IκBζ is a key transcriptional regulator of IL-36–driven psoriasis-related gene expression in keratinocytes

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Edited by Sankar Ghosh, College of Physicians and Surgeons, Columbia University, New York, NY, and accepted by Editorial Board Member Tadatsugu Taniguchi August 17, 2018 (received for review January 24, 2018)

Proinflammatory cytokine signaling in keratinocytes plays a crucial role in the pathogenesis of psoriasis, a skin disease characterized by hyperproliferation and abnormal differentiation of keratinocytes and infiltration of inflammatory cells. Although IL-17A and TNFα are effective therapeutic targets in psoriasis, IL-36 has recently emerged as a proinflammatory cytokine. However, little is known about IL-36 signaling and its downstream transcriptional responses. Here, we found that exposure of keratinocytes to IL-36 induced the expression of IκBζ, an atypical IκB member and a specific transcriptional regulator of selective NF-κB target genes. Induction of IκBζ by IL-36 was mediated by NF-κB and STAT3. In agreement, IL-36–mediated induction of IκBζ was found to be required for the expression of various psoriasis-related genes involved in inflammatory signaling, neutrophil chemotaxis, and leukocyte activation. Importantly, IκBζ–knockout mice were protected against IL-36–mediated dermatitis, accompanied by reduced proinflammatory gene expression, decreased immune cell infiltration, and a lack of keratinocyte hyperproliferation. Moreover, expression of IκBζ mRNA was highly up-regulated in biopsies of psoriasis patients where it coincided with IL36G levels. Thus, our results uncover an important role for IκBζ in IL-36 signaling and validate IκBζ as an attractive target for psoriasis therapy.

NFKBIZ | IκBζ | IL-36 | keratinocytes | psoriasis

Transcription factor NF-κB has been implicated in several inflammatory diseases, including psoriasis, by activating various proinflammatory target genes (1). The classical activation of NF-κB is controlled by cytoplasmic inhibitory proteins, such as IκB, which sequester NF-κB in the cytoplasm (2). Inflammatory stimulation of cells results in the rapid activation of IκB kinase (IKK), which triggers the phosphorylation-induced degradation of IκBα, leading to NF-κB’s nuclear translocation and transcriptional activation. Recent evidence, however, suggests that the activation of NF-κB target genes is more complex and is dependent on the particular gene context or stimulus, which is thought to facilitate selective gene regulation in distinct physiological settings (3). Whereas the rapid activation of primary response genes is directly induced by the classical NF-κB pathway, expression of so-called “secondary-response genes” requires prior protein synthesis of additional NF-κB regulators (4). In this context, we and others have identified IκBζ, an atypical nuclear IκB protein, which functions not only as a repressor but, more importantly, also as an activator of a selective subset of NF-κB target genes (5–8). The mechanisms of this differential gene regulation by IκBζ remain largely unknown, but increasing evidence suggests that the transcriptional activity of IκBζ is mainly mediated at the level of chromatin remodeling (6, 9, 10).

In keratinocytes (KCs), IL-17A and, more potently, its combination with TNFα induce IκBζ expression (11). Subsequently, IκBζ mediates the induction of important psoriasis-related gene products, including chemokines (e.g., CXCL8 and CCL20), cytokines (e.g., IL22 and IL17C), and antimicrobial proteins, such as S100 calcium-binding proteins (e.g., S100A9, β-defensin-2 (DEFB4A), or lipocalin-2 (LCN2)). Antagonists of TNFα and IL-17A have therefore been approved for the treatment of psoriasis (12). Moreover, NFKBIZ, the gene encoding IκBζ, has been identified as a psoriasis-susceptibility locus (13). Global Nfkbia KO mice are resistant to imiquimod (IMQ)- or IL-23–induced psoriasis–like skin inflammation (11). In contrast, Tnfa– or Il17a–KO mice, which are only partially protected against IMQ-induced psoriasis, still show elevated IκBζ mRNA levels in inflamed skin areas (11). These observations imply an additional IL-17A/TNFα–independent pathway which drives IκBζ expression and thereby contributes to inflammatory gene expression in psoriasis.

Recently, IL-36 cytokines have received attention as therapeutic targets for psoriasis (14). This subfamily of IL-1–related cytokines consists of three proinflammatory members, IL-36α (encoded by IL1F6/IL36α), IL-36β (encoded by IL1F8/IL36B), and IL-36γ (encoded by IL1F9/IL36G) (15–17). All family members bind to a common heterodimeric receptor, composed of IL-36R (also termed “IL-1RL2”) and IL-1RACp, leading to the recruitment of the adapter MyD88 and subsequent activation of NF-xB and MAPK (18). A fourth IL-36 member, IL-36RN, acts as a natural antagonist of IL-36 signaling, as it binds to IL-36R but does not recruit the coreceptor IL-1RAcP (19, 20).

