Psoriasis is a T lymphocyte–driven systemic inflammatory disease. Regulatory T cells (Tregs) are essential for establishing and maintaining immune tolerance. In this study, we found that patients with psoriasis and healthy controls had comparable percentages of circulating CD4⁺CD25⁺FOXP3⁺ Tregs, but psoriatic Tregs had reduced suppressive function. Thereafter, mRNA arrays were performed to study the gene expression profile of psoriatic Tregs. Psoriatic Tregs expressed high levels of a T helper type 1–like transcription factor and cytokines such as T-bet and IFN-γ. Furthermore, we found that FOXP1 can bind to the promoter of TBX21 to inhibit its expression, thus keeping the suppressive function of Tregs. However, an increase in protein kinase B–mediated phosphorylation of FOXP1 was observed in psoriatic Tregs, which subsequently caused FOXO1 inactivation by nuclear exclusion. In addition, incubation of healthy Tregs with psoriatic serum led to the activation of protein kinase B, nuclear exclusion of FOXO1, and the loss of FOXP1 transcription activity. The role of FOXO1 in regulating the function of Tregs was corroborated using a psoriasis-like mouse model in which Foxo1-deficient Tregs failed to protect mice from developing psoriasis. In conclusion, our findings reveal that the dysregulation of the protein kinase B—FOXO1 pathway may be a critical cause of Treg dysfunction in psoriasis.


INTRODUCTION
Psoriasis is a T lymphocyte–driven autoimmune disorder. It is characterized by the accumulation of inflammatory cells in the dermis, which leads to epidermal keratinocyte hyper-proliferation. There is currently no known cure for this disease; therefore, it causes an extensive psychological and physical burden. Psoriasis was previously considered a T helper type (Th) 1 cell–mediated disease. In the last decade, the role of Th17 cells in the pathogenesis of psoriasis has been widely accepted as the key factor. The association between regulatory T cells (Tregs) and immune pathogenesis of psoriasis was recognized as well (Mattozzi et al., 2013; Soler and McCormick, 2011).

Tregs with a CD4⁺Foxp3⁺ phenotype play an indispensable role in establishing and maintaining immune homeostasis by suppressing self-destructive T-cell responses. Tregs are CD127 negative, express a high level of CD25, and do not express IL-2 and inflammatory cytokines such as IFN-γ, tumor necrosis factor-α, and IL-17. Foxp3 is a lineage-specific transcription factor of Tregs and is believed to play a key role in regulating their development and suppressive function (Ziegler, 2006). Recent studies reported that other transcription factors are also crucial in regulating the function of Tregs (Fu et al., 2012).

Foxo1, a member of the Forkhead transcription factor O family, is crucial for regulating T-cell activation, as well as the development and function of Tregs (Hedrick et al., 2012; Ouyang and Li, 2011). Foxo1 is highly expressed in naïve T cells and keeps them in a quiescent state. When T cells are activated, TCR signaling–mediated protein kinase B (Akt) activation induces the phosphorylation of Foxo1 at Thr24, Ser256, or Ser319, leading to the translocation of Foxo1 from the nucleus to the cytoplasm and preventing Foxo1-mediated transcription activity (Ouyang et al., 2009). Mice with a Treg cell–specific deletion of Foxo1 develop a fatal inflammatory disorder that is similar in severity to that observed in Foxp3-deficient mice (Ouyang et al., 2012). Genome-wide analyses identified 300 Foxo1-bound target genes in Tregs, including the proinflammatory cytokine IFN-γ, suggesting that Foxo1 controls a novel genetic program that is indispensable for Treg function (Ouyang et al., 2012). However, it remains unclear whether dysregulation of the Akt-FOXO1 pathway...
could be a mechanism leading to Treg dysfunction in the context of human autoimmune diseases.

