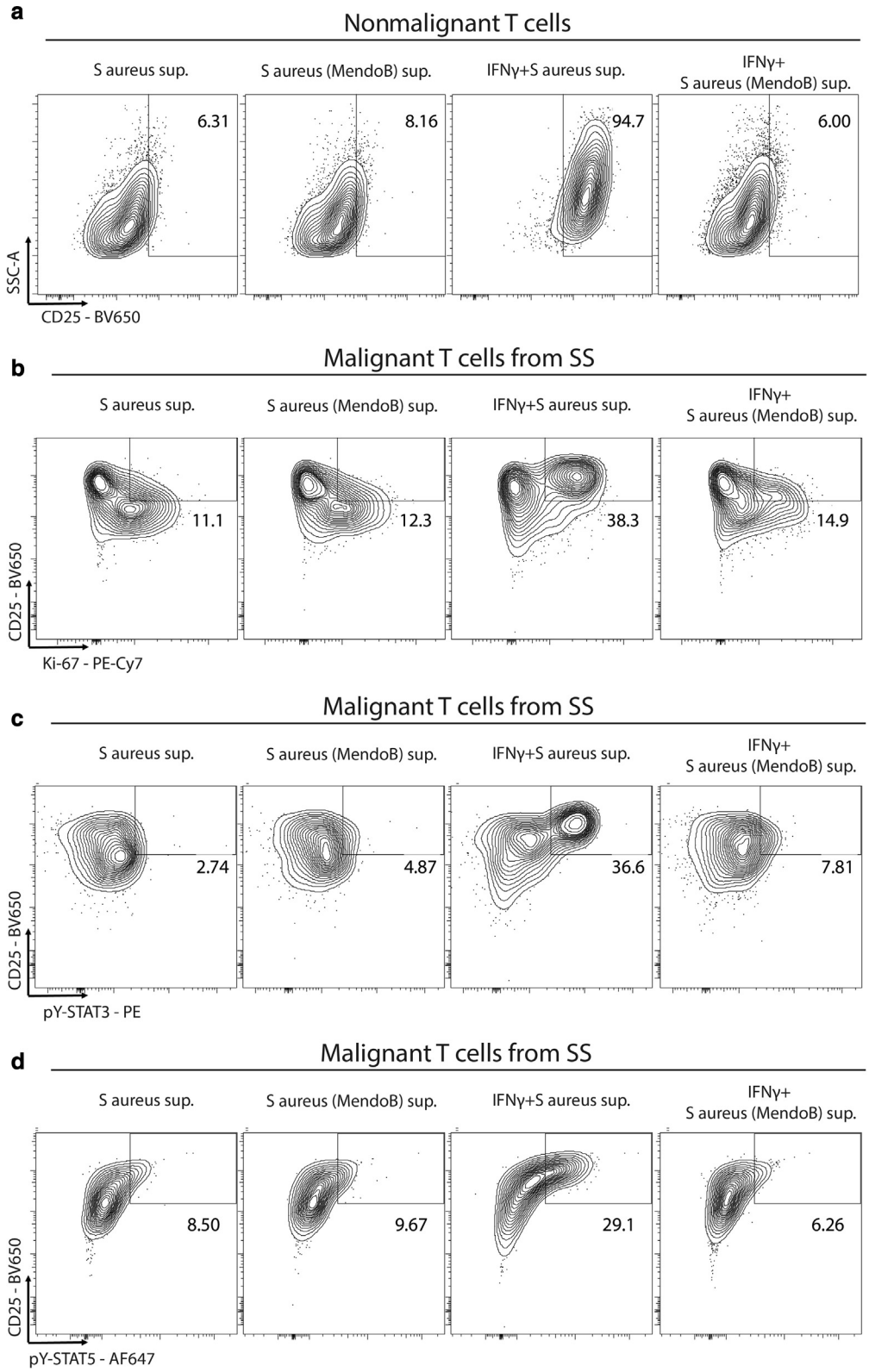
Supplementary Figure S6. Keratinocytes present SEs from patient-derived *S aureus* isolates to induce proliferation of nonmalignant T cells (correspond to Figure 5b and c). Nonmalignant T cell (Myla1850) cultured with malignant T cell and keratinocyte (HaCaT) for 24 hours were used. Cells were run on flow cytometry analysis to determine pY(705)-STAT3 and pY(694)-STAT5 accompanied with CD25 expression. sup denotes supernatant. PE, phycoerythrin; SE, staphylococcal enterotoxin; STAT, signal transducer and activator of transcription.