Significance

Psoriasis is an autoinflammatory disease characterized by cytokine-driven keratinocyte proliferation and infiltration of immune cells. While IL-17A and TNFα are established targets in psoriasis therapy, IL-36 is emerging as an important cytokine in this disease. The mechanisms of IL-36–driven proinflammatory responses are largely unknown. Here we identified IκBζ, a transcriptional regulator of selective NF-κB target genes, as a crucial mediator of IL-36 action. In keratinocytes, IκBζ was required for the expression of several psoriasis-related cytokines and chemokines. Moreover, genetic deletion of IκBζ prevented IL-36–mediated dermatitis induction in mice. Since IκBζ is essential not only for IL-36 but also for IL-17 signaling, our results suggest that inhibition of IκBζ function could be a future strategy in psoriasis therapy.
Importantly, while full-length IL-36 proteins seem to be biologically inactive, activation of IL-36 signaling requires their N-terminal proteolytic processing (19, 21).

IL-36 contributes to skin inflammation by acting on KCs and immune cells. Interestingly, IL-36 can induce a subset of proinflammatory target genes similar to those induced by IL-17A in KCs, including CXCL8, IL-23A, DEFBA, or LCN2 (22–24). Vice versa, IL-17, which is typically expressed by immune cells, induces IL-36y expression in KCs (25, 26). Therefore, IL-36 appears to have a central position in the interplay between immune cells and KCs. In patients with psoriasis vulgaris, IL-36α and IL-36γ are overexpressed, whereas inactivating mutations of IL-36RN are enriched in a psoriasis subtype, called “generalized pustular psoriasis” (22, 23, 27, 28). In agreement, mice overexpressing IL-36α in basal KCs exert skin inflammation at 3 wk of age, which is augmented in an IL-36RN-deficient background (20, 29). In contrast, mice deficient for the IL-36R are fully protected against IMQ-induced psoriasis (30).

Despite its involvement in psoriasis, little is known about IL-36 signaling and its transcriptional responses. In the present study, we found that IL-36α and IL-36γ are potent inducers of IkBζ expression. Moreover, we identified MyD88, NF-κB and STAT3 as crucial components for IL-36-induced IkBζ expression. Silencing of IkBζ in primary human KCs prevented IL-36-mediated up-regulation of multiple psoriasis-associated genes, while a global knockout of IkBζ protected against IL-36-mediated psoriasis-like dermatitis in mice. These results and our finding of a strong correlation of NFKBIZ and IL-36G expression in psoriatic lesions uncover an important role for IkBζ in IL-36 signaling and thus validate IkBζ as an attractive target for psoriasis therapy.

Results

IL-36 Induces IkBζ Expression in KCs. To investigate the relationship between IL-36 and IkBζ, we treated the keratinocyte cell line HaCaT and primary human KCs with recombinant IL-36α for 1–24 h. Whereas untreated KCs lacked IkBζ expression, 1 h of stimulation with IL-36α was sufficient to induce sustained IkBζ expression on the mRNA and protein level (Fig. L4). As revealed by the addition of actinomycin D to IL-36α-treated cells, the increased NFKBIZ mRNA levels resulted from transcriptional up-regulation of NFKBIZ rather than from mRNA stabilization (SI Appendix, Fig. S1A). Importantly, full-length IL-36α, which supposedly lacks biological activity, failed to induce IkBζ expression, whereas IL-17A, either alone or combined with TNFα, induced IkBζ expression with kinetics similar to those of truncated IL-36α (Fig. L4 and SI Appendix, Fig. S1 B and C). As some reports implied distinct target gene regulation by the different IL-36 members (14, 24, 25), we also stimulated HaCaT cells and primary KCs with IL-36γ. IL-36γ induced NFKBIZ mRNA and protein expression with kinetics and potency similar to that of IL-36α (Fig. 1B).

We next investigated whether other psoriasis-associated cytokines, such as IL-1β, IL-17A, TNFα, or IFNγ, could potentiate the effect of IL-36α on IkBζ protein expression (Fig. 1C). Although certain differences were noted between HaCaT cells and primary KCs, most of the tested cytokines enhanced IL-36α-mediated IkBζ expression. Importantly, the combination of IL-17A and IL-36α was clearly more effective in triggering IkBζ expression than were the single cytokines alone.