Previous studies have shown that the frequency and function of Tregs were decreased in patients with psoriasis (Richetta et al., 2011; Sugiyama et al., 2005; Yang et al., 2016). However, the mechanism underlying the impaired Treg function in these patients remains unknown. In this study, we found that the average frequency of circulating CD4⁺CD25⁺FOXP3⁺ Tregs in patients with psoriasis was comparable to that of healthy controls, but Tregs of the patients showed reduced suppressive function. Gene expression profiling and signaling studies revealed dysregulation of the Akt-FOXO1 axis in psoriatic Tregs that highly express Th1-like transcription factor and cytokines. The knockdown of FOXO1 promoted the conversion of Tregs into Th1 cells by initial TBX21 transcription, leading to the dysfunction of Tregs. In a psoriasis-like mouse model, Foxo1-deficient Tregs failed to protect mice from developing psoriasis. Taken together, these findings reveal that the dysregulation of the Akt-FOXO1 axis may be a critical cause of Treg dysfunction in psoriasis.

RESULTS

Frequency and function of circulating Tregs in patients with psoriasis

Blood samples were obtained from 81 patients with psoriasis and 46 healthy controls. Flow cytometry analysis showed that there was no significant difference in the frequency of circulating CD4⁺CD25⁺FOXP3⁺ Tregs between patients with psoriasis and healthy controls (mean ± standard error of the mean [SEM], 6.43% ± 0.19% vs. 6.81% ± 0.24%; P = 0.1365) (Figure 1a and b). However, the absolute number of CD4⁺CD25⁺FOXP3⁺ Tregs in peripheral blood mononuclear cells was increased in patients with psoriasis (P = 0.241). This may be caused by the activation of immune response, including the proliferation of responder T cells (Tresps) and Tregs in patients with psoriasis. In addition, the frequency of circulating CD4⁺CD25⁺FOXP3⁺ Tregs in the patients was positively correlated with the Psoriasis Area and Severity Index score (R² = 0.2580, P < 0.001) (Figure 1d). Thymus-derived Tregs were determined by the expression of the Helios protein. The frequency of Helios⁺ T cells within the CD25⁺FOXP3⁺ Treg cell population was comparable between patients with psoriasis and healthy controls (mean ± SEM, 53.61% ± 2.17% vs. 54.58% ± 2.02%; P = 0.7514) (Figure 1a and e).

The suppressive function of Tregs was assessed by coculturing CD4⁺CD25⁺CD127⁻ Tregs from patients with psoriasis or healthy controls with CD4⁺CD25⁺CD127⁻ Tresps from third-party healthy control subjects. Thereafter, we detected the proliferation of Tresps to reflect the suppressive function of Tregs. We found that compared with healthy control Tregs, Tresps show increased proliferation when cocultured with psoriatic Tregs (Figure 1f and g). These results suggested that the suppressive function of psoriatic Tregs was diminished as compared to that of healthy Tregs.

Taken together, these results suggest that although there was no difference in the frequency of circulating Tregs and Tregs generated from the thymus between patients with psoriasis and healthy controls, the suppressive function of Tregs from patients with psoriasis was impaired.

Increased expression of Th1 signature genes in psoriatic Tregs

To investigate the mechanism underlying the dysfunction of psoriatic Tregs, CD4⁺CD25⁺CD127⁻ Tregs were isolated from patients with psoriasis and healthy controls for transcriptome study using microarray by FACS (Supplementary Figure S1 online). Tregs from 15 patients or 15 healthy controls were pooled because it was difficult to get enough Tregs from one patient to perform the experiment. Gene ontology analysis of the microarray data showed that the expression of genes associated with the immune and proinflammatory responses was increased in psoriatic Tregs compared with healthy Tregs (Figure 2a and Supplementary Figure S2a online). In contrast, the expression of genes associated with transcription regulation was decreased (Supplementary Figure S2b). By quantitative PCR and flow cytometry, we confirmed that psoriatic Tregs expressed comparable levels of suppressive function—related molecules to healthy Tregs but expressed higher levels of proinflammatory cytokines such as IFN-γ and tumor necrosis factor-α, as well as T-bet (Figure 2b–d). T-bet is the key transcription factor for Th1-cell differentiation. High expression of T-bet and IFN-γ suggests the dysregulation of the Th1 program in psoriatic Tregs.