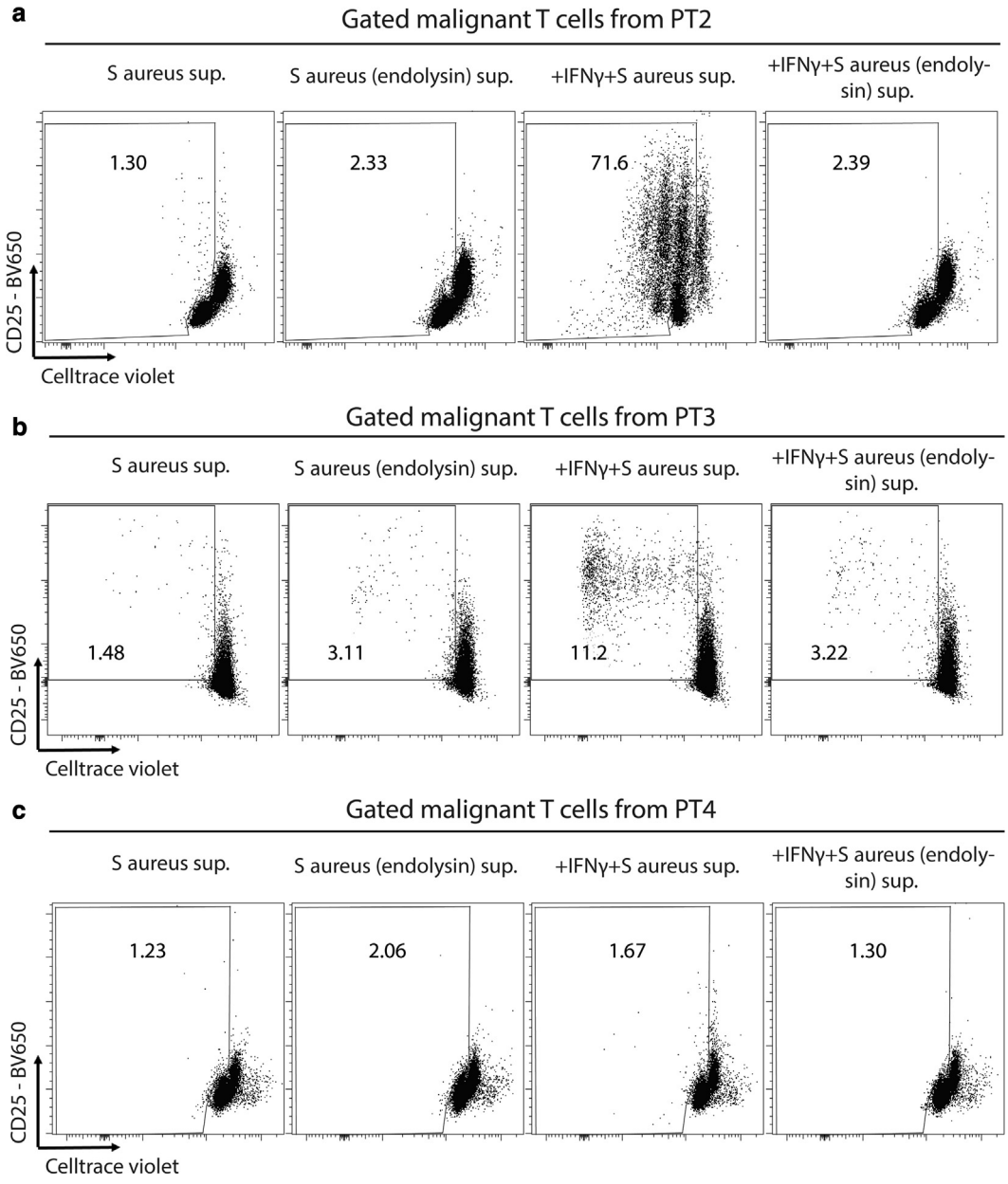
Supplementary Figure S7. Keratinocytes present SEs from patient-derived *S aureus* isolates to induce proliferation of nonmalignant T cells (correspond to Figure 5d). Nonmalignant T cell (Myla1850) cultured with malignant T cell and keratinocyte (HaCaT) for 24 hours were used. Cells were run on flow cytometry analysis to determine Ki-67 expression. sup denotes supernatant. FSC-H, forward scatter height; PE, phycoerythrin; SE, staphylococcal enterotoxin.



