nature medicine

The microRNA miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TAB2, TAB3 and IKK-α

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Inflammatory cytokines such as interleukin-17 (IL-17) promote inflammatory autoimmune diseases. Although several microRNAs (miRNAs) have been shown to regulate autoimmune pathogenesis by affecting lymphocyte development and function, the role of miRNAs in resident cells present in inflammatory lesions remains unclear. Here we show that miR-23b is downregulated in inflammatory lesions of humans with lupus or rheumatoid arthritis, as well as in the mouse models of lupus, rheumatoid arthritis or multiple sclerosis. IL-17 downregulates miR-23b expression in human fibroblast-like synoviocytes, mouse primary kidney cells and astrocytes and is essential for the downregulation of miR-23b during autoimmune pathogenesis. In turn, miR-23b suppresses IL-17–, tumor necrosis factor α (TNF-α)– or IL-1β–induced NF-κB activation and inflammatory cytokine expression by targeting TGF-β–activated kinase 1/MAP3K7 binding protein 2 (TAB2), TAB3 and inhibitor of nuclear factor κ-B kinase subunit α (IKK-α) and, consequently, represses autoimmune inflammation. Thus, IL-17 contributes to autoimmune pathogenesis by suppressing miR-23b expression in radio-resident cells and promoting proinflammatory cytokine expression.

Abnormal inflammatory responses promote tissue damage in autoimmune diseases, including rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (SLE)[1–3]. Most of our understanding of the pathogenesis of autoimmune diseases comes from investigations of the relevant mouse models, such as collagen-induced arthritis (CIA), experimental autoimmune encephalomyelitis (EAE) and the autoimmune-prone MRL/*lpr* mouse strain[4]. Expression of proinflammatory cytokines, including TNF-α, IL-lβ and IL-17, has been shown to be elevated in patients with rheumatoid arthritis, multiple sclerosis or SLE, and these cytokines contribute to the pathogenesis of autoimmune diseases[5–8]. The use of blocking antibodies that inhibit the functions of these cytokines is beneficial in several preclinical models and in patients with autoimmune diseases[9–12], suggesting shared signaling pathways and common inflammatory components in the pathogenesis of seemingly disparate autoimmune diseases.

MiRNAs have been shown to have a central role in the development and regulation of the immune system[13–24]. Several miRNAs, including miR-155, miR-146a and miR-326, affect the functions of T and B cells, thereby modulating autoimmune pathogenesis[13–16,23]. However, the function of miRNAs in local resident cells, such as fibroblast-like synoviocytes (FLSs) and kidney cells, during autoimmune pathogenesis is unclear. IL-17 can promote secretion of proinflammatory cytokines by local resident cells in autoimmune diseases, but it remains unclear whether IL-17 regulates miRNAs to contribute to disease pathogenesis. Here, using comparative miRNA profilings of local inflammatory lesions of humans or mice with autoimmune disease, we identified miR-23b as a commonly downregulated miRNA in patients with rheumatoid arthritis or SLE and in the mouse models of CIA, MRL/*lpr* and EAE. IL-17 is responsible for downregulating miR-23b. MiR-23b suppresses the pathogenesis of multiple autoimmune diseases by targeting proinflammatory cytokine-mediated signaling. Thus, miR-23b may be a new common target for inflammatory autoimmune diseases.

RESULTS

MiR-23b is downregulated in autoimmune disease

miRNA screening studies performed to date have focused on individual autoimmune diseases and mainly on peripheral blood cells[25–30]. To determine whether there are commonly regulated miRNAs in the inflammatory lesions of different autoimmune diseases, we performed comparative miRNA screenings of affected tissues of humans with rheumatoid arthritis or SLE, as well as of the relevant mouse models, including CIA for rheumatoid arthritis, MRL/*lpr* for SLE and EAE for multiple sclerosis, using human or mouse miRNA arrays (**Fig. 1a** and

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Received 15 December 2011; accepted 25 April 2012; published online 3 June 2012; doi:10.1038/nm.2815

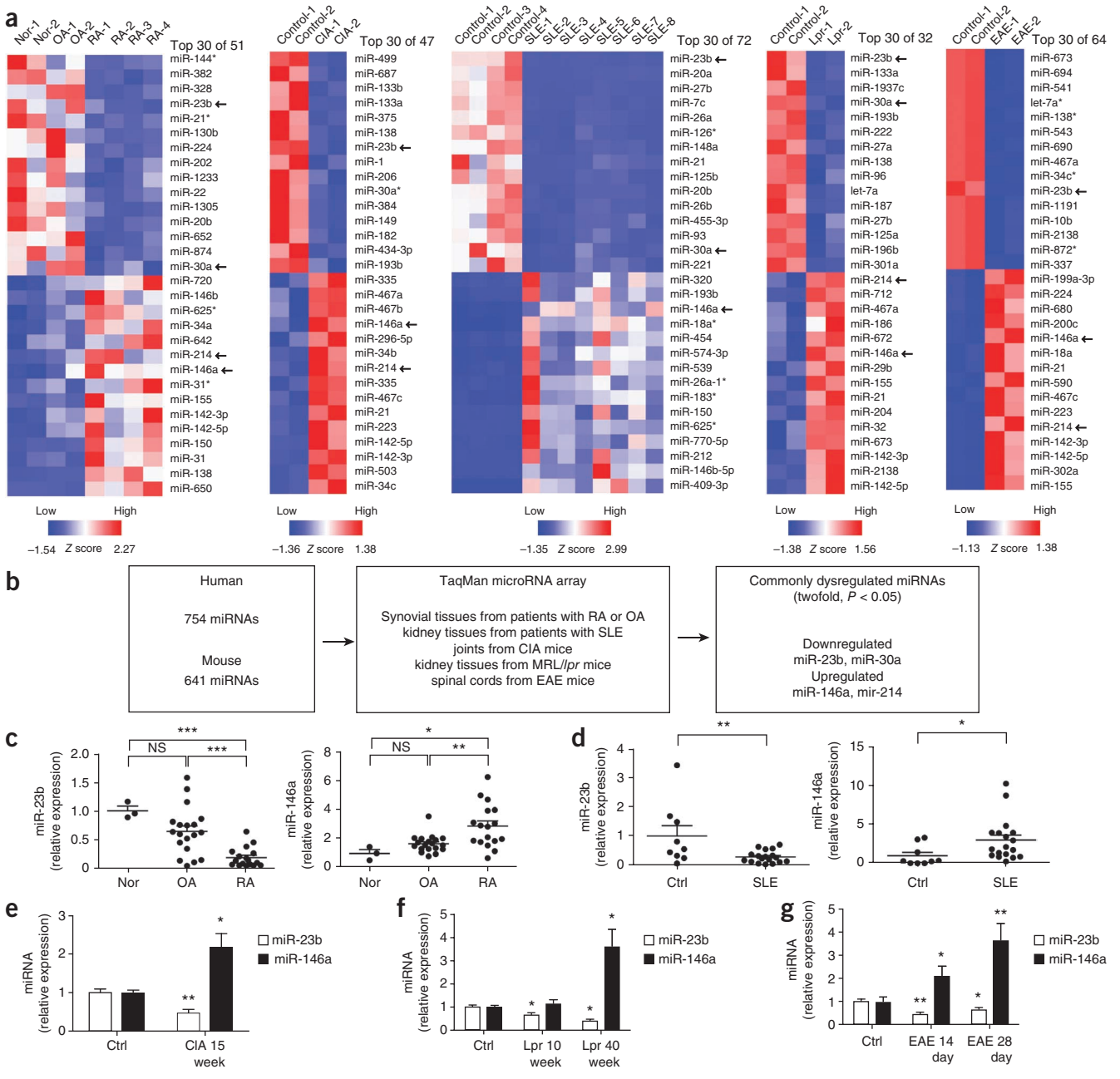

Figure 1 MiR-23b expression is downregulated in inflammatory lesions in autoimmune disease. (**a**) Screening of miRNAs differentially expressed in inflammatory tissue from human autoimmune diseases and the related mouse models. Shown are heatmaps of the 15 most upregulated and 15 most downregulated miRNAs in synovia from four individuals with rheumatoid arthritis (RA) (RA-1–RA-4) compared to two individuals with osteoarthritis (OA) (OA-1 and OA-2) and two healthy controls (Nor-1 and Nor-2); of two samples from CIA mice (CIA-1 and CIA-2) compared to two samples from naive control mice (control-1 and control-2); of renal biopsy samples from eight individuals with SLE (SLE-1–SLE-8) compared to tumor-adjacent tissues from four individuals with kidney cancer (control-1–control-4); of two samples from MRL/*lpr* mice (Lpr-1 and Lpr-2) compared to two samples from control mice (control-1 and control-2); and of two samples from EAE mice (EAE-1 and EAE-2) compared to two samples from naive control mice (control-1 and control-2). The mouse samples represent pooled tissues from 3–6 mice. All miRNAs with a reliable value (Ct < 30) and a significant difference between the tissues of autoimmune diseases and their respective controls (twofold, $P < 0.05$) are listed in **Supplementary Table 3**. (**b**) Scheme showing the screening procedure for the commonly dysregulated miRNAs that are indicated by arrows in **a** or are colored red in **Supplementary Table 3**. (**c**,**d**) qPCR validation of miR-23b expression and miR-146a as a positive control in synovia from 17 individuals with rheumatoid arthritis, 19 individuals with osteoarthritis or 3 healthy controls (nor) (**Supplementary Table 1**) (**c**) or in renal biopsy samples from 18 individuals with SLE or tumor-adjacent kidney tissues of 9 individuals with kidney cancer (ctrl) (**Supplementary Table 2**) (**d**). (**e**–**g**) qPCR validation of miR-23b expression and miR-146a as positive control in joints pooled from 12 CIA mice or 6 naive DBA/1J mice (ctrl) (**e**); in kidney tissues pooled from 6 10-week-old MRL/*lpr* (Lpr), 10 40-week-old MRL/*lpr* or 6 40-week-old MRL control mice (**f**); or in spinal cords pooled from 6 EAE mice 14 d after immunization, 6 EAE mice 28 d after immunization or 6 naive control C57BL/6 mice (**g**). The relative miRNA expressions were normalized to the expression of RNU48 (**c**,**d**) or snoRNA202 (**e**–**g**). All data are means ± s.e.m. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Student's *t* test), NS, no significant difference between the indicated groups or relative to the control group (**c**–**g**) in three independent experiments (**e**–**g**).