FOXO1 knockdown promotes the conversion of Tregs to Th1 cells by directly regulating TBX21 transcription

Previous studies demonstrated that Foxo1 is a crucial transcription factor in maintaining the suppressive function of Tregs (Ouyang et al., 2012). Foxo1 can also repress T-bet expression in CD8⁺ T cells (Rao et al., 2012). To investigate whether FOXO1 is involved in the dysregulation of the Th1 program of psoriatic Tregs, we compared gene expression profiles of psoriatic Tregs and Foxo1⁻/⁻ Tregs. Nearly 30% of the genes upregulated in Foxo1⁻/⁻ Tregs were also increased in psoriatic Tregs (Figure 3a). Further, we predicted the FOXO1 binding sites of the TBX21 promoter sequences by using the JASPAR database (Supplementary Figure S3 online) and performed chromatin immunoprecipitation (ChIP) assay and luciferase reporter system to prove this (Supplementary Figure S4 online). FOXO1 can bind to the promoter sequences between −491 and −707 of TBX21 and repress its transcription (Figure 3b and c).

In addition, Tregs isolated from healthy controls were infected with lentiviral FOXO1 short hairpin RNA (shRNA) to knock down FOXO1. Transduced Tregs were marked by the red fluorescent protein that was expressed by the lentiviral shRNA plasmid (Supplementary Figure S5a online). The reduction of FOXO1 in shRNA-transduced Tregs was confirmed by western blot (Supplementary Figure S5b). Consistent with the findings in Foxo1-deficient mouse models (Ouyang et al., 2012), the expression of T-bet and IFN-γ was upregulated significantly in Tregs in which FOXO1 had been knocked down (Figure 3d–g). In addition, Tregs that were transduced with FOXO1 shRNA exhibited impaired suppressive function as compared to the vehicle control—transduced Tregs (Figure 3h and i). Thus, these results suggest that inactivation of FOXO1 promotes the conversion of Tregs to Th1 cells by directly regulating TBX21 transcription.
transcription, which may subsequently lead to the dysfunction of Tregs in psoriasis.

**Dysregulation of the Akt-FOXO1 axis in psoriatic Tregs**

Next, we examined dysfunction of FOXO1 in psoriatic Tregs. We found that the expression of FOXO1 mRNA was comparable between psoriatic and healthy Tregs, but the levels of Akt1 mRNA were increased in psoriatic Tregs (Figure 4a). The transcription activity of FOXO1 was regulated by phosphorylation at a different site. Akt phosphorylates FOXO1 at Thr24, Ser256, and Ser319, thereby inactivating FOXO1 by promoting its translocation from nucleus to cytosol (Zhao et al., 2011). Therefore, we examined the expression levels of FOXO1, phospho-FOXO1, Akt, and phospho-Akt in psoriatic and healthy Tregs. Psoriatic Tregs had high expression levels of phospho-Akt and phospho-FOXO1, and a higher ratio of phospho-Foxo1/Foxo1 and phospho-Akt/Akt than Tregs from healthy controls (Figure 4b and c). In line with the high phosphorylation of Akt and FOXO1, FOXO1 was mainly located in the cytoplasm of psoriatic Tregs, in contrast to its main nuclear location in healthy Tregs (Figure 4d).

To further investigate whether psoriatic microenvironment can affect the Akt-FOXO1 axis, Tregs isolated from healthy
controls were incubated with psoriatic or healthy serum in culture. Immunofluorescence staining results indicated that incubation of healthy Tregs with psoriatic serum promoted the translocation of FOXO1 from the nucleus into the cytoplasm (Figure 5a and b). The incubation also increased the ratio of phospho-FOXO1/FOXO1 and phospho-Akt/Akt in healthy Tregs (Figure 5c). Taken together, these results revealed that Akt activation mediated FOXO1 inactivation in Tregs of patients.
with psoriasis, suggesting that FOXO1 inactivation may contribute to the dysfunction of psoriatic Tregs.