Induction of IkBζ by IL-36 Is Mediated by MyD88, NF-κB, and STAT3. As IkBζ is also induced by IL-17A, we further dissected the mechanism of IkBζ expression induced by IL-36 compared to IL-17A. IL-17A binds and activates the IL-17RA/IL-17RC receptor, followed by the recruitment of the adapter protein Act1 and the activation of MAPK and NF-κB (31). In contrast, IL-36 utilizes a divergent proximal signaling cascade by binding to the IL-36 receptor complex, composed of IL1R2 and its coreceptor IL1RAP, leading to the recruitment of MyD88 and activation of MAPK and NF-κB (17). Indeed, knockdown of MyD88 revealed that it was indispensable for IkBζ expression upon IL-36α stimulation, while it had no effect in IL-17A–treated cells (Fig. 2A and SI Appendix, Fig. S2A).

As IkBζ is transcriptionally induced by IL-36, we explored the NFKBIZ promoter region to identify relevant transcription factors. Two major IkBζ isoforms have been described, including a long isoform (IkBζL) of 718 aa and a N-terminally truncated isoform (IkBζS) of 618 aa that is thought to be generated by alternative splicing (8, 32). By analyzing published DNase I and Pol II ChIP-sequencing (ChIP-seq) data (33), we identified that the two isoforms arise not only from alternative splicing but also from two different promoter regions with distinct transcriptional start sites (Fig. 2B). Moreover, our own RNA-sequencing (RNA-seq) data revealed that KCs use only the proximal promoter 2 that is translated into the IkBζL isoform. Previous promoter
analyses, however, had examined only promoter 1, which is located ~20 kb upstream of promoter 2 (32, 34, 35). This distal promoter is used in several cell types for transcription of NFKBIZ variant 2, which lacks exon 3 and thus is translated to the IkBζ variant.

Bioinformatic analysis of the NFKBIZ promoter 2 revealed putative binding sites for STAT3, NF-κB, API, KLF4, and STAT1. To uncover the contribution of these sites to NFKBIZ induction, we cloned the promoter region (~1.5 kb upstream of the transcription start site of IkBζ1) into a luciferase construct and generated deletions lacking one of the predicted binding sites. Expression of the constructs was analyzed after transfection of HaCaT cells followed by stimulation with IL-36α. Indeed, expression of the NFKBIZ promoter 2 was significantly increased by IL-36α, whereas deletion of the STAT3- or the NF-κB-binding site inhibited NFKBIZ promoter expression (Fig. 2C). In accordance, ChIP identified a direct physical binding of NF-κB p65 and STAT3 to NFKBIZ promoter 2, along with the binding of phosphorylated RNA polymerase II as a marker for active transcription (Fig. 2D). IL-36α also triggered the early activation of STAT3, NF-κB, and MAPK in HaCaT cells or primary KCs (Fig. 2E and SI Appendix, Fig. S2B). Interestingly, a similar activation of STAT3 and NF-κB was detected in IL-17A-treated cells (SI Appendix, Fig. S2C). Whereas inhibition of MAPK did not affect IkBζ expression in IL-36α-treated HaCaT cells (SI Appendix, Fig. S2D), the blocking of NF-κB activation by IKK inhibition or knockdown of p65 efficiently prevented IkBζ expression upon IL-36α stimulation (Fig. 2F and G). Moreover, depletion of STAT3 by two different shRNAs strongly inhibited IkBζ mRNA and protein expression (Fig. 2H). Similarly, depletion of p65 or STAT3 impaired IkBζ induction after stimulation with IL-17A (SI Appendix, Fig. S2 E and F). Thus, IL-36α and IL-17A both employ NF-κB and STAT3 for IkBζ induction.

IkBζ Is a Key Mediator of IL-36-Induced Gene Expression in KCs. Next, we investigated the function of IkBζ in IL-36 signaling and therefore first explored the time course of IkBζ-modulated gene expression. We stimulated control and NFKBIZ-knockdown HaCaT cells for 0–24 h with IL-36α and analyzed selected IL-36 target genes. IL-36α stimulation led to the induction of IL36G, IL17C, CXCL5, or S100A49 with different kinetics (SI Appendix, Fig. S3A). Surprisingly, NFKBIZ silencing not only prevented the induction of late-responsive genes such as S100A49 but also affected early gene induction, e.g., of IL36G or IL17C.