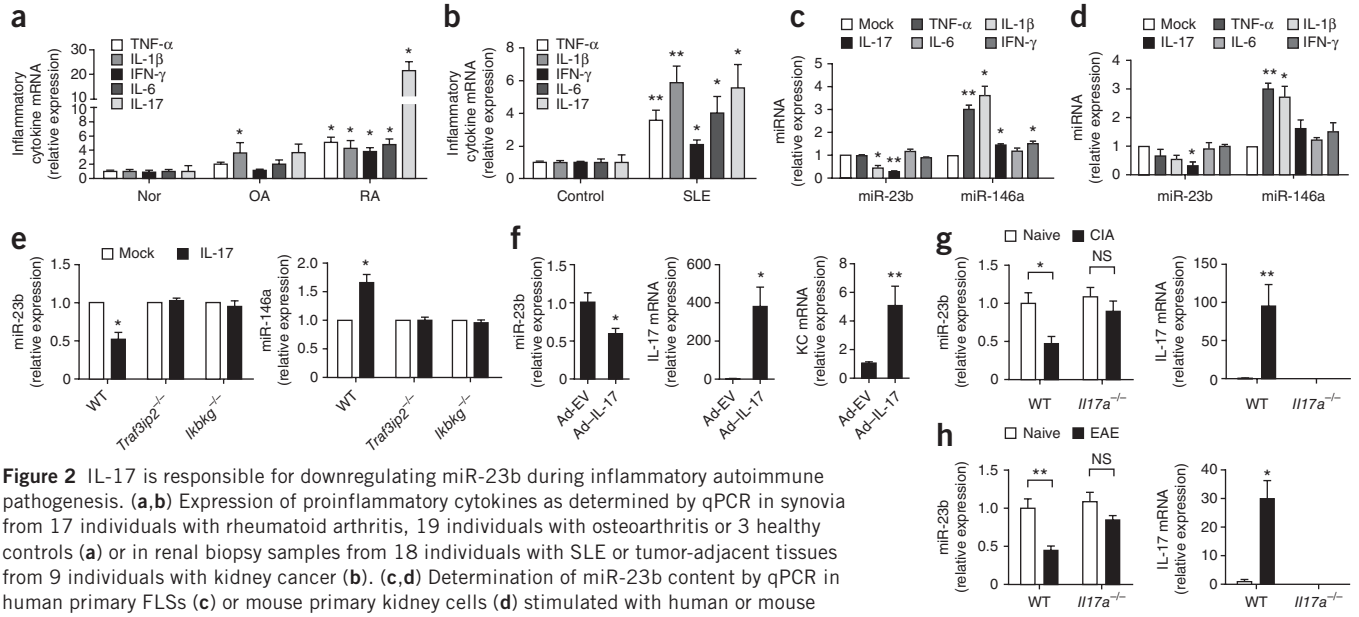

Figure 2 IL-17 is responsible for downregulating miR-23b during inflammatory autoimmune pathogenesis. (**a**,**b**) Expression of proinflammatory cytokines as determined by qPCR in synovia from 17 individuals with rheumatoid arthritis, 19 individuals with osteoarthritis or 3 healthy controls (**a**) or in renal biopsy samples from 18 individuals with SLE or tumor-adjacent tissues from 9 individuals with kidney cancer (**b**). (**c**,**d**) Determination of miR-23b content by qPCR in human primary FLSs (**c**) or mouse primary kidney cells (**d**) stimulated with human or mouse TNF-α, IL-1β, IL-17, IL-6 or IFN-γ. Data are pooled from four (**c**) or three (**d**) independent experiments. (**e**) Expression of miR-23b as determined by qPCR in wild-type, *Traf3ip2*$^{-/-}$ or *Ikbkg*$^{-/-}$ mouse embryonic fibroblasts stimulated with IL-17. Data are representative of three independent experiments. (**f**) Expression of miR-23b, IL-17 or KC mRNA as determined by qPCR in joints treated with adenovirus expressing IL-17 (Ad–IL-17) or empty virus (Ad-EV). $n = 4$ mice per group in three independent experiments. (**g**,**h**) Determination of miR-23b or IL-17 mRNA expression by qPCR in joints of wild-type or *Il17a*$^{-/-}$ mice 2 months after induction of CIA (**g**) or in spinal cord tissue from EAE mice two weeks after immunization (**h**). $N = 4$–5 mice per group in two independent experiments. Cytokine and chemokine expressions were normalized to the expression of the housekeeping gene *Rpl13a* in **a**, **b** and **f**–**h**. The relative miRNA expressions were normalized to the expression of RNU48 in **c** and of snoRNA202 in **d**–**h**. All data represent means ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's *t* test), NS, no significant difference relative to the control group.

Supplementary Tables 1 and **2**). We found that miR-23b and miR-30a were commonly downregulated and that miR-146a and miR-214 were upregulated in all the autoimmune disease samples compared to their representative controls (**Fig. 1a**,**b** and **Supplementary Table 3**). Apart from miR-146a, the other three commonly regulated miRNAs that we found have not previously been reported to participate in the pathogenesis of autoimmune inflammatory diseases. Considering that the downregulation of miR-23b was much stronger than that of miR-30a in the humans and mouse models we studied and there have been few studies on downregulated miRNAs in the pathogenesis of inflammatory autoimmune diseases, we selected miR-23b for further investigation.

We confirmed the downregulation of miR-23b and the upregulation of miR-146a by quantitative real time PCR (qPCR) in a larger number of human samples, including synovial tissues from 17 individuals with rheumatoid arthritis or 22 controls (19 individuals with osteoarthritis and 3 individuals without arthritis) (**Fig. 1c** and **Supplementary Table 1**) and kidney tissues from 18 individuals with SLE or tumor-adjacent kidney tissues of 9 individuals with kidney cancer as relative controls (**Fig. 1d** and **Supplementary Table 2**), as well as mouse samples, including joint tissues of CIA mice compared to naive mice (**Fig. 1e**), kidney tissues of MRL/*lpr* mice compared to MRL control mice (naive mice on an MRL background) (**Fig. 1f**) and spinal cords of EAE mice compared to naive mice (**Fig. 1g**).

IL-17 is responsible for the downregulation of miR-23b

Chronic inflammation and elevated production of TNF-α and IL-1β are common features of several autoimmune diseases, both of which can regulate miRNA expression in cell culture systems[31,32]. Although the expression of miR-23b was markedly lower in the affected tissues from individuals with rheumatoid arthritis or SLE (**Fig. 1c**,**d**), the concentrations of inflammatory cytokines, including TNFα, IL-1β, IL-17, IL-6 and interferon γ (IFNγ), were markedly higher in those tissues compared to the respective control individuals (**Fig. 2a**,**b**). Linear correlation analyses of transcripts of miR-23b and the cytokines showed that miR-23b expression inversely correlated with amount of IL-17 in samples from patients with rheumatoid arthritis and their controls ($R^2 = 0.306$, $P < 0.001$) and in samples from patients with SLE and their controls ($R^2 = 0.211$, $P<0.05$), although the correlations were not strong (**Supplementary Fig. 1**). IL-17 downregulated the expression of miR-23b in human primary FLSs or in mouse primary kidney cells, whereas the other cytokines had a weaker effect or had no effect on miR-23b expression (**Fig. 2c**,**d**). IL-17 promotes the activation of nuclear factor of κ light polypeptide gene enhancer in B cells 1 (NF-κB) through the adaptor TRAF3-interacting protein 2 (Act1) and the IκB kinase (IKK) complex[33–35]. MiRNAs have been shown to be regulated by the cytokine-mediated NF-κB activation pathway[32,36]. We found that deficiency of Act1 (*Traf3ip2*$^{-/-}$) or IKK-γ (*Ikbkg*$^{-/-}$) blunted the IL-17–mediated regulation of miR-23b and miR-146a (**Fig. 2e**), suggesting that IL-17–mediated NF-κB activation is key for its regulation of miRNAs. Indeed, knockdown of p65 blocked the IL-17–mediated regulation of miR-23b and miR-146a (**Supplementary Fig. 2a**). We then performed chromatin immunoprecipitation (ChIP) assays and found that IL-17 stimulation led to the recruitment of NF-κB subunits, p50 and p65, to the miR-23b promoter (**Supplementary Fig. 2b**)[37]. Bioinformatic analyses revealed that there is a consensus NF-κB binding site in the miR-23b promoter (**Supplementary Fig. 2c**). In cells expressing a luciferase construct containing the wild-type miR-23b promoter, IL-17 markedly reduced luciferase activity, whereas mutation of the NF-κB binding sites in the promoter inhibited

IL-17–triggered suppression of reporter activity (**Supplementary Fig. 2d**), indicating that NF-κB binding to the promoter is crucial for the regulation of miR-23b expression by IL-17.