**Foxo1-deficient Tregs failed to prevent the development of psoriasis in vivo**

Imiquimod (IMQ) has been used to induce a psoriasis-like pathology on the ears of mice. The application of IMQ induced psoriasis-like inflammation on the ears of wild type (WT) but not Rag2−/− mice (Supplementary Figure S6a and b online). However, IMQ application together with the intradermal injection of WT-derived CD4+ T cells into the ears of Rag2−/− mice successfully induced psoriasis-like inflammation (Supplementary Figure S6c and d). We then used this Rag2−/− mouse model to investigate whether Foxo1 deficiency of Tregs contributes to the development of psoriasis in vivo. To this end, estrogen receptor-Cre recombinase with Foxo1fl/fl mice were treated with tamoxifen to delete Foxo1. The deletion of Foxo1 was
confirmed by genotyping PCR and western blot (Supplementary Figure S7 online).

CD4⁺CD25⁺CD127⁻ Tregs were isolated from WT and tamoxifen-treated ER-cre Foxo1<sup>[f/f]</sup> and ER-cre Foxo1<sup>[f/+]</sup> mice, and CD4⁺CD25⁺CD127⁻ Tresps were harvested from WT mice. After local treatment with IMQ on both ears once a day for 7 days consecutively, Rag<sup>2⁻/⁻</sup> mice were injected intradermally with Tresps together with Tregs isolated from ER-cre Foxo1<sup>[f/f]</sup> mice, ER-cre Foxo1<sup>[f/+]</sup> mice, or WT mice into the right ears, or with phosphate buffered saline into the left ears as a control. Then, IMQ was applied locally once a day for 7 days consecutively (Figure 6a). Foxp<sup>3⁺</sup> Tregs were observed in the dermis after injection; however, the number of Tregs decreased significantly in ER-cre Foxo1<sup>[f/f]</sup> Treg-transferred mice (Figure 6b and c). Hematoxylin and eosiin staining showed that the thickness of the epidermis decreased significantly in mice having received the Tregs from WT or ER-cre Foxo1<sup>[f/+]</sup> mice together with Tresps as compared to those that received Tresps alone (mean ± SEM, 39.89 ± 2.920 μm vs. 60.78 ± 4.457 μm, P = 0.0015; and 40.90 ± 3.722 μm vs. 60.78 ± 4.457 μm, P = 0.0041, respectively) (Figure 6d–f), suggesting that Tregs may prevent the development of psoriasis in this model. The epidermis was significantly thicker in mice injected with Tresps together with Tregs isolated from ER-cre Foxo1<sup>[f/+]</sup> mice as compared to those injected with Tresps mixed with Tregs from ER-cre Foxo1<sup>[f/-]</sup> mice (mean ± SEM, 51.71 ± 3.195 μm vs. 40.90 ± 3.722 μm, P = 0.0447) (Figure 6d–f), indicating that the protective function of Foxo1-deficient Tregs was impaired. In addition, WT-derived and ER-cre Foxo1<sup>[f/+]</sup> Tregs suppressed the expression of inflammatory cytokines including tumor necrosis factor-α, IFN-γ, and IL-17, but ER-cre Foxo1<sup>[f/-]</sup> Tregs could not (Figure 6g). Taken together, these in vivo data suggest that Foxo1 is essential for Tregs in preventing the development of psoriasis, and Foxo1 inactivation—mediated Treg dysfunction may contribute to the development of psoriasis.