To reveal a global picture of IL-36-driven gene expression by IkBζ, we generated control and NFKBIZ-depleted primary KCs and performed transcriptome analyses after 1.5 and 24 h of IL-36α stimulation (Fig. 3 A and B). Silencing of IkBζ resulted in the deregulation of several hundred target genes in IL-36α-stimulated primary human KCs (SI Appendix, Tables S1 and S2). Interestingly, early after IL-36α stimulation most genes were down-regulated by IkBζ, including genes for antiinflammatory phosphatases (DUSP2 and DUSP9). In contrast, after 24 h most IkBζ-modulated genes were positively regulated and hence were down-regulated by the NFKBIZ knockdown. Many of these IkBζ-inducible genes are typically overexpressed in psoriasis, including genes for antimicrobial proteins (DEFB4 and LCN2), S100 proteins (S100A47, S100A8, and S100A9), and chemokines (CFSF2, CFS3, CXCL8, IL23A, and IL36A).

Principal component analysis (PCA) revealed that the gene-expression profile not only differed between untreated and IL-36α-stimulated cells but was also divergent after 1.5 and 24 h of IL-36α stimulation (SI Appendix, Fig. S3B). Moreover, as shown in the Venn diagrams in Fig. 3 C and D, only a subset of the IL-36α–regulated genes was IkBζ-dependent (83 of 607 genes after 1.5 h and 86 of 800 genes after 24 h of IL-36α stimulation). Gene ontology (GO) term analysis of the affected genes uncovered that IkBζ is mostly regulated inflammatory responses, neutrophil chemotaxis, and leukocyte function downstream of IL-36 (Fig. 3D). We also compared our RNA-seq analyses with a previously defined IL-36 core signature comprising 182 genes that were regulated by IL-36 after 24 h in human KCs (14). The comparison not only revealed a high overlap with our RNA-seq analyses but also identified 39 of the 182 IL-36 core target genes as IkBζ-dependent (SI Appendix, Fig. S3 C and D).

The IkBζ-dependent gene regulation by IL-36α in primary KCs at early and late time points was confirmed by qPCR of
select genes, such as IL36G, S100A9, LCN2, DEFB4, CXCL8, and CCL20 (Fig. 3E). Importantly, regulation of these IkBζ target genes was conserved in IL-17A− and TNFα−treated primary KCs as well as in IL-36α−, IL-36γ−, and IL-18−treated HaCaT cells (Fig. 3F and SI Appendix, Fig. S4 A–C). These findings thus implicate IkBζ as a master regulator of proinflammatory gene expression not only in IL-36−stimulated but also in IL-17A−, TNFα−, or IL-18−treated KCs.

IkBζ Promotes IL-36-Driven Psoriasis-Like Disease in Vivo. Global Nfkbiz−KO mice are protected against IMQ-induced psoriasis-like skin inflammation (11). Since the TLR7 agonist IMQ directly activates the innate immune response, it is difficult to discriminate between the contribution of IL-17 and IL-36 to the disease onset. Moreover, global Nfkbiz−KO mice develop an autoinflammatory phenotype in adulthood (36, 37), which could influence the skin inflammation of IMQ-treated mice. We therefore generated a mouse model using tamoxifen-inducible Nfkbiz−KO mice that received intradermal injections of active IL-36α into the ears. Tamoxifen-induced Cre recombinase activation just before IL-36α application led to an effective KO of IkBζ, thereby preventing potential congenital off-target effects (Fig. 4A). Intradermal injection of IL-36α into the ears of control animals induced Nfkbiz transcription (Fig. 4A) and, moreover, triggered ear swelling, scaling, epidermal thickening, KC hyperproliferation and increased infiltration of immune cells (Fig. 4B and C). Intradermal injection of IL-36α into the ears of control animals induced Nfkbiz transcription (Fig. 4A) and, moreover, triggered ear swelling, scaling, epidermal thickening, KC hyperproliferation and increased infiltration of immune cells (Fig. 4B and C).
and C and SI Appendix, Fig. S5A). These alterations were nearly absent in the IL-36α–treated KO mice. Histological and flow cytometric analyses revealed a marked increase in infiltrating CD45+ immune cells, macrophages, and neutrophils in the IL-36α–treated control animals, which was significantly blocked in the KO mice (Fig. 4 D and E). T cell infiltration was reduced in the KO animals as well, although the degree of T cell infiltration was generally low in the ears of IL-36α–treated mice (SI Appendix, Fig. S5B). Importantly, expression of several psoriasis-associated target genes, similar to those identified by transcriptome analysis of IL-36α–treated KCs (Fig. 3), was up-regulated in the ears of IL-36α–treated control but not in IL-36α–treated KO mice (Fig. 4E). Likewise, the expression of IkBζ-dependent proteins involved in granulocyte and leukocyte chemotaxis was also decreased in the KO mice (SI Appendix, Fig. S5C). Thus, IkBζ KO strongly protected against IL-36–driven psoriasis-like disease in vivo, which could be mediated by effects of Nfkbia deficiency in KCs as well as in immune cells.