Although both TNF-α and IL-1β promote NF-κB activation and NF-κB is essential for miR-23b regulation, IL-17 more effectively downregulates miR-23b expression, and the degree of NF-κB activation did not correlate well with the amount of miR-23b downregulation in three different primary cell types (**Supplementary Fig. 3**), suggesting that NF-κB is not the only regulator of miR-23b and that other factors must contribute to the regulation of miR-23b. One potential factor might be IκB-ζ, which associates with and modulates NF-κB activity[38] and has been reported to be strongly induced by IL-17 (refs. 39,40). We found that upregulation of IκB-ζ was associated with miR-23b downregulation (**Supplementary Fig. 3**),

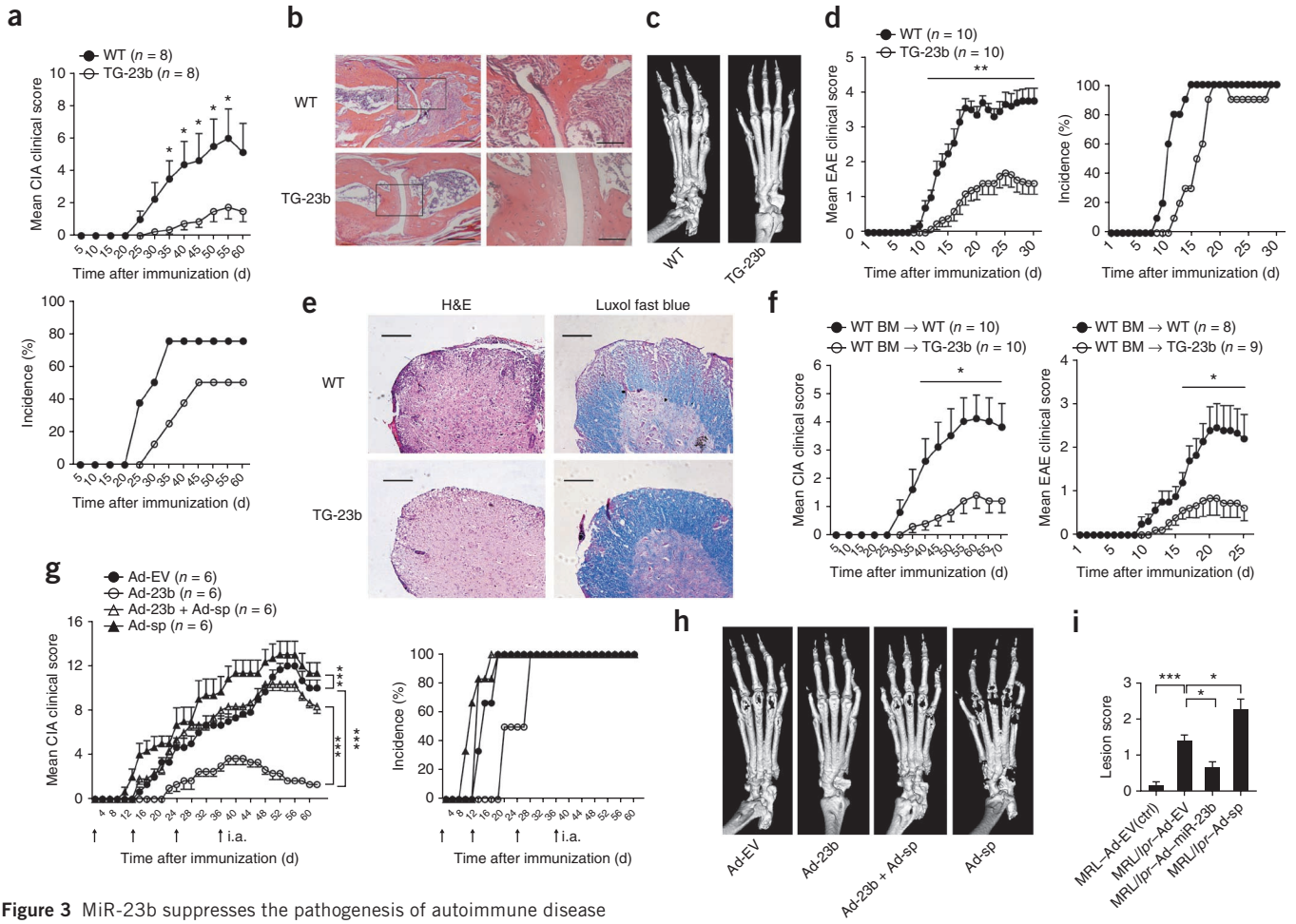

Figure 3 MiR-23b suppresses the pathogenesis of autoimmune disease in mouse models. (**a**) Mean clinical scores (± s.e.m.) and disease incidence (bottom) of CIA induced in wild-type (WT) or miR-23b transgenic (TG-23b) mice. (**b**,**c**) Representative H&E staining of ankle sections (**b**) or representative CT scan photographs (**c**) of the hind paws of the mice from the experiments in **a** at day 50 (**b**) or day 80 (**c**) after the second immunization. In **b**, the images on the right are enlargements of the boxed areas in the images on the left, and scale bars represent 100 μm (left) or 25 μm (right). (**d**) Mean clinical scores (± s.e.m.) and disease incidence of EAE. (**e**) Representative H&E staining or Luxol fast blue staining of spinal cord sections at day 16 after immunization from the mice in the experiments in **d**. Scale bars represent 100 μm. (**f**) Mean clinical scores (± s.e.m.) of CIA (left) or EAE (right) in WT or TG-23b mice reconstituted with WT bone marrow cells (WT BM). (**g**) Mean clinical scores (± s.e.m.) and disease incidence of CIA in DBA/1J mice treated with intra-articular (i.a.) injection of adenovirus control (Ad-EV), adenovirus encoding miR-23b (Ad-23b), adenovirus encoding miR-23b sponge (Ad-sp) or adenovirus encoding miR-23b and miR-23 sponge together (Ad-23b + Ad-sp) at the times indicated by the arrows. (**h**) Representative CT scan photographs of the hind paws at day 80 after the second immunization from the mice in the experiments in **g**. (**i**) The renal histological lesion scores of MRL/*lpr* mice intravenously injected with Ad-EV (MRL/*lpr*–Ad-EV), Ad-23b (MRL/*lpr*–Ad-23b) or Ad-sp (MRL/*lpr*–Ad-sp) every 3 weeks from 6–24 weeks of age. Control 24-week-old MRL mice injected with Ad-EV (MRL–Ad-EV) were used as controls. $n = 5$–6 mice per group. (**j**) Representative H&E staining of kidney sections of the treated mice in **i** at 24 weeks of age showing glomerular cell proliferation (top and middle) and perivascular mononuclear cell infiltration in the tubulointerstitium (bottom). All data represent means ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t* test in **a**,**d**,**f** and **i** or two-way analysis of variance in **g**) in two (**f**) or three (**a**,**d**,**g**,**i**) independent experiments.