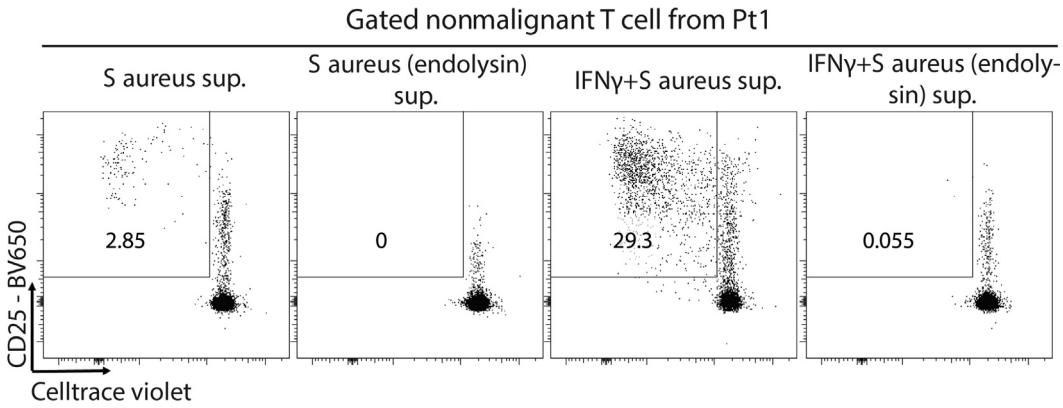
Supplementary Figure S8. Phage-derived *S aureus*–specific endolysin (MEndoC) blocks the proliferation effect on malignant T cells when cultured with nonmalignant T cells and keratinocytes. Malignant T cells from SS (SEAx) and nonmalignant T cells (Myla1850) cultured for 24 hours with keratinocyte (HaCaT) treated with ±10 ng/ml IFN-γ and *S aureus* (±1 μg/ml MEndoC) supernatant were used. (**a**) Flow cytometric contour plots shows the CD25 expression level on nonmalignant T cells (Myla1850) after culturing. Flow cytometric contour plots shows the (**b**) Ki-67, (**c**) pY(705)-STAT3, and (**d**) pY(694)-STAT5 accompanied with CD25 expression level on malignant T cells (SEAx) after culturing. sup denotes supernatant. FSC-H, forward scatter height; PE, phycoerythrin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome; STAT, signal transducer and activator of transcription.



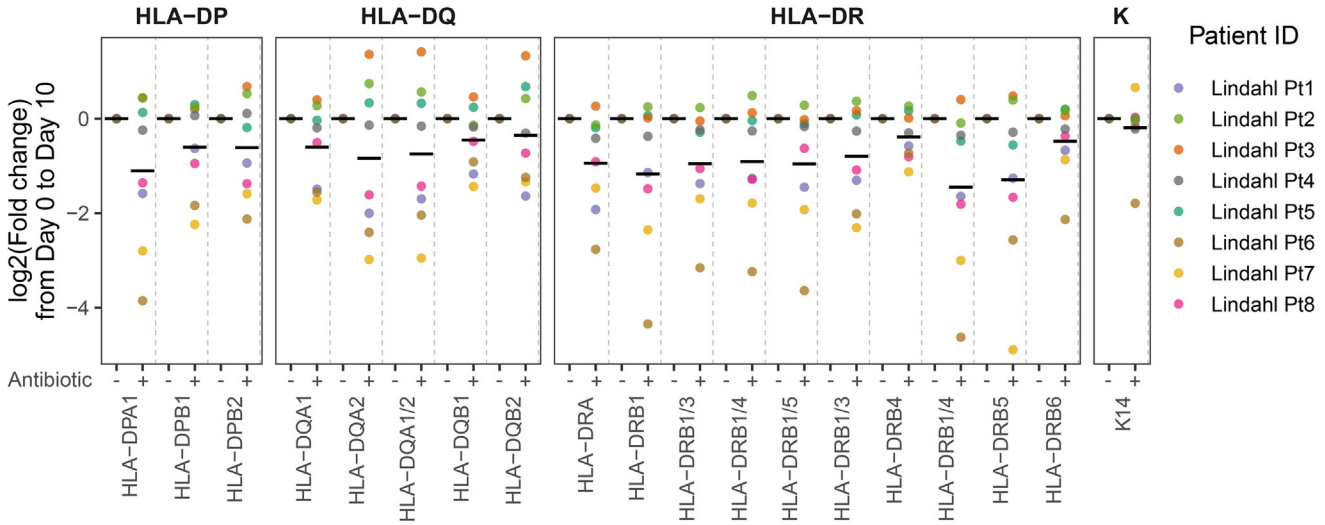
Supplementary Figure S9. Phage-derived *S aureus*–specific endolysin (MEndoB) blocks the proliferation effect on malignant T cells when cultured with nonmalignant T cells and keratinocytes. Malignant T cells from SS (SEAx) and nonmalignant T cells (Myla1850) cultured for 24 hours with keratinocyte (HaCaT) treated with ±10 ng/ml IFN-γ and *S aureus* (±1 μg/ml MEndoB) supernatant were used. (**a**) Flow cytometric contour plots show the CD25 expression level on nonmalignant T cells (Myla1850) after culturing. Flow cytometric contour plots show the (**b**) Ki-67, (**c**) pY(705)-STAT3, and (**d**) pY(694)-STAT5 accompanied with CD25 expression level on malignant T cells (SEAx) after culturing. sup denotes supernatant. PE, phycoerythrin; SEA, staphylococcal enterotoxin type A; SS, Sézary syndrome; SSC-A, side scatter area; STAT, signal transducer and activator of transcription.