**DISCUSSION**

This study investigated the dysfunction of Tregs in patients with psoriasis and the underlying mechanisms (Supplemental Figure S8 online). We found that Tregs of patients with psoriasis showed a reduced suppressive function although the percentages of circulating CD4⁺CD25⁺FOXP3⁺ Tregs were similar to that of healthy controls. Gene expression profiling and signaling studies revealed dysregulation of the Akt-FOXO1 axis in psoriatic Tregs and high expression levels of Th1-like transcription factor and cytokines. The ChIP assay and luciferase reporter system proved that FOXO1 can bind to the promoter sequences of TBox21 and repress its transcription. Knockdown of FOXO1 promoted the conversion of human Tregs into Th1 cells, leading to dysfunction of Tregs. In a psoriasis-like mouse model, Foxo1-deficient Tregs failed to protect mice from developing psoriasis. These findings reveal that the dysregulation of the Akt-FOXO1 axis may be a critical cause of Treg dysfunction in psoriasis.

Psoriasis is believed to be caused by excessive activation of T cells. Tregs, which are pivotal in controlling the immune response, are also associated with psoriasis (Mattozzi et al., 2013). Some studies showed that the frequency of Tregs was decreased in psoriasis (Chen et al., 2008), whereas others found that the frequency was comparable to that observed in healthy controls (Sugiyama et al., 2005). Nonetheless, Treg function has been consistently reported to be impaired in psoriasis (Sugiyama et al., 2005). The inconsistent results regarding the frequency of Tregs in psoriasis could be attributable to several factors, for example, differences observed in the disease severity, disease stage, infection history, and treatment (Mattozzi et al., 2013). In this study,
Figure 5. Serum from patients with psoriasis activated Akt and induced FOXO1 cytoplasm translocation in Tregs from healthy controls.

CD4^+CD25^+CD127^− Tregs from HC were cultured with serum from HC or Pso at 37 °C for 20 minutes, and then stained with indicated immunofluorescence-labeled antibodies or extracted for western blot. (a) Representative immunostaining images of FOXO1. (b) Quantitative analysis of FOXO1 localization in the cytosol and nuclei of Tregs. Data were collected from five fields, and the total number of cells (232–445 cells) was counted (n = 5). (c) Expression of p-FOXO1 Thr24, total FOXO1, p-Akt Ser473, and total Akt proteins in Tregs. Akt, protein kinase B; cyto, cytosol; HC, healthy control; Nuc, nuclei; p-Akt, phospho-Akt; p-FOXO1, phospho-FOXO1; Pso, patients with psoriasis; Treg, regulatory T cell.

The aberrant frequency and function of Tregs were observed not only in the blood of patients with psoriasis but also in the psoriatic lesions (Goodman et al., 2009). Recent studies showed that skin-resident Tregs were highly proliferative and produced low levels of IL-17 in the inflamed skin of patients with psoriasis and IMQ-induced psoriasis-like dermatitis (Sanchez Rodriguez et al., 2014). In this study, the intradermal injection of Tregs could alleviate IMQ-induced psoriasis-like dermatitis (Sanchez Rodriguez et al., 2014). In this study, the intradermal injection of Tregs could alleviate IMQ-induced psoriasis-like dermatitis more significantly than systemic injection, suggesting that lesional Tregs play a more important role in psoriasis. Nevertheless, these lesional Tregs are not only derived from resident Tregs but also recruited from the periphery; thus, dysfunction of both subsets of Tregs may contribute to the development of psoriasis (Sanchez Rodriguez et al., 2014; Sugiyama et al., 2005).

Previous studies have mainly focused on the aberrant frequency and function of Tregs in psoriasis. The molecular mechanism remains unclear. Tregs derived from blood samples and lesions from patients with psoriasis produce IL-17, which could be induced by IL-6 in the lesion (Bovenschen et al., 2011; Goodman et al., 2009; Sanchez Rodriguez et al., 2014). Tregs and Th17 cells are two different subsets that can be converted into each other under immune environments (Koenen et al., 2008; Lochner et al., 2008). A variety of evidence has suggested that Tregs can also be converted into other T cell subsets, such as Th1 and Th2 (Yu et al., 2015; Zheng et al., 2011). Here, a transcriptome study demonstrated that psoriatic Tregs produced more Th1-like transcription factor and cytokines than healthy Tregs, including T-bet, IFN-γ, and tumor necrosis factor-α. Thus, Tregs could be converted into both Th17 and Th1 cells in psoriasis.