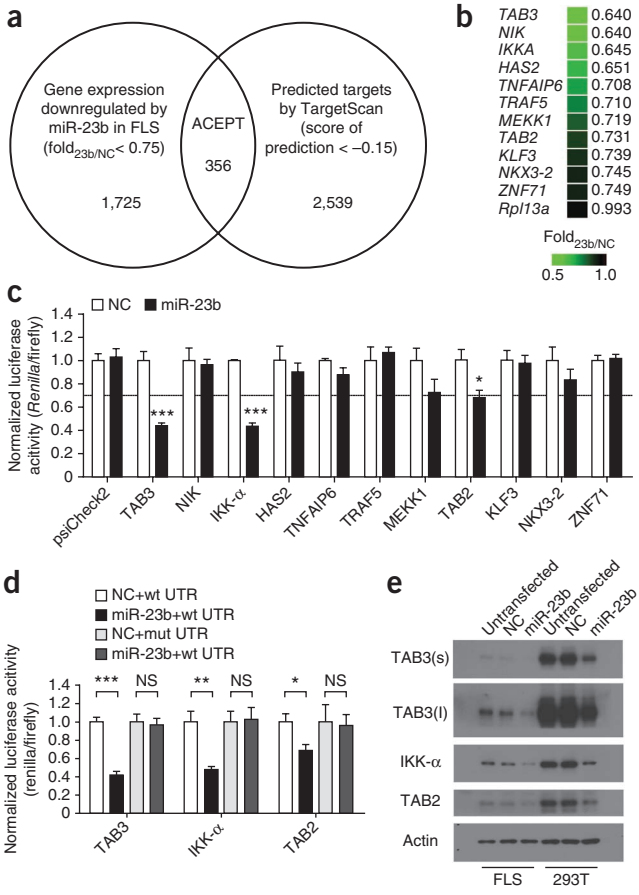

Figure 4 MiR-23b targets TAB2, TAB3 and IKK-α. (**a**) Total RNAs of FLSs transduced with the miR-23b mimic or control mimic (NC) were used for a microarray analysis (38,500 genes total). Transcripts of 1,725 genes were found to be downregulated by 25% by the miR-23b mimic. TargetScan predicted 2,539 potential targets of miR-23b. The overlapping 356 genes are defined as ACEPT genes. The ACEPT list is shown in **Supplementary Table 4**. $Fold_{23b/NC}$, Fold change of gene expression in FLSs transfected with miR-23b mimic compared to control mimic. (**b**) Eleven selected ACEPT genes that were related to the inflammatory response according to ingenuity pathway analysis (**Supplementary Fig. 5**). *Rpl13a* is shown as negative control. The gradually changing colors (green indicates downregulated, and black indicates no change) and the numbers indicate the gene expression in FLSs transduced with the miR-23b mimic compared to those transduced with the control mimic, as determined by microarray. (**c**,**d**) Luciferase activity determined in HEK293T cells that were first transfected with the indicated 3′ UTR reporter constructs (**c**) or with the indicated wild-type or point-mutated 3′ UTR reporter constructs (wt UTR or mut UTR) (**d**) and then transduced with miR-23b or control mimic. Data are representative of four independent experiments (means ± s.e.m.). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t* test), NS, no significant difference relative to the control group or between the indicated groups. (**e**) Immunoblotting of endogenous TAB2, TAB3 and IKK-α expression in FLSs or HEK293T cells untransfected, transfected with the control mimic or with the miR-23b mimic. (s), short exposure; (l), long exposure. Data represent three independent experiments.

suggesting that IκB-ζ may be contributing to the regulation of miR-23b. Indeed, IL-17 stimulation led to the recruitment of IκB-ζ to the miR-23b promoter (**Supplementary Fig. 4a**), and siRNA-mediated gene knockdown of IκB-ζ blocked IL-17–mediated regulation of miR-23b (**Supplementary Fig. 4b**), confirming that IκB-ζ contributes to IL-17–mediated miR-23b regulation. Although other uncharacterized factors may also be involved in miR-23b regulation, IκB-ζ seemed to be specific for miR-23b regulation, as IκB-ζ did not affect IL-17–mediated regulation of miR-146a (**Supplementary Fig. 4b**,**c**). Consistent with the NF-κB–mediated suppression of the miR-23b promoter, IL-17 also inhibited the expression of primary miR-23b (pri-miR-23b) (**Supplementary Fig. 5**), suggesting that IL-17 mediates miR-23b regulation at the transcriptional level.

To determine the *in vivo* effect of IL-17 on miR-23b regulation, we injected adenovirus encoding mouse IL-17 into mouse joints. Consistent with the results in the primary cells, IL-17 repressed miR-23b expression in the mouse joints *in vivo* (**Fig. 2f**). Although miR-23b was repressed after the induction of CIA and EAE in wild-type mice that express IL-17, downregulation of miR-23b was considerably blocked in *Il17*-deficient mice (**Fig. 2g**,**h**), suggesting that IL-17 was responsible for miR-23b regulation during autoimmune pathogenesis.

MiR-23b suppresses autoimmune pathogenesis

To investigate its potential function during autoimmune pathogenesis, we generated miR-23b transgenic (TG-23b) mice (**Supplementary Fig. 6**). The transgenic mice were born normally with the expected Mendelian frequency and did not show any abnormality in body size and weight and in lymphocyte populations in age-matched mice (data not shown). Notably, the onset of CIA was delayed and the clinical score and incidence of CIA were lower in the transgenic mice compared to wild-type mice (**Fig. 3a**). H&E staining and micro computed tomography (micro-CT) confirmed reduced inflammatory infiltration and bone erosion in the transgenic mice compared to the wild-type mice (**Fig. 3b**,**c**). Similarly, the onset of EAE was delayed and the EAE clinical score and amounts of inflammatory infiltration and demyelination were substantially lower in the transgenic mice compared to the wild-type mice (**Fig. 3d**,**e**). Furthermore, lethally irradiated miR-23b transgenic mice reconstituted with wild-type bone marrow had delayed onset and much lower clinical scores of EAE and CIA as compared to irradiated wild-type recipient mice reconstituted with wild-type bone marrow (**Fig. 3f**), similar to those seen in miR-23b transgenic mice compared to WT mice (**Fig. 3a**,**d**). We also found that there was no significant difference in clinical scores of EAE or CIA between the *Rag1*-deficient mice transferred with splenocytes from miR-23b transgenic mice or wild-type control mice (data not shown). These data suggest that miR-23b expression in radio-resistant resident cells is crucial for its suppression of autoimmune pathogenesis.

Adenoviral-mediated, ectopic expression of miR-23b in mouse ankles markedly reduced CIA disease severity, incidence and bone erosion (**Fig. 3g**,**h** and **Supplementary Fig. 7**), similar to the results obtained from miR-23b transgenic mice (**Fig. 3a**). In contrast, administration of an adenovirus expressing a sponge of miR-23b that antagonizes miR-23b substantially enhanced disease severity and bone erosion during CIA (**Fig. 3g**,**h**), confirming the suppressive effect of miR-23b on CIA. In addition, in a spontaneous lupus nephritis model, adenoviral-mediated overexpression of miR-23b reduced and overexpression of the miR-23b sponge increased the severity of renal lesions, including the proliferation of glomerular cells and the infiltration of periglomerular, perivascular and interstitial mononuclear cells in MRL/*lpr* mice (**Fig. 3i**,**j**), suggesting that miR-23b also has a suppressive role in lupus nephritis.

We next sought to determine whether miR-23b inhibits inflammatory cytokine expression during autoimmune pathogenesis. The expression of several inflammatory genes was markedly suppressed in the joints or spinal cords of miR-23b transgenic mice