Supplementary Figure S10. Proliferation on primary $CD4^+$ T cells isolated from the blood of patients with SS cultured with primary keratinocytes treated with IFN-γ and *S aureus* supernatant derived from patients with CTCL. (**a**–**c**) Flow cytometric contour plots were shown on gated malignant T cells from 3 patients with SS. Proliferation was analyzed using Celltrace violate proliferation dye stained on primary T cells isolated from 3 patients with SS after 5-day culturing with NHEKs treated with ±10 ng/ml IFN-γ and *S aureus* supernatant (±1 μg/ml endolysin XZ.700) derived from patients with CTCL. (**a**) Malignant T cells were gated as $CD3^+CD4^+TCRC\beta^-CD26^-$. (**b**) Malignant T cells were gated as $CD3^+CD8^-TCRC\beta^-CD26^-$. (**c**) Malignant T cells were gated as $CD3^+CD4^+TCRV\beta1^+CD26^-$. sup denotes supernatant. PT2, PT3, and PT4 denote patients 2, 3, and 4, respectively. CTCL, cutaneous T-cell lymphoma; NHEK, normal human epidermal keratinocyte; SS, Sézary syndrome.



Supplementary Figure S11. Keratinocytes present SEs from patient-derived *S aureus* isolates to induce activation and proliferation of nonmalignant T cells (correspond to Figure 5h). Flow cytometric contour plots were shown on gated nonmalignant T cells from Pt1. Proliferation was analyzed using Celltrace violate proliferation dye stained on primary T cells isolated from 3 patients with SS after 5-day culturing with NHEKs treated with ±10 ng/ml IFN-γ and *S aureus* supernatant (±1 μg/ml endolysin XZ.700) derived from patients with CTCL. Nonmalignant T cells were gated on $CD3^{+}CD4^{+}TCR\nu\beta18^{-}$. CTCL, cutaneous T-cell lymphoma; NHEK, normal human epidermal keratinocyte; Pt1, patient 1; SE, staphylococcal enterotoxin; SS, Sézary syndrome.



Supplementary Figure S12. HLA class II gene expression on lesional site of 10 patients with CTCL before and after antibiotic treatment. HLA class II and K14 expressions on samples from lesional site of 8 patients with CTCL from public gene chip (Affymetrix) were analyzed (Lindahl et al, 2019). CTCL, cutaneous T-cell lymphoma; ID, identification; K, keratin; Pt, patient.

Supplementary Table S1. Pt Characteristics

Pt	Sex	Age	Diagnosis	Year of Diagnosis	Stage/Classification	Treatment[1]	Previous Treatment
Pt1	M	82	SS	2018	B2	ECP	NA
Pt2	F	74	SS	2016	B2	ECP	Prednisolone, methotrexate, PUVA
Pt3	M	85	SS	2012	NA	ECP, IFN-α	PUVA, IFN-α, bexarotene
Pt4	M	77	SS	2017	B2	ECP, Brentuximab vedotin	PUVA, UVB, methotrexate, IFN, bexarotene

Abbreviations: ECP, extracorporeal photopheresis; F, female; M, male; NA, not available; Pt, patient; PUVA, psoralen and UVA; SS, Sezary syndrome.
[1]Treatment indicates the Pt clinical treatments at the time point of blood collection where PBMCs were isolated and used for experiments.