Previous studies have shown that Foxo1-deficient Tregs also produce high levels of Th1-like cytokines (Ouyang et al., 2012). We proved that FOXO1 controls the conversion of Tregs to Th1 cells by directly repressing TBX21 transcription. In addition, overlapping gene expression profiling between psoriatic and Foxo1-deficient Tregs suggests that FOXO1 may be inactivated in psoriatic Tregs. Although the mRNA levels of FOXO1 were equivalent in psoriatic and healthy Tregs, FOXO1 phosphorylation at Thr24 was increased in psoriatic Tregs, which led to FOXO1 cytosolic location. In addition, incubation of normal Tregs with serum from patients with psoriasis promoted FOXO1 phosphorylation and its nuclear exclusion. These findings support that FOXO1 inactivation cannot repress TBX21 transcription, leading to Th1-like Tregs in patients with psoriasis.

The transcription factor Foxo1 plays a crucial role in regulating T-cell activation, as well as the development and function of Tregs (Hedrick et al., 2012; Ouyang and Li, 2011). Several downstream mediators of Foxo1 have been identified in Tregs in mice, including Foxp3 and CTLA-4 (Bothur et al., 2015; Kerdiles et al., 2010; Ouyang et al., 2010). Foxo1 activation induces Foxp3 expression, which is essential for
the initiation of Treg differentiation and maintenance of the suppressive immune function of Tregs (Harada et al., 2010; Ohkura and Sakaguchi, 2010). This study demonstrates that FOXO1 also plays an important role in the function of human Tregs. It must be noted that although the gene expression profile was similar in psoriatic Tregs and Foxo1-knockout Tregs, the expression of FOXP3 and CTLA-4 was not decreased in psoriatic Tregs.

However, there still exist some limitations. The markers to distinguish Tregs are not precise enough. CD25, CD127, and FOXP3 were considered the best markers to distinguish Tregs from activated Tresp. However, CD25 can be positively expressed in activated Tresp. TCR/CD28-activation can downregulate CD127 expression, leading to CD127εT cells (Alves et al., 2008). Activated Tresp can also express FOXP3 instantaneously upon polyclonal TCR activation.

Figure 6. Foxo1-deficient Tregs failed to ameliorate psoriasis in mouse models. (a) Schematic of the animal experimental protocol. (b, c) Representative immunostaining images of Foxo1 (green) and Foxp3 (red). (d, e) H&E staining. (f) Mean thickness of the epidermis (n = 5). (g) The mRNA expression of TNF-α, IFN-γ, and IL-17 in the lesion (n = 5). *P < 0.05 and **P < 0.01. H&E, hematoxylin and eosin; Treg, regulatory T cell. Scale bar = 100 μm.
To distinguish FOXP3+ Tregs from FOXP3+ Tresp, Rag2−/−Foxp3GFP/creROSA26RFP mice models were used to define Foxp3+ Tregs as Foxp3GFPRFP and Foxp3+ Tresp as Foxp3−RFP (Miyao et al., 2012). However, in humans, it is too difficult to distinguish between CD4+CD25+CD127−FOXPO3+ Tregs and CD4+CD25+CD127−FOXPO3+ Tresp. Although the specific markers for Tregs are controversial, FOXP3 is still widely considered a well-recognized characteristic of Tregs (Lu et al., 2017). Thus, in this study, we analyzed the role of CD4+CD25+FOXP3+ Tregs in patients with psoriasis. In addition, we think the best way to define Tregs is that they exhibit suppressive immune function. In our study, we performed coculture experiments to provide strong evidence.