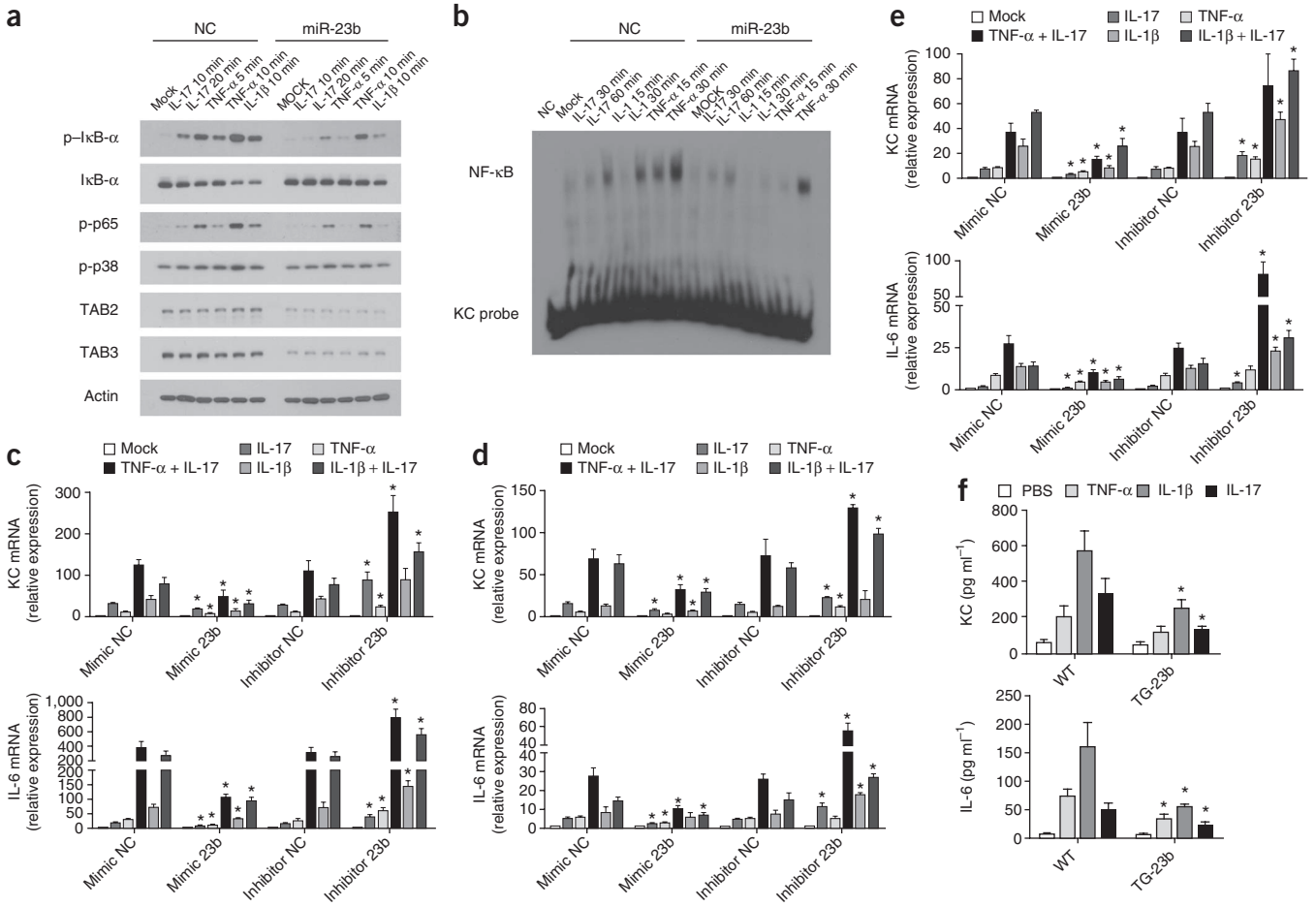

Figure 5 MiR-23b inhibits inflammatory cytokine-mediated signaling and gene expression. (**a**,**b**) Immunoblotting of whole-cell lysates (**a**) or electrophoretic mobility shift assay of nuclear extracts (**b**) from HeLa cells transfected with the miR-23b mimic or control mimic (NC) and then left untreated (Mock) or incubated with IL-17, TNF-α or IL-1β for the indicated times. The NF-κB binding site from the KC gene was used as a probe. Data represent four (**a**) or two (**b**) independent experiments. p- indicates the phosphorylated form of the protein. (**c**–**e**) The expression of KC or IL-6 mRNA determined by qPCR in primary FLSs (**c**), mouse primary kidney cells (**d**) or mouse primary astrocytes (**e**), which were transfected with the miR-23b mimic, miR-23b inhibitor or the relative controls (mimic NC and inhibitor NC) and then incubated with IL-17, TNF-α, IL-1β or a combination thereof. (**f**) The concentrations of KC or IL-6 determined by ELISA in serum from 8-week-old WT and TG-23b mice that were injected intravenously with the indicated cytokines or PBS. Data are representative of four (**c**) or three (**d**–**f**) independent experiments (means ± s.e.m.). $*P < 0.05$ (Student's *t* test) relative to the control group.

compared to those of wild-type mice during CIA or EAE, respectively (**Supplementary Fig. 8a**,**b**). Similarly, adenoviral-mediated overexpression of miR-23b inhibited the expression of proinflammatory genes in MRL/*lpr* mice, whereas overexpression of the miR-23b sponge increased their expression (**Supplementary Fig. 8c**). These results suggest that miR-23b suppresses autoimmune pathogenesis, probably through the suppression of inflammatory cytokine expression.

MiR-23b targets multiple genes in proinflammatory signaling

To identify potential targets of miR-23b, we combined target prediction through TargetScan and microarray gene-expression analyses to look for gene transcripts downregulated by miR-23b overexpression. Using a combination of the two approaches, we identified 356 genes (**Fig. 4a** and **Supplementary Table 4**), which we defined as anti-correlated expressed predicted target (ACEPT) genes[41]. To assess the potential roles of the proteins encoded by these 356 genes in inflammatory signaling pathways, we predicted the biological functions and mapped biomolecular networks based on known pathways, gene ontology and interactions using ingenuity pathway analysis. Predicted genes targeted by miR-23b were associated with biological networks related to the inflammatory response and cell death or canonical pathways containing tumor necrosis factor receptor superfamily, member 1 (TNFR1) signaling and NF-κB signaling (**Supplementary Table 5**). As we found that miR-23b suppressed proinflammatory gene expression (**Supplementary Fig. 8**), we chose 11 inflammation-related genes that belong to the biological function network of inflammatory response and cell death to validate because they were ranked first and are functionally consistent with the suppressive function of miR-23b in autoimmune disease models (**Fig. 4b** and **Supplementary Fig. 9**). MiR-23b markedly repressed the reporter activity of the 3′ untranslated regions (UTRs) of three genes, encoding TAB3, IKK-α and TAB2, whereas reporter activity of constructs containing the 3′ UTRs of the other eight genes were either only slightly affected or were not affected by miR-23b (**Fig. 4c**). Mutation of the 3′ UTRs of the three repressed genes confirmed they are targets of miR-23b (**Fig. 4d** and **Supplementary Fig. 10**). Consistent with the reporter assays, the expressions of endogenous TAB3, IKK-α and

TAB2 protein were decreased in primary FLSs or HEK 293T cells by overexpression of the miR-23b mimic (**Fig. 4e**).

To avoid potentially significant genes from being excluded as a result of the 3′ UTR consideration only, we also used RNA22 to predict potential target sites of miR-23b in the 11 inflammation-related genes and found additional sites in the coding sequence (CDS) regions of mitogen-activated protein kinase kinase kinase 1 (MEKK1), mitogen-activated protein kinase kinase kinase 14 (NIK), hyaluronan synthase 2 (HAS2), Kruppel-like factor 3 (Klf3) and zinc finger protein 71 (ZNF71) (**Supplementary Fig. 11a**). However, a miR-23b mimic did not reduce the amounts of the five proteins encoded by their cloned CDS regions (**Supplementary Fig. 11b**), indicating that none of the five CDS regions was targeted by miR-23b.

MiR-23b suppresses inflammatory cytokine-mediated signaling

TAB2 and TAB3, two targets of miR-23b, are essential for signaling mediated by TNF-α or IL-1β[42]. We therefore determined whether miR-23b affects TNF-α– or IL-1β–mediated NF-κB activation.