As previously reported (11, 13), we validated increased NFKBIZ expression in lesions from psoriasis patients, as compared with nonlesional skin areas or unaffected individuals (SI Appendix, Fig. S5D). Expression of IL17A and especially IL36G was elevated in psoriatic lesions. We then correlated the expression of NFKBIZ, IL36G, and IL17A in nonlesional and lesional samples in the individual patients to obtain an idea of the relevance of the two cytokines in driving NFKBIZ expression in psoriatic tissue. The correlation of IL36G and NFKBIZ was stronger than the link between IL17A and NFKBIZ, implicating IL-36 as an important driver of NFKBIZ expression in psoriasis (Fig. 4G). Moreover, as IL-36–mediated NFKBIZ induction could account for increased expression of psoriasis-related cytokines, we correlated the expression of LCN2, a bona fide IkBζ target gene (38), to IL36G, NFKBIZ, and IL17A expression. Indeed, the expression level of LCN2 matched strongly IL36G and NFKBIZ expression, whereas it was only weakly correlated to IL17A expression patterns in psoriatic lesions (SI Appendix, Fig. S5E). These findings support a major role of IkBζ in IL-36 signaling in KCs and psoriasis and suggest IkBζ as an attractive therapeutic target which mediates proinflammatory signaling downstream of IL-17A and IL-36.

Discussion

Previous studies by us and others found that IkBζ is overexpressed in psoriatic lesions, whereas Nfkbia KO mice are protected against IMQ-induced psoriatic skin inflammation (11, 13). In these and follow-up studies, IkBζ was identified as a major mediator of IL-17A signaling, leading to the induction of proinflammatory signaling in KCs (11, 39). Interestingly, in Il17a- or Il17a–KO mice neither induction of Nfkbia nor skin inflammation were fully blocked after IMQ treatment (11, 40), implying additional pathways of NFKBIZ induction and promotion of psoriasis.

Recently, IL-36α and IL-36γ have been identified as being overexpressed in psoriatic lesions (22, 23). In agreement, IL-36 treatment of KCs induced proinflammatory signaling (14), whereas KO of the IL-36 receptor inhibited IMQ-induced skin inflammation in mice. Our results show that IkBζ provides an important link between IL-36 signaling and psoriasis-associated inflammatory gene expression. We revealed that IL-36 mediates IkBζ expression in HaCaT cells and primary KCs, which followed kinetics similar to those seen with IL-17A/TNFα treatment, implying similar signaling pathways in IkBζ induction.

By ChIP-seq data and our own RNA-seq analyses we identified that KCs induce transcription of NFKBIZ from the yet uncharacterized proximal promoter 2, which contains several conserved binding sites for proinflammatory transcription factors. Indeed, IL-36 and IL-17A stimulation led to the activation of NF-κB, whereas knockdown of the NF-κB subunit p65 prevented IkBζ induction.

Besides NF-κB, we identified STAT3 as a regulator of IkBζ expression, as its depletion was sufficient to block IL-36– and IL-17A–mediated induction of IkBζ. These findings are intriguing, as STAT3 itself can drive proinflammatory gene expression in psoriasis (41). Constitutively active STAT3 in the epidermis of psoriatic lesions is often detectable, whereas pharmacological inhibition of STAT3 ameliorated psoriasis-like skin lesions in mice (42, 43). Moreover, STAT3 was proposed to control IkBζ expression in T cells (44). As STAT3 is especially involved in IL-36–driven induction of IkBζ expression, STAT3 inhibitors could be promising agents for the effective treatment of general pustular psoriasis, which is caused by mutations of IL36RN and hyperactivation of the IL-36 pathway (27, 28).