Psoriasis is a complex disease that is mediated by an excessive immune response. Foxo1-deficient Tregs failed to protect mice from developing psoriasis in the IMQ-induced psoriasis-like mouse model, which strongly suggests that dysfunction of Tregs may promote the development of psoriasis. These findings reveal that the dysregulation of the Akt-FOXO1 axis may be a critical cause of Treg dysfunction in psoriasis. Thus, this study provides further insights into the understanding of the pathogenesis of psoriasis.

MATERIALS AND METHODS

Patients and samples
This study was approved by the Ethics Committee of the Fourth Military Medical University of Xijing Hospital. All healthy volunteers and patients with psoriasis agreed to participate in this study and signed informed consent forms. The information of all healthy volunteers and patients with psoriasis can be seen in Supplementary Methods and Supplementary Table S1 online.

In vitro Treg suppression assay
The coculture of CD4+CD25+CD127+ Tresp and CD4+CD25+CD127+ Tregs were performed as described in Supplementary Methods.

Microarrays
CD4+CD25−CD127− Tregs were isolated from 15 patients with psoriasis and 15 healthy controls by FACS. Sorted Tregs from the patients or the healthy controls were pooled to ensure each group had enough cells for the microarray study. Total RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA). Microarrays were performed using Affymetrix Chips (Thermo Fisher Scientific, Waltham, MA), and the resulting data were analyzed by Bio-Medlab (Chatswood, Australia). These approaches are detailed in Supplementary Methods.

Real-time quantitative PCR
These approaches are detailed in Supplementary Methods and Supplementary Table S2 online.

Flow cytometry
To detect cytokine production, peripheral blood mononuclear cells were incubated with lipopolysaccharide for 12 hours followed by a cell stimulation cocktail for 4 hours. Thereafter, flow cytometry was performed as described in detail in Supplementary Methods.

Chromatin immunoprecipitation assay
ChIP was performed using the ChIP Assay Kit (MilliporeSigma, Burlington, MA) according to the manufacturer’s instructions. These approaches are detailed in Supplementary Methods.

Luciferase reporter system
Jurkat cells (1 × 105 per well) were cultured in a 24-well plate and transfected with TBX21 luciferase reporter plasmid and the internal control vector pRL-TK (Promega, Madison, WI). These approaches are detailed in Supplementary Methods.

shRNA knockdown
shRNA targeting human FOXO1 was carried by lentiviral vectors (GenePharma, Shanghai, China). The sequences of the oligonucleotides are shown in Supplementary Table S2. These approaches are detailed in Supplementary Methods.

Animal models
All animal studies were performed in compliance with the institutional guidelines for the care and use of laboratory animals and were approved by the Laboratory Animal Resource Center of the Fourth Military Medical University. The source of mice is outlined in detail in Supplementary Methods. Psoriasis-like models were established in Rag2−/− mice by the adoptive transfer of CD4+ T cells combined with the local application of IMQ cream (iNova Pharmaceuticals, Chatswood, Australia). These approaches are detailed in Supplementary Methods.

Western blot
Tregs were lysed in radioimmunoprecipitation assay buffer (Cell Signaling Technology, Danvers, MA) supplemented with protease and phosphatase inhibitor cocktails (Thermo Fisher Scientific). Western blot was performed to detect the activation of Akt-FOXO1 pathways in Tregs. These approaches are detailed in Supplementary Methods.

Immunofluorescence microscopy
Tregs were cultured in plates coated with poly-L-lysine (50 ng/ml). The plates were then centrifuged at 10,000 r.p.m. for 5 minutes to make the cells adhere to the bottom of the wells. Immunofluorescence staining was performed to detect the FOXO1 translocation in Tregs as detailed in Supplementary Methods.

Statistical analysis
The data were analyzed using GraphPad Prism 5 (GraphPad Software, San Diego, CA) and are presented as mean ± SEM. P < 0.05 was considered to be statistically significant.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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