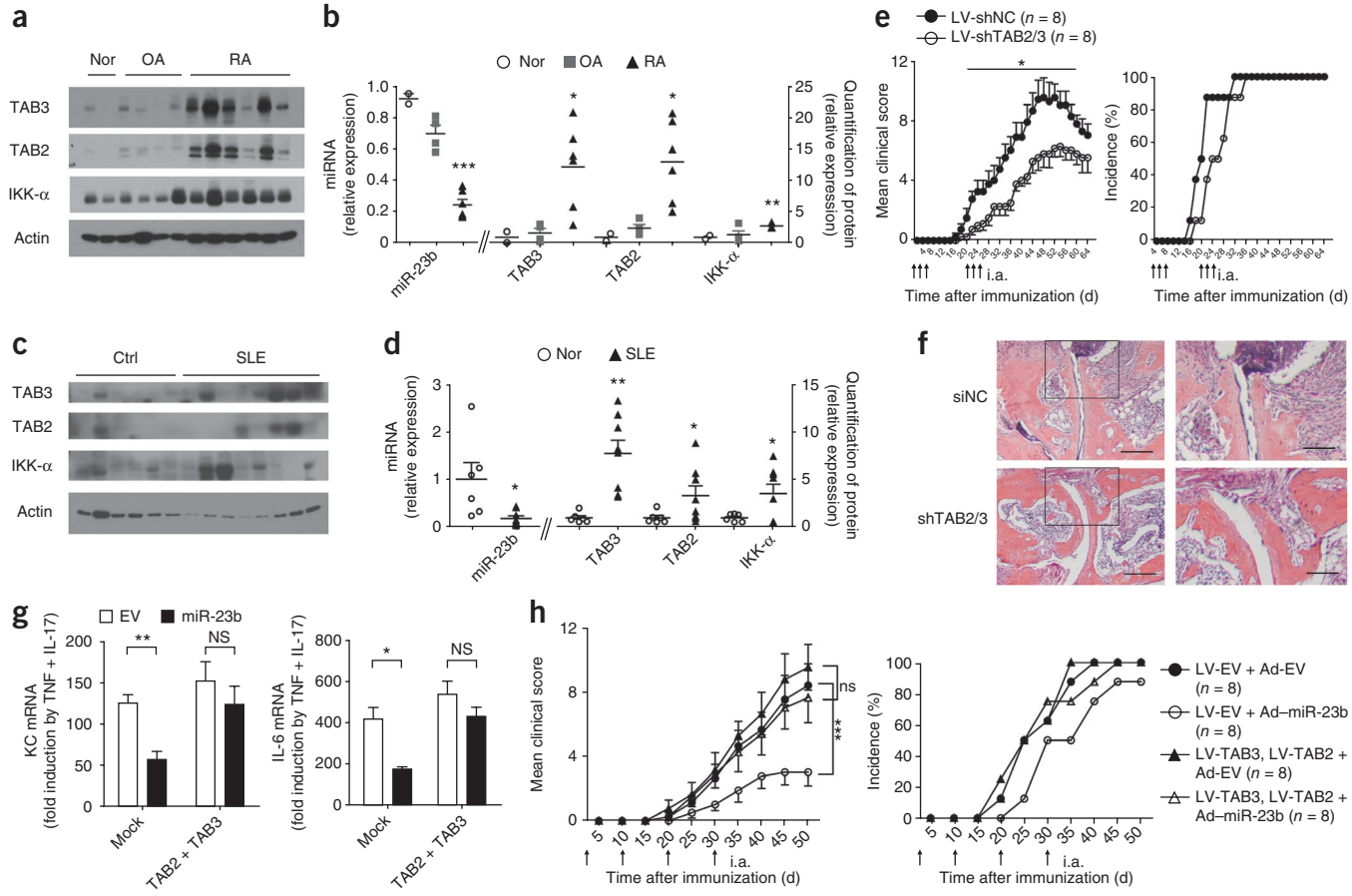

Figure 6 TAB2 and TAB3 are functional targets of miR-23b. (**a**–**d**) Immunoblotting (**a**,**c**) and quantification of the protein expression (**b**,**d**) of endogenous TAB3, TAB2 and IKK-α or expression of miR-23b in the same set of synovial samples from individuals with osteoarthritis or rheumatoid arthritis and healthy controls (**a**,**b**) or in kidney samples from individuals with SLE and controls (**c**,**d**). Protein expression was normalized to the expression of actin. (**e**) Mean clinical scores and disease incidence of CIA in DBA/1J mice injected intra-articularly with lentiviral vectors encoding shRNA directed against both TAB2 and TAB3 (LV-shTAB2/3) or nontargeting shRNA (LV-shNC) at the times indicated by the arrows. (**f**) Representative H&E staining of ankle sections at day 50 after the second immunization from the mice in the experiments in **e**. In **f**, the images on the right are enlargements of the boxed areas in the images on the left., and **s**cale bars represent 100 μm (left) or 50 μm (right). (**g**) Expression of KC or IL-6 mRNA determined by qPCR in FLSs transfected with constructs encoding TAB2 and TAB3 containing mutated miR-23b binding sites or empty construct and then infected with empty virus or adenovirus encoding miR-23b and stimulated with TNF-α plus IL-17. Data are representative of four independent experiments. (**h**) Mean clinical scores and disease incidence of CIA in DBA/1J mice after intra-articular injection of the indicated combination of adenoviruses encoding miR-23b (Ad–miR-23b) or control (Ad-EV) and lentiviruses encoding TAB2 (LV-TAB2) plus TAB3 (LV-TAB3) with mutated miR-23b binding sites or control lentivirus (LV-EV). The injection was done several times, as indicated by the arrows. Data represent three independent experiments (**a**,**c**,**e**,**h**) and are means ± s.e.m. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Student's *t* test in **b**,**d**,**e** and **g** or two-way analysis of variance in **h**), NS, no significant difference relative to the control group or between the indicated groups (**b**,**d**,**e**,**g**,**h**). (**i**) Schematic model of the regulation and function of miR-23b in inflammatory autoimmune pathogenesis. NEMO, inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ; TAB2/3, TAB2 or TAB3.

We confirmed that a synthetic miR-23b mimic or its inhibitor was active using a luciferase reporter assay, which we then used for further experiments (**Supplementary Fig. 12**). Overexpression of miR-23b suppressed both TNF-α– and IL-1β–induced NF-κB activation in HeLa cells (**Fig. 5a**,**b** and **Supplementary Fig. 13**). Because IL-17 is crucial for the pathogenesis of autoimmune diseases[43–45] and regulates miR-23b expression (**Fig. 2c**–**h**), we tested whether miR-23b affects IL-17–mediated signaling, and whether TAB2 and TAB3 are involved in IL-17–mediated signaling. MiR-23b suppressed TAB2 and TAB3 expression and IL-17–induced NF-κB activation (**Fig. 5a**,**b** and **Supplementary Fig. 13**). We then performed coimmunoprecipitation and found that TNF receptor-associated factor 6 (TRAF6) was associated with TAB2 and TAB3 in a manner that was dependent on IL-17 (**Supplementary Fig. 14a**). Given the redundant roles of TAB2 and TAB3 in TNF-α– and IL-1β–mediated signaling, we then knocked down both TAB2 and TAB3 and found that they were required for IL-17–induced NF-κB activation but not MAPK activation (**Supplementary Fig. 14b**). Knockdown of TGF-β activated kinase 1 (TAK1) also suppressed IL-17–mediated NF-κB activation but not MAPK activation (**Supplementary Fig. 14c**). Consistent with the function of the TAK1 complex (TAK1-TAB2-TAB3) in IL-17–mediated signaling, knockdown of TAB2 and TAB3 together but not individually markedly reduced IL-17–mediated induction of IL-6 and chemokine (C-X-C motif) ligand 1 (KC, also known as CXCL1), similar to the roles of TAB2 and TAB3 in TNF-α– or IL-1β–mediated gene induction (**Supplementary Fig. 14d**). In addition to TRAF6-dependent NF-κB activation, IL-17 also induces mRNA stability in inflammatory genes[46–48]. We found that miR-23b did not affect the IL-17–mediated mRNA stabilization of KC (**Supplementary Fig. 15**), suggesting that miR-23b specifically targets IL-17–induced NF-κB signaling. Our data show that NF-κB suppresses miR-23b expression and that miR-23b also inhibits NF-κB activation, suggesting there may be a feedback loop. Indeed, we found that the miR-23b mimic upregulated and the miR-23b inhibitor downregulated primary miR-23b expression both in the basal state and in an IL-17–dependent manner (**Supplementary Fig. 16**).

Next, we sought to determine whether miR-23b suppresses the induction of KC and IL-6 expression by TNF-α, IL-1β or IL-17 in physiologically relevant cell types. The miR-23b mimic inhibited and the miR-23b inhibitor enhanced the induction of KC and IL-6 in primary FLSs (**Fig. 5c**), mouse kidney cells (**Fig. 5d**) and mouse astrocytes (**Fig. 5e**). Consistent with the findings *in vitro*, TNF-α–, IL-1β– or IL-17–mediated induction of KC and IL-6 was lower *in vivo* in miR-23b transgenic mice as compared to wild-type mice (**Fig. 5f**).

TAB2 and TAB3 are functional targets of miR-23b *in vivo*

To determine whether TAB2 and TAB3 are functional targets of miR-23b *in vivo* during autoimmune pathogenesis, we investigated their expression in humans with rheumatoid arthritis or SLE. The expression of TAB2 and TAB3 were indeed markedly higher in synovial tissue from individuals with rheumatoid arthritis as compared to normal or osteoarthritis samples (**Fig. 6a**,**b**). The expression of IKK-α, another miR-23b target, was also higher in tissue from individuals with rheumatoid arthritis compared to the normal and osteoarthritis tissues (**Fig. 6a**,**b**). Expression of TAB2, TAB2 and IKK-α protein was also higher in kidney tissue from individuals with SLE compared to normal controls (**Fig. 6c**,**d**). Elevated expression of these proteins was associated with lower expression of miR-23b in both rheumatoid arthritis and SLE tissue (**Fig. 6b**,**d**). In mouse models of autoimmune disease, the amounts of TAB2, TAB3 and IKK-α were also higher in the joints of CIA mice, the kidneys of MRL/*lpr* mice and the spinal cords of EAE mice compared to the samples from the representative control mice (**Supplementary Fig. 17a**–**c**). Further, we found that adenoviral-mediated overexpression of IL-17 *in vivo* in mouse joints increased the expression of TAB2, TAB3 and IKK-α (**Supplementary Fig. 17d**). These results indicate TAB2, TAB3 and IKK-α may be targeted by miR-23b.

To investigate the potential pathological functions of TAB2 and TAB3, we used lentiviral vectors expressing shRNAs to silence the *Tab2* and *Tab3* genes. A panel of siRNAs targeting *Tab2* or *Tab3* (siTAB2 or siTAB3, respectively) was screened for effective gene silencing in mouse melanoma B16 cells (**Supplementary Fig. 18a**). We then chose sequences of siTAB2-4, siTAB2-5, siTAB3-1 and siTAB3-3 for further *in vivo* experiments. Administration of the combined lentiviruses reduced TAB2 and TAB3 protein expression in the joint tissues of mice (**Supplementary Fig. 18b**). Knockdown of TAB2 and TAB3 *in vivo* markedly reduced the severity of CIA (**Fig. 6e**), although the effect of TAB2 and TAB3 knockdown on CIA pathogenesis was weaker than that of miR-23b overexpression (**Fig. 3a**). This may be the result of residual expression of TAB2 and TAB3 or other potential targets of miR-23b. H&E staining also showed reduced lymphocyte infiltration and bone erosion (**Fig. 6f**).

To further show that TAB2 and TAB3 are functional targets of miR-23b in autoimmune pathogenesis, we transfected FLSs with constructs encoding TAB2 and TAB3 containing mutated miR-23b target sites. These vectors reversed the miR-23b–imposed inhibitory effect on IL-17– and TNF-α–mediated induction of KC and IL-6. (**Fig. 6g**). Subsequent injection of mice with lentiviruses encoding TAB2 or TAB3 containing mutated miR-23b target sites markedly reduced the miR-23b–mediated suppression of CIA (**Fig. 6h** and **Supplementary Fig. 19**), suggesting that TAB2 and TAB3 are key targets of miR-23b *in vivo* and contribute to the beneficial effects of miR-23b in mouse models of autoimmune disease.

DISCUSSION

MiRNA profilings performed to date have been on individual autoimmune diseases and focused mainly on peripheral blood cells[25–30]. Here we determined whether miRNAs are commonly regulated in local inflammatory lesions of multiple human autoimmune diseases, as well as their relevant mouse models, using miRNA microarrays. Apart from miR-146a, which was commonly elevated and has been well characterized previously[13,32,49], our study indentified three previously uncharacterized miRNAs (miR-23b, miR-30a and miR-214) that are commonly altered in the three autoimmune diseases studied. We further characterized miR-23b because it was markedly downregulated and there was a lack of information regarding downregulated miRNAs during autoimmune pathogenesis. Although both TNF-α and IL-1β were more potent than IL-17 at inducing miR-146a expression, IL-17 more potently suppressed miR-23b expression, suggesting that miRNAs are differentially regulated by different cytokines. The induction of IκB-ζ by IL-17 and its recruitment to the miR-23b promoter may account for the specific effects of IL-17 on miR-23b. Although we showed that IL-17 regulated miR-23b expression at the transcriptional level, we cannot exclude the possibility that miR-23b may also be regulated post-transcriptionally.