Our gene-expression profiling revealed that IL-36 affected the expression of hundreds of genes at early and late stimulation time points. As early effects of IL-36 stimulation on gene expression have not been investigated before in KCs, we could not only validate defined IL-36 target genes (14) but also identify previously unknown IL-36 dependent genes (e.g., IL17C, CSF2, CSF3) that encode important psoriasis-promoting cytokines (45–46). Of note, NFKBIZ knockdown led to the deregulation of a specific subset of IL-36 target genes at early and late stimulation time points. Most of these IkBζ-dependent IL-36 target genes regulate antimicrobial and proinflammatory responses, neutrophil chemotaxis, and leukocyte activation and hence have been implicated in the pathogenesis of psoriasis. Moreover, IkBζ–dependent gene expression seems to be highly conserved, as we found similar changes in the expression of IkBζ–dependent genes (e.g., DEF64, CCL20, SI100A7, SI100A9, and LCN2) in HaCaT cells and primary KCs as well as upon IL-36α, IL-36γ, or IL-17A/TNFα stimulation.

Employing an inducible Nfkbia–KO model, we further demonstrate that the absence of IkBζ also impaired psoriasis-related gene expression under in vivo conditions of IL-36α stimulation. Nfkbia–KO mice exhibited significantly reduced skin pathology, including less ear swelling and KC proliferation, and a strongly reduced infiltration of immune cells, in particular neutrophils. The results are consistent with findings in Il36α-deficient mice that are also protected in the IMQ psoriasis model (30). Notably, the present study demonstrated that Nfkbia–KO mice were even more protected than Il17a–deficient mice (11), supporting the idea that IkBζ might also be involved in IL-17–independent effects of psoriasis development. Of note, Il36α-deficient mice also showed stronger protection in the IMQ model than Il17a–KO mice (30).

In agreement with our findings in cultured KCs and Nfkbia–KO mice, expression data from psoriasis patients validated elevated NFKBIZ and IL36G levels in psoriatic lesions as compared with nonaffected skin areas or skin from unaffected healthy individuals. Moreover, the expression of IL36G and NFKBIZ was strongly correlated with the IkBζ target gene LCN2 compared with a much weaker correlation of IL17A with NFKBIZ and LCN2 expression levels. These data strengthen the hypothesis that IL-36 is an initial driver for NFKBIZ expression in psoriasis.

While our results clearly position IkBζ downstream of IL-36, IL-17A and IL-36, in turn, are also transcriptional targets downstream of IL-36 (SI Appendix, Fig. S4D). Likewise, IL-17A, especially in combination with TNF, is a strong inducer of IkBζ expression but can be also induced downstream of IkBζ. Thus, the strong expression of NFKBIZ in psoriasis patients might be caused not only by elevated IL-36 expression but also by increased IL-17–type responses. The exact contribution of each cytokine is complicated by the existence of multiple members of the IL-17 and IL-36 families. Because IL-17 and IL-36 can mutually reinforce each other (25, 26), the two cytokines drive
complex autoamplification loops in which IkBζ seems to have an integral role in promoting skin inflammation (for a scheme see SI Appendix, Fig. S6). In fact, our present and previous results (11) suggest a dual requirement for IkBζ in IL-36 signaling of innate epithelial cells, such as KCs, as well as in IL-17A signaling of T cells, both of which might be necessary to drive full-blown psoriasis.

In conclusion, our findings reveal that two major cytokines, IL-36 and IL-17A, promote psoriasis by inducing IkBζ expression. While IL-17A antibodies have proven therapeutic efficacy, blocking of IL-36 might represent an alternative for patients resistant to anti–IL-17A therapy. Moreover, targeting their common mediator IkBζ might lead to future approaches for efficient long-term treatment of psoriasis patients.

**Materials and Methods**

Detailed information on cell culture experiments, generation of knockdown cells, luciferase reporter assays, ChIP, analyses of RNA and protein expression, RNA-seq, cytokine antibody arrays, generation of Nfkbia-KO mice, flow cytometry, histology, and analysis of patient data is provided in SI Appendix.

**Acknowledgments**

We thank C. Schönfeld, C. Resch, and J. Leefler for assistance and A. Witten and M. Stoll (University of Münster) for transcriptome profiling. Murine IL-36α was generously provided by Amgen. This work was supported by the grants Sonderforschungsbereich/Transregio SFB/TR 156 (to S.H. and D.K.), SFB/TR 209 (to K.S.-O. and H.S.), the Emmy-Noether program of the Deutsche Forschungsgemeinschaft (to S.H.), and the German Ministry for Education and Research Grant of the Network for Autoinflammatory Disorders in Children and Adolescents 01FP090148B (to K.S.-O.).