Although miR-23b regulates cell metabolism and cancer development[50,51], a role for miR-23b in autoimmune pathogenesis was previously unknown. Here we show that miR-23b suppresses the development and pathogenesis of multiple autoimmune diseases, consistent with its common downregulation. Although miR-23b has

been found to target glutaminase and regulate glutamine metabolism[51], we identified additional targets of miR-23b, including TAB2, TAB3 and IKK-α, that act in signaling cascades downstream of inflammatory cytokines. Whereas miR-23b overexpression downregulated these targets, TAB2, TAB3 and IKK-α were upregulated in human autoimmune diseases, as well as in the relevant mouse models, in which miR-23b was downregulated. Although TAB2 and TAB3 have been shown to be crucial for TNF-α– and IL-1β–mediated NF-κB activation, as well as MAPK activation, their involvement in IL-17–mediated signaling was previously unknown[35,42,52–56]. We found that TAB2 and TAB3 were specifically required for IL-17–mediated NF-κB activation but not MAPK activation. Our finding that miR-23b regulates inflammatory cytokine-mediated (TNF-α, IL-1β and IL-17) signaling suggests that miR-23b may have regulatory roles in other inflammatory diseases that involve these inflammatory cytokines, such as infectious diseases, septic shock and type 2 diabetes. We also found that miR-23b regulated lipopolysaccharide-mediated inflammatory responses and septic shock (S.Z. and Y.Q., unpublished data), suggesting that miR-23b inhibits inflammatory responses in multiple disease models.

Although previous studies have shown that several miRNAs, including miR-146a and miR-155, regulate autoimmune pathogenesis through their effects on T and B cells[13,14], the role of miRNAs in radio-resident cells during autoimmune pathogenesis is unclear. Our study found that miR-23b regulates autoimmunity through its effect on local resident cells by targeting proinflammatory cytokine-mediated signaling (**Fig. 6i**). Although miR-23b overexpression can inhibit NF-κB activation in immune cells (**Supplementary Fig. 20**), miR-23b expression in these cells may not be crucial for autoimmune pathogenesis because miR-23b has lower expression in lymphoid tissues than in stromal cells, and transgenic overexpression of miR-23b in radio-resistant cells was crucial for its suppression of autoimmune pathogenesis. As there has been no previous *in vivo* study of downregulated miRNAs during autoimmune pathogenesis, to the best of our knowledge, miR-23b is the first downregulated miRNA to be functionally characterized during the pathogenesis of autoimmune disease. Thus, miR-23b may be a common therapeutic target for inflammatory disease.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Gene Expression Omnibus: GSE37276.

Note: Supplementary information is available in the online version of the paper.

ACKNOWLEDGMENTS

We thank R. Flavell (Yale University) for providing *Il17a*-deficient mice. This work is supported by grants from National Natural Science Foundation of China and 973 program (30930084, 2010CB529705, 91029708 and 30871298), Chinese Academy of Sciences (KSCX2-YW-R-146) and the Science and Technology Commission of Shanghai Municipality (10JC1416600) to Y.Q.; as well as National Natural Science Foundation of China (30971632 and 81025016), Chinese Ministry of Health (201202008) and the Program of the Shanghai Commission of Science and Technology (10JC1409300) to N.S.

AUTHOR CONTRIBUTIONS

S.Z. and Y.Q. designed the experiments and wrote the manuscript. S.Z. and W.P. conducted most of the experiments and analyzed the data. W.P., N.S. and J.B.H. edited the manuscript. X. Song and Y.L. helped with mouse experiments. X. Shao helped with molecular cloning. Y.S., Y.T. and D.L. provided technical support. H.W. provided *Il17a*$^{-/-}$ mice. W.L. performed Ingenuity Pathway Analyses. N.S. and D.H. provided clinical samples. Y.Q. and N.S. supervised the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/doifinder/10.1038/nm.2815.
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1. Firestein, G.S. Evolving concepts of rheumatoid arthritis. *Nature* **423**, 356–361 (2003).
2. Compston, A. & Coles, A. Multiple sclerosis. *Lancet* **372**, 1502–1517 (2008).
3. Rahman, A. & Isenberg, D.A. Systemic lupus erythematosus. *N. Engl. J. Med.* **358**, 929–939 (2008).
4. Zhu, S. & Qian, Y. IL-17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential. *Clin. Sci. (Lond.)* **122**, 487–511 (2012).
5. Feldmann, M., Brennan, F.M. & Maini, R.N. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* **14**, 397–440 (1996).
6. Smolen, J.S., Steiner, G. & Aringer, M. Anti-cytokine therapy in systemic lupus erythematosus. *Lupus* **14**, 189–191 (2005).
7. Sospedra, M. & Martin, R. Immunology of multiple sclerosis. *Annu. Rev. Immunol.* **23**, 683–747 (2005).
8. Williams, R.O., Paleolog, E. & Feldmann, M. Cytokine inhibitors in rheumatoid arthritis and other autoimmune diseases. *Curr. Opin. Pharmacol.* **7**, 412–417 (2007).
9. Hueber, W. *et al.* Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci. Transl. Med.* **2**, 52ra72 (2010).
10. Lipsky, P.E. *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med.* **343**, 1594–1602 (2000).
11. Geyer, M. & Muller-Ladner, U. Actual status of antiinterleukin-1 therapies in rheumatic diseases. *Curr. Opin. Rheumatol.* **22**, 246–251 (2010).
12. Genovese, M.C. *et al.* LY2439821, a humanized anti–interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled, proof-of-concept study. *Arthritis Rheum.* **62**, 929–939 (2010).
13. Lu, L.F. *et al.* Function of miR-146a in controlling T_{reg} cell–mediated regulation of Th1 responses. *Cell* **142**, 914–929 (2010).
14. O'Connell, R.M. *et al.* MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. *Immunity* **33**, 607–619 (2010).
15. Du, C. *et al.* MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. *Nat. Immunol.* **10**, 1252–1259 (2009).
16. Stittrich, A.B. *et al.* The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. *Nat. Immunol.* **11**, 1057–1062 (2010).
17. Xiao, C. *et al.* MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell* **131**, 146–159 (2007).
18. Rodriguez, A. *et al.* Requirement of bic/microRNA-155 for normal immune function. *Science* **316**, 608–611 (2007).
19. Thai, T.H. *et al.* Regulation of the germinal center response by microRNA-155. *Science* **316**, 604–608 (2007).
20. Johnnidis, J.B. *et al.* Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* **451**, 1125–1129 (2008).
21. Lu, L.F. *et al.* Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity* **30**, 80–91 (2009).
22. Xiao, C. *et al.* Lymphoproliferative disease and autoimmunity in mice with increased miR-17–92 expression in lymphocytes. *Nat. Immunol.* **9**, 405–414 (2008).
23. Ponomarev, E.D., Veremeyko, T., Barteneva, N., Krichevsky, A.M. & Weiner, H.L. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. *Nat. Med.* **17**, 64–70 (2011).
24. O'Connell, R.M., Rao, D.S., Chaudhuri, A.A. & Baltimore, D. Physiological and pathological roles for microRNAs in the immune system. *Nat. Rev. Immunol.* **10**, 111–122 (2010).
25. Nakamachi, Y. *et al.* MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum.* **60**, 1294–1304 (2009).
26. Junker, A. *et al.* MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. *Brain* **132**, 3342–3352 (2009).
27. Dai, Y. *et al.* Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. *Rheumatol. Int.* **29**, 749–754 (2009).
28. Pan, W. *et al.* MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. *J. Immunol.* **184**, 6773–6781 (2010).
29. Li, J. *et al.* Altered microRNA expression profile with miR-146a upregulation in CD4+ T cells from patients with rheumatoid arthritis. *Arthritis Res. Ther.* **12**, R81 (2010).
30. De Santis, G. *et al.* Altered miRNA expression in T regulatory cells in course of multiple sclerosis. *J. Neuroimmunol.* **226**, 165–171 (2010).

31. Tili, E. *et al.* Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-α stimulation and their possible roles in regulating the response to endotoxin shock. *J. Immunol.* **179**, 5082–5089 (2007).
32. Taganov, K.D., Boldin, M.P., Chang, K.J. & Baltimore, D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc. Natl. Acad. Sci. USA* **103**, 12481–12486 (2006).
33. Chang, S.H., Park, H. & Dong, C. Act1 adaptor protein is an immediate and essential signaling component of interleukin-17 receptor. *J. Biol. Chem.* **281**, 35603–35607 (2006).
34. Sønder, S.U. *et al.* IL-17-induced NF-κB activation via CIKS/Act1: physiologic significance and signaling mechanisms. *J. Biol. Chem.* **286**, 12881–12890 (2011).
35. Qian, Y. *et al.* The adaptor Act1 is required for interleukin 17-dependent signaling associated with autoimmune and inflammatory disease. *Nat. Immunol.* **8**, 247–256 (2007).
36. Ma, F. *et al.* The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. *Nat. Immunol.* **12**, 861–869 (2011).
37. Rodriguez, A., Griffiths-Jones, S., Ashurst, J.L. & Bradley, A. Identification of mammalian microRNA host genes and transcription units. *Genome Res.* **14**, 1902–1910 (2004).
38. Yamamoto, M. *et al.* Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IκBζ. *Nature* **430**, 218–222 (2004).
39. Shen, F., Ruddy, M.J., Plamondon, P. & Gaffen, S.L. Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17– and TNF-α–induced genes in bone cells. *J. Leukoc. Biol.* **77**, 388–399 (2005).
40. Kao, C.Y., Kim, C., Huang, F. & Wu, R. Requirements for two proximal NF-κB binding sites and IκB-ζ in IL-17A–induced human β-defensin 2 expression by conducting airway epithelium. *J. Biol. Chem.* **283**, 15309–15318 (2008).
41. Rossi, R.L. *et al.* Distinct microRNA signatures in human lymphocyte subsets and enforcement of the naive state in CD4+ T cells by the microRNA miR-125b. *Nat. Immunol.* **12**, 796–803 (2011).
42. Ishitani, T. *et al.* Role of the TAB2-related protein TAB3 in IL-1 and TNF signaling. *EMBO J.* **22**, 6277–6288 (2003).
43. Qian, Y., Kang, Z., Liu, C. & Li, X. IL-17 signaling in host defense and inflammatory diseases. *Cell. Mol. Immunol.* **7**, 328–333 (2010).
44. Onishi, R.M. & Gaffen, S.L. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* **129**, 311–321 (2010).
45. Iwakura, Y., Ishigame, H., Saijo, S. & Nakae, S. Functional specialization of interleukin-17 family members. *Immunity* **34**, 149–162 (2011).
46. Hartupee, J. *et al.* IL-17 signaling for mRNA stabilization does not require TNF receptor–associated factor 6. *J. Immunol.* **182**, 1660–1666 (2009).
47. Bulek, K. *et al.* The inducible kinase IKKi is required for IL-17–dependent signaling associated with neutrophilia and pulmonary inflammation. *Nat. Immunol.* **12**, 844–852 (2011).
48. Sun, D. *et al.* Treatment with IL-17 prolongs the half-life of chemokine CXCL1 mRNA via the adaptor TRAF5 and the splicing-regulatory factor SF2 (ASF). *Nat. Immunol.* **12**, 853–860 (2011).
49. Nakasa, T. *et al.* Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* **58**, 1284–1292 (2008).
50. Zhang, H. *et al.* Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. *Nat. Commun.* **2**, 554 (2011).
51. Gao, P. *et al.* c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* **458**, 762–765 (2009).
52. Zhu, S. *et al.* Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. *J. Exp. Med.* **207**, 2647–2662 (2010).
53. Kanayama, A. *et al.* TAB2 and TAB3 activate the NF-κB pathway through binding to polyubiquitin chains. *Mol. Cell* **15**, 535–548 (2004).
54. Liu, C. *et al.* Act1, a U-box E3 ubiquitin ligase for IL-17 signaling. *Sci. Signal.* **2**, ra63 (2009).
55. Shen, F. *et al.* IL-17 receptor signaling inhibits C/EBPβ by sequential phosphorylation of the regulatory 2 domain. *Sci. Signal.* **2**, ra8 (2009).
56. Shi, P. *et al.* Persistent stimulation with interleukin-17 desensitizes cells through SCFβ-TrCP–mediated degradation of Act1. *Sci. Signal.* **4**, ra73 (2011).

ONLINE METHODS

Human subjects. Tissue specimens from arthritic joints of individuals with rheumatoid arthritis or osteoarthritis were obtained at the time of arthroplastic surgery. Rheumatoid arthritis and osteoarthritis were diagnosed according to the criteria of the American College of Rheumatology. Normal joint tissue specimens were obtained from healthy people with knee joint injury. FLSs were also isolated from synovial joint tissues collected from these individuals. The clinical characteristics of the individuals are shown in **Supplementary Table 1**. For the lupus nephritis samples, a total of 18 individuals with lupus nephritis were included in the study. Nine samples of kidney tissue were taken adjacent to kidney tumors in individuals with kidney cancer and were used as controls. All individuals with SLE fulfilled the American College of Rheumatology classification criteria for SLE. SLE clinical activity was assessed using the SLE Disease Activity Index. Any individuals with concurrent infections were excluded from the study. For additional clinical information about the subjects, please see **Supplementary Table 2**. All individuals provided informed consent. The study was approved by the Research Ethics Board of Shanghai Renji Hospital and Shanghai Guanghua Hospital.

Mice. To generate TG-23b mice, 282 bp of genomic DNA encoding mmu-miR-23b was cloned into the plasmid pCAGGS, as previously described[52], using the forward primer 5′- ATACTCGAGAGCAGGCTGCACTGCTAGC -3′ and the reverse primer 5′- TGTAGATCTTCGAAGGCTGTTGGCTTCT -3′. The mice were created and maintained on the C57BL/6 background by Shanghai Biomodel Organism Science and Technology Development. The primer sequences used for genotyping are as follows: sense primer 5′- GGGGTTCGGCTTCTG -3′ on the pCAGGS promoter and antisense primer 5′- CGTGGTTGCGTGGTAAT -3′ on the mouse miR-23b coding sequence. TG-23b mice and their littermate controls (male and female) were used for the experiments at 6–12 weeks of age. *Il17a*-deficient mice (male and female) on a C57BL/6 background were provided by R. Flavell (Yale University). C57BL/6 (male and female), DBA/1J (male), MRL/MpJ (female) and MRL/MpJ-*Fas*^{lpr}/J (MRL/*lpr*) (female) mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. All mice were maintained in specific pathogen-free conditions. All animal experiments were performed in compliance with the guide for the care and use of laboratory animals and were approved by the institutional biomedical research ethics committee of the Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences).

MiRNA microarray and DNA microarray. Total RNA was isolated using TRIzol reagent (Invitrogen). RNA quality was assessed with an Agilent 2100 BioAnalyzer (Agilent), and only samples with RNA integrity number over eight were used. For the miRNA expression analyses, a TaqMan Low Density Array v3.0 (Applied Biosystems) was used to detect and quantify up to 754 human miRNAs or 641 mouse miRNAs using an Applied Biosystems real-time instrument, according to the manufacturer's protocol. Global mRNA expression of FLSs transfected with miR-23b mimic or control mimic were assayed with the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array, and data were analyzed further with Agilent GeneSpring GX 10 Microarray Data Analysis Software.

EAE. The induction of EAE was described previously[52]. Briefly, C57BL/6 mice at 6–8 weeks of age were immunized subcutaneously with 300 μg of the MOG (35–55) peptide in complete Freund's adjuvant (CFA) (Difco) in the back region. Pertussis toxin (List Biological Laboratories) at a dose of 200 μg per mouse in PBS was administered intravenously on the day of immunization and once more 48 h later. Disease severity was evaluated as previously described[52], and the observers scoring the mice were blinded to the treatment.

CIA. C57BL/6 Mice at 8–10 weeks of age were injected intradermally at several sites into the base of the tail with 100 μg CII (Sigma-Aldrich) emulsified with CFA on day 1 and day 21. Male DBA/1J mice at 8–10 weeks of age were immunized subcutaneously with 100 μg CII (Sigma-Aldrich) emulsified with CFA on day 1 and incomplete Freund adjuvant (Difco) on day 21. Mice were observed every 2–5 d for signs of joint inflammation and scored for clinical signs, and the observers scoring the mice were blinded to the treatment.

Bone marrow chimeras. Recipient wild-type control mice or TG-23b mice were lethally irradiated with 800 cGy before injection in the tail vein of 5×10^6 mixed bone marrow cells from donor wild-type mice. The transplanted mice were fed with drinking water containing 2 mg/ml neomycin sulfate (Bioshop Canada Inc.) for 2 weeks. After 8 weeks of bone marrow reconstitution, EAE or CIA was induced as described.

Histology and renal morphology. Tissues for histological analyses were dissected from MOG-immunized mice, CII-immunized mice and MRL/*lpr* mice and immediately fixed in 4% paraformaldehyde. Paraffin-embedded 5-μm sections of spinal cord were stained with H&E or Luxol fast blue and then examined by light microscopy. Mouse ankles were decalcified in 10% formic acid in water for 2 weeks, embedded in paraffin and sectioned at a 5-μm thickness before staining with H&E. Paraffin-embedded 2-μm sections of kidney were stained with H&E, periodic acid Schiff or Masson's trichrome staining and examined by light microscopy. For renal morphology, the severity of mouse renal lesions was graded from 0–3 (for normal (0), mild (1), moderate (2) or severe (3)) using the activity index described for human lupus nephritis[57,58].

Statistics. A two-tailed Student's *t* test was used for the statistical comparison of two groups or, where appropriate, a two-way analysis of variance followed by Bonferroni's *post hoc* test for multiple comparisons and a Mann-Whitney test for nonparametric data was used. $P \leq 0.05$ was considered significant.

Additional methods. qPCR, cell culture, micro-CT scans, adenovirus-mediated gene expression, miRNA mimics and inhibitors, ELISAs, luciferase assays, immunoblots, lentivirus-mediated gene expression and knockdown, electrophoretic mobility shift assays, ChIP, siRNA, bioinformatic prediction of transcription factor binding sites and details of EAE, CIA and renal morphology are described in the **Supplementary Methods**.

57. Austin, H.A. III., Muenz, L.R., Joyce, K.M., Antonovych, T.T. & Balow, J.E. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int.* **25**, 689–695 (1984).
58. Moreth, K. *et al.* The proteoglycan biglycan regulates expression of the B cell chemoattractant CXCL13 and aggravates murine lupus nephritis. *J. Clin. Invest.* **120**, 4251–4272 (